In situ Enzymatic Conversion of Biomass into Glucose in Biodegradable Enzyme Compatible Ionic Liquids

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work, and 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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List of Abbreviations:

A

[Amim] Cl: 1-allyl-3-methyl-1H-imidazol-3-ium chloride

[Amim] Cl Rad: wood chips radial direction expansion in [Amim] Cl

[Amim] Cl Tan: wood chips tangential direction expansion in [Amim] Cl

[AA]: Amino acid

[AAE]: amino acid ester

[AAE]Cl: amino acid ester chloride

[AAE]NO_3: amino acid ester nitrate

ATCC: American type culture collection

B

$[BF_4]^-$: tetrafluoroborate

Bmpy BF_4: 1-butyl-4-methylpyridinium tetrafluoroborate

Bmph OTs: triisobutylmethylphosphonium tosylate

Bmim NTf_2: 1-butyl-3-methylimidazolium bis(trifluoromethane)sulfonimide

Bmpyro NTf_2: 1-Butyl-1-methylpyrrolidinium bis(trifluoromethane)sulfonimide

BmimCl: 1-butyl-3-methyl imidazolium chloride

BnmimCl: 1-methyl-3-benzylimidazolium chloride

BnomimCl: 1-methyl-3-m-methoxylbenzylimidazolium chloride

BnmmimCl: 1-methyl-3-methylbenzylimidazolium chloride

BnmimDCA: 1-methyl-3-benzyl-imidazolium dicyanamide

[Bmim] Ace: 1-butyl-3-methylimidazolium acesulfamate

[Bmim] Ac: 1-butyl-3-methylimidazolium acetate

[BMIM]BF_4: 1-Butyl-3-methylimidazolium tetrafluoroborate

[Bu_4N][L-Pro]: tetrabutyl ammonium L-prolinate

[BMIM][PF_6]: 1-Butyl-3-methylimidazolium hexafluorophosphate

BOD: biochemical oxygen demand

C

Cbz: Carboxybenzyl

CILs: Chiral ILs

[C_2C_1im][$MeCO_2$]: 1-butyl-3-methylimidazolium methyl sulfate

[C_4C_1im][HSO_4]: 1-butyl-3-methylimidazolium hydrogen sulfate

$[C_4\text{-mim}]^+PF_6^-$: 1-butyl-3-methylimidazolium hexafluorophosphate

$[C_2\text{-mim}]^+BF_4^-$: 1-ethyl-3-methylimidazolium tetrafluoroborate

$[C_4\text{-mim}]^+BF_4^-$: 1-butyl-3-methylimidazolium tetrafluoroborate

COD: chemical oxygen demand

D

DCM: dichloromethane

DCU: Dublin City University

DDA: didecyldimethylammonium

DMF: dimethylformamide

DMSO: dimethylsulfoxide

DOM: N, N-dimethyl-N-(2-phenoxyethyl)-1-dodecanaminium]

DSC: differential scanning calorimetry

[dca]: dicyanamide

dr: diastereo ratio

E

[Emim] Ac: 1-Ethyl-3-methylimidazolium acetate

EtOAc: ethyl acetate

[Emim] Ace: 1-ethyl-3-methylimidazolium acesulfamate

[Emim][Ac]Tan: wood chips tangential direction expansion in [Emim] Ac

[Emim][Ac]Rad: wood chips radial direction expansion in [Emim] Ac

ee: enantio excess

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

G

GC-MS: gas chromatography–mass spectrometry

H

HPLC: high performance liquid chromatography

HEMA: tris-(2-hydroxylethyl)methylammonium methylsulfate

HMF: 5-hydroxylmethyl-2-furfural

HOBt: 1-hydroxylbenzotriazole

Cp: heat capacity

I

ILs: ionic liquids

IR: infrared

IC: inhibitory concentration

M

MCC: microcrystalline cellulose

MIC: minimum inhibitory concentration

[Mmim][DMP]: 1,3-dimethylimidazolium dimethyl phosphate

[Mmim][DMP]Rad: wood chips radial direction expansion in [Mmim][DMP]

[Mmim][DMP]Tan: wood chips tangential direction expansion in [Mmim][DMP]

MS: mass spectrometry

MRSA: methicillin resistant Staphylococcus aureus

N

NMR: nuclear magnetic resonance

O

OECD: organisation for economic cooperation and development

P

PTCs: phase transfer catalysts

[p1,4]: N, N-dialkylpyrrolidinium

[p_{4444}]Br: tetrabutyl phoshonium bromide

[p_{4444}]OH: tetrabutyl phoshonium hydroxyl

[P_{4444}][AA]: tetrabutyl phoshonium amino acid based ILs

[P_{4444}][Val]: tetrabutyl phoshonium valine based ILs

[P_{4444}][Ala]: tetrabutyl phoshonium alanine based ILs

[P_{4444}][Gly]: tetrabutyl phoshonium glycine based ILs

[P_{4444}][Ser]: tetrabutyl phoshonium serine based ILs

[P_{4444}][Pro]: tetrabutyl phoshonium prolinate ILs

[$P_{6,6,6,14}$]: tetra-alkylphosphonium

[PF_6]: hexafluorophosphate

R

RT: room temperature

RTILs: room temperature ionic liquids

T

TBAB: tetrabutylammonium bromide

TBACl: tetrabutylammonium chloride

TBAI: tetrabutylammonium iodide

TBA: tetrabutylammonium

TPA: tetrapropylammonium

TEA: tetraethylammonium

TFA: trifluoroacetic acid

TMA: tetramethylammonium

T_g: glass transition temperature

T_d: decomposition temperature

TMP: thermo-mechanical pulp

TGA: thermo-gravimetric analysis

[tcm]: tricyanomethide

TLC: thin layer chromatography

[TPA][Pro]: tetrapopylammonium prolinate

V

VOC: volatile organic compound

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Project Aims:

To investigate the ability of typical ionic liquids for wood swelling

To investigate the wood regeneration in typical ionic liquids

To investigate the enzymatic hydrolysis of regenerated wood from ILs to correlate wood swelling with the structure of various ionic liquids.

To synthesis and characterize a library of novel sustainable, low-toxicity, biodegradable, prolinate chiral ionic liquids (ILs)

To investigate the physical properties of the chiral IL library

To investigate the toxicity and biodegradation properties of the chiral ILs library

To investigate the ability of chiral prolinate ILs for wood swelling

To investigate the wood regeneration in chiral prolinate ILs

Abstract:

This thesis is focused on the wood swelling, dissolution and regeneration in ionic liquids and cellulase digestibility after wood pretreatment in ionic liquids. VariousILs and their mixtures were used to swell wood chips of accurately measured linear dimensions. The change in the swelling of the wood chips was correlated to cellulase digestibility.

A series of novel chiral prolinate ionic liquids were designed, synthesized and characterized in order to measure their thermal stability, biodegradability and anti-microbial toxicity. The amino acids and natural alcohol derivatives (i.e. proline, *trans*-4-hydroxyl-proline and *cis*-4-hydroxyl-proline are raw materials for green synthesis of ionic liquids. Tetrabutyl ammonium hydroxide solution, tetraethyl ammonium hydroxide solution, tetrapropyl ammonium hydroxide solution and tetramethyl ammonium pentahydrate are the other starting materials for the ionic liquid synthesis. The synthesis of these ILs was carried out conveniently in one pot in air; a single-step, followed by purification. All the prolinate ILs made were in the thesis were characterized by a range of spectroscopic techniques including: ^{1}H and ^{13}C NMR, IR and HRMS. The biodegradability of 24 synthesized prolinate chiral ILs were analyzed by using with a wastewater microorganism the medium for the target chemicals. Antifungal activity of the 24 prolinate chiral ILs were determined against twelve fungal strains, and antimicrobial toxicity was determined against eight bacterial strains. All prolinated based chiral ILs were found to be low toxic towards eight strains of bacteria and twelve strains of fungi. The effects of stereochemistry and cation structure on the toxicity also have been discussed.

A series prepared chiral prolinate ILs have also been applied in wood expansion, dissolution and regeneration. Cellulose and lignocellulose dissolution studies with ionic liquids have shown the ionic liquid cation plays a significant role, with the tetraalkylammonium prolinate series yielding good results for lignocellulose pretreatment, before enzymatic hydrolysis of the complex biomass structure.

Chapter 1

Literature Review

Ionic Liquids

1.0 Literature Review

1.1 Introduction:

Lignocellulosic biomaterials are regarded as a renewable resource of carbon-based feedstock for fuel and chemical production in the same way that crude oil serves as the carbon feedstock in polymer production and in petrochemical and biological refineries.[1] In recent research, an increasing number applications of lignocellulosic biomass have been reported.[2] Bioethanol has been used to replace gasoline to reduce the global carbon footprint. Therefore, the conversion of lingocellulosic biomass such as wood and agriculture feedstock to bio-fuels could be a good choice for bio-fuel production.[3] However, there are problems with this kind of application such as the separation of wood components and glucose release from lignocellulosic materials. This is mainly because the major components of lignocellulose are cellulose, hemicellulose and lignin which are covalently cross-linked to hemicellulose.[4] Therefore, it is almost impossible to dissolve wood in conventional solvents in its native state. The aim of applying ionic liquids (ILs) in wood chemistry is to destroy the hydrogen bonds and open the compact structure of lignocelluloses to enhance the conversion of cellulose to glucose in the hydrolysis step.[5] Consequently, green and novel solvents with high efficiency are being designed for application in this research field.

Today, much of the recent progress in the field of ILs have arisen from the rather simple observation.[6] ILs can be used as green solvents in condensation,[7] Carbon-Ferrier rearrangements[8] and 1,3-Cycloaddition of nitrones.[9] Various researches into synthesis of chiral ionic liquids and their applications in catalysis of green chemistry synthesis have also been reported.[10] Biodegradation and metabolic pathways of different types of ILs have also been studied.[11] Physical properties such as viscosity, melting point and glass transition temperature have been reported.[12] It has already been shown that ionic liquids can dissolve a large number of bio-macromolecules, such as cellulose[13] silk fibroin,[14] lignin[15] and zein protein[16] with high efficiency.

Ionic liquids (ILs) have been defined as molten salts that composed of ions completely.[17] Ionic liquids (ILs) contain both cations and anions. The melting point of ILs is usually under 100 ^{o}C and some of them are liquids at room temperature called 'room temperature ionic liquids' (RTILs) mainly because the highly unsymmetrical organic cations lead to weak intermolecular interaction to reduce melting point.[18]

1.2 Application of Ionic liquids in Wood Chemistry:

There are several efficient methods of biomass pretreatment.[19] These include chemical hydrolysis, (such as alkali or acid), oxidizing agents and organic solvent treatments; physical pretreatment, (e.g. pulverization and irradiation) and dissolution in an IL or mixtures for delignification, regeneration and modification.[20] The physical and chemical properties of ILs can be easily adjusted by changing the types of the anions/cations; so many ILs can be designed to fit different demands of biomass pretreatment. Certain ILs have been proven to dissolve cellulose and other biopolymers under relatively mild conditions.[21]

In this chapter, the first aim is to demonstrate the versatility of ionic liquids in wood and biomass application, such as wood dissolution and regeneration, sugar conversion and enzymatic hydrolysis. The second main point is to elaborate the synthesis of the chiral amino acid based chiral ILs and their applications in organic synthesis, particular in asymmetric catalysis. We are also going to discuss the toxicity and biodegradation study progress and their physical properties of glass transition temperature and decomposition temperature are discussed briefly.

1.2.1 Application in Biomass Dissolution and Regeneration:

Cellulose dissolution was carried out by Rogers at 2002.[22] 1-butyl-3-methylimidazolium cation based ILs with different types of anions such as [Cl^{-}, Br^{-},

$[PF_6]^-$, SCN^- and $[BF_4]^-$] were applied to dissolve cellulose under the heating at 110 ^oC and water, or other anti-solvent like methanol and acetone could be added into the solution to precipitate the cellulose. Over 10 wt% of cellulose could be dissolved in the ILs with the anions which are strong hydrogen bond acceptors. The ILs contain the chloride and bromide can solubilize cellulose more efficient than other ILs contain $[PF_6]^-$, SCN^- and $[BF_4]^-$, mainly because the hydrogen bonding formation between the hydroxyl function group in the cellulose with the anions in the solution.

A range of imidazolium-based ionic liquids (ILs) (Figure 1.1) have been already synthesized and used to dissolve both hardwoods and softwoods (Norway spruce sawdust and Norway spruce and Southern pine thermomechanical pulp fibers) under gentle conditions.[23]

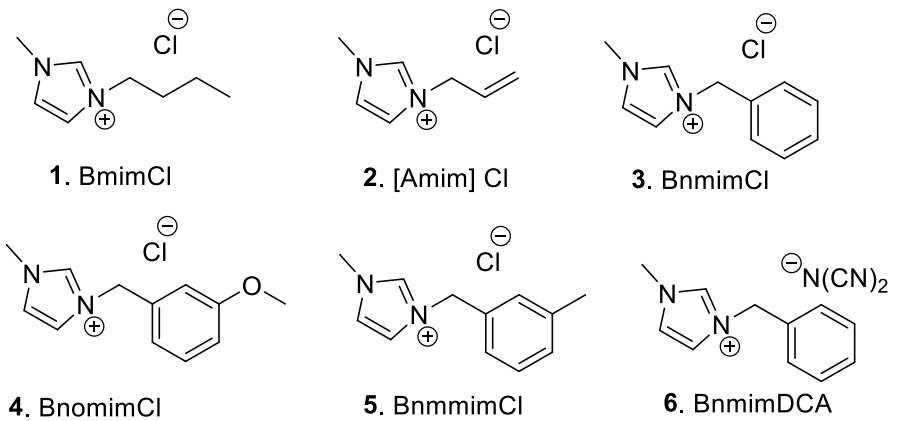


Figure 1.1: Structures and abbreviations of the examined ILs

The components of wood are described in Figure 1.2. The experiment was based on the interaction of the aromatic property of the cation in the ionic liquid with the lignin in the wood. After dissolution, wood can be regenerated as an amorphous mixture of its original components. Addition of distilled water or methanol leads to precipitation of the cellulose. The solubilization step involves heating the pre-dried wood with ILs at about 110 ^oC for 15 hrs. The percentage of weight dissolved is up to 8%. Several factors such as wood density, pulverization effects and regeneration solvents affect the wood dissolution and its regeneration in ILs.[24]

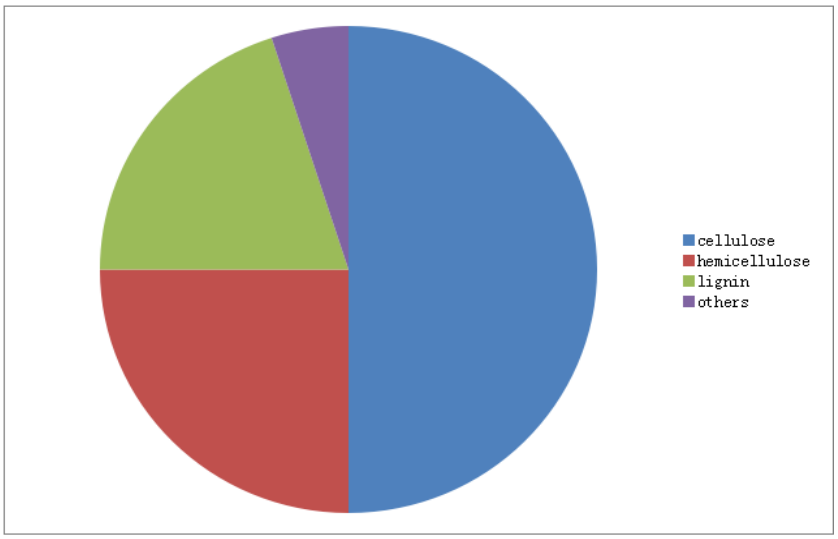


Figure 1.2: Components of wood

The weight percentage of dissolved wood is only 8% because of the more complicated structures of lignin and cellulose. Therefore, a high-throughput screening system has been introduced by Dr. Spiess.[25] ILs can lead to a competition with the lignocellulose components for hydrogen bonding; this then destroys its three-dimensional structure. In the ILs screening system, 1-Ethyl-3-methylimidazolium-acetate has been proved to be the most efficient solvent for cellulose dissolution (Figure 1.3).

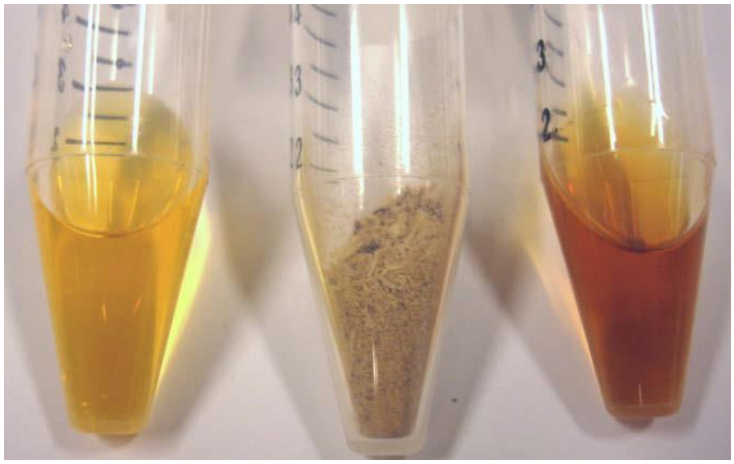


Figure 1.3: Dissolution of wood chips from common beech in [Emim] Ac. [Emim] Ac (left) and wood chips from common beech (center) were heated up and shaken for 1 h until a homogeneous brownish solution was obtained (right)

Rogers *et al*. reported that complete dissolution and partial delignification of wood can be carried out in the ILs 1-ethyl-3-methylimidazolium acetate. Red oak and southern yellow pines were the two starting materials used. Acetone/water (1:1 v/v) was used as suitable co-solvent to precipitate the cellulose-rich materials and carbohydrate-free lignin. The effects of wood particle size, wood species, initial wood concentration and pretreatment were discussed in the paper. Recovered insoluble lignin and cellulose-rich material were quantified from the original wood (Figure 1.4).[26]

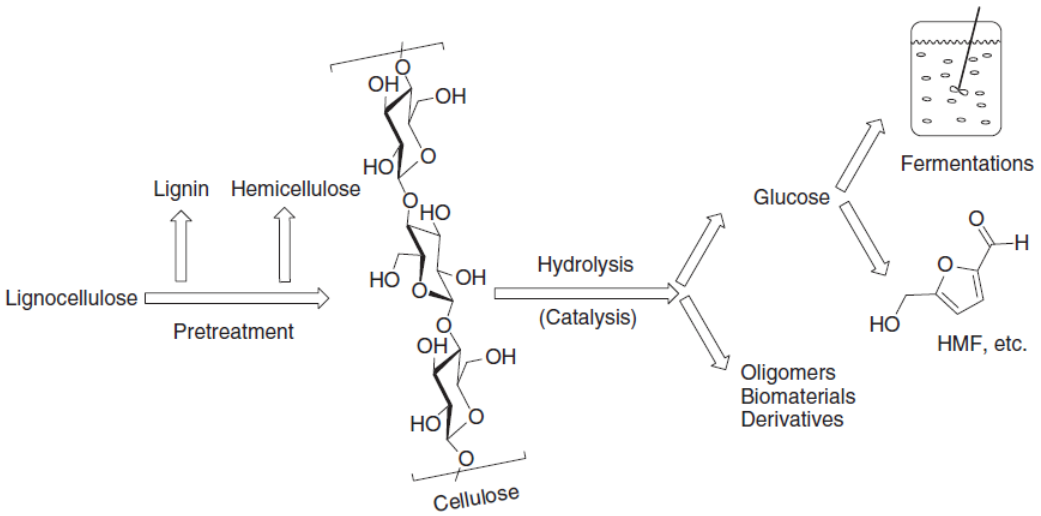


Figure 1.4: Flowchart for the process of dissolution and regeneration of wood in IL

Pang and co-workers reported using ILs to extract lignin from wood without cellulose dissolving; regeneration and degradation.[27] Lignin is the second most abundant natural polymer on earth.[28] There are three basic units in the structure: *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 1.5).

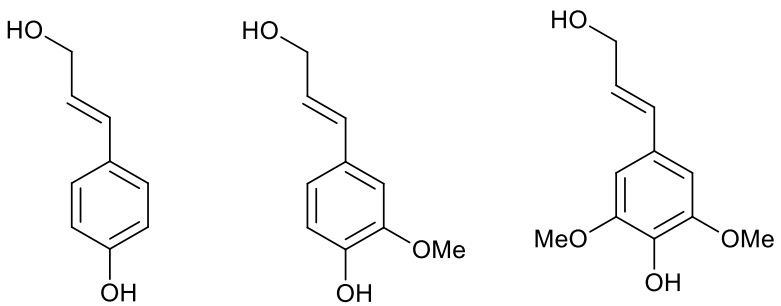
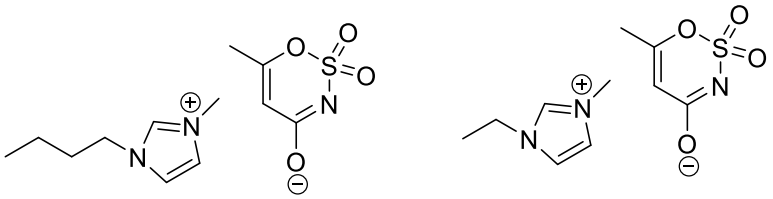


Figure 1.5: Three fundamental unites in lignin

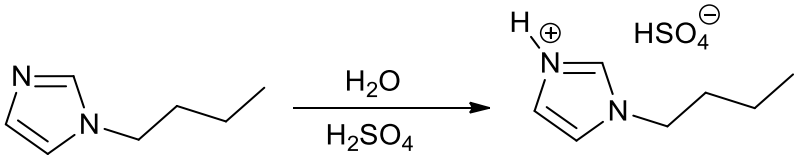
Imidazolium acesulfamate (Figure 1.6) which can be derived from the food-additive were used for extraction. Wood flour extraction with [Bmim] Ace yielded both cellulosic-rich wood residue with a high degree of crystallinity and extracted lignin. Extraction time, temperature, amount of wood load, water content, wood particle size and wood species were all controlled parameters during the procedure.



10. [Bmim] Ace **11.** [Emim] Ace

Figure 1.6: The structure of 1-butyl-3-methylimidazolium acesulfamate ([Bmim] Ace) and 1-ethyl-3-methylimidazolium acesulfamate ([Emim] Ace)

Welton research group has synthesized acidic ILs 1-butylimidazolium hydrogen sulphate (Scheme 1.1) and applied this ILs to investigate the pretreatment and fractionation of lignocellulosic biomass.[29]



12 **13**

Scheme 1.1: Synthesis of 1-butylimidazolium hydrogen sulfate

Lignin fraction and cellulose component are afforded separated. The cellulose part was performed to enzymatic digestion which could release the variety of monosaccharide. The acidity of the ILs could be adjusted by vary the ratio of ILs with sulfuric acid. Several conditions such as acidity of the ILs solution, pretreatment time and the water content of ILs solution have been examined for the remove of the lignin, recovery of hemicellulose and yield of enzymatic hydrolysis for monosaccharide. Longer pretreatment time could result in better remove lignin and hemicellulose but will lead to the loss of glucose into the solution. It is also noted that the existence of water in the ILs solution could improve the saccharification yields.

Sakurai *et al* reported that 1-butyl-3-methylimidazolium acetate ([Bmim] Ac) was used as a solvent to dissolve and regenerate chitin, which is similar to cellulose, and is also abundant polysaccharide (Figure 1.7).[30] Chitins with different origins (α- and β-chitins) were dissolved in [Bmim] Ace under heating and then cooling in the ILs solution to room temperature. Corresponding chitin materials were regenerated by adding distilled water or methanol.

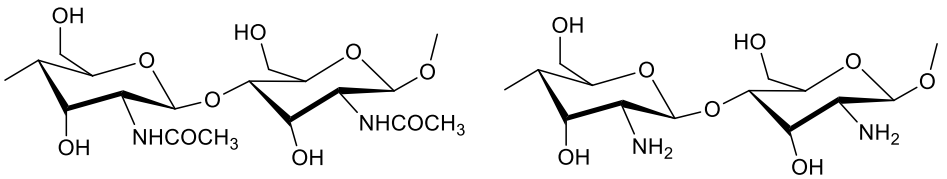


Figure 1.7: Structure of chitin and chitosan

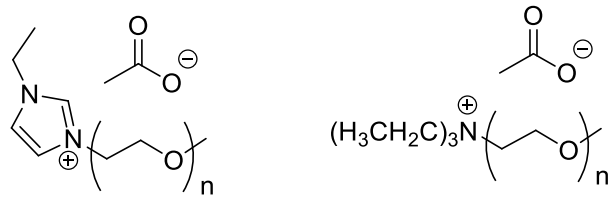
In summary, the research of ionic liquids (ILs) application with dissolution and regeneration pretreatment of lignocellulosic biomaterials has been improved significantly in the decades. However, the mechanism of the ionic liquids interact with lignocellulosic has still been in doubt. Wang *et al*. have investigated the dissolution mechanism of cations and anions with structures in ILs interaction with biomass.[31] For the cation and anion, acidic protons could be the most critical factor for cellulose solubility due to the formation of hydrogen bonds with hydroxyl group or ether oxygen in the cellulose structure. Another factor probably exerts negative influence on the solubility is the existence of large substituted group in cation or anion, like phenyl group or long alkyl side chain.

1.2.2 Application in Saccharification and Hydrolysis of Lignocellulose Biomass:

The saccharification and hydrolysis are two key steps for the conversion from biomass to bio-fuel and the pretreatment of the cellulose has been proven to be the critical step

for enzymatic hydrolysis.[32] Because of the dissolution and precipitation, amorphous cellulose is formed and shows more accessibility for the enzyme digestion than untreated natural cellulose.[33]

Person and co-workers reported that acetate based ILs were used to dissolved more than 10 wt% cellulose and regenerated cellulose is less crystallinity (58–75%) than natural cellulose. Regenerated Avicel from ILs was hydrolyzed 2–10 times faster than the respective untreated cellulose and more than twice accessible by cellulase (>2 times) than the untreated substrate (Figure 1.8).[34]



16 **17**

Figure 1.8: Imidazolium and ammonium-based ILs consisting of an alkyloxyalkyl-substituted cation and acetate anion (n=2 or 3)

Petrich *et al* used several ILs (Figure 1.9) as the media for enzymatic hydrolysis. *Tricoderma reesei* was used for hydrolysis in both the ILs and buffer solution.[35] The ILs tris-(2-hydroxylethyl) methyl ammonium methylsulfate (HEMA) was suitable for enzymatic hydrolysis, including buffer solution. The reason for this is that the enzyme is irreversibly deactivated at 50 °C in buffer solution whereas they are stable to temperatures as high as 115 ^{o}C in HEMA due to the high viscosity of the media.

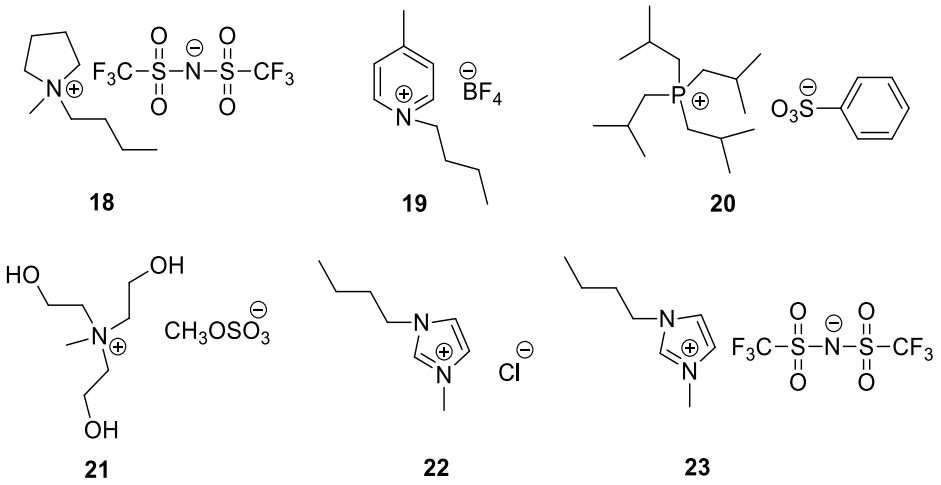


Figure 1.9: Structures of ionic liquids studied: (**18**) Bmpyro NTf_2, (**19**) Bmpy BF_4, (**20**) Bmph OTs, (**21**) HEMA, (**22**) BmimCl and (**23**) Bmim NTf_2

Recently, Welton *et al* reported that use of the ILs-water mixtures 1-butyl-3-methylimidazolium methyl sulfate and 1-butyl-3-methylimidazolium hydrogen sulfate for both ground pine and willow biomass digestion.[36] This was followed by enzymatic hydrolysis into glucose and hemicellulose sugars. Figure 1.10 has illustrated the relationship about saccharification time, types of ILs and the possible yield of the glucose released. The glucose released yield after $[C_4C_1im][HSO_4]$ pretreatment of willow was almost same as obtained for Miscanthus and the willow with $[C_2C_1im][MeCO_2]$ pretreated has obvious lower glucose yield. The significant lowest glucose yield was the for the pine pretreatment with both $[C_4C_1im][HSO_4]$ and$[C_2C_1im][MeCO_2]$ after enzymatic hydrolysis.

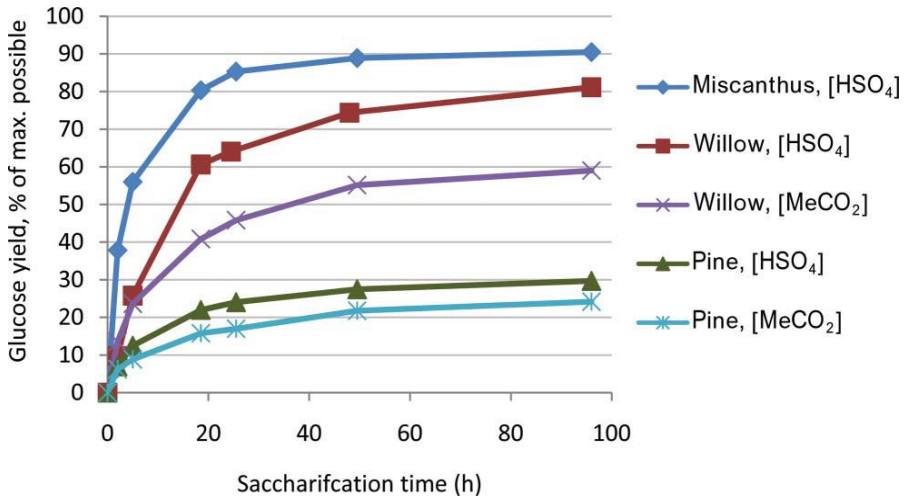


Figure 1.10: Enzymatic saccharification of biomass after pretreatment with $[C_4C_1im][HSO_4]$ and $[C_2C_1im][MeCO_2]$ at 120 ^oC

Sun and co-workers have studied crystalline structures which could have influence on enzymatic hydrolysis behaviors of microcrystalline cellulose (MCC) regenerated from ionic liquid 1-butyl-3-methylimidazolium chloride.[37] Only 4% (w/w) microcrystalline cellulose was added into ILs for dissolution then water. These MCC samples were regenerated from water, ethanol and acetone then dried in a vacuum oven at 60 ^oC overnight, which were labelled as DRC-w, DRC-e and DRC-a. The sample regenerated from water and dried in a freeze drier was labeled as FDRC-w. The intensity of regenerated MCC was evaluated by X-ray diffraction in Figure 1.11. Microcrystalline cellulose regenerated from acetone displayed a much lower crystallinity than other samples due to lower polarity than water and ethanol. As expected, higher enzymatic hydrolysis conversion ratio was obtained with the regenerated sample from acetone as well. The results have proved that low crystallinity of cellulose was the critical factor to improve the enzymatic hydrolysis efficiency and the yield of released glucose.

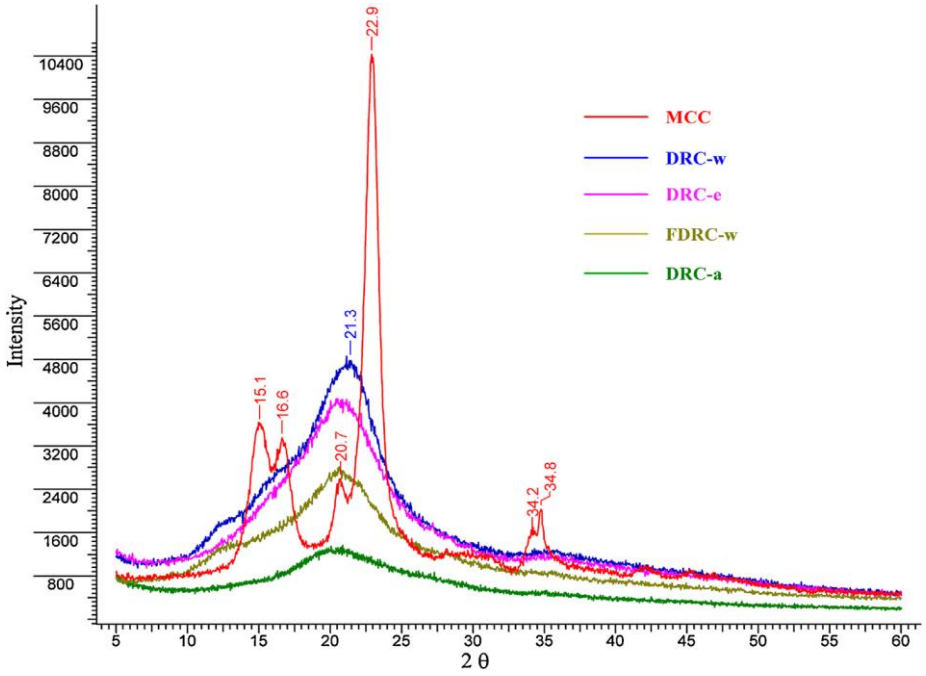


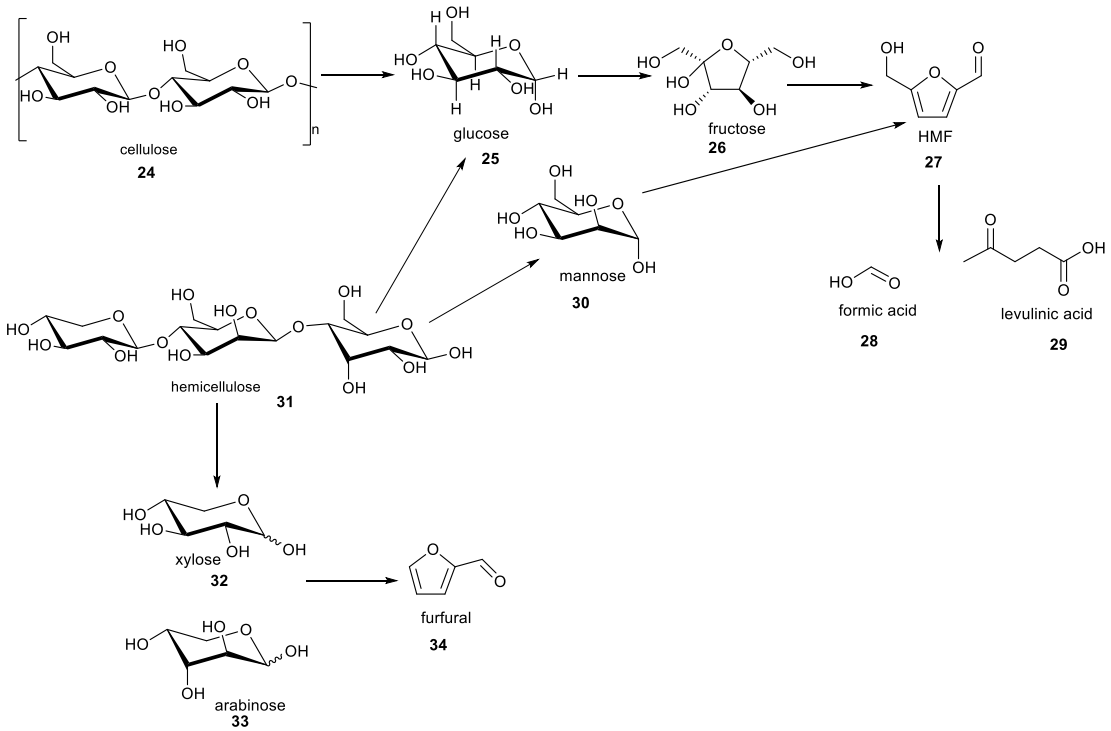
Figure 1.11: X-ray diffraction of MCC and regenerated cellulose samples

1.2.3 Application of Sugar Conversion and Platform Chemicals Synthesis:

As mentioned above, biomass dissolution and decrystallization in ionic liquids increase the monosaccharides conversion in the enzymatic hydrolysis and afforded monosaccharides could be processed or converted into platform molecules in the presence of ionic liquids.[38] Consequently, biomass conversion into useful chemicals and materials has been extensively investigated.[39] The two major platform chemicals from biomass feed-stocks are bioethanol and carboxylic acids such as levulinic and succinic acids.[40]

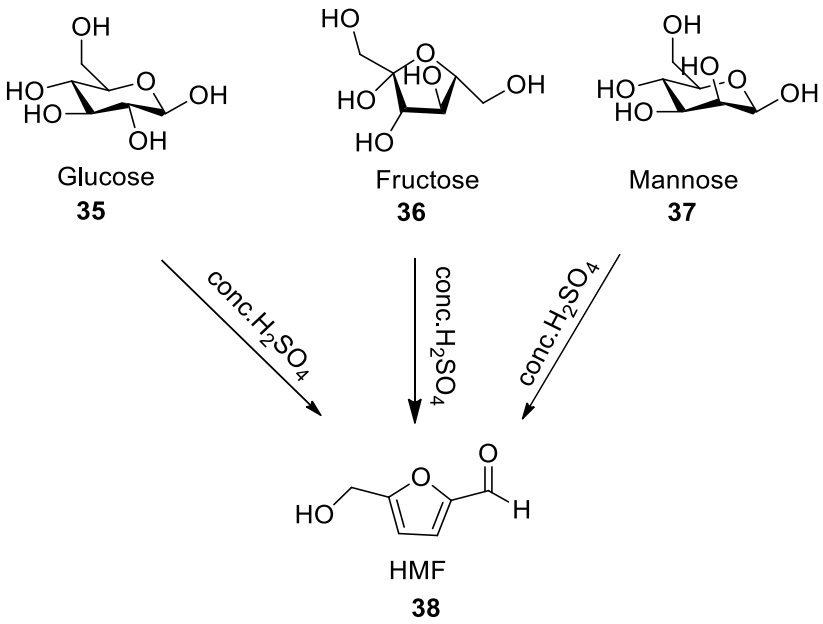
De Jong *et al* have reported that cellulose biomass could be covert into levulinic acid and furanic products by hydrolysis into glucose and dehydration to 5-hydroxylmethyl-2-furfural (HMF). Additionally, hemicelluloses could be degraded into both hexoses (glucose and mannose) and pentoses (xylose and arabinose). Glucose and mannose can

be converted into HMF; xylose and arabinose can be dehydrated into furfural under acidic condition (Scheme 1.2).[41]



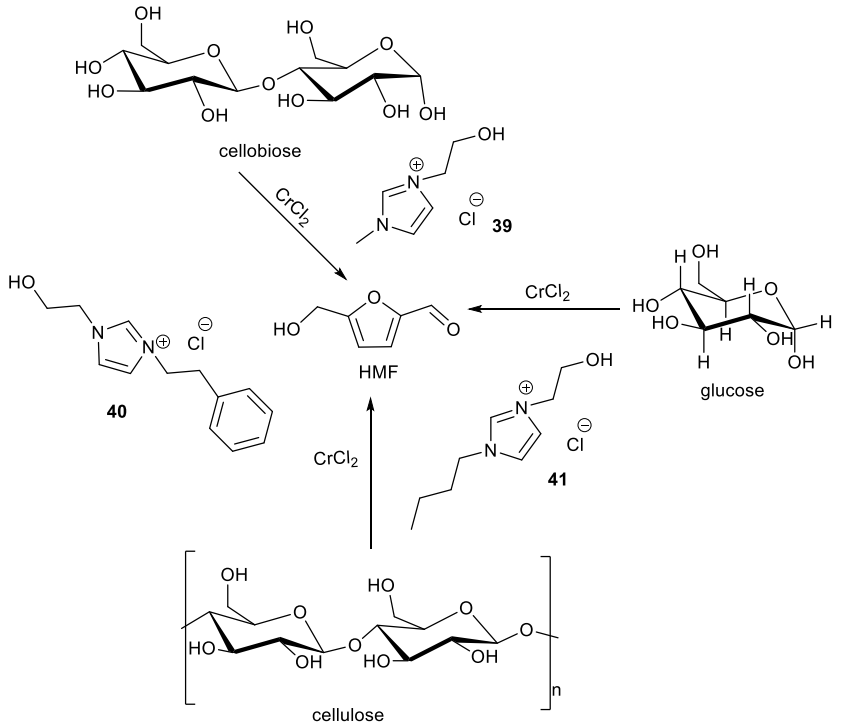
Scheme 1.2: Dehydration of lignocellulosic derived sugars into furanic compounds and levulinic acid

Conversion from mono-saccharides such as glucose, mannose, fructose and xylose to furfural and 5-hydroxylmethyl-2-furfural (HMF) which is a key platform chemical that may be obtained from various cellulosic (biomass) derivatives in an ionic-liquid phase was successfully carried out by Jones and co-workers (Scheme 1.3).[42] 1-butyl-3-methylimidazolium chloride (BmimCl) was used as a reaction medium at 120 ^{o}C in the presence of a Brønsted acid because of its ability to dissolve cellulose and stabilize monosaccharides. The yield of HMF conversion from fructose was higher than other mono-saccharides also when in the absence of a Brønsted acid.



Scheme 1.3: Reaction pathways for the conversion of glucose, fructose, and mannose into HMF

Dyson *et al* has appiled ILs/$CrCl_2$ system to improve the conversion from glucose, cellulose and cellobiose to 5-hydroxylmethyl-2-furfural (HMF) by dehydration (Scheme 1.4).[43] The yield of conversion from cellobiose to HMF in ILs **39**, **40** and **41** with hydroxyl group in the presence of $CrCl_2$ are 68%, 67% and 36% respectively, which are higher than normal imidazolium chlorides (36%). The reason for this is attributed to the presence of hydroxyl group of the imidazolium cation could coordinate with the OH group of cellobiose to form stronger hydrogen bond than the acidic proton in the simple imidazolium cation, which lead to better activity of cellobiose. However, the significantly lower conversion was obtained from ILs **41** mainly because the long alkyl chain on cation. Another factor is the introduction of $CrCl_2$ catalyst, which can interact with the hydroxyl group in cellobiose to enhance the ring opening for the hexose.



Scheme 1.4: ILs/$CrCl_2$ examined in dehydration of cellobiose, glucose and cellulose into HMF

1.2.4 Biomass Functionalization and Modification in ILs:

Heinze *et al* applied pyridinium, imidazolium and ammonium base ILs as reaction medium for cellulose carboxymethylation (Figure 1.12).[44] Homogeneous solution of cellulose in ILs was afforded with heating 12 hrs and precipitated cellulose were freeze dried. $ClCH_2COONa$ was the reagent for cellulose carboxymethylation in the presence of sodium hydroxide powder.

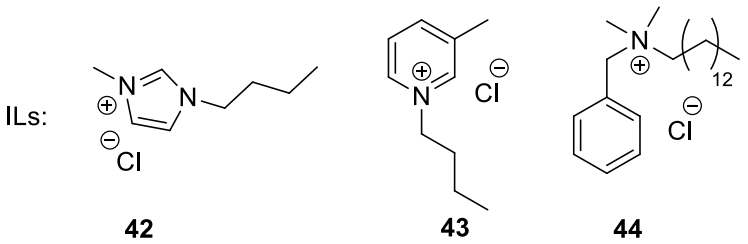
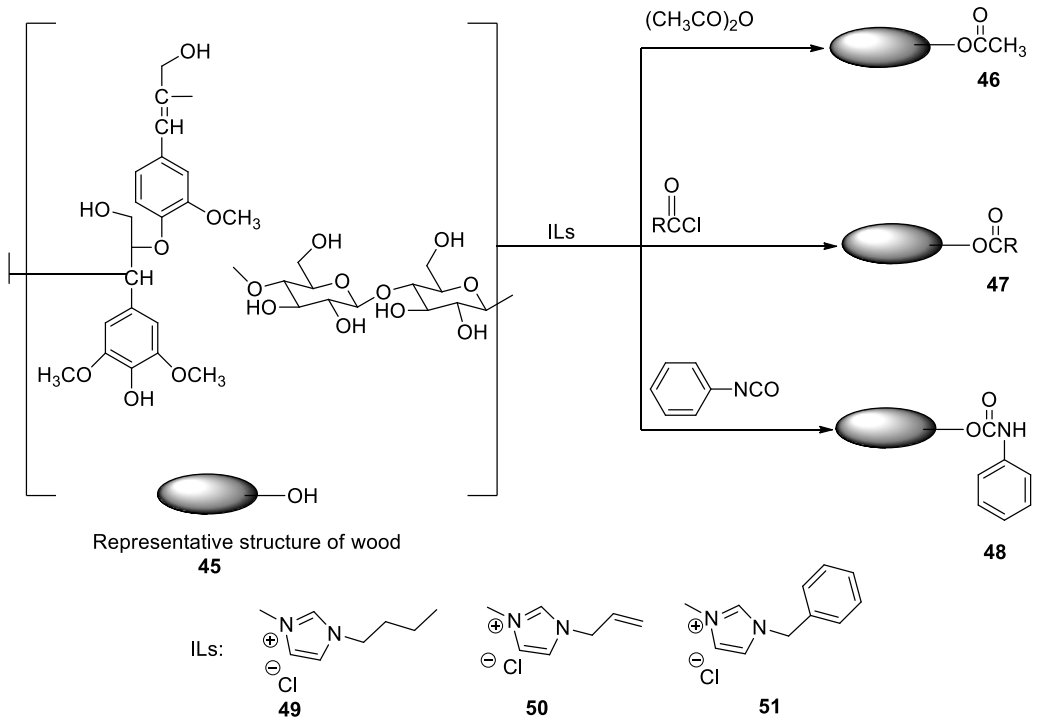


Figure 1.12: The structure of the ILs used in cellulose carboxymethylation

Xie and co-workers reported the homogenous acetylation, benzoylation, and carbanilation for wood-based lignocellulosic materials in ILs. Wood was firstly dissolved in 1-butyl-3-methylimidazolium chloride (BmimCl), 1-allyl-3-methyl-imidazolium chloride ([Amim] Cl) and 1-benzyl-3-methylimidazolium chloride (BnmimCl) then acetyl chloride, benzoyl chloride, and phenyl isocyanate were added into the ILs solution in the presence of pyridine respectively (Scheme 1.5). Finally, highly substituted lignocellulosic compounds were obtained by filtration and washing. Different thermal stability was displayed after modification in ILs by thermogravimetric analysis and new thermal transitions for these compounds were determined by differential scanning calorimetry.[45]



Scheme 1.5: Structures of wood and the homogenous reactions in ILs

Argyropoulos *et al* reported two consecutive conversions from wood-based lignocellulosic materials to thermoplastic materials.[46] The first step used benzoyl chloride and lauroyl chloride as acylation reagents to synthesize benzoylated spruce and lauroylated spruce respectively in BmimCl. Secondly, benzoylated and lauroylated spruce were used to make thermoplastic composites by mixing with poly (styrene) and

poly (propylene) under high temperature and high rotation speed. TGA results have shown that the addition definitively improved the thermal stability of the new wood composites. The reason for this improvement is that the compatibility between wood-based materials and poly (styrene) or poly (propylene) was enhanced by benzoylation and lauroylation in ILs so resulting in woody fibrous material evenly dispersed in the wood thermal composite.

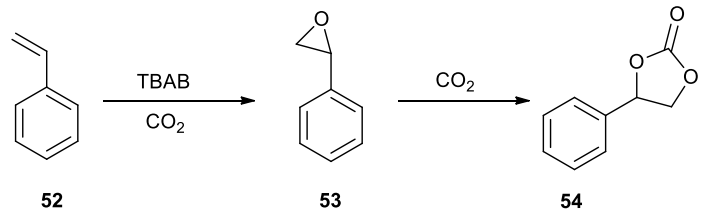
In summary, various modifications of wood or cellulose in ILs have been studied for the synthesis bio-polymers, which can contribute to the manufacture of biomedical-devices. However, all of the above wood chemistry applications are performed in traditional imidazolium and pyridinium based non-chiral ILs. The applications in novel amino acid based ILs are not carried out commonly. Only cholinium amino acid based ILs were applied in the pretreatment of microcrystalline cellulose[47] and rice straw biomass but the effect of stereochemistry of ILs have not been researched.[48]

1.3 Tetraalkyl Ammonium and Chiral Amino Acid Ionic Liquids Preparation and Application in Organic Synthesis

Over the last two decades, ILs have attracted increasing attention both in organic chemistry[49] and bio-chemistry.[50] This is mainly because of their physical properties, such as high polarity, very low vapor pressure, and solubility profile for a wide range of small organic compounds, enzymes and biomass.[51] Combined with green chemistry principles, ILs have been used in a wide range of industrial applications and green chemistry reactions.[52] This includes ammonium- and pyrrolidinium-based cations combined with methyl sulphate or methyl sulphonate investigated as lubricants or lubrication additives in chemical industry[53] and as reaction medium to improve the asymmetric CO_2 cycloaddition[54] and as a lubricant for rubbing process.[55]

1.3.1 Tetraalkyl Ammonium Ionic Liquids Applications in Organic Synthesis:

It has been reported that quaternary tetra-alkyammonium based ILs have also been used for hydrogen peroxide oxidation.[56] Two consecutive reactions of epoxidation and cycloaddition were performed in one pot reaction (Scheme 1. 6).



Scheme 1.6: One-pot synthesis of styrene carbonate from styrene with CO_2

Six different ILs (tetrabutylammonium bromide (TBAB), tetrabutylammonium chloride (TBACl), tetrabutylammonium iodide (TBAI), 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4\text{-mim}]^+PF_6^-$), 1-ethyl-3-methylimidazolium tetrafluoroborate ($[C_2\text{-mim}]^+BF_4^-$) and 1-butyl-3-methylimidazolium tetrafluoroborate ($[C_4\text{-mim}]^+BF_4^-$) were selected as both catalysts and solvents for the synthesis to examine the influences of nucleophilicity of anions on the reaction yield.

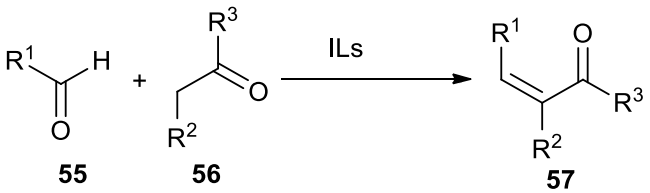
Table 1.1: Results of synthesis of styrene carbonate using various ionic liquids

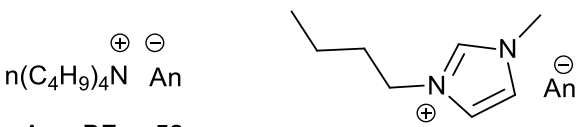
Entry	ILs	Amount (mmoL)	Conversion[a] (%)	Yield[b]	
				SC	SO
1	TBAB	2	74	33	2
2	TBAI	2	72	16	7
3	TBACl	2	84	8	3
4	$[C_4\text{-mim}]^+BF_4^-$	10.8	57	0	0
5	$[C_4\text{-mim}]^+PF_6^-$	10.6	17	0	0
6	$[C_2\text{-mim}]^+BF_4^-$	13.0	36	0	0

[a]: Total conversion of styrene
[b]: Yield is moles of the product formed against initial moles of styrene used. SC: styrene carbonate and SO: styrene epoxide.

Results in Table 1.1 have shown that the highest conversion was observed in TBAB mainly because the excellent nucleophilicity of bromide anion to attack epoxide group leading to ring opening. The lower conversion with TBAI than TBAB was due to the formation of iodine by oxidant.

Tetraalkylammonium and 1, 3-dialkylimidazolium tetrafluoroborates were introduced into solid base-promoted cross-aldol condensations as phase transfer catalysts with high yield and the catalysts kept their activity after several time recycles (Scheme 1.7).[57] Phase transfer catalysts whose anions like BF_4^- and PF_6^- anions with weakly solvation, have higher efficiency than halide based PTCs. Furthermore, electronic property of substrates had an influence on the reaction yield. Aldehydes with electron-releasing groups in the aromatic ring decreased the product, while electron-donating group elevated the yield.





An: BF_4, **58a**
PF_6, **58b**
Br, **58c**

An: BF_4: **59a**
PF_6: **59b**
Br: **59c**
Cl: **59d**

Scheme 1.7: Cross-aldol condensations of aromatic aldehydes with carbonyl compounds

Tetraalkylammonium oleate and linoleate based ionic liquids which are a new class of ILs due to the existence of unsaturated fatty acids for anions have been applied in the metal removal from aqueous phases.[58] Kroon and co-workers has synthesized this greener and simple ILs with low toxicity from renewable resources in 2013 (Figure 1.13).

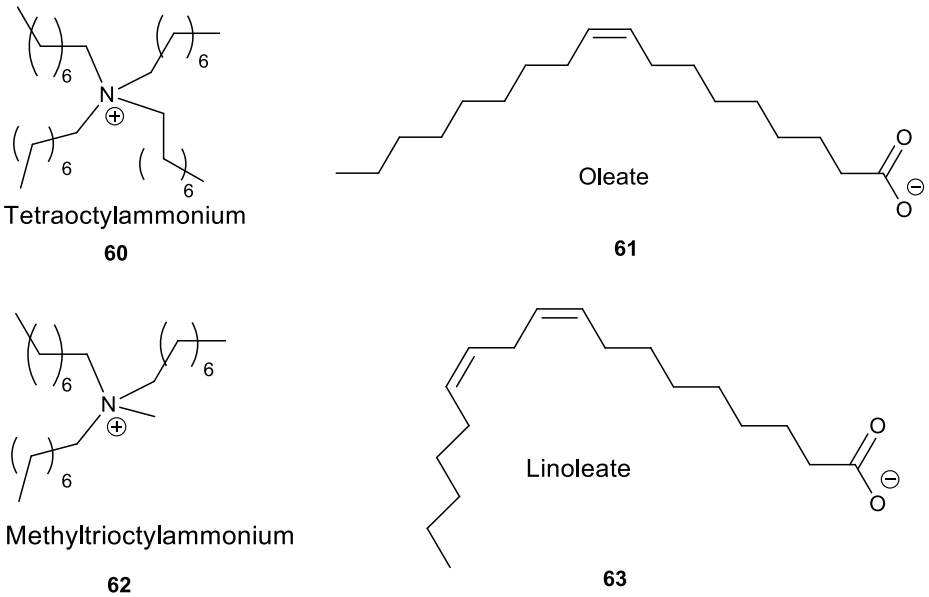


Figure 1.13: Ions used to synthesize oleate and linoleate based ionic liquids used for metal extraction

Metal extraction properties were examined against these types ILs by extraction Mn (II), Fe (II), and Zn (II) chlorides from aqueous solutions. As expected, the kind ILs have showed good extraction results (>90%) for the IV group transition metal salts (Figure 1.14). Another advantage of this approach is that metal extraction process was based on ion extraction instead of ion exchange, which could minimize IL leakages into the water phase.

