Imidazolium-based achiral and chiral Ionic Liquids; Synthesis, Antimicrobial Toxicity and Biodegradation Studies

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Dedicated to Mam and Dad

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph.D is entirely my own work, that I have exercised reasonable care to ensure that the work is original, and does not to the best of my knowledge breach any law of copyright, and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

A series of novel (a)chiral imidazolium based Ionic Liquids (ILs) were synthesised, characterised and screened for biodegradation and antimicrobial toxicities. The effect of incorporating ester and amide moieties into the IL side chain, on these biological systems was studied. 18 achiral imidazolium ILs were prepared containing alkyl (C5-C14) ester or amide functionalities in the cation side chain. A range of amino acid ester based ILs (48 examples) were also synthesised and fully characterised. Similar synthetic methodology was employed for the preparation of both these class of ILs. 17 Chiral Ionic Liquids (CILs) with dipeptidyl moieties in the side chain were also designed, prepared and characterised. All compounds reported in the thesis were characterized by a range of spectroscopic techniques including: ^{1}H, ^{13}C, DEPT 135 and HMQC NMR in addition to IR and MS.

Toxicity and biodegradation studies were carried out on the novel ILs. Minimum Inhibitory Concentration assays were used to screen ILs toxicity against several strains of bacteria (gram positive and gram negative strains). Most ILs displayed relatively low levels of toxicity towards the isolated strains, with inhibition only evident at higher test concentrations (200 mM) in these tests. Antimicrobial studies were also performed against clinically resistant strains of fungi and bacteria. Inhibition of the resistant MRSA bacterial strain was noted for some examples. An Activated Sludge assay was also set up in order to investigate the biodegradation of ILs. A modified OECD 301 A (Die-Away test, 28 day test) was employed using inoculum from a waste water treatment facility in a pharmaceutical company in south Co. Dublin. The breakdown of some ILs was monitored *via* Direct Infusion ElectroSpray Mass Spectrometry (DI-ESI MS). Some promising results were achieved from this study, and possible metabolite structures have been elucidated using the MS data.

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Table of Contents

Abbreviations

A

AAILs: Amino Acid Ionic Liquids

Ace: Acesulfamate

ACN: Acetonitrile

B

BF_4: Tetrafluoroborate

bmim: 1-Butyl-3-methylimidazolium

BOC: *Tert*-butoxycarbonyl

BOD: Biochemical Oxygen Demand

BOP: Benzotriazolyl-1-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate

Bpoc: Diphenylisopropyloxycarbonyl

C

CBD: Calgary Biofilm Device

Ch: Choline

CILs: Chiral Ionic Liquids

COD: Chemical Oxygen Demand

COSY: Correlation Spectroscopy

D

DCC: Dicyclohexylcarbodiimide

DCM: Dichloromethane

DCU: *N, N'*-Dicyclohexylurea

DEPT: Distortionless enhancement by polarization transfer

DIC: Diisopropylcarbodiimide

dmim: 2,3-Dimethylimidazolium

DMSO: Dimethyl sulfoxide

DOC: Dissolved Organic Carbon

E

EDC: *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride

EIC: Extracted Ion Count

emim: 1-Ethyl-3-methylimidazolium

EPS: Extracellular polymeric substance

ESI-MS: Electrospray Ionisation Mass spectrometry

F

FMOC: 9-Fluorenylmethoxycarbonyl

H

$HgCl_2$: Mercuric Chloride

HILIC: Hydrophilic interaction chromatography

hmim: 1-Hexyl-3-methylimidazolium

HMQC: Heteronuclear multiple quantum coherence

HOAt: 1-Hydroxyazobenzotriazole

HOBt: 1-Hydroxybenzotriazole

HPLC: High Performance Liquid Chromatography

I

IC: Inorganic Carbon

IL: Ionic Liquid

IR: Infrared

L

Lac: L-lactate

M

m.p.: Melting point

m/z: Mass to charge ratio

MBC: Minimum bactericidal concentration

MBEC: Minimum biofilm eradication concentration

MIC: Minimum inhibitory concentration

mim: 1-Methyl imidazolium

MRSA: Methicillin Resistant Staphylococcus aureus

MS: Mass spectrometry

N

$N(CN)_2$: Dicyanamide

NAILs: Naphthenic acid ionic liquids

NMR: Nuclear Magnetic Resonance

NTf_2: Bis(trifluoromethyl)sulfonyl amide

O

$OctOSO_3$: Octyl sulfate

OECD: Organisation for Economic Co-operation and Development

omim: 1-Octyl-3-methylimidazolium

P

PF_6: Hexafluorophosphate

PIC: Phenyl isopropyl carbodiimide

POPs: Persistent Organic Pollutants

PyBOP: Benzotriazol-1yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate

R

RP-HPLC: Reverse Phase High Performance Liquid Chromatography

RT: Room temperature

S

Sac: Saccharinate

SbF_6: Hexafluoroantimonate

SDS: *n*-Dodecyl sulfate

SS: Suspended solids

T

TEA: Triethylamine

TFA: Trifluoroacetic acid

THF: Tetrahydrofuran

TIC: Total Ion Count

TOC: Total Organic Carbon

TSILs: Task Specific Ionic Liquids

V

VOC: Volatile organic compound

Z

Z/CBz: Benzyloxycarbonyl

Chapter 1: Literature Review

Biodegradation studies of Ionic Liquids

1.1 Introduction

Ionic liquids (ILs) have been described as molten salts that are entirely ionic in nature, comprising both a cationic and anionic species and by definition having a melting point below 100 °C.[1]

ILs usually consist of a large unsymmetrical organic cation associated with a polyatomic anion that may be either organic or inorganic[1] (Figure 1.1). Symmetrical species tend to pack more effectively in the solid state, and so highly symmetrical anions and cations tend to form salts with higher melting points. However, for most applications a lower melting point (by definition < 100 °C) is preferred for ILs. This is one of the reasons that in many cases the cation is designed with a reduced symmetry (e.g. 1-butyl-3-methylimidazolium cation is preferred over 1,3-dibutyl or 1,3-dimethylimidazolium cations).[2]

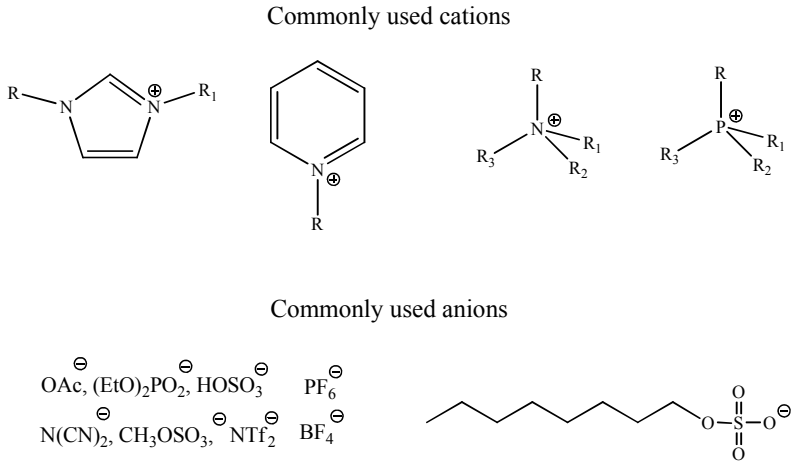


Fig. 1.1: Cations and anions frequently used in Ionic Liquid formation.

The modular nature of ILs means that structural modifications can either be made to the anion, the cationic core or substituents on the anion or cation. Hence, a wide diversity in IL structure is possible and by altering either the cationic or anionic component of an IL, the physical properties of the IL can readily be fine-tuned. Physical properties which can be tailored to the requirements of a process include the melting point, viscosity, density, solubility and hydrophobicity of the ionic liquid. Moreover, reaction products can often be separated more easily from an ionic liquid than from conventional solvents. These benefits make ILs an attractive choice of solvent in many important chemical processes, with

examples reported in the areas of catalysis,[3] biocatalysis,[4] synthetic chemistry[5] and electrochemistry.[6] A number of detailed reviews have demonstrated the advantages of using ILs as new solvent types.[3,5,7,8]

In addition to the favourable physical and chemical properties of ILs, their low vapour pressures and near lack of flammability make them interesting as potentially 'green' solvents. In general, the negligible volatility of these ILs means that air pollution by gaseous release is not a concern. However, the potential release of ionic liquid vapours (or decomposition products) must be considered when ILs are used at elevated temperatures.[9,10] In addition, Seddon *et al.* reported that 1-alkyl-3-methylimidazolium ILs can be distilled at 200-300 °C.[11] Even though the release of ILs into the environment from gaseous waste streams is not expected to be a significant cause of pollution[9] many ILs are water soluble and also thermally and chemically stable. Hence, the environmental impact of these new solvents could prove to be a cause for concern if they should escape into the environment *via* waste-water effluents.

Hazard assessment of ILs has now become an important area of research and many groups have reported toxicity, ecotoxicity and biodegradation studies on ILs. The toxicity of ILs has been evaluated in systems involving microorganisms[12,13,14,15] as well as terrestrial invertebrates such as earthworms,[16] aquatic species, including the zebrafish (*Danio rerio*)[17] as well as waterfleas, (*Daphnia magna*)[18,19] and algae.[20] The toxicity of ILs towards terrestrial plants has also been investigated and more recent studies have even screened human cells.[21] From the accumulated toxicity data, trends have emerged in terms of structural features that give rise to increased toxicity in ILs. Notably, ILs that possess a substituted cation with an alkyl chain of greater than 8 carbons, and also those with lipophilic anions, have been shown to display undesirable toxicity.[18,22,23] A recent comprehensive review of toxicity studies of ILs was reported by Zhao.[24]

Many classes of ILs are water soluble, with the exceptions of those containing lipophilic anions, such as bis(trifluoromethyl)sulfonyl amide [NTf_2], or hexafluorophosphate [PF_6] anions. A highly desirable property in the design of the first generation of 1-butyl-3-

methylimidazolium [bmim] ILs was chemical stability, and where possible, inertness. These features have given ILs the versatility to be used in a wide variety of chemical reactions. However, this stability has led to concern over whether ILs might also prove resistant to biological breakdown and hence accumulate in the environment. Due to the vastness of the library of ILs that might conceivably be synthesised, it is important at the design stage to consider factors which may influence the toxicity and biodegradation of the ILs. The pioneering work of Boethling[25,26] in the design of biodegradable chemicals has greatly assisted researchers in the field of ILs by giving guidelines for the synthesis of environmentally benign solvents.

Boethling highlighted a number of factors that can improve the mineralization of organic compounds by mixed microbial communities. An increase in aerobic biodegradation is usually observed for those compounds that contain the following structural features:

- benzene rings, and unsubstituted linear alkyl chains (> 4 carbons in chain length)
- groups that provide possible sites for enzymatic hydrolysis (especially oxygen atoms in the form of hydroxyls, aldehydes, or carboxylic acids)

However, increased resistance to aerobic biodegradation is generally observed for those compounds which contain the following structural motifs:

- halogens; with chlorine and fluorine being particularly persistent
- chain branching; particularly where tertiary nitrogens or quaternary carbons are part of the structure, or where multiple branches are present in the same molecule
- nitro, nitroso, azo and arylamino groups
- polycyclic frameworks of the kind encountered in fused aromatic hydrocarbons (e.g. benzo[a]pyrene)
- heterocycles (e.g. pyridine rings)
- aliphatic ethers

It should be noted that these 'rules of thumb' are only guidelines and that the presence of a single desirable or undesirable structural feature within a molecule does not guarantee either biodegradability or persistence in the environment. While structural motifs that are less commonly encountered by enzymes in nature may result in poor biodegradability, even xenobiotics (molecular species completely foreign to a biological system) can be biodegraded either by 'fortuitous' metabolism (in the presence of a natural substrate, a 'co-metabolite') or by 'gratuitous' metabolism[25] (which takes advantage of an existing pathway).

A wide variety of esterase enzymes (EC 3.1.5-EC 3.1.15) are present in the environment and many of these exhibit broad substrate specificity.[27] These enzymes are ubiquitously found in microbial communities which ensure that biodegradation is frequently improved if the substrate contains ester moieties. In certain cases, such as that of the alkylsulfate surfactants, etherase and sulfatase enzymes may also play a role in biodegradation. Enzymatic oxidation provides another important means by which living systems can mineralize foreign molecules, converting them into water-soluble species by hydroxylation or epoxidation. The oxidase enzymes can even act upon species that would normally be considered inert, such as unsubstituted alkyl chains and aromatic rings. In the environment this step is carried out by bacteria and is frequently the rate-limiting step in the degradation of organic molecules.[27] In particular, unsubstituted alkyl chains with greater than four carbons and benzene rings provide possible sites for attack by oxygenases. These substituents are particularly beneficial when hydroxylation is required to increase solubility and aid breakdown of a potential toxin.

Biodegradability data gathered from decades of analysis of pesticide, pollutant and surfactant residues[27] enabled Boethling[25] to identify molecular features that impede biodegradability, leading to persistence in the environment. Persistent Organic Pollutants (POPs) typically contain halogens (especially chlorine and fluorine), chain branching (in particular problems associated with quaternary carbons), tertiary amines, polycyclic systems (more than three fused ring systems) or heterocycles – all features that tend to decrease the biodegradability of chemicals.

A compromise must arise in the design of biodegradable ionic liquids in order to balance the required stability of the solvent with favourable biodegradability. Such considerations are especially important when groups introduced into the IL to improve biodegradation may limit its practical applications. An example of this bottleneck is observed by the requirement of a biodegradable ionic liquid for catalytic hydrogenation reactions. Whilst examples of pyridinium cations with good biodegradability[28,29] have been reported, they are unsuitable as solvents for hydrogenation reactions because of the lower activity of the catalyst and in some cases, the susceptibility of the pyridinium ring to reduction. In imidazolium solvated hydrogenation reactions, maintenance of catalyst efficiency is obtained, while the heterocycle is robust enough even for high pressure reductions.

1.2 Biodegradation Assays

Biodegradation assays are generally carried out according to OECD Guidelines for Testing of Chemicals: A series of guidelines laid down by the Organisation for Economic Co-operation and Development with the aim of reproducibly assessing the effects of chemicals on workers and the environment.

Commonly used terms and abbreviations in accordance with OECD Guidelines[30] include

- *Biodegradation:* conversion or breakdown of a chemical structure catalysed by enzymes *in vitro* or *in vivo*, resulting in loss of specific properties, especially biological activity.

- *Readily Biodegradable:* an arbitrary classification of chemicals that have passed certain specified screening tests for ultimate biodegradability; these tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environments under aerobic conditions.

- *Ultimate Biodegradation:* the level of degradation achieved when the test compound is totally utilised by micro-organisms resulting in the production of

carbon dioxide, water, mineral salts and new microbial cellular constituents (biomass).

- *Primary Biodegradation:* an alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance.

- *Mineralization:* the complete degradation of an organic compound to small molecules, such as carbon dioxide and water under aerobic conditions and carbon dioxide, water and methane under anaerobic conditions.

- *Inoculum:* the source of microorganisms used to carry out biodegradation of the test substance; typically this may be derived from a variety of sources: activated sludge; sewage effluents (unchlorinated); surface waters and soils; or from a mixture of these. For the DOC Die-Away (301 A), CO_2 Evolution (301 B) and Manometric Respirometry (301 F) methods if activated sludge is used, it should be taken from a treatment plant or laboratory-scale unit receiving predominantly domestic sewage. Inocula from other sources, usually yielding lower cell densities, have been found to give higher scattering of results. For the Modified OECD Screening (301 E) and Closed Bottle (301 D) methods, a more dilute inoculum without sludge flocs is needed and the preferred source is a secondary effluent from a domestic waste water treatment plant or laboratory-scale unit. For the MITI (I) (301 C) method, the inoculum is derived from a mixture of sources. Details of the sources and preparation of inocula are described under the headings of the specific test methods.[30]

- *Bioaccumulation*: Gradual build up over time of a chemical in a living organism.

- *DOC:* Dissolved Organic Carbon (DOC/L), is the organic carbon present in solution or that which passes through a 0.45 μm filter or remains in the supernatant after centrifuging at approximately 4,000 g (around 40,000 ms^{-2}) for 15 minutes.

- *TOC:* Total Organic Carbon, is the sum of the organic carbon in solution and in suspension.

- *BOD:* Biochemical Oxygen Demand is the amount (mg) of oxygen consumed by micro-organisms when metabolising a test substance.

- *COD:* Chemical Oxygen Demand is the amount (mg) of oxygen consumed during the oxidation of a test compound with hot acidic dichromate; it provides a measure of the amount of oxidisable matter present.

- *10-day window:* The ten days immediately following the attainment of 10 % biodegradation.[30]

The biodegradability of ionic liquids has been evaluated using a number of standard methods. The most commonly used tests are the Modified Sturm and Closed Bottle Tests (OECD 301 B and D respectively), the DOC Die-Away Test (OECD 301 A) and also the CO_2 Headspace Test (ISO 14593), which is the reference method for laboratory testing of ultimate biodegradability.

Each method has a relative principle of the test, where by the degradation is monitored by the determination of parameters such as Dissolved Organic Carbon (DOC), carbon dioxide production and oxygen uptake. Measurements of these parameters are taken at sufficiently frequent intervals, in order to identify the beginning and end of the biodegradation. Typically the tests are performed over a 28 day period; however, tests may be ended before the 28 day time frame if the biodegradation curve has reached a plateau for the last three measurements. Also, the tests can be extended beyond the 28 days when the biodegradation curve has shown that biodegradation has started but the plateau has not been reached by the last day (day 28). In the later case, the chemical would not be deemed *readily biodegradable*. The method used to assess the biodegradation of organic chemicals depends on several fundamental physical properties of the compound in question, i.e. whether the test substance is soluble in water (to at least 100 mg/L), volatile or adsorbing in nature. Table 1.1 illustrates the suitability of the test methods based on these properties of the test compound. However, each test method varies depending on the parameters investigated

during the test (DOC, CO_2 evolution or O_2 consumption) and also on the general procedure and preparations followed.

Table 1.1: Applicability of OECD test methods.

Test	Analytical method	Suitability for compounds which are:		
		poorly soluble	volatile	Adsorbing
DOC Die-Away (301 A)	Dissolved organic carbon	-	-	-/+
CO_2 evolution (301 B)	Respirometry: CO_2 evolution	+	-	+
MITI (I) (301 C)	Respirometry: Oxygen consumption	-	-/+	+
Closed bottle (301 D)	Respirometry: Dissolved oxygen	-/+	+	+
Modified OECD Screening (301 E)	Dissolved organic carbon	-	-	-/+
Manometric respirometry (301 F)	Oxygen consumption	+	-/+	+
CO_2 Headspace Test (ISO 14593)	CO_2 evolution	+	+	+
OECD 309	^{14}C Labelling	-/+	+	+
ASTM 5988	CO_2 production/BOD	-	-	-/+

Suitable method to screen compound: +; Unsuitable method to screen compound: -

1.2.1 Die-Away Test (OECD 301 A)

In the Die-Away Test[30] the compound under investigation should ideally be non-volatile and possess a water solubility of at least 100 mg/L. It is also desirable to know the carbon content and the purity of the compound prior to testing. A measured volume of inoculated

mineral medium, along with a known concentration of the test substance (10-40 mg DOC/L) as the main source or organic carbon, is aerated in the dark at 22 ± 2 °C. The breakdown of the compound is monitored by DOC analysis at frequent time intervals over 28 days. The degree of biodegradation is calculated by expressing the concentration of DOC removed (corrected for the DOC in the blank control) as a percentage of the concentration initially added. The percentage biodegradation is calculated using the following equation:

$$D_t = \left[1 - \frac{C_t - C_{b(t)}}{C_o - C_{b(o)}} \right] \times 100$$

Where:

- D_t = the percentage biodegradation at time t
- C_o = mean starting concentration of DOC in the inoculated culture medium containing the test substance (mg DOC/L)
- C_t = mean concentration of DOC in the inoculated culture medium containing test substance at time t (mg DOC/L)
- $C_{b(o)}$ = mean starting concentration of DOC in the blank mineral medium
- $C_{b(t)}$ = mean concentration of DOC blank inoculated mineral medium at time t (mg DOC/L)

The test system should be set up so that the there are:

- Two flasks containing the test suspension (inoculum and test compound)
- Two flasks containing the inoculum blank (inoculum only) and a number of controls may be run also. The controls used are the procedure control, which is the reference compound (these are compounds which have been classified as readily

biodegradable, such as aniline, sodium *n*-dodecyl sulfate (SDS) and sodium acetate) present with the inoculum.

- An adsorption control can be used where the flask contains the inoculum, test compound and a sterilising reagent.

- Finally an abiotic control is run in order to investigate the possible abiotic breakdown of the test compound. This control consists of the inoculum and a sterilising agent which inactivates the metabolising inoculum.

Sampling should be carried out so that a sufficient amount of samples are taken to allow the percentage removal in the 10-day window (10 days immediately following the attainment of 10 % biodegradation) to be assessed. If subsequent analysis is carried out on the day of sampling, the next sampling day can be determined by considering the result of the analysis. If the samples are preserved (stored at low temperatures i.e. 2-4 °C for a maximum of 48h, or below -18 °C for longer amount of time) samples should be taken daily or every two days.

Water soluble compounds can be screened by the DOC Die-Away method, while poorly water soluble compounds (alkanes, fats, oils, hydrophobic ionic liquids) and those which are adsorbing in nature are not suitable for this test.

1.2.2 CO_2 Evolution (*Modified Sturm Test*) (OECD 301 B)

This well established method has extensively been applied to assess the readily biodegradability of organic compounds. It is based on the original tests carried out by Sturm[31] and the principle is to measure the carbon dioxide produced as a result of microbial respiration. This method has been used to screen compounds which are poorly soluble, and those which strongly adsorb. Test species must also be non-volatile, as the production of carbon dioxide is considered to be the primary indication of microbial activity.

A measured amount of inoculated mineral medium, with a known concentration of the test compound (10-20 mg DOC or Total Organic Carbon, TOC/L) as the main source of organic carbon, is aerated with carbon dioxide free air at a controlled rate in the dark, over

28 days. Biodegradation is determined over this time frame by measuring the carbon dioxide produced. Carbon dioxide formed is trapped in barium or sodium hydroxide and is then measured by titration of the residual OH or as inorganic carbon (CO_2). The amount of carbon dioxide evolved from the test substance (relative to that obtained from blanks) is expressed as a percentage of theoretical maximum carbon dioxide production $ThCO_2$.

The experimental set up of this test should include:

- two vessels containing the test substance and inoculum (test suspension)
- two vessels with only the inoculum present (inoculum blank)
- a procedure control vessel (in which the reference compound (e.g. sodium n-dodecyl sulfate) and inocula are present)
- an abiotic control (containing the test substance and a sterilising reagent)
- a toxicity control can be set up (test substance, reference standard and inoculum) in order to check the possible inhibitory effect of the tested chemical.

To determine the carbon dioxide produced, it is suggested that the relevant analysis be carried out every second or third day and following this, at least every fifth day until the last day of testing (day 28). This is to ensure that the 10-day window is identified.

The percentage biodegradation is calculated from the following equation:

$$\% \text{ degradation} = \frac{\text{mg } CO_2 \text{ produced}}{ThCO_2 \times \text{mg test substance added}} \times 100$$

Biodegradation curves should be plotted and the 10-day window should be clearly indicated. An example of a general biodegradation curve is seen in Figure 1.2. In this curve, three different types of substances are being investigated. Three test substances reach the pass level (60 %) before or on the 10 day period. Peptones, which are water soluble compounds that are obtained by acid or enzyme hydrolysis of natural proteins, and are used as nutrients in culture media, 100 % degradation is achieved within 5 days of the test

period. >60 % biodegradation is observed for the glycol ether compound just within the 10 day window. Adsorption of the aliphatic amine to the activated sludge is evident from the biodegradation curve (Figure 1.2). This physicochemical phenomenon is indicated when there is complete or significant removal of the test compound in the first 3 hours and the difference between test and blank samples remains at an unexpectedly low value.

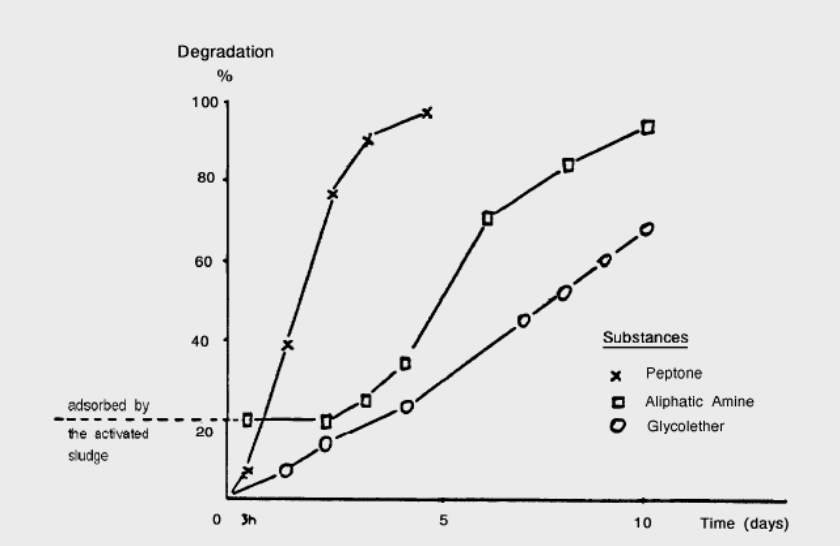


Fig. 1.2: An example of a plot of biodegradation curves.[32]

Water soluble or insoluble compounds may be tested using OECD 301 B CO_2 Evolution Test, while volatile and adsorbing compounds are less suited to this test. The degradation of non-adsorbing pollutants, such as trichlorobenzenes has been assessed using this method.[33]

1.2.3 Closed Bottle Test (301 D)

In this method, insoluble and volatile compounds may be tested provided that the necessary precautions are taken. The degradation for insoluble test substance can be mistakenly low if the bottles are not agitated periodically for the duration of the incubation. In the Closed Bottle Test a solution of the sampled chemical in mineral medium (2-5 mg/L), is inoculated with a relatively small number of microbes from a mixed population and maintained in completely full, closed bottles in the dark at a constant temperature. Biodegradation is monitored by analysis of dissolved oxygen over 28 days. The amount of oxygen consumed

by the micro-organisms (corrected for the uptake of O_2 by the blank inoculum) is expressed as a percentage of the ThOD. The system should be set up as follows:

- At least ten bottles containing the test compound and the inoculum
- At least ten bottles containing just the inoculum (test blank)
- At least ten bottles containing a reference compound and the inoculum

Test bottles (at least in duplicate) should be withdrawn for dissolved oxygen analysis (see Figure 1.3) at weekly time intervals over the 28 days.

Fig. 1.3: Dissolved oxygen analysis carried out on the closed bottles.[34]

Weekly sampling establishes the percentage removal in a 14-day window, and sampling every 3-4 days allows the 10-day window to be known. In the case of nitrogen-containing test compounds, corrections to the uptake of oxygen by any nitrification occurring should be considered. This is usually completed by analysing the concentration of oxygen uptake using the electrode method as before, and then samples taken from the BOD bottles for nitrites and nitrates analysis. The percentage degradation is calculated by dividing the specific Biochemical Oxygen Demand BOD as mg of O_2 per mg of the test compound, by

the specific ThOD or in some cases the COD. The following equations are used to carry out these calculations:

$$\% \text{ biodegradation} = \frac{\text{BOD (mg } O_2 \text{ / mg test substance)}}{\text{ThOD (mg } O_2 \text{ / mg test substance)}} \times 100$$

$$\% \text{ biodegradation} = \frac{\text{BOD (mg } O_2 \text{ / mg test substance)}}{\text{COD (mg } O_2 \text{ / mg test substance)}} \times 100$$

The degradation of aromatic, hydroaromatic and aliphatic organic compounds can be screened using this test. Compounds which are volatile and adsorbing can be studied; however those lipophilic examples are not ideally suited to this method. Degradation of antibiotics[35] and perfluoroalkylated substances (PFAS)[36] has also been investigated using the Closed Bottle Test.

1.2.4 CO_2 Headspace Test (ISO 14593)

Biodegradation assessment by the ISO 14593 considers the extent to which an organic compound is mineralized by a microbial community to evolve carbon dioxide and gives a measure of *ultimate biodegradation*. The CO_2 Headspace Test is the preferred method for screening poorly soluble and highly adsorbent species. Several tests study carbon dioxide evolution as a parameter of degradation assessment, such as work by Sturm and Gledhill[37] to assess the readily biodegradation of chemicals. However, there are a number of drawbacks to these methods, and improvements have been made to these tests. In the Sturm methodology[31], it has been reported that inorganic carbon (IC) can accumulate in the mineral medium during the application of the reference standard. The collection of carbon dioxide therefore does not give a true reflection of the amount of carbon dioxide evolved as a result of microbial metabolism. Thus, the specification that >60 % of the $ThCO_2$ must be collected within a 10-day window for a compound to be classified as readily biodegradable would not be met for some test compounds which can be regarded as readily biodegradable if only the DOC removal method is used. The Sturm Test can also be time-consuming, cumbersome, and prone to experimental error and is inapplicable to volatile compounds.

However, independent studies by Struijs and Stoltenkamp,[38] and Birch and Fletcher[39] have resulted in improved methods for studying CO_2 evolution, using more compact test systems with the significant advantage over the Sturm Test that volatile compounds can be assessed, and any delay in measuring carbon dioxide can be minimised. These two tests were combined to give the ISO Headspace CO_2 Biodegradation Test, and this was ring tested in 1995.[40]

In the CO_2 Headspace Test, the sample chemical (at a concentration of 20 mg/L) as the main source of energy and carbon, is incubated in a mineral medium inoculated with a mixed population of microbes. The test is then carried out in sealed bottles with a headspace of air which provides a reservoir of oxygen. The percentage biodegradation is expressed as a percentage of the Theoretical maximum Inorganic Carbon evolved (TIC) based on the concentration of the test compound initially added. The percentage biodegradation is calculated from the following equation:

$$\% \text{ biodegradation} = \frac{(TIC_t - TIC_b)}{TOC} \times 100$$

Where:

- TIC_t = mg TIC in bottle at time t.
- TIC_b = mean amount (mg) of TIC in blank bottles at time t
- TOC = mg TOC initially added to bottle.

A biodegradation curve can then be plotted of the percentage biodegradation versus time, (see Figure 1.4).

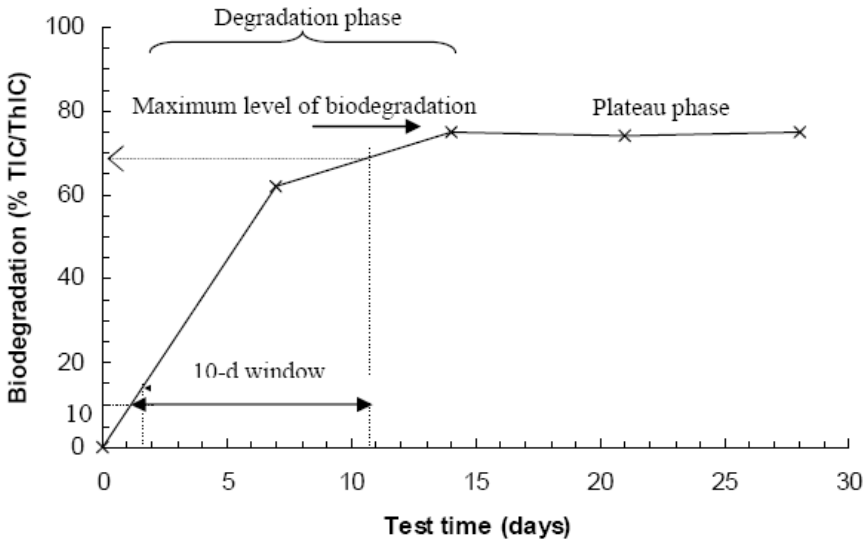


Fig. 1.4: An example of a biodegradation curve using the CO_2 Headspace Test.[41]

Hydrophilic and hydrophobic substances are suitable for biodegradation analysis by the CO_2 Headspace Test, including organic solvents, Volatile Organic Compounds VOCs, toluene, hexane, oils, fats, nitrates, acetates and ammonium containing compounds. Surfactants biodegradation has also been assessed using the CO_2 Headspace method.[42]

1.2.5 OECD 309-Aerobic mineralization in Surface Water: Simulation Biodegradation Test

The OECD 309 test measures the biodegradation of a test substance over time at low concentrations in aerobic natural water and to quantify the observations in kinetic rate expressions. The test is performed in batch by "pelagic test" (i.e. in batch by incubation of the test compound with surface water only) or "suspended sediment test" (test substance incubated with surface water amended with suspended solids or sediment) to simulate a water body with suspended solids or re-suspended sediment.[43]

The test vessels are incubated in the dark at an environmental temperature (e.g. 20-25 °C) under aerobic conditions and agitated for 60 days. At least two different concentrations of test substance are required. The maximum test concentration should be below 100 µg/L and

the lowest test concentration in the range of <1-10 µg/L. Two samples are taken from each test flask at each sampling time. Degradation is traced at appropriate time intervals by measuring either the residual ^{14}C or the residual concentration of the test substance. The total mineralization and primary biodegradation are determined by separate ^{14}C labelling experiments within the molecule. ^{14}C labelling of the most stable part of the compound allows the determination of the total mineralization. Isotopic labelling of a less stable region of the molecule enables the assessment of only primary biodegradation. Periodic measurements of pH and oxygen concentration in the test system must be also reported.

This test is applicable for substances with low volatility or are non-volatile (with Henry's law constants less than 1 Pa · m^3/mol (approx. 10^{-5} atm · m^3/mol). Reference compounds are also used to confirm there is an active microbial population in the surface water.

1.2.6 ASTM D 5988- Standard method for determining Aerobic Biodegradation in Soil of Plastic Materials or Residual Plastic Materials after composting

Biodegradability of synthetic plastic materials in soil or a mixture of soil and mature compost under laboratory conditions is determined with the ASTM D 5988 test.[44] Soil is a species-rich source of inocula for the assessment of biodegradation of plastics in the environment. While soil provides an example of a typical medium where spillage of a substance might occur, its biological activity is lower than that of other inocula, such as activated sludge or compost.

The test species should be of known weight and have sufficient carbon content. Tested substances should be in the form of films, pieces, fragments, powders or formed articles, or in aqueous media, and added directly to the soil matrix. A reference substance (for example starch or cellulose) is used to test the activity of the soil. If <70 % biodegradation is obtained for the reference control after six months, the test must be reported as invalid and should be repeated with fresh soil inoculum. Carbon dioxide production measured for a tested material is expressed as a fraction of the measured or calculated carbon content and is reported with respect to time, from which the biodegradation is assessed. Biochemical Oxygen Demand (BOD) can also be determined, and the level of biodegradation can be

reported by comparing the BOD with the ThOD. The percentage biodegradation from oxygen consumption is determined by first calculating the specific biochemical oxygen demand (BODs) of the test substance using the equation:

$$BODs = \frac{B_t - B_{bt}}{C_T}$$

Where:

- B_t = the BOD of the flasks containing test material at time t, in mg/kg of the test soil
- B_{bt} = the BOD of the blank control at time t, in mg/kg of the test soil
- C_T = the concentration of the test material in the test flasks in mg/kg of the test soil

The percentage biodegradation as a ratio of the specific biochemical oxygen demand to the ThOD, in mg/g of the test substance is calculated as shown below:

$$\% \text{ biodegradation} = \frac{BODs}{ThOD} \times 100$$

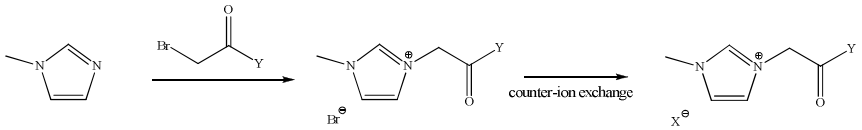
This test has been applied to the investigation of biodegradability of imidazolium ionic liquids in soil (see Section 1.3.5).

1.3 Biodegradation studies of Ionic Liquids

Despite ionic liquids appearing increasingly in the research literature for over a decade,[5] biodegradation data for this class of compounds have only appeared in recent years.[45,46] Biodegradation studies of imidazolium, pyridinium and ammonium ionic liquids have emerged in the literature as of late. Convincing examples of biodegradable phosphonium ionic liquids **66**-**67** have yet to emerge, and so these are only briefly referred to in Table 1.5. Primary biodegradation and metabolite profiling of ionic liquids has also been investigated, including methods of identifying metabolites arising from ionic liquid biodegradation. Elucidation of possible biodegradation pathways occurring during the biological breakdown has also been examined by some groups.

1.3.1 Biodegradation of imidazolium-based ionic liquids

In 2002 Gathergood and Scammells[46] were the first to undertake biodegradation studies of ionic liquids when they introduced functional groups which would be susceptible to enzymatic hydrolysis (i.e. ester/amides) into the ionic liquid cation side chain. Biodegradation of the resulting 3-methyl-1-(alkyloxycarbonylmethyl)imidazolium ionic liquids was then compared with that of the commonly used dialkylimidazolium salts, [bmim][BF_4] and [bmim][PF_6] using the modified Sturm and Closed Bottle Tests (OECD 301 B and D respectively). Compounds which reached a biodegradation level higher than 60 % are referred to as ‘‘readily biodegradable’’. The ionic liquids were prepared by the route depicted in Figure 1.5 and Table 1.2.[46]



1

2 Y = OMe
3 Y = OEt
4 Y = OPr
5 Y = OBn
6 Y = OHex
7 Y = OOct
8 Y = NHBu
9 Y = NMeBu
10 Y = NEt_2

11 Y = OMe, X = BF_4
12 Y = OEt, X = BF_4
13 Y = OPr, X = BF_4
14 Y = OOct, X = BF_4
15 Y = NHBu, X = BF_4
16 Y = NMeBu, X = BF_4
17 Y = NEt_2, X = BF_4
18 Y = OMe, X = PF_6
19 Y = OEt, X = PF_6
20 Y = OPr, X = PF_6
21 Y = OOct, X = PF_6
22 Y = NHBu, X = PF_6
23 Y = NMeBu, X = PF_6
24 Y = NEt_2, X = PF_6
25 Y = OMe, X = NTf_2
26 Y = OEt, X = NTf_2
27 Y = OPr, X = NTf_2
28 Y = OOct, X = NTf_2
29 Y = NHBu, X = NTf_2
30 Y = NEt_2, X = NTf_2
31 Y = OEt, X = $N(CN)_2$
32 Y = NMeBu, X = $N(CN)_2$

Fig. 1.5: Preparation of ester and amide-functionalised ionic liquids[46]

Table 1.2: Ester and amide-functionalised ionic liquids - parentheses indicate ILs that are solids at RT.

Y =	**X = Br**	**X = BF_4**	**X = PF_6**	**X = NTf_2**	**X = $N(CN)_2$**
OMe	(**2**) [MeOCO CH_2mim][Br]	**11** [MeOCO CH_2mim][BF_4]	(**18**) [MeOCO CH_2mim][PF_6]	**25** [MeOCO CH_2mim][NTf_2]	**-**
OEt	**3** [EtOCO CH_2mim][Br]	**12** [EtOCO CH_2mim][BF_4]	**19** [EtOCO CH_2mim][PF_6]	**26** [EtOCO CH_2mim][NTf_2]	**31** [EtOCO CH_2mim] [$N(CN)_2$]
O^{n}Pr	**4** [PrOCO CH_2mim][Br]	**13** [PrOCO CH_2mim][BF_4]	**20** [PrOCO CH_2mim][PF_6]	**27** [PrOCO CH_2mim][NTf_2]	**-**
O^{n}Bu	**5** [BuOCO CH_2mim][Br]	**-**	**-**	**-**	**-**
O^{n}Hex	**6** [HexOCO CH_2mim][Br]	**-**	**-**	**-**	**-**
O^{n}Oct	**7** [OctOCO CH_2mim][Br]	**14** [OctOCOCH_2mi m][BF_4]	**21** [OctOCO CH_2mim][PF_6]	**28** [OctOCO CH_2mim][NTf_2]	**-**
NH^{n}Bu	(**8**) [BuNHCO CH_2mim][Br]	**15** [BuNHCO CH_2mim][BF_4]	(**22**) [BuNHCO CH_2mim][PF_6]	**29** [BuN(CH_3)CO CH_2mim][NTf_2]	**-**
NMe^{n}Bu	**9** [BuN(CH_3)CO CH_2mim][Br]	**16** [BuN(CH_3) $COCH_2$mim] [BF_4]	(**23**) [BuN(CH_3)COC H_2mim][PF_6]	**-**	**32** [BuN(CH_3) OCH_2mim] [$N(CN)_2$]
NEt_2	(**10**) [Et_2NCO CH_2mim][Br]	**17** [Et_2NCO CH_2mim][BF_4]	**[24]** [Et_2NCO CH_2mim][PF_6]	**[30]** [Et_2NCO CH_2mim][NTf_2]	**-**

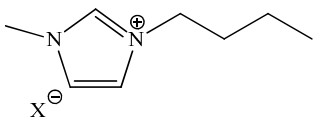
During the design of these novel ionic liquids, the effect on the physical properties of these imidazolium based ionic liquids following modification to the cation was a concern. The introduction of an ester or amide alkyl chain may affect the melting points and solubility of

the parent ionic liquids. Imidazolium ionic liquids with ester side chains were generally found to be liquids at room temperature (21 out of 23 examples). Ionic Liquids [$MeOCOCH_2$mim][BF_4] **11** and [$EtOCOCH_2$mim][BF_4] **25** demonstrated that by changing the counter ion to BF_4 or NTf_2, analogues containing the methyl ester can be prepared which are liquid at room temperature. The amide derivatives were seen to have higher melting points than the ester containing ionic liquids. It was predicted that the cisoid-transoid mixture of isomers for the amide examples, would lead to a depressed melting point, and might give rise to ILs that are liquid at room temperature. However, although the BF_4 ionic liquids [$BuNHCOCH_2$mim][BF_4] **15**, [$BuN(CH_3)COCH_2$mim][BF_4] **16** and [Et_2NCOCH_2mim][BF_4] **17** were liquid at room temperature, [$BuNHCOCH_2$mim][Br] **8**, [Et_2NCOCH_2mim][Br] **10**, [$BuNHCOCH_2$mim][PF_6] **22,** [$BuN(CH_3)COCH_2$mim][PF_6] **23** Et_2NCOCH_2mim][PF_6] **24** and [Et_2NCOCH_2mim][NTf_2] **30** were solids. Significantly the diethyl amide ionic liquid (in which the prospect of cisoid-transoid rotamers is removed) [Et_2NCOCH_2mim][NTf_2] **30** was the only NTf_2 salt prepared that was solid at room temperature. A general trend is that the NTf_2 counter ion gives lower viscosity and decreased melting point compared with bromide precursors. The symmetry of the diethyl amide is also a contributing factor in the observed elevated melting point.

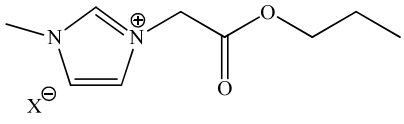
A preliminary biodegradation investigation was carried out on the two ester-containing ionic liquids ([$EtOCOCH_2$mim][Br] **3** and [$EtOCOCH_2$mim][BF_4] **12**, Table 1.2) and [bmim][PF_6] using the modified Sturm Test protocol. All three ionic liquids were close to the pass level (>60 % readily biodegradable), with [$EtOCOCH_2$mim][Br] **3**= 48 %, [$EtOCOCH_2$mim][BF_4] **12** = 59 %, and [bmim][PF_6] = 60 %.[46] Following this study a Closed Bottle Test was utilised to screen a larger library of ionic liquids with ester and amide side chains. In these tests, the ionic liquid (in a concentration of 2 mg/L) was added to an aerobic mineral medium inoculated with wastewater sludge and the depletion of dissolved oxygen was measured over 28 days. A control inoculum was run in parallel to determine oxygen blanks and sodium *n*-dodecyl sulfate (SDS) used as the reference standard. Incorporation of an ester into the ionic liquid side chain significantly improved the biodegradation, whereas the amide derivatives displayed poor biodegradability. The presence of an ester bond in the side chain provides a site for possible enzymatic cleavage

to give the parent imidazolium fragment and the corresponding primary alcohol that may be readily metabolised via fatty acid β-oxidation. Esters with an alkyl side-chain of ≥4 carbons proved to be the most biodegradable of the series.

These encouraging initial results led Gathergood *et al.* to elucidate the effect of the anion on the biodegradation of ionic liquids.[19] A series of ester-functionalised ionic liquids with a variety of different anions were compared with [bmim][Br] examples. Again, the Closed Bottle Test was used to compare the biodegradability of the two classes of ionic liquid (Figure 1.6).



35 X=Br [bmim][Br]
36 X=BF_4 [bmim][BF_4]
37 X =PF_6 [bmim][PF_6]
38 X=NTf_2 [bmim][NTf_2]
39 X=$N(CN)_2$ [bmim][$N(CN)_2$]
40 X=$C_8H_{17}OSO_3$ [bmim][$OctOSO_3$]



4 X=Br [$PrOCOCH_2mim$][Br]
13 X=BF_4 [$PrOCOCH_2mim$][BF_4]
20 X=PF_6 [$PrOCOCH_2mim$][PF_6]
27 X=NTf_2 [$PrOCOCH_2mim$][NTf_2]
33 X=$N(CN)_2$ [$PrOCOCH_2mim$][$N(CN)_2$]
34 X=$C_8H_{17}OSO_3$ [$PrOCO_2CH_2mim$][$OctOSO_3$]

Fig. 1.6: Ionic liquids screened in the Closed Bottle Test by Gathergood *et al.*[19]

From the data it could be seen that the 3-methyl-1-(propoxycarbonyl)imidazolium series showed higher levels of biodegradation, compared with the 1-butyl-3-methylimidazolium derivatives (see biodegradation curves- Figures 1.7 and 1.8). In particular, when the octylsulfate ($C_8H_{17}OSO_3$) anion was incorporated into the ionic liquid structure, an increase in biodegradation was observed (49 % degradation after 28 days).

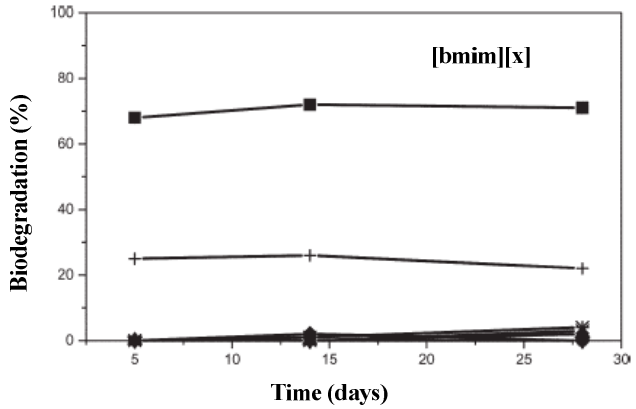


Fig. 1.7: Biodegradation curves for the 3-butyl-1-methylimidazolium salts (**35**-**40**) [bmim][Br] **35** (●), [bmim][BF_4] **36** (▼), bmim][PF_6] **37** (♦), [bmim][$OctOSO_3$] **40** (+),[bmim][$N(CN)_2$] **39** (**x**), [bmim][NTf_2] **38** (*), reference substance SDS (■). Figure reproduced.[19]

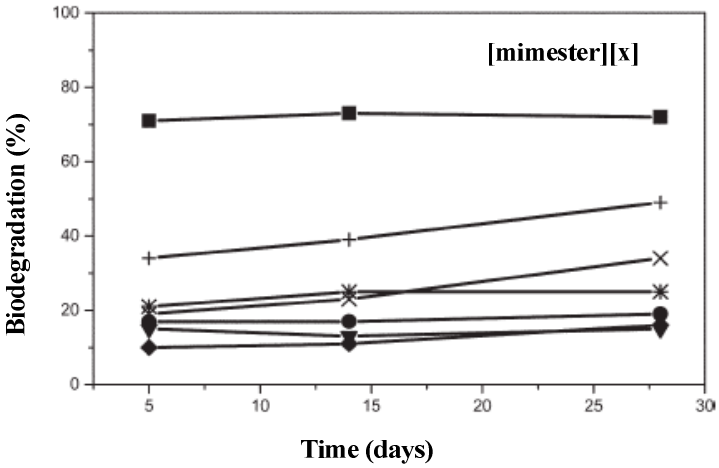
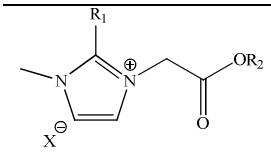


Fig. 1.8: Biodegradation curves for the 3-methyl-1-(propoxycarbonyl)imidazolium ionic liquids (**4**,**13**,**20**,**27**,**33**, and **34**). [$PrOCOCH_2$mim][Br] **4** (●), [$PrOCOCH_2$mim][BF_4] **13** (▼), [$PrOCOCH_2$mim][PF_6] **20** (♦), [$PrOCOCH_2$mim][NTf_2] **27** (*), [$PrOCOCH_2$mim][$N(CN)_2$] **33** (**x**), [$PrOCO_2CH_2$mim][$OctOSO_3$] **34** (+); reference substance SDS (■). Figure reproduced.[19]

Introduction of an additional methyl group at the C2 position of the imidazolium ring was carried out in an attempt to improve enzymatic oxidation of the imidazole core. However,

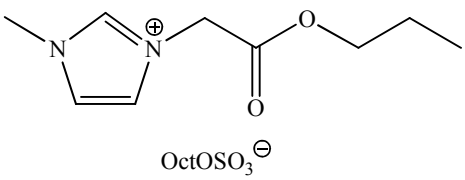
biodegradability remained approximately the same as for the C2-unsubstituted ILs. Finally this work led to the first reported readily biodegradable ionic liquids, with ILs [$PrOCO_2CH_2$mim][$OctOSO_3$] **34**, [1-($PrOCOCH_2$)dmim][$OctOSO_3$] **44**, [$PnOCOCH_2$mim][$OctOSO_3$] **45** and [1-($PnOCOCH_2$)dmim][$OctOSO_3$] **46** showing biodegradabilities above 60 % in the CO_2 Headspace Test[47] (Table 1.3).

Table 1.3: 3-Methyl and 2,3-dimethylimidazolium ionic liquids investigated for biodegradability using the CO_2 Headspace Test.

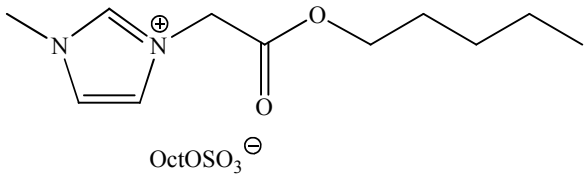
				Biodegradability (%)	
	R_1	R_2	X	CO_2 Headspace (%)	Closed Bottle (%)
4 [$PrOCOCH_2$ mim][Br]	H	C_3H_7	Br	24	24
41 [1-($PrOCOCH_2$) dmim][Br]	CH_3	C_3H_7	Br	nd	23
42 [$PnOCOCH_2$ mim][Br]	H	C_5H_{11}	Br	41	32
43 [1-($PnOCOCH_2$) dmim][Br]	CH_3	C_5H_{11}	Br	n.d.	33
34 [$PrOCO_2CH_2$ mim][$OctOSO_3$]	H	C_3H_7	$OctOSO_3$	64	49
44 [1-($PrOCOCH_2$) dmim][$OctOSO_3$]	CH_3	C_3H_7	$OctOSO_3$	62	55
45 [$PnOCOCH_2$ mim][$OctOSO_3$]	H	C_5H_{11}	$OctOSO_3$	67	54
46 [1-($PnOCOCH_2$) dmim][$OctOSO_3$]	CH_3	C_5H_{11}	$OctOSO_3$	61	56

nd= not determined

For this groups study into the effect of the anion, the CO_2 Headspace Test (ISO 14593) was selected as the method for monitoring biodegradation. The ionic liquid was added to a mineral medium[30] at a concentration of 40 mg/L, inoculated with activated sludge and then incubated in a sealed vessel with a headspace of air for 28 days. Mineralization of the compound to carbon dioxide was determined by measuring the net increase in TOC over 28 days and comparing with blanks. The levels of biodegradation recorded using the CO_2 Headspace Test were notably higher than those obtained by the Closed Bottle Test. These differences may be attributed to the higher bacterial cell density in the inoculum used in the CO_2 Headspace Test.[47] The ionic liquids [$PrOCO_2CH_2mim$][$OctOSO_3$] **34** and [$PnOCOCH_2mim$][$OctOSO_3$] **45** (Figure 1.9) were both found to be readily biodegradable according to the CO_2 Headspace Test. This demonstated that the inclusion of an ester bond into a short hydrocarbon carbon chain (c.f. dehydrogenated tallow dimethyl ammonium compounds) still gave improved biodegradation over the butylimidazoilum salts screened.



34 [$PrOCO_2CH_2mim$][$OctOSO_3$]



45 [$PnOCOCH_2mim$][$OctOSO_3$]

Fig. 1.9: Readily biodegradable ionic liquids, 3-methyl-1-(propoxycarbonyl)imidazolium octylsulfate (**34**) and 3-methyl-1-(pentoxycarbonyl)imidazolium octylsulfate (**45**).

In 2009 Morrissey *et al*. reported biodegradation studies for a library of 66 ionic liquids containing oxygenated side chains and also amides.[48] A toxicity screen of the ionic liquids was also reported, in which seven strains of bacteria were used to assess the antimicrobial activity of the ionic liquids. Four Gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella sp.*, *Salmonella sp.*) and three Gram positive (*Staphylococcus aureus*, *Enterococcus sp.*, *Bacillus subtilis*) were used in these tests. A significant reduction in toxicity for the ionic liquids containing ether or poly ether side-chains (MIC values >20

mg/mL, corresponding to low toxicity at concentrations of >27 mM to >75 mM) compared with those bearing long chain alkylimidazolium salts was observed. 15 Ionic liquids (Figures 1.10 and 1.11) were studied for biodegradation and 6 of these were classified as readily biodegradable by the CO_2 Headspace Test.

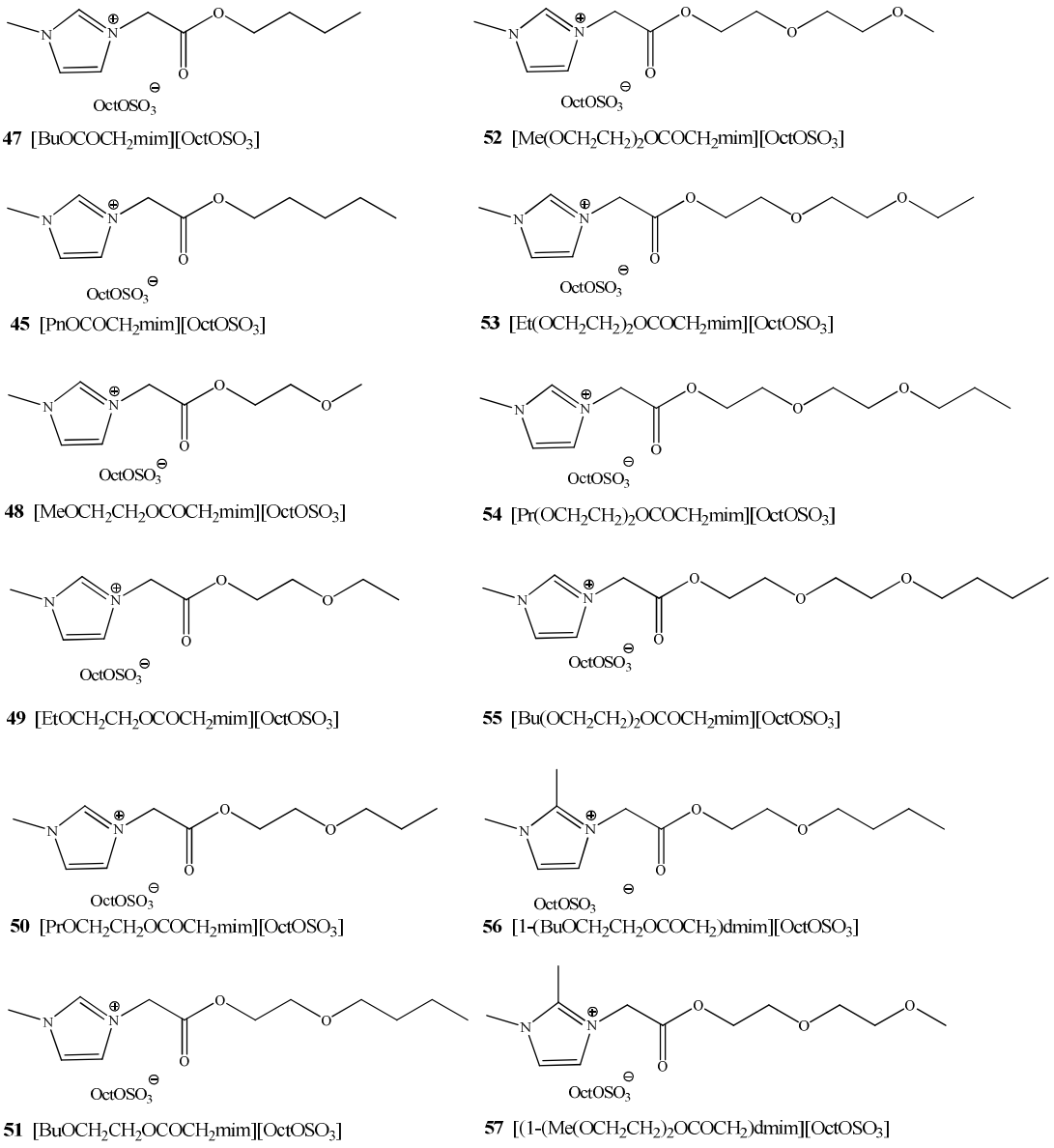


Fig. 1.10: Oxygen-functionalised ionic liquids prepared and screened for biodegradation by Morrissey *et al.*[48]

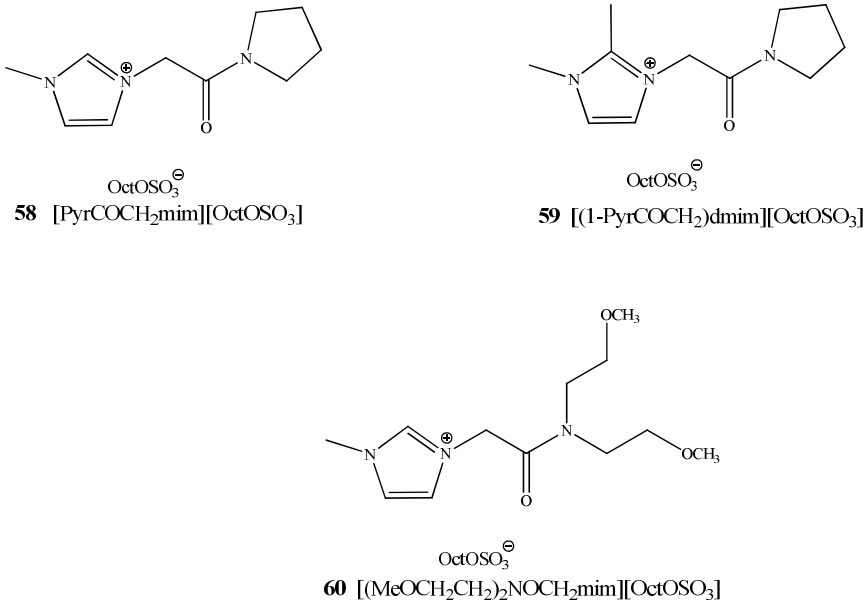
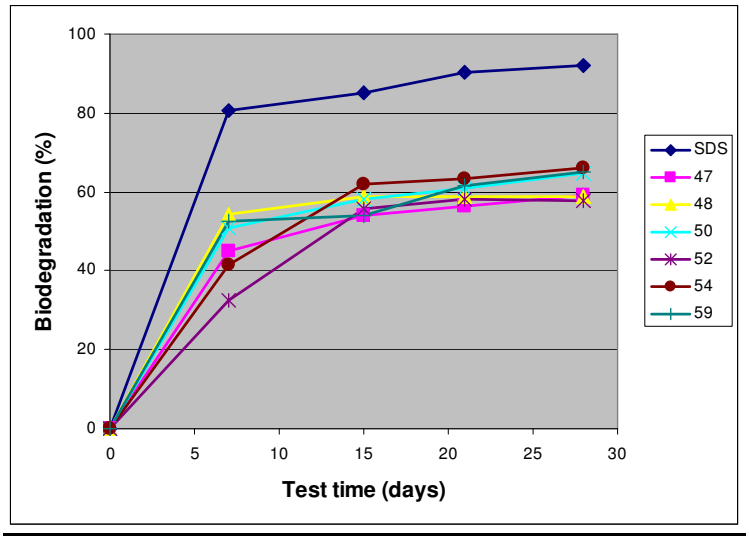


Fig. 1.11: Amide functionalized ionic liquids investigated using the CO_2 Headspace Test.

Ionic liquids [PnOCOCH$_2$mim][OctOSO$_3$] **45,** [PrOCH$_2$CH$_2$OCOCH$_2$mim][OctOSO$_3$] **50,** [BuOCH$_2$CH$_2$OCOCH$_2$mim][OctOSO$_3$] **51,** [Pr(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim][OctOSO$_3$] **54,** [Bu(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim][OctOSO$_3$] **55** and [1- (BuOCH$_2$CH$_2$OCOCH$_2$)dmim] [OctOSO$_3$] **56** passed the CO_2 Headspace Test (% biodegradation of >60 % over 28 days) and therefore could be classified as readily biodegradable.ILs [BuOCOCH$_2$mim][OctOSO$_3$] **47,** [MeOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **48,** [EtOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **49,** [Me(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **52** and [Et(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **53** displayed lower levels of biodegradation (between 55-59 %, Figure 1.12) while the biodegradation values for the amides (Figure 1.11) did not exceed 40 %. Incorporation of one or two ether groups into the side chain of the ionic liquid did not have a detrimental effect on the biodegradation of the ionic liquids compared to the alkyl ester analogues. Ionic liquids with propoxy or butoxy terminus [PrOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **50**, [BuOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **51**, [Pr(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **54**, [Bu(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **55** and [1-(BuOCH$_2$CH$_2$OCOCH$_2$)dmim] [OctOSO$_3$] **56** all passed the CO_2 Headspace Test. Methoxy [MeOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **48** and [Me(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **52** and ethoxy [EtOCH$_2$CH$_2$OCOCH$_2$mim] [OctOSO$_3$] **49** and [Et(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim] [OctOSO$_3$] **53** capped ionic liquids failed the CO_2

Headspace Test. This data shows close agreement to Boethling's rules of thumb where a greater or equal to C4 chain is preferred for improved biodegradation.

(a)



(b)

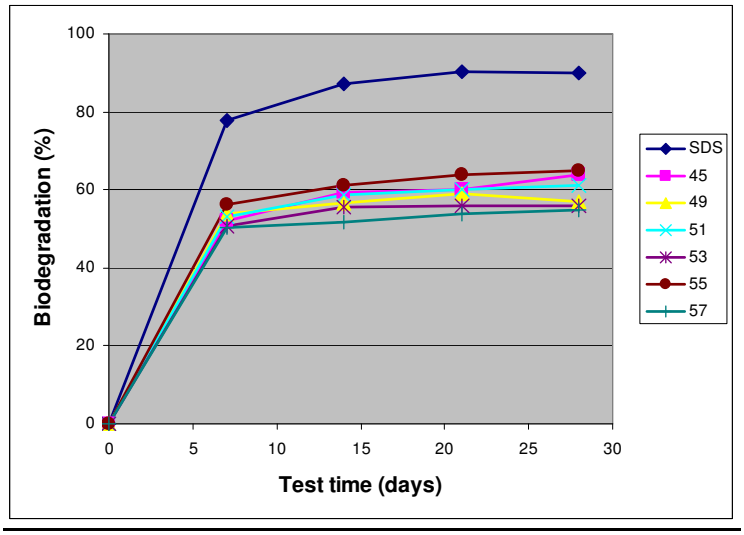


Fig. 1.12: Biodegradation curves (a) for compounds [$BuOCOCH_2mim$] [$OctOSO_3$] **47**, [$MeOCH_2CH_2OCOCH_2mim$] [$OctOSO_3$] **48**, [$PrOCH_2CH_2OCOCH_2mim$] [$OctOSO_3$] **50**, [$Me(OCH_2CH_2)_2OCOCH_2mim$][$OctOSO_3$] **52**, [$Pr(OCH_2CH_2)_2OCOCH_2mim$] [$OctOSO_3$] **54** and [(1-$PyrCOCH_2$)dmim] [$OctOSO_3$] **59**; (b) for compounds [$PnOCOCH_2mim$] [$OctOSO_3$] **45**, [$EtOCH_2CH_2OCOCH_2mim$] [$OctOSO_3$] **49**, [$BuOCH_2CH_2OCOCH_2mim$]

[$OctOSO_3$] **51**, [$Et(OCH_2CH_2)_2OCOCH_2mim$] [$OctOSO_3$] **53**, [$Bu(OCH_2CH_2)_2OCOCH_2mim$][$OctOSO_3$] **55** and [(1-$(Me(OCH_2CH_2)_2OCOCH_2)$dmim] [$OctOSO_3$] **57**. Reference compound used SDS (●).

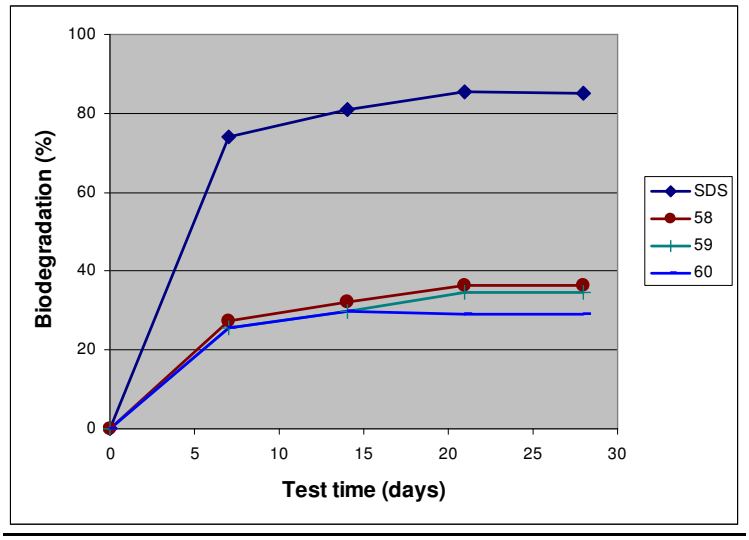


Fig. 1.13: Biodegradation curves for compounds [$PyrCOCH_2mim$] [$OctOSO_3$] **58**, [(1-$PyrCOCH_2$)dmim] [$OctOSO_3$] **59** and [$(MeOCH_2CH_2)_2NOCH_2mim$] [$OctOSO_3$] **60.**

Wells and co-workers studied the biodegradation of each of the major classes of IL cation - ammonium, imidazolium, phosphonium and pyridinium ions, and also screened related salts or acids (i.e. $NaPF_6$, $NaCH_3OSO_3$, $HN(SO_2CF_3)_2$) to test for counter anion effects.[18] In Wells' 2006 study the biochemical oxygen demand (BOD_5) for the library of ionic liquids was measured (Table 1.4) and the biodegradability results compared (Table 1.5).

Table 1.4: Ionic liquids investigated for biodegradation by Wells *et al.*.[18]

IL	Cation	R_1	R_2	MWt	Anion
37	imidazolium	C_4H_9	CH_3	139	PF_6
61 [bmim][Cl]	imidazolium	C_4H_9	CH_3	139	Cl
62 [dodecmim][Cl]	imidazolium	$C_{12}H_{25}$	CH_3	251	Cl
63 [hexadecmim][Cl]	imidazolium	$C_{16}H_{33}$	CH_3	307	Cl
64 [octadecmim][Cl]	imidazolium	$C_{18}H_{37}$	CH_3	335	Cl
65 [1-Bupy][Cl]	pyridinium	C_4H_9	-	136	Cl
66 [1-Bu-1-$EtPH_2$][$(EtO)_2PO_2$] CY169™	phosphonium	C_4H_9	C_2H_5	231	$(EtO)_2PO_2$
67 [1-Hex-1-tetradecylPH_2][Cl] CY101™	phosphonium	C_6H_{13}	$C_{14}H_{29}$	483	Cl
68 [1-Oct-1-MethylNH_2][NTf_2]	ammonium	C_8H_{17}	CH_3	368	$N(SO_2CF_3)_2$
69 EcoEng500™	ammonium[a]	$\{C_2H_4O(C_2H_4O)_4Me\}_2C_{14}H_{29}$	CH_3	696	CH_3OSO_3

[a] contains two PEG5 groups plus C14 chain as R1

Table 1.5: Biodegradation of ionic liquids screened by Wells *et al.*.[18]

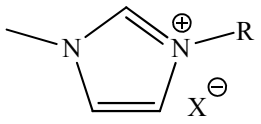
	% inhibition of glucose / glutamate biodegradation at a given test substance concentration:						
IL	100 mg/L	µM	10 mg/L	µM	1 mg/L	µM	Measured biodegradation [a]
37	9	720	8	72.0	28	7.20	0
61	0	720	18	72.0	15	7.20	0
62	97	398	59	39.8	3	3.98	Not tested [b]
63	100	325	100	32.5	16	3.25	Not tested [b]
64	100	298	100	29.8	100	2.98	Not tested [b]
65	4	735	13	73.5	21	7.35	0
66	19	433	15	43.3	16	4.33	9
67	100	207	100	20.7	78	2.07	Not tested [b]
68	23	272	37	27.2	26	2.72	0
69	100	144	100	14.4	47	1.44	Not tested [c]
$HN(SO_2CF_3)_2$	-		-		-		0[d]

[a] % oxygen uptake (biodegradation) after 28 days = BOD / measured COD
[b] Inhibitory at test concentration; [c] BOD_5 result shows material is readily biodegradable
[d] note low carbon content of substrate

In Wells' experiment the ionic liquids were screened at different concentrations (expressed as µM) to investigate the inhibition of glucose/glutamate as part of the BOD biodegradation test. However, the difference in concentration did not appear to influence the biodegradability of the ionic liquid itself. For example ionic liquids [1-Bupy][Cl] **65** (pyridinium cation / chloride anion) and **66** [1-Bu-1-$EtPH_2$][$(EtO)_2PO_2$] were tested at concentrations of 100 mg/L, 10 mg/L and 1 mg/L with no difference in biodegradability. [1-Bupy][Cl] **65** did not undergo biodegradation, whilst [1-Bu-1-$EtPH_2$][$(EtO)_2PO_2$] **66**

was biodegradable at all concentrations. However, when concentrations are expressed in mass / volume, it must be remembered that the molar concentration may vary considerably – although [1-Bupy][Cl] **65** has a concentration of 735 μM at 100 mg/L, EcoEng500™ **69** has a concentration of just 144 μM. Notably, both sodium methylsulfate and ditriflimide (which were used as controls) exhibited no measurable biodegradation. Furthermore, while the phosphonium ionic liquid, [1-Bu-1-EtPH$_2$][(EtO)$_2$PO$_2$] **66** (CY169™) appears to have a modest, but encouraging biodegradability of 9 %, on closer examination, Wells noted that this roughly corresponds to degradation of the carbon content of the diethyl phosphate counter anion, and it is entirely possible that no biodegradation of the ethyl tributylphosphonium cation has taken place. Cations with short alkyl chains (C ≤ 4) did not undergo biodegradation over the period of the test. Only one short-chain test compound, sodium methylsulfate gave any detectable BOD during the test. However, it was apparent that imidazolium and pyridinium ILs with longer alkyl chains (C8, C12 and C18) exhibit an increase in toxicity to the tested species and most of the ionic liquids displayed resistance to biological breakdown.

Romero has studied the acute toxicity and biodegradability of several imidazolium-based ionic liquids (Figure 1.14) in the aqueous phase.[49]



60 R = $(CH_2)_3CH_3$,X = Cl [bmim][Cl]
70 R = CH_3, X = CH_3OSO_3 [mmim][$MeOSO_3$]
71 R = CH_2CH_3, X = $CH_3CH_2OSO_3$ [emim][$EtOSO_3$]
72 R = $(CH_2)_4CH_3$, X = Cl [pentmim][Cl]
73 R = $(CH_2)_6CH_3$, X = Cl [hepmim][Cl]
74 R = $(CH_2)_4CH_3$, X = PF_6 [pentmim][PF_6]
75 R = $(CH_2)_6CH_3$, X = PF_6 [hepmim][PF_6]

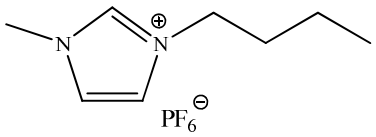
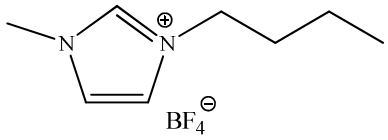
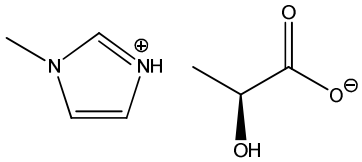
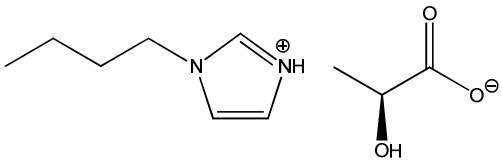
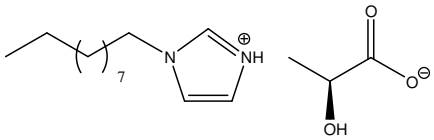
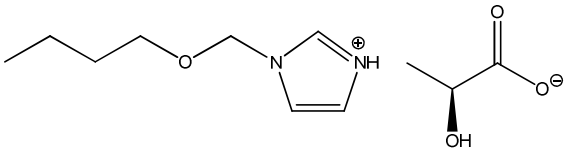
Fig. 1.14: Imidazolium based ionic liquids prepared and screened by Romero *et al.*.[49]

The Microtox® Protocol was used to investigate the EC_{50} and acute toxicity of these compounds. The group reported a correlation between alkyl chain length and toxicity; in agreement with Wells' study.[18] It was found that the shorter the alkyl chain of R the lower

the toxic effect. Biodegradability was analysed using the biochemical oxygen demand over 5 days (BOD_5) with aqueous samples containing known initial concentrations of the compounds and/or D-glucose (as a carbon source). In this test, solutions containing 100 mg/mL of the tested ionic liquid and / or 100 mg/mL of glucose were prepared in aerated media. 244 mL of each of these solutions were inoculated with 1 mL of effluent from a wastewater treatment plant and were dispensed into BOD bottles and incubated at 20 ± 1°C in the dark for 5 days. The final concentration of D-glucose and ionic liquid in the samples were analysed using an enzymatic kit or by UV-spectrometry. The glucose concentration decreased, indicating that it was being consumed by the inoculum, while the concentrations of the ionic liquids remained similar to the initial values, indicating that the ionic liquids were poorly biodegradable in the presence of glucose. Tests were also run on the ionic liquid samples in the absence of an extra carbon source, in order to see if the microorganisms could consume the ionic liquids if glucose was excluded. The concentrations of the compounds after 10 days were almost identical to the initial concentrations used, confirming that the ionic liquids were poorly biodegradable, in either the presence or the absence of an extra carbon source (i.e. glucose).

1-Butyl-3-methylimidazolium hexafluorophosphate **37**, 1-butyl-3-methylimidazolium tetrafluoroborate **36**, 1-methylimidazolium L-lactate **76**, 1-butylimidazolium L-lactate **77**, 1-decylimidazolium L-lactate **78** and 1-butoxymethylimidazolium L-lactate **79** (Table 1.6) were tested for biodegradability by Garbaczewska[50] according to OECD guidelines (OECD 309). In the biodegradation test the ionic liquids were inoculated with river surface water and then incubated in the dark at 20 °C. Samples were removed at various time intervals and the concentration of the ionic liquid present in the sample was determined *via* HPLC analysis (Table 1.6).

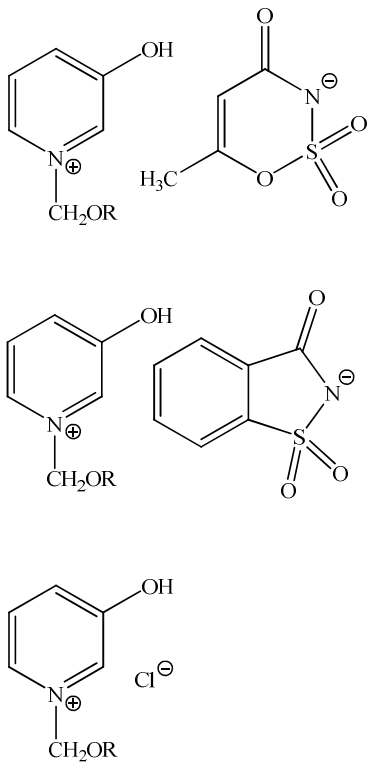
Table 1.6: Concentrations (determined by RP-HPLC peak areas) of imidazolium ionic liquids in surface water at sampling times.[50]

Ionic Liquid	Time (day)	Conc. (%). x 10^{-5}
 **37** [bmim][PF_6]	1	45
	14	41
	33	41
	48	36
	62	35
	98	34
 **36** [bmim][BF_4]	1	43
	14	38
	33	40
	48	36
	62	35
	98	33
 **76** [mimH][Lac]	1	13
	13	13
	32	11
	43	11
	55	12
	77	11
 **77** [bimH][Lac]	1	23
	13	21
	32	20
	43	20
	55	20
	77	19
 **78** [decimH][Lac]	1	17
	13	10
	32	0
	43	0
	55	0
	77	0
 **79** [$BuOCH_2imH$][Lac]	1	14
	13	13
	32	12
	43	11
	55	12
	77	10

The 1-Butoxymethylimidazolium lactate ionic liquid [$BuOCH_2$imH][Lac] **79** displayed increased biodegradability (reflected in a lower concentration remaining in the surface water sample) compared with 1-butylimidazolium lactate [bimH][Lac] **77**. The C10 imidazolium cation **78** was completely undetectable in the sample after day 13, indicating that in favourable cases longer alkyl chains can dramatically increase the rate of biodegradation.

1.3.2 Biodegradation studies on pyridinium-based ionic liquids

A range of pyridinium ionic liquids with environmentally benign anions (i.e. saccharinates and acesulfamates) have been screened for biodegradation.[51] Saccharin and acesulfamate anions are widely used in the food industry as non-nutritive food sweeteners and are non-toxic in nature. Combining these non-toxic, benign anions with organic cations can lead to the production of greener ionic liquids. The general principle of bringing both non-toxic parent moieties together is to form a resulting non-toxic compound. The design of low toxicity and environmentally benign ionic liquids based on trends observed experimentally or calculated from molecular modelling can assist in the rapid determination of preferred target ionic liquids. However, every ionic liquid is unique and has a distinct individual toxicity and biodegradation. It is therefore preferable to screen an ionic liquid individually before it is applied as a solvent type in various applications. The chloride salts were also screened as reference compounds (Figure 1.15).



80 R = C_3H_7 [3-HO-1-PrOCH_2py][Ace]
81 R = C_4H_9 [3-HO-1-BuOCH_2py][Ace]
82 R = C_6H_{13} [3-HO-1-HexOCH_2py][Ace]
83 R = C_7H_{15} [3-HO-1-HeptOCH_2py][Ace]
84 R = $C_{11}H_{23}$ [3-HO-1-UndecOCH_2py][Ace]
85 R = $C_{18}H_{37}$ [3-HO-1-OctadecOCH_2py][Ace]

86 R = C_3H_7 [3-HO-1-PrOCH_2py][Sac]
87 R = C_4H_9 [3-HO-1-BuOCH_2py]][Sac]
88 R = C_6H_{13} [3-HO-1-HexOCH_2py][Sac]
89 R = C_7H_{15} [3-HO-1-HeptOCH_2py][Sac]
90 R = $C_{11}H_{23}$ [3-HO-1-UndecOCH_2py][Sac]
91 R = $C_{18}H_{37}$ [3-HO-1-OctadecOCH_2py][Sac]

92 R = C_3H_7 [3-HO-1-PrOCH_2py][Cl]
93 R = C_7H_{15} [3-HO-1-HeptOCH_2py][Cl]
94 R = $C_{11}H_{23}$ [3-HO-1-UndecOCH_2py][Cl]
95 R = $C_{18}H_{37}$ [3-HO-1-OctadecOCH_2py][Cl]

Fig. 1.15: 1-Alkoxymethyl-3-hydroxypyridinium ionic liquids with acesulfamate, saccharinate and chloride anions.

Ionic liquids (**80-95,** Figure 1.15) were tested using the Closed Bottle Test (OECD 301 D) in the dark at an incubation temperature of 20 ± 1 ˚C. Compounds which reached a biodegradation level higher than 60 % are referred to as ‘‘readily biodegradable’’. The ionic liquid and nutrient solution was inoculated with the supernatant liquor. The ionic liquid was added in a concentration of 4 mg/L, for an incubation period of 28 days. For the duration of the test the Dissolved Oxygen Concentration (DOC) was measured each day for the initial 7 days, and henceforth every seventh day. Biodegradation of the 1-alkoxymethyl-3-hydroxypyridinium ionic liquids ranged from 21 % to 72 % (Table 1.7).

Table 1.7: Biodegradation data for 1-alkoxymethyl-3-hydroxypyridinium ionic liquids.

Cation	**Counter Anion**		
1-Alkoxymethyl-3-Hydroxypyridinium	Saccharinate [Sac]	Acesulfamate [Ace]	Chloride [Cl]
	% Biodegradation after 28 days		
$C_3H_7OCH_2$	43.2	24.4	39.6
	86	**80**	**92**
$C_4H_9OCH_2$	12.7	21.5	-
	87	**81**	
$C_6H_{13}OCH_2$	31.2	38.5	-
	88	**82**	
$C_7H_{15}OCH_2$	31.7	41.2	43.5
	89	**83**	**93**
$C_{11}H_{23}OCH_2$	72.2	48.7	47.7
	90	**84**	**94**
$C_{18}H_{37}OCH_2$	20.2	32.4	25.4
	91	**85**	**95**

As can be seen from the table of biodegradation data, the biodegradability of the ionic liquids depends on both the anion and the length of the alkyl side chain appended to the cation. In 4 of the 6 examples, the acesulfamate derivates gave better biodegradation than the saccharinates, with values ranging from 21.5-48.7 %. However, with each of the 3 anions the highest biodegradability was obtained with a C11 chain attached to the pyridine ring, and it was 1-undecanoxy-3-hydroxypyridinium saccharinate, [3-HO-1-UndecOCH_2py][Sac] **90** which proved the outstanding example, with a biodegradation of 72.2 %.

Scammells *et al.* have also studied the biodegradability of pyridinium ionic liquids using the CO_2 Headspace Test (ISO 14593).[28,29] Compounds which reached a biodegradation level higher than 60% are referred to as ‘‘readily biodegradable’’. These ionic liquids were prepared either from pyridine or nicotinic acid, which are cheap and readily available. Ionic liquids with a C4 alkyl chain at the 1-position (Figure 1.16) gave poor biodegradabilities with values of 1-3 % biodegradation obtained after 28 days. The 1-butylpyridinium salt with octylsulfate incorporated as the anion gave higher levels of biodegradation (37-40 %). The introduction of a methyl group to the 3-position of the pyridinium core (Figure 1.16) did not give rise to increased biodegradation of the ionic liquids.

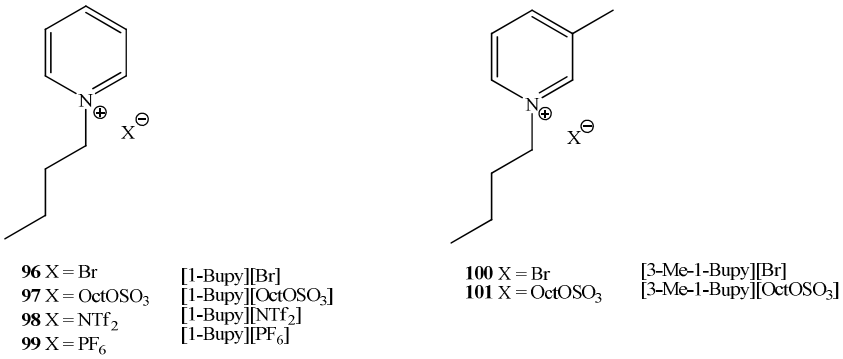


Fig. 1.16: 1-Butylpyridinium ionic liquids prepared by Scammells *et al.*[28]

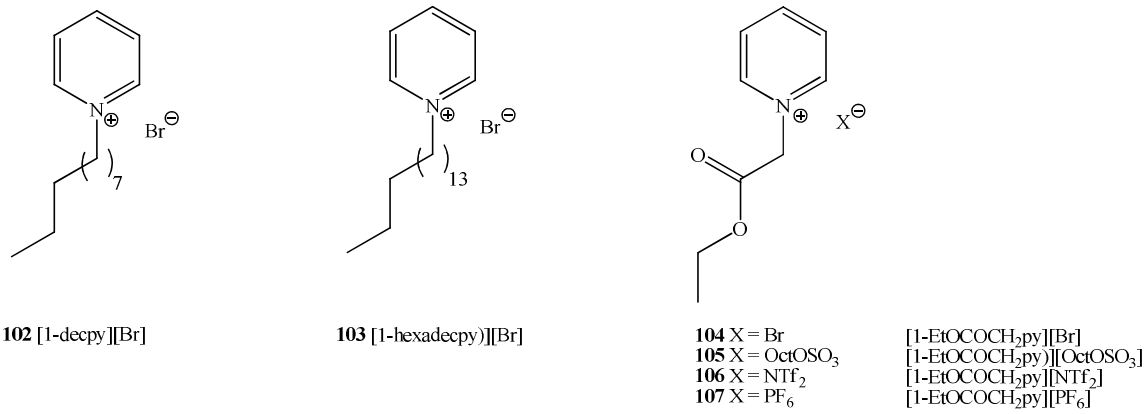


Fig. 1.17: Pyridinium ionic liquids with 1-alkyl and 1-alkylester side chains.

Ionic liquids with long alkyl chains and ester moieties at the 1-position (Figure 1.17) were also synthesised and evaluated for biodegradation. 1-Alkyl pyridinium bromides [1-decpy][Br] **102** and [1-hexadecpy)][Br] **103** gave poor biodegradabilities (9 % and 0 % respectively). Figure 1.18 illustrates the biodegradation curves obtained for ionic liquids **96-103**.

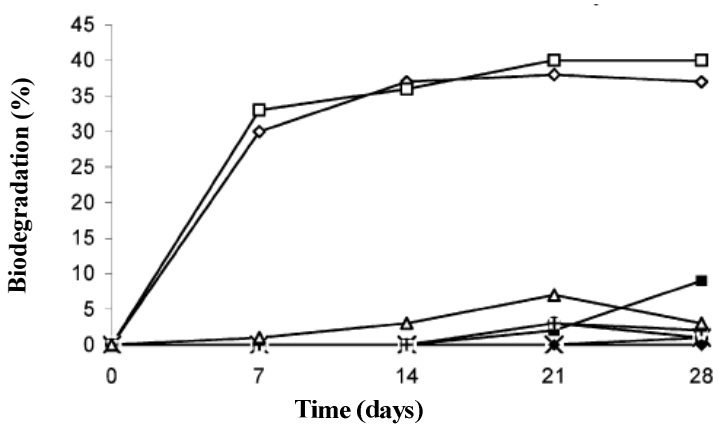


Fig. 1.18: Biodegradation curves for [1-Bupy][Br] **96** (○), [1-Bupy][OctOSO$_3$] **97** (□), [1-Bupy][NTf$_2$] **98** (+), [1-Bupy][PF$_6$] **99** (Δ), 3-Me-1-Bupy][Br]**100** (x), [3-Me-1-Bupy][OctOSO$_3$] **101** (◊), [1-decpy][Br] **102** (■) and [1-hexadecpy)][Br] **103** (♦).

Incorporation of an ester group at the 1-position of the pyridinium core resulted in ionic liquids [1-EtOCOCH$_2$py][Br] **104,** [1-EtOCOCH$_2$py)][OctOSO$_3$] **105,** [1-EtOCOCH$_2$py][NTf$_2$] **106** and [1-EtOCOCH$_2$py][PF$_6$] **107** (Figure 1.17). These salts were screened using the CO_2 Headspace Test and classified as 'readily biodegradable', with biodegradations of 60-89 % (Figure 1.19).

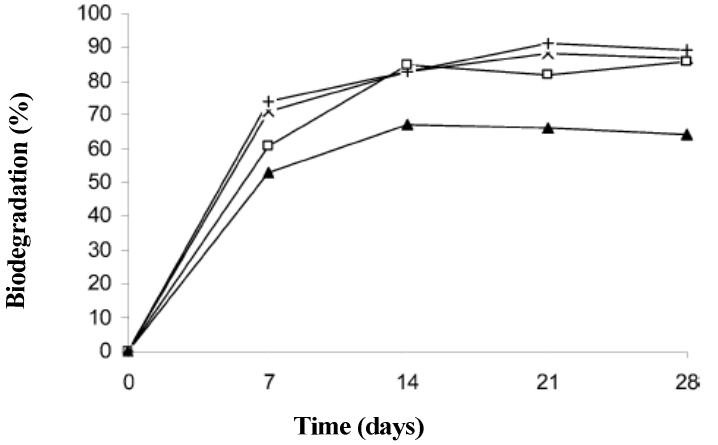
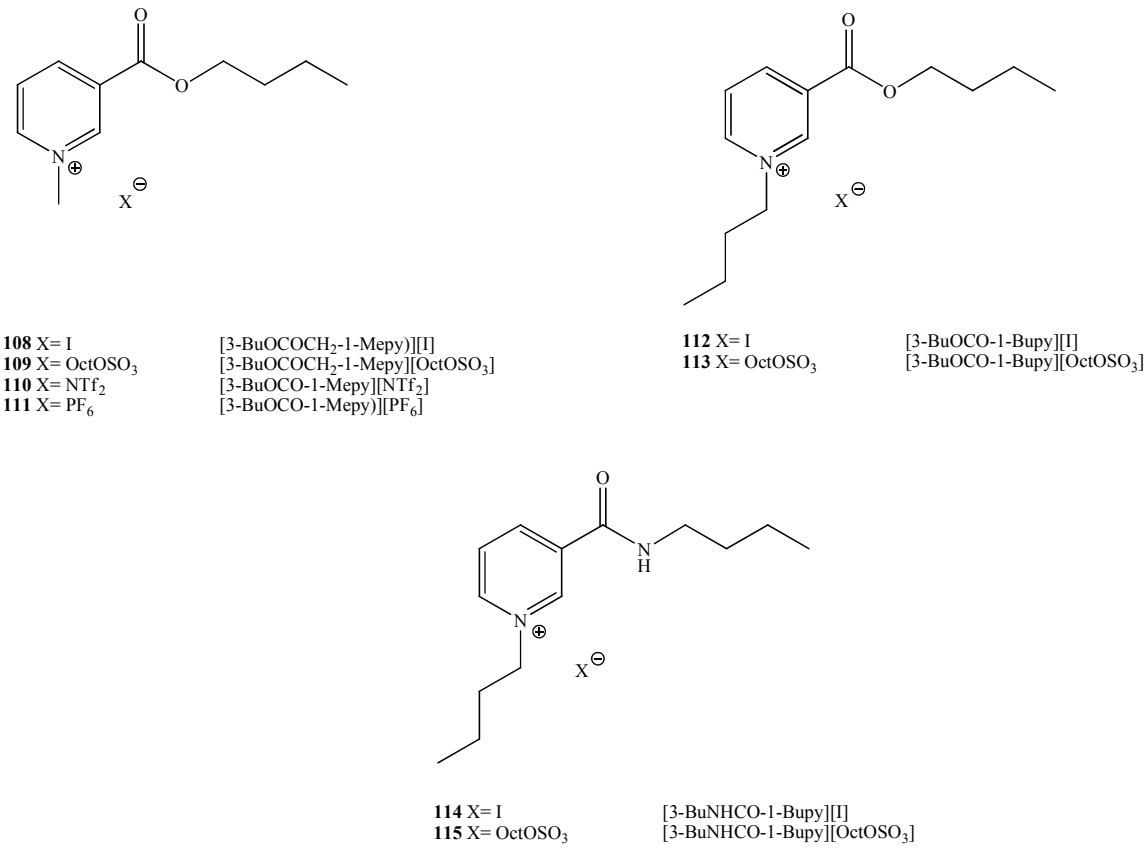


Fig. 1.19: Biodegradation curves for [1-EtOCOCH$_2$py][Br] **104** (x), [1-EtOCOCH$_2$py)][OctOSO$_3$] **105** (+), [1-EtOCOCH$_2$py][NTf$_2$] **106** (▲) and [1-EtOCOCH$_2$py][PF$_6$] **107**(□).



108 X= I [3-BuOCOCH$_2$-1-Mepy)][I]
109 X= OctOSO$_3$ [3-BuOCOCH$_2$-1-Mepy][OctOSO$_3$]
110 X= NTf$_2$ [3-BuOCO-1-Mepy][NTf$_2$]
111 X= PF$_6$ [3-BuOCO-1-Mepy)][PF$_6$]

112 X= I [3-BuOCO-1-Bupy][I]
113 X= OctOSO$_3$ [3-BuOCO-1-Bupy][OctOSO$_3$]

114 X= I [3-BuNHCO-1-Bupy][I]
115 X= OctOSO$_3$ [3-BuNHCO-1-Bupy][OctOSO$_3$]

Fig. 1.20: Pyridinium-based ionic liquids derived from nicotinic acid.

Ionic liquids [3-BuOCOCH$_2$-1-Mepy][I] **108,** [3-BuOCOCH$_2$-1-Mepy][OctOSO$_3$] **109, [**3-BuOCO-1-Mepy][NTf$_2$] **110,** [3-BuOCO-1-Mepy)][PF$_6$] **111,** [3-BuOCO-1-Bupy][I] **112,** [3-BuOCO-1-Bupy][OctOSO$_3$] **113,** [3-BuNHCO-1-Bupy][I] **114** and [3-BuNHCO-1-Bupy][OctOSO$_3$] **115** were prepared from nicotinic acid by formation of an ester or amide linkage from the 3-carboxy group on the pyridine ring (Figure 1.20). These ionic liquids were assessed for levels of biodegradability with the ester derivatives being classified as readily biodegradable. Ionic liquids [3-BuOCOCH$_2$-1-Mepy][I] **108** and [3-BuOCO-1-Bupy][OctOSO$_3$] **113** gave biodegradation levels of 72 and 84 % respectively. The octylsulfate [3-BuOCOCH$_2$-1-Mepy][OctOSO$_3$] **109**, the triflimide [3-BuOCO-1-Mepy][NTf$_2$] **110** and the hexafluorophosphate [3-BuOCO-1-Mepy][PF$_6$] **111** ionic liquids showed biodegradabilities of 75, 68 and 75 % respectively. The nicotinamide based ionic liquid [3-BuNHCO-1-Bupy][OctOSO$_3$] **115** gave biodegradation of 30 % after the 28 day

duration of the test. Figure 1.21 illustrates the biodegradation curves obtained for ionic liquids [3-BuOCOCH$_2$-1-Mepy][I] **108**, [3-BuOCOCH$_2$-1-Mepy][OctOSO$_3$] **109**, [3-BuOCO-1-Mepy][NTf$_2$] **110**, [3-BuOCO-1-Mepy][PF$_6$] **111**, [3-BuOCO-1-Bupy][I] **113** and [3-BuOCO-1-Bupy][OctOSO$_3$] **115.**

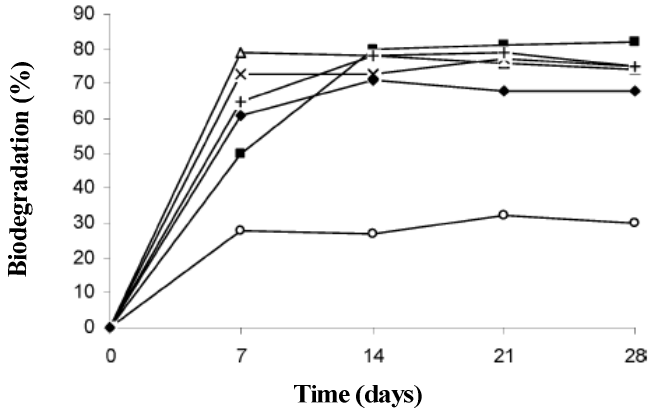
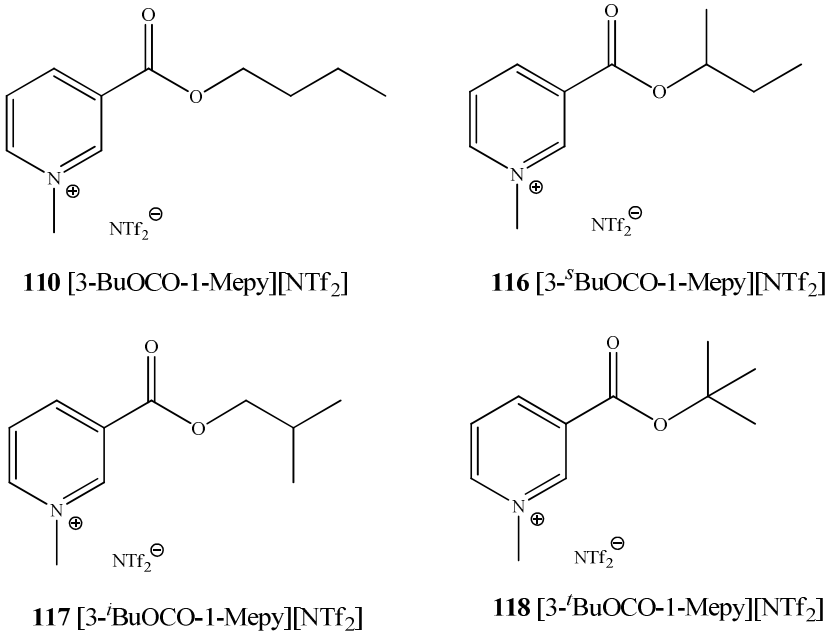


Fig. 1.21: Biodegradation curves of ionic liquids [3-BuOCOCH$_2$-1-Mepy][I] **108** (Δ), [3-BuOCOCH$_2$-1-Mepy][OctOSO$_3$] **109** (**x**), [3-BuOCO-1-Mepy][NTf$_2$] **110** (♦), [3-BuOCO-1-Mepy][PF$_6$] **111** (**+**),[3-BuOCO-1-Bupy][OctOSO$_3$] **113** (■) and [3-BuNHCO-1-Bupy][OctOSO$_3$] **115** (○).

In a more recent study by the same group, a range of ester analogues of [3-BuOCO-1-Mepy][NTf$_2$] **110** were designed and prepared[52] for use as solvents in Sonogashira coupling reactions (Section 1.4.3). The presence of the n-butyl ester in **110** could limit its use in coupling reactions and other related palladium-catalysed carbon-carbon bond formation reactions. Such reactions require the use of bases and sometimes harsh reaction conditions, which could potentially lead to cleavage of the ester group in the IL structure. Hence, more hindered ester groups such as *iso*-butyl, *sec*-butyl and *tert*-butyl esters were introduced into the IL side chain (Figure 1.22).



110 [3-BuOCO-1-Mepy][NTf_2] **116** [3-^{s}BuOCO-1-Mepy][NTf_2]

117 [3-^{i}BuOCO-1-Mepy][NTf_2] **118** [3-^{t}BuOCO-1-Mepy][NTf_2]

Fig. 1.22: Biodegradable 3-Butoxycarbonyl-1-methylpyridinium triflimide ionic liquids prepared by Scammels *et al*..[52]

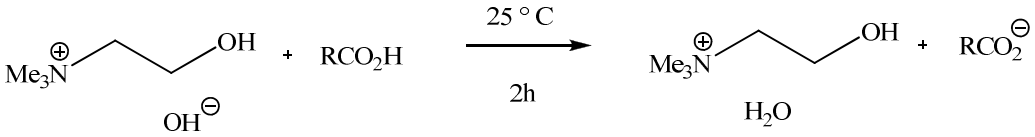
The biodegradability of these ionic liquids (**110**, **116**-**118**) was assessed using the CO_2 Headspace Test (OECD 310). It was found that IL **110** displayed 61 % biodegradation after 1 week and therefore could be termed "readily biodegradable". Salts **116**-**118** reached the pass level (60 %) at a slower rate but did so within the 28-day time frame of the test (Table 1.8).

Table 1.8: Biodegradation (%) of ILs (**110**-**118**).

Ionic Liquid	Biodegradation (%)
[3-BuOCO-1-Mepy][NTf_2] **110**	68
[3-^{s}BuOCO-1-Mepy][NTf_2] **116**	70
[3-^{i}BuOCO-1-Mepy][NTf_2] **117**	69
[3-^{t}BuOCO-1-Mepy][NTf_2] **118**	72

1.3.3 Biodegradation studies on ammonium-based ionic liquids

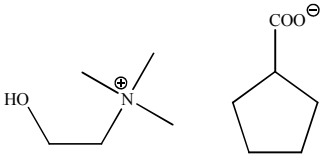
Recently, Yu and co-workers reported the preparation of ten biodegradable choline-based ionic liquids with naphthenic acid derivatives as counter anions.[53] These naphthenic acid ionic liquids (NAILs) were prepared in a one-pot neutralisation of the respective acids with choline hydroxide (Scheme 1.1).



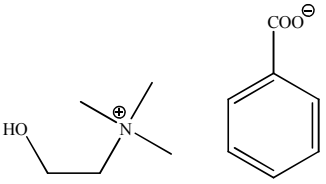
Scheme 1.1: Synthesis of NAILs.

This one-pot synthesis is advantageous over other commonly used metathesis reactions in the formation of ionic liquids, as it results in the pure form of the salt with negligible halogen contamination. It is also a greener alternative in that it is atom efficient with minimal waste produced.

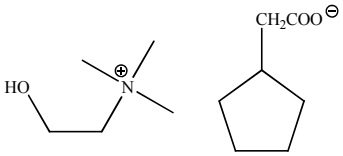
This group chose naphthenic acid surrogates which were previously shown to biodegrade to carbon dioxide and methane. Biodegradation studies on these NAILs using the Closed Bottle Test showed that 8 of the 10 ionic liquids prepared reached the pass level for readily biodegradability (% biodegradation >60 %, Figure 1.23).



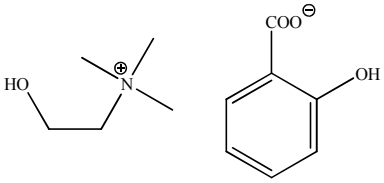
119 [Ch][CPC]



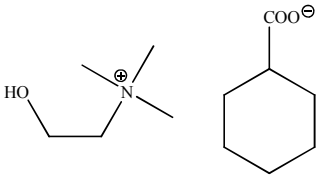
123 [Ch][Be]



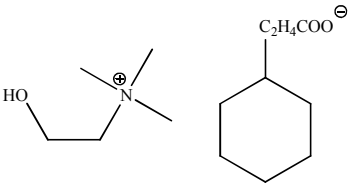
120 [Ch][CPA]



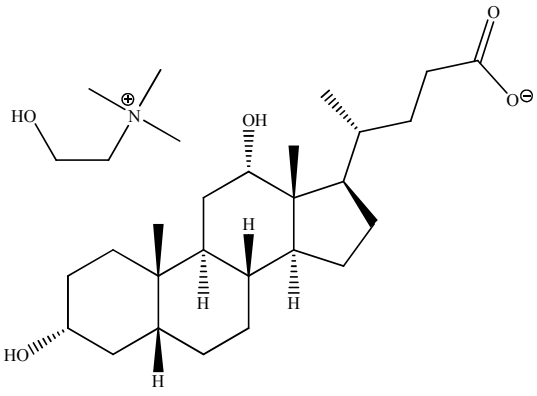
124 [Ch][Sa]



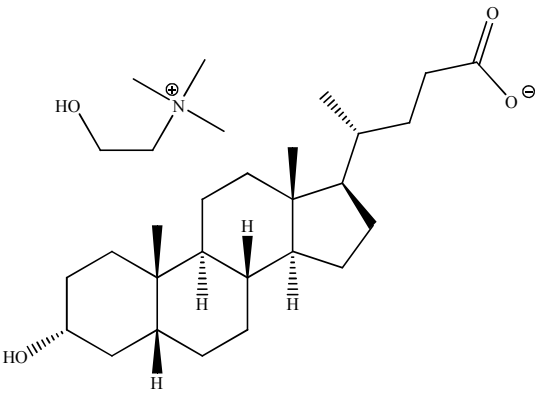
121 [Ch][CHC]



125 [Ch][CHP]



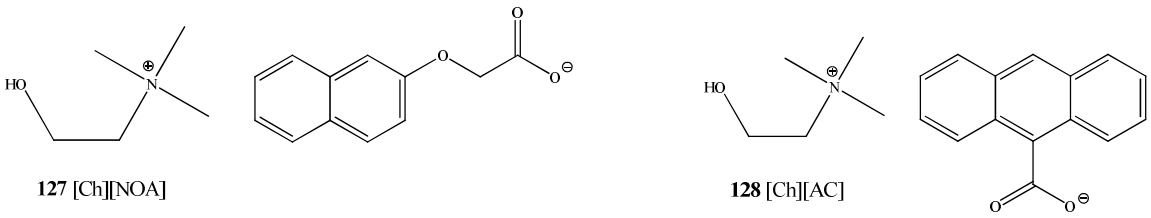
122 [Ch][DOC]



126 [Ch][LC]

Fig. 1.23: NAILS **119**-**126** which passed the Closed Bottle Test.

The two ionic liquids which failed the biodegradability test were choline 2-naphthoxyacetate [Ch][NOA] **127** and choline anthracene-9-carboxylate [Ch][AC] **128** (Figure 1.24).



127 [Ch][NOA] **128** [Ch][AC]

Fig. 1.24: NAILS [Ch][NOA] **127** and [Ch][AC] **128** which failed the Closed Bottle Test.

This low biodegradability is in accordance with Boethling's rule of thumb that polycyclic residues (especially polycyclic aromatic hydrocarbons) can lead to an increase in resistance to biological breakdown.[27] However, salts containing anions with four fused ring systems as part of a steroid skeleton passed the biodegradation tests, a result which is unsurprising for a molecular architecture commonly encountered in biochemical pathways (Figure 1.24). In addition to testing these novel NAILs, this group also screened a number of commercially available ionic liquids and organic solvents. From these results, the NAILs displayed biodegradability comparable with ethanol (a highly biodegradable solvent) while the commonly used ionic liquids gave levels of low biodegradation.

Choline-based quaternary ammonium salts of this kind are highly promising biodegradable ionic liquids and have even been demonstrated to have a stimulating affect on activated sludge in wastewater treatment plants.[54] Considerable data are already available for the biodegradation of related quaternary ammonium based surfactants[40] but are beyond the scope of this review. Design features which have been incorporated into ionic liquids based on the precedent from the surfactant industry are: avoiding branched hydrocarbon chains, including hydrolysable groups (e.g esters and amides), and the presence of ether groups and linear alkyl chains greater or equal to C4. As a general rule all these features have lead to improved biodegradation, except the inclusion of an amide bond.

1.3.4 Primary biodegradation studies

It is evident from the reported biodegradation data that work to determine possible pathways for the biodegradation of ionic liquids is still at an early stage. However, steps are

now being made towards this objective and a variety of analytical techniques have been used to identify possible metabolites of ionic liquids. These studies are especially important because a possible metabolite may display toxicity and persist in the environment, even if the parent ionic liquid is non-toxic and appears to be biodegradable.

Docherty has recently used ^{1}H NMR analysis to investigate the biodegradation of a number of commonly used ionic liquids.[55] In her study six ionic liquids consisting of imidazolium and pyridinium bromide salts with 1-butyl, 1-hexyl and 1-octyl alkyl side chains were subjected to biodegradation by an activated sludge (Figure 1.25).

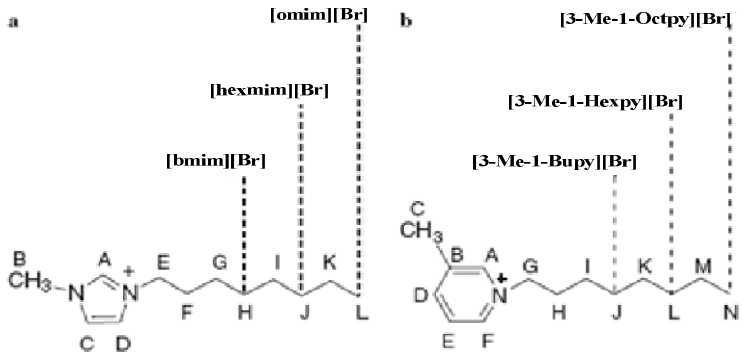


Fig. 1.25: Chemical structures of the six (a) imidazolium and (b) pyridinium ILs examined by Docherty *et al*..[55]

A modified OECD guideline for the Testing of Chemicals standard dissolved organic carbon (DOC) Die-Away Test was used to perform the biodegradation analysis of the ionic liquids. The ionic liquids were tested at a concentration of 40 mg C L^{-1} and were inoculated with 10.38 mL of activated sludge sample, yielding a final concentration of 30 mg suspended solids per 1 L bottle. Biotic controls were also prepared whereby three replicate bottles contained no ionic liquid and only inoculated media. An abiotic control was also set up (flasks which contained mineral medium and the test compound, but no inoculum). Sodium acetate was also used as a positive control (as a carbon source, to test sludge viability). All the test vessels were shaken aerobically at room temperature. 10 mL of sample was removed four times per week from all flasks for DOC analysis. When a ≥ 20 % decrease in DOC was observed, 10 mL samples were removed everyday for 14 days or until DOC concentrations became constant. NMR analysis on initial and final chemical

structures of the ionic liquids was also carried out. At the beginning of the test one set of 1 L bottles of inoculated IL-Medium for all the ILs and one blank were prepared. These were immediately filtered (0.22 µm) and analysed by NMR. An NMR study was also carried out after the last incubation day where the samples were evaporated to remove water and then dissolved in 2 mL of D_2O. The re-suspended samples were syringe filtered into NMR tubes. Figures 1.26 and 1.27 illustrate the NMR data obtained for pyridinium and imidazolium based salts.

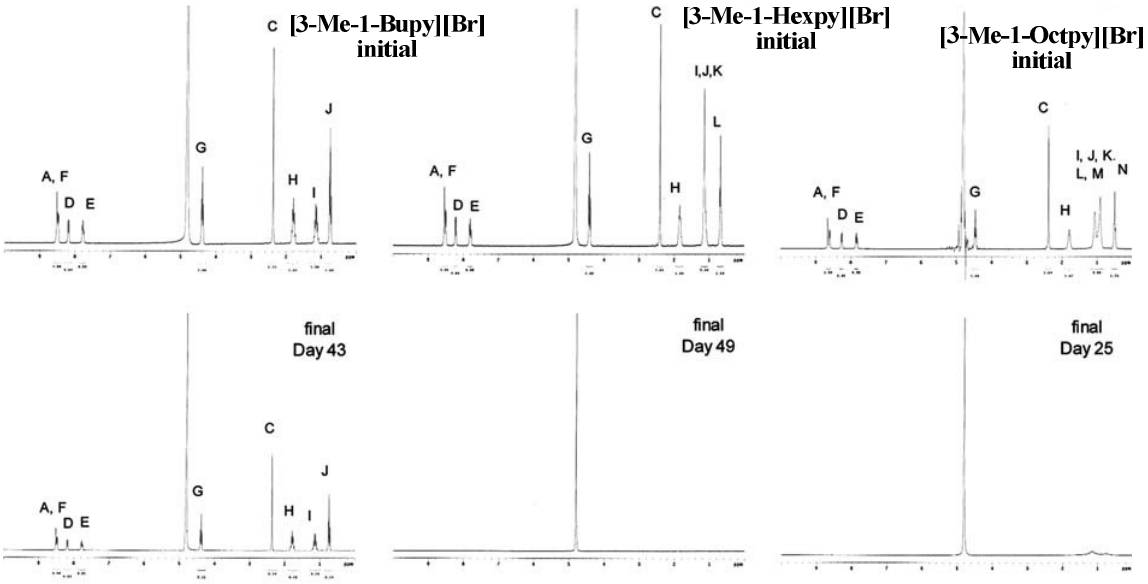


Fig. 1.26: ^1H NMR data of pyridinium based ionic liquids at initial and final sampling times during biodegradation screening (for NMR assignments, see Figure 1.24). Figure reproduced.[55]

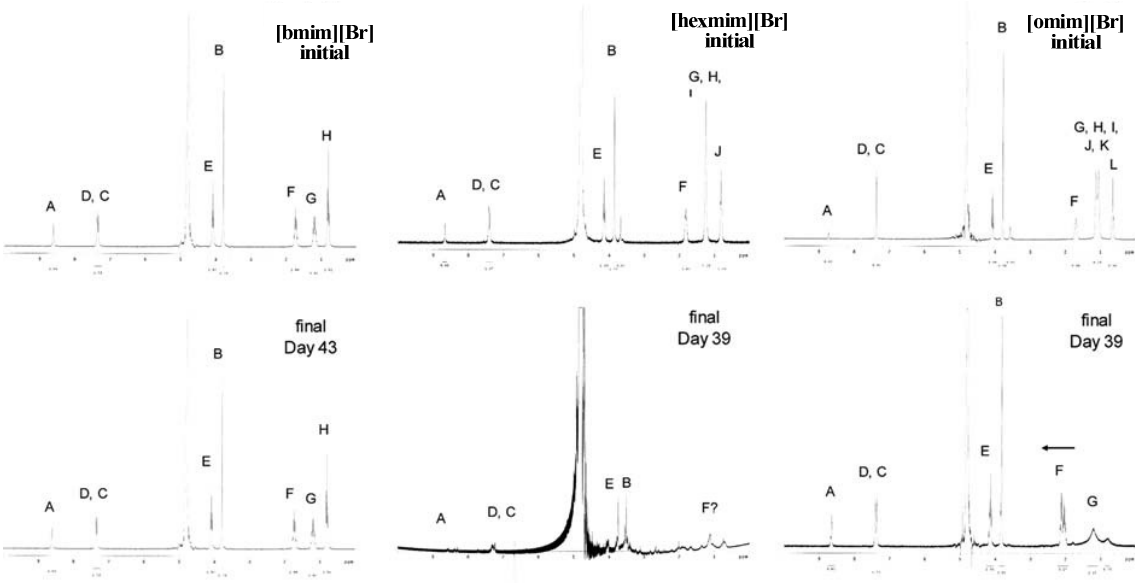


Fig. 1.27: ^{1}H NMR data of imidazolium-based ionic liquids at initial and final sampling times during biodegradation screening (for NMR assignments, see Figure 1.25). Figure reproduced.[55]

Ionic liquids most commonly contain a large organic cation substituted with one of a variety of possible alkyl side chains. The length of the alkyl side chain alters the lipophilicity of the ionic liquid, which can also have a pronounced effect on toxicity.[18] Docherty determined that pyridines alkylated with octyl chains are more biodegradable than the corresponding *N*-hexyl or *N*-butylpyridinium ions. Pyridinium cations also proved to be generally more biodegradable than comparable imidazolium ions. Of the six ionic liquids screened, only one example [3-Me-1-Octpy][Br] could be classified as readily biodegradable according to the DOC Die-Away Test guidelines (% biodegradation of [3-Me-1-Octpy][Br] = 96 % after 25 days). Extended incubation times (up to 49 days) saw partial mineralization of imidazolium ionic liquids, while the pyridinium ionic liquids were fully mineralized in this time frame.

Recently Docherty and co-workers further investigated the primary biodegradation of these *N*-alkyl substituted methyl-pyridines.[56] Ionic liquids containing 1-Butyl-3-methyl pyridinium [3-Me-1-Bupy], 1-Hexyl-3-methylpyridinium [3-Me-1-Hexpy], and 1-Octyl-3-

methylpyridinium [3-Me-1-Octpy] cations were studied using an activated sludge mixed microbial community. Primary biodegradation of these cations was traced using HPLC-MS analysis and Tandem MS (MS/MS) was also employed as a means of elucidating the possible routes of IL degradation. All 3 pyridinium cations were seen to be fully mineralised by the microbes but only the octyl-derivative could be classified as "readily biodegradable" according to OECD protocol. From previous results[55] however the *N*-butyl methyl pyridine was seen not to be metabolised after 43 days, using similar methods and inoculum from the same waste water treatment plant. It was postulated that the activated sludge samples used in the latter study may have varied from that studied in earlier tests (i.e. microbial species may have varied between different batches of activated sludge inoculum).

A number of possible metabolites and degradation pathways of these ILs were suggested by Docherty based on the results achieved from MS analysis. The MS spectrum of [3-Me-1-Bupy]$^+$ on day 41 gave three degradation products at 148 (*m/z*), 166 (*m/z*) and 164 (*m/z*). MS/MS was carried out on each isolated mass as a means of elucidating the metabolite structure. From this analysis it could be determined that the butyl pyridinium cation was degrading to yield products where the pyridinium ring was hydroxylated at one of the ring carbons (exact position on the ring was not known). The butyl side chain also appeared to lose two protons (148 (*m/z*)), leading to unsaturation of the alkyl chain. Similar results were observed for the hexyl derivative (178 (*m/z*)), where unsaturation of the alkyl side chain occurred (176 (*m/z*)). Biodegradation of the parent IL by hydroxylation in the side chain (192 (*m/z*)) and in the aromatic ring (194 (*m/z*)) was also concluded. Degradation of the *N*-octyl substituted methyl pyridinium cation (206 (*m/z*)) yielded breakdown products with double bond insertion in the alkyl side chain (204 (*m/z*)) and hydroxylation of the side chain (222 (*m/z*)).

Zhang *et al*.[57] observed degradation of pyridinium ILs *via* ring opening of the pyridinium core by a soil bacterium which was isolated by an enrichment-culture technique. *Corynebacterium* sp. was the identified bacterium isolated. The ionic liquids studied were *N*-ethylpyridinium tetrafluoroborate [1-Etpy][BF_4], *N*-ethylpyridinium trifluoroacetate [1-

Etpy][CF_3COO] and 1-Butyl-3-methylimidazolium hexafluorophosphate [bmim][PF_6]. The experimental set-up involved addition of 10 mL of tested ILs to 50 mL of mineral salt medium in 125 mL flasks with subsequent inoculation with 2 mL of an early-log-phase bacterial culture. Triplicate samples were prepared and incubated at 28 °C in the dark. At various time intervals, 5 mL samples were taken and bacterial growth was investigated using optical density (OD) measurements at 600 nm. Samples were also analysed using UV-Vis spectrometry to determine the concentration of *N*-ethylpyridinium cation in biological samples. The aromatic pyridinium ring has a characteristic absorbance at 210 and 259 nm in UV spectrum. After 24 hours the peaks at these absorbances disappeared suggesting biodegradation of the pyridinium cation. Further analysis was carried out by electrospray ionisation mass spectrometry (ESI-MS). In the MS spectra obtained, two metabolite structures could be indentified with molecular weights of 157.9 and 143.9 (*m/z*) respectively. These two metabolites were believed to be formed upon ring opening of the pyridinium cation. MS/MS analysis further identified these breakdown products as *N*-ethyl-(4-carboxyamino)but-3-enoic acid semialdehyde and (4-(carboxyamino)but-3-enoic acid, which are oxidised further to acetic acid and glyoxylate. Peaks at 73 (*m/z*) and 60 (*m/z*) in the MS were evident in low concentrations, corresponding to these end products. The proposed degradation pathway is summarised in Figure 1.28.

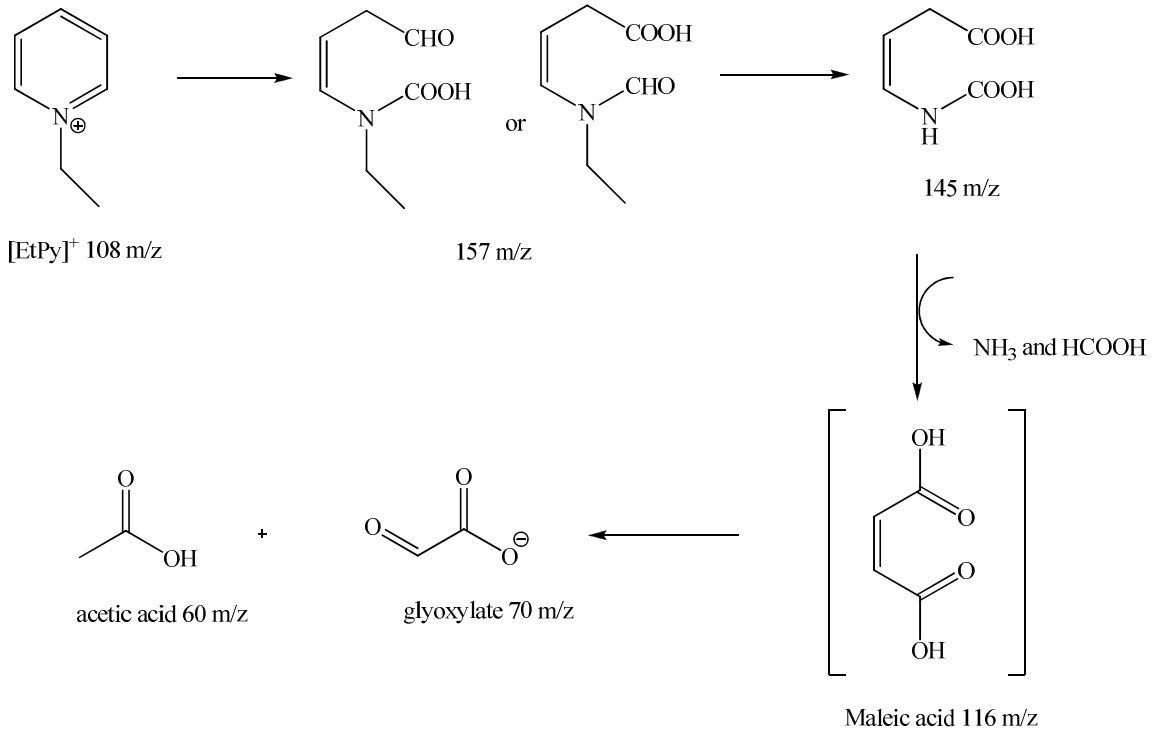


Fig. 1.28: Proposed degradation pathway of *N*-ethyl pyridinium cation via ring cleavage.[57]

An isolate fungal strain has also been used in biodegrading ionic liquids.[58] The primary biodegradation of a series of novel cholinium based ionic liquids was reported by ^{1}H NMR analysis. Environmentally benign cholinium $[NMe_3(CH_2CH_2OH)]^+$ was combined with a range of alkanoate anions ($[C_nH_{2n+1}CO_2]^-$, n= 1-9) (Figure 1.29) and was challenged against the isolate strain *Penicillium Corylophium* sp.

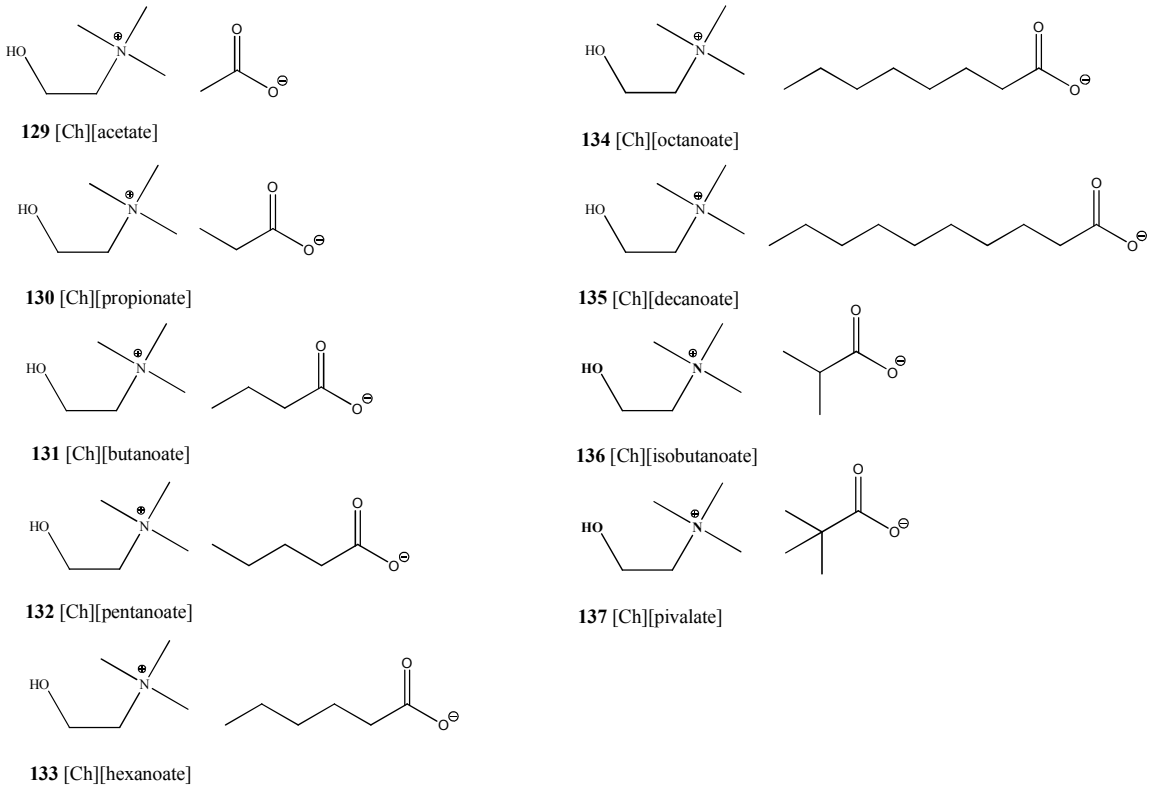
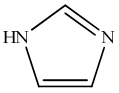


Fig. 1.29: Biodegradable cholinium alkanoate ionic liquids.

In this assay the fungal cultures (20 mL) were incubated in the dark at 25 °C under agitation (at 90 rpm) for 28 days. On sampling days, 1 mL aliquots were taken and syringe filtered (through 0.20 μm membrane) then freeze dried to remove water. Samples were then subsequently analysed by ^{1}H NMR spectroscopy in D_2O solvent. From this analysis it could be seen that complete degradation of the long alkyl anions occurred, i.e. butanoate, pentanoate, hexanoate and octanoates. Complete disappearance of the spectral peaks due to these groups in the ^{1}H NMR spectra demonstrated this degradation. The shorter linear chain anions, namely acetate and proponoate, were not fully degraded and this was believed to be due to their high concentration in the test media (0.375 and 0.5 M respectively). These ionic liquids were also screened for their toxicity properties (see Chapter 5).

Stolte *et al*.[59] reported a study on the primary biodegradation of different *N*-imidazoles, imidazolium, pyridinium and 4-(dimethylamino)pyridinium ionic liquids with various alkyl side chains. The biodegradation study used was based on OECD guideline 301D.

The compounds to be tested were prepared at a concentration of 200 μM (200 ppm) in inoculated test media with a total volume of 100 mL. Blank samples were prepared, consisting of inoculated media without the test compound, abiotic controls (200 μM of the test substances in inoculated media poisoned with $HgCl_2$), and also positive controls (inoculum with 200 μM of imidazole). Replicates of all the samples and controls were kept in the dark at 20 ± 1˚C. The samples were tested at various time periods (day 4, day 9, day 17, day 24, day 31) and 500 μL samples were taken and centrifuged (5000 rpm, 15 min). These samples were then analysed using HPLC-MS to determine possible metabolites formed upon mineralization by the activated sludge community with the aim of deducing pathways of IL degradation. Adsorption of the test compounds to the sludge was investigated using an abiotic control (in which the sludge was inactivated by the $HgCl_2$). Stolte and co-workers found that of the 27 substances screened (i.e. three aromatic head groups [4-(dimethylamino)pyridinium, pyridinium and 1-methylimidazolium] substituted with various alkyl side chains (C2-C8) and also some simple mono *N*-substituted imidazoles (Figures 1.30 and 1.31) there was no indication of sorption to the sludge. It was also noted that for the biotic samples a decrease in concentration of the test compounds was observed, which would suggest that biological degradation of the chemicals had occurred. This result was of significance because adsorption to the sludge is a primary concern in the choice of experimental method to be adopted.



