Synthesis and Evaluation of Polystyrene Based Soluble Polymeric Supports for Organic Synthesis

Nafisa Hafiz Rehman

Doctor of Philosophy

ASTON UNIVERSITY

May 2007

Aston University

Synthesis and Evaluation of Polystyrene Based Soluble Polymeric Supports for Organic Synthesis

A thesis submitted by Nafisa Hafiz Rehman for the degree of Doctor of Philosophy

May 2007

Summary:

Several copolymers of linear polystyrene were prepared for evaluation as soluble polymeric supports for organic synthesis. These polymers were utilized for the synthesis of Δ^2-isoxazoline compounds. The target compounds were synthesized *via* 1,3-dipolar cycloaddition reactions between polymer bound alkenes and nitrile oxides generated *in situ* from their corresponding aldoximes. The cleaved Δ^2-isoxazoline compounds were tested for biological activity against *Mycobacterium fortuitum*. To compare the success of these linear polystyrene copolymers, some of the Δ^2-isoxazoline compounds synthesized on soluble polymeric supports were also prepared *via* traditional crosslinked polymer supports.

The polymer-bound Δ^2-isoxazolines were also tested for antimicrobial activity. In addition attempts were made to prepare polymers containing the Δ^2-isoxazolines but anchored by non-hydrolysable bonds.

Although the copolymers of polystyrene gave good loading capacity in mmol/g, and being soluble in chlorinated solvents it was possible to monitor the reactions by ^{1}H NMR spectroscopy, the cleavage of the polymer bound products proved to be quite troublesome. Product purification was not as straightforward as it was anticipated. Isolation of the cleaved target compounds proved to be time consuming and laborious when compared to the traditional organic synthesis and solid phase organic synthesis (SPOS).

Polymer-bound Δ^2-isoxazolines close to the polymer backbone exhibited some biological activity against *Staphylococcus aureus*. Polymers with substitution at the *para*-position of the aryl substituent at position 3 of isoxazoline ring showed antimicrobial activity.

Keywords: Δ^2-isoxazoline, nitrile oxides, 1,3-dipolar cycloaddition, solid phase organic synthesis (SPOS).

Acknowledgements:

I would like to thank my supervisor Dr. Daniel Rathbone for his constant support and guidance throughout the course of this programme.

I would like to thank Mike Davis, for his help around the Lab, Karen Farrow for technical assistant and help in processing mass spectrums. Thanks to everyone in the medicinal chemistry lab, Nasim, Aisha, Katy, Laila and Beth for their support. Thanks to Dr Bill Fraser for his help at various occasions.

Many thanks to Professor P. Lambert for his support and guidance for biological studies, and Shaina for biological screening of my compounds.

I would also like to thank my mother Fahmida Khanum, father Mohammed Rafiq, sisters Rehana, Shamaila and Isma and brothers Abdul Qudoos and Mohammed Waqas for their continuous support.

A huge thanks to my brother-in-law Atiq Rehman for sorting out my computer, printer and several other IT related problems through out the course of this degree. Many thanks to my sisters-in-law Samina, Seema and Fauzia for their support and putting up with me throughout this PhD course. A special thank you to Fauzia for teaching me ins and outs of typing and for sorting out the references section.

A sincere and very huge thank you to my dear husband Hafiz Rehman for continuous support and putting up with me through out the PhD programme and standing by my side through thick and thin.

A massive thanks to my sweet children, daughter Maria and son Hassan for giving me strength and stamina to successfully complete this degree.

Last but not least a big thanks to my mother-in-law Zaida Pervin for her help and support throughout the PhD programme, and for looking after Maria and Hassan while I spent my days and nights to complete my study. I truly believe that without my mother-in law's support and help I couldn't have achieved my goal.

Dedicated to my Parents and Parents-in-Law (especially my late father-in-law, Fazal Rehman)

Contents:

List of Figures:

List of Tables:

List of Schemes:

List of Graphs:

Abbreviations:

AMA	Allyl methacrylate
EDMA	Ethylene glycol dimethacrylate
GTP	Group transfer polymerisation
CCl_4	Tetrachloromethane
DCM	Dichloromethane
$CHCl_3$	Chloroform
AIBN	2,2-Azobis(2-methyl-propiontrile) or (2,2'-azobis(isobutyronitrile)
THF	Tetrahydrofuran
$MgSO_4$	Magnesium sulphate
$CDCl_3$	Deuterated chloroform
TFA	Trifluoroacetic acid
INH	Isoniazid
RIF	Rifampicin
TB	Tuberculosis
MDR-TB	Multidrug resistant tuberculosis
NMR	Nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
IR	Infra red
UV	Ultraviolet
DMAP	Dimethylaminopyridine
1,3-DC	1,3-Dipolar cycloaddition
MO	Molecular orbital
EtOAc	Ethyl acetate
SPOS	Solid phase organic synthesis
LPOS	Liquid phase organic synthesis
SPS	Soluble polymeric supports
HF	Hydrofluoric acid
PS	Polystyrene
PEG-PS	Polystyrene-polyethylene glycol co-polymer
PEG	Polyethylene glycol
PEO	Polyethylene oxide
POE	Polyoxyethylene
MeO-PEG	Polyethylene glycol monomethyl ether
MAPc	Mycolic acids arabinogalactan peptidoglycan complex
Ac-INH	*N*-Acetylisoniazid
NAT2	*N*-Acetyltransferase2
PAS	*p*-Aminosalicylic acid
MIC	Minimum inhibitory concentration
DMF	Dimethylformamide
PGE_2	Prostaglandin E_2 methyl ester

VAN	Vancomycin
AEEA	8-amino-3,6-dioxaoctanoate (aminoethyoxyethoxyacetate)
APTS	aminopropyltriethoxysilane
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
P. aeruginosa	*Pseudomon aeruginosa*
E. coli	*Escherichia coli*

Aims and objectives

The aims of this project were to explore the use of styrene-based copolymers as soluble supports for organic synthesis. In addition the biological properties of the intact polymers and isolated cleaved final products were to be investigated.

Individual objectives included;

- Optimisation of the solubilities and loading capacities of the soluble supports.
- The synthesis of potentially antimicrobial Δ^2-isoxazolines using the soluble supports and comparison to similar synthesis using conventional crosslinked solid supports as well as traditional solution phase synthesis.
- Biological evaluation Δ^2-isoxazolines as potential inhibitors of mycobacterial mycolic acid biosynthesis.
- Biological evaluation of the functionalised styrene copolymers.

CHAPTER 1

Introduction

1: Introduction:

Synthesis has been the backbone of organic chemistry since WÖhler's work on production of urea from ammonium cyanate in 1828. During the last hundred years chemists have been challenged time and again to new achievements by the products of nature. The modern technology of today enables synthesis of molecules of almost any complexity. However, despite all this progress, the goal of organic synthesis has remained the same, the most efficient and selective synthesis possible of a single target molecule of defined structure.[1]

Currently, there is a tremendous effort in the pharmaceutical industry being directed toward the discovery of lead compounds possessing activity against an increasing number of biological targets. This has led to a dramatic increase in the demand for novel compounds.[2] The synthesis of small molecule libraries is currently the limiting step in the drug discovery process. Traditional conventional methods are time consuming and synthetic chemists are always searching for alternative methods to achieve more in less time.[3] Synthesis of small organic molecules can be divided into two main categories; a) polymer supported synthesis, b) non-polymer supported synthesis.

1.1: Polymer supported synthesis:

Polymer supported synthesis is an important tool for the development of new synthetic strategies in organic chemistry.[4] In this form of synthesis a polymer is employed, where a substrate molecule is temporarily attached to the polymer *via* a linker. Synthesis is performed upon the substrate. The modified substrate is released from the polymeric support by specific chemical conditions. A pictorial representation of polymer supported synthesis is shown in **Figure 1**. Polymeric supports can be divided into two main groups; a) solid-phase organic synthesis (SPOS), b) liquid-phase organic synthesis (LPOS). A third class of organic synthesis, introduced fairly recently, employed the polymer supported reagents known as scavenger resins, this will be discussed in section 1.6, page 55.

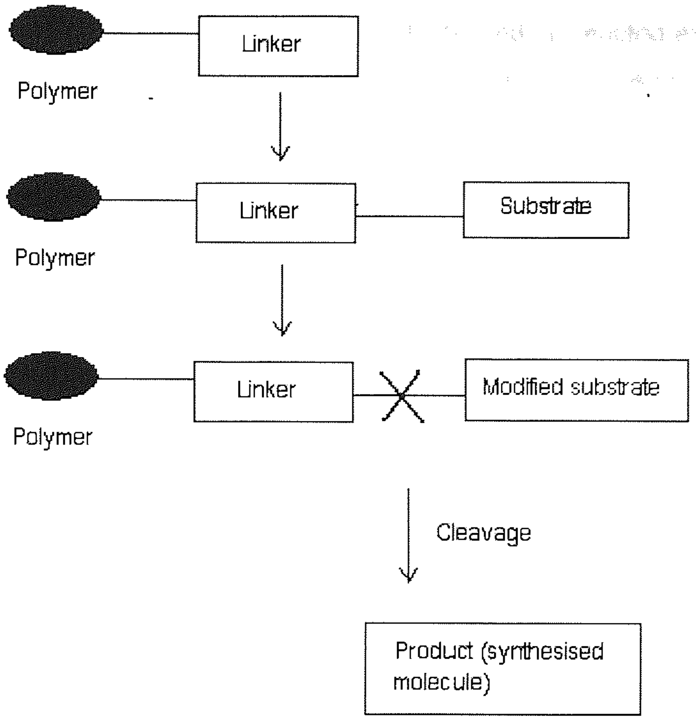


Figure 1: A pictorial representation of synthesis facilitated by polymeric supports.

1.2: Non-polymer supported synthesis:

Non-polymer supported synthesis is the traditional solution-phase organic synthesis. All reagents used for the reaction are in solution-phase and are not anchored to any from of scaffold or support. The reactions are carried out in homogeneous conditions. However attempts to drive reactions to completion may require the tedious purification of intermediates.

1.3: Solid-phase organic synthesis (SPOS):

Solid-phase organic synthesis (SPOS) has moved on from its first use as a solid support for peptides and then nucleotides synthesis to the synthesis of small organic molecules. It is an area of great importance for the synthesis of chemical libraries, both in academia and in the pharmaceutical industry.[5] Solid phase synthesis is now a key component of the high-throughput synthesis and screening approach to, and is recognized as a valuable tool for, drug discovery.[6,7]

The vast majority of published work on small molecules library synthesis has been performed using solid-phase synthesis.[8] Development of the chemistry on the solid-phase is often the rate limiting step, but an increasing number of synthetic transformations that are routine in traditional solution-phase organic chemistry are being established on various solid supports.

There are many advantages that accrue from conducting reactions by solid phase synthesis. These include ease of purification, where non-resin bound by-products, and un-reacted excess reagents can simply be removed by washing the resins in the appropriate solvent, thus making it easier to perform multi-step reactions. Excess reagents may be used to drive reactions to completion. The products may be obtained in high purity avoiding time consuming and costly chromatographic purification. Solid-phase resins give 'site isolation', whereby a reactive site on a resin has in effect infinite dilution. If a molecule with two equally active functions is reacted with the resin, only one site can attach to the resin, the other will remain free to react with other molecules in solution.[6,8,9]

Although highly successful, solid-phase synthesis still exhibits several shortcomings due to the nature of heterogeneous reaction conditions.[10] The main disadvantages of solid-phase chemistry are the extra labour (i.e. attachment of substrate and cleavage of product), large excesses of reagents, lower reactivity due to steric hindrance at the polymer interface and therefore longer reaction times, often required to force reactions to completion.[11] The use of excess reagents is particularly undesirable in the case of costly or commercially unavailable reagents.[5] There are limited means of monitoring reactions and characterization of intermediates attached to the support.[12] Ordinary spectroscopic approaches fail and chromatography analysis is not possible. Solid-phase routes also necessitate additional steps, to link the substrate and cleave off the product from the support. The amounts of products produced are often small because of the limited loading capacity of solid-phase resins. Generally, solid-phase synthesis is used to prepare very small amounts of the final product, and cost factors usually prohibit the production of large quantities of product.

1.3.1: Solid-phase supports for the synthesis of small organic molecules:

Various supports have been developed for solid phase synthesis. For the synthesis of small organic molecules, the most commonly used resins are polystyrene-divinylbenzene copolymers with ~ 1-2% cross-linking, and a polystyrene-polyethylene glycol co-polymer (PEG-PS) also known as Tentagel™. One major limitation of polystyrene resin is that the resin beads are not well solvated in polar protic solvents, such as alcohol and water, resulting in poor reaction site accessibility and diminished reaction rates. In contrast PEG-PS is well solvated in both protic and aprotic solvents, because the PEG is not cross-linked the reaction sites may be more accessible. PEG-PS has the disadvantage of being more expensive, lower loading capacity and it is mechanically less stable than polystyrene.[6] The presence of linear PEG impurities have also been found in small molecule products cleaved from the resins. PEG resins have the tendency to become sticky therefore as the synthesis progresses they become difficult to handle.

1.3.2: Solid-phase linkers:

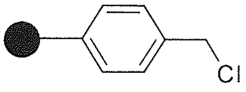
In SPOS the critical step is the attachment of the molecule to the solid phase, which is achieved through a cleavable linker. In broader definitions a linker can be described as a connection between the molecule being synthesized and the solid phase polymer that is cleaved to release the desired product. Some synthetic chemists may describe it as a bi-functional protecting group that is attached to the molecule being synthesized through a bond labile to the cleavage conditions and is attached to the solid phase polymer through a more stable bond. However not all linkers do fit into the latter definition.[13]

The ideal linker should fulfil a number of criteria that includes, it should be cheap and readily available, the attachment of starting material should be readily achieved in high yield, it should survive the conditions of the synthesis, be readily cleaved from the resins without destroying the synthesised molecule once the reaction is complete and the cleavage method should be easily worked up in large numbers and should not introduce impurities that are difficult to remove.[13]

1.3.3: Linkers for immobilization of carboxylic acids:

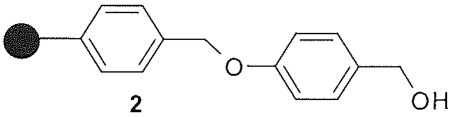
The attachment of the molecule to the solid support, and the cleavage of the product from it, depends on the type of linker. A large number of linkers for solid support are available commercially. For the immobilization of carboxylic acids on solid supports, the original polymer developed by Merrifield for the synthesis of peptide, was a chloromethylated copolymer of styrene and divinylbenzene (Merrifield resin) **(1)**.[14] The product is cleaved from the resin by hydrofluoric acid (HF) which is very corrosive and toxic. An improvement upon Merrifield is Wang resin **(2)**. Wang resin is a polymer functionalized with an alkoxybenzyl group. It is now the most widely used resin in SPOS. Cleavage of the product from resin is achieved by much milder conditions compared to the Merrifield resin. A solution of 50% trifluoroacetic acid (TFA) in DCM affords the cleavage from solid support. Similar to the Wang resin, with an additional methoxy substituent at the ortho position of the phenyl ring, is Sasrin resin **(3).** The presence of an additional methoxy group makes it more sensitive to acid cleavage such that the product can now be cleaved with just 5% TFA in DCM.[8] Trityl linker **(4)** and rink linker **(5)** are useful linkers for the immobilization of carboxylic acids. The products can be cleaved from the resins by using 1% and 20% TFA in DCM respectively. Some of the most common linkers used for immobilization of carboxylic acids on solid-phase synthesis are shown in **Figure 2** below.

The acid labile linkers for carboxylic acids may also be cleaved by nucleophilic conditions. e.g. with NaOMe to give the methyl carboxylic ester, or by unhindered amines such as methylamine to give the corresponding amide. Post-cleavage work-up procedures, i.e. chromatography, also become necessary to obtain pure products under these conditions.[8]



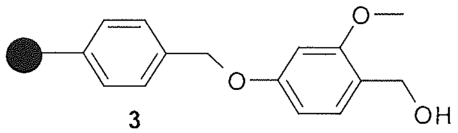
1

Merrifield Linker



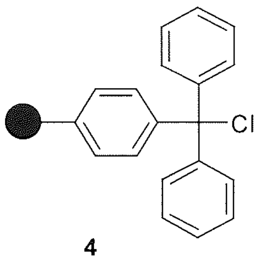
2

Wang Linker



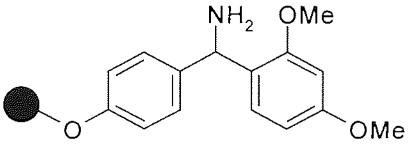
3

Sasrin Linker



4

Trityl Linker (Chlorotrityl resin)



5

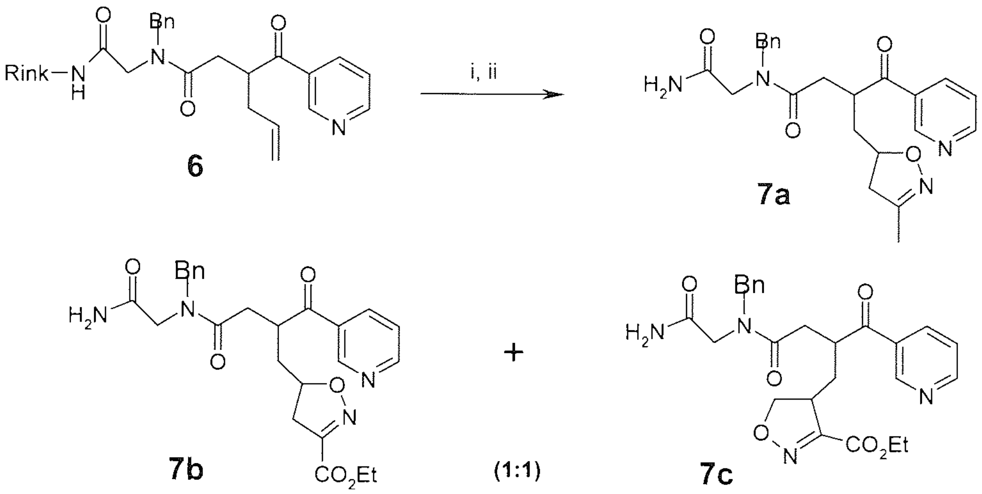
Rink Linker

Figure 2: **Common linkers used for the immobilization of carboxylic acids on solid-phase synthesis.**

1.3.4: Solid-phase synthesis of Δ^2-isoxazolines:

A vast amount of literature is present on the synthesis of isoxazolines. First example of a 1,3-dipolar cycloaddition (1,3-DC) of nitrile oxides to polymer-bound alkynes was published by Yedida and Lenzof in 1980. In the last decade numerous publications concerning 1,3-DC reactions of nitrile oxides to alkenes and alkynes on solid supports have been published. However only a few of these publications are known for the successful application of 1,3-DC of nitrones with alkenes in solid-phase organic synthesis of isoxazolidines.[11] Some of these examples are mentioned in this thesis.

In 1994 Pei *et al.*[15], reported a one-step formation of isoxazoles and isoxazolines on solid-phase, through [3+2] cycloaddition of nitrile oxides with alkynes and alkenes respectively. The resin bound peptoid **(6)** was prepared, which was then reacted with nitrile oxides, generated *in situ* by reacting the nitroalkyl compounds with phenyl isocyanate and triethylamine (TEA) or by oxidizing oximes with sodium hypochlorite in the presence of triethylamine. The synthesised isoxazoline compounds **(7)** were cleaved from the resin with 20% TFA in DCM. Peptoid **(6)** gave a single isomer of isoxazoline with the nitrile oxides from nitroethane **(7a)**. However, a mixture of two regioisomers was reported **(7b** and **7c)** in 1:1 ratio, with the nitrile oxides from ethylnitroacetate It has been documented that the presence of electron-withdrawing groups (in this case -CO_2Et), decreased the regioselectivity and stability of nitrile oxides[15] **(Figure 3)**.



i = RCH_2NO_2, Ph-N=C=O, Et_3N, toluene 100 °C (R= alkyl, aryl)
ii = 20% CF_3CO_2H in DCM

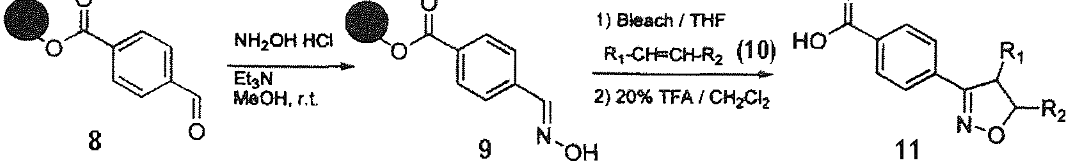
Figure 3: Synthesis of isoxazolines on solid-phase. Adapted from Pie *et al*[15].

In 1998 Cheng *et al.*[16] and Shankar *et al.*[17] independently reported the synthesis of Δ^2-Isoxazolines on solid phase through 1,3-DC of nitrile oxides. A number of Δ^2-Isoxazolines was prepared.

Cheng *et al* utilized the Wang resin as a solid support and used two different approaches. Approach one furnished isoxazolines by reacting resin bound nitrile oxides with dipolarophiles from solution. Approach two achieved the target compounds by reacting resin bound diplarophiles with nitrile oxides from solution. In both cases, nitrile oxides were generated *in situ* by the treatment of aldoximes with sodium hypochlorite in the form of commercially available bleach.

In order to generate the resin bound nitrile oxides, the benzylic alcohol of Wang's resin was coupled with the 4-formyl benzoic acid in a standard DIC/DMAP coupling procedure to give resin bound aldehyde **(8)**. Reaction of aldehyde resin **(8)** with hydroxylamine in the presence of triethylamine in methanol (MeOH) at room temperature gave the desired resin-bound aldoxime **(9)**. The resin-bound aldoxime **(9)** was suspended in THF and treated with an excess of commercially available bleach to form nitrile oxides, which were reacted *in situ* with alkenes **(10)** in one pot to furnish the corresponding resin-bound Δ^2-Isoxazolines **(11a-f)**. The cleavage of the resin-bound product was achieved by treating the resin with 20% TFA in DCM **(scheme 1)**. It was observed that nitrile oxides cycloaddition to terminal alkenes gave 5-substituted Δ^2-isoxazolines. Several Δ^2-isoxazolines compounds were synthesized. In all cases a single regioisomer was obtained, except where the alkenes were a

1,2-disubstituted (**11f (i)** and **11f (ii)**), which led to a mixture of regioisomers in approximately a 1:1 ratio. The **Table 1** below shows the compounds synthesized and the yields obtained.



Scheme 1: Synthesis of isoxazoline on solid support. Nitrile oxides anchored to the solid support. Adapted from Cheng *et al.*[16]

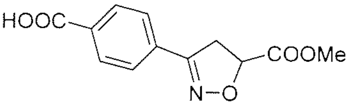
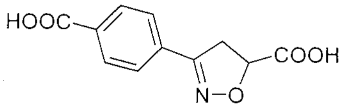
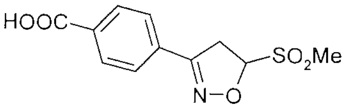
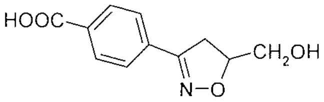
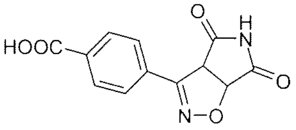
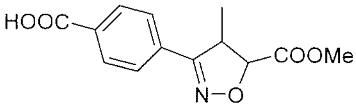
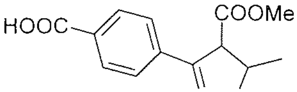
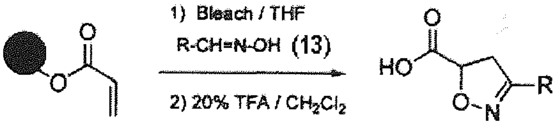
Entry	Dipolarophile	Δ^2-Isoxazolines	Yield (%)
11a	Methyl acrylate		94
11b	Acrylic acid		90
11c	Methyl vinylsulfonate		66
11d	Allyl alcohol		92
11e	Maleic imide		60
11f	Methyl crotonate	 i	45
		 ii	40

Table 1: Δ^2-Isoxazolines synthesized on solid support. The nitrile oxides were immobilized onto solid support. Adapted from Cheng *et al.*[16]

In the second approach the resin bound alkenes **(12)** were reacted with aldoximes **(13)** in the presence of bleach, to generate, *in situ,* nitrile oxides, in THF to give resin bound products **(scheme 2)**. The target Δ^2-isoxazolines compounds **(14a-d)** were cleaved with 20% TFA in DCM. On the basis of regiospecificity of 1,3-DC, only single regioisomers were isolated and compounds were obtained with over 90% purity. The regiochemistry of 1,3-DC would be discussed in detail in section 1.7 and 2.6 page 61 and 122 respectively. The compounds prepared by Cheng *et al* are given in **Table 2** below.



12 14

Scheme 2: Synthesis of Δ^2-isoxazolines on solid supports. Dipolarophiles anchored to the solid support. Adapted from Cheng *et al.*[16]

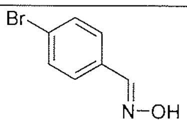
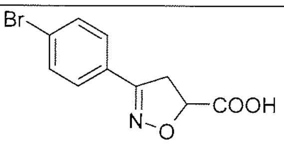
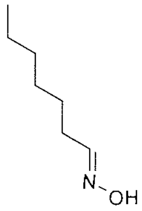
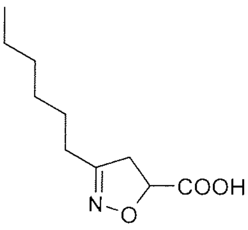
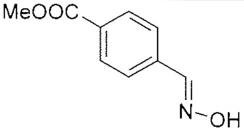
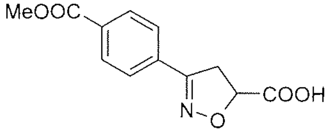
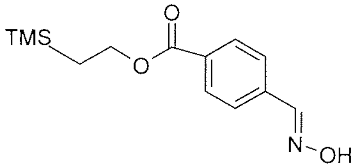
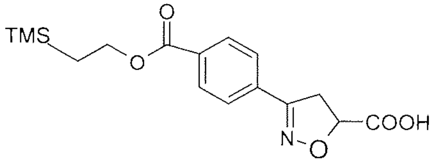
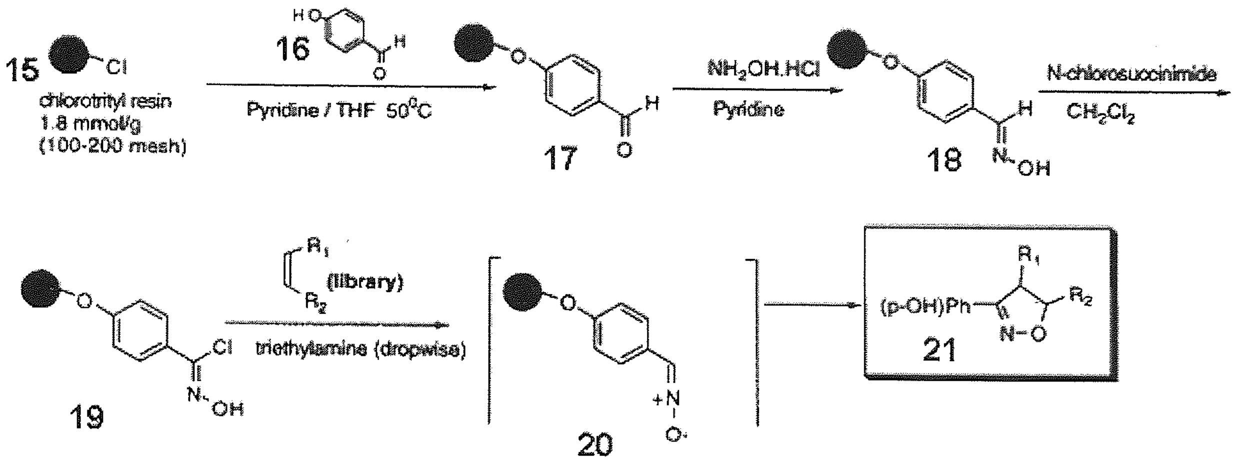
Entry	Aldoxime	Δ^2-Isoxazolines	Yield (%)
14a			98
14b			95
14c			95
14d			90

Table 2: Synthesis of Δ^2-isoxazolines compounds on solid support. In this approach the dipolarophile was immobilized onto solid support and the nitrile oxides were generated *in situ* in solution. Adapted from Cheng *et al.*[16]

Shankar *et al.* used a similar strategy to Cheng *et al.* and anchored the nitrile oxides precursor on to the solid phase. To synthesize the target compounds *para*-hydroxy benzaldehyde **(16)** was attached on chlorotrityl resin **(15)**. The aldoxime was prepared by treating resin **(17)** with an excess of hydroxylamine hydrochloride in pyridine at room temperature (RT). To achieve resin **(19)**, a precursor for nitrile oxides, the oxime resin **(18)** was chlorinated with 4-eq. *N*-chlorosuccinimide in DCM for two hours. A ten fold excess of dipolarophile (olefin) in DCM was added to the resin **(19)**, followed by slow addition of TEA, to generate the resin-bound nitrile oxides **(20)**. The cycloadduct was cleaved off the resin with 1% TFA in DCM. The **Scheme 3** below shows the route followed to achieve the target compounds **(21)**. A library of isoxazolines **(21a-21h)** was synthesized by trapping *in situ* generated nitrile oxides with appropriate olefins and acetylenes. The yields obtained were in the range of 60 - 80% and greater than 90% in purity. The compounds synthesized are shown in **Table 3**, below.



Scheme 3: **One pot synthesis of isoxazolines on solid phase.**

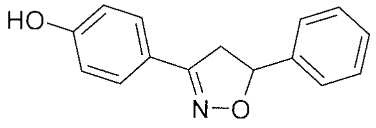
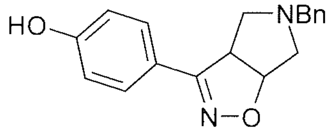
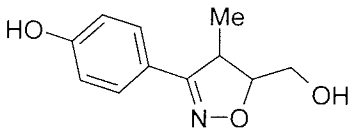
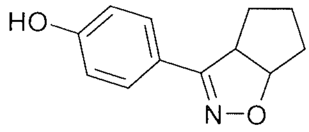
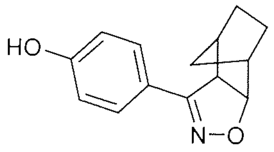
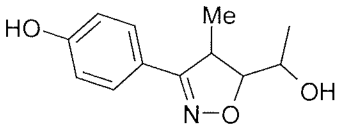
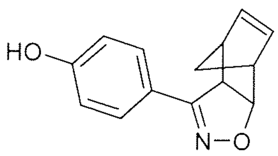
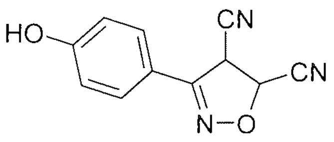
Entry	Isoxazoline Compound	Entry	Isoxazoline Compound
21a		**21e**	
21b		**21f**	
21c		**21g**	
21d		**21h**	

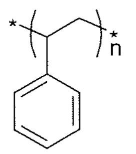
Table 3: **The library of isoxazolines synthesized on solid phase, by trapping *in situ* generated nitrile oxides with appropriate olefins. Adapted from Shankar et *al.*[17]**

1.4: Liquid-phase organic synthesis (LPOS):

Liquid-phase organic synthesis (LPOS) employs soluble polymeric supports (SPS) as a scaffold for the synthesis of organic molecules. The first examples of liquid-phase synthesis were simple modification of the Merrifield method of peptide synthesis, and employed linear soluble polystyrene to achieve homogeneous reaction conditions while, attempting to preserve many aspects of solid-phase synthesis. However, this methodology did not gain much attention from the synthetic chemists until mid 1990s, when Janda *et al.*[10] reintroduced the methodology of liquid-phase organic synthesis by investigating a number of polymeric supports for organic synthesis.

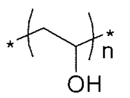
In LPOS, a polymeric support is employed which is soluble in some solvents and insoluble in others. It couples the advantages of homogeneous solution chemistry with those of solid-phase synthesis. Excess reagents may be used to drive a reaction to completion. Purification is simply achieved by the precipitation of the polymer in an appropriate solvent, followed by filtration to remove excess reagents and by-products. With synthesis on SPS the general difficulties of heterogeneous solid-phase chemistry, namely lower reactivity and characterization of the polymer-bound intermediates are overcome. LPOS permits product characterization on soluble polymer supports by routine analytical methods. UV-visible, IR, and NMR (C^{13} and ^{1}H NMR) spectroscopy may be used to monitor reactions without requiring preliminary cleavage from the polymer supports. Moreover, aliquots taken for characterization may be returned to the reaction flask upon recovery from these non-destructive analytical methods.[3,10,18,19]

Several soluble polymeric supports were evaluated by Janda *et al.*[10,19] including, polystyrene **(22)**, polyvinyl alcohol **(23)**, polyethylene imine **(24)**, polyacrylic acid **(25)**, polymethylene oxide **(26)**, polyethylene glycol **(27)**, polypropylene oxide **(28)**, polyacrylamide **(29)**, cellulose **(30)**, and various copolymers. These polymers are shown in **Figure 4** and their applications are listed in **Table 4**.



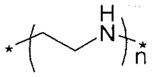
Polystyrene

22



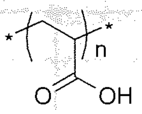
Polyvinyl alcohol

23



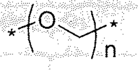
Polyethylene imine

24



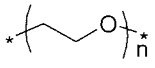
Polyacrylic acid

25



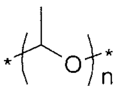
polymethylene oxide

26



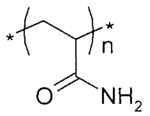
Polyethylene glycol

27



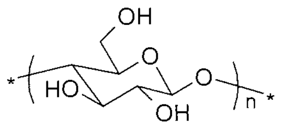
Polypropylene oxide

28



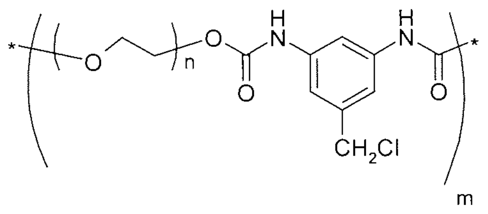
Polyacrylamide

29



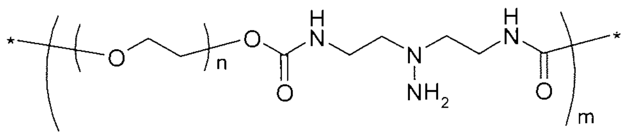
cellulose

30



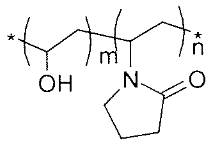
PEG with 3,5-diisocyanatobenzyl chloride

31



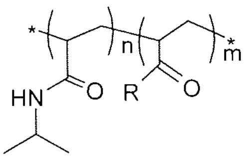
PEG with 3-nitro-3-azapentane-1,5-diisocyanate (and after hydrogenation)

32



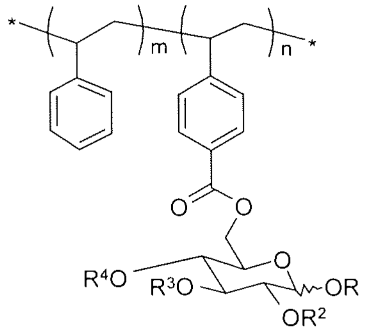
polyvinyl alcohol-poly (1-vinyl-2-pyrrolidinone)

33



Poly(*N*-isopropylacrylamide)-poly (acrylic acid derivatives)

34



Polystyrene-poly (vinyl-substituted monosaccharides)

35

Figure 4: **Soluble polymeric supports utilized in liquid-phase synthesis by Janda *et al*[10].**

Polymer	Application*			
Homopolymers	Peptide synthesis	Oligonucleotide synthesis	Oligosaccharide synthesis	Small molecules
Polystyrene (non-cross-linked)	√	√	√	
Polyvinyl alcohol	√	√	√	√
Polyethylene imine	√			
Polyacrylic acid	√			
Polymethylene oxide	√			
Polyethylene glycol (PEG)	√	√	√	√
Polypropylene oxide	√			
Cellulose		√		
Polyacrylamide			√	
Copolymers				
PEG with 3,5-diisocyanatobenzyl chloride	√			
PEG with 3-nitro-3-azapentane 1,5-diisocyanate	√			
Polyvinyl alcohol-poly(1-vinyl-2-pyrrolidinone)	√	√		
Polystyrene-poly(vinyl-substituted monosaccharides)			√	
Pol(*N*-isopropylacrylamide)-poly(acrylic acid derivatives)				√

Table 4: Table shows different soluble polymeric supports that were tried by Janda *et al* for various applications. Table adapted from Janda *at el.*[10]

In much the same way that Merrifield's 1-2% divinyl benzene (DVB) cross-linked polystyrene resin dominated the developing field of solid-phase chemistry, poly(alkene oxide)s such as PEG have been the most studied polymers for soluble polymer supported organic synthesis. Therefore the vast majority of examples of synthesis using soluble polymeric supports has utilised PEG as the polymeric support. Other supports, such as poly(ethylene) oligomers and poly(styrene)s have been used only in more specialised cases.[18,20]

1.4.1: Polyethylene glycol (PEG):

Polyethylene glycol (PEG), polyethylene oxide (PEO), and polyoxyethylene (POE) all represent the same linear polymers from the polymerisation of ethylene oxide. PEG usually indicates the polyether of molecular weight less than 20,000; PEO signifies polymers of higher molecular weights and POE have been applied to polymers of a wide range of molecular weights. Therefore it should be noted that these polymers do not exhibit one discrete molecular weight, but exist as a distribution of molecular weights. However, the polydispersity of commercially produced PEGs is quite narrow. PEGs of molecular weight 2,000 to 20,000 are crystalline at RT with a loading capacity of 1 to 0.1 mmol/g, whilst the lower molecular weight PEGs exist as liquids at room temperature.[10]

PEG and its derivatives are non-toxic, non-immunogenic, and are used as chemical modifying reagents in the pharmaceutical industry.[21,22] Chemical modification of protein drugs by PEG can improve the stability of these drugs in the blood circulation. Therefore drug conjugation with PEG can enhance the stability of a drug during storage and use. The conjugate may reduce toxicity as well as a reduced rate of biotransformtion in the liver and kidney excretion thus prolonging circulation time.[22]

The most common use of PEG in synthetic chemistry is as a soluble polymeric support for the synthesis of organic molecules.

1.4.2: PEG as a soluble polymeric support:

PEG, a linear homopolymer, exhibits solubility in a wide range of organic solvents including dimethylformamide (DMF), dichloromethane (DCM), toluene, acetonitrile, methanol and water, but it is insoluble in diethyl ether, hexane, *tert*-butyl methyl ether, and *i*-propyl alcohol. The latter solvents are used to induce PEG precipitation. PEG is also insoluble in THF (below room temperature).[18,23] Diethyl ether is most commonly used for the precipitation of polymer, however being relatively non-polar, polar impurities are often associated with polymer precipitation from this solvent. The use of *tert*-butyl methyl ether to dissolve these impurities is undesirable due to it is being environmentally unfriendly. Careful precipitation conditions or cooling of polymer solutions in ethanol and methanol yields crystalline PEG due to the helical structure of the polymer that produces a strong propensity to crystallize. Therefore as long as the polymer backbone remains unchanged during the synthesis, purification by precipitation can be achieved at each step of the synthesis.[10] However being insoluble in diethyl ether and THF (at low temperatures) limits the use of PEG in reactions requiring these solvents. In addition, the solubility of PEG in water further complicates the removal of inorganic materials and by-products during product isolation.[23] The Instability of the PEG backbone to strong Lewis acids also restricts its use in reactions requiring these reagents.[18]

Several PEG derivatives are used in synthetic chemistry. Depending on polymerisation conditions, PEG termini may consist of hydroxyl groups or may be selectively functionalised. A bifunctional PEG posses hydroxyl groups at both ends, and PEG possessing a methoxy group at one end and a hydroxyl group at the other is considered monofunctional as typically the methoxy group of MeO-PEG remains unchanged throughout chemical manipulation.[10]

The solubilizing power of PEG allows homogeneous reactions under numerous reaction conditions as well as allowing the individual reaction steps to be monitored without requiring cleavage of the product from the polymer support. The characterization of the PEG-bound organic moieties is often straightforward as the polymer does not interfere with spectroscopic or chemical methods of analysis. PEG has sp^3 hybridized bonds and its very flexible and can compact together and gives a very simple and sharp signal in ^1H NMR spectroscopy. Monitoring reactions by ^1H NMR is easily done as the ethyl protons of the PEG backbone give a single peak at δ=3.64 ppm, which can also be used as an internal standard,[10], as demonstrated in **Figure 5** below. As it can be seen that most of the ^{1}H NMR spectrum is a clear 'window', where product spectra can be observed. (MeO-PEG contains an additional methoxy group at δ=3.38ppm).

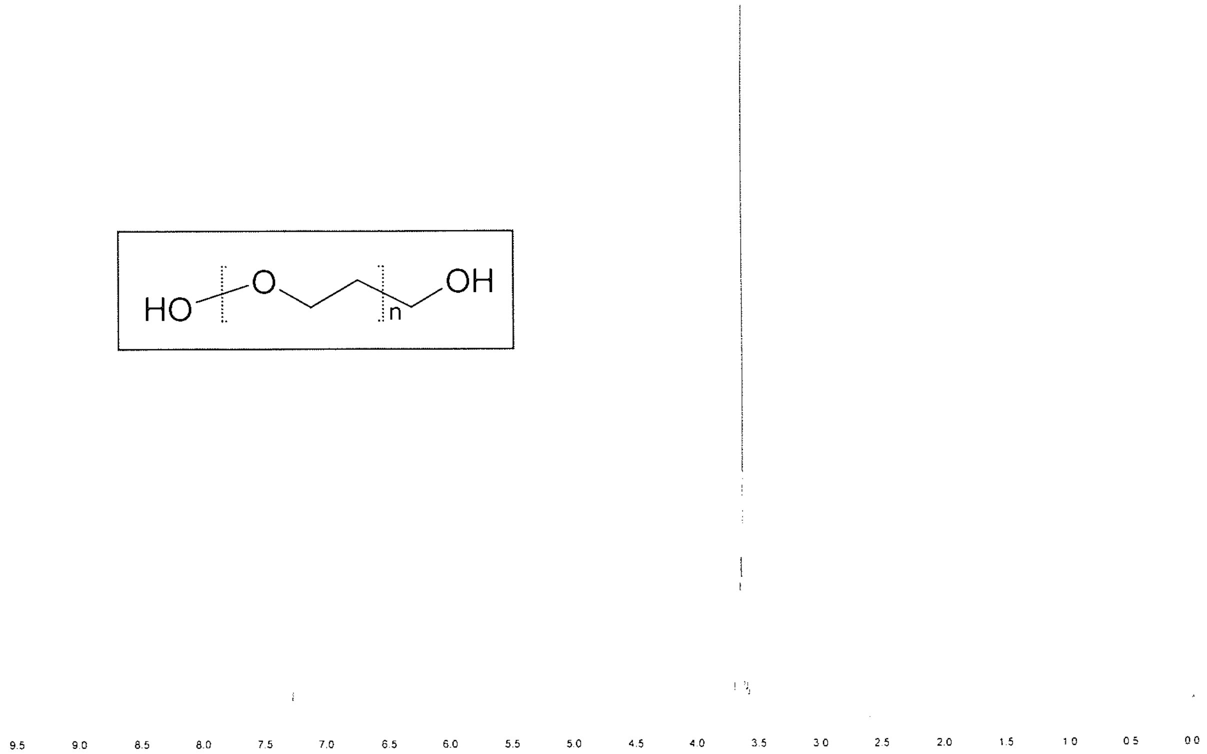
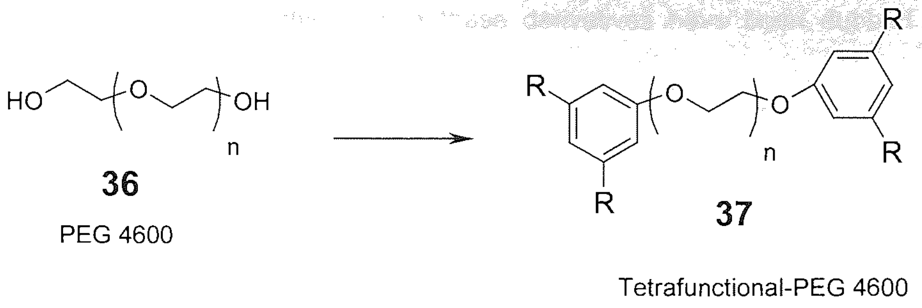


Figure 5: **The ^{1}H NMR spectrum of PEG-OH 4000 in $CDCl_3$.**

1.4.3: Optimisation of loading capacity of PEG:

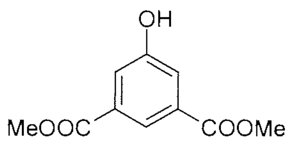
One of the main criticisms levelled at soluble polymer supports for organic synthesis is their low loading. The limiting factor when developing high-loading supports is the compromise between loading and solubility profile.[18] PEG has proven to be a versatile soluble polymeric support for organic synthesis, though the use of PEG has been limited by it relatively low loading. Cozzi and coworkers[24] linked dendrimer and PEG chemistry to produce new soluble PEG-supports with expanded functional group capacity. A dihydroxy-PEG 4600 core **(36)** (0.43 mmol/g) was functionalised as a *m*-dicarboxyphenyl derivative which yielded a tetrahydroxyaryl-PEG 4600 **(37c)** with a loading capacity of 0.86 mmol/g **(Figure 6)**.To demonstrate the possibility of performing some synthetic chemistry, this high loading PEG-derivative was utilized in a synthetic scheme to generate β-lactams. All of the intermediates were purified by precipitation into diethyl ether with excellent polymer recovery. This suggests that the solubility profile of PEG-4600 was not modified by the higher terminal substitution.[18,24]



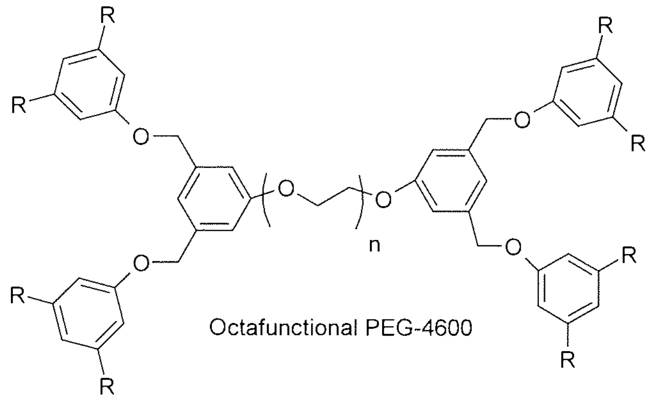
R= COOMe **(37a)**
COOH **(37b)**
CH_2OH **(37c)**
CHO **(37d)**
CH_2Cl **(37e)**

Figure 6: Tetrafunctional PEG-4600 with increased loading capacity.[18]

The possibility of further expanding the loading capacity of these new PEG materials was investigated by Cozzi *et al.*[24] Tetrachloride PEG-4600 (e) was reacted with dimethyl 5-hydroxyisophalate **(38)** in the presence of Cs_2CO_3, to afford the octaester **(39)** in 71% yield. Reduction of **(39)** with an excess of DIBALH gave the octaol **(40)** in 65% yield **(Figure 7)**.



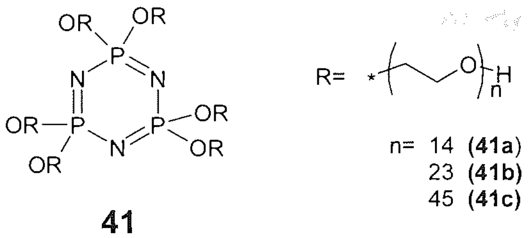
38



R= COOMe **(39)**
R= CH_2OH **(40)**

Figure 7: Octafunctional PEG-4600 with further increased loading capacity.[24]

In another literature report,[25] to improve the loading of PEG-based polymers while maintaining their solubility profile, Janda *et al.*[18] explored the development of PEG-star polymers containing a cyclotriphosphazene core **(Figure 8)**, and produced a PEG with loading capacity of 1 mmol/g. The cyclotriphosphazene core is invisible to ^1H NMR and so does not complicate the analysis of any polymer-bound intermediates. For this reason these derivatives have been dubbed 'stealth star' polymers **(41)**.[18,25]


R=

n= 14 **(41a)**
23 **(41b)**
45 **(41c)**

41

Figure 8: Stealth star PEG-polymers (41a-c).[18]

1.4.4: Peptide synthesis on PEG:

One of the first examples of PEG used as a soluble polymeric support was in peptide synthesis. A large number of peptides and peptide libraries has been synthesized using different molecular weight PEGs and PEG derivatives. Only a few examples of PEG utilization as soluble polymeric support for peptide synthesis will be mentioned here as the aim of this project was not the synthesis of peptides.[10,19]

A library of 1,024 pentapeptides was synthesized by Janda *et al.*[10] on PEG 10,000. Linkage was made by esterification of the first amino acid to PEG mediated by 1,3-dicyclohexylcarbodiimide (DCC) **(Figure 9)**. Peptide coupling yields, determined by ninhydrin and dansyl methods, were greater than 99%. PEG-peptide purification was achieved by simple filtration after addition of greater than 5-fold excess diethyl ether to PEG solutions in DCM. The cleaved peptides were screened for ligands to an antibody to β-endorphin.[10,19]

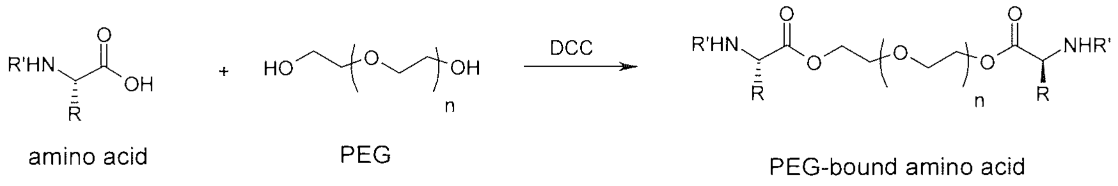


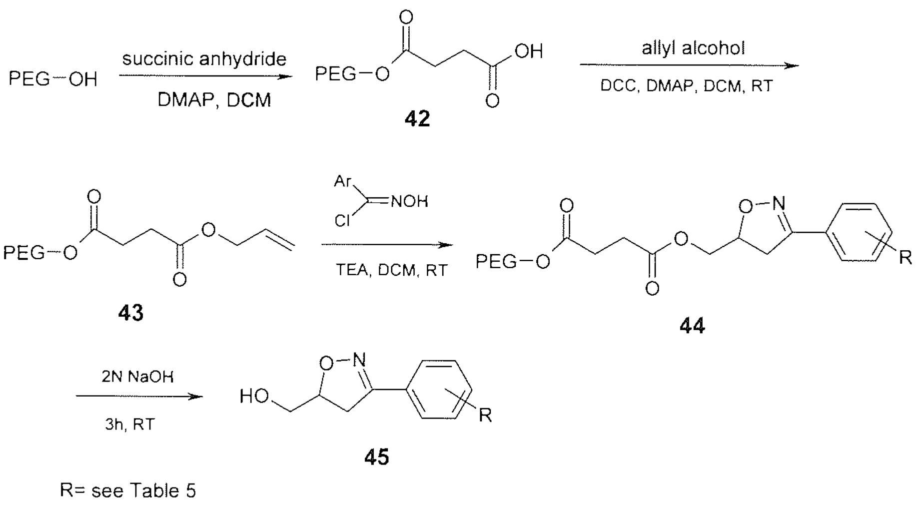
Figure 9: General procedure adopted by Janda *et al*, for direct esterification of PEG with amino acids. Adapted from Janda *et al.*[10]

Optimisation of soluble polymer-supported peptide synthesis succeeded in the stepwise synthesis of a 14-mer on PEG 6000. A 33% yield of analytically pure peptide was reported. It has been reported[10] that although increasing the molecular weight of PEG may help keep longer single-chain peptides in solution, the solubilizing power of PEG may not be strong enough to prevent aggregation of peptide chains. Another drawback of larger molecular weight PEG would be the decreased loading capacity, especially where MeO-PEG-OH is used, as it has been mentioned earlier (section 1.4.2, page 35) that the MeO-PEG remains unchanged during synthesis.

