The design and synthesis of peptide/protein based ATRP initiators

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The Design and Synthesis of Peptide/Protein based ATRP Initiators

(Hetero-bifunctional Linkers)

by

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ABBREVIATIONS

d	Doublet
dd	Doublet of doublets
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DMF	Dimethylformamide
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EI	Electron ionisation
ES	Electrospray
Et	Ethyl
Ether	Diethyl ether
FAB	Fast atom bombardment
Fmoc	9-Fluorenylmethoxycarbonyl
HCl	Hydrochloric acid
HOBt	5-Hydroxybenzitriazole
HPLC	High pressure liquid chromatography
HRMS	High resolution mass spectrometry
Hz	Hertz
IR	Infra red
m	multiplet
M	Molar
m/z	Mass to charge ratio
MALDI-tof	Matrix assisted laser desorption/ionisation time of flight
Me	Methyl

mmol	Millimole
mol	Mole
m.p	Melting point
NMR	Nuclear magnetic resonance
o/n	Over night
PBS	Phosphate buffered saline
Pd/C	Palladium on carbon
Ph	Phenyl
ppm	Parts per million
q	Quartet
rt	Room temperature
s	Singlet
SPPS	Solid phase peptide synthesis
t	Triplet
Tf	Trifluoromethanesulphonyl
TFA	Trifluoroacetic acid
TfO	Triflate (Trifluromethanesulfonate, $-SO_2CF_3$)
TG	TentaGel®
THF	Tetrahydrofuran
TLC	Thin layer chromatography

DECLARATION

The work contained in this thesis was carried out in the Department of Chemistry, University of Durham between October 2001 and December 2004. All the work is my own unless otherwise indicated. It has not previously been submitted for a degree at this or any other university.

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Design and synthesis of peptide and protein-based ATRP initiators

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D.Phil., Sept 2005

A series of novel hetero-bifunctional linkers functionalised as ATRP initiators and protein and peptide-reactive agents has been prepared using standard synthetic techniques. A protein-based initiator has been applied to the initiation of living polymerisation in the synthesis of a novel bioconjugate.

The linkers were designed based on the properties of polyethylene glycol and short alkyl chains coupled to either amine selective or thiol selective moieties for chemoselectivity, and bromoisobutyryl esters to facilitate atom transfer living polymerisation.

The bi-functional linkers have also been coupled to short peptides based on the RGD bio-recognition sequence synthesised by standard solid phase peptide synthesis and the protein, Human Serum Albumin (HSA) using standard conditions to prepare peptide/protein-based ATRP initiators.

Attempts at functionalising peptides with the N succinimidyl 4-(2-bromo-2-methylpropionyloxy)butanoate linker were unsuccessful.

Model test of the protein based ATRP initiators in living polymerisation towards a novel strategy for bioconjugate synthesis was inconclusive as premature termination of the polymer chain was observed by MALDI analysis.

APPENDIX

Research Conferences Attended

Sep 2004	Celebration of Organic Chemistry, Warwick University, UK *
May 2004	3rd Year PhD Presentation, University of Durham, UK **
May 2004	Boron Chemistry, University of Durham, UK
Apr 2004	RSC North East, Organic Division, University of Leeds, UK
Mar 2004	Avecia Poster Competition, University of Durham, UK
Mar 2003	Perkin Division, North East, Regional Meeting, University of Newcastle upon Tyne, UK
Dec 2002	Modern Aspects of Stereochemistry, Sheffield University, UK
Apr 2002	Perkin Division, North East Regional Meeting, University of York, UK
Dec 2001	Modern Aspects of Stereochemistry, Sheffield University, UK

* Poster presentation by the author
** Oral presentation by the author

Chapter 1

GENERAL INTRODUCTION

1.1 Introduction

Increased bioavailability of active agents in a diseased area will not only reduce toxicity in healthy cells; higher drug efficacy will be expected as well. Therefore various combinations of means for effective delivery, stabilisation and localisation of administered drugs have been investigated by scientists. Perhaps the most versatile and modern approach involves the use of water-soluble biopolymers with targeted interactions. Biopolymers are (in general) polymers conjugated to one or more recognition molecules which are usually bioactive (or mimics) for targeted delivery - they are sometimes referred to as "smart" conjugates when coupled macromolecules synergistically respond to external stimuli while exhibiting a particular and/or expected state of readiness, usually site-recognition. These bioactive molecules can be proteins, oligosaccharides, phospholipids, and other synthetic molecules, which because of their large size promote a prolonged half-life and efficient accumulation in the affected area. These unique properties of polymeric conjugates result in improved efficacy and make them effective tools for therapeutic studies. This had already been reviewed by Ringsdorf[1] in relation to polymer-anticancer drug conjugates before the novel developments in polymerisation techniques. The components and concept of bioconjugation in association with potentially novel therapeutics available by living polymerisation techniques will hence be explored.

1.2 Conjugation

While the focus of traditional approaches to pharmaceutical research is on the discovery of new compounds, one of the frequent problems with this approach is the almost ubiquitous association with low drug efficacy arising from the low molecular weight, low solubility and consequently low bio-distribution, resulting in high toxicity. However, by conjugation of drugs to other macromolecules, increased efficacy and inter-entity

cooperation can be employed to improve on current drugs. The principle of cooperation takes advantage of the ability to design, synthesise and modify natural and synthetic macromolecules with the required, perhaps novel characteristics (micellisation or hydrogel formation), to improve, for example, drug bioavailability within the bloodstream or increased half-life of a reporter molecule by introducing better targeting capability. A recent illustration is the development of nanocrystal technology, which originally had little biomedical value, until conjugated, yet has already brought about the development of a new area of biomedical science.

Quantum Dots[2] (Qdot®) are nanoscopic inorganic crystals with cores of cadmium sulfide (CdS), cadmium selenide (CdSe), or cadmium telluride (CdTe); their bioconjugates are Qdot nanocrystals coupled to small biomolecules[3] (sugars, proteins, *etc*) to aid site-specific recognition as a replacement for conventional dyes to achieve lower limits of detection, greater quantitative results, and more photo-stable samples. Due to their remarkable fluorescence-imaging sensitivity and the small quantities required, they can have biomedical use. The advantages of bioconjugation become more obvious when two different types of macromolecules are involved, such as drug conjugates and peptide/protein conjugates (further discussed in section 1.3) or oligonucleotides with fluorescence dye[4], metal chelates[5], photochemical crosslinkers[6], redox probes[7] or lipids[8].

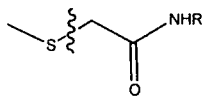
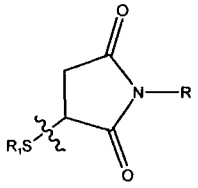
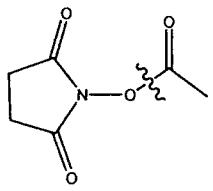
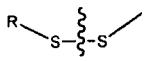
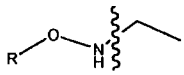
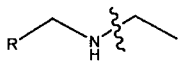
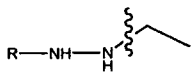
However, as enhanced capabilities are realised in the preparation of conjugates of drug and polymeric macromolecules with well defined structures, greater directions are being sought from biologically compatible systems (or mimetics) leading to the formation of biologically active conjugates (bioconjugates). Therefore convenient synthetic methods for the preparation of simple organic bioconjugates are of importance. They also include chemical tools which can aid the amalgamation of two or more complex substrates covalently.

The different areas of research into conjugation have contributed different specialised reagents to the pool of bioconjugation reagents from which certain methods and reagents are generally used or associated e.g. radio-labelling, purification, assay development or protein chemistry. Due to continued advances in protein chemistry, it has been possible to chemically modify proteins and peptides in order to protect or functionalise substrates effectively for use in conjugation using a variety of reagents.

Chemically reactive groups have helped to crosslink various compounds depending on their eventual use and the associated advantages resulting in sequence-selective crosslinking (see section 1.5.2 for definition and details) or cleavage of linkages. For the past 15-20 years, there have been a plethora of functional groups capable of crosslinking but few have become standard reagents. Some examples are shown in (Table 1):

- Thiol-selective reagents – using i.e. the thiol of cysteine to covalently conjugate with either a maleimide or a pyridiyl disulphide respectively, iodoacetal being the most active. Hence selectivity is highly dependent on the pH of reaction. If no such residues are available i.e. in a native protein, thiol group can be incorporated with Traut's reagent (2-iminothiolane[9]).
- Amide bond formation using a primary amine and activated carboxylic acids e.g. succinimide and aminooxyacetamide esters; or hydrazine/amine with carbonyl (i.e. aldehyde) to form a condensation product hydrazone before hydride reduction.
- Biotin / Avirdin - a non covalent coupling (by association) using the ligand biotin and avirdin associated to a receptor protein, particularly useful in the detection and localisation of antigens, glycoconjugates, and nucleic acids by employing biotinylated[10] antibodies, lectins, or nucleic acid[11] agents.

Table 1. *Examples of functional groups /reagents for bioconjugation.*

Reagents	Selectivity	Product	Examples/Refs
Iodoacetamide	Thiol		12,13,14
Maleimide			15,16,17,18
N-hydroxy succinimide	Amine		19
Thiol	Disulphide (Cystine)		20,21,22,23
Aldehyde	Aminooxy		24, 25,26
	Amine		
Hydrazone then reduction	Aldehyde		16,27,28

Notably, various promising gene and protein-related drugs are limited by their inability to penetrate the cell membrane and be delivered inside the cell. In order to enhance the effectiveness of any drug, an appropriate conjugation methodology to improve targeting and the mode for sustained release of the active drug are most often considered.

Hence the use of linkers has become prevalent for aiding the targeting and release mechanisms in drug and diagnostic studies of disease states. However, there is still a great demand for more robust methods or alternatives, to compensate for problems that arise in the biofunctionalisation and subsequent conjugation of polymers. In particular, the harsh reaction conditions that are often required tended to lead to the degradation and inactivation of sensitive biological compounds or drugs. Moreover, the synthesis of stoichiometrically defined polymers - macrobiomolecule conjugates - is a challenge, and is particularly important from a biomedical point of view to generate well-defined (architecture and composition) macromolecules, and the methods and material to achieve such macromolecules are now more varied and better developed - especially with the advent of living radical polymerisation.

1.3 Bioconjugates

1.3.1 The Principles of Conjugation

The rationale for conjugating a drug or other low molecular weight entity to a macromolecule for biomedical use is that the resultant increase in molecular weight significantly alters the biodistribution of the active agents in the conjugate and curtails the indiscriminate uptake of the drug into cells, thus achieving targeting at either tissue or cellular levels. This not only improves the therapeutic index of an otherwise toxic drug, but also obviates the need for repeated high doses (predictable drug strategy, drug uptake and activity). The polymer–drug conjugate exhibits decreased clearance—this prolonged circulation provides sufficient time for the polymer–drug conjugate (better pharmacokinetics and biodistribution) to reach and concentrate at its target site[29], a strategy already employed in various commercially available drug conjugates often associated with the enhanced permeation and retention effect of the increased molecular weight.

Camptothecin (CPT) derivatives[30] have advanced to become standard components in the treatment of several malignancies. However, as a class of anti-cancer compounds, camptothecins pose many challenges that need to be overcome before they can be widely used in anticancer therapy. One of the principal chemical features of this class of agents is the presence of the lactone functionality, which is not only essential for anti-cancer activity, but also confers a degree of instability to these agents in aqueous solution.

A variety of different strategies are being investigated to modulate the systemic delivery of CPTs, such as the development of prodrugs and polymer conjugates[31]. Among all the approaches currently under investigation, the polymer conjugates have been studied the most since they can lead to alterations in biological distribution, longer retention times within the body, reduction in systemic toxicity and improvements in therapeutic efficacy. There are several types of CPT-Polymer conjugates reported in the literature to overcome the delivery challenges

such as poly-(L-glutamic acid)-CPT[32], HPMA co-polymer-CPT[33], PEG-CPT[34], cyclodextrin-CPT[35] and carboxymethyl dextran-CPT[36]. The immediate goal of these approaches is often focused on the treatment of dose-limiting toxicity in tumors[37]. Targeted delivery systems to cell surface receptors[38,39] is another approach that could be used in order to treat diseased cells but spare healthy ones. One of the more studied approaches utilises macromolecules. In general, conjugation of natural (protein, polysaccharides, DNA *etc*) and synthetic polymer macromolecules (which exist in various forms: emulsions, hydrogels, adsorbed or grafted on a surface or dissolved in solution) to carefully selected biopolymers or drugs provides improved drug stabilisation, cytotoxicity, solubility, localisation and controlled release with direct implications on efficacy. Bioconjugation approaches[40,41] to therapeutic studies have therefore recently focussed on the development of biocompatible functional polymers of different architecture provided by versatile and robust polymerisation techniques. These further allowed access to the variety of molecular organisation required to produce sophisticated bioconjugates.

Although scientists have always explored the possibilities that macromolecule conjugation can afford, only recently has the term "bio-conjugation" been introduced to describe the amalgamation (either by physiosorption or covalent association) of biological moieties to synthetic macromolecules to develop novel polymers[42] with specific biological capabilities[43]. In addition, the non-selective nature of many drugs and their resulting toxicity has been a major hindrance in biomedical approaches to the treatment of various diseases (e.g. cancer). As a result, various potential anticancer drugs have now been re-engineered via conjugation to create novel chemotherapy (i.e. immunotoxins[44]) and tumour-selective cytotoxic conjugates with polymeric carriers[45] and targeted with carbohydrates or antibodies to reduce toxicity to non-cancerous cells during chemotherapy. However, since polymeric material can be engineered with in-built but predictable mechanical properties as necessary for the various biomedical requirements by effecting simple principles for conjugation. The various

combinations of method of conjugation have already been shown to offer benefits to potential drug candidates; such as

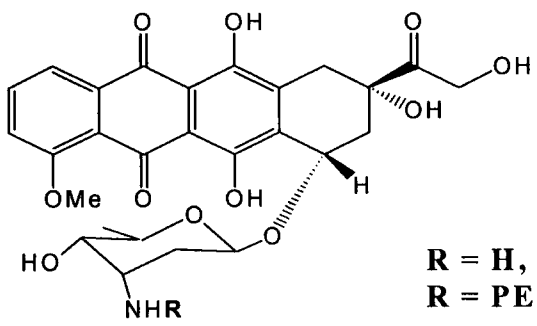
1. Esterification of functional groups on drugs[46] (aspirin, ibuprofen with PEG; or CPT) with a simple polymer stabilises some drugs in their active state, which may otherwise be hydrolysed under physiological conditions leading to inactive or toxic by-products
2. The use of hydrophilic polymers leads to highly water-soluble formulations of conjugates of low/high molecular weight with hydrophobic substrates for greater bio-distribution (EPR effect).
3. Incorporation of spacer/linker units ensures ease of conjugation to sites deep below the surface of macromolecules and a controlled release of drug in response to stimulus.

Despite the sophistication and prominence shown by conjugation techniques, inappropriately designed components can often still lead to bioconjugates of insufficient stability or adversely altered antigenic properties of substrates (i.e. peptides[47]) due to the inappropriate design and method of construction of intended substrates, even with the application of commonly used conjugation components and methods. However, as scientists learn more about disease progression and the function(s) of specific natural biomolecules in any given process, the more prominent the role of bioconjugates as therapeutics and diagnostic tools has been in understanding the precise and required design of conjugates. However, one of the more prominent hurdles in bioconjugation is the high reactivity of some molecules, such as protein and nucleic acids, because of their varied reactive sites and the difficulties of chemoselectivity[48], and this has therefore been more routinely studied.

As such, there are only a few classes of compounds that enjoy routine use as substrates for conjugation and they include proteins and low molecular weight drugs. For this reason, this discussion will focus on the conjugation methods and reports pertaining to these classes of biologically important molecules.

1.3.2 Drug conjugation to macromolecules

There are more than 200 different types of cancer, but four of them – breast, lung, large bowel (colorectal) and prostate – account for over half of all new cases.



R = H, Doxorubicin (DOX HCl)
R = PEG (i.e Doxil)

Figure 1.1: *Structure of an anticancer drug already in use but with high toxicity.*

Doxorubicin[49] (Adriamycin) is an anthracycline antibiotic with a broad spectrum anti-cancer capability used to treat many types of tumour by inhibiting cell growth and inducing cell death. Unfortunately, side effects from doxorubicin include nausea and vomiting which may last up to 24-48 hours after treatment, loss of appetite, difficulty swallowing, thinned or brittle hair and skin irritation.

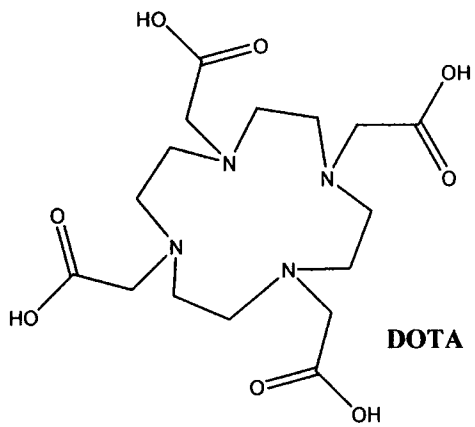
The poly(ethylene glycol)-modified derivative of doxorubicin "DOXIL®" encapsulated with liposomes[50,51] (polymeric carriers) to reduce the side effects via non-specific drug delivery has found use in the treatment of Kaposi sarcoma (a type of cancer common in HIV victims) and is recommended to patients with low tolerance to traditional chemotherapy treatments because of side effects. The main side effects associated with the modified drug are heart problems and neutropenia (low white blood cell count). While liposomal encapsulation was the simplest and initially the most widely used form of drug delivery system used to improve the pharmacological properties of a variety of drugs, the size and charge was thought to hinder their ability to effectively distribute and therefore has a limited use. However the use of liposome

encapsulation has shown that conjugation with specially designed components may be a promising concept to improve efficacy.

Some other covalent conjugates described the use of hydrophilic polymers (e.g., N-(2-hydroxypropyl) methacrylamide (HPMA) or poly(ethylene glycol)[52](PEG) conjugates of doxorubicin to reduce doxorubicin toxicity[53] when compared with the free doxorubicin drug. The same technology has been used to produce diagnostic and therapeutic products when polymeric products are conjugated with folates[54]and other biomacromolcules.

1.3.3 Peptide/Protein - Polymer Bioconjugates.

Several peptide conjugates have been designed to exploit the relative ease with which peptides can be recognised and actively transported across cell membranes and hence be used as antisense carriers into the cell and in targeting/release in a dual functional role.



DOTA

Figure 1.2: *DOTA[55] , which can be conjugated with peptides for use as a metal ion carrier*

Shively *et al* in 1994[56] conjugated DOTA to achieve high specific activity and high chelate stability in radiolabelled immunoconjugates and in 2001[57] developed a simple, water-soluble procedure for conjugation of 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid (DOTA) to the human/murine chimeric anti-carcinoembryonic antigen antibody. This improved method results in a 6-fold increase in conjugation efficiency, a 3-7-fold decrease in antibody crosslinking, a more homogeneous population of conjugate species, and a 5-fold decrease in the quantities of

reagents needed for conjugation. The DOTA conjugate was labelled to high specific activity with ^{111}In, ^{90}Y, ^{88}Y, ^{64}Cu, and ^{67}Cu, affording near-quantitative incorporation of the majority of radio-metals.

This improved conjugation procedure was proposed to facilitate large-scale production and radio-metal labelling of cT84.66-DOTA for clinical radio-immunotherapy. The use of peptides is not restricted to labelling or targeting but can also act as a release mechanism in response to a predetermined trigger (i.e. pH assisted hydrolysis).

Dubowchik et al[58] utilised the ability of enzymes to recognise and rapidly cleave the dipeptide Phe-Lys in their design of an efficient linker and targeting moiety. Furthermore, the linker was designed such that the enzyme cleaves the amide bond, which attaches the lysine residue[59] to *p*-aminobenzyl alcohol (PABA). Such conjugates have shown considerable importance in the field of contrast agent carriers, catalytically active metal ions and radioactive markers where the use of purely instrumental techniques is unsuitable for detection of low levels of contrast agent in biological matrices.

The use of peptide sequences as a targeting moiety has a similar principle to that involved in most physiological processes, including the immune response, cell differentiation, and cell-to-cell interactions. They are also involved in the disease state, such as cancer cell proliferation[60]and various inflammatory processes like rheumatoid arthritis and asthma[61]. The specific sequence required, where known, has been fully exploited in bioconjugate synthesis.

The integrins are a superfamily of cell surface proteins (first examples described were fibronectin and vitronectin receptors) which bind to an RGD (Arg-Gly-Asp) ligand sequence in the protein, the main receptor by which cells attach to the extracellular matrix on which their subsequent ability to facilitate cellular functions including disease states are highly dependent; for these reasons, the proteins and their application or influence in therapeutic approaches has been of great interest.

Fibronectin type III (in the same integrin-receptor family) is a cell adhesion protein widely distributed in the tissues of all vertebrates and is present as a soluble polymeric fibrillar network in the extracellular matrix.

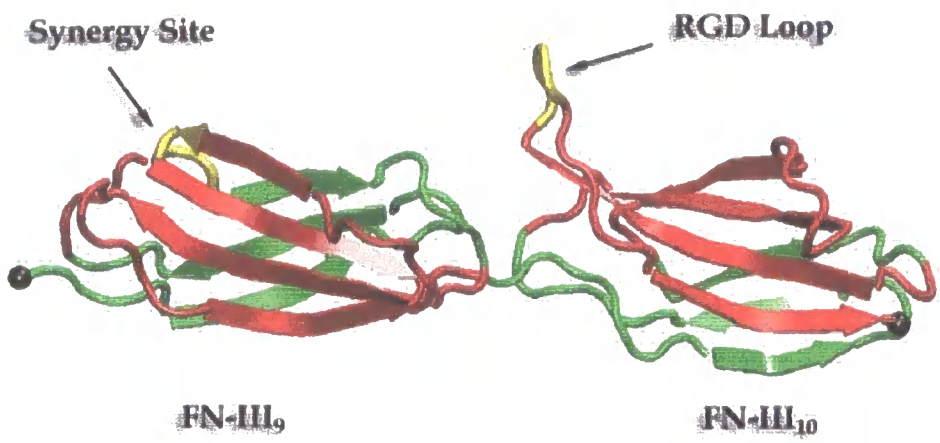


Figure 1.3. *Cartoon representation of the functionally decoupled state*[62]

Fibronectin has two major domains that help with cell adhesion; they both have a peptide motif found within fibronectin with a minimal peptide sequence of (leucine-aspartic acid-valine) LDV motif. This is analogous to the arginine-glycine-aspartic acid (RGD[62]) motif and their importance in the adhesion process has been substantiated[63]. Vitronectin and laminin are two other proteins that use this sequence to induce adhesion on surfaces that are otherwise non-adhesive[64]. The affinity for these tripeptides compared with fibronectin is, however, low. Tambunan *et al*[65] had also shown (using cyclic RGD) that hydrophobic residues adjacent to the RGD sequence are important for an improved affinity of the sequence[66]. An elegant but carefully constructed polymeric capsule (considering the micellar size and biocompatibility) for improved biocompatibility, labelled with the RGD sequence should therefore be a viable vehicle for target delivery and thus of therapeutic importance. The concept has already been applied and recently Mitra *et al*[67] further discussed the application of water-soluble, N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer carrying cyclised

Arg-Gly-Asp motifs (HPMA copolymer-RGD conjugate) which showed greater in vivo accumulation than the control Arg-Gly-Glu (HPMA copolymer-RGE conjugate) conjugate, which they proposed to provide a foundation that should support targeted delivery of radionucleides and drugs to solid tumours for diagnostic and therapeutic applications in specific tumor angiogenesis targeting. The above information has therefore contributed to the design of short, hydrophilic and unique RGD-incorporated peptide[68] sequence with the ability to mimic the natural protein binding activity hence avoiding all the intrinsic problems associated with lengthy chain peptide synthesis.

1.4 Advantages of Polymer Bioconjugates

1.4.1 Introduction

The use of liposomes, spherical vesicles made of phospholipids commonly used in combating cytotoxicity by encapsulation and their ability to permeate cell membranes, forms perhaps one of the simplest methods of drug delivery and has led the way for other more sophisticated methods.

As Maeda *et al*[69] recently reviewed, the process known as enhanced permeability and retention (EPR) usually associated with lipid and macromolecular agents has been exploited to provide the opportunity for more selective targeting of lipid or polymer-conjugated drugs. The improved permeability and delivery of such macromolecular drugs are highly dependent on the choice of polymer macromolecule. These are easily obtained either by natural (liposome) or synthetic polymerisation methodologies.

The use of polymer carriers has several advantages over other delivery methods, such as liposomes and antibodies. Liposomes, for example can be taken up by macrophages or move to the tissue from the blood vessels where they are not necessarily wanted and antibodies have the disadvantage that most receptors on tumour cells are also present on

normal cells making it difficult to find ones that are unique to cancer cells.

In contrast, the use of water-soluble polymers of PEG and *N*-(2-hydroxypropyl)methacrylamide allows the use of a simpler molecule rather than a large particle while still acting as an effective protective/delivery system in vivo. This constitutes a macromolecular prodrug.

Such polymer-biomolecules have formed a group of conjugates with applications in varied areas of medicine and biotechnology, which has recently been reviewed by Alexander *et al*[70]. They explored the influence of smart polymers in medicine especially as controlled drug release vehicles, the types of stimulus response used in therapeutic applications and the main classes of responsive materials developed to date.

- To summarise, polymeric bioconjugation provides approaches to deliver intact low molecular weight substrates to required sites by:
- conjugation with polymers to provide a stimulus-sensitive coating or targeting with biodegradable polymers
- embedding biodegradable matrices, micelles and hydrogels to provide stealth and hence
- *Via* high molecular weight drug development to produce enhanced EPR effect.

There are various reasons why polymer bioconjugation has been vital to research especially when specially designed smart polymers[71] are conjugated. The enhanced permeability and retention (EPR) effect of macromolecules in tumors by plasma proteins and their various synthetic polymers conjugates have shown accumulation in solid tumors for prolonged periods. More recently, the key mechanism for the EPR effect[72] for macromolecules in solid tumors was found to be retention, whereas low-molecular-weight substances were not retained but were returned to circulating blood by diffusion[73].

The above unique behaviour and the ability to design smart polymers that can be manipulated to respond to a predetermined stimulus has

revolutionised various aspects of research, for example as biomedical tools, illustrated below with some examples.

1.4.2 Diagnostic tools and Immunoassays

The wide use of monoclonal antibodies (mAB) as efficient toxin delivery agents and their capabilities in antibody-mediated cytotoxicity has ensured rejuvenated interest for various cancer therapies as immunotoxin bioconjugates[74] but, they have shown side effects as a result of processes which are difficult to monitor without genetic engineering, hence are not as advanced in application.

The use of fluorescence tagging has a wide-ranging influence both in the area of high throughput screening[75] and as a diagnostic tool in biological systems. Simon *et al*[76] reported the use of luminescent quantum dot conjugates as an alternative to current organic dyes. The new conjugate was biocompatible and allows for simultaneous studies of multiple cells over a long period of time. The corresponding conjugates can be proteins (synthetic and natural), biocompatible polymers or antibodies with high immunoassay stability[77].

1.4.3 Gene Therapy

Genetic material is more often optimally transported into host cells by naturally evolved vectors, such as viruses and bacteria. While the development and use of mimics or alternatives is gaining ground, improvement on nature's design still proves to be the most practical. They are increasingly sophisticated while remaining non-toxic to the host.

Ideally, vectors should allow efficient gene insertion and prolonged transgene expression, absence of viral host integration and excellent immune stimulation. By using information collected on the limitations and difficulties associated with such technique, reasonable progress has been made in recent years. Focusing on a conjugate-related approach, the report[78] that recombinant adenovirus, non-covalently complexed to cationic polymers and cationic lipids with adenovirus have been shown to increase adenovirus uptake and transgene expression in cells that were

inefficiently infected by adenovirus, suggested that there are benefits from well designed and conjugated novel approach to gene therapy.

Gene delivery by vector systems, approached by modification of adenoviruses with PEG/peptide complexes[79] has also been investigated. The bioconjugates serve to partially overcome the barrier of inefficient gene transfer associated with gene delivery by vectors, by incorporating cell-specific ligands into the virion to aid vector active site recognition. The increased affinity of viral receptors on the surface of cells was introduced by incorporating screened peptide recognition sequences.

The new approach[80] of using non-viral[81] methods (or mimics),-that is, cationic lipids and polymers - is catching up on the more traditional gene therapy method of using cell-infectious viral RNA/DNA delivered into a cell, allowing the cell to develop the therapeutic protein. The problem of immunogenic responses associated with gene delivery by the virus is thus avoided. Santanu Bhattacharya *et al*[82] have investigated the transfection ability of a number of cytofectins based on cholesterol. They follow on the success of cationic cholesterol derivatives capable of DNA transfection in cells with high efficiency; several other groups have also prepared poly(ethylene glycol, PEG) and sugars conjugated with peptides, some of which have the ability to form a DNA condensate. They are receiving attention for their possible use in DNA complexation[83] and as gene transfer agents.

Another emerging strategy uses non-invasive delivery of drugs and genes with microbubbles[84] using ultrasound-mediated microbubble destruction, encapsulation of drugs in microbubbles induced by ultrasound application, and also the direct delivery of substances bound to microbubbles[85] in the absence of ultrasound. Different drugs and genetic material can be incorporated into the ultrasound contrast agent system.

1.4.4 Controlled Release

Controlled release is effected when a required substrate (or drug) is either

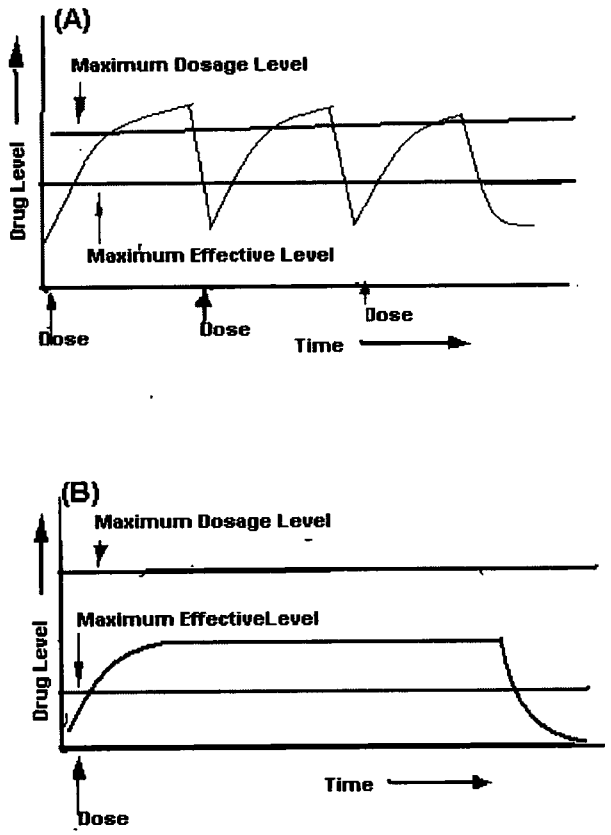


Figure 1.4. *Schematics for the behaviour of drug levels in the blood by (a) traditional drug dosing and (b) controlled-delivery dosing.*

covalently bound to a polymer (natural or synthetic) or associated non-covalently in order to facilitate a predetermined action/release mechanism. The release of the drug can be triggered over a period of time and the action initiated by a predetermined stimulus. The advantages of such controlled-delivery systems can include the maintenance of drug levels within a desired range (Figure 1.4), the need for fewer administrations to maintain a level of therapeutic effect, and increased patient compliance.

Many of the approaches to the development of novel material useful for controlled release are focused on the preparation of polymers with specially designed features. Such systems include

- Block copolymers (see section 1.8)
- Copolymers with desirable amphiphilic interactions
- Biodegradable polymers
- New blends of hydrocolloids and polymers (natural and synthetic)

Block copolymers offer a unique but simple way to address drug release and bioavailability of drugs. Figure 1.5(**A**) shows a typical basic cationic copolymer that swells at pH 5-8 but is soluble at pH 2.5. Figure 1.5(**B**) shows an anionic copolymer resistant to gastric juice yet soluble in water in the small intestine, but insoluble and impermeable to water at a pH below 6. They therefore can be tailored to provide protection for substrates in specific in-vivo environments.

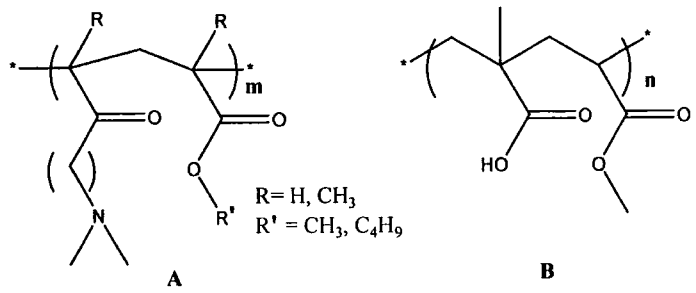


Figure 1.5: *(A) Typical cationic copolymer (B) Typical anionic polymer used in drug release*[86]

Block copolymers of amino acids have been designed to increase the residence time at the active site of hydrophilic drugs, and can be designed to respond to a thermally induced stimulus (controlled release). The controlled release capabilities (over a period of one month) of Fmoc-L-tryptophan-Boc conjugation with a ABA triblock copolymer (of lactic acid and glycolic acid, PLGA) was investigated by Park and co-workers[87] for PLGA microspheres.

In recent years, more polymers specially designed for medical applications have entered the arena of controlled release.

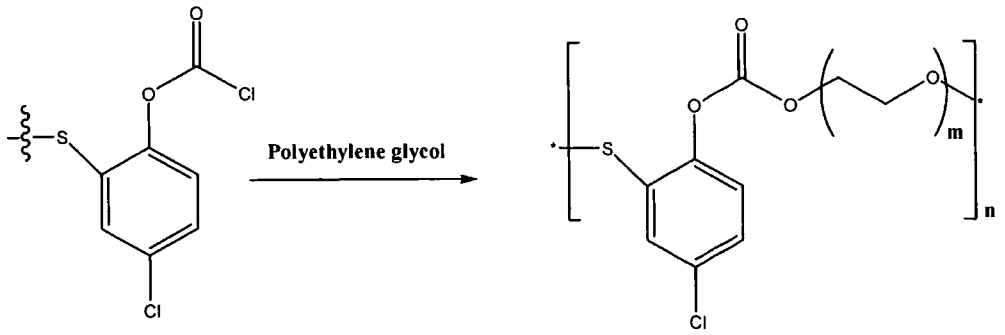


Figure 1.6. *Polycarbonate of bithionol with poly (ethylene) glycol as spacer group*

Many of these materials are designed to degrade within the body. Some are based on polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides and polyorthoesters. Vogl and co workers[88,89] have succesfully modulated the solubility of bithionol (Figure 1.6) by conjugating an alternating copolycarbonate of bithionol and poly (ethylene glycol) of molecular weight 4000 with an inherent viscosity of more than 1.2 dl/g. Alternating copolymers of bithionol (antibacterial agent) and other hindered bisphenols have been synthesised with an antioxidant in the same polymer chain. These polymers have the potential to act as very useful materials for the controlled release of bithionol (bactericide), as they undergo degradation under physiological conditions by hydrolysis.

1.4.5 Targeted Delivery

Delivery systems for targeting fall into two basic categories: drug conjugate systems, in which individual drug molecules are chemically modified to target them directly to the site of action; and carrier-based systems, in which the drug or gene is first packaged non-covalently into a synthetic carrier that is then targeted to the tumour. In both cases, the objective is to maximise exposure of the target cells to the drug yet minimise side effects that may result from non-specific toxicity in normal tissues.

The use of polymeric micelles for targeted delivery has been widely studied. Targeted delivery can often be achieved by controlling particle

size and incorporating biological, chemical or biochemical selectivity mechanisms. As a result, the conjugate should show specificity. When a polymer carrier, conjugated to an active drug, is further modified with a natural polymer (e.g. polysaccharides and proteins) with known and specific properties (including recognition sites) as a targeting component, thereby rendering the drug inactive elsewhere in the body, the result should be a site directed (targeted) delivery system.

Active intracellular targeting systems use protein transduction, a recently developed technique that enables proteins to be directly introduced into cells. The substrate (e.g. peptides, small proteins, full-length enzymes, DNA oligomers, peptide-nucleic acid oligomers, liposomes, and magnetic nanoparticles) of interest can be "transduced" to any type of cell and will rapidly enter each cell so that every cell will contain an equivalent amount of transduced protein. Protein transduction-based approaches circumvent many of the obstacles associated with gene therapy. It is not difficult to envision treatments for cancer, other infectious diseases, genetic diseases and the degenerative diseases of ageing arising from this new technique. Recent examples of the range of potential applications have been reviewed by Kendrick and co-workers[90]and several cell-penetrating peptides that enable the intracellular delivery of polar, biologically active compounds in vitro and in vivo have been described[91].

The use of ionic polymers for protein transduction via an ionic interaction of specially defined molecules (e.g. sialic acids) with cell membranes has also generated interest. Most importantly, such systems are capable of internalising hydrophilic cargoes by a receptor-independent mechanism much like the natural equivalent. Polysaccharides are valuable in the recognition by proteins called 'lectins' on cell surfaces; hence, polymers incorporating sugar residues (for example) have been investigated[92] as practical components of synthetic target delivery vehicles that may promote i.e. gene transfer independently of the classical endocytic pathway.[93,94.]

1.5 Bioconjugate Assembly (Peptide Conjugation)

1.5.1 Strategy for Conjugate Assembly

In general, bioconjugation is often performed by covalently reacting biological moieties with preformed polymer macromolecules, after activation or assisted by traditional coupling reagents (DCC, PyBOP *etc*) or by physical interaction (i.e. Van der Waals interaction). However, various other strategies[95] for conjugation culminating in more efficient release mechanism of material are employed in the design of conjugates.

In contrast to the synthesis of low molecular weight natural products and drugs, which is aided by a plethora of specific reactions and protecting group chemistry, site-specific modification of unprotected peptides and proteins to prepare well-defined bioconjugates is more challenging.

The demands placed on the synthesis of various conjugates are influenced by their intended end use, as extremely stringent requirements on the purity and homogeneity apply to the bioconjugates especially those intended for use as intravenous polymeric drugs. However, the nature of the linkage between the components of conjugates has often been critically important in determining the efficacy of the resulting drug and indeed the success of conjugation for both random site or site-specific conjugation.

For site-specific conjugation, it is desired that only the intended site in a peptide or protein be modified in the presence of other functional groups, thus leading to the formation of a well-defined bioconjugate aided by the different available methods and reagents (see Table 1). This is generally achieved by providing favourable covalent conjugation conditions (usually pH or temperature) for respective functional groups, for thiol (cysteine), which is 100 times more reactive at pH less than 6 than N-terminal amine or indeed pendant lysine amine.

Although degradable substrates (i.e. peptide) sequences are often incorporated, drug release from macromolecules in many cases is highly inefficient, because the polymeric structure sterically hinders the drug-

cleavage mechanism. The above problems of site-specific conjugation and inefficient release mechanism have prompted an increased interest in the use of linkers as a simple but effective medium to enhance efficacy. There are two general methods for conjugating macromolecules:

a) Traditional approach – This process often does not require the use of linkers but traditional activating/coupling agents (i.e. carbodiimide[96]) to form carbonates or carbamates[97]. Such conjugation approaches to macromolecule synthesis often results in multiple bonds and by-product formation, a process that can lead to diminished potency of the final bioconjugate[98], a problem further exacerbated by the fact that the site of modification of substrates often dramatically affects pharmacokinetics[99]. A well-defined structure will be difficult but not impossible to obtain, often only after further purification.

b) Linker/Spacer approach - Macromolecules are sterically demanding, and ease of accessibility to target sites either for cleavage or conjugation is a prerequisite for efficient drug release. The use of linkers has been shown to be suitable for creating effective biomacromolecule-drug conjugates, which ensure efficient drug release characteristics. In the design of a suitable linker it has to be taken into account that conjugation to active sites (which are often located well below the surface of the required bio-macromolecule, due to secondary interactions, for example) may require the use of a linker with an appropriate spacer length and compatibility to discourage unfavourable interactions with the recognition moiety, while at the same time providing direct access to the intended conjugation functional group.

The use of linkers has now become a prerequisite for efficient conjugation of chemotherapeutics (where high molecular weight natural macromolecules are prevalent i.e. Bovine Serum Albumin and Immunoglobulin).

While chemotherapeutics and macromolecules can be designed to exercise reasonable tolerance using various recent polymerisation techniques, a sizeable number of such polymer bioconjugate drugs often display reduced potency for reasons that can be attributed to a less than efficient conjugation and release mechanism of the potent drug, sometimes highly influenced by the type, length and function of the linker/spacer used[100]. Kruger *et al*[101] have already described the influence of an appropriately chosen linker length in a complex system. They found that potency was dependent on the spacer length used to conjugate receptor binding domains and synthetic bivalent antagonists of adrenocorticotropic hormone (ACTH), synthesised by coupling the terminal carbonyl with the cysteine of synthetic ACTH to form a peptide-spacer-cysteine conjugate.

Further studies by other research groups have also shown that efficacy of chemotherapeutic drugs, for example Calicheamicin[102,103], an anticancer agent, already approved by US Food and Drug Administration (FDA) can be improved on, mainly by appropriate choice and design of linker unit[104].

1.5.2 Biological Hetero-bifunctional Reagents.

1.5.2.1 Types and Examples of Linker Units

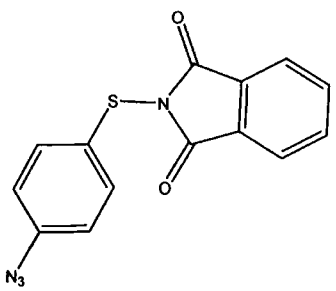
By definition "linkers" are functional agents consisting of a spacer (cleavable or non-cleavable) and two reactive end groups. These reactive groups are required for direct, facile and selective access to functional groups for anchoring two different entities (e.g. proteins, polymers and surfaces). For biomedical applications, linkers with the ability to site selectively release compounds (e.g. drug, polymer or peptide) usually by hydrolysis from a conjugated polymer *via* predetermined stimuli (or stimulus) in an aqueous environment and predetermined conditions have generated greater application.

In vivo, this property can be utilised in the controlled release of drugs for treating various ailments or *in vitro*, to study surface receptors of cell culture as a model system[105]. The use of cleavable linkers has been widely reported in various capacities. However for cancer chemotherapy the advantages are more readily exploited. Kukis *et al*[106] reported an increase in the tumour-to-liver and tumour-to-kidney radiation dose ratios in preclinical studies of radiolabelled proteins conjugated to antibodies, designed for cleavage in the liver and kidney.

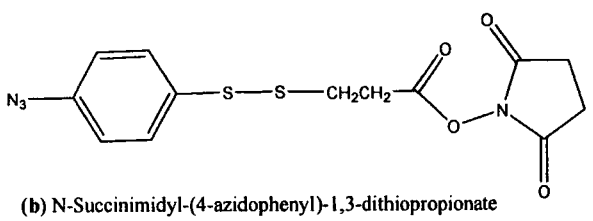
To crosslink biological (i.e. protein or antigens[107]) macromolecules, it is necessary to use a bifunctional reagent, which has two functional groups linked by a spacer arm. These reagents fall into two classes:

1) Homo-bifunctional with two identical functional groups.
2) Hetero-bifunctional with two different functional groups.

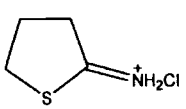
Bifunctional reagents can have varying lengths of spacer and different reactive side chains on a protein. The most useful reagents are those that possess a cleavable crosslinker to regenerate the subunits, often by incorporating disulphide links, which can be cleaved by introducing a reducing environment or hydrazine - cleavable in acidic environment.



(a) N-(4-azidopheny)thiopthalimide



(b) N-Succinimidyl-(4-azidophenyl)-1,3-dithiopropionate



(C) 2-Iminothiolane

Figure 1.7. *Example of functional reagents:* ***a****, Mono functional cysteine specific reagent.* ***b****, Bifunctional cysteine/lysine specific reagent.* ***c****, Traut's reagent*[108,109] *Hetero-bifunctional linker that converts the terminal amine of lysine into a thiol derivative*

Spacers are other vital components of a good linker as the linkage can be designed to control where and when substrates are released. Some of the shorter linkers are simple to prepare but highly prone to unfavourable interaction of the conjugated macromolecules.

However, the use of polyethylene glycol (PEG[110]) and other synthetic polymeric carriers has recently been realised as almost essential, as more and more PEG-conjugates perform better in clinical trials.

Other advantages of PEG in bioconjugation also include:

- Non reactive (relatively) and easily functionalised end groups
- Cheap and commercially available
- Water soluble, very stable and can be modified to be cleavable.
- Renal clearance highly dependent on molecular weight (tuneable property)
- High molecular weight – *in vivo* degradation by-products are non-toxic
- FDA approved, already been used in food and drugs.

In spite of all the advantages offered by both PEG and ATRP (especially their high tolerance to other functional groups), very few ATRP systems have utilised or exploited the concept of hetero-bifunctional linkers as initiators for synthesis of biologically active polymer conjugates to establish a protocol for large-scale synthesis of peptide/protein polymer bioconjugates. It was therefore envisaged that an ideal system would consist of bifunctional linkers to take advantage of living polymerisation and bioconjugation.

- A PEG Linker to enable tuneable biological interactions and aid hydrophilicity and reduce unfavourable macromolecular interaction by reducing steric interaction[111] but encouraging aggregation where and when expected of the bioconjugate

- Two functional groups: Archetype alkyl halide for living polymerisation and a stable functional group for sequence conjugation to ($-NH_2$) or (-SH) of RGD-based peptide or protein (Human Serum Albumin) (see Table 1).

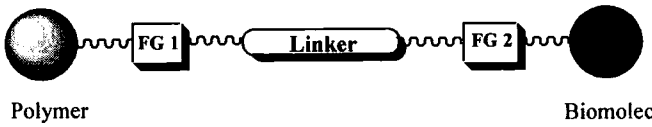


Figure 1.8. *A representative structure typical of the proposed hetero-bifunctional linker conjugated system*

The above system would be synthesised and tested under physiological conditions with the hope of being utilised in biological systems and, to allow for large-scale synthesis, by incorporating a versatile and robust macromolecule synthesis method, i.e. living polymerisation.

1.6 Living Radical Polymerisation and Macromolecules.

1.6.1 Macromolecules as Drug Carriers

The discovery of the ability of macromolecules to localise to subcellular compartments known as lysosomes heralded their evolution as drug carriers[112]. In 1975, Ringsdorf formulated the rationale for targetable polymeric drug delivery based on pharmacologically active polymers[1]. Since then, polymers of natural and synthetic origin have been widely used for the delivery of low and high molecular weight drugs further enhanced by (for instance), the research by R. Duncan *et al* (1984) into the use of synthetic polymers as potential drug carriers to offset the extremely rapid biodegradation, immunogenicity and instability towards modification with drugs of naturally occurring polymers (e.g., oligosaccharides, proteins)

In a polymeric drug delivery system, the drug is generally but not exclusively included either as a unit of the polymer backbone or as a pendant group, part of the carrier side chains. The drug can be introduced by either copolymerization with a monomeric drug derivative (e.g., N-(4-aminobenzensulfonyl)butylurea), or by a polymer-analogous reaction with side chains carrying functionalised groups, such as active esters. Intended for synthetic polymers, such as polyethylene glycol (PEG) and biodegradable poly(l-glutamic acid) (PGA), which are currently being evaluated in clinical trials, e.g. Pegasys-branched PEG-IFN-a2a, have been extensively reviewed[113,114,115].

The synthesis of novel synthetic material with improved properties and functionalities is an ever-expanding frontier in science. The increased interest in the synthesis of functionalised macromolecules can be attributed to the recent advances in polymerisation techniques and the need for desirable polymer composites and conjugates of high dispersibilty in aqueous systems, and which are highly uniform (in size and composition) and reproducible.

A notable advance was made in 1995, when Percec *et al*[116], Matyjaszewski and Sawamoto independently reported the use of controlled/living radical polymerisation (instead of anionic, cationic, co-ordination or ring opening methodology) to design different architectural polymers, and the subsequent synthesis of an increasingly large number of functional synthetic polymers with characteristics similar to natural polymers both in complexity and function (recent reviews[117,118,119]). Chemists can therefore - using the chemistry established by Matyjaszewski, Sawamoto and Percec - design macromolecules and combine several synthetic macromolecules (conjugation) of predictable properties to suit the specific field of research.

While the developments in polymerisation chemistry may have been brought about by the need to synthesise novel biologically responsive macromolecules, the need to overcome certain intrinsic drawbacks (e.g. solubility, cytotoxicity and low bio-availability) and the availability of polymerisation method(s) for well defined polymers, which can mimic natural and improve on synthetic polymers, that have revolutionised the polymerisation process and the chemistry. However, most research conducted on the mechanism and kinetic studies of living polymerisation in contrast with conventional radical polymerisation, was inconclusive in determining the conventional nature[120,121]of the living process (e.g. similar rate constant, tacticity, region, stereo and chemoselectivity). The indirect/direct influence of the "persistent radical effect" (PRE)[122,123,124] in living radical polymerisation as the predominant (a non self terminating by-product) side reaction may also play a vital role in distinguishing conventional from living polymerisation.

The development of the living radical polymerisation technique has led to improved polymer technology and to the efficient production of different polymer architectures with applications in various areas of research.

1.6.2 Living Radical Polymerisation - Classification.

Despite the simplicity, robustness and mechanistic similarities of conventional polymerisation - which consequently led to the wide industrial usage of conventional radical polymerisation techniques - the resulting polymers of ill-defined structure, unpredictable molecular weight distribution, all directly linked to lack of control over the termination step (by combination or disproportionation) in conventional polymerisation has helped encourage the fostering of more efficient alternative techniques.

Other traditional polymerisation techniques (ionic[125] and group transfer) have also played vital roles in industry, but exercise limited monomer compatibility and the need for stringent conditions[126,127](Figure 1.9). This has possibly hindered advances in novel polymer products and other areas for which collaboration with polymer chemistry is now vital (i.e. synthetic natural polymers and biopolymer science). The last few decades have seen the rapid growth in the development and understanding of other techniques, especially controlled/living radical polymerisation.