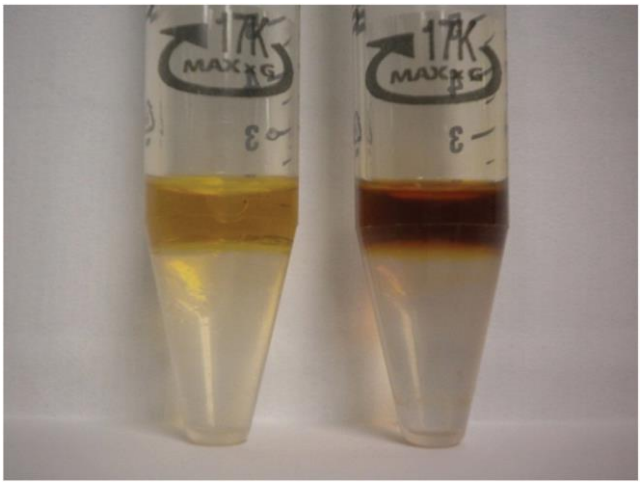
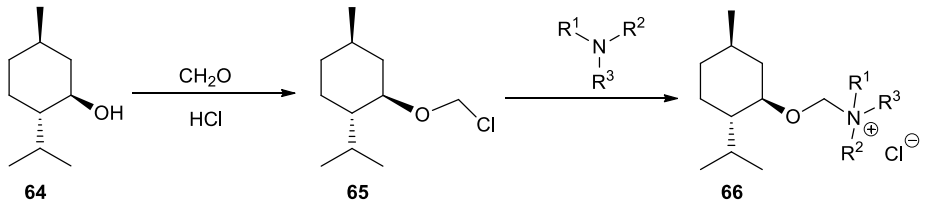


Figure 1.14: Color change during period IV transition metal extraction. The left tube shows ILs tetraoctylammonium linoleate before metal extraction the right tube was the same IL after metal extraction

1.3.2 Synthesis and Application of Chiral Ionic Liquids:

Chiral ILs (CILs) either as solvents or as chiral catalysts in asymmetric technologies, have been studied in a wide range of research fields[59] and their applications in chiral recognition abilities in spectroscopic techniques such as NMR have also been developed.[60]

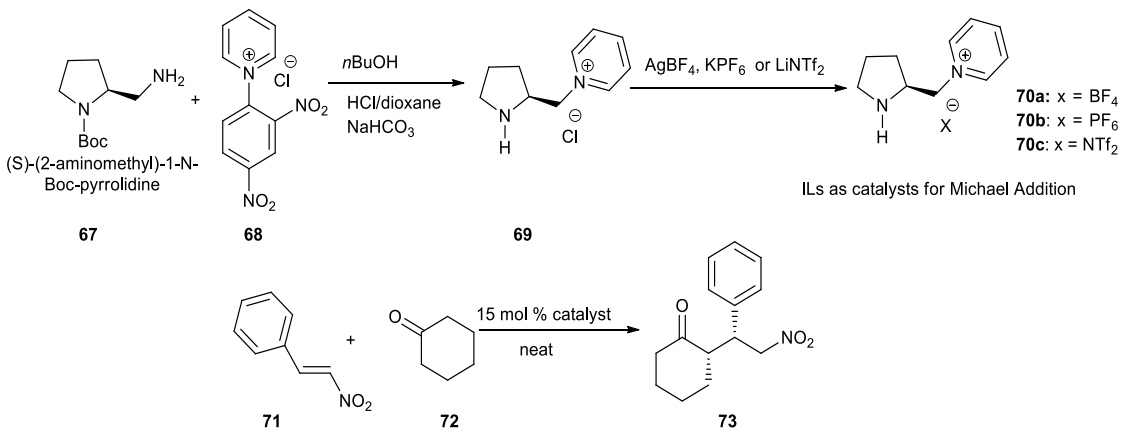
Menschutkin quaternization had been applied by Pernak *et al* to prepare a series chiral ammonium based ILs under mild condition.[61] Firstly, the intermediate quaternization reagent **65** was synthesized by chloromethylation of starting chiral alcohol and followed by reacted with trialkyl amine to afford the chiral ammonium based ILs (Scheme 1.8). The physical properties such as glass transition temperature, thermal degradation temperature, viscosity and density have been determined. Furthermore, antimicrobial screening tests were carried out to conclude that these ILs with more than five carbons on the alkyl chain have wide range of antimicrobial activities.



R^1: C_2H5, *i*-C_3H_7, C_4H_9, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}, $C_{10}H_{21}$, CH_2Ph
R^2: C_2H_5, CH_3
R^3: C_2H_5, CH_3

Scheme 1.8: Chloromethylation and Menschutkin quaternization for preparation of chiral ammonium ILs

Headley and co-workers designed and prepared a series of pyrrolidine-based chiral pyridinium ionic liquids from commercial starting material (*S*)-(2-aminomethyl)-1-*N*-Boc-pyrrolidine reacted with Zincke's salt then was deprotected to afford pyrrolidine-based chiral pyridinium chloride.[62] The other ILs could be converted by anion exchange from the chlorides. Asymmetric Michael addition of cyclohexanone and nitro styrene was performed in the presence of above pyrrolidine-based chiral pyridinium ILs (Scheme 1.9). All the catalysis results were listed in Table 1.2.



Scheme 1.9: Synthesis of pyrrolidine-based chiral ILs and catalysis in Michael addition of cyclohexanone and nitrostyrene

From the results in Table 1.2, the diastereo-selectivity for the *cis*-isomer (dr value) was

high with no significant difference between the several of ILs with different anions. The enantio-selectivity altered with the change of structures of the anions as well.

Table 1.2: Results of ILs as catalysts in the Michael addition of cyclohexanone and nitrostyrene

Entry	ILs	TFA[a] mol%	*T* °C	Yield[b] (%)	ee[c](%) (*syn*)	dr[d] (*syn/anti*)
1	**69**	none	rt	74	99	98/2
2	**69**	5	rt	91	99	97/3
3	**70a**	none	rt	75	94	99/1
4	**70a**	5	rt	95	98	99/1
5	**70a**	5	4 °C	92	99	99/1
6	**70b**	none	rt	90	93	95/5
7	**70c**	5	rt	92	86	96/4
8	**70c**	none	rt	81	95	96/4
9	**70a**	5	rt	95	93	99/1

[a]: TFA (trifluoroacetic acid)
[b]: Isolated yield.
[c]: Determined by Chiral HPLC
[d]: Determined by ^{1}H NMR

Pernak *et al* had prepared a class of quaternary ammonium mandelates ionic liquids (ILs) with long chain in ammonium cations, which were didecyldimethylammonium (DDA), benzalkonium with $C_{12}H_{25}$ and $C_{14}H_{29}$ alkyl groups equal to 60% and 40%, (BA) and domiphen [*N*, *N*-dimethyl-*N*-(2-phenoxyethyl)-1-dodecanaminium] (DOM) (Figure 1.15).[63] Mandelic acid can be converted from biomaterials with well-known antibacterial activities. The biological activities of these ILs were determined by anti-microbial and anti-fungal screening. Thermogravimetric analysis results showed that mandelated ILs start to decompose at 155 °C.

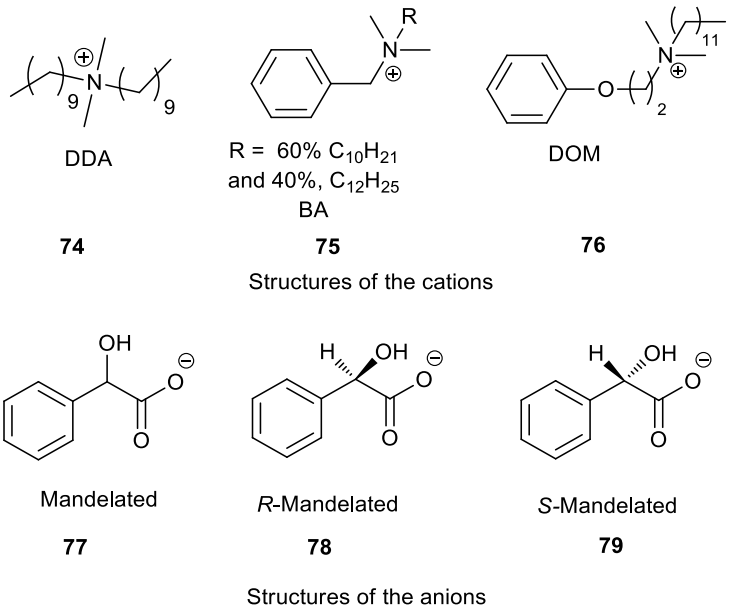
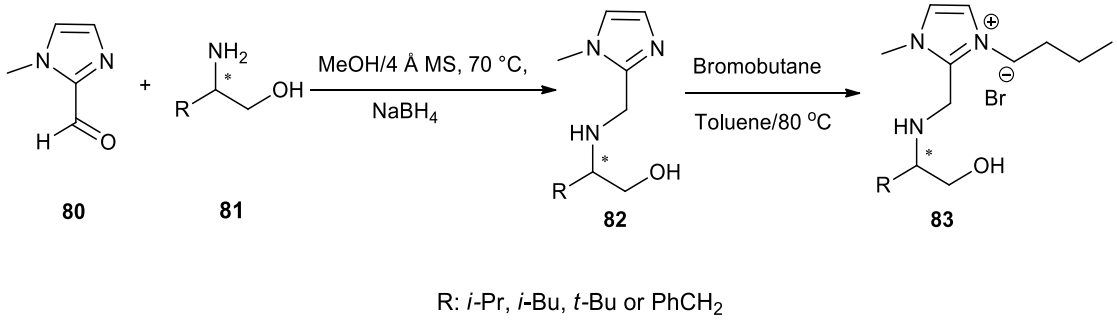


Figure 1.15: The structures of cations and anions in the mandelated based ILs

It has been well known that the proton on the C-2 position of the imidazolium cation in ILs has a kind of acidity.[64] Hence, when imidazolium based ILs were used as reaction medium in basic condition, the proton on C-2 position would be deprotonated and side products would be formed.[65] Li and coworkers designed and prepared a novel C-2 substituted chiral imidazolium ILs with more stability and less activity to minimize the above disadvantages.[66] The synthesis route is depicted in Scheme 1.10. First step is the condensation of 1-methyl imidazole aldehyde with β-NH_2 alcohol compound in methanol to form Schiff base followed by reduction with $NaBH_4$ to get the imidazolium amino alcohol, which was converted into target C-2 substituted chiral imidazolium ILs by alkylation with bromobutane with good yield (75%-90%).



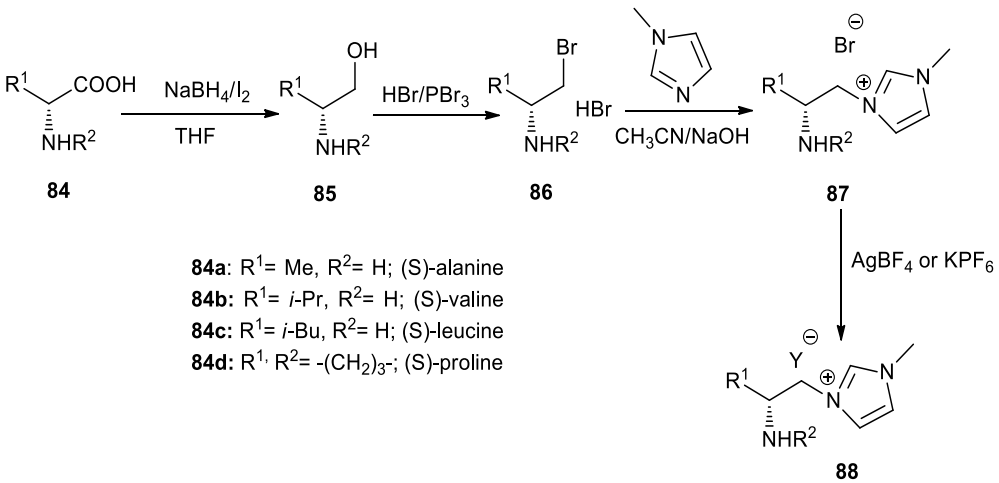
Scheme 1.10: Synthesis of Chiral Imidazolium Ionic Liquids

1.3.3 Synthesis and Application of Chiral Amino Acid Derived Ionic Liquids:

Recently, amino acid based ILs, especially examples from the chiral pool, have attracted interest within the green chemistry community.[67] The reason is that there are two basic functional groups (carboxylic and amino groups) in one molecule so that amino acids have a wide range of modification to be converted into various derivatives with new function groups as cations or anions in ILs preparation.[68] Imidazolium cation-based natural amino acid ILs were synthesized by Ohno group and their physical properties such as glass transition or melting temperature, viscosity and thermal stability had been investigated.[69]

1.3.3.1 Synthesis of Chiral Amino Acid Derived ILs

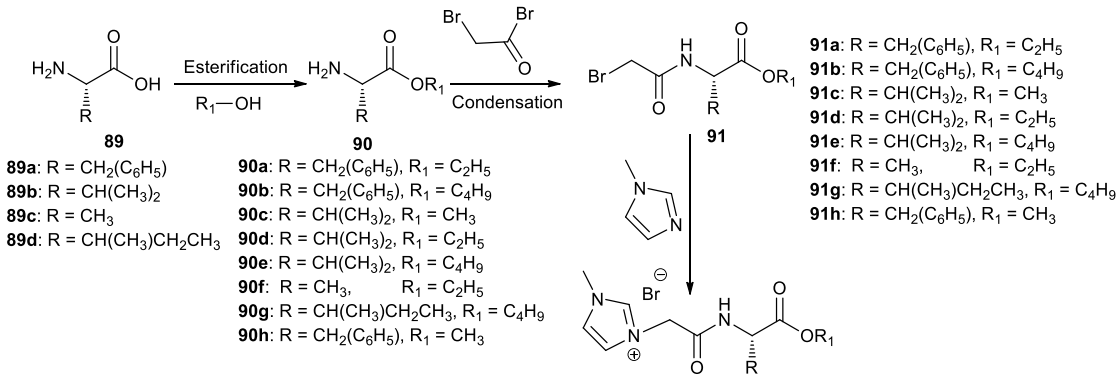
Chiral amino acid modified ILs were designed and synthesized by Xu *et al*.[70] (*S*)-Alanine, (*S*)-valine, (*S*)-leucine and (*S*)-proline were the starting materials and were reduced by $NaBH_4/I_2$ followed by bromination with PBr_3/HBr to form intermediate salt. These ILs were obtained by the alkylation of N-methylimidazole with the intermediate salt and NaOH neutralization. $AgBF_4$ or KPF_6 were used to ion exchange to get BF_4 and PF_6 based ILs (Scheme 1.11). These amino acid modified ILs possess a highly efficient chiral environment, which was investigated and proved by ^{19}F-NMR chiral discrimination study of ILs with racemic Mosher's acid mixtures.



Scheme1.11: Synthesis of chiral amino based ILs

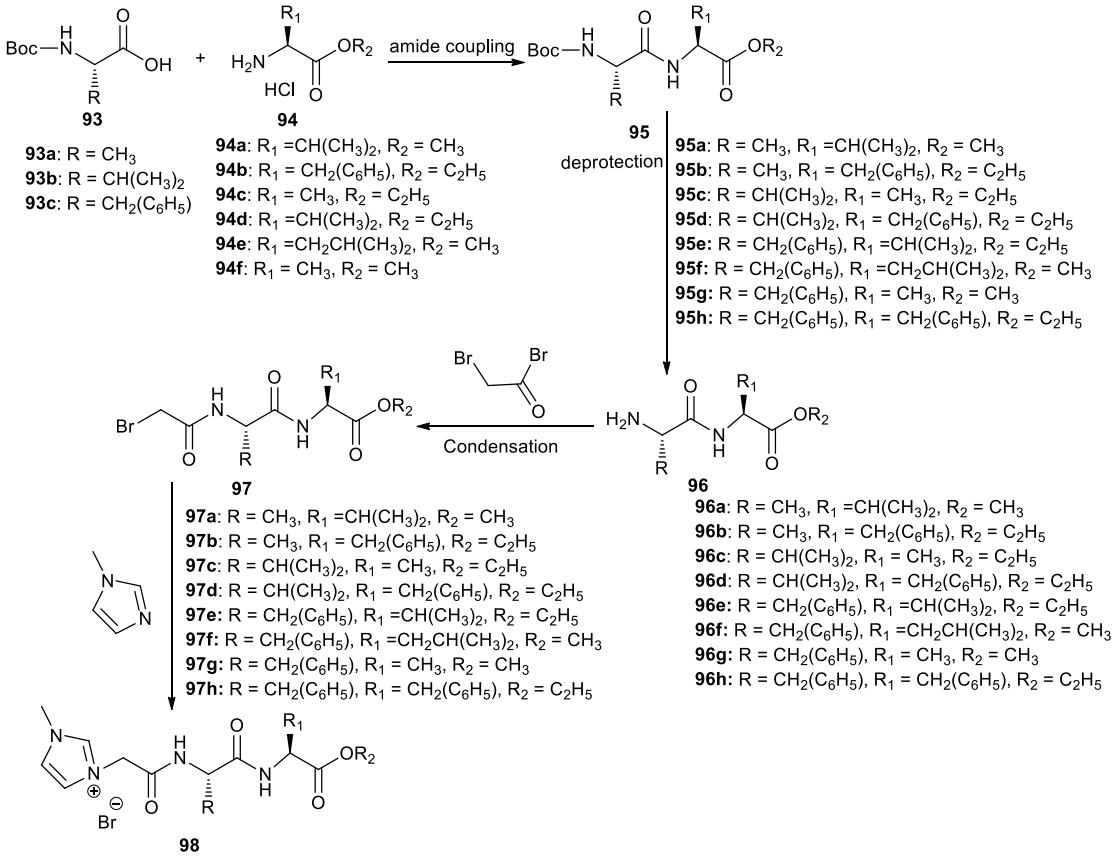
Maschmeyer and co-workers had synthesized new chiral ionic liquids by the reaction of an amino acid with tetrabutylammonium hydroxide in water.[63, 71] The cation moiety was from tetrabutylammonium hydroxide, which is a strong base and which could easily deprotonates carboxylic acid of amino acids to form a carboxylate salt. The another advantage of using tetrabutylammonium hydroxide is the bulky cation group would decrease the intermolecular interaction to enhance the potential of ILs being a liquid at room temperature, which could open the application range in chemistry field.

Gathergood's research group had designed chiral ILs by amino acid building blocks. Ester, amide and dipeptidyl were introduced into ILs cation by simple synthetic route.[72] Ester group were prepared from natural amino acid reacted with alcohol in the presence of thionyl chloride by esterification and followed by condensation with bromoacetyl bromide with triethylamine in DCM to form the bromo substituted amide compound which was alkylated with 1-methylimidazole to get targeted amino acid derived ILs (Scheme 1.12).



Scheme 1.12: Synthetic route of amino acid amide CILs

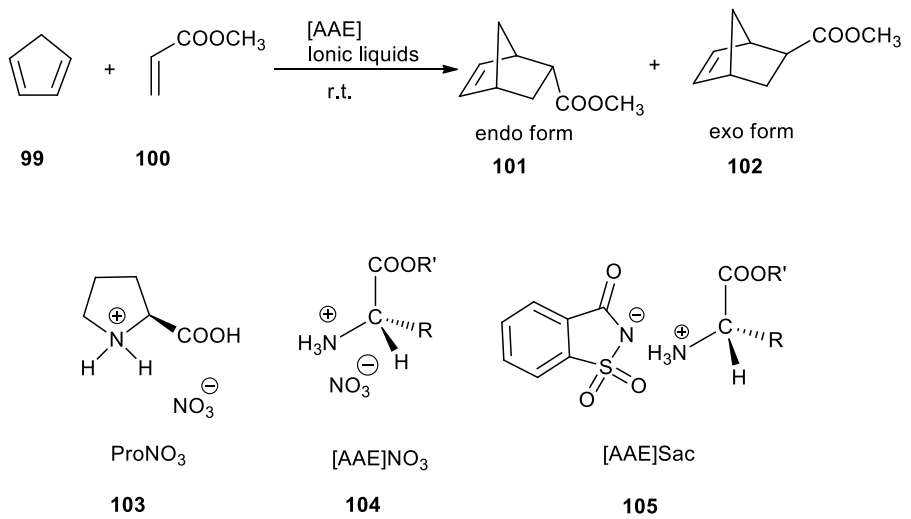
Another novel of chiral ILs with dipeptidyl side chain in cation were also synthesized from natural amino acid.[72] Amino acid ester hydrochloride was obtained by amino acid ester reacted with 1-hydroxyl benzotriazole (HOBt) and N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC) in DCM. Amino acid ester hydrochloride was coupled with Boc protected amino acid to form dipeptidyl compound, which was deprotected in the acidic condition to be converted to the intermediate with amino group on the terminal. The compound was converted into alkylated agent by the condensation with bromoacetyl bromide on the terminal amino group. Last step was alkylation with 1-methylimidazole to afford chiral amino acid derived dipeptidyl ILs (Scheme 1.13).



Scheme 1.13: Synthetic route of amino acid based dipeptidyl CILs

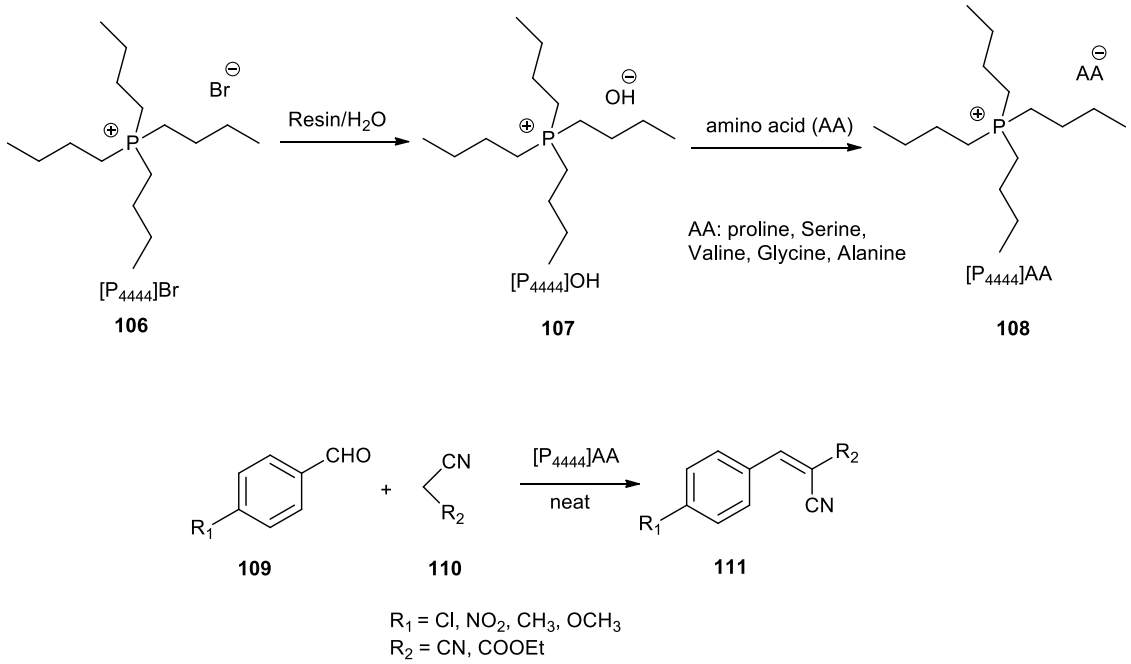
1.3.3.2 Application of Chiral Amino Acid Derived ILs in Organic Synthesis

Amino acid [AA] and amino acid ester [AAE] derived ILs were designed and synthesized by Kou *et al*.[73] These cations were from natural amino acid and the anions were from NO_3 and saccharide (Sac) which are non-toxic. ILs ProNO_3 was obtained straight forward by mixing proline with HNO_3 in water. [AAE]NO_3 was afforded by the reaction of [AAE]Cl and $AgNO_3$ in methanol after filtration of sliver chloride. The reaction of sodium saccharin with [AAE]Cl formed ILs [AAE]Sac in acetone. Then Diels–Alder reaction was carried out in the presence of [AAE]NO_3 and [AAE]Sac (Scheme1.14). The yield of the cycloaddition of cyclopentadiene to methyl acrylate was around 95% with catalyst load 30 mol%; the ratio of *endo/exo* was from 3.3 to 3.9.



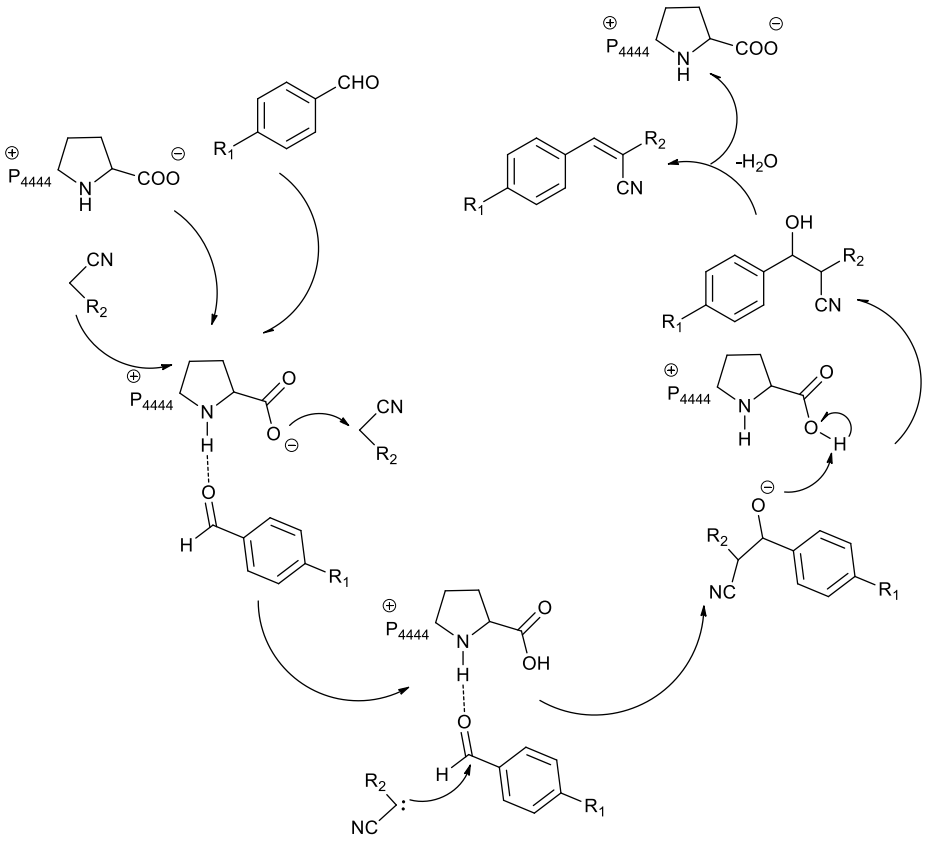
Scheme 1.14: The structures of amino acid ester based ILs and as catalysts in Diels–Alder reaction

Five tetrabutyl phosphonium amino acid based ionic liquids were synthesized by two straightforward steps and used as catalysts in Knoevenagel condensation of aromatic aldehyde with methylene compounds with acidic proton under neat condition (Scheme 1.15).[74]



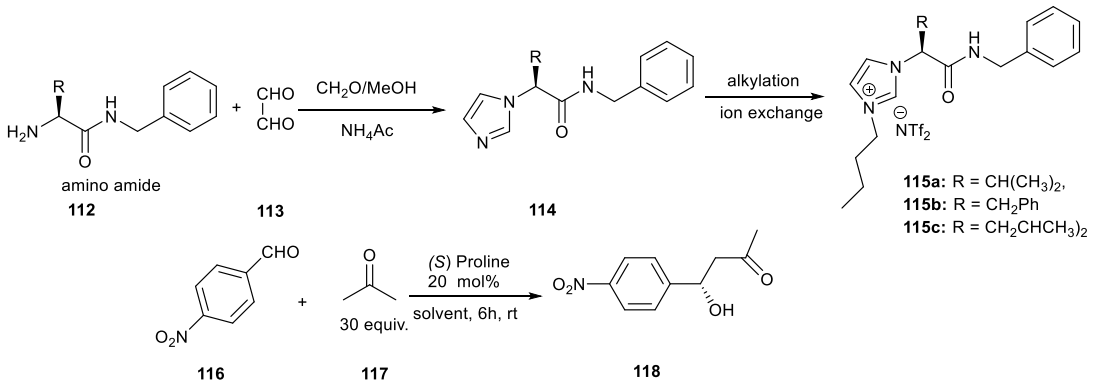
Scheme 1.15: Synthesis route for the of $[P_{4444}][AA]$ ILs and as catalysts in Knoevenagel condensation

Knoevenagel condensation was performed by above five ILs with catalyst loading (1 wt% based on aromatic aldehyde) under 60 ^{o}C for 20 minutes. GC-MS analysis results showed that yield of the reaction catalyzed by tetrabutyl phosphonium prolinate was the highest (93%) in all tested ILs mainly because prolinate anion moiety has higher alkalinity (pH: 9.67 under 25 ^{o}C) than other four ILs with pH 9.57 ($[P_{4444}][Val]$), 9.55 ($[P_{4444}][Ala]$, 9.15 ($[P_{4444}][Gly]$ and 8.56 ($[P_{4444}][Ser]$). Reactions were developed by using various substituted aromatic aldehydes and activated methylene compounds under same condition catalyzed by tetrabutyl phosphonium prolinate. The results revealed that reaction time and yield with -CN group for R_2 was shorter and higher than the -COOEt group for R_2 in the activated methylene compounds due to the stronger electron withdrawing effect of cyano. In additionally, substituted groups with electron donation effect like $-CH_3$ and $-OCH_3$ lead to longer reaction time and lower yield, which were accordance with the mechanism in (Scheme 1.16).



Scheme 1.16: Plausible mechanism for Knoevenagel condensation by [P_{4444}][Pro]

Another useful application of amino acid derived ILs is playing a role as chiral solvent for for asymmetric Aldol reaction. Luis and co-workers used amino acid to synthesis amide which was the precursor to react with ammonium acetate, formaldehyde and glyoxal to form the imidazolium moiety in the amide structure followed by alkylation and ion exchange to afford designed ILs.[75] These ILs was used as chiral reaction medium in the presence of (*S*)-proline to catalyze asymmetric Aldol reaction (Scheme 1.17).



Scheme 1.17: Synthesis of chiral amino acid derived ILs and be medium for asymmetric Aldol Reaction

ILs **115a** was used as chiral medium with (*S*)-proline as catalyst to promote the asymmetric Aldol reaction. The conversion, selectivity and *ee* value were compare with the reaction which were used other traditional organic solvents with (*S*)-proline. Results showed in Table 1.3 were illustrated that both conversion and selectivity were relatively high in all examined solvents except water. However, the *ee* value was only 9 in the methanol and 62 in Bmim NTf_2. As the solvent was changed to **115a**, highest *ee* value with 77 was observed due to the chiral center in the amide moiety.

Table 1.3: Solvents screening for Aldol reaction between acetone and *p*-nitrobenzaldehyde using (*S*)-proline as catalyst with different solvents[a]

Entry	Solvent	Conversion[%][b]	Selectivity [%][c]	*ee* [%][d]
1	toluene	99	89	71
2	DMSO	99	90	71
3	None	95	97	71
4	DCM	98	99	67
5	H_2O	0	0	0
6	MeOH	98	90	9
7	Bmim NTf_2	99	99	62
8	CIL 2	80	99	77

[a] Conditions: Aldehyde/acetone/cat. = 1:30:0.2, solvent/ acetone = 1:1 v/v, r.t. 6 h

[b] Conversion [%]: evaluated by ^{1}H NMR analysis of the crude reaction mixture

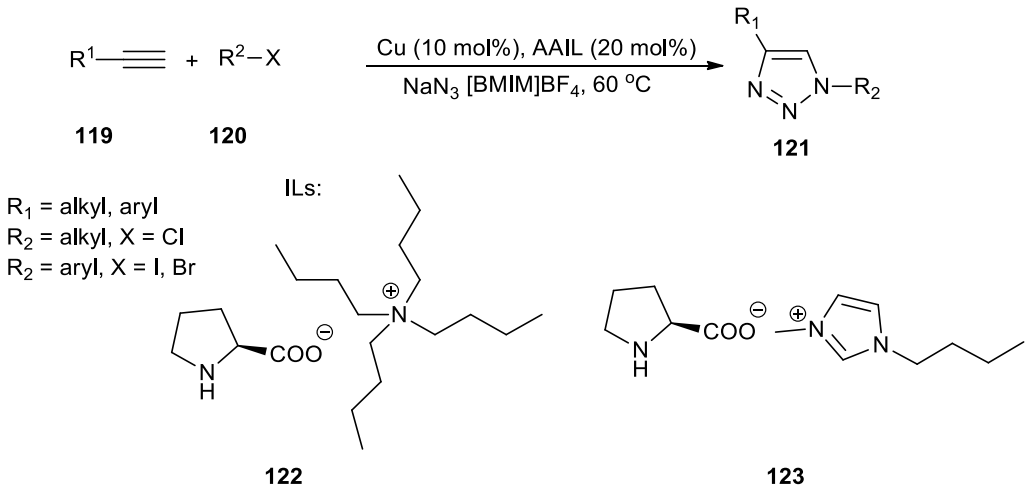
[c] Selectivity [%]: determined by ^{1}H NMR analysis of the crude reaction mixture and according to the ratio between aldol products and dehydration products

[d] *ee* value calculated by HPLC analysis

In summary, apart from the synthesis of imidazolium-based chiral ILs (**98a** to **98h**) from L-, D- and D/L-amino acid by our group,[72] it has not been reported synthesis of corresponding enantiomers of chiral amino acid based ILs and their applications are only limited on being chiral base and mediums in organic synthesis without the effect of stereochemistry study.

1.3.4 Application of Chiral Prolinate Ionic Liquids in Organic Synthesis:

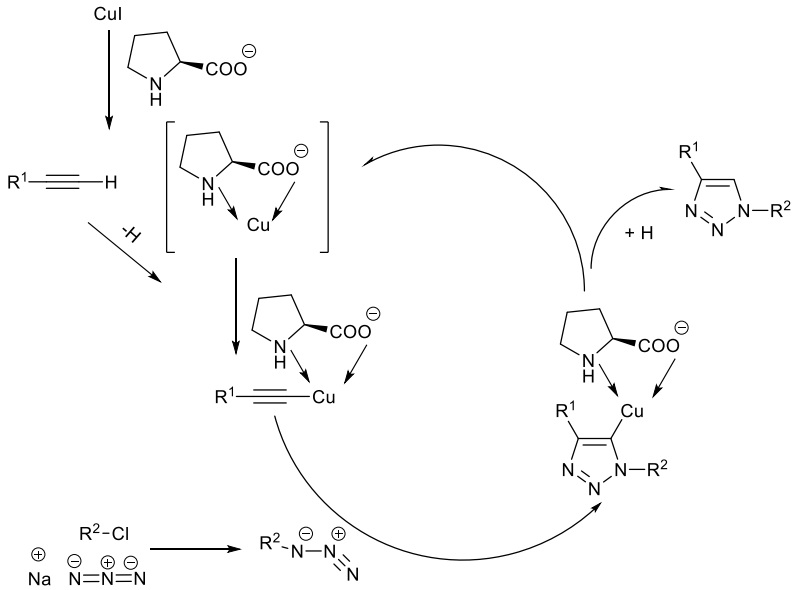
Wang *et al* designed and synthesized prolinate ILs and applied these ILs with Cu(I) as co-catalyst system to promote the synthesis of 1,4-disubstituted 1,2,3-triazoles compounds from aryl iodides or alkyl chlorides, sodium azide and terminal alkynes with high yield 85% (Scheme 1.18).[76] The reaction was carried out under the mole ratio of terminal alkynes, aryl iodides, and sodium azide in a 1:1:2 molar ratio with CuI (10 mol%) and prolinate ILs (20 mol%) in [BMIM]BF_4 as solvent at 60 °C for 10 hrs.



Scheme 1.18: Synthesis of 1,4-Disubstituted 1,2,3-triazoles catalyzed by Copper(I) and prolinate ILs catalytic system

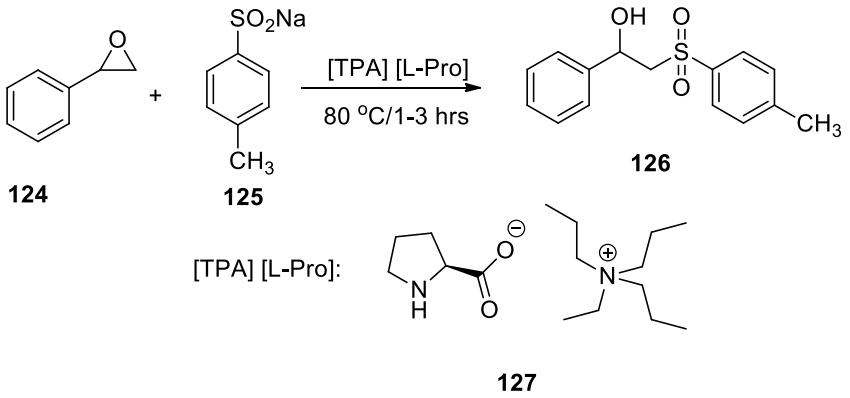
The plausible mechanism in Scheme 1.19 revealed the prolinate ILs played a role as N, O-ligand in the reaction. Cu(I) was coordinated with proline moiety to form the

intermediate compound followed by the cycloaddition with aryl or alkyl azide to generate 1,2,3-triazoles coordinated with Cu(I) prolinate ligand. Designed product was obtained by gain one proton to lose the Cu(I) prolinate ligand.



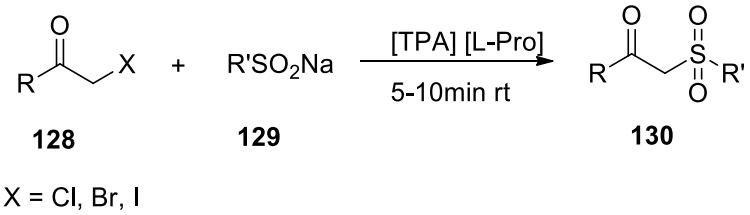
Scheme 1.19: Plausible mechanism for synthesis of 1,4-Disubstituted 1,2,3-triazoles

Venkateswarlu group had used prolinates ILs as high efficient reaction medium for a few organic reactions. Tetrapopylammonium prolinate ([TPA][Pro]) was applied as a solvent for synthesis of β-hydroxyl-sulfones with high yield (Scheme 1.20).[77] The reason for choosing [TPA][Pro] as reaction medium was that poor yields were obtained with a few protic solvents and less polar solvents owing to their low dissolubility for sulfinate salt. [TPA][Pro] aqueous mixture can not only dissolve the substrates completely but also force sulfinate nucleophile prior attack the epoxide from less hindered position to generate the β-hydroxyl-sulfones with no formation of sulfinate esters.



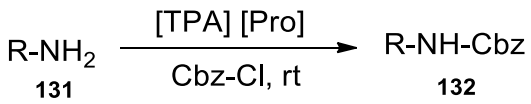
Scheme 1.20: synthesis of β -hydroxyl-sulfones

In the same year, [TPA][Pro] was used as a reusable reaction medium for the synthesis of β -keto-sulfones from α-haloketone with sodium alkyl/aryl sulphinates (Scheme 1.21).[78] Compared with reaction time of using traditional organic liquids as solvent, [TPA][Pro] and [TPA][Pro]/CH_3CN made reaction complete very quickly because of its phase transfer catalysis property. [TPA][Pro] also can be recovered and reused for next run without loss of efficiency.



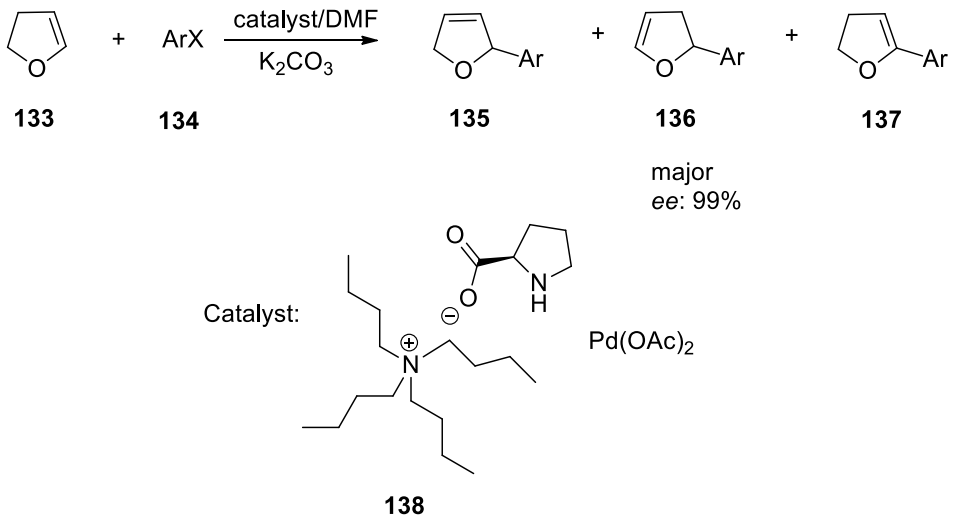
Scheme 1.21: Synthesis of β -keto-sulfones

Venkateswarlu *et al* have introduced [TPA][Pro] into amino group protection reaction.[79] This prolinates ILs had been used as solvent for reaction of amines with Cbz-Cl and both N-Cbz protected aliphatic and aromatic amines were afforded (Scheme 1.22). Chemical-selectivity was also tested by using equal moles of aliphatic and aromatic amine mixture reacted with Cbz-Cl. The result was that only aliphatic amine was reacted with Cbz-Cl and aromatic amine was intact. Furthermore, when the amines contain hydroxyl group such as 4-aminophenol, only amino group was protected.



Scheme 1.22: Amino Protection by Cbz-Cl

Phosphorous ligands have been studied deeply for stereo-selectivity of the Heck arylation for obtaining the high *ee* value of product **135** without formation of product **136**.[80] Recently, the combination of chiral prolinate ILs with palladium was employed in the asymmetric Heck arylation of 2,3-dihydrofuran by Pernak to obtain product **136**.[81] 2,3-dihydrofuran with aryl iodides were used as substrates and the 2-aryl-2,3-dihydrofuran was afforded as the major product (**136**) with high *ee* value 99% without phosphorous ligands (Scheme 1.23).



Scheme1.23: Heck cross-coupling of 2,3-dihydrofuran with iodobenzene

From the results showed in Table 1.4, best conversion and yield of the major product **136** with the highest enantio-selectivity was gained on the basis of the mole ratio of $[Bu_4N][L\text{-}Pro]$ (CIL) with $Pd(OAc)_2$ 2 after hrs. The lower conversion, yield and enantio-selectivity were obtained from the large amounts of $[Bu_4N][L\text{-}Pro]$.

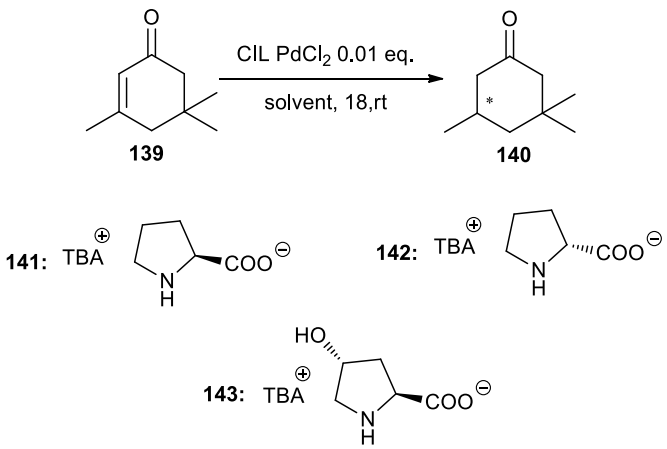
Table 1.4: Heck arylation of DHF with $Pd(OAc)_2$ and different amounts of $[Bu_4N][L\text{-}Pro]$ (CIL)[a]

Time (h)	CIL:Pd =2			CIL:Pd =4		
	Conversion [b] (%)	b yield (%) [ee%]	a yield (%) [ee%]	Conversion [b] (%)	b yield (%) [ee%]	a yield (%) [ee%]
2	14.4	9.4 [7.5]	5.0 [5.7]	17.9	7.4 [3.0]	10.5 [3.2]
3	56.7	39.5 [>99]	12.7 [1.4]	39.3	27.7 [13.3]	11.2 [2.6]
6	83.0	51.8 [>99]	26.4 [2.2]	41.0	25.6 [9.3]	14.9 [1.1]

[a] Conditions: $Pd(OAc)_2$ (1 mol%), 70 °C, solvent DMF (6 mL), DHF (8.6 mmol), PhI (3.57 mmol), K_2CO_3 (4.34 mmol)

[b] Conversion of PhI

Our research group has prepared three prolinate tetra-alkyl ammonium based ILs which were tetrabutylammonium-(*S*)-prolinate, tetrabutylammonium-(*R*)-prolinate and tetrabutylammonium *trans*-4-hydroxyl-(*S*)-prolinate with low antimicrobial toxicity to a wide range of bacteria and fungi. Three prolinate ILs were applied with $PdCl_2$ to promote hydrogenation of α, β-unsaturated ketones with enantio-selectivity (Scheme 1.24).[82]



Scheme 1.24: hydrogenation of α, β-unsaturated ketones

Solvents impact on the conversion and enantio-selectivity were investigated by performing hydrogenation in several protic and aprotic organic solvents. The results were displayed in Table 1.5. Both high yield 98% and selectivity 94% of hydrogenation

were observed in the H_2O as solvent but without ee value. Same situations were obtained in other three aprotic solvents which are THF, DMF and 1,4-dioxane. When protic reaction mediums were choosed, like absolute EtOH, methanol, *t*-BuOH and *i*-PrOH, almost 100% conversion was achieved; the selectivity was relatively lower than in the aprotic solvents due to the formation of trimethylcyclohexanol as by-product. For the enantio-selectivity, the highest *ee* was observed in *i*-PrOH with 47% (*S*).

Table 1.5: Influences of the solvent to the hydrogenation[a]

Solvents	Conversion[b] (%)	Selectivity[b] (%)	ee[c] (%)
H_2O	98	94	—
Absolute EtOH	100	70	24 (*S*)
MeOH	100	85	32 (*S*)
t-BuOH	97	62	17 (*S*)
i-PrOH	100	59	47 (*S*)
THF	98	98	—
DMF	89	99	—
1,4-Dioxane	94	98	—

[a] Conditions: ILs **141** (400 mg), $PdCl_2$ (0.01 eq.), mass ratio solvent/ILs **141** = 5, 18 h, RT. —: no *ee* observed. [b] Determined by GC. [c] Determined by GC by a chiral column.

The configuration of the prolinate ILs also had an infiuence on the enantio-selectivity of the ketone. (Table 1.6) Tetrabutylammonium-(*S*)-prolinate (ILs **141**) and tetrabutylammonium *trans*-4-hydroxyl-(*S*)-prolinate resulted in the (*S*) configuration of ketone product, while tetrabutylammonium-(*R*)-prolinate lead to the (*R*) configuration of saturated ketone.

Table 1.6: Influence of the nature of ILs[a]

ILs	Conversion[b] (%)	Selectivity[b] (%)	ee[c] (%)
—	100	95	—
141	100	59	47(*S*)
142	100	58	26 (*R*)
142	75	85	3 (*S*)

[a] Conditions: CIL (400 mg), $PdCl_2$ (0.01 eq.), *i*-PrOH (2.5 mL), 18 h, RT.
—: no ee observed. [b] Determined by GC. [c] Determined by GC with a chiral column.

Based on the above statements, most of the chiral prolinates are synthesized from the natural abundant L-proline and the enantiomers of unnatural D-proline with 4-hydroxyl group have not been prepared (with the single exception of **143**). The major applications of prolinates are focused in organic synthesis as chiral additives and medium, such as being catalyst in α-amination of aldehydes to obtain the inversion of enantio-selectivity.[83] Consequently, information lacking here is the study of stereochemistry effect of the prolinate enantiomers, especially for the *trans*/*cis* 4-hydroxyl-L/D prolinates.

1.4. Biodegradation and Toxicity Study of Ionic Liquids:

An increasing amount of biological and biochemical research has been focused on evaluation and testing the toxicity and biodegradability of ILs.[84] ILs are viewed as an environmental friendly non-VOC solvent. They have been applied in catalysis chemistry, biochemistry, wood chemistry and organometallic chemistry. Hence, the issue regarding the release of ILs into the environment safely has become more and more urgent.[85] Recently, bacteria, fungi and algae have been used to study the biodegradation of imidazolium and pyridinium-based research.[11] A new series of imidazolium ILs which contain ester or amide groups in the alkyl side chain were designed by Gathergood.[86] The biodegradability was tested by the CO_2 headspace test. The aromatic ring and substituted functional group have been proved to be very important for the biodegradability of the ILs for the reason that aromatic rings, especially benzene rings and unsubstituted linear alkyl side chains offer several positions for enzymatic hydrolysis and are thus attacked more readily by oxygenases. Scammells and co-workers have used pyridine and nicotinic acid as cations source to synthesize another type of ILs which has already proven to be biodegradable.[87] A few additional factors which could influence the biodegradability of ILs have been also studied.[88] Ionic liquids with longer alkyl chains have been proven to be more toxic. This may the reason for some of the poor biodegradation results observed, as the toxicity of ILs may be effecting on the biological activity of the aerobic microorganism.[89]

1.4.1 Biodegradation studies of ILs:

There are several biodegradation study methods certificated by the Organization of Economic Cooperation and Development (OECD) (See Table 1.7).

Table 1.7: Biodegradation Methodology in Application

Test No.	Name	Analytical method
OECD 301 A	DOC Die-Away	Dissolved organic carbon
OECD 301 B	CO_2 evolution	CO_2 evolution
OECD 301 C	MITI (Ministry of International Trade and Industry, Japan)	Oxygen consumption
OECD 301 D	Closed bottle	Dissolved oxygen
OECD 301 E	Modified OECD screening	Dissolved organic carbon
OECD 301 F	Manometric respirometry	Oxygen consumption
ISO 14593	CO_2 headspace test	CO_2 evolution
OECD 309	OECD 309	^{14}C labelling
ASTM 5988	ASTM 5988	CO_2 production / Biochemical oxygen demand

According to OECD guidelines, there are three categories to classify biodegradation level of chemicals. (**a**) Ultimate biodegradation: tested chemicals can be degraded or mineralized by microorganisms to water, carbon dioxide, biomass and inorganic materials completely, which can be classified as 'biocompatibility'. (**b**) Readily biodegradable: positive results can be observed under aerobic conditions in screening tests by mineralization, elimination and alteration, such as hydrolysis, oxidation and photolysis. (**c**) Primary biodegradation: An elimination or alteration of the test sample by microorganisms, in order to lose its specific properties.

New pyridine and nicotinic acid based ILs with 1-(2-hydroxyethyl) side chain were synthesized by Scammells *et al* and their biodegradability was also evaluated the CO_2 headspace test under aerobic conditions.[90] The results showed that several of the ILs

can be classified as readily biodegradable (Figure 1.15). However, significantly low levels of biodegradability were afforded when methyl or ethyl ether groups were present on the side chain. Furthermore, low levels of biodegradability for pyridinium ILs with acetal and carbamate functional groups and thiazolium-based salts were also observed.

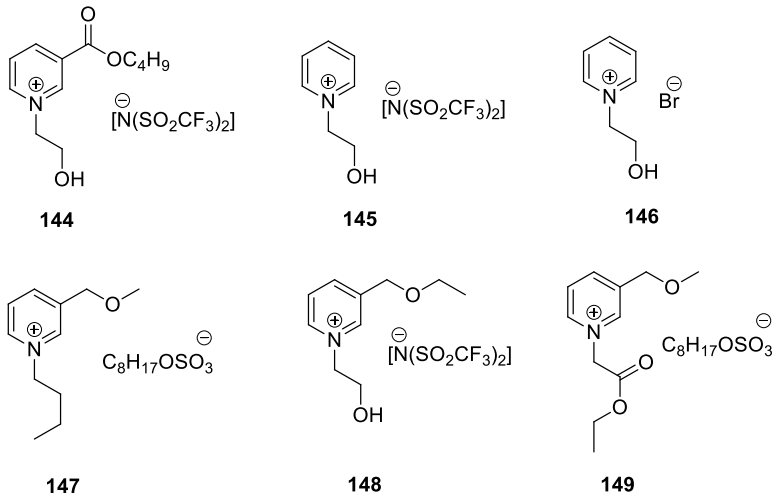
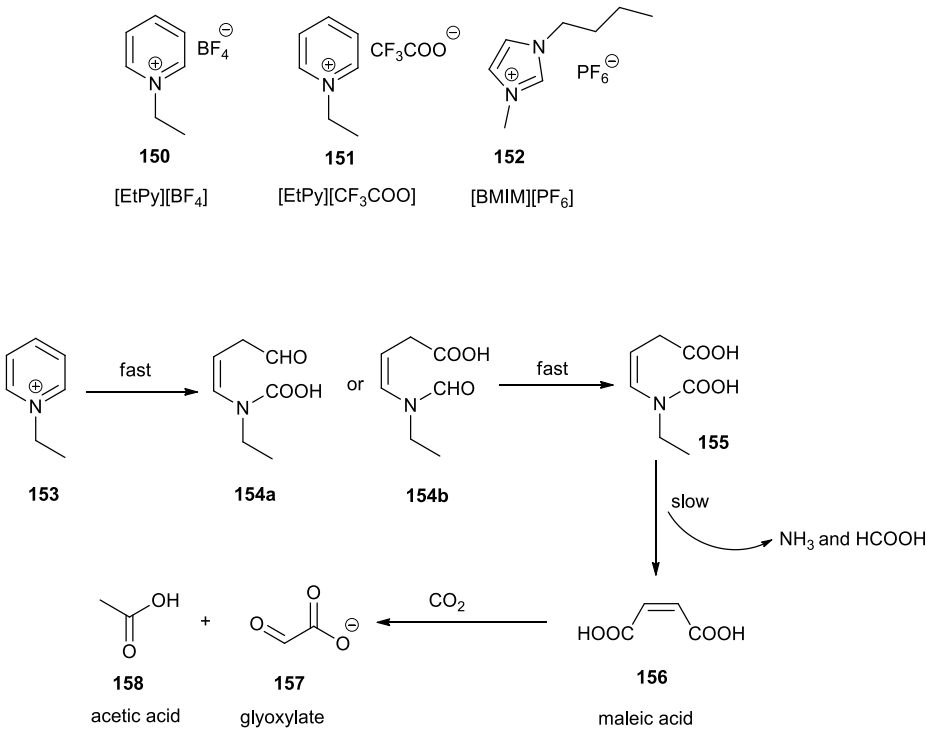


Figure 1.15: ILs with 1-(2-hydroxylethyl) side chain from pyridine or nicotinic acid derivatives

Francis *et al* investigated the biodegradability of three pyridinium and imidazolium based ILs in the presence of an axenic culture of soil *Corynebacteria* up to 40 hrs.[91] The biodegradation results showed that the bacteria *Corynebacterium* was capable to decompose *N*-ethylpyridinium cation while had no positive effects on the anions. But no biodegradation results of [BMIM][PF_6] were obtained. ESI/MS/MS were used to analyze the possible metabolites from the pyridinium moiety and to confirm the reasonable degradation pathway illustrated in Scheme 1.25. The first degradation step was to open the pyridinium ring between C2 and C3 positions to form carbamic acid with aldehyde group followed by losing ammonia and formic acid to the intermediate maleic acid. The final step is the oxidization of maleic acid to acetic acid and glyoxylate.



Scheme 1.25: Pyridinium and imidazolium based ILs in biodegradation and reasonable degradation pathway

The majority of the previous former studies were focused on aerobic biodegradation conditions, limiting the results to aerobic microorganism. Stolte and co-workers have introduced anaerobic condition for biodegradability testing based on the theory of anaerobic respiration, especially in the aspect of a nitrogen reducing environment. The reason for the anaerobic environment is that many microorganisms can survive under anaerobic condition such as are present in soil and sediments.[92] A few different imidazolium, pyridinium and dimethylaminopyridinium based IL cations were tested under anaerobic condition (Figure 1.16). Anaerobic conditions were established using mineral salt medium compositions in anoxic media and HPLC/UV was applied to monitor the denitrifying conditions for the test.

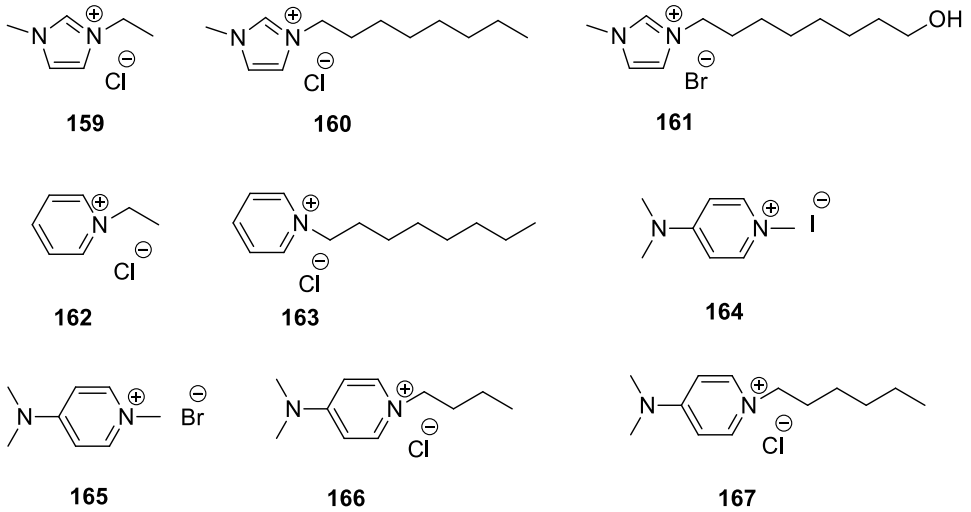


Figure 1.16: Structures of IL used in anaerobic biodegradation study

Additionally, Stolte and co-workers have improved both aerobic and anaerobic biodegradation analysis condition.[93] Owing to lack of complete biodegradation and toxicity tests of fluoroorganic and cyano-based IL anions such as $(CF_3SO_2)_2N^-$, $(C_2F_5)_3PF_3^-$ and $N(CN)_2^-$, $C(CN)_3^-$ $B(CN)_4^-$, biological degradation with both aerobic and denitrifying conditions, followed by inhibition under both aerobic and denitrifying conditions were carried out on the above IL anions. It was concluded that it was hard to classify the studied IL anions as readily biodegradable in both aerobic and denitrifying conditions.

Recently, Stolte and co-workers have studied the biodegradability of ILs with pyrrolidinium, morpholinium, piperidinium, imidazolium and pyridinium based cations and substituted by different alkyl or functionalized side chains with halide anions comprehensively (Figure 1.17).[94] Primary degradability and their ultimate biodegradability were applied biochemical oxygen demand tests according to OECD guideline 301F. Based on the biodegradation results, when hydroxyl group was introduced into the side chain in the cation, complete mineralization (side chain and core) was observed with enough time (60 days). Furthermore, the biodegradability increased with lengthening side chain. For example, the biodegradability classification of 1-alkyl-1-methylpyrrolidinium ILs improved from "not readily biodegradable" with

ethyl to "inherently biodegradable" with butyl and finally change to "readily biodegradable" with octyl side chain. Pyrrolidinium based ILs are most biodegradable in all tested ILs with five different cations and followed by pyridinium core based ILs and the biodegradability of piperidinium and morpholinium based ILs were much lower than pyrrolidinium and pyridinium core based ILs. Imidazolium seems to be the most refractory head group against biodegradation.

