Synthesis of New 5-Fluorouracil Photosensitiser Conjugates for use as Prodrugs in the Photodynamic Therapy for Cancer

DECLARATION

I herby certify that this material, which I now submit for assessment on the programme of study leading to the award of Doctor of Philosophy, is entirely my own work 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.

Signed:  Student Number: 50161784

Ian J. Gray

Date: 20th Sep 2005

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DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
$(DHQ)_2PHAL$	1,4-bis(9-O-dihydroquininyl)-phthalazine
Dtmp	2'-deoxythymidine 5-monophosphate
E.coli	*Escherichia coli*
Equiv.	equivalence
ESI	electrospray ionisation
$EtNH_2$	ethylamine
EtOH	ethanol
FDA	Food and Drug Administration
$FeCl_3$	iron chloride
FMN	flavin mononucleotide
FUDR	5-fluoro-2-deoxyuridine
GDEPT	gene directed enzyme prodrug therapy
HCFU	1-Hexylcarbamoyl-5-fluorouracil
HCl	hydrochloric acid
HpD	hematoporphyrin derivative
H_2O	water
IC_{50}	inhibitory concentration that killed 50% of the cells
IPA	isopropyl alcohol
$K_2OsO_2(OH)_4$	potassium osmate dihydrate
KBr	potassium bromide
K_2CO_3	potassium carbonate
KOH	potassium hydroxide

LDL	low density lipoprotein
LiOH	lithum hydroxide
MeOH	methanol
$MgSO_4$	magnesium sulphate
mmol	millimoles
MP	melting point
MS	mass spectrometry
mV	millivolts
Na^+	sodium ion
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	sodium hydrogen carbonate
NaOH	sodium hydroxide
Na_2SO_4	sodium sulphate
NBA	nitrobenzyl acetamide
NBB	nitrobenzyl bromide
NBC	nitrobenzyl chloroformate
nm	nanometre
NMR	nuclear magnetic resonance
NTR	nitroreductase enzyme
OEt	ethoxy
OMe	methoxy
Pc	phthalocyanine
PcSi	silicon (IV)-phthalocyanine

Pd/C	palladium/carbon
PDT	photodynamic therapy
PEG	polyethylene glycol
ppm	parts per million
PPTS	4-toluenesulfonic acid monohydrate
Ps	photosensitiser
PSA	prostrate –specific antigen
Rf	relative mobility
RNA	ribonucleic acid
ROS	reactive oxygen species
RT	room temperature
SGC	silica gel chromatography
SO_2Cl_2	thionyl chloride
tBuOH	t-butyl alcohol
TS	thymidylate synthase
TAP	tumour activated prodrugs
TEA	triethylamine
THF	tetrahydrofuran
TLC	thin layer chromatography
TsOH	toluenesulphonic acid
µg/ml	microgram per millilitre
µM	micromolar
uv/vis	ultraviolet/visable
Vol.	volume

v/v	volume per volume

AMINO ACID ABBREVIATIONS

Name	Abbreviation	Symbol
Glutamine	Gln	Q
Histidine	His	H
Leucine	Leu	L
Lysine	Lys	K
Serine	Ser	S

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ABSTRACT

A major limitation to cancer chemotherapy is the non-selectivity of the drug. One such drug that is used extensively to combat cancer is the pyrimidine derivative 5-fluorouracil (5-FU). However, its associated high toxicity and side effects has limited its clinical use. This in turn has challenged chemists to explore 5-FU prodrug derivatives in order to find a selective, non-toxic form. Although libraries of 5-FU prodrugs have been reported, selectivity still remains a problem and needs improvement. It was therefore our goal to try and improve 5-FU selectivity by designing a 5-FU prodrug that could be selectively delivered to the cancerous tissue where it could be activated. To achieve this we designed a 5-FU produg based on the general tumour activated prodrug (TAP) model concept i.e. triggers, linker and effector.

It is known that nitro-aromatic moieties can be reduced by the hypoxic conditions associated with tumour cells hence, we can use these moieties as triggers to activate the 5-FU prodrug. Seven such compounds were synthesised and incubated with 4T1 cancer cell lines for 72 hrs, however only two of the synthesised compounds (52) and (53) showed significant increase in cytotoxicity under hypoxic conditions, thus indicating that activation by endogenous one-electron reductases was occurring.
Photosensitisers used in photodynamic therapy (PDT) have been found to selectively accumulate in the cellular organelles of cancerous tissue. By exploiting this phenomena we coupled 5-FURD and the four synthesised nitro-5-FURD prodrugs to the photosensitiser dihydroxysilicon phthalocyanine. This in turn generated the five novel Bis(5-fluorouridine) phthalocyaninosilicon derivatives, thus allowing the photosensitiser to selectively carry the nitro-aromatic-5-FURD prodrug to the cancerous tissue, where the 5-FURD prodrug is activated thus releasing the 5-FU. However, this not only allows for the selective delivery of the 5-FU prodrug but generates a third generation photosensitiser that can be used in PDT application. However, the tumour reduction conditions may not be suitable for optimal activation of our nitro-aromatic prodrugs via the nitroreductase enzymes in the "dark", we therefore propose two alternative possibilities for activation via (1) the action of gene directed enzyme prodrug therapy (GDEPT) and *E.coli* or (2) activation through the consequence of oxidative stress on the cell via the release of excess cellular reductase.

It was found that of eleven 5-FU prodrugs tested on the SW480 and 4T1 cancer cell lines two (59) and (58) gave similar IC_{50} values as that for the 5-FU drug and that three of the prodrugs (53), (59) and (69) gave lower anti-invasion activity then the 5-FU drug.

We report the synthesis of a novel prodrug 2',3'-isopropylidene-5'-O-((-4''S,5''R)-N-acetyl-2'',2''-dimethyl-4''-phenyl-1'',3''-oxazolidine-5''-carboxylate)-fluorouridine 102). This design incorporates a lipophillic handle coupled to 5-fluorouridine. This compound was tested on SW480 and 4T1 cancer cell lines, however no cell death was observed at the concentrations used, instead growth inducement behaviour was exhibited. Following a SAR study this was attributed to (1) 5-fluorouridine being in the protected form of 2,3-isoprpoylidene-5-fluorouridine, thus preventing the formation of 5-fluorouracil metabolites and (2) the presence of the lipophillic ester slowing down its activation.

We also report that during our synthesis of prodrug (102) we discovered a novel isopropylidene deprotection procedure for the deprotection of 2,3-isopropylidene-5-fluorouridine and 2,3-isopropylideneuridine nuclosides to their parent nucleosides. This procedure also allowed for selective cleavage of trityl group in the presence of the isopropylidene moiety. The following optimised reaction conditions allow for reaction completion; 30% hydrogen peroxide 200μl, a 1:1:1 stoichiometry of acetonide, benzyl chloroformate in the presence or absence of base, in DMSO at 85°C. Yields ranging between 42-58% were obtained.

I. Cancer

What is cancer? Cancer is the name given to a family of diseases that vary in rate of growth, state of cellular differentiation, diagnostic detectability, invasiveness, metastatic potential and response to treatment and prognosis.[1] Cancer occurs when cells become abnormal and keep dividing, forming new cells without any control or order. All organs of the body are made up of cells. Normally, cells divide to form new cells only when the body needs them. When cells divide uncontrollably a mass of excess tissue is formed called a tumour. Tumours can be non-cancerous (benign) or cancerous (malignant). If cells are malignant they can invade and damage neighboring tissue and organs. These malignant cells can also travel into the blood stream to form new tumours in other parts of the body, this process is known as metastasis.

Cancer is the world's biggest killer. It is a disease that can occur at any time and is impartial of age and sex. However, through better diagnostic techniques and improved treatment millions of people have been treated and cured.

I.0.1. Causes of cancer

In many cases, the causes of cancer are not clear, both external and internal factors play a role. Cigarette smoking is a major casual factor of lung cancer. Diet, genetic mutation, infection, sexual behaviour, exposure to ultraviolet light and carcinogenic chemicals have also been implicated in other forms of cancer. However, a hereditary link may be involved with cancers such as those which affect the breast.

I.0.2. Cancer prevention

The best way to reduce deaths from cancer is to prevent it. Doctors generally agree that about one third of all human cancers are directly related to cigarette smoking.[1] For

smokers, the risk of cancer is much higher than that of a nonsmoker. Excluding the ultraviolet rays of sun light that cause skin cancer, the next most common cited cancer-causing factor is diet. The National Cancer Institute and the American Cancer Society recommended a diet low in fat, high in fiber, and rich in fruits and vegetables. Chemoprevention on the other hand is simply prevention with drugs. The word " drugs" is used to include dietary supplements, hormones, and vitamins etc., as well as real drugs, synthetic and natural agents used for therapeutic purposes. The number of chemoprevention agents is increasing.[2]

I.0.3. Cancer Treatments

Surgery is still the oldest and most common treatment method for the removal of malignant tumours. Radiation therapy is another method used to treat cancer [3]. It involves the irradiation of cancerous tissue with high energy X-rays, which damage the cancer cells, and stops them from growing and spreading. It can be used to shrink a tumour before surgery, however this treatment is often used after surgery. Like surgery it is a localised treatment affecting cells only in the area to be treated.

I.0.4. Cancer chemotherapy

Chemotherapy is the use of drugs to kill cancer cells. Unlike surgery and radiation therapy it is systemic; it works through the body. A single drug or a combination of drugs may be used. It is often used after surgery to kill any hidden cancer cells that may remain in the body. It works by interfering with the cells ability to replicated DMA thus preventing cells from multiplying. However most common cancers e.g. breast, lung, colorectal and prostrate cancer are not curable by chemotherapy alone, and often require a combination of surgery and radiation treatment.

In order for an effective chemotherapy drug to be developed its mechanism of action needs to be known as well as knowledge on normal cell division in comparison to cancerous " abnormal" cells. Both of these features in conjunction with the ongoing advances in molecular and cellular biology should help in development of an effective chemotherapy drug.

1.0. Chapter 1. The development of Cancer chemotherapy drugs

Throughout history, mankind has been interested in the properties of naturally occurring compounds. Simple extraction of these compounds from flowers and plants has allowed their properties to be used for various purposes, e.g. dyes, flavourings and perfumes.[1] However, it has been the medicinal properties of these compounds that have received the most attention. Classic examples of medicinal natural product extracts used in modern medicine are viscristine, teniposide, camptotheca and paclitaxel. Viscristine (Oncovin®)(FIGURE 1) is isolated from periwinkle (catharaanthus roseus) and is an antimiotic agent that is used in combination with other agents for the treatment of a wide range of cancers, including leukemia, bladder cancer, testicular cancer and lymphomas such as Hodgkin's diseases.[2,3] Teniposide (Vumon®) (FIGURE 2) a chemical analog of the natural product podophyllotoxin shows activity against Hodgkin's diseases, malignant lymphomas, paediatric refactory neuroblastoma, and brain tumors in adults.[4,5,6,7] The alkaloid camptothecin was first isolated from the tree camptotheca acuminata. It has good activity against various cancers in the laboratory; but is too insoluble for clincial use.[8,9,10,11] However, various water-soluble analogs of camptothecin (FIGURE 3) have been developed, and have found significant clinical use. Perhaps the most important member of the clinically useful natural anticancer agents is paclitaxel (Taxol®) (FIGURE 4) that was discovered in the bark of the western yew (Taxus brevifolia). The United States Food and Drug Administration (FDA) approved Taxol, for refractory ovarian cancer in December 1992 and refractory breast cancer in April 1994.[12,13]

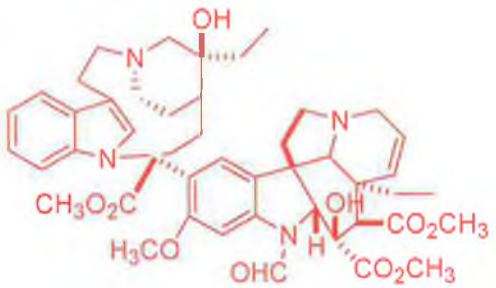


FIGURE 1. VINCRISTINE

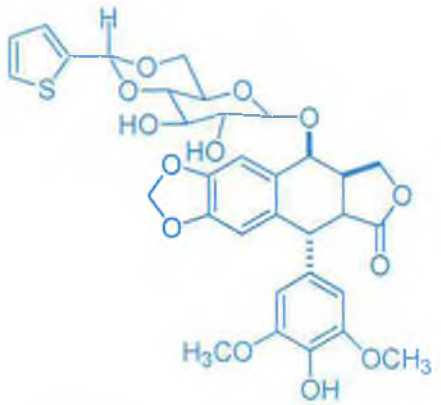


FIGURE 2. TENIPOSIDE

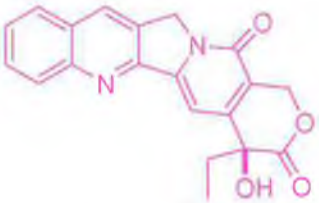


FIGURE 3. CAMPTOTHECIN

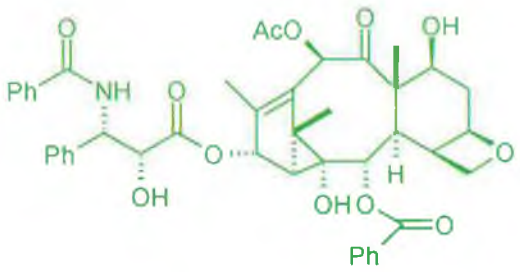


FIGURE 4. PACLITAXEL

A drug can be defined as a chemical used for the treatment/curing or prevention of disease in human beings or in animals.[14] Unfortunately, many conventional drugs suffer from various drawbacks as outlined in TABLE 1. However, many of these drawbacks have been eradicated with the development of prodrugs.[15]

TABLE 1. CONVENTIONAL DRUG DRAWBACKS.

PHARMACEUTICAL	**Pharmacokinetic**
Unpleasant smell	Poor bio-availability
Pain on injection	Short duration
Poor solubility	High first-pass metabolism
Instability	Toxicity/side effects
Slow dissolution rate	Non-specificity

1.1. The Prodrug Concept

Prodrugs were defined in the 1950's by professor Adrien Albert, an Australian medicinal chemist, as pharmacologically inactive chemical derivatives that could be used to alter the physicochemical properties of drugs, in a temporary manner, to increase their usefulness and/or to decrease associated toxicity. Subsequently, such drug-derivatives have also been referred to as 'latentiated drugs', 'bio reversible derivatives' and 'congeners', however, 'prodrug' is now the most commonly accepted term.[16]

Prodrugs can be defined as agents that are transformed after administration, either by metabolism or by spontaneous chemical breakdown, to form a pharmacologically active species. Ideally, the prodrug is converted to the original drug as soon as it reaches the site of action (FIGURE 5), followed by the rapid elimination of the released carrier without causing side effects in the process.[17]

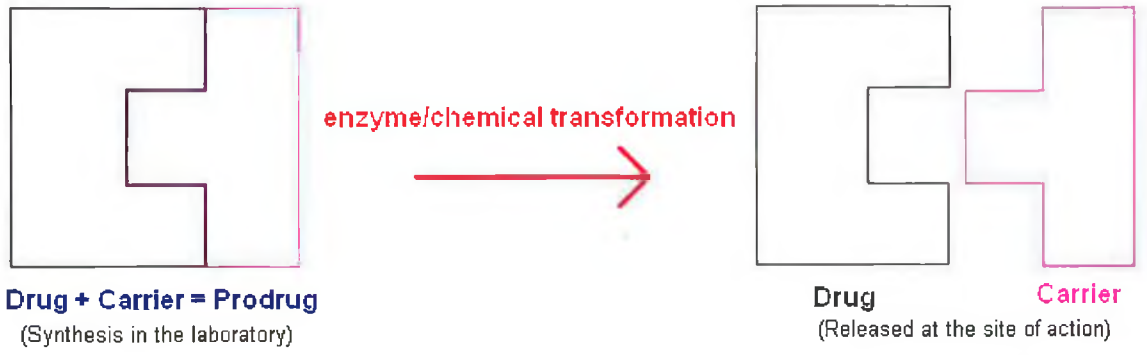


FIGURE 5. ENZYMATIC/CHEMICAL TRANSFORMATION OF A PRODRUG TO ACTIVE DRUG AND CARRIER

Although a number of prodrugs have been designed and developed to eradicate problems associated with drug utilisation (see; TABLE 1.), three common attributes are associated to all prodrugs:

1) A prodrug must be readily transported to the site of action.
2) A prodrug must be converted to the active drug.
3) Once the prodrug is generated at the site of action, the tissue must retain the active drug without further degradation.

Fortunately, there is a complement between bodily conditions and prodrug activation mechanisms, and it is this feature that has been instrumental in the design and development of prodrugs. This in turn has lead to the design of two types of prodrugs:

1) Prodrug design based on site-specific conditions, which utilises site-specific body conditions such as pH and oxygen content. This class of prodrug is commonly refered to as a carrier linked prodrug.
2) Prodrug design based on tissue/enzyme specific conditions. This class of prodrug is commonly refered to as a bioprecursor prodrug. [17]

1.1.1. Carrier linked prodrug

As the name indicates a carrier-linked prodrug consists of an active drug linked to an easily removed non-toxic and bio-inactive carrier. The linkage between the two must be a labile functional group, which can be cleaved to release the active drug. Examples of such a linkage are esters, which can be readily cleaved by either enzymatic or hydrolysis conditions. Carrier-linked prodrugs can be further subdivided into bipartate and tripartate prodrugs. Where a bipartate prodrug consists of a carrier to drug conjugate, while a tripartate prodrug consists of a carrier conjugated via a linker to the drug (FIGURE 6).

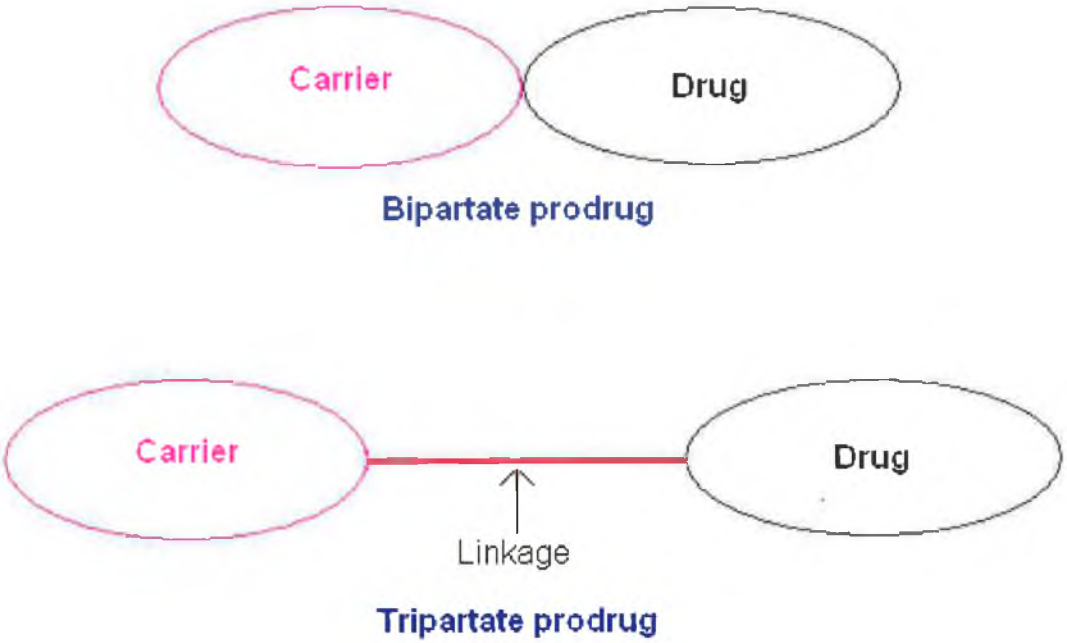


FIGURE 6. BIPARTATE AND TRIPARTATE PRODRUG DESIGN

1.1.2. Bioprecursor prodrug

This type of prodrug design relies on the concept that a biologically inactive molecule can be metabolised into an active drug *in-vivo* (FIGURE 7).

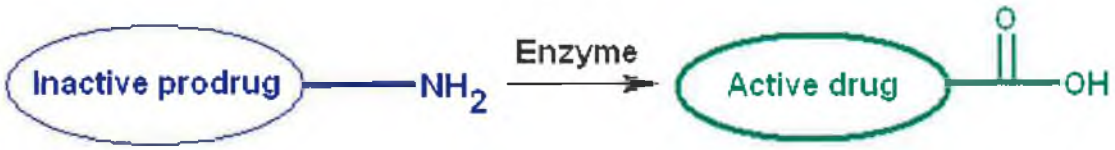


FIGURE 7. BIOPRECURSOR PRODRUG DESIGN

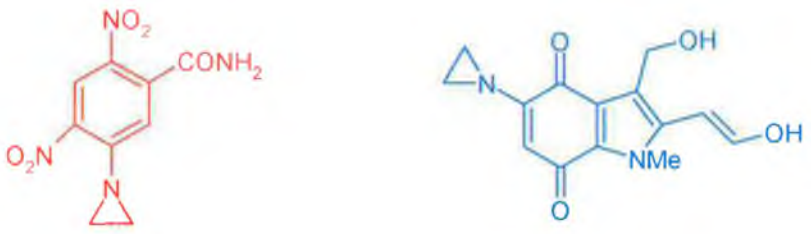
Various prodrug strategies in cancer therapy have utilised both of the above designs, with success.[18]

1.2. Prodrug Strategies in Cancer Therapy

It is known that the majority of clinically used anticancer drugs are cytotoxins, that preferentially kill dividing cells, primarily by attacking DNA at some level (synthesis, replication or processing). [17,22] However, these drugs are not truly selective for cancer cells as they may also damage normal proliferating cells and hence their use is limited. This has lead to the development of prodrugs that are selectively activated in tumour tissue, and are often referred to as "tumour activated prodrugs"(TAP). These prodrugs are selectively activated by tumour tissue by utilising the subtle metabolic and physiological properties unique to tumour tissue.[20,23]

1.2.1. Prodrug activation via enzyme expression selective to tumour cells

Because certain enzymes are expressed in elevated levels (eg. carbonyl reductase, amidases and DT-diaphorase) within tumour tissue many produgs have been designed to incorporate a functional group that can be selectively metabolised by these enzymes. Examples of such prodrugs are 5-aziridinyl-2,4-dinitrobenzamide CB1954 **1** and aziridoquinone EO9 **2** (FIGURE 8). Both were designed to take advantage of the high levels of DT-diaphorase expressed by tumours, but unfortunately they had limited success.



1 **2**

FIGURE 8. 5-AZIRIDINYL-2,4-DINITROBENZAMIDE CB1954 **1** AND AZIRIDOQUINONE EO9 **2**

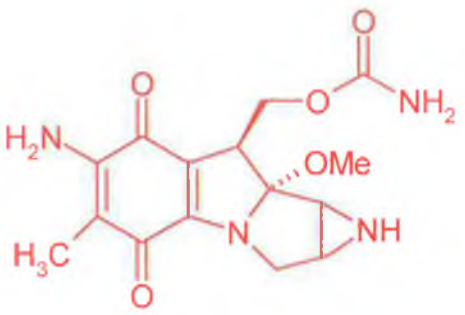
Conjugated peptide drug designs have also been developed to take advantage of this phenomenon. In this case activation is via the selective cleavage of the peptide bond by enzymes that are secreted by tumour cells, (e.g. prostate-specific antigens (PSA) from protase tumour), to release the active drug at the tumour site.[24-33]

1.2.2. Prodrug activation via tumour hypoxia

Hypoxia in cancer tissue is a result of the blood vessels in the tumour tissue lacking regularity and systematic connectivity, which results in unvascularised areas in the tumour as a result of inadequate blood flow. Cells that do not have a sufficient supply of blood die as a consequence of oxygen deficiency. This, and the high and variable interstitial pressures caused by the growing tumour lead to the presence of a variable but high proportion of hypoxia cells. Hypoxia can be classified into two broad groups:

1) Chronic or diffusion hypoxia. This type of hypoxia keeps cells sufficiently distant from the nearest blood capillary hypoxia for long periods.
2) Transient or perfusion hypoxia. This type of hypoxia results from the temporary shut down of blood vessels, therefore placing sections of the tissue under hypoxia for short periods of time. [34,35,36]

Hypoxia appears to be a common and unique property of solid tumours, and has therefore been a target for cancer prodrug development. Consequently, prodrug activation via hypoxic conditions has been a widely studied and used mechanism to generate the effector from hypoxia-selective TAP's. Tai-Shun Lin *et al.* (1986) hypothesised that reactive electrophiles could be generated by prodrugs requiring reductive activation. They called this class of drugs bioreductive alkylating agents. This concept was first reported for the quinone antibiotic mitomycin C **3** that may be considered a prototype bioreductive-alkylating agent (FIGURE 9).[17,25,37-39]



3

FIGURE 9. MITOMYCIN C

Once under hypoxic cellular or chemically reducing ($Na_2SO_2O_4$ or H_2/PtO_2) conditions it undergoes a cascade of transformations, which unmask its highly electrophilic reactivity that facilitates DNA alkylation.[40] Its mode of action is believed to involve bioreduction to the hydroquinone **4** which is followed by the elimination of methanol and aziridine ring opening thus generating the vinologous quinone methide **5,** which reacts exclusively with the N^2 of guanine in DNA. The carbamate can be easily eliminated at this stage generating the conjugated iminium ion **6** situated near the guanine residue on opposing or separate strands, which trap the electrophile to form inter and intrastrand crosslinks **7** (FIGURE 10).[41,42,25]

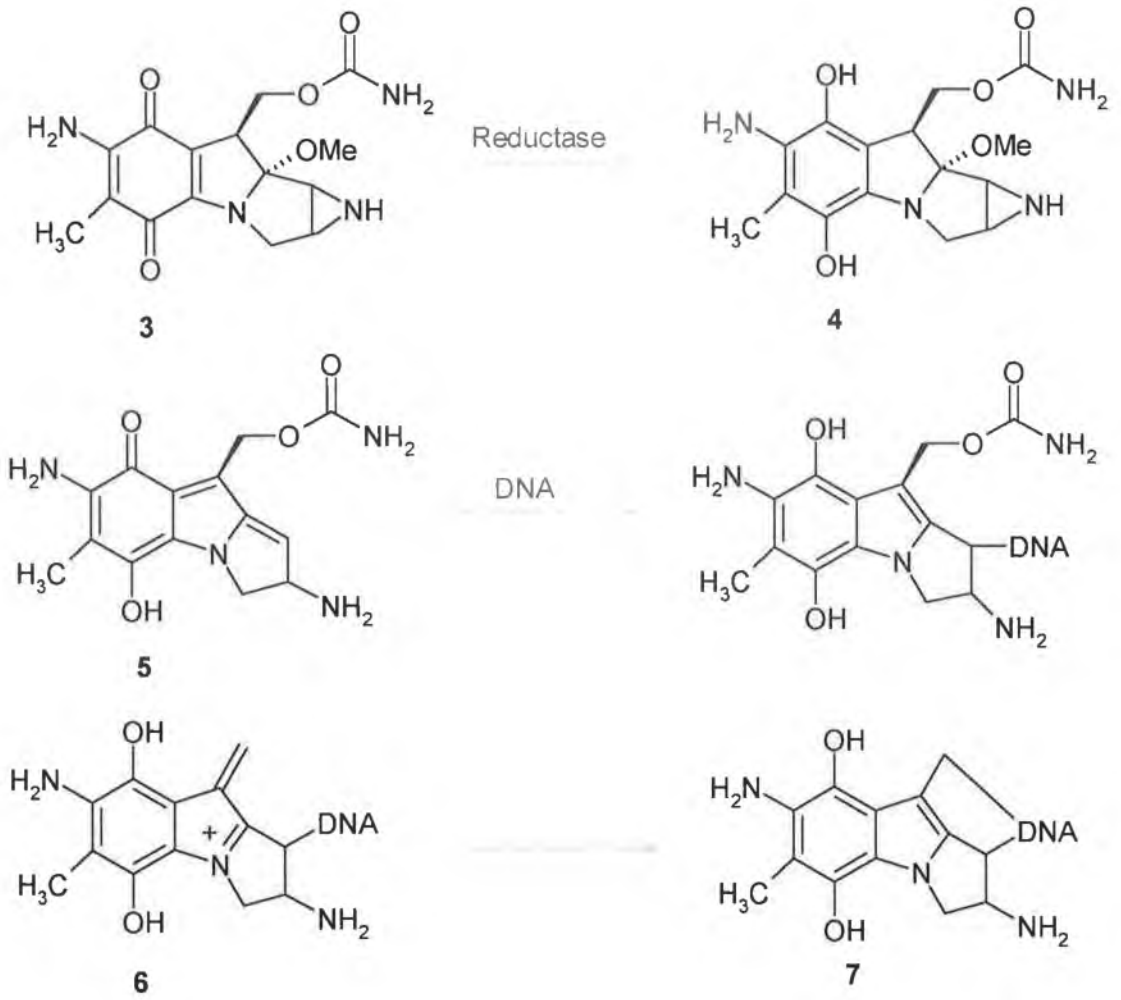


FIGURE 10. ACTIVATION MECHANISM FOR MITOMYCIN C

Nitroaromatic heterocycles have also shown toxicity to hypoxic cells. The toxicity is a result of the complete enzymatic reduction activation of the nitro heterocyclic compounds. The Sartorelli A.C group demonstrated this by synthesising a number of nitroreductase-catalysed bioreductive alkylating agents that incorporated both nitrobenzyl and nitrobenzylcarbamate moieties. They hypothesised that their toxicity to EMT6 tumour cells was due to the formation of a reactive methide species following the bioreduction by a nitroreductase enzyme system.[43] It has been postulated that the nitroreductase activity may involve the following enzymes: NADPH-cytochrome P-450 reductase, xanthine oxidase, DT-diaphorase cytochrome P-450 or carbonyl reductase.

The nitro moiety of nitroaromatic prodrugs has been used to trigger these types of TAP's.[44-47]

1.2.3. Bioreduction via nitroreductase.

Although naturally occurring nitro-aromatic compounds are rare, most classes of organisms, including bacteria, fungi, protozoa, plants and mammals can metabolise them using nitroreductase enzymes to reduce the nitro-group (FIGURE 12). This is because the reductive metabolism is carried out by widely distributed constitutive flavo-enzymes that are able to use nitro-aromatic compounds as alternative electron acceptors.[48] Three of the cellular components in mammalian cells selectively produce a varity of nitroreductase enzymes. These cellular organelles consist of the cytoplasm, mitochondria and microsomes. The enzymes that are produced from these organelles include the following: aldehyde oxidase, DT-diaphorase and xanthine oxidase in the cytoplasm, NADPH-cytochrome reductase and cytochrome P-450 in the microsomes and the enzymes dihydrolipoamide dehydrogenase, cytochrome b5 reductase, NADH-dehydrogenase and succinate dehydrogenase in the mitochondria (TABLE 2.).[49-53]

TABLE 2. NITROREDUCTASES SELECTIVE FOR THE CYTOPLASM, MICROSOMES AND MITOCHONDRIAL.

Cytoplasm	Microsomes	Mitochondrial
aldehyde oxidase	NADPH-cytochrome reductase	dihydrolipoamide dehydrogenase
DT-diaphorase	cytochrome P-450	cytochrome b5 reductase
xanthine oxidase		NADH-dehydrogenase
		succinate dehydrogenase
		carbonyl reductase

Although an exact bioreductive activation pathway still remains elusive, the nitro group of nitro-aromatic compounds would seem to undergo reduction by one of the endogenous enzymes listed above to their cytotoxic moieties in both aerobic and hypoxic cells. However, these drugs are only cytotoxic under hypoxia conditions as a result of their back oxidation in the presence of oxygen (FIGURE 11). Either a one two or a combined one two enzyme reducing cycle can achieve the bioreductive drug activation of nitro-aromatic moieties via the endogenous enzymes listed above. For example the enzymes cytochrome P450s, cytochrome P450 reductase, xanthine oxidase and cytochrome b_5 reductase occur via a one electron reducing enzymatic pathway. While, DT-diaphorase occurs via a two-electron reducing enzymatic pathway, and the carbonyl reductase by a one and two electron reducing enzymatic pathway. Studies have shown that activity levels of carbonyl reductase and DT-diaphorase can be significantly elevated within human tumours (biopsies), whereas cytochrome P450 reductase and cytochrome b5 reductase levels were equivalent to those in normal tissue.[55,56]

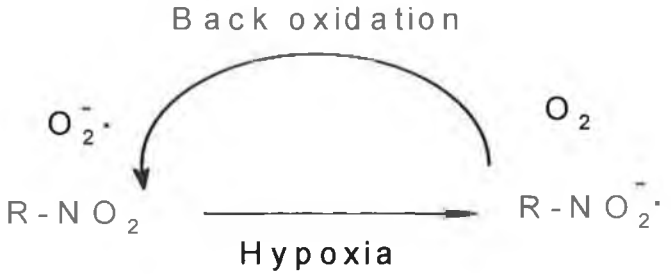


FIGURE 11. BACK OXIDATION TO PRODRUG UNDER AEROBIC CONDITIONS

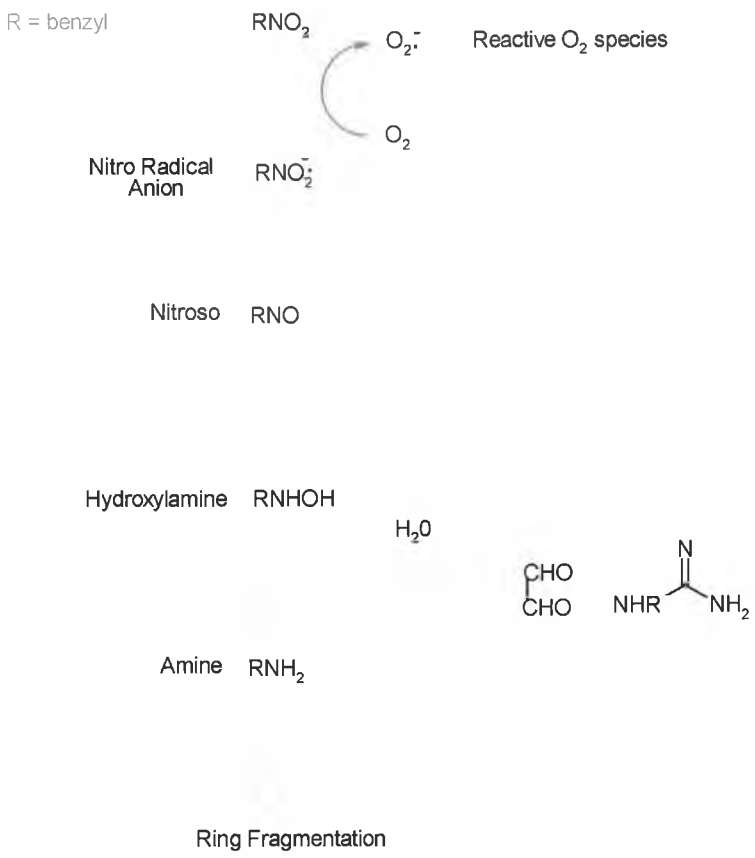


FIGURE 12. BIOREDUCTIVE METABOLISM OF A NITRO- MOIETY

Of the different nitroaromatic prodrugs studied, it has been found that the presence of an electron withdrawing group (i.e. amides, acids and esters) facilitates the reduction of the nitro group.[57] These types of bioreductive agents have therefore received the most attention as TAP triggers.[58] An example of one such nitroaromatic prodrug are the nitrobenzyl carbamates (FIGURE 13) which undergoes a multi electron reduction to generate an electron donating hydroxylamine or amine species. It is the release of electrons to the π-system that stabilises the developing positive charge on the benzylic carbon that allows the hydroxylamine to readily fragment and release the amine.[59] This prodrug design exemplifies well the trigger-linker-effector concept for TAP (section 1.4.).

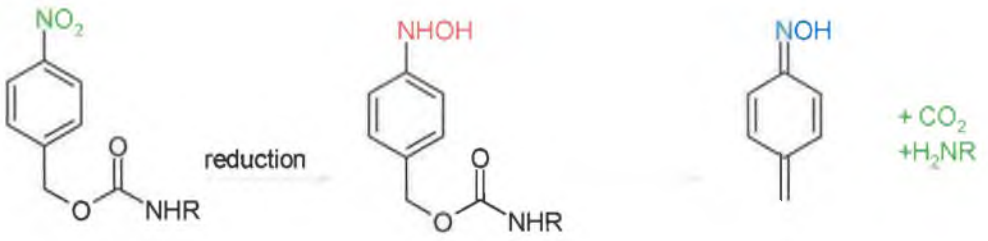


FIGURE 13. CARBAMATE FRAGMENTATION

However, it has been reported that a reduction potential of approximately –500 mV is necessary for the nitro trigger to be reduced. This requirement has lead to many potential nitroaromatic prodrugs falling short of the required potential to be reduced by the cellular nitroreductase listed in TABLE 2.[60]

The discovery of an *E. coli* B nitroreductase enzyme (NTR) has however generated renewed interest into these bioreductive agents. It was found that when the NTR enzyme was used in conjunction with NADH or NADPH, the low reduction potential of typical nitrobenzyl carbamates (ca.-490mV), could be reached and undergo reduction to the corresponding unstable hydroxylamine.[61-64]

1.2.4. Radiation activited prodrugs

When the water in cells is exposed to ionising radiation the reduced species generated can be used to activate prodrugs (FIGURE 14).[19]

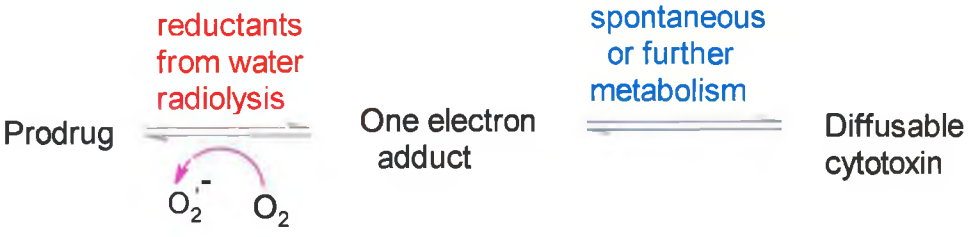
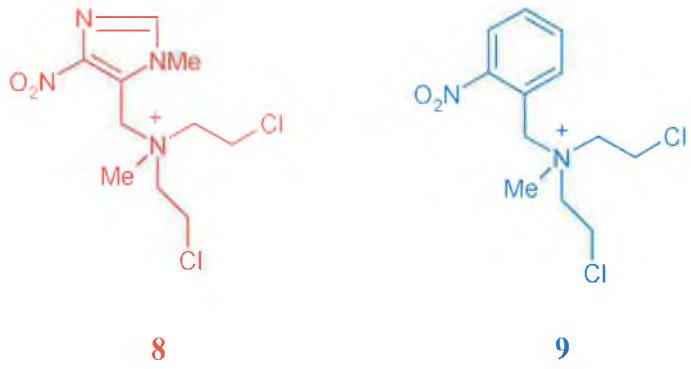


FIGURE 14. MECHANISM OF PRODRUG ACTIVATION BY THERAPEUTIC RADIATION

Radiation activated prodrugs are selective since they are activated in the hypoxia regions within the radiation field. The advantage of this approach is that prodrug activation can occur without the presence of enzymes, therefore allowing activation to occur in all the hypoxia regions of the tumour. However, as a consequence of small amounts of reducing equivalent been delivered via a dose amount of radiation considerable demand is placed on the prodrug design. Despite this shortfall radiation activated prodrugs are commonly used in cancer therapy. The quaternary ammonium salts of mechlorethane **8** and **9** have been reported as potential radiation-activated prodrugs (FIGURE 15). [65,66,19]



8 **9**

FIGURE 15. MECHLORETHANE DERIVATIVES USED AS RADIATION-ACTIVATED PRODRUGS

1.2.5. Tumour specific antigens ("armed antibody" approach)

Many tumour cell surfaces possess characteristic tumour associated antigens. It is possible to take advantage of this and attach prodrugs to "targeting" antibodies. Once the antibody binds to the tumour cell the prodrug would then be converted to the active drug.[67] An adaption of this concept is Antibody directed enzyme prodrug therapy (ADEPT). In ADEPT, a prodrug is cleaved by a specific non-human enzyme; this enzyme, which can activate the prodrug, is attached to the targeting antibody, instead of the prodrug. The idea behind this is to localise the prodrug-activating enzyme at the

tumour, the prodrug is then administered and will only be activated by the enzymes that have been targeted to the tumour.[68] At this point the activated prodrug may diffuse from the cell where it is generated to enter and kill surrounding tumour cells. The efficacy of this approach depends on a number of factors related to choice of antibody, enzyme and prodrug.[69-75]

1.2.6. Gene directed enzyme prodrug therapy

This type of prodrug offers an alternative to ADEPT; where the enzyme is targeted to tumour cells by integrating the gene that produces it into the genome of the tumour cells. This therapy retains the advantages of ADEPT in terms of selectivity and access of the drug to tumour cells; it also increases the class of enzymes available to those that require endogenous (human) cofactors.[76-78,19]

1.3. Tumour-Activated Prodrug Design

In order for a tumour-activated prodrug (TAP) to be successful there are a number of factors that need to be incorporated into its design including the following:

- to have efficient distribution to remote hypoxia regions in the tumour tissue.
- to undergo selective cellular metabolism in tumour tissue to generate a cytotoxin capable of diffusing a limited distance to kill surrounding tumour cells that may lack the ability to activate the prodrug. [19]

It is thought that in order to fulfil these criteria that the TAP design can be considered to encompass three separate units: trigger, linker and effector [tripartate design](FIGURE 16).[20,21] The role of the trigger unit is to undergo efficient metabolism by one of the above tumour specific mechanisms. The role of the linker is to deactivate the prodrug until the trigger undergoes metabolism thus rapidly transmitting the change to the effector in such a way as to cause rapid and significant activation. Consequently, the role of the effector is to kill the tumour cells rapidly, and be able to diffuse a sufficient distance to cause a substantial by-stander effect.

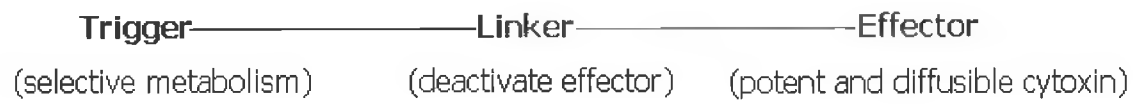
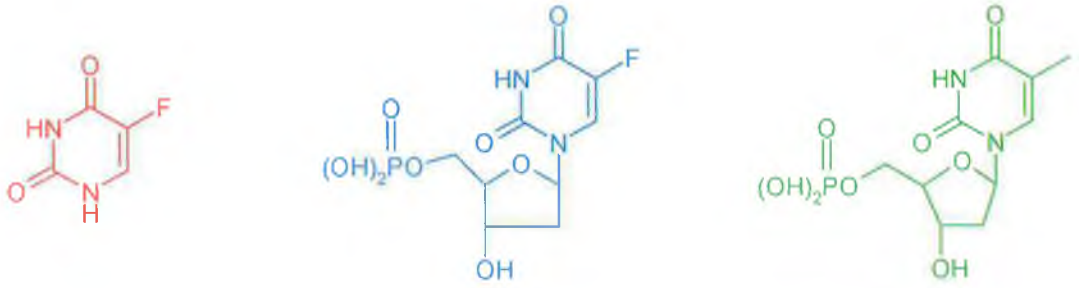


FIGURE 16. TRIGGER-LINKER-EFFECTOR CONCEPT FOR TRIPARTATE TAP DESIGN

1.4. 5-Fluorouracil

Because there is such a vast quantity of available anti-cancer drugs, it would have proved a mammoth task to try and decide the most suitable cytotxoxin for our study. For this reason, we decided to work on one of the most extensively studied and commonly used drugs in cancer therapy, 5-fluorouracil. In 1959 5-fluorouracil (5-FU) **10** (FIGURE 17) was synthesised by Duschinsky and co. workers [79] while Heildelberger and co. workers [80] demonstrated its anti-tumor activity. Since then the fluorinated pyrimidine has been used as a chemotherapeutic agent in the treatment of a large number of malignant tumours. Some of these include colon, rectal, breast, stomach, pancreatic, ovarian, cervical and bladder cancers. The 5-FU can follow three pathways to induce cell death: 1) thymidylate synthase inhibition, 2) formation of 5-FU-RNA complexes and 3) 5-FU-DNA complexes. Once *in-vivo*, the 5-fluorouracil is converted into 2'-deoxy-5-fluorouridine-5'-monophosphate (5-FdUMP) **11** (FIGURE 17).[81] This acts as a powerful inhibitor of thymidylate synthase (TS), thus depleting the intra cellular stores of 2'-deoxythymidine-5'-monophosphate (dTMP) **12** (FIGURE 17) inhibiting DNA production and cell division[81]. Therefore, if a cell cannot make thymidine, it cannot synthesise DNA. Alternatively, 5-FU may be anabolised to 5-fluorouridine monophosphate, which is further metabolised to 5-fluorouridine triphosphate (5-FUTP). The latter can be incorporated into RNA or converted into the 5-FdUMP. Finally, the 5-FdUMP may subsequently be phosphorlyated to 5-fluoro-2'-deoxyuridine-5'-triphosphate (5-FdUTP), which is incorporated into DNA.[82,83]



10 **11** **12**

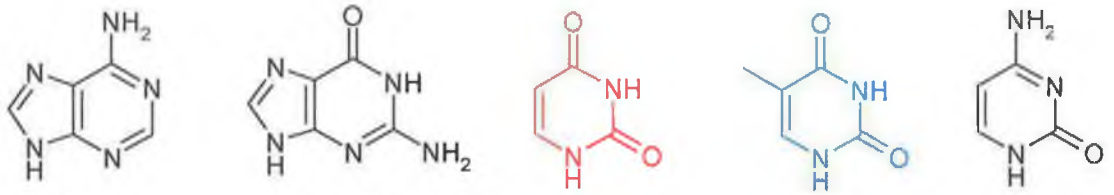
FIGURE 17. 5-FU **10**, FdUMP **11** and DTMP **12**

1.4.1. Mechanism of Action

In order to understand the mechanism for inhibition of 5-fluorouracil thyimdylate synthase, we need to first look at its parent base uracil **15** (FIGURE 18). As can be seen from TABLE 3, one difference between DNA and RNA is that RNA contains the heterocyclic base uracil while DNA contains the heterocyclic base thymine **16** (FIGURE 18). The thymine used in the biosynthesis of DNA is synthesised by thymidylate synthase, an enzyme that requires N^5, N^{10}-methylene-tetrahydrofolate as a coenzyme.

TABLE 3. SHOWING THE DIFFERENT BASES ASSOCIATED WITH RNA AND DNA.

RNA BASES	**DNA Bases**
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Uracil	Thymine



adenine	guanine	uracil	thymine	cytosine
13	**14**	**15**	**16**	**17**

FIGURE 18. STRUCTURES OF RNA AND DNA BASES

Outlined in (FIGURE 19). is the reaction mechanism involved in the conversion of uracil to thymine by thymidylate synthase. When the reaction is complete the dihyrofolate must be converted back to N^5, N^{10}-methylene-tetrahydrofolate so that the coenzyme can undergo another catalytic cycle.

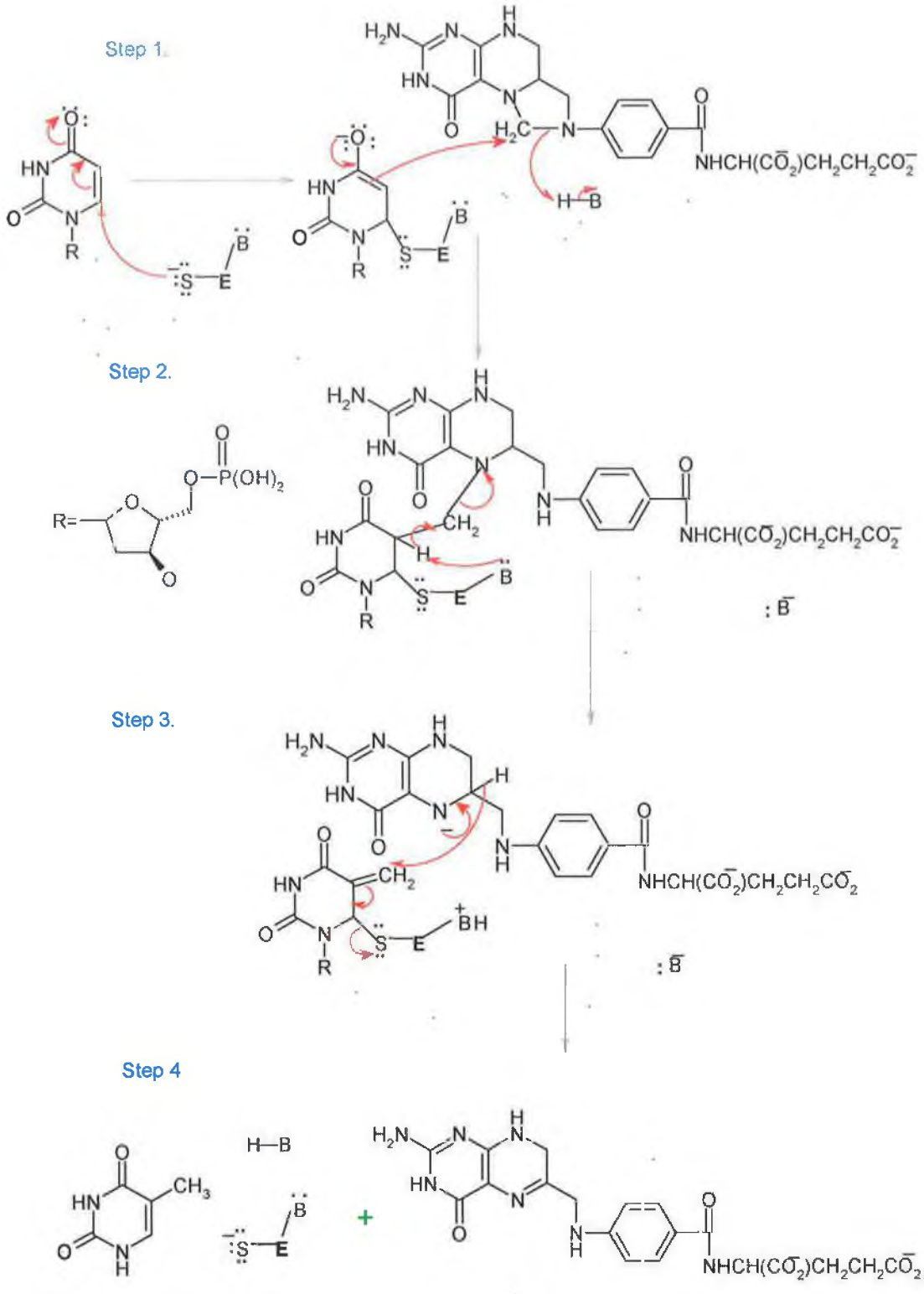


FIGURE 19. MECHANISM FOR THE GENERATION OF THYMINE FROM URACIL BY THYMIDYLATE SYNTHASES

Although uracil and 5-fluorouracil react with thymidylate synthase in the same way, they differ only in their susceptibility to methylation at C-5 as outlined in (FIGURE 20). Since, the fluorine cannot be removed, the reaction stops at this point, leaving the enzyme permanently attached to the substrate, consequently, the synthesis of DNA is stopped.