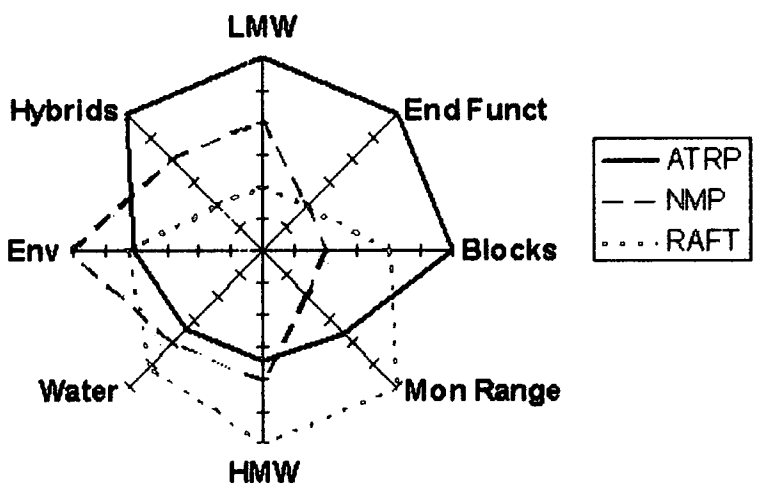
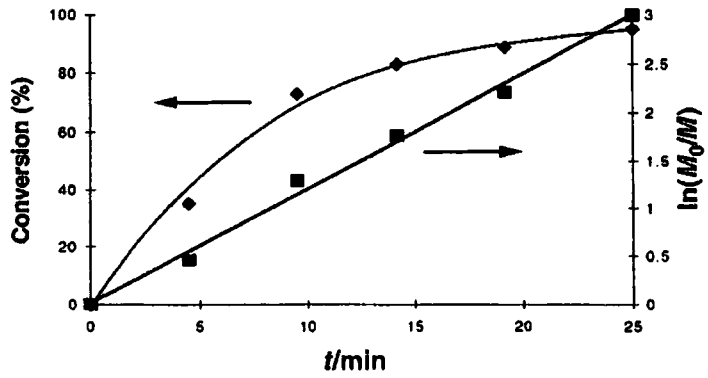


Figure 1.9: *Schematic shows one interpretation of the relative advantages and limitations of ATRP, NMP and RAFT as applied to the synthesis of low (LMW) and high molecular weight polymers (HMW), range of polymerisable monomers (Mon Range), preparation of block copolymers (Blocks), end-functional polymers (End Funct), Hybrids , aqueous systems (Water) and some environmental issues (Env).* http://www.chem.cmu.edu/groups/maty/crp_2001-2005/

The major mechanistic difference between conventional and controlled radical polymerisation is the lifetime of the propagating radical species during the polymerisation process – attributed to the dynamic equilibrium created between the active and inactive radical species in conjunction with a radical moderator, which has the net effect of prolonged radical half-life of up to hours for a typical living polymerisation system. This is unlike the conventional process where the propagating radicals merely persist for seconds after the initial radical initiator decomposition-propagation step resulting in a lack of control over the polymerisation process and its polymer product. A living process of an efficient polymerisation possesses unique and investigated characteristics[128] (experimentally or mechanistically) easily observed in order to predict the progress or the outcome of a typical polymerisation i.e.

- Narrow molar mass distribution (Mw/Mn < 1.1-1.3[128])

- Linear plot of ln[1/(1-x)] vs. time through the origin. Where x=conversion. Indicative of constant concentration of active species.

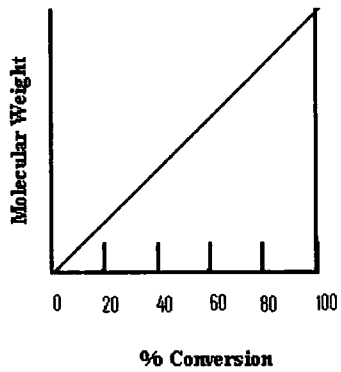


- Predictable polymer molar mass/degree of polymerisation.

$$DP = [M]_0/[initiator]_0 \times conversion$$

$[M]_0$ = initial concentration of monomer

- Linear plot of Mn vs conversion through the origin[129].



While the favoured system for polymerisation depends highly on the requirements of the resulting polymer and the capabilities of such techniques (see Figure 1.9, page 30), the types of "living" system generated mainly depend on the type of radical transfer agent used to assist or maintain the equilibrium generated (i.e. nitroxide or transition metal) of the living polymerisation, techniques that can be described (with general mechanism) as:

- Stable free radical polymerisation (SFRP) e.g. Nitroxide-mediated radical process (NMRP)

$$P\text{-}X \underset{K_d}{\overset{K_a}{\rightleftharpoons}} P^* + X^* \qquad \textit{Dissociation- Combination}$$

- Degenerative transfer e.g. Reversible addition-fragmentation chain transfer (RAFT)

$$P\text{-}X + Z^* \underset{K_d}{\overset{K_a}{\rightleftharpoons}} P^* + X\text{-}Z \qquad \textit{Degenerative Transfer}$$

- Metal-mediated atom transfer radical polymerisation (ATRP).

$$P\text{-}X + Y \underset{K_d}{\overset{K_a}{\rightleftharpoons}} P^* + XY^* \qquad \textit{Atom Transfer}$$

They are easily differentiated by the choice of capping agent (radical moderator) employed to maintain the reversible activation process of a typical living "equilibrium" system.

1.6.3 Nitroxide-Mediated Radical Polymerisation (NMRP)

SFRP processes are conducted under polymerisation conditions comprising a radical source (which can be conventional i.e. iodine or AIBN), a stable free radical (alkoxyamine-based radical regulator, see Fig 1.10 and 1.11) and at an appropriate temperature to provide the effective exchange between the active and dormant species. The radical source and the stable free radical may be combined in one species. NMRP processes are typical of SFRP processes. NMRP processes include the initiation of the polymerisation in the presence of a nitroxide, such as, 2,2,6,6-tetramethyl-1-piperidinyloxyl ("TEMPO")[130], or its derivatives (Figure 1.11A, B-D), or phosphorus-containing nitroxides (designed by Gnanou *et.al*[131]), for acrylate polymerisation, or may employ a preformed alkoxyamine as the radical source/control agent. The most popular nitroxide used for NMRP in the past has been TEMPO. However, TEMPO is limited in the range of compatible monomers, mostly due to the stability of the radical. Hawker *et. al*[132] discovered that by replacing the α-tertiary carbon atom with a secondary carbon atom, the stability of the nitroxide radical decreased which lead to an increased effectiveness in polymerisation for

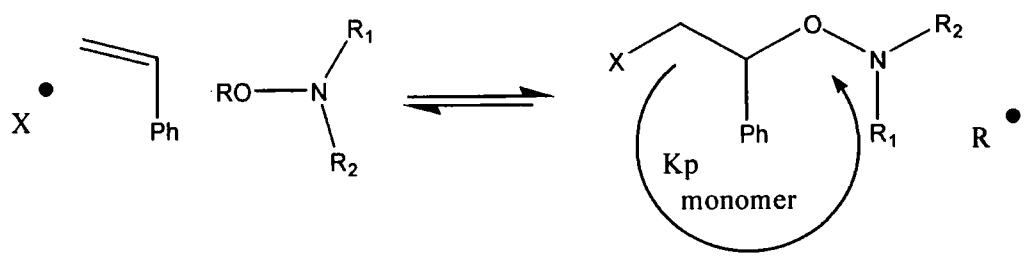


Figure 1.10. *General mechanism for NMRP*

many monomers in which TEMPO was inefficient. This had the net effect of increasing the range of moderator (hence reduced operating temperature), and monomer (from styrene only to various acrylate, acrylamide and acrylonitrile).

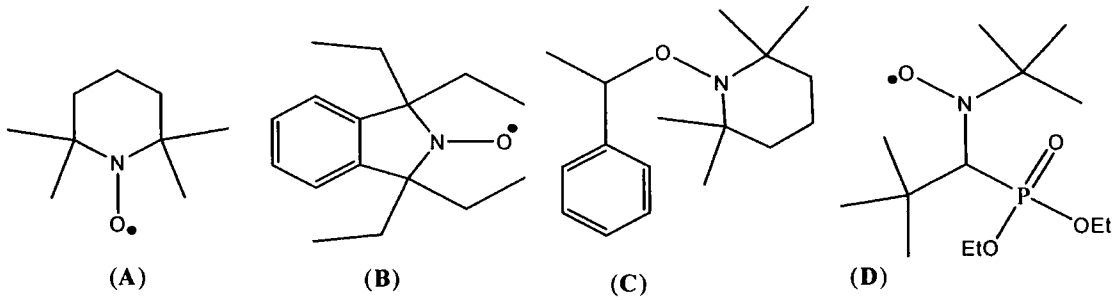


Figure 1.11. *Polymerisation mediators for use in Nitroxide mediated radical polymerisation.*

The use of radical stabilizers (i.e. nitroxide and its derivatives) ensures that irreversible terminations and PRE are suppressed while the equilibrium can also be activated and propagated (Kp) to maintain the essence of living polymerisation.

Unfortunately, thermally induced polymerisation, especially for a bimolecular approach using TEMPO, also means high temperatures are often required (>60°C) for the initial dissociation to release nitroxide, a highly unfavourable condition for large-scale industrial usage or biological substrates. However, the choice of nitroxide moderator especially of the second and third generation alicyclic TEMPO design has permitted the use of a wider range of monomer with moderate or better control of the resulting polymer. The process requires stringent conditions and the choice of monomers is still comparatively restricted.

1.6.4 Reversible Addition-Fragmentation Transfer (RAFT)

Degenerative transfer or RAFT[133,134,135] is the newest LRP technique, developed in 1998. RAFT polymerisation conditions allow the polymerisation of a wide range of monomers including functional monomers, such as, but not limited to, styrenes, acrylates, methacrylates, acrylic cid, DMAEMA, and HEMA to give functional polymers with a wide range of architectures, including block, star, and gradient polymers.

The mechanism follows the addition of thioesters, (the efficient reversible addition-fragmentation chain transfer agent) to conventional free-radical polymerisation, subsequently; addition-fragmentation of the propagating

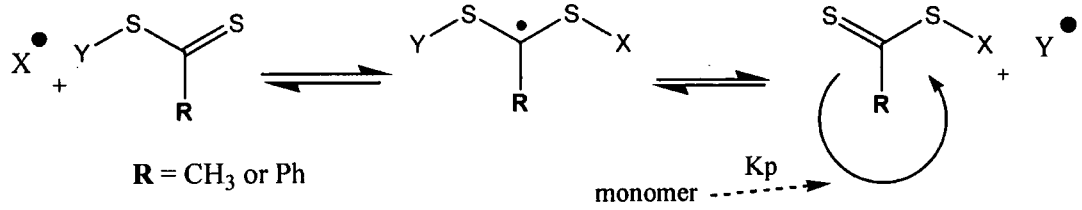


Figure 1.12: *General mechanism for RAFT*

carbon-centred radicals, which is reversibly stabilised by the dithioester, and generates the active radical species. The reaction of thioesters with initiator-radical species (fast trapping) is faster than the rate of propagation and the fragmentation process that generates the active radical species, hence, suppressing bimolecular termination.

In RAFT polymerisation, the molecular weight is predetermined by the ratio of consumed monomer to the consumed initiator and the quantity of added transfer agent. While the monomer compatibility and the operating temperature (approx. 60°C) for RAFT are considerably better than for NMRP, the reported work of McCormick et al discussed the use of RAFT

as a viable technique for the synthesis of acryloamido water soluble polymers since ATRP failed to give controlled molecular weights for acrylamides, and NMP methods are usually run in organic solvents with nonionic monomers, RAFT was the only valid technique for these types of systems[136,137]

However, there are limitations. For example: -

1. Some of the RAFT agent can be relatively difficult to synthesise
2. Reactions can be slow
3. The process involves sulphur compounds, bad odour.
4. Polymers tend to be coloured.
5. Efficient (fast) fragmentation i.e. good choice of thioesters is essential
6. The end-group (i.e. thioether) after polymerisation tends to lead to toxicity and displacement usually requires further polymerisation.

1.7 Atom Transfer Radical Polymerisation (ATRP[138,139, 140])

1.7.1 Introduction

The performance of any polymer is largely determined by the composition and architecture (e.g. micelle forming block copolymer). Therefore the ability of any living polymerisation system to afford the wide variety of scientifically interesting materials is highly dependent on the ease of access to sophisticated architecture. The process can easily be approached by using living radical polymerisation to directly effect the:

- Copolymerisation of new material with predictable properties (e.g. micelle-forming block copolymer)
- Accommodation of novel initiators/monomer to facilitate new architecture (e.g. functionalised[141] or bio-macroinitiators)
- Control over the architecture (e.g. grafted or statistical copolymer)
- Post-polymerisation (e.g. with functional groups)

1.7.2 Mechanism

ATRP is more complex than other living polymerisation method because it involves a complex, often heterogeneous catalytic system. The solubility and structure of the catalyst in solution may change not only the catalyst composition but also the compatibility of each monomer, solvent, and temperature conditions with the process of living polymerisation. Various groups have studied (recently Fischer 2001) the kinetics and mechanics[142] involved to understand the fundamental

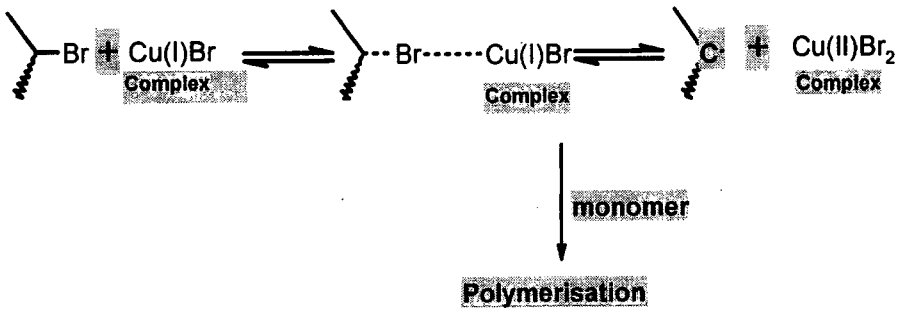


Figure 1.13: *A general schematic mechanism of ATRP*

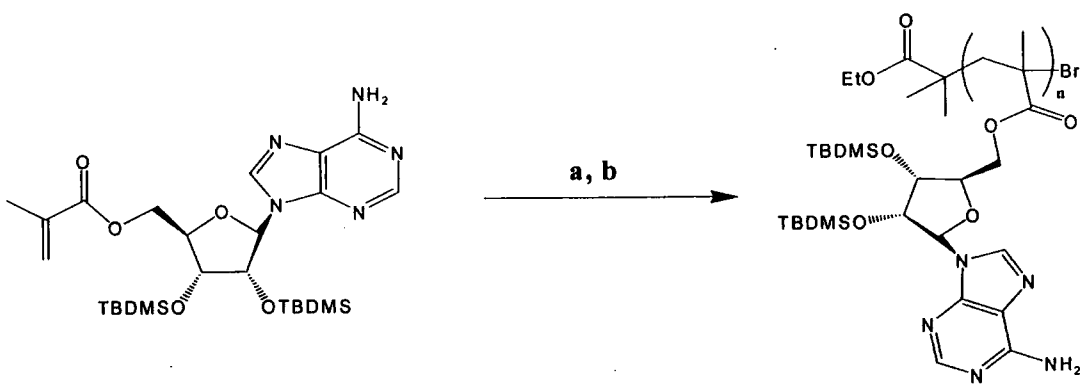
chemistry, scope, and limitations of ATRP (living polymerisation and PRE[143]), which has formed the basis for the rapid advance in the development of the technique. They showed that it follows first-order kinetics with respect to monomer concentration, indicating a living process. Fakuda *et al*[144] also recently produced a more comprehensive literature based on an experimental and theoretical approach to the study of the kinetics of living radical polymerisation. ATRP is based on the ability of the metal complex to efficiently maintain the radical species' transformation and concentration via a fast and reversible redox from active to dormant (and *vice versa*) at any given time and temperature

1.7.3 Components and their Influence on ATRP.

ATRP is probably the most versatile living radical polymerisation technique that can easily afford all of the above conditions. It is the most conducive to producing biopolymers with biomedical applications by virtue of its tolerance to different monomers and homopolymer properties.

1.7.3.1 Monomers

The prerequisite for a plausible monomer is the ability to produce the active radical species fast from the capped growing polymer chain



a. CuBr, Toluene, TREN **b.** Bromoisobutyric ethyl ester

No of monomer (**Mn**) = 3,400; Molecular Weight (**Mw**) = 4,600;
Polydispersity (**PD**) = 1.35

Figure 1.14:.An example of adenosine derived monomer used in living polymerisation developed by Andrew Marsh, Warwick[162]

effected by using a phenyl or carbonyl (stabilising) alkyl halide adjacent to a carbon radical complexed to a metal centre. This process is, however, less accessible in systems like acrylates (in general), except dienes and carboxylic acids as they possess acidic protons, which can lead to poisoning of the metal catalyst[145]. However, the ATRP of MMA using 2-bromoisobutyric acid has been demonstrated[146]. With the appropriate monomer (acrylate or methacrylate) regardless of the tandem structures (except acrylamido *etc*) for example, nucleoside (Figure 1.14) or PEG, can undergo polymerisation.

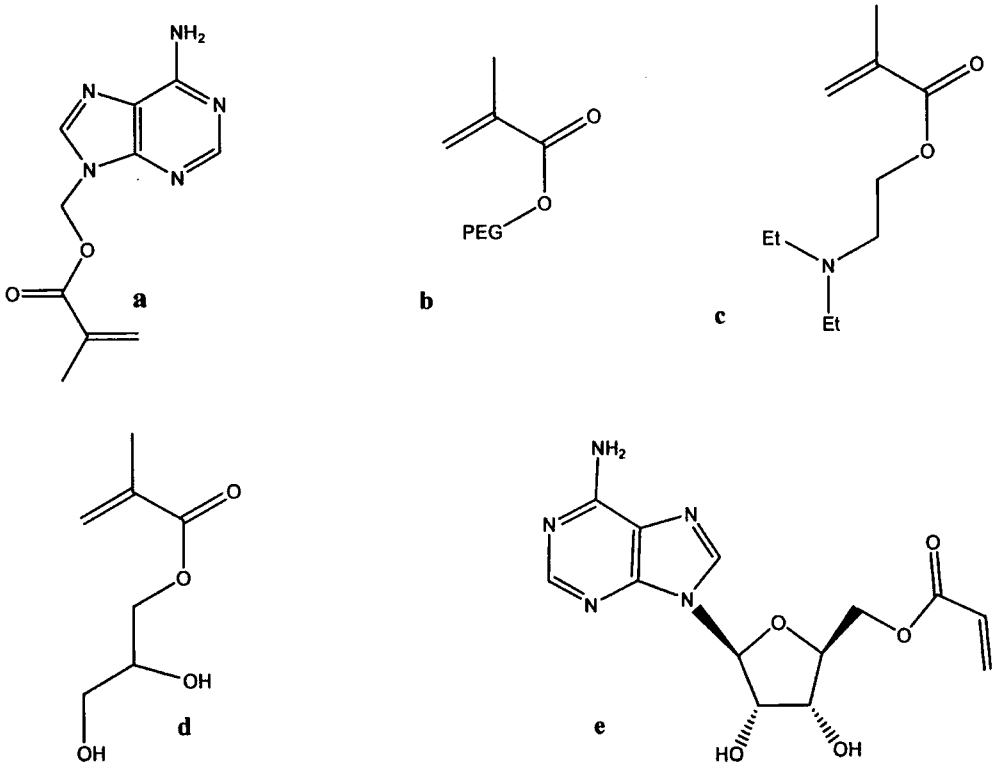


Figure 1.15. *Example of functional monomers in ATRP.* ***a.*** *Adenine methyl methacrylates,* ***b.*** *Poly(ethyleneglycol) methacrylate,* ***c.*** *(Diethylamino)ethyl methacrylate,* ***d.*** *Dihydroxymethylmethacrylate and* ***e.*** *Adenosine acrylate.*

The practical advantages of ATRP can be ascribed to:

- The ability to accommodate many varied monomer structures and functionalities to create functional polymers. These monomers include methacrylates[147](examples, Figure 1.15 **a-d**), acrylates[148,149] (Figure 1.15**e**), styrenes[150,151,152], acrylonitrile[153], hydroxyls, amines, and ethers.
- Operational at temperatures as low as room temperature[154].
- It can be carried out in the presence of water[155,156] or other protogenic[147] reagents.
- Its livingness can lead to novel polymer architectures (block copolymers *etc*)

Most significant is the ability to optimise and modify any of the reagents to suit the purpose of polymerisation (i.e. the design of novel materials) and the efficiency of the systems. The optimal operational conditions that best satisfy a specific atom transfer radical polymerisation system are affected by various factors, such as reagents, solvents systems and catalyst. Careful studies have been carried out on the roles of the components in the polymerisation process. It is known that, for example, while various monomer/initiator combinations have been used successfully in ATRP, each monomer exhibits particular rate behaviour even at the same reaction conditions.

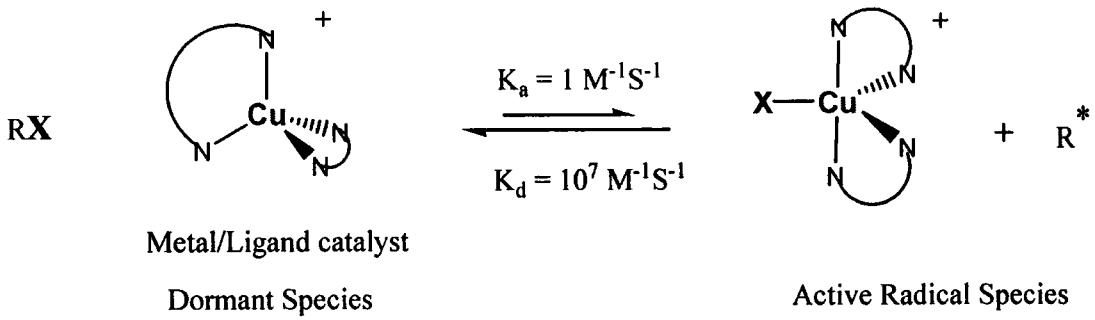


Figure 1.16. *Showing the equilibrium between dormant and active system*

As a result, only components with a predictable and manageable influence are manipulated to ensure efficient polymerisation, which often

involves the transition metal catalyst. Perhaps the more interesting component for the purpose of this project and as a synthetic chemist (in general) is the ease of manipulation and the range of initiators applicable in producing biopolymers.

1.7.3.2 Catalysts (transition metals) and Initiators

ATRP utilises a transition metal as radical initiator *via* a halogen transfer (usually bromide) cycle; deactivation of the chain is by reversible transfer of the halogen, and reactivation by halogen abstraction.

The inherent stability of a carbon halide initiator helps to control the equilibrium constants involving the initiation and propagation step on which the concept of "livingness" depend with the basic requirement for a transition metal catalyst for use in ATRP being the access to an empty d-orbital, the ability to accommodate coordination expansion to form a stable complex with ligands and display no Lewis acid characteristics. While a number of group VI – X transition metal catalysts have been applied in ATRP (they include, molybdenum, chromium, rhenium, ruthenium, iron, rhodium, nickel, palladium and copper), copper catalysts have been the most successful and widely used because of their relative low cost and versatility.

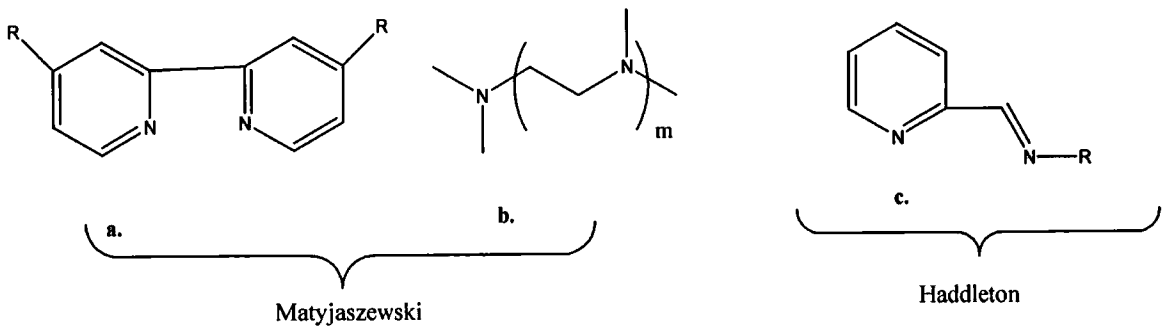


Figure 1.17. ***a.*** *Substituted bipy and* ***b.*** *TREN ligands developed by Matyjaszewski.* ***c.*** *Pyridyl methanimine ligand developed by Handdleton for use in ATRP.*

An efficient catalyst (for ATRP) depends on the use of an appropriate ligand to give the complex a more reducing property and therefore make

it more active. The ligands help to form soluble catalysts, which greatly increases the rate of polymerisation while maintaining good control.
These Schiff base ligands are easily prepared, amine-based, bi-or multidentate, which lends itself to modification by changing the **R** group as required (Figure 1.17. **a-c**).

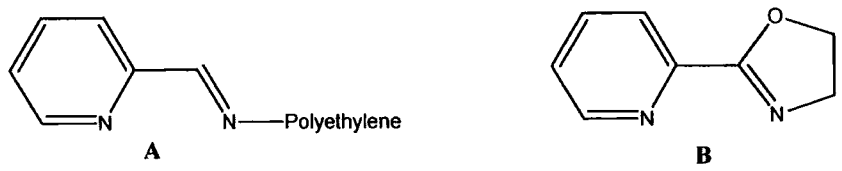


Figure 1.18

The disadvantages of using these catalysts are due to the catalyst not being often recycled, and copper contamination in products does occur. Some publications have shown the removal of contaminant effectively performed with the use of the polyethylene-segmented pyridinimine ligand (**A**) in the polymerisation of methylmethacrylate and CuBr[157]. Similarly, 2-(2'-pyridyl)-4,5-dihydro-oxazole (**B**) in the CuCl-mediated polymerisation of methacrylates[158]

1.7.3.3 Initiators

Living polymerisations are well known for their ability to accommodate various functional groups. Hence, by using specifically derivatised initiators, living polymerisation can produce functionalised[159] end-group polymers tailored to particular requirements, and therefore offers one of the best routes to biologically active conjugates. For example, polymeric vectors for the delivery of bio-agents (bioconjugates) or the delivery of oligonucleotide conjugates and the synthesis of polymers coupled directly to drugs and pro-drugs (therapeutic agents) or simply functionalised with antibiotics, cholesterol, sugars and other biologically important functionalities, have received increased interest as means of generating structures capable of recognition, binding and intelligent delivery of

materials, hence directly affecting drug administration[160], methodology and increasing the pool of research tools.

While any alkyl halide can potentially be an initiator provided it can reasonably stabilise the radical species to minimise side reactions, the need for copolymerisation and polymerisation of functional macromolecules meant that alkyl halides have been substituted (using synthetic organic chemistry) with more useful functionalities like mono and oligosaccharides, cholesterol, benzylic halides, nucleosides, miscellaneous initiators and dendritic initiators for ATRP (Figure 1.19 **a-h**). Haddleton et al[161] successfully transformed β-cyclodextrin into an initiator for polymerisation with various methacrylate-based monomers

The main role of the initiator is to determine the number of propagating polymer species with the initial concentration of initiator in a living polymerisation determining the theoretical molecular weight or degree of polymerisation (DP) which increases reciprocally. If initiation is fast and transfer and termination negligible, then the number of growing chains is constant and equal to the initial initiator concentration.

$$DP = [M]_0/[\text{initiator}]_0 \times \text{conversion}$$

$$[M]_0 = \text{initial concentration of monomer}$$

In ATRP, alkyl halides are typically used as the initiator and the rate of the polymerization is first order with respect to the concentration of alkyl halides. To obtain well defined polymers with narrow molecular weight distributions, the halide group must rapidly and selectively migrate between the growing chain and the transition-metal complex. Thus far, when halide is either bromine or chlorine, the molecular weight control is the best. Iodine works well for acrylate polymerisations in copper-mediated ATRP and has been found to lead to controlled polymerisation of styrene in rhenium-based ATRP.

The use of macroinitiators (in-situ or isolated) is crucial in the synthesis of block copolymers of various topologies or composition (see block copolymer synthesis).

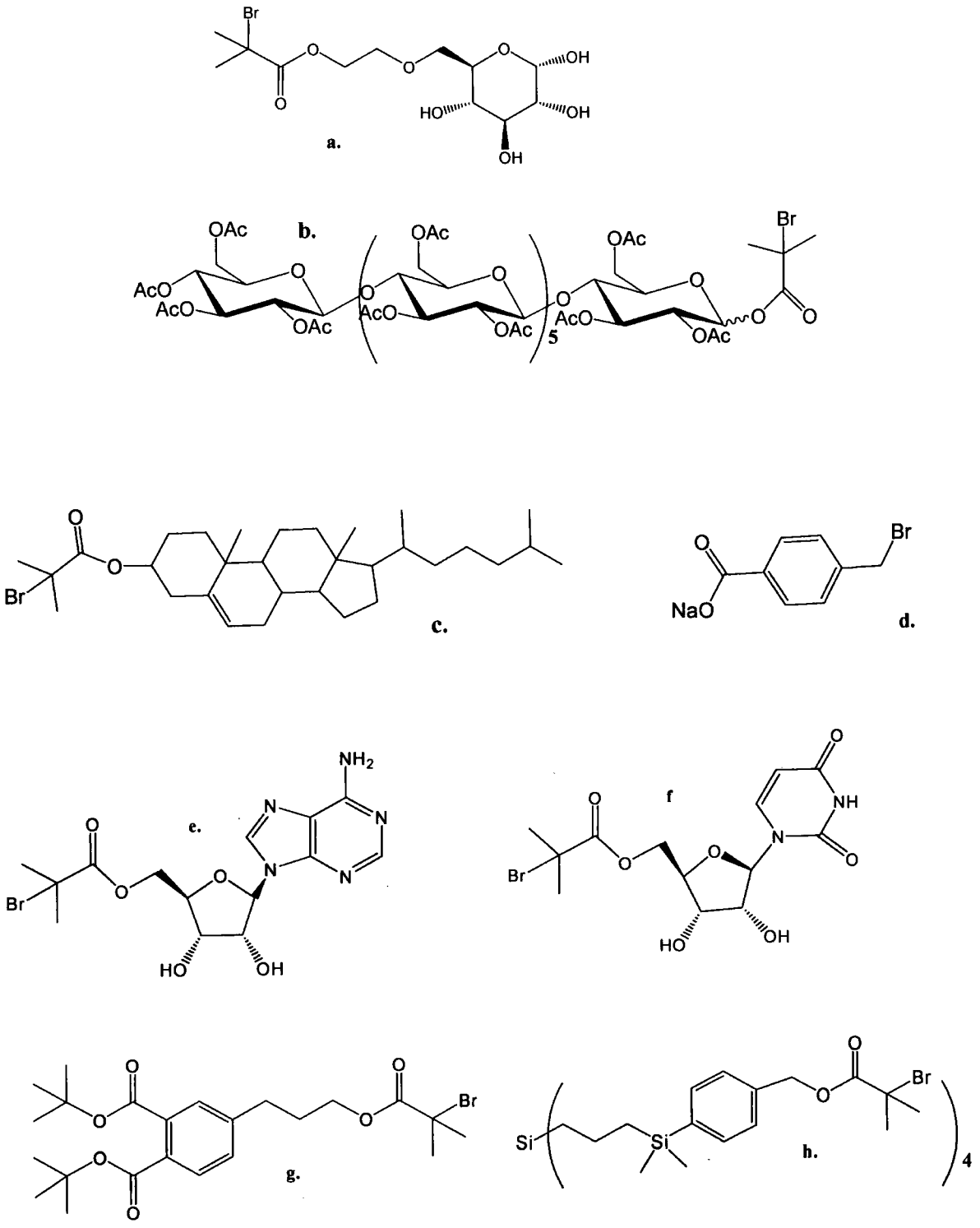


Figure 1.19: *Showing the diversity in ATRP initiators under current usage* ***a*** *.monosaccharides* [162], ***b.*** *oligosaccharides* [162], ***c.*** *cholesterol* [162], ***d.*** *benzylic halide* [154], ***e.*** *adenosine*[163], ***f.****uridine*[163], ***g.*** *miscellaneous* [164], ***h*** *dendritic* [162]

Allyl bromide as an initiator offered a narrower molecular weight distribution than the chloride counterpart in the polymerisation of styrene. This is due to the stability offered by electronic conjugation of the radical to the vinyl[165]. However, the irreversible activation of the carbon-chlorine bond in CCl_4 with $Fe(OAc)_2$ to generate the CCl_3 radical has been used to induce polymerisation of less conjugated vinyl acetate[166]. The diversity in the initiators tolerated by ATRP has helped to generate a renewed interest in areas of collaboration between polymer and biomedical scientists. The versatility of ATRP has also led to novel polymeric systems with applications in existing areas of research, as well as allowing the development of new branches of polymer science, such as the collaboration between polymer scientists and biological chemists in the development of biopolymer conjugates.

1.7.3.4 Other Factors (Solvent, Temperature)

Solution polymerisations are slower than bulk polymerisation due to the reaction order of each component except at high temperatures (90° C – 130° C) where a proportional increase in the level of side reaction can also be observed i.e. the evolution of aqueous HCl from the propagating species accompanied by unsaturated substrates and the oxidation of copper (I) to inactive copper (II) by oxidants (i.e. oxygen).

In general, protic solvents[167] can poison the catalyst. However, most chain transfer inert solvents with the ability to solvate the initiator/catalyst complex can be suitable. Some researches have also shown water to accelerate the rate[168,169] of living polymerisation. Also, in solution, the behaviour of the living process differs in both heterogeneous and homogeneous mixtures, which are in-turn greatly influenced by the ligand used to complex the metal.

1.8 Block Copolymers

Block copolymers are one of the macromolecular architectures possible from the polymerisation of monomers. They can be presented in different forms, such as di-, tri-, four-arm, graft, star-block and random multi-block copolymers.

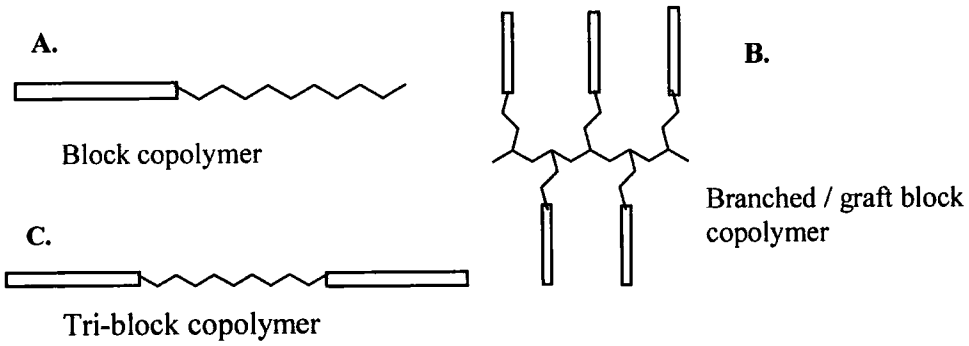


Figure 1.20. *A representation of three different polymer architecture based on block copolymers*

1.8.1 Synthesis

Block copolymers can be synthesised[170] from a monofunctionalised radical initiator (or macroinitiator) by sequential homopolymerisation of its constituent monomers. However, when bifunctional initiators are used, ABA or ABC triblock[171] copolymers are formed, the best known example being the PEO-PPO-PEO triblock copolymer (pluronics). Multifunctionalised initiators are required for multi-arm and star block copolymers[171]. Polymers with similarities to natural polymers (for example, polyelectrolytic polymers as substitute for DNA, RNA and some proteins) can be designed with this process. Zwitterionic block[172] copolymers are similar electronically to proteins and may therefore be useful as synthetic polymeric substitutes for proteins.

1.8.2 Properties of Block Copolymers

As one of the best-known smart functional block copolymers, micelles containing a specific hydrophobic block have been found to be more effective as carriers of some specific drug. Some of the other sought after properties of block copolymers are: -

- Solubilisation; The ability to solubilise an otherwise insoluble substance into an incompatible environment
- It is possible to synthesise block copolymers with high structural definition and predictable properties, using living radical polymerisation. This is useful for defining the quality and subsequent use of the polymer produced.
- Synthesis of block copolymer conjugates with dual solubility (amphipathic) and micellisation capability under predetermined biochemical conditions (e.g. pH and temperature).
- Possibilities for reverse micelles, ionic micelles and hydrophilic – hydrophobic block copolymer formation

In studies, various groups have investigated many of the physico–chemical parameters of block copolymer micelles in a very systematic way[173]. Several reviews have also (indirectly or directly) resulted from this work.

They are typically polymers that respond reversibly or irreversibly to changes in biological, physical or chemical stimuli, a property often exploited in block copolymer design (usually from hydrophilic-hydrophilic or hydrophilic – hydrophobic block copolymers) for biomedical applications.

The polybasic hydrophilic-hydrophilic (AB) block copolymer of N, N'(dimethylaminoethyl) methacrylate and N, N'(diethylaminoethyl) methacrylate has already been synthesised where the resulting polymer micellised under pH-induced condition

Two properties for characterising the phase behaviour of block copolymers in solution are micellisation and gelation.

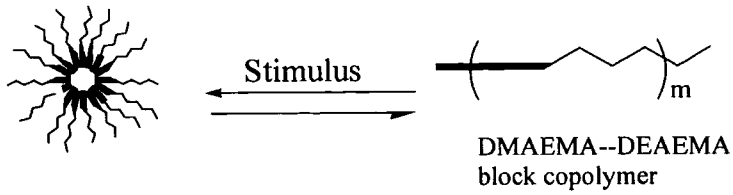


Figure 1.21. *Shows a general schematic for a smart polymer sensitive to stimulus and forming micelles (Chem. Comm., 1997,671-2)*

Upon aggregation[174] or association of block copolymers in solution beyond a certain critical point termed critical micelle concentration, micelles[175] are formed. The aggregation usually forms spherical micelles (sometimes elongated) with two distinct regions, the corona (or shell), and the hydrophobic core in aqueous solution, which can incorporate a hydrophobic substance.

Attention has been attracted by the development of unique hydrophilic-hydrophobic micelle forming combinations with possible applications in biological systems under physiological conditions. Armes and co-workers have described the properties and characterisations of micelles/ micellization in a range of copolymers.

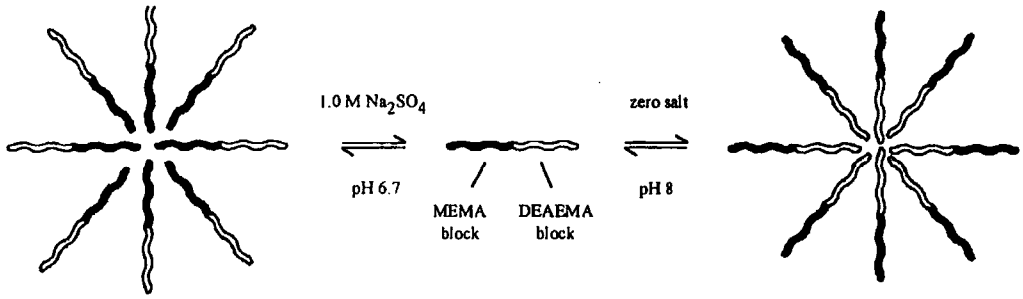


Figure 1.22: *Schematic representation of the formation of micelles and reverse micelles for an AB diblock copolymer.*

They have also described the synthesis of micelles with an interchangeable (with temperature) core/corona using a partially quaternised DMAEMA-MEMA block copolymer[176].Optimum biopolymer mimetics capabilities are strongly related to the balance in polymer compositions, size and shape. Allen *et al* [177] has reviewed the determinant for the ability of micelles to deliver various drugs, and factors that influence or control each of these individual properties. i.e. loading capacity, release kinetics, circulation time, biodistribution, size, size population distribution and stability, and morphology of block copolymer aggregates as another potential performance related parameter. Micelles of amphiphillic block copolymers in aqueous solutions at room temperature have been synthesised by a number of research groups, which can precipitate at elevated temperature due to the relative solubility of the constituent homopolymers. For polyelectrolytic block copolymers[178], the efficient distribution of electric charges, which consequently contributes to their biocompatibility, defines their ability to accurately and efficiently participate in various gene transfer processes. Further investigations into these properties should ensure that block copolymers are at the forefront of biologically useful materials development, perhaps most significantly as delivery systems.

Unfortunately, the synthesis and manipulation of many of the biocompatible block copolymers is difficult. For this reason, few systematic studies have been performed whereby one parameter (e.g. core block length) is varied and the effect of this variation on the micelle's characteristics is measured due to the fact that there are such a large number of variables, which influence micelle properties. Haddleton and co-workers[179] recently polymerised DMAEMA with macroinitiators to yield *n*-BMA and DMAEMA (60–80 mol %) blocks of different architectures (diblock, triblock and star block copolymers). They investigated by different techniques as a function of the composition and architecture of the compound, the micellization behaviour of the amphiphilic block copolymers in acidic water. However, the PPO–PEO-based block copolymers are available commercially, and for this reason many studies have been done on this system.

1.9 Project Strategy

1.9.1 Design and Synthesis of ATRP Initiator/linker reagents

The aims of the above project are to design and synthesise hetero bifunctional linkers for functionalising bioactive substrates with ATRP initiators for polymerisation to form water-soluble biocompatible bioconjugates. Attachment of a chemoselective group (See Table 1) and an ATRP initiator unit to either end of a medium length polyethylene glycol (PEG) spacer will give a suitable reagent for such a purpose.

The anchored hetero-bifunctionality on the PEG will possess a functionality (α-bromoisobutyryl ester) for ATRP polymerisation and maleimide or succinimide selective towards amines or thiols in proteins and peptides; they can also, in the absence of the primary target groups, be used with others, functionalities (e.g. phenols). These reagents will be soluble and stable in a wide range of synthetic and aqueous conditions. After the desired protein/peptide-ATRP initiator synthesis has been achieved, they will be subjected to bioconjugate synthesis by exploiting the reactivity of the tert-alkyl halide in a metal assisted living polymerisation process as previously discussed.

The project was envisaged to apply established methodology (Merrified's Fmoc peptide synthesis and ATRP *etc*) in the development of a standard protocol, for the preparation of bioconjugates.

1.9.2 Structure of the Linkers

Two classes of novel hetero-bifunctional reagents, containing thiol or amine chemoselective groups as targeting moiety tethered to an alkyl or (tri, tetra and penta) ethylene glycol spacer functionalised with an ATRP initiator of the isobutyryl bromide variety were intended. The masking of the ATRP moieties to prevent deleterious side reactions would be investigated. The medium length ethyleneglycol was expected to provide increased aqueous solubility for the reagent and the conjugates; and where the bioconjugate is a complex macromolecule (i.e. proteins), the spacer should improve access[180] of the targeting moiety to the protein

receptor and in some case suppress non-specific binding[181]. A shorter length linker would also be synthesised with a minimum length of alkyl spacer to reduce the hydrophobicity of the resulting linker/initiator.

These two groups of biocompatible ATRP initiator/ linker were intended to be further amenable to encompass a variable number of available targeting moieties (i.e. RGD, HSA *etc*) in order to develop a simple protocol for encouraging large-scale synthesis of ATRP polymer bioconjugates.

1.9.2.1 Polymerisation

The block copolymerisation and the subsequent conjugated macromonomers using the uniquely developed initiators to satisfy certain technological or biomedical requirements will also posses the ability to form micelles on exposure to appropriate conditions. These initiator labelled substrates (of amino acid, peptide or protein) would be subjected to ATRP conditions according to published procedure to construct the final peptide/protein polymer bioconjugates.

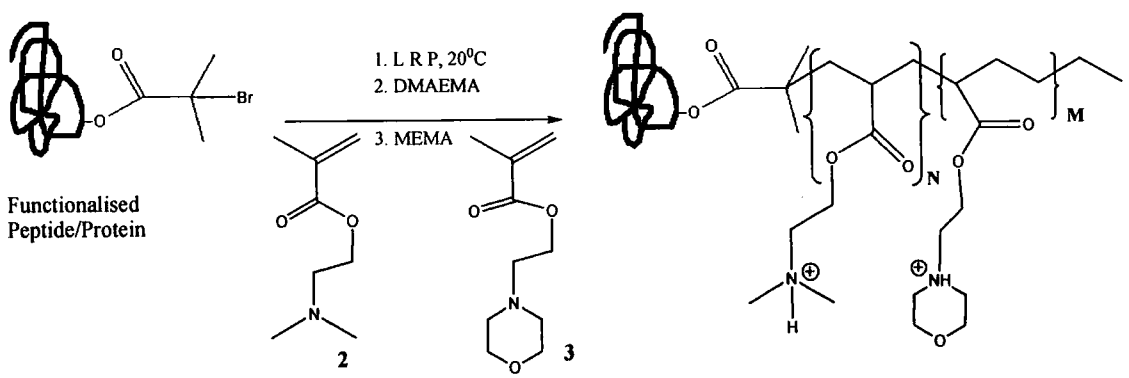


Figure 1.23.

1.10 References

1 H. Ringsdorf, *J. Pharm. Sci. Polymer Symp.* **1975**, *51*, 135.

2 D. Geho, N. Lahar, P. Gurnani, M. Huebschman, P. Herrmann, V. Espina, A. Shi, J. Wulfkuhle, H. Garner, E. Petricoin, L. A. Liotta, K. P. Rosenblatt, *Bioconjugate Chem.* **2005**, *16*, 559.

3 S.Y. Ding, M. Jones, M. P. Tucker, J. M. Nedeljkovic, J. Wall, M. N. Simon, G. Rumbles, M. E. Himmel, *Nano Lett.* **2003**, *3*, 1581.

4 M. Adamczyk, C. M. Chan, J. R. Fino, P. G. Mattingly, *J. Org. Chem.* **2000**, *65*, 596.

5 J. L. Czlapinski, T. L. Sheppard, *J. Am. Chem. Soc.*, **2001**, *123*, 8618.

6 K. G. Bendinskas, A. Harsh, R. M. Wilson, W. R. Midden, *Bioconjugate Chem.* **1998**, *9*, 555.

7 M. T. Tierney, M. W. Grinstaff, *J. Org. Chem.* **2000**, *65*, 5355.

8 V. A. Efimov, A. L. Kalinkina, O. G. Chakhmakhcheva, *Nucleic Acid Res.* **1993**, *21*, 5337.

9 S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chem.*, **1990**, *1*, 71.

10 S. Connolly, D. Fitzmaurice, *Adv. Materials.* **1999**, *11*, 1202.

11 S. Kumar, K. K. Dubey, S. Tripathi, M. Fujii, K. Misra *Nucleic Acids Symp. Ser.*, **2000**, *44*:75.

12 Mie Park, K. Y. Kwok, C. Boukarim, K. G. Rice, *Bioconjugate Chem.* **2002**, *13*, 232.

13 G. Pinghua, P. R. Selvin, *Bioconjugate Chem.* **2003**, 14, 870.

14 F. Bergström, P. Hägglöf, J. Karolin, T. Ny, L. B. Johansson, *Proc. Natl. Acad. Sci. U S A.* **1999**, *96* 12477.

15 N. D. Sonawane, A. S. Verkman, *J. Cell Biol.*, **2003**, *160*, 1129.

16 S. O. Doronina, B. E. Toki, M. Y. Torgov, B. A. Mendelsohn, C. G. Cerveny, D. F. Chace, R. L. DeBlanc, R P. Gearing, T. D. Bovee, C. B. Siegall, J. A. Francisco, A. F. Wahl, D. L. Meyer, P. D. Senter, *Nature Biotech.* **2003**, *21*, 778.

17 M. Ali, M. Amon, N. Kurosaka, N. Manolios, *J. Rheumatology.* **2004**, *7*, 11.

18 H. Seok Kang, S. R. Yang, Jong-Duk Kim, Sang-Hoon Han, S. Chang, *Langmuir*, **2001,** *17*, 7501.

19 N. D. Sonawane, and A.S. Verkman, *J. Cell Biol.*, **2003**, *160*, 1129.

20 B. Wetzwer, G. Byk, M. Fredricke, M. Airiau, F. Blanche, Bruno Pitard and D. Scherman. *Biochem. J.*, **2001,** *356*,747.

21. C. W. Hafner, S. Allwardt, R. Dorothee, B. Angelika, O. Scheiner, H. Pehamberger, H. Breiteneder, *Melanoma Research*, April **2005,** *15*, 111.

22 R. V. Chari, K. A. Jackel, L. A.Bourret, S. M. Derr, B. M. Tadayoni, K. M.Mattocks, S. A.Shah, C. Liu, W. A. Blattler, V. S. Goldmacher, *Cancer Res.*, **1995**, *55*, 4079.

23 Zhan-Yun Guo, Catherine C. Y. Chang, Xiaohui Lu, Jiang Chen, Bo-Liang Li, and Ta-Yuan Chang., *Biochemistry*, **2005**, *44*, 6537.

24 G. Thumshirn, U. Hersel, S. L. Goodman, H. Kessler, *European Journal*, **2003**, *9*, 2717.

25 B. J. Berger, *Antimicrobial Agents and Chemotherapy*, **2000**, *44*, 2540.

26 M. Ali, M. I. Amon, N. Kurosaka and N. Manolios., *J. Rheumatology.*, **2004,** *7*, 11.

27 R. S. Greenfield, T. Kaneko, A. Daues, M. A. Edson, K. A. Fitzgerald, L. J. Olech, J. A. Grattan, G. L. Spitalny G. R. Braslawsky, *Cancer Research*, **2001**, *50*, 6600.

28 B. A. Froesch, R. A. Stahel, U. Zangemeister-Wittke, *Cancer Immunol Immunother.*, **1996**, *42*, 55.

29 D. Putnam, J. Kopecek, *Adv. Polym. Sci.*, **1995**, *122*, 55.

30 M. Harel, J. L. Hyatt, B. Brumshtein, C.. L. Morton, K. Jin P. Yoon, R. M. Wadkins, I. Silman, J. L. Sussman, P. M. Potter, *Mol. Pharma.*, **2005**, *67*, 1874.

31 A. Hatefi, B. Amsden, *Pharmaceutical Research,* **2002,** *19* 1389.

32 J. W. Singer, R. Bhatt, J. Tulinsky, K. R. Buhler, E. Heasley, P. Klein, P. De Vries, *J. Controlled Release,* **2001**, *74*, 243.

33 S. Sakuma, Z. Lu, P. Kopeckova, J. Kopecek, *J. Controlled Release*, **2001**, *75*, 365.

34 C. D. Conover, R. B. Greenwald, A. Pendri, C. Gilbert, K. L. Shum, *Cancer Chemotherap. Pharmacology*, **1998,** *42*, 407.

35 J. Cheng, K. T. Khin, G. S. Jensen, A. Liu, M. E. Davis, *Bioconjugate Chem.*, **2003**, *14* 1007.

36 S. Okuno, M. Harada, T. Yano, S. Yano, S. Kiuchi, N. Tsuda, Y. Sakuma, J. Imai, T. Kawaguchi, K. Tsujihara, *Cancer Research* **2000**, *60*, 2988.

37 W. Maison, J. V. Frangioni, *Angewandte Chemie. Int. Ed.*, **2003**, *42*, 4726.

38 H. Han, G. Amidon, *AAPS Pharm Sci.,* **2000**, *2* 1.

39 T. M. Allen, *Nature Reviews Cancer*, **2002**, *2*, 750.

40 K. Ulbrich, V. Šubr, J. Strohalm, D. Plocová, M. Jelínková, B. Říhová, *J. Controlled Release,* **2000,** *64*, 63.

41 D. G. Williams, *J. Immunological Methods,* **1984**, *72*, 3.

42 T. Shimoboji, Z. Ding, P. S. Stayton, A. S. Hoffman *Bioconjugate Chem.*, **2001**, *12*, 314.

43 K. Petrak, *Polym. J.*, **1990**, *22*, 213.

44 A. A. Hertler, A. E. Frankel, *J. of Clinical Oncology*, **1989**, *7*, 1932.

45 R. Duncan, *Anticancer Drugs,* **1992**, *3*, 175.

46 E. W. P. Damen, P. H. G. Wiegerinck, L. Braamer, D. Sperling, D. de Vos, H. W. Scheeren., *Bioorg, and Med. Chem.*, **2000**, *8*, 427.