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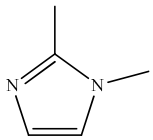

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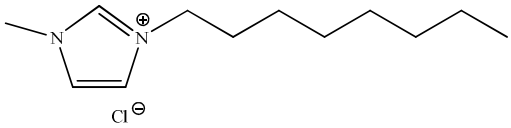
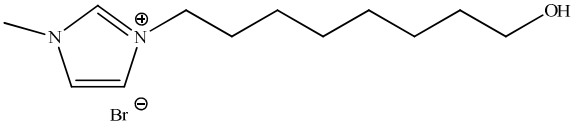
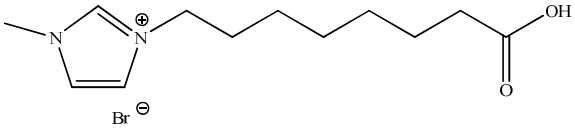


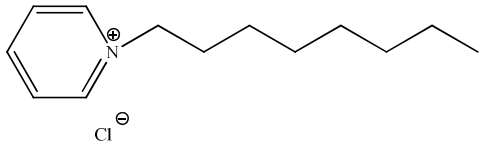
140 [omim][Cl]



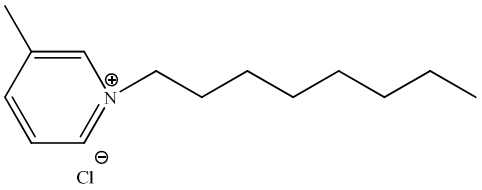
141 [$HO(CH_2)_8$mim][Br]



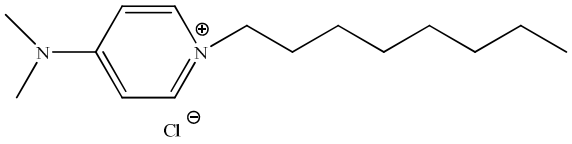
142 [$HOCO(CH_2)_7$mim][Br]



143 [1-Octpy][Cl]



144 [3-Me-1-Octpy][Cl]



145 [4-Me_2N-1-Octpy][Cl]

Fig. 1.30: Ionic liquids and imidazole controls that gave 100 % primary biodegradation within 31 days.[59]

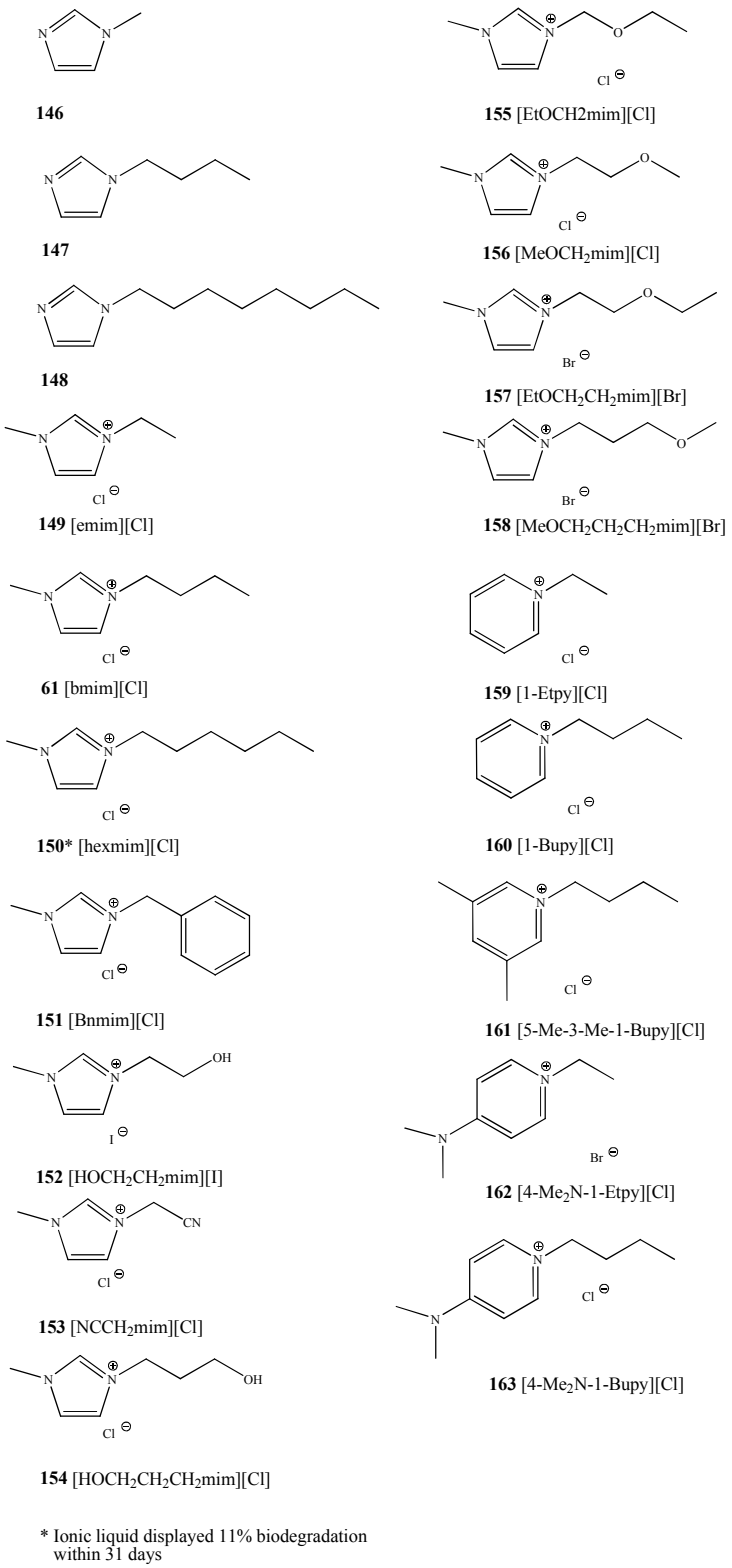


Fig. 1.31: Ionic liquids for which no primary biodegradation was recorded after 31 days.[59]

Two different types of inoculum were used for the evaluation of the general biodegradability of the test substances. A commercially available freeze-dried mix of bacteria and an activated sludge community from a wastewater treatment plant were studied. The freeze-dried bacterial mixture was used because it is a standardised product, and therefore should give high reproducibility in the test. In addition to this, the mixture gave an acceptable biological matrix, compared with the activated sludge, causing less interference in the HPLC-MS analysis. However, the limited diversity of microorganisms present in the freeze-dried bacterial mixture made it unsuitable for biodegradation studies, compared with the activated sludge.

In the case of the 1-octyl-3-methylimidazolium (omim) cation, biodegradation followed by HPLC-MS analysis established that the mass ions from the original test sample of omim chloride were completely absent, and Stolte proposed two possible metabolic pathways[59] for the biodegradation based on the mass ions observed (Figure 1.32).

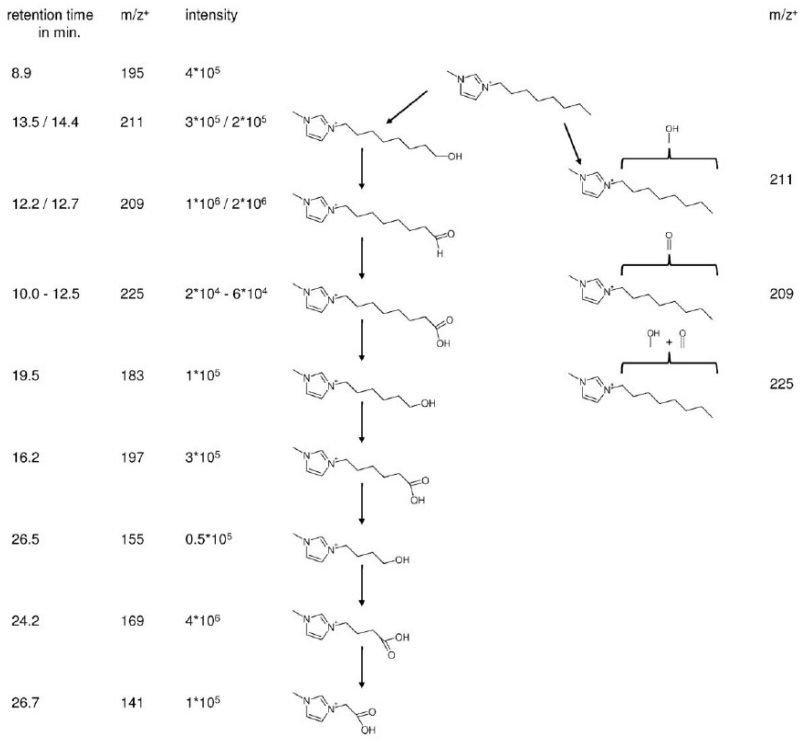


Fig. 1.32: Proposed chemical structures and degradation pathways for 1-octyl-3-methylimidazolium chloride [omim][Cl] **140** (Figure reproduced).[59]

These researchers recently carried out a biodegradation study of these ionic liquids in the absence of molecular oxygen i.e. anaerobic biodegradation.[60] Anaerobic biodegradation is a process used in water treatment facilities and soil remediation and has been shown to breakdown compounds which are reluctant to aerobic degradation. In these studies, the primary biodegradation of imidazolium and pyridinium ionic liquids under denitrifying conditions was monitored *via* HPLC-UV analysis over an 11 month period (328 days). In the experimental set-up, the ILs were added as the only source of carbon (200 μM) to the inoculated activated sludge media under a nitrogen atmosphere. An equimolar amount of acetate was added to two extra parallel flasks of inoculated media in order to test for co-metabolism. Additional controls were also set up where no test substance was present in the inoculated sample (Blank control), and a known biodegradable compound was added to sludge (Positive control). All test vessels were stored at room temperature in the dark. At each testing day 1.3 mL samples were taken and centrifuged at 14,500 rpm for 15 min. Analytical work was subsequently carried out using HPLC-UV measurements. From the study, the concentration of compound **141** was the only noticeable concentration decrease observed over the 328 day test period. Approximately 52 to 54 % degradation of this IL was recorded after 9 days and after 34 days no more [$HO(CH_2)_8$mim][Br] (**141**) was detected. MS analysis indicated the various mass to charge ratios of structures present in the [$HO(CH_2)_8$mim][Br] samples. At the initial day of the test the parent [$HO(CH_2)_8$mim] mass (211 *m/z*) was detected. In the early phase samples masses of 225 and 197 *m/z* were evident. These correspond to metabolite structures 1-(7-carboxyheptyl)-3-methylimidazolium cation and 1-(5-carboxypentyl)-3-methylimidazolium cation respectively. In the last day of analysis (day 328), a peak of mass 169 *m/z* was detected as from MS/MS analysis was proposed to be 3-(3-carboxypropyl)-1-methylimidazolium cation. Figure 1.33 depicts the parent IL [$HO(CH_2)_8$mim][Br] and its proposed metabolite products.

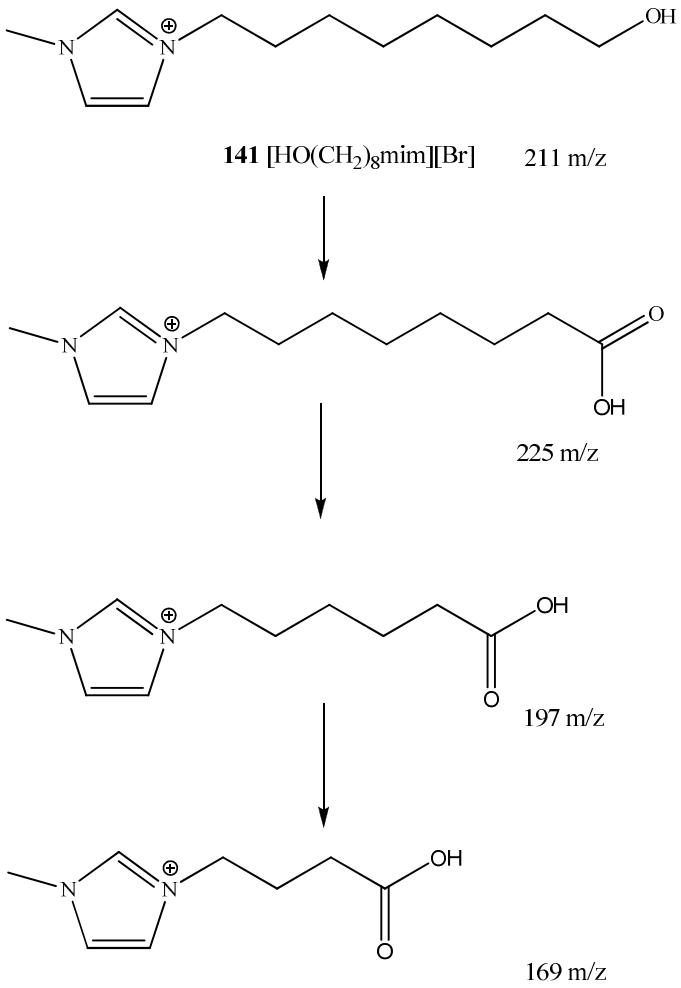


Fig. 1.33: Anaerobic biodegradation of **141** [$HO(CH_2)_8$mim][Br].

Pham *et al.*[61] were the first group to investigate the metabolites formed as a result of biodegradation of 1-butyl-3-methylpyridinium bromide and its biodegradation was studied using an activated sludge assay (OECD 301E). This primary biodegradation was analysed *via* HPLC-MS. Figure 1.34 depicts an example of a HPLC-MS chromatograph showing the biodegradation of *N*-butyl-3-methylpyridinium cation after 18, 21 and 28 days incubation with activated sludge.

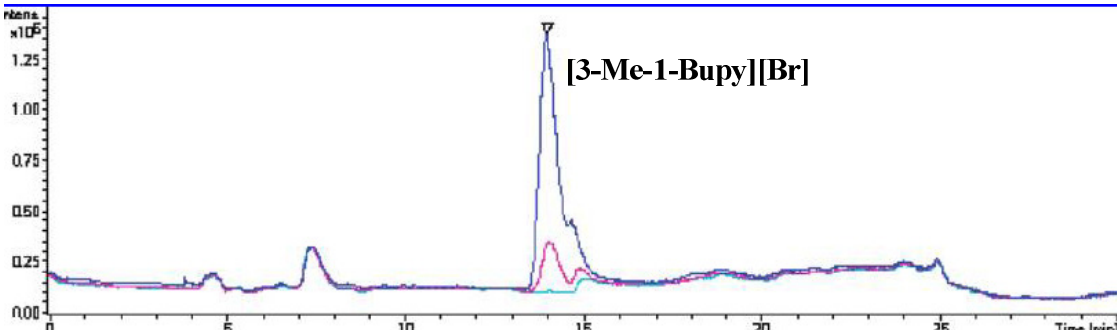


Fig. 1.34: HPLC-MS chromatogram showing the biodegradation of *N*-butyl-3-methylpyridinium cation after 18 (Blue), 21 (Red) and 28 (Green) days incubation with activated sludge.[61]

From the HPLC-MS analysis degradation pathways were proposed and possible metabolites hypothesized. Two possible pathways for the biodegradation of *N*-butyl-3-methylpyridinium bromide were suggested (Figure 1.35). In pathway (I), the ionic liquid may first be converted into an *N*-hydroxybutyl-4-(3-methylpyridinium) cation by enzymatic oxidation. Further oxidation of the C1 hydroxyl group of the butyl side chain to an aldehyde, and hydroxylation at C3 would give the *N*-(2-hydroxy-4-oxo-butyl)-3-methylpyridinium cation as a final metabolite. Pham proposed that this metabolite may fragment under HPLC-MS conditions to give the *N*-(2-hydroxyethyl)-3-methylpyridinium cation and acetaldehyde.

Putative pathway (II) on the other hand invokes a simpler mode of degradation, in which hydroxylation of C2 of the alkyl chain gives an *N*-(2-hydroxybutyl)-3-methylpyridinium cation. Under HPLC-MS conditions this metabolite may lose ethane, and subsequently ethanol, to give mass ions corresponding to the *N*-(2-hydroxyethyl)-3-methylpyridinium cation.

At the end of either pathway (I) or (II) a final fragmentation of the *N*-(2-hydroxyethyl)-3-methylpyridinium fragment under HPLC-MS conditions may occur in which loss of ethane gives the 3-methylpyridinium cation. However, the authors were unable to confirm whether this ion was merely an artefact from 3-methylpyridine contaminating the IL stock solution.

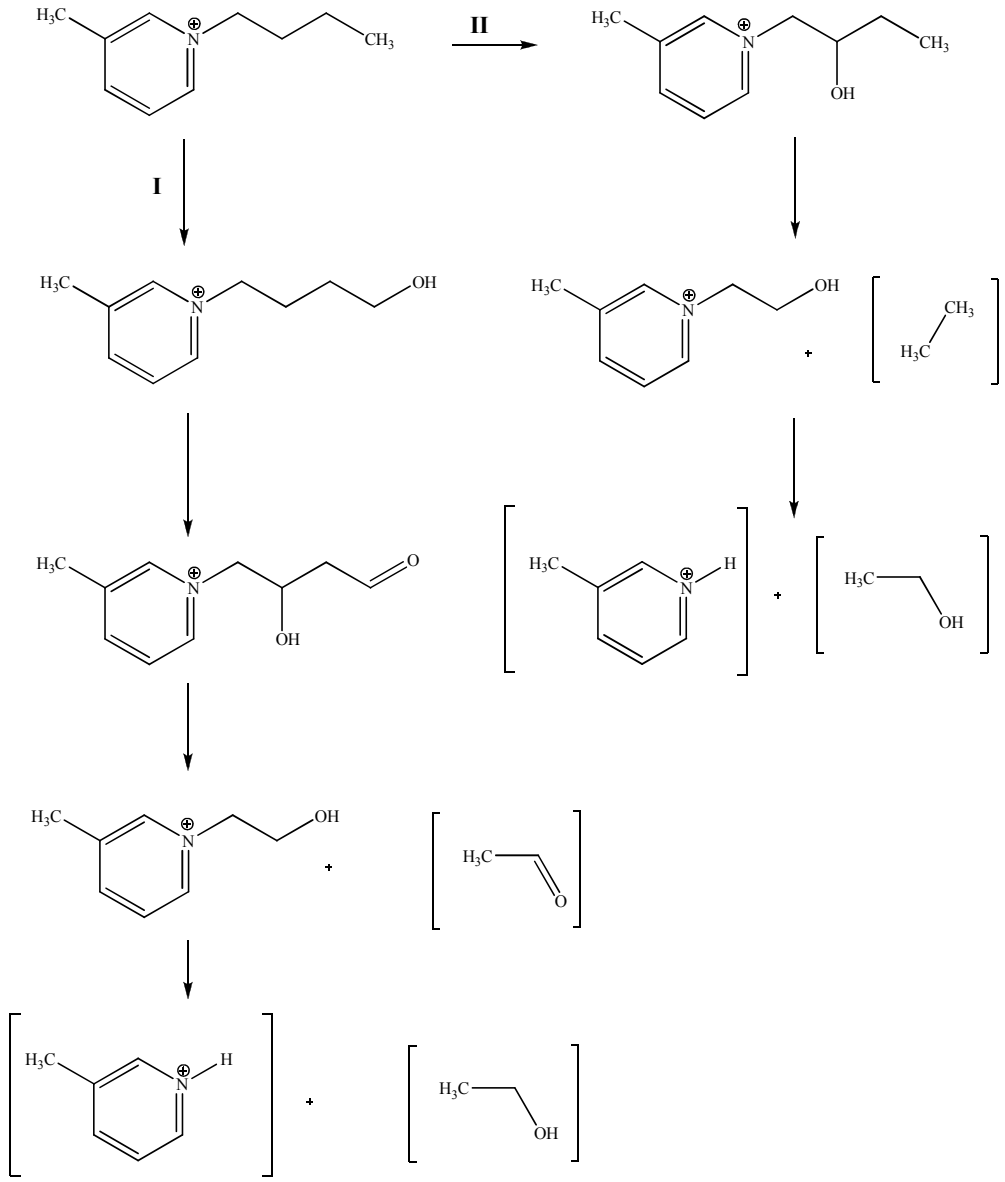
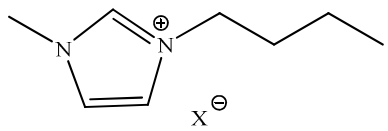


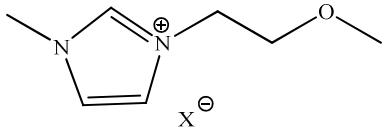
Fig. 1.35: The proposed biodegradation pathways for the *N*-butyl-3-methylpyridinium cation. The intermediates in brackets were not detected or confirmed in the study.[61]

1.3.5 Biodegradation studies of ionic liquids in soil

Biodegradation of ionic liquids in soil has also been addressed Mondelli[62] who reported the first aerobic biodegradation of imidazolium ionic liquids in soil. Two cations, 1-butyl-3-methylimidazolium [bmim] and 1-methoxyethyl-3-methyl imidazolium [$MeOCH_2CH_2mim$], with BF_4 and $N(CN)_2$ anions (Figure 1.36) were tested.



36 X = BF_4 [bmim][BF_4]
39 X = $N(CN)_2$ [bmim][$N(CN)_2$]



164 X = BF_4 [$MeOCH_2CH_2mim$]][BF_4]
165 X = $N(CN)_2$ [$MeOCH_2CH_2mim$][$N(CN)_2$]

Fig. 1.36: Oxygenated and non-oxygenated ionic liquids screened for biodegradation in soil.

The tests were carried out over six months according to the ASTM D 5988-96 protocol. 0.5 g of each ionic liquid was mixed with 300 g of soil, sieved to 2 mm particle size and tested in triplicate in a single batch. CO_2 was measured by titrations every 2-3 days for the initial two months of the test period and then weekly over the remaining 4 months. It was found from these studies that *n*-butyl derivatives were biodegradable, with the dicyanamide ionic liquid being less biodegradable than the tetrafluoroborate. No significant CO_2 evolution was observed with the oxygenated ionic liquids [$MeOCH_2CH_2mim$][BF_4] **164** and [$MeOCH_2CH_2mim$][$N(CN)_2$] **165** over the six months.

Atomic charge distributions and frontier orbital structures of the 1-alkyl-3-methylimidazolium cations with B3LYP/6-31G(d) were calculated using the Mulliken approximation. Changes in electron distribution resulting from replacement of a methylene group in the ester side chain by an ether linkage were estimated using this computational method. The localisation properties of the HOMO of the oxygen derivative were very different from those of the *n*-butylimidazolium cation, but until the pathway of biodegradation is more firmly established, the role which electron distribution may play in improving biodegradability remains uncertain.

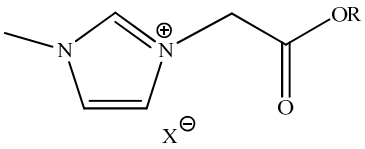
1.4 Applications of Biodegradable ionic liquids

Though a worthwhile goal is to obtain biodegradable ionic liquids, it is also important that these ionic liquids can be applied as green alternative solvents. One such application is as reaction media in chemical synthesis. A few publications have investigated the potential of known biodegradable ionic liquids as new solvents. Comparison with other ionic liquids

and VOCs provides researchers with valuable data on whether a performance advantage can be obtained in addition to the enhanced biodegradation properties.

1.4.1 Diels-Alder reactions

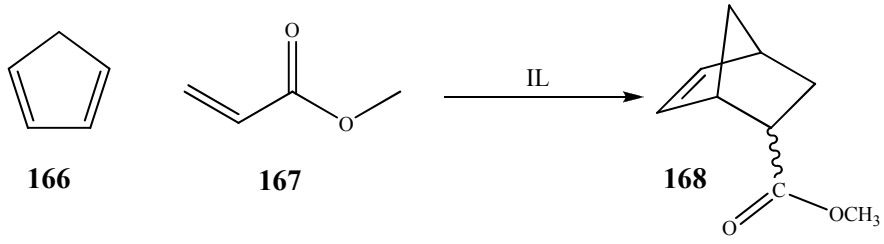
Scammells and co-workers[63] evaluated the readily biodegradable ionic liquids 3-methyl-1-(propoxycarbonylmethyl)imidazolium octylsulfate and 3-methyl-1-(pentoxycarbonylmethyl)imidazolium octylsulfate (Figure 1.37) as reaction solvents for Diels-Alder reactions.



34 R = C_3H_8, X = $OctOSO_3$ [$PrOCO_2CH_2mim$][$OctOSO_3$]
45 R = C_5H_{12}, X = $OctOSO_3$ [$PnOCOCH_2mim$][$OctOSO_3$]

Fig. 1.37: Biodegradable ionic liquids 3-methyl-1-(propoxycarbonylmethyl)imidazolium octylsulfate **34** and 3-methyl-1-(pentoxycarbonylmethyl)imidazolium octylsulfate **45**.

The cycloaddition reaction between cyclopentadiene and methyl acrylate was carried out in biodegradable ionic liquids and the results compared with those using conventional 1,3-dialkylimidazolium salts (Table 1.9). The *endo*-selectivity was clearly enhanced in the biodegradable examples when compared with those performed in conventional molecular organic solvents or in water. Ionic liquids [$PrOCOCH_2mim$][$N(CN)_2$] **33**, [$PrOCO_2CH_2mim$][$OctOSO_3$] **34** and [$PnOCOCH_2mim$][$OctOSO_3$] **45** gave high yields (72-82 %) of product and *endo*/*exo* selectivities of 2.1-3.4 after 72 hours reaction time.



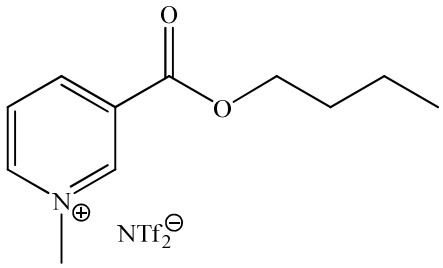
166 **167** **168**

Table 1.9: Reaction of cyclopentadiene with methyl acrylate in imidazolium ILs.

Entry	Ionic Liquid	Time (h)	Yield (%)	*Endo*-to-*exo* ratio
1	**169** [hmim][BF_4]	1[d]	16[a]	3.8
2	**170** [emim][BF_4]	72 [d]	91[c]	4.3
3	**36** [bmim][BF_4]	24 [d]	97[b]	3.5
4	**37** [bmim][PF_6]	24 [d]	97[b]	3.8
5	**171** [bmim][SbF_6]	24 [d]	94[b]	4.2
6	**38** [bmim][NTf_2]	24 [d]	99[b]	4.2
7	**172** [bmim][CF_3CO_2]	24 [d]	96[b]	4.4
8	**33** [$PrOCOCH_2$mim][$N(CN)_2$]	72 [e]	72[c]	3.4
9	**34** [$PrOCO_2CH_2$mim][$OctOSO_3$]	72 [e]	88[c]	2.8
10	**45** [$PnOCOCH_2$mim][$OctOSO_3$]	72 [d]	82[c]	2.1

[a]Not isolated, estimated by ^{1}H NMR
[b]Yield calculated from gas chromatography
[c]Isolated yield
[d] Reaction carried out at RT
[e] Reaction carried out at 40 °C

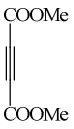
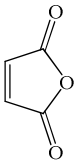
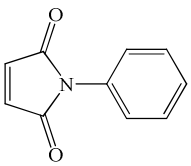
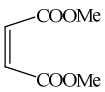
In a more recent publication, this group reported the use of the biodegradable pyridinium based ionic liquid [3-BuOCO-1-Mepy][NTf_2] **110** as the reaction solvent in Diels-Alder reactions[28] (Table 1.10):



110 [3-BuOCO-1-Mepy][NTf_2]

Fig. 1.38: 3-(Butoxycarbonyl)-1-methylpyridinium triflimide **110** used as reaction media in the Diels-Alder reaction by Scammells *et al*..[28]

Table 1.10: Diels–Alder reactions in the biodegradable ionic liquid 3-(butoxycarbonyl)-1-methylpyridinium ditriflimide **110**.[28]

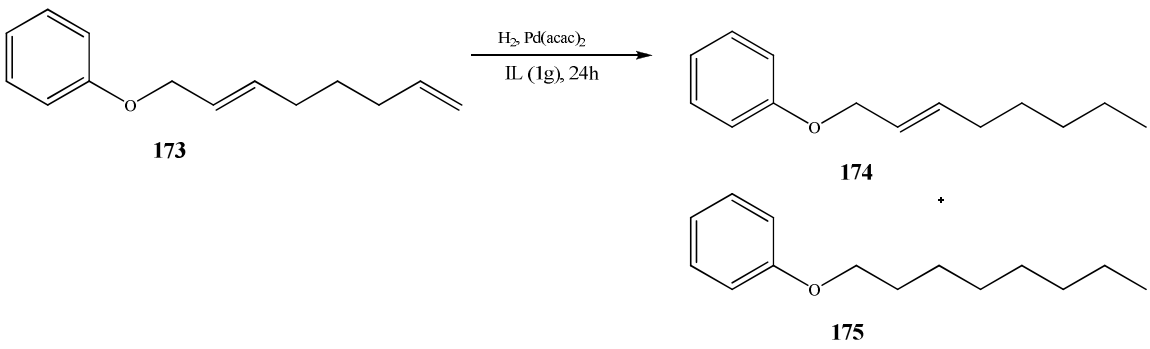
Entry	Dienophile	Reaction time	Isolated yield[a]	*endo*:*exo* ratio[b]
1		72 h	97%	76:24
2		72 h	57%	79:21
3		2 h	97%	100:0
4		10 min	100%	100:0
5		10 min	97%	100:0
6		18 h	95%	95:5

[a]Isolated by column chromatography. [b] Determined by ^{1}H NMR.

Cyclopentadiene reacted with a range of dienophiles, including methyl vinyl ketone (Entry 2), dimethylacetylene dicarboxylate (Entry 3), maleic anhydride (Entry 4), *N*-phenylmaleimide (Entry 5) and dimethyl maleate (Entry 6). The reaction between cyclopentadiene and methyl acrylate in ionic liquid [3-BuOCO-1-Mepy][NTf_2] **110** gave the expected Diels-Alder adduct in 70 % yield after 24 hours and in 97 % after 72 hours. All the dienophiles reacted readily with cyclopentadiene in [3-BuOCO-1-Mepy][NTf_2] **110** to give the corresponding cycloaddition products in good to very good yields (57-100 % yield). It was also noted that reactivity was directed by the electron deficient character of the dienophile, indirectly affecting the reaction time and selectivity of the reaction.

1.4.2 Hydrogenation reactions

Bouquillon *et al.*[63] described a selective hydrogenation of 1-phenoxyoctadiene in which reduction of the terminal double bond occurs as the major process, leaving the internal olefin intact. The hydrogenation was carried out at 0.1 MPa hydrogen pressure using palladium acetylacetonate as the precatalyst and a range of imidazolium ionic liquids, including readily biodegradable 3-methyl-1-(pentoxycarbonylmethyl)imidazolium octylsulfate **45** were screened as solvents (Figure 1.39, Table 1.11).



173 **174** **175**

Fig. 1.39: Hydrogenation of phenoxyocta-2,7-diene (**173**)

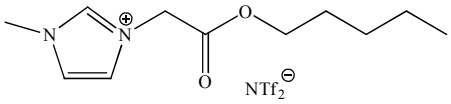
Table 1.11: Hydrogenation of phenoxyocta-2,7-diene (**173**) in ionic liquids.

Entry	Ionic liquid	Conversion[a] (%)	**174** yield (%)	**175** yield (%)
1	**35** [bmim][Br]	76	57	13
2[b]	**35** [bmim][Br]	48	36	5
3	**176** [PnOCOCH$_2$mim][BF$_4$]	85	64	18
4	**177** [PnOCOCH$_2$mim][NTf$_2$]	75	44	28
5	**178** [PrOCH$_2$CH$_2$OCOCH$_2$mim][NTf$_2$]	75	40	30
6	**45** [PnOCOCH$_2$mim][OctOSO$_3$]	85	70	12
7[b]	**45** [PnOCOCH$_2$mim][OctOSO$_3$]	55	48	5

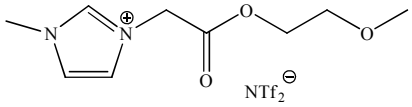
[a]Conditions: Pd(acac)$_2$(0.03 equiv.), H$_2$ (0.1 MPa), 24h
[b]Recycling experiment

The highest conversion for the hydrogenation of 1-phenoxyocta-2,7-diene was obtained using [PnOCOCH$_2$mim][OctOSO$_3$] **45** as the solvent (85 % conversion) with the desired product, 1-phenoxyoct-2-ene obtained in 70 % yield. This reaction provides a clear example of how a biodegradable solvent can not only replace a conventional one, but can actually give an improvement in performance.

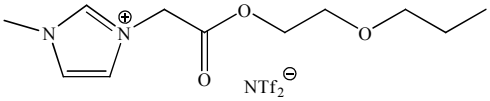
Another selective hydrogenation was recently reported by Morrissey, in which *trans*-cinnamaldehyde was reduced to hydrocinnamaldehyde using ionic liquids, including biodegradable examples.[64] In this study, a range of imidazolium ionic liquids with ester groups in the side chain were used as reaction solvents (Figure 1.40).



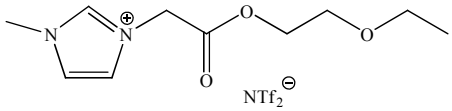
177 [PnOCOCH$_2$mim][NTf$_2$]



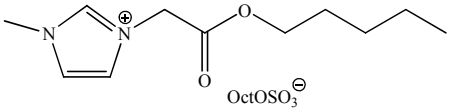
181 [MeOCH$_2$OCOCH$_2$mim][NTf$_2$]



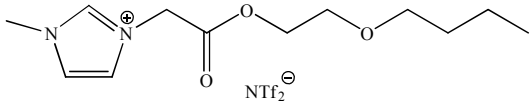
178 [PrOCH$_2$CH$_2$OCOCH$_2$mim][NTf$_2$]



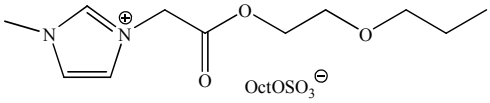
182 [EtOCH$_2$CH$_2$OCOCH$_2$mim][NTf$_2$]



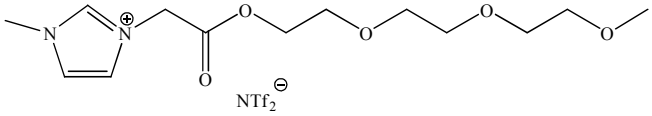
45 [PnOCOCH$_2$mim][OctOSO$_3$]



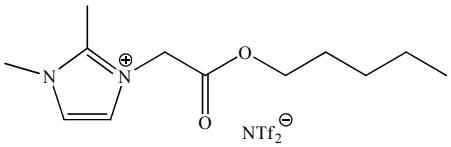
183 [BuOCH$_2$CH$_2$OCOCH$_2$mim][NTf$_2$]



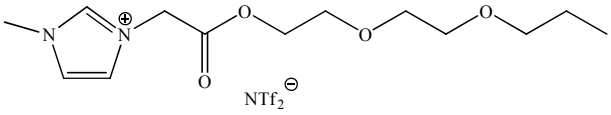
50 [PrOCH$_2$CH$_2$OCOCH$_2$mim][OctOSO$_3$]



184 [Me(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim[NTf$_2$]



179 [1-PnOCOCH$_2$dmim][NTf$_2$]



180 [Pr(OCH$_2$CH$_2$)$_2$OCOCH$_2$mim][NTf$_2$]

Fig. 1.40: Ionic liquids used as reaction solvents in the hydrogenation of *trans*-cinnamaldehyde by Morrissey *et al*..[64]

Selectivities towards hydrocinnamaldehyde ranged from 90-100 % when the imidazolium pentyl esters **177** and **179** were employed as the reaction media. When the dimethyl imidazolium ionic liquid was used a 100 % conversion and selectivity was monitored after 24 hours. Recycling of the catalyst/ionic liquid mixture was investigated, with almost the same reaction efficiency recorded up to the fourth recycle. Table 1.12 illustrates the overall

results obtained when the reaction was carried out in these ionic liquids [PnOCOCH$_2$mim][NTf$_2$] **177** and [1-PnOCOCH$_2$dmim][NTf$_2$] **179**.

Table 1.12: Results obtained in catalytic hydrogenation using [PnOCOCH$_2$mim][NTf$_2$] **177** and [1-PnOCOCH$_2$dmim][NTf$_2$] **179** ILs.

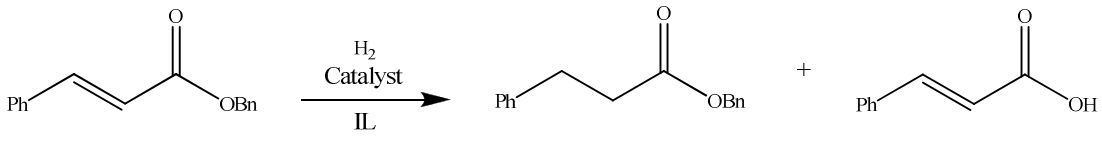
Solvent	Experiment (E) / Recycle (R)	Time (h)	Conversion(%)	Selectivity (%)
179 [1-PnOCO CH$_2$dmim][NTf$_2$]	E1	24	8	100
		48	36	100
	R1	24	100	100
		48	100	93
	R2	24	48	73
		48	97	98
	R3	24	79	99
		48	100	96
	R4	24	89	100
		48	97	100
177 [PnOCO CH$_2$mim][NTf$_2$]	E1	48	98	94
	R1	48	100	93

Trans-cinnamaldehyde was also hydrogenated in the commercially available ionic liquids [bmim][NTf$_2$] and [bmim][OctOSO$_3$] and in toluene (Table 1.13). 100 % Conversion was also possible using these solvents, but the selectivities (67-87 %) were not as high as those achieved using the biodegradable ionic liquids and ionic liquids [PnOCOCH$_2$mim][NTf$_2$] **177** and [1-PnOCOCH$_2$dmim][NTf$_2$] **179** (93-100 % selectivity).

Table 1.13: Hydrogenation of *trans*-cinnamaldehyde in commercially available solvents.

Solvent	Time (h)	% conversion	% selectivity
38 [bmim][NTf_2]	24	100	87
40 [bmim][$OctOSO_3$]	24	100	69
Toluene	24	100	67

Selective hydrogenation of benzyl cinnamate without hydrogenolysis of the benzyl ester (Figure 1.41) was also reported by Morrissey *et al*.[64]



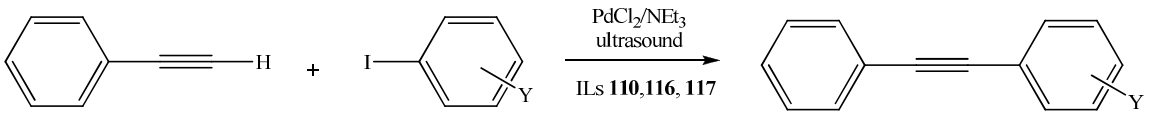
185 **186** **187**

Fig. 1.41: Reduction of benzyl cinnamate.

Solvent effects and catalyst loading were investigated during the hydrogenation of benzyl cinnamate. Using 0.005g of catalyst / 4 mmol substrate under 1 atmosphere of hydrogen 100 % conversion was observed, while only 32 % conversion was achieved if half this mass of catalyst was used. Selectivities were retained when the reaction was performed over 48 hours, suggesting that competing hydrogenolysis of the reduced benzyl ester **186** did not take place. This finding was further supported by a separate experiment in which the reduced ester **186** (benzyl 3-phenylpropanoate) was subjected to the same hydrogenation conditions using an increased amount of catalyst, at 1 atm. H_2 pressure and no hydrogenolysis occurred. This result indicates that the ionic liquid may play a role in suppressing hydrogenolysis of the benzyl ester, both in the substrate, and the product.

1.4.3 Sonogashira coupling reaction

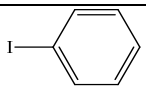
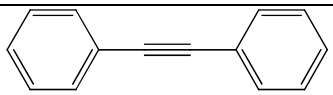
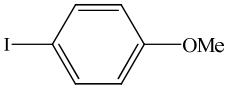
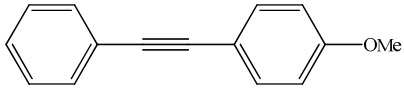
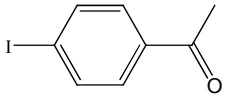
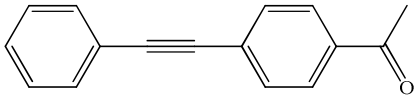
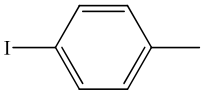
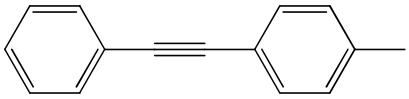
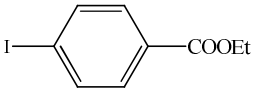
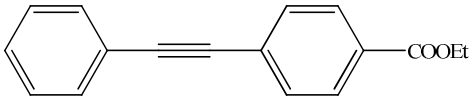
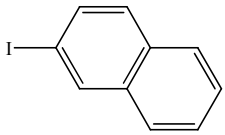
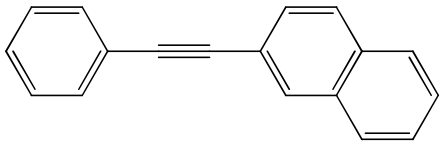
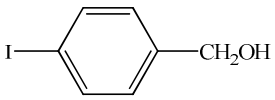
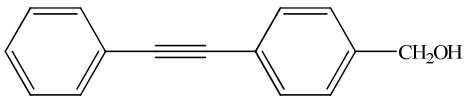
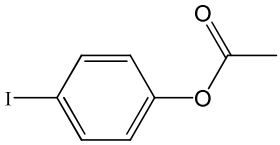
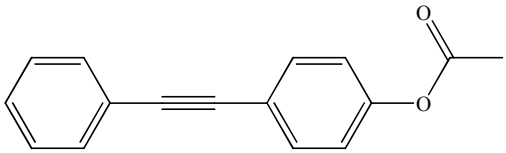
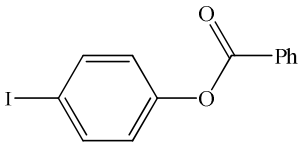
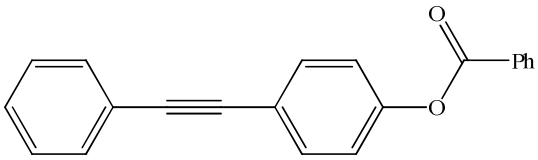
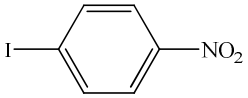
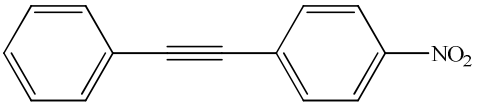
Biodegradable ionic liquids **110**, **116**, **117** were investigated as solvents for Sonogashira coupling reactions in the absence of a copper co-catalyst or a phosphine (Scheme 1.2).[52]

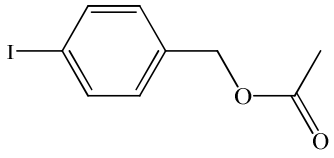
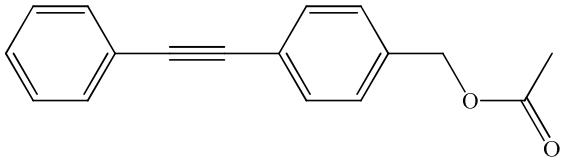
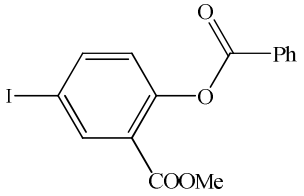
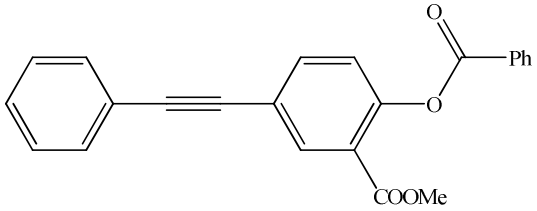
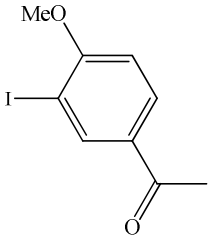
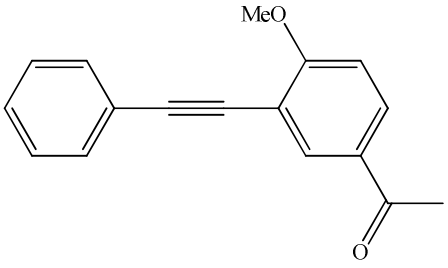
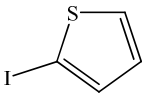
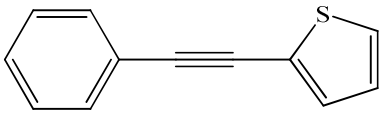
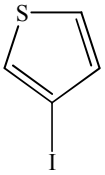
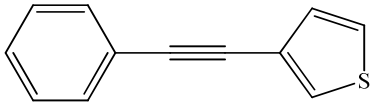
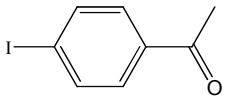
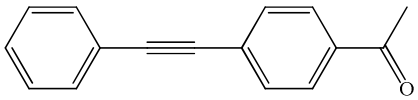
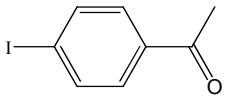
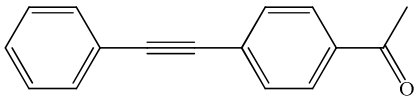


Scheme 1. 2: Sonogashira coupling reaction in ionic liquids **110**, **116** and **117**.[52]

Ultrasonic irradiation was also used in these reactions in order to encourage catalytic activity of the palladium catalyst in the absence of phosphines. It also allowed the reactions to be conducted at room temperature for shorter reaction times. Table 1.34 summarises results obtained whilst using the biodegradable ionic liquid **110** in this type of reaction. The Sonogashira coupling reaction of iodobenzene with diphenylacetylene gave very good yields of 86 % of diphenylacetylene product. The lowest yields were obtained when using iodoarenes with electron donating *para*-substituted groups (Table 1.14, Entries 2 and 4). Biodegradable ILs **116** and **117** were also employed as solvents in these coupling reactions and gave comparable results to IL **110**. The reaction between 1-(4-iodophenyl)ethanone and diphenylacetylene in **116** and **117** gave very good yields of the coupled product (77 and 85 % yield respectively). A similar result was achieved when using **110** as the reaction media (Entry 3, Table 1.14).

Table 1.14: Results obtained in Sonogashira coupling reactions in biodegradable ionic liquids by Scammels *et al*..[52]

Entry	Arene	Product	Yield (%)
1			86
2			47
3			88
4			60
5			93
6			78
7			72
8			81
9			76
10			86

11			93
12			79
13			78
14			98
15			84
16			85
17			77

1.5 Conclusions

Biodegradable ionic liquids have been designed and prepared based on the principles used to improve biodegradation of surfactants. Initial studies aimed at manipulation of the side chain of the ionic liquid structure. The incorporation of ester groups into long alkyl chains reduced toxicity and improved ecotoxicity of ionic liquids.[19,45-48] Further introduction of

ether moieties into the side chain improves the biodegradability of imidazolium-based ionic liquids. Exchanging halide anions with an octylsulfate anion has a further beneficial effect on ionic liquid biodegradation.

Recent work on pyridinium-based ionic liquids demonstrated how the heteroaromatic cationic core can be modified to produce biodegradable examples. As with the imidazolium examples, the inclusion of an ester group in the cation side chain led to improved biodegradability.[28,29] Introduction of the octyl sulfate anion also facilitated improved pyridinium-based ionic liquid breakdown. High levels of biodegradability have also been achieved in examples where environmentally benign anions such as saccharinate and acesulfamate are included.[51] Several ammonium ionic liquids based on choline have been introduced which are biodegradable and simple to prepare.[53-58]

The use of analytical tools such as HPLC-MS, MS/MS and NMR has been employed to assess the primary biodegradation of a large library of ionic liquids. Techniques such as these allow for the identification of possible metabolites formed during the biological breakdown of ionic liquids. Possible pathways of biodegradation can be traced using HPLC-MS, indicating which part of the ionic liquid is being targeted during the process of mineralization.

Biodegradable ionic liquids have been investigated as solvents in synthetic organic reactions.[52,63,64] In particular, superior product selectivities have been observed compared with conventional organic solvents and commercially available ionic liquids when biodegradable imidazolium-based ionic liquids are used as reaction media for Diels-Alder and hydrogenation reactions. Catalyst performance has also been enhanced in selective hydrogenations of cinnamaldehyde and benzyl cinnamate using biodegradable ionic liquids when recycling of the solvent / catalyst system could be achieved without any significant loss in activity of the catalyst.[64] Biodegradable pyridinium-based ionic liquids have been employed as solvent media in Sonagashira coupling reactions, with high yields of aryl products obtained.[52]

We have recently published a review on Biodegradation studies of Ionic Liquids (Appendix III).[65]

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Chapter 2: Results and discussion

Ester and Amide functionalised achiral Ionic Liquids, Synthesis and Characterisation

2.1 Introduction

A conclusion can be drawn from a review of the literature on Ionic Liquid (IL) research, that a vast number of Task Specific Ionic Liquids (TSILs) have been designed, prepared and studied for various applications. The ILs prepared herein are based primarily on the imidazolium cation. Preparation of imidazolium-cation based ILs has been widely researched due to their facile preparation, low melting points and relatively favorable viscosities.[1] Incorporation of various functional groups into the side chain of the imidazolium cation has been studied in this work, with a view of reducing the toxicity and improving biodegradability of the prepared ILs.

2.1.1 Aim

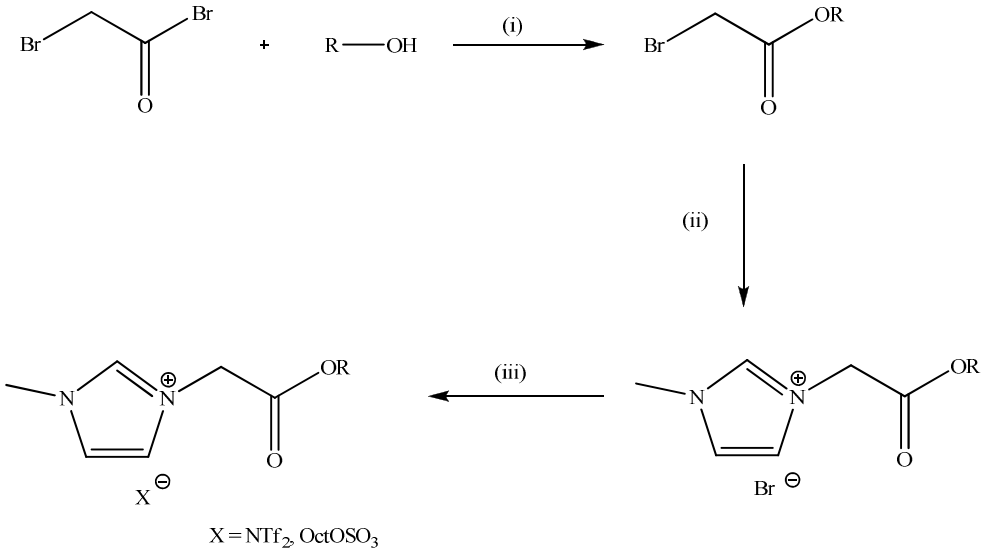
The main objective of the work outlined in this chapter was to synthesise a range of model ester and amide achiral ionic liquids, with various alkyl side chains. The introduction of these functional groups has previously been achieved.[2,3] By adding esters and amides to the IL structure, the physical and biological properties of the salts can be altered. Improved biodegradation and toxicity were reported for ILs with ester containing side chains.[2] Ester groups provide sites for possible enzymatic hydrolysis when exposed to the environment. The introduction of amide functionalities on the other hand, can improve the performance of an IL when employed as reaction media. However the presence of amide groups in IL structures also results in elevated melting points and an increased stability towards biodegradation (more stable to hydrolysis than esters). The presence of long alkyl chains can also assist in IL environmental breakdown,[4] but can also lead to high levels of toxicity.[5]

However, from a clinical point of view this toxicity may be beneficial in the production of antimicrobial agents (against known pathogenic bacteria such as MRSA). These achiral ILs were prepared in their pure form and a toxicity screen was performed on these pure salts. The synthesis of the achiral derivatives involved a three step preparation process, with the formation of the bromide salt followed by anion metathesis reactions.

2.2 Synthesis of achiral Ionic Liquids

2.2.1 Synthesis of achiral ester Ionic Liquids

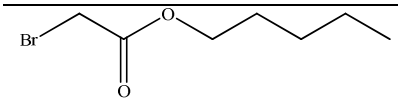
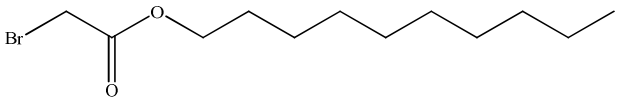
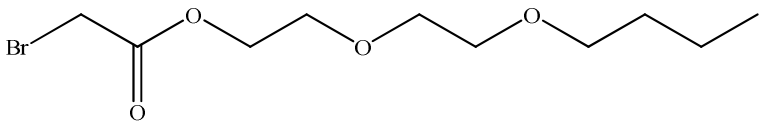
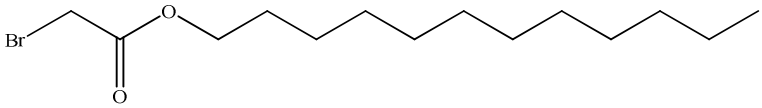
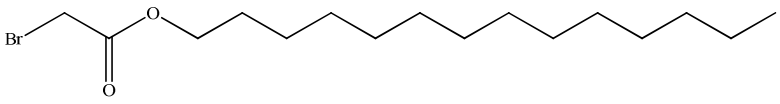
The general scheme for the three step synthesis of achiral ionic liquids is illustrated in Scheme 2.1. The first step *(i)* of the achiral IL synthesis is an esterification reaction, which involves the formation of an alkylating intermediate. This was carried out by the reaction of bromoacetyl bromide with various alkyl alcohols (with increasing side chain lengths; C5, C10, C12 and C14) in the presence of a base. Low temperature ranges of -15 and -78 °C were used in these reactions, in particular during addition of bromoacetyl bromide.



Scheme 2.1: General scheme for achiral ester ionic liquid preparation; (i) Bromoacetyl bromide, Et_3N, (ii) 1-methyl imidazole, (iii) $NaOctOSO_3$ or $LiNTf_2$.

These α-bromoester derivatives were obtained in average yields, Table 2.1 below summarises the yields obtained for all the alkylating agents prepared.

Table 2.1: Yields obtained for achiral α-bromoester intermediates.

Alkylating reagent	Compound No.	Yield (%)
	**188**[10]	64
	**189**[2]	52
	**190**[2]	61
	**191**	51
	**192**	42

These reactions yielded crude products which contained some coloured impurities (typically dark brown or black in colour). From ^{1}H NMR analysis these crude compounds showed little impurity. However, it was necessary to remove these coloured impurities, even though only present on a small scale, from the intermediate in order to prepare pure ILs of limited colour. Figure 2.1 (a) and (b) demonstrate the difference between the pure and crude bromoesters.

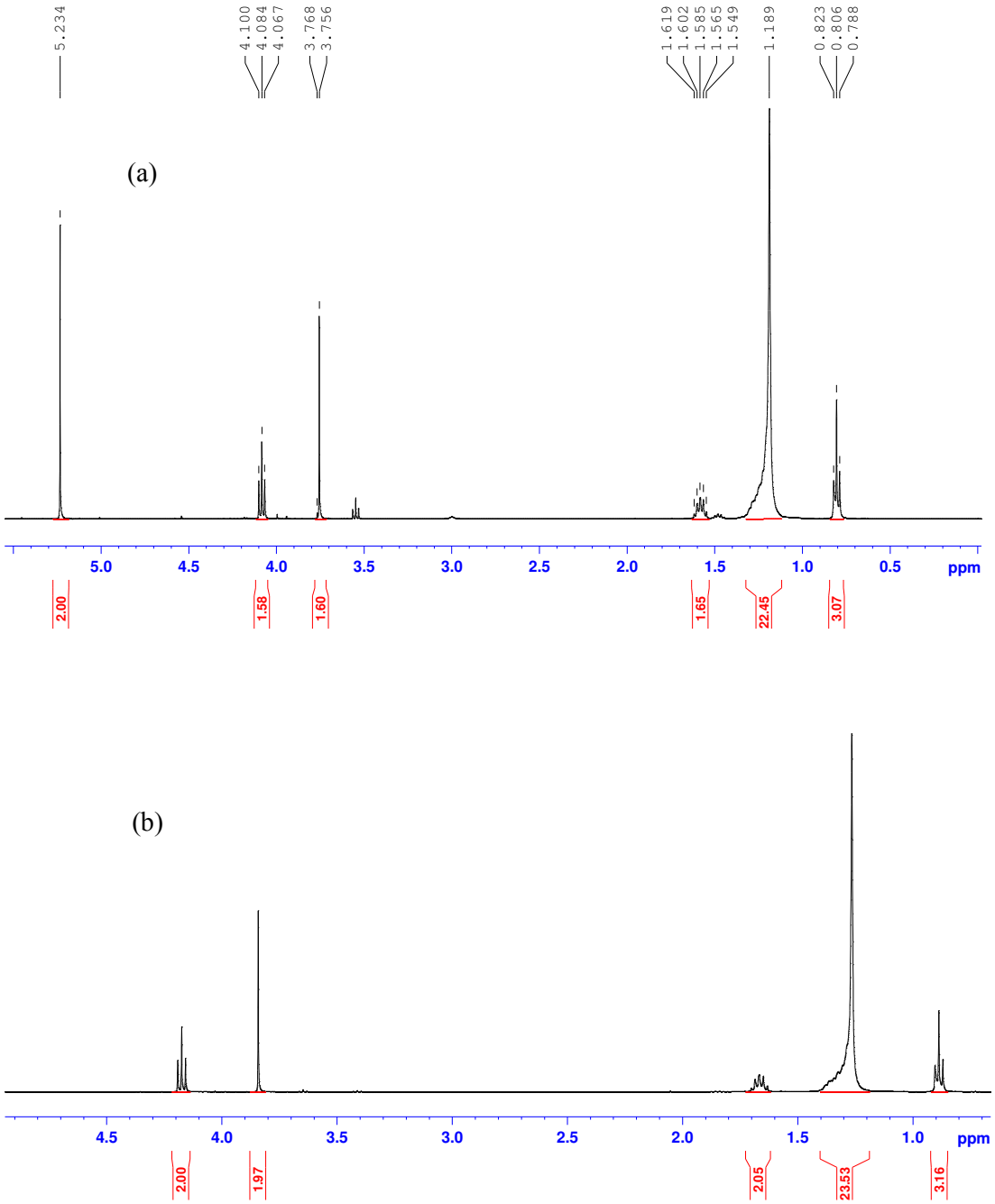


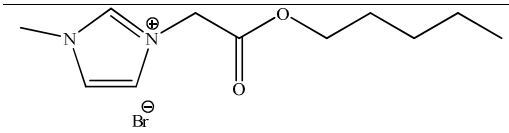
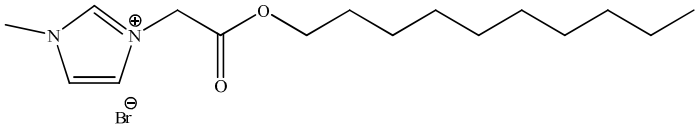
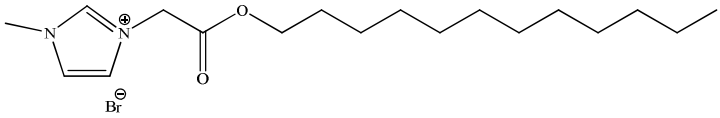
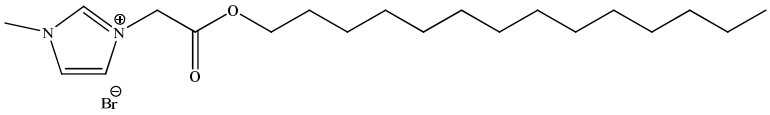
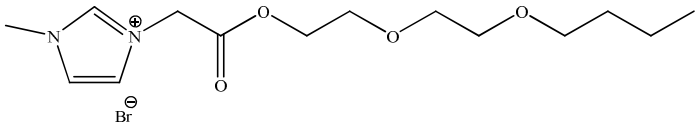
Fig. 2.1: ^{1}H NMR of crude tetradecyl-2-bromoacetate (a), and of pure tetradecyl-2-bromoacetate (b).

In the ^{1}H NMR of the crude tetradecyl-2-bromoacetate (Figure 2.1 (a)) some peaks which cannot be assigned as protons from the product can be seen. Namely, a singlet peak at δ 5.24 is observed and can be assigned as dichloromethane solvent which can be easily removed *in vacuo*. At ~ δ 3.51 and 1.49 some minor multiplet peaks can be noted and are attributed to the unreacted tetradecyl alcohol starting material. This alcohol has a high boiling point, and is therefore difficult to remove. Drying under high vaccum did not remove these signals from the ^{1}H NMR spectrum.

The crude α-bromoesters were purified by vacuum distillation and the pure products were obtained as colourless to pale yellow liquids. However purification by distillation of some of the bromoesters became increasingly difficult. The alkylating agents with longer alkyl chains were difficult to purify by distillation, presumably due to their increased molecular weight, which lead in an increase in their boiling point. This resulted in lower yields obtained for these compounds with increased alkyl side chains. This can be seen in Table 2.1, yields for decyl, dodecyl and tetradecyl bromoesters are slightly lower than those obtained for the other shorter chain derivatives. Some of these reactions were carried out on a large scale synthesis (120-400 mmol). In such cases an increase in the equivalents of bromoacetyl bromide starting material used was employed to ensure the reaction reached completion. Reaction times were also increased while maintaining a low temperature (- 78 °C) to encourage completion of the alkylating reagent formation. In the synthesis of dodecyl-2-bromoacetate and tetradecyl-2-bromoacetate a higher reaction temperature of -15 °C was utilised. Initially, during dropwise addition of bromoacetyl bromide to the alkyl alcohols at - 78 °C, the reaction mixture solidified and adequate stirring could not be achieved. Increasing the reaction temperature to -15 °C during addition of bromoacetyl bromide solved this problem.

The next step in the IL synthesis *(ii)* involved alkylation of 1-methyl imidazole with these α-bromoesters to produce the bromide salts. The yields obtained from these reactions are tabulated in Table 2.2.

Table 2.2: Summary of yields and melting points obtained for the bromide salts prepared.

Ionic Liquid	Compound No.	Yield (%)	m.p (°C)
	**42**[10]	96	n/a
	**193**[2]	97	51-53
	**194**	55	56-58
	**195**	82	64-66
	**196**[2]	89	50-52

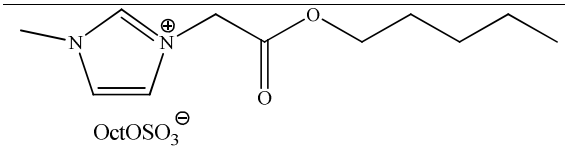
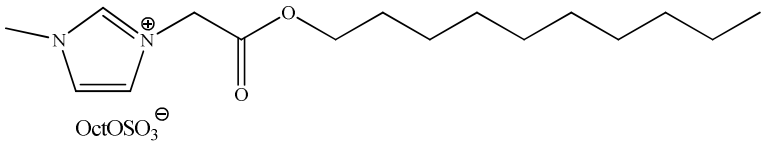
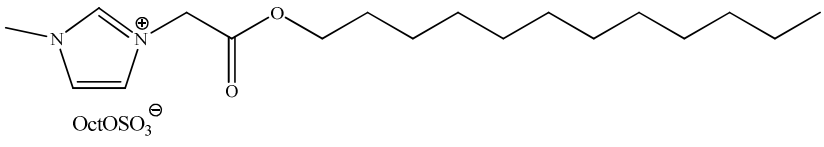
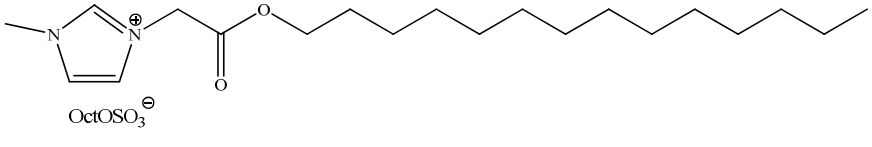
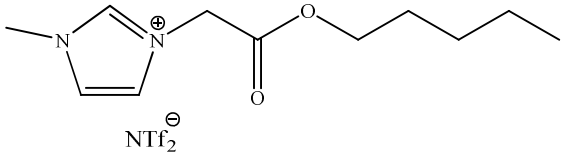
n/a liquid at R.T.

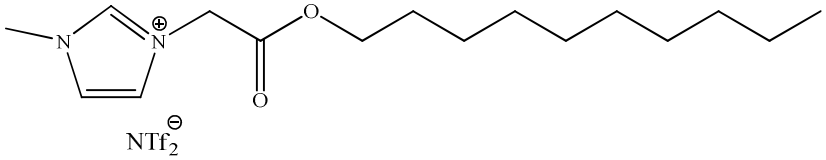
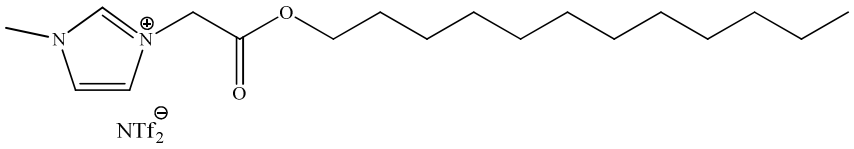
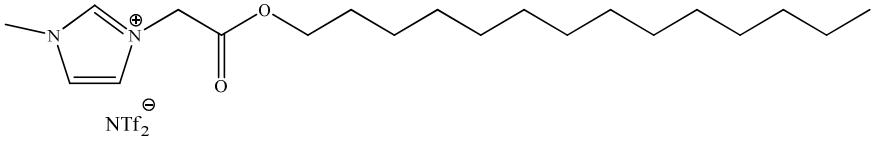
In this reaction, the α-bromoester was added drop wise to a stirring solution of 1-methylimidazole in diethyl ether under an inert atmosphere. Diethyl ether was the solvent of choice in these experiments as the bromide salt precipitated facilitating isolation. The ether layer could then be decanted and the IL was then washed numerous times with diethyl ether. In some cases the IL was stirred in ether overnight in order to achieve thorough washing of the salt. The ILs were then usually dried under high vacuum to remove residual solvent and drying times varied depending on solvent(s) present in different IL species. Good to very good yields were obtained for the bromide salts (55-97%). Most of the

bromide ILs formed were solids at room temperature (i.e. decyl, dodecyl, tetradecyl and 2-(2-butoxyethoxy)ethyl derivatives). All the salts prepared could be classified as ionic liquids, due to their low melting points (i.e. < 100 °C) ranging from 50-66 °C (Table 2.2)

After the bromide salt preparation, the final step *(iii)* in synthesis of ester achiral ionic liquids was anion exchange reactions. A series of octyl sulfate ($OctOSO_3$) and bistriflimide (and NTf_2) salts were prepared (Table 2.3). Anion metathesis reactions were performed using sodium and lithium salts (i.e. sodium octylsulfate and lithium trifluoromethanesulfonimide).

Table 2.3: Results obtained for counter ion exchange reactions.

Ionic liquid	Compound No.	Yield (%)
	**45**[2]	85
	**197**	71
	**198**	79
	**199**	95
	**177**[2]	90

	**200**	79
	**201**	89
	**202**	88

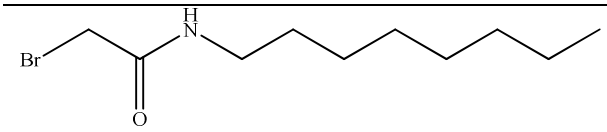
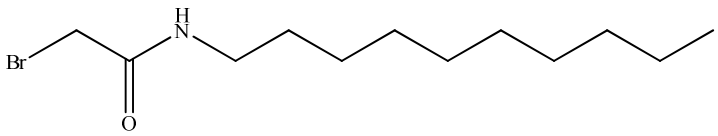
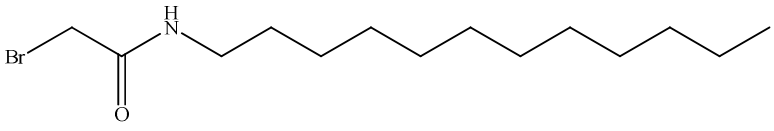
The ILs were obtained in good to excellent yields (71-95%). All the salts prepared were liquids at room temperature. Therefore, by changing the anion of the bromide ionic liquid the physico-chemical properties of the salt were altered.[6,7,8] It was noted that the NTf_2 salts (**177**-**202**) were hydrophobic and liquid in nature. In these ILs the lattice energy can be reduced as a result of charge dispersion due to the SO_2CF_3 groups present in the anion species. The $OctOSO_3$ derivatives were prepared in order to improve the biodegradability of the ionic liquids as seen previously within the group.[9,10]

To prepare the NTf_2 salts, the bromide ILs were stirred with lithium salt ($LiNTf_2$) in distilled water at room temperature. Hydrophobic NTf_2 ILs precipitated out of solution and were washed several times with water to yield salts in very good yields (79-90%). In the case of the $OctOSO_3$ salts the method was not as straightforward.[11] The bromide salts were stirred with sodium octylsulfate in distilled water at 60 °C for 2 hours. Following this reaction time, the water was removed slowly *via* rotary evaporation. An oily residue was obtained, re-dissolved in dichloromethane and washed with distilled water (three times) to remove excess sodium salt. Residual solvent was then removed under reduced pressure. The yields of the $OctOSO_3$ salts decreased after the water washings. This is due to the increased solubility of the $OctOSO_3$ product in water and DCM layers, compared to the NTf_2 ILs.

2.2.2 Synthesis of achiral amide Ionic Liquids

In addition to the ester derivatives described above, novel amide achiral ionic liquids were prepared. The synthesis of these ILs employed the same methodology previously used to prepare the ester ILs (Scheme 2.1). In the first step *(i)* of the IL synthesis, alkyl amines (octyl, decyl and dodecyl amines) were reacted with bromoacetyl bromide to form bromoamide alkylating intermediates. This reaction yielded crude products which contained some coloured impurities. Vacuum distillation was carried out on the crude bromoamides, to give the purified products in moderate to good yields (44-92 %) (Table 2.4).

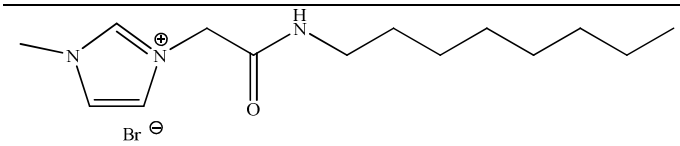
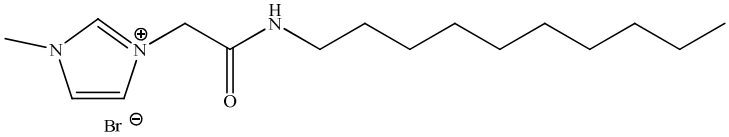
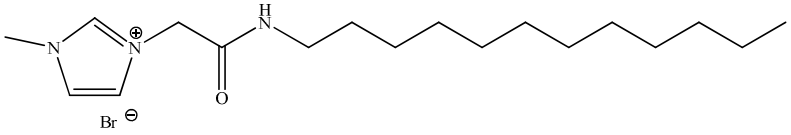
Table 2.4: 2-Bromo-*N*-alkylacetamide intermediates.

Alkylating reagent	Compound No.	Yield (%)
	**203**	44
	**204**	71
	**205**	92

Step *(ii)* involved the alkylation reaction of these intermediates with 1-methylimidazole using the same synthethic protocol as before for the ester compounds. In this, the α-

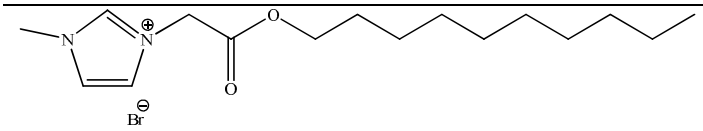
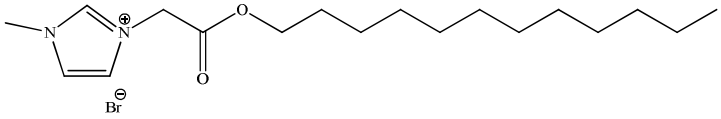
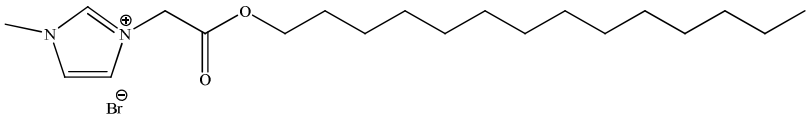
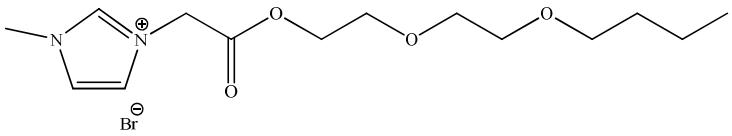
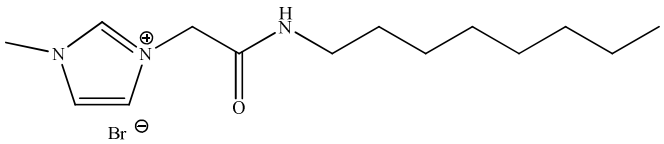
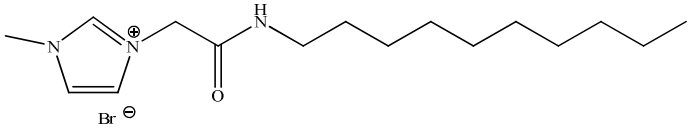
bromoamide intermediate was added dropwise to a stirring solution of 1-methylimidazole in diethyl ether solvent at – 15 °C and allowed to proceed under an inert atmosphere overnight. The amide ILs precipitated and purification was hence simplified, with ether washings required to remove the imidazole starting material impurity. Excellent yields (94-98%) were achieved for the achiral amide bromide salts. All the ILs were solids at room temperature, however with melting points below 100 °C (Table 2.5).

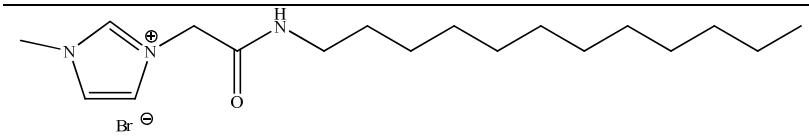
Table 2.5: Achiral amide Ionic Liquids; yields and melting points.

Ionic liquid	Compound No.	Yield (%)	m.p (°C)
	**206**	98	82-84
	**207**	94	85-87
	**208**	94	89-91

It is apparent from the accumulated experimental data that manipulation of the ionic liquid structures results in a direct effect on their corresponding melting points. Upon anion exchange from bromide to NTf_2 and $OctOSO_3$ salts, depression of melting point values was observed. Furthermore, changes to the IL cation structures also lead to noticeable differences in the melting points measured. Table 2.6 summarises the achiral ionic liquids synthesised and their obtained melting points.

Table 2.6: Melting points of achiral ionic liquids*.

Ionic Liquid	m.p (°C)
 **193**	51-53
 **194**	56-58
 **195**	64-66
 **196**	50-52
 **206**	82-84
 **207**	85-87



89-91

208

*Note: ILs **42**, **45**, **197**-**202** were liquids at RT

2.3 NMR studies of achiral Ionic Liquids

All the achiral ester and amide salts prepared were characterised using various spectroscopic techniques; including ^{1}H NMR, ^{13}C NMR, DEPT 135, COSY and HMQC 2D NMR.

2.3.1 ^{1}H NMR studies of achiral Ionic Liquids

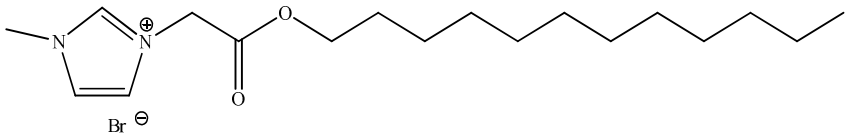
Novel achiral ester (**42**, **45**, **177**, **193**-**202**) and amide ionic liquids (**206**-**208**) were characterized by ^{1}H NMR spectroscopy. ^{1}H NMR experiments were conducted in deuterated chloroform ($CDCl_3$) solvent. The ^{1}H NMR data obtained for known ILs (**42**, **45**, **177** and **193**) was in agreement with that previously reported in the group.[2,10] The acidic proton[12] of the imidazolium ring (-NC*H*N-) gives a singlet peak whose chemical shift differs depending on the anion species present in the IL structure. In the case of bromide ILs, the acidic imidazolium C*H* appears downfield at ~ δ 9.73-10.28. NTf_2 achiral ILs give signals shifted upfield towards ~ δ 8.75 and the $OctOSO_3$ derivatives are only slightly more upfield to the bromide derivatives at ~ δ 9.23-9.41.

Another common signal in the ^{1}H NMR spectra of these compounds is due to the methylene group adjacent to the imidazolium core. This group gives rise to signals at ~ δ 5.29-5.47 for bromide achiral ester and amide ILs. Achiral $OctOSO_3$ salts produce signals that are similar in chemical shift at ~ δ 5.12. In the NTf_2 analogues, the methylene peak is again shifted slightly further upfield at ~ δ 4.90.

Table 2.7: Selected ^{1}H NMR data (δ, $CDCl_3$) of achiral Ionic Liquids (**194, 195, 198, 199, 201, 202, 207** and **208**).

Compound No.	Acidic imidazolium C*H*	Imidazolium C*H*'s	Methylene NCH_2
194	10.26	7.56,7.43	5.47
195	10.17	7.58,7.47	5.47
198	9.23	7.35,7.33	5.12
199	9.41	7.30,7.22	5.12
201	8.74	7.30,7.25	4.93
202	8.75	7.29,7.24	4.94
207	9.79	7.65,7.26	5.37
208	9.73	7.57,7.15	5.29

2.3.1.1 ^{1}H NMR spectroscopic study of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium bromide (194)



(**194**)

In the ^{1}H NMR spectrum of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium bromide (in $CDCl_3$ solvent), the acidic proton of the imidazolium ring (-NC*H*N-) appears at δ 10.26. The other imidazolium protons (-NC*H*-C*H*N-) appear at δ 7.56 and 7.43 as triplet peaks with coupling constants of 1.8 Hz (Figure 2.2). It would be expected to observe a set of doublet peaks for these protons as they are coupling with each other. However it appears that coupling between these protons with the acidic hydrogen, through the aromatic ring, is

occurring giving rise to triplet signals. At δ 5.47 a singlet peak is evident with an integration of 2, and can be assigned as the methylene group between the alkyl ester side chain and the imidazolium core. A triplet occurring at δ 4.20 and integrating as two protons with a coupling constant of 6.8 Hz, is due to the OCH_2 methylene group of the ester side chain. This type of splitting is as expected for these protons as they are coupling to the neighbouring methylene protons of the alkyl chain. At δ 4.10 a singlet peak can be seen with an integration of three protons and is due to the *N*-methyl group of the imidazolium ring. In the upfield region of the ^{1}H NMR spectrum, the protons of the long alkyl chain can be seen. A triplet of triplet (tt) peak is observed at δ 1.67 with coupling constants of 6.8 and 7.2 Hz. This is due to the second methylene group of the alkyl ester side chain. All of the remaining protons of the dodecyl alkyl chain appear together as a broad multiplet peak at δ 1.36-1.23 with an integration of 18 protons. The last peak appearing in the aliphatic region corresponds to the terminal methyl group of the long alkyl chain, giving a triplet signal at δ 0.89 with coupling constant of 7.2 Hz.

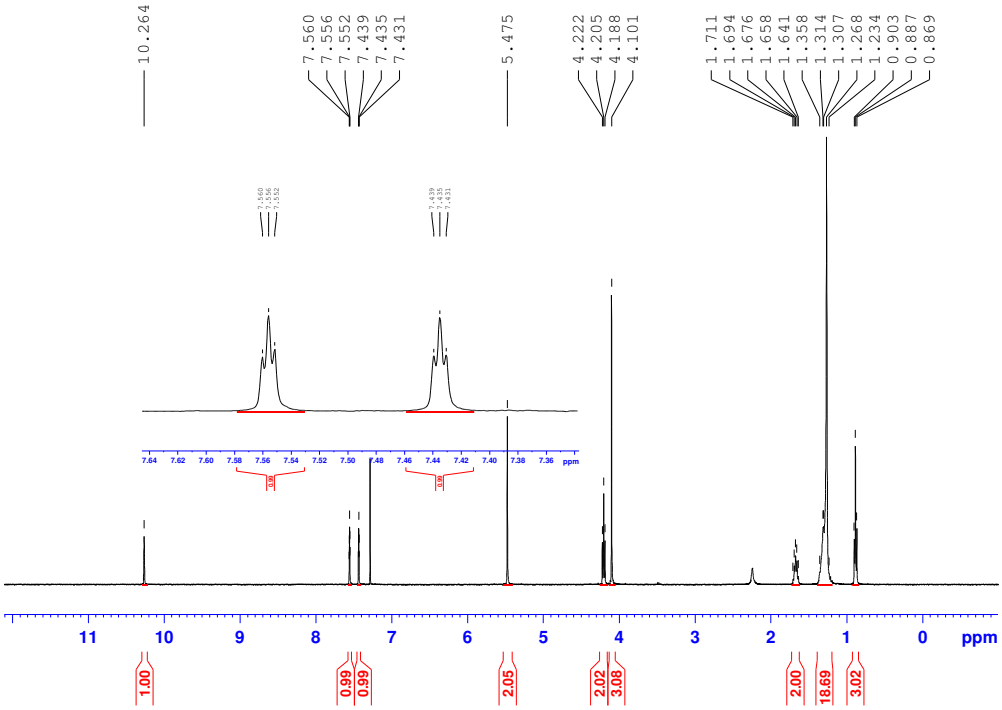
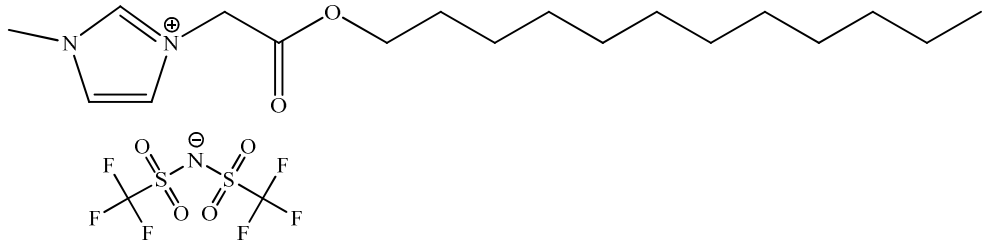


Fig. 2.2: ^{1}H NMR spectrum ($CDCl_3$) of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium bromide (**194**).

2.3.1.2 ^{1}H NMR spectroscopic study of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium NTf_2 (201)



(**201**)

Studying the ^{1}H NMR spectra in terms of chemical shift investigations, can prove useful when analysing ILs with varying anionic species. For bistriflimide examples no additional peaks are observed in the ^{1}H NMR spectra due to the lack of protons in the inorganic anion. Hence one way of differentiating between NTf_2 ILs and those with a bromide anion is by studying differences in the chemical shifts of various protons of the cation in the NMR spectra. Differences in these proton chemical shifts can also be due to an ion concentration effect.[13] However, this concentration effect on chemical shift is not as pronounced as that observed as a result of anion exchange from the bromide salt. In the ^{1}H NMR spectrum of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium NTf_2, the acidic proton of the imidazolium cation appears as a singlet shifted upfield at δ 8.74 compared to the bromide counterpart (δ 10.26). The methine groups of the imidazolium ring can be seen as a set of two triplets with coupling constants of 1.8 Hz. These peaks again appear shifted upfield at δ 7.30 and 7.25, relative to the bromide salts (peaks at δ 7.56 and 7.43). At δ 4.93 a singlet due to the methylene group adjacent to imidazolium ring is evident. When comparing the chemical shift of this peak to the bromide IL it can be noted that this signal has been shifted upfield (methylene peak appears at δ 5.47 for bromide salt). At δ 4.13 a triplet with a coupling constant of 6.8 Hz can be seen and is assigned as the OCH_2 methylene of alkyl ester side chain. A change in chemical shift of the peak due to the *N*-methyl group of the imidazolium cation is evident. A singlet peak at δ 3.88 can be seen in the spectrum (Figure 2.3), and has been shifted when compared to signal seen in the spectrum of the bromide IL (δ 4.10). The peaks corresponding to the linear alkyl chain all appear at the most upfield area of the NMR spectrum but with slightly different chemical shifts than those of the IL

containing bromide anion. A more upfield shift is observed for these signals, further highlighting the effect of the NTf_2 anion on the cation component. A triplet of triplets (tt) is seen at δ 1.59 with coupling constants of 6.8 and 7.2 Hz and are due to methylene group neighbouring the O*C*H_2 group. A broad multiplet at δ 1.24-1.18 arises from the remaining protons of the alkyl chain (integration of 18 protons). A triplet at δ 0.81 with a coupling constant of 7.0 Hz, can be assigned as the methyl group of the dodecyl chain.

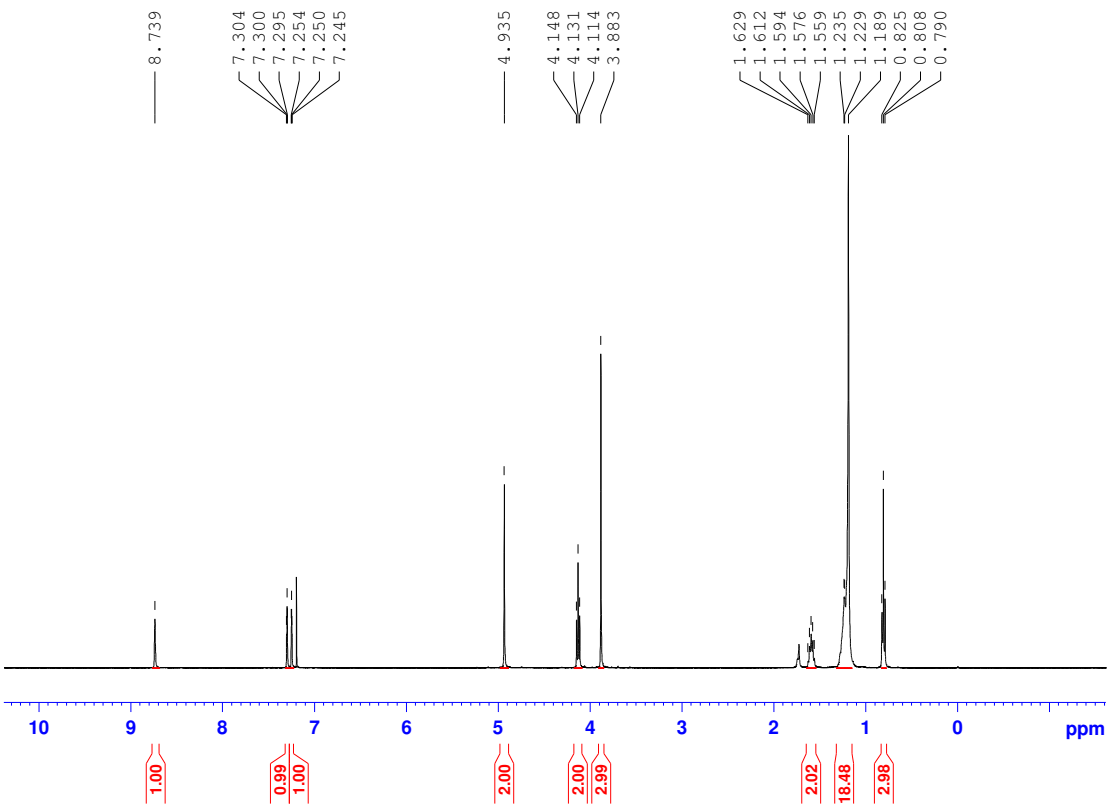
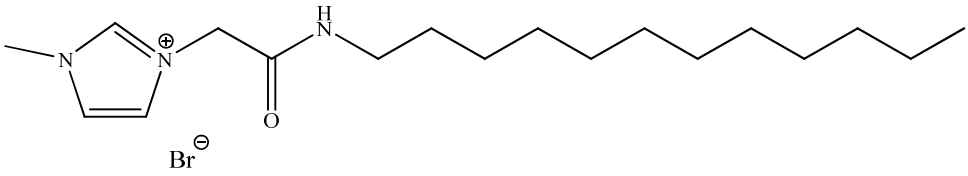


Fig. 2.3: ^{1}H NMR spectrum ($CDCl_3$) of 3-methyl-1-(dodecoxycarbonylmethyl) imidazolium NTf_2 (**201**).

2.3.1.3 ^{1}H NMR spectroscopic study of 3-methyl-1-(2-(dodecylamino)-2-oxoethyl)-imidazolium bromide (208)



(**208**)

Changes in the IL cation are clearly evident in the corresponding ^{1}H NMR spectrum. The difference between ester and amide functionalities in the achiral ILs side chain results in different NMR spectra. In the ^{1}H NMR spectrum of 3-methyl-1-(2-(dodecylamino)-2-oxoethyl)-imidazolium bromide (**208**), a singlet at δ 9.73 can be seen. This signal is due to the acidic proton of the imidazolium ring and is slightly shifted upfield compared to the ester IL (δ 10.26). At δ 8.49, a triplet resides with a coupling constant of 5.4 Hz, and can be assigned as the amide proton of the IL side chain. This proton is coupling to the neighbouring methylene protons of the dodecyl alkyl side chain. In the aromatic region of the ^{1}H NMR spectrum, the imidazolium methine protons are evident. Two triplet signals appear at δ 7.58 and 7.15 with coupling constants of 1.6 Hz and are due to the aromatic imidazolium protons. A singlet peak at δ 5.29 with an integration of two protons is due to the methylene (NCH_2) group linking the imidazolium cation to the alkyl amide side chain. The *N*-methyl group of the imidazole is evident at δ 3.96 and appears as a singlet signal, integrating as three protons. At δ 3.16 an overlapping doublet of triplet signal arises with a coupling constant of 6.0 and 7.2 Hz and with an integration of 2. This signal is due to the methylene group adjacent to the amide moiety in the IL side chain. Further upfield the hydrogens of the remaining alkyl side chain are detected. A triplet of triplets is noted at δ 1.50 with coupling constants of 7.2 and 7.8 Hz. This signal integrates as two protons and can be allocated as the second methylene group from the amide functionality. The remaining protons of the dodecyl side chain appear together as a broad multiplet at δ 1.22-1.17. A triplet at δ 0.81 with a coupling constant of 7.0 Hz is due to the terminal methyl group of the long alkyl chain.

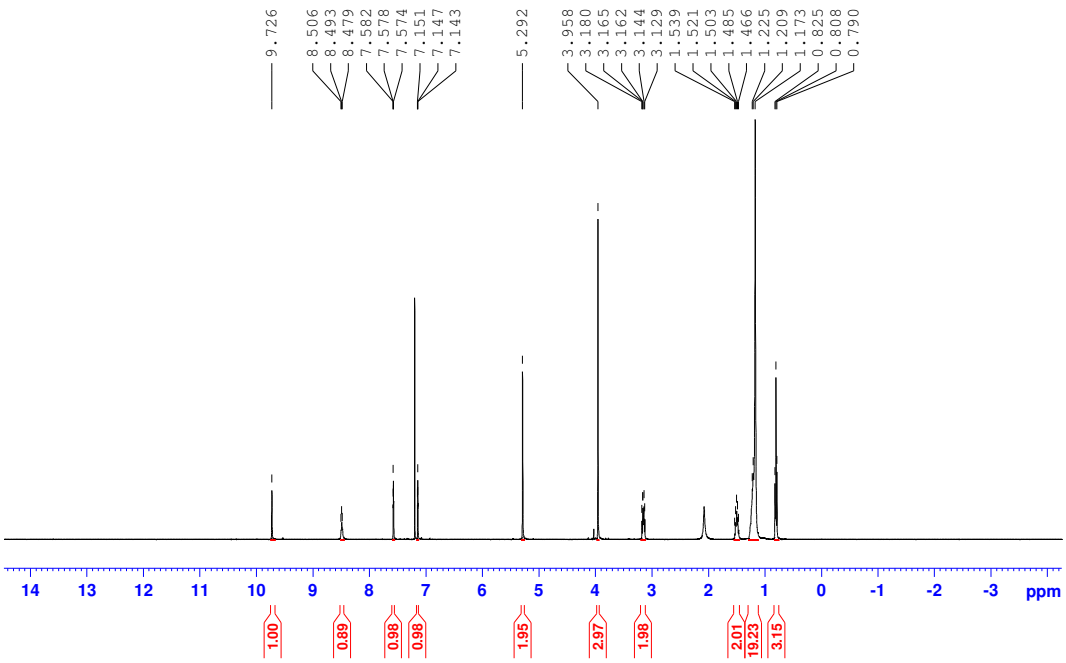


Fig. 2.4: ^{1}H NMR spectrum ($CDCl_3$) of 3-methyl-1-(2-(dodecylamino)-2-oxoethyl)-imidazolium bromide (**208**).

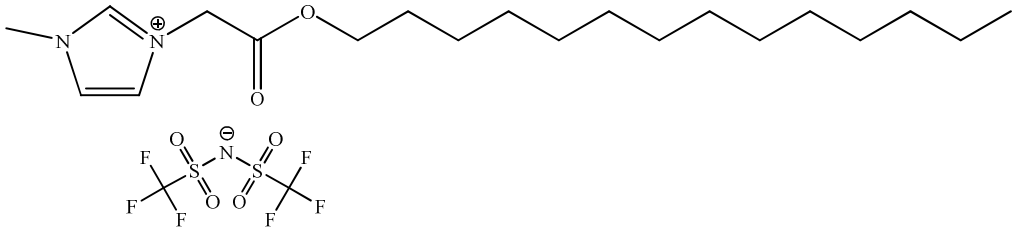
2.3.2 ^{13}C NMR and DEPT spectroscopic studies of achiral Ionic Liquids

^{13}C NMR and DEPT 135 experiments were also carried out on all novel achiral ionic liquids in deuterated chloroform. In the ^{13}C NMR spectra of the achiral ester derivatives the ester carbonyl group appears at ~ δ 165.7-166.5. The amide carbonyls of the amide ILs reside at ~ δ 164.2. In the aromatic region of the ^{13}C spectrum the carbons of the imidazolium ring can be seen, namely at ~ δ 137.5-138.6, 123.7 and 119-123.2. The methylene group between the cation core and the achiral side-chain is seen at ~ δ 49.9-51.8 in the ^{13}C spectra and gives a negative peak in the DEPT 135 spectra. The *N*-methyl of the imidazolium cation appears up-field at ~ δ 36.5-36.9. Further up-field in the carbon spectra the carbons of the linear alkyl chains are observed and give corresponding negative mode peaks in the DEPT 135. Table 2.8 illustrates some selected ^{13}C NMR data of achiral ionic liquids.

Table 2.8: Selected ^{13}C data (δ, $CDCl_3$) of amide and ester functionalised achiral ionic liquids (**193, 194, 195, 198, 201, 202, 206, 207** and **208**)

Compound	*C*=O ester	*C*=O Amide	N*C*HN imidazolium	N*C*H imidazolium	N*C*H_2 methylene	N*C*H_3 imidazolium
193	166.2		138.2	123.9	50.1	36.8
				122.8		
194	166.1		138.3	123.8	50.3	36.9
				123.0		
195	166.1		138.5	123.7	50.3	36.9
				122.9		
198	166.5		137.7	123.6	49.9	36.6
				123.0		
201	165.8		137.5	123.8	49.9	36.5
				123.3		
202	165.7		137.7	123.8	49.9	36.6
				123.2		
206		164.2	137.5	123.9	51.9	36.8
				122.3		
207		164.2	137.5	122.6	51.8	36.8
				119.7		
208		164.2	137.5	123.9	51.9	36.8
				122.2		

2.3.2.1 ^{13}C NMR and DEPT 135 study of 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium NTf_2 (202)



(**202**)

In the ^{13}C spectrum of 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium NTf_2 (**202**), a carbonyl group can be seen at δ 165.7. This corresponds to the ester carbonyl of the achiral ester side chain of the IL cation. In the DEPT 135 NMR spectrum this peak is not observed. The imidazolium carbons can be seen at δ 137.7, 123.8 and 123.2. These can be assigned as the carbon of the acidic proton (-N*C*HN-) and the two ring methine carbons (N*C*H) respectively. Also appearing in the aromatic region of the ^{13}C spectrum is a quartet signal with a coupling constant of 319.0 Hz (Figure 2.5). This quartet is assigned as the two quaternary carbons present in the bistriflimide anion which is further supported by their absence in the DEPT 135 spectrum (Figure 2.6). These carbons appear as a quartet signal due to their coupling to the neighbouring fluorines and appear as one quartet due to the anion symmetry.[2] At δ 67.3 a signal appears and gives a negative peak (opposite resonance to aromatic *C*H at δ 123.8) in the DEPT 135 NMR. This carbon is assigned as the methylene ester O*C*H$_2$ of the IL alkyl ester side chain. The methylene group adjacent to the imidazolium core can be seen at δ 49.9 and appears as negative peak in the corresponding DEPT 135 spectrum. A signal at δ 36.6 can be noted and is due to the *N*-methyl group of the imidazolium ring. A series of peaks can be seen in the aliphatic region of the ^{13}C spectrum and appear as negative peaks in the DEPT 135 spectrum between δ 31.9-22.7. These signals represent the methylene carbons of the tetradecyl alkyl side chain. In the most upfield region of the ^{13}C spectrum a signal at δ 14.1 is evident and corresponds to the methyl group of the alkyl ester side chain.

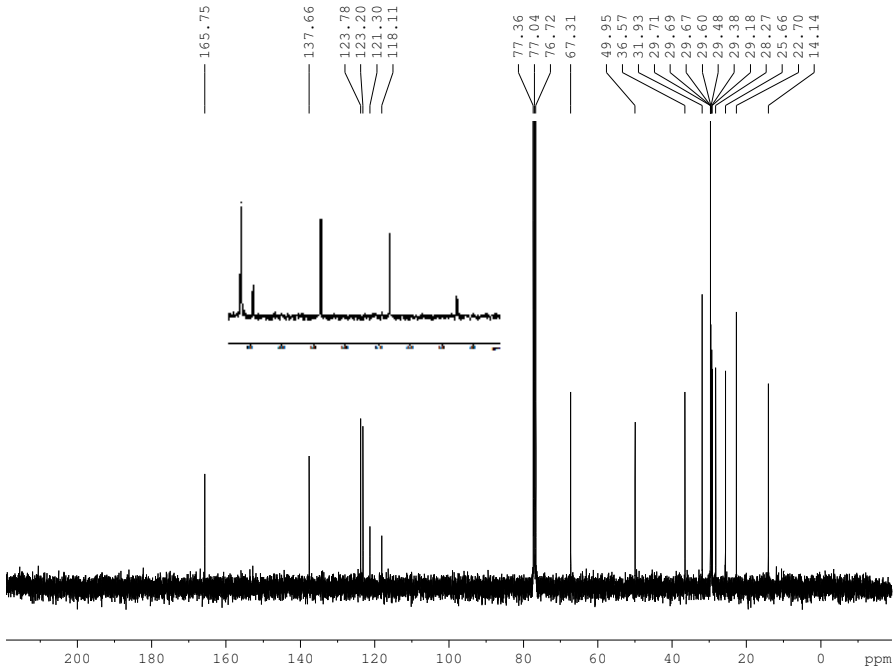


Fig. 2.5: ^{13}C NMR spectrum of 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium NTf_2 (**202**)

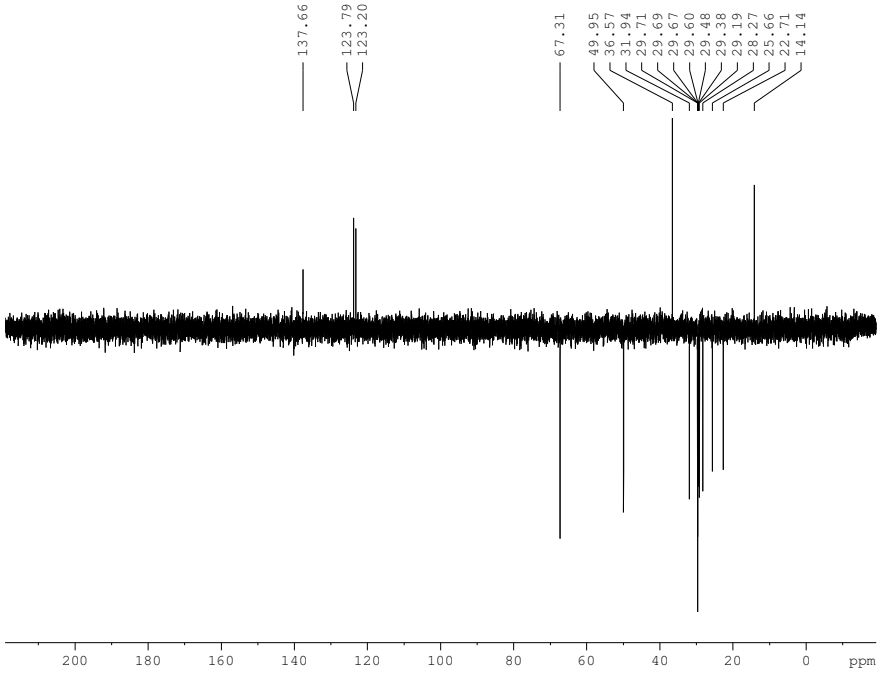


Fig. **2.6**: DEPT 135 spectrum of 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium NTf_2 (**202**).

2.3.3 COSY study of 3-methyl-1-(2-(decylamino)-2-oxoethyl)-imidazolium bromide achiral Ionic Liquid (207)

Two-Dimensional NMR experiments were obtained for novel achiral ionic liquids. One such 2D NMR technique employed was Correlation Spectroscopy (COSY). In a COSY NMR spectrum the frequencies of a ^{1}H NMR experiment are shown along both axes and the third dimension (within the box) shows the intensity of the observed signals. COSY NMR spectroscopy enables elucidation of the connectivity of a molecule by determining which protons are spin-spin coupled.[14] In the COSY NMR spectrum of 3-methyl-1-(2-(decylamino)-2-oxoethyl)-imidazolium bromide (Figure 2.8), the acidic proton **1** (δ 9.77) couples to the methine protons **3** and **4** of the imidazolium ring (δ 7.65 and 7.26). It is evident in the spectrum (Figure 2.9) that the amide proton **6** (δ 8.60) is coupled to the methylene group **7** (δ 3.24) of the alkyl side chain. Further coupling of this methylene **7** to an adjacent methylene moiety **8** (δ 1.58) in the linear alkyl chain can also be seen. Coupling is observed between the imidazolium methine protons **3** and **4** (δ 7.65 and 7.26) and between methylene groups **8** (δ 1.58) and **9**-**15** (δ 1.31-1.20). The terminal methyl group **16** (δ 0.88) of the alkyl chain also displays correlation to the multiplet representing the alkyl protons **9**-**15** in the COSY spectrum.

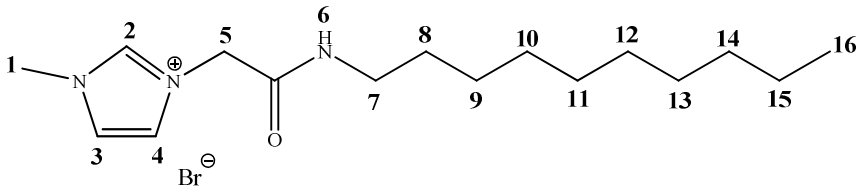


Fig. 2.7: 3-Methyl-1-(2-(decylamino)-2-oxoethyl)-imidazolium bromide (**207**) achiral ionic liquid.

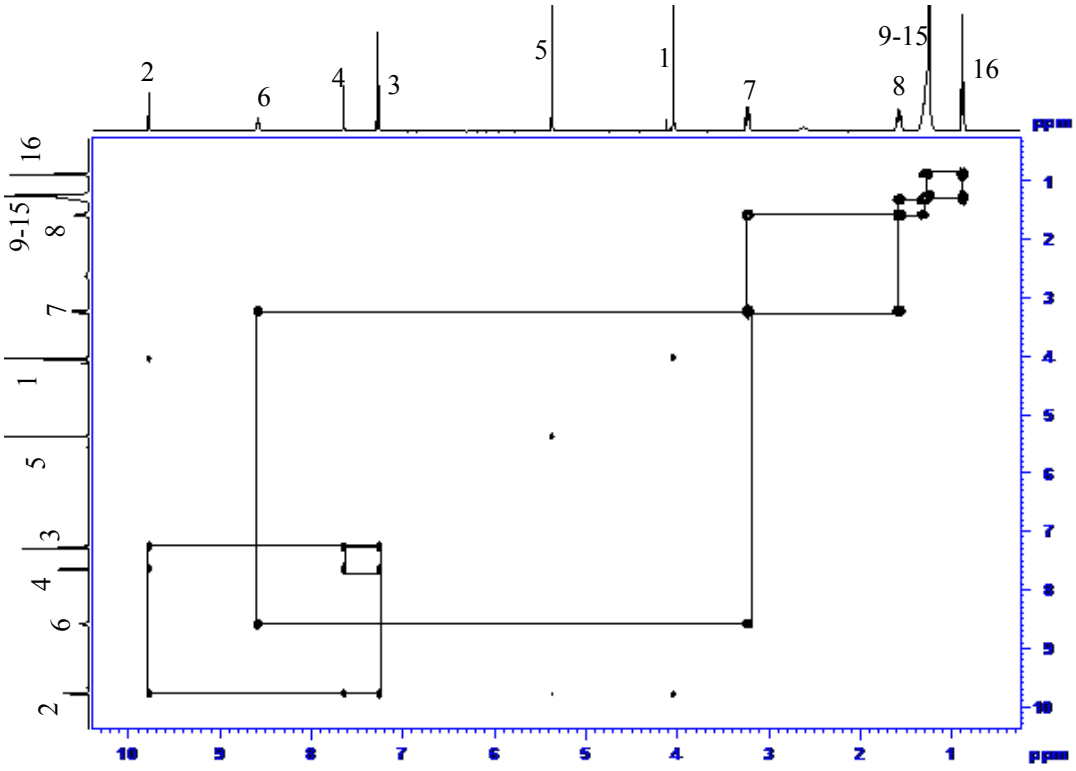


Fig. 2.8: COSY spectrum of 3-methyl-1-(2-(decylamino)-2-oxoethyl)-imidazolium bromide (**207**) achiral ionic liquid.

2.3.4 HMQC study of 3-methyl-1-(tetradecylcarbonylmethyl) imidazolium bromide achiral Ionic Liquid (195)

Another two-dimensional NMR method used to characterize novel achiral ionic liquids was Heteronuclear Multiple Quantum Coherence (HMQC). HMQC is a 2D inverse correlation technique that allows determination of correlations between proton and carbon atoms. It typically involves a pulse sequence with a delay time set at half the value of the ^{13}C-^{1}H coupling constant (100-200 Hz). This type of experiment results in a correlation between protons and the carbon to which they are attached.[14,15] Therefore as expected quaternary carbons are not evident in HMQC spectrum. Table 2.9 and Figure 2.9 describe a C-H correlation study of 3-methyl-1-(tetradecylcarbonylmethyl) imidazolium bromide achiral IL (**195**).

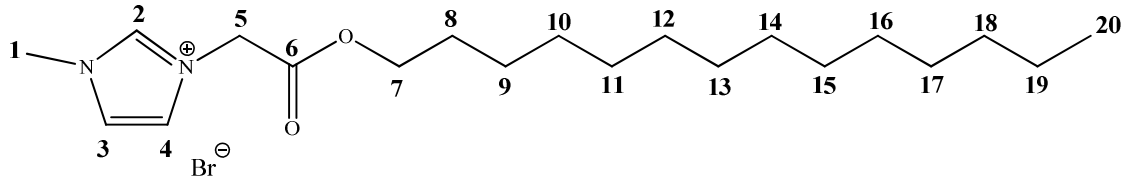


Table 2.9: C-H correlation data from HMQC spectrum of 3-methyl-1-(tetradecylcarbonylmethyl) imidazolium bromide (**195**).

Carbon No.	^{1}H NMR	^{13}C NMR	HMQC
1	4.09		36.95
2	10.17		138.53
3	7.47		122.92
4	7.58		123.71
5	5.47		50.32
6		166.15	
7	4.19		67.15
8	1.67		28.35
9	1.31-1.21		31.91-22.70
10	1.31-1.21		31.91-22.70
11	1.31-1.21		31.91-22.70
12	1.31-1.21		31.91-22.70
13	1.31-1.21		31.91-22.70
14	1.31-1.21		31.91-22.70
15	1.31-1.21		31.91-22.70
16	1.31-1.21		31.91-22.70
17	1.31-1.21		31.91-22.70
18	1.31-1.21		31.91-22.70
19	1.31-1.21		31.91-22.70
20	0.88		14.14

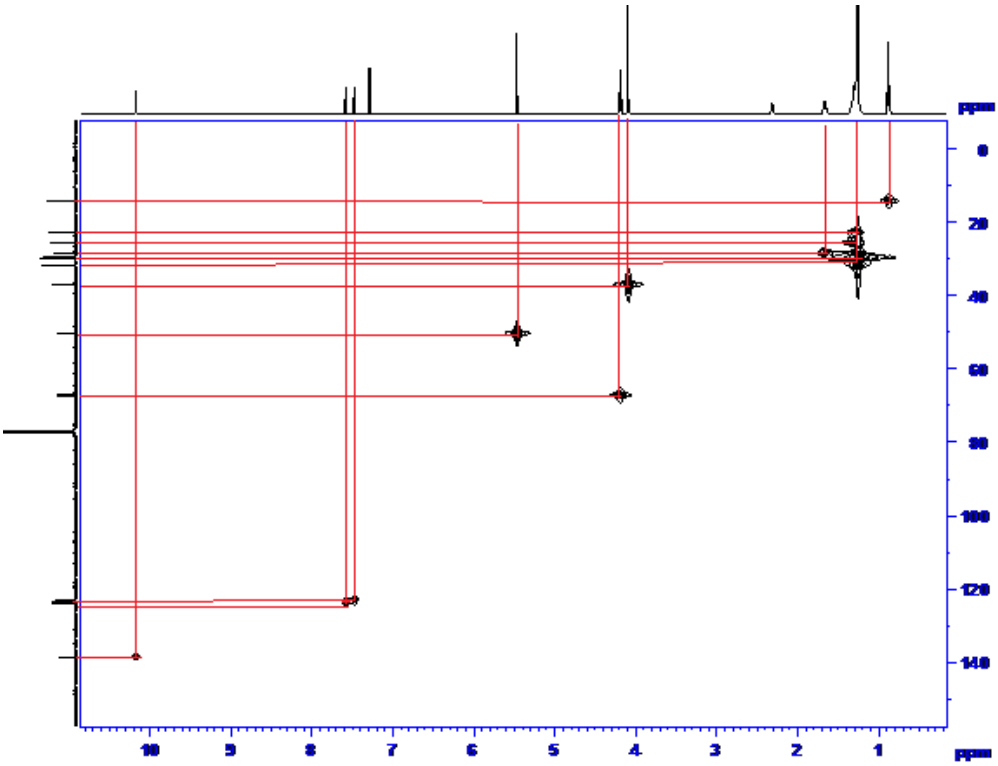


Fig. 2.9: HMQC NMR spectrum of 3-methyl-1-(tetradecylcarbonylmethyl) imidazolium bromide (**195**).

2.4 Conclusions

A range of imidazolium based ionic liquids, and associated intermediates, were successfully designed and prepared with various functionalised achiral side chains. Five α-bromoester intermediates (**188**-**192**) were synthesised and five bromide achiral ester ILs (**42**, **193**-**196**) were subsequently prepared. Four NTf_2 (**177**, **200**-**202**) and four $OctOSO_3$ (**45**, **197**-**199**) ILs were also produced. Additionally, three achiral α-bromoamide intermediates (**203**-**205**) were prepared and then used in the synthesis of three bromide achiral amide ILs (**206**-**208**). These novel compounds were characterized by a range of spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC, IR and MS. All compounds gave data in accordance with their proposed chemical structures. The accumulated spectroscopic data also allowed for differentiation between cations with

varying anionic components. Chemical shifts studies could be conducted of ^{1}H NMR spectra of ILs with various anions (i.e. Br, NTf_2 and $OctOSO_3$). Additionally, by altering the anionic species of the ILs, their physico-chemical properties can be also be changed (Table 2.6). Viscosities and depression of melting points are examples of some IL properties which can be manipulated upon anion exchange.

2.5 References

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Chapter 3: Results and discussion

Amino Acid Ester Chiral Ionic Liquids, Synthesis and Characterisation

3.1 Introduction

Chiral Ionic Liquids (CILs) are a class of ionic liquids whereby either the cation or anion bears at least one stereogenic centre. This field of IL research is still in its relative infancy, with the first papers reported just over a decade ago.[1] Libraries of novel CILs exist in the literature today, and some comprehensive reviews have expounded their preparation and applications.[2-4]

An efficient and relatively easy way to synthesis enantiomerically pure ionic liquids is to use precursors derived from the chiral pool. Many research groups have used amino acids as chiral precursors and as a means of preparing functionalised ionic liquids. Amino acids contain both a carboxylic acid site and an amino group which allows for their use as either cations or anions in ionic liquids. Many groups have incorporated amino acids as anionic species. Fukumoto and Ohno were first to report the synthesis of Amino acid ionic liquids (AAILs).[5,6] Different natural amino acids were employed as anions with the commonly used 1-ethyl-3-methylimidazolium [emim] cation. Figure 3.1 illustrates the preparation of these AAILS.

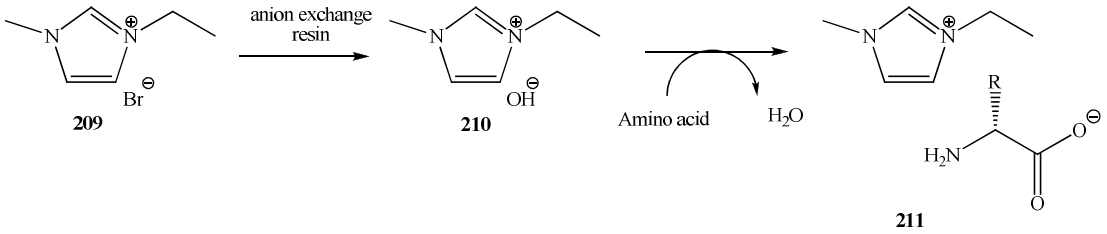


Fig. 3.1: Synthesis of amino acid ionic liquids by Fukumoto *et al.*.[5]

A neutralization method was used whereby 1-ethyl-3-methylimidazolium hydroxide [emim][OH] was vigourously mixed with an aqueous solution of amino acid. All the imidazolium AAILs were obtained as liquids and displayed low thermal stability (decomposition occurred at 200 °C). To investigate the effect of the ionic liquid cation on its relative thermal stability, a series of Alanine salts were prepared using phosphonium, ammonium, pyridinium and pyrolidinium cations. From this list of novel AAILs, the

phosphonium based ionic liquid exhibited the highest thermal stability and lowest glass transition temperature (T_g, temperature lower than melting temperature). As a consequence, a series of tetraalkylphosphonium amino acid salts were further prepared by the same group, to determine the effects of the alkyl chain of the cation on thermal stability, density and glass transition temperature. A three step synthesis was required to obtain the novel asymmetric phosphonium AAILs. The first step involved the quaternisation of tri-alkylphosphine with various alkyl halides. Following this, the halide salt was passed through an anion exchange resin in order to attain the hydroxide derivative IL. Finally the $[P_{n,n,n,m}][OH]$ ILs were neutralised with aqueous solutions of various L-amino acids. A number of physico-chemical properties, namely glass transition temperature, decomposition temperature, density and viscosity, were measured for tetraalkylphosphonium AAILs. Whilst all AAILs exhibited high levels of thermal stability, a trend was observed in terms of alkyl chain length of cation and IL viscosity. The viscosity increased upon increasing the carbon number in the alkyl chain.

A series of tetraalkylammonium AAILs have been synthesised recently.[7] Preparation of these AAILs was achieved using similar methods previously described in the literature.[5] Symmetry of the cation had an evident effect on the IL viscosity. The asymmetric tetraalkylammonium derivatives gave lowest viscosity values (lowest value observed 29 mPa s). The potential application of these AAILs as CO_2 absorbents was also investigated. Absorbtion of carbon dioxide has become an important research issue recently as increased levels in atmospheric CO_2 has lead to global warming. Ionic liquids have been studied as possible alternatives to CO_2 scavengers commonly used in industry. The low viscosity of the tetraalkylammonium amino acid ionic liquids was seen to enhance CO_2 mass transfer. Up to 90 % of absorbed CO_2 could be released from AAIL system, with high recovery levels of IL observed after repeated cycles of CO_2 uptake and release.

Amino acids have also been incorporated as cationic components in IL structures. Shreeve and co-workers have recently reported the synthesis of a range of *N*-alkyl substituted glycine ester ionic liquids.[8] These AAILs were prepared in two steps (Figure 3.2). Direct acidification of glycine and glycine ester starting materials, followed by anion exchange reactions yielded *N*-unsubstituted and *N*,*N*-dialkyl substituted glycine ionic liquids. *N*-

trialkyl glycine halide ionic liquids were synthesised by alkylation of glycine esters with bromo and iodoalkanes. Viscosities and thermal stabilities were again investigated in this work. A similar trend in cation symmetry effecting AAILs viscosity and melting points was observed here, with asymmetric cations yielding salts with low viscosities.

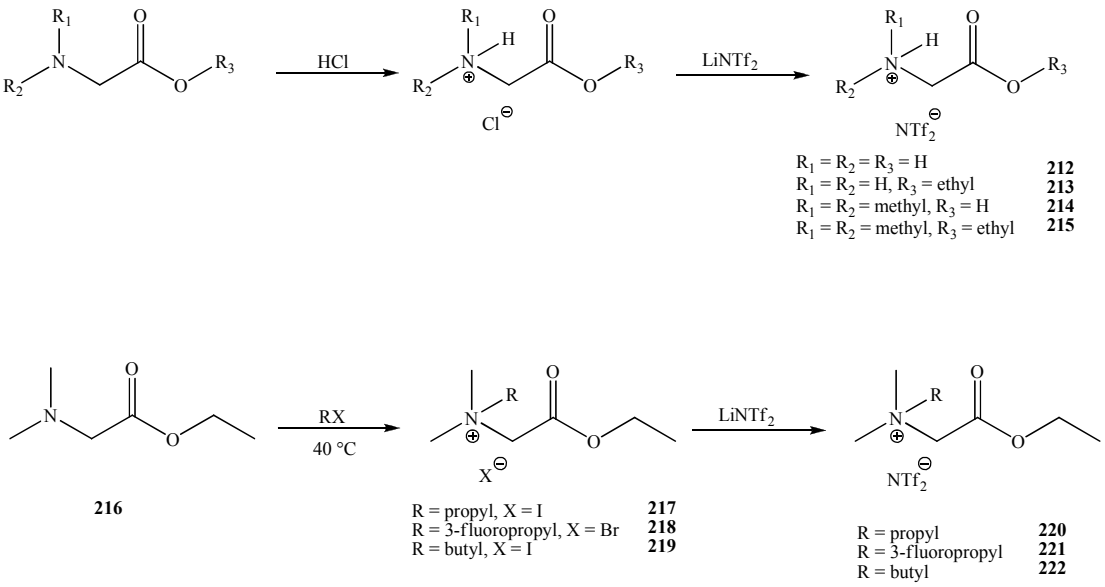


Fig. 3.2: Preparation of Glycine-based ionic liquids.[8]

In our work chirality and functionalisation was incorporated into the ionic liquid structure by introducing various amino acid esters into the imidazolium cation side chain.

3.1.1 Aim

L-, D- and DL- Amino acid esters (methyl, ethyl and butyl) were selected as chiral building blocks and were coupled to the imidazolium cation. Incorporation of these derivatives provides the IL side chain with several possible biodegradation sites. The ester forms of these amino acids were to be synthesized in order to successfully form the chiral bromoamide intermediates which could then be used to prepare the desired chiral bromide salt. Figure 3.3 below demonstrates the general structure of these CILs.

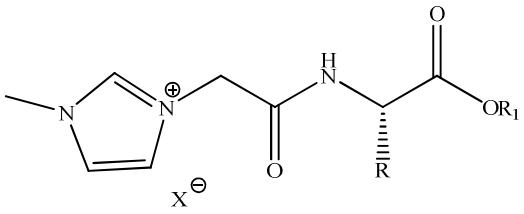
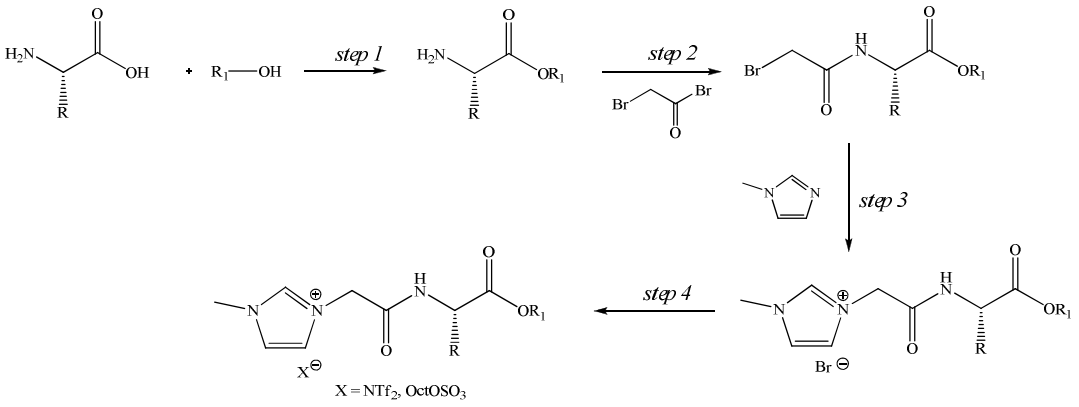


Fig. 3.3: General structure of Amino acid based chiral ionic liquids.

As seen in Figure 3.3, there is an amide and ester moiety present in the CIL side chain. This strategy provides two possible sites for enzymatic cleavage, hence leading to improved IL biodegradability. The goal was to prepare a library of novel amino acid ester ionic liquids for toxicity and ecotoxicity studies. A series of Br salts were prepared and screened for antimicrobial toxicity. NTf_2 and $OctOSO_3$ ILs produced derivatives with various physico-chemical properties (i.e. liquid/solid, melting point studies). The $OctOSO_3$ salts have also been known to improve IL biodegradation.

3.2 Synthesis of Chiral Ionic Liquids

The synthethic route utilized in CIL formation is similar to that previously employed for the achiral examples (Chapter 2). An extra step is required at the start of the four step synthesis. This involved the esterification of the C-terminus of the amino acids in order to add a protecting group to the carboxylic acid.[9] Scheme 3.1 illustrates the synthetic pathway involved in the formation of these CILs.



Scheme 3.1: Synthesis of amino acid ester chiral ionic liquids; (1) $SOCl_2$, (2) Bromoacetyl bromide, Et_3N, -78 °C, (3) 1-methyl imidazole, (4) $NaOctOSO_3$ or $LiNTf_2$.

3.2.1 Step 1:Preparation of Amino Acid esters

A range of L-, D- and DL- Amino acid ester (AAE) derivatives were prepared from their Amino acid (AA) precursors. The amino acids used were;

- L-, D-, DL-Phenylalanine
- L-, D-, DL-Valine
- L-Alanine
- L-Isoleucine
- L-Leucine

Methyl, ethyl and butyl esters were prepared. Figure 3.4 illustrates the reaction conditions used in this step.

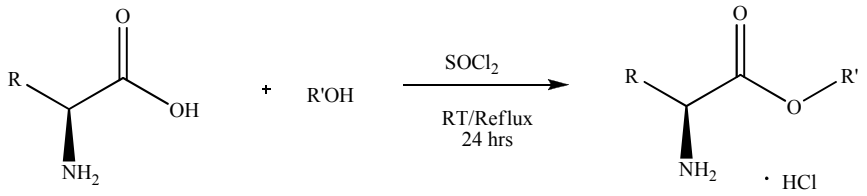


Fig. 3.4: Preparation of Amino Acid esters.

The amino acids were allowed to react with thionyl chloride in the presence of alkyl alcohols at ambient temperatures. However in some cases (namely for the ethyl and butyl derivatives **230**, **231**, **233**, **234**, **239** and **243**) reflux conditions were used to obtain the alkyl ester hydrochloride salts. The esters were prepared in very good yields (Table 3.1). In some cases the esters were commercially available (**223**, **224**, **235**, **236**) and were therefore not synthesized as above.

Table 3.1: Amino acid esters synthesized.

Amino acid ester	Compound no.	Yield (%)
L-phenylalanine methyl ester hydrochloride	**223**	n/a*
L-phenylalanine ethyl ester hydrochloride	**224**	n/a*
L-phenylalanine butyl ester hydrochloride	**225**†	66
L-alanine methyl ester hydrochloride	**226**†	94
L-alanine ethyl ester hydrochloride	**227**†	60
L-alanine butyl ester hydrochloride	**228**†	94
L-valine methyl ester hydrochloride	**229**†	96
L-valine ethyl ester hydrochloride	**230**†	87
L-valine butyl ester hydrochloride	**231**†	65
L-isoleucine methyl ester hydrochloride	**232**†	96
L-isoleucine ethyl ester hydrochloride	**233**†	94
L-isoleucine butyl ester hydrochloride	**234**†	89
L-leucine methyl ester hydrochloride	**235**	n/a*
L-leucine ethyl ester hydrochloride	**236**	n/a*
L-leucine butyl ester hydrochloride	**237**†	66
D-valine methyl ester hydrochloride	**238**†	95
D-valine ethyl ester hydrochloride	**239**†	77
D-phenylalanine methyl ester hydrochloride	**240**†	97
D-phenylalanine ethyl ester hydrochloride	**241**†	97
DL-valine methyl ester hydrochloride	**242**†	90
DL-valine ethyl ester hydrochloride	**243**†	64
DL-phenylalanine methyl ester hydrochloride	**244**†	85
DL-phenylalanine ethyl ester hydrochloride	**245**†	96
D-alanine methyl ester hydrochloride	**246**	n/a*

n/a* Amino acid esters purchased from chemical supplier, † known compound, see Appendix I

3.2.2 Step 2: α-bromoamide intermediates

Synthesis of the alkylating reagents was carried out as before (Chapter 2) for the achiral derivatives (Figure 3.5). The amino acid ester was dissolved in DCM and stirred at -78 °C in the presence of Et_3N under inert atmospheric conditions. Bromoacetyl bromide was then added and stirring was continued for 4-5 hours at - 78°C.

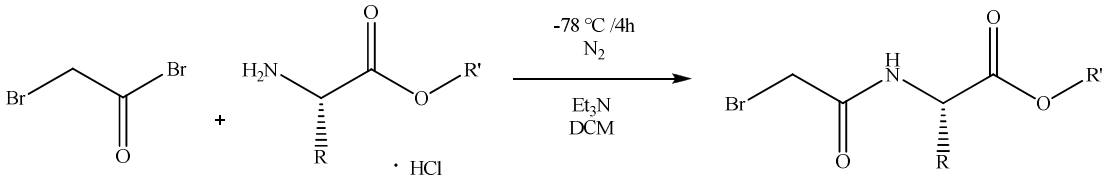
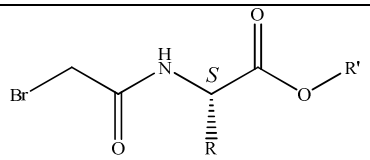


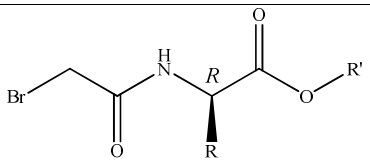
Fig. 3.5: Formation of α-bromoamide intermediates.

Column chromatography was used to purify these bromoamides. The eluant used was 50:50 ethyl acetate: hexane. The products (**247**-**269**) were obtained in moderate to good yields. Table 3.2 summarises the isolated yields obtained for the pure forms (by ^{1}H and ^{13}C NMR) of the alkylating intermediates.

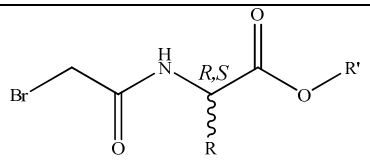
Table 3.2: Synthesis of chiral α-bromoamide intermediates.



R	R'	Compound No.	Yield (%)
$-CH_2-(C_6H_5)$	$-CH_3$	**247**	66
$-CH_2-(C_6H_5)$	$-C_2H_5$	**248**	64
$-CH_2-(C_6H_5)$	$-C_4H_9$	**249**	79
$-CH_3$	$-CH_3$	**250**	65
$-CH_3$	$-C_2H_5$	**251**	40
$-CH_3$	$-C_4H_9$	**252**	74
$-CH(CH_3)_2$	$-CH_3$	**253**	60
$-CH(CH_3)_2$	$-C_2H_5$	**254**	40
$-CH(CH_3)_2$	$-C_4H_9$	**255**	58
$-CH_2CH(CH_3)_2$	$-CH_3$	**256**	62
$-CH_2CH(CH_3)_2$	$-C_2H_5$	**257**	61
$-CH_2CH(CH_3)_2$	$-C_4H_9$	**258**	54
$-CH(CH_3)CH_2CH_3$	$-CH_3$	**259**	56
$-CH(CH_3)CH_2CH_3$	$-C_2H_5$	**260**	59
$-CH(CH_3)CH_2CH_3$	$-C_4H_9$	**261**	68



$-CH_2-(C_6H_5)$	$-CH_3$	**262**	65
$-CH_2-(C_6H_5)$	$-C_2H_5$	**263**	70
$-CH(CH_3)_2$	$-CH_3$	**264**	45

-$CH(CH_3)_2$	-C_2H_5	**265**	59
			
-$CH(CH_3)_2$	-CH_3	**266**	82
-$CH(CH_3)_2$	-C_2H_5	**267**	62
- CH_2-(C_6H_5)	-CH_3	**268**	66
- CH_2-(C_6H_5)	-C_2H_5	**269**	69

An alternative method of α-bromoamide formation was also investigated for some intermediates. In this method the amino acid ester was stirred in DCM with potassium carbonate at 0 °C. Bromoacetyl bromide was added dropwise and the reaction was stirred at room temperature for 24 hours (Figure 3.6).[10]

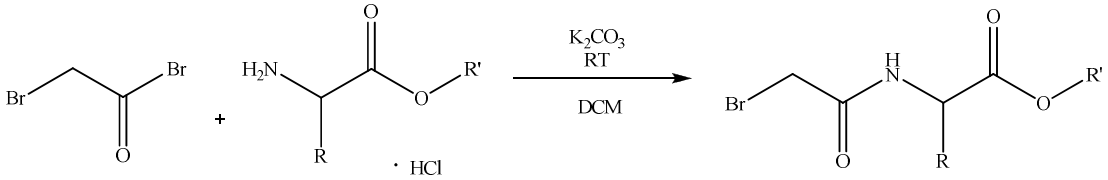


Fig. 3.6: Formation of bromoamide intermediates using K_2CO_3 method.