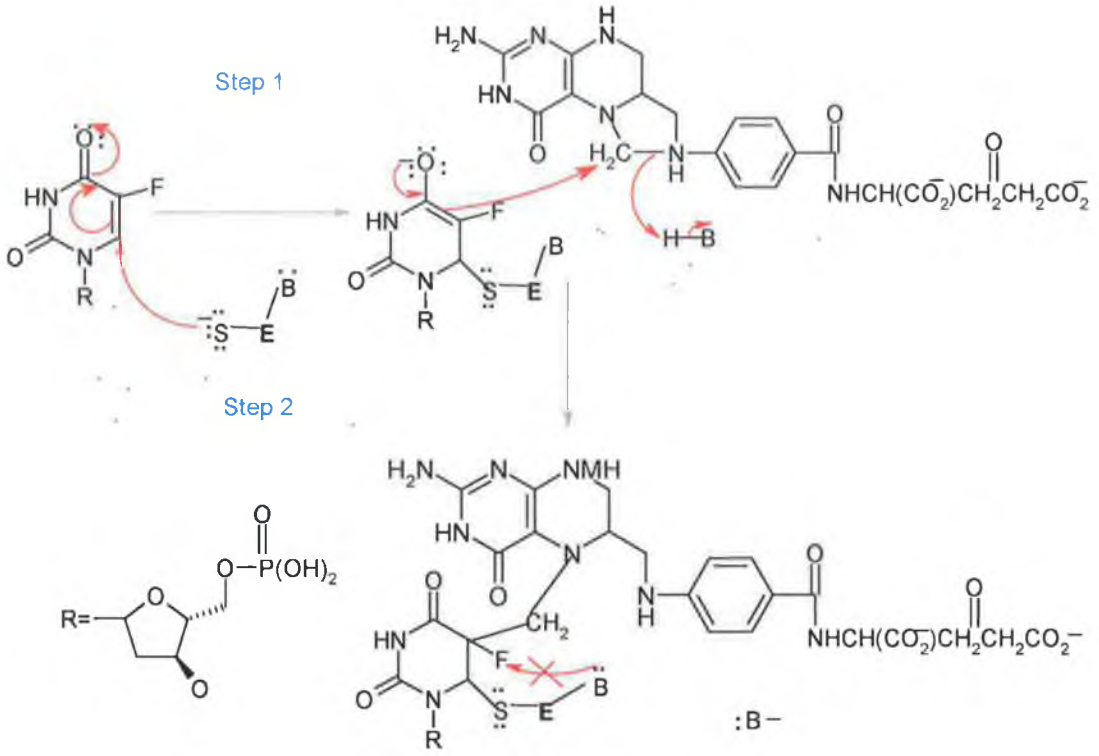


FIGURE 20. MECHANISM FOR 5-FU INHIBITING THYMIDYLATE SYNTHASE

Even though 5-fluorouracil is well known to have strong anti-tumour activity its chief disadvantage, like most anticancer drugs, is its inability to discriminate between diseased and normal cells. As a result, it is toxic to both normal cells and diseased cells, resulting in a series of side effects which include: drying or darkening skin or nails, sensitivity of skin to sun light, thinned or brittle hair, headaches, loss of appetite, weight loss, tingling or numbness in hands, chills, fever, sore throat, cough, nausea and vomiting, mouth blisters, fatigue, diarrhoea, stomach pain, painful urination, red or coloured urination, unusual brushing or bleeding, yellowing of skin, rashes, black/tarry

stools and joint pain. Therefore, numerous attempts to prepare prodrugs of 5-fluorouracil have been attempted to diminish these unwanted side effects.[84]

1.4.2. 5-Fluorouracil prodrug derivatives

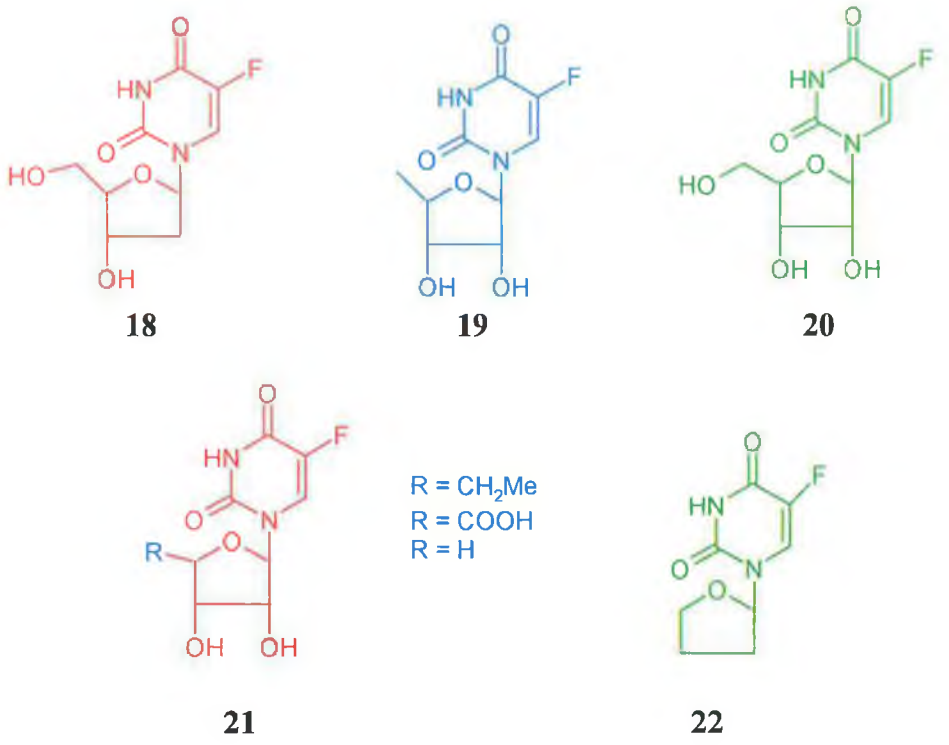
Over the years 5-fluorouracil prodrugs possessing a broader spectrum of anti-tumor activity and fewer toxic side effects have been sought diligently in a number of laboratories. For classification purposes we have decided to divide the various 5-fluorouracil prodrugs into nucleoside prodrug derivatives and base prodrug derivatives.

1.4.2.1. NUCLEOSIDE TYPE 5-FLUOROURACIL PRODRUG DERIVATIVES

As it was known that 5-fluoro-2-deoxyuridine (FUDR) **18** (FIGURE 21) is an active metabolite of 5-fluorouracil many groups used this knowledge to prepare prodrug derivatives possessing the same structural features. The 5-fluoro-2-deoxyuridine, although showing superior anticancer activity in some test systems, had no advantage over 5-fluorouracil when used clinically. This was attributed to its rapid metabolic break down to 5-fluorouracil *in-vivo*, and as the 5-fluoro-2-deoxyuridine [81] cytotoxicity is time dependent this allows for only moderate activity. Another of its metabolites, 5-fluoro-2-deoxyuridine-5-phosphate **11** (FIGURE 17), was synthesised but this also proved not to be an effective chemotherapeutic agent, because it does not penetrate into the cells in sufficiently high concentration.

5-Deoxy-5-fluorouridine (DFUR) **19** (FIGURE 21) [85,86] a structural analogue of the commercial available 5-fluorouridine (5-FURD) **20** (FIGURE 21), exhibits anti-tumor activity with low host toxicity. Its favourable therapeutic index is considered to reflect preferential bioactivation in tumour cells as opposed to normal proliferative tissues. Activation results from cleavage of 5-deoxy-5-fluorouridine to 5-fluorouracil.

Rosowsky *et al.* (1982) synthesised analogues of DFUR to examine the effect of structural modification of the 5'-region of the ribofuranose moiety on the activity of potential pro-drug derivatives of 5-fluorouracil. They showed that a number of the 5'-modified DFUR analogues that they prepared were active *in-vivo* with lower cytotoxicity **21** (FIGURE 21). Another derivative that has received attention over the years is 1-(2-tetrahydrofuryl)-5-fluorouracil (ftorafur) **22** [86] (FIGURE 21) and is used clinically as an effective anti-tumour agent.



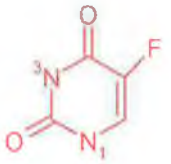
18 **19** **20**

21 **22**

FIGURE 21. STRUCTURES FOR FUDR, DFUR, 5-FURD, DFUR DERIVATIVES AND FTORAFUR

1.4.2.2. Base prodrug derivatives of 5-fluorouracil

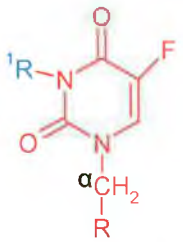
Over the years a series of 5-FU derivatives have been reported. These have been prepared through N^1, N^3 or bis N^1 and N^3 modification **10** (Figure 22). However, because N^1 is the synthetically more reactive amine the majority of 5-FU prodrugs generated have favoured substitution at this position.



10

Figure 22. The location of reactive nitrogen's in 5-fluorouracil

It has been found that lipophilicity can increase the bioavailability of a drug. With this in mind a number of alkylated 5-fluorouracil derivatives **23** (Figure 23) were prepared and tested for anti-tumour activity[87]. These substituted alkyl-5-fluorouracil derivatives were found to possess no anti-tumour activity, presumably because the bond between the 1-nitrogen of 5-fluorouracil and the α-carbon of the alkyl group in such compounds is too strong to break in *in-vivo*.

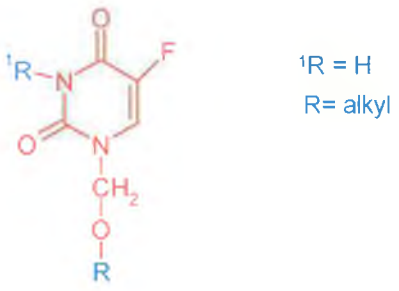


23

^1R = H
R= alkyl

Figure 23. Alkylated 5-fluorouracil derivatives

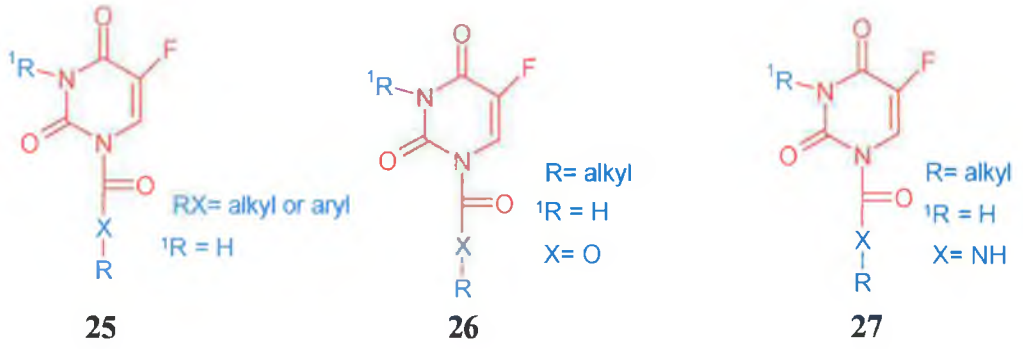
This work led to the preparation of 5-fluorouracil derivatives where an ether oxygen was introduced at the α position of the alkyl group **24** (FIGURE 24), this in turn allowed for the bond between nitrogen and carbon to become more labile to chemical or enzyme hydrolysis conditions, therefore generating 5-fluorouracil.



24

FIGURE 24. ETHER DERIVATIVES OF 5-FLUOROURACIL

The 1-tetrahydrofuryl-, 1-ethoxymethyl- and acyloxyalkyl-5-fluorouracil derivatives belong to this class, and were found to possess moderate anti-tumour activity [88]. Furthermore, it was found that compounds having a carbonyl group bound directly to the N^1 of 5-fluorouracil show very strong anti-tumour activity. Examples of such compounds are 1-acyl-5-fluorouracils (RX= alkyl or aryl) **25**,[86] 1-alkoxy-carbonyl-5-fluorouracils (X=O, R= alkyl) **26**[89] and 1-carbamoyl-5-fluorouracils (X=NH, R=alkyl) **27**[90] (FIGURE 25). But the 1-acyl-5-fluorouracils and 1-alkoxy-carbonyl-5-fluorouracils were unstable in water, and decompose readily[91]. Therefore, both of these prodrug designs are unsuitable for drug use.

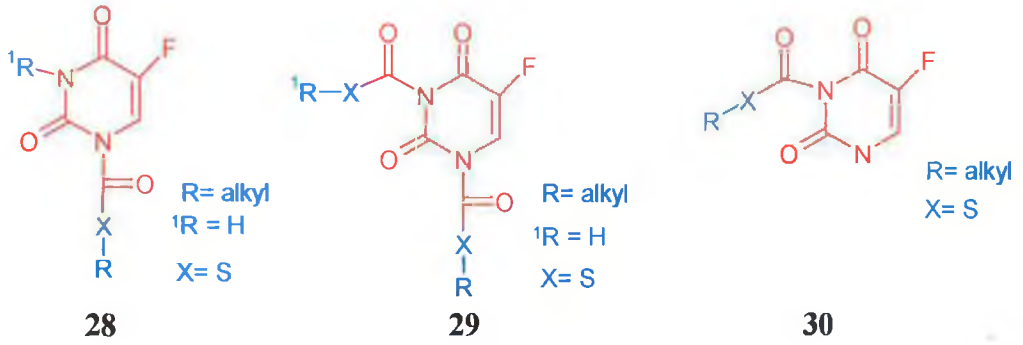


25 **26** **27**

FIGURE 25. CARBONYL DERIVATIVES OF 5-FLUOROURACIL

However, moderate success was found with 1-carbamoyl-5-fluorouracil **27** type prodrugs due to their stability in acidic media and moderate decomposition in neutral media. 1-hexylcarbamoyl-5-fluorouracil (HCFU, Carmofur) has been used extensively on a number of cancers and ranks as one of the top anti-tumour agents used clinically. Nevertheless, it is still accompanied by side effects.

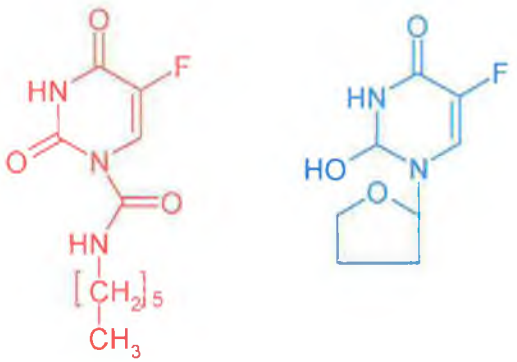
Ozaki *et al.* (1986) synthesised a series of 5-fluorouracil derivatives possessing (alkylthio) carbonyl groups at the 1-, bis-1, 3 and, 3- position **28**, **29** and **30**, respectively (FIGURE 26).[92]



28 **29** **30**

FIGURE 26. ALKYLTHIO CARBONYL 5-FLUOROURACIL DERIVATIVES

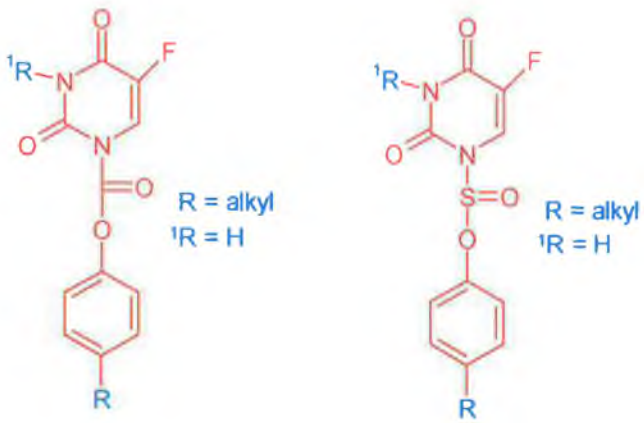
Among them 1-(octylthio) carbonyl-5-fluorouracil showed high activity and a greater *in-vivo* life span when compared to 1-hexylcarbamoyl-5-fluorouracil (Carmofur) **31** and (tetrahydrofuryl)-5-fluorouracil (Tegafur) **32**, which are both in clinical use (FIGURE 27).



31 **32**

FIGURE 27. STRUCTURES FOR CARMOFUR AND TEGAFUR

Other groups have found that the introduction of an alkoxybenzyl **33** and alkoxybenzesulfonyl **34** substituent into 5-fluorouracil at the N^{1}position gave anti-tumour activity equal to 5-fluorouracil and with lower toxicity[93] (FIGURE 28).



33 **34**

FIGURE 28. ALKOXY 5-FLUOROURACIL DERIVATIVES

1.5. 5-fluorouracil as a Tumour Activated Prodrug

While some of the 5-fluorouracil prodrugs discussed have had varying degrees of success, they are still accompanied by adverse side effects due to their poor selectivity for cancer cells. One approach designed to improve 5-fluorouracils selectivity, is to develop a tumour activated prodrug.

1.5.1. 5-Fluorouracil as a TAP activated by therapeutic radiation

Although radiotherapy is one of the most common cancer treatments available, only two general types of radiation-activated prodrugs have been reported. These include prodrug derivatives of 5-fluorouracil and prodrugs that possess an aromatic quaternary salt.[17] Nishimoto *et al.* (1992) reported the electrochemical synthesis of a novel N(1)-C(5')-linked dimer 1-(5'-fluoro-6'-hydroxy-5',6'-dihydrouracil-5'-yl)-5-fluorouracil (FIGURE 29), by anodic oxidation of 5-fluorouracil in an argon purged aqueous solution containing NaCl as supporting electrolyte. They subsequently reported that the 5-fluorouracil dimer underwent a one-electron reduction by hydrated electrons on irradiation of the anoxic aqueous solution, thereby causing the N(1)-C(5') bond to cleave releasing the 5-fluorouracil in high yield.[94] Following on from this they synthesised a series of related 5-fluorouracil dimer derivatives i.e. 5-fluoro-1-(2'-oxocycloalkyl)uracil derivatives (FIGURE 30), in the hope to find a new class of radiation activated prodrugs that release more efficient antitumour 5-fluorouracil under anoxia or hypoxia irradiation. Their study concluded that the 2'-oxo group present in the 5-fluoro-1-(2'-oxocycloalkyl)uracil derivative is a crucial feature in the prodrug design. Its presence allowed for efficient C(1')-N(1) bond dissociation thereby releasing 5-fluorouracil in relatively high yields 47-96%. An open chain derivative (FIGURE 30) **35** was also reported to have shown similar results for 5-fluorouracil

release under both hypoxia and aerobic conditions, but no significant therapeutic effects were observed.[95, 96]

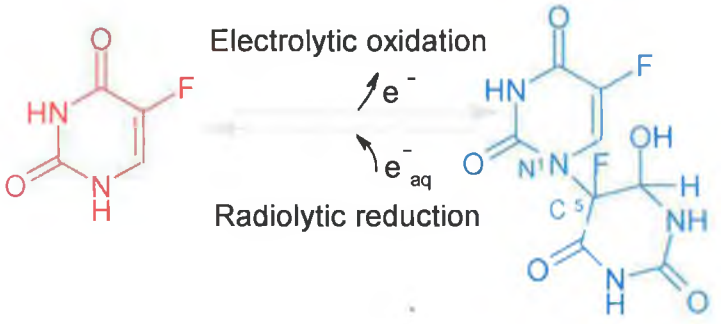
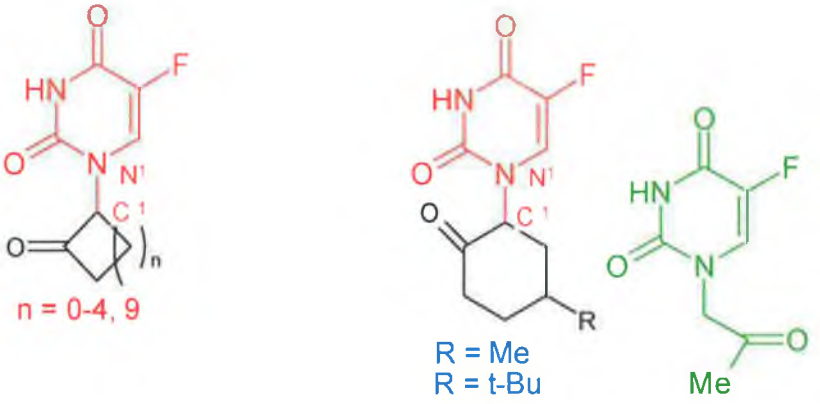


Figure 29. N(1)-C(5')-linked-dimer,1-(5'-fluoro-6'-hydroxy-5',6'-dihydrouracil-5'-yl)-5-fluorouracil



35

FIGURE 30. SERIES OF 5-FLUORO-1-(2'-OXOCYCLOALKYL)URACIL DERIVATIVES AND AN OPEN CHAIN DERIVATIVE **35**

1.5.2. 5-Fluorouracil as a TAP activated by tumour specific enzymes

As mentioned earlier, this concept relies on the prodrug (active drug-peptide) being selectively activated to the drug by enzymes specific to the targeted area. The role of the conjugated peptide is not to physically locate the prodrug on to the tumour cells, but to serve as a substrate for (target) enzymes that are produced and secreted preferentially by the tumour cells.[17] One example of this was reported by Khan *et al.* (2002) were

they attempted to target deliver 5-fluorodeoxyuridine (FUDR) by taking advantage of the unique ability of prostate cancer cells to produce PSA.[97,98,99]. It has been reported that the peptide His-Ser-Ser-Lys-Leu-Gln-OH (HSSKLQ) (FIGURE 31) is a PSA specific peptide substrate and that it is efficiently and selectively cleaved by the enzymatic activity of protease.[100] On the basis of this they synthesised the prodrug by coupling the active drug 5-FUDR to the PSA-peptide via a self-cleaving diamino acid linker to produce the peptide-conjugate prodrug HSSKLQ-Leu-Aib-FudR (FIGURE 32). They have shown that this approach permitted efficient conversion of the inactive 5-fluorouracil derivative back to the active 5-fluorouracil derivative by the enzymatic activity of PSA, which is highly expressed in prostate cells.[101,97]

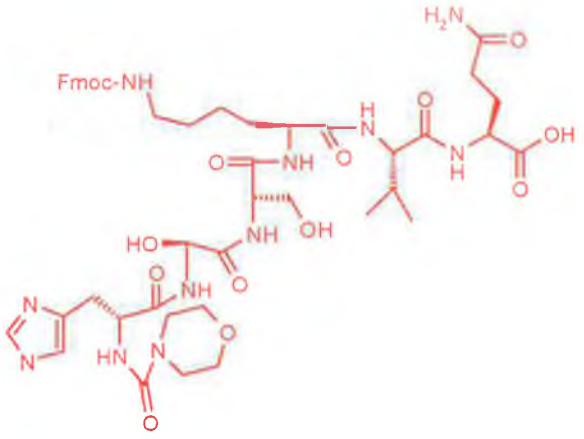


FIGURE 31. PSA SPECIFIC PEPTIDE HIS-SER-SER-LYS-LEU-GLU-OH

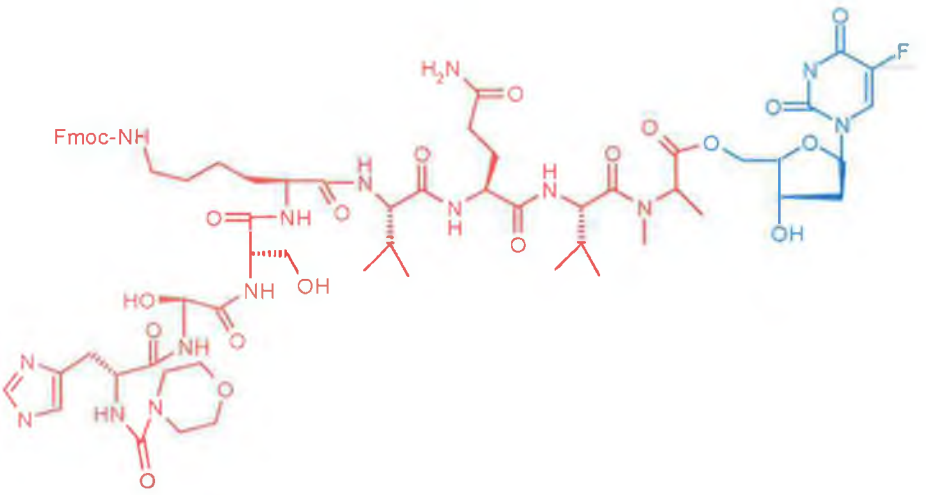


FIGURE 32. PEPTIDE CONJUGATED PRODRUG HIS-SER-SER-LYS-LEU-GLU-LEU-AIB-FUDR.

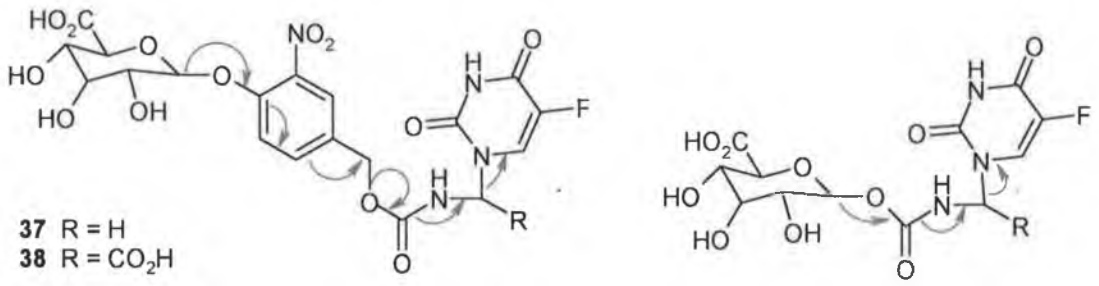
1.5.3. 5-Fluorouracil as a TAP activated by ADEPT

5-Fluorouracil has seen modest use in ADEPT. One group that initially investigated 5-FU use in ADEPT was Wallace *et al.* (1994)[102]. They took advantage of the microbial enzyme cytosine deaminase (CD) which converted the non-toxic anticancer prodrug 5-fluorocytosine (5-FC) (FIGURE 33) **36** into the antitumour agent 5-fluorouracil.[103] They chemically conjugated the CD enzyme to the L6 monoclonal antibody, forming a conjugate that bound to antigens on the H2981 lung adenocarcinoma. In doing this they generated a method where 5-FC **36** could be converted into 5-FU **10** at the tumour site.

36 **10**

FIGURE 33. CONVERSION OF 5-FLUOROCYTOSINE **36** TO 5-FU **10** VIA THE MICROBIAL ENZYME CYTOSINE DEAMINASE (CD).

Another group that used 5-FU as an antigen directed prodrug was the group of Florent *et al.* (1998).[104] They continued on from their initial work on doxorubicin prodrugs, which had seen them utilise one of the glycosidase enzymes, β-glucuronidase, the most widely used activating enzyme in ADEPT. They incorporated the 5-FU into three glucuronide-based prodrugs **37**, **38** and **39** (FIGURE 34). The three prodrugs were designed to be activated at the tumour site, through cleavage of the glucuronly moiety by *E. coli* glucuronidase to afford a rapid release of the corresponding 5-FU. The prodrug design was related to that of a peptide cleavage based mechanism with the liberation of 5-FU, initialised by a 1,6-elimination following enzymatic hydrolysis, to generate an unstable carbamic acid that decomposes to a free amino group (FIGURE 34).[105]

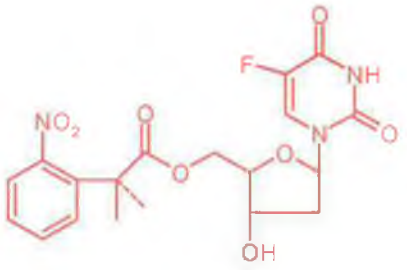


39

FIGURE 34. FLORENT'S GLUCURONIDE-BASED PRODRUGS.

ADEPT has also been used to reduce nitro benzyl carbamates to their corresponding amines or hydroxyamines using the bacterial nitroreductase from *Escherichia coli* B in

conjunction with cofactors NADH or NADPH i.e. 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine (FIGURE 35) **40**.[106, 107]



40

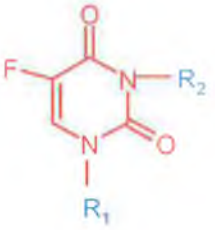
FIGURE 35. 5'-[2-(2-NITROPHENYL)-2-METHYLPROPIONYL]-2'-DEOXY-5-FLUOROURIDINE **40**

1.5.4. 5-Fluorouracil as a TAP activated by GDEPT

With the development of enzyme-prodrug gene therapy some success has been found with the cytotoxin 5-FU when used in GDEPT. This development utilised the non-mammalian enzyme cytosine deaminase (CD) and the 5-FU prodrug 5-fluorocytosine. As in ADEPT the CD enzyme found in bacteria, fungi and yeast selectively converts the non-toxic 5-fluorocytosine to the thymidylate synthase inhibitor 5-FU through hydrolytic deamination. Therefore, when the gene that encodes the CD enzyme is introduced into the tumour cells it possesses the ability to convert the 5-FC prodrug into 5-FU, thereby generating the anti-metabolites 5-FdUMP, 5-FdUTP and 5-FUTP while reducing the toxicity to normal tissue. GDEPT has also been used to reduce nitro benzyl carbamates to their corresponding amines or hydroxyamines using the bacterial nitroreductase from *E. coli* B in conjunction with cofactors NADH or NADPH i.e. 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine**40**.[107-110]

1.5.5. 5-Fluorouracil and TAP activated by tumour hypoxia

Lin *et al.* (1986) first introduced the cytotoxin 5-FU to the bioreductive alkylating agent concept.[111] They synthesised a series of (o-and p-nitrobenzyloxycarbonyl) and (o- and p-nitrobenzyl)-5-fluorouracil derivatives (FIGURE 36) with the potential to be activated preferentially by hypoxia cells using cellular nitroreductase to produce a highly reactive quinone methides and 5-fluorouracil.



R_1 = H, R_2 =$OCOCH_2C_6H_4NO_2$-p

R_1 =R_2 = $OCOCH_2C_6H_4NO_2$-p

R_1 = R_2 $CH_2C_6H_4NO_2$-p

R_1 = $CH_2C_6H_4NO_2$-p, R_2 = H

R_1 = tetrahydrofuran-2-yl, R_2 = $CH_2C_6H_4NO_2$-p

R_1 = 2' deoxyribofuranosyl, R_2 = $CH_2C_6H_4NO_2$-p

FIGURE 36. SERIES OF 5-FU NITROBENZYL AND NITROBENZYLOXYCARBONYL DERIVATIVES.

The group reported that one of the prodrugs that they synthesised, 3-(p-nitrobenzyloxycarbonyl)-5-fluourouracil (FIGURE 37), appeared to be superior to 5-FU in prolonging the survival time of mice bearing intraperitoneal implants of the P388 leukaemia and Sarcoma 180. But, it was later reported that the 3-(p-nitrobenzyloxycarbonyl)-5-fluourouracil showed no hypoxic selectivity in cells, because the reduction potential of the benzene based compound was too low for efficient reduction by cellular NTRs (FIGURE 38).[19] This problem has been some what eradicated since the discovery of the bacterial nitroreductase from *E. coli* B. This FMN containing flavoprotein is capable of reducing certain nitro-groups to the corresponding amines or hydroxylamines in the presence of a cofactor NADH or NADPH. One prodrug of 5-FU which shows significant hypoxia selectivity is 5'-[2-(2-nitrophenyl)-2-methylpropionyl]-2'-deoxy-5-fluorouridine **40**. It was found to release quickly the parent drug FUDR in quantitative yield when placed in reduction conditions so as to

mimic the bioreduction in a hypoxia tumour cell. Numerous nitrobenzyl carbamate derivatives have been metabolised to their corresponding amines with *E. coli* B NTR. A variety of these have been proposed as GDEPT prodrugs in conjunction with NTR.[107] Therefore, 5-FU prodrugs possessing a nitrobenzyl or nitrobenzyl carbamate moiety could be reduced. [60]

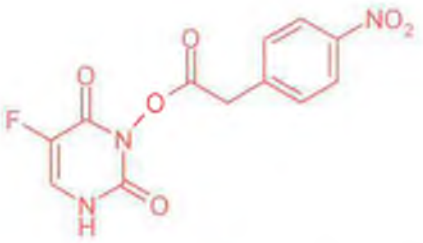


FIGURE 37. PRODRUG 3-(P-NITROBENZYLOXYCARBONYL)-5-FLUOUROURACIL

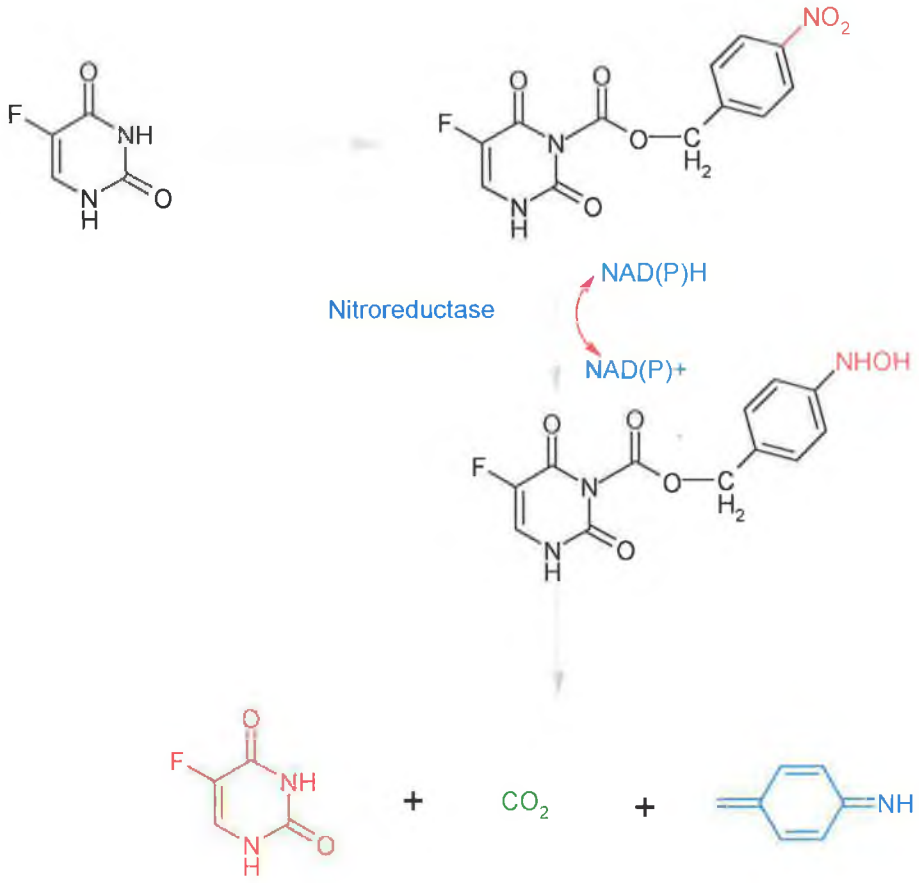


FIGURE 38. REDUCTION OF 3-(P-NITROBENZYLOXYCARBONYL)-5-FLUOUROURACIL BY NITROREDUCTASE

1.6. Photodynamic therapy (PDT)

Like many natural products, the use of light for its therapeutic properties has been well documented throughout history. The Egyptians used light to treat vitiligo and psoriasis by exposing the plaques to sunlight after topical application of bergamot oil. Despite this early awareness, advancement in phototherapeutic techniques had been considerably slow, and it was only in the second half of the last century that real progress was achieved. This development can be credited to advances in the understanding of the interactions between light and biological tissues, along with the arrival of sophisticated optical techniques that helped to illuminate the photophysical, photochemical and photobiological aspects of phototherapy. The two-phototherapeutic techniques available for the treatment of cancer at present are photochemotherapy and phototherapy. In phototherapy, endogenous molecules in the diseased tissue absorb the incident light; while with photochemotherapy the diseased tissue is irradiated at suitable intervals after the topical or systemic administration of a photosensitiser, which should preferentially accumulate in the diseased area.

An example of a relatively new photochemotherapy technique used for the treatment of cancers is photodynamic therapy (PDT). Over the past 25 years results from studies conducted worldwide have established photodynamic therapy as a useful treatment for certain cancer types. Since 1993, the use of Photofrin® (FIGURE 39) a partially purified, commercially available hematoporphyrin derivative has been regulatory approved as a photodynamic therapy agent for cancers of the lung, digestive tract, and genitourinary tract in Canada, The Netherlands, France, Germany, Japan, and the United States.[112-114]

This technique involves the administration of a tumour-localising photosensitising agent, followed by activation of the agent by light of a specific wavelength. This therapy results in a sequence of photochemical and photobiologic processes that cause irreversible damage to tumor tissues.[115]

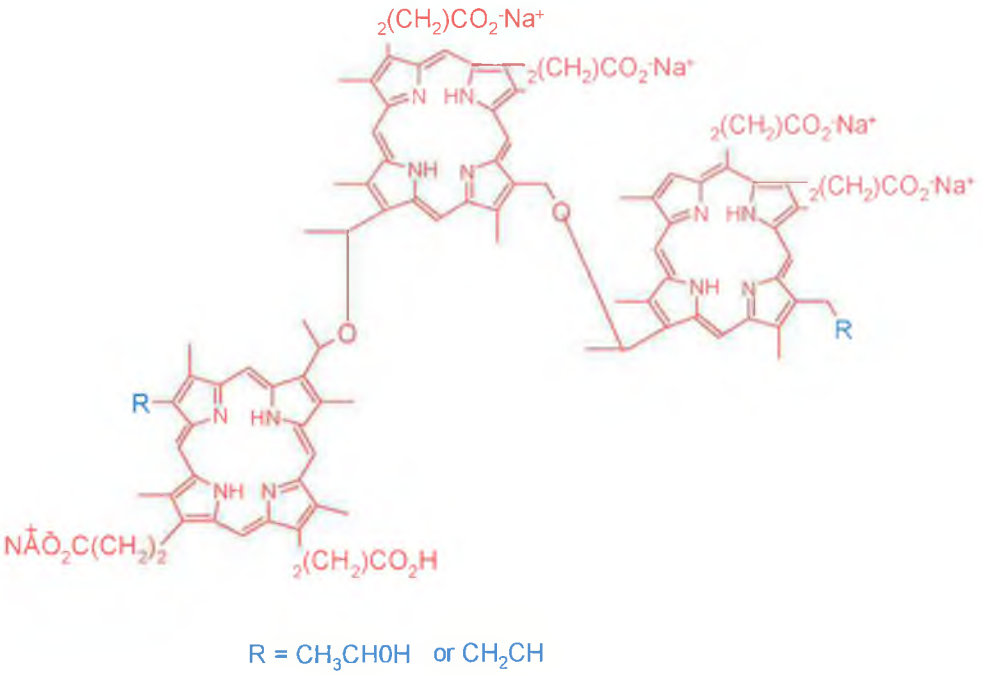


FIGURE 39. STRUCTURE OF PHOTOFRIN®

1.6.1. Basic principles

It has been found that hydrophobic or amphiphilic dyes can readily cross cell membranes and partition in apolar compartments, such as mitochondrial, lysosomal or cytoplasmic membranes, from which they are cleared at a slow rate, owing to tumour tissue typically having poor lymphatic drainage.[116-118] Reports have also shown that the hydrophobic photosensitisers are largely transported in the blood by serum lipoproteins. One component of the lipoprotein family, namely low-density lipoproteins (LDL), play a significant role in the accumulation of these photosensitisers in tumours. However, neoplastic cells express larger amounts of the LDL receptors than most normal cells.

As a result more LDL's containing photosensitisers are taken up by carrier cells via receptor-mediated endocytosis.[119-121]

However, the actual amount of administered photosensitiser is limited since lipoproteins account for approximately 10% of the over all serum proteins, while at least 70% of the lipoproteins are represented by the high-density class. This limitation has been eradicated somewhat by the incorporation of the photosensitiser into a suitable liposomal vesicles, which selectively releases the drug to the serum lipoproteins.[126-129]

Thus, the higher concentration of LDL's and poor lymphatic drainage associated with tumour tissue when compared to normal tissue, could possibly explain why photosensitisers are more selective and retained longer in malignant tissue.

1.6.2. Mechanism of PDT

The photosensitizer (Ps) is illuminated with light of a suitable wavelength to excite it to its lowest excited singlet state So→S1 (FIGURE 40) The excited sensitizer (^{1}Ps*) can then either decay back to ground state S1→So (emitting fluorescence in the process) or undergo intersystem crossing (electron spin conversion) to the long lived reactive triplet state S1→T1 generating the triplet state photosensitiser (^{3}Ps*). At this stage the ^{3}Ps* can undergo two oxidative mechanisms. Type I or Type II mechanisms (SCHEME 1.).[113,126]

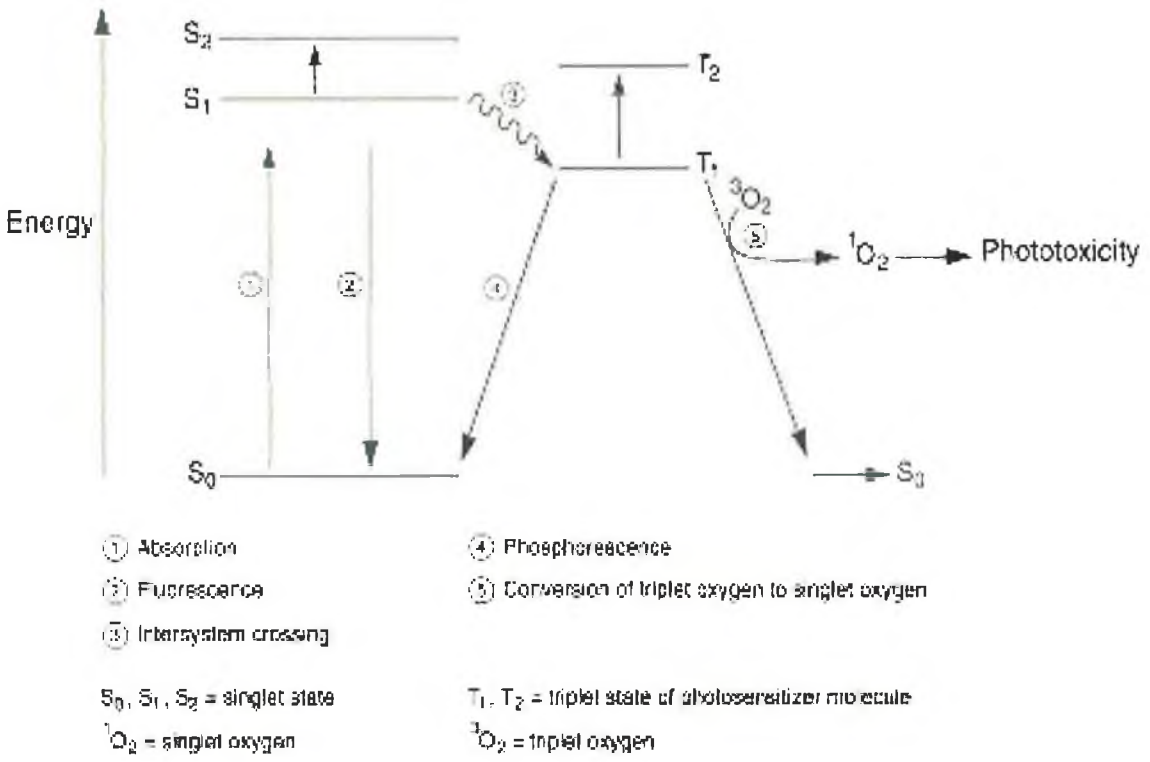


FIGURE 40. POTENTIAL PDT MECHANISM.

Type II mechanism

$Ps \rightarrow {}^1Ps^* \rightarrow {}^3Ps^*$

${}^3Ps^* + {}^3O_2 \rightarrow Ps + {}^1O_2$

${}^1O_2$ + substrate → oxidized substrate

Type I mechanism

${}^3Ps^* + O_2 \rightarrow Ps^{\bullet+} + O_2^{\bullet}$

${}^3Ps^* + \text{substrate} \rightarrow Ps^{\bullet} + \text{substrate}^{\bullet+}$

$Ps^{\bullet} + O_2 \rightarrow Ps + O_2^{\bullet}$

$O_2^{\bullet} \rightarrow HO_2^{\bullet}$

$HO_2^{\bullet}$ + substrate-H → H_2O_2 + substrate$^{\bullet}$

SCHEME 1. TYPE I AND TYPE II MECHANISMS.

In the Type I mechanism the ${}^3Ps^*$ interacts with biological substrates, by proton or electron transfer, to form radicals and radical ions, which react with molecular oxygen to form oxidized products by irreversible oxidation. In the Type II mechanism singlet oxygen (${}^1O_2$) is generated as a result of electronic energy transfer from the triplet excited state of the photosensitiser (${}^3Ps^*$) to the triplet ground state of the oxygen molecule(${}^3O_2$) $T_1 \rightarrow S_0$. Singlet oxygen then attacks electron rich sites in the surrounding medium, thus giving rise to oxidised substrates. While it is believed that

both of the oxidative mechanisms are involved in the photodestruction of malignant tissue it is considered that the Type II mechanism to be the main process in photodynamic therapy.[113,126,127] The oxidative products of both Type I and Type II reactions are highly reactive, with very short lifetimes in biological systems i.e. 40 nanoseconds. Therefore, damage caused by these species occurs locally i.e. radius of action of 0.02μm, tissue damage will be limited to the illuminated areas containing both the photosensitizer and oxygen.[128,129]

1.6.3. Mechanisms of tumour destruction

It has been postulated that three mechanisms could be involved in the irreversible damage of tumour tissue when treated with PDT. These include the following:[112]

1) Direct killing of malignant cells, largely as a consequence of impairment of mitochondrial functions and the alteration of mitochondrial/cytoplasmic membranes.
2) Indirect cell killing due to photodynamic damage or shutdown of the (neo) vascular with loss of oxygen and nutrients to the tumour owing to disruption of the endothelium.
3) Additional anti-tumour contributions from the inflammatory and immune response of the host.

Although all three are thought necessary for the best long term response, their relative roles will vary considerably depending on the photosensitiser, its sub cellular and tissue distribution, the tumour type and its microvasculature, and the type and duration of the inflammatory and immune response elicited in the host.

Also, as a consequence of PDT the singlet oxygen generated by the photosensitiser will affect practically all of the cellular organelles due to the wide and varied sensitiser distribution within the cells. Subsequently, because there are multiple cellular targets

for the singlet oxygen it has been proved difficult to distinguish between the events leading up to cell death. However, with the ongoing development in this area, more sensitive techniques are becoming available for the detection of photosensitiser in cells. With the use of innovative molecular and biochemical strategies the mapping of cellular events before and after photosensitisation, is bringing some clarity to the process of cell death via toxic oxygen treatment. Although there are multiple cellular targets available to the toxic oxygen one of the cellular components that has been studied and targeted by PDT in preference to others is the mitochondria.[130]

1.6.4. General properties of PDT sensitisers

In general, the majority of photosensitisers employed in cancer phototherapy have been based on porphyrins (FIGURE 41).

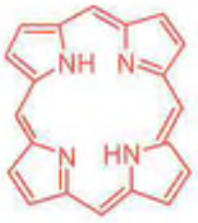


FIGURE 41. PORPHYRIN SKELETON

Hematoporphyrin and its chemical derivatives; hematoporphyrin derivative (HpD), and its purified forms Photofrin®, Photosan® and Photochem®, were the first photosensitisers employed in PDT and are referred to as first generation photosensitisers (FIGURE. 42).[113] However, although these photosensitisers are known to selectively accumulate in a variety of solid tumours for prolonged periods of time, they do suffer from three major drawbacks.[129]

1) Low selectivity between tumours and peritumoral tissue, consequently, skin photosensitivity is a major side effect.

2) Absorption in the red region is weak i.e. 630nm, and so deep tumours are difficult to treat.

3) The photosensitisers are a mixture of at least 12 components.