47 J. P. Briand, S. Muller, M. H. V. Van Regenmortel, *J. Immunological Methods,* **1985**, *78*, 1.

48 M. Adamczyk, J. C. Gebler, R. E. Reddy, Z. Yu. *Bioconjugate Chem.* **2001**, *12*, 139.

49 M. Isaksson, D. Willner, I. Hellstrom, K. E. Hellstrom P. A. Trail, *Cancer Research*, **1997**, *57*, 4530.

50 O. P. Medina, Ying Zhu, K. Kairemo, *Curr. Pharmceutical Design*, **2004**, *10*, 2981.

51 K. Moribe, K. Maruyama *Curr. Pharmaceutical Design*, **2002**, *8*, 441.

52 M. Pechar, K. Ulbrich, V. Šubr, L.W. Seymour, E. Schacht. *Bioconjugate Chem.*, **2000**, *11*, 131.

53 T. Etrych, P. Chytil, M. Jelínková, B. Říhová, K. Ulbrich., *Macromol. Biosci.*, **2002**, *2*, 43-52.

54 K. Kono, M. liu, J. M. Frechet, *J Bioconjugate Chem.*, **1999**, *10*, 1115.

55 J. J. Peterson, R. H. Pak, C. F. Meares, *Bioconjugate Chem.* **1999**, *10*, 316.

56 M. R. Lewis, A. Raubitschek, and J. E. A. Shively, *Bioconjugate Chem.*, **1994**, *5*, 565.

57 M. R. Lewis, J. Y. Kao, Anne-Line J. Anderson, J. E. Shively, and A. Raubitschek. *Bioconjugate Chem.*, **2001**, *12*, 320.

58 G. M. Dubowchik, R. A. Firestone, *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3341.

59 E. Dolusic, M. Kowalczyk, V. Magnus, G. Sandberg, and J. Normanly. *Bioconjugate Chem.*, **2001**, *12*, 152.

60 S. M. Akula, N. P. Pramod, F. Wang, and B. Chandran, *Cell*, **2002**, *108*, 407.

61 S. A. Mousa and D. A. Cheresh, *Drug Discovery Today*. **1997**, *2*, 187.

62 A. Krammer, D. Craig, W. E. Thomas, K. Schulten, V. Vogel, *Matrix Biology*, **2002**, *21*, 139.

63 M. D. Pierschbacher, E. Ruoslahti, *Nature*, **1984**, *309*, 30.

64 A. M. Krezel, J. S. Ulmar, G. Wagner. R. A. Lazarus, *Science*, **1987**, *238*, 491.

65 S. D. S. Jols, U. S. F. Tambunan, S. Chakrabarti T. J. Slahaan. *J. Biomolecular Structure & Dynamics*, **1996**, *14*, 1.

66 R. M. Scarborough, J. W. Rose, M. A. Naughton, D. R. Phillips, L. Nannzzi, A. Arfsten, A. N. Campbell, I. F. Charo, *J. Biol. Chem.*, **1993**, *263*, 1058.

67 A. Mitra, J. Mulholland, A. Nan, E. McNeill, H. Ghandehari, B. R. Line, *J. of Controlled Release*, **2005**, *102*, 191.

68 E. Ruoslanhti, *Annual Rev. Cell Dev. Biol.*, **1996**, *12*, 697.

69 H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, *J Control Release*. **2000**, *65*, 271.

70 C. de las Heras Alarcón, S. Pennadam and C. Alexander, *Chemical Soc. Revs.*, **2005**, *34*, 276.

71 T. Shimoboji, Z. Ding, P. S. Stayton, A. S. Hoffman *Bioconjugate Chem.*, **2001**, *12*, 314.

72 R. Duncan, *Pharm. Sci. Technol. Today,* **1999**, *2*, 441.

73 Y. Noguchi, J. Wu, R. Duncan, J. Strohalm, K. Ulbrich, T. Akaike, H. Maeda, *Japan J. Cancer Res.*, **1998**, *89*, 307.

74 Min Wu, *J. Immunopharmac.*, **1997**, *19*, 83.

75 R. Fernandes, *Biomolec. Screening*, **2003**, *8*, 264.

76 J. K. Jaiswal, H. Mattoussi, J. M. Mauro S. M. Simon, *Nature Biotech.*, **2002**, *21*, 47.

77 J.M. Mauro, Anal. Chem., **2002**, *74*, 841.

78 A. Fasbender, J. Zabner, M. Chillon, T. O. Moninger, A. P. Puga, B. L. Davidson, M. J. Welsh, *J Biol Chem.*, **1997**, *272*, 6479.

79 H. Romanczuk, C. E. Galer, J. Zabner, G. Barsomian, S. C. Wadsworth, C. R. O'Riordan, *Human. Gene Therapy,* **1999**, *10*, 2575.

80 A. G. Gomez-Valades, M. Molas, A. Vidal-Alabro, J. Bermudez, R. Bartrons, J. C. Perales, *J. Controlled Release*, **2005**, *102*, 277.

81 D. Trentin, J. Hubbell, H. Hall, *J. Controlled Release,* **2005**, *102*, 263.

82 F. L. Sorgi, S. Bhattacharya, L. Huang, *Gene Therapy,* **1997**, *4*, 961.

83 J. S. Choi, D. K. Joo, C. H. Kim, K. Kim, J. S. Park, *J. Am. Chem. Soc.*, **2000**, *122*, 474.

84 K. Wei, D. M. Skyba, C. Firschke, A. R. Jayaweera, J. R. Lindner, S. Kaul, *J Am. Coll Cardiologists,* **1997**, *29*, 1081.

85 R. V. Shohet, S. Chen, Y. T. Zhou, Z. Wang, R. S. Meidell, R. H. Unger, *Circulation,* **2000**, *101*. 2554.

86 J. Kalal, *Makromol. Chem. Macromol. Symp.*, **1987,** *12*, 259.

87 J. E. Nam , Y. S. Lee , K. H. Park , *J. Controlled Release*, **1999**, *57*, 269; *ibid.*, **1998**, *55*, 181.

88 W. Deits, O. Vogl, *J. Polymer Sci.: Polymer Chem. Ed.*, **1981**, *19*, 403.

89 O. Vogl, G.D. Jaycox, K. Hatada, *J Macromol. Sci. Chem.*, **1990,** *A27*, 1781.

90 M. A. Bogoyevitch, T. S. Kendrick, D. C. Ng, R. K. Barr, *DNA Cell Biol.*, **2002,** *21*, 879.

91 J. Temsamani, P. Vidal, *Drug Discovery Today*,**2004**, *9*(23), 1012.

92 S. Olofsson, M, T. Bergstra, *Annals of Medicine*, **2005**, *37*, 154.

93 Y. Kawamata, Y. Nagayama, K. Nakao, H. Mizuguchi, T. Hayakawa, T. Sato, N. Ishii, *Biomat.*, **2002**, *23*, 4573.

94 A. Kichler , C. Leborgne , P. B. Savage, O. Danos., *J. Controlled Release*, **2005**, IN PRESS

95 F. M. Huenneken, *Adv. Enzyme. Regulation*, **1997**, *37*, 77.

96 Y. Cliquet, E. Cox, C. Van Dorpe, E. Schacht, and B. M. Goddeeris., *J. Agric. Food Chem.*, **2001**, *49*, 3349.

97 F. M. H. de Groot, L. W. A. van Berkom, and H. W. Scheeren. *J. Med. Chem.*, **2000**, *43*, 3093.

98 V. Olivier, I. Meisen, B. Meckelein, T. R. Hirst, J. P. Katalinic, M. A. Schmidt, and Frey, *Bioconjugate Chem.*, **2003**, *14*, 1203.

99 J. M. Harris, N. E. Martin, M. Modi, *Clin. Pharmacokinectics*, **2001**, *40*, 539.

100 R. B. Greenwald, A. Pendri, C . D. Conover, C. Lee, Y. H. Choe, C. Gilbert, A. Martinez, J. Xia, D. Wu, M. M. Hsue., *Bioorg. Med. Chem.*, **1998**, *6*, 551.

101 R. J. Kruger, J. A. Frank, Chaomei-Lin and Gauter Sarath. *Biochemical Pharmacology*, **1991**, *41*, 789.

102 P. R. Hamann, L. M. Hinman, I. Hollander, C. F. Beyer, D. Lindh, R. Holcomb, W. Hallett, H. R. Tsou, J. Upeslacis, D. Shochat, A. Mountain, D. A. Flowers, I. Bernstein, *Bioconjugate Chem.*, **2002** *13*, 47.

103 P. V. Paranjpe, Yu Chena, V. Kholodovych, W. Welsh, S. Stein, P. J. Sinko. *J. Controlled Release*, **2004,** *100*, 275.

104 P. R. Hamann, L. M. Hinman, C. F. Beyer, D. Lindh, J. Upeslacis, D. A. Flowers, *Bioconjugate Chem.*, **2002**, *13*, 40.

105 A. Frey, B. Meckelein, and M. A. Schmidt, *J. Bioconjugate Chem.*, **1999**, *10*, 562.

106 D. L. Kukis, I. Novak-Hofer, S. J. DeNardo, *Cancer Biotherapy Radiopharmaceuticals,*. **2001**, *16*, 457.

107 N. D. Heindel, H. R. Zhao, R. A. Egolf, C. H. Chang, K. J. Schray, J. G. Emrich, J. P. McLaughlin, D. V. Woo, *Bioconjugate Chem,*. **1991**, *2*, 427.

108 S. S. Ghosh, P. M. Kao, A. W. McCue, H. L. Chappelle, *Bioconjugate Chem.*, **1990**, *1*, 71.

109 M. E. Annunziato, U. S. Patel, M. Ranade, P. S. Palumbo, *Bioconjugate Chem.*, **1993**. *4*, 212.

110 S. Zalipsky, *Adv. Drug. Delivery. Rev.*, **1995**, *16*, 157.

111 P. L. Carl, P. K. Charkravarty, J. A. Katzenellenbogen, *J. Med. Chem.*, **1981**, *24*, 479.

112 C. De Duve, T. De Barsy, B. Poole, A. Trouet, P. Tulkens, F. Van Hoof, *Biochem. Pharmacol.*, **1974,** *23* 2495.

113 J. M. Harris, B. C. Robert, *Nat. Rev., Drug Discov.*, **2003,** *2*, 214

114 C. Li, *Adv. Drug Deliv. Rev.*, **2002,** *54,* 695.

115 R. B. Greenwald, Y. H. Choe, J. McGuire, C. D. Conover, *Adv.Drug Deliv. Rev.*, **2003,** *55*, 217.

116 V. Percec, B. Barboiu, *Macromolecules*, **1995**, *28*, 7970.

117 K. Matyjaszewski, J. Xia, *Chem. Rev.*, **2001**, *101*, 2921.

118 M. Kamigaito, T. Ando, M. Sawamoto, *Chem. Rev.*, **2001**, *101*, 3689.

119 C. J. Hawker, A. W. Bosman, E. Harth, *Chem. Rev.*, **2001**, *101*, 3661.

120 J. P. A. Heuts, R. Mallesch, T. P. Davis, *Macromol. Chem. Phys.*, **1999**, *200*, 1380.

121 S. G. Roos, A. H. E. Muller, *Macromol. Rapid Commun.*, **2000**, *21*, 864.

122 J. Lutz, Lacroix-Desmazes and B. Boutevin, *Macromol. Rapid Commun.*, **2001**, *22*, 189.

123 T. Pintauer, B. McKenzie, K. Matyjaszewski, *ACS Symposium Series,* **2003**, *854*, 130-147

124 W. Braunecker, E. Collange, R. Poli, K. Matyjaszewski, *Macromolecules,* **2004**, *37*, 2679.

125 A. P. Zagala and T. E. Hogen-Esch, *Macromolecules*, **1996**, *29*, 3038.

126 a). D. Kunkel, A. H. E. Muller, L. Lochmann M. Janata, *Makromol. Chem., Macromol. Symp.*, **1992**, *60*, 315; b). M. Janata, L. Lochmann, P. Vicek, J. Dybal A. H. E. Muller, *Makromol. Chem.*, **1992**, *193*, 101.

127 T. Ishizone, K. Yoshimura, A. Hirao and S. Nakahama, *Macromolecules*, **1998**, *31*, 8706.

128 X. S. Wang, S. F. Lascelles, R. A. Jackson S. P. Armes. *Chem. Commun.*, **1999**, 1817;

129 Odian, G., *Principles of Polymerization*, John Wiley & Sons, Inc, **1991**, 8.

130 L. I. Gabaston, S. A. Furlong, R. A. Jackson, S. P. Armes, *Polymer*, **1999**, *40*, 4505.

131 D. Benoit, S. Grimaldi, S. Robin, J.P. Finet, P. Tordo, Y. Gnanou, *J. Am. Chem. Soc.*, **2000**, *122*, 5929.

132 D. Benoit, V. Chaplinski, R. Braslau, C. J. Hawker, *J. Am. Chem. Soc.*, **1999**, *121*, 3904.

133 L. F. J. Noël, J. L. Van Altveer, M. D. F. Timmermans, A. L. German, *J. Polym. Sci. Polym. Chem.*, **1994**, *32*, 2223.

134 M. A. Dubé, A. Penlidis, *Polymer*, **1995**, *36*, 587.

135 Y. K. Chong, T. P. Le, E. R. Moad, S. H. Thang, *Macromolecules*, **1999**, *32*, 2071.

136 Y. Mitsukami, M. S. Donavon, A. B. Lowe, C. L. McCormick, *Macromolecules*, **2001**, *34*, 2248.

137 B. S. Sumerlin, M. S. Donavon, Y. Mitsukami, A. B. Lowe, C. L. McCormick, *Macromolecules,* **2001**, *34*, 6561.

138 (a) K. Matyjaszewski, *Chem. Eur. J.*, **1999**, *5*, 3095. (b) T. E. Patten, K. Matyjaszewski, *Acc. Chem. Res.*, **1999**, *32*, 895.

139 M. Kato, M. Kamigaito, M. Sawamoto and T. Higashimura., *Macromolecules*, **1995**, *28*, 1721.

140 K. Matyjaszewski and J. Xia, *Chem. Rev.*, **2001**, *101*, 2921.

141 R. Jordan, K. Martin, H. J. Rader, K. K. Unger., *Macromolecules*, **2001**, *34*, 8858.

142 H. Fischer, *Chem Rev.*, **2001**, *101*, 3581.

143 R. B. Grubbs, C. J. Hawker, J. Dao, J. M. J. Frechet, *Angew. Chem. Int. Ed. Engl.*, **1997**, *36*, 270.

144 T. Fakuda, A. Goto, *Progress In Polymer Sci.*, **2004**, *29*, 329.

145 K. Matyjaszewski, S. Coca, Y. Nakagawa, J. Xia, *J. Polym. Mater. Sci. Eng.*, **1997**, *76*, 147.

146 D. M. Haddleton, A. M. Heming, D. Kukulj, D. J. Duncalf, A. J. Shooter, *Macromolecules*, **1998**, *31*, 2016.

147 Nishikawa, T., Ando, T., Kamigaito, M., Sawamoto, M., *Macromolecules*, **1997**, *30*, 2244.

148 K. Matyjaszewski, T. Grimaud, *Macromolecules*, **1997**, *30*, 2216.

149 K. Matyjaszewski, T. E. Patten, J. Xia, T. Abernathy, *Science*, **1996**, *272*, 866.

150 J. S. Wang, K. Matyjaszewski, *J. Am. Chem. Soc.*, **1995**, *117*, 5614.

151 J. S. Wang, K. Matyjaszewski, *J. Am. Chem. Soc.*, **1995**, *38*, 7901.

152 K. Matyjaszewski, T. E. Patten, J. Xia, *J. Am. Chem. Soc.*, **1997**, *119*, 674.

153 K. Matyjaszewski, S. M. Jo., H. J. Paik, D. A. Shipp, *Macromolecules*, **1999**, *32*, 6431.

154 X. S. Wang, and S. P. Armes, *Macromolecules,* **2000**, *33*, 6640.

155 T. Nishikawa, M. Kamigaito, M. Sawamoto, *Macromolecules*, **1999**, *32*, 2204.

156 W. Huang, Jong-Bum Kim, M. L. Bruening, and G. L. Baker., *Macromolecules*, **2002**, *35*, 1175.

157 S. Liou, J. T. Rademacher, D. Malaba, M. E. Pallack, W. J. Brittain, *Macromolecules*, **2000**, *33*, 4295.

158 M. Van der Sluis, B. Barboiu, N. Pesa, V. Percec, *Macromolecules,* **1998**, *31*, 9409.

159 R. Jordan, K. Martin, H. J. Rader, K. K. Unger, *Macromolecules*, **2001**, *34*, 8858.

160 K. Petrak, *British Polymer Journal*, **1990,** *22*, 213.

161 D. M. Haddleton, K. Ohno., *Biomacromolecules*, **2000**, *1*, 152.

162 N. J. Hovestad, G. van Koten, S. A. F. Bon, D. M. Haddleton., *Macromolecules*, **2000**, *33*, 4048.

163 A. Marsh, A. Khan, D. M. Haddleton, J. M. Hannon, *Macromolecules*, **1999**, *32*, 8725.

164 B. Moon, T. R. Hoye, C. W. Macosko, *Macromolecules*, **2001**, *34*, 7941.

165 Y. Nakagawa, K. Matyjaszewski, *Polym. J.*, **1998**, *30*, 138.

166 J. Xia, H.J. Paik, K. Matyjaszewski, *Macromolecules*, **1999**, *32*, 8310.

167 T. Nishikawa, T. Ando, M. Kamigaito, M. Sawamoto, *Macromolecules*, **1997**, *30*, 224.

168 T. Nishikawa, M. Kamigaito, M. Sawamoto, *Macromolecules*, **1999**, *32*, 2204.

169 W. Huang, Jong-Bum Kim, M. L. Bruening, and G. L. Baker. *Macromolecules*, **2002**, *35*, 1175.

170 I. Q. Li, B. A. Howell, M. T. Dineen, P. E. Kastl, J. W. Lyons, D. M. Meunier, P. B. Smith, D. Priddy, *Macromolecules*, **1997**, *30*, 5195.

171 J. S. Choi, D. K. Joo, C. H. Kim, J. S. Park., *J. Am. Chem. Soc.*, **2000**, *122*, 474.

172 A. B. Lowe, N. C. Billingham, S. P. Armes, *Macromolecules,* **1998**, 31, 5991.

173 A. P. Narrainen, S. Pascual, D. M. Haddleton., *J. Polymer. Sci. Part A: Polymer Chemistry,* **2002**, *40*, 439.

174 V. Bütün, N. C. Billingham, S. P. Armes, *J. Am. Chem. Soc.*, **1998**, *120*, 11818.

175 H. Harada, K. Kataoka, *Macromolecules*, **1998**, *31*, 288.

176 V. Bütün, N. C. Billingham, S. P. Armes, *J. Am. Chem. Soc.*, **1998**, *120*, 12135.

177 C. Allen , D. Maysinger, A. Eisenberg, *Colloids and Surfaces B: Biointerfaces*, **1999**, *16*, 3.

178 D. V. Pergushov, E. V. Remizova, J. Feldthusen, A. B. Zezin,, A. H. E. Mu1ller, V. A. Kabanov., *J. Phys. Chem. B*, **2003**, *107*, 8093.

179 A. P. Narrainen, S. Pascual, D. M. Haddleton., *J. Polymer Sci, Polymer Chemistry* **2002**, *40*, 439.

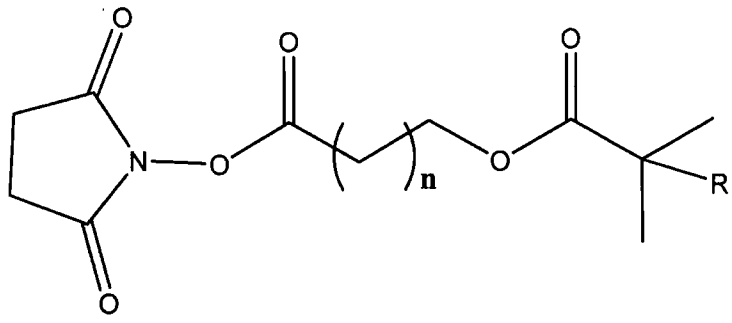
180 V. Marchi-Artzner, L. Julien, T. Gulik-Krzymick, J. M.Lehn. *Chem Comm.*, **1997**, 117.

181 C. Pale-Grosdemange, E. S. Simon, K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.*, **1991**, *113*, 12.

CHAPTER 2

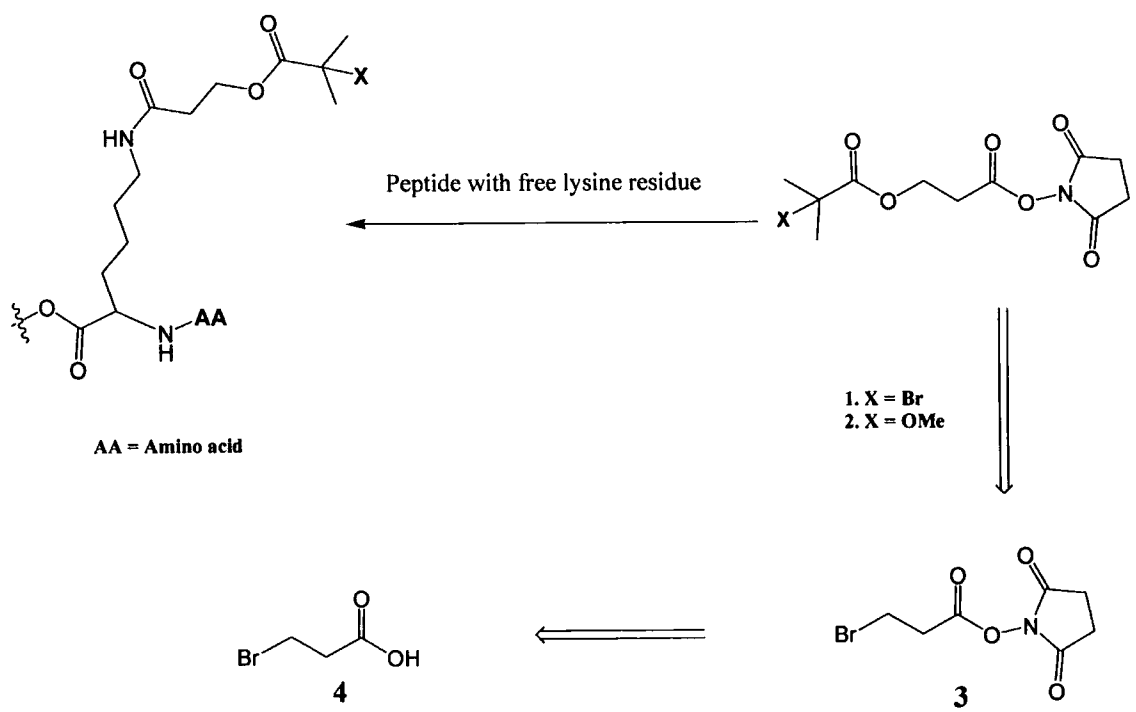
SYNTHETIC APPROACHES TO AN ALKYL ATRP INITIATOR

(Amine-Selective Hetero-bifunctional Linker)



2.1 Introduction

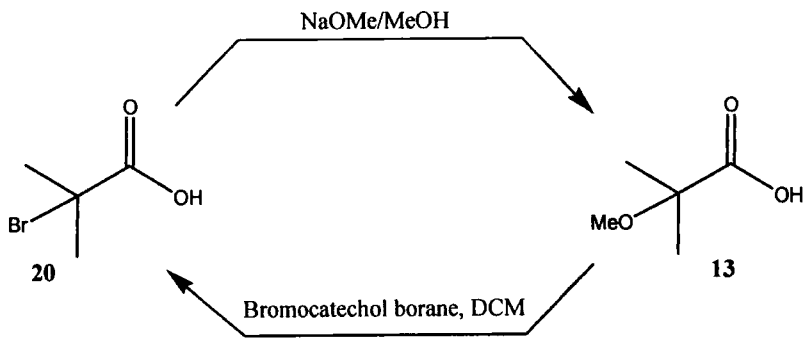
The main focus of our synthetic strategy to succinimidyl ester **1** is the construction of the diester in an activated form suitable for selective functionalisation of substrates in a mild environment thereby avoiding complications associated with conditions intolerable to biological substrates. The synthesis relies on the successive coupling/condensation reaction based on standard reagents and conditions to give **1**, Scheme 2.1. The retrosynthetic analysis in Scheme 2.1 shows the key intermediate **3**, and the early introduction of the succinimide ester to **3** will also ensure easy access to the required final substrate in a single step. The final compound would then be loaded onto a pre-prepared and appropriately functionalised amino acid residue (i.e. lysine) to produce the required ATRP initiator-labelled peptide (or protein) for use in living polymerisation.



Scheme 2.1: *Suggested retrosynthesis of N-alkyl succinimidyl-based ATRP reagent* **1**

2.1.1 AIMS

The main aim of this work was to undertake a model study based on our strategy to afford a unique ATRP initiator with minimum hydrophobic or contributive hydrophilic properties to the constructed bioconjugate consisting of, for example, a peptide/protein coupled to ATRP generated polymer. Our first synthetic target was the diester **1** with the initial retrosynthetic procedure proposed shown in Scheme 2.1. The succinimide **3** would be prepared by reacting the commercially available 3-bromopropionic acid **4** with *N*-hydroxysuccinimide. The resulting activated ester would be condensed with an appropriately α–substituted isobutyric acid (Scheme 2.2) to produce the diester **1.** The reagent **1** was designed to incorporate a minimum length of hydrophobic alkyl chain to reduce hydrophobicity. Also, preparation of the α-methoxy **2** equivalent to α-bromo compound **1** when unmasked (Scheme 2.2) was also investigated to provide an alternative route to functionalisation with **2** in environments less conducive to the halogenated equivalent. Consequently, this report will be in two sections describing the synthetic approaches to **1** and **2** and the attempted interconversion.



Scheme 2.2:*Interconversion of the masked halogen under mild conditions.*

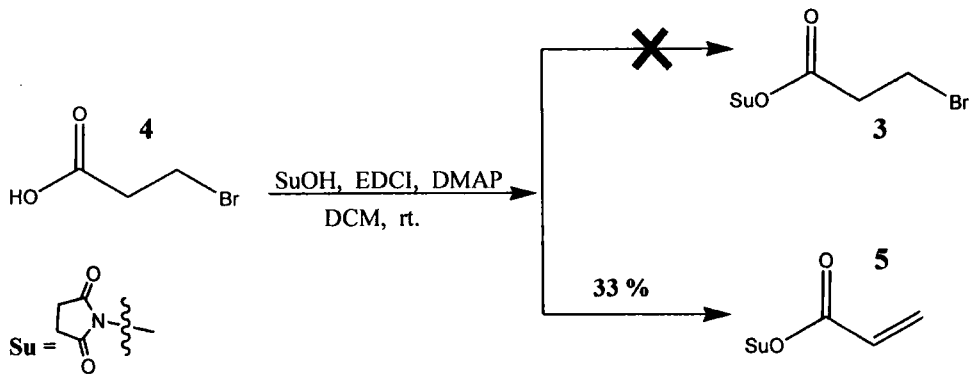
2.2 Synthesis of succinimidyl /amine-reactive diester – ATRP initiator/linkers

2.2.1 Section A: Attempted synthesis via 3 bromopropionic acid

Starting from commercially available 3-bromopropionic acid **4**, the synthesis of the key succinimidyl intermediate **3** would be expected to be approached in several simple and sequential condensation reactions as shown in Scheme 2.1.

The attempted coupling of unprotected 3-bromopropionic acid was carried out using standard coupling reagents, that is, treatment of **4** with 1.4 equivalents of N-hydroxysuccinimide in the presence of EDCI with or without a catalytic amount of N,N-dimethylaminopyridine in dry dichloromethane at room temperature. After complete consumption of the acid, the mixture was subjected to aqueous workup and *in vacuo* removal of solvent to give a crude compound. Upon analysis of the crude compound by ^{1}H NMR, the presence of at least three different compounds was observed. The mixture was further subjected to either distillation or column chromatography both of which resulted in the degradation of the mixture.

The mixture was then triturated in methanol to give the acrylate **5** as a colourless solid in 33 % yield. This was confirmed by the characteristic broad four-proton signal in the ^{1}H NMR corresponding to the (CH_2CH_2) of *N*-succinimide at δ 2.88 ppm suggesting the incorporation of succinimide in the product, while the presence of three sets of

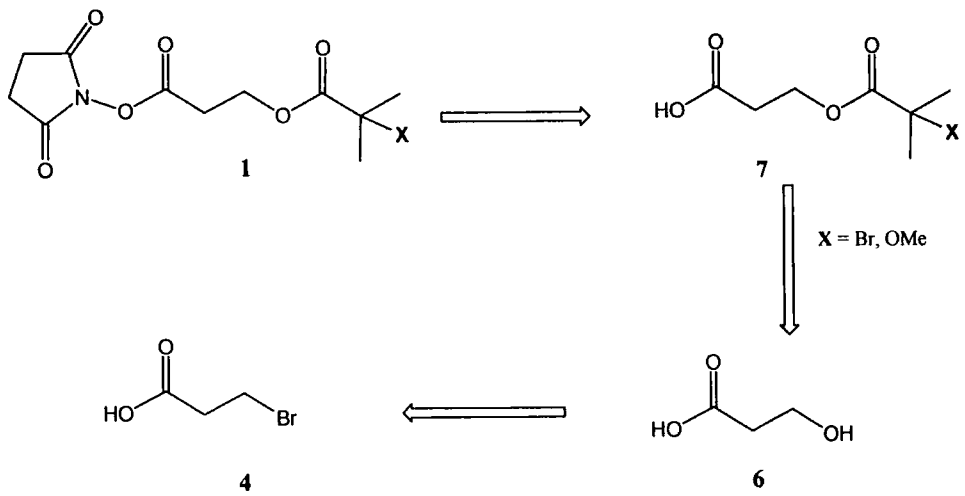


Scheme 2.3

doublets of doublets at δ 6.3 ppm, δ 6.5 ppm and at δ 6.7 ppm corresponding to a vicinal proton adjacent to a pair of geminal protons were also observed, suggesting that the acrylate **5** had been formed. However, the expected product, succinimidyl 3-bromopropionate **3** which had been observed as two triplets at δ 2.9 ppm and δ 3.5 ppm (CH_2CH_2Br) in the ^1H NMR of the crude product (with other by-products) during preliminary analysis was not identifiable thereafter either in the methanol triturate or after column chromatography. The presence and identity of the acrylate was further confirmed by comparing the observed melting point 67 – 69 °C and other data with the data published[1] for N-succinimidyl acrylate **3** to further confirm the identity of the unexpected elimination product. The elimination product *via* the loss of HBr was thought to be facilitated by the tertiary amine present on EDCI, which may have deprotonated the highly base labile α-proton to the carbonyl and further accelerated by the potential to form a stable conjugate after such elimination. Therefore, the use of a substrate with less potential as a leaving group than bromide anion was investigated.

2.2.2 Attempted synthesis via 3-hydroxypropionic acid

The retrosynthesis below was therefore proposed to incorporate the functional



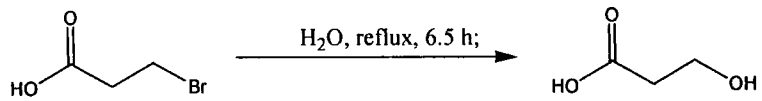
Scheme 2.4. *New retrosynthetic procedure to target compound* **1**

group interconversion to an hydroxy substrate **6** (Scheme 2.4) with a poorer leaving group than alkyl halide **4** necessary to give the new key intermediate **7**.

Discussed below are the attempts to synthesise the intermediate **7** and to proceed to the target compound **1**. The proposed approach for the new strategy was based on modified published[2] methodology for the conversion of various haloalkyl carboxylic acids into the equivalent hydroxypropionic acids in moderate to good yields.

2.2.2.1 Synthesis of 3-hydoxypropionic acid

Using the method of Kowski and Lossen[2], 3-bromopropionic acid was refluxed in water for 6.5 h, Scheme 2.5. Upon completion, as determined by TLC, the reaction mixture was extracted with ethyl acetate and concentrated under reduced pressure. Unfortunately, analysis of the reaction mixture by mass spectroscopy showed that it consisted of several products. The product resulting from dehydration was detected as an ion at m/z (-ES) 73, and at m/z (-ES) 45, a



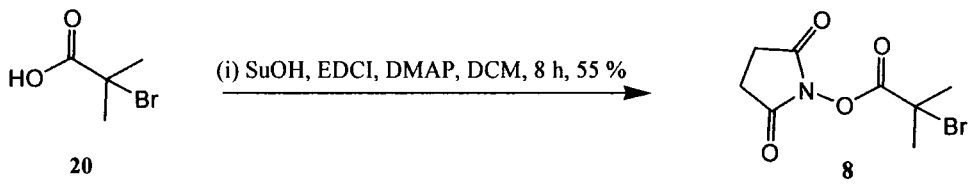
4 **6**

Scheme 2.5

product resulting from decarboxylation. A signal at m/z 89 ($M - H^+$) corresponding to the expected product was also seen in the mass spectrum. Attempts at precipitation of the crude mixture or column chromatography almost always resulted in accelerated process of elimination to give acrylic acid and subsequent formation of polymer by-products. The reaction mixture was instead subjected to kugelrohr distillation, giving the product 3-hydroxypropionic acid **6** in 30 % yield as an orange/pink oil which solidified on standing. The presence of the expected new methylene group of hydroxyl methylene ($C\underline{H}_2OH$) was

observed as a ^{1}H NMR signal at δ 4.37 ppm with the expected characteristic infrared signal at 3264 cm^{-1} for the hydroxyl group.

To synthesise the key intermediate **7**, synthesis of the required bromoisobutyryl ester **8** activated with *N*-hydroxysuccinimide was necessary to reduce reaction time and consequently reduce exposure to a possibly deleterious environment.



20 **8**

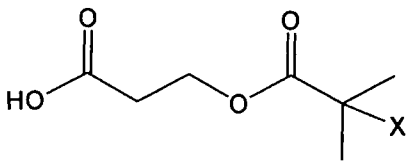
Scheme 2.6

Using traditional coupling reagents to produce the aqueous soluble urea, 1.2 equivalents of EDCI, N,N-dimethylaminopyridine (catalytic) in dichloromethane and α-bromoisobutyric acid **20** was then stirred with 1.2 equivalents of *N*-hydroxysuccinimide in dry dichloromethane at room temperature and the reaction was monitored by TLC until complete consumption of the investigated carboxylic acid occurred, after 8 hours. After aqueous workup and removal of the solvent *in vacuo*, further purification gave the desired product **8** in 55 % yield, Scheme 2.6. The structure of the product was further identified by the presence of the characteristic ^{1}H NMR signals for succinimide and substituted isobutyryl units. The structure of α – bromosuccinimide ester **8** was confirmed by the presence of the unperturbed signal for a six-proton singlet at δ 2.08 ppm and a broad four-proton singlet signal at δ 2.86 ppm in the ^{1}H NMR spectrum corresponding to α–bromodimethyl and *N*-substituted succinimidyl group respectively. A molecular ion for (M^+) at m/z 263 in the mass spectrum was also observed.

The lower yield for the formation of **8** was thought to be due to the formation of the elimination product, hydrogen bromide, exacerbated by a lengthy reaction time, although, only the unreacted acid **20** (20 %) was identifiable (total recovered reactant mass was less than expected) as the by-product.

2.2.3 Attempted synthesis of 3-(2-bromo-2-methylpropionyloxy)propionic acid

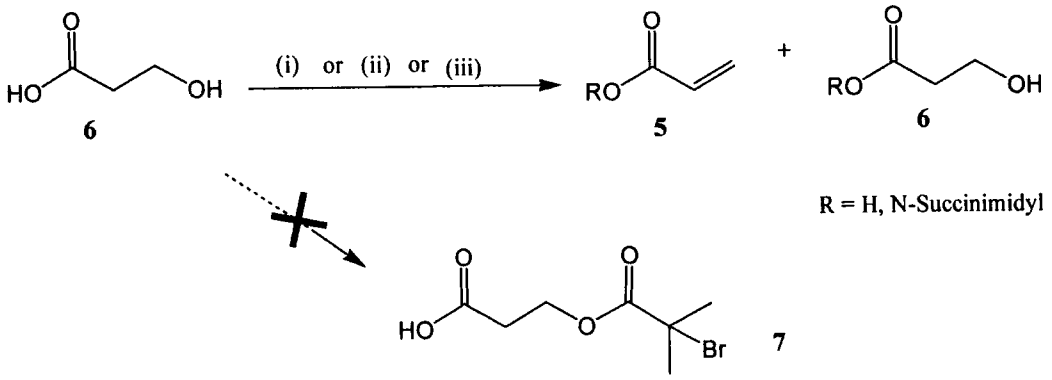
The next stage of the proposed strategy, and with both activated α–substituted isobutyryl and the hydroxyethyl carboxylic acid reagents in hand, is the synthesis of the key intermediates (Scheme 2.7) by the coupling of an isobutyryl to the free hydroxyl group. With the use of succinimide **8**, it was hoped that the milder environment would discourage or reduce the formation of elimination products.



7 (X = Br)

Scheme 2.7

To form the 3-(2-methoxy-2-methylpropionyloxy) carboxylic acid **7**, a solution of 3-hydroxypropionic acid **6** was stirred with 1.2 equivalents of succinimidyl 2-bromo-2-methylpropionate **8** and a catalytic amount of N,N-dimethylaminopyridine, Scheme 2.8. Unfortunately, the resulting elimination

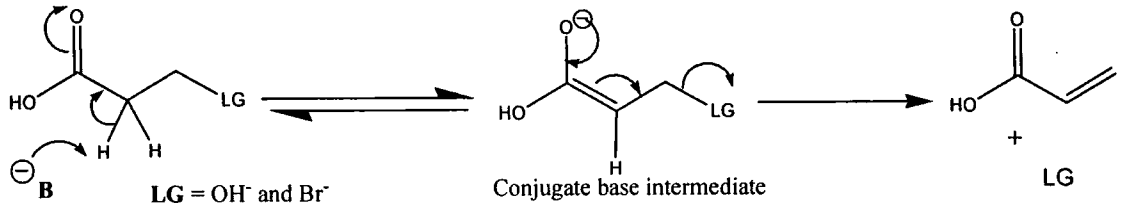


(i) SuOH, DMAP, EDCI; (iia) Ph_3CCl, TEA; (b) **8** (c) Dioxane/H_2O (1:1);

(iii) PMBCl, NaI, DMF, TEA

Scheme 2.8. *Schematic of attempts to synthesise the key intermediate* **7**.

compound **5** was the only isolated product in 30 % yield after purification. This was increased to 40% yield whenever DMAP is used in the reaction. The by-product **5** was further confirmed by comparing with previously isolated acrylate product, Scheme 2.3. The crude reaction mixture, after interpretation of the spectral data showed the presence of a hydroxyethyl group, which differed from that in the starting carboxylic acid **6** although with a higher proportion of the characteristic ABX coupling pattern in the ^{1}H NMR signal attributed to a proton adjacent to germinal alkenes. A number of other unsuccessful (Scheme 2.8) attempts were made at synthesising the key intermediate, some of which included *in-situ* protection/deprotection of the carboxylic acid prior to hydroxyethyl functionalisation and changes in reaction time and temperature. The observed by-product was proposed to be due to the highly acidic proton (pKa ~ 20) adjacent



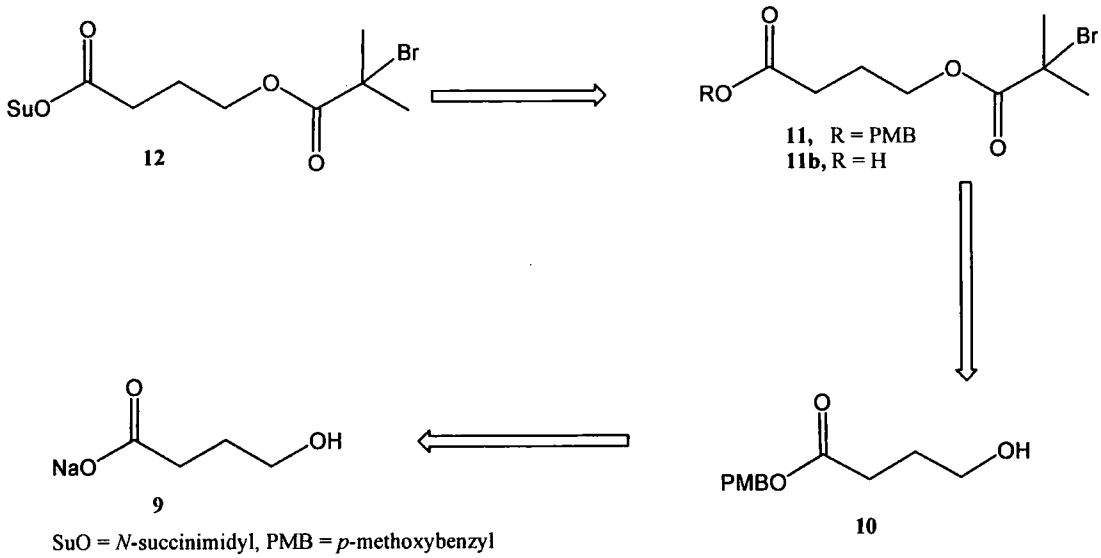
Scheme 2.9. *The stabilised elimination reaction mechanism*

to a carbonyl and the influence of a conjugated potential leaving group in the β–position, which could have facilitated the elimination via enolate intermediate formation to produce an α, β - unsaturated carbonyl compound, Scheme 2.9. Hence, to reduce the potential for leaving group conjugation assisted elimination ($E1_{CB}$), an extension of the alkyl chain from 3-hydroxypropionyl to its hydroxybutyl equivalent was proposed. The hydroxybutyl should disfavour the formation of α,β-unsaturated carbonyl even if a conjugate base intermediate (via enolisation) was formed. Due to our unsuccessful attempts to efficiently synthesise succinimidyl ester of **7** or **3**, the approach starting from 3-hydroxypropanoic acid was now abandoned in favour of the newly proposed strategy below based on hydroxybutanoic acid.

2.3 Synthesis of succinimide substituted n-butylester

2.3.1 Synthesis of *p*-methoxybenzyl 4-hydroxybutanoate

The synthesis of the alkyl succinimide reagent **12** was attempted based on the procedure outlined below (Scheme 2.10). The new approach would employ the commercially available sodium salt of 4-hydroxybutanoate **9** to synthesise its

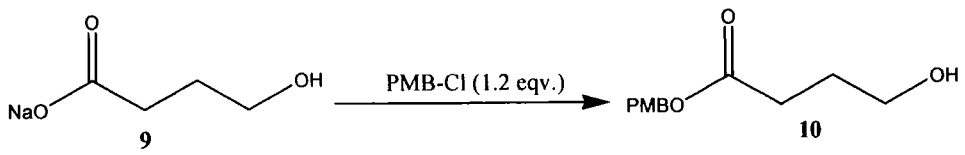


Scheme 2.10

4-methoxybenzyl ester **10** by reacting with 4-methoxybenzyl halide based on general[3] methodologies for benzylation of carboxylic acids.

The reaction of *p*-methoxybenzyl chloride (1.2 eqv) with the sodium salt of 4-hydroxybutanoate in dry DMF and a catalytic amount of sodium iodide with activated powdered sieves was monitored by TLC, the level of reactant consumption was the same after the first 4 h as after 72 h (5% MeOH/ dichloromethane, R_f 0.35). The presence of two different ^{1}H NMR signals (MeOPh$\underline{CH_2}$, δ 5.07 ppm and δ 4.86 ppm) for the same benzyl methylene proton were thought to represent both the product and *p*-methoxybenzyl hydroxide from the incomplete reaction, accompanied by other unassigned signals. On workup, the low yield of 40 % for **10** was attributed to the ease of hydrolysis of *p*-

methoxybenzyl iodide and also could have resulted from the choice of solvent (DMF) and the consequent decomposition of the highly reactive *p*-methoxybenzyl iodide.



Scheme 2.11

Reaction No	Reagents & Solvent	Condition(s)	Yields %	Comment(s)/ Others (%)
01	NaI, DMF	25 °C, 72 h	40	**Decomposition
02	TBAB, DCM	25 °C, 72 h	51	4-hydroxybutanoic acid 40 %
03	TBAB, THF	25 °C, 72 h	66	4-hydroxybutanoic acid 30 %
04*	TBAI, acetone	Reflux, 24 h	**80-92**	**Decomposition
*Literature[4], ** including 4-hydroxybutanoic acid amongst the products				

Table 2.1

The solvent system (DMF) was therefore substituted with THF and dichloromethane respectively in subsequent reactions (Table 2.1) without sodium iodide in order to reduce possible hydrolysis. However in both cases after 72 h only a limited reaction was observed without NaI. When a catalytic amount of tetrabutylammonium bromide (phase transfer agent) was added and NaI was

omitted from the reaction mixture for a 72 hours (monitored by TLC) reaction period, the reaction gave **10** as a colourless oil in 51 % in dry dichloromethane and 66 % in dry tetrahydrofuran with 40 % and 34 % of 4-hydroxybutanoic acid recovered respectively from the resulting brown oil after chromatography. It was noted that on addition of catalytic NaI to the reaction, the yields were significantly reduced and increased decomposition that is, increased quantity of by-products were observed.

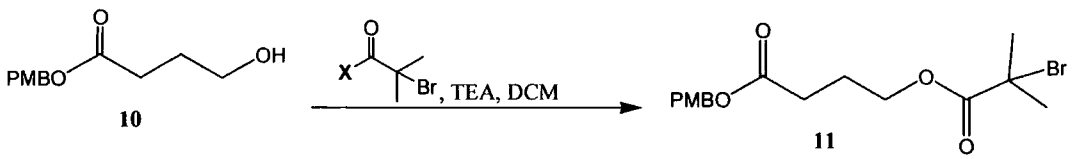
The above observations suggested that while increased solubility of reagents/reactants increased the rate of reaction, the increased reactivity associated with introducing a more reactive substituent (Table 1) also increases the rate of side reactions including hydrolysis. The process leading to the decomposition material was not comprehensively investigated but minimised by avoiding NaI.

During the course of our attempt to efficiently use *p*-methoxybenzyl chloride in alkylating sodium 4-hydroxybutanoate, a different and successful approach was reported[4] involving the reflux of *p*-methoxybenzyl chloride and sodium 4-hydroxybutanoate in acetone to afford the protected carboxylic acid in good yield. We therefore attempted the reaction in accordance with literature, 1.2 equivalents of *p*-methoxybenzyl chloride was stirred with the 4-hydroxybutanoate salt in acetone before adding a catalytic amount of tetrabutylammonium iodide and stirred for 24 hours. The reaction mixture was then filtered, concentrated and diluted with dichloromethane before being washed with water. The resulting brown oil was dried and purified by flash column chromatography to give **10** in 92 % yield - a result that was further reproduced on multigram scale synthesis.

2.3.2 Synthesis of 4-methoxybenzyl 4-(2-bromo-2-methylpropionyloxy)butanoate

Introduction of the ATRP initiator moiety on to the linker was achieved by converting the free 4-hydroxyl group on 4-methoxybenzyl 4-hydroxybutanoate **10** to the key intermediate **11** by reacting respectively with bromoisobutyryl bromide **21** and N,N-dimethylaminopyridine (cat.) at room temperature overnight. The progress was monitored by TLC and column chromatography

gave the product (**11**) 54 % yield. When **8** was used instead of bromoisobutyryl bromide **21** to react with 4-methoxybenzyl 4- hydroxybutanoate **10**, the yield was improved by 20 % (to 72 %) of **11**, Scheme 2.12.



X = Br (**21**), 54%,
X = oxy succinimidyl ester (**8**), 72 %

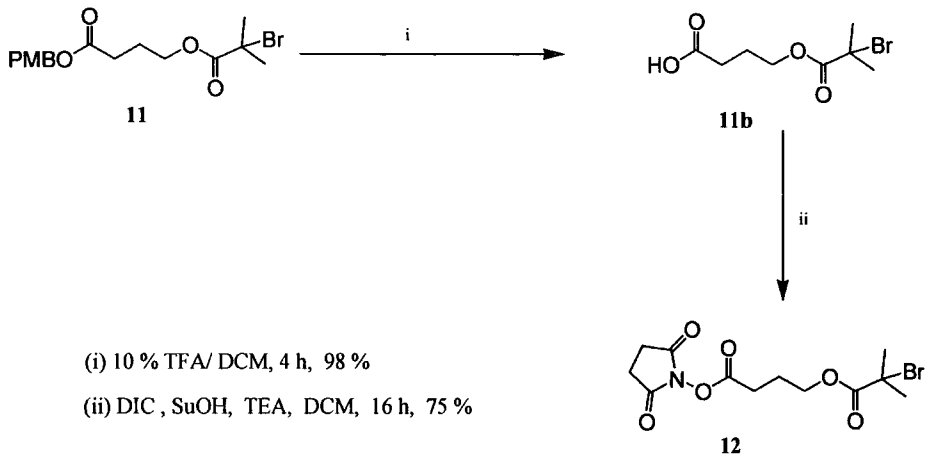
Scheme 2.12

The increased yield can be attributed to the mildness of the liberated spectator molecules and their influence on the system (product and reagents). The liberation of hydrogen bromide (strong acid) when α-bromoisobutyryl bromide was used with the absence of an adequate quantity of triethylamine to neutralise the reaction mixture, had the probable effect of deprotecting the *p*-methoxybenzyl esters **10** and **11** to their carboxylic acids. The problem can be overcome by using a greater number of equivalents of more hindered base (i.e. DBU) or as proven above, Scheme 2.12, the use of **8** that produces a less hazardous spectator species - *N*-hydroxysuccinimide.

2.3.3 Synthesis of N-succinimidyl 4-(2-bromo-2-methylpropionyloxy)butanoate

The final stage for the synthesis of the described alkyl linker was approached as shown below, using standard deprotection and coupling methodology. The *p*-methoxybenzyl protected bromoalkyl spacer **11** was treated with 10 % TFA/ dichloromethane (1:1, v/v) for approximately 4 hours at room temperature during which a dark red colouration was observed. The consumption of **11** was monitored by TLC. Upon completion, the mixture was quenched with water and saturated $NaHCO_3$ – further treatment of the aqueous extract gave the

intermediate carboxylic acid **11b** in 90 % yield confirmed by molecular ions at m/z 274.9912/276.9894 in high resolution mass spectroscopy which was in agreement with the calculated value of m/z 275.000/276.998 for $C_8H_{13}BrO_4Na$. The free carboxylic acid **11b** was treated under standard coupling conditions with 1.2 equivalents of DIC, 1.2 equivalents of N-hydroxysuccinimide and 2.0 equivalents of triethylamine in dry dichloromethane.



Scheme 2.13

The mixture was stirred for 16 h at room temperature, monitoring of the reaction by TLC showed the appearance of a new spot and further investigation by observing the ^{1}H NMR spectrum also showed a new four-proton succinimidyl signal at δ 2.85 ppm after 12 h. Purification by column chromatography gave the target succinimidyl product **12** in 75 % yield (Scheme 2.13), this was confirmed by HRMS which agreed with the calculated values.

No	Reagents/Condition	Duration (h)	Analysis (^{1}H NMR & ES/MS)
1	20% Piperidine/ DMF	0.5	No decomposition
2	TFA/ Water/ TIS	4	No decomposition
3	TFA/ Water/ TIS/ EDT 32 : 2 : 2 : 1 (ratio)	4	No decomposition

Table 2.2

However, our model test (Table 2.2) to determine the stability of the bifunctional linker **12** in a peptide synthesis environment, showed the substrate to be unaffected within a specific period of time representative of the intended solid phase peptide synthesis (SPPS) protocol in Table 2.