Depending on the molecular weight of PEG used as soluble polymeric supports, the reaction may need to be switched between solvents in order to maintain the solubility profiles of PEG. For example, PEG 3000 was utilized in stepwise synthesis of a peptide corresponding to the insulin segment B 13-20. The use of this relatively low molecular weight PEG required the changing of solvent from DCM to DMF during the peptide elongation to maintain homogeneity[10].

1.4.5: Δ^2-Isoxazolines Synthesis on PEG:

Liquid-phase synthesis of isoxazolines and related heterocycles *via* 1,3-DC reactions, (namely isoxazoles, oxazoles) is well documented. There are a few literature reports on the synthesis of these compounds on solid-phase. However, the synthesis of isoxazolines by liquid-phase organic synthesis has been reported by only one group, Wang *et al.*[26] Six isoxazolines compounds were prepared on PEG 4000, from the resin-bound alkenes **(43)** and corresponding aldoximes as shown in **Scheme 4**. In order to achieve the target compounds, aldoxime was added to a solution of *N*-chlorosuccinimide, in anhydrous DCM. After the chlorination was over, the polymer-supported alkenes were added in one portion. The reaction mixture was stirred at RT for about 30 min, and then TEA was added drop-wise and mixture was stirred overnight at RT. A five fold excess of anhydrous benzene was added to remove the triethylamine hydrochloride formed. The mixture was filtered and the filtrate was concentrated. Addition of diethyl ether to the residue precipitated the PEG, which was then collected by filtration and washed with diethyl ether to afford the PEG-supported isoxazolines **(44)**. The cleavage of the polymer-supported compounds was accomplished by treating the polymer with 2M sodium hydroxide (NaOH). Δ^2-Isoxazolines compounds **(45)** were purified by chromatography on a silica gel column. See **Table 5** for cleaved compounds, and the yields and purity obtained. Purity of the compounds was based on GC-MS analysis of cleaved samples.



Scheme 4: **PEG-bound synthesis of Δ^2-isoxazolines.**

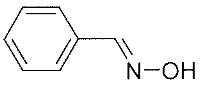
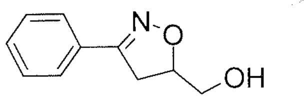
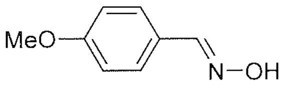
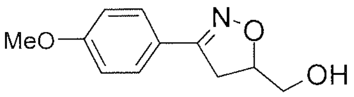
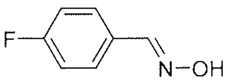
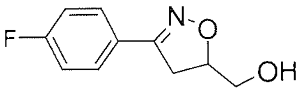
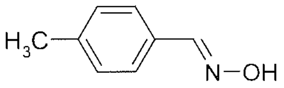
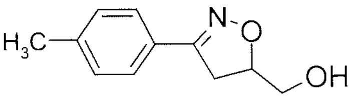
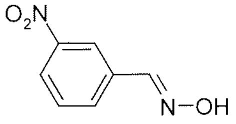
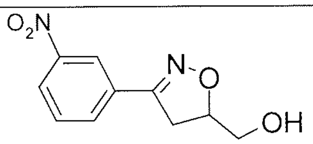
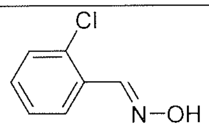
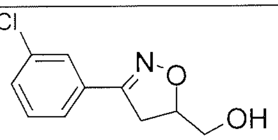
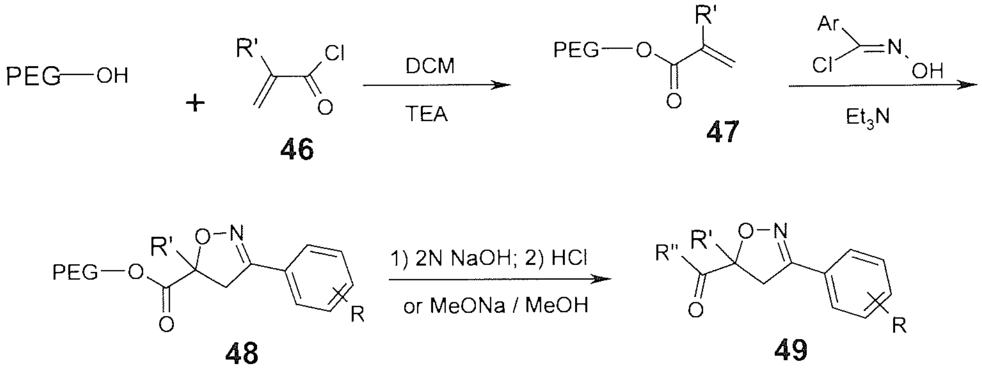
Compounds	Aldoxime	Isoxazoline	Yield (%)	Purity (%)
45a			88	99
45b			96	100
45c			86	98
45d			93	100
45e			57	95
45f			91	99

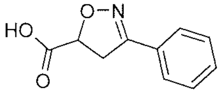
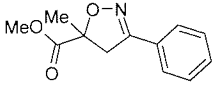
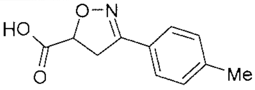
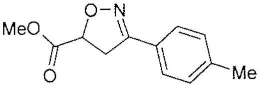
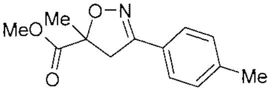
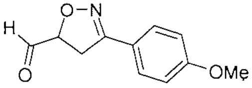
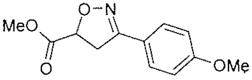
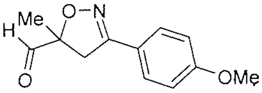
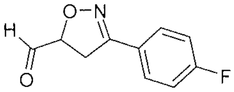
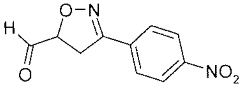
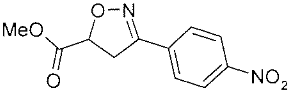
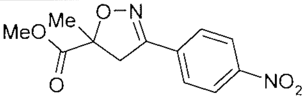
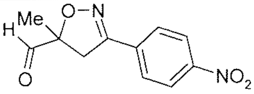
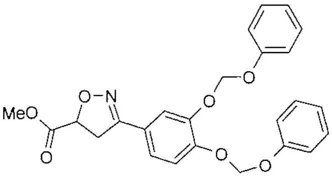
Table 5: **Δ^2-Isoxazolines compounds synthesized on PEG 4000 by Wang *et al.*[26]**

In a more recent report, Wang *et al.*[27] again reported the synthesis of isoxazoline compounds using PEG-4000-supported acrylate. Several isoxazolines (**Table 6**) were prepared by trapping *in situ* nitrile oxides, generated from corresponding aldoximes, with the PEG-supported acrylates. (**Scheme 5**). Good yields (85-95%) and purity greater than 95%, were reported. In each case only one isomer was obtained. In a similar procedure, a number of isoxazoline compounds (**Table 6**) were prepared from resin-bound methyacrylate and corresponding aldoximes (**Scheme 5**). Again satisfactory yields (68-98%) and purity (greater than 95%) were reported.



R = H, Me, OMe, F, NO_2
R' = H, Me
R''= H, OH, OMe

Scheme 5: **Synthesis of isoxazoline compounds on PEG-supported acrylate and methacrylate. Adapted from Wang *et al.*[27]**

Compounds	Isoxazoline compound	Yield (%)	Purity(%)
49a	 (i)	90	97
	 (ii)	95	99
49b	 (i)	93	99
	 (ii)	95	96
	 (iii)	98	98
49c	 (i)	94	95
	 (ii)	95	96
	 (iii)	96	100
49d		90	98
49e	 (i)	85	95
	 (ii)	95	95
	 (iii)	72	100
	 (iv)	67	99
49f	 (i)	92	98

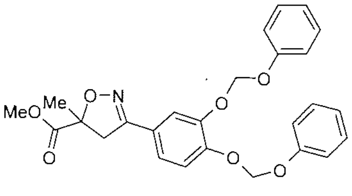
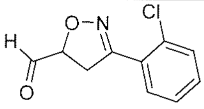
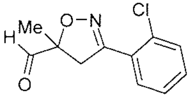
	 (ii)	68	95
49g	 (i)	86	95
	 (ii)	82	99

Table 6: Isoxazoline compounds synthesized on soluble polymeric support. Adapted from Wang *et al.*[27]

1.5: Polystyrene as soluble polymeric support:

Introduction of solid-phase synthesis methodology by Merrifield gained tremendous attention and interest from the synthetic chemists, but the excitement was soon over. Soon it became obvious that along its advantages, this method of synthesis was far from perfect and had its shortcomings, (Section 1.3, page 24). Some laboratories sought out alternative methods where the advantages of both traditional solution chemistry and the solid-phase chemistry could be combined. Several synthetic chemists investigated the use of soluble polymeric supports.

A soluble non-cross linked copolymer of chloromethylated polystyrene was the first reported soluble polymeric support used as a scaffold in synthetic chemistry. It is essentially a soluble form of Merrifield's polymer (resin). Earlier examples of soluble polystyrene used as a synthetic support, included the synthesis of polypeptides and oligonucleotides only.[28,29,30]

Linear polystyrene (LPS) is simple to prepare with a wide variety of functional groups and is compatible with a broad spectrum of chemistries.[31] The functional group content can be easily controlled by using varying ratios of starting monomers and then quantified by NMR. Non-cross linked polystyrene has soluble properties that are amenable to organic chemistry. It is soluble in tetrahydrofuran (THF), DCM, chloroform and ethyl acetate even at low temperatures (-78 °C) and is insoluble in water and methanol.[32] These features allow implementation of solvent extraction techniques used in traditional organic synthesis in conjunction with the polymer crystallization techniques currently used in the PEG liquid-phase approach.[10]

LPS has a non-polar hydrophobic nature and the polymer-bound products can be diluted with DCM and aqueous extractions can be carried out on the organic layer. Methanol is used to precipitate the polymer and polymer-bound products to remove excess reactants and by-products. LPS has an advantage over PEG here, because the insolubility of PEG in THF at low temperature and its potential to complex metal cations preclude its use as a support for modern synthesis where the use of organic and organometallic compounds is necessary. In contrast LPS being insoluble in water and methanol is the ideal polymeric support for such chemistry. The analysis of polymer-bound intermediates and products can be carried out by the routine analytical methods especially NMR in a non-destructive manner without the need for any specialized NMR techniques.[10,32] The ^{1}H NMR spectrum of linear polystyrene takes up more space in contrast to the ^{1}H NMR spectrum of PEG, **(Figure 10)** as proton signals both from the aromatic protons as well as polystyrene backbone are observed. However the reaction analysis can easily be done, since most of the region in the spectrum (δ2.5 – 6.5 ppm) is clear.

An additional advantage of LPS is that the loading level of polymer is not limited to twice the inverse of its molecular weight, as it is with PEG. The linear nature of PEG dictates that a maximum of two molecules can be attached to each polymer molecule, one at each terminus. Therefore a compromise has to be reached between the loading level and the molecular weight of PEG. For example, the maximum achievable loading level of PEG 2000 is 1 mmol/g. The loading level of LPS can be adjusted as desired by changing the ratio of monomers used in the polymerisation reaction and can approach the theoretical maximum of un-functionalized polystyrene (9.6 mmol/g). Chloromethylated polystyrene can give the theoretical maximum loading of 6.6 mmol/g. However, a limitation to the issue of loading is that, at a point when loading is too high or the molecule attached are too large, the polymer begins to exhibit the solubility properties of the attached molecules and may not precipitate properly. Furthermore, if there are many molecules attached closely on the polymer, they may interact with one another in undesirable ways.[31]

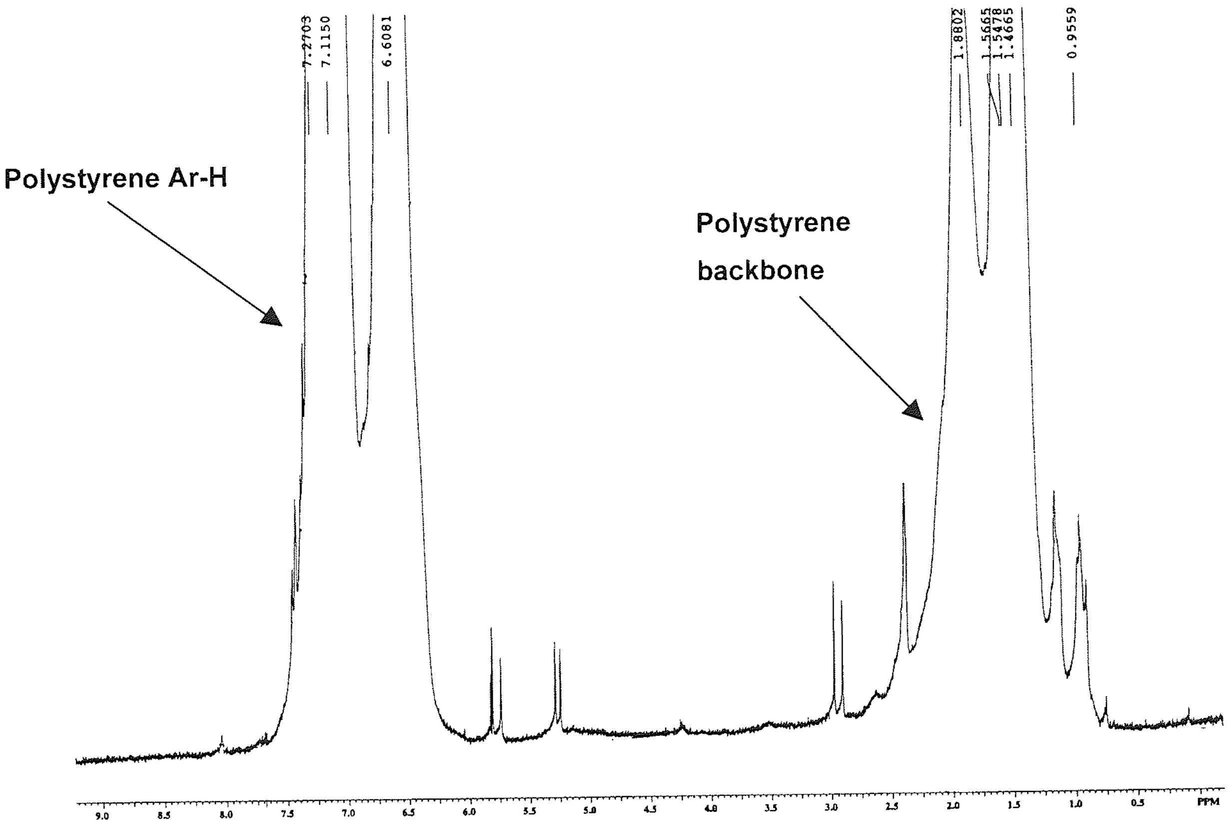
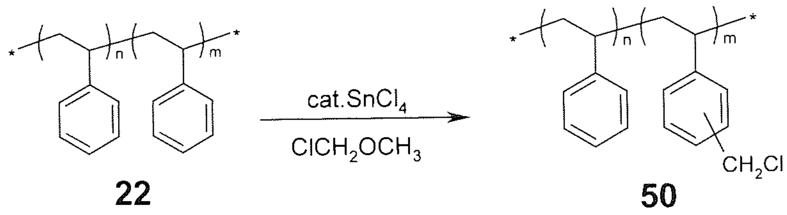


Figure 10: **The ^1H NMR spectrum of linear polystyrene in deuterated chloroform ($CDCl_3$).**

1.5.1: Early examples of linear polystyrene used as a synthetic support:

1.5.1.1: Peptide synthesis on linear polystyrene:

In 1965 Shemyakin *et al.*[28] synthesized a tetrapeptide on soluble linear polystyrene. Non-cross linked polystyrene of 200,000 molecular weight served as a soluble polymeric support for the synthesis of glycylglycyl-L-leucylglycine (Gly-Gly-Leu-Gly) with an overall yield of 65%. Linkage sites were made by chloromethylation of the polymer (**Scheme 6**)[10], achieving a loading capacity of 0.91 mmol of glycine/g. Chloromethylated polystyrene **(50)** was achieved by the treatment of the polymer **(22)** with monochlorodimethyl ether in the presence of tin chloride. The peptide chain was built up with the aid of *N*-hydroxysuccinimide esters of *tert*-butyloxycarbonylamino acids. Homogeneous reactions were carried out in dioxane or DMF and the polymer was precipitated by addition of water. The peptide was cleaved from the polymer by means of HBr in trifluoroacetic acid.[28] Water-induced precipitation of polystyrene resulted in some co-precipitation of reagents. Phase separation of the polymer during coupling reactions was observed after several synthetic steps due to the cross-linking of chloromethyl groups that remained after incomplete esterification of the first amino acid to the polymer support.[31] Synthesis of the hexapeptide Val-Tyr-Val-His-Pro-Phe was achieved in overall 61% after the optimisation of reagents and reaction conditions. However, as the peptide was elongated, incomplete precipitation from dioxane by addition of water resulted in increased lose of polymer peptide.[10] In an attempt to increase the loading capacity of linear polystyrene, Maher *et al.*[33] extensively chloromethylated linear polystyrene to provide a polymer support, where 62.2% of the phenyl groups were chloromethylated, and after reacting with protected glycine, 1.49 mmol of glycine/g was obtained. The resulting glycine-terminated polymer was insoluble in DMF and chloroform and subsequent peptide synthesis was carried out under heterogeneous conditions.



Scheme 6: Chloromethylation of linear polystyrene. Adapted from Janda *at el.*[10]

Several groups, between 1978 and 1980, reported the synthesis of chloromethylated polystyrene (**Figure 11**) for liquid phase peptide synthesis by copolymerising a 9:1 ratio of styrene **(51)** and chloromethylstyrene (vinylbenzyl chloride) **(52)** (7:3 *m* and *p*-isomers respectively). In contrast to functionalising polystyrene with chloromethyl groups, the copolymerisation resulted in virtually no cross-linking. Reactions were performed in DMF with methanol induced polymer precipitation gave >93% recovered polymer. It was also reported that in peptide synthesis, as the loading capacity was increased, reduced yields were obtained at each coupling step due to decreased solubility of the peptide-bound support.

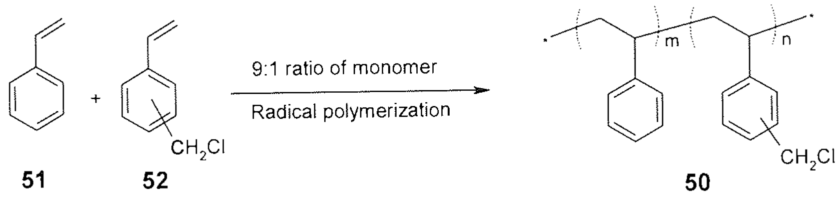
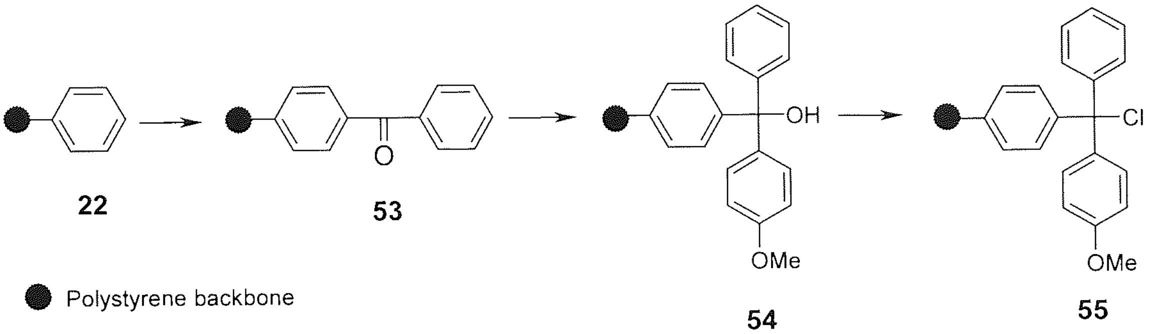


Figure 11: Chloromethylated polystyrene obtained by copolymerisation of styrene and vinyl benzyl chloride. Adapted from Janda *et al.*[10]

1.5.1.2: Oligonucleotide Synthesis on Linear Polystyrene:

In an attempt to extend the techniques developed in the early phases of liquid-phase peptide synthesis, soluble linear polystyrene was used as the support for oligonucleotide synthesis in pyridine. In 1966, two groups Carmer *et al.* [29]and Khorana *et al.*[30] independently, reported the synthesis of a trinucleotide (thymidylyl-(3'→ 5')-thymidylyl-(3'→5')-thymidine) on linear polystyrene of molecular weight of 170,000 dervatized to contain about 20% *p*-monomethoxytrityl chloride groups **(Figure 12)**. To obtain the trityl linker, polystyrene was first subjected to a Friedel-Crafts reaction with benzoyl chloride and aluminium chloride to give **(53)**. The product **(53)** was then reacted with *p*-methoxyphenylmagnesium bromide and substituted trityl alcohol **(54)** was obtained. Treatment of **(54)** with acetyl chloride gave a soluble polymer with loading capacity of 0.4mmol/g of *p*-monomethoxytrityl chloride polystyrene **(55)**.[29] The trinucleotide was cleaved from the polymer with a mixture of TFA in dioxane followed by chromatographic purification in a yield of 11%.

In another literature report, an attempt to synthesise a pentanucleotide was not very productive as the polystyrene supported oligonucleotides had significant solubility in water that prevented significant precipitation of polymer from pyridine.[34] As it is seen in liquid phase synthesis, polystyrene was abandoned to be replaced by more hydrophilic polymer supports for oligonucleotide synthesis.[10]



Trityl-functionalized polystyrene

Figure 12: **Trityl-functionalized polystyrene. Adapted from Cramer et al.**[29]

Other examples of the utilization of soluble polystyrene supports in the initial phase of liquid phase organic synthesis includes the synthesis of oligosaccharides.[10,35] As seen in other methodologies employing polystyrene, recovered yields were anticipated to decrease as the oligosaccharide elongates due to increased solubility of the sugar-polymer conjugate in polar solvents.[10]

Due to the progressively decreasing yields obtained for longer peptides and other compounds that play essential roles in biological processes, the use of linear polystyrene as soluble polymeric supports was abandoned.

1.5.2: More recent examples of linear polystyrene as soluble support:

In the early days of liquid-phase organic synthesis, the use of polystyrene was explored for the synthesis of peptides and other biologically important compounds only. In late 1990, a group of chemists, led by Kim. D. Janda, investigated liquid phase organic synthesis and explored the utility of PEG and linear polystyrene as soluble polymeric supports. A small library of prostanoids was synthesized on non-cross linked polystyrene support.

1.5.2.1: Synthesis of prostanoids on soluble polystyrene support:

Prostaglandins constitute one of the most physiologically potent families of non-protein molecules found in mammals. These natural products play an important role in the processes of inflammation, tissue repair and in the immunoresponse.[32,36]

A small library of five and six membered ring prostanoids, prostaglandin E_2 methyl ester and prostaglandin $F_{2\alpha}$ was synthesized on linear polystyrene support by Janda *et al.*[20,31,32,36] The non-cross linked polystyrene support **(22)** as prepared by copolymerising styrene with 4-vinylbenzoyl chloride. Polymer-bound (*R*)-(+)-4-Hydroxy-2-cyclopentene-1-one **(56)** **(Figure 13)** was used as a precursor for the synthesis of prostanoids library and prostaglandins. The soluble support was able to resist the aggressive cleavage conditions needed to liberate the product. 48% Aqueous HF-THF at 45 °C, was used for the efficient liberation of the product from soluble support. The prostanoids isolated, after the chromatographic methods, were in the range of 19-24% overall yield.

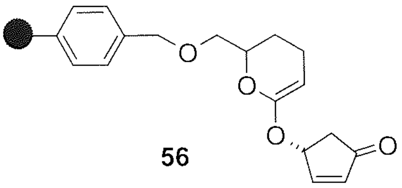

56

Figure 13: Polymer-bound (*R*)-(+)-4-Hydroxy-2-cyclopentene-1-one.

1.5.2.2: Synthesis of prostaglandin E_2 methyl ester on soluble polystyrene support:

A prostaglandin E_2 methyl ester **(63)** was successfully prepared in a six step synthesis, using **(56)** as a precursor **(Scheme 7)**. A copolymer with a loading value of 0.3mmol/g was used in the synthesis. It was observed that the polymer mass balance for each step in the synthesis was ≥ 97%, and only a single polymer-bound species was detected by NMR in all cases. The cleavage of PGE_2 methyl ester from the soluble support was afforded with 48% aqueous HF-THF at 45 °C for 6 hours, also used for the removal of the TBS protecting group in one step. HF was removed by washing the reaction medium with saturated $NaHCO_3$ solution. After flash chromatography, an overall yield of 37% was obtained.[32]

1.5.2.3: Synthesis of prostaglandin $F_2\alpha$ on soluble polystyrene support:

Total synthesis of naturally occurring prostaglandin $F_{2\alpha}$ **(64)** was achieved in eight chemical steps synthesis from precursor **(56)**. A procedure similar to the synthesis of prostaglandin E_2 methyl ester **(63)** was followed. **(Scheme 7)**. Following cleavage from the support the product was purified by flash chromatography. Approximately 80% of the polymer was recovered after seven chemical steps. The overall yield obtained was about 30% after eight chemical steps on the polymer support. **Figure 14** shows the ^1H NMR spectrum of the polymer-bound prostaglandin $F_{2\alpha}$. As mentioned previously (section 1.5, page 43), despite the broad peaks of polymer backbone taking up some of the spectral area, it is still possible to analyse the product by NMR spectroscopy.

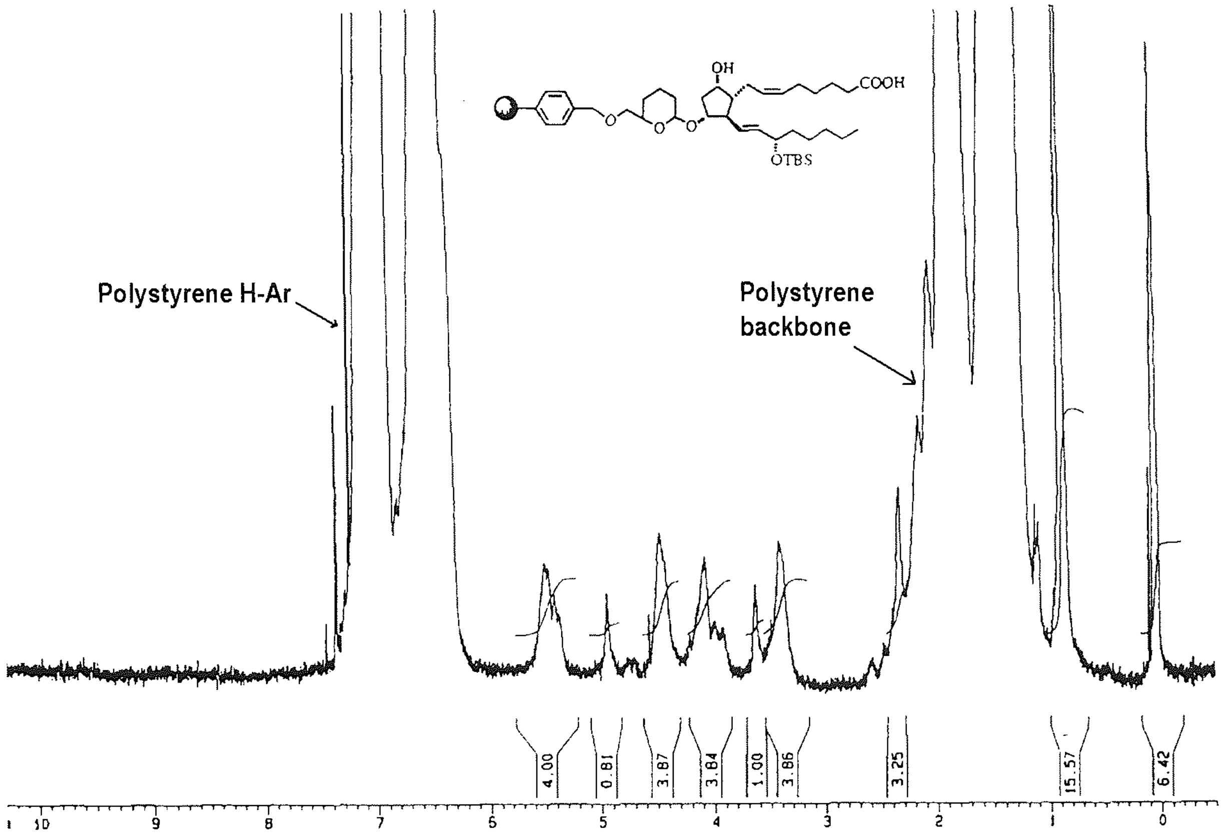
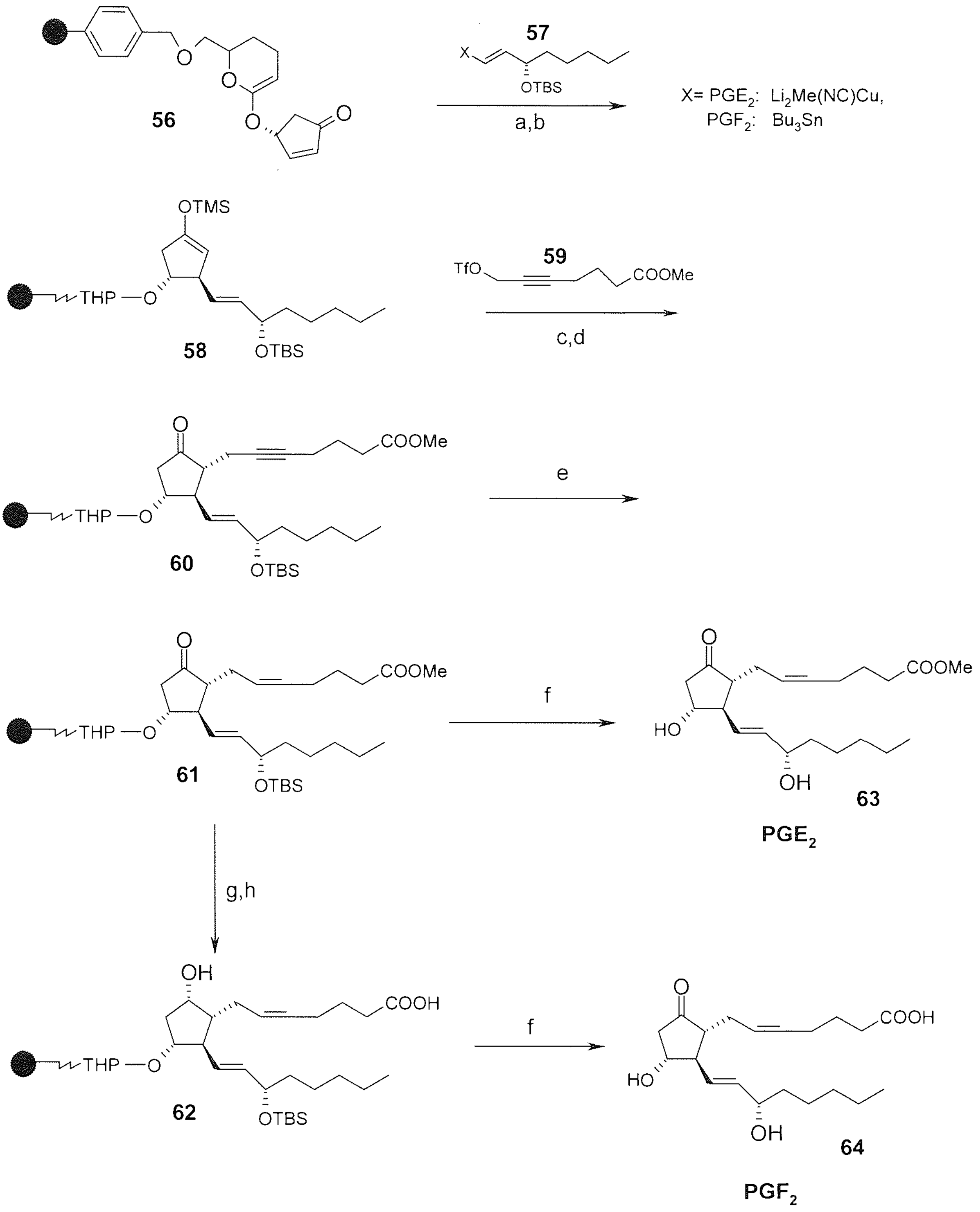


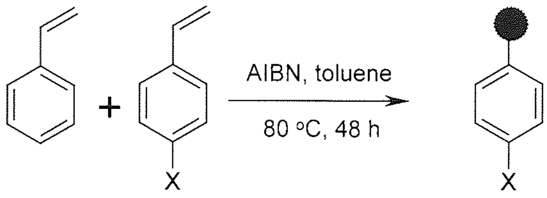
Figure 14: ^1H NMR spectrum of polymer bound Prostaglandin $F_{2\alpha}$. Adapted from Janda *et al.*[20]



Scheme 7: Polymer supported synthesis of Prostaglandin E_2 methyl ester and Prostaglandin $F_{2\alpha}$. Adapted from Janda *et al.* Conditions: (a), THF, –78 °C, 15 min; (b) (*i*) TMSCl,–78 °C, 30 min, (ii) TEA 0 °C, 15 min; (c) MeLi, THF –23 °C, 20 min; (d) – 78 °C, 10 min then –23 °C, 6 h;(e) H_2, 5% Pd-$BaSO_4$, quinoline, benzene/cyclohexane (1:1), RT, 48 h; (f) 48% aqueous HF/THF (3:20, v/v), 45 °C, 6h; (g) L-Selectride, THF, –78 °C, 1h; (h) 3N LiOH, THF, MeOH, rt, 48 h. [20,31,32]

1.5.3: Soluble polystyrene supports with various functional groups for LPOS:

Several copolymers of styrene have been reported and most of the work was focused on the modification of the polystyrene bearing a chloromethyl substituent. Several copolymers of styrene with various monomers were prepared by Malagu *et al.*[37]have been reported. These copolymers were prepared using the standard procedure shown in **Scheme 8** and reported by Janda *et al.* The radical copolymerisation of monomers with substituted styrene, even in the presence of other unsaturations was successful. The yields obtained were in the range of 20 to 55%. The results are shown in **Table 7.**



X=See Table 7 below

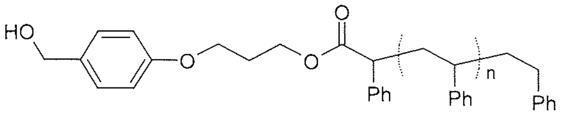
Scheme 8: Preparation of Copolymers of styrene with various monomers. Adapted from Malagu *et al.* [37]

X=	Loading (mmol/g)	Yield(%)
CH_2Cl	0.32	52
CH_2OH	0.24	52
CH_2N_3	0.18	40
CH_2NH_2	0.30	37
$CH_2O\textit{p}C_6H_4CH_2OH$	0.28	25
$(CH_2)_2CH{=}CH_2$	0.50	44
$=CO_2H$	0.37	35
$SO_3{}^{i}Pr$	1.1	20
$SO_2N(H)CH_2CH{=}CH_2$	0.32	55
$SO_2N(CH_2CHCH_2)\text{-}CH_2C{=}CH)$	0.32	49

Table 7: Loading and yield obtained for various copolymers of styrene. Adapted from Malagu *et al.* [37]

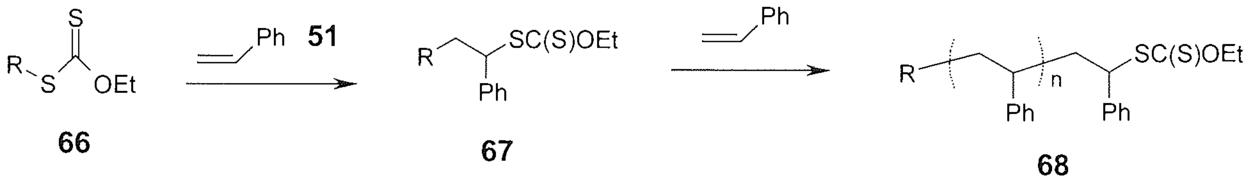
1.5.4: Other polystyrene based soluble polymeric supports:

As mentioned previously, most of the work on soluble copolymers of polystyrene involved either chloromethylation of the styrene aromatic ring, or, copolymerization of a derivatised styrene monomer with styrene. A new type of polystyrene soluble copolymer **(65)** was reported by Dublanchet *et al.*[38] which could be compared to PEG in the sense that the linker is at the terminal of the polymer. By functionality it could be regarded as an analogue of Wang resin.



65

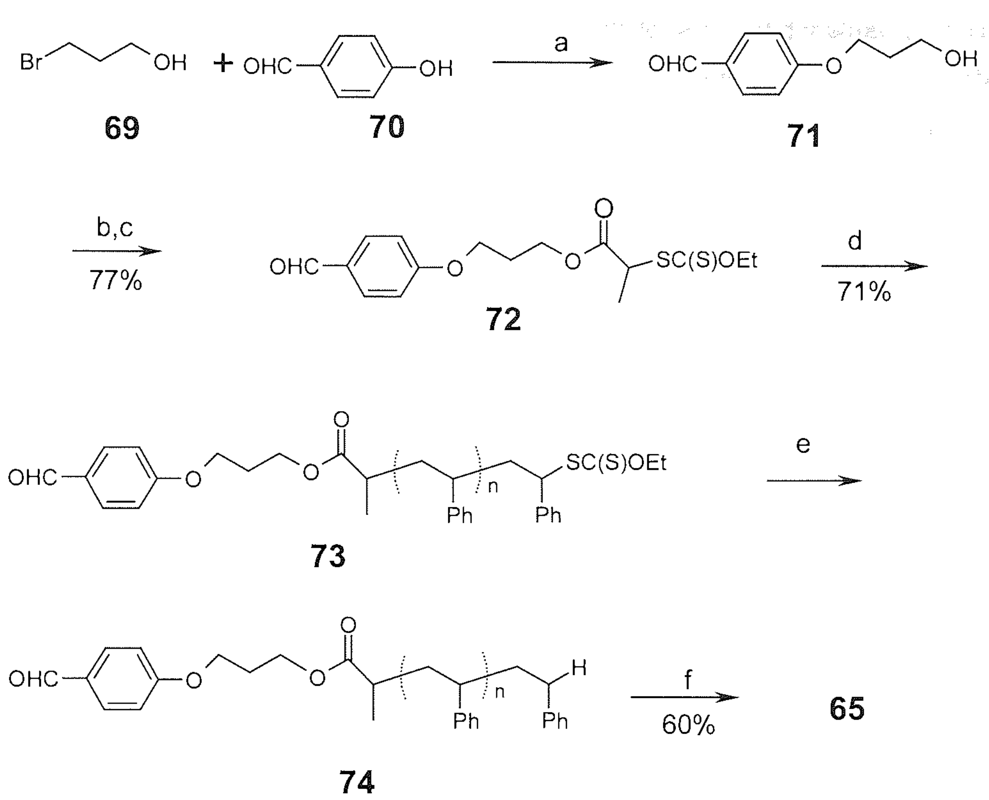
This copolymer was prepared by reacting together a xanthate moiety and polystyrene in the presence of a radical initiator. In a general procedure, heating a mixture of xanthate **(66)** and styrene **(51)** in toluene in the presence of radical initiator gives an adduct **(67)**. The adduct itself a xanthate, can act as a starting point for addition of another styrene molecule, resulting in the formation of a polymer **(68)** **(Scheme 9)**.



R= alkyl, aryl

Scheme 9: **Coplymerization between xanthate and styrene.**

The synthesis of this new polymer began with reaction of *p*-hydroxybenzaldehyde with 3-bromopropanol **(69)** (**Scheme 10**). The resulting alcohol **(70)** was converted into the xanthate *via* a chloroacetate ester derivative. The xanthate was heated with 20 equiv. of styrene in toluene with lauroyl peroxide, followed by concentration under vacuum and precipitation to give the polymeric derivative in 71% yield. Due to the presence of the aldehyde moiety, the polymerisation conditions had to be kept mild. To make the polymer suitable for further utilization in polymer chemistry, the terminal xanthate moiety had to be cleanly removed. The complete elimination of the xanthate moiety was achieved by means of tributyltin hydride and the target polymer was achieved. The reduction of aldehyde afforded the new soluble polymer containing the Wang linker with the loading of 0.24 mmol/g as judged by ^1H NMR spectroscopy.[38]



Scheme 10: Synthesis of soluble copolymer of styrene with xanthate. Conditions; (a) K_2CO_3, DMF, reflux 8h, 82%; (b) DCM, 2-chloropropionyl chloride, pyridine, RT, 2h; (c) KSC(S)Oet, acetone, RT, 2h, 77%; (d) styrene 20equiv, toluene, 90 °C, 13 mol % lauroyl peroxide, 71%; (e) Bu_3SnH, AIBN, C_6H_6, reflux, 30 min; (f) $NaBH_4$, THF, EtOH, RT 1h, 60%from 73.

1.5.5: Soluble polymeric supports from polystyrene and co-monomer allyl methacrylate:

Free-radical homopolymerizations of allylic monomers, general structure $CH_2{=}CR{-}CH_2{-}X$, are well known to proceed at low rates and to yield polymers of low molecular mass. It is believed that "autoinhibition" arises from hydrogen atom abstraction from the allylic methylene (monomer) followed by loss of the resulting allylic radicals *via* radical coupling reaction[39]. Autoinhibition limits the achievable degrees of polymerisation therefore restricting the preparation of useful linear homopolymers from allylic monomers. Despite this restriction, monomers containing allylic C=C bonds are the basis of certain thermosetting resins and are often used at low levels in free-radical copolymerization[39]. Bifunctional vinyl monomers with two double bonds of identical reactivity such as divinyl benzene, ethylene glycol dimethacrylate (EDMA) or diethylene glycol dimethacrylate (DegDMA) are mainly used as cross-linking agents. Allyl methacrylate (AMA), though frequently used as a cross-linker, belongs to this class of monomers since it contains both methacrylic and an allylic double bond.[40,41]

AMA has been widely used as an alternative cross-linker to ethylene glycol dimethacrylate (EDMA) in the preparation of coatings, resin foams and materials of high water absorption.[40,42] AMA is an unsymmetrical difunctional monomer.[43] It contains two types of vinyl groups, methacryloyl and allyl both having quite different reactivities.[43] The copolymerisation involving bifunctional monomers such as AMA and EDMA are really terpolymerizations allowing, in principle, additional reactions of the pendent double bonds, (e.g. crosslinking, cyclization) not consumed in straight chain propagation.[42]

However there are reports in the literature[44] on obtaining soluble linear polymers employing the AMA as monomer. Such linear polymers are made by either group transfer polymerisation (GTP), or free radical polymerization. In free radical polymerisation, AMA was polymerized in solution in tetrachloromethane (CCl_4), toluene, dioxane, benzene, acetone or diallyl ether, with benzoyl peroxide initiator. It is reported that in CCl_4 solution, at low concentrations, conversions of up to 40% soluble polymer were obtained before gelation occurred. In dioxane solutions, at concentrations of up to 2.2 mol/L monomer, high yield of soluble polymer were obtained. In diallyl ether solution at very low monomer concentrations, almost 100% soluble polymer was obtained. In general, it was found that as the concentration of the monomer was increased the gelation occurred at lower conversions. It is also reported that the elemental analyses of the polymers of allyl methacrylate formed in dioxane and diallyl ether solutions confirmed that little or no copolymerisation had occurred and that the allyl methacrylate had formed homopolymers.[44]

A soluble copolymer of allyl methacrylate styrene was prepared in a free radical polymerization using AIBN as radical initiator by Mennicken *et al.*[45] To confirm the presence of free alkenes (that it is not cross linked), the copolymer was subjected to bromination. After the bromination, the ^1H NMR spectrum of the copolymer showed no peaks around $\delta 4.5 - 5.8$ (peaks d, and e) confirming that the alkenes are present and the polymer is not cross-linked **(Figure 15)**. Therefore it can be concluded that it is possible to make linear copolymers of allyl methacrylate and styrene, and may have a possible use in synthetic chemistry.

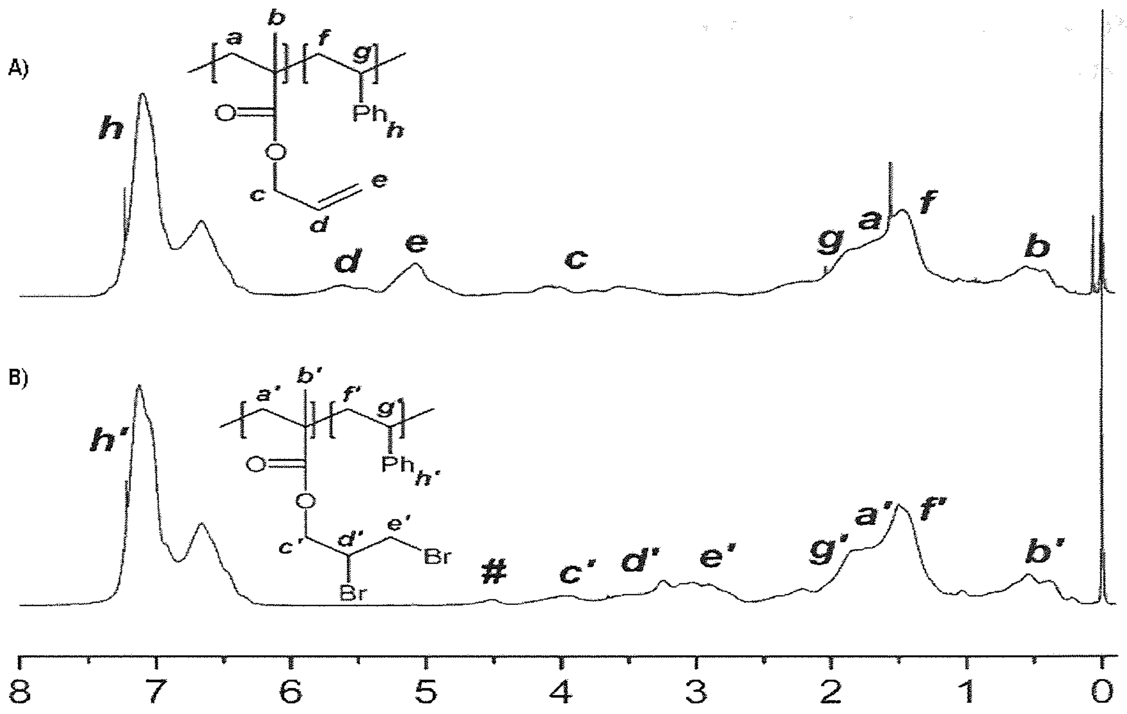


Figure 15: **Poly (styrene-co-allyl-methacrylate) copolymer, prepared commercially. It was subjected to bromination to show the peaks arising from the terminal alkene are indeed free and not cross linked. A) ^{1}H NMR spectrum of copolymer allyl methacrylate and styrene, B) Same copolymer after it was subjected to bromination.**

1.5.6: Analytical methods for characterization of linear polymers:

The characterization of a polymer requires several parameters to be specified. This is because a polymer actually consists of chains of varying lengths, and each chain consists of monomer residues which affect its properties.[46]

A soluble linear polymer may be characterized by routine analytical methods, i.e. UV-visible, IR and NMR spectroscopy can be used to determine the composition of the polymers. Other analytical methods, routinely used for characterization of small organic molecules, such as mass spectroscopy (MS) and thin layer chromatography (TLC) often fail for the characterization of polymer and polymer bound products due to a range of sizes of the polymer chains. Another technique for polymer characterization is gel permeation chromatography (GPC). Also known as size exclusion chromatography (SEC), GPC is a high performance liquid chromatography technique for the separation of components based on their molecular size in solution. It is used to analyze the molecular weight and polydispersity of synthetic polymers, and it is the only proven technique for characterizing the complete molecular weight distribution of a polymer.[46,47]

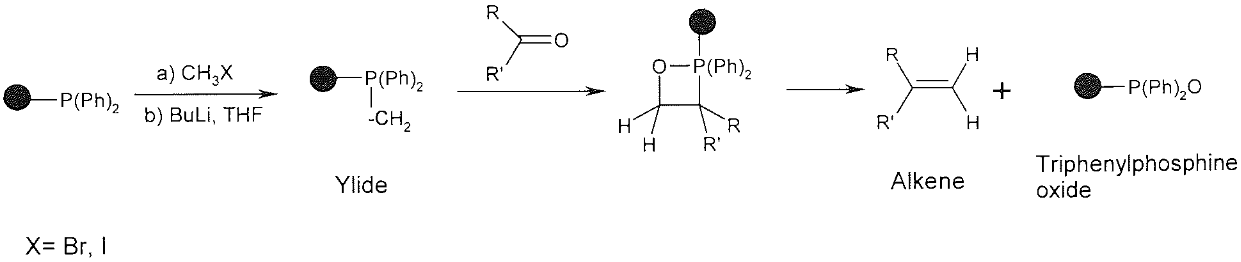
In the GPC approach, there is both a mobile phase and a stationary phase The mobile phase, comprising a solvent that moves past the stationary phase. The stationary phase consists of a column containing finely divided, porous particles such as silica or cross-linked polystyrene. Polymer molecules that are smaller than the pore sizes in the particles can enter the pores, and therefore have a longer path and longer transit time than larger molecules that cannot enter the pores. Thus, the larger molecules elute earlier in the chromatogram, while the smaller molecules elute later. Thermal analysis (TA), a branch of materials science, refers to a collection of analytical techniques which provide information about materials by measuring structural and property changes occurring in response to changes in temperature. However, TA may not be very useful for the analysis of linear polymers, that are explored in this thesis as polymeric supports in organic synthesis, because TA approaches provide physical properties of the solid materials which was not the aim of this thesis. To determine the crystalline structure of polymers, techniques such as X-ray scattering and neutron scattering can also be used.[46,47,48,49,]

1.6: Polymer supported reagents:

For the synthetic organic chemist, solid supported reagents are highly valuable as they reduce or eliminate the need for difficult labour-intensive purification steps.[50] Several names for supported reagents have been used in the literature, including solid-phase scavenging, polymer supported quench (PQS) and complementary molecular reactivity and molecular recognition (CMR/R).[51] The use of solid supported reagents to facilitate purification was first reported by Kaldor *et al.*[52] in 1996, however the ion-exchange resins, which serve similar purpose, have been used for a long time in organic synthesis.[53]

A key strength of solid phase organic synthesis (SPOS) is the ease with which reaction work-up and purification can be conducted. The use of supported reagents can be regarded as a complementary solution phase approach for assisting purification. The concept is similar to SPOS, since, as with SPOS, an excess of one starting material can be utilized to drive a reaction to completion without fear of complicating the isolation and purification of the final product. The supported reagents are easily removed from reaction by filtration and, in theory, the recovered reagents can be recycled which is especially important for expensive reagents, e.g. Pd catalysts. In addition the handling of toxic, explosive and noxious reagents is often much safer when contained on solid support. However, there could be a number of downsides to solid supported reagents including slow and/or poor interaction of some reagents with solid supports, poor stability of supported reagents under harsh conditions and ability to recycle reagents contained on supported reagents is not assured. A major drawback of supported reagents is that they can be very expensive to prepare.[54,55]

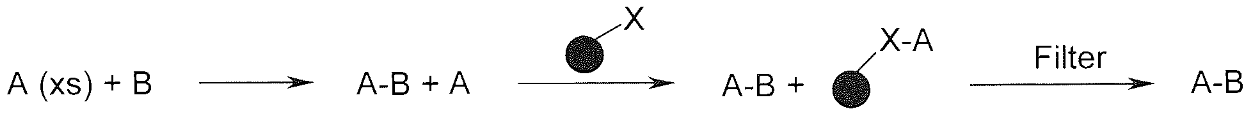
While SPOS and combinatorial synthesis methods provide the principle source of lead prospecting libraries to high-throughput screening in many emerging biotech companies, established pharmaceutical companies often rely on historical collections of drug-like molecules for prospecting. Since the typical lead from historical collection was originally prepared by solution phase method, adapting that synthesis to automation is usually less time consuming than developing an SPOS route, provided that one can simplify purification steps. Short syntheses are more practical in solution than on a solid phase when an efficient purification strategy exists since no resin attachment and cleavage reactions are required. An excellent example of the use of solid supported reagents can be seen in Wittig reaction.[56,57] Witting reaction is one of the most valuable reaction in synthetic chemistry, for it gives regiospecific introduction of carbon-carbon double bond. Removal of the byproduct triphenylphosphine oxide from the alkene products, is often difficult. To facilitate the product purification, a polymer bound triphenylphosphine is used and at the end of the reaction the byproduct triphenylphosphine oxide, which is contained on the polymer support, is simply removed by filtration, leaving behind pure product.[57] A general reaction for the preparation of alkene by Wittig reaction is shown below in **Scheme 11**.