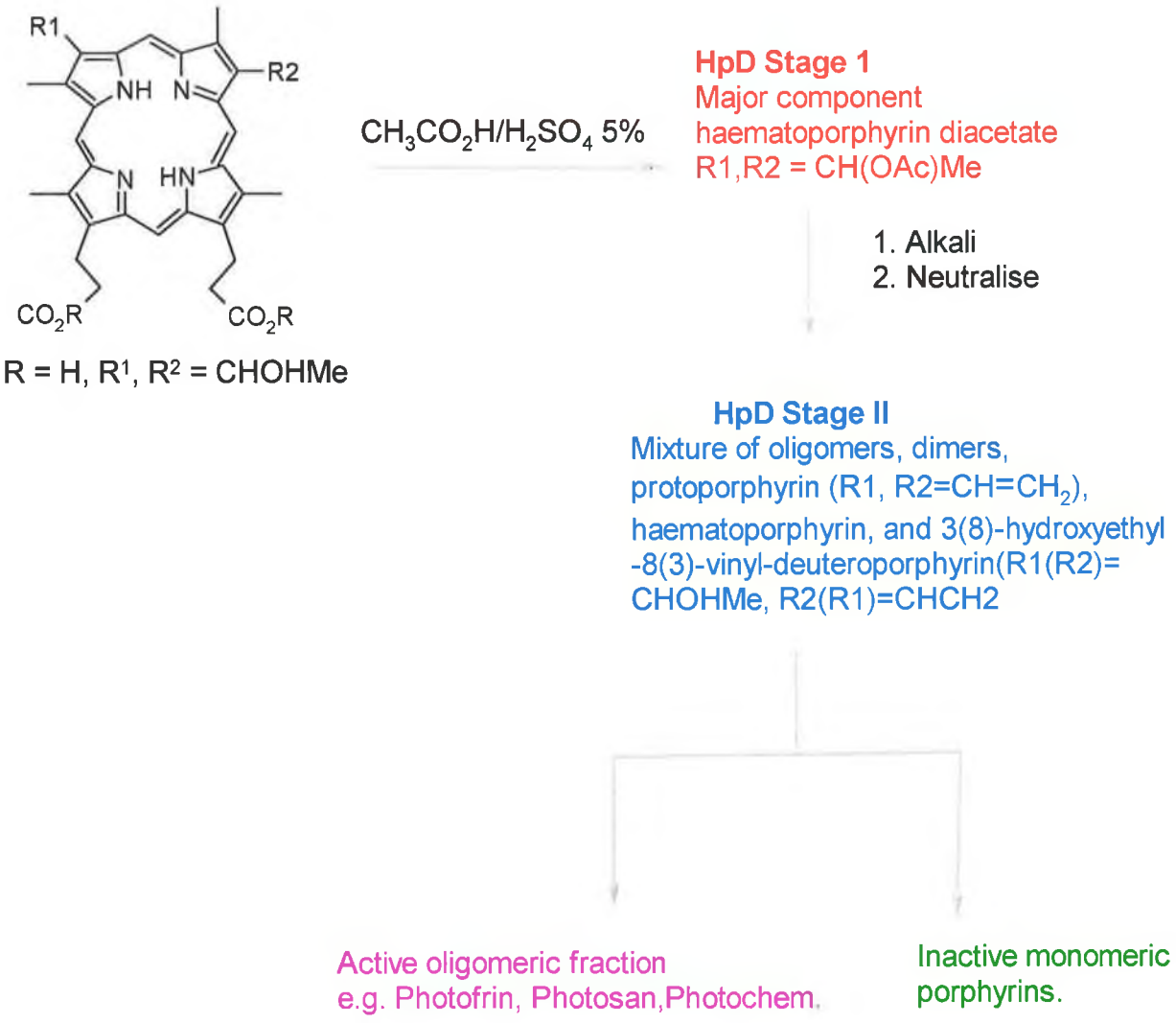
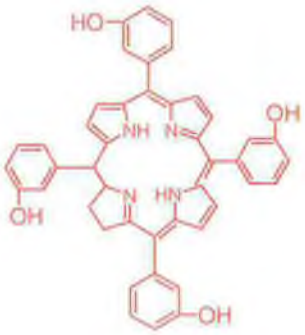


FIGURE 42. SYNTHESIS OF HPD

As a result numerous new photosensitisers, referred to as second-generation photosensitisers, have been developed to try and overcome these limitations. This development has in turn led to the synthesis of a large number of novel photosensitisers with a variety of structures ranging from porphyrin-type compounds e.g. chlorins (41) and bacteriochlorins **42** to phthalocyanines **43**, porphycenes **44** methylene blue **45** and texaphyrins **46** (FIGURE 43). In designing an improved photosensitiser, a number of factors need to be considered and are outlined in TABLE 4:[131-133]

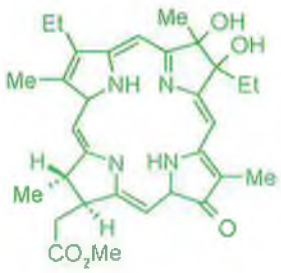
TABLE 4. SOME OF THE PROPERTIES A SECOND GENERATION PHOTOSENSITISER SHOULD POSSESS.

Physico-chemical	A photosensitiser should be easy to synthesise.
	Have high chemical purity.
	Possess a constant composition.
	Large molar extinction coefficient in the red to absorb beyond the biological window of 650nm.
	Low tendency to aggregation in an aqueous environment.
Photophysical	Possess a high triplet quantum yield.
	Long triplet lifetime.
	Have a triplet energy >94kjmol^{-1} with efficient transfer to produce singlet oxygen.
Pharmacological	Efficient and high selective targeting for tumour tissue.
	Fast clearance from the serum and healthy tissue i.e. easy elimination from the body.
	Possess little or no dark toxicity.
	Low systemic toxicity.
Photo-therapeutic	Efficient and preferential killing of malignant cells.
	Lack of side effects.
	Lack of mutagenic potential.



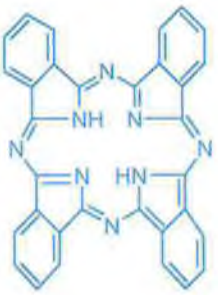
Chlorine

41



Bacteriochlorine

42

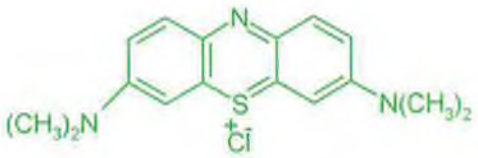


Phtalocyanine

43

Porphycene

44



Methylene blue

45

Texaphyrin

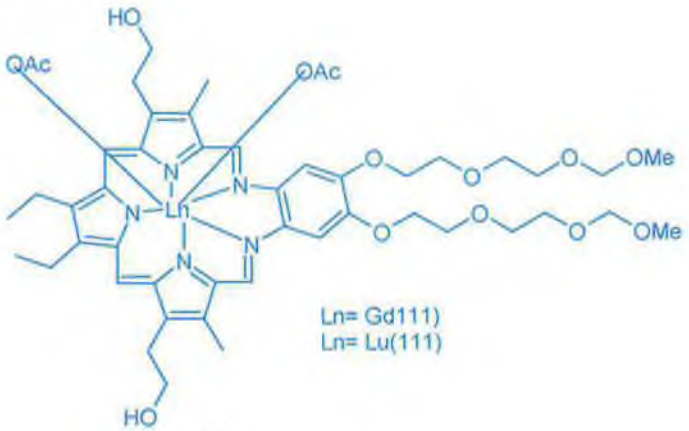


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FIGURE 43. 2ND GENERATION PHOTOSENSITISERS

Of the numerous photosensitisers available for PDT the phthalocyanines (Pc) **43** (FIGURE 43) have received much attention.[133-136] This has been partially due to their associated advantages when compared to Photofrin®. These include: purity, hydrophobicity (which has been reported to increase the affinity of photosensitiser for neoplastic tissue), higher extinction coefficients, excellent absorption range 650-800 nm (FIGURE 44), and no dark toxicity. However, even with these advantages they are associated with poor water solubility thus limiting their use for cancer therapy.

1.6.4.1. IMPROVING THE WATER SOLUBILITY OF PHTHALOCYANINES

Because Pcs can chelate to both transition and non-transition metals, namely aluminium, gallium, zinc and silicon, substitution of axial ligands can occur and be placed orthogonal to the molecular plane. Not only does this prevent aggregation but, it also allows for ligand modification, thus influencing the lipo/hyro-solubility of the Pc so its partition among the various compartments of the tumour tissue can be somewhat controlled. For example in order to increase the hydrophilicity of the Pc so as to allow for direct injection polar substituents have been attached to the peripheral positions of the macrocycle e.g. sulphonic acid, carboxylic acid and hydroxy groups. Also the macrocyclic photosensitiser silicon (IV)-phthalocyanine **47** (FIGURE 45) bearing two axial ligands to the centrally coordinated metal ion e.g. poly(ethylene glycol) chains, has been demonstrated to have improved the physicochemical properties and pharmacokinetic behaviour of the PDT photsensitiser. This is attributed to the lowering of aggregation between Pc rings thus increasing the lifetime of the sensitiser. Alternatively, hydrophobic photosensitisers can be administered into different delivery systems such as liposomes or oil emulsions.[137,138,134]

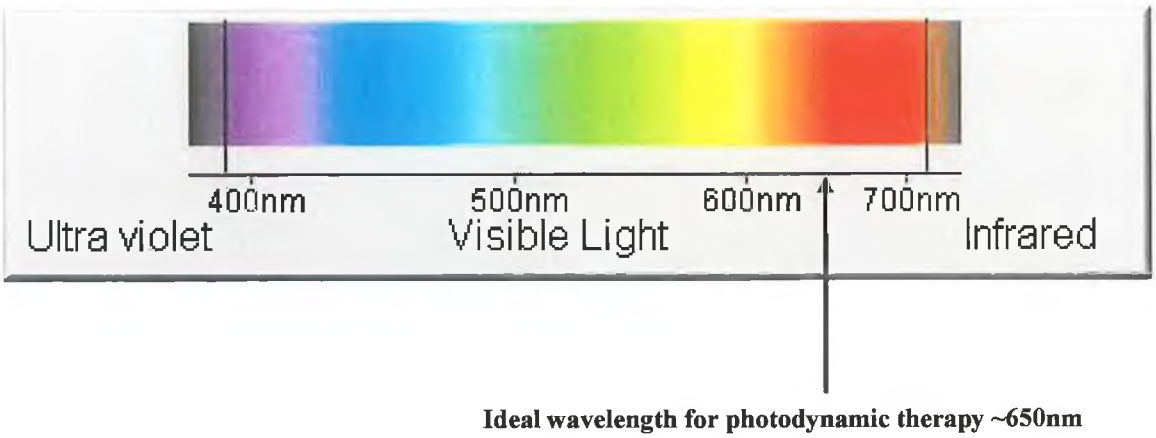
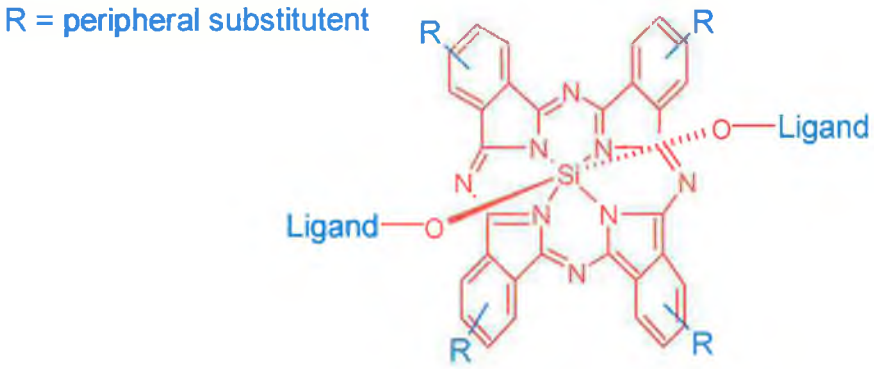


FIGURE 44: IDEAL WAVELENGTH FOR PHOTODYNAMIC THERAPY



47

FIGURE 45. SILICON(IV)-PHTHALOCYANINE

1.7. Project Proposal

It is a common fact that when PDT is used on tumour tissue, the cells that localise the photosensitiser become terminal as a consequence of the toxic oxygen generated. As previously mentioned (section 1.6.3.) it had been thought that these cells die via one or as a result of all three of the aforementioned mechanisms. A shortcoming associated with the use of PDT treatment is its duration as once exposure to light is "ceased" the treatment stops. Therefore, it has been found that the entire tumour mass may not be destroyed during the light treatment, and as a result will only shrink in size, consequently redeveloping over time, which is obviously an undesirable outcome.

Ideally, it would be desirable to have a normal "dark" cytotoxin present with the photosensitiser. Thus, when exposure to light has been "turned off" the dark cytotoxin can be released and destroy any remaining living tumour tissue. Hence, it is the goal of this research to explore and develop ways in which to accomplish this. One possible way to achieve this is to develop a photosensitiser-prodrug that can be activated by the conditions generated during PDT treatment.

1.7.1 Cell response to oxidative stress

Cells constantly generate reactive oxygen species (ROS) during aerobic metabolism. To counter this effect, the cell possesses a number of antioxidant enzymes and repair activities that detoxify the ROS and repair damage caused by them. During normal cell growth most of these enzymes are expressed at low levels. However, bacterial, yeast and mammalian cells all have adaptive responses to deal with elevated levels of oxidative stress and the expression of many antioxidant enzymes are induced (TABLE 5). PDT causes oxidative stress within the environment where the photosensitisers are localised, thereby causing the cell to produce increased levels of reductase enzymes [139-148]. We believe that we can manipulate this oxidative stress to our advantage. What we

proposed to do is to link a "redox" activated prodrug into the photosensitiser. This prodrug will be activated by the reductase produced by the tumour to combat the oxidative stress of PDT, resulting in the release of a dark therapeutic. When the light therapy ceases the dark therapeutic will remain behind to destroy surviving tumour tissue. Outlined in FIGURES **46**, **47** and **48** are the target compounds we are interested in. FIGURE 46 shows the trigger-linker-effector design with 5-FU, with the mechanism of reductase activation shown in FIGURE 47. FIGURE 48 shows the final target with the trigger-linker-effector conjugated into a phthalocynin photosensitiser.

TABLE 5. LIST OF SOME OF THE ANTIOXIDANT ENZYMES EXPRESSED TO PROTECT THE CELL AGAINST DAMAGE CAUSED BY OXIDATIVE STRESS.

ANTIOXIDANT ENZYMES
Manganese superoxide dismutase
Fumarase C
Aconitase A
Glucose-6-phosphate dehydrogenase
Ferredoxin reductase
Endonuclease IV
Iron superoxide dismutase
Copper-zinc super oxide dismutase
Glutathione reductase
Hydroperoxidase I and II
Exonuclease III
8-hydroxyguanine endonuclease
Exonuclease III
Methionine sulfoxide reductase

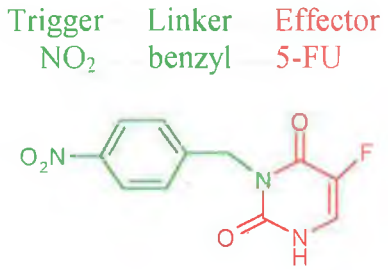


FIGURE 46. ORIGINAL DRUG DESIGN.

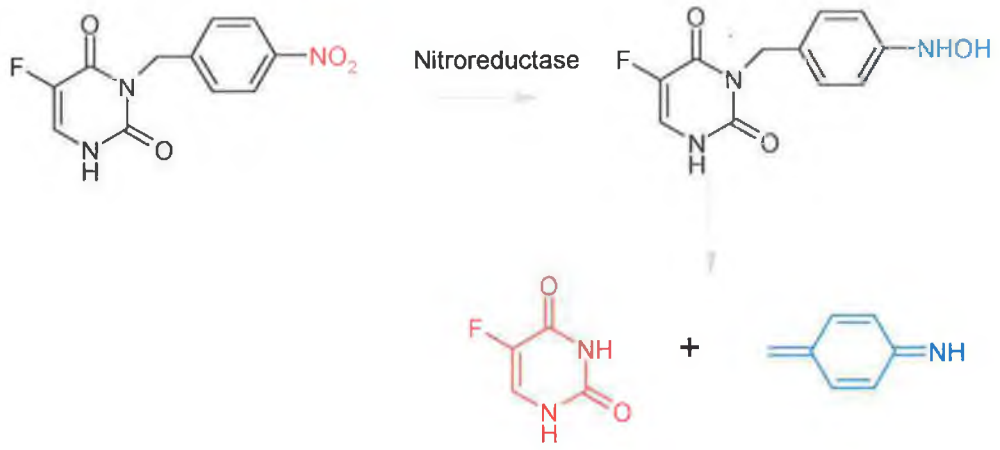
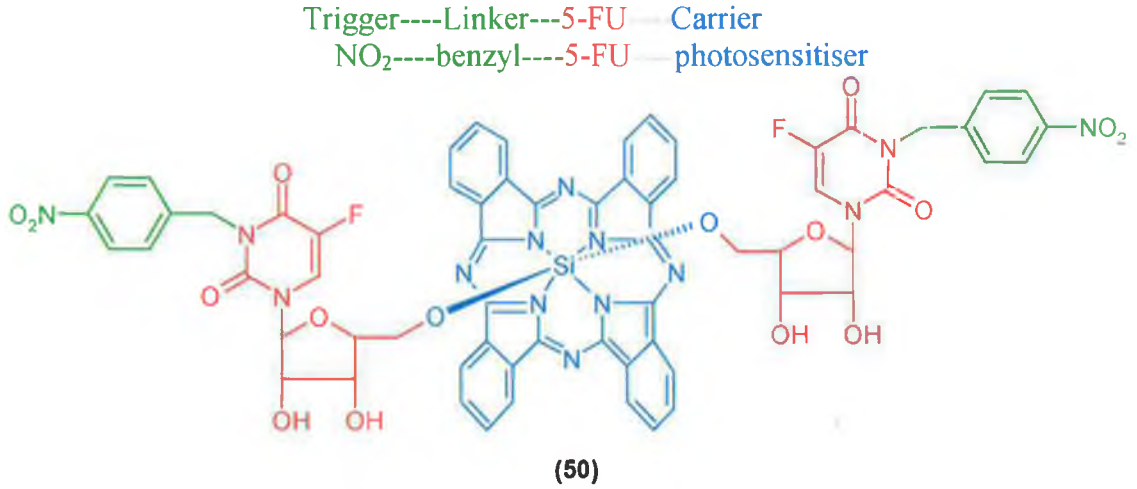


FIGURE 47. SCHEME FOR REDUCTASE OF NITRO BENZYL 5-FU.



(50)

FIGURE 48. MODIFIED DRUG DESIGN FOR (50).

However, we envisaged a potential problem with the usage of a nitro-aromatic trigger; do human reductase enzymes (TABLE 2.) possess the necessary redox potential to

efficiently reduce nitro-aromatics? As a result we deemed it necessary to prepare a series of nitro-aromatics with varying redox potentials and conjugate them to the appropriate photosensitizer. However, if the human reductases are not able to reduce our trigger it will still be possible to activate our prodrug through the action of GDEPT and *E. coli* nitro reductase (FIGURE 49).

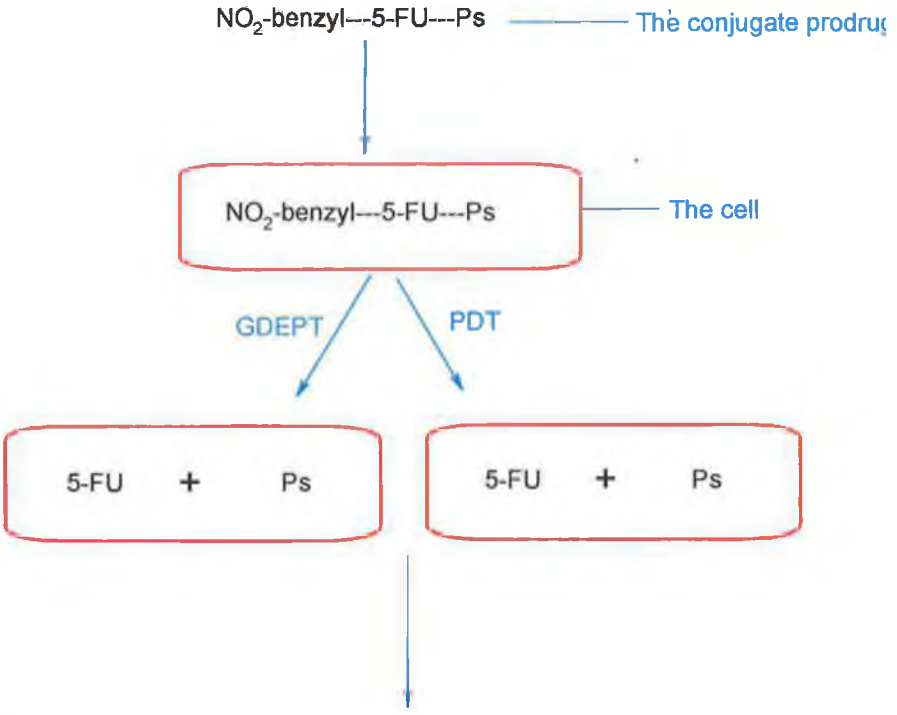


FIGURE 49. GENERAL SCHEME FOR 5-FU PRODRUG ACTIVATION.

2.0. Discussion – Prodrug Photosensitisers

The strategy we propose for preparing the target compound **50A** (FIGURE 50), involves the conjugation of a trigger-effector to a photosensitiser. FIGURE 50 outlines a general approach to the trigger-effector-linker and the final target.

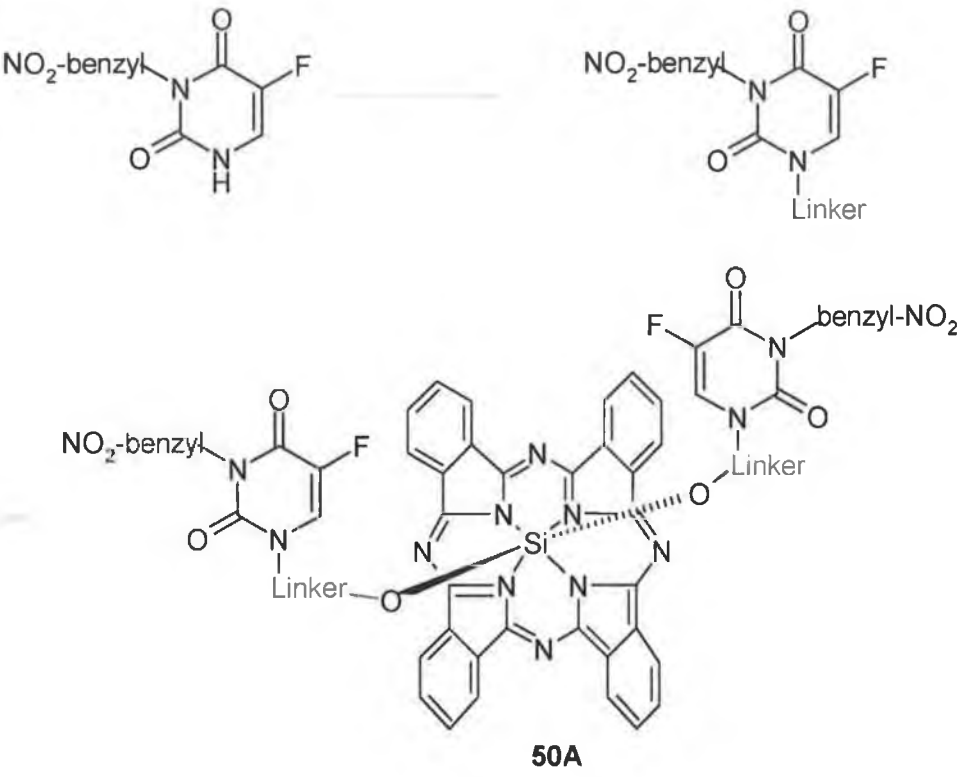


FIGURE 50. PROPOSED SYNTHETIC STRATEGY FOR THE NITROBENZYL-5-FU PRODRUG

2.0.1. Prodrugs of 5-fluorouracil containing the nitro-aromatic moiety

We decided to start our study with the preparation of nitro-aromatic-5-FU-prodrugs that would best suit our prodrug design (FIGURE 50). After much review our attention was turned to Lin *et al.* (1986) and their work on nitro-aromatic-5-fluorouracil derivatives. The prodrugs that they designed seemed ideal for our study, as they were simple to prepare and provided a means to test our hypothesis. In order for our prodrug to be linked to a photosensistiser the N^1 of the 5-fluorouracil needed to be free for substitution. Lin *et al.* (1986) accomplished this by selectively synthesising a series of

nitro-aromatic-5-fluorouracil-bioreductive agents. However, of the 5-FU derivatives reported we were only interested in two, namely, 3-(p-nitrobenzyloxycarbonyl)-5-fluorouracil **51** and 3-(p-nitrobenzyl)-5-fluorouracil **52** (FIGURE 51).

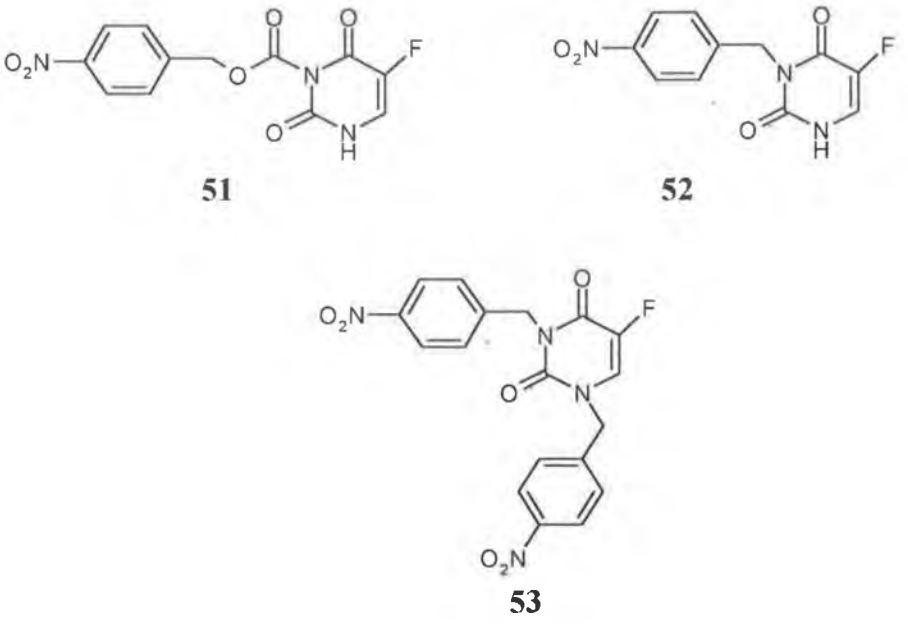
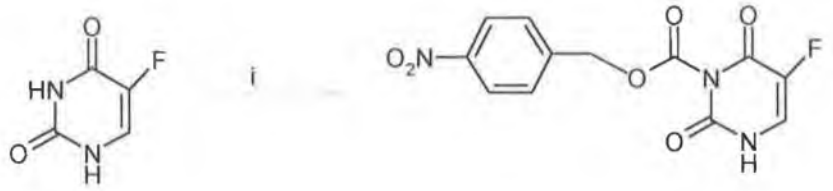


FIGURE 51 STRUCTURE OF 3-(NITROBENZYLOXYCARBONYL)-5-FU, 3-(NITROBENZYL)-5-FU AND 1,3-(NITROBENZYL)-5-FU PRODRUGS

2.0.2. Preparation of 3-(p-nitrobenzyloxycarbonyl)-5-fluorouracil

The preparation of 3-(p-Nitrobenzyoxycarbonyl)-5-Fluorouracil **51** was attempted using the Lin *et al.* (1986) protocol for selective N^3 substitution (FIGURE 52). An equimolar amount of 5-FU and triethylamine (TEA) were dissolved in dry DMSO and stirred at room temperature. To this reaction a solution of nitrobenzyl chloroformate in dry dioxane was added drop-wise over 10 minutes at room temperature. The reaction mixture was allowed to stir at room temperature for approximately 4 hours. TLC monitoring indicated that the reaction was completed within 3 hours. The $Et_3N{\cdot}HCl$ salt that was formed during this time was filtered and washed with a minimum amount of DMSO. The solution was then poured into a mixture of ice and water to precipitate the crude white product. TLC analysis indicated the presence of product plus some

unreacted nitrobenzyl chloroformate. The latter was removed by washing the product mixture with dry chloroform, as the benzyl chloroformate is soluble in chloroform while the product is not. This method was repeated once more until TLC analysis showed only product, which was dried to yield the product **51** in 58% yield.

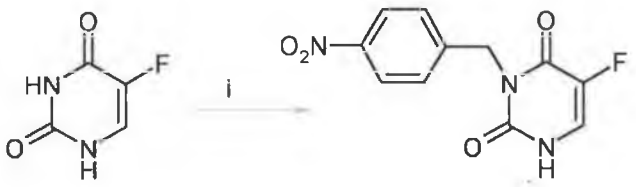


10 **51**

FIGURE 52. PREPARATION OF **51**. Reagents i) DMSO, Et_3N, NBC at RT.

2.0.3. Synthesis of 3-(p-nitrobenzyl)-5-fluorouracil

We decided to use 1 mole equivalents of potassium carbonate, p-nitrobenzyl bromide and 5-FU, these were dissolved in DMSO. The reaction was heated to 100°C for one hour, TLC analysis showed the presence of two spots. To the hot reaction mixture was added a 10% potassium hydroxide solution, crystals formed and were collected, washed with H_2O and ethanol to yield 1,3-Bis- (p-nitrobenzyl)-5-fluorouracil **53** in 20% yield. The mother liquor was acidified to a pH of 2 with HCl that resulted in the formation of white crystals that were collected and washed with H_2O and ethanol to yield 1-(p-nitrobenzyl)-5-fluorouracil **52** in 20% yield .



10 **52**

FIGURE 53. PREPARATION OF **52**. Reagents i) DMSO, K_2CO_3, NBB AT 100°C.

2.0.4. Synthesis of N^1 methoxy ether-5-fluorouracil

Both of these prodrugs were then examined for their suitability for linkage to a photosensitiser. Although a number of possible routes were available to achieve this we decided to use a methyl hydroxy linker (FIGURE 54). Since 5-FU possesses two acidic NH moieties we can generate a bis hydroxymethyl 5-FU prodrug derivative (FIGURE 55).

FIGURE 54. GENERAL SCHEME FOR N-HYDROXYMETHYLATION

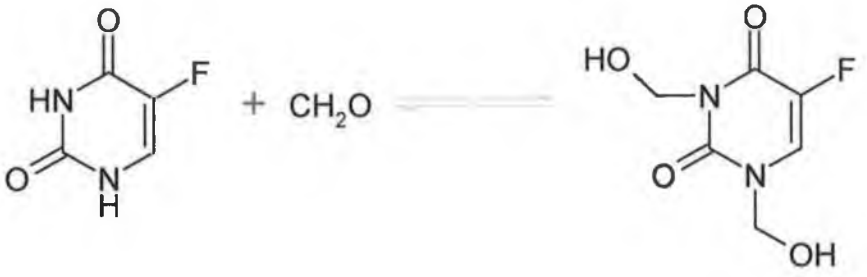


FIGURE 55. REACTION SCHEME FOR N-HYDROXYMETHYLATION OF 5-FU

The hydroxy moieties can act as handles for the 5-FU prodrug to be attached to the photosensitiser, furthermore this methyl ester handle can be fragmented in vivo releasing the active 5-FU and the Pc (FIGURE 56).

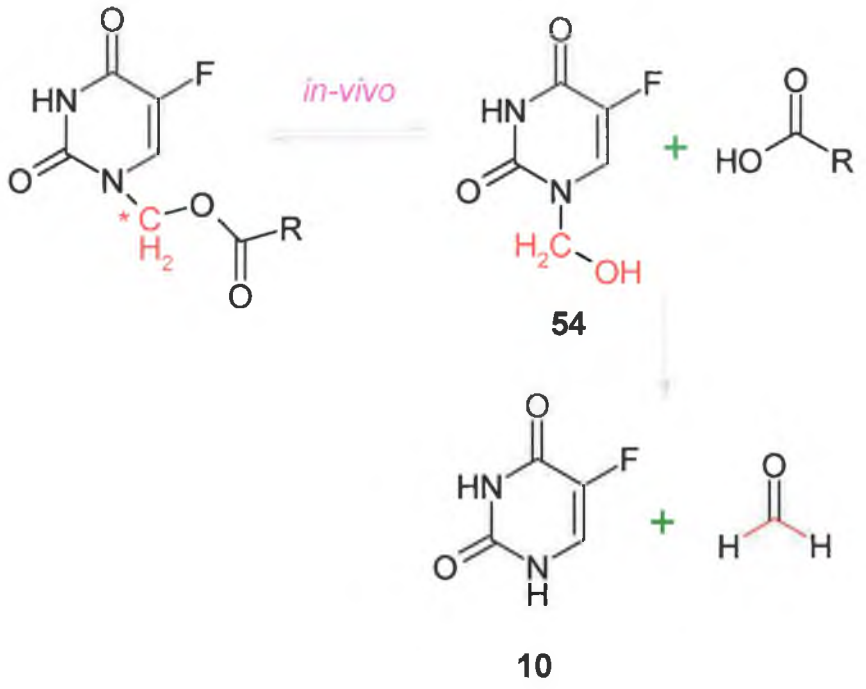


FIGURE 56. *IN-VIVO* DECOMPOSITION OF A TYPICAL N-HYDROXYMETHYL 5-FU PRODRUG

The synthesis and suitability of such methyl hydroxy prodrug derivatives were investigated.

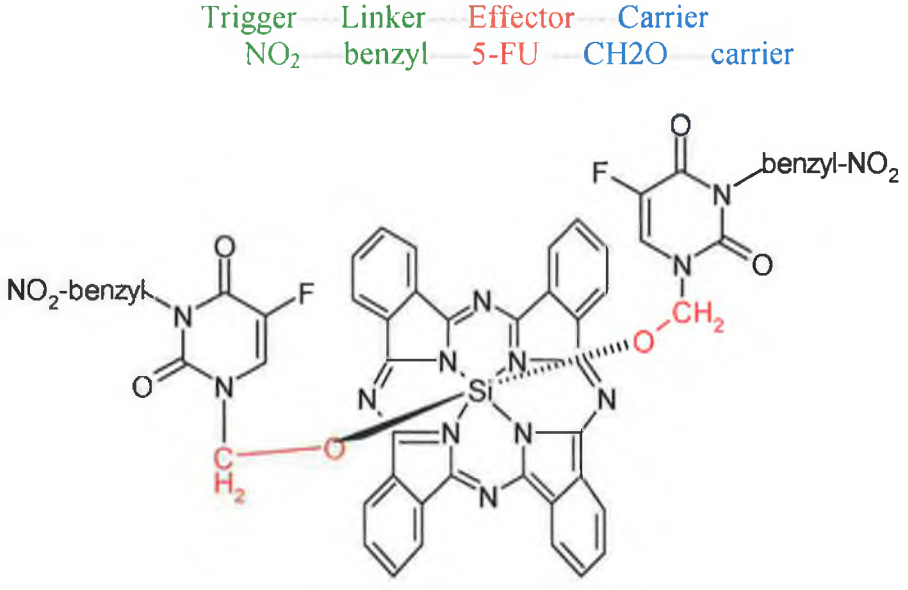
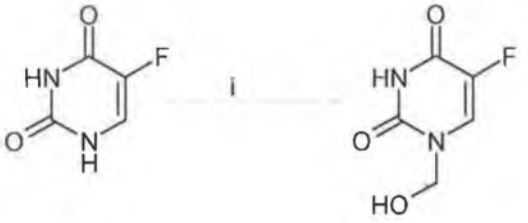


FIGURE 57. PRODRUG WITH METHYL HYDROXYL LINKERS

2.0.5. Preparation of N^1 methoxy ether-5-fluorouracil

The synthesis of the N^1 methoxy ether-5-fluorouracil **54** (FIGURE 58) was achieved using 30% formalin. The temperature was not allowed to exceed 60°C as this led to decomposition of the product back to the starting material 5-FU (polymerisation has also been reported).[157]



10 **54**

FIGURE 58. REACTION SCHEME FOR THE FORMATION OF 5-FU. Reagents i) 30% formalin at 60°C.

However, although the prodrug design is theoretically sound the synthesis of the target hydroxy methyl derivative-nitrobenzyl-5-FU prodrugs **55** and **56** (FIGURE 59) was unsuccessful. Instead of obtaining the desired products we obtained the fragmented prodrug starting materials for the reaction of **51**, while **52** did not react at all.

To try and overcome this problem a temperature study was carried out. TABLE 6 outlines the results of these studies. Unfortunately, changing temperature was deemed unsuccessful as neither **55** or **56** were generated.

TABLE 6. OUTLYING RESULTS FROM TEMPERATURE STUDY

Required Prodrug	Temperature°C	Prodrug formation
55/56	Ambient	No/No
55/56	30°C	No/No
55/56	40°C	No/No
55/56	70°C	No/No
55/56	Reflux	No/No

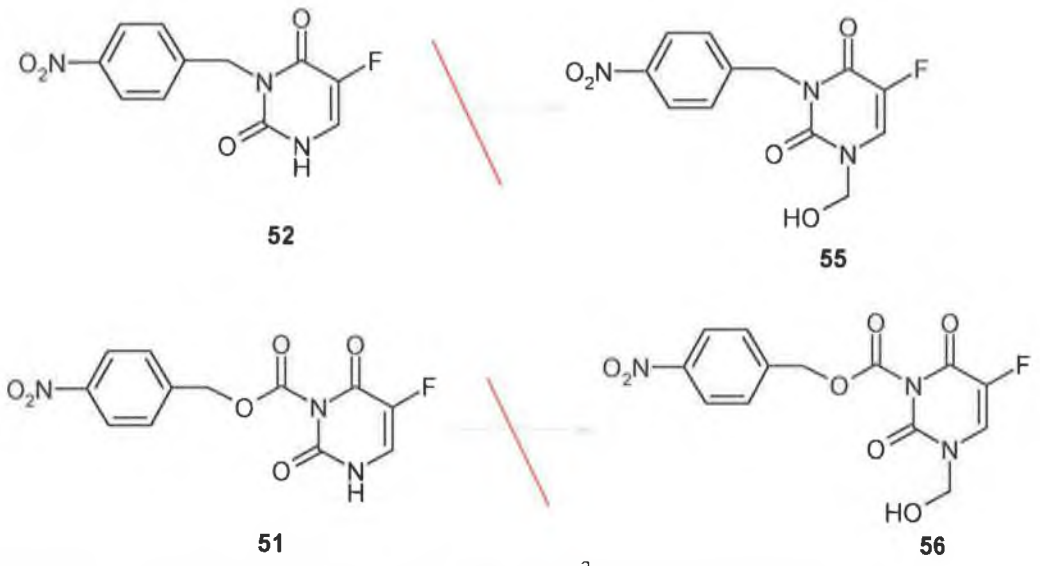


FIGURE 59. UNSUCCESSFUL SYNTHESIS USING N^3-AROMATIC-5-FU.

Following on from this we decided to use an alternative pathway (FIGURE 60) by starting with the hydroxy methyl-5-FU prodrug **54**. Following a modified procedure to the one Lin *et al.* (1986)[37] used for the synthesis of **51** we set up two reactions, one containing a solution of nitrobenzyl chloroformate in dry dioxane while the other contained the nitrobenzyl bromide. Both were added dropwise to a solution of hydroxy methyl-5-FU **54** in dry DMSO containing the base triethylamine. The reactions were allowed stir at room temperature while monitored by TLC. After 5-6 hours only the nitrobenzyl chloroformate reaction showed evidence of product formation. At this stage we quenched the reactions by pouring onto ice-cold water. The precipitate that formed was isolated and collected. On analysis it was observed that the reaction had in fact not worked and that the new spots were that of the starting materials. The reaction was repeated using the same reagents in different stiochiometries (TABLE 7). However, none of these changes were successful.

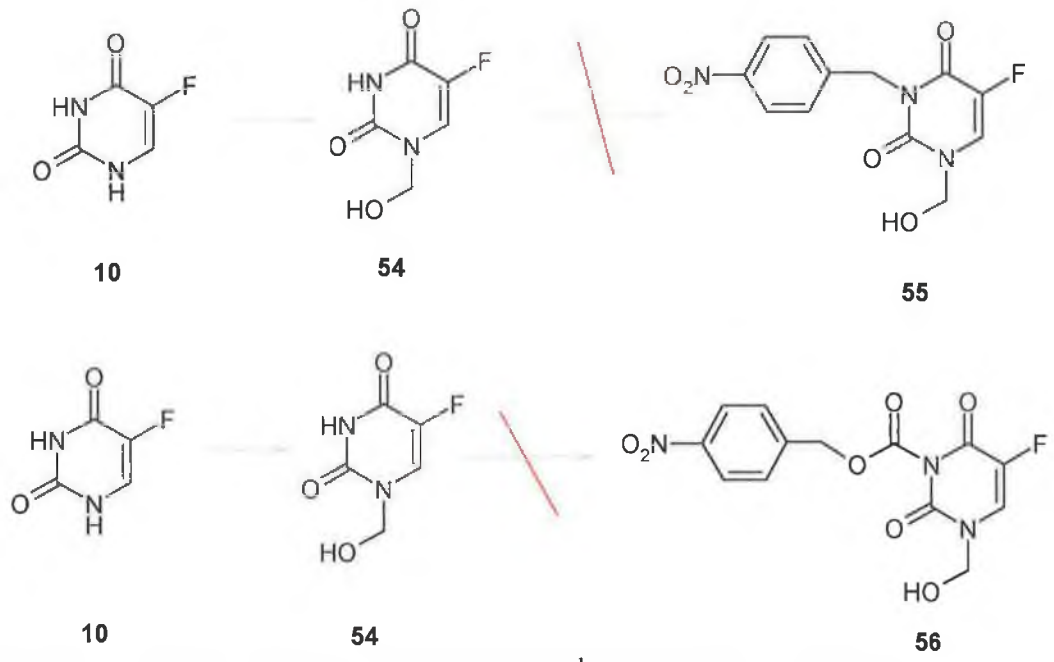


FIGURE 60. UNSUCCESSFUL SYNTHESIS USING N^1 METHOXY ETHER-5-FU

TABLE 7. RESULTS FOR STIOCHMETRIC ADDITION OF NITRO BENZYL DERIVATIVES.

Nitrobenzyl chloroformate Equiv. Amt.	Nitrobenzyl bromide Equiv. Amt.	Prodrug formation
1	1	No
2	2	No
3	3	No
4	4	No
5	5	No

TABLE 8. RESULTS FROM DILUTION STUDY.

Nitrobenzyl chloroformate DMSO vol	Nitrobenzyl bromide DMSO vol.	Prodrug formation
2.5	2.5	No
5	5	No
10	10	No
15	15	No
30	30	No

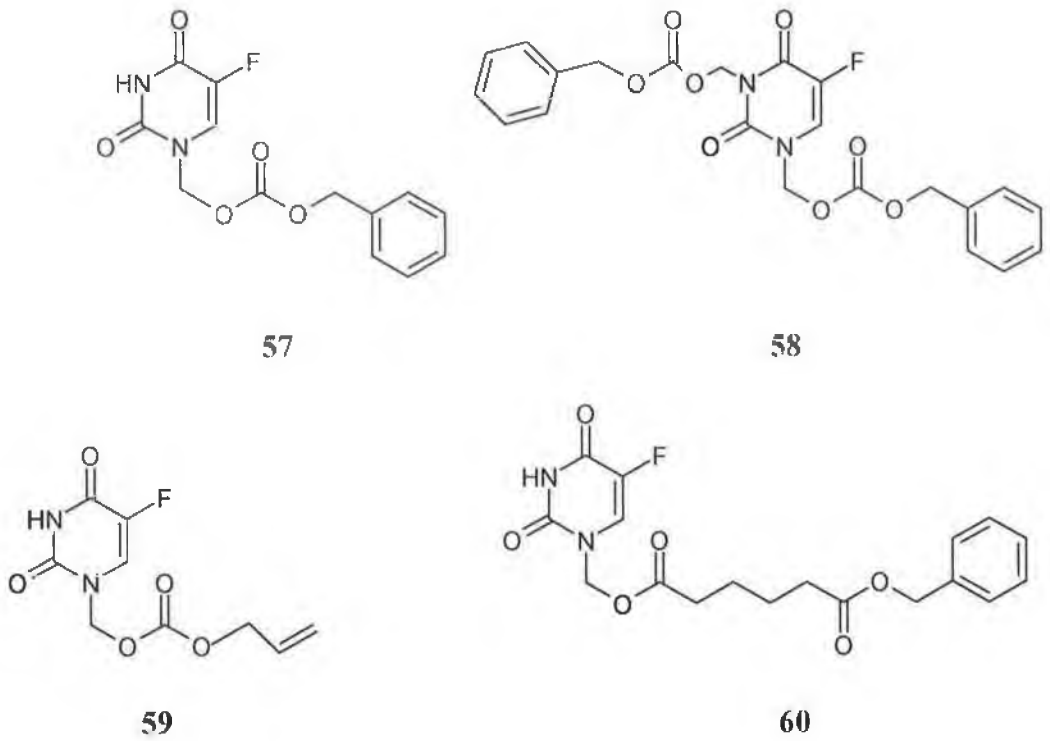
TABLE 9. RESULTS FOR BASE STUDY.

Base type	Nitrobenzyl chloroformate Prodrug formation	Nitrobenzyl bromide Prodrug formation
K_2CO_3	No	No
KOH	No	No
Pyridine	No	No
DBU	No	No
NaOH	No	No
TEA	No	No

Thus, both pathways to prepare the target N^1 methoxy 5-FU prodrugs **55** and **56** failed. The failure to generate prodrug **56** was attributed to the heat that is required to induce the formation of the N-hydroxy methyl group, as this consequently cleaves the nitrobenzyl group from **56**. This then led us to believe that by forming the N-hydroxy methyl prodrug **54** first, we could then avoid using heat. However, the methoxy seemed to be sensitive to the conditions used as only the products **51** and **52** were obtained. Therefore, we abandoned this approach and decided to look at more stable linkers.

We turned our attention to the possibility of introducing a linker that possesses a labile terminal functional group, which may allow for the attachment of a photosensitiser with out the use of heat. We decided to incorporate an ester derivative that would allow for linkage through a reactive terminal functional group. To test this we decided to prepare three types of 5-FU esters, 1-benzyloxycarbonylmethyl-5-fluorouracil **57**, 1-alloxycarbonyloxymethyl-5-fluorouracil **59** and 1-hexanedioic acid benzylester- (5-fluorouracil) methyl ester **60** (FIGURE 61).

2.0.6. Synthesis of ^{1}N ester derivatives of 5-fluorouracil



57 **58**

59 **60**

FIGURE 61. ESTER DERIVATIVES OF 5-FU.

It was thought that the 1-alloxycarbonyloxymethyl-5-fluorouracil **59** (FIGURE 61) could possibly be linked to the photosensitiser **59A** through a metathesis reaction (FIGURE 62). While the esters 1-benzyloxycarbonylmethyl-5-fluorouracil **57** and 1-hexanedioic acid benzylester-(5-fluorouracil) methyl ester **60** (FIGURE 61), can be linked through the transesterfication reaction (FIGURE 63)or an esterfication reaction (FIGURE 64) once the benzyl group is deprotected.

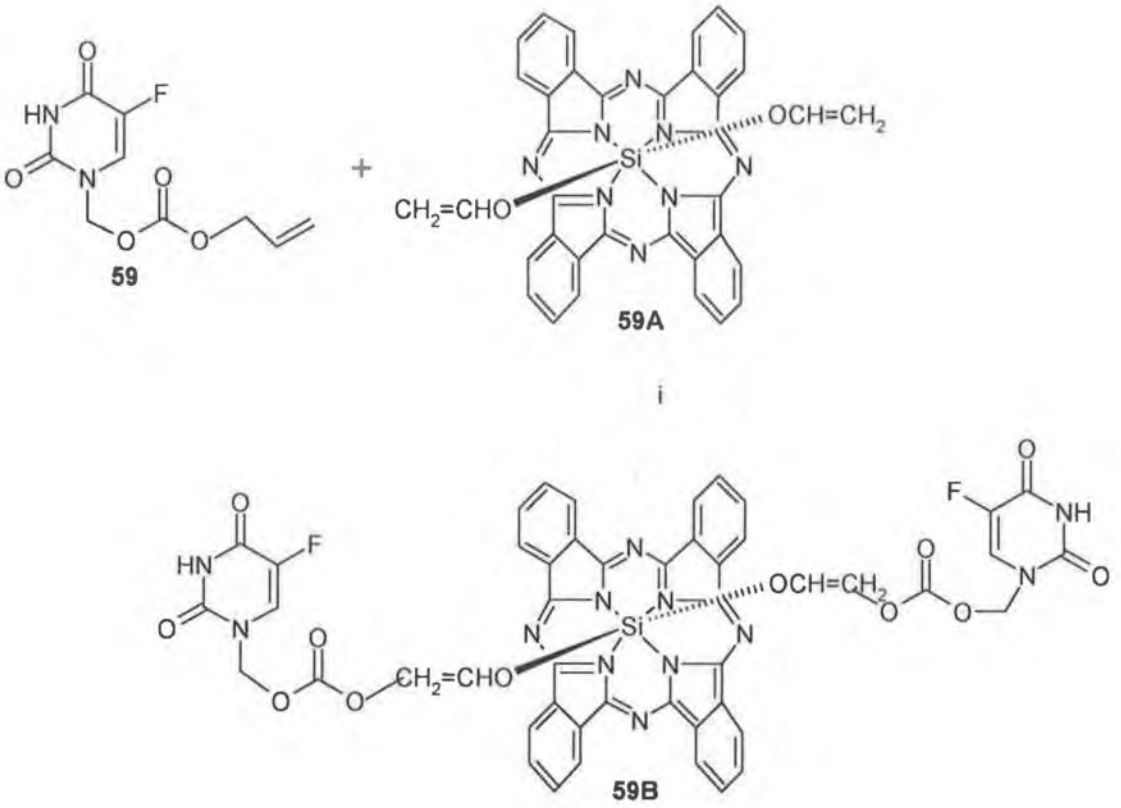


FIGURE 62. REACTION SCHEME FOR THE SYNTHESIS OF **59** USING A METATHESIS REACTION. Reagents i) Grubbs Catalyst.

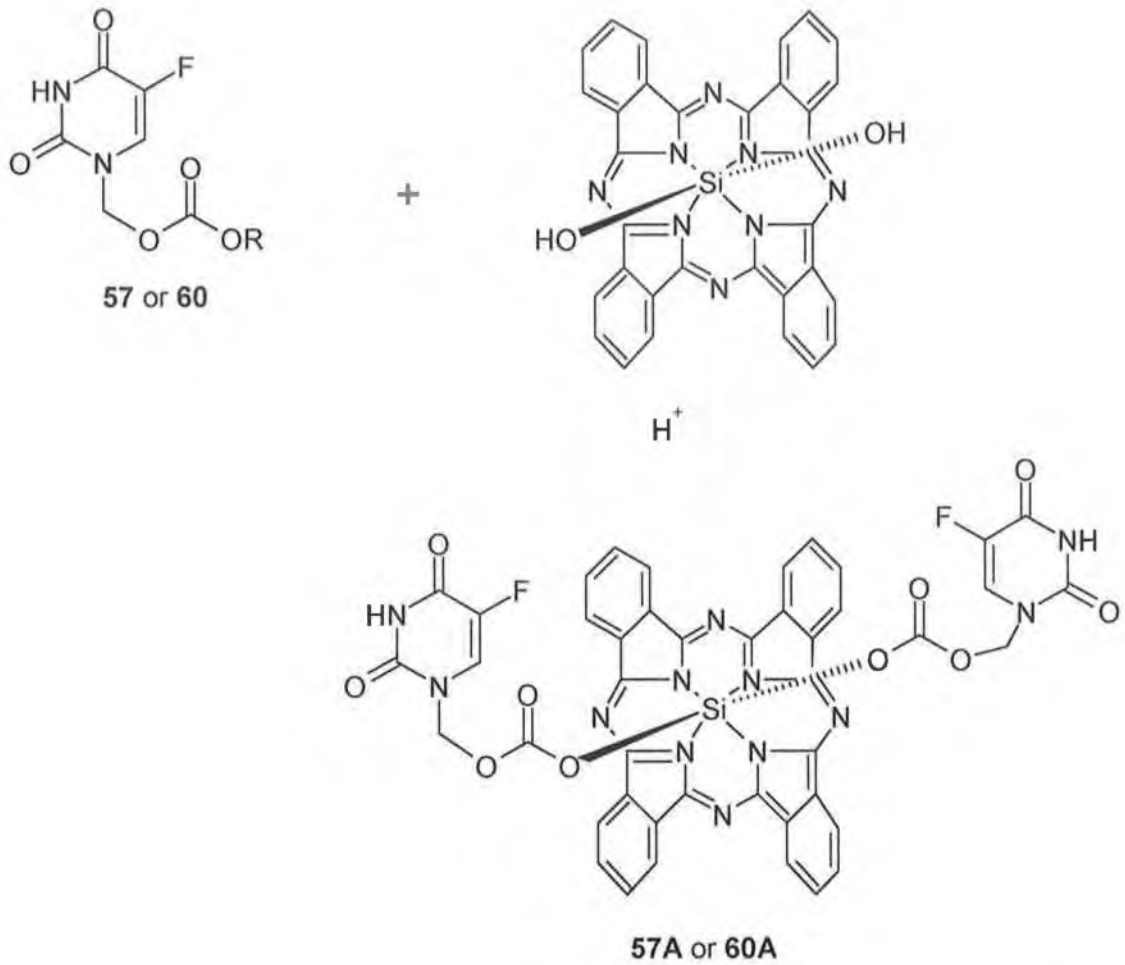


FIGURE 63. REACTION SCHEME FOR THE SYNTHESIS OF **57A** OR **60A** USING TRANSESTERFICATION REACTION CONDITIONS.