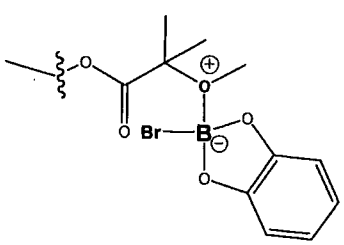
2.4 Section B: Synthesis of α-methoxy masked diester

2.4.1 Introduction

As demonstrated during the synthesis of **8** (Scheme 2.6) and **11** (Scheme 2.10) of Section A, the α-bromoisobutyryl unit was sensitive and therefore susceptible to deleterious reactions induced by either the reaction environment or by co-reagents involved in a transformation. Although the model test above eliminated the need for such action during solid phase peptide synthesis (SPPS) for short duration based on the conditions above however, the examples in Section A proved otherwise especially with time. The investigation would hence focus on a simple but routinely effective method of interconverting masked α-halide functionality for use in (for example) prolonged and potentially detrimental transformation/functionalisation processes.

2.4.2 AIMS

This is to synthesise **15** (Scheme 2.16) and perform an efficient functional group interconversion of the more stable masked substrate **15** to its active species **12** based on Scheme 2.2. The masking functional group would be of the form of an



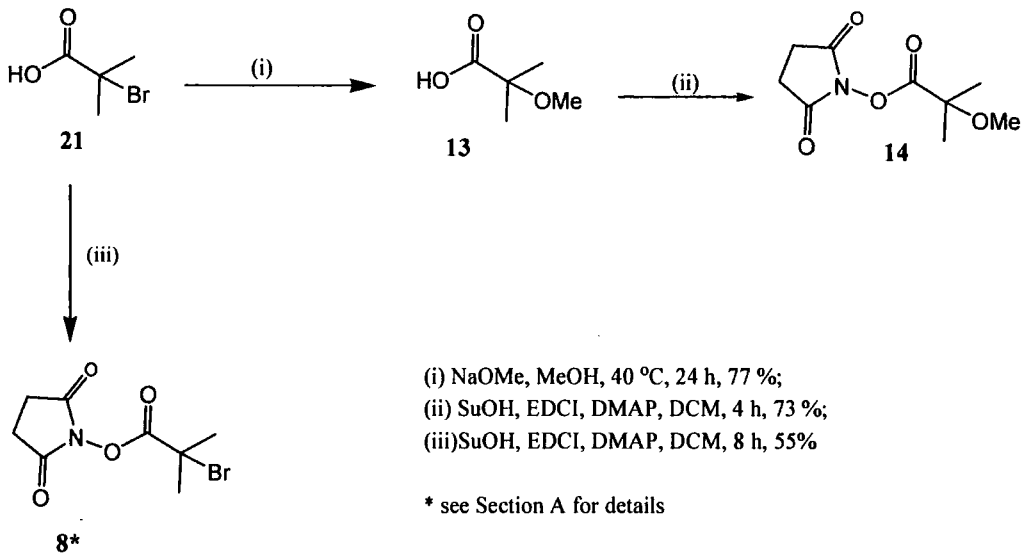
Nucleophilic Boron intermediate

Scheme 2.14

alkyl ether synthesised as shown in Scheme 2.2 (and elaborated on later), and transformed using agents based on Lewis acidic organoboranes. Monofunctional organoboranes of the general formula R_2BBr were envisaged to be suitable halogenating agents because of their steric and electronic nature (Figure 2), which should influence the S_N1 vs S_N2 reactivity of an appropriate ether-type substrates and consequently the expected transformation. The attempts are discussed in the next section.

2.4.3 Synthesis of N-Succinimidyl 2-methoxy-2-methylpropionate

Starting from the commercially available 2-bromo-2-methylpropionyl bromide **21**, the syntheses of N-succinimidyl 2-methoxy-2-methylpropionate **14** (Scheme 2.15) would be established from its carboxylic acid based on modified literature procedure.[5] The masking group (α-methoxy) was expected to form a more stable substrate but easily convertible to its corresponding alkylbromo moiety under mild conditions[6] using a simple procedure proposed to involve a nucleophilic boronic intermediate species (Figure 2), thus α-methoxy was chosen as a suitable substitute.



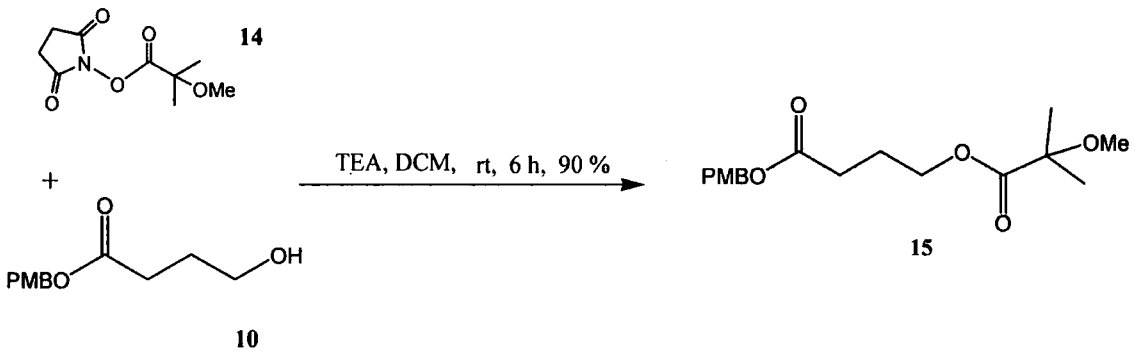
(i) NaOMe, MeOH, 40 °C, 24 h, 77 %;
(ii) SuOH, EDCI, DMAP, DCM, 4 h, 73 %;
(iii)SuOH, EDCI, DMAP, DCM, 8 h, 55%

* see Section A for details

Scheme 2.15

The nucleophilic substitution of the bromide in the commercial 2-bromo-2-methylpropionic acid was achieved by stirring with freshly prepared NaOMe (2.2 eqv) in methanol at 40°C, and the resulting mixture was monitored by TLC over a period of 24 h, and the diminishing ^{1}H NMR signal at δ 1.92 ppm for the α substituted tertiary halide was monitored. The reaction was then acidified to pH 1, filtered and the methanolic filtrate concentrated and subjected to further workup to give **13** in 77% yield, Scheme 2.15. The structure was observed in ^{1}H NMR spectrum as signal for six proton singlet at δ 1.4 ppm and three proton singlet at δ 3.3 ppm corresponding to the dimethyl and methoxy respectively, adjacent to the carboxylic acid. The structure was further confirmed by the presence of a molecular ion [M+Na$^+$] at m/z 141 in the mass spectrum.

Attempts at increasing the yield by varying time and temperature proved unsuccessful, often resulting mainly in reduced yield and the recovery of the bromoisobutyric acid especially at temperatures below 40 °C, while decomposition products became more prominent at temperatures above 60 °C. The carboxylic acid obtained was then treated with standard coupling reagents as described for **8** (Scheme 2.6) in Section A with N-hydroxysuccinimide to yield the activated ester **14** in 73% yield. The compound was confirmed by the presence of the new four-proton singlet



Scheme 2.16

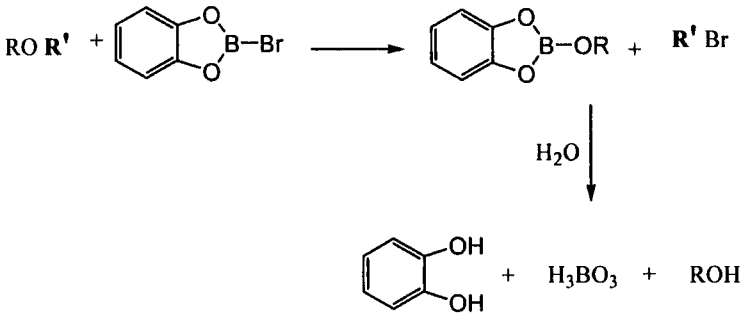
signal at δ 2.8 ppm corresponding to CH_2CH_2 of succinimide in the ^{1}H NMR spectrum and the presence of a molecular ion [M+Na$^+$] at m/z 238 in the mass spectrum.

The synthesis of **15** was easily accomplished by reacting 1.1 equivalents of **14** with **10** in 90% yield following the procedure described for **11** (Scheme 2.13). The replacement of the two proton multiplet at δ 3.6 ppm for CH_2OH with two proton triplets at δ 3.8 ppm corresponding to the acylated hydroxyl group in the ^1H NMR was used to monitor the reaction progress and the structure was further confirmed by presence of the molecular ion $[M+Na^+]$ at m/z 347 in the mass spectrum. With the substrate **15** in hand, the next section discusses the attempts at the proposed interconversion with organoborane reagents.

2.4.4 Model conversion using organoborane reagents, Part 1

2.4.4.1 Attempted synthesis of α-bromoisobutyryl ester from α-methoxyisobutyryl units using organoborane reagents.

The use of boron in synthesis often takes advantage of their high selectivity[6] (especially monofunctionalised organoborane) in the cleavage of carbon – oxygen bond, and their method of reactivity (S_N1 Vs S_N2) can be modified based on electronic

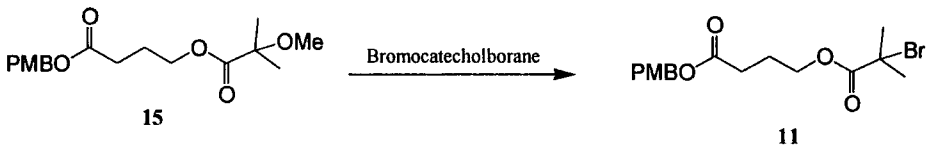


Scheme 2.17

and steric control of the boron/ligand composition. Diphenylboron bromide and dimethylboron bromide[7]are the most prominent boron reagents for such studies.

Other literature[8] had already described how with or without a co-reagent ($BF_3.OEt_2$), various commonly used ethers on azo dyes have been converted into bromides using catecholboron bromide at room temperature based on the mechanism shown below, Scheme 2.17. Published data showed catecholboron bromide to represent an even milder but equally efficient reagent with reactions often completed in one to two hours (but varies from 5 min, 0.5 h to 36 h), which after an aqueous workup gives the products in excellent yields (84 – 95 %).

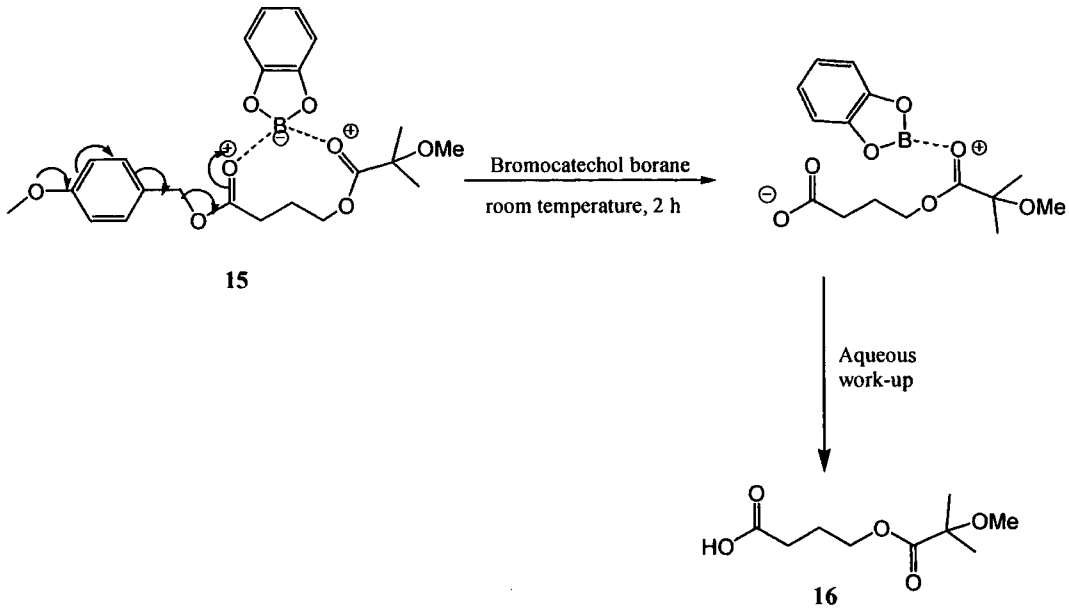
The unmasking via borane-assisted functional group interconversion (FGI) of the masked ATRP substrate **15** to its bromoalkyl ATRP functional group in **11** (Scheme 2.13) was intended to occur under mild conditions to protect the surrounding potentially sensitive biomacromolecule (e.g. peptide, protein). The α-methoxy alkyl **15** was therefore subjected to the literature conditions by the treatment with 1.1 equivalents of bromocatechol borane at 0°C in dry dichloromethane.



15 **11**

Scheme 2.18

However, no reaction was observed by TLC analysis after a period of 4 hour. The reaction was then allowed to stir at room temperature for a further 4 hour, during which several new spots not corresponding to the expected product were observed as major components of the crude mixture, hence the reaction was quenched with water and aqueous $NaHCO_3$. After an aqueous workup, the column chromatography gave unreacted **15** in 60 % with other unassigned by-products.

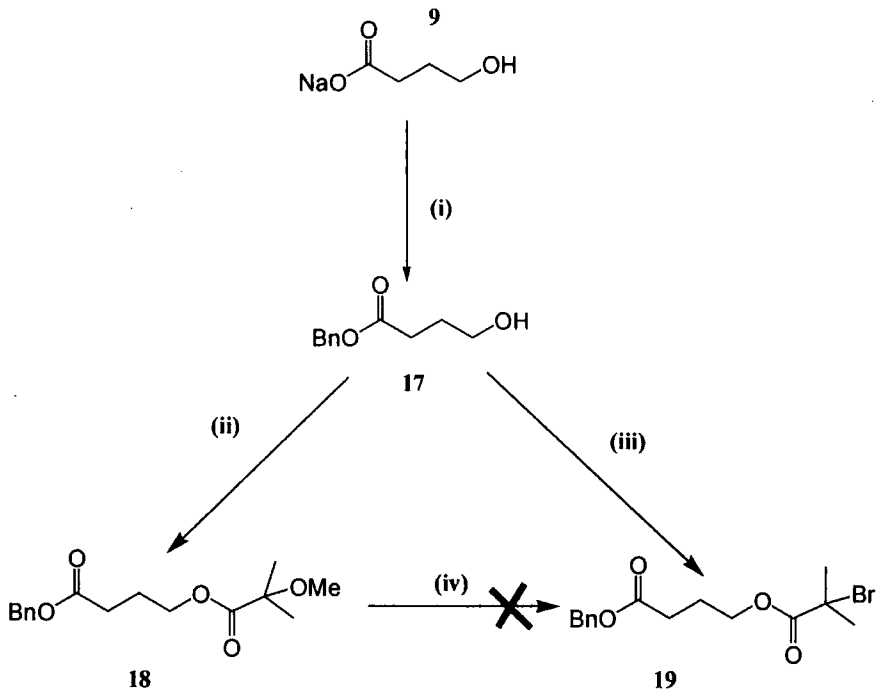


15 **16**

Scheme 2.19

When the reaction temperature was reduced to –78 °C for 6 h, analysis by TLC showed patterns similar to that described above with 50 % of **15** and 25 % unassigned by-products recovered after workup. The observed results were proposed to have been facilitated by the high affinity for electrons by catecholborane in the neutral environment that aided the facile removal of the *p*-methoxybenzyl ester instead of the other ester or indeed the desired ether.

Hence, attempts to reduce the electronic contributions from the potential conjugating system present in the *p*-methoxybenzyl ester (Scheme 2.19) were



(i) BnBr (1.2 eqv.), DMF, rt, 48 h, 97 %; (ii) 2-methoxy-2-methylpropionic acid (1.2 eqv.), EDCI (1.2 eqv.), DMAP (1.2 eqv), DCM, 51 %; (iii) 2-bromo-2-methylpropionyl bromide (1.2 eqv), TEA, DCM, 5 h, 56 %.; (iv) Bromo catechol borane.

Scheme 2.20

proposed. They would ensure the adjacent carbonylate was preferentially and adversely transformed. A benzyl-protected alkyl ester was therefore proposed and synthesised using standard methodology.

The phenyl protecting group without electron donating substituents would be a less labile protecting group and therefore avoid intramolecular hydrolysis even in the presence of species capable of hydrolysis.

The benzyl ester was synthesised by treating the sodium salt of 4-hydroxybutanoic acid **9** in dry DMF with tetrabutylammonium bromide (cat.) and 1.2 equivalents of benzyl bromide for 48 hours at room temperature. The crude mixture was subjected to *in vacuo* removal of DMF before being subjected to an aqueous workup and column chromatography. This gave **17** in 97% yield. The identity of the compound was further confirmed by the presence of a two-

proton singlet at δ 5.15 ppm for benzyl methylene in the ^{1}H NMR spectrum and by the molecular ion at 217 ($M + Na^+$), which agreed with calculated value of 217 for $C_{11}H_{14}BrO_3Na$ (Scheme 2.14). Subsequent treatment with 1.2 equivalents of 2-methoxy-2-methylproionic acid **13** or 1.2 equivalents of α-bromoisobutyryl bromide **21** was carried out according to previously described methods for **11** and **15** to give **18** in 51 % yield and **19** in 56 % yield respectively with high recovery of **17** in both cases. The yield could possibly be improved by using a greater excess of reagents relative to **17**. However, as the aforementioned compounds **18** and **19** were only initially required in milligram quantities for test conversion purposes, it was not considered necessary to attempt to improve the yield.

2.4.5 Model conversion using organoborane reagents, Part 2.

Compared with the standard approaches that used strong acid or base cleavage methodology, the mildness and neutral nature of the above methodologies therefore made catecholboron bromide our first choice as a reagent for converting α-methoxyether into its α-bromo equivalent. One equivalent of bromocatecholborane was stirred in dry dichloromethane containing **18** at room temperature. The reaction was monitored by TLC over a period of 24 hours but

18 **19**

Scheme 2.21: *Some of the attempt at unmasking using mild organo-boron based reagents*

unfortunately showed no change. The same result was observed when the ratio of borane to substrate was increased to 4:1 in order to compensate for the necessity

for coordination. The above approach also showed no reaction over a period of 24 hours in dry dichloromethane as judged by TLC with the substrate recovered quantitatively. While the temperature was kept constant at room temperature and the duration maintained at 24 hours, three different boron reagents and conditions were employed to no avail. They included the use of boron tribromide (one and four equivalents), dimethylboron bromide (one and four equivalents) in toluene, acetonitrile or dry dichloromethane.

Due to time constraints the required transformation was not realised.

2.5 References

1 M. Mammen, G. Dahmann, G. M. Whitesides, *J. Med. Chem.* **1995,** *38*, 4179.

2 W. Lossen, E. Kowski, *Justus Liebigs Ann. Chemie*, **1905**, *342*, 127.

3 J. M. Sanderson, P. Singh, W. G. Colin, J. B. C. Finlay, *J. Chem. Soc., Perkin Trans 1*, **2000**, 3227.

4 J. A. Ellman, T. P. Tang, *J. Org. Chem.* **2002**, *67*, 7819.

5 Von P. Ruedi, C. H. Eugster, *Helv Chim Acta,* **1971,** *54*, 1606.

6 R. K. Boeckman, J. C. Potenza, *Tetrahedron Letters*, **1985**, *26*, 1411.

7 Y. Guidon, C. Yaokim, H. E. Morton, *Tetrahedron Letters*. **1983**, *24*, 2969.

8 P. F. King, S. G. Stroud, *Tetrahedron Letters* **1985**, *26*, 1415.

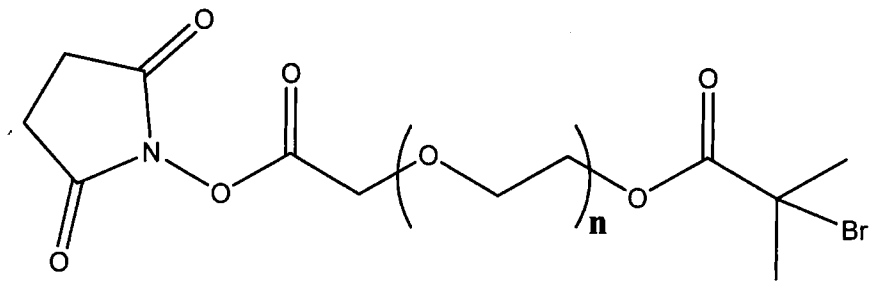
CHAPTER 3

SYNTHETIC APPROACH TO MEDIUM

LENGTH POLYETHYLENE GLYCOL BASED

ATRP INITIATORS

(A Hydrophilic Hetero-bifunctional Linker)



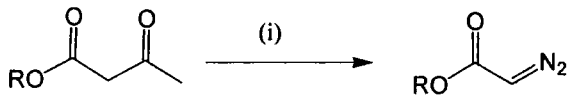
3.1 Introduction

Our target linker reagent, inspired by the synthesis of zwitterionic (or Huisgen ylid) intermediates of carboxymethyl esters, is a succinimidyl ester that incorporates a medium length polyethylene glycol (of the tri, tetra and penta series) unit as spacer in the developed potential ATRP initiators. Polyethylene glycols are degradable by both enzymatic and chemical hydrolysis, and have been described[1] as effective component of bioconjugation strategy as observed to also enhance half life, solubility and biocompatibility (and consequently biodistribution) of, for example, drug bioconjugates[2] than the drug's native form e.g. pegylated recombinant interferon[3]

The pre-requisite α-bromoisobutyryl unit has already been utilised by various groups in the presence of polyethylene glycol for living polymerisation; furthermore, Matyjazewski and Armes for example, have both independently described the effect of aqueous media on living polymerisation with favourable effect on polymerisation and resulting polymer properties i.e. narrow polydispersity index (see Section 1.4).

3.1.1 AIMS

In this chapter we are concentrating on the development and synthesis of N-succinimidyl functionalised polyethylene glycol of the α-bromoisobutyryl substrate, a precursor of potential ATRP initiators unit for polymerisation.

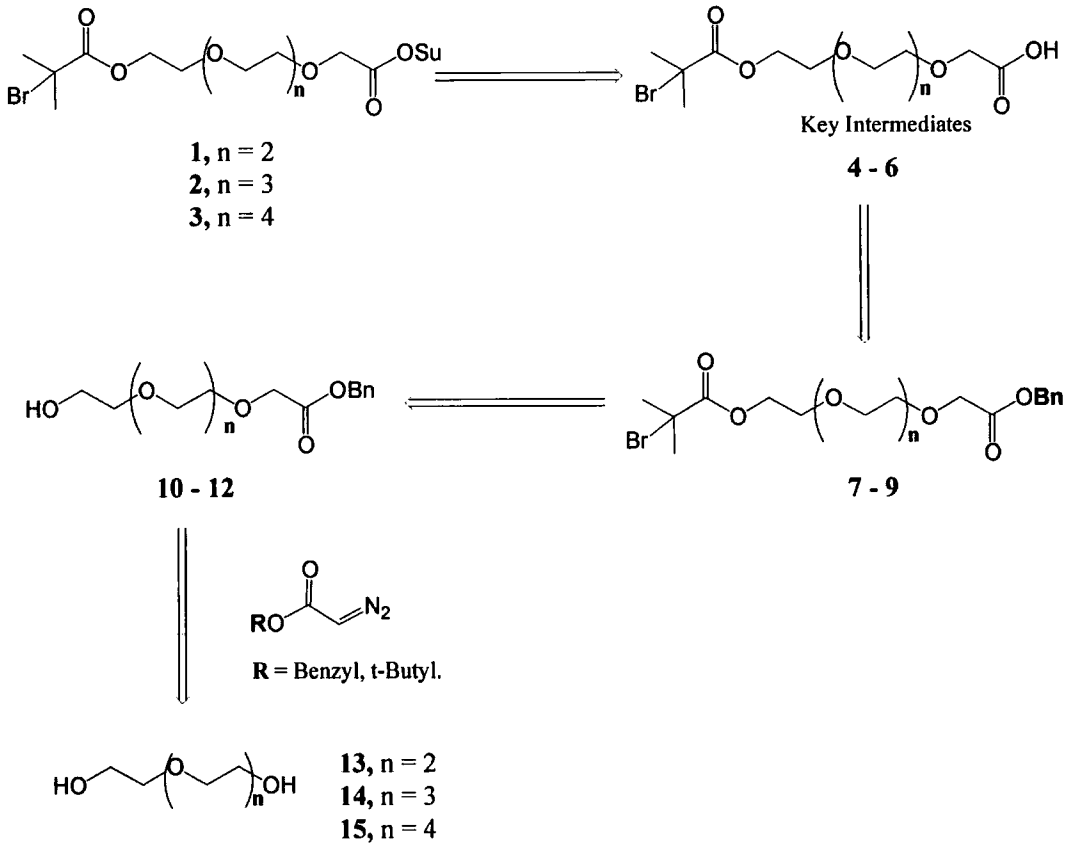


(i) R' N_3, Base, DCM

Scheme 3.1: *Proposed alternative to haloacetate approach.*

The synthesis of the linker reagents (**1** - **3**) was based on the Lewis acid assisted insertion of carbene into OH σ-bond using diazonium transfer agents, Scheme 3.1, R = Bn, *tert*-Bu, R'=Me, Tol.

This approach provides access to the carboxymethyl unit under milder reaction conditions and better yield than the alternative methodology[4,5]that (for example) utilises bromoacetic acid. The protecting group prevents complication with the monofunctionalised PEG while the preferred benzyloxycarbonyl protecting group of the diazo is ideally suited to our goal, being stable to the reaction condition employed and easily removed in near neutral conditions. Access to the key intermediate (**4** - **6**) was expected to be *via* the condensation of an appropriately activated α-substituted isobutyryl substrate with the free hydroxyl group to yield (**7** - **9**) prior to debenzylation. The applicaton of a traditional coupling technique to the key intermediate should then afford the target compound, Scheme 3.2.



Scheme 3.2: *Retrosynthesis of the carboxymethyl of N-Succinimidyl ester (1-3).*

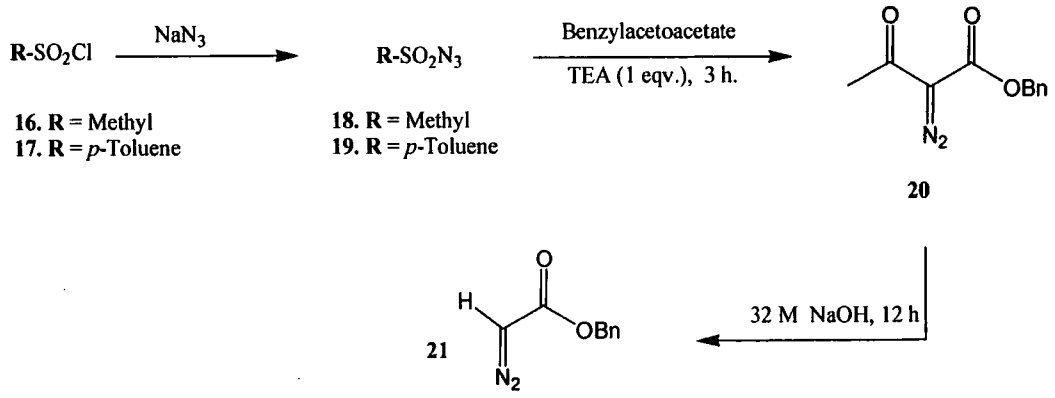
This methodology would allow the fast and convenient large-scale synthesis of a series of compounds for the chemoselective labelling of biomacromolecules for use in living polymerisation of bioconjugates.

3.2 Synthesis of carboxymethyl ester substituted PEGs as reagents for ATRP

The basic design principle of **12**, (chapter 2) was extended to the synthesis of a more hydrophilic and more flexible ATRP agent utilising the intrinsic advantages (discussed above) offered by medium length polyethylene glycol groups, namely – ease of functionalisation and aqueous solubility[6]. One such functionalisation technique utilises a diazonium-based transfer approach to create a new carbon – carbon bonds or carbon - oxygen bonds *via* a carbene insertion reaction. To this end the first intermediate to be synthesised was benzyl 11-hydroxy-3,6,9-trioxaundecanoate **10**. We therefore first needed to synthesise the required diazo transfer agent, benzyl diazoacetate – which was achieved according to literature methods[7].

3.2.1 Synthesis of benzyl diazoacetate (via diazo transfer agent)

The diazo sources (methyl and *p*-toluenesulphonyl azide) were prepared according to literature method[8] by treating 1.4 equivalents of sodium azide with methanesulphonyl chloride **16** in acetone at room temperature over a period of



16. R = Methyl
17. R = *p*-Toluene

18. R = Methyl
19. R = *p*-Toluene

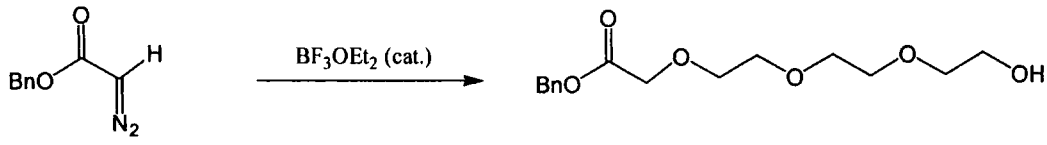
Scheme 3.3

2.5 hours to give the azide **18** in quantitative yield. This was confirmed by characteristic infrared signals at 2146 cm^{-1} (N=N). Alternatively, 1.5 equivalents of sodium azide in a mixture of water and ethanol solution was added to the solution of *p*-toluenesulphonyl chloride **17** in ethanol maintained at 45°C, after twelve hours gave the tosylazide **19** in quantitative yield confirmed by infra red signals at 2132 cm^{-1} (N=N, str) The azide **18** or **19**, was treated with triethylamine and benzyl acetoacetate in anhydrous acetonitrile for three hours at room temperature before adding potassium hydroxide, and stirred for a further twelve hours. After purification by silica gel chromatography, the reaction gave **21** in quantitative yield. The intermediate **20** was often recovered after adding and stirring with triethylamine but before the addition of KOH in attempt to initially improve the yield of **21**. However, it was observed that the prolonged one-pot reaction (described) with KOH had similar effects (similar yields) thereby negating the need for additional isolation-purification step. The diazoacetate was further confirmed by ^1H NMR spectroscopy and characteristic diazo infrared stretch at 2115 cm^{-1} corresponding to nitrogen (N=N).

3.2.2 Monofunctionalisation of ethyleneglycol

With the diazo at hand, we next attempted to functionalise the polyethylene glycol by Lewis acid assisted degradation and the subsequent carbene sigma bond insertion.

Benzyl diazoacetate **21** in dichloromethane was treated with three equivalents of triethylene glycol under Lewis acid catalyst condition and at room temperature to



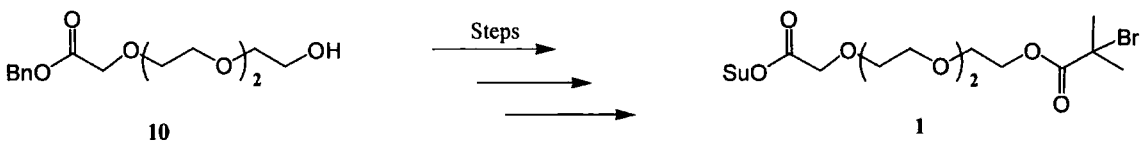
21 **10, n = 88%**

Scheme 3.4

yield the monobenzylacetyl substituted triethylene glycol **10** in 88% yield after flash chromatography, Scheme 3.1. The product was confirmed by the presence of the distinctive two proton singlet and the five proton multiplet ^{1}H NMR signals at δ 5.2 ppm and 7.3 ppm in the spectrum corresponding to the benzyl methylene (CH_2Ph) and phenyl protons (CH_2Ph) respectively. The observed [M + Na^+] ion at m/z 321 in the low resolution mass spectroscopy was in agreement with calculated value of m/z 321 for $C_{15}H_{22}BrO_6Na$.

3.2.3 Deprotection of (α-bromoisobutyryl)benzyl decanoate

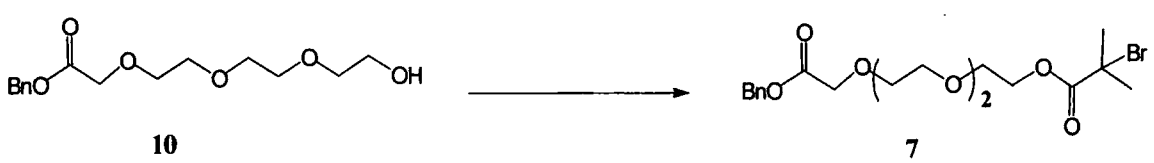
Having functionalised the polyethylene glycol, we then chose a route that involved simple deprotection and condensation steps to introduce the desired α-bromoisobutyryl and N-succinimidyl ester functionality for ATRP initiation onto the proposed water- soluble substrate 1, Scheme 3.5.



10 **1**

Scheme 3.5

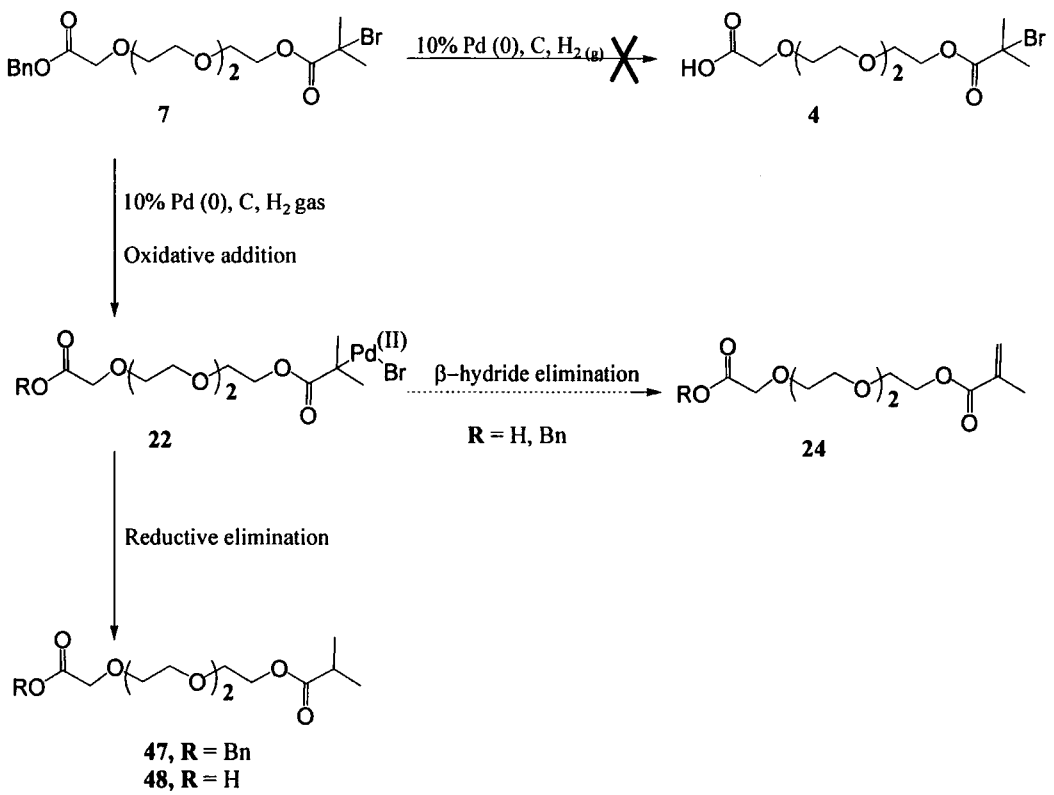
The key intermediate, carboxylic acid **4** was attempted from the corresponding alcohol **10** by treating with 1.2 equivalents of 2-bromo-2-methylpropionyl bromide and triethylamine in dichloromethane, initially at 0°C but then allowed to warm up to room temperature and stirred for eight hours. After workup and silica gel chromatography the benzyl diester **7** was obtained in 78% yield. Formation of the benzyl diester was confirmed by the presence of a new six-proton singlet



10 **7**

Scheme 3.6

at δ 1.9 ppm corresponding to the isobutyryl unit and by the presence of the molecular ion [M+Na$^+$] at 469.0940/471.0920 in HRMS, which was in agreement with the calculated value of 469.0843/471.0826 for $C_{19}H_{27}{}^{79/81}BrO_7Na$. However, the reaction of benzyl diester **7** with a combination of 10% palladium on charcoal, atmosphere of hydrogen gas and in methanol showed an incomplete level of consumption (as judged by TLC) which remained even after stirring overnight at room temperature. After removal of the insoluble/solid materials by filtration, the filtrate was concentrated and dried *in vacuo*.



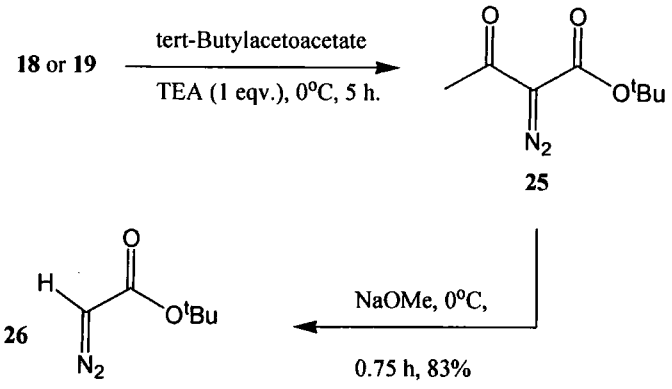
Scheme 3.7

Observed in the resulting ^{1}H NMR spectrum of the crude product was the lack of α-bromo substituted product (a common feature for two of the three possible components of **4** and **7**) as judged by the distinctively absent signal for the associated six proton singlet at δ 1.9 ppm but the appearance of a doublet at δ 1.2 ppm corresponding to an isopropyl unit in addition to the presence of a one

proton multiplet at δ 2.6 ppm. Further analysis by mass spectrometry proved difficult due to presence of large quantities of by-products. However, the infrared spectrum showed the presence of both carboxylic acid (OH) at 3208 cm^{-1} and carboxylate carbonyl at 1735 cm^{-1}. The presence of two of the components was identified by comparing with prepared authentic samples of **47** and **48**. Conceivably, the oxidative addition of the alkyl halide onto palladium (0) may have resulted in the reductive elimination product **22**, and where the concentration of dissolved hydrogen is low, a β-hydrogen abstraction may ensue to reveal the methacrylate **24**, although such process usually proceed or accelerated in the presence of base, Scheme 3.7. Methacrylates are highly reactive polymerisation monomer hence, once formed, could have contributed to the level of by-product observed. Attention was therefore turned to exploring processes of protecting the α–halide by masking with substitutes prior to debenzylation. The various processes of investigations into substitutes for α-bromoisobutyryl subtrate has been described in section 3.4; and discussed below is the actual alternative procedure followed to accomplish the synthesis of target compounds **1** – **3**.

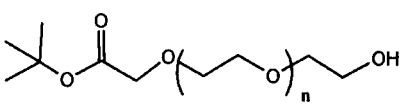
3.3 Synthesis of 'butyl diazoacetate (via diazo transfer agent)

Our next approach relies on the ability to form the *tert*-butyl diazoacetate via the methodology used to prepare the benzyl equivalent (Scheme 3.3) although the prepared *tert*-butyl diazo compound may also be incompatible with our intended target compound.



Scheme 3.8

'Butyl diazoacetate **25** was prepared according to the published method[7] *via* a diazo transfer from an azide onto an active methylene of tert-butylacetoacetate to obtain the diazoacetoacetate in 80% yield, with further decomposition by base assisted deacetylation in acetonitrile at 0°C and distillation at reduced pressure to give the diazoacetate **26** in 83% yield, Scheme 3.8. The formation of the diazoacetate was verified by the presence of a one-proton singlet in the ^{1}H NMR spectrum at δ 2.5 ppm corresponding to the α-hydrogen and a nine-proton singlet



27, n = 2 (71 %)
28, n = 3 (87%)
29, n = 4 (70 %)

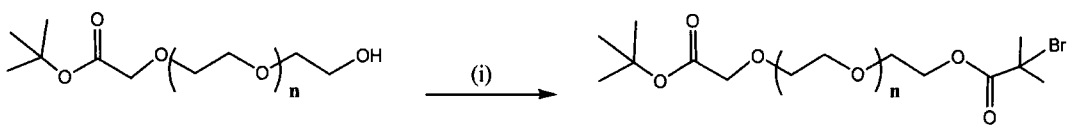
Scheme 3.9

at δ 1.5 ppm corresponding to the tertiary butyl protons. The structure was further confirmed by observation in the infrared spectrum that revealed a nitrogen and carbonyl stretch at 2132 cm^{-1} and 1714cm^{-1} respectively.

We then attempted to monofunctionalise the free hydroxyl group of the selected series of polyethylene glycol with the diazo **26** according to the previously described method for **10** (Scheme 3.4) to give the carboxymethyl of tri, tetra and penta ethylene glycol. The procedure was therefore repeated on tri, tetra and penta ethylene glycol until after 3 hours, the reaction was judged complete, as observed by TLC and subjected the reaction mixture to flash chromatography to give the expected compounds in good yields. The structure of the compounds were confirmed in the ^1H NMR spectrum by the appearance of a new two proton singlet signal at δ 4.0 ppm corresponding to carboxy methylene protons and twelve proton multiplet (for n = 2) at δ 3.6 – 3.7 ppm confirming the presence of the protons of oxyethylene repeating units. The [M+Na^+] molecular ions were also observed at m/z 287 (**27**, 71% yield), 331 (**28**, 87% yield) and 375 (**29**, 70% yield) for tri, tetra and pentaethylene glycol respectively in the mass spectrum.

3.3.1 Synthesis of the series of ATRP initiators.

Proceeding *via* a series of general deprotection and condensation conditions of the robust tertiary butyl ester protecting the required substrates, the title compound was therefore obtained with the desired functionalities and in good yield as described below.



27, n = 2
28, n = 3
29, n = 4

30, n = 2 (94%)
31, n = 3 (94%)
32, n = 4 (80%)

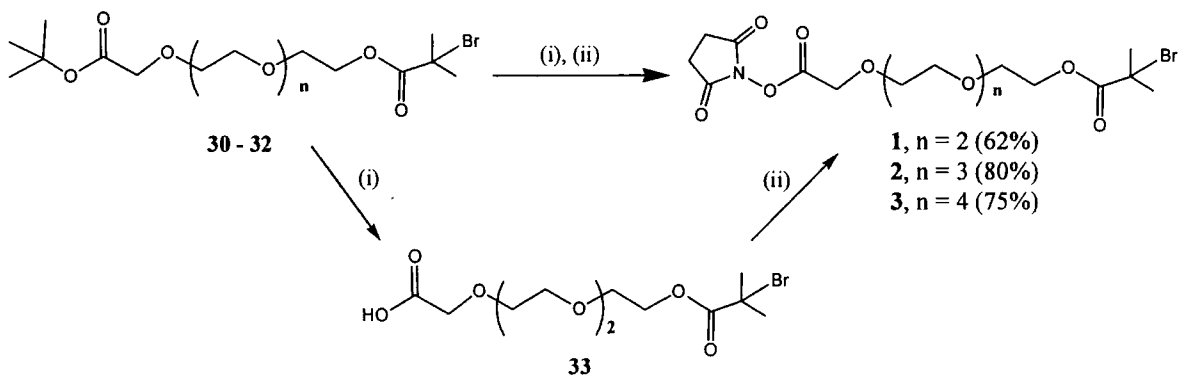
(i) Bromoisobutyryl bromide (1.2 eqv.), TEA (1.2 eqv), DMAP (cat.), 8 h

Scheme 3.10

The next step towards the synthesis of diester (**1** - **3**) was the conversion of the free hydroxyl esters to the corresponding substituted isobutyryl ester.

The isobutyryl ester **30** was obtained in quantitative yield by reacting the hydroxy ester **27** with 1.2 equivalents of triethylamine, N,N-dimethylaminopyridine for one hour at room temperature then reaction was stirred at 0° C for eight hours with α-bromoisobutyryl bromide, Scheme 3.10. Purification of the crude by flash chromatography after workup gave the expected diester **30** in 94% yield. The procedure was then repeated on substrates **28** and **29** to give their esters in 94% and 80% yields respectively. The structure of the compounds were confirmed in the ^{1}H NMR spectrum by the appearance of a new six proton singlet at δ 1.9 ppm and a nine proton singlet at δ 1.5 ppm corresponding to α-bromodimethyl and *tert*-butyl respectively. The compounds were further confirmed by the presence of the [M+Na$^+$] ion in the HRMS spectrum corresponding to $C_{16}H_{29}{}^{79/81}BrO_7Na$, $C_{18}H_{33}{}^{79/81}BrO_8Na$ and $C_{20}H_{37}{}^{79/81}BrO_9Na$, which were in agreement with the expected values

Deprotection of the *tert*-butyl ester **31** (n=2) using a solution of trifluoroacetic acid (30%) in dichloromethane gave the acid **33** in quantitative yield after flash column chromatography. The ^{1}H NMR spectrum no longer exhibited the nine proton singlet for the *tert*-butyl diester, instead showed the presence of the free carboxylic acid proton as a broad singlet at δ 4.6 ppm.



(i), 30% TFA/DCM, 3 h, rt, 95% (n = 2), (ii), SuOH (1.2 eqv), EDC (1.2 eqv), TEA, DMAP, 16 h, DCM.

Scheme 3.11

Coupling of the intermediate carboxylic acid **4** with *N*-hydroxysuccinimide gave the succinimide ester **1** (n=2) in 62 % yield. We observed in the ^{1}H NMR spectroscopic analysis the presence of a new (broad) four proton signal at δ 2.8 ppm corresponding to the succinimidyl cyclic ethylene protons. The compound was further confirmed by the presence of the [M+Na$^+$] ion in the HRMS at m/z 476.0493/478.0553 corresponding to $C_{16}H_{24}{}^{79/81}BrO_9Na$, which was in agreement with the expected value m/z 476.0634/478.0614 for the succinimidyl diester **1**.

The same procedure as for **1** (except for the isolation of the intermediate carboxylic acids) was applied to the synthesis of the succinimidyl esters **2** and **3** in 80% and 75% yields respectively. Their structures were also observed and confirmed by ^{1}H NMR and HRMS analysis to be corresponding to expected signals and calculated values.

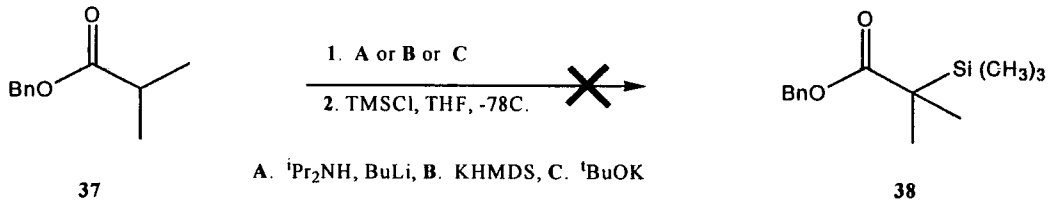
3.4 Investigated synthetic strategies as masking substitutes for α–bromoisobutyryl unit

3.4.1 Silyl electrophile based attempts

In an attempt to develop masked substitutes for the α-bromoisobutyryl unit during the deprotection of benzyl ester **7** (Scheme 3.7) we therefore focussed on commonly used hetero-atoms in order to eliminate or reduce any side reactions i.e. evoked by palladium.

The range and ease with which silyl ethers can be displaced by nucleophilic substitution[9] made this approach attractive and hereafter discussed are our attempts to α-substitute isobutyryl unit, first by exploring silyl compounds.

The ambidental nature of enamide and enol nucleophiles has been exploited in various syntheses to prepare enolates of electrophiles of silyl chloride and derivatives of sulphonic acid where O-silylations are readily formed as the preferred regio-isomeric product of silylation. However, except *via* an alkylhalide, the possibilities and application of silyl chloride in forming C-Si has comparatively rarely been exploited and so initial work concentrated in this area. Discussed below are the attempts made to synthesise α-silylated ester by enolisation with hindered, non-nucleophilic base of firstly an *in-situ* prepared



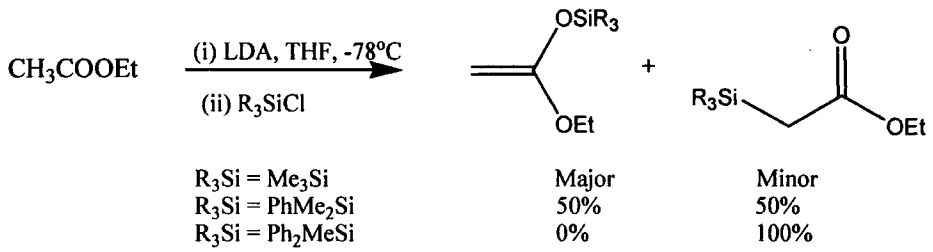
37 38

Scheme 3.12

lithiumdiisipropylethylamine quenched with trimethylsilylchloride at various number of equilvalents. Unfortunately, observed in the ^{1}H NMR of the crude mixture was the one proton sextet, before only isolating the hydrolysis product after chromatography. Similar results were observed when potassium

hexamethyldisilisane or potassium *tert*-butoxide was employed in the process under the same conditions. The observed results were thought to be due to the unstable nature of the electrophile to form the more stable substrate (C-Si) hence, during aqueous workup, the preferred reversible kinetic product (O-Si) decomposed. We therefore turned our attention to a more stable silyl electrophile.

However, studies[10,11] (Scheme 3.13) have shown that the influence of the alkyl/aryl substituents on the silyl electrophile can be exploited to preferentially prepare C-silylation or O-silylation; and that the process is independent of sterics, rather the basicity induced on silylchloride by its substituents; a phenyl substituent renders the silyl center softer thereby reactive towards the corresponding site on the substrate. As a result, diphenylmethylsilylchloride proved to be the suitable silyl electrophile, Scheme 3.13.

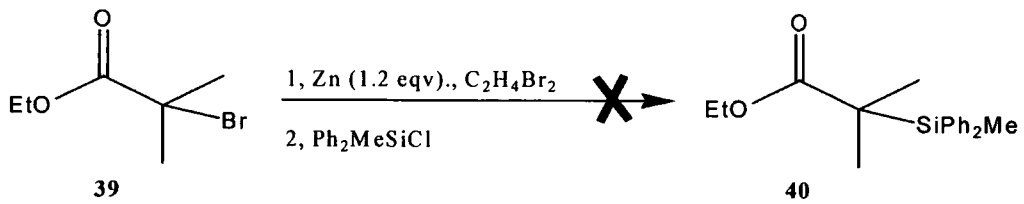


Scheme 3.13: *Literature precedence for C-silylation.*

We proceeded to attempt the synthesis of dimethylsilylbenzyl ester **38** by reacting isopropylbenzyl ester **37** with lithium diisopropylethylamine at –78° C, quenched the lithium enolate with diphenylmethylsilyl chloride and worked the reaction up according to literature procedure however, only the starting benzyl ester and hydrolysed silyl reagent was recovered. Attempts at silylating n-propionylbenzyl ester with diphenylmethylsilylchloride were also unsuccessful as observed by LC/MS-MS analysis. Other variables were also investigated but proved unsuccessful.