Scheme 11: The use of polymer supported triphenylphosphine in Wittig reaction. Adapted from Ford *et al.*[57]

1.6.1: Scavenger resins:

Scavenger resins are solid-supported reagents and facilitate purification in organic synthesis. The general concept involves adding one or more solid-supported reagents which selectively binds to impurities on the basis of chemical reactivity. Either covalent bond forming reactions or ionic interactions or a mixture thereof serves as an acceptable means of attaching the impurities to the solid supported reagent. A subsequent filtration completes the separation, leaving a solution of desired products(s) of enhanced purity.[52] A general concept of scavenger resins is shown in **Scheme 12** below.



Scheme 12: Scavenging excess reagents in a solution phase synthesis. Adapted from Kaldor *et al.*[52]

The use of scavenger resins is a valuable strategy for product purification in solution phase parallel organic synthesis. For the approach to be effective, the material to be scavenged must contain a functional group not present in the target molecule, which can be utilized as a handle to chemoselectively immobilize the compound to a resin bearing complementary functionality. Both covalent and ionic bonding can be utilized for compound sequestration, although the former is preferred since there is less risk of leaching during resin wash steps. In cases where the desired product is contaminated with only excess starting material, purification using scavenger resins may just involve treatment of the crude mixture with the appropriate resins, filtration and evaporation. Unlike conventional purification methods, scavenger resins can be used for the purification of intentional mixtures of related compounds. Scavenger resins are most frequently used for the removal of electrophiles and neucleophiles. Many supports are designed such that the immobilization reaction does not liberate any soluble by-products or require any additional reagents. Due to site isolation effects, it is possible to use simultaneously scavenger resins which contain incompatible functional groups.[52,54,56,57]

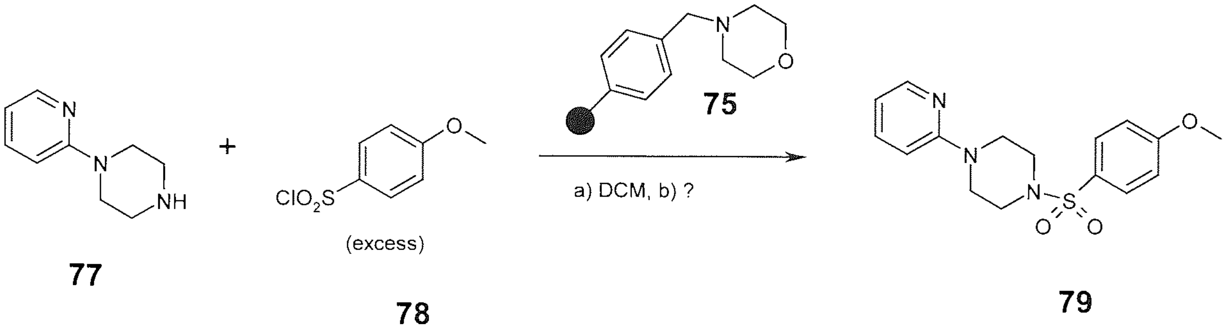
A number of scavenger resins is available today to assist compound purification in solution phase synthesis. However there are disadvantages associated with these reagents namely the resins being very expensive, except the proton scavenger resins, and the large amount of resins needed for purification. In contrast to solid-phase synthesis where resins of limited loading offer the tactical advantage of site isolation, resins which have high loading functional groups per gram are preferred in purification application. A high loading both facilitates the use of excess solid-supported reagents in a small volume and minimizes costs due to the amount of resins and solvent needed per reaction.[51]

1.6.1.1: Proton scavenger resins:

A large number of proton scavenger resins is commercially available, **(75)** and **(76)** **(Figure 16)**, most of them very cheap when compared with other scavenger resins such as nucleophilic and electrophilic resins. Acylation and sulfonylation reactions are conveniently carried out when a polymer bound base such as resin **76** is used as shown in **Scheme 13**.[51] A single filtration step can be used to remove the reagent base, the HCl byproduct, the excess acylating or sulfonylating reagent and the purification resin.

75 **76**

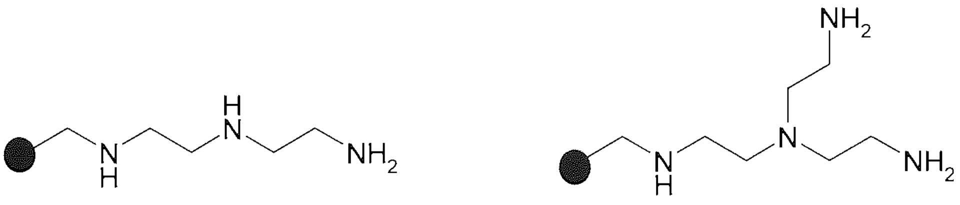
Figure 16: Commercially available proton scavenger resins.[51]



Scheme 13: **Proton scavenger resins for use in sulfonylation.**[51]

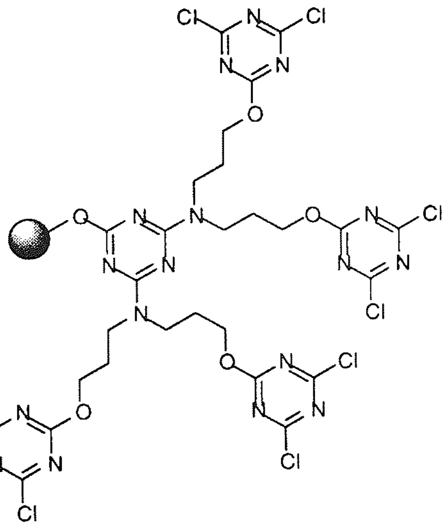
1.6.1.2: Nucleophilic scavenger resins:

Commercially available nucleophilic scavenger resins such as **(80)** and **(81)** (**Figure 17**) have been applied for the removal of electrophiles such as acid chlorides, sulfonyl chlorides and isothiocyanates, by covalentely attaching them to the nucleophilic resins. As mentioned previously (section 1.6.1, page 56) that the cost factor is the major disadvantage of scavenger resins. A solution for this problem could lie in preparation of high loading resins. Such high loading scavenger resins to scavenge nucleophiles and protons have been prepared by Marsh *et al.*[58] employing the strategy that has been used for the synthesis of dendrimers. The efficacy of these resins for chemical synthesis was demonstrated for the preparation of secondary amides. It has been reported that products were obtained between 77–83% pure. One such high loading nucleophilic scavenger resin **(82)** is shown in **Figure 18**.



80 **81**

Figure 17: **Commercially available nucleophilic resins.**[51]

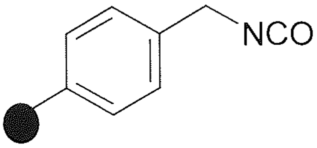


82

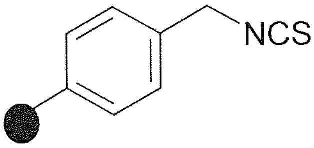
Figure 18: **High loading nucleophilic scavenger resin. (Adapted from Marsh *et al*[58])**

1.6.1.3: Electrophilic scavenger resins:

Polymer bound isocyanate (**83**) and isothiocyanate **(84) (Figure 19)** are excellent for removing nucleophiles such as excess amine from a reaction. Scavenger resin **(83)** has been successfully used to remove excess primary and secondary amines from tertiary amino alcohols.[51]



83

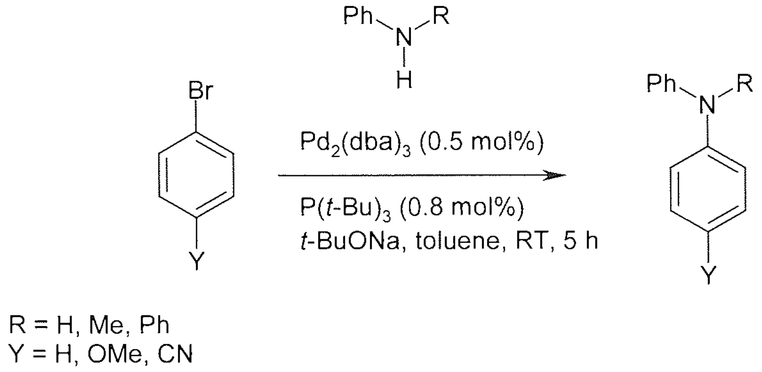


84

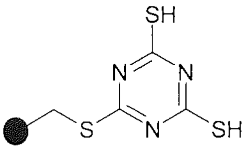
Figure 19: **Commercially available electrophilic resins.[51]**

1.6.1.4: Other scavenger resins:

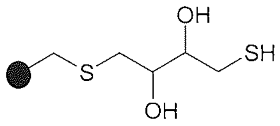
Palladium catalysis is powerful tool in synthetic chemistry for C-C and C-X bond formation. The removal and recovery of the precious metal from the products have become an important issue, due to the significant cost of palladium. Several palladium catalyst scavengers have been marketed in recent years including several polymer resins **(85** and **86) (Figure 20)**. The phosphine functionalised polymer supports **(87**, **88** and **89) (Figure 21)** as scavengers of Pd catalysts were investigated by Guino *et al.*[59] in Buchwald-Hartwig aryl animation reactions **(Scheme 14)**. The reactions were repeated without scavenging techniques. It has been reported that the product arylamines were obtained between 60-90% yield with good product purity, either completely free from Pd catalyst or containing less than 0.2% Pd catalyst residue when compared with the products prepared without the use of scavenger resins, where between 1.44–0.63% Pd catalyst residue was present. It was reported that the **(88)** was most effective in removing the palladium catalyst from these reactions.



Scheme 14: Buchwald-Hartwig aryl animation reaction (Adapted from Guino *et al*[59])

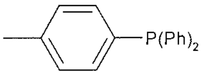


85



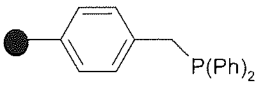
86

Figure 20: commercially available Pd scavenger resins.[59]



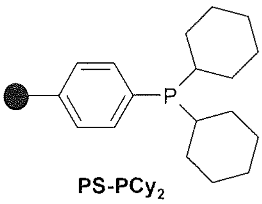
(PS-TPP)

87



PS-P(Ph)$_2$

88



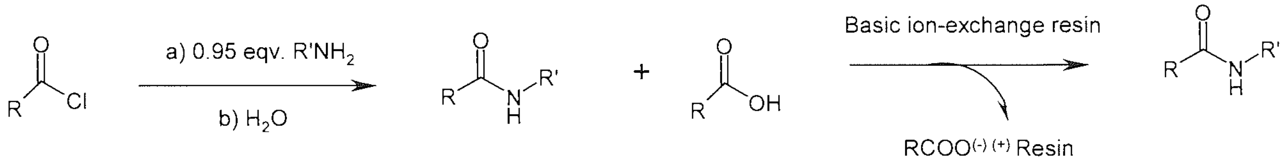
PS-PCy$_2$

89

Figure 21: Phosphine functionalised polymer resins.[59]

1.6.1.5: Ion-exchange resins:

A large number of ion exchange resins is commercially available ranging from strongly acidic to strongly basic. These resins have been used for a long time in solution phase parallel synthesis as ion-exchange resins. In these reactions, there may be little or no by products, but often these reactions require aqueous work-up, which is tedious if manually preparing large libraries. Ion-exchange resins can be used to eliminate the aqueous work-up and remove specific by products in solution phase synthesis of combinatorial libraries. For example, as illustrated in **Scheme 15** below, in a typical amide formation, the amine is reacted with slight excess of the acid chloride to provide the amide. Following the reaction a small amount of water is added to quench any unreacted acid chloride. The carboxylic acid and associated HCl is then absorbed by the resin, leaving only clean product in solution.[53,60]



Scheme 15: A general reaction with the use of ion-exchange resins. Adapted from Gayo *et al.*[53]

1.7: 1,3-Dipolar cycloaddition reactions:

The addition of a 1,3-dipole to an alkene or alkyne for the synthesis of five-membered rings is a classic reaction in organic chemistry. The 1,3-dipolar cycloaddition (1,3-DC) reactions are used for the preparation of molecules of fundamental importance for both academia and industry. The history of 1,3-dipoles goes back to Curtius, who discovered diazoacetic ester in 1883. A few years later his younger colleague Buchner studied the reaction of diazoacetic ester with α,β-unsaturated esters and described the first 1,3-DC reaction.[61] The general application of 1,3-dipoles in organic chemistry was first established by the systemic studies by Huisgen in the 1960s.

Closely related to Diels-Alder reactions, 1,3-DC reactions provide a versatile route to a wide variety of five membered heterocyclic compounds. 1,3-DC reactions are 6π-electron pericyclic reactions. The 4π-electron component, called the 1,3-dipole, is a three atom system *abc*, at least one of which is a heteroatom represented by zwitterionic octet. The 2π–electron component is called the dipolarophile.[62] In most reactions the 1,3-dipole is not isolated but generated *in situ* in presence of the dipolarophile. In many cases the dipolarophile is an alkene or an alkyne, but this is not essential and dipolarophiles containing hetero-multiple bonds can also be used for example imines, nitriles and carbonyl compounds. A typical example of well known reaction between diazomethane the 1,3-dipole, and an α,β-unsaturated ester to form a pyrazoline.

A 1,3-dipole is defined as an *a–b–c* structure that undergoes 1,3-DC reactions. 1,3-Dipoles can be divided into two different types: the allyl anion type and the propargyl/allenyl anion type. The allyl anion type is characterized by four electrons in three parallel p_z orbitals perpendicular to the plane of the dipole. This type of dipole is bent. Two resonance structures in which the three centres (*a,b,c*), have an electron octet, and two structures in which *a* or *c* has an electron sextet, can be drawn. The central *b* atom can be nitrogen, oxygen or sulphur. The propargyl/allenyl anion type has an extra orbital located in the plane orthogonal to the allenyl anion type molecular orbital (MO). This type of 1,3-dipole is linear and the central atom *b* is limited to nitrogen.[61,62]

1,3-DC reactions are both region selective as well as highly stereo selective, features which enhance their synthetic usefulness In the addition reactions of nitrones and nitrile oxides (1,3-dipoles) to mono and 1,1-disubstituted alkenes (dipolarophiles), except those carrying powerful electron withdrawing groups, the oxygen of the 1,3-dipole attacks the more highly substituted carbon of the double bond. Although the reaction of an unsymmetrical 1,3-dipole with an unsymmetrical dipolarophile could in theory give two products, in general one is formed predominantly or even exclusively. In reactions with geometrically isomeric alkenes, the steric disposition of the substituents about the double bond is retained in the heterocycle.[62]

1.7.1: 1,3-Dipolar cycloaddition reactions and frontier molecular orbitals (FMO):

Frontier molecular orbital (FMO) considerations are frequently used in organic chemistry to explain experimentally observed reactivities and selectivities of cycloaddition reactions. A general rationalization of the regiochemistry of 1,3-dipolar cycloadditions, based on the FMO theory states that some 1,3-DC reactions are controlled mainly by the HOMO (dipole) – LUMO (dipolarophile) interactions and others by the LUMO (dipole) – HOMO (dipolarophile) interaction. The smaller the energy gap between the controlling orbitals the faster the reaction. Reactions controlled by HOMO (dipole) – LUMO (dipolarophile) are accelerated by electron donating substituents in the dipole and the electron withdrawing substituents in the dipolarophile, and those controlled by LUMO (dipole) – HOMO (dipolarophile) are accelerated by electron withdrawing substituents on the dipole and electron donating substituents on the dipolarophile. In each case the energy gap between the two components is small. Furthermore in most 1,3-DC reactions, both the HOMO (dipole) – LUMO (dipolarophile) and LUMO (dipole) – HOMO (dipolarophile) interactions may be important. The controlling interactions depend on the nature of substituents on the dipolarophile and the electronic nature of substituents on the dipole. These reactions can be accelerated by both electron donating and electron withdrawing substituents on either components.[63,64]

More recent studies, however, suggest that this is not, in fact, the case. In a recent study, conducted by Wagner *et al.*[65] on the reaction mechanism and regioselectivity between benzonitrile oxide and propene, it was reported that the benzonitrile oxide does not interact through its FMO, as traditionally assumed. Instead, the FMO±2 are involved in the reaction, indicating that the classical FMO concept should be applied with care as it might lead to wrong conclusions.

Calculated transition state geometries revealed that benzonitrile oxide generally does not involve its FMOs in the reaction but rather the orthogonal FMO±2 orbitals. (The relevant MO for benzonitrile oxide is shown in **Figure 22**). These orbitals are energetically less favourable, but at the same time their involvement in the bond formation does not cause any loss of conjugation of the CNO unit of nitrile oxide with the phenyl substituent. If interactions with FMO±2 are prevented by steric interactions, only then the reaction occurs under involvement of the FMOs and on expenses of the resonance energy that is lost by interrupting the conjugation of the phenyl group with CNO moiety. Such a change of orbital interaction can complicate the application of the FMO concept to assess reactivities and selectivities of cycloaddition. [63,65]

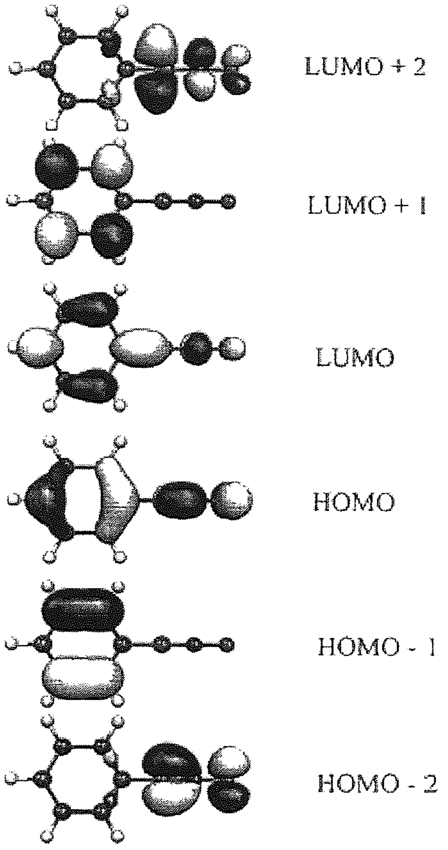
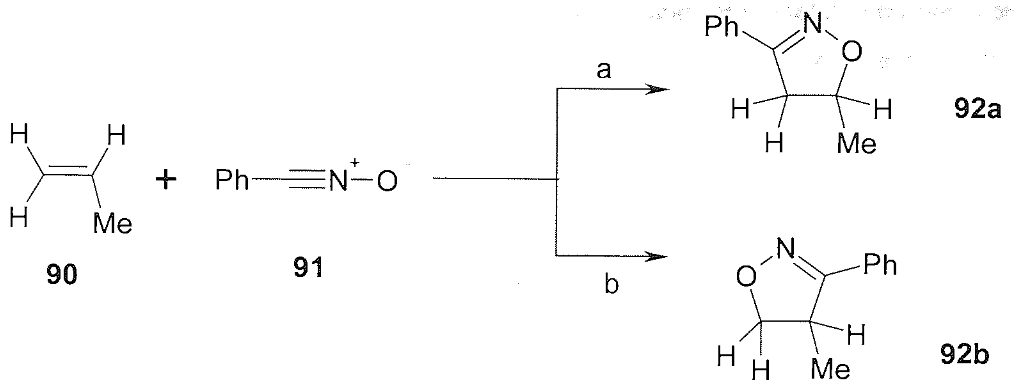


Figure 22: Relevent orbitals of benzonitrile oxide. Adapted from Wagner *et al.*[65]

Relative energies for the reaction of propene **(90)** with, benzonitrile oxide **(91)** for the two possible regioisomers **(92a and 92b) (Scheme 16)**, were calculated. It has been reported that the relative energies, both transition state and the product, for **92a** were found to be lower than **92b**.[65]



Scheme 16: The two possible stereoisomers, when propene (an alkene) and benzonitrile oxide reacts together. Adapted from Wagner *et al.*[65]

In two other similar studies, a) conducted by Rastelli *et al.*[63] for the reactions between 1,3-dipoles, diazomethane and formonitrile oxide, and dipolarophiles methyl vinyl ether, acrylonitrile and propene, b) conducted by Corsaro *et al.*[66] for the reaction of mesitonitrile oxide with amino- and nitrostilbenes, similar results have been reported. In summery, the FMO theory cannot arrive at the correct prediction of the regiochemistry for the 1,3-dipolar cycloaddition reactions between dipoles and dipolarophiles mentioned above. Therefore the FMO theory should be applied with care as it might lead to wrong conclusions.

1.8: Isoxazolines:

Isoxazolines belong to a family of five membered hereocylces, having a nitrogen atom plus one another heteroatom in a 1,2-relationship in the ring. There are two types of isoxazoline compounds, Δ^2-isoxazolines and Δ^4-isoxazolines. Δ^2-Isoxazolines **(95)** are also known as 4,5-dihydroisoxazolines or 2-isoxazolines. The standard synthesis for the preparation of Δ^2-isoxazolines is *via* 1,3-DC of nitrile oxides **(93)** to alkenes **(94)** as shown in **Figure 23**.[67]

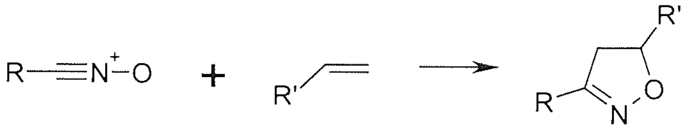


Figure 23: 1,3-DC reaction between a nitrile oxide (93) and alkenes (94) resulting in the formation of Δ^2-isoxazolines (95).[67]

The Δ^2-isoxazolines are an important class of hetrocycles, and are useful intermediates in the synthesis of many biologically important compounds,[68,69] They can be selectively transformed into 1,3-amino alcohols, β-hydroxy ketones, 1,3-diols, β-hydroxynitriles and unsaturated oximes which are useful intermediates in the total synthesis of a wide variety of natural products. It is known that the monosubstituted alkenes exhibit high regioselectivity in 1,3-DC reaction with nitrile oxides to give 5-substituted-2-isoxazolines as the major product. However a mixture of Δ^2-isoxazolines regioisomers generally results if unsymmetrical disubstituted alkenes including α,β-unsaturated esters and ketones are used. The use of α,β-unsaturated aldehydes is restricted due to the formation of a bis cycloadduct resulting from the addition of nitrile oxide to the carbaldehyde group of the initial Δ^2-isoxazoline product.[70]

Δ^2-Isoxazolines are found in a number of pharmaceutical agents such as glycoprotein IIb/IIIa (GPIIb/IIIa) inhibitors and human leukocyte elastase inhibitors.[68] The Δ^2-isoxazoline structure is found in some pharmacologically interesting natural products, e.g. in the antibiotic cycloserin **(96)**, which is used in the treatment of tuberculosis. It is also found in the antibiotic acivincine **(97)** **(Figure 24)**, possising an α-amino acid, with antitumour action. Δ^4-isoxazolines are also known as 2,3-dihydroisoxazolines or 4-isoxazoline. Its standard synthesis is *via* the 1,3-dipolar cycloaddition of nitrones to alkynes, also known as Husigen synthesis.[67] Δ^4-Isoxazolines are unstable and very reactive compounds. They readily undergo thermal isomerization to give aziridines.

96 **97**

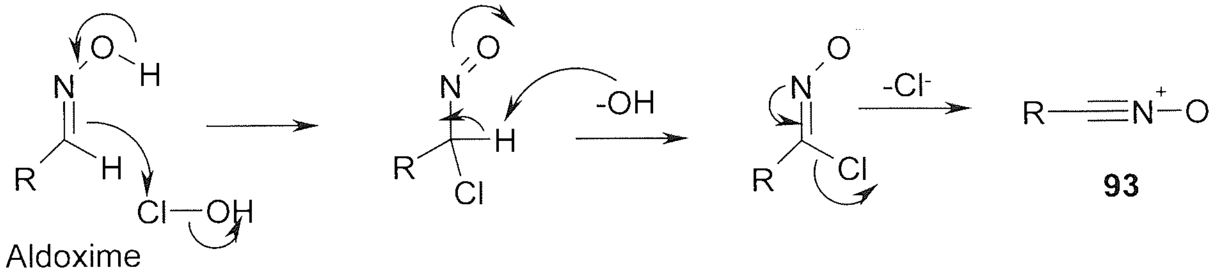
Figure 24: **Δ^2-Isoxazolines found in pharmaceutical agents.[67]**

1.9: Nitrile oxides:

Nitrile oxides are versatile intermediates for the preparation of heterocyclic compounds *via* 1,3 dipolar cycloaddition. However they are highly reactive and unstable species that undergo cyclic dimerizations to form furoxane, hence they are usually generated *in situ.*[71]

Two common methods are used to generate the nitrile oxides. The Mukaiyama method is based on reaction of primary nitroalkanes with phenyl isocyanate and catalytic amount of TEA. A modification upon this procedure may utilize benzensulfonyl chloride or ethyl chloroformate in the presence of TEA or *p*-toluenesulfonic acid dehydrating agents. The second frequently used method is base mediated dehydrohalogenation of hydroximoyl halides obtained by reaction of aldoximes with *N*-chlorosuccinimide or *N*-bromosuccinimide, halogens, 1-chlorobenzotriazol, sodium hypochlorite or chloramines.

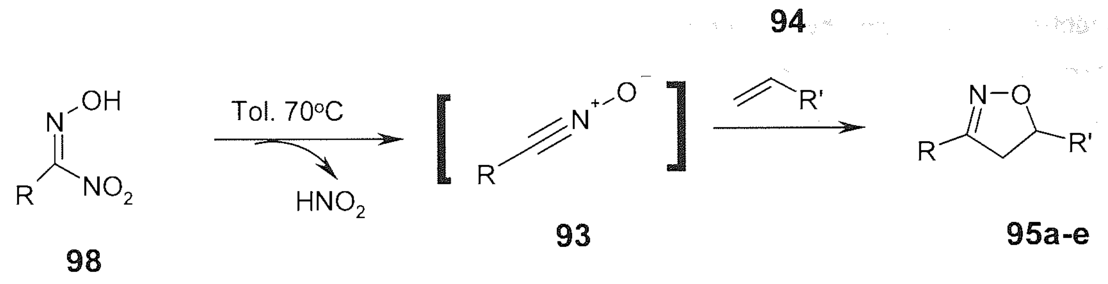
The direct methods for the oxidation of aldoximes to nitrile oxides includes the use of lead tetraacetate which requires cooling (-78 C) and only (*Z*)-aldoximes react this way. Hypervalent iodine compounds as oxidizing agents require alakaline workup and that limits the range of substrates to alkaline-resistanc compounds. Potassium ferricyanide oxidation proceeds only in aqueous media and ceric ammonium nitrate has been used on aromatic aldoximes only and produces a number of undesired by-products. A mechanism for the generation of nitrile oxides from aldoximes is shown below in **Scheme 17**.



Scheme 17: **Nitrile oxides generated from aldoxime in the presence of sodium hypochlorite.**

1.9.1: Other methods for generating nitrile oxides:

Nitrolic acids are known to be highly unstable, difficult to handle and prone to decomposition upon heating *via* formation of the corresponding nitrile oxides. Mioskoswski *et al.*[72] in 2000 reported preparation of nitrile oxides from nitrolic acids to synthesize isoxazolines under neutral conditions. **(Scheme 18)**.



R and R' = See **Table 8** below

Scheme 18: Nitrile oxides generated under neutral conditions.[72]

A series of nitrolic acids was prepared from their nitro or bromo precursors by treating with mixture of acetic acid and sodium nitrate in DMSO. The stability of nitrolic acids was found to be dependent on their substituents, as alkylnitrolic acids were easily isolated and purified by silica gel chromatography in around 90% yield and stored for several months at –20 °C. The alkoxycabonyl- and arylnitrolic acids were found to be unstable and started to decompose during column chromatography. Furthermore they have strong tendency to give rise to the corresponding nitrile oxides witnessed by the formation of furoxanes. To perform the cycoadditions the THF solutions of nitrolic acids were simply heated under reflux, in the presence of dipolarophiles.[72] The resulting isoxazolines are shown in **Table 8** below.

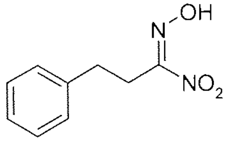
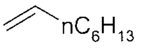
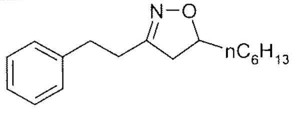
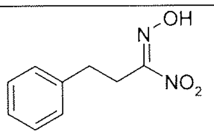
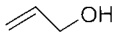
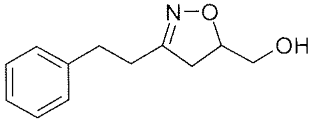
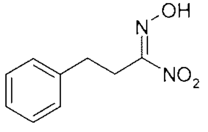
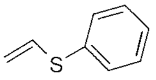
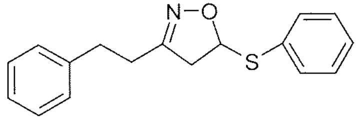
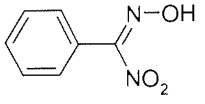
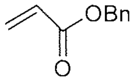
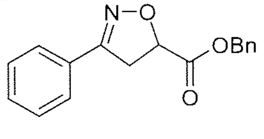
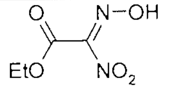
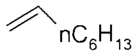
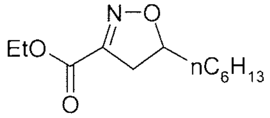
Compound	Nitrolic acids	Dipolarophiles	Cycoladduct	Yield %
95a				95
95b				97
95c				95
95d				53
95e				55

Table 8: **Δ^2-Isoxazoline compounds prepared from nitrile oxides, generated *in situ* from their corresponding nitrolic acids, and dipolarophiles under neutral conditions.**[72]

Since the reaction conditions are neutral and do not require strong dehydrants or strong oxidants like classical methods for generation of nitrile oxides, unprotected alcohols (b) and highly oxidizable sulphur moieties (c) are compatible with the reaction conditions. Classical methods for the preparation of nitrile oxides use large excess of reagents that lead to the formation of by-products. Thermolytic generation of nitrile oxides operated under stoichiometric and neutral conditions which avoid these drawbacks. At the end of the reaction no work-up is necessary the cycloadduct was directly obtained after concentration of the crude mixture.[70,72]

Oxidation of aldoximes to generate nitrile oxides, where simplicity of procedure and mild reaction conditions are required, could be carried out using MnO_2. It has been reported by Kiegiel *et al*,[71] that the 1,3-DC reactions proceed very effectively for oximes bearing electron withdrawing groups when MnO_2 is used as an oxidising agent.

1.10: Oximes:

Oximes are isonitroso compounds containing the group CNOH. They are formed by reaction of an aldehyde or ketone with hydroxylamine to form Aldoxime and Ketoxime respectively as shown in **Figure 25** below.

$$R^1R^2C{=}O \;+\; H_2N\text{-}OH \longrightarrow R^1R^2C{=}N\text{-}OH \;+\; H_2O$$

Figure 25: Reaction of aldehydes or ketones with hydroxylamine to give oximes.

Oximes crystallize well, and for this reason they are often used to characterize aldehydes and ketones. Aldoximes are also used in industries to generate other starting materials/intermediates for organic synthesis, i.e. dehydration of *Z*-aldoximes by acetic anhydride produces nitrites, which are intermediates for several organic synthesis.[73]

1.11: Antimicrobial polymers:

Implant-associated infections are serious complications of medical insertions. Suffering and additional health costs due to implant infections are a major public health problem. Serious complications, which may result from these infections, include tissue destruction, premature device failure and the spread of the infection to other areas. Such infections are difficult to treat for two principal reasons. Firstly, the implant is surrounded by a fibrous coating, as is typical in response to a foreign body. This encapsulation provides a protected environment for bacterial growth that is largely inaccessible to the host immune system. Secondly, bacteria such as *Staphylococcus aureus* and *Staphylococcus* *epidermidis* adhere readily to implant surfaces. Therefore, the implant surface, which is coated with a host of extra-cellular matrix serum proteins, provides an ideal environment to facilitate bacterial adhesion and proliferation. Implant associated infections can arise in weeks immediately following surgery due to contamination. Despite aggressive antibiotic treatment, eradication of established implant-associated infections often fails and in severe cases can lead to death.[74,75,76]

Antimicrobial agents used for the treatment of different infections are usually low molecular weight compounds. Most of these compounds are toxic and their applications are short lived.

1.11.1: Antimicrobial biomaterials:

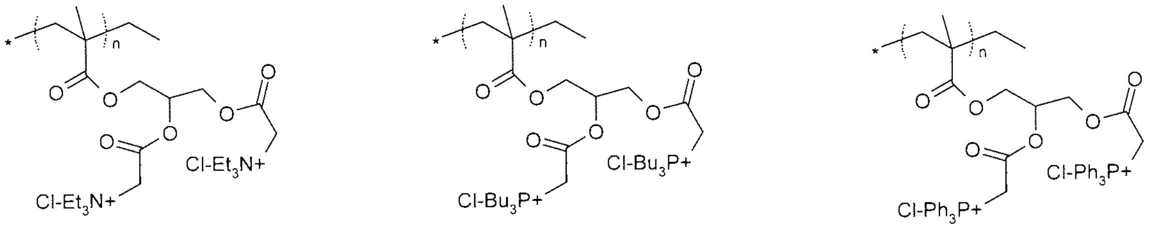
Ordinary implant device materials are not anti-microbial. One way to prevent the establishment of infection would be to render the implant surface bactericidal *via* covalent modification.[74] Such materials could also be utilized in variety of applications such as to coat the surfaces of common objects touched by people in everyday life (e.g. door knobs, children's toys, computer keyboards, telephones etc), food packaging, medical applications and health care related materials, to render them antiseptic and thus help to prevent the cross infections.[77] For example, surfaces chemically modified with PEG and certain other synthetic polymers can repel but not kill the micro-organisms. One of the earliest antimicrobial compound to be incorporated into a polymer for the prevention of infection was gentamicin. Gentamicin has also been incorporated into bone cement or formulated as beads for the prevention of infections of prostheses.[78]

Currently much research has been directed towards making anti-bacterial biomaterials. One of the earlier ideas to make antimicrobial materials, was to impregnate the biomaterials with anti-microbial agents, such as antibiotics, antiseptics, quaternary ammonium compounds or metallic ions.[74,78] The impregnated device, when immersed in fluids gradually releases the antimicrobial agent into the surrounding solution and kills microorganisms therein. However this strategy is only short lived, because once the antibacterial agents supply is exhausted, the antibacterial activity would also end there. This strategy has been verified in aqueous solutions but would not be expected to be effective against airborne bacteria in the absence of a liquid medium.[77,78]

1.11.2: Antibacterial polymers containing polycations:

Various polycations possess antibacterial properties in solution, presumably by interacting with and disrupting bacterial cell membranes. However, this antibacterial activity vanishes when theses polycations are crosslinked or otherwise insolubilized. It has been reported by several groups that their antibacterial properties can be preserved, even after insolubilization, and expressed in a dry state, if the immobilized polycationic chains are sufficiently long and flexible to be able to penetrate the bacterial cell walls.

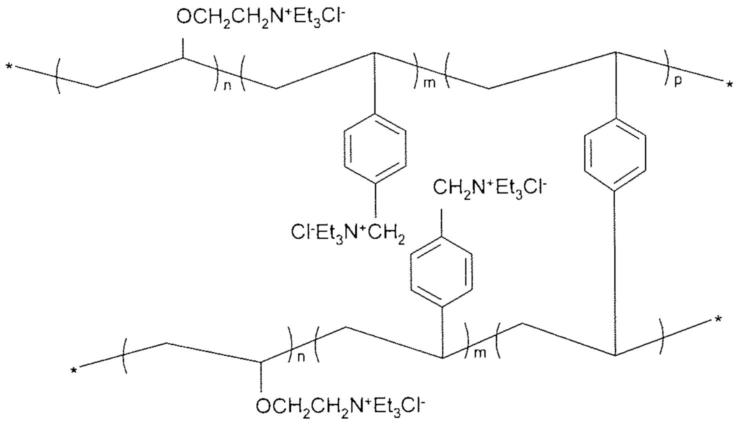
In the last decade several groups reported the antimicrobial activity of polymers containing quaternary ammonium compounds, bonded to the polymer through non-hydrolysable covalent bonds. A polymer comprising a polymethylene backbone with amine or ammonium functional groups attached to the polymer backbone *via* aliphatic spacer groups was reported to posses the antibacterial properties by Mandeville III *et al.*[79] Another group of scientists led by Kenawy *et al.*[80] reported the synthesis and antimicrobial activity of glycidyl methacrylate polymers covalently bonded to quaternary ammonium and phosphonium groups **(Figure 26**, polymers **99**, **100** and **101)**. It is reported that these polymers posses antibacterial activity against a number of microorganisms including *Escherichia coli*, *Salmonella typhae, Bacillus cereus* and *Pseudomon aeruginosa* and that the bacterial growth was increasingly inhibited by increasing the polymer concentration.



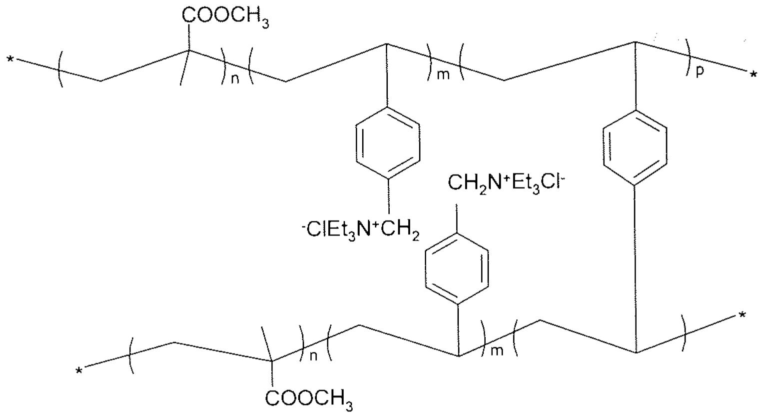
99 **100** **101**

Figure 26: Glycidyl methacrylate polymers covalently bonded to quaternary ammonium and phosphonium groups, possessing antimicrobial activity against a number of microorganisms.[79,80]

Recently the same group of researchers[81] reported the synthesis of cross-linked polymers containing quaternary ammonium and phosphonium groups **(102**, **103**, **104 and 105) (Figure 27)**, possessing antimicrobial activity. Vinylbenzyl chloride (VBC) was copolymerised with 2-chloroethyl vinyl ether (CEVE) or methylmethacrylate (MMA) using the 5% divinylbenzene as crosslinker, in a free radical polymerisation.

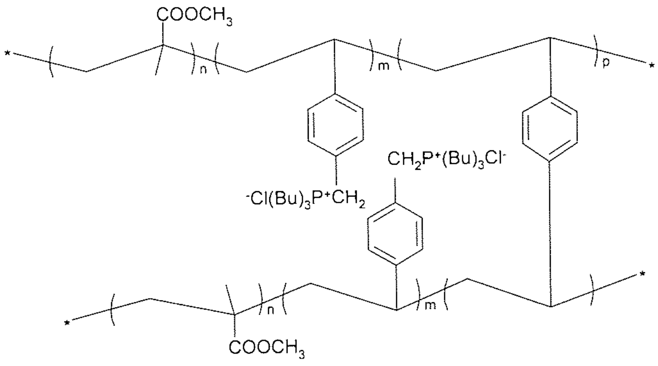


102



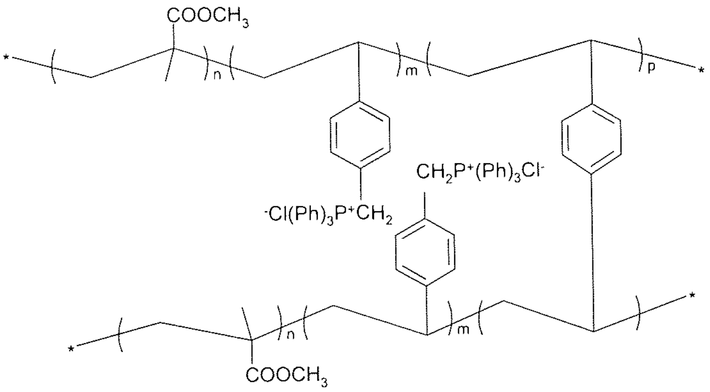
n = 1,
m = 3,

103



n = 1,
m = 3,

104



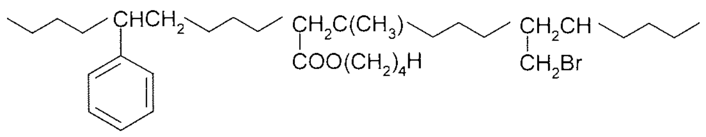
n = 1,
m = 3,

105

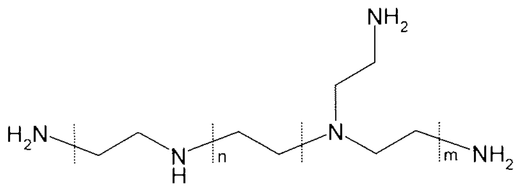
Figure 27: **Cross-linked polymers containing quaternary ammonium and phosphonium groups, possessing antimicrobial activity. Adapted from Kenawy *et al.*[81]**

These copolymers were tested for their antimicrobial activity against a number of fungi including *Candida albicans, Fusarium oxysporum and Aspergillus flavus,* and bacteria including *S. aureus, B. subtilis* and *E. coli.* It was found that all copolymers showed some antimicrobial activity, with the compound with the triphenylphosphonium salt **(105)** being the most effective against both bacteria and fungi.

Klibanov *et al*[82], has recently reported the synthesis of a polystyrene copolymer covalently linked to hydrophobic polycations with highly bactericidal activities. Styrene was copolymerized with butyl methacrylate and allyl bromide in methyl ethyl ketone in the presence of AIBN in a free radical polymerisation under standard conditions and copolymer-Br **(106)** was made **(Figure 28)**. The copolymer-Br was precipitated in methanol. The copolymer-Br was *N*-alkylated to polyethylenimine (PEI) **(107),** followed by *N*-alkylation and *N* methylation to give *N*-copolymer **(108)**, *N*-hexyl **(109)**, *N*-methyl-PEI **(110)**. See **Scheme 13** below.

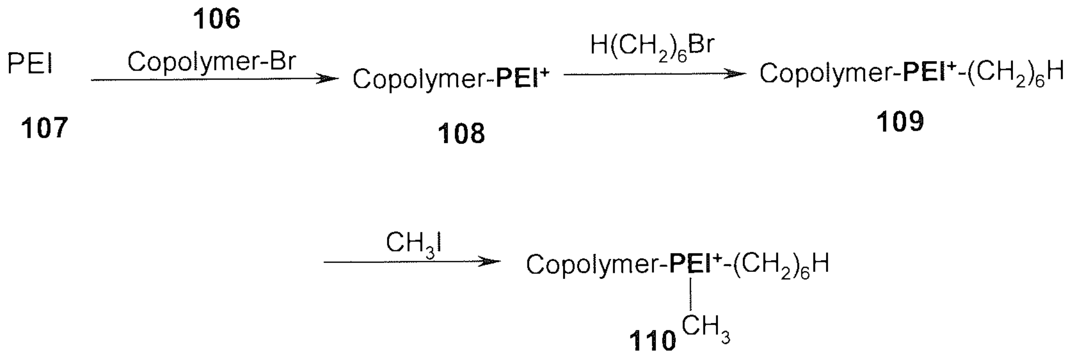


Copolymer-Br **106**



PEI= Polyethylenimine (PEI) **107**

Figure 28: **Structures of polystyrene copolymer-Br and polyethylenimine (PEI).82**[82]



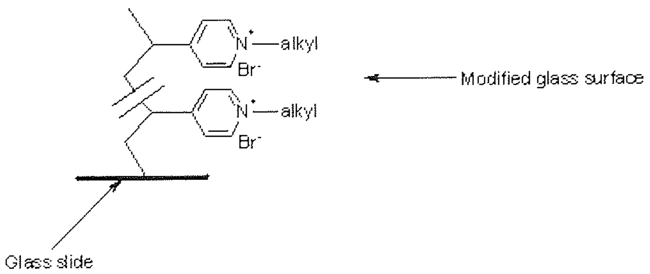
Scheme 19: **Synthesis of a polystyrene copolymer *N*-hexyl, *N*-methyl-PEI.**[82]

To test the *N*-copolymer, *N*-hexyl-*N*-methyl-PEI for any antibacterial activity, commercially available glass slides and polyethylene slides were dipped for 1 min into a solution of 100 mg of *N*-copolymer, *N*-hexyl, *N*-methyl-PEI per mL of methyl ethyl ketone followed by air drying for two minutes. A *S. aureus* bacterial suspension in PBS with a cell density of 5 x 10^6 cells/mL was sprayed onto slides at a rate of approximately 10 mL/min in a fume hood. After 2-min drying in air, the slide was placed in a Petri dish and immediately covered with a layer of 1.5% solid growth agar. The Petri dish was sealed and incubated at 37°C overnight. A control test was performed on noncoated glass and polyethylene slides following the above procedure. On noncoated control slides, typically some 120 bacterial colonies per cm^2 were detected. However on the polymer coated slides, not a single colony was observed. Therefore, it can be concluded that either the coated surface kills the bacteria on contact or the coating's polycations leach from the surface, thus killing the bacteria.[82]

1.11.3: Antibacterial agents covalently linked to the glass surfaces:

Long chains of *N*-alkylated poly(4-vinylpyridine) (PVP) were attached covalently to a glass surface **(111) (Figure 29)** by Kilbanov *et al.*[77] The resultant glass slides killed, on contact, a number of airborne gram-negative and gram-positive bacteria.

To attach PVP to glass slide, an NH_2 functionalised glass slide (aminopropyltrimethoxysilane-coated microscope slide), was immersed in a mixture of dry DCM and TEA. After cooling to 4 °C, acryloyl chloride was added and stirred in a cold room for overnight and at RT for 2 h. The acylated NH_2 glass slide was rinsed with methanol/TEA mixture and methanol. The glass bonded acryloyl moieties were then copolymerised with 4-vinylpyridine. The acryloylated glass slide was placed in a solution of perchloric acid with $Ce(SO_4)_2$ and 4-vinylpyridine was added. After washing away any unattached PVP, the slide was placed in a solution of an alkyl bromide in nitromethane. The reaction mixture was stirred at 75 °C. The resultant polycation-derivatized PVP slide was rinsed with methanol and distilled water and air dried. The polymer's pyridine rings were *N*-alkylated by seven linear alkyl bromides, with chain lengths varying from propyl to hexadecyl.