The purification of the α-bromoamide was facile, requiring only a base wash on the crude reaction product. An advantage to this was that the use of column chromatography was eliminated, hence making the reaction a greener alternative to the previously used method. Figure 3.7 demonstrates a ^1H NMR spectrum of a representative purified bromoamide intermediate (**254**). An improvement in product yields was also observed. Table 3.3 draws the comparison between yields obtained of alkylating intermediates using both methods.

Table 3.3: Et_3N alkylation method vs K_2CO_3 method.

α-bromoamide intermediate	Et_3N method (yield %)	K_2CO_3 method (yield %)
247	66	86
249	79	82
251	40	70
253	60	77
254	40	71
255	58	78
256	62	98
257	61	75
264	40	66
265	59	73
266	82	67
267	62	68

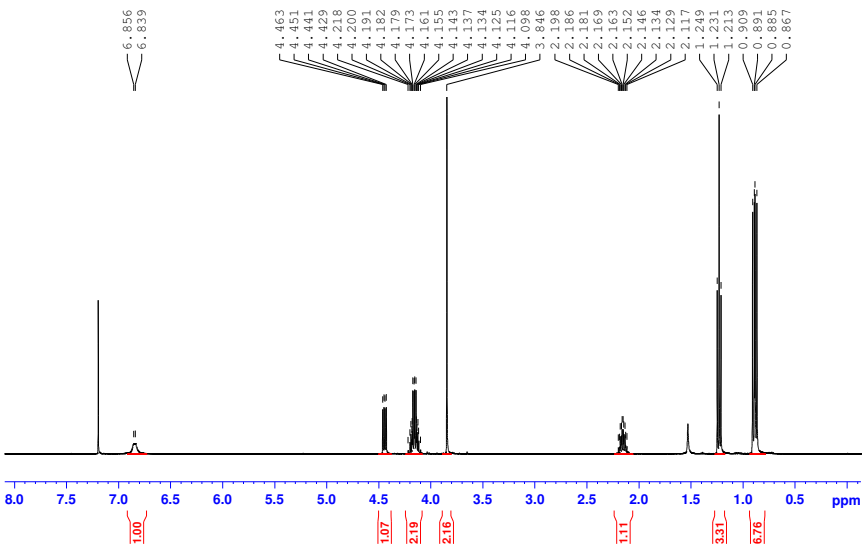


Fig. 3.7: ^1H NMR of L-valine ethyl ester bromoacetate (**254**).

In the ^{1}H NMR spectrum of L-valine ethyl ester bromoacetate (**254**), a doublet at δ 6.84 with a coupling constant of 6.8 Hz, represents the proton of the bromo-amide. A doublet of doublets appears at δ 4.44 with coupling constants of 8.8 and 4.8 Hz. This signal is due to the proton at the L-valine chiral center. A large peak at δ 4.22-4.09 with an integration of 2 is noted. This peak can be assigned to be the methylene of the ethyl ester group. The protons of this methylene group appear inequivalent and as a result are seen to couple to each other and to the adjacent protons of the terminal methyl group. Two overlapping doublet of quartets are evident for these protons. The singlet at δ 3.85 corresponds to the methylene protons adjacent to the terminal bromide atom. Upfield in the proton NMR spectrum, the aliphatic protons of the L-valine side chain arise. The methine and methyl groups of the branched alkyl side chain appear at δ 2.15 and 0.88 respectively. The triplet at δ 1.23 is due to the methyl group of the ethyl ester.

3.2.3 Step 3: Formation of bromide salts

The next stage of the synthesis involved the alkylation of 1-methyl imidazole to form the chiral bromide salts (**270**-**292**). Etheral solvents (e.g. diethyl ether or tetrahydrofuran) are used extensively in the group for the preparation of bromide ILs.[11] These reactions were performed initially using diethyl ether (Et_2O) as reaction solvent. However, in some cases the IL did not precipitate as a separate phase to the Et_2O. In others, poor solubility of the alkylating reagents was observed when using diethyl ether as the reaction media. A considerable amount of starting material was observed in the ^{1}H NMR spectra following the reaction in ether. This was evident in the spectrum of 3-methyl-1-D-valine ethyl ester imidazolium bromide (**288**). A singlet at δ 3.70 indicates the presence of the *N*-methyl group of the imidazolium ring and a set of doublets at δ 6.89 and 7.16 shows the imidazolium methine protons. The removal of this starting material is difficult, as methyl imidazole has a high boiling point and therefore will not be removed *via* rotary evaporation. In addition column chromatography was attempted in some cases; however the product adhered to the silica stationary phase and was difficult to remove. Copious amounts of methanol were usually required to remove the IL from the column. Et_2O washings were also employed with only slight imidazole removal observed in a number of cases. As most

of the CILs were liquids at RT recrystallisation was also a purification option which could not be used. Therefore the reaction conditions were altered in order to optimize the product yields and purity. Tetrahydrofuran (THF) replaced diethyl ether as the reaction media, as the starting material is more soluble in this solvent. It was clear from the ^{1}H NMR data that the amount of residual starting material was noticeably reduced by replacing diethyl ether with THF (Figure 3.8).

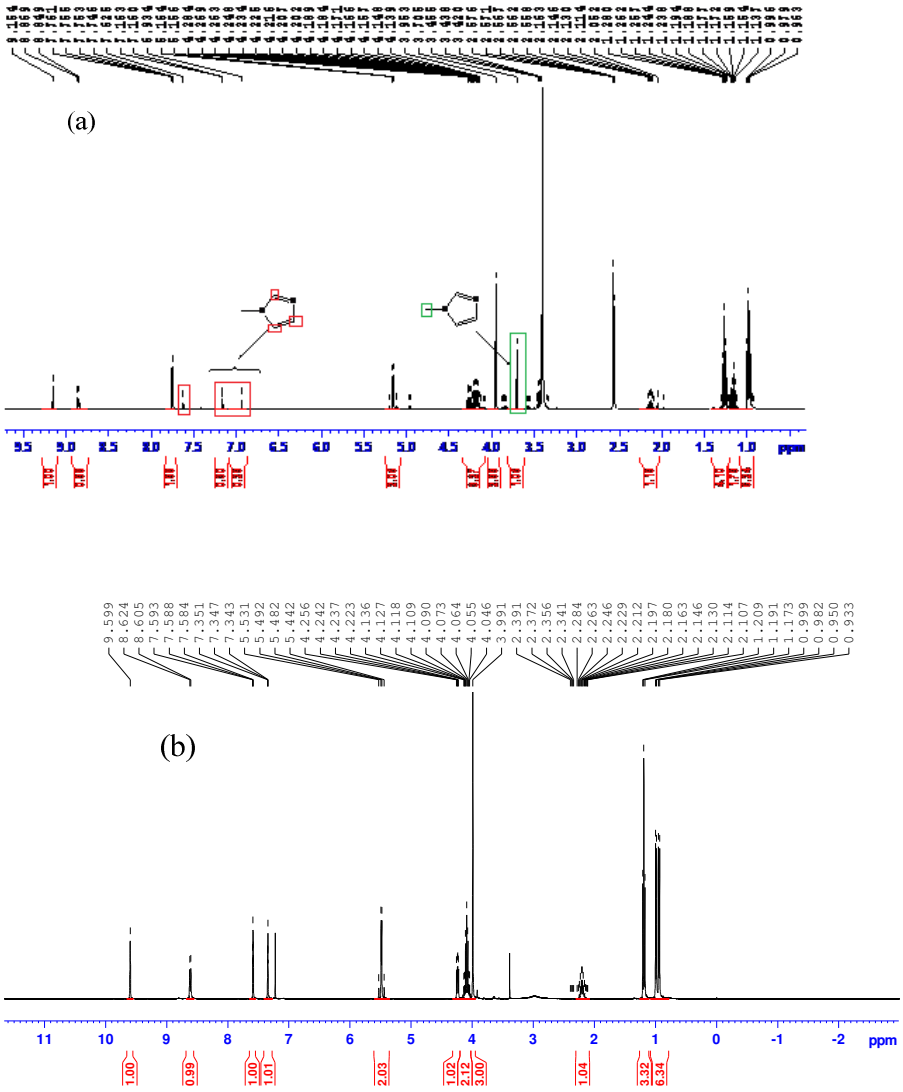
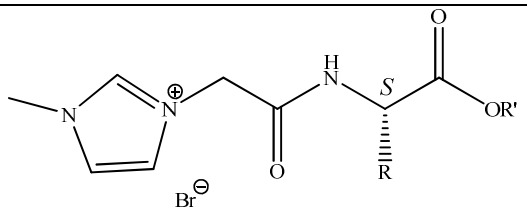


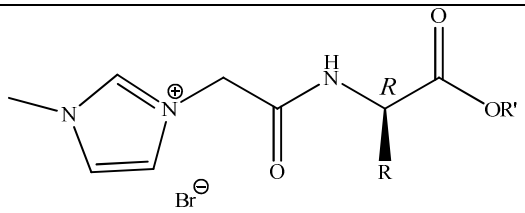
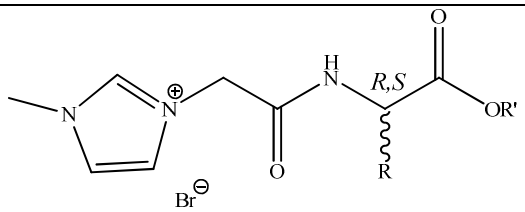
Fig. 3.8: (a) 1-methyl imidazole starting material evident in the NMR spectrum of the CIL (**288**) using diethyl ether as solvent; (b) and the imidazole peaks removed upon replacement of diethyl ether with THF.

The bromide CILs were obtained in good to very good yields (Table 3.4) as a range of liquids or solids.

Table 3.4: CILs synthesised and yields obtained.



Compound no.	R	R'	Yield (%)
270	$-CH_2(C_6H_5)$	$-CH_3$	98
271	$-CH_2(C_6H_5)$	$-C_2H_5$	98
272	$-CH_2(C_6H_5)$	$-C_4H_9$	99
273	$-CH_3$	$-CH_3$	95
274	$-CH_3$	$-C_2H_5$	90
275	$-CH_3$	$-C_4H_9$	82
276	$-CH(CH_3)_2$	$-CH_3$	85
277	$-CH(CH_3)_2$	$-C_2H_5$	98
278	$-CH(CH_3)_2$	$-C_4H_9$	96
279	$-CH(CH_3)CH_2CH_3$	$-CH_3$	67
280	$-CH(CH_3)CH_2CH_3$	$-C_2H_5$	94
281	$-CH(CH_3)CH_2CH_3$	$-C_4H_9$	98
282	$-CH_2(CH_3)_2$	$-CH_3$	95
283	$-CH_2(CH_3)_2$	$-C_2H_5$	69
284	$-CH_2(CH_3)_2$	$-C_4H_9$	86

			
285	$-CH_2(C_6H_5)$	$-CH_3$	87
286	$-CH_2(C_6H_5)$	$-C_2H_5$	84
287	$-CH(CH_3)_2$	$-CH_3$	89
288	$-CH(CH_3)_2$	$-C_2H_5$	79
			
289	$-CH_2(C_6H_5)$	$-CH_3$	84
290	$-CH_2(C_6H_5)$	$-C_2H_5$	79
291	$-CH(CH_3)_2$	$-CH_3$	87
292	$-CH(CH_3)_2$	$-C_2H_5$	77

3.2.4 Step 4: Anion metathesis

3.2.4.1 NTf_2 salt formation

Following formation of the bromide salts, anion exchange reactions were carried out yielding a library of novel CILs. Chiral NTf_2 ionic liquids were prepared using lithium trifluoromethanesulfonimide as used in the synthesis of the achiral derivatives. This method involved stirring the bromide salt with lithium trifluoromethanesulfonimide in distilled water at room temperature overnight. After this time the hydrophobic NTf_2 salt formed as a separate phase. Water washings of the precipitate gave the pure CILs in good to excellent yields, all as liquids.

Table 3.5: Chiral NTf_2 ionic liquids (**293**-**307**).

Compound name	Compound No.	Yield (%)
3-methyl-1-L-isoleucine methyl ester imidazolium NTf_2	**293**	84
3-methyl-1-L-isoleucine ethyl ester imidazolium NTf_2	**294**	67
3-methyl-1-L-isoleucine butyl ester imidazolium NTf_2	**295**	77
3-methyl-1-L-alanine methyl ester imidazolium NTf_2	**296**	54
3-methyl-1-L-alanine ethyl ester imidazolium NTf_2	**297**	83
3-methyl-1-L-alanine butyl ester imidazolium NTf_2	**298**	72
3-methyl-1-L-leucine methyl ester imidazolium NTf_2	**299**	78
3-methyl-1-L-leucine ethyl ester imidazolium NTf_2	**300**	85
3-methyl-1-L-leucine butyl ester imidazolium NTf_2	**301**	86
3-methyl-1-L-phenylalanine methyl ester imidazolium NTf_2	**302**	65
3-methyl-1-L-phenylalanine ethyl ester imidazolium NTf_2	**303**	94
3-methyl-1-L-phenylalanine butyl ester imidazolium NTf_2	**304**	84
3-methyl-1-L-valine methyl ester imidazolium NTf_2	**305**	88
3-methyl-1-L-valine ethyl ester imidazolium NTf_2	**306**	93
3-methyl-1-L-valine butyl ester imidazolium NTf_2	**307**	94

3.2.4.2 $OctOSO_3$ CILs

In the synthesis of $OctOSO_3$ CILs, the bromide salt was stirred in distilled water and sodium octyl sulfate. The reaction was then allowed to proceed overnight. The water was then removed slowly *via* rotary evaporation. The precipitate obtained was dissolved in DCM and washed with small aliquots of distilled water. After these washings, DCM solvent was removed yielding CILs in good to very good yields (up to 96 %). Suppression in CIL melting points was observed as all $OctOSO_3$ salts (compared to the bromide analogues) were obtained as liquids at RT.

Table 3.6: Yields obtained for $OctOSO_3$ CILs (**308**-**317**).

Compound name	Compound no.	Yield (%)
3-methyl-1-L-leucine methyl ester imidazolium $OctOSO_3$	**308**	93
3-methyl-1-L-leucine ethyl ester imidazolium $OctOSO_3$	**309**	76
3-methyl-1-L-leucine butyl ester imidazolium $OctOSO_3$	**310**	75
3-methyl-1-L-phenylalanine ethyl ester imidazolium $OctOSO_3$	**311**	96
3-methyl-1-L-phenylalanine butyl ester imidazolium $OctOSO_3$	**312**	95
3-methyl-1-L-alanine methyl ester imidazolium $OctOSO_3$	**313**	86
3-methyl-1-L-alanine ethyl ester imidazolium $OctOSO_3$	**314**	85
3-methyl-1-L-valine methyl ester imidazolium $OctOSO_3$	**315**	87
3-methyl-1-L-valine ethyl ester imidazolium $OctOSO_3$	**316**	67
3-methyl-1-L-valine butyl ester imidazolium $OctOSO_3$	**317**	77

3.3 NMR studies of Chiral Ionic Liquids

3.3.1 ^{1}H NMR studies of chiral ionic liquids

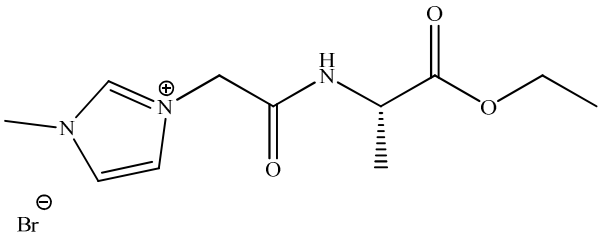
^{1}H NMR experiments of all novel amino acid ester ionic liquids (**270**-**317**) were performed in deuterated chloroform or deuterated dimethyl sulfoxide. The amide protons of the peptide side chain appear typically as doublets due to coupling with the α-hydrogen at the chiral centre. These signals reside downfield, with chemical shifts dependent on the different counter ions present in the salts. For bromide derivatives (**270**-**292**), the amide signals appear between ~ δ 8.50-9.63. In the case of the NTf_2 salts (**293**-**307**), amide protons are evident between ~ δ 7.16-7.22 and for the $OctOSO_3$ examples (**308**-**317**) these protons can be assigned at ~ δ 8.31-8.55. The acidic proton of the imidazolium ring gives a singlet whose chemical shift differs depending on the anion present in the IL structure. For the bromide ILs, the acidic imidazolium C*H* appears downfield at ~ δ 9.10-9.85. NTf_2 CILs give singlets shifted upfield towards ~ δ 8.50-8.75 and the $OctOSO_3$ salts are slightly more

upfield to the bromide derivatives at ~ δ 8.98-9.35. The methylene group adjacent to the imidazolium core gives rise to signals at ~ δ 5.02-5.48 for bromide CILs. The $OctOSO_3$ counterparts produce signals that are similar in chemical shift at ~ δ 5.02-5.28. Whilst the methylene groups of the NTf_2 salts are again shifted slightly further upfield at ~ δ 4.06-4.98.

Table 3.7: Selected ^1H NMR spectral data (δ, $CDCl_3$) for 3-methyl-1-L-alanine ester imidazolium bromide ionic liquids (**273**, **274**, **296**, **29**7, **313** and **314**).

Compound no.	**Amide N*H***	**Acidic imidazolium C*H***	**Imidazolium C*H*'s**	**Methylene NCH_2**
273	9.04	9.15	7.77,7.45	5.12
274	8.90	9.63	7.58,7.33	5.39
296	7.19	8.74	7.26,7.17	4.95
297	7.24	8.67	7.38,7.20	4.93
313	8.63	9.22	7.49,7.24	5.16
314	8.52	9.13	7.48,7.27	5.13

3.3.1.1 ^{1}H NMR spectroscopic study of 3-methyl-1-L-alanine ethyl ester imidazolium bromide (274)



(**274**)

The ^{1}H NMR spectrum of 3-methyl-1-L-alanine ethyl ester imidazolium bromide (**274**) was obtained in $CDCl_3$. In this deuterated solvent the acidic proton (-NC*H*N-) of the imidazolium ring appears at δ 9.63 as a singlet, which is expected as it is not neighbouring any other protons. The amide of the L-alanine side chain moiety occurs as a doublet at δ 8.90 with a coupling constant 6.4 Hz. This proton appears as a doublet due to coupling with the α-hydrogen at the chiral centre. Two triplets appear at δ 7.58 and 7.33 with coupling constants of 1.8 and 1.6 Hz respectively. These peaks can be assigned as the NC*H* protons of the imidazolium ring for which doublet peaks would be expected. However, it is predicted that coupling between these hydrogens with the acidic hydrogen may be occurring through the aromatic system. A singlet peak at δ 5.39 in the ^{1}H NMR spectrum is due to the methylene group alpha to the imidazolium cation core. At δ 4.33 a peak resembling a quintet arises, corresponding to the hydrogen at the chiral centre. However, this proton is coupled to inequivalent protons, namely, the methyl protons of the L-alanine side chain and the amide proton. Coupling to these protons results in a doublet of quartets which appear at the same chemical shift with the same coupling constants of 7.2 Hz. Therefore the signal observed in the ^{1}H NMR spectrum appears similar to a quintet. The methylene of the ethyl ester group appears as two overlapping doublet of quartets δ 4.09 and 4.08. The *N*-methyl group gives a singlet occurring at δ 3.99. At δ 1.44 a doublet is noted with a coupling constant of 7.2 Hz. This doublet is assigned to the methyl of L-alanine, and is split due to coupling with the proton of the chiral centre. The most upfield signal observed at δ 1.18 and is due to the CH_3 of the ethyl ester group and appears as a triplet with a coupling constant of 7.2 Hz.

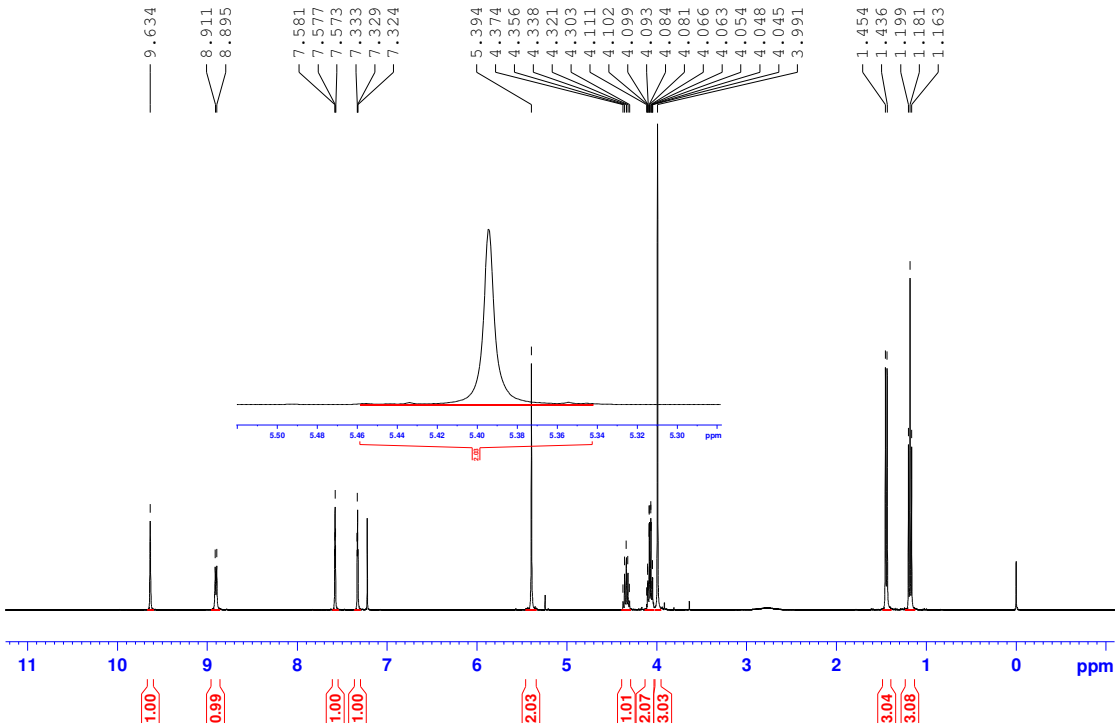
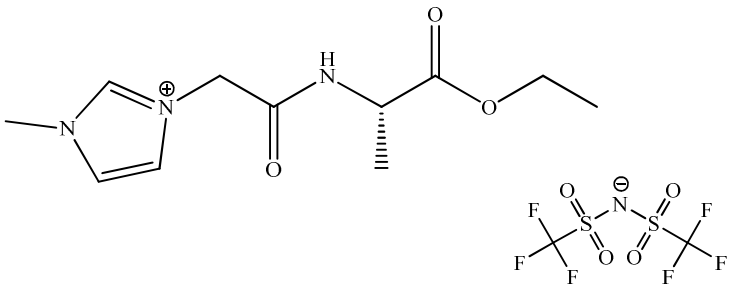


Fig. 3.9: ^{1}H NMR spectrum of 3-methyl-1-L-alanine ethyl ester imidazolium bromide (**274**).

3.3.1.2 ^{1}H NMR spectroscopic study of 3-methyl-1-L-alanine ethyl ester imidazolium NTf_2 (297)



(**297**)

Triflimide anions are inorganic and do not possess any hydrogen atoms. Hence these ions do not give signals in ^{1}H NMR spectra. Their presence however in an ionic liquid salt can be seen in a chemical shift study of various protons of the imidazolium cation. The amide protons of the L-alanine in the cation side chain appear slightly shifted up-field to δ 7.24. In the case of the bromide salt (**274**) this peak occurred more downfield at δ 8.90. The acidic proton of the imidazolium cation gives a singlet at δ 8.67 whereas the bromide derivatives also gave a singlet peak shifted down at δ 9.16. The two methine protons of the imidazolium ring give rise to two triplet signals at δ 7.38 and 7.20, with one triplet overlapping with the residual $CHCl_3$ solvent peak, and the other with a coupling constant of 1.8 Hz. The protons of the methylene group appear to be diastereotopic in the spectrum of the NTf_2 salt. Here, coupling is occurring between the two protons of the methylene of the cation side chain giving rise to an AB system with a coupling constant of 10.8 Hz. This type of coupling suggests that these protons are chemically different and hence a roofing effect is observed. Similar to the imidazolium protons, the methylene signal is shifted in a more up-field direction. This indicates that the cation of the triflimide salt is less de-shielded than that of the bromide example. Notable changes in chemical shifts of these signals in the ^{1}H NMR spectra can aid in differentiating between salts with varying anions, in particular when an inorganic counter ion is present. A change in chemical shift is also seen for the *N*-Methyl group of the imidazolium ring (gives a singlet at δ 3.86; δ 3.99 for bromide salt) and the methyl group of the L-alanine side chain (a doublet occurring at δ 1.36; δ 1.44 for bromide salt).

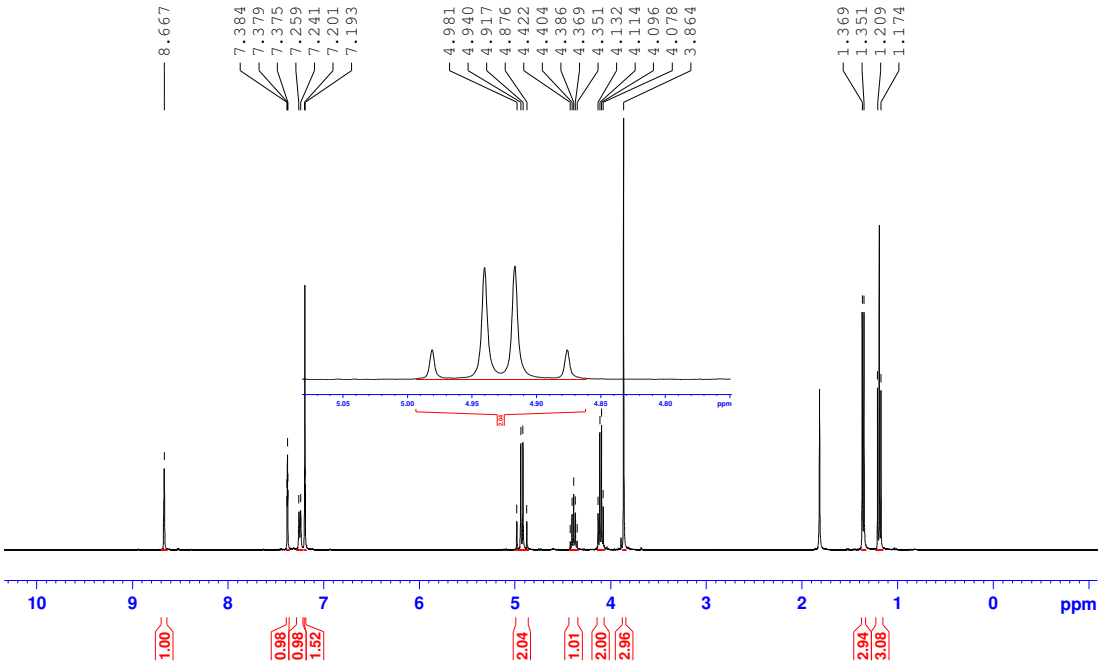
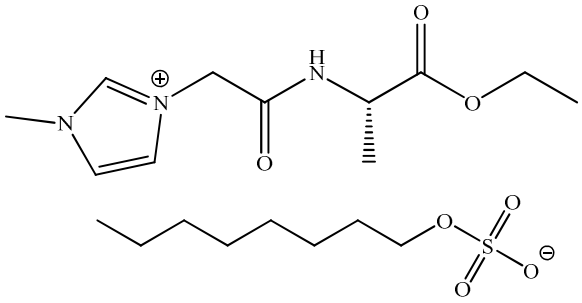


Fig. 3.10: ^{1}H NMR spectrum of 3-methyl-1-L-alanine ethyl ester imidazolium NTf_2 (**297**).

3.3.1.3 ^{1}H NMR spectroscopic study of 3-methyl-1-L-alanine ethyl ester imidazolium $OctOSO_3$ (314)



(**314**)

The amide proton of the CIL side chain appears at δ 8.52 as a doublet with a coupling constant of 6.8 Hz. At δ 9.13 a singlet is present indicating the presence of the acidic proton of the imidazolium moiety. The chemical shift of this proton is almost similiar to that

observed in the bromide derivative (δ 9.16). Two singlets appear at δ 7.48 and 7.27 which are assigned to be the methine groups of the imidazolium core. When compared to the bromide counterpart, it can be noted that the chemical shifts of these signals are slightly up-field (δ 7.58, 7.37 for bromide CILs). The NCH_2 group between the imidazolium cation and the amino acid containing side chain gives a singlet at δ 5.13. Again, this is slightly shifted towards the up-field end of the ^{1}H NMR spectrum with respect to the bromide example (δ 5.39) and can be correlated to a shielding effect of the anion. Unlike the triflimide CIL, the octyl sulfate anion is evident in the ^{1}H NMR spectrum. A triplet can be seen at δ 3.92 and overlaps with the *N*-methyl of the imidazolium group. This triplet is due to the OCH_2 of the octyl sulfate anion. Further evidence of the octyl sulfate anion can be seen in the aliphatic region of the NMR spectrum. The protons in the octyl chain of the anion give multiplet signals at δ 1.57-1.40 and 1.26-1.16.

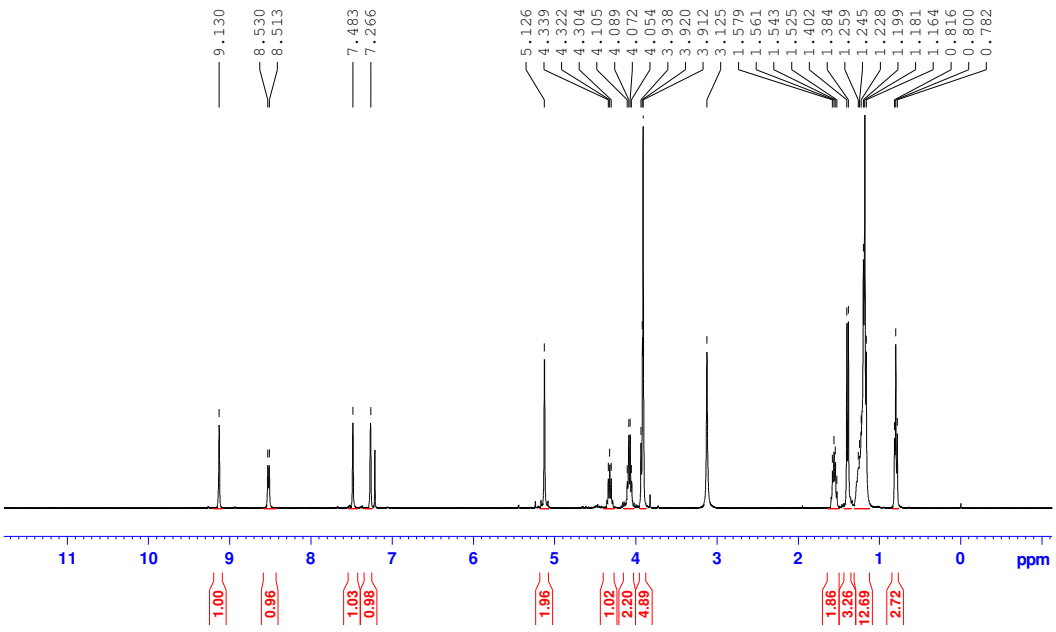


Fig. 3.11: ^{1}H NMR spectrum of 3-methyl-1-L-alanine ethyl ester imidazolium $OctOSO_3$ (**314**).

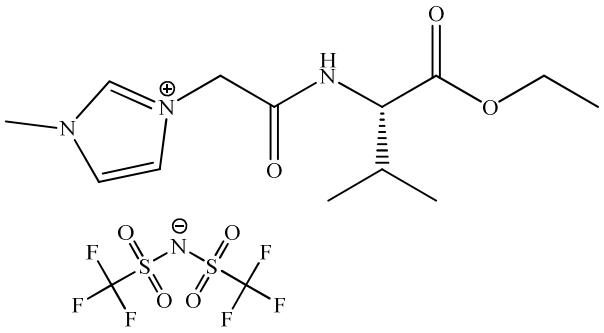
3.3.2 ^{13}C NMR and DEPT spectroscopic studies of chiral ionic liquids

^{13}C NMR and DEPT 135 experiments were also performed for all novel chiral ionic liquids. In the ^{13}C NMR spectrum the amide carbonyl carbon appears between ~ δ 171.2-171.9 while the ester carbonyl groups reside between ~ δ 164.8-165.2. The carbons of the imidazolium ring give signals in the aromatic region of the ^{13}C spectrum; ~ δ 137.7, 123.7 and 122.6. The *N*-methyl of the imidazolium cation appears up-field at ~ δ 35.7-37.4. The methylene group bridging the cation core to the chiral side-chain is seen at ~ δ 50.2-52.4 in the carbon spectra and gives a negative peak (relative to aromatic *C*H) in the DEPT 135 spectra. Table 3.8 illustrates some selected ^{13}C NMR data of amino acid ester chiral ionic liquids.

Table 3.8: Selected ^{13}C data (ppm, in $CDCl_3$) for Amino acid ester chiral ionic liquids (**276, 277, 283, 284, 306** and **310**).

Compound no.	*C*=O ester	*C*=O amide	N*C*HN Imidazolium	N*C*H imidazolium	N*C*H$_2$ Methylene	N*C*H$_3$ Imidazolium
276	171.9	165.2	137.79	123.9	52.2	36.8
				122.3		
277	170.9	165.3	137.7	123.7	50.2	35.8
				122.9		
283	172.5	165.0	137.7	123.7	51.7	36.8
				122.6		
284	172.5	164.8	137.8	123.8	51.9	36.8
				122.3		
306	171.3	164.5	137.1	123.9	50.9	36.4
				122.9		
310	172.7	165.2	137.8	123.6	51.0	36.4
				122.8		

3.3.2.1 ^{13}C NMR and DEPT 135 spectroscopic study of 3-methyl-1-L-valine ethyl ester imidazolium NTf_2 (306)



(**306**)

In the ^{13}C spectrum of 1-methyl-3-L-valine ethyl ester imidazolium NTf_2 (**306**) two carbonyl carbons can be seen in the low field region of the NMR at δ 171.3 and 164.5. These correspond to the ester and amide carbonyls of the L-valine ethyl ester moiety of the CIL side chain respectively. In the DEPT 135 spectrum these carbonyl groups are not evident. In the aromatic region at δ 137.1, 123.9 and 122.9 the carbons of the imidazolium ring appear. Also present in this region, at δ 118.0, is a quartet of a coupling constant of 318 Hz. This quartet is assigned to be the two quaternary carbons present in the bistriflimide anion which is further supported by their absence in the DEPT 135 spectrum. These carbons appear as a quartet signal due to their coupling to the neighbouring fluorine groups and appear as one quartet as the anion is symmetrical. This type of coupling has previously been reported in the group[12] with coupling constants of ~ 320 Hz typically observed. The methylene carbon of the ethyl ester side chain appears at δ 61.6, and gives a negative peak in the DEPT 135 NMR spectrum. The methylene group adjacent to the imidazolium cation also shows a negative resonance peak at δ 50.9. The *N*-methyl group of the imidazolium cation occurs at δ 36.4. The other methyl groups of the branched L-valine side chain and the ethyl ester chain appear in the upfield region at δ 18.7, 17.6 and 14.0 respectively.

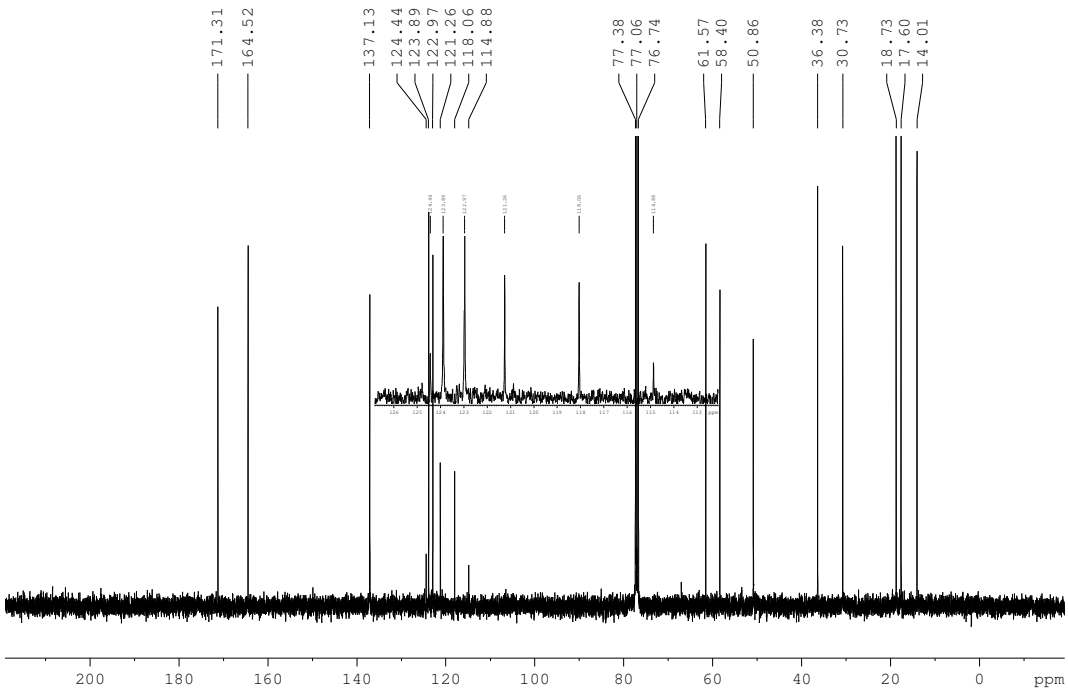


Fig. 3.12: ^{13}C NMR spectrum of 3-methyl-1-L-valine ethyl ester imidazolium NTf_2 (**306**).

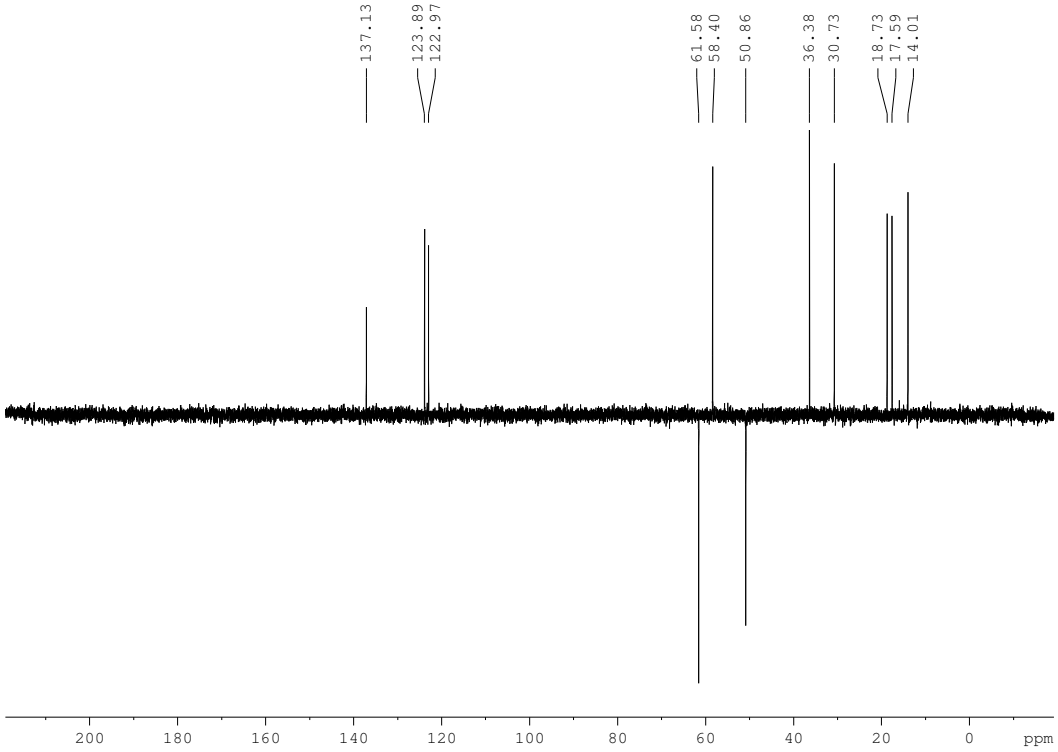
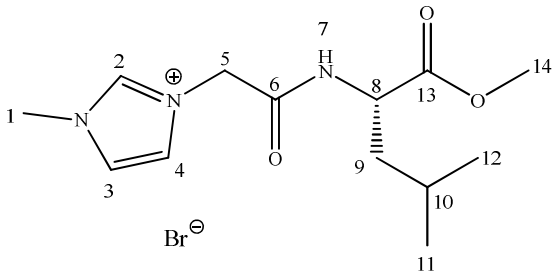


Fig. 3.13: DEPT 135 spectrum of 3-methyl-1-L-valine ethyl ester imidazolium NTf_2 (**306**)

3.3.3 COSY study of 3-methyl-1-L-leucine methyl ester imidazolium bromide chiral ionic liquid (282)

Correlation Spectroscopy (COSY) was preformed on novel amino acid ester chiral ionic liquids. This technique is the simplest form of two dimensional NMR. The proton NMR chemical shifts are plotted along both axes and the spectrum shows distinct spots on the diagonal with each spot corresponding to the same peak on each coordinate axis.[13] In the COSY NMR spectrum of 3-methyl-1-L-leucine methyl ester imidazolium bromide (**282**), it can be seen that the acidic proton of the imidazolium cation **2** (δ 9.62) correlates with the methine protons of the ring **3** and **4** (δ 7.55 and 7.24). It is also clear that amide proton **7** (δ 8.89) couples with the methine proton **8** (δ 4.35) at the chiral centre of the L-leucine side chain. This proton correlates further with the methylene **9** (δ 1.74) and methine **10** (δ 1.59) groups of the ^{i}butyl side chain of L-leucine. Coupling of these protons to the methyl groups **11** and **12** (δ 0.88 and 0.83) of the ^{i}butyl side chain is also noted from the COSY spectrum. Coupling is evident with the methine protons of the imidazolium ring **3** and **4**, and also between protons **9** and **10** in the 2D NMR spectrum (Figure 3.15).



(**282**)

Fig.3.14: 3-methyl-1-L-leucine methyl ester imidazolium bromide (**282**) chiral ionic liquid.

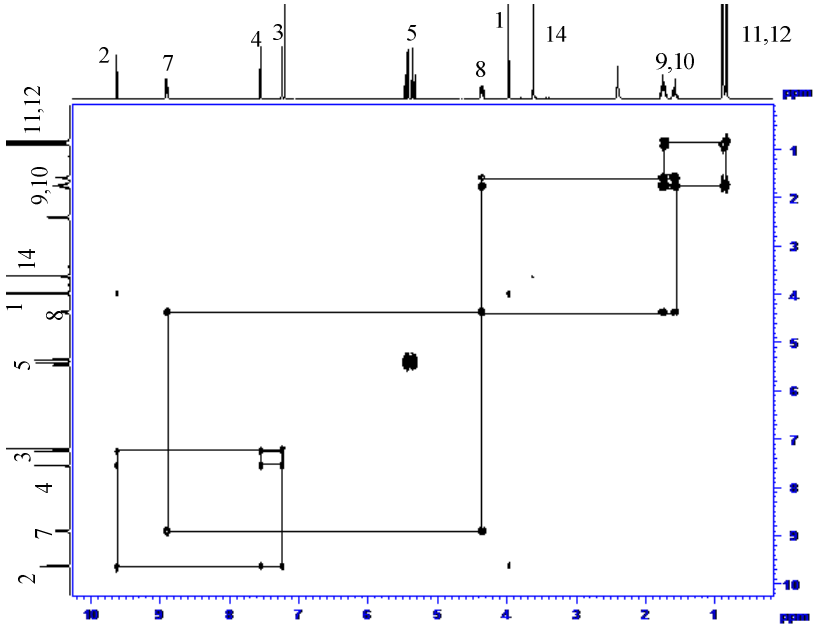
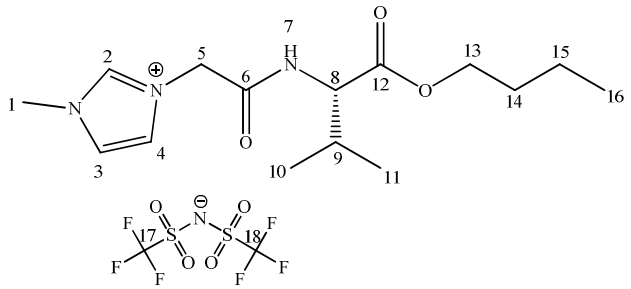


Fig. 3.15: COSY spectrum of 3-methyl-1-L-leucine methyl ester imidazolium bromide (**282**).

3.3.4 HMQC study of 3-methyl-1-L-Valine butyl ester imidazolium NTf_2 chiral ionic liquid (307)

Heteronuclear multiple quantum coherence (HMQC) is a 2D inverse C-H correlation technique. It allows for the determination of carbon to hydrogen connectivity. This NMR technique is extremely useful in resolving overlapping proton peaks in complex chemical structures. As it is a C-H correlation method, quaternary and carbonyl carbons do not appear in HMQC spectra.[13,14] Table 3.9 and Figure 3.16 describes a C-H correlation study of 3-methyl-1-L-valine butyl ester imidazolium NTf_2 (**307**).



(**307**)

Table 3.9: C-H correlation data from HMQC spectrum of 3-methyl-1-L-valine butyl ester imidazolium NTf_2 (**307**).

Site	^{1}H NMR	^{13}C NMR	HMQC
1	3.97		36.56
2	8.71		137.23
3	7.25		121.26
4	7.45		122.72
5	5.12		51.03
6		164.35	
7	7.17		
8	4.42		58.43
9	2.14		30.46
10	0.99-0.93*		18.84-13.63
11	0.99-0.93*		18.84-13.63
12		171.26	
13	4.12-4.04		65.46
14	1.66		30.71
15	1.38		19.06
16	0.99-0.93*		18.84-13.63
17		121.26	
18		121.26	

*appear within a multiplet in ^{1}H NMR spectrum

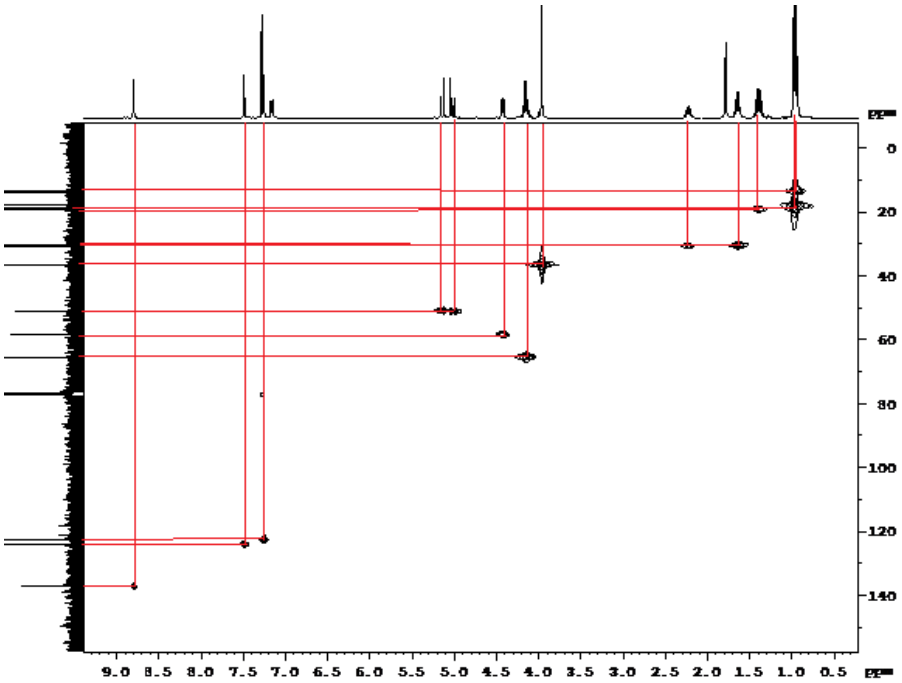


Fig. 3.16: HMQC NMR spectrum of 3-methyl-1-L-valine butyl ester imidazolium NTf_2 (**307**).

3.4 Infra red spectroscopic studies of chiral ionic liquids

Infra red spectroscopy (IR) is commonly used by organic chemists to characterise novel compounds. It is mainly used to identify various functional groups within a molecule.[14] When a molecule absorbs infrared radiation, different vibrational modes (stretching, twisting bending and rocking) are induced. Most structural information of a compound can be obtained in the region of the IR spectrum above 1400 cm^{-1}. In the IR spectra of amino acid ester chiral ionic liquids, the amide N-H appears as two bands above 3000 cm^{-1}. This is due to the two possible configurations which the amide groups can undertake (Figure 3.17). Two amide carbonyl bands (Amide I and II) can be seen between 1660 and 1530 $cm^{-1.}$ The ester group can be observed at 1743 cm^{-1} in the IR spectrum, and C-O stretches give rise to bands at 1171 $cm^{-1.}$

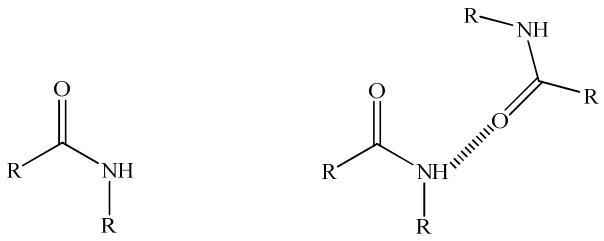


Fig. 3.17: Two amide configurations observed in IR spectra.

Table 3.10: IR frequencies of amino acid ester chiral Ionic Liquids (cm^{-1}).

Compound no.	N-H	C=O amide I and II	C=O ester	C-O ester
271	3210	1658,1534	1730	1172
272	3236	1660,1543	1738	1172
279	3278	1640,1553	1727	1266,1173
280	3220	1661,1534	1737	1206,1172
282	3220	1656,1533	1739	1205,1171
283	3187	1651,1532	1751	1175

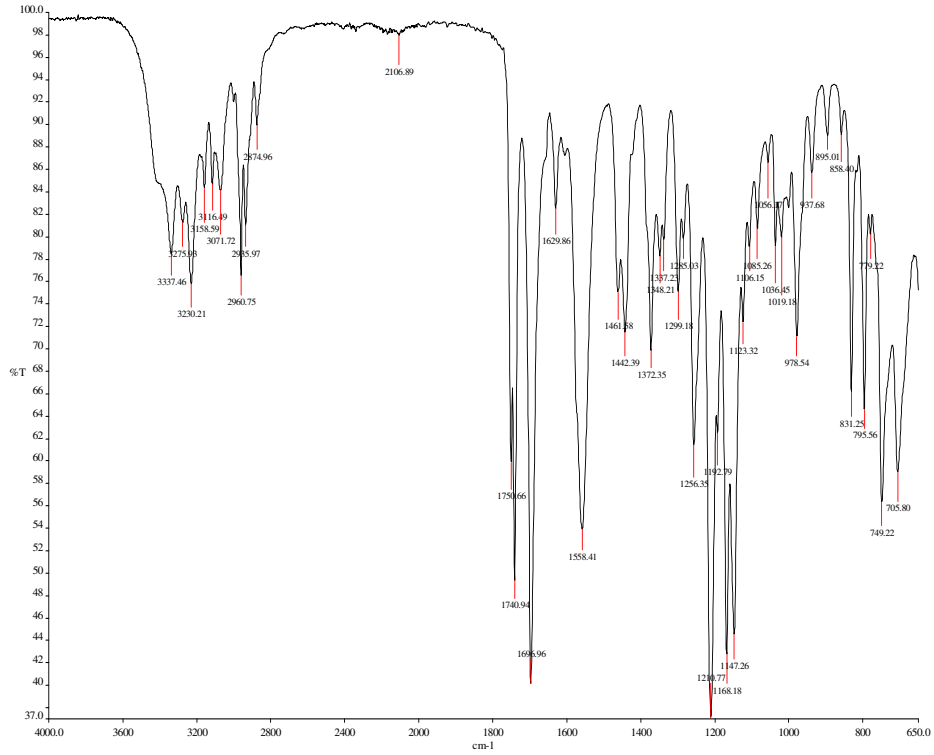


Fig. 3.18: IR spectrum of 3-methyl-1-L-isoleucine methyl ester imidazolium bromide (**279**).

3.5 Conclusions

A panel of 48 novel chiral ionic liquids with highly functionalised side chains were sucessfully synthesised. 23 α-bromoamide intermediates (**247**-**269**) were prepared from amino acid ester precursors. Product yields and purities were improved for these intermediates using a modified synthetic method. A method employing milder reaction conditions (i.e. use of K_2CO_3 base, at RT) than previously used (i.e. Et_3N, -78 °C), gave enhanced yields for various chiral alkylating intermediates (**247**, **249**, **251**, **253**, **254**, **255**, **256**, **257**, **264**, **265**, **267**).

23 bromide CILs (**270**-**292**) were synthesised from these α-bromoamide intermediates. THF replaced diethyl ether as reaction media in this synthesis in order to optimise bromide salt formation. All the bromide CILs prepared were screened for various antimicrobial activities (see Chapter 5). Bromide CIL (**272**) was also investigated in an Activated Sludge

biodegradation assay (Chapter 6). A series of anion metathesis reactions yielded 15 NTf_2 CILs (**293**-**307**), and 10 $OctOSO_3$ (**308**-**317**) derivatives. All the CILs (NTf_2 and $OctOSO_3$) were obtained as liquids at RT. Hence, as seen in Chapter 2, exchanging the bromide anion in the IL structures leads to notable changes in the physical properties of the salts. All novel chiral ILs were successfully characterised *via* a range of spectroscopic techniques, namely ^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC, IR and MS.

3.6 References

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Chapter 4: Results and discussion

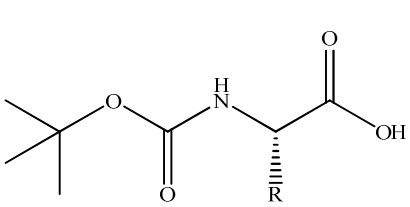
Dipeptidyl Chiral Ionic Liquids, Synthesis and Characterisation

4.1 Introduction

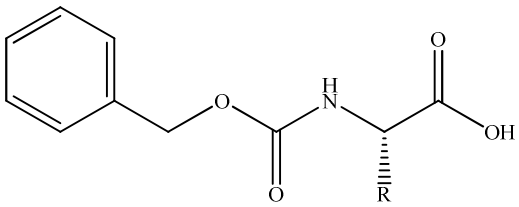
A series of dipeptidyl ionic liquids were designed and prepared to further add functionality to the IL side chain of the CILs. By incorporating additional sites for possible enzymatic cleavage (compared to the amino acid ester derivatives), it was hoped that improved biodegradation could be achieved. It was also hypothesised that an ionic liquid containing a peptidyl moiety may be more readily recognised by metabolising enzymes (e.g. amidases). This could therefore improve IL breakdown in the environment. The side chains of these novel ILs possess amide-amide-ester fragments. The first of these amide bonds is attached directly to the imidazolium cation. The subsequent amide moiety is the peptide bond between two amino acids. As one of the amino acids of the dipeptide requires protection of its *C*-terminus, this gives rise to the terminal ester of the side chain fragment. The introduction of peptide bonds to surfactants[1] and polymers[2] has been studied to improve biodegradability. Peptide chemistry was applied in order to synthesise these novel CILs.

4.1.1 Peptide bond formation

A peptide bond is a covalent bond formed between two amino acids, when the *C*-terminus (carboxyl group) of one amino acid reacts with the *N*-terminus (amino group) of the other amino acid. To ensure that regiospecific coupling occurs between the amino acids, protection of the amine and carboxyl groups not involved in the peptide bond formation is required. A commonly used amine protecting group employs a carbamate moiety. These carbamate units display low nucleophilic reactivity and can be easily removed *via* a decarboxylation reaction. Common examples of these protecting groups are *t*-butoxycarbonyl (BOC) (**318**) and benzyloxycarbonyl (Cbz) (**319**) groups.



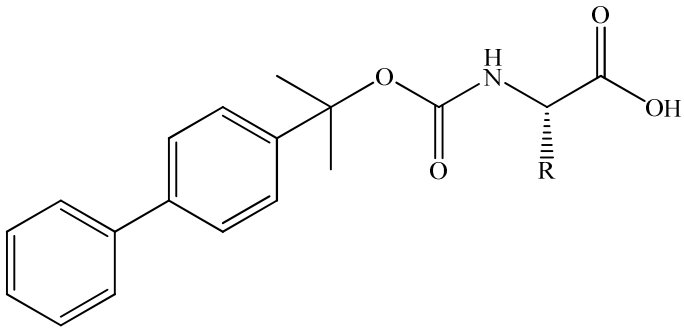
(**318**)



(**319**)

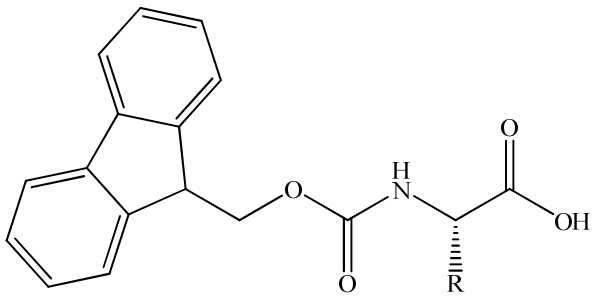
These groups can be introduced onto the amino acid amine using the corresponding chloroformates and can be removed under acidic conditions.[3]

A modified version of the BOC group is the diphenylisopropyloxycarbonyl (Bpoc) derivative (**320**). The presence of the aromatic rings enables stabilization of the adjacent carbocation in the deprotection reaction.



(**320**)

9-Fluorenylmethyoxycarbonyl (Fmoc) (**321**) is an example of a base labile and acid stable amine protecting group. Removal can be achieved using ammonia, piperidine or morpholine. The diphenylfulvene formed is trapped by the deprotecting base.[3]



(**321**)

Protection of the carboxylic acid group of an amino acid is carried out by conversion to its corresponding ester. Reacting the amino acid with thionyl chloride ($SOCl_2$) in an alkyl alcohol yields the alkyl ester hydrochloride salts. These hydrochloride salts can be neutralised to give the free base form of the peptide. However, these generally deteriorate rapidly and therefore the free base is generally generated *in situ* when required, usually by

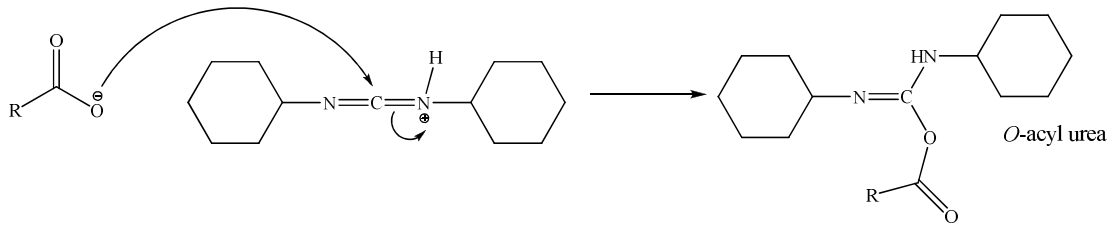
using a tertiary amine. Deprotection reactions of these groups are typically conducted in basic conditions.

4.1.2 Coupling reagents

The reaction between an amine and a carboxylic acid to form an amide bond cannot occur spontaneously at ambient temperatures. Therefore it is necessary to activate the carboxylic acid, by converting the –OH into a good leaving group. One method of carboxylic acid activation is to use peptide coupling reagents. These reagents can form reactive intermediates such as acid chlorides, anhydrides and active esters.

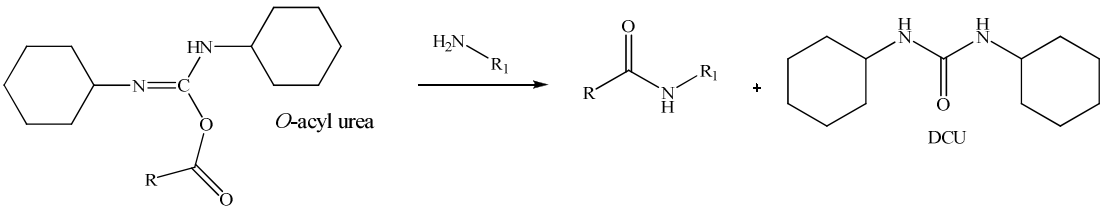
4.1.2.1 Carbodiimides

Carbodiimides are the most commonly used coupling reagents in peptide synthesis. Dicyclohexylcarbodiimide (DCC) was the first carbodiimide reagent synthesised in 1955. In coupling reactions using DCC, the initial step involves the reaction of DCC with the carboxylic acid group. This leads to the formation of the potent acylating intermediate *O*-acylurea.[4]

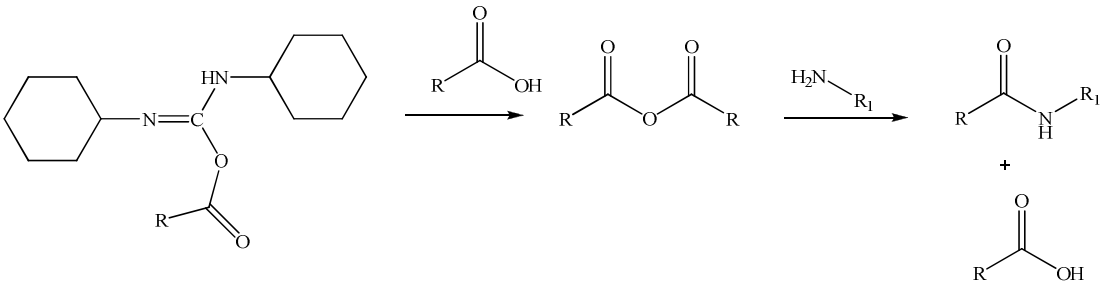


O-acylurea can further undergo several reactions;

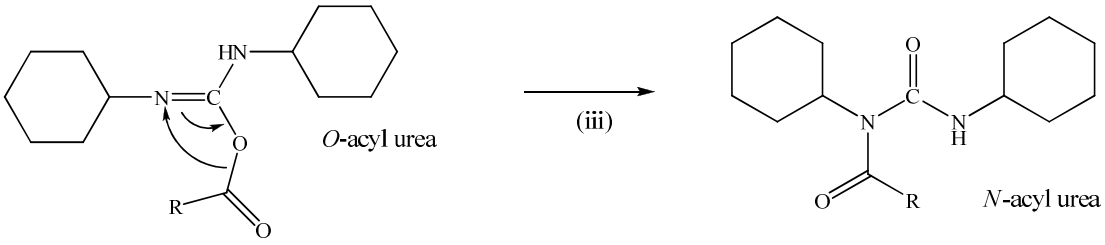
i. Amide formation by direct reaction with the amine. In this reaction the by-product formed is Dicyclohexylurea (DCU). This product is poorly soluble in organic solvents and can be easily removed by filtration.



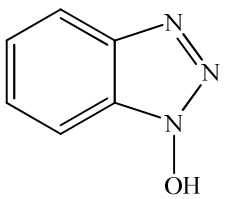
ii. Aminolysis can be achieved *via* a symmetrical anhydride.



iii. *O*-acylurea can undergo an intramolecular acyl transfer to form the *N*-acyl urea by product.

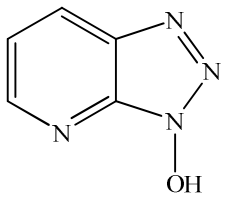


Moreover, the *O*-acylourea intermediate can undergo racemization when an intramolecular proton transfer from the chiral carbon to the basic centre of the urea occurs.[4] To overcome this problem of epimerization, a secondary nucleophile such as 1-hydroxy-1*H*-benzotriazole (HOBt) (**322**) can be added. 1-Hydroxy-7-azobenzotriazole (HOAt) (**323**) is another commonly used additive in peptide synthesis.



(**322**)

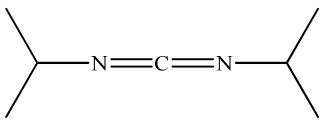
1-Hydroxy-1*H*-benzotriazole (HOBt)



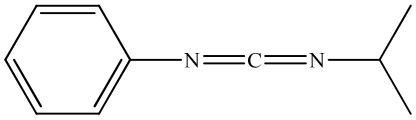
(**323**)

1-Hydroxy-7-azobenzotriazole (HOAt)

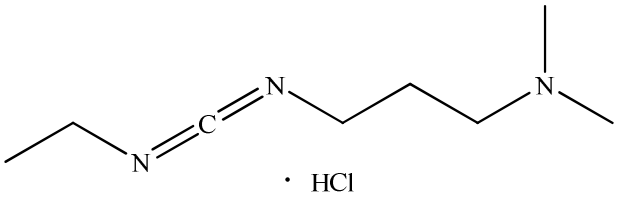
HOBt reacts with the *O*-acylurea to form a benzotriazole (OBt) activated ester. This improves the reactivity of the resulting active ester by stabilizing the amine approach *via* hydrogen bonding. HOBt is also regenerated *in situ*, hence only catalytic amounts are required in coupling reactions. Many other carbodiimide coupling reagents are commercially available and widely used in the area of peptide bond formation. Other carbodiimides include diisopropylcarbodiimide (DIC) (**324**), phenyl isopropyl carbodiimide (PIC) (**325**) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (**326**).



diisopropylcarbodiimide (DIC) (**324**)



phenyl isopropyl carbodiimide (PIC) (**325**)



N-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC) (**326**)

EDC is commonly used as a coupling reagent in peptide synthesis, as the urea by-product formed, 1-{3-(dimethylamino)propyl}-3-ethyl urea, is water soluble. This removes the difficulties associated with the separation of the undesired DCU by-product from the DCC coupling reaction. The peptide bond formation, using EDC coupling reagent, in the presence of HOBt is illustrated in Figure 4.1.

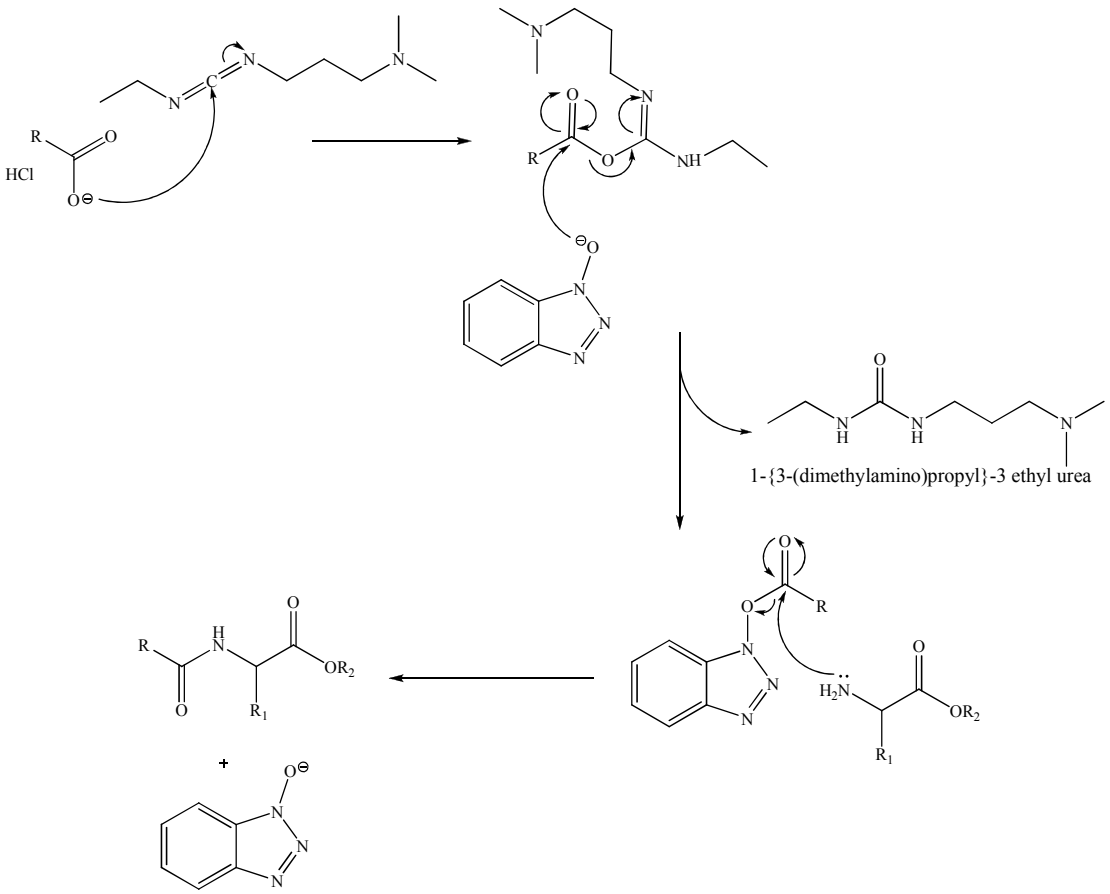


Fig. 4.1: Amide bond formation using EDC/HOBt.

4.1.2.2 Phosphonium coupling reagents

A number of coupling reagents based on the 1*H*-benzotriazole structure can be used in amide bond formation. An example of such coupling reagents are based on HOBt/HOAt structures and contain a phosphonium group. The first phosphonium coupling reagent reported was benzotriazolyl-1-oxy-*tris*(dimethylamino) phosphonium hexafluorophosphate (BOP) (**327**). In coupling reactions, BOP reacts with the carboxylic acid to form an active ester species. Aminolysis then occurs between the activated intermediate and the amine (Figure 4.2).

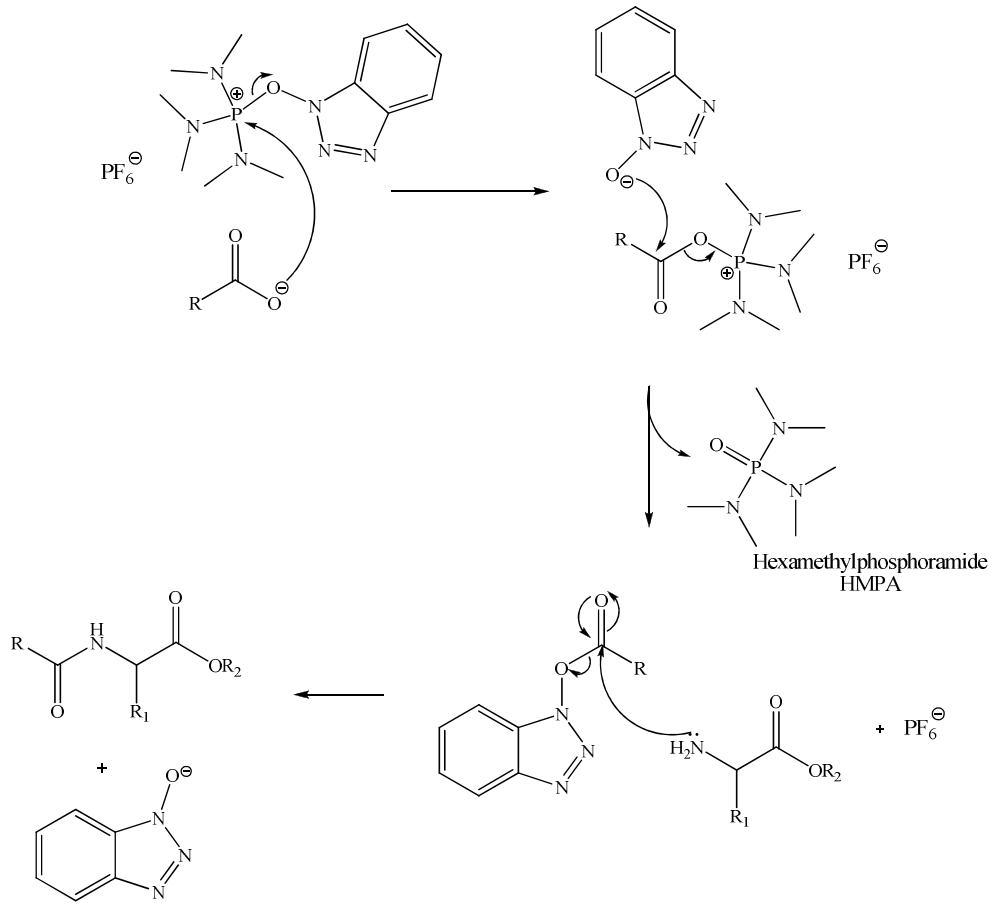
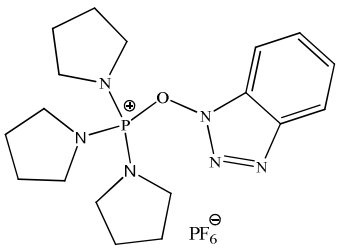


Fig. 4.2: Peptide coupling using benzotriazolyl-1-oxy-*tris*(dimethylamino) phosphonium hexafluorophosphate (BOP).

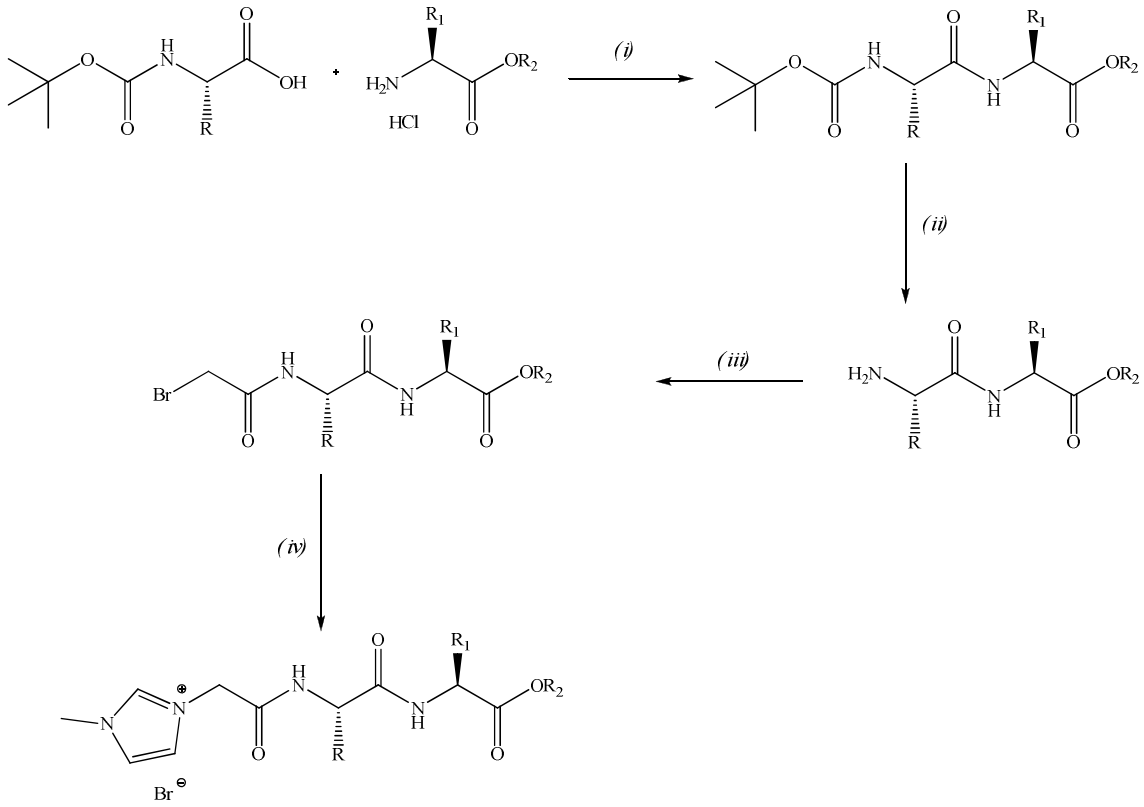
The use of BOP as a coupling reagent in peptide synthesis has been limited due to the formation of the carcinogenic by-product hexamethylphosphoramide (HMPA). Less toxic derivatives have been developed, such as Benzotriazol-1-yl-oxy-*tris*-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (**328**).[5]



PyBOP (**328**)

4.2 Synthesis of dipeptidyl Chiral Ionic Liquids

These CILs contain an imidazolium cation core, with a dipeptide fragment in the side chain. The synthesis of these CILs involves a 4 step experimental procedure (Scheme 4.1); *(i)* amide bond formation of the dipeptide species (using the EDC/HOBt protocol), *(ii)* subsequent BOC removal from the *N*-terminus of the dipeptide, to form the free amine species (using 4M hydrochloric acid in dioxane), *(iii)* nucleophilic substitution reaction with bromoacetyl bromide to form the alkylating intermediate, and *(iv) N*-alkylation reaction with 1-methyl imidazole to yield the bromide salt. An extra step was required in some cases where *C*-protection of one amino acid (i.e. preparation of amino acid esters) was performed.



Scheme 4.1: Synthesis of imidazolium based dipeptide chiral ionic liquids (i) EDC, HOBt, Et_3N, (ii) 4M HCl in dioxane, (iii) Bromoacetyl bromide, Et_3N, -78 °C (iv) 1-methyl imidazole.

4.2.1 Peptide synthesis

The dipeptide fragments were prepared using conventional peptide coupling chemistry as introduced in Section 4.1 of the chapter. The synthesis involved treating the BOC protected amino acids (i.e. *N-tert*-butyloxycarbonyl-L-alanine, L-valine and L-phenylalanine) with 1-hydroxybenzotriazole (HOBt), *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC), and triethylamine (Et_3N) in dichloromethane at 0 ^oC in the presence of various amino acid esters. The dipeptides synthesized according to this method are summarized in Table 4.1.

Table 4.1: Dipeptides prepared and obtained yields.

Dipeptide	Compound no.	Yield (%)
N-tert-butyloxycarbonyl-L-phenylalanine-L-phenylalanine methyl ester	**329**	64
N-tert-butyloxycarbonyl-L-phenylalanine-L-leucine methyl ester	**330**	90
N-tert-butyloxycarbonyl-L-phenylalanine-D-phenylalanine ethyl ester	**331**	87
N-tert-butyloxycarbonyl-L-phenylalanine-L-phenylalanine butyl ester	**332**	85
N-tert-butyloxycarbonyl-L-phenylalanine-L-valine ethyl ester	**333**	71
N-tert-butyloxycarbonyl-L-phenylalanine-L-alanine methyl ester	**334**	61
N-tert-butyloxycarbonyl-L-valine-L-alanine ethyl ester	**335**	77
N-tert-butyloxycarbonyl-L-valine-L-leucine methyl ester	**336**	82
N-tert-butyloxycarbonyl-L-valine-L-valine methyl ester	**337**	78
N-tert-butyloxycarbonyl-L-valine-L-phenylalanine ethyl ester	**338**	84
N-tert-butyloxycarbonyl-L-valine-D-valine ethyl ester	**339**	87
N-tert-butyloxycarbonyl-L-valine-L-alanine methyl ester	**340**	80
N-tert-butyloxycarbonyl-L-alanine-L-valine methyl ester	**341**	85
N-tert-butyloxycarbonyl-L-alanine-L-phenylalanine ethyl ester	**342**	76
N-tert-butyloxycarbonyl-L-alanine-L-alanine butyl ester	**343**	76
N-tert-butyloxycarbonyl-L-alanine-L-isoleucine methyl ester	**344**	46
N-tert-butyloxycarbonyl-L-alanine-L-leucine methyl ester	**345**	60

Purification of these dipeptides was achieved by washing with a dilute acid to remove the urea by-product from the EDC coupling reagent, 1-{3-(dimethylamino)propyl}-3 ethyl urea. Recrystallisation using ethyl acetate/pet. ether 40-60 °C was then employed yielding the peptides in good to very good yields of 46-90 % (Table 4.1).

The next step of the synthesis *(ii)* involved the removal of the BOC protecting group from the dipeptide. This reaction was conducted in the presence of an acid (i.e. trifluoroacetic acid or hydrochloric acid), with formation of a carbamic acid followed by loss of carbon dioxide as depicted in Figure 4.3.

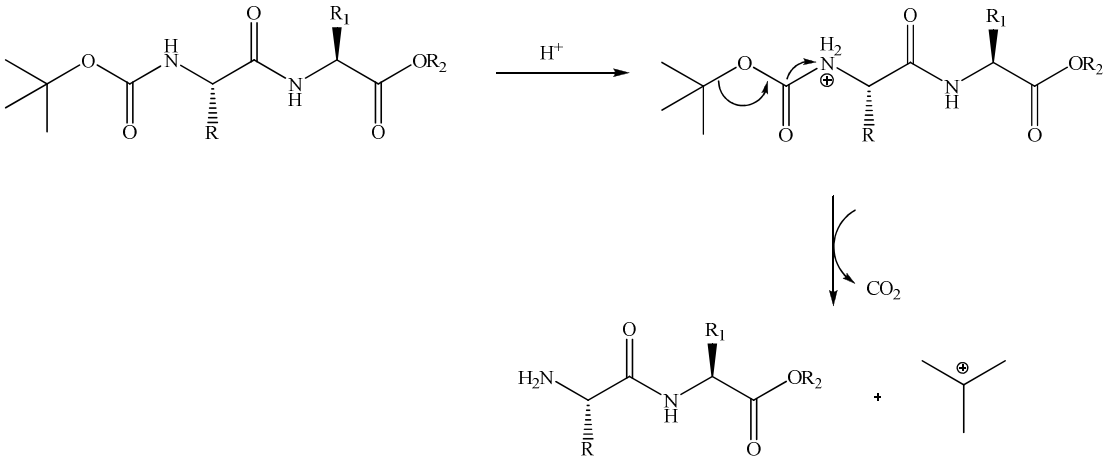


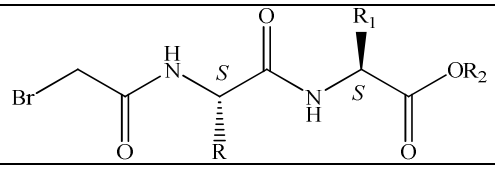
Fig. 4.3: Removal of BOC protecting group from dipeptides.[4]

Initially trifluoroacetic acid (TFA) was used in these reactions, where the BOC-dipeptide was stirred in DCM and TFA was added dropwise at 0 °C. However the TFA salts of the dipeptides formed did not react successfully in the next step of the synthesis (with bromoacetyl bromide). As a result the hydrochloric salt forms of the peptides were prepared using 4M HCl in dioxane solution. This involved treating the BOC-dipeptide in 4M HCl in dioxane solution and allowing to stir for 4-5 hours at room temperature. Removal of the dioxane solvent *via* rotary evaporation and further drying under vacuum with heating overnight yielded the dipeptide hydrochloride salt.

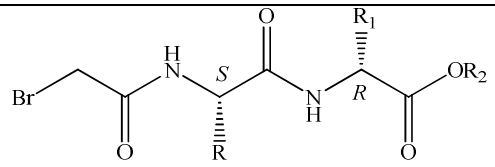
4.2.2 Formation of alkylating intermediates

Following removal of the BOC protecting group from the dipeptide moiety, the free amine terminus can react with bromoacetyl bromide to form the bromo-alkylating reagents (Scheme 4.1, step *(iii)*). In this reaction the peptide was stirred in DCM, in the presence of Et_3N. Bromoacetyl bromide was added dropwise at -78 °C. The reaction was allowed to proceed at this temperature for 5-6 hours and then warmed to room temperature. Initial attempts to prepare these intermediates were carried using the TFA salts of the dipeptides. These reactions yielded products in very low yields (16-42 %), or in some cases the reaction did not proceed and the desired product was not obtained. Reacting bromoacetyl bromide with the dipeptide hydrochloride salts gave the bromoamides in good yields. Tables 4.2 and 4.3 outlines the results obtained from these reactions using both the TFA and HCl dipeptide salts.

Table 4.2: Bromo-alkylating intermediates obtained from dipeptide HCl salts.



R	R_1	R_2	Compound No.	Yield (%)
CH_3	CH_3	C_4H_9	**346**	77
CH_3	$CH(CH_3)_2$	CH_3	**347**	67
CH_3	$CH(CH_3)CH_2CH_3$	CH_3	**348**	63
CH_3	CH_2-(C_6H_5)	C_2H_5	**349**	76
CH_3	$CH_2CH(CH_3)_2$	CH_3	**350**	73
$CH(CH_3)_2$	CH_2-(C_6H_5)	C_2H_5	**351**	77
$CH(CH_3)_2$	CH_3	CH_3	**352**	49
$CH(CH_3)_2$	CH_3	C_2H_5	**353**	84
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	CH_3	**354**	68
$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	**355**	60
CH_2-(C_6H_5)	$CH_2CH(CH_3)_2$	CH_3	**356**	74
CH_2-(C_6H_5)	CH_3	CH_3	**357**	73
CH_2-(C_6H_5)	$CH(CH_3)_2$	C_2H_5	**358**	72
CH_2-(C_6H_5)	CH_2-(C_6H_5)	C_4H_9	**359**	84
CH_2-(C_6H_5)	CH_2-(C_6H_5)	CH_3	**360**	62



R	R_1	R_2	Compound No.	Yield (%)
CH_2-(C_6H_5)	CH_2-(C_6H_5)	C_2H_5	**361**	69
$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	**362**	72

Table 4.3: Bromo-alkylating intermediates (**346, 348, 351, 353, 356, 357, 359, 360** and **361**) obtained from dipeptide TFA salts.

Compound no.	Yield (%)
346	n/a
348	n/a
351	46
353	n/a
356	44
357	16
359	22
360	34
361	30

n/a indicates where reaction did not proceed and product was not obtained

Column chromatography was employed to purify the dipeptidyl bromoamide intermediates. The mobile phase used was a gradient system of 100 % hexane to 50:50 hexane:ethyl acetate. Yields of 60-84 % were obtained of pure products and ^{1}H NMR spectroscopy was used to confirm product purity (Figure 4.4).

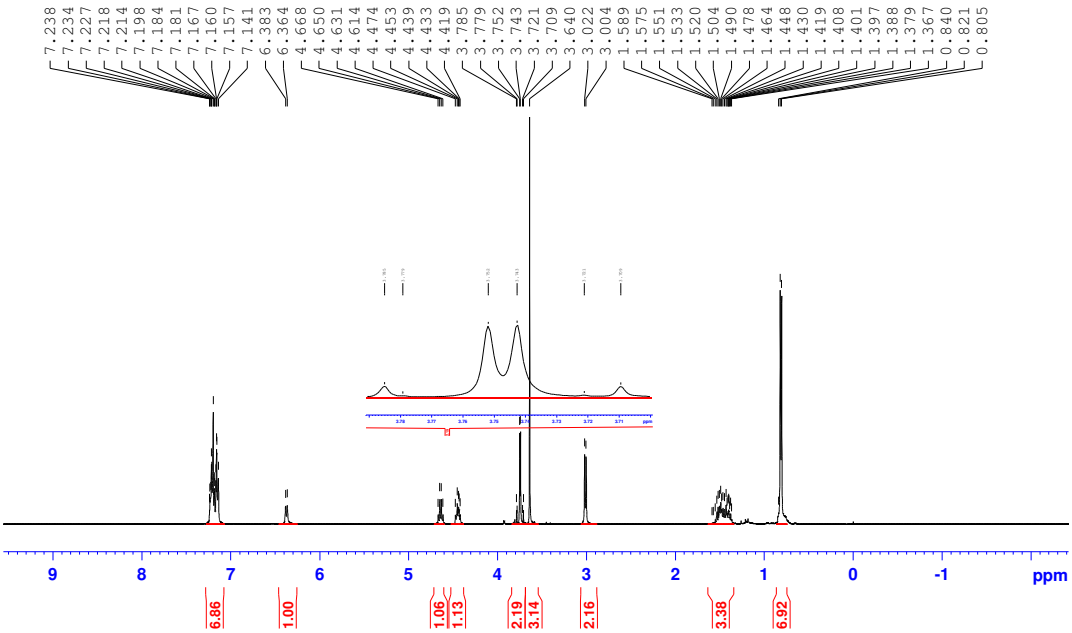


Fig. 4.4: ^{1}H NMR of pure L-phenylalanine-L-leucine methyl ester bromoacetate (**356**).

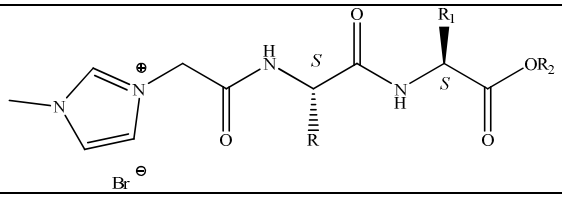
The multiplet at δ 7.24-7.14 in the aromatic region of the ^{1}H NMR spectrum is due to the aromatic protons of the L-phenylalanine benzyl group. Also residing in this multiplet is one of the amide protons of the dipeptide fragment (integration of 6). A doublet at δ 6.37 with a coupling constant of 7.6 Hz corresponds to the amide proton of the L-phenylalanine fragment. This proton appears as a doublet as it is coupling with the α-proton of the chiral centre of the L-phenylalanine. Subsequent coupling of this proton to the methylene protons of the benzyl group of L-phenyalanine is also observed. A doublet at δ 3.01 with a coupling constant of 7.2 Hz can be assigned as this methylene group. The methylene adjacent to the bromide terminus of the alkylating product gives rise to a doublet peak at δ 3.74. Evidence of a roofing effect can be seen with this signal (Figure 4.4). In the aliphatic area of the ^{1}H NMR the protons of the ^{i}butyl side chain of L-leucine emerge. A multiplet integrating as three protons corresponds to the methine and methylene hydrogens of the ^{i}butyl moiety. The methyl groups of the L-leucine side chain result in a doublet signal at δ 0.81, with a coupling constant of 6.8 Hz.

4.2.3 Bromide salt formation

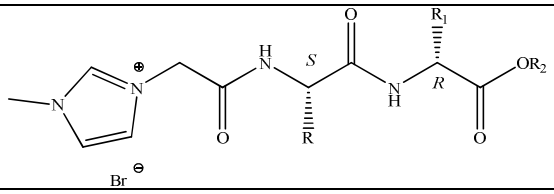
Once the dipeptidyl alkylating intermediate was purified, *N*-alkylation of 1-methyl imidazole was carried out. In this reaction THF was used as the reaction solvent. To a stirring solution of 1-methyl imidazole in THF, the bromo-alkylating reagents were added dropwise (at -15 °C). Stirring at room temperature overnight furnished the end product (i.e. the bromide salt). In many cases, the CIL did not form as a separate phase to the THF. Removal of the reaction solvent *via* rotary evaporation yielded a residue. ^{1}H NMR indicated that this residue contained both the desired CIL and some unreacted imidazolium starting material.

Washing with THF or diethyl ether removed the imidazole starting material. For some examples, the crude residue was dissolved in a small portion of dichloromethane. Washings with small aliquots of water resulted in the pure CIL. The CILs were obtained as a range of liquids, hygroscopic solids and solids. Table 4.4 summarises the yields and melting points of the prepared CILs. All CILs prepared can be classified as ionic liquids as they possess melting point <100 °C, with one exception (Table 4.4, **377** has a melting point of 130-132 °C).

Table 4.4: Dipeptidyl Chiral Ionic Liquids.



R	R_1	R_2	Compound no.	Yield (%)	m.p (°C)
CH_3	CH_3	C_4H_9	**363**	88	60-63
CH_3	CH_2-(C_6H_5)	C_2H_5	**364**	84	nd[b]
CH_3	$CH(CH_3)_2$	CH_3	**365**	97	nd[a]
CH_3	$CH(CH_3)CH_2CH_3$	CH_3	**366**	98	nd[a]
CH_3	$CH_2CH(CH_3)_2$	CH_3	**367**	75	nd[a]
CH_2-(C_6H_5)	$CH(CH_3)_2$	C_2H_5	**368**	96	nd[b]
CH_2-(C_6H_5)	CH_2-(C_6H_5)	C_4H_9	**369**	91	nd[b]
CH_2-(C_6H_5)	CH2CH(CH_3)$_2$	CH_3	**370**	75	nd[b]
CH_2-(C_6H_5)	CH_3	CH_3	**371**	97	nd[b]
CH_2-(C_6H_5)	CH_2-(C_6H_5)	CH_3	**372**	83	97-99
$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	**373**	91	nd[b]
$CH(CH_3)_2$	CH_2-(C_6H_5)	C_2H_5	**374**	98	nd[b]
$CH(CH_3)_2$	CH_3	CH_3	**375**	70	80-82
$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	CH_3	**376**	95	nd[b]
$CH(CH_3)_2$	CH_3	C_2H_5	**377**	95	130-132



R	R_1	R_2	Compound no.	Yield (%)	m.p (°C)
CH_2-(C_6H_5)	CH_2-(C_6H_5)	C_2H_5	**378**	98	93-95
$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3	**379**	93	82-84

a = liquid at RT, b = very hygroscopic, nd = not determined

4.3 NMR studies of dipeptidyl Chiral Ionic Liquids

4.3.1 ^{1}H NMR studies of dipeptidyl chiral ionic liquids

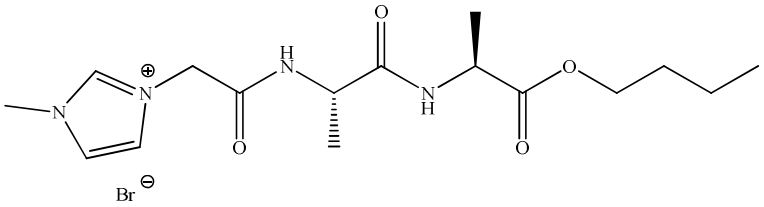
All novel peptidyl chiral ionic liquids (**363**-**379**) were characterised by various spectroscopic methods. ^{1}H NMR experiments were conducted in deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO) as some examples were not soluble in chloroform. In other cases proton NMR was obtained in both solvents in order to investigate solvent effects (in terms of hydrogen bonding behavior of dipeptide species) on NMR spectra. In $CDCl_3$, the amide protons of the dipeptide side chain appear between δ 7.59-9.35. The protons of the imidazolium ring reside in the aromatic region of the ^{1}H NMR spectra. The acidic proton (-NC*H*N-) is observed at ~ δ 9.48-9.87, whilst the methine protons of the ring can be seen at ~ δ 7.22-7.69. The methylene group which connects the chiral side chain to the imidazolium cation can be noted at ~ δ 5.00-5.98. Table 4.5 illustrates selected ^{1}H NMR spectral data of dipeptidyl chiral ionic liquids.

Table 4.5: Selected ^{1}H NMR spectral data (δ, $CDCl_3$) for imidazolium dipeptidyl chiral ionic liquids (**363, 364, 366, 368, 369, 373** and **375**).

Compound	Amide NH's	Acidic imidazolium CH	Imidazolium CH's	Methylene NCH_2
363	8.86, 7.59	9.56	7.61-7.25	5.12
364	8.94, 7.57	9.69	7.46, 7.27	5.66
366	8.69, 8.33	9.07	7.69, 7.66	5.00
368	9.37, 7.45-7.17*	9.45	7.45-7.17*	5.02
369	9.48, 8.12	9.75	7.34-7.10*	5.56
373	9.10, 7.27	9.60	7.59, 7.26	5.65
375	9.07, 7.88	9.87	7.58, 7.22	5.98

*protons appear within a multiplet

4.3.1.1 ^{1}H NMR spectroscopic study of 3-methyl-1-L-alanine-L-alanine butyl ester imidazolium bromide (363)



(**363**)

^{1}H NMR experiments were carried out of 3-methyl-1-L-alanine-L-alanine butyl ester imidazolium bromide (**363**) in both $CDCl_3$ and *d*$_6$-DMSO. The ^{1}H NMR spectrum of the IL differed between the two solvents used. In the ^{1}H NMR spectrum in $CDCl_3$, the acidic proton of the imidazolium ring appears as a singlet peak at δ 9.56. In the spectrum obtained in DMSO this proton also appears as a singlet peak, but more upfield (δ 9.12). The amide protons of the dipeptide side chain appear at δ 8.86 and δ 7.57 in chloroform, with the latter signal overlapping with one of the imidazolium methine peaks. In *d*$_6$-DMSO however the amide protons give distinctive peaks at δ 8.77 and 8.54 respectively. In both cases the amide hydrogens appear as two doublets with coupling constants of 7.2 and 7.6 Hz. Doublet peaks are observed due to coupling of the amide proton with the α-hydrogens at the chiral centres. The methine protons of the imidazolium cation give rise to a set of two triplets both with coupling constants of 1.8 Hz in both spectra. These peaks can be seen at δ 7.61 and 7.25 in the spectrum obtained in $CDCl_3$, whilst they appear at δ 7.74 and 7.73 in *d*$_6$-DMSO. Another very obvious difference between the two spectra is the appearance of the peak due to the methylene group adjacent to the imidazolium core. In the $CDCl_3$ spectrum these protons appear as two doublet peaks at δ 5.52 and δ 5.13, both with coupling constants of 15.6 Hz. In *d*$_6$-DMSO the methylene moiety appears as a singlet peak at δ 5.07 (Figure 4.6). The protons of the chiral centres give overlapping signals at δ 4.47-4.31 in the spectrum obtained in $CDCl_3$. However, when the ^{1}H NMR experiment is carried out in DMSO two doublet of quartet peaks at δ 4.45 and 4.31 can be seen for the chiral protons. Further overlapping is evident for the signals corresponding to the methylene ester protons (OC*H*$_2$) of the butyl chain and the *N*-methyl group of the imidazolium ring in the $CDCl_3$ spectrum. These peaks appear at δ 4.05-3.93 in the proton NMR. In *d*$_6$-DMSO, no

overlapping occurs between these two signals. Two overlapping doublet of quartet peaks at δ 4.13 and 4.12 integrating as two protons is due to the OCH_2 protons and a singlet at δ 3.95 is assigned as the protons of the *N*-methyl group. The protons of the linear butyl ester chain give signals in the aliphatic region of the spectrum. Also evident in this area of the NMR spectrum are signals due to the methyl groups of the L-alanine side chains and the terminal methyl group of the alkyl ester. The L-alanine methyls appear as two doublets with coupling constants of 7.2 and 7.6 Hz at δ 1.37 and 1.33 (in d_6-DMSO solvent). A triplet at δ 0.96 with a coupling constant of 7.2 Hz corresponds to the methyl terminus of the butyl chain.

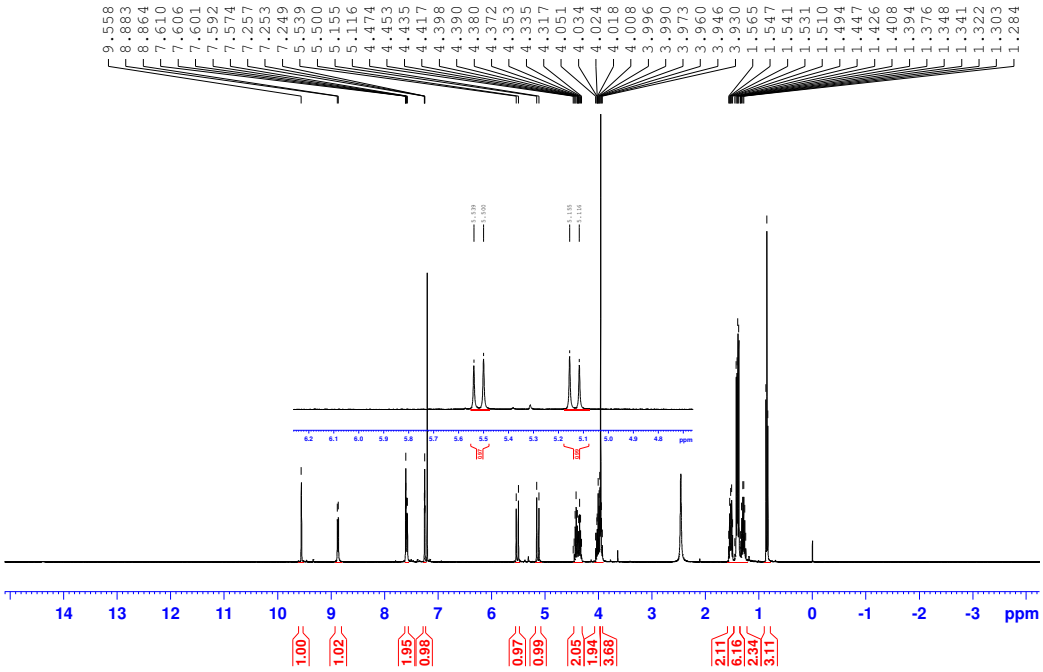


Fig 4.5: ^{1}H NMR spectrum of 3-methyl-1-L-alanine-L-alanine butyl ester imidazolium bromide (**363**) in $CDCl_3$.

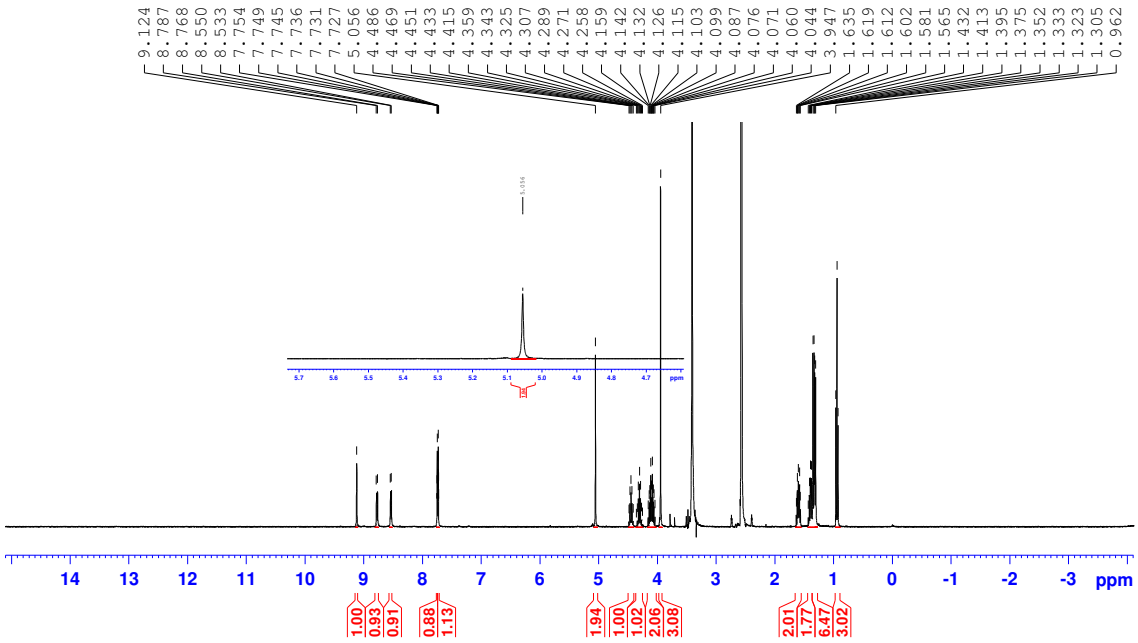
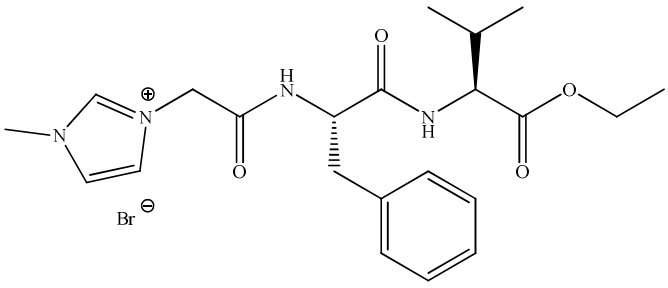


Fig. 4.6: ^{1}H NMR spectrum of 3-methyl-1-L-alanine-L-alanine butyl ester imidazolium bromide (**363**) in d_6-DMSO.

4.3.1.2 ^{1}H NMR spectroscopic study of 3-methyl-1-L-phenylalanine-L-valine ethyl ester imidazolium bromide (368)



(**368**)

In the proton NMR of 3-methyl-1-L-phenylalanine-L-valine ethyl ester imidazolium bromide (**368**) chiral ionic liquid in $CDCl_3$, the acidic proton of the imidazolium cation appears at δ 9.45 as a singlet peak. In the spectrum obtained in d_6-DMSO, this peak can be seen at δ 9.01. The amide protons of the dipeptide moiety appear as two doublets, both with coupling constants of 8.0 Hz at δ 8.75 and 8.49 when the spectrum was acquired in DMSO.

In $CDCl_3$ these protons give a doublet at δ 9.37 and a signal which appears within a multiplet at δ 7.40-7.15. The imidazolium protons (C*H*) are observed in the aromatic region of the NMR spectra. In chloroform, a triplet signal with a coupling constant of 1.6 Hz corresponds to one of the imidazolium methine protons. The other methine group gives a signal which overlaps with the multiplet at δ 7.40-7.15, where the protons of the L-phenylalanine ring also reside. In the spectrum obtained in d_6-DMSO, two triplet peaks at δ 7.66 and 7.54 both with coupling constants of 1.6 Hz can be noted. These peaks are assigned as the imidazolium methine protons. At δ 5.38 and 4.98 two doublets signals arise with coupling constants of 15.2 Hz each and when integrated together give two protons. These signals are due to the methylene group adjacent to the imidazolium ring. A different signal appears in the d_6-DMSO ^{1}H NMR spectrum, where these protons give a doublet at δ 4.93 with evidence of a roofing effect occurring (Figure 4.8). In the same spectrum, a doublet of doublets at δ 4.74 with coupling constants of 8.4 and 4.8 Hz corresponds to the proton of the L-valine chiral centre. At δ 4.17-4.07 a multiplet resides which integrates as three protons. These correspond to the methylene group of the L-valine ethyl ester and the chiral proton of the L-phenylalanine residue. In the ^{1}H NMR spectrum carried out in $CDCl_3$ a multiplet at δ 4.66-4.59 and a doublet of doublets at δ 4.38 are due to the chiral protons of the L-phenyalanine and L-valine groups respectively. The *N*-methyl group of the imidazolium ring gives a singlet peak at δ 3.96 in $CDCl_3$ and at δ 3.86 in d_6-DMSO. In the up-field region of the NMR spectra the methine of the L-valine moiety can be seen at approximately δ 2.05 (DMSO) and δ 2.19 ($CDCl_3$). In both spectra the methyl moiety of the ethyl ester group arises as a triplet at approximately δ 1.20 with a coupling constant of 7.2 Hz. The methyl groups of the branched L-valine side chain give a doublet of doublets at δ 0.89 with coupling constants of 8.4 and 6.8 Hz for each signal (in d_6-DMSO spectrum). A doublet of doublets at δ 0.95 is also evident for these protons in the $CDCl_3$ spectrum.

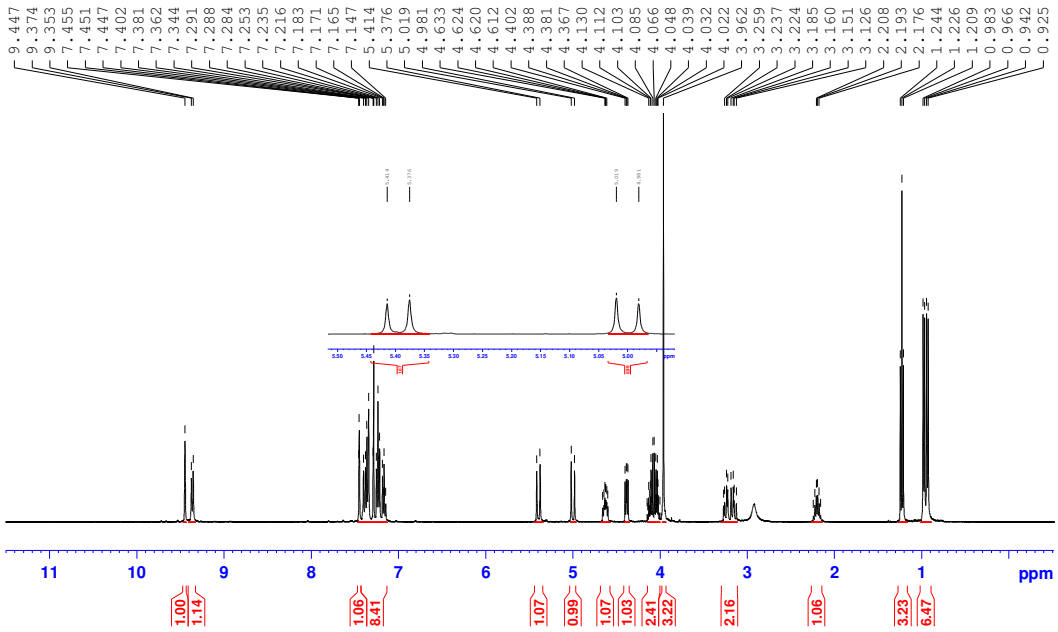


Fig. 4.7: ^{1}H NMR spectrum of 3-methyl-1-L-phenylalanine-L-valine ethyl ester imidazolium bromide (**368**) chiral ionic liquid in $CDCl_3$.

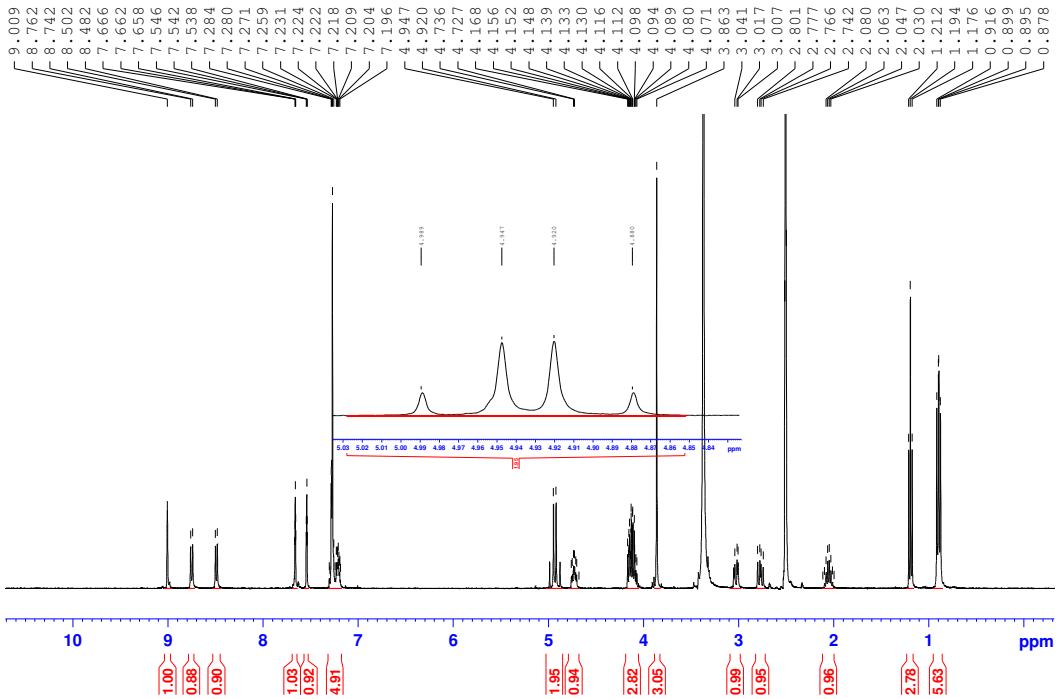


Fig. 4.8: ^{1}H NMR spectrum of 3-methyl-1-L-phenylalanine-L-valine ethyl ester imidazolium bromide (**368**) chiral ionic liquid in d_6-DMSO.

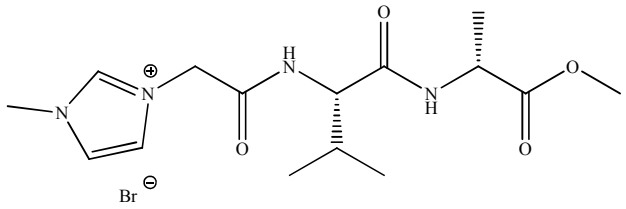
4.3.2 ^{13}C NMR and DEPT spectroscopic studies of dipeptidyl chiral ionic liquids

^{13}C NMR and DEPT 135 studies were also carried out on each of the compounds prepared. The amide and ester carbonyl moieties of the dipeptide side chain appear between δ 173.8 and δ 164.7. In the aromatic region of the ^{13}C spectra the carbons of the imidazolium reside, typically at ~ δ 137.6, 123.8 and 122.4. The *N*-methyl carbons of the imidazolium ring give rise to peaks at ~ δ 35.7-37.7 in the carbon spectra. At ~ δ 50.4-53.6 the signal due to the methylene group between the IL cation and side chain occurs. As expected this peak appears as a negative peak in the DEPT 135 spectra.

Table 4.6: Selected ^{13}C data for dipeptidyl chiral ionic liquids (**365, 369, 370, 371, 372, 374, 375, 376** and **377**).

Compound No.	*C*=O ester	*C*=O amide	N*C*HN Imidazolium	N*C*H imidazolium	N*C*H$_2$ Methylene	N*C*H$_3$ Imidazolium
365	172.9	172.6 164.8	137.8	124.1 122.5	52.0	36.7
369	172.5	171.1 164.7	137.9	123.9 122.2	51.9	36.6
370	173.8	171.5 165.0	137.6	123.8 122.7	51.6	36.7
371	173.7	171.7 164.8	137.1	123.9 122.4	51.8	36.7
372	172.6	171.1 164.9	137.7	123.8 122.5	53.6	37.7
374	172.1	171.3 165.4	137.7	123.9 122.8	51.7	36.7
375	172.8	170.3 164.8	137.7	123.7 122.9	50.4	35.8
376	174.3	171.4 165.0	137.9	124.0 122.4	52.0	36.7
377	172.3	170.3 164.7	137.7	123.7 122.9	50.4	35.8

4.3.2.1 ^{13}C NMR and DEPT 135 spectroscopic study of 3-methyl-1-L-valine-L-alanine methyl ester imidazolium bromide (375)



(**375**)

In the ^{13}C spectrum of 3-methyl-1-L-valine-L-alanine methyl ester imidazolium bromide (**375**) peaks at δ 172.8, 170.3 and 164.8 are evident. These peaks correspond to the amide and ester carbonyls of the dipeptide side chain are their absence in the DEPT 135 spectrum is also notable. The imidazolium carbons occur at δ 137.7, 123.7 and 122.9 in the ^{13}C spectrum. At δ 50.4 the methylene group bridging the IL cation and side chain is observed. Both these signals appear as negative peaks in the DEPT 135 spectrum. The *N*-methyl carbon of the imidazolium can be seen at δ 35.8 in the ^{13}C spectrum and remains as a positive peak in the DEPT NMR. In the most up-field region of the ^{13}C and DEPT spectra signals due to the methyl carbons of the dipeptide arise. At δ 18.9 and 17.9 the methyl carbons of the L-Valine side chain are observed. The methyl carbon of the L-Alanine moiety gives a peak at δ 16.7 in the carbon spectrum.

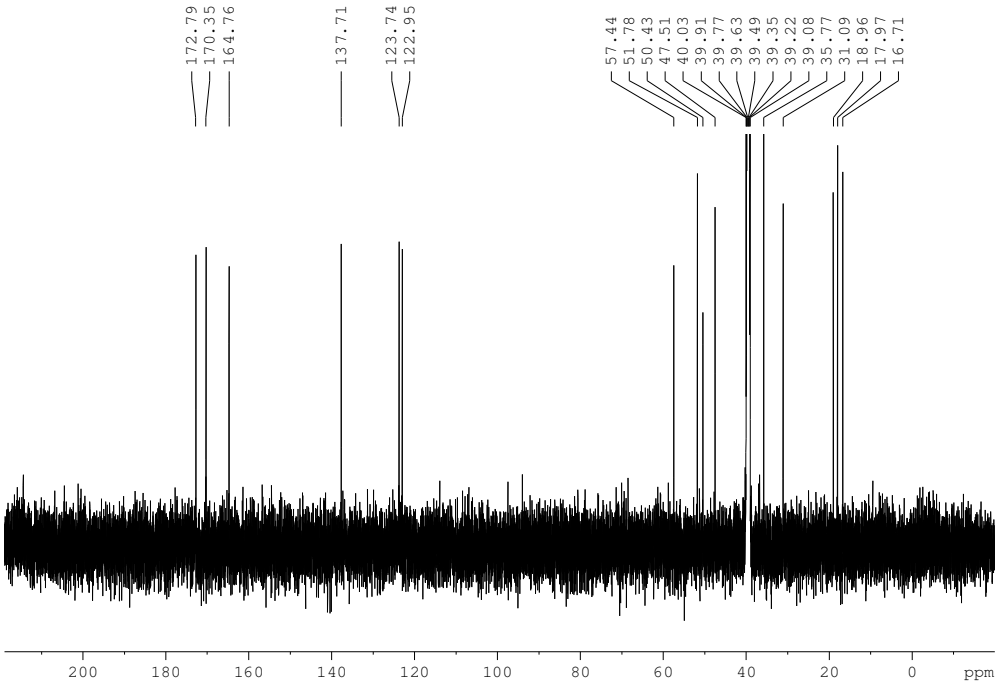


Fig. 4.9: ^{13}C NMR spectrum of 3-methyl-1-L-valine-L-alanine methyl ester imidazolium bromide (**375**).

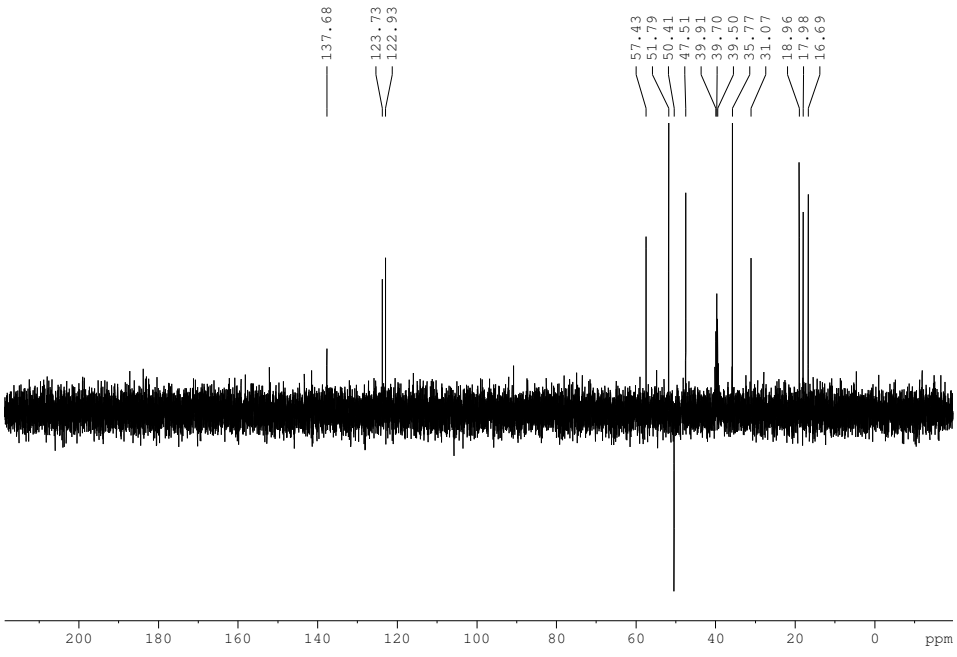


Fig. **4.10**: DEPT 135 spectrum of 3-methyl-1-L-valine-L-alanine methyl ester imidazolium bromide (**375**).

4.3.3 COSY study of 3-methyl-1-L-phenylalanine-L-leucine methyl ester imidazolium bromide chiral ionic liquid (370)

^{1}H-^{1}H correlation techniques (COSY) were used to characterise novel dipeptidyl chiral ionic liquids. In the COSY spectrum of 3-methyl-1-L-phenylalanine-L-leucine methyl ester imidazolium bromide (**370**), the amide proton **7** (δ 9.15) correlates with the methine proton **8** (δ 4.48) at the L-phenylalanine chiral center. This methine group subsequently couples with the methylene protons **9** (δ 3.26-3.08) in the COSY spectrum. The amide proton **17** (δ 7.87) couples with proton **18** at the L-Leucine chiral center (δ 4.50) which further couples to the methylene protons **19** (δ 1.86-1.69). These methylene protons are also seen to correlate with the methine proton **20** (δ 1.61-1.54). Coupling occurs between the imidazolium protons **2** (δ 9.38), **3** and **4** (δ 7.54-7.24) in the COSY spectrum. The protons due to the methylene group **5** (δ 5.42 and 4.95) are also seen to couple with each other (Figure 4.11).

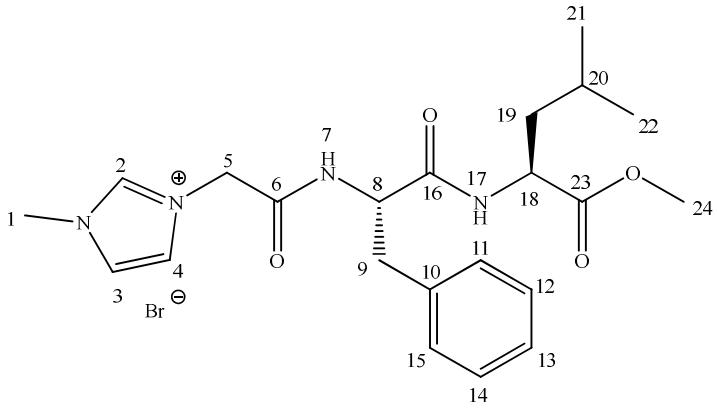


Fig. 4.11: 3-methyl-1-L-phenylalanine-L-leucine methyl ester imidazolium bromide (**370**).

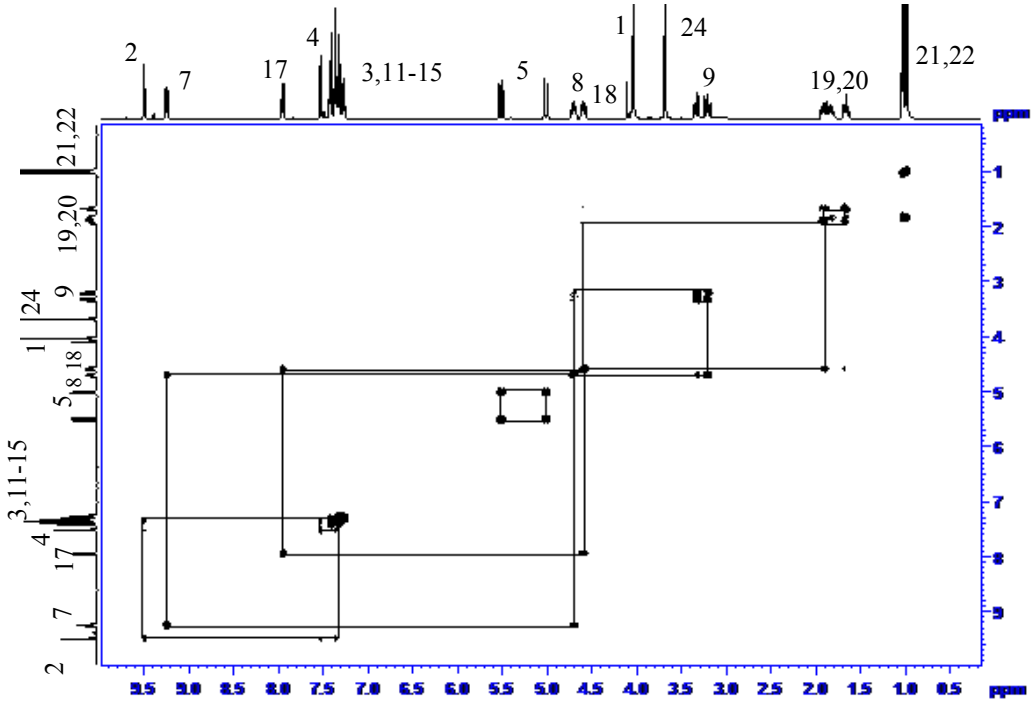


Fig. 4.12: COSY spectrum of 3-methyl-1-L-phenylalanine-L-leucine methyl ester imidazolium bromide (**370**).

4.3.4 HMQC study of 3-methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide Chiral Ionic Liquid (377)

^{1}H-^{13}C two-dimensional NMR experiments were carried out on novel dipeptidyl CILs. The HMQC technique allows for assignment of carbon and proton signals of the compounds.[6] Table 4.7 summarizes the results obtained from the HMQC spectroscopic study of 3-methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide (**377**)

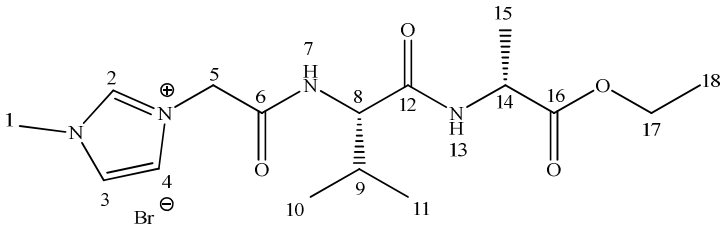


Fig. 4.13: Numbered structure of 3-methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide (**377**).

Table 4.7: C-H correlation data from HMQC spectrum of 3-methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide (**377**).

Site	^{1}H NMR	^{13}C NMR	HMQC
1	3.95		35.78
2	9.16		137.70
3	7.74		122.95
4	7.75		123.73
5	5.14		50.44
6		164.76	
7			
8	4.32		57.45
9	2.07		31.07
10	0.94/0.97		17.99/19.01
11	0.94/0.97		17.99/19.01
12		170.29	
13			
14	4.28		47.62
15	1.33		16.72
16		172.79	
17	4.16-4.10		60.41
18	1.24		13.98

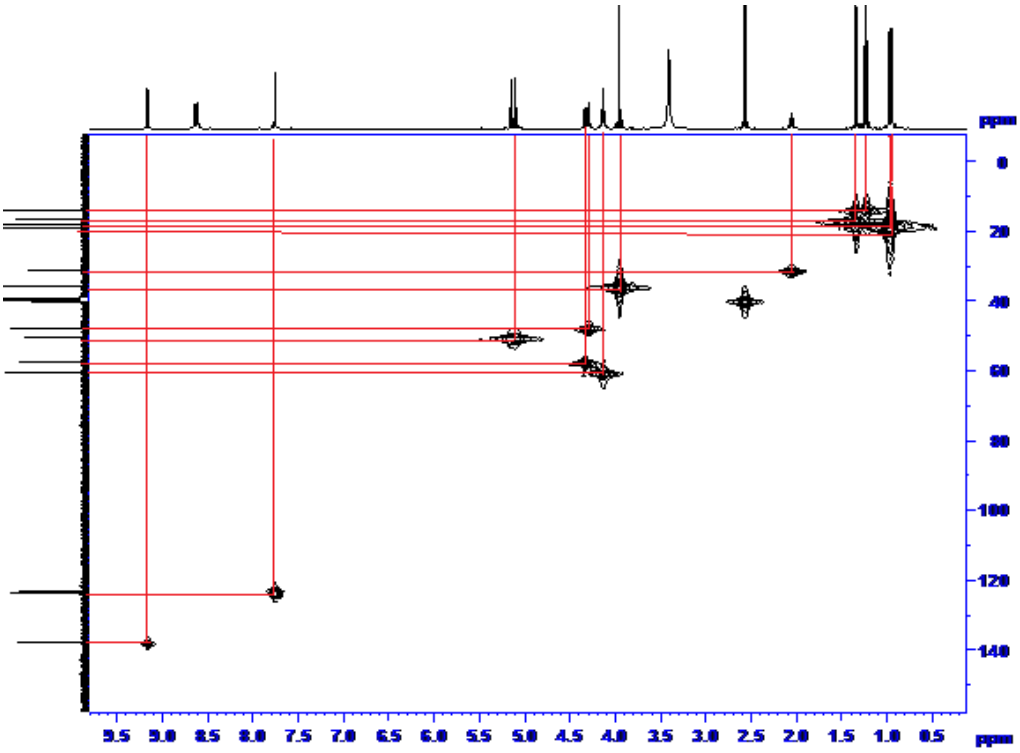


Fig. 4.14: HMQC NMR spectrum of 3-methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide (**377**).

4.4 Infra red spectroscopic studies of dipeptidyl chiral ionic liquids

The presence of different functional groups within a structure can be identified by IR spectroscopy. At certain wavelengths molecular vibrations of various functional groups can be induced, after IR radiation.[7] In the IR spectra dipeptidyl chiral ionic liquids, the amide N-H appears as two bands above 3000 cm^{-1}. The amide carbonyl stretching vibration band (Amide I) and the localized N-H bending vibration (Amide II) can be seen between 1660 and 1530 $cm^{-1.}$ The ester group can be observed at 1740 cm^{-1} in the IR spectrum, and C-O stretches give rise to bands at 1170 $cm^{-1.}$

Table 4.8: IR frequencies of dipeptidyl chiral Ionic Liquids (cm^{-1}).