Having established literature precedence for the viability of C-silylation, it was reasoned that the required dimethyl substituents provided steric hinderance at the α-carbon centre that could have prevented such reaction. We therefore

proposed the use of the Reformatsky reaction because of the different site of attack (on carbonyl oxygen) utilised, which as a result negates the need for initial steric considerations. Reformatsky methodology[12,13] was therefore attempted, in order to prepare a zinc enolate from the α-bromoester **39** and zinc at reflux based on literature procedure[14]



39 **40**

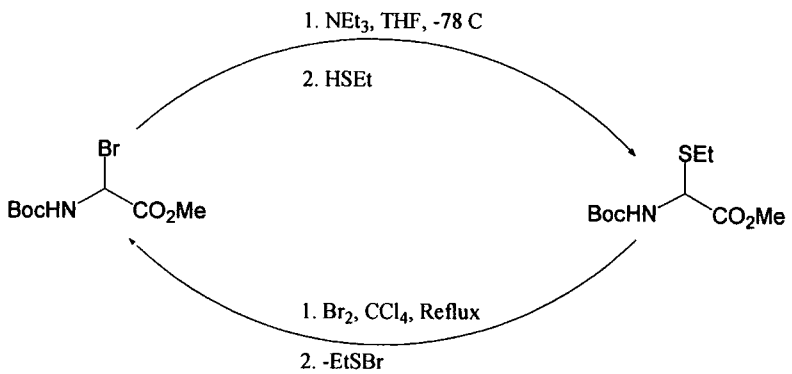
Scheme 3.14

The mixture of **39** and freshly prepared zinc solid was quenched with the diphenylmethylsilyl chloride in order to yield **40** from **39** based on modified literature procedure. On work-up, the unreacted zinc was filtered and the filtrate treated to recover the α-bromoester as identified by ^{1}H NMR. The reaction was then repeated with dibromoethane as catalyst for zinc and after 3 hours refluxing in DMF the zinc had dissolved. The reaction was then quenched with diphenylmethylsilyl chloride to recover the hydrolysed silyl products after aqueous workup and chromatography as the only recognisable material. As all the observations may have suggested, the formation and subsequent decomposition of the enol without the formation of the expected stable C-silyl adduct can be due to the incompabtibility of the electrophile-nucleophile substrate combination. Although with further investigation, greater compatibility can be developed however a different electrophilic heteroatom approach was pursued.

3.4.2 Sulphuryl based attempts

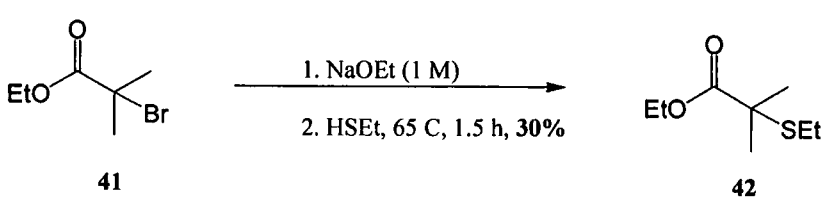
We had described our unsuccessful attempt to synthesise the α-silyl substituted isobutyryl substrates based on literature procedures with the conclusion that incompatibility of electrophile was the probable reason. The formation of α-substituted thiol ether was attractive because of the stability to palladium transformation processes of thiol derivatives and the variety of sulphur-based compounds already employed in synthesis demonstrating versatility.

Furthermore, the inter-conversion of Boc-α-ethylthioglycinate to the corresponding bromo derivatives (Scheme 3.15) had already been described[15] so, initial work concentrated on methodologies based on this strategy



Scheme 3.15

This chemistry was repeated on our α-thioether substrate **42**, which was prepared by treating the corresponding α-bromo propionate **41** with triethylamine before adding ethanethiol based on literature methods[15], Scheme 3.15. The mixture was then monitored with TLC, which showed no reaction after 24 h at room



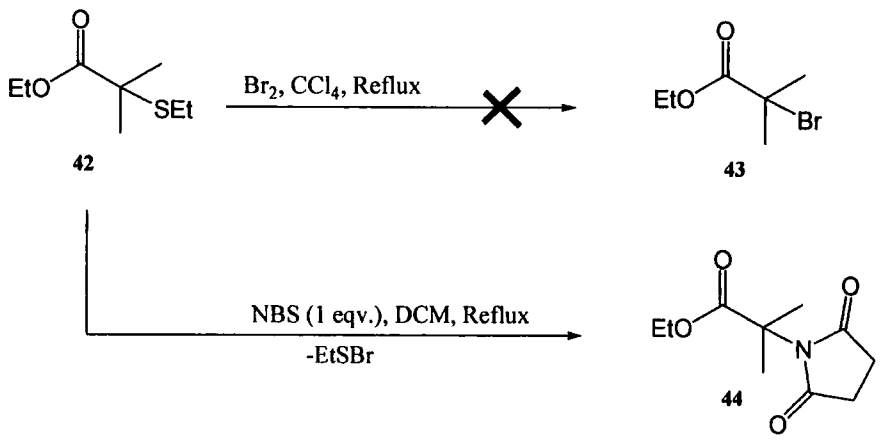
41 **42**

Scheme 3.16

temperature while, on subjecting the reaction mixture to refluxing condition resulted in the recovery of unacceptable level of by-products. The reaction was unsuccessfully repeated under the same conditions with different solvents (THF and DMF) and base (TEA and pyridine). The substitution reaction was then repeated based on the method developed for the synthesis of α-methoxy **8** equivalent (Chapter 2) by stirring with 1.2 equivalents of ethanethiol and one mole of freshly prepared sodium ethoxide. After stirring for 1.5 hours at 65°C, the reaction showed the complete consumption of the ethyl ester as judged by TLC. The precipitates formed were then removed by filtration, concentrated the mother liquor before distillation to give the ethylthio propionate **42** in 31% yield. This was confirmed by the presence of the six-proton singlet at δ 1.6 ppm corresponding to the thioisobutyl units in the ^{1}H NMR spectrum of the product and a carbonyl stretch at 1728 cm^{-1} in the infrared spectrum.

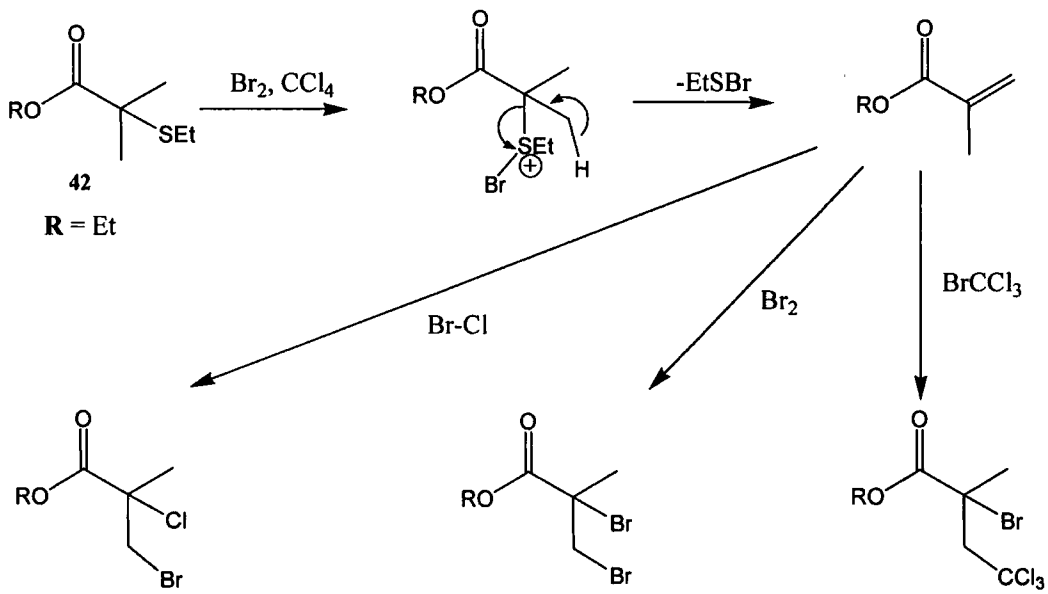
3.4.2.1 Molecular bromine based bromination strategy

However, treatment of α-ethylthioisobutyrate **42** with excess bromine solution in carbon tetrachloride under reflux conditions to evoke the conversion to the α-bromo derivative. After three hours, the conditions resulted in the observation of various halogenated mixtures (Scheme 3.18) as judged by the isotopic



Scheme 3.17

composition and observed by positive electrospray mass spectroscopy. The solvent was replaced with a less reactive dichloromethane but similar results were observed at reflux. The halogenating agent (bromine) was replaced with bromocatechol borane or boron tribromide in carbon tetrachloride or dichloromethane, none of which yielded the expected α-halogenated isobutyryl ester **41** but rather resulted in hydrolysis and degradation products at elevated temperatures. The observed isotopic composition of the mixture using reactive solvents with multiple halogens (i.e. carbon tetrachloride, bromine, dichloromethane) was proposed to have instigated the multiple halogenation process that was further exacerbated by the harsh reaction conditions.



Scheme 3.18

The high temperature could have resulted in the homolytic cleavage of bromine bond, which further cleaved the C-Cl bond of the solvent to result in a multitude of interactions *via* radical chain reaction, Scheme 3.18. Since reactions were only observed at reflux, it was then decided to attempt the conversion with milder, mono-halogenated reagent, for example *N*-bromosuccunimide in carbon tetrachloride.

3.4.2.2 N-Bromosuccinimide based bromination strategy

The use of *N*-bromosuccunimide (NBS) as a highly selective allylic bromination agent in carbon tetrachloride has been documented,[16] furthermore, the substitution of a carbon-hydrogen bond with a carbon-bromide bond has also been discussed.[17,18] Other factors contributing to the selectivity of bond formation process were concluded[19] to include the chain carrier property of the imidyl radical, often resulting in a ring opened imidyl radical accounting for up to 60% of by-product, and hydrogen abstraction by imidyl radical with high selectivity.

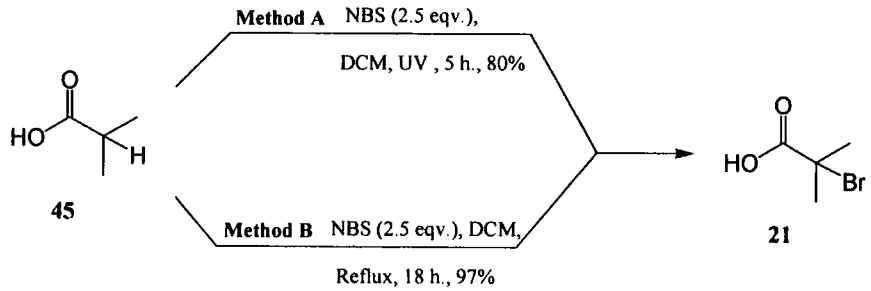
Our initial strategy was to derive the α-bromoisobutyryl substrate by coupling the cheap and commercially available isobutyric acid using standard techniques to the free hydroxyl on a monofunctionalised PEG before introducing the α–halogen to the isopropyl unit post debenzylation depending on conditions and substrate tolerance.

In order to determine the feasibility of the strategy, the radical reaction of one equivalent of NBS with isobutyric acid was carried out in dry, argon degassed CCl_4 under either reflux or radiation conditions under an atmosphere of argon. The reaction was then monitored with TLC to show complete consumption of the acid. Unfortunately the crude material was impossible to identify by ^{1}H NMR, and electrospray mass spectroscopy showed the presence of multiple halogenated products (combinations of chlorine and bromine isotopes, Scheme 3.18. To reduce deleterious influence of the reactive solvent (i.e. CCl_4) on the process, a less reactive solvent (methylene chloride) was therefore proposed. The reaction was then repeated in freshly distilled dichloromethane with one equivalent of NBS and monitored with TLC until complete consumption was observed. The reaction mixture, after aqueous workup and drying *in vacuo* was examined by electrospray spectroscopy to reveal little or no halogenated isotopes. It was speculated that the observation was due to the relative low solubility and ring opening potential of NBS that has proven more favourable to the formation of NBS open ring adducts with the isopropyl unit as evident by the presence of m/z 214 ($M+H^+$) corresponding to ethyl 2-methyl-2-(N-succinimidyl) propionate **44** (Scheme 3.17) and four proton singlet at δ 2.8 ppm corresponding to succinimidyl proton in ^{1}H NMR of the crude sample. Attempts at purification proved difficult.

We therefore decided to increase the ratio of NBS to increase the rate of hydrogen abstraction[20] and/or the bromine radical concentration and perhaps consequentially reduce unwanted succinimidyl adducts formation. By evaluation with authentic commercial sample, α–bromoisobutyric acid **21** was observed as the product of the reaction of 2.5 equivalents of NBS with isopropionic acid in dry dichloromethane under an argon atmosphere after refluxing for 18 hours or UV radiation for 5 hours as judged by TLC. After filtration, aqueous workup and drying *in vacuo*, the crude product **21** was obtained in yields of 97% (by reflux) and 80% (by UV) respectively. Analysis without further purification of the compound by ^{1}H NMR showed a six-proton singlet at δ 1.9 ppm for the α-bromoisobutyryl unit that compared favourably with an authentic sample. With the model conversion procedure of C-H to C-Br established, we therefore attempted to repeat the process of bromination on the intended PEG substrate in order to further access the viability of the strategy.

3.4.3 Model functionalisation of isopropyl unit by NBS

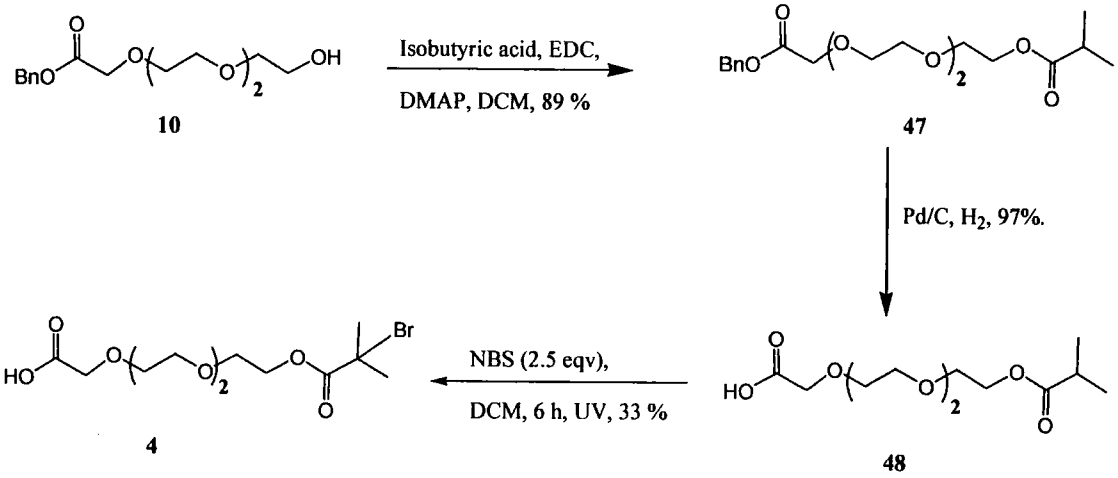
We were able to demonstrate with a simple model, the efficient bromination of an isopropyl unit under two different conditions. This chemistry was expected to be repeated on the intended substrate **48**, to obtain the expected product **4** in



Scheme 3.19

good yield. First, the precursor benzyl isopropyl diester **47** was prepared by coupling the free hydroxyl group on the monofunctionalised **10** with two

equivalents of isobutyric acid in dry dichloromethane with N,N-dimethylaminopyridine and EDCI. The isopropyl diester **47** was obtained in 89% yield after column chromatography. The product was confirmed by molecular ion at m/z 391 in the mass spectrum, which was in agreement with the calculated value of 391 for $C_{19}H_{28}O_7Na$ and ^1H NMR. Treatment of the isopropyl **47** with 10% palladium on carbon in methanol under an atmosphere of hydrogen at room temperature gave a quantitative conversion to the carboxylic acid **48**, as observed by the disappearance of the five proton multiplet at δ 7.4 ppm corresponding to the phenyl protons and the appearance of an infra red stretch at 3208 cm^{-1} in the spectrum for OH corresponding to the liberated carboxylic acid



Scheme 3.20

With the carboxylic acid at hand, we therefore attempted the model bromination reaction (Scheme 3.20) on the key intermediate **48** to produce the final product after activation with N-hydroxysuccinimide. Using the exact concentration as determined from the model system above, 2.5 equivalents of NBS in dry dichloromethane was reacted with 11-(2-methylpropionyloxy)3,6,9-trioxaundecanoic acid **48** under radiation condition. After 8 h, no further consumption was observed of the acid and the crude reaction was filtered and analysed. ^1H NMR showed the presence of a mixture of isopropyl and substituted α-bromoisobutyryl unit (3:1) as determined from the relative integral of the corresponding dimethyl unit at δ 1.1 ppm and δ 1.9 ppm respectively. Unfortunately, even after changing the variables for example, time, glassware

(silicate or borate) and distance of UV source, the reaction showed no significant improvement. When the reflux strategy established above was applied, indiscriminate/multiple bromination product was observed by mass spectroscopy. The route was therefore not pursued further.

It was expected that generating α-bromo isobutyryl unit from the more stable isobutyric acid would be, as demonstrated above, more practical and stable to palladium assisted debenzylation and this was achieved however, in a poor 33% yield.

3.5 References

1 S. Zalipsky, *Adv. Drug. Delivery.* Rev., **1995**, *16*, 157.

2 M. Pechar, K. Ulbrich, V. Šubr, L.W. Seymour, E. Schacht, *Bioconjugate Chemistry*, **2000**, *11*, 131.

3 Yu-Sen Wang, S. Youngster, M. Grace, J. Bausch, R. Bordensc, D. F. Wyss, *Adv. Drug Del. Revs,* **2002**, *54*,547.

4 A. K. Mishra, M. Hosono, K. Chuttani, P. Mishra, R. K. Sharma, J. F. Chatal, *J Drug Target*, **2004**; *12*, 559.

5 L. Morandeau, E. Benoist, A. Loussouarn, A. Ouadi, P. Lesaec, M. Mougin, A. Faivre-Chauvet, J. Le Boterff, J. F. Chatal, J. Barbet, J. F. Gestin, *Bioconjugate Chemistry,* **2005**, *16*, 184.

6 S. Zalipsky, *Bioconjugate Chem.,* **1995**, *6*, 150.

7 M. Regitz, J. Hocker, A. Liedhegener, *Organic Syntheses*, Collected Vol. *5*, 179.

8 R. L. Danheiser, R. F. Miller, R. G. Brisbois, S. Park, *J. Org. Chem.*, **1990**, 55, 1959.

9 A. P. Smith, S. A. Savage, C. L. Fraser, *J. Org. Chem.*, **1998**, 39, 8643.

10 G. L. Larson, *Pure & Appl. Chem.*, **1990**, *62*, 2021.

11 G. L. Larson, L. M. Fuentest, *J. Am. Chem. Soc.*, **1981**, *103,* 2418.

12 J. D. Parrish, D. R. Shelton, R. D. Little, *Org. Lett.*, **2003**, *5*, 3615.

13 A.Mi, Z.Wang, J. Zhang, Y. Jiang, *Synth. Commun.*, **1997**, *27*, 1469.

14 V. V. Shchepin, D. V. Fotin, M. I. Vakhrin., *Russian J. Org. Chem.*, **2004**, *40*, 834.

15 G. Apitz, W. Steglich, *Tetrahedron Letters*, **1991**, *32*, 3163.

16 C. Djerassi, *Chem Review*, **1948**, *43*, 271.

17 J. C. Day, M. G. Katsaros, W. D. Kocher, A. E. Scott, P. S. Skell, *J. Am. Chem. Soc.*, **1978**, *100*, 1950.

18 P. S. Skell, J. C. Day, *Acc. Chem. Res.*, **1978**, *11*, 381.

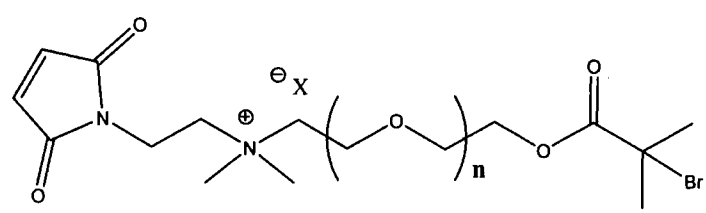
19 U. Luning, P. S. Skell., *Tetrahedron,* **1985**, *41*, 4289.

20 R. L. Tlumak, J. C. Day, J. P. Slanga, P. S. Skell, *J. Am. Chem. Soc.* **1982**, *104*, 7257.

CHAPTER 4

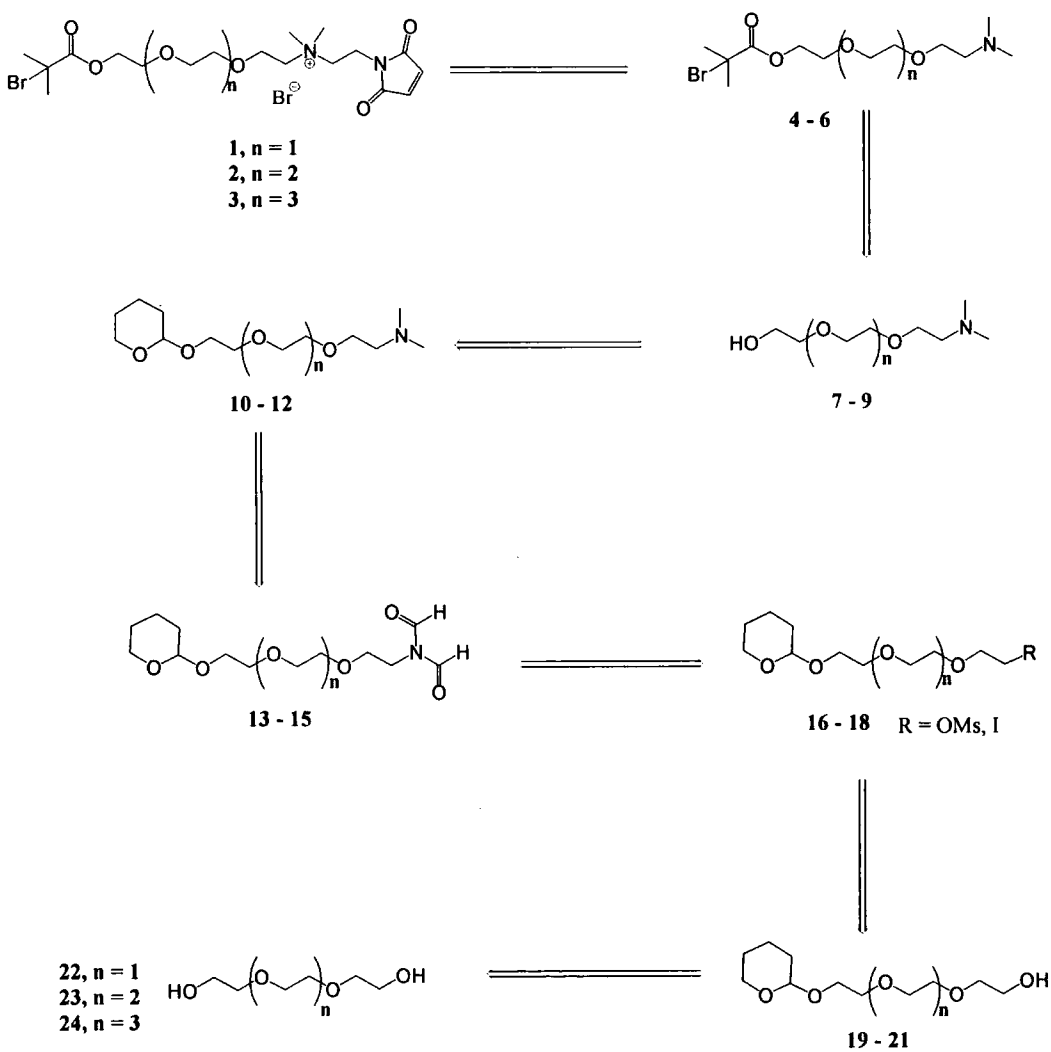
SYNTHETIC APPROACHES TO MEDIUM LENGTH POLYETHYLENE GLYCOL MALEIMIDE BASED ATRP INITIATORS

(Thiol-Selective Hetero-bifunctional Linker)



4.1 Introduction

In this chapter our target ATRP initiators consist of a series of medium length PEG units, which incorporate a quaternary ammonium centre introduced by either one of two dimethylamino-functionalised substituents as determined by substrate compatibility. The synthetic objectives involved the coupling of one end of a PEG (previously protected as a tetrahydropyranyl) to an α-bromoisobutyryl unit, a required ATRP functionality; the second free hydroxyl group would be functionalised with appropriately reactive group to facilitate an S_N2 displacement



Scheme 4.1

reaction with a labile leaving group (i.e. iodo or pseudohalide functionalities) on an *N*-alkyl substituted maleimide, Scheme 4.1. The simplest approach to the key intermediates (**4 - 6**) was by selective coupling of isobutyryl bromide to the free hydroxyl and then of substituted *N*-alkylmaleimide to the hydroxylamine precursor (**7 – 9**) prepared by a modified Gabriel synthesis. The use of an acid labile protecting group (i.e. tetrahydropyran) was expected to reduce complication of homo-bifunctionalised PEG formation and encourage region- and chemoselectivety during the application of a modified Gabriel synthesis.

4.2 Synthesis of PEG based maleimide/thiol-reactive linkers as ATRP initiators linker unit

4.2.1 Synthesis of a functionalised PEG for dimethylamino substrates

Formation of the tetrahydropyranyl **19** was achieved by treating dihydropyran with *p*-toluenesulphonic acid and three equivalents of azeotropically treated triethylene glycol at 70° C for 2 hours.

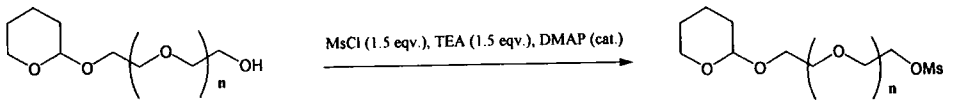
19, n = 2 (69 %)
20, n = 3 (64 %)
21, n = 4 (52 %)

Scheme 4.2

Removal of the solvent *in vacuo* gave the mono functionalised product **19** in 69% yield after column chromatography. This was confirmed by the presence of six proton multiplets at δ 1.4 - 1.8 ppm in the ^{1}H NMR spectrum corresponding to the THP methylene and the molecular [M+Na$^+$] ion at m/z 257 in the mass

spectrum. The procedure was employed to also synthesise the tetra and pentaethylene glycol in yields of 64% and 52% respectively. The typical signal for cyclic methylene (as for triethylene glycol) was observed by ^{1}H NMR analysis for both **20** and **21**. The respective molecular [M+Na$^+$] ion at m/z 301 and m/z 345 of the mass spectrum was observed. Examination by mass spectroscopy of the samples from column chromatography showed the exclusive formation of di-substituted polyethylene glycol as the only by-product in the above reaction, hence the low yield.

The free hydroxyl on monofunctionalised triethylene glycol **16** was then stirred overnight with 1.5 equivalents of methanesulphonyl chloride and after the removal of the solvent *in vacuo,* aqueous workup and purification by flash column chromatography, and the desired mesylate **16** was obtained in 89% yield.



16, n = 2 (89 %)
17, n = 3 (77 %)
18, n = 4 (67 %)

Scheme 4.3

The structure of the product was confirmed by the presence of a three proton singlet at δ 3.0 ppm in the ^{1}H NMR spectrum corresponding to the methanesulphonyl group and an ion for [M+Na$^+$] at m/z 335 in the mass spectrum. The procedure was repeated in the synthesis of the mesylates of tetra and pentaethylene glycol in yields of **17** and **18**. They were confirmed by ^{1}H NMR spectrum and the molecular [M+Na$^+$] ion at m/z 379 and m/z 423 in the mass spectrum.

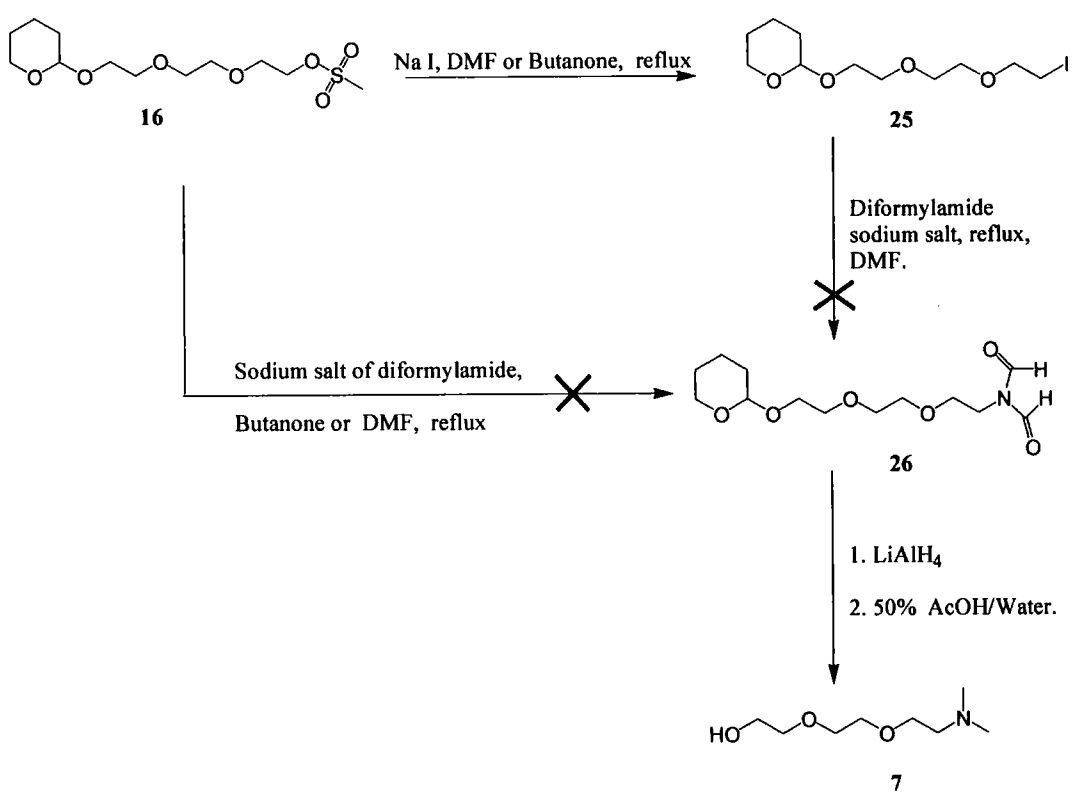
Further attempts were made to improve the yield for pentaethylene glycol mesylate **18** by conducting the reaction in the presence of freshly activated sieves suspension. Unfortunately, the approach tended to evoke a side reaction, which resulted in an increased level of by-products being isolated after column chromatography. Flash chromatography was also employed to reduce the effect of the acidic silica gel. The workup that only involved neutralisation with

aqueous sodium bicarbonate before extraction to avoid solvent induced complications was also unable to consistently increase the yield.

4.2.2 Synthesis of a tertiary amine substituted PEG

4.2.2.1 Synthesis of a tertiary amine *via* a modified Gabriel reagent

The introduction of tertiary amine was first envisaged to exploit the modification of a known procedure for the formation of primary amines *via* an *N*-alkylphthalimide by substituting the base hydrolysis to reveal the primary amine (Gabriel synthesis[1]) with a different process (i.e. hydride reduction) to expectedly liberate a dimethyl tertiary amine and eventually afford a quaternary ammonium salt after S_N2 displacement of an alkylhalide functionalised maleimide.



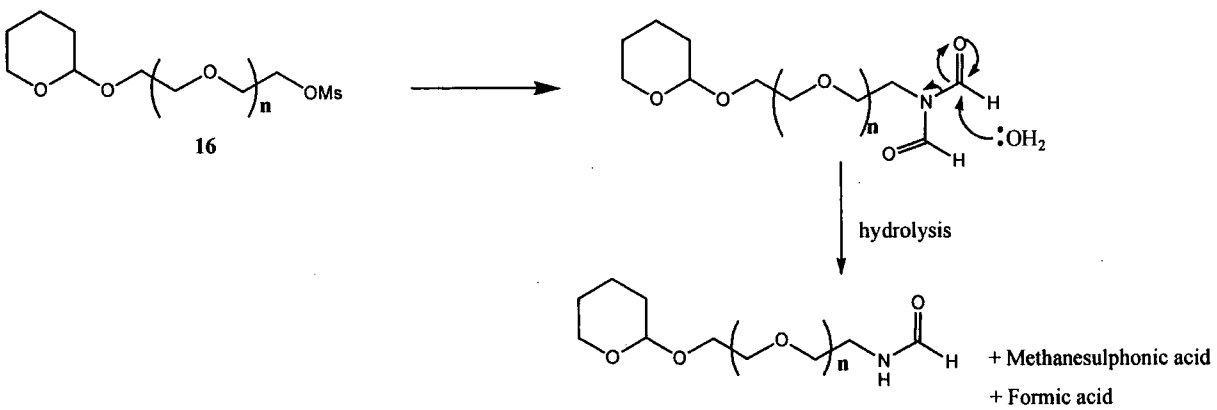
Scheme 4.4

However, a reported literature[2] had applied a convenient and modified Gabriel synthesis reagent substitute employing sodium diformylamides to N-alkylate

halides or sulphonates, and after hydrolysis this gave the primary amine. We therefore developed a strategy based on this approach, Scheme 4.4.

We attempted the S_N2 reaction described above by treating the mesylate **16** under reflux conditions with two equivalents of diformylamide sodium salt in butanone. The reaction was monitored by TLC until complete consumption of the mesylate (after 48 h) was observed which upon analysis of the crude, observed a mixture of products. The mixture proved difficult to separate cleanly by column chromatography however, during ^{1}H NMR analysis of the crude and the several fractions, mono and diformylated products and diminished tetrahydropyranyl constituents as mixtures were observed. This was also evident in the crude TLC analysis as multiple spots, and the observation of one-proton broad singlet at δ 1.9 ppm of the crude ^{1}H NMR spectrum corresponding to the new, free hydroxyl group. The monoformylated products were also observed as a significant component in the ^{1}H NMR spectrum with broad singlet signal at δ 8.9 ppm in the spectrum for the secondary amine. A similar result was observed when butanone was replaced with DMF.

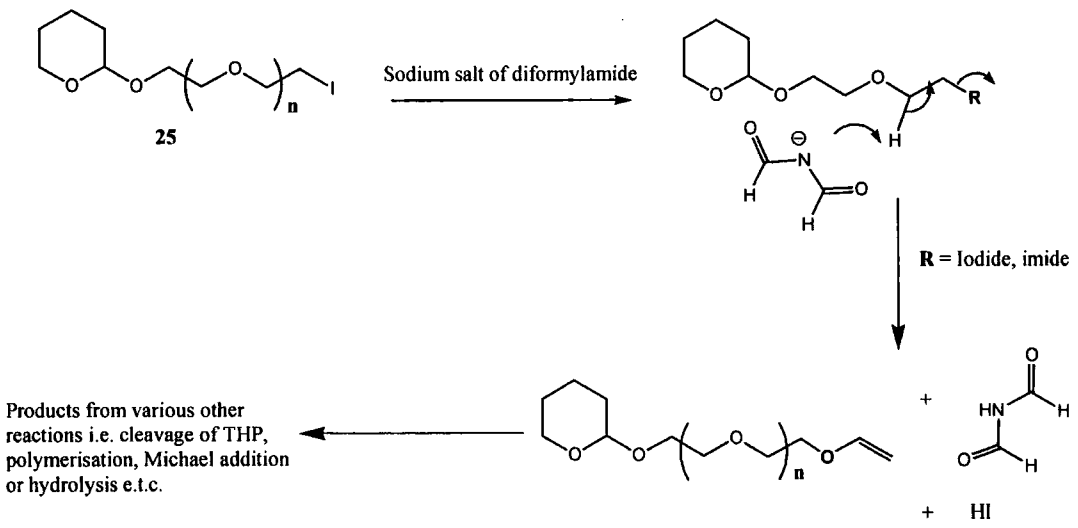
We assumed that the spectator ion, the mesylate, became more active in the deformylation process by acting as the source of acidity (protonation of



Scheme 4.5

carbonyl) and due to the forceful hydrolysis conditions induced by the prolonged, high reaction temperature, resulted in several by-products, Scheme 4.5. To reduce the forceful nature of the reaction condition (i.e. 24/48 h and reflux), it was proposed that the synthesis of the intermediate **25** consisting of primary iodoalkyl would accelerate the rate of reaction thereby reducing monoformylated products probably originating from hydrolysis. The iodide was then prepared by treating 1.5 equivalents of sodium iodide with the mesylate **16** in butanone overnight. The crude reaction mixture was then examined by TLC and confirmed by ^{1}H NMR only, to exhibit the expected two proton triplet at δ 3.2 ppm corresponding to the CH_2I of **25** (Scheme 4.4) having replaced the two proton multiplet signal at δ 4.2 ppm corresponding to CH_2OMs of the starting mesylate. Unfortunately, attempts at further analysis of the intermediate iodide resulted in hydrolysis product; and therefore we filtered the reaction mixture to remove the solids or salt content (with syringe through cotton wool plug) before adding 1.5 equivalents of diformylamide (assuming quantitative yield) in portions and heating at 70° C for 18 hours in DMF or butanone. The crude mixture by TLC showed multiple spots and the attempted column chromatography resulted in the recovery of material difficult to identify by mass spectroscopy or ^{1}H NMR.

The reaction of diformylamide with the iodide intermediate **25** was repeated with commercial or freshly prepared diformylamide in butanone or DMF but yielded

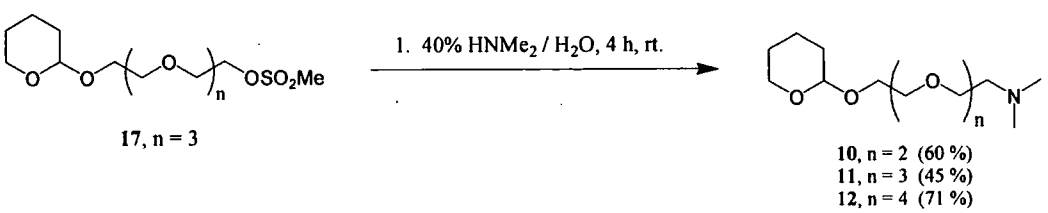


Scheme 4.6

results similar to described above; as did varying the reaction temperatures. The speculated method of degradation was envisaged to involve the reductive elimination of the alkyl diformylamide to give an aza-enolate (amide tautomer) and the resulting acidic environment (*via* elimination of HI) further degrades the substrate, Scheme 4.6. At this stage it was apparent that the simple diformylamide unit is inherently sensitive to the employed reaction conditions. We therefore proposed the introduction of tertiary amine *via* a more practical source of dimethylamine. To avoid the use of gaseous dimethylamine, which is difficult to handle, or the hydrochloride salt of dimethylamine, which may be deleterious to the substrate if a reflux condition is required. The use of dimethylamine dissolved in water would take advantage of the dual solubility of polyethylene glycol in both aqueous and organic solvents and avoid the use of gaseous amino compounds. The work generated in this area is discussed in the next section.

4.2.2.2 Synthesis of a tertiary amine substrate *via* an alternative method

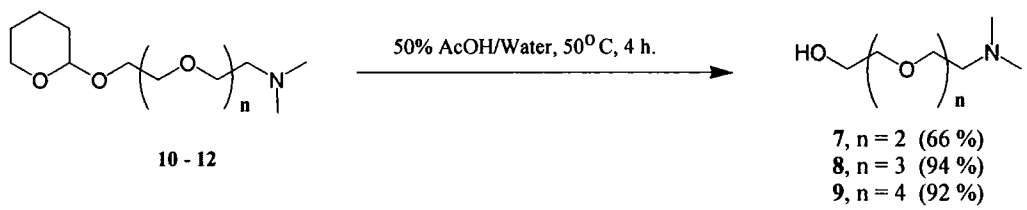
The nucleophilic substitution of mesylate **17** in dichloromethane was effected by treating with dimethylamine (40% in water) initially at 0° C then by stirring at room temperature. The reaction was monitored by ^{1}H NMR spectroscopy and quantitative conversion was observed by the replacement of the methylene and methyl of CH_2OSO_2Me with the two proton triplet at δ 2.5 ppm and six proton singlet at δ 2.2 ppm corresponding with methylene and the new dimethyl of CH_2NMe_2 respectively.



Scheme 4.7

The reaction mixture, after aqueous treatment was subjected to column chromatography to reveal the tertiary amine **11** in 45% yield with the structure further confirmed by a molecular ion at 306.2202 in the HRMS which was in agreement with the expected value of 306.2297 for $C_{13}H_{33}NO_5Na$. The procedure was also repeated for the mesylate of **16** and **18** to reveal their equivalent tertiary amine in yields of 60% and 71% respectively, which were confirmed by ^1H NMR and mass spectroscopy.

Treatment of the tertiary amines with an acid catalysed deprotection condition for tetrahydropyran to liberate the hydroxyl amine **8,** Scheme 4.8, was observed as the replacement of the six-proton multiplet signal at δ 1.4 – 1.8 ppm corresponding to tetrahydropyran with two proton multiplet signal at δ 3.8 ppm corresponding to the new CH_2OH in the ^1H NMR spectrum was used to confirm the conversion.



10 - 12

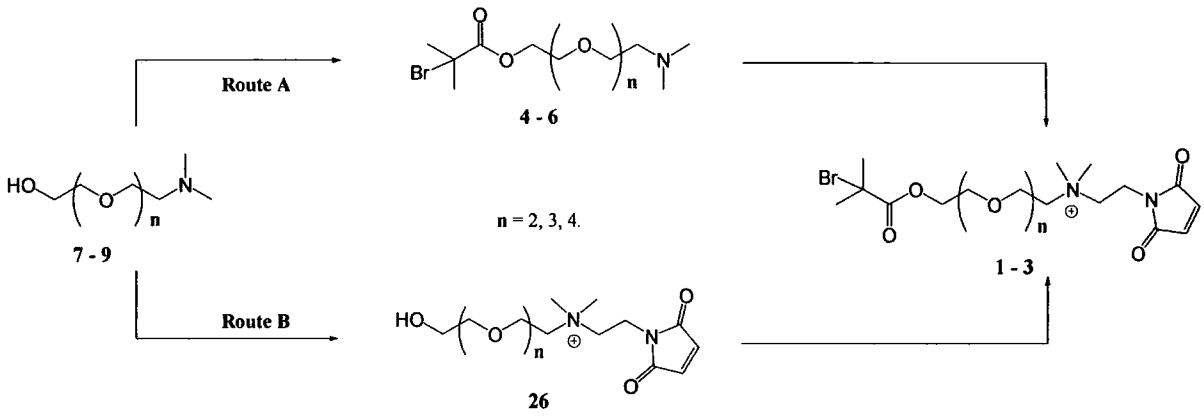
7, n = 2 (66 %)
8, n = 3 (94 %)
9, n = 4 (92 %)

Scheme 4.8

The amino tetrahydropyranal **10** was reacted either with 30% hydrochloric acid in diethyl ether or 50% acetic acid in water and heated at 50° C for 4 hours. The resulting mixture was either filtered and washed with cold ether or passed through a plug of neutral alumina before drying *in vacuo* and over phosphorus pentoxide to give the hydroxyamine **7** in 66% yield. The structure was further confirmed by the hydroxy (OH) absoption at 3409 cm^{-1} in the IR spectrum and a molecular ion [$M+Na^+$] at m/z 178 in the mass spectrum.

4.2.3 Attempted synthesis of dimethylammonium substituted maleimides

We now envisaged a bilateral approach to the preparation of the target ammonium salt. The process would involve the nucleophilic displacement reaction of a suitably functionalised *N*-alkylmaleimide (i.e. halide or pseudohalide) relying on both sterics and the relative nucleophilicity of tertiary



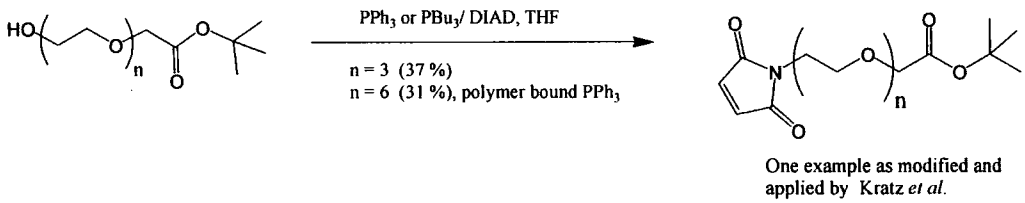
Scheme 4.9

amine before or after affording the condensation product with α – bromoisobutyryl bromide to obtain the target molecule *via* route A or route B, Scheme 4.9. Unfortunately, the various attempts made to prepare the bromoisobutyryl amine (**4 – 6**) *via* route A had resulted in decomposition product being recovered. The reaction was performed in the presence of N,N-dimethylaminopyridine or potassium carbonate then bromoisobutyryl bromide in different solvents (i.e. MeCN, DCM, DMF and THF) and at different temperatures (0° C and rt only) while been monitored with TLC. As observed in the mass spectroscopy and ^{1}H NMR, the crude reaction mixture was unassigned and so attention turned to route B. In order to explore the possibilities of functionalising the spacer units (**7 – 9**) by coupling to appropriately configured maleimide substrate, it was essential before reacting with α-bromoisobutyryl functionalities to test the possible condition to ensure the generation of the

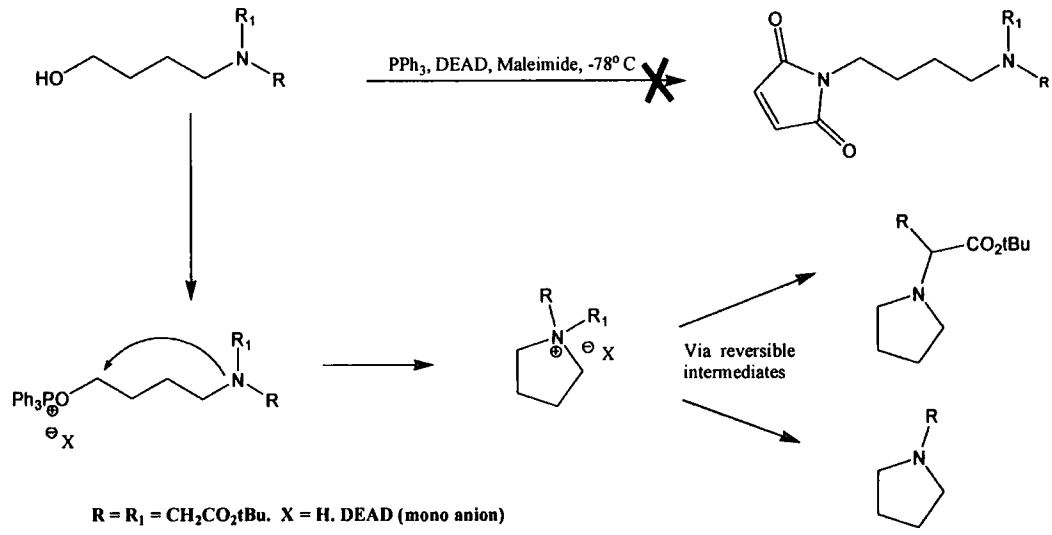
required ammonium salt. The preparation of the required and varied *N*-alkyl substituted maleimide was therefore attempted and discussed below.

4.2.4 Synthesis of a *N*-substituted alkyl maleimide

Direct alkylation of maleimide represents a convenient procedure for the synthesis of N-alkylmaleimides under mild or neutral condition as represented by Mitsunobu[3] reaction. However, some reports have demonstrated the process to be low yielding and deleterious to certain substrates, i.e. substituted polyethylene



Scheme 4.10. Low yields from Mitsunobu as demonstrated by Kratz *et al*[4]

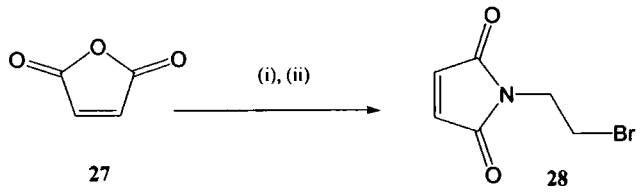


Scheme 4.11. Stevens rearrangement as a possible side reaction as reported by Walker *et al*[5].

glycol[6] and for tertiaryamino butanol[5], because of the various competing reactions and reagents present, Scheme 4.10 and 4.11.

Another method towards the synthesis of bifunctional *N*-substituted maleimide involves the condensation cyclisation of the corresponding maleamic acid under various conditions[7,8,9,10] which generally proceed in modest yield for *N*-arylmaleimide but less satisfactorily for N-alkyl maleimide[11,12].

Based on the literature precedents, in order to realise the successful construction of the target ammonium salts (**1** - **3**) we therefore pursued the requirement to establish an efficient means to prepare *N*-alkylmaleimides compatible with our substrates and in good yields.



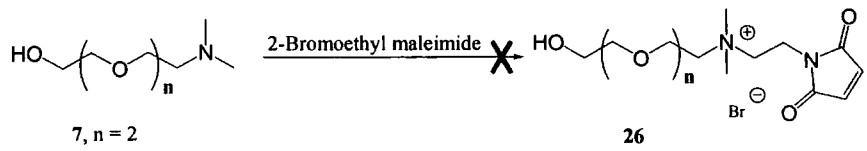
27 28

(i). 2-bromoethylamine .HCl, $NaHCO_3$, Water, 2 h, 77%.

(ii). Ac_2O, NaOAc, 80°C, 10 min, 85°C, 10 min, 14%.

Scheme 4.12

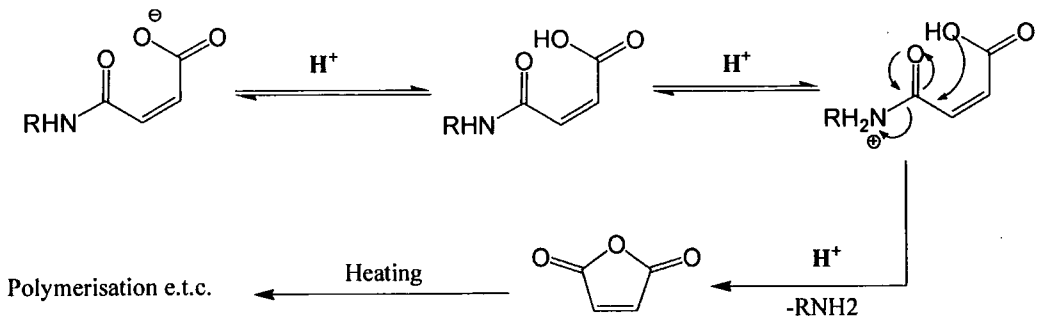
Other literature[13]preparation had already shown that haloalkyl maleimide could be prepared in moderate yields in a two stage cyclo-condensation process of the maleamic acid - by treating with a combination of NaOAc and acetic anhydride at 80° C for 15 min. However, our attempt at repeating the procedure resulted in a poor yields (14%, after two steps) after further purification of the maleimide **28**, Scheme 4.12. The maleimide was confirmed by the presence of a two-proton singlet signal at δ 6.7 ppm and two proton triplet signal at δ 3.5 ppm corresponding to (CH=CH) and CH_2Br respectively in the ^1H NMR spectrum; and by carbonyl stretch at 1770 cm^{-1} in the IR spectrum.



7, n = 2 **26**

Scheme 4.13

We proceeded to examine the compatibility of the two substrates by stirring the maleimide (1.1 eqv.) with the tertiary amine **7** in dry diethylether at room temperature overnight. The resulting precipitate was filtered, concentrated and dried *in vacuo* to yield an unidentified product possessing no recognisable signal observable by ^{1}H NMR and mass spectrum. The reaction was repeated with or without Schiff's base; and varying the solvents (THF and MeCN) was unsuccessful.