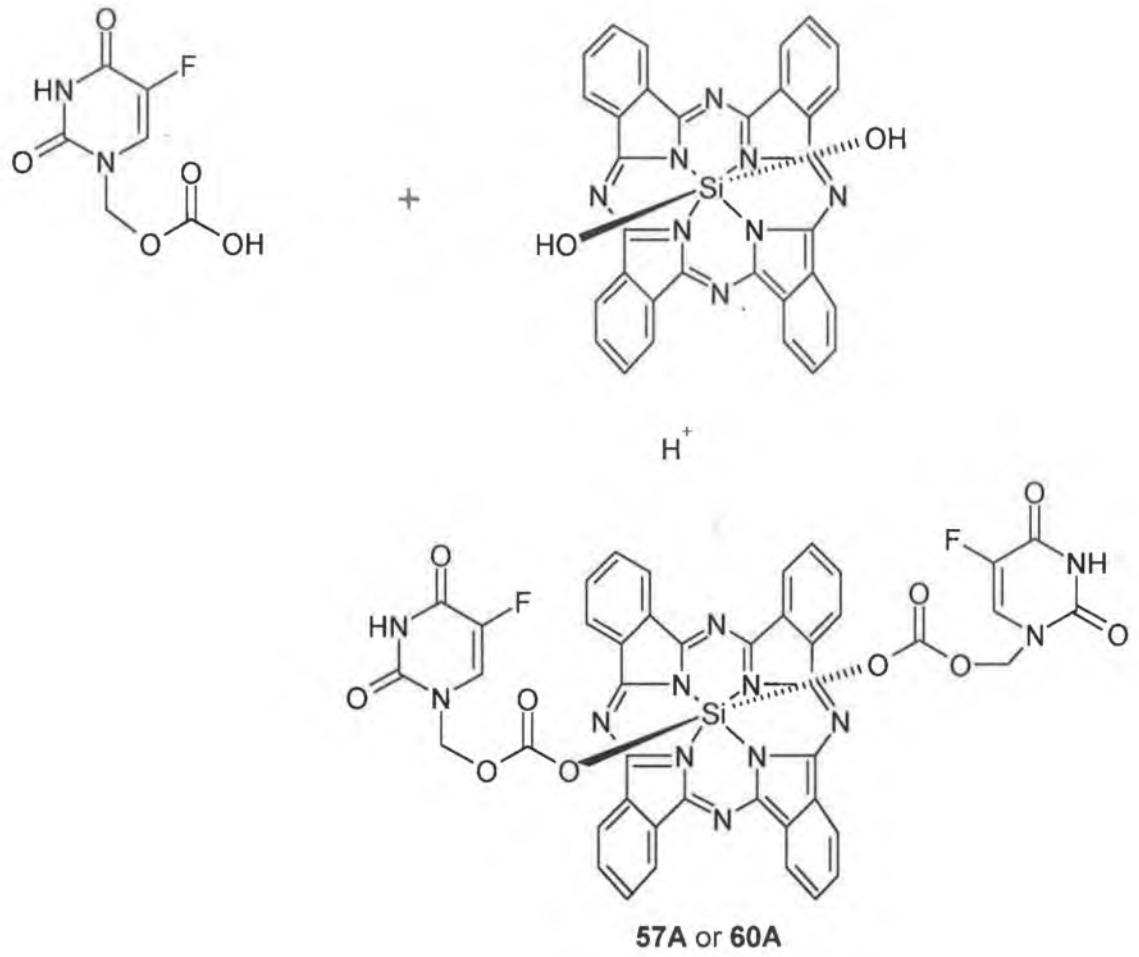


FIGURE 64. REACTION SCHEME FOR THE SYNTHESIS OF **57A** OR **60A** USING ESTERFICATION REACTION CONDITIONS.

Both 1-alloxycarbonyloxymethyl-5-fluorouracil and 1-benzyloxycarbonylmethyl-5-fluorouracil were prepared in the following manner.[149] The 5-FU was allowed to react with 37% formalin at 60°C for 45 minutes. All traces of water were removed and the residue was dissolved in dry acetonitrile. An equimolar amount of triethylamine and the corresponding chloroformate were added to the solution, to afford both 1-alloxycarbonyloxymethyl-5-fluorouracil **59** and 1-benzyloxycarbonylmethyl-5-fluorouracil **57** in yields of 74% and 69% respectively. The disubstituted by-product 1,3-benzyloxycarbonylmethyl-5-fluorouracil **58** was also generated in a yield of 18%.

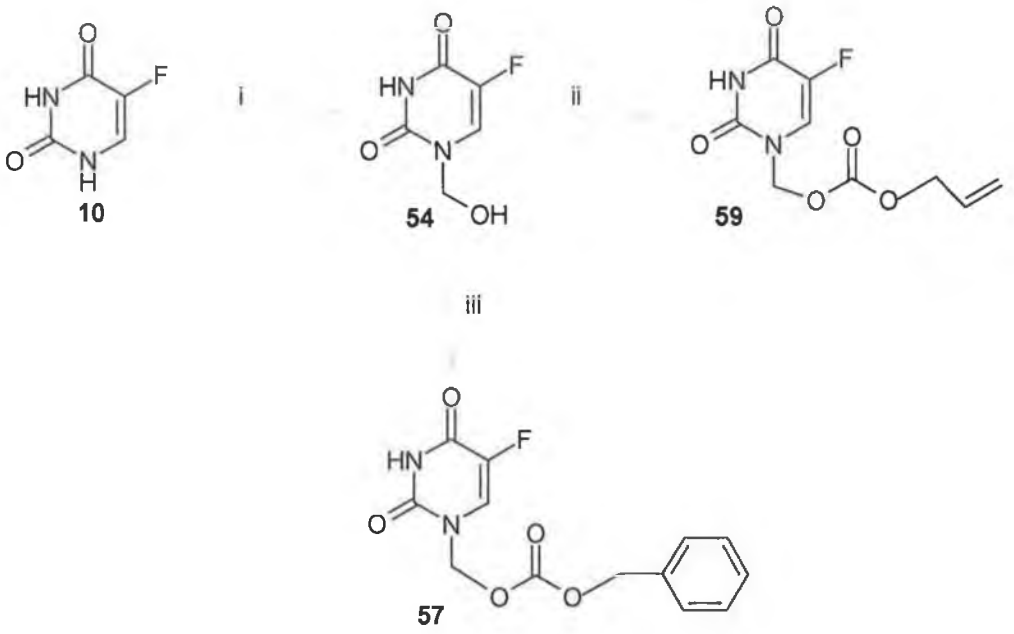


FIGURE 65. GENERAL SCHEME FOR THE SYNTHESIS OF **57** AND **59**. Reagents i) 37% formalin at 60°C, ii) acetonitrile, Et_3N allylchloroformate , iii) acetonitrile, Et_3N and benzylchloroformate.

Not only would the synthesis of 1-hexanedioic acid benzylester- (5-fluorouracil) methyl ester **60** allow for a non-thermal mild linkage reaction, but it would also enable us to examine the effect linker length has on the cleavage of the drug from the photosensitiser. The synthesis of **60** was similar to that reported by Menger *et al.* (1997).[151] Initial preparation required the synthesis of a hexanedioic acid benzyl ester methyl ester. This was carried out by refluxing (using a dean stark apparatus) a mixture of adipic acid **61** (FIGURE 66), benzyl alcohol in toluene with acid catalyst, to give the hexanedioic acid benzyl **62** (FIGURE 66) in 69% yield after work-up.[150] This monoester was then dissolved in dry DMF containing cesium carbonate in order to abstract the acidic hydrogen prior to the addition of chloromethyl phenyl sulfide. This afforded the product hexanedioic acid benzyl ester(phenylsulfanyl)methyl ester **63** (FIGURE 66) in a yield of 71%. Following on from this the hexanedioic acid benzylester(phenylsulfanyl)methyl ester was dissolved up in dichloromethane. To this a

solution of sulfuryl chloride made up in dichloromethane was added slowly. The colour changed from clear to orange indicating the generation of the desired chloromethyl ester plus the very reactive benzenesulfenyl chloride side product. In order to trap this reactive side product we used cis-4-cyclohexene-1,2-dicarboxylic acid.[151] This trapping reaction was signified by the orange colour been converted back to its original clear colour. After work-up the product hexanedioic acid benzyl ester chlormethyl ester **64** (FIGURE 66) was obtained in a yield of 92%. To a stirred solution of 5-FU in dry DMF, a 3-fold excess of triethylamine was added. To this reaction mixture a solution of hexanedioic acid benzyl ester chlormethyl ester in DMF was slowly added and allowed to stir over-night. The triethylamine hydrochloride formed was filtered and the DMF was removed using a high vacuum pump. The product hexanedioic acid benzyl ester – 5-fluorouridine-methylester **60** (FIGURE 66) was obtained in 47% yield prior to SGC purification.

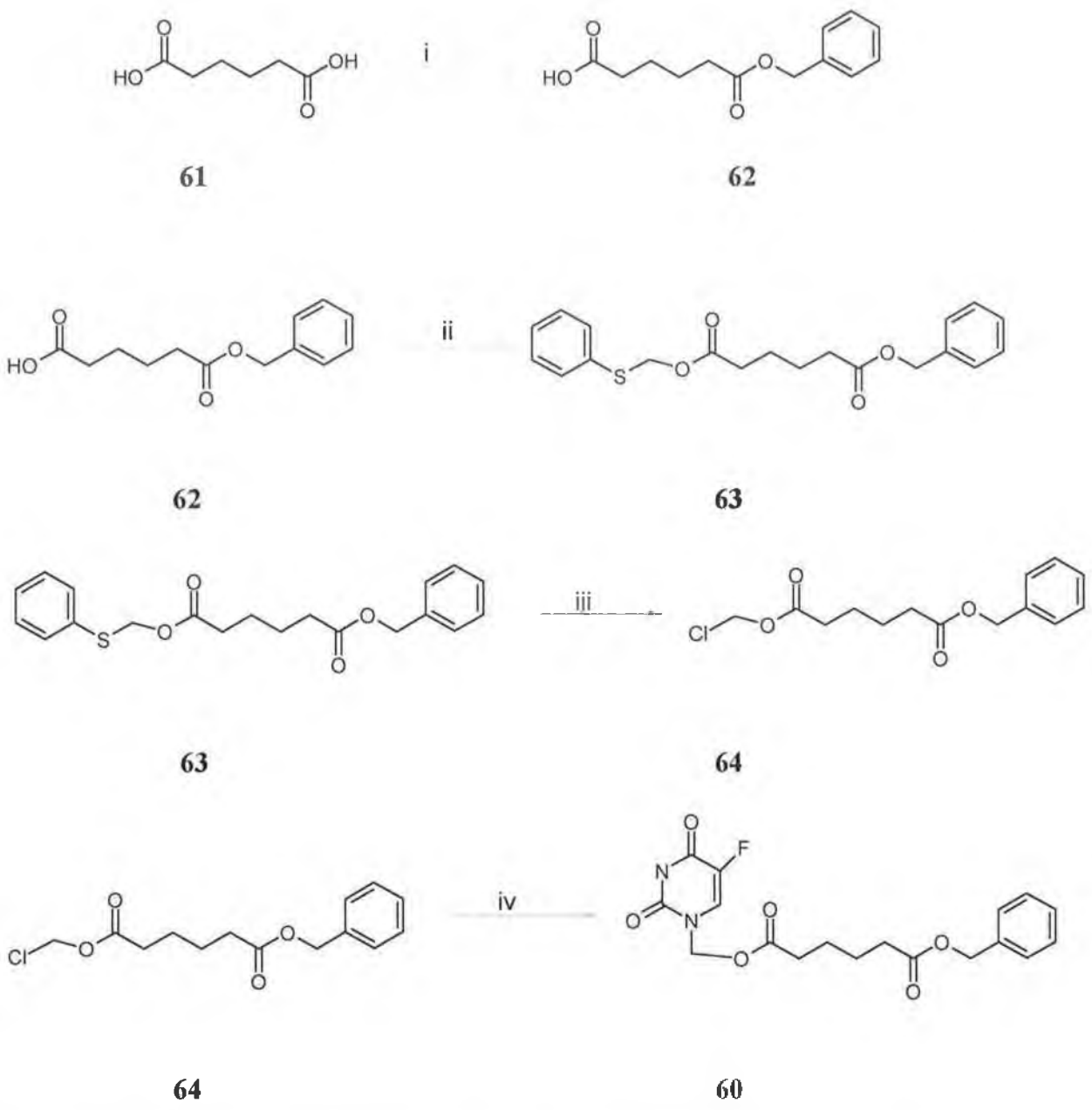


FIGURE 66. SYNTHESIS OF HEXANEDIOIC ACID BENZYL ESTER-5-FLUOROURIDINE-METHYLESTER. Reagents: (i) benzyl alcohol, p-toluenesulfonic acid, toluene; (ii) Cs_2CO_3, $PhSCH_2Cl_2$, DMF; (iii) SO_2Cl_2, cis-4-cyclohexen-1, 2-dicarboxylic acid, CH_2Cl_2; (iv) 5-FU, EtN_3, DMF.

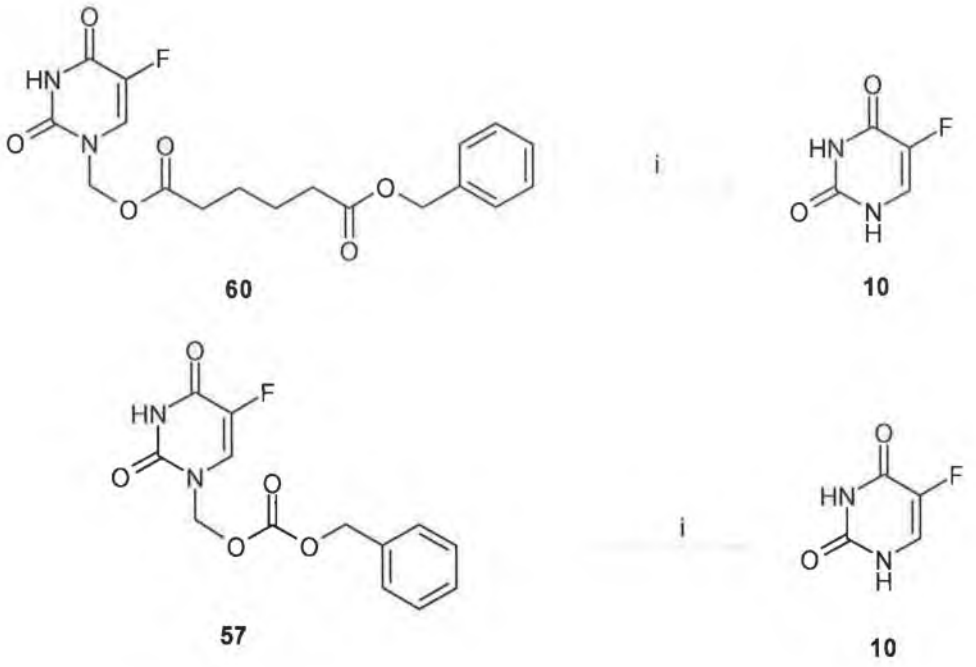


FIGURE 67. DEBENZYLATION OF **57** AND **60**. Reagents i) acetic acid, 10% Pd/C and 1,4-cyclohexadiene.

We attempted to then generate the acid derivative of the 5-FU prodrugs **57** and **60** by selectively cleaving the benzyl moiety. Using both Felix *et al.* (1978) and Menger *et al.* (1997) procedures we dissolved the esters in acetic acid and stirred until completely dissolved (FIGURE 67).[158,151] To this we added 10% Pd/C and 1,4-cyclohexadiene (hydrogen source) and allowed the reaction to stir. After approximately 2 hours TLC monitoring showed the formation of a new base line spot. The reaction was filtered through celite and washed with acetic acid and evaporated till dry. However, on analysis of the white solid we discovered that the entire benzyl ester was cleaved leaving only the 5-FU (FIGURE 68).

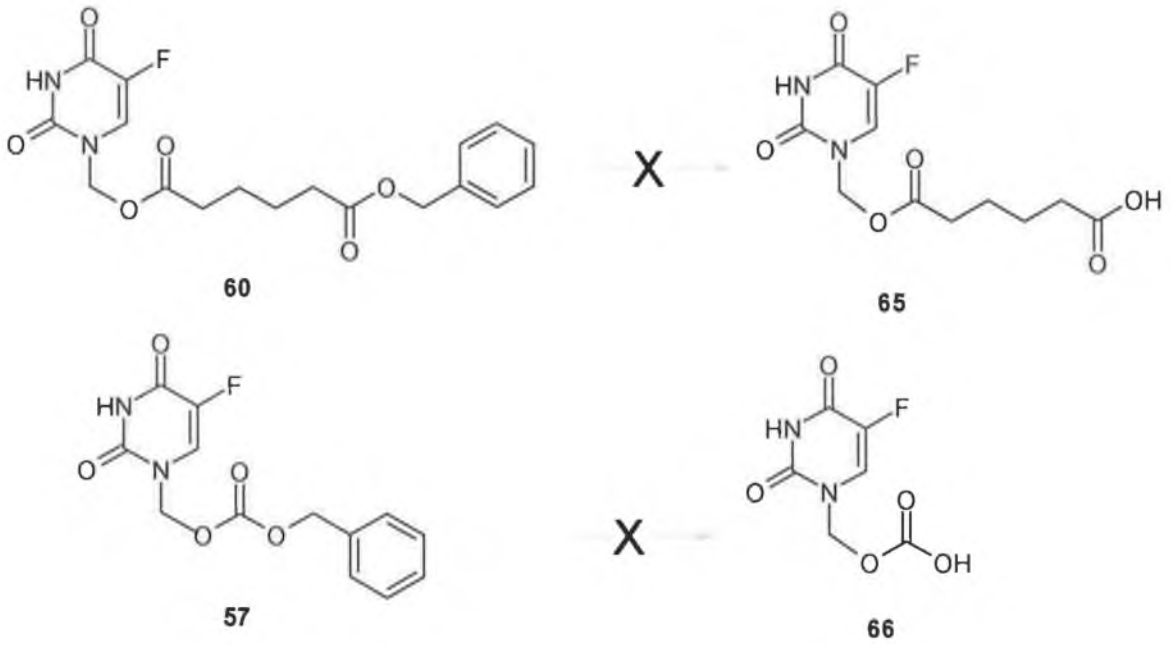


FIGURE 68. UNSUCCESSFUL DEBENZYLATION REACTION OF **57** AND **60**.

This led us to try the Tsuji procedure (1979), [159] which uses anisole and aluminium trichloride as reagent (FIGURE 69). Mechanistically, the aluminium trichloride has a high affinity for oxygen hence coordinating itself to the carbonyl oxygen, which in turn assists in the generation of the benzyl cation that is then trapped by anisole.

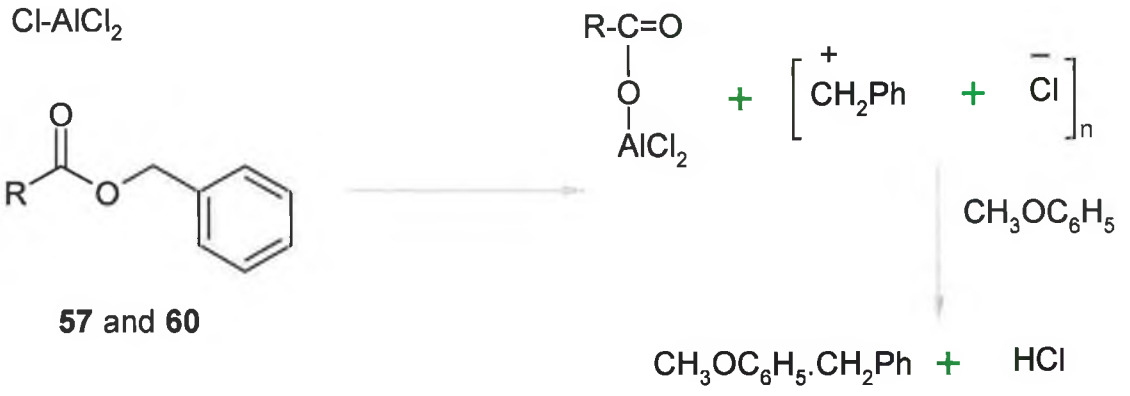


FIGURE 69. TSUJI DEBENZYLATION REACTION MECHANISM.

However, as with the Felix *et al.* (1978) and Menger's *et al.* (1997) methods a base line spot formed after approximately 10 minutes. The reaction was diluted with ethyl acetate and treated with dilute HCl. The organic layer was separated and concentrated to give a white residue. On analysis it was discovered that the reaction was again yielding only 5-FU.

2.0.7. Substitution of nitroaromatic moiety

Due to the unforeseen problems associated with the synthesis of a suitable nitrobenzyl 5-FU prodrug with an appropriate linker we decided to abandon this approach. An alternative approach to link our prodrug into PcSi was to use the nucleoside 5-fluorouridine **20** (FIGURE 70). Since not only is 5-fluorouridine a metabolite of 5-FU, it also possesses a sugar attachment with a primary hydroxy group that could act as a possible linker to the photosensitiser.

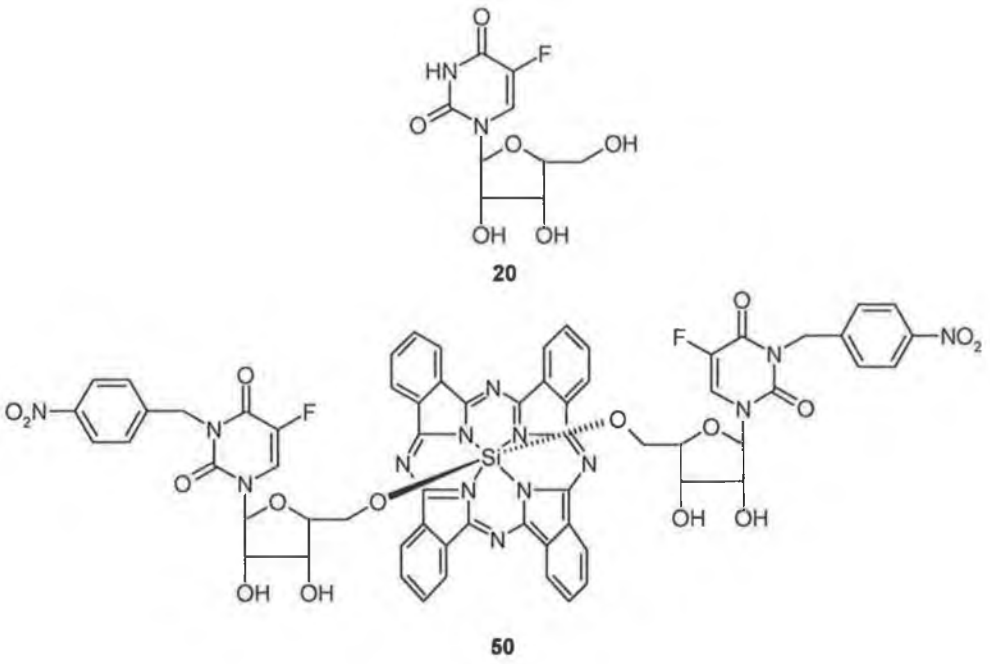


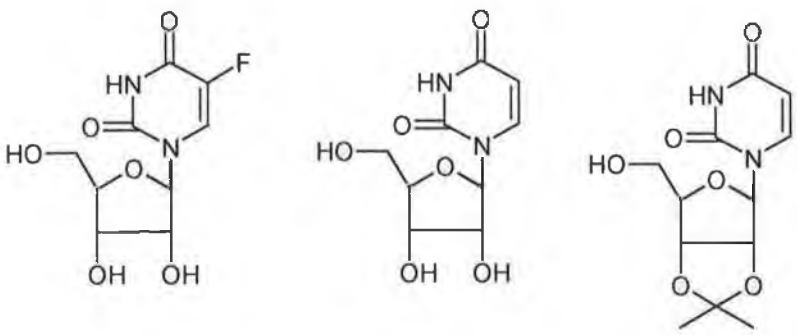
FIGURE 70. 5-FURD 20 AND PS CONJUGATED 5-FU PRODRUG **50**.

Given that the prodrug 3-(p-Nitrobenzyoxycarbonyl)-5-fluorouracil is too labile for further modifications to its structure, we decided to concentrate on the more stable 3-(p-nitrobenzyl)-5-fluorouracil prodrug. Therefore, with the p-nitrobenzyl moiety acting as the trigger for the prodrug we set about synthesising a series of 3-(p-nitrobenzyl)-5-fluorouridine prodrug derivatives that would eventually be linked to a photosensitiser **50** (FIGURE 70).

2.1. 5-Fluorouridine protection

Due to the expense of 5-fluorouridine **20** (FIGURE 70) it was decided to initially work on the inexpensive parent nucleoside uridine **67** (FIGURE 71). This provided us with a cheap starting material to use in our protocol design.

We attempted to eliminate any possibility of unwanted side reactions by selectively protecting the two secondary hydroxyl groups C-2 and C-3, while leaving the primary hydroxyl at C-5 free to react with the photosensitiser. Of the numerous protection groups available for this selective protection [i.e. cyclic acetals and ketals, chiral ketones, cyclic ortho esters, silyl derivatives, cyclic carbonates and cyclic boronates][160] it was decided to use the isopropylidene group **68** (FIGURE 71), because it has been proven to be a cheap and reliable method of simultaneously masking the C- 2 and C-3 hydroxyls of the nucleoside.



20 **67** **68**

FIGURE 71. STRUCTURES OF 5-FURD, URIDINE AND ACETONIDE.

2.1.1. Nucleoside protection using tosic acid

We decided to apply a method developed by Reese *et al.* (1975) [161, 154] to protect the 2', 3' hydroxy groups of both uridine and 5-fluorouridine. This method involved the stirring of the nucleoside overnight with p-toluenesulfonic acid in the presence of 2,2-dimethoxypropane in dry acetone. The nucleosides were successfully converted to the protected nucleosides **68** and **69** (FIGURE 72). After recrystallisation the protected nucleosides **68** and **69** were obtained in 83% and 70.1% yields, respectively.

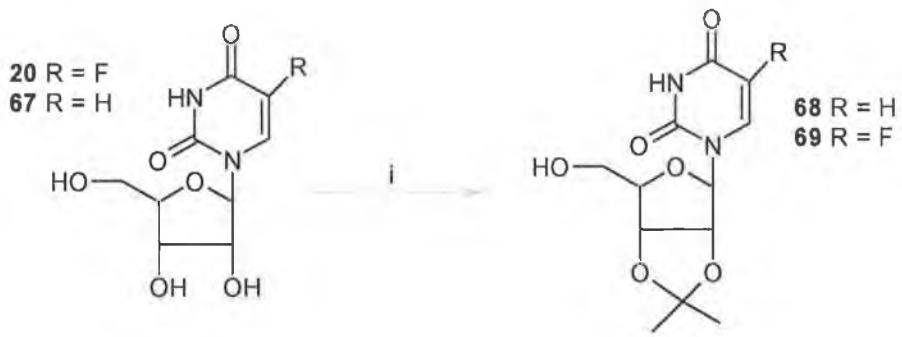


FIGURE 72. PREPARATION OF ACETONIDES **68** AND **69**. Reagents: i) TsOH, 2,2-dimethoxypropane, acetone, 25°C, 16 hours.

2.2. Nitro-aromatic nucleoside synthesis

The conjugation of the nitrobenzyl moiety to **20**, **68** and **69** was attempted using a similar procedure to that of Lin *et al.* (1986) were he reported the synthesis of the prodrug 3-(p-nitrobenzyl)-deoxy-5-fluorouridine.

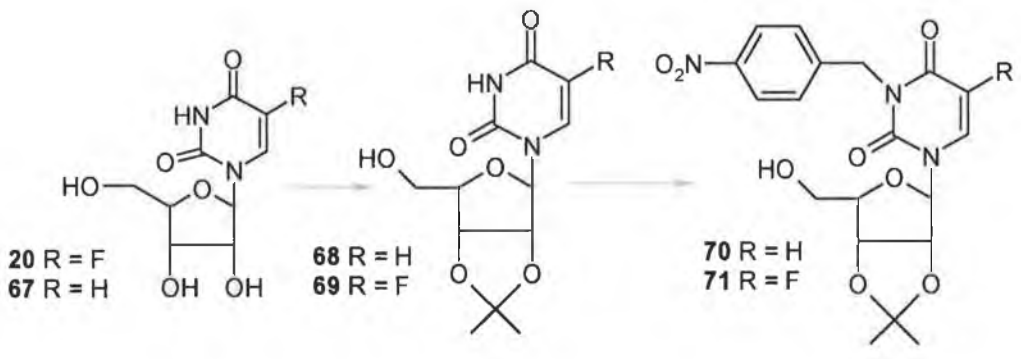
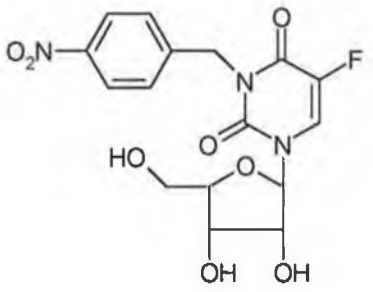


FIGURE 73. REACTION SEQUENCE FOR THE SYNTHESIS OF 2'3-ISOPROPYLIDENE-N^3-(P-NITROBENZYL)-URIDINE.

The procedure we followed involved treating the protected nucleoside with nitrobenzyl bromide and potassium carbonate in DMF. The reaction was allowed to stir at room temperature until all starting materials were consumed (monitored by TLC). The reaction mixture was filtered and concentrated; further washes with ethyl acetate were required to remove the remaining potassium bromide. The crude product was further purified by silica gel chromatography to generate 2'3-isopropylidene-N^3-(p-nitrobenzyl)-uridine **70** (FIGURE 73) and 2'3-isopropylidene-N^3-(p-nitrobenzyl)-5-fluorouridine **71** (FIGURE 73), in 66% and 78% yields, respectively.

Using a similar procedure as above we generated the N^3-(p-nitrobenzyl)-5-fluorouridine prodrug **72** (FIGURE 74 in 78% yield from the unprotected 5-fluorouridine.



72

FIGURE 74. STRUCTURE OF N^3- (P-NITROBENZYL)-5-FLUOROURIDINE **72**.

2.2.1. Enhancing the reduction potential of nitro-benzyl-5-FURD prodrugs

With the successful development of a synthetic protocol to **72** we now turned our attention to the introduction of other funtionalized nitro aromatics.

It has been reported that the reduction potential of nitro aromatic compounds can be increased by the presence of electron withdrawing substituents (FIGURE 75). So substitutents like acetyls, nitro, halogens and to some extent methyl groups should enhance the reduction potential of the nitro group.

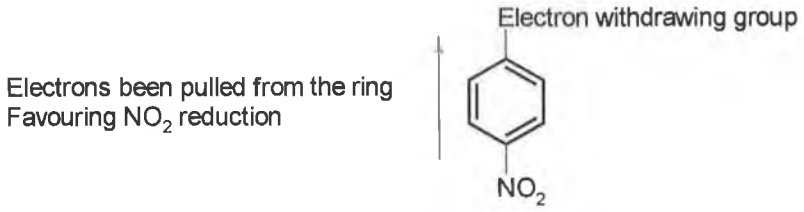


FIGURE 75. EFFECT OF ELECTRON WITHDRAWING SUBSTITUENT ON NITRO-AROMATIC RING.

The position of the electron-withdrawing group (ewg) in relation to the nitro moiety has also a major influence over the ease of reduction. It has been reported that ewg positioned at the 4 position of the benzene ring allows for the highest reduction potential, while the ewg postioned at the 2 positions generates the lowest (FIGURE 76).

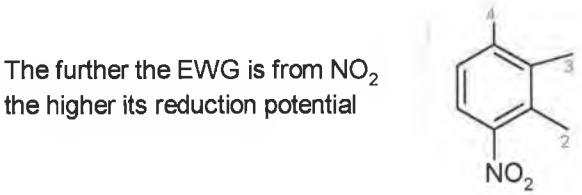


FIGURE 76. THE EFFECT OF EWG POSITIONING ON THE NITRO-AROMATIC RING.

This phenomenon can be partially explained by way of resonance, where the 4-postion substituent allows for extended resonance of the reduction intermediate followed by the 3 and 2 postion. It should be noted that substituents at the 3 and 2 position can interfere with reduction via steric hindrance.

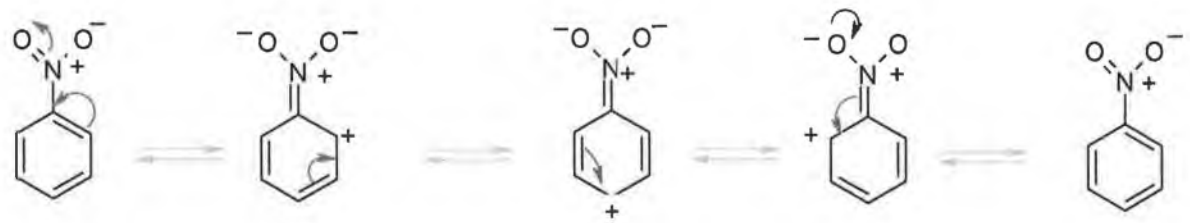


FIGURE 77. NITROAROMATIC RING RESONANCE.

With this in mind we attempted to synthesise a series of nitro-aromatic prodrug derivatives that should possess more desirable reduction potentials. Since, we have already synthesised a 4-nitrobenzyl-5-fluorouridine derivative, we believed it possible to prepare the new derivatives using these similar reaction conditions (FIGURE 78). The only problem that could arise is that the substitutents could sterically hinder the benzyl halide therefore inhibiting the reaction from occurring.

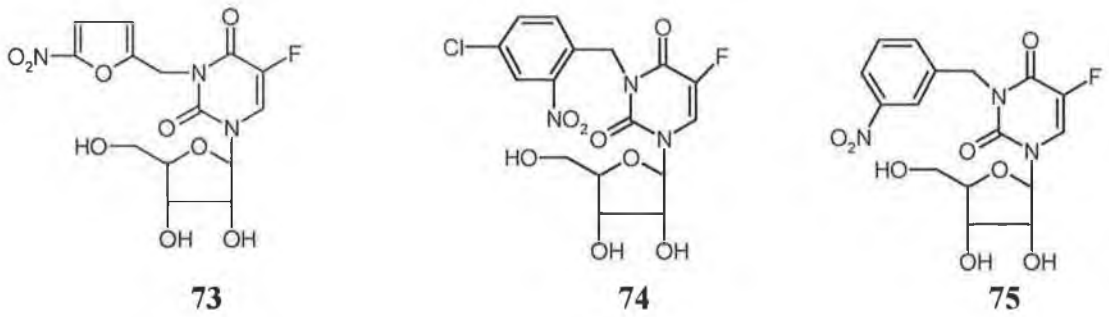


FIGURE 78. SERIES OF POTENTIAL NITRO-AROMATIC –5-FU PRODRUGS.

Using the same procedure for the synthesis of **72**, we generated N^3-(4-nitrobenzylfuran)-5-fluorouridine **73** in 48% yield, N^3-(4-chloro-2-nitrobenzyl)-5-fluorouridine **74** in 54% yield and N^3-(3-nitrobenzyl)-5-fluorouridine(75) in 70% yield (FIGURE 79). Thus, steric hinderence was not a problem.

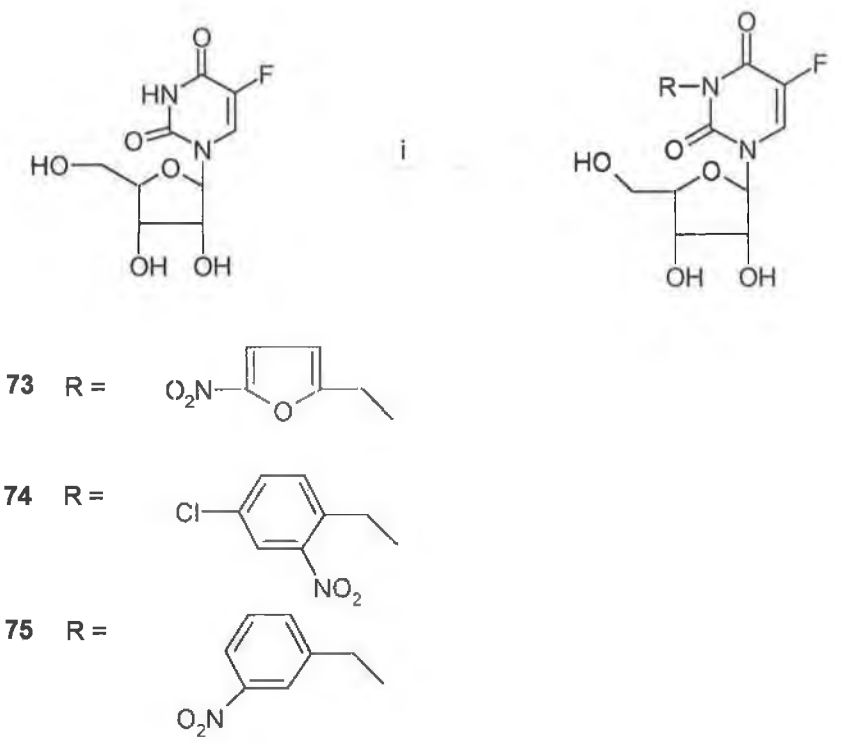
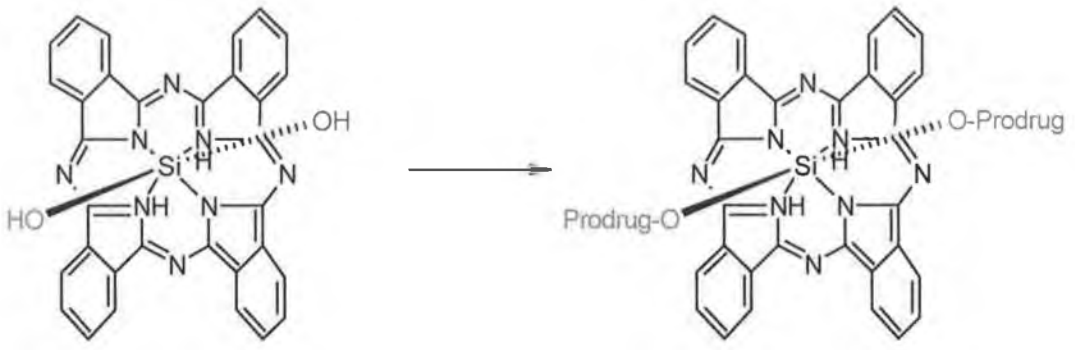


FIGURE 79. REACTION SCHEME FOR THE GENERATION OF PRODRUGS **73**, **74** AND **75**. Reagents i) appropriate benzyl halide, DMF, K_2CO_3 .

2.3. Choice of Photosensitiser

Although we could have picked a number of photosensitisers suitable for PDT we decided to limit our search to one that was commercially available and would allow for the direct substitution of our prodrug in a single step. In doing so we found that the photosensitiser dihydroxysilicon phthalocyanine **76** (FIGURE 80) offers each of the afore mentioned criteria.



76 **77**

FIGURE 80. DIHYDROXYSILICON PHTHALOCYANINE AND ITS PRODRUG DERIVATIVE.

Not only is this phthalocyanine commercially available but it also offers two free hydroxyl groups for prodrug linkage **77** (FIGURE 80). Thus, the decisive advantage of using this class of photosensitiser is that it allows for double substitution of the prodrug, thereby delivering two parts prodrug to one part photosensitiser to the cell.

To test if this compound could be synthesised we decided to use the cheaper compound 2'3-isopropylidene-N^3-(p-nitrobenzyl)-uridine **70** (FIGURE 73) and attempt to couple it to the phthalocyanine carrier **76** using a typical condensation reaction and sodium hydride reaction. [134, 137, 162] To test that our prodrug-photosensitiser could be synthesised we examined both procedures.

2.3.1. Condensation reaction

A condensation reaction was reported for the conjugation of PEG groups to dihydroxysilicon phthalocyanine **76**. [162] Therefore, we initially attempted to synthesise the target compound using these conditions. This involved refluxing the 2'3-isopropylidene-N^3-(p-nitrobenzyl)-uridine **70** with dihydroxysilicon phthalocyanine **76** (FIGURE 81) in dry DMF while monitoring by TLC. After approximately 1 hour a fast eluting spot formed that became more intense as the reaction proceeded. However, this spot appeared to be a co-elution of two fractions. This was clarified when the mobile phase was adjusted. The reaction was stopped after 3 hours. An identical reaction was allowed to run over-night but the resulting TLC profile was indistinguishable to that of the 3-hour reaction. The reaction was cooled to room temperature, filtered, concentrated to give a blue residue that was re-dissolved in dry THF. The fractions were separated and collected by flash chromatography, to give the product **78** in 26% yield.

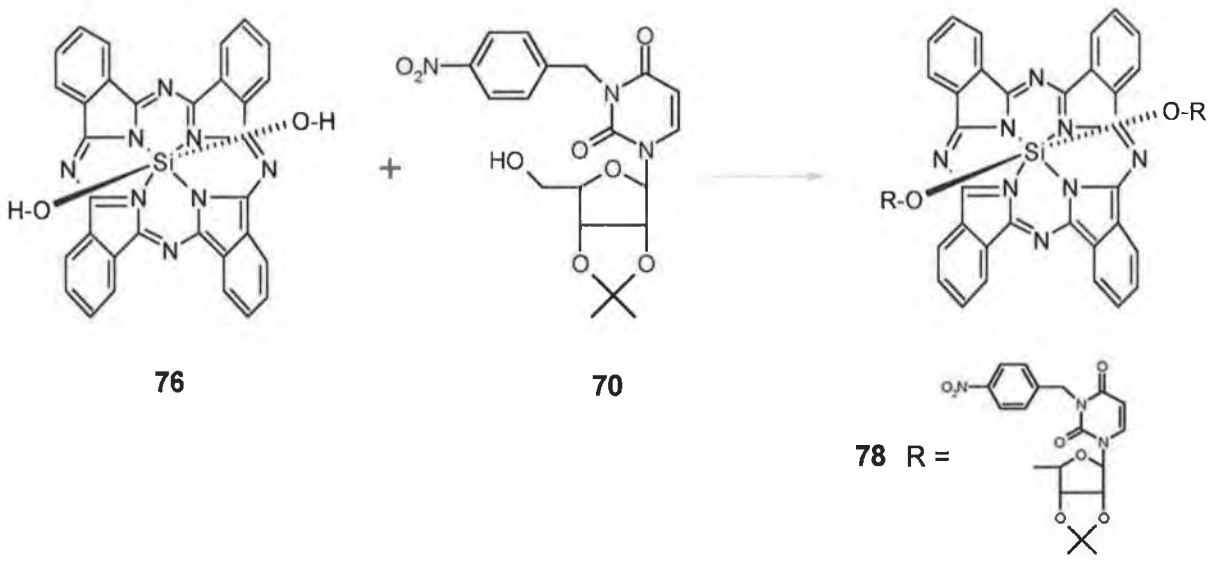


FIGURE 81. THE PREPARATION OF **78** USING BOTH CONDENSATION AND HYDRIDE PROCEDURES.

2.3.2. Sodium hydride reaction

This reaction type was reported for the conjugation of large dendritic substitutents to the phthalocyanine.[134] The compounds **70**, **76** and sodium hydride were dissolved in toluene and heated to 80°C for 4 days and monitored by TLC. Multiple spots were formed after a couple of hours and no change was observed after 4 days. To ensure that the reaction had gone to completion an identical reaction was allowed run for 7 days but no difference was observed in the TLC profile of the 4 day and 7 day reactions. The purification of the crude product involved initially removing the unreacted Pc by flushing through a silica gel (DCM: MeOH v/v 10:0.5) column prior to dissolving the crude material in THF and passing it through a size exclusion column (SX-3 polystyrene) to isolate the product. Since our product is the largest entity in the mixture we concluded that it should be the first to elute. However, on TLC analysis of the eluted product more than one spot was observed. Therefore, the eluted crude mixture was dissolved in dry THF and gravity filtered to remove any insoluble starting material. The crude product was filtered twice more and concentrated down to a minimum to allow for it to be applied to the silica-gel column (SGC). The first fraction to elute off the column was collected along with the other fractions. The first fraction was analysed by MS and UV-Vis and it was determined to be the prodrug derivative dioxysilicon-2'3-isopropylidene-N^3-(p-nitrobenzyl)-uridine-phthalocyanine **73** in 32% yield. The UV-Vis spectrum (FIGURE 83) showed a distinctive red shift of 10nm, which is highly characteristic for axially alkoxy substituted PcSi (TABLE 10). Electrospray ionization (ESI) for Pc's is rare but compound **78** did ionize by ESI with a molecular parent ion peak at 1399, which takes into account the ionized product and Na^+ (FIGURE 82).

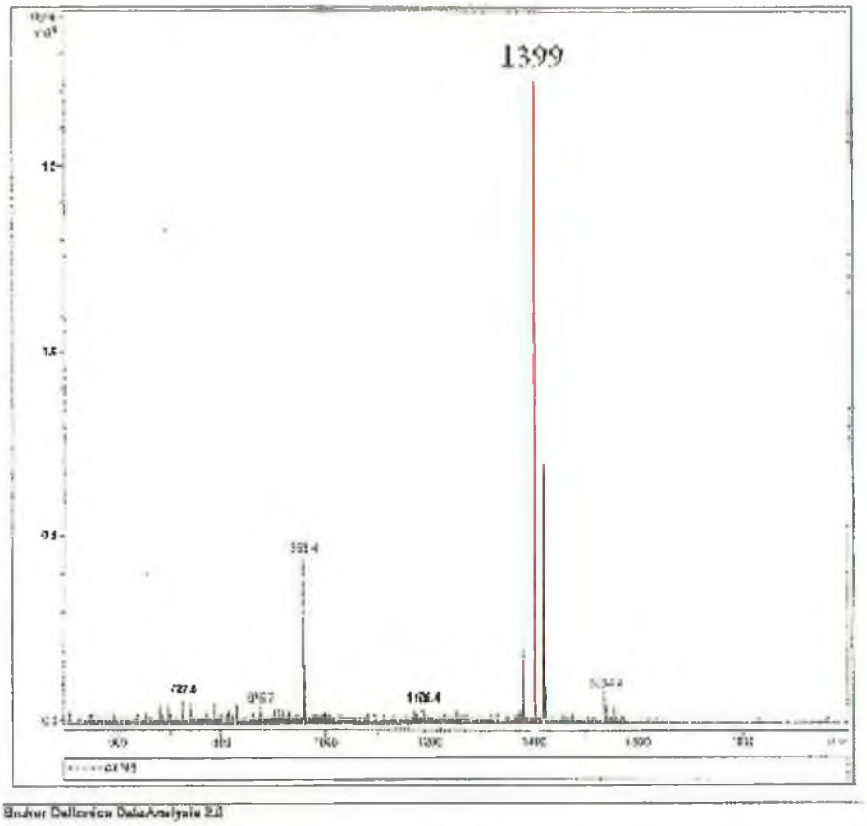


FIGURE 82. MASS SPECTRA OF COMPOUND 78

TABLE 10. UV-VIS DATA FOR THE PRODRUG 78.

Compound (in DMSO)	Wavelength (nm)
76	672
78	682

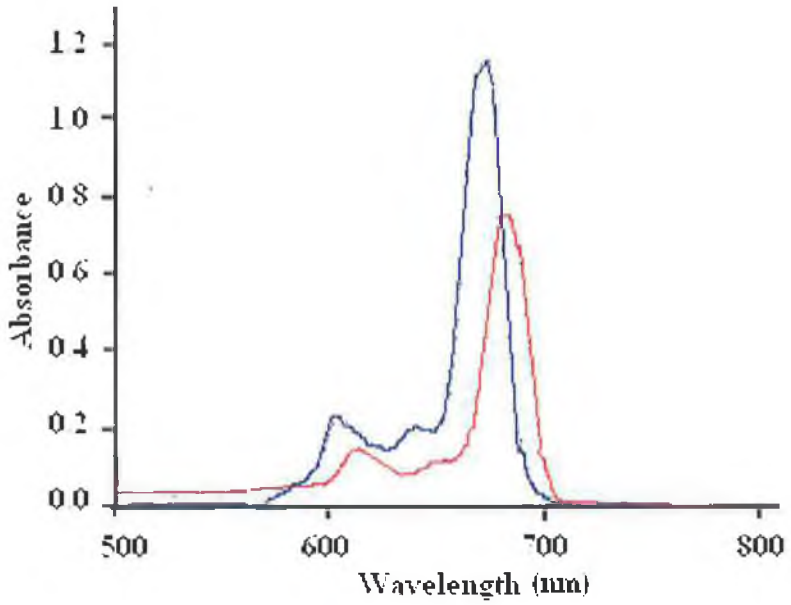


FIGURE 83 UV-VIS OF 76 AND 78

Although both methods, condensation and hydride, gave the targeted conjugated Pc-5-FU prodrug **78** obvious advantages were noted for the condensation method over the hydride method. As can be seen from TABLE 11 the condensation method has the added advantage of not only generating the Pc-5-FU prodrug in 26% yield but the reaction time is short, 3 hours with purification only taking a single silica-gel column (SGC). In comparison apart from the slightly superior yield of 32% the hydride reaction procedure requires longer reaction times and is more tedious to purify.