Compound no.	N-H	C=O amide I and II	C=O Ester	C-O ester
378	3210	1658,1534	1730	1172
367	3236	1660,1543	1738	1172
379	3278	1640,1553	1727	1266,1173
365	3220	1661,1534	1737	1206,1172
376	3220	1656,1533	1739	1205,1171
364	3187	1651,1532	1751	1175
375	3281	1637,1553	1731	1171

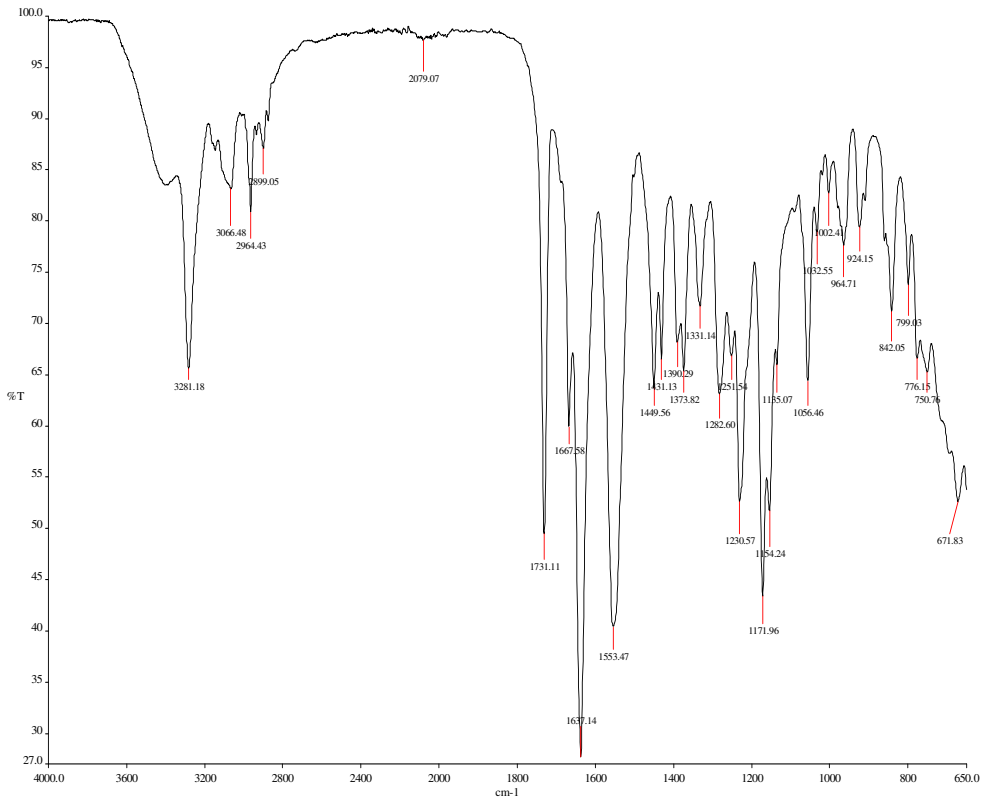


Fig 4.15: IR spectrum of 3-methyl-1-L-valine-D-valine methyl ester imidazolium bromide (**379**).

4.5 Mass spectrometric studies of dipeptidyl chiral ionic liquids

Mass spectrometry (MS) is a frequently used analytical technique for the determination of relative molecular masses of a molecules.[8] A mass spectrometer consists of three parts, namely an ion source, mass analyser and a detector. In the ion source the sample is converted into ions, the ions are then sorted by their mass/charge ratios (*m/z*). The separated ions are detected and subsequently displayed as the mass spectrum.

Electrospray Ionisation (ESI) mass spectrometry was employed to analyse dipeptidyl chiral ionic liquids. ESI is an Atmospheric Pressure Ionisation (API) technique which can be used to analyse polar molecules.[8] The sample (in a polar volatile solvent) is introduced to the ion source through a narrow stainless steel capillary, which is surrounded by a nebulising gas (nitrogen). A high voltage is applied to the tip of the capillary. As the sample solution exits the capillary, an aerosol is formed composed of charged droplets. The charged droplets are directed to the mass spectrometer by a flow of nebulising gas. Droplets in the aerosol diminish in size as the solvent evaporates hence concentrating the charged ions. An electrostatic repulsion occurs between these ions and the droplet undergoes a 'Coulombic explosion'. The sample ions are then released into a vapour phase and are then directed to the mass analyser using various sampling orfices.[8] In the ESI-MS spectrum of the 3-methyl-1-L-valine-L-phenylalanine ethyl ester imidazolium cation, the molecular ion $[M]^+$ is noted at *m/z* 415 (Figure 4.16). This ion was further fragmented using MS/MS in order to study any possible sequence specific fragment ions.

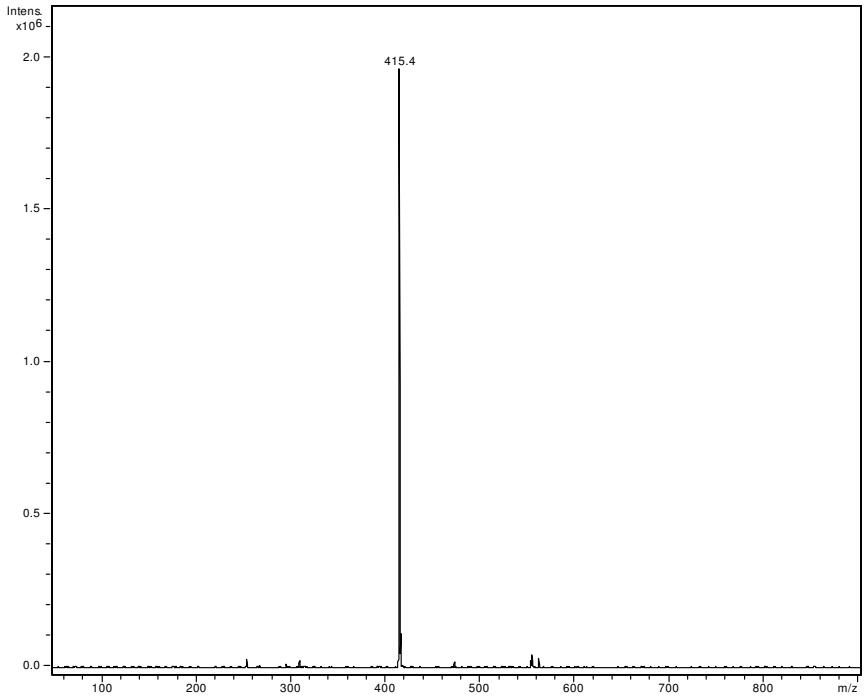


Fig. 4.16: ESI mass spectrum of 3-methyl-1-L-valine-L-phenylalanine ethyl ester imidazolium bromide (**374**).

MS/MS (or Tandem MS) is commonly used for peptide identification. Peptidyl species can be elucidated by fragmentation in the mass spectrometer. When peptides collide with a gas within the MS they can dissociate into fragments at their peptide bonds.[8] Peptidyl fragmentation results in the formation of *b* and *y* ions which correspond to the residue masses of the respective amino acids. A nomenclature system has been developed for identifying peptide fragments that result from a MS/MS spectrum.[9,10] This system distinguishes fragment ions according to the amide bond that fragments and also the terminal of the peptide that retains a charge after the fragmentation process. Fragmentation can occur at three different bonds along the amino acid sequence, namely at the NH-CH, CH-CO and CO-NH bonds (Figure 4.17). Peptidyl fragment ions are indicated by *a*, *b* or *c* if the charge is retained on the N-terminal fragment, and by *x*, *y* or *z* if the charge is maintained on the C-terminal fragment (the subscript indicates the number of amino acids in the fragment).[9]

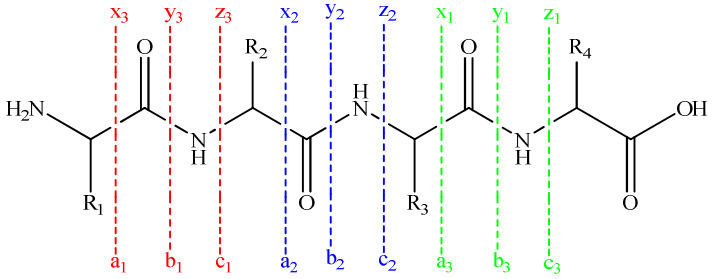


Fig. 4.17: Peptide fragmentation nomenclature.[9]

In the MS/MS spectrum of 3-methyl-1-L-valine-L-phenylalanine ethyl ester imidazolium bromide (Figure 4.18), an intense peak at *m/z* 416 is present. This mass is attributed to the $[M+H]^+$ ion. A peak of largest intensity is observed at *m/z* 194, which represents a *b* ion fragment (i.e. the L-phenylalanine ethyl ester $[PheOEt]^+$, Figure 4.18).

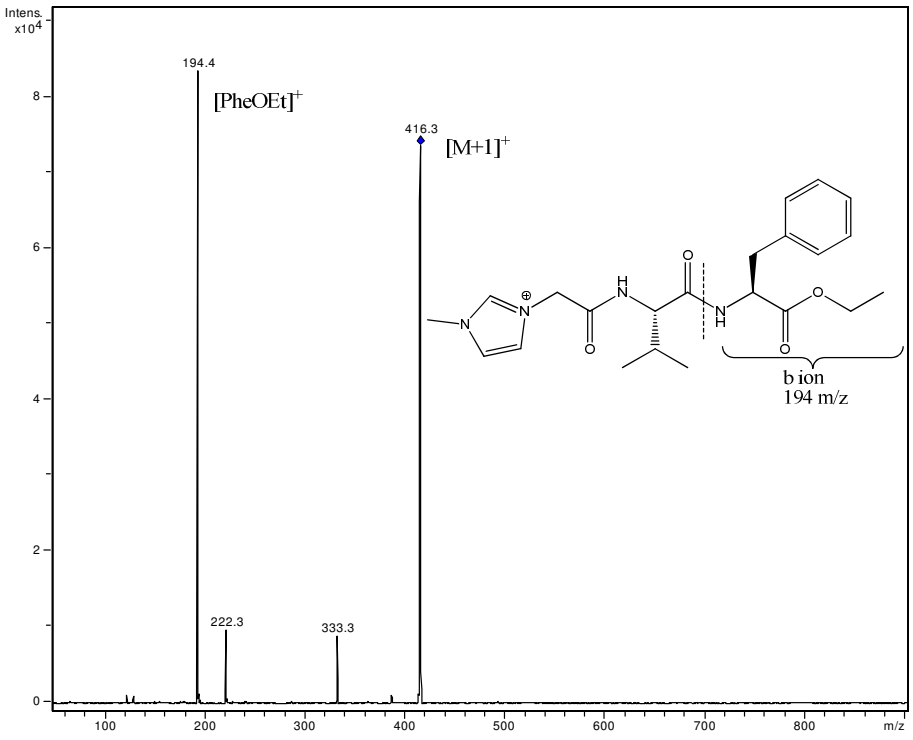
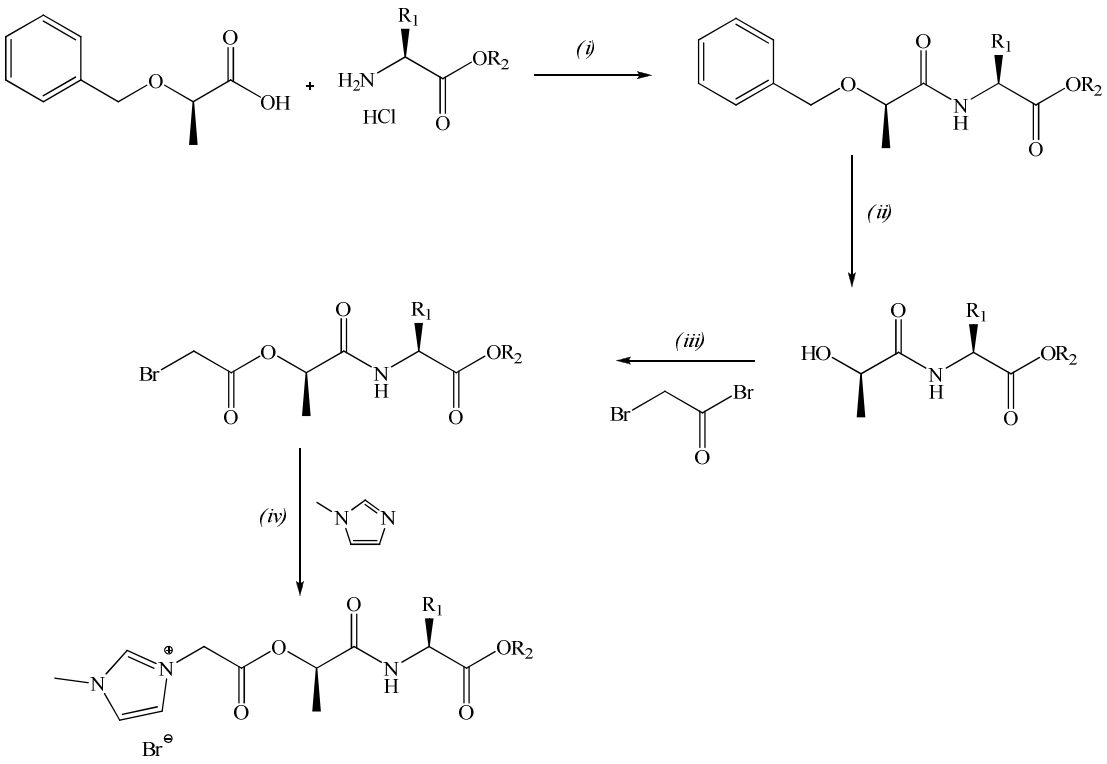


Fig. 4.18: MS/MS spectrum of 3-methyl-1-L-valine-L-phenylalanine ethyl ester imidazolium bromide (**374**).

4.6 Synthesis of Lactate-peptidyl Chiral Ionic Liquids

Manipulation of the dipeptidyl side chain of the chiral ionic liquids was also attempted. In these examples, one of the peptides was replaced with a lactate moiety. By doing this, the order of the chiral fragment was altered so as to obtain an ester-amide-ester sequence. The former ester functionality originated from the lactate group. Coupling of the benzyloxy protected lactate with an amino acid ester, using EDC/HOBt, yielded the peptidyl fragment. A four step synthetic route was thus employed to prepare these derivatives (Scheme 4.2).



Scheme 4.2: Lactate-peptidyl Chiral Ionic liquid synthesis. (i) EDC, HOBt, Et_3N (ii) 5% Pd/C, H_2 (iii) Bromoacetyl bromide, K_2CO_3 (iv) 1-methyl imidazole.

The first step in the preparation of these chiral ILs, involved a peptide coupling reaction between *R*-(benzyloxy)-Lactic acid and various amino acid esters. Peptide bond formation was achieved using the standard EDC/HOBt coupling protocol (as described in Section 4.2). Column chromatography was employed to purify the crude reaction products (mobile phase 50:50 hexane:ethyl acetate). The coupling products were obtained in good yields (Table 4.9).

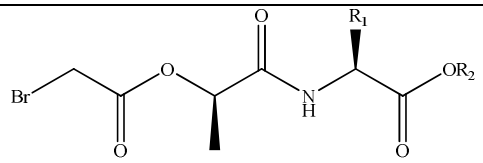
Table 4.9: Lactate-peptides prepared.

Compound	Compound no.	Yield (%)
Benzyloxy-R-lactate- L-alanine methyl ester	**380**	83
Benzyloxy-R-lactate- L-alanine ethyl ester	**381**	81
Benzyloxy-R-lactate- D-alanine methyl ester	**382**	68
Benzyloxy-R-lactate- L-phenylalanine methyl ester	**383**	98
Benzyloxy-R-lactate- L-phenylalanine ethyl ester	**384**	61

Following these coupling reactions, the benzyloxy protecting group was removed from the peptidyl fragment. Cleavage of these protecting groups is carried out under hydrogenation conditions, using 5 % Pd/C catalyst. The reaction was monitored by TLC (50:50 hexane:ethyl acetate) and completion times varied between 3 to 5 days. When the reaction reached completion, the catalyst was removed by filtration.

Bromoalkylating reagents were prepared in the following stage of the CIL synthesis. The experimental procedure used differed to that employed for the dipeptidyl derivatives. In these reactions, the *R*-Lactate-peptidyl products (obtained from hydrogenation reaction) were stirred in DCM, with K_2CO_3 (Et_3N was the base used previously in the dipeptidyl derivatives) at 0 °C and bromoacetyl bromide was added dropwise. The reaction was monitored by TLC, with extra equivalents of bromo acetyl bromide required to force the reactions to completion. There are a number of advantages in using this method. In this method the K_2CO_3 base is present as a separate phase to the reaction mixture, hence allowing for facile removal (i.e by filtration). Also, in using this reaction, compared to Et_3N method, less reaction impurities may arise. In some cases when using Et_3N as the reaction base, deprotonation of the α-methylene protons adjacent to the bromine of bromoacetyl bromide can occur. This leads to the formation of side products and impurities. The reaction products typically required purification before being used in the final step of the CIL synthesis. Column chromatography (60:40 hexane:ethyl acetate) furnished the desired chiral α-bromo esters in moderate yields (Table 4.10).

Table 4.10: Lactate-peptidyl alkylating intermediates.



R_1	R_2	Compound No.	Yield (%)
CH_3	CH_3	**385**	73
CH_3	C_2H_4	**386**	54
CH_3*	CH_3	**387**	35
CH_2-(C_6H_5)	CH_3	**388**	37
CH_2-(C_6H_5)	C_2H_4	**389**	55

* Amino acid in D enantiomeric form

Figure 4.19 represents an example of a ^{1}H NMR spectrum obtained for purified *R*-Lactate-L-Alanine ethyl ester bromoacetate alkylating intermediate. A doublet peak resides at δ 6.71 with a coupling constant of 6.8 Hz and corresponds to the amide proton of the amide bond. At δ 5.22, a quartet with a coupling constant of 6.8 Hz appears. This signal is due to the chiral methine proton of the *R*-lactate chiral centre. The proton adjacent to the amide bond (of the L-alanine fragment) gives a peak at δ 4.50 as a doublet of quartets. A quartet at δ 4.15, with an integration of two protons, correlates to the methylene of the ethyl ester group. The methylene group adjacent to the bromo terminus gives rise to two doublets mutually coupled at δ 3.80 and 3.90. The methyl groups then appear upfield in the spectrum. Two doublet peaks at δ 1.45 and 1.36 correspond to the methyl groups of the lactate and amino acid respectively. A triplet at δ 1.23 represents the methyl protons of the ethyl ester side chain.

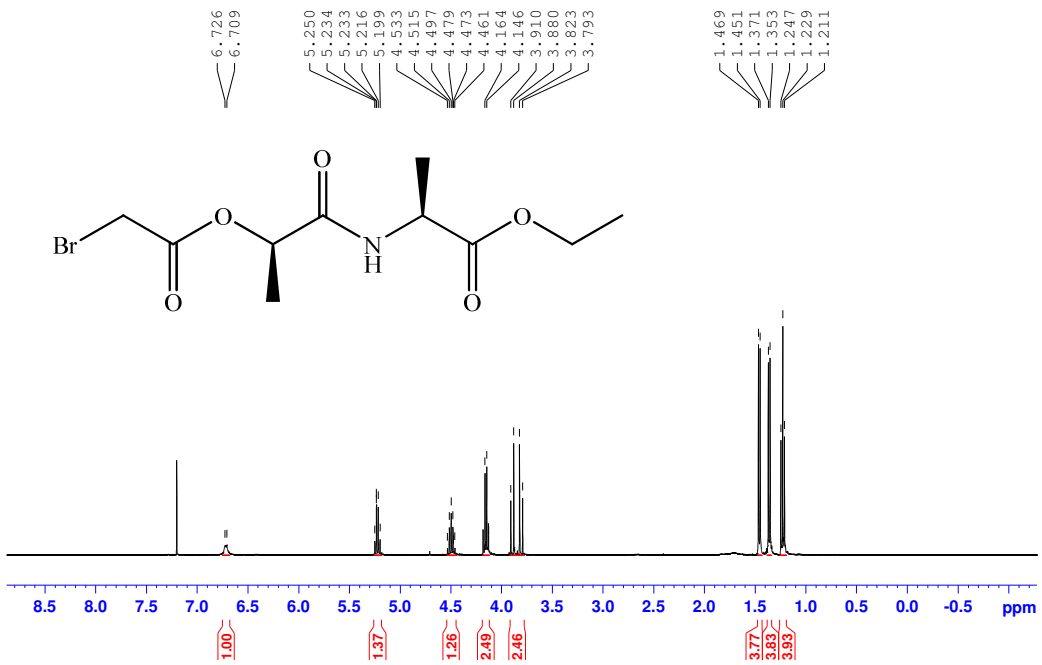


Fig. 4.19: ^{1}H NMR of purified *R*-lactate-L-alanine ethyl ester bromoacetate alkylating intermediate (**386**).

After successful purification of the above intermediates, the next step required reacting them with 1-methyl imidiazole to yield to final CIL product. These reactions were carried out as before, by adding the chiral α-bromo esters to 1-methylimidazole in THF. The CILs typically precipitated out of solution, therefore the THF could be decanted off. ^{1}H NMR analysis of these reaction products indicated high levels of impurities, in particular imidazolium based contaminants were noted. Ether washings were carried out initially, as this has previously been used to remove imidazolium impurities from ionic liquids. Up to ten 20 mL aliquots of ether were used in this washing process, and some washing sequences were left overnight. THF washes were also adapted as these imidazolium impurities are more soluble in this solvent. However, this failed to remove the imidazolium contamination. A number of purification techniques were then attempted in order to remove the evident impurities. Column chromatography using DCM: Methanol was used. Lower percentages of methanol were used initially in the mobile phase system, however a final ratio of 90:10 DCM:methanol was required to move the compound along the stationary phase. The column chromatography separation proved unsuccessfully, from the ^{1}H NMR

analysis of the column product, it was clear that the chiral product had broke down on the stationary phase (Figure 4.20 (a) and (b)).

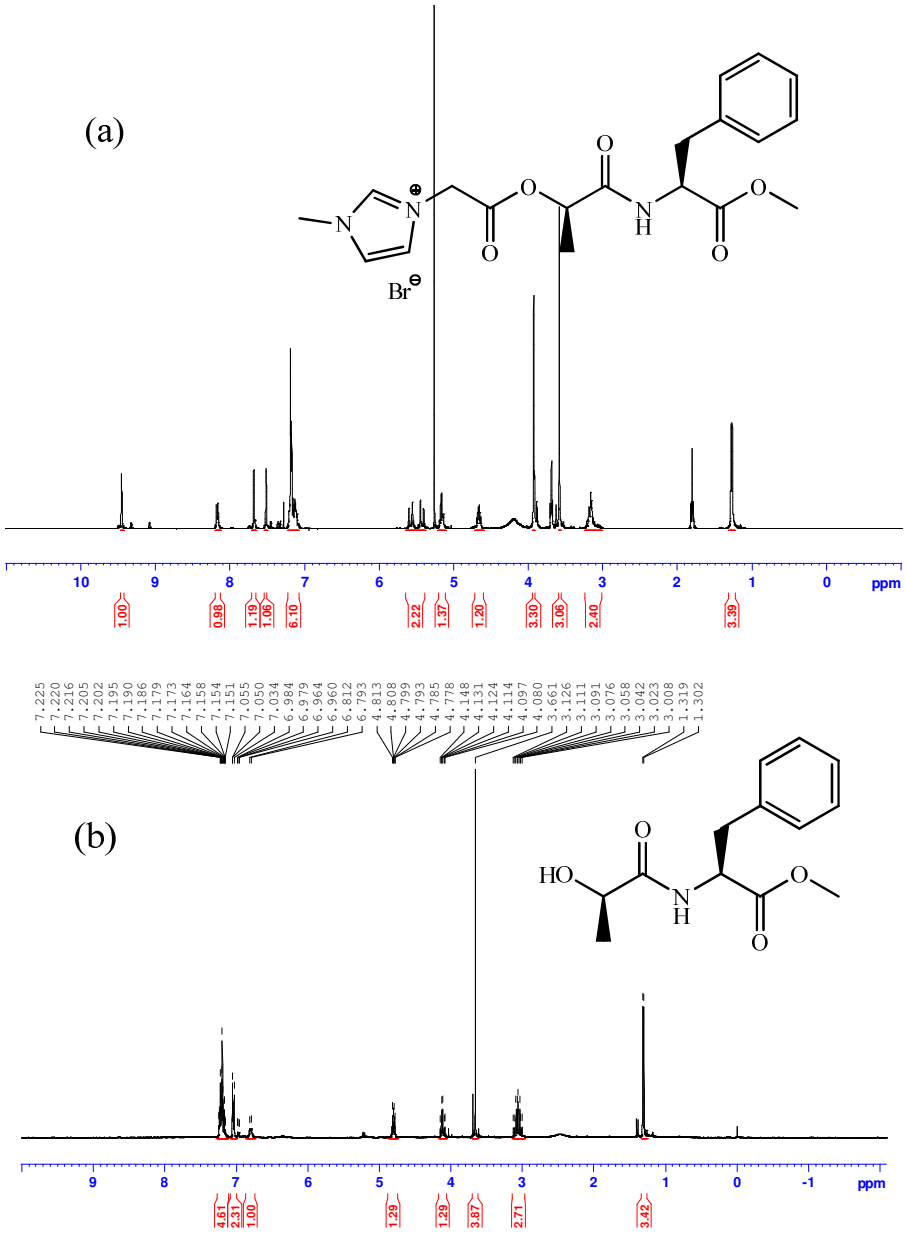
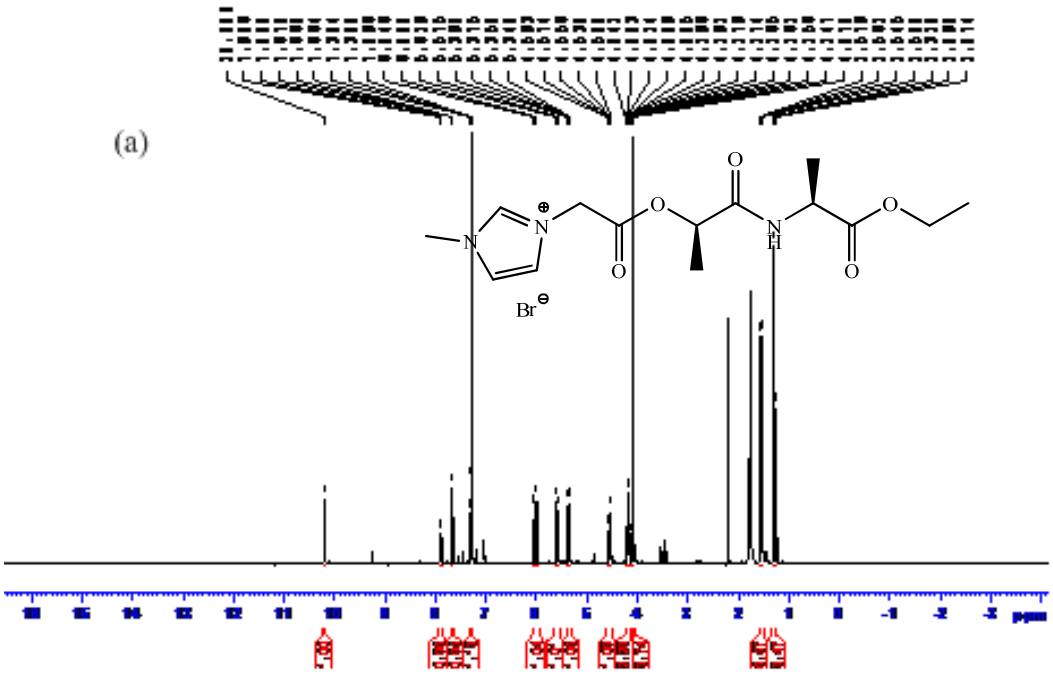


Fig. 4.20: (a) ^{1}H NMR spectrum of crude 3-methyl-1-*R*-lactate-L-phenylalanine methyl ester imidazolium bromide (**390**); (b) ^{1}H NMR spectrum of 3-methyl-1-*R*-lactate-L-phenylalanine methyl ester imidazolium bromide product following column chromatography.

It is noteworthy from the ^{1}H NMR data, the absence of the imidazolium protons from the product obtained from the column. This would suggest that cleavage occurred at the chiral ester side chain of the CIL.

Column chromatography using 90:10 DCM: ethanol was also attempted in isolating the desired chiral ionic liquids. However this gave the same result as using the previous mobile phase systems. Preparative Thin Layer Chromatography (Prep TLC) was also trialled as a means of product purification and also proved unsuccessful. Recrystalisation could not be performed on the CILs as they were all present in a liquid or oil form. It was also found that when the CILs were left for a period of time, even during refrigeration, they continued to degrade. Figure 4.21 illustrates a ^{1}H NMR spectrum of 3-methyl-1-*R*-lactate-L-alanine ethyl ester imidazolium bromide obtained initially (a) and after two weeks (b)



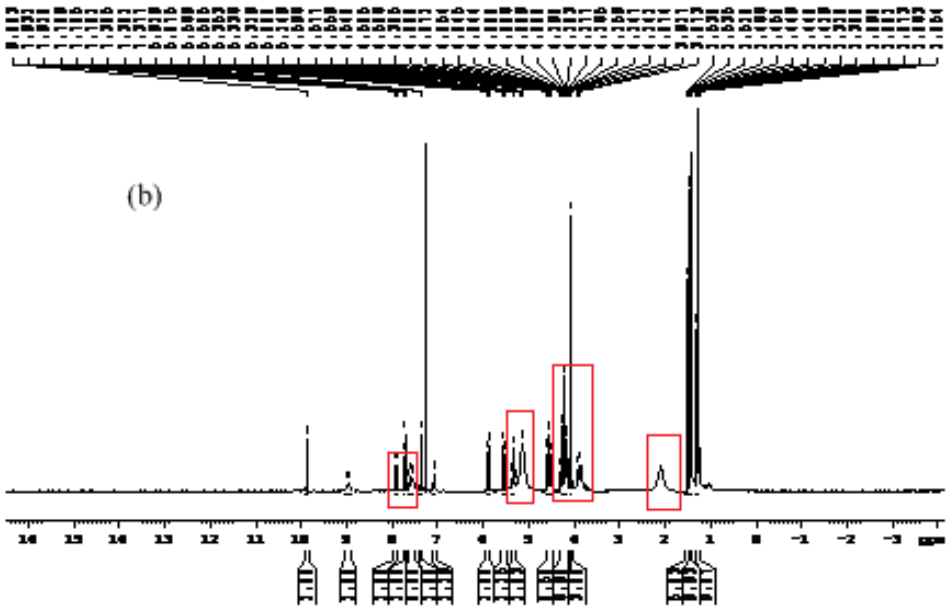


Fig. 4.21: (a) ^{1}H NMR spectrum of 3-methyl-1-*R*-lactate-L-alanine ethyl ester imidazolium bromide (**391**) obtained initially and (b) degradation observed after two weeks (stored at -4 °C); the peaks in the highlighted areas indicate presence of degraded product.

One example, however, was successfully obtained in its pure form and could be fully characterized. 3-Methyl-1-*R*-lactate-L-alanine methyl ester imidazolium bromide was synthesized as a white solid, and from the ^{1}H NMR spectrum, did not require further purification (Figure 4.22). The purity of this CIL compared to the other examples may be explained in terms of the difference in physical states observed for the CILs. 3-Methyl-1-*R*-lactate-L-alanine methyl ester imidazolium bromide was attained in a solid state, whilst the other CILs were present as viscous liquids. The increase in viscosity of the CILs can allow for impurities to become trapped and hence more cumbersome to remove from the CIL.

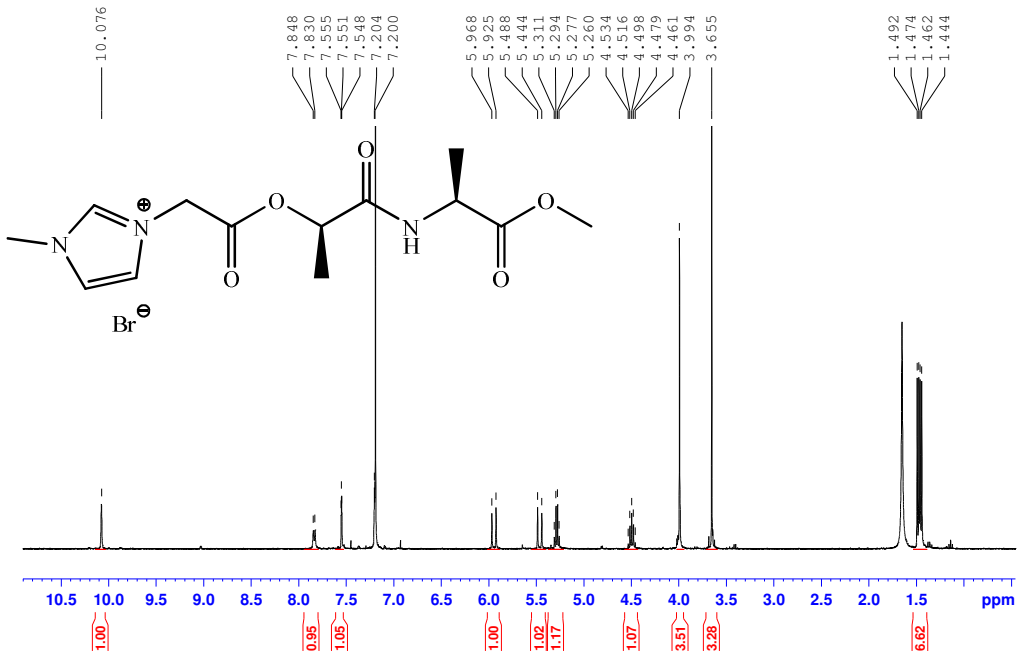


Fig. 4.22: ^{1}H NMR spectrum of 3-methyl-1-*R*-lactate-L-alanine methyl ester imidazolium bromide (**392**).

In the ^{1}H NMR spectrum of 3-methyl-1-*R*-lactate-L-alanine methyl ester imidazolium bromide (Figure 4.22), the acidic proton of the imidazolium cation is noted at δ 10.08 as a singlet peak. The protons of the amide bond, which links the lactate and amino acid moieties, gives rise to a doublet at δ 7.84, with a coupling constant of 7.6 Hz. These protons evidently couple with the adjacent methine group of L-alanine, which appear as a doublet of quartet signal with coupling constants of 7.2 Hz. At δ 7.55 and 7.20 the methine protons of the imidazolium ring reside as triplet signals, with the latter overlapping with the residual solvent ($CHCl_3$) peak. The bridging methylene group between the imidazolium cation and the chiral side chain appears as a set of peaks between δ 5.94 and 5.46. A singlet peak at δ 3.99 which integrates as three protons, corresponds to the *N*-methyl protons of the imidazolium cation. Another singlet appears as δ 3.65 which can be assigned as the protons of the methyl ester of the L-alanine side chain. At the most upfield region of the ^{1}H NMR spectrum, the protons of the lactate and alanine methyl moieties reside as doublets, with coupling constants of 7.2 Hz.

4.7 Conclusions

A series of dipeptidyl chiral ionic liquids (**363**-**379**) were successfully designed and synthesised. 17 dipeptidyl bromo-alkylating intermediates (**346**-**362**) were prepared from the corresponding dipeptides. Subsequent formation of 17 bromide salts from these chiral intermediates was achieved. Various protecting group and peptide chemistry was employed in this synthetic route. All novel chiral ILs were successfully characterised *via* a range of spectroscopic techniques, namely ^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC, IR and MS (MS/MS). By preparing these ILs, various functionalities were successfully introduced onto the cation scaffold. Furthermore, addition of peptide moieties in the IL structure may contribute to biodegradability. The biodegradation of CIL **378** was further studied using an activated sludge assay (Chapter 6). All dipeptidyl CILs were also screened against a range of bacterial and fungal strains (Chapter 5). A series of chiral ester ILs was also attempted, using Lactic acid and peptide precursors. 5 chiral bromoesters were successfully prepared (**385**-**389**). The chiral ester imidazolium bromide salts prepared were unstable to column chromatography conditions and evidently decomposed further under storage conditions (-4 °C). However one bromide salt (**392**) was successfully synthesised from the intermediates and fully characterised *via* the aforementioned spectroscopic techniques.

4.8 References

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Chapter 5: Results and discussion

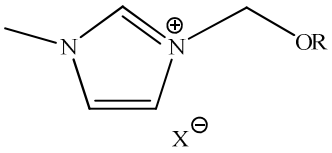
Toxicity studies of (a)chiral ionic liquids

5.1 Introduction

The terms 'green' and 'benign' have been used *ad infinitum* to describe ionic liquids in the literature, with their negligible vapour pressures and flammabilities lending to these classifications. Recently however, ionic liquid researchers have begun to employ this terminology with caution. In order to confidently label ILs as 'green', their environmental impact must be known. Since many ILs are water soluble, the potential for their release into the environment *via* wastewater effluents becomes a major concern. As a consequence, a combination of toxicity, biodegradation and bioaccumulation data for these compounds is pertinent. Environmental hazard assessment of ILs has now become an important area of research with many groups reporting toxicity, ecotoxicity and biodegradation studies on ILs. The toxicity of ILs has been evaluated in numerous biological systems including; antimicrobial studies on bacterial and fungal strains, acute toxicity studies on terrestrial invertebrates[1] (e.g. earthworms), aquatic species (zebrafish[2] (*Danio rerio*)), waterfleas[3] (*Daphnia magna*), algae and terrestrial plants.[4] In the following sections, the toxicological assessment of ILs will be introduced and discussed, with particular focus on antibacterial, antifungal and antibiofilm studies on novel ILs.

5.1.1 Antibacterial and antifungal studies of ILs

The antimicrobial activities of a series of 3-alkoxymethyl-1-methylimidazolium ionic liquids (Figure 5.1) were evaluated by Pernak *et al*.[5] These ILs were tested against various microbial strains, namely rod (*Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*) and cocci (*Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Enterococcus hirae*) shaped bacteria and fungal isolates (*Candida albicans*, *Rhodotorula rubra*).

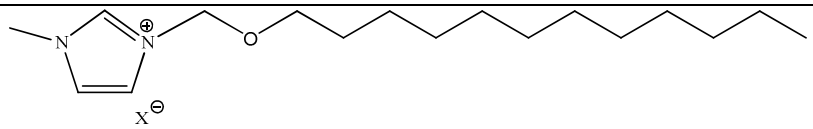


	X =		
R =	Cl	BF_4	PF_6
CH_3	**393**	**408**	**424**
C_2H_5	**155**	**409**	**425**
C_3H_7	**394**	**410**	**426**
C_4H_9	**395**	**411**	**427**
C_5H_{11}	**396**	**412**	**428**
C_6H_{13}	**397**	**413**	**429**
C_7H_{15}	**398**	**414**	**430**
C_8H_{17}	**399**	**415**	**431**
C_9H_{19}	**400**	**416**	**432**
$C_{10}H_{21}$	**401**	**417**	**433**
$C_{11}H_{23}$	**402**	**418**	**434**
$C_{12}H_{25}$	**403**	**419**	**435**
$C_{13}H_{27}$	**404**	**420**	**436**
$C_{14}H_{29}$	**405**	**421**	**437**
$C_{15}H_{31}$	**406**	**422**	**438**
$C_{16}H_{33}$	**407**	**423**	**439**

Fig. 5.1: 3-Alkoxymethyl-1-methylimidazolium ionic liquids screened by Pernak.[5]

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) were reported for the ILs and also for Benzalkonium chloride (BAC), a commonly used ingredient in biocides.[5] The most active IL was that which contained a dodecyl alkoxy side chain. All three salts (**403**, **419** and **435**) containing this cation approached the activity of BAC (Table 5.1)

Table 5.1: MIC (μM) values of 3-dodecyloxymethyl-1-methylimidazolium vs. BAC.[5]



Strain	X = Cl	X = BF_4	X = PF_6	BAC
	403	**419**	**435**	
M. Luteus	25	21	37	7
S. epidermidis	25	21	18	3
S. aureus	25	21	18	7
MRSA	99	85	73	7
E. hirae	99	85	37	11
E. coli	99	170	73	7
P. vulgaris	197	170	147	22
K. pneumonia	197	170	147	11
P. aeruginosa	395	340	587	54
C. albicans	197	340	587	7
R. rubra	45	21	37	11

In a later study, the same research group reported the antimicrobial activities of 1-alkylimidazolium and 1-alkoxymethyl imidazolium lactate ILs (Figure 5.2).[6] A range of bacterial strains were challenged against the ILs; *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Serratia marcescens* (Rods), *Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Enterococcus hirae* (cocci) and fungal isolates *Candida albicans*, *Rhodotorula rubra*.

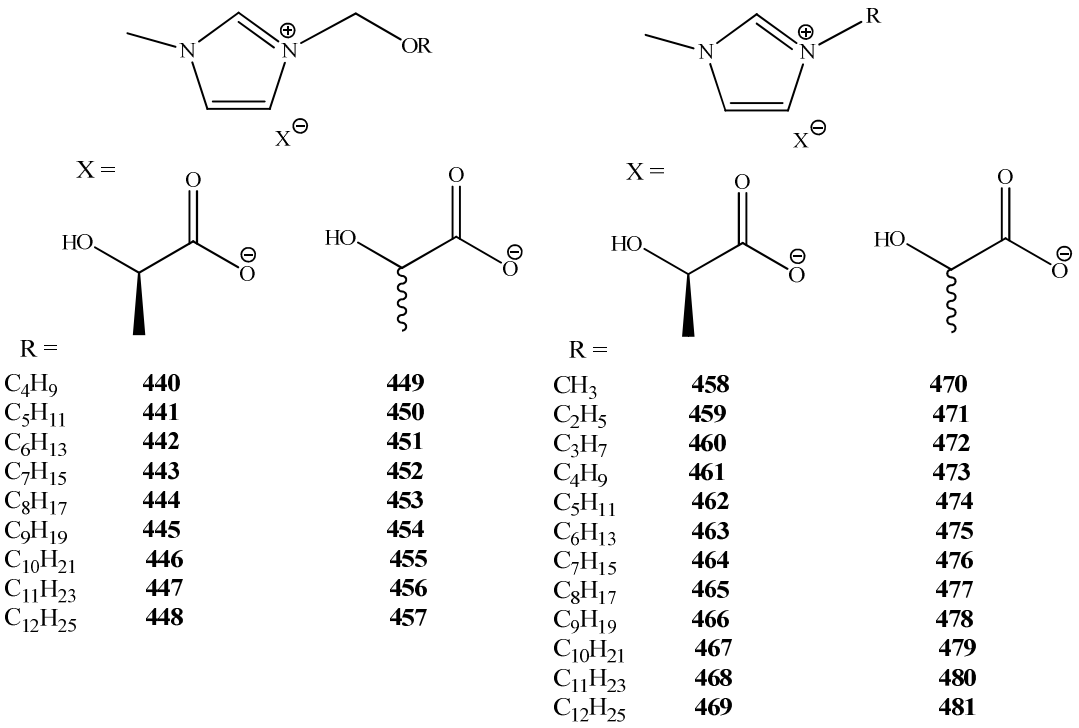


Fig. 5.2: 1-Alkoxymethyl and 1-alkylimidazolium imidazolium lactate ILs.

It was generally observed that the L-lactate ILs displayed the highest anti-microbial activity (lower MIC values) than the DL-lactate derivatives. Additionally, L-lactates with undecyl and dodecyl alkyl side chains proved most toxic towards the tested microorganisms. MIC and MBC values reported for these ILs were similar for those obtained for BAC. Shorter alkyl chain salts typically proved inactive towards the microbes.

The growth of three microorganisms *Escherichia coli*, *Pichia pastoris* and *Bacillus cerieus*, in the presence of [bmim][BF_4] and [PF_6] was studied by Bornscheuer.[7] These commonly used ILs gave similar inhibitory effects as several organic solvents (e.g. ethanol, methanol and dimethylsulfoxide) against the tested bacterial strains.

In 2009 anti-bacterial quaternary ammonium cations (QACs) were combined with artificial sweetener anions, namely acesulfamates and saccharinates, to form a new class of ILs[8] (Figure 5.3). These dual function salts were screened for antimicrobial activities.

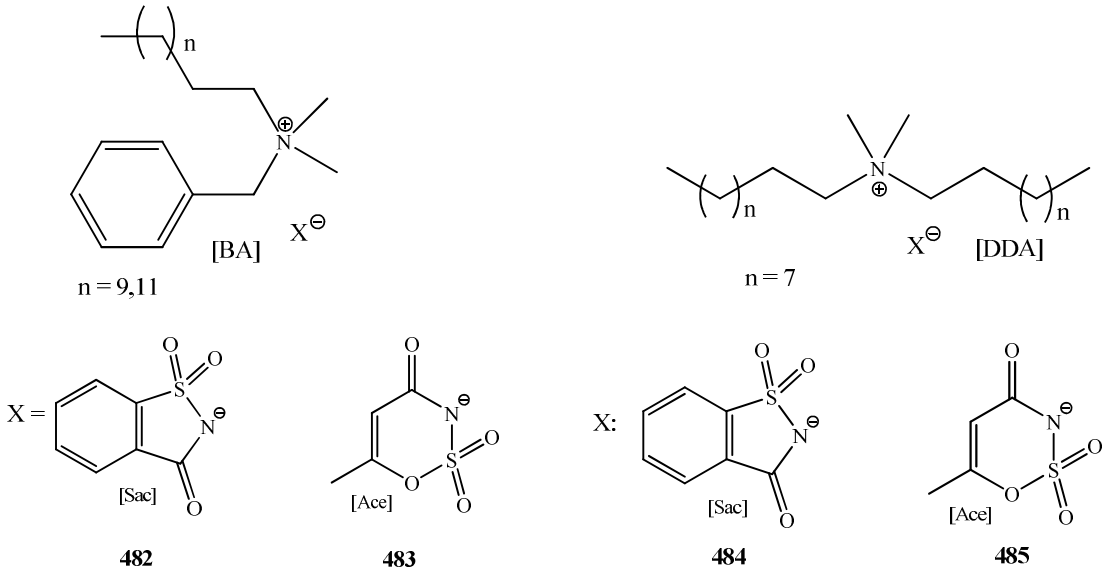


Fig. 5.3: QAC based ILs.[8]

Toxicity of these QAC ILs (**482**-**485**) was assessed against various bacterial strains; *Staphylococcus aureus*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Enterococcus faecium*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, *Streptococcus mutans* and fungi *Candida albicans*, *Rhodotorula rubra*,. MIC and MBC values were reported for the ILs, and also for the commercially available staring materials BAC and didecyldimethylammonium chloride (DDAC). From this data (Table 5.2 and 5.3) it was clear that the activities of the ILs approach those of the toxic starting materials, with [BA][Sac] producing highest toxicity against all strains. This IL displayed higher activity than the synthetic precursor chloride in some cases (Table 5.2, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Streptococcus mutans*).

Table 5.2: MIC (μM) values of QAC ILs.

Strain	**482**	**483**	**484**	**485**	BAC	DDAC
S. aureus	7.8	8.3	7.9	16.4	5.4	5.5
MRSA	7.8	8.3	7.9	8.2	5.4	5.5
E. faecium	15.6	16.7	15.8	16.4	10.9	11.0
E. coli	31.1	64.7	31.5	32.8	21.8	22.1
M. luteus	7.8	16.7	7.9	16.4	10.9	5.5
S. epidermidis	7.8	8.3	7.9	8.2	5.4	5.5
K. pneumoniae	7.8	16.7	7.9	8.2	10.9	11.0
C. albicans	31.1	33.4	31.5	32.8	21.8	22.1
R. rubra	31.1	33.4	31.5	32.8	21.8	11.0
S. mutans	0.2	2.1	61.0	32.8	5.4	5.5

Table 5.3: MBC (μM) values of QAC ILs.

Strain	**482**	**483**	**484**	**485**	BAC	DDAC
S. aureus	60.3	64.7	122.0	32.8	5.4	5.5
MRSA	60.3	64.7	61.0	63.5	5.4	5.5
E. faecium	31.1	64.7	31.5	63.5	10.9	11.0
E. coli	120.6	260.9	31.5	127.0	21.8	22.1
M. luteus	120.6	129.4	61.0	127.0	10.9	5.5
S. epidermidis	60.3	129.4	31.5	63.5	5.4	5.5
K. pneumonia	120.6	64.7	31.5	63.5	10.9	11.0
C. albicans	60.3	64.7	31.5	63.5	21.8	22.1
R. rubra	120.6	125.2	61.0	127.0	21.8	11.0
S. mutans	0.9	33.4	122.0	256.1	5.4	5.5

A study of the biological activity of alkyl imidazolium ionic liquids towards the luminescent bacterium *Vibrio fischeri* (Figure 5.4) was undertaken by Ranke et al.[9] V. *fischeri* is a rod shaped bacterium which is found in marine environments. Free living *V.*

fischeri can survive on decaying organic matter. The *V. fischeri* acute bioluminescence inhibition assay is a frequently used standard ecotoxicological bioassay in Europe (DIN EN ISO 11348). This assay is utilized for routine screening of river and lake water industry effluents and for screening chemical toxicity. Toxicity towards *V. fischeri* can be determined by measuring the difference in light output from the organism. A decrease in observed light output indicates an increase in toxicity. Increase in alkyl chain length of the imidazolium cation, displayed a subsequent increase in toxicity (a decrease in light production of bacterium). A slight anion effect was also noted, whereby the PF_6 anions appeared slightly toxic compared to BF_4 and Cl derivatives. However, one exception was observed from this trend. The decyl imidazolium cation combined with the BF_4 anion, demonstrated higher toxicity than its Cl and PF_6 counterparts.

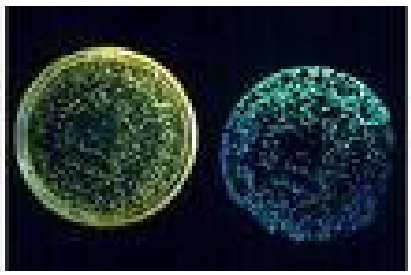


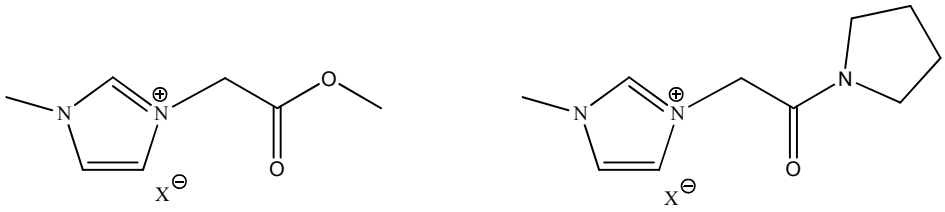
Fig. 5.4: *Vibrio fischeri* bacteria plates are visible with ambient light (left), and also in the dark (right), due to their production of blue-green bioluminescence.[10]

Fungal isolates have also been subjected to ionic liquid environments as a means of measuring their tolerance to these chemical entities. In 2009 a group of researchers investigated the toxicity of a series of imidazolium, pyridinium and cholinium based ILs towards filamentous fungi (of Penicilium genus).[11] The species tested were *Penicillium breviocompactum*, *Penicillium olsonii*, *Penicillium janczewskii*, *Penicillium glandicola*, *Penicillium corylophilum*, *Penicillium glabrum*, *Penicillium restictum*, *Penicillium adametzii*, *Penicillium variabile*, and *Penicillium diversum*. The ionic liquid medium (50 mM) was inoculated with the fungal spores (10^5 spores per mL), and incubated in the dark at 25 °C. In addition to testing the ILs, a number of experimental controls were also set up. An osmotic stress control (media treated with aqueous NaCl or CsCl) and starting material controls (media with aqueous 1-methylimidazole and pyridine) were run in the test. Fungal

growth (or lack thereof) was studied by measuring the absorbance of the medium at 600 nm. An increase in absorbance indicated fungal growth. The authors reported the fungal isolates to display the least tolerance towards the imidazolium based ionic liquids. The well reported observation of increased alkyl chain length of an IL cation resulting in increased toxicity was also evident. The environmentally benign cation cholinium unsurprisingly exhibited the lowest fungal toxicity (highest MIC and Minimum Fungicidal Concentrations (MFC)). Moreover, the IL staring materials namely 1-methylimidazole and pyridine inhibited fungal growth in 100 and 60 % of cases respectively. Thus proving to be more toxic than their corresponding ionic liquid derivatives. Studies were also conducted to examine if the ILs could alter the metabolic profile (by MS analysis) in the fungal species. Fungal cultures responded to specific ILs by altering their cell biochemistry, and therefore leading to variations in the secondary metabolite pattern.

Continuing on from this work[11], this group probed the toxic effect of the anion species of cholinium based ILs against filamentous fungi.[12] A series of cholinium ILs with alkanoate anions $[C_nH_{2n+1}CO_2]^-$ (where n = 1-9) were screened against a range of fungal isolates; *Penicillium glandicola, Penicillium brevicompactum* and *Penicillium diversum*. Fungal tolerance decreased with an increase in the alkyl chain length of the IL anion. Branching of anion side chain lead to lower toxicities, which was evident for IL s containing 2-methylproponoate and 2,2-dimethylproponoate anions. In comparison to their linear counterparts (butanoate and pentanoate respectively) much lower MIC and MFC values were observed.

Gathergood and Connon *et al*. recently reported antifungal studies of imidazolium derived ILs[13] (Figure 5.5). These ILs were challenged against a large array of fungal isolates; *Candida albicans* (ATCC 44859 and ATCC 90028), *Candida parapsilosis*, *Candida krusei* (ATCC 6258 and E28), *Candida tropicalis*, *Candida glabrata*, *Candida lusitaniae*, *Trichosporon beigelii*, *Aspergillus fumigatus*, *Absidia corymbifera* and *Trichophyton mentagrophytes*. No fungal inhibition was observed against the test strains when exposed to the ILs (highest test concentration of 2000 μM).



X = BF_4 **486**
Br **487**

X = Br **488**

Fig. 5.5: Imidazolium-based ILs with low antifungal activities.[13]

5.1.2 Antibiofilm studies

(Eco)toxicological screening of ionic liquids reported hitherto, have predominately employed organisms in a planktonic mode of growth (single cells which may float or swim in liquid medium). However, the main mode of growth of microorganisms in pathogenic and environmental scenarios is as biofilms. Biofilm is a mode of microbial growth, whereby cells adhere to each other or to a surface within a self-produced matrix of extracellular polymeric substance (EPS). Microorganisms can form biofilms in response to many external factors and signify a key survival mechanism for these microbial communities. Numerous chronic plant, animal and human infections have been caused by biofilms, in addition to other environmental and clinical problems. Therefore antibiofilm investigations are of environmental and clinical importance. Albeit toxicity of ionic liquids has been viewed as a negative environmental effect, it can also be exploited to a number of beneficial applications (in new antiseptic agents or as anti-fouling media).

Gilmore and co-workers were the first researchers to study the antibiofilm properties of ionic liquids.[14] In this work a range of 1-alkyl-3-methylimidazolium chloride ionic liquids were assessed for antibiofilm activity against clinically relevant pathogens. The strains of resistant bacteria and fungi used included *Staphylococcus aureus* (ATCC 29213), *Methicillin-resistant Staphylococcus aureus* (MRSA), *Epidemic Methicillin-resistant Staphylococcus aureus* (E-MRSA 15), *Staphylococcus epidermidis* (ATCC 35984), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Bacillus cenocepacia*, *Pseudomonas mirabilis* and *Candida tropicalis*. Biofilms of each strain were grown on

polystyrene pegs of a Calgary Biofilm Device (CBD), and were grown for 24 hours. 24 hour biofilm viable counts were carried out to in order to measure viable cell counts for each strain biofilm. MIC and MBEC (minimum biofilm eradication concentration) were obtained for the tested ILs (Table 5.4).

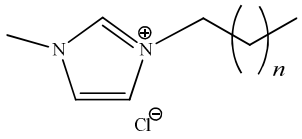


Table 5.4: MIC and MBEC (μM) values of 1-alkyl-3-methylimidazolium chloride ILs.

Strain		n = 6 **140**	8 **489**	10 **62**	12 **490**
S. aureus	**MIC**	722	40	18	16
	MBEC	2708	2415	272	124
E-MRSA	**MIC**	722	40	18	16
	MBEC	2708	1207	272	248
MRSA	**MIC**	1444	160	36	16
	MBEC	21666	4829	545	124
S. epidermidis	**MIC**	722	40	36	7.75
	MBEC	10833	4829	272	124
E. coli	**MIC**	722	321	73	33
	MBEC	21666	9659	1089	124
P.aeruginosa	**MIC**	5416	2415	580	264
	MBEC	21666	2415	1089	496
K. aerogenes	**MIC**	1444	643	73	33
	MBEC	43331	19318	2179	248
B. cenocepacia	**MIC**	>1444	1287	290	132
	MBEC	43331	19318	2179	496
P. mirabilis	**MIC**	1444	1287	580	264
	MBEC	43331	9659	4357	1984
C. tropicalis	**MIC**	1444	321	73	66
	MBEC	>43331	19318	8714	248

From this data it was found that for each strain the MBEC value decreased (increase in antibiofilm activity) with an increase in the alkyl chain length of the IL side chain. The decyl derivative (**489**) displayed antibiofilm activity against all Gram positive and most

Gram negative bacteria tested, with tolerance observed against *K. aerogenes* and *B. Cenopacia*. Overall, it appeared that the tetradecyl substituted IL (**490**) gave the greatest activity against all biofilm strains (i.e. higher potency). This group further investigated the antibiofilm activities if 1-alkylquinolium bromide ILs against pathogen biofilm microbes.[15] The authors reported a similar trend of higher antibiofilm potency with increased alkyl chain length of the IL side chains.

5.2 Toxicity Studies of (a)chiral imidazolium ionic liquids

The following sections detail the work undertaken in our research on the antimicrobial activites of novel (a)chiral imidazolium based ionic liquids.

5.2.1 Antibacterial studies of achiral ionic liquids

A panel of achiral imidazolium-based ionic liquids (**42**, **45**, **193**-**199**) were screened for antibacterial activities against four strains of bacteria. Two Gram negative bacteria, all rod-shaped, (*Salmonella sp*., *Klebsiella sp.*) and two Gram positive rod and cocci, (*Bacillus Subtilis*, *Micrococcus sp.*) were challenged against the ILs over a wide concentration range (0-20 mg/mL). The minimum inhibitory concentrations (MIC) were measured visibly for ILs which displayed activity against the bacterial strains after 24 hour incubation (at 37 °C). Table 5.5 outlines the MIC values determined in these studies.

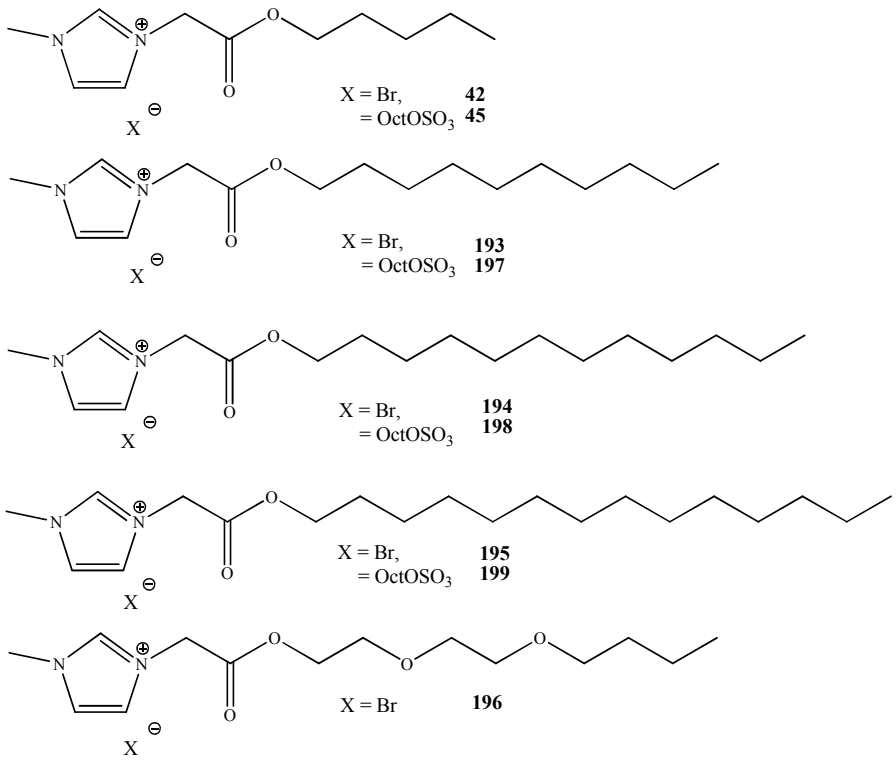


Table 5.5: MIC (μM) values of achiral imidazolium based ionic liquids.

	IL								
Organism	**42**	**193**	**196**	**194**	**195**	**45**	**197**	**198**	**199**
Salmonella sp.	>6872	>5540	>5479	**2570**	**2398**	>4761	**2040**	>3861	>3663
Klebsiella sp.	>6872	>5540	>5479	**1285**	**1199**	>4761	**4081**	**1930**	**915**
B. subtilis	>6872	**2770**	>5479	**1285**	**149**	>4761	**4081**	**965**	**1831**
Micrococcus sp.	>6872	>5540	>5479	**2570**	**1199**	>4761	**2040**	**3861**	**1831**

From the obtained data (Table 5.5), MIC values greater than 20 mg/mL correspond to a lack of IL toxicity against the bacterial strains at concentration ranges from >3663 μM to >6872 μM (depending on the molecular weight of the IL). In general, inhibition was

observed for those ILs containing cations with long hydrocarbon side chains. Low MIC (high toxicity) values were measured for decyl, dodecyl and tetradecyl examples, whilst high MIC values (low toxicities) were noted for ILs with pentyl and ethereal side chains.[16] The most toxic ILs were 3-methyl-1-(dodecoxycarbonylmethyl)imidazolium bromide (**194**) and 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium bromide (**195**), displaying activity against all bacterial strains, and in some cases even at lower concentrations (Table 5.5, *B. subtilis*). This result is in accordance with data reported in the literature[5,8,14] where increased alkyl chain length of ILs cation and/or anion side chain results in an increase of toxicity. An increase in IL lipophilicity means that these compounds can cross biomembranes readily and therefore enter the cell. Varying the IL anion did not seem to influence toxicity overall (bromide vs $OctOSO_3$), with the exception of the decyl derivative. The $OctOSO_3$ salt (**197**) appeared more toxic than the bromide form against certain strains of bacteria (Table 5.5, *Salmonella sp*., *Klebsiella sp.*, *Micrococcus sp.*) most likely due to an increase in IL lipophilicity. Furthermore, no clear trend was observed between toxicity and the nature of bacterial species (i.e. Gram negative and Gram positive), with toxicity seen across a broad range of microorganisms.

The pentyl, dodecyl and tetradecyl ester achiral ILs (**42**, **194**, **195**) were further screened against a range of clinically resistant bacterial strains, these included; *Staphylococcus aureus* (CCM 4516/08), *Escherichia coli* (CCM4517), *Pseudomonas aeruginosa* (CCM 1961), a range of clinical isolates were also challenged; *Staphylococcus aureus* MRSA (H 5996/08), *Staphylococcus epidermidis* (H 6966/08), *Enterococcus sp.* (J 14365/08), *Klebsiella pneumoniae* (D 11750/08), *Klebsiella pneumonia*-ESBL positive (J 14368/08). This work was undertaken in collaboration with Dr. Marcel Špulák of Charles University, Czech Republic. The MIC values obtained are given in Table 5.6.

Table 5.6: MIC (μM) values of ILs **42**, **194** and **195**.†

Organism	Time (h)	IL		
		42	**194**	**195**
S. aureus	24	>2000	**15.62**	**7.81**
	48	>2000	**15.62**	**15.62**
MRSA	24	>2000	**15.62**	**7.81**
	48	>2000	**15.62**	**7.81**
S. epidermidis	24	>2000	**15.62**	**31.25**
	48	>2000	**31.25**	**62.50**
Enterococcus sp.	24	>2000	**31.25**	**31.25**
	48	>2000	**62.50**	**62.50**
E. coli	24	>2000	**31.25**	**15.62**
	48	>2000	**62.50**	**62.50**
K. pneumonia	24	>2000	**31.25**	**62.50**
	48	>2000	**125**	**62.50**
K. pneumoniae-ESBL	24	>2000	**31.25**	**62.50**
	48	>2000	**125**	**125**
P. aeruginosa	24	>2000	**31.25**	**1000**
	48	>2000	**125**	**1000**

†Results obtained by collaborator

Similar to the results obtained in previous antibacterial screens (Table 5.5), ILs **194** and **195** displayed high levels of bacterial activity, whilst the pentyl derivative **42** proved non-toxic. From the results obtained (Table 5.6) in this study, relatively low MIC values (corresponding to high toxicities) were obtained for **194** and **195** against all the test

organisms (Gram negative and Gram positive). These alkyl ester ILs exhibit similar potency as the biocide Benzalkonium chloride, BAC (Tables 5.1, 5.2). From an environmental prospective these results are not desirable as highly antimicrobial toxic ILs cannot be classified as 'green'. However, in terms of clinical applications these ILs have the potential to act as new classes of antibacterial or antiseptic agents towards, in particular, resistant bacterial strains (e.g. MRSA).

5.2.2 Antifungal studies of achiral ionic liquids

Three achiral bromide salts (**42**, **194** and **195**, Figure 5.6) were tested for antifungal activities against four ATCC strains (*Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258), eight clinical isolates of yeast (*Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 11880) and three filamentous fungi (*Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445). This work was also carried out by our collaborator, Dr. Marcel Špulák. The results retrieved from these studies are reported in Table 5.7.

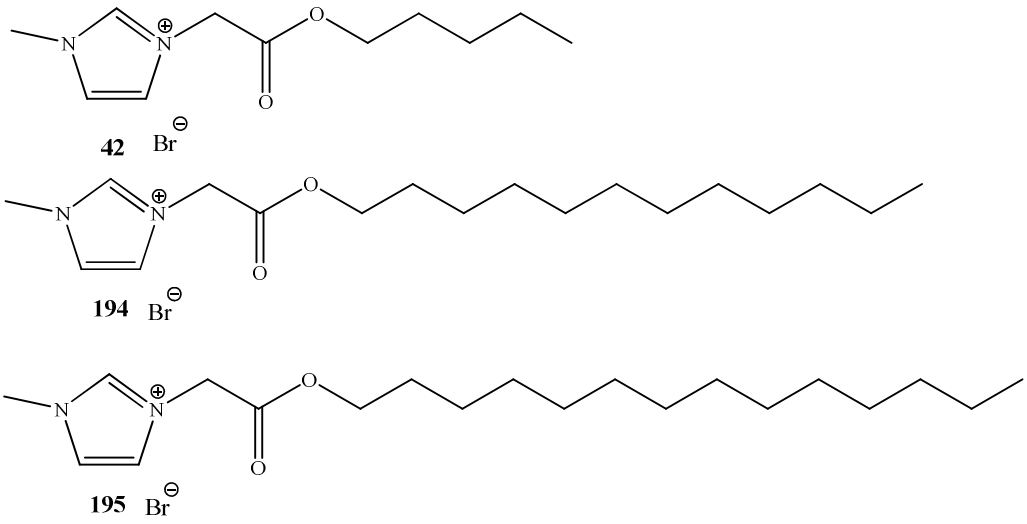


Fig. 5.6: Achiral ILs screened for antifungal activities.

Table 5.7: Antifungal activities of achiral ILs **42**, **194** and **195**

Organism	Time (h)	IL **42**	**194**	**195**
C. albicans (ATCC44859)	24	>2000	**7.81**	**3.90**
	48	>2000	**31.25**	**7.81**
C. albicans (ATCC 90028)	24	>2000	**7.81**	**3.90**
	48	>2000	**31.25**	**7.81**
C. parapsilosis (ATCC 22019)	24	>2000	**31.25**	**7.81**
	48	>2000	**62.50**	**7.81**
C. krusei (ATCC 6258)	24	>2000	**1.95**	**1.95**
	48	>2000	**3.90**	**3.90**
C. krusei (E28)	24	>2000	**1.95**	**1.95**
	48	>2000	**3.90**	**3.90**
C. tropicalis (156)	24	>2000	**3.90**	**3.90**
	48	>2000	**7.81**	**7.81**
C. glabrata (20/I)	24	>2000	**1.95**	**1.95**
	48	>2000	**3.90**	**3.90**
C. lustaniae (2446/I)	24	>2000	**31.25**	**7.81**
	48	>2000	**62.50**	**7.81**
T.asahii (1188)	24	>2000	**15.62**	**1.95**
	48	>2000	**31.25**	**7.81**
A. fumigates (231)	24	>2000	**15.62**	**1.95**
	48	>2000	**31.25**	**7.81**
A. corymbifera (272)	24	>2000	>2000	>2000
	48	>2000	>2000	>2000
T. mentagrophytes (445)	72	>2000	**15.62**	**7.81**
	120	>2000	**31.25**	**7.81**

As can be seen from the accumulated data, inhibition was observed for ILs **194** and **195** against the tested fungal strains. Notably the filamentous fungal isolate *Absidia corymbifera* 272 was an exception, and proved resistant to the most toxic ILs (Table 5.7). High antifungal activity of these compounds can be related to the structural features of the IL cation. In ILs, **194** and **195**, the cation side chains contain dodecyl and tetradecyl esters respectively. Introduction of long alkyl chains to an IL structure has been well known to increase their bioactivities.[5,6,14] Similarly the less lipophilic ILs, namely those which contain short alkyl side chains, lack in ability to inhibit microorganism growth. Achiral IL **42** displayed no inhibition towards the twelve fungal species, and therefore can be described as 'green'.

5.2.3 Antibacterial studies of chiral ionic liquids (CILs)

The toxicity of various novel chiral ionic liquids (Figure 5.7) was investigated against environmentally relevant bacteria. The bacterial strains employed were *Escherichia coli*, *Bacillus subtilis* (commonly found in soil samples), *Pseudomonas fluorenscens* (ubiquitous in soil and water systems), *Pseudomonas putida*-CP1, and *Pseudomonas putida*-KT2440 (known to biologically degrade chemical compounds, e.g. toluene). MIC values were measured both visually and photometrically at 450 nm. The results obtained from these tests are outlined in Table 5.8.

Table 5.8: MIC (μM) values of CILs (**274, 283, 284, 363** and **364**).

	IL				
Strain	**274**	**283**	**284**	**363**	**364**
E. coli	**22800**	**43500**	**77000**	>90000	>142000
B. subtilis	**22800**	**43500**	**77000**	>90000	>142000
P. fluorescens	**91000**	>174000	**77000**	>90000	>142000
P. putida (CP1)	**91000**	>174000	**154000**	>90000	>142000
P. putida (KT2440)	**91000**	>174000	**154000**	>90000	>142000

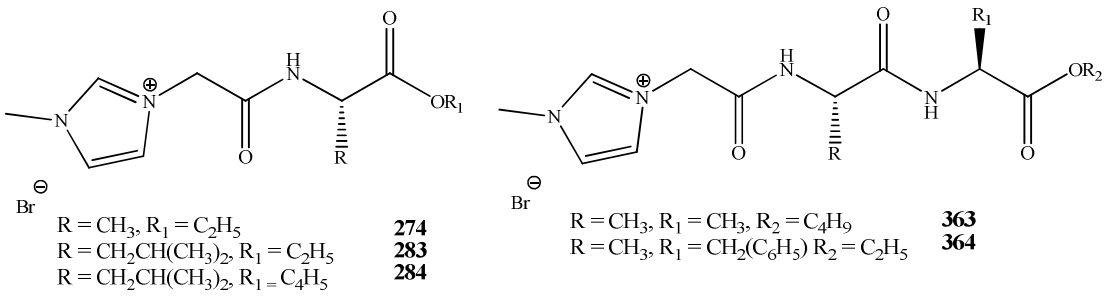


Fig. 5.7: CILs challenged against environmental bacteria *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorenscens*, *Pseudomonas putida*-CP1 and *Pseudomonas putida*-KT2440.

The dipeptidyl derivatives **363** and **364** appeared the least toxic against the tested isolates giving MIC values higher than the test concentration (90000 and 142000 μM respectively). Compounds **274**, **283** and **284** displayed inhibition against the bacteria screened. However the concentration required to inhibit these microorganisms was relatively large (Table 5.8). Taking the example of *Bacillus subtilus*, the lowest test concentration (MIC) capable of inhibiting the growth of this bacterium was 22800 μM (for compound **274**). However, in a previous screen of the achiral derivatives, an MIC value of 149 μM (Table 5.5) was

measured for IL **195** against this bacterial strain. All other CILs give MIC values at or near to their upper limit tested concentrations.

In vitro antibacterial activities of a number of novel chiral ionic liquids (Figure 5.8, 5.9) were also investigated against more clinically relevant microbes. The microorganisms employed in these studies were *Staphylococcus aureus* (CCM 4516/08), *Escherichia coli* (CCM4517), *Pseudomonas aeruginosa* (CCM 1961), a range of clinical isolates were also challenged; *Staphylococcus aureus* MRSA (H 5996/08), *Staphylococcus epidermidis* (H 6966/08), *Enterococcus sp.* (J 14365/08), *Klebsiella pneumoniae* (D 11750/08), *Klebsiella pneumonia*-ESBL positive (J 14368/08). The results in Table 5.9 represent the MIC values obtained for those dipeptidyl CILs which displayed antibacterial inhibition.

Table 5.9: MIC (μM) values obtained for dipeptidyl CILs (**368, 370, 371** and **378**).†

Organism	Time (h)	IL			
		368	**370**	**371**	**378**
S. aureus	24	**500**	>2000	>2000	**1000**
	48	**1000**	>2000	>2000	**1000**
MRSA	24	**125**	>2000	>2000	**2000**
	48	**500**	>2000	>2000	**2000**
S. epidermidis	24	**500**	**2000**	**1000**	**2000**
	48	>2000	>2000	>2000	>2000
Enterococcus sp.	24	**2000**	>2000	>2000	**2000**
	48	>2000	>2000	>2000	**2000**
E. coli	24	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000
K. pneumonia	24	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000
K. pneumoniae-ESBL	24	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000
P. aeruginosa	24	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000

†Results obtained by collaborator

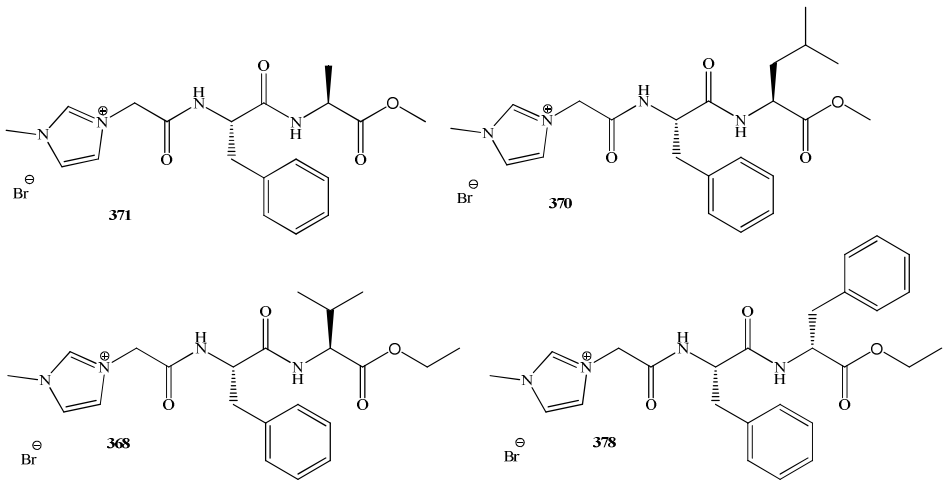


Fig. 5.8: Dipeptidyl CILs displaying antibacterial activities.

Out of the 17 dipeptidyl CILs screened, only four examples (**368**, **370**, **371** and **378**) exhibited activity towards various bacterial strains (<2000 μM). **371** and **370** were relatively non toxic overall, but both demonstrated activity towards *Staphylococcus epidermidis* (Table 5.9, MIC values of 1000 and 2000 μM respectively). CILs **368** and **378** appeared most toxic, inhibiting the growth of four resistant isolates, namely, *Staphylococcus aureus*, MRSA, *Staphylococcus epidermidis*, and *Enterococcus sp*.. The lowest MIC value was obtained for **368** against the MRSA strain (125 μM after 24 hour incubation and 500 μM after 48 hours). Some minor trends can be noted from the results (Table 5.9). Structurally, all four CILs possess a phenylalanine residue adjacent to the cation core. ILs bearing this structural motif may have increased lipophilicities, and hence increased bioactivities. Interestingly, some trends can be noted between CIL activity and the bacterial strains tested. The CILs (**368**, **370**, **371** and **378**) displayed increased toxicity towards the Gram positive type bacteria (namely *Staphylococcus aureus*, *Staphylococcus aureus*-MRSA, *Staphylococcus epidermidis*, and *Enterococcus sp*.). Whilst the Gram negative species appeared the most tolerant towards the test compounds. This result may be understood by considering the different structural features present in both types of bacteria. Gram positive bacterial cell walls lack the outer membrane found in Gram negative species. Certain components of the Gram negative bacterial cell well (e.g. lipopolysaccharide layer) can protect the bacteria from various antibiotics and chemical entities.[17]

23 amino acid ester CILs (**247**-**269**) were also screened against these resistant bacteria, with antimicrobial activity evident for only 5 examples (**272**, **277**, **281**, **285** and **287**, Figure 5.9). The CILs which displayed activities are summarized in Table 5.10.

Table 5.10: MIC (μM) values† obtained for amino acid ester CILs (**272**, **277**, **281**, **285** and **287**). †Results obtained by collaborator

		IL				
Organism	Time (h)	**272**	**277**	**281**	**285**	**287**
S. aureus	24	**2000**	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000
MRSA	24	>2000	>2000	**2000**	**2000**	>2000
	48	>2000	>2000	>2000	>2000	>2000
S. epidermidis	24	>2000	**2000**	>2000	>2000	**2000**
	48	>2000	>2000	>2000	>2000	>2000
Enterococcus sp.	24	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000
E. coli	24	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000
K. pneumonia	24	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000
K. pneumoniae-ESBL	24	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000
P. aeruginosa	24	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000

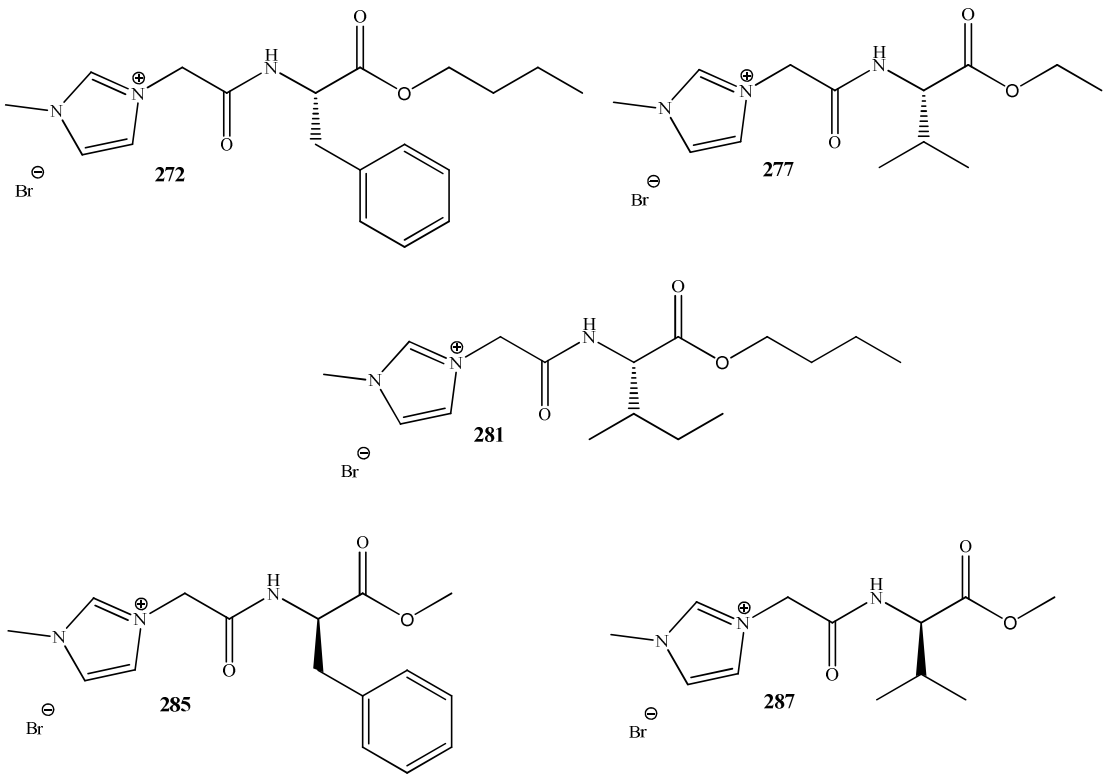


Fig. 5.9: Amino acid ester CILs displaying antibacterial activities.

An overall lack in toxic activity was noted for the amino acid ester CILs (Table 5.10). Only compounds **272**, **277**, **281**, **285** and **287** showing toxicity towards the tested bacteria. No clear trends can be noted between IL structure and antibacterial activity in this study. Moreover, the stereochemistry of the IL side chain does not seem to play a significant role in the activity of the compound. CILs containing both L and D enantiomeric amino acid groups displayed toxicity towards the bacterial isolates. Inhibition of *Staphylococcus aureus* at 2000 μM was evident for **272**. CILs **281** and **285** both demonstrated toxicity towards the resistant strain MRSA at a high MIC value of 2000 μM (after 24 hour incubation). As seen previously for the dipeptidyl derivatives, the Gram positive bacteria are more susceptible to the test compounds than the Gram negative strains (Table 5.10). This relationship between IL activity and bacterial class is believed to be due to differences in biochemical properties between the two classes.

5.2.4 Antifungal studies of chiral ionic liquids (CILs)

In vitro antifungal activities of several chiral ionic liquids were evaluated on a range of filamentous fungi and yeasts isolates. Novel CILs were screened against *Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Candida krusei* E28, *Candida tropicalis* 156, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 11880, *Aspergillus fumigatus* 231, *Absidia corymbifera* 272, *Trichophyton mentagrophytes* 445. The results obtained for those dipeptidyl and amino acid ester CILs which indicated inhibition are summarized in Tables 5.11 and 5.12.

Table 5.11: Antifungal activities* of dipeptidyl CILs (**364, 365, 368, 371, 374, 377** and **378**).†

		IL						
Organism	Time (h)	**364**	**365**	**368**	**371**	**374**	**377**	**378**
C. albicans	24	>2000	>2000	**2000**	>2000	>2000	>2000	>2000
(ATCC44859)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. albicans	24	>2000	>2000	**2000**	>2000	>2000	>2000	>2000
(ATCC 90028)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. parapsilosis	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(ATCC 22019)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. krusei	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(ATCC 6258)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. krusei	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(E28)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. tropicalis	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(156)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. glabrata	24	>2000	>2000	>2000	>2000	>2000	>2000	**2000**
(20/I)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. lustaniae	24	>2000	>2000	**2000**	>2000	>2000	>2000	>2000
(2446/I)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
T.asahii	24	**2000**	**2000**	>2000	>2000	**2000**	**2000**	**2000**
(1188)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000

A. fumigates (231)	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
A. corymbifera (272)	24	>2000	>2000	>2000	**2000**	>2000	>2000	>2000
	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000
T. mentagrophytes (445)	72	>2000	>2000	>2000	>2000	>2000	>2000	>2000
	120	>2000	>2000	>2000	>2000	>2000	>2000	>2000

* reported as MIC (μM) values

†Results obtained by collaborator

In this study 17 dipeptidyl chiral ionic liquids were screened for antifungal activities, with inhibition observed for 7 examples. Figure 5.10 illustrates the most active CILs from this class.

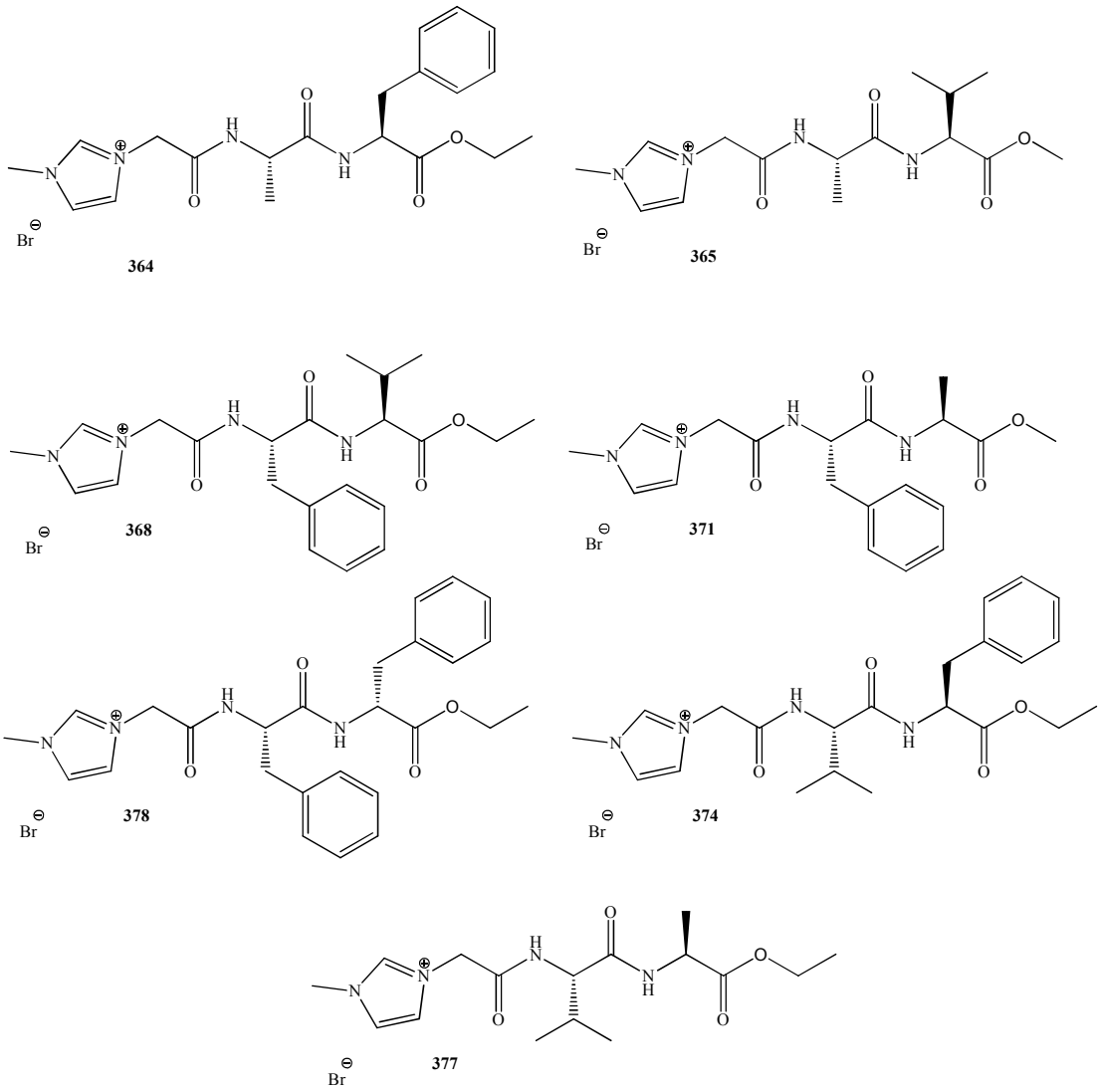


Fig. 5.10: Dipeptidyl CILs with antifungal activities.

The dipeptidyl CILs appeared moderately toxic towards the fungal species. Inhibition was observed for compounds **364**, **365**, **368**, **371**, **378**, **374** and **377** against the various fungal strains. CIL **368** inhibited the growth of three fungi, namely, *Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028 and *Candida lusitaniae* 2446/I. The remaining ILs were seen to inhibit only one or two isolates, all at higher concentrations (MIC values of 2000 μM). In terms of structure activity relationships (SAR), no strong trends can be deduced from the obtained data. The presence of phenylalanine groups in the IL structure (i.e. an increase in IL lipophilicity) could increase its toxicity towards the tested fungi.

However **365** and **377** do not possess an aromatic functionality in the IL side chain, and inhibition was measured for both CILs.

Amino acid ester CILs were also studied for their antifungal toxicities. 23 bromide chiral salts were screened and activity was determined for 10 derivatives. Figure 5.11 depicts the chemical structures of these CILs.

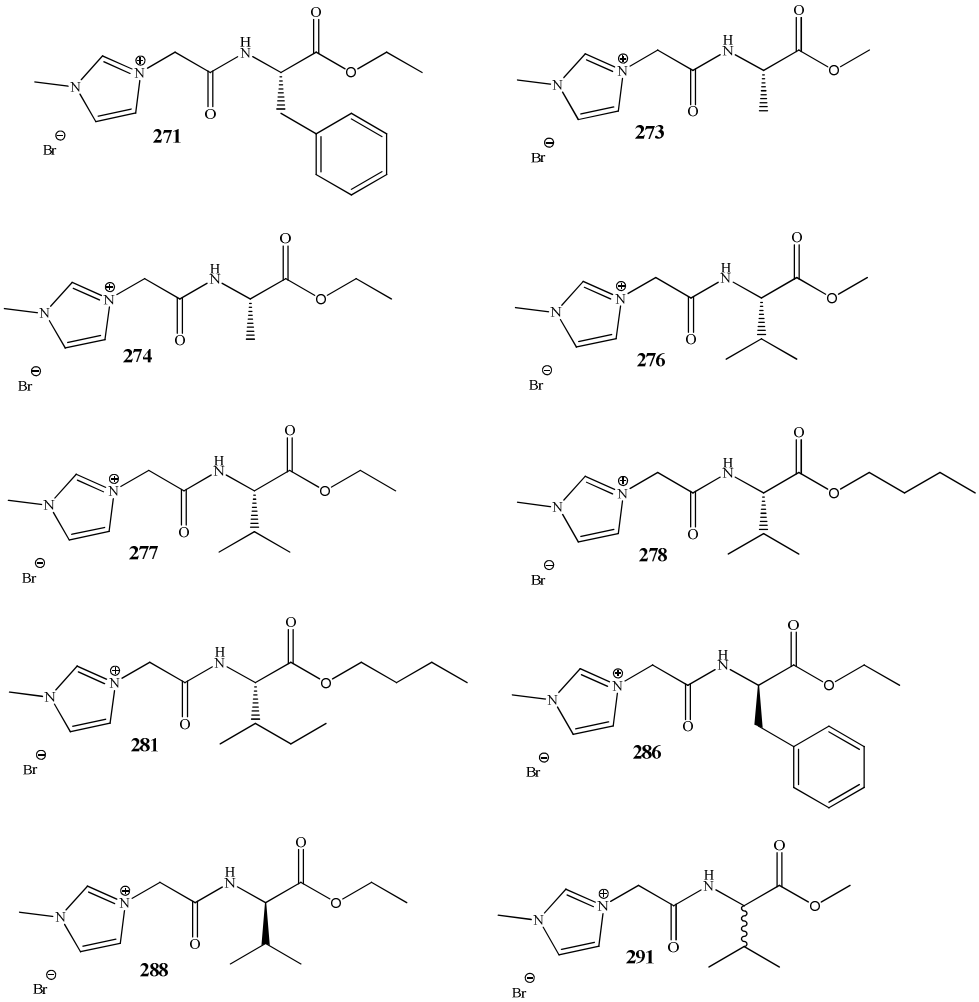


Fig. 5.11: Amino acid ester CIL with antifungal properties.

Table 5.12: Antifungal activities† of Amino acid ester CILs. †Results obtained by collaborator

Organism	Time (h)	IL **271**	**273**	**274**	**276**	**278**	**277**	**281**	**286**	**288**	**291**
C. albicans	24	**2000**	>2000	>2000	>2000	>2000	>2000	**500**	>2000	>2000	>2000
(ATCC 44859)	48	>2000	>2000	>2000	>2000	>2000	>2000	**2000**	>2000	>2000	>2000
C. albicans	24	**2000**	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(ATCC 90028)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. parapsilosis	24	**2000**	>2000	>2000	>2000	>2000	>2000	**2000**	>2000	>2000	>2000
(ATCC 22019)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. krusei	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(ATCC 6258)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. krusei	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(E28)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. tropicalis	24	>2000	>2000	>2000	>2000	>2000	>2000	**2000**	>2000	>2000	>2000
(156)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000

Table 5.12: (continuation) Antifungal activities† of Amino acid ester CILs. †Results obtained by collaborator

Organism	Time (h)	IL **271**	**273**	**274**	**276**	**278**	**277**	**281**	**286**	**288**	**291**
C. glabrata	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(20/I)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
C. lustaniae	24	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
(2446/I)	48	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000	>2000
T.asahii	24	>2000	>2000	>2000	>2000	**500**	>2000	**1000**	>2000	>2000	**2000**
(1188)	48	>2000	>2000	>2000	>2000	**500**	>2000	>2000	>2000	>2000	>2000
A. fumigates	24	>2000	>2000	>2000	>2000	**500**	>2000	>2000	>2000	>2000	>2000
(231)	48	>2000	>2000	>2000	>2000	**1000**	>2000	>2000	>2000	>2000	>2000
A. corymbifera	24	>2000	>2000	**2000**	>2000	>2000	**250**	**1000**	**2000**	**2000**	>2000
(272)	48	>2000	>2000	**2000**	>2000	>2000	**250**	>2000	>2000	>2000	>2000
T. mentagrophytes	72	**1000**	**500**	**500**	**1000**	**62.5**	>2000	**1000**	**1000**	**1000**	**250**
(445)	120	**2000**	**1000**	**500**	**1000**	**62.5**	>2000	**2000**	>2000	>2000	**250**

It can be seen from these studies, that both classes of CILs are relatively non-toxic towards fungal isolates. However, in general the amino acid ester derivatives appear to inhibit more strains of fungi than the dipeptidyl CILs (Tables 5.11 and 5.12). **281** proved to be the most active of the ILs tested, inhibiting six fungal strains (Table 5.12), *Candida albicans* (ATCC 44859), *Candida parapsilosis* (ATCC 22019), *Candida tropicalis* (156), *Trichosporon asahii* (1188), *Absidia corymbifera* (272), *Trichopyton mentagrophytes* (445)). The lowest MIC value obtained for **281** was at 500 μM against *Candida albicans* (ATCC44859) after 24 hour incubation, and further inhibited growth after 48 hours (MIC 2000 μM). However **278** gave the lowest measured MIC value (highest toxicity) of 62.50 μM against *Trichopyton mentagrophytes* (445) even after 48 hours incubation. Compound **271** displayed activity against four isolates, namely *Candida albicans* (ATCC 44859), *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019) and *Trichopyton mentagrophytes* (445). The lowest MIC value measured for this IL was at 1000 μM against the dermatophytic strain *Trichopyton mentagrophytes* (445) after 24 hour incubation. This fungal strain (*Trichopyton mentagrophytes* (445)) appeared the most susceptible isolate overall, being inhibited by 9 of the 10 active test compounds (Table 5.12).

5.3 Conclusions

A range of toxicity studies were carried out on novel achiral and chiral ionic liquids. Minimum Inhibitory Concentrations (MIC) of the ILs were measured against bacterial and fungal strains. A panel of achiral ionic liquids (**42**, **45**, **193**-**199**) was screened for antimicrobial activity. High levels of toxicity were observed for ILs containing dodecyl (**194**) and tetradecyl (**195**) ester side chains. These compounds displayed a broad range of activity towards resistant bacterial and fungal isolates. The potency of these ILs can be related to structural features of the IL side chain. Substitution of the ILs cation with long alkyl chains results in more lipophilic examples. This increase in lipophilicity results in a corresponding increase in toxicity.

Dipeptidyl (**363**-**379**) and Amino acid ester (**247**-**269**) chiral ionic liquids were challenged against a range of bacteria and fungi, of both clinical and environmental relevance. Both classes of CILs displayed relatively low toxicities (high measured MIC values) against the

test microorganisms. CIL **368** was capable of inhibiting the resistant bacterial strain MRSA at 125 μM concentration (Table 5.9). Inhibition of other resistant bacterial strains was also observed for compounds **370**, **371** and **378**. All the aforementioned toxic CILs possessed a phenylalanine moiety in the cation side chain. The presence of this group in IL side chains leads to increased lipophilicity and toxicity. A trend was also noted between IL activity and the bacterial strains. Inhibition was predominately against Gram positive bacteria, whilst the Gram negative species proved the most tolerant. The amino acid ester CILs gave higher toxicities (lower MIC values) towards the fungal strains. 23 bromide CILs were screened with 10 examples (**273**-**291**) demonstrating antifungal activities (Table 5.12).

ILs displaying toxicity in these studies cannot be classified as 'green'. However, an inhibitory effect towards a microbe, in particular resistant examples, may allow for medicinal applications of these compounds.

5.4 References

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Chapter 6: Results and discussion

Primary biodegradation and metabolite profiling of chiral ionic liquids-initial studies

6.1 Introduction

Chapter one of the thesis provides an in depth review of the literature on work which has been carried out to date on biodegradation investigations of ionic liquids. Interest in this area of research has escalated, with many research groups reporting biodegradable ionic liquids. Various types of biodegradation and the assays consequently used to measure these biodegradations are also discussed (Chapter one, Section 1.2). Many techniques measure respirometric parameters such as oxygen depletion (OECD 301 D), carbon dioxide evolution (OECD 301 B), dissolved organic carbon (DOC) content (OECD 301 A/E) and biochemical oxygen demand (BOD) (OECD 301 C, ASTM 5988). By analyzing these parameters, compounds can be classified as various levels of biodegradable, namely as '*readily biodegradable*' or '*ultimately biodegradable*'. These classifications describe the level of degradation achieved when the test compound is totally utilised by micro-organisms resulting in the production of carbon dioxide, water, mineral salts and biomass. '*Primary biodegradation*', on the other hand, results in an alteration in the chemical structure of a substance, brought about by biological action of active microbes. Analytical techniques such as High Performance Liquid Chromatography (HPLC), High Performance Liquid Chromatography Mass Spectrometry (HPLC-MS), Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) have been employed in the determination of ILs '*primary biodegradation*'.[1-6] In this work, HPLC-MS (quantitative) and ESI-MS (qualitative) analysis was used to analyze the products of biodegradation of two novel chiral ionic liquids, namely 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (**272**) and 3-methyl-1-L-phenylalanine-D-phenylalanine ethyl ester imidazolium bromide (**378**).

6.2 HPLC-MS method development for the identification of novel chiral ionic liquids and their metabolite products

The first step in these studies was to develop a suitable High Performance Liquid Chromatography (HPLC) system in order to conduct quantitative analysis of ionic liquid biodegradation samples. Two different HPLC methods have been reported in the literature for identification of ionic liquids and their corresponding metabolites. Hydrophilic

interaction chromatography (HILIC)[4,5] and reverse-phase chromatography[2,6,7] have both been used in the chemical analysis of ionic liquids. HILIC is a normal-phase chromatography method that involves the use of organic solvents that are miscible in water, typically methanol or acetonitrile. HILIC is an especially useful method for hydrophilic or ionisable compounds and can improve obtained mass spectra. The stationary phase is polar and is generally made of a silica, hybrid, amide, cyano, amino or diol material. The mobile phase requires a high percentage of organic solvent and a much lower volume of aqueous solvent. The aqueous solvent acts as the eluting solvent in this type of chromatography. Retention of ILs on reverse-phase columns is based on hydrophobicity properties of the ILs. The stationary phase in reverse-phase chromatography is non-polar, composed of a modified silica based packing with C8 or C18 functionalities incorporated on the silica surface. A highly polar mobile phase system is then employed, generally containing a high water content. The organic solvent in this case acts as the eluting solvent.

6.2.1 Method development of HILIC HPLC system

Hydrophilic interaction chromatography (HILIC) has been employed by other groups as a means of quantitatively identifying ionic liquids and their biodegradation products.[4,5] HILIC chromatography was investigated in this work along with UV and MS detection. A UV spectrometric analysis of **272** CIL gave an absorbance maximum at the wavelength (λ) 210 nm. Ionic liquids typically display low absorbance maxima around 200 nm.[7,12] The use of acetonitrile (ACN) as a organic modifier can improve the low wavelength in UV detection of ionic liquids.[12] Furthermore, in HILIC chromatography a high volume of organic solvent is required, in order to elute the desired solute product. ACN acts as a suitable organic modifier in this type of liquid chromatography. Development of a suitable HILIC method was conducted using two columns; Waters Atlantis® HILIC Silica column (5 μm packing, 4.6 × 150 mm, Column A) and Waters XBridge™ Amide column (3.5 μm packing, 4.6 × 150 mm, Column B). Work was initially carried out on the Amide column (Column B), as a manufacturing error was encountered with Column A. Standard solutions of **272** (in ACN) at various concentrations were prepared and used as the standard injections for the method development process. Fresh standards were prepared regularly

and refrigerated until required. Initial methods involved the use of the UV detector at 210 nm. A mobile phase system composed of 50 % ACN and 50 % water (0.1 % formic acid) was attempted initially to gauge the relative retention of the CIL. This resulted in a large peak eluting off the column before the void volume at a retention time of 4.105 min (Figure 6.1).

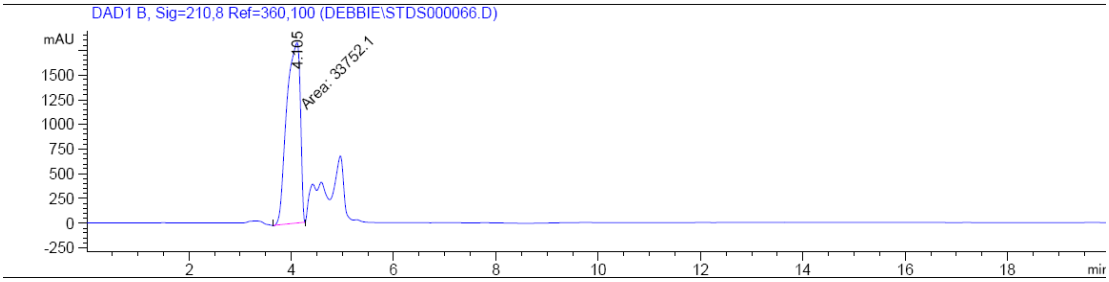


Fig. 6.1: HPLC-UV chromatogram of **272** (1 mg/mL) eluted from Column B using 50:50 ACN:Water (0.1 % formic acid).

Changing the mobile phase to 70:30 ACN:Water (0.1 % formic acid) an increase in retention time to 4.443 min was observed so that the peak was eluting just after the void volume. Increasing the volume of organic solvent (90:10 ACN:Water) and the flow rate of the system resulted in the product peak eluting at 3.517 min, however it was well resolved from the void volume (Figure 6.2).

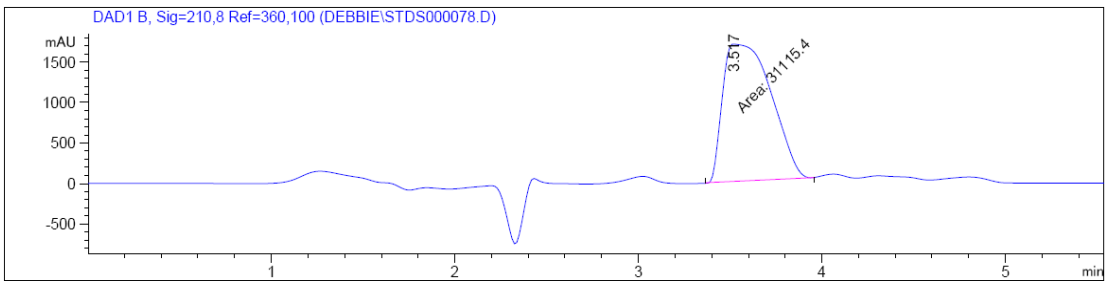


Fig. 6.2: HPLC-UV chromatogram of **272** (1 mg/mL) eluted from Column B using 90:10 ACN:Water (0.1 % formic acid).

However, the observed peak was quite broad and not of favourable shape. The mobile phase was further manipulated in an attempt to improve the peak shape. A binary mixture of 80 % ACN and 20 % 5mM ammonium formate buffer solution as the aqueous phase was

run. The analyte peak gave a retention time 5.351 min when using these system conditions, with improved peak shape also noted (Figure 6.3).

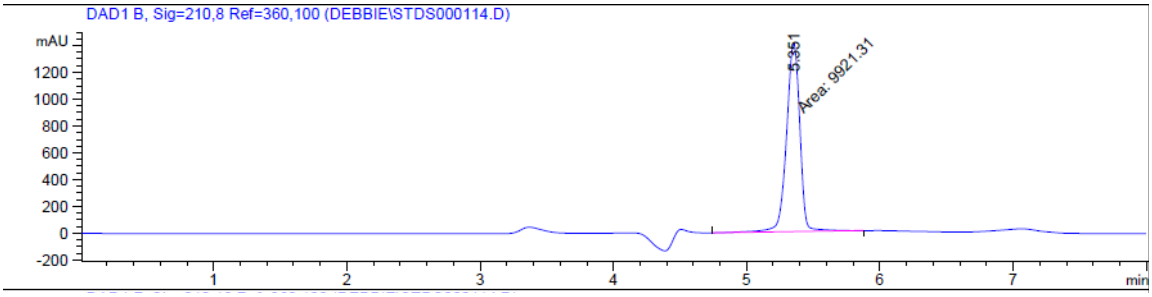


Fig. 6.3: HPLC-UV chromatogram of **272** (1 mg/mL) eluted from Column B using 80:20 ACN: 5mM ammonium formate buffer.

The mobile phase was once again altered, this time to obtain improved retention (longer retention time was desirable) and consequently better separation from the void volume. By changing the amount of buffer phase, the analyte was retained for a longer time, eluting at 6.812 min. The observed peak was very sharp with no evidence of fronting. Table 6.1 summarises the parameters employed in this method.

Table 6.1: HILIC-UV method used for identifying **272**, using Column B.

Column	Waters XBridge™ Amide column
Mobile phase	85:15, ACN: 5 mM Ammonium formate buffer
Flow rate	0.5 mL/min
Detection method	UV at 210 nm
Temperature	25°C
Run time	10 min
Injection volume	50 µL

This method was deemed suitable for the HPLC-UV analysis of the CIL **272**, and validation was therefore required. A calibration curve was constructed using the aforementioned method. A calibration curve is a plot of how an instrumental response changes with the concentration of the analyte. Various concentrations of **272** were injected and a plot of peak area versus concentration was obtained (Figure 6.4).

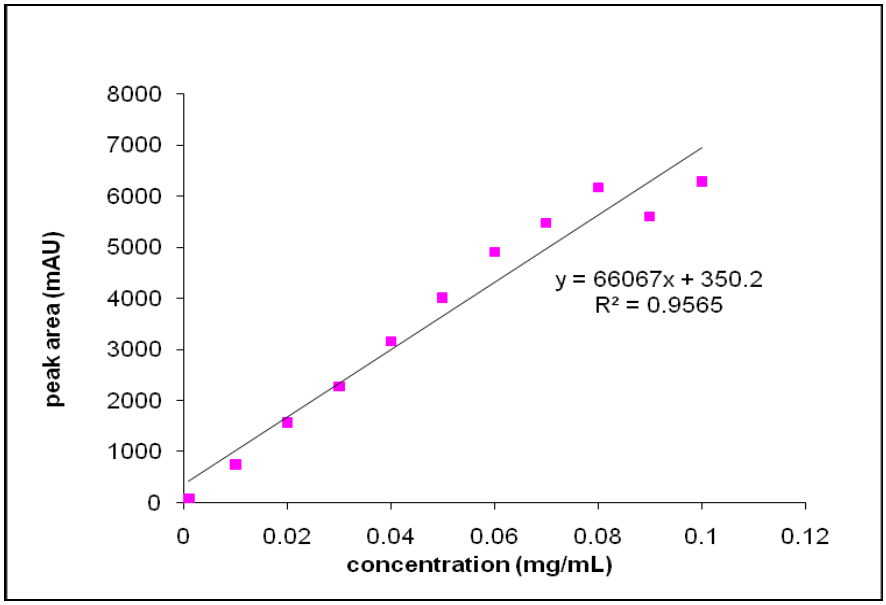


Fig. 6.4: Calibration curve using HILIC-UV method.

As can be seen in Figure 6.4, the plot obtained appeared linear up until standard concentrations of 0.08 - 0.10 mg/ml. The linearity of the calibration curve diminishes between these concentrations ranges. The reproducibility of the system was also studied as a means of validating the method. This test involved multiple injections of the same 0.10 mg/mL standard and subsequently monitoring reproducible peak areas. Peak areas and retention times both showed lack of precision from these standard injections. UV peak areas were obtained ranging from 5883.39 – 8205.79 mAU after multiple injections. Due to this lack of reproducibility using this mode of detection, MS detection was therefore investigated.

During the development of this method some issues arose with the HILIC column. The system pressure appeared to notably fluctuate. A regeneration of the column was performed as follows;

1. 25 ml of LCMS grade water was run through column.
2. 100 ml of Tetrahydrofuran (THF) was run through column.
3. 50 ml of Methanol was run through column.
4. 50 ml of water was run through column.
5. Equilibrated with mobile phase.

An extended equilibration time was required in order to stabilise the fluctuation in pressure. Following this a mobile phase system composed of 90:10 ACN:Water (0.1 % formic acid) was used for injections to the MS detector. Smaller injection volumes were also utilised so as to avoid overloading and consequently contaminating the MS system. Table 6.2 indicates the system parameters employed in this analytical method.

Table 6.2: HILIC-MS method used for identifying **272**, using Column B.

Column	Waters XBridge™ Amide column
Mobile phase	90:10, ACN: Water (0.1% F.A.)
Flow rate	0.4 mL/min
Detection method	MS detector
Temperature	25°C
Run time	15 min, 6 min post time
Injection volume	10 μL

Once this system was established as being a suitable method to analyse the IL samples, validation was sought. This required multiple injections of the CIL standard into the MS detector to give reproducible peak areas and retention times. As previously observed for the UV detection method, a very noticeable lack in reproducibility was observed. Figure 6.5 demonstrates an overlay TIC chromatogram of the standard injections.

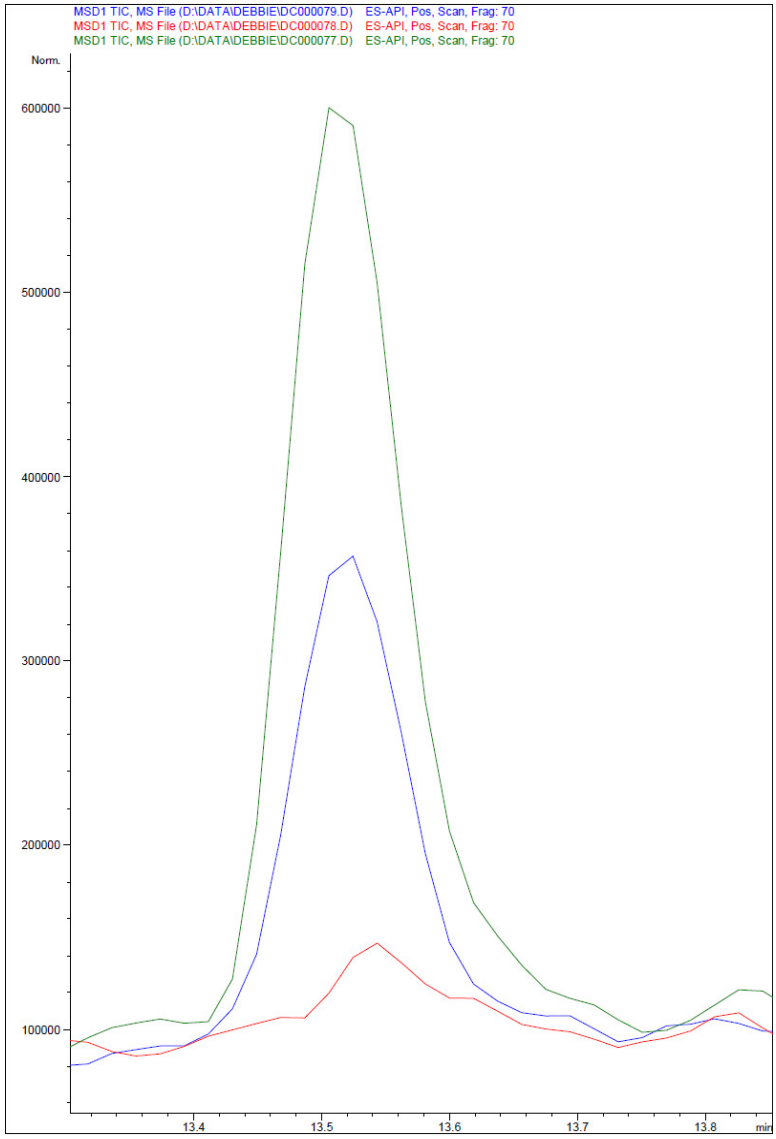


Fig. 6.5: Overlay MS TIC chromatogram of analyte peak (0.1 mg/ml).

Upon further consideration of this method (Table 6.2), a number of parameters were deemed unsuitable. Though the HILIC method has a short run time, 90 % ACN is used

throughout the run at flow rate of 0.4 mL/min. This results in the generation of copious amounts of organic waste. The use of high volumes of ACN in mobile phase systems is a growing concern due to the global shortage of this organic solvent. To try reduce the amount of organic waste produced during this analysis, and to also investigate further the lack of reproducibility, a second HPLC system was studied.

6.2.2 Method development of Reverse-Phase HPLC system

Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) is a commonly used analytical technique in many fields of chemistry. In this type of chromatography the stationary phase is typically composed of a non-polar material. In this work, columns containing octadecylsilica (C18) stationary phase packing were employed. The two RP columns employed were an Agilent XDB-C18 1.8 µm packing, 4.6 × 50 mm column (Column C) and an Agilent Eclipse XDB-C18 5 µm packing, 4.6 × 150 mm column (Column D). A polar mobile phase system is typically used in RP-methods. Binary mixtures containing a high percentage of water or aqueous buffers with a small percentage of organic solvents (namely acetonitrile or methanol) are generally employed.

The isocratic methods developed for the HILIC system were initially incorporated using the Agilent Eclipse XDB-C18 5 µm, 4.6 × 150 mm column (Column D). The eluted peaks appeared poorly resolved with equally poor peak shapes. In some cases the CIL standard eluted as two separate peaks as seen in Figure 6.6.

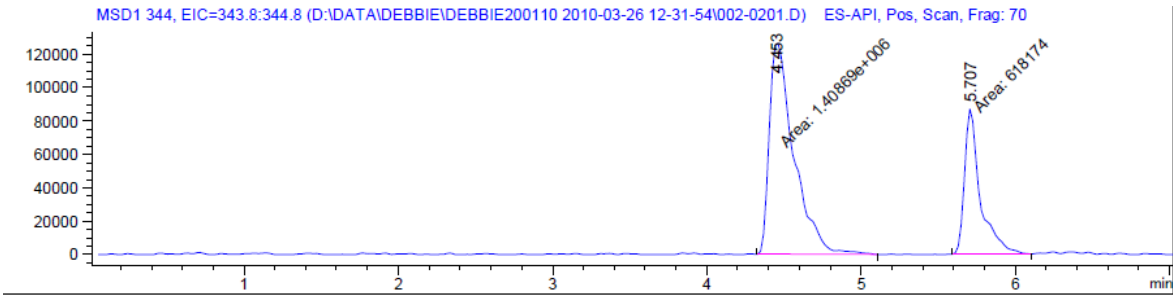


Fig. 6.6: Extracted ion count (EIC) MS chromatogram for **272**, using an isocratic method with 95:5 ACN: Water (0.1 % formic acid).

Previous HPLC work carried out in our research group involved the use of RP methods with gradient solvent systems. These solvent gradients were investigated in this work using the two C18 columns since the isocratic methods gave poor results. From this study, Column D gave superior peak resolution and shape over Column C, and was therefore chosen in developing a suitable RP system. Figures 6.7 and 6.8 illustrate TIC chromatograms obtained of **272** using Column C and Column D respectively.

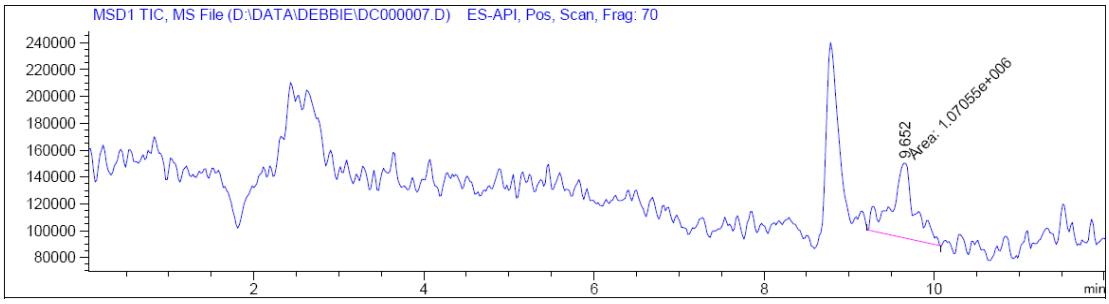


Fig. 6.7: TIC chromatogram of **272** using Agilent XDB-C18 1.8 µm, 4.6 × 50 mm column (Column C).

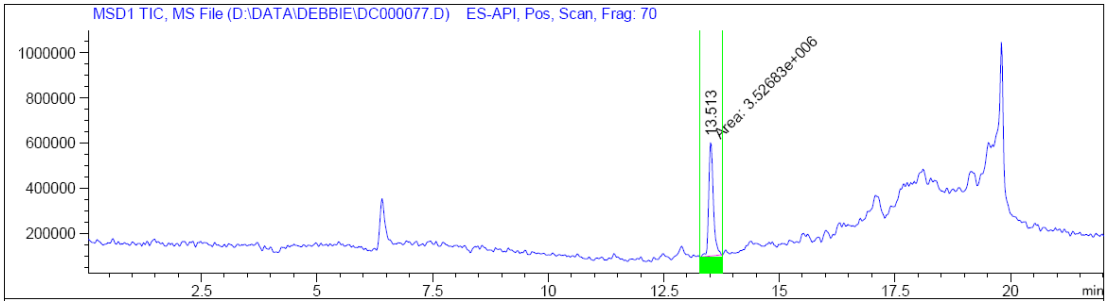


Fig. 6.8: TIC chromatogram of **272** using Agilent Eclipse XDB-C18, 5 µm 4.6 × 150 mm column (Column D).

A longer run time was employed when using the longer RP-column; 22 minute run time with an additional 6-7 minute pre-equilibration time. A gradient solvent phase consisting of a high volume of water initially (for 10 mins), then 100 % ACN (for 2 min) and then returning to 95:5 water:ACN (for final 10 minutes) was used. The final optimized method for the RP-HPLC method is summarized in Table 6.3. Once the method was developed, validation studies were conducted.

Table 6.3: RP-HPLC method developed to identify CIL **272**.

Column	Column D
Mobile phase	Gradient
	Time (*min*) / ACN:H_20 (*0.1%F.A.*)
	- 0 — 5:95
	- 10 — 100:0
	- 11 — 100:0
	- 12 — 5:95
Flow rate	0.25 mL/min
Detection method	MS detector
Temperature	25°C
Run time	22 min
Injection volume	10 µl and needle wash

As encountered previously with the HILIC systems, after the development of a suitable method reproducibility could not be achieved using the RP-method (Table 6.3). This issue of obtaining reproducibility and method validation could therefore be associated with instrumental errors.

6.3 Primary biodegradation and metabolite profiling studies of 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (272) CIL

6.3.1 Activated Sludge assay

A preliminary primary biodegradation test was conducted using a modified OECD method (OECD 301 A).[8] The inoculum used was an activated sludge mixed microbial community, collected from a pharmaceutical company's wastewater treatment facility (Dublin). The

sludge sample was pre-conditioned by aeration at room temperature for 5 days. After aeration, the activated sludge was washed three times with mineral nutrient medium (prepared according to OECD guideline 301 A).[8] The final supernatant was decanted and the solid sludge was re-suspended in mineral medium to give a concentration of 5 g/L suspended solids (SS). The CIL **272** was tested in duplicate at a concentration of 240 μM. All controls flasks were also tested in duplicate. Abiotic control flasks were prepared whereby a flask containing 240 μM of the test substance was subsequently poisoned by adding $HgCl_2$ at a concentration of 50 mg/L. Positive control flasks containing reference standard sodium-n-dodecyl sulfate (SDS) (240 μM) and inoculated medium, were also set up. Blank controls contained no test substrate only inoculated mineral medium. All the test flasks were capped with cotton plugs and subsequently incubated aerobically at 80 rpm in the dark at 25°C.[6] Figure 6.9 illustrates the experimental set-up. During the 28 day incubation period 500 μl duplicate samples were retrieved from the flasks every 3 to 4 days. All samples were then centrifuged (8000 rpm for 15 mins) before being analysed *via* ESI-MS.

Fig. 6.9: Activated sludge experimental set up.

Following analysis of the results obtained from this preliminary screen (see section 6.3.2) several adjustments were made to the activated sludge experimental procedure. These

changes included the addition of three abiotic controls; flasks containing the test substance and autoclaved inoculum (sterile control), flasks containing the test substance in mineral medium in the absence of activated sludge inoculum and flasks containing the test substance in distilled water (no inoculated medium) were all prepared. Additionally the flask agitation was also increased to 100 rpm to ensure a more homogenous mixing of the aqueous supernatant with the solid sludge particles.

6.3.2 ESI-MS analysis of CIL metabolites

Electro-spray ionisation mass spectrometry (ESI-MS) was used to qualitatively analyse the preliminary biodegradation samples of 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (**272**). Samples obtained from the biological experiments were directly infused (DI) into the MS detector using a Cole Palmer 749000 Series 100 µl syringe at an injection rate of 300 µL/hour (5 µL/min). Positive ion mode was generally used to obtain MS spectral data of biological samples. Figure 6.10 depicts the MS spectrum obtained of the initial testing day activated sludge sample (day 0). An intense peak at *m/z* 344 can be noted and indicates the presence of the parent CIL cation $[M\text{-}Br]^+$.

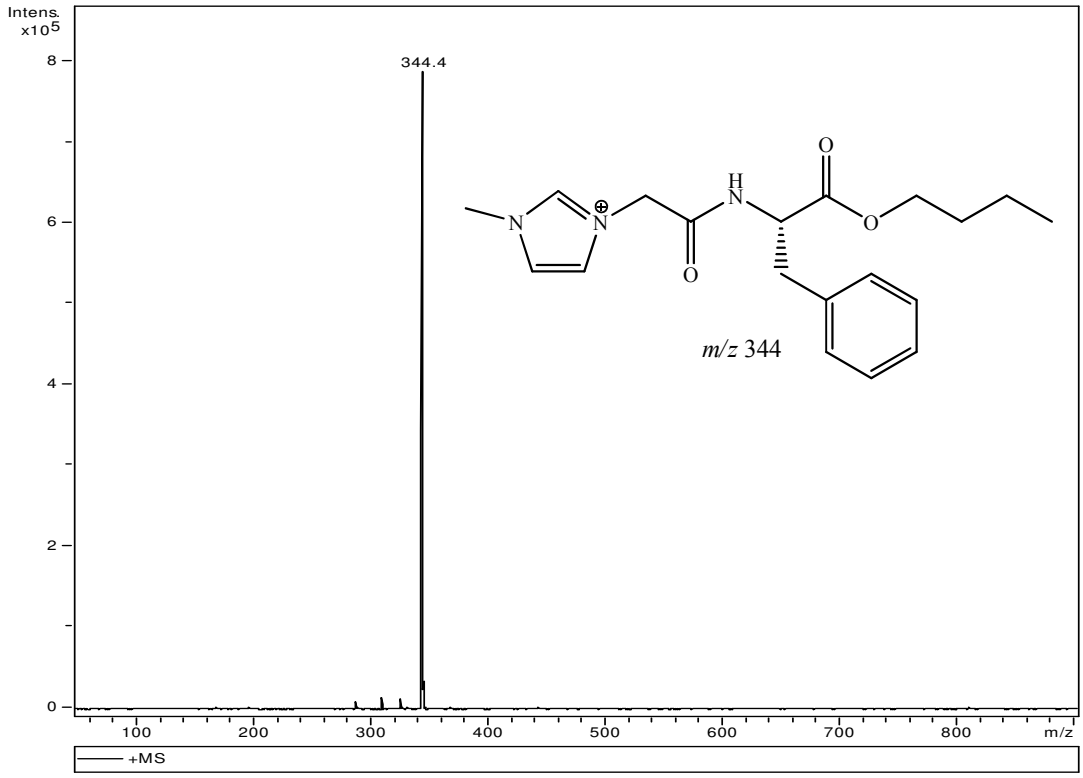
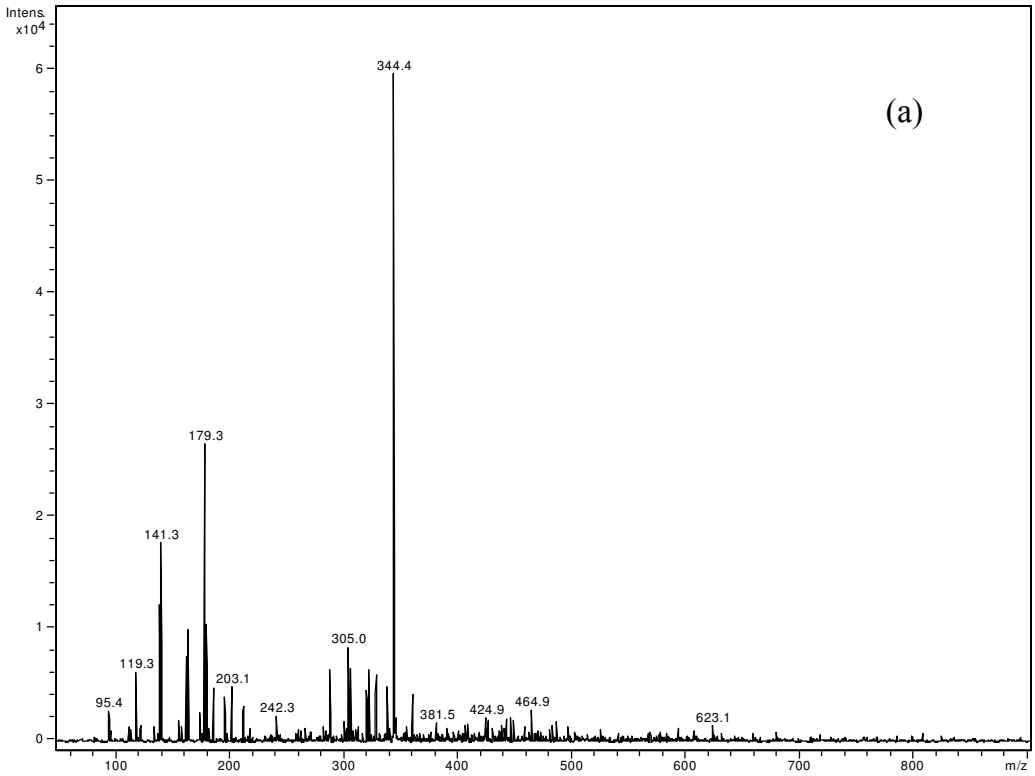


Fig. 6.10: MS spectrum of day 0 activated sludge sample of **272**.

This mass peak remained evident in the MS spectra until day 15 of the activated sludge test. However, the relative intensity of this peak decreases over time, and the appearance of a secondary mass peak becomes apparent. Figure 6.11 illustrates the MS spectra obtained for day 3, day 7 and day 15 of the assay.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141.3
305.0
119.3
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242.3
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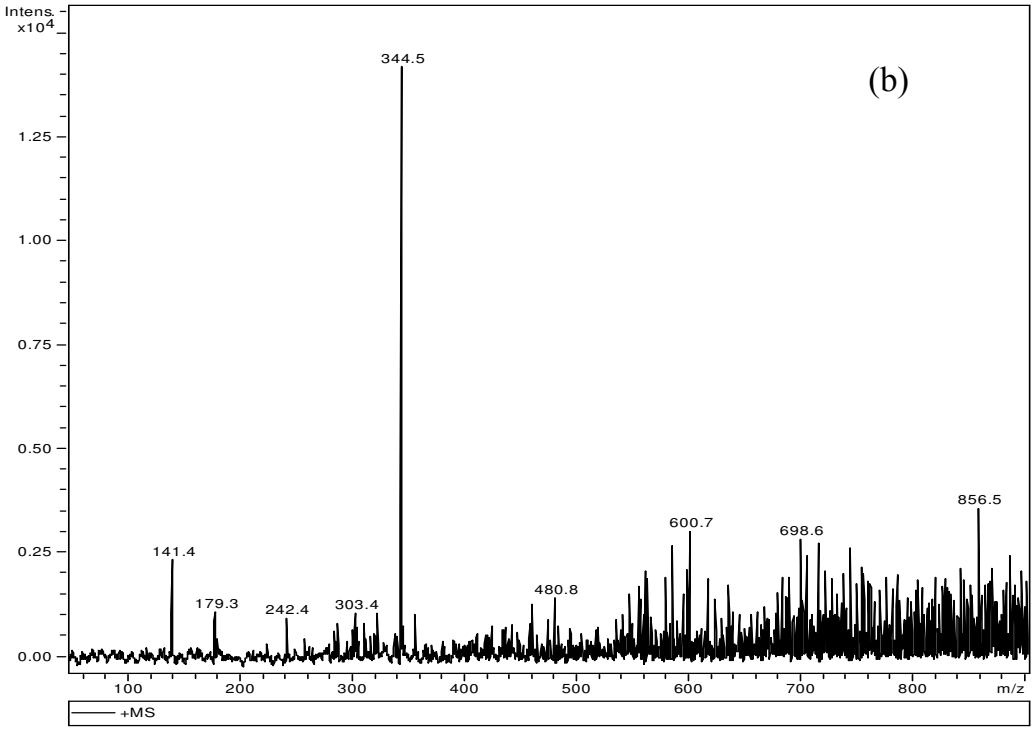
700
800
m/z
(a)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141.4
179.3
242.4
303.4
480.8
600.7
698.6
856.5
100
200
300
400
500
600
700
800
m/z
+MS
(b)

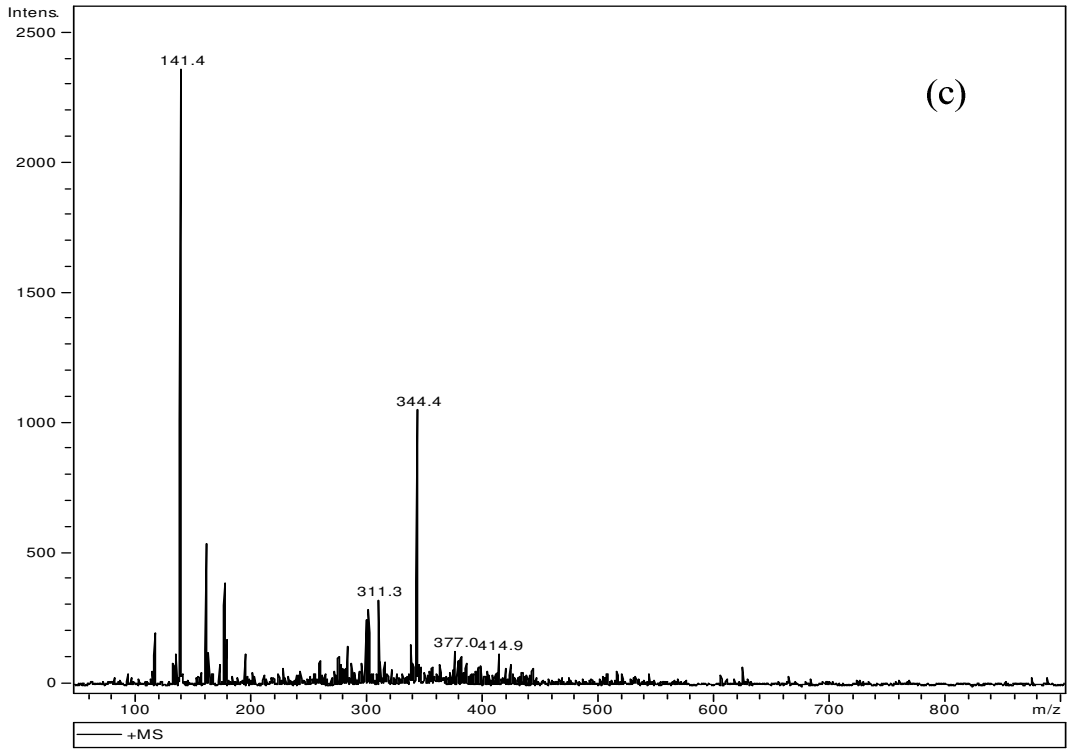


Fig. 6.11: MS spectra of (a) day 3, (b) day 7 and (c) day 15 of biodegradation test.