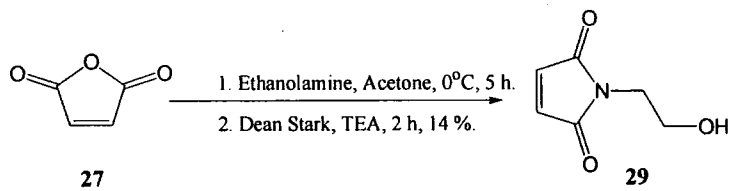
Scheme 4.14

The attempted synthesis at elevated temperature of the quaternary ammonium salt using maleimide **28** could not be attempted due to the unsuccessful attempt at synthesising 2-bromoethyl maleimide by the method above; and various other acid catalysed cyclo-condensation based on literature methodology i.e. *p*TsOH, PhMe, reflux, 8 h or polyphosphoric acid, PhMe, reflux, 18 h or ethanol/HCl, reflux – as they all gave unidentified white polymeric solids, as observed by mass spectrum and ^{1}H NMR. The observed results can be attributed to polymerisation product at high temperature of maleic anhydride or maleic

anhydride reformed from the maleamates *via* intramolecular cleavage of the amide bond at low pH, Scheme 4.14[14]. Unfortunately, when the cyclo-condensation reaction was attempted in the presence of a varied amount of triethylamine (catalytic or stoichiometric amount) in toluene to obtain polymeric material attributed to the probable formation of an allylic product, which would have polymerised. Similar results were observed irrespective of the PEG substrate subjected to the conditions as described above.

To reduce the rate of formation of the proposed elimination product during attempted cyclisation, consequently increasing the yield for the maleimide, a pseudohalide (i.e. triflate or mesylate) was proposed as a substitute for the highly labile bromine and reactive hydrolysis product (aq. HCl) as a practical alternative and work in this area will be discussed next.

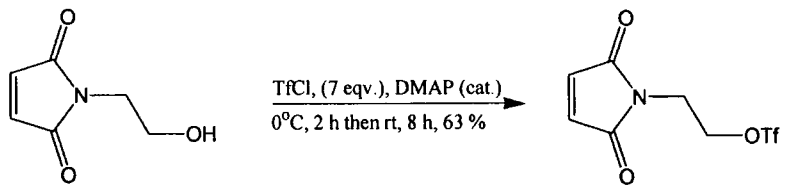
Recent literature by Kratz *et al*[15] showed precedence for the base assisted cyclo-condensation of maleimide *via* an amidoacid intermediate. They were able to



27 29

Scheme 4.15

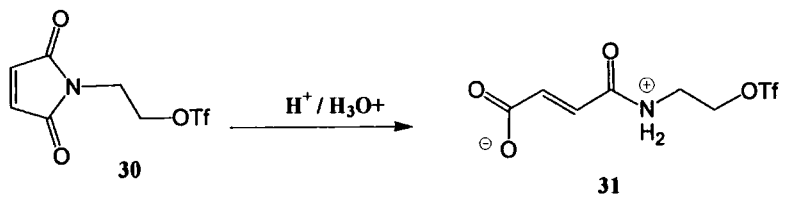
couple ethanolamine to maleic anhydride to give the expected hydroxyethyl amidoacid in moderate yields before condensation to give *N*-(2-hydroxyethyl) maleimide. This chemistry was repeated and ethanolamine was treated with



29 30

Scheme 4.16

maleic anhydride **27** in acetone at 0° C for 4 h and the zwitterionic acid precipitated at 4° C in quantitative yield. This was confirmed by melting point value of 99° C which agrees with the expected value 98° C[16]. Without further purification, the amidoacid was allowed to dissolve in hot toluene before refluxing for 2 hours with one equivalent of triethylamine in a Dean Stark setup. The bifunctional maleimide **29** was obtained in 14% yield after silica column chromatography. The ^{1}H NMR spectrum showed the appearance of a two-proton triplet signal at δ 3.7 ppm and a two proton singlet at δ 6.7 ppm corresponding to the signal for CH_2OH and CH=CH respectively; and the molecular ion $[M+NH_4^+]$ at m/z 158 as viewed with mass spectroscopy. Treatment of the hydroxy maleimide **29** with seven equivalents of trifluoromethanesulphonyl chloride in the presence of a catalytic amount of N,N-dimethylaminopyridine for 2 h at 0° C then at room temperature for 8 h gave the triflate **30** in 63% yield. This was observed by the shift in the ^{1}H NMR signal from δ 3.7 ppm to δ 3.9 ppm corresponding to the formation of CH_2OTf from CH_2OH and the molecular ion $[M+H^+]$ at m/z 274 in the mass spectrum. At lower equivalents of trifluoromethanesulphonyl chloride (i.e. less than 2.5 eqv.) the reaction was observed to be very slow at room temperature (as observed by TLC) and after 24 h, resulted in the recovery of the expected triflate in less than 30% yield.



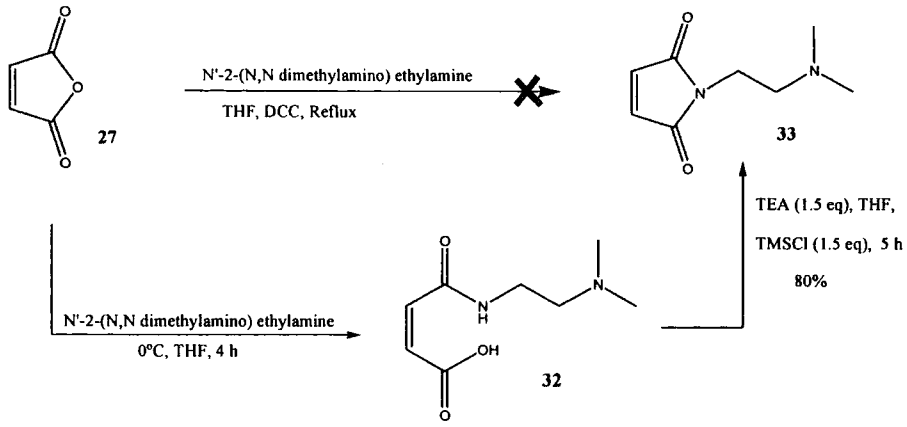
Scheme 4.17

The use of triflic anhydride was less successful than all comparable equivalents. The acidic environment exacerbated by prolonged reaction time had probably encouraged the generation of an intramolecularly cleaved zwitterionic amidoacid **31**, Scheme 4.17. With the triflate at hand, attention was then turned to the proposed quarternisation *via* an S_N2 displacement of the triflate with the required

tertiary amine. This was attempted by stirring two equivalents of the hydroxyamine **7** with the triflate **30** in dichloromethane at room temperature over five days during which observations by ^{1}H NMR showed minimal reaction. Refluxing the reaction resulted in disappearance of the unsaturation of the maleimide in the ^{1}H NMR spectrum. The reaction was further attempted by conducting the reaction in methanol or ethanol at room temperature or at reflux based on published procedures[17,18] for the formation of alkyl ammonium chloride and alkyl ammonium tosylate respectively. Unfortunately, the above and further attempts remained unsuccessful as the halide and pseudohalide alkylmaleimide proved to be unstable and so easily decompose before the substitution reaction was effected. Although these reactions demonstrated a stability problem, quarternisation is an established chemistry and was therefore suggested as an approach using a different combination of bifunctional quaternising agents of N'-(N-dimethyl)ethyl maleimide. The dimethylamino on maleimide was considered an attractive alternative approach as this provides an inherent stabilizing agent for the maleimide by maintaining a basic environment (high pKa) in solution (Scheme 4.14), while exhibiting less lability towards elimination reaction (relative to halides or pseudohalides). Progress with dimethylethyl maleimide is described in the next section.

4.2.5 Synthesis of a bifunctional *N*-substituted alkyl amino maleimide

Several procedures had been published[19] for the condensation of various alkylamines with aryl substituted maleic anhydride. They also showed that the reaction of phthalic acids with dialkylaminoalkyl amines in the presence of dicyclohexaycarbodiimide (DCC) in THF could be used to synthesise their corresponding phthalimides in high yields. We therefore applied the procedure to our substrate by refluxing the mixture of maleic anhydride in THF with an equivalent amount of *N,N*-dimethylethylenediamine.



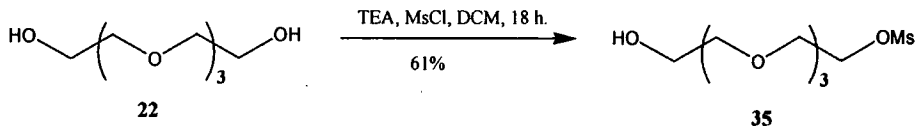
Scheme 4.18

After two hours refluxing, the urea formed was filtered and the organic phase subjected to precipitation with warm ether. Although total consumption of the diamine was observed during the reaction by TLC, three other distinct types of diamine products including the expected diamine (as a minor content) had been formed as observed in the ^{1}H NMR. We suspected the formation of acyclic diimide. We therefore decided to prepare and isolate the intermediate amidoacid prior to cyclo-condensation to ensure efficient cyclo-condensation. The addition of an equimolar amount of dimethylethylenediamine to maleic anhydride to give the expected intermediate **32** in quantitative yield after drying *in vacuo*. No further purification was required to confirm the compound by the two one-proton doublets at δ 5.9 ppm and δ 6.1 ppm corresponding to CH_A=CH and CH=CH_B respectively, and a two proton triplet at δ 3.2 for CH_2NMe_2 in the ^{1}H NMR spectrum and a molecular ion [M+H^+] at m/z 187 in the mass spectrum. When the amidoacid **32** was subjected to triethylamine assisted Dean Stark condensation procedure, a very poor yield of the expected compound **33** was observed in ^{1}H NMR. However, on changing the solvent from toluene to THF, a significant reduction in the amount of unidentifiable constituents was seen by ^{1}H NMR. When DCC was added to the condensation mixture to accelerate the process, decomposition product was observed as the main component by ^{1}H NMR. However employing trimethylsilyl chloride resulted in a significantly improved yield. Hence, the maleimide **33** was prepared by stirring the suspended mixture of amidoacid **32** and 1.2 equivalents of triethylamine in THF for half an hour under reflux conditions before treating the mixture with 1.2 equivalents of

trimethylsilyl chloride at reflux for 5 hours. The supernatant was concentrated *in vacuo* to obtain an orange powder that was recrystalised from dichloromethane in 80% yield. The maleimide was confirmed by a two-proton singlet at δ 6.7 ppm and a six proton singlet at δ 2.2 ppm, corresponding to CH=CH and dimethylamino units respectively, in the ^{1}H NMR spectrum, and the presence of a molecular ion [M+H$^+$] at m/z 169 in the mass spectrum.

4.2.5.1 Model quaternisation reaction

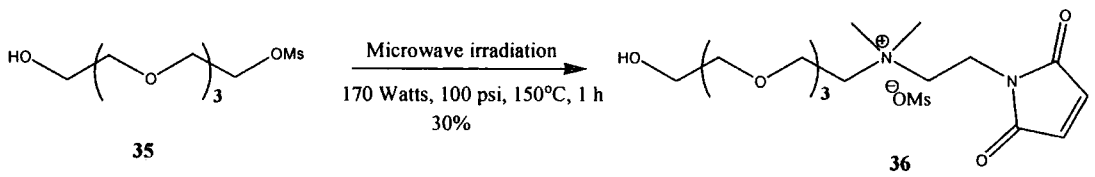
As the synthesis of maleimide **33** was expected to allow access to the generation of a quaternary ammonium salt, it was desirable to establish condition(s) conducive to functionalised tetraethylene glycol with stable but S_N2 labile groups (i.e. mesylates). The strategy was to subject the maleimide to quarternisation



22 **35**

Scheme 4.19

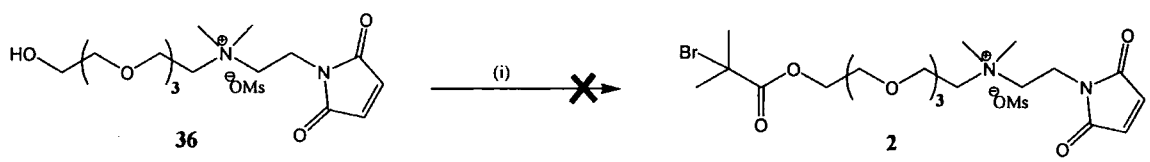
conditions based on a modified procedure[17] with mesylated tetraethylene glycol **35**. The mono-mesylated tetraethylene glycol **35** was synthesised by stirring methanesulphonyl chloride with one equivalent of triethylamine and five equivalents of tetraethylene glycol in dichloromethane and after flash column chromatography obtained the mono-mesylate **35** in 61% yield as seen in ^{1}H NMR spectrum as a three proton singlet at δ 3.1 ppm corresponding to the methyl of mesylate and a molecular ion [M+Na$^+$] at m/z 295 in the mass spectrum. However, treatment of mesylate **35** with maleimide **33** under several



Scheme 4.20

room temperature conditions and reagents showed only unreacted, hydrolysed maleimide or at reflux, showed no recognisable material and none of the expected ammonium salt.

We therefore applied microwave irradiation chemistry to attempt to obtain the ammonium salt. The expected hydroxyammonium salt **36** was obtained in 30% yield by dissolving tetraethylene glycol monomesylate **35** in a solution of N'-(N,N-dimethylamino)ethyl maleimide **33** in dry THF under argon. The mixture was subjected to microwave irradiation (superheating) for one hour, Scheme 4.20.



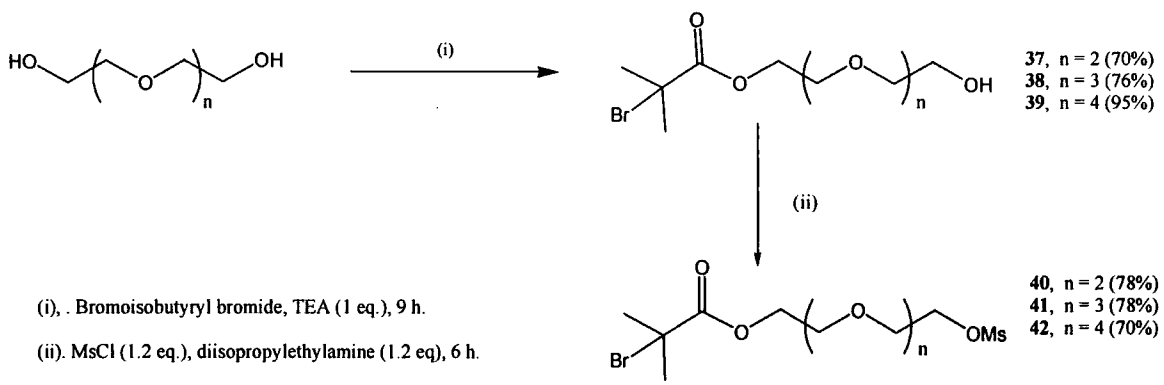
(i), Bromoisobutyryl bromide, TEA, DMAP, MeCN

Scheme 4.21

The precipitate formed was collected and washed with cold THF, and dried *in vacuo* before being characterised. The salt was observed in the ^{1}H NMR spectrum with signals at δ 3.6-3.9 ppm corresponding to the twelve-proton multiplet of tetraethylene glycol and the molecular ion at 345.2020 in the HRMS, which was in agreement with the expected value of 345.2007 for $C_{16}H_{29}N_2O_6Na$. To improve on the yield, numerous attempts were carried out, altering the conditions (i.e. wattage, pressure, time) and reagents (i.e. organic/inorganic base and various stoichiometric ratios) but all were unsuccessful and were unable to improve the yield. We therefore preceded to the reaction of hydroxy ammonium

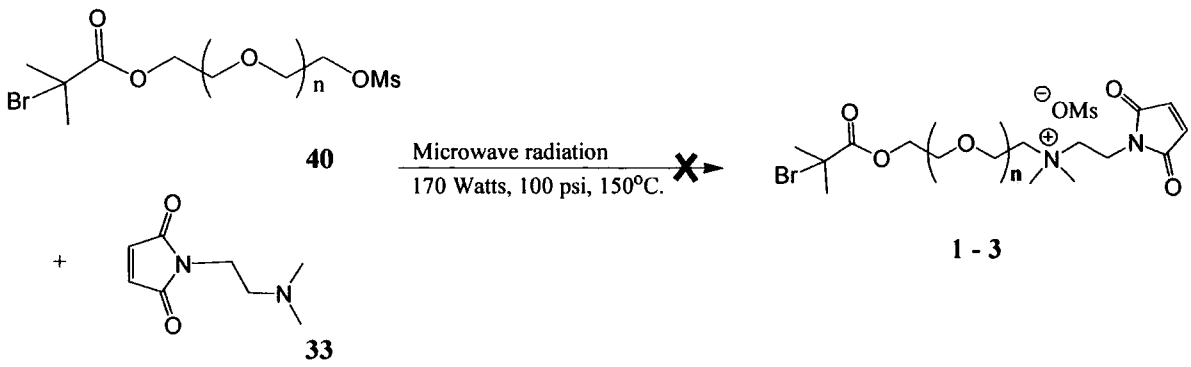
salt **36** with bromoisobutyryl bromide in acetonitrile as observed by ^{1}H NMR, which showed none of the expected bromoisobutyryl ammonium salt **2**.

At this stage it was apparent that reacting the isobutyryl unit with the free hydroxy of ethylene glycol post any other modification was incompatible, we therefore decide to install the isobutyryl unit prior to mesylation (or other equivalents) and then quaternisation. Hence, we proceeded by stirring 2-bromo-2-methylpropionyl bromide with triethylamine and triethylene glycol in dichloromethane to obtain the functionalised PEG **37** after flash chromatography, in 70% yield. The compound was observed by ^{1}H NMR spectrum as a six-proton singlet at δ 1.9 ppm and a ten-proton multiplet at δ 3.7-3.8 ppm corresponding to the α-bromo dimethyl unit and ethylene glycol repeating unit respectively.



Scheme 4.22

The structure was further confirmed by the observed [M+Na$^+$] molecular ion at 321/323 in the mass spectrum. The procedure was also repeated for tetraethyleneglycol of **38** and pentaethylene glycol **39** to give their equivalent isobutyrate esters in yields of 76% and 95% respectively, which were also observed by ^{1}H NMR and mass spectroscopy and confirmed to be in agreement with expected data. The condensation to produce the mesylate **41** was achieved in 78% by treating with methanesulphonyl chloride according to the previously described procedure. The mesylate was confirmed in the ^{1}H NMR spectrum by a six proton singlet signal at δ 1.9 ppm and a three proton singlet at 3.0 ppm corresponding to the α-bromo dimethyl unit and the methanesulphonyl unit respectively.

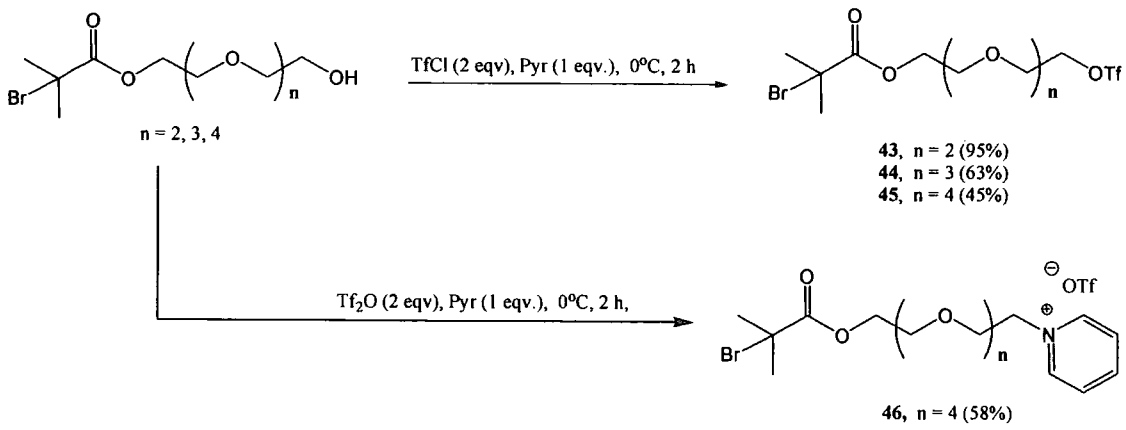


Scheme 4.23

Further clarification was observed in the mass spectrum for the molecular ion [M+Na$^+$] at m/z 399/401. The procedure was repeated for tetraethylene glycol **38** and pentaethylene glycol **39** to reveal their equivalent mesylates **41** and **42** in yields of 78% and 70% respectively, which were also observed by ^{1}H NMR and mass spectroscopy and confirmed to be in agreement with expected data. With the mesylates at hand, attention was therefore turned to the microwave reaction with N'-(N,N-dimethylamino)ethyl maleimide **33** according to the previously successfully attempted procedure and conditions described above in order to afford the ammonium salts (**1 - 3**) as the final product. However, treatment of the mesylates under microwave conditions with maleimide **33** did not yield expected ammonium salt (**1 - 3**) but rather a mixture of products possessing degraded mixtures of substrates and hydrolysed maleimide signal observed in the ^{1}H NMR spectrum but unidentified by mass spectroscopy.

It was speculated that the weak C-Br bond of the isobutyryl unit was also unstable to the microwave irradiation conditions and therefore resulted in unwanted side reactions and the eventual degradation of the reagents.

A different strategy was consequently proposed based on substituting the methanesulphonate with its triflate equivalent as a more labile moiety for S_N2 displacement with dimethylaminoethylmaleimide; and relying on the steric hindrance around α-bromoisobutyryl to prevent a parallel nucleophilic substitution at room temperature at this centre. We therefore proceeded to

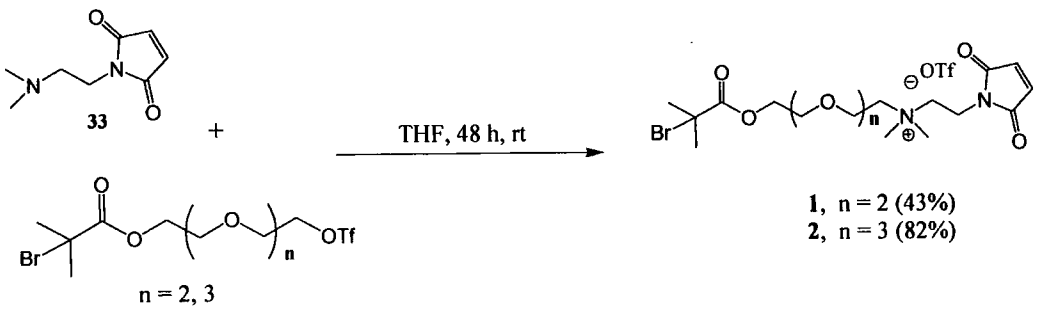


Scheme 4.24

synthesise the triflates by treating the free hydroxyl of the propionate **37** with two equivalents of trifluoromethanesulphonyl chloride and one equivalent of pyridine to obtain the triflate **43** in 95% yield as seen by ^{19}F NMR spectrum at δ −78 ppm corresponding to CF_3 unit and further confirmed by the observed molecular ion [M+Na$^+$] at m/z 453/455 in the mass spectrum. The procedure was repeated for tetraethylene glycol to obtain their equivalent triflates **44** and **45** in yields of 63% and 45% respectively, which were also observed by ^1H NMR and mass spectroscopy and confirmed to be in agreement with expected data although it was not possible to confirm the triflates by HRMS. The lower yield for **45** was attributed to relatively greater hygroscopic nature of pentaethylene glycol. In order to improve the yields, different conditions were attempted without much improvement, however whenever trifluoromethanesulphonyl anhydride was used instead of trifluoromethanesulphonyl chloride under similar conditions, the pyridinium salt **46** was isolated in 58% yield as seen in the ^1H NMR spectrum by the signal at δ 8.0 ppm (two proton triplet), δ 8.5 ppm (one proton triplet) and δ 9.0 ppm (two proton triplet) corresponding to the pyridinium protons (Ph-H) and the molecular ion [M+Na$^+$] for 404/406 in the mass spectrum (Scheme 4.24).

The triflate **43** (1.1 equivalents) was then stirred with dimethylamino maleimide **33** in dry THF at room temperature for 48 hours, Scheme 4.25. The reaction mixture was monitored by ^1H NMR to reveal the replacement of the

CH_2OTf signal at δ 4.6 ppm with a (CH_2N^+) signal as overlapping multiplet at δ 3.7-4.1 ppm in the spectrum.



Scheme 4.25

The supernatant was then filtered, washed with ice-cold ethylacetate or ether and dried *in vacuo* over phosphorus pentoxide to obtain the triflate ammonium salt **1** as brown syrup in 43% yield. The salt was further confirmed by a molecular ion at 449.1320/451.1312 in HRMS, which was in agreement with the calculated value of 449.1287/451.1267 for $C_{18}H_{30}{}^{79/81}BrN_2O_6Na$. The procedure was repeated for tetraethylene glycol triflate to give the ammonium salt **2** in 82% yield, which was also observed by ^1H NMR and as molecular ion [M^+] at m/z 493/495 in the mass spectrum. The hetero-bifunctional-polymerisation initiators **1** and **2** are stable brown syrups stored at –4 °C for several weeks.

The initial attempts to prepare the ammonium salt of pentaethylene glycol **3** were unsuccessful and due to time constraints we were unable to explore the preparation attempts further.

4.3 Coupling of selected linkers to peptides and proteins.

4.3.1 Introduction

This section discusses the attempts to functionalise the pre-prepared biological entities (RGD-based peptide and the protein, HSA) with some of the previously prepared initiators (from chapters two, three and four) to give ATRP initiators with bio-recognition properties. They (the bio-initiators) will then be subjected to polymerisation process according to published methodology, and after purification would produce peptides and protein-based water-soluble bioconjugates.

The peptide was designed based on the ubiquitous RGD sequence modified with strategically chosen and positioned amino acid residue (see introduction chapter 1) to aid bio-recognition.

4.3.1.1 Peptide synthesis

The polypeptide **52** (Figure 4.1) was prepared by standard solid phase Fmoc chemistry on TentaGel-S RAM Fmoc resin. (0.1 g, 0.2-0.3 mmol/g substitution). The last Fmoc deprotection was performed as a bench reaction with 20% piperidine/DMF and a small sample of the resin-bound polypeptide **51** was cleaved from the resin (95% TFA) and the polypeptide precipitated with ice-cold ether overnight. The polypeptide **52** was confirmed by the presence of molecular ion [M^+] at m/z 745 in the mass spectroscopy corresponding to the calculated value. The initial design involved introducing protected lysine residue strategically in the sequence during synthesis.

Parent Peptide	G	R	G	D	S	P	A	S	**TentaGel Resin**
Analogue 1	X	R	G	D	S	P	A	S	**TentaGel Resin**

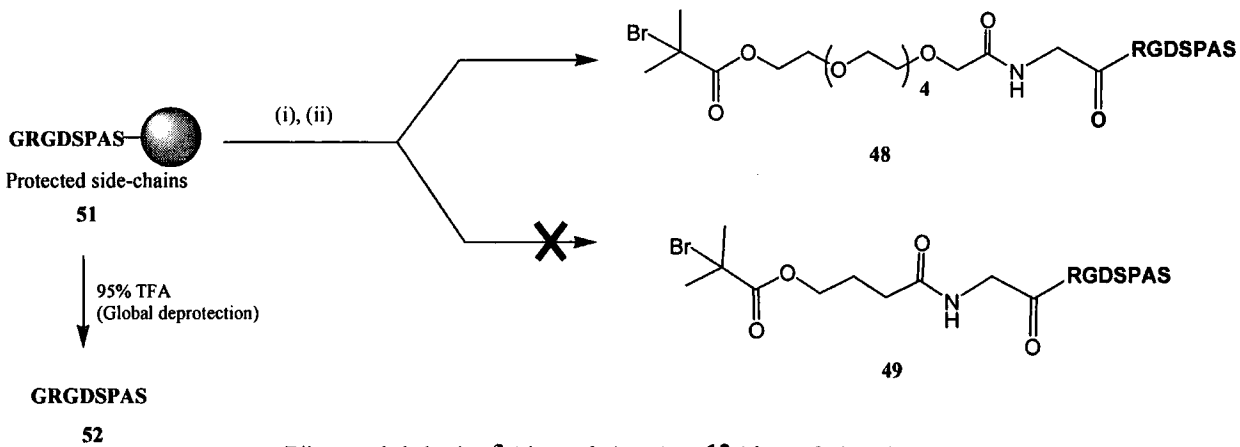
X = Glycine(NH)-- ATRP initiators

Figure 4.1

The lysine residue was then deprotected and coupling attempted with the succinimidyl-based initiators using standard coupling techniques and the terminal Fmoc left intact to reduce complications. However, on analysis, we were unable to recover any molecular ion corresponding to the calculated values but based on the isotopic distribution, we were able to propose that the unexpected deprotection of the terminal Fmoc might have resulted in di or multiple functionalisation of the peptide.

4.3.2 Synthesis of Peptide-initiator complex

The peptide-initiator was prepared from the condensation reaction of the parent polypolypeptide below (0.1g, free $-NH_2$, ~25%) with succinimidyl 17-(2-bromo-2-methylpropionyloxy)-3, 6, 9, 12, 15-pentaoxaheptadecanoate **3.3** (Compound **3**, Chapter **3**) (62 mg, 4 eq) to give the analogue peptide (Figure 4.1) after TFA cleavage and precipitation with cold ether. The resulting initiator was further analysed by mass spectroscopy as observed by the presence of $[M^+]$ at m/z 1171/1173 corresponding to expected values. Attempted coupling of N-succinimidyl 4-(2-bromo-2-methylpropionyloxy)butanoate **12** (chapter 2) with the polypeptide using the strategy stated above was unsuccessful, only the unmodified polypeptide was observed by mass spectroscopy



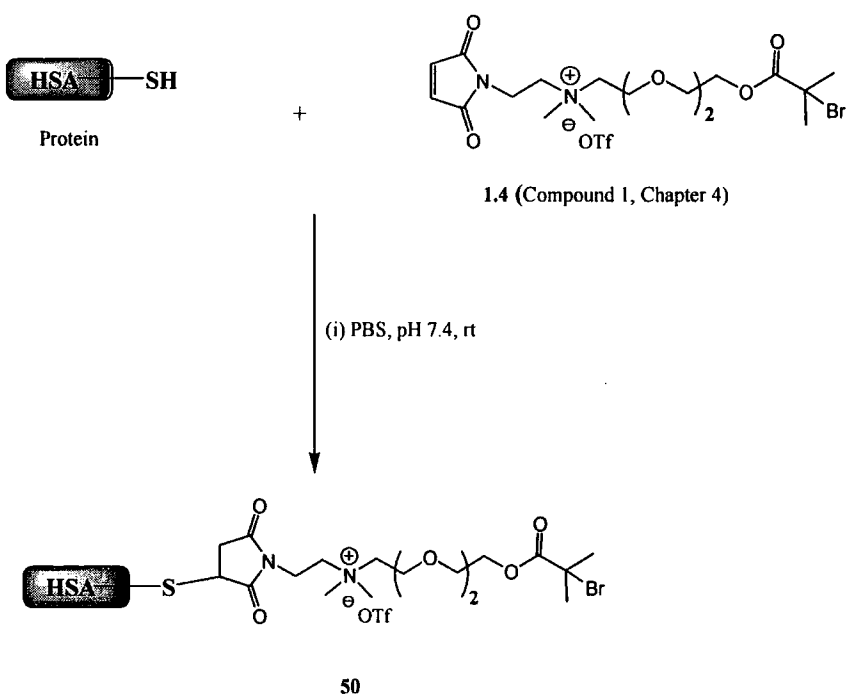
(i) Diisopropylethylamine, **3** (chapter 3; 4 eqv.) **or 12** (chapter 2; 4 eqv.)

(ii) 95% TFA, 5% TIS/Water.

Scheme 4.26

4.3.3 Synthesis of Protein (HSA)-initiator complex

The protein-initiator complex **50** was prepared by first treating the protein Human Serum Albumin (HSA) with ten molar equivalents of ethanethiol to cleave disulphide bonds after 24 hours at 4° C. By observing the Ellman's anion in the UV-VIS, the concentration of the free thiol was determined (0.6 - 0.8 SH/HSA) after being purified by gel filteration on Sephadex PD-10 against a phosphate buffer eluent. The protein was further observed by TOF mass spectroscopy to show the molecular ion $(M + 50H)^{50+}$ at m/z 1328, which was comparable with expected value of m/z 1331. The protein was then treated with the maleimide functionalised initiator **1.4** (compound 1, chapter 4) in PBS solution at room temperature. After completion of the reaction as adjudged by observing Ellman's anion, the reaction was subjected to gel filtration and the fractions collected analysed after PBS elution. The protein-based initiator **50** was further observed by TOF mass spectroscopy to show the molecular ion $(M + 47H)^{47+}$ at m/z 1425, which was comparable with expected value of m/z 1423.



Scheme 4.27

4.3.4 Model polymerisation of protein-initiator complex

The model polymerisation with the monomer monomethoxy capped oligo(ethylene oxide) methacrylate initiated by the initiator **50** was attempted based on the literature procedure[20]. The initiator **50** (0.01 mM) in PBS was dissolved in doubly distilled de-ionised water (2 mL) before adding bipyridine ligand (2.2 eqv. of Cu^{+}) followed by CuBr (15 mg) and the monomer (0.02 g, 100 eqv. of initiator). The reaction was vortexed under a stream of nitrogen at room temperature and the progress of the reaction was observed as the mixture became dark brown and viscous. The reaction was then purified with Sephadex PD 10 eluted with water to yield a colourless liquid. Attempts to analyse by GPC (aqueous or organic solvent based) proved difficult, while analysis by MALDI was more productive (Figure 4.2) in giving the m/z signals at 33628 and 67541 which did not correspond to the expected value(s) but were indicative of premature termination.

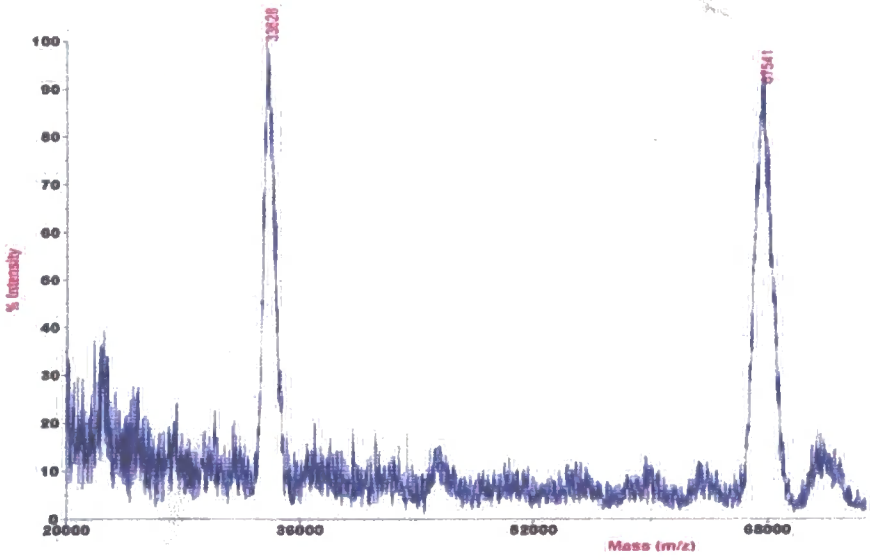
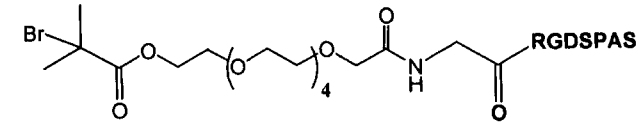


Figure 4.2

4.4 Summary

We have demonstrated that three different classes of bio-macromolecule linker can be prepared that incorporate the ability to initiate living polymerisation process with reactivity to biomolecules.



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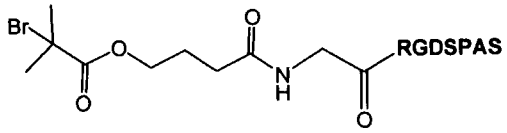
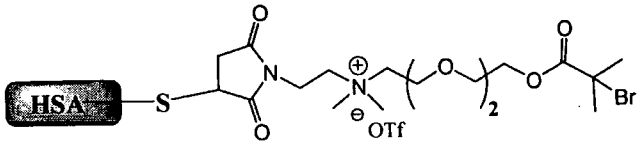


49 (Unsuccessful)



50

Figure 4.3

They were designed to facilitate chemoselectivity for peptide and protein residues; unfortunately, compound **49** proved to be unstable in an aqueous environment and as such polymerisation with **49** was not realised. Compound **50** on the other hand, after initial attempts, showed promising living polymerisation properties (initiation and limited propagation). These will be improved upon in further studies. Compound **48** was successfully prepared but due to time constraints was not investigated as an ATRP initiator.

4.5 References

1 J. C. Sheehan, V. A. Bolhofer, *J. Am. Chem. Soc.*, **1950**, *72*, 2786.

2 Han Yinglin, Hu Hongwen, *Synthesis,* **1990**, 122.

3 J. M. Takacs, Z. Xu et al., *Org. Lett.*, **2002**, *4*, 3843.

4 A. Warnecke, F. Kratz, *Bioconjugate Chemistry*, **2003**, *14*, 377.

5 M. A. Walker, *Tetrahedron Letters,* **1996**, *37*, 8133.

6 A. Warnecke, F. Kratz, *Bioconjugate Chemistry,* **2003**, *14*, 377.

7 A. I. Meyer, B. A. Lefker, T. J. Sowin, L. J. Westrum, *J. Org. Chem.*, **1989**, *54*, 4243.

8 T. F. Braish, D. E. Fox, *Synlett*, **1992**, 979.

9 O. Nielsen, O. Buchardt, *Synthesis*, **1991**, 819.

10 R. A. Nadzhafova, *Russian Journal of Org. Chem.*, **2002**, *38*, 858.

11 N. B. Metha, A. P. Phillips, F. F. Lui, R. E. Brooks, *J. Org. Chem.*, **1960**, *25*, 1012.

12 O. Keller, J. Rudinger, *Helv. Chim. Acta.*, **1975**, *58*, 531.

13 M. Aponte, G. B. Butler, *J. of Polymer Sci., Polymer Chem. Ed.,* (**1984**, *22*, (11 part 1), 2841.

14 (a) M. L. Bender, *Journal of the American Chemical Society,* **1957**, *79*, 1258.

(b) S. Fletcher, M. R. Jorgensen, A. D. Miller, *Org. Lett.*, **2004**, *6*, 4245.

15 U. Beyer, M. Kruger, P. Schumacher, C. Unger, F. Kratz., *Monatsh Chem,* **1997**, *128*, 91.

16 E. Koyama, F. Sanda, T. Endo., J. *Polymer Sci. A, Polymer chem.*, **1997**, *35*, 345.

17 M. Nasreddine, S. Szonyi, A. Cambon, *J. Fluorine Chem.*, **1994**, *66*, 293.

18 Yue-Ling Wong, M. P. Hubieki, C. L. Curfman, G. F. Doncel, T. C. Dudding, P. S. Savle, R. D. Gandour, *Bioorg. Med. Chem.*, **2002**, *10*, 599.

19 I. Ganjian, M. Khorsshidi, I. Lalezari, *J. Heterocyclic Chem.*, **1991**, *28*, 1173.

20 X. S. Wang, S. F. Lascelles, R. A. Jackson, S. P. Armes, *Chem. Comm.*, **1999**, 1817.

CHAPTER 5 – EXPERIMENTAL

EXPERIMENTAL PROCEDURES

5.1 General Procedures

Reactions were carried out under an atmosphere of argon in dry glassware unless otherwise stated.

Reagents

Reagents were used as supplied unless otherwise stated.

Melting Points

Recorded using Cambridge Instrument Gallen^{TM} III Kofler melting point apparatus and were uncorrected.

Solvents

Dry DMF was obtained from the Aldrich Chemical Company and used as received. THF was distilled from sodium benzophenone ketyl and dry DCM was distilled over calcium hydride under an atmosphere of nitrogen. 40-60 Petroleum-ether refers to the fraction of petroleum ether, which boils between 40 and 60 °C and was redistilled prior to use. All other solvents were used as received. In the cases where mixtures of solvents were used, ratios given refer to volumes used.

Solvents were dried according to standard procedures: diethyl ether and tetrahydrofuran were distilled under N_2 over Na, immediately prior to use. All solvents were removed by evaporation under reduced pressure.

Chromatography

Flash chromatography was carried out using silica gel (Fluorochem 60A, 40-60μ). Analytical thin layer chromatography (TLC) was performed using aluminium plates precoated with silica gel (60 F_{254}), and materials were visualized by UV radiation at 254 nm, or by staining with potassium permanganate in aqueous sodium carbonate or phosphomolybdic acid in ethanol.

IR Spectroscopy

IR spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrometer. Liquids were recorded as thin layers (neat) between KBr plates and solids were recorded as compression formed discs made using KBr unless otherwise specified.

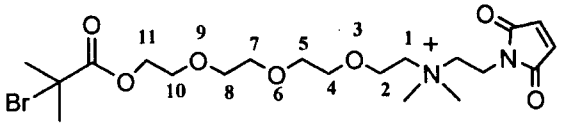
NMR Spectroscopy

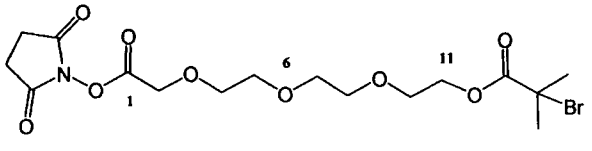
^1H NMR spectra were analysed in D_2O, $CDCl_3$ or CD_3OD on Varian Oxford 200, Bruker AM250, Varian Unity 300, Varian VXR 400 or Varian Inova 500 spectrometers and are reported as follows: chemical shift δ (ppm) (number of protons, multiplicity, coupling constant J (Hz), assignment). ^{13}C NMR spectra were recorded at 63 MHz on the Bruker AM250 spectrometer or 126 MHz on the Varian Inova 500 spectrometer. The central resonance of $CDCl_3$ (δ_C = 77.00 ppm) was used as an internal reference. ^{19}F NMR spectra were analysed in $CDCl_3$ at 188 MHz on the Varian Oxford 200 spectrometer. ^{19}F spectral data are referenced to $CFCl_3$ (δ_F = 0 ppm). Chemical shifts are quoted in parts per million relative to tetramethylsilane (δ_H = 0 ppm) and coupling constants are quoted in Hertz.

Mass Spectrometry

Low-resolution mass spectra were obtained on VG Analytical 7070E or VG Autospec Organic Mass Spectrometer. High-resolution mass spectra were obtained from the Durham University Chemistry Department microanalysis service. In both cases electrospray (ES), chemical ionisation (CI) and electron ionisation (EI) methods were used to generate ions. MALDI-tof spectrum was recorded on the Applied Biosystems Voyager-DE STR-MALDI system.

The numbering system used in reporting/naming the compounds





5.2 Compounds from Chapter 2

5.2.1 N – succinimidyl acrylate (5)

1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI, 0.82 g, 4.30 mmol) was added to a stirred mixture of N hydroxysuccinimide (0.48 g, 4.15 mmol) and 3 – bromopropionic acid (0.57 g, 3.75 mmol) in dichloromethane (10 mL) at room temperature under a nitrogen atmosphere. The mixture was then stirred for 2 h, washed with potassium bicarbonate solution (2 x 20 mL), dilute HCl (2 x 20 mL) and dried (Na_2SO_4), before being concentrated *in vacuo* to give the crude product. The crude product was then triturated with MeOH to give a colourless crystalline solid (0.197 g, 30%).

δ_H ($CDCl_3$; 500 MHz) 2.88 (4H, s, br, CH_2CH_2), 6.18 (1H, d, J_{AB} = 6.5 Hz, CH_X=CH_BH_A), 6.32 (1H, dd, J_{BX} = 11 Hz, C=OCH_X=CH_2 and J_{BA} = 6.5 Hz, CH_X=CH_BH_A), 6.71 (1H, d, J_{AX} = 18.5 Hz, CH_X=CH_BH_A).

m/z (EI) 170 (50 %, M + H) and 56 (100 %) M.P 67 – 69 °C

5.2.2 3-Hydroxypropanoic acid (6)

3-Bromopropionic acid (2.5g, 16.3 mmol) was refluxed in water (32 mL) for 6.5 h. Upon completion, as determined by TLC (R_f = 0.5 in 100 % MeOH) the reaction was extracted with ethyl acetate and the organic extracts concentrated under reduced pressure. Kulgerohr distillation (2 mbar, 150 – 152 °C) furnished the product (0.43 g, 30 %) as an orange/pink oil which solidified on standing.

δ_H ($CDCl_3$; 400 MHz) 2.66 (2H, t, J = 6.6 Hz, CH_2COOH), 4.37 (2H, t, J = 6.6 Hz, CH_2OH)

δ_C ($CDCl_3$; 100 MHz) 176.92 (C=O), 37.66 (CH_2OH) and 25.23 ($\underline{C}H_2COOH$).

m/z (ES -) 89 (40 %, M - H); (CI+) 108 (40 %, M + NH_4), 234 (100%); (EI+) 107 (M + NH_4), 91 (100 %, M+H)

ν_{max} (liquid film, cm^{-1}) 3264 (OH), 2928 (COOH, br), and 1648 (C=O).

C: H: N.: $C_3H_6O_3$ Requires C40.45, H5.62 Found C45.66, H5.40.

M.P 57 – 58 °C.

5.2.3 N-Succinimidyl-(2-bromo-2-methyl)propionate (8)

1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide iodide (EDCI, 0.82 g, 4.30 mmol) was added in portions to a stirred solution of 2-bromo-2-methylpropionic acid (0.618 g, 3.7 mmol) and N-hydroxysuccinimide (0.48 g, 4.15 mmol) in dry dichloromethane (10 mL) at room temperature under a nitrogen atmosphere. The mixture was stirred for 8 h before being washed with saturated aq. sodium bicarbonate (2 x 20 mL), dil HCl (3 x 20 mL) and water (2 x 20 mL). After drying of the organic phase with Na_2SO_4 and concentration *in vacuo*, the crude product was purified by column chromatography (50 % dichloromethane / petrol 40-50,) to yield the title compound (R_f 0.5, 0.562 g, 55%) as a pale brown syrup.

δ_H ($CDCl_3$; 400 MHz) 2.08 (6H, s, $(CH_3)_2$), 2.86 (4H, s, br, CH_2-CH_2)

δ_C ($CDCl_3$; 100 MHz) 169 (C=O), 168 (C=O), 52 (C-Br), 31.5 (Me) and 26 (CH_2).

(EI) 263 (5%, M +) and 184 (M - Br).

C: H: N.; Found C 36.42, H 3.79, N 5.55; Required C 36.39, H, 3.82, N 5.30

5.2.4 4-Methoxybenzyl-4-hydroxybutanoate (10)

To a solution of sodium 4-hydroxybutyrate (0.5 g, 4.00 mmol) and tetrabutyl-ammonium iodide (120 mg, catalytic) in dry tetrahydrofuran (30 mL) was added 4- methoxybenzyl chloride (0.74 g, 0.64 mL, 4.76 mmol). The reaction mixture was heated at reflux for 24 h. The reaction was allowed to cool to room temperature before being concentrated *in vacuo* to a volume of 2 mL and then dilute with dichloromethane (30 mL) and washed with water (3 x 20 mL). The dichloromethane extracts were combined and the aqueous layer further extracted with dichloromethane (2 x 15 mL). The organic extracts were combined, dried $MgSO_4$ and filtered. Purification by silica gel column chromatography (20% ethyl acetate/hexane) yielded the title compound (0.8 g, 92 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.82 (2H, m, CH_2), 2.41 (2H, t, J = 7.8 Hz CH_2C=O), 3.60 (2H, m, CH_2OH), 3.76 (3H, s, MeO), 5.01 (2H, s, CH_2Ph), 6.82 (2H, d, J = 8.5 Hz, Ph), 7.22 (2H, d, J = 8.4, Ph)

δ_C ($CDCl_3$; 50 MHz) 174.10 (C=O), 159.85 (Ph-C-O), 130.31 (Ph-C), 128.24 (C-H), 114.17 (C-H), 66.41 (CH_2O), 62.08 (CH_2OH), 55.49 (MeO), 31.25 (CH_2), 27.91.

m/z (ES) 247 (M + Na) ν_{max} (liquid film, cm^{-1}) 1712 (C=O)

5.2.5 4-Methoxybenzyl 4-(2-bromo-2-methylpropionyloxy)butanoic acid (11)

To a solution of 4-methoxybenzyl 4-hydroxybutanoate (2.0 g, 8.93 mmol), and triethylamine (1.1 g, 10.71 mmol, 1.2 eqv) in dichloromethane (25 mL) was added dimethylaminopyridine (0.22 g, 20 mol %) and the reaction was allowed to stir at room temperature for 0.5 h. The mixture was then cooled to 0°C before the dropwise addition of 2-bromo-2-methylpropionyl bromide (2.5 g, 1.76 mL, 1.2 eqv), and stirred for 4 h at 0°C and overnight at room temperature. The reaction mixture was washed with dil HCl, water and dried ($MgSO_4$) before concentration *in vacuo* and purification by column chromatography (10 % ethyl acetate/hexane). The title compound (1.8 g, 54 %) was obtained as a colourless oil.

δ_H ($CDCl_3$; 200 MHz) 1.92 (6H, s, C $[CH_3]_2$ Br), 2.0 (2H, m, CH_2), 2.47 (2H, t, J = 7.4 Hz, CH_2C=O), 3.80 (3H, s, MeO), 4.20 (2H, t, J = 6.4 Hz, CH_2O), 5.06 (2H, s, $PhCH_2O$), 6.87 (2H, d, J = 8.5 Hz, Ph), 7.34 (2H, d, J = 8.5 Hz, Ph).

δ_C ($CDCl_3$; 50 MHz) 175.0, 173.0 (C=O), 160.0, 130.0 (Ph-C), 128.0 (CH), 114.0 (CH), 66.0, 65.0 (CH_2O), 55.0 (MeO), 30.0 (CH_2) and 24.0 (Me).

m/z (ES) 395/397 (M + Na).

HRMS (ES) $C_{16}H_{21}{}^{79}BrO_5Na$ requires 395.0572 found 395.0469

$C_{16}H_{21}{}^{81}BrO_5Na$ requires 397.0552 found 397.0452.

5.2.6 4-(2-Bromo-2-methylpropionyloxy) butanoic acid (11b)

A solution of 10 % TFA/ DCM (10 mL) was added slowly with stirring to a solution of 4-methoxybenzyl 4-(2-bromo-2-methylpropionyloxy) butyrate (2.0 g, 5.4 mmol) in dichloromethane (1 mL) at room temperature. The dark red solution was stirred for 4 h at room temperature, and monitored by TLC (25 % ethyl acetate/ hexane, R_f = 0.6 of ester). The reaction was diluted with water (5

mL) and then saturated aq. $NaHCO_3$ (20 mL) was added with stirring. The resulting mixture was stirred for 10 min before being extracted with DCM (3 x 20 mL) and the aqueous extracts combined, acidified (1M HCl) and the resulting solution further extracted with DCM (3 x 20 mL), concentrated and dried over P_2O_5 under reduced pressure to obtain the title compound (1.33 g, 98 %) as a colourless oil, which turned pale brown on standing.