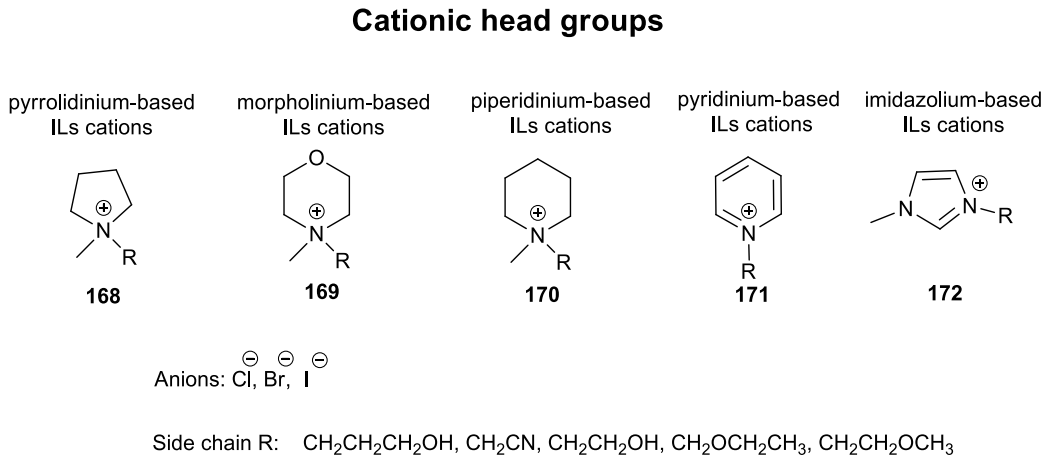


Figure 1.17: Structures of five cationic head based ILs for biodegradation study

As can be seen from above biodegradation results of ILs, most of tested ILs composed by nitrogen heterocyclic cations and halide or other inorganic salts anions. Few biodegradation tests have been carried out for chiral ILs to determine the effect of stereochemistry. Only the biodegradations of two chiral imidazolium amino acid amide ILs (**92b** and **98f**) and L/D prolinate ILs (**141**,**142** and **143**) were determined by Gathergood.[72] Biodegradation of chiral prolinates enantiomers so far have not been evaluated.

1.4.2 Toxicity Study of ILs:

In the light of the above applications in both wood chemistry and organic, it is very obvious that ILs have been a promising alternative to traditional organic solvents.[95] However, the potential environment hazards, contaminations and toxicity[96] to aquatic organism have attracted more concern.[97] Consequently, aerobic and anaerobic biodegradation and eco-toxicity evaluation have been studied on a wide range of ILs.[98]

For the purpose of designing and preparing 'green and safe' ILs, our group has formulated a few strategies of ILs toxicity screening. Gathergood *et al* designed and synthesized imidazolium based ILs with an ether group incorporated into the ester side chain (Figure 1.18). Seven different bacterial strains (four Gram negative and three Gram positive) were used to screen the toxicity of ILs.[86] For the anti-toxicity studies, MIC (minimum inhibitory concentration) values for antifungal study was defined as 80% inhibition (IC_{80}) of the control growth for yeast and 50% inhibition (IC_{50}) of the control growth for filamentous fungi. Furthermore, for the antibacterial study, MIC values were defined as 95% inhibition (IC_{95}) of the control growth. The results of anti-toxicity screening tests showed that ILs contain ether or poly ether in long alkyl side chains in cations lead to obvious low toxicity with higher MIC value compared to the ILs with same length of alkyl side chains in cations.

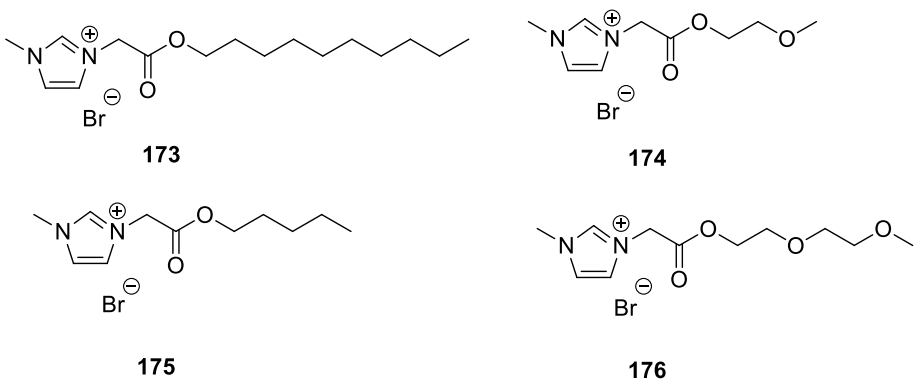


Figure 1.18: Oxygen functionalized ILs

Gathergood and co-workers designed and synthesized both imidazolium-based chiral ILs and dipeptidyl chiral ILs (Figure 1.19) and used them to carry out biodegradation and anti-microbial screening.[72] Twelve fungi strains and eight bacteria strains such as *Candida krusei* E28, *Candida tropicalis 156*, *Candida glabrata* 20/I, *Candida lusitaniae* 2446/I, *Trichosporon asahii* 1188 and *Aspergillus fumigatus* 231 were used for antimicrobial and antifungal toxicity studies. The results showed that these series chiral ionic liquids have low antifungal activities (MIC values of >2000 μM against *Candida albicans* ATCC 44859, *Candida albicans* ATCC 90028 and *Candida*

lusitaniae 2446/I). Furthermore, biodegradation data was afforded at weekly intervals over 28 days under aerobic conditions and most of them were over 60% biodegradable. The main reason for this significant achievement presented by Gathergood was that the ester and amide groups in the imidazolium cation offered two possible positions for enzymatic attack.

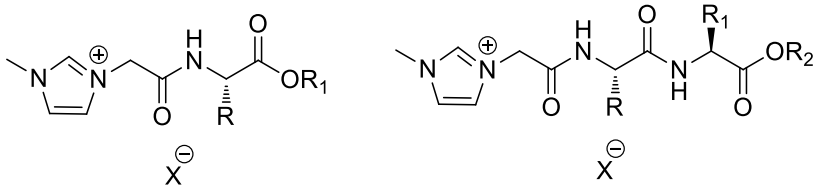
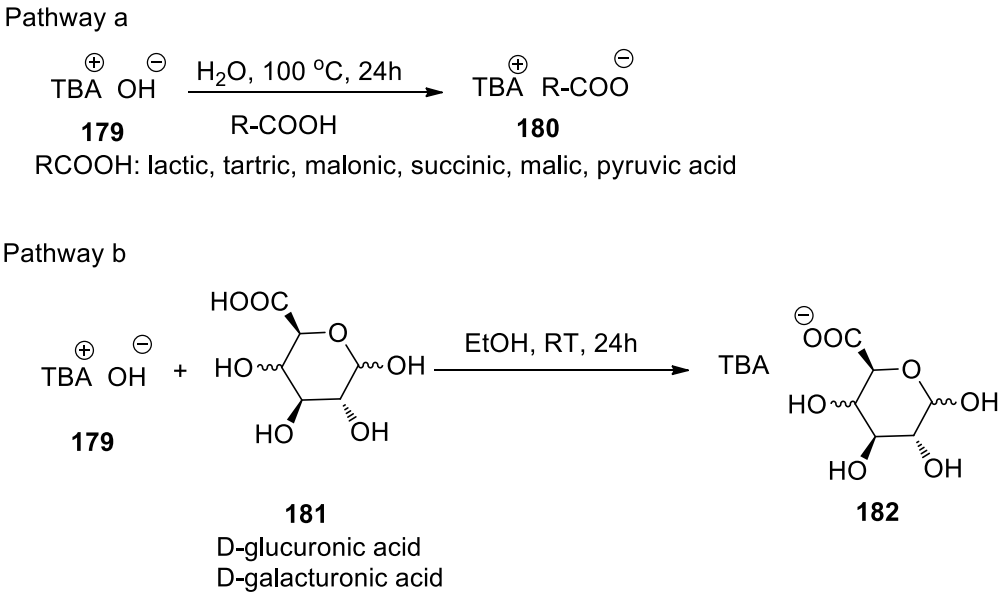


Figure 1.19: General structure of amino acid based and dipeptdyl chiral ILs

Gathergood *et al* have synthesized biomass derived ILs from natural organic acids and their toxicity and biodegradation have been also analyzed.[99] The reaction is start from cheap commercial tetrabutylammomium hydroxide solution react with sustainable acids obtained available from the biomass (Scheme 1.26).



Scheme 1.26: Reaction of TBA based ILs

As previously reported, three prolinate tetra-alkyl ammonium based ILs were synthesized and applied in the enantio-selectivity hydrogenation (Figure 1.20). Next step was to evaluate the toxicity of three ILs.[82] Both Gram positive and Gram negative organisms for the antibacterial; yeast strains and flamentous fungi were used for anti-fungi assessment. All of the three prolinate tetra-alkyl ammonium based ILs do not have high antimicrobial toxicity to the 20 bacteria and fungi strains tested. Further IC_{50} values were obtained in the range of 3 to 50 mM for (*Escherichia coli* ATCC 8739, *P.fluorescens*, *P. putida* (CP1), *P. putida* (KT2440) and *B. subtilis* against three ILs.

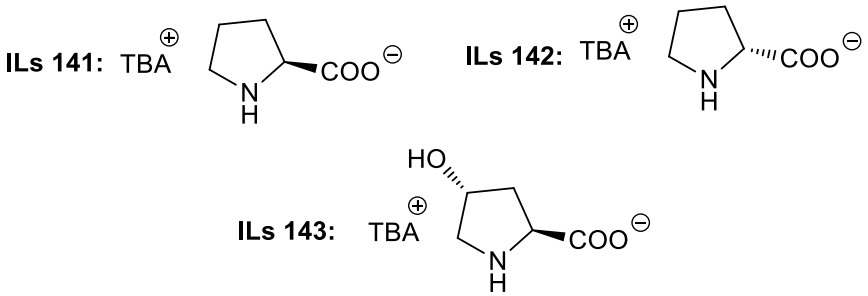


Figure 1.20: Three tested ILs for antimicrobial toxicity

1.5. Thermal Stability Study of ILs:

The thermal property of imidazolium based ILs had been reported by McEwen in 2000.[100] However, due to its low biodegradability and non–chirality, amino acid based chiral ILs have been designed and synthesized as the environmental friendly ILs.[101] In order to applied amino acid based ILs in the organic synthesis and wood chemistry application, the assessments of their thermal stability have been developed widely in recent years.[102] TGA and DSC analysis are the most frequently used methods to determin the thermal stability of ILs.

Differential Scanning Calorimetry (DSC), which is a thermal analysis method that measure how a material's heat capacity (Cp) is altered with temperature changing. This technique is usually applied to detect the melting, glass transitions, phase changes transitions of materials. Because of its flexibility and multi-functions, DSC is used in many industries including pharmaceuticals, polymers and food industries.

Thermogravimetric Analysis is a technique to measure a sample's weight when it is heated or cooled in a furnace within a controlled atmosphere.

Scott *et al* had carried out TGA studies on a range of cyano anion containing ILs. N, N-dialkylpyrrolidinium [$p_{1,4}$] and tetra-alkylphosphonium [$P_{6,6,6,14}$] cations were combined with dicyanamide [dca] and tricyanomethide [tcm] anions (Figure 1.21).[103]

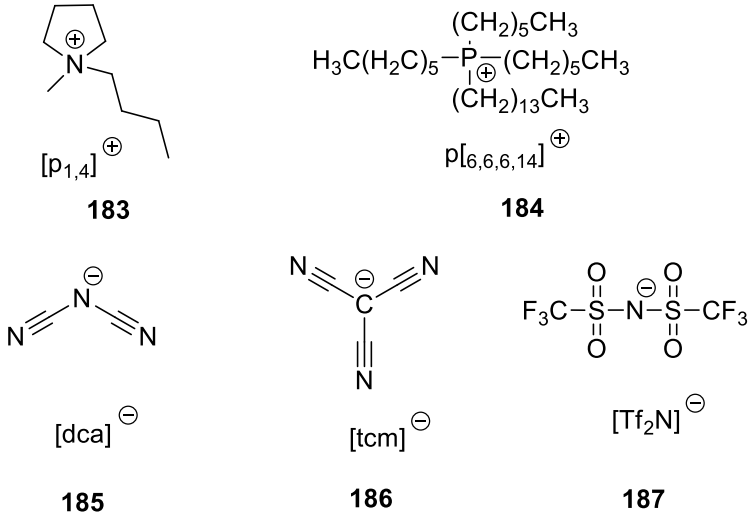


Figure 1.21: Cations and anions used in TGA analysis

The results displayed in Figure 1.22 showed there were distinct differences in the four decomposition temperatures. It was demonstrated that the cyano group and N-based cation ILs to polymerize during decomposition. However, phosphonium based ILs decomposed to volatile compounds completely.

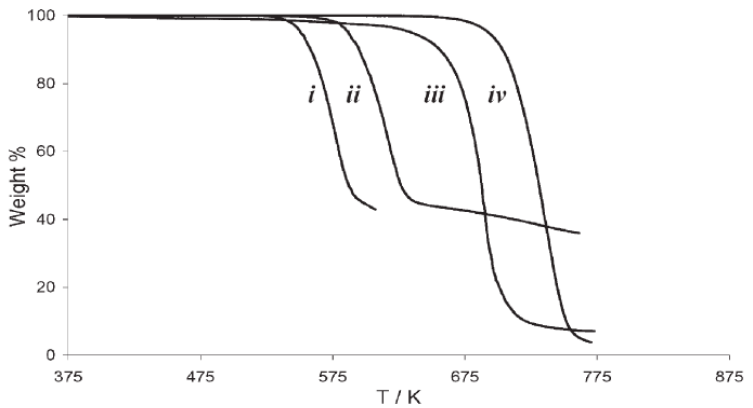


Figure 1.22: TGA trace of (i) [$p_{1,4}$][dca], (ii) [$p_{1,4}$][tcm], (iii) [$P_{6,6,6,14}$][dca] and (iv) [$p_{1,4}$][Tf_2N]

Zhang and co-workers had determined the decomposition and glass transition temperature for several asymmetric tetraalkylammonium amino acid based ILs. Trimethylbutylammonium, triethylmethylammonium and triethylbutylammonium were three kinds of cations; glycine, L-alanine, β-alanine and valine were the anion moiety. Glass transition temperatures were in the range of –76 ^{o}C to –100 ^{o}C ions. These results are due to the alkyl chain length and asymmetry of the cations. In other word, longer the alkyl and the more asymmetric structure of the cation lead to the lower glass transition temperature.[104]

Ohno *et al* investigated the thermal stabilities of tetraalkyl phosphonium-based amino-acid ionic liquids [TBP][amino acid].[105] The glass transition decomposition temperatures were both determined. According to the results, the decomposition temperatures of [TBP][amino acid] were around 220 ^{o}C, which meant better thermal stability than tetraalkyl ammonium-based amino-acid ILs due to the stability of phosphonium cation. The glass temperatures of [TBP][amino acid] were much lower than imidazolium based amino acid ILs were both analyzed. The reason was that protons at the 2, 4 and 5 positions in imidazolium ring could form hydrogen bond with the functional groups on the amino acid moiety, like amino group, carboxyl groups.

Rahman and co-workers reported several novel amino acids based chiral ILs and reported their physical properties.[106] Tetraethylammonium hydroxide cation and several natural amino acids were used as the substrates. The TGA results showed that the range of decomposition temperatures of the series of targeted ILs were from 160–210 °C (Figure 1.23). These values were a little lower than the tetra-butylphosphonium based amino acids (T_d 210–320 °C), which was previously introduced in the front of this section.

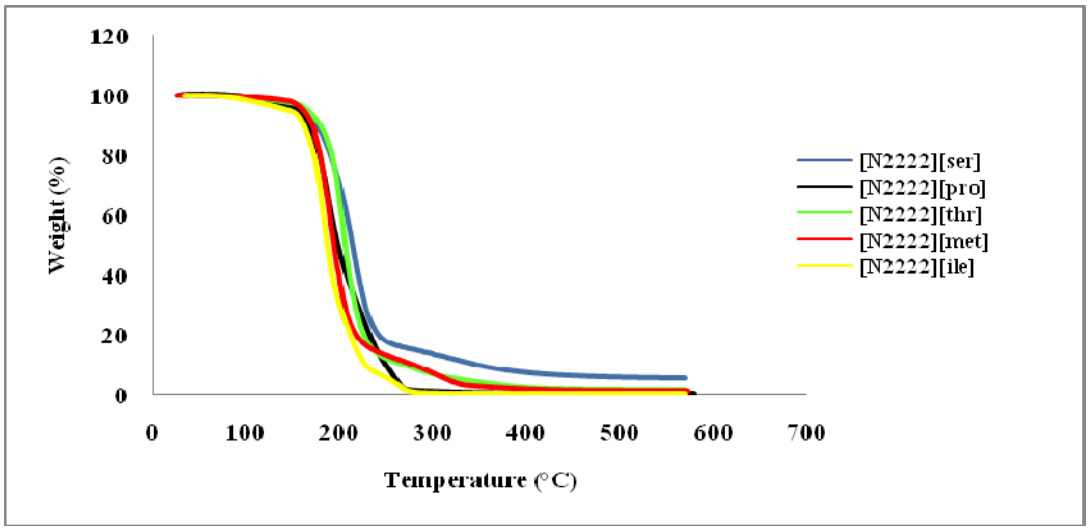


Figure 1.23: TGA Trace of several tetraethylammonium-based amino acids chiral ILs

Mu *et al* have carried out comprehensive investigation to determine the thermal stability of 66 ILs by Thermogravimetric Analysis.[107] The cations of tested ILs were imidazolium, pyridinium and tetraphosphonium based with different length of alkyl side chains substitutions; the anions were tetrafluoroborate, hexafluorophosphate, amino acid, acetate, chloride, bromide, iodide, nitrate and hydrogen sulfate. According to the obtained results, several effects of ILs cations and anions' structures on thermal stability were studied. Anion type mainly determines the thermal stability of ILs. For chains length of the cations like imidazolium and pyridinium, thermal stability increases with the chains length decrease. The thermal stability also increases with increasing degree of substitution on the cations. In summary, Imidazolium based ILs have the best thermal stability independent of anion. However, the thermal stability of tetraalkyl ammonium, piperidinium, and pyridinium based ILs are more dependent on their anions..

1.6 Conclusion:

All above literatures have shown that a wide range of ILs have been proven to have certain efficacy for the pre-treatments for lignocellulosic biomass. Both hardwood and softwoods can be dissolved partially and completely in certain ILs followed by regenerated from ILs, which can enhance the efficiency of enzymatic hydrolysis for release of monosaccharides. However, the study of wood expansion in ILs has been reported by Welton only with traditional non-chiral ILs.[108] Additionally, the correlation of the ILs ability for wood expansion, dissolution, regeneration and sugar release have not researched yet.

Biodegradable ILs with a range of cations and anions have been designed and synthesized and biodegradability has been evaluated by the CO_2 head space test. Toxicity testing, anti-microbial screening and physical property analysis have also been carried out. Although the synthesis, application, biodegradation, toxicity and thermal stability of ILs have been studied widely, most of them are focused on non-chiral nitrogen heterocyclic based ILs instead of chiral amino acid based ILs. The effects of prolinates stereochemistry for the wood dissolution, toxicity, biodegradation and toxicity have not been determined, particular for the 4-hydroxyl-*trans*/*cis*-L/D prolinate. Although our group has performed the biodegradation and toxicity tests for chiral imidazolium amide based ionic liquids, the multi-step of synthesis is the major limitation.

Considering the potential advantages of chiral ILs application and thermal stability, we planned to use traditional ILs first in wood expansion, dissolution, regeneration and enhancement for sugar release after enzymatic hydrolysis then correlate the ability of tested ILs for wood application. Secondly, we planned to synthesize a class of chiral prolinate ILs from both natural and un-natural L/D proline with 4-hydroxyl group. All the enantiomers will be synthesized and characterized; biodegradation, toxicity and thermal stability will be evaluated as well. The forthcoming chapter will describe the application of prolinat ILs in wood chemistry.

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Chapter 2

Experimental

2.1 Introduction

2.1.1 Wood Sample Method:

Material: soft pine (genus is Pinus) was obtained from a local North Carolina source. All specimens were from a single board-board chosen for growth ring orientation-board planed to 10 mm thickness with a Watkin planer with helical carbide cutter head 10 mm wide strips on an in-line rip saw strip with best growth ring orientation selected (best radial/tangential orientation). Selected strip was cross-cut on a micro table saw into 5 mm sections. Final specimens are 5 mm longitudinal × 10 mm radial × 10 mm tangential, all cut from the same 10 mm × 10 mm strip (specimens are axially aligned, sampling the same growth rings). Three dimensions of wood chips sample were measured by a calliper with an accuracy of ±0.001 mm. Unbleached Norway spruce thermomechanical (TMP) fiber (particle size: 40 mesh) were used as starting materials for dissolution and regeneration procedure in ILs. All the wood samples and TMP fiber are kept in vacuum less than 50 ^{o}C until they reached constant weight before used in all tests.

2.1.2 Chemicals:

All chemicals were purchased from Sigma Aldrich, with the exception of D-proline ($\geqslant$ 98%), *cis*-4-hydroxyl-D-proline (98%), *cis*-4-hydroxyl-L-proline (>97%) and tetra-ethyl ammonium hydroxide (35% in H_2O) which were purchased at TCI Fine Chemicals. Thin layer chromatography (TLC) was carried out on aluminum sheets covered with 0.20 mm of fluka silica gel containing fluorescent indicator 254 nm. Riedel de Haën silica gel was used for flash chromatography.

2.1.3 NMR:

NMR analysis were carried out on Bruker 400 MHz spectrometer in deuterated chloroform or deuterated oxide, operating at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR. Some samples were also analyzed by Bruker 600 MHz spectrometer,

operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR in deuterated chloroform or deuterated oxide as well. Chemical shifts are measured in parts per million (ppm) and coupling constants (*J*) are measured in Hertz (Hz). Splitting patterns were recorded by using following abbreviations: s: singlet; d: doublet; t: triplet; q: quartet; dd: doublet of doublets; tt: triplet of triplets; tq: triplet of quartets; m: multiplet.

2.1.4 Optical Rotation:

All optical rotation values were determined by a Perkin Elmer 341 polarimeter in chloroform at 25 ºC.

2.1.5 IR Analysis:

IR analysis was performed on a Perkin Elmer FT-IR spectrum GX spectrometer with neat samples.

2.1.6 Water Content Analysis:

Water content analysis was evaluated by Karl Fischer titration. Karl Fischer Moisture MKS-500 was used to determine all water content values.

2.1.7 LC/MS:

Liquid chromatography mass spectrometry was carried out on an Agilent Technologies 1200 Series Liquid 252 Chromatography system, with an Agilent Technologies 6110 Mass Spectrometer (Quadrupole G6110A). High Performance Liquid Chromatography (HPLC) analysis of released sugar after enzymatic hydrolysis of cellulose was performed in our collaborator's laboratory at North Carolina State University, Raleigh, USA, using (Agilent 1200 HPLC system) a Shodex sugar SP-0810 column at 80 °C with a refractive index detector and mobile phase is Milli-Q water with 0.5 mL/min flow rate. Calibration is with six standard solutions of glucose, xylose, galactose, arabinose, and mannose, ranging between 0.1 to 20 mg/mL.

2.1.8 Mass Spectrometry:

The HRMS analysis was carried out in our collaborator's laboratory at Lüneburg Germany. Determination of the exact mass was performed on LTQ Orbitrap XL via syringe pump injection. The value is an average of approximately 50 single spectra. The anions were determined in negative ion mode, the cations in positive ion mode.

2.1.9 Glass Transition Temperature (T_g) Determination:

The glass transition temperature (T_g) was determined by Differential Scanning Calorimetry (Perkin Elmer's DSC 400) with a heating rate of 20 ^oC min^{-1} and cooling the samples from −60 ^oC to 200 ^oC under nitrogen.

2.1.10 Dynamic Thermal Decomposition Temperature (T_d) Determination:

Dynamic thermal decomposition temperature (T_d) was evaluated by Thermo-gravimetric Analysis (TGA, TA Instruments Q500) under N_2 atmosphere (50 mL min^{-1}) to prevent any oxidation of the sample during the process. The decomposition temperature is defined as the intersection of the baseline weight (after the drying step) and decomposition curve. All samples were placed in a platinum pan at a heating rate of 10 °C min^{-1} and the temperature was programmed from 20 ^oC to 500 °C.

2.2 Experimental Procedure for Toxicity Study of ILs

2.2.1 Experimental Method for Antifungal Toxicity Screening

Antifungal toxicity screening was performed in our collaborator's laboratory at Charles University in Czech Republic. *In vitro* antifungal activities[1] of the chiral prolinate ILs were evaluated on a panel of four types of ATCC strains (CA1: *Candida albicans* ATCC 44859, CA2: *Candida albicans* ATCC 90028, CP: *Candida parapsilosis* ATCC 22019, CK1: *Candida krusei* ATCC 6258) and eight types of clinical isolates of yeasts (CK2: *Candida krusei* E28, CT: *Candida tropicalis* 156, CG: *Candida glabrata* 20/I,

CL: *Candida lusitaniae* 2446/I, TA: *Trichosporon asahii* 1188). The rest of three are filamentous fungi (AF: *Aspergillus fumigatus* 231, AC *Absidia corymbifera* 272 and TM: *Trichophyton mentagrophytes* 445). All the twelve fungal strains were deposited at Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. Three ATCC strains were used as the quality control strains. All the isolates were maintained on Sabouraud dextrose agar prior to being tested.

During all the antifungal toxicity screening procedure, Dimethyl sulfoxide (100%) was applied to be diluent for all ILs, the final concentration of which did not exceed 2%. RPMI 1640 (Sevapharma, Prague) medium supplemented with *L*-glutamine and buffered with 0.165 M morpholinepropanesulfonic acid (Serva) to pH 7.0 by 10 M NaOH was used as the test medium. The wells of the microdilution tray contained 200 µL of the RPMI 1640 medium with 2-fold serial dilutions of the compounds (2000 to 0.488 µmol/L for the new compounds) and 10 µl of inoculum suspension. Fungal inoculum in RPMI 1640 was prepared to give a final concentration of $5 \times 10^3 \pm 0.2$ cfu.mL^{-1}. The trays were incubated at 35 °C. In the case of the T. mentagrophytes, readings were taken after 72 and 120 h and MIC/IC_{50} values were calculated while for all other strains MIC/IC_{80} values were calculated after 24 and 48 h of exposure to ILs. Minimal inhibitory concentration (MIC) is employed to determine the anti-fungi toxicity of ILs to the fungal isolates. The inhibition of ILs against fungi is defined as 80% inhibition of the growth of control for yeasts and as 50% inhibition of the growth of control for filamentous fungi. MIC values were determined twice and in duplicate.

2.2.2 Experimental Procedure for Antibacterial Studies in Czech Republic

Antibacterial toxicity screening was carried out in our collaborator's laboratory at Charles University in Czech Republic. In *vitro* antifungal activities[2] of the chiral prolinate ILs were evaluated on a panel of three ATCC strains (SA: *Staphylococcus aureus* ATCC 6538, EC: *Escherichia coli* ATCC 8739, PA: *Pseudomonas aeruginosa*

ATCC 9027) and five clinical isolates (MRSA *Staphylococcus aureus* HK5996/08, SE: *Staphylococcus epidermidis* HK6966/08, EF: *Enterococcus sp*. HK14365/08, KP: *Klebsiella pneumoniae* HK11750/08, KPE: *Klebsiella pneumoniae* ESBL HK14368/08) from the collection of bacterial strains deposited at the Department of Biological and Medical Sciences, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic. The above-mentioned ATCC strains also served as the quality control strains. All the isolates were maintained on Mueller-Hinton agar prior to being tested.

During all the antifungal toxicity screening procedure, the antimicrobial toxicity of the ILs involves the regulation of the growth rate of bacteria while interfering with their productivity. Dimethyl sulfoxide (100%) served as a diluent for all compounds; the final concentration did not exceed 2%. Mueller-Hinton agar (MH, HiMedia, Čadersky-Envitek, Czech Republic) buffered to *p*H 7.4 (± 0.2) was used as the test medium. The wells of the microdilution tray contained 200 µl of the Mueller-Hinton medium with 2-fold serial dilutions of the compounds (2000 to 0.488 µmol/L) and 10 µL of inoculum suspension. Inoculum in MH medium was prepared to give a final concentration of 0.5 McFarland scale (1.5×10^8 cfu.mL^{-1}). The trays were incubated at 37 °C and MICs were read visually after 24 h and 48 h. The MICs were defined as 95% inhibition of the growth of control. MICs were determined twice and in duplicate. The deviations from the usually obtained values were no higher than the nearest concentration value up and down the dilution scale.

2.2.3 Experimental Procedure for Further Antibacterial Studies in DCU

Antibacterial toxicity screening at higher concentration was carried out at our collaborator's laboratory at School of Biotechnology and National Institute of Cellular Biotechnology, Dublin City University. Four kinds of Gram-negative bacteria (*E. coli* (DSMZ 498), *P. fluoroscens* (DSMZ 50090), *P. putida* (CP1), and *P. putida* (KT 2440) and one Gram-positive bacterium strain (*Bacillus subtilis (B. subtilis;* DSMZ 10) were used to carried out the further anti-bacteria toxicity screening tests. Mueller-Hinton

broth was purchased from Oxoid for the strains growing. The five bacteria strains were grown in the uni-vials with 10 mL nutrient broth and incubated overnight in a shaking machine at 30 °C. The next day the cultures were centrifuged at 4000 rpm for 15 minutes and the pellets washed with 10ml 0.01 M sodium phosphate buffer. Optical density of cultures was adjusted to afford an optical density about 0.07 at 660 nm. The chemical stock solution was prepared in Eppendorf tubes with a concentration of 2 M. The chemical was introduced to the tube and the tube was filled to the 1 mL mark with Mueller-Hinton broth to allow compound to be fully dissolved. The antimicrobial activity of the ILs was tested on a 96 well microplate. Mueller-Hinton broth was transferred to the wells by use of a multi-pipette: 180 µL of broth was pipetted into **column** 1 of wells and 100 µL into wells **columns** 2-8 and 11. 20 µL of the chemical solution was transferred into **column** 1; this gives a concentration of 200 mM from 2 M stock solution and the solution was then mixed. 100 µL of the solution from **column** 1 is then transferred to the next **column** and mixed. The procedure is repeated up to and including **column** 8 where 100 µL of solution is discarded. Each well was inoculated with 5 µL of bacterial culture overnight and the optical density was measured the next day using an ELISA reader. The results were checked using a microplate reader at a wavelength of 405 nm. The IC_{50} values were determined as the concentration or range of concentrations that caused a 50% reduction in growth.

2.3 Experimental Procedure for Biodegradation Study of ILs

To determine the biodegradability of synthesized chiral prolinate ILs, OECD 301D 'Closed Bottle Test' was applied.[2] This method allows for the determination of the aerobic biodegradability of an organic compound in an aqueous medium at a given concentration of microorganism, by analysis of the chemical oxygen demand. In this test, Sodium *n*-dodecyl sulfate (SDS) was used as reference substance. The stock solution was prepared by the test ILs (2-5 mg/L) in mineral medium inoculated with a small number of micro-organisms from a mixed population and kept in completely full, closed BOD bottles in the dark at constant temperature. Strongly aerate mineral medium for at least 20 minutes before use. Making parallel inoculum of BOD bottles with the

absence of test ILs was for the determination of oxygen blanks for the reference substances. Duplicate bottles of each series were analyzed immediately for dissolved oxygen and the remaining bottles were incubated at 20 °C±1 °C in the dark. Withdraw at least duplicate bottles of all series for dissolved oxygen analysis at time intervals (at least weekly) over the 28 days incubation. After 28 days test run, oxygen depletion (O_2 mg/L) of ILs was calculated by using oxygen depletion (O_2 mg/L) of test ILs minus oxygen depletion (O_2 mg/L) from the blank then oxygen depletion (O_2 mg/L) of ILs was divided by the concentration (mg/L) of the test ILs to afford the BOD as mg oxygen per mg test ILs. Finally, the biodegradation percentage was calculated by dividing the specific BOD by COD; Where: BOD is the biochemical oxygen demand; COD is the mean chemical oxygen demand.

2.4 Reference

1 D. Coleman, M. Špulák, M. T. Garcia and N. Gathergood, *Green Chemistry*, 2012, **14**, 1350-1356

2 (a) N. Gathergood, M. T. Garcia and P. J. Scammells, *Green Chemistry*, 2004, **6**, 166-175; E. L. Bizukojc and D. Gendaszewska, *Journal of Bioscience and Bioengineering*, 2013, **115**, 71-75; (c) T. P. Pham, C. Cho, C. Jeon , Y. J. Chung, and Y. S. Yun, *Environ. Sci. Technol.* 2009**, 43**, 516-521.

Chapter 3

Application of ILs in Wood Chemistry

Contributors

Prof. Dimitris S. Argyropoulos (North Carolina State University) performed HPLC sugar analysis

3.1 Introduction:

Safer solvents and auxiliaries, use of renewable feedstocks and design for energy efficiency are three principles of green chemistry.[1] Lignocellulosic biomaterials are a renewable resource of carbon-based feedstock for fuel and chemical production from biorefineries,[2] a sustainable alternative to crude oil which provides organic compounds from the petrochemical refineries.[3] Recently a large number of applications of lignocellulosic biomass have been reported,[4-6] but the challenge in this research field is the separation of wood components. Lignocellulosic biomass is composed of three major components: cellulose, hemicellulose, and lignin which is covalently cross-linked to hemicellulose.[7] Development of green and high performance solvents for biomass processing is a worthwhile goal.[2]

Ionic liquid (IL) based technologies for biomass manipulation are proposed as a solution.[8] Rogers *et al.* reviewed IL dissolution of lignocellulose using ILs highlighting the high loadings achieved.[9] Gathergood and others have reported the synthesis of low antimicrobial toxicity (e.g. **1**, Fig 3.1.)[10-12] and/or biodegradable ILs[13-15] and their applications in green chemistry catalytic methodologies.[16-21] Rogers[9] and Gathergood[8] have stressed the importance of toxicity and biodegradation studies for ILs envisaged to be utilized on large scale in biorefineries.

It has already been shown that ionic liquids can dissolve a large number of biomacromolecules, such cellulose,[22–24] silk fibroin,[25] lignin,[26] starch and zein protein,[27] chitin/chitosan,[28,29] with high efficiency. Wood dissolution, modification, regeneration and enzymatic hydrolysis also have been reported by Argyropoulos using imidazolium ILs.[30-31] 1-Allyl-3-methyl-1H-imidazol-3-ium chloride [Amim] Cl (**2**), 1,3-dimethylimidazolium dimethyl phosphate [Mmim][DMP] (**3**) and 1-ethyl-3-methylimidazolium acetate [Emim] Ac (**4**) in complete dissolution and partial delignification of wood,[32] dissolution behaviour of chitin[33] and pre-treatment of cellulosic biomass[34] has previously been studied. According to Welton,[35-37] deconstruction and fractionation of lignocellulosic biomass have been processed in ILs

or ILs-water mixtures. Furthermore, a convenient and easily assessable screening method for biomass dissolution by ILs ranking was also described by Welton using Bmim and Emim ILs and pine wood chips.[38]

In order to introduce ILs applications into wood chemistry, it is necessary to discuss key references found in the literatures.[32] The application of IL technology has a large potential in the fuel and biochemical industry.[6] According to the literature, three traditional ILs which are 1-allyl-3-methyl-1*H*-imidazol-3-ium chloride [Amim] Cl, 1, 3-dimethylimidazolium dimethyl phosphate [Mmim][DMP], and 1-ethyl-3-methylimidazolium acetate [Emim] Ac have been selected as targeted substrates and regeneration solvents for this project. The major objective of this project was to research the ability of traditional ILs to cause wood chip swelling and then wood regeneration, followed by enzymatic hydrolysis. They were performed to correlate the swelling ability of ILs on wood chips.

We disclose herein ILs, [Amim] Cl (**2**), [Mmim][DMP] (**3**) and [Emim] Ac (**4**), and their 1:1 mixtures ability to swell wood chips. The effect of adding a low microbial and algal toxicity[10] benzylpyridinium derivative salt **1** to the bulk ILs (**2**-**4**) to study the influence the aromatic groups have on interaction with the aromatic lignin structure of wood will be investigated. The relationship between wood chip expansion in IL and release of sugars on digestion of pre-treated wood will be determined.

3.2 Project Aims:

- Synthesis of chiral mandelate ILs
- To investigate the ability of typical ionic liquids for wood expansion
- To investigate the wood dissolution and regeneration in typical ionic liquids
- To investigate the enzymatic hydrolysis of regenerated wood from ILs to correlate wood swelling with the structure of various ionic liquids.

3.3 Experimental and Procedures

3.3.1 Materials

Allyl chloride and 3-methylimidazole were purchased from Aldrich and freshly distilled prior to use. [Emim] Ac was purchased from Aldrich and purified by using acetone extraction and dried under high vacuum at 60 ^{o}C for 24 hrs. Pyridinium salt (**1**) was synthesized according to published procedures by Gathergood *et al*.[10] Viscozyme was purchased from Aldrich.

Soft wood (soft pine from a local North Carolina source) and unbleached Norway spruce thermomechanical (TMP) fiber are used as wood starting materials. All specimens from a single board-board chosen for growth ring orientation-board planed to 10 mm thickness with a Watkin planer with helical carbide cutter head 10 mm thick plank cut into 10mm wide strips on an in-line rip saw strip with best growth ring orientation selected (best radial/tangential orientation). Selected strip was cross-cut on a micro table saw into 5 mm sections. Three dimensions were measured by a calliper with an accuracy of $\pm$0.01 mm. Final specimens are 5 mm longitudinal × 10 mm radial × 10 mm tangential, all cut from the same 10 mm × 10 mm strip (specimens are axially aligned, sampling the same growth rings). All the wood samples used (Norway TMP fibre and small wood chips were kept in vacuum less than 50 ^{o}C until they reached constant weight before used in all tests.

3.3.2 Synthesis of ILs

All ionic liquids used in test are listed in **Figure 3.1.**

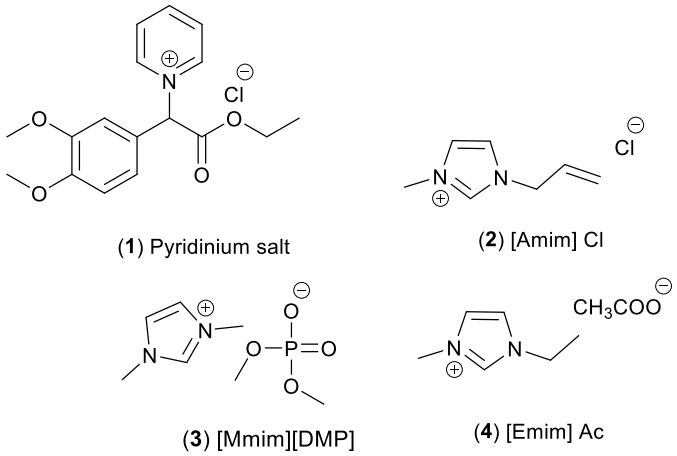

Figure 3.1 Structures of the Ionic Liquids Applied

All ionic liquids used in test are listed in Figure 3.1. [Amim] Cl (***2***) was prepared according to the general route of Li,[39] and dried at 60°C *in vacuo* for 72 hrs prior to use. 1,3-Dimethylimidazolium dimethyl phosphate (**3**) was prepared according to Liu[40] method. A flask was charged with trimethylphosphate (0.25 mol) and 1-(methyl) imidazole (0.25 mol) and heated at reflux (80 °C) for 24 hrs protected by an inert atmosphere (Ar). After the reaction finished, the cooled mixture was extracted with ethyl acetate (4 x 25 mL) then ethyl acetate was removed under reduced pressure. The title compound (**3**) was dried for at 60 °C for 24 hrs *in vacuo* to afford [Mmim][DMP] as a low viscosity slightly yellow liquid.

3.3.3 Wood Chips Swelling Test in ILs

Soft pine was obtained from a local North Carolina source. Based on recommendations from Welton's research,[38] all the swelling tests were conducted in three parallel experiments because of sample heterogeneity. Tests were conducted in a high vacuum oven (525 mmHg) at either 50 °C or 80 °C. All the ILs were dried under high vacuum at 60 °C. Small wood chips (0.23 - 0.27 g) were immersed in 2.3 g – 2.7 g IL in small vials (10% by weight). Wood chips were taken out then measured by a calliper quickly every 24 hrs and the parameter a/a_0, the ratio of woods chips' swelling was calculated. For the parameter a/a_0, a_0 is the original length of three dimensions (tangential, radial and axial, See Fig 3.2.) of wood chips; a is the length after 24 hrs intervals.

3.3.4 Wood Dissolution, Regeneration, Enzymatic Hydrolysis, Glucose Determination.

Unbleached Norway spruce thermomechanical (TMP) fiber (particle size: 40 mesh) was selected as the substrate of wood dissolution and regeneration studies. TMP fiber (1.00 g, 7.1% by weight%) was added into ionic liquid (13.00 g) in a round bottom flask then mixture was stirred rapidly at 120 ^{o}C for 12 hrs. The colour of the mixture was turned to dark yellow or brown. Wood solution was poured into distilled water (500 mL) with strong agitation. The precipitate was separated by reduced pressure filtration, followed by washing with distilled water (5 x 100 mL) to remove ionic liquid residue. Wet regenerated wood was collected and then a small portion of regenerated wood was dried at 80 ^{o}C in oven for 16h to determine solids content thus allowing the regenerated wood yield to be calculated. Next step is the wet regenerated wood was treated by Viscozyme (Aldrich, 100 FBG/g, 1.21 g/mL). The ratio of Viscozyme is 40 FBG/g of wood. CH_3COONa solution with pH 5.2; 5% consistency for starting woods was chosen as buffer solvent. Enzymatic hydrolysis was performed in small vial in water bath shaker at 40 ^{o}C for 48 hrs then using distilled water (20 mL x 4) to wash the mixture and separated by centrifuge. Supernatant liquor of four times was collected and the monomeric sugars in the solution were determined by HPLC analysis using an Agilent 1200 HPLC system with a Shodex sugar SP-0810 column at 80 °C with a refractive index detector; mobile phase was Milli-Q water with a 0.5 mL/min flow rate. Calibration is with six standard solutions of glucose, xylose, galactose, arabinose, and mannose, ranging between 0.1 to 20 mg/mL. Regenerated wood residue after enzymatic hydrolysis was freeze dried and yield of residue was calculated.

3.4 Results and Discussions

3.4.1 Wood chips Swelling Test

[Amim] Cl (**2**), [Mmim][DMP] (**3**) and [Emim] Ac (**4**) were investigated as sole IL solvents for the wood swelling test at 50 ^{o}C (Fig. 3.3) and 80 ^{o}C (Fig. 3.4 and 3.5), followed by 1:1 IL mixtures (**2**):(**3**), (**2**):(**4**), and (**4**):(**3**) at 50 ^{o}C (Fig. 3.8) and 80 ^{o}C (Fig. 3.9 and 3.10). Pyridinium salt (**1**) (20 mol%) was dissolved in [Amim] Cl (**2**),

[Mmim][DMP] (**3**) and [Emim] Ac (**4**) at 80 ^{o}C (Fig. 3.4 and 3.5) and wood swelling evaluated. There are three different sides of the wood chips, which are shown in (Fig 3.2.).

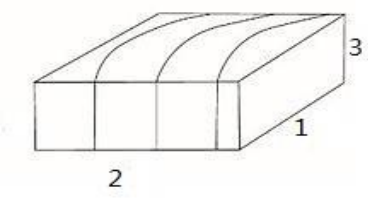

Fig. 3.2 Wood chip with annual ring and definition of three dimension **1** (tangential), **2** (radial) and **3** (axial) relative to growth rings

[Emim] Ac (**4**) gave superior results for wood swelling along radial and tangential sides compared to ILs (**2**) and (**3**) at 50 ^{o}C (Fig. 3.3). Tangential side expanded to a maximum of a/a_0 1.20 and 1.13 for radial side. For IL [Mmim][DMP] (**3**), expansion extent of tangential and radial is a/a_0 1.15 and 1.13; while the lowest swelling ratio was obtained in [Amim] Cl (**2**) with a/a_0 1.15 of tangential and 1.11 of radial at 50 ^{o}C. There was no measurable expansion in axial direction (even at 80 ^{o}C) for any wood chips. Sample break did not occur at 50 ^{o}C.

Radial sides of wood chips in (**3**) and (**4**) expanded from initially a/a_0 1.00 to 1.11 within the first 24 hrs, then onto a maximum expansion of 1.13 after 3 days. Using IL (**2**) 3 days was also required to achieve the maximum expansion of radial side a/a_0 1.10, however a slower expansion rate was observed with only a/a_0 values of 1.07 and 1.09, for 24hrs and 48 hrs respectively. For (**3**) tangential side expanded more slowly than radial with only a/a_0 1.05 after 1 day. The same phenomenon was observed for (**2**). However (**4**) exhibited a large tangential expansion in 24 hrs, with a/a_0 1.13 found.

Comparing the pine wood chip expansion extent at a similar temperature in Welton's research,[38] tangential expansion ratio a/a_0 1.11 was afforded in IL [Bmim] Ac after 40 hrs at 60 ^{o}C. Our study gives a tangential expansion ratio a/a_0 1.20 for [Emim] Ac (**4**) after 48 hrs at 50 ^{o}C (Fig. 3.3). ILs [Bmim][DMP], [Bmim][Me_2SO_4], [Bmim][OTf] and [Bmim][$N(CN)_2$], were less effective, with the expansion ratio a/a_0 is in the range

1.00 to 1.05 for tangential side after 40 hrs at 60 °C using soft pine wood chips.[38] In agreement with Welton's results the greatest expansion of wood chips was achieved using acetate ILs as well as highest rate of swelling. [Bmim] Ac achieved tangential expansion ratio a/a_0 1.10 in 10 hrs at 60 °C. A slower tangential expansion rate was reported for the [DMP] analogue in the same study (a/a_0 1.04 in 10 hrs at 60 °C)[38] which we also observed for (**3**) (a/a_0 1.05 in 24 hrs at 50 °C, Fig. 3.3).

In the Fig. 3.8, radial side expanded to a/a_0 1.10 in 1:1 (**2**):(**3**) and 1:1 (**2**):(**4**), which is slightly lower than expansion in single ILs **3** and **4** with a/a_0 1.13. Tangential side expanded to a/a_0 1.16 in 1:1 (**2**):(**3**), which is almost same expansion a/a_0 1.15 in single ILs **3**; Tangential side expanded to a/a_0 1.18 in 1:1 (**2**):(**4**), which is slightly lower than and a/a_0 1.20 in ILs **4**. Consequently, the increased concentration of chloride ion do not enhance the wood expansion extent even has a slight inhibition on swelling in IL mixture 1:1 (**2**):(**4**).

Wood chip swelling studies at 80 °C for ILs (**2**-**4**) are shown in Fig. 3.4 and 3.5. Radial expansion a/a_0 values at 80 °C and 50 °C are very similar for (**3**) 1.12 vs 1.13. A significant change is observed in (**4**) as temperature increases, radial a/a_0 values increase from 1.13 at 50 °C (over 6 days, Fig. 3.3) to 1.19 at 80 °C (over 3 days, then sample break occurring, Fig. 3.4). No increase in radial or tangential side expansion for (**2**) was found on raising temperature from 50 °C to 80 °C, Fig 3.3 vs Fig 3.4 and 3.5). A significant change in tangential expansion is observed in (**3**) and (**4**) as temperature increases; (**3**) a/a_0 values increase from 1.15 at 50 °C (over 6 days, Fig. 3.3) to 1.31 at 80 °C (over 6 days, no sample break) and (**4**) a/a_0 values increase from 1.20 at 50 °C (over 6 days, Fig. 3.3) to 1.24 at 80 °C (over 3 days, then sample break occurring, Fig. 3.5) Wood sample breaking in [Bmim] Ac was also reported by Welton *et al*.[38] Also as the increased wood swelling temperature, best wood expansion extent was obtained in IL [Emim] Ac with a/a_0 1.21 of tangential and 1.06 of radial at 120 °C after 36 hrs, which indicated higher temperature has the positive effect in wood expansion in ILs.

Fig. 3.9 and 3.10 also display the expansion extent of radial and tangential sides in three single ILs mixtures at 80 ^{o}C. Compare the swelling extent at same temperature in single ILs (**2**), (**3**) and (**4**), radial side expand with larger ratio a/a_0 1.18 in 1:1 (**4**):(**3**) and 1.17 in 1:1 (**2**):(**4**) than a/a_0 1.12 in IL (**3**) and 1.10 in IL (**2**), but is slightly lower in (**4**) a/a_0 1.19. For the tangential side swelling, expansion extent with a/a_0 1.25 in 1:1 (**2**):(**4**) and 1.23 in 1:1 (**2**):(**3**) is significantly higher than a/a_0 1.14 in (**2**); there is no obviously positive or negative effects on tangential side expansion between a/a_0 1.25 in 1:1 (**2**):(**4**) and 1.29 in 1:1 (**4**):(**3**) with a/a_0 1.31 in (**3**) and 1.23 (**4**) due to the sample splitting eventually. Hence, the expansion of radial side in ILs (**2**) and (**3**) can be promoted by the addition of IL (**4**); swelling extent of tangential in IL (**2**) is enhanced by the addition of ILs (**3**) and (**4**) at 80 ^{o}C as well.

Three equal mole ratio mixtures of [Emim] Ac, [Mmim][DMP] and [Amim] Cl were tested their ability for swelling and 20% mol per-cent pyridinium salt **1** was also mixed with each three single ionic liquids at 80 ^{o}C (Fig. 3.4, 3.5, 3.9 and 3.10). As shown in Fig. 3.5 and S2, sample break only occurred in [Emim] Ac, 1:1 (**2**):(**4**) and 1:1 (**4**):(**3**). From these four pictures, [Emim] Ac is the best ionic liquid in the all tested ILs for wood swelling both from tangential and radial direction.

For pyridinium salt **1** mixture ILs 1:4 (**1**):(**4**) and 1:4 (**1**):(**3**), both tangential and radial sides expand less than in only the ILs mixture [Emim] Ac (**4**) and [Mmim][DMP] (**3**), because the weak ability of wood swelling chloride anion (c.f. BMIM Cl only gave an a/a_0 1.10 of tangential and 1.02 of radial expansion at the highest temperature studied, 120 ^{o}C after 180 hrs).[38] Additionally, the radial sides expanded with same extent in the pyridinium salt **1** mixed with [Amim] Cl; but tangential side swelled to the extent of a/a_0 from 1.14 to 1.21. It could be attributed into the addition of pyridinium cation to facilitate the wood expansion only in IL [Amim] Cl.

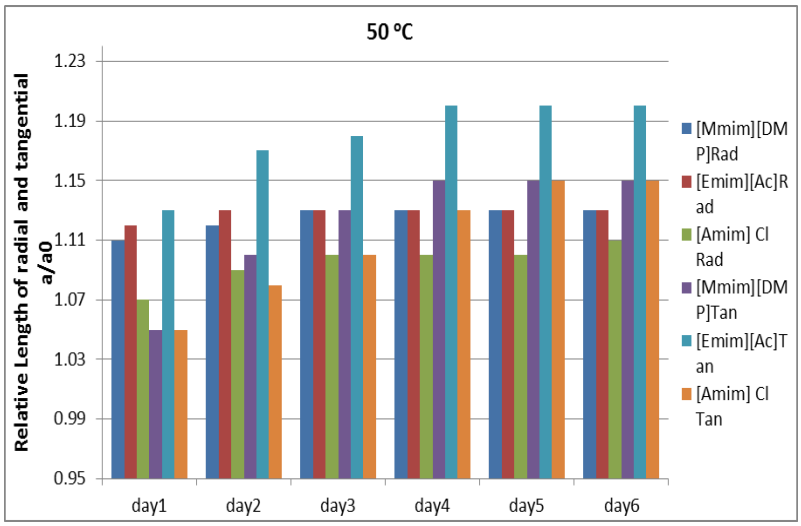

Fig. 3.3 Swelling of radial and tangential direction of wood chips at 50 ºC temperatures in three single ILs. Each time point was averaged in three samples.

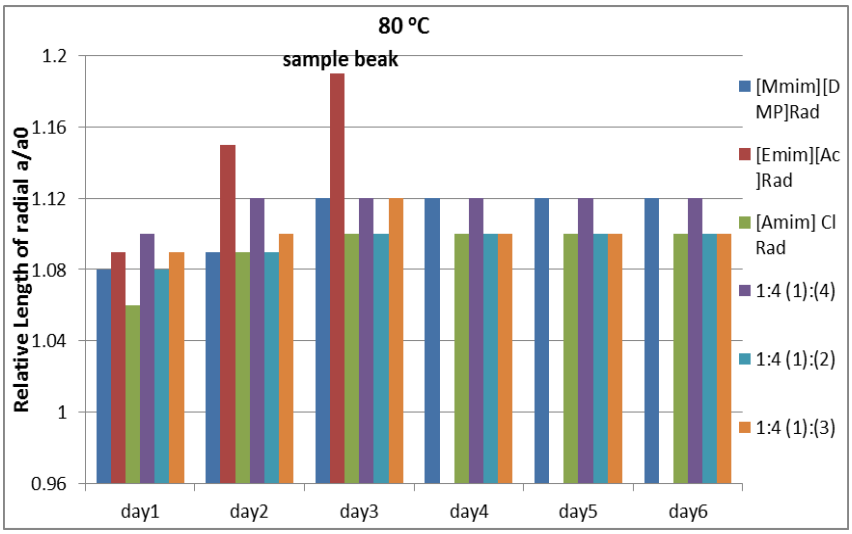

Fig. 3.4 Swelling of radial direction of wood chips at 80 ºC temperatures in three single ILs and three mixtures with pyridinium salt **1**. Each time point was averaged in three samples.

Another interesting point is the time of sample break occurred. In the Fig. 3.4 and 3.5, sample break took place at the third testing day in [Emim] Ac. However, sample break occurred in the fourth testing day in two kinds of ILs mixtures which are 1:1 (**2**):(**4**) and

1:1 (**4**):(**3**) (Fig. 3.9 and 3.10**)**. The addition of [Mmim][DMP] and [Amim] Cl still had the negative affect of slowing the swelling procedure.

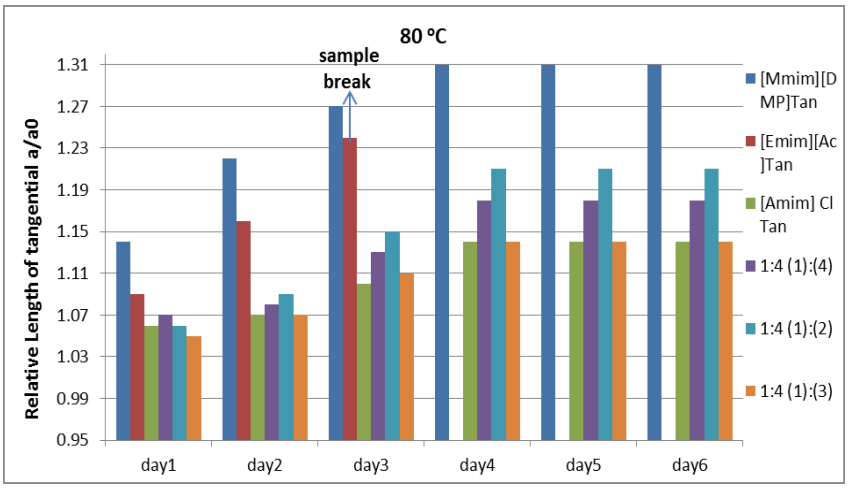

Fig. **3.5** Swelling of tangential direction of wood chips at 80 ^{o}C temperatures in three single ILs and three mixtures with pyridinium salt **1**. Each time point was averaged in three samples.

Three different stages during the wood swelling test are depicted in (Fig. 3.6). There are sharp contrasts between original wood, swelling wood (picture A) and sample break wood (picture B). A significant conclusion can be drawn that wood swelling and expanding to break along with the annual ring and the final wood chips in (Fig. 3.6) are more different with one in (Fig. 3.7). Hence, [Emim] Ac is the most useful and critical ionic liquids in the tests for wood swelling even to sample break.

Fig. 3.11 and Fig. 3.12 are the swelling test for wood chips without growing ring in three single ionic liquids. The starting wood chips were cut along with growing ring then immersed in ionic liquids at 80 ^{o}C. There is no sample break occurred in these small wood chips. Consequently, temperature, annual ring and appropriate ionic liquid are three main reasons for wood swelling and sample break. Another reason for [Emim] Ac is that it can open the inside structures of wood chips more than other ionic liquids so that wood chips swell best in pure [Emim] Ac.