The mass peak at *m/z* 141 increases in intensity over time whilst the parent ion at *m/z* 344 appears to decrease. By day 28 the peak at *m/z* 344 is no longer evident and the most intense peak is seen at *m/z* 141 (Figure 6.12).

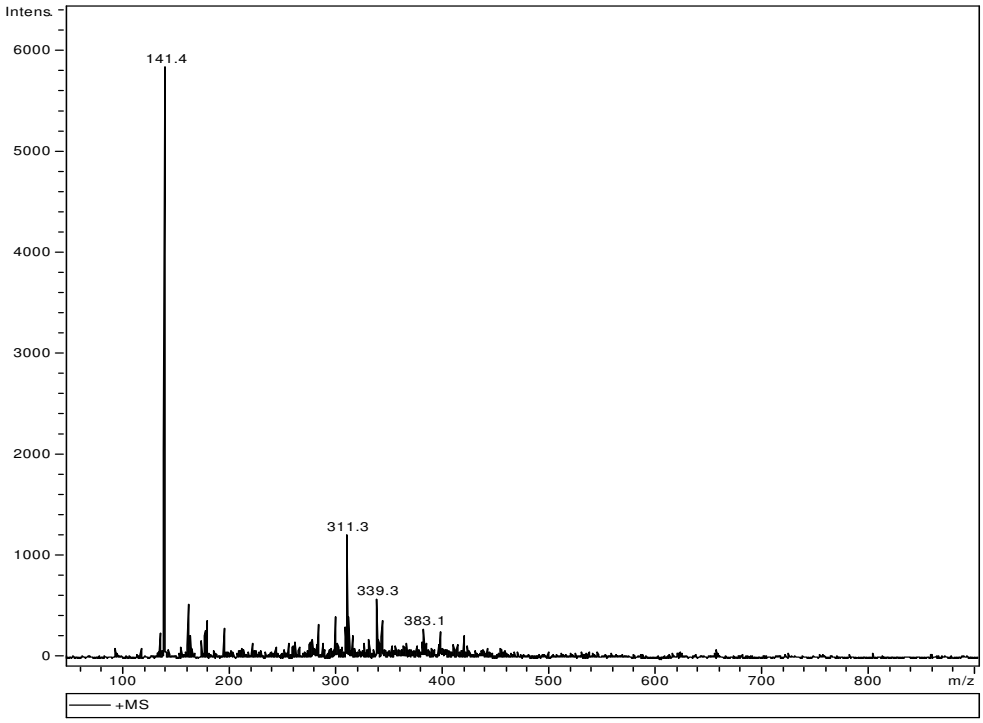


Fig. 6.12: MS spectra of day 28 activated sludge sample.

This mass peak may represent the presence of a metabolite structure of 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide. Enzymatic hydrolysis at the peptide bond of the chiral side chain of the IL may have occurred (Figure 6.13). This demonstrates a significant result as amide bonds are known to be more resistant to biological breakdown than, in particular, ester bonds.[9,10,11] As can be seen in Figure, the side chain of CIL **272** possesses both an ester and an amide functionality. Hydrolysis at the ester moiety can be noted, but gives a peak of very low intensity in the MS spectrum. The peak at *m/z* 311 can be attributed to the sodium adduct of the ester hydrolysed parent ion $[M\text{-}Br\text{-}C_4H_9]^+$.

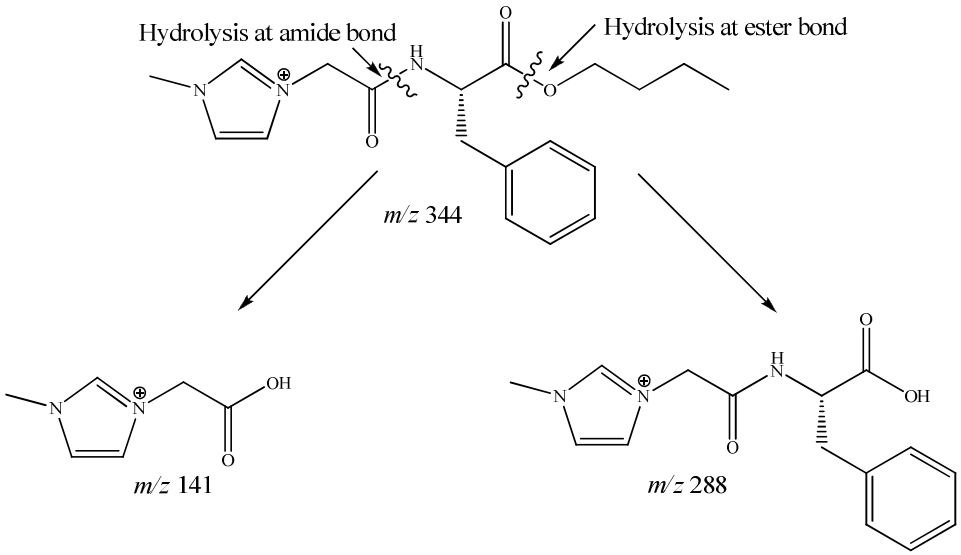
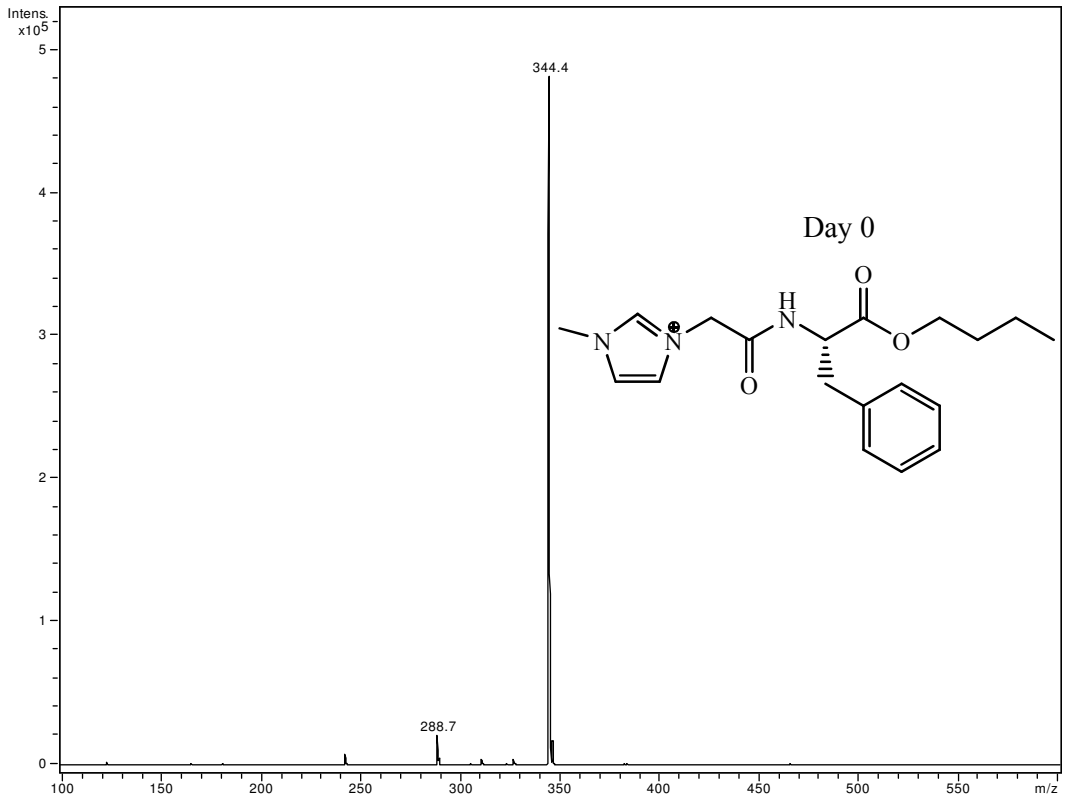


Fig. 6.13: Possible biodegradation mechanism of **272**.

However, degradation of the compound was also observed in the abiotic control samples. In these controls, the sludge was inactivated or poisoned by the addition of $HgCl_2$. Therefore biodegradation of the CIL is not expected in these flasks. The MS spectra of these samples indicate the disappearance of the intact parent cation (*m/z* 344) over time and the formation of the mass ion at *m/z* 141(Figure 6.14).


Intens.
x10^5
5
4
3
2
1
0
344.4
288.7
Day 0
N
N
H
N
O
O
O
100
150
200
250
300
350
400
450
500
550
m/z

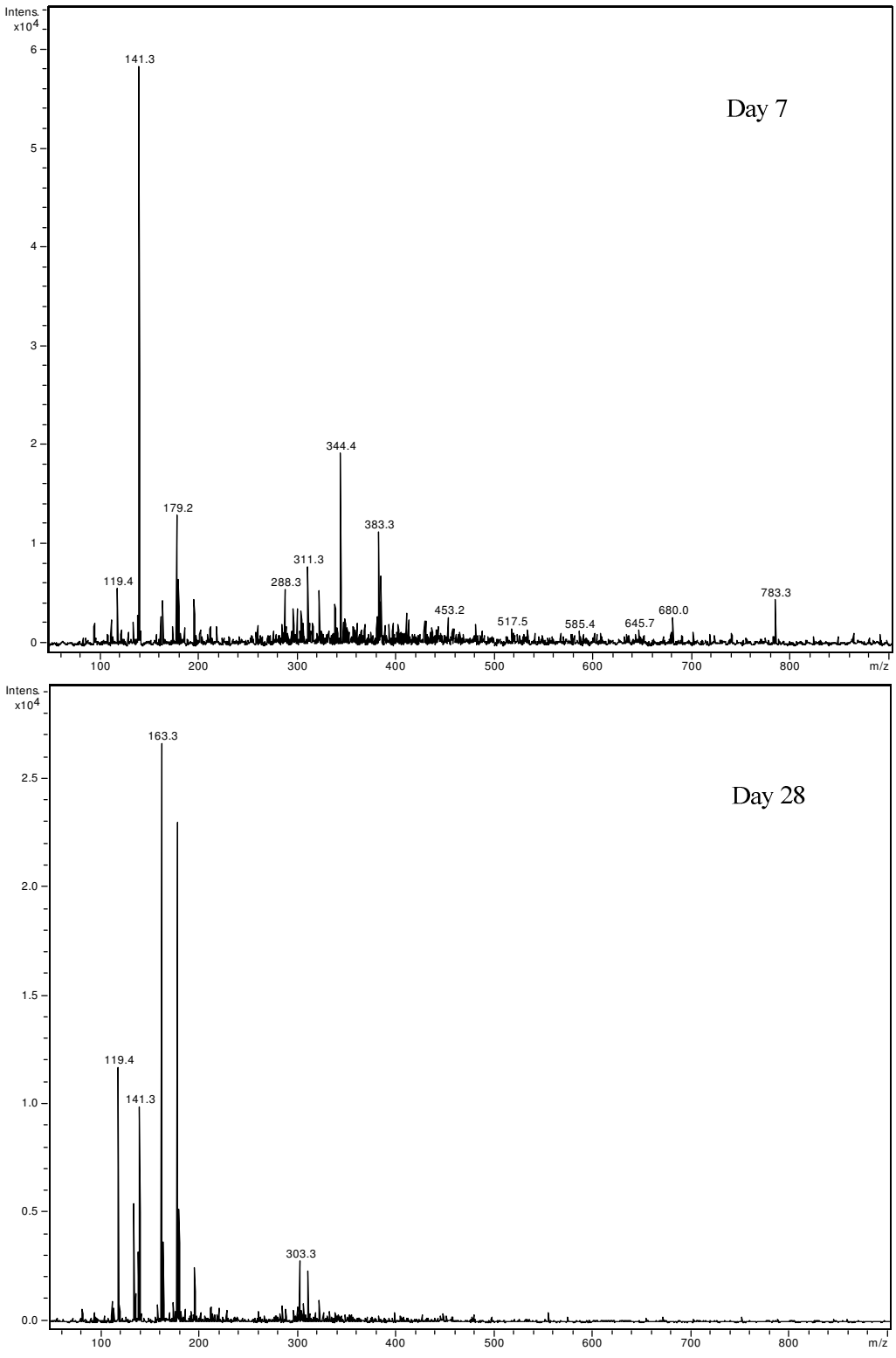


Fig. 6.14: Abiotic control samples after day 0, day 7 and day 28.

In the day 28 abiotic sample (Figure 6.14) the sodium adduct of the hydrolysis product can be seen as a high intensity peak (*m/z* 163) and the IL cation mass is no longer evident. This result would hence suggest that an abiotic degradation of the CIL occurred. The concentration of the $HgCl_2$ added to the control flask may not have been efficient in inactivating the sludge inoculum. Adsorption of the test substance to the solid sludge particles or a chemical hydrolysis of the IL side chain in aqueous media may also explain the disappearance of the parent molecule in both biotic and abiotic controls. Taking these factors into consideration, several additional abiotic controls were prepared in the subsequent experiments.

The experiments were repeated with several alterations made to the experimental procedure. A panel of additional controls was prepared in order to gauge a better understanding of the observed IL degradation. Sterile controls (test IL in the presence of autoclaved inoculated test media), controls with no activated sludge inoculum present (IL in mineral medium only) and a control containing the test IL in distilled water. The speed of the flask agitation was increased also to 100 rpm to ensure a homogenous mixing during incubation.

The MS data acquired from these sets of experiments yielded results similar to those observed in the preliminary tests. The mass ion at *m/z* 141 or its corresponding sodium and/or potassium adducts (*m/z* 163 and 179 respectively) was noted in both biotic and abiotic controls. The disappearance of the parent cationic species in the test samples from these experiments was rapid, compared to those noted in the preliminary data. The cation of **272** can be seen on the initial day of sampling (Figure 6.15) but is no longer observed in MS spectra of the next sampling day (Figure 6.16, Day 3). This may be due to the fact that the agitation speed of the incubated flasks was increased (from 80 to 100 rpm). A more homogenous mixture of the aqueous supernatant and the solid sludge could lend to this significant difference in degradation rates. However when the assay was repeated again at the higher agitation speed, the rate of degradation remained rapid (disappearance of parent cation after day 3 of testing). The difference in degradation rates between the various experimental runs may be as a result of different activated sludge samples employed. The

mixed microbial community of the activated sludge can vary from one sludge sample to the next.[1,2]

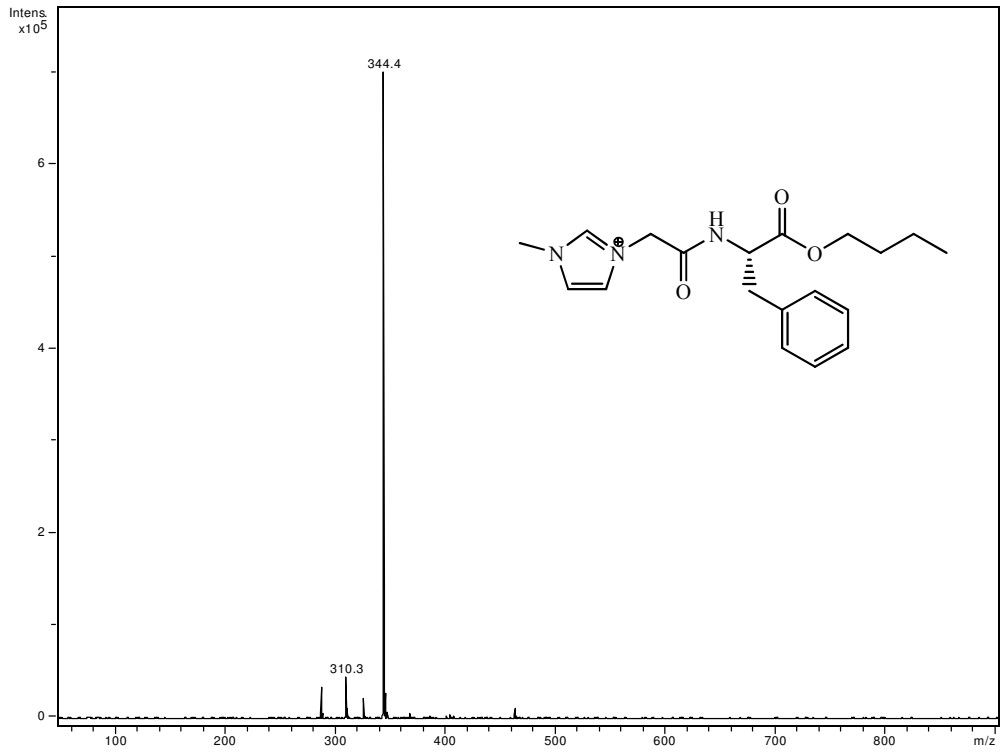


Fig. 6.15: MS spectrum of **272** (Day 0 activated sludge sample).

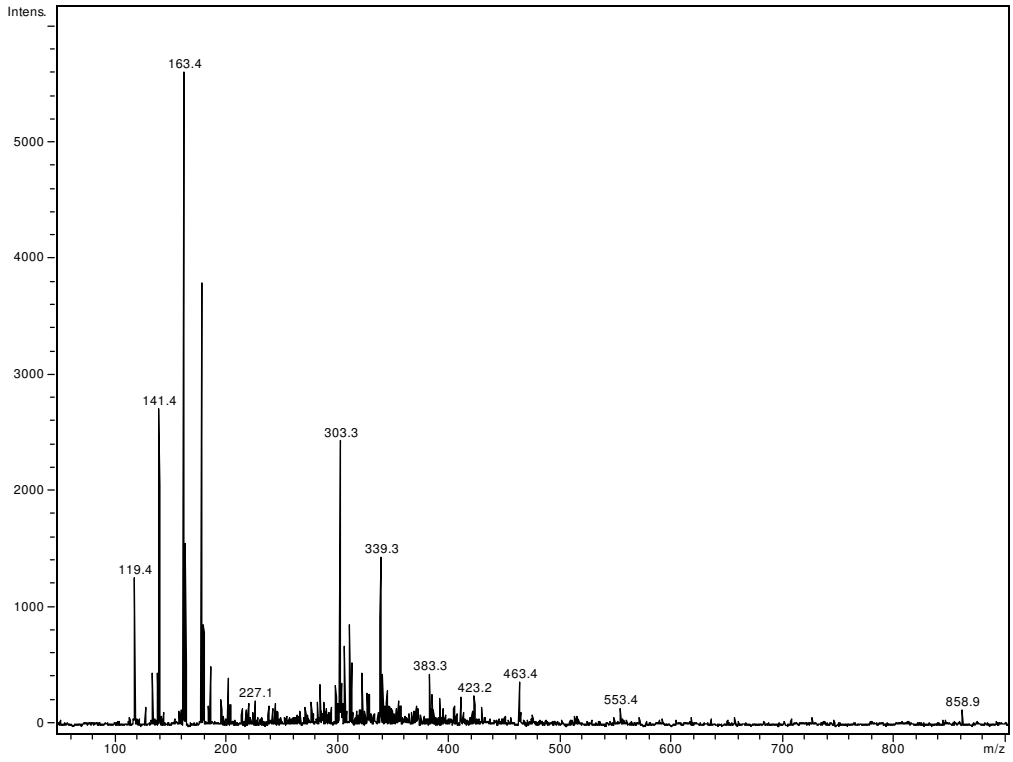


Fig. 6.16: MS spectrum of **272** (Day 3 activated sludge sample).

The abiotic controls all displayed similar MS data to the abovementioned biotic data with a clear absence of the parent ion at *m/z* 344. In the sterile activated sludge controls (where sludge was autoclaved prior to treatment with tested IL) removal of **272** occurred after day 3 of the test. This result was not anticipated since sterilising the inoculum would ensure that all the microorganisms in the sludge would be killed. Therefore biological mineralization of the IL would not be possible. The MS spectra of the abiotic controls containing no sludge inoculum indicated the presence of this mass ion (*m/z* 344) up until day 10 of the experiment. Additionally, the MS data of the test controls containing the CIL in distilled water only demonstrated removal of the parent cation after day 12 of the study (Figure 6.18).

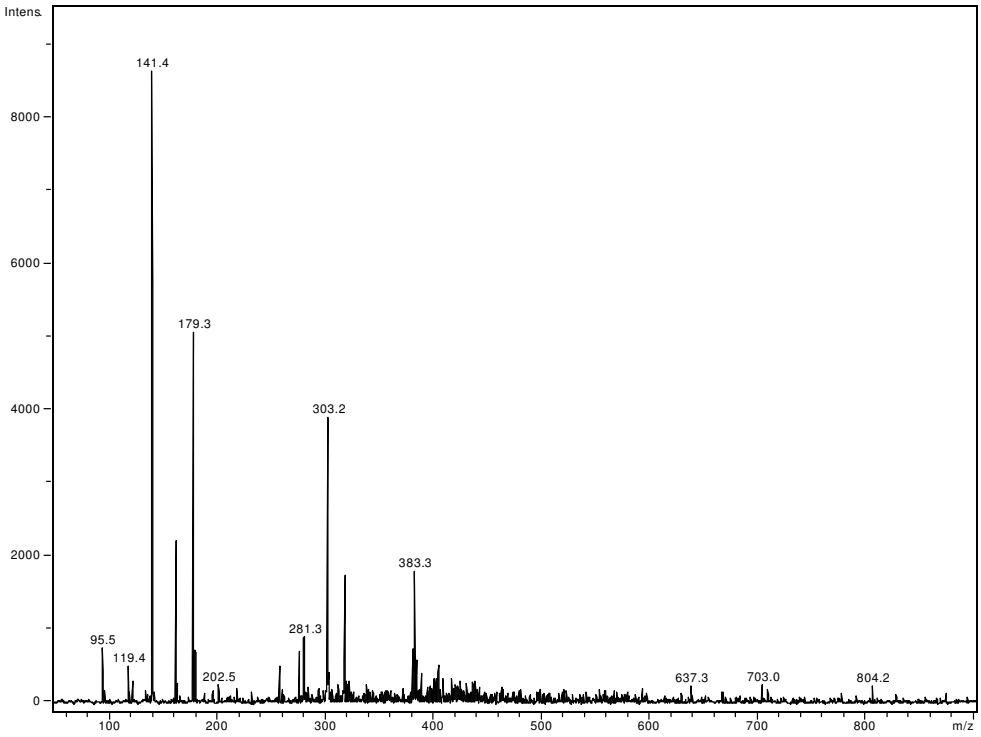


Fig. 6.17: MS spectrum of **272** in mineral nutrient medium after day 10.

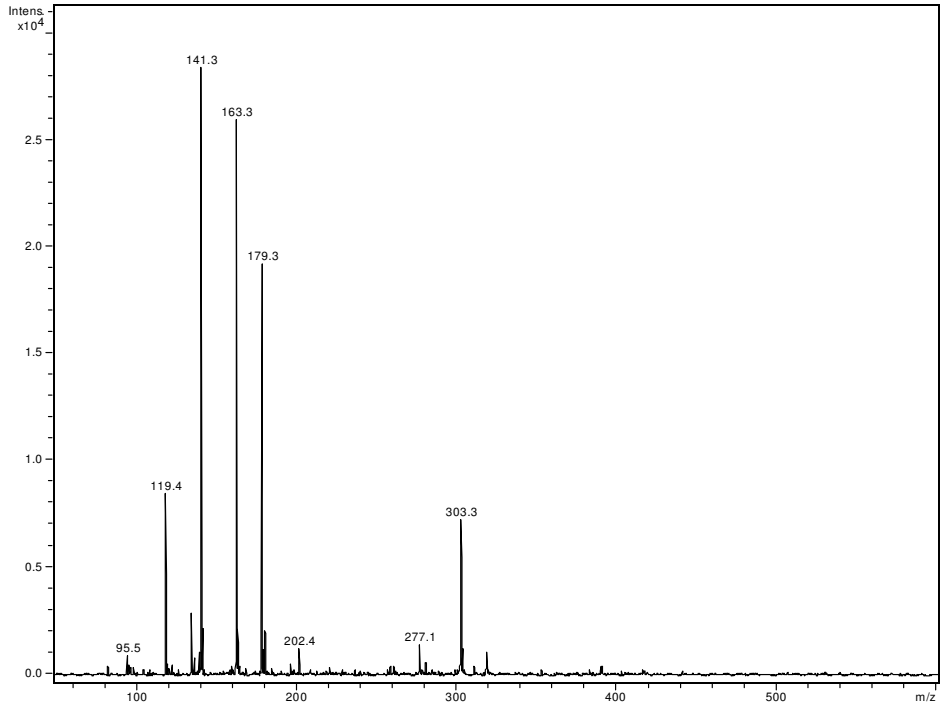


Fig. 6.18: MS spectrum of **272** in distilled water after day 12.

This data clearly indicates that biotic degradation of **272** did not occur in the test controls. The results would also suggest that the CIL **272** breaks down in aquatic environments over time. Although this CIL cannot be described as biodegradable from the obtained experimental results, it is envisaged that its persistence in aquatic environments may not be a concern.

6.4 Primary biodegradation and metabolite profiling studies of 3-methyl-1-L-phenylalanine-D-phenylalanine ethyl ester imidazolium bromide (378) CIL

6.4.1 Activated Sludge assay

The primary biodegradation of 3-methyl-1-L-phenylalanine-D-phenylalanine ethyl ester imidazolium bromide (**378**) CIL was studied using an activated sludge assay. The AS inoculum was treated as previously described (Section 6.3.1). In order to investigate possible abiotic degradation of the compound, a series of controls were run in parallel. These abiotic controls contained the same concentration of the test substance (240 μM) however in various test environments. Control flasks were prepared in which the activated sludge inoculum was inactivated or sterilized (poisoned by the addition of $HgCl_2$ or autoclaved respectively). Controls in which no sludge inoculum was present (i.e. control flasks containing the test CIL in mineral nutrient medium or in distilled water) were also set up. All the test vessels and controls was prepared in duplicate and kept at 100 rpm in the dark at 25 °C. The flasks were maintained under these conditions for the 28 day test period. Sampling was performed every 3 or 4 days and subsequently analysed *via* ESI-MS.

6.4.2 ESI-MS analysis of CIL metabolites

Following sampling from the biological test vessels, analysis was performed using ESI-MS. This technique allowed for qualitative analysis of the CIL biodegradation samples and identification of any possible metabolite products. Samples were introduced to the MS detector *via* a direct infusion method. Figure 6.19 depicts an MS spectrum obtained of an initial **378** biodegradation sample (day 0).

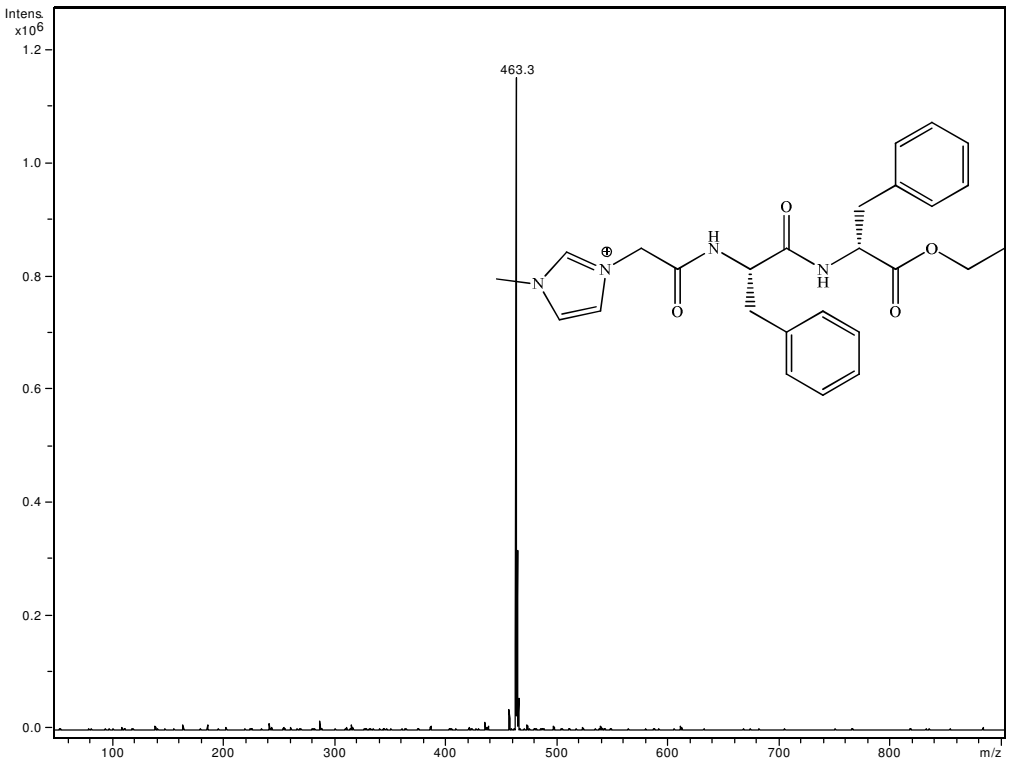


Fig. 6.19: MS spectrum of **378** activated sludge sample (Day 0).

An intense peak at *m/z* 463 indicates the presence of the intact parent cation of **378** [M-Br]$^+$. However this mass ion disappears after the next sampling day (Figure 6.20, Day 3) and other peaks become evident. This rapid removal of the CIL may be due to the organisms present in the activated sludge sample (may contain a higher diversity of microbes compared to previous batches). A physical interaction between the test chemical and the activated sludge may also explain the disappearance of the cation so quickly.

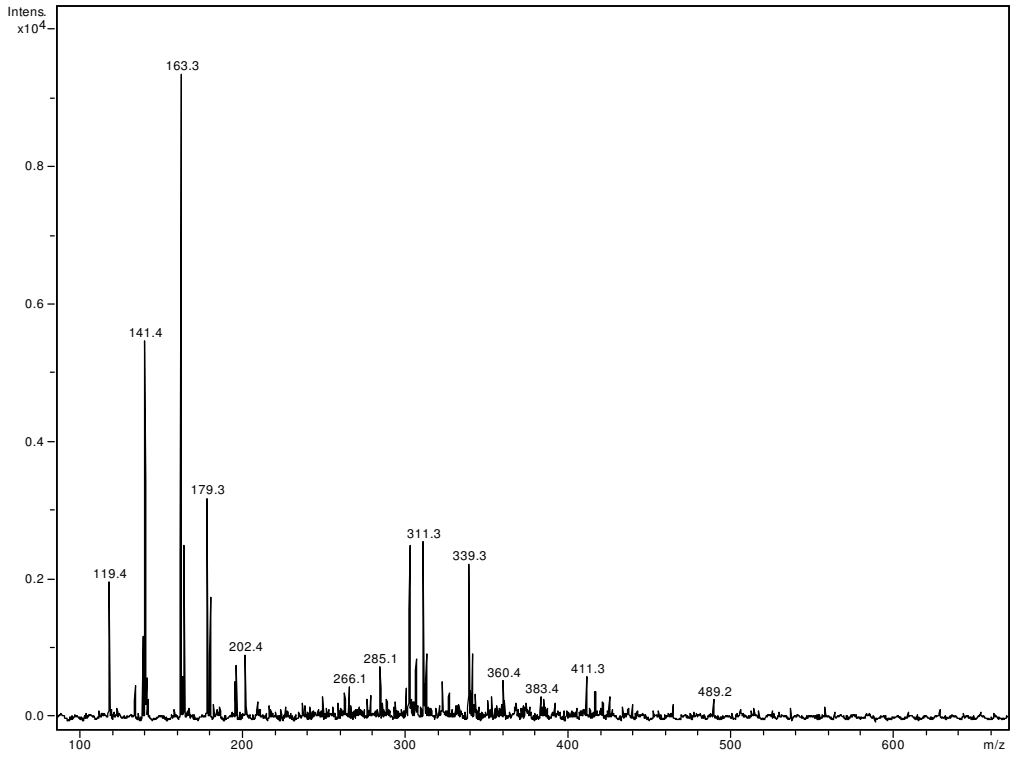


Fig. 6.20: MS spectrum of **378** activated sludge sample (Day 3).

From the spectra a peak at *m/z* 141 can be seen (Figure 6.20). This mass peak was seen previously in the biodegradation samples of the CIL 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (**272**). This mass arises from hydrolysis of the amide bond adjacent to the imidazolium cation (Figure 6.21). The peaks at *m/z* 163 and *m/z* 179 correspond to the sodium and potassium metal adducts. Figure 6.21 schematically depicts the chemical structure of **378**. The side chain of the CIL bears an ester and two amide functionalities which provide sites for possible enzymatic hydrolysis. However, it appears from the obtained MS data that hydrolysis mainly occurs at the bridging amide moiety.

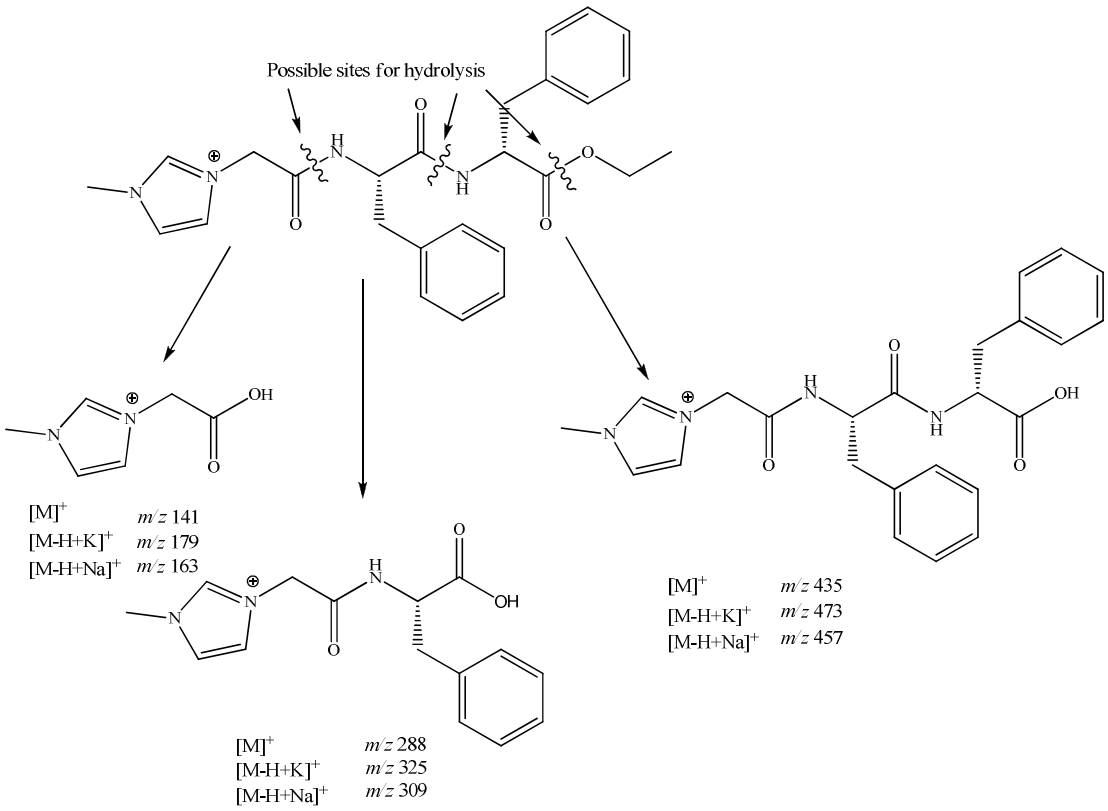


Fig. 6.21: Chemical structure of **378**, indicating possible sites for hydrolysis.

Similar MS data was obtained for the abiotic controls, with the removal of the CIL cation (*m/z* 463) observed after 3 days of the test. The disappearance of this mass ion was noted in the assay flasks containing poisoned (inactivated) and sterilized sludge inoculum. Moreover, degradation of the CIL (disappearance of parent ion) was also noted in the test vessels which contained no sludge inoculum (IL in distilled water or mineral medium). The rate of degradation in these samples was slower than inoculated abiotic controls. After ten days incubation, samples from these controls indicated the presence of the hydrolysis product at *m/z* 141 (Figure 6.22).

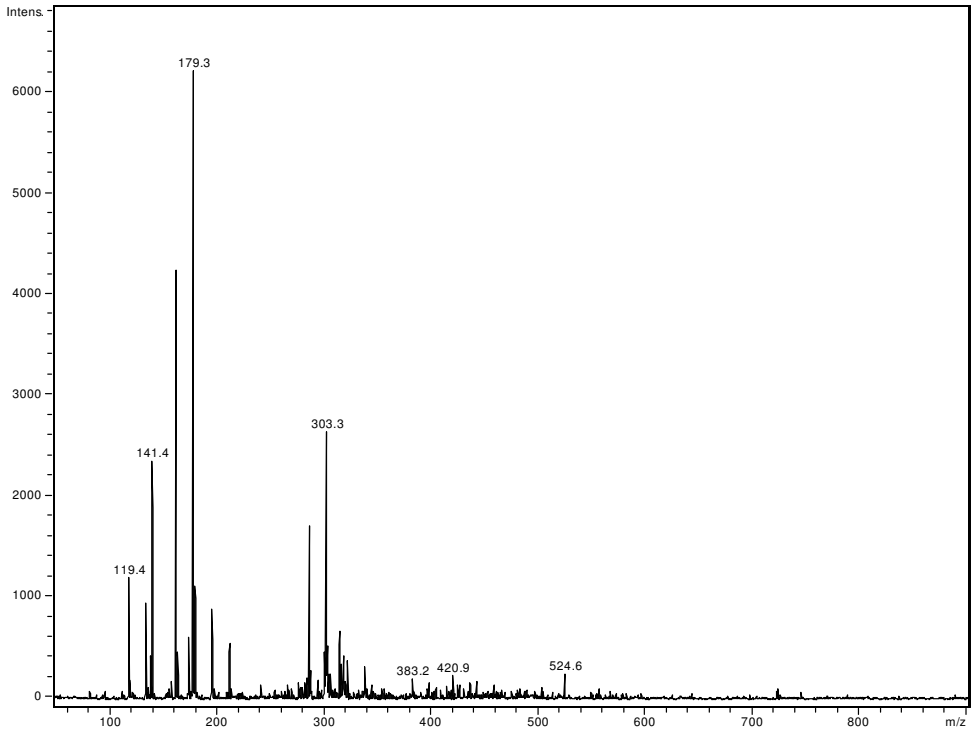


Fig. 6.22: MS spectrum of **378** in mineral medium (after 10 days).

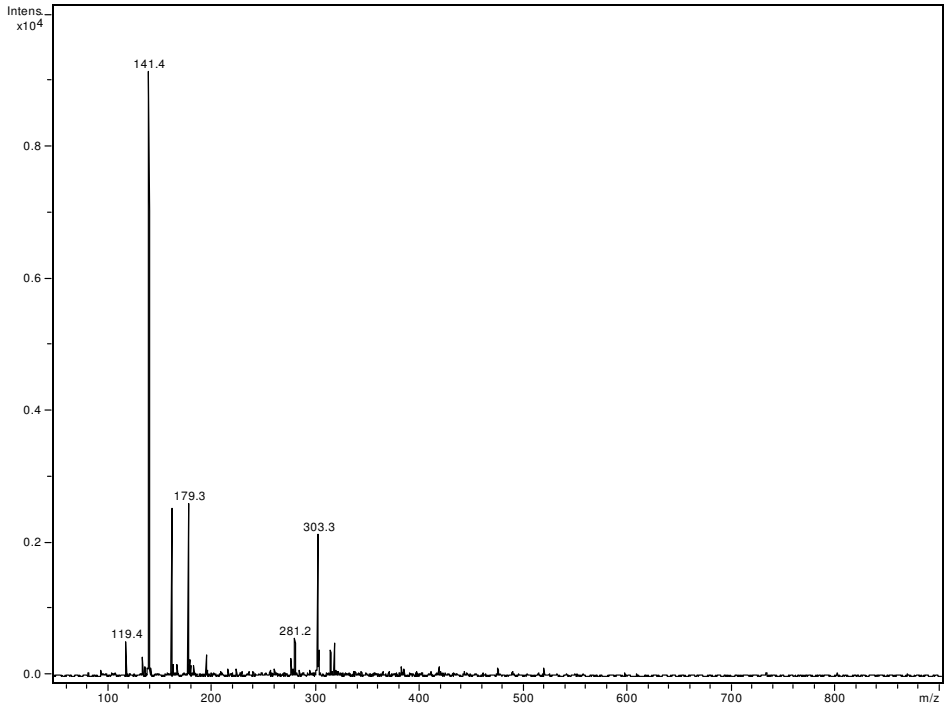
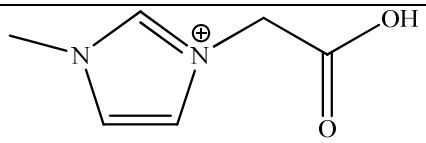


Fig. 6.23: MS spectrum of **378** in distilled water (after 10 days).

From screening both of these CILs for biodegradation, one metabolite structure can be proposed. 3-Carboxymethyl-1-methylimidazolium (*m/z* 141) is formed as a result of hydrolysis of the chiral side chain of the CIL. Albeit the CILs **272** and **378** displaying relatively low levels of antibacterial and antifungal activities (Chapter 5), the toxicity of the metabolite product must be known. As a result, 3-carboxymethyl-1-methylimidazolium bromide was synthesized[13] in the laboratory (see experimental, Chapter 7) and subsequently challenged against various strains of bacteria. This compound displayed low levels of toxicity against bacterial strains, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorenscens*, *Pseudomonas putida* (CP1), and *Pseudomonas putida* (KT2440). High MIC values (low toxicities) of 100000 μM (100 mM) were observed (Table 6.4) for all bacterial strains.

Table 6.4: MIC (μM) values of proposed metabolite, 3-carboxymethyl-1-methylimidazolium.



Strain	MIC (μM)
E. coli	100000
Bacillus subtilis	100000
P. fluorenscens	100000
P. putida (CP1)	100000
P. putida (KT2440)	100000

6.5 Conclusions

Two High Performance Liquid Chromatography (HPLC) methods were developed to identify novel chiral ionic liquids and their corresponding metabolite structures. Reverse-Phase (RP) and Hydrophilic interaction chromatography (HILIC) systems were studied with Mass Spectrometry (MS) employed as the detection method in both cases. However validation of the developed methods was not successful, most notably due to a lack of reproducibility. This lack of reproducibility was evident for both methods therefore indicating potential instrumental errors (i.e. lack of obtaining an analytical system). A Direct Infusion Electro-Spray Ionisation Mass Spectrometry (DI ESI-MS) method was hence employed to analyse IL biodegradation samples.

The biodegradation of two novel CILs (**272** and **378**) were studied using an activated sludge assay. In this assay an activated sludge inoculum (sampled from a wastewater treatment facility) was challenged against the test ILs over a 28-day period. Samples were withdrawn periodically (every 3-4 days) and subsequently analysed by MS. From the accumulated data obtained, it appeared that both CILs broke down (disappearance of parent ion masses from mass spectra) in the test vessels over the sampling period. However, this degradation could not be classified as a biotic breakdown of the test compounds as similar MS data was achieved in the experimental abiotic controls (i.e. sludge inoculum was poisoned, sterilised or absent from test flasks). A metabolite structure was proposed, namely 3-carboxymethyl-1-methylimidazolium, and synthesized in the laboratory. An antibacterial screen was performed on the metabolite compound (Table 6.4) in order to investigate its possible toxicity in the environment. Relatively high MIC values (low toxicities) were noted from this test.

6.6 References

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[13] J. Li, Y. Peng and G. Song, *Catal. Lett*., 2005, **102**, 159-162.

Chapter 7: Experimental

7.1 Introduction

7.1.1 Chemicals

All chemicals used in Chapters 2, 3 and 4 were purchased from Sigma Aldrich, with the exceptions of lithium bis(trifluromethanesulfonyl) imide ($LiNTf_2$) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (EDC) which were purchased from Solvionic and TCI Europe respectively. Methanol, ethanol, hexane and triethylamine were dried over molecular sieves and distilled before use. 1-butanol, 1-pentanol, 1-decanol were dried over molecular sieves and used without further purification. THF and diethyl ether were dried over sodium wire, then sodium benzophenone, and distilled before use. DCM was dried over calcium hydride, and distilled before use. Riedel de Haën silica gel was used for flash and thin layer chromatography.

Müeller Hinton broth, 96 well plates and nutrient broth were received from Sigma Aldrich and Oxoid and used as instructed (Chapter 5). In Chapter 6, ammonium formate, formic acid, water with 0.1% formic acid (HPLC-MS grade), acetonitrile with 0.1% formic acid (HPLC-MS grade) and $HgCl_2$ were obtained from Fluka. Potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium hydrogen orthophosphate (K_2HPO_4), disodium hydrogen orthophosphate dehydrate ($Na_2HPO_4{\cdot}2H_2O$), Iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) and bromoacetic acid were received from Aldrich. Ammonium chloride (NH_4Cl), calcium chloride anhydrous ($CaCl_2{\cdot}2H_2O$) and Magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) were obtained from Riedel de Haën. and Fluka respectively.

7.1.2 NMR

All NMR analysis was preformed on a Bruker AC 400 MHz spectrometer in deuterated chloroform or dimethyl sulfoxide (DMSO-d_6), operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. A 600 MHz spectrometer, operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR, was also used for analysis of some examples. Chemical shifts are reported in parts per million (ppm) are relative to the internal standard TMS and coupling constants (*J*) in Hertz (Hz).

Numbering of specific protons and carbons of the compounds is assigned for all alkylating agents, bromide and octylsulfate ($OctOSO_3$) salts. Numbering is then assigned for the first outlined compound of each bistriflimide anion (NTf_2). When stating multiplicity of peaks in NMR the following abbreviations are used; s-singlet, d-doublet, t-triplet, q-quartet, qt-quintet, dd-doublet of doublets, dt doublet of triplets, dq-doublet of quartets, tt-triplet of triplets, tq-triplet of quartets, m-multiplet, br-broad.

7.1.3 Optical Rotation

Optical rotations were measured using a Perkin Elmer 343 Polarimeter in chloroform, water or ethanol at 20 °C.

7.1.4 Melting point

Melting points were determined using a Griffin melting point apparatus and the values are expressed in degrees celcius (°C).

7.1.5 IR analysis

All IR analysis was carried out on a Perkin Elmer 100 FT-IR spectrometer with ATR. The strength of reported peaks are described as weak (w), medium (m), broad (b), strong (s) and very strong (vs).

7.1.6 MS

Mass spectrometry (MS) characterisation was obtained for novel (a)chiral ILs (from Chapters 2, 3 and 4). High resolution mass spectrometry was obtained for all bromide ILs. Low resolution mass spectrometry was obtained for all NTf_2 and $OctOSO_3$ ILs. Mass spectrometry analysis was not obtained for the starting materials (namely α-bromoester and amide intermediates) due to the reactivity and rapid hydrolysis of these compounds.

7.1.7 LC/MS

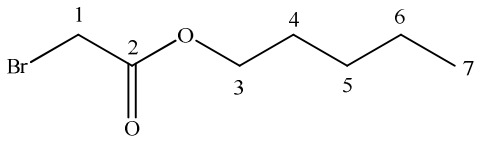
High Performance Liquid Chromatography (HPLC) analysis of biodegradation samples (Chapter 6) was carried out on an Agilent Technologies 1200 Series Liquid

Chromatography system, with a degasser and quaternary pump (G1311A), and Dual Loop Autosampler (DLA-G2258A). Agilent Chemstation software was also utilised in processing obtained spectral data (Agilent ChemStation Rev.B.03.01-SR1 [317]). MS analysis was preformed on an Agilent Technologies 6110 Mass Spectrometer (Quadrupole G6110A) and a Bruker Esquire 3000 Mass spectrometer. Direct Infusion Electrospray Ionization (DI-ESI MS) was carried out using a Cole Palmer 749000 Series 100 µl syringe pump.

7.2 Chapter 2 experimental-Preparation of Achiral Ionic liquids

7.2.1 Preparation of achiral bromoesters

***Representative procedure for the preparation of achiral bromoesters*; Pentyl-2-bromoacetate (188)**



To a stirred solution of DCM (300 mL), pentanol (26.421 g, 300.0 mmol), and triethylamine (55.30 mL, 400.0 mmol) under a nitrogen atmosphere at -78 ºC was added dropwise bromoacetyl bromide (72.648 g, 360 mmol). After stirring at -78 ºC for 3 h, the reaction mixture was allowed warm up to -20 ºC and quenched by addition of water (60 mL). The organic phase was washed with distilled water (3 x 60 mL), saturated ammonium chloride (3 x 60 mL), saturated sodium bicarbonate (3 x 60 mL) and brine (3 x 60 mL). The organic phase was then dried over magnesium sulfate, filtered and solvents removed *via* rotary evaporation to yield to give a brown liquid at RT in 79 % yield. The crude product was then distilled to give the title compound (**188**) as a pale yellow oil at RT in 64 % yield (40.223 g, 192.0 mmol).

Molecular formula $C_7H_{13}BrO_2$

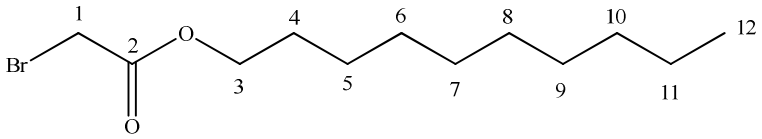
Molecular weight 209 $gmol^{-1}$

^{1}H NMR* (400 MHz, $CDCl_3$) δ (ppm) 4.04 (t, *J* = 6.8 Hz, 2H, *H3*), 3.74 (s, 2H, *H1*), 1.60 (tt, *J* = 7.2, 6.8 Hz, 2H, *H4*), 1.30-1.21 (m, 4H, *H5,6*), 0.82 (t, *J* = 7.2 Hz, 3H, *H7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.35 (*C*O,*C2*), 65.54 (O*C*H$_2$,*C3*), 28.69 (*C*H$_2$,*C4*), 27.37 (*C*H$_2$,*C5/C6*), 25.03 (*C*H$_2$,*C1*), 22.37 (*C*H$_2$,*C5*/*C6*), 13.98 (*C*H$_3$,*C7*)

The NMR data in agreement with literature[1]

Decyl-2-bromoacetate (189)



The title compound (**189**) was prepared from decan-1-ol (31.651 g, 200.0 mmol) and bromoacetyl bromide (48.440 g, 240.0 mmol) according to the general procedure (Section 7.2.1, page 252) as a colourless oil in 52 % yield (29.008 g, 103.97 mmol).

Molecular formula $C_{12}H_{23}BrO_2$

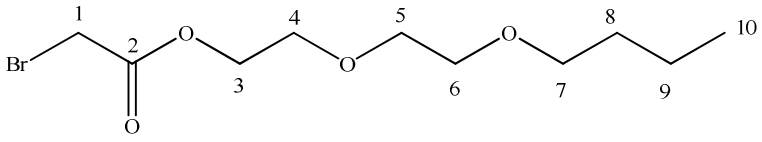
Molecular weight 279 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.10 (t, *J* = 6.6 Hz, 2H, *H3*), 3.76 (s, 2H, *H1*), 1.62 (tt, *J* = 7.4, 6.6 Hz, 2H, *H4*), 1.48-1.24 (m, 14H, *H5-11*), 0.81 (t, *J* = 6.8 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.45 (*C*O,*C2*), 67.52 *(*O*C*H$_2$,*C3)*, 62.49 (*C*H$_2$,*C4*), 34.16 (*C*H$_2$), 32.95 (*C*H$_2$), 32.77 (*C*H$_2$), 28.69 (*C*H$_2$), 28.58 (*C*H$_2$), 28.34 (*C*H$_2$), 28.19 (*C*H$_2$,*C1*), 22.64 (*C*H$_2$), 14.12 (*C*H$_3$,*C12*)

The NMR data in agreement with literature[2]

2-(2-butoxyethoxy)ethyl-2-bromoacetate (190)



The title compound (**190**) was prepared from butoxy ethoxyethanol (28.253 g, 200.0 mmol) and bromoacetyl bromide (48.339 g, 240.0 mmol) according to the general procedure (Section 7.2.1, page 252) as a pale yellow liquid in 61 % yield (34.561 g, 122.12 mmol).

Molecular formula $C_{10}H_{19}BrO_4$

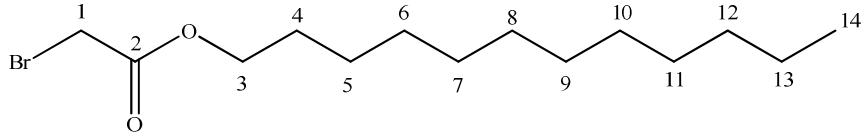
Molecular weight 283 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.30 (t, *J* = 6.0 Hz, 2H, *H3*), 3.82 (s, 2H, *H1*), 3.60 (t, *J* = 6.0 Hz, 2H, *H4*), 3.51 (t, *J* = 4.6 Hz, 2H, *H5*), 3.44 (t, *J* = 4.6 Hz, 2H, *H6*), 3.33 (t, *J* = 6.8 Hz, 2H, *H7*), 1.56 (tt, *J* = 7.6, 6.8Hz, 2H, *H8*), 1.38 (tq, *J* = 7.6, 7.2 Hz, 2H, *H9*) 0.90 (t, *J* = 7.2 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 168.28 (*C*O,*C2*), 71.23 (O*C*H_2,*C7*), 71.12 (O*C*H_2,*C6*), 70.69 (O*C*H_2,*C5*), 68.81 (O*C*H_2,*C4*), 64.38 (O*C*H_2,*C3*), 31.64 (*C*H_2,*C8*), 24.78 (*C*H_2,*C1*), 19.22 (*C*H_2,*C9*), 14.06 (*C*H_3,*C10*)

NMR data in agreement with literature[2]

Dodecyl-2-bromoacetate (191)



The title compound (**191**) was prepared from 1-dodecanol (18.626 g, 120.00 mmol) and bromoacetyl bromide (30.273 g, 150.00 mmol) according to the general procedure (Section 7.2.1, page 252) as a pale yellow liquid in 51 % yield (18.901 g, 61.76 mmol).

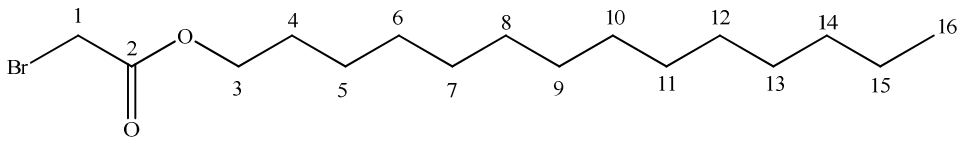
Molecular formula $C_{14}H_{27}BrO_2$

Molecular weight 307 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.08 (t, *J* = 6.8 Hz, 2H, *H3*), 3.75 (s, 2H, *H1*), 1.62 (tt, *J* = 7.6, 6.8 Hz, 2H, *H4*), 1.32-1.20 (m, 18 H, *H5-13*), 0.82 (t, *J* = 7.0 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 167.35 (*C*O,*C2*), 66.48 (O*C*H_2,*C3*), 31.99 (*C*H_2), 29.68 (*C*H_2), 29.63 (*C*H_2), 29.56 (*C*H_2), 29.49 (*C*H_2), 29.19 (*C*H_2), 28.58 (*C*H_2), 28.19 (*C*H_2,*C4*), 25.96 (*C*H_2,*C1*), 25.74 (*C*H_2), 22.70 (*C*H_2), 14.13 (*C*H_3,*C14*).

Tetradecyl-2-bromoacetate (192)



The title compound (**192**) was prepared from 1-tetradecanol (24.401 g, 144.00 mmol) and bromoacetyl bromide (28.259 g, 172.80 mmol) according to the general procedure (Section 7.2.1, page 252) as a pale yellow liquid in 42 % yield (20.45 g, 61.23 mmol).

Molecular formula $C_{16}H_{31}BrO_2$

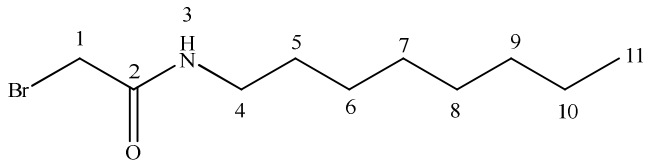
Molecular weight 335 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 4.11 (t, *J* = 6.8 Hz, 2H, *H3*), 3.84 (s, 2H, *H1*), 1.62 (tt, *J* = 7.4, 6.6 Hz, 2H, *H4*), 1.32-1.19 (m, 22H, *H5-15*), 0.83 (t, *J* = 6.8 Hz, 3H, *H16*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.35 (*C*O,*C2*), 64.48 (O*C*H_2,*C3*), 30.93 (*C*H_2), 29.79 (*C*H_2), 29.73 (*C*H_2), 29.68 (*C*H_2), 29.65 (*C*H_2), 29.58 (*C*H_2), 29.50 (*C*H_2), 29.19 (*C*H_2), 28.60 (*C*H_2), 28.18 (*C*H_2,*C4*), 25.86 (*C*H_2), 25.70 (*C*H_2,*C1*), 22.80 (*C*H_2), 14.16 (*C*H_3,*C16*)

7.2.2 Preparation of achiral bromoamides

***General procedure for the preparation of achiral bromoamides*; 2-bromo-*N*-octylacetamide (203)**



To a stirred solution of DCM (90 mL), octylamine (4.080 g, 32.0 mmol), and triethylamine (4.155 g, 41.0 mmol) under a nitrogen atmosphere at -78 ºC was added dropwise bromoacetyl bromide (7.728 g, 38.0 mmol). After stirring at -78 ºC for 30 mins, the reaction mixture was allowed warm up to room temperature and allowed to stir for 5 hours. The reaction was then quenched by addition of water (60 mL). The organic phase was washed with distilled water (3 x 60 mL), saturated ammonium chloride (3 x 60 mL),

saturated sodium bicarbonate (3 x 60 mL) and brine (3 x 60 mL). The organic phase was then dried over magnesium sulfate, filtered and solvents removed *via* rotary evaporation to yield to give a brown liquid at RT in 57 % yield. The crude product was distilled to yield the title compound (**203**) in 44 % yield (3.548 g, 14.19 mmol) as a pale yellow liquid.

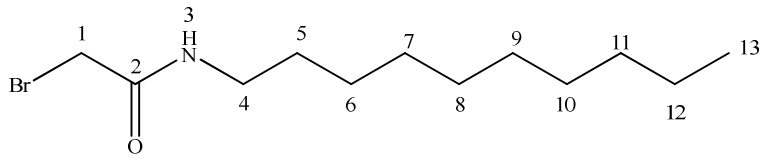
Molecular formula $C_{10}H_{20}BrNO$

Molecular weight 250 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.92 (t, *J* = 8.0 Hz, 1H, *H3*), 3.89 (s, 2H, *H1*), 3.26 (dt, *J* = 7.2, 7.0 Hz, 2H, *H4*), 1.57 (tt, *J* = 7.2, 6.8 Hz, 2H, *H5*), 1.32-1.25 (m, 10H, *H6-10*), 0.86 (t, *J* = 7.0 Hz, 3H, *H11*)

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 165.69 (*C*O,*C2*), 40.29 (HN*C*H_2,*C4*), 31.74 (*C*H_2), 29.23 (*C*H_2,*C5*), 29.19 (*C*H_2), 29.14 (*C*H_2), 26.90 (*C*H_2,*C1*), 26.57 (*C*H_2), 22.60 (*C*H_2), 14.06 (*C*H_3,*C11*)

2-bromo-*N*-decylacetamide (204)



The title compound (**204**) was prepared from decylamine (5.011 g, 32.0 mmol) and bromoacetyl bromide (7.691 g, 38.0 mmol) according to the general procedure (Section 7.2.2, page 255) as a pale orange solid in 71 % yield (6.315 g, 22.71 mmol).

m.p. 58-60 °C

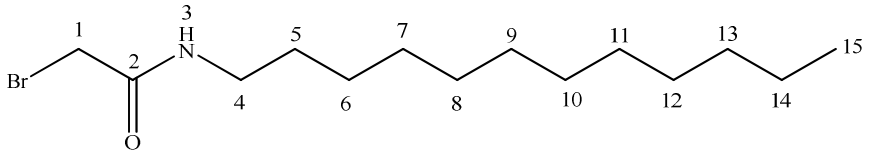
Molecular formula $C_{12}H_{24}BrNO$

Molecular weight 278 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.43 (t, *J* = 8.0 Hz, 1H, *H3*), 3.84 (s, 2H, *H1*), 3.24 (dt, *J* = 7.0, 7.0 Hz, 2H, *H4*), 1.47 (tt, *J* = 7.4, 7.0 Hz, 2H, *H5*), 1.23-1.17 (m, 14 H, *H6-12*), 0.83 (t, *J* = 6.8 Hz, 3H, *H13*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.27 (*C*O,*C2*), 40.31 (HN*C*H$_2$,*C4*), 31.90 (*C*H$_2$,*C5*), 29.61 (*C*H$_2$), 29.54 (*C*H$_2$), 29.53 (*C*H$_2$), 29.45 (*C*H$_2$), 29.32 (*C*H$_2$), 29.26 (*C*H$_2$,*C1*), 26.83 (*C*H$_2$), 22.71 (*C*H$_2$), 14.16 (*C*H$_3$,*C13*)

2-bromo-*N*-dodecylacetamide (205)



The title compound (**205**) was prepared from dodecylamine (15.002 g, 80.90 mmol) and bromoacetyl bromide (19.381 g, 97.10 mmol) according to the general procedure (Section 7.2.2, page 255) as a pale orange solid in 92 % yield (22.885 g, 74.78 mmol).

m.p. 64-66 °C

Molecular formula $C_{14}H_{28}BrNO$

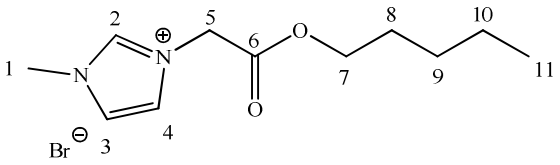
Molecular weight 306 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.41 (t, *J* = 8.8 Hz, 1H, *H3*), 3.82 (s, 2H, *H1*), 3.21 (dt, *J* = 7.0, 6.6 Hz, 2H, *H4*), 1.47 (tt, *J* = 7.4, 6.6 Hz, 2H, *H5*), 1.23-1.14 (m, 18H, *H6-14*), 0.81 (t, *J* = 6.8 Hz, 3H, *H15*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.29 (*C*O,*C2*), 40.30 (HN*C*H$_2$,*C4*), 31.92 (*C*H$_2$,*C5*) 29.63 (*C*H$_2$), 29.57 (*C*H$_2$), 29.51 (*C*H$_2$), 29.39 (*C*H$_2$), 29.35 (*C*H$_2$), 29.25 (*C*H$_2$), 29.05 (*C*H$_2$,*C5*), 26.82 (*C*H$_2$,*C1*), 26.70 (*C*H$_2$), 22.70 (*C*H$_2$), 14.13 (*C*H$_3$,*C15*)

7.2.3 Preparation of achiral bromide salts

***General procedure for the preparation of achiral bromide salts*; 3-Methyl-1-(pentoxycarbonylmethyl) imidazolium bromide (42)**



To a stirred solution of 1-methylimidazole (9.032 g, 110.0 mmol) in diethyl ether (200 mL) at -78 ºC under a nitrogen atmosphere pentyl bromoacetate (**188**) (37.011 g, 132.0 mmol) was added dropwise. The reaction mixture was stirred vigorously at -15 ºC for 2 h, then at RT overnight. The diethyl ether top phase was decanted and the IL washed with diethyl ether (3 x 10 mL). The residual solvent was removed on the rotary evaporator and the product was dried under high vacuum for 24 h to yield the title compound (**42**) as a yellow/orange viscous liquid at RT in 96 % yield (30.769 g, 105.73 mmol).

Molecular formula $C_{11}H_{19}BrN_2O_2$

Molecular weight 291 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.01 (s, 1H, *H2*), 7.72 (t, *J* = 1.8 Hz, 1H, *H4*), 7.66 (t, *J* = 1.6 Hz, 1H, *H3*), 5.41 (s, 2H, *H5*), 4.11 (t, *J* = 7.0 Hz, 2H, *H7*), 4.05 (s, 3H, *H1*), 1.63 (tt, *J* = 7.2, 7.0 Hz, 2H, *H8*), 1.28-1.23 (m, 4H, *H9,10*), 0.85 (t, *J* = 6.8 Hz, 3H, *H11*)

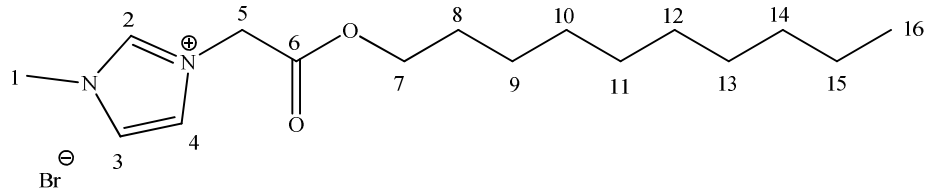
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.17 (*C*O,*C6*), 137.94 (N*C*HN,*C2*), 123.00 (N*C*H,*C4*), 122.87 (N*C*H,*C3*), 66.91 (O*C*H_2,*C7*), 50.17 (N*C*H_2,*C5*), 36.84 (N*C*H_3,*C1*), 27.91 (*C*H_2,*C8*), 27.70 (*C*H_2,*C9*), 22.14 (*C*H_2,*C10*), 13.87 (*C*H_3,*C11*)

IR (neat) (cm^{-1}) 3060 (w), 2957 (w), 2932 (w), 1743 (s), 1225 (s), 1202 (s), 1173 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 211.1440, $C_{11}H_{19}N_2O_2^+$ requires 211.1441

The spectral data are in agreement with literature[1]

3-Methyl-1-(decoxycarbonylmethyl) imidazolium bromide (193)



The title compound (**193**) was prepared from 1-methylimidazole (6.733 g, 82.0 mmol) and decyl bromoacetate (**189**) (29.010 g, 99.0 mmol) according to the general procedure (Section 7.2.3, page 257) as a white solid in 97 % yield (28.811 g, 79.81 mmol).

m.p 51-53 °C

Molecular formula $C_{16}H_{29}BrN_2O_2$

Molecular weight 361 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.01 (s, 1H, *H2*), 7.72 (t, *J* = 1.6 Hz, 1H, *H4*), 7.66 (t, *J* = 1.6 Hz, 1H, *H3*), 5.41 (s, 2H, *H5*), 4.11 (t, *J* = 7.0 Hz, 2H, *H7*), 4.05 (s, 3H, *H1*), 1.63 (tt, *J* = 7.2, 6.8 Hz, 2H, *H8*), 1.28-1.15 (m, 14H, *H9-15*), 0.85 (t, *J* = 7.2 Hz, 3H, *H16*)

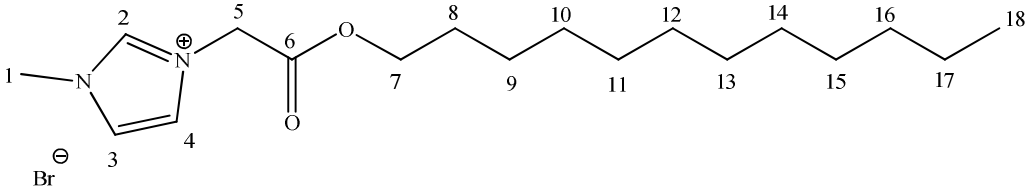
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.19 (*C*O,*C6*), 138.20 (N*C*HN,*C2*), 123.90 (N*C*H,*C4*), 122.81 (N*C*H,*C3*), 67.05 (O*C*H_2,*C7*), 50.12 (N*C*H_2,*C5*), 36.81 (N*C*H_3,*C1*), 31.84 (*C*H_2), 29.50 (*C*H_2), 29.45 (*C*H_2), 29.27 (*C*H_2), 29.18 (*C*H_2), 28.31 (*C*H_2,*C8*), 25.68 (*C*H_2), 22.65 (*C*H_2), 14.11 (*C*H_3,*C16*)

IR (neat) (cm^{-1}) 2921 (m), 2850 (m), 1748 (s), 1218 (s), 1176 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 281.2216, $C_{16}H_{29}N_2O_2^+$ requires 281.2223

The spectral data are in agreement with literature[2]

3-Methyl-1-(dodecylcarbonylmethyl) imidazolium bromide (194)



The title compound (**194**) was prepared from 1-methylimidazole (4.187 g, 51.0 mmol) and dodecyl bromoacetate (**191**) (18.362 g, 60.0 mmol) according to the general procedure (Section 7.2.3, page 257) as a pale orange solid in 55 % yield (10.868 g, 27.94 mmol).

m.p 56-58 °C

Molecular formula $C_{18}H_{33}BrN_2O_2$

Molecular weight 389 $gmol^{-1}$

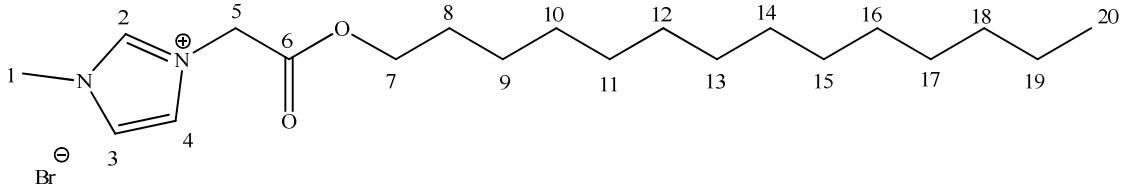
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.26 (s, 1H, *H2*), 7.57 (t, *J* = 1.8 Hz, 1H, *H4*), 7.43 (t, *J* = 1.8 Hz, 1H, *H3*), 5.47 (s, 2H, *H5*), 4.20 (t, *J* = 6.8 Hz, 2H, *H7*), 4.10 (s, 3H, *H1*), 1.67 (tt, *J* = 7.2, 6.8 Hz, 2H, *H8*), 1.36-1.23 (m, 18H, *H9-17*), 0.89 (t, *J* = 7.2 Hz, 3H, *H18*)

^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.15 (*C*O,*C6*), 138.33 (N*C*HN,*C2*), 123.79 (N*C*H,*C4*), 123.05 (N*C*H,*C3*), 67.10 (O*C*H_2,*C7*), 50.28 (N*C*H_2,*C5*), 36.92 (N*C*H_3,*C1*), 31.90 (*C*H_2), 29.63 (*C*H_2), 29.58 (*C*H_2), 29.48 (*C*H_2), 29.34 (*C*H_2), 29.29 (*C*H_2), 29.19 (*C*H_2), 28.33 (*C*H_2,*C8*), 25.70 (*C*H_2), 22.68 (*C*H_2), 14.13 (*C*H_3,*C18*)

IR (neat) (cm^{-1}) 3420 (b), 2916 (s), 1746 (s), 1578 (m), 1470 (m) , 1224 (s), 1177 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 309.2540, $C_{18}H_{33}N_2O_2^+$ requires 309.2536

3-Methyl-1-(tetradecylcarbonylmethyl) imidazolium bromide (195)



The title compound (**195**) was prepared from 1-methylimidazole (4.243 g, 51.30 mmol) and tetradecyl bromoacetate (**192**) (20.451 g, 62.0 mmol) according to the general procedure (Section 7.2.3, page 257) as a white solid in 82 % yield (17.472 g, 41.90 mmol).

m.p 64-66 °C

Molecular formula $C_{20}H_{37}BrN_2O_2$

Molecular weight 417 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.17 (s, 1H, *H2*), 7.58 (t, *J* = 1.8 Hz, 1H, *H4*), 7.47 (t, *J* = 1.8 Hz, 1H, *H3*), 5.47 (s, 2H, *H5*), 4.13 (t, *J* = 6.8 Hz, 2H, *H7*), 4.02 (s, 3H, *H1*), 1.62 (tt, *J* = 7.2, 6.8 Hz, 2H, *H8*), 1.30-1.19 (m, 22H, *H9-19*), 0.83 (t, *J* = 6.8 Hz, 3H, *H20*)

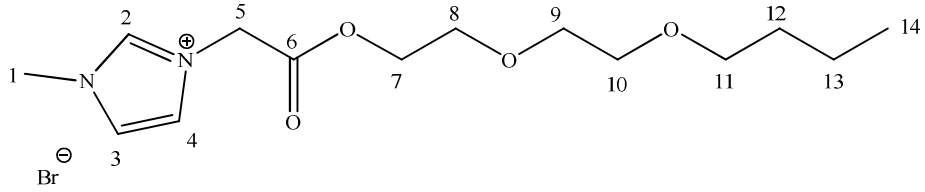
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.15 (*C*O,*C6*), 138.53 (N*C*HN,*C2*), 123.71 (N*C*H,*C4*), 122.92 (N*C*H,*C3*), 67.15 (O*C*H_2,*C7*), 50.32 (N*C*H_2,*C5*), 36.95 (N*C*H_3,*C1*), 31.91 (*C*H_2,*C18*), 29.65 (*C*H_2), 29.64 (*C*H_2), 29.59 (*C*H_2), 29.49 (*C*H_2), 29.35 (*C*H_2), 29.20

(CH_2), 29.19 (CH_2), 29.14 (CH_2), 28.35 (CH_2,*C8*), 25.72 (CH_2), 22.69 (CH_2), 14.14 (CH_3,*C20*)

IR (neat) (cm^{-1}) 3332 (w), 2915 (s), 2850 (s), 1747 (s), 1579 (m), 1471 (m), 1230 (s), 1178 (vs)

MS (*m/z*): Found $[M\text{-}Br^-]^+$ 337.2841, $C_{20}H_{37}N_2O_2^+$ requires 337.2849

3-Methyl-1-(butoxyethoxycarbonylmethyl) imidazolium bromide (196)



The title compound (**196**) was prepared from 1-methylimidazole (14.779 g, 180.0 mmol) and di(ethyleneglycol)butyl ether bromoacetate (**190**) (61.331 g, 217.0 mmol) according to the general procedure (Section 7.2.3, page 257) as a white solid in 89 % yield (58.256 g, 159.60 mmol).

m.p 50-52 °C

Molecular formula $C_{14}H_{25}BrN_2O_4$

Molecular weight 365 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 10.04 (s, 1H, *H2*), 7.64 (t, *J* = 1.8 Hz, 1H, *H4*), 7.47 (t, *J* = 1.6 Hz, 1H, *H3*), 5.46 (s, 2H, *H5*), 4.30 (t, *J* = 4.8 Hz, 2H, *H7*), 4.03 (s, 3H, *H1*), 3.68 (t, *J* = 4.6 Hz, 2H, *H8*), 3.60 (t, *J* = 4.6 Hz, 2H, *H9*), 3.53 (t, *J* = 4.8 Hz, 2H, *H10*), 3.40 (t, *J* = 6.8 Hz, 2H, *H11*), 1.52 (tt, *J* = 7.6, 6.8 Hz, 2H, *H12*), 1.33 (tq, *J* = 7.6, 7.4 Hz, 2H, *H13*), 0.86 (t, *J* = 7.4 Hz, 3H, *H14*)

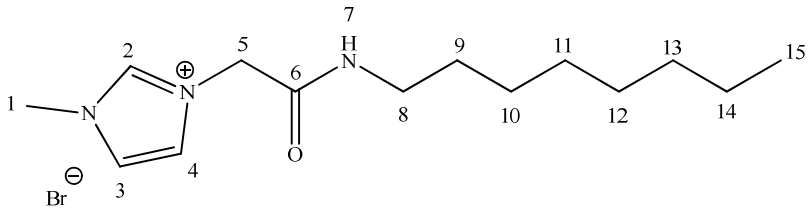
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.16 (*C*O,*C6*), 137.90 (N*C*HN,*C2*), 123.92 (N*C*H,*C4*), 123.24 (N*C*H,*C3*), 71.03 (O*C*H_2,*C11*), 70.45 (O*C*H_2,*C10*), 69.93 (O*C*H_2,*C9*), 69.79 (O*C*H_2,*C8*), 68.40 (O*C*H_2,*C7*), 50.08 (N*C*H_2,*C5*), 36.81 (N*C*H_3,*C1*), 31.50 (CH_2,*C12*), 19.10 (CH_2,*C13*), 13.84 (CH_3,*C14*)

IR (neat) (cm^{-1}) 3440 (w), 2870 (m), 1752 (s), 1576 (m), 1567 (m), 1219 (s), 1095 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 285.1811, $C_{14}H_{25}N_2O_2^+$ requires 285.1808

The spectral data are in agreement with literature[2]

3-Methyl-1-(2-(octylamino)-2-oxoethyl)-imidazolium bromide (206)



The title compound (**206**) was prepared from 1-methylimidazole (0.623 g, 7.60 mmol) and 2-bromo-*N*-octylacetamide (**203**) (2.271 g, 9.10 mmol) according to the general procedure (Section 7.2.3, page 257) as a beige solid in 98 % yield (2.489 g, 7.52 mmol).

m.p 82-84 °C

Molecular formula $C_{14}H_{26}BrN_3O$

Molecular weight 332 $gmol^{-1}$

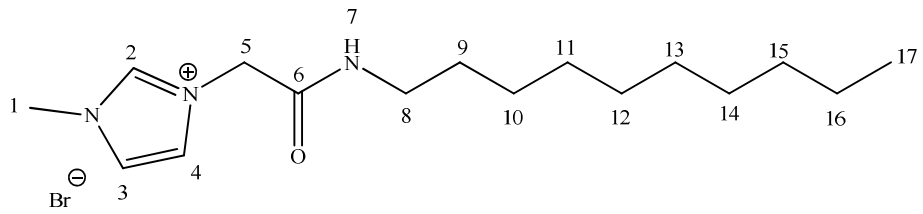
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.89 (s, 1H, *H2*), 8.60 (t, *J* = 4.7 Hz, 1H, *H7*), 7.68 (s, 1H, *H4*), 7.06 (s, 1H, *H3*), 5.38 (s, 2H, *H5*), 4.03 (s, 3H, *H1*), 3.23 (dt, *J* = 6.8, 6.4 Hz, 2H, *H8*), 1.59 (tt, *J* = 7.0, 6.8 Hz, 2H, *H9*), 1.31-1.21 (m, 10H, *H10-14*), 0.88 (t, *J* = 6.8 Hz, 3H, *H15*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.22 (*C*O,*C6*), 137.52 (N*C*HN,*C2*), 123.87 (N*C*H,*C4*), 122.31 (N*C*H,*C3*), 51.87 (N*C*H_2,*C5*), 40.16 (HN*C*H_2,*C8*), 36.80 (N*C*H_3,*C1*), 31.92 (*C*H_2,*C9*), 29.58 (*C*H_2), 29.37 (*C*H_2), 29.28 (*C*H_2), 27.07 (*C*H_2), 22.70 (*C*H_2), 14.14 (*C*H_3,*C15*)

IR (neat) (cm^{-1}) 3071 (w), 2920 (m), 2852 (m), 1667 (vs), 1686 (m), 1560 (s), 1267 (s), 1183 (s), 1165 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 252.2088, $C_{14}H_{26}N_3O^+$ requires 252.2070

3-Methyl-1-(2-(decylamino)-2-oxoethyl)-imidazolium bromide (207)



The title compound (**207**) was prepared from 1-methylimidazole (1.448 g, 17.60 mmol) and 2-bromo-*N*-decylacetamide (**204**) (5.871 g, 21.10 mmol) according to the general procedure (Section 7.2.3, page 257) as a beige solid in 94 % yield (5.982 g, 16.62 mmol).

m.p. 85-87 °C

Molecular formula $C_{16}H_{30}BrN_3O$

Molecular weight 360 $gmol^{-1}$

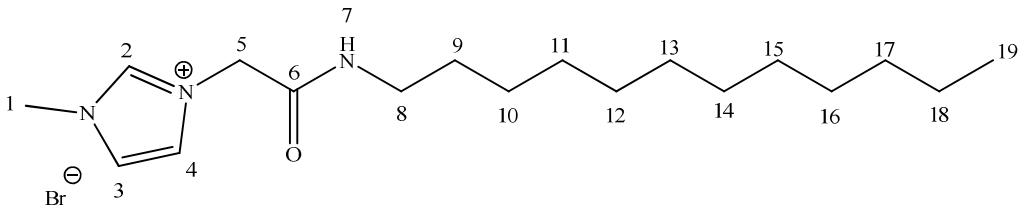
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.79 (s, 1H, *H2*), 8.60 (t, *J* = 5.7 Hz, 1H, *H7*), 7.65 (t, *J* = 1.6 Hz, 1H, *H4*), 7.26 (t, *J* = 1.6 Hz, 1H, *H3*), 5.37 (s, 2H, *H5*), 3.99 (s, 3H, *H1*), 3.24 (dt, *J* = 7.2, 7.0 Hz, 2H, *H8*), 1.58 (tt, *J* = 7.8, 7.2 Hz, 2H, *H9*), 1.31-1.20 (m, 14H, *H10-16*), 0.88 (t, *J* = 7.2 Hz, 3H, *H17*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.25 (*C*O,*C6*), 137.47 (N*C*HN,*C2*), 122.64 (N*C*H,*C4*), 119.65 (N*C*H,*C3*), 51.82 (N*C*H_2,*C5*), 40.10 (HN*C*H_2,*C8*), 36.83 (N*C*H_3,*C1*), 31.86 (*C*H_2,*C9*), 29.57 (*C*H_2), 29.55 (*C*H_2), 29.29 (*C*H_2), 29.26 (*C*H_2), 29.14 (*C*H_2), 27.07 (*C*H_2), 22.65 (*C*H_2), 14.10 (*C*H_3,*C17*)

IR (neat) (cm^{-1}) 3211 (w), 3071 (w), 2918 (m), 2850 (m), 1667 (vs), 1686 (m), 1561 (s), 1263 (m), 1182 (s), 1164 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 280.2389, $C_{16}H_{30}N_3O^+$ requires 280.2383

3-Methyl-1-(2-(dodecylamino)-2-oxoethyl)-imidazolium bromide (208)



The title compound (**208**) was prepared from 1-methylimidazole (0.262 g, 3.20 mmol) and 2-bromo-*N*-dodecylacetamide (**205**) (1.171 g, 3.80 mmol) according to the general procedure (Section 7.2.3, page 257) as a beige solid in 94 % yield (1.170 g, 3.01 mmol).

m.p 89-91 °C

Molecular formula $C_{18}H_{34}BrN_3O$

Molecular weight 388 $gmol^{-1}$

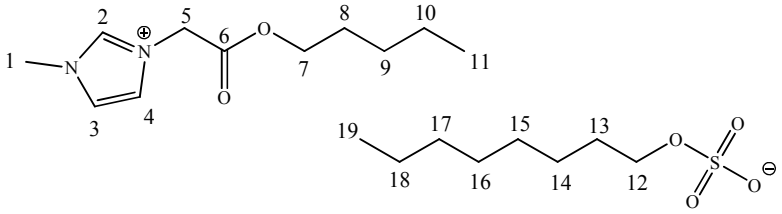
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.73 (s, 1H, *H2*), 8.49 (t, *J* = 5.4 Hz, 1H, *H7*), 7.57 (t, *J* = 1.6 Hz, 1H, *H4*), 7.15 (t, *J* = 1.6 Hz, 1H, *H3*), 5.29 (s, 2H, *H5*), 3.96 (s, 3H, *H1*), 3.16 (dt, *J* = 7.2, 6.0 Hz, 2H, *H8*), 1.50 (tt, *J* = 7.8, 7.2 Hz, 2H, *H9*), 1.22-1.17 (m, 18H, *H10-18*), 0.81 (t, *J* = 7.0 Hz, 3H, *H19*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 164.20 (*C*O,*C6*), 137.49 (N*C*HN,*C2*), 123.90 (N*C*H,*C4*), 122.25 (N*C*H,*C3*), 51.88 (N*C*H_2,*C5*), 40.15 (HN*C*H_2,*C8*), 36.79 (N*C*H_3,*C1*), 31.93 (*C*H_2,*C9*), 29.66 (*C*H_2), 29.58 (*C*H_2), 29.37 (*C*H_2), 29.28 (*C*H_2), 29.20 (*C*H_2), 29.10 (*C*H_2), 27.07 (*C*H_2), 22.70 (*C*H_2), 14.15 (*C*H_3,*C19*)

IR (neat) (cm^{-1}) 3212 (w), 3071 (w), 2917 (vs), 2850 (s), 1667 (vs), 1686 (m), 1561 (s), 1266 (m), 1183 (m), 1165 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 308.2698, $C_{18}H_{34}N_3O^+$ requires 308.2696

7.2.4 Preparation of achiral $OctOSO_3$ salts- *General procedure for the preparation of $OctOSO_3$ achiral salts*: 3-methyl-1-(pentoxycarbonylmethyl)imidazolium $OctOSO_3$ (45)



To a solution of 3-methyl-1-(pentoxycarbonylmethyl)imidazolium bromide (**42**) (0.580 g, 2.00 mmol) in distilled water (7 mL) was added in one portion sodium octyl sulphate (0.521 g, 2.20 mmol) and stirred at 60 ºC for 2 h. The water was then slowly removed under vacuum and a brown solid precipitated was obtained. The precipitate was dissolved in DCM (8 mL) and washed with distilled water (2 x 1 mL). The DCM was removed on the rotary evaporator to yield the title compound (**45**) as a viscous pale yellow liquid at RT in 85 % yield (0.712 g, 1.69 mmol).

Molecular formula $C_{19}H_{36}N_2O_6S$

Molecular weight 420 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.29 (s, 1H, *H2*), 7.39 (t, *J* = 1.6 Hz, 1H, *H4*), 7.33 (t, *J* = 1.6 Hz, 1H, *H3*), 5.14 (s, 2H, *H5*), 4.12 (t, *J* = 7.0 Hz, 2H, *H7*), 3.95 (t, *J* = 6.8 Hz, 2H, *H12*), 3.94 (s, 3H, *H1*), 1.89-1.63 (m, 4H, *H8,13*), 1.32-1.19 (m, 14H, *H9-10,14-18*), 0.88 (t, *J* = 7.0 Hz, 3H, *H11*), 0.82 (t, *J* = 6.8 Hz, 3H, *H19*)

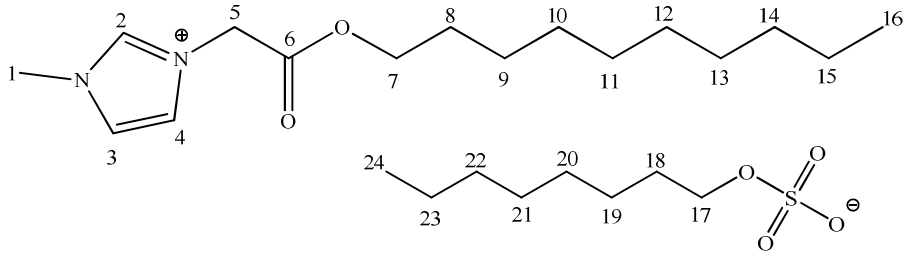
^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 166.50 (*C*O,*C6*), 138.79 (N*C*HN,*C2*), 123.69 (N*C*H,*C4*), 123.07 (N*C*H,*C3*), 67.94 (O*C*H_2,*C12*), 66.95 (O*C*H_2,*C7*), 49.93 (N*C*H_2,*C5*), 36.56 (N*C*H_3,*C1*), 31.83 (*C*H_2), 29.49 (*C*H_2), 29.37 (*C*H_2), 29.23 (*C*H_2,*C8*), 28.03 (*C*H_2,*C13*), 27.81 (*C*H_2), 25.87 (*C*H_2), 22.66 (*C*H_2), 22.26 (*C*H_2), 14.12 (*C*H_3,*C11/C19*), 13.94 (*C*H_3,*C11/C19*)

IR (neat) (cm^{-1}) 3050 (w), 2955 (w), 2922 (w), 1747 (m), 1223 (vs), 1057 (m), 1017 (m)

LRMS (*m/z*) 211.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

Spectral data in agreement with literature[2]

3-Methyl-1-(decoxycarbonylmethyl)imidazolium $OctOSO_3$ (197)



The title compound (**197**) was prepared from 3-methyl-1-(decoxycarbonylmethyl)imidazolium bromide (**193**) (1.310 g, 4.00 mmol) and sodium octyl sulfate (1.401 g, 4.20 mmol) according to the general procedure (Section 7.2.4, page 265) as a pale yellow viscous liquid in 71 % yield (1.401 g, 2.85 mmol).

Molecular formula $C_{24}H_{46}N_2O_6S$

Molecular weight 491 $gmol^{-1}$

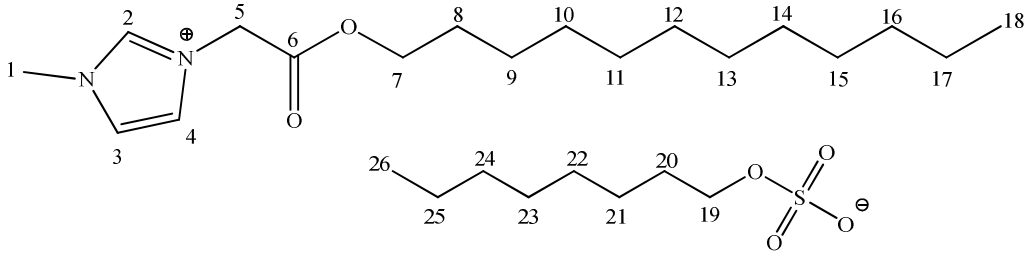
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.17 (s, 1H, *H2*), 7.35 (t, *J* = 1.8 Hz, 1H, *H4*), 7.28 (t, *J* = 1.6 Hz, 1H, *H3*), 5.12 (s, 2H, *H5*), 4.11 (t, *J* = 6.8 Hz, 2H, *H7*), 3.95 (t, *J* = 6.8 Hz, 3H, *H17*), 3.94 (s, 3H, *H1*), 1.62-1.57 (m, 4H, *H8,18*), 1.28-1.10 (m, 24H, *H9-15,19-23*), 0.83-0.78 (m, 6H, *H16,24*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.42 (*C*O,*C6*), 139.59 (N*C*HN,*C2*), 123.73 (N*C*H,*C4*), 122.93 (N*C*H,*C3*), 67.93 (O*C*H_2,*C17*), 67.03 (O*C*H_2,*C7*), 49.96 (N*C*H_2,*C5*), 36.60 (N*C*H_3,*C1*), 31.90 (*C*H_2), 31.84 (*C*H_2), 29.97 (*C*H_2), 29.75 (*C*H_2), 29.61 (*C*H_2), 29.56 (*C*H_2), 29.51 (*C*H_2), 29.37 (*C*H_2), 29.32 (*C*H_2), 29.28 (*C*H_2,*C8*), 29.22 (*C*H_2,*C18*), 28.26 (*C*H_2), 25.88 (*C*H_2), 25.73 (*C*H_2), 14.14 (*C*H_3,*C16/C24*), 13.91 (*C*H_3,*C16/C24*)

IR (neat) (cm^{-1}) 3048 (w), 2919 (m), 2852 (m), 1749 (m), 1223 (vs), 978 (m)

LRMS (*m/z*) 281.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-(dodecoxycarbonylmethyl)imidazolium $OctOSO_3$ (198)



The title compound (**198**) was prepared from 3-methyl-1-(dodecoxycarbonylmethyl)imidazolium bromide (**194**) (0.741 g, 1.90 mmol) and sodium octyl sulfate (0.538 g, 2.30 mmol) according to the general procedure (Section 7.2.4, page 265) as a viscous yellow liquid in 79 % yield (0.776 g, 1.49 mmol).

Molecular formula $C_{26}H_{50}N_2O_6S$

Molecular weight 519 $gmol^{-1}$

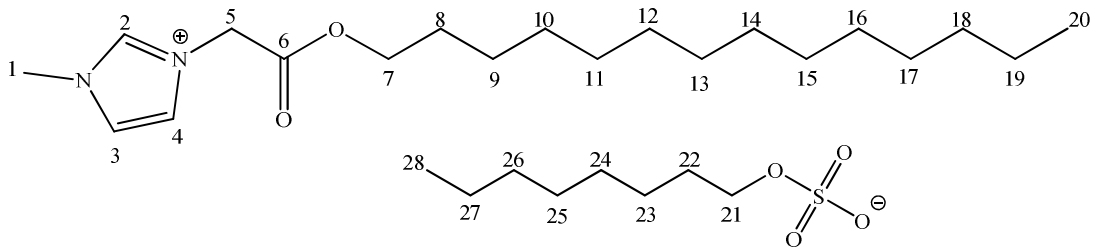
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.23 (s, 1H, *H2*), 7.35 (t, *J* = 1.8 Hz, 1H, *H4*), 7.33 (t, *J* = 1.6 Hz, 1H, *H3*), 5.12 (s, 2H, *H5*), 4.11 (t, *J* = 7.0 Hz, 2H, *H7*), 3.92 (t, *J* = 6.8 Hz, 2H, *H19*), 3.91 (s, 3H, *H1*), 1.61-1.56 (m, 4H, *H8,20*), 1.27-1.14 (m, 28H, *H9-17,21-25*) 0.83-0.78 (m, 6H, *H18,26*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.55 (*C*O,*C6*), 137.73 (N*C*HN,*C2*), 123.58 (N*C*H,*C4*), 123.30 (N*C*H,*C3*), 68.10 (O*C*H_2,*C19*), 66.99 (O*C*H_2,*C7*), 49.94 (N*C*H_2,*C5*), 36.57 (N*C*H_3,*C1*), 31.93 (*C*H_2), 31.86 (*C*H_2), 29.98 (*C*H_2), 29.84 (*C*H_2), 29.74 (*C*H_2), 29.68 (*C*H_2), 29.54 (*C*H_2), 29.50 (*C*H_2), 29.47 (*C*H_2), 29.39 (*C*H_2,*C8*), 28.37 (*C*H_2,*C20*), 25.86 (*C*H_2), 25.73 (*C*H_2), 24.88 (*C*H_2), 22.70 (*C*H_2), 22.68 (*C*H_2), 14.14 (*C*H_3,*C18/26*), 14.12 (*C*H_3,*C18/26*)

IR (neat) (cm^{-1}) 3123 (w), 2922 (s), 2853 (m), 1747 (m), 1175 (vs), 1046 (vs)

LRMS (*m/z*) 309.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-(tetradecoxycarbonylmethyl)imidazolium $OctOSO_3$ (199)



The title compound (**199**) was prepared from 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium bromide (**195**) (0.752 g, 1.80 mmol) and sodium octyl sulfate (0.514 g, 2.20 mmol) according to the general procedure (Section 7.2.4, page 265) as a colourless viscous liquid in 95 % yield (0.938 g, 1.71 mmol).

Molecular formula $C_{28}H_{54}N_2O_6S$

Molecular weight 547 $gmol^{-1}$

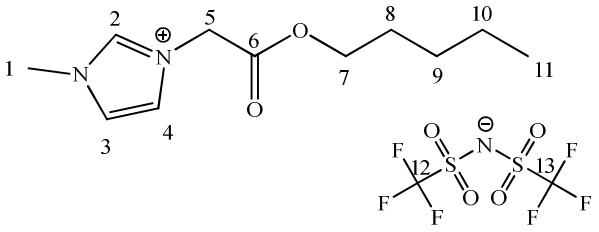
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.41 (s, 1H, *H2*), 7.30 (t, *J* = 1.8 Hz, 1H, *H4*), 7.22 (t, *J* = 1.6 Hz, 1H, *H3*), 5.12 (s, 2H, *H5*), 4.11 (t, *J* = 7.0 Hz, 2H, *H7*), 3.94 (t, *J* = 6.8 Hz, *H7*), 3.93 (s, 3H, *H1*), 1.62-1.57 (m, 4H, *H8,22*), 1.29-1.18 (m, 32H, *H9-19,22-27*) 0.83-0.78 (m, 6H, *H20,28*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 166.46 (*C*O,*C6*), 138.88 (N*C*HN,*C2*), 123.65 (N*C*H,*C4*), 123.02 (N*C*H,*C3*), 67.91 (O*C*H_2,*C21*), 67.00 (O*C*H_2,*C7*), 49.92 (N*C*H_2,*C5*), 36.56 (N*C*H_3,*C1*), 31.94 (*C*H_2), 31.84 (*C*H_2), 29.92 (*C*H_2), 29.81 (*C*H_2), 29.71 (*C*H_2), 29.68 (*C*H_2) 29.64 (*C*H_2), 29.54 (*C*H_2), 29.51 (*C*H_2), 29.38 (*C*H_2), 29.28 (*C*H_2), 29.25 (*C*H_2,*C8*), 28.37 (*C*H_2,*C22*), 25.88 (*C*H_2), 25.74 (*C*H_2), 24.22 (*C*H_2), 22.71 (*C*H_2), 22.67 (*C*H_2), 14.15 (*C*H_3,*C20*/*C28*), 14.13 (*C*H_3,*C20*/*C28*)

IR (neat) (cm^{-1}) 3114 (w), 2914 (vs), 2850 (vs), 1756 (s), 1575 (w), 1471 (m), 1366 (w), 1236 (vs), 1203 (s), 1059 (m)

LRMS (*m/z*) 337.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

7.2.5 Preparation of achiral NTf_2 salts- ***General procedure for the preparation of achiral NTf_2 salts*****; 3-Methyl-1-(pentoxycarbonylmethyl)imidazolium NTf_2 (177)**



A flask was charged with 3-methyl-1-(pentoxycarbonylmethyl)imidazolium bromide (**42**) (40.891 g, 141.0 mmol) and distilled water (100 mL). Lithium trifluoromethanesulfonimide (48.101 g, 169.0 mmol) was added in one portion and the suspension stirred vigorously overnight at RT. The top aqueous layer was removed, the IL washed with water (3 x 30 mL) then the solvent removed on the rotary evaporator. The product was dried under high vacuum to give the title compound (**177**) as a yellow/orange slightly viscous liquid at RT in 90 % yield (62.141 g, 126.56 mmol).

Molecular formula $C_{13}H_{19}F_6N_3O_6S_2$

Molecular weight 491 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.68 (s, 1H, *H2*), 7.34 (t, *J* = 1.8 Hz, 1H, *H4*), 7.28 (t, *J* = 1.8 Hz, 1H, *H3*), 4.92 (s, 2H, *H5*), 4.13 (t, *J* = 7.0 Hz, 2H, *H7*), 3.96 (s, 3H, *H1*), 1.17 (tt, *J* = 7.2, 6.8 Hz, 2H, *H8*), 1.33-1.27 (m, 4H, *H9,10*), 0.91 (t, *J* = 6.8 Hz, 3H, *H11*)

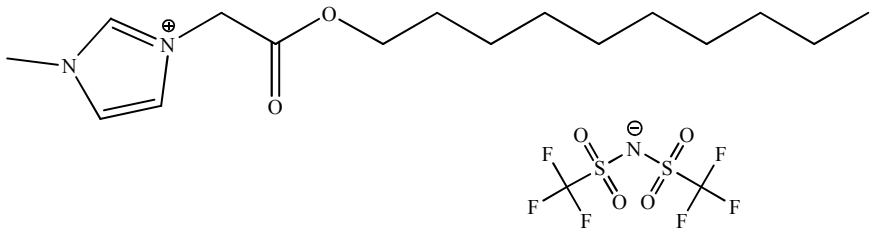
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.81 (*C*O,*C6*), 137.34 (N*C*HN,*C2*), 123.90 (N*C*H,*C4*), 123.32 (N*C*H,*C3*), 118.09 (q, *J* = 319 Hz, 2*C*F_3,*C12,C13*), 67.09 (O*C*H_2,*C7*), 49.80 (N*C*H_2,*C5*), 36.36 (N*C*H_3,*C1*), 27.85 (*C*H_2,*C8*), 27.69 (*C*H_2,*C9*), 22.14 (*C*H_2,*C10*), 13.76 (*C*H_3,*C11*)

IR (neat) (cm^{-1}) 3167 (w), 2917 (w), 2848 (w), 1760 (m), 1342 (m), 1324 (s), 1173 (vs), 1120 (vs), 1059 (m)

LRMS (*m/z*) 211.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

The spectral are data in agreement with literature[1]

3-Methyl-1-(decoxycarbonylmethyl)imidazolium NTf_2 (200)



The title compound (**200**) was prepared from 3-methyl-1-(decoxycarbonylmethyl)imidazolium bromide (**193**) (0.612 g, 1.70 mmol) and Lithium trifluoromethanesulfonimide (0.571 g, 2.00 mmol) according to the general procedure (Section 7.2.5, page 269) as a yellow liquid in 79 % yield (0.757 g, 1.35 mmol).

Molecular formula $C_{18}H_{29}F_6N_3O_6S_2$

Molecular weight 561 $gmol^{-1}$

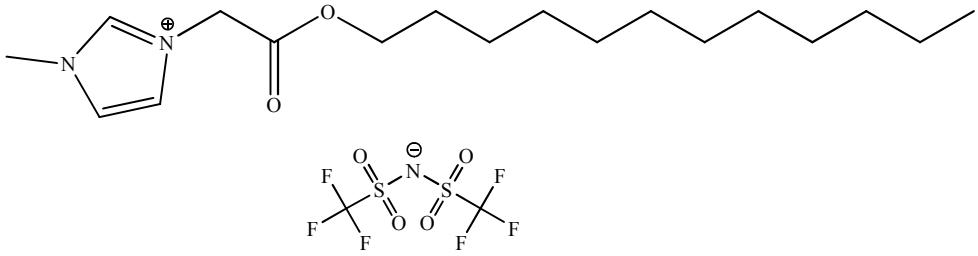
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.82 (s, 1H), 7.38 (t, *J* = 1.8 Hz, 1H), 7.28 (t, *J* = 1.8 Hz, 1H), 5.02 (s, 2H), 4.21 (t, *J* = 7.0 Hz, 2H), 3.89 (s, 3H), 1.59 (tt, *J* = 7.2, 6.8 Hz, 2H), 1.23-1.18 (m, 14H), 0.89 (t, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.71, 137.61, 123.61, 122.96, 118.29 (q, *J* = 318 Hz, 2*C*F_3), 67.45, 59.52, 37.43, 36.75, 31.88, 29.52, 29.45, 29.30, 29.15, 28.29, 22.69, 14.13

IR (neat) (cm^{-1}) 3177 (w), 2919 (w), 2854 (w), 1759 (m), 1341 (s), 1329 (s), 1176 (vs), 1129 (vs), 1058 (m)

LRMS (*m/z*) 281.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-(dodecoxycarbonylmethyl)imidazolium NTf_2 (201)



The title compound (**201**) was prepared from 3-methyl-1-(dodecoxycarbonylmethyl)imidazolium bromide (**194**) (0.660 g, 1.70 mmol) and Lithium trifluoromethanesulfonimide (0.581 g, 2.00 mmol) according to the general procedure (Section 7.2.5, page 269) as a pale yellow liquid in 89 % yield (0.889 g, 1.51 mmol).

Molecular formula $C_{20}H_{33}F_6N_3O_6S_2$

Molecular weight 590 $gmol^{-1}$

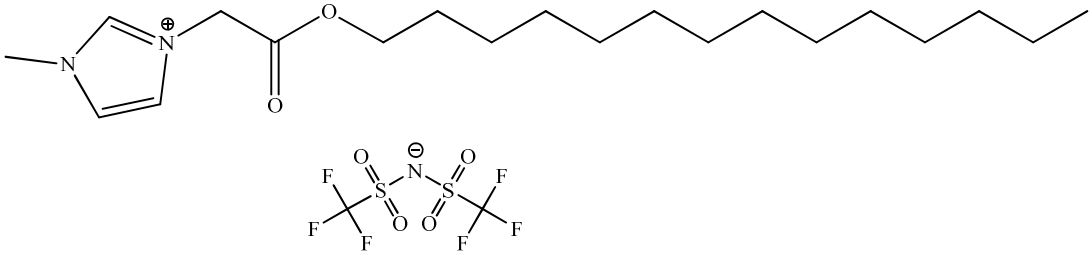
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.74 (s, 1H), 7.30 (t, *J* = 1.8 Hz, 1H), 7.25 (t, *J* = 1.8 Hz, 1H), 4.93 (s, 2H), 4.13 (t, *J* = 6.8 Hz, 2H), 3.88 (s, 3H), 1.59 (tt, *J* = 7.0, 6.8 Hz, 2H), 1.24-1.18 (m, 18H), 0.81 (t, *J* = 7.0 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.78, 137.53, 123.82, 123.26, 118.10 (q, *J* = 319 Hz, 2*C*F_3), 67.25, 49.89, 36.50, 31.92, 29.65, 29.64, 29.58, 29.47, 29.36, 29.18, 28.25, 25.64, 22.70, 14.13

IR (neat) (cm^{-1}) 3176 (w), 2916 (m), 2851 (m), 1761 (s), 1342 (s), 1329 (s), 1177 (vs), 1129 (vs), 1061 (s)

LRMS (*m/z*): 309.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-(tetradecoxycarbonylmethyl)imidazolium NTf_2 (202)



The title compound (**202**) was prepared from 3-methyl-1-(tetradecoxycarbonylmethyl)imidazolium bromide (**195**) (0.707 g, 1.70 mmol) and Lithium trifluoromethanesulfonimide (0.581 g, 2.00 mmol) according to the general procedure (Section 7.2.5, page 269) as a pale yellow liquid in 88 % yield (0.925 g, 1.50 mmol).

Molecular formula $C_{22}H_{37}F_6N_3O_6S_2$

Molecular weight 618 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.75 (s, 1H), 7.29 (t, *J* = 1.8 Hz, 1H), 7.24 (t, *J* = 1.6 Hz, 1H), 4.94 (s, 2H), 4.16 (t, *J* = 6.8 Hz, 2H), 3.88 (s, 3H), 1.59 (tt, *J* = 6.9, 6.8 Hz, 2H), 1.24-1.18 (m, 22H), 0.81 (t, *J* = 6.8 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 165.75, 137.66, 123.78, 123.20, 119.65 (q, *J* = 320 Hz, 2*C*F_3), 67.31, 49.95, 36.57, 31.93, 29.91, 29.71, 29.69, 29.67, 29.60, 29.48, 29.38, 29.18, 28.27, 25.66, 22.70, 14.14

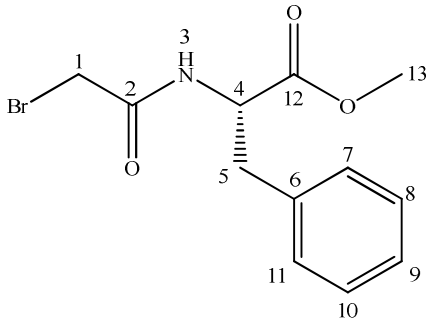
IR (neat) (cm^{-1}) 3169 (w), 2916 (m), 2851 (m), 1761 (s), 1471 (w), 1343 (s), 1329 (s), 1179 (vs), 1129 (vs), 1062 (s)

LRMS (*m/z*) 337.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

7.3 Chapter 3 experimental-Preparation of Chiral ionic liquids-AAE CILs

7.3.1 Preparation of Chiral bromoamide intermediates

Representative procedure for the preparation of chiral α-bromoamides **L-phenylalanine methyl ester bromoacetate (247)**



To a stirred solution of DCM, L-phenylalanine methyl ester hydrochloride (**223**) (2.092 g, 10.40 mmol), and triethylamine (1.368 g, 13.50 mmol), under a nitrogen atmosphere at -78 ºC was added dropwise bromoacetyl bromide (2.519 g, 12.50 mmol). After stirring at -78 ºC for 5 h, the reaction mixture was allowed warm up to -20 ºC and quenched by addition of water (10 mL). The organic phase was washed with distilled water (3 x 10 mL), saturated ammonium chloride (3 x 10 mL), saturated sodium bicarbonate (3 x 10 mL) and brine (3 x 10 mL). The organic phase was then dried over anhydrous magnesium sulfate, filtered and volatiles removed *via* rotary evaporation to give a crude product in 72 % yield (2.233 g, 7.45 mmol). Column chromatography was performed on the crude product (50:50 hexane:ethyl acetate) to yield the title product (**247**) as a white solid in 66 % yield (2.063 g, 6.88 mmol).

m.p. 82-84 ^{o}C, $[\alpha]_D^{20} =$ +48.3 ° (1.0 c, $CHCl_3$)

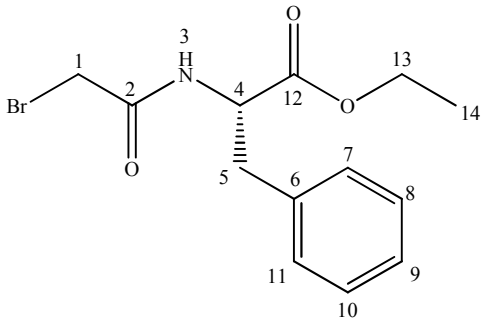
Molecular formula $C_{12}H_{14}BrNO_3$

Molecular weight 300 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.26-6.99 (m, 5H, *H7-11*), 6.78 (d, *J* = 7.6 Hz, 1H, *H3*), 4.88 (ddd, *J* = 8.0, 5.6, 5.6 Hz, 1H, *H4*), 3.90 (d, *J* = 3.6 Hz, 2H, *H1*), 3.67 (s, 3H, *H13*), 3.11 (dd, *J* = 13.6, 5.6 Hz, 1H, *H5*), 3.07 (dd, *J* = 14.0, 6.0 Hz, 1H, *H5*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.29 (*C*O,*C2*), 165.18 (*C*O,*C12*), 135.30 (Ar*C*,*C6*), 129.29 (Ar*C*H), 128.72 (Ar*C*H), 127.67 (Ar*C*H,*C9*), 53.72 (*C*H,*C4*), 52.54 (O*C*H_3,*C13*), 37.72 (*C*H_2,*C5*), 25.52 (*C*H_2,*C1*)

L-Phenylalanine ethyl ester bromoacetate (248)



The title compound (**248**) was prepared from L-phenylalanine ethyl ester hydrochloride (**224**) (2.220 g, 10.30 mmol) and bromoacetyl bromide (2.503 g, 12.40 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 64 % yield (2.078 g, 6.62 mmol).

m.p. 65-67 ^{o}C, $[\alpha]_D^{20}$ = +40.7 ° (0.9 c, $CHCl_3$)

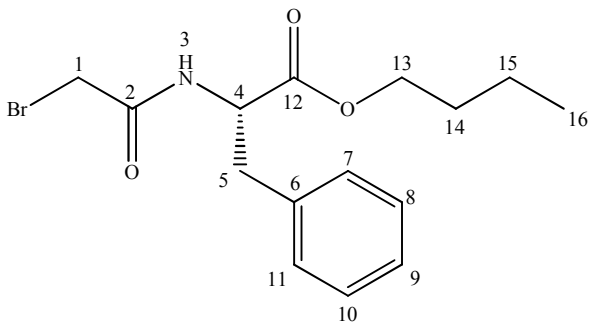
Molecular formula $C_{13}H_{16}BrNO_3$

Molecular weight 314 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.25-7.03 (m, 5H, *H7-11*), 6.82 (d, *J* = 7.2 Hz, 1H, *H3*), 4.78 (ddd, *J* = 8.0, 5.6, 5.6 Hz, 1H, *H4*), 4.14 (q, *J* = 7.0 Hz,, 2H, *H13*), 3.78 (d, *J* = 2.8 Hz, 2H, *H1*), 3.09 (dd, *J* = 14.0, 6.0 Hz, 1H, *H5*), 3.04 (dd, *J* = 14.0, 6.0 Hz, 1H, *H5*), 1.20 (t, *J* = 7.0 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.85 (*C*O,*C2*), 165.15 (*C*O,*C12*), 135.39 (Ar*C*,*C6*), 129.36 (Ar*C*H), 128.64 (Ar*C*H), 127.31 (Ar*C*H,*C9*), 61.78 (O*C*H_2,*C13*), 53.76 (*C*H,*C4*), 37.77 (*C*H_2,*C5*), 28.69 (*C*H_2,*C1*), 14.11 (*C*H_3,*C14*)

L-Phenylalanine butyl ester bromoacetate (249)



The title compound (**249**) was prepared from L-phenylalanine butyl ester hydrochloride (**225**) (2.001 g, 7.80 mmol) and bromoacetyl bromide (1.881 g, 9.30 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 79 % yield (2.112 g, 6.17 mmol).

m.p. 66-68 ^{o}C, $[\alpha]_{D}^{20}$ = +28.9 ^{o} (0.9 c, $CHCl_3$)

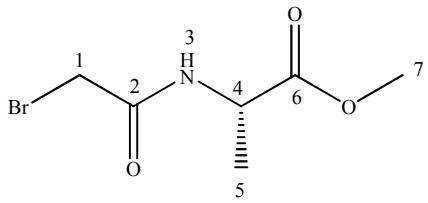
Molecular formula $C_{15}H_{20}BrNO_3$

Molecular weight 342 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.23-7.03 (m, 5H, *H7,11*), 6.98 (d, *J* = 7.6 Hz, 1H, *H3*), 4.77 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H4*), 4.06 (q, *J* = 6.0 Hz, 2H, *H13*), 3.87 (d, *J* = 2.0 Hz, 2H, *H1*), 3.09 (dd, *J* = 14.0, 6.0 Hz, 1H, *H5*), 3.05 (dd, *J* = 14.0, 6.0 Hz, 1H, *H5*), 1.52 (tt, *J* = 7.0, 6.8 Hz, 2H, *H14*), 1.26 (tq, *J* = 7.6, 7.0 Hz, 2H, *H15*), 0.94 (t, *J* = 7.4 Hz, 3H, *H16*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.90 (*C*O,*C2*), 165.04 (*C*O,*C12*), 135.40 (Ar*C*,*C6*), 129.33 (Ar*C*H), 128.65 (Ar*C*H), 127.30 (Ar*C*H,*C9*), 65.61 (O*C*H$_2$,*C13*), 53.78 (*C*H,*C4*), 37.84 (*C*H$_2$,*C5*), 30.45 (*C*H$_2$,*C14*), 28.70 (*C*H$_2$,*C1*), 19.05 (*C*H$_2$,*C15*), 13.65 (*C*H$_3$,*C16*)

L-Alanine methyl ester bromoacetate (250)



The title compound (**250**) was prepared from L-alanine methyl ester hydrochloride (**226**) (1.002 g, 6.80 mmol) and bromoacetyl bromide (1.642 g, 8.10 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 65 % yield (0.997 g, 4.45 mmol).

$[\alpha]_D^{20} = +12.0^{o}$ (0.7 c, $CHCl_3$)

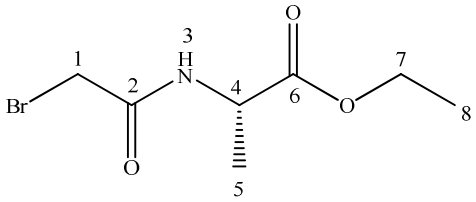
Molecular formula $C_6H_{10}BrNO_3$

Molecular weight 224 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.28 (d, *J* = 7.8 Hz, 1H, *H3*), 4.64 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 3.91 (d, *J* = 2.0 Hz, 2H, *H1*), 3.80 (s, 3H, *H7*), 1.48 (d, *J* = 7.2 Hz, 3H, *H5*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.83 (*C*O,*C2*), 165.15 (*C*O,*C6*), 53.70 (O*C*H_3,*C7*), 48.75 (*C*H,*C4*), 28.71 (*C*H_2,*C1*), 18.22 (*C*H_3,*C5*)

L-Alanine ethyl ester bromoacetate (251)



The title compound (**251**) was prepared from L-alanine ethyl ester hydrochloride (**227**) (1.012 g, 6.80 mmol) and bromoacetyl bromide (1.642 g, 8.10 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 40 % yield (0.647 g, 2.72 mmol).

$[\alpha]_D^{20} = +14.4^{o}$ (0.8 c, $CHCl_3$)

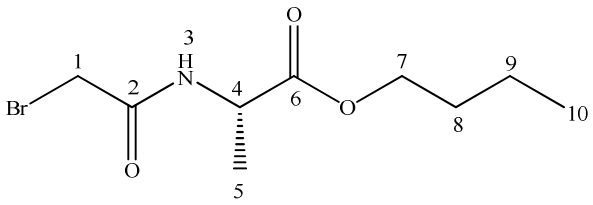
Molecular formula $C_7H_{12}BrNO_3$

Molecular weight 238 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.96 (d, *J* = 7.8 Hz, 1H, *H3*), 4.52 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 4.19 (q, *J* = 7.0 Hz, 2H, *H7*), 3.82 (s, 2H, *H1*), 1.39 (d, *J* = 7.2 Hz, 3H, *H5*), 1.25 (t, *J* = 7.0 Hz, 3H, *H8*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.83 (*C*O,*C2*), 162.64 (*C*O,*C6*), 59.51 (O*C*H_2,*C7*), 46.27 (*C*H,*C4*), 26.17 (*C*H_2,*C1*), 19.03 (*C*H_3,*C5*), 15.69 (*C*H_3,*C8*)

L-Alanine butyl ester bromoacetate (252)



The title compound (**252**) was prepared from L-alanine butyl ester hydrochloride (**228**) (1.034 g, 5.50 mmol) and bromoacetyl bromide (1.341 g, 6.60 mmol) according to the general procedure (Section 7.3.1, page 273) as a colourless liquid in 74 % yield (1.086 g, 4.08 mmol).

$[\alpha]_D^{20} = +22.0^{o}$ (0.8 c, $CHCl_3$)

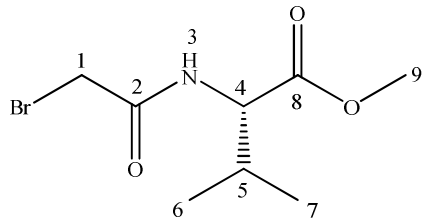
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.20 (d, *J* = 6.2 Hz, 1H, *H3*), 4.51 (dq, *J* = 6.8, 6.0 Hz, 1H, *H4*), 4.13 (dq, *J* = 7.2, 7.0 Hz, 1H, *H7*), 4.12 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.82 (s, 2H, *H1*), 1.58 (tt, *J* = 7.2, 6.8 Hz, 2H, *H8*), 1.39 (d, *J* = 6.8 Hz, 3H, *H5*), 1.29 (tq, *J* = 7.2, 7.0 Hz, 2H, *H9*), 0.89 (t, *J* = 7.4 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.43 (*C*O,*C2*), 164.97 (*C*O,*C6*), 65.63 (O*C*H$_2$,*C7*), 48.58 (*C*H,*C4*), 30.51 (*C*H$_2$,*C8*), 28.77 (*C*H$_2$,*C1*), 19.03 (*C*H$_2$,*C9*), 18.33 (*C*H$_3$,*C5*), 13.65 (*C*H$_3$,*C10*)

L-Valine methyl ester bromoacetate (253)



The title compound (**253**) was prepared from L-valine methyl ester hydrochloride (**229**) (1.042 g, 6.00 mmol) and bromoacetyl bromide (1.464 g, 7.20 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 60 % yield (0.904 g, 3.60 mmol).

$[\alpha]_D^{20} = +21.0^{o}$ (0.9 c, $CHCl_3$)

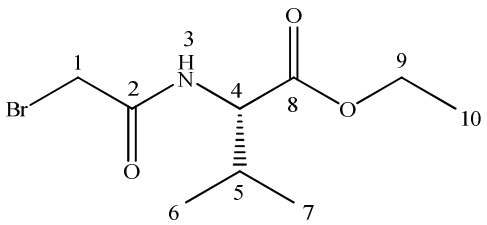
Molecular formula $C_8H_{14}BrNO_3$

Molecular weight 252 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.90 (d, *J* = 7.2 Hz, 1H, *H3*), 4.48 (dd, *J* = 8.8, 4.8 Hz, 1H, *H4*), 3.86 (s, 2H, *H1*), 3.70 (s, 3H, *H9*), 2.13 (qqd, *J* = 6.8, 6.4, 4.8 Hz, 1H, *H5*), 0.88 (dd, *J* = 9.6, 6.8 Hz, 6H, *H6,7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.51 (*C*O,*C2*), 169.46 (*C*O,*C8*), 59.02 (*C*H,*C4*), 50.23 (O*C*H$_3$,*C9*), 35.78 (*C*H,*C5*), 30.11 (*C*H$_2$,*C1*), 18.85 (*C*H$_3$,*C6*/*C7*), 18.06 (*C*H$_3$,*C6*/*C7*)

L-Valine ethyl ester bromoacetate (254)



The title compound (**254**) was prepared from L-valine ethyl ester hydrochloride (**230**) (2.001 g, 11.0 mmol) and bromoacetyl bromide (2.649 g, 13.20 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 40 % yield (1.166 g, 4.38 mmol).

$[\alpha]_D^{20} = +18.0^{o}$ (1.0 c, $CHCl_3$)

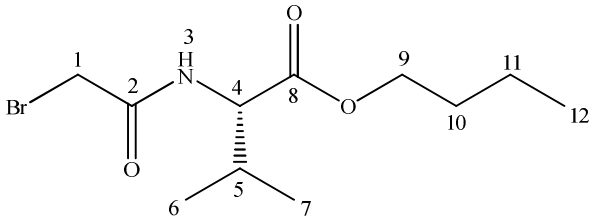
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.84 (d, *J* = 6.8 Hz, 1H, *H3*), 4.44 (dd, *J* = 8.8, 4.8 Hz, 1H, *H4*), 4.17 (dq, *J* = 7.2, 7.2 Hz, 1H, *H9*), 4.14 (dq, *J* = 7.2, 7.0 Hz, 1H, *H9*), 3.85 (s, 2H, *H1*), 2.15 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H5*), 1.23 (t, *J* = 7.2 Hz, 3H, *H10*), 0.88 (dd, *J* = 9.2, 6.8 Hz, 6H, *H6,7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.29 (*C*O,*C2*), 165.36 (*C*O,*C8*), 61.51 (O*C*H_2,*C9*), 57.68 (*C*H,*C4*), 31.37 (*C*H,*C5*), 29.01 (*C*H_2,*C1*), 18.87 (*C*H_3,*C6*/*C7*), 17.66 (*C*H_3,*C6*/*C7*), 14.22 (*C*H_3,*C10*)

L-Valine butyl ester bromoacetate (255)



The title compound (**255**) was prepared from L-valine butyl ester hydrochloride (**231**) (2.010 g, 9.60 mmol) and bromoacetyl bromide (2.321 g, 11.50 mmol) according to the general procedure (Section 7.3.1, page 273) as a colourless liquid in 58 % yield (1.640 g, 5.58 mmol).

$[\alpha]_D^{20} = +15.1^{o}$ (1.0 c, $CHCl_3$)

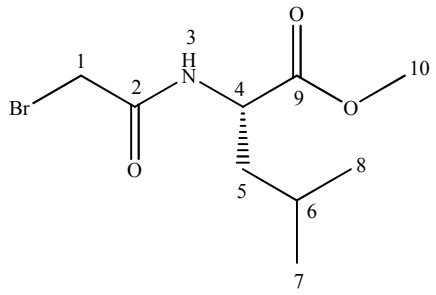
Molecular formula $C_{11}H_{20}BrNO_3$

Molecular weight 294 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.97 (d, *J* = 8.0 Hz, 1H, *H3*), 4.57 (dd, *J* = 8.0, 4.8 Hz, 1H, *H4*), 4.28 (q, *J* = 7.2 Hz, 2H, *H9*), 3.94 (s, 2H, *H1*), 2.15 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H5*), 1.63 (m, 2H, *H10*), 1.46-1.36 (m, 2H, *H11*), 0.99-0.94 (m, 9H, *H 6,7,12*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.40 (*C*O,*C2*), 165.45 (*C*O,*C8*), 65.41 (O*C*H_2,*C9*), 57.73 (*C*H,*C4*), 31.41 (*C*H_2,*C10*), 30.43 (*C*H,*C5*), 29.02 (*C*H_2,*C1*), 19.11 (*C*H_3,*C6/C7*), 19.00 (*C*H_3,*C6/C7*), 17.65 (*C*H_2,*C11*), 13.66 (*C*H_3,*C12*)

L-Leucine methyl ester bromoacetate (256)



The title compound (**256**) was prepared from L-leucine methyl ester hydrochloride (**235**) (3.050 g, 16.50 mmol) and bromoacetyl bromide (4.011 g, 19.80 mmol) according to the general procedure (Section 7.3.1, page 273) as a colourless liquid in 62 % yield (2.731 g, 10.26 mmol).

$[\alpha]_D^{20}$ = +5.5 ° (0.7 c, $CHCl_3$)

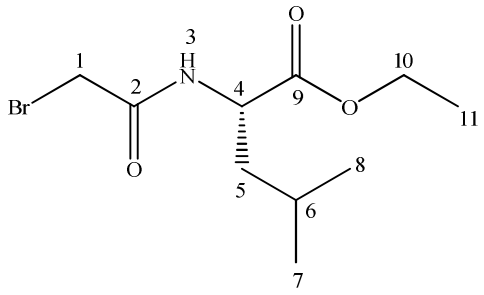
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.73 (d, *J* = 8.4 Hz, 1H, *H3*), 4.56 (ddd, *J* = 8.4, 8.4, 5.6 Hz, 1H, *H4*), 3.83 (d, *J* = 1.2 Hz, 2H, *H1*), 3.68 (s, 3H, *H10*), 1.67-1.50 (m, 3H, *H5,6*), 0.88 (d, *J* = 6.4 Hz, 6H, *H7,8*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.89 (*C*O,*C2*), 166.38 (*C*O,*C9*), 52.57 (*C*H,*C4*), 51.09 (O*C*H$_3$,*C10*), 41.16 (*C*H$_2$,*C5*), 28.59 (*C*H$_2$,*C1*), 24.84 (*C*H,*C6*), 22.79 (*C*H$_3$,*C7*/*C8*), 21.84 (*C*H$_3$,*C7*/*C8*)

L-Leucine ethyl ester bromoacetate (257)



The title compound (**257**) was prepared from L-leucine ethyl ester hydrochloride (**236**) (3.002 g, 15.30 mmol) and bromoacetyl bromide (3.714 g, 18.40 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 61 % yield (2.620 g, 9.36 mmol).

$[\alpha]_D^{20}$ = +6.0 ° (1.0 c, $CHCl_3$)

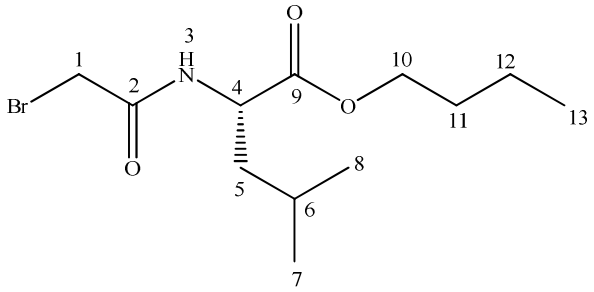
Molecular formula $C_{10}H_{18}BrNO_3$

Molecular weight 280 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.76 (d, *J* = 7.6 Hz, 1H, *H3*), 4.52 (ddd, *J* = 8.4, 8.4, 5.2, Hz, 1H, *H4*), 4.17 (q, *J* = 7.2 Hz, 2H, *H10*), 3.83 (s, 2H, *H1*), 1.65-1.51 (m, 3H, *H5,6*), 1.24 (t, *J* = 7.2 Hz, 3H, *H11*), 0.90 (d, *J* = 6.4 Hz, 6H, *H7,8*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.39 (*C*O,*C2*), 165.45 (*C*O,*C9*), 61.54 (O*C*H$_2$,*C10*), 51.40 (*C*H,*C4*), 41.37 (*C*H$_2$,*C5*), 28.79 (*C*H$_2$,*C1*), 24.67 (*C*H,*C6*), 22.79 (*C*H$_3$,*C7*/*C8*), 21.95 (*C*H$_3$,*C7*/*C8*), 14.12 (*C*H$_3$,*C11*)

L-Leucine butyl ester bromoacetate (258)



The title compound (**258**) was prepared from L-leucine butyl ester hydrochloride (**237**) (2.012 g, 9.00 mmol) and bromoacetyl bromide (2.169 g, 10.75 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow oil in 54 % yield (1.490 g, 4.84 mmol).