TABLE 11. COMPARISON OF THE CONDENSATION AND HYDRIDE PROCEDURES TO GENERATE PC-5-FU CONJUGATE PRODRUG.

DATA	CONDENSATION	**Hydride**
Yield	26%	32%
Time	3 hours	4-7 days
Purification	SGC	SGC, gel permeation, SGC

2.3.3. Synthesis of the 5-FU prodrug pthalocyanine conjugate

Now that we have developed a robust method to prepare our Pc-conjugates we set about generating a series of 5-FU-prodrug conjugates, using the condensation reaction protocol. We therefore, refluxed N^3-(p-nitrobenzyl)-5-fluorouridine **72** (FIGURE 74) with the dihydroxysilicon phthalocyanine **76** in dry DMF while monitoring by TLC, the molecular model of which is shown in FIGURE 85. The reaction was stopped after 3 hours had elapsed as no other spots were formed; we again noted two co-eluting spots. The reaction was cooled to room temperature, filtered and concentrated down to a minimal volume were it was purified by column chromatography. Separation of the fractions proved cumbersome with only one fraction being isolated. The UV-Vis spectrum (FIGURE 86) for this sample gave an absorption at 678nm, which showed a red-shift of 8 nm when compared to the absorbance of the phthalocyanine starting material (TABLE 12). However, on analysis of this fraction via mass spectrometry we obtained a cluster with a molecular mass of 1347 (FIGURE 87), this value takes into account the molecular mass of 1332 for the compound bis(3-(p-nitrobenzyl)-5-fluorouridine)phthalocyaninosilicon (79) (FIGURE 84), which still leaves a mass difference of 15. This difference could be attributed to the addition of a methyl group to one of the hydroxyl groups in the nucleoside. Thus, 1347 would be an M^{+1} peak for such a compound. An alternative explanation for the mass difference of 15 would be the possiblity of two Li^+ being present. We believe this to be *very unlikely* since this would lead to an M^{+2} ionisation that would give an M/2 cluster. This same peak was observed a second time from a second reaction, thus it is indeed a real peak, but a reliable assignment can't be made.

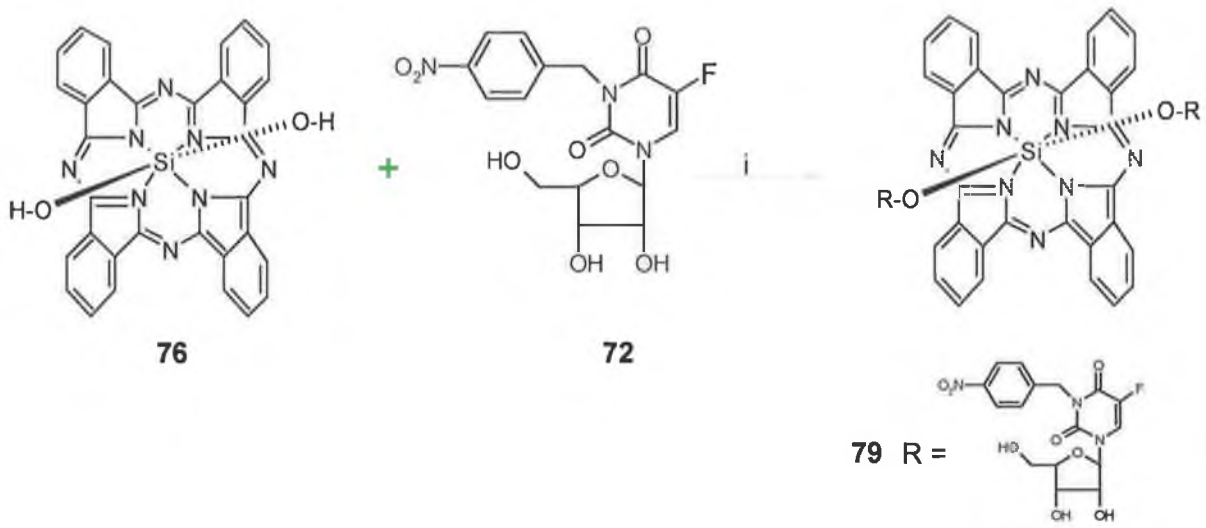


FIGURE 84. SYNTHESIS OF **79**. Reagents for reaction: i) DMF and reflux 3hours.

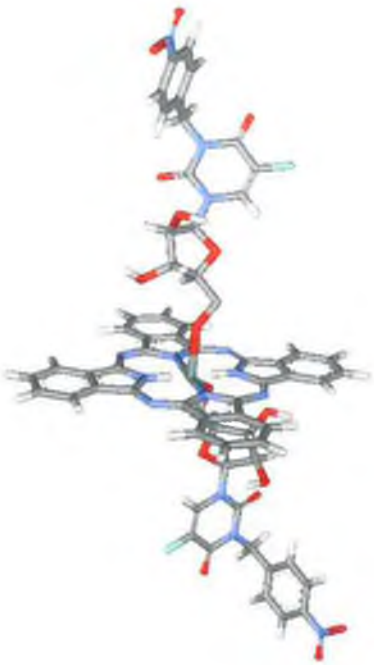


FIGURE 85. MOLECULAR MODEL OF **79** (HYPERCHEM MM+)

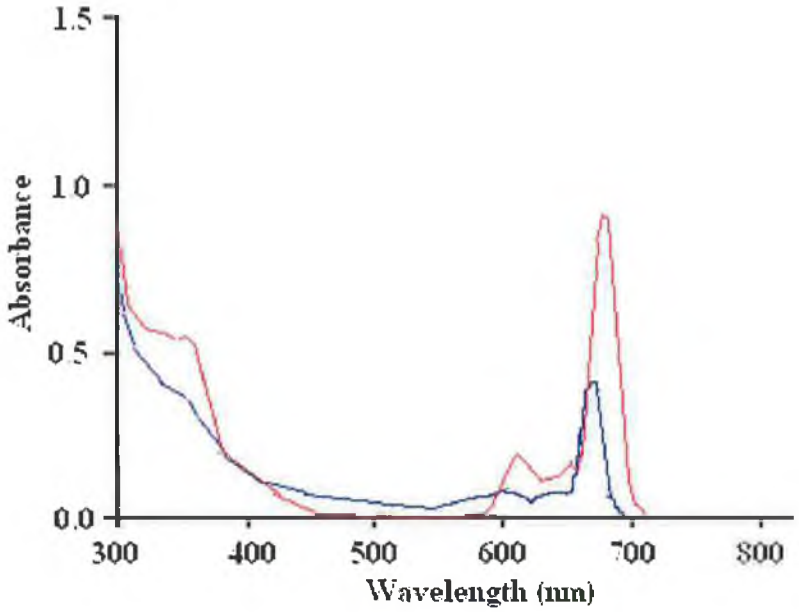


FIGURE 86. UV-VIS SPECTRA OF 76 AND 79

TABLE 12. UV-VIS DATA FOR THE PRODRUG **79**

Compound (in DMSO)	Wavelength (nm)
76	670
79	678

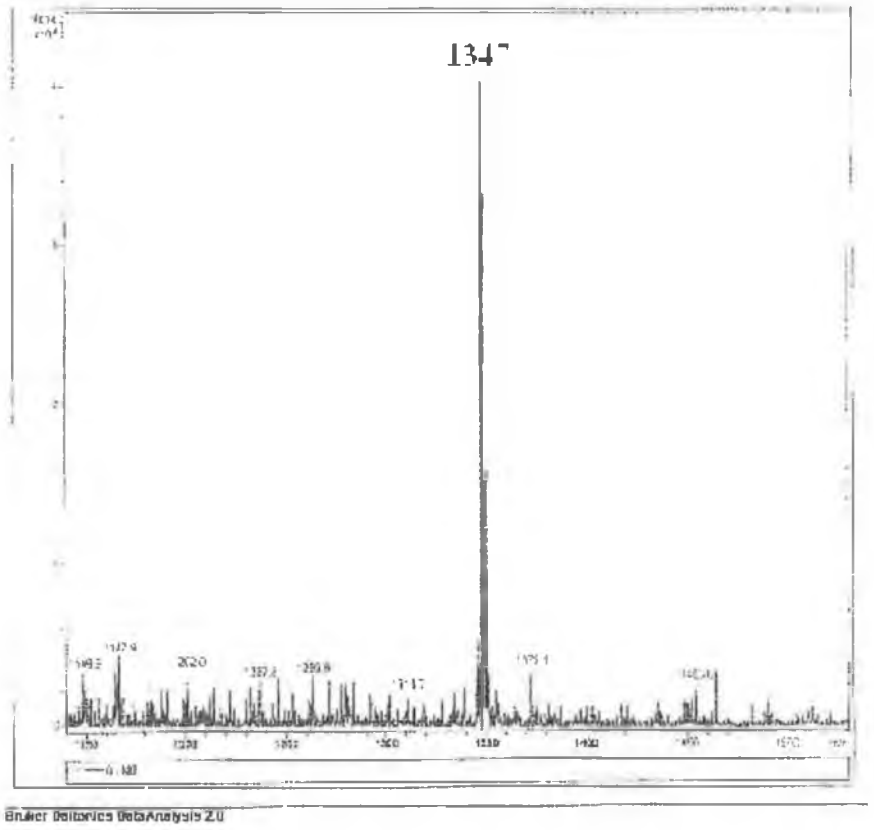


FIGURE 87. MASS SPECTRUM OF 79

We also used the same procedure to prepare the 5-FURD prodrug bis-(5-fluorouridine) phthalocyaninosilicon **80** from 5-flurouridine **20** and dihydroxyoxysilicon phthalocyanine **76** (FIGURE 88), to generate the product **80** in a 10% yield, the molecular model of which is shown in FIGURE 89. This product was confirmed again via mass spectrometry and UV-Vis analysis. The UV-Vis spectrum (FIGURE 90) gave a peak red shifted by 10nm in the red (TABLE 13). Mass spectral analysis (FIGURE 91) showed a molecular ion peak of 1089, which takes into account the molecular mass of 1066 for the product bis-5-fluorouridine-phthalocyaninosilicon **80** plus a sodium ion (M+Na).

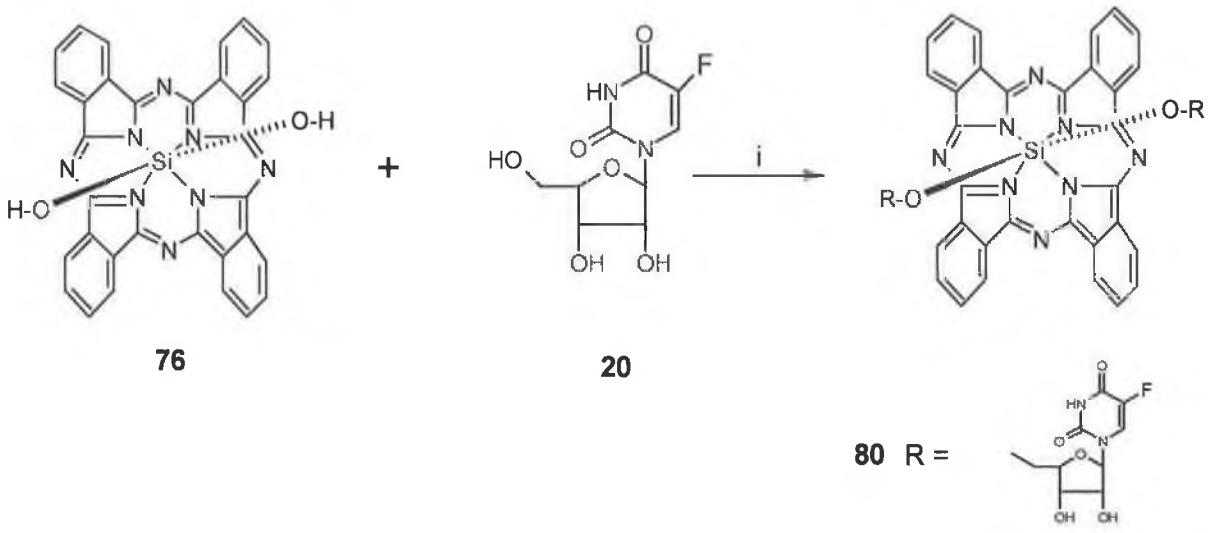


FIGURE 88. SYNTHESIS OF **80**. Reagents for reaction: i) DMF and reflux 3 hours.

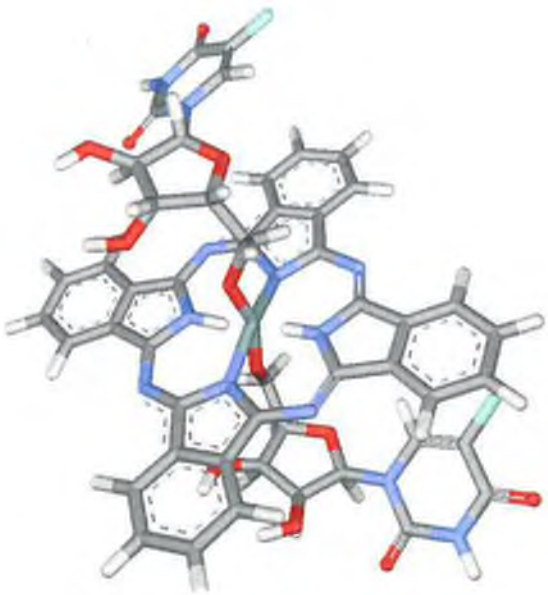


FIGURE 89. MOLECULAR MODEL OF **80** (HYPERCHEM MM+)

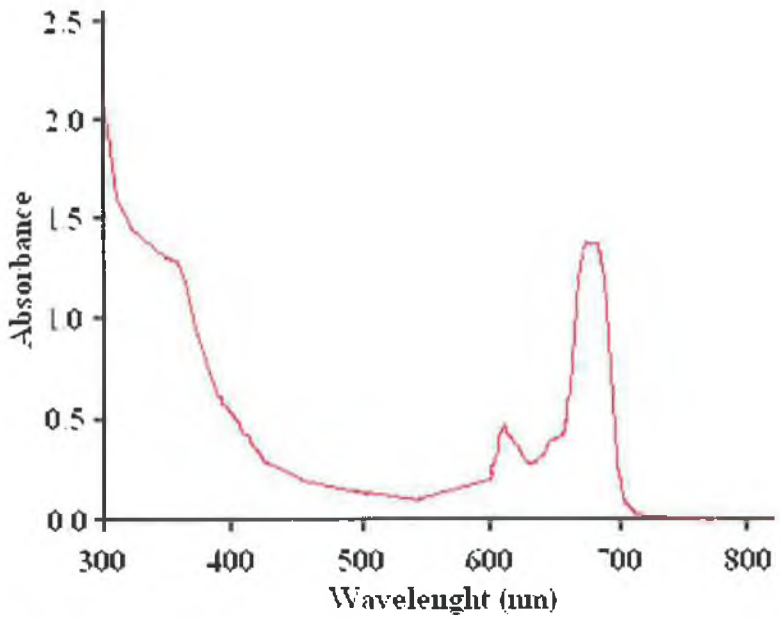


FIGURE 90. UV-VIS SPECTRA FOR COMPOUND **80**

TABLE 13. UV DATA FOR THE PRODRUG **80**

Compound (in DMSO)	Wavelength (nm)
80	680

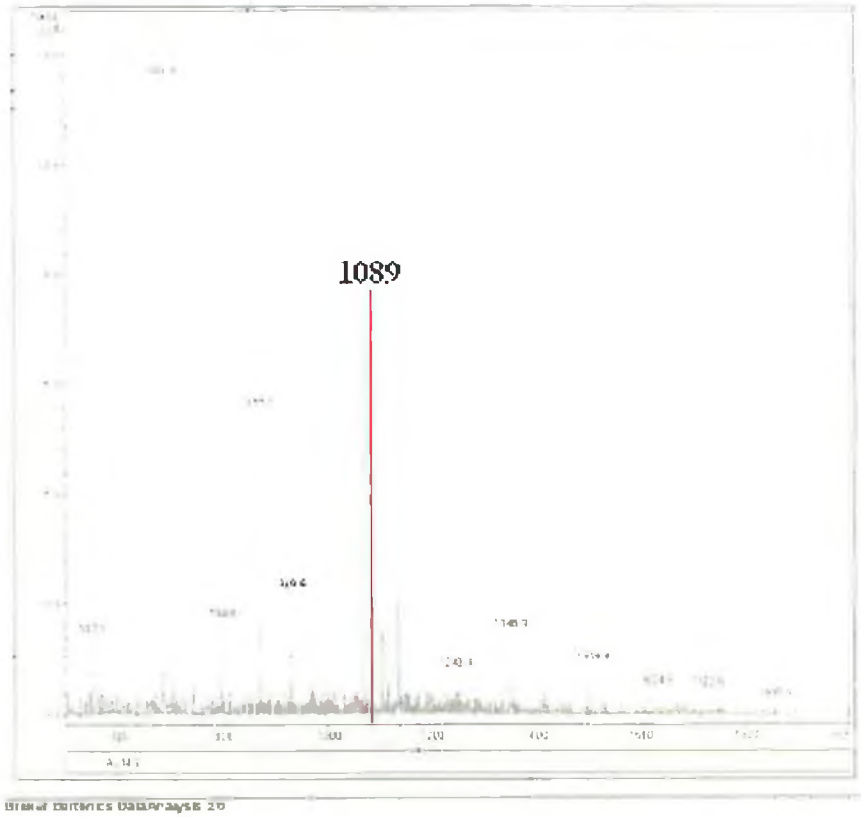


FIGURE 91. MASS SPECTRUM OF **80**.

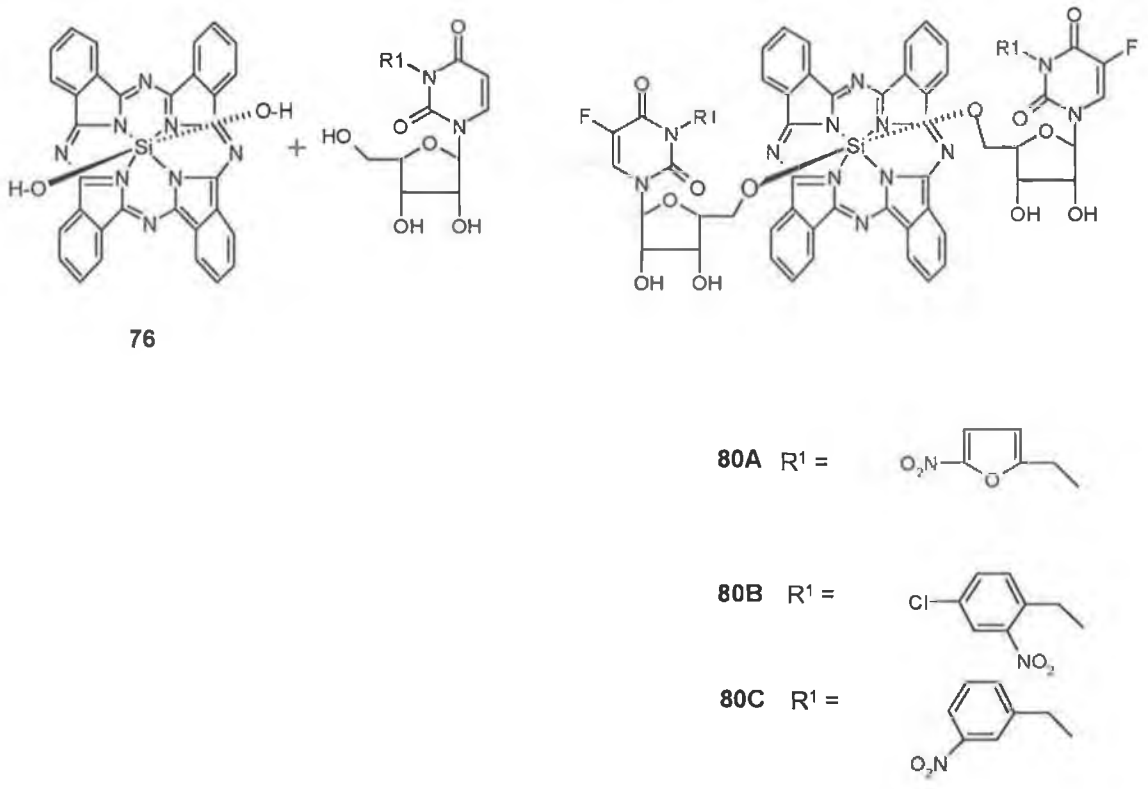


FIGURE 92. SYNTHESIS OF **80A**, **80B** AND **80C**. Reagents for reaction: i) DMF and reflux 3 hours.

A similar procedure was used to prepare prodrugs **80A**, **80B** and **80C** from N^3-(4-nitrobenzylfuran)-5-fluorouridine **73**, N^3-(4-chloro-2-nitrobenzyl)-5-fluorouridine **74** and N^3-(3-nitrobenzyl)-5-fluorouridine**75** respectively (FIGURE 92). The appropriate 5-FU prodrug was refluxed with dihydroxyoxysilicon phthalocyanine **76** (FIGURE 92). To generate the respective products **80A**, **80B** and **80C** in a 41%, 28% and 30% yield. These products were confirmed by UV-Vis analysis (TABLE 14.). Like prodrugs **80**, **79** and **78** a 10nm red shift peak was observed for each prodrug. However, when mass spectral analysis was attempted using electrospray ionisation no molecular ion peaks were found.

TABLE 14. UV DATA FOR THE PRODRUGS **80A, 80B** AND **80C**

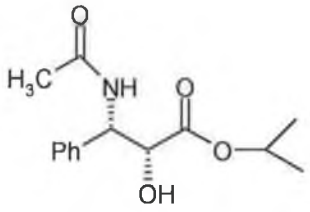
Compound (in DMSO)	Wavelength (nm)
80A	682
80B	682
80C	682

2.4. Conclusion.

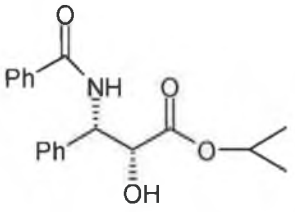
The ultimate aim of this project was the preparation of a trigger-effector - photosensitiser, which was achieved through the synthesis of the five 5-FU-photosensitisers prodrugs **79**, **80**, **80A**, **80B** and **80C**. This was achived with ease when the condensation procedure was used resulting in yields ranging from 10% to 41%.

3.0. More Selective 5-FU Prodrugs: The Synthesis of a Novel 5-FU Prodrug

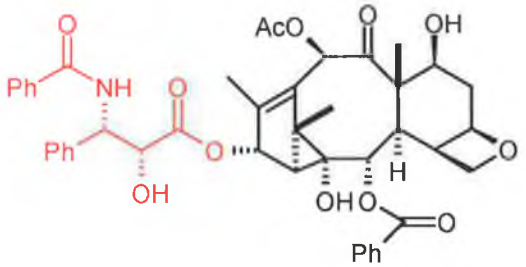
To improve 5-FU anticancer selectivity we attempted to prepare a 5-FU prodrug that incorporates both a nitro benzyl trigger and a lipophilic handle. We believed that this type of 5-FU prodrug may possess enhanced cellular uptake through the action of the lipophillic handle, while the nitro moiety would be activated by cancerous tissue. Although various 5-FU lipophillic prodrugs have been prepared in the past (SECTION 1.4.2.2) we believed that a lipophilic carrier ester linked to 5-FU would have enhanced lipophilicity and good *in-vivo* cleavage lability. Our choice of carrier is the isopropyl-(2R, 3S)-3-(N-acetyl) amino-2-hydroxy-3-phenylpropionate **83** (FIGURE 93),[165] a derivative of the Taxol **85** side chain (2R, 3S)-N-benzoyl-3-phenylisoserine **84** (FIGURE 93).[1]



83



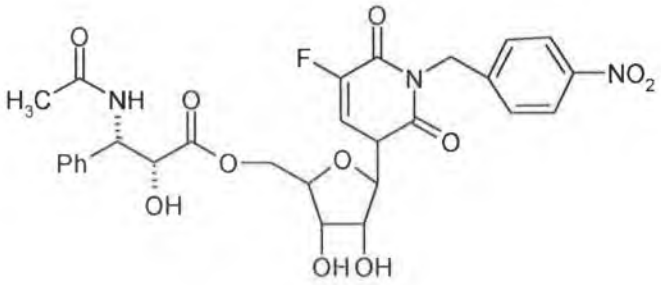
84



85

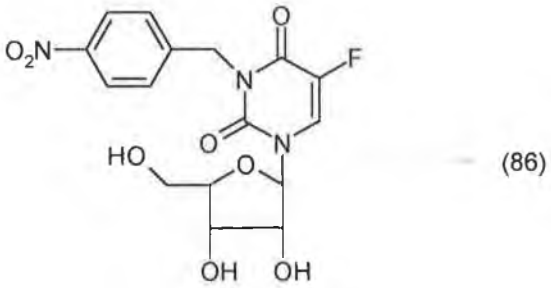
FIGURE 93. POSSIBLE LIPOPHILIC HANDLES AND TAXOL MOLECULE

The proposed 5-FU prodrug that incorporates the taxol side-chain ester **76** is outlined in FIGURE 94, and its preparation is outlined in FIGURE 95.



86

FIGURE 94. 5-FU PRODRUGS **86**.



(86)

72

FIGURE 95. 5-FU PRODRUG **72**.

We believe that **86** (FIGURE 95) is an interesting prodrug candidate since, the nitro benzyl group will deactivate the 5-FU until it reaches the target area where it can be activated either by the reduction environment of the hypoxic cancerous tissue or through the administration of nitroreductase. Furthermore, the synthetic strategy we are employing requires the ^{3}N-amine to be protected to prevent unwanted side reactions and the nitro benzyl group will act as the protection group.

To prepare **86**, we needed to conjugate ester **83** to the nitrobenzyl-5-FU prodrug **72**. A retrosynthetic analysis of our target **86** is outlined in FIGURE 96.

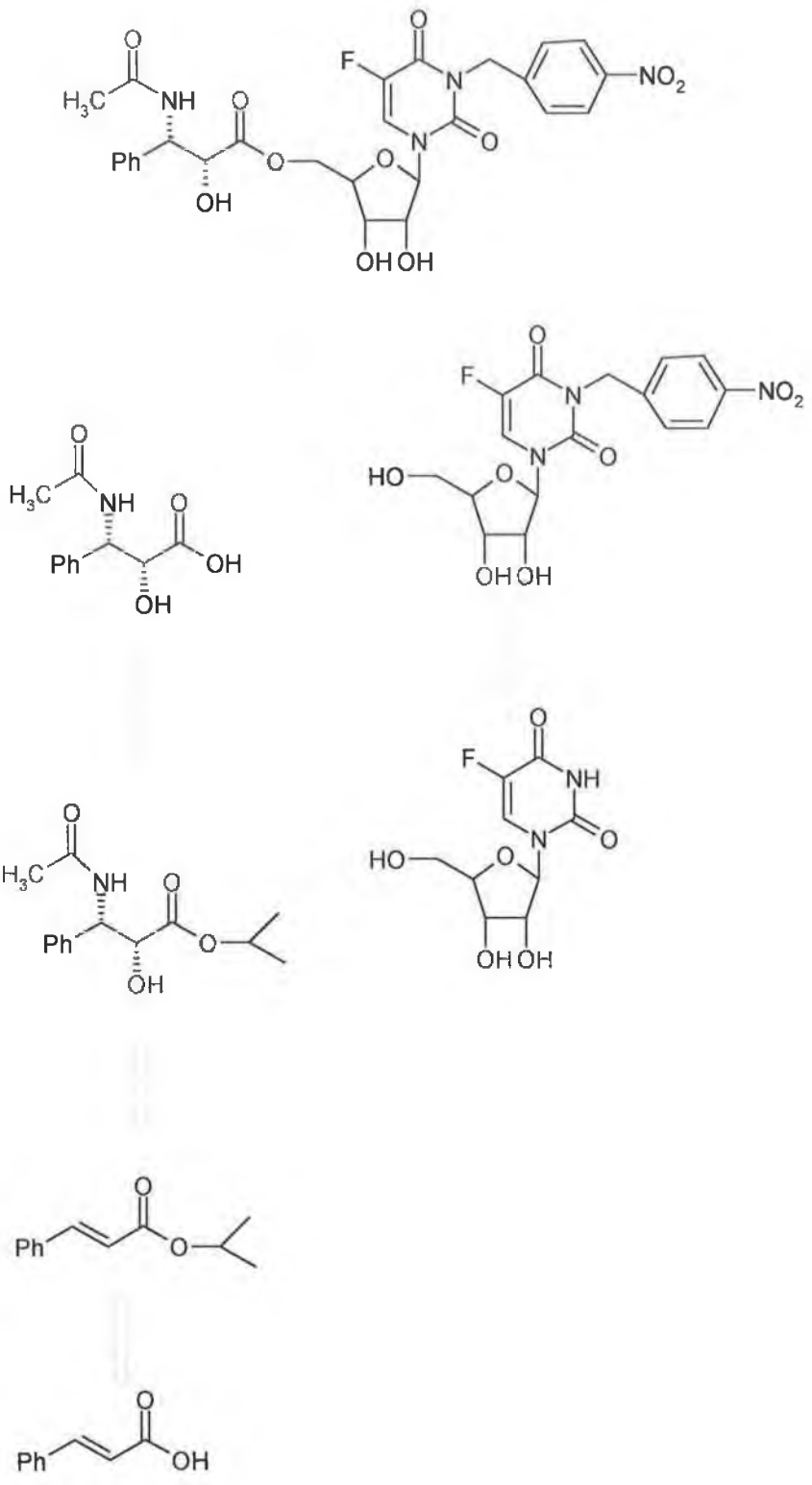


FIGURE 96. PROPOSED RETROSYNTHETIC STRATEGY FOR **86**.

Prior to the synthesis of **86** we needed to prepare ester **83**, once prepared we believed that it can be readily conjugated to **72**. Outlined in FIGURE 97 is a proposed synthetic route to **83** involving an asymmetric Sharpless aminohydroxylation.

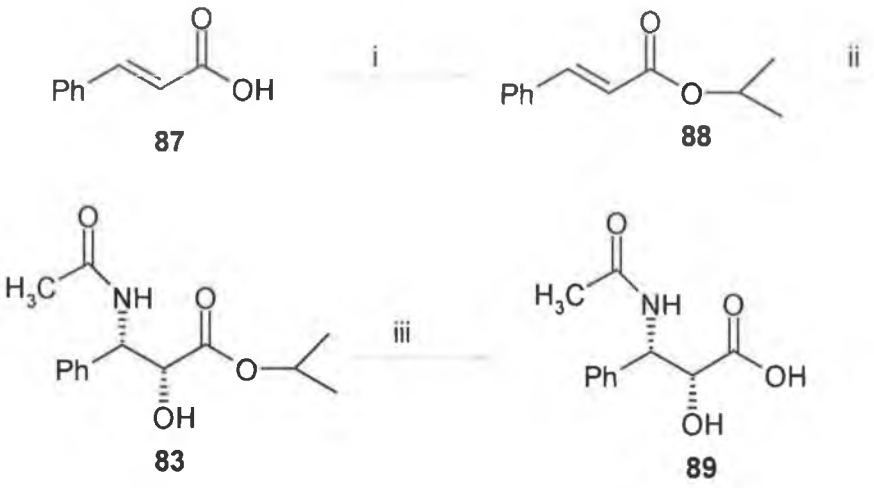


FIGURE 97. PROPOSED SYNTHETIC SCHEME FOR **83**. Reagents i) IPA, trimethylsilyl chloride, reflux 4hr, ii) K_2OsO_4, $(DHQ)_2PHAL$, $LiOH.H_2O$, NBA, $tBuOH/H_2O$,4°C, iii) $LiOH.H_2O$, IPA/H_2O, HCl, 0°C.

3.1. Preparation of Ester 83

Using the Sharpless asymmetric aminohydroxylation reaction[166,167] we set about synthesising **83**. Using cinnamic acid **87** as starting material we carried out an esterification reaction through the use of trimethylsilyl chloride mediated acidic dehydrating conditions to obtain the isopropyl cinnamate **88** in 71% yield (FIGURE 98).

87 **88**

FIGURE 98. REACTION SCHEME FOR THE SYNTHESIS OF ISOPROPYL CINNAMATE **88**. Reagents: i) propan-2-ol, trimethylsilyl chloride, reflux 4hr.

The Nitrogen source in an asymmetric aminohydroxylation reaction has two fundamental roles:[166]

(1) To act as a co-oxidant thereby aiding the aminohydroxylation reaction.

(2) To incorporate a primary amide moiety into the structure of the ester.

We attempted to synthesise primary bromo-amides from di-bromoisocyanuric acid **90**,[168] which was prepared from cyanuric acid **91**, LiOH and bromine (FIGURE 99), this reagent can then be used in the aminohydroxylation reaction to generate ester **83**.

91 **90**

FIGURE 99. PREPARATION OF DI-BROMOISOCYANURIC ACID **90**. Reagents i) $LiOH,H_2O$ and Br_2.

When the di-bromoisocyanuric acid was refluxed with the appropriate primary amide in the dark for 5-6 hours a N-bromo primary amide resulted in moderate to high yield. The two primary amides we prepared by this method were N-bromoactamide (NBA) **92** and N-bromobenzamide (NBB) **93** which were prepared in yields of 85% and 91%, respectively.

N-bromoactamide **92** (FIGURE 100) could also be prepared by dissolving the acetamide in bromine and subsequently treating it with potassium hydroxide until the solution turned yellow, an excess of calcium hydroxide was then added to the mixture.[169] Hot dichlormethane was then used to extract the NBA from a brine solution, which precipitated upon treatment of hexane to give NBA in 37% yield. Both bromoamides proved to be quite stable to storage below 0°C over several weeks.

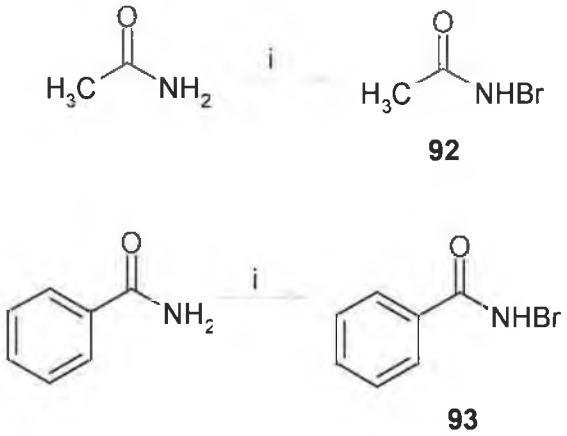


FIGURE 100. SYNTHESIS OF NBA AND NBB. Reagents : i) di-bromoisocyanuric acid **90** in DCM.

3.1.1. Asymmetric aminohydroxylation

The synthesis of the isopropyl **83** methyl **95** and t-butyl **96** esters was achieved by using standard aminohydroxylation conditions (FIGURE 101).[167] An alkaline solution of potassium osmate was treated with *tert*-butyl alcohol and chiral ligand $(DHQ)_2PHAL$ at room temperature followed by dilution, which resulted in a homogenous solution. This solution was cooled with rapid stirring to 4°C. Subsequently, N-bromoacetamide was added in one portion, followed by either one of the esters. Overnight stirring resulted in the formation of **83**, **95** and **96**, respectively. Isolation was adapted from the Sharpless procedure. Addition of sodium sulphite was essential to allow for the effective ethyl acetate extraction of organics. Silica chromatography (1:1 ethyl acetate:hexane)

followed by ethyl acetate/hexane crystallisation provided each of the aforementioned esters in respective yields of 75% **83**, 58% **95** and 88% **96**.

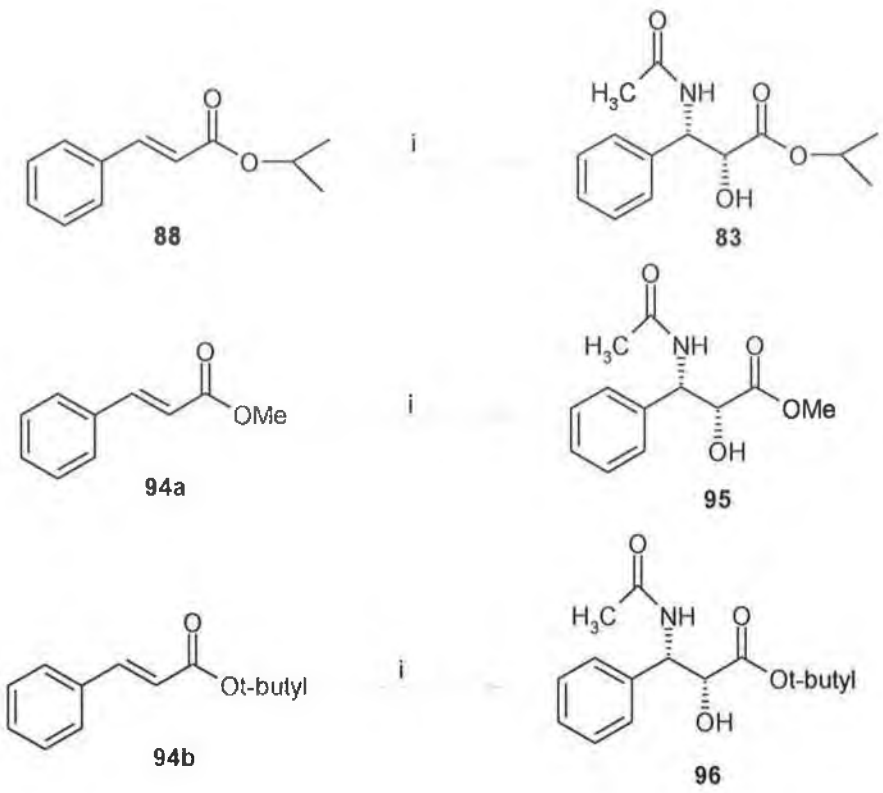


FIGURE 101. REACTION SCHEME FOR SYNTHESIS OF SIDE CHAIN DERIVATIVES. Reagents: i) K_2OsO_4, $(DHQ)_2PHAL$, $LiOH.H_2O$, NBA, $tBuOH/H_2O$,4°C.

3.1.2. β-Benzamido side chain formation

We also decided to prepare the benzamido derivative **97** (FIGURE 102) with the intention to couple it to **72**. This was achieved using two different procedures outlined in FIGURES 103 and 104.

The first procedure involved using Sharpless asymmetric aminohydroxylation with NBB **93** instead of NBA **92** to generate **84** in 30% yield. Followed by hydrolysis to its respective acid via sapanofication yielding **97** in 10% yield. (FIGURE 102).[170, 176]

In the second procedure, compound **83** was prepared via asymmetric aminohydroxylation and was then hydrolysed in boiling 10% HCl over 3 to 4 hours. The organic impurities were isolated through an isopropyl ether/hexane wash, to yield the acid **98**. Conversion of acid **98** to the hydrolysed benzamido product **97** (FIGURE 103) proceeded efficiently via direct benzoylation, although the formation of sodium benzoate gum did hamper the isolation leading to a yield of 72%. [165, 170,176]

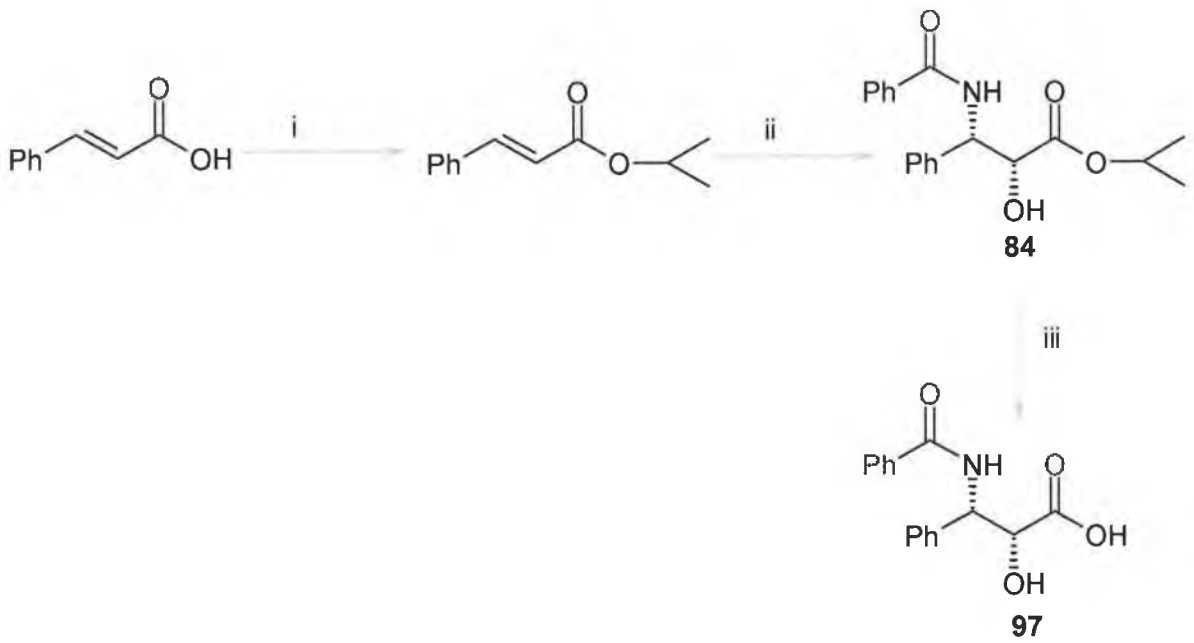


FIGURE 102. REACTION SCHEME FOR THE PREPARATION OF **97**. Reagents i) IPA, trimethylsilyl chloride, reflux 4hr, ii) K_2OsO_4, $(DHQ)_2PHAL$, $LiOH.H_2O$, NBB, t-$BuOH/H_2O$,4°C, iii) $LiOH.H_2O$, IPA/H_2O, HCl, 0°C.

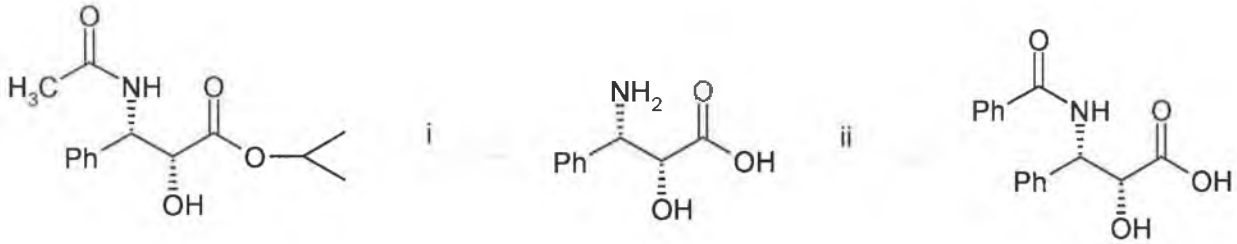


FIGURE 103. REACTION SCHEME FOR THE PREPARATION OF **97**. Reagents i) HCl, refux, 4 hrs, ii) BzCl, NaOH, H_2O.

3.1.3. Protection of **83** prior to coupling

The next step toward the preparation of **83** involved a DCC coupling. Since epimerisation is known to occur under DCC conditions we envisaged a need to protect **83** prior to its use.[172] Therefore, a decision had to be made whether to start the laborious task of selectively protecting the C'-2 hydroxy group (FIGURE 104) of the side chain with various protecting groups prior to coupling, or to try to find an easier quicker alternative.

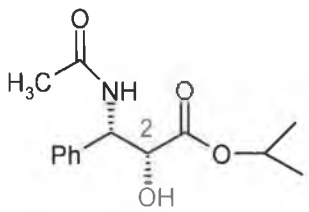
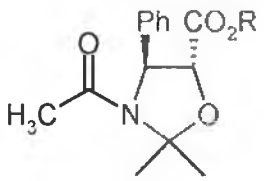


FIGURE 104. HYDROXYL GROUP OF **83** THAT NEEDS TO BE PROTECTED PRIOR TO COUPLING

We decided to convert the isoserine side chain to an oxazolidine **99** (FIGURE 105), as this structure holds the configuration during the esterification reaction and protects the C'-2 hydroxy group from unnecessary side reactions.[166]



R = $CH(CH_3)_2$

99

FIGURE 105. OXAZOLIDINE **99**.

3.1.4. Oxazolidine formation

Following a modified Commercon procedure[173] for the synthesis of oxazolidines we successfully generated our lipophilic oxazolidine **99**. This was achieved by preparing a toluene solution of **83** (FIGURE 106) and treating it with PPTS and 2-methoxypropene. Another quantity of PPTS was added and the reaction mixture was heated to 80°C while collecting the distillate in a dean stark flask to help drive the reaction to completion. A constant reaction volume was maintained with the addition of a toluene/2-methoxypropene solution. Upon completion of the 2-methoxypropene additions, a small quantity of PPTS was added to ensure product formation and the mixture concentrated. Isolation of the product was achieved by silica gel chromatography to give **100** in a 10-15% yield (FIGURE 106).

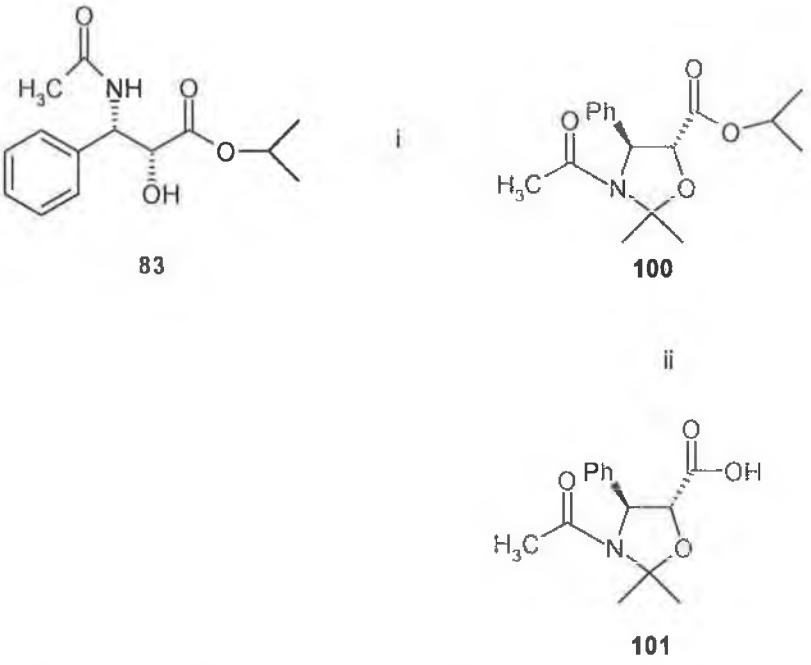
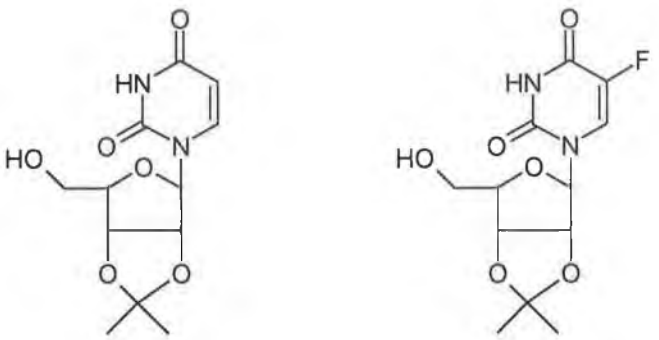


FIGURE 106. REACTION SCHEME FOR THE SYNTHESIS OF OXAZOLIDINE **100** AND **101**. Reagents: i) 2-methoxypropene, PPTS, toluene, 80°C; ii) $LiOH.H_2O$, IPA/H_2O, HCl, 0°C

Hydrolysis of the ester was achieved by saponification, with lithium hydroxide. Careful work up of the resulting salt was achieved through low temperature protonation with hydrochloric acid, and then extraction with ethyl acetate gave the desired acid. The resultant product **101** was stored below 0°C until required for esterification.

3.2. Synthesising the Lipophilic-5FU Prodrug

We attempted the synthesis of **86** using DCC coupling conditions. However, prior to this it was decided to first test the reaction conditions using the uridine and 5-FURD derivatives **68** and **69**, respectively (FIGURE 107).



68 **69**

FIGURE 107. URIDINE **68** AND 5-FURD **69** DERIVATIVES.

By using a modified esterfication procedure[174] we attempted to generate the desired prodrugs (FIGURE 108). The oxazolidine acid **101** was activated with DCC in toluene at room temperature. The reaction was brought to 80°C followed by the addition of DMAP and nucleosides **68** and **69** (FIGURE 108). The mixture was stirred for approximately 2 hours until the reaction was complete (reaction monitored by TLC). However, the isolation/purification of the product proved tedious because there was an appreciably amount of urea contamination, as a result of the urea being partially soluble in both the aqueous and organic layers. Therefore, yields ranged from 25% for (102) and 45% for **103**. The molecular model of **102** is shown in FIGURE 109.

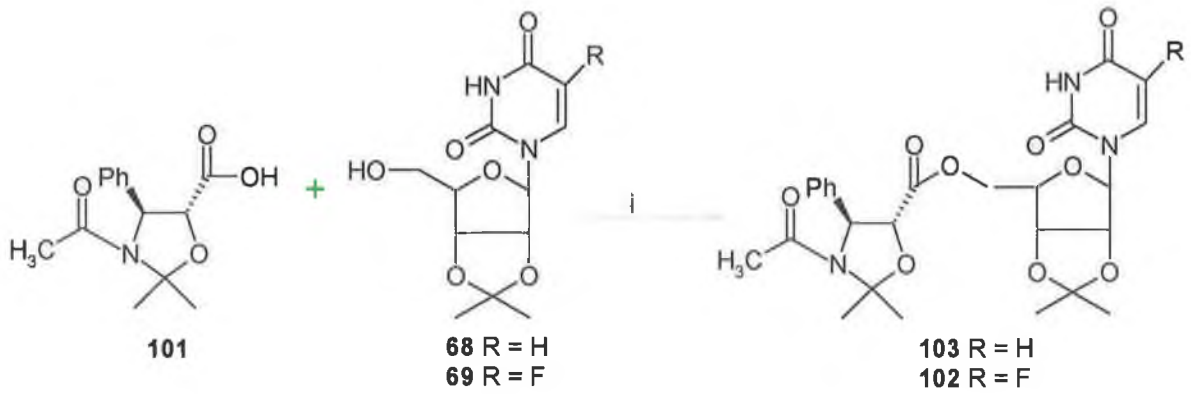


FIGURE 108. REACTION SCHEME FOR SYNTHESIS OF 5-FU PRODRUG **102** AND **103**.
Reagents: i) DCC, DMAP, toluene, 80°C, 2hr.