111

Figure 29: Modified surface of glass surface showing cation (quaternary ammonium ion). Adapted from J. C. Tiller *et al.*[77]

The antibacterial activity of the polycation-derivatized PVP glass slide was tested against several microrganisms including *S. aureu, S. epidermidis, P. aeruginosa* and *E. coli.* A bacterial suspension was sprayed onto the glass slide and air dried. The slide was placed in a petri dish, and growth agar was added. The petri dish was incubated at 37°C. It was found that propylated, butylated, hexylated and octylated immobilized PVP chains were effective in markedly reducing the number of viable bacterial cells, with the most effective hexyl-PVP affording a 94 ± 4% reduction. The results are shown in **Table 9** below. **Figure 30** shows the photograph of control and PVP modified glass slides with bacterial growth. It can be seen the very few colonies grew on hexyl-PVP-modified slide in contrast to the NH_2 glass slide.

Bacterium	Percentage of bacteria killed
S. aureu	94 ± 4
S. epidermidis	>99
P. aeruginosa	>99.8
E. coli	>99

Table 9: The ability of hexyl-PVP attached to glass slides to kill various airborne bacteria on contact. Adapted from J. C. Tiller *et al.*[77]

Figure 30: Photographs of a commercial NH_2 glass slide (*Left*) and a hexyl-PVP-modified slide (*Right*) onto which aqueous suspensions (≈ 10^6 cells per mL of distilled water) of *S. aureus* cells were sprayed, air dried for 2 min, and incubated under 0.7% agar in a bacterial growth medium at 37 °C overnight. Adapted from J. C. Tiller *et al.*[77]

To test whether the antibacterial polymers immobilized can leach from glass surface, the hexyl-PVP slide was placed into polystyrene petri dish, and aqueous suspension of *S. aureus* cells was sprayed on the slide as well as on nonbacterial polystyrene dishes. After air drying and incubation under growth agar, it was observed that only a few colonies were growing on hexyl-PVP glass slide. In contrast a large number of colonies grew on the surrounding Petri dishes, even in the immediate proximity of the slide, suggesting that the immobilized polymer does not leach from the slide.

To test how effective the ability to kill airborne bacteria was among dry surfaces, *S. aureus* cell suspension was sprayed on various common materials, including metals, synthetic and natural polymers and ceramics. The number of colonies counted for each material was compared with NH_2 glass. As it can be seen from **Table 10**, none of the materials significantly reduced the bacterial growth.

Material	Relative number of viable bacterial cells, %
Plain glass	83 ±10
Polystyrene	105 ±15
Polypropylene	97 ±9
Aluminium	72 ±7
Steel	95 ±15
Paper	77 ±8
Wood (birch)	102 ±10
Porcelain	85 ±10
PVP slide	115 ±16
Hexyl-PVP slide	6 ±4

Table 10: Number of *S. aureus* colonies obtained after their aqueous suspension have been sprayed onto various common materials. Adapted from Tiller *et al.*[77]

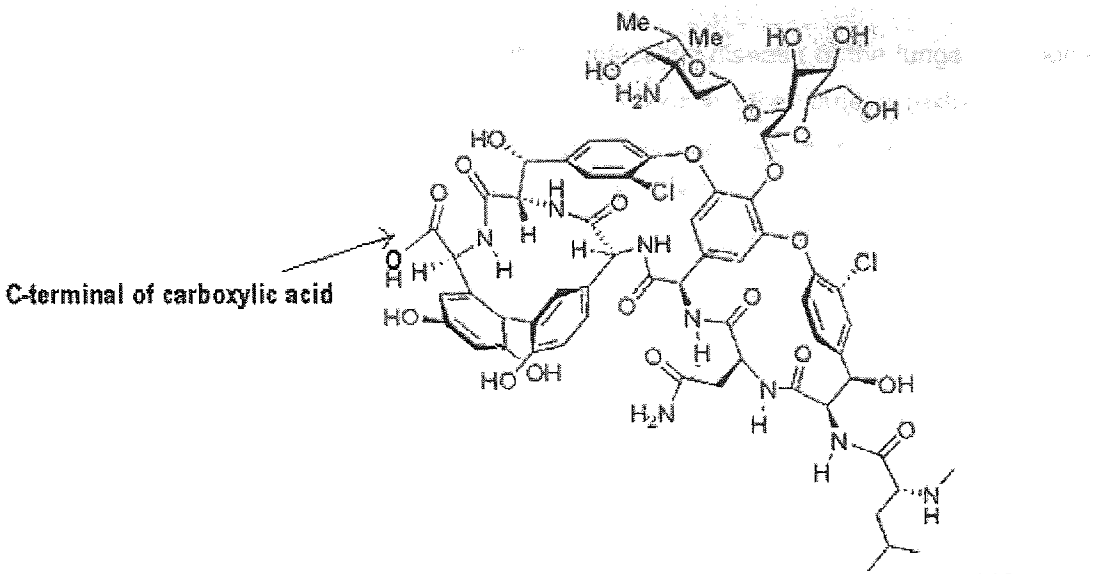
1.11.4: Antibiotics covalently bonded to metal surfaces:

Self protecting implants could lead to the beginning of a new implants generation that could lower the incident of implant-associated infections and prevent bacterial biofilm formation. One way to prevent the establishment of implant-associated infections, would be to make the implant surface bactericidal by covalently attaching the antibiotics to it. This methodology has been tried and tested by Wickstorm *et al.*[74] by covalently attaching the Vancomycin (VAN) antibiotic to titanium beads.

Titanium (Ti) is a metal with very good biocompatibility and it is commonly used both in its pure state as well as an alloy in the fabrication of orthopaedic and dental implants. Ti biocompatibility appears to depend on the Ti-OH surface layer.

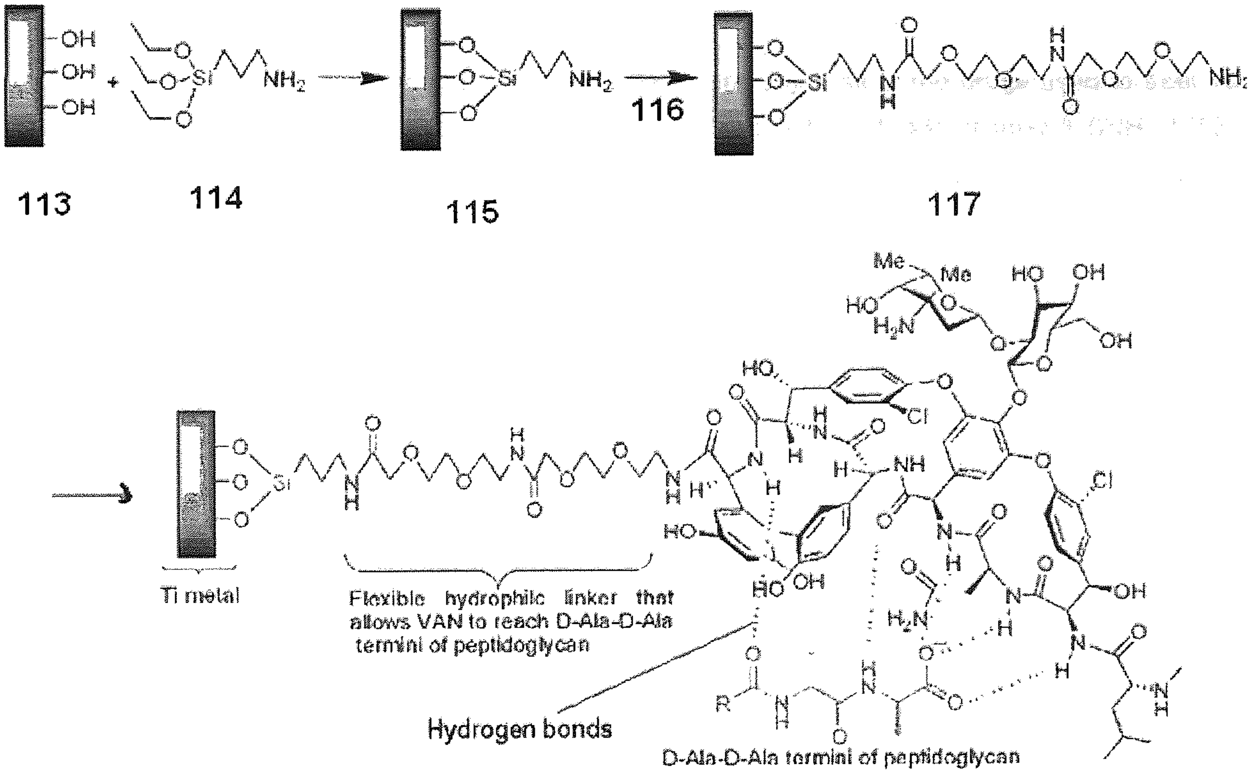
Vancomycin **(112)** is an antibiotic with a broad spectrum of activity. It provides a potent treatment for a large spectrum of bacterial infections, especially prescribed for serious life threatening infections caused by gram-positive bacteria. It blocks steps in the biosynthesis of the peptidoglycan layer of the cell wall of gram-positive bacteria. It is known that the C-terminal carboxylic acid of VAN **(Figure 31)** is not necessary for antibiotic activity. Hence it could be used as a covalent coupling site to implant surface.

To couple VAN to Ti metal, the Ti surface **(113)** was first derivatised with 3-aminopropyltriethoxysilane **(114)** and gave aminopropylated Ti surface **(115)**. To extend VAN away from the silanized Ti surface, two molecules of 8-amino-3,6-dioxaoctanoate (aminoethyoxyethoxyacetat; AEEA) **(116)** were linked to aminopropylated Ti to give **(117)**. The AEEA is a flexible linker that is hydrophilic due to the presence of ether linkages in the molecule, which was attached to the aminopropylated Ti *via* an amide linkage. The vancomycin was also, covalently coupled to the AEEA linker by an amide linkage **(118)**. **Scheme 21** shows sequences of attachment of VAN to titanium beads. It is believed that the cell wall synthesis is inhibited *via* the hydrogen bond interactions between the D-Ala-D-Ala termini of peptidoglycan and vancomycin.



112

Figure 31: Structure of Vancomycin



118

Scheme 20: Schematic of, step by step, proposed VAN-tethering to Ti implant material. Also shown is the inhibition of cell wall synthesis by VAN through the binding of the D-Ala-D-Ala termini of peptidoglycan, with VAN by five hydrogen bonds. Adapted from Wickstorm *et al.*[74]

1.12: Tuberculosis:

The term tuberculosis (TB) is used primarily to signify an infectious disease of the lungs, (pulmonary TB), caused by *Mycobacterium tuberculosis*, but may also occur in other organs (extra pulmonary TB).[83,84] TB has been around for centuries and the disease has long been a major killer of mankind. The disease reached epidemic proportions in Western Europe in the early 19th century when the industrialisation fuelled a massive growth in the urban poor population. It is estimated that one-third of all deaths in London during the first 40 years of the 19th century were as a result of TB. Although it was most rampant among the poor, TB also infected the more privileged.[85]

In 1882, Robert Koch identified the tubercle bacillus. The realisation that TB was transmissible prompted a strategy of confinement either in patients' homes, hospitals or purpose-built sanatorium on hillsides where a strict regimen of bed rest and fresh air was often imposed and surgical procedures such as thoracoplasty was also tried, which was aimed to halt the disease process.[85,86,87]

1.12.1: Onset of drug development for treatment of TB:

The development of Bacille Calmette-Guerin (BCG) vaccine, by Albert Calmette and Camille Guerin, from an attenuated strain from *Mycobacterium bovis*, and the discovery of streptomycin (STM) in 1944 was the beginning of a golden age in TB drug development, when all of the drugs used to treat TB today were introduced. These includes p-aminosalicylic acid (PAS, 1946), isoniazid (INH, 1952), cycloserine (1955), rifampicin (RIF, 1965), ethionamide (1966), ethambutol (1968), and pyrazinamide (1970).[84,87,88] The introduction of radiological services for screening, improved surveillance, milk pasteurisation, treatment of contacts and the widespread use of BCG vaccine accelerated the rate of decline. It was not until 1970s, however, that the real revolution took place, when the key clinical trials conducted by the British Medical Research Council established the multi-drug regimen that forms the basis of the treatment recommended by the World Health Organisation (WHO) today. As a result, TB drug development stopped altogether as it was assumed that the effective drug regimen, combined with the mass BCG vaccination programmes, would lead to the steady elimination of TB as a public health threat.[88]

However, the late 1980s saw a resurgence of the disease. In 1993 the WHO declared TB a global health emergency. TB is now a worldwide pandemic. According to the WHO's 2006 report, TB kills nearly 2 million people around the world every year. The global TB incidence is still growing at 1% a year because of the rapid increase in Africa. About 2 billion people, that is almost one third of the world's population, are now infected with the TB. Over 95% of the people infected with TB come from the developing world.[85,89,90] Reasons for resurgence of TB is multifunctional. But it can be largely attributed to the human immunodeficiency virus (HIV), increased world poverty and the emergence of multi-drug resistance strains of TB (MDR-TB).

As mentioned above TB is generally known as the disease of the lungs, causing chronic infection of the lower respiratory tract, but it can affect almost any organ (most commonly the lymph nodes), bones and joints, the central nervous system (CNS), gastrointestinal and genitourinary system.[85,91]

1.12.2: Transmittance of TB disease:

M. tuberculosis is transmitted primarily *via* the respiratory route and almost exclusively by people with the active pulmonary or laryngeal form of the disease. The tubercle bacilli appears in water droplets expelled during coughing, sneezing or talking. Either in the droplet form or as the desiccated airborne bacilli, following inhalation, the mycobacteria, upon reaching the alveoli in the lung periphery are taken up by alveolar macrophages where they survive and multiply. Circulating microphages and lymphocytes, attracted to the site, carry the organism to local lymph nodes, where a cell mediated immune response is triggered. The host, unable to eliminate the pathogen, contains them within small granulomas or tubercles. If high numbers of mycobacteria are present then the cellular responses can result in tissue necrosis. The tubercles contain viable pathogens, which may persist for the remaining life of the host.[85,91,92]

About 10 per cent of the people infected with TB bacilli would develop the active TB in their lifetime. People with active form of TB have following symptoms, a cough of more than two weeks' duration, weight loss, fever, night sweats, fatigue, shortness of breath, chest pain, haemoptysis (coughing up blood) in later stages.

1.12.3: Mycobacteria:

There are over 80 named species of bacteria belonging to genus *Mycobacterium*. This can be divided into two major groups, the slow and the rapid growers, although the growth rate of latter is slow relative to that of most other bacteria. Members of the *Mycobacterium tuberculosis* complex are pathogens responsible for the disease called Tuberculosis (TB).[83,84]

1.12.4: Mycobacterium tuberculosis complex:

The term *Mycobacterium tuberculosis* complex refers to a group of very closely related species which are a variation of a single species. All of them cause tuberculosis. The complex includes:

M. tuberculosis:	causes most human tuberculosis,
M. canetti:	vey rare varient of *M. tuberculosis* with smooth colonies,
M. bovis:	the principle cause of tuberculosis in cattle and many other mammels,
	a caprine (goat) variant of *M. bovis*,
M. microti:	pathogn of voles and other small mammals,

M. africanum: appears to be an intermediate in form between the human and bovine type. It causes human tuberculosis and is mainly found in Equatorial Africa.

Members of the *M. tuberculosis complex* (tubercle bacilli) are gram-positive, non-motile, non-sporing, non-capsulate, straight or slightly curved rods. They are

- Obligate aerobes growing most successfully in tissues with a high oxygen content, such as lungs.
- Slow-growing with a generation time of 12 to 18 hours.
- Hydrophobic with a high lipid content in the cell wall. They tend to clump togather in small clusters, and are resistant ot drying out.
- acid fast bacilli resist the decolorization with acidified organic solvents.
- Faacultative intracellular pathogens usually infecting mononuclear phagocytes

M. tuberculosis is also unusual as it is able to survive inside macrophages. It predominantly grows inside them, rather than extracellulary.[83,84,87]

1.12.5: The Mycobacterial cell wall:

The cell wall of mycobacteria species, including *M. tuberculosis* is essential for growth and survival of the pathogen in the infected host. It differes from other bacteria cell walls as it has a thick waxy coat comprising complex lipids and carbohydrates, which makes it impermeable to many drugs.[93] The cell envelope of *M. tuberculosis* is made up of three major componenet: a plasma membrane, covalentely linked mycolic acids, arabinogalactan and peptidoglycan complex (MAPc), and a polysaccharide rich capsule like material. MAPc is the basis of many of physiological and pathogenic features of *M. tuberculosis* and the site of susceptibility and resistance to many anti-tuberculosis drugs.[94,95] **Figure 32** below shows the cell envelop of *M. tuberculosis.*

The unique structure of the MAPc led the scientists to come to the conclusion that the enzymes that synthesize this structure should yield potential drug targets. This has led recent research in this field to be focused on the structure of MAPc and any potential drug targets within the MAPc.[94] **Figure 32** shows a model structure of MAPc.

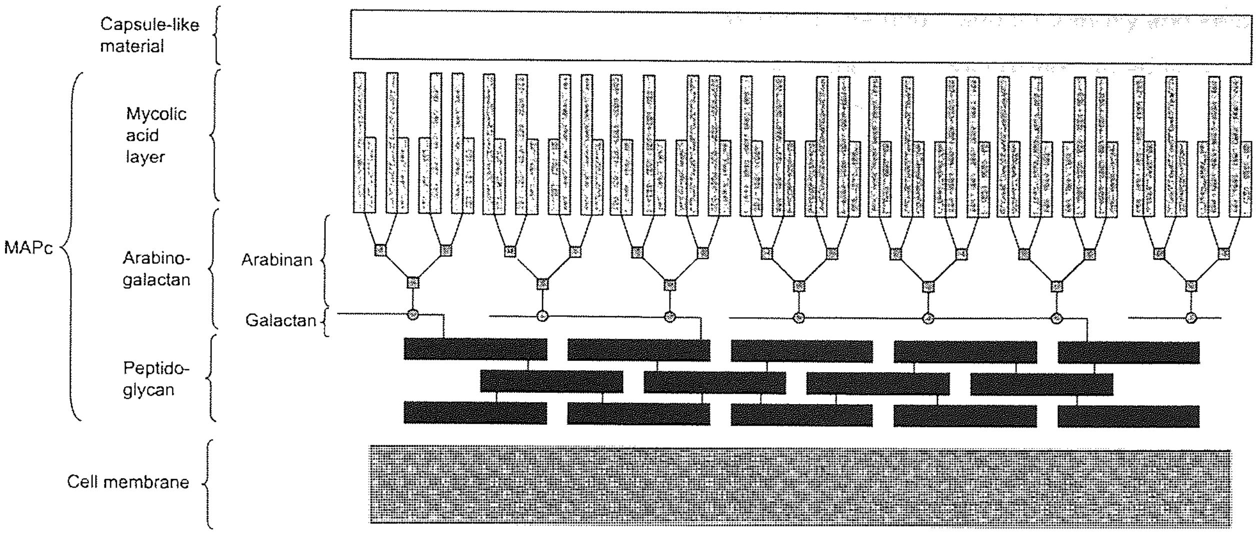


Figure 32: **Schematic cross-section of the *M. tuberculosis* cell envelope. The model shown emphasizes the mycolic acid–arabinogalactan-peptidoglycan complex (MAPc). Figure adapted from Crick *et al*[94]**

1.12.5.1: Peptidoglycan:

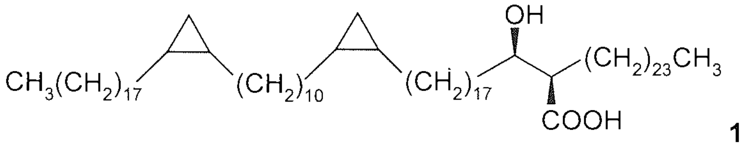
Peptidoglycan is a vital component of the cell wall of virtually all bacteria. It is a macromolecule composed of sugar (glycan) chains cross-linked by short peptide chains. The peptidoglycan plays a vital role in maintaining the shape and mechanical strength of the bacterial cell. If there is any interruption in the synthesis, or any damage to peptidoglycan, the shape of the cell becomes distorted and eventually bursts due to the increase in internal osmotic pressure.[96,97]

1.12.5.2: Arabinogalactan:

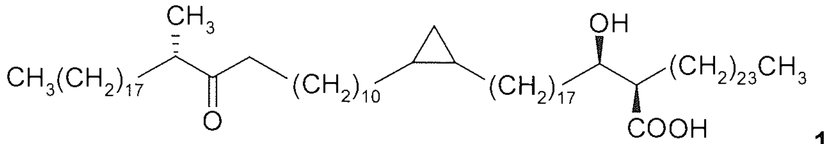
A major cell wall polysaccharide of mycobacteria is a branched-chain arabinogalactan with the arabinose residues forming the reducing termini. It is esterified with mycolic acid and linked, *via* an acid labile phosphodiester bridge to an *N*-glycolyllmyramic acid residue n the peptidoglycan.[94,98]

1.12.5.3: Mycolic acids:

The cell wall contains a high proportion of lipids in the form of mycolic acids. Mycolic acids are characteristic molecules of *Mycobacteria*. They are essential for the integrity of the cell envelope. They are long-chain α-alkyl branched, and β-hydroxylated fatty acids that range in size in mycobacteria from C_{70} – C_{90} and contain a variety of functional groups on the long chain. These functional groups are either nonpolar moieties, such as olefins, methyl branches and cyclopropanes, or polar moieties, such as ketones, methoxy groups, epoxides and esters. In *M. tuberculosis* three distinct classes of mycolic acids are produced, α-mycolates **(119)** containing two cis-cyclopropane rings, and a methoxy and keto series **(120)**, each of which contains a cis- or trans-cyclopropane in the proximal position, in addition to the distal oxygen function with an adjacent methyl branch.[99] (**Figure 33**).



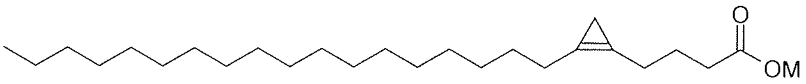
119



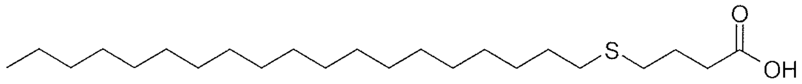
120

Figure 33: Structures of mycolic acids from *M. tuberculosis*, (1) α-mycolate (2) ketomycolate.

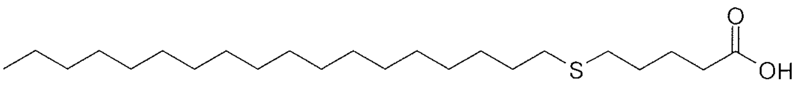
Mycolic acid synthetic pathways offer potential drug targets. There are several reports in the literature which taken together suggest that long chain fatty acids analogues conatining an interrupter group near the carboxyl end may inhibit mycolic acid production and thereby act as anti-tuberculsosis agents. **Figur 34** shows some of such fatty analogues.



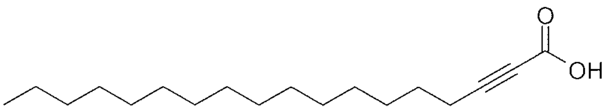
121



122



123



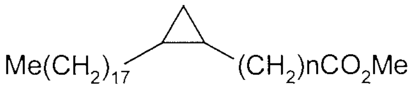
124

Figure 34: Structures of some antimycobacterial fatty acid analogues containing an "interrupter" group near the carboxyl end. These analogues were literature reported by a) besra, Wheeler *et al*, b+c) Barry *et al*[100] and d) Sacchettini *et al*[101],[102].

Investigation into mycolic acids biosynthesis in *M. tuberculosis* by Takayama and Qureshi (1994) indicated that (*Z*)-tetracos-5-enoic acid **(125)** **(Figure 28)** is a key intermediate. This acid was shown to stimulate α-mycolic acid synthesis in extracts of the environmental agent, *Mycobacterium smegmatics*. (*Z*)-Tetracos-5-enoic acid **(125)** was considered to be formed by Δ^5-desaturase from tetracosanoate which was supported by the inhibition of mycolate synthesis by the cyclopropane analogue **(126)** of **(125)** (**Figure 35**). Fatty acid desaturase had previously shown to be inhibited by cyclopropene fatty acid derivatives. Racemic *cis*-cyclopropane acids **(127)** **(Figure 36)** have been synthesized by Hartmann *et al* (1994) and have shown to be weak inhibitors of α-mycolate synthesis in *M. smegmatis.*[103]

125 **126**

Figure 35: (*Z*)-tetracos-5-enoic acid (2) **an intermediate for α-mycolic acid synthesis and its cyclopropane analogue (3).**[103]



n= 3,4

127

Figure 36: *Cis*-cyclopropane acids, shown to be weak inhibitors of of α-mycolate synthesis in *M. smegmatis.* [103]

1.12.6: Current drugs available for the treatment of TB:

The biggest problem with the treatment of the TB is the time it takes to treat it effectively. TB is treated in two stages and a cocktail of between three and nine drugs is administrated for anything between three and nine months. Therefore the patience compliance with the full course of treatment is often unrealistic. During the first few weeks of the treatment TB, when TB symptoms subside as the drugs exert their effect, sufferers feel much better and therefore and may stop taking the medication, particularly if they have to walk many miles to a treatment station, as is often the case in the developing world. This enables the bacteria to mutate and become resistance to that antibiotic, producing the multi-drug resistance strain of TB (MDR-TB).[85]

The MDR-TB is another biggest challenge to treat the TB. In some parts of the world some strains of MDR-TB are resistant to up to ten antibiotics and MDR of this type would be resistant to even the most intensive chemotherapy. It can be rightly said that in reality a poorly administrated treatment is worse than no treatment at all. A partially treated patient actually remains highly infectious and would pass on the MDR-TB. Therefore cheaper faster acting drugs that reduce the treatment time for TB are urgently required.

The Two stages in the treatment of TB are outlined below.

Stage one:

At least three drugs are used to reduce the bacterial population as rapidly as possible and to prevent the emergence of drug resistance. The stage one usually lasts two months. A combination of three to four drugs administrated daily. The dose administrated is dependent on the patient's weight. The drugs of choice are isoniazid, rifampicin, pyrazinamide, ethambutol.[86]

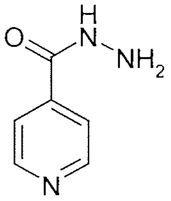
Ethambutol is usually omitted if the risk of resistance to isoniazid is low. Isoniazid resistance is considered likely if the patient had previously been treated for TB, if the patient is immunocompromised or if exposure to a resistant strain is known.[86]

Stage two:

A continuation phase of four to seven months with isoniazid and rifampicin therapy. The treatment can be modified if resistance develops.[86]

1.12.7: Antituberculosis drugs:

1.12.7.1: Isoniazid (INH):

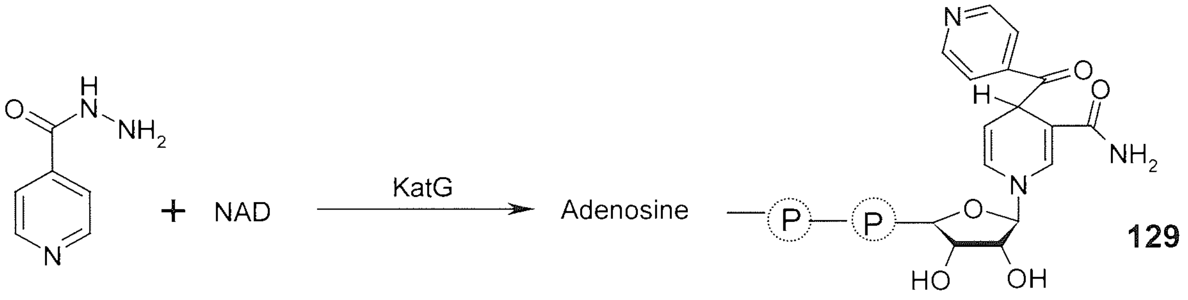


128

Isoniazid (INH) is the hydrazide of isonicotonic acid. It is a synthetic antibacterial agent prepared by reacting the methyl ester of the isonicotonic acid with hydrazine[92]. Isoniazid has high activity against *M.tuberculosis* and is still most widely used drug in antituberculos regimens.[104,105] Isoniazid is bactericidal against replicating tubercle bacilli but bacteriostatic against the nonreplicating organisms.[86] Isoniazid is freely soluble in water, readily absorbed from the gasterointestinal tract or after parentel injection. It is widely distributed throughout the tissues and body fluids including the cerebrospinal fluid. Its ability to penetrate into phagocytic cells makes it active against both extracellular and interacellular organisms.[92,106]

The mechanism of action of INH has only become clearer over the past few years.[86] It owes its activity against *M. tuberculosis* by inhibiting the synthesis of mycolic acids.[86]

Isoniazid is a prodrug that has to be activated in *vivo* to exert its antimycrobial effects. It is susceptible to oxidative reactions catalysed by KatG. KatG is a dual function, catalase-peroxidase, heme enzyme found in *M. tuberculosis.* Genetic studies have shown that the KatG is the only catalase in the *M.tuberculosis*, therefore it is a key enzyme responsible for mycobacterial survival during attack by reactive oxygen intermediates from the host. An isoniazid-NAD (IN-NAD) **(129)** adduct molecule is formed, which is catalayzed by the KatG **(Scheme 21)**, and it is a very tight binding inhibitor of InhA. InhA is the *M. tuberculosis* enoyl-acyl carrier protein reductase, a key enzyme involved in the biosynthesis of mycolic acid[107]. Inactivation of InhA alone is sufficient to inhibit mycolic acid biosynthesis and induces cell lysis very rapidly after exposure of bacteria to INH.



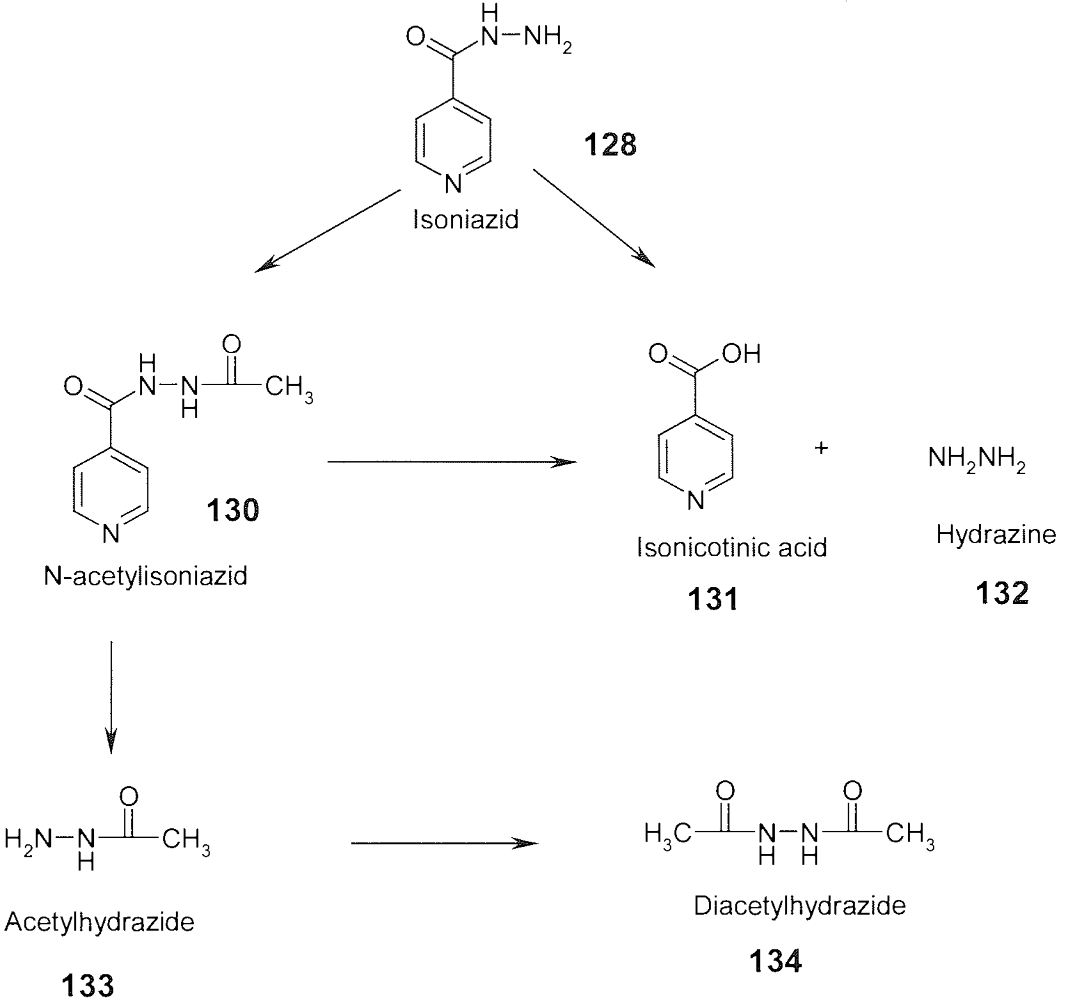
128

129

Scheme 21: The structure of IN-NAD adduct is adapted from ref[104].

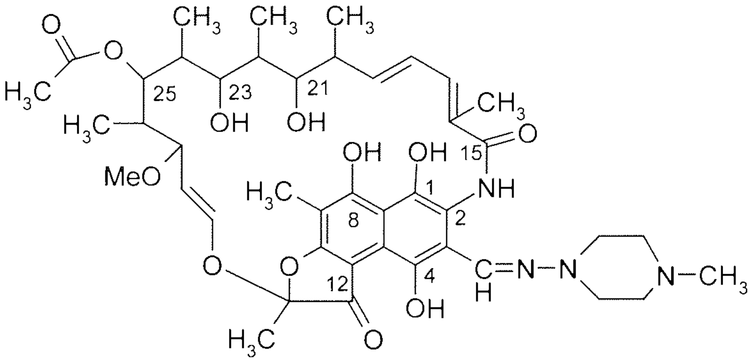
After the treatment with INH, the *M. tuberculosis* loses its acid fastness, which can be interpreted as indicating that the drug interferes with the cell wall development. The INH is extensively metabolised to inactive metabolites (**Scheme 22**). The major metabolite is *N*-acetylisoniazid (AcINH) **(130)**. The enzyme responsible for acetylation, *N*-Acetyltransferase2 (NAT2), is produced under genetic control in an inherited autosomal fashion. Individuals who posses high concentration of the enzyme are rapid acetylators whereas those with low concentrations are slow acetylators. Under similar dosages, some fast acetylators cannot obtain ideal effect, however the slow acetylators may be at risk of side effects.[108] This may result in a need to adjust the dosage for fast acetylators.

Adverse effects are relatively uncommon and are more frequent in people who are slow acetylators. Neurological side effects are the most common and include the restlessness, insomnia, muscle twitcing and difficulty in initiating micturition. More serious but less frequently encountered, neurological side effects include optic neuritis, encephalopathy, anxiety, depression and peripheral neuropathy. Other less common side effects include an influenza like syndrome, hypersensitivity reactions, and rashes.



Scheme 22: **Some of the metabolites of isoniazid.**

1.12.7.2: Rifampin (RIF):



135

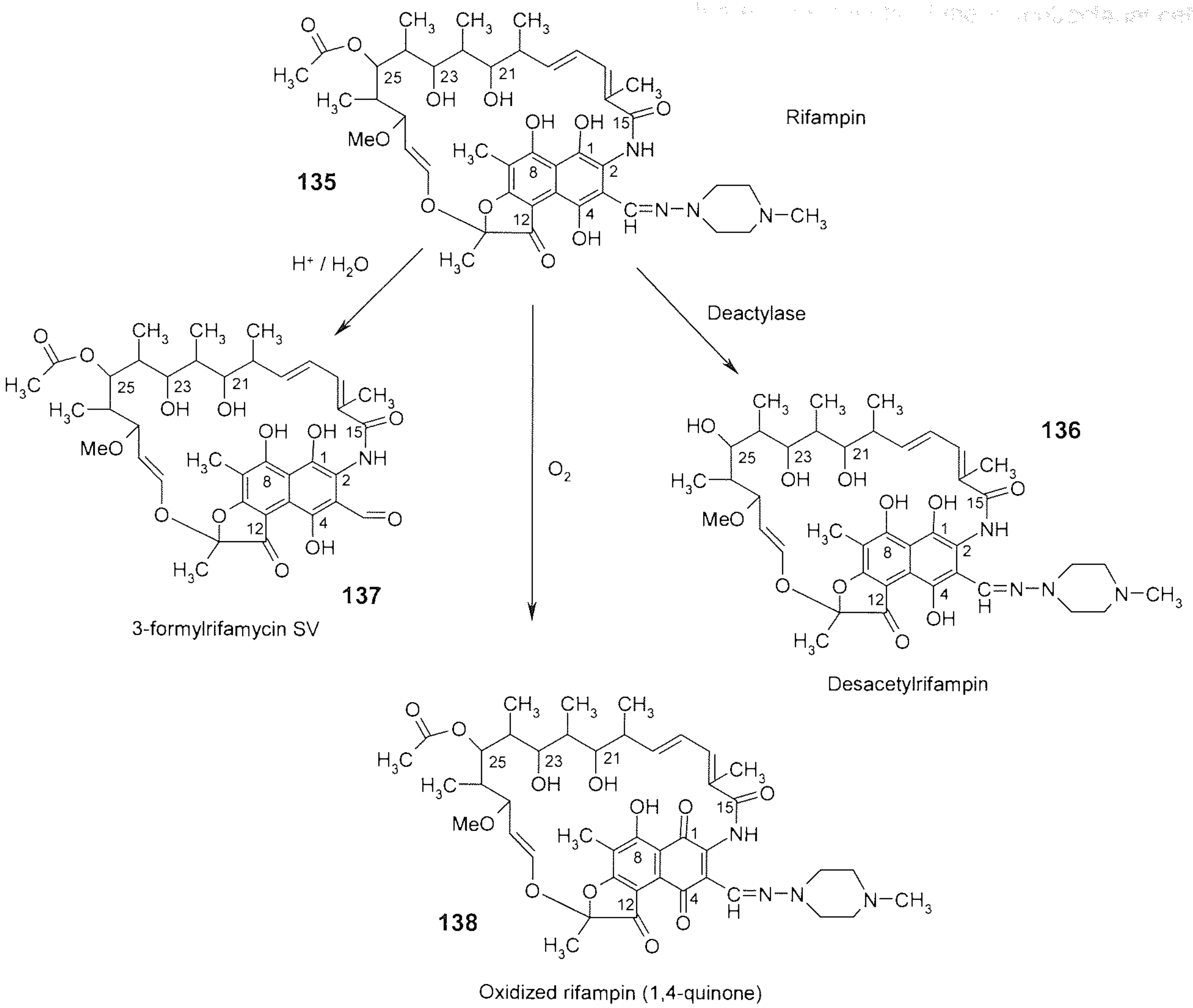
Rifampin (RIF) **(135)** is a semisynthetic derivitive of rifampicin B, an antibiotic produces by *Streptomyces mediterranei*. It belongs to a class of natural antibiotics called ansamycins. This class of compounds is characterized by molecules with an aliphatic chain forming a bridge between two nonadjacent positions of an aromatic moiety.[92,106,109]

Rifampin is bactericidal against a wide range of micro-organisms, including mycobacteria. It has the ability to kill extra- and intracellular organism. Rifampin interferes with the nucleic acid synthesis[86]. It binds strongly to the β-subunit of bacterial DNA-dependent RNA polymerase and thereby inhibits RNA synthesis.[92,106,109] Binding of just one molecule of rifampin inhibits the initiation stage of transcription in which the first nucleic acid is incorporated in the RNA chain. It has been suggested that the structure of rifampin resembles that of two adenosine nucleotides in the RNA, this may form the basis of the binding of the antibiotic to the β-subunit.[96] The action of rifampin is specific for the microbial RNA polymerase and the mammalian enzyme is not affected.[92,96,106,109]

The use of rifampin is complicated by the fact that it is a potent inducer of hepatic enzymes. The metabolism of several drugs (e.g, oestrogen, phenytoin, corticosteriods, sulphonylureas, methadone and anticoagulants) is therefore accelerated.[86]

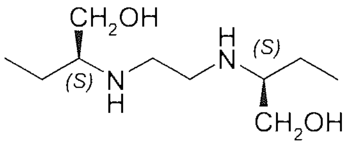
The major metabolism of RIF is by deacetylation, occurs at the C-25 acetate **(Scheme 23).** The metabolite desacetylrifampin **(136)** is also an active antibacterial agent. Another metabolite, 3-formylrifamycin SV **(137)** also posses broad spectrum of activity, but less effective than the RIF.[92,109,110] Rifampin is excreted in urine, bile, saliva, sweat and tears.[92,106,109]

The adverse side affects are rare, however the most common unwanted affects of rifampin includes skin reactions (flushing with/out rash), gasterointestinal disturbances, disturbances of hepatic functions and influenza-like symptoms.[86]



Scheme 23: Metabolism and in vitro reactions of rifampin. The structures of rifampin metabolites are adapted from *ref*[82]

1.12.7.3: Ethambutol:

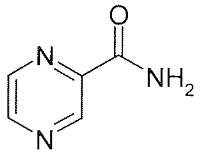


Ethambutol **139**

Ethambutol ((+)-2,2'-(ethylenediimino)di-1-butanol) **(139)**, is a slow acting bacteriostatic agent and its antimicrobial activity is specific to mycobacteria.[86,92,109,111] The *S,S* – absolute stereochemistry is essential and only the dextrorotatory isomer has antituberculosis activity.[96,106,109] The antimycobacterial activity of ethambutol is due to the inhibition of mycobacterial arabinofuranosyl transferases. These enzymes are responsible for glycosylation steps in the biosynthesis of lipoarabinomannan (LAM) and arabinogalactan (AG), which are constituents of the mycobacterial cell wall.[86,111]

The ethambutol is rapidly absorbed after oral administration. It is mainly excreted in urine as unchanged. The unwanted effects are uncommon, the most important being optic neuritis, which can be irreversible if treatment is not promptly discontinued. Other side effects include peripheral neuritis, hyperureicaemia and rarely, thrombocytopenia and jaundice.[86,106]

1.12.7.4: **Pyrazinamide:**



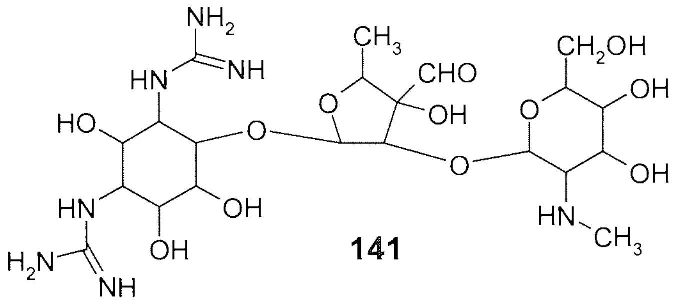
Pyrazinamide **140**

Pyrazinamide **(140)** is an important front-line sterilizing drug that plays a unique role in shortening the TB therapy from previously 9 – 12 months to 6 months.[86],[112]. It is a pro-drug that is converted into the active form pyrazinoic acid (POA) by bacterial nicotinamidase / pyrazinamidase (Pzase).[112]

Pyrazinamide is less active against young growing tubercle bacilli and kills a population of semi-dormant old non-growing bacilli in acidic pH environment that are not killed by other TB drugs. The mechanism of action of pyrazinamide is not fully understood and it has been reported only recently that the principle metabolite pyrazinoic acid, disrupts the cell membrane energetics and inhibits the membrane transport function in *M. tuberculosis*.[112]

Pyrazonamide is usually well tolerated. Pyrazinoic acid inhibits renal excretion of uric acid, occasionally causing gout. Some less common side effects include anorexia, nausea, mild skin flushing and photosensitisation.[86]

1.12.7.5: Streptomycin:



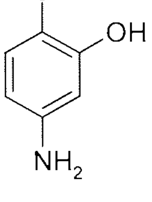
141

Streptomycin (STM) **(141)** is a member of the aminoglycoside-aminocyclitol group of antibiotics.[113] It has a relatively broad-spectrum of antibacterial activity for most gram-positive and negative bacteria and mycobacterial species. STM is water-soluble with basic properties. The hydrophilic nature of the STM results in very poor absorbance from the gasterointestinal tract and it is usually administrated intravenously.[86,109]

STM is taken into cells by active transport where it binds to the bacterial 30S ribosomal subunits, inhibiting initiation of protein synthesis and generating errors in the transcription of the genetic code.[86,113]

Fever, skin rashes and other allergic reactions may result from hypersensitivity to streptomycin.[109] Pain and irritation at the site of injection is also relatively common.[86]

1.12.7.6: Aminosalicylic acid (PAS):

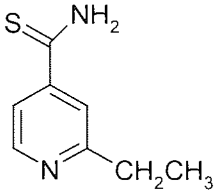


142

p-Aminosalicylic acid (PAS) **(142)** is active almost exclusively against *M. tuberculosis*. Once a popular component in TB therapy PAS is used primarily in case of resistance, re-treatment and intolerance of other agents. A combination of bacterial resistance and severe side effects has greatly reduced its value.[92,106,113]

PAS is readily absorbed from the gastrointestinal tract and it is widely distributed into most of body fluids and tissues. It is believed that PAS prevents the incorporation of p-aminobenzoic acid (PABA) into the dihydrofolic acid molecule catalysed by the enzyme dihydrofolate synthetase.[106]

1.12.7.7: Ethionamid:



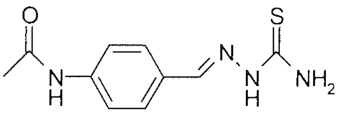
143

Ethionamide **(143)** is used as a second line drug in the treatment of TB and it is active against several species of mycobacteria. It is most frequently used in conjunction with other agents for disease caused by the MDR-TB and leprosy.[114]

The exact mechanism of action of the drug is not fully elucidated, but it appears to inhibit the peptide synthesis in susceptible organisms. Ethionamide is extensively metabolized to active and inactive metabolites. Metabolism is presumed to occur in the liver and six metabolites have been isolated, which are: 1) 2-ethylisonicotinamide, 2) carbonyl-dihydropyridine, 3) thiocarbonyl-dihydropyridine, 4) *S*-oxocarbamoyl dihydropyridine, 5) 2-ethylthioiso-nicotinamide, and 6) ethionamide sulphoxide.

The ethionamide sulphoxide metabolite has been demonstrated to have antimicrobial activity against *Mycobacterium tuberculosis.* The unwanted effects of the drug include stomach upset, loss of appetite, metallic taste in mouth and excessive salivation.[110,114]

1.12.7.8: Thiacetazone:



144

Thiacetazone **(144)** is a member of thiosemicarbazones. It is reported to be specific anti-mycobacterial agent and exhibits potent in vitro activity against *M. tuberculosis* and *M. avium.* It was introduced as a cheap and effective substituent for PAS in combined chemotherapy for tuberculosis and has been used mostly in developing countries where the cost of medicines for the treatment of tuberculosis ia an important factor. The mode of action of thiacetazone is unknown. However some thiacetazone-resistant strains of *M. tuberculosis* exhibit cross-resistance to ethionamide. It has been suggested that thiacetazone, like ethionamide might inhibit mycolic acid biosynthesis.[113]

1.12.7.9: Pyridoxine:

Pyridoxine (vitamin B6) is not an anti-tuberculosis drug. It is usually co-prescribed to patients at an increased risk of peripheral neuropathy (e.g, patients with hepatic impairment, pregnant women, alcoholics, renal dialysis patients, HIV positive patients, malnourished and the elderly).[86]

CHAPTER 2

Results and Discussions on synthesis

2: Results and Discussion on functionalised soluble polymers and their use as soluble polymeric supports in organic synthesis:

Note: Prefix **Pol** in results and discussions and experimental sections, refers to the soluble polystyrene copolymers that were investigated in this thesis. Prefix **W** referrers to the Wang resin polymer of solid-phase synthesis also investigated as acomparasion to soluble polymers.

As mentioned previously (section 1.5, page 43) linear polystyrene polymers as soluble polymeric supports offer advantages over PEG-soluble polymeric supports, by being insoluble in MeOH and water. Therefore it should be possible to perform a vast majority of organic reactions on these soluble polymeric supports. Based on these findings it was decided to prepare and explore a range of linear polystyrene copolymers as soluble polymeric supports in organic synthesis. A {} bracket contains the section number (s) and page number (p) a particular polymer/compound is mentioned in experimental section.

2.1: Investigation on ally methacrylate copolymers as a soluble polymeric support for the synthesis of Δ^2-isoxazoline compounds:

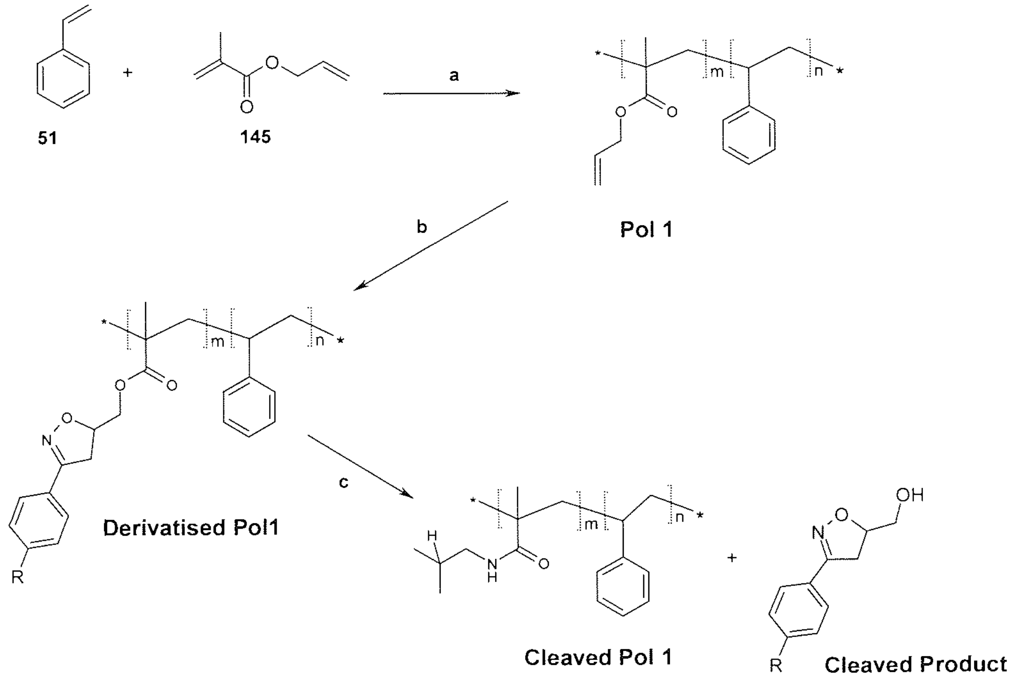
In order to develop polystyrene based soluble copolymers as synthetic supports in organic synthesis, a set of criteria was developed. It was decided to choose a monomer that would,

- give good solubility profile,
- gives a good cleavage yield,
- be inexpensive.