The compound was analysed and used without further purification.

δ_H ($CDCl_3$; 400 MHz) 1.92 (6H, s, C $[CH_3]_2$ Br), 2.11 (2H, m, CH_2), 2.51 (2H, t, J = 7.4 Hz, $CH_2C{=}O$), 4.24 (2H, t, J = 6.4 Hz, CH_2O).

m/z (+ES) 274/276 (100 %, M + Na).

HRMS (+ES) $C_8H_{13}{}^{79}BrO_4Na$ requires 275.000 found 274.9912.

$C_8H_{13}{}^{81}BrO_4Na$ requires 276.998 found 276.9894

5.2.7 N-Succinimidyl 4-(2-Bromo-2-methylpropionyloxy) butanoate (12)

A solution of 4-(2-bromo-2-methylpropionyloxy) butyric acid (1.00 g, 4 mmol), and DIC (0.60 g, 0.56 mL, 1.2 eq.) in DCM (10 mL) was stirred for 1 h at room temperature. A solution of N-hydroxysuccinimide (0.55 g, 1.2 eq.) and triethylamine (0.80 g, 2 eq.) in DCM (10 mL) was then added. The mixture was further stirred at room temperature for 16 h. Upon completion (TLC, 50 % ethyl acetate / hexane, R_f = 0.45, $KMnO_4$), dilute HCl (2 x 20 mL) and water (2 x 20 mL) were used to wash the mixture before drying $MgSO_4$. After evaporation, the residue was subjected to column chromatography (30 % ethyl acetate / hexane). The title compound (1.1 g, 75 %) was recovered as a colourless oil.

δ_H ($CDCl_3$; 400 MHz)_1.94 (6H, s, C $[CH_3]_2$ Br), 2.11 (2H, m, CH_2), 2.77 (2H, m, $CH_2C{=}O$), 2.84 (4H, s, br, CH_2-CH_2), 4.27 (2H, m, CH_2O).

δ_C ($CDCl_3$; 100 MHz)_171.51, 169.00 (C=O), 167.93 (NC=O), 60.41(CH_2O), 55.61 (C-Br), 30.69 (CH_2), 27.56, 25.59 (Me), 23.59, 21.06 (CH_2).

m/z (+ES) 372 (100 %, M + Na) and 374 (80 %, M + Na)

HRMS (+ES) $C_{12}H_{16}{}^{79}BrNO_6Na$ requires 372.0161 found 372.0064

$C_{12}H_{16}{}^{81}BrNO_6Na$ requires 374.0195 found 374.0050

IR (liquid film, cm^{-1}) 1795 (C=O), 1784 (C=O).

5.2.8 2-Methoxy-2-methyl-propionic acid (13)

2-Bromo-2-methylpropionic acid (3 g, 18 mmol) was finely ground and added in portions to a freshly prepared solution of NaOMe (0.9 g sodium, 39.5 mmol, 2.2 eqv) in methanol (60 mL) at room temperature. The mixture was then allowed to stir overnight at 40 °C under a nitrogen atmosphere. On completion of the reaction (TLC, 10 % MeOH / DCM, $R_{f\ new}$ 0.25, $R_{f\ starting\ acid}$ 0.8, $KMnO_4$), the mixture was acidified (1M HCl) to pH 1. The precipitate formed was decanted off and the methanolic filtrate concentrated and extracted with dichloromethane (3 x 20 mL). The combined dichloromethane extracts were concentrated *in vacuo* and further washed with brine (2 x 25 mL), water (2 x 25 mL) and dried Na_2SO_4. The organic phase was then concentrated and dried under reduced pressure before being dried over P_2O_5. The product (2.1 g, 77%) was obtained as a brown syrup, which was used in subsequent reactions without further purification.

δ_H ($CDCl_3$; 300 MHz) 1.46 (6H, s, C $[CH_3]_2$ OMe), 3.32 (3H, s, C $[CH_3]_2$ OMe), 8.7 (1H, s, br, COOH).

δ_C ($CDCl_3$; 100 MHz) 167.0 (C=O), 72.5 (C $[CH_3]_2$), 61.0 (MeO), 24.0 (Me_2).

m/z (ES) 141 (100 %, M + Na) m/z (CI+) 136 (100 %, M + NH_4)

m/z (EI+) 59 (80 %, M - COOH), ν_{max} (liquid film, cm^{-1}) 2988 (br, OH) and 1724 (C=O).

Literature: (see Chap. 2, Ref 4, p80.)

δ_H (CCl_4) 1.41 (6H, s), 3.21 (3H, s), 11.6 (1H, s). ν_{max} (liquid film, cm^{-1}) 3300-3100 (br), 2985, 1735, 1715.

5.2.9 N-Succinimidyl -2-methoxy-2-methyl-propionate (14)

A solution of 2-methoxy-2-methyl-propionic acid (1.3 g, 11 mmol), EDCI (2.3 g, 12 mmol, 1.1 eqv) and *N,N*-dimethylaminopyridine (catalytic) in dichloromethane (15 mL) was stirred for 0.5 h at room temperature under an atmosphere of nitrogen before the addition of N-hydroxysuccinimide (1.39 g, 12 mmol, 1.1 eqv). The mixture was then allowed to stir for a further 6 h at room temperature. The reaction mixture was washed with 1M HCl (3 x 15 mL), water (3 x 20 mL) and dried with $MgSO_4$ before being concentrated *in vacuo*, column

chromatography ([1:2] DCM, petrol ether 40/60) before drying over P_2O_5 under reduced pressure to yield the title product (1.76 g, 73 %) as a brown syrup.

δ_H ($CDCl_3$; 200 MHz) 1.54 (6H, s, C $[CH_3]_2$ OMe), 2.80 (4H, s, cyclic-CH_2CH_2), 3.36 (3H, s, C $[CH_3]_2$ OMe); m/z (ES) 238 (M + Na).

5.2.10 4-Methoxybenzyl 4-(2-methoxy-2-methyl-propionyloxy)butanoate (15)

Triethylamine (90 mg, 0.893 mmol, 0.125 mL) wad added to a solution of 4-methoxybenzyl 4-hydroxybutanoate (100 mg, 0.446 mmol) and succinamidyl 2-methoxy-2-methyl-propionate (106 mg, 0.491 mmol, 1.1 eq) in dichloromethane (3 mL) at room temperature before being allowed to stir for 6 h at room temperature under a nitrogen atmosphere. The reaction mixture was stirred and monitored with TLC (50 % ethyl acetate/hexane $R_{f\ new}$ 0.8 in $KMnO_4$). The reaction mixture was diluted with dichloromethane (20 mL) washed with dilute HCl (2 x 20 mL) and water (2 x 20 mL), concentrated and dried $MgSO_4$ before purification by column chromatography (25 % ethyl acetate/hexane). The title compound (130 mg, 90 %) was obtained as a colourless oil.

δ_H ($CDCl_3$; 200 MHz) 1.41 (6H, s, C $[CH_3]_2$ OMe), 2.00 (2H, m, CH_2), 2.42 (2H, t, J = 7.4 Hz, CH_2C=O), 3.26 (3H, s, C $[CH_3]_2$ O<u>Me</u>), 3.82 (3H, s, CH_2 PhO<u>Me</u>), 4.20 (2H, t, J = 6.5 Hz, CH_2O), 5.12 (2H, s, $PhCH_2O$), 6.87 (2H, d, J = 8.5 Hz, Ph), 7.34 (2H, d, J = 8.5 Hz, Ph).

δ_C ($CDCl_3$; 50 MHz) 175.0, 173.0 (C=O), 160.0, 130.0 (Ph-C), 128.0 (CH), 114.2, 114.3 (CH), 66.4, 66.5(CH_2O), 64.1, 55.0, 52.0 (MeO), 30.9, 30.8 (CH_2), 24.3, 24.2 (Me).

m/z (ES) 347 (M + Na) HRMS (ES) $C_{17}H_{24}O_6Na$ requires 347.1573 found 347.1487.

ν_{max} (liquid film, cm^{-1}) 1732 (C=O), 1613 (C=O), 1515 (C-O) and 1166 (C-O).

5.2.11 Benzyl 4-hydroxybutanoate (17)

The sodium salt of 4-hydroxybutyrate (200 mg, 1.59 mmol), tetrabutylammonium bromide (0.102 mg, 20 mol%) and benzyl bromide (0.227 mL, 1.91 mmol) were added to DMF (5 mL) and allowed to stir at room temperature. After completion of the reaction (48 h), as monitored by TLC (40 % ethyl acetate/hexane $R_{f\ new}$ 0.15, UV and $KMnO_4$), the mixture was concentrated *in vacuo*, diluted with DCM (15 mL), washed with water (5 x 20 mL) and dried over $MgSO_4$. The crude compound was further subjected to high vacuum rotary evaporation before purification by chromatography (10 % ethyl acetate/hexane) to yield the title compound (220 mg, 97%) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.65 (1H, s, OH), 1.92 (2H, m, CH_2), 2.52 (2H, m, CH_2), 3.70 (2H, m, CH_2), 5.15 (2H, s, $PhCH_2$), 7.28 (5H, m, Ph).

δ_C ($CDCl_3$; 100 MHz) 174 (C=O), 136 (Ph-C), 129 (CH), 128 (CH), 67 (CH_2O), 62 (CH_2OH), 32 (CH_2), 28 (CH_2).

m/z (+ES) 217 (80 %, M + Na)

ν_{max} (liquid film, cm^{-1}) 3279 (OH) and 1736 (C=O).

HRMS (+ES) $C_{11}H_{14}O_3Na$ requires 217.0943 found 217.0823.

5.2.12 Benzyl 4-(2-methoxy-2-methyl-propionyloxy)butanoate (18)

A mixture of EDCI (130 mg, 1.2 eqv, 0.680 mmol), 2–methoxy-2-methylpropionic acid (80 mg, 0.680 mmol, 1.2 eqv) and N, N-dimethylaminopyridine (85 mg, 1.2 eqv, 0.680 mmol) in DCM (3 mL) was stirred for 1 h before a solution of benzyl-4-hydroxybutanoate (110 mg, 0.567 mmol) in DCM (5 mL) was added dropwise with continued stirring. The reaction was monitored by TLC (40 % ethyl acetate / hexane, $R_{f\ new}$ 0.75, $R_{f\ starting\ benzyl}$ 0.3, both UV and $KMnO_4$ visible) and left to stir overnight at room temperature under a nitrogen atmosphere. The reaction mixture was washed with 1M HCl (2 x 25 mL), water (2 x 25 mL), extracted and concentrated *in vacuo* before purification with silica gel chromatography (10 % and 50 % ethyl acetate /

hexane respectively) to yield the title compound (83 mg, 51 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.40 (6H, s, C $[CH_3]_2$ OMe), 2.0 (2H, m, CH_2O), 2.42 (2H, m, CH_2), 3.34 (3H, s, MeO), 4.21 (2H, m, CH_2), 5.10 (2H, s, Ph CH_2O), 7.42 (5H, m, Ph).

δ_C ($CDCl_3$; 100 MHz) 175.0 (C=O), 173.0 (C=O), 136.0 (Ph-C), 128.7, 128.6, 128.4, 128.3(CH), 72 ((C_$[CH_3]_2$), 67.0, 65.0 (CH_2O), 61 (MeO), 52.0, 31.0 (CH_2), 25.2, 25.0 (Me)

m/z (ES) 317 (100 %, M + Na) HRMS (ES), $C_{16}H_{22}O_5Na$ requires 317.1467 found 317.1361, ν_{max} (liquid film, cm^{-1}) 2940 (CH_2) and 1738(C=O).

5.2.13 Benzyl 4-(2-bromo-2-methyl-propionyloxy)butanoate (19)

A solution of benzyl 4-hydroxybutanoate (108 mg, 0.557 mmol) and triethylamine (0.09 mL, 0.668 mmol, 1.2 eqv) in DCM (5 mL) was stirred at room temperature under a nitrogen atmosphere for 0.5 h. The solution was then cooled to 0 °C before the dropwise addition of 2-bromo-2-methylpropionyl bromide (0.109 mL, 0.668 mmol 1.2 eqv.). The reaction mixture was further stirred at room temperature for 5 h during which some white precipitate formed. After completion of the reaction, as determined by TLC (40 % ethyl acetate / hexane, $R_{f\ new}$ = 0.9 two spots, $R_{f\ sm}$ = 0.3 consumed, both UV and $KMnO_4$ visible), the mixture was diluted with DCM (20 mL), washed with 1M HCl (2 x 20 mL), water (2 x 15 mL) and concentrated under reduced pressure. The resulting oily mixture was purified by silica gel chromatography (20 % ethyl acetate / hexane) to produce the title compound (101 mg, 56 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.95 (6H, s, C $[CH_3]_2$ Br), 2.05 (2H, m, CH_2O), 2.51 (2H, m, CH_2), 4.22 (2H, m, CH_2), 5.18 (2H, s, $PhCH_2O$), 7.4 (5H, m, Ph).

δ_C ($CDCl_3$; 100 MHz) 173.0 (C=O), 171.0 (C=O), 135.0 (Ph-C), 128.7, 128.6, 128.4, 128.3(CH), 67.0, 65.0 (CH_2O), 55.0 (C), 31.0 (CH_2), 25.2, 25.0 (Me)

m/z (ES) 365/367 (M + Na)

HRMS (ES) $C_{13}H_{19}{}^{79}BrO_4$ requires 365.0467 found 365.0378.

$C_{13}H_{19}{}^{81}BrO_4$ requires 367.0446 found 367.0338

ν_{max} (liquid film, cm^{-1}) 1735 (C=O)

5.3 Compounds from Chapter 3

5.3.1 Methanesulphonyl Azide (18)

A three-necked flask equipped with a nitrogen inlet and a powder funnel was filled with methanesulphonyl chloride (15 mL, 194 mmol) in acetone (Analar grade, 100 mL). Sodium azide (18.9 g, 290 mmol) was then added through the funnel over 0.5 h under a constant flow of nitrogen at room temperature to the solution and stirred for 2 h. The mixture was then filtered, dried over Na_2SO_4 and concentrated under reduced pressure for 2 h. Methanesulphonyl azide (22.3 g, 94 %) was obtained as a colourless oil which was used without further purification.

ν_{max} (liquid film, cm^{-1}) 2146 (N=N), 1362 and 1174.

C:H:N : $CH_3SO_2N_3$ requires C60.27; H5.06; N12.78; found C60.32; H4.65; N12.31.

5.3.2 Toluenesulphonyl Azide (19)

A solution of sodium azide (10.0 g, 196 mmol) in a mixture of water (28.5 mL) and ethanol (57 mL, 90 %) was added to a solution of tosyl chloride (27.2 g, 143 mmol) in ethanol (143 mL, 90 %) at 45 °C. After 12 h at room temperature, the reaction was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure. The product (22.76 g, 98 %) was obtained as a colourless oil, and was not further purified.

δ_H ($CDCl_3$; 400 MHz) 2.42 (3H, s, CH_3Ph), 7.33 (2H, d, Ph), 7.78 (2H, d, Ph)

δ_C ($CDCl_3$; 100 MHz) 146 (C-Me), 135(C-S), 130 (CH), 127 (CH) and 21(Me).

ν_{max} (liquid film, cm^{-1}) 2132 (N=N).

C: H: N: S: $C_7H_7N_3S$ requires: C42.63, H3.58, N21.31, S16.26 found: C41.44, H3.46, N20.81, S15.73.

5.3.3 Benzyl diazoacetate (21)

Benzyl acetoacetate (13 mL, 14.5 g, 75.5 mmol) was added to tosyl azide (15 g, 75.5 mmol, 1 eqv.) in anhydrous acetonitrile (92 mL) in the presence of triethylamine (7.6 g, 75.5 mmol, 1 eqv.) over 3 h at room temperature. A solution of KOH (9 g) in water (7 mL) was then added and the mixture stirred for a

further 12 h during which the reaction turned reddish brown in colour. The reaction mixture was extracted with ether, washed with a solution of KOH (3 g) in water (25 mL) and then with water. After drying (Na_2SO_4), filtration and evaporation of the solvent under reduced pressure, the crude product was obtained as a reddish brown oil. Purification by silica gel chromatography (90 % ethyl acetate / hexane; then 2 % methanol in ethyl acetate) gave the title compound (6.0 g, 94 %) as a yellow oil.

δ_H ($CDCl_3$; 200 MHz) 4.78 (1H, s), 5.19 (2H, s, CH_2Ph), 7.39 (5H, s, Ph)

ν_{max} (liquid film, cm^{-1}) 2115 (N=N), 1690 (C=O)

5.3.4 Benzyl 11-hydroxy-3,6,9-trioxaundecanoate (10)

Benzyl diazoacetate (5.5 g, 31 mmol) in dichloromethane (20 mL) was added slowly to a stirred solution of triethyleneglycol (20 g, 155 mmol, 3 eqv.) and boron trifluoride etherate (2 drops, catalytic) at room temperature in dichloromethane (10 mL), and a steady evolution of gas was observed The colourless reaction mixture was stirred under nitrogen at room temperature for 3 h, before being diluted with dichloromethane (20 mL) and washed with water (4 x 30 mL). The aqueous washings were washed with DCM (1 x 20 mL) and the combined organic extracts were dried ($MgSO_4$) before being concentrated *in vacuo* to give the crude product as a yellow oil. Purification by flash chromatography (50 % hexane/ ethyl acetate) then produced the title compound (8.2 g, 88 %) as a pale yellow oil.

δ_H ($CDCl_3$; 300 MHz) 2.5 (1H, s, br, OH), 3.6 – 3.8 (12H, m, 5 x OCH_2 and CH_2OH), 4.3 (2H, m, BnOCOC$\underline{H_2}$), 5.2 (2H, s, C$\underline{H_2}$Ph) 7.32 (5H, s, Ph)

δ_C ($CDCl_3$; 75 MHz) 170.0 (C=O), 135.0 (Ph-C), 128.0 (2 x CH), 128.4 (2 x CH), 72.0 ($PhCH_2O$), 70.9 (2 x OCH_2), 70.6 (2 x OCH_2), 70.3 (2 x OCH_2), 66.0 (CH_2OH).

m/z (+ES) 321 (100%, M + Na), HRMS (+ES) $C_{15}H_{22}O_6Na$ requires 321.1416 found 321.1329.

ν_{max} (liquid film, cm^{-1}) 3385 (-OH), 1749 (C=O), 1116 (C-O).

5.3.5 Benzyl (11(-2-bromo-2-methylpropionyloxy))-3,6,9-trioxaundecanoate (7)

A solution of 2-bromo-2-methylpropionyl bromide (0.92 g, 0.49 mL, 1.2 eq.) in dichloromethane (5 mL) previously cooled to 0 °C was introduced to a solution of benzyl 11-hydroxy-3, 6, 9-trioxaundecanoate (1.00 g. 3.36 mmol) and triethylamine (0.72 mL, 1.2 eq.) in dichloromethane (15 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 8 h and after a further 8 h at room temperature, before being washed successively with saturated aq. $NaHCO_3$ (3 x 20 mL) and water (2 x 20 mL). The extracts were combined and dried ($MgSO_4$), concentrated *in vacuo* and the crude material purified by silica gel flash column chromatography (20 % ethyl acetate then 50 % ethyl acetate / hexane). The title compound (1.2 g, 75 %) was recovered as pale brown oil.

δ_H ($CDCl_3$; 400 MHz) 1.95 (6H, s, Me_2), 3.6 – 3.8 (10H, m, 5 x OCH_2), 4.19 (2H, s, $BnOCOC\underline{H}_2$), 4.3 (2H, m, CH_2 $C\underline{H}_2OC{=}OC[CH_3]_2Br$), 5.19 (2H, s, $C\underline{H}_2Ph$) 7.38 (5H, m, Ph)

δ_C ($CDCl_3$; 100 MHz) 172.0(C=O), 171.0 (C=O), 137.0 (CH), 129.0 - 127.0 (4 x Ph-C), 72.5 - 72.0 (6 x OCH_2), 69.0 (OCH_2), 67.0 (OCH_2), 56.0 (C-Br), 32.0 (2 x Me).

m/z (+ES) 469 / 471 (100 %, M + Na)

HRMS (+ES) $C_{19}H_{27}{}^{79}BrO_7Na$ requires 469.0940 found 469.0843.

$C_{19}H_{27}{}^{81}BrO_7Na$ requires 471.0920 found 471.0826.

ν_{max} (liquid film, cm^{-1}) 1735 (C=O).

5.3.6 t-Butyl α-diazoacetate (26)

A. A mixture of

anhydrous acetonitrile (110 mL), *t*-butyl acetoacetate (13.4 g, 84.8 mmol), and triethylamine (11.4 mL, 84.8 mmol) was cooled to 0°C. The exothermic reaction mixture was stirred for 0.5 h before the dropwise addition of methanesulphonyl azide (10 g, 84.8 mmol) in acetonitrile (20 mL) (faster addition of reagents resulted in a rapid increase in temperature) over a period of 0.5 h with vigorous stirring at 0°C. The resulting yellow reaction mixture was then allowed to warm to room temperature and stirred for a further 3.5 h. The solvent was evaporated

under reduced pressure and the compound dissolved in ether (100 mL). The ethereal solution was washed aq. KOH (23 g in 250 mL, water x 2), KOH (4 g in 120 mL water, twice) and water (3 x 150 mL, twice). The organic phase was dried (Na_2SO_4). and concentrated under reduced pressure to gave the product (12.5 g, 80 %) as a yellow oil.

B. A freshly prepared solution of sodium methoxide (3.0 g sodium in 50 mL of methanol) was added dropwise with stirring to a solution of *t*-butyl α-diazoacetoacetate (12.5 g) in methanol (50 mL) cooled to 0 °C. The temperature of the reaction was at 0 °C by regulating the rate of addition of sodium methoxide. After the addition, the reaction was stirred at room temperature for 0.75 h. The resulting reddish/brown mixture was poured into iced water (500 mL), stirred for 0.5 h and extracted with ether (2 x 150 mL). The aqueous phase was saturated with sodium chloride and extracted with ether (3 x 120 mL). The combined organic phase was washed with water (2 x 200 mL) and dried (Na_2SO_4). The ether was concentrated using a rotary evaporator and slightly reduced pressure at 40 °C water bath temperature.

The resulting yellow oil was distilled in portions under the same conditions using 6 mbar/35 °C in a temperature controlled water bath to gave the yellow diazoacetate (8 g, 83 %) as a yellow oil.

δ_H ($CDCl_3$; 200 MHz) 1.48 (9H, s, $[CH_3]_3$) and 2.5 (1H, s, $CH{=}N_2$)

ν_{max} (liquid film, cm^{-1}) 2132 (N=N), 1714 (C=O)..

B.p 51 – 53 °C (12 mmHg).

5.3.7 t-Butyl 11-hydroxy-3,6,9-trioxaundecanoate.(27)

t-Butyl α-diazoacetate (1.49 g, 0.95 g/cm^3, 9.43 mmol) in dichloromethane (5 mL) at room temperature, was added slowly using a syringe to a stirred solution of triethyleneglycol (20 g) and boron trifluoride etherate (2 drops, catalytic) in dichloromethane (10 mL). A steady evolution of gas was observed. The colourless reaction mixture was stirred under a nitrogen atmosphere at room temperature for 3 h. The reaction was then diluted with dichloromethane (50 mL) and washed with water (4 x 30 mL). The aqueous washings were further

combined and extracted with dichloromethane (1 x 20 mL) and the combined organic extracts were dried (Na_2SO_4) before being concentrated under reduced pressure to reveal the crude product as a yellow oil. Purification by flash chromatography (50 % hexane/ethyl acetate then 100 % ethyl acetate) furnished the title compound (1.77 g, 71%) as a pale yellow oil.

δ_H ($CDCl_3$; 300 MHz) 2.30 (1H, s, br, OH), 1.5 (9H, s, C $[CH_3]_3$), 3.68 – 3.7(12H, m, 8 x OCH_2), 4.0 (2H, s, $OCH_2C{=}O$).

δ_C ($CDCl_3$; 125 MHz) 170 (C=O), 73 (C-Me), 73.4 - 72.2 (6 x CH_2O), 69 (CH_2O), 62 (CH_2OH), 28 (2 x Me).

m/z (ES) 287 (100 %, 264 + Na). HRMS (ES) $C_{12}H_{24}O_6Na$ requires: 287.1573 found 287.1494.

ν_{max} (liquid film, cm^{-1}) 3416 (OH) and 1745(C=O).

5.3.8 t-Butyl 14-hydroxy-3,6,9,12-tetraoxatetradecanoate (28).

Using the method described for **27** above, a solution of ^{t}butyl α-diazoacetate (2.0 g, 0.95 g/cm^3, 12.7 mmol) in dichloromethane (10 mL) was added slowly over a period of 0.25 h to the mixture of tetraethylene glycol (5.0 g) and boron trifluoride etherate (2 drops, catalytic) in dichloromethane (10 mL) before being stirred for 4h at room temperature under a nitrogen atmosphere. The reaction was then diluted with dichloromethane (50 mL) and washed with water (4 x 30 mL). The aqueous wash was further extracted with dichloromethane (1 x 20 mL) and the combined organic extracts dried over Na_2SO_4, before being concentrated under reduced pressure to reveal the crude product as a yellow oil. Purification by flash chromatography (50 % hexane/ethyl acetate then 100 % ethyl acetate) to furnish the title compound (2.9 g, 87 % based on diazo ester) as a pale yellow oil.

δ_H ($CDCl_3$; 300 MHz) 2.50 (1H, s, br, OH), 1.50 (9H, s, C $[CH_3]_3$), 3.56 - 3.68 (16H, m, 8 x OCH_2), 4.0 (2H, s, $OCH_2C{=}O$).

δ_C ($CDCl_3$; 75 MHz) 170.0 (C=O), 73.5 (C-Me_3), 72.0 - 70.7 (8 x CH_2O), 69.0 (CH_2O), 61.9 (CH_2OH), 28.0 (3 x Me).

m/z (ES) 331 (100 %, M+ Na). HRMS (ES) $C_{14}H_{28}O_7Na$ requires 331.1835 found 331.1725.

ν_{max} (liquid film, cm^{-1}) 3398 (OH), 1750 (C=O).

5.3.9 t-Butyl 17-hydroxy-3,6,9,12,15-pentaoxaheptadecanoate (29)

Using the method described for **27** above, a solution of *t*-butyl α-diazoacetate (3.6 g, 0.95 g/cm^3, 22.9 mmol) in dichloromethane (10 mL) was stirred with pentaethyleneglycol (5.0 g) and boron trifluoride etherate (2 drops, catalytic) in dichloromethane (10 mL) at room temperature to obtain the title compound (6.3 g, 71 % based on diazo ester) as a pale yellow oil.

δ_H ($CDCl_3$; 400 MHz) 2.40 (1H, s, br, OH), 1.50 (9H, s, C $[CH_3]_3$), 3.67 - 3.78 (20H, m, 10 x OCH_2), 4.0 (2H, s, $OCH_2C{=}O$).

δ_C ($CDCl_3$; 100 MHz) 170.0 (C=O), 74.2 - 70.5 (10 x CH_2O), 73.8 (C-Me), 69.0 (CH_2O), 61.9 (CH_2OH) and 28.0 (3 x Me).

m/z (+ES) 375 (80 %, M + Na) HRMS (+ES) $C_{16}H_{32}O_8Na$ requires: 375.2097 found 375.2009.

ν_{max} (liquid film, cm^{-1}) 3380 (OH) and 1748 (C=O).

5.3.10 t-Butyl 11-(2-bromo-2-methylpropionyloxy)-3,6,9-trioxa undecanoate (30)

To a solution of *t*-butyl 11-hydroxy-3,6,9-trioxaundecanoate (1.75 g, 6.71 mmol), triethylamine (0.81 mL, 8.05 mmol, 1.2 eqv.) and N, N-dimethylaminopyridine (200 mg, catalytic) in dichloromethane (10 mL) previously stirred for 1 h at room temperature was added dropwise 2 bromo-2-methylpropionyl bromide (0.99 mL, 8.05 mmol, 1.2 eqv.). Following the addition, the reaction was stirred for 8 h at 0 °C. The mixture was then washed with dilute HCl (2 x 20 mL), and extracted with dichloromethane (3 x 20 mL). The organic phase was washed with saturated aq. sodium bicarbonate (2 x 20 mL) and brine (2 x 20 mL), and dried (Na_2SO_4). Filtration and removal of the solvent under reduced pressure resulted in a crude product, which was purified by flash chromatography (20 % then 80 % ethyl acetate/hexane) to give the title compound (1.86 g, 94 %) as an oil.

δ_H ($CDCl_3$; 300 MHz) 1.47 (9H, s, C $[CH_3]_3$), 1.95 (6H, s, C $[CH_3]_2$ Br), 3.65 - 3.74 (10H, m, 5 x OCH_2), 4.08 (2H, s, $OCH_2C{=}O$), 4.28 (2H, m, CH_2 $\underline{CH_2}OC{=}OC$ $[CH_3]_2$ Br)

δ_C ($CDCl_3$; 75 MHz) 170.0 (2 x C=O), 72.0 (C-Me), 70.9 - 70.7 (7 x CH_2O), 69.0 (2 x CH_2O), 56.0 (C-Br) and 28.0 (2 x Me).

m/z (+ES) 435 / 437 (100 %, M + Na)

HRMS (+ES) $C_{16}H_{29}{}^{79}BrO_7Na$ requires 435.1097 found 435.0959.

$C_{16}H_{29}{}^{81}BrO_7Na$ requires 437.1097 found 437. 2372

ν_{max} (liquid film, cm^{-1}) 1742 (C=O).

5.3.11 t-Butyl 14-(2-bromo-2-methylpropionyloxy)-3, 6, 9, 12-tetraoxatetradecanoate. (31)

Using the method described above for **30** , a solution of *t*-butyl 14-hydroxy-3, 6, 9, 12-tetraoxatetradecanoate (2.90 g, 6.71 mmol) was reacted with 2-bromo-2-methylpropionyl bromide (3.44 mL, 1.2 eqv.). Filtration and removal of the solvent under reduced pressure resulted in a crude product, which was purified by flash chromatography (20%, 80% then 100% ethyl acetate/ hexane) to give a colourless oily product (4.03 g, 94%).

δ_H ($CDCl_3$; 300 MHz) 1.47 (9H, s, C $[CH_3]_3$), 1.95 (6H, s, C $[CH_3]_2$Br), 3.62 – 3.78 (14H, m, 7 x OCH_2), 4.05 (2H, s, $OCH_2C{=}O$), 4.38 (2H, m, CH_2 $\underline{CH_2}OC{=}OC$ $[CH_3]_2$ Br)

δ_C ($CDCl_3$; 75 MHz) 171.9, 169.9 (2 x C=O), 72.8 (C-Me), 70.9 - 69.2 (9 x CH_2O), 68.9 (2 x CH_2O), 55.9 (C-Br) and 28.0 (2 x Me).

m/z (ES) 479 / 481 (30 %, M + Na)

HRMS (ES) $C_{18}H_{33}{}^{79}BrO_8Na$ requires 479.1359 found 479.1269

$C_{18}H_{33}{}^{81}BrO_8Na$ requires 481.1339 found 481.1210

ν_{max} (liquid film, cm^{-1}) 1750 (C=O).

5.3.12 t-Butyl 17-(2-bromo-2-methylpropionyloxy)-3, 6, 9, 12, 15-pentaoxaheptadecanoate (32)

Using the method described above for **30**, a solution of *t*-butyl 17-hydroxy-3, 6, 9, 12, 15-pentaoxaheptadecanoate (1.5 g, 4.26 mmol) was reacted with 2- bromo-2- methylpropionyl bromide (2.20 mL, 1.2 eqv.). Purified of the crude product by flash chromatography (50 % ethyl acetate/ hexane, 100 % ethyl acetate then 2% methanol/ethyl acetate) gave a colourless oily product (1.72 g, 80 %).

δ_H ($CDCl_3$; 300 MHz) 1.48 (9H, s, C $[CH_3]_3$), 1.95 (6H, s, C $[CH_3]_2Br$), 3.66 – 3.81 (18H, m, 9 x OCH_2), 4.00 (2H, s, $OCH_2C{=}O$), 4.28 (2H, m, CH_2 $C\underline{H_2}OC{=}OC\ [CH_3]_2\ Br\ OCH_2C{=}O$)

δ_C ($CDCl_3$; 75 MHz) 171.0, 169.0 (C=O), 73.9 (C-Me), 70.8 - 69.2 (11 x CH_2O), 68.9 (2 x CH_2O), 55.0 (C-Br) and 28.0 (2 x Me).

m/z (ES) 523 / 525 (100 %, M + Na)

HRMS (ES) $C_{20}H_{37}{}^{79}BrO_9Na$ requires 523.1621 found 523.1516.

$C_{20}H_{37}{}^{81}BrO_9Na$ requires 525.1620 found 525.1575.

ν_{max} (liquid film, cm^{-1}) 1736 (C=O).

5.3.13 11-(2-bromo-2-methylpropionyloxy)-3,6,9-trioxa undecanoic acid.(4)

The solution of *t*-butyl 11-(2-bromo-2-methylpropionyloxy)-3,6,9-trioxa undecanoate (3.9 g) was added and stirred with a solution of 30 % TFA/ DCM (Analar grade; 15 mL), for 3 hours at room temperature, and upon completion (TLC, 40 % ethyl acetate/ hexane, R_f = 0.6 of ester), the reaction was diluted with water (1 mL) and stirred for 10 min. The mixture was dried (Na_2SO_4) and concentrated under reduced pressure, and the resulting oil passed through a silica plug. The column was eluted with a gradient of 80 to 100 % ethyl acetate / hexane then 5 % methanol / ethyl acetate) and the eluents concentrated and dried over P_2O_5 *in vacuo* to yield the title compound (2.84 g, 95%) as a colourless oil.

δ_H ($CDCl_3$; 300 MHz) 1.95 (6H, s, C $[CH_3]_2Br$), 3.65 - 3.80 (10H, m, 5 x OCH_2), 4.18 (2H, s, $OCH_2C{=}O$), 4.29 (2H, m, $OCH_2C\underline{H_2}OC{=}O\ [CH_3]_2\ Br$) 4.6 (1H, br, COOH)

5.3.14 Succinimidyl 11-(2-bromo-2-methylpropionyloxy)-3,6,9-trioxa undecanoate.(1)

A solution of 11-(2-bromo-2-methylpropionyloxy)-3,6,9-trioxaundecanoic acid (2.4 g, 6.74 mmol) and EDCI (1.55 g, 8.09 mmol 1.2 eq.) in DCM (10 mL) was stirred for 1 hour at room temperature before the addition of a solution of N-hydroxysuccinimide (0.95 g, 8.09 mmol, 1.2 eq.) and triethylamine (1.0 mL, 1.3 mmol) in DCM (10 mL). The mixture was stirred at room temperature for a further 16 hours. Upon completion of the reaction (TLC, 80 % ethyl acetate / hexane, R_f = 0.5), dilute HCl (3 x 25 mL) and water (2 x 25 mL) was used to wash the mixture, before the organic phase was dried with $MgSO_4$ and thy solvent removed *in vacuo*. Purification by column chromatography (40 % then 80 % ethyl acetate / hexane then 5 % methanol / ethyl acetate) produced the title compound (1.9 g, 62%) as a colourless oil.

δ_H ($CDCl_3$; 300 MHz) 1.91 (6H, s, C $[CH_3]_2Br$), 2.82 (4H, br, s, CH_2-CH_2) 3.62 - 3.79 (10H, m, 5 x OCH_2), 4.16 (2H, s, OCH_2C=O), 4.29 (2H, m, OCH_2 $C\underline{H_2}OC$=O $[CH_3]_2$ Br)

δ_C ($CDCl_3$; 100 MHz) 172.0 - 166.0 (4 x C=O), 72.0 -, 67.0 (7 x CH_2O), 56.0 (C-Br), 31.0 (2 x Me) and 26.0 (2 x CH_2).

m/z (ES) 476 / 478 (100 %, M + Na)

HRMS (ES) $C_{16}H_{24}{}^{79}BrNO_9Na$ requires 476.0634found 476.0493

$C_{16}H_{24}{}^{81}BrNO_9Na$ requires 478.0614 found 478.0553

ν_{max} (liquid film, cm^{-1}) 1790 (C=O), 1738 (C=O).

5.3.15 Succinimidyl 14-(2-bromo-2-methylpropionyloxy)-3, 6, 9, 12-tetraoxatetradecanoate. (2)

Using the method described for **1** above, a solution of *t*-butyl 14-(2-bromo-2-methylpropionyloxy)-3,6 9,12-tetraoxa-tetradecanoate. (1.5 g, 4.26 mmol) was stirred with N-hydroxysuccinimide. (0.5 g, 1.2 eq) to give the title compound (1.72 g, 80 %) as an oil.

δ_H ($CDCl_3$; 500 MHz) 1.94 (6H, s, C $[CH_3]_2Br$), 2.92 (4H, br, s, CH_2-CH_2) 3.65 - 3.79 (14H, m, 7 x OCH_2), 4.18 (2H, s, OCH_2C=O), 4.38 (2H, m, CH_2 $C\underline{H_2}OC$=OC $[CH_3]_2$ Br).

δ_C ($CDCl_3$; 125 MHz) 172.0 - 166.0 (4 x C=O), 72.0, - 67.0 (9 x CH_2O), 56.0 (C-Br), 31.0 (2 x Me) and 26.0 (2 x CH_2).

m/z (ES) 520 / 522 (100 %, M + Na)

HRMS (ES) $C_{18}H_{28}{}^{79}BrNO_{10}Na$ requires: 520.0897 found 520.0840.

$C_{18}H_{28}{}^{81}BrNO_{10}Na$ requires: 522.0876 found 522.0817.

ν_{max} (liquid film, cm^{-1}) 1787 (C=O), 1744(C=O)

5.3.16 Succinimidyl 17-(2-bromo-2-methylpropionyloxy)-3,6,9,12,15-pentaoxaheptadecanoate.(3)

A solution of *t*-butyl 14-(2-bromo-2-methylpropionyloxy)-3,6,9 12,15-pentaoxa-heptadecanoate (3.2 g, 7.64 mmol) was stirred with N-hydroxysuccinimide (1.0 g, 1.2 eq) according to the method described for **1** above to give the title compound (3.2 g, 75 %) as a colourless oil.

δ_H ($CDCl_3$; 500 MHz) 1.95 (6H, s, C $[CH_3]_2Br$), 2.82 (4H, br, s, CH_2-CH_2) 3.62 - 3.79 (18H, m, 9 x OCH_2), 4.16 (2H, s, OCH_2C=O), 4.28 (2H, m, CH_2 $C\underline{H_2}OC$=OC $[CH_3]_2$ Br)

δ_C ($CDCl_3$; 125 MHz) 172.0 - 166.0 (C=O), 72.0 - 67.0 (11 x CH_2O), 56.0 (C-Br), 31.0 (2 x Me) and 26.0 (2 x CH_2).

m/z (ES) 564 / 566 (50 %, M + Na).

HRMS (ES) $C_{20}H_{32}{}^{79}BrNO_{11}Na$ requires: 564.1159 found 564.1033.

$C_{20}H_{32}{}^{81}BrNO_{11}Na$ requires: 566.1138 found 566.0961.

ν_{max} (liquid film, cm^{-1}) 1785 (C=O), 1739 (C=O).

5.3.17 Benzyl (11(-2-methyl-propionyloxy))-3,6,9-trioxaundecanoate (47)

A solution of benzyl 11-hydroxy-3,6,9-trioxaundecanoate (80 mg, 0.268 mmol) in dichloromethane (5 mL) was stirred with N, N-dimethylaminopyridine (103 mg, 0.539 mmol, 2 eqv) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (103 mg, 0.539 mmol, 2 eqv) for 0.5 h at room temperature. A mixture of

isobutyric acid (47 mg, 0.539 mmol, 2 eqv) and N, N-diisopropylethylamine (70 mg, 0.539 mmol, 2 eqv) in dichloromethane (3 mL) was then added and the mixture stirred overnight under a nitrogen atmosphere. The reaction was then washed with dil.HCl (3 x 30 mL), water (2 x 25 mL) and the organic phase dried ($MgSO_4$.) The organic phase was then concentrated under reduced pressure, and the crude product purified by silica gel column chromatography (40 % ethyl acetate / hexane) to obtain the product (88 mg, 89 %) as a colourless oil

δ_H ($CDCl_3$; 500 MHz) 1.15 (6H, d, $[CH_3]_2$), 2.60 (1H, m, $C[CH_3]_2\underline{H}$), 3.60-3.80 (10H, m, 5 x OCH_2), 4.20 (4H, m, $BnOCOC\underline{H}_2$ and $C\underline{H}_2OCOCH$ (CH_3)), 5.19 (2H, s, $C\underline{H}_2Ph$), 7.38 (5H, m, Ph)

δ_C ($CDCl_3$; 100 MHz) 177.0, 170.0 (C=O), 135.0 (Ph-C), 127.0 - 128.0 (5 x CH), 71.0 - 72.0 (6 x OCH_2), 68.0, 66.0 (OCH_2), 34.0 (CH), 19.0 (2 x Me).

m/z (+ES) 391 (100 %, $M+Na^+$) m/z (CI+) 369 (10 %, $M+H^+$); 386 (20 %, $M+NH_3^+$).

HRMS (+ES) $C_{19}H_{28}O_7Na$ requires 391.1835 found 391.1749.

ν_{max} (liquid film, cm^{-1}) 1732 (C=O), 1733 (C=O).

5.3.18 11(2-methylpropionyloxy)-3,6,9-trioxaundecanoic acid (48)

A suspension of 10 % palladium on charcoal (catalytic) was stirred at room temperature with a solution of benzyl (11(-2-methylpropionyloxy))-3,6,9-trioxaundecanoate (60 mg, 0.163 mmol) in dry methanol (1 mL), previously purged and refilled with hydrogen gas. The reaction completion as determined by TLC (100 % ethyl acetate, $R_{f\ new}$ = 0.3, $KMnO_4$) after 7 h before proceeding to diluting the mixture with methanol (5 mL) and filtered through a celite plug, dried ($MgSO_4$.) and the filtrate concentrated *in vacuo* to yield the title compound (44 mg, 97 %) as an oil. This was used without further purification.

δ_H ($CDCl_3$; 400 MHz) 1.15 (6H, d, $[CH_3]_2$), 2.60 (1H, m, $C[CH_3]_2\underline{H}$), 3.0 - 3.70 (10H, m, 5 x OCH_2), 4.15 (2H, s, $BnO(CO)C\underline{H}_2$) 4.20 (2H, s, br, $C\underline{H}_2O(CO)C(CH_3)$ H).

δ_C ($CDCl_3$; 100 MHz) 177, 170 (C=O), 72.6 - 72.0 (6 x OCH_2), 68, 63 (OCH_2), 34 (CH), 19(Me).

m/z (-ES) 277 (100 %, M - H).

ν_{max} (liquid film, cm^{-1}) 3208 (COOH, br), 2876, 1735 (C=O).

5.3.19 Ethyl(2-methyl-2-thioethyl)propionate (42)

A solution of sodium ethoxide (1.06 M, 1.2 eqv), prepared by dissolving sodium (1.1 g) in dry ethanol (45 mL), was stirred with ethanethiol (5.68 mL, 2 eqv) and ethyl (2-bromo-2-methyl) propionate (7.0 g, 36 mmol) at 65 °C for 1.5 h. The white precipitate formed was removed by filtration, and the mother liquor concentrated to approximately 15 mL *in vacuo*. Distillation (50 °C at 7 atm) of the resulting mixture furnished the title compound (1.96g, 31 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.32 (3H, m, $SCH_2C\underline{H}_3$), 1.45 (3H, m, $OCH_2C\underline{H}_3$), 1.67 (6H, s, $(CH_3)_2$), 2.78 (2H, m, SCH_2), 4.3 (2H, m, OCH_2)

δ_C ($CDCl_3$; 100 MHz) 174.29 (C=O), 61.11 (OCH_2), 46.91 (C-S), 25.72 (2 x Me), 23.71 (SCH_2), 14.16 ($SCH_2\underline{C}H_3$), 14.12 ($OCH_2\underline{C}H_3$).

m/z (+ES) 199 (10 %, M + Na) and 469 (100 %)

ν_{max} (liquid film, cm^{-1}) 1728 (C=O)

5.4 Compounds from Chapter 4

5.4.1 8-(Tetrahydropyranyl-2-oxy)-3,6-dioxaoctanol (19)

Dihydropyran (19 mL, 18 g, 0.21 mol) was added dropwise into a vigorously stirred mixture of *p*-toluenesulphonic acid monohydrate (catalytic, 40 mol%) and triethyleneglycol (100 mL, 115 g, 0.76 mol) at 70 °C over 2 h under nitrogen. The pale brown reaction mixture was then allowed to cool to room temperature, diluted with brine (100 mL) and extracted with hexane (2 x 200 mL) and DCM (2 x 200 mL) successfully. The DCM extracts were further washed with brine (2 x 100 mL) and dried with Na_2SO_4 before concentration under reduced pressure. Silica gel flash chromatography, using a gradient elution (50 % to 100 % ethyl acetate/hexane) gave the pure title compound (35 g, 69%) as a pale brown oil.

δ_H ($CDCl_3$; 400 MHz) 1.40 - 1.80 (6H, m, THP), 3.40 - 3.70 (12H, m, 6 x CH_2O), 3.80 (2H, m, $C\underline{H}_2OH$), 4.58 (1H, t, J=3.2 Hz, CH, cyclic).

δ_C ($CDCl_3$; 100 MHz) 99.0 (OCH, cyclic), 73.0, 72.0, 71.6, 71.0, 67.0 (CH_2O), 62.0 (CH_2O, cyclic), 61.3 (CH_2OH), 30.0, 25.0, 19.0 (CH_2, cyclic).

m/z (+ES) 257 (100%, M + Na)

ν_{max} (liquid film, cm^{-1}) 3446 (OH), 2939 (CH_2, str)., 2869 (CH_2, str).

5.4.2 11-(Tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanol (20)

Dihydropyran (8.0 g, 95.2 mmol) and tetraethyleneglycol (60 mL) were reacted following the method described for 8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanol. The crude product was purified by flash chromatography (gradient eluent, 50 % to 100% ethyl acetate/hexane) to yield the title compound (18 g, 64 %) as pale brown oil

δ_H ($CDCl_3$; 500 MHz) 1.40 - 1.80 (6H, m, THP), 3.40 - 3.70 (16H, m, 8 x CH_2O), 3.90 (2H, m, HO-$C\underline{H}_2$), 4.58 (1H, t, J = 3.2 Hz, CH-O cyclic-).

δ_C ($CDCl_3$; 125 MHz) 99.0 (CH-O, cyclic), 72.8 (CH_2O) , 72.0 (overlapping signals), 71.6 (CH_2O), 71.0 (CH_2O), 67.0 (CH_2O), 62.0 (CH_2O, cyclic), 61.3 (CH_2OH), 31.0 (CH_2, cyclic), 25.5 (CH_2, cyclic), 20.0(CH_2, cyclic).

m/z (ES) 301 (80 %, M + Na) and 217 (100%)

ν_{max} (liquid film, cm^{-1}) 3488 (OH), 2941 (CH/CH_2, str), 2870 (CH/CH_2, str).

5.4.3 14-(Tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanol (21)

Dihydropyran (2.1 g, 25 mmol) was added dropwise with vigorous stirring to a mixture of *p*-toluenesulphonic acid monohydrate (40 mol%, catalytic) and triethyleneglycol (5.0 g, 4.3 mL; previously dried by azeotropic distillation) and over phosphorus pentoxide under reduced pressure. The reaction was then heated to 60 °C and gently stirred for 2 h under nitrogen. The resulting dark brown reaction mixture was then allowed to cool to room temperature and subsequently diluted with brine (25 mL), extracted with hexane (2 x 25 mL) and DCM (2 x 25 mL) respectively. The DCM extracts were further washed with brine (2 x 25 mL), dried the organic extracts with Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography (gradient eluent, 50 % to 100% ethyl acetate/hexane; and a final wash with 10 % methanol/ethyl acetate) gave the title compound (4.2 g, 52 %) as a pale brown oil.

δ_H ($CDCl_3$; 200 MHz) 1.40 - 1.90 (6H, m, THP), 3.40 - 3.80 (20H, m, 10 x CH_2O), 3.80 – 3.90 (2H, m, HO-C$\underline{H_2}$), 4.62 (1H, t, J = 3.2 Hz, CH-O cyclic).

δ_C ($CDCl_3$; 75 MHz) 99.0 (CH, cyclic), 72.8, 72.0, 71.6, 71.0 (overlapping signals), 65(CH_2O), 62.0 (CH_2O, cyclic), 61.3 (CH_2OH), 30.0, 25.0, 19.0(CH_2, cyclic).

m/z (+ES) 345 (100%, M + Na) HRMS (+ES) $C_{15}H_{30}O_7Na$ requires 345.1992, found 345.1889

ν_{max} (liquid film, cm^{-1}) 3446 (OH), 2939 (CH/CH_2, str), 2869 (CH/CH_2, str).

General Procedure for preparation of methanesulphonates

5.4.4 8-(Tetrahydropyranyl-2-oxy)-3,6-methanesulphonate (16)

Methanesulphonyl chloride (0.75 g, 6.50 mmol, 1.5 eq.) was added in a dropwise manner to a stirred mixture of 8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanol **19** (1.0 g, 4.27 mmol), triethylamine (2 mL), and N, N-dimethylaminopyridine (5 mg, catalyst) in dichloromethane (10 mL) at 0 °C. The resulting clear solution was stirred at 0 °C for 5 h under a nitrogen atmosphere, and at room temperature for a further 18 h during which a brown solution containing white solids was observed. The reaction was diluted with dichloromethane (25 mL), and washed with dilute HCl (2 x 25 mL) and water (2 x 25 mL) respectively. The organic phase was further dried with magnesium sulphate, concentrated *in vacuo* and purified by flash chromatography [50% to 100% ethyl acetate/hexane] to yield the title compound (1.2 g, 89 %) as a pale brown oil.

δ_H ($CDCl_3$; 400 MHz) 1.38 - 1.78 (6H, m, THP), 3.0 (3H, s, OMs), 3.40 – 3.90 (12H, m, 6 x CH_2O), 4.21 (2 H, m, CH_2O), 4.50 (1H, t, J = 3.2 Hz, CH-O cyclic).

δ_C ($CDCl_3$; 100 MHz) 99.0 (CH, cyclic), 72.8, 72.0, 71.6, 71.0, 67.0, 63.0 (CH_2O), 62.0 (CH_2O, cyclic), 38.0 (SO_3Me), 31.0, 25.5, 20.0(CH_2, cyclic).

m/z (+ES) 335 (100%, M + Na).

HRMS (+ES) $C_{12}H_{24}O_7SNa$ requires 335.1243 found 335.1151.

5.4.5 11-(Tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanyl methanesulphonate (17)

Using method described above for **16**, a solution of 11-(tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanol **20** (13.0 g, 46.8 mmol) and methanesulphonyl chloride (11.8 mL, 8 g, 70 mmol) were reacted to yield the title compound (12.8 g, 77 %)as an oil after chromatography with (50 % to 100 % ethyl acetate/ hexane) gradient elution.

δ_H ($CDCl_3$; 400 MHz) 1.40 - 1.80 (6H, m, THP), 3.06 (3H, s, OMs), 3.40 – 3.90 (16H, m, 8 x CH_2O), 4.32 (2 H, m, CH_2O), 4.46 (1H, t, J = 3.2 Hz, CH-O).