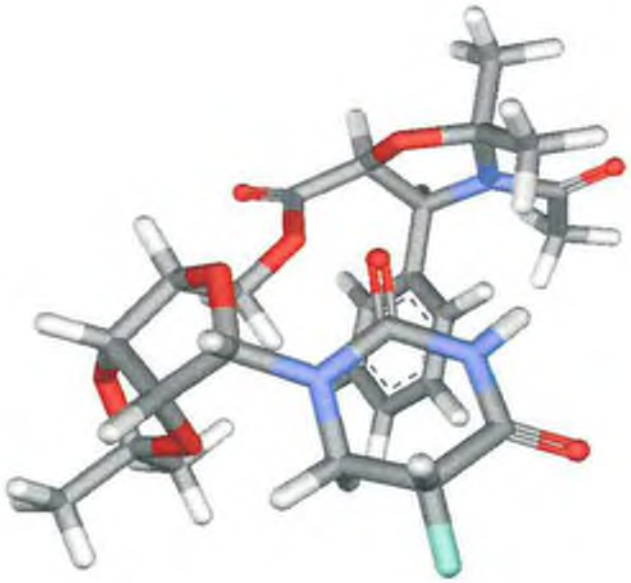


FIGURE 109 MOLECULAR MODEL OF **102**.

3.3. Deprotection of the Nucleoside Esters

At this point we decided to try to deprotect the hydroxyls in a one step reaction thus generating the coupled prodrug (FIGURE 110). If this could be achieved we would then turn our attention to the synthesis of prodrug **86**.

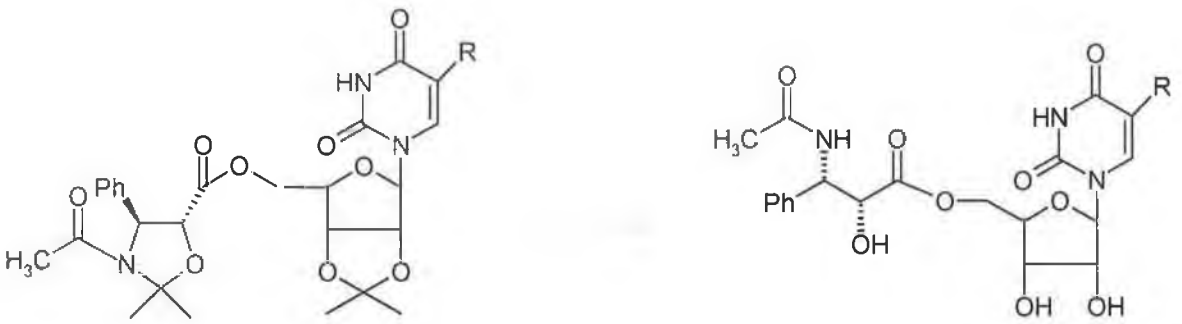


FIGURE 110. ONE POT DEPROTECTION OF ACETONIDES.

It has been found that several Bronsted acid reagents such as acetic acid, HBr(aq), HCl(aq) and H_2SO_4(aq) can be used to selectively cleave terminal isopropylidene groups.[175] However, although these acidic conditions resulted in cleavage of the isopropyl groups the unfavourable α-hydroxyl activated ester hydrolysis was found to be unavoidable. (FIGURE 111).

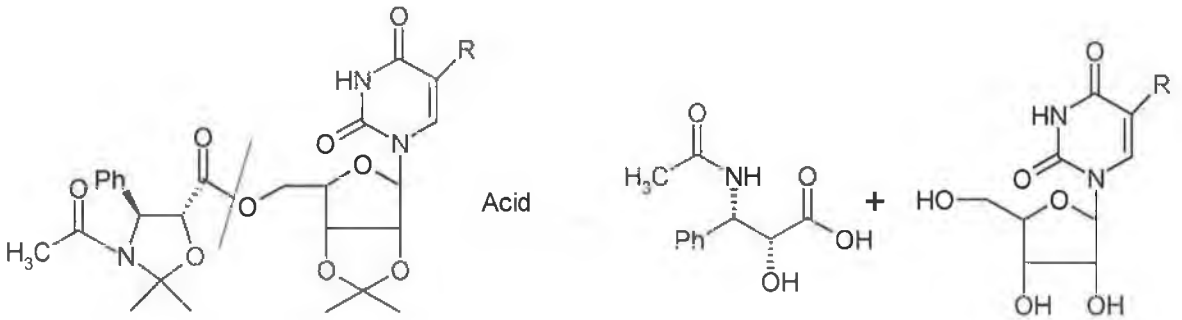


FIGURE 111. UNAVOIDABLE HYDROXYL ACTIVATED ESTER HYDROLYSIS.

Therefore, these reagents could not be used for the deprotection of our target prodrugs. Milder reaction conditions would be needed for its deprotection. Compared to protic conditions, Lewis acid based reagents are scarce. A number of Lewis acids have been reported for the cleavage of the terminal isopropylidenes in the presence of an internal one. Some of these Lewis acids include $FeCl_3 \cdot 6H_2O/SiO_2$, $CuCl_2 \cdot H_2O$ in ethanol and $Zn(NO)_3 \cdot 6H_2O$ in acetonitrile (FIGURE 112). It would seem that *in-situ* hydrolysis of these salts provide the necessary protic medium for hydrolysis. We attempted to take advantage of a gentler Lewis acid $Zn(NO)_3 \cdot 6H_2O$ in acetonitrile[175] which has been used previously for the selective cleavage of terminal isopropylidenes.

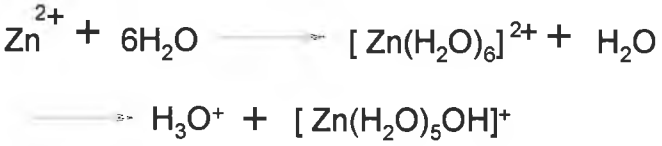


FIGURE 112. FORMATION OF ACIDIC CONDITIONS USING $Zn(NO)_3 \cdot 6H_2O$.

However, before we attempted to selectively deprotect our prodrugs we decided to first test the conditions on the oxazolidine **101** and 2',3'-isopropylideneuridine **68** (FIGURE

113). We dissolved 68 in acetonitrile upon which the $Zn(NO)_3 \cdot 6H_2O$ was added and stirred at 27°C. The reaction was monitored by TLC until only a single baseline spot remained, after 50 hours a spot that was consistent with uridine remained and was confirmed by ^{1}HNMR. This reaction was repeated at 50°C with uridine formation after 8 hours (TABLE 15.).

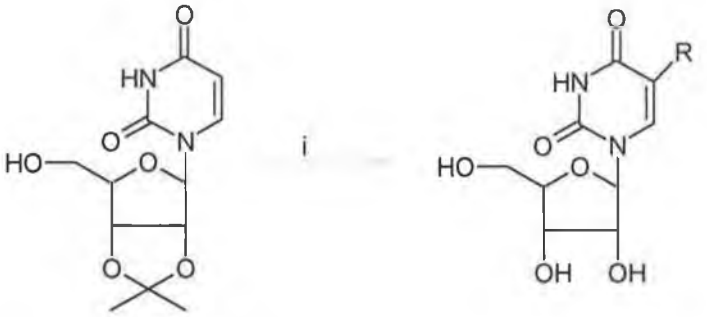


FIGURE 113. GENERATION OF URIDINE.

Reagents i) acetonitrile, $Zn(NO)_3 \cdot 6H_2O$ heated to 50°C.

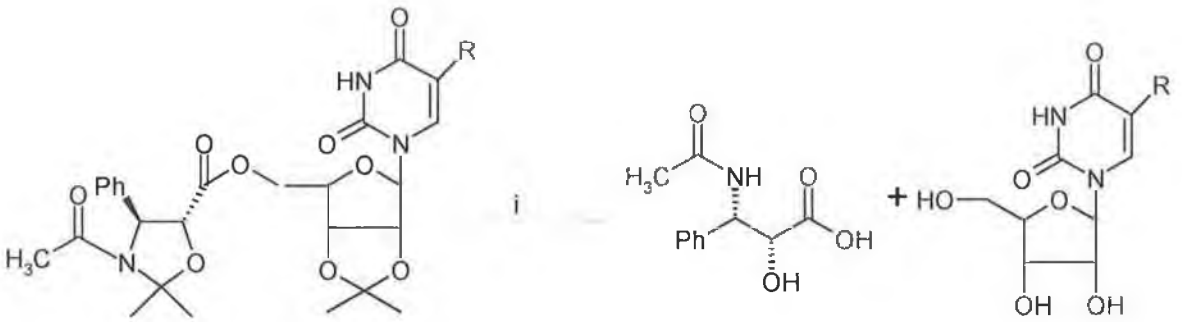
Like-wise, oxazolidine **101** was dissolved in acetonitrile with $Zn(NO)_3 \cdot 6H_2O$ and stirred at 27°C (FIGURE 114). The reaction was monitored over approximately 20 hours until only the unprotected side chain spot remained by TLC. Again this reaction was repeated at 50°C to yield the acid in 10 hours (TABLE 15.).

FIGURE 114. DEPROTECTION OF OXAZOLIDINE **88**.

Reagents i) acetonitrile, $Zn(NO)_3 \cdot 6H_2O$ heated to 50°C.

TABLE 15. RESULTS FOR DEPROTECTION OF **68** AND **101** USING $Zn(NO_3)_2 6H_2O$ AT 27°C AND 50°C.

Compound number	27°C.	50°C.
101	20 hours	10 hours
68	50 hours	8 hours



103 R = H
102 R = F

FIGURE 115. REACTION SCHEME FOR DEPROTECTION OF 5-FU PRODRUG. Reagents i) acetonitrile, $Zn(NO_3)_2 6H_2O$ heated to 50°C.

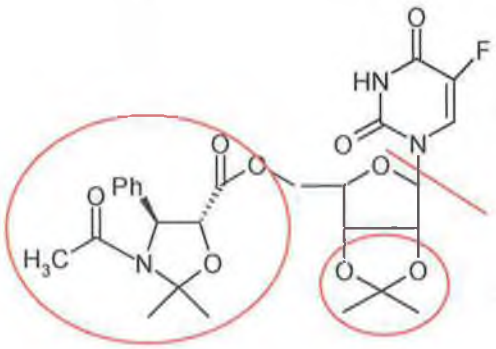
Following this, the prodrug derivatives **102** and **103** (FIGURE 115) were treated as above and monitored by TLC. However, after approximately 30 hours at 27°C and 16 hours at 50°C two TLC spots indicative of the products of ester hydrolysis. Confirming not only the isopropylidene protections have been eliminated, but that the unfavourable ester hydrolysis reaction had occurred (TABLE 16.). Therefore, the one-pot deprotection reaction was unsuccessful, since ester hydrolysis occurred simultaneously to isopropylidene cleavage.

TABLE 16. RESULTS FOR DEPROTECTION OF **102** AND **103** USING $Zn(NO_3)_2 6H_2O$ AT 27°C AND 50°C.

Compound number	27°C.	50°C.
102	30 hours	16 hours
103	30 hours	16 hours

As a result of this we decided to abandon the synthesis of **86**, and instead use prodrug **102** to examine the effect of the ester side chain **83** had on 5-FURD activity. When prodrug **102** was tested against SW480 and 4T1 cell lines over a 3 and 5 day period at a concentration between 0.1-0.7μg/ml we obtained an IC_{50} value of >7μg/ml. Instead of killing the cells the prodrug has in fact promoted their growth. When this value is compared to the 5-FURD **20** and the acetonide derivative of 5-FURD **69** we see that a similar value to **69** is obtained. It would seem that the cytoxicity is inhibited through the protection of the nucleoside diols, therefore, deactivating the drug. This can be explained by the fact that the nucleosides **68** and **69** cannot be converted directly to the phosphate metabolites, i.e. pathway is blocked by the acetonide protecting group, unlike nucleoside **20**. It also appears that the cytotoxicty is further decreased by substitution of the oxazolidine moiety. Again this is probably due to the fact that the primary alcohol of the prodrug is protected, therefore, prohibiting the direct generation of the phosphate metabolites.

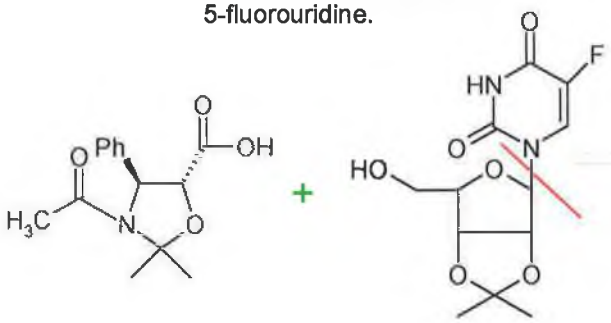
Lipophilic agent enhances drugs diffusion into the cell were 5-FU can be released.



5-FU released in cell generating the metabolites:
5-fluorouridine-trl-phosphate,
2,deoxyfluororidine mono-phosphate
2-deoxy-5-fluorouridine-3-phosphate

Prevents formation of phosphate metabolites.

Invitro hydrolysis of ester releasing oxazolidene and 2',3'-isopropylidene-5-fluorouridine.



5-FU released in cell generating the metabolites:
5-fluorouridine-tri-phosphate,
2,deoxyfluororidine mono-phosphate
2-deoxy-5-fluorouridine-3-phosphate

FIGURE 116. POSSIBLE REACTION PATHWAY FOR THE 5-FU PRODRUG **102**.

3.4. Conclusion

Using prodrug **102** as our initial isopropyl deprotection model we found that the ester linkage of **102** was too labile to the Lewis acid conditions used, resulting in ester hydrolysis simultaneous to isopropyl deprotection. Because of this we abandoned the preparation of prodrug **86** until a more subtle deprotection condition could be found. However, because the prodrug possesses some lipophilic character through its oxazolidine attachment its cellular uptake should be somewhat enhanced when compared to 5-FU. This was tested by examing the cytotoxicity of **102** on SW480 and 4T1 cell lines. The 3 and 5-day cytotoxicity study resulted in IC_{50} values of >7μg/ml for both studies. However, because the highest concentration tested was 7μg/ml further test would need to be carried to determine the cytotoxic dose of the prodrug. Also the synthesis of prodrug **86** in its protected form could also be carried out and tested under hypoxia conditions to examine its activation and activity.

4.0 Novel Deprotection of Acetonides.

As discussed in section 2 the initial design of our nitrobenzyl prodrug **50** incorporated a photosensistiser linked via the N^1 of the 5-fluorouracil. In order to prepare this we needed to selectively substitute a nitrobenzyl moiety at the N^3 of 5-FU hence leaving a single hydroxy free for substitution into the photosensitiser. To achieve this we used the procedure developed by Lin *et al.* (1986), which utilises the following conditions, 1,4-dioxane, benzyl chloroformate, triethylamine dissolved in DMSO at RT (FIGURE 117).

However, it was discovered that the conditions used by Lin *et al.* (1986) did not work for 2,3-isopropylidene-5-fluorouridine **69**. On investigation it was discovered that when the reaction was heated above 50°C an unexpected transformation took place that yielded the removal of the isopropylidine protection group to give the parent nucleoside, 5-fluorouridine **20**.

It was therefore initially thought that this new cleavage condition could be used to selectively cleave our prodrug **102** thereby overcoming the problem of ester hydrolysis (FIGURE 118).

With this in mind it was decided to carry out a study to determine the optimum cleavage reaction conditions; by examining the effect of base, temperature, solvent and reagents, and secondly to use these results to obtain an understanding of the reaction mechanism.

Due to the expense of 5-FU we decided to use the less expensive 2,3-isopropylideneuridine **68** in these studies.

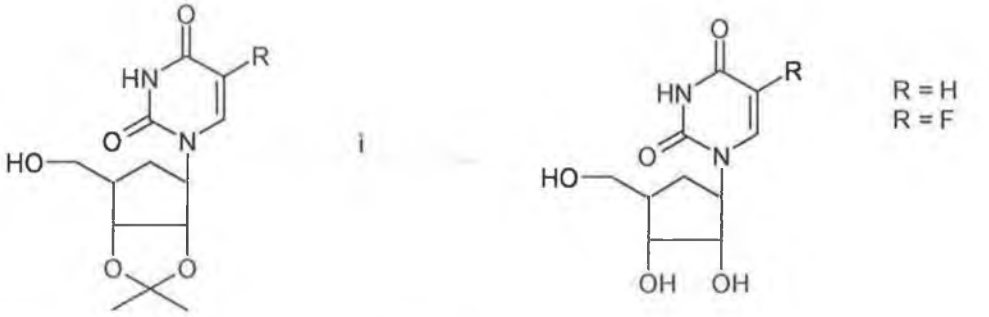


FIGURE 117. GENERAL DEPROTECTION SCHEME.

Reagents i) 1,4-dioxane, benzyl chloroformate,triethylamine and DMSO at >50°C

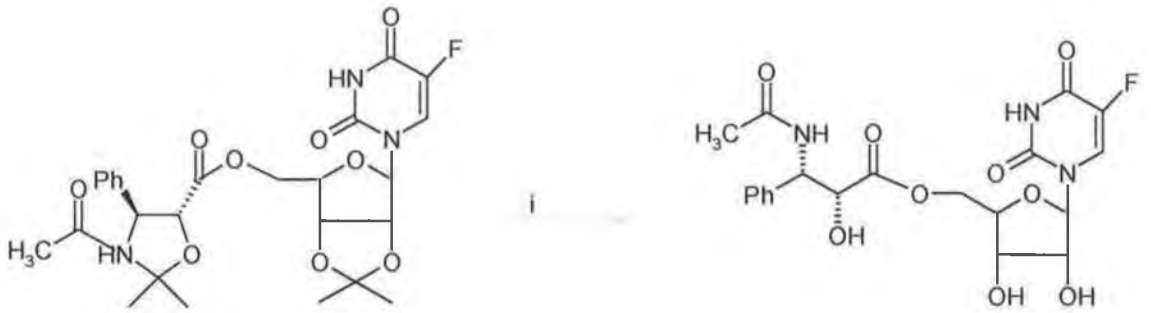


FIGURE 118. GENERAL DEPROTECTION SCHEME FOR PRODRUG **102**.

Reagents i) 1,4-dioxane, benzyl chloroformate,triethylamine and DMSO at >50°C

4.1. Base and Temperature Study

Since the organic base triethylamine (TEA) was used in the initial reaction we decided to investigate the effect of various bases on the reaction. We first attempted the same reaction except at room temperature with the following bases 1,8-diazabicyclo[5.4.0]undecene (DBU), K_2CO_3 , pyridine and 4-dimethylaminopyridine (DMAP). However, as can be seen from the results in TABLE 17 no reaction occurred after 24 hours at room temperature (RT).

TABLE 17. RESULTS FROM BASE STUDY AT ROOM TEMPERATURE

Base	stoichiometry mMole	Temp °C	Time hrs	Reaction Completion
TEA	1	RT	24	No
DMAP	1	RT	24	No
DBU	1	RT	24	No
K_2CO_3	1	RT	24	No
Pyridine	1	RT	24	No

Consequently, we decided to repeat these reaction conditions at higher temperatures with TEA. We initially increased the temperature to 30°C and the reaction was monitored over a two-hour period, with no observable change. The temperature was then increased to 50°C and then gradually to 85°C. It was at this temperature that a base line spot was observed by TLC while the spot indicative of 2,3-isopropylidene-uridine started to disappear. After approximately 12 hours only two spots were observed by TLC. At which point the solution was filtered, concentrated and the fractions were isolated by flash chromatography (2:3 chloroform:ethyl acetate). Analysis of the two spots by NMR showed that the faster eluting spot was the benzyl carboxylic acid and the base line spot was in fact uridine.

We then investigated the other bases (DMAP, DBU, K_2CO_3 and pyridine) at 85°C. A solution of 2,3-isopropylideneuridine in dry DMSO with 1 equivalent of base was prepared and allowed to dissolve. To this a 1 equivalent benzyl chloroformate-dioxane solution was added drop-wise, and allowed to stir at room temperature for 12 hours. All reactions were monitored by TLC. The results of these reactions are shown in Table 18.

TABLE 18. RESULTS FROM BASE STUDY AT 85°C.

Base	**Stoichiometry mMole**	**Temp°C**	**Time hrs**	**Reaction Completion**
Triethylamine	1	RT-85	36	Yes
DMAP	1	RT-85	36	Partial
DBU	1	RT-85	36	Trace
K_2CO_3	1	RT-85	36	No
Pyridine	1	RT-85	36	Partial

However, while monitoring the reaction by TLC we noticed the formation of a new spot for both the DMAP and pyridine reactions. Also, on addition of the DMAP a base line spot formed and therefore masked the formation of the product. Even though each of the reactions were allowed to run for a total of 36 hours, only the TEA reaction went to completion, while both the DMAP and pyridine reactions went to partial completion [i.e. 2,3-isopropylideneuridene spot still evident on TLC]. The reactions with DBU and K_2CO_3 gave no product. Using the same conditions we then examined the effect of base stoichiometry on the reaction. We used the two bases TEA and DMAP since these bases gave the best results (TABLE 18.). A series of reactions were set up using different equivalents of base (0.5-6.0 equivalents) (TABLE 19.). Again only the TEA gave complete reaction while the DMAP showed partial reaction. Therefore, not only did this demonstrate that TEA is the best base for this cleavage reaction but also that its stoichiometry has no effect on the reaction.

TABLE 19. RESULTS FROM TEA AND DMAP BASE STUDY.

Base Type	Stoichiometry mMole	Time hrs	Result RT	Result 85°C
TEA	0.5	36	N	Yes
TEA	1.0	36	N	Yes
TEA	2.0	36	N	Yes
TEA	3.0	36	N	Yes
TEA	6.0	36	N	Yes
DMAP	0.5	36	N	Partial
DMAP	1.0	36	N	Partial
DMAP	2.0	36	N	Partial
DMAP	3.0	36	N	Partial
DMAP	6.0	36	N	Partial

We now decided to eliminate specific reagents so as to determine the effect of the reagents on the reaction. As a result we decided to first assess the combined role of benzyl chloroformate and dioxane. To do this we needed to set up both a "control reaction" and a "test reaction". The control reaction had all the reagents present while the test reaction had dioxane removed. As TABLE 20. below shows the control reaction went to completion while the test reaction did not react at all. This led us to believe that the presence of dioxane is crucial for reaction success.

TABLE 20. REACTION DEPENDENCE ON DIOXANE

Reaction type	Temp°C	Time hrs	Reaction Completion
Control	RT-85	36	Yes
Test	RT-85	36	No

A second reaction was set up to examine the effect of benzyl chloroformate, the control had all reagents present, while the test reaction had benzyl chloroformate removed. As TABLE 21. shows the control reaction gave product formation while the test reaction did not, even after 36 hours had elapsed. It would appear that the presence of both dioxane and benzyl chloroformate is imperative for selective acetonide cleavage.

TABLE 21. REACTION DEPENDENCE ON BENZYLCHLOROFORMATE.

Reaction type	Temp°C	Time hrs	Result
Control	RT-85	36	Completion
Test	RT-85	36	None

This subsequently led us to assess the role of dioxane and benzyl chloroformte in this reaction.

4.2. Dioxane study

While studying the role of 1.4-dioxane on the reaction we discovered that when a freshly purchased bottle of 1,4-dioxane was used no reaction occurred. However, when we reverted back to the aged 1,4-dioxane a complete conversion to the parent nucleoside resulted. From this we concluded that the 1,4-dioxane must generate a species when left over time that is essential for reaction success. However, as it is known that 1,4-dioxane generates peroxides over time we needed to examine if the cleavage reaction was in fact due to the peroxides formed in the 1,4-dioxane. We therefore decided to set-up a series of reactions using different grades of 1,4-dioxane (i.e. shelf live), and also a single reaction containing 30% solution of hydrogen peroxide. The results for which are shown in TABLE 22.

TABLE 22. DIOXANE/PEROXIDE STUDY

Reaction	Time (hour)	Reaction Completion
Fresh batch of dioxane	48	No
Anhydrous batch of dioxane	48	No
Original dioxane batch	12	Yes
30% Hydrogen peroxide 0.5ml	2	Yes

As TABLE 22. shows when the anhydrous and fresh 1,4-dioxane was used no reaction occurred, as opposed to the original 1,4-dioxane batch, which resulted in deprotection. Furthermore, the reaction containing peroxides also gave cleavage. This would indicate that the peroxides formed in the old batch were indeed driving the reaction. Therefore, supporting our hypotheses that the peroxides formed over time in the 1,4-dioxane were influencing the original reaction. However, it was also observed that when the benzyl chloroformate was omitted from the reaction containing peroxide no reaction occured. From these studies it would appear that the required reagents for this cleavage reaction are peroxide, tertiary amine and acid chloride. This hypothesis was further tested and confirmed by a series of experiments outlined in TABLE 23

TABLE 23. BENZYL CHLOROFORMATE AND PEROXIDE ADDITION STUDY.

Benzyl derivative	Peroxide	Solvent	TEA	Reaction Completion
Yes	Yes	DMSO	Yes	Yes
Yes	Yes	DMSO	No	Yes
No	Yes	DMSO	Yes	No
No	Yes	DMSO	No	No
Yes	No	DMSO	Yes	No
Yes	No	DMSO	No	No

An investigation into the effect of peroxide stoichiometry was carried out and results are outlined in TABLE 24. We discovered that 100µl of 30% peroxide was required to deprotect the acetonide (0.33mmol). It was also discovered that when the peroxide quantity was increased to 2ml that the reaction still went to completion (TABLE 24.). However, as demonstrated previously when the peroxide was removed no reaction took place. This study demonstrates that the cleavage reaction is independent of peroxide stoichiometry.

TABLE 24. RESULTS FROM PEROXIDE STUDY ON 0.33MMOL ACETONIDE

Peroxide amt	Benzyl amt mMole	DMSO ml	Reaction Completion
100µl	0.33	3	Yes
200µl	0.33	3	Yes
0.5ml	0.33	3	Yes
1.0ml	0.33	3	Yes
2.0ml	0.33	3	Yes
0ml	0.33	3	No
100µl	0	3	No

4.3. Benzyl Chloroformate Study

Following on from the previous study we decided to investigate the effect of the stoichiometry of benzyl chloroformate on the reaction. Reactions with 0.5, 1.0, 1.5, 2.0 and 5 equivalents of benzyl chloroformate were examined and the results are outlined in TABLE 25. No obvious difference was observed when the reactions were compared by TLC. This study indicates that the benzyl chloroformate may be acting as a catalyst since the reaction is independent of benzyl chloroformate stoichiometry, and all of the benzyl chloroformate is isolated from the reaction mixture as the acid.

TABLE 25. RESULTS FROM BENZYL STUDY (IN H_2O_2, TEA, 85°C AND DMSO).

Acid chloride (equiv)	Time/temperature	Reaction completion
0.5	2 hr /85°C	Yes
1.0	2 hr /85°C	Yes
1.5	2 hr /85°C	Yes
2.0	2 hr /85°C	Yes
5.0	2 hr /85°C	Yes

We also examined the order of addition of reagents, (i.e. peroxide first followed by benzyl chloroformate and also the reverse order). It was noticed that a specific order needed to be followed; benzyl chloroformate first followed by peroxide and heat. When we added the peroxide before the benzyl chloroformate and heated to 80°C the reaction did not go to completion and did not change much after 3 hours. We also tried to add the peroxide to the benzyl chloroformate direct but it would not mix so it had to be diluted with DMSO and left to react. But again no reaction was observed after 12 hours.

4.4. Solvent Study

We set up a solvent study to determine the effect of solvent on both reaction rate and yield. It was also found that when we changed the solvent from DMSO to DMF no reaction occured (TABLE 26.). It would appear from these results that DMSO is the solvent of preference for this cleavage reaction. This fact was further verified when we used methanol, acetonitrile and toluene, no reaction took place. We subsequently decided to study the effect of dilution on the reaction. A minimum amount of DMSO was added just to keep the solutes in solution and followed the reaction protocol, again no reaction was observed.

TABLE 26. RESULTS OF SOLVENT STUDY.

Solvent	Time / temperature	Reaction Completion
DMSO	1 hr /85 °C	Yes
DMF	1 hr /85 °C	No
Methanol	12 h/ 85°C	No
acetonitrile	12 h/ 85°C	No
toluene	12 h/ 85°C	No

4.5. pH Effects

We suspected that the reaction medium turns from neutral to acidic over the course of the reaction, we decided to test this by monitoring the pH of the reaction by taking aliquots at different stages. The initial pH was 6-7 when the reaction was initially heated to 85°C. As the reaction started, an exotherm occurred and the temperature increased to110° C, and the pH was 5. When the reaction temperature settled down to 85°C the pH was found to have dropped to a value of 3. This result demonstrates that as the reaction progresses acid is being generated, thus the deprotection reaction is aided

by acidic conditions. This result may also explain why the addition of base lowers the rate of reaction (TABLE 27.).

TABLE 27. pH STUDY (IN PEROXIDE, 85°C AND DMSO).

Stage	Temperature °C	pH
1	85	6-7
2	110	5
3	85	3

Therefore, because an acid species is being generated during the course of the reaction it would seem logical to assume that the acid chloride could supply the required acid moiety, via hydrolysis (FIGURE 119). This assumption was supported by the confirmation of the presence of the carboxylic acid via NMR analysis.

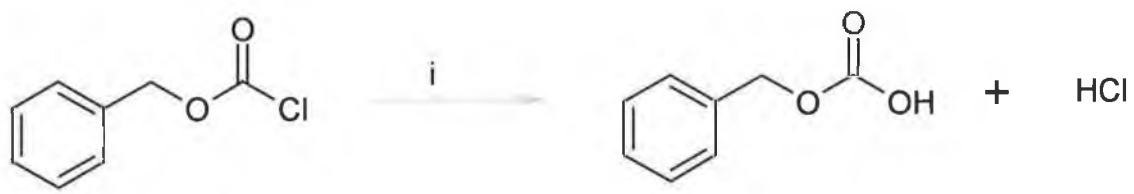


FIGURE 119. HYDROLYSIS OF ACID CHLORIDE.

Reagents i) H_2O.

However, because the reaction uses peroxides for its progress we cannot rule out a radical reaction. We decided to test this theory. If the reaction is indeed a radical reaction then it should be inhibited by the presence of a radical scavenger. We decided to test this hypothesis by setting up 5 reactions using four different scavengers. As can be seen (TABLE 28.) all reactions were inhibited by the scavengers. These results further support that the reaction may indeed be a radical reaction.

TABLE 28. EFFECT OF ADDITION OF SCAVENGER.

Scavenger	Time/temperature	Reaction Completion
Methanol	2 hr /85°C	No
Pyrogallol	2 hr /85°C	No
1,4-cyclohexadiene	2 hr /85°C	No
Tritylmethylchloride	2 hr /85°C	No
Blank	2 hr /85°C	Yes

*Each reaction was allowed to run for 36 hours

But the question remains as to the role of benzyl chloroformate in the radical reaction. We believed that it had to be playing a vital part in the reaction because without it the reaction would not proceed. Given that benzyl groups can form stable radicals we decided to test if other benzyl derivatives could work in this reaction, this will aid us in determining if benzyl chloroformate is behaving as a radical. Results of these studies are outlined in TABLE 29. When phenyl chloroformate was used no reaction occurred, this reagent possesses an acid chloride but is lacking a benzyl carbon. These results indicate that a radical intermediate may indeed be involved in this reaction.

TABLE 29. EFFECT OF DIFFERENT BENZYL REAGENTS.

Radical	Base	Time	Reaction Completion
p-nitrobenzyl- chloroformate	Yes	< 1hr	Yes
p-nitrobenzyl-chloroformate	No	<20 min	Yes
Benzyl- chloroformate	Yes	< 1hr	Yes
Benzyl-chloroformate	No	< 20 min	Yes
p-nitrophenyl-chloroformate	Yes	36 hr	No
p-nitrophenyl-chloroformate	No	36 hr	No

4.6 Reaction Mechanism

Prior to the study our initial understanding of the reaction mechanism was inconclusive. However, after post-test analysis we believe that the reaction may be occurring through two possible mechanisms, 1) a hydrolysis reaction or 2) a free radical reaction. This was founded on the following observations:

4.6.1. Hydrolysis reaction mechanism

(1) An acid chloride is necessary for reaction success. (Section 4.3.)

(2) The acid chloride is hydrolysed generating an acid. (Section 4.5.). Acidic medium is generated as the reaction progresses. (Section 4.5.)

(3) The reaction rate is slower when TEA is present.

(4) The possible formation of a peroxyacetic acid **81** (FIGURE 120).

It has been reported that a peroxyacetic acid can be prepared from acetic acid and hydrogen peroxide in the presence of an acid catalyst.[163, 164]

(81)

FIGURE 120. FORMATION OF PEROXYACETIC ACID.

Therefore, it is plausible to assume the acidic conditions necessary for deprotection is provided through the formation of a peroxy acetic acid and subsequent HCl by-products. This would explain points 1-4 above. Thus, there are possibly three acid sources present 1) hydrolysised acid, 2) HCl and 3) peroxyacid. (FIGURE 121)

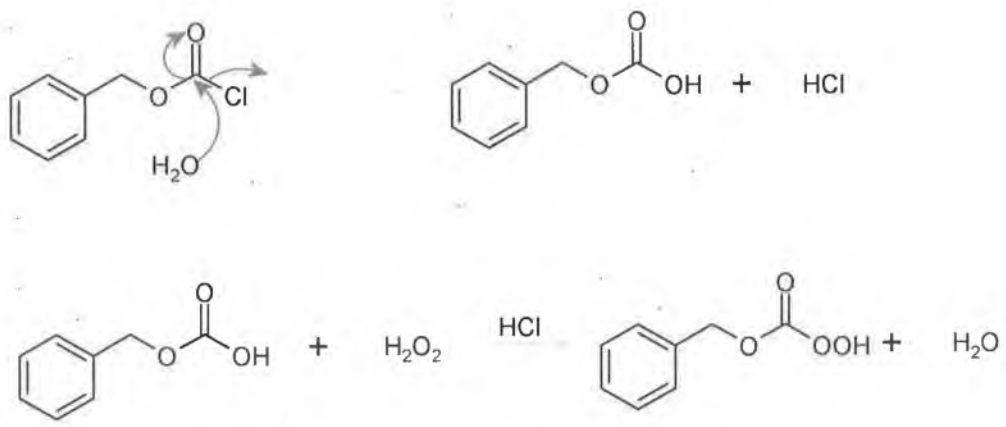


FIGURE 121. GENERATION OF AN ACIDIC ENVIRONMENT THROUGH HYDROLYSIS AND PEROXYACID FORMATION.

4.6.2. Possible free radical role?

(1) Hydrogen peroxide was necessary for reaction success. (Section 4.2.)

(2) Heat was required to initiate the reaction (Section 4.1.)

(3) The addition of radical scavengers inhibited the reaction. (Section 4.5.)

(4) Reaction was dependent on the formation of stable radical intermediates. (Section 4.5.)

It is possible that radicals are formed during the reaction and may ultimately lead to acetonide deprotection. The fact that radical scavengers inhibit the reaction (TABLE 28.) and that the presence of a benzyl carbon is required (TABLE 29.) indicates that a radical species maybe formed. Outlined in FIGURE 122 is a proposed mechanism as to the possible role of a radical species in this reaction. This mechanism does explain the formation of acidic species as the reaction progresses.

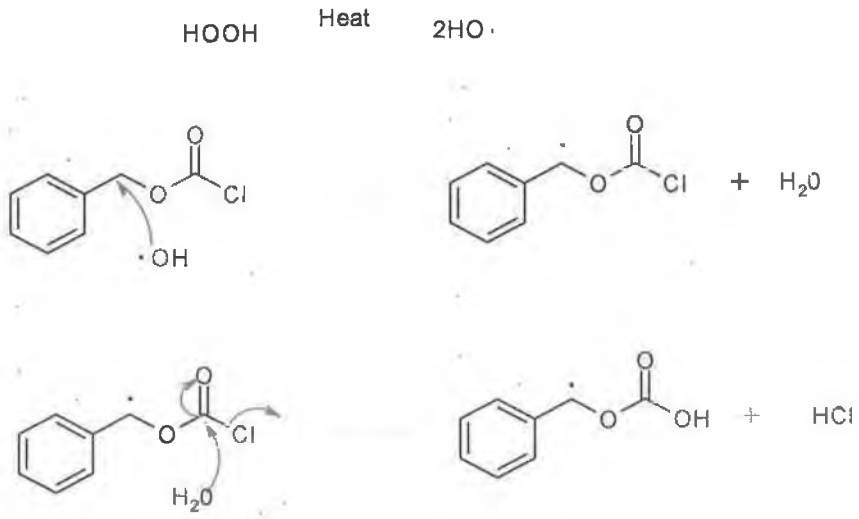


FIGURE 122. POSSIBLE RADICAL/HYDROLYSIS REACTION MECHANISM.

The key to the success of this reaction depends on the generation of acid, whether it occurs by hydrolysis or radical or both (FIGURE 123).

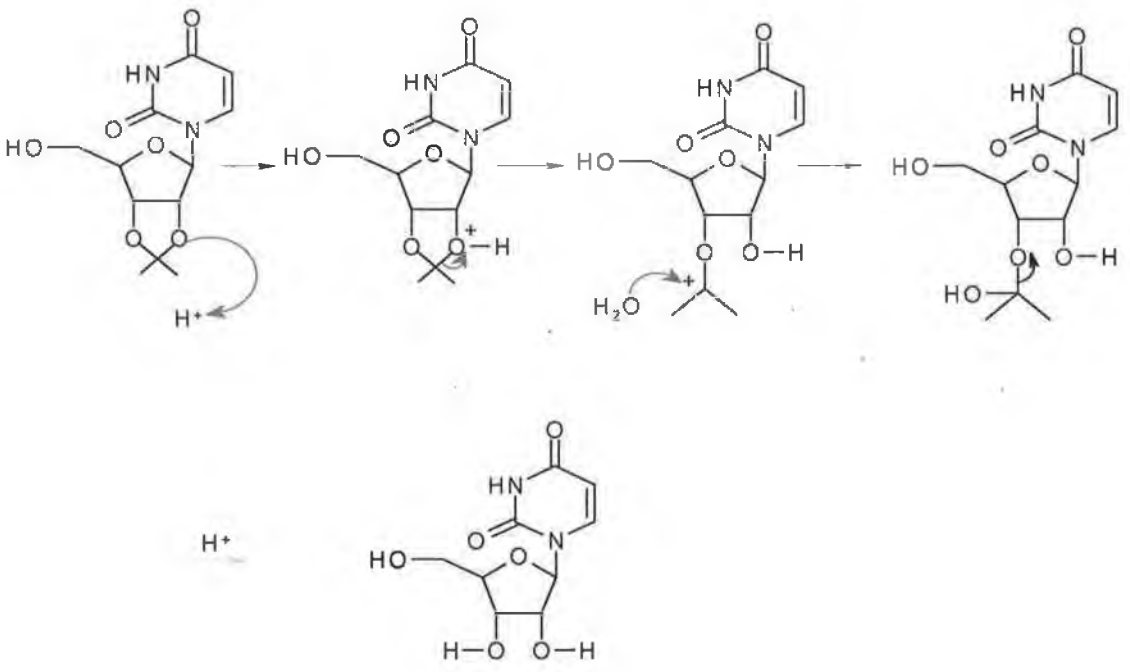
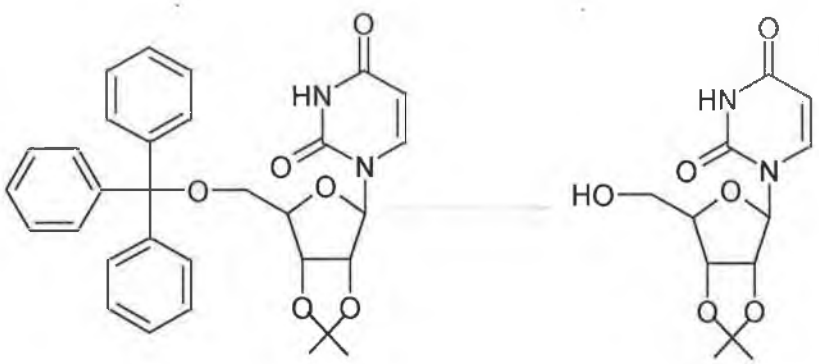


FIGURE 123. SCHEME FOR THE DEPROTECTION OF 2,3-ISOPROPYLIDENEURIDINE.

A final study was carried out to determine the selectivity of the acetonide group over other labile protecting groups using our reaction conditions. We decided to use 5-trityl-2, 3-isopropylideneuridine **82** (FIGURE 124) which is labile to both acidic and basic conditions. Following the aforementioned deprotection reaction conditions (with and without the presence of base) we discovered that the trityl group was selectively cleaved while the acetonide remained unaffected (48% and 34% yield, respectively).



82 **68**

FIGURE 124. REACTION SCHEME FOR SELECTIVE DEPROTECTION OF 5-TRITYL-2, 3-ISOPROPYLIDENEURIDINE **82**.

4.7. Conclusion

In conclusion, while preparing prodrug **50** we discovered a new deprotection procedure to deprotect the nucleosides 2,3-isopropylidene-5-fluorouridine and 2,3-isopropylideneuridine to their parent nucleosides. This was achieved by developing and optimising the reaction conditions; 30% hydrogen peroxide 200µl, a 1:1:1 stoichometry of acetonide, benzyl chloroformate in the presence or absence of base, dissolved in DMSO at 85°C. Yields ranged between 42-58% (TABLE 30. & 31.) after 20 minutes when no base was present, while similar yields were obtained with base after 1 hour.

However, although we initially thought that this procedure could selectively remove the isopropyl moiety of prodrug **102**, thus generating prodrug **105**. We deemed that the reaction conditions would be too harsh, thereby cleaving the more labile ester.

We also found that by using our reaction conditions we could selectively remove the trityl group of 5-trityl-2,3-isopropylideneuridine thus generating 2,3-isopropylideneuridine, in a 48% yield, leaving the isopropylidene intact.

The reaction scheme is believed to follow that of a free radical/acid hydrolysis mechanism, since both reactions produce the acidic environment necessary for acetonide cleavage. However, further test would be needed for a definitive answer.

TABLE 30. Yields for reaction with in the absence of base.

Protected Nucleoside	PRODUCT	YIELD
2,3-isopropylidene-5-fluorouridine	5-fluorouridine	45%
2,3-isopropylideneuridine	Uridine	58%
5-trityl-2,3-isopropylideneuridine	2,3-isopropylideneuridine	48%

TABLE 31. Yields for reaction in the presence of TEA

Protected Nucleoside	PRODUCT	YIELD
2,3-isopropylidene-5-fluorouridine	5-fluorouridine	42%
2,3-isopropylideneuridine	Uridine	50%
5-trityl-2,3-isopropylideneuridine	2,3-isopropylideneuridine	34%

5.0. BIOLOGICAL DATA

The biological data reported within this thesis was carried out by Aisling Redmond of Dr. Susan Mc Donald's cellular biology research group at DCU.

It was decided to test the 5-FURD and 5-FU prodrugs for cytotoxicity using normal oxygenated conditions prior to their hypoxic cytotoxicity evaluation using human colon cancer cell line and 4T1, a murine mammary cancer cell line. The cell lines were cultured under standard conditions, allowed to attach overnight in 96-well plates and then treated for three and five days to determine the IC_{50} values (inhibitory concentration that killed 50% of the cells). From TABLE 34 it can be seen that most toxic compounds were the derivatives of 5-FU, specifically the ester derivatives, with IC_{50} values ranging from 1.53μM to >250μM for three day treatment in the SW480 cell line and 0.25μM to >302μM for five day treatment. The IC_{50} values were lower for the 4T1 cell line, ranging from 0.035μM to >250μM for three day treatment and 0.013μM to 150μM for five day treatment.

TABLE 34. SHOWING IC_{50} VALUES FOR 4T1 AND SW480 CELL LINES

Compd.	IC_{50} Values 4T1 cell Line				IC_{50} Values SW480 Cell Line			
	3 Days		5 Days		3 Days		5 Days	
	μg/ml	μM	μg/ml	μM	μg/ml	μM	μg/ml	μM
5-FU 10	0.05	0.38	0.02	0.15	0.80	6.15	0.40	3.08
FU 51	0.25	0.81	0.01	0.03	3.50	11.33	1.00	3.24
FU 52	60	226	5	19	40	151	80	302
FU 53	ND >100	>250	60	150	ND >100	>250	ND >100	>250
FU 57	0.10	0.45	0.01	0.04	2.50	11.26	0.70	3.15
FU 58	0.09	0.20	0.06	0.13	0.70	1.53	0.60	1.31
FU 59	0.009	0.035	0.005	0.018	0.40	1.64	0.06	0.25
FU 60	0.3	0.79	0.005	0.013	4.50	11.90	2.00	5.29
FURD 20	0.005	0.02	ND <0.001	<0.004	0.10	0.38	0.06	0.23
FURD 69	ND >100	>332	70	233	ND >100	>332	ND >100	>332
FURD 71	ND >7	>16	ND >7	>16	ND >7	>16	ND >7	>16
FURD 72	ND >3	>8	ND >3	>8	ND >3	>8	ND >3	>8
FURD 102	ND >7	>13	3.5	6.40	ND >7	>13	ND >7	>13

*ND = Not Determined

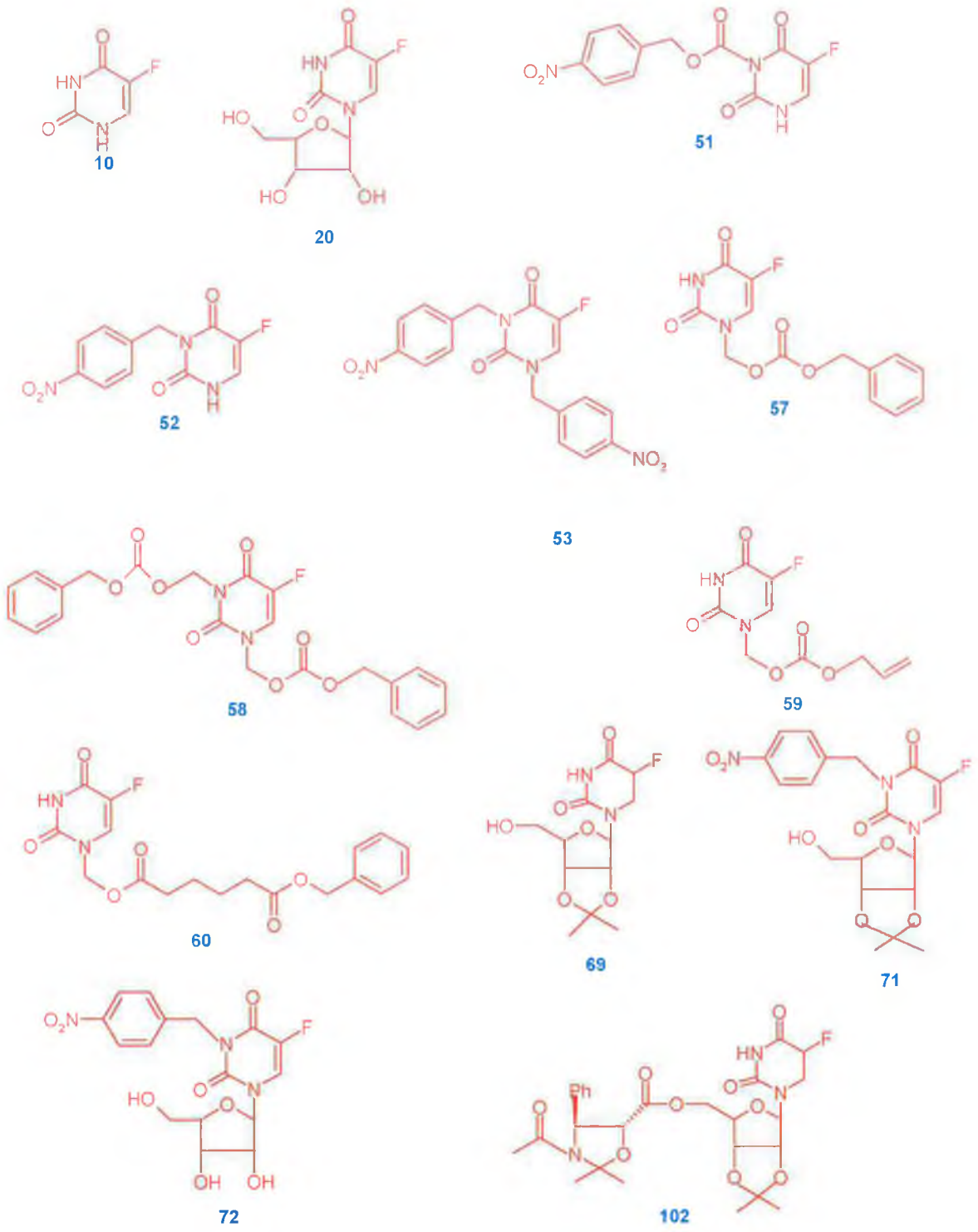
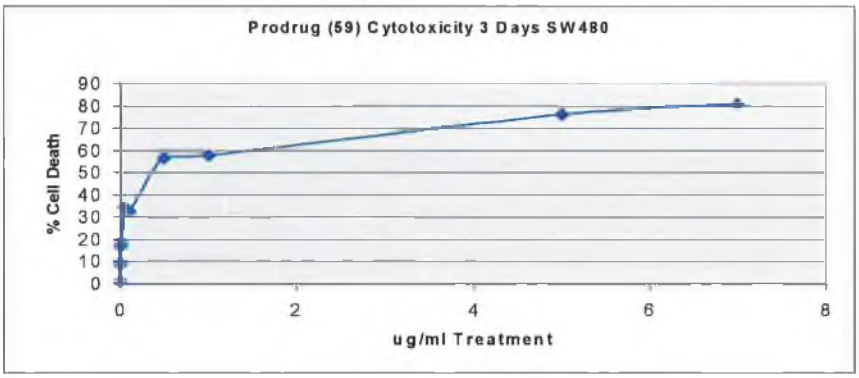
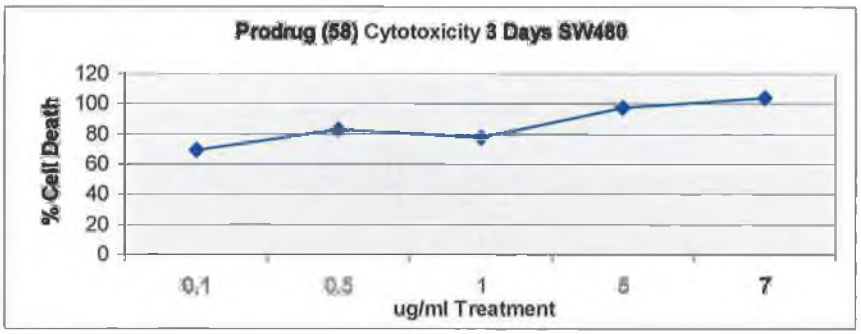


FIGURE 125. STRUCTURES FOR THE BIOLOGICALLY TESTED PRODRUGS.