2.1.1: Poly (styrene-co-allyl-methacrylate) copolymers:

As discussed in (section 1.5.5, page 52), there have been reports in the literature for synthesis of of linear copolymers of styrene with allyl methacrylate. Therefore in the light of these literature reports, and as an extension to these findings it was decided to investigate the soluble linear polymers of polystyrene with the allyl methacrylate as a comonomer. **Scheme 24** shows preparation of **(Pol 1)** {s. 5.3.1, p. 159} and its derivatised polymers and the attempted synthesis of Δ^2-isoxazolines.

A polystyrene based polymer (**Pol 1**) was synthesised as a polymeric support for the synthesis of potential anti-mycrobacterial agents. The copolymer was obtained by reacting together styrene and ally methacrylate, in the presence of the radical initiator AIBN, in toluene in a free radical polymerization. Four different batches of the copolymer (**Pol 1**) were made **(Table 11)**. Each of the four batches contained a different ratio of styrene to ally methacrylate. This was done so that the variation of copolymer solubility with co-monomer ratio could be studied. This was undertaken with a view to establishing an appropriate compromise between solubility and the functional group loading.



Scheme 24: **Reagents and conditions: (a) AIBN and toluene, heated at 70 °C for 72 hours, (b) RC=NOH (5 equiv), aqueous sodium hypochlorite (commercially available bleach containing 4.5% w/w chlorine), DCM, (c) 20% v/v isobutylamine, DCM, stirred 24h at RT.**

Batch No (**Pol 1**)	Ratio of styrene and allyl methacrylate		Functional unit loading mmol/g	$CHCl_3$ (mL) needed to dissolve 500mg of polymer.
	Styrene	Allyl methacrylate		
1	2	1	3.00	33
2	3	1	2.28	25
3	4	1	1.85	21
4	5	1	1.55	15

Table 11: **The table above shows the ratios of styrene to allyl methacrylate and the loading of functional units in mmol per gram for Pol 1.**

It was found that all four batches had low solubility. Batch No 1 in particular had very poor solubility making it virtually impossible to obtain a good interpretable ^1H NMR spectrum in $CDCl_3$ or d_6-DMSO. This was also true for the volume of toluene needed to synthesise the polymer, which is again directly related to the solubility of the polymer. As the ratio of the allyl methacrylate to styrene was increased the volume of toluene used also increased. It was also directly proportional to the volume of methanol required to precipitate the polymer. Again as the ratio of ally methacrylate was increased, more toluene was used to keep the polymer in solution therefore more methanol was needed to precipitate the polymer.

Batch No 4 was chosen to carry out further reactions on copolymer **(Pol 1)**, because it gave the best solubility of the group. 1,3-Dipolar cycloaddition reactions **(Scheme 24, step b)** were carried out using nitrile oxides generated, *in situ,* by the action of sodium hypochlorite on RC=NOH. Aqueous sodium hypochlorite was used as commercially available bleach, containing 4.5% w/v chlorine. For every gram of oxime used 18mL of bleach was used. The step b had practical difficulties, which reduced the overall suitability of the polymer. The main problem was associated with the solubility of the polymer. The reaction was performed in a biphasic system using commercially available bleach (the aqueous system) and the DCM (organic system). The presence of aqueous layer made it more difficult to keep the polymer in solution. Almost three times more DCM was required to perform this reaction when compared to a reaction performed in a monophasic system, otherwise a cloudy reaction medium was achieved. As a result the overall reaction medium was more dilute, and this possibly, slowed down the overall reaction kinetics.

At the end of step b, the two layers were separated, and DCM layer was dried over magnesium sulphate. The filtration process to remove the magnesium sulphate was extremely slow. The polymer solution was diluted to about 100mL per gram of polymer to achieve this step, but even then it was time consuming.

Initially two aldoximes, 4-*tert*-butyl benzaldehyde oxime and 4-chlorobenzaldehyde oxime, were reacted with the polymer-bound alkenes on **Pol 1**, and produced **Pol 12** {s. 5.5.1 p.174} and **Pol 13** {s. 5.5.2 p.174} respectively. It was anticipated that the lipophilicity of 4-*tert*-butyl group would help to increase the solubility profile of the derivatised copolymer.

A comparison between the ^1H NMR spectrum of the un-derivatised copolymer **Pol 1 (Figure 37, Pol 1)** and derivatised **Pol 12 (Figure 37, Pol 12)** shows that polymer **Pol 1** gave a very narrow window in ^1H NMR spectrum to monitor the reaction. The only evidence that the 1,3-dipolarcycloaddition had taken place came from the disappearance of the peaks between δ4.0 and δ5.0 ppm **(Pol 1).** Any new peaks corresponding to the formation of a new compound were not observable. This can be compared to the literature reported ^1H NMR spectrum of the copolymer of allyl methacrylate (section 1.5.5, page 54).

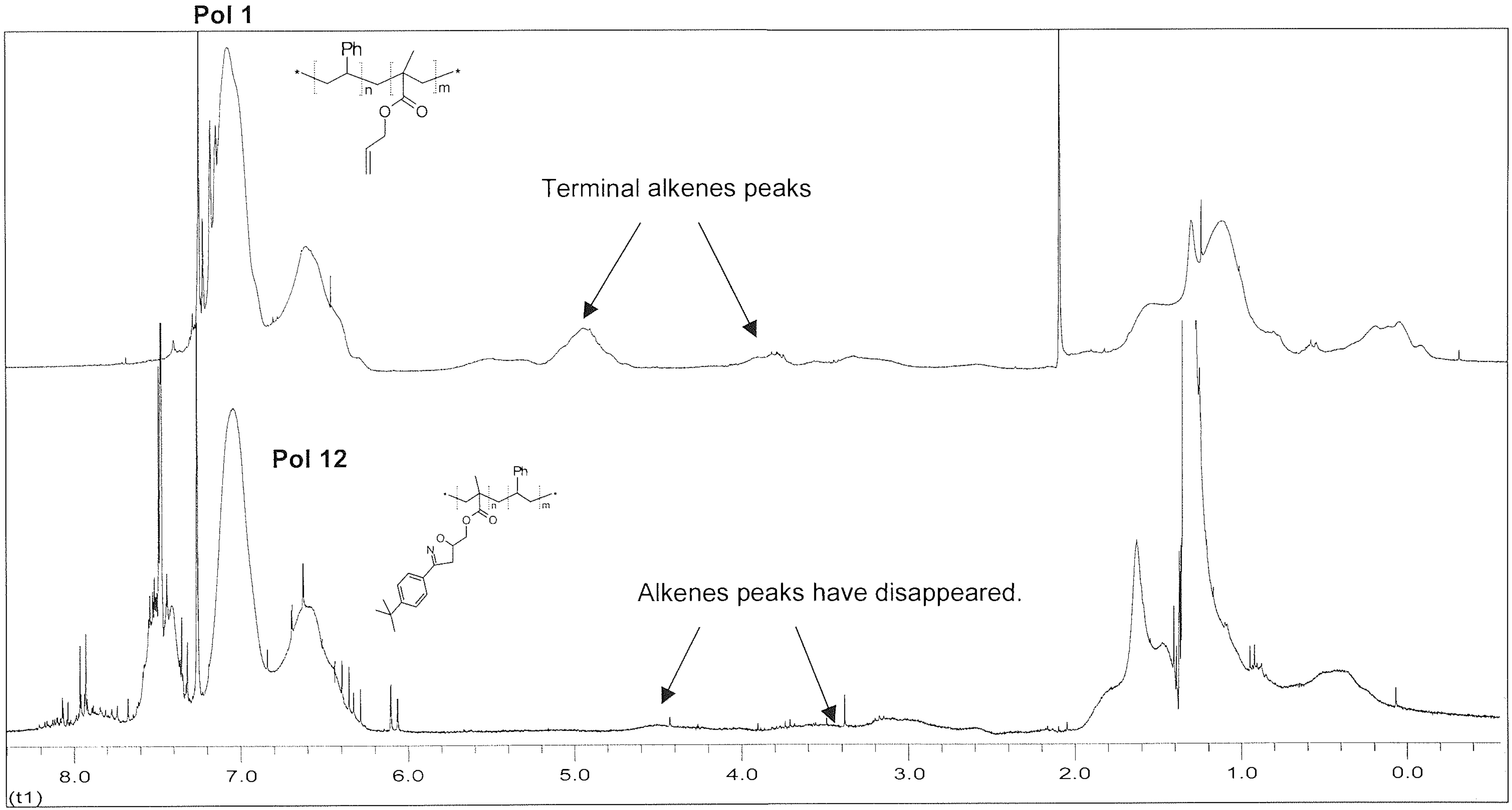


Figure 37: Following the progress of reaction on polymeric support. Derivatised copolymer Pol 1 and derivatised copolymer Pol 2. It is clear from Pol 1 spectrum that there is a very narrow ^{1}H NMR window available to monitor the progress of a reaction on this polymer.

Step c highlighted another unfavourable characteristic of the polymer (**Pol 1**). The cleavage point at the ester group being too close to the polymer backbone presented steric hindrance for nucleophilic attack by the isobutyl amine. Therefore the cleavage of the polymer-bound compounds **(146a)** and **(146b)** was not very successful. It is apparent from the ^{1}H NMR spectrum of the cleaved products, **(Figures 38** and **39)**, the target compounds were not obtained. However the mass spectrum of the crude products show little evidence for the presence of the target compounds as for both compounds **146a** and **146b** peaks were observed at the M+H (234) and (211) respectively. It was very difficult to purify the compounds of interest as the R_f values of impurities in crude products were very close with the product in several different solvent systems. The expected ^{1}H NMR of the cleaved compounds were obtained using the CHEM Draw Ultra 8.6 version, for comparison purposes. When compared with the ^{1}H NMR of the crude products, it is clear that the target compounds were not obtained.

Based on these results it was decided to abandon the polymer **Pol 1** and move onto the synthesis of other soluble polymeric supports where the cleavage point is possibly away from the polymer backbone and which gives more useful solubility profiles.

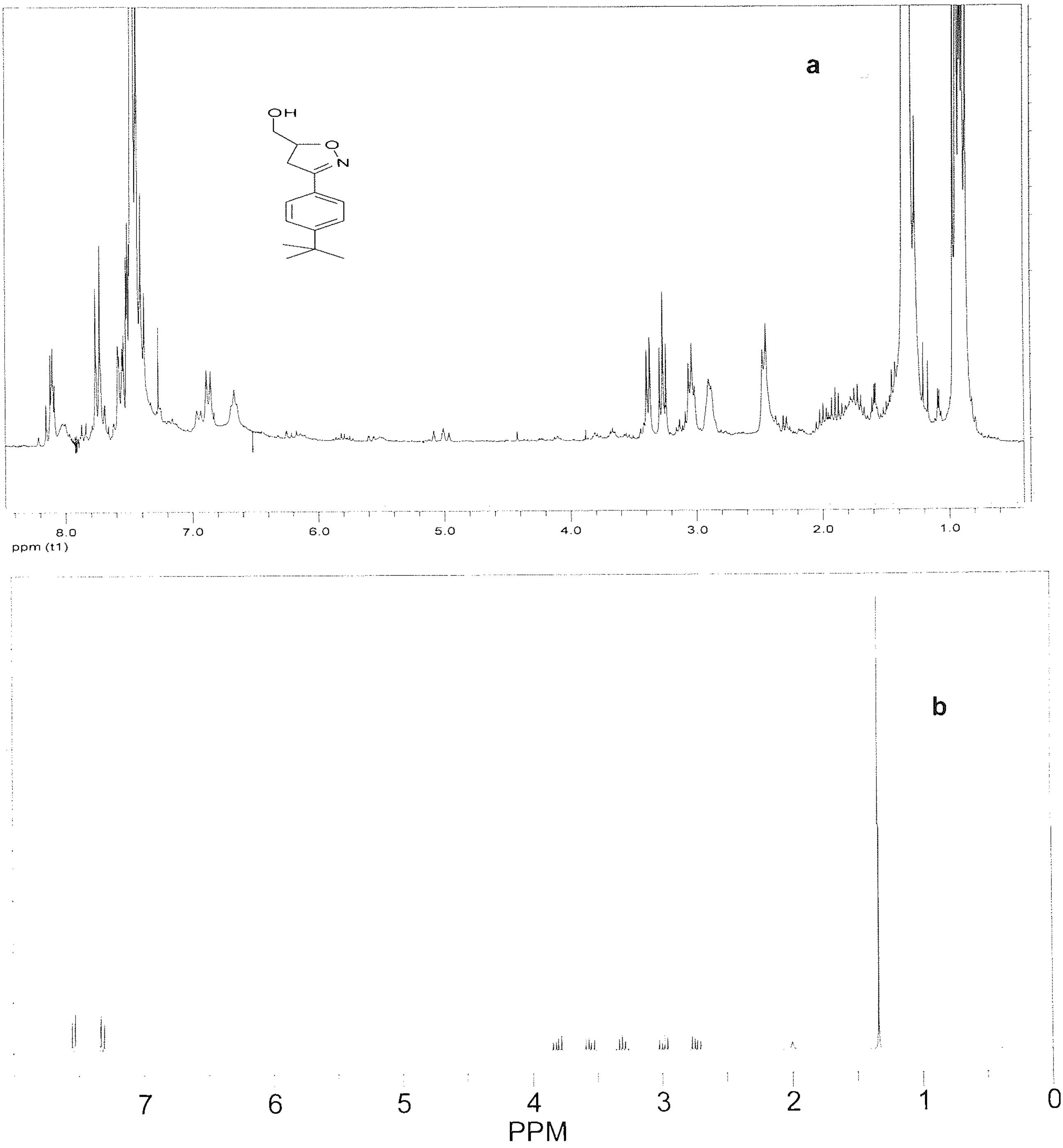


Figure 38: The structure and the ^{1}H NMR spectrum of crude [3-(4-tert-Butyl-phenyl)-4,5-dihydro- isoxazol-5-yl]-methanol (146a) obtained by isobutylamine mediated cleavage from the polymeric support. The expected spectrum for this compound is obtained from the CHEM Draw Ultra 8.6 as a comparison.

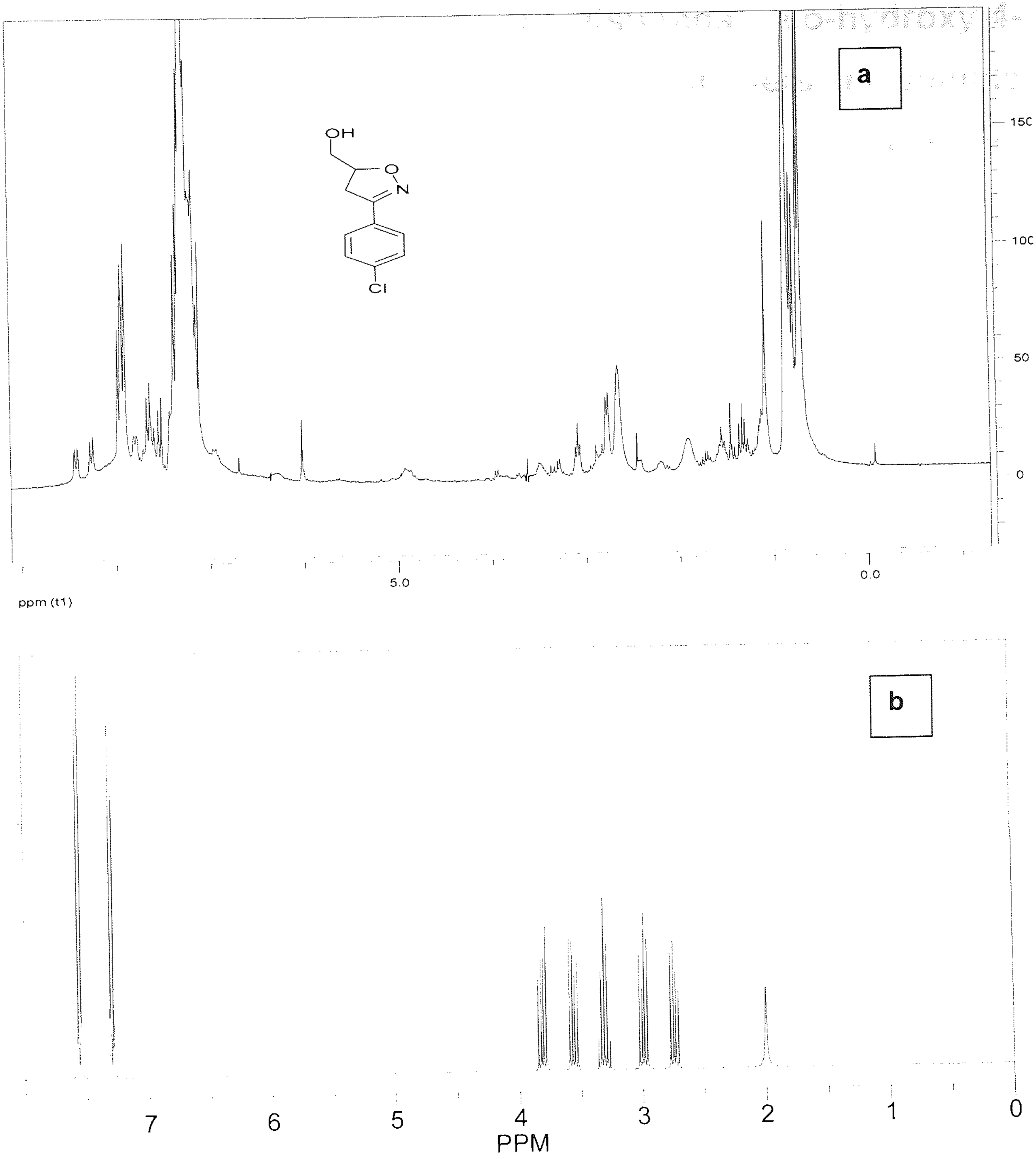
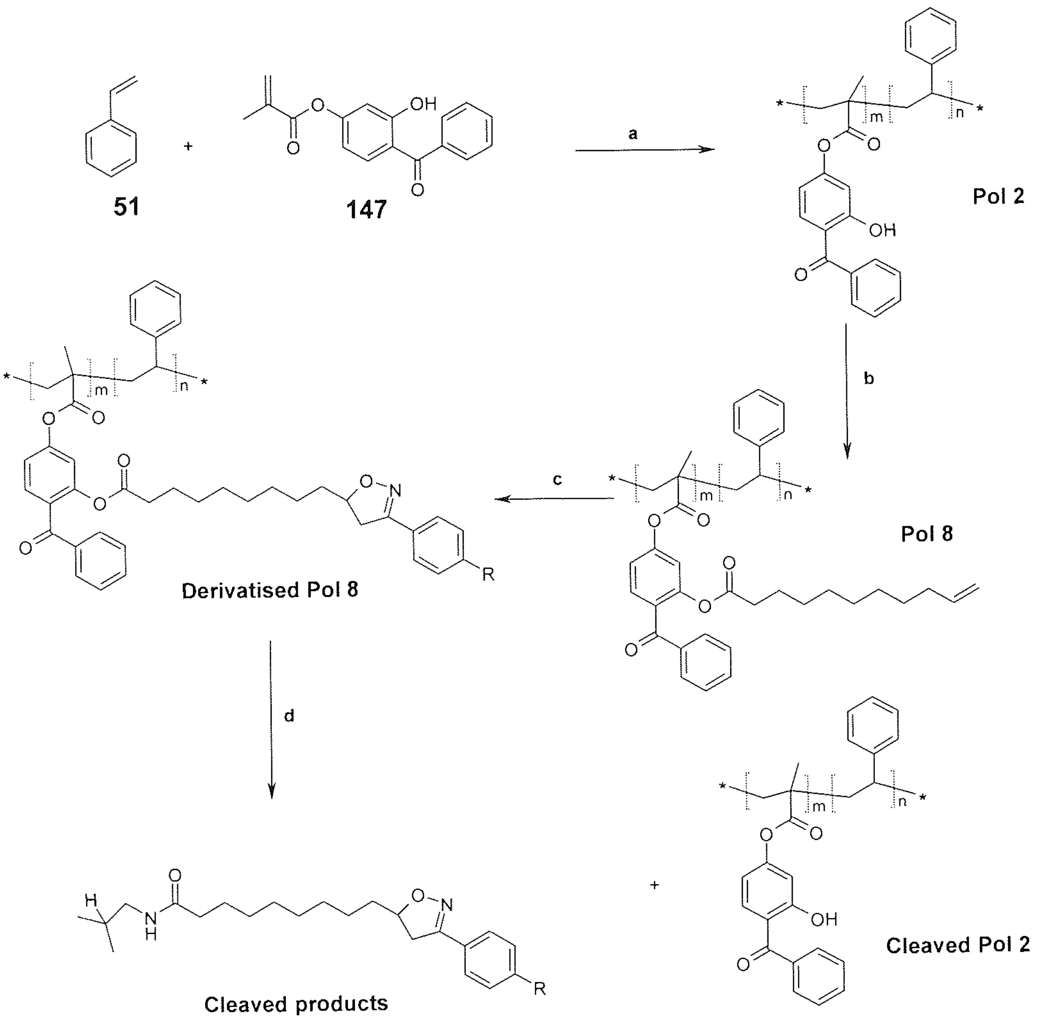


Figure 39: The structure and the ^{1}H NMR spectrum of crude [3-(4-Chloro-phenyl)-4,5-dihydro-isoxazol-5-yl]-methanol (146b)obtained by isobutylamine mediated cleavage from the polymeric support and expected ^{1}H NMR spectra is obtained from CHEM Draw Ultra 8.6.

2. 2: Investigation on poly(styrene co-hydroxy-4-(methacryloyloxy)) benzophenone copolymers as soluble polymeric support for the synthesis of Δ^2-isoxazoline compounds:

After the unsuccessful attempts with copolymers of allyl methacrylate and styrene it was decided to prepare soluble polymeric supports with between solubility profiles. Therefore a soluble polymeric support **(Pol 2)** {s.5.3.2, p. 160} was synthesised by copolymerising the styrene and 2-hydroxy-4-(methacryloyloxy) benzaphenone. It was anticipated that the presence of two aromatic groups, a ketone group and a hydroxyl group would give a better solubility profile as well as a greater chance to cleave the product from the resin. The **Scheme 25** depicts the procedure followed for preparation of **Pol 2** and its derivatised polymers.



Scheme 25: (a) AIBN, toluene heated under reflux at 70°C, (b) anhydrous $CHCl_3$, 10-undecenoyl chloride, Amberlite IRA-67, D-map, heated under reflux 70°C under argon, (c) DCM, aldoxime, sodium hypochlorite, (d) isobutylamine, DCM.

The solubility of polymer **Pol 2** was slightly better than polymer **Pol 1**. The addition of an 11 carbon chain further enhanced the solubility. The ^{1}H NMR spectrum gave a better window to monitor the reaction at each step. **Figure 40** shows spectrums from the parent as well as the derivatised co-polymers. The acylated polymer **(Pol 8),** {s. 5.4.1, p. 163} shows peaks at δ4.9 and δ5.7-5.9 ppm, a triplet and an overlapping multiplet respectively, arising from the presence of polymer bound terminal alkenes. **Pol 8** was reacted with two aloximes, 4-*tert*-butyl benzaldehyde oxime and 4-chlorobenzaldehyde oxime. As a result derivatised polymers **Pol 14** {s. 5.5.3, p. 175} and **Pol 15** {s. 5.5.4, p. 175} were made, respectively. The derivatised Polymer **(Pol 14)** spectrum showed no peaks at the region of δ4.9 and δ5.7 ppm indicating that a reaction had taken place at the polymer-bound terminal alkenes.

Pol 2, however also had similar practical difficulties as those mentioned for **Pol 1**. The main limiting factor once again was the cleavage step to obtain the target compound. The cleavage point being quite close to the polymer backbone caused the steric hindrance for the cleavage step to take place effectively and no cleaved products were obtained from **Pol 14** and **Pol 15**. However **Figure 41** shows ^{1}H NMR spectrum of the cleaved compound **(148)** from **Pol 14** and it can be seen the desired compound was not obtained.

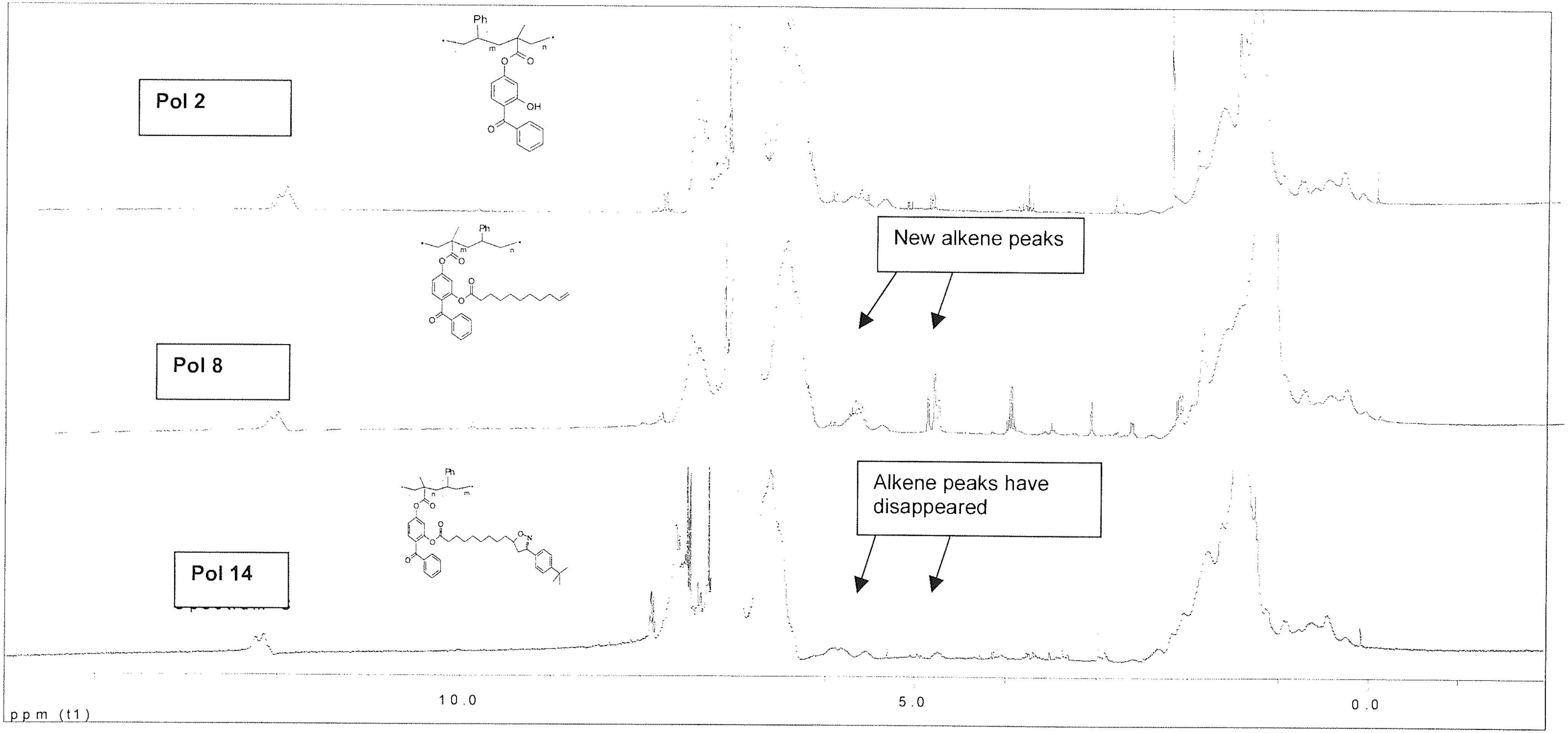


Figure 40: Follow up of reactions on polymer Pol 2 after each step was carried out (except the cleavage step). Parent polymer (Pol 2), acylated polymer after reaction with 10-undecenoyl chloride (Pol 8), and (Pol 14) is the polymer that has successfully undergone 1,3-dipolarcycloaddition reactions.

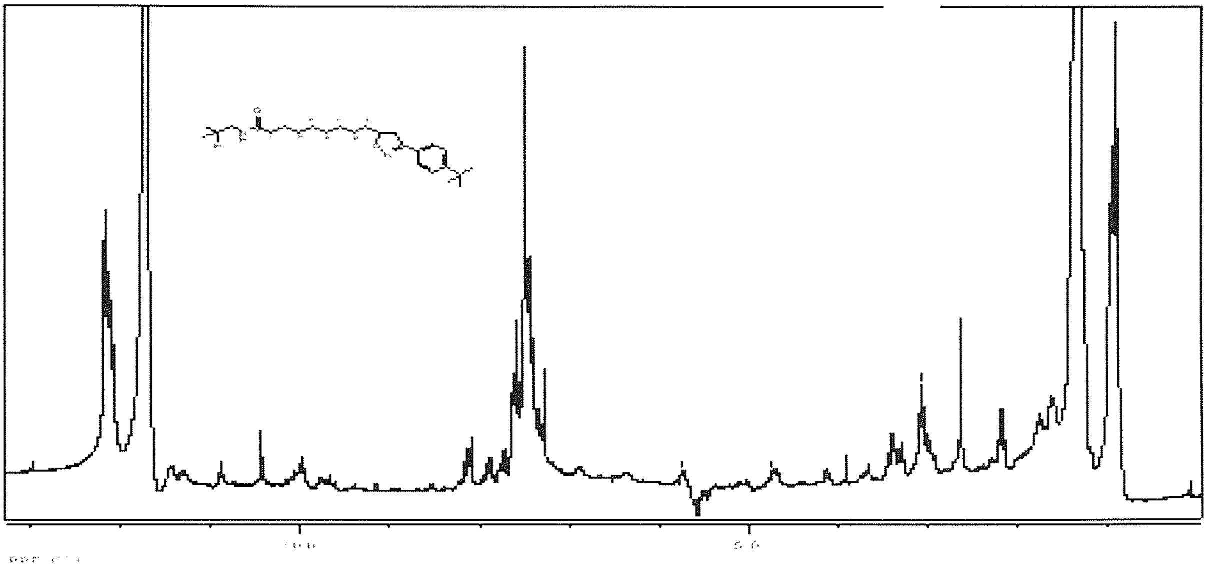


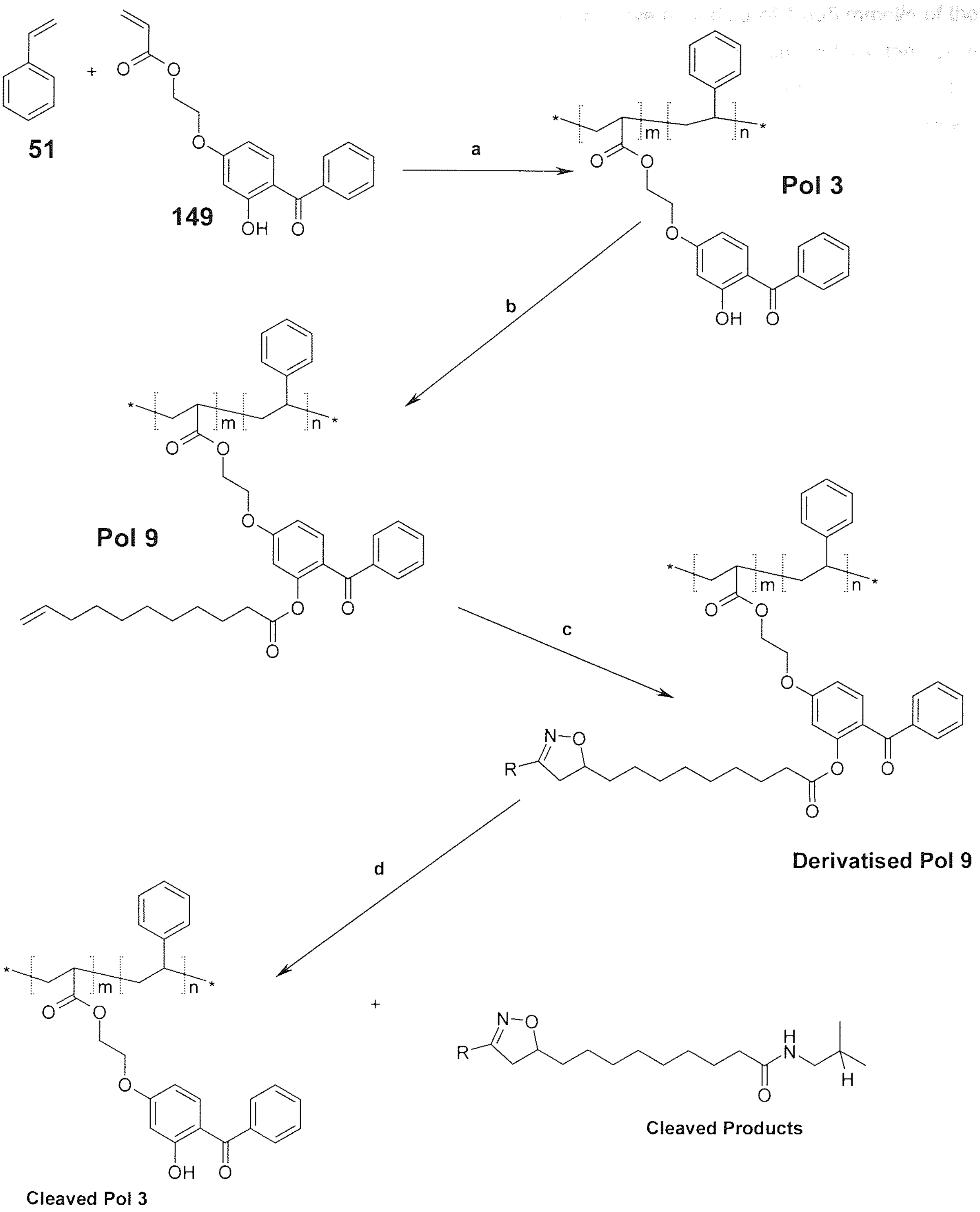
Figure 41: **A ^{1}H NMR spectrum of the crude product (147) cleaved from copolymer Pol 14.**

2.3: Poly (Styrene-co-undec-10-enoic acid -5-(2-acryloyloxy ethoxy)-2-benzoyl phenyl ester) copolymers:

In the light of above attempts and to overcome the chemical and physical difficulties mentioned above, the criteria for the synthesis of soluble polymeric supports was revised and It was decided to choose a monomer that would,

- give better solubility profile,
- have reactive site away from the polymer backbone,
- gives a good cleavage yield,
- be inexpensive.

The monomer 2-(4-benzoyl-3-hydroxyphenoxy) ethyl acrylate was chosen, because it contains a two-carbon spacer group (C–C–O) that places the functional site further away from polymer backbone. Therefore the site should be more accessible to reagents. It should also give better solubility profile from those polymers made above. Having the hydroxyl group directly attached to the aromatic group should also stabilize the leaving group in the hydrolytic cleavage step, thus should give a good cleaved yield. **Scheme 26** below shows the steps involved to synthesise the potential anti-mycobacterial agents on polymeric support.



Scheme 26: Synthesis of modified soluble polymeric support and its use to synthesise the target compound. (a) AIBN, toluene heated under reflux at $70^{\circ}C$ (b) anhydrous $CHCl_3$, 10-undecenoyl chloride, Amberlite IRA-67, D-map, heated under reflux $70^{\circ}C$ under argon, (c) DCM, aldoxime, sodium hypochlorite, (d) isobutylamine, DCM.

To establish an appropriate compromise between functional group loading and the solubility of the copolymer **Pol 3**, {s. 5.3.3, p. 160} four different batches were made, see **Table 12** below. Each of the four batches contained different ratio of styrene to 2-(4-benzoyl-3-hydroxy-phenoxy) ethyl acrylate.

Batch N° 3 was found to be the most appropriate of the group, gave a loading of 1.335 mmol/g of the functional unit and a 98% yield. Although batches Nos 1 and 2 gave good solubility profiles, they gave very low theoretical loadings of the functional unit and low percentage yields. On the other hand the batches Nos 4 and 5 gave higher theoretical loadings of the functional unit, 2.401 and 3.20 mmol/g respectively, but they gave poor solubility. Therefore batch No 3 was employed to carry out further reactions to synthesise our target compounds.

Pol 3 Batch No	Styrene (mmol)	Monomer (mmol)	Theoretical loading of functional group (mmol/g)	Actual loading of functional group (mmol/g)	Yield (%)
1	40.5	1	0.221		54
2	21	1	0.4	0.608	54
3	5	1	1.186	1.335	98
4	2	1	1.923	1.194	71
5	0.0	1	3.20	3.20	69

Table 12: The ratios of styrene to 2-(4-benzoyl-3-hydroxy-phenoxy) ethyl acrylate used to obtain different batches of co-polymer Pol 3. The loading of functional group in mmol/g of co-polymer is also shown, both theoretical and calculated from the integration of the ^{1}H NMR spectrum.

The polymer was soluble in chlorinated solvents i.e. DCM and $CHCl_3$, toluene and THF. It was sparingly soluble in ethyl acetate and acetonitrile, and insoluble in methanol, ethanol, acetone, water, diethyl ether and petroleum ether.

2.3.1: Acylation of polymer bound phenol.

The acylation of the polymer bound phenol (**Pol 3**) was carried out with three different acid chlorides and gave esters derived from,

1.	10-Undecenoyl chloride,	**Pol 9** {s. 5.4.2, p. 165}
2.	4-Pentenoyl chloride,	**Pol 10** {s. 5.4.3, p. 166}
3.	Acryloyl chloride.	**Pol 11** {s. 5.4.4, p. 166}

The acylation of the polymer bound phenol was carried out in the presence of the basic catalyst 4-*N,N*-dimethylaminopyridine (DMAP), under argon in anhydrous conditions. To remove the HCl, the by-product formed in the reaction, triethylamine (TEA), pyridine and Amberlite™ IRA 67 resin (resin-bound tertiary amine) were investigated. It was found that the former two were difficult to get rid off and a trace amount was always present in the ^{1}H NMR spectra of the polymer, even after several re-precipitations. The use of Amberlite™ IRA 67 resin meant that unnecessary re-precipitations of the acylated polymers were no longer needed. The resins were simply removed by filtration, and clean uncontaminated polymers were obtained.

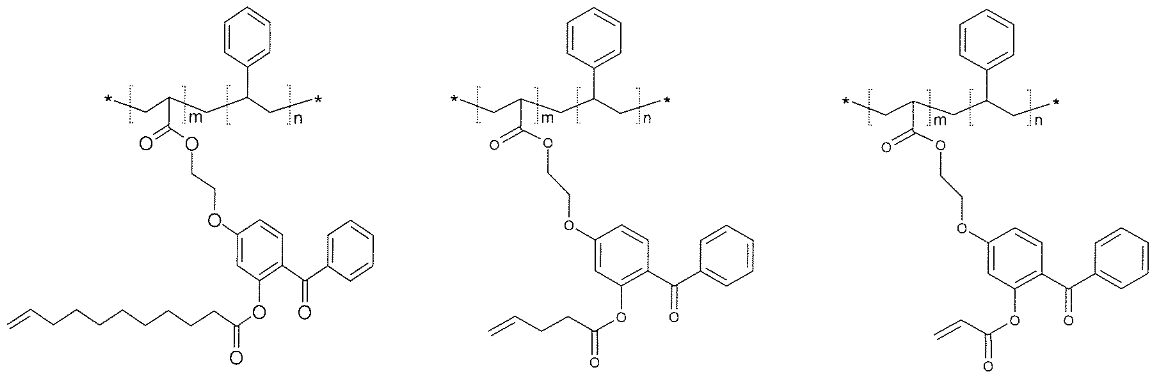
Initially the acylation step, with all acid chlorides used, was carried out at RT. It was found that the time and the temperatures required for the acylation were directly related to the molecular weight of the acid chlorides. Acylation with acryloyl chloride was carried at room temperature and the reaction vessel was wrapped up in aluminium foil to prevent any light induced self-polymerisation of the acryloyl chloride. Reactions with 4-pentenoyl chloride required slightly higher temperatures ($40^{o}C$) and twice as long (36 hours) as the acryloyl chloride (18 hours). 10-Undecenoyl chloride required heating under reflux in chloroform for up to 48 hours for the same reaction.

From the ^{1}H NMR spectra of the acylated polymers, in deuterated chloroform, it was noted that 100% acylation of the polymer bound phenol was not achieved with any of the acid chlorides used. This was investigated with different reaction temperatures, for the reaction with 10-undecenoylchloride. The reaction time and reaction concentrations were kept constant. In most cases between 25–50 % of polymer bound phenol was acylated, as estimated by the ^{1}H NMR integration. See **Table 13** below.

Due to the nature of the copolymers made, it was difficult to monitor the progress of the reactions. The only options available were ^{1}H NMR spectroscopy and the IR spectroscopy. Mass spectrometry, UV spectroscopy and thin layer chromatography (TLC) didn't work with the polymers. It was found that even IR spectroscopy was not very helpful to analyse the reaction progress. Therefore the only real option was ^{1}H NMR spectroscopy. In ^{1}H NMR spectra any new peaks in the regions of δ1.00–δ2.30, δ3.60 – δ4.10 and δ6.20 – δ7.70 ppm were not easy to see as the peaks due to the polymer backbone covered these regions. The window available to monitor the reaction was around the regions of δ2.30 – δ3.60 and δ4.10 – δ6.30 and downfield to δ7.70 ppm. **Pol 9** gave a better ^{1}H NMR spectrum to view the progress of further reactions carried out on the polymer when compared to **Pol 10** and **Pol 11**. Peaks arising from the terminal alkene of the derivatised polymers were the only measure to monitor the reaction progress. As the aliphatic chain length was decreased the peaks corresponding to the terminal alkene moved down field closer to the peaks coming from the polymer backbone in the aromatic region. For **Pol 9** and **Pol 10** the alkene peaks were observed at δ4.92 – 5.03 and δ5.74 – 5.90, and for **Pol 11** these were seen at δ5.80 and δ6.04 ppm. This could be explained from the structure of these polymers. See **Figure 42**. For **Pol 9** and **Pol 10** the alkene double bond is not adjacent to the carbonyl carbon. Whereas for **Pol 11** the alkene double bond and the carbonyl group are in conjugation, making it electron deficient therefore it is deshielded from the applied magnetic field. Hence signals from these protons are observed further downfield. It was also observed that **Pol 9** gave sharp peaks and **Pol 11** gave broad peaks for the terminal alkene. Again this is due to the difference in the carbon chain length between the carbonyl group and terminal alkene. For **Pol 9** the alkene bond is further away from the polymer backbone, therefore better resolved than **Pol 11**, which has alkene double bond much closer to the polymer backbone. See **Figure 43** below for a comparison between the peaks arising from terminal alkenes at the end of varying length of aliphatic chains.

Pol 9 (1.335 mmol/g)	T (°C)	Acylated phenol (%)	Actual loading of the functional group (mmol/g)
Batch a	55	25	0.27
Batch b	60	31	0.33
Batch c	70	40	0.44
Pol 10	40	44	0.48
Pol 11	RT	49	0.54

Table 13: The table shows acylated phenol in % and the subsequent loading of the functional group in mmol/g of the polymer, when started with a functional group loading of 1.335 mmol/g under different conditions.



Pol 9 **Pol10** **Pol 11**

Figure 42: Structures of acylated Polymers bearing terminal alkenes, Pol 9, Pol 10 and Pol 11

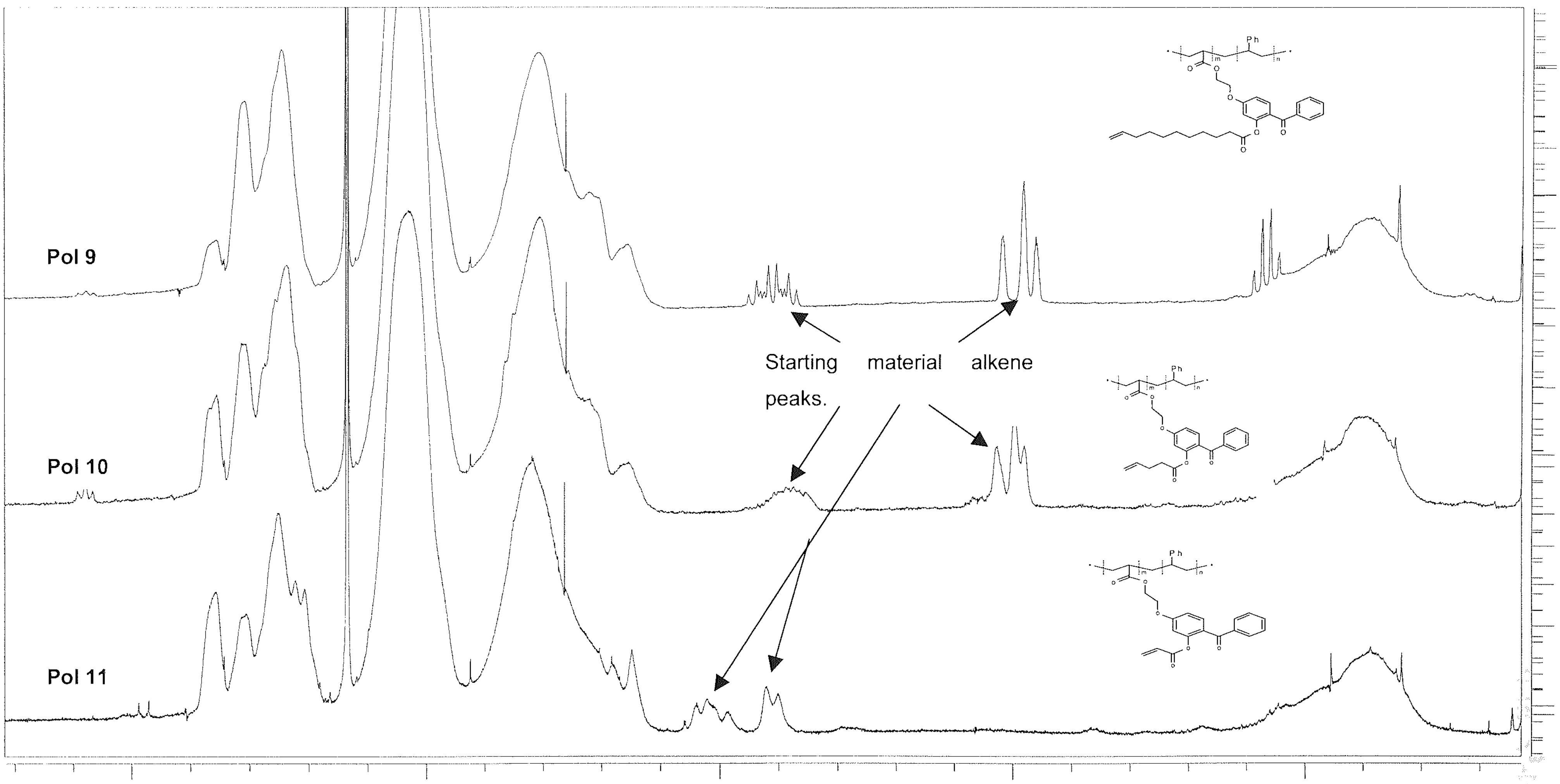


Figure 43: A ^{1}H NMR spectrum of Pol 9, Pol 10 and Pol 11 showing the signals coming from the terminal alkenes of each polymer.

2.3.2: Synthesis of polymer bound Δ^2-isoxazoline compounds *via* 1,3-dipolar cycloaddition reaction between polymer bound terminal alkene and nitrile oxides:

The 1,3-dipolar cycloaddition reactions between nitrile oxides and terminal alkenes and alkynes have been studied extensively in solution phase. They have also been studied in the solid-phase where the dipolarophile is either resin bound or suspended in solution.[115,116,117] However the study of 1,3-dipolar cycloaddition reactions between the polymer bound terminal alkene and the nitrile oxides, generated *in situ* from their corresponding aldoximes, in polystyrene soluble polymeric supports this date have not been reported.

2.3.4 1,3-Dipolar cycloaddition reactions on polymer bound alkenes on Pol 9:

The polymer bound Δ^2-isoxazoline compounds were afforded *via* the 1,3-dipolar cycloaddition reactions. The polymer was dissolved in DCM. Aqueous sodium hypochlorite, in the form of commercially available bleach, was added to the reaction vessel. The reaction was stirred vigorously. The aldoxime solution, in DCM, was added drop-wise, to form the nitrile oxide, which reacted *in situ* with the polymer-bound alkene in a one pot to furnish the corresponding polymer bound Δ^2-isoxazoline compounds. Cleavage of the product from the polymer was achieved by treating the polymer with isobutylamine (20% in the overall reaction medium) in DCM.

Once the polymer solution had been dried over magnesium sulphate, after separating it from the biphasic system, the filtration step to remove the magnesium sulphate proved to be very time consuming. The polymer solution had to be diluted first to facilitate faster filtration and then the filtrate was concentrated under reduced pressure and the polymer was precipitated by pouring the filtrate onto rapidly stirred methanol.

The ^{1}H NMR spectra of the polymers revealed that if the polymer solution was poured fast and/or if the methanol was not stirred rapidly the polymer obtained was not clean and it retained un-reacted starting materials and by-products. To obtain clean polymers, the polymers were re-precipitated up to three times, and if necessary the polymer solution was added drop-wise to rapidly stirred methanol, so that polymer was precipitated slowly and hence should be free of any unreacted, unbound reagents that otherwise could get trapped with the polymer.

The reactions were analysed by ^{1}H NMR spectrum observing new peaks that could correspond to the formation of new compound and also disappearance of alkene peaks at δ4.92–5.03 and δ5.74–5.90 ppm. It was not possible to observe new peaks in the aromatic and aliphatic regions as well as around δ3.55-δ4.30 ppm, because of the peaks coming from the polymer backbone. The disappearance of alkene peaks gave the indication that a reaction has taken place at this group. A successful 1,3-

dipolar cycloaddition reactions on **Pol 9** gave following observations. A weak signal of a multiplet observed around δ4.50-δ4.80 ppm corresponded to the isoxazoline chiral proton. Two individual multiplets between δ2.80-δ3.60 ppm came from the prochiral protons from the isoxazoline ring. Any other peaks arising from the substituted groups at 3 position of the isoxazoline ring were observable sometimes only, depending on the nature of the substituted group.