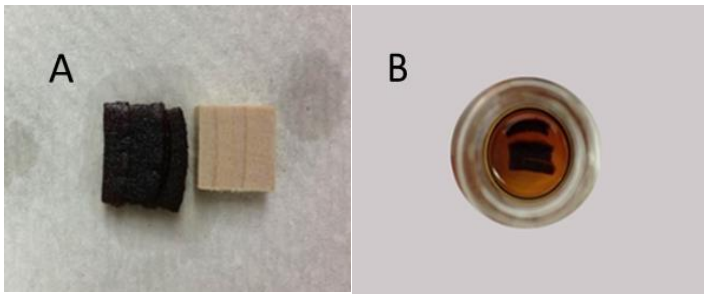

Fig. 3.6 Three states of wood chips during swelling test

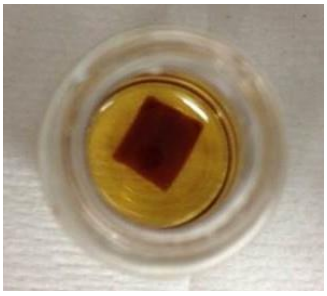

Fig. 3.7 Wood chips swelling state in other ionic liquids without the presence of [Emim] Ac

3.4.2 Wood Regeneration Enzymatic Hydrolysis

Argyropoulos had reported that using imidazolium based ILs to dissolve wood sample at 110 ^{o}C then regenerate wood via adding distilled water.[37] Furthermore, [Amim] Cl was applied to extract cellulose from pine and catalpa wood chips by Li.[38] In consideration of above methods, [Emim] Ac, [Mmim][DMP] and [Amim] Cl were used as three regeneration solvents for the pre-treatment. The wood regeneration yield is illustrated in (Table 3.1**)**.

Yield of wood regeneration in [Emim] Ac is the lowest among the entire tests, followed by [Mmim][DMP] , next is [Amim] Cl. In the swelling part, [Emim] Ac has been proven to have the best ability to make wood chips swell because its anion can open the structures inside well. In this test, Norway TMP fibre was dissolved in ionic liquids with strong agitation under 120 ^{o}C so that it was easy for ionic liquids come in insides of wood chips and open their structures. The lowest regeneration yield of [Emim] Ac

means that more cellulose and hemicellulose were dissolved in distilled water in the precipitation process than other two experiments which used [Mmim][DMP] and [Amim] Cl as solvents. Because more structure of wood inside was opened by [Emim] Ac during dissolution, more cellulose and hemicellulose were dissolved in water phase and removed by water. The regeneration tests results have also proved [Emim] Ac is the best one in the tested ILs for opening structure of wood.

Table 3.1 Yields of regenerated wood with three ILs at 120 °C for 12 hrs using water as the non-solvent

Ionic Liquids	Yield of Wood Regeneration %[a]
[Emim] Ac	91.0
[Mmim][DMP]	93.5
[Amim] Cl	95.0

[a] Yield of regeneration was average with two experiments

Table 3.2 Enzymatic hydrolysis data of ILs pre-treated wood.

	Starting regenerated wood (g)	Glucose Released (g)	Actual Yield of Glucose (%)	Yield of wood Residue (%)	All the sugar released (g)
[Emim] Ac	0.795	0.156	28.5	55.4	0.204
[Mmim][DMP]	0.800	0.110	19.75	62.8	0.153
[Amim] Cl	0.835	0.073	12.1	71.45	0.153

In comparison with Argyropoulos's research,[37] the yield of wood regeneration was not stated and there is no comparison between each tested ILs. In the research which has

been reported by Zhao,[39] 1-butyl-3-methylimidazolium chloride was applied for cellulose regeneration but there is no weight percentage of cellulose in ILs and yield of regeneration either. The remaining regenerated woods without dried were treated by Viscozyme in the buffer solution. The results are listed in Table 3.2.

The yield of glucose released by enzymatic hydrolysis and residual wood after enzymatic hydrolysis was calculated according to the equation.[35] Generally speaking, ionic liquids pre-treatment can improve the actual glucose amount released from enzymatic hydrolysis. Results in Table 3.3 are the data of enzymatic hydrolysis of original TMP fibre without IL pre-treatment. Compared with the data in Table 3.2, both of the yields of glucose and all the sugar released without ILs pre-treatment are much lower than [Emim] Ac, [Mmim][DMP] and [Amim] Cl pre-treatment. It should be mentioned here that much higher yields of regenerated residue were observed after the enzymatic hydrolysis of untreated wood. As can be seen in Table 3.2, the yield of glucose released after [Emim] Ac pre-treatment is the highest in the tests. It meant that regenerated TMP fibre which was pre-treatment by [Emim] Ac can be hydrolysed by Viscozyme more efficiently because of more opened structure inside. It is worth noting that starting regenerated wood for hydrolysis should not be via freeze or high temperature dry prior to enzymatic hydrolysis mainly because drying process will make microporosity which was generated by ILs collapse, which can reduce the efficiency of enzyme.

Table 3.3 Enzymatic hydrolysis data without ILs pre-treatment.

Starting TMP fibre without pre-treatment in ILs (g)	Glucose Released (g)	Actual yield of glucose (%)	Yield of Residue (%)	All of Sugar Released (g)
0.56	0.019	5	89.7	0.028

3.5 Conclusions

In this chapter, we have demonstrated that three single ILs and their mixtures were analysed about their ability of making wood swelling and results were proved by two consecutive experiments which were wood regeneration and enzymatic hydrolysis. In consistent with Welton,[29] it is found that three dimensions of dry wood chips have changed obviously after immersed in dry ILs for consecutive days. Sizes of wood chip expanded best in [Emim] Ac and sample break occurred in ionic liquids with the presence of [Emim] Ac at 80 ^{o}C, which is lower than the temperature (120 ^{o}C) in Welton's procedure.[29] The tangential side has expanded up to 1.31(a/a_0) under 80 ^{o}C, which is better that Welton's results (a/a_0 = 1.23). Furthermore, it is concluded from the study that the addition of pyridinium salt **1** has negative effect in wood swelling. Wood regeneration in [Emim] Ac resulted in lower yields than [Mmim][DMP] and [Amim] Cl but with more glucose was released after the enzymatic hydrolysis than others. The study correlated the ability of ILs for wood swelling with wood regeneration and using regenerated wood in enzymatic hydrolysis. Consequently, [Emim] Ac was viewed as most suitable swelling, dissolution and regeneration medium among all investigated ILs and their different mixtures. Above all, after the pre-treatment of [Emim] Ac, the amount of glucose released by enzymatic hydrolysis have been increased.

All in all, this research is to exploit various efficient ILs to improve and optimise the enzymatic hydrolysis process. However, the wood swelling and regeneration method in this paper still have some limitations such as, the toxicity of chloride and phosphate and low biodegradability. Consequently, varieties of new kinds of ILs which are low toxic and partial biodegradable are currently under investigation in our laboratories and focused on exploring the full potential of application between biomass with ILs.

Nonetheless, there are a few drawbacks in ILs which are used in series wood applications. Firstly, three ILs and their mixtures are non-chiral and the addition of chiral pyridinium salt 1 does not exert positive effect in wood expansion. Secondly, three ILs used are not biodegradable and contain the toxic anions chloride and

phosphate. Hence, it is essential to design and prepare chiral readily biodegradabile ILs with low toxicity and determine their thermal stability for the application in wood expansion, regeneration and enzymatic hydrolysis.

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Chapter 4

Synthesis of Chiral Prolinate ILs with physical properties, biodegradability and anti-toxicity activity Study

Contributors

Prof. Klaus Kümmerer (Leuphana University of Lüneburg) performed ILs biodegradation study

Dr. Marcel Špulák (Charles University in Prague) performed antifungal and antimicrobial toxicity tests

Dr. Brid Quilty (Dublin City University) performed further anti-bacteria toxicity tests

4.1 Introduction

Over the last two decades, ILs have attracted increasing attention both in organic chemistry and bio-chemistry. This is mainly because of their physical properties, such as high polarity, very low vapour pressure, and solubility profile for a wide range of small organic compounds, enzymes and biomass. Combined with green chemistry principles, ILs have been used in a wide range of industrial applications and green chemistry reactions.[1] This includes ammonium- and pyrrolidinium- based cations combined with methylsulphate or methylsulphonate investigated as lubricants or lubrication additives in chemical industry.[2] Commonly, general ILs can be easily prepared by anion exchange of halide salts with metal salts.[64] However, this method has limitations in the preparation of pure ILs due to contaminations by metal halide salts. Furthermore, these kind of ILs are always more toxic than chiral ILs without metal cations. Nonetheless, chiral ILs can be synthesized from readily available starting materials containing chiral anions or cations to produce chiral products.[69]

Quaternary tetra-alkyammonium based ILs have also been used as solvents for hydrogen peroxide oxidation[3], in palladium-catalysed conversion of allylic alcohols to ketones[4] and BF_4 and PF_6 derivatives employed as reusable phase transfer catalysts in crossed-aldol condensations.[5] In addition, saccharides, cellulose and biopolymers can be dissolved in quaternary ammonium based ILs due to the interaction between carbohydrate and ILs.[6] Recently, tetraalkyl ammonium oleate and linoleate based ILs were synthesized by metathesis reaction and used for extraction of metal from aqueous phases.[7]

Chiral ILs (CILs) as additives and solvents in asymmetric technologies, have been studied in a wide range of research fields.[8, 9] For instance, quaternary ammonium mandelates CILs[10] and pyrrolidine-based CILs[11] were applied in the asymmetric Michael addition of ketones to nitrostyrene, and CILs have been also used as both solvent and catalyst for Diels–Alder reactions to enhance the reaction rate and selectivity.[12] In the aspect of spectroscopic applications, Wasserscheid[13] has reported a

method where CILs are applied to determine the excess enantiomers of samples by NMR studies.

Recently, amino acid based ILs, especially examples from the chiral pool, have attracted interest within the green chemistry community.[14–16] Applications in chiral discrimination, asymmetric synthesis, and the resolution of racemates have been reported.[17] Chiral amino acid based ILs have been synthesized from natural amino acid via short routes with desirable properties, such as low toxicity, high biodegradability and excellent biocompatibility observed.[18, 19]

Chiral amino acid based ILs have been used as green reaction mediums for organic reactions such as synthesis of β-keto-sulfones[20] and amino group protection reaction.[21] A series of amino acid based CILs were reported to be used as reaction medium to improve the asymmetric CO_2 cycloaddition.[22] It has also been reported that five tetra-butyl ammonium-based amino acids ILs have been synthesized with good lubricating properties for rubbing process.[23] In addition, Wang *et al.* applied chiral ILs with copper (I) salts in a co-catalytic system to successfully promote the reaction of synthesis of 1,4-disubstituted 1,2,3-triazoles.[24] Blackmond *et al.* have performed stereoselective amination reactions in the presence of either tertiary amine bases or preformed prolinate salts.[25]

Prolinate CILs in combination with palladium was employed in the asymmetric Heck arylation of 2,3-dihydrofuran by Pernak.[26] Our group has prepared tetra-butyl ammonium prolinate ILs and used them in an asymmetric reaction catalysed by palladium.[27] Furthermore, tetra-ethyl ammonium amino acid based ILs have been prepared and their physical properties determined[28] and amino acid based tetra-butyl phosphonium IL synthesized and used as catalysts in the Konevenagel condensations of aromatic aldehydes with active methylene compounds.[29]

Evaluating the toxicity and biodegradability of ILs, as part of a green chemistry metrics based classification of an environmental friendly non-VOC solvent, is paramount.

Bacteria, fungi and algae are frequently used to study IL toxicity.[30–32] Gathergood, Garcia and Scammells carried out a series of studies to test biodegradability and toxicity of ILs.[33–36] A new class of imidazolium ILs which contain ester or amide groups in the alkyl side chain were designed by Gathergood.[37] Their biodegradability was tested by the CO_2 headspace test. Gathergood *et al.* designed and synthesized imidazolium-based chiral ILs and dipeptidyl chiral ILs and used them to carry out biodegradation and anti-microbial screening.[38] New pyridine and nicotinic acid based ILs were synthesized and their biodegradability were also evaluated by the CO_2 headspace test under aerobic conditions.[39]

Herein, on the basis of the results obtained previously in our group, we planned to synthesize chiral prolinate based quaternary ammonium ILs, which are expected to show low toxicity to a number of bacteria and fungi. Proline has been previously shown to be a very useful starting material in organic catalysis, especially in asymmetric synthesis.[75]

4.2 Project Aims

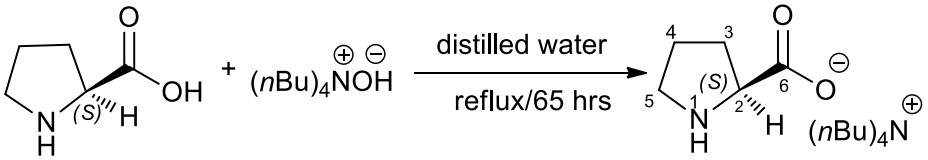
- To synthesis and characterize a library of novel sustainable, low-toxicity, biodegradable, prolinate chiral ionic liquids (ILs) with enantiomers
- To investigate the physical properties of the chiral ILs library
- To investigate the biodegradation and toxicity properties of the chiral ILs and enantiomers library

4.3 Results and Discussion

4.3.1 Synthesis

A series of chiral prolinate ILs have been designed and synthesized (See Table 4.1).[16, 17, 27, 28] Six different kinds of proline and four different types of tetra-alkyl ammonium hydroxide were selected as substrates for the synthesis. The synthetic routes for the chiral ILs only include one simple step. L-proline and D-proline were reacted with tetra-alkylammonium hydroxide respectively.[16] During the synthesis, distilled water was

used to dissolve the proline and the reaction was heated to keep reflux for 65 hrs. The general synthetic route is illustrated in (Scheme 4.1). Chiral ILs **1a-2d** are synthesized from L/D proline with four tetra-alkyl ammonium hydroxide (Fig 4.1).



Scheme 4.1 Synthesis of L-proline and D-proline based ILs

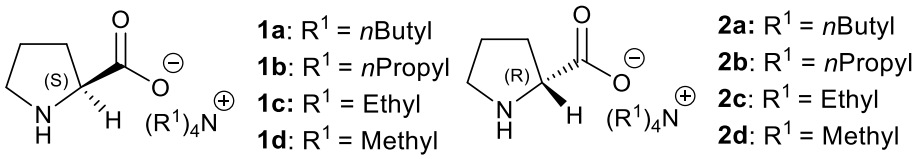


Fig 4.1 L/D proline based ILs

Additionally, when the substrates were changed from proline to 4-hydroxy-proline, the reaction condition was the same as mentioned above. *Trans*-4-hydroxy-D/L-proline were dissolved in distilled water and heated with tetra-alkyl ammonium hydroxide under reflux for 65 hrs (Fig 4.2).

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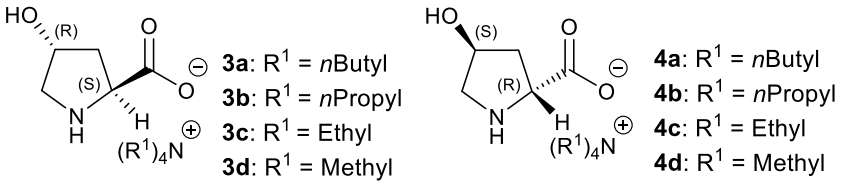


Fig 4.2 *Trans*-4-hydroxy-D/L-proline based ILs

Consequently, *cis*-4-hydroxy-D/L-proline were also performed in the same reaction condition with tetra-alkylammonium hydroxide to afford the desired chiral prolinate ILs (Fig 4.3).

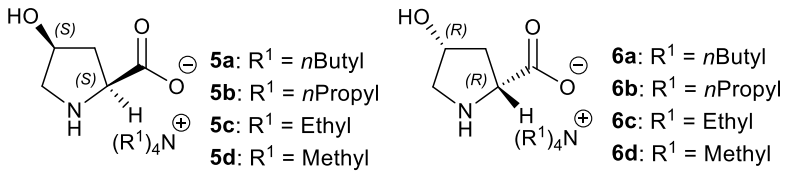


Fig 4.3 *Cis*-4-hydroxy-D/L-proline based ILs

NMR, IR and mass spectrometry were used to characterize the structures of targeted compounds. All 24 products were obtained with high yield which ranging from 84% to 96% (Table 4.1). Besides ^{1}H, ^{13}C NMR, IR and mass spectrometry, optical rotation and water content were also analysed on the pure and dry chiral ILs.

4.3.2 Physicochemical properties

Optical rotation and water content data of the prepared ILs are displayed in Table 4.1. Optical rotation values of the 24 compounds were analysed by a Perkin Elmer 341 polarimeter. Distilled water was used as the solvent to make the chiral ILs solutions. The absolute value of optical rotation between each enantiomer is supposed to be almost same as seen in Table 4.1. Another parameter is water content, which was tested by Karl Fischer titration.

Dynamic thermal decomposition temperatures (T_d) were investigated by thermo-gravimetric analysis (TGA, TA Instruments) under N_2 atmosphere (50 mL min^{-1}) and constant heating rate of 10 ^{o}C min^{-1} to prevent any oxidation of the sample during the process. Table 1 also summarizes the decomposition temperature of the 24 compounds. It can be seen from Table 4.1 that all the ILs are stable up to 190 ^{o}C to 200 ^{o}C and part of the ILs (8 out of 24) which contain the tetra-methyl ammonium cation had higher decomposition temperature are stable up to 240 ^{o}C-260 ^{o}C and the highest T_d is 264.4 ^{o}C, corresponding to a weight loss of 86%.

Fig 4.4 describes the TGA traces of four L-proline based ILs and four D-proline based ILs respectively. In the Table 4.1, compound **1d** (tetramethyl ammonium L-prolinate

ILs) has the highest decomposition temperature (T_d = 248.2 ^oC) than other three ILs which contain tetrabutyl, tetrapropyl and tetraethyl ammonium cations. The reason for this obtained results can attributed to the tetra-methyl side chain in the cation. It is deduced that tetra-butyl, tetra-propyl and tetra-ethyl side chain are more vulnerable to decompose than tetra-methyl side chain under heat condition. Consequently, the other 16 ILs which contain tetra-butyl, tetra-propyl and tetra-ethyl side chain in their cations have a lower T_g value than the eight ILs.

As can be seen the structures of ILs in the synthetic route, van der Waals forces could be enhanced by longer alkyl side chains in anions, like tetra-butyl and tetra-propyl chains. However, the entirely lower decomposition temperatures of ILs with tetra-butyl and tetra-propyl chains were due to the weaker intramolecular electrostatic interaction, which was decreased by long alkyl chains.[40, 41] Carbocation and carbon radicals could be formed during the thermal decomposition process. Consequently, the longer alkyl chains increase the stability of the carbocation and carbon radicals, which make the ILs decompose more easily.[42]

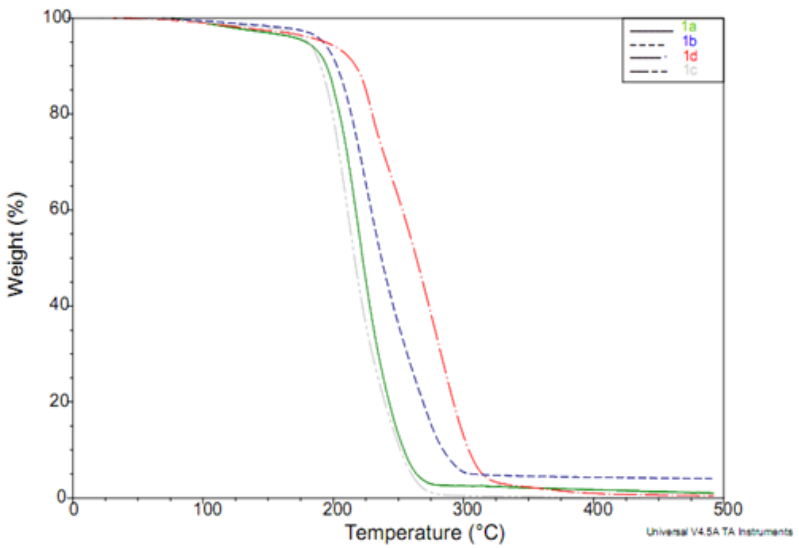


Figure 4.4 Thermo-gravimetric analysis traces of four L-proline based ILs under nitrogen flow.

Another aspect, as discussed above, the T_d value for each pair enantiomers with the same cation and anion are supposed to be almost the same in theory. It can be seen from the Table 4.1 that the difference of decomposition temperatures between each enantiomer is very small whilst the introduction of hydroxyl group in the proline ring exerted no obvious effect on the T_d (Table 4.1). As described in Table 4.1, all the ILs are liquids under room temperature. The glass transition temperature values of the series of chiral prolinates ILs are from –49.5 ^oC to –36.1 ^oC. The glass transition temperature of each pair of enantiomers is expected to be identical. As the results show in Table 4.1, the T_g value of most enantiomers are very close. The difference of T_g between compound **1a** and **2a** are only 3 ^oC. However, T_g differences between two pairs enantiomers of ILs **1c** with **2c** and **3a** with **4a** are 13 ^oC and 11 ^oC, which could be result from the absorption of moisture during the sampling procedure before DSC analysis exposure to the air.

It can be concluded from above discussions that the length of alkyl chains of anions is the dominant factor for the thermal stability of prolinate ILs. Consequently, the order of the thermal stability of prolinate based ILs is tetra-butyl ammonium ILs < tetra-propyl ammonium ILs < tetra-ethyl ammonium ILs < tetra-methyl ammonium ILs.

Table 4.1 Synthesis of chiral prolinate based ILs and physical properties

Entry	ILs	D or L proline	Absolute Stereo-chemistry	*Cis* or *trans* 4-hydroxy	Optical rotation [a]	Yield (%)	Water Content (%)[b]	T_g/^oC[c]	T_d/^oC[d]
1	**1a**	L	2*S*	n/a	–30.4 (c = 0.62)	85	0.59	–46.3	198.7
2	**1a**[27]	L	2*S*	n/a		93[27]		–53.8[27]	174.3[27]
3	**1a**[19]	L	2*S*	n/a	–28.5 (c = 10)[19]	97[19]			
4	**2a**	D	2*R*	n/a	+30.5 (c = 0.62)	87	0.34	–49.5	190.9
5	**2a**[27]	D	2*R*	n/a	+28.50 (c = 10)[28]	94[27]		–49.6[28]	167.4[27]
7	**2b**	D	2*R*	n/a	+32.6 (c = 0.58)	90	1.18	–46.2	199.1
8	**1c**	L	2*S*	n/a	–30.1 (c = 0.50)	94	1.80	–49.4	192.8
9	**1c**[28]	L	*2S*	n/a	–42.9 (c = 1)[29]	90[28]			172.0[28]

10	**2c**	D	2*R*	n/a	+28.9 (c = 0.50)	89	1.75	–36.1	199.0
11	**1d**	L	2*S*	n/a	–30.2 (c = 0.55)	88	1.16	–46.4	248.2
12	**2d**	D	2*R*	n/a	+30.4 (c = 0.55)	86	0.61	–49.5	255.5
13	**3a**	L	2*S*, 4*R*	*trans*	–32.7 (c = 0.47)	87	0.97	–36.4	217.8
14	**3a**[27]	L	2*S*, 4*R*	*trans*	–7.83 (c = 12)[27]	90[27]		–38.0[27]	168.6[27]
15	**4a**	D	2*R*, 4*S*	*trans*	+30.8 (c = 0.47)	84	1.17	–47.4	199.5
16	**3b**	L	2*S*, 4*R*	*trans*	–24.2 (c = 0.40)	92	1.33	–49.4	211.2
17	**4b**	D	2*R*, 4*S*	*trans*	+22.8 (c = 0.40)	84	1.70	–46.4	208.8
18	**3c**	L	2*S*, 4*R*	*trans*	–31.5 (c = 0.48)	91	1.65	–48.5	191.7
19	**4c**	D	2*R*,4*S*	*trans*	+29.6 (c = 0.48)	84	1.65	–46.2	192.1
20	**3d**	L	2*S*, 4*R*	*trans*	–32.4 (c = 0.35)	90	1.51	–49.4	252.5
21	**4d**	D	2*R*,4*S*	*trans*	+33.9 (c = 0.35)	90	1.10	–49.5	252.5
22	**5a**	L	2*S*, 4*S*	*cis*	–19.7 (c = 0.50)	94	0.87	–46.1	214.2
23	**6a**	D	2*R*, 4*R*	*cis*	+21.0 (c = 0.50)	89	1.40	–46.3	207.2
24	**5b**	L	2*S*, 4*S*	*cis*	–25.3 (c = 0.25)	90	0.55	–46.4	238.5
25	**6b**	D	2*R*, 4*R*	*cis*	+24.1 (c = 0.25)	96	1.70	–46.4	241.8
26	**5c**	L	2*S*, 4*S*	*cis*	–26.0 (c = 0.25)	91	1.08	–48.7	214.8
27	**6c**	D	2*R*, 4R	*cis*	+24.8 (c = 0.25)	91	1.14	–49.5	211.4
28	**5d**	L	2*S*, 4*S*	*cis*	–30.5 (c = 0.21)	92	0.79	–49.4	264.4
29	**6d**	D	2*R*, 4*R*	*cis*	+30.1 (c = 0.21)	92	1.16	–46.4	257.2

[a] At 25 °C solution in H_2O

[b] Water content were analysed by Karl Fischer titration

[c] Glass transition temperatures (T_g) were determined by DSC with a heating rate of 20 °C min^{-1} and cooling the samples from −60 °C to 200 °C under nitrogen.

[d] Decomposition temperatures (T_d) were measured by TGA with a heating rate of 10 °C min^{-1} from 40 °C to 500 °C under nitrogen

4.3.3 Biodegradation and Toxicity Study

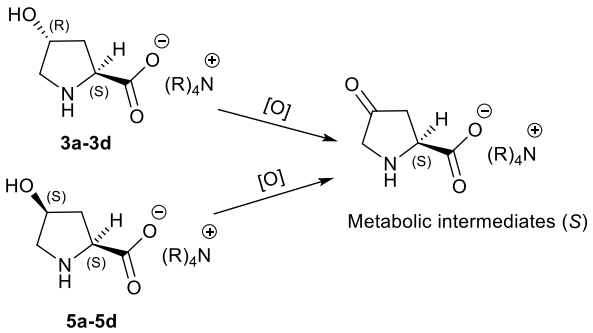
4.3.3.1 Closed Bottle Test

The biodegradability of 24 synthesized prolinate chiral ILs was performed by an aerobic aqueous with wastewater microorganisms is used to be the medium for the target chemicals. The depletion of dissolved molecular oxygen is measured for a defined period of time and output as a percentage of the theoretical maximum. Chemicals which reach a biodegradation level higher than 60% are referred to as ‘‘readily biodegradable’’.

4.3.3.2 Biodegradation data

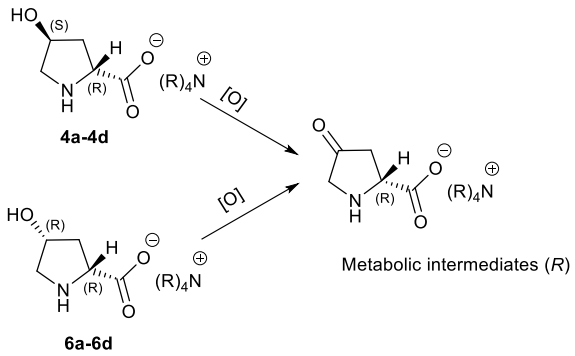
Results for biodegradation of the 24 prolinate ILs over 28 days were illustrated in Fig 4.5. Unfortunately, none of these ILs have passed the biodegradation test (at least 60% over 28 days duration). Compounds, **1d**, **2d**, **4d** and **6d** which contain tetra-methyl ammonium cation showed better biodegradability which are 55.4%, 50.3%, 50.7% and 55.7% respectively. **1a**, **2a**, **3a**, **4a**, **4b** and **5a** which contain tetra-butyl or tetra-propyl ammonium cation show low biodegradability and the tetraethyl ammonium ILs cations have ca. 40% biodegradation results. ILs with tetra-methyl ammonium cation have the larger weight percentage for the proline ring than other ILs due to the tetra-methyl ammonium group has the lowest molecule weight in four tetra-alkyl ammonium groups.

These results suggest that the proline ring in the ILs is biodegradable and tetra-alkyl ammonium cation recalcitant. Biodegradability of 4-hydroxy prolinate ILs **3d** and **5d** are below 35%, compared to above 50% for **4d** and **6d**. The plausible metabolic intermediates of **3d**, **5d**, **4d** and **6d** are shown in Scheme 4.2 and 4.3. It can be seen clearly that the metabolic intermediates of above four ILs are same except the stereo chemistry. **3d** and **5d** have the lower biodegradability because they go through a common kinetic metabolite with *S* configuration which is poorly biodegradable. On the contrary, the common kinetic metabolite with *R* configuration from **4d** and **6d** can be more easily metabolised into CO_2 under test conditions.



Scheme 4.2 Metabolic intermediates (*S*) of ILs **3a**–**3d** and **5a**–**5d**

Biodegradation was dependant on the stereochemistry at the 2 position of the 4-hydroxyprolinate. The diastereomers with the (2*R*) sterogenic centre, **4d** and **6d,** were more biodegradable than the (2*S*) diastereomers, **3d** and **5d**. Relative sterochemistry (*syn* vs *anti*) did not significantly effect biodegradation. Biodegradation was independent of absolute stereochemistry of prolinate ILs (e.g. **1d** vs **2d**). These results emphasise the significance of the proline ring and its substituents on biodegradation for chiral ILs.



Scheme 4.3 Metabolic intermediates (*R*) of ILs **4a**–**4d** and **6a**–**6**

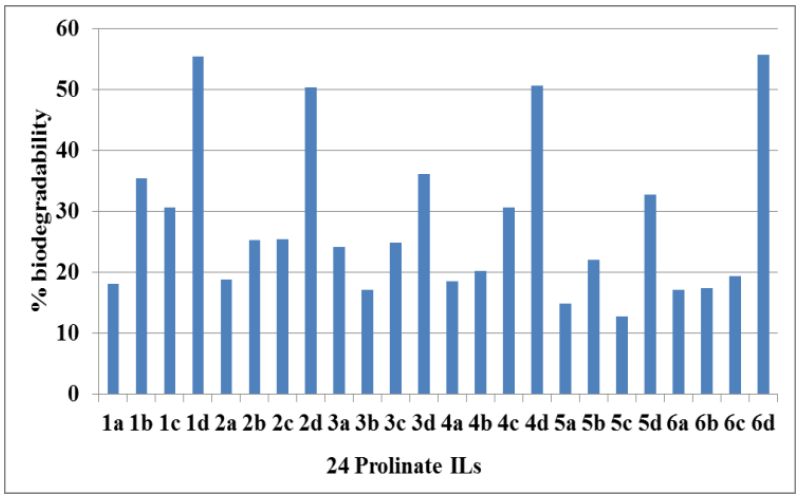


Figure 4.5 Biodegradation of 24 ILs

4.3.3.3 Antifungal and antimicrobial toxicity

Twelve strains of fungal were employed to evaluate the antifungal activity of the 24 prolinate chiral IL. In vitro antifungal activities[35, 36] of the chiral ILs, four types of ATCC strains (CA1: *Candida albicans* ATCC 44859, CA2: *Candida albicans* ATCC 90028, CP: *Candida parapsilosis* ATCC 22019, CK1: *Candida krusei* ATCC 6258) and eight types of clinical isolates of yeasts (CK2: *Candida krusei* E28, CT: *Candida tropicalis* 156, CG: *Candida glabrata* 20/I, CL: *Candida lusitaniae* 2446/I, TA: *Trichosporon asahii* 1188). The rest of three are filamentous fungi (AF: *Aspergillus fumigatus* 231, AC *Absidia corymbifera* 272 and TM: *Trichophyton mentagrophytes* 445)

Minimal inhibitory concentration (MIC) is employed to determine the fungi toxicity of ILs to the fungal isolates. The inhibition of ILs against fungi is defined as 80% inhibition of the growth of control for yeasts and as 50% inhibition of the growth of control for filamentous fungi. MIC values were determined twice and in duplicate. In the case of the *T. mentagrophytes*, readings were taken after 72 and 120 hrs and MIC/IC_{50} values were calculated while for all other strains.

MIC/IC_{80} values were calculated after 24 and 48 hrs of exposure to ILs. These results gained for 24 chiral prolinate tetra-alkyl ammonium based ILs were illustrated in Table 4.2. These results show that the chiral prolinate tetra-alkyl ammonium based ILs (**1a-6d**) do not have high antifungal acMIC/IC_{80} values were calculated after 24 and 48 hrs of exposure to ILs. These results gained for 24 chiral prolinate tetra-alkyl ammonium based ILs were illustrated in Table 4.2. These results show that the chiral prolinate tetra-alkyl ammonium based ILs (**1a-6d**) do not have high antifungal activity with IC_{50}/IC_{80} values over 2.0 mM determined, except for **3c**, **4c** and **6d**, where the lower solubility in the media lead to a maximum test concentration of 0.5 mM. At this solubility limit **3c** and **4c** gave IC_{50} values of 0.5 mM for two of the 12 fungal strains (CP and CK1) after 24h, however after 48 hrs IC_{50} values for CP and CK1 were greater than 0.5 mM in agreement with other 10 fungal strains in test. We propose that Table 4.2 demonstrates that the general trend is ILs (**1a-6d**) do not exhibit high antifungal toxicity to the 12 strains screened under the test conditions. Within the concentration range screened no effect due to modification of tetraalkylammoninm cation or prolinate anion structure could be ascertained. MIC values at 24 hrs and 48 hrs (or 72 hrs and 120 hrs for TM strain) were the same for all ILs, except for the **3c** and **4c** CP and CK1 data points stated earlier. Any breakdown products due to fungal metabolism are not present at a concentration where their toxicity to the fungal strain inhibits growth enough for an IC_{50} value lower than 2 mM (or 0.5 mM) to be measured.

For the antimicrobial toxicity screening tests, **1a-6d** were evaluated[45] against eight bacteria which are ATCC strains (SA: *Staphylococcus aureus* ATCC 6538, EC: *Escherichia coli* ATCC 8739, PA: *Pseudomonas aeruginosa* ATCC 9027) and clinical isolates (MRSA *Staphylococcus aureus* HK5996/08, SE: *Staphylococcus epidermidis* HK6966/08, EF: *Enterococcus sp*. HK14365/08, KP: *Klebsiella pneumoniae* HK11750/08, KPE: *Klebsiella pneumoniae* ESBL HK14368/08).

Table 4.2 Antifungal activities of prolinate ILs

ILs	Organism[a] –MIC/IC_{80} IC_{50} ($mmol.L^{-1}$)																			
	CA1		CA2		CP		CK1		CK2		CT		CG		CL		TA		TM	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	72h	120h
1a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	2	2
1b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	2	2
2b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
1c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
1d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3c	>0.5	>0.5	>0.5	>0.5	0.5	>0.5	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
4c	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
3d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6b	>2	>2	>2	>2	2	2	2	2	2	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6d	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5

[a] CA1: *Candida albicans* ATCC 44859, CA2: *Candida albicans* ATCC 90028, CP: *Candida parapsilosis* ATCC 22019, CK1: *Candida krusei* ATCC 6258, CK2: *Candida krusei* E28, CT: *Candida tropicalis* 156, CG: *Candida glabrata* 20/I, CL: *Candida lusitaniae* 2446/I, TA: *Trichosporon asahii* 1188, AF: *Aspergillus fumigatus* 231, AC *Absidia corymbifera* 272 and TM: *Trichophyton mentagrophytes* 445

Table 4.3 MIC (mM, IC_{95}) values obtained for Prolinate ILs

ILs	Organism[a] –MIC/IC_{95} (mmol.L^{-1})															
	SA		MRSA		SE		EF		EC		KP		KP-E		PA	
	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h	24h	48h
1a	>2	>2	>2	>2	2	>2	2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2a	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
1b	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
1c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
1d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
2d	>2	>2	>2	>2	2	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3b	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
3c	0.5	>0.5	0.5	>0.5	0.5	0.5	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
4c	0.5	>0.5	0.5	>0.5	>0.5	>0.5	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5
3d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
4d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5a	2	2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6a	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6b	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6c	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
5d	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2	>2
6d	>0.5	>0.5	0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5	>0.5

[a] SA: *Staphylococcus aureus* ATCC 6538, EC: *Escherichia coli* ATCC 8739, PA: *Pseudomonas aeruginosa* ATCC 9027, MRSA: *Staphylococcus aureus* HK5996/08, SE: *Staphylococcus epidermidis* HK6966/08, EF: *Enterococcus sp* HK14365/08, KP: *Klebsiella pneumoniae* HK11750/08, KP-E: *Klebsiella pneumoniae* ESBL HK14368/08

The minimal inhibitory concentration (IC_{95}) is applied to measure the antimicrobial toxicity of ILs against eight kinds of strains. The results of antimicrobial toxicity for 24 targeted ILs were described in Table 4.3. Similar results were obtained as the antifungal toxicity screening test. Twenty one targeted ILs do not display inhibition activity up to 2 mM/L concentration to all tested microorganisms after 24 hrs and 48 hrs. However, ILs **3c**, **4c** and **6d** still have a little lower MIC value which is up to 0.5 mM/L concentration to the tested system. The overall results show that all the prolinate based tetra-alkyl ammonium ILs are non-toxic towards both fungal strains and micro-organisms with some exception.

4.3.3.4 Further anti-bacteria toxicity tests

Four Gram-negative bacteria (*E. coli* (DSMZ 498), *P. fluoroscens* (DSMZ 50090), *P. putida* (CP1), and *P. putida* (KT 2440) and one Gram-positive bacterium strain (*Bacillus subtilis (B. subtilis;* DSMZ 10) were used to determine IC_{50} values for **1a-6d** (Table 4.4). The five bacteria strains were grown in the uni-vials with 10 mL nutrient broth and incubated overnight in a shaking machine and the chemical stock solution is prepared with a concentration of 2 M in Eppendorf tubes. The antimicrobial activity tests of the ILs are tested on a 96 well microplate. ILs **1c**, **1d**, and **2d** have the lowest toxicity, of all ILs tested, against three of the bacterial strains, *E. coli* 50 - 200 mM, *P. putida* (CP1) 50 - >200 mM, and *P. putida* (KT 2440) 50 - >200 mM. Compound **3d** also showed very low toxicity (IC_{50} >200 mM) against both *P. putida* (CP1), and *P. putida* (KT 2440). ILs **5d** and **6d** exhibited low toxicity (IC_{50} >200 mM) to *P. putida* (KT 2440) only. **4d** and **5d** were the most toxic of the tetramethylammonium ILs series, **1d-6d**. This is exemplified by the toxicity to *Bacillus subtilis* (IC_{50} **4d** and **5d** 25-50 mM; **1d-3d** and **6d** 75-100 mM). However, the general trend is that the lowest antibacterial toxicity prolinate ILs contain the tetramethyl ammonium cation (Table 4.4). Increasing the size of the tetra-alkyl ammonium cation from tetramethyl to tetrabutyl leads to an increase in bacterial toxicity (*c.f.* IC_{50} *E. coli* 15−25 mM (ILs **1a**) vs 150−200 mM (ILs **1d**); IC_{50} *P. putida* (KT 2440) 12.5−25 mM (ILs **6a**) vs >200 mM (ILs **6d**)). Tetrabutylammonium IL **6a** also has exhibited the highest toxicity (of the series 1a-6d, albeit still moderate toxicity IC_{50} values ca. 10 mM) to all five bacteria

strains, while the tetrabutylammonium **5a** showed similar level of moderate toxicity except low toxicity to *P. putida* (KT 2440) IC_{50} 50-100 mM. Determining general trends in antibacterial activity based on stereochemistry from data in Table 4.4 is difficult. Analysis of results for series of enantiomers (**1** vs **2**; **3** vs **4**; **5** vs **6**) gives no clear result. For instance, enantiomers **1a**,**2a**; **1d**,**2d**; **3c**,**4c** and **5c**, **6c** exhibit very similar IC_{50} values against all five bacteria strains independent of absolute stereochemistry. However, considerable variation from this trend is observed across Table 4.4, e.g **1b**, **2b**; **3a**, **4a**; **3d**, **4d**; **5b**, **6b** and **5d**, **6d**. The effect on antibacterial toxicity on addition of a hydroxyl group to the 4 position of proline can be determined. Comparing series L-prolinate **1** with **3**((*R*)-4-OH) and **5**((*S*)-4-OH). No change in IC_{50} values was observed for **3a-d**((*R*)-4-OH) vs **1a-d**. However, an increase in antibacterial toxicity was found when **1a-d** was compared to **5a-d**((*S*)-4-OH) analogues. On comparison of D-prolinate **2** with **4**((*S*)-4-OH) and **6**((*R*)-4-OH) the general trend is that introduction of the hydroxyl group as either stereoisomer leads to an increase in bacterial toxicity, although in some cases no increase or even a decrease in toxicity was observed due to the large number of possible combinations to support a general trend in this dataset.

The effect of relative stereochemistry of the 2 chiral centres in the 4-OH prolinate anion can be probed by comparing (2*S*,4*R*)-**3a-d** vs (2*R*,4*R*)-**6a-d** and (2*R*,4*S*)-**4a-d** vs (2*S*,4*S*)-**5a-d**. **6a-d** are more toxic than **3a-d,** thus changing the stereochemistry of (2*R*) to (2*S*), when (4*R*) is maintained reduces antimicrobial toxicity. We propose that this shows the *anti* diastereoisomer has a lower antimicrobial toxicity than the *syn.* **5a-d** are more toxic than **4a-d,** thus changing the stereochemistry of (2*S*) to (2*R*), when (4*S*) is maintained reduces antimicrobial toxicity. This also supports our hypothesis that the *anti* diastereoisomer has a lower antimicrobial toxicity than the *syn.* Table 4.4 shows Gram-positive bacterium strain (*B. subtilis*; DSMZ 10) and Gram-negative bacteria (*P. fluoroscens* DSMZ 50090) are the most sensitive to the class of compounds in this study **1a-6d** with IC_{50} values lower than 100 mM determined. The preliminary microbial toxicity results (Table 4.2 and 4.3) show that the ILs do not have a high toxicity to a wide range of bacteria and fungi. While Table 4.4 show the ILs have a moderate (IC_{50} 6.25−2.5 mM) to very low toxicity (IC_{50} >200 mM) across all 5 strains of bacteria.

Table 4.4 IC_{50} value for twenty ILs Synthesized and Five Different Bacteria Strains

	IC_{50} (mM)				
ILs	*E. coli*	*B. subtilis*	*P. putida* (CP1)	*P. putida* (KT 2440)	*P. fluorescens*
1a	15–25	25–50	75–100	100–150	50–75
2a	15–25	25–50	50–75	75–100	25–50
1b	25–50	50–75	75–100	175–200	75–100
2b	15–25	15–25	15–30	25–50	15–30
1c	100–150	75–100	>200	>200	75–100
2c	50-100	25-50	50-100	50-100	25-50
1d	150–200	75–100	175–200	>200	75–100
2d	100–150	75–100	>200	>200	75–100
3a	25–50	60–90	50–100	50–100	75–90
4a	15–30	15–25	25–50	50–75	15–25
3b	25–50	75–100	75–100	150–200	50–75
4b	15–30	25–50	30–50	75–100	25–50
3c	75–100	75–100	75–100	75–100	75–100
4c	100–125	75–100	75–100	100–120	75–100
3d	75–100	75–100	>200	>200	75–100
4d	50-100	25-50	50-100	50-100	25-50
5a	6.25-12.5	6.25-12.5	6.25-12.5	50-100	6.25-12.5
6a	7.5–12.5	7.5–12.5	12.5–17.5	12.5–25	7.5–12.5
5b	15–25	25–50	75–100	50–75	25–50
6b	15–25	15–25	15–25	25–50	15–25
5c	25-50	25-50	50-100	50-100	25-50
6c	15.5–22.5	15.5–22.5	50–75	75–90	15.5–22.5
5d	15–25	25–50	75–100	>200	25–50
6d	75–100	75–100	150–200	>200	75–100

4.4 Conclusion

The synthesis of a series of tetraalkylammonium chiral ILs which are based on all enantiomers of proline and 4-hydroxy-proline is presented. The route selected to prepare these ILs is concise, one step, and uses water as the solvent. Tetramethylammonium (**1d**-**6d**) ILs have the greatest stability to thermal degradation compared to ethyl, propyl and butyl analogues. Glass transition temperatures for all stereoisomers (prolinate and 4-hydroxyprolinates) were within a narrow range.

The biodegradation of 24 ILs (**1a**-**6d**) have been performed via Closed Bottle Test, however none passed, so therefore cannot be defined as readily biodegradable, based on this study. The highest biodegradability was obtained for the tetramethylammonium

ILs series, **1d**-**6d** (upto 55.7%) although **3d** and **5d** gave significantly lower biodegradation (30-35%) within the 28 days of the test. Biodegradation was dependant on the stereochemistry at the 2 position of the 4-hydroxyprolinate. The diastereomers with the (2*R*) sterogenic centre, **4d** and **6d,** were more biodegradable than the (2*S*) diastereomers, **3d** and **5d**. Relative sterochemistry (*syn* vs *anti*) did not significantly effect biodegradation. We propose that an oxidation step of the 4-hydroxy group is dependent on the stereochemistry at the 2- position, with the 2*R* epimers (**4d** and **6d**) more rapidly converted to CO_2. For the unsubstituted prolinates ((2*S*)-**1d** and (2*R*)-**2d**), both enantiomers gave equal conversion to CO_2 under biodegradation conditions, thus no influence of stereochemistry in these aminoacid based ILs was observed. However as many breakdown pathways are possible under the test conditions, further metabolite characterisation is required. Biodegradation was independent of absolute stereochemistry of prolinate ILs (e.g. **1d** vs **2d**). As the tetramethylammonium cation in the **1d**-**6d** series contained the lowest carbon content of the tetraalkylammonium group screened, analysis of the effect of the prolinate anion on the CBT results was more straightforward. Where organic cations are required in an IL design we recommend the use of tetramethylammonium cation when assessing biodegradability.

All the prolinate based tetra-alkyl ammonium ILs do not have a high toxicity to a wide range of bacteria and fungae used in our preliminary screen. Very low to moderate toxicity towards 5 bacteria strains was established, with even IC_{50} >200 mM determined in some cases. The lowest bacterial toxicity were observed in ILs **1c**, **1d**, and **2d**. Stereochemistry did not influence the bacterial toxicity of prolinate series **1** and **2**. In contrast, the order of increasing toxicity for the 4-OH prolinate diasteromers was, **3**, **4**, **6** then **5**. The *syn* isomer series **4** and **5** are more toxic to bacteria than *anti* isomer series **3** and **6**; (4*S*) isomers more toxic to bacteria than (4*R*). Increasing the lipophilicity of ILs by changing the size of the tetra-alkyl ammonium cation from tetramethyl (1**d**-6**d**) to tetrabutyl (**1a**-**6a**) also resulted in an increase in bacterial toxicity. Based on the biodegradation data (**3d** and **5d**, lowest values for **1d**-**6d**) and bacteria toxicity data (highest toxicity for **4** and **5** series), the **5** series (2*S*,4*S*) hyrdoxyprolinate is the least desirable stereomer for further study in asymmetric methodologies. Our

recommendation that the **4** series (2*R*,4*S*) hyrdoxyprolinate is the preferred diastereoisomer is based on high biodegradation (**1d**, **2d**, **4d** ,**6d**) and bacteria toxicity (lowest toxicity for **3** series and **4** series). Within this study both enantiomers of prolinate Ils have similar high biodegradation (50-55%) and low toxicity to bacteria and fungae, with no preference for one enantiomer over the other for future investigations based on this data. As modified proline compounds establish their role in asymmetric catalysis, the use of the 4-hydroxy analogue as a building block is a popular choice. This study presents the first examination of the effect of stereochemistry on biodegradation and antimicrobial toxicity of this class of aminoacids. Applications of these chiral solvents in dissolution of biomass studies, including the effect of the stereochemistry of solvent on the asymmetric structure of lignicelluose is ongoing and will be reported in due course.

4.5 References

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Chapter 5

Application of Chiral Prolinate ILs in Wood Chemistry

5.1 Introduction:

As mentioned at the end of chapter 1, most of the ILs applied in wood dissolution, modification and conversion to monosaccharide are achiral. It has only been reported that chiral cholinium amino acid based ILs have been prepared and applied in the dissolution of lignin, cellulose and xylan.[1] Another reported application of cholinium amino acid based ILs is as a solvent for the pretreatment of rice straw biomass.[48] However, the stereochemistry effect was not discussed in this application. In chapter 3, three kinds of ILs and their mixtures were applied to correlate wood swelling, dissolution and regeneration in ILs and cellulase digestibility. Due to the low biodegradability and high toxicity of ILs used in chapter 3, a new class of chiral tetraalkyl ammonium prolinate ILs was synthesized with thermal stability, biodegradation and toxicity screening studies detailed in chapter 4. In this chapter, twelve commercial available tetra-alkyl ammonium prolinate ILs and three kinds of cholimium based ILs were choosen as a media for wood expansion, dissolution and regeneration.

5.2 Project Aims:

- To investigate chiral prolinate ILs for wood expansion
- To investigate wood dissolution and regeneration in typical ILs
- To investigate stereochemistry effect of chiral prolinate isomers in wood expansion, dissolution and regeneration.

5.3 Pretreatment of Pine Wood Chips in Prolinate ILs

5.3.1 Method and Procedure:

The primary material of the wood chips welling test is wood (soft pine from a local North Carolina source). Specimens are from a single board which is chosen for annual ring orientation. Final specimens are 5 mm longitudinal*10 mm radial*10 mm tangential, all cut from the same 10 mm*10 mm strip (specimens are axially aligned, sampling the same growth rings). All the wood samples used (Norway TMP fiber and

small wood chips) were kept in a vacuum oven under 50 °C until they reached constant weight before use in all tests. Fifteen kinds of ILs were used in the swelling test and are displayed in Figure 5.1.

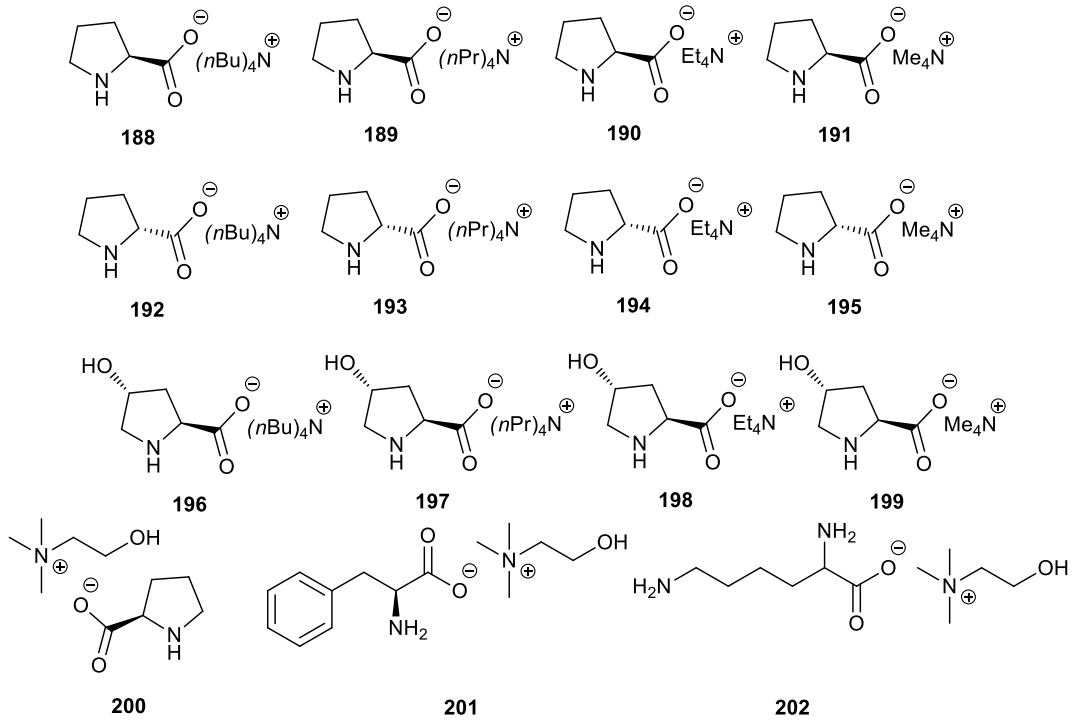


Figure 5.1: Structures of the chiral ILs Applied in wood swelling.

The preparation of twelve chiral prolinate ILs were explained in chapter 4 and three cholinium based ILs were synthesized according to the synthetic route.[1] The three dimensional size of tested wood chips are 10 mm for tangential, 10 mm for radial and 5 mm for axial as displayed in Figure 5.2.

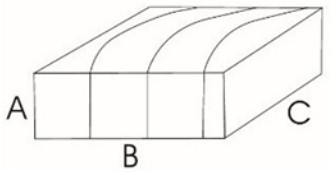


Figure 5.2: Wood chip with annual rings. Definition of three dimensions relative to growth rings; C (tangential), B (radial) and A (axial).

Three original dimensions of tested wood chips were measured by calipers with an accuracy of ±0.001 mm before performing the swelling tests. All the swelling tests were conducted in three parallel experiments because of sample heterogeneity. All the ILs were dried under high vacuum at 55 °C to remove moisture. Small wood chips (0.2 g - 0.3 g) were immersed in approximate 2 g - 3 g of ILs in small vials (10% by weight). As mentioned in chapter 3, wood chips had less expansion at 50 °C than 80 °C. Hence, swelling tests were carried out in the vacuum oven at 80 °C. The wood chips were taken out and measured by calipers quickly every 24 hrs to measure the parameter a/a_0 and calculate the woods chips' swelling ratio. For the parameter a/a_0, a_0 is the original length of three dimensions (tangential, radial and axial) and a is the length after each day's measurement.

5.3.2 Results and Discussions:

As mentioned in the toxicity screening section of chapter 4, chiral prolinate ILs **188-199** were non-toxic up to 2.0 mM concentration and all of these ILs are liquids at RT with decomposition temperatures above 190 °C, facilitating these ILs to be ideal media in wood applications. The first section of wood expansion test was carried out in four kinds of tetra-alkyl ammonium L-prolinate ILs at 80 °C. The expansions of radial and tangential directions are described in Figures 5.3 and 5.4.

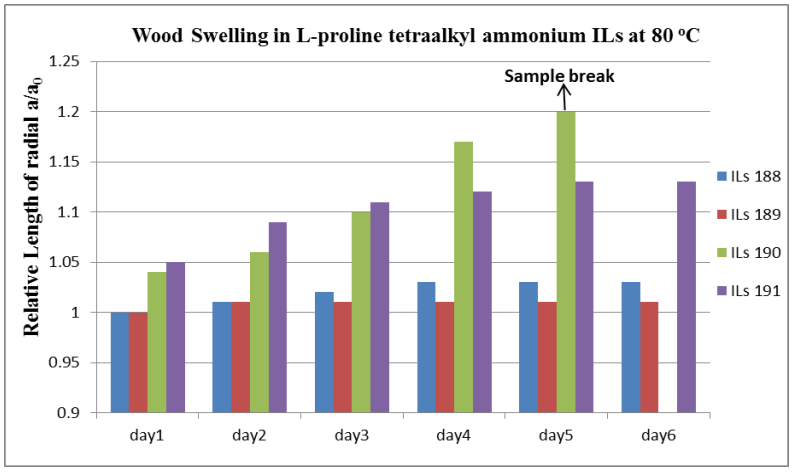


Figure 5.3: Swelling of radial direction of wood chips at 80 °C in four tetra-alkyl ammonium L-prolinate ILs. Each time point was averaged in three samples.

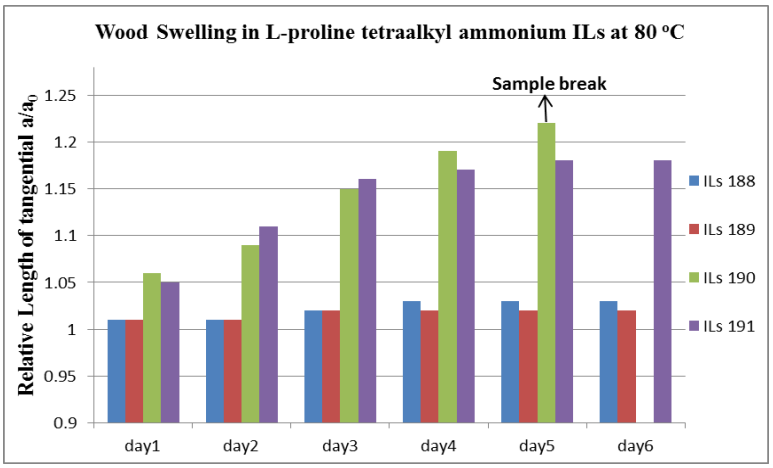


Figure 5.4: Swelling of tangential direction of wood chips at 80 °C in four tetra-alkyl ammonium L-prolinate ILs. Each time point was averaged in three samples.

From the above two figures it can be seen that IL **190** (tetra-ethyl ammonium L-prolinate) was the best of four ILs tested in wood swelling along radial and tangential directions at 80 °C. The tangential side in the three dimensions expanded to a maximum of 1.22 a/a_0 in IL **190**, while the radial side only expanded to 1.2 a/a_0. When using IL **191** as a medium, tangential and radial sides expanded to 1.18 and 1.14 a/a_0 respectively. It is obvious that ILs **188** and **189** have a negligible effect on wood swelling even after six days (Figure 5.5).