$[\alpha]_D^{20}$ = +9.2 ° (1.0 c, $CHCl_3$)

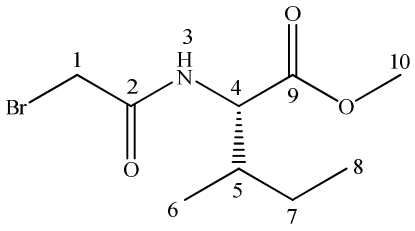
Molecular formula $C_{12}H_{22}BrNO_3$

Molecular weight 308 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.71 (d, *J* = 8.0 Hz, 1H, *H3*), 4.30 (ddd, *J* = 8.0, 8.0, 5.2 Hz, 1H, *H4*), 4.12 (dq, *J* = 7.0, 7.0 Hz, 1H, *H10*), 4.11 (dq, *J* = 7.4, 7.2 Hz, 1H, *H10*), 3.88 (d, *J* = 5.6 Hz, 2H, *H1*), 1.70-1.46 (m, 5H, *H5,6,11*), 1.35 (m, 2H, *H12*), 0.91-0.84 (m, 9H, *H7,8,13*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.06 (*C*O,*C2*), 166.20 (*C*O,*C9*), 64.20 (O*C*H_2,*C10*), 54.89 (*C*H,*C4*), 50.74 (*C*H_2,*C11*), 30.07 (*C*H_2,*C12*), 28.80 (*C*H_2,*C1*), 24.20 (*C*H,*C6*), 22.72 (*C*H_3,*C7*/*C8*), 21.25 (*C*H_3,*C7*/*C8*), 18.49 (*C*H_2,*C12*), 13.51 (*C*H_3,*C13*)

L-Isoleucine methyl ester bromoacetate (259)



The title compound (**259**) was prepared from L-isoleucine methyl ester hydrochloride (**232**) (0.579 g, 3.20 mmol) and bromoacetyl bromide (0.785 g, 3.85 mmol) according to the general procedure (Section 7.3.1, page 273) as a yellow liquid in 59 % yield (0.501 g, 1.88 mmol).

$[\alpha]_D^{20} = +30.0^{o}$ (0.9 c, $CHCl_3$)

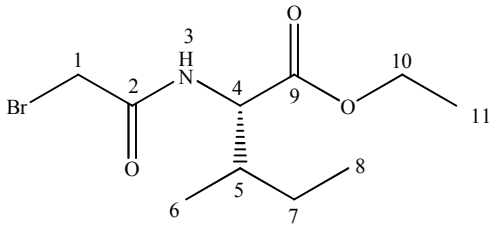
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.93 (d, *J* = 7.2 Hz, 1H, *H3*), 4.53 (dd, *J* = 8.4, 4.8 Hz, 1H, *H4*), 3.85 (s, 2H, *H1*), 3.69 (s, 3H, *H10*), 1.90 (dddq, *J* = 8.0, 8.0, 6.8, 4.4 Hz, 1H, *H5*), 1.41 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H7*), 1.18 (ddq, *J* = 8.0, 8.0, 7.0 Hz, 1H, *H7*), 0.89 (t, *J* = 7.2 Hz, 3H, *H8*), 0.84 (d, *J* = 6.8 Hz, 3H, *H6*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.78 (*C*O,*C2*), 165.33 (*C*O,*C9*), 57.08 (*C*H,*C4*), 52.31 (O*C*H_3,*C10*), 37.91 (*C*H,*C5*), 28.96 (*C*H_2,*C1*), 25.13 (*C*H_2,*C7*), 15.39 (*C*H_3,*C6*), 11.56 (*C*H_3,*C8*)

L-Isoleucine ethyl ester bromoacetate (260)



The title compound (**260**) was prepared from L-isoleucine ethyl ester hydrochloride (**233**) (0.663 g, 3.40 mmol) and bromoacetyl bromide (0.807 g, 4.00 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 59 % yield (0.558 g, 2.00 mmol)

$[\alpha]_D^{20} = +29.6^{o}$ (0.7 c in $CHCl_3$)

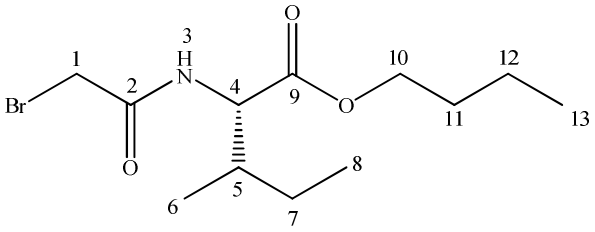
Molecular formula $C_{10}H_{18}BrNO_3$

Molecular weight 280 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.88 (d, *J* = 7.6 Hz, 1H, *H3*), 4.50 (dd, *J* = 8.0, 4.4 Hz, 1H, *H4*), 4.15 (dq, *J* = 7.2, 7.2 Hz, 1H, *H10*), 4.14 (dq, *J* = 7.2, 7.2 Hz, 1H, *H10*), 3.84 (s, 2H, *H1*), 1.96 (dddq, *J* = 8.0, 8.0, 6.8, 4.8 Hz, 1H, *H5*), 1.49 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H7*), 1.22 (m, 4H, *H7,11*), 0.89 (t, *J* = 7.2 Hz, 6H, *H6,8*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.24 (*C*O,*C2*), 165.20 (*C*O,*C9*), 61.47 (O*C*H_2,*C10*), 57.06 (*C*H,*C4*), 37.91 (*C*H,*C5*), 28.23 (*C*H_2,*C1*), 25.16 (*C*H_2,*C7*), 15.37 (*C*H_3,*C6*), 14.21 (*C*H_3,*C11*), 11.62 (*C*H_3,*C8*)

L-Isoleucine butyl ester bromoacetate (261)



The title compound (**261**) was prepared from L-isoleucine butyl ester hydrochloride (**234**) (1.528 g, 6.85 mmol) and bromoacetyl bromide (1.65 g, 8.20 mmol) according to the general procedure (Section 7.3.1, page 273) as a pale yellow liquid in 68 % yield (1.443 g, 4.68 mmol)

$[\alpha]_D^{20} = +22.0^{o}$ (0.7 c in $CHCl_3$)

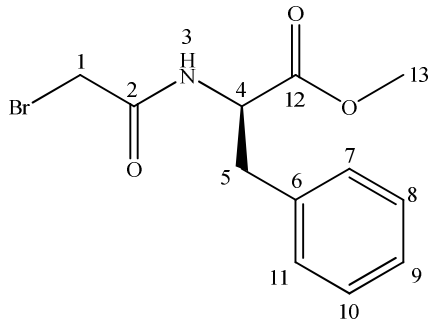
Molecular formula $C_{12}H_{22}BrNO_3$

Molecular weight 308 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.86 (d, *J* = 7.6 Hz, 1H, *H3*), 4.50 (dd, *J* = 8.4, 4.8 Hz, 1H, *H4*), 4.12 (dq, *J* = 7.0, 7.0 Hz, 1H, *H10*), 4.11(dq, *J* = 7.2, 7.0 Hz, 1H, *H10*), 3.84 (s, 2H, *H1*), 1.92 (dddq, *J* = 8.0, 8.0, 6.6, 4.8 Hz, 1H, *H5*), 1.49 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H7*), 1.25-1.11 (m, 5H, *H7,11,12*), 0.97 (t, *J* = 7.2 Hz, 3H, *H13*), 0.89 (t, *J* = 7.2 Hz, 6H, *H6,8*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.56 (*C*O,*C2*), 164.98 (*C*O,*C9*), 61.37 (O*C*H$_2$,*C10*), 56.66 (*C*H,*C4*), 37.92 (*C*H,*C5*), 31.42 (*C*H$_2$,*C11*), 28.33 (*C*H$_2$,C1), 25.17 (*C*H$_2$,*C7*), 22.34 (*C*H$_2$,*C12*), 15.35 (*C*H$_3$,*C6*/*C8*), 14.18 (*C*H$_3$,*C6*/*C8*), 11.60 (*C*H$_3$,*C13*)

D-Phenylalanine methyl ester bromoacetate (262)



The title compound (**262**) was prepared from D-phenylalanine methyl ester hydrochloride (**240**) (2.096 g, 9.75 mmol) and bromoacetyl bromide (2.509 g, 11.70 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 65 % yield (1.901 g, 6.34 mmol).

m.p. 92-94 °C $[\alpha]_D^{20}$ = -47.3 ° (0.9 c, $CHCl_3$)

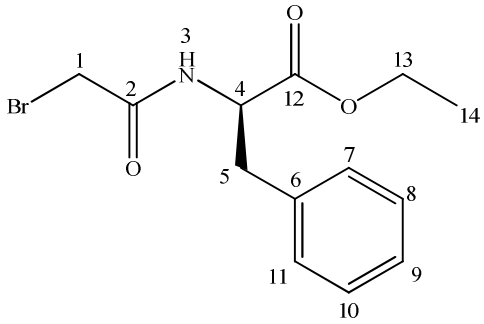
Molecular formula $C_{12}H_{14}BrNO_3$

Molecular weight 300 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.26-7.04 (m, 5H, *H7-11*), 6.77 (d, *J* = 6.8 Hz, 1H, *H3*), 4.78 (ddd, *J* = 7.0, 5.6, 5.6 Hz, 1H, *H4*), 3.78 (d, *J* = 3.6 Hz, 2H, *H1*), 3.68 (s, 3H, *H13*), 3.12 (dd, *J* = 13.6, 5.6 Hz, 1H, *H5*), 3.09 (dd, *J* = 13.6, 5.6 Hz, 1H, *H5*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.30 (*C*O,*C2*), 165.28 (*C*O,*C12*), 135.28 (Ar*C*,*C6*), 129.31 (Ar*C*H), 128.72 (Ar*C*H), 127.70 (Ar*C*H,*C9*), 53.68 (*C*H,*C4*), 52.52 (O*C*H$_3$,*C13*), 37.71 (*C*H$_2$,*C5*), 25.50 (*C*H$_2$,*C1*)

D-Phenylalanine ethyl ester bromoacetate (263)



The title compound (**263**) was prepared from D-phenylalanine ethyl ester hydrochloride (**241**) (1.721 g, 8.00 mmol) and bromoacetyl bromide (1.942 g, 9.60 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 70 % yield (1.750 g, 5.57 mmol).

m.p. 94-96 °C, $[\alpha]_D^{20}$ = -40.3 ° (0.9 c, $CHCl_3$)

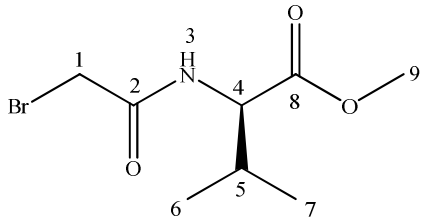
Molecular formula $C_{13}H_{16}BrNO_3$

Molecular weight 314 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.19-7.03 (m, 5H, *H7-11*), 6.86 (d, *J* = 6.8 Hz, 1H, *H3*), 4.73 (ddd, *J* = 8.0, 6.4, 6.4 Hz, 1H, *H4*), 4.08 (q, *J* = 7.2 Hz, 2H, *H13*), 3.71 (d, *J* = 2.0 Hz, 2H, *H1*), 3.14 (dd, *J* = 13.6, 5.6 Hz, 1H, *H5*), 3.02 (dd, *J* = 13.6, 5.6 Hz, 1H, *H5*), 1.14 (t, *J* = 7.2 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 170.95 (*C*O,*C2*), 165.82 (*C*O,*C12*), 135.58 (Ar*C*,*C6*), 129.17 (Ar*C*H), 128.33 (Ar*C*H), 127.22 (Ar*C*H,*C9*), 61.70 (O*C*H_2,*C13*), 53.81 (*C*H,*C4*), 37.70 (*C*H_2,*C5*), 28.37 (*C*H_2,*C1*), 14.13 (*C*H_3,*C14*)

D-Valine methyl ester bromoacetate (264)



The title compound (**264**) was prepared from D-valine methyl ester hydrochloride (**238**) (2.031 g, 15.30 mmol) and bromoacetyl bromide (3.673 g, 18.20 mmol) according to the general procedure (Section 7.3.1, page 273) as a slightly viscous yellow liquid in 45 % yield (1.750 g, 6.94 mmol).

$[\alpha]_D^{20}$ = -22.7 ° (0.9 c, $CHCl_3$)

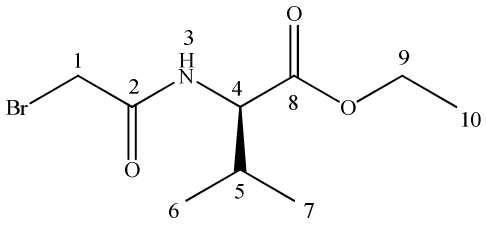
Molecular formula $C_8H_{14}BrNO_3$

Molecular weight 252 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.16 (d, *J* = 8.4 Hz, 1H, *H3*), 4.44 (dd, *J* = 8.0, 4.8 Hz, 1H, *H4*), 3.88 (s, 2H, *H1*), 3.68 (s, 3H, *H9*), 2.17 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H5*), 0.90 (d, *J* = 6.8, Hz, 3H, *H6/7*), 0.84 (d, *J* = 6.8, Hz, 3H, *H6/7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.84 (*C*O,*C2*), 166.32 (*C*O,*C8*), 57.59 (*C*H,*C4*), 52.35 (O*C*H_3,*C9*), 31.09 (*C*H,*C5*), 28.93 (*C*H_2,*C1*), 18.84 (*C*H_3,*C6*/*C7*), 17.55 (*C*H_3,*C6*/*C7*)

D-Valine ethyl ester bromoacetate (265)



The title compound (**265**) was prepared from D-valine ethyl ester hydrochloride (**239**) (2.001 g, 11.00 mmol) and bromoacetyl bromide (2.664 g, 13.20 mmol) according to the

general procedure (Section 7.3.1, page 273) as a colourless liquid in 59 % yield (1.731 g, 6.50 mmol).

$[\alpha]_D^{20}$ = -18.4 ° (0.9 c, $CHCl_3$)

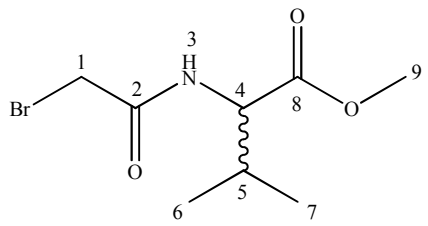
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.69 (d, *J* = 8.0 Hz, 1H, *H3*), 4.48 (dd, *J* = 8.8, 4.4 Hz, 1H, *H4*), 4.18 (dq, *J* = 7.2, 7.0 Hz, 1H, *H9*), 4.16 (dq, *J* = 7.0, 7.0 Hz, 1H, *H9*), 3.85 (s, 2H, *H1*), 2.17 (qqd, *J* = 6.4, 6.4, 4.4 Hz, 1H, *H5*), 1.23 (t, *J* = 7.0 Hz, 3H, *H10*), 0.91 (d, *J* = 6.8, Hz, 3H, *H6/7*), 0.87 (d, *J* = 6.8, Hz, 3H, *H6/7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.34 (*C*O,*C2*), 165.82 (*C*O,*C8*), 61.28 (O*C*H_2,*C9*), 57.60 (*C*H,*C4*), 31.27 (*C*H,*C5*), 28.82 (*C*H_2,*C1*), 18.81 (*C*H_3,*C6*/*C7*), 17.61 (*C*H_3,*C6*/*C7*), 14.15 (*C*H_3,*C10*)

DL-Valine methyl ester bromoacetate (266)



The title compound (**266**) was prepared from DL-valine methyl ester hydrochloride (**242**) (2.011 g, 12.00 mmol) and bromoacetyl bromide (2.901 g, 14.40 mmol) according to the general procedure (Section 7.3.1, page 273) as a yellow liquid in 82 % yield (2.471 g, 9.84 mmol).

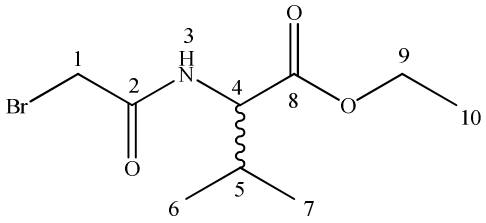
Molecular formula $C_8H_{14}BrNO_3$

Molecular weight 252 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.69 (d, *J* = 8.8 Hz, 1H, *H3*), 4.56 (dd, *J* = 8.8, 4.8 Hz, 1H, *H4*), 3.94 (s, 2H, *H1*), 3.78 (s, 3H, *H9*), 2.15 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H5*), 0.98 (d, *J* = 6.8 Hz, 3H, *H6/7*), 0.90 (d, *J* = 6.8 Hz, 3H, *H6/7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.85 (*C*O,*C2*), 166.88 (*C*O,*C8*), 57.53 (*C*H,*C4*), 52.40 (O*C*H_3,*C9*), 31.11 (*C*H,*C5*), 28.89 (*C*H_2,*C1*), 18.88 (*C*H_3,*C6*/*C7*), 17.69 (*C*H_3,*C6*/*C7*)

DL-Valine ethyl ester bromoacetate (267)



The title compound (**267**) was prepared from DL-valine ethyl ester hydrochloride (**243**) (2.591 g, 14.30 mmol) and bromoacetyl bromide (3.451 g, 17.10 mmol) according to the general procedure (Section 7.3.1, page 273) as a yellow liquid in 62 % yield (2.361 g, 8.87 mmol).

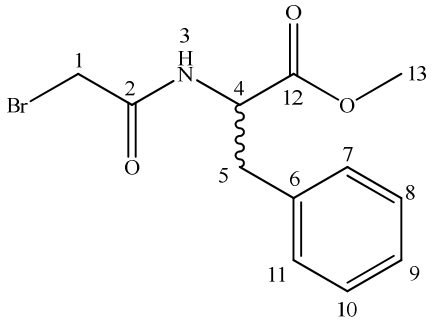
Molecular formula $C_9H_{16}BrNO_3$

Molecular weight 266 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.99 (d, *J* = 8.0 Hz, 1H, *H3*), 4.54 (dd, *J* = 8.0, 4.8 Hz, 1H, *H4*), 4.19 (dq, *J* = 7.0, 7.0 Hz, 1H, *H9*), 4.17 (dq, *J* = 7.2, 7.0 Hz, 1H, *H9*), 3.92 (s, 2H, *H1*), 2.17 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H5*), 1.30 (t, *J* = 7.2 Hz, 3H, *H10*), 0.93 (dd, *J* = 9.6, 6.8 Hz, 6H, *H6,7*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.87 (*C*O,*C2*), 166.27 (*C*O,*C8*), 61.16 (O*C*H_2,*C9*) 57.54 (*C*H,*C4*), 31.12 (*C*H,*C5*), 28.89 (*C*H_2,*C1*), 18.85 (*C*H_3,*C6*/*C7*), 17.54 (*C*H_3,*C6*/*C7*), 14.20 (*C*H_3,*C10*)

DL-Phenylalanine methyl ester bromoacetate (268)



The title compound (**268**) was prepared from DL-phenylalanine methyl ester hydrochloride (**244**) (2.501 g, 11.60 mmol) and bromoacetyl bromide (2.821 g, 13.95 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 66 % yield (2.301 g, 7.67 mmol).

m.p. 68-70 ° C

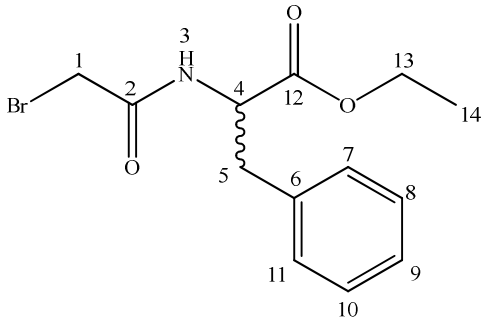
Molecular formula $C_{12}H_{14}BrNO_3$

Molecular weight 300 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.35-7.13 (m, 5H, *H7-11*), 6.86 (d, *J* = 7.2 Hz, 1H, *H3*), 4.87 (dd, *J* = 8.0, 6.8, 6.8 Hz, 1H, *H4*), 3.88 (d, *J* = 3.6 Hz, 2H, *H1*), 3.77 (s, 3H, *H13*), 3.14 (dd, *J* = 14.0, 6.8 Hz, 1H, *H5*), 3.09 (dd, *J* = 14.0, 6.4 Hz, 1H, *H5*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.29 (*C*O,*C2*), 165.16 (*C*O,*C12*), 135.35 (Ar*C*,*C6*), 129.14 (Ar*C*H), 128.70 (Ar*C*H), 127.35 (Ar*C*H,*C9*), 53.74 (*C*H,*C4*), 52.49 (O*C*H_3,*C13*), 37.74 (*C*H_2,*C5*), 28.63 (*C*H_2,*C1*)

DL-Phenylalanine ethyl ester bromoacetate (269)



The title compound (**269**) was prepared from DL-phenylalanine ethyl ester hydrochloride (**245**) (2.011 g, 8.70 mmol) and bromoacetyl bromide (2.113 g, 10.45 mmol) according to the general procedure (Section 7.3.1, page 273) as a white solid in 69 % yield (1.890 g, 6.02 mmol).

m.p. 71-73 ° C

Molecular formula $C_{13}H_{16}BrNO_3$

Molecular weight 314 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.35-7.15 (m, 5H, *H7-11*), 6.67 (d, *J* = 7.2 Hz, 1H, *H3*), 4.87 (ddd, *J* = 8.4, 6.8, 6.8 Hz, 1H, *H4*), 4.14 (q, *J* = 7.0 Hz, 2H, *H13*), 3.84 (d, *J* = 3.5 Hz, 2H, *H1*), 3.18 (dd, *J* = 14.0, 6.8 Hz, 1H, *H5*), 3.11 (dd, *J* = 13.8, 6.4 Hz, 1H, *H5*), 1.28 (t, *J* = 7.2 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.37 (*C*O,*C2*), 166.31 (*C*O,*C12*), 135.75 (Ar*C*,*C6*), 129.53 (Ar*C*H), 128.33 (Ar*C*H), 126.86 (Ar*C*H,*C9*), 61.74 (O*C*H_2,*C13*), 53.89 (*C*H,*C4*), 37.63 (*C*H_2,*C5*), 28.58 (*C*H_2,*C1*), 14.11 (*C*H_3,*C14*)

7.3.2 Representative procedure for the preparation of chiral α-bromoamides (K_2CO_3 method); L-phenylalanine methyl ester bromoacetate (247)

To a stirred solution of DCM, L-phenylalanine methyl ester hydrochloride (**223**) (2.780 g, 12.88 mmol), and potassium carbonate (2.671 g, 19.33 mmol) at room temperature was added dropwise bromoacetyl bromide (3.901 g, 19.33 mmol). After stirring at this temperature for 24 h, the reaction was filtered to remove the potassium carbonate. The organic phase was washed with distilled water (3 x 10 mL) and saturated ammonium chloride (3 x 10 mL). The organic phase was then dried over anhydrous magnesium sulfate, filtered and volatiles removed *via* rotary evaporation to give title product (**247**) in 86 % yield (3.337 g, 11.16 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Phenylalanine butyl ester bromoacetate (249)

The title compound (**249**) was prepared from L-phenylalanine butyl ester hydrochloride (**224**) (1.680 g, 6.52 mmol) and bromoacetyl bromide (1.974 g, 9.78 mmol) according to the general procedure in 82 % yield (1.829 g, 5.36 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Alanine ethyl ester bromoacetate (251)

The title compound (**251**) was prepared from L-alanine ethyl ester hydrochloride (**226**) (1.433 g, 9.34 mmol) and bromoacetyl bromide (2.828 g, 14.01 mmol) according to the general procedure in 70 % yield (1.558 g, 6.57 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Valine methyl ester bromoacetate (253)

The title compound (**253**) was prepared from L-valine methyl ester hydrochloride (**229**) (1.270 g, 7.60 mmol) and bromoacetyl bromide (2.302 g, 11.40 mmol) according to the general procedure in 77 % yield (1.480 g, 5.89 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Valine ethyl ester bromoacetate (254)

The title compound (**254**) was prepared from L-valine ethyl ester hydrochloride (**230**) (1.057 g, 5.82 mmol) and bromoacetyl bromide (1.763 g, 8.74 mmol) according to the general procedure in 71 % yield (1.096 g, 4.12 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Valine butyl ester bromoacetate (255)

The title compound (**255**) was prepared from L-valine butyl ester hydrochloride (**231**) (2.622 g, 12.95 mmol) and bromoacetyl bromide (3.921 g, 19.42 mmol) according to the general procedure in 78 % yield (2.992 g, 10.17 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Leucine methyl ester bromoacetate (256)

The title compound (**256**) was prepared from L-leucine methyl ester hydrochloride (**235**) (1.012 g, 5.57 mmol) and bromoacetyl bromide (1.680 g, 8.35 mmol) according to the general procedure in 98 % yield (1.449 g, 5.45 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

L-Leucine ethyl ester bromoacetate (257)

The title compound (**257**) was prepared from L-leucine ethyl ester hydrochloride (**236**) (2.049 g, 10.47 mmol) and bromoacetyl bromide (3.170 g, 15.70 mmol) according to the general procedure in 75 % yield (2.189 g, 7.82 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

D-Valine methyl ester bromoacetate (264)

The title compound (**264**) was prepared from D-valine methyl ester hydrochloride (**238**) (0.499 g, 2.98 mmol) and bromoacetyl bromide (0.901 g, 4.46 mmol) according to the general procedure in 66 % yield (0.498 g, 1.98 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

D-Valine ethyl ester bromoacetate (265)

The title compound (**265**) was prepared from D-valine ethyl ester hydrochloride (**239**) (1.837 g, 9.49 mmol) and bromoacetyl bromide (2.874 g, 14.25 mmol) according to the general procedure in 73 % yield (1.842 g, 6.95 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

DL-Valine methyl ester bromoacetate (266)

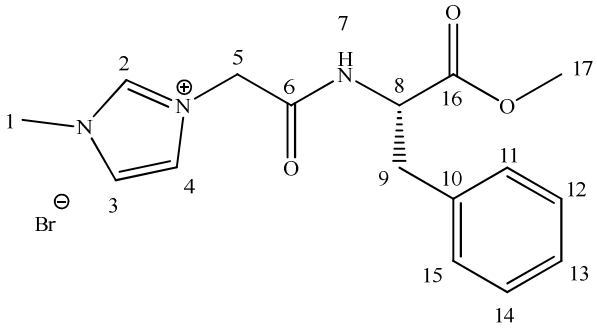
The title compound (**266**) was prepared from DL-valine methyl ester hydrochloride (**242**) (1.717 g, 10.25 mmol) and bromoacetyl bromide (3.105 g, 15.38 mmol) according to the general procedure in 67 % yield (1.738 g, 6.92 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

DL-Valine ethyl ester bromoacetate (267)

The title compound (**267**) was prepared from DL-valine ethyl ester hydrochloride (**243**) (1.084 g, 5.97 mmol) and bromoacetyl bromide (1.810 g, 8.95 mmol) according to the general procedure in 68 % yield (1.071 g, 4.04 mmol). ^{1}H and ^{13}C NMR spectral data was in accordance with that previously reported for the triethylamine method (Section 7.3.1).

7.3.3 Preparation of Chiral Br ILs

***General procedure for the Preparation of Chiral Amino Acid ester bromide salts*; 3-methyl-1-L-phenylalanine methyl ester imidazolium bromide (270)**



To a stirred solution of 1-methylimidazole (0.472 g, 5.75 mmol) in tetrahydrofuran (60 mL) at -78 ºC under a nitrogen atmosphere L-phenylalanine methyl ester bromoacetate (**247**) (2.061 g, 6.90 mmol) was added dropwise. The reaction mixture was stirred vigorously at -15 ºC for 2h, then at RT overnight. The THF top phase was decanted and the IL washed with diethyl ether (3 x 10 mL). The residual solvent was removed on the rotary evaporator and the product was dried under high vacuum for 24 h to yield the title product (**270**) as a colourless liquid at RT in 98 % yield (2.160 g, 5.65 mmol).

$[\alpha]_D^{20} = +18.0^{\circ}$ (1.0 c, $CHCl_3$)

Molecular formula $C_{16}H_{20}BrN_3O_3$

Molecular weight 382 $gmol^{-1}$

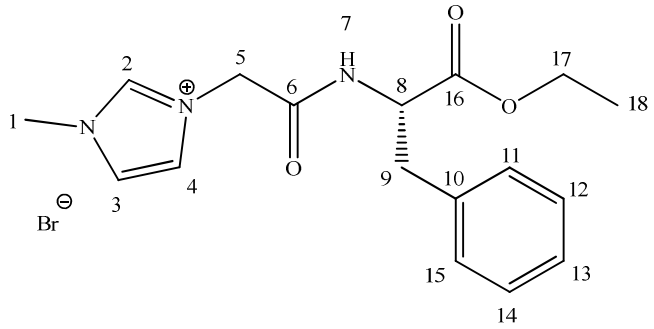
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.74 (s, 1H, *H2*), 9.06 (d, *J* = 8.0 Hz, 1H, *H7*), 7.68 (t, *J* = 1.6 Hz, 1H, *H4*), 7.61 (t, *J* = 1.6 Hz, 1H, *H3*), 7.40-7.12 (m, 5H, *H11-15*), 5.02 (d, *J* = 6.0 Hz, 2H, *H5*), 4.62 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H8*), 3.91 (s, 3H, *H1*), 3.58 (s, 3H, *H17*), 3.14 (dd, *J* = 13.0, 6.0 Hz, 1H, *H9*), 3.09 3.14 (dd, *J* = 13.0, 6.0 Hz, 1H, *H9*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.73 (*C*O,*C16*), 164.91 (*C*O,*C6*), 137.74 (N*C*HN,*C2*), 136.64 (Ar*C*,*C10*), 129.47 (Ar*C*H), 126.83 (Ar*C*H), 123.68 (Ar*C*,*C13*), 123.06 (N*C*H,*C4*), 122.46 (N*C*H,*C3*), 54.74 (*C*H,*C8*), 52.45 (N*C*H_2,*C5*), 51.49 (O*C*H_3,*C17*), 37.30 (*C*H_2,*C9*), 36.76 (N*C*H_3,*C1*)

IR (neat) (cm^{-1}) 3199 (w), 3026 (w), 1773 (s), 1672 (vs), 1528 (m), 1262 (m), 1219 (s), 1173 (vs), 764 (m), 701 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 302.1497, $C_{16}H_{20}N_3O_3^+$ requires 302.1499

3-Methyl-1-L-phenylalanine ethyl ester imidazolium bromide (271)



The title compound (**271**) was prepared from 1-methylimidazole (0.454 g, 5.55 mmol) and L-phenylalanine ethyl ester bromoacetate (**248**) (2.081 g, 6.60 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 98 % yield (2.152 g, 5.43 mmol).

$[\alpha]_D^{20} = +17.0^{o}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{17}H_{22}BrN_3O_3$

Molecular weight 396 $gmol^{-1}$

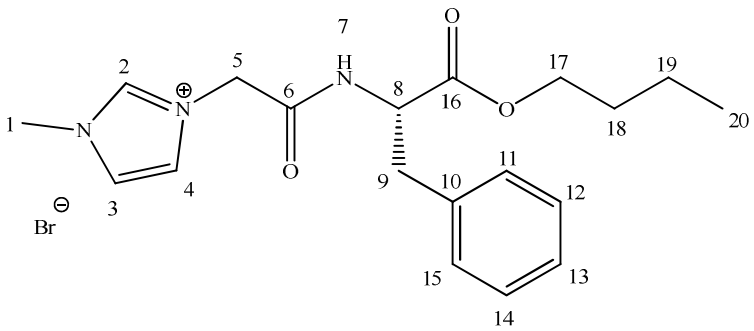
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.04 (s, 1H, *H2*), 8.99 (d, *J* = 7.6 Hz, 1H, *H7*), 7.68 (t, *J* = 1.6 Hz, 1H, *H4*), 7.60 (t, *J* = 1.8 Hz, 1H, *H3*), 7.33-7.22 (m, 5H, *H11-15*), 5.02 (d, *J* = 5.6 Hz, 2H, *H5*), 4.46 (ddd, *J* = 7.8, 5.6, 5.6 Hz, 1H, *H8*), 4.04 (q, *J* = 7.2, Hz, 2H, *H17*), 3.92 (s, 3H, *H1*), 3.07 (dd, *J* = 13.0, 6.0 Hz, 1H, *H9*), 2.96 (dd, *J* = 10.0, 8.8 Hz, 1H, *H9*), 1.14 (t, *J* = 7.2 Hz, 3H, *H18*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.22 (*C*O,*C16*), 164.80 (*C*O,*C6*), 137.78 (N*C*HN,*C2*) 136.66 (Ar*C*,*C10*), 129.52 (Ar*C*H), 128.48 (Ar*C*H), 126.79 (Ar*C*H,*C13*), 123.69 (N*C*H,*C4*), 122.39 (N*C*H,*C3*), 61.50 (O*C*H_2,*C17*), 54.81 (*C*H,*C8*), 51.54 (N*C*H_2,*C5*), 37.40 (*C*H_2,*C9*), 36.76 (N*C*H_3,*C1*), 14.06 (*C*H_3,*C18*)

IR (neat) (cm^{-1}) 3160 (b), 3034 (w), 1735 (m), 1682 (s), 1548 (s), 1172 (vs), 747 (m), 701 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 316.1653, $C_{17}H_{22}N_3O_3^+$ requires 316.1655

3-Methyl-1-L-phenylalanine butyl ester imidazolium bromide (272)



The title compound (**272**) was prepared from 1-methylimidazole (0.401 g, 4.90 mmol) and L-phenylalanine butyl ester bromoacetate (**249**) (1.991 g, 5.85 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 99 % yield (2.072 g, 4.89 mmol).

$[\alpha]_D^{20} = +20.0^{o}$ (0.9 c, $CHCl_3$)

Molecular formula $C_{19}H_{26}BrN_3O_3$

Molecular weight 424 $gmol^{-1}$

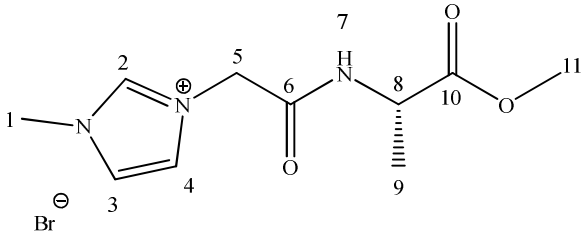
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.68 (s, 1H, *H2*), 8.95 (d, *J* = 7.6 Hz, 1H, *H7*), 7.36 (t, *J* = 1.6 Hz, 1H, *H4*), 7.30-7.12 (m, 6H, *H3,11-15*), 5.33 (d, *J* = 8.4 Hz, 2H, *H5*), 4.63 (ddd, *J* = 7.8, 5.2, 5.2 Hz, 1H, *H8*), 4.10 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 3.92 (s, 3H, *H1*), 3.11 (dd, *J* = 13.6, 6.0 Hz, 1H, *H9*), 3.07 (dd, *J* = 13.6, 5.6 Hz, 1H, *H9*), 1.45 (tt, *J* = 7.0, 6.8 Hz, 2H, *H18*), 1.24 (tq, *J* = 7.2, 7.0 Hz, 2H, *H19*), 0.83 (t, *J* = 7.4 Hz, 3H, *H20*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.33 (*C*O,*C16*), 164.76 (*C*O,*C6*), 137.77 (N*C*HN,*C2*), 136.64 (Ar*C*,*C10*), 129.49 (Ar*C*H), 128.44 (Ar*C*H), 126.79 (Ar*C*H,*C13*), 123.69 (N*C*H,*C4*), 122.38 (N*C*H,*C3*), 65.39 (O*C*H_2,*C17*), 54.80 (*C*H,*C8*), 51.55 (N*C*H_2,*C5*), 37.43 (*C*H_2,*C9*), 36.77 (N*C*H_3,*C1*), 35.48 (*C*H_2,*C18*), 19.00 (*C*H_2,*C19*), 13.70 (*C*H_3,*C20*)

IR (neat) (cm^{-1}) 3423 (b), 3214 (w), 2959 (w), 1735 (m), 1683 (s), 1561 (m), 1174 (vs), 744 (m), 700 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 344.1964, $C_{19}H_{26}N_3O_3^+$ requires 344.1968

3-Methyl-1-L-alanine methyl ester imidazolium bromide (273)



The title compound (**273**) was prepared from 1-methylimidazole (0.250 g, 3.45 mmol) and L-alanine methyl ester bromoacetate (**250**) (0.971 g, 3.45 mmol) according to the general procedure (Section 7.3.3, page 295) as a pale yellow slightly viscous liquid in 96 % yield (1.009 g, 3.30 mmol).

$[\alpha]_D^{20} = -11.0\ ^o$ (1.0 c, $CHCl_3$)

Molecular formula $C_{10}H_{16}BrN_3O_3$

Molecular weight 306 $gmol^{-1}$

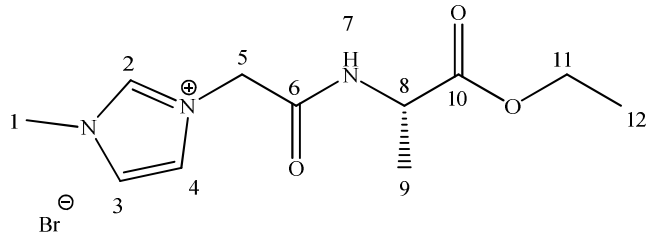
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.15 (s, 1H, *H2*), 9.04 (d, *J* = 7.2 Hz, 1H, *H7*), 7.77 (t, *J* = 1.6 Hz, 1H, *H4*), 7.45 (t, *J* = 1.8 Hz, 1H, *H3*), 5.12 (d, *J* = 2.4 Hz, 2H, *H5*), 4.42 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 3.95 (s, 3H, *H1*), 3.71 (s, 3H, *H11*), 1.40 (d, *J* = 7.2 Hz, 3H, *H9*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.50 (*C*O,*C10*), 164.83 (*C*O,*C6*), 137.72 (N*C*HN,*C2*), 123.73 (N*C*H,*C4*), 122.98 (N*C*H,*C3*), 52.05 (N*C*H_2,*C5*), 51.90 (O*C*H_3,*C11*), 47.88 (*C*H,*C8*), 35.78 (N*C*H_3,*C1*), 17.02 (*C*H_3,*C9*)

IR (neat) (cm^{-1}) 3385 (b), 3069 (w), 1732 (m), 1679 (s), 1563 (m), 1219 (m), 1208 (m), 1173 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 226.1195, $C_{10}H_{16}N_3O_3^+$ requires 226.1186

3-Methyl-1-L-alanine ethyl ester imidazolium bromide (274)



The title compound (**274**) was prepared from 1-methylimidazole (0.190 g, 2.70 mmol) and L-alanine ethyl ester bromoacetate (**251**) (0.653 g, 2.70 mmol) according to the general procedure (Section 7.3.3, page 295) as a pale yellow liquid in 90 % yield (0.781 g, 2.44 mmol).

$[\alpha]_D^{20} = -16.0^{o}$ (1.0 c, $CHCl_3$)

Molecular formula $C_{11}H_{18}BrN_3O_3$

Molecular weight 320 $gmol^{-1}$

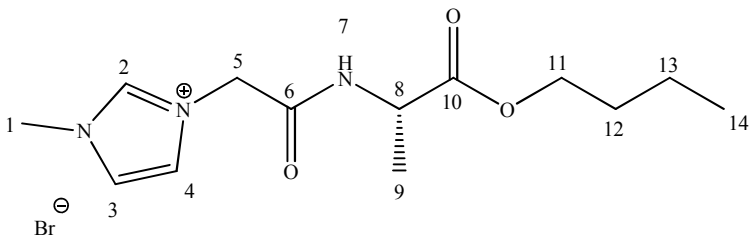
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.63 (s, 1H, *H2*), 8.90 (d, *J* = 6.4 Hz, 1H, *H7*), 7.58 (t, *J* = 1.8 Hz, 1H, *H4*), 7.33 (t, *J* = 1.6 Hz, 1H, *H3*), 5.39 (s, 2H, *H5*), 4.33 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 4.08 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 3.99 (s, 3H, *H1*), 1.44 (d, *J* = 7.2 Hz, 3H, *H9*), 1.18 (t, *J* = 7.2 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.49 (*C*O,*C10*), 164.76 (*C*O,*C6*), 137.75 (N*C*HN,*C2*), 123.85 (N*C*H,*C4*), 122.67 (N*C*H,*C3*), 61.48 (O*C*H_2,*C11*), 51.52 (N*C*H_2,*C5*), 49.03 (*C*H,*C8*), 36.88 (N*C*H_3,*C1*), 17.05 (*C*H_3,C9), 14.15 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3406 (b), 2985 (w), 1731 (m), 1681 (s), 1564 (m), 1210 (s), 1183 (s), 1173(vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 240.1342, $C_{11}H_{18}N_3O_3^+$ requires 240.1342

3-Methyl-1-L-alanine butyl ester imidazolium bromide (275)



The title compound (**275**) was prepared from 1-methylimidazole (0.227 g, 2.77 mmol) and L-alanine butyl ester bromoacetate (**252**) (1.032 g, 3.88 mmol) according to the general procedure (Section 7.3.3, page 295) as a pale yellow oil in 82 % yield (0.789 g, 2.27 mmol).

$[\alpha]_D^{20} = -20.0\ ^{o}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 $gmol^{-1}$

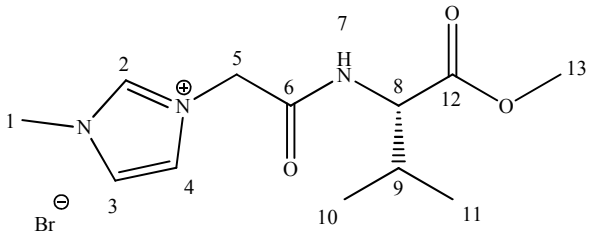
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.11 (s, 1H, *H2*), 8.96 (d, *J* = 7.2 Hz, 1H, *H7*), 7.71 (t, *J* = 1.6 Hz, 1H, *H4*), 7.68 (t, *J* = 1.6 Hz, 1H, *H3*), 5.06 (s, 2H, *H5*), 4.32 (dq, *J* = 7.2, 7.0 Hz, 1H, *H8*), 4.08 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 4.07 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 3.89 (s, 3H, *H1*), 1.56 (tt, *J* = 6.9, 6.8 Hz, 2H, *H12*), 1.34-1.28 (m, 5H, *H9,13*), 0.89 (t, *J* = 7.4 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.45 (*C*O,*C10*), 164.80 (*C*O,*C6*), 137.72 (N*C*HN,*C2*), 123.72 (N*C*H,*C4*), 122.99 (N*C*H,*C3*), 64.30 (O*C*H_2,*C11*), 54.52 (N*C*H_2,*C5*), 48.00 (*C*H,*C8*), 35.78 (N*C*H_3,*C1*), 30.05 (*C*H_2,*C12*), 18.47 (*C*H_2,*C13*), 17.04 (*C*H_3,*C9*), 13.50 (*C*H_3,*C14*)

IR (neat) (cm^{-1}) 3416 (b), 3060 (w), 2960 (w), 1736 (m), 1682 (vs), 1563 (m), 1204 (s), 1172 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 268.1656, $C_{13}H_{22}N_3O_3^+$ requires 268.1655

3-Methyl-1-L-valine methyl ester imidazolium bromide (276)



The title compound (**276**) was prepared from 1-methylimidazole (0.123 g, 1.45 mmol) and L-valine methyl ester bromoacetate (**253**) (0.552 g, 2.20 mmol) according to the general procedure (Section 7.3.3, page 295) as a pale yellow liquid in 85 % yield (0.412 g, 1.23 mmol).

$[\alpha]_D^{20}$ = -9.6 ° (0.7 c, $CHCl_3$)

Molecular formula $C_{12}H_{20}BrN_3O_3$

Molecular weight 334 $gmol^{-1}$

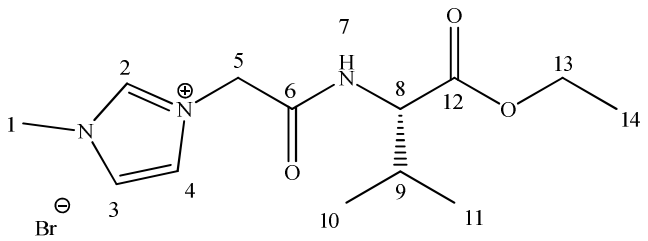
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.55 (s, 1H, *H2*), 8.53 (d, *J* = 7.6 Hz, 1H, *H7*), 7.45 (t, *J* = 1.6 Hz, 1H, *H4*), 7.27 (t, *J* = 1.6 Hz, 1H, *H3*), 5.36 (s, 2H, *H5*), 4.30 (dd, *J* = 8.8, 4.8 Hz, 1H, *H8*), 3.86 (s, 3H, *H1*), 3.52 (s, 3H, *H13*), 2.15 (qqd, *J* = 6.8, 6.4, 4.8 Hz, 1H, *H9*), 0.90 (d, *J* = 6.8 Hz, 3H, *H10*/*11*), 0.85 (d, *J* = 7.2 Hz, 3H, *H10*/*11*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.96 (*C*O,*C12*), 165.28 (*C*O,*C6*), 137.79 (N*C*HN,*C2*), 123.95 (N*C*H,*C4*), 122.31 (N*C*H,*C3*), 58.90 (*C*H,*C8*), 52.26 (N*C*H_2,*C5*), 51.68 (O*C*H_3,*C13*), 36.80 (N*C*H_3,C1), 30.32 (*C*H,*C9*), 19.18 (*C*H_3,*C10*/*C11*), 18.55 (*C*H_3,*C10*/*C11*)

IR (neat) (cm^{-1}) 3386 (b), 3232 (b), 2965 (w), 1733 (m), 1678 (s), 1545 (m), 1209 (s), 1174 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 254.1493, $C_{12}H_{20}N_3O_3^+$ requires 254.1499

3-Methyl-1-L-valine ethyl ester imidazolium bromide (277)



The title compound (**277**) was prepared from 1-methylimidazole (0.418 g, 5.10 mmol) and L-valine ethyl ester bromoacetate (**254**) (1.532 g, 6.15 mmol) according to the general procedure (Section 7.3.3, page 295) as a pale yellow liquid in 98 % yield (1.744 g, 5.01 mmol).

$[\alpha]_D^{20}$ = -11.2 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 $gmol^{-1}$

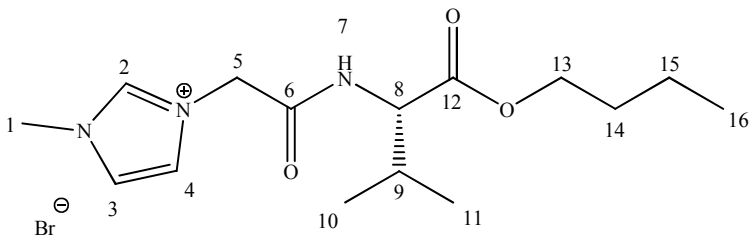
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.10 (s, 1H, *H2*), 8.82 (d, *J* = 8.0 Hz, 1H, *H7*), 7.64 (t, *J* = 1.8 Hz, 1H, *H4*), 7.38 (t, *J* = 1.8 Hz, 1H, *H3*), 5.53 (d, *J* = 4.0 Hz, 2H, *H5*), 4.44 (dd, *J* = 8.0, 4.8 Hz, 1H, *H8*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H13*), 4.17 (dq, *J* = 7.2, 7.0 Hz, 1H, H13), 4.04 (s, 3H, *H1*), 2.16 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H9*), 1.21 (t, *J* = 7.2 Hz, 3H, *H14*), 1.06 (d, *J* = 6.8 Hz, 3H, *H10*/*11*), 0.96 (d, *J* = 6.8 Hz, 3H, *H10*/*11*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.96 (*C*O,*C12*), 165.33 (*C*O,*C6*), 137.74 (N*C*HN,*C2*), 123.75 (N*C*H,*C4*), 122.95 (N*C*H,*C3*), 60.56 (O*C*H_3,*C13*), 57.72 (*C*H,*C8*), 50.26 (N*C*H_2,*C5*), 35.78 (N*C*H_3,*C1*), 30.11 (*C*H,*C9*), 18.84 (*C*H_3,*C10*), 18.03 (*C*H_3,*C11*), 14.07 (*C*H_3,*C14*)

IR (neat) (cm^{-1}) 3401 (b), 3219 (b), 2967 (w), 1733 (s), 1681 (vs), 1545 (s), 1373 (m), 1173 (vs), 1022 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 268.1661, $C_{13}H_{22}N_3O_3^+$ requires 268.1655

3-Methyl-1-L-valine butyl ester imidazolium bromide (278)



The title compound (**278**) was prepared from 1-methylimidazole (0.399 g, 4.86 mmol) and L-valine butyl ester bromoacetate (**255**) (1.641 g, 5.85 mmol) according to the general procedure (Section 7.3.3, page 295) as a viscous pale yellow liquid in 96 % yield (1.755 g, 4.67 mmol).

$[\alpha]_D^{20}$ = -5.0 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{15}H_{26}BrN_3O_3$

Molecular weight 376 $gmol^{-1}$

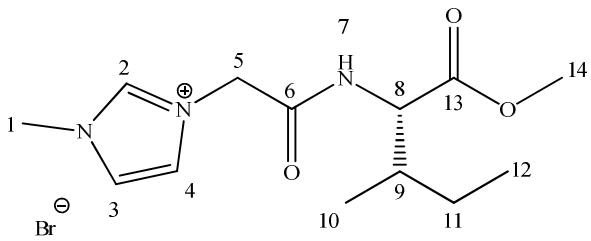
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.09 (s, 1H, *H2*), 8.81 (d, *J* = 8.0 Hz, 1H, *H7*), 7.70 (t, *J* = 1.8 Hz, 1H, *H4*), 7.64 (t, *J* = 1.6 Hz, 1H, *H3*), 5.10 (d, *J* = 4.0 Hz, 2H, *H5*), 4.23 (dd, *J* = 8.8, 4.4 Hz, 1H, *H8*), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H, *H13*), 4.08 (dq, *J* = 7.2, 7.2 Hz, 1H, *H13*), 3.92 (s, 3H, *H1*), 2.12 (qqd, *J* = 6.8, 6.0, 4.8 Hz, 1H, *H9*), 1.58 (tt, *J* = 7.0, 6.8 Hz, 2H, *H14*), 1.33 (tq, *J* = 7.2, 7.0 Hz, 2H, *H15*), 0.93-0.87 (m, 9H, *H10,11,16*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.51 (*C*O,*C12*), 165.22 (*C*O,*C6*), 137.77 (N*C*HN,*C2*), 123.85 (N*C*H,*C4*), 122.44 (N*C*H,*C3*), 65.15 (O*C*H_2,*C13*), 58.91 (*C*H,*C8*), 51.65 (N*C*H_2,*C5*), 36.84 (N*C*H_3,*C1*), 30.54 (*C*H_2,*C14*), 30.35 (*C*H,*C9*), 19.23 (*C*H_2,*C15*), 19.13 (*C*H_3,*C10*/*C11*), 18.51 (*C*H_3,*C10*/*C11*), 13.71 (*C*H_3,*C16*)

IR (neat) (cm^{-1}) 3386 (b), 3197 (w), 2961 (m), 1733 (s), 1678 (vs), 1545 (s), 1209 (m), 1174 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 296.1967, $C_{15}H_{26}N_3O_3^+$ requires 296.1968

3-Methyl-1-L-isoleucine methyl ester imidazolium bromide (279)



The title compound (**279**) was prepared from 1-methylimidazole (0.236 g, 3.46 mmol) and L-isoleucine methyl ester bromoacetate (**259**) (0.922 g, 3.46 mmol) according to the general procedure (Section 7.3.3, page 295) as a yellow viscous liquid in 67 % yield (0.813 g, 2.33 mmol).

$[\alpha]_D^{20}$ = -7.2 ° (0.6 c, $CHCl_3$)

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 $gmol^{-1}$

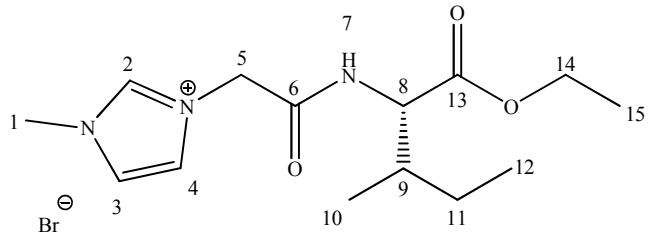
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.70 (s, 1H, *H2*), 8.69 (d, *J* = 7.6 Hz, 1H, *H7*), 7.56 (t, *J* = 1.8 Hz, 1H, *H4*), 7.19 (t, *J* = 1.8 Hz, 1H, *H3*), 5.48 (s, 2H, *H5*), 4.34 (dd, *J* = 8.0, 6.4 Hz, 1H, *H8*), 3.96 (s, 3H, *H1*), 3.64 (s, 3H, *H14*), 1.94 (dddq, *J* = 8.0, 8.0, 7.2, 6.0 Hz, 1H, *H9*), 1.48 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H11*), 1.30 (ddq, *J* = 8.0, 8.0 , 7.2 Hz, 1H, *H11*), 0.89 (t, *J* = 7.2 Hz, 3H, *H12*), 0.85 (d, *J* = 7.2 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.81 (*C*O,*C13*), 165.11 (*C*O,*C6*), 137.82 (N*C*HN,*C2*), 123.90 (N*C*H,*C4*) 122.30 (N*C*H,*C3*), 57.88 (*C*H,*C8*), 52.13 (N*C*H_2,*C5*), 51.64 (O*C*H_3,*C14*), 36.82 (*C*H,*C9*), 36.79 (N*C*H_3,*C1*), 25.55 (*C*H_2,*C11*), 15.77 (*C*H_3,*C10*), 11.55 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3337 (w), 3230 (w), 2960 (w), 1750 (s), 1740 (s), 1696 (vs), 1558 (s), 1216 (m), 1168 (vs), 1147 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 268.1654, $C_{13}H_{22}N_3O_3^+$ requires 268.1655

3-Methyl-1-L-isoleucine ethyl ester imidazolium bromide (280)



The title compound (**280**) was prepared from 1-methylimidazole (0.123 g, 1.50 mmol) and L-isoleucine ethyl ester bromoacetate (**260**) (0.587 g, 2.11 mmol) according to the general procedure (Section 7.3.3, page 295) as a viscous yellow liquid in 94 % yield (0.510 g, 1.41 mmol).

$[\alpha]_D^{20} = -4.0\ ^{o}$ (1.0 c, $CHCl_3$)

Molecular formula $C_{14}H_{24}BrN_3O_3$

Molecular weight 362 $gmol^{-1}$

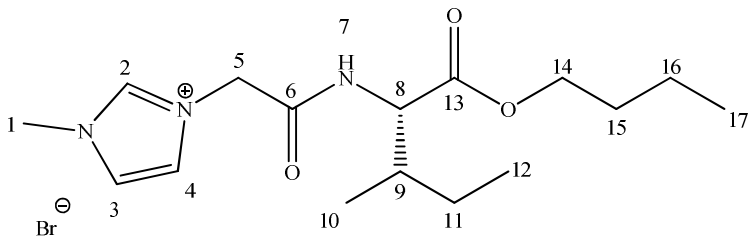
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.71 (s, 1H, *H2*), 8.62 (d, *J* = 7.6 Hz, 1H, *H7*), 7.56 (t, *J* = 1.4 Hz, 1H, *H4*), 7.23 (t, *J* = 1.4 Hz, 1H, *H3*), 5.46 (s, 2H, *H5*), 4.32 (dd, *J* = 8.0, 6.0 Hz, 1H, *H8*), 4.14 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 3.96 (s, 3H, *H1*), 1.98 (dddq, *J* = 8.0, 8.0, 7.2, 6.6 Hz, 1H, *H9*), 1.49 (ddq, *J* =, 8.0, 8.0, 7.2 Hz, 1H, *H11*), 1.32 (ddq, *J* = 8.0, 8.0, 7.0 Hz, 1H, *H11*), 1.19 (t, *J* = 7.0 Hz, 3H, *H15*), 0.88 (d, *J* = 7.2 Hz, 3H, *H12*), 0.85 (t, *J* = 7.2 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.28 (*C*O,*C13*), 165.06 (*C*O,*C6*), 137.82 (N*C*HN,*C2*), 123.88 (N*C*H,*C4*) 122.33 (N*C*H,*C3*), 61.19 (O*C*H_2,*C14*), 57.87 (*C*H,*C8*), 51.65 (N*C*H_2,*C5*), 36.89 (*C*H,*C9*), 36.80 (N*C*H_3,*C1*), 25.57 (*C*H_2,*C11*), 15.78 (*C*H_3,*C10*), 14.25 (*C*H_3,*C15*), 11.62 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3406 (b), 2967 (w), 1733 (s), 1681 (vs), 1545 (s), 1373 (m), 1173 (vs), 1022 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 282.1816, $C_{14}H_{24}N_3O_3^+$ requires 282.1812

3-Methyl-1-L-isoleucine butyl ester imidazolium bromide (281)



The title compound (**281**) was prepared from 1-methylimidazole (0.276 g, 3.37 mmol) and L-isoleucine butyl ester bromoacetate (**261**) (1.451 g, 4.72 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 98 % yield (1.284 g, 3.29 mmol).

$[\alpha]_D^{20} = -3.2\ ^{o}$ (0.9 c, $CHCl_3$)

Molecular formula $C_{16}H_{28}BrN_3O_3$

Molecular weight 390 $gmol^{-1}$

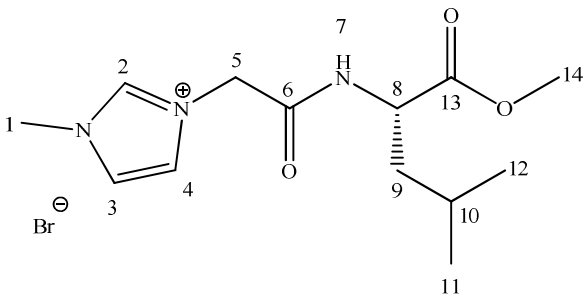
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.42 (s, 1H, *H2*), 8.55 (d, *J* = 7.6 Hz, 1H, *H7*), 7.54 (t, *J* = 1.8 Hz, 1H, *H4*), 7.29 (t, *J* = 1.8 Hz, 1H, *H3*), 5.38 (s, 2H, *H5*), 4.34 (dd, *J* = 8.8, 6.8 Hz, 1H, *H8*), 4.09-3.98 (m, 2H, *H14*), 3.95 (s, 3H, *H1*), 1.97 (dddq, *J* = 8.0, 8.0, 7.2, 6.8 Hz, 1H, *H9*), 1.46 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H11*), 1.38 (ddq, *J* = 8.0, 8.0, 7.0 Hz, 1H, *H11*), 1.45-1.23 (m, 4H, *H15,16*), 0.92 (d, *J* = 6.8 Hz, 3H, *H10*), 0.87-0.82 (m, 6H, *H12,17*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.70 (*C*O,*C13*), 165.33 (*C*O,*C6*), 137.69 (N*C*HN,*C2*), 123.84 (N*C*H,*C4*), 122.64 (N*C*H,*C3*), 65.16 (O*C*H_2,*C14*), 57.75 (*C*H,*C8*), 51.52 (N*C*H_2,*C5*), 36.96 (*C*H,*C9*), 36.84 (N*C*H_3,*C1*), 30.51 (*C*H_2), 25.51 (*C*H_2,*C11*), 19.11 (*C*H_2), 15.78 (*C*H_3,*C10*), 13.66 (*C*H_3,*C17*), 11.63 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3405 (b), 2961 (m), 1735 (s), 1682 (vs), 1545 (s), 1382 (w), 1174 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 310.2119, $C_{16}H_{28}N_3O_3^+$ requires 310.2125

3-Methyl-1-L-leucine methyl ester imidazolium bromide (282)



The title compound (**282**) was prepared from 1-methylimidazole (0.279 g, 3.40 mmol) and L-leucine methyl ester bromoacetate (**256**) (1.081 g, 4.10 mmol) according to the general procedure (Section 7.3.3, page 295) as a white solid in 95 % yield (1.131 g, 3.25 mmol).

m.p 60-62 ^{o}C $[\alpha]_{D}^{20}$ = -17.5 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 gmol^{-1}

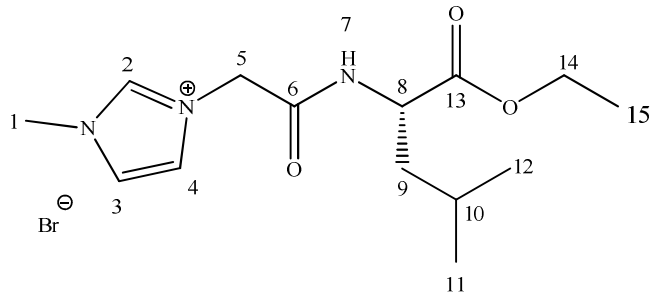
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.62 (s, 1H, *H2*), 8.89 (d, *J* = 7.2 Hz, 1H, *H7*), 7.55 (t, *J* = 1.8 Hz, 1H, *H4*), 7.24 (t, *J* = 1.8 Hz, 1H, *H3*), 5.49 (d, *J* = 11.6 Hz, 2H, *H5*), 4.35 (ddd, *J* = 8.0, 8.0, 5.2 Hz, 1H, *H8*), 4.94 (s, 3H, *H1*), 3.58 (s, 1H, *H14*), 1.77-1.58 (m, 3H, *H9,10*), 0.88 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.83 (d, *J* = 6.4 Hz, 3H, *H11/12*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.95 (*C*O,*C13*), 165.01 (*C*O,*C6*), 137.83 (N*C*HN,*C2*), 123.83 (N*C*H,*C4*), 122.50 (N*C*H,*C3*), 52.36 (N*C*H$_2$,*C5*), 51.85 (*C*H,*C8*), 51.60 (O*C*H$_3$,*C14*), 39.87 (*C*H$_2$,*C9*), 36.82 (N*C*H$_3$,*C1*), 24.88 (*C*H,*C10*), 22.79 (*C*H$_3$,*C11/C12*), 21.59 (*C*H$_3$,*C11/C12*)

IR (neat) (cm^{-1}) 3160 (m), 3019 (w), 2952 (m), 1743 (s), 1668 (vs), 1537 (s), 1273 (m), 1208 (s), 1183 (s), 1171 (s)

MS (*m/z*) Found [M-Br^{-}]$^{+}$ 268.1656, $C_{13}H_{22}N_3O_3^{+}$ requires 268.1655

3-Methyl-1-L-leucine ethyl ester imidazolium bromide (283)



The title compound (**283**) was prepared from 1-methylimidazole (0.281 g, 3.45 mmol) and L-leucine ethyl ester bromoacetate (**257**) (1.152 g, 4.15 mmol) according to the general procedure (Section 7.3.3, page 295) as a white solid in 69 % yield (0.866 g, 2.39 mmol).

m.p 67-69 ºC $[\alpha]_D^{20}$ = -11.0 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{14}H_{24}BrN_3O_3$

Molecular weight 362 $gmol^{-1}$

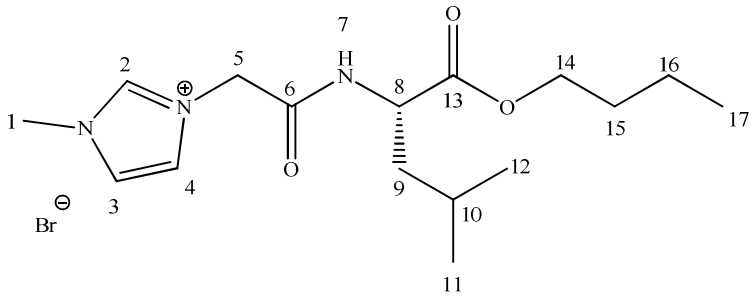
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.89 (s, 1H, *H2*), 8.89 (d, *J* = 7.6 Hz, 1H, *H7*), 7.62 (t, *J* = 1.6 Hz, 1H, *H4*), 7.19 (t, *J* = 1.8 Hz, 1H, *H3*), 5.43 (d, *J* = 17.6 Hz, 2H, *H5*), 4.40 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H8*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.15 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.03 (s, 3H, *H1*), 1.79-1.56 (m, 3H, *H9,10*), 1.29 (t, *J* = 7.0 Hz, 3H, *H15*), 0.90 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.84 (d, *J* = 6.0 Hz, 3H, *H11/12*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.54 (*C*O,*C13*), 165.02 (*C*O,*C6*), 137.75 (N*C*HN,*C2*), 123.77 (N*C*H,*C4*), 122.67 (N*C*H,*C3*), 61.33 (O*C*H_2,*C14*), 51.92 (*C*H,*C8*), 51.71 (N*C*H_2,*C5*), 39.92 (*C*H_2,*C9*), 36.86 (N*C*H_3,*C1*), 24.88 (*C*H,*C10*), 22.78 (*C*H_3,*C11/C12*), 21.63 (*C*H_3,*C11/C12*), 14.17 (*C*H_3,*C15*)

IR (neat) (cm^{-1}) 3164 (m), 3106 (w), 2954 (m), 1743 (s), 1687 (vs), 1547 (m), 1373 (m), 1273 (m), 1186 (vs), 1155 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 282.1813, $C_{14}H_{24}N_3O_3^+$ requires 282.1812

3-Methyl-1-L-leucine butyl ester imidazolium bromide (284)



The title compound (**284**) was prepared from 1-methylimidazole (0.307 g, 3.75 mmol) and L-leucine butyl ester bromoacetate (**258**) (1.382 g, 4.50 mmol) according to the general procedure (Section 7.3.3, page 295) as a white solid in 86 % yield (1.252 g, 3.21 mmol).

m.p 78-80 ºC $[\alpha]_D^{20}$ = -10.7 º (0.8 c, $CHCl_3$)

Molecular formula $C_{16}H_{28}BrN_3O_3$

Molecular weight 390 $gmol^{-1}$

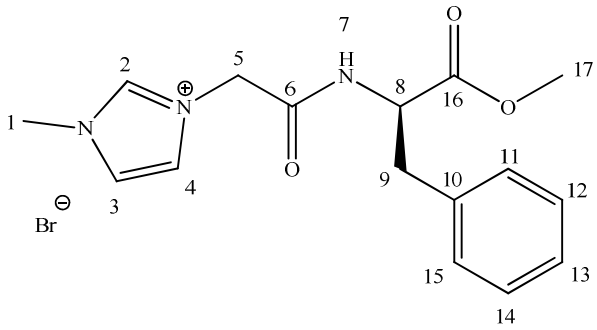
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.68 (s, 1H, *H2*), 8.84 (d, *J* = 8.0 Hz, 1H, *H7*), 7.53 (t, *J* = 1.6 Hz, 1H, *H4*), 7.18 (t, *J* = 1.8 Hz, 1H, *H3*), 5.52 (d, *J* = 18.9 Hz, 2H, *H5*), 4.35 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H8*), 4.04 (dq, *J* = 7.2, 7.0 Hz, 1H, *H14*), 4.02 (dq, *J* = 7.2, 7.0 Hz, 1H, *H14*), 3.96 (s, 3H, *H1*), 1.71 (tt, *J* = 7.0, 6.8 Hz, 2H, *H15*), 1.62-1.50 (m, 3H, *H9,10*), 1.28 (tq, *J* = 7.2, 6.8 Hz, 2H, *H16*), 0.91-0.84 (m, 9H, *H11,12,17*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.53 (*C*O,*C13*), 164.85 (*C*O,*C6*), 137.81 (N*C*HN,*C2*), 123.85 (N*C*H,*C4*), 122.35 (N*C*H,*C3*), 65.23 (O*C*H_2,*C14*), 51.97 (N*C*H_2,*C5*), 51.66 (*C*H,*C8*), 39.94 (*C*H_2,*C9*), 36.81 (N*C*H_3,*C1*), 30.52 (*C*H_2,*C15*), 24.91 (*C*H,*C10*), 22.78 (*C*H_3,*C11*/*C12*), 21.66 (*C*H_3,*C11*/*C12*), 19.08 (*C*H_2,*C16*), 13.74 (*C*H_3,*C17*)

IR (neat) (cm^{-1}) 3165 (m), 3023 (w), 2955 (m), 1741 (s), 1686 (vs), 1545 (m), 1274 (m), 1226 (m), 1185 (s), 1172 (s), 1157 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 310.2127, $C_{16}H_{28}N_3O_3^+$ requires 310.2125

3-Methyl-1-D-phenylalanine methyl ester imidazolium bromide (285)



The title compound (**285**) was prepared from 1-methylimidazole (0.432 g, 5.25 mmol) and D-phenylalanine methyl ester bromoacetate (**262**) (1.881 g, 6.30 mmol) according to the general procedure (Section 7.3.3, page 295) as an off white solid in 87 % yield (1.753 g, 4.59 mmol).

m.p. 56-58 °C, $[\alpha]_D^{20}$ = -19.0 ^{o} (1.0 c, $CHCl_3$)

Molecular formula $C_{16}H_{20}BrN_3O_3$

Molecular weight 382 $gmol^{-1}$

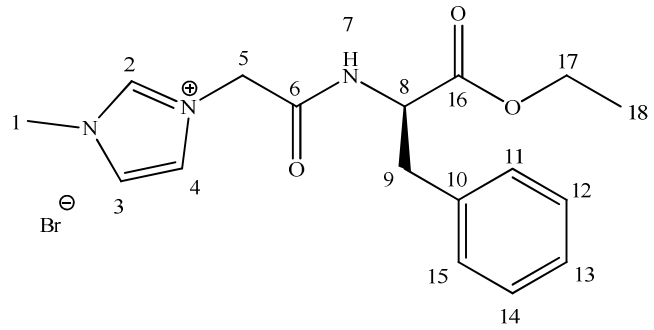
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.44 (s, 1H, *H2*), 8.98 (d, *J* = 7.6 Hz, 1H, *H7*), 7.35 (s, 1H, *H4*), 7.26-7.19 (m, 6H, *H3,11-15*), 5.28 (d, *J* = 10.0 Hz, 2H, *H5*), 4.64 (ddd, *J* = 7.4, 5.6, 5.6 Hz, 1H, *H8*), 3.91 (s, 3H, *H1*), 3.57 (s, 3H, *H17*), 3.19 (dd, *J* = 13.6, 6.0 Hz, 1H, *H9*), 3.08 (dd, *J* = 13.0, 6.6 Hz, 1H, *H9*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.74 (*C*O,*C16*), 164.93 (*C*O,*C6*), 137.65 (N*C*HN,*C2*) 136.65 (Ar*C*,*C10*), 129.98 (Ar*C*H), 128.48 (Ar*C*H), 126.85 (Ar*C*H,*C13*), 123.66 (N*C*H,*C4*), 122.65 (N*C*H,*C3*), 54.81 (*C*H,*C8*), 52.48 (NCH_2,*C5*), 51.51 (OCH_3,*C17*), 37.31 (NCH_3,*C1*), 36.85 (CH_2,*C9*).

IR (neat) (cm^{-1}) 3194 (w), 3025 (m), 1736 (s), 1672 (vs), 1529 (m), 1220 (s), 1174 (vs), 1111 (m), 765 (m), 701 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 302.1500, $C_{16}H_{20}N_3O_3^+$ requires 302.1499

3-Methyl-1-D-phenylalanine ethyl ester imidazolium bromide (286)



The title compound (**286**) was prepared from 1-methylimidazole (0.381 g, 4.65 mmol) and D-phenylalanine ethyl ester bromoacetate (**263**) (1.753 g, 5.60 mmol) according to the general procedure (Section 7.3.3, page 295) as a white solid in 84 % yield (1.549 g, 3.91 mmol).

m.p. 63-65 °C $[\alpha]_D^{20}$ = -15.5 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{17}H_{22}BrN_3O_3$

Molecular weight 396 $gmol^{-1}$

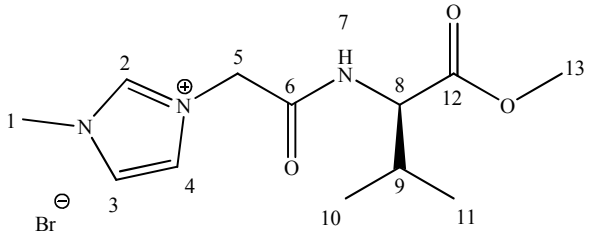
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.54 (s, 1H, *H2*), 8.98 (d, *J* = 8.0 Hz, 1H, *H7*), 7.35 (s, 1H, *H4*), 7.30-7.07 (m, 6H, *H3,11-15*), 5.42 (d, *J* = 24.1 Hz, 2H, *H5*), 4.61 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H8*), 4.05 (dq, *J* = 7.2 , 7.2 Hz, 1H, *H17*), 4.05 (dq, *J* = 7.2 , 7.2 Hz, 1H, *H17*), 3.92 (s, 3H, *H1*), 3.17 (dd, *J* = 13.6, 6.0 Hz, 1H, *H9*), 3.07 (dd, *J* = 13.0, 6.6 Hz, 1H, *H9*), 1.11 (t, *J* = 7.2 Hz, 3H, *H18*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.24 (*C*O,*C16*), 164.82 (*C*O,*C6*), 137.72 (N*C*HN,*C2*) 136.67 (Ar*C*,*C10*), 129.54 (Ar*C*H), 128.43 (Ar*C*H), 126.81 (Ar*C*H,*C13*), 123.67 (N*C*H,*C4*), 122.49 (N*C*H,*C3*), 61.52 (O*C*H_2,*C17*), 54.84 (*C*H,*C8*), 51.54 (N*C*H_2,*C5*), 37.41 (N*C*H_3,*C1*), 36.80 (*C*H_2,*C9*), 14.08 (*C*H_3,*C18*)

IR (neat) (cm^{-1}) 3201 (w), 3027 (m), 2937 (w), 1732 (s), 1675 (vs), 1527 (m), 1373 (m), 1218 (s), 1177 (vs), 1108 (s), 749 (s), 703 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 316.1655, $C_{17}H_{22}N_3O_3^+$ requires 316.1655

3-Methyl-1-D-valine methyl ester imidazolium bromide (287)



The title compound (**287**) was prepared from 1-methylimidazole (0.283 g, 3.45 mmol) and D-valine methyl ester bromoacetate (**264**) (1.035 g, 4.12 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 89 % yield (1.028 g, 3.08 mmol).

$[\alpha]_D^{20} = +9.3\ ^{o}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{12}H_{20}BrN_3O_3$

Molecular weight 334 $gmol^{-1}$

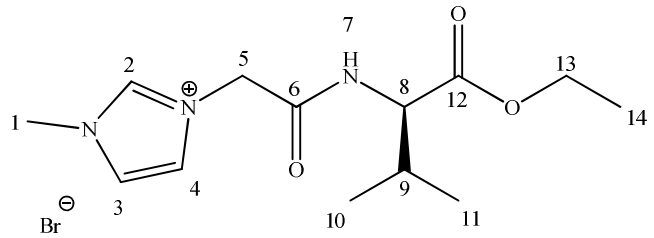
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.67 (s, 1H, *H2*), 8.60 (d, *J* = 7.6 Hz, 1H, *H7*), 7.47 (t, *J* = 1.8 Hz, 1H, *H4*), 7.17 (t, *J* = 1.6 Hz, 1H, *H3*), 5.42 (s, 2H, *H5*), 4.18 (dd, *J* = 8.0, 4.4 Hz, 1H, *H8*), 3.88 (s, 3H, *H1*), 3.55 (s, 3H, *H13*), 2.18 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H9*), 0.91 (d, *J* = 6.8 Hz, 3H, *H10/11*), 0.85 (d, *J* = 6.8 Hz, 3H, *H10/11*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.87 (*C*O,*C12*), 165.27 (*C*O,*C6*), 137.78 (N*C*HN,*C2*), 123.83 (N*C*H,*C4*), 122.51 (N*C*H,*C3*), 58.98 (*C*H,*C8*), 52.15 (N*C*H_2,*C5*), 51.66 (O*C*H_3,*C13*), 36.82 (N*C*H_3,C1), 30.29 (*C*H,*C9*), 19.19 (*C*H_3,*C10*/*C11*), 18.66 (*C*H_3,*C10*/*C11*)

IR (neat) (cm^{-1}) 3456 (b), 3148 (w), 2966 (w), 1736 (s), 1672 (vs), 1551 (m), 1203 (s), 1174 (s), 1147 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 254.1499, $C_{12}H_{20}N_3O_3^+$ requires 254.1499

3-Methyl-1-D-valine ethyl ester imidazolium bromide (288)



The title compound (**288**) was prepared from 1-methylimidazole (0.382 g, 4.65 mmol) and D-valine ethyl ester bromoacetate (**265**) (1.609 g, 6.04 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless liquid in 79 % yield (1.282 g, 3.68 mmol).

$[\alpha]_D^{20} = +10.0^{\circ}$ (0.9 c, $CHCl_3$)

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 $gmol^{-1}$

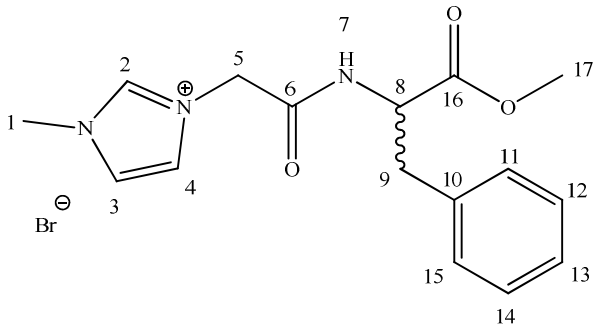
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.51 (s, 1H, *H2*), 8.61 (d, *J* = 7.6 Hz, 1H, *H7*), 7.63 (t, *J* = 1.8 Hz, 1H, *H4*), 7.40 (t, *J* = 1.8 Hz, 1H, *H3*), 5.48 (d, *J* = 4.0 Hz, 2H, *H5*), 4.27 (dd, *J* = 8.0, 4.4 Hz, 1H, *H8*), 4.12 (dq, *J* = 7.2, 7.0 Hz, 1H, *H13*), 4.11 (dq, *J* = 7.0, 7.0 Hz, 1H, *H13*), 4.01 (s, 3H, *H1*), 2.21 (qqd, *J* = 6.6, 6.8, 4.4 Hz, 1H, *H9*), 1.22 (t, *J* = 7.0 Hz, 3H, *H14*), 0.98 (d, *J* = 6.8 Hz, 3H, *H10/11*), 0.91 (d, *J* = 6.8 Hz, 3H, *H10/11*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.40 (*C*O,*C12*), 165.26 (*C*O,*C6*), 137.60 (N*C*HN,*C2*), 123.82 (N*C*H,*C4*), 122.73 (N*C*H,*C3*), 61.25 (O*C*H_2,*C13*), 58.76 (*C*H,*C8*), 50.30 (N*C*H_2,*C5*), 36.74 (N*C*H_3,C1), 30.37 (*C*H,*C9*), 19.09 (*C*H_3,*C10*/*C11*), 18.36 (*C*H_3,*C10*/*C11*), 14.19 (*C*H_3,*C14*)

IR (neat) (cm^{-1}) 3047 (b), 2967 (w), 1733 (s), 1682 (vs), 1544 (s), 1198 (s), 1173 (s), 1150 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 268.1657, $C_{13}H_{22}N_3O_3^+$ requires 268.1655

3-Methyl-1-DL-phenylalanine methyl ester imidazolium bromide (289)



The title compound (**289**) was prepared from 1-methylimidazole (0.405 g, 4.92 mmol) and D-phenylalanine methyl ester bromoacetate (**268**) (1.922 g, 6.40 mmol) according to the general procedure (Section 7.3.3, page 295) as an off white solid in 84 % yield (1.581 g, 4.14 mmol).

m.p. 55-57 °C

Molecular formula $C_{16}H_{20}BrN_3O_3$

Molecular weight 382 $gmol^{-1}$

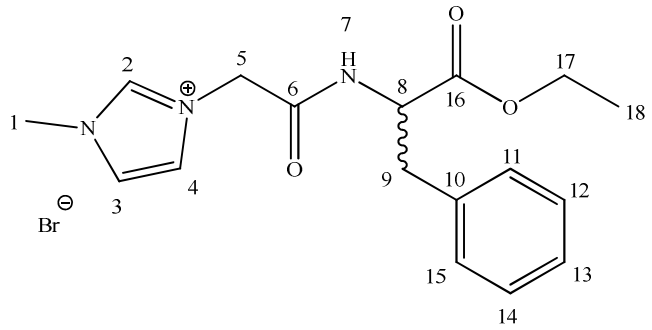
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.84 (s, 1H, *H2*), 9.05 (d, *J* = 8.0 Hz, 1H, *H7*), 7.44 (t, *J* = 1.6 Hz, 1H, *H4*), 7.44-7.23 (m, 5H, *H11-15*), 7.14 (t, *J* = 1.8 Hz, 1H, *H3*), 5.37 (d, *J* = 5.6 Hz, 2H, *H5*), 4.75 (ddd, *J* = 7.6, 5.6, 5.6 Hz, 1H, *H8*), 4.00 (s, 3H, *H1*), 3.70 (s, 3H, *H17*), 3.18 (dd, *J* = 14.0, 5.6 Hz, 1H, *H9*), 3.09 (dd, *J* = 13.0, 5.6 Hz, 1H, *H9*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.84 (*C*O,*C16*), 165.13 (*C*O,*C6*), 136.95 (N*C*HN,*C2*) 136.66 (Ar*C*,*C10*), 129.88 (Ar*C*H), 128.54 (Ar*C*H), 126.65 (Ar*C*H,*C13*), 123.58 (N*C*H,*C4*), 122.60 (N*C*H,*C3*), 54.80 (*C*H,*C8*), 52.52 (NCH_2,*C5*), 51.50 (OCH_3,*C17*), 37.30 (NCH_3,*C1*), 36.79 (*C*H_2,*C9*)

IR (neat) (cm^{-1}) 3229 (w), 3033 (m), 1740 (m), 1728 (s), 1672 (s), 1528 (m), 1217 (s), 1173 (s), 764 (m), 704 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 302.1498, $C_{16}H_{20}N_3O_3^+$ requires 302.1499

3-Methyl-1-DL-phenylalanine ethyl ester imidazolium bromide (290)



The title compound (**290**) was prepared from 1-methylimidazole (0.314 g, 3.82 mmol) and D-phenylalanine ethyl ester bromoacetate (**269**) (1.556 g, 4.97 mmol) according to the general procedure (Section 7.3.3, page 295) as a beige solid in 79 % yield (1.203 g, 3.04 mmol).

m.p. 60-62 °C

Molecular formula $C_{17}H_{22}BrN_3O_3$

Molecular weight 396 $gmol^{-1}$

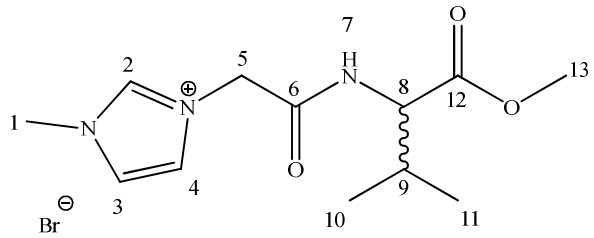
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.74 (s, 1H, *H2*), 8.91 (d, *J* = 7.6 Hz, 1H, *H7*), 7.36 (s, 1H, *H4*), 7.30-7.07 (m, 5H, *H11-15*), 7.06 (s, 1H, *H3*), 5.42 (d, *J* = 10.0 Hz, 2H, *H5*), 4.64 (ddd, *J* = 7.6, 5.2, 5.2 Hz, 1H, *H8*), 4.07 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 4.06 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 3.91 (s, 3H, *H1*), 3.18 (dd, *J* = 13.0, 5.6 Hz, 1H, *H9*), 3.08 (dd, *J* = 13.0, 5.6 Hz, 1H, *H9*), 1.12 (t, *J* = 7.2 Hz, 3H, *H18*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.10 (*C*O,*C16*), 165.80 (*C*O,*C6*), 138.05 (N*C*HN,*C2*) 136.60 (Ar*C*,*C10*), 129.58 (Ar*C*H), 128.30 (Ar*C*H), 126.78 (Ar*C*H,*C13*), 123.64 (N*C*H,*C4*), 122.47 (N*C*H,*C3*), 61.50 (O*C*H_2,*C17*), 54.81 (*C*H,*C8*), 51.51 (N*C*H_2,*C5*), 37.39 (N*C*H_3,*C1*), 36.80 (*C*H_2,*C9*), 14.04 (*C*H_3,*C18*)

IR (neat) (cm^{-1}) 3218 (b), 3029 (w), 1733 (s), 1671 (vs), 1533 (m), 1371 (s), 1215 (s), 1178 (vs), 749 (s), 704 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 316.1655, $C_{17}H_{22}N_3O_3^+$ requires 316.1655

3-Methyl-1-DL-valine methyl ester imidazolium bromide (291)



The title compound (**291**) was prepared from 1-methylimidazole (0.283 g, 3.45 mmol) and DL-valine methyl ester bromoacetate (**266**) (1.035 g, 4.12 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 87 % yield (1.003 g, 3.00 mmol).

Molecular formula $C_{12}H_{20}BrN_3O_3$

Molecular weight 334 $gmol^{-1}$

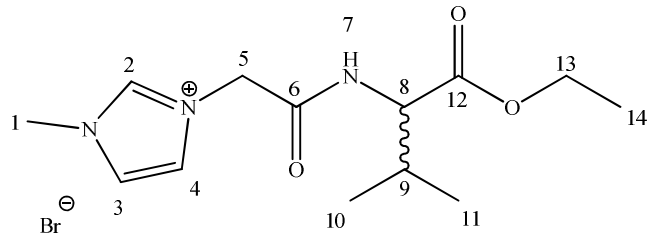
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.75 (s, 1H, *H2*), 8.68 (d, *J* = 7.6 Hz, 1H, *H7*), 7.56 (t, *J* = 1.8 Hz, 1H, *H4*), 7.17 (t, *J* = 1.8 Hz, 1H, *H3*), 5.50 (s, 2H, *H5*), 4.27 (dd, *J* = 8.0, 5.6 Hz, 1H, *H8*), 3.96 (s, 3H, *H1*), 3.64 (s, 3H, *H13*), 2.16 (qqd, *J* = 6.8, 6.8, 5.2 Hz, 1H, *H9*), 0.96 (d, *J* = 6.8 Hz, 3H, *H10*/*H11*), 0.88 (d, *J* = 6.8 Hz, 3H, *H10*/*H11*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.91 (*C*O,*C12*), 164.88 (*C*O,*C6*), 137.66 (N*C*HN,*C2*), 123.91 (N*C*H,*C4*), 122.43 (N*C*H,*C3*), 59.04 (*C*H,*C8*), 52.02 (N*C*H_2,*C5*), 51.68 (O*C*H_3,*C13*), 36.81 (N*C*H_3,C1), 30.28 (*C*H,*C9*), 19.17 (*C*H_3,*C10*/*C11*), 18.54 (*C*H_3,*C10*/*C11*)

IR (neat) (cm^{-1}) 3149 (w), 2966 (m), 1736 (s), 1672 (vs), 1552 (m), 1203 (s), 1175 (s), 1147 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 254.1501, $C_{12}H_{20}N_3O_3^+$ requires 254.1499

3-Methyl-1-DL-valine ethyl ester imidazolium bromide (292)



The title compound (**292**) was prepared from 1-methylimidazole (0.347 g, 4.22 mmol) and DL-valine ethyl ester bromoacetate (**267**) (1.461 g, 5.50 mmol) according to the general procedure (Section 7.3.3, page 295) as a colourless viscous liquid in 77 % yield (1.134 g, 3.26 mmol).

Molecular formula $C_{13}H_{22}BrN_3O_3$

Molecular weight 348 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.82 (s, 1H, *H2*), 8.67 (d, *J* = 7.6 Hz, 1H, *H7*), 7.65 (s, 1H, *H4*), 7.23 (s, 1H, *H3*), 5.58 (s, 2H, *H5*), 4.32 (dd, *J* = 8.0, 4.4 Hz, 1H, *H8*), 4.18 (dq, *J* = 7.2, 7.0 Hz, 1H, *H13*), 4.17 (dq, *J* = 7.2, 7.2 Hz, 1H, *H13*), 4.04 (s, 3H, *H1*), 2.29 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H9*), 1.28 (t, *J* = 7.0 Hz, 3H, *H14*), 1.08 (d, *J* = 6.8 Hz, 3H, *H10/H11*), 1.03 (d, *J* = 6.8 Hz, 3H, *H10/11*)

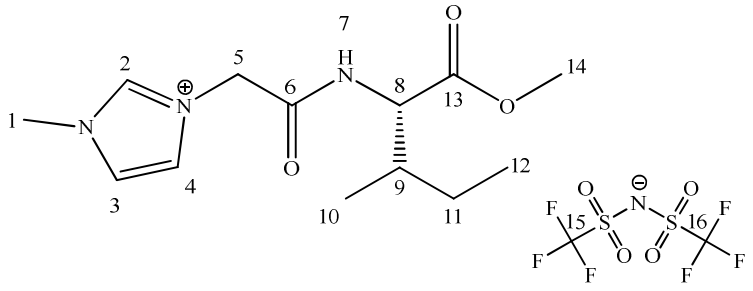
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.26 (*C*O,*C12*), 165.14 (*C*O,*C6*), 137.82 (N*C*HN,*C2*), 123.87 (N*C*H,*C4*), 122.34 (N*C*H,*C3*), 61.21 (O*C*H_2,*C13*), 58.96 (*C*H,*C8*), 53.46 (N*C*H_2,*C5*), 36.80 (N*C*H_3,*C1*), 30.33 (*C*H,*C9*), 19.19 (*C*H_3,*C10/C11*), 18.55 (*C*H_3,*C10/C11*), 14.25 (*C*H_3,*C14*)

IR (neat) (cm^{-1}) 3047 (b), 2964 (w), 2927 (w), 1733 (s), 1681 (vs), 1544 (s), 1173 (vs), 1149 (s), 1022 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 268.1650, $C_{13}H_{22}N_3O_3^+$ requires 268.1655

7.3.4 Preparation of Chiral NTf_2 ILs

***General procedure for the Preparation of Chiral Amino Acid ester NTf_2 salts*; 3-methyl-1-L-isoleucine methyl ester imidazolium NTf_2 (293)**



A flask was charged with 3-methyl-1-L-isoleucine methyl ester imidazolium bromide (**279**) (0.201 g, 0.58 mmol) and distilled water (2 mL). $LiNTf_2$ (0.182 g, 0.64 mmol) was added in one portion and the suspension was stirred vigorously for overnight at RT. The top aqueous layer was removed and the IL was washed with distilled water (3 x 1 mL). The solvent was then removed on the rotary evaporator and under high vacuum for 5 h to give the title material (**293**) as a pale yellow liquid at RT in 84 % yield (0.266 g, 0.48 mmol)

$[\alpha]_D^{20} =$ -6.6 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{15}H_{22}F_6N_4O_7S_2$

Molecular weight 548 $gmol^{-1}$

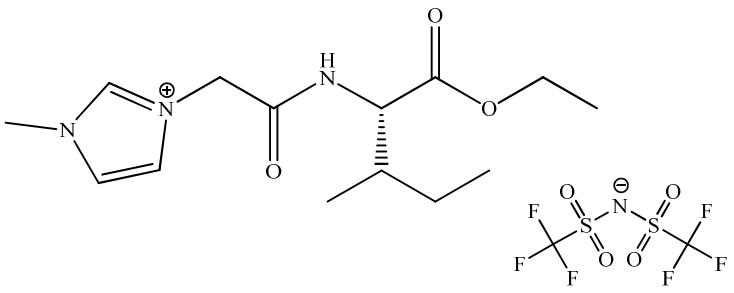
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.65 (s, 1H, *H2*), 7.37 (s, 1H, *H4*), 7.17 (s, 1H, *H3*), 7.16 (d, *J* = 8.0 Hz, 1H, *H7*), 4.95 (d, *J* = 18.6 Hz, 2H, *H5*), 4.38 (dd, *J* = 8.0, 4.8 Hz, 1H, *H8*), 3.85 (s, 3H, *H1*), 3.65 (s, 3H, *H14*), 1.85 (dddq, *J* = 8.0, 8.0, 6.8, 4.4 Hz, 1H, *H9*), 1.42 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H11*), 1.30 (ddq, *J* = 8.4, 8.4, 7.0 Hz, 1H, *H11*), 0.89 (t, *J* = 7.2 Hz, 3H, *H12*), 0.85 (d, *J* = 7.2 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.63 (*C*O,*C13*), 163.36 (*C*O,*C6*), 136.13 (N*C*HN,*C2*), 122.93 (N*C*H,*C4*) 121.89 (N*C*H,*C3*), 119.70 (q, *J* = 319.5 Hz, 2*C*F_3, *C15*,*C16*), 56.54 (*C*H,*C8*), 52.43 (N*C*H_2,*C5*), 49.39 (O*C*H_3,*C14*), 36.30 (*C*H,*C9*), 35.42 (N*C*H_3,*C1*), 24.04 (*C*H_2,*C11*), 14.24 (*C*H_3,*C10*), 10.31 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3363 (b), 2968 (w), 1735 (m), 1689 (s), 1539 (m), 1347 (s), 1178 (vs), 1132 (vs), 1053 (vs)

LRMS (*m/z*) 268.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-isoleucine ethyl ester imidazolium NTf_2 (294)



The title compound (**294**) was prepared from 3-methyl-1-L-isoleucine ethyl ester imidazolium bromide (**280**) (0.200 g, 0.55 mmol) and $LiNTf_2$ (0.170 g, 0.60 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow liquid in 67 % yield (0.206 g, 0.37 mmol).

$[\alpha]_D^{20} =$ -10.4 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{16}H_{24}F_6N_4O_7S_2$

Molecular weight 562 $gmol^{-1}$

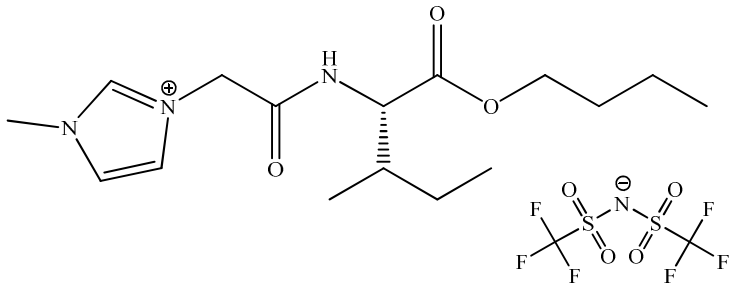
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.62 (s, 1H), 7.35 (s, 1H), 7.23 (s, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 4.97 (d, *J* = 17.8 Hz, 2H), 4.35 (dd, *J* = 8.4, 4.4 Hz, 1H), 4.18-4.07 (m, 2H), 3.84 (s, 3H), 1.86 (dddq, *J* = 8.0, 8.0, 7.0, 4.4 Hz, 1H), 1.41 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H), 1.22 (ddq, *J* = 8.0, 8.0, 7.4 Hz, 1H), 1.19 (t, *J* = 7.2 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H), 0.83 (d, *J* = 7.2 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.25, 165.35, 137.12, 123.88, 122.85, 118.60 (q, *J* = 319.5 Hz, 2*C*F_3), 61.54, 57.54, 50.87, 36.38, 28.95, 25.10, 15.24, 14.00, 11.37

IR (neat) (cm^{-1}) 3356 (b), 2970 (w), 1732 (m), 1688 (s), 1544 (m), 1347 (s), 1179 (vs), 1133 (vs), 1053 (vs)

LRMS (*m/z*) 282.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-isoleucine butyl ester imidazolium NTf_2 (295)



The title compound (**295**) was prepared from 3-methyl-1-L-isoleucine butyl ester imidazolium bromide (**281**) (0.202 g, 0.51 mmol) and $LiNTf_2$ (0.161 g, 0.56 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow oil in 77 % yield (0.231 g, 0.39 mmol).

$[\alpha]_D^{20} = -6.0\ ^\circ$ (0.7 c, $CHCl_3$)

Molecular formula $C_{18}H_{28}F_6N_4O_7S_2$

Molecular weight 590 $gmol^{-1}$

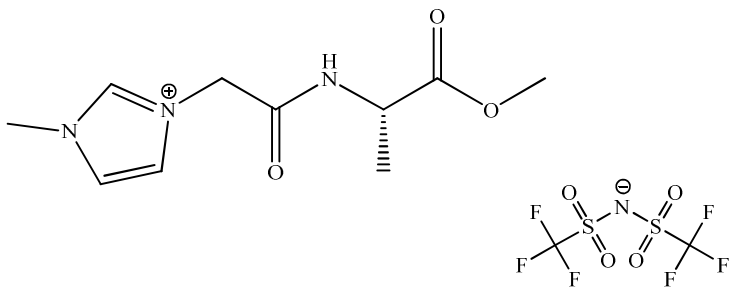
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.63 (s, 1H), 7.36 (s, 1H), 7.23 (s, 1H), 7.14 (d, *J* = 7.8 Hz, 1H), 4.93 (d, *J* = 17.8 Hz, 2H), 4.38 (dd, *J* = 8.0, 4.4 Hz, 1H), 4.09-4.00 (m, 2H), 3.85 (s, 3H), 1.85 (dddq, *J* = 8.0, 8.0, 7.2, 5.0 Hz, 1H), 1.59-1.50 (m, 2H), 1.40-1.26 (m, 4H), 0.86-0.80 (m, 9H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.37, 164.30, 137.13, 123.92, 122.87, 118.62 (q, *J* = 318 Hz, 2*C*F_3), 65.38, 57.55, 50.89, 37.41, 36.41, 30.51, 25.08, 19.04, 15.31, 13.60, 11.41

IR (neat) (cm^{-1}) 3360 (b), 3160 (w), 2966 (w), 1732 (m), 1689 (s), 1538 (m), 1347 (s), 1180 (vs), 1133 (vs), 1054 (vs)

LRMS (*m/z*) 310.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-alanine methyl ester imidazolium NTf_2 (296)



The title compound (**296**) was prepared from 3-methyl-1-L-alanine methyl ester imidazolium bromide (**273**) (0.202 g, 0.69 mmol) and $LiNTf_2$ (0.220 g, 0.76 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow liquid in 54 % yield (0.189 g, 0.37 mmol).

$[\alpha]_D^{20} = -10.2^{\circ}$ (0.4 c, $CHCl_3$)

Molecular formula $C_{12}H_{16}F_6N_4O_7S_2$

Molecular weight 506 $gmol^{-1}$

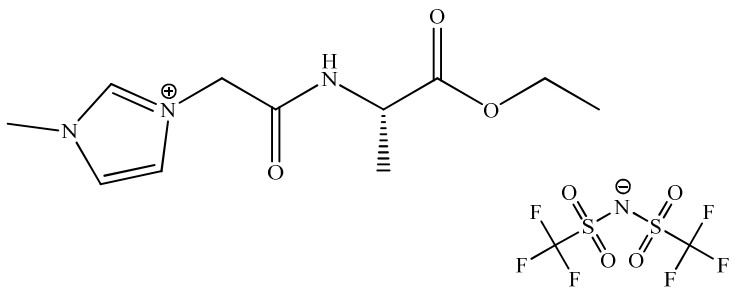
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.74 (s, 1H), 7.26 (t, *J* = 1.8 Hz, 1H), 7.19 (d, *J* = 6.8 Hz, 1H), 7.17 (t, *J* = 1.6 Hz, 1H), 4.95 (d, *J* = 10.8 Hz, 2H), 4.31 (dq, *J* = 7.2, 7.2 Hz, 1H), 3.86 (s, 3H), 3.32 (s, 3H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.17 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.22, 164.58, 136.78, 123.87, 122.88, 119.08 (q, *J* = 319.0 Hz, $2CF_3$), 61.70, 51.01, 48.10, 36.59, 16.89

IR (neat) (cm^{-1}) 3362 (b), 3163 (w), 1739 (m), 1686 (s), 1546 (m), 1346 (vs), 1176 (vs), 1132 (vs), 1051 (vs)

LRMS (*m/z*) 226.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-alanine ethyl ester imidazolium NTf_2 (297)



The title compound (**297**) was prepared from 3-methyl-1-L-alanine ethyl ester imidazolium bromide (**274**) (0.200 g, 0.66 mmol) and $LiNTf_2$ (0.201 g, 0.72 mmol) according to the general procedure (Section 7.3.4, page 318) as a colourless liquid in 83 % yield (0.284 g, 0.55 mmol).

$[\alpha]_D^{20} =$ -14.1 ° (0.4 c, $CHCl_3$)

Molecular formula $C_{13}H_{18}F_6N_4O_7S_2$

Molecular weight 520 $gmol^{-1}$

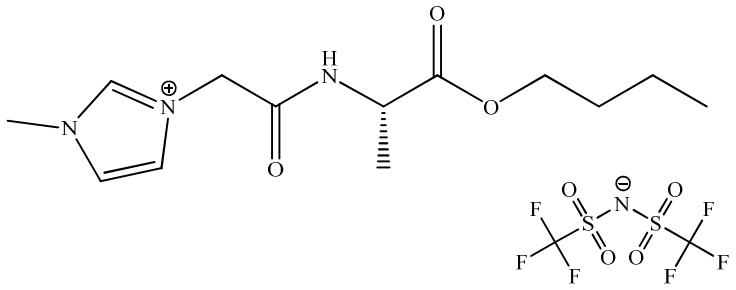
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.67 (s, 1H), 7.38 (t, *J* = 1.8 Hz, 1H), 7.24 (d, *J* = 6.8 Hz, 1H), 7.20 (t, *J* = 1.6 Hz, 1H), 4.93 (d, *J* = 10.8 Hz, 2H), 4.38 (dq, *J* = 7.2, 7.2 Hz, 1H), 4.10 (q, *J* = 7.2 Hz, 2H), 3.86 (s, 3H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.19 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.12, 163.89, 137.18, 123.97, 122.83, 118.08 (q, *J* = 319.0 Hz, $2CF_3$), 61.70, 51.01, 48.09, 36.48, 17.20, 13.09

IR (neat) (cm^{-1}) 3360 (w), 3161 (w), 2992 (w), 1735 (m), 1685 (s), 1543 (m), 1347 (s), 1329 (s), 1176 (vs), 1132 (vs), 1051 (vs)

LRMS (*m/z*) 240.3 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-alanine butyl ester imidazolium NTf_2 (298)



The title compound (**298**) was prepared from 3-methyl-1-L-alanine butyl ester imidazolium bromide (**275**) (0.171 g, 0.51 mmol) and $LiNTf_2$ (0.162 g, 0.56 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow oil in 72 % yield (0.201 g, 0.37 mmol).

$[\alpha]_D^{20} =$ -16.2 ° (0.4 c, $CHCl_3$)

Molecular formula $C_{15}H_{22}F_6N_4O_7S_2$

Molecular weight 548 $gmol^{-1}$

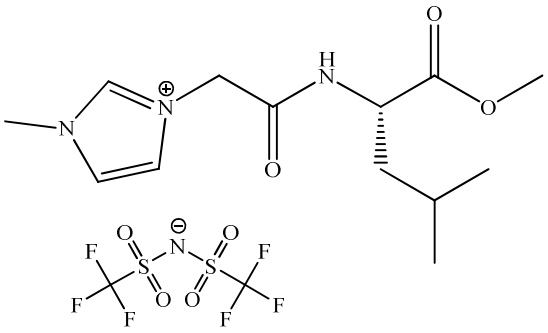
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.75 (s, 1H), 7.46 (t, *J* = 1.8 Hz, 1H), 7.28 (t, *J* = 1.6 Hz, 1H), 7.26 (d, *J* = 6.8 Hz, 1H), 4.95 (d, *J* = 11.2 Hz, 2H), 4.44 (dq, *J* = 7.2, 7.2 Hz, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.95 (s, 3H), 1.59 (tt, *J* = 7.2, 7.0 Hz, 2H), 1.43 (d, *J* = 7.6 Hz, 3H), 1.37 (tq, *J* = 7.6, 7.2 Hz, 2H), 0.95 (t, *J* = 7.6 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.12, 163.84, 137.16, 123.97, 122.83, 118.08 (q, *J* = 319.0 Hz, $2CF_3$), 65.55, 51.00, 48.99, 36.50, 30.44, 18.98, 17.35, 13.62

IR (neat) (cm^{-1}) 3549 (b), 3357 (w), 1736 (m), 1693 (s), 1565 (m), 1345 (s), 1181 (vs), 1130 (vs), 1052 (vs)

LRMS (*m/z*) 268.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-leucine methyl ester imidazolium NTf_2 (299)



The title compound (**299**) was prepared from 3-methyl-1-L-leucine methyl ester imidazolium bromide (**282**) (0.201 g, 0.58 mmol) and $LiNTf_2$ (0.180 g, 0.63 mmol) according to the general procedure (Section 7.3.4, page 318) as a colourless liquid in 78 % yield (0.248 g, 0.45 mmol)

$[\alpha]_D^{20} = -11.8^{o}$ (0.9 c, $CHCl_3$)

Molecular formula $C_{15}H_{22}F_6N_4O_7S_2$

Molecular weight 548 $gmol^{-1}$

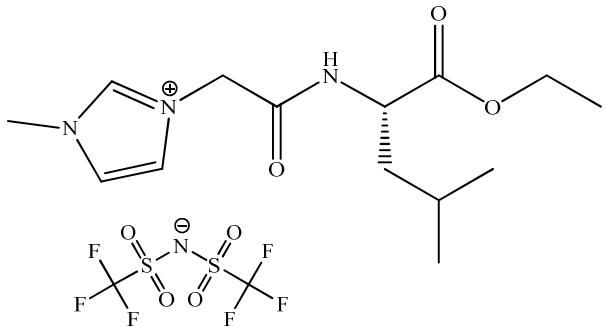
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.67 (s, 1H), 7.42 (s, 1H), 7.32 (s, 1H), 7.22 (d, *J* = 7.6 Hz, 1H), 4.98 (s, 2H), 4.46 (ddd, *J* = 8.0, 8.0, 5.2 Hz, 1H), 3.92 (s, 3H), 3.70 (s, 3H), 1.71-1.62 (m, 3H), 0.91 (dd, *J* = 8.6, 5.6 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.74, 164.36, 137.09, 123.21, 123.07, 118.06 (q, *J* = 318 Hz, $2CF_3$), 52.42, 51.64. 50.81, 40.47, 36.38, 24.64, 22.16, 21.52

IR (neat) (cm^{-1}) 3359 (b), 2962 (w), 1739 (m), 1688 (s), 1542 (m), 1347 (s), 1176 (vs), 1132 (vs), 1053 (vs)

LRMS (*m/z*) 268.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-leucine ethyl ester imidazolium NTf_2 (300)



The title compound (**300**) was prepared from 3-methyl-1-L-leucine ethyl ester imidazolium bromide (**283**) (0.203 g, 0.56 mmol) and $LiNTf_2$ (0.175 g, 0.61 mmol) according to the general procedure (Section 7.3.4, page 318) as a yellow liquid in 85 % yield (0.269 g, 0.48 mmol).

$[\alpha]_D^{20} = -8.5^{\circ}$ (0.5 c, $CHCl_3$)

Molecular formula $C_{16}H_{24}F_6N_4O_7S_2$

Molecular weight 562 $gmol^{-1}$

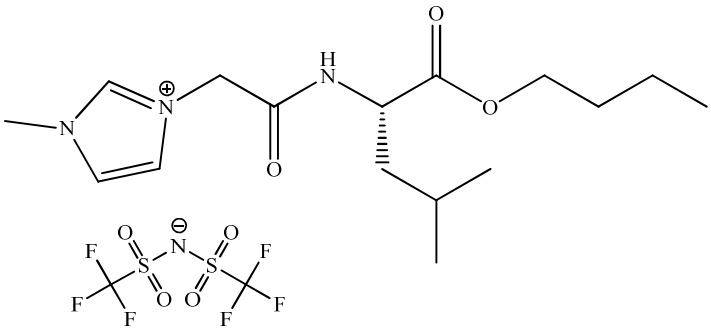
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.62 (s, 1H), 7.38 (s, 1H), 7.20 (s, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 4.91 (d, *J* = 15.4 Hz, 2H), 4.45 (ddd, *J* = 7.8, 7.8, 5.6 Hz, 1H), 4.17 (dq, *J* = 7.2, 7.2 Hz, 2H), 4.15 (dq, *J* = 7.2, 7.2 Hz, 2H), 3.82 (s, 3H), 1.65-1.51 (m, 3H), 1.18 (t, *J* = 7.0 Hz, 3H), 0.91 (dd, *J* = 6.0, 6.0 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.21, 163.22, 136.13, 123.87, 122.84, 119.77 (q, *J* = 319 Hz, $2CF_3$), 60.58, 50.74, 49.95, 39.65, 35.48, 23.88, 21.74, 20.58, 13.09

IR (neat) (cm^{-1}) 3359 (b), 3162 (w), 2964 (w), 1734 (m), 1691 (s), 1543 (m), 1347 (s), 1179 (vs), 1132 (vs), 1053 (vs)

LRMS (*m/z*) 282.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-leucine butyl ester imidazolium NTf_2 (301)



The title compound (**301**) was prepared from 3-methyl-1-L-leucine butyl ester imidazolium bromide (**284**) (0.202 g, 0.51 mmol) and $LiNTf_2$ (0.160 g, 0.56 mmol) according to the general procedure (Section 7.3.4, page 318) as a colourless liquid in 86 % yield (0.260 g, 0.44 mmol).

$[\alpha]_D^{20} = -6.2^{\circ}$ (0.3 c, $CHCl_3$)

Molecular formula $C_{18}H_{28}F_6N_4O_7S_2$

Molecular weight 590 $gmol^{-1}$

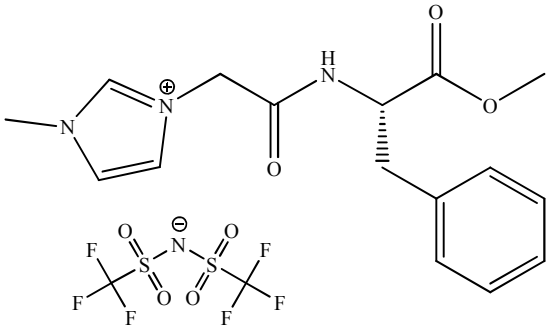
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.59 (s, 1H), 7.33 (s, 1H), 7.25 (s, 1H), 7.16 (d, *J* = 7.6 Hz, 1H), 4.91 (d, *J* = 5.6 Hz, 2H), 4.41 (ddd, *J* = 7.8, 7.8, 5.2 Hz, 1H), 4.05-3.99 (m, 2H), 3.84 (s, 3H), 1.64-1.49 (m, 5H), 1.30 (tq, *J* = 7.6, 7.6 Hz, 2H), 0.86-0.81 (m, 9H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.44, 164.34, 137.05, 123.74, 123.11, 118.04 (q, *J* = 319 Hz, $2CF_3$), 65.47, 51.77, 49.79, 40.53, 36.33, 30.14, 24.66, 22.40, 21.58, 18.94, 13.42

IR (neat) (cm^{-1}) 3357 (b), 2963 (w), 1733 (m), 1691 (s), 1544 (m), 1347 (s), 1777 (vs), 1133 (vs), 1054 (vs)

LRMS (*m/z*) 310.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-phenylalanine methyl ester imidazolium NTf_2 (302)



The title compound (**302**) was prepared from 3-methyl-1-L-phenylalanine methyl ester imidazolium bromide (**270**) (0.270 g, 0.71 mmol) and $LiNTf_2$ (0.221 g, 0.78 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow liquid in 65 % yield (0.267 g, 0.46 mmol).

$[\alpha]_D^{20} = +14.0^{\circ}$ (0.4 c, $CHCl_3$)

Molecular formula $C_{18}H_{20}F_6N_4O_7S_2$

Molecular weight 582 $gmol^{-1}$

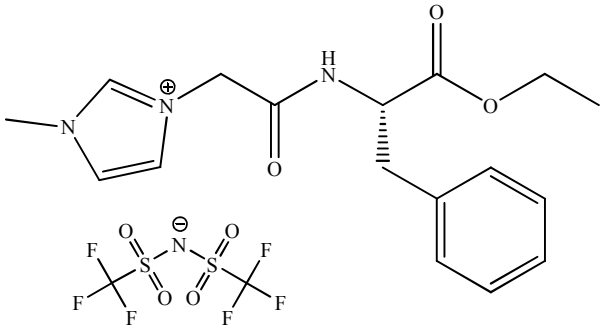
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.54 (s, 1H), 7.33 (d, *J* = 7.6 Hz, 1H), 7.20-7.04 (m, 7H), 4.83 (d, *J* = 4.8 Hz, 2H), 4.64 (ddd, *J* = 7.8, 6.0, 6.0 Hz, 1H), 3.78 (s, 3H), 3.59 (s, 3H), 3.07 (dd, *J* = 13.0, 6.0 Hz, 1H), 2.92 (dd, *J* = 14.0, 6.0 Hz, 1H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.54, 164.28, 137.06, 135.94, 129.94, 128.61, 127.14, 123.77, 122.92, 118.64 (q, *J* = 319 Hz, $2CF_3$), 54.36, 52.54, 50.97, 50.83, 37.62, 36.27

IR (neat) (cm^{-1}) 3357 (w), 1740 (m), 1689 (s), 1540 (m), 1347 (s), 1175 (vs), 1132 (vs), 1052 (vs), 739 (m), 703 (m)

LRMS (*m/z*) 302.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-phenylalanine ethyl ester imidazolium NTf_2 (303)



The title compound (**303**) was prepared from 3-methyl-1-L-phenylalanine ethyl ester imidazolium bromide (**271**) (0.200 g, 0.51 mmol) and $LiNTf_2$ (0.151 g, 0.55 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow viscous liquid in 94 % yield (0.284 g, 0.48 mmol)

$[\alpha]_D^{20} = +13.1\ ^{o}$ (0.9 c, $CHCl_3$)

Molecular formula $C_{19}H_{22}F_6N_4O_7S_2$

Molecular weight 596 $gmol^{-1}$

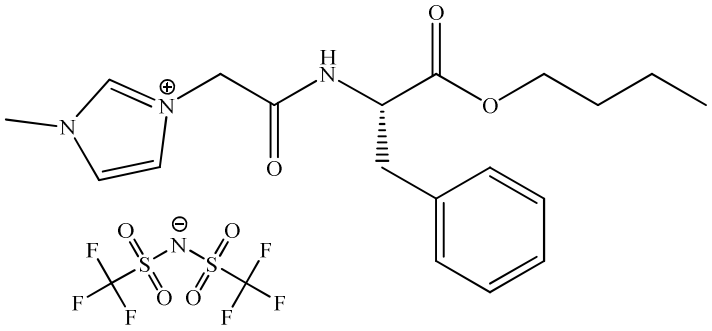
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.51 (s, 1H), 7.24-7.04 (m, 8H), 4.81 (d, *J* = 4.8 Hz, 2H), 4.66 (ddd, *J* = 7.6, 5.8, 5.8 Hz, 1H), 4.06 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 3.07 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.92 (dd, *J* = 13.0, 7.8 Hz, 1H), 1.03 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 169.94, 163.01, 136.01, 134.81, 128.32, 127.55, 126.10, 122.74, 121.88, 117.57 (q, *J* = 319 Hz, $2CF_3$), 60.77, 53.34, 49.90, 36.67, 35.43, 12.84

IR (neat) (cm^{-1}) 3160 (b), 1735 (m), 1692 (s), 1539 (m), 1347 (s), 1177 (vs), 1132 (vs), 1053 (vs), 738 (m), 703 (m)

LRMS (*m/z*) 316.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-phenylalanine butyl ester imidazolium NTf_2 (304)



The title compound (**304**) was prepared from 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (**272**) (0.202 g, 0.49 mmol) and $LiNTf_2$ (0.152 g, 0.54 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow oil in 84 % yield (0.256 g, 0.41 mmol).

$[\alpha]_D^{20} = +11.2^{\circ}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{21}H_{26}F_6N_4O_7S_2$

Molecular weight 624 $gmol^{-1}$

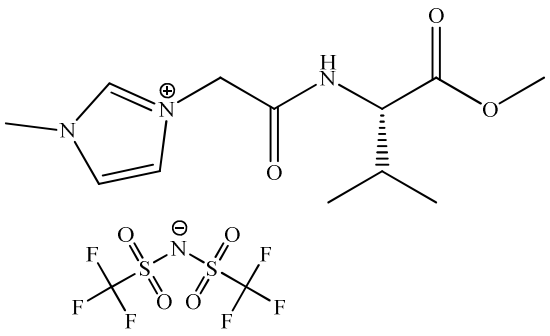
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.50 (s, 1H), 7.20-7.08 (m, 8H), 4.81 (s, 2H), 4.67 (ddd, *J* = 7.8, 6.0, 6.0 Hz, 1H), 4.07 (q, *J* = 7.2 Hz, 2H), 3.98 (s, 3H), 3.07 (dd, *J* = 16.6, 6.6 Hz, 1H), 2.93 (dd, *J* = 14.0, 7.8 Hz, 1H), 1.48 (tt, *J* = 6.8, 7.0 Hz, 2H), 1.21 (tq, *J* = 7.2 Hz, 2H), 1.03 (t, *J* = 7.2 Hz, 3H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.07, 164.01, 137.02, 135.89, 129.25, 128.59, 127.12, 123.75, 122.89, 118.64 (q, *J* = 319 Hz, $2CF_3$), 65.69, 54.38, 50.92, 37.77, 36.38, 30.32, 18.95, 13.75

IR (neat) (cm^{-1}) 3356 (b), 3160 (w), 2963 (w), 1734 (m), 1692 (s), 1541 (m), 1347 (s), 1178 (vs), 1132 (vs), 1053 (vs), 738 (m), 702 (m)

LRMS (*m/z*) 344.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-valine methyl ester imidazolium NTf_2 (305)



The title compound (**305**) was prepared from 3-methyl-1-L-valine methyl ester imidazolium bromide (**276**) (0.202 g, 0.60 mmol) and $LiNTf_2$ (0.190 g, 0.66 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow liquid in 88 % yield (0.282 g, 0.53 mmol).

$[\alpha]_D^{20} = -4.7\ ^{o}$ (0.3 c, $CHCl_3$)

Molecular formula $C_{14}H_{20}F_6N_4O_7S_2$

Molecular weight 534 $gmol^{-1}$

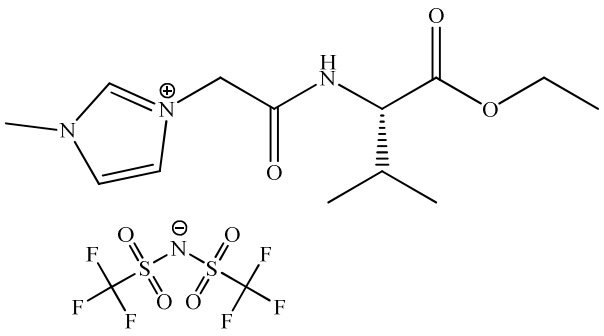
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.66 (s, 1H), 7.38 (t, *J* = 1.8 Hz, 1H), 7.23 (t, *J* = 1.6 Hz, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 5.00 (d, *J* = 14.0 Hz, 2H), 4.32 (dd, *J* = 8.4, 5.2 Hz, 1H), 3.84 (s, 3H), 3.65 (s, 3H), 2.11 (qqd, *J* = 6.8, 6.4, 5.6 Hz, 1H), 0.86 (dd, *J* = 6.8, 3.6 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.70, 164.51, 137.16, 123.97, 122.86, 118.07 (q, *J* = 319 Hz, $2CF_3$), 58.46, 52.32, 50.91, 36.45, 30.68, 18.78, 17.69

IR (neat) (cm^{-1}) 3353 (b), 2968 (w), 1736 (m), 1686 (s), 1543 (m), 1347 (s), 1176 (vs), 1132 (vs), 1052 (vs)

LRMS (*m/z*) 254.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-valine ethyl ester imidazolium NTf_2 (306)



The title compound (**306**) was prepared from 3-methyl-1-L-valine ethyl ester imidazolium bromide (**277**) (0.200 g, 0.57 mmol) and $LiNTf_2$ (0.181 g, 0.63 mmol) according to the general procedure (Section 7.3.4, page 318) as a yellow liquid in 93 % yield (0.293 g, 0.53 mmol).

$[\alpha]_D^{20} =$ -5.0 ^{o} (0.2 c, $CHCl_3$)

Molecular formula $C_{15}H_{22}F_6N_4O_7S_2$

Molecular weight 548 $gmol^{-1}$

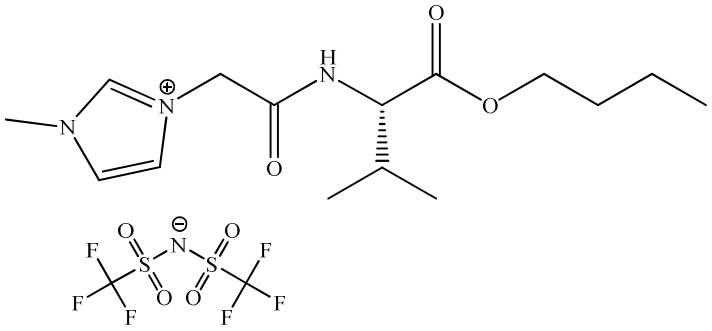
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.63 (s, 1H), 7.36 (t, *J* = 1.6 Hz, 1H), 7.23 (t, *J* = 1.8 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 4.98 (d, *J* = 13.6 Hz, 2H), 4.31 (dd, *J* = 8.0, 5.2 Hz, 1H), 4.11 (dq, *J* = 7.0, 7.0 Hz, 1H), 4.10 (dq, *J* = 7.2, 7.0 Hz, 1H), 3.84 (s, 3H), 2.10 (qqd, *J* = 7.0, 6.8, 5.6 Hz, 1H), 1.19 (t, *J* = 7.0 Hz, 3H), 0.86 (dd, *J* = 6.8, 5.6 Hz, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.31, 164.52, 137.13, 123.89, 122.97, 118.06 (q, *J* = 318 Hz, $2CF_3$), 61.57, 58.40, 50.86, 36.38, 30.73, 18.73, 17.60, 14.01

IR (neat) (cm^{-1}) 3361 (b), 3162 (w), 2970 (w), 1732 (m), 1688 (s), 1541 (m), 1347 (s), 1177 (vs), 1132 (vs), 1053 (vs)

LRMS (*m/z*) 268.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

3-Methyl-1-L-valine butyl ester imidazolium NTf_2 (307)



The title compound (**307**) was prepared from 3-methyl-1-L-valine butyl ester imidazolium bromide (**278**) (0.202 g, 0.53 mmol) and $LiNTf_2$ (0.167 g, 0.59 mmol) according to the general procedure (Section 7.3.4, page 318) as a pale yellow liquid in 94 % yield (0.288 g, 0.50 mmol).

$[\alpha]_D^{20} =$ -7.4 ^{o} (0.4 c, $CHCl_3$)

Molecular formula $C_{17}H_{26}F_6N_4O_7S_2$

Molecular weight 576 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.71 (s, 1H), 7.45 (t, *J* = 1.8 Hz, 1H), 7.25 (t, *J* = 1.8 Hz, 1H), 7.17 (d, *J* = 8.4 Hz, 1H), 5.12 (d, *J* = 21.6 Hz, 2H), 4.42 (dd, *J* = 8.0, 5.2 Hz, 1H), 4.07 (dq, *J* = 7.0, 7.0 Hz, 1H), 4.06 (dq, *J* = 7.2, 7.0 Hz, 1H), 3.96 (s, 3H), 2.14 (qqd, *J* = 7.0, 6.8, 5.2 Hz, 1H), 1.66 (tt, *J* = 7.4, 6.8 Hz, 2H), 1.38 (tq, *J* = 7.6, 7.4 Hz, 2H), 0.99-0.93 (m, 9H)

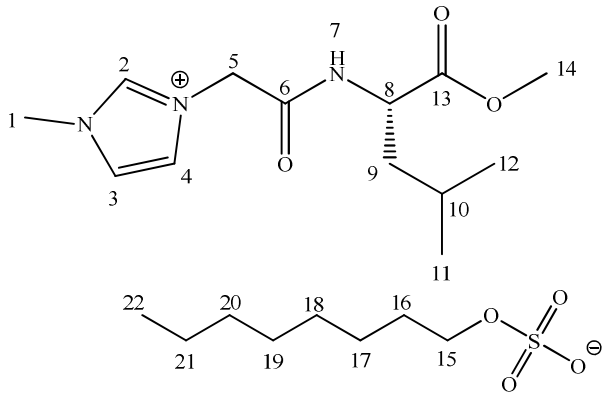
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.26, 164.35, 137.23, 124.07, 122.72, 121.26 (q, *J* = 319 Hz, 2*C*F_3), 65.46, 58.43, 51.03, 36.56, 30.71, 30.46, 19.06, 18.84, 17.59, 13.63.

IR (neat)(cm^{-1}) 3355 (b), 2966 (w), 1732 (m), 1688 (s), 1541 (m), 1347 (s), 1178 (vs), 1133 (vs), 1053 (vs)

LRMS (*m/z*) 296.4 $[M\text{-}NTf_2]^+$; 279.9 $[NTf_2]^-$

7.3.5 Preparation of Chiral $OctOSO_3$ ILs

***General procedure for the Preparation of Chiral Amino Acid ester $OctOSO_3$ salts*; 3-methyl-1-L-leucine methyl ester imidazolium $OctOSO_3$ (308)**



To a stirred solution of 3-methyl-1-L-leucine methyl ester imidazolium bromide (**282**) (0.200 g, 0.59 mmol) in distilled water (3 mL) was added in one portion sodium octyl sulfate (0.171 g, 0.72 mmol). The mixture was left stirring overnight, and then the water was evaporated on the rotary evaporator. The remaining residue was dissolved in DCM (4 mL) and washed with water (2 x 1 mL). The product was then dried on the rotary evaporator and under high vacuum for 10 h to give the title product (**308**) as a colourless liquid at RT in 93 % yield (0.262 g, 0.55 mmol).

$[\alpha]_D^{20} =$ -14.1 ^{o} (0.9 c, $CHCl_3$)

Molecular formula $C_{21}H_{39}N_3O_7S$

Molecular weight 478 $gmol^{-1}$

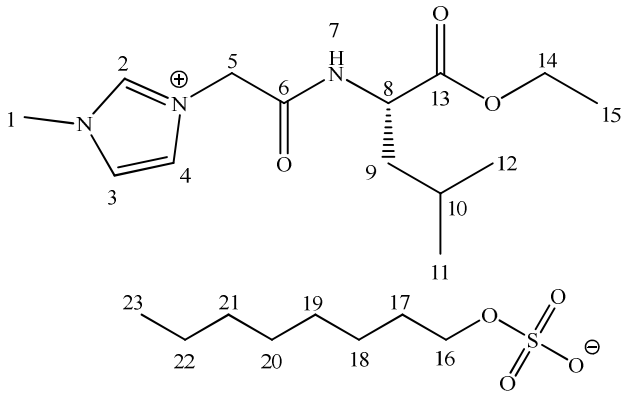
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.91 (s, 1H, *H2*), 8.44 (d, *J* = 7.2 Hz, 1H, *H7*), 7.43 (s, 1H, *H4*), 7.33 (s, 1H, *H3*), 5.08 (d, *J* = 3.6 Hz, 2H, *H5*), 4.34 (ddd, *J* = 8.4, 8.4, 5.6 Hz, 1H, *H4*), 3.90 (t, *J* = 6.8 Hz, 2H, *H15*), 3.87 (s, 3H, *H1*), 3.62 (s, 3H, *H14*), 1.73-1.52 (m, 5H, *H9,10,16*), 1.27-1.18 (m, 10H, *H17-21*), 0.88 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.83 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.78 (t, *J* = 7.0 Hz, 3H, *H22*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.26 (*C*O,*C13*), 165.51 (*C*O,*C6*), 137.70 (N*C*HN,*C2*), 123.66 (N*C*H,*C3*), 122.69 (N*C*H,*C4*), 68.28 (O*C*H_2,*C15*), 52.36 (O*C*H_3,*C14*), 51.57 (*C*H,*C8*), 50.96 (N*C*H_2,*C5*), 40.01 (*C*H_2,*C9*), 36.41 (N*C*H_3,*C1*), 31.79 (*C*H_2), 29.37 (*C*H_2), 29.33 (*C*H_2), 29.22 (*C*H_2), 25.79 (*C*H_2), 24.71 (*C*H,*C10*), 22.72 (*C*H_2), 22.60 (*C*H_3,*C11*/*C12*), 21.47 (*C*H_3,*C11*/*C12*), 14.05 (*C*H_3,*C22*)

IR (neat) (cm^{-1}) 3159 (w), 3087 (w), 2957 (m), 2927 (m), 2857 (w), 1731 (m), 1682 (s), 1564 (m), 1175 (vs), 1052 (m)

LRMS (*m/z*) 268.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-leucine ethyl ester imidazolium $OctOSO_3$ (309)



The title compound (**309**) was prepared from 3-methyl-1-L-leucine ethyl ester imidazolium bromide (**283**) (0.250 g, 0.70 mmol) and sodium octyl sulfate (0.190 g, 0.85 mmol) according to the general procedure (Section 7.3.5, page 333) as a pale yellow slightly viscous liquid in 76 % yield (0.260 g, 0.53 mmol).