2.3.5: 1,3-Dipolar cycloaddition reactions on polymer bound alkenes on Pol 10:

The polymer bound Δ^2-isoxazoline compounds were made in the same way as described above in section 2.3.4. Similar problems to **Pol 9** were encountered during these reactions and reactions were monitored in the same way as mentioned above. Disappearance of the alkenes peaks and presence of any new peaks in the regions stated above were the only measure to analyse the progress of polymer bound compounds. It was noted that the observance of peaks corresponding to the polymer bound compounds were not as clear and noticeable as with the derivatised polymers made from **Pol 9**. This can be attributed to the fact that for **Pol 9** there is an aliphatic chain comprising of eight carbons between the ester linkage and the isoxazoline ring, which places the polymer bound compound further away from the polymer backbone. Therefore giving this group of polymer bound compounds better resolved ^{1}H NMR spectrum. Whereas for **Pol 10** there is only a two-carbon chain between the ester linkage and the isoxazoline ring. This places **Pol 10** polymer-bound compounds much closer to the polymer backbone, when compared to **Pol 9**. Hence the ^{1}H NMR spectrum for **Pol 10** polymer bound compounds is less resolved than **Pol 9** polymer bound compounds. However the observance of peaks corresponding to the polymer bound compounds is depended on the 3-substituted groups.

2.3.6: 1,3-Dipolar cycloaddition reactions on polymer bound alkenes on Pol 11:

To achieve **Pol 11** polymer-bound Δ^2-isoxazoline compounds, the procedure mentioned in sections 2.3.4 and 2.3.5 was adopted. The alkene peaks at δ5.80 and δ6.04 ppm in the ^{1}H NMR spectrum of **Pol 11** were very close to the peaks observed for polymer bound styrene and other aromatic groups. As with **Pol 9** and **Pol 10**, for **Pol 11** also, disappearance of the alkene peaks were the only evidence that a reaction has take place at the polymer bound alkene. Only this time the isoxazoline ring would be directly attached to the ester linkage, placing it very close to the polymer backbone. Therefore no additional peaks were observed which could correspond to the polymer bound isoxazoline compounds.

2.4: Polymer-bound isoxazoline compounds:

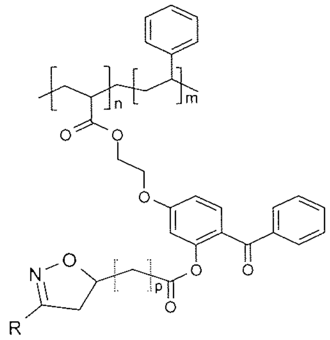


Figure 44: **a general structure for polymer-bound Δ^2-isoxazolines.**

As discussed above that it was not possible to say whether a polymer bound target compound was achieved. It could only be said if a reaction had taken place or not. Therefore the **Table 14** below shows the results obtained from the 1,3-dipolar cycloaddition reactions of polymer bound alkenes and nitrile oxides, generated *in situ* from their corresponding aldoximes, as a summary only. If terminal alkenes had disappeared completely it was assumed that the starting material had been consumed therefore a reaction must have taken place. However if the starting material was still present it was apparent that a complete reaction was not achieved. (**Figure 44** above, shows a general structure of polymer-bound isoxazoline compounds)

Polymer No		p= 0, 2, 8	R =	Starting material consumed completely
Pol 16	{s. 5.5.5, p. 174}	8	4-*tert*-Butylphenyl	Yes
Pol 17	{s. 5.5.6, p. 174}	8	4-Chlorophenyl	Yes
Pol 18	{s. 5.5.7, p. 175}	8	Benzyl	Yes
Pol 19	{s. 5.5.8, p. 175}	8	Butyl	No
Pol 20	{s. 5.5.9, p. 176}	8	4-(Chlorophenyl-3-sulphonyl)phenyl	No
Pol 21	{s. 5.5.10, p. 176}	8	3-Methoxy-4-benzyloxyphenyl	Yes
Pol 22	{s. 5.5.11, p. 177}	8	2,6-Dichlorophenyl	Yes
Pol 23	{s. 5.5.12, p. 177}	8	2,5-Di-methoxyphenyl	Yes
Pol 24	{s. 5.5.13, p. 178}	8	4-Nitrophenyl	Yes
Pol 25	{s. 5.5.14, p. 178}	8	3-Methoxy-4-ethoxyphenyl	Yes
Pol 26	{s. 5.5.15, p. 179}	8	Dodecyl	No
Pol 27	{s. 5.5.16, p. 179}	8	4-Dimethylaminophenyl	No
Pol 28	{s. 5.5.17, p. 180}	8	3-Pyridyl	No
Pol 29	{s. 5.5.18, p. 180}	8	3-Hydroxy-4-methoxyphenyl	Yes
Pol 30	{s. 5.5.19, p. 181}	2	4-*tert*-Butylphenyl	Yes
Pol 31	{s. 5.5.20, p. 181}	2	Benzyl	No
Pol 32	{s. 5.5.21, p. 182}	2	4-Chlorophenyl	Yes
Pol 33	{s. 5.5.22, p. 182}	2	2,6-Dichlorophenyl	Yes
Pol 34	{s. 5.5.23, p. 183}	2	4-Nitrophenyl	Yes
Pol 35	{s. 5.5.24, p. 183}	2	Butyl	No
Pol 36	{s. 5.5.25, p. 184}	2	Dodecyl	No
Pol 37	{s. 5.5.26, p. 184}	2	4-Dimethylaminophenyl	No
Pol 38	{s. 5.5.27, p. 185}	0	4-*tert*-Butylphenyl	Yes
Pol 39	{s. 5.5.28, p. 185}	0	4-Chlorophenyl	Yes
Pol 40	{s. 5.5.29, p. 186}	0	Benzyl	Yes
Pol 41	{s. 5.5.30, p. 186}	0	Butyl	Yes
Pol 42	{s. 5.5.31, p. 187}	0	4-Dimethylaminophenyl	Yes
Pol 43	{s. 5.5.32, p. 187}	0	Dodecyl	Yes

Table 14: **A summary of the results obtained from the 1,3-dipolar cycloaddition reaction between the polymer bound alkene and nitrile oxides.**

2.4.1: Polymer-bound Δ^2-isoxazoline compounds on Pol 9:

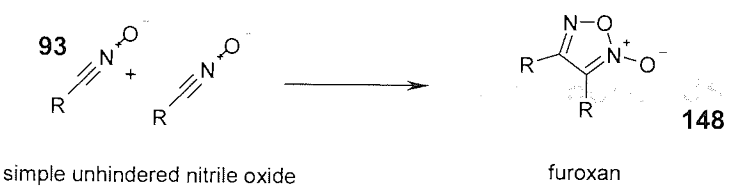
A general trend was observed for 1,3-dipolar cycloaddition reactions between polymer bound alkenes and nitrile oxides. It was observed that for reaction at the alkenes at the end of a long aliphatic chain, it was possible to see two sets of individual multiplets corresponding to the isoxazoline prochiral protons. From ^1H NMR spectrum of purified cleaved compounds and compounds prepared in the solution phase, these multiplets are doublets of doublets. Since the two prochiral protons are not equivalent, one being *syn* and other *anti* to the chiral proton, they couple individually to the chiral proton as well as coupling to each other, thus giving a doublet of doublets. However when the compound is still attached to the polymeric support it is not possible to resolve the doublet of doublets, but rather a broader multiplet. It was also observed that when the phenyl group attached to position 3 of the isoxazoline carried substituents like methoxy, ethoxy or benzyloxy it was possible to see OCH_2 peaks.

Where the R was a substituted phenyl with an electron withdrawing group i.e. nitro, chloro it was observed that peaks coming from the starting material had disappeared in the ^1H NMR spectrum. This was the indication that the starting reagents had been consumed and a reaction had taken place at the polymer bound alkene. However it could not be said with certainty that the compound formed is the compound of interest until the compound was cleaved from the polymeric support.

On the other hand, having an electron donating group i.e. butyl (**Pol 19**, **Pol 35** and **Pol 41**), and dodecyl (**Pol 26**, **Pol 36** and **Pol 43**) as the R group and N,N-dimethylamino (**Pol 27**, **Pol 37** and **Pol 42**) as a substituent on aromatic ring of R group, the starting material failed to be consumed completely and only a partial reaction was observed. A lot of the starting material was always present with very weak signals appearing around the regions where signals from chiral and prochiral protons of isoxazoline ring would appear. Thus indicating that only a partial reaction of some sort took place.

There does not seem to be any great steric hindrance from the polymer bound dipolarophile (alkene), as the dipolarophile is at the end of a long aliphatic chain away from the polymer backbone. The 1,3-dipole (nitrile oxide) also doesn't seem to have much of a steric problem that could prevent the 1,3-dipolar cycloaddition reactions from taking place. If steric hindrance was the factor for failure of these reactions, then nitrile oxides namely, 2,6-dichlorobenzonitrile oxide (**Pol 22**), and 2,5-dimethoxybenzonitrile oxide (**Pol 23**) would have failed too. Therefore there must be other reasons for failure of these reactions.

The unsuccessful 1,3-dipolar cycloaddition reactions between the nitrile oxides from their corresponding aldoximes and polymer bound alkene could be due to any one or a combination of following factors. Simple aliphatic and unhindered nitrile oxides such as butyronitrile *N*-oxide and dodecanenitrile *N*-oxide are extremely unstable and readily dimerize to furaxons even at 0°C in dilute solutions within seconds.[118] **(Scheme 27).** It is possible that most of the aliphatic nitrile oxides were consumed in side reactions forming furoxans and a small percentage was used up in the actual 1,3-dipolar cycloaddition reaction to give the desired compound, hence very weak signals were observed for chiral and prochiral protons of isoxazoline ring.



Scheme 27: Dimerization of simple unhindered nitrile oxides into furoxans. [118]

For aromatic aldoximes such as pyridine-3-carbeldehyde oxime, dimethylamino benzaldehyde oxime and 3-hydroxy-4-methoxy benzaldehyde oxime, the solubility of these compounds in DCM was a major issue. Since the 1,3-dipolar cycloaddition reactions were carried out under same conditions in a biphasic solvent system these oximes precipitated out when added to the reaction medium. Therefore to keep all reagents dissolved, the volume of DCM was considerably increased when compared to other reactions such as with **Pol 16**. This made the overall reaction medium very dilute, which possibly affected the overall kinetics for the formation of nitrile oxides from their corresponding aldoximes and for a reaction to take place between the desired 1,3-dipoles and the dipolarophiles. This explains the presence of polymer bound un-reacted dipolarophiles in the ^{1}H NMR spectrum of **Pol 27**, **Pol 28** and **Pol 29**. **Figure 45** below, shows comparasion between alkene peaks corresponding to polymer bound alkene and and disappearance of alkene peaks after the 1,3-DC reaction carried out.

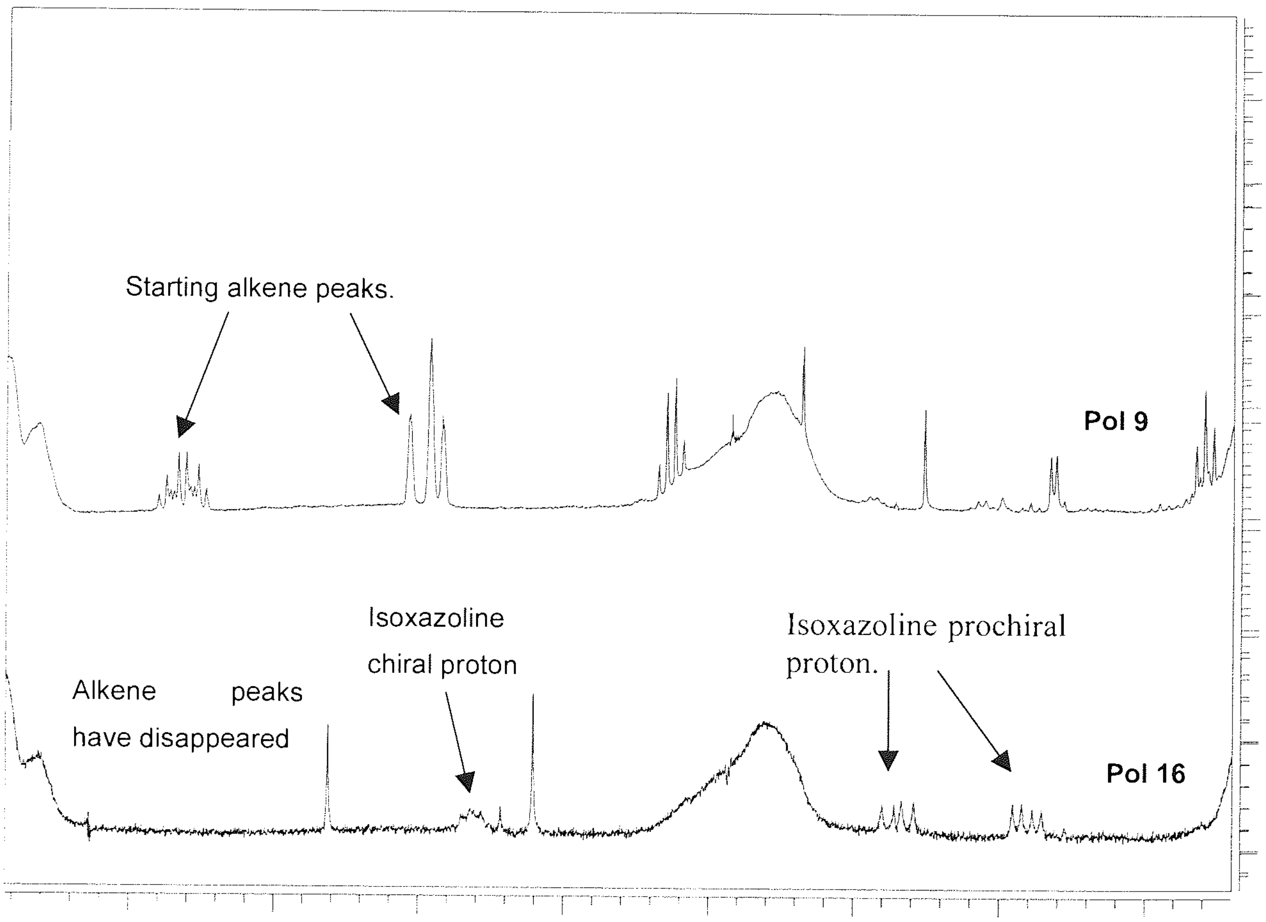


Figure 45: A comparison between Pol 9 and Pol 16 ^{1}H NMR Spectra. The top spectrum (Pol 9) between δ2-6 ppm showing peaks from starting material. Lower spectrum (Pol 16) shows the disappearance of starting material and presence of isoxazoline chiral and prochiral protons suggesting that 1,3-dipolar reaction has taken place.

2.4.2: Polymer-bound Δ^2-isoxazoline compounds on Pol 10:

1,3-Dipolar cycloaddition reactions on **Pol 10** gave similar trends to **Pol 9**. The observance of signals coming from the isoxazoline prochiral protons were not as clear as they were in derivatised polymers from **Pol 9**. This observation is consistence with the ^{1}H NMR spectrum for acylated polymers that as a group is moved further away from the polymer backbone it is well resolved and signals coming from that particular group are much sharper and clear than from a group that is moved closer to the polymer backbone.

For the reaction between 4-*tert*-butylbenzonitrile N-oxide and the polymer bound alkenes, **Pol 30**, the signals coming from the alkene had disappeared indicating that alkene had been consumed in a reaction, but no additional peaks were observed. However upon vertical expansion of the spectra, it was noted that a small broad peak was present at δ4.62-4.79 ppm, possibly the proton at the chiral carbon of the isoxazoline ring. A triplet at δ5.16-5.26 ppm and a multiplet at δ5.48-5.58 ppm were also observed, indicating that the starting material was not completely consumed.

4-chlorobenzonitrile *N*-oxide (**Pol 32**), 2,6-dichlorobenzonitrile *N*-oxide (**Pol 33**), 4-nitrobenzonitrile *N*-oxide (**Pol 34**), gave similar observations to the derivatised polymers from **Pol 9**. i.e. the disappearance of alkenes peaks and weak signals for the isoxazoline chiral and prochiral protons. See **Figure 46** below.

The reaction between benzonitrile *N*-oxide and alkene (**Pol 31**) was expected to have worked same as **Pol 18**, but it didn't work very well. A lot of the starting material could be seen in the ^{1}H NMR of the derivatised **Pol 31**. Some new peaks could be seen around regions where isoxazoline chiral and prochiral peaks would appear. However these new signals are ambiguous and it cannot be said with certainty that they are coming from the target compound.

Reaction of butyronitrile *N*-oxide (**Pol 35**), dodecanenitrile *N*-oxide (**Pol 36**) and dimethylamino benzonitrile *N*-oxide (**Pol 37**), all failed to work. For **Pol 35** and **Pol 36** a partial reaction was observed from the ^{1}H NMR spectrum of these polymers. For **Pol 37** there was no reaction possibly due to the same solubility reasons explained above.

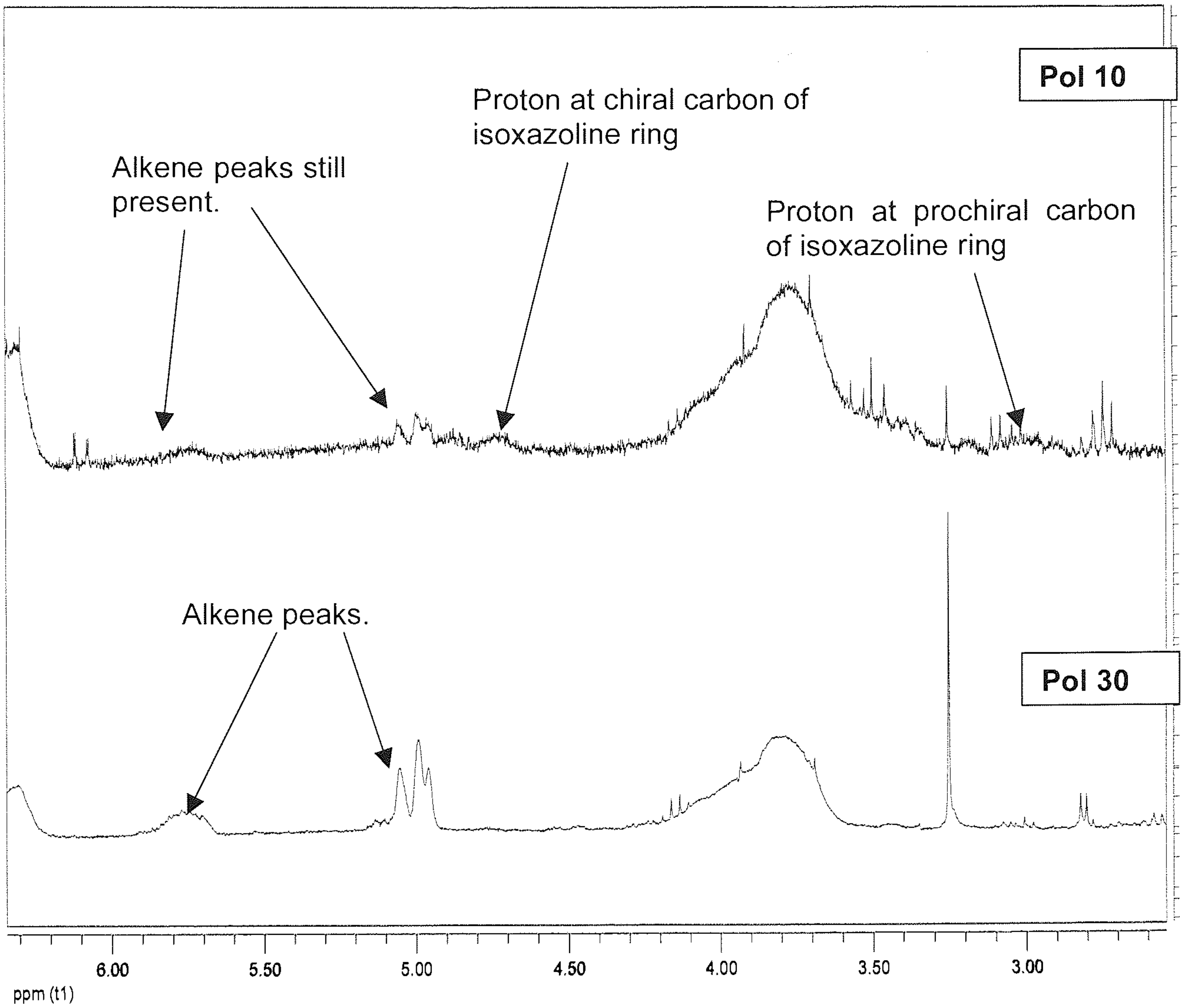
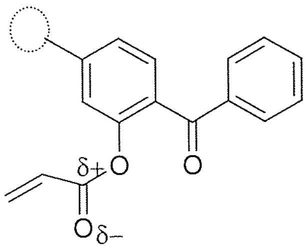


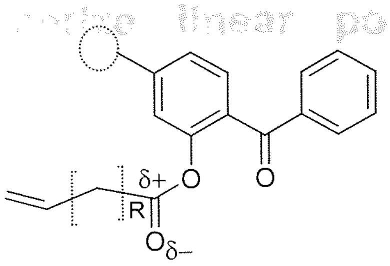
Figure 46: A ^{1}H NMR spectrum for Pol 10 (top spectra) and Pol 30 (bottom spectra). The starting material alkene peaks can be seen in top spectra. In the bottom spectra, the starting material can still be seen, suggesting that the reaction didn't go to completion.

2.4.3: Polymer bound Δ^2-isoxazoline compounds on Pol 11:

As mentioned above it was not possible to analyse the progress of reaction even with the ^{1}H NMR spectrum for this class of polymers. Again the only evidence was the disappearance of the alkene peaks. See **Figure 41**. Nitrile oxides such as dimethylamine benzonitrile N-oxide, butyronitrile N-oxide and dodecanenitrile *N*-oxide that failed to react with polymer bound alkenes for **Pol 9** and **Pol 10**, reacted successfully with alkenes on **Pol 11**. This can be explained from the chemical structure of acylated **Pol 11**. Presence of a double bond at α position to the carbonyl carbon makes **Pol 11** chemically different from **Pol 9** and **Pol 10** as shown below in **Figure 47**. It makes the alkene bond electron deficient, being next to the carbonyl carbon, when compared to the alkene bonds for **Pol 10** and **Pol 11**.



Pol 11



R= 2 (**Pol 10**) and 8 (**Pol 9**)

Figure 47: Structure of Pol 9, Pol 10 and Pol 11. It can be seen that in Pol 11 the alkene peaks are adjacent to the carbonyl group therefore making it chemically different from the other two.

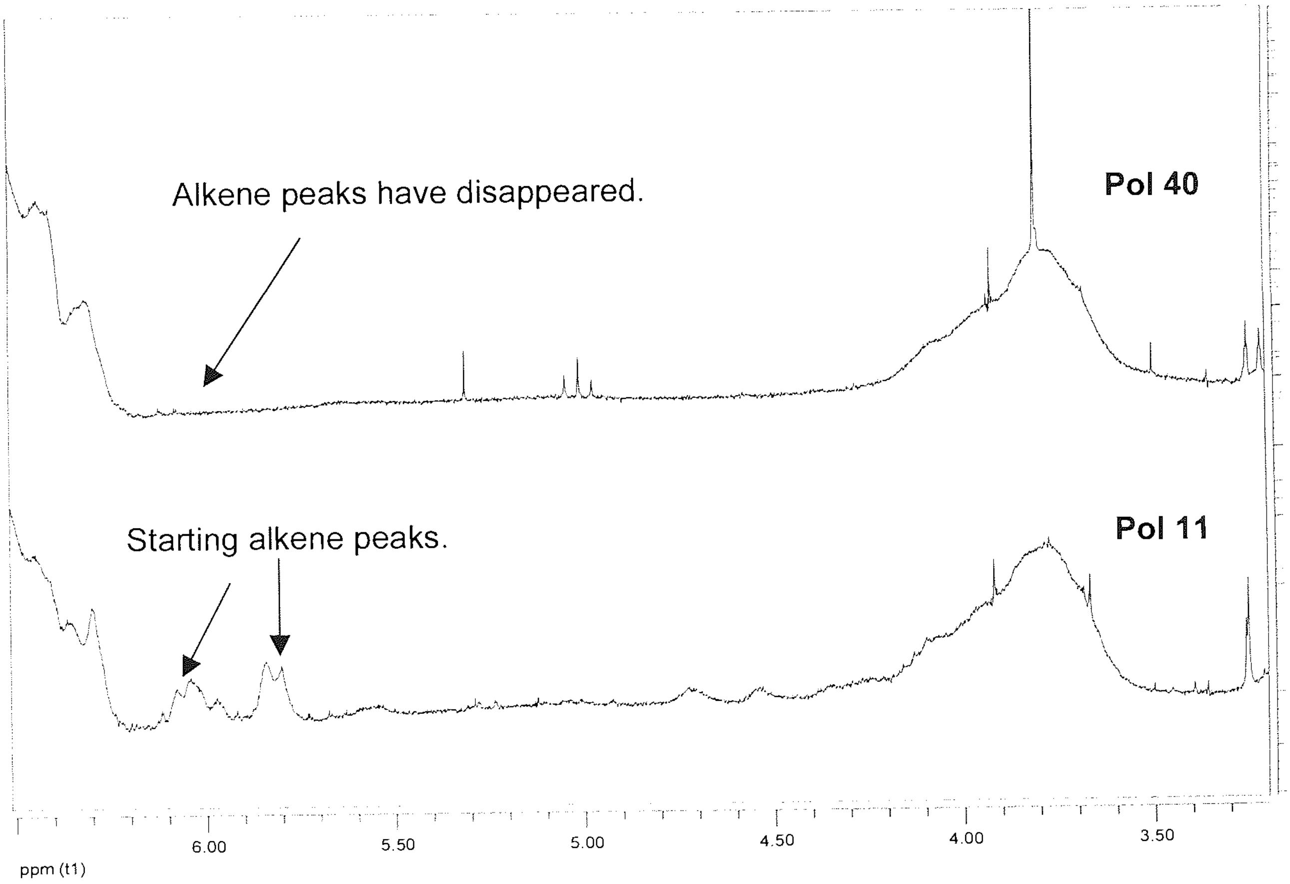


Figure 48: A comparison between Pol 11 (lower spectrum) and Pol 40 (top spectrum). The starting alkene peaks can be seen on top spectrum. After the 1,3-dipolar cycloaddition reaction starting material peaks have disappeared.

2.5: Methods used to characterize linear polystyrene copolymers in this thesis:

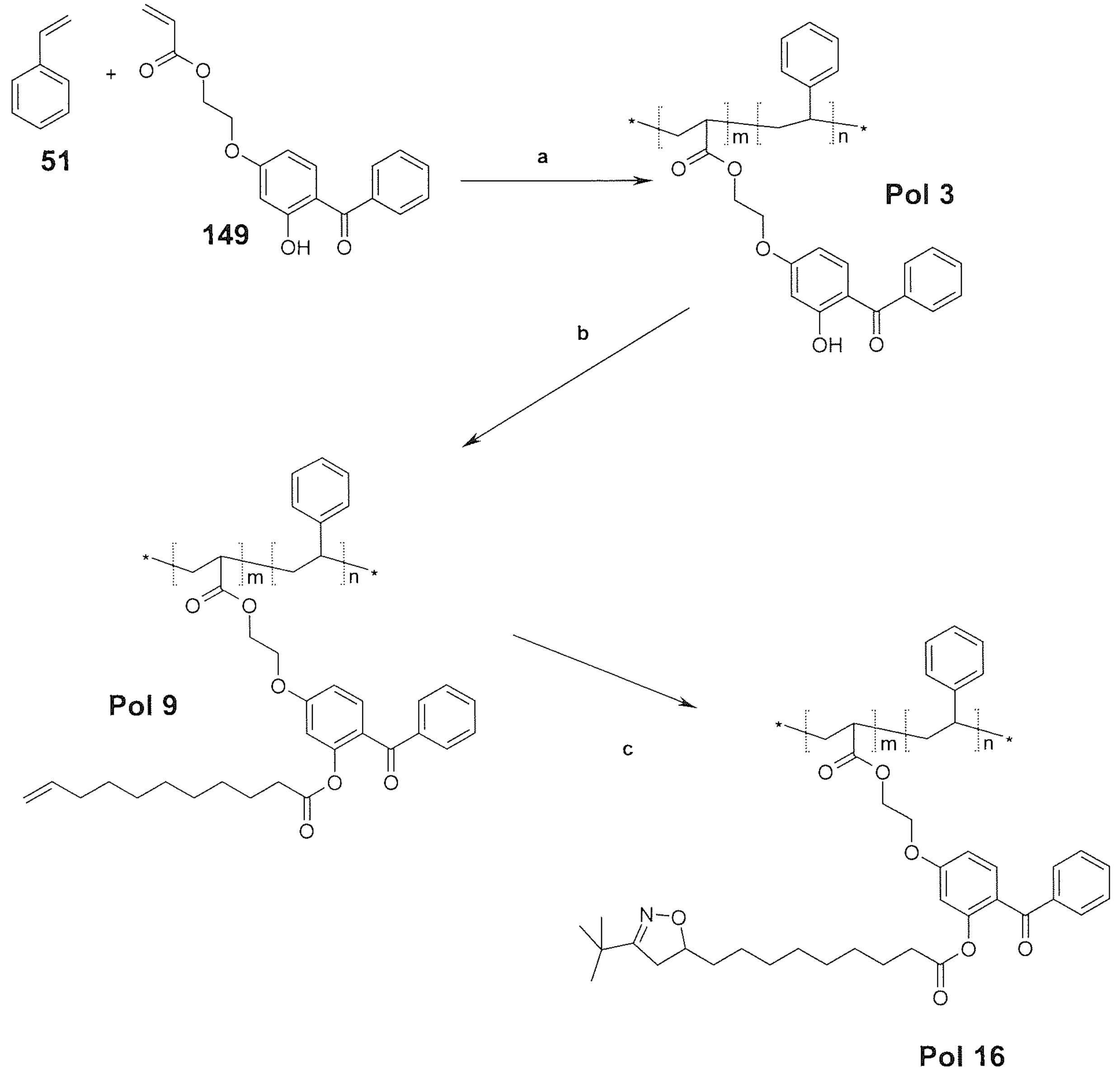
As mentioned previously, the aim of this thesis was to prepare suitable soluble linear polystyrene based polymeric supports for the synthesis of organic compounds. The solubility profile of these polymers was used to characterize the physical properties of the polymers. Since polymers are soluble in toluene, ethyl acetate, THF and chlorinated solvents and insoluble in solvents such as ethanol, methanol, petroleum ether and water.

To determine the chemical composition of these soluble linear polymers, ^{1}H NMR spectroscopy was the main technique used (sections 2.2-2.4.3,). This method was the most useful of all the techniques used. Although the peaks corresponding to the polymer backbone and aromatic group of the polystyrene, covered a large area of the ^{1}H NMR spectrum, both at high and low field, it was still possible to characterize the part of the polymer where the synthetic modifications were undertaken. Even though it was not possible to characterize the polymer bound compounds completely, comparison between the ^{1}H NMR spectrum of new polymer and the ^{1}H NMR spectrum of the starting polymer provided sufficient evidence that a reaction had taken place, this was judged by the disappearance of the peaks corresponding to the starting material.

Attempts were also made to characterize these polymers by using ^{13}C NMR spectroscopy. ^{13}C NMR spectra were obtained for a complete set of reaction **(Scheme 28)** i.e. **Pol 3** (basic polymer) and the **Pol 7** (polystyrene) polymers, **Pol 9** (acylated polymer) and **Pol 16** (polymer-bound isoxazoline compounds). All ^{13}C spectra were obtained at a frequency of 62.9 MHz, and were left overnight obtaining more than 15,000 scans. The results obtained were rather ambiguous which was probably due to several reasons. As it can be seen from **Figure 49** below, that despite such a large number of scans the signal to noise was very poor. The acylation process, as supported by ^{1}H NMR spectrum, was never achieved to 100%, thus making the ^{13}C spectrum more complex because the acylated polymer (**Pol 9**) contained a mixture of **Pol 9** and **Pol 3**. Therefore, peaks were observed for both, **Pol 9** and **Pol 3**. This gave a rather complex ^{13}C NMR spectrum and gave impractical results. In ^{13}C NMR spectra of polymers containing polymer-bound isoxazoline compounds **(Pol 16)** peaks corresponding to the carbonyl carbons were not observed, see **Pol 16** ^{13}C NMR spectrum in **Figure 49**. Also, the tertiary carbons especially the chiral carbon of the isoxazoline ring was not easy to detect (in ^{1}H NMR spectrum, the peaks from the H attached to tertiary carbon was one of the main indications for a 1,3-DC to have taken place). Therefore it was decided not to make ^{13}C NMR spectroscopy the main focus for polymer characterization.

Attempts were also made to characterize the polymer bound products by Infra Red (IR) spectroscopy. IR spectra were obtained for **Pol 1 - Pol 11,** (parent polymers and acylated polymers with polymer-bound alkene) **Pol 16, Pol 17** and **Pol 24** (polymers that underwent 1,3-dipolar cycloaddition reactions and carried polymer-bound Δ^2-isoxazoline compounds). The IR spectra obtained for **Pol 16**, **Pol 17** and **Pol 24** were essentially identical to those from acylated polymer **Pol 9.** The C=O stretch was observed, at around 1720-1740 cm^{-1}, for all acylated polymers as well as those had undergone the

1,3-dipolar cycloaddition reaction (regardless whether the reaction was successful or unsuccessful), however the N-O, N=C or O-C stretch of the polymer bound heterocycle were not observed. This could be due to several reasons, i.e. the peaks overlapped with those corresponding to the dominant polymer backbone and other peaks from polymer bound products, or the absorbance was very low which made it difficult to see these stretches. Therefore it was decided that obtaining IR spectrum for other polymers would be unfruitful. TLC on silica was also attempted in chlorinated solvents, but it was unsuccessful in moving the polymers from baseline. The GPC would have been helpful to find out the polydispersity of these polymers, but as these facilities were not available these couldn't be performed.



Scheme 28: Synthesis of Pol 3 and its derivatised polymers (Pol 9 and Pol 16). (a) AIBN, toluene heated under reflux at 70°C (b) anhydrous $CHCl_3$, 10-undecenoyl chloride, Amberlite IRA-67, D-map, heated under reflux 70°C under argon, (c) DCM, aldoxime, sodium hypochlorite, (d) isobutylamine, DCM.

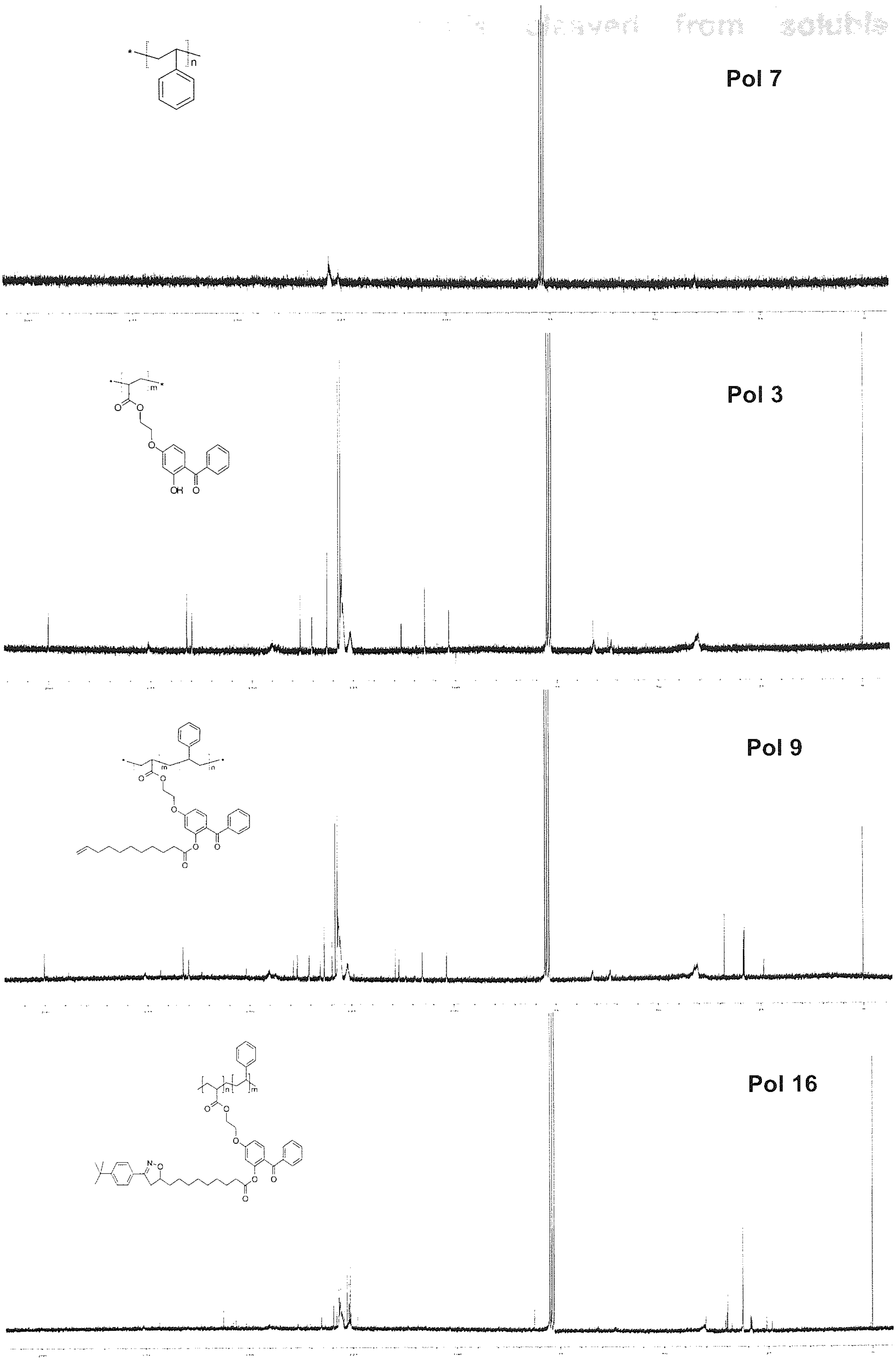
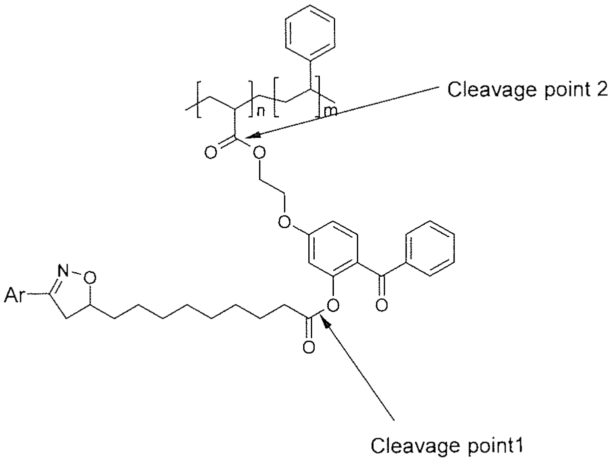


Figure 49: ^{13}C NMR spectrum for a complete set of reactions (CDCl3 as solvent) plotted 0-200ppm.

2.6: Δ^2-Isoxazoline compounds cleaved from soluble polymeric support:

To cleave off the polymer bound Δ^2-isoxazoline compounds from their soluble polymeric support, the polymer was first dissolved in DCM and was treated with isobutylamine (20 % in the overall reaction medium). The reaction was stirred at RT for overnight. At the end of reaction time the isobutylamine was removed under high vacuum. The polymer was re-dissolved in minimum amount of DCM and was poured on rapidly stirred methanol. The polymer precipitate was collected by filtration. The filtrate was collected and solvent was removed under vacuum to obtain an oily residue.



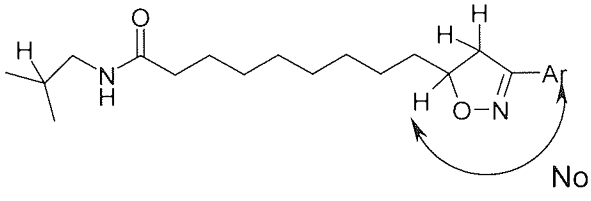
149b

Figure 50: The two possible cleavage points in polystyrene based soluble polymeric support.

The polymeric support has two cleavage points (see **Figure 50** above). It was anticipated that most of the cleavage would take place at point 1, resulting in the formation of desired compounds. In theory it is possible to obtain more than one product from the cleavage of the polymer bound compounds. i) cleavage at point 1, forming an amide at that end, ii) cleavage at point 2, giving an alcohol functionality, iii) cleavage at both point 1 and point 2 giving a phenol functionality at one end and an alcohol at the other end. Depending on the regioselectivity of the 1,3-DC reactions, the number of products obtained can further increase.

Nitrile oxide cycloadditions to terminal alkenes can result in two regioisomers. However there is ample literature precedent to support that the reaction proceed regioselectively to give 5-substituted Δ^2-isoxazolines as single products. The preference of one isomer over the other is due to the steric hindrance. The attachment of oxygen to the most hindered position at mono alkene results in a compound where the substituent at carbon end on nitrile oxide is further away from the substituent on alkene therefore no steric problems are faced. However if the oxygen atom attacks the end carbon of the alkene a compound is formed where the substituent can cause steric hindrance between the substituent and the atom/group at alkene. The oxygen end of the nitrile oxide becomes joined to the most hindered end of mono-substituted alkene (dipolarophile). This is due to the steric hindrance arising from the substituent attached to the carbon end of the nitrile oxide. The reaction proceeds in a concerted manner and can be compared to the Diels Alder reactions for the reaction mechanisms. On the other hand, reactions with 1,2-disubstituted internal alkenes lead to a mixture of regioisomers in approximately 1:1 ratio.[115] (section. 1.3.4, page. 27). This statement is in agreements with the results reported for solution phase synthesis of Δ^2-isoxazolines compounds, because only single regioisomer was observed in these reactions. **(Figure 51)**

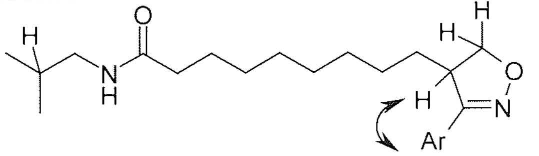
Thermodynamically preferred isomer



150

No steric interaction

Thermodynamically least preferred isomer



151

Steric interaction

Figure 51: **Thermodynamically most and least preferred isomers of Δ^2-isoxazolines.**

The ^{1}H NMR, mass spectroscopic and TLC analysis was performed on the crude cleaved compound. The ^{1}H NMR spectrum indicated the presence of an isoxazoline ring by the signals coming from the chiral and prochiral isoxazoline protons, which resonate at δ4.5-δ4.85 ppm as a multiplet, δ2.75-3.4 ppm as two individual doublets of doublets or multiplet respectively, along with signals arising from the presence of aliphatic prochiral protons and protons at α-carbon. The mass spectroscopy confirmed the presence of the target compound according to the ion corresponding to the protonated molecule $(M+H)^+$. The isoxazolines were considered absent, either when the $(M+H)^+$ ion was not detected or when the chiral and prochiral isoxazoline protons were not observed in ^{1}H NMR spectrum. Based on these findings it was then decided whether a purification method should be attempted on the crude cleaved product. For a ^{1}H NMR spectrum of a typical cleaved crude product see **Figure 52** below.

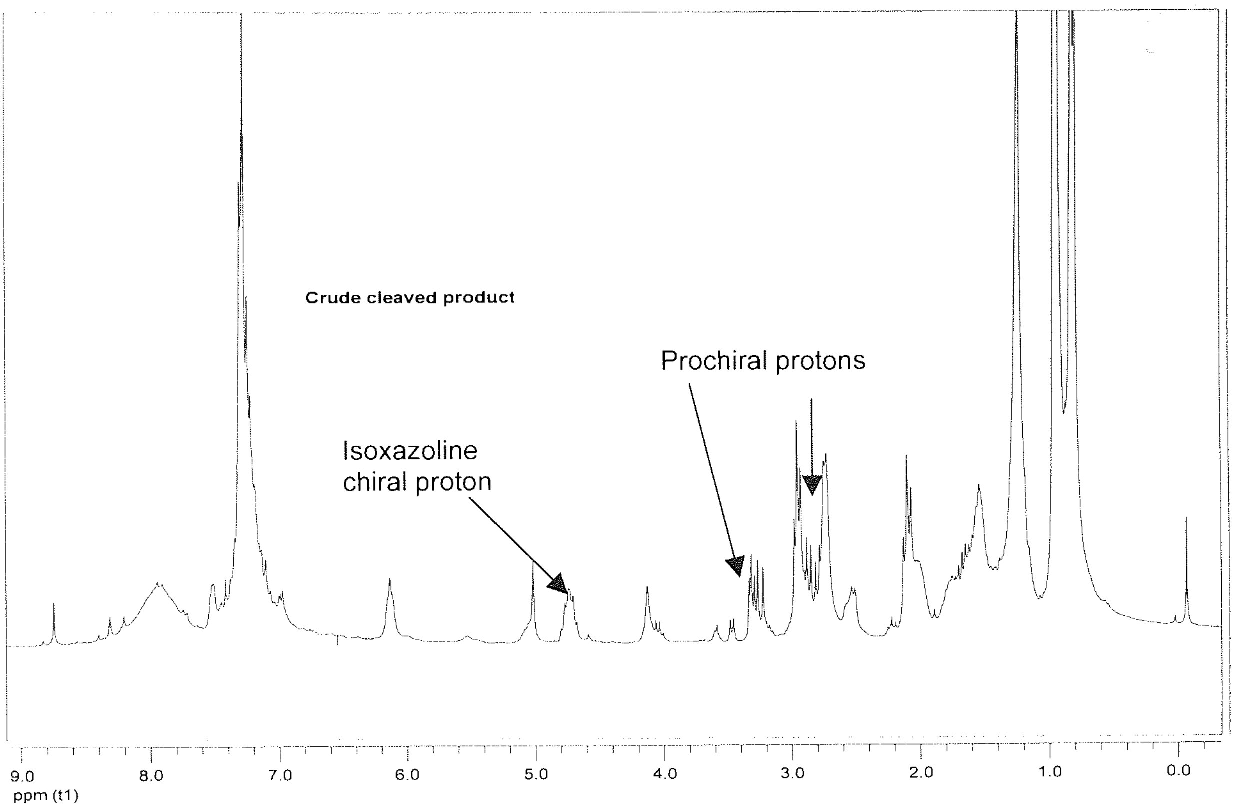


Figure 52: **A ^{1}H NMR spectrum of a typical cleaved product from Pol 9.**

A ^{1}H NMR spectrum was also obtained for the cleaved polymer that was recovered from the filtration. The spectra looked identical to the parent polymer **Pol 3** as it can be seen in **Figure 53** and **Figure 54** below. In theory this recovered polymer could be used again as a soluble polymeric support. However this was not attempted due to the difficulties and problems encountered during the course of this project as mentioned previously.

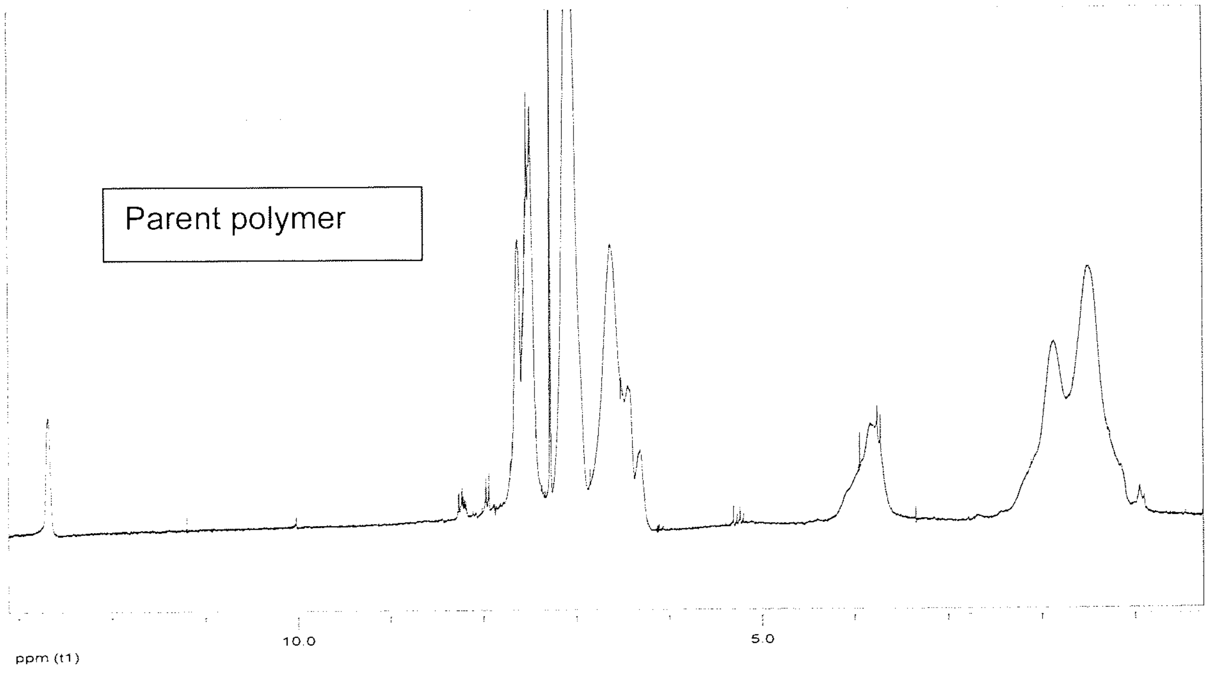


Figure 53: **^{1}H NMR spectrum of parent polymer containing OH linker (Pol 3)**

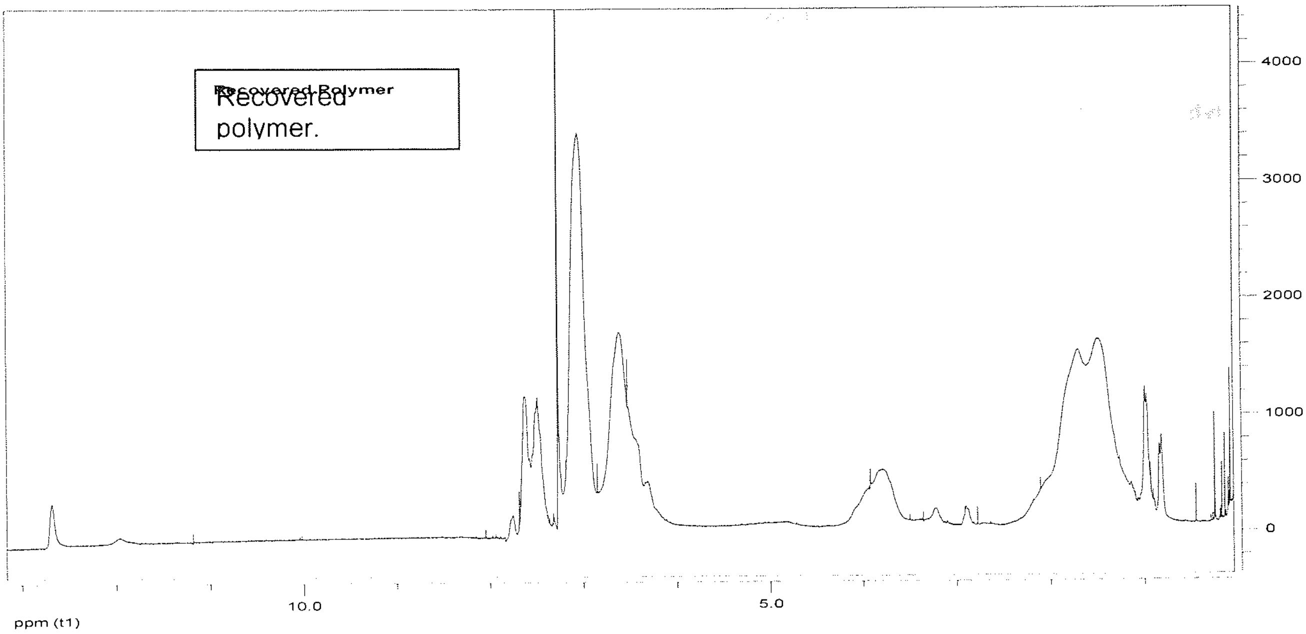


Figure 54: ^{1}H NMR spectrum of recovered polymer, after the product was cleaved off from the polymer.