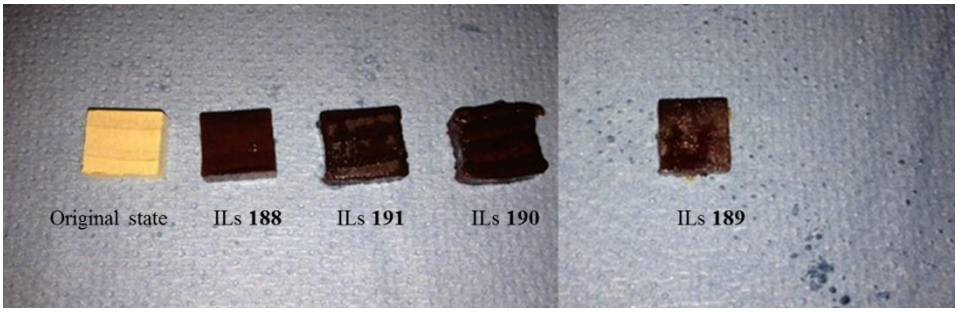


Figure 5.5: Intermediate states of wood chips in ILs **188**-**189** during swelling test

A significant phenomenon which was observed at 80 °C was sample break. The wood chip cracked along with the annual ring in IL **190** after five days. There are obvious differences between the original wood, swelled wood and sample break wood (Figure 5.6).

Figure 5.6: Wood expansion states in ILs **190** at 80 ^{o}C

In the following wood expansion section, D-prolinate tetra-alkyl ammonium based ILs **192** to **195** were applied as media at 80 ^{o}C. The expansion curves of radial and tangential directions in ILs **192** to **195** are described in Figures 5.7 and 5.8.

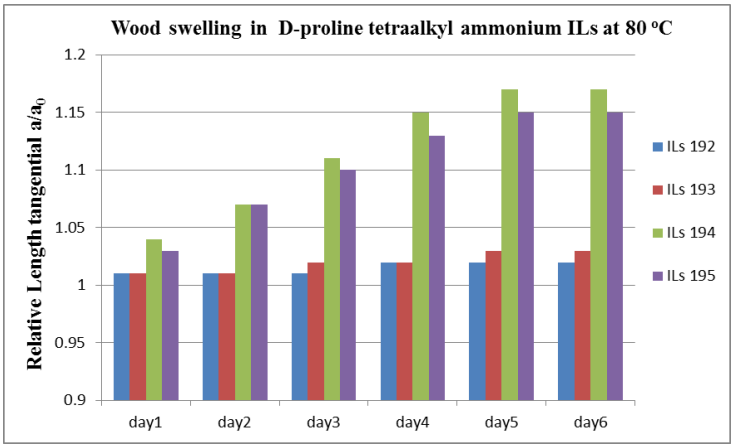


Figure 5.7: Swelling of tangential direction of wood chips at 80 ^{o}C in four tetra-alkyl ammonium D-prolinate ILs. Each time point was averaged in three samples.

As shown in Figures 5.7 and 5.8, wood expansion extent in D-prolinate ILs was significantly lower than in L-prolinate ILs. The highest tangential direction expansion ratio was 1.17 a/a_0 in IL **194** (tetraethyl ammonium D-prolinate), followed by 1.15 a/a_0 in IL **195**.

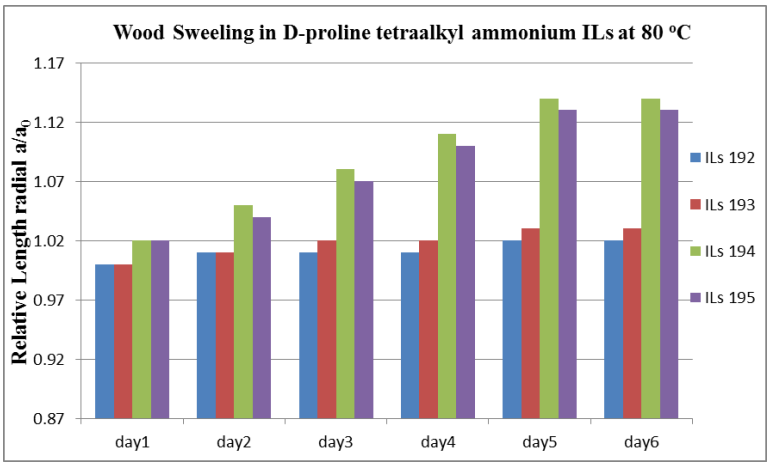


Figure 5.8: Swelling of radial direction of wood chips at 80 °C in four tetra-alkyl ammonium D-prolinate ILs. Each time point was averaged in three samples.

The tangential side expansion ratio of the 1.17 a/a_0 was still higher than the radial side at 1.14 a/a_0 in IL **194**. The order of the radial side expansion ratio was the same as the order of the tangential side expansion ratio: IL **194** > IL **195** > IL **193** ≥ IL **192**. There was almost no swelling of wood chips in both ILs **193** and **192,** which are tetraproply and tetrabutyl D-prolinate ILs respectively. The final results of wood swelling after six days are illustrated in Figure 5.9.

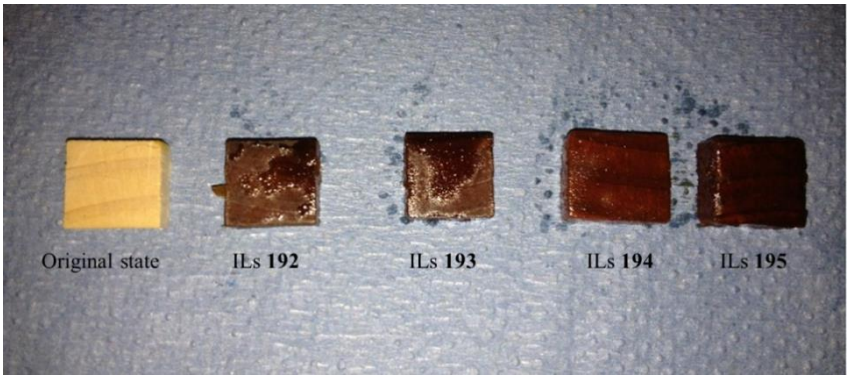


Figure 5.9: Final state of wood chips in ILs **192-195** after the six days swelling test

The next swelling section was performed in four tetra-alkyl ammonium *trans*-4-hydroxy-L-prolinate ILs **196** to **199** at 80 °C. Figures 5.10 and 5.11 display the tangential and radial direction expansion ratio curve obtained from the six days swelling test.

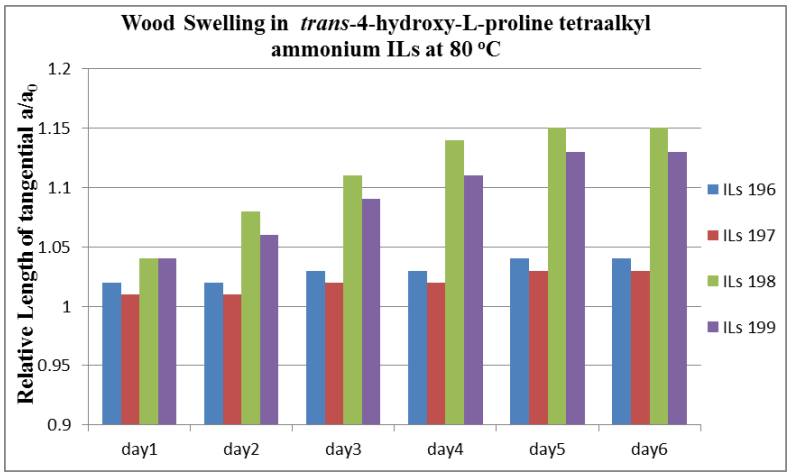


Figure 5.10: Swelling of tangential direction of wood chips at 80 °C in tetra-alkyl ammonium *trans*-4-hydroxy-L-prolinate ILs. Each time point was averaged in three samples

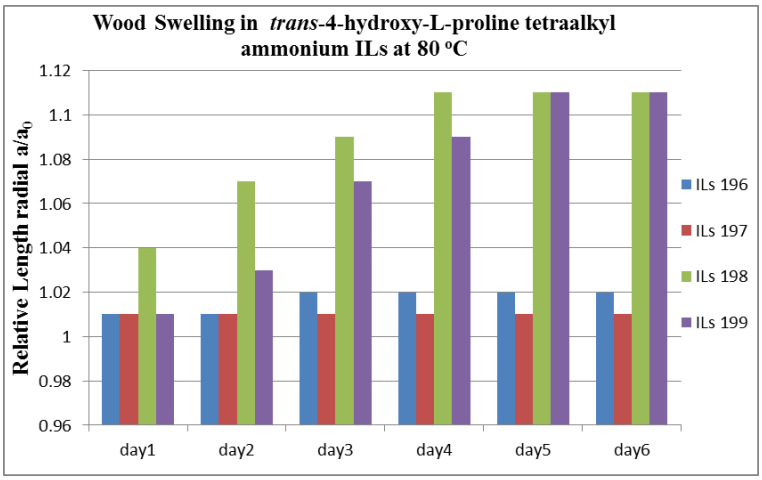


Figure 5.11: Swelling of radial direction of wood chips at 80 °C in tetra-alkyl ammonium *trans*-4-hydroxy-L-prolinate ILs. Each time point was averaged in three samples

As can be seen in Figures 5.10 and 5.11, the expansion ratio of the tangential side is up to 1.15 a/a_0 in both ILs **198** and **199** which was a little lower than 1.17 a/a_0 in IL **194**. The result of radial side expansion was obtained around 1.11 a/a_0, which is lower than 1.17 a/a_0 in IL **194** (tetraethyl ammonium D-prolinate)**.** The order of the radial side

expansion ratio was the same as the order of the tangential side expansion ratio: IL **198** = IL **199** > IL **196** ⩾ IL **197**. No obvious expansion results were observed in ILs **196** and **197**.

In order to compare the wood expansion ability of prolinate ILs with other chiral amino acid based ILs, another series of wood swelling tests were carried out in three cholinium based ILs, **200**-**202** and **198** at 80 °C (Figures 5.12 and 5.13).

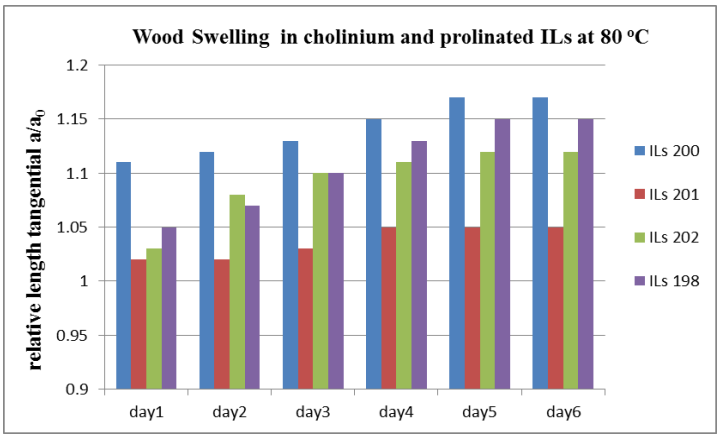


Figure 5.12: Swelling of tangential direction of wood chips at 80 °C in cholinium ILs and ILs **198** Each time point was averaged in three samples

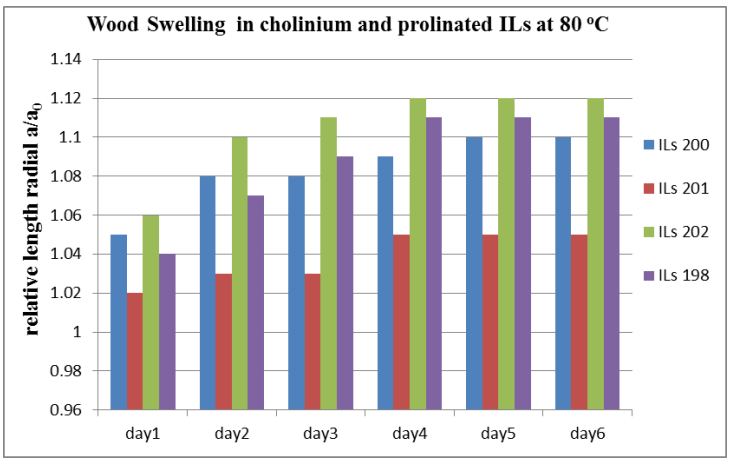


Figure 5.13: Swelling of radial direction of wood chips at 80 °C in cholinium ILs and ILs **198** Each time point was averaged in three samples

In the Figure 5.12, IL **202** had a neligible capability of swelling wood at 80 ^{o}C with 1.05 a/a_0. The tangential side expansion ratio of IL **202** was 1.12 a/a_0; 1.15 a/a_0 in IL **198** and 1.17 a/a_0 in IL **200**. However, the order of expansion ratio of the radial side (Figure 5.13) was different from the order of tangential side expansion ratio. IL **202** had the highest expansion ratio with 1.12 a/a_0, followd by 1.11 a/a_0 in IL **198** and finally IL **200** with 1.11 a/a_0. The final swelling state appearance of the wood chips are displayed in figure 5.14.

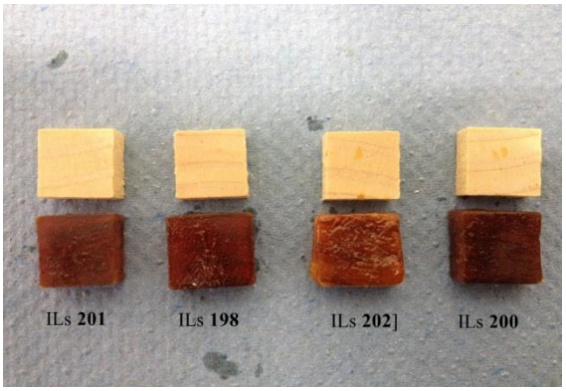


Figure 5.14: Final state of wood chips in cholinium and prolinate ILs after the six days swelling test

From the four final states of wood chips in tested ILs, we can see that three wood chips have swelled obviously along both the radial and tangential sides. However, no radial swelling was observed in IL **201** [Ch][Phe]. It can be concluded that the capabilities of radial side swelling in ILs **198, 200 and 202** to induce radial side swelling are nearly equal with IL **201** as an exception. In other words, the cholinium amino acid based ILs have moderate capabilities for wood swelling, similar to prolinated based ILs, but they do not induce sample break.

On the basis of the above results, the tangential direction is more susceptible to swelling than the radial side in all tested chiral ILs at 80 ^{o}C. For the impact of ILs structure on wood swelling, all the tetra-butyl and tetra-propyl ammonium prolinate and cholinium ILs showed significantly low ability for wood expansion, mainly because longer side chains in the cations reduce the solubility of wood components in the ILs. In contrast,

tetra-methyl and tetra-ethyl ammonium prolinate ILs have been proven to be as suitable media for wood expansion. It is worth mentioning that sample break along the annual ring of the wood chip occurred in IL **190** which has a tetra-ethyl ammonium cation and L-prolinate anion. Except for the ILs with tetra-butyl and tetra-propyl side chains, L-prolinate IL **190** and **191** had the best ability in wood swelling, followed by D-prolinate ILs **194** and **195** and finally *trans*-4-hydroxy-L-prolinate ILs **198** and **199**. Consequently, the stereochemistry effect of prolinate ILs can be summarized that an L-prolinate (*S*) IL is better than D-prolinate (*R*) IL. Additionally, the introduction of a hydroxyl group on the proline moiety did not increase the wood expansion extent. When changing to a cholinium based ILs as media, better wood expansion results were not obtained.

5.4 Wood Dissolution and Regeneration in Prolinate ILs

5.4.1 Method and Procedure:

In order to correlate the ability of chiral ILs for wood swelling, wood dissolution and regeneration studies were performed in thirteen commercial available chiral ILs and regeneration yield was calculated. Unbleached Norway spruce thermomechanical pulp (TMP) fiber (particle size: 40 mesh) was chosen as the substrate for wood dissolution and regeneration. Thirteen commercial available ILs were choosed as media to dissolve the wood fiber (Figure 5.15).

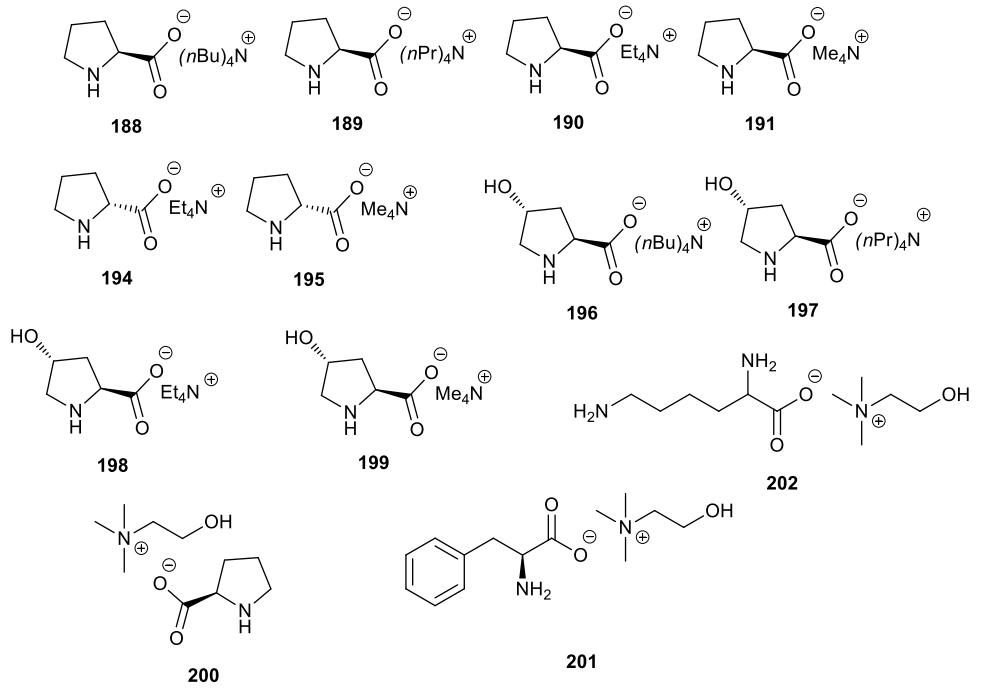


Figure 5.15: Structures of the Chiral ILs applied in wood dissolution and regeneration

TMP fiber (1 g, 7% by weight) was added into the ILs (13 g) in a round bottom flask and stirred rapidly at 120 ^{o}C using an oil bath to control the temperature for 12 hrs. The color of the mixture turned brown or dark yellow. The wood solution was poured into distilled water with strong agitation. A large amount of material precipitated out of the solution. The precipitate was then collected by reduced pressure filtration, followed by washing with distilled water to remove IL residue. Wet regenerated wood was collected and a small portion of the regenerated wood was dried under 100 ^{o}C in the oven overnight to determine the solid content, allowing the regenerated wood yield to be calculated. The flowchart displaying the wood dissolution and regeneration procedure is illustrated in Figure 5.16.

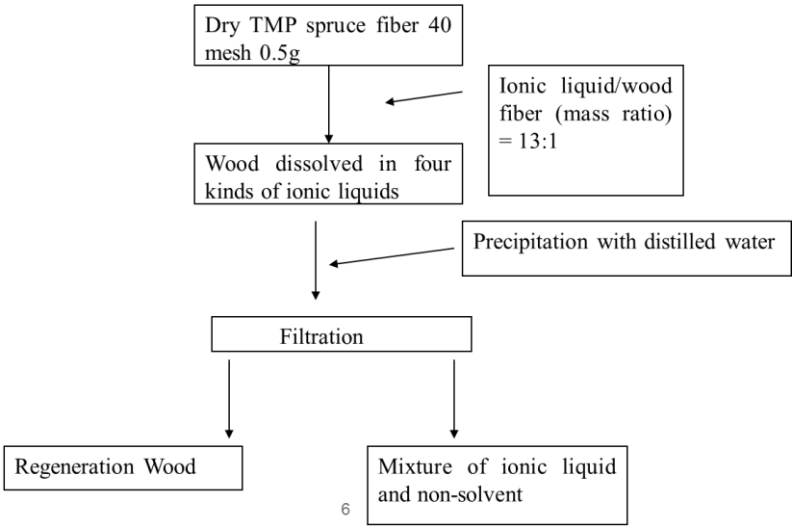


Figure 5.16: Flowchart of wood dissolution and regeneration procedure

5.4.2 Results and Discussions:

In consideration of previous research carried out by Argyropoulos[2] and Rogers,[3] which did not state regeneration yields or comparison between each tested ILs, wood regeneration yields of the thirteen tested ILs were calculated and are shown in Table 5.1.

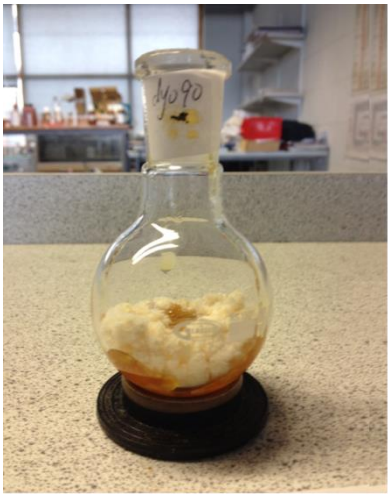
In this test, Norway TMP fiber was dissolved in ILs with strong agitation under 120 ^{o}C so the ILs can easily permeate the wood fiber and open the wood structure more easily than in the swelling procedure at 80 ^{o}C. As the yield showed in Table 5.1, the lowest regeneration yield (89.1%) was obtained in IL **190** (tetra-ethyl ammonium L-prolinate ILs), while the highest regeneration yields were observed in ILs **196** and **197** both with 97.8% regeneration. The yields of other ILs **188**, **189**, **200** and **201** were close to 97% as well. For the rested of test ILs, the regeneration yields are in the range of 91 to 95%. The precipitated materials were rich in cellulose and hemicellulose; meanwhile most of lignin, hemicellulose and a small fraction of cellulose were remained in the water solution.

Table 5.1: Yields of regenerated wood with thirteen ILs at 120 °C for 12 h using water as the co-solvent.

ILs	Yield of Wood Regeneration %[a]
188	97.2
189	96.8
190	89.1
191	91.8
194	93.0
195	93.7
196	97.8
197	97.8
198	94.4
199	95.6
200	96.8
201	97.1
202	94.4

[a] Yield of regeneration was average with two experiments

The raw wood fiber before dissolution in the tested ILs and the regenerated wood from the IL is shown in Figure 5.17.



Dry wood fiber was added into tested ILs before mixing and heating

Wet regenerated wood from filtration

Figure 5.17: State of raw wood fiber mix with ILs and regenerated wood from ILs

A lower wood regeneration yield from the IL means that more cellulose, hemicellulose and lignin were dissolved because of the reduced crystallinity and more open structures of the ILs solutions. Another reason for a lower regeneration yield is that the IL proline moiety and tetra-alkyl ammonium cation are able to break the hydrogen bonding network. However, the solubility of wood components in ILs decreases with the increase in length of the tetra-alkyl side chains of the cation. The regeneration test results have also proved that IL **190** is the best of the tested ILs for opening the structure of wood.

5.5 Conclusion

Wood Swelling tests were carried out in fifteen chiral ILs followed by wood dissolution and regeneration performed in thirteen chiral ILs. It was found that tangential and radial directions of dry wood chips have changed obviously after immersion in dry ILs for six consecutive testing days at 80 ^{o}C. The best swelling results were obtained in IL **190** with 1.22 a/a_0 of tangential side swelling and 1.20 a/a_0 for radial direction swelling. Wood chips expanded better in L-prolinate ILs than in other D-prolinate, *trans*-4-hydroxy L-prolinate and cholinium ILs. Better swelling results were also obtained in the prolinate ILs with a tetra-methyl and tetra-ethyl ammonium cation. Sample breaking was observered in IL **190** only. However, minimal expansions were observed in the prolinate ILs with a tetra-butyl and tetra-propyl ammonium cation. In the wood dissolution and regeneration procedure, the lowest yield (89.1%) was afforded in IL **190**, which highlighted that IL **190** is the best suitable solvent for wood dissolution. All the regeneration yields were consistent with the results of wood swelling, with lower wood regeneration yields obtained from the ILs which have a better capability for wood expansion. Comparing these results with chapter 3, chiral prolinate ILs with low toxicity can replace the highly toxic imidazolium ILs as a medium in wood expansion and dissolution. Like [Emim] Ac, sample break was achieved in IL **190** at 80 ^{o}C. Furthermore, the regeneration yield (89.1%) in ILs 190 was lower than in [Emim] Ac (91%). Additionally, from a toxicity point of view our ILs have been proved to be

'greener' and safer in chapter 3. In addition, there is the potential for the ILs to be further studied for enhancement of their biodegradability, improvement in solubility of wood components and used in sugar conversion by enzymatic hydrolysis.

5.6 References

1. Q. P. Liu, X. D. Hou, N. Li and M. H. Zong, *Green Chemistry*, 2012, **14**, 304-307.
2. I. Kilpeläinen, H. Xie, A. King, M. Granstrom, S. Heikkinen and D. S. Argyropoulos, *Journal of Agricultural and Food Chemistry*, 2007, **55**, 9142-9148.
3. R. P. Swatloski, S. K. Spear, J. D. Holbrey and R. D. Rogers, *J Am Chem Soc*, 2002, **124**, 4974-4975.

Chapter 6

Conclusion and Future Work

Wood application, especially wood regeneration and glucose released by enzymatic hydrolysis from regenerated wood components are important aspects in green and sustainable chemistry. The wood dissolution rather than cellulose dissolution have offered a wider range of methods for biomass utilization. Direct dissolution and regeneration of lignocellulosic biomass are the basis for deep exploitation of this kind of carbon-based feedstock instead of using fuel in chemicals industry to produce key platform molecules, biofuel and biopolymers.

Three single ILs [Amim] Cl, [Mmim][DMP], [Emim] Ac and their mixtures were applied for their abilities to swell wood and these results were proved by two consecutive experiments which were wood regeneration and enzymatic hydrolysis. The changes of three dimensions of wood chips after swelling in ILs were measured. Tangential side expanded to a maximum of 1.22 a/a_0 and 1.13 a/a_0 for radial side and tangential and radial directions of the wood chip expanded most in [Emim] Ac; sample break occurred in single ILs and mixtures with the presence of [Emim] Ac at 80 ^oC.

In aim of correlation of ILs ability for wood swelling, unbleached Norway spruce thermomechanical (TMP) fiber was used in wood dissolution in three single ILs at 120 ^oC followed by adding distilled water to afford the regenerated wood components. This kind of wood pretreatment increases the access of enzymes to hydrolyze cellulose to release kinds of monosaccharides. Yield of wood regeneration and the amount of glucose released after enzymatic hydrolysis was calculated. Wood regeneration in [Emim] Ac resulted in lower yields (91.0%) than in [Amim] Cl (95%) and [Mmim][DMP] (93.5%). However, more glucose was released from the regenerated wood in [Emim] Ac than others after the enzymatic hydrolysis. The actual yield of glucose released of [Emim] Ac, [Mmim][DMP] and [Amim] Cl were 28.5%, 19.8% and 12.1% respectively. These studies correlated the performance of ILs for wood swelling with wood regeneration in three traditional ILs and using regenerated wood for enzymatic hydrolysis.

In view of certain flaws in above three single ILs used in wood application, like the toxicity of imidazolinium ILs, non-biodegradability and achirality, 24 chiral tetra-alkyl ammonium prolinate ILs were designed and easily prepared from tetra-alkylammonium hydroxide and proline or 4-hydroxy-proline. Before introducing these prolinate ILs in wood application, their characterizations were realized through routine ^{1}H, ^{13}C NMR, IR, and Mass Spectrometry. Optical rotation was analyzed by a Perkin Elmer 341 polarimeter. Furthermore, the optical rotation values between each enantiomer were very close. In addition, the water content of the 24 chiral ILs were quantified by Karl Fischer titration. All the water content value were below 2% and some of them were under 1% in order to minimize the negative affect caused by water in thermal stability analysis and wood application.

Thermal stabilities, glass transition temperature and decomposition temperature, were analyzed by DSC and a TGA machine respectively. The effect of 24 prolinate ILs structures on their thermal stability has been discussed. Prolinate ILs with tetra-butyl and tetra-propyl side chains on cations result in lower decomposition temperature than ILs with tetra-ethyl and tetra-methyl side chains. All the ILs are stable over 190 ^{o}C, which means that all of them are suitable for dissolving wood fiber at 120 ^{o}C heat condition.

Based on the consideration of green chemistry, all the prolinate were tested in both general toxicity and high concentration toxicity screening. The prolinate ILs possessing tetra-methyl side chain on cation (**1d, 2d, 3d, 4d, 5d** and **6d**) were found to be least toxic (high MIC values) against both bacteria (8 strains) and fungi (12 strains). Whilst the prolinate ILs possessing tetra-butyl side chain on cation (**1a, 2a 3a, 4a, 5a** and **6a**) were found to be most toxic (low MIC values) to the eight strains of bacteria and twelve strains of fungi. The reason for that could be attributed to the enhanced lipophilic property with increased length of alkyl side chain in cations could elevate the toxicity of ILs.

All the twenty four prolinate ILs were tested in biodegradation studies via CO_2 Headspace test but all the ILs cannot defined readily biodegradable (higher than 60%). Better biodegradability results were obtained in ILs compounds **1d**, **2d**, **4d** and **6d**, which contain tetra-methyl ammonium cation with biodegradation percentage 55.4%, 50.3%, 50.7% and 55.7% respectively and other ILs which contain tetra-butyl or tetra-propyl ammonium cation show very low biodegradability and the ILs whose cations are tetraethyl ammonium have about 40% biodegradation results. It could be concluded that the biodegradation of prolinate ILs were determined by the size of cation structures. More bulky tetra-alkyl ammonium cations lead to lower biodegradability of ILs.

Based on the above research findings, twelve commercial chiral prolinate ILs and three cholinium ILs were used to carry out wood expansion, dissolution and regeneration. IL **190** was found to be the best ILs in wood swelling and wood components dissolution. Wood chips were cracked at 80 ^{o}C in IL **190**. Compared with the research in chapter 3, prolinate ILs are proven to be as equal effective in wood application as [Emim] Ac. Furthermore, prolinate ILs with low toxicity, chirality and relative low biodegradability are much 'greener' and more promising mediums than traditional imidazolium ILs.

Finally, we conclude that our aim of introducing ILs into wood application to converse biomass into glucose was achieved successfully. Three single ILs were used in wood expansion and pre-treatment. Glucose conversion was completed from pre-treatment wood with elevated yield. 24 low toxicity prolinate ILs were prepared successfully with good thermal stability. Fifteen chiral prolinate and cholinium ILs were chosen to be employed as media in wood expansion and pretreatment as well. Further exploitation of prolinate ILs should be studied in structure modification for the improvements of biodegradability and application in enzymatic hydrolysis for glucose conversion.

Appendix 1

Figures of Wood Expansion Results in Chapter 3

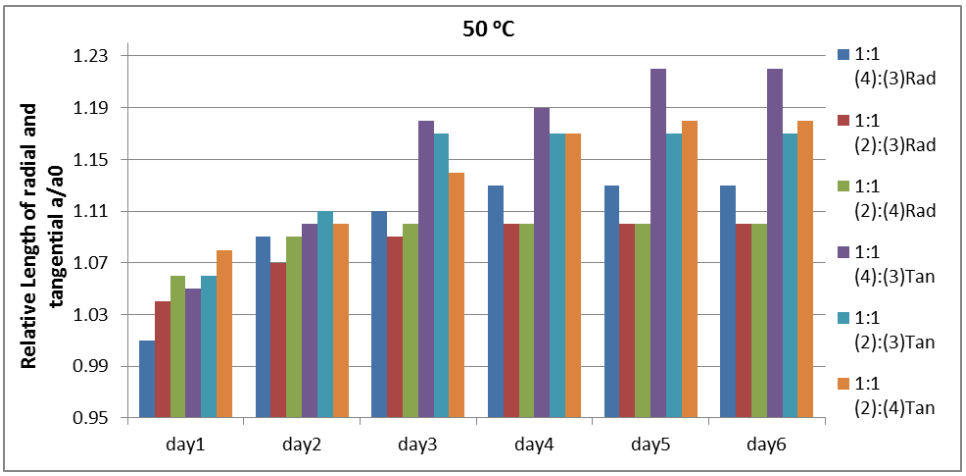


Fig. 3.8 Swelling of radial and tangential direction of wood chips at 50 °C temperatures in three ionic liquids mixtures. Each time point was averaged in three samples.

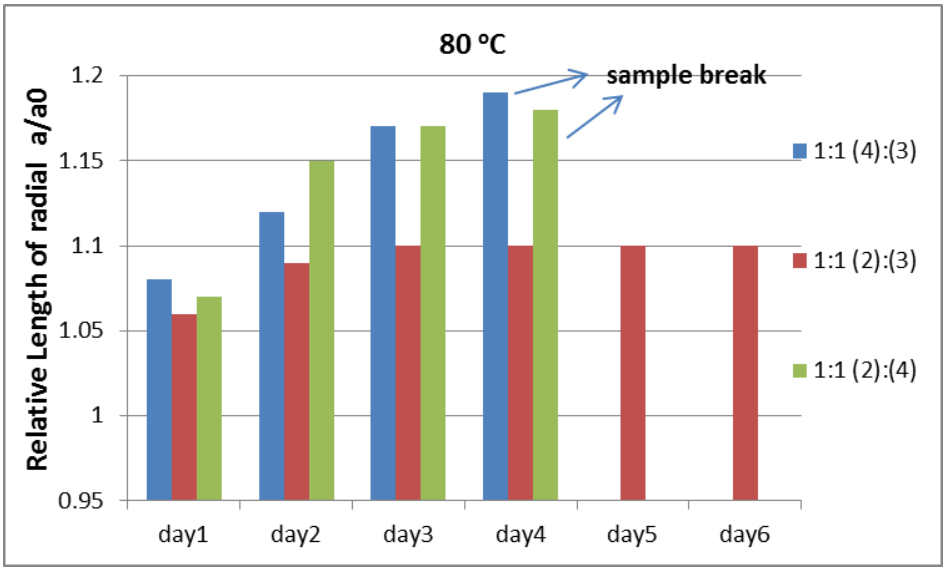


Fig. 3.9 Swelling of radial direction of wood chips at 80 °C temperatures in three ionic liquids mixtures. Each time point was averaged in three samples.

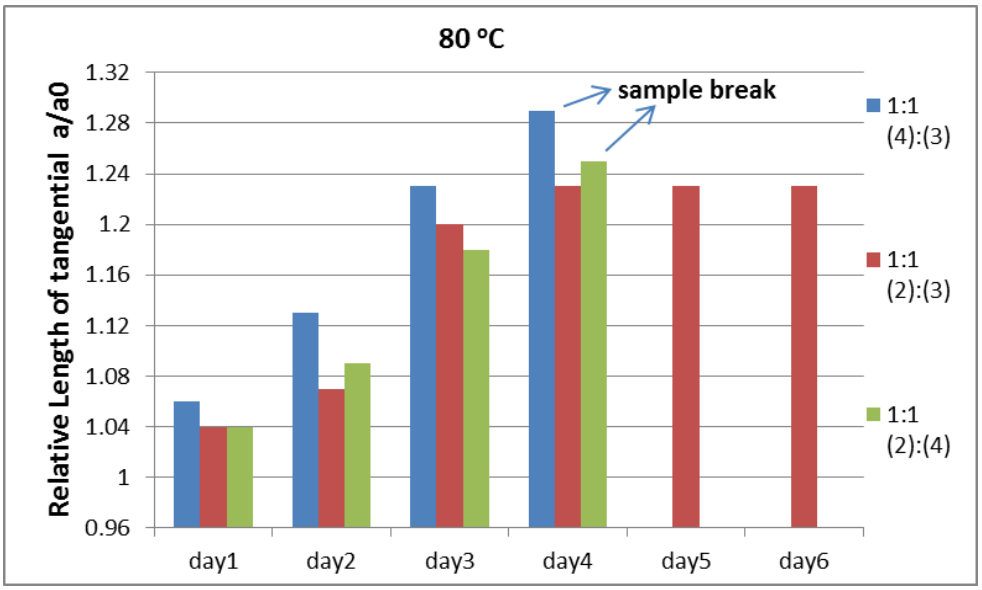


Fig. **3.10** Swelling of tangential direction of wood chips at 80 ºC temperatures in three ionic liquids mixtures. Each time point was averaged in three samples.

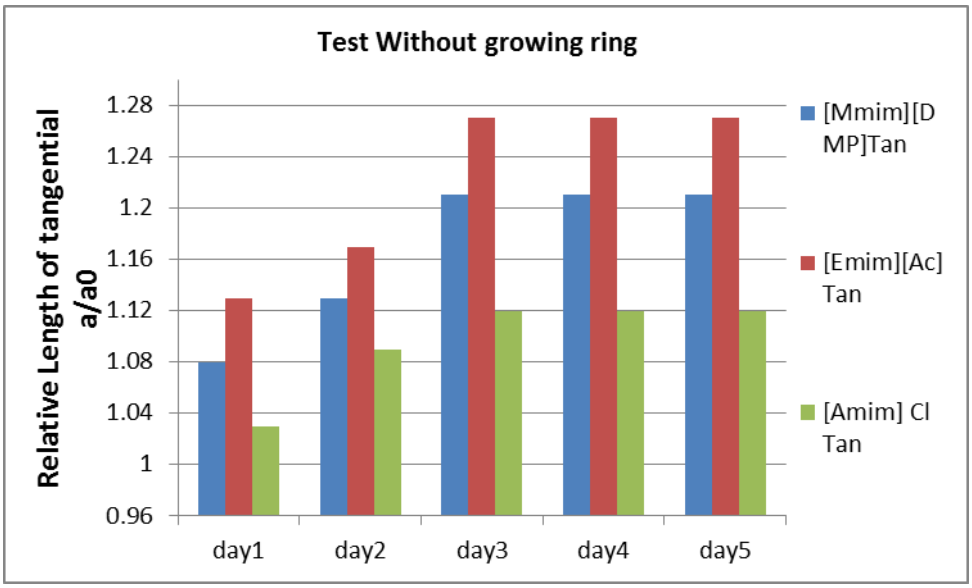


Fig. **3.11** Swelling of tangential direction of wood chips without growing ring at 80 ºC temperatures in three single ILs. Each time point was averaged in three samples.

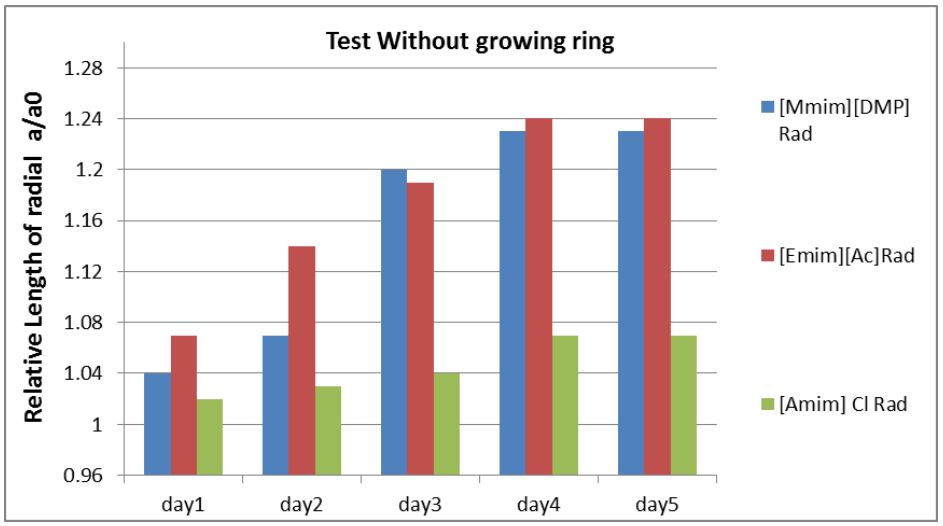
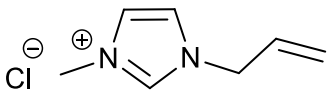


Fig. 3.12 Swelling of radial direction of wood chips without growing ring at 80 oC temperatures in three single ILs. Each time point was averaged in three samples.

Synthesized ILs Used in Chapter 3

Synthesis of 1-allyl-3-methylimidazolium chloride [Amim] Cl[24]



[Amim] Cl

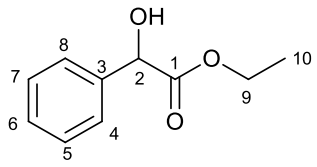
A 50 mL RB flask was charged with solution of freshly distilled methylimidazole (1 equivalent) in dry acetone. To this solution was added freshly distilled allyl chloride (0.95 equivalents). The reaction mixture was stirred overnight at 55 °C under a nitrogen atmosphere. After cooling, the acetone phase was separated and the excess unreacted methylimidazole was removed by extraction with more acetone. The crude product was added into acetone slowly and the mixture was stirred for 5 hrs at room temperature (in order to decrease the viscosity, the mixture was allowed to be heated to 40 °C) then the acetone phase was separated. This procedure was repeated five times. The ILs layer was separated and condensed by rotary evaporation. The crude product was further purified using active carbon in boiling methanol. After filtration and further evaporation of the volatiles, the product was dried under vacuum at 40 °C for 48 hrs prior to use.

Chemical Formula: $C_7H_{11}ClN_2$

Molecular Weight: 158.63 g/mol

^{1}H NMR (300 MHz, $CDCl_3$, δ) 3.83 (3 H, s), 4.73 (2H, d, J = 6.3 Hz), 5.10-5.20 (2H, m), 5.65–5.79 (1H, m), 7.30 (1H, s), 7.54 (1H, s), 10.10 (1H, s). ^{13}C NMR (300 MHz, $CDCl_3$, δ) 136.58, 129.92, 123.47, 121.86, 121.34, 50.98, 35.89

Synthesis of ethyl 2-hydroxy-2-phenylacetate (203)



203

To a stirred solution of mandelic acid (2 g, 13.1 mmol) in ethanol (35 mL) was added thionyl chloride (779 mg, 6.55 mmol, 0.49 mL) at RT. After stirring for 6 hrs the $NaHCO_3$ solution was added into reaction mixture and the product was extracted with ethyl acetate (5 × 50 mL). The combined extracts were dried over anhydrous $MgSO_4$, filtered and concentrated under high vacuum. The crude product was purified by column chromatography (SiO_2, Hexane: EtOAc, 80:20) to obtain a colourless liquid **203** in 92% yield (2.17 g, 12.0 mmol).

Chemical Formula: $C_{10}H_{12}O_3$

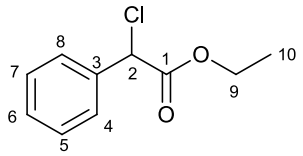
Molecular Weight: 180.20 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.92-6.80 (m, 3H, *H4*, *H8*, *H6*), 6.0 (s, 2H, *H5*, *H7*), 5.07 (s, 1H, *H2*), 4.29-4.20 (m, 2H, *H9*), 3.45 (bs, 1H, *OH* at C_2), 1.26 (t, J = 7.2 Hz, 3H, *H10*).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.69 (COO, *C1*), 138.46 (ArC, *C3*), 128.57 (2ArCH, *C5*, *C7*), 128.40 (ArCH, *C6*), 126.57 (2ArCH, *C4*, *C8*), 72.92 (CHOH, *C2*), 62.21(OCH_2, *C9*), 14.03 (CCH_3, *C10*).

^{1}H and ^{13}C-NMR spectra are in agreement with the literature data.[110]

Synthesis of ethyl 2-chloro-2-phenylacetate (204)



204

A 50 mL RB flask was charged with ethyl 2-hydroxy-2-phenylacetate (**203**) (2.00 g, 11.10 mmol) and CH_2Cl_2 (20 mL) at 0 ^{o}C. To this solution was added thionyl chloride (1.32 g, 0.81 mL, 11.10 mmol) dropwise and followed by adding TEA (1.37 mL, 11.10 mmol) dropwise and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred under at room temperature for 3 hrs. After that, reaction mixture was washed with distilled water (3 × 10 mL) and $NaHCO_3$ (aq) (3 × 10 mL). The organic phase was separated and dried by anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane: EtOAc, 80:20) to give the title product **204** as a light yellow oil at RT in 52% yield (1.14 g, 5.78 mmol).

Chemical Formula: $C_{10}H_{11}ClO_2$

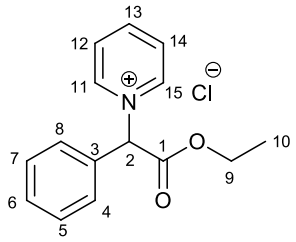
Molecular Weight: 198.65 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.05 (s, 1H, *H4*), 6.9 (m, 1H, *H8*), 6.78 (d, J = 8.0 Hz 1H, *H5*), 6.00 (s, 2H, *H6*, *H7*), 5.28 (s, 1H, *H2*), 4.29-4.10 (m, 2H, *H9*), 1.28 (t, J = 7.2 Hz, 3H, *H10*).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 177.64 (COO, *C1*), 139.74 (ArC, *C3*), 130.51 (2ArCH, *C5*, *C7*), 127.12 (ArCH, *C6*), 126.34 (2ArCH, *C4*,*C8*), 73.87 (CH, *C2*), 61.77 (OCH_2, *C9*), 14.83 (CCH_3, *C10*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[111]

Synthesis of 1-(2-ethoxy-2-oxo-1-phenylethyl) pyridin-1-ium chloride (205)



205

A 10 mL RB flask was charged with compound **(204)** (0.99 g, 5.00 mmol) followed by adding pyridine (0.38 g, 0.39 mL, 4.80 mmol). The reaction mixture was stirred under the protection of N_2 for 24 hrs. After that, crude light yellow solid was collected and washed by diethyl ether (5 × 10 mL) to afford white powder by filtration. Product was dried under high vaccum to afford compound **205** in 77% yield (1.03 g, 3.70 mmol).

Chemical Formula: $C_{15}H_{16}ClNO_2$

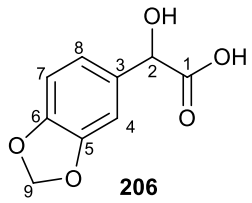
Molecular Weight: 277.75 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.74 (s, 2H, *H11, H15*), 8.59 (s, 1H, *H13*), 8.53 (t, J = 7.6 Hz 1H, *H6*), 8.06 (t, J = 7.2 Hz 2H, *H12, H14*), 7.77-7.75 (m, 2H, *H7, H5*), 7.49-7.47 (m, 3H, *H4, H8, H2*), 4.42-4.29 (m, 2H, *H9*), 1.29 (t, J = 7.2 Hz, 3H, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 179.44 (COO, *C1*), 150.45 (ArC, *C13*), 146.92 (ArC, *C11, C15*), 129.49 (ArC, *C12, C14*), 124.46 (ArC, *C3*), 122.98 (ArCH, *C8*), 118.73 (ArCH, *C4*), 111.58 (ArCH, *C7*), 109.23 (ArCH, *C5*), 106.73 (ArCH, *C6*),73.32 (NCH, *C2*), 63.77 (OCH_2, *C9*), 17.83 (CCH_3, *C10*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-3,4-Methylenedioxymandelic acid (206)[109]



206

A 50 mL RB flask was charged with glyoxylic acid monohydrate (1.83 g, 20.00 mmol), water (1.90 mL) at −10 ºC then conc. sulfuric acid (2.18 mL, 41.00 mmol) was added dropwise to the reaction mixture. After 10 mins stirring, 1,2-(methylenedioxy)benzene (2.20 g, 18.10 mmol) was added dropwise over 2 hrs at −10 ºC. The reaction mixture was allowed to warm to room temperature and stirring for 30 hrs. After that, water (7 mL) was added into mixture and the mixture was stirred for another 10 mins then white solid was precipitated. The solid was collected by filtration then dissolved in 30 mL of water and 10 mL of toluene was added and mixture was heated to 85 ºC. Ethyl acetate (6 × 25 mL) was used to extract product. The organic phase was dried by anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to obtain product **206** as a white solid in 70% yield (2.50 g, 12.67 mmol).

Chemical Formula: $C_9H_8O_5$

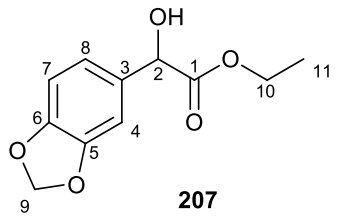
Molecular Weight: 196.16 g/mol

^{1}H NMR (400 MHz, DMSO-d_6, δ): 6.94 (s, 1H, *H4*), 6.90 (d, J = 8.0, Hz, 1H, *H8*), 6.87 (d, J = 8.0 Hz, 1 H, *H7*), 5.94 (s 2H, *H9*), 5.88 (bs, 1H, OH, *C2*), 4.94 (s, 1H, *H2*).

^{13}C NMR (100 MHz, DMSO-d_6, δ): 173.98 (COO, *C*1), 146.78 (ArC, *C5/C6*), 145.78 (ArC, *C5/C6*), 134.78 (ArC, *C3*), 120.45 (ArCH, *C7/C8*), 106.98(ArCH, *C7/C8*), 105.96 (ArCH, *C4*), 101.90 (OCH_2O, *C9*), 73.02 (HCOH, *C2*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[111]

Synthesis of Ethyl 2-(2H-1,3-benzodioxol-5-yl)-2-hydroxyacetate (207)[109]



207

A 50 mL RB flask was charged with a solution of compound (**206**) (1.96 g, 10.0 mmol) in ethanol (30 mL). Thionyl chloride (0.42 g, 0.26 mL, 3.4 mmol) was added into reaction mixture dropwise at RT. After stirring for 3 hrs, the reaction was quenched by adding water and $NaHCO_3$ (aq) and the reaction mixture was extracted with ethyl acetate (5 × 40 mL). The combined organic layer was washed with saturated $NaHCO_3$ (aq) (50 mL), then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the compound (**207**) as a light yellow oil in 81% yield (1.82 g, 8.1 mmol).

Chemical Formula: $C_{11}H_{12}O_5$

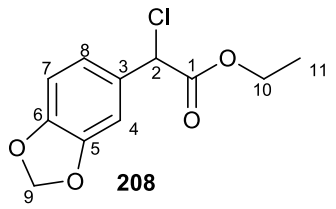
Molecular Weight: 224.21 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.91 (d, J = 6.0 Hz, 2H, *H8*, *H4*), 6.81 (d, J = 8.4 Hz, 1H, *H7*), 5.98 (s, 2H, *H9*), 5.07 (d, J = 5.6 Hz, 1H, *H2*), 4.27 (dt, J = 7.2, 6.0 Hz, 1H, *H10*), 4.20 (dt, J = 7.2, 6.0 Hz, 1H, *H10*), 3.46 (d, J = 5.6 Hz, 1H, *OH*, C2), 1.26 (t, J = 7.2 Hz, 3H, *H11*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 174.83 (COO, *C1*), 146.88 (ArC, *C5/C6*), 145.89 (ArC, *C5/C6*), 133.7 (ArC, *C3*), 122.91 (ArCH, *C4/C8*), 108.89 (ArCH, *C7*), 107.77 (ArCH, *C4/C8*), 101.99 (OCH_2O, *C9*), 72.89 (CHOH, *C2*), 63.44 (OCH_2, *C10*), 13.87 (CH_3, *C11*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[111]

Synthesis of (*RS*)-Ethyl 2-chloro-2-(3,4-methylenedioxyphenyl) acetate (208)[109]



208

A 50 mL RB flask was charged with compound (**207**) (1.60 g, 7.10 mmol) and CH_2Cl_2 (20 mL) at 0 ºC. To this stirred solution was added thionyl chloride (0.84 g, 0.53 mL, 7.10 mmol) dropwise and followed by adding TEA (0.89 mL, 7.10 mmol) dropwise and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred under at room temperature for 4 hrs. After that, reaction mixture was washed with distilled water (3 × 10 mL) and $NaHCO_3$ (aq) (3 × 10 mL). The organic phase was separated and dried by anhydrous MgSO4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane: EtOAc, 80:20) to give the product **208** as a yellow oil at RT in 50% yield (0.86 g, 3.55 mmol).

Chemical Formula: $C_{11}H_{11}ClO_4$

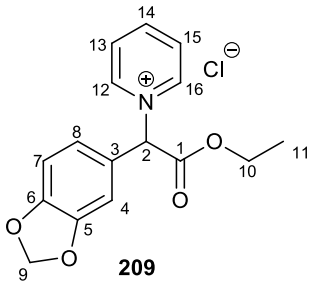
Molecular Weight: 242.66 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.04 (d, J = 2.0 Hz, 1H, *H4*), 6.94 (dd, 1H, J = 8.0, 2.0 Hz, *H8*), 6.78 (d, J = 8.0 Hz, 1H, *H7*), 5.99 (s, 1H, *H9*), 5.28 (s, 1H, *H2*), 4.29-4.19 (m, 2H, *H10*), 3.78 (t, J = 7.2, 3H, *H11*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.74 (*C*OO, *C1*), 147.9 (Ar*C*, *C5/C6*) 147.11 (Ar*C*, *C5/C6*) 130.10 (Ar*C*, *C3*) 123.34 (Ar*C*H, *C8*) 108.79 (Ar*C*H, *C4*) 108.02 (Ar*C*H, *C7*) 102.34 (O*C*H_2O, *C9*), 73.79 (CHOH, *C2*), 64.12 (OCH_2, *C10*), 14.34 (CH_3, *C11*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-Ethyl 2-(3,4-methylenedioxyphenyl)-2-pyridinium acetate, chloride salt (209)[109]



209

A 10 mL RB flask was charged with compound (**208**) (0.72 g, 3.00 mmol) followed by adding pyridine (0.22 g, 0.23 mL, 2.90 mmol). The reaction mixture was stirred under the protection of N_2 for 24 hrs. After that, crude brown solid was collected and washed by diethyl ether (5 × 10 mL) to afford light brown powder by filtration. Product was dried under high vacuum to afford compound **209** in 70% yields (0.67 g, 2.10 mmol).

Chemical Formula: $C_{16}H_{16}ClNO_4$

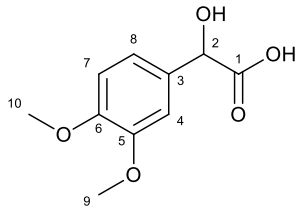
Molecular Weight: 321.76 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.63 (d, *J* = 5.6 Hz 2H, *H12*, *H16*), 8.58 (t, *J* = 7.6 Hz, 1H, *H14*), 8.36 (s, 1H, *H2*), 8.12-8.07 (m, 2H, *H13*, *H15*), 7.21-7.27 (m, 2H, *H4*, *H8*), 6.84 (d, 1H, *J* = 8.8 Hz, *H7*), 5.98 (d, *J* = 1.6 Hz, 1H, *H9*), 4.36-4.25 (m, 2H, *H10*), 1.25 (t, *J* = 7.2 Hz 3H, *H11*).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 168.10 (*C*OO, *C1*), 148.99 (Ar*C C6*), 148.88 (Ar*C C5*), 146.87 (Ar*C*, *C14*), 144.47 (Ar*C*, *C12*, *C16*), 127.44 (Ar*C*, *C13*, *C15*), 123.89 (Ar*C*, *C3*), 123.11 (Ar*C*H, *C8*), 108.89 (Ar*C*H, *C4*), 107.93 (Ar*C*H, *C7*), 102.03 (O*C*H_2O, *C9*), 72.99 (CHOH, *C2*), 63.89 (OCH_2, *C10*), 14.11 (CH_3, *C11*).

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-3,4-Dimethoxymandelic acid (210)[109]



210

A 50 mL RB flask was charged with glyoxylic acid (50% aq. solution, 4.34 mL, 38.87 mmol) at −10 ºC then conc. sulfuric acid (2.83 mL, 53.17 mmol) was added dropwise to the reaction mixture. After 10 mins stirring, then 1,2-dimethoxybenzene (4.97 g, 36.00 mmol) was added dropwise over 1 hrs at −10 ºC. After stirring for an additional 2.5 hrs at −10 ºC water (6 mL) was added, followed by 30% aquoues NaOH (9.60 mL, 11.30 g, 85.00 mmol). The reaction mixture was washed with toluene (6 × 20 mL) and extracted with ethyl acetate (5 x 25 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the compound **210** as a yellow solid in 60% yield (4.58 g, 21.60 mmol).

Chemical Formula: $C_{10}H_{12}O_5$

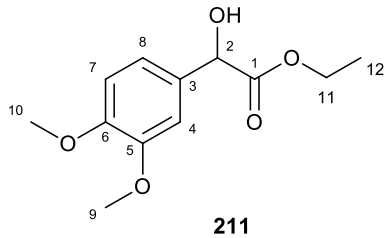
Molecular Weight: 212.20 g/mol

^{1}H NMR (400 MHz, DMSO-d_6, δ): 12.49 (s, 1H, COOH), 7.04-6.92 (m, 3H, *H4*, *H7*, *H8*), 4.90 (s, 1H, *H2*), 3.71 (s, 6H, *H9*, *H10*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 178.01 (*C*OO, *C1*), 150.12 (Ar*C*, *C5*/*C6*), 149.78 (Ar*C*, *C5/C6*), 131.34 (Ar*C*, *C3*), 118.98 (Ar*C*H, *C8*), 111.77 (Ar*C*H, *C7*), 109.34 (Ar*C*H, *C4*), 72.89 (*C*HOH, *C2*), 56.88(O*C*H$_3$, *C9*/*C10*), 57.03 (O*C*H$_3$, *C9*/*C10*)

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-Ethyl-3,4-dimethoxymandelate (211)[109]



211

A 100 mL RB flask was charged with a solution of compound (**210**) (3.18 g, 15.0 mmol) in ethanol (50 mL). Thionyl chloride (0.69 g, 0.43 mL, 5.8 mmol) was added into reaction mixture dropwise at RT. After stirring for 3 hrs, the reaction was quenched by adding water and $NaHCO_3$ (aq) and the reaction mixture was extracted with ethyl acetate (5 × 40 mL). The combined organic layer was washed with saturated $NaHCO_3$ (aq) (50 mL), then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford the compound (**211**) as a yellow oil in 83% yield (2.99 g, 12.4 mmol).