The metabolites of 5-FU **10** are 5-fluorouridine-tri-phosphate, 2'-deoxy-5'-fluororidine mono-phosphate, and 2-deoxy-5-fluorouridine-triphosphate (SEE SECTION 1.4). These are intracellular toxic forms of 5-FU and result in both DNA and RNA directed damage. The 5-FURD derivative **20** shows higher toxicity at lower concentrations than 5-FU **10** this can be ascribed to **20** being in its nucleotide form, and therefore "skipping" a metabolic step. The 5-FU ester derivatives **60** and **57** show increased activity at increased concentrations. Demonstrating that the protection groups have lowered the activity of 5-FU. The 5-FURD prodrug **72** does not have as good activity as **20** and this is presumably due to the presence of the nitro-aromatic group, which would appear to be deactivating the 5-FU. No activity is observed for prodrugs **69** and **71** at the concentrations used due to the hydroxyls being protected therefore preventing the formation of the tri-phosphate metabolite. We also believe that prodrug **71** is further inhibited due to the presence of nitro-aromatic groups as was the case with **72**.

Prodrug **59** has comparable activity to **20**, we believe that the carbonate ester linkage is very labile and releases 5-FU rapidly. The prodrug derivative **58** containing two carbonate esters shows good cytotoxicity but with one order of magnitude less than **59**. We believe that 5-FU is being released more slowly in **58** than in **59** since two groups must be hydrolysed instead of one (FIGURE 126).


Prodrug (59) Cytotoxicity 3 Days SW480
% Cell Death
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
ug/ml Treatment



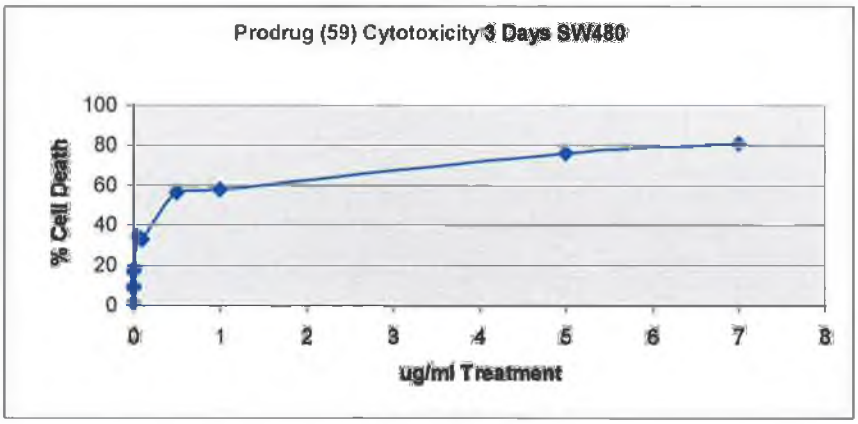


Figure 126. Graph of prodrugs **20**, **58** and **59** cytoxicities

Both the nitrobenzyl prodrugs **52** and **53** show similar cytotoxicity within the studied concentrations. They both have similar cytotoxicity profiles to **72**. Thus, when compared to the standard 5-FU **10** it can be seen that the aromatic nitro group is inhibiting the cytotoxicity of 5-FU perhaps by slowing down its reaction with thymidylate synthase. However, because our interest remains with the generation of hypoxic sensitive nitrobenzyl 5-FU prodrugs that can be linked into our Pc, it was decided to next evaluate the nitrobenzyl prodrugs cytotoxicity under hypoxic conditions. As mentioned in the introduction, hypoxia results in elevated levels of reductase, we believe that such conditions could be used to determine if mammalian reductase can reduce the nitro benzyl protection group. Thus, cells were cultured under hypoxic conditions (2% O_2) for 24, 48 and 72hrs to determine their sensitivity to hypoxia treatment. Derivatives **52** and **53** were tested under hypoxia. Both prodrugs were incubated with the 4T1 cells for 72hr and showed a significant increase in cytotoxicity under hypoxic conditions, indicating that activation by endogenous one-electron reductases may be occurring (FIGURE 127).

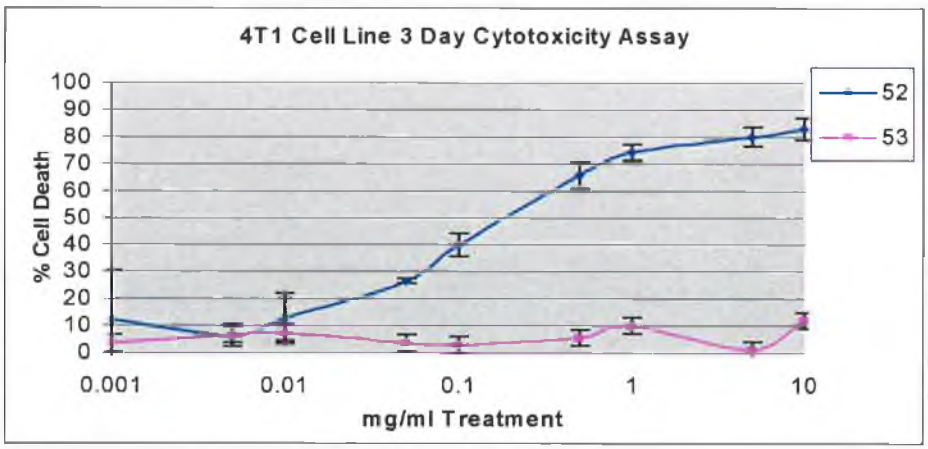
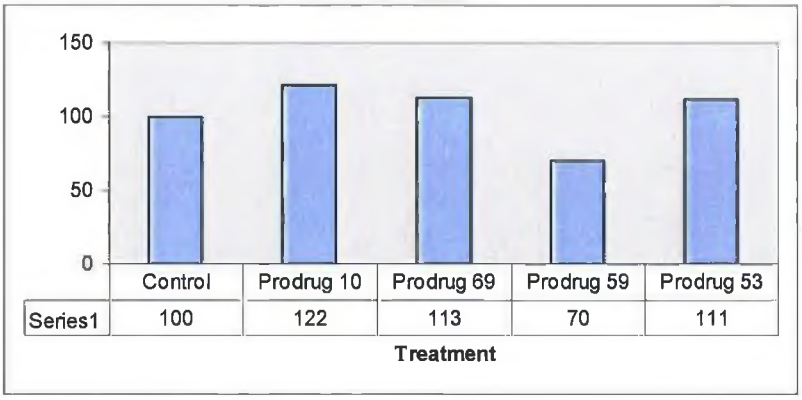


FIGURE 127. GRAPHIC PLOT SHOWING PRODRUGS **52** AND **53** CYTOTOXICITIES UNDER HYPOXIC CONDITIONS OVER 3 DAYS.

5.1. Metastasis Results

Perhaps the greatest killer of most cancer patients results from cancer metastasis, this is where cancer cells from the original tumour invade other areas of the body. Normally metastasised cells are immune to chemotherapy, in some cases the chemotherapy may actually enhance invasion.



.(% CELL GROWTH Y AXIS)

FIGURE 128.METASTASIS RESULTS FOR PRODRUGS **10**, **69**, **59** AND **53**

Research into metastasis inhibition, that is the prevention of invasion by metastasised cells, is becoming a very hot topic. An unexpected result involving metastasis inhibition was discovered for prodrugs **53**, **59** and **69**. The cellular assays used in these studies involve using BD Biocoat matrigel invasion chambers. Cells which, migrate through the chambers, are stained with crystal violet and detected by UV-visible spectroscopy. It was discovered that wells containing **53**, **59** and **69** showed lower invasion than the 5-FU **10** (FIGURE 128). From these results it would appear that prodrugs **53**, **59** and **69** are potential invasion inhibitors for metastasis. This result is also of interest since these compounds could aid in developing models to explain why cancer cells are activated to metastasis.

5.2. FUTURE WORK

We are currently awaiting (a new high sensitivity MALDI system will be installed here at DCU in early 2005) to analyse the metabolites from the above cellular cultures by mass spectrometry to determine/prove that the nitrobenzyl groups are indeed being converted to the respective quinmethides/fluorouracil via reduction. Parallel to this evaluation of the potential activation of these compounds *in-vitro* using a bacterial nitroreductase will be assesed in the next six months. If these experiments demonstrate that nitroreductase can indeed convert our prodrugs to the active form then we have also opened up the door for the potential use of Gene-Directed Enzyme Prodrug Therapy (GDEPT) with our compounds.

A SAR study will also be initiated on prodrugs **59** and **58** to try and determine why they exhibit such excellent activity. These same compounds are to be tested against 5-FU resistant cancer cell lines, and their cytotoxicity against normal mammalian cell lines will also be evaluated. If activity is observed against 5-FU resistant cell lines then **59** and/or **58** have excellent potential as new therapeutics! Furthermore, if the cytotoxicity of **59** and **58** is the same or lower than 5-FU then both of these compounds have excellent potential as cancer treatment candidates!

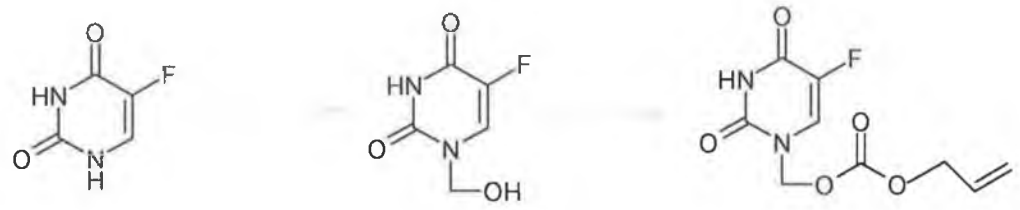
Thus, our initial idea of linking a deactivated nitrobenzyl 5-FU prodrug to a Pc, were it could be activated by reductases under oxidative stress (or hypoxic conditions) appears to be promising based on the results obtained to date. The Pc–nitrobenzyl 5-FU prodrugs are to be tested soon under light irradiance conditions.

General Methodology

^{1}H NMR spectra were measured at 400 MHz, using a Brucker AM 400 spectrometer. ^{13}C NMR spectra were measured on the same instrument at 100 MHz. IR spectra were recorded using a Perkin Elmer 2000 FT-IR spectrometer. Mass spectra were analysed on an Esquire-Bruker/Hewlett Packard LC-MS 1100 series, equipped with an electro-spray ion source. Optical rotations were measured on a Perkin Elmer 343 Polarimeter. Thin layer chromatography was conducted using Riedel de Haen silica 60F254 plates in the solvent systems specified in the text. TLC detection was achieved through UV absorbency. Flash chromatography was carried out using Riedel de Haen silica gel 60. Butanols and lower molecular weight alcohols were dried over magnesium, followed by distillation onto activated molecular sieves. Ethers were dried by sodium/benzophenone reflux, followed by distillation: diethyl ether was pre-columned through alumina. Chlorinated solvents, aliphatic hydrocarbons and benzene were dried over calcium hydride, followed by distillation. Toluene was purified by treatment with ice cold-concentrated sulfuric acid, 30% caustic soda solution, brine followed by pre-drying over $MgSO_4$, before fractional distillation over P_2O_5. Triethylamine and pyridine were stored over KOH, before decanting and distilling from calcium hydride and lithium aluminum hydride respectively. Acetone was stored over $CaSO_4$, prior to distillation. Acetonitrile was dried over P_2O_5, before fractional distillation from calcium hydride. DMF and DMSO were dried over calcium hydride before distillation on to activated molecular sieves. Ethyl acetate was purified by distillation. Distilled solvents were stored under nitrogen gas.

6.0. Potential 5-Fluorouracil Prodrug

6.0.1. Preparation of 1-alloxycarbonyloxymethyl-5-fluorouracil **59**[149]



59

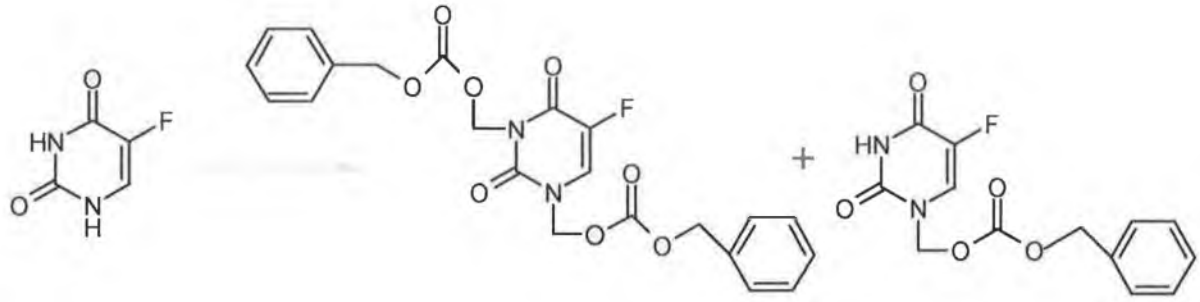
5-FU (1.3g, 10mmol) and 37w% formaldehyde in aqueous solution (2.0g, 25mmol) were added to water. The reaction was heated to 60°C under agitation for 30 minutes. The resultant solution was concentrated and dried in a vacuum oven at 60°C for 48 hours. The oily product was then dissolved in dry acetonitrile (30ml) preceeded by the addition of triethylamine (1.37g 13.5mmol). The solution was then agitated under nitrogen at room temperature, followed by the dropwise addition of allyl chloroformate (1.54g, 12.4mmol). After stirring at room temperature for 3 hours the mixture was filtered and acetonitrile removed under reduced pressure. The crude product was then dissolved in dichloromethane (30ml) and washed three times with HCl (1N), saturated $NaHCO_3$ aq (30ml x 2) and water (30ml x 2). The organic layer was dried over sodium sulfate overnight, suction filtered and concentrated by rotary evaporation. The product was isolated using silica gel chromatography (methanol-dichloromethane, 1:10, v/v) to yield (1.8g, 74%) of **59** as a white solid.

TLC, $R_f = 0.71$ (methanol-dichloromethane, 1:10, v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 4.60-4.63 (m, 2H, O-CH_2-CH=), 5.25-5.37(m, 2H, CH=CH_2), 5.60(s, 2H, N-CH_2-O), 5.89-5.98(m, 1H, CH=CH_2), 8.14(d, 1H, J= 8.0Hz,

CH-CF), 12.03 (bs, 1H, NH). $\delta^{13}C$ (100MHz, DMSO-d_6) δ (ppm) 68.71, 73.68, 119.15, 129.74 (d, J= 34 Hz) 132.06, 140.94, 149.59, 153.71, 157.85, (d, J= 26 Hz), $\delta^{19}F$ (DMSO-d_6) – 169.03(d, 1F, J= -6.3Hz).

6.0.2. Preparation of 1-benzyloxycarbonyloxymethyl-5-fluorouracil **57** and 1,3-benzyloxycarbonyloxymethyl-5-fluorouracil **58**



10 **58** **57**

5-FU (1.3g, 10mmol) and formalin (1.78g, 22mmol) were stirred at approximately 60°C for 45mins. The oily product was dried under vacuum and was then dissolved in dry acetonitrile (30ml) followed by the addition of triethylamine (1.37g 13.5mmol). The solution was then agitated under nitrogen at room temperature, followed by the dropwise addition of benzylchloroformate (2.12g, 12.4mmol). After stirring at room temperature for 3 hours the mixture was filtered and acetonitrile removed under reduced pressure. The crude product was then dissolved in dichloromethane (30ml) and washed three times with HCl (1N), saturated $NaHCO_3$ aq (30ml x 2) and water (30ml x 2). The organic layer was dried over sodium sulfate overnight, suction filtered and concentrated under vaccum. The product was further purified using silica gel chromatography (5:2 chloroform:ethyl acetate) to yield **57** (2.03g in 69%) as a white solid and **58** in 18%.

1-Benzyloxycarbonyloxymethyl-5-fluorouracil **57**

TLC, R_f 0.39 (ethyl acetate-hexane, 1:1v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 5.18(s, 2H, -CH_2-Ph), 5.61 (s, 2H, N-CH_2-O), 7.38-7.42-7.46(m, 5H, aromatic), 8.14(d, 1H, J_F = 6.55, CH-CF), 12.03 (bs, 1H, NH). δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 69.92, 73.74, 128.78, (d, J= 34 Hz) 129.56, 129.91, 135.22, 138.62, 140.92, 149.63, 153.86, 157.98 (d, J= 26 Hz).δ^{19}F (DMSO-d_6) – 169.02(s, 1F).

1,3-Benzyloxycarbonyloxymethyl-5-fluorouracil **58**

TLC, R_f 0.32 (ethyl acetate-hexane, 1:1v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 5.19(d, 4H, J =2.0Hz, -CH_2-Ph + -CH_2-Ph), 5.70 (s, 2H, ^{1}N-CH_2-O), 5.85 (s, 2H, ^{3}N-CH_2-O), 7.37-7.40(m, 10H, aromatic), 8.32(d, 1H, J_F = 6.4, CH-CF). δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 68.70, 123.95, (d, J= 7.9 Hz), 124.67 (d, J= 38H z), 129.02, 139.42, 141.76, 142.80, 146.33, 147.60, 149.83, 157.35 (d, J= 27H z). δ^{19}F (DMSO-d_6) – 167.50(d, 1F, J= -6.2 Hz).

6.0.3. Preparation of aliphatic diacid monoester **62**[150]

61 **62**

Adipic acid **61** (7.3g, 0.05mole), benzyl alcohol (8.1g, 0.075mole), p-toluenesulfonic acid (0.095g, .05mmole) and toluene (40ml) were combined in a flask equipped with a dean-stark trap and heated to reflux until 1.35ml of water was collected. The reaction was then cooled to room temperature, diluted with water (30ml) and the pH adjusted to 8 with 6N NaOH (10ml). The aqueous layer was separated and washed with diethyl

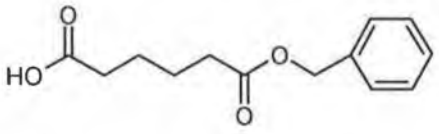
ether (2 x 25ml). Fresh ether (20ml) was added to the aqueous phase and the pH was adjusted to 2 by acidifying with 6N HCl (6ml). The organic portion was then separated, dried over $NaSO_4$ and concentrated under vaccum to give 8.2g in 69.5% of **62** as a clear oil.

TLC, $R_f = 0.70$ (ethyl acetate-hexane, 1:1, v/v)

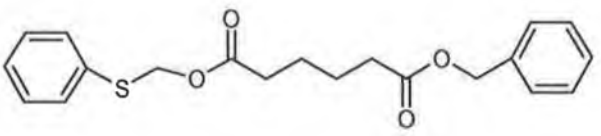
δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.64 (m, 4H, CH_2-CH_2), 2.39 (m, 4H C=OCH_2 + C=OCH_2), 5.14 (s, 2H, CH_2-Ph), 7.28-7.41 (m, 5H, aromatic), 8.84(bs, 1H, OH). $\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 24.47, 34.26, 66..72, 127.46, 127.93, 128.32, 128.69, 129.42, 136.35, 173.78, 179.47.

6.0.4. Preparation of hexanedioc acid benzyl ester (phenylsulfanyl) methyl ester **63**

151



(62)



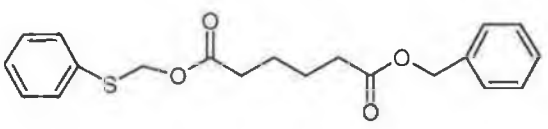
63

Aliphatic diacid monoester **62** (3.9g, 16.5mmol) and cesium carbonate (5.37g, 16.5mmol) were combined in DMF (50ml). The reaction was stirred for 30 minutes at room temperature and then at 70°C for 15 minutes. Following this the reaction was cooled to room temperature and stirred for 30 minutes, and chloromethyl phenyl sulfide (2.37g, 15mmol) was added via a syringe. The mixture was stirred for 3 hours at room temperature and then at 70°C for 15 minutes. The reaction mixture was then cooled to room temperature and diluted with water (100ml). The reaction was allowed to stir until the cesium carbonate went into solution (10min). The DMF/H_20 mixture was extracted by washing the solution with diethyl ether (4 x 50ml). The ether extracts were

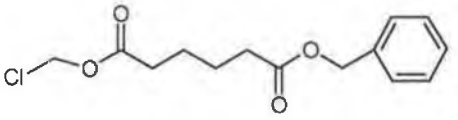
then washed with 1N NaOH (50ml), brine (4 x 50ml) and dried over $MgSO_4$. The product was concentrated on the rotary evaporator to yield 4.2g in 71% of **63** as a clear oil.

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.67-1.76 (m, 4H, CH_2-CH_2), 2.35-2.44 (m, 4H C=OCH_2 + C=OCH_2), 5.14 (s, 2H, CH_2-Ph), 5.45 (s, 2H, O-CH_2-S), 7.28-7.51 (m, 10H, aromatic). $\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 24.62, 24.66, 34.23, 34.31, 66.60, 68.30,127.78, 128.67, 129.02, 129.60, 130.72, 135.20,136.48, 172.92, 173.40.

6.0.5. Preparation of hexanedioic acid benzyl ester chloromethyl ester **64**



63



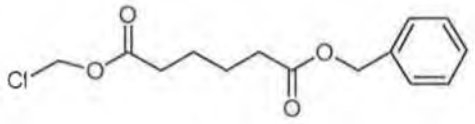
64

The diester **63** (2.83g, 10mmol) was dissolved in CH_2Cl_2 (40ml) and was purged with argon for 5 minutes. Sulfuryl chloride (1.63g, 12mmol) in 1N CH_2Cl_2 (24ml) was added slowly to the reaction mixture. A colour change was observed from clear to dark orange after 1 hour of stirring at room temperature. Cis-4-cyclohexen-1, 2-dicarboxylic acid (1.7g, 10mmol) was added to the reaction mixture. The colour reverted back to its original clear colour after 30 minutes of stirring. The white precipitate that formed was filtered and the solution was concentrated in vacuo to an oily residue. The residue was dissolved in diethyl ether (25ml) washed with 10% $NaHCO_3$ (1 x 25ml), H_2O (2 x25ml), dried over $MgSO_4$ and filtered to yield 2.6g (92%) of **64** as a white solid.

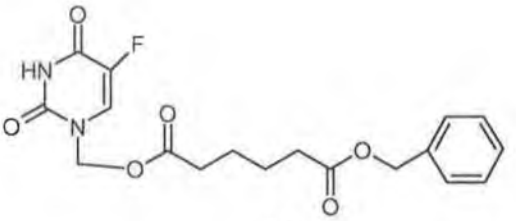
δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.59-1.61(m, 4H, CH_2-CH_2), 2.29-2.31 (m, 4H C=OCH_2 + C=OCH_2), 5.13 (s, 2H, CH_2-Ph), 5.70 (s, 2H, O-CH_2-Cl), 7.33 (s, 5H,

aromatic). $\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 24.30, 24.52, 33.93, 34.18, 66.66, 69.04, 128.627, 128.652, 128.980, 136.36, 171.68, 173.45.

6.0.6. Preparation of hexandioic acid benzyl ester (5-fluoro-2,4-dioxo-3,4-dihydro-2H-pyrimidin-1-ly)-methyl ester **60**[151]



64



60

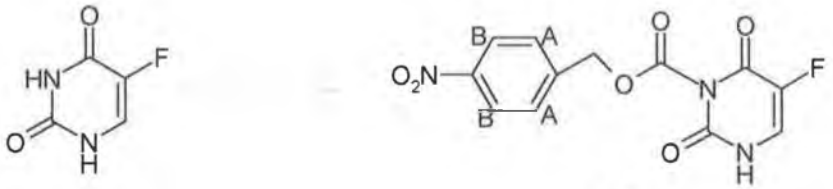
To DMF (5ml) 5-FU (0.50g, 4.05mmol) was added and stirred for 10 minutes. To this solution a 3-fold excess of triethylamine (1.2g, 12.15mmol) was added via a syringe and stirred at room temperature for 30 minutes. Hexanedioic acid benzyl ester chloromethyl ester (1.14g, 4.05mmol) was then dissolved **64** in DMF (5ml) this solution was then added dropwise to the reaction mixture and allowed to stir overnight. The precipitate that formed was gravity filtered and the DMF was removed via high vacuum pump assisted rotary evaporator to yield a yellow residue gum. The product was isolated using silica gel chromatography (40% ethyl acetate/ dichloromethane) to yield 0.7g (45%) of **60** as a white solid.

TLC, R_f 0.70 (ethyl acetate-dichloromethane, 40%,).

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.66-1.70(m, 4H, CH_2-CH_2), 2.37-2.42 (m, 4H $C{=}OCH_2$ + $C{=}OCH_2$), 5.12 (s, 2H, CH_2-Ph), 5.64 (s, 2H, O-CH_2-N), 7.36(s, 5H, aromatic), 7.64 (d, 1H, J_F = 5.4 Hz, CH-CF), 10.11 (sb, 1H, NH). $\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 24.28, 24.46, 33.76, 34.09, 66.71, 70.16, 128.63, 128.66, 128.98(d, J = 5.4 Hz), 136.29, 140.58(d, J = 239Hz), 150.00, 157.74(d, J = 27 Hz), 173.52, 173.84. $\delta^{19}F$ ($CDCl_3$ -d_6) – 164.93.(d, J= 5 Hz)

6.1. Synthesis of Nitro-Aromatic Precursors of 5-FU

6.1.1. Preparation of 3-(p-nitrobenzyloxycarbonyl)-5-fluorouracil **51**[37]



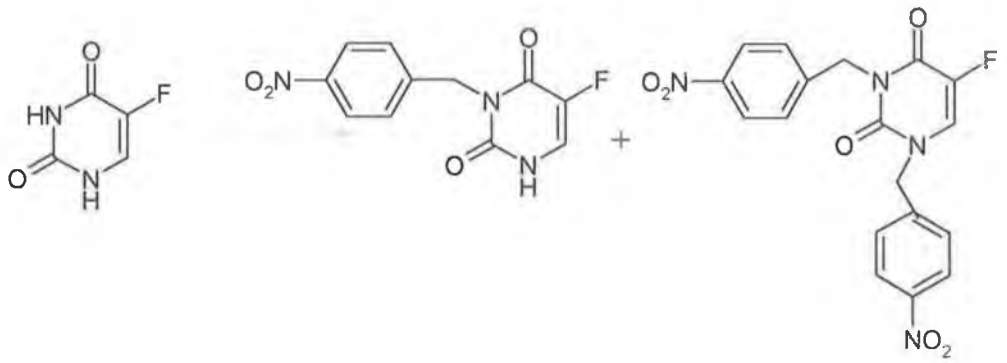
51

Nitrobenzyl chloroformate (1.42g, 6.66mmol) in dioxane (6ml) was added dropwise to a solution of 5-FU (0.84g, 6.66mmol) and triethylamine (0.66g, 6.66mmol) in DMSO (5ml). The reaction was stirred for 5 hours, filtered and poured into a solution of ice water (100ml). The white solid precipitate that formed was collected and washed with ice water. The solid was allowed to dry over night and was suspended in $CHCl_3$ (25ml) with stirring for 1 hour. The residue was suction filtered and washed twice more with $CHCl_3$. The product was allowed to dry overnight under vacuum to yield 0.98g (48%) of **51** as a white solid.

TLC, R_f 0.41 (chloroform-ethyl acetate, 2:1.5, v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 5.50(s, 2H, O-CH_2), 7.74 (d, 2H, J_{AB} = 8.6, ^{A}H's on aromatic ring,) 8.22 (d, 2H, J_F = 7.2 Hz, CH-CF), 8.25 (d, 2H, J_{AB} = 8.6, ^{B}H's on aromatic ring), 12.05 (s, 1H, NH). δ^{13}C (100MHz, $CDCl_3$ -d_6) δ (ppm) 68.70, 123.90, 128.82 (d, J = 21Hz), 139.42, 142.80, 146.33, 147.60, 149.83, 157.44 (d, J = 27 Hz). δ^{19}F ($CDCl_3$ -d_6) δ (ppm) – 165.24 (d, 1F, J = 7.4Hz).

6.1.2. Preparation of 3-(p-nitrobenzyl)-5-fluorouracil(52) and 1,3-Bis-(p-nitrobenzyl)-5-fluorouracil **53**[152]



52 **53**

Potassium carbonate (1.38g, 0.01mole), p-nitrobenzyl bromide (2.16g, 0.01mole) and 5-FU (1.3g, 0.01mole) were dissolved in DMSO (15ml). The reaction mixture was heated to 80°C for 1 hour under agitation. A 10% potassium hydroxide solution (50ml) was then added to the hot solution. The warm solution was suction filtered, the crystals that remained on the filter paper were collected and washed with H_2O (2 x 25ml) and ethanol (2 x 10ml), to yield (0.8g, 20%) of 1,3-Bis- (p-nitrobenzyl)-5-fluorouracil **53** as an orange/red powder. The orange/red mother liquor was acidified to a pH of 2 with HCl(conc), white crystals formed that were suction filtered and washed with H_2O (2 x 10ml) and ethanol (2 x 15ml). To yield 0.4g (20%) of 1-(p-nitrobenzyl)-5-fluorouracil **51** as a white/orange solid.

3-(p-nitrobenzyl)-5-fluorouracil **52**.

TLC, R_f 41 (chloroform-ethyl acetate, 2:3,v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 4.96 (s, 2H, O-CH_2-^1N), 7.57 (d, $2H_A$, J_{HA} = 8.7 Hz aromatic ring), 8.19-8.28 (m, 3H, $2H_B$ in aromatic ring and CH-CF), 11.92 (bs, 1H, ^3NH). $\delta^{13}C$ (100MHz, DMSO-d_6) δ (ppm) 50.70, 124.04, 128.77, 130.38 (d, J = 21 Hz),.

144.52, 147.27, 150.0.3, 157.78 (d, J = 27 Hz). $\delta^{19}F$ (DMSO-d_6) δ (ppm) – 169.06(d, 1F, J= -6.6Hz).

1,3-Bis- (p-nitrobenzyl)-5-fluorouracil **53**.

TLC, R_f 0.62 (chloroform-ethyl acetate, 2:3,v/v).

δ^1H (400MHz, DMSO-d_6) δ (ppm) 5.08 (d, 4H, O-CH_2-^{1}N and O-CH_2-^{3}N), 7.53(d, 2H, H_A aromatic), 7.60 (d, 2H, H_B aromatic), 8.14(d, 2H, H_A aromatic), 8.18 (d, 2H, H_B aromatic), 8.41 (d. 1H, CH-CF). (100MHz, DMSO-d_6) δ (ppm) 44.38, 51.95,123.95(d, J = 18 Hz). 128.79, 129.87(d, J = 27 Hz), 144.17, 144.46, 150.13, 157.16(d, J = 27 Hz). . $\delta^{19}F$ (DMSO-d_6) δ (ppm) – 167.12 (d, 1F, J= -6.4Hz).

6.2. Protected Nucleosides

6.2.1. Preparation of 2',3'-isopropylideneuridine **68**[154,161]

67 **68**

To a solution of uridine **67** (10.4g, 42.5mmol) and 4-toluenesulfonic acid monohydrate (1.2g, 6.3mmol) in dry acetone (200ml) one portion of 2,2-Dimethoxypropane (27.5g, 264.0mmol) was added. The reaction mixture was allowed to stir for 12 hours at room temperature. Afterwhich time it was neturalised with 2.5M sodium methoxide in

methanol and filtrered through Celite. The solvent was removed via rotary evaporation and recrystallised from water to yield 11.19g (83%) of **68**.

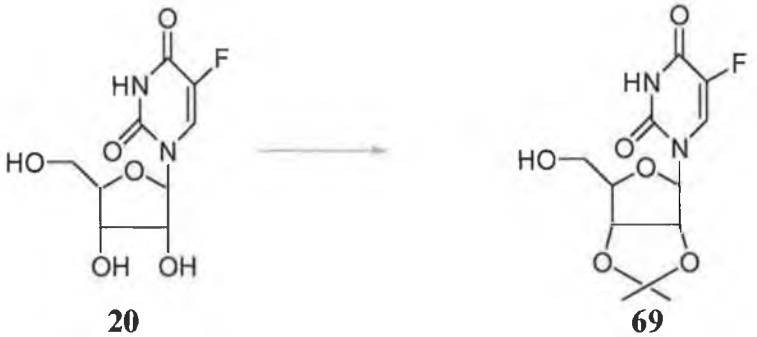
TLC, R_f 0.25 (ethyl acetate-chloroform, 1:1, v/v)

V_{max} (KBr) 3244, 1704, 1675 cm^{-1}.

δ^1H (400MHz, DMSO-d_6) δ (ppm) 1.28 (s, 3H, $(CH_3)_2C$), 1.48 (s, 3H, $(CH_3)_2C$), 3.57 (m, 2H, H-5'), 4.06-4.08 (m, 1H, H-4'), 4.74 (dd, 1H, $J_{3'-2'}$ =6.4 Hz, $J_{3'-4'}$ = 3.8 Hz, H-3'), 4.88 (dd, 1H, $J_{2'-3'}$ =6.4 Hz, $J_{2'-1'}$ = 2.6 Hz, H-2'), 5.16 (bs, 1H, OH), 5.62 (d, 1H, J= 7.9 Hz, NCH=CHCO) 5.82 (d, 1H, $J_{1'-2'}$ =2.4 Hz, H-1'), 7.78 (d, 1H, J= 7.9 Hz, NCH=CHCO), 11.38 (bs, 1H, NH).

$\delta^{13}C$ (100MHz, DMSO-d_6) δ (ppm) 25.46, 27.34, 61.58 (C-5'), 80.79 (C-2'), 84.00 (C-3'), 86.82 (C-4'), 91.46 (C-1'), 102.06 (C-5), 113.34(C-Me_2), 142.29(C-6), 150.68(C-2), 163.63(C-4).

6.2.2. Preparation of 5-fluoro-2',3'-isopropylideneuridine **69**



20 **69**

Compound **20** (1.10g, 4.2mmol) and 4-toluenesulfonic acid monohydrate (0.12g, 0.63mmol) were dissolved in dry acetone (20ml). One portion of 2,2-Dimethoxypropane (2.75g, 26.40mmol) was added to the reaction mixture and stirred for 12 hours at room temperature. The reaction was neturalised with 2.5M sodium

methoxide in methanol. The reaction mixture was filtered through Celite, and solvent removed under vacuum to yield (69) (0.89g, 70.1%) as a white solid.

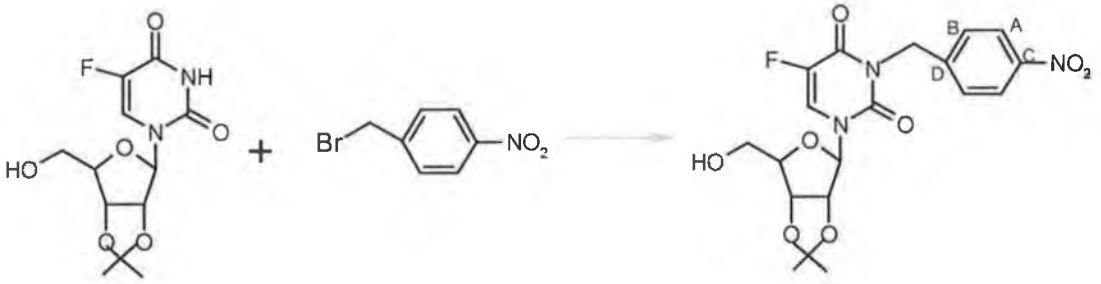
δ^1H (400MHz, DMSO-d_6) δ (ppm) 1.27 (s, 3H, $(CH_3)_2C$), 1.48 (s, 3H, $(CH_3)_2C$), 3.62 (m, 2H, H-5'), 4.10 (m, 1H, H-4'), 4.74 (dd, 1H, $J_{3'-2'}$ =9.6 Hz, $J_{3'-4'}$ = 3.3 Hz, H-3'), 4..87 (dd, 1H, $J_{2'-3'}$ =8.9 Hz, $J_{2'-1'}$ = 2.6 Hz, H-2'), 5.29(bs, 1H, OH), 8.18 (d, 1H, J= 7.1 Hz, NCH=CFCO), 11.92 (bs, 1H, NH).

δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 25.47, 27.33, 61.45 (C-5'), 80.54(C-2'), 84.09(C-3'), 86.83(C-3'), 91.30(C-1'), 113.29(C-Me_2), 126.16(d, J= 27 Hz, C-6), 141.40(C-2), 149.36(C-5), 157.51(d, J = 21Hz, C-4)

δ^{19}F(DMSO-d_6) δ (ppm) -167.99 (d, J= -6.7Hz)

6.3. Synthesis of Nitro-Aromatic Precursors of 5-FURD Prodrugs

6.3.1. Preparation of N^3-(4-nitrobenzyl)-2', 3'-isopropylidene-5-fluorouridine 71



69 **71**

GENERAL PROCEDURE

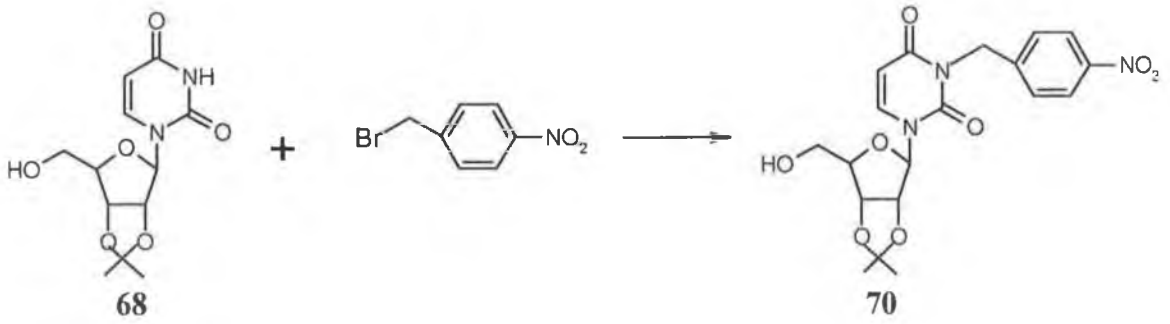
A mixture of 2,3-isopropylidene-5-fluorouridine **69** (302.2mg, 1mmol), 4-nitrobenzyl bromide (248.44mg, 1.15mmol) and K_2CO_3 (250mg, 1.18mmol) were stirred in dry DMF (12.5ml) at room temperature for 3 hours. The solution was gravity filtered and concentrated *in-vacuo* and allowed to cool. To the residue a minimum amount of ethyl

acetate was added to precipitate any remaining salt. The solution was suction filtered and the collected precipitate was washed with ethyl acetate. The collected precipitate was purified by silica gel chromatography (ethyl acetate-hexane, 1:1, v/v) to yield 310 mg (78%) of **71** as a clear syrup that was solidified to a white powder by the addition ethyl acetate

Yield 195.6 mg (84%) as a clear syrup;

TLC, R_f 0.41 (ethyl acetate-hexane, 1:1, v/v); H^1NMR (400MHz, DMSO-d_6) δ (ppm) 1.04(s, 3H, CCH_3), 1.23(s, 3H, CCH_3), 3.41(m, 2H, H-5'), 3.94(m, 1H, H-4'), 4.52(d, 1H, H-3'), 4.65(dd, 1H, H-2'), 4.86(s, 2H,benzyl CH_2), 5.08(d, 1H, 5'-OH), 5.64(s, 1H, H'-1), 7.32(d, 2H, H_A-aromatic ring), 7.94(d, 2H, H_B-aromatic ring), 8.11(d, 1H, H-6). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 25.48(CCH_3), 27.31(CCH_3), 44.25(benzyl), 61.39(C-5'), 80.59(C-3'), 84.38(C-2'), 87.20(C-4'), 92.52(C-1'), 113.18($C(CH_3)_2$), 123.90(2C, C_C-aromatic ring), 125.60(C-6), 128.99(2C, C_B-aromatic ring), 140.91(C-2), 144.43(1C, C_D-aromatic ring), 147.11(1C, C_A-aromatic ring), 158.50(C-3). F^{19}NMR (DMSO-d_6) δ (ppm) -166.42 (d, J= -6.7Hz) m/z (EI) 460.3 (M^++Na $C_{19}H_{20}O_8N_3F_1$ requires 437.3)

6.3.2. Preparation of N^3-(4-nitrobenzyl)-2', 3'-isopropylidene-uridine **70**

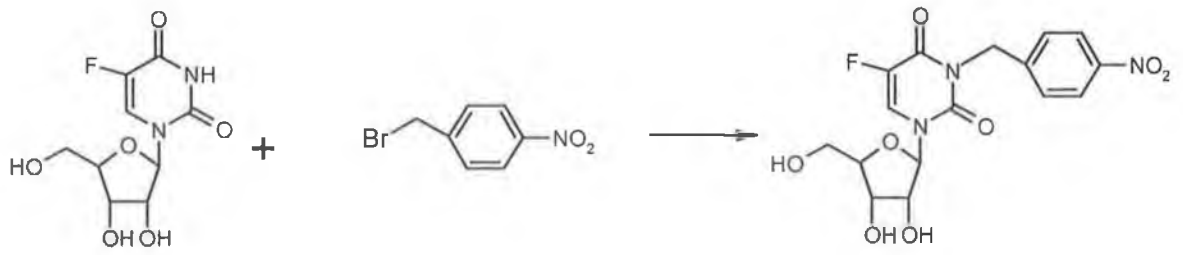


68 **70**

The general procedure was employed to prepare **70** using 2,3-isopropylidene-uridine **68** (283.2mg, 1mmol), 4-nitrobenzyl bromide (248.44mg, 1.15mmol). The collected product was purified by silica gel chromatography solvent system.

Yield **70** 280mg (64%) as a clear syrup; TLC, R_f 0.26 (ethyl acetate-hexane, 2:1, v/v); H^1NMR (400MHz, DMSO-d_6) δ (ppm) 1.28(s, 3H, CCH_3), 1.47(s, 3H, CCH_3), 3.56(m, 2H, H-5'), 4.13(d, 1H, H-4'), 4.75-4.76(m, 1H, H-3'), 4.91-4.92(m, 1H, H-2'), 5.08(benzyl CH_2), 5.18(d, 1H, 5'-OH), 5.85-5.87(m, 2H, H-1' and H-6), 7.52(d, 2H, H_A-aromatic ring), 7.93(d, 1H, H-6), 8.18(d, 2H, H_B-aromatic ring). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 25.50(CCH_3), 27.34(CCH_3), 43.47(benzyl, CH_2), 61.52(C-5'), 80.82(C-3'), 84.30(C-2'), 87.20(C-4'), 91.72(C-1'), 101.10(C-5), 113.21($C(CH_3)_2$), 123.92(2C, C_C-aromatic ring), 128.86(2C, C_B-aromatic ring), 141.16(C-6), 145.06(1C, C_D-aromatic ring), 147.08(1C, C_A-aromatic ring), 150. m/z (EI) 442.2 (M^++Na $C_{19}H_{20}O_8N_3F_1$ requires 419.2)

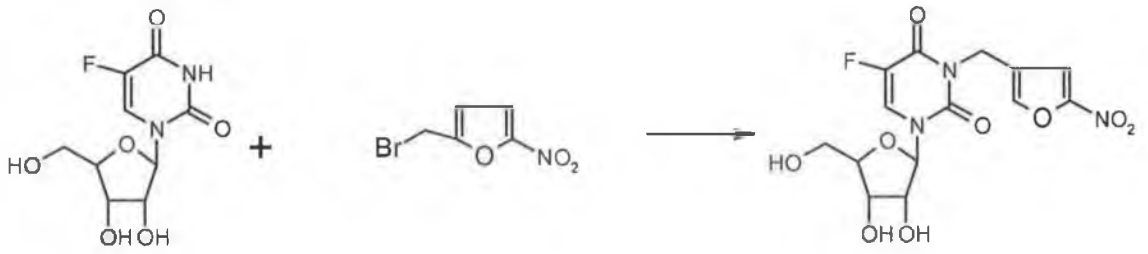
6.3.3. Preparation of N^3-(4-nitrobenzyl)-5-fluorouridine **72**



20 **72**

The general procedure was employed to prepare **72** using 5-fluorouridine **20** (262.2mg, 1mmol), 4-nitrobenzyl bromide (248.44mg, 1.15mmol) and K_2CO_3 (250mg, 1.18mmol). The collected residue was purified by silica gel chromatography (4:1 ethyl acetate-hexane v/v) to yield **72**, 310 mg (78%) of a clear syrup that was solidified to a white powder from ethyl acetate: mp 156-158 °C; TLC, R_f 0.42 (ethyl acetate-hexane, 4:1, v/v) H^1NMR (400MHz, DMSO-d_6) δ (ppm) 3.59-3.64(m, 1H, H-4'), 3.72-3.76(m, 1H, H-3'), 3.88-3.89(m, 1H, H-2'), 4.00-4.07(m, 2H, H-5'), 5.11-5.20(m, 3H, 5'-OH and benzyl CH_2), 5.36(t, 1H, 3'-OH), 5.50(d, 1H, 2'-OH), 5.77-5.78(m, 1H, H-1'), 7.58(d, 2H, H_A-aromatic ring), 8.18(d, 2H, H_B-aromatic ring), 8.53(d, 1H, H-6). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 44.22 (benzyl), 60.21 (C-5'), 69.17(C-3'), 74.34(C-2'), 84.85(C-4'), 89.95(C-1'), 123.89(2C, C_B-aromatic ring), 124.48(d, J= 27Hz, C-6), 128.97(2C, C_C-aromatic ring), 140.92(C-2), 144.54(1C, C_D-aromatic ring), 147.09(1C, C_A-aromatic ring), 149.52(C-5), 156.88(d, J= 21 Hz,C-1). F^{19}NMR (DMSO-d_6) δ (ppm) -166.38. m/z (EI) 437.3 (M^++K $C_{16}H_{16}O_8N_3F_1$ requires 398.3)

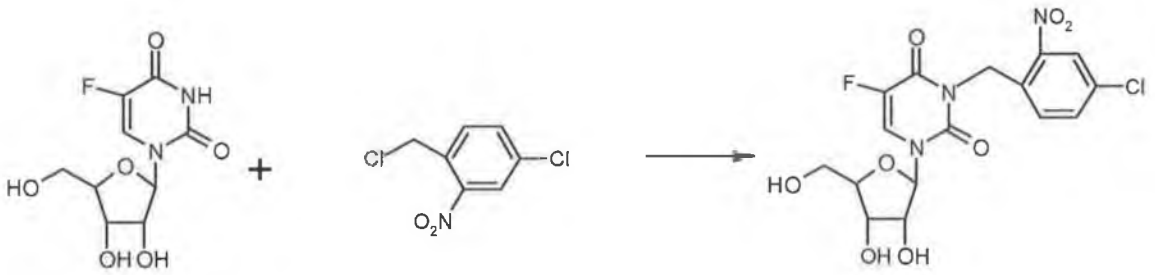
6.3.4. Preparation of N^3-(4-nitrobenzylfuran)-5-fluorouridine **73**



73

The general procedure was employed to prepare **73** using 5-fluorouridine (262.2mg, 1mmol), 2-bromomethyl-5-nitrofuran (248.44mg, 1.15mmol) and K_2CO_3 (250mg, 1.18mmol). The collected residue was purified by silica gel chromatography (4:1 ethyl acetate-hexane v/v) to yield **73**, 310 mg (78%) of a clear syrup that was solidified to a white powder from ethyl acetate; TLC, R_f 0.42 (ethyl acetate-hexane, 4:1, v/v) H^1NMR (400MHz, DMSO-d_6) δ (ppm) 3.59-3.61(m, 1H, H-4'), 3.70-3.75(m, 1H, H-3'), 3.86-3.90(m, 1H, H-2'), 4.01-4.04(m, 2H, H-5'), 5.01-5-09(m, 3H, 5'-OH and benzyl CH_2), 5.55(bs, 1H, 3'-OH), 5.75(d, 1H, 2'-OH), 7.0-7.66(m, 1H, H-1'), 7.72(d, 1H), 8.12(d, 2H,), 8.40(d, 1H, H-6). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 44.01 (benzyl), 60.29 (C-5'), 69.31(C-3'), 74.22(C-2'), 84.85(C-4'), 89.69(C-1'), 122.84, 130.37, 134.86, 138.73, 148.04, 149.59, 156.93(d, J= 21Hz)

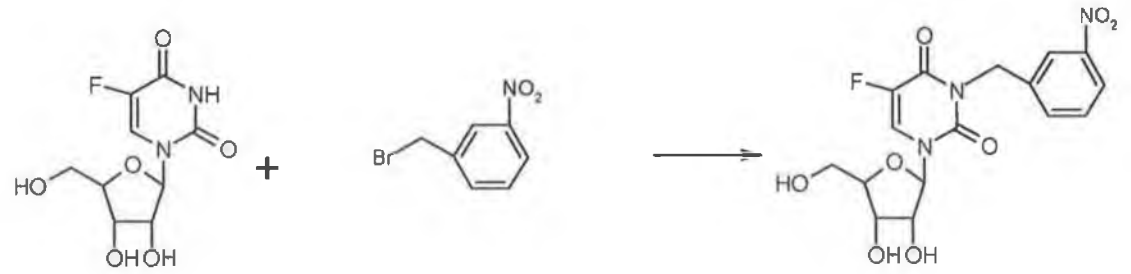
6.3.5. Preparation of N^3-(4-chloro-2-nitrobenzyl)-5-fluorouridine **74**



74

The general procedure was employed to prepare **74** using 5-fluorouridine (262.2mg, 1mmol 4-chloro-2-nitrobenzyl bromide (248.44mg, 1.15mmol) and K_2CO_3 (250mg, 1.18mmol). The collected residue was purified by silica gel chromatography (4:1 ethyl acetate-hexane v/v) to yield **74**, 310 mg (78%) of a clear syrup that was solidified to a white powder from ethyl acetate: TLC, R_f 0.62 (ethyl acetate-hexane, 4:1, v/v) H^1NMR (400MHz, DMSO-d_6) δ (ppm) 3.58-3.60(m, 1H, H-4'), 3.72-3.77 (m, 1H, H-3'), 3.88-4.01(m, 1H, H-2'), 4.16-4.19(m, 3H, H-5'), 5.40-5.42(m, 3H, 5'-OH and benzyl CH_2), 5.55(t, 1H, 3'-OH), 5.72(d, 1H, 2'-OH), 7.43(d, 1H, H-1'), 7.77(dd, 2H,aromatic), 8.16(d, 1H, aromatic), 8.55(d, 1H, aromatic). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 41.22 (benzyl), 60.08 (C-5'), 69.01(C-3'), 74.37(C-2'), 84.75(C-4'), 90.00(C-1'), 124.45, 124.84(d, J=27 Hz), 130.39, 132.95, 134.06, 149.14, 149.46, 156.90 (d, J= 21Hz), 170.75.