Post cleavage purification was not performed on all of the cleaved Δ^2-isoxazoline compounds. Compounds isolated, were subjected to flash chromatography. Prior to the chromatography, some cleaved crude products had to be subjected to paper filtration to remove the small amount of polymer that probably came through in the previous filtration process. The presence of polymer was also confirmed by the ^{1}H NMR spectrum of the cleaved crude product.

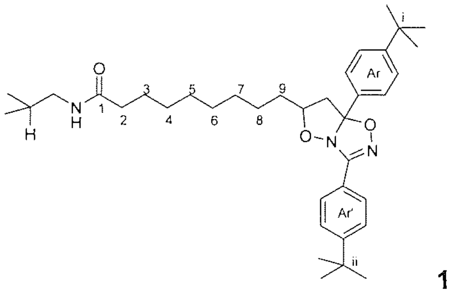
The TLC of the crude products showed multiple components in almost all solvents tried, both in a single solvent system and dual-solvent systems. The solvent systems tried ranged from methanol to petroleum ether including DCM, chloroform, ethyl acetate, acetone, toluene, ethanol, diethyl ether and acetonitrile. Often it was necessary to perform more than one flash column using different solvent polarities, on a particular crude product i.e.**152** (s. 5.6.10, p.198). This was necessary to widen the gap between two consecutive spots. In this way it was possible to separate the target compound.

2.6.1: 9-[3-(4-*tert*-Butylphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic isobutylamide (150):

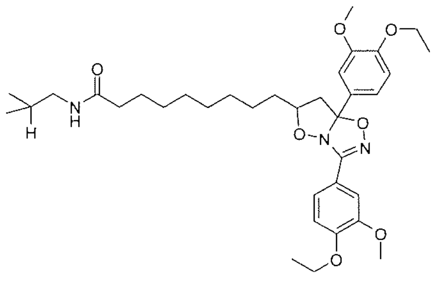
The above compound **(150)** {s. 5.6.1, p. 195} was synthesized by two different routes. It was established that it was much easier and less time consuming to synthesise the compound using traditional solution phase chemistry (route A) rather than synthesizing it on a soluble polymeric support (route B). In solution phase two synthesis steps achieved the target compound in less than a week's time. The compound made by route A was purified in very little time in comparison to route B, and fewer by-products (two in total) were separated in flash column chromatography. Route B took almost two week to achieve the same compound. The purification of the target compound took two days, and 18 by-products were collected from the flash column chromatography.

Apart from the target compound 9-[3-(4-*tert*-butyl-phenyl)-4,5-dihydro-isoxazol-5-yl]-nonanoic-isobutyl-amide **(153)**, {s. 5.6.2, p. 196} a bicyclic adduct was also separated in the flash column chromatography form crude products for both routes. The bicyclic adduct was discovered from the ^{1}H NMR analysis of the fraction collected immediately before the target compound. However the bicyclic adduct was not isolated as a pure compound as there were some impurities still present as evident by the TLC and ^{1}H NMR spectrum. The mass spectroscopy analysis also confirmed the presence of molecular ion at 590 for the bicyclic adduct.

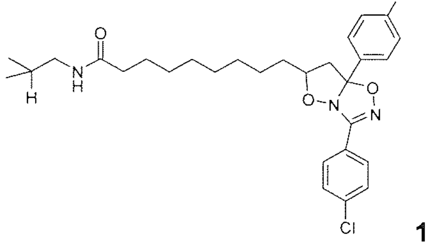
However the bicyclic adduct deteriorated upon standing at RT, with in a few days. When it was tried to further purify it, the TLC of bicyclic adduct showed multiple components which were absent in TLC taken at the time of target compound isolation. The presence of bicyclic adduct in other cleaved products was confirmed by the presence of the molecular ion in mass spectroscopy. Compounds **(153)**, **(154b)** {s. 5.6.8, p. 199} and **(155)** {s. 5.6.4, p. 197} gave a peak at bicyclic adduct molecular ion at 546 and 626 respectively, but could not be isolated in pure form. Hence it can only be assumed that a bicyclic adduct was made in these reaction. Two points are worthy of comment. I) A bicyclic adduct was only found for the compounds that contained a long aliphatic chain substituent at atom 5 of the isoxazoline ring.



153



154b

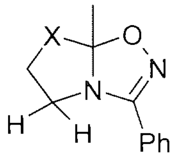


155

The isoxazole bicyclic adducts

The bicyclic adduct was seen for the compounds that had a substituent at *para* and/or *meta* position of the phenyl ring attached to the atom 3 of the isoxazoline ring. However there are other compounds with a substituent at *para* position of the phenyl group where the bicyclic adduct was not found. i.e

(156) {s. 5.6.15, p. 203}. Miller *et al.* have reported the formation of oxazole and thiozole bicyclic adducts from the reaction of nitrile oxides with 4,5-dihydrooxazole and 4,5-dihydrothiazole derivatives respectively.[119] **(Figure 55)**.



X= O , S **157**

Figure 55: An oxazole bicyclic adduct (oxadiazole or amide acetal compound).

The bicyclic adducts obtained by Miller *et al* can be regarded as bicyclic amide acetals. Though the two compounds cannot be compared chemically, it is obvious that the both compounds are formed in 1,3-dipolar cycloaddition reaction between the nitrile oxides and the 4,5 dihydro oxazole / dihydro isoxazole. There is literature precedent to show that, in accordance with the principle of maximum gain in σ–bond energy, the electronegative end of a hetero-polarophile becomes joined to the carbon end of the nitrile oxide. Miller *et al* also reported their compounds to be unstable and the ring opening occurs giving rise to an open chain structure $PhCOXCH_2CH_2N(CN)COMe$.[119]

2.6.2: 9-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (158):

The compound **(158)** was cleaved off from the soluble polymeric support as mentioned above. The crude cleaved product contained a lot of the undesired products which is the characteristic of 1,3 dipolar cycloaddition reactions between nitrile oxides and the alkenes. Only single region-isomer was obtained. A bicyclic adduct **(155)** was also detected in the crude mixture of the compound, but was not isolated as a clean compound.

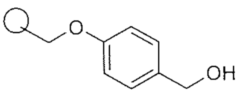
2.6.3: Other compounds cleaved from the polymeric support:

A number of compounds were cleaved from the polymeric supports by treatment of polymeric supports with isobutylamine. Compounds **159**, **160**, **161** {s. 5.6.5-5.6.7, p.196-199} and **162** {s.5.6.10, p. 200}, were isolated as pure compounds by flash column chromatography. Compounds **163**, **164** {s. 5.6.11, 5.6.12, p. 201, 203}, **165** {s. 5.6.14, p. 203}, **166** and **167** {s. 5.6.16, 5.6.17, p. 204} were not isolated as pure compounds because of the difficulties mentioned above.

2.7: Wang-Resin as insoluble polymeric support:

2.7.1: Investigation on Wang-resin as insoluble polymeric support for the synthesis of Δ^2-isoxazoline compounds: A comparison between the polystyrene soluble polymeric support and Wang-resin insoluble polymeric support in organic synthesis:

A comparative study was performed between the in-house built soluble polystyrene polymeric supports and commercially available insoluble cross-linked polystyrene polymeric support. For this purpose, commercially available Wang-Resins with hydroxyl linker and a functional loading of 1.7mmol/g was chosen (**W1**) {s. 5.10.3, p. 222}. Resin with 1.7mmol/g functional was chosen because the soluble polymeric support that was prepared for organic synthesis had a theoretical loading of the functional group of 1.18mmol/g and the actual loading, as calculated from the integration of ^{1}H NMR spectra, was 1.34mmol/g.



W1

W1 was subjected to acylation with 10-undecenoyl chloride under similar conditions as for the acylation of **Pol 3**. The difference being that **Pol 3** being soluble dissolved completely in $CHCl_3$, whereas **W1** being insoluble was allowed to swell completely in $CHCl_3$. The base used for **Pol 3** was resin bound triethylamine (Amberlite IRA 67 ™) and for **W1** free isobutylamine (in liquid form) was used. Since both **W1** and Amberlite IRA 67 are insoluble resins, it would have been impossible to separate the one from the other. An IR spectrum was obtained for **W1** before and after the acylation (**W2**). { s. 5.10.3, p. 223} A C=O stretch at 1736 cm^{-1} was observed for **W2** indicating that an acylation process has taken place. However an O-H stretch was also present at 3446 cm^{-1}, indicating that 100% acylation was not achieved. The 1,3-dipolar cycloaddition reaction was performed under similar conditions as for soluble polymeric support. An IR spectrum of resin-bound isoxazoline compound (**W4**) was ambiguous. Therefore IR spectrum for other resin-bound compounds were not obtained. At the end of 1,3-DC reactions, to wash the resins to remove excess reagents and by-products by filtration, proved very difficult and time consuming.

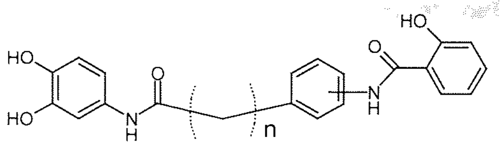
Resin-bound isoxazoline compounds were released by treating the resins with 20% trifluoroacetic acid (TFA) in DCM. Carboxylic acids were obtained. ^{1}H NMR study of the cleaved compounds proved the presence of the target compounds. It was discovered that the crude cleaved products from Wang-resins were cleaner then those cleaved from the soluble polymeric support, which was supported by the TLC, mass spectroscopy and ^{1}H NMR spectroscopy. For any biological activity related study, further purification would be necessary. However, no further purification was done on these compounds because the aim here was to compare the two types of polymeric supports (insoluble cross-linked polymeric support and soluble linear polymeric supports) for organic synthesis.

As a comparison a small amount of **W4** (1.0 g) {s. 5.10.3, p. 220} was treated with 20% isobutylamine to release the resin-bound isoxazoline compound. From the ^{1}H NMR spectrum of the two identical compounds prepared on different polymeric supports, it was clearly obvious that the compound prepared *via* Wang-resin was much cleaner than the one prepared on soluble polystyrene copolymers. As mentioned previously (section 2.6, page 122) this is due to the fact that the soluble polymers had more than one cleavage point. In theory it is possible to get a number of compounds. On the other hand the insoluble polymers had only one cleavage point. Even if there were any other cleavage points, being insoluble the isobutylamine/TFA could only work on the surface of resins. Compounds **169 -176** {s. 5.10.5.1 -5.10.5.8, p. 228-231} were cleaved from the solid suuports but were not isolated, because the aim of this project was to prepare these compounds on soluble polymeric supports. These compounds were prepared purely for comparasion purposes.

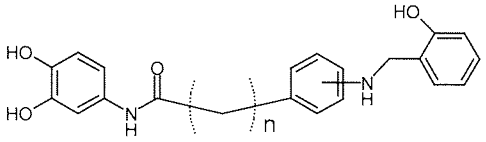
1,3-Dipolar cycloaddition reaction between *N,N*-dimethylamine oxime and the polymer bound-alkene failed for the soluble polymeric support. However a peak in mass spectroscopy of the same compound but on insoluble polymeric support

2.8: Investigating the synthesis of compounds containing catechol moiety on soluble polystyrene polymeric supports:

A small library of compounds containing a catechol moiety **(177, 178)** was synthesized by Simpson[120] Rathbone *et al*[121] and showed promising biological activity as lipoxygenase inhibitors. These compounds were prepared by traditional solution phase synthesis. A crucial step in synthesis of these compounds was sequential reduction of nitro group on aromatic ring followed by acid chloride coupling. Many problems were encountered during these steps and low yields were obtained. Synthesis on Merrifield resin was also attempted which proved to be impractical due to the problems encountered with difficulty in analysis of the intermediate products. Biological evaluation suggested that the catechol moiety was essential for the biological activity.



177



178

It was anticipated that the problems encountered in traditional solution phase synthesis and solid phase synthesis could be avoided by preparing these compounds on soluble polymeric supports.

It was decided that the catechol moiety could be attached to a soluble support by the formation of an acid-labile cyclic ketal. This would give the advantage of protecting the hydroxyl groups whilst linking them to the soluble polymeric supports. A polystyrene copolymer **(Pol 46)**, prepared by Simpson, containing ketone functionality was heated under reflux in toluene with excess 4-nitrocatechol in the presence of *p*-toluene sulphonic acid (*p*TSA). **(Scheme 28** step **f)** The reaction was unsuccessful as judged by ^{1}H NMR spectroscopy.

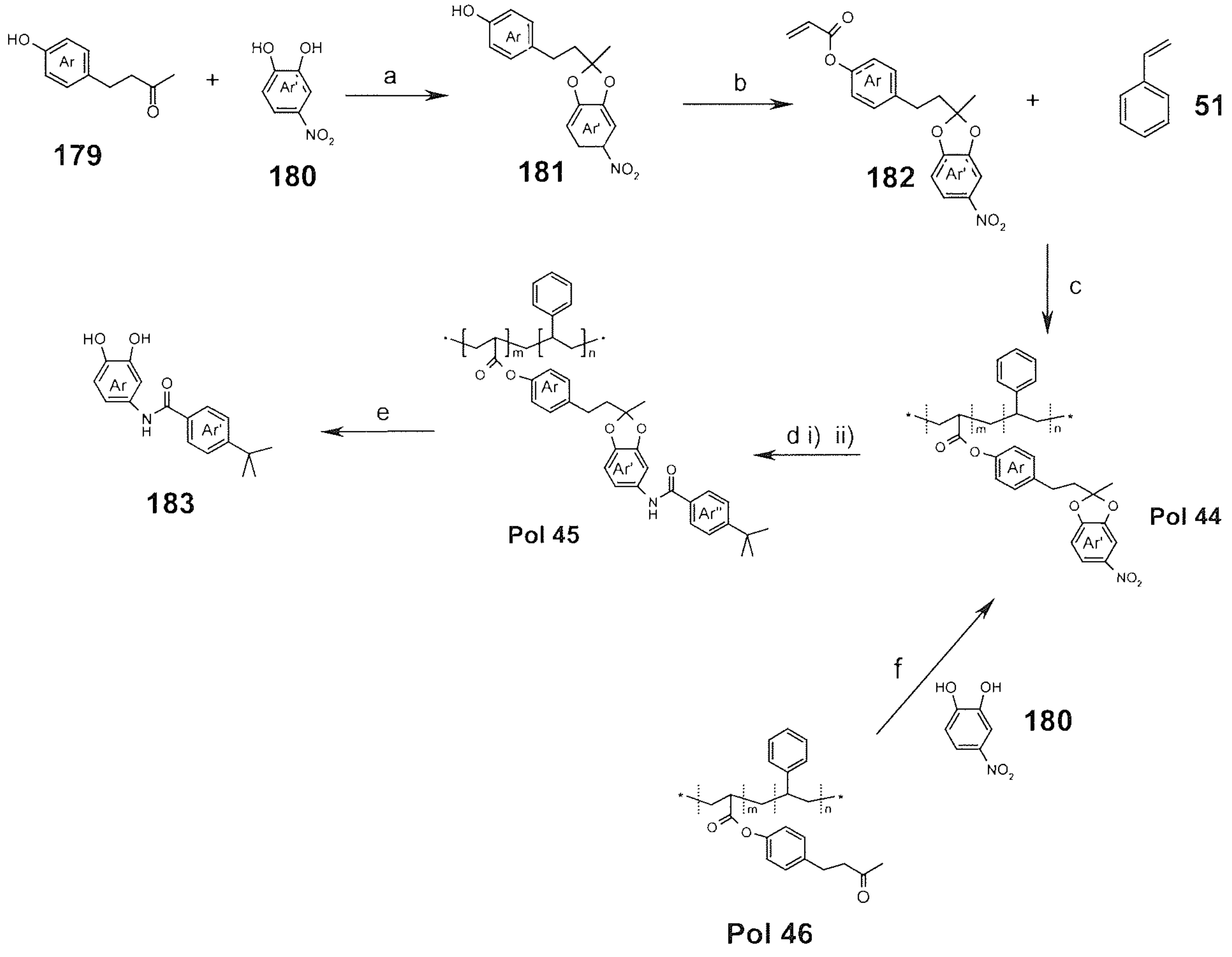
A different approach to the synthesis of catechol compounds was attempted. (**Scheme 29**). A mixture of 1-(4-hydroxyphenyl)-3-butanone **(179)** and 4-nitrocatechol **(180)** was heated under reflux in toluene in catalytic amount of *p*TSA for 4 days. TLC on the crude product revealed the presence of 4-nitrocatechol. Product **(181)** was isolated by flash chromatography in 75% yield. Compound **(181)** was reacted with acryloyl chloride under anhydrous conditions in the presence of triethylamine and reaction was cooled in an ice bath. The reaction mixture was added to ice water and washed with sodium hydrogen carbonate solution. Separation of organic layer from the aqueous layer and evaporation of solvents gave a crude product bearing polymerisable functionality. Product isolation by flash chromatography yielded 65% pure monomer (x). A copolymer of styrene and monomer **(182)** was prepared following the standard procedure in a free radical polymerisation. The polymer **Pol 44** was collected by methanol induced precipitation of the polymer followed by filtration. A copolymer bearing nitro functionality was obtained in 27% yield with a loading capacity of 1.14mmol/g.

Several attempts were made for catalytic hydrogenation of aromatic nitro group with 10% Pd-C followed by immediate coupling with *p-tert*-butyl benzoyl chloride to give **Pol 45**. It was observed by Simpson that the amine formed by reduction of nitro group was very unstable in air and decomposed immediately to give a complex mixture. Therefore the subsequent coupling with acid chloride had to be performed as soon as possible or in *situ* after reduction. For this reason the product after reduction of nitro group was not isolated and acid chloride coupling was performed in *situ* in each attempt in the presence of TEA. The reaction mixture was filtered and washed with THF. The polymer was precipitated by pouring onto methanol and collected by filtration. No new peaks were observed in the ^{1}H NMR analysis of polymer.

The reduction of aromatic nitro group was investigated at various temperatures including room temperature, 30, 35, 40, 45 and 50 °C and at various pressures including, 80, 90, 100, 110 and 125 psi.

To determine any success of the reduction and coupling reactions, attempts were made to cleave the pendant molecule from the soluble polymeric support. Cleavage of the polymer-bound compound was attempted using 5M HCl in DME and water under reflux. In a second attempt the polymer was treated with 20% TFA in THF at room temperature for three hours. TLC analysis of the reaction did not show any new product formation. Therefore it was decided to stop any further work on this particular polymer for two reasons. Firstly there was no evidence that an amide bond was created in the reduction and acylation step, secondly the cleavage of the product at ketal group was unsuccessful under mild conditions.

The reaction was performed under heterogeneous conditions. It is possible the reaction sites were not accessible by the catalyst, therefore the reduction could not take place hence acylation could not be carried out.

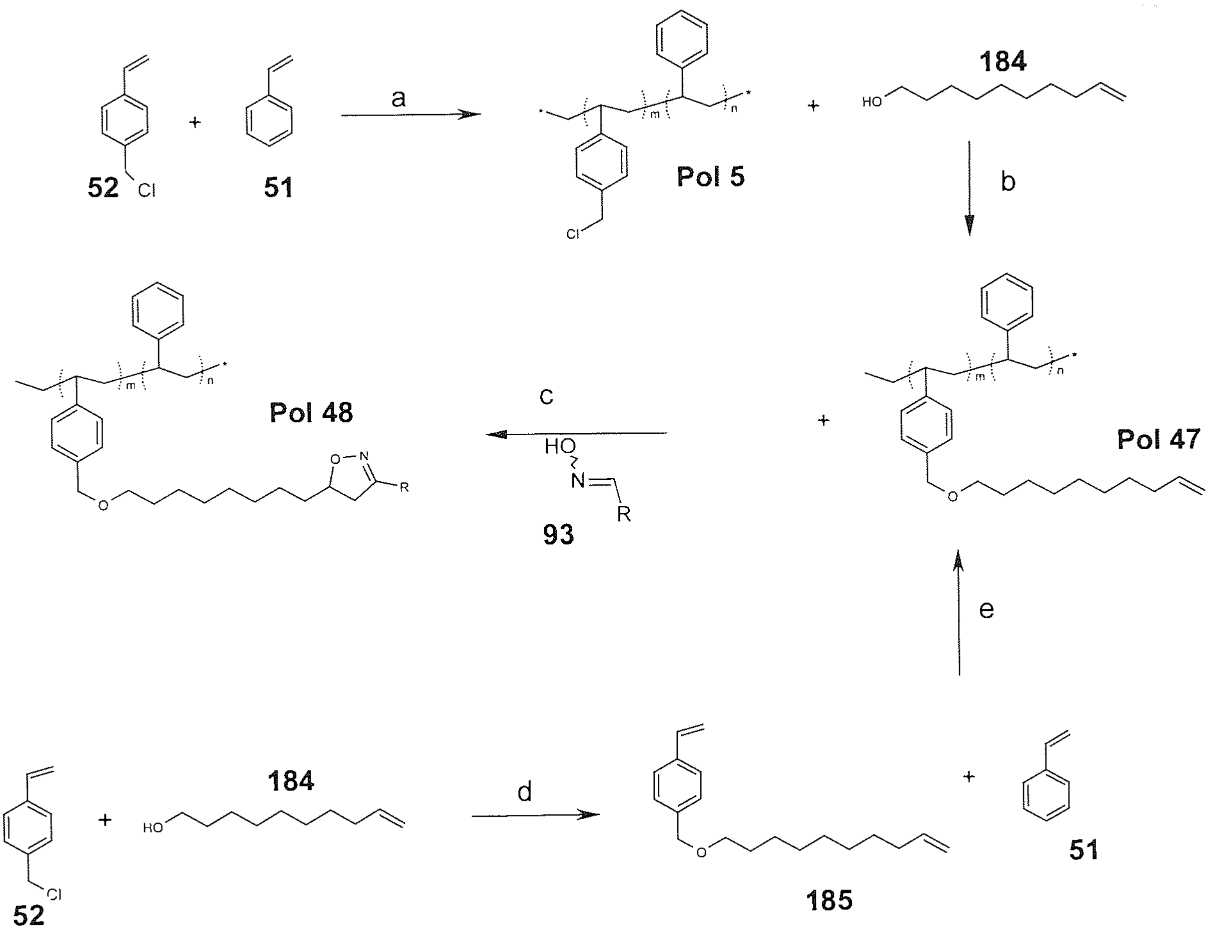


Scheme 29: **Soluble polymeric support assisted, possible synthesis of compounds containing catechol moiety.**

2.9: Attachment of Δ^2-isoxazolines to polystyrene soluble polymeric supports *via* non-hydrolysable ether linkage to prepare antimicrobial polymers:

Ethers are relatively stable and unreactive compounds. The R'–O–R bonds are non hydrolysable and stable under common reaction conditions. The common method generally used for the laboratory preparation of ethers is known as Williamson ether synthesis. Metal oxides react with primary alkyl halides by an S_N2 mechanism to yield ethers. The alkoxides needed in the Williamson reaction are normally prepared by the reaction of an alcohol with a strong base.

With a view to anchor Δ^2-isoxazolines to polystyrene based soluble polymeric supports by a non-hydrolysable ether linkage in order to prepare potential antimicrobial polymers, two possible routes were explored (**Scheme 30**). Route 1 involved the preparation of chloromethylated linear polystyrene by copolymerisation of styrene and vinyl benzoyl chloride. To immobilize alkene to soluble polymeric support *via* an ether linkage, 10-undecene-1-ol and **Pol 5** were heated under reflux, under anhydrous conditions, in the presence of a strong base and catalytic amount of sodium iodide (step b). The use of different solvents namely anhydrous acetone, anhydrous acetonitrile, bases including sodium hydride, and potassium carbonate, and different reaction times 6, 24, 48, 72, 96 hours were investigated. TLC analysis of the reaction mixture did not give any new spot that could correspond to the formation of new product. The ^{1}H NMR analysis also showed unsuccessful reaction. Route 2 explored the formation of ether linkage first followed by copolymerisation of this derivatised vinyl benzene with styrene. As mentioned earlier the formation of ether linkage was unsuccessful between **Pol 5** and 10-undecene-1-ol, and it also failed between 10-undecene-1-ol and vinyl benzoyl chloride. Therefore no further chemistry was performed on this polymer. Hence the attachment of Δ^2-isoxazolines to soluble polymeric support *via* non-hydrolysable bond, as potential antimicrobial polymers was not achieved.



R= alkyl, aryl

Scheme 30: Possible synthesis of soluble polymeric supports attached to the Δ^2-isoxazolines via non-hydrolysable ether bond. a & e) AIBN, toluene, 70°C, b & d) anhydrous solvents, strong base, see text, c) DCM, aldoxime, bleach.

CHAPTER 3

Biological evaluation of soluble linear polymers

3.1: Biological evaluation of the soluble linear polystyrene based copolymers:

Biological evaluation of the soluble linear polystyrene copolymers synthesized, was performed. The aim was to test the copolymers for any anti-microbial and/or anti-adhesion activity that would decrease the number of live bacteria that would adhere to the glass surface coated with polymer. This would suggest that activity would be specific to the polymer with anti-bacterial and anti-adhesion activity.

To test the anti-microbial activity of the polymers, a number of approaches was investigated before the procedure used was adopted. Many experimental problems were encountered during the experiment. These are discussed below.

Initially the biological testing was attempted by using a procedure similar to the one reported in reference (sec 1.6.3, p. 73). Glass slides were coated with the polymer, air dried for 2 minutes and placed in a solution of *S. aureus* cell suspension with gentle agitating for overnight (~18 hours). Slides were then removed from the cell suspension and immersed in fresh distilled water three times to remove any loose bacterial cells. Excess water was absorbed by gentle touch of paper towels. Slides were placed in the Petri dish and covered with molten nutrient agar, almost at its setting temperature and incubated at 37°C overnight. However this approach did not work as no colonies were observed. This could be due to two reasons, a) the agar was too hot for the bacterial cells and it killed all bacterial cells on the slides, b) the bacterial colonies would have been between the glass slide and agar instead of being on the surface of the agar, therefore would have been difficult to observe.

All polystyrene copolymers prepared were tested for anti-microbial activity. The procedure that was used is a modification of widely used clinical biological method to investigate the contamination of medical devices and implants, the Maki roll plate method.[122,123] This was modified from published methods[124,125,126] and performed under the supervision of Professor P. Lambert (Life and Health Sciences, Aston University). Prior to the microbiological testing, all polymers were re-precipitated by dissolving in a minimal amount of DCM and pouring onto rapidly stirred methanol. The precipitate collected was used to test for biological activity.

The procedure that was followed involved the coating of glass microscope slides with polymer film (polymer solution in chloroform was used to coat the slides), followed by air drying. The slides were then submerged in bacterial cell suspension solution overnight. Loosely bound bacterial cells were washed away with water leaving only firmly-adhering cells as microcolonies. The number of microcolonies on the slides were then determined by placing the slides on the surface of solid growth medium. In this process, as in the Maki roll plate sampling method for medical devices removed from patients, organisms are transferred from the slide to the solid medium in the Petri dish. Following incubation of the plates at 37°C overnight the number of colonies reflected the number of micrpcolonies present on the glass slides.

Four different polymer concentrations of polymer solutions were tested for the antibacterial activity against *S. aureus*. The polymer concentrations tried included, 10 % w/v, 5 % w/v, 1 % w/v, 0.1 % w/v.

At 10 % w/v concentration, polymers **Pol 12, Pol 13, Pol 14** and **Pol 15** had solubility problems such that they didn't dissolve completely. Other polymers that didn't have any solubility problem worked well with the coating of glass slides. But as soon as the slides were submerged in the cell suspension, almost all of the polymer films came off the slides. This suggests that the co-polymers prepared do not have good adhesion properties.

At 1 % and 0.1 % w/v concentrations, problems like poor solubility and removal of polymer films upon submergence of coated glass slides in cell suspension were not encountered, but the results were very unpredictable, such that for any given polymer, one (sometimes even two) out of five slides would have very high growth so that the counting colonies, was not feasible. Similarly for some polymers a few slides of the same replicate didn't show any growth at all. This experiment was repeated three times, but every time the results were as ambiguous as before. Therefore it was decided not to work with 1 and 0.1 % w/v concentrations.

At 5 % w/v concentration, the removal of polymer films, once the polymer coated slides were placed in cell suspension, was not as problematic as the 10 % w/v concentration. Some polymers showed partial removal of the polymer film while others remained fully intact. Also the bacterial growth obtained was much more manageable than the one obtained for 1 or 0.1 %. Therefore it was decided to explore the anti-microbial activity of the polymers working with the 5% w/v concentrations.

The volume of polymer solution used to coat the glass slides was also explored. Initially 100, 70 and 50 μL were tried. Since the polymer films were removed once they came in touch with the cell suspension, it was observed that the removal of polymer film was much more pronounced on slides coated with 100 and 70 μL than the 50 μL.

For the preparation of cell suspension, different concentrations of inoculum were tried with *S. aureus*. An inoculum containing ~ 10^9 cfu mL^{-1} was used to prepare the cell suspension. 16mL, 8mL, 4mL, 2mL and 1mL per litre of the inoculum were explored. Imprints made from the polymer coated slides treated with 16, 8, and 4 mL per litre cell suspension gave a very dense growth of *S. aureus*. In contrast 1mL per litre cell suspension gave growth fewer than 50 colonies per plate, whereas 2mL per litre cell suspension gave more dense growth then 1 mL per litre and less dense growth than 4mL per litre of cell suspension. Based on these results it was decided to try 1.6mL per litre of the inoculum. This gave good manageable results therefore 1.6 mL per litre of inoculum for all microbes were used.

All copolymers prepared, were subjected to initial screening for biological activity against *S. aureus*. Most copolymers exhibited more or similar growth to the control. Based on the findings of these test a total number of 7 polymers was selected for further tests with other microbes. The polymers selected were, **polystyrene** (control 2), **Pol 16, Pol 17, Pol 29, Pol 31, Pol 38** and **Pol 39**. Chloroform was also used as a control (control 1). The above procedure was repeated for these copolymers against *S.*

epidermidis, *Micrococcus luteus*, and *Micrococcus roseus*. The structures of these polymers are shown in **Table 15 below.**

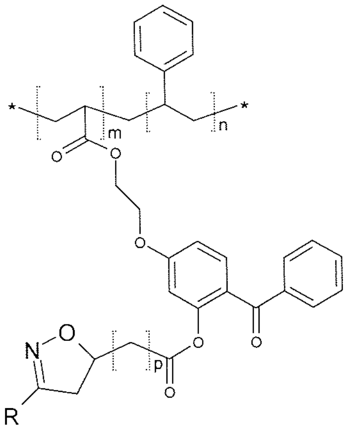
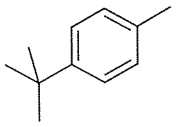
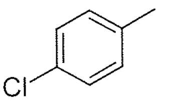
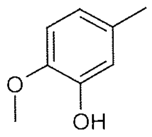
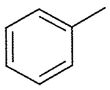
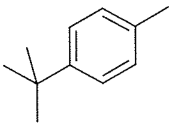
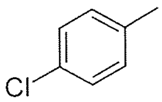
	Polymer	R	p
	**Pol 16**		8
	Pol 17		8
	Pol 29		8
	Pol 31		2
	Pol 38		0
	Pol 39		0

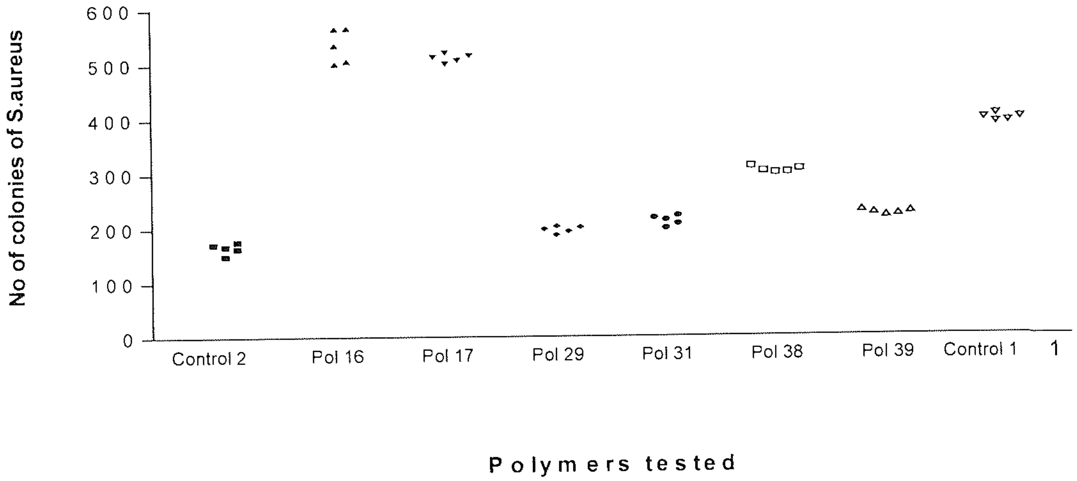
Table 15: Structure of copolymers that were tested for anti-microbial activity against various micro-organisms.

A Mann-Whitney (non-parametric) test was performed on the results obtained for each polymer tested against all microbes. If the P value obtained from the statistical analysis gave a value smaller than 0.05, then there was a significant difference in bacterial attachment to the polymers. However if the P value was greater than 0.05, then there was not any significant difference in bacterial attachment.

When tested against *S.aureus*, the polystyrene polymer (control 2) showed the most activity. Control 1 gave less activity when compared to control 2. The polymers **Pol 29**, **Pol 31**, **Pol 38** and **Pol 39** gave more activity than control 1 and less than control 2. **Pol 16** and **Pol 17** both showed an enhanced bacterial growth (no biological activity) when compared with both control 1 and 2, as it can be seen from **Graph 1** and **Figure 56**.

Statistical analysis suggested that there was a significant difference in the bacterial growth obtained for all copolymers tested. As it can be seen from **Table 16** that the P value is smaller than 0.05, when compared with both controls i.e. $CHCl_3$ and **Pol 7**.

Polymers that gave reduced bacterial growth (biological activity), both **Pol 38** and **Pol 39** have a substituent at the *para* position (*tert*-butyl and chloro group respectively) of the aromatic group and the isoxazoline group is directly attached to the linker. **Pol 31** does not have any substituent on aromatic ring and the isoxazoline group has two a carbon chain at C5 before it is attached to the linker. **Pol 29** has a methoxy substituent at *para* and a hydroxy group at *meta* position of the aromatic group. The isoxazoline group has an eight carbon chain between the C5 of the ring and the linker. In contrast **Pol 16** and **Pol 17** gave no biological activity. Both these polymers are comparable with **Pol 38** and **Pol 39** chemically, as **Pol 16** (same as **Pol 38**) has a *tert*-butyl and **Pol 17** (same as **Pol 39**) has chloro group at *para* position of aromatic group. The only difference is the presence of an eight carbon chain between the isoxazoline C5 and the linker through which the molecule is attached to the polymeric support.



Graph 1: **The number of colonies of *S. aureus* counted in each plate for the polymers that were selected to do further tests.**

	Control 1	Control 2	Pol 16	Pol 17	Pol 29	Pol 31	Pol 38	Pol39
Mean (n = 5)	394.8	166.2	534.6	511.4	196.6	212.6	303.6	224.6
Standard deviation	5.975	10.26	31.05	7.668	6.348	9.138	4.930	4.278
Coeffient of variance	0.015	0.062	0.058	0.015	0.032	0.043	0.016	0.019
P value (Mann Whitney test)*		0.0079	0.0079	0.0079	0.0079	0.0079	0.0079	0.0079
P value (Mann Whitney test)**	0.0079		0.0079	0.0079	0.0079	0.0079	0.0079	0.0079

*= When everything compared with control 1,

**= When compared with control 2 (polystyrene polymer).

Table 16: **Mann Whitney test results performed on the number of colonies of *S. aureus* counted for the polymers that were selected to do further tests on selected polymers**

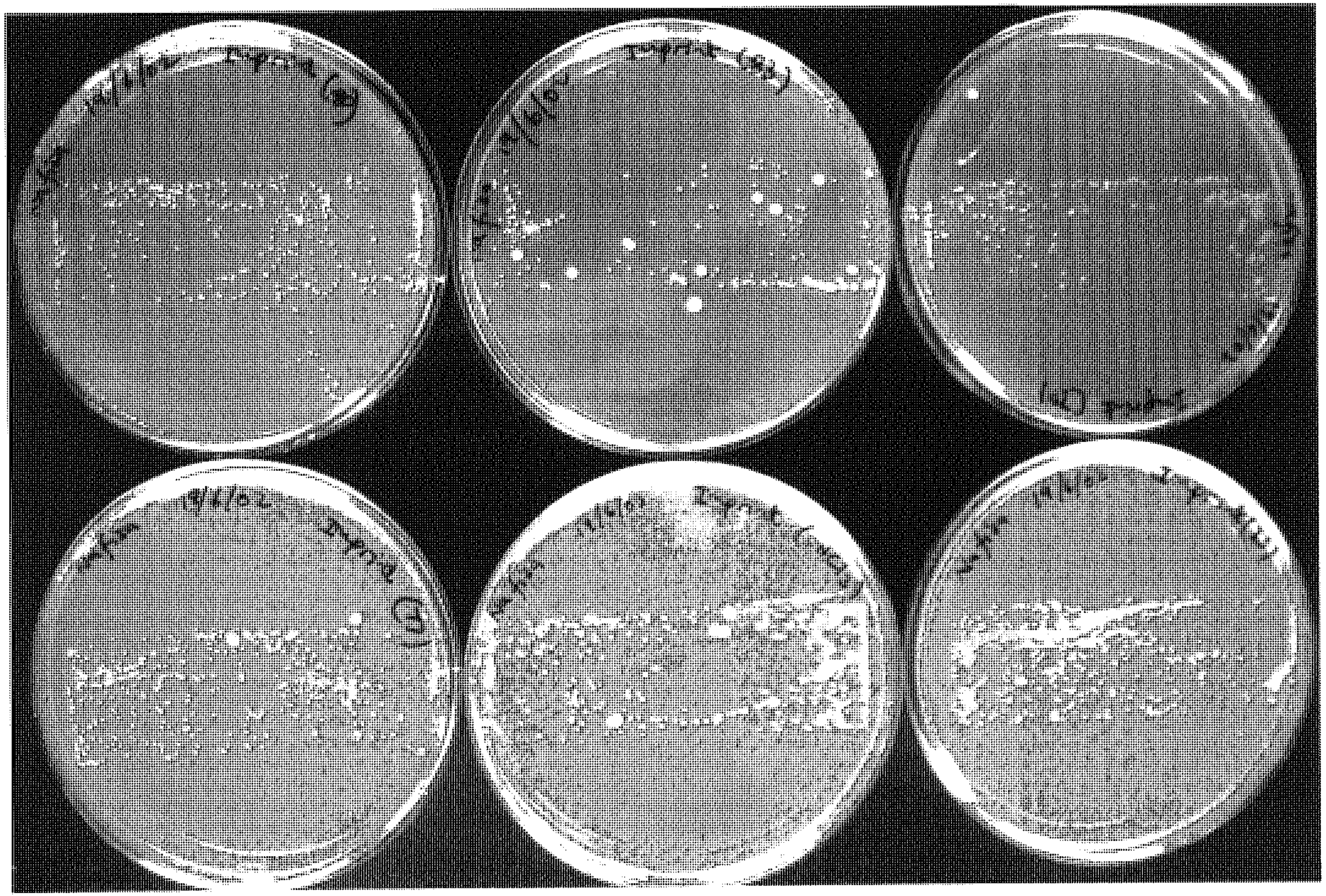


Figure 56: **A Picture of the colony growth obtained for *S. aureus*, when treated with controlled and a some other copolymers.**

Upper left **Pol 31** Upper middle **Control 2** Upper right **Pol 29**
Lower left **Pol 17** Lower middle **Control 1** Lower right **Pol 16**

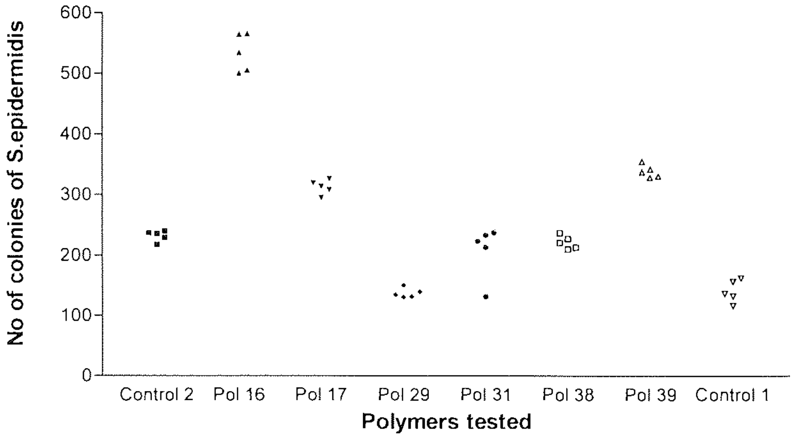
It can be seen from **Graph 2, 3** and **4** and **Figure 57, 58** and **59** below, that **Pol 16** and **Pol 17** exhibited mixed activity when tested against other micro-organisms, such as *S. epidermidis*, *M. luteus* and *M. roseus*. **Pol 16** showed no activity against *S. epidermidis* and *M. roseus* and showed weak activity against *M. leuteus.* **Pol 17** showed weak activity against *S. epidermidis* and *M. luteus.* **Pol 31** showed weak activity against *M. luteus.* **Pol 38** gave only weak biological activity against *S. epidermidis* and *M. luteus* only. **Pol 39** and **Pol 29** gave no activity for any of the micro-organisms.