Chemical Formula: $C_{12}H_{16}O_5$

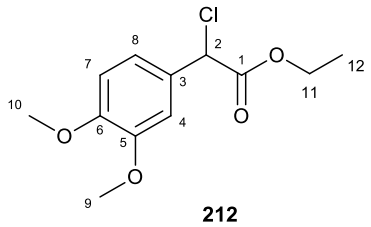
Molecular Weight: 240.25 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 6.98-6.95 (m, 2H, *H4*, *H8*), 6.87 (d, *J* = 8.0 Hz, 1H, *H7*), 5.13 (s, 1H, *H2*), 3.85 (s, 3H, *H9/H10*), 3.84 (s, 3H, *H9/H10*), 3.89 (m, 2H, *H11*), 1.24 (t, *J* = 7.2 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.97 (*C*OO, *C1*), 149.78 (ArC, *C5*/*C6*), 149.32 (ArC, *C5/C6*), 131.89 (Ar*C*, *C3*), 120.21 (Ar*C*H, *C8*), 112.11 (Ar*C*H, *C7*), 109.78 (Ar*C*H, *C4*), 72.43 (*C*HOH, *C2*), 60.21 (O*C*H$_2$, *C11*), 56.15 (O*C*H$_3$, *C9/C10*), 56.13 (O*C*H$_3$, *C9/C10*), 15.21 (CH$_3$, *C12*)

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-Ethyl 2-chloro-2-(3, 4-dimethoxyphenyl) acetate (212)[109]



212

A 50 mL RB flask was charged with compound (**211**) (2.40 g, 10.00 mmol) and CH_2Cl_2 (20 mL) at 0 °C. To this stirred solution was added thionyl chloride (1.19 g, 0.74 mL, 10.00 mmol) dropwise and followed by adding TEA (1.25 mL, 10.00 mmol) dropwise and the reaction mixture was allowed to warm to room temperature. The reaction mixture was stirred under at room temperature for 4 hrs. After that, reaction mixture was washed with distilled water (3 × 15 mL) and $NaHCO_3$ (aq) (3 × 15 mL). The organic phase was separated and dried by anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane: EtOAc, 80:20) to give the product **212** as a yellow oil at RT in 54% yield (1.34 g, 5.40 mmol).

Chemical Formula: $C_{12}H_{15}ClO_4$

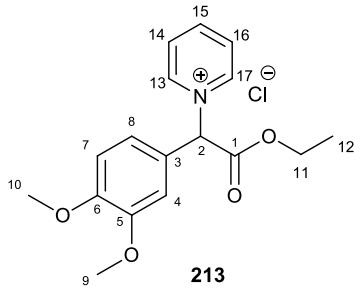
Molecular Weight: 258.70 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.04-6.99 (m, *2*H, *H4*, *H7*), 6.81 (d, 1H, *J* = 8.4 Hz, *H8*), 5.29 (s, 1H, *H2*), 4.24-4.18 (m, 2H, *H11*), 3.88 (s, 3H, *H9*/*H10*), 3.86 (s, 3H, *H9*/*H10*), 1.24 (t, *J* = 7.2 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 168.14 (*C*OO, *C1*), 150.22 (Ar*C*, *C5/C6*), 149.78 (Ar*C*, *C5/C6*), 129.11 (Ar*C*, *C3*), 121.78 (Ar*C*H, *C8*), 111.89 (Ar*C*H, *C4*), 110.34 (Ar*C*H, *C7*), 73.78 (*C*HOH, *C2*), 64.78 (O*C*H$_2$, *C11*), 57.29 (O*C*H$_3$, *C9/C10*), 57.11 (O*C*H$_3$, *C9/C10*), 15.72 (CH$_3$, *C12*)

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Synthesis of (*RS*)-Ethyl 2-(3,4-dimethoxyphenyl)-2-pyridinium acetate, chloride salt (213)[109]



213

A 10 mL RB flask was charged with compound (**212**) (0.77 g, 3.00 mmol) followed by adding pyridine (0.22 g, 0.23 mL, 2.90 mmol). The reaction mixture was stirred under the protection of N_2 for 24 hrs. After that, crude brown solid was collected and washed by diethyl ether (5 × 10 mL) to afford light brown powder by filtration. Product was dried under high vaccum to afford compound **213** in 74% yield (0.75 g, 2.22 mmol).

Chemical Formula: $C_{17}H_{20}ClNO_4$

Molecular Weight: 337.80 g/mol

^{1}H NMR (400 MHz, $CDCl_3$, δ): 9.65 (d, *J* = 5.6 Hz 2H, *H13*, *H17*), 8.49-8.47 (m, 1H, *H14*), 8.38 (s, 1H, *H2*), 8.02 (t, *J* = 7.2 Hz, 2H, *H14*, *H16*), 7.75 (d, *J* = 2.0 Hz, 1H, *H4*), 7.28 (s, 2.0 Hz, 1H, *H8*), 6.93 (d, *J* = 8.2 Hz, 1H, *H7*), 4.36-4.33 (m, 2H, *H11*), 3.94 (s, 3H, *H9*/*H10*), 3.92 (s, 3H, *H9*/*H10*), 1.34 (t, *J* = 6.8 Hz, 3H, *H12*)

^{13}C NMR (100 MHz, $CDCl_3$, δ): 168.17 (*C*OO, *C1*), 151.19 (Ar*C*, *C6*), 150.02 (Ar*C*, *C5*), 146.78 (Ar*C*, *C15*), 145.28 (Ar*C*, *C13*, *C17*), 128.16 (Ar*C*, *C14*, *C16*), 122.91 (Ar*C*, *C3*), 121.89 (Ar*C*H, *C8*), 112.87 (Ar*C*H, *C4*), 111.98 (Ar*C*H, *C7*), 74.12 (N*C*H, *C2*), 56.42 (O*C*H$_3$, *C9*/*C10*), 56.96 (O*C*H$_3$, *C9*/*C10*), 54.98 (O*C*H$_3$, *C11*), 15.98 (CH$_3$, *C12*)

^{1}H NMR spectra and ^{13}C NMR are in agreement with the literature data.[112]

Appendix 2

Supporting Information for Chapter 4

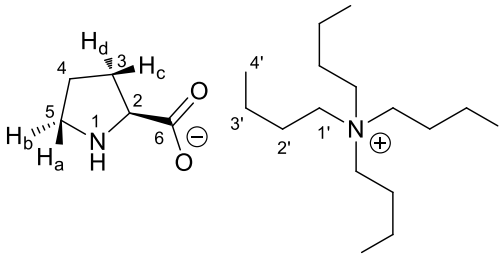
Materials

L-proline (99%), *trans*-4-hydroxy-L-Proline (⩾99%), *trans*-4-hydroxy-D-Proline (97%), tetra-butyl ammonium hydroxide solution (54.0-56.0% in H_2O), tetra-propyl ammonium hydroxide solution (1.0 M in H_2O) and tetra-methyl ammonium hydroxide pentahydrate (⩾97%) were purchased at Sigma-Aldrich. D-proline (⩾98%), *cis*-4-hydroxy-D-proline (98%), *cis*-4-hydroxy-L-proline (>97%) and tetra-ethyl ammonium hydroxide (35% in H_2O) were purchased at TCI Fine Chemicals. All NMR spectra were recorded in deuterium oxide (99.9%) on a Bruker 400 MHz or 600 MHz spectrometer (NMR spectra described in Supporting Information). ILs **1a–6d** synthesized in this work were presented in Tables 1. Optical rotation values were determined by a Perkin Elmer 341 polarimeter. Water content was evaluated by Karl Fischer Moisture Titrator MKS-500. The glass transition temperature (T_g) was determined by Differential Scanning Calorimetry (Perkin Elmer's DSC 400) and the decomposition temperature (T_d) was evaluated by Thermo-gravimetric Analysis (TA Instruments Q500).

Methods

Synthesis and purification of ILs

Preparation of (2S)-Pyrrolidine-2-carboxylate tetrabutylazanuide (IL 1a):



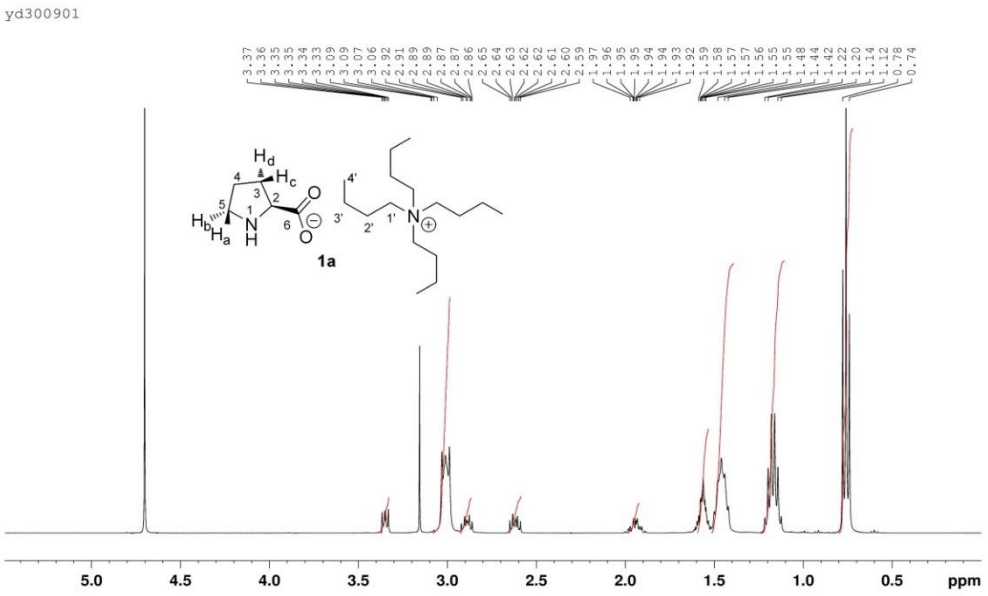
A 50 mL RB flask was charged with L–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetrabutyl ammonium hydroxide solution (3.57 g, 7.56 mmol and 3.60 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was evaporated under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation

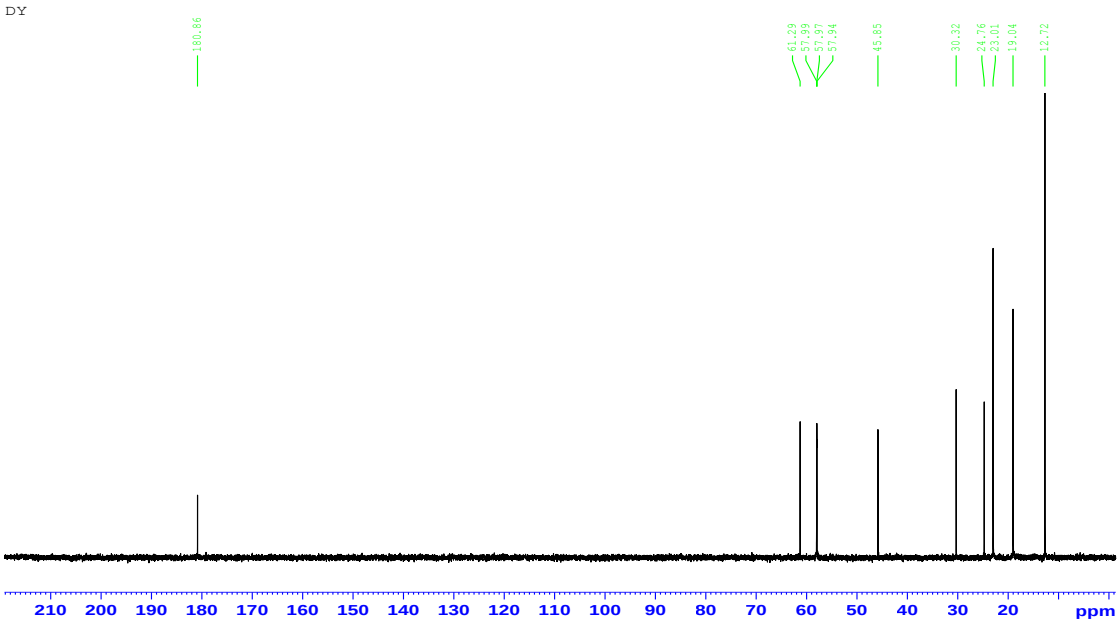
and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **1a** as light yellow oil at RT in 85% yield (2.29 g, 6.43 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_2$

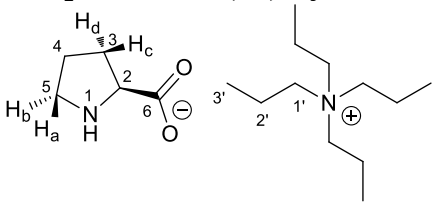
Molecular Weight: 352.55 g/mol

$[\alpha]_D^{20}$ = –30.4 (0.62 c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.36–3.33 (m, 1H, *H2*), 3.07 (t, J = 7.2 Hz, 8H, *H1'*), 2.92–2.86 (m, 1H, $H5_a$), 2.65–2.59 (m,1H, $H5_b$), 1.97–1.92 (m, 1H, $H3_c$), 1.58–1.54 (m, 3H, $H3_d$, *4*), 1.47–1.42 (m, 8H, *H2'*), 1.15 (tq, J = 7.6, 7.6 Hz, 8H, *H3'*), 0.77 (t, J = 7.6 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.86 (CO, *C6*), 61.29 (NCH_2, *C1'*), 57.97 (NHCH, *C2*), 45.85 (NCH_2, *C5*), 30.46 (CH_2, *C3*), 24.76 (CH_2, *C4*), 23.01 (CH_2, *C2'*), 19.05 (CH_2, *C3'*), 12.75 (CH_3, *C4'*). IR (neat, cm^{-1}): 3245, 2960, 2936, 2874, 1592, 1463, 1376, 1287, 1156, 1107, 1042, 881. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2834. Water Content = 0.59%. T_g = –46.3 °C. T_d = 198.7 °C.





Preparation of (2*S*)-Pyrrolidine-2-carboxylate tetrapropylazanuide (IL 1b):



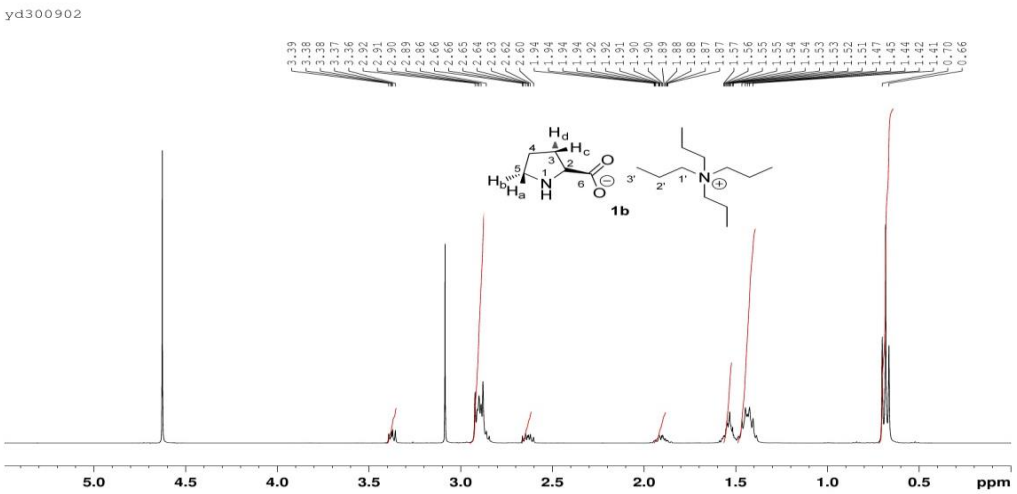
A 50 mL RB flask was charged with L–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetrapropyl ammonium hydroxide solution (6.83 g, 7.56 mmol and 6.67 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **1b** as light yellow oil at RT in 90% yield (2.04 g, 6.81 mmol).

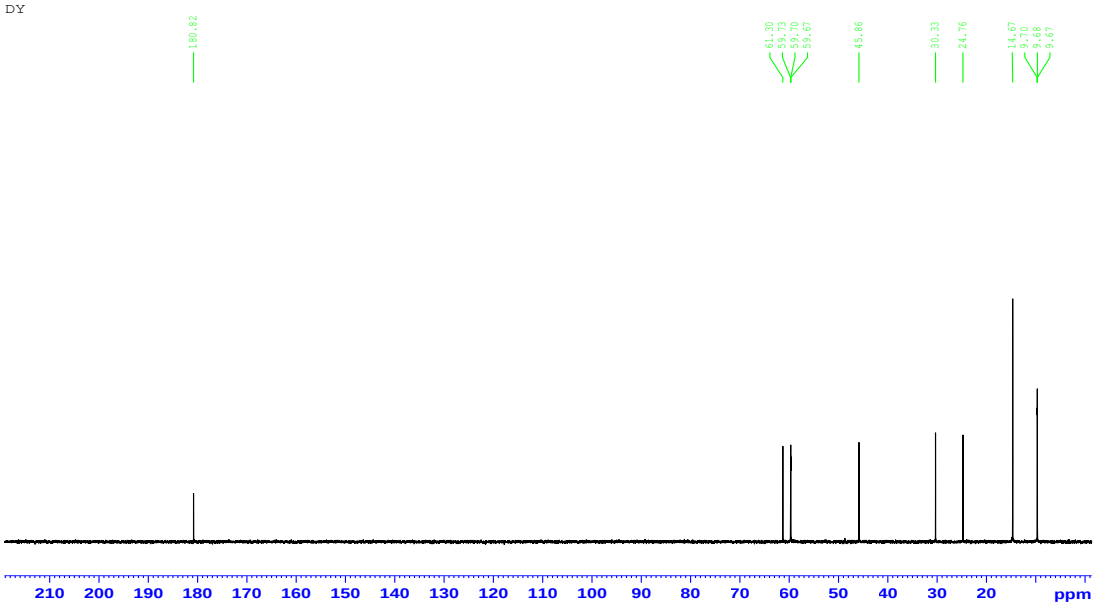
Chemical Formula: $C_{17}H_{32}N_2O_2$

Molecular Weight: 296.45 g/mol

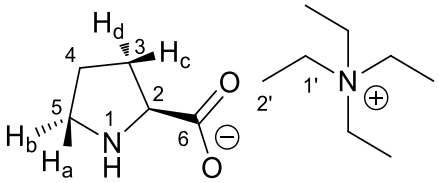
$[\alpha]_D^{20}$ = –32.8 (0.58 c, H_2O). ^1H NMR (400 MHz, D_2O, δ): 3.39–3.35 (m, 1H, *H2*), 2.92–2.86 (m, 9H, *H1', 5$_a$*), 2.63–2.60 (m, 1H, *H5$_b$*), 1.94–1.87 (m, 1H, *H3$_c$*), 1.56–1.51

(m, 3H, *H4*, 3_d), 1.43 (tq, J = 7.6, 7.6 Hz, 8H, *H2'*), 0.68 (t, J = 7.6 Hz, 12H, *H3'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.82 (CO, *C6*), 61.30 (NHCH, *C2*), 59.70 (NCH_2, *C1'*), 45.86 ($NHCH_2$, *C5*), 30.33 (CH2, *C3*), 24.76 (CH_2, *C4*), 14.67 (CH_2, C*2'*), 9.6 (CH_3, *C3'*). IR (neat, cm^{-1}): 3273, 2968, 2879, 2809, 1589, 1475, 1460, 1371, 1283, 1171, 1042, 969. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2210. Water Content = 1.43%. T_g = –46.7 °C. T_d = 201.6 °C.





Preparation of (2*S*)-Pyrrolidine-2-carboxylate tetraethylazanuide (IL 1c):

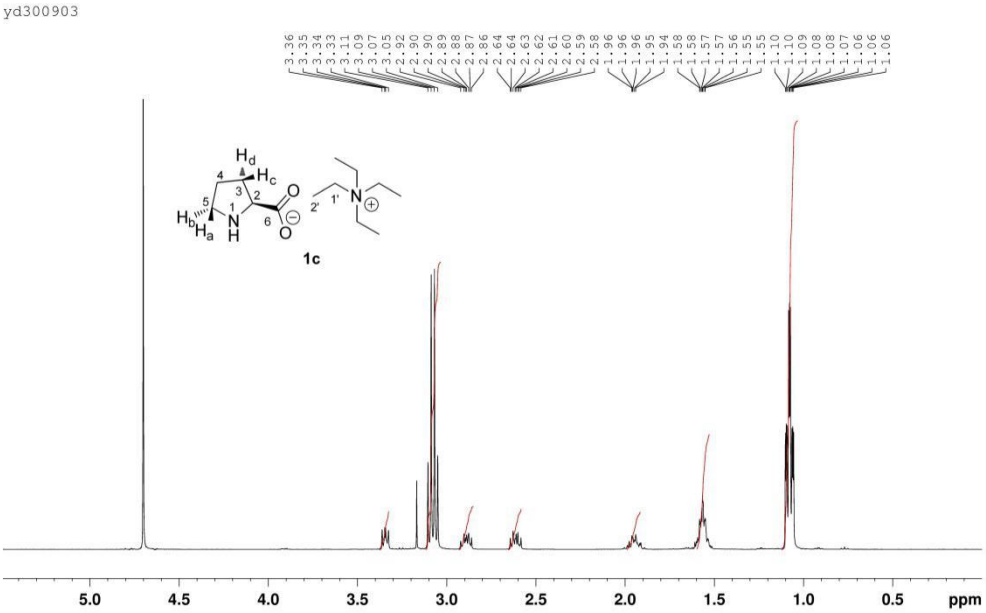


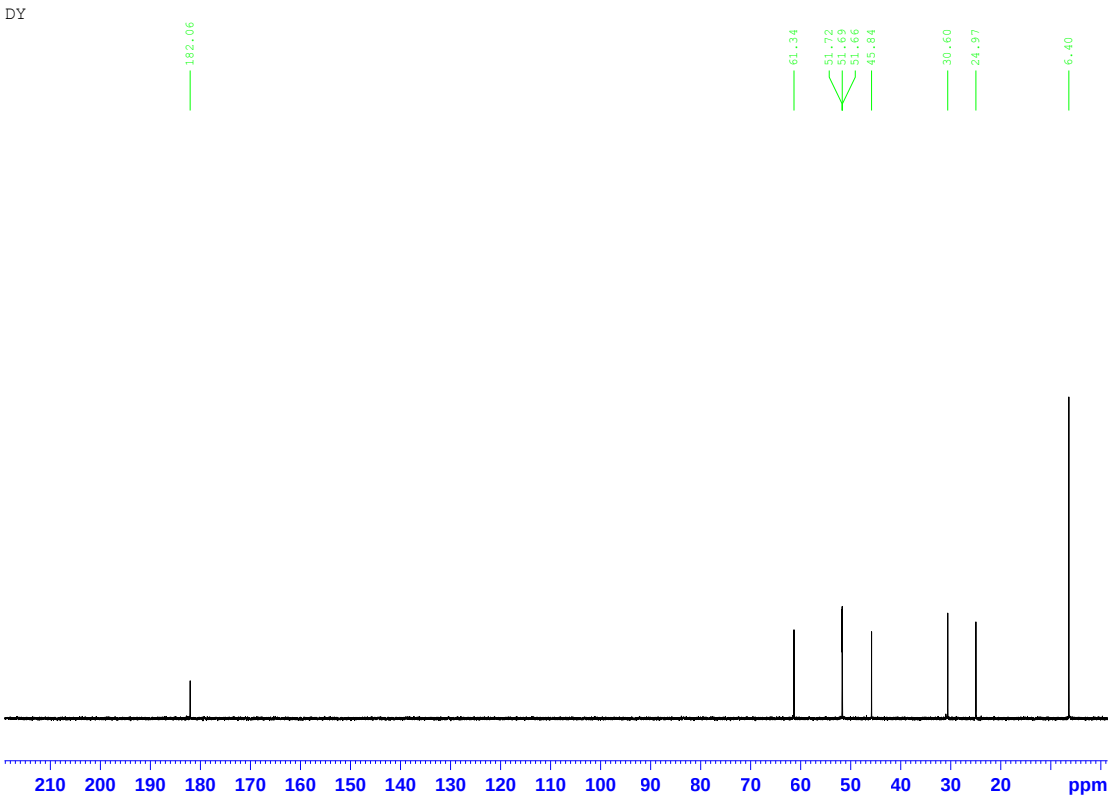
A 50 mL RB flask was charged with L–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetraethyl ammonium hydroxide solution (3.18 g, 7.56 mmol and 3.11 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **1c** as light yellow oil at RT in 94% yield (1.74 g, 7.11 mmol).

Chemical Formula: $C_{13}H_{24}N_2O_2$

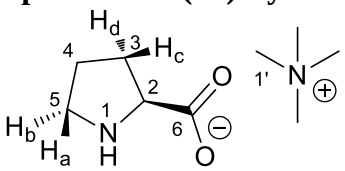
Molecular Weight: 240.34 g/mol

$[\alpha]_D^{20}$ = –30.1º (0.50 c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.34 (q, J = 6.4 Hz, 1H, *H2*), 3.07 (q, J = 7.2Hz, 8H, *H1'*), 2.92–2.86 (m, 1H, $H5_a$), 2.64–2.58 (m, 1H, $H5_b$), 1.96–1.94 (m, 1H, $H3_c$), 1.58–1.55 (m, 3H, $H3_d$, *4*), 1.07 (tq, J = 7.6, 7.2 Hz, 12H, *H2'*). ^{13}C NMR (100 MHz, D_2O, δ): 182.18 (CO, *C6*), 61.34 (NHCH, *C2*), 51.69 (NCH_2, *C1'*), 45.83 ($NHCH_2$, *C5*), 30.62 (CH_2, *C3*), 24.99 (CH_2, *C4*), 6.39 (CH_3, *C2'*). IR (neat, cm^{-1}): 3295, 2952, 2868, 2258, 1583, 1486, 1373, 1173, 1077, 1002, 936, 784. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1586. Water Content = 1.80%. T_g = –49.4 ºC. T_d = 192.8 ºC.


yd300903
3.36 3.35 3.34 3.33 3.11 3.09 3.07 3.05 2.92 2.90 2.90 2.89 2.88 2.87 2.86 2.64 2.64 2.63 2.62 2.61 2.60 2.59 2.58 1.96 1.96 1.96 1.95 1.94 1.58 1.58 1.57 1.57 1.56 1.55 1.55 1.10 1.10 1.09 1.08 1.08 1.07 1.06 1.06 1.06 1.06
1c
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


DY
182.06
61.34
51.72
51.69
51.66
45.84
30.60
24.97
6.40
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

Preparation of (2*S*)-Pyrrolidine-2-carboxylate tetramethylazanuide (IL 1d):

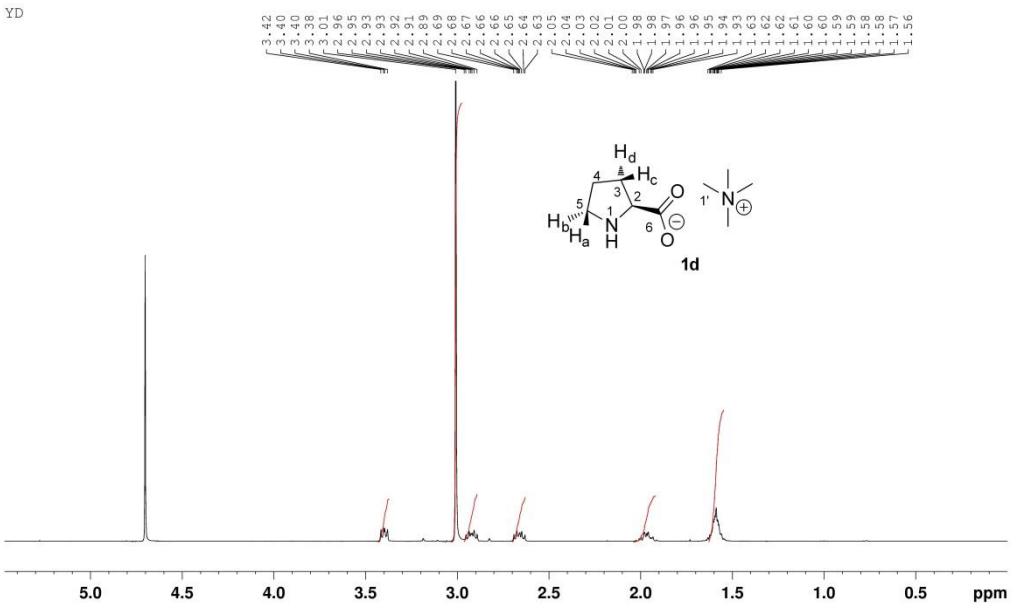


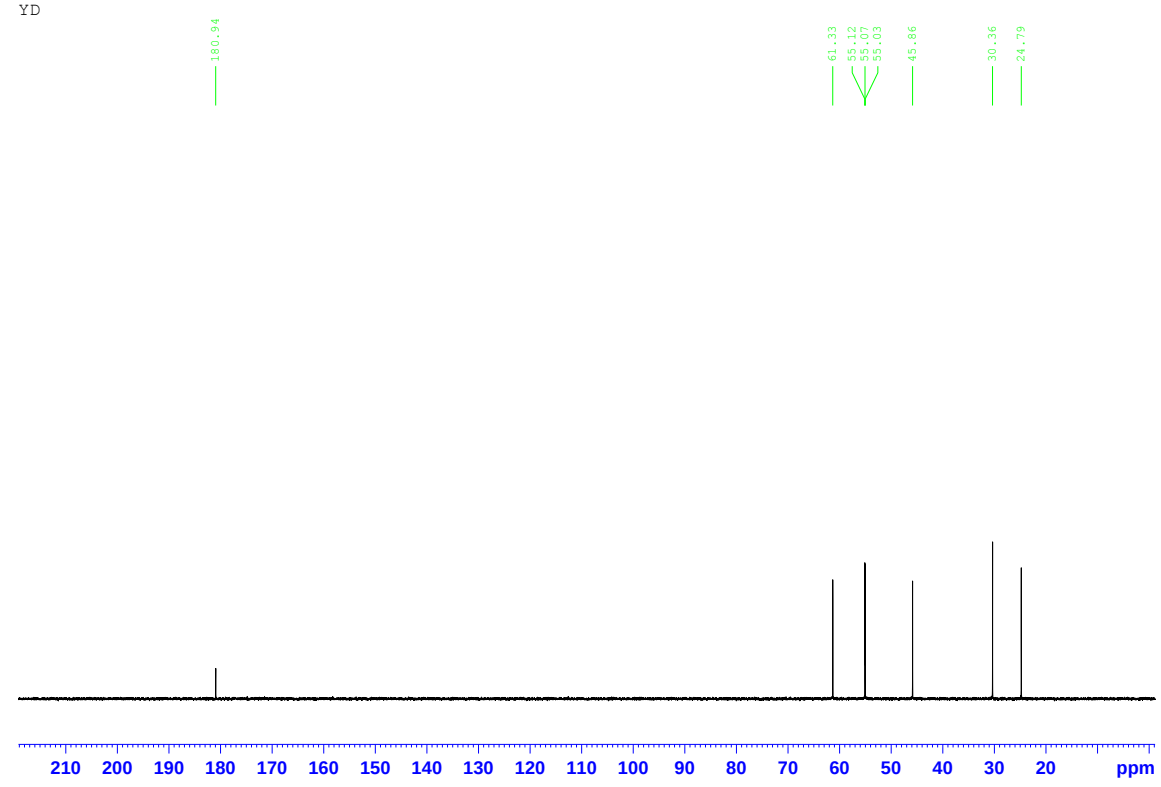
A 50 mL RB flask was charged with L–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetramethyl ammonium hydroxide pentahydrate (1.37 g, 7.56 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **1d** as light orange oil at RT in 88% yield (1.25 g, 6.65 mmol).

Chemical Formula: $C_9H_{16}N_2O_2$

Molecular Weight: 184.24 g/mol

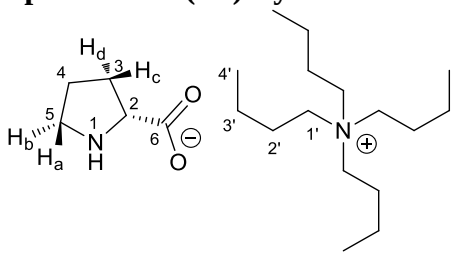
$[\alpha]_D^{20}$ = –30.2 (0.55c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.40 (q, J = 6.8 Hz, 1H, *H2*), 3.00 (s, 12H, *H1'*), 2.93–2.91 (m, 1H, $H5_a$), 2.69–2.63 (m, 1H, $H5_b$), 1.98–1.95 (m, 1H, $H3_c$), 1.60–1.57 (m, 3H, $H3_d$, *4*). ^{13}C NMR (100 MHz, D_2O, δ): 180.94 (CO, *C6*), 61.33 (NHCH, *C2*), 55.07 (NCH_3, *C1'*), 46.86 ($NHCH_2$, *C5*), 30.36 (CH_2, *C3*), 24.79 (CH_2, *C2*). IR (neat, cm^{-1}): 3292, 3030, 2961, 2873, 1580, 1489, 1377, 1291, 1176, 1093, 1035, 950. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_4H_{12}N^+$, calculated = 74.0964, found = 74.0962. Water Content = 1.16%. T_g = –46.4 °C. T_d = 248.2 °C.


YD
3.42 3.40 3.40 3.38 3.01 2.96 2.95 2.93 2.93 2.92 2.91 2.89 2.69 2.68 2.67 2.66 2.66 2.65 2.64 2.63 2.05 2.04 2.03 2.02 2.01 2.00 1.98 1.98 1.97 1.96 1.96 1.95 1.94 1.93 1.63 1.62 1.62 1.61 1.60 1.60 1.59 1.59 1.58 1.58 1.57 1.56
Hd
Hc
Hb
Ha
N
H
O
O
1'
N
1d
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


YD
180.94
61.33
55.12
55.07
55.03
45.86
30.36
24.79
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

5

Preparation of (2*R*)-Pyrrolidine-2-carboxylate tetrabutylazanuide (IL 2a):



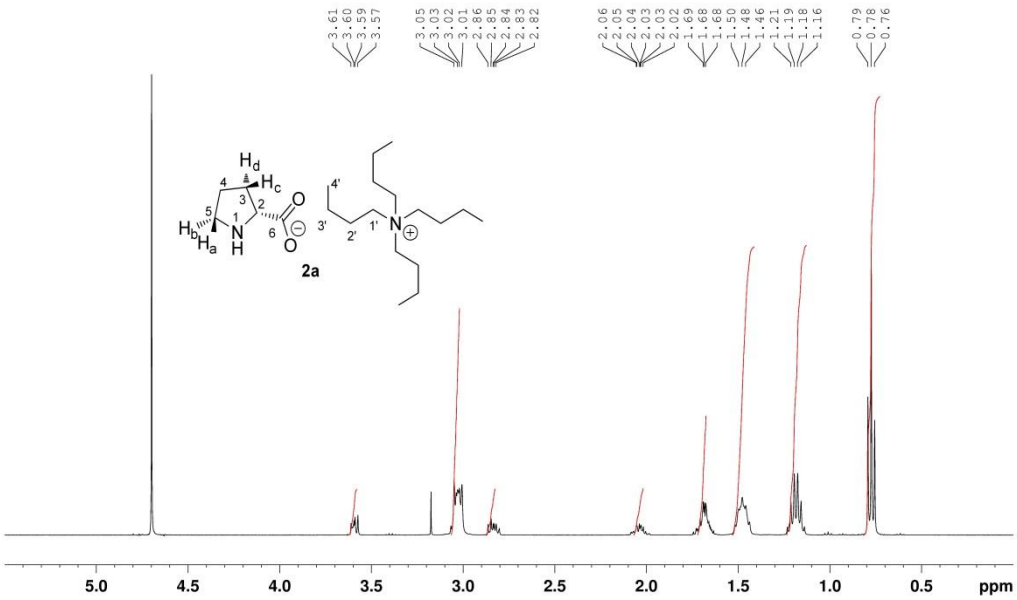
A 50 mL RB flask was charged with D–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetrabutyl ammonium hydroxide solution (3.57 g, 7.56 mmol and 3.60 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **2a** as yellow oil at RT in 87% yield (2.35 g, 6.58 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_2$

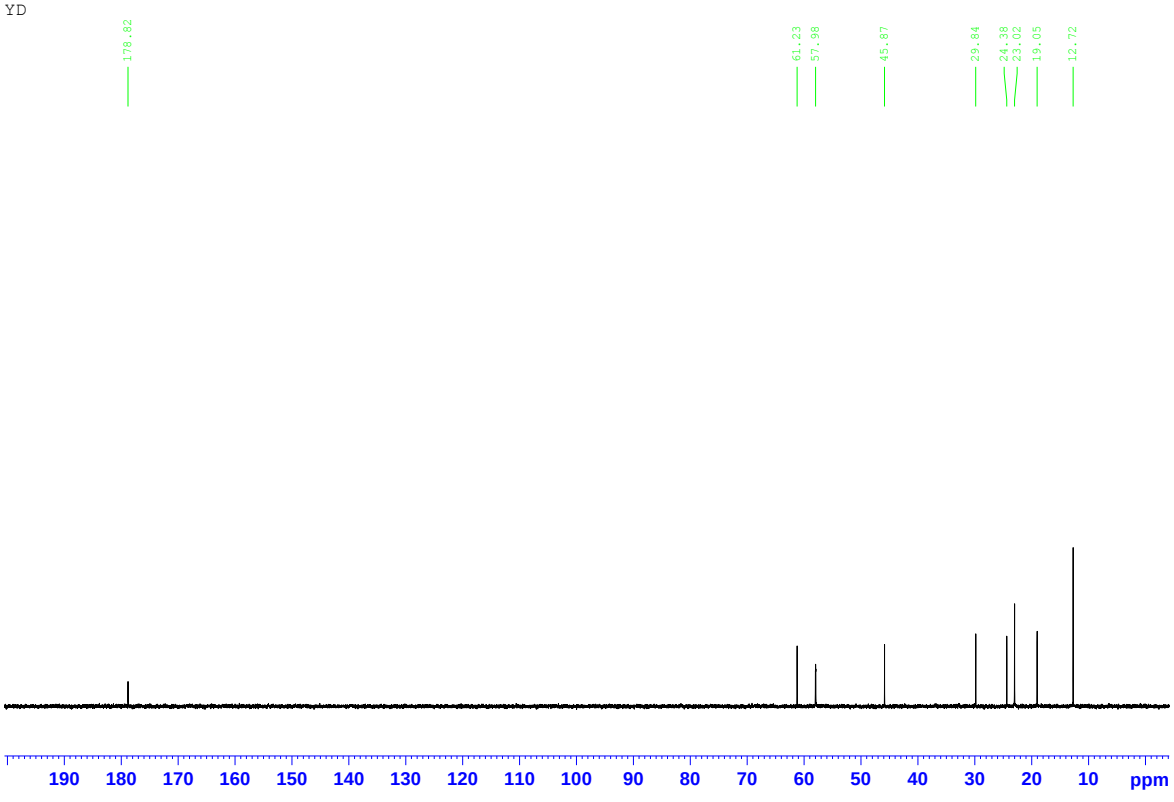
Molecular Weight: 352.55 g/mol

$[\alpha]_D^{20}$ = +30.5 (0.62c, H_2O). ^{1}H–NMR (400 MHz, D_2O, δ): 3.59 (q, J = 6.4 Hz, 1H, *H2*), 3.02 (q, J = 7.6 Hz, 8H, *H1'*), 2.86–2.82 (m, 1H, $H5_a$), 2.05–2.01 (m, 1H, $H5_b$), 1.69–1.67 (m, 3H, *H3*$_{c, d}$, *4*), 1.49–1.45 (m, 8H, H*2'*), 1.18 (tq, J = 7.6, 7.2 Hz, 8H, *H3'*), 0.78 (t, J = 7.2 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 178.82 (CO, *C6*), 61.23 (NHCH, *C2*), 57.98(NCH_2, *C1'*), 45.87 ($NHCH_2$, *C5*), 29.84 (CH_2, *C3*), 24.38 (CH_2, *C4*), 23.02 (CH_2, *C2'*), 19.05 (CH_2, *C3'*), 12.72 (CH_3, *C4'*). IR (neat, cm^{-1}): 3218, 2959, 2873, 2807, 1590, 1463, 1371, 1328, 1162, 1106, 1047, 881. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2833. Water Content = 0.34%. T_g = –49.5 °C. T_d = 190.9 °C.

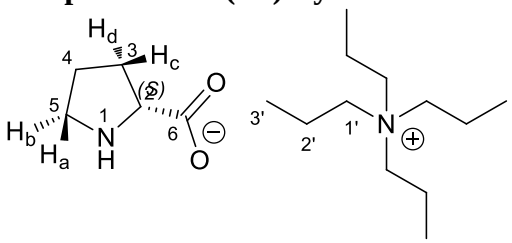
yd280902


3.61
3.60
3.59
3.57
3.05
3.03
3.02
3.01
2.86
2.85
2.84
2.83
2.82
2.06
2.05
2.04
2.03
2.03
2.02
1.69
1.68
1.68
1.50
1.48
1.46
1.21
1.19
1.18
1.16
0.79
0.78
0.76
Hd
Hc
Hb
Ha
N
H
O
O
2a
4'
3'
2'
1'
N
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

YD


178.82
61.23
57.98
45.87
29.84
24.38
23.02
19.05
12.72
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

Preparation of (2*R*)-Pyrrolidine-2-carboxylate tetrapropylazanuide (IL 2b):



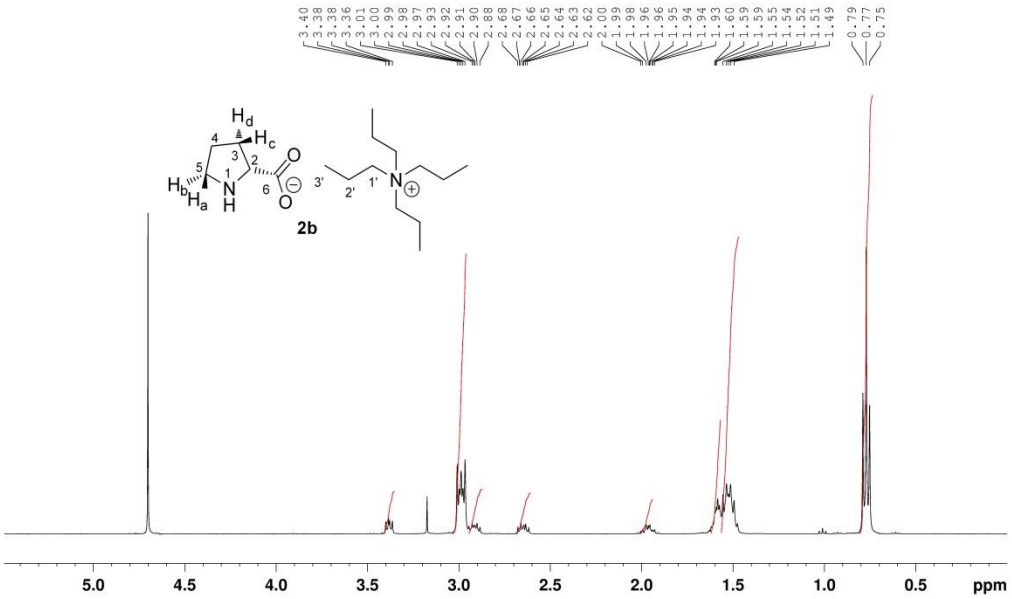
A 50 mL RB flask was charged with D–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetrapropyl ammonium hydroxide solution (6.83 g, 7.56 mmol and 6.67 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **2b** as yellow oil at RT in 90% yield (2.04 g, 6.81 mmol).

Chemical Formula: $C_{17}H_{32}N_2O_2$

Molecular Weight: 296.45 g/mol

$[\alpha]_D^{20}$ = +32.6 (0.58c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.38 (q, J = 6.0 Hz, 1H, *H2*), 2.98 (q, J = 8.0 Hz 8H, *H1'*), 2.93–2.88 (m, 1H, $H5_a$), 2.68–2.62 (m, 1H, $H5_b$), 2.00–1.93 (m, 1H, $H3_c$), 1.61–1.59 (m, 3H, $H3_d$, *4*), 1.52 (tq, J = 7.6, 7.2 Hz, 8H, *H2'*), 0.76 (t, J = 7.2 Hz, 12H, *H3'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.84 (CO, C_6), 61.31 (NHCH, C_2), 59.72 (NCH_2, *C1'*), 45.86 ($NHCH_2$, *C5*), 30.31(CH_2, *C3*), 24.75 (CH_2, *C4*), 14.68 (CH_2, *C2'*), 9.68 (CH_3, *C3'*). IR (neat, cm^{-1}): 3301, 2969, 2879, 2811, 1587, 1488, 1460, 1377, 1295, 1169, 1040, 970. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2210. Water Content = 1.18%. T_g = –46.2 °C. T_d = 199.1°C.

YD-0011


3.40
3.38
3.38
3.36
3.01
3.00
2.99
2.98
2.97
2.93
2.92
2.91
2.90
2.88
2.68
2.67
2.66
2.65
2.64
2.63
2.62
2.00
1.99
1.98
1.96
1.96
1.95
1.94
1.94
1.93
1.60
1.59
1.59
1.55
1.54
1.52
1.51
1.49
0.79
0.77
0.75
Hd
Hc
Hb
Ha
N
H
O
O
3'
2'
1'
N
2b
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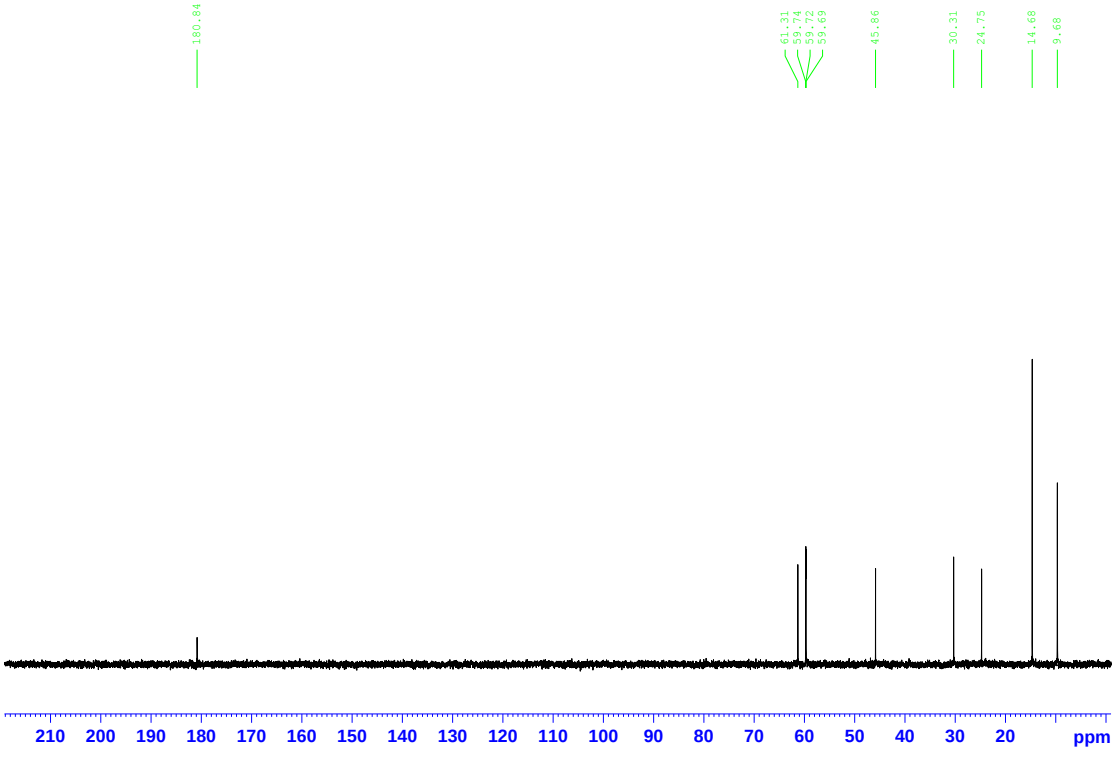
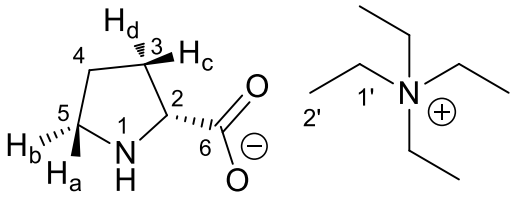
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm


180.84
61.31
59.74
59.72
59.69
45.86
30.31
24.75
14.68
9.68
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

5

Preparation of (2*R*)-Pyrrolidine-2-carboxylate tetraethylazanuide (IL 2c):



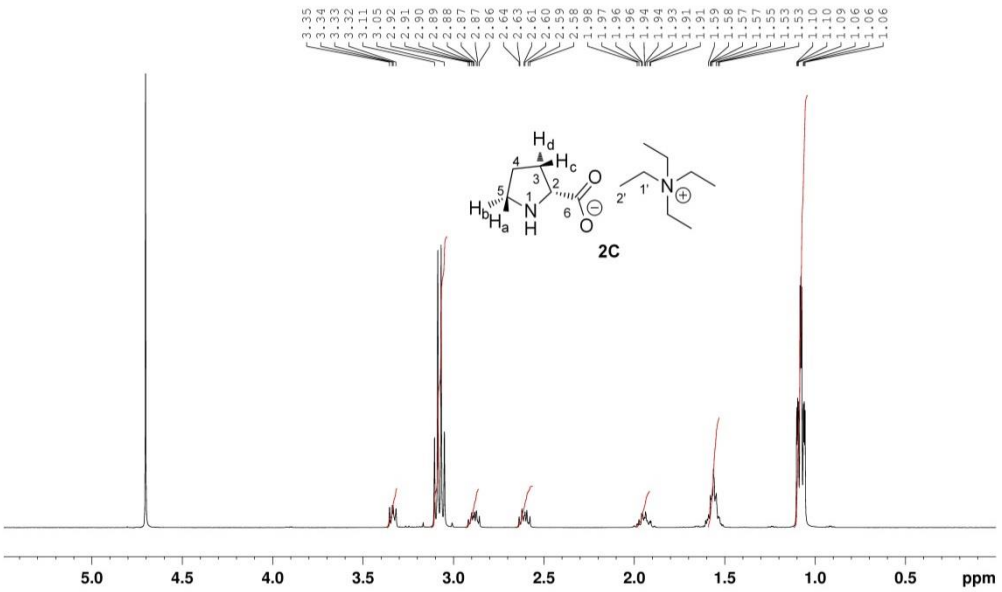
A 50 mL RB flask was charged with D–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetraethyl ammonium hydroxide solution (3.18 g, 7.56 mmol and 3.11 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **2c** as transparent oil at RT in 89% yield (1.64 g, 6.73 mmol).

Chemical Formula: $C_{13}H_{24}N_2O_2$

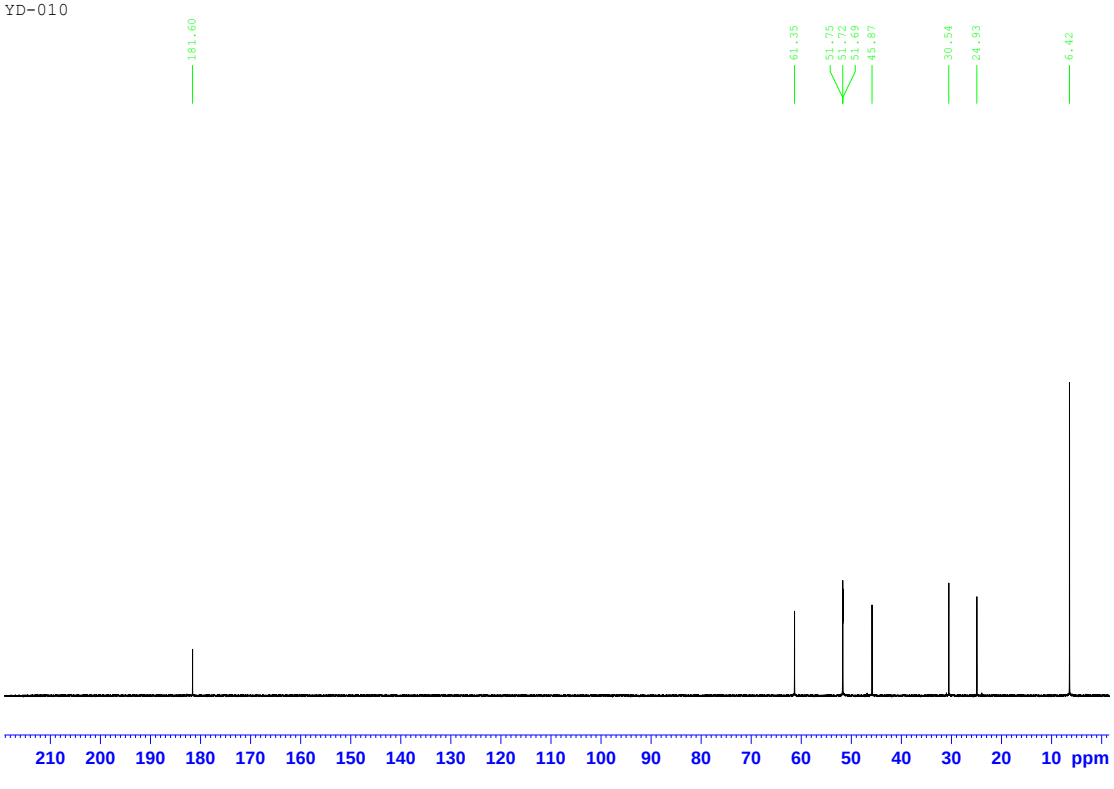
Molecular Weight: 240.34 g/mol

$[\alpha]_D^{20}$ = +28.9 (0.50c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.34 (q, J = 6.0 Hz, 1H, *H2*), 3.07 (q, J = 8.0 Hz, 8H, *H1'*), 2.92–2.86 (m, 1H, $H5_a$), 2.64–2.58 (m, 1H, $H5_b$), 1.98–1.91 (m, 1H, $H3_c$), 1.59–1.53 (m, 3H, $H3_d$, *4*), 1.07 (tq, J = 7.6, 7.2 Hz, 12H, *H2'*).^{13}C NMR (100 MHz, D_2O, δ): 181.60 (CO, *C6*), 61.35 (NHCH, *C2*), 51.72 (NCH_2, *C1'*), 45.87 ($NHCH_2$, *C5*), 30.54 (CH_2, *C3*), 24.93 (CH_2, *C4*), 6.42 (CH_3, *C2'*). IR (neat, cm^{-1}): 3302, 2954, 2870, 1581, 1486, 1456, 1307, 1377, 1174, 1097, 1002, 784. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1586. Water Content = 1.75%. T_g = –36.1 °C. T_d = 199.0 °C.

YD-010

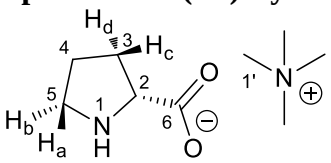


YD-010



5

Preparation of (2*R*)-Pyrrolidine-2-carboxylate tetramethylazanuide (IL 2d):



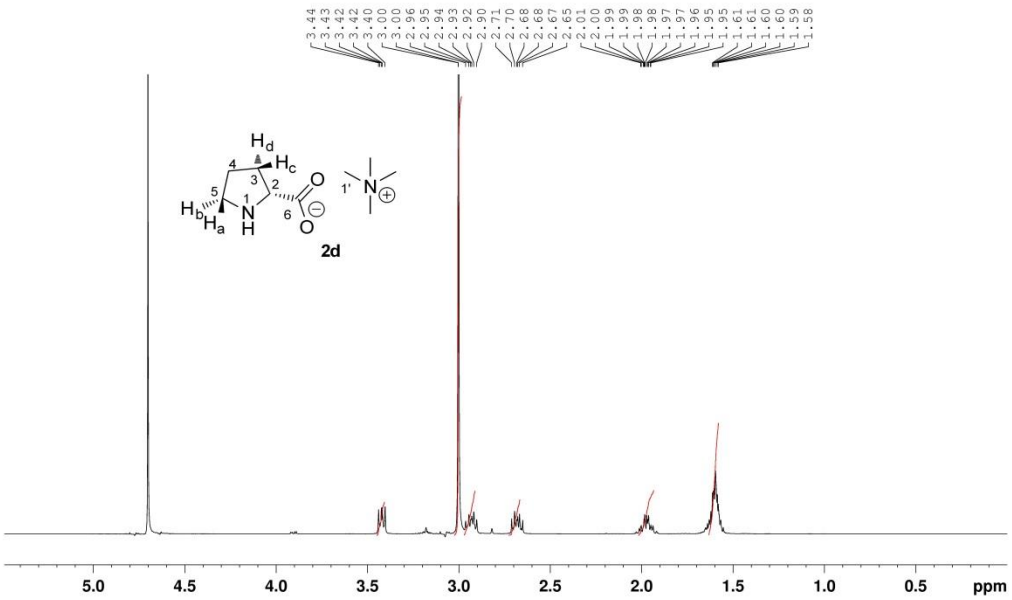
A 50 mL RB flask was charged with D–proline (1.00 g, 8.70 mmol) and distilled water (15 mL). To this solution was added tetramethyl ammonium hydroxide pentahydrate (1.37 g, 7.56 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **2d** as light yellow oil at RT in 86% yield (1.22 g, 6.65 mmol).