$[\alpha]_D^{20} = -9.0^{\circ}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{22}H_{41}N_3O_7S$

Molecular weight 492 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.03 (s, 1H, *H2*), 8.43 (d, *J* = 7.2 Hz, 1H, *H7*), 7.45 (s, 1H, *H4*), 7.27 (s, 1H, *H3*), 5.08 (s, 2H, *H5*), 4.36 (ddd, *J* = 8.8, 8.8, 6.0 Hz, 1H, *H4*), 4.09 (dq, *J* = 7.2, 7.0 Hz, 1H, *H14*), 4.08 (dq, *J* = 7.0, 7.0 Hz, 1H, *H14*), 3.91 (t, *J* = 7.0 Hz,

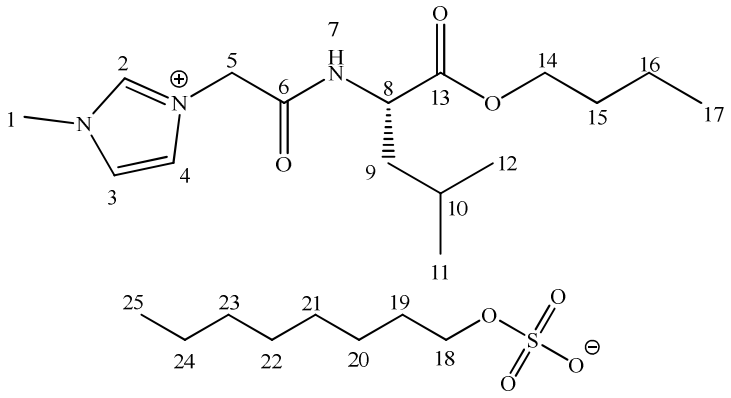
2H, *H16*), 3.89 (s, 3H, *H1*), 1.73-1.52 (m, 5H, *H9,10,17*), 1.28-1.16 (m, 13H, *H15,17-22*), 0.88 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.84 (d, *J* = 6.4 Hz, 3H, *H11/12*), 0.79 (t, *J* = 6.8 Hz, 3H, *H23*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.56 (*C*O,*C13*), 164.20 (*C*O,*C6*), 136.97 (N*C*HN,*C2*), 122.77 (N*C*HN,*C4*), 121.80 (N*C*HN,*C3*), 68.07 (O*C*H_2,*C15*), 61.29 (O*C*H_2,*C14*), 51.69 (*C*H,*C8*), 51.07 (N*C*H_2,*C5*), 40.07 (*C*H_2,*C9*), 36.46 (N*C*H_3,*C1*), 31.80 (*C*H_2), 29.45 (*C*H_2), 29.33 (*C*H_2), 29.23 (*C*H_2), 25.84 (*C*H_2), 24.73 (*C*H_3,*C11*/*C12*), 22.75 (*C*H_2), 22.62 (*C*H_2), 20.65 (*C*H_3,*C11*/*C12*), 14.11 (*C*H_3,*C15*), 14.08 (*C*H_3,*C23*)

IR (neat) (cm^{-1}) 3268 (b), 2957 (m), 2927 (m), 1739 (m), 1689 (s), 1565 (m), 1198 (vs), 1019 (m)

LRMS (*m/z*) 282.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-leucine butyl ester imidazolium $OctOSO_3$ (310)



The title compound (**310**) was prepared from 3-methyl-1-L-leucine butyl ester imidazolium bromide (**284**) (0.201 g, 0.55 mmol) and sodium octyl sulfate (0.150 g, 0.65 mmol) according to the general procedure (Section 7.3.5, page 333) as a pale yellow slightly viscous liquid in 75 % yield (0.216 g, 0.41 mmol).

$[\alpha]_D^{20} = -8.3^{\circ}$ (0.6 c, $CHCl_3$)

Molecular formula $C_{24}H_{45}N_3O_7S$

Molecular weight 520 $gmol^{-1}$

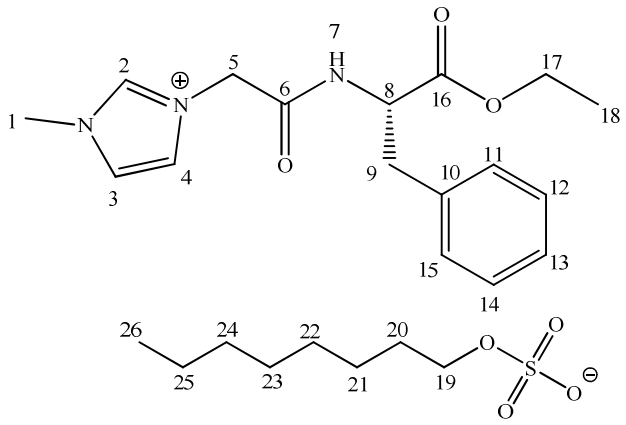
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.00 (s, 1H, *H2*), 8.43 (d, *J* = 7.2 Hz, 1H, *H7*), 7.46 (t, *J* = 1.6 Hz, 1H, *H4*), 7.29 (t, *J* = 1.8 Hz, 1H, *H3*), 5.08 (s, 2H, *H5*), 4.31 (ddd, *J* = 7.8, 7.8, 5.6 Hz, 1H, *H4*), 4.01 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.00 (dq, *J* = 7.2, 7.0 Hz, 1H, *H14*), 3.91 (t, *J* = 6.8 Hz, 2H, *H18*), 3.89 (s, 3H, *H1*), 1.72-1.50 (m, 7H, *H9,10,15,19*), 1.35-1.18 (m, 12H, *H16,20-24*), 0.88-0.78 (m, 12H, *H11,12,17,25*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.73 (*C*O,*C13*), 165.24 (*C*O,*C6*), 137.87 (N*C*HN,*C2*), 123.65 (N*C*HN,*C4*), 122.86 (N*C*HN,*C3*), 68.10 (O*C*H_2,*C15*), 65.18 (O*C*H_2,*C14*), 51.67 (*C*H,*C8*), 51.02 (N*C*H_2,*C5*), 40.13 (*C*H_2,*C9*), 36.43 (N*C*H_3,*C1*), 31.79 (*C*H_2), 30.48 (*C*H_2), 29.43 (*C*H_2), 29.33 (*C*H_2), 29.22 (*C*H_2), 25.82 (*C*H_2), 24.74 (*C*H_3,*C11*/*C12*), 22.71 (*C*H_2), 22.61 (*C*H_2), 21.68 (*C*H_3,*C11*/*C12*), 19.02 (*C*H_2), 14.06 (*C*H_3,*C17*), 13.66 (*C*H_3,*C25*)

IR (neat) (cm^{-1}) 3274 (b), 2958 (m), 2928 (m), 2872 (w), 1738 (m), 1689 (s), 1565 (m), 1347 (w), 1200 (s), 1058 (m)

LRMS (*m/z*) 310.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-phenylalanine ethyl ester imidazolium $OctOSO_3$ (311)



The title compound (**311**) was prepared from 3-methyl-1-L-phenylalanine ethyl ester imidazolium bromide (**271**) (0.202 g, 0.50 mmol) and sodium octyl sulfate (0.130 g, 0.55

mmol) according to the general procedure (Section 7.3.5, page 333) as a colourless liquid in 96 % yield (0.254 g, 0.48 mmol).

$[\alpha]_D^{20} = +15.5$ ° (0.9 c, $CHCl_3$)

Molecular formula $C_{25}H_{39}N_3O_7S$

Molecular weight 526 $gmol^{-1}$

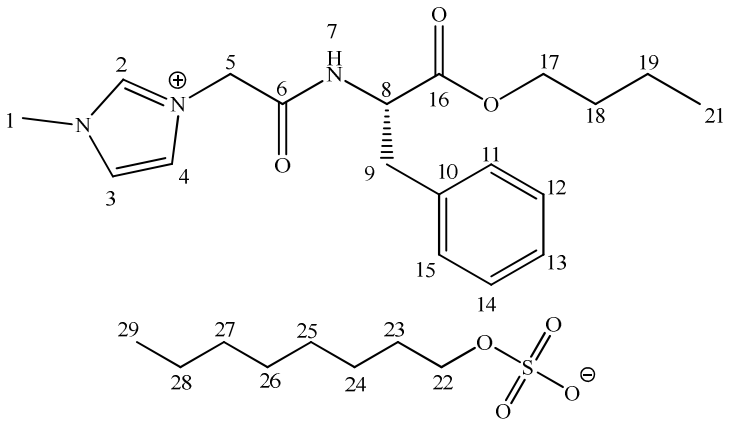
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.98 (s, 1H, *H2*), 8.53 (d, *J* = 7.8 Hz, 1H, *H7*), 7.29 (t, *J* = 1.8 Hz, 1H, *H4*), 7.21 (t, *J* = 1.8 Hz, 1H, *H3*), 7.17-7.09 (m, 5H, *H11-15*), 5.00 (d, *J* = 5.4 Hz, 2H, *H5*), 4.57 (ddd, *J* = 8.0, 6.6, 6.6 Hz, 1H, *H8*), 4.01 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 3.98 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 3.94 (t, *J* = 6.9 Hz, 2H, *H19*), 3.84 (s, 3H, *H1*), 3.09 (dd, *J* = 12.0, 6.0 Hz, 1H, *H9*), 2.99 (dd, *J* = 12.0, 6.0 Hz, 1H, *H9*), 1.56 (tt, *J* = 7.2, 7.0 Hz, 2H, *H20*), 1.28-1.23 (m, 2H, *H21*), 1.20-1.13 (m, 8H, *H22-25*), 1.07 (t, *J* = 7.2 Hz, 3H, *H18*), 0.78 (t, *J* = 7.2 Hz, 3H, *H26*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.26 (*C*O,*C16*), 164.99 (*C*O,*C6*), 137.92 (N*C*HN,*C2*), 136.64 (Ar*C*,*C10*), 129.38 (Ar*C*H), 128.60 (Ar*C*H), 127.69 (Ar*C*H,*C13*), 123.56 (N*C*H,*C4*), 122.78 (N*C*H,*C3*), 68.08 (O*C*H_2,*C19*), 61.41 (O*C*H_2,*C17*), 54.68 (*C*H,*C8*), 51.12 (N*C*H_2,*C5*), 37.74 (*C*H_2,*C9*), 36.42 (N*C*H_3,*C1*), 31.79 (*C*H_2), 29.49 (*C*H_2), 29.34 (*C*H_2), 29.22 (*C*H_2), 28.68 (*C*H_2,*C20*), 25.87 (*C*H_2), 22.61 (*C*H_2), 14.06 (*C*H_3,*C18*), 13.98 (*C*H_3,*C26*)

IR (neat) (cm^{-1}) 3158 (w), 2926 (m), 2856 (m), 1735 (m), 1682 (s), 1564 (m), 1174 (s), 1048 (m), 746 (m), 700 (m)

LRMS (*m/z*) 316.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-phenylalanine butyl ester imidazolium $OctOSO_3$ (312)



The title compound (**312**) was prepared from 3-methyl-1-L-phenylalanine butyl ester imidazolium bromide (**272**) (0.198 g, 0.47 mmol) and sodium octyl sulfate (0.121 g, 0.52 mmol) according to the general procedure (Section 7.3.5, page 333) as a colourless liquid in 95 % yield (0.244 g, 0.45 mmol).

$[\alpha]_D^{20} = +9.0^{o}$ (0.8 c, $CHCl_3$)

Molecular formula $C_{27}H_{43}N_3O_7S$

Molecular weight 554 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.02 (s, 1H, *H2*), 8.55 (d, *J* = 7.8 Hz, 1H, *H7*), 7.30 (t, *J* = 1.8 Hz, 1H, *H4*), 7.23 (t, *J* = 1.8 Hz, 1H, *H1*), 7.20-7.11 (m, 5H, *H11-15*), 5.03 (s, 2H, *H5*), 4.58 (ddd, *J* = 8.4, 6.0, 6.0 Hz, 1H, *H8*), 3.96-3.92 (m, 4H, *H17,22*), 3.85 (s, 3H, *H1*), 3.09 (dd, *J* = 13.0, 6.0 Hz, 1H, *H9*), 3.01 (dd, *J* = 13.8, 6.0 Hz, 1H, *H9*), 1.57 (tt, *J* = 7.2, 7.0 Hz, 2H, *H23*), 1.47-1.39 (m, 2H, *H18*), 1.28-1.24 (m, 2H, *H19*), 1.22-1.12 (m, 10H, *H24-28*), 0.82 (t, *J* = 7.2 Hz, 3H, *H21*), 0.78 (t, *J* = 7.2 Hz, 3H, *H29*)

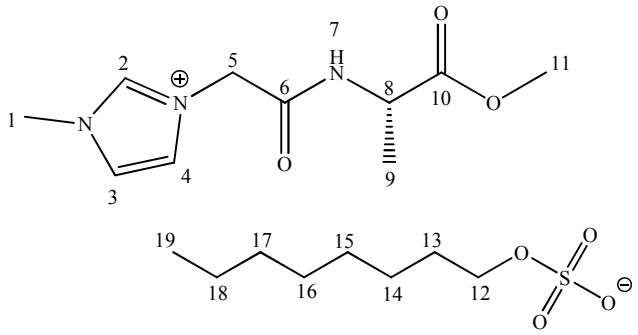
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.27 (*C*O,*C16*), 164.88 (*C*O,*C6*), 137.90 (N*C*HN,*C2*), 136.66 (Ar*C*,*C10*), 129.37 (Ar*C*H), 128.39 (Ar*C*H), 126.77 (Ar*C*H,*C13*), 123.51 (N*C*H,*C4*), 122.80 (N*C*H,*C3*), 68.02 (O*C*H_2,*C19*), 65.20 (O*C*H_2,*C17*), 54.71 (*C*H,*C8*), 51.18 (N*C*H_2,*C5*), 37.58 (*C*H_2,*C9*), 36.48 (N*C*H_3,*C1*), 31.79 (*C*H_2), 30.40 (*C*H_2),

29.51 (*C*H_2), 29.34 (*C*H_2), 29.22 (*C*H_2), 28.36 (*C*H_2,*C23*), 25.84 (*C*H_2), 25.77 (*C*H_2), 22.64 (*C*H_2), 18.97 (*C*H_2), 14.05 (*C*H_3,*C21*), 13.64 (*C*H_3,*C29*)

IR (neat) (cm^{-1}) 3066 (w), 2927 (m), 1737 (m), 1688 (s), 1563 (m), 1202 (vs), 1018 (m), 748 (m), 700 (m)

LRMS (*m/z*) 344.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-alanine methyl ester imidazolium $OctOSO_3$ (313)



The title compound (**313**) was prepared from 3-methyl-1-L-alanine methyl ester imidazolium bromide (**273**) (0.244 g, 0.80 mmol) and sodium octyl sulfate (0.280 g, 0.96 mmol) according to the general procedure (Section 7.3.5, page 333) as a colourless liquid in 86 % yield (0.298 g, 0.68 mmol).

$[\alpha]_D^{20} = -9.0^{o}$ (0.6 c, $CHCl_3$)

Molecular formula $C_{18}H_{33}N_3O_7S$

Molecular weight 435 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.22 (s, 1H, *H2*), 8.63 (d, *J* = 6.4 Hz, 1H, *H7*), 7.49 (t, *J* = 1.8 Hz, 1H, *H4*), 7.24 (t, *J* = 1.8 Hz, 1H, *H3*), 5.16 (s, 2H, *H5*), 4.34 (dq, *J* = 7.8, 7.6 Hz, 1H), 3.95-3.92 (m, 5H, *H1,12*), 3.63 (s, 3H, *H11*), 1.57 (tt, *J* = 7.6, 7.4 Hz, 2H, *H13*), 1.41 (d, *J* = 7.8 Hz, 3H, *H9*), 1.28-1.18 (m, 10H, *H14-18*), 0.79 (t, *J* = 6.2 Hz, 3H, *H19*)

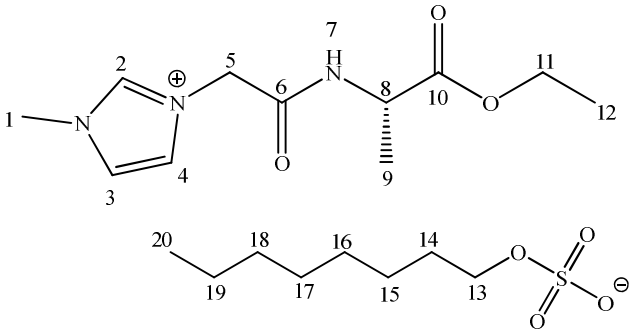
^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.92 (*C*O,*C10*), 164.90 (*C*O,*C6*), 138.00 (N*C*HN,*C2*), 123.79 (N*C*H,*C4*), 122.71 (N*C*HN,*C3*), 68.19 (O*C*H_2,*C12*), 52.43

(O*C*H$_3$,*C11*), 51.26 (N*C*H$_2$,*C5*), 48.82 (*C*H,*C8*), 36.61 (*C*H$_3$,*C1*), 31.80 (*C*H$_2$), 29.43 (*C*H$_2$), 29.32 (*C*H$_2$), 29.23 (*C*H$_2$,*C13*), 25.83 (*C*H$_2$), 22.62 (*C*H$_2$), 16.94 (*C*H$_2$), 14.07 (*C*H$_3$,*C19*)

IR (neat) (cm^{-1}) 3080 (w), 2955 (m), 2927 (m), 1741 (m), 1684 (s), 1564 (m), 1208 (vs), 1176 (vs), 1056 (m)

LRMS (*m/z*) 226.3 [M-OctOSO$_3$]$^+$; 209.1 [OctOSO$_3$]$^-$

3-Methyl-1-L-alanine ethyl ester imidazolium OctOSO$_3$ (314)



The title compound (**314**) was prepared from 3-methyl-1-L-alanine ethyl ester imidazolium bromide (**274**) (0.220 g, 0.63 mmol) and sodium octyl sulfate (0.171 g, 0.75 mmol) according to the general procedure (Section 7.3.5, page 333) as a pale yellow oil in 85 % yield (0.240 g, 0.53 mmol).

$[\alpha]_D^{20} = -12.1^{\circ}$ (0.5 c, CHCl$_3$)

Molecular formula $C_{19}H_{35}N_3O_7S$

Molecular weight 449 gmol^{-1}

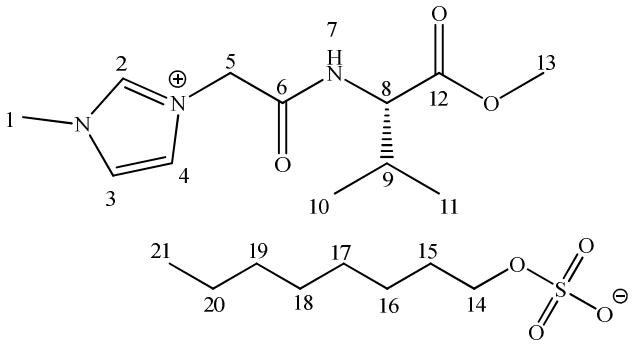
^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) 9.13 (s, 1H, *H2*), 8.52 (d, *J* = 6.8 Hz, 1H, *H7*), 7.48 (s, 1H, *H4*), 7.27 (s, 1H, *H3*), 5.13 (s, 2H, *H5*), 4.32 (dq, *J* = 7.2, 6.8 Hz, 1H, *H8*), 4.07 (q, *J* = 6.4 Hz, 2H, *H11*), 3.93-3.90 (m, 5H, *H1,13*), 1.55 (tt, *J* = 7.2, 7.0 Hz, 2H, *H14*), 1.39 (d, *J* = 7.2 Hz, 3H, *H9*), 1.26-1.16 (m, 13H, *H12,15-19*), 0.80 (t, *J* = 6.6 Hz, 3H, *H20*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.47 (*C*O,*C10*), 163.99 (*C*O,*C6*), 136.94 (N*C*HN,*C2*), 122.74 (N*C*H,*C4*), 121.99 (N*C*HN,*C3*), 67.10 (O*C*H_2,*C13*), 60.69 (O*C*H_2,*C11*), 50.14 (N*C*H_2,*C5*), 47.88 (*C*H,*C8*), 36.49 (*C*H_3,*C1*), 31.77 (*C*H_2), 29.41 (*C*H_2), 29.30 (*C*H_2), 29.19 (*C*H_2,*C13*), 25.81 (*C*H_2), 22.56 (*C*H_2), 16.94 (*C*H_2), 15.97 (*C*H_3,*C12*), 14.07 (*C*H_3,*C20*)

IR (neat) (cm^{-1}) 3079 (w), 2958 (w), 2930 (m), 1742 (m), 1686 (s), 1567 (m), 1210 (vs), 1174 (vs), 1056 (s)

LRMS (*m/z*) 240.3 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-valine methyl ester imidazolium $OctOSO_3$ (315)



The title compound (**315**) was prepared from 3-methyl-1-L-valine methyl ester imidazolium bromide (**276**) (0.202 g, 0.63 mmol) and sodium octyl sulfate (0.180 g, 0.76 mmol) according to the general procedure (Section 7.3.5, page 333) as a colourless liquid in 87 % yield (0.256 g, 0.55 mmol).

$[\alpha]_D^{20} =$ -8.1 ^{o} (0.4 c, $CHCl_3$)

Molecular formula $C_{20}H_{37}N_3O_7S$

Molecular weight 463 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.10 (s, 1H, *H2*), 8.39 (d, *J* = 8.0 Hz, 1H, *H7*), 7.49 (t, *J* = 1.8 Hz, 1H, *H4*), 7.29 (t, *J* = 1.8 Hz, 1H, *H3*), 5.22 (d, *J* = 2.8 Hz, 2H, *H5*), 4.25 (dd, *J* = 7.6, 5.6 Hz, 1H, *H8*), 3.93 (t, *J* = 6.4 Hz, 2H, *H14*), 3.91 (s, 3H, *H1*), 3.64 (s, 3H, *H13*),

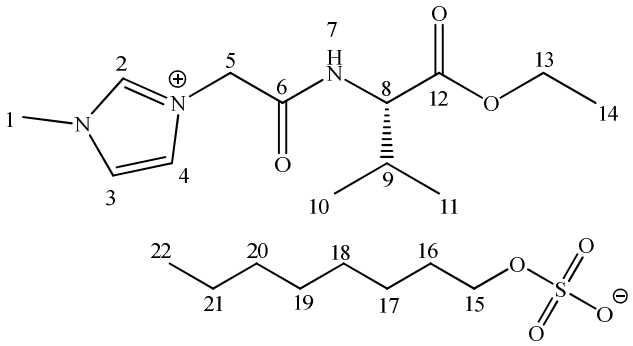
2.19 (qqd, *J* = 7.0, 7.0, 5.2 Hz, 1H, *H9*), 1.55 (tt, *J* = 6.9, 6.8 Hz, 2H, *H15*), 1.40-1.32 (m, 10H, *H16-20*), 1.02 (d, *J* = 7.0 Hz, 3H, *H10/11*), 0.98 (d, *J* = 7.0 Hz, 3H, *H10,11*), 0.88 (t, *J* = 6.8 Hz, 3H, *H21*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.11 (*C*O,*C12*), 165.56 (*C*O,*C6*), 137.88 (N*C*HN,*C2*), 123.77 (N*C*H,*C4*), 122.77 (N*C*HN,*C3*), 68.21 (O*C*H_2,*C14*), 58.65 (*C*H,*C8*), 52.20 (N*C*H_2,*C5*), 51.12 (O*C*H_3,*C13*), 36.56 (*C*H_3,*C1*), 31.82 (*C*H_2), 30.32 (*C*H_2), 29.41 (*C*H_2), 29.36 (*C*H_2), 29.26 (*C*H_2,*C14*), 25.83 (*C*H_2), 22.63 (*C*H_2), 19.03 (*C*H_3,*C10/C11*), 18.24 (*C*H_3,*C10/C11*), 14.09 (*C*H_3,*C21*)

IR (neat) (cm^{-1}) 2959 (w), 2927 (w), 2856 (m), 1735 (m), 1687 (s), 1563 (m), 1203 (vs), 1020 (m)

LRMS (*m/z*) 254.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-valine ethyl ester imidazolium $OctOSO_3$ (316)



The title compound (**316**) was prepared from 3-methyl-1-L-valine ethyl ester imidazolium bromide (**277**) (0.203 g, 0.61 mmol) and sodium octyl sulfate (0.170 g, 0.72 mmol) according to the general procedure (Section 7.3.5, page 333) as a viscous colourless liquid in 67 % yield (0.198 g, 0.41 mmol).

$[\alpha]_D^{20} = -10.0^{\circ}$ (0.6 c, $CHCl_3$)

Molecular formula $C_{21}H_{39}N_3O_7S$

Molecular weight 478 $gmol^{-1}$

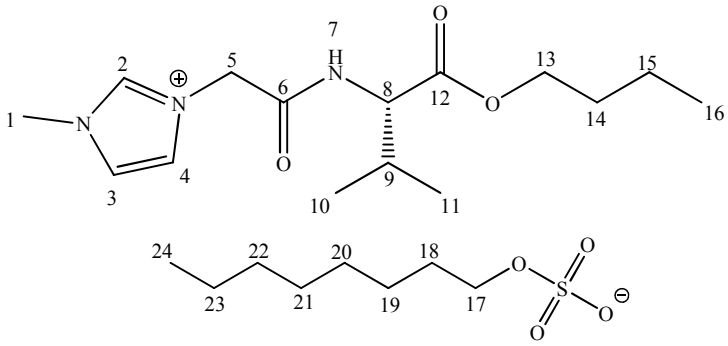
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.36 (s, 1H, *H2*), 8.44 (d, *J* = 7.6 Hz, 1H, *H7*), 7.60 (t, *J* = 1.8 Hz, 1H, *H4*), 7.21 (t, *J* = 1.8 Hz, 1H, *H3*), 5.28 (d, *J* = 12.8 Hz, 2H, *H5*), 4.30 (dd, *J* = 7.6, 5.6 Hz, 1H, *H8*), 4.16 (dq, *J* = 7.2, 7.0 Hz, 1H, *H13*), 4.14 (dq, *J* = 7.0, 7.0 Hz, 1H, *H13*), 4.05 (t, *J* = 6.4 Hz, 2H, *H15*), 3.98 (s, 3H, *H1*), 2.19 (qqd, *J* = 7.0, 6.8, 5.2 Hz, 1H, *H9*), 1.66 (tt, *J* = 7.0, 6.8 Hz, 2H, *H16*), 1.39-1.26 (m, 13H, *H14,17-21*), 1.02 (dd, *J* = 7.0, 6.8 Hz, 6H, *H10/11*), 0.88 (t, *J* = 6.8 Hz, 3H, *H22*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.57 (*C*O,*C12*), 165.23 (*C*O,*C6*), 138.13 (N*C*HN,*C2*), 123.93 (N*C*H,*C4*), 122.28 (N*C*HN,*C3*), 68.17 (O*C*H_2,*C15*), 61.24 (O*C*H_2,*C13*) 58.69 (*C*H,*C8*), 51.20 (N*C*H_2,*C5*), 36.54 (*C*H_3,*C1*), 31.83 (*C*H_2), 30.32 (*C*H_2), 29.44 (*C*H_2), 29.34 (*C*H_2), 29.26 (*C*H_2,*C14*), 25.85 (*C*H_2), 22.66 (*C*H_2), 19.03 (*C*H_3,*C10/C11*), 18.06 (*C*H_3,*C10/C11*), 14.21 (*C*H_3,*C14*), 14.09 (*C*H_3,*C22*)

IR (neat) (cm^{-1}) 2956 (w), 2920 (m), 2852 (w), 1739 (m), 1685 (s), 1548 (m), 1214 (vs), 1080 (vs), 1019 (s)

LRMS (*m/z*) 268.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

3-Methyl-1-L-valine butyl ester imidazolium $OctOSO_3$ (317)



The title compound (**317**) was prepared from 3-methyl-1-L-valine butyl ester imidazolium bromide (**278**) (0.204 g, 0.57 mmol) and sodium octyl sulfate (0.161 g, 0.71 mmol) according to the general procedure (Section 7.3.5, page 333) as a colourless liquid in 77 % yield (0.221 g, 0.44 mmol).

$[\alpha]_D^{20} = -13.1^{\circ}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{23}H_{43}N_3O_7S$

Molecular weight 506 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 9.35 (s, 1H, *H2*), 8.31 (d, *J* = 7.6 Hz, 1H, *H7*), 7.52 (s, 1H, *H4*), 7.13 (s, 1H, *H3*), 5.26 (d, *J* = 34.0 Hz, 2H, *H5*), 4.26 (dd, *J* = 7.6, 5.2 Hz, 1H, *H8*), 4.10-3.95 (m, 4H, *H13/17*), 3.91 (s, 3H, *H1*), 2.19 (qqd, *J* = 6.8, 6.8, 5.6 Hz, 1H, *H9*), 1.63-1.51 (m, 4H, *H14,18*), 1.34-1.19 (m, 12H, *H15,19-23*), 0.96 (dd, *J* = 8.4, 6.8 Hz, 6H, *H10/11*), 0.85 (t, *J* = 7.4 Hz, 3H, *H16*), 0.80 (t, *J* = 7.0 Hz, 3H, *H24*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.69 (*C*O,*C12*), 165.51 (*C*O,*C6*), 137.86 (N*C*HN,*C2*), 123.74 (N*C*H,*C4*), 122.79 (N*C*HN,*C3*), 68.17 (O*C*H_2,*C17*), 65.13 (O*C*H_2,*C13*) 58.58 (*C*H,*C8*), 51.08 (N*C*H_2,*C5*), 36.50 (*C*H_3,*C1*), 31.82 (*C*H_2), 30.51 (*C*H_2), 30.37 (*C*H_2), 29.58 (*C*H_2), 29.40 (*C*H_2), 29.36 (*C*H_2), 29.25 (*C*H_2,*C14*), 25.85 (*C*H_2), 22.63 (*C*H_2), 19.08 (*C*H_3,*C10/C11*), 18.09 (*C*H_3,*C10/C11*), 14.21 (*C*H_3,*C16*), 14.11 (*C*H_3,*C24*)

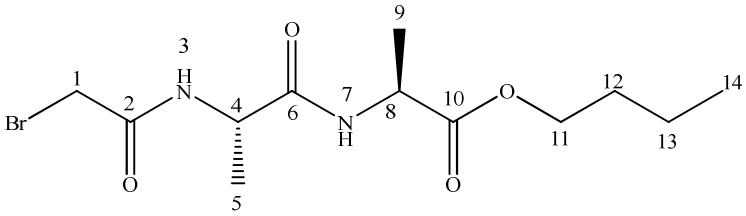
IR (neat) (cm^{-1}) 3159 (w), 2960 (m), 2930 (m), 1735 (m), 1686 (s), 1563 (m), 1201 (vs), 1059 (m)

LRMS (*m/z*) 296.4 $[M\text{-}OctOSO_3]^+$; 209.1 $[OctOSO_3]^-$

7.4 Chapter 4 experimental-Preparation of dipeptidyl Chiral Ionic Liquids

7.4.1 Preparation of Chiral dipeptidyl α-bromoamides

***Representative procedure for the preparation of chiral dipeptide α-bromoamides*: L-alanine-L-alanine butyl ester bromoacetate (346)**



To a stirred solution of DCM, L-alanine-L-alanine butyl ester hydrochloride (1.180 g, 4.70 mmol), and triethylamine (0.660 g, 6.55 mmol), under a nitrogen atmosphere at -78 ºC was added dropwise bromoacetyl bromide (1.131 g, 5.60 mmol). After stirring at -78 ºC for 5 h,

the reaction mixture was allowed warm up to -20 ºC and quenched by addition of water (10 mL). The organic phase was washed with distilled water (3 x 10 mL), saturated ammonium chloride (3 x 10 mL), saturated sodium bicarbonate (3 x 10 mL) and brine (3 x 10 mL). The organic phase was then dried over anhydrous magnesium sulfate, filtered and volatiles removed *via* rotary evaporation to give a crude product (1.42 g). The crude product was purified by column chromatography (eluant, ethyl acetate:hexane, 50:50) to give the title compound (**346**) as a white solid at RT in 77 % yield (1.221 g, 3.62 mmol).

m.p. 78-80 °C, $[\alpha]_D^{20}$ = -42.5 ° (0.8 c, $CHCl_3$)

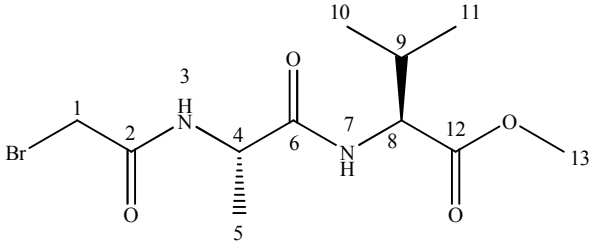
Molecular formula $C_{12}H_{21}BrN_2O_4$

Molecular weight 337 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.12 (d, *J* = 7.2 Hz, 1H, *H3*), 6.58 (d, *J* = 7.2 Hz, 1H, *H7*), 4.50 (dq, *J* = 7.2, 7.0 Hz, 1H, *H4*), 4.39 (dq, *J* = 7.2, 7.2 Hz, *H8*), 4.13 (q, *J* = 7.4 Hz, 2H, *H11*), 3.80 (s, 2H, *H1*), 1.61 (tt, *J* = 7.2, 6.8 Hz, 2H, *H12*), 1.39 (d, *J* = 7.0 Hz, 3H, *H5*), 1.36 (d, *J* = 7.2 Hz, 3H, *H9*), 1.27 (tq, *J* = 7.2, 7.2 Hz, 2H, *H13*), 0.87 (t, *J* = 7.2 Hz, 3H, *H14*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.64 (*C*O, *C2*), 171.06 (*C*O,*C10*), 165.48 (*C*O, *C6*), 65.51 (O*C*H_2,*C11*), 49.39 (*C*H,*C4*), 48.35 (*C*H,*C8*), 36.53 (*C*H_2,*C12*), 28.61 (*C*H_2,*C1*), 19.02 (*C*H_2,*C18*), 18.48 (*C*H_3,*C5*), 18.27 (*C*H_3,*C9*), 13.62 (*C*H_3,*C14*)

L-Alanine-L-valine methyl ester bromoacetate (347)



The title compound (**347**) was prepared from L-alanine-L-valine methyl ester hydrochloride (1.411 g, 5.95 mmol) and bromoacetyl bromide (1.433 g, 7.10 mmol) according to the

general procedure (Section 7.4.1, page 344) as a beige solid in 67 % yield (1.295 g, 4.01 mmol).

m.p. 94-96 °C $[\alpha]_D^{20}$ = -22.0 ° (0.8 c, $CHCl_3$)

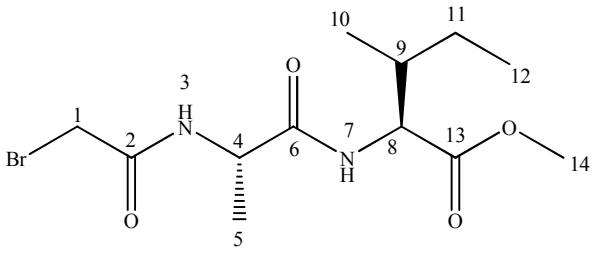
Molecular formula $C_{11}H_{29}BrN_2O_4$

Molecular weight 323 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.96 (d, *J* = 7.6 Hz, 1H, *H3*), 6.31 (d, *J* = 8.4 Hz, 1H, *H7*), 4.48 (dd, *J* = 8.8, 4.8 Hz, 1H, *H8*), 4.40 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 3.81 (s, 2H, *H1*), 3.69 (s, 3H, *H13*), 2.17 (qqd, *J* = 6.6, 6.8, 4.8 Hz, 1H, *H9*), 1.38 (d, *J* = 7.0 Hz, 3H, *H5*), 0.90 (d, *J* = 6.8 Hz, 3H, *H10/11*), 0.87 (d, *J* = 6.8 Hz, 3H, *H10/11*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.05 (*C*O,*C2*), 171.38 (*C*O,*C12*), 165.55 (*C*O,*C6*), 57.35 (*C*H,*C8*), 52.21 (*C*H,*C4*), 49.48 (O*C*H_3,*C13*), 31.12 (*C*H,*C9*), 28.55 (*C*H_2,*C1*), 18.93 (*C*H_3,*C10/C11*), 17.92 (*C*H_3,*C10/C11*), 17.71 (*C*H_3,*C5*)

L-Alanine-L-isoleucine methyl ester bromoacetate (348)



The title compound (**348**) was prepared from L-alanine–L-isoleucine methyl ester hydrochloride (2.331 g, 9.20 mmol) and bromoacetyl bromide (2.240 g, 11.10 mmol) according to the general procedure (Section 7.4.1, page 344) as a pale orange solid in 63 % yield (1.969 g, 5.84 mmol).

m.p. 75-77 °C, $[\alpha]_D^{20}$ = -13.0° (1.0 c, $CHCl_3$)

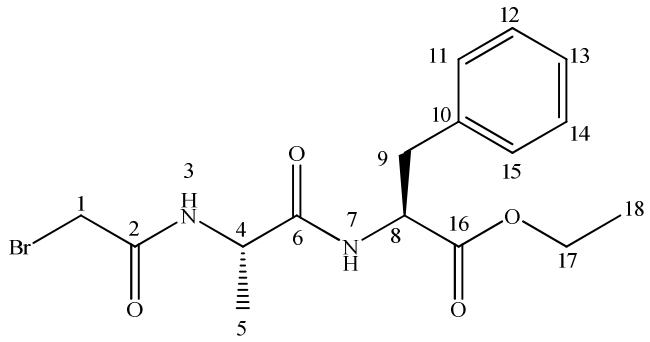
Molecular formula $C_{12}H_{21}BrN_2O_4$

Molecular weight 337 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.99 (d, *J* = 7.2 Hz, 1H, *H3*), 6.67 (d, *J* = 8.4 Hz, 1H, *H7*), 4.51 (dd, *J* = 8.0, 4.8 Hz, 1H, *H8*), 4.39 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 3.81 (s, 2H, *H1*), 3.68 (s, 3H, *H14*), 1.84 (dddq, *J* = 8.0, 8.0, 6.8, 4.8 Hz, 1H, *H9*), 1.35 (d, *J* = 6.8 Hz, 3H, *H5*), 1.30 (ddq, *J* = 8.8, 8.0, 7.2 Hz, 1H, *H11*), 1.13 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H11*), 0.87 (t, *J* = 7.2 Hz, 3H, *H12*) 0.83 (d, *J* = 7.2 Hz, 3H, *H10*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.12 (*C*O,*C2*), 171.57 (*C*O,*C13*), 165.65 (*C*O,*C6*), 56.71 (*C*H,*C8*), 52.24 (*C*H,*C4*), 49.38 (O*C*H_3,*C14*), 37.68 (*C*H,*C9*), 28.56 (*C*H_2,*C1*), 25.09 (*C*H_2,*C11*), 18.44 (*C*H_3,*C5*), 15.49 (*C*H_3,*C10*), 11.61 (*C*H_3,*C12*)

L-Alanine-L-phenylalanine ethyl ester bromoacetate (349)



The title compound (**349**) was prepared from L-alanine–L-phenylalanine ethyl ester hydrochloride (1.862 g, 6.20 mmol) and bromoacetyl bromide (1.503 g, 7.45 mmol) according to the general procedure (Section 7.4.1, page 344) as an off-white solid in 76 % yield (1.828 g, 4.75 mmol).

m.p. 90-92 °C, $[\alpha]_D^{20}$ = +22.4° (1.4 c, $CHCl_3$)

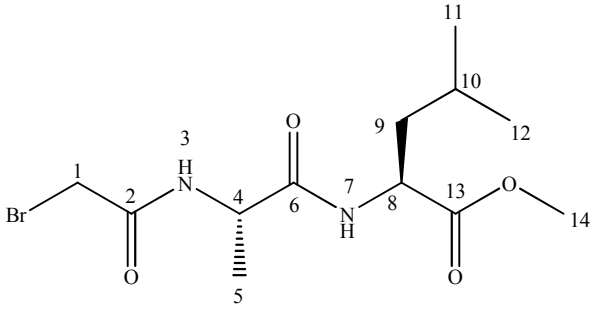
Molecular formula $C_{16}H_{21}BrN_2O_4$

Molecular weight 385 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.23-7.03 (m, 6H, *H7,11-15*), 6.54 (d, *J* = 8.0 Hz, 1H, *H7*), 4.78 (ddd, *J* = 8.0, 6.4, 6.0 Hz, 1H, *H8*), 4.40 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 4.12 (q, *J* = 7.2 Hz, 2H, *H17*), 3.76 (d, *J* = 8.4 Hz, 2H, *H1*), 3.09 (dd, *J* = 14.0, 6.0 Hz, 1H, *H9*), 3.01 (dd, *J* = 13.6, 6.4 Hz, 1H, *H9*), 1.31 (d, *J* = 7.2 Hz, 3H, *H5*), 1.18 (t, *J* = 7.2 Hz, 3H, *H18*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.16 (*C*O,*C2*), 168.13 (*C*O,*C16*), 165.64 (*C*O,*C6*), 135.60 (Ar*C*,*C10*), 129.29 (Ar*C*H), 128.60 (Ar*C*H), 127.22 (Ar*C*H,*C13*), 61.73 (O*C*H_2,*C17*), 53.34 (*C*H,*C8*), 49.34 (*C*H,*C4*), 37.73 (*C*H_2,*C9*), 28.60 (*C*H_2,*C1*), 18.09 (*C*H_3,*C5*), 14.11 (*C*H_3,*C18*)

L-Alanine-L-leucine methyl ester bromoacetate (350)



The title compound (**350**) was prepared from L-alanine–L-leucine methyl hydrochloride (2.411 g, 9.55 mmol) and bromoacetyl bromide (2.232 g, 11.50 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 73 % yield (2.351 g, 6.98 mmol).

m.p 128-130 °C, $[\alpha]_D^{20} = +14.9^\circ$ (1.0 c, $CHCl_3$)

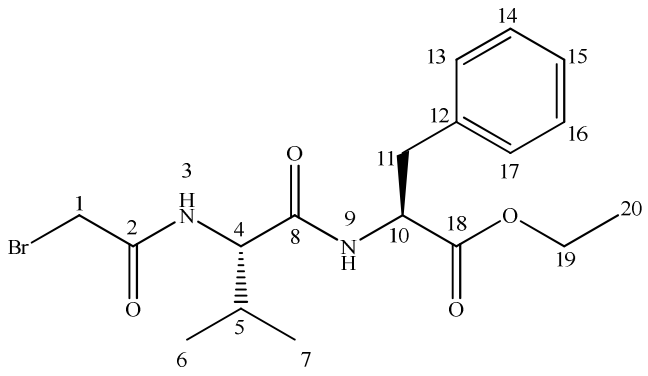
Molecular formula $C_{12}H_{21}BrN_2O_4$

Molecular weight 337 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.13 (d, *J* = 7.2 Hz, 1H, *H3*), 6.49 (d, *J* = 8.0 Hz, 1H, *H7*), 4.53 (ddd, *J* = 8.4, 8.0, 5.4 Hz, 1H, *H8*), 4.45 (dq, *J* = 7.2, 7.2 Hz, 1H, *H4*), 3.80 (s, 2H, *H1*), 3.68 (s, 3H, *H14*), 1.63-1.48 (m, 3H, *H9,10*), 1.37 (d, *J* = 7.2 Hz, 3H, *H5*), 0.87 (d, *J* = 6.0 Hz, 3H, *H11/12*), 0.84 (d, *J* = 6.0 Hz, 3H, *H11/12*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.13 (*C*O,*C2*), 171.42 (*C*O,*C13*), 165.58 (*C*O,*C6*), 52.45 (*C*H,*C8*), 50.95 (*C*H,*C4*), 49.33 (O*C*H_3,*C14*), 41.22 (*C*H_2,*C9*), 28.61 (*C*H_2,*C1*), 24.84 (*C*H,*C10*), 22.79 (*C*H_3,*C11/C12*), 21.84 (*C*H_3,*C11/C12*), 18.32 (*C*H_3,*C5*)

L-Valine-L-phenylalanine ethyl ester bromoacetate (351)



The title compound (**351**) was prepared from L-valine–L-phenylalanine ethyl ester hydrochloride (1.720 g, 5.24 mmol) and bromoacetyl bromide (1.24 g, 7.44 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 77 % yield (1.66 g, 4.02 mmol).

m.p. 166-168 °C, $[\alpha]_D^{20}$ = -20.0 ° (0.7 c, $CHCl_3$)

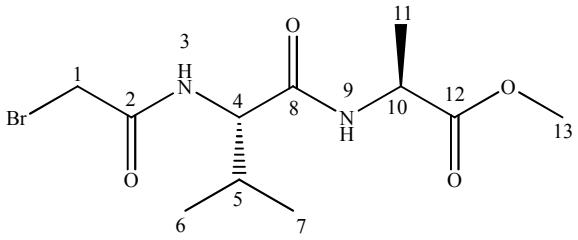
Molecular formula: $C_{18}H_{25}BrN_2O_4$

Molecular weight 413 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.23-7.03 (m, 5H, *H13-17*), 7.12 (d, *J* = 6.8 Hz, 1H, *H9*), 6.89 (d, *J* = 8.8 Hz, 1H, *H3*), 4.79 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H10*), 4.22 (dd, *J* = 8.8, 6.8 Hz, 2H, *H4*), 4.12 (q, *J* = 7.2 Hz, 2H, *H19*), 3.80 (d, *J* = 9.6 Hz, 2H, *H1*), 3.09-2.94 (m, 2H, *H11*), 2.12 (qqd, *J* = 6.8, 6.8, 6.4 Hz, 1H, *H5*), 1.16 (t, *J* = 7.2 Hz, 3H, *H20*), 0.86 (dd, *J* = 9.8, 6.8 Hz, 6H, *H6,7*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.12 (*C*O,*C2*), 170.19 (*C*O,*C18*), 165.78 (*C*O,*C8*), 135.55 (Ar*C*,*C12*), 129.31 (Ar*C*H), 128.3 (Ar*C*H), 127.25 (Ar*C*H,*C15*), 61.63 (*C*H,*C4*), 58.81 (O*C*H_2,*C19*), 53.22 (*C*H,*C10*), 37.85 (*C*H_2,*C11*), 31.32 (*C*H,*C5*), 28.83 (*C*H_2,*C1*), 19.03 (*C*H_3,*C6/C7*), 18.02 (*C*H_3,*C6/C7*), 14.11 (*C*H_3,*C20*)

L-Valine-L-alanine methyl ester bromoacetate (352)



The title compound (**352**) was prepared from L-valine–L-alanine methyl ester hydrochloride (1.592 g, 6.70 mmol) and bromoacetyl bromide (1.622 g, 8.02 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 49 % yield (1.072 g, 3.32 mmol).

m.p. 70-72 °C, $[\alpha]_D^{20}$ = -14.0 ° (0.6 c, $CHCl_3$)

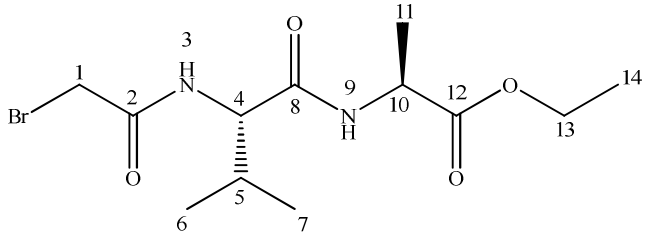
Molecular formula: $C_{11}H_{19}BrN_2O_4$

Molecular weight 323 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.92 (d, *J* = 8.0 Hz, 1H, *H3*), 6.17 (d, *J* = 6.8 Hz, 1H, *H9*), 4.49 (dq, *J* = 7.2, 7.2 Hz, 1H, *H10*), 4.17 (dd, *J* = 8.8, 6.4 Hz, 1H, *H4*), 3.83 (s, 2H, *H1*), 3.69 (s, 3H, *H13*), 2.14 (qqd, *J* = 6.8, 6.8, 6.4 Hz, 1H, *H5*), 1.37 (d, *J* = 7.2 Hz, 3H, *H11*), 0.92 (dd, *J* = 6.8, 3.2 Hz, 6H, *H6,7*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.83 (*C*O,*C2*), 170.44 (*C*O,*C12*), 165.95 (*C*O,*C8*), 57.31 (*C*H,*C4*), 51.74 (O*C*H_3,*C13*), 47.52 (*C*H,*C10*), 31.05 (*C*H,*C5*), 29.35 (*C*H_2,*C1*), 18.90 (*C*H_3,*C6/C7*), 17.87 (*C*H_3,*C6/C7*), 16.66 (*C*H_3,*C11*)

L-Valine-L-alanine ethyl ester bromoacetate (353)



The title compound (**353**) was prepared from L-valine–L-alanine ethyl ester hydrochloride (1.901 g, 7.55 mmol) and bromoacetyl bromide (1.830 g, 9.06 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 84 % yield (2.151 g, 6.38 mmol).

m.p. 75-77 °C, $[\alpha]_D^{20}$ = -10.6 ° (0.6 c, $CHCl_3$)

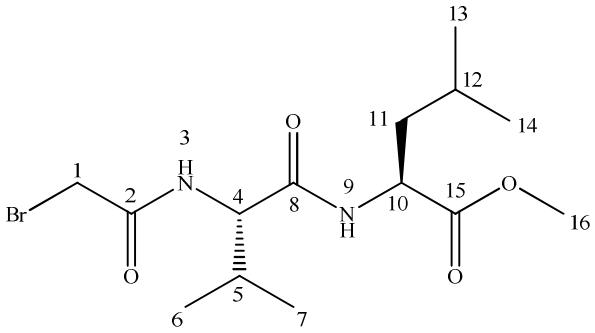
Molecular formula: $C_{12}H_{21}BrN_2O_4$

Molecular weight 337 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.96 (d, *J* = 8.4 Hz, 1H, *H3*), 6.20 (d, *J* = 6.8 Hz, 1H, *H9*), 4.49 (dq, *J* = 7.2, 7.2 Hz, 1H, *H10*), 4.32 (dd, *J* = 8.0, 4.8 Hz, 1H, *H4*), 4.17 (q, *J* = 7.2 Hz, 2H, *H13*), 3.83 (s, 2H, *H1*), 2.16 (qqd, *J* = 7.0, 6.8, 4.8 Hz, 1H, *H5*), 1.37 (d, *J* = 7.2 Hz, 3H, *H11*), 1.22 (t, *J* = 7.2 Hz, 3H, *H14*), 0.92 (dd, *J* = 7.2, 3.6 Hz, 6H, *H6,7*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.35 (*C*O,*C2*), 170.37 (*C*O,*C12*), 165.93 (*C*O,*C8*), 60.38 (*C*H,*C4*), 57.30 (*C*H_2,*C13*), 47.62 (*C*H,*C10*), 31.06 (*C*H,*C5*), 29.35 (*C*H_2,*C1*), 18.95 (*C*H_3,*C6/C7*), 17.98 (*C*H_3,*C6*/*C7*), 16.67 (*C*H_3,*C11*), 13.95 (*C*H_3,*C14*)

L-Valine-L-leucine methyl ester bromoacetate (354)



The title compound (**354**) was prepared from L-valine–L-leucine methyl ester hydrochloride (1.981 g, 7.10 mmol) and bromoacetyl bromide (1.722 g, 8.53 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 68 % yield (1.773 g, 4.86 mmol).

m.p 111-113 °C, $[\alpha]_D^{20}$ = -19.3 ° (1.1 c, $CHCl_3$)

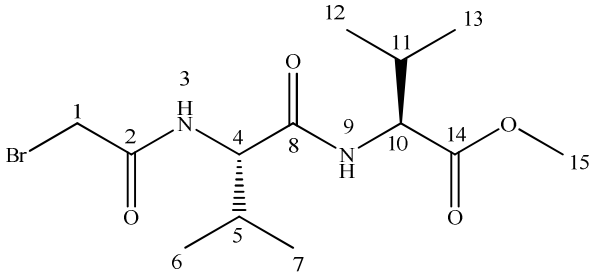
Molecular formula: $C_{14}H_{27}BrN_2O_4$

Molecular weight 365 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.98 (d, *J* = 8.4 Hz, 1H, *H3*), 6.18 (d, *J* = 8.0 Hz, 1H, *H9*), 4.52 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H10*), 4.22 (dd, *J* = 8.8, 7.2 Hz, 1H, *H4*), 3.82 (s, 2H, *H1*), 3.67 (s, 3H, *H16*), 2.14 (qqd, *J* = 6.8, 6.8, 6.4 Hz, 1H, *H5*), 1.71-1.43 (m, 3H, *H11,12*), 0.91 (dd, *J* = 6.8, 6.8 Hz, 6H, *H6,7*), 0.85 (dd, *J* = 6.0, 4.0 Hz, 6H, *H13,14*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.07 (*C*O,*C2*), 170.68 (*C*O,*C15*), 166.05 (*C*O,*C8*), 58.77 (*C*H,*C4*), 52.32 (*C*H,*C10*), 50.98 (O*C*H_3,*C16*), 41.00 (*C*H_2,*C11*), 31.58 (*C*H,*C5*), 30.05 (*C*H,*C11*), 28.75 (*C*H_2,*C1*), 24.82 (*C*H,*C12*), 22.73 (*C*H_3,*C13/C14*), 21.90 (*C*H_3,*C13*/*C14*), 18.99 (*C*H_3,*C6/C7*), 18.15 (*C*H_3,*C6*/*C7*).

L-Valine-L-valine methyl ester bromoacetate (355)



The title compound (**355**) was prepared from L-valine–L-valine methyl ester hydrochloride (1.453 g, 5.44 mmol) and bromoacetyl bromide (1.322 g, 6.53 mmol) according to the general procedure (Section 7.4.1, page 344) as a pale yellow liquid in 60 % yield (1.151 g, 3.28 mmol).

$[\alpha]_D^{20} = -9.3\ ^{o}$ (0.5 c, $CHCl_3$)

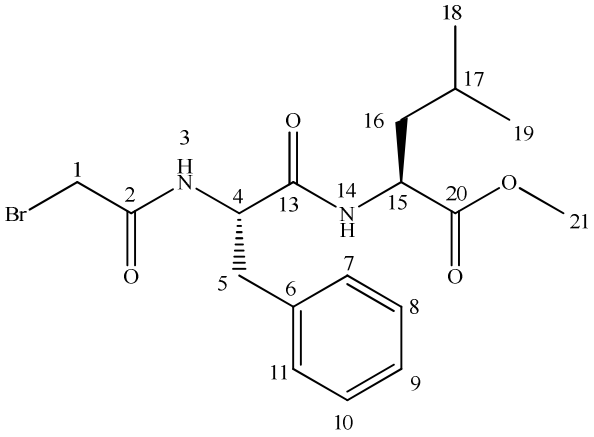
Molecular formula: $C_{13}H_{23}BrN_2O_4$

Molecular weight 351 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.22 (d, *J* = 8.8 Hz, 1H, *H3*), 6.70 (d, *J* = 8.4 Hz, 1H, *H9*), 4.44 (dd, *J* = 8.8, 5.2 Hz, 1H, *H4*), 4.33 (dd, *J* = 8.4, 6.8 Hz, 1H, *H10*), 3.82 (s, 2H, *H1*), 3.68 (s, 3H, *H15*), 2.14-2.03 (m, 2H, *H5,11*), 0.92-0.83 (m, 12H, *H6,7/12,13*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.20 (*C*O,*C2*), 170.83 (*C*O,*C14*), 166.07 (*C*O,*C8*), 58.97 (*C*H,*C4*), 57.38 (*C*H,*C10*), 52.25 (O*C*H$_3$,*C15*), 31.43 (*C*H,*C5*), 30.98 (*C*H,*C11*), 28.73 (*C*H$_2$,*C1*), 19.04 (*C*H$_3$), 18.94 (*C*H$_3$), 18.18 (*C*H$_3$), 17.85 (*C*H$_3$,*C6/C7/C12/C13*)

L-Phenylalanine-L-leucine methyl ester bromoacetate (356)



The title compound (**356**) was prepared from L-phenylalanine–L-leucine methyl ester hydrochloride (1.681 g, 5.12 mmol) and bromoacetyl bromide (1.238 g, 6.14 mmol) according to the general procedure (Section 7.4.1, page 344) as a pale orange liquid in 74 % yield (1.558 g, 3.77 mmol).

$[\alpha]_D^{20} = +14.3^{o}$ (0.9 c, $CHCl_3$)

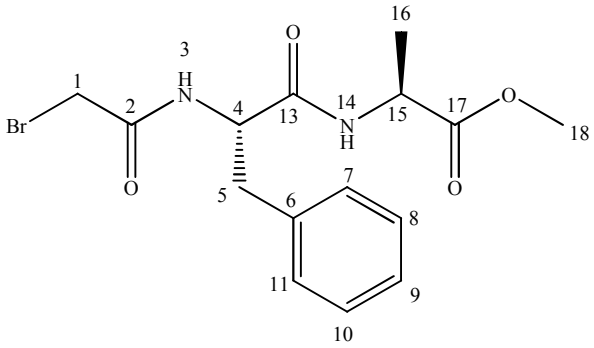
Molecular formula: $C_{18}H_{25}BrN_2O_4$

Molecular weight 413 gmol^{-1}

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.24-7.14 (m, 6H, *H7-11,14*), 6.37 (d, *J* = 7.6 Hz, 1H, *H3*), 4.63 (dt, *J* = 7.2, 7.0 Hz, 1H, *H4*), 4.44 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H15*), 3.74 (d, *J* = 3.6 Hz, 2H, *H1*), 3.64 (s, 3H, *H16*), 3.01 (d, *J* = 7.2 Hz, 2H, *H5*), 1.59-1.37 (m, 3H, *H16,17*), 0.80 (d, *J* = 6.4 Hz, 6H, *H18,19*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.72 (*C*O,*C2*), 170.16 (*C*O,*C20*), 165.85 (*C*O,*C13*), 135.96 (Ar*C*,*C6*), 129.46 (Ar*C*H), 128.69 (Ar*C*H), 127.19 (Ar*C*H,*C9*), 54.90 (*C*H,*C4*), 52.39 (*C*H,*C15*), 51.03 (O*C*H$_3$,*C21*), 41.26 (*C*H$_2$,*C16*), 38.27 (*C*H$_2$,*C5*), 28.59 (*C*H$_2$,*C1*), 24.77 (*C*H,*C17*), 22.68 (*C*H$_3$,*C18/C19*), 21.95 (*C*H$_3$,*C18/C19*)

L-Phenylalanine-L-alanine methyl ester bromoacetate (357)



The title compound (**357**) was prepared from L-phenylalanine–L-alanine methyl ester hydrochloride (1.768 g, 6.32 mmol) and bromoacetyl bromide (1.532 g, 7.58 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 73 % yield (1.710 g, 4.61 mmol).

m.p. 128-130 °C, $[\alpha]_D^{20} = +24.4^{o}$ (0.8 c, $CHCl_3$)

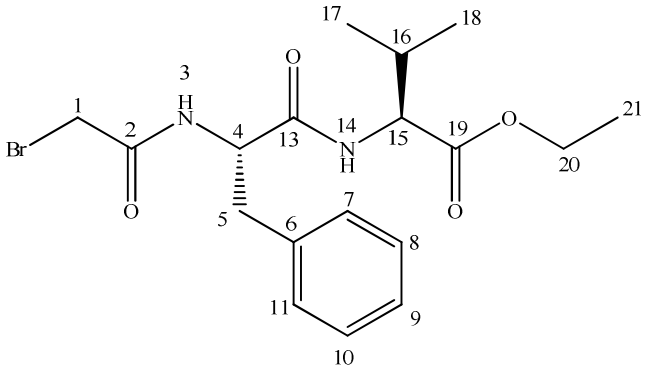
Molecular formula: $C_{15}H_{19}BrN_2O_4$

Molecular weight 371 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.27-7.16 (m, 5H, *H7-11*), 7.05 (d, *J* = 7.6 Hz, 1H, *H3*), 6.14 (d, *J* = 6.8 Hz, 1H, *H14*), 4.52 (ddd, *J* = 8.0, 6.4, 6.0 Hz, 1H, *H4*), 4.53 (dq, *J* = 7.4, 7.2 Hz, 1H, *H15*), 3.79 (d, *J* = 4.8 Hz, 2H, *H1*), 3.66 (s, 3H, *H18*), 3.08 (dd, *J* = 14.0, 6.4 Hz, 1H, *H5*), 2.98 (dd, *J* = 14.0, 7.6 Hz, 1H, *H5*), 1.29 (d, *J* = 7.2 Hz, 3H, *H16*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.72 (*C*O,*C2*), 169.90 (*C*O,*C17*), 165.78 (*C*O,*C13*), 135.91 (Ar*C*,*C6*), 129.42 (Ar*C*H), 128.71 (Ar*C*H), 127.23 (Ar*C*H,*C9*), 54.91 (*C*H,*C4*), 52.58 (O*C*H_3,*C18*), 48.30 (*C*H,*C15*), 38.44 (*C*H_2,*C5*), 28.63 (*C*H_2,*C1*), 18.16 (*C*H_3,*C16*)

L-Phenylalanine-L-valine ethyl ester bromoacetate (358)



The title compound (**358**) was prepared from L-phenylalanine–L-valine ethyl ester hydrochloride (1.688 g, 5.79 mmol) and bromoacetyl bromide (1.382 g, 6.84 mmol) according to the general procedure (Section 7.4.1, page 344) as a pale yellow liquid in 72 % yield (1.733 g, 4.20 mmol).

$[\alpha]_D^{20} = +13.9^{\circ}$ (1.0 c, $CHCl_3$)

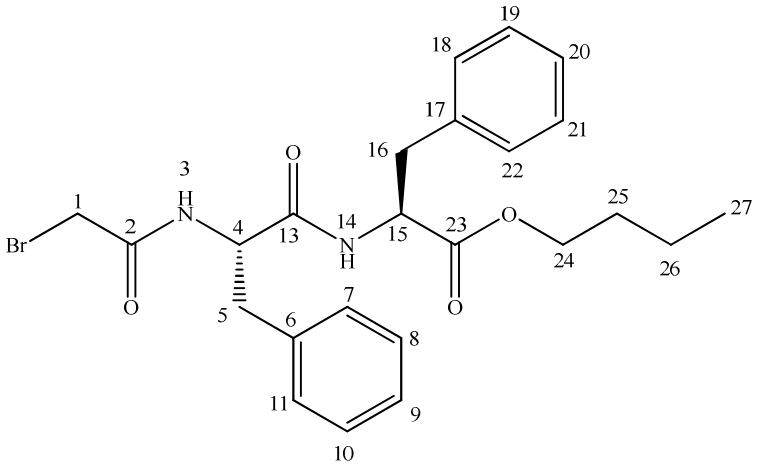
Molecular formula: $C_{18}H_{25}BrN_2O_4$

Molecular weight 413 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.24-7.14 (m, 6H, *H3,7-11*), 6.31 (d, *J* = 8.4 Hz, 1H, *H14*), 4.63 (ddd, *J* = 8.0, 6.4, 6.0 Hz, 1H, *H4*), 4.33 (dd, *J* = 8.8, 5.2 Hz, 1H, *H15*), 4.09 (q, *J* = 7.2 Hz, 2H, *H20*), 3.75 (d, *J* = 4.4 Hz, 2H, *H1*), 3.09 (dd, *J* = 14.0, 6.4 Hz, 1H, *H5*), 2.98 (dd, *J* = 14.0, 6.4 Hz, 1H, *H5*), 2.02 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H16*), 1.20 (t, *J* = 7.2 Hz, 2H, *H21*), 0.78 (dd, *J* = 6.8, 6.8 Hz, 6H, *H17,18*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.15 (*C*O,*C2*), 170.17 (*C*O,*C19*), 165.69 (*C*O,*C13*), 135.96 (Ar*C*,*C6*), 129.39 (Ar*C*H), 128.73 (Ar*C*H), 127.20 (Ar*C*H,*C9*), 61.33 (O*C*H_2,*C20*), 57.47 (*C*H,*C15*), 55.08 (*C*H,*C4*), 38.25 (*C*H_2,*C5*), 31.45 (*C*H_2,*C16*), 28.61 (*C*H_2,*C1*), 18.82 (*C*H_3,*C17/C18*), 17.74 (*C*H_3,*C17/C18*), 14.24 (*C*H_3,*C21*)

L-Phenylalanine-L-phenylalanine butyl ester bromoacetate (359)



The title compound (**359**) was prepared from L-phenylalanine–L-phenylalanine butyl ester hydrochloride (1.461 g, 3.61 mmol) and bromoacetyl bromide (0.872 g, 4.33 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 84 % yield (1.483 g, 3.03 mmol).

m.p. 93-95 °C $[\alpha]_D^{20}$ = -79.0 ° (0.8 c, $CHCl_3$)

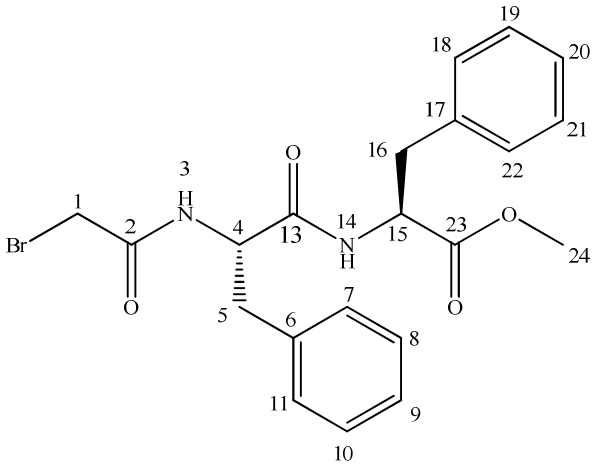
Molecular formula: $C_{24}H_{29}BrN_2O_4$

Molecular weight 489 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.24-6.90 (m, 11H, *H3,7-11,18-22*), 6.10 (d, *J* = 7.6 Hz, 1H, *H14*), 4.68 (ddd, *J* = 7.6, 6.0, 6.0 Hz, 1H, *H4*), 4.53 (ddd, *J* = 7.8, 6.6, 6.0 Hz, 1H, *H15*), 4.02 (dq, *J* = 7.2, 7.2 Hz, 1H, *H24*), 4.01 (dq, *J* = 7.2, 7.0 Hz, 1H, *H24*), 3.72 (s, 2H, *H1*), 3.07-2.89 (m, 4H, *H5,16*), 1.51 (tt, *J* = 7.4, 7.2 Hz, 2H, *H25*), 1.24 (tq, *J* = 7.2, 7.0 Hz, 2H, *H26*), 0.85 (t, *J* = 7.2 Hz, 3H, *H27*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.88 (*C*O,*C2*), 169.56 (*C*O,*C23*), 165.46 (*C*O,*C13*), 135.85 (Ar*C*,*C6/C17*), 135.46 (Ar*C*,*C6C17*), 129.43 (Ar*C*H), 129.21 (Ar*C*H), 128.78 (Ar*C*H), 128.59 (Ar*C*H), 127.29 (Ar*C*H,*C9/C20*), 127.19 (Ar*C*H,*C9/C20*), 65.33 (O*C*H_2,*C24*), 54.83 (*C*H,*C4*), 53.40 (*C*H,*C15*), 37.94 (*C*H_2,*C5/C16*), 37.87 (*C*H_2,*C5/C16*), 30.45 (*C*H_2,*C25*), 28.67 (*C*H_2,*C1*), 19.05 (*C*H_2,*C26*), 13.69 (*C*H_3,*C27*)

L-Phenylalanine-L-phenylalanine methyl ester bromoacetate (360)



The title compound (**360**) was prepared from L-phenylalanine–L-phenylalanine methyl ester hydrochloride (2.031 g, 5.41 mmol) and bromoacetyl bromide (1.312 g, 6.49 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 62 % yield (1.511 g, 3.38 mmol).

m.p. 105-107 °C, $[\alpha]_D^{20} = +18.0^{\circ}$ (0.7 c, $CHCl_3$)

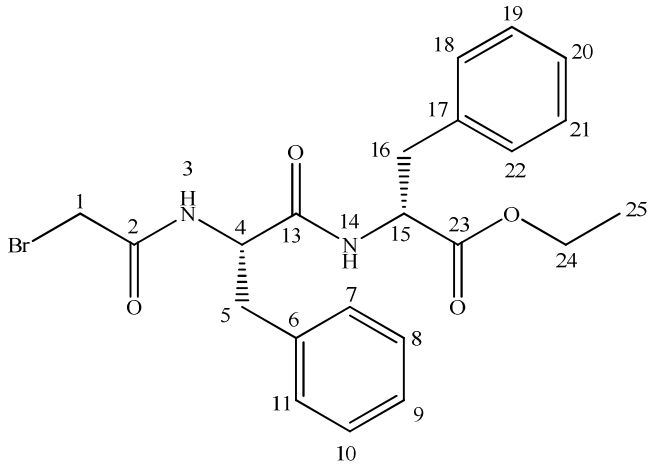
Molecular formula: $C_{21}H_{23}BrN_2O_4$

Molecular weight 447 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.22-6.88 (m, 10H, *H3,7-11,18-22*), 6.03 (d, *J* = 7.6 Hz, 1H, *H14*), 4.71 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H4*), 4.49 (ddd, *J* = 7.6, 7.0, 6.0 Hz, 1H, *H15*), 3.72 (s, 2H, *H1*), 3.64 (s, 3H, *H24*), 3.06-2.88 (m, 4H, *H5,16*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.24 (*C*O,*C2*), 169.66 (*C*O,*C23*), 165.49 (*C*O,*C13*), 135.88 (Ar*C*,*C6/C17*), 135.42 (Ar*C*,*C6/C17*), 129.44 (Ar*C*H), 129.16 (Ar*C*H), 128.76 (Ar*C*H), 128.63 (Ar*C*H), 127.26 (Ar*C*H,*C9/C20*), 127.24 (Ar*C*H,*C9/C20*), 54.81 (*C*H,*C4*), 53.36 (*C*H,*C15*), 52.44 (O*C*H_3,*C24*), 37.96 (*C*H_2,*C5/C16*), 37.76 (*C*H_2,*C5/C16*), 28.67 (*C*H_2,*C1*)

L-Phenylalanine-D-phenylalanine ethyl ester bromoacetate (361)



The title compound (**361**) was prepared from L-phenylalanine–D-phenylalanine ethyl ester hydrochloride (2.050 g, 5.40 mmol) and bromoacetyl bromide (1.322 g, 6.50 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 69 % yield (1.731 g, 3.75 mmol).

m.p. 110-112 °C, $[\alpha]_D^{20}$ = -30.0 ° (0.9 c, $CHCl_3$)

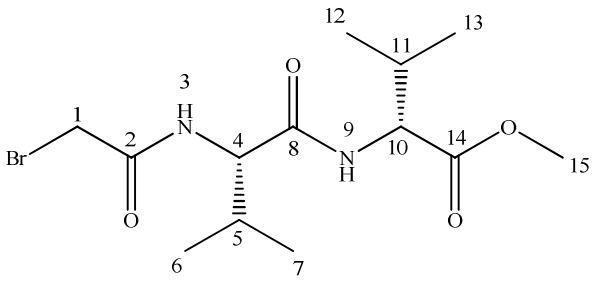
Molecular formula: $C_{22}H_{25}BrN_2O_4$

Molecular weight 461 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.35-7.18 (m, 10H, *H7-11,18-22*), 6.99 (d, *J* = 8.0 Hz, 1H, *H3*), 6.13 (d, *J* = 8.4 Hz, 1H, *H14*), 4.83 (ddd, *J* = 8.0, 6.4, 6.0 Hz, 1H, *H4*), 4.64 (ddd, *J* = 8.4, 6.0, 6.0 Hz, 1H, *H15*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H24*), 4.16 (dq, *J* = 7.2, 7.2 Hz, 1H, *H24*), 3.83 (s, 2H, *H1*), 3.11-2.89 (m, 4H, *H5,16*), 1.22 (t, *J* = 7.2 Hz, 3H, *H25*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 170.01 (*C*O,*C2*), 169.81 (*C*O,*C23*), 165.59 (*C*O,*C13*), 135.94 (Ar*C*,*C6/C17*), 135.56 (Ar*C*,*C6*/*C17*), 129.42 (Ar*C*H), 129.34 (Ar*C*H), 128.76 (Ar*C*H), 128.60 (Ar*C*H), 127.27 (Ar*C*H,*C9/C20*), 127.22 (Ar*C*H,*C9/C20*), 61.63 (O*C*H_2,*C24*), 55.28 (*C*H,*C4*), 53.23 (*C*H,*C15*), 38.37 (*C*H_2,*C5/C16*), 37.91 (*C*H_2,*C5/C16*), 28.28 (*C*H_2,*C1*), 14.07 (*C*H_3,*C25*)

L-Valine-D-valine methyl ester bromoacetate (362)



The title compound (**362**) was prepared from L-valine–D-valine methyl ester hydrochloride (1.462 g, 6.46 mmol) and bromoacetyl bromide (1.562 g, 7.75 mmol) according to the general procedure (Section 7.4.1, page 344) as a white solid in 72 % yield (1.630 g, 4.64 mmol).

m.p 76-78 °C, $[\alpha]_D^{20} = -9.0^{o}$ (0.5 c, $CHCl_3$)

Molecular formula: $C_{13}H_{23}BrN_2O_4$

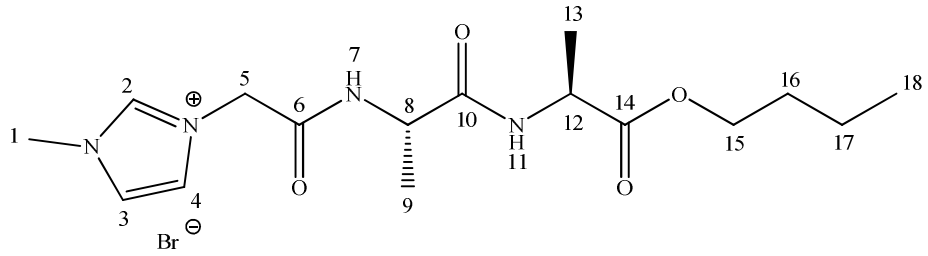
Molecular weight 351 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.18 (d, *J* = 8.4 Hz, 1H, *H3*), 6.62 (d, *J* = 8.8 Hz, 1H, *H9*), 4.44 (dd, *J* = 8.4, 4.8 Hz, 1H, *H4*), 4.31 (dd, *J* = 8.8, 6.8 Hz, 1H, *H10*), 3.84 (s, 2H, *H1*), 3.68 (s, 3H, *H15*), 2.17-2.07 (m, 2H, *H5,11*), 0.92-83 (m, 12H, *H6,7/12,13*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.89 (*C*O,*C2*), 168.83 (*C*O,*C14*), 165.12 (*C*O,*C8*), 57.96 (*C*H,*C4*), 58.22 (*C*H,*C10*), 52.65 (O*C*H_3,*C15*), 31.76 (*C*H,*C5*), 30.94 (*C*H,*C11*), 28.18 (*C*H_2,*C1*), 19.22 (*C*H_3), 18.94 (*C*H_3), 18.19 (*C*H_3), 17.87 (*C*H_3, *C6*/*C7*/*C12*/*C13*)

7.4.2 Preparation of dipeptidyl chiral Br ILs

Representative procedure for the preparation of chiral dipeptide bromide salts: **3-Methyl-1-L-alanine-L-alanine butyl ester imidazolium bromide (363)**



To a stirred solution of 1-methylimidazole (0.233 g, 2.86 mmol) in tetrahydrofuran (20 mL) at -15 ºC under an inert atmosphere was added dropwise L-alanine-L-alanine butyl ester bromoacetate (**346**) (1.101 g, 3.44 mmol). The reaction mixture was stirred vigorously at -15 ºC for 2 h, then at RT overnight. The solvent was removed on the rotary evaporator and the residual product was washed with diethyl ether. The product was dried under high vacuum for 48 h to yield the title product (**363**) as an off white solid in 88 % yield (1.059 g, 2.53 mmol).

m.p. 60-63 °C, $[\alpha]_D^{20}$ = -34.4 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{16}H_{27}BrN_4O_4$

Molecular weight 419 $gmol^{-1}$

^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.12 (s, 1H, *H2*), 8.79 (d, *J* = 7.6 Hz, 1H, *H7*), 8.54 (d, *J* = 7.2 Hz, 1H, *H11*), 7.75 (t, *J* = 1.8 Hz, 1H, *H4*), 7.73 (t, *J* = 1.8 Hz, 1H, *H3*), 5.06 (s, 2H, *H5*), 4.45 (dq, *J* = 7.6, 7.2 Hz, 1H, *H8*), 4.31 (dq, *J* = 7.2, 7.2 Hz, 1H, *H12*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H15*), 4.12 (dq, *J* = 7.2, 7.2 Hz, 1H, *H15*), 3.94 (s, 3H, *H1*), 1.56 (tt, *J* = 7.2, 6.8 Hz, 2H, *H16*), 1.33 (tq, *J* = 7.6, 6.8 Hz, 2H, *H17*), 1.37 (d, *J* = 7.6 Hz, 3H, *H9*), 1.33 (d, *J* = 7.2 Hz, 3H, *H13*), 0.96 (t, *J* = 7.2 Hz, 3H, *H18*)

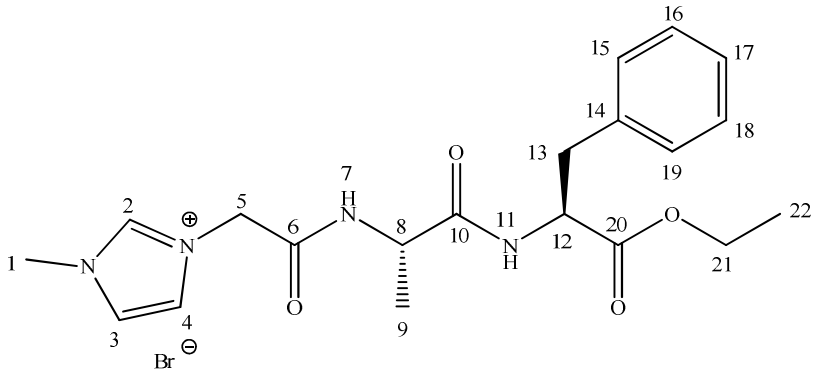
^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.35 (*C*O,*C14*), 171.59 (*C*O,*C10*), 164.34 (*C*O,*C6*), 137.68 (N*C*HN,*C2*), 123.70 (N*C*H,*C4*), 122.96 (N*C*H,*C3*), 64.07 (O*C*H_2,*C15*),

50.45 (*C*H,*C8*), 48.07 (N*C*H$_2$,*C5*), 47.61 (*C*H,*C12*), 35.78 (N*C*H$_3$,*C1*), 30.10 (*C*H$_2$,*C16*), 18.39 (*C*H$_2$,*C17*), 17.94 (*C*H$_3$,*C9*), 16.78 (*C*H$_3$,*C13*), 13.47 (*C*H$_3$,*C18*)

IR (neat) (cm^{-1}) 3415 (m), 3314 (m), 2960 (w), 1730 (s), 1654 (vs), 1566 (s), 1535 (s), 1212 (s), 1177 (s)

MS (*m/z*) Found [M-Br$^-$]$^+$ 339.2018, $C_{16}H_{27}N_4O_4^+$ requires 339.2026

3-Methyl-1-L-alanine-L-phenylalanine ethyl ester imidazolium bromide (364)



The title compound (**364**) was prepared from 1-methylimidazole (0.298 g, 3.65 mmol) and L-alanine-L-phenylalanine ethyl ester bromoacetate (**349**) (1.673 g, 4.35 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow hygroscopic semi-solid in 84 % yield (1.440 g, 3.08 mmol).

$[\alpha]_D^{20} = -11.4^{\circ}$ (0.6 c, $CHCl_3$)

Molecular formula $C_{20}H_{27}BrN_4O_4$

Molecular weight 467 gmol^{-1}

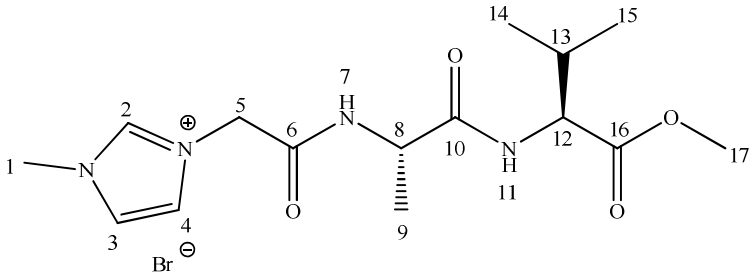
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.69 (s, 1H, *H2*), 8.94 (d, *J* = 7.0 Hz, 1H, *H7*), 7.57 (d, *J* = 8.0 Hz, 1H, *H11*), 7.46 (d, *J* = 1.4 Hz, 1H, *H4*), 7.27-7.17 (m, 6H, *H3,15-19*), 5.66 (d, *J* = 14.8 Hz, 1H, *H5*), 4.89 (d, *J* = 14.4 Hz, 1H, *H5*), 4.73 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H12*), 4.62 (d, *J* = 14.4 Hz, 1H, *H5*), 4.24 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 4.11 (q, *J* = 6.8 Hz, 2H, *H21*), 3.92 (s, 3H, *H1*), 3.15 (dd, *J* = 14.0, 6.0 Hz, 1H, *H13*), 3.05 (dd, *J* = 13.8, 6.4 Hz, 1H, *H13*), 1.24 (d, *J* = 7.2 Hz, 3H, *H9*), 1.12 (t, *J* = 7.0 Hz, 3H, *H22*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.50 (*C*O,*C20*), 171.93 (*C*O,*C10*), 165.05 (*C*O,*C6*), 137.62 (N*C*HN,*C2*), 136.57 (Ar*C*,*C14*), 129.53 (Ar*C*H), 128.38 (Ar*C*H), 126.81 (Ar*C*H,*C17*), 123.98 (N*C*H,*C4*), 122.83 (N*C*H,*C3*), 61.37 (O*C*H_2,*C21*), 53.58 (*C*H,*C12*), 51.58 (N*C*H_2,*C5*), 50.79 (*C*H,*C8*), 37.57 (*C*H_2,*C13*), 36.75 (N*C*H_3,*C1*), 17.60 (*C*H_3,*C9*), 14.08 (*C*H_3,*C22*)

IR (neat) (cm^{-1}) 3423 (w), 3301 (w), 2983 (w), 1751 (s), 1679 (vs), 1650 (vs), 1532 (vs), 1212 (m), 1175 (vs), 749 (m), 699 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 387.2018, $C_{20}H_{27}N_4O_4^+$ requires 387.2026

3-Methyl-1-L-alanine-L-valine methyl ester imidazolium bromide (365)



The title compound (**365**) was prepared from 1-methylimidazole (0.261 g, 3.35 mmol) and L-alanine-L-valine methyl ester bromoacetate (**347**) (1.290 g, 4.00 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow viscous liquid in 97 % yield (1.320 g, 3.26 mmol).

$[\alpha]_D^{20}$ = -23.5 ^{o} (0.7 c, $CHCl_3$)

Molecular formula $C_{15}H_{25}BrN_4O_4$

Molecular weight 405 $gmol^{-1}$

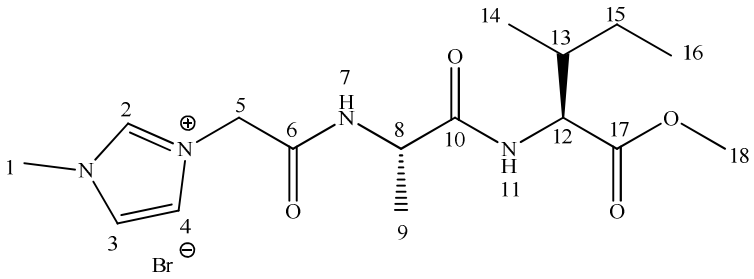
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.65 (s, 1H, *H2*), 9.24 (d, *J* = 7.6 Hz, 1H, *H7*), 7.59 (t, *J* = 1.8 Hz, 1H, *H4*), 7.25-7.20 (m, 2H, *H3,11*), 5.58 (d, *J* = 15.2 Hz, 1H, *H5*), 5.01 (d, *J* = 15.2 Hz, 1H, *H5*), 4.37 (dd, *J* = 8.8, 4.8 Hz, 1H, *H12*), 4.32 (dq, *J* = 7.2, 7.2 Hz, *H8*), 3.97 (s, 3H, *H1*), 3.10 (s, 3H, *H17*), 2.17 (qqd, *J* = 7.0, 7.0, 4.8 Hz, 1H, *H13*), 0.94 (d, *J* = 6.8 Hz, 3H, *H9*), 0.89 (d, *J* = 7.2 Hz, 3H, *H14/15*), 0.85 (d, *J* = 7.2 Hz, 3H, *H14/15*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.97 (*C*O,*C16*), 172.66 (*C*O,*C10*), 164.83 (*C*O,*C6*), 137.84 (N*C*HN,*C2*), 124.06 (N*C*H,*C4*), 122.47 (N*C*H,*C3*), 57.28 (*C*H,*C12*), 52.01 (N*C*H_2,*C5*), 51.73 (*C*H,*C8*), 51.28 (O*C*H_3,*C17*), 36.74 (N*C*H_3,*C1*), 31.06 (*C*H,*C13*), 19.09 (*C*H_3,*C14/C15*), 18.33 (*C*H_3,*C14/C15*), 17.69 (*C*H_3,*C9*)

IR (neat) (cm^{-1}) 3220 (b), 3053 (w), 2966 (w), 1737 (m), 1661 (vs), 1534 (s), 1206 (s), 1173 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 325.1861, $C_{15}H_{25}N_4O_4^+$ requires 325.1870

3-Methyl-1-L-alanine-L-isoleucine methyl ester imidazolium bromide (366)



The title compound (**366**) was prepared from 1-methylimidazole (0.141 g, 1.70 mmol) and L-alanine-L-isoleucine methyl ester bromoacetate (**348**) (0.692 g, 2.05 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow viscous liquid in 98 % yield (0.701 g, 1.67 mmol).

$[\alpha]_D^{20} = -19.0^{\circ}$ (0.6 c, $CHCl_3$)

Molecular formula $C_{16}H_{27}BrN_4O_4$

Molecular weight 419 $gmol^{-1}$

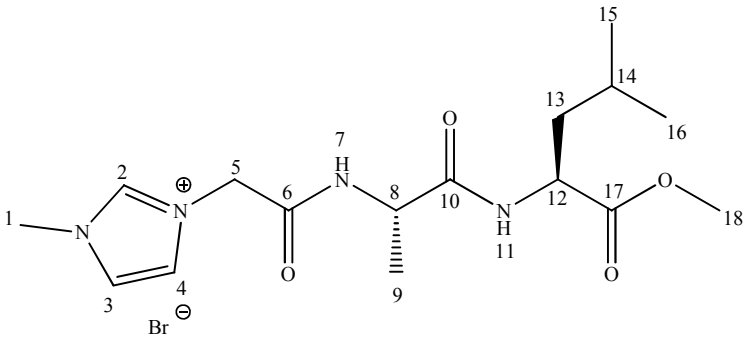
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.07 (s, 1H, *H2*), 8.69 (d, *J* = 7.2 Hz, 1H, *H7*), 8.33 (d, *J* = 8.0 Hz, 1H, *H11*), 7.69 (t, *J* = 1.8 Hz, 1H, *H4*), 7.66 (t, *J* = 1.8 Hz, 1H, *H3*), 5.00 (s, 2H, *H5*), 4.48 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 4.20 (dd, *J* = 8.0, 6.0 Hz, 1H, *H12*), 3.88 (s, 3H, *H1*), 3.63 (s, 3H, *H18*), 1.84 (dddq, *J* = 8.0, 8.0, 6.8, 6.0 Hz, 1H, *H13*), 1.41 (ddq, *J* = 8.8, 8.0, 7.2 Hz, 1H, *H15*), 1.23 (d, *J* = 7.2 Hz, *H9*), 1.21 (dd, *J* = 8.0, 8.0, 7.2 Hz, 1H), 0.86 (t, *J* = 7.0 Hz, 3H, *H16*), 0.82 (d, *J* = 7.2 Hz, 3H, *H14*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.83 (*C*O,*C17*), 172.62 (*C*O,*C10*), 164.95 (*C*O,*C6*), 137.78 (N*C*HN,*C2*), 124.04 (N*C*H,*C4*), 122.70 (N*C*H,*C3*), 56.61 (*C*H, *C12*), 51.99 (N*C*H_2,*C5*), 51.60 (*C*H,*C8*), 51.07 (O*C*H_3,*C18*), 37.35 (*C*H,*C13*), 36.74 (N*C*H_3,*C1*), 25.34 (*C*H_2,*C15*), 17.67 (*C*H_3,*C9*), 15.60 (*C*H_3,*C14*), 11.45 (*C*H_3,*C16*)

IR (neat) (cm^{-1}) 3064 (w), 3040 (w), 2965 (w), 1732 (m), 1659 (vs), 1537 (s), 1175 (s), 1205 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 339.2033, $C_{16}H_{27}N_4O_4^+$ requires 339.2026

3-Methyl-1-L-alanine-L-leucine methyl ester imidazolium bromide (367)



The title compound (**367**) was prepared from 1-methylimidazole (0.411 g, 5.05 mmol) and L-alanine-L-leucine methyl ester bromoacetate (**350**) (2.042 g, 6.10 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow viscous liquid in 75 % yield (1.590 g, 3.79 mmol).

$[\alpha]_D^{20}$ = -25.8 ^{o} (0.7 c, $CHCl_3$)

Molecular formula $C_{16}H_{27}BrN_4O_4$

Molecular weight 419 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.54 (s, 1H, *H2*), 8.92 (d, *J* = 7.8 Hz, 1H, *H7*), 7.61 (d, *J* = 8.0 Hz, 1H, *H11*), 7.34 (t, *J* = 1.8 Hz, 1H, *H4*), 7.29 (t, *J* = 1.8 Hz, 1H, *H3*), 5.51 (d, *J* = 15.0 Hz, 1H, *H5*), 5.09 (d, *J* = 15.0 Hz, 1H, *H5*), 4.46 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H12*), 4.33 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 3.96 (s, 3H, *H1*), 3.58 (s, 3H, *H18*), 1.72-1.50 (m,

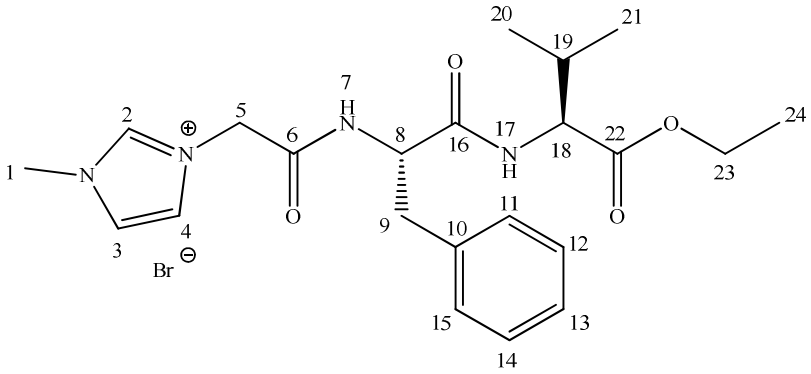
3H, *H13,14*), 1.42 (d, *J* = 7.2 Hz, 3H, *H9*), 0.87 (d, *J* = 6.6 Hz, 3H, *H15/16*), 0.83 (d, *J* = 6.6 Hz, 3H, *H15/16*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.95 (*C*O,*C17*), 172.79 (*C*O,*C10*), 164.91 (*C*O,*C6*), 137.86 (N*C*HN,*C2*), 124.09 (N*C*H,*C4*), 122.63 (N*C*H,*C3*), 65.86 (*C*H,*C8*), 52.17 (*C*H,*C12*), 51.76 (N*C*H_2,*C5*), 51.14 (O*C*H_3,*C18*), 40.54 (*C*H_2,*C13*), 36.77 (N*C*H_3,*C1*), 24.90 (*C*H,*C14*), 22.90 (*C*H_3,*C15/C16*), 21.58 (*C*H_3,*C15/C16*), 17.84 (*C*H_3,*C9*)

IR (neat) (cm^{-1}) 3236 (b), 3056 (w), 2957 (m), 1738 (m), 1660 (s), 1534 (s), 1206 (m), 1172 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 339.2021, $C_{16}H_{27}N_4O_4^+$ requires 339.2026

3-Methyl-1-L-phenylalanine-L-valine ethyl ester imidazolium bromide (368)



The title compound (**368**) was prepared from 1-methylimidazole (0.266 g, 3.25 mmol) and L-phenylalanine-L-valine ethyl ester bromoacetate (**358**) (1.592 g, 3.87 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow hygroscopic semi-solid in 96 % yield (1.544 g, 3.12 mmol).

$[\alpha]_D^{20}$ = -22.0 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{22}H_{31}BrN_4O_4$

Molecular weight 495 $gmol^{-1}$

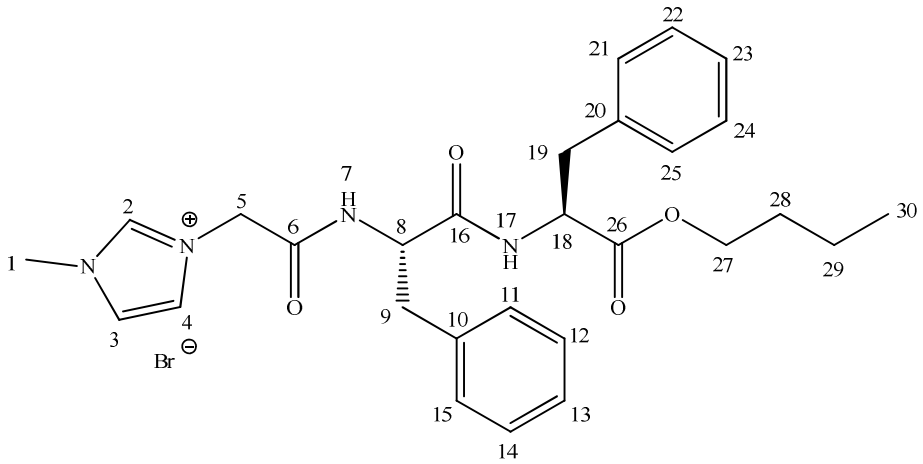
^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.01 (s, 1H, *H2*), 8.75 (d, *J* = 8.0 Hz, 1H, *H7*), 8.49 (d, *J* = 8.4 Hz, 1H, *H17*), 7.66 (t, *J* = 1.6 Hz, 1H, *H4*), 7.54 (t, *J* = 1.6 Hz, 1H, *H3*), 7.30-7.20 (m, 5H, *H11-15*), 4.93 (d, *J* = 14.4 Hz, 2H, *H5*), 4.74 (dd, *J* = 8.4, 4.8 Hz, 1H, *H18*), 4.17-4.07 (m, 3H, *H8,23*), 3.86 (s, 3H, *H1*), 3.04 (dd, *J* = 13.6, 4.0 Hz, 1H, *H9*), 2.78 (dd, *J* = 14.0, 7.6 Hz, 1H, *H9*), 2.05 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H19*), 1.19 (t, *J* = 7.2 Hz, 3H, *H24*), 0.89 (dd, *J* = 8.4, 6.8 Hz, 6H, *H20,21*).

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.17 (*C*O,*C22*), 171.48 (*C*O,*C16*), 165.03 (*C*O,*C6*), 137.56 (N*C*H$_2$,*C2*), 137.12 (Ar*C*,*C10*), 129.47 (Ar*C*H), 128.46 (Ar*C*H), 126.74 (Ar*C*H,*C13*), 123.78 (N*C*H,*C4*), 122.69 (N*C*H,*C3*), 61.10 (O*C*H$_2$,*C23*), 57.66 (*C*H,*C18*), 56.99 (*C*H,*C8*), 51.57 (N*C*H$_2$,*C5*), 37.81 (*C*H$_2$,*C9*), 36.70 (N*C*H$_3$,*C1*), 30.94 (*C*H,*C19*), 19.09 (*C*H$_3$,*C20/C21*) 18.47 (*C*H$_3$,*C20/C21*), 14.19 (*C*H$_3$,*C24*)

IR (neat) (cm^{-1}) 3210 (b), 3044 (w), 2965 (w), 1730 (m), 1658 (s), 1534 (m), 1172 (s), 1206 (m), 744 (m), 701 (m)

MS (*m/z*) Found [M-Br$^-$]$^+$ 415.2236, $C_{22}H_{31}N_4O_4^+$ requires 415.2239

3-Methyl-1-L-phenylalanine-L-phenylalanine butyl ester imidazolium bromide (369)



The title compound (**369**) was prepared from 1-methylimidazole (0.167 g, 2.05 mmol) and L-phenylalanine-L-phenylalanine butyl ester bromoacetate (**359**) (1.192 g, 2.45 mmol) according to the general procedure (Section 7.4.2, page 360) as a white hygroscopic semi-solid in 91 % yield (1.060 g, 1.86 mmol).

$[\alpha]_D^{20}$ = -31.4 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{28}H_{35}BrN_4O_4$

Molecular weight 571 $gmol^{-1}$

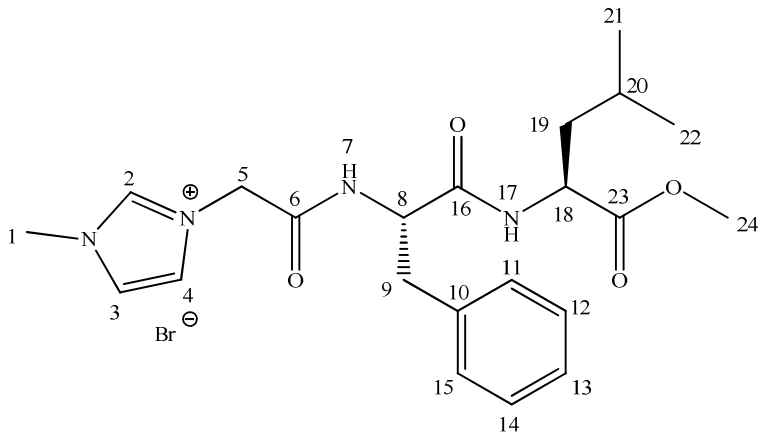
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.75 (s, 1H, *H2*), 9.48 (d, *J* = 8.0 Hz, 1H, *H7*), 8.12 (d, *J* = 8.8 Hz, 1H, *H17*), 7.34-7.10 (m, 12H, *H4,11-15,21-25*), 7.02 (s, 1H, *H3*), 5.56 (d, *J* = 14.4 Hz, 2H, *H5*), 4.76 (ddd, *J* = 8.0, 6.6, 6.6 Hz, 1H, *H8*), 4.56 (d, *J* = 14.0 Hz, 1H, *H5*), 4.40 (ddd, *J* = 8.4, 6.0, 6.0 Hz, 1H, *H18*), 3.94 (dq, *J* = 7.2, 7.2 Hz, 1H, *H27*), 3.93 (dq, *J* = 7.2, 7.2 Hz, 1H, *H27*), 3.90 (s, 3H, *H1*), 3.04-2.66 (m, 4H, *H9,19*), 1.52 (tt, *J* = 7.2, 6.9 Hz, 2H, *H28*), 1.24 (tq, *J* = 7.4, 6.8 Hz, 2H, *H29*), 0.84 (t, *J* = 7.4 Hz, 3H, *H30*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.55 (*C*O,*C26*), 171.07 (*C*O,*C16*), 164.77 (*C*O,*C6*), 137.97 (N*C*HN,*C2*), 137.18 (Ar*C*,*C10/C20*), 136.73 (Ar*C*,*C10/C20*), 129.76 (Ar*C*H), 129.29 (Ar*C*H), 128.40 (Ar*C*H), 127.81 (Ar*C*H), 126.80 (Ar*C*H,*C13/C23*), 126.60 (Ar*C*H,*C13/C23*), 123.86 (N*C*H,*C4*), 122.19 (N*C*H,*C3*), 65.06 (O*C*H_2,*C27*), 57.20 (*C*H,*C8*), 53.39 (*C*H,*C18*), 51.97 (N*C*H_2,*C5*), 38.09 (*C*H_2,*C9/C19*), 37.76 (*C*H_2,*C9/C19*), 36.60 (N*C*H_3,*C1*), 30.43 (*C*H_2,*C28*), 19.11 (*C*H_2,*C29*), 13.73 (*C*H_3,*C30*)

IR (neat) (cm^{-1}) 3202 (b), 3030 (w), 2959 (w), 1732 (m), 1661 (s), 1547 (m), 1497 (m), 1454 (m), 1175 (s), 743 (m), 699 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 491.2639, $C_{28}H_{35}N_4O_4^+$ requires 491.2652

3-Methyl-1-L-phenylalanine-L-leucine methyl ester imidazolium bromide (370)



The title compound (**370**) was prepared from 1-methylimidazole (0.242 g, 2.95 mmol) and L-phenylalanine-L-leucine methyl ester bromoacetate (**356**) (1.451 g, 3.55 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow hygroscopic semi-solid in 75 % yield (1.091 g, 2.20 mmol).

$[\alpha]_D^{20} = -44.9^{\circ}$ (0.8 c, $CHCl_3$)

Molecular formula $C_{22}H_{31}BrN_4O_4$

Molecular weight 495 $gmol^{-1}$

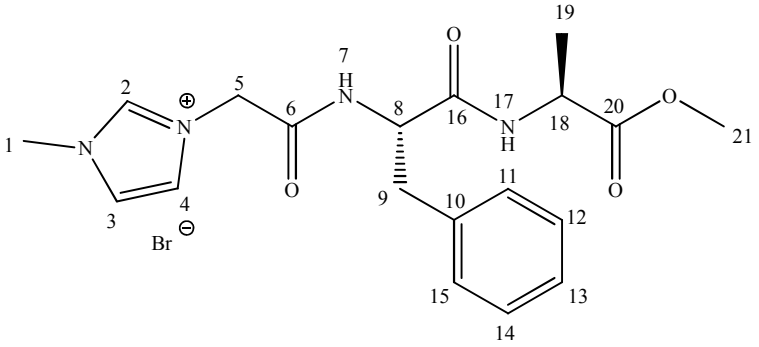
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.38 (s, 1H, *H2*), 9.15 (d, *J* = 8.4 Hz, 1H, *H7*), 7.87 (d, *J* = 8.0 Hz, 1H, *H17*), 7.44 (s, 1H, *H4*), 7.40-7.24 (m, 7H, *H3,11-15*), 5.42 (d, *J* = 15.2 Hz, 1H, *H5*), 4.95 (d, *J* = 15.6 Hz, 1H, *H5*), 4.58 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H8*), 4.47 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H18*), 3.94 (s, 3H, *H1*), 3.61 (s, 3H, *H24*), 3.26 (dd, *J* = 9.0, 5.2 Hz, 1H, *H9*), 3.11 (dd, *J* = 10.4, 6.0 Hz, 1H, *H9*), 1.86-1.58 (m, 3H, *H19,20*), 0.94 (d, *J* = 6.4 Hz, 3H, *H21/22*), 0.88 (d, *J* = 6.4 Hz, 3H, *H21/22*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.87 (*C*O,*C23*), 171.59 (*C*O,*C17*), 165.04 (*C*O,*C6*), 137.59 (N*C*HN,*C2*), 137.00 (Ar*C*,*C10*), 129.45 (Ar*C*H), 128.47 (Ar*C*H), 126.79 (Ar*C*H,*C13*), 123.83 (N*C*H,*C4*), 122.70 (N*C*H,*C3*), 56.97 (*C*H, *C8*), 52.20 (*C*H,*C18*), 51.61 (N*C*H_2,*C5*), 50.91 (O*C*H_3,*C24*), 40.33 (*C*H_2,*C19*), 37.88 (*C*H_2,*C9*), 36.69 (N*C*H_3,*C1*), 24.86 (*C*H,*C20*), 22.93 (*C*H_3,*C21/C22*), 21.51 (*C*H_3,*C21/C22*)

IR (neat) (cm^{-1}) 3196 (w), 3037 (w), 2956 (w), 1738 (m), 1660 (vs), 1537 (s), 1436 (m), 1202 (s), 1171 (vs), 745 (m), 700 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 415.2339, $C_{22}H_{31}N_4O_4^+$ requires 415.2339

3-Methyl-1-L-phenylalanine-L-alanine methyl ester imidazolium bromide (371)



The title compound (**371**) was prepared from 1-methylimidazole (0.272 g, 3.31 mmol) and L-phenylalanine-L-alanine methyl ester bromoacetate (**357**) (1.594 g, 4.31 mmol) according to the general procedure (Section 7.4.2, page 360) as a white hygroscopic semi-solid in 97 % yield (1.455 g, 3.21 mmol).

$[\alpha]_D^{20} =$ -22.7 ^{o} (0.8 c, $CHCl_3$)

Molecular formula $C_{19}H_{25}BrN_4O_4$

Molecular weight 453 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.36 (s, 1H, *H2*), 8.96 (d, *J* = 8.4 Hz, 1H, *H7*), 7.77 (d, *J* = 7.2 Hz, 1H, *H17*), 7.34 (t, *J* = 1.6 Hz, 1H, *H4*), 7.32-7.12 (m, 6H, *H3,11-15*), 5.35 (d, *J* = 10.8 Hz, 1H, *H5*), 4.88 (d, *J* = 10.6 Hz, 1H, *H5*), 4.59 (ddd, *J* = 8.0, 5.6, 5.6 Hz, 1H, *H8*), 4.42 (dq, *J* = 7.2, 7.2 Hz, 1H, *H18*), 3.89 (s, 3H, *H1*), 3.57 (s, 3H, *H21*), 3.18 (dd, *J* = 13.8, 4.8 Hz, 1H, *H9*), 3.02 (dd, *J* = 14.4, 10.2 Hz, 1H, *H9*), 1.38 (d, *J* = 7.2 Hz, 3H, *H19*)

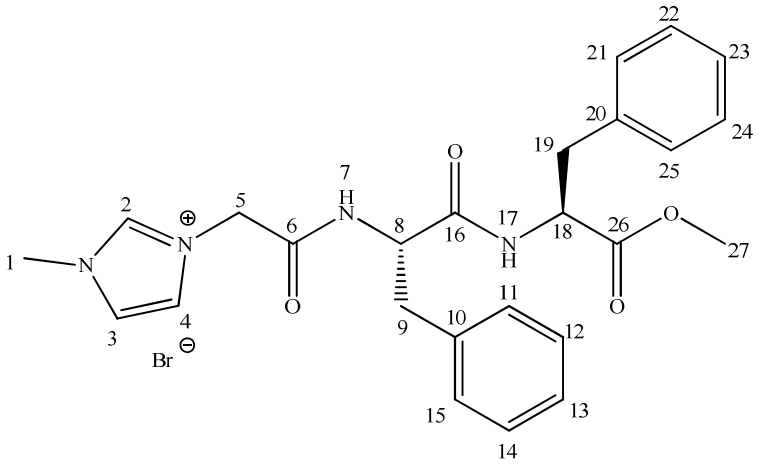
^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.75 (*C*O,*C20*), 171.75 (*C*O,*C16*), 164.85 (*C*O,*C6*), 137.11 (Ar*C*,*C10*), 137.08 (N*C*HN,*C2*), 129.46 (Ar*C*H), 128.48 (Ar*C*H), 126.74

(Ar*C*H,*C13*), 123.86 (N*C*H,*C4*), 122.36 (N*C*H,*C3*), 56.69 (*C*H,*C8*), 52.57 (O*C*H_3,*C21*), 51.79 (N*C*H_2,*C5*), 48.13 (*C*H,*C18*), 37.93 (*C*H_2,*C9*), 36.70 (N*C*H_3,*C1*), 17.79 (*C*H_3,*C19*)

IR (neat) (cm^{-1}) 3198 (b), 3046 (w), 2952 (w), 1737 (m), 1659 (vs), 1537 (s), 1210 (s), 1169 (s), 726 (m), 701 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 373.1862, $C_{19}H_{25}N_4O_4^+$ requires 373.1870

3-Methyl-1-L-phenylalanine-L-phenylalanine methyl ester imidazolium bromide (372)



The title compound (**372**) was prepared from 1-methylimidazole (0.211 g, 2.72 mmol) and L-phenylalanine-L-phenylalanine methyl ester bromoacetate (**360**) (1.461 g, 3.27 mmol) according to the general procedure (Section 7.4.2, page 360) as a white solid in 83 % yield (1.201 g, 2.27 mmol).

m.p. 97-99 °C, $[\alpha]_D^{20}$ = -33.6 ^{o} (0.5 c, $CHCl_3$)

Molecular formula $C_{25}H_{29}BrN_4O_4$

Molecular weight 529 $gmol^{-1}$

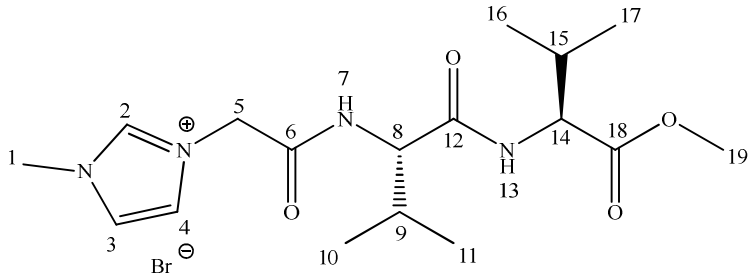
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.72 (s, 1H, *H2*), 9.18 (d, *J* = 8.4 Hz, 1H, *H7*) 7.96 (d, *J* = 8.8 Hz, 1H, *H17*), 7.44-7.14 (m, 12H, *H3,4,11-15,21-25*), 5.68 (d, *J* = 14.4 Hz, 1H, *H5*), 4.85 (ddd, *J* = 8.0, 5.6, 5.6 Hz, 1H, *H8*), 4.68 (d, *J* = 14.4 Hz, 1H, *H5*), 4.50 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H18*), 3.99 (s, 3H, *H1*), 3.65 (s, 3H, *H27*), 3.26-2.85 (m, 4H, *H9/19*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.61 (*C*O,*C26*), 171.12 (*C*O,*C16*), 164.95 (*C*O,*C6*), 137.70 (N*C*HN,*C2*), 137.04 (Ar*C*,*C10/C20*), 136.65 (Ar*C*,*C10/C20*), 129.65 (Ar*C*H), 129.33 (Ar*C*H), 128.53 (Ar*C*H), 128.43 (Ar*C*H), 126.86 (Ar*C*H,*C13/C23*), 126.68 (Ar*C*H,*C13/C23*), 123.88 (N*C*H,*C4*), 122.47 (N*C*H,C3), 67.97 (*C*H,*C8*), 57.14 (*C*H,*C18*), 53.57 (N*C*H_2,*C5*), 52.19 (O*C*H_3,*C27*), 37.68 (N*C*H_3,*C1*), 37.60 (*C*H_2,*C9/C19*), 36.64 (*C*H_2,*C9/C19*)

IR (neat) (cm^{-1}) 3205 (w), 3031 (w), 2952 (w), 1737 (m), 1661 (s), 1533 (s), 1215 (m), 1172 (s), 744 (m), 700 (vs)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 449.2177, $C_{25}H_{29}N_4O_4^+$ requires 449.2183

3-Methyl-1-L-valine-L-valine methyl ester imidazolium bromide (373)



The title compound (**373**) was prepared from 1-methylimidazole (0.203 g, 2.50 mmol) and L-valine-L-valine methyl ester bromoacetate (**355**) (1.052 g, 3.00 mmol) according to the general procedure (Section 7.4.2, page 360) as a colourless hygroscopic semi-solid in 91 % yield (0.989 g, 2.28 mmol).

$[\alpha]_D^{20}$ = -48.5 ^{o} (0.4 c, $CHCl_3$)

Molecular formula $C_{17}H_{29}BrN_4O_4$

Molecular weight 433 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.60 (s, 1H, *H2*), 9.10 (d, *J* = 8.8 Hz, 1H, *H7*), 7.59 (t, *J* = 1.6 Hz, 1H, *H4*), 7.27 (d, *J* = 8.4 Hz, 1H, *H13*), 7.26 (t, *J* = 1.8 Hz, 1H, *H3*), 5.65 (d, *J* = 15.2 Hz, 1H, *H5*), 5.05 (d, *J* = 15.2 Hz, 1H, *H5*), 4.37 (dd, *J* = 8.8, 4.8 Hz, 1H, *H18*), 4.10 (dd, *J* = 8.0, 4.4 Hz, 1H, *H8*), 3.96 (s, 3H, *H1*), 3.59 (s, 3H, *H19*), 2.29-2.10 (m, 2H,

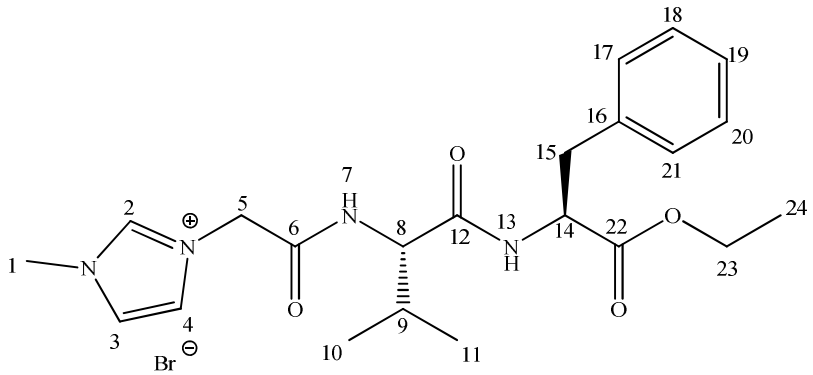
H9,15), 0.99 (dd, *J* = 6.8, 6.8 Hz, 6H, *H10,11/16,17*), 0.93 (dd, *J* = 6.8, 6.4 Hz, 6H, *H10,11/16,17*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.07 (*C*O,*C18*), 172.62 (*C*O,*C12*), 165.29 (*C*O,*C6*), 137.80 (N*C*HN,*C2*), 124.02 (N*C*H,*C4*), 122.54 (N*C*H,*C3*), 61.47 (*C*H,*C8*), 57.60 (*C*H,*C14*), 51.98 (N*C*H_2,*C5*), 51.82 (O*C*H_3,*C19*), 36.73 (N*C*H_3,*C1*), 30.77 (*C*H,*C9/C15*), 30.27 (*C*H,*C9/C15*), 19.43, 19.2, 19.13, 18.64 (*C*H_3,*C10/11/16/17*)

IR (neat) (cm^{-1}) 3225 (b), 3053 (w), 2964 (w), 1737 (m), 1657(vs), 1532 (s), 1206 (m), 1172 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 353.2181, $C_{17}H_{29}N_4O_4^+$ requires 353.2183

3-Methyl-1-L-valine-L-phenylalanine ethyl ester imidazolium bromide (374)



The title compound (**374**) was prepared from 1-methylimidazole (0.245 g, 3.00 mmol) and L-valine-L-phenylalanine ethyl ester bromoacetate (**351**) (1.481 g, 3.60 mmol) according to the general procedure (Section 7.4.2, page 360) as a pale yellow hygroscopic semi-solid in 98 % yield (1.450 g, 2.93 mmol).

$[\alpha]_D^{20} =$ -20.7 ^{o} (0.9 c, $CHCl_3$)

Molecular formula $C_{22}H_{31}BrN_4O_4$

Molecular weight 495 $gmol^{-1}$

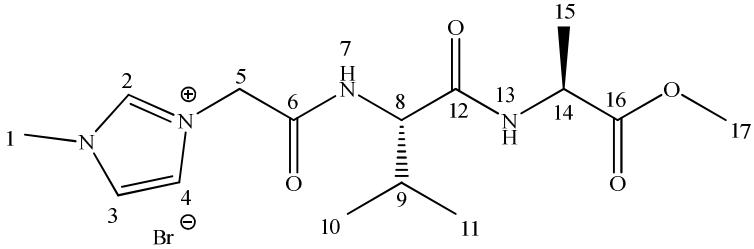
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.68 (s, 1H, *H2*), 8.88 (d, *J* = 6.8 Hz, 1H, *H13*), 7.76 (d, *J* = 8.8 Hz, 1H, *H7*), 7.49 (t, *J* = 1.6 Hz, 1H, *H4*), 7.39-7.23 (m, 6H, *H3,17-21*), 5.78 (d, *J* = 14.8 Hz, 1H, *H5*), 4.89 (d, *J* = 14.8 Hz, 1H, *H5*), 4.75 (ddd, *J* = 8.0, 6.4, 6.4 Hz, 1H, *H14*), 4.06-3.94 (m, 3H, *H8,23*), 3.92 (s, 3H, *H1*), 3.16-3.06 (m, 2H, *H15*), 2.16 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H9*), 1.13 (t, *J* = 7.2 Hz, 3H, *H24*), 0.82 (d, *J* = 6.8 Hz, 3H, *H10/11*), 0.58 (d, *J* = 6.8 Hz, 3H, *H10/11*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.13 (*C*O,*C22*), 171.26 (*C*O,*C12*), 165.37 (*C*O,*C6*), 137.68 (N*C*HN,*C2*), 136.70 (Ar*C*,*C16*), 129.43 (Ar*C*H), 128.56 (Ar*C*H), 126.77 (Ar*C*H,*C19*), 123.95 (N*C*H,*C4*), 122.80 (N*C*H,*C3*), 65.82 (O*C*H_2,*C23*), 61.29 (*C*H,*C8*), 53.67 (*C*H,*C14*), 51.72 (N*C*H_2,*C5*), 37.51 (*C*H_2,*C15*), 36.69 (N*C*H_3,*C1*), 30.31 (*C*H,*C9*), 19.06 (*C*H_3,*C10/C11*), 18.95 (*C*H_3,*C10/C11*), 15.25 (*C*H_3,*C24*)

IR (neat) (cm^{-1}) 3055 (w), 2963 (w), 1731 (m), 1659 (vs), 1535 (s), 1173 (vs), 745 (m), 700 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 415.2339, $C_{17}H_{29}N_4O_4^+$ requires 415.2339

3-Methyl-1-L-valine-L-alanine methyl ester imidazolium bromide (375)



The title compound (**375**) was prepared from 1-methylimidazole (0.152 g, 1.84 mmol) and L-valine-L-alanine methyl ester bromoacetate (**352**) (0.651 g, 2.21 mmol) according to the general procedure (Section 7.4.2, page 360) as a white solid in 70 % yield (0.522 g, 1.29 mmol).

m.p. 80-82 °C $[\alpha]_D^{20}$ = -42.5 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{15}H_{25}BrN_4O_4$

Molecular weight 405 $gmol^{-1}$

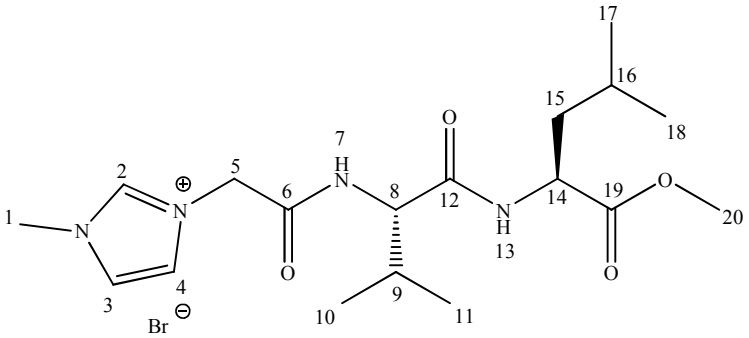
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.87 (s, 1H, *H2*), 9.07 (d, *J* = 9.2 Hz, 1H, *H7*), 7.88 (d, *J* = 8.0 Hz, 1H, *H13*), 7.58 (s, 1H, *H4*), 7.22 (s, 1H, *H3*), 5.98 (d, *J* = 14.4 Hz, 1H, *H5*), 4.86 (d, *J* = 14.8 Hz, 1H, *H5*), 4.71 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.61 (dd, *J* = 9.2, 6.2 Hz, 1H, *H8*), 4.05 (s, 3H, *H1*), 3.68 (s, 3H, *H17*), 2.20 (qqd, *J* = 6.8, 6.8, 6.0 Hz, 1H, *H9*), 1.51 (d, *J* = 7.6 Hz, 3H, *H15*), 1.07 (d, *J* = 6.8 Hz, 3H, *H10/11*), 1.02 (d, *J* = 6.8 Hz, 3H, *H10/11*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.81 (*C*O,*C16*), 170.39 (*C*O,*C12*), 164.83 (*C*O,*C6*), 137.63 (N*C*HN,*C2*), 123.63 (N*C*H,*C4*), 122.95 (N*C*H,*C3*), 57.56 (*C*H,*C8*), 51.78 (*C*H,*C14*), 50.43 (N*C*H_2,*C5*), 47.55 (O*C*H_3,*C19*), 35.80 (N*C*H_3,*C1*), 31.09 (*C*H,*C9*), 18.95 (*C*H_3,*C10/C11*), 17.91 (*C*H_3,*C10/C11*), 16.66 (*C*H_3,*C15*)

IR (neat) (cm^{-1}) 3281 (w), 2964 (w), 1731 (m), 1667 (m), 1637 (vs), 1553 (s), 1230 (m), 1171 (s), 1056 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 325.1868, $C_{15}H_{25}N_4O_4^+$ requires 325.1870

3-Methyl-1-L-valine-L-leucine methyl ester imidazolium bromide (376)



The title compound (**376**) was prepared from 1-methylimidazole (0.310 g, 3.77 mmol) and L-valine-L-leucine methyl ester bromoacetate (**354**) (1.632 g, 4.53 mmol) according to the general procedure (Section 7.4.2, page 360) as a colourless liquid in 95 % yield (1.602 g, 3.58 mmol).