δ_C ($CDCl_3$; 100 MHz) 99.0 (CH, cyclic) 70.0, 70.1, 69.0, 68.0, 68.4, 67.0 (CH_2O, overlapping), 62.0 (CH_2O, cyclic), 38.0 (SO_3Me), 31.0, 25.0, 19.0 (CH_2, cyclic)

m/z (ES) 379 (M + Na). HRMS (ES) $C_{14}H_{28}O_8SNa$ requires 379.1505 found 379.1414 .

ν_{max} (liquid film, cm^{-1}) 2938 (CH_2, str), 2869 (CH_2, str), 1354 (S=O, asym-str), 1178 (S=O, sym-str).

5.4.6 14-(Tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanyl methanesulphonate (18)

Using method described above for **16**, a solution 14-(tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanol **21** (3.7 g, 11.5 mmol) and methanesulphonyl chloride (2.0 g, 2.9 mL, 17.3 mmol) were reacted to yield the title compound (3.1 g, 67 %) as an oil.

δ_H ($CDCl_3$; 400 MHz) 1.40 - 1.80 (6H, m, THP), 3.10 (3H, s, OMs), 3.40 – 3.90 (20H, m, 10 x CH_2O), 4.40 (2 H, m, CH_2O), 4.60 (1H, t, J = 3.2 Hz, CH-O).

δ_C ($CDCl_3$; 100 MHz) 99.0 (CH, cyclic) 70.0, 70.1, 69.0, 69.2, 68.0, 68.4, 67.0 (CH_2O, overlapping), 62.0 (CH_2O, cyclic), 38.0 (SO_3Me), 31.0, 25.0, 19.0 (CH_2, cyclic).

m/z (ES) 423 (100 %, M + Na).

ν_{max} (liquid film, cm^{-1}) 2938 (CH_2, str), 2869 (CH_2, str), 1354 (S=O, asym-str), 1178 (S=O, sym-str).

5.4.7 N,N-Dimethyl-8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanylamine (10)

Dimethylamine (40% in water, 10 mL) was added slowly to a solution of 8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanyl methanesulphonate **16** (2.0 g, 6.4 mmol) in DCM (5 mL) at 0 °C. After the addition, the resulting exothermic mixture was then stirred for a further 4 h at room temperature. The reaction was then diluted with saturated $NaHCO_3$ solution (20 mL), extracted with dichloromethane (3 x 25 mL) and the organic phase was washed with saturated sodium carbonate solution (2 x 25 mL) before being dried using magnesium sulphate and concentrated *in vacuo*. Purification by flash chromatography ([7: 3] ethyl acetate/ hexane) and subsequent flushing of the column with a methanol/

ethyl acetate gradient (maximum 5% methanol/ ethyl acetate) yielded the title compound (1.0 g, 60%) as pale brown oil.

δ_H ($CDCl_3$; 400 MHz) 1.20 - 1.80 (6H, m, THP), 2.26 (6H, s, NMe_2), 2.50 (2H, t, J = 5.7 Hz, CH_2N), 3.20 - 3.70 (10H, m, 5 x CH_2O), 3.90 (2H, m, cyclic $OC\underline{H_2}$), 4.60 (1H, m, J = 3.1 Hz, CH-O).

δ_C ($CDCl_3$; 100 MHz) 99.18 (CH, cyclic), 70.83, 70.77, 70.60, 69.40, (CH_2O, overlapping), 62.50 (CH_2O, cyclic), 58.90 (CH_2N), 46.00 (NMe_2), 30.80, 25.70 and 19.70 (CH_2, cyclic).

m/z (+ES) 284 (M + Na, 90%), HRMS (+ES) $C_{13}H_{28}NO_4$ requires 262.1940 found 262.2034

IR (liquid film, cm^{-1}) 2941, 2868, 2770 (CH_2, str) and 1454.

5.4.8 N,N-Dimethyl-11-(tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanyl amine (11)

Using the method employed for *N, N*-dimethyl-8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanylamine, the solution of 11-(tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanyl methanesulphonate (6 g, 17 mmol) was treated with dimethylamine (25 mL) to yield the title compound (2.3g, 45 %) as a pale brown oil.

δ_H ($CDCl_3$; 400 MHz) 1.40 - 1.90 (6H, m, THP), 2.27 (6H, s, NMe_2), 2.50 (2H, t, J = 5.7 Hz, CH_2N), 3.40- 3.80 (14H, m, 7 x CH_2O), 3.90 (2H, m, cyclic $OC\underline{H_2}$), 4.60 (1H, m, J = 3.2 Hz, CH-O).

δ_C ($CDCl_3$; 100 MHz) 100.0 (CH, cyclic), 72.0, 71.2, 71.0, 69.0 (CH_2O, overlapping), 62.0 (CH_2O, cyclic), 59.0 (CH_2N), 46.0 (NMe_2), 30.0, 25.0, 19.0 (CH_2, cyclic).

m/z (+ES) 306 (M + H^+); HRMS (+ES) $C_{13}H_{32}NO_5$ requires 306.2202 found 306.2297

IR (liquid film, cm^{-1}). 2948, 2872, 2772 (CH_2, str) and 1456.

5.4.9 N,N-Dimethyl-14-(tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanylamine.(12)

Using the method employed for *N, N*-dimethyl-8-(tetrahydropyranyl-2-oxy) -3,6-dioxaoctanylamine, a solution 14-(tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanyl methanesulphonate (2.1 g, 5 mmol) was treated with dimethylamine (12 mL) to yield the title compound (1.3 g, 71 %) as an oil.

δ_H ($CDCl_3$; 500 MHz) 1.40 - 1.90 (6H, m, THP), 2.30 (6H, s, NMe_2), 2.50 (2H, t, J 5.7 Hz, CH_2N), 3.35- 3.90 (18H, m, 9 x CH_2O), 3.90 (2H, m, cyclic $OC\underline{H_2}$), 4.60 (1H, m, J = 3.2 Hz, CH-O).

δ_C ($CDCl_3$; 125 MHz) 99 .0 (CH, cyclic), 72.0, 71.2, 71.0, 69.0 (CH_2O, overlapping), 63.0(CH_2O, cyclic), 59.0(CH_2N), 46.0 (NMe_2), 31.0, 26.0, 19.0 (CH_2, cyclic)

m/z (+ES) 350 ($M + H^+$)

IR (liquid film, cm^{-1}) 2940, 2868, 2769 (CH_2, str) and 1454.

5.4.10 1-(N,N-dimethylamino)-3,6-dioxa-octan-8-ol (7)

A mixture of AcOH/water (5 mL, 1:1, v/v) was introduced dropwise into a solution of *N,N*-dimethyl-8-(tetrahydropyranyl-2-oxy)-3,6-dioxaoctanylamine (0.5 g, 2 mmol) in absolute ethanol (1 mL) and the solution stirred for 4 h at 50 °C. The reaction was allowed to cool to room temperature before being concentrated *in vacuo.* The product was treated azeotropically with toluene before passing through a plug of neutral alumina. The plug was washed successively with ethyl acetate (50 mL) and 2 % methanol/ethyl acetate (2 x 20 mL). The methanolic ethyl acetate wash was then concentrated under reduced pressure and the resulting oil further dried *in vacuo* over phosphorus pentoxide to yield the title product (0.23 g, 66%) as colourless oil, which turned brown on standing.

No further purification was attempted.

δ_H (MeOD; 300 MHz) 2.81 (6H, s, NMe_2), 3.42 (2H, t, J= 5.6 Hz, CH_2NMe_2), 3.2 (8H, m, 4 x OCH_2), 3.81 (2H, m, CH_2OH).

δ_C (MeOD; 100 MHz) 71 (OCH_2), 66 (CH_2OH), 58 (CH_2N), 44 (NMe_2)

m/z (+ES) 178 (100%, M + H) HRMS (+ES) $C_8H_{20}NO_3$ requires 178.1365 found 177.9423.

ν_{max} (liquid film, cm^{-1}) 3409 (OH), 2507, 2243, 2027(CH_2) and 1119(C-O).

5.4.11 1-(N,N-dimethylamino)-3,6,9-trioxa-undeca-11-ol.(8)

Using the method employed for 1-(*N,N*-dimethylamino)-3,6-dioxa-octan-8-ol, N,N-dimethyl-11-(tetrahydropyranyl-2-oxy)-3,6,9-trioxaundecanylamine (0.1 g, 0.33 mmol) was treated with AcOH/ Water (2 mL, 1:1, v/v) to yield the product as a pale brown oil (0.7 g, 94 %).

δ_H ($CDCl_3$; 500 MHz) 2.41 (1H, s, OH), 2.92 (6H, s, NMe_2), 3.21 (2H, t, J = 5.7 Hz, CH_2NMe_2) 3.55-3.72 (12H, m, 6 x OCH_2), 3.89 (2H, m, CH_2OH).

δ_C ($CDCl_3$; 125 MHz) 73.0, 71.5, 71.2, 70.0 (CH_2O), 66.0 (CH_2OH), 58.0 (CH_2N), 44.0 (NMe_2).

m/z (+ES) 222 (100%, M + 1), 224 (50 %, M + Na)

ν_{max} (liquid film, cm^{-1}) 3376 (OH), 2872, 2693, 2473 (CH_2), and 1118 (C-O).

5.4.12 1-(N,N-dimethylamino)-3,6,9,12-tetraoxa-tetradeca-14-ol.(9)

Using the method employed for 1-(N,N-dimethylamino)-3,6-dioxa-octan-8-ol, N,N-dimethyl-14-(tetrahydropyranyl-2-oxy)-3,6,9,12-tetraoxatetradecanylamine (100 mg, 0.29 mmol) was treated with AcOH/ Water (2 mL, 1:1, v/v) to yield the title product (70 mg, 92 %)as a brown oil.

δ_H ($CDCl_3$; 400 MHz) 2.40 (1H, Br, s, OH), 2.82 (6H, s, NMe_2), 3.30 (2H, t, J = 5.7 Hz, CH_2NMe_2) 3.61 - 3.80 (16H, m, 8 x OCH_2), 3.90 (2H, m, CH_2OH).

δ_C ($CDCl_3$; 100 MHz) 72.7, 72.8, 70.5, 70.5, 70.3, 70.1(CH_2O), 65.6 (CH_2OH), 56.0 (CH_2N), 43.0 (NMe_2).

m/z (+ES) 266 (100%, M + H) HRMS (+ES) $C_{12}H_{28}NO_5$ requires 266.1889 found 266.1982

ν_{max}(liquid film, cm-1) 3406 (OH), 2878, 1472 (CH_2) and 1113 (C-O).

5.4.13 N-(2-bromoethyl)maleimide (28)

To a stirred solution of sodium bicarbonate (2.6 g, 31 mmol) in water (30 mL) was added 2-bromoethylamine (6.3 g, 31 mmol). As the latter compound dissolved, some effervescence was observed. Freshly crushed maleic anhydride (3.0 g, 31 mmol) was added slowly with stirring to the clear solution. After an increase in the amount of effervescence, it completely dissolved and a white precipitate was formed. After stirring for 2 h, the precipitate that had formed was removed by filtration and dried over phosphorus pentoxide under reduced pressure to obtain the amido-acrylic acid intermediate (5.27 g, 77 %) as a white solid without further purification.

A suspension of sodium acetate (0.69 g) in acetic anhydride (17 mL) and was heated to 80 °C for 10 min using an oil bath before 3-(*N*-2-bromoethyl carbamoyl) acrylic acid (3 g) was slowly added. The reaction mixture was further heated with vigorous stirring at 85 °C for 10 min, after which the mixture was poured into a ice-cold water (20 mL) and extracted with chloroform (2 x 15 mL). The organic extract was washed with water and dried ($MgSO_4$) before being concentrated and recrystallised from cyclohexane to yield the product (220 mg, 14 %) as a white powder/ solid.

δ_H ($CDCl_3$; 300 MHz) 3.54 (2H, t, J = 6.71 Hz, CH_2Br), 3.96 (2H, t, J = 6.71 Hz, CH_2N), 6.76 (2H, s, CH=CH)

δ_C ($CDCl_3$; 100 MHz) 169.00 (C = O), 134.24 ($CH_2 = CH_2$), 39.16 (CH_2N) and 28.18 (CH_2Br);

m/z (ES) 227 (40 %, M + Na). M.P 60 – 61 °C

ν_{max} (liquid film, cm^{-1}) 1770 (C=O), 1710 (C=C, str) and 1406 (C-H, bend).

Literature. (Chapter 4, Ref. 14, p124)

δ_H ($CDCl_3$) 3.53 (2H, t,), 3.95 (2H, t,), 6.75 (2H, s, CH=CH)

δ_C ($CDCl_3$) 170.09, 134.22, 39.14 and 28.17.

Lit (2) 59 – 62 °C.

ν_{max} (, cm^{-1}) 3090 (w),1770 (m), 1700 (m), 1580 (m).

5.4.14 N- (2-Hydroxyethyl)maleimide (29)

Solutions of ethanolamine (20.0 g, 0.32 mmol) in acetone (100 mL) and maleic anhydride in acetone (100 mL) both at room temperature, were added simultaneously using a dropping funnel over a period of 2 h into a flask charged with acetone (200 mL) at 0 °C with vigorous stirring. The reaction was stirred for a further 90 min, the volume reduced by half and cooled to 4°C. The precipitate formed was washed with chilled acetone and dried under reduced pressure to yield the hydroxy maleimic acid intermediate (40.0 g, M.P 99 °C, Lit (1) 98 °C) as a white solid.

10 g (63 mmol) of the maleimic acid was allowed to dissolved in a warm mixture of toluene (400 mL) and triethylamine (17.42 mL, 126 mmol) with stirring. The flask was then equipped with a Dean Stark condenser, and heating continued for 2 h. The reaction was then allowed to cool before the toluene phase was decanted from the residue and the residue was further extracted with hot toluene (4 x 30 mL). All toluene extracts were combined and concentrated, leaving another yellow residue. This residue was extracted with hot ether (3 x 50 mL). The extracts were concentrated under reduced pressure and after chromatography (3:1 ethyl acetate / hexane), the compound (R_f = 0.35, 1.2 g, 14%) was obtain as a yellow syrup.

δ_H ($CDCl_3$; 300 MHz) 3.73 (2H, t, J = 15 Hz, CH_2N), 3.79 (2H, t, J = 17 Hz, CH_2OH), 6.76 (2H, s, CH=CH)

δ_C ($CDCl_3$; 100 MHz) 171 (C=O), 135 (C=C), 61.5 (CH_2OH) and 41.8 (CH_2N)

m/z (ES) 115 (10 %), 215 (100 %) and 158 (10 %, M + NH_4)

ν_{max} (liquid film, cm^{-1}) 3464 (OH) and 1711(C=O).

Literature. (Chapter 4, Ref. 16, p126)

δ_H (DMSO-d_6; 300 MHz) 3.47 (m, 4H, 2 x CH_2), 4.07 (br s, 1H, OH), 7.01 (2H, s, CH=CH), δ_C ($CDCl_3$; 100 MHz) 171 (C=O), 134 (C=C), 57 (CH_2OH) and 39 (CH_2N), Ms (EI) 111 (100 %) and 114 (2 %)

5.4.15 N- (2-trifluoromethanesulphonyloxy)ethyl maleimide.(30)

To a solution of N-(2-hydroxy) ethylmaleimide (700 mg, 15.0 mmol) and *N, N*-dimethylamino pyridine (100 mg, cat.) in dichloromethane (10 mL) at 0 °C, was added trifluoromethane- sulphonyl chloride (1.60 mL, 7 eq) and the solution allowed to stir for 2 h at 0 °C. The pale yellow solution was allowed to warm to room temperature and stirred for a further 8 h. Upon completion of the reaction, (TLC, 50 % ethyl acetate / hexane, R_{fnew} 0.8, $KMnO_4$) the reaction was concentrated under reduced pressure, diluted with dichloromethane (20 mL), washed with water (2 x 20 mL), dil HCl (2 x 20 mL), water (20 mL) and dried ($MgSO_4$) before being concentrated under reduced pressure. After column chromatography (25 % ethyl acetate / hexane) the title product (0.476 g, 63 %) was obtained as a white (fluffy) solid.

δ_H ($CDCl_3$; 300 MHz) 3.69 (2H, t, J = 6.3 Hz, NCH_2), 3.89 (2H, t, J = 6.4 Hz, OCH_2) and 6.75 (2H, s, CH_2=CH_2)

δ_C ($CDCl_3$; 125 MHz) 170 (C=O), 134 (CH_2=CH_2), 41 (CH_2O) and 39 (CH_2N).

m/z (ES) 274 (M + H) (EI) 293 (25 %)

ν_{max} (liquid film, cm^{-1}) 1709 (C=O).

5.4.16 3-(2-Dimethylamino-ethylcarbamoyl)acrylic acid (32)

To a solution of maleic anhydride (5g, 51 mmol) in dry THF (100 mL) at 0 °C, was added *N,N*-dimethylethylamine (5.48 mL, 52 mmol) in THF (100 mL) over a period of 2 h. The reaction was stirred for a further 2 h at 0 ° C, the white solids removed by filtration and the organic solvent partially reduced *in vacuo* to induce further precipitation by titurating. The combined white solid was washed with ice-cold THF and dried under reduced pressure. The product (9.4 g, 99 %) was obtained as a white solid, which was used without further purification.

δ_H (D_2O; 200 MHz) 2.81 (6H, s, N $(CH_3)_2$), 3.22 (2H, t, J = 6.6 Hz, $NCH_2C\underline{H}_2N$ $(CH_3)_2$), 3.52 (2H, t, J_{XA} = 5.4 Hz, $NC\underline{H}_2CH_2N$ $(CH_3)_2$), 5.97 (1H, d, J_{AX}, = 12 Hz, =C<u>H</u> (CO) NH), 6.16 (1H, d, J_{XA}, = 12 Hz, =$C\underline{H}CO_2H$)

m/z (ES) 187 (30 %, M + H) and 169 (100 %, M – H_2O)

C: H: N: $C_8H_{14}N_2O_3$. Requires C 51.05, H 7.58, N 14.88. Found C 51.04, H 7.47, N 14.67.

5.4.17 N^1- (N,N-dimethylamino)ethyl maleimide.(33)

The amido-acid (9g, 48.3 mmol) was suspended in THF (50 mL) with triethylamine (7.8 mL, 1.2 eqv., 53 mmol) and refluxed for 30 min before trimethylsilyl chloride (7.3 mL, 1.2 eqv, 6.3 g, 58 mmol) was added in portions and the solution was allowed to reflux for 5 h under a nitrogen atmosphere. After cooling to room temperature, the solids were filtered off and the mother liquor was concentrated (solidifies on standing). The solid was dissolved in dichloromethane (10 mL) and washed with cold water (2 x 20 mL) and the aqueous wash was further extracted with hexane (20 mL). The organic extracts were combined and concentrated *in vacuo* to yield the product (2.3 g, 80 %) as a orange oil which solidified on standing.

The solid was always re-extracted with dichloromethane before use to reduce hydrolysis by-product.

δ_H ($CDCl_3$; 500 MHz) 2.25 (6H, s, N $(CH_3)_2$), 2.49 (2H, t, J_{AX} = 6.6 Hz, $NCH_2\underline{CH_2}N(CH_3)_2$), 3.64 (2H, t, J_{XA} = 6.63 Hz, $NC\underline{H_2}CH_2N(CH_3)_2$), 6.69 (2H, s, CH=CH)

δ_C ($CDCl_3$; 125 MHz) 171 (C=O), 134.4 (2 x =CH), 57 ($N\underline{C}H_2CH_2N(CH_3)_2$), 46 ($NCH_2CH_2N(\underline{C}H_3)_2$), 37 ($NCH_2\underline{C}H_2N(CH_3)_2$)

m/z (ES) 169 (100 %, M + 1)

ν_{max} (liquid film, cm^{-1}) 1720 and 1414.

5.4.18 Tetraethyleneglycol monomesylate (35)

A solution of methanesulphonyl chloride (8 g, 70 mmol) in dichloromethane (20 mL) at 0 °C was stringed over a period of 0.5 h into a solution of tetraethylene glycol (10 g, 77 mmol), TEA (9.6 mL, 70 mmol) and DMAP (0.8 g, catalytic) under nitrogen atmosphere in dry dichloromethane (20 mL) at 0 °C. The mixture

was then stirred at 0 °C for an additional 18 hours under a nitrogen atmosphere. The reaction mixture was then washed with water (2 x 50 mL), brine (2 x 50 mL) and dried ($MgSO_4$) to give the crude. The crude compound was then column chromatographed on silica gel to give the title compound as a pale brown syrup (11.6 g, 60 %).

δ_H ($CDCl_3$; 300 MHz) 3.08 (3H, s, OMs), 3.59 – 3.80 (12H, m, 6 x CH_2O), 4.20 (4 H, m, 2 x CH_2O), δ_C (D_2O; 75 MHz) 72.8, 72.0, 71.6, 71.0, 67.0, 63.0 (overlapping CH_2O), 37.6 (Me)

m/z (ES) 295 (100 %, M + Na), ν_{max} (liquid film, cm^{-1}) 3442 (OH)

5.4.19 N,N-dimethylammonium-N-ethylmaleimide-N-3, 6, 9-trioxaundeca-11- octanyl methanesulphonate salt (36)

Tetraethyleneglycol monomesylate (0.10 g, 0.39 mmol) was dissolved in a solution of N^1-(*N, N*-dimethyl)ethylmaleimide (0.06 g, 1 eqv) in dry THF (3 mL) under argon. The mixture was subjected to microwave radiation (170 watt, 100 psi) at 150 °C for 1 h. The mixture was allowed to reach room temperature and the THF was decanting off. The brown solid was collected, washed with THF and dried with phosphorus pentoxide under a reduced pressure to give the title compound (0.052g, 30 %) as a brown solid/syrup.

δ_H (D_2O; 300 MHz) 2.41 (1H, s, OH), 2.93 (6H, d, br, $CH_2N(C\underline{H}_3)_2$), 3.58 – 3.64 (4H, s, br, $C\underline{H}_2N(CH_3)_2C\underline{H}_2$) 3.64 – 3.9 (14H, m, 6 x OCH_2 and $N(CH_3)_2CH_2C\underline{H}_2N$), 3.95 – 4.15 (2H, m, $C\underline{H}_2N(CH_3)_2$), 6.3 (2H, s, CH= CH).

δ_C (D_2O; 75 MHz) 170.00 (C=O), 135.00, 71.85, 69.56, 69.61(CH_2O), 67.99(CH_2OH), 60.50 (CH_2N+), 38.63 ($(CH_3)_2N$+) and 25.16 ($\underline{C}H_2NC$=O).

m/z (ES) 345.2 (100 %, M+), HRMS (+ES) $C_{16}H_{29}N_2O_6$: requires 345.2020 found 345.2007.

5.4.20 2-Bromo-2-methylpropionyloxy-3, 6, 9 trioxaundecanyl-11-pyridinium salt (46)

To a mixture of 11-hydroxy-3,6,9-trioxaundecanyloxy(2-bromo-2-methyl) propionate (350 mg, 1.023 mmol) and a suspension of freshly activated powdered sieves in dry dichloromethane (5 mL) was added pyridine (81 mg, 1.0 eqv) at room temperature, and the mixture stirred for 1 h. The reaction was then cooled to 0 °C before adding trifluoromethanesulphonyl anhydride (0.207 mL, 1.2 eqv) and left to stir for 6 h at 0 °C. The mixture was then washed with ice-cold 1M HCl, concentrated *in vacuo* and chromatographed (10 % to 90 % ethyl acetate / hexane eluent) to obtain the title compound (241 mg, 58 %) as a yellow oil.

δ_H ($CDCl_3$; 400 MHz) 1.96 (6H, s, C $[CH_3]_2$ Br), 3.52 – 3.8 (10H, s, 5 x OCH_2), 4.0 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 4.36 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 4.82 (2H, m, $OCH_2C\underline{H_2}$, $^{(+)}$NPyr), 8.05 (2βH, t, J =7.19 Hz, pyr), 8.5 (1γ H, t, J = 7.88 Hz, pyridine), 9.02 (2αH, t, J = 5.8 Hz, pyr)

δ_C ($CDCl_3$; 100 MHz) 172.00 (C=O), 146.00, 128.00 (CH, Ar), 70.80, 69.86, 69.92, 69.61 (CH_2O), 65.59 $C\underline{H_2}O$ CH_2N+, 61.70 (CH_2N^+), 56.00 (C-Br) and 31.00 (CMe_2). m/z (ES) 404 / 406 (100 %, M + Na).

HRMS (+ES) $C_{17}H_{27}{}^{79}BrNO_5{}^+$ requires 404.1073 found 404.1086

$C_{17}H_{27}{}^{81}BrNO_5{}^+$ requires 406.1052 found 406.1044

ν_{max} (liquid film, cm^{-1}) 2873 (CH), 1731(C=O).

5.4.21 8-Hydroxy-3, 6-dioxaoctanyloxy (2-bromo-2-methyl)propionate (37)

A solution of triethyleneglycol (5.0 g) and triethylamine (1.8 mL, 16 mmol) in dichloromethane (30 mL) was stirred for 1 h at room temperature and cooled to 0 °C. 2-Bromo-2-methylpropionyl bromide (2 mL, 3.72 g, 16 mmol) was added dropwise over a 15 min period. The reaction was stirred for a further 8 h at 0 °C. The reaction mixture was washed with dilute HCl (2 x 30 mL) and water (2 x 30 mL), and the organic phase dried (Na_2SO_4). Filtration and removal of the solvent *in vacuo* resulted in a crude product which was purified by flash chromatography

(50 % then 100 % ethyl acetate / hexane) to obtain the title compound (4.2 g, 70 %). as a colourless oil.

δ_H ($CDCl_3$; 300 MHz) 1.95 (6H, s, C $[CH_3]_2Br$), 3.69 - 3.79 (10H, m, 5 x OCH_2), 4.29 (2H, m, $C\underline{H}_2OC{=}O$)

δ_C ($CDCl_3$; 75 MHz) 171.9 (C=O), 72.5, 70.7, 70.4, 70, 69.0, 62.0 (CH_2OH), 61.6, 55.9 (C-Br), and 26.9 (Me).

m/z (+ES) 321/323 (80%, M + Na), ν_{max} (liquid film, cm^{-1}) 3438 (OH), 1735(C=O)

5.4.22 11-Hydroxy-3, 6, 9-trioxaundecanyloxy (2-bromo-2-methyl) propionate (38)

A solution of tetraethylene glycol (6.0 g) in dichloromethane (30 mL) was reacted with 2-bromo-2-methylpropionyl bromide (2.0 mL, 3.72 g, 16 mmol) according to the method described above for **37** to obtain the title compound (4.5 g, 76 %) as a colourless oil.

δ_H ($CDCl_3$; 200 MHz) 1.93 (6H, s, C $[CH_3]_2Br$), 3.67 – 3.81 (14H, m, 7 x OCH_2), 4.34 (2H, m, $C\underline{H}_2OC{=}O$)

δ_C ($CDCl_3$; 100 MHz) 175 (C=O), 72.5, 70.9, 70.7, 70.4, 70, 68.8, 65.1(CH_2O), 61.8 (CH_2OH), 55.7(C-Br), 57.2 and 28.8 (Me), m/z (ES) 365/367 (M + Na).

HRMS (+ES) $C_{12}H_{23}{}^{79}BrO_6$ requires 365.0678 found 365.0588.

$C_{12}H_{23}{}^{81}BrO_6$ requires 367.0658 found 367.0510

ν_{max} (liquid film, cm^{-1}) 3441 (OH), , 1735 (C=O).

5.4.23 14-Hydroxy-3, 6, 9, 12-tetraoxatetradecanyloxy (2-bromo-2-methyl) propionate (39)

A solution of pentaethylene glycol (5.0 g) in dichloromethane (30 mL) was reacted with 2- bromo-2- methylpropionyl bromide (2.0 mL, 3.72 g, 16 mmol) according to the method described above for **37** to give the title compound (5.8 g, 95 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.91 (6H, s, C $[CH_3]_2Br$), 3.69 (18H, m, 9 x OCH_2), 4.30 (2H, m, $C\underline{H_2}OC{=}O$)

δ_C ($CDCl_3$; 100 MHz) 172.0 (C=O), 73.2, 72.7, 70.9, 70.8, 70.7, 70.5, 68.9, 65.3, 61.9 (CH_2OH),, 56.0 (C-Br) and 28.0 (Me), m/z (+ES) 409 / 411 (80%, M + Na^+)

HRMS (+ES) $C_{14}H_{27}{}^{79}BrO_7Na$ requires 409.0940 found 409.0847

$C_{14}H_{27}{}^{81}BrO_7Na$ requires 411.0920 found 411.0825

ν_{max} (liquid film, cm^{-1}) 3454 (OH), 1735(C=O)

5.4.24 General method for methanesulphonates 8- Methanesulphonyloxy -3, 6-dioxaoctanyloxy (2-bromo-2-methyl) propionate (40)

A solution of 8-hydroxy-3,6-dioxaoctanyloxy (2-bromo-2-methyl) propionate (1.2 g, 4.0 mmol) was stirred with methanesulphonyl chloride (0.81 mL, 4.8 mmol, 1.2 eq) according to the method described below for **41** to give the title compound (1.2 g, 78 %) as a colourless oil.

δ_H ($CDCl_3$; 400 MHz) 1.94 (6H, s, C $[CH_3]_2Br$), 3.07 (3H, s, OSO_2CH_3), 3.47 (4H, s, 2 x OCH_2), 3.65 (2H, s, $OC\underline{H_2}CH_2OSO_2CH_3$), 3.72 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 4.35 (2H, m, $CH_2OSO_2CH_3$), 4.39 (2H, m, $OCH_2C\underline{H_2}OC{=}O$)

δ_C ($CDCl_3$; 100 MHz) 172 (C=O), 72, 69, 68, 67(CH_2O), 65 ($COSO_2$), 56 (C-Br), 38 (SO_2Me) and 28 (Me).m/z (ES) 399 / 401 (M + Na)

HRMS (+ES) $C_{11}H_{21}{}^{79}BrO_7SNa$ requires 399.0191 found 399.0093.

$C_{11}H_{21}{}^{81}BrO_7SNa$ requires 401.0171 found 401.0093.

ν_{max} (liquid film, cm^{-1}) 1728 (C=O)

5.4.25 11-Methanesulphonyloxy-3, 6, 9-trioxaundecanyloxy (2-bromo-2-methyl)propionate (41)

11-Hydroxy-3, 6, 9-trioxaundecanyloxy(2-bromo-2-methyl) propionate (1.0 g, 3.0 mmol) in dichloromethane (25 mL) was stirred with *N, N*-diisopropylethylamine (2.0 mL, 1.48 g, 1.2 eq) for 1 h at room temperature. The mixture was then cooled to 0 °C before the dropwise addition of methanesulphonyl chloride (1.0 mL, 1.48 g, 1.2 eq) with stirring. The reaction was stirred at 0°C for a further 5 h and at room temperature for 1 h under a

nitrogen atmosphere. Upon completion of the reaction (TLC, R_{Fnew} = 0.9, 50 % ethyl acetate/hexane, $KMnO_4$), the mixture was washed with 1M HCl (3 x 25 mL), brine (2 x 25 mL) and dried with $MgSO_4$. Removal and concentration of the organic phase resulted in a crude product, which was purified by flash chromatography (30 % ethyl acetate/hexane) to give the title compound (0.95 g, 78 %) as a colourless oil.

δ_H ($CDCl_3$; 500 MHz) 1.94 (6H, s, C $[CH_3]_2Br$), 3.05 (3H, s, OSO_2CH_3), 3.65 – 3.70 (8H, m, 4 x OCH_2), 3.64 (2H, s, $OC\underline{H_2}CH_2OSO_2CH_3$), 3.68 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 4.35 (2H, m, $CH_2OSO_2CH_3$), 4.39 (2H, m, $OCH_2C\underline{H_2}OC{=}O$)

δ_C ($CDCl_3$; 125 MHz) 172 (C=O), 72, 69, 68 (CH_2O), 65 ($COSO_2$), 56 (C-Br), 38 (SO_2Me) and 28 (Me). m/z (ES) 443 /445 (M + Na)

HRMS(+ES) $C_{13}H_{25}{}^{79}Br\ O_8S$ requires 443.0454 found 443.0342.

$C_{13}H_{25}{}^{81}Br\ O_8S$ requires 445.0433 found 445.0241

ν_{max} (liquid film, cm^{-1}) 1732 (C=O), 1357 (S=O), 1173 (S=O).

5.4.26 14-Methanesulphonyloxy-3, 6, 9, 12-tetraoxatetradecanyloxy (2-bromo-2-methyl)propionate.(42)

14-Hydroxy-3, 6, 9, 12-tetraoxatetradecanyloxy(2-bromo-2-methyl) propionate (1.0 g, 2.6 mmol) was stirred with methanesulphonyl chloride (1.2 eq), according to the method for 11-Methanesulphonyloxy-3, 6, 9-trioxaundecanyloxy (2-bromo-2-methyl) propionate, purification by flash chromatography (30 % ethyl acetate/hexane)to give the title compound (0.80 g, 70 %) as a colourless oil.

δ_H ($CDCl_3$; 500 MHz) 1.93 (6H, s, C $[CH_3]_2Br$), 3.07 (3H, s, OSO_2CH_3), 3.62 – 3.70 (12H, m, 6 x OCH_2), 3.64 (2H, s, $OC\underline{H_2}CH_2OSO_2CH_3$), 3.78 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 4.35 (2H, m, $CH_2OSO_2CH_3$), 4.39 (2H, m, $OCH_2C\underline{H_2}OC{=}O$)

δ_C ($CDCl_3$; 125 MHz) 172.0 (C=O), 71.0, 70.8, 70.5, 70.0, 69.5 (CH_2O), 65.0 ($COSO_2$), 56.0 (C-Br), 38.0 (SO_2Me) and 31.0 (Me).

m/z (ES 464 / 466 (70 %, M+)

HRMS (+ES) $C_{15}H_{29}{}^{79}Br\ O_9SNa$ requires 487.0716 found 487.0609

$C_{15}H_{29}{}^{81}Br\ O_9SNa$ requires 489.0695 found 489.0572.

ν_{max} (liquid film, cm^{-1}) 2870 (CH), 1742 (C=O), 1352 (S=O), 1173 (S=O).

5.4.27 General method for trifluoromethanesulphonates 8-Trifluoromethanesulphonyloxy-3, 6,-dioxaoctanyloxy(2-bromo-2-methyl)propionate (43)

A solution of trifluoromethanesulphonyl chloride (0.56 g, 2 eq) in dichloromethane (10 mL) at 0 °C was cannulated over a period of 0.5 h into a solution of 8- hydroxy -3,6-dioxaoctanyloxy(2-bromo-2-methyl) propionate (300 mg, 0.1 mmol) and pyridine (80 mg, 1 eq) suspended over activated powdered sieves in dichloromethane (10 mL) at 0 °C. The mixture was then stirred at 0 °C for an additional 1.5 h under a nitrogen atmosphere. The reaction mixture was then filtered through a cotton wool plug then a celite plug before being washed with ice-cold water (2 x 20 mL), dried ($MgSO_4$) to give the title compound as a brown syrup (412 mg, 95 %). The compound was used without further purification.

δ_H ($CDCl_3$; 400 MHz) 1.94 (6H, s, C $[CH_3]_2$ Br), 3.66 (4H, s, 2 x OCH_2), 3.71 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 3.82 (2H, m, $OC\underline{H_2}CH_2OSO_2CF_3$), 4.36 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 4.6 (2H, m, $CH_2OSO_2CF_3$

δ_C ($CDCl_3$; 100 MHz) 172.0 (C=O), 76.0, 70.4, 70.2, 69.0, 67.0, 62.0 (CH_2O), 56.0 (C-Br) and 31.0 (Me)

δ_F ($CDCl_3$; 188 MHz) –75.08 (3F, s, OSO_2CF_3)

m/z (ES) 453/455 (10 %, M + Na), 335/337(65 %), 193/195 (100 %).

ν_{max} (liquid film, cm^{-1}) 2932 (CH), 1750 (C=O)

5.4.28 11-Trifluoromethanesulphonyloxy-3, 6, 9 - trioxaundecanyloxy (2-bromo-2-methyl)propionate (44)

11-Hydroxy-3, 6, 9-trioxaundecanyloxy(2-bromo-2-methyl) propionate (0.20 g, 0.59 mmol) was reacted with trifluoromethanesulphonyl chloride (0.20 g, 0.32 mL, 2 eq) according to the method described above for **43** to give the title compound (0.31 mg, 63 %) as a brown syrup.

δ_H ($CDCl_3$; 400 MHz) 1.94 (6H, s, C $[CH_3]_2$ Br), 3.66 (8H, s, 4 x OCH_2), 3.71 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 3.82 (2H, m, $OC\underline{H_2}CH_2OSO_2CF_3$), 4.36 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 4.6 (2H, m, $CH_2OSO_2CF_3$)

δ_C ($CDCl_3$; 100 MHz) 172.0 (C=O), 76.0, 70.6, 70.4, 70.2, 70.1, 69.0, 67.0, 62.0 (CH_2O), 56.0 (C-Br) and 31.0 (Me)

δ_F ($CDCl_3$; 188 MHz) –75.02 (3F, s, OSO_2CF_3)

m/z (ES+) 404/406 (100%, M^+ - CF_3)

ν_{max} (liquid film, cm^{-1}) 1750 (C=O).

5.4.29 14-Trifluoroethanesulphonyloxy-3, 6, 9, 12-tetraoxatetradecanyloxy (2-bromo-2-methyl)propionate (45)

14-Hydroxy-3, 6, 9, 12-tetraoxatetradecanyloxy(2-bromo-2-methyl) propionate (0.49 g, 1.27 mmol) was reacted with trifluoromethanesulphonyl chloride (0.43 g, 0.68 mL, 2 eq) according to the method described above for **43** to give the title compound (0.30 g, 45 %) as a brown syrup.

δ_H ($CDCl_3$; 400 MHz) 1.94 (6H, s, C $[CH_3]_2$ Br), 3.66 (12H, s, 6 x OCH_2), 3.71 (2H, m, $OC\underline{H_2}CH_2OC{=}O$), 3.82 (2H, m, $OC\underline{H_2}CH_2OSO_2CF_3$), 4.36 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 4.6 (2H, m, $CH_2OSO_2CF_3$)

δ_C ($CDCl_3$; 100 MHz) 172.0 (C=O), 73.0, 72.0, 70.9, 70.8, 70.7, 70.5, 69.0, 65.0, 62.0 (CH_2O), 56.0 (C-Br) and 31.5 (Me).

δ_F ($CDCl_3$; 188 MHz) –75.09 (3F, s, OSO_2CF_3)

ν_{max} (liquid film, cm^{-1}) 1754 (C=O)

5.4.30 *N, N*-dimethylammonium-*N*-3,6–dioxaoctanyl (8-[2-bromo-2-methylpropionyloxy])*N*-ethylmaleimide trifluoromethanesulphonate.(1)

A solution of 2-(*N, N* dimethylamino)ethylmaleimide (70 mg, 0.417 mmol) and 8-trifluoromethanesulphonyloxy-3, 6,-dioxaoctanyloxy (2-bromo-2-methyl) propionate (200 mg, 1.12 eqv) in dry THF (2 mL) was stirred for 48 h under a nitrogen atmosphere at room temperature. The reaction mixture was then filtered through a cotton wool plug and the plug washed with THF (20 mL). The organic

washings were combined and concentrated under reduced pressure to produce a brown syrup. The syrup was further extracted with cold ethyl acetate. The ethyl acetate extracts were evaporated and dried over phosphorus pentoxide *in vacuo* for 8 h to give the product (85 mg, 43 %) as a brown syrup.

δ_H ($CDCl_3$; 400 MHz) 1.92 (6H, s, C $[CH_3]_2$ Br) 3.30 (6H, s, N $(CH_3)_2$), 3.64 (4H, s, 2 x OCH_2), 3.74 – 3.80 (6H, m, $NC\underline{H_2}CH_2N$ $(CH_3)_2$ $CH_2C\underline{H_2}O$ and $OC\underline{H_2}CH_2OC{=}O$), 3.84 – 4.15 (4H, m, $NCH_2C\underline{H_2}N$ $(CH_3)_2$ $C\underline{H_2}CH_2O$), 4.3 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 6.8 (2H, s, CH = CH).

δ_C ($CDCl_3$; 100 MHz) 172.6, 172, 170 (C=O), 135 (C=C), 72, 72.3, 71, 65 (CH_2O),, 65.2 (CH_2N+), 56 (C-Br), 44 (NMe), 32 (CH_2N) and 31 (C-Me).

m/z (ES) 449 / 451 (100 %, M + Na^+)

HRMS (+ES) $C_{18}H_{30}{}^{79}BrN_2O_6Na$ requires 449.1287 found 449.1320

$C_{18}H_{30}{}^{81}BrN_2O_6Na$ requires 451.1267 found 451.1318.

ν_{max} (liquid film, cm^{-1}) 1716 (C=O).

5.4.31 N,N-dimethylammonium-N-3, 6, 9 –trioxaundecanyl (11-[2-bromo-2-methylpropionyloxy])N-ethylmaleimide trifluoromethanesulphonate.(2)

11-Trifluoromethanesulphonyloxy-3,6,9-trioxaundecanyloxy(2-bromo-2-methyl) propionate (57 mg, 1.1 eq) was reacted with 2-(*N, N* dimethylamino) ethylmaleimide (19 mg, 0.11 mmol) in dry THF (2 mL) according to the method described above for **1** to give the title compound (47.5 mg, 82 %) as a brown syrup.

δ_H ($CDCl_3$; 400 MHz) 1.92 (6H, s, C $[CH_3]_2$ Br), 2.35 (6H, s, N $(CH_3)_2$), 3.64 – 3.8 (8H, m, 4 x OCH_2), 3.73 – 3.81 (6H, m, $NC\underline{H_2}CH_2N$ $(CH_3)_2$ $CH_2C\underline{H_2}O$ and $OC\underline{H_2}CH_2OC{=}O$), 3.95 – 4.15 (4H, m, $C\underline{H_2}N$ $(CH_3)_2$ $C\underline{H_2}CH_2O$), 4.37 (2H, m, $OCH_2C\underline{H_2}OC{=}O$), 6.69 (2H, s, CH = CH).

δ_C ($CDCl_3$; 100 MHz) 171.01, 170.31, 170.28 (C=O), 134.88, 134.46 (C=C), 70.96, 70.89, 70.62, 68.97, 67.3, 65.36 (CH_2O), 65.25 (CH_2N+), 56.95 (C-Br), 45.24 (NMe), 35.38 (CH_2N), 30.97 and 30.85 (C-Me).

m/z (ES) 493 / 495 (15 %, M^+), 405 / 407 (100 %)

ν_{max} (liquid film, cm^{-1}) 1712 (C=O)

5.4.32 Peptide Synthesis and Modification with Initiators

General methods for the preparation of the peptide/protein initiator complex

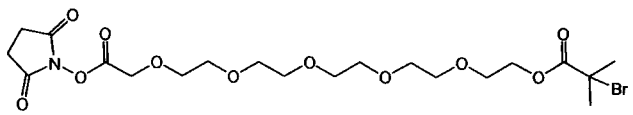
5.4.32.1 Peptide Synthesis

FmocNH--GRGDSPAS---RESIN

Was performed on an Advanced ChemTech peptide synthesiser (36 wells) using Fmoc synthesis procedure. The resin, TentaGel-S RAM Fmoc (0.2-0.4 mmol/g substitution), Fmoc-protected amino acids and HOBt and PyBOP used to facilitate the coupling reactions, were purchased from Advanced ChemTech (KY) and used as received. The resin-bound peptide was isolated first treated with 20% piperidine/ DMF mixture for 4 h at room temperature to remove the terminal Fmoc protected amine. The resin was then washed with DMF (6 x 15 mL) and dichloromethane (6 x 15 mL). The cleavage of solid supported peptide for analysis was evoked by treating with 95% TFA (5% TIS and water) for 4 hours with constant but gentle vortexing under a nitrogen atmosphere. The resin was filtered and the filtrate containing the peptide concentrated and the peptide precipitated into ice-cold ether at 4°C overnight. The precipitate was analysed by TOF MS (+ES) spectroscopy.

TOF MS (+ES) The parent peptide m/z 745 (100%, M^+)

5.4.32.2 Preparation of analogue peptide modified with initiator.



Initiator 3.3

Initiator---GRGDSPAS---Resin

The free ($-NH_2$) was first confirmed by positive ninhydrin test (blue-purple colouration) before the activated initiator, N-Succinimidyl 17-(2-bromo-2-methylpropionyloxy)-3, 6, 9, 12, 15-pentaoxaheptadecanoate **3.3** (Compound **3**, Chapter **3**) (62 mg, 4 eq) was vortexed with resin-bound peptide (0.1 g, 0.029 mMol, ~30% NH_2 functionality) and N,N-diisopropylethylamine (0.020 mL, 15.0 mg, 4 eq) in dry DMF (4 mL, HPLC grade) at room temperature for 6 h under an atmosphere of argon. The resin was then washed with DMF (6 x 15 mL), dichloromethane (6 x 15 mL) and dried *in vacuo*.

5.4.32.3 The cleavage reaction

Initiator---GRGDSPAS

The resin was cleaved from the peptide-initiator complex by stirring with a mixture of trifluoroacetic acid (TFA, 95%), triisopropylsilane (TIS, 2.5%) and water (2.5%) for 4 hours at room temperature under a nitrogen atmosphere. The solution was filtered and washed with neat TFA (3 x 3 mL) and the resulting solution concentrated *in vacuo* before the peptide was precipitated into ice-cold ether at 4°C overnight, to obtain the complex in 20% (7 mg over three steps) yield.

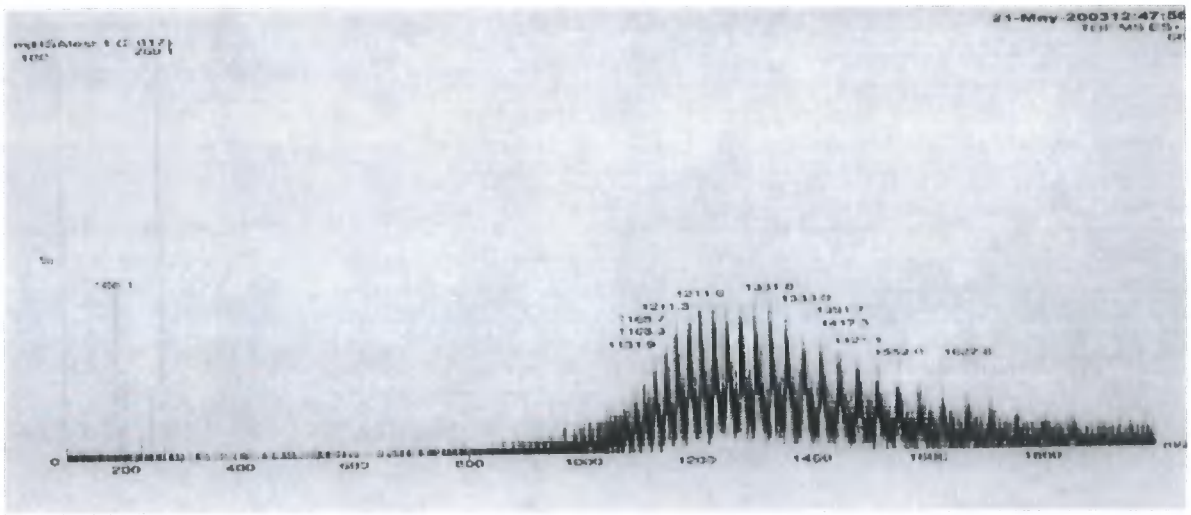
TOF MS (+ES) m/z 1171/1173 (55%, M^+).

5.4.33 HSA modification with ammonium maleimide salt

5.4.33.1 Human Serum Albumin (Unmodified)

Human Serum Albumin (0.5 mL, 2.6 mM) was treated with ethanethiol (20 mM) to stir under a nitrogen atmosphere at 4 °C for 24 h in phosphate buffer (pH 7.4, 200 mM). The mixture was purified by gel filtration on sephadex PD-10 against a phosphate buffer eluent. HSA thus prepared had a concentration of 10-50 μM and typically 0.6 - 0.8 SH/HSA. By observing Ellman's anion[1,2], the concentration of HSA was determined from the absorbance at 412 nm (ε =13,600 M^{-1}, cm^{-1}) assuming a molecular weight of 66,440 Da[3].

TOF MS (+ES) expected m/z 1331, found m/z 1328 (48%, M + 50H) $^{50+}$

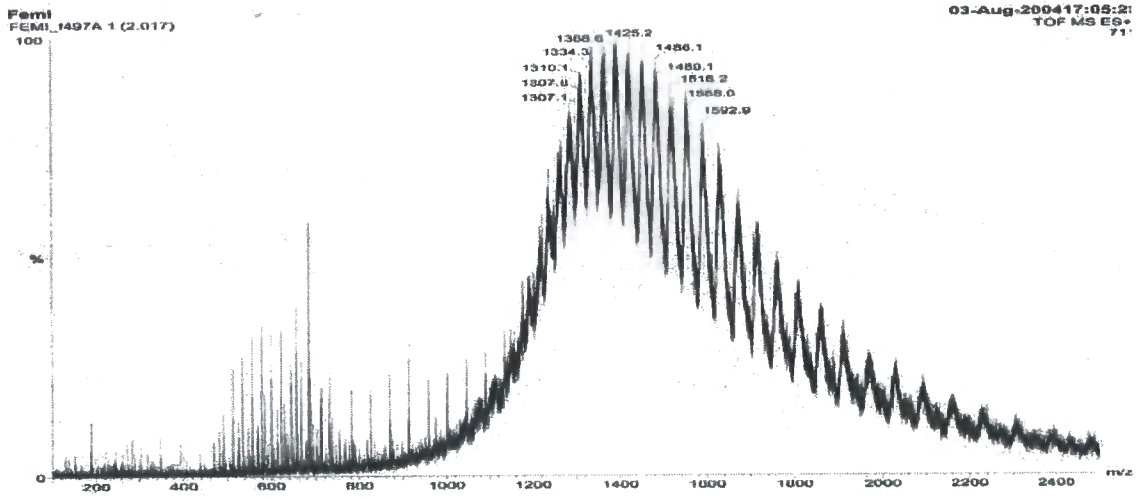


5.4.33.2 Human Serum Albumin (modified)

A solution of *N,N*-dimethylammonium-N-3,6–dioxaoctanyl(8-[2-bromo-2-methyl-propionyloxy])*N*-ethylmaleimide trifluoromethanesulphonate

(1.4) (compound 1, Chapter 4, 4 eqv., 2.6 mM) in phosphate buffer (pH 7.4, 200 mM) was gently vortexed with HSA (2.6 mM.) under a nitrogen atmosphere at room temperature. Upon completion (UV analysis of Ellman's anion) the reaction mixture was treated with gel filtration on sephadex PD-10 at room temperature.

TOF MS (+ES) expected m/z 1423, found m/z 1425 (100%, M + 47H)$^{47+}$



5.5 References

1 G. Ellman, *Archives Biochem. Biophysics*. **1959**, *82*, 70.

2 R. A. Moss, S. Swarup, H. Zhang, *J. Am. Chem. Soc.*, **1988**, *110*, 2914.

3 D. C. Carter, J. X. Ho, *Adv. Protein Chem.*, **1984**, *120*, 8253.