Chemical Formula: $C_9H_{16}N_2O_2$

Molecular Weight: 184.24 g/mol

$[\alpha]_D^{20}$ = +30.4 (0.55c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 3.43 (q, J = 6.0 Hz, 1H, *H2*), 3.00 (s, 12H, *H1'*), 2.96–2.90 (m, 1H, $H5_a$), 2.71–2.65 (m, 1H, $H5_b$), 2.00–1.95 (m, 1H, $H3_c$), 1.61–1.58 (m, 3H, $H3_d$, *4*). ^{13}C NMR (100 MHz, D_2O, δ): 180.45 (CO, *C6*), 61.34 (NHCH, *C2*), 55.12 (NCH_3, *C1'*), 46.89 ($NHCH_2$, *C5*), 30.36 (CH_2, *C3*), 24.70 (CH_2, *C2*). IR (neat, cm^{-1}): 3282, 3031, 2958, 2874, 2819, 1581, 1489, 1378, 1293, 1200, 1033, 950. HRMS (ESI^-, *m/z*): $C_5H_8NO_2^-$, calculated = 114.0561, found = 114.0559, (ESI^+, *m/z*): $C_4H_{12}N^+$, calculated = 74.0964, found = 74.0962. Water Content = 0.61%. T_g = –49.5 ºC. T_d = 255.5 ºC.

dy012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1.97
1.97
1.96
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1.95
1.61
1.61
1.60
1.60
1.59
1.58
Hd
Hc
Hb
Ha
N
H
O
O
1'
N
2d
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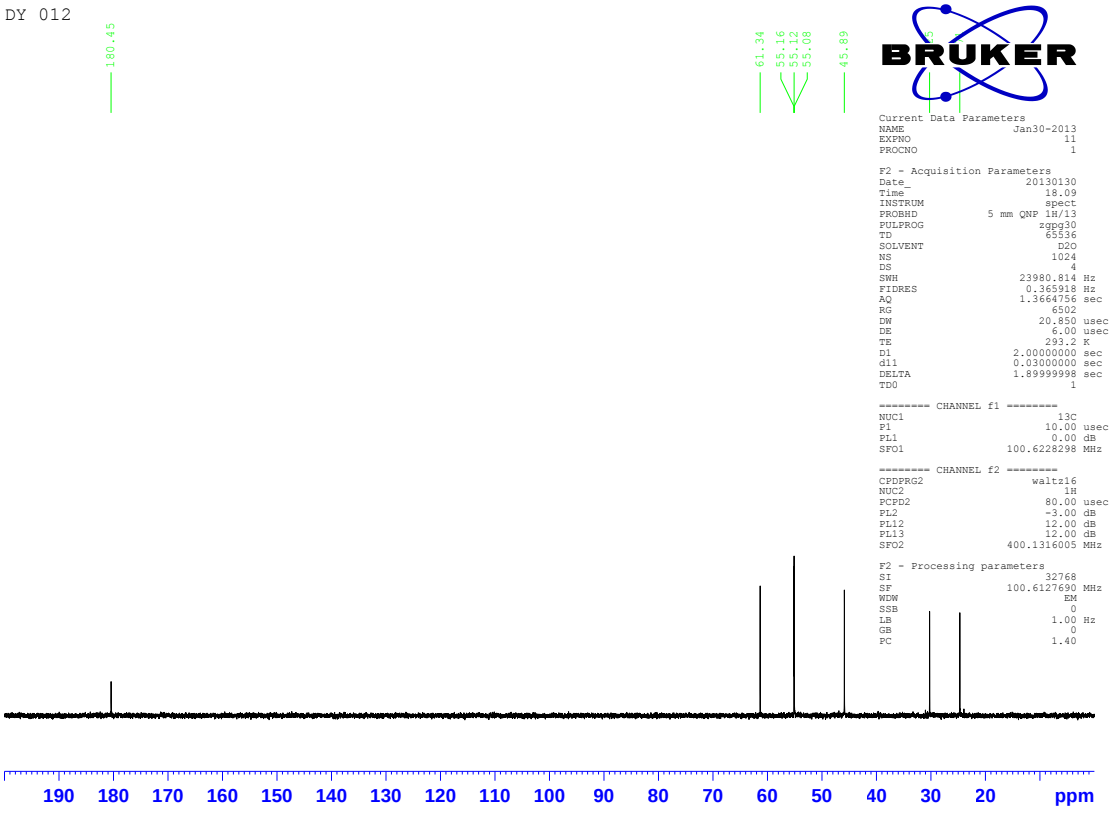
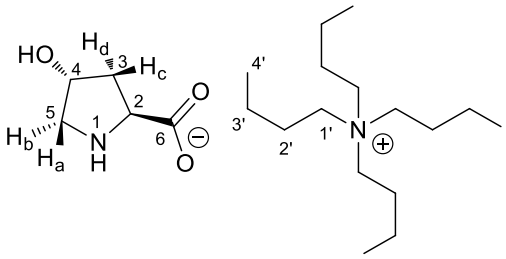
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

DY 012


180.45
61.34
55.16
55.12
55.08
45.89
BRUKER
Current Data Parameters
NAME Jan30-2013
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130130
Time 18.09
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 1024
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 6502
DW 20.850 usec
DE 6.00 usec
TE 293.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 0.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 12.00 dB
PL13 12.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm

Preparation of (*2S, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetrabutylazanuide (IL 3a):



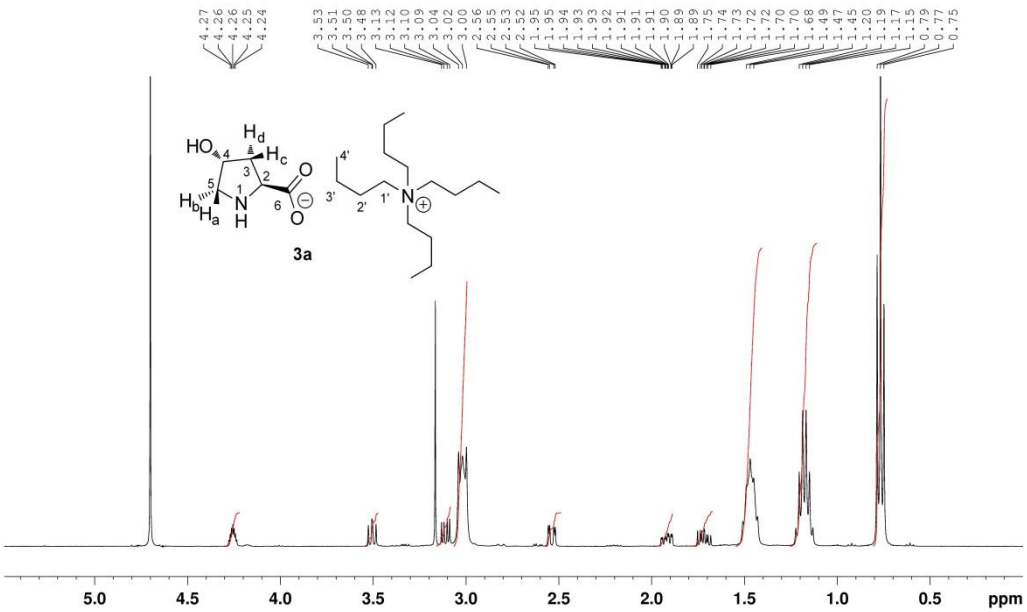
A 50 mL RB flask was charged with *trans*-4-hydroxy-L–proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrabutyl ammonium hydroxide solution (3.13 g, 6.61 mmol and 3.16 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **3a** as transparent oil at RT in 87% yield (2.15 g, 5.77 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_3$

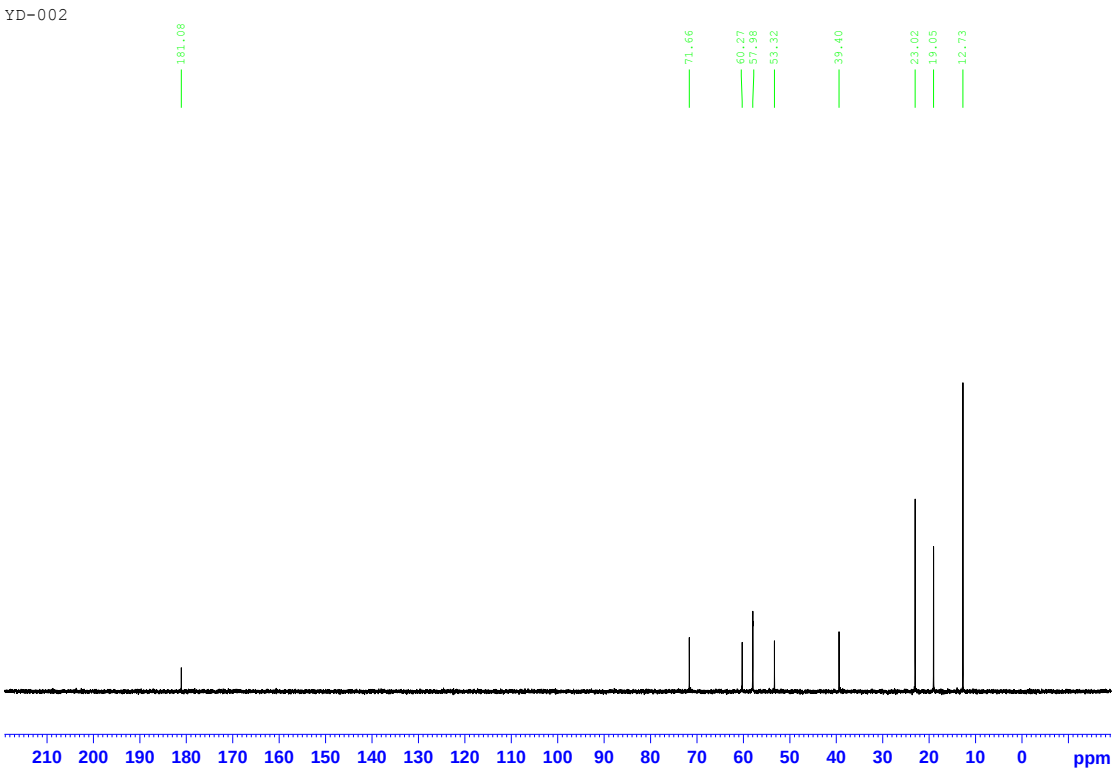
Molecular Weight: 368.55 g/mol

$[\alpha]_D^{20}$ = –32.7 (0.47c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.27–4.24 (m, 1H, *H2*), 3.50 (q, J = 7.6 Hz, 1H, *H4*), 3.11 (dd, J = 6.0, 5.6 Hz, 1H, $H5_a$), 3.02 (t, J = 7.2 Hz 8H, *H1'*), 2.53 (dd, J = 6.0, 5.6 Hz, 1H, $H5_b$), 1.95–1.89 (m, 1H, $H3_c$), 1.75–1.68 (m, 1H, $H3_d$), 1.48–1.45 (m, 8H, *H2'*), 1.17 (tq, J = 7.6, 7.2 Hz, 8H, *H3'*), 0.77 (t, J = 7.2 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.08 (CO, *C6*), 71.66 (CHOH, *C4*), 60.27 (NHCH, *C2*), 57.98 (NCH_2, *C1'*), 53.32 ($NHCH_2$, *C5*), 39.40 (CH_2, *C3*), 23.02 (CH_2, *C2'*) 19.05 (CH_2, *C3'*), 12.73 (CH_3, *C4'*). IR (neat, cm^{-1}): 3229, 2960, 2935, 2874, 1587, 1487, 1283, 1170, 1091, 1040, 968, 881. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*) $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2834. Water Content = 0.97%. T_g = –36.4 °C. T_d = 217.8 °C.

YD280901

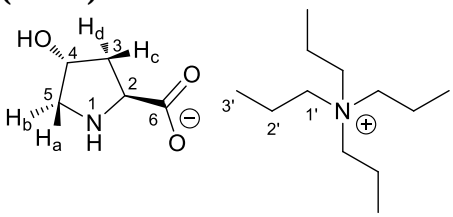


YD-002



5

Preparation of (*2S, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetrapropylazanuide (IL 3b):



Procedure:

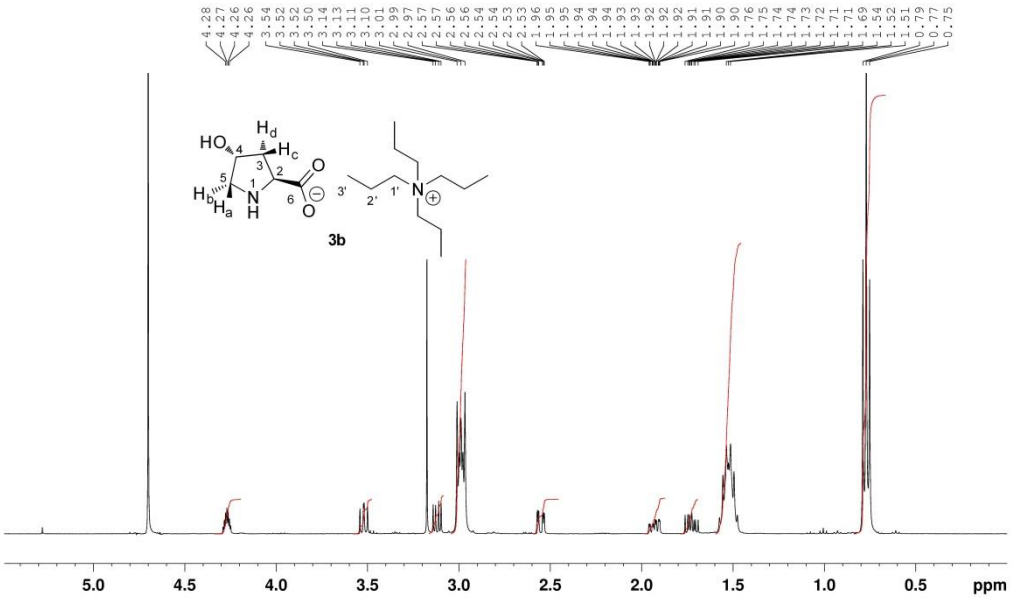
A 50 mL RB flask was charged with *trans*-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrapropylammonium hydroxide solution (5.98 g, 6.61 mmol and 5.84 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **3b** as yellow oil at RT in 92% yield (1.93 g, 6.09 mmol).

Chemical Formula: $C_{17}H_{32}N_2O_3$

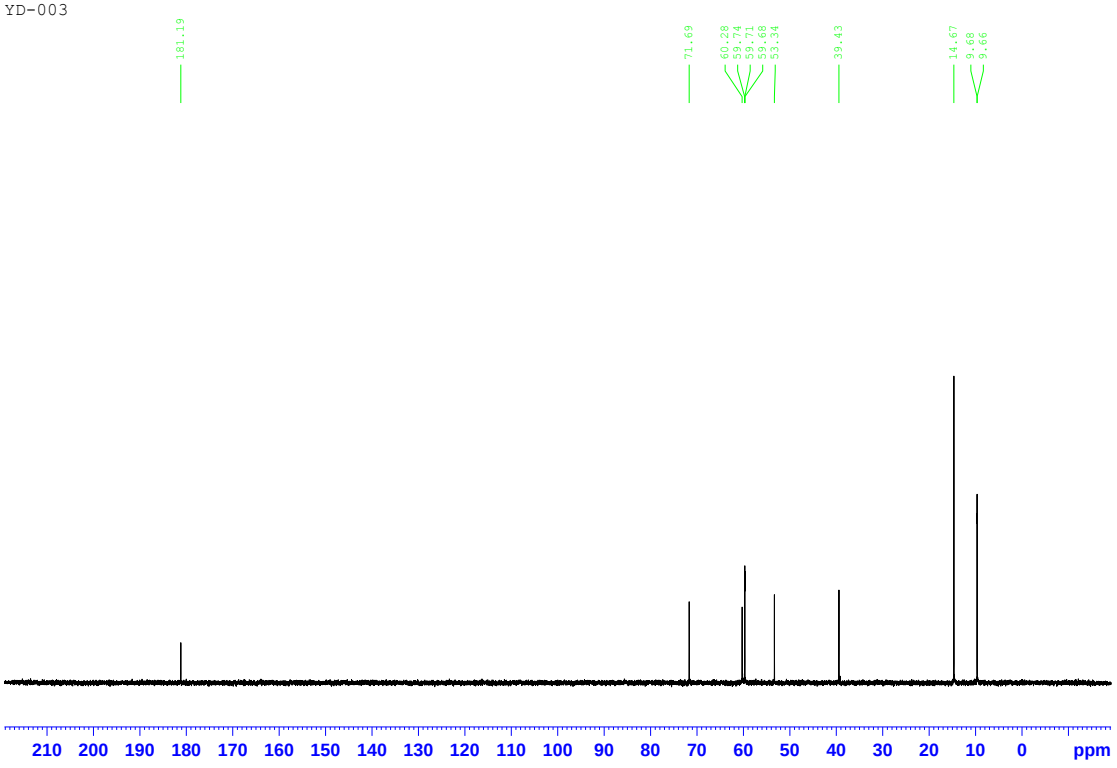
Molecular Weight: 312.45 g/mol

$[\alpha]_D^{20}$ = –24.2 (0.40c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.26 (q, J = 5.6 Hz, 1H, *H2*), 3.52 (q, J = 7.6 Hz, 1H, *H4*), 3.13 (dd, J = 5.6, 5.2 Hz, 1H, *H5$_a$*), 2.98 (t, J = 8.0 Hz, 8H, *H1'*), 2.55 (dd, J = 5.6, 5.2 Hz, 1H, *H5$_b$*), 1.96-1.90 (m, 1H, *H3$_c$*), 1.76–1.70 (m, 1H, *H3$_d$*), 1.52 (tq, J = 7.6, 7,2 Hz, 8H, *H2'*), 0.77 (t, J = 7.6 Hz, 12H, *H3'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.19 (CO, *C6*), 71.69 (CHOH, *C4*), 60.28 (NHCH, *C2*), 59.71 (NCH_2, *C1'*), 53.34 ($NHCH_2$, *C5*), 39.43 (CH_2, *C3*), 14.67 (CH_2, *C2'*), 9.67 (CH_3, *C3'*). IR (neat, cm^{-1}): 3238, 2971, 2938, 2880, 1585, 1475, 1461, 1376, 1284, 1173, 1089, 989, 757. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2210. Water Content by = 1.33%. T_g = –49.4 °C. T_d = 211.2 °C.

YD280903

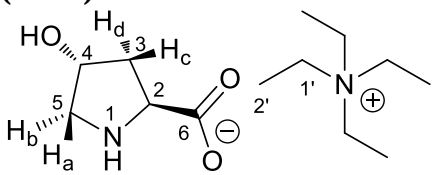


YD-003



5

Preparation of (*2S, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetraethylazanuide (IL 3c):



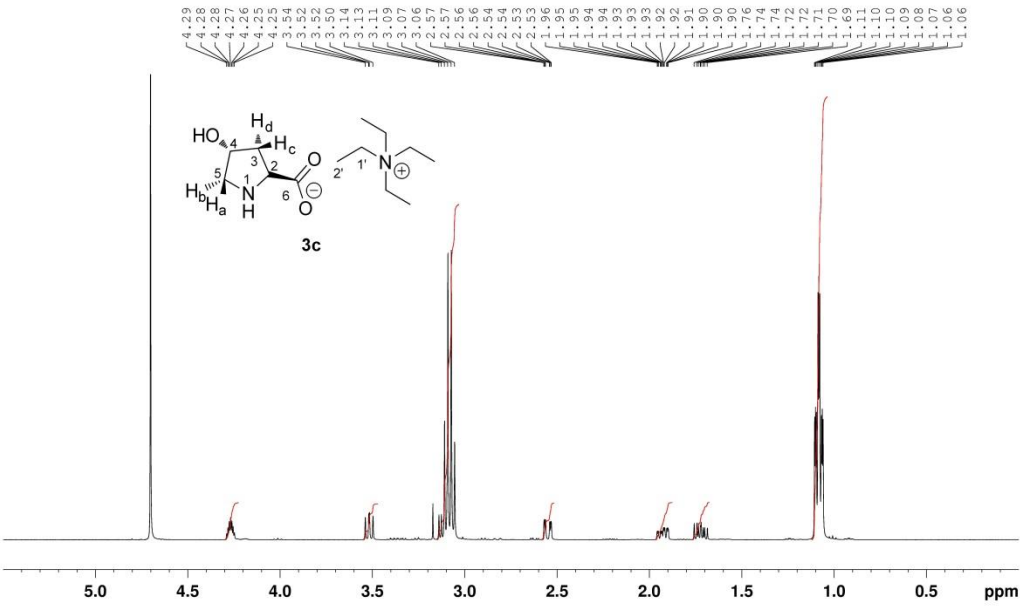
A 50 mL RB flask was charged with *trans*-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetraethyl ammonium hydroxide solution (2.79 g, 6.61 mmol and 2.73 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **3c** as orange oil at RT in 91% yield (1.47 g, 6.01 mmol).

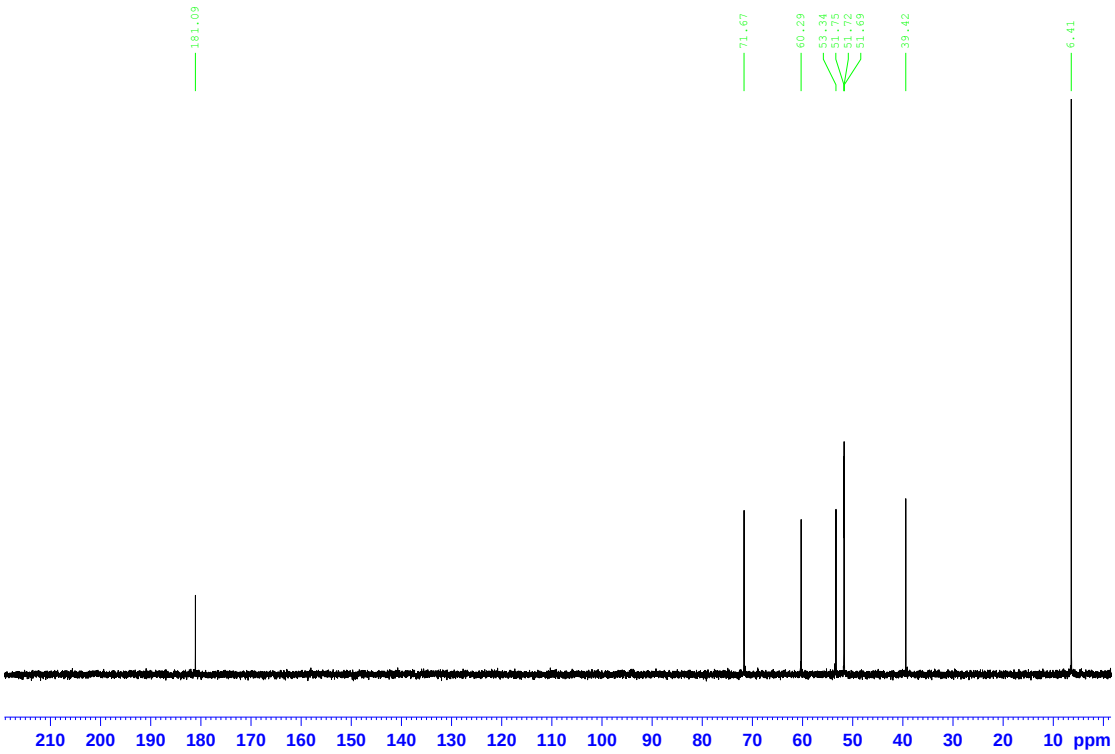
Chemical Formula: $C_{13}H_{24}N_2O_3$

Molecular Weight: 256.34 g/mol

$[\alpha]_D^{20}$ = –31.5 (0.48c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.29–4.25 (m, 1H, *H2*), 3.52 (q, J = 7.2 Hz, 1H, *H4*), 3.12 (dd, J = 5.2, 5.6 Hz 1H, $H5_a$), 3.06 (q, J = 7.6 Hz 8H, *H1'*), 2.54 (dd, J = 5.2, 5.6 Hz, 1H, $H5_b$), 1.96–1.90 (m, 1H, $H3_c$), 1.76–1.69 (m, 1H, $H3_d$), 1.08 (tq, J = 7.6, 7.2 Hz, 12H, *H2'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.09 (CO, *C6*), 71.67 (CHOH, *C4*), 60.29 (NHCH, *C2*), 53.34 (NCH_2, *C1'*), 51.72 ($NHCH_2$, *C5*), 39.42 (CH_2, *C3*), 6.41 (CH_2, *C2'*). IR (neat, cm^{-1}): 3279, 2982, 1584, 1486, 1455, 1374, 1173, 1090, 1002, 967, 839, 785. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.05210, found = 130.0506, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1586. Water Content = 1.65%. T_g = –48.5 ºC. T_d = 191.7 ºC.

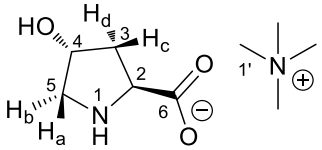
YD-009


4.29 4.28 4.28 4.27 4.26 4.25 4.25 3.54 3.52 3.52 3.50 3.14 3.13 3.11 3.09 3.07 3.06 2.57 2.56 2.56 2.54 2.54 2.53 2.53 1.96 1.95 1.95 1.94 1.94 1.93 1.93 1.93 1.92 1.92 1.91 1.90 1.90 1.90 1.76 1.74 1.74 1.72 1.72 1.71 1.70 1.69 1.11 1.10 1.10 1.09 1.08 1.07 1.06 1.06
HO
4
H d
H c
3
2
O
5
1
2'
1'
N
H b
H a
N H
6
O
3c
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


181.09
71.67
60.29
53.34
51.75
51.72
51.69
39.42
6.41
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

5

Preparation of (*2S, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetramethylazanuide (IL 3d):



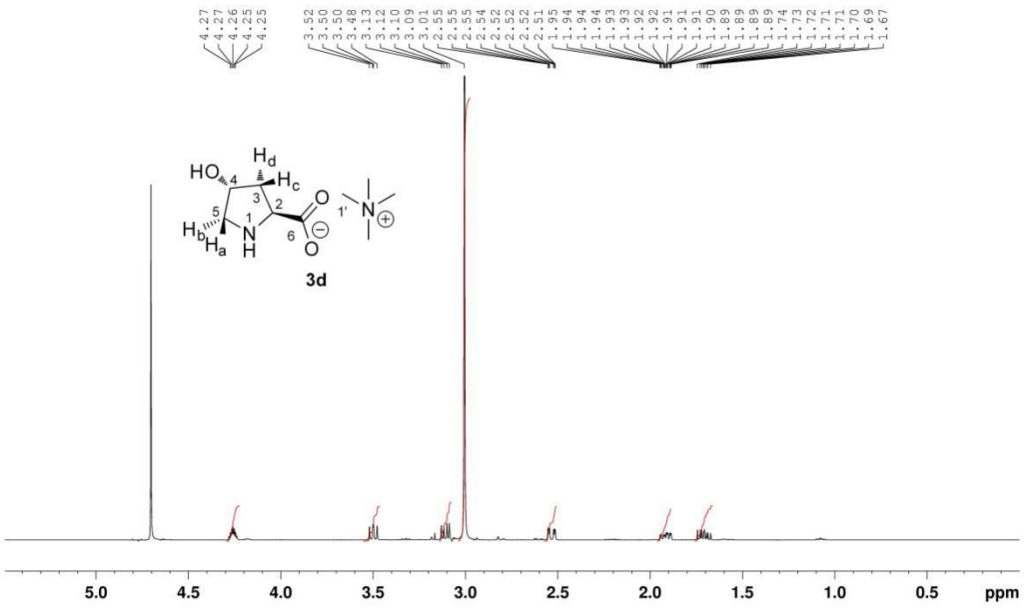
A 50 mL RB flask was charged with *trans*-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetramethyl ammonium hydroxide pentahydrate (1.20 g, 6.61 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to light yield **3d** as orange oil at RT in 90% yield (1.21 g, 6.01 mmol).

Chemical Formula: $C_9H_{16}N_2O_3$

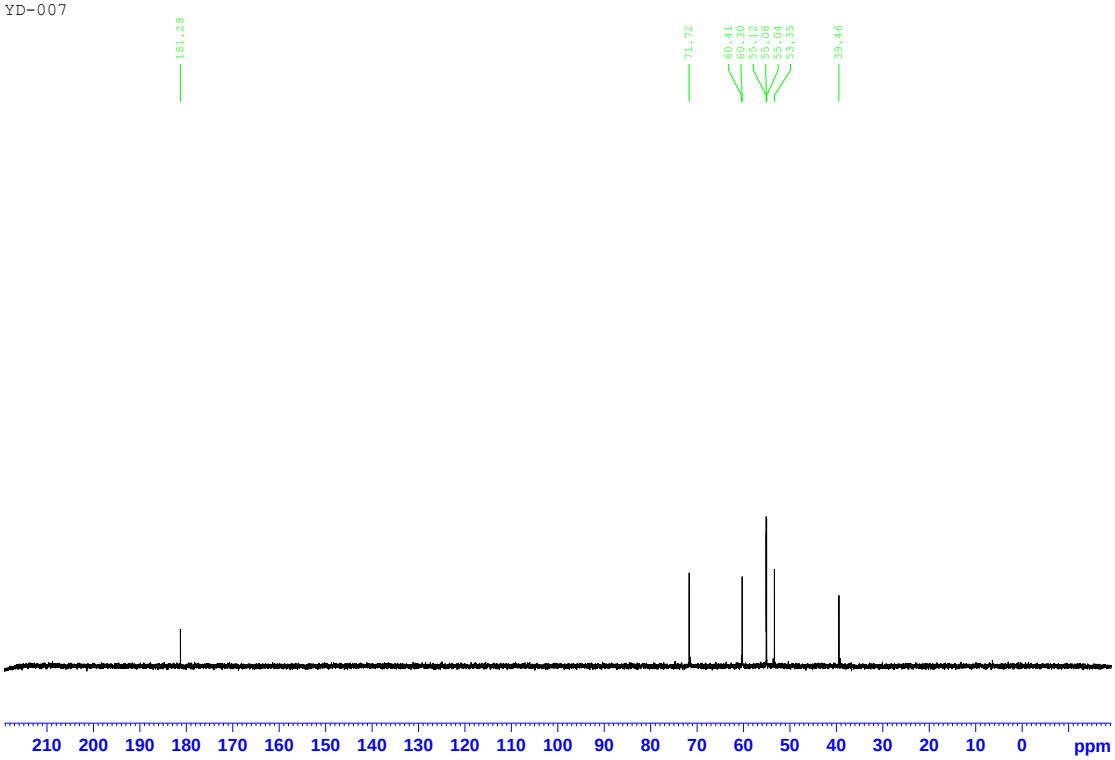
Molecular Weight: 200.23 g/mol

$[\alpha]_D^{20}$ = –32.4 (0.35c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.27–4.24 (m, 1H, *H2*), 3.50 (q, J = 7.6 Hz, 1H, *H4*), 3.10 (dd, J = 5.6, 5.2 Hz, 1H, $H5_a$), 3.00 (s, 12H, *H1'*), 2.53 (dd, J = 5.6, 5.2 Hz 1H, $H5_b$), 1.95–1.89 (m, 1H, $H3_c$), 1.74–1.67 (m, 1H, $H3_d$). ^{13}C NMR (100 MHz, D_2O, δ): 181.28 (CO, *C6*), 71.72 (CHOH, *C4*), 60.35 (NHCH, *C2*), 55.08 (NCH_3, *C1'*), 53.35 ($NHCH_2$, *C5*), 39.46 (CH_2, *C3*). IR (neat, cm^{-1}): 3257, 3030, 2928, 2219, 1581, 1489, 1377, 1289, 1207, 1179, 1090, 1032. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0501, found = 130.0506, (ESI^+, *m/z*): $C_4H_{12}N^+$, calculated = 74.0964, found = 74.0962. Water Content = 1.51%. T_g = –49.4 °C. T_d = 252.5 °C.

dy007

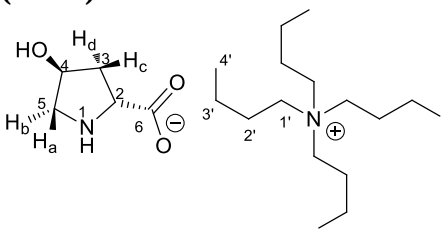


YD-007



5

Preparation of (*2R, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetrabutylazanuide (IL 4a):



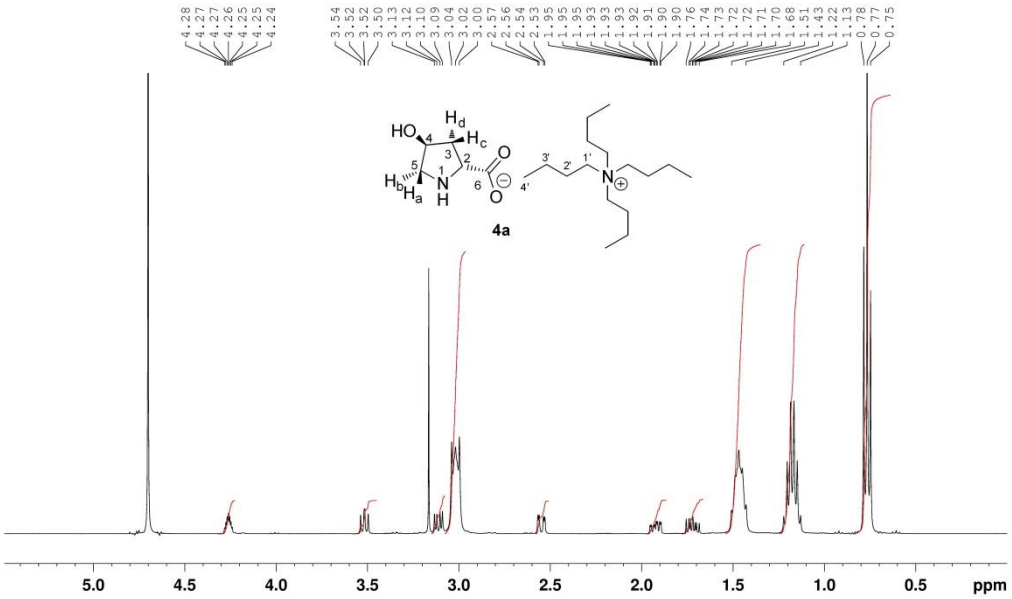
A 50 mL RB flask was charged with *trans*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrabutylammonium hydroxide solution (1.72 g, 6.61 mmol and 3.12 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **4a** as light brown oil at RT in 84% yield (1.76 g, 5.55 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_3$

Molecular Weight: 368.55 g/mol

$[\alpha]_D^{20}$ = +30.8 (0.47c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.28–4.24 (m, 1H, *H2*), 3.52 (t, J = 8.0 Hz, 1H, *H4*), 3.51 (dd, J = 5.2, 5.2 Hz, 1H, $H5_a$), 3.02 (t, J = 8.0 Hz, 8H, *H1'*), 2.54 (dd, J = 2.4, 2.4 Hz, 1H, $H5_b$), 1.95–1.90 (m, 1H, $H3_c$), 1.75–1.69 (m, 1H, $H3_d$), 1.50–1.42 (m, 8H, *H2'*), 1.21 (tq, 8H, J = 7.2, 7.6 Hz, *H3'*), 0.76 (t, J = 7.6 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.35 (CO, *C6*), 61.31 (CHOH, *C4*), 57.96 (NHCH, *C2*), 45.85 (NCH_2, *C1'*), 30.44 ($NHCH_2$, *C5*), 25.35 (CH_2, *C3*), 23.01 (CH_2, *C2'*), 19.04 (CH_2, *C3'*), 6.73 (CH_3, *C4'*). IR (neat, cm^{-1}): 3268, 2890, 2830, 1684, 1574, 1378, 1366, 1250, 988, 945, 854, 756. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2833. Water Content = 1.17%. T_g = –47.4 °C. T_d = 199.5 °C.

DY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3.54 3.52 3.52 3.50 3.13 3.12 3.10 3.09 3.04 3.02 3.00 2.57 2.56 2.54 2.53 1.95 1.95 1.93 1.93 1.93 1.92 1.91 1.90 1.90 1.76 1.74 1.73 1.72 1.72 1.71 1.70 1.68 1.51 1.43 1.22 1.13 0.78 0.77 0.75
HO
Hd
Hc
Hb
Ha
N
H
O
O
4a
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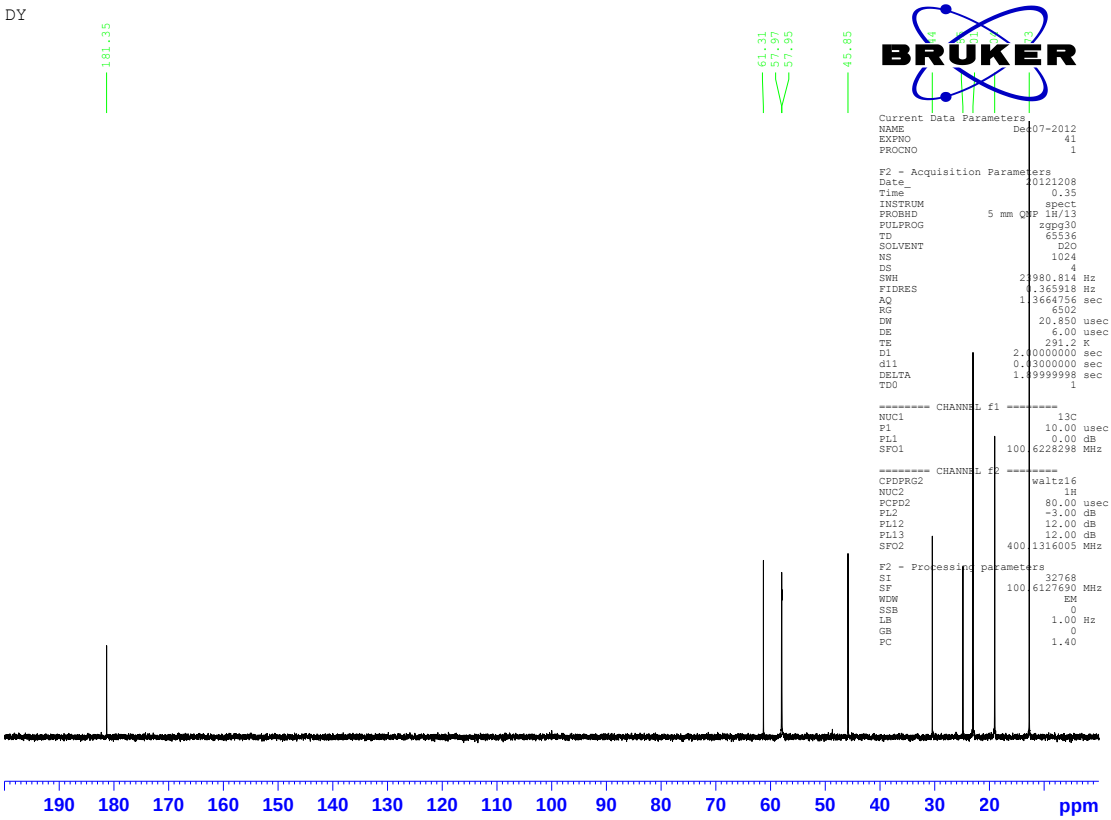
DY


181.35
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57.97
57.95
45.85
BRUKER
Current Data Parameters
NAME De107-2012
EXPNO 41
PROCNO 1
F2 - Acquisition Parameters
Date_ 20121208
Time 0.35
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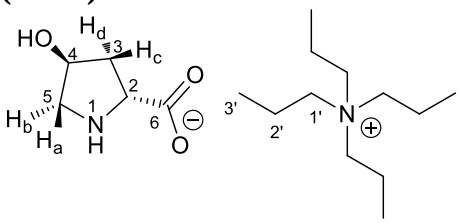
SOLVENT D2O
NS 1024
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 6502
DW 20.850 usec
DE 6.00 usec
TE 291.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 0.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 12.00 dB
PL13 12.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

Preparation of (*2R, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetrapropylazanuide (IL 4b):



Procedure:

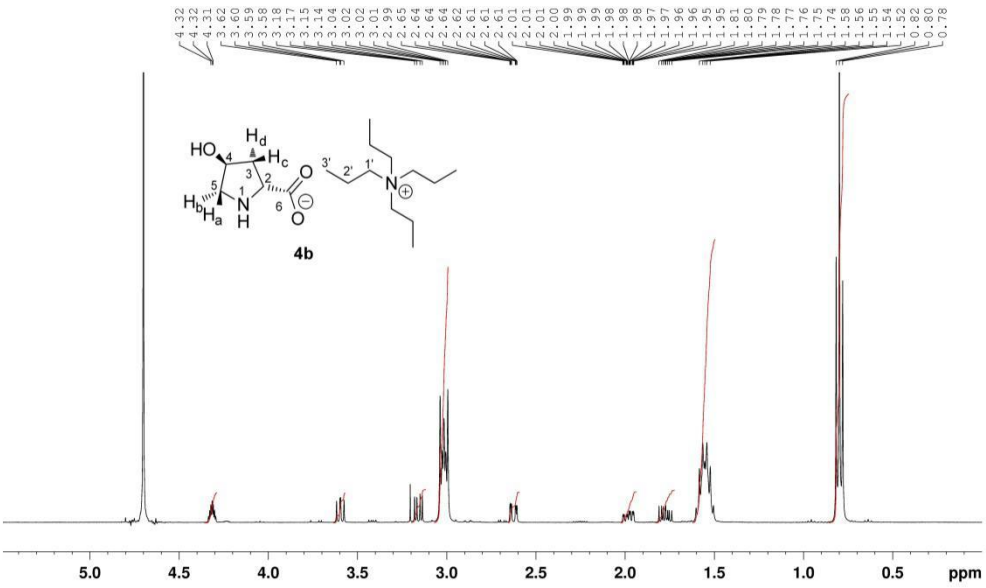
A 50 mL RB flask was charged with *trans*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrapropylammonium hydroxide solution (5.98 g, 6.61 mmol and 5.84 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **4b** as light brown oil at RT in 84% yield (1.76 g, 5.55 mmol).

Chemical Formula: $C_{17}H_{32}N_2O_3$

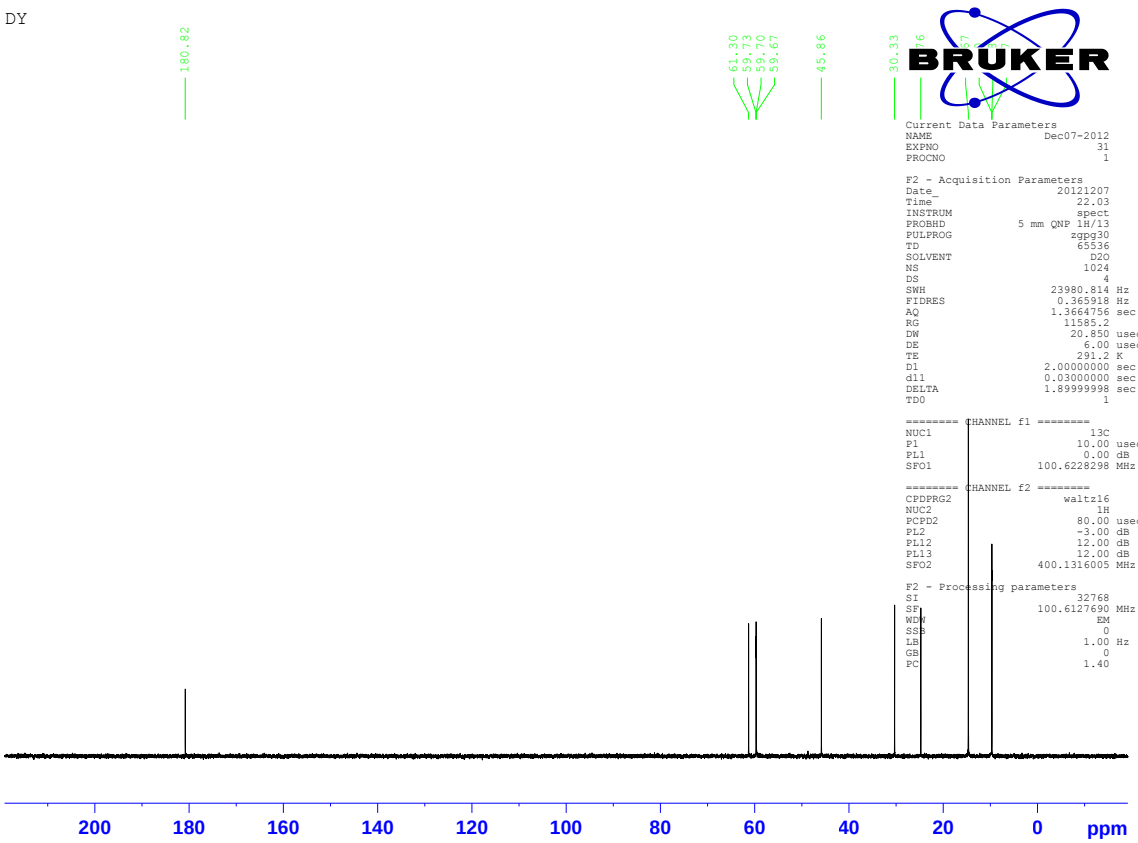
Molecular Weight: 312.45 g/mol

$[\alpha]_D^{20}$ = +22.8 (0.40c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.31 (t, J = 6.8, 1H, *H2*), 3.60 (q, J = 7.6 Hz, 1H, *H4*), 3.15 (dd, J = 5.2, 5.2 Hz, 1H, $H5_a$), 3.01 (t, J = 7.6 Hz, 8H, *H1'*), 2.62 (dd, J = 4.0, 4.0 Hz, 1H, $H5_b$), 2.01–1.95 (m, 1H, $H3_c$), 1.81–1.74 (m, 1H, $H3_d$), 1.55 (tq, J = 7.6, 7.2 Hz, 8H, *H2'*), 0.80 (t, J = 7.2 Hz, 12H, *H3'*) ^{13}C NMR (100 MHz, D_2O, δ): 180.82 (CO, *C6*), 61.30 (CHOH, *C4*), 59.70 (NHCH, *C2*), 45.86 (NCH_2, *C1'*), 30.33 ($NHCH_2$, *C5*), 24.76 (CH_2, *C3*), 16.67 (CH_2, *C2'*), 9.78 (CH_3, *C3'*). IR (neat, cm^{-1}): 3252, 2971, 2880, 1584, 1474, 1378, 1331, 1207, 1038, 969, 844, 757. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2209. Water Content = 1.70%. T_g = –46.4 °C. T_d = 208.8 °C.

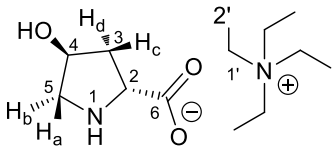
DY-0022


4.32 4.32 4.31 3.62 3.60 3.59 3.58 3.18 3.17 3.15 3.14 3.04 3.02 3.01 2.99 2.65 2.64 2.64 2.62 2.61 2.61 2.01 2.01 2.00 1.99 1.99 1.98 1.98 1.97 1.96 1.95 1.81 1.80 1.79 1.78 1.77 1.76 1.75 1.74 1.58 1.56 1.55 1.54 1.52 0.82 0.80 0.78
HO
Hd
Hc
Hb
Ha
N
H
O
O
N
4b
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

DY


180.82
61.30
59.73
59.70
59.67
45.86
30.33
BRUKER
Current Data Parameters
NAME Dec07-2012
EXPNO 31
PROCNO 1
F2 - Acquisition Parameters
Date_ 20121207
Time 22.03
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 1024
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 11585.2
DW 20.850 usec
DE 6.00 usec
TE 291.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 0.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 12.00 dB
PL13 12.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

Preparation of (*2R, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetraethylazanuide (IL 4c):



Procedure:

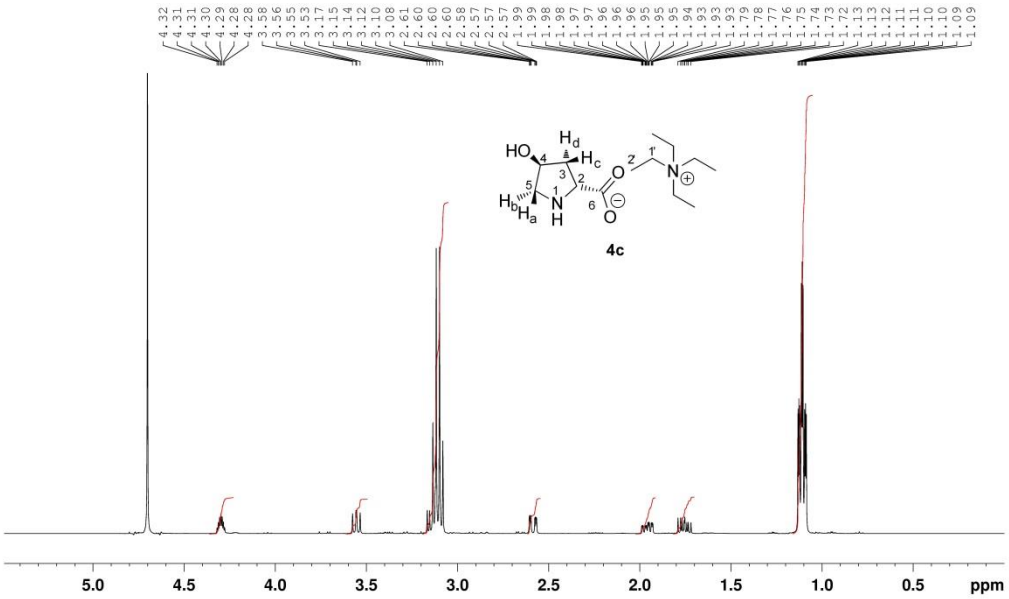
A 50 mL RB flask was charged with *trans*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetraethylammonium hydroxide solution (2.79 g, 6.61 mmol and 2.73 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **4c** as yellow oil at RT in 84% yield (1.44 g, 5.55 mmol).

Chemical Formula: $C_{12}H_{21}N_2O_3$

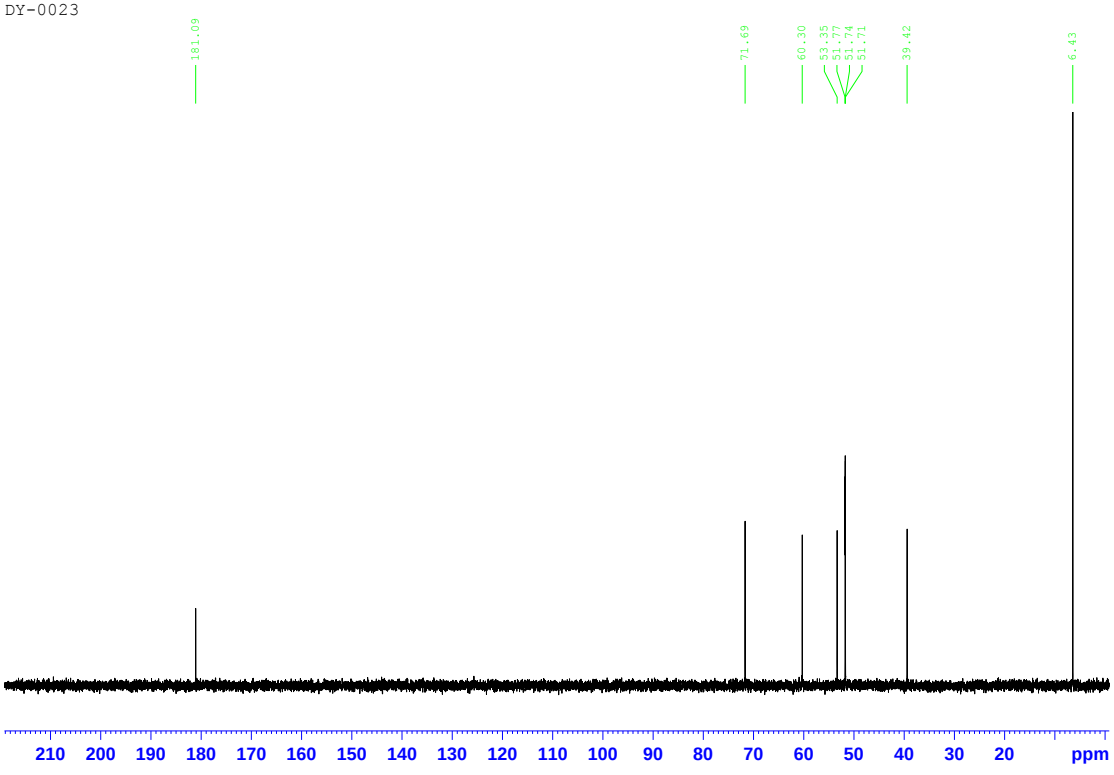
Molecular Weight: 241.31 g/mol

$[\alpha]_D^{20}$ = +29.6 (0.48c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.32–4.28 (m, 1H, *H2*), 3.56 (q, J = 7.6 Hz, 1H, *H4*), 3.15 (dd, 1H, J = 5.2, 5.2 Hz, $H5_a$), 3.10 (q, 8H, J = 7.6 Hz, *H1'*), 2.58 (dd, J = 2.8, 2.8 Hz, 1H, $H5_b$), 1.99–1.93 (m, 1H, $H3_c$), 1.79–1.72 (m, 1H, $H3_d$), 1.09 (tq, J = 7.6, 7.2 Hz, 12H, *H2'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.09 (CO, *C6*), 71.69 (CHOH, *C4*), 60.30 (NHCH, *C2*), 53.35 (NCH_2, *C1'*), 51.74 ($NHCH_2$, *C5*), 39.42 (CH_2, *C3*), 6.43 (CH_3, *C2'*). IR (neat, cm^{-1}): 3210, 2982, 2932, 1585, 1485, 1374, 1173, 1085, 1002, 966, 785, 687. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1586. Water Content = 1.65%. T_g = –46.2 °C. T_d = 192.1 °C

DY-0023

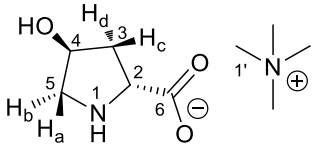


DY-0023



5

Preparation of (*2R, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetramethylazanuide (IL 4d):



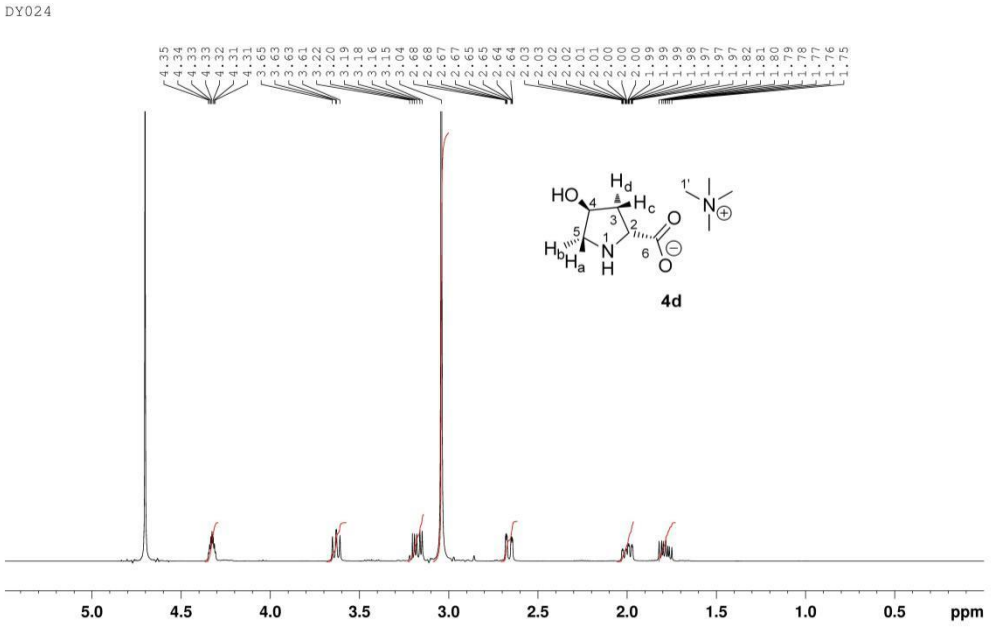
A 50 mL RB flask was charged with *trans*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetramethylammonium hydroxide pentahydrate (1.20 g, 6.61 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **4d** as yellow oil at RT in 90% yield (1.18 g, 5.71 mmol).