6.3.6. Preparation of N^3-(3-nitrobenzyl)-5-fluorouridine **75**

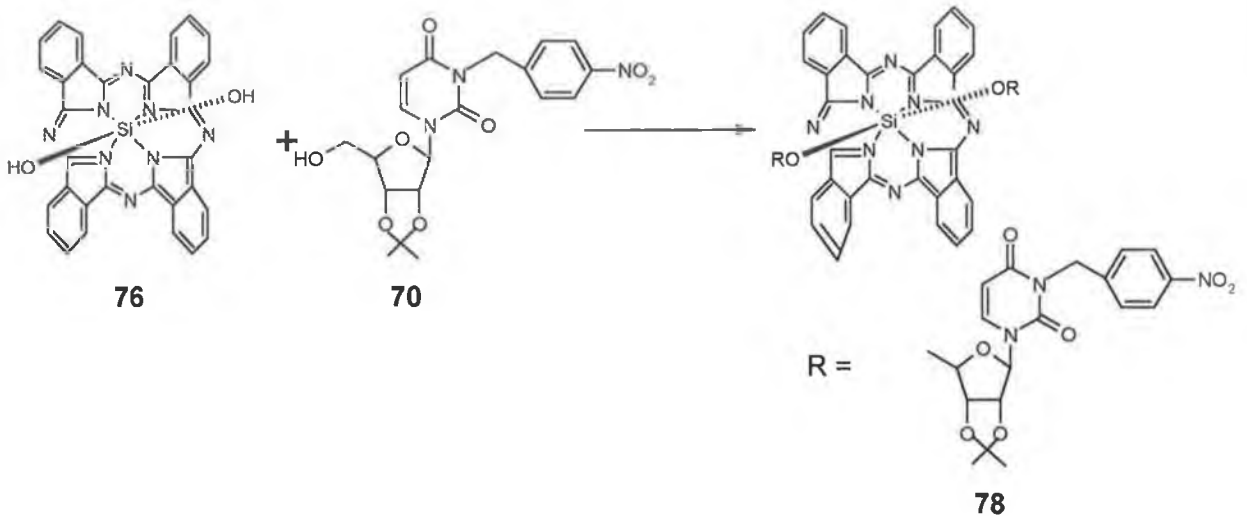


75

The general procedure was employed to prepare **75** using 5-fluorouridine (262.2mg, 1mmol), 3-nitrobenzyl bromide (248.44mg, 1.15mmol) and K_2CO_3 (250mg, 1.18mmol). The collected residue was purified by silica gel chromatography (4:1 ethyl acetate-hexane v/v) to yield **75**, 310 mg (78%) of a clear syrup that was solidified to a white powder from ethyl acetate: TLC, R_f 0.68 (ethyl acetate-hexane, 4:1, v/v) H^1NMR (400MHz, DMSO-d_6) δ (ppm) 3.60-3.61(m, 1H, H-4'), 3.69-3.70(m, 1H, H-3'), 3.72-3.75(m, 1H, H-2'), 4.00-4.15(m, 2H, H-5'), 5.09-5.21(m, 2H, 5'-OH and benzyl CH_2), 5.76(d, 1H, 2'-OH), 6.74(m, 1H, H-1'), 7.55(d, 2H, aromatic), 7.89(d, 2H, aromatic), 8.43(d, 1H, aromatic). C^{13}NMR (100MHz, DMSO-d_6) δ (ppm) 37.95 (benzyl), 60.26 (C-5'), 69.28(C-3'), 74.23(C-2'), 84.86(C-4'), 89.74(C-1'), 113.03,114.64,124.41 (d, J= 21Hz), 138.51, 140.79(C-2), 153.92, 156.59(d, J=21Hz), 163.15.

6.4. Preparation of 5-FURD-Photosensitiser Conjugates

6.4.1. Preparation of Bis(3-(p-nitrobenzyl)-2',3- isopropylideneuridine) phthalocyaninosilicon **78** [method 1-condensation]



76 **70** **78**

Dihydroxysilicon phthalocyanine **76** (16.1mg, 0.028mmol) and 4-nitrobenzyl-uridine **70** (92.44mg, 0.28mmol) were dissolved in dry DMF (10ml). The solution was refluxed for 3 hours, cooled and gravity filtered. The filtrate was concentrated by rotary evaporation and purified by silica gel chromatography, (CHCl3-MeOH, 10:0.5, v/v). Three fractions were collected. Fraction 1 yielded product Bis(3-(p-nitrobenzyl)-uridine)phthalocyaninosilicon **78** in 10mg (26%). Fraction 2 was un-reacted (70) while the final fraction was unreacted dihydroxysilicon phthalocyanine **76**.

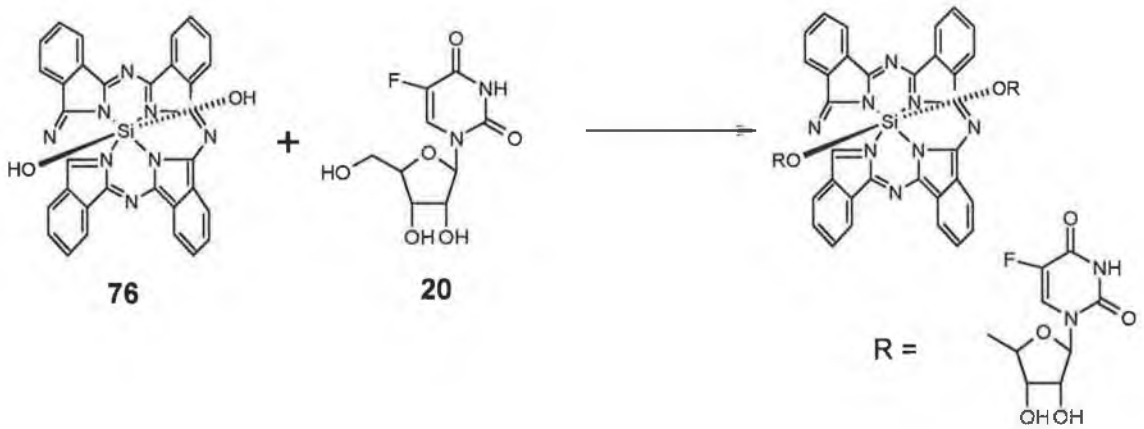
6.4.2. Preparation of Bis(3-(p-nitrobenzyl)-2',3- isopropylideneuridine) phthalocyaninosilicon **78** [Method 2-Sodium Hydride]

Sodium hydride (12.0mg, 0.28mmol) was added to a mixture of dihydroxysilicon phthalocyanine **76** (16.1mg, 0.028mmol) and 3-(p-nitrobenzyl)-uridine **70** (92.44mg, 0.28mmol) dissolved in dry Toluene (10ml). This solution was heated to 80°C for 96 hours. The reaction mixture was poured into water (10ml), extracted with toluene and the toluene fraction was concentrated under vacuum. The crude product was purified by a silica gel column (DCM: MeOH v/v 10:0.5). The collected Pc was then purified by size exclusion column chromatography (SX-3 polystyrene) to give 12mg (32%) of **78**.

UV/Vis (DMSO): λmax =682nm

m/z (EI) 1399 (M^++Na ,requires 1376)

6.4.3. Preparation of Bis(5-fluorouridine)phthalocyaninosilicon **80**



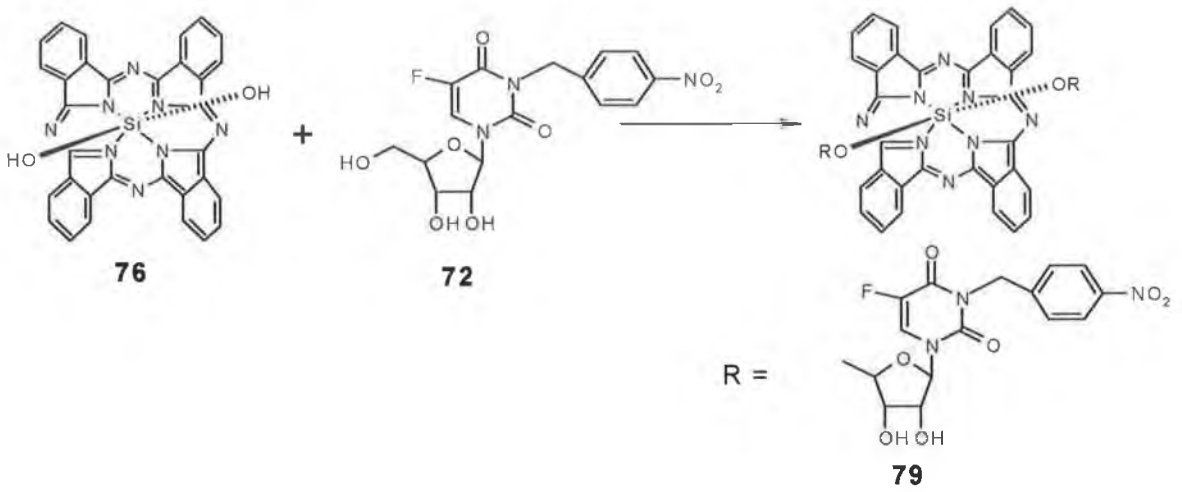
76 **20** **80**

Method I was employed using **76** (12.66mg, 0.022mmol) and 5-fluorouridine **20** (250mg, 0.95mmol). The crude product was purified by silica gel chromatography ($CHCl_3$-MeOH, 10:1.0, v/v). Three fractions were collected. Fraction 1 yielded 14.3mg (61%) of **80**. Fraction 2 yielded 5-fluorouridine and fraction 3 was un-reacted dihydroxysilicon phthalocyanine.

UV/Vis (DMSO): λmax =680nm

m/z (EI) 1089 (M^++Na ,requires 1066)

6.4.4. Preparation of Bis(3-(p-nitrobenzyl)-5-fluorouridine)phthalocyaninosilicon 79



Method I was employed using **76** (16.1mg, 0.028mmol) and 3-(p-nitrobenzyl)-5-fluorouridine **72** (111.44mg, 0.28mmol) were dissolved in dry DMF (10ml). The crude product was purified by silica gel chromatography, ($CHCl_3$-MeOH, 10:0.5, v/v). Three fractions were collected. Fraction 1 yielded 15.8mg (42.3%) of **79**. Fraction 2 was un-reacted p-nitrobenzyl-5-fluorouridine while the final fraction was unreacted dihydroxysilicon phthalocyanine.

UV/Vis (DMSO): λmax =678nm

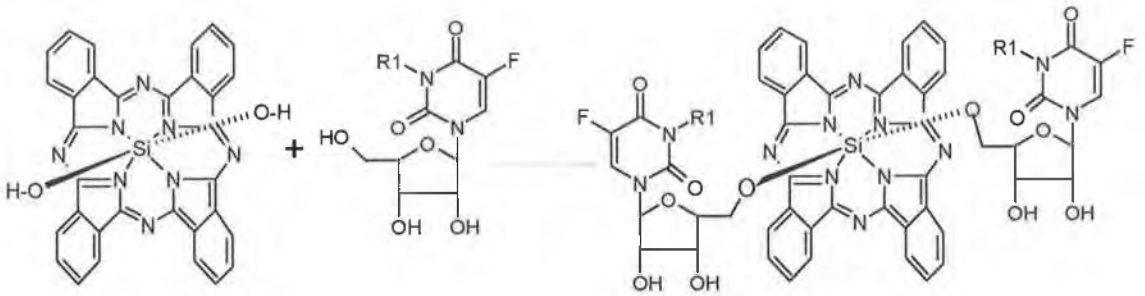
m/z (EI) 1347 (M^+ requires 1319)

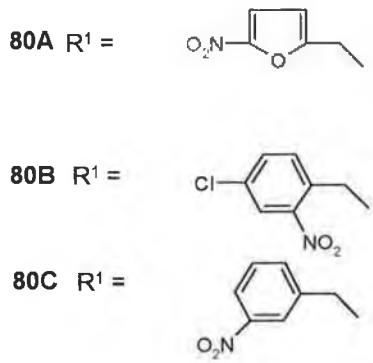
6.4.5. Preparation of :

Bis(N^3- (4-nitrobenzylfuran)-5-fluorouridine) phthalocyaninosilicon **80A**

Bis(N^3- (4-chloro-2-nitrobenzyl)-5-fluorouridine) phthalocyaninosilicon **80B**

Bis(N^3-(3-nitrobenzyl)-5-fluorouridine) phthalocyaninosilicon **80C**





Method I was employed using **76** (16.1mg, 0.028mmol) and appropiate 5-fluorouridine derivative **73** (112mg, 0.28mmol), **74** (120mg, 0.28mmol) and **75** (111.44mg, 0.28mmol) were dissolved in dry DMF (10ml). The crude product was purified by silica gel chromatography, ($CHCl_3$-MeOH, 10:0.5, v/v). Three fractions were collected. Fraction 1 for each yielded **80A** in 15.4mg (41%) **80B** in 11.0mg (28%) and **80C** in 11.4mg(30%). Fraction 2 was un-reacted 5-fluorouridine derivatives **73**, **74** and **75** while the final fraction was unreacted dihydroxysilicon phthalocyanine **76**.

6.5. Novel Deprotection of Acetonide Derivative

GENERAL PROCEDURE

The appropriate acetonide (0.33mmol) and benzyl derivative (0.33mmol) were dissolved in DMSO (4.0ml) in the presence of *triethyamine (0.33mmol). To this 30% hydrogen peroxide was added (200ul). The reaction was allowed to stir at 85°C.

The benzyl derivative was removed by chloroform extraction (5x7ml). The peroxide was removed by stirring the reaction in activated alumina for 2 hours. The reaction mixture was filtered and concentrated to give an oily residue. The residue was titurated with dry acetonitrile, to yield the parent nucleoside in moderate yield.

*triethyamine can be either present or not present during reaction.

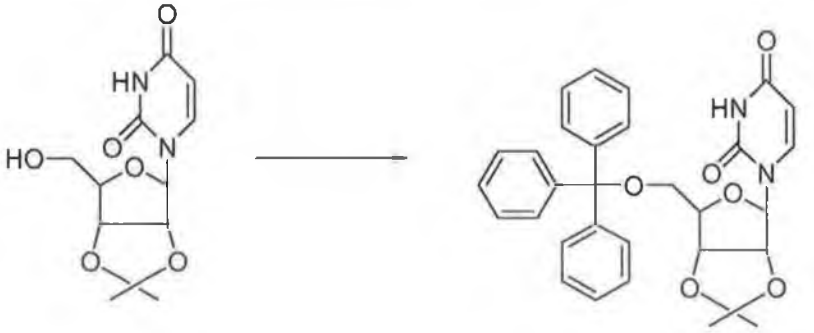
TABLE 32. SHOWING YIELDS FOR REACTION WITH IN THE ABSENCE OF BASE.

Protected Nucleoside	PRODUCT	YIELD
2,3-isopropylidene-5-fluorouridine	5-fluorouridine	45%
2,3-isopropylideneuridine	Uridine	58%
5-trityl-2,3-isopropylideneuridine	2,3-isopropylideneuridine	48%

TABLE 33. SHOWING YIELDS FOR REACTION IN THE PRESENCE OF TEA

Protected Nucleoside	PRODUCT	YIELD
2,3-isopropylidene-5-fluorouridine	5-fluorouridine	42%
2,3-isopropylideneuridine	Uridine	50%
5-trityl-2,3-isopropylideneuridine	2,3-isopropylideneuridine	34%

6.5.1. Preparation of 5'-O-trityl-2',3'-isopropylideneuridine **94**[155]



(68) **82**

Acetonide **68** (1.8796g, 6.0mmol), trityl chloride (1.84g, 6.6mmol), triethylamine (1.5ml) and DMAP (58.2mg, 0.3mmol) were dissolved in dichloromethane (15ml). The reaction mixture was stirred overnight at room temperature under nitrogen. After which time ice cold water (20ml) was added to the reaction mixture and the product was extracted with dichloromethane (20ml). The aqueous phase was washed twice with dichloromethane (10ml), and the organic layers were combined and washed with saturated ammonium chloride (20ml), water (20ml), then dried over $NaSO_4$. The solvent was removed by evaporation and the resulting residue was recrystallised from water to yield **82** (2.25g, 71%)

δ^1H (400MHz, DMSO-d_6) δ (ppm)1.26 (1H, s, $(C\underline{H}_3)_2C$), 1.47 (3H, s, $(C\underline{H}_3)_2C$), 3.33 (2H, m, H-5'), 4.14-4.15 (1H, m, H-4'), 4.70 (1H, dd, $J_{3'-2'}$ =6.2 Hz, $J_{3'-4'}$ = 3.4 Hz, H-3'), 4.98 (1H, dd, $J_{2'-3'}$ =6.2 Hz, $J_{2'-1'}$ = 2.6 Hz,), 5.51(1H, d, J= 7.9 Hz, NC$\underline{H}$=CHCO, H-5), 5.85 (1H, d, $J_{1'-2'}$ =2.4 Hz, H-1'), 7.21-7.38 (15H, m, aromatic), 7.73 (1H, d, J= 7.9 Hz, NCH=C$\underline{H}$CO, H-6), 11.42 (1H, bs, NH).

δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 18.89, 25.56, 27.36, 56.39, 64.42, 80.8, 84.01, 86.35, 92.22, 102.01, 113.58, 126.98, 127.26, 127.48, 127.87, 128.12, 128.21, 128.28, 128.47, 128.58, 128.98, 143.79, 148.11, 150.56, 163.58.

6.6. Preparation of a More Lipophillic 5-FU Prodrug

6.6.1 Initial preparation

6.6.1.1. Preparation of dibromoisocyanuric acid **82**[168]

91 **90**

Cyanuric acid **91** (12.9g, 100mmol) and LiOH (8.4g, 200ml) were added to water (1000ml). The solution was stirred until all reagents fully dissolved. At this time bromine (63.9g, 400 mmol) was slowly added. After the bromine was dissolved, the solution was placed in the refrigerator overnight. The solution was then suction filtered, and the filtrate was dried in vacuo to yield **90** as a white powder (22.02g, 86.4%).

δ^{1}H (400MHz, DMSO-d_6)δ (ppm) 11.25 (bs, 1H, OH)

δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 61.82, 150.26.

6.6.1.2. PREPARATION OF N-BROMOACETAMIDE **92**

92

To a solution of acetamide (4.48g 92.8mmol) in dichloromethane (500ml) was added dibromoisocyanuric acid **90** (16.00g, 111.4 mmol) and the suspension was refluxed in the dark for 6 hours. The reaction mixture was extracted with dichloromethane (2 x 150ml) and the solvent removed under reduced pressure to afford the crude product as an orange precipitate. The crude product **92** was purified by recrystallisation with ethyl acetate to yield (10.8g, 85%)

6.6.1.3. ALTERNATIVE PREPARATION OF N-BROMOACETAMIDE **92**[169]

Acetamide (20.00g, 338.58mmol) was dissolved in bromine (54.0g, 337.90mmol). The solution was cooled to 0°C. An ice cold aqueous 50% potassium hydroxide solution was then added in portions with swirling and cooling until the mixture became light yellow in appearance. The near solid reaction mixture was allowed to stand at 0°C for 3 hours. The mixture was then treated with sodium chloride (40g) and dichloromethane (200ml). The top layer of the biphasic layer that formed was decanted while boiling. The extraction was repeated twice more with 200ml and 100ml portions of dihloromethane. The combined extracts were dried over Na_2SO_4 gravity filtered and the filtrate was diluted with hexane (500ml) (gentle stirring was required). The white needles of **92** were collected by suction filtration, washed with hexane and air dried to give a yield of 17.14g (37%) (lit. m.p. 102-105). M.p (lit. m.p. 102-105°C) 103-105°C

6.6.1.4. PREPARATION OF N-BROMOBENZAMIDE **93**

93

To a solution of benzamide (5.62g 46.4mmol) in dichloromethane (300ml) was added dibromoisocyanuric acid (90) (8.00g, 92.8 mmol). The suspension was refluxed in the dark for 5 hours. The reaction mixture was suction filtered and the collected precipitate was washed with dichloromethane (2 x 150ml). The filtrate was collected and the solvent was removed under reduced pressure to yield **93** as a white powder. (8.9g, 96%). M.pt. (lit. m.pt. 129-131°C) 129-131°C

6.6.2. Amino hydroxylation derivatives

6.6.2.1 PREPARATION OF ISOPROPYL CINNAMATE **88**

87 **88**

Cinnamic acid **87** (14.80g, 99.89mmol) was dissolved in dry propan-2-ol (500ml). To this solution was added trimethylsilyl chloride (24.00g 220.91mmol). The mixture was refluxed for 4 hours and the solvent was removed under reduced pressure. The residue was dissolved in ether (150ml), washed with 10% aqueous sodium carbonate (2 x

50ml), and once with brine (50ml). The organic layer was dried over $MgSO_4$, gravity filtered and concentrated under vacumm to yield 13.3g, (71%) of **88**.

δ^1H (400MHz, $CDCl_3$-d_6) δ (ppm) 1.27 (d, 6H, J = 6.3 Hz, $(CH_3)_2CH$), 5.14 (sept, 1H, J =6.3 Hz $(CH_3)_2CH$), 6.38 (d, 1H, CH=CHCO, J_{trans} = 16Hz), 7.29-7.46 (m, 5H, aromatic), 7.63 (d, 1H, CH=CHCO, J_{trans} = 16Hz)

δ^{13}C (100MHz, $CDCl_3$-d_6) δ (ppm) 22.28, 68.01, 119.11, 128.36, 129.20, 130.50, 134.834, 144.61, 166.68.

6.6.2.2. Preparation of isopropyl-(2R,3S)-3-(N-acetyl)amino-2-hydroxy-3-phenylpropinoate **83**[170]

88 **83**

Lithium hydroxide monohydrate (2.83g, 67.49mmol) was dissolved in water (190ml). To this solution was added potassium osmate dihydrate (0.35g, 0.95mmol) and the solution was then further diluted with tert-butanol (380ml). $(DHQ)_2PHAL$ (0,49g, 0.63mmol) was then added and the reaction mixture stirred for 10 minutes at 20°C. The reaction mixture was diluted with water (379ml) and cooled to below 4°C. Isopropyl cinnamate **88** (12.00g 63.08mmol) was poured into the reaction mixture, followed by N-bromoactamide (92) (11.00g, 79.73mmol). The reaction mixute was then stirred for 20 hours at 4°C. After this time sodium sulfite (10g) was added and the reaction mixture was allowed stir for an additional 30 minutes. The reaction mixture was then extracted with ethyl acetate (3x150ml). The organic extracts were combined, washed with brine

and dried over $MgSO_4$. The solvent was removed in vacuo to yield a brown gum, which was purified by flash chromatography (1:1 ethyl acetate in hexane) and recrystallised from ethyl acetate to yield **83** (11.95g, 75%).

R_f 0.27 (ethyl acetate-hexan, 1:1, v/v)

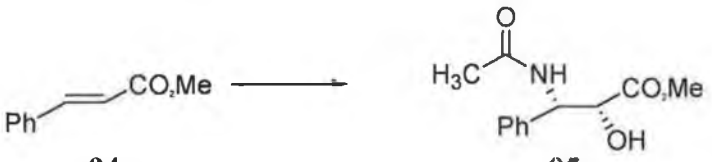
$[\alpha]_D^{20}$ –25.1 (0.61, $CHCl_3$)

Vmax (KBr) 3302, 3067, 2988, 2923, 2890, 2716, 1742, 1655, 15001 cm^{-1}

δ^1H (400MHz $CDCl_3$-d_6) δ (ppm) 1.30 (dq, 6H, $J_{2'-2''}$= 8.4 Hz, $J_{2'-1'}$= 6.3 Hz, H-2', H-2''), 1.99 (s, 3H, CH_3CONH), 3.46 (d, 1H, J_{OH-1} = 4.0 Hz, OH), 4.49 (dd, 1H, J_{1-OH} = 4.0 Hz, J_{1-2} = 2.2 Hz, H-1), 5.12, (qq, 1H, $J_{1'-2'}$= 6.3 Hz, H-1'), 5.56 (dd, 1H, $J2_{-NH}$= 9.3Hz, J_{2-1}= 2.3 Hz, H-2),6.45, (d, 1H, J_{NH-2}= 9.3 Hz, NH), 7.28-7.38 (m, 5H, aromatic)

$\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 21.88, 22.7, 23.37, 54.94, 70.94, 73.83, 127.31, 128.08, 128.94, 139.28, 170.14, 172.76.

6.6.2.3. PREPARATION OF METHYL- (2R,3S)-3-(N-ACETYL)AMINO-2-HYDROXY-3-PHENYLPROPINOATE **95**



94a **95**

The same procedure used to prepare **83** was used to prepare **95** using lithium hydroxide monohydrate (482mg, 11.50mmol), water (30ml), potassium osmate dihydrate (147mg, 0.399mmol),$(DHQ)_2PHAL$ (390mg, 0.50mmol) and methy cinnamate **94a** (1.75g, 10.80mmol).

Yield 1.5g (58%)

TLC, R_f 0.25 (ethyl acetate-hexane, 2:1, v/v)

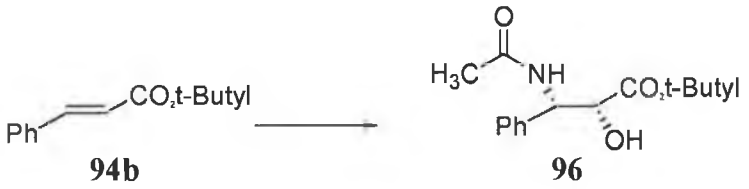
$[\alpha]_D^{20}$ +8.2 (0.4, EtOH)

δ^1H (400MHz DMSO-d_6) δ (ppm) 1.76 (s, 3H, CH_3CONH), 3.59 (s, 3H, $CH_3C{=}OCH$), 4.54 (dd, 1H, $J_{1\text{-}OH}$ = 8.8 Hz, $J_{1\text{-}2}$ =3.6 Hz, H-2), 5.08 (d, 1H, $J_{OH\text{-}1}$ = 3.6 Hz, H-1), 5.88 (br, 1H, OH), 7.23-7.38 (m, 5H, aromatic), 8.20 (d, 1H, J = 8.8 Hz NH)

δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 22.48, 52.21, 58.79, 72.60, 126.58, 127.56, 128.12, 142.12, 170.04, 171.26.

ES-MS m/z 260.2 (M + Na), $C_{12}H_{15}N_1O_4$ requires M = 237

6.6.2.4. Preparation of t-butyl- (2R,3S)-3-(N-acetyl)amino-2-hydroxy-3-phenylpropinoate **96**



The same procedure used to prepare **95** was used to prepare **96** using lithium hydroxide monohydrate (42.8mg, 10.2mmol), water (3ml), potassium osmate dihydrate (147mg, 0.399mmol), $(DHQ)_2PHAL$ (39mg, 0.5mmol) and t-butyl cinnamate **94b** (204mg, 0.68mmol).

Yield 180mg (88%)

TLC, R_f 0.27 (ethyl acetate-hexane, 1:1, v/v)

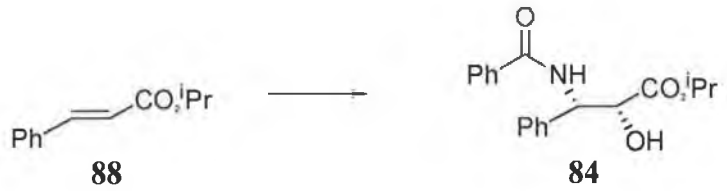
$[\alpha]_D^{20}$ –21.9 (0.32, $CHCl_3$)

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.43 (s, 9H, $C(CH_3)_3$), 1.93 (s, 3H, CH_3CO), 3.22 (br, 1H, OH), 4.35 (dd, 1H, $J_{1\text{-}OH}$ = 3.0 Hz, $J_{1\text{-}2}$ =2.3 Hz, H-1), 5.49 (dd, 1H, $J_{2\text{-}NH}$ = 7.4 Hz, H-2), 6.27 (d, 1H, J= 9.3 Hz NH), 7.21-7.32 (m, 5H, aromatic)

$\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 23.62, 28.19, 54.57, 73.72, 84.43, 127.24, 128.06, 128.99, 139.47, 169.54, 172.44.

ES-MS m/z 302.4 (M + Na), $C_{15}H_{21}N_1O_4$ requires M = 279

6.6.2.5. Preparation of isopropyl- (2R,3S)-3-benzamido-2-hydroxy-3-phenylpropinoate **84**[171]



88 **84**

In 30ml of an aqueous solution of $LiOH/H_2O$ (806mg, 10.2mmol), $K_2OsO_2(OH)_4$ (147mg,0.4mmol) was dissolved by stirring. After the addition of acetonitrile (120ml), $(DHQ)_2PHAL$ (1.558g,2.mmol) was added, and the mixture stirred for 10 minutes to give a clear solution. Water (30ml) was subsequently added, and the mixture cooled to 0°C. After addition of isopropyl cinnamate **88** (1.902g, 10mmol), N-bromobenzamide (4.0g, 20mmol) was added in one portion, and the mixture was stirred at 0°C for 10 hours. Over the course of the reaction, the colour changed from a deep green to yellow colour. The reaction was worked-up by the addition of saturated aqueous sodium sulphite (30ml) (5%w/v) and the reaction mixture was let stir for an additional hour. The two phases were separated, and the aqueous phase was extracted with ethyl acetate. Both the acetonitrile and ethyl acetate phases were combined and washed with 3N HCl, water, brine and the organic layer was then dried over anhydrous MgSO4. The solvent was removed by rotary evapotartion to afford a crude product that was purified by silica gel chromatography (ethyl acetate: hexane 1:1) and recrystallised from ethyl acetate to yield (84) (1.34g, 40%).

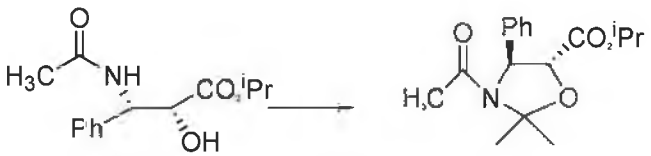
TLC, R_f 0.68 (ethyl acetate-hexane 1:1, v/v)

$[\alpha]_D^{20}$ –20.6 (0.6, $CHCl_3$)

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.28 (q, 3H, J= 6.2 Hz H-2'), 1.32 (q, 3H, J= 6.2 Hz H-2''), 3.38 (d, 1H, J= 3.6 Hz, OH), 4.61 (dd, 1H, $J_{1\text{-}OH}$ = 2.2 Hz, $J_{1\text{-}2}$ = 5.8 Hz, H-1), 5.12-5.20 (m, 1H, H-1'), 5.78 (dd, 1H, $J_{2\text{-}NH}$= 11.0 Hz, $J_{2\text{-}1}$= 1.8 Hz, H-2), 7.1 (d, 1H, J= 8.9 Hz, NH), 7.3-7.5 (m, 8H, aromatic), 7.83 (d, 2H, J=7.7 Hz, aromatic)

$\delta^{13}C$ (100MHz, $CDCl_3$ -d_6) δ (ppm) 21.99, 22.09, 54.98, 71.49, 73.75, 127.27, 127.41, 128.26, 129.08, 132.14, 134.62, 139.13, 167.14, 172.87.

6.6.2.6. Preparation of isopropyl-(4S,5R)-N-acetyl-2,2-dimethyl-4phenyl-1,3-oxazolidin-5-yl carboxylate **100**[153]



97 **100**

Compound **97** (1.89g, 6.83mmol) was dissolved in toluene (20ml). 2-Methoxypropene (0.6ml) and 4-toluenesulfonic acid monohydrate (0.06g, 0.32mmol) were then added and the reaction mixture was let stir at room temperature. After one hour more 4-toluenesulfonic acid monohydrate (0.06g) was added and the reaction was then heated to 80°C. The distillate was collected in a scale-receiving flask. A solution of 2-methoxypropene (3.1ml) in toluene (14ml) was added dropwise to the reaction mixture to maintain constant reaction volume. Subsequently, more 4-toluenesulfonic acid monohydrate (0.06g) was added to the reaction mixture and the mixture stirred for 10 minutes at 80°C. The reaction was cooled to room temperature whereupon saturated sodium bicarbonate (10ml) was added to the reaction mixture. The aqueous layer was

separated and extracted with dichloromethane (2 x 10ml). The combined organics were dried over $MgSO_4$, gravity filtered and evaporated under vacuum (<40°C). Flash chromatography (1:2 ethyl acetate: hexane) yielded (100) as a white wax (0.56g, 26%)

TLC, R_f 0.35 (ethyl acetate-hexane, 1:1, v/v)

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.31 (dq, 6H, $J_{2'-2''}$= 2.0 Hz, $J_{2'-1}$= 6.2 Hz, H-2', H-2''), 1.72 (s, 3H, CH_3), 1.80 (s, 1H, CH_3), 1.85 (s, 1H, CH_3) 4.46 (d, 1H, J= 3.9 Hz, H-1), 5.12-5.19(m, 1H, H-1'), 5.31 (d, 1H, J= 3.9Hz, H-2), 7.28-7.42 (m, 5H, aromatic)

δ^{13}C (100MHz, $CDCl_3$ -d_6) δ (ppm) 21.97, 22.14, 25.17, 26.26, 26.30, 64.61, 70.30, 82.52, 98.68, 126.51, 128.61, 129.53, 140.58, 168.88, 170.35.

6.6.2.7. Preparation of isopropyl-(4S,5R)-N-acetyl-2,2-dimethyl-4phenyl-1,3-oxazolidin-5-yl carboxylic acid **101**

100 **101**

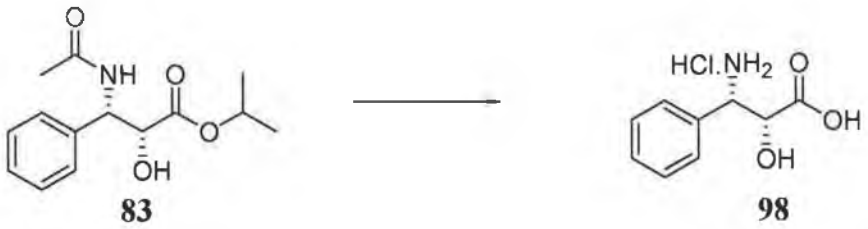
Oxazolidine **100** (0.4g, 1.31mmol) was dissolved in proan-2-ol (0.6ml). Lithium hydroxide monohydrate (0.12g in 0.2ml water, 2.86mmol) was added dropwise over 10 minutes at 0°C. The reaction mixture was stirred for a further 10 minutes. The reaction mixture was then concentrated by rotary evaporation and then diluted with water (2ml) and washed with isopropyl ether (2ml). The aqueous layer was acidified to pH 3 with HCl (1N) and extracted with dichloromethane (3x3ml). The organic extracts were washed with brine (2ml), and dried over $MgSO_4$ and evaporated to yield **101** (250mg, 52%) as a white solid.

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.70 (s, 3H, CH_3), 1.76(s, 3H, CH_3), 1.78(s, 3H, CH_3), 4.49 (d, 1H, $J_{2\text{-}1}$= 4.0 Hz, H-2), 5.26 (d, 1H, $J_{1\text{-}2}$= 4.1Hz, H-1), 6.20 (bs, 1H, CO_2OH) 7.28-7.36 (m, 5H, aromatic).

δ^{13}C (100MHz, $CDCl_3$ -d_6) δ (ppm) 24.93, 26.26, 26.33, 64.62, 81.97, 99.19, 126.44, 128.83, 129.71, 140.12, 169.59, 173.00

ES-MS m/z 286.3 (M + Na), $C_{14}H_{17}N_1O_4$ requires M = 263

6.6.2.8. Preparation of (2R,3S)-3-amino-2-hydroxy-3-phenylpropanoic acid hydrochloride **98**[165]



83 **98**

The isopropylidene ester **83** (1.195g, 4.50mmol) was added to 1:2 hydrochloric acid: watermixture (100ml) and the mixture was heated to reflux for 2 hours. The reaction mixture was then concentrated by rotary evaporation and washed with isopropyl ether: hexane (1:1, 3x5ml). The aqueous layer was collected and evaporated to dryness via a vacuum oven to yield **98** (0.94g, 95%) as a white solid.

M.p(lit. m.p. 224-226°C) 223-225°C

$[\alpha]_D^{20}$ = -14.1° (c 0.32 MeOH)

6.6.2.9. PREPARATION OF (2R,3S)-N-BENZYL-3PHENYLISOSERINE **97**[176]

98 **97**

To a solution of **98** (219mg, 1.00ml) in water (10ml) containing sodium bicarbonate (500mg) was added a solution of benzoyl chloride (0.14ml, 1.2mmol) in dichloromethane (5.0ml). The mixture was stirred for 16 hours at room temperature. The reaction mixture was acidified with 0.1N HCl and the crude product was extracted with ethyl acetate (3x40ml). The combined organic extracts were dried over anhydrous $MgSO_4$ gravity filtered and concentrated by rotary evaporation to yield a crude residue. The residue was purified by silica gel chromatography (ethyl acetate in hexane, 2:1) to give **97** (205mg, 72%) as a white solid.

TLC, R_f 0.30 (ethyl acetate-hexane, 2:1, v/v)

m.p. 166-168°C

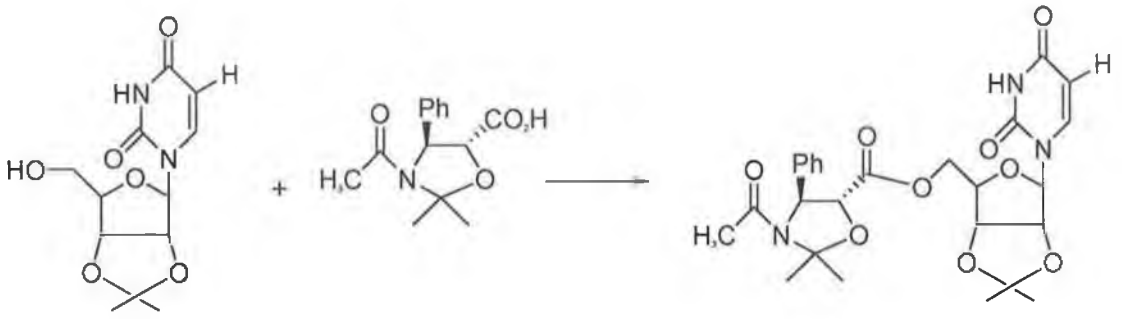
$[\alpha]_D^{20}$-35.5° (0.50, EtOH)

δ^1H (400MHz DMSO-d_6) δ (ppm) 4.38 (d, 1H, J= 4.3Hz, H-1), 5.46(dd, 1H, J= 8.8 Hz, J= 4.2 Hz, H-2), 5.65 (bs, 1H, OH), 7.22-7.57 (m, 8H, aromatic), 7.83 (d, 2H, aromatic, J=7.2 Hz), 8.61 (d, 1H, J= 8.9 Hz, NH), 12.73 (br, 1H, CO_2H).

δ^{13}C (100MHz, DMSO-d_6) δ (ppm) 56.16, 73.92, 127.38, 127.48, 127.66, 128.44, 128.76, 131.88, 134.52, 140.49, 166.54, 173.82.

ES-MS m/z 308.5 (M + Na), $C_{15}H_{21}N_1O_4$ requires M = 285

6.6.2.10. PREPARATION OF 2',3'-ISOPROPYLIDENE-5'-O-((-4''S,5''R)-N-ACETYL-2'',2''-DIMETHYL-4''-PHENYL-1'',3''-OXAZOLIDINE-5''-CARBOXYLATE)-URIDINE **103**[174]



68 **101** **103**

Oxazolidine **101** (0.298g, 1.13mmol) and dicyclohexylcarboiimide (0.259g, 1.26mmol) were stirred in toluene (1ml) for 5mins. Uridine isopropylidene **68** (0.30g, 1.06mmol) and 4-dimethylaminopyridine (0.069g, 0.056mmol) were added as a slurry in toluene (15ml), the mixture was heated at 80°C for 20 minutes. The reaction was cooled to 20°C and saturated sodium bicarbonate (12ml) was added. The aqueous layer was extracted with dichloromethane (3x5ml) and the combined organics were dried (MgSO4), gravity filtered and concentrated under vacuum. **103** was isolated by silica gel chromatography (5:1 ethyl acetate) as a white powder (0.245g, 45%).

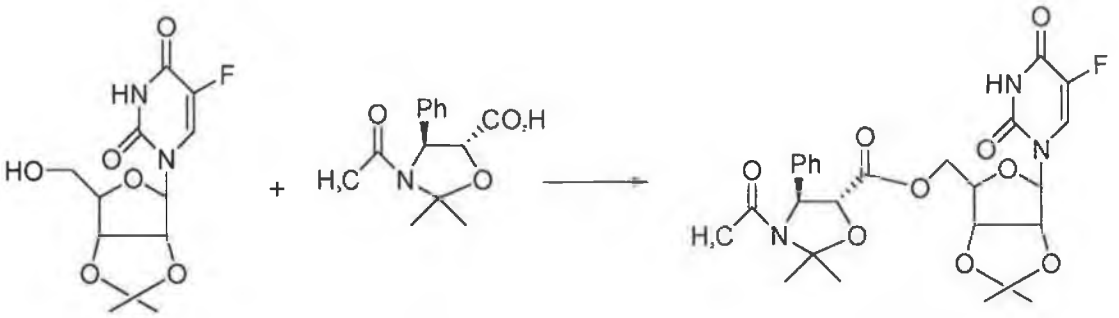
TLC, R_f 0.42 (ethyl acetate-hexane, 5:1,v/v)

$[\alpha]_D^{20}$ = -40.5° (0.06, chloroform)

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.18 (s, 3H, acetonide), 1.29 (s, 3H, acetonide), 1.50 (s, 3H, CH_3CON), 1.73 (s, 3H, oxazolidine) 1.76 (s, 3H, oxazolidine) 4.29-4.31 (m, 1H, H-5'), 4.33-4.38 (m, 2H, H-4', H-5') 4.47 (d, 1H, J= 4.0Hz, H-2''), 4.80 (dd, 1H, $dJ_{3'-2'}$ =6.4 Hz, $J_{3'-4'}$ =4.4Hz, H-3'), 4.97 (dd, 1H, $J_{2'-3'}$ =6.4 Hz, $J_{2'-1'}$ =4.4Hz, H-2'), 5.25 (d, 1H, $J_{1''-2''}$ =4.0Hz, H-1''), 5.55 (d, 1H, $J_{1'-2'}$ = 2.0Hz, H-1'), 5.62 (dd, 1H, J_{5-1} =2.0Hz, NCH=CHCO,), 7.21-7.28 (m, 5H, aromatic), 7.32(d, 1H, J= 7.5 Hz, NCH=CHCO), 8.54 (s, 1H, NH).

$\delta^{13}C$ (100MHz, $CDCl_3$-d_6) δ (ppm) 24.77, 25.13, 25.24, 26.30, 27.53, 64.53, 65.41, 81.38, 84.76, 85.36, 95.08, 103.10, 115.12, 126.31, 128.35, 129.69, 137.44, 142.55, 149.95, 163.10, 163.63, 169.95.

6.6.2.11. PREPARATION OF 2',3'-ISOPROPYLIDENE-5'-O-((-4''S,5''R)-N-ACETYL-2'',2''-DIMETHYL-4''-PHENYL-1'',3''-OXAZOLIDINE-5''-CARBOXYLATE)-FLUOROURIDINE **102**.



69 **101** **102**

Oxazolidene **101** (0.130g, 0.49mmol) and dicyclohexylcarboiimide (0.113g, 0.55mmol) were stirred in toluene (1ml) for 5mins. The 5-fluoro-2', 3'-isopropylideneuridine **69** (0.139g, 0.46mmol) and 4-dimethylaminopyridine (0.069g, 0.056mmol) were added as a slurry in toluene (5ml), the mixture was then heated to 80°C for 20 minutes. The reaction was cooled to room temperature and saturated sodium bicarbonate (2ml) was added. The aqueous layer was extracted with dichloromethane (5x 2.5ml) and the combined organics were dried over $MgSO_4$, gravity filtered and concentrated under vacuum. The product **102** was isolated by silica gel chromatography (1:3chloroform ethyl acetate) as a white wax (0.065g, 25%).

TLC, R_f 0.42 (chloroform-ethyl acetate, 1:3, v/v)

$[\alpha]_D^{20}$ = -39.5° (0.06, chloroform)

δ^1H (400MHz, $CDCl_3$ -d_6) δ (ppm) 1.29 (s, 3H, acetonide), 1.51 (s, 3H, acetonide), 1.64 (s, 3H, CH_3CON), 1.71 (s, 3H, oxazolidine) 1.76 (s, 3H, oxazolidine) 4.34-4.38 (m, 1H, H-5'), 4.42-4.4.43 (m, 2H, H-4', H-5') 4.44-4.48 (d,1H, J = 4.0Hz, H-2''), 4.75 (dd,1H, $J_{3'-2'}$ = 6.4 Hz, $J_{3'-4'}$ = 4.4Hz, H-3'), 4.90 (dd,1H, $J_{2'-3'}$ = 6.4 Hz, $J_{2'-1'}$ = 4.4Hz, H-2'), 5.24 (d,1H, $J_{1''-2''}$ = 4.0Hz, H-1''), 5.63 (d,1H, $J_{1'-2'}$ = 2.0Hz, H-1'), 7.20-7.35 (m, 5H, aromatic), 7.38 (d,1H, J = 7.5 Hz, NCH=CFCO), 9.01 (s,1H, NH).

δ^{13}C (100MHz, $CDCl_3$ -d_6) δ (ppm) 23.82, 24.25, 24.67, 24.96, 26.11, 63.14, 63.79, 79.76, 80.74, 83.12, 83.37, 93.08, 97.51, 125.12 (d, J= 27Hz), 127.44, 128.29, 138.66, 147.52, 155.63, 167.69, 168.76, 170.18.

δ^{19}F ($CDCl_3$ -d_6) – 164.81(d, J=-6.7Hz)

ES-MS m/z 548.2 (M^+ + H), $C_{26}H_{30}N_3O_9$ requires M = 547

6.6.2.12. PREPARATION OF URIDINE **67** FROM 2',3'-ISOPROPYLIDENEURIDINE **68**.[175]

68 **67**

To a solution of 2', 3'-isopropylideneuridine **68** (0.284g, 1mmol) in acetonitrile (5ml) was added $Zn(NO_3)_2.H_2O$ (0.95g, 5.0mmol) and the reaction mixture was stirred at 50°C overnight. After which time it was analysed by TLC (5:1 ethyl acetate:hexane), a spot consistent with uridine **67** was evident. The solvent was evaporated under vacuum, the residue diluted with dichloromethane (15ml), dried ($MgSO_4$) gravity filtered and

solvent removed via rotary evaporation. Recrystallised from water to obtain (67) (0.144g, 60%)

6.6.2.13. Preparation of isopropyl-(2R,3S)-3-(acetyl)amino-2-hydroxy-3-phenylpropinonat e from isopropyl-(4S,5R)-N-acetyl-2,2-dimethyl-4phenyl-1,3-oxazolidin-5-yl carboxylic acid **89**.

101 **89**

To a solution of **101** (0.263g, 1mmol) in acetonitrile (5ml) was added $Zn(NO_3)_2.H_2O$ (0.95g, 5.0mmol) and the reaction was stirred at 50°C overnight. TLC analysis (5:1 ethyl acetate:hexane), showed a spot consistent with (95). The solvent was then removed by evaporation, the residue diluted with dichloromethane (15ml), washed with water (10ml) and dried over $MgSO_4$ and gravity filtered. The solvent was removed under vacuum. The recovered residue was recrystallised from ethyl acetate to obtain **89** (0.13g, 49%).

7.0. Conclusion

The main objective of this body of work was to design and prepare a reductase activated phthalocyanine conjugate prodrug. We were also interested in exploring the potential use of an isoserine side chain as a potential carrier for 5-fluorouracil. To achieve both goals a large body of synthesis was carried out, and many new prodrug derivatives of fluorouracil were both synthesised and evaluated for bioactivity. This work led to the following:

1. The successful synthesis of an axial nucleotide – silicon phthalocyanine conjugate.
2. Synthesis of new 5-FU derivatives that demonstrate both enhanced bioactivity against cancer cell lines and potential anti-invasion activity.
3. The development of new 5-FU prodrugs, which possess no bioactivty under normal cellular conditions, but are activated under reductase rich hypoxic conditions.
4. The total synthesis of a novel 5-FU prodrug conjugated to an isoserine side chain. Unfortunately, this new derivative possesses no bioactivity against cancer lines and cannot be activated to any extent under hypoxic conditions.
5. The synthetic work in Chapter 4 lead to the development of a new cleavage technology, which we had hoped to solve a synthetic hurdle with the synthesis of the 5-FU isoserine prodrug **102**. These new cleavage conditions, although unsuccessful in the selective cleavage of **102**, have been successfully applied in the selective cleavage of trityl groups in the presence of acetonide protection groups **82**.

The next stage of this research program is the biological evaluation of the new 'reductase activated' prodrug **79**. If light therapy does indeed activate the prodrugs to yield 5-FU and quinone methides then a new third generation photosensitizer is born. If

failure occurs with light therapy, that is human reductases cannot convert prodrug **79** into 5-FU and quinone methides, then the genetherapy route which incorporates the gene for nitroreductase becomes the next necessary step for the application of these new and novel photosensitiser prodrugs. This will be also tested on prodrugs **80A**, **B** and **C**. A SAR study should also be undertaken in the future to determine why prodrugs **58** and **59** are showing such excellent biological activity, once determined then perhaps better leads can be developed to replace 5-FU.

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