$[\alpha]_D^{20}$ = -45.2 ^{o} (0.7 c, $CHCl_3$)

Molecular formula $C_{18}H_{31}BrN_4O_4$

Molecular weight 447 $gmol^{-1}$

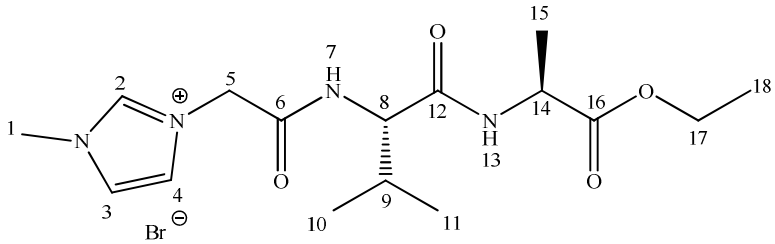
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.64 (s, 1H, *H2*), 8.54 (d, *J* = 9.0 Hz, 1H, *H7*), 8.52 (d, *J* = 8.4 Hz, 1H, *H13*), 7.55 (t, *J* = 1.6 Hz, 1H, *H4*), 7.23 (t, *J* = 1.8 Hz, 1H, *H3*), 5.73 (d, *J* = 14.4 Hz, 1H, *H5*), 4.89 (d, *J* = 14.6 Hz, 1H, *H5*), 4.50 (ddd, *J* = 8.0, 8.0, 5.6 Hz, 1H, *H14*), 4.10 (dd, *J* = 9.0, 7.2 Hz, 1H, *H8*), 3.95 (s, 3H, *H1*), 3.56 (s, 3H, *H20*), 2.18 (qqd, *J* = 6.8, 6.8, 6.8 Hz, 1H, *H9*), 1.84-1.74 (m, 1H, *H15*), 1.74-1.67 (m, 1H, *H16*), 1.54-1.48 (m, 1H, *H15*), 0.97 (d, *J* = 7.2 Hz, 3H, *H10/11*), 0.92 (d, *J* = 6.6 Hz, 3H, *H10/11*), 0.88 (d, *J* = 6.6 Hz, 3H, *H17/18*), 0.84 (d, *J* = 6.6 Hz, 3H, *H17/18*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 174.31 (*C*O,*C19*), 171.40 (*C*O,*C12*), 165.05 (*C*O,*C6*), 137.90 (N*C*HN,*C2*), 124.05 (N*C*H,*C4*), 122.40 (N*C*H,*C3*), 61.62 (*C*H,*C8*), 52.05 (*C*H,*C14*), 52.03 (N*C*H_2,*C5*), 50.64 (O*C*H_3,*C20*), 40.43 (*C*H_2,*C15*), 36.70 (N*C*H_3,*C1*), 30.40 (*C*H,*C9*), 24.89 (*C*H,*C16*), 23.01 (*C*H_3,*C17/18*), 21.30 (*C*H_3,*C17/18*), 19.40 (*C*H_3,*C10/11*), 19.10 (*C*H_3,*C10/11*)

IR (neat) (cm^{-1}) 3220 (b), 3049 (w), 2959 (w), 1739 (m), 1656 (vs), 1533 (s), 1205 (m), 1171 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 367.2351, $C_{18}H_{31}N_4O_4^+$ requires 367.2339

3-Methyl-1-L-valine-L-alanine ethyl ester imidazolium bromide (377)



The title compound (**377**) was prepared from 1-methylimidazole (0.101 g, 1.32 mmol) and L-valine-L-alanine ethyl ester bromoacetate (**353**) (0.579 g, 1.72 mmol) according to the general procedure (Section 7.4.2, page 360) as a white solid in 95 % yield (0.526 g, 1.25 mmol).

m.p. 130-132 °C $[\alpha]_D^{20}$ = -46.2 ^{o} (0.5 c, $CHCl_3$)

Molecular formula $C_{16}H_{27}BrN_4O_4$

Molecular weight 419 $gmol^{-1}$

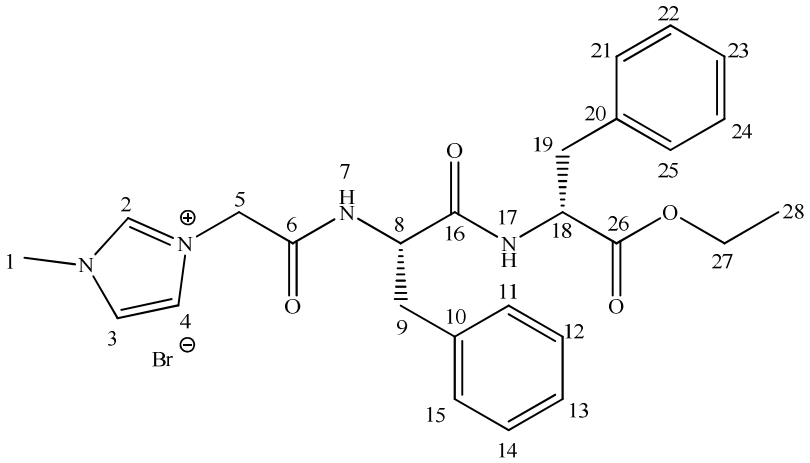
^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 9.16 (s, 1H, *H2*), 8.63 (d, *J* = 9.0 Hz, 1H, *H7*), 8.47 (d, *J* = 6.6 Hz, 1H, *H13*), 7.76 (t, *J* = 1.8 Hz, 1H, *H4*), 7.49 (t, *J* = 1.8 Hz, 1H, *H3*), 5.14 (d, *J* = 16.2 Hz, 2H, *H5*), 4.32 (dd, *J* = 8.4, 6.0 Hz, 1H, *H8*), 4.28 (dq, *J* = 7.2, 7.2 Hz, 1H, *H14*), 4.14 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H17*), 3.95 (s, 3H, *H1*), 2.06 (qqd, *J* = 6.8, 6.8, 5.6 Hz, 1H, *H9*), 1.33 (d, *J* = 7.2 Hz, 3H, *H15*), 1.24 (t, *J* = 7.2 Hz, 3H, *H18*), 0.97 (d, *J* = 6.6 Hz, 3H, *H10/11*), 0.94 (d, *J* = 6.6 Hz, 3H, *H10/11*)

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 172.29 (*C*O,*C16*), 170.29 (*C*O,*C12*), 164.76 (*C*O,*C6*), 137.70 (N*C*HN,*C2*), 123.73 (N*C*H,*C4*), 122.95 (N*C*H,*C3*), 60.41 (O*C*H_2,*C17*), 57.45 (*C*H,*C8*), 50.44 (N*C*H_2,*C5*), 47.62 (*C*H,*C14*), 35.78 (N*C*H_3,*C1*), 31.07 (*C*H,*C9*), 19.01 (*C*H_3,*C10/C11*), 17.99 (*C*H_3,*C10/C11*), 16.72 (*C*H_3,*C15*), 13.98 (*C*H_3,*C18*)

IR (neat) (cm^{-1}) 3280 (m), 3258 (m), 2964 (w), 1727 (m), 1669 (m), 1646 (vs), 1557 (s), 1219 (s), 1174 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 339.2024, $C_{16}H_{27}N_4O_4^+$ requires 339.2026

3-Methyl-1-L-phenylalanine-D-phenylalanine ethyl ester imidazolium bromide (378)



The title compound (**378**) was prepared from 1-methylimidazole (0.170 g, 2.05 mmol) and L-phenylalanine-D-phenylalanine ethyl ester bromoacetate (**361**) (1.151 g, 2.50 mmol) according to the general procedure (Section 7.4.2, page 360) as a white solid in 98 % yield (1.098 g, 2.02 mmol).

m.p. 93-95 °C $[\alpha]_D^{20} =$ -23.7 ^{o} (0.7 c, $CHCl_3$)

Molecular formula $C_{26}H_{31}BrN_4O_4$

Molecular weight 543 $gmol^{-1}$

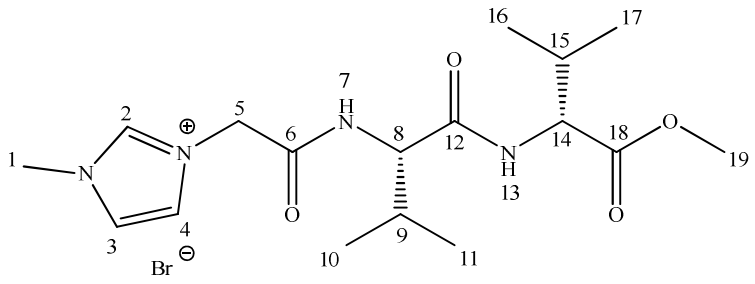
^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm) 8.99 (s, 1H, *H2*), 8.75 (d, *J* = 8.4 Hz, 1H, *H7*), 8.66 (d, *J* = 9.0 Hz, 1H, *H17*), 7.64 (t, *J* = 1.6 Hz, 1H, *H4*), 7.53 (t, *J* = 1.6 Hz, 1H, *H3*), 7.31-7.16 (m, 10H, *H11-15,21-25*), 4.87 (d, *J* = 21.6 Hz, 2H, *H5*), 4.63 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H8*), 4.52 (ddd, *J* = 8.8, 5.6, 5.6 Hz, 1H, *H18*), 4.01 (dq, *J* = 7.2, 7.2 Hz, 1H, *H27*), 4.00 (dq, *J* = 7.2, 7.2 Hz, 1H, *H27*), 3.85 (s, 3H, *H1*), 3.11-2.94 (m, 4H, *H9,19*), 1.11 (t, *J* = 7.2 Hz, 3H, *H28*)

^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm) 171.34 (*C*O,*C26*), 170.40 (*C*O,*C16*), 164.42 (*C*O,*C6*), 137.54 (N*C*HN,*C2*), 137.12 (Ar*C*,*C10/*C*20*), 136.99 (Ar*C*,*C10/C20*), 129.19 (Ar*C*H), 129.17 (Ar*C*H), 128.21 (Ar*C*H), 128.01 (Ar*C*H), 126.61 (Ar*C*H,*C13/C23*), 126.32 (Ar*C*H,*C13/C23*), 123.51 (N*C*H,*C4*), 122.97 (N*C*H,*C3*), 66.99 (O*C*H_2,*C27*), 60.64 (*C*H,*C8*), 53.87 (*C*H,*C18*), 50.44 (N*C*H_2,*C5*), 38.14 (*C*H_2,*C9/C19*), 36.99 (N*C*H_3,*C1*), 35.77 (*C*H_2,*C9/C19*), 13.93 (*C*H_3,*C28*)

IR (neat) (cm^{-1}) 3300 (m), 1736 (s), 1677 (m), 1645 (vs), 1536 (s), 1219 (s), 1181 (s), 746 (m), 695 (s)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 463.2338, $C_{26}H_{31}N_4O_4^+$ requires 463.2339

3-Methyl-1-L-valine-D-valine methyl ester imidazolium bromide (379)



The title compound (**379**) was prepared from 1-methylimidazole (0.290 g, 3.65 mmol) and L-valine-D-valine methyl ester bromoacetate (**362**) (1.531 g, 4.37 mmol) according to the general procedure (Section 7.4.2, page 360) as an off white solid in 93 % yield (1.471 g, 3.40 mmol).

m.p. 82-84 °C $[\alpha]_D^{20}$ = -36.1 ° (1.2 c, $CHCl_3$)

Molecular formula $C_{17}H_{29}BrN_4O_4$

Molecular weight 433 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 9.63 (s, 1H, *H2*), 8.71 (d, *J* = 7.8 Hz, 1H, *H7*), 7.61 (s, 1H, *H4*), 7.25 (s, 1H, *H3*), 7.15 (d, *J* = 8.4 Hz, 1H, *H13*), 5.50 (d, *J* = 15.6 Hz, 1H, *H5*), 5.30 (d, *J* = 15.6 Hz, 1H, *H5*), 4.30 (dd, *J* = 8.4, 6.0 Hz, 1H, *H14*), 4.19 (dd, *J* = 7.8, 7.2 Hz, 1H, *H8*), 3.97 (s, 3H, *H1*), 3.64 (s, 3H, *H19*), 2.11-1.95 (m, 2H, *H9,15*), 0.95 (dd, *J* = 6.6, 6.0 Hz, 6H, *H10,11/16,17*), 0.84 (dd, *J* = 6.3, 6.0 Hz, 6H, *H10,11/16,17*)

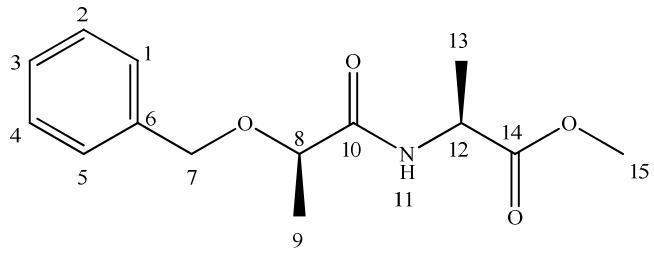
^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.46 (*C*O,*C18*), 171.36 (*C*O,*C12*), 165.32 (*C*O,*C6*), 137.96 (N*C*HN,*C2*), 123.97 (N*C*H,*C4*), 122.51 (N*C*H,*C3*), 60.40 (*C*H,*C8*), 57.92 (*C*H,*C14*), 52.17 (N*C*H_2,*C5*), 51.65 (O*C*H_3,*C19*), 36.82 (N*C*H_3,*C1*), 30.58 (*C*H,*C9/C15*), 30.45 (*C*H,*C9/C15*), 19.43 (*C*H_3), 19.11 (*C*H_3), 18.57 (*C*H_3), 18.44 (*C*H_3, *C*H_3,*C10/C11/ C16/C17*)

IR (neat) (cm^{-1}) 3402 (b), 3278 (w), 2964 (w), 1727 (m), 1670 (m), 1640 (vs), 1553 (s), 1266 (m), 1173 (m), 1022 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 353.2172, $C_{17}H_{29}N_4O_4^+$ requires 353.2183

7.5 Preparation of Chiral Lactate dipeptidyl Ionic liquids- Chapter 4

7.5.1 General procedure for the preparation of starting materials for Lactate peptidyl Chiral Ionic Liquids: **Benzyloxy-*R*-lactate- L-alanine methyl ester (380)**



R-Benzyloxy-Lactic acid (1.290 g, 7.16 mmol) was dissolved in DCM (20 mL) with *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.372 g, 7.16 mmol), 1-hydroxybenzotriazole (0.966 g, 7.16 mmol), and triethylamine (0.724 g, 7.16 mmol). The reaction mixture was then cooled to 0 °C. L-alanine methyl ester hydrochloride (**227**) (1.020 g, 7.16 mmol) was added. After 30 mins the reaction was raised to room temperature and the reaction was allowed to proceed for 24 h. After this time, the organic phase was washed with distilled water, then dried over $MgSO_4$. The organic solvent was then removed *via* rotary evaporation yielding a white solid. The crude product was purified *via* column chromatography (50:50 ethyl actetate:hexane), and the title compound (**380**) was obtained in 83 % yield (1.570 g, 5.92 mmol).

m.p 40-44 ° C, $[\alpha]_D^{20} =$ +15.0 ° (1.0 c, $CHCl_3$)

Molecular formula $C_{14}H_{19}NO_5$

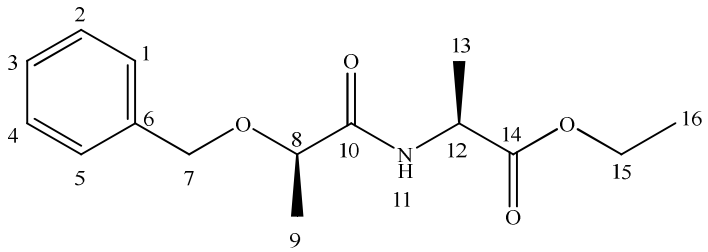
Molecular weight 265 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.41-7.28 (m, 5H, *H1-5*), 7.12 (d, *J* = 7.2, 1H, *H11*), 4.65-4.55 (m, 3H, *H7,12*), 3.99 (q, *J* = 6.8 Hz, 1H, *H8*), 3.77 (s, 3H, *H15*), 1.44 (d, *J* = 6.8 Hz, 3H, *H9*), 1.42 (d, *J* = 7.2 Hz, 3H, *H13*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.08 (*C*O,*C14*), 172.61 (*C*O,*C10*), 137.47 (Ar*C*,*C6*), 128.62 (Ar*C*H), 128.10 (Ar*C*H), 127.83 (Ar*C*H,*C3*), 76.00 (*C*H,*C8*), 71.99

(O*C*H$_2$,*C7*), 52.47 (*C*H,*C12*), 47.39 (O*C*H$_3$,*C15*), 18.50 (*C*H$_3$,*C13*), 18.45 (*C*H$_3$,*C9*). MS (*m/z*) Found [M+H]$^+$ 266.1380, $C_{14}H_{10}NO_4^+$ requires 266.1392

Benzyloxy-*R*-lactate-L-alanine ethyl ester (381)



The title compound (**381**) was prepared from *R*-Benzyloxy lactic acid (2.046 g, 11.35 mmol), L-alanine ethyl ester hydrochloride (**227**) (1.742 g, 11.35 mmol), 1-hydroxybenzotriazole (1.533 g, 11.35 mmol), and triethylamine (1.148 g, 11.35 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.175 g, 11.35 mmol) according to the general procedure (Section 7.5.1, page 379) as a pale yellow solid in 81 % yield (2.574 g, 9.22 mmol).

m.p 39-41 °C, $[\alpha]_D^{20}$ = +18.1 ° (0.9 c, $CHCl_3$)

Molecular formula $C_{15}H_{21}NO_4$

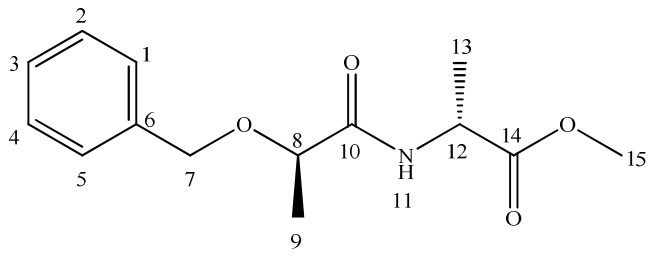
Molecular weight 279 gmol^{-1}

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.41-7.30 (m, 5H, *H1-5*), 7.15 (d, *J* = 7.2 Hz, 1H, *H11*), 4.63-4.55 (m, 3H, *H7,12*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H15*), 4.17 (dq, *J* = 7.2, 7.2 Hz, 1H, *H15*), 3.99 (q, *J* = 6.8 Hz, 1H, *H8*), 1.45 (d, *J* = 6.8 Hz, 3H, *H9*), 1.31 (d, *J* = 7.2 Hz, 3H, *H13*), 1.29 (t, *J* = 7.0 Hz, 3H, *H16*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.00 (*C*O,*C10*), 172.62 (*C*O,*C14*), 137.30 (Ar*C*,*C6*), 128.63 (Ar*C*H), 128.09 (Ar*C*H), 127.82 (Ar*C*H,*C3*), 73.74 (*C*H,*C8*), 71.99 (O*C*H$_2$,*C7*), 61.48 (O*C*H$_2$,*C15*), 47.62 (*C*H,*C12*), 18.48 (*C*H$_3$,*C13*), 18.40 (*C*H$_3$,*C9*), 14.12 (*C*H$_3$,*C16*)

MS (*m/z*) Found [M+H]$^+$ 280.1538, $C_{15}H_{22}NO_4^+$ requires 280.1549

Benzyloxy-*R*-lactate- D-alanine methyl ester (382)



The title compound (**382**) was prepared from *R*-Benzyloxy lactic acid (2.581 g, 14.30 mmol), D-alanine methyl ester hydrochloride (**246**) (2.001 g, 14.30 mmol), 1-hydroxybenzotriazole (1.801 g, 14.30 mmol), and triethylamine (1.449 g, 14.30 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.740 g, 14.30 mmol) according to the general procedure (Section 7.5.1, page 379) as a colourless viscous liquid in 68 % yield (2.583 g, 9.75 mmol).

$[\alpha]_D^{20} = +33.4\ ^\circ$ (1.2 c, $CHCl_3$)

Molecular formula $C_{14}H_{29}NO_4$

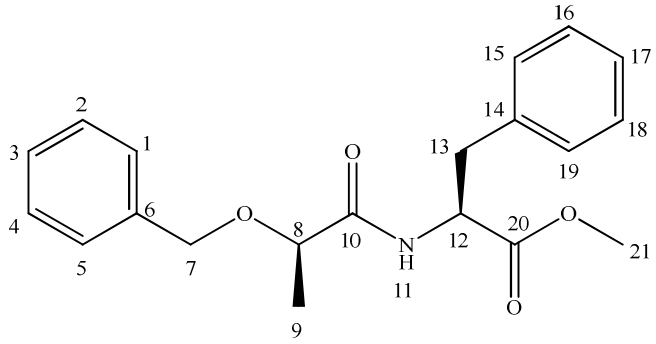
Molecular weight 265 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.44-7.31 (m, 5H, *H1-5*), 7.16 (d, *J* = 7.2 Hz, 1H, *H11*), 4.68-4.59 (m, 3H, *H7,12*), 3.98 (q, *J* = 6.8 Hz, 1H, *H8*), 3.78 (s, 3H, *H15*), 1.43 (d, *J* = 6.8 Hz, 3H, *H9*), 1.41 (d, *J* = 7.2 Hz, 3H, *H13*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.29 (*C*O,*C14*), 173.02 (*C*O,*C10*), 137.23 (Ar*C*,*C6*), 128.62 (Ar*C*H), 128.04 (Ar*C*H), 127.82 (Ar*C*H,*C3*), 76.06 (*C*H,*C8*), 72.21 (O*C*H_2,*C7*), 52.48 (*C*H,*C12*), 47.36 (O*C*H_3,*C15*), 18.78 (*C*H_3,*C13*), 18.43 (*C*H_3,*C9*)

MS (*m/z*) Found $[M+H]^+$ 266.1380, $C_{14}H_{20}NO_4^+$ requires 266.1392

Benzyloxy-*R*-lactate- L-phenylalanine methyl ester (383)



The title compound (**383**) was prepared from *R*-Benzyloxy lactic acid (2.044 g, 11.35 mmol), L-phenylalanine methyl ester hydrochloride (**223**) (2.446 g, 11.35 mmol), 1-hydroxybenzotriazole (1.532 g, 11.35 mmol), and triethylamine (1.147 g, 11.35 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.174 g, 11.35 mmol) according to the general procedure (Section 7.5.1, page 379) as a white solid in 98 % yield (3.819 g, 11.20 mmol).

m.p. 37-39 °C $[\alpha]_D^{20}$ = +24.3 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{20}H_{23}NO_4$

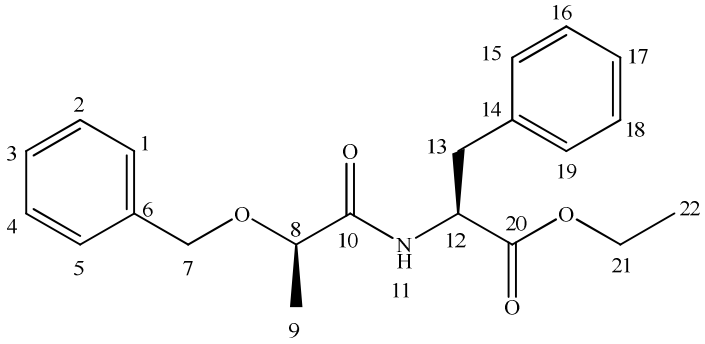
Molecular weight 341 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.38-7.22 (m, 10H, *H1-5,15-19*), 7.01 (d, *J* = 8.0 Hz, 1H, *H11*), 4.87 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H12*), 4.39 (d, *J* = 1.2 Hz, 2H, *H7*), 3.94 (q, *J* = 6.8 Hz, 1H, *H8*), 3.76 (s, 3H, *H21*), 3.25 (dd, *J* = 14.0, 7.2 Hz, 1H, *H13*), 3.12 (dd, *J* = 14.4, 7.2 Hz, 1H, *H13*), 1.42 (d, *J* = 6.8 Hz, 3H, *H9*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.11 (*C*O,*C10*), 171.77 (*C*O,*C20*), 137.21 (Ar*C*,*C6/C14*), 135.87 (Ar*C*,*C6/C14*), 129.27 (Ar*C*H), 128.68 (Ar*C*H), 128.53 (Ar*C*H), 127.99 (Ar*C*H), 127.79 (Ar*C*H,*C3/C17*), 127.66 (Ar*C*H,*C3/C17*), 72.12 (*C*H,*C8*), 71.75 (O*C*H_2,*C7*), 53.46 (*C*H,*C12*), 52.52 (O*C*H_3,*C21*), 37.75 (*C*H_2,*C13*), 18.80 (*C*H_3,*C9*)

MS (*m/z*) Found $[M+H]^+$ 342.1707, $C_{20}H_{24}NO_4^+$ requires 342.1705

Benzyloxy-*R*-lactate- L-phenylalanine ethyl ester (384)



The title compound (**384**) was prepared from *R*-Benzyloxy lactic acid (1.010 g, 5.55 mmol), L-phenylalanine ethyl ester hydrochloride (**224**) (1.261 g, 5.55 mmol), 1-hydroxybenzotriazole (0.686 g, 5.55 mmol), and triethylamine (0.556 g, 5.55 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.059 g, 5.55 mmol) according to the general procedure (Section 7.5.1, page 379) as a white solid in 61 % yield (1.198 g, 3.37 mmol).

m.p. 33-35 °C $[\alpha]_D^{20} =$ -24.5 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{21}H_{25}NO_4$

Molecular weight 355 $gmol^{-1}$

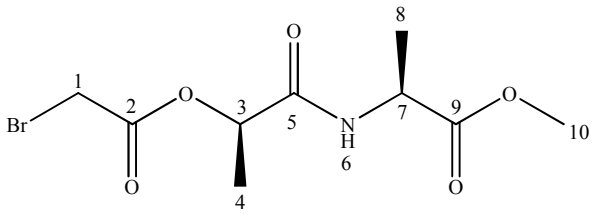
^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.29-7.03 (m, 10H, *H1-5,15-19*), 6.95 (d, *J* = 8.0 Hz, 1H, *H11*), 4.85 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H12*), 4.34 (s, 2H, *H7*), 4.11 (q, *J* = 7.2 Hz, 2H, *H21*), 3.84 (q, *J* = 6.8 Hz, 1H, *H8*), 3.16 (dd, *J* = 14.4, 8.4 Hz, 1H, *H13*), 3.10 (dd, *J* = 13.8, 6.8 Hz, 1H, *H13*), 1.33 (d, *J* = 6.8 Hz, 3H, *H9*), 1.18 (t, *J* = 7.2 Hz, 3H, *H22*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.05 (*C*O,*C10*), 171.29 (*C*O,*C20*), 137.22 (Ar*C*,*C6/C14*), 135.95 (Ar*C*,*C6/C14*), 129.23 (Ar*C*H), 128.62 (Ar*C*H), 127.97 (Ar*C*H), 127.64 (Ar*C*H), 127.14 (Ar*C*H,*C3/C17*), 127.56 (Ar*C*H,*C3/C17*), 75.91 (*C*H,*C8*), 71.75 (O*C*H_2,*C7*), 61.52 (O*C*H_2,*C21*), 52.55 (*C*H,*C12*), 37.78 (*C*H_2,*C13*), 18.52 (*C*H_3,*C9*), 14.12 (*C*H_3,*C22*)

MS (*m/z*) Found $[M+H]^+$ 356.1860, $C_{21}H_{26}NO_4^+$ requires 356.1862

7.5.2 Preparation of Chiral Lactate-peptidyl α-bromoamides

***Representative procedure for the preparation of chiral Lactate-peptidyl α-bromoamides*: *R*-lactate-L-alanine methyl ester bromoacetate (385)**



To a stirred solution of DCM, *R*-lactate-L-alanine methyl ester (0.444 g, 2.53 mmol), and potassium carbonate (0.520 g, 2.53 mmol), bromoacetyl bromide (0.766 g, 3.80 mmol) was added dropwise. The reaction was continued stirring overnight at room temperature. After this time the potassium carbonate was removed by filtration. The organic phase was washed with distilled water (3 x 10 mL), and saturated sodium bicarbonate (3 x 10 mL). The organic phase was then dried over anhydrous magnesium sulfate, filtered and volatiles removed *via* rotary evaporation to give a crude product (0.685 g). The crude product was purified by column chromatography (eluant, ethyl acetate:hexane, 50:50) to give the title compound (**385**) as a colourless liquid in 73 % yield (0.546 g, 1.84 mmol).

$[\alpha]_D^{20} = +35.1\ ^\circ$ (0.8 c, $CHCl_3$)

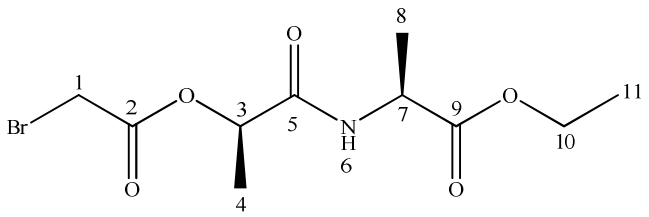
Molecular formula: $C_9H_{14}BrNO_5$

Molecular weight 296 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.74 (d, *J* = 7.4 Hz, 1H, *H6*), 5.24 (q, *J* = 6.8 Hz, 1H, *H3*), 4.51 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.86 (d, *J* = 16.2 Hz, 2H, *H1*), 3.70 (s, 3H, *H10*), 1.47 (d, *J* = 6.8 Hz, 3H, *H4*), 1.36 (d, *J* = 7.2 Hz, 3H, *H8*)

^{13}C NMR (150 MHz, $CDCl_3$) (δ ppm) 173.10 (*C*O,*C9*), 169.31 (*C*O,*C5*), 165.74 (*C*O, *C2*), 71.79 (*C*H,*C3*), 53.49 (O*C*H_3,*C10*), 47.09 (*C*H,*C7*), 25.44 (*C*H_2,*C1*), 18.32 (*C*H_3,*C8*), 17.50 (*C*H_3,*C4*)

_R_-Lactate-L-alanine ethyl ester bromoacetate (386)



The title compound (**386**) was prepared from *R*-lactate–L-alanine ethyl ester (0.753 g, 4.00 mmol) and bromoacetyl bromide (1.207 g, 6.00 mmol) according to the general procedure (Section 7.5.2, page 384) as a pale yellow liquid in 54 % yield (0.669 g, 2.16 mmol).

$[\alpha]_D^{20} = +28.0\ ^\circ$ (1.0 c, $CHCl_3$)

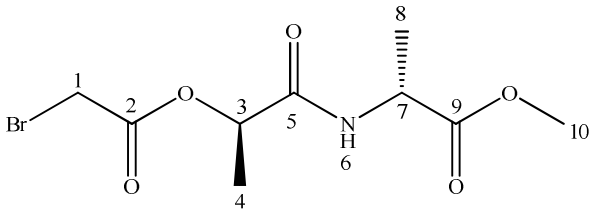
Molecular formula: $C_{10}H_{16}BrNO_5$

Molecular weight 310 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.71 (d, *J* = 6.8 Hz, 1H, *H6*), 5.22 (q, *J* = 6.8 Hz, 1H, *H3*), 4.50 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 4.15 (q, *J* = 7.2 Hz, 2H, *H10*), 3.86 (d, *J* = 16.2 Hz, 2H, *H1*), 1.44 (d, *J* = 6.8 Hz, 3H, *H4*), 1.36 (d, *J* = 7.2 Hz, 3H, *H8*), 1.23 (t, *J* = 7.2 Hz, 3H, *H11*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 172.59 (*C*O,*C5*), 169.15 (*C*O,*C9*), 165.71 (*C*O, *C2*), 71.96 (O*C*H_2,*C10*), 61.69 (*C*H,*C3*), 47.97 (*C*H,*C7*), 25.39 (*C*H_2,*C1*), 18.36 (*C*H_3,*C8*), 17.57 (*C*H_3,*C4*), 14.13 (*C*H_3,*C11*)

_R_-Lactate-D-alanine methyl ester bromoacetate (387)



The title compound (**387**) was prepared from *R*-lactate–D-alanine methyl ester (0.540 g, 3.10 mmol) and bromoacetyl bromide (0.926 g, 4.65 mmol) according to the general

procedure (Section 7.5.2, page 384) as a colourless liquid in 35 % yield (0.321 g, 1.08 mmol).

$[\alpha]_D^{20}$ = +30.0 ° (0.6 c, $CHCl_3$)

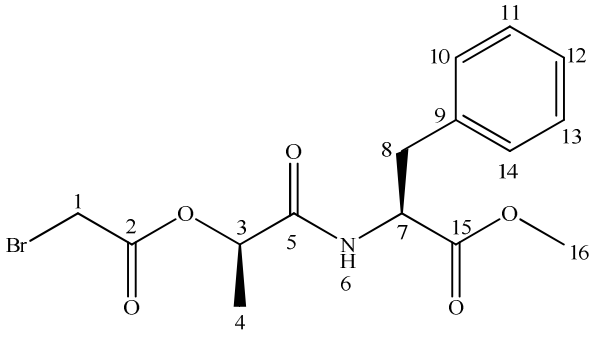
Molecular formula: $C_9H_{14}BrNO_5$

Molecular weight 296 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 6.79 (d, *J* = 7.0 Hz, 1H, *H6*), 5.28 (q, *J* = 6.8 Hz, 1H, *H3*), 4.61 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.90 (d, *J* = 16.4 Hz, 2H, *H1*), 3.78 (s, 3H, *H10*), 1.54 (d, *J* = 6.8 Hz, 3H, *H4*), 1.46 (d, *J* = 7.2 Hz, 3H, *H8*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.18 (*C*O,*C5*), 169.02 (*C*O,*C9*), 165.17 (*C*O, *C2*), 71.79 (*C*H,*C3*), 53.48 (O*C*H_3,*C10*), 47.07 (*C*H,*C7*), 25.45 (*C*H_2,*C1*), 18.30 (*C*H_3,*C8*), 17.54 (*C*H_3,*C4*)

_R_-Lactate-L-phenylalanine methyl ester bromoacetate (388)



The title compound (**388**) was prepared from *R*-lactate–L-phenylalanine methyl ester (0.664 g, 2.70 mmol) and bromoacetyl bromide (0.350 g, 4.00 mmol) according to the general procedure (Section 7.5.2, page 384) as a pale yellow liquid in 37 % yield (0.373 g, 1.00 mmol).

$[\alpha]_D^{20}$ = +23.0 ° (0.8 c, $CHCl_3$)

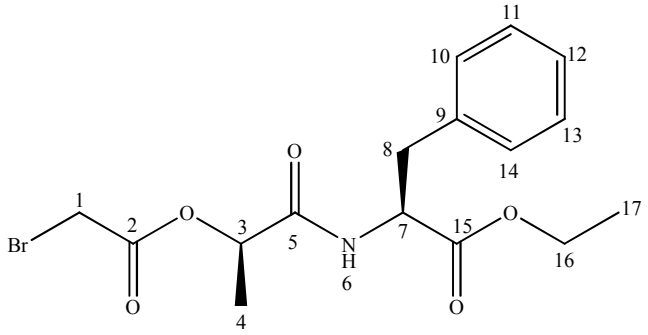
Molecular formula: $C_{15}H_{18}BrNO_5$

Molecular weight 372 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.24-7.00 (m, 5H, *H10-14*), 6.52 (d, *J* = 7.6 Hz, 1H, *H6*), 5.18 (q, *J* = 6.8 Hz, 1H, *H3*), 4.81 (ddd, *J* = 8.0, 6.8, 6.8 Hz, 1H, *H3*), 3.72 (d, *J* = 15.4 Hz, 2H, *H1*), 3.68 (s, 3H, *H16*), 3.12-3.02 (m, 2H, *H8*), 1.41 (d, *J* = 6.8 Hz, 3H, *H4*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.57 (*C*O,*C5*), 169.36 (*C*O,*C15*), 165.65 (*C*O, *C2*), 135.37 (Ar*C*,*C9*), 129.29 (Ar*C*H), 128.68 (Ar*C*H), 127.68 (Ar*C*H,*C12*), 71.87 (*C*H,*C7*), 52.80 (O*C*H_3,*C16*), 52.54 (*C*H,*C3*), 37.69 (*C*H_2,*C8*), 25.24 (*C*H_2,*C1*), 17.63 (*C*H_3,*C4*)

_R_-Lactate-L-phenylalanine ethyl ester bromoacetate (389)



The title compound (**389**) was prepared from *R*-lactate–L-phenylalanine ethyl ester (0.434 g, 1.65 mmol) and bromoacetyl bromide (0.390 g, 2.00 mmol) according to the general procedure (Section 7.5.2, page 384) as a pale yellow liquid in 55 % yield (0.350 g, 0.91 mmol).

$[\alpha]_D^{20} = +15.1°$ (0.8 c, $CHCl_3$)

Molecular formula: $C_{16}H_{20}BrNO_5$

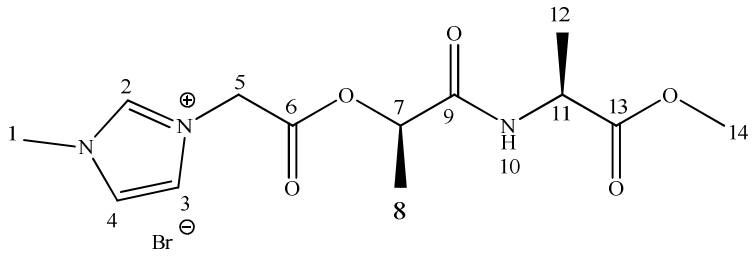
Molecular weight 386 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.21-7.01 (m, 5H, *H10-14*), 6.53 (d, *J* = 7.6 Hz, 1H, *H6*), 5.21 (q, *J* = 7.2 Hz, 1H, *H3*), 4.79 (ddd, *J* = 7.8, 6.4, 6.4 Hz, 1H, *H7*), 4.15 (q, *J* = 7.2 Hz, 2H, *H16*), 3.74 (d, *J* = 15.4 Hz, 2H, *H1*), 3.10-3.04 (m, 2H, *H8*), 1.44 (d, *J* = 6.8 Hz, 3H, *H4*), 1.19 (t, *J* = 7.2 Hz, 3H, *H17*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 171.09 (*C*O,*C5*), 169.31 (*C*O,*C15*), 165.64 (*C*O, *C2*), 135.46 (Ar*C*,*C9*), 129.37 (Ar*C*H), 128.62 (Ar*C*H), 127.62 (Ar*C*H,*C12*), 71.88 (O*C*H_2,*C16*), 61.76 (*C*H,*C3*), 52.84 (*C*H,*C7*), 37.72 (*C*H_2,*C8*), 25.25 (*C*H_2,*C1*), 17.65 (*C*H_3,*C4*), 14.14 (*C*H_3,*C17*)

7.5.3 Preparation of lactate-peptidyl chiral Br ILs

3-Methyl-1-*R*-lactate-L-alanine methyl ester imidazolium bromide (392)



To a stirred solution of 1-methylimidazole (0.095 g, 1.16 mmol) in tetrahydrofuran (20 mL) at -15 ºC under a nitrogen atmosphere was added dropwise *R*-lactate-L-alanine methyl ester bromoacetate (**385**) (0.445 g, 1.50 mmol). The reaction mixture was stirred vigorously at -15 ºC for 2 h, then at RT overnight. The IL precipitated separate from the THF phase. The solvent was removed on the rotary evaporator and the residual product was washed with diethyl ether. The product was dried under high vacuum for 48 hrs to yield the title product (**392**) as an off white solid in 87 % yield (0.382 g, 1.01 mmol).

m.p. 68-70 °C, $[\alpha]_D^{20}$ = +18.1 ° (0.8 c, $CHCl_3$)

Molecular formula $C_{13}H_{20}BrN_3O_5$

Molecular weight 378 $gmol^{-1}$

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) 10.08 (s, 1H, *H2*), 7.84 (d, *J* = 7.6 Hz, 1H, *H10*), 7.55 (t, *J* = 1.8 Hz, 1H, *H4*), 7.20 (t, *J* = 1.8 Hz, 1H, *H3*), 5.94 (d, *J* = 17.2 Hz, 1H, *H5*), 5.46 (d, *J* = 17.6 Hz, 1H, *H5*), 5.28 (q, *J* = 6.8 Hz, 1H, *H7*), 4.94 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 3.99 (s, 3H, *H1*), 3.65 (s, 3H, *H14*), 1.48 (d, *J* = 7.2 Hz, 3H, *H12*), 1.45 (d, *J* = 6.8 Hz, 3H, *H8*)

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 173.42 (*C*O,*C9*), 169.57 (*C*O,*C13*), 165.92 (*C*O,*C6*), 138.08 (N*C*HN,*C2*), 123.20 (N*C*H,*C4*), 122.96 (N*C*H,*C3*), 72.28 (*C*H,*C7*), 65.86 (N*C*H_2,*C5*), 52.37 (*C*H,*C11*), 50.81 (O*C*H_3,*C14*), 36.92 (N*C*H_3,*C1*), 17.59 (*C*H_3,*C8*), 17.29 (*C*H_3,*C12*)

IR (neat) (cm^{-1}) 3269 (w), 3043 (w), 1761 (m), 1742 (vs), 1653 (s), 1525 (s), 1188 (s), 1173 (s), 1086 (s),1026 (m)

MS (*m/z*) Found $[M\text{-}Br^-]^+$ 298.1393, $C_{13}H_{20}N_3O_5^+$ requires 298.1397

7.6 Chapter 5 experimental-toxicity studies

7.6.1 Minimum Inhibitory Concentration (MIC) Assay- Microtiter broth dilution technique

Minimum inhibitory concentrations (MICs) for compounds were determined by serial two-fold dilutions in Mueller-hinton or nutrient broth using the microtiter broth dilution technique described by Amsterdam.[3] All assays were done in triplicate.

Method

Test strains were grown in nutrient broth at 30 °C overnight. Next day, cultures were centrifuged at 5000 rpm for 10 minutes. The pellet formed was washed twice with 10 ml 0.01 M sodium phosphate buffer (pH 7.0). Optical density of cultures was adjusted to give an optical density of 0.07 at 660 nm.

The compound solution and 96 well plates were ready before the cultures were adjusted to the desired optical density.

For stock solution of chemical, the compound to be tested was dissolved in 1 ml of sterile water or organic solvents such as methanol and DMSO dependant on the chemicals solubility.

For microplate preparation, 190 µl of Müeller-hinton broth was dispensed into wells in column 1. 100 µl of Müeller-hinton broth was dispensed into all wells from column 2 to column 12. 10 µl of the compound solution was pipetted into wells in column 1 (far left of

plate). The compound was mixed into the wells in column 1 by pipetting up and down 6-8 times. 100 µl was withdrawn from column 1 and added to column 2. This made column 2 a two-fold dilution of column 1. This was mixed up and down 6-8 times. 100 µl was transferred to column 3. This procedure was repeated down to column 10 only. 100µl was discarded from column 10 rather than putting it in column 11. 5µl of the strain to be tested was dispensed into wells in columns 11 to 1 in that order. Column 11 was used as a growth control and column 12 was the sterility control. The plates were incubated at 30 °C overnight. Growth on the plates was noted and optical density measured after 24 hours.

7.7 Chapter 6 experimental-Primary biodegradation studies

7.7.1 Primary Biodegradation Assay

For the Primary Biodegradation assay a modified OECD Guideline for Testing of Chemicals (OECD 301A- Die-Away Test) was used.[4] Activated sludge (AS) collected from a pharmaceutical company was used for the mixed microbial community inoculum. The AS sample was pre-conditioned by aeration at room temperature for 5 days. After aeration, the total suspended solids (SS) concentration of the AS used was 5g/L SS in mineral nutrient medium. The mineral medium was composed as follows; 0.085 g/L KH_2PO_4, 0.2175 g/L K_2HPO_4, 0.334 g/L $Na_2HPO_4{\cdot}2H_2O$, 5 mg/L NH_4Cl, 36.4 mg/L $CaCl_2{\cdot}2H_2O$, 22.5 mg/L $MgSO_4{\cdot}7H_2O$, 0.25 mg/L $FeCl_3{\cdot}6H_2O$. The ILs were tested in duplicate at a concentration of 240 µM. All controls were also tested in duplicate. Abiotic control flasks containing 240 µM of the chemical and $HgCl_2$ at a concentration of 50 mg/L were prepared. Flasks containing the test substance and autoclaved inoculum (sterile control), flasks containing the test substance in mineral medium in the absence of AS inoculum and flasks containing the test substance in distilled water (no inoculated medium) were all prepared. The positive control flask contained the standard Sodium-n-dodecyl sulfate (SDS) at a concentration of 240 µM. The blank controls contained no test substrate only mineral medium and AS. All the test flasks were incubated at 25°C for 28 days at 100 rpm; 500 µl samples were

retrieved from the flasks every 3 to 4 days, these samples were taken in duplicate per flask. All samples were subsquently centrifuged (8000 rpm, 15 min) prior to analysis.

7.7.2 Preparation of proposed metabolite, 3-carboxymethyl-1-methylimidazolium bromide

Bromacetic acid (2.001 g, 14.50 mmol) was added in small portions over a 30 min time period to 1-methylimidazole (1.188 g, 14.50 mmol). The reaction was then heated to 70 °C for 4 hours.[5] After this time the reaction mixture was cooled to room temperature and washed with acetonitrile (5 x 10 mL). Drying *in vacuo* yielded the product as a white solid in 76 % yield (2.439 g, 11.13 mmol).

^{1}H NMR (400 MHz, D_2O) δ (ppm) 8.65 (s, 1H), 7.36 (s, 2H), 4.84 (s, 2H), 4.65 (s, 3H)

^{13}C NMR (100 MHz, D_2O) δ (ppm) 170.68, 137.21, 123.59, 123.47, 51.00, 36.07

7.8 References

[1] N. Gathergood, M. T. Garcia and P. J. Scammells, *Green Chem.*, 2005, **7**, 9-14.

[2] S. Morrissey, B. Pegot, D. Coleman, M. T. Garcia, D. Ferguson, B. Quilty and N. Gathergood, *Green Chem.*, 2009, **11,** 475-483.

[3] D. Amsterdam, Susceptibility testing of Antimicrobials in liquid media. "*Antibiotics in Laboratory Medicine*", Lorian, V., ed. Fourth Edition, 1996, 52-111.

[4] OECD *guidelines for testing chemicals* 301 A-F, Organisation for Economic Co-operation and Development, Paris, France, 1992.

[5] J. Li, Y. Peng and G. Song, *Catal. Lett.*, 2005, **102**, 159-162.

Thesis Conclusions and Future Work

A series of achiral imidazolium based ionic liquids, and associated intermediates, were successfully designed and prepared (Chapter 2). Alkyl ester and amide moieties (alkyl chains C5-C14) were incorporated into the imidazolium cation side chain. Introduction of these functionalities was achieved previously in the research group with superior biodegradation and decreased toxicity observed for ester examples, whilst ILs containing amide side chains gave poor biodegradabilities. A number of anion exchange reactions yielded a range of salts with various physico-chemical properties (i.e. melting points and viscosities). It was generally found that ILs containing $OctOSO_3$ and NTf_2 anions were liquids at room temperature.

A library of novel chiral ionic liquids containing amino acid ester groups in the side chain was also designed and synthesised (Chapter 3). Introduction of these chiral building blocks provides the IL side chain with several possible biodegradation sites. 23 bromide CILs (**270**-**292**) were synthesised. THF replaced diethyl ether as reaction medium in this synthesis in order to optimise bromide salt formation. All the bromide CILs prepared were screened for various antimicrobial activities (Chapter 5). Bromide CIL **272** was also investigated in an activated sludge biodegradation assay (Chapter 6). A range of NTf_2 (**293**-**307**) and $OctOSO_3$ (**308**-**317**) CILs was also successfully synthesised. As seen in Chapter 2, exchanging the bromide anion in the IL structures leads to notable changes in the physical properties of the salts (all NTf_2 and $OctOSO_3$ CILs were obtained as liquids).

The final approach in preparing highly functionalised ionic liquids, involved the coupling of dipeptidyl species into the IL side chain. This resulted in salts containing ester and amide functionalities, at various positions, in the side chain. It was hoped that improved biodegradation could be achieved by using this strategy. By including amide bonds adjacent to ester groups, biodegradation of this typically stable moiety was envisaged. It was also hypothesised that an ionic liquid containing a peptidyl moiety may be more readily recognised by metabolising enzymes. This could therefore lead to improved environmental breakdown of ionic liquids. 17 dipeptidyl examples were successfully

synthesised and characterised. Peptide species were prepared using the standard EDC/ HOBt coupling protocol. All novel achiral and chiral ionic liquids described in the thesis were structurally characterised by a range of spectroscopic techniques including ^{1}H, ^{13}C, DEPT 135 and HMQC NMR. IR and MS data was also reported.

Biological assessment of the novel ionic liquids was also a prime objective of this research. Antimicrobial screening of various achiral and chiral ionic liquids was carried out both in DCU and in collaborative work with Dr. Marcel Špulák (Charles University, Czech Republic). A range of environmentally relevant bacteria and clinically resistant strains of bacteria and fungi were challenged against novel ILs. Achiral ionic liquids (**42**, **45**, **193**-**199**) were screened for antimicrobial activity. High levels of toxicity were observed for ILs containing dodecyl (**194**) and tetradecyl (**195**) ester side chains. These compounds displayed a broad range of activity towards resistant bacterial and fungal strains. The potency of these ILs can be related to structural features of the IL side chain. Substitution of the ILs cation with long alkyl chains results in more lipophilic examples. Increase in lipophilicity results in a corresponding increase in toxicity. Dipeptidyl (**363**-**379**) and Amino acid ester (**247**-**269**) chiral ionic liquids were challenged against a range of bacterial and fungal isolates, of both clinical and environmental relevance. CILs **274**, **283**, **284**, **363** and **364** displayed relatively high MIC values (Minimum Inhibitory Concentration) corresponding to low toxicities against several strains of bacteria ubiquitous in the environment. CIL **368** was capable of inhibiting the resistant bacterial strain MRSA at 125 μM concentration. Inhibition of other resistant bacterial strains was also observed for compounds **370**, **371** and **378**. All the abovementioned toxic CILs possessed a phenylalanine moiety in the cation side chain. The presence of this group in IL side chains leads to increased lipophilicity and toxicity. A trend was also noted between IL activity and the bacterial strains. Inhibition was predominately against Gram positive bacteria, whilst the Gram negative species proved the most tolerant.

The primary biodegradation of two novel ILs (**272** and **378**) was also investigated (Chapter 6). High Performance Liquid Chromatography with Mass Spectrometry (HPLC-MS) and Electrospray Ionisation Mass Spectrometry (ESI MS) analysis was employed to monitor IL breakdown. Two HPLC methods were successfully developed to identify novel CILs and

their corresponding metabolite structures. Reverse-Phase (RP) and Hydrophilic interaction chromatography (HILIC) systems were studied with MS employed as the detection method in both cases. However, validation of the developed methods could not be achieved (presumably due to instrumental errors). An activated sludge assay (a modified OECD 301 A, 28 day test) was set up in order to investigate biological breakdown of the novel ILs. Over the 28 day test period, samples were retrieved and subsequently analysed by ESI-MS. The results obtained from this assay indicated an evident disappearance of the parent IL cation masses over the 28 days. Nonetheless, the results also suggested that an abiotic degradation of the novel salts had occurred in the test vessels. Similar MS data was obtained from both these biotic and abiotic controls. A metabolite structure was proposed, namely 3-carboxymethyl-1-methylimidazolium, and synthesized in the laboratory. An antibacterial screen was performed on the metabolite compound in order to investigate its possible toxicity in the environment. Relatively high MIC values (low toxicities) were noted from this test. Although these ILs cannot be described as biodegradable from the obtained experimental results, it is envisaged that their persistence in aquatic environments may not be a concern.

Due to the vast number of possible ionic liquid structures which may be formed (by the variation of the anion or cation within an IL structure) and of possible peptide moieties which may also be prepared, a further library of novel ILs could be attained. Examples may be prepared with the inclusion of other biodegradable functionalities (i,e hydroxyls, aldehydes or aromatic esters). Ionic liquids have gained much attention in the literature as possible replacements for organic solvents in various chemical reactions. Therefore, it would be of interest to examine the possible application of the novel ILs prepared in this work in organic chemical reactions (e.g. Diels Alder, Hydrogenation).

Inhibition of various resistant strains of bacteria and fungi have been obtained for some of the novel ILs. Even though these particular examples cannot be fully classified as ‘green’, their relative toxicities may be exploited to a number of beneficial applications (as new antiseptic or antibacterial agents). Gilmore *et al.* have recently described the antibiofilm activities of imidazolium based ionic liquids. Numerous chronic plant, animal and human infections have been caused by bacterial biofilms. Therefore antibiofilm screenings are of

environmental and clinical importance. Further work may involve challenging the novel ILs prepared in this research in biofilm environments.

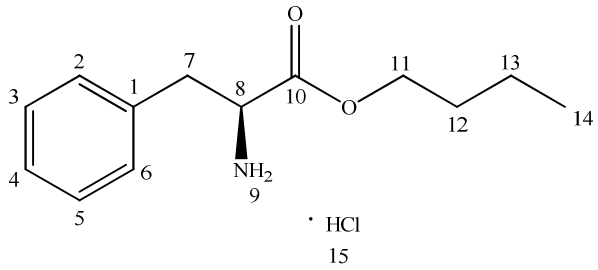
The biodegradation of all the prepared ILs may also be examined in the future. The use of respirometric analysis along with the primary biodegradation studies would allow for a better understanding of the IL breakdown. Validation of the developed HPLC methods discussed in this thesis (Chapter 6) is necessary in order to report quantitative analysis of degradation samples. Therefore work should be carried out to obtain reproducible results using the developed methods.

Appendix I

General procedures for the preparationof known starting materials for L-Amino acid ester Chiral Ionic Liquids

(Chapter 3)

***General procedure for the preparation of starting materials for L-Amino acid ester Chiral Ionic Liquids*; Preparation of L-Phenylalanine butyl ester hydrochloride (225)**



L-Phenylalanine (2.010 g, 12.0 mmol) was added slowly to a solution of butanol (30 mL) and thionyl chloride (2.88 mL) at 0 °C. The solution was stirred under reflux for 48 hours. After this time the reaction was allowed to cool to room temperature, and evaporation of solvent furnished the desired product (**225**) as a white powder in 66 % yield (2.040 g, 7.91 mmol).

m.p. 124-126 °C, lit. m.p. 128-130 °C,[1] $[\alpha]_D^{20}$ = -28.0 ° (0.8 c, MeOH), lit. $[\alpha]_D^{20}$ = -33.0 ° (1.0 c, EtOH)[1]

Molecular formula $C_{13}H_{20}ClNO_2$

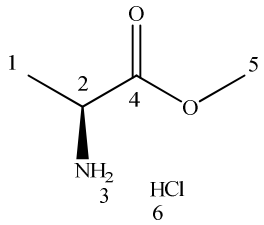
Molecular weight 258 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.69 (s_{Br}, 3H, *H9,15*), 7.24-7.15 (m, 5H, *H2-6*), 4.32 (dd, *J* = 8.4, 5.2 Hz, 1H, *H8*), 4.00 (t, *J* = 6.8 Hz, 2H, *H11*), 3.43 (dd, *J* = 7.2, 4.4 Hz, 1H, *H7*), 3.30 (dd, *J* = 7.2, 6.6 Hz, 1H, *H7*), 1.45-1.32 (tt, *J* = 7.2, 6.8 Hz, 2H, *H12*), 1.18 (tq, *J* = 7.6, 7.2 Hz, 2H, *H13*), 0.78 (t, *J* = 7.2 Hz, 3H, *H14*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 168.99 (*C*O,*C10*), 134.15 (Ar*C*,*C1*), 129.62 (Ar*C*H), 128.86 (Ar*C*H), 127.58 (Ar*C*H,*C4*), 66.31 (O*C*H_2,*C11*), 54.35 (*C*H,*C8*), 36.40 (*C*H_2,*C7*), 30.17 (*C*H_2,*C12*), 18.87 (*C*H_2,*C13*), 13.60 (*C*H_3,*C14*)

NMR data was in agreement with the literature.[2]

L-Alanine methyl ester hydrochloride (226)



L-Alanine (0.510 g, 5.60 mmol) was added slowly to a solution of methanol (20 mL) and thionyl chloride (0.81 mL) at 0 °C. The solution was stirred at room temperature for 48 hours. Evaporation of solvent furnished the desired product (**226**) as a white powder in 94 % yield (0.736 g, 5.29 mmol).

m.p. 106-108 °C, lit. m.p. 106-108 °C,[3] $[\alpha]_D^{20}$ = +6.9 ° (0.8 c, MeOH), lit. $[\alpha]_D^{20}$ = +6.5 ° (1.0 c, MeOH)[4]

Molecular formula $C_4H_{10}ClNO_2$

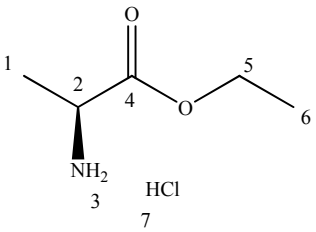
Molecular weight 139 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.49 (s_{Br}, 3H, *H3,6*), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H, *H2*), 3.75 (s, 3H, *H5*), 1.42 (d, *J* = 7.2 Hz, 3H, *H1*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 170.34 (*C*O,*C4*), 54.70 (O*C*H_3,*C5*), 46.72 (*C*H,*C2*), 15.49 (*C*H_3,*C1*)

NMR data was in agreement with the literature.[5]

L-Alanine ethyl ester hydrochloride (227)



L-Alanine (0.997 g, 11.20 mmol) was added slowly to a solution of ethanol (20 mL) and thionyl chloride (1.63 mL) at 0 °C. The solution was stirred at room temperature for 48

hours. Evaporation of solvent furnished the desired product (**227**) as a white solid in 60 % yield (1.036 g, 6.73 mmol).

m.p. 74-76 °C, lit. m.p. 75-77 °C,[6] $[\alpha]_D^{20}$ = +2.6 ° (0.9 c, H_2O), lit. $[\alpha]_D^{20}$ = +3.1 (2.0 c, H_2O)[7]

Molecular formula $C_5H_{12}ClNO_2$

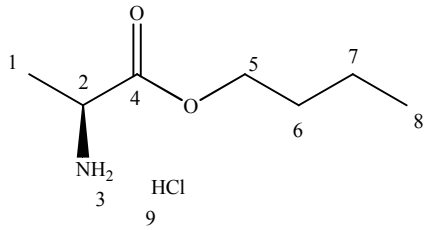
Molecular weight 154 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.55 (s_{Br}, 3H, *H3,7*), 4.17 (dq, J = 7.0, 7.0 Hz, 1H, *H5*), 4.16 (dq, *J* = 7.2, 7.0 Hz, 1H, *H5*), 4.05 (dq, *J* = 7.2, 7.2 Hz, 1H, *H2*), 1.42 (d, *J* = 7.2 Hz, 3H, *H1*), 1.25 (t, *J* = 7.2 Hz, 3H, *H6*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.30 (*C*O,*C4*), 59.53 (O*C*H_2,*C5*) 54.78 (*C*H,*C2*), 19.30 (*C*H_3,*C1*), 13.49 (*C*H_3,*C6*)

NMR data was in agreement with the literature.[8]

L-Alanine Butyl ester hydrochloride (228)



L-Alanine (0.997 g, 11.20 mmol) was added slowly to a solution of butanol (20 mL) and thionyl chloride (1.63 mL) at 0 °C. The solution was stirred at room temperature for 48 hours. Evaporation of solvent furnished the desired product (**228**) as a white solid in 94 % yield (1.919 g, 10.54 mmol).

m.p. 88-90 °C, $[\alpha]_D^{20}$ = +12.2 ° (0.9 c, H_2O) lit. $[\alpha]_D^{20}$ = +15.0 ° (0.8 c, MeOH)[9]

Molecular formula $C_7H_{16}ClNO_2$

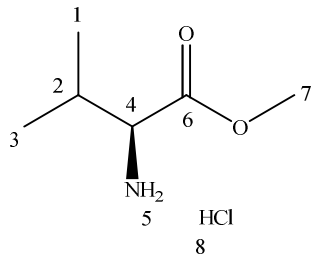
Molecular weight 182 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.48 (s_{Br}, 3H, *H3,9*), 4.14 (dq, *J* = 7.2, 7.0 Hz, 1H, *H5*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H5*), 4.09 (dq, *J* = 7.2, 7.2 Hz, 1H, *H2*), 1.60 (tt, *J* = 7.2, 7.0 Hz, 2H, *H6*), 1.32 (tq, *J* = 7.2, 7.0 Hz, 2H, *H7*), 0.91 (t, *J* = 7.2 Hz, 3H, *H1*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.02 (*C*O,*C4*), 65.25 (O*C*H_2,*C5*), 47.80 (*C*H,*C2*), 29.96 (*C*H_2,*C6*), 18.40 (*C*H_2,*C7*), 15.70 (*C*H_3,*C1*), 13.39 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[9]

L-Valine methyl ester hydrochloride (229)



L-Valine (1.001 g, 8.50 mmol) was added slowly to a solution of methanol (30 mL) and thionyl chloride (1.24 mL) at 0 °C. The solution was stirred at room temperature for 48 hours. Evaporation of solvent furnished the desired product (**229**) as a white solid 96 % yield (1.369 g, 8.15 mmol).

m.p. 169-171 °C, lit. m.p. 168-170 °C,[10] $[\alpha]_D^{20}$ = +15.0 ° (0.9 c, H_2O), lit. $[\alpha]_D^{20}$ = +15.6 ° (1.0 in H_2O)[10]

Molecular formula $C_6H_{14}ClNO_2$

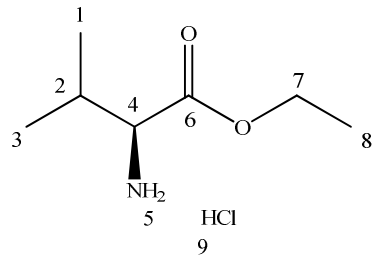
Molecular weight 168 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.64 (s_{Br}, 3H, *H5,8*), 3.83 (d, *J* = 4.0 Hz, 1H, *H4*), 3.72 (s, 3H, *H7*), 2.18 (qqd, *J* = 7.0, 6.8, 4.4 Hz, 1H, *H2*), 0.97 (d, *J* = 7.2 Hz, 3H, *H1/3*), 0.93 (d, *J* = 7.2 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.07 (*C*O,*C6*), 57.20 (*C*H,*C4*), 52.49 (O*C*H_3,*C7*), 29.25 (*C*H,*C2*), 18.24 (*C*H_3,*C1/C3*), 17.50 (*C*H_3,*C1/C3*)

NMR data was in agreement with the literature.[11]

L-Valine ethyl ester hydrochloride (230)



L-Valine (5.011 g, 42.70 mmol) was added slowly to a solution of ethanol (60 mL) and thionyl chloride (6.20 mL) at 0 °C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**230**) as a white solid 87 % yield (6.794 g, 37.32 mmol).

m.p. 99-101 °C, lit. m.p 102-104 °C,[12] $[\alpha]_D^{20} = +7.0^{\circ}$ (1.0 c, H_2O), lit. $[\alpha]_D^{20} = +6.6^{\circ}$ (2.0 c, H_2O)[12]

Molecular formula $C_7H_{16}ClNO_2$

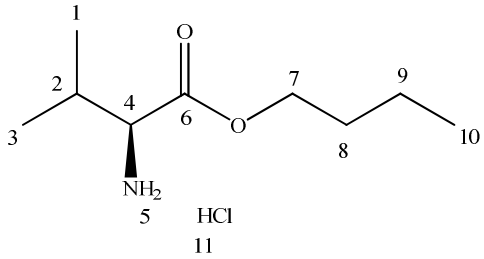
Molecular weight 182 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.64 (s_{Br}, 3H, *H5,9*), 4.16 (dq, *J* = 7.0, 7.0 Hz, 1H, *H7*), 4.15 (dq, *J* = 7.2, 7.0 Hz, 1H, *H7*), 3.79 (d, *J* = 4.0 Hz, 1H, *H4*), 2.17 (qqd, *J* = 7.0, 6.8, 4.0 Hz, 1H, *H2*), 1.23 (t, *J* = 7.0 Hz, 3H, *H8*), 1.00 (d, *J* = 6.8 Hz, 3H, *H1/3*), 0.95 (d, *J* = 7.2 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.46 (*C*O,*C6*), 61.53 (O*C*H_2,*C7*), 57.20 (*C*H,*C4*), 29.27 (*C*H,*C2*), 18.20 (*C*H_3,*C1*/*C3*), 17.66 (*C*H_3,*C1*/*C3*), 14.00 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[13]

L-Valine Butyl ester hydrochloride (231)



L-Valine (3.001 g, 25.60 mmol) was added slowly to a solution of butanol (60 mL) and thionyl chloride (1.25 mL) at 0 °C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**231**) as an off white solid 65 % yield (3.510 g, 16.71 mmol).

m.p. 50-52 °C, lit. m.p. 53-54 °C,[9] $[\alpha]_D^{20}$ = +15.6 ° (0.7 c, H_2O) lit. $[\alpha]_D^{20}$ = +12.5 °(0.8c, MeOH)[9]

Molecular formula $C_9H_{20}ClNO_2$

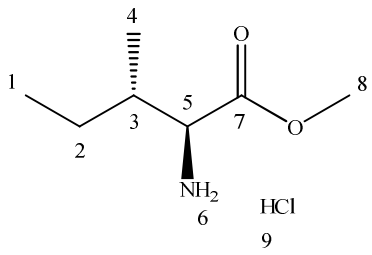
Molecular weight 210 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.41 (s_{Br}, 2H, *H5,11*), 4.00-3.89 (m, 2H, *H7*), 3.72 (d, *J* = 4.0 Hz, 1H, *H4*), 2.17 (qqd, *J* = 6.8, 6.4, 4.4 Hz, 1H, *H2*), 1.62 (tt, *J* = 6.8, 6.6 Hz, 2H, *H8*), 1.38 (tq, *J* = 6.8, 6.8 Hz, 2H, *H9*), 0.96 (t, *J* = 6.8 Hz, 3H, *H10*), 0.91 (d, *J* = 6.8 Hz, *H1/3*), 0.84 (d, *J* = 6.8 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.20 (*C*O,*C6*), 61.69 (O*C*H_2,*C7*), 59.03 (*C*H,*C4*), 30.90 (*C*H_2,*C8*), 28.98 (*C*H,*C2*), 18.40 (*C*H_2,*C9*), 18.14 (*C*H_3,*C1/C3*), 17.68 (*C*H_3,*C1/C3*), 13.38 (*C*H_3,*C10*)

NMR data was in agreement with the literature.[9]

L-Isoleucine methyl ester hydrochloride (232)



L-Isoleucine (1.010 g, 7.70 mmol) was added slowly to a solution of methanol (45 mL) and thionyl chloride (1.12 mL) at 0 °C. The solution was stirred at room temperature for 48 hours. Evaporation of solvent furnished the desired product (**232**) as a white solid in 96 % yield (1.340 g, 7.36 mmol).

m.p. 98-100 °C, lit. m.p. 98-100 °C,[4] $[\alpha]_D^{20}$ = +21.0 ° (0.9 c, H_2O), lit. $[\alpha]_D^{20}$ = +27.0 ° (0.2c, H_2O)[4]

Molecular formula $C_7H_{16}ClNO_2$

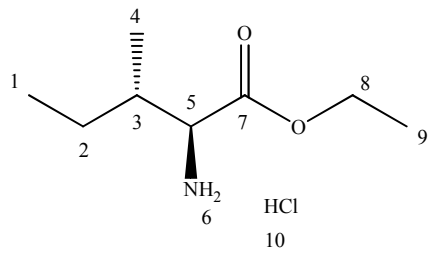
Molecular weight 182 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.12 (s_{Br}, 3H, *H6,9*), 3.93 (d, *J* = 4.0 Hz, 1H, *H5*), 3.68 (s, 3H, *H8*), 1.96 (dddq, *J* = 8.0, 8.0, 6.8, 4.4 Hz, 1H, *H3*), 1.48 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H2*), 1.27 (ddq, *J* = 8.8, 8.0, 7.2 Hz, 1H, *H2*), 0.89 (t, *J* = 7.2 Hz, 3H, *H1*), 0.85 (d, *J* = 6.8 Hz, 3H, *H4*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.12 (*C*O,*C7*), 55.95 (*C*H,*C5*), 52.54 (O*C*H_3,*C8*), 39.65 (*C*H,*C3*), 25.23 (*C*H_2,*C2*), 14.38 (*C*H_3,*C4*), 11.57 (*C*H_3,*C1*)

NMR data was in agreement with the literature.[14]

Preparation of L-Isoleucine ethyl ester (233)



L-Isoleucine (1.96g, 15.34 mmol) was added slowly to a solution of ethanol (30 mL) and thionyl chloride (2.00 mL) at 0 ^{o}C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**233**) as a yellow liquid in 94 % yield (2.821 g, 14.39 mmol).

$[\alpha]_D^{20}$ = +18.4 ° (0.8 c, H_2O), lit. $[\alpha]_D^{20}$ = +27.8 °(neat)[15]

Molecular formula $C_7H_{16}ClNO_2$

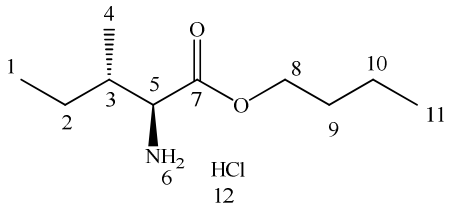
Molecular weight 195 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.53 (s_{Br}, 3H, *H6,10*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 4.17 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 3.89 (d, *J* = 4.0 Hz, 1H, *H5*), 1.98 (dddq, *J* = 7.8, 7.8, 6.8, 4.0 Hz, 1H, *H3*), 1.49 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H2*), 1.29 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H2*), 0.91 (t, *J* = 7.2 Hz, 3H, *H1*), 0.89 (t, *J* = 7.2 Hz, 3H, *H9*), 0.85 (d, *J* = 6.8 Hz, 3H, *H4*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.05 (*C*O,*C7*), 61.54 (O*C*H_2,*C8*), 56.05 (*C*H,*C5*), 35.63 (*C*H,*C3*), 25.35 (*C*H_2,*C2*), 15.61 (*C*H_3,*C4*), 14.20 (*C*H_3,*C1*), 11.56 (*C*H_3,*C9*)

NMR data was in agreement with the literature.[15]

L-Isoleucine Butyl ester hydrochloride (234)



L-Isoleucine (1.061 g, 7.70 mmol) was added slowly to a solution of butanol (30 mL) and thionyl chloride (1.12 mL) at 0 ^{o}C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**234**) as a pale yellow liquid was obtained in 89 % yield (1.531 g, 6.83 mmol).

$[\alpha]_D^{20} = +8.0^{o}$ (0.8 c, H_2O),

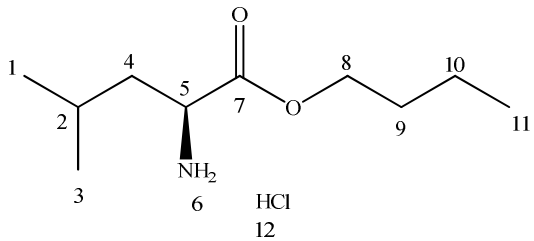
Molecular formula $C_{10}H_{22}ClNO_2$

Molecular weight 224 gmol^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.53 (s_{Br}, 3H, *H6,12*), 4.19 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 4.18 (dq, *J* = 7.2, 7.2 Hz, 1H, *H8*), 3.90 (d, *J* = 4.0 Hz, 1H, *H5*), 1.96 (dddq, *J* = 7.8, 7.8, 6.8, 4.0 Hz, 1H, *H3*), 1.50 (ddq, *J* = 8.0, 8.0, 7.2 Hz, 1H, *H2*), 1.28 (ddq, *J* = 8.0, 8.0, 6.8 Hz, 1H, *H2*), 0.96-0.83 (m, 9H, *H1,4,11*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.05 (*C*O,*C7*), 61.54 (O*C*H$_2$,*C8*), 56.05 (*C*H,*C5*), 35.63 (*C*H,*C3*), 31.18 (*C*H$_2$,*C9*), 25.35 (*C*H$_2$,*C2*), 15.10 (*C*H$_3$,*C4*), 14.20 (*C*H$_3$,*C1*), 11.56 (*C*H$_3$,*C11*)

L-Leucine Butyl ester hydrochloride (237)



L-Leucine (3.067 g, 23.40 mmol) was added slowly to a solution of butanol (40 mL) and thionyl chloride (3.41 mL) at 0 °C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**237**) as a white solid precipitate was obtained in 66 % yield (3.456 g, 15.43 mmol).

m.p. 103-105 °C, lit. m.p. 113-115 °C,[16] $[\alpha]_D^{20}$ = +13.6 ° (0.9 c, EtOH), lit. $[\alpha]_D^{20}$ = +6.0 ° (1.0 c, aq. HCl)[16]

Molecular formula $C_{10}H_{22}ClNO_2$

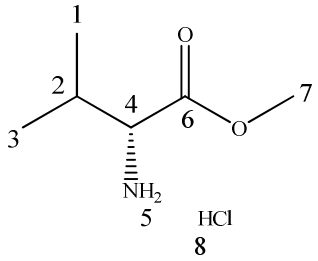
Molecular weight 224 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.64 (s_{Br}, 3H, *H6,12*), 4.14 (dq, *J* = 7.2, 7.0 Hz, 1H, *H8*), 4.13 (dq, *J* = 7.2, 7.0 Hz, 1H, *H8*), 3.88 (t, *J* = 7.0 Hz, 1H, *H5*), 1.78-1.56 (m, 5H, *H2,4,9*), 1.42-1.26 (m, 2H, *H10*), 0.91-0.86 (m, 9H, *H1,3,11*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.88 (*C*O,*C7*), 65.21 (*C*H_2,*C8*), 50.47 (*C*H,*C5*), 40.09 (*C*H_2, *C4*), 29.93 (*C*H_2,*C9*), 23.76 (*C*H,*C2*), 22.17 (*C*H_3,*C1/C3*), 21.89 (*C*H_3,*C1/C3*), 18.60 (*C*H_2,*C10*), 13.46 (*C*H_3,*C11*)

NMR data was in agreement with the literature.[16]

D-Valine methyl ester hydrochloride (238)



D-Valine (2.030 g, 17.35 mmol) was added slowly to a solution of methanol (30 mL) and thionyl chloride (2.50 mL) at 0 °C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**238**) as a white solid 95 % yield (2.765 g, 16.46 mmol).

m.p. 166-168 °C, lit. m.p. 167-169 °C,[17] $[\alpha]_D^{20}$ = -13.0 ° (0.8 c, H_2O), lit, $[\alpha]_D^{20}$ = -15.6° (2.0c, H_2O)[17]

Molecular formula $C_6H_{14}ClNO_2$

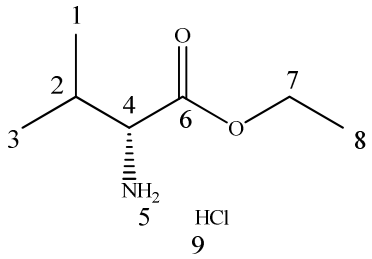
Molecular weight 168 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.75 (s_{Br}, 3H, *H5,8*), 3.93 (d, *J* = 4.0 Hz, 1H, *H4*), 3.75 (s, 3H, *H7*), 2.17 (qqd, *J* = 7.0, 7.0, 4.4 Hz, 1H, *H2*), 0.98 (d, *J* = 7.2 Hz, 3H, *H1/3*), 0.92 (d, *J* = 7.2 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.02 (*C*O,*C6*), 57.18 (*C*H,*C4*), 52.44 (O*C*H_3,*C7*), 29.29 (*C*H,*C2*), 18.21 (*C*H_3,*C1*/*C3*), 17.58 (*C*H_3,*C1*/*C3*)

NMR data was in agreement with the literature.[18]

D-Valine ethyl ester hydrochloride (239)



D-Valine (2.050 g, 17.50 mmol) was added slowly to a solution of ethanol (30 mL) and thionyl chloride (2.50 mL) at 0 °C. The solution was stirred under reflux for 48 hours. Evaporation of solvent furnished the desired product (**239**) as a white solid 77 % yield (2.465 g, 13.54 mmol).

m.p. 96-98 °C, lit. m.p 102-104 °C,[19] $[\alpha]_D^{20}$ = -10.0 ° (1.0 c, H_2O), lit. $[\alpha]_D^{20}$ = -6.7 ° (2.0 c, H_2O)[19]

Molecular formula $C_7H_{16}ClNO_2$

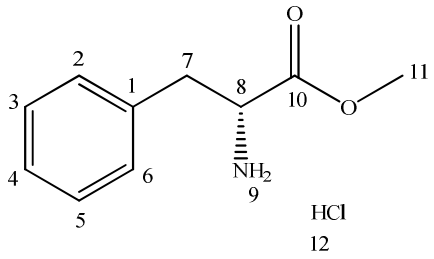
Molecular weight 182 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.74 (s_{Br}, 3H, *H5,9*), 4.18 (dq, *J* = 7.2, 7.0 Hz, 1H, *H7*), 4.16 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.80 (d, *J* = 4.0 Hz, 1H, *H4*), 2.16 (qqd, *J* = 6.8, 6.8, 4.0, 1H, *H2*), 1.25 (t, *J* = 7.0 Hz, 3H, *H8*), 0.99 (d, *J* = 6.8 Hz, 3H, *H1/3*), 0.93 (d, *J* = 6.8 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.90 (*C*O,*C6*), 61.49 (O*C*H_3,*C7*), 57.14 (*C*H,*C4*), 29.29 (*C*H,*C2*), 18.18 (*C*H_3,*C1*/*C3*), 17.50 (*C*H_3,*C1*/*C3*), 14.06 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[13]

D-Phenylalanine methyl ester hydrochloride (240)



D-Phenylalanine (2.020 g, 10.05 mmol) was added slowly to a solution of methanol (30 mL) and thionyl chloride (2.00 mL) at 0 °C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**240**) as a white solid 97 % yield (2.115 g, 9.79 mmol).

m.p. 155-157 °C, lit. m.p. 160-162 °C,[20] $[\alpha]_D^{20}$ = +28.1 ° (1.0 c, H_2O), lit. $[\alpha]_D^{20}$ = +35.7 ° (2.0 c, H_2O)[20]

Molecular formula $C_{10}H_{14}ClNO_2$

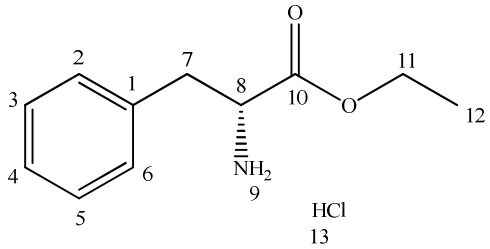
Molecular weight 216 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.71 (s_{Br}, 3H, *H9,12*), 7.37-7.23 (m, 5H, *H2-6*), 4.28 (dd, *J* = 8.0, 6.6 Hz, 1H, *H8*), 3.66 (s, 3H, *H11*), 3.18 (dd, *J* = 10.4, 7.2 Hz, 1H, *H7*), 3.08 (dd, *J* = 10.0, 7.2 Hz, 1H, *H7*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.34 (*C*O,*C10*), 134.63 (Ar*C*,*C1*), 129.37 (Ar*C*H), 128.51 (Ar*C*H), 127.25 (Ar*C*H,*C4*), 53.17 (*C*H,*C8*), 52.52 (O*C*H$_3$,*C11*), 35.80 (*C*H$_2$,*C7*)

NMR data was in agreement with the literature.[21]

D-Phenylalanine ethyl ester hydrochloride (241)



D-Phenylalanine (2.018 g, 10.00 mmol) was added slowly to a solution of ethanol (30 mL) and thionyl chloride (2.00 mL) at 0 °C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**241**) as a white solid 97 % yield (2.240 g, 9.74 mmol).

m.p. 148-150 °C, lit. m.p. 154-155 °C,[22] $[\alpha]_D^{20}$ = +27.0 ° (1.0 c, H_2O), lit. $[\alpha]_D^{20}$ = +32.7 ° (2.0 c, H_2O)[22]

Molecular formula $C_{11}H_{16}ClNO_2$

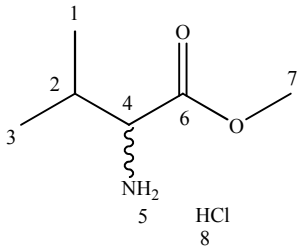
Molecular weight 230 gmol^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.77 (s_{Br}, 3H, *H9,13*), 7.35-7.24 (m, 5H, *H2-6*), 4.18 (dd, *J* = 8.0, 6.6 Hz, 1H, *H8*), 4.03 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 4.02 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 3.24 (dd, *J* = 7.8, 5.6 Hz, 1H, *H7*), 3.06 (dd, *J* = 8.0, 5.6 Hz, 1H, *H7*), 1.08 (t, *J* = 7.2 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.89 (*C*O,*C10*), 134.74 (Ar*C*,*C1*), 129.42 (Ar*C*H), 128.49 (Ar*C*H), 127.18 (Ar*C*H,*C4*), 61.50 (O*C*H$_2$,*C11*), 53.15 (*C*H,*C8*), 35.90 (*C*H$_2$,*C7*), 13.71 (*C*H$_3$,*C12*)

NMR data was in agreement with the literature.[22]

DL-Valine methyl ester hydrochloride (242)



DL-Valine (2.010 g, 17.20 mmol) was added slowly to a solution of methanol (30 mL) and thionyl chloride (2.50 mL) at 0 ^{o}C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**242**) as a white solid 90 % yield (2.616 g, 15.57 mmol).

m.p. 161-163 °C, lit. m.p. 167-169 °C[17]

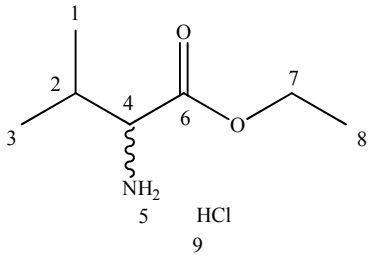
Molecular formula $C_6H_{14}ClNO_2$

Molecular weight 168 gmol^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.65 (s_{Br}, 3H, *H5,8*), 3.87 (d, *J* = 4.0 Hz, 1H, *H4*), 3.68 (s, 3H, *H7*), 2.17 (qqd, *J* = 7.0, 7.0, 4.4 Hz, 1H, *H2*), 1.01 (d, *J* = 7.2 Hz, 3H, *H1/3*), 0.98 (d, *J* = 7.2 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.88 (*C*O,*C6*), 57.20 (*C*H,*C4*), 52.40 (O*C*H$_3$,*C7*), 29.31 (*C*H,*C2*), 18.17 (*C*H$_3$,*C1/C3*), 17.62 (*C*H$_3$,*C1/C3*)

DL-Valine ethyl ester hydrochloride (243)



DL-Valine (2.001 g, 17.10 mmol) was added slowly to a solution of ethanol (30 mL) and thionyl chloride (2.50 mL) at 0 ^{o}C. The solution was stirred under reflux for 48 hours.

Evaporation of solvent furnished the desired product (**243**) as a white solid 64 % yield (2.010 g, 11.04 mmol).

m.p. 95-97 ^{o}C, lit. m.p 101-103 ^{o}C[19]

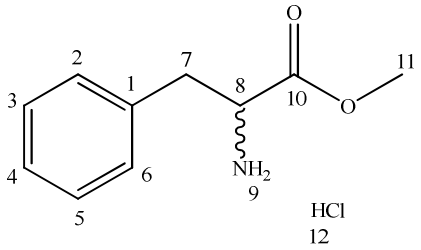
Molecular formula $C_7H_{16}ClNO_2$

Molecular weight 182 gmol^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.42 (s_{Br}, 3H, *H5,9*), 4.17 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 4.16 (dq, *J* = 7.2, 7.0 Hz, 1H, *H7*), 3.88 (d, *J* = 4.0 Hz, 1H, *H4*), 2.15 (qqd, *J* = 7.0, 7.0, 4.0 Hz, 1H, *H2*), 1.23 (t, *J* = 7.2 Hz, 3H, *H8*), 0.98 (d, *J* = 7.2 Hz, 3H, *H1/3*), 0.94 (d, *J* = 7.2 Hz, 3H, *H1/3*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 170.02 (*C*O,*C6*), 60.90 (O*C*H$_2$,*C7*), 57.16 (*C*H,*C4*), 29.28 (*C*H,*C2*), 18.17 (*C*H$_3$,*C1*/*C3*), 17.48 (*C*H$_3$,*C1*/*C3*), 14.02 (*C*H$_3$,*C8*)

DL-Phenylalanine methyl ester hydrochloride (244)



DL-Phenylalanine (3.002 g, 18.19 mmol) was added slowly to a solution of methanol (30 mL) and thionyl chloride (2.65 mL) at 0 ^{o}C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**244**) as a white solid 85 % yield (3.336 g, 15.44 mmol).

m.p. 152-153 °C, lit. m.p. 158-160 °C[20]

Molecular formula $C_{10}H_{14}ClNO_2$

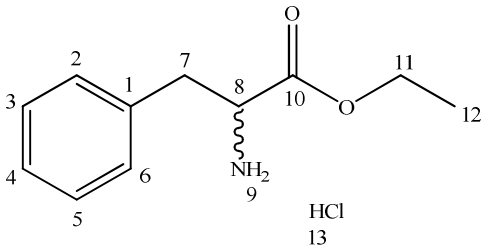
Molecular weight 216 gmol^{-1}

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.58 (s_{Br}, 3H, *H9,12*), 7.37-7.25 (m, 5H, *H2-6*), 4.24 (dd, *J* =7.8, 4.4 Hz, 1H, *H8*), 3.63 (s, 3H, *H11*), 3.22 (dd, *J* = 8.8, 6.6 Hz, 1H, *H7*), 3.07 (dd, *J* = 8.0, 6.4 Hz, 1H, *H7*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.40 (*C*O,*C10*), 134.68 (Ar*C*,*C1*), 129.50 (Ar*C*H), 128.49 (Ar*C*H), 127.19 (Ar*C*H,*C4*), 53.15 (*C*H,*C8*), 52.50 (O*C*H_3,*C11*), 35.80 (*C*H_2,*C7*)

NMR data was in agreement with the literature.[20]

DL-Phenylalanine ethyl ester hydrochloride (245)



DL-Phenylalanine (2.100 g, 10.45 mmol) was added slowly to a solution of ethanol (30 mL) and thionyl chloride (2.50 mL) at 0 °C. The solution was stirred at room temperature for 24 hours. Evaporation of solvent furnished the desired product (**245**) as a white solid 96 % yield (2.314 g, 10.06 mmol).

m.p. 140-142 °C, lit. m.p. 152-154 °C[22]

Molecular formula $C_{11}H_{16}ClNO_2$

Molecular weight 230 $gmol^{-1}$

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm) 8.73 (s_{Br}, 3H, *H9,13*), 7.36-7.24 (m, 5H, *H2-6*), 4.21 (dd, *J* = 8.0, 6.6 Hz, 1H, *H8*), 4.14 (dq, *J* = 7.2, 7.0 Hz, 1H, *H11*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H11*), 3.22 (dd, *J* = 8.8, 5.6 Hz, 1H, *H7*), 3.04 (dd, *J* = 8.0, 5.0 Hz, 1H, *H7*), 1.06 (t, *J* = 7.2 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 169.91 (*C*O,*C10*), 134.69 (Ar*C*,*C1*), 129.42 (Ar*C*H), 128.51 (Ar*C*H), 127.14 (Ar*C*H,*C4*), 61.53 (O*C*H_2,*C11*), 53.13 (*C*H,*C8*), 35.92 (*C*H_2,*C7*), 13.72 (*C*H_3,*C12*)

NMR data was in agreement with the literature.[22]

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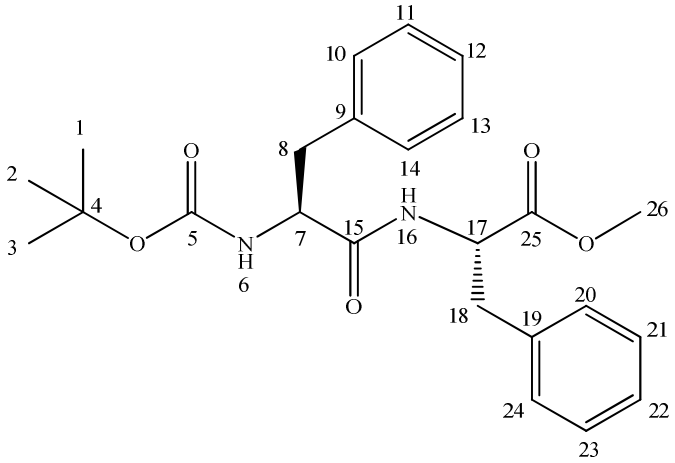
Appendix II

General procedures for the preparation of known starting materials for dipeptidyl Chiral Ionic Liquids (Chapter 4)

***General procedure for the preparation of starting materials for dipeptidyl Chiral Ionic Liquids*: *N-tert*-butyloxycarbonyl-L-phenylalanine-L-phenylalanine methyl ester (329)**



N-tert-butyloxycarbonyl-L-phenylalanine (3.011 g, 11.30 mmol) was dissolved in DCM (90mL) with *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.159 g, 11.30 mmol), 1-Hydroxybenzotriazole (1.526 g, 11.30 mmol), and triethylamine (1.139 g, 11.30 mmol). The reaction mixture was then cooled to 0 °C. L-phenylalanine methyl ester hydrochloride (**224**) (2.432 g, 11.30 mmol) was added after 30 mins and the reaction was stirred at room temperature for 24 hours. After this time, the organic phase was washed with distilled water, 10 % potassium hydrogen carbonate and 5 % citric acid then dried over $MgSO_4$. The organic solvent was then removed *via* rotary evaporation yielding a white solid. The crude product was recrystallised from ethyl acetate/pet. ether 40-60 °C, yielding the title compound (**329**) in 64 % yield (3.092 g, 7.26 mmol).

m.p. 115-117 °C, lit. m.p 114-116 °C,[1] $[\alpha]_D^{20}$ = -12.3 ° (0.9 c, EtOH) lit. $[\alpha]_D^{20}$ = -13.3 ° (1.0 c, MeOH)[1]

Molecular formula $C_{24}H_{30}N_2O_5$

Molecular weight 426 $gmol^{-1}$

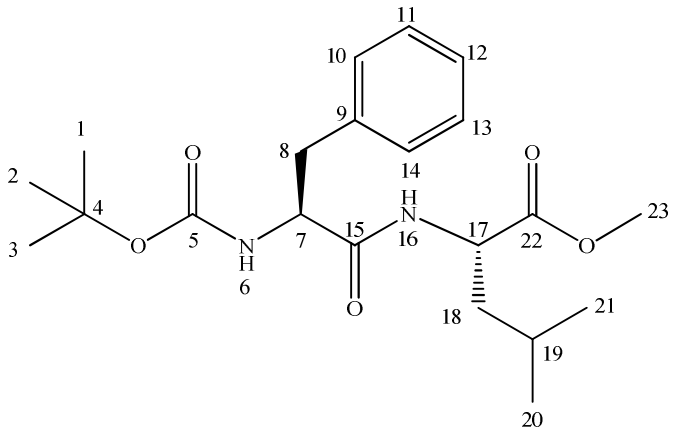
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.31-6.99 (m, 10H, *H10-14,20-24*), 6.29 (d, *J* = 7.2 Hz, 1H, *H6*), 4.95 (ddd, *J* = 7.4, 5.2, 5.2 Hz, 1H, *H7*), 4.81 (d, *J* = 6.0 Hz, 1H, *H16*), 4.34

(ddd, *J* = 6.4, 4.0, 4.0 Hz, 1H, *H17*), 3.69 (s, 3H, *H26*), 3.12-3.02 (m, 4H, *H8,18*), 1.42 (s, 9H, *H1-3*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.35 (*C*O,*C15*), 170.76 (*C*O,*C25*), 155.59 (*C*O,*C5*), 136.47 (Ar*C*,*C9*/*C19*), 135.62 (Ar*C*,*C9*/*C19*), 129.49 (Ar*C*H), 129.23 (Ar*C*H), 128.69 (Ar*C*H), 127.70 (Ar*C*H), 127.14 (Ar*C*H,*C12*/*C22*), 127.00 (Ar*C*H,*C12/C22*), 80.24 (*C_q*,*C4*), 55.69 (*C*H,*C7*), 53.29 (*C*H,*C17*), 52.31 (O*C*H_3,*C26*), 38.26 (*C*H_2,*C8*/*C18*), 37.97 (*C*H_2,*C8*/*C18*), 28.24 ((*C*H_3)$_3$,*C1*,*C2*,*C3*)

NMR data was in agreement with the literature.[1]

***N-tert*-Butyloxycarbonyl-L-phenylalanine-L-leucine methyl ester (330)**



The title compound (**330**) was prepared from *N-tert*-butyloxycarbonyl-L-phenylalanine (2.001 g, 7.55 mmol), L-leucine methyl ester hydrochloride (**235**) (1.367 g, 7.55 mmol), 1-hydroxybenzotriazole (1.020 g, 7.55 mmol), and triethylamine (0.755 g, 7.55 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.442 g, 7.55 mmol) according to the general procedure as a white solid in 90 % yield (2.651 g, 6.76 mmol).

m.p 92-94 °C, lit. m.p. 101-103 °C,[2] $[\alpha]_D^{20}$ = -14.9 ° (0.7 c, EtOH), lit. $[\alpha]_D^{20}$ = -17.5 ° (1.2 c, MeOH)[2]

Molecular formula $C_{21}H_{32}N_2O_5$

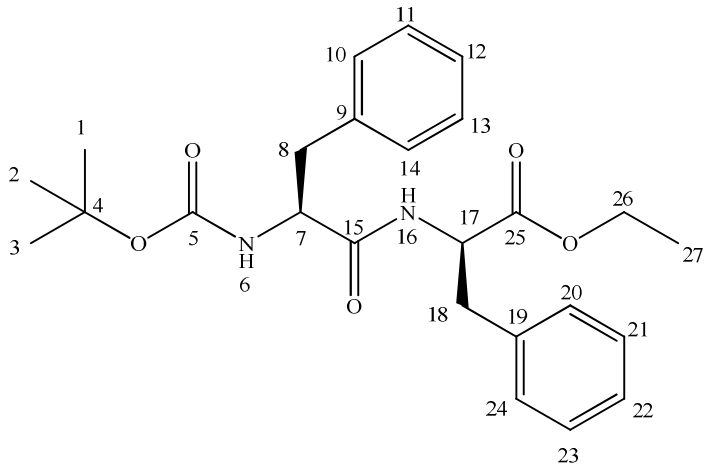
Molecular weight 392 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.25-7.12 (m, 5H, *H10-14*), 6.21 (d, *J* = 8.8 Hz, 1H, *H6*), 5.04 (d, *J* = 7.4 Hz, 1H, *H16*), 4.68 (ddd, *J* = 7.4, 7.4, 5.6 Hz, 1H, *H17*), 4.42 (ddd, *J* = 8.8, 6.0, 6.0 Hz, 1H, *H7*), 3.75 (s, 3H, *H23*), 2.97 (d, *J* = 6.8 Hz, 2H, *H8*), 1.66-1.53 (m, 3H, *H18,19*), 1.48 (s, 9H, *H1-3*), 0.98 (d, *J* = 6.8 Hz, 3H, *H20/21*), 0.91 (d, *J* = 6.8 Hz, 3H, *H20/21*).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.84 (*C*O,*C22*), 170.98 (*C*O,*C15*), 155.41 (*C*O,*C5*), 129.39 (Ar*C*,*C9*), 128.65 (Ar*C*H), 126.76 (Ar*C*H), 126.94 (Ar*C*H,*C12*), 80.14 (C_q,*C4*), 58.86 (*C*H,*C7*), 52.29 (*C*H,*C17*), 50.74 (O*C*H_3,*C23*), 41.62 (*C*H_2,*C18*), 37.40 (*C*H_2,*C8*) 28.25 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 24.65 (*C*H,*C19*), 22.76 (*C*H_3,*C20*/*C21*), 21.89 (*C*H_3,*C20*/*C21*)

NMR data was in agreement with the literature.[2]

***N-tert*-Butyloxycarbonyl-L-phenylalanine-D-phenylalanine ethyl ester (331)**



The title compound (**331**) was prepared from *N-tert*-butyloxycarbonyl-L-phenylalanine (1.013 g, 3.75 mmol), D-phenylalanine ethyl ester hydrochloride (**241**) (0.895 g, 3.75 mmol), 1-hydroxybenzotriazole (0.510 g, 3.75 mmol), and triethylamine (0.377 g, 3.75 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (0.722 g, 3.75 mmol) according to the general procedure as a colourless oil in 87 % yield (1.441 g, 3.27 mmol).

$[\alpha]_D^{20}$ = +8.0 ^{o} (0.7 c, EtOH), lit. $[\alpha]_D^{20}$ = +2.4 (4.0 c, in MeOH)[3]

Molecular formula $C_{25}H_{32}N_2O_5$

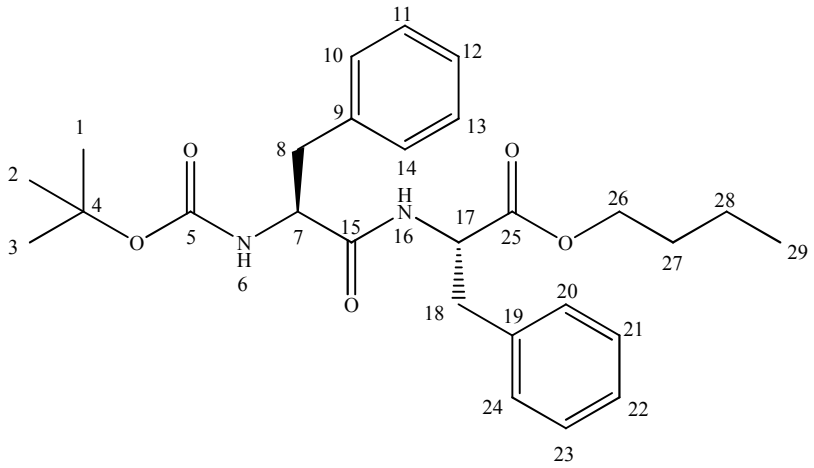
Molecular weight 440 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.29-6.91 (m, 10H, *H10-14,20-24*), 6.37 (d, *J* = 7.6 Hz, 1H, *H6*), 5.04 (d, *J* = 8.0 Hz, 1H, *H16*), 4.81 (ddd, *J* = 7.5, 5.2, 5.2 Hz, 1H, *H7*), 4.41 (ddd, *J* = 7.8, 6.0, 6.0 Hz, 1H, *H17*), 4.14 (dq, *J* = 7.2, 7.2 Hz, 1H, *H26*), 4.13 (dq, *J* = 7.2, 7.2 Hz, 1H, *H26*), 3.11-2.91 (m, 4H, *H8,18*), 1.39 (s, 9H, *H1-3*), 1.23 (t, *J* = 7.0 Hz, 3H, *H27*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.11 (*C*O,*C15*), 170.32 (*C*O,*C25*), 155.35 (*C*O,*C5*), 136.61 (Ar*C*,*C9*/*C19*), 135.61 (Ar*C*,*C9*/*C19*), 129.35 (Ar*C*H), 129.25 (Ar*C*H), 128.67 (ArCH), 128.56 (Ar*C*H), 127.12 (ArCH,*C12/C22*), 126.98 (Ar*C*H, *C12/C22*), 80.22 (*C_q*,*C4*), 61.49 (O*C*H_2,*C26*), 55.76 (*C*H,*C7*), 53.11 (*C*H,*C17*), 38.45 (*C*H_2,*C8*/*C18*), 37.96 (*C*H_2,*C8*/*C18*), 28.24 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 14.07 (*C*H_3,*C27*)

NMR data was in agreement with the literature.[4]

***N-tert*-Butyloxycarbonyl-L-phenylalanine-L-phenylalanine butyl ester (332)**



The title compound (**332**) was obtained from *N-tert*-butyloxycarbonyl-L-phenylalanine (2.011 g, 7.50 mmol), L-phenylalanine butyl ester hydrochloride (**225**) (1.781 g, 7.50 mmol), 1-hydroxybenzotriazole (1.012 g, 7.50 mmol), and triethylamine (0.755 g, 7.50 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.444 g,

7.50 mmol) according to the general procedure as a white solid in 85 % yield (2.987 g, 6.38 mmol).

m.p 102-104 °C, $[\alpha]_D^{20} = +30.8^{o}$ (0.7 c, $CHCl_3$)

Molecular formula $C_{27}H_{36}N_2O_5$

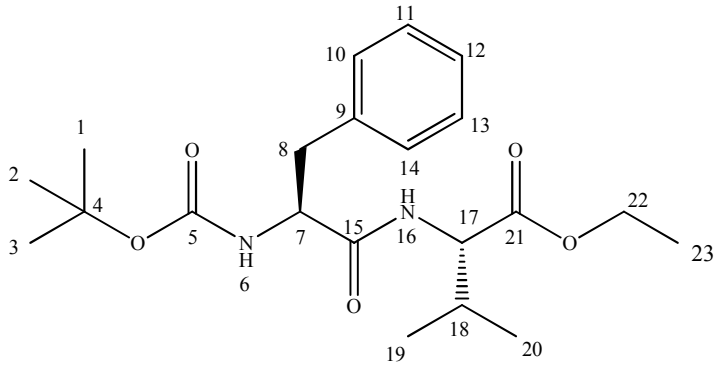
Molecular weight 468 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.20-6.91 (m, 10H, *H10-14,20-24*), 6.23 (d, *J* = 6.2 Hz, 1H, *H6*), 4.85 (ddd, *J* = 6.0, 5.0, 5.0 Hz, 1H, *H7*), 4.71 (d, *J* = 6.8 Hz, 1H, *H16*), 4.26 (ddd, *J* = 7.0, 6.0, 6.0 Hz, 1H, *H17*), 4.09 (q, *J* = 7.2 Hz, 2H, *H26*), 3.03-3.92 (m, 4H, *H8,18*), 1.55-1.39 (m, 2H, *H27*), 1.32 (s, 9H, *H1-3*), 1.25- 1.14 (m, 2H, *H28*), 0.87 (t, *J* = 7.4 Hz, *H29*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.03 (*C*O,*C15*), 170.81 (*C*O,*C25*), 155.30 (*C*O,*C5*), 136.52 (Ar*C*,*C9*/*C19*), 135.71 (Ar*C*,*C9*/*C19*), 129.38 (Ar*C*H), 129.29 (Ar*C*H), 128.66 (Ar*C*H), 128.51 (Ar*C*H), 127.07 (Ar*C*H,*C12*/*C22*), 126.98 (Ar*C*H,*C12*/*C22*), 80.21 (C_q,*C4*), 65.36 (O*C*H_2,*C26*), 55.69 (*C*H,*C7*), 53.37 (*C*H,*C17*), 38.29 (*C*H_2,*C8*/C18), 38.09 (*C*H_2,*C8*/*C18*), 30.43 (*C*H_2,*C27*), 28.25 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.02 (*C*H_2,*C28*), 13.67 (*C*H_3,*C29*)

MS (*m/z*) Found $[M+H]^+$ 469.2689, $C_{27}H_{37}N_2O_5^+$ requires 469.2702

***N-tert*-Butyloxycarbonyl-L-phenylalanine-L-valine ethyl ester (333)**



The title compound (**333**) was prepared from *N-tert*-butyloxycarbonyl-L-phenylalanine (2.001 g, 7.55 mmol), L-valine ethyl ester hydrochloride (**229**) (1.261 g, 7.55 mmol), 1-Hydroxybenzotriazole (catalytic amount), and triethylamine (0.755 g, 7.55 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.442 g, 7.55 mmol) according to the general procedure as a colourless oil in 71 % yield (2.122 g, 5.41 mmol).

$[\alpha]_D^{20} = -18.0\ ^{o}$ (0.7 c, EtOH), lit. $[\alpha]_D^{20} = -15.5\ ^{o}$ (2.0 c, MeOH)[5]

Molecular formula $C_{21}H_{32}N_2O_5$

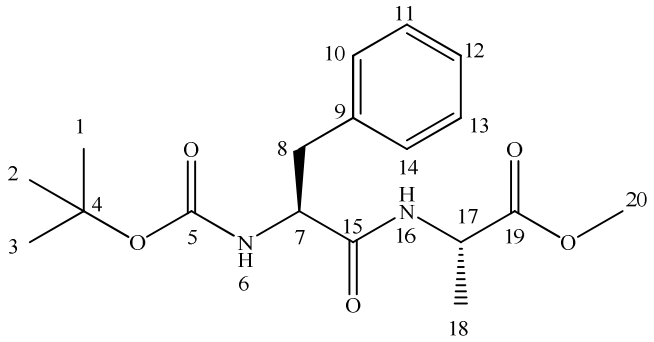
Molecular weight 392 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.34-7.23 (m, 6H, *H6,10-14*), 6.37 (d, *J* = 8.4, 1H, *H16*), 4.97 (ddd, *J* = 7.0, 5.0, 5.0 Hz, 1H, *H7*), 4.46 (dd, *J* = 8.4, 4.8 Hz, 1H, *H17*), 4.18 (q, *J* = 7.2 Hz, 2H, *H22*), 3.11 (dd, *J* = 14.1, 6.8 Hz, 1H, *H8*), 3.05 (dd, *J* = 14.1, 6.8 Hz, 1H, *H8*), 2.16 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H18*), 1.44 (s, 9H, *H1-3*), 1.21 (t, *J* = 7.0 Hz, 3H, H23), 0.90 (d, *J* = 6.8 Hz, 3H, *H19/20*), 0.86 (d, *J* = 6.8 Hz, 3H, *H19/20*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 171.79 (*C*O,*C15*), 171.16 (*C*O,*C21*), 155.46 (*C*O,*C5*), 136.61 (Ar*C*,*C9*), 129.34 (Ar*C*H), 128.68 (Ar*C*H), 126.92 (Ar*C*H,*C12*), 80.23 (C_q,*C4*), 61.01 (O*C*H_2,*C22*), 57.24 (*C*H,*C17*), 55.86 (*C*H,*C7*), 37.98 (*C*H_2,*C8*), 31.29 (*C*H,*C18*), 28.26 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 18.82 (*C*H_3,*C19/C20*), 17.75 (*C*H_3,*C19/C20*), 14.03 (*C*H_3,*C23*).

NMR data was in agreement with the literature.[5]

***N-tert*-Butyloxycarbonyl-L-phenylalanine-L-alanine methyl ester (334)**



The title compound (**334**) was prepared from *N*-tert-butyloxycarbonyl-L-phenylalanine (2.653 g, 10.00 mmol), L-alanine methyl ester hydrochloride (**226**) (1.395 g, 10.00 mmol), 1-hydroxybenzotriazole (catalytic amount), and triethylamine (1.018 g, 10.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.911 g, 10.00 mmol) according to the general procedure as a white solid in 61 % yield (2.142 g, 6.12 mmol).

m.p 96-98 °C, lit. m.p. 99-101 °C,[6] $[\alpha]_D^{20}$ = -10.0 ^{o} (0.8 c, EtOH), lit. $[\alpha]_D^{20}$ = -18.0 (1.0 c, MeOH)[7]

Molecular formula $C_{18}H_{26}N_2O_5$

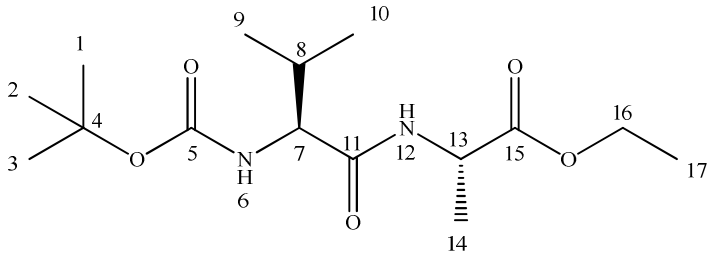
Molecular weight 350 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.25-7.13 (m, 6H, *H6,10-14*), 6.37 (d, *J* = 7.2, 1H, *H16*), 4.93 (ddd, *J* = 8.0, 6.4, 6.0 Hz, 1H, *H7*), 4.48 (dq, *J* = 7.2, 7.0 Hz, 1H, *H17*), 3.65 (s, 1H, *H20*), 3.10 (dd, *J* = 13.0, 6.6 Hz, 2H, *H8*), 3.04 (dd, *J* = 13.0, 6.0 Hz, 2H, *H8*), 1.34 (s, 9H, *H1-3*), 1.28 (d, *J* = 6.8 Hz, 3H, *H18*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.85 (*C*O,*C15*), 170.71 (*C*O,*C19*), 155.36 (*C*O,*C5*), 136.49 (Ar*C*,*C9*), 129.34 (Ar*C*H), 128.67 (Ar*C*H), 126.98 (Ar*C*H,*C12*), 80.10 (C_q,*C4*), 57.48 (*C*H,*C7*), 52.47 (O*C*H_3,*C20*), 48.11 (*C*H,*C17*), 38.35 (*C*H_2,*C8*), 28.25 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 18.42 (*C*H_3,*C18*)

NMR data was in agreement with the literature.[6]

***N-tert*-Butyloxycarbonyl-L-valine-L-alanine ethyl ester (335)**



The title compound (**335**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.171 g, 10.00 mmol), L-alanine ethyl ester hydrochloride (**227**) (1.536 g, 10.00 mmol), 1-

hydroxybenzotriazole (1.340 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a colourless oil in 77 % yield (2.449 g, 7.75 mmol).

$[\alpha]_D^{20}$ = -12.4 ^{o} (0.5 c, $CHCl_3$), lit. $[\alpha]_D^{20}$ = -8.3 (1.0 c, $CHCl_3$)[8]

Molecular formula $C_{15}H_{28}N_2O_5$

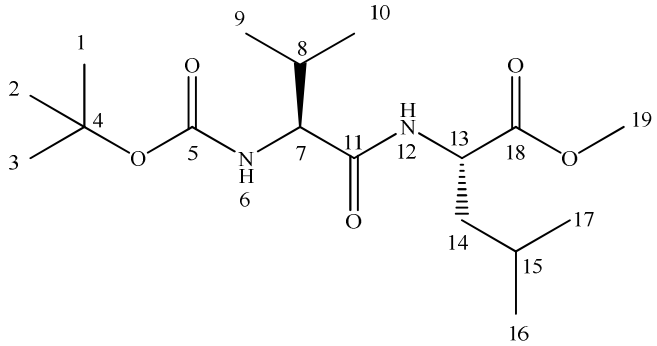
Molecular weight 316 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.52 (d, *J* = 6.8 Hz, 1H, *H12*), 5.10 (d, *J* = 8.8 Hz, 1H, *H6*), 4.50 (dq, *J* = 7.2, 7.0 Hz, 1H, *H13*), 4.24 (q, *J* = 7.2 Hz, 2H, *H16*), 3.88 (dd, *J* = 8.4, 4.8 Hz, 1H, *H7*), 2.18 (qqd, *J* = 6.8, 6.8, 4.2 Hz, 1H, *H8*), 1.37 (s, 9H, *H1-3*), 1.33 (d, *J* = 7.0 Hz, 3H, *H14*), 1.20 (t, *J* = 7.2 Hz, *H17*), 1.00 (d, *J* = 6.8 Hz, 3H, *H9/10*), 0.94 (d, *J* = 6.8 Hz, 3H, *H9/10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 174.45 (*C*O,*C15*), 171.22 (*C*O,*C11*), 155.88 (*C*O,*C5*), 79.84 (C_q,*C4*), 61.45 (*C*H,*C7*), 59.81 (O*C*H_2,*C16*), 48.08 (*C*H,*C13*), 31.19 (*C*H,*C8*), 28.30 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.16 (*C*H_3,*C9/C10*), 18.28 (*C*H_3,*C9/C10*), 17.78 (*C*H_3,*C14*), 14.08 (*C*H_3,*C17*)

NMR data was in agreement with the literature.[8]

***N-tert*-Butyloxycarbonyl-L-valine-L-leucine methyl ester (336)**



The title compound (**336**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.001 g, 9.20 mmol), L-leucine methyl ester hydrochloride (**235**) (1.666 g, 9.20 mmol), 1-hydroxybenzotriazole (1.240 g, 9.20 mmol), and triethylamine (0.929 g, 9.20 mmol) and *N*-

[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.76 g, 9.20 mmol) according to the general procedure as a white solid in 82 % yield (2.614 g, 7.60 mmol).

m.p 122-124 °C, lit m.p 126-128 °C,[3] $[\alpha]_D^{20}$ = -36.0 ^{o} (0.2 c, EtOH), lit. $[\alpha]_D^{20}$ = -53.0 (1.0 c, MeOH)[3]

Molecular formula $C_{17}H_{32}N_2O_5$

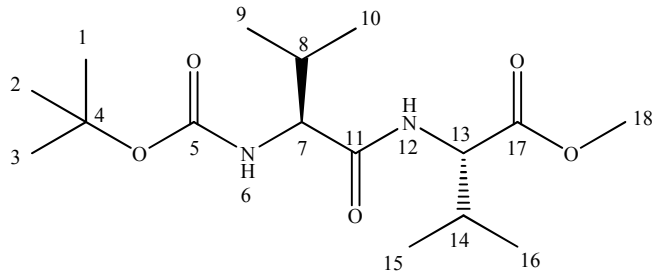
Molecular weight 344 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.18 (d, *J* = 7.2 Hz, 1H, *H12*), 4.99 (d, *J* = 8.0 Hz, 1H, *H6*), 4.53 (dd, *J* = 8.0, 4.8 Hz, 1H, *H7*), 3.74 (ddd, *J* = 7.2, 7.0, 5.2 Hz, 1H, *H13*), 3.62 (s, 1H, *H19*), 2.19 (qqd, *J* = 6.8, 6.8, 4.4 Hz, 1H, *H8*), 1.68-1.56 (m, 3H, *H14,15*), 1.46 (s, 9H, *H1-3*), 1.00 (dd, *J* = 6.8, 6.0 Hz, 6H, *H9,10*), 0.96 (d, *J* = 6.8 Hz, 3H, *H16/17*), 0.88 (d, *J* = 6.8 Hz, 3H, *H16/17*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.17 (*C*O,*C18*), 171.71 (*C*O,*C12*), 155.86 (*C*O,*C5*), 79.67 (C_q,*C4*), 59.76 (*C*H,*C7*), 52.15 (*C*H,*C13*), 50.66 (O*C*H$_3$,*C19*), 41.17 (*C*H$_2$,*C14*), 30.92 (*C*H,*C8*), 28.26 ((*C*H$_3$)$_3$,*C1*,*C2*,*C3*), 24.70 (*C*H,*C15*), 22.73 (*C*H$_3$,*C16/C17*), 21.79 (*C*H$_3$,*C16*/*C17*), 17.95 (*C*H$_3$,*C9/C10*), 17.42 (*C*H$_3$,*C9/C10*)

NMR data was in agreement with the literature.[9]

***N-tert*-Butyloxycarbonyl-L-valine-L-valine methyl ester (337)**



The title compound (**337**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.170 g, 10.0 mmol), L-valine methyl ester hydrochloride (**229**) (1.666 g, 10.00 mmol), 1-hydroxybenzotriazole (1.352 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and

N-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.0 mmol) according to the general procedure as a white solid in 78 % yield (2.561 g, 7.76 mmol).

m.p. 152-154 °C, lit. m.p. 157-159 °C,[5] $[\alpha]_D^{20}$ = -40.0 ^{o} (0.6 c, $CHCl_3$) lit. $[\alpha]_D^{20}$ = -44.0 ^{o} (0.4 c, $CHCl_3$)[10]

Molecular formula $C_{16}H_{30}N_2O_5$

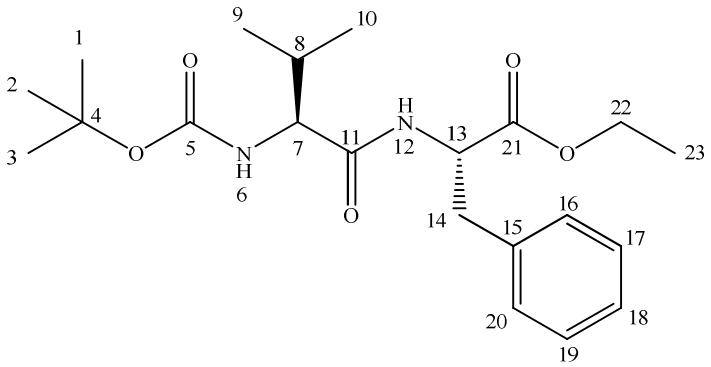
Molecular weight 330 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.29 (d, *J* = 7.2 Hz, 1H, *H12*), 4.96 (d, *J* = 8.0 Hz, 1H *H6*), 4.49 (dd, *J* = 8.8, 6.8 Hz, 1H, *H7*), 3.82 (dd, *J* = 7.8, 5.8 Hz, 1H, *H13*), 3.67 (s, 3H, *H18*), 2.22-2.03 (m, 2H, *H8,14*), 1.38 (s, 9H, *H1-3*), 0.90-0.82 (m, 12H, *H9,10,15,16*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.16 (*C*O,*C17*), 171.57 (*C*O,*C11*), 153.84 (*C*O,*C5*), 79.93 (C_q,*C4*), 60.19 (*C*H,*C7*), 57.04 (*C*H,*C13*), 52.17 (O*C*H_3,*C18*), 31.26 (*C*H,*C8/C14*), 30.63 (*C*H,*C8/C14*), 28.30 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.28, 18.94, 17.88, 17.73 (*C*H_3, *C9,C10,C15,C16*)

NMR data was in agreement with the literature.[11]

***N-tert*-Butyloxycarbonyl-L-valine-L-phenylalanine ethyl ester (338)**



The title compound (**338**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.170 g, 10.00 mmol), L-phenylalanine ethyl ester hydrochloride (**224**) (2.297 g, 10.00 mmol), 1-hydroxybenzotriazole (1.352 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and

N-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a white solid in 84 % yield (3.278 g, 8.36 mmol).

m.p 100-102 °C, lit. m.p. 110-112 °C,[5] $[\alpha]_D^{20}$ = +32.2 ° (0.6 c, $CHCl_3$) lit. $[\alpha]_D^{20}$ = +31.1 ° (1.0 c. CH_2Cl_2)[5]

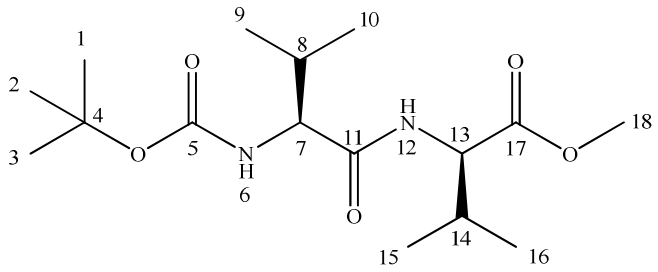
Molecular formula $C_{21}H_{32}N_2O_5$

Molecular weight 392 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.24-6.99 (m, 5H, *H16-20*), 6.34 (d, *J* = 7.8 Hz, 1H, *H12*), 5.00 (d, *J* = 8.4 Hz, 1H *H6*), 4.76 (dd, *J* = 8.4, 4.8 Hz, 1H, *H7*), 4.06 (q, *J* = 7.0 Hz, 2H, *H22*), 3.82 (ddd, *J* = 8.0, 6.0, 6.0 Hz, 1H, *H13*), 3.09 (dd, *J* = 8.0, 5.2 Hz, 1H, *H14*), 2.99 (dd, *J* = 13.6, 6.4 Hz, 1H, *H14*), 2.00 (qqd, *J* = 7.0, 6.8, 4.4 Hz, 1H, *H8*), 1.37 (s, 9H, *H1-3*), 1.14 (t, *J* = 7.2 Hz, 3H, *H23*), 0.84 (d, *J* = 7.2 Hz, 3H, *H9/H10*), 0.79 (d, *J* = 6.0 Hz, 3H, *H9*/*H10*)

^{13} C NMR (100 MHz, $CDCl_3$) δ (ppm) 171.53 (*C*O,*C21*), 170.70 (*C*O,*C11*), 155.77 (*C*O,*C5*), 135.78 (Ar*C*,*C15*), 129.31 (Ar*C*H), 128.55 (Ar*C*H), 127.10 (Ar*C*H,*C18*), 79.86 (C_q,*C4*), 61.48 (*C*H,*C7*), 59.94 (O*C*H_2,*C22*), 53.51 (*C*H,*C13*), 38.05 (*C*H_2,*C14*), 31.23 (*C*H,*C8*), 28.30 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.14 (*C*H_3,*C9/C10*), 17.74 (*C*H_3,*C9*/*C10*), 14.05 (*C*H_3,*C23*)

***N-tert*-Butyloxycarbonyl-L-valine-D-valine methyl ester (339)**



The title compound (**339**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.170 g, 10.0 mmol), D-valine methyl ester hydrochloride (**238**) (1.666 g, 10.00 mmol), 1-hydroxybenzotriazole (1.352 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and

N-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a white solid in 87 % yield (2.863 g, 8.67 mmol).

m.p. 118-120 °C, lit. m.p. 110-112 °C,[12] $[\alpha]_D^{20}$ = -18.4 ^{o} (0.7 c, $CHCl_3$), lit. $[\alpha]_D^{20}$ = -23.7 ^{o} (1.0 c, $CHCl_3$)[12]

Molecular formula $C_{16}H_{30}N_2O_5$

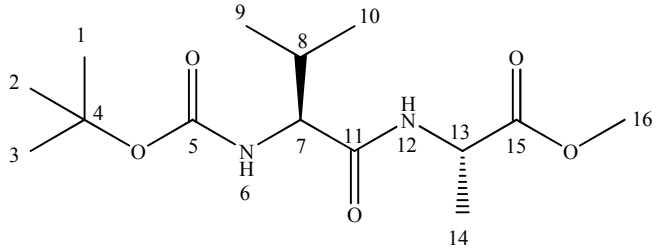
Molecular weight 330 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.61 (d, *J* = 8.0 Hz, 1H, *H6*), 5.09 (d, *J* = 6.8 Hz, 1H *H12*), 4.49 (dd, *J* = 8.0, 4.0 Hz, 1H, *H7*), 3.86 (dd, *J* = 6.8, 4.8 Hz, 1H, *H13*), 3.66 (s, 3H, *H18*), 2.17-2.08 (m, 2H, *H8,14*), 1.38 (s, 9H, *H1-3*), 0.92-0.82 (m, 12H, *H9,10,15,16*)

^{13} C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.29 (*C*O,*C17*), 171.65 (*C*O,*C11*), 155.81 (*C*O,*C5*), 79.92 (C_q,*C4*), 59.89 (*C*H,*C7*), 57.03 (*C*H,*C13*), 52.16 (O*C*H_3,*C18*), 31.22 (*C*H,*C8*), 30.72 (*C*H,*C14*), 28.28 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.36, 19.00, 17.74, 17.49 (*C*H_3,*C9,C10,C15,C16*)

NMR data was in agreement with the literature.[12]

***N-tert*-Butyloxycarbonyl-L-valine-L-alanine methyl ester (340)**



The title compound (**340**) was prepared from *N-tert*-butyloxycarbonyl-L-valine (2.170 g, 10.00 mmol), L-alanine methyl ester hydrochloride (**226**) (1.390 g, 10.00 mmol), 1-hydroxybenzotriazole (1.352 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a white solid in 80 % yield (2.424 g, 8.05 mmol).

m.p. 103-105 °C, lit m.p 112-116 °C,[13] $[\alpha]_D^{20}$ = -53.3 ^{o} (0.4 c, $CHCl_3$) lit. $[\alpha]_D^{20}$ = -60.9 ^{o} (1.0 c, H_2O)[13]

Molecular formula $C_{14}H_{26}N_2O_5$

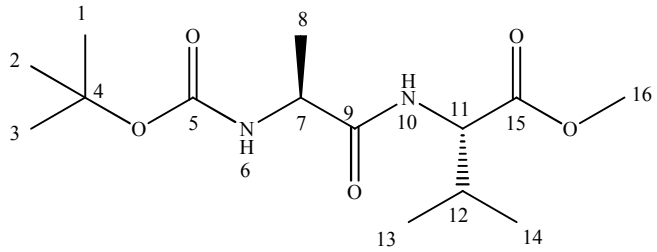
Molecular weight 302 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.73 (d, *J* = 6.4 Hz, 1H, *H12*), 5.20 (d, *J* = 8.8 Hz, 1H, *H6*), 4.54 (dd, *J* = 8.4, 4.4 Hz, 1H, *H7*), 3.92 (dq, *J* = 7.4, 7.2 Hz, 1H, *H13*), 3.66 (s, 3H, *H16*), 2.09 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H8*), 1.37 (s, 9H, *H1-3*), 1.34 (d, *J* = 7.2 Hz, 3H, *H14*), 0.91 (d, *J* = 6.8 Hz, 3H, *H9/10*), 0.87 (d, *J* = 6.8 Hz, 3H, *H9/10*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.18 (*C*O,*C15*), 171.37 (*C*O,*C11*), 155.90 (*C*O,*C5*), 79.77 (C_q,*C4*), 59.67 (*C*H,*C7*), 52.37 (O*C*H_3,*C16*), 47.94 (*C*H,*C13*), 31.13 (*C*H,*C8*), 28.28 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.13 (*C*H_3,*C9/C10*), 18.07 (*C*H_3,*C9/C10*), 17.81 (*C*H_3,*C14*)

NMR data was in agreement with the literature.[14]

***N-tert*-Butyloxycarbonyl-L-alanine-L-valine methyl ester (341)**



The title compound (**341**) was prepared from *N-tert*-butyloxycarbonyl-L-alanine (1.891 g, 10.00 mmol), L-valine methyl ester hydrochloride (**229**) (1.666 g, 10.00 mmol), 1-hydroxybenzotriazole (1.350 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a white solid in 85 % yield (2.561 g, 8.48 mmol).

m.p. 78-80 °C, lit. m.p. 72-75 °C,[15] $[\alpha]_D^{20}$ = -37.4 ^{o} (0.3 c, EtOH) lit. $[\alpha]_D^{20}$ = -45.5 ^{o} (2.8 c, MeOH)[15]

Molecular formula $C_{14}H_{26}N_2O_5$

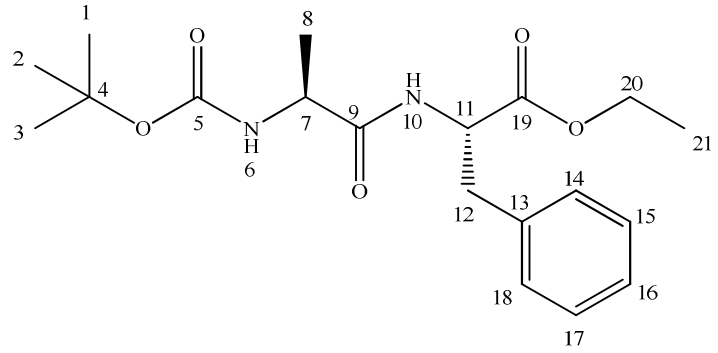
Molecular weight 302 $gmol^{-1}$

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.55 (d, *J* = 7.4 Hz, 1H, *H6*), 4.87 (d, *J* = 8.8 Hz, 1H *H10*), 4.48 (dd, *J* = 8.0, 4.4 Hz, 1H, *H11*), 4.01 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.66 (s, 3H, *H16*), 2.11 (qqd, *J* = 6.8, 6.8, 4.8 Hz, 1H, *H12*), 1.38 (s, 9H, *H1-3*), 1.30 (d, *J* = 7.2 Hz, 3H, *H8*), 0.92 (d, *J* = 6.8 Hz, 3H, *H13/14*), 0.85 (d, *J* = 6.8 Hz, 3H, *H13/14*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.62 (*C*O,*C15*), 172.18 (*C*O,*C9*), 155.58 (*C*O,*C5*), 80.07 (C_q,*C4*), 57.06 (*C*H,*C11*), 52.06 (*C*H,*C7*), 50.01 (O*C*H_3,*C16*), 31.22 (*C*H,*C12*), 28.28 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 18.89 (*C*H_3,*C13/*C14), 18.71 (*C*H_3,*C13/C14*), 17.62 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[15]

***N-tert*-Butyloxycarbonyl-L-alanine-L-phenylalanine ethyl ester (342)**



The title compound (**342**) was prepared from *N-tert*-butyloxycarbonyl-L-alanine (1.891 g, 10.00 mmol), L-phenylalanine ethyl ester hydrochloride (**224**) (2.289 g, 10.00 mmol), 1-hydroxybenzotriazole (1.350 g, 10.00 mmol), and triethylamine (1.009 g, 10.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.922 g, 10.00 mmol) according to the general procedure as a white solid in 76 % yield (2.767 g, 7.60 mmol).

m.p 93-95 °C, lit. m.p. 100-102 °C,[16] $[\alpha]_D^{20}$ = +24.1 ^{o} (1.0 c, $CHCl_3$), lit. $[\alpha]_D^{20}$ = +30.0 ^{o} (0.9 c, $CHCl_3$)[16]

Molecular formula $C_{19}H_{28}N_2O_5$

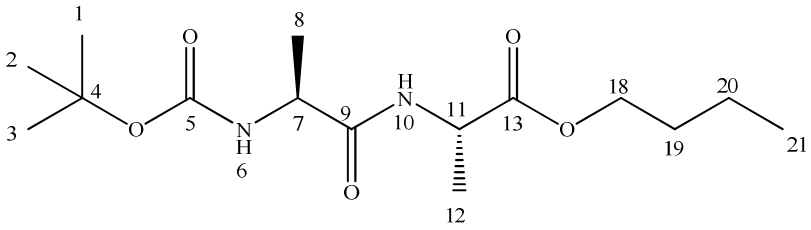
Molecular weight 364 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.28-7.05 (m, 5H, *H14-18*), 6.47 (d, *J* = 7.2 Hz, 1H, *H6*), 4.90 (s_B, 1H *H10*), 4.74 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 4.13-4.03 (m, 3H, *H11,20*), 2.16-2.05 (m, 1H, *H12*), 1.36 (s, 9H, *H1-3*), 1.24 (d, *J* = 7.2 Hz, 3H, *H8*), 1.15 (t, *J* = 7.2 Hz, 3H, *H21*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.19 (*C*O,*C19*), 171.23 (*C*O,*C9*), 155.32 (*C*O,*C5*), 135.86 (Ar*C*,*C13*), 129.36 (Ar*C*H), 128.50 (Ar*C*H), 127.07 (Ar*C*H,*C16*), 80.05 (C_q,*C4*), 61.48 (O*C*H_2,*C20*), 53.22 (*C*H,*C11*), 50.10 (*C*H,*C7*), 37.98 (*C*H_2,*C12*), 28.30 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 18.35 (*C*H_3,*C8*), 14.07 (*C*H_3,*C21*)

NMR data was in agreement with the literature.[16]

***N-tert*-Butyloxycarbonyl-L-alanine-L-alanine butyl ester (343)**



The title compound (**343**) was prepared from *N-tert*-butyloxycarbonyl-L-alanine (1.131 g, 6.00 mmol), L-alanine butyl ester hydrochloride (**228**) (1.082 g, 6.00 mmol), 1-hydroxybenzotriazole (0.810 g, 6.00 mmol), and triethylamine (0.609 g, 6.00 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (1.154 g, 6.00 mmol) according to the general procedure as a white solid in 76 % yield (1.445 g, 4.57 mmol).

m.p 68-70 °C, $[\alpha]_D^{20}$ = -17.0 ^{o} (1.0 c, $CHCl_3$)

Molecular formula $C_{15}H_{28}N_2O_5$

Molecular weight 316 gmol^{-1}

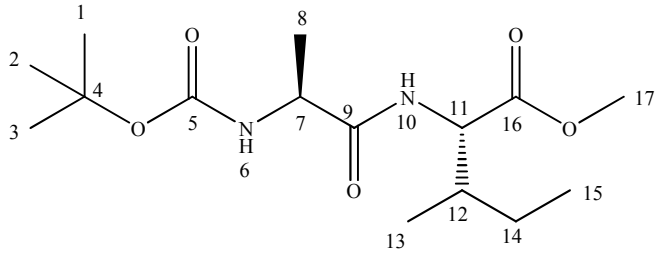
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.82 (d, *J* = 7.6 Hz, 1H, *H6*), 5.20 (d, *J* = 7.2 Hz, 1H *H10*), 4.74 (dq, *J* = 7.4, 7.2 Hz, 1H, *H7*), 4.21-4.08 (m, 3H, *H11,18*), 1.62 (tt, *J* = 7.2, 7.0

Hz, 1H, *H19*), 1.38 (s, 9H, *H1-3*), 1.40 (d, *J* = 7.4 Hz, 3H, *H8*), 1.37 (d, *J* = 6.8 Hz, 3H, *H12*), 1.30 (tq, *J* = 7.4, 7.2 Hz, 2H, *H20*), 0.92 (t, *J* = 7.4 Hz, 3H, *H21*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 172.82 (*C*O,*C13*), 172.32 (*C*O,*C9*), 155.45 (*C*O,*C5*), 80.00 (C_q,*C4*), 65.31 (O*C*H_2,*C18*), 49.93 (*C*H,*C11*), 48.09 (*C*H,*C7*), 30.50 (*C*H_2,*C19*), 28.28 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 19.00 (*C*H_2,*C20*), 18.45 (*C*H_3,*C8*), 18.31 (*C*H_3,*C12*), 13.64 (*C*H_3,*C21*)

MS (*m/z*) Found $[M+H^-]^+$ 317.2066, $C_{15}H_{29}N_2O_5^+$ requires 317.2076

***N-tert*-Butyloxycarbonyl-L-alanine-L-isoleucine methyl ester (344)**



The title compound (**344**) was prepared from *N-tert*-butyloxycarbonyl-L-alanine (2.428 g, 12.90 mmol), L-isoleucine methyl ester hydrochloride (**232**) (2.330 g, 12.90 mmol), 1-hydroxybenzotriazole (1.741 g, 12.90), and triethylamine (1.301 g, 12.90 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.474 g, 12.90 mmol) according to the general procedure as a white solid in 46 % yield (1.898 g, 6.01 mmol).

m.p 90-92 °C, $[\alpha]_D^{20}$ = -40.1 ^{o} (0.8 c, $CHCl_3$), lit. $[\alpha]_D^{20}$ = -47.8 ^{o} (1.1 c, $CHCl_3$)[17]

Molecular formula $C_{15}H_{28}N_2O_5$

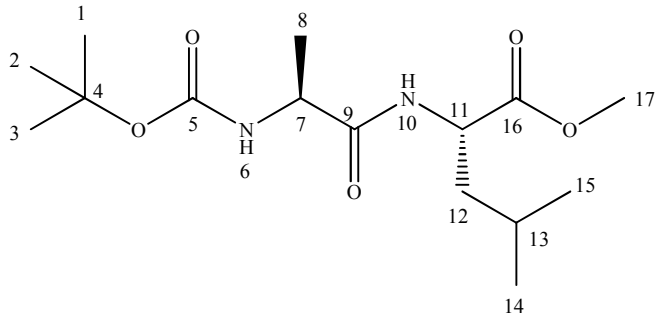
Molecular weight 316 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 6.46 (d, *J* = 6.8 Hz, 1H, *H6*), 4.91 (d, *J* = 8.8 Hz, 1H, *H10*), 4.40 (dq, *J* = 7.2, 7.0 Hz, 1H, *H7*), 4.04 (dd, *J* = 8.8, 6.8 Hz, 1H, *H11*), 3.55 (s, 3H, *H17*), 1.52-1.35 (m, 3H, *H12,14*), 1.27 (s, 9H, *H1-3*), 1.16 (d, *J* = 6.8 Hz, 3H, *H8*), 0.89 (t, *J* = 7.2 Hz, 3H, *H15*), 0.85 (d, *J* = 6.8 Hz, 3H, *H13*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.23 (*C*O,*C9*), 172.40 (*C*O,*C16*), 155.52 (*C*O,*C5*), 80.11 (C_q,*C4*), 52.29 (*C*H,*C11*), 50.63 (*C*H,*C7*), 49.86 (O*C*H_3, *C17*), 41.47 (*C*H,*C12*), 28.27 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 24.73 (*C*H_2,*C14*), 22.84 (*C*H_3,*C13*), 21.79 (*C*H_3, *C15*), 17.88 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[17]

***N-tert*-Butyloxycarbonyl-L-alanine-L-leucine methyl ester (345)**



The title compound (**345**) was prepared from *N-tert*-butyloxycarbonyl-L-alanine (2.001 g, 10.60 mmol), L-leucine methyl ester hydrochloride (**235**) (1.923 g, 10.60 mmol), 1-hydroxybenzotriazole (1.350, 10.60 mmol), and triethylamine (1.067 g, 10.60 mmol) and *N*-[(3-dimethylamino)propyl]-*N*'-ethyl carbodiimide hydrochloride (2.031 g, 10.60 mmol) according to the general procedure in 60 % yield (2.013 g, 6.37 mmol).

m.p 69-71 °C, lit. m.p. 66-68 °C,[18] $[\alpha]_D^{20}$ = -24.5 ^{o} (0.8 c, $CHCl_3$), lit. $[\alpha]_D^{20}$ = -32. ^{o} (1.2 c, CH_2Cl_2)[19]

Molecular formula $C_{15}H_{28}N_2O_5$

Molecular weight 316 gmol^{-1}

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 8.12 (d, *J* = 7.0 Hz, 1H, *H6*), 6.89 (d, *J* = 8.0 Hz, 1H, *H10*), 4.32 (ddd, *J* = 8.0, 8.0, 5.4 Hz, 1H, *H11*), 3.98 (dq, *J* = 7.2, 7.2 Hz, 1H, *H7*), 3.61 (s, 3H, *H17*), 1.70-1.44 (m, 2H, *H12,13*), 1.37 (s, 9H, *H1-3*), 1.16 (d, *J* = 6.8 Hz, 3H, *H8*), 0.89 (d, *J* = 6.4 Hz, *H14/15*), 0.83 (d, *J* = 6.8 Hz, *H14/15*)

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 173.23 (*C*O,*C16*), 172.51 (*C*O,*C9*), 155.52 (*C*O,*C5*), 79.97 (C_q,*C4*), 53.45 (*C*H,*C11*), 52.24 (*C*H,*C7*), 50.61 (O*C*H_3,*C17*), 41.35 (*C*H_2,*C12*), 28.25 ((*C*H_3)$_3$,*C1*,*C2*,*C3*), 24.27 (*C*H,*C13*), 22.81 (*C*H_3,*C14/C15*), 21.74 (*C*H_3,*C14/C15*), 18.02 (*C*H_3,*C8*)

NMR data was in agreement with the literature.[18]

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Appendix III

Publications