Chemical Formula: $C_9H_{16}N_2O_3$

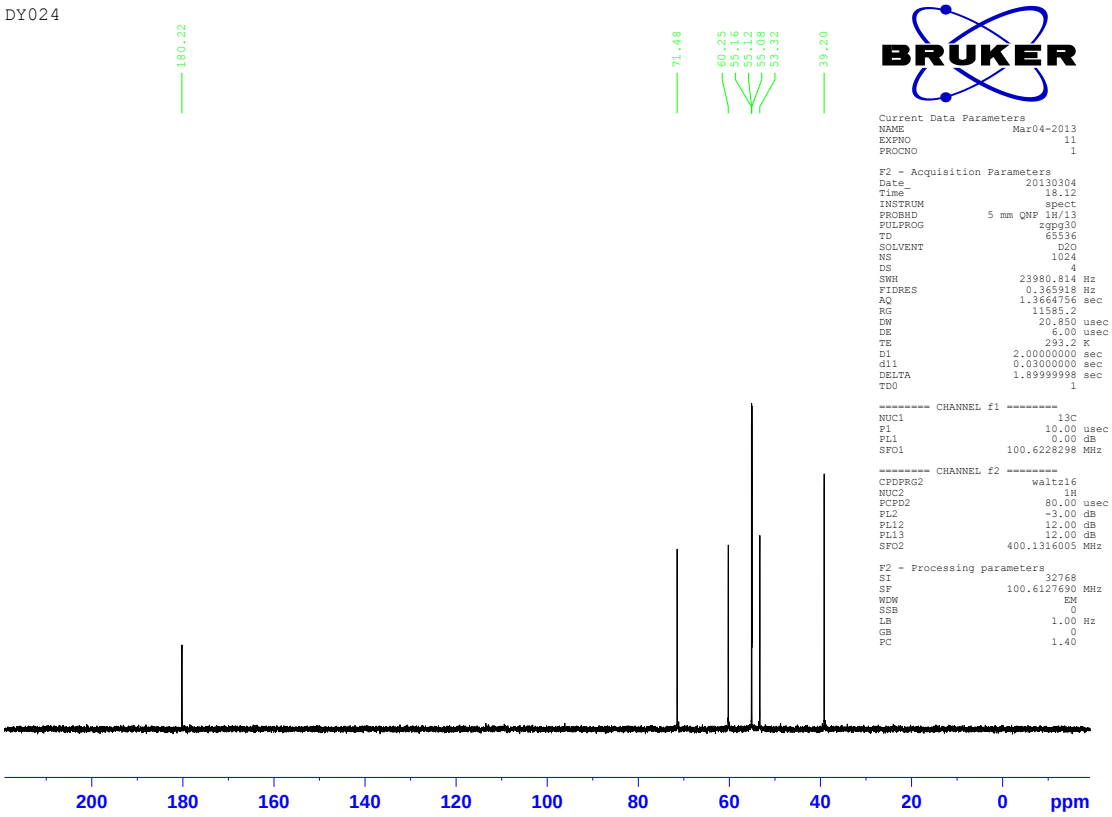
Molecular Weight: 200.23 g/mol

$[\alpha]_D^{20}$ = +33.9 (0.35c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.35–4.31 (m, 1H, *H2*), 3.63 (q, J = 7.6 Hz, 1H, *H4*), 3.22–3.16 (dd, 1H, $H5_a$), 3.04 (s, 12H), 2.68–2.64 (m, 1H, $H5_b$), 2.03–1.97 (m, 1H, $H3_c$), 1.79–1.75 (m, 1H, $H3_d$). ^{13}C NMR (100 MHz, D_2O, δ): 180.22 (CO, *C6*), 71.48 (CHOH, *C4*), 60.25 (NHCH, *C2*), 55.12 (NCH_2, *C1'*), 53.32 ($NHCH_2$, *C5*), 39.20 (CH_2, *C3*). IR (neat, cm^{-1}): 3195, 2978, 2900, 1613, 1485, 1374, 1154, 1097, 1002, 953, 735, 657. Water Content = 1.10%. T_g = –49.5 °C. T_d = 252.5 °C.

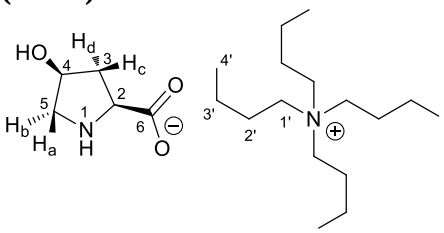
DY024


4.35 4.34 4.33 4.33 4.32 4.31 4.31 3.65 3.63 3.63 3.61 3.22 3.20 3.19 3.18 3.16 3.15 3.04 2.68 2.68 2.67 2.67 2.65 2.65 2.64 2.64 2.03 2.03 2.02 2.02 2.01 2.01 2.00 2.00 2.00 1.99 1.99 1.99 1.98 1.97 1.97 1.97 1.82 1.81 1.80 1.79 1.78 1.77 1.77 1.76 1.75
HO
Hd
Hc
Hb
Ha
N
H
1'
N
O
O
4d
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

DY024


180.22
71.48
60.25
55.16
55.12
55.08
53.32
39.20
BRUKER
Current Data Parameters
NAME Mar04-2013
EXPNO 11
PROCNO 1
F2 - Acquisition Parameters
Date_ 20130304
Time 18.12
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 1024
DS 4
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664756 sec
RG 11585.2
DW 20.850 usec
DE 6.00 usec
TE 293.2 K
D1 2.00000000 sec
d11 0.03000000 sec
DELTA 1.89999998 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.00 usec
PL1 0.00 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 -3.00 dB
PL12 12.00 dB
PL13 12.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6127690 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 180 160 140 120 100 80 60 40 20 0 ppm

Preparation of (*2S, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetrabutylazanuide (IL 5a):



Procedure:

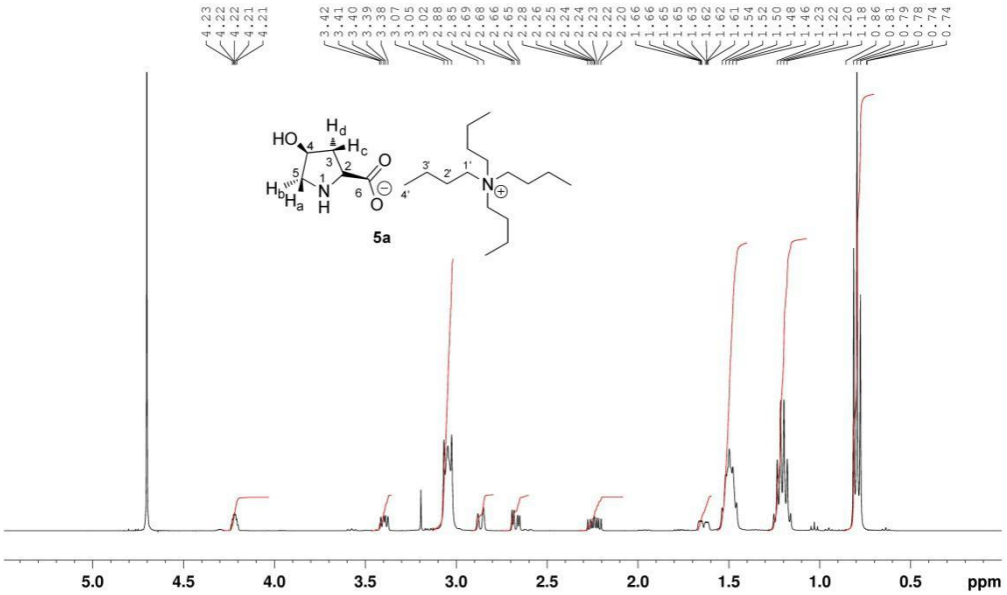
A 50 mL RB flask was charged with *cis*–4–hydroxy–L–proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrabutyl ammonium hydroxide solution (3.13 g, 6.61 mmol and 3.16 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **5a** as transparent oil at RT in 94% yield (2.31 g, 6.21 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_3$

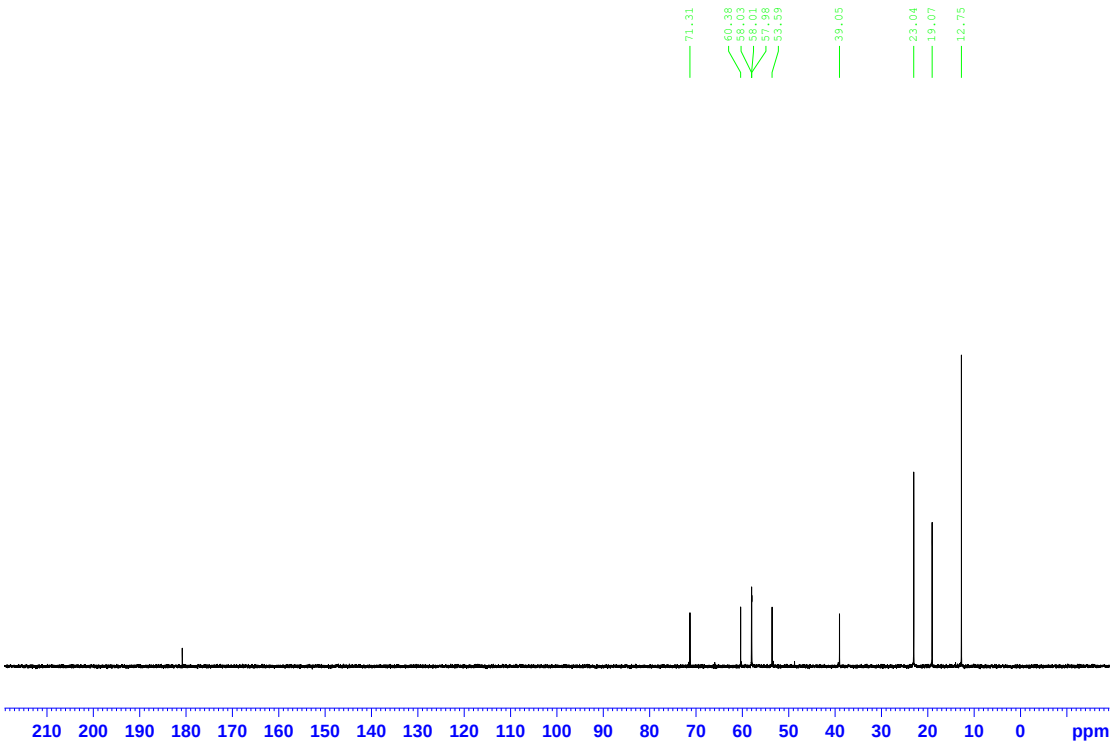
Molecular Weight: 368.55 g/mol

$[\alpha]_D^{20}$ = –19.7 (0.50c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.23–4.21 (m, 1H, *H2*), 3.42–3.37 (m, 1H, *H4*), 3.04 (t, J = 8.4 Hz, 8H, *H1'*), 3.00 (dd, J = 4.0, 4.4 Hz, 1H, $H5_a$), 2.67 (dd, J = 4.0, 4.4 Hz, 1H, $H5_b$), 2.28–2.20 (m, 1H, $H3_c$), 1.65–1.61 (m, 1H, $H3_d$), 1.53–1.46 (m, 8H, *H2'*), 1.20 (tq, J = 7.6, 7.6 Hz, 8H, *H3'*), 0.79 (t, J = 7.6 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.11 (CO, *C6*), 71.31 (CHOH, *C4*), 60.38 (NHCH, *C2*), 58.01 (NCH_2, *C1'*), 53.59 (NHCH, *C5*), 39.05 (CH_2, *C3*), 23.04 (CH_2, *C2'*), 19.07 (CH_2, *C3'*), 12.75 (CH_3, *C4'*). IR (neat, cm^{-1}): 3252, 2961, 2936, 2875, 1586, 1487, 1464, 1334, 1298, 1229, 1107, 140. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2833. Water Content = 0.87%. T_g = –46.1 °C. T_d = 214.2 °C.

DY-0019

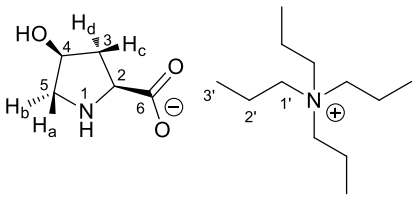


DY-0019



5

Preparation of (*2S, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetrapropylazanuide (IL 5b):



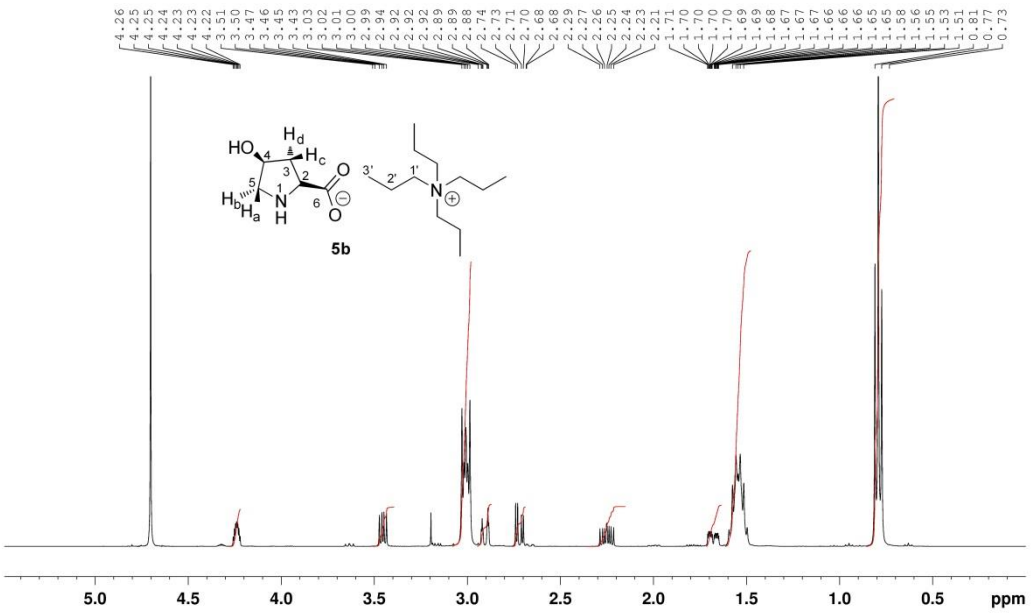
A 50 mL RB flask was charged with cis-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrapropyl ammonium hydroxide solution (5.98 g, 6.61 mmol and 5.84 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **5b** as yellow oil at RT in 90% yield (1.88 g, 5.95 mmol).

Chemical Formula: $C_{17}H_{32}N_2O_3$

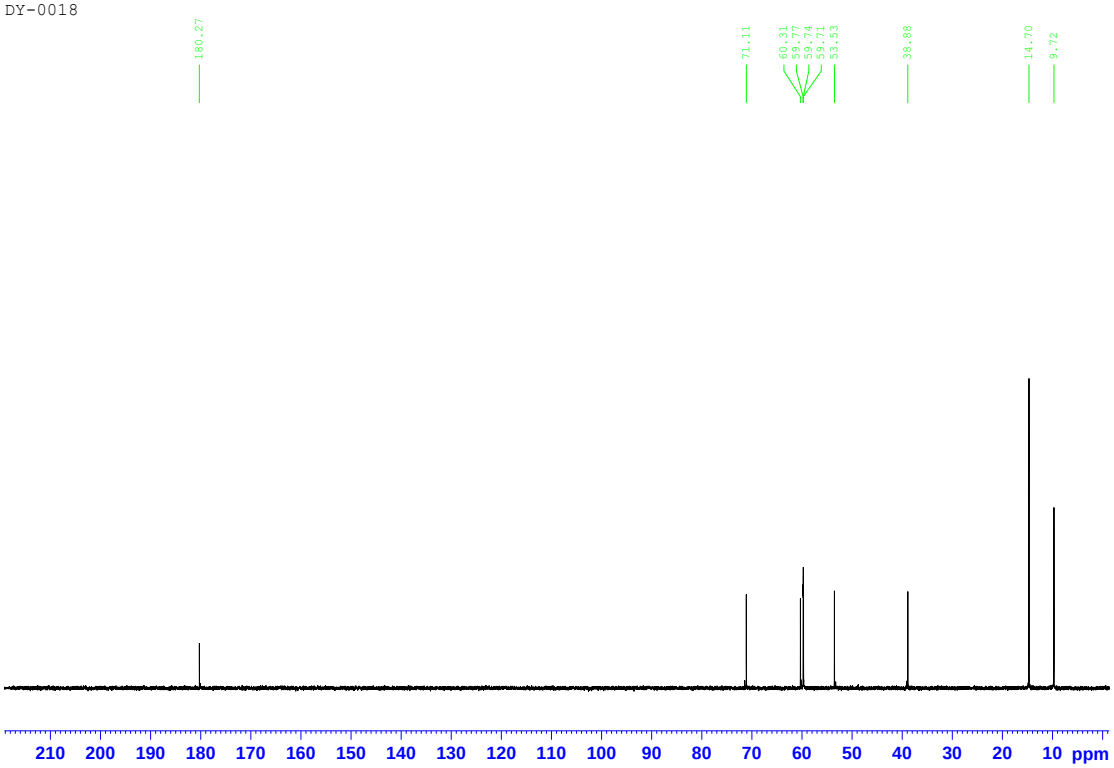
Molecular Weight: 312.45 g/mol

$[\alpha]_D^{20}$ = –25.3 (0.25c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.26–4.22 (m, 1H, *H2*), 3.44 (q, J = 5.6 Hz, 1H, *H4*), 3.02–2.91 (m, 8H, *H1'*), 2.92 (dd, J= 4.0, 4.0 Hz, 1H, $H5_a$), 2.72 (dd, J= 4.0, 4.4 Hz, 1H, $H5_b$), 2.29–2.21 (m, 1H, $H3_c$), 1.71–1.65 (m, 1H, $H3_d$), 1.54 (tq, J = 7.6, 7.6 Hz, 8H, *H2'*), 0.80 (t, J = 7.6 Hz, 12H, *H3'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.27 (CO, *C2*), 71.11 (CHOH, *C4*), 60.31 (NHCH, *C2*), 59.74 (NCH_2, *C1'*), 53.53 ($NHCH_2$, *C5*), 38.88 (CH_2, *C3*), 14.70 (CH_2, *C2'*), 9.72 (CH_3, *C3'*). IR (neat, cm^{-1}): 3256, 2971, 2881, 1585, 1487, 1378, 1230, 1082, 969, 865, 757, 673. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2209. Water Content by Karl Fischer = 0.55%. T_g = –46.4 °C. T_d = 238.5 °C.

DY-0018

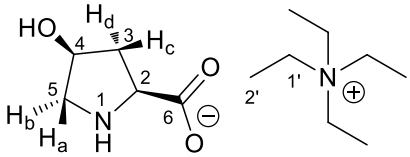


DY-0018



5

Preparation of (*2S, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetraethylazanuide (IL 5c):



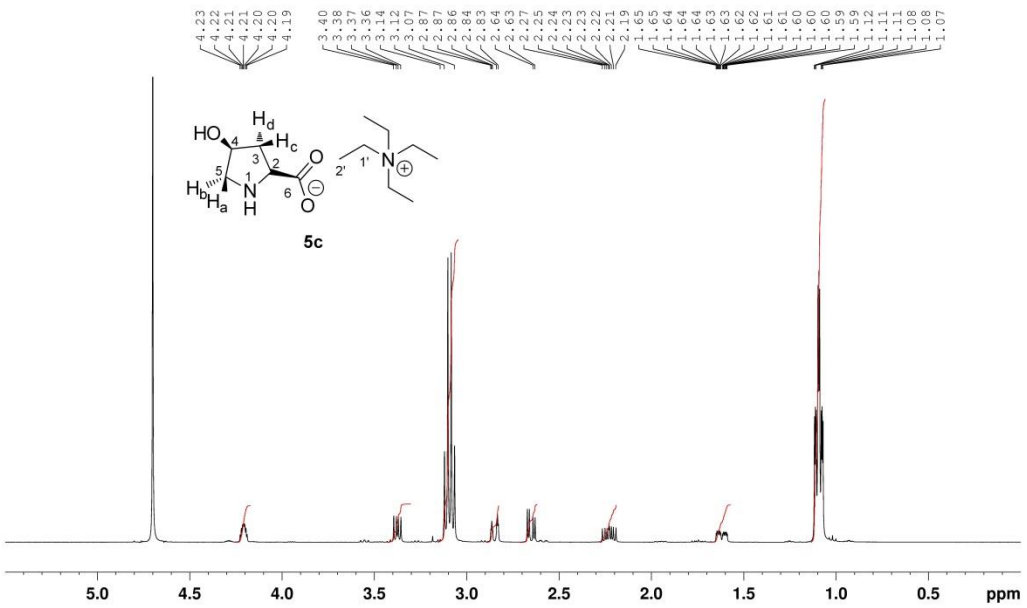
A 50 mL RB flask was charged with *cis*-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetraethyl ammonium hydroxide solution (2.79 g, 6.61 mmol and 2.73 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **5c** as light yellow oil at RT in 91% yield (1.47 g, 6.01 mmol).

Chemical Formula: $C_{13}H_{24}N_2O_3$

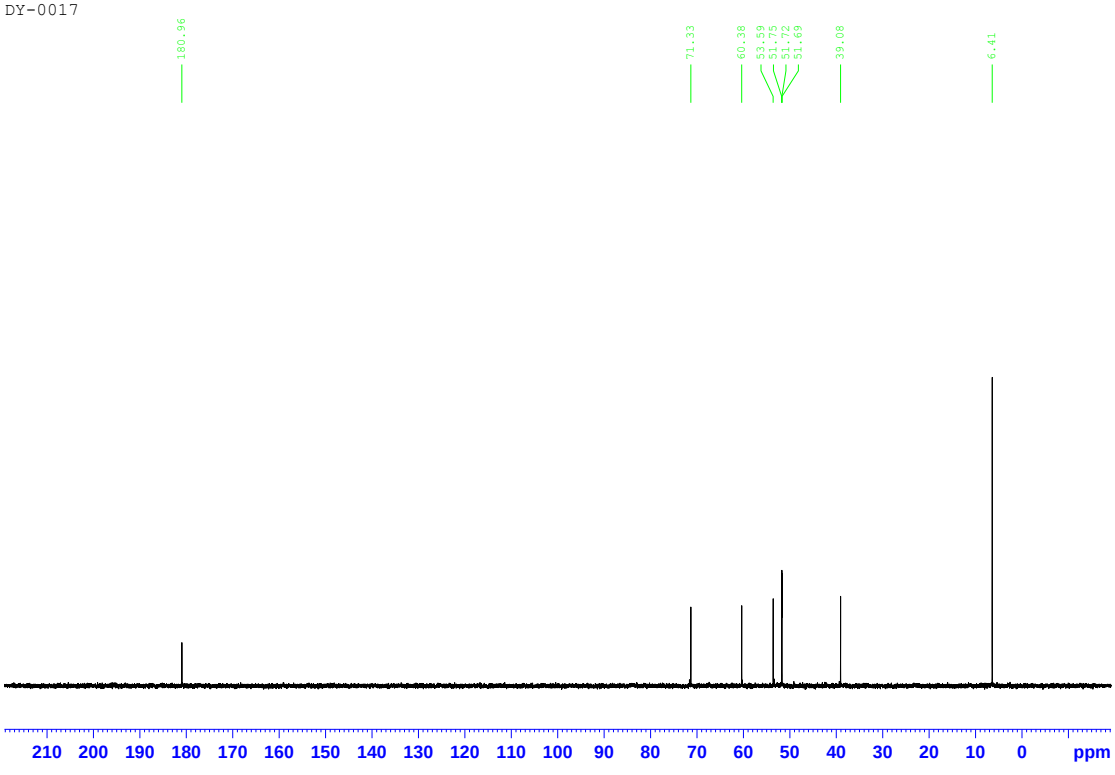
Molecular Weight: 256.34 g/mol

$[\alpha]_D^{20}$ = –26.0 (0.25c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.23–4.19 (m, 1H, *H2*), 3.37 (q, J = 6.0 1H, *H4*), 3.11 (q, J = 8.4 Hz, 8H, *H1'*), 2.86 (dd, J = 3.6, 3.6 Hz, 1H, $H5_a$), 2.63 (dd, J = 4.4, 4.4 Hz, 1H, $H5_b$), 2.27–2.20 (m, 1H, $H3_c$), 1.65–1.59 (m, 1H, $H3_d$), 1.09 (tq, J = 7.6, 8.0 Hz, 12H, *H2'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.96 (CO, *C6*), 71.33 (CHOH, *C4*), 60.38 (NHCH, *C2*), 53.59 (NCH_2, *C1'*), 51.72 ($NHCH_2$, *C5*), 39.08 (CH_2, *C3*), 6.41(CH_3, *C2'*). IR (neat, cm^{-1}): 3288, 2983, 2945, 1582, 1385, 1486, 1334, 1173, 1080, 1002, 977, 784. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1585. Water Content = 1.08%. T_g = –48.7 °C. T_d = 214.8 °C.

DY-0017

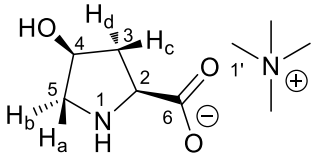


DY-0017



5

Preparation of (*2S, 4S*)-4-hydroxypyrrolidine-2-carboxylate tetramethylazanuide (IL 5d):



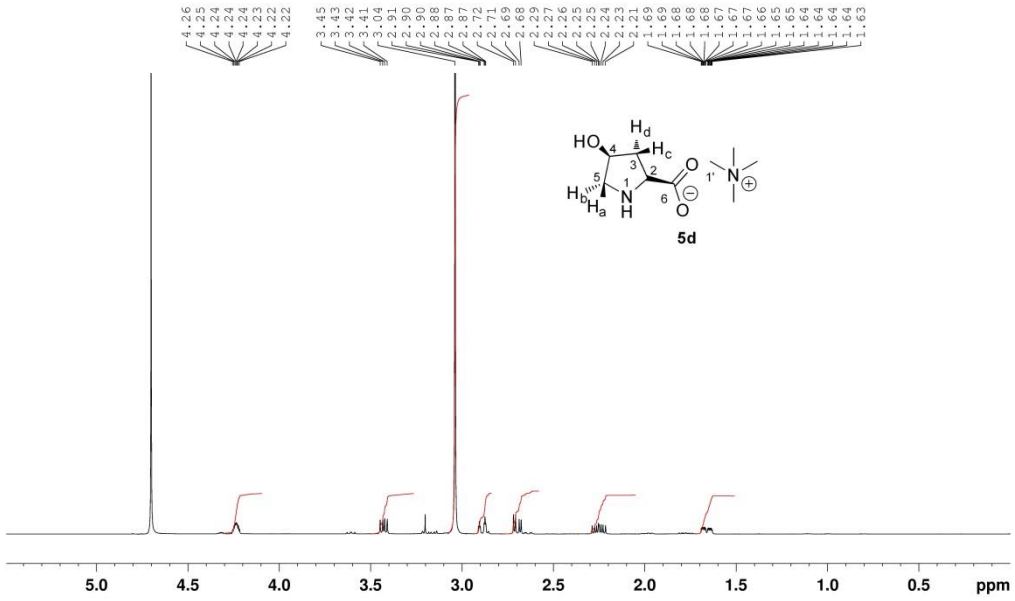
A 50 mL RB flask was charged with *trans*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetramethylammonium hydroxide pentahydrate (1.20 g, 6.61 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **5d** as yellow oil at RT in 92% yield (1.24 g, 6.01 mmol).

Chemical Formula: $C_9H_{16}N_2O_3$

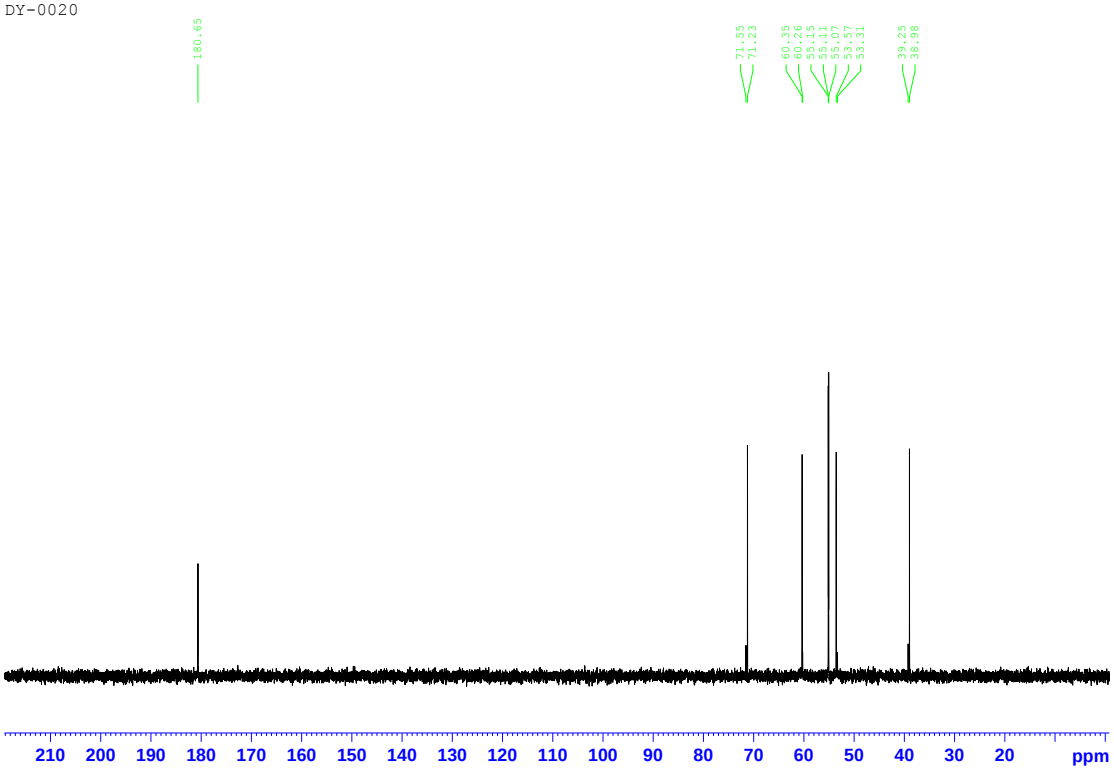
Molecular Weight: 200.23 g/mol

$[\alpha]_D^{20}$ = –30.5 (0.21c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.25–4.22 (m, 1H, *H2*), 3.42 (q, J = 5.2 Hz, 1H, *H4*), 3.04 (s, 12H, *H1'*), 2.90 (dd, J = 2.0, 3.6 Hz, 1H, $H5_a$), 2.69 (dd, J = 4.4, 4.0 Hz, 1H, $H5_b$), 2.29–2.22 (m, 1H, $H3_c$), 1.69–1.63 (m, 1H, $H3_d$). ^{13}C NMR (100 MHz, D_2O, δ): 180.65 (CO, *C6*), 71.35 (CHOH, *C4*), 60.30 (NHCH, *C2*), 55.11 (NCH_3, C1'), 53.45 ($NHCH_2$, *C5*), 39.09 (CH_2, *C3*). IR (neat, cm^{-1}): 3254, 3032, 2850, 1567, 1458, 1378, 1333, 1296, 1055, 950, 869, 815. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_4H_{12}N^+$, calculated = 74.0964, found = 74.0962. Water Content = 0.79%. T_g = –49.4 °C. T_d = 264.4 °C.

DY-0020

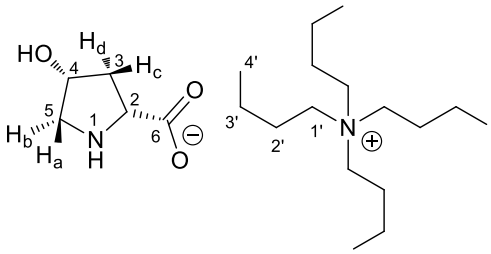


DY-0020



5

Preparation of (*2R, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetrabutylazanuide (IL 6a):



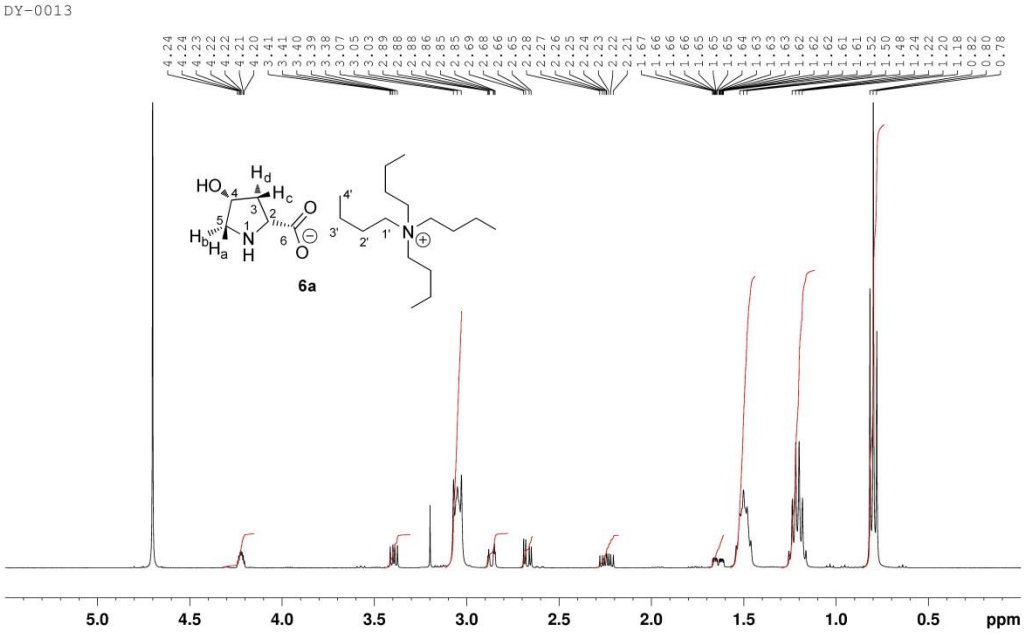
A 50 mL RB flask was charged with *cis*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrabutylammonium hydroxide solution (3.13 g, 6.61 mmol and 3.16 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **6a** as transparent oil at RT in 89% yield (2.20 g, 5.90 mmol).

Chemical Formula: $C_{21}H_{40}N_2O_3$

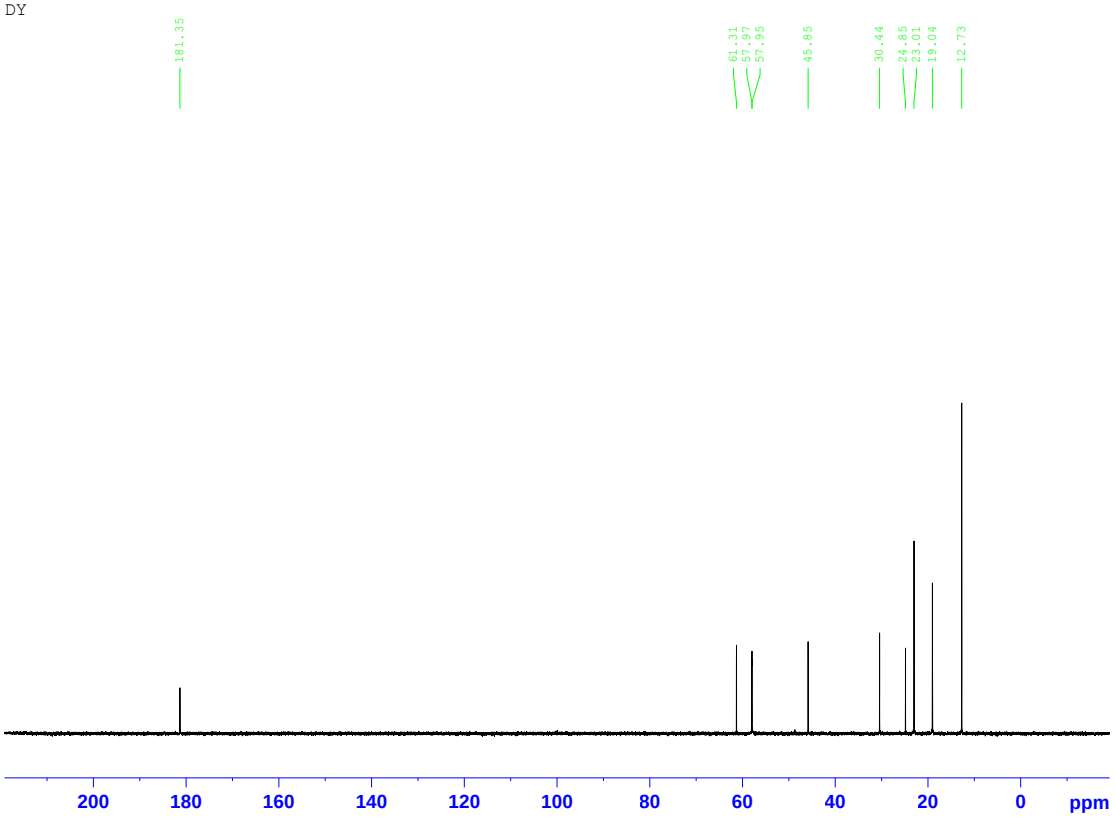
Molecular Weight: 368.55 g/mol

$[\alpha]_D^{20}$ = +21.0 (0.50c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.24–4.20 (m, 1H, *H2*), 3.40 (q, J = 6.0 Hz, 1H, *H4*), 3.05 (t, J = 8.4 Hz, 8H, *H1'*), 2.87 (dd, J = 5.6, 6.0 Hz, 1H, $H5_a$), 2.67 (dd, 1H, J = 5.6, 6.0 Hz, $H5_b$), 2.28–2.20 (m, 1H, $H3_c$), 1.67–1.61 (m, 1H, $H3_d$), 1.52–1.48 (m, 8H, *H2'*), 1.21 (tq, J = 7.2, 7.6 Hz, 8H, *H3'*), 0.80 (t, J = 7.2 Hz, 12H, *H4'*). ^{13}C NMR (100 MHz, D_2O, δ): 181.35 (CO, *C6*), 61.31 (CHOH, *C4*), 57.96 ($NHCH_2$, *C2*), 45.84 (NCH_2, *C1'*), 30.44 ($NHCH_2$, *C5*), 24.85 (CH_2, *C3*), 23.01 (CH_2, *C2'*), 19.04 (CH_2, *C3'*), 12.75 (CH_3, *C4'*). IR (neat, cm^{-1}): 3235, 2959, 2874, 1589, 1487, 1380, 1207, 1152, 1068, 1041, 970, 882. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{16}H_{36}N^+$, calculated = 242.2842, found = 242.2834. Water Content = 1.40%.T_g = –46.3 °C. T_d = 207.2 °C.

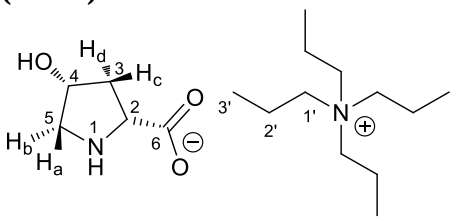
DY-0013


4.24 4.24 4.23 4.22 4.22 4.21 4.20 3.41 3.41 3.40 3.39 3.38 3.07 3.05 3.03 2.89 2.88 2.88 2.86 2.85 2.85 2.69 2.68 2.66 2.65 2.28 2.27 2.26 2.25 2.24 2.23 2.22 2.21 1.67 1.66 1.66 1.66 1.65 1.65 1.64 1.63 1.63 1.62 1.62 1.62 1.61 1.61 1.52 1.50 1.48 1.24 1.22 1.20 1.18 0.82 0.80 0.78
HO
4
H_d
H_c
3
2
5
1
N
H
6
O
O
H_b
H_a
4'
3'
2'
1'
N
6a
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

DY


181.35
61.31
57.97
57.95
45.85
30.44
24.85
23.01
19.04
12.73
200
180
160
140
120
100
80
60
40
20
0
ppm

Preparation of (*2R, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetrapropylazanuide (IL 6b):

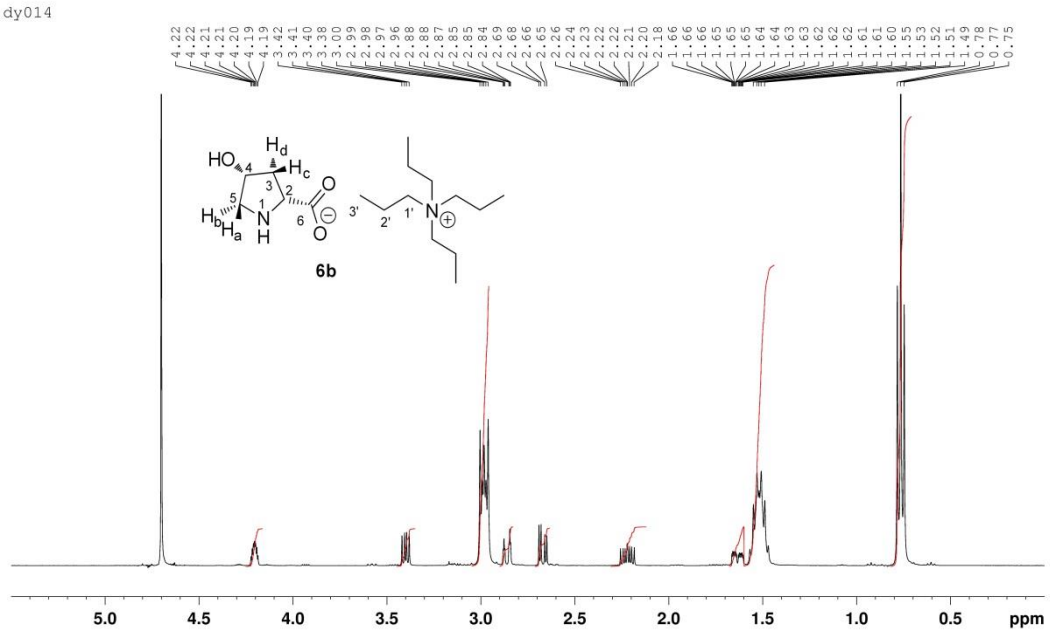


A 50 mL RB flask was charged with *cis*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetrapropyl ammonium hydroxide solution (5.98 g, 6.61 mmol and 5.84 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **6b** as transparent oil at RT in 96% yield (2.00 g, 6.09 mmol).

Chemical Formula: $C_{17}H_{32}N_2O_3$

Molecular Weight: 312.45 g/mol

$[\alpha]_D^{20}$ =+24.1 (0.25c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.22–4.18 (m, 1H, *H2*), 3.40 (q, J = 5.6 Hz 1H, *H4*), 2.97 (t, J = 8.0 Hz, 8H, *H1'*), 2.86 (dd, J = 3.6, 3.6 Hz, 1H, $H5_a$), 2.67 (dd, J = 4.4, 4.4 Hz, 1H, $H5_b$), 2.26–2.22 (m, 1H, $H3_c$), 1.66–1.61 (m, 1H, $H3_d$), 1.53 (tq, J = 8.4, 7.6 Hz, 8H, *H2'*), 0.76 (t, J = 7.6 Hz, 12H, *H3'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.49 (CO, *C6*), 71.18 (CHOH, *C4*), 60.32 (NHCH, *C2*), 59.72 (NCH_2, *C1'*), 53.54 ($NHCH_2$, *C5*), 39.94 (CH_2, *C3*), 14.69 (CH_2, *C2'*), 9.70 (CH_3, *C3'*). IR (neat, cm^{-1}): 3224, 2969, 2938, 2880, 1587, 1475, 1383, 1329, 1296, 1207, 1083, 1040. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_{12}H_{28}N^+$, calculated = 186.2216, found = 186.2210. Water Content = 1.70%. T_g = –46.4 °C. T_d = 241.8 °C.


dy014
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3.5
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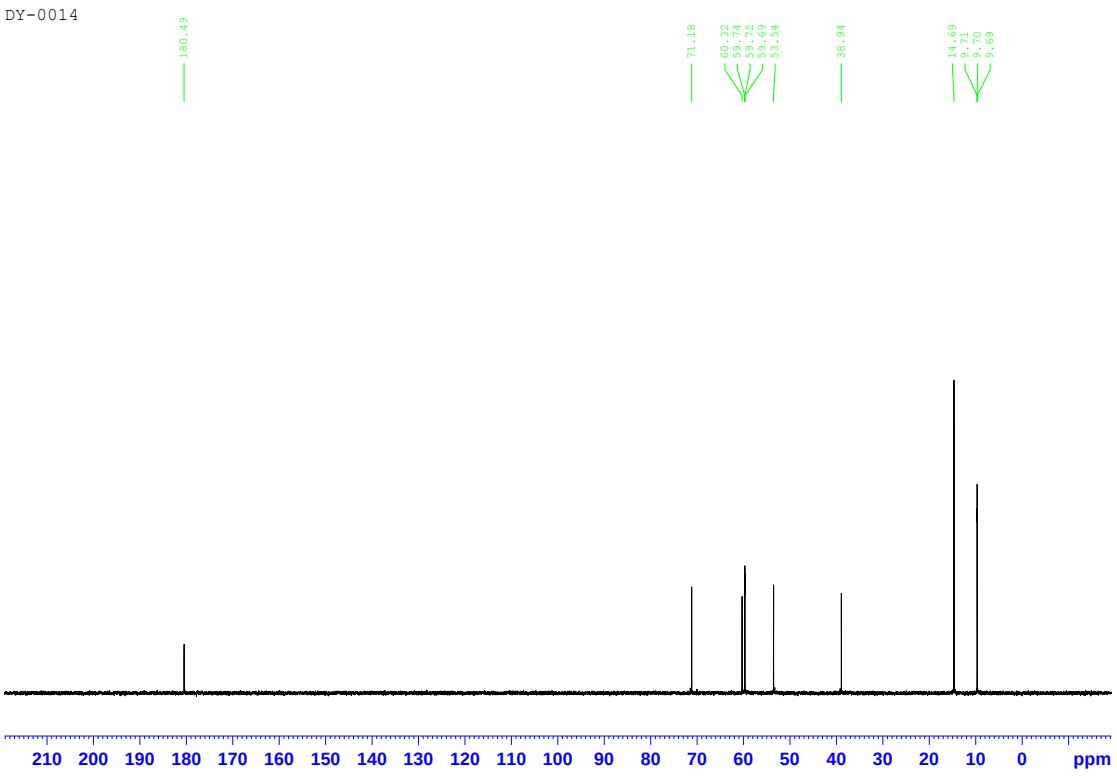
2.5
2.0
1.5
1.0
0.5
ppm


DY-0014
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59.74
59.72
59.69
53.54
38.94
14.69
9.71
9.70
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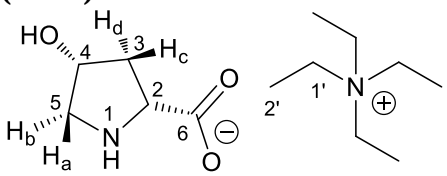
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

Preparation of *(2R, 4R)*-4-hydroxypyrrolidine-2-carboxylate tetraethylazanuide (IL 6c):



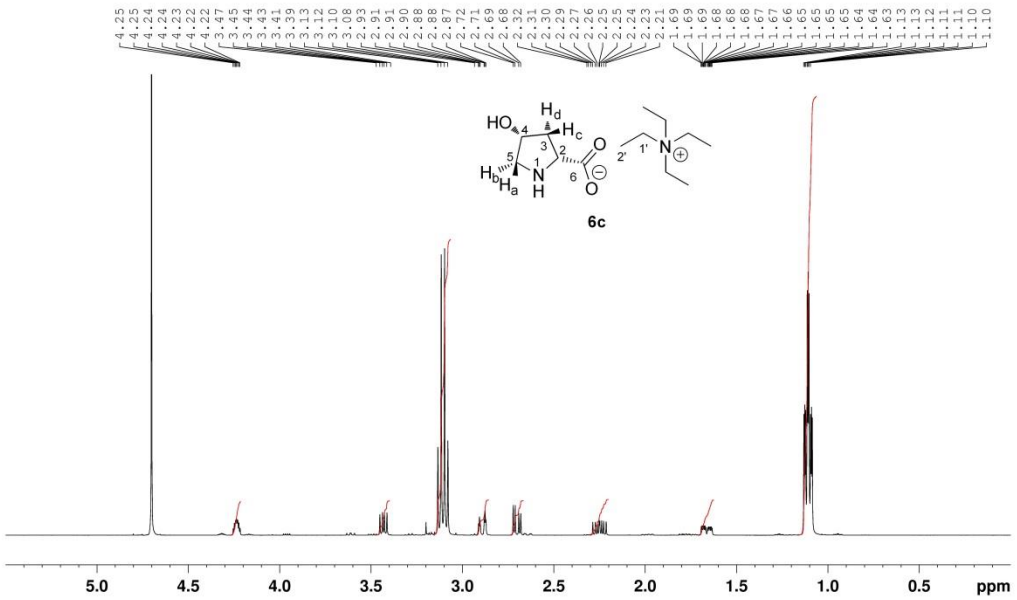
A 50 mL RB flask was charged with *cis*-4-hydroxy-D-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetraethyl ammonium hydroxide solution (2.79 g, 6.61 mmol and 2.73 mL). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **6c** as light yellow oil at RT in 91% yield (1.47 g, 6.01 mmol).

Chemical Formula: $C_{13}H_{24}N_2O_3$

Molecular Weight: 256.34 g/mol

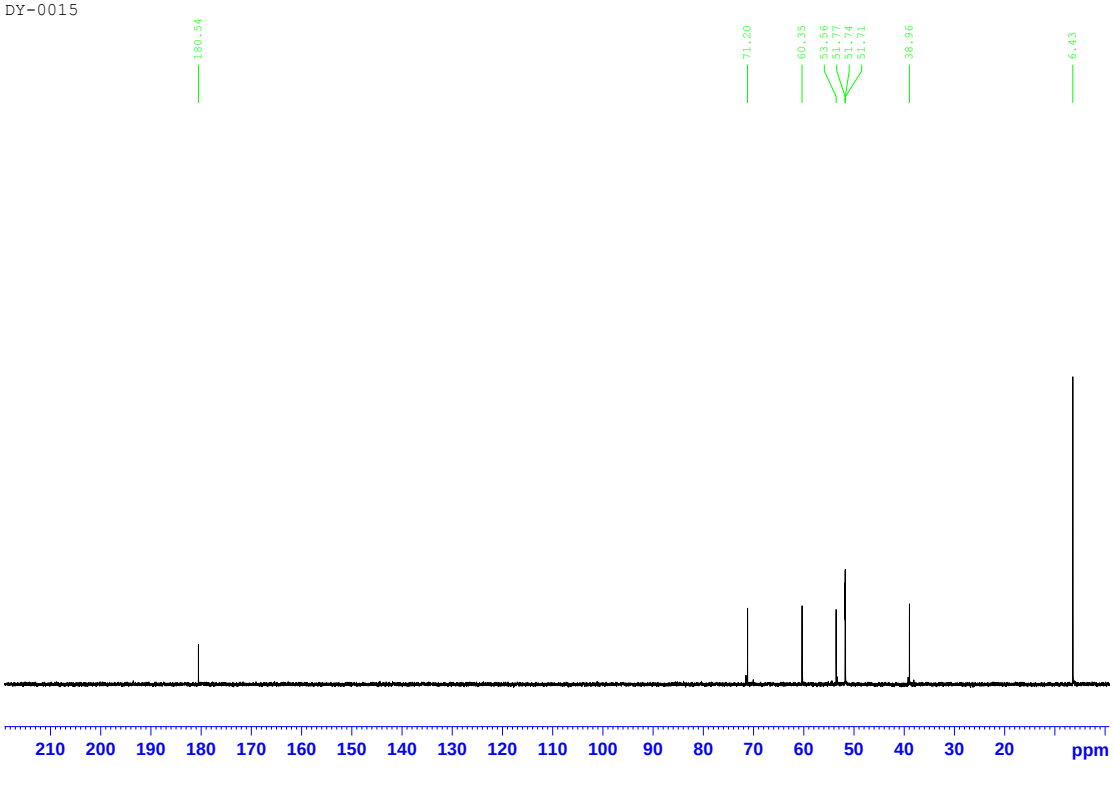
$[\alpha]_D^{20}$ = +24.8 (0.25c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.22–4.19 (m, 1H, *H2*), 3.37 (q, J = 5.6 Hz, 1H, *H4*), 3.08 (q, J = 7.6 Hz, 8H, *H1'*), 2.86 (dd, J = 3.6, 3.6 Hz, 1H, $H5_a$), 2.65 (dd, J = 3.6, 4.4 Hz, 1H, $H5_b$), 2.26–2.19 (m, 1H, $H3_c$), 1.65–1.59 (m, 1H, $H3_d$), 1.08 (tq, J = 7.2, 7.2 Hz, 12H, *H2'*). ^{13}C NMR (100 MHz, D_2O, δ): 180.54 (CO, *C6*), 71.20 (CHOH, *C4*), 60.35 (NHCH, *C2*), 53.56 (NCH, *C1'*), 51.74 ($NHCH_2$, *C5*), 38.96 (CH_2, *C3*), 6.43 (CH_3, *C2'*). IR (neat, cm^{-1}): 3252, 2983, 2935, 1585, 1487, 1376, 1295, 1174, 1078, 1035, 979, 784. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, calculated= 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_8H_{20}N^+$, calculated = 130.1590, found = 130.1586. Water Content = 1.14%. T_g = –49.5 ºC. T_d = 211.4 ºC.

DY-0015



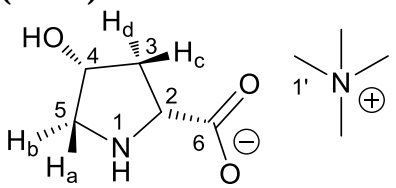
6c

DY-0015



5

Preparation of (*2R, 4R*)-4-hydroxypyrrolidine-2-carboxylate tetramethylazanuide (IL 6d):



Procedure:

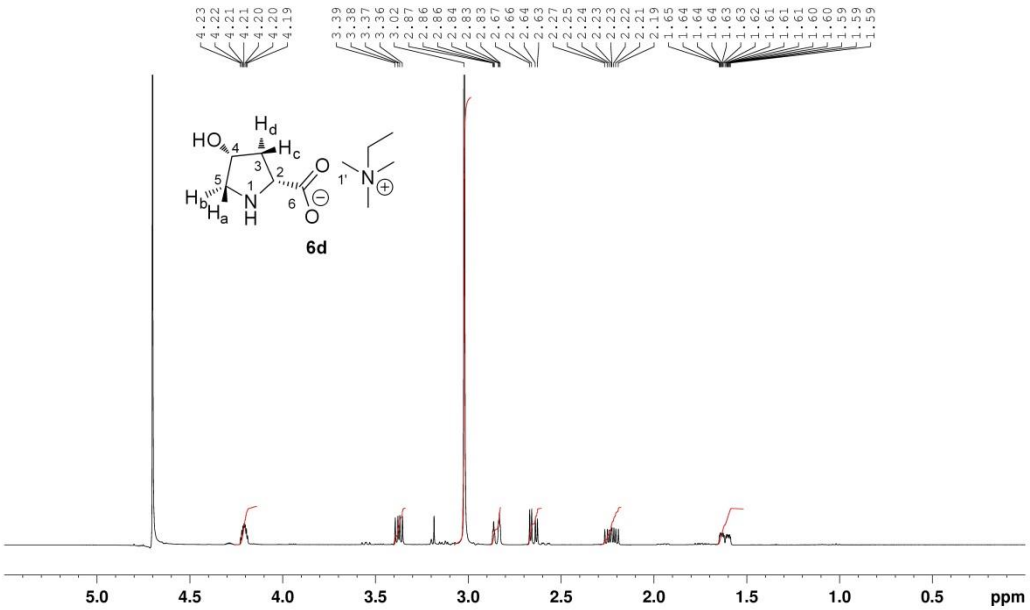
A 50 mL RB flask was charged with *trans*-4-hydroxy-L-proline (1.00 g, 7.60 mmol) and distilled water (15 mL). To this solution was added tetramethylammonium hydroxide pentahydrate (1.20 g, 6.61 mmol). The reaction mixture was stirred under reflux for 65 hrs. After cooling, the solvent was removed under reduced pressure and the crude product was purified by precipitation of the amino acid in cold methanol (5 mL) and then filtrate was washed by diethyl ether (5*15 mL). Solvent was removed via rotary evaporation and the product was dried in *vacuo* at 50 °C during 48 hrs to yield **6d** as orange oil at RT in 92% yield (1.24 g, 6.01 mmol).

Chemical Formula: $C_9H_{16}N_2O_3$

Molecular Weight: 200.23 g/mol

$[\alpha]_D^{20}$ = +30.1 (0.21c, H_2O). ^{1}H NMR (400 MHz, D_2O, δ): 4.23–4.19 (m, 1H, *H2*), 3.37 (q, J = 5.6 Hz, 1H, *H4*), 3.02 (s, 12H, *H1'*), 2.84 (dd, J = 3.6, 3.6 Hz, 1H, $H5_a$), 2.64 (dd, J = 4.4, 4,4 Hz, 1H, $H5_b$), 2.26–2.19 (m, 1H, $H3_c$), 1.65–1.59 (m, 1H, $H3_d$). ^{13}C NMR (100 MHz, D_2O, δ): 180.97(CO, C6), 71.33(CHOH, *C4*), 60.33(NHCH, *C2*), 55.09 (NCH_3, *C1'*), 53.60 ($NHCH_2$, *C5*), 39.08(CH_2, *C3*). IR (neat, cm^{-1}): 3252, 3033, 2936, 1581, 1489, 1377, 1294, 1209, 1079, 1047, 950, 676. HRMS (ESI^-, *m/z*): $C_5H_8NO_3^-$, requires = 130.0510, found = 130.0506, (ESI^+, *m/z*): $C_4H_{12}N^+$, calculated = 74.0964, found = 74.0962. Water Content = 1.16%. T_g = –46.4 °C. T_d = 257.2 °C.

DY-0016



DY-0016