Statistical analysis showed that for *S. epidermidis*, there was no significant difference in the growth obtained for **Pol 31** when compared to control $CHCl_3$, and **Pol 29** when compared with polystyrene as control 2 as the P value is greater than 0.05. (**Graph 2**, **Table 17** and **Figure 57**)

For *M. luteus*, there was a significant difference for growth obtained for all polymers, except the **Pol 39** when compared to $CHCl_3$ as a control as the P value is larger than 0.05. (**Graph 3, Table 18** and **Figure 58**)

For *M. roseus*, there was no significant difference in the bacterial growth obtained for **Pol 17, Pol 29, Pol 39,** when compared to $CHCl_3$ as a control, and for **Pol 31** when compared to **Pol 7** (polystyrene) as control. (**Graph 4, Table 19** and **Figure 59**)

S.epidermidis,



Graph 2: **Number of colonies counted for *S. epidermidis* for selected copolymers.**

PAGE 142 BLANK

	Control 1	Control 2	Pol 16	Pol 17	Pol 29	Pol 31	Pol 38	Pol 39
Mean (n = 5)	141.4	232.0	534.6	313.2	137.8	208.4	221.6	339.2
Standard deviation	18.68	8.803	31.05	11.73	8.167	43.71	10.90	10.45
Coeffient of variance	0.132	0.038	0.058	0.037	0.059	0.210	0.049	0.031
P value (Mann Whitney test)*		0.0079	0.0079	0.0079	0.6905	0.0952	0.0079	0.0079
P value (Mann Whitney test)**	0.0079		0.0079	0.0079	0.6905	0.0952	0.0079	0.0079

*= When everything compared with control 1,

**= When compared with control 2 (polystyrene polymer).

Table 17: **Mann Whitney test results performed on the number of colonies of *S. epidermidis* for selected co-polymers.**

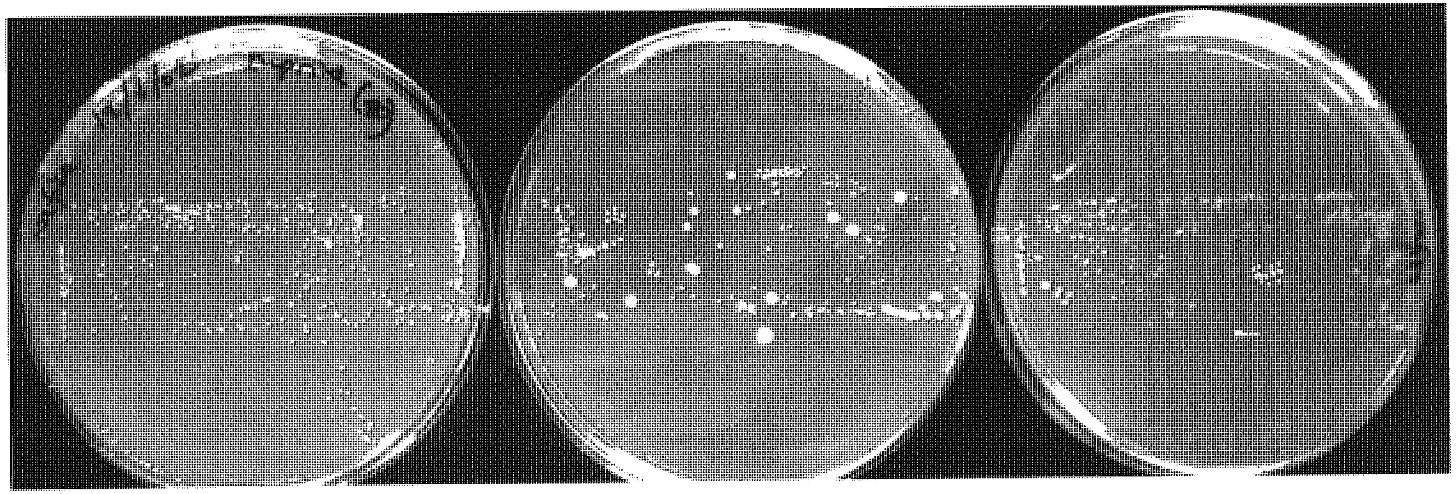
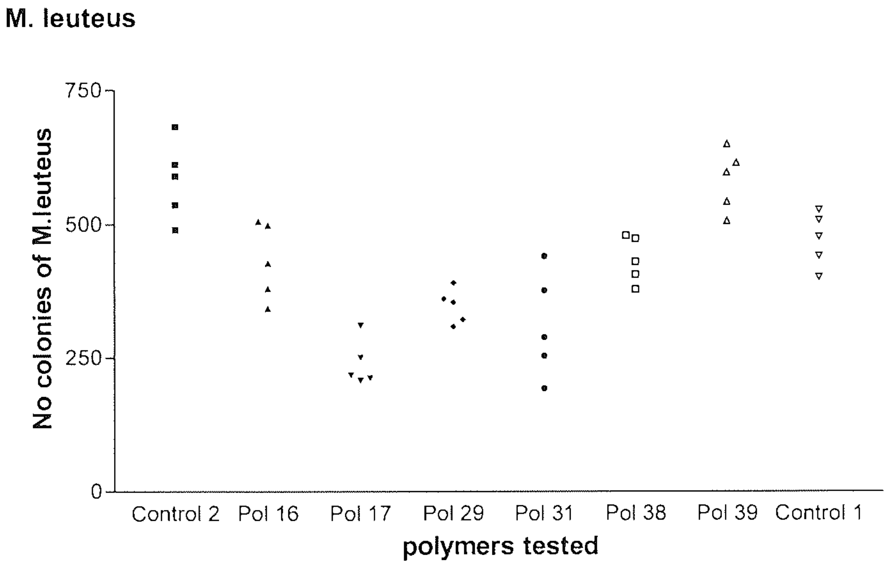


Figure 57: **A picture of colony growth obtained for *S.epidermidis* when treated against Pol 29, Pol 38 and control 1 (in middle)**

Left **Pol 38** Middle: **Control 1** Right **Pol 29**

Page 144 - BLANK



Graph 3: **Number of colonies counted for *M. luteus* for selected copolymers.**

	Control 1	Control 2	Pol 16	Pol 17	Pol 29	Pol 31	Pol 38	Pol 39
Mean (n = 5)	470.8	538	431.2	239.8	347	310.8	434.2	582.4
Standard deviation	50.84	73.39	71.77	43.11	32.59	98.63	43.24	57.19
Coeffient of variance	0.108	0.136	0.166	0.180	0.094	0.317	0.099	0.098
P value (Mann Whitney test)*		0.0317	0.3095	0.0079	0.0079	0.0159	0.3095	0.0317
P value (Mann Whitney test)**	0.0317		0.0317	0.0079	0.0079	0.0159	0.0079	0.8413

*= When everything compared with Control 1,

**= When compared with Control 2 (polystyrene polymer)

Table 18: **Mann Whitney test results performed on the number of colonies of *M. luteus* for selected co-polymers.**

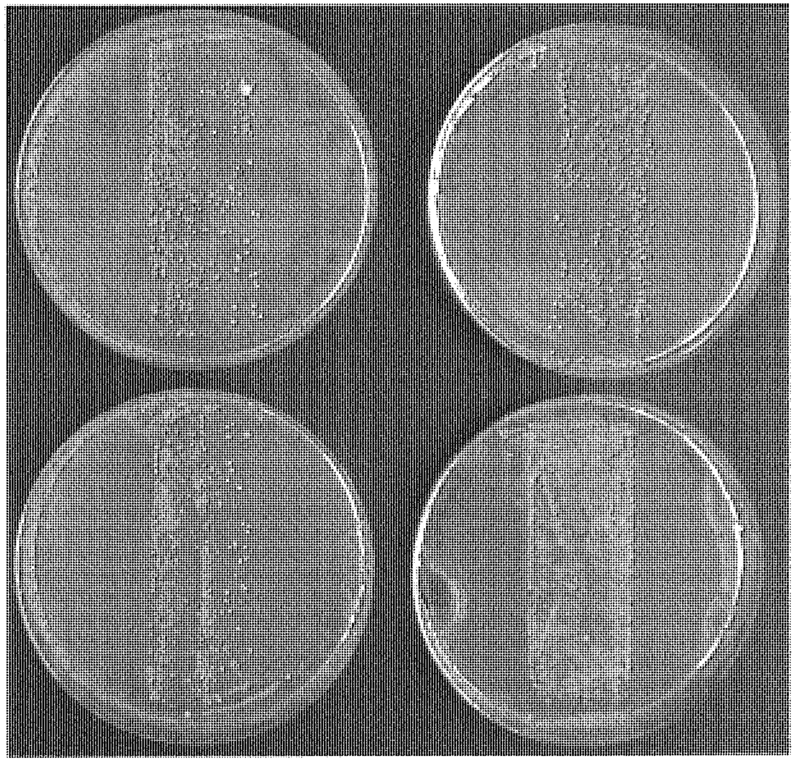
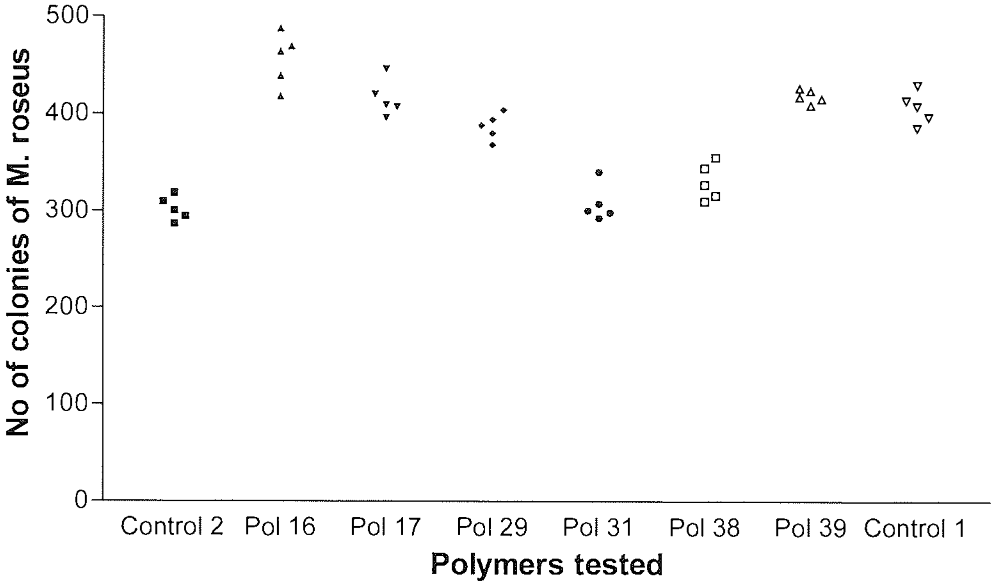


Figure 58: **A picture of the colony growth obtained for *M. luteus*, when treated with control 2 and other copolymers:**

Upper Left: **Pol 29** Upper right: **Pol 17**
Lower Left: **Pol 31** Lower right: **Control 2**

M. roseus



Graph 4: **Number of colonies counted for *M. roseus* for selected copolymers**

PAGE 148 BLANK

	Control 1	Control 2	Pol 16	Pol 17	Pol 29	Pol 31	Pol 38	Pol 39
Mean (n = 5)	408	302.4	456.8	416.6	387.8	308.4	331.4	419.8
Standard deviation	16.73	12.52	27.46	19.01	13.68	18.99	18.88	7.050
Coeffient of variance	0.041	0.041	0.060	0.046	0.035	0.062	0.057	0.017
P value (Mann Whitney test)*		0.0079	0.0159	0.6905	0.0952	0.0079	0.0079	0.2222
P value (Mann Whitney test**	0.0079		0.0079	0.0079	0.0079	0.8413	0.0317	0.0079

*= When everything compared with Control 1,

**= When compared with Control 2 (polystyrene polymer).

Table 19: **Mann Whitney test results performed on the number of colonies of *M. roseus* for selected co-polymers.**

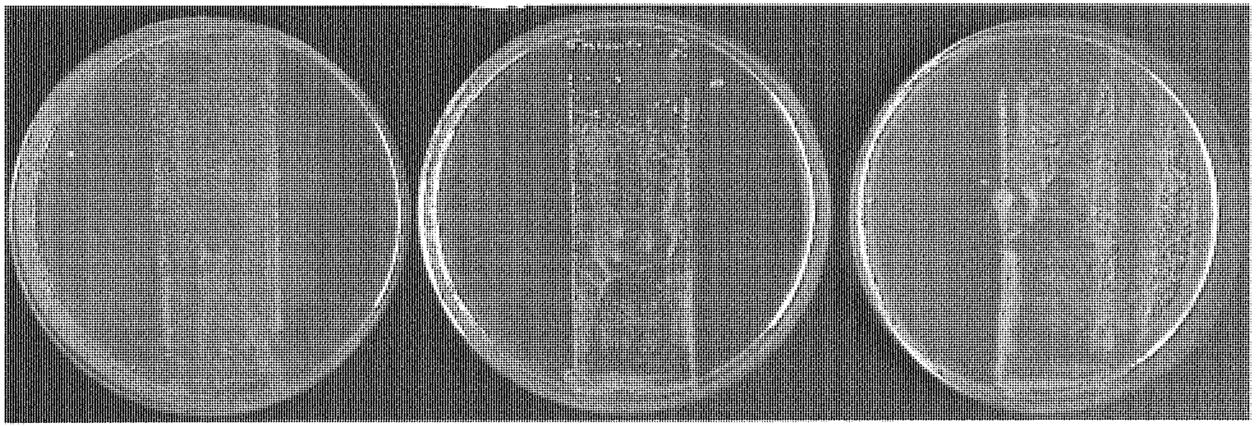


Figure 59: **A picture of the growth obtained for *M. roseus*, when treated against control 1 and some copolymers**

Left: **Pol 31** Middle: **Control 1** Right: **Pol 16**

3.2: Antimicrobial activity of Δ^2-isoxazoline compounds:

The purified isoxazoline compounds, synthesized both, on polymeric support and by traditional solution phase chemistry (compounds **150 - 158**, **162** and **163** {section 5.6.1 - 5.6.11, p. 196-200}), were subjected to an initial biological screening against *Mycobacterium fortuitum* (NCTC 10394).

For the biological testing of Δ^2-isoxazoline compounds, as a first screen the compounds were tested against *M. fortuitum* at a fixed concentration of 32 μg/mL. Those compounds exhibiting antimicrobial activity at this concentration were to be screened further over a range of lower drug concentrations. However, none of the Δ^2-isoxazoline compounds that were tested, exhibited any inhibitory activity against the *M. fortuitum* (NCTC 10394). Almost all compounds gave more or similar growth to the control. Therefore no further tests were done on these compounds.

CHAPTER 4

Conclusion

4.1: Soluble linear polymeric supports for organic synthesis:

A selection of soluble linear polystyrene copolymers was prepared with a view to exploring the utility of these copolymers as soluble supports in organic synthesis. Initially **Pol 1**, carrying an ester linker, was prepared. This polymer was utilized in 1,3-dipolar cycloaddition reactions, between polymer bound terminal alkenes and nitrile oxides generated in *situ* from their corresponding aldoximes, to produce polymer bound isoxazoline compounds (**Pol 12** and **Pol 13**). Although, it was supported by the ^{1}H NMR that a reaction had taken place at the terminal alkenes, the attempts to cleave the product from the polymeric support were unsuccessful. Therefore, no further reactions were carried out on this polymer. Further progress was made by preparing another soluble polymer, **Pol 2**, which carried an OH functionality bounded to an aromatic ring of the polymer pendant group, which itself, was connected to another aromatic ring through another carbonyl functionality. This polymer gave better solubility profiles than the **Pol 1**. **Pol 2** then had to be, first treated with 10-undeconyl chloride, to yield a polymer bounded terminal alkene (**Pol 8**). **Pol 8** was further utilized in 1.3-DC reactions to give polymer-bound isoxazoline compounds (**Pol 14** and **Pol 15**). Attempts to cleave the polymer-bound products were unsuccessful. This was probably due to the steric hindrance from the polymer backbone and the aromatic groups of the pendant group that gave very poor cleaved product yields. Therefore no further reactions were performed on **Pol 2**.

To further advance progress in the preparation of soluble linear polymeric supports, another soluble polymer **Pol 3** was prepared. This polymer carried a similar functionality, where the synthetic modification took place, to that of **Pol 2**. In addition it carried an ethylene glycol spacer group between the connection point at the polymer backbone and the aromatic groups of the linker (chapter 2). **Pol 3** gave the best solubility profile of the range of polymers prepared. **Pol 3** was reacted with three different aliphatic acid chlorides of various lengths, to yield **Pol 9**, **Pol 10** and **Pol 11** polymers carrying terminal alkenes attached *via* an ester linkage to the aromatic ring of the pendant group. These polymers were further utilized in 1,3-DC reactions with various nitrile oxides generated in *situ* from their corresponding aldoximes, to give polymer bound isoxazoline compounds.

It was discovered that **Pol 9** gave the best results in terms of analysing the progress of a reaction and obtaining the cleaved products, followed by **Pol 10** and **Pol 11** respectively. This can be explained from the chemical nature of these polymers. **Pol 9** had terminal alkenes at the end of a long chain of aliphatic carbons. This placed the site for chemical modification furthest away from the polymer backbone and aromatic groups of polymer pendant group, thus making it more accessible for the reagents to react. Being away from the polymer backbone also made it easier to observe any changes in ^{1}H NMR spectrum of the starting and synthetically modified polymers, especially the terminal alkene peaks of **Pol 9**. It was also discovered that the nitrile oxides with electron withdrawing groups were most successful in reacting with the terminal alkenes, followed by the neutral and weakly electron donating groups. Strongly electron donating groups, e.g. *N,N*-dimethyl amine, failed to give successful 1,3-DC reactions. Aliphatic nitrile oxides were also unsuccessful at reacting with the terminal alkenes, especially lower molecular mass one. A similar pattern of results was obtained for **Pol 10**. It carried the terminal alkenes at the end of a two carbon chain. Still being away from the polymer backbone, it was possible to analyse the reactions carried out in the ^{1}H NMR spectrum. However, it was not as obvious to see changes in the starting and synthetically modified polymers from this polymer as it was with **Pol 9** and its synthetically modified polymers. On the other hand, **Pol 11** being different in chemical nature to the other two polymers, i.e. carried an acrylate, reacted with both types of nitrile oxides, i.e. those carrying an electron donating group and those carrying an electron withdrawing groups. This was supported by the ^{1}H NMR spectrum of the polymers.

Cleavage of the products from the polymeric supports exhibited a similar pattern as mentioned above. There were two points of cleavage present in the polymers studied. One very close to the polymer backbone and the other attached to the aromatic group of the polymer pendant group that was the point of interest. The one close to the polymer backbone would present strong steric hindrance therefore cleavage at this site would be very low. Derivatised polymers from **Pol 9** gave the best yield for cleaved products. i.e. **Pol 16**, **Pol 17**, **Pol 18** etc, followed by those derivatised from **Pol 10** i.e. **Pol 29**, **Pol 30**. Derivatised polymers from **Pol 11**, however failed to gave any cleaved products. Again this can be explained from the chemical nature of these polymers. **Pol 9** would have caused the least steric hindrance from the polymer bound isoxazoline groups, therefore making it more accessible for the reagents to act at the site of cleavage. **Pol 10** would have presented some steric hindrance depending on the substituents on polymer-bound isoxazoline groups. Derivatised polymers from **Pol 11** with polymer-bound isoxazoline groups, being very close to the point of cleavage, would have caused most steric hindrance dependent on the nature of the substituent present on C3 of the isoxazoline ring, thus preventing the reagents to act on the site of cleavage.

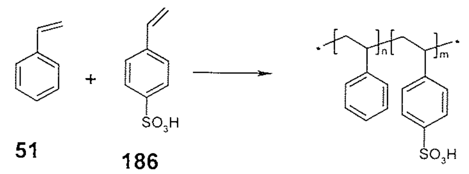
Overall from the studies performed on the three basic polymers **Pol 1**, **Pol 2** and **Pol 3**, it can be concluded that the **Pol 3** was the most suitable as a soluble linear polymeric support for organic synthesis. The derivatised polymers derived from **Pol 3**, **Pol 9** and its derivatised polymers gave the best results both in terms of the yield obtained and the chemical analysis by analytical methods.

4.1.1: Future work:

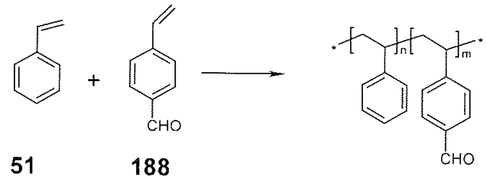
In this thesis, one possible use of linear polystyrene copolymers as soluble polymeric supports was explored. There could be other ways in which the use of these polymers could be explored. e.g as scavenger resins. Majority of scavenger resins have only been used in the form of insoluble cross linked polystyrene polymers. A new type of soluble scavenger resin could be exploited, where the reaction, including the scavenger resins, would take place under complete homogeneous reaction conditions. This would avoid the use of lager volumes of solvents that are required for scavenging techniques during a reaction. (However, a larger volume of solvent would be required in the workup to precipitate the polymer). Another advantage this type of scavenger resins would provide over the conventional scavenger resins is the cost factor. Scavenger resins are very expensive and a large amount is often needed for scavenging. In the case of soluble form of scavenger resins the loading could be fine tuned according to the chemist's need for a particular reaction. Some examples of potential soluble form of scavenger resins that could be exploited are shown below in **Figure 60**.

The **Pol 3**, as it stands, can be used as nucleophilic scavenger resin, to remove excess acid chlorides

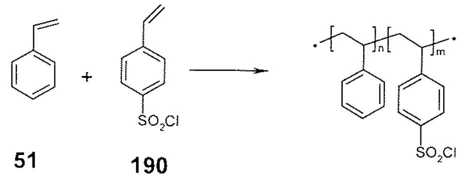
Electrophilic scavenger resins:



51 186 187

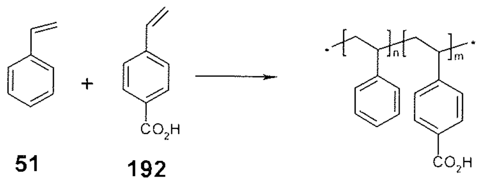


51 188 189



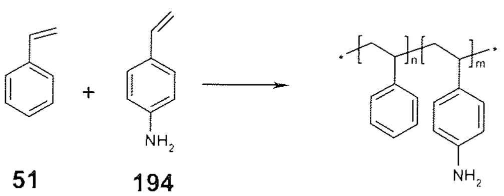
51 190 191

Ion-exchange resins:

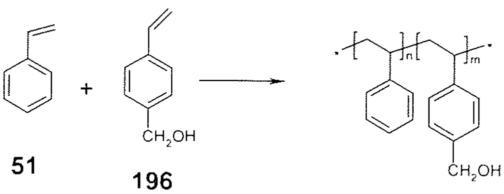


51 192 193

Nucleophilic scavenger resisn:



51 194 195



51 196 197

Figure 60: Some potential soluble scavenger resins.

4.2: Antimicrobial activity of polystyrene copolymers:

A range of linear soluble polystyrene based polymers were prepared and tested for biological activity. The method used to study the biological activity of these polymers was adapted from literature reports and is a modification of widely used clinical method the Maki roll plate method which is used to investigate the contamination of medical devices and implants. The biological study was performed under the supervision of Professor P. Lambert (Life and Health Sciences, Aston University).

After initial screening of all polymers prepared, seven polymers in total were selected for further tests which included **Pol 7**, **Pol 16**, **Pol 17**, **Pol 29**, **Pol 31**, **Pol 38** and **Pol 39**. A range of polymer concentrations (w/v) and cell suspensions were investigated, against four different types of bacteria, *S. aureus, S. epidermidis*, *M. luteus*, and *M. roseus* It was found that a 5% w/v polymer solution in chloroform and 1.6mL bacterial inoculum per litre gave the best results. Polystyrene coated slides and those coated with chloroform were used as control. It was discovered that polymers exhibited a range of activity against different bacteria.

When tested against *S. aureus*, **Pol 29**, **Pol 31**, **Pol 38** and **Pol 39** gave reduced activity and **Pol 16** and **Pol 17** gave enhanced activity when compared against controls. Tests against *S. epidermidis* showed **Pol 29**, **Pol 31** and **Pol 38** with reduced activity and rest similar or enhanced activity as controls. Against *M. leuteus* except **Pol 39** all polymers showed reduced activity. None of the polymers gave reduced biological activity when compared with controls except **Pol 31** and **Pol 38** which gave similar growth to that was obtained for controls.

Having done the glass slide coated experiments on soluble polymers and in the light of results obtained, it can be concluded that **Pol 31** and **Pol 38** gave the smallest number of bacterial growth therefore preventing the adhesion of live bacterial cells to the glass surface coated with these polymers. **Pol 16** and **Pol 17** do not prevent the adhesion of the live bacterial cells to the glass surfaces coated with these bacteria therefore gave a large bacterial growth.

However one cannot purely rely on these results, as there is room for improvement in the experimental procedure, especially the adhesion difficulties that were encountered.

Future work:

In order to obtain more reliable results, it would be advisable to have covalent attachment of related polymers to the surfaces as carried out in literature work (p. 73, ref. 77 by Kilbanov) and repeat the above biological testings.

CHAPTER 5

EXPERIMENTAL

5.1: Chemicals:

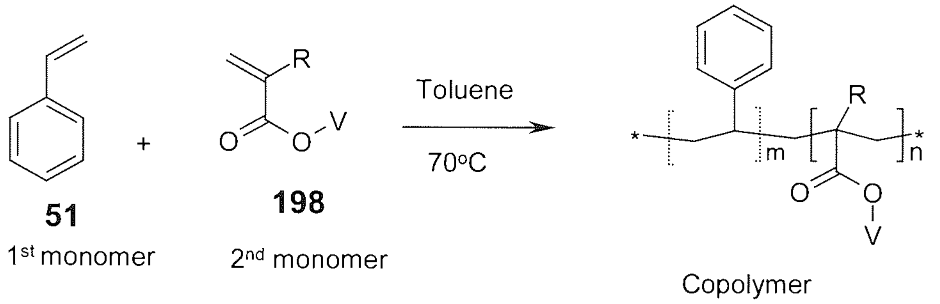
N,N-Diisopropylethylamine (Hünig's base) (99.5%), *p*-toluenesulfonic acid monohydrate (99%), Amberlite™ IRA 67, methyl-10-undecenoate (96%), 4-vinyl benzyl chloride (90%) and 1-(4-hydroxyphenyl)-3-butanon (99%) were purchased from Sigma-Aldrich. 4-Nitrocatechol (98%), 4-hydroxyacetophenone (99%), styrene, butyraldehyde oxime (98%), trifluoroacetic acid (99%), 10-undecenoic acid, 10-undecen-1-ol (99%) and palladium on carbon (10%) were purchased from Avocado Research Chemicals Ltd. 2,2'-Azobis(2-methylpropionitrile) (AIBN) (98%), *syn*-benzaldehyde oxime (99%) and acryloyl chloride (98%) were purchased from Acros Organics. Aqueous sodium hypochlorite was used in the form of commercially available bleach containing 4.5% w/v chlorine and was purchased from Fische Chemicals. The Wang-resins were kindly donated by Avecia Life Science Molecules as pre-production batch.

5.2: Instrumentation:

Thin layer chromatography plates, aluminium-backed silica gel 60 F_{254}, were obtained from Merck. All proton NMR and ^{13}C NMR spectra were obtained on a Bruker AC 250 instrument operating at 250 MHz as solutions in $CDCl_3$ and referenced from $\delta CDCl_3$ = 7.27ppm and 77.0 ppm respectively, unless otherwise stated. Infrared spectra were recorded as KBr discs. unless otherwise stated, on a Mattson 3000 FTIR spectrophotometer. Atmospheric pressure chemical ionisation mass spectrometry (APCI-MS) was carried out on a Hewlett-Packard 5989B quadrupole instrument connected to an electrospray 59987A unit with an APCI accessory and automatic injection using a Hewlett-Packard 1100 series autosampler. Melting points were obtained using a Reichert-Jung Thermo Galen hot stage microscope and are corrected. Elemental composition electrospray (+) mass spectroscopy was carried out on a Walter LCT premier ToF (Time of flight) Mass spectrometer. Samples were dissolved in 100% methanol and injected into flow of 100% methanol (0.25mL/min). Leucine enkephaline was used as lock mass at 556.2771.

5.3: Preparation of soluble copolymers of linear polystyrene:

A general procedure for the preparation of a linear styrene copolymer with various monomers:



1st monomer: = Styrene
2nd monomer: A = Allyl methacrylate,
B = 2-hydroxy-4-(methacryloyloxy) benzaphenone,
C = 2-4-benzoyl-3-(hydroxyphenoxy) ethyl acrylate,
D = vinylbenzylchloride,
E = styrene,
F = Acrylic acid 4-[2-(5-nitro-2-methyl-benzo[1,3]dioxol-2-yl)-ethyl]-phenyl-ester,

R = H or methyl group,
V = variable aromatic and aliphatic groups,
m + n variable repeated units.

Scheme 31: **A general reaction for the polymerisation of a copolymer from styrene and a co-monomer:**

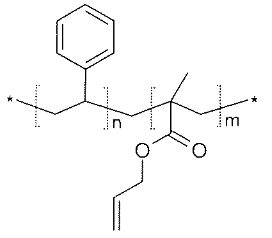
A solution of a co-monomer, AIBN, and styrene in toluene was de-oxygenated by alternate applications of vacuum and argon (x 5) with rapid stirring. The solution was heated at 70°C, for 4 days under an argon atmosphere. The toluene was removed under vacuum distillation until there was just enough toluene to keep everything in solution. The solution was poured onto vigorously stirred methanol. The resulting precipitate was collected by filtration, re-dissolved in a small amount of dichloromethane and re-precipitated onto methanol. The final precipitate was collected by filtration and dried under vacuum to give the product as a solid.

For the ratio of each monomer to styrene and other reagents used, please see **Table 20** below. For the individual spectral analysis of each type of copolymer also see sections 5.3.1 to 5.3.7.

Copolymer prepared	Co-monomer			Styrene		AIBN (mg)	Toluene (mL)	Methanol (mL) to precipitate the polymer
	Nº	Quantity						
		mmol	g	mmol	g			
Pol 1	A	80.01	8.32	400	41.60	500	150	500
Pol 2	B	1.77	0.18	33.7	3.50	16	15	250
Pol 3	C	48.00	6.05	240	24.96	300	150	500
Pol 4		16.01	2.02	0.00	0.00	100	35	350
Pol 5	D	23.68	2.98	157.7	16.40	200	100	500
Pol 6	E	0.00	0.00	96.0	9.98	200	100	500
Pol 7	F	2.06	0.26	10.925	1.14	36	20	250

Table 20: **The ratio of co-monomers to styrene and the other reagents used to obtain a copolymer.**

5.3.1: Poly (styrene-co-allylmethacrylate) copolymer (Pol 1):



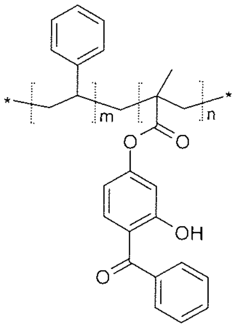
(**Pol 1**)

Yield: 33.54g, 65%, 1.39 mmol/g loading of functional unit, a pale yellow / cream coloured solid:

^{1}H NMR: δ_H: 0.23–1.02(br m CH$_3$), 1.10–2.29(overlapping br m, polymer back bone CH$_2$), 3.19–4.23 (overlapping br m, 2H, $OC\underline{H}_2CH{=}CH_2$), 4.78–5.31(overlapping br m, 2H, $OCH_2CH{=}C\underline{H}_2$), 5.33–5.80(overlapping br m, 1H, $OCH_2C\underline{H}{=}CH_2$), 6.23-7.25 (overlapping br m, Ar-H from polymer bonded styrene units) ppm.

IR ν = 2921, 1728 (C=O), 1598, 1490, (Ar ring stretch)

5.3.2: Poly (styrene-co-2-hydroxy-4-(methacryloyloxy) benzophenone copolymer (Pol 2):



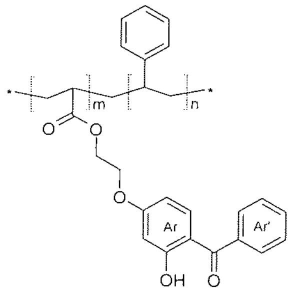
(**Pol 2**)

Yield: 2.15g, 53.8%, 0.44mmol/g loading of functional unit, a pale yellow coloured solid.

^{1}H NMR: δ_H: 0.31–1.05(overlapping br m, polymer backbone CH_3), 1.16-2.53(overlapping br m, polymer backbone CH_2), 6.21-7.77(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

IR ν = 3418 (O-H), 3027, 2919, 1941, 1754 (C=O), 1625, 1494 (Ar ring stretch), 1447 cm^{-1}.

5.3.3: Poly (styrene-co-2-(4-benzoyl-3-hydroxyphenoxy) ethoxy acryloyloxy) copolymer (Pol 3):



(**Pol 3**)

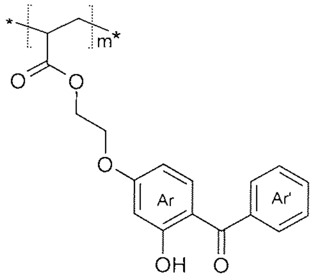
Yield: 26.60g, 98%, 1.34mmol/g loading of functional unit, yellow solid.

^{1}H NMR δ_H: 1.09-2.30(overlapping br m, polymer back bone CH_2), 3.55-4.22(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 6.15-7.75(overlapping br m, Ar-H from polymer backbone styrene and polymer pendant groups), 12.70(s, 1H, Ar-OH) ppm.

^{13}C NMR δ_C: 39.7-41.2(broad peaks, polymer back bone), 61.9-62.($\underline{C}H_2OAr$), 66.13, 101.62, 107.51, 113.33, 127.60-128.79(broad peaks, aromatic carbons), 135.20, 38.19, 145.00(broad peaks), 164.85, 166.25, 175.68. 199.98(carbonyl carbon) ppm.

IR ν = 3440 (O–H), 3027, 2925, 1943, 1826, 1729 (C=O), 1598, 1490 (Ar ring stretch) cm^{-1}.

5.3.4: Poly(2-(4-benzoyl-3-hydroxyphenoxy) ethoxy acryloyloxy polymer (Pol 4):



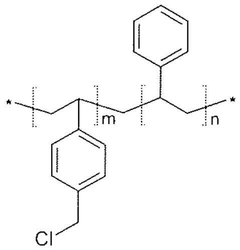
(Pol 4)

Yield: 3.47g, 69%, 3.20 mmol/g functional group loading, pale yellow solid.

^{1}H NMR δ_H: 1.32-2.45(overlapping br m, CH_2 from polymer back bone), 3.81-4.50 (overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 6.19-6.56 (overlapping br m, 2,6-ArH) 7.13-7.70(overlapping br m, 5-Ar and Ar' protons), 12.6 (OH) ppm.

IR ν = 3450 (O–H), 3060, 2934, 1739 (C=O), 1621 cm^{-1}.

5.3.5: Poly (styrene-co-vinylbenzyl chloride) coplymer (Pol 5):



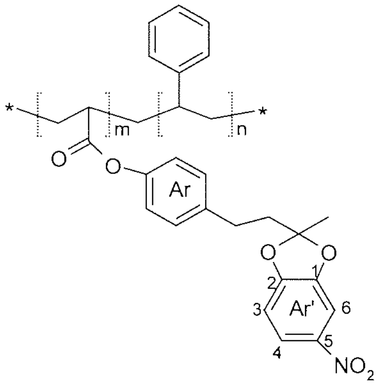
(Pol 5)

Yield: 9.33g, 46.65%, 1.20 mmol/g loading of functional group, white solid.

^{1}H NMR δ_H: 1.20-2.25(overlapping br m, CH_2 and CH from the polymer backbone), 4.25-4.63(overlapping br d, 2H, $ArC\underline{H}_2Cl$), 6.25-7.27(overlapping br m, Ar-H from the aromatic groups of the polymer) ppm.

IR ν = 3024, 2920, 1949, 1601, 1492, 1451, 1368, 1265, 1027 cm^{-1}.

5.3.6: Poly (styrene-co-4-[2-(5-nitro-2-methylbenzo[1,3]dioxol-2-yl) ethyl] phenyl ester) copolymer (Pol 6):



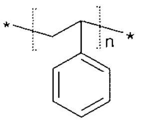
(Pol 6)

Yield: 0.49g, 27%, 1.37 mmol/g loading of the functional group, pale yellow solid.

^{1}H NMR δ_H: 0.94-2.57(overlapping br m, $C\underline{H}_2$ from polymer back bone and Ar-$CH_2C\underline{H}_2COOCH_3$), 2.58-2.90(br m, 2H, Ar-$C\underline{H}_2CH_2COOCH_3$), 6.0-7.35(overlapping br m, containing aromatic protons from Ar, Ar' and styrene from polymer back bone), 7.63(br s, 1H, 6-Ar'-H), 7.85-7.95(br d, J= 8.47 Hz, 1H, 4-Ar'H) ppm.

IR ν = 3417, 3023, 2921, 1949, 1752 (C=O), 1604 (Ar ring stretch), 1492 (NO_2)

5.3.7: Polystyrene.



(Pol 7)

Yield: 5.58g, 55%, white powder solid.

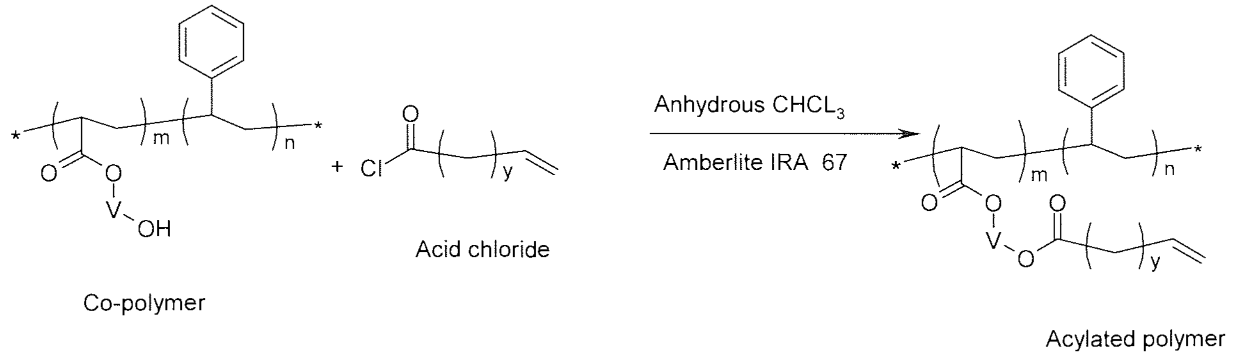
^{1}H NMR δ_H: 1.46-2.25(overlapping br m, CH_2 and CH from polymer backbone), 6.05-7.55(overlapping br m, Ar-H from the aromatic groups of the polymer) ppm.

^{13}C NMR δ_C: 40.33-40.73(broad peaks, polymer back bone carbons), 77.0 ($CDCl_3$), 125.62-127.94(aromatic carbons)ppm.

IR ν = 3435, 3025 (Ar-H), 1492 (Ar ring stretch) cm^{-1}.

5.4: Acylation of polymer-bound phenol:

A general procedure for acylation of polymer bound phenol of a styrene copolymer:



Acid chlorides:
y = 0 - acroloyl chloride,
4 - pentonyl chloride,
8 - 10-undecenoyl chloride,

V = variable aromatic and aliphatic groups

Scheme 32: A general reaction for the acylation of co-polymers having an OH linker.

A mixture of the polymer containing an OH as the functional group, Amberlite™ IRA 67 and D-MAP in anhydrous chloroform was stirred under argon until the polymer had dissolved. The acid chloride was added drop-wise *via* a syringe and the reaction was cooled in an ice-bath. (Please see **Table 21** below for the reaction times)

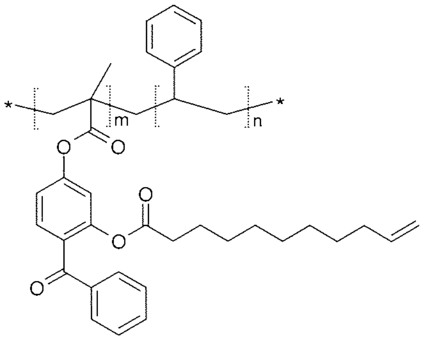
The Amberlite™ IRA 67 was removed by filtration. The filtrate was collected and the reaction mixture was concentrated by removing most of the chloroform under vacuum, until the reaction contained just enough chloroform to keep everything in solution. The polymer was precipitated by pouring the solution onto vigorously stirred methanol. The precipitate was collected by filtration and dried under vacuum.

For the quantities of the reagents used for this reaction, please see **Table 21** below.

Co-polymer		Polymer prepared	acid chlorides			Amberlite IRA 67 (1.5mmol/g) (g)	D-MAP (mg)	$CHCl_3$ anhydrous (mL)	Time taken to add the acid chloride (min)	Cooling time (in an ice bath) (min)	Reaction temperature.
			Acid chlorides	mmol	(g)						
Pol 2	7.504	**Pol 8**	10-undecenoyl chloride	12.40	2.51	10.21	150	200	15	15	Refluxed at 70°C for 48 hours.
Pol 3	10.00	**Pol 9**	10-undecenoyl chloride	47.44	9.59	60.80	500	150	20	20	Refluxed at 70°C for 48 hours.
	10.03	**Pol 10**	Pentonyl chloride	29.65	3.52	32.62	500	150	25	20	Refluxed at 40°C for 36 hours.
	10.05	**Pol 11**	Acryloyl chloride	42.95	3.87	45.0	300	150	30	30	Left at RT (18°C) for overnight.

Table 21: **The table shows the quantities of different types of copolymers against different types of acid chlorides used, along with other reagents used for this reaction. The table also shows the reaction times as well as the temperature for each reaction.**

5.4.1: Poly (styrene-co-undec-10-enoic acid 5-acryloyloxy-2-benzyloxy) phenyl ester copolymer (Pol 8):

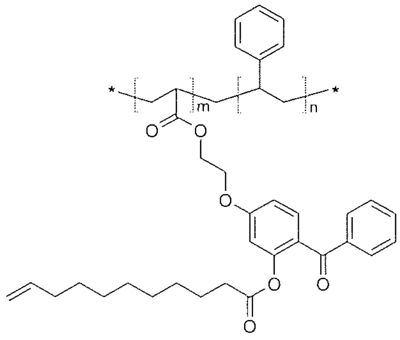


(Pol 8)

Yield: 7.20g, pale yellow solid.

^{1}H NMR δ_H: 0.31-1.01(br m, CH_3), 1.16-2.53(br m, polymer backbone CH_2 and CH_2 from the aliphatic pendent chain), 4.58-5.10(overlapping m, 2H, CH=C$\underline{H_2}$). 5.68-5.97(overlapping m, 1H, C$\underline{H}$=CH_2) 6.25-7.83(br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.4.2: Poly (styrene-co-undec-10-enoic acid-5-(2-acryloyloxy ethoxy)-2-benzoyl phenyl ester) copolymers (Pol 9):



(Pol 9)

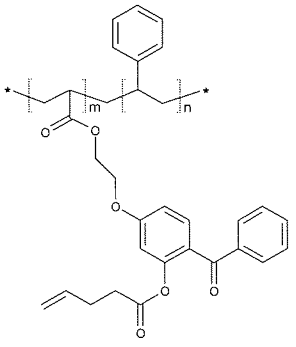
Yield : 10.42g, 0.99mmol/g loading of the functional group, pale yellow solid.

^{1}H NMR δ_H: 1.05-2.45(overlapping br m, polymer back bone C$\underline{H_2}$ and C$\underline{H_2}$ from the aliphatic chain of pendant), 3.55-4.35(br m, 4H, OC$\underline{H_2}$C$\underline{H_2}$O-Ar), 4.90-5.07(overlapping m, 2H CH=C$\underline{H_2}$), 5.72-5.95(m, 1H, C$\underline{H}$=CH_2), 6.15-7.80(overlapping br m, Ar-H from polymer backbone styrene and polymer pendant groups),12.70(s, 1H, unreacted Ar-OH) ppm.

^{13}C NMR δ_C: 24.14, 28.83, 33.72, 39.7-41.21(broad peaks, polymer back bone), 61.49-627.($\underline{C}H_2$OAr)65.5-66.0(COO$\underline{C}H_2CH_2$OAr), 77.0(CDCl3), 122.33, 124.16, 125.87, 126.38, 127.00-129.52((broad peaks, aromatic carbons), 132.42, 135.35, 138.16, 139.18, 144.55-145.76(broad peaks), 150.76, 164.68, 171.77, 175.47, 199.98(carbonyl carbon) ppm.

IR ν = 3445, 3027(=C-H), 2925, 1812, 1733(C=O), 1637(C=C), 1490, 1454 cm^{-1}.

5.4.3: Poly (styrene-co-pent-4-enoic acid 5-(2-acryloyloxy-ethoxy)-2-benzoylphenyl ester) copolymer (Pol 10):



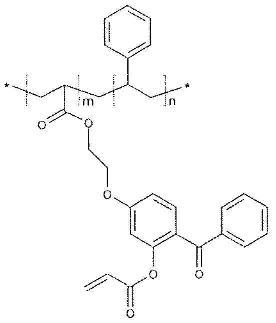
(Pol 10)

Yield: 8.94g, pale yellow powdered solid

^{1}H NMR δ_H: 1.04-2.45(overlapping br m, polymer back bone CH_2 and CH_2 from $O{=}CC\underline{H}_2C\underline{H}_2CH{=}CH_2$), 3.58-4.22(br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.92-5.09(overlapping m, 2H, $CH{=}C\underline{H}_2$), 5.65-5.89(br m, 1H, $C\underline{H}{=}CH_2$), 6.19-7.82(overlapping br m, Ar-H from polymer backbone styrene and polymer pendant groups), 12.70(s, 1H, unreacted Ar-OH) ppm.

IR ν = 3443, 3027(=C-H), 2917, 2847, 1735(C=O) 1655 (C=C) cm^{-1}.

5.4.4: Poly (styrene-co-acrylic acid 5-(2-acryloyloxyethoxy)-2-benzoylphenyl ester) copolymer (Pol 11):



(Pol 11)

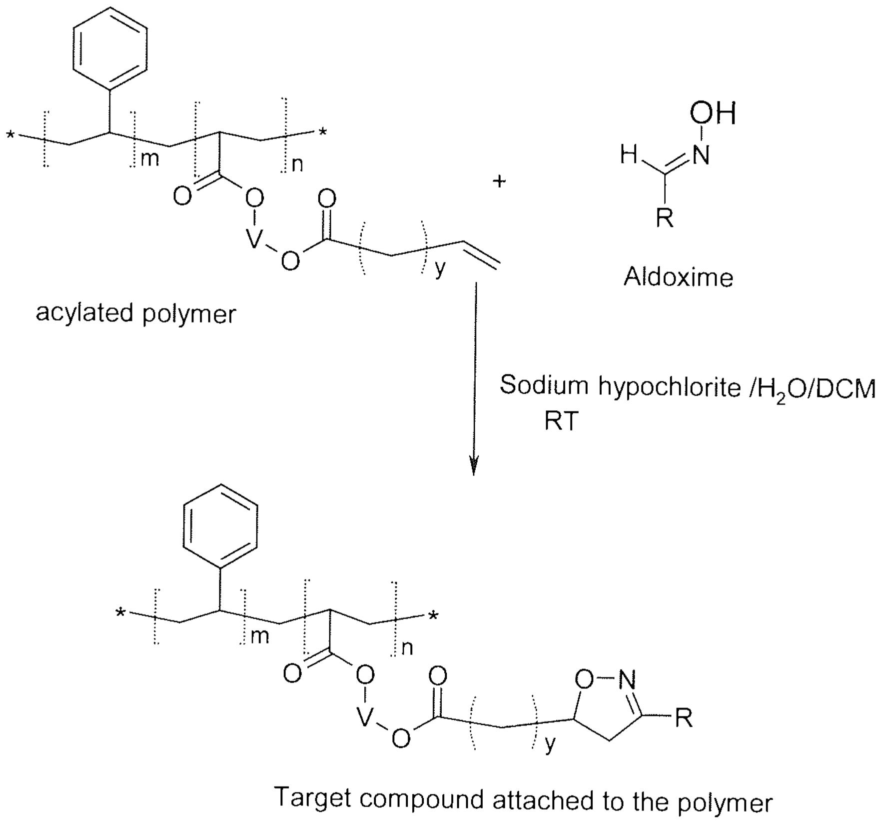
Yield: 6.78g, pale yellow powdered solid.

^{1}H NMR δ_H: 1.03-2.35(overlapping br m, polymer back bone CH_2) 3.51-4.23(br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 5.74-5.90(br m, 2H, $CH{=}C\underline{H}_2$), 5.94-6.17(overlapping br m, 1H, $C\underline{H}{=}CH_2$), 6.23-7.81(overlapping br m, Ar-H from polymer backbone styrene and polymer pendant groups), 12.70(s, 1H, unreacted Ar-OH) ppm.

IR ν = 3450 (=C-H), 3027, 2925, 2360, 1737(C=O), 1639 (C=C) cm^{-1}.

5.5: 1,3-Dipolar-cycloaddition reactions between nitrile oxides and polymer-bound alkenes:

A general procedure for 1,3-Dipolar-cycloaddition reactions between nitrile oxides and polymer bound alkenes:



V = aliphatic and aromatic groups
R = aryl, alkyl groups

y = 0, 4, 8

n + m, variable repeated units of styrene and 2nd monomer, respectively.

Scheme 33: A general reaction to show 1,3-dipolar cycloaddition reactions between nitrile oxide and polymer bound terminal alkenes.

The acylated polymer was dissolved in DCM. Aqueous sodium hypochlorite in the form of bleach was added to the reaction and reaction was stirred vigorously, to make sure that the DCM layer and aqueous layers were being mixed thoroughly. A solution of the aldoxime in DCM was added to the reaction drop-wise. The reaction was stirred for 24 hours at room temperature.

The DCM layer was separated from the aqueous layer. The aqueous layer was washed with DCM (x 3). The DCM layers were combined, dried over magnesium sulphate, concentrated in volume under vacuum, and poured onto rapidly stirred methanol. The precipitated polymer was collected by filtration and dried under vacuum.

For the quantities of different copolymers, aldoximes and other reagents used for the 1,3-dipolar-cycloaddition reactions please see **Tables 22** to **26** below.

PAGE 169 – BLANK

Copolymer **Pol 1** Quantity (g)	Copolymer prepared (*)	The Aldoxime			DCM (mL) for reaction	Bleach (sodium hypochlorite) (mL)	DCM to wash the aqueous layer (3 x mL)	Methanol to precipitate polymer (mL)
		Name of the oxime	Quantity					
			(mmol)	(g)				
9.10	**Pol 12**	4-*tert*-Butylbenzaldehyde oxime	45.3	8.02	500	145	300	500
5.0g	**Pol 13**	4-Chlorobenzaldehyde oxime	31.25	4.84	300	86	200	300

(*) = For the structures and chemical analysis of these polymers refer to sections. 5.5.1 and 5.5.2.

Table 22: **The table shows the quantities of reagents and solvents used for the 1,3-dipolarcycloaddition reactions of copolymer Pol 1 with various aldoximes:**

Copolymer **Pol 8** Quantity (g)	Copolymer prepared. (*)	The Aldoxime			DCM (mL) for reaction	Bleach (sodium hypochlorite) (mL)	DCM to wash the aqueous layer (3 x mL)	Methanol to precipitate polymer (mL)
		Name of the oxime	Quantity					
			(mmol)	(g)				
1.5g	**Pol 14**	4-*tert*-Butylbenzaldehyde oxime	1.95	0.35	60	6.5	50	175
5.03g	**Pol 15**	4-Chlorobenzaldehyde oxime	8.85	1.37	150	25	200	500

(*) = For the structures and chemical analysis of these polymers refer to sections.5.5.3 and 5.5.4.

Table 23: **The table shows the quantities of reagents and solvents used for the 1,3-dipolarcycloaddition reactions of copolymer Pol 4 with various aldoximes:**

Polymer **Pol 9** Quantity (g)	Polymer prepared (*)	The Aldoxime			DCM (mL) / ($CHCl_3$) for reaction	sodium hypochlorite (Bleach) (mL)	DCM to wash the aqueous layer (3 X mL)	Methanol to precipitate polymer (mL)
		Name of the oxime	Quantity					
			(mmol)	(g)				
10.0	**Pol 16**	4-*tert*-Butylbenzaldehyde oxime	45.01	7.97	400	160	70	450
5.01	**Pol 17**	4-Chlorobenzaldehyde oxime	23.32	3.61	250	66	50	500
4.0	**Pol 18**	*syn*-Benzaldehyde oxime	18.89	2.2.9	150	42	40	450
4.0	**Pol 19**	Butyraldehyde oxime	18.89	1.64	125	30	40	450
4.0	**Pol 20**	3-(4-Chloro-phenylsulfanyl)-benzaldehyde oxime	8.36	2.20	100 / (50)	40	40	450
5.0	**Pol 21**	4-Benzyloxy-3-methoxy-benzaldehyde oxime	22.96	5.90	350	107	50	500
5.0	**Pol 22**	2,6-Dichlorobenzaldehyde oxime	23.6	4.46	200 / (100)		50	
5.0	**Pol 23**	2,5-Dimethoxy-benzaldoxime	23.6	4.27	350	77	50	500
1.992	**Pol 24**	4-Nitrobenzaldehyde oxime	5.94	0.99	90 / 20-ethylacetate	18	20	250
4.0	**Pol 25**	3-Methoxy-4-methoxymethyl-benzaldehyde oxime	18.88	3.68	200	66	40	400
2.03	**Pol 26**	Dodecyl aldehyde oxime	10.03	2.00	110	36	20	500
5.0	**Pol 27**	4-Dimethylamine-benzaldehyde oxime	22.26	3.65	350	66	50	500
1.52	**Pol 28**	Pyridine-3-carbaldehyde oxime	12.29	1.50	100	27	15	250

2.01	**Pol 29**	3-Hydroxy-4-methoxy benzaldehyde oxime	11.98	2.00	110	36	30	300

(*)~= For the structures and further analysis of these polymers refer to sections 5.5.5 – 5.5.18.

Table 24: The table shows the quantities of different reagents and solvents used for the 1,3-dipolar cycloaddition reactions of copolymer Pol 5 with various aldoximes:

Polymers **Pol 10** Quantity (g)	Polymer prepared (*)	The Aldoxime			DCM (mL) / ($CHCl_3$) for reaction	Bleach (sodium hypochlorite) (mL)	DCM to wash the aqueous layer (3 x mL)	Methanol precipitate polymer (mL)
		Name of the oxime	Quantity					
			(mmol)	(g)				
9.05g	**Pol 30**	4-tert-butylbenzaldehyde oxime	53.1	9.40	400	170	300	400
5.0	**Pol 31**	*syn*-benzaldehyde oxime	18.89	2.29	150	42	200	450
5.0	**Pol 32**	4-Chlorobenzaldehyde oxime	23.65	3.67	275	75	200	300
1.99	**Pol 33**	2,6-Dichlorobenzaldehyde oxime	10.58	2.00	100	36	100	350
1.98	**Pol 34**	4-Nitrobenzaldehyde oxime	10.53	1.75	110 / 25-ethylacetate	31.5	100	350
4.75	**Pol 35**	Butyraldehyde oxime	22.53	1.96	150	35	200	500
2.05	**Pol 36**	Dodecyl aldehyde oxime	10.04	2.00	110	36	150	350
3.75	**Pol 37**	4-Dimethylamine-benzaldehyde oxime	12.20	2.50	150	50	200	500

(*) = For the structures and chemical analysis of these polymers refer to sections. 5.5.19 – 5.5.26.

Table 25: The table shows the ratio of oxime to linear copolymer Pol 6, along with other solvents, for 1,3-dipolar cycloaddition reactions:

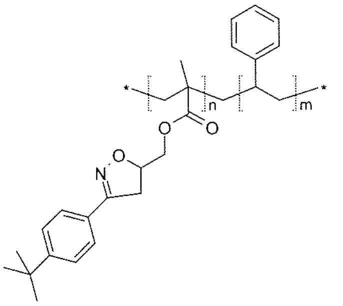
Co-polymer **Pol 11** Quantity (g)	Copolymer prepared. (*)	The Aldoxime			DCM (mL) / ($CHCl_3$) for reaction.	Bleach (sodium hypochlorite) (mL)	DCM to wash the aqueous layer (3 x mL)	Methanol precipitate polymer (mL)
		Name of the oxime	Quantity					
			(mmol)	(g)				
5.0	**Pol 38**	4-*tert*-butylbenzaldehyde oxime	23.60	4.18	250	75	200	300
4.0	**Pol 39**	4-Chlorobenzaldehyde oxime	18.90	2.93	250	55	200	300
4.04	**Pol 40**	*syn*-benzaldehyde oxime	18.93	2.29	150	42	200	500
4.5	**Pol 41**	Butyraldehyde oxime	21.35	1.86	100	33	200	500
2.07	**Pol 42**	4-Dimethylamin benzaldehyde oxime	12.38	2.03	150	36	200	500
1.99	**Pol 43**	Dodecyl aldehyde oxime	10.04	2.00	110	36	200	500

(*) = For the structures and chemical analysis of these polymers refer to sections.5.5.27 – 5.5.32.

Table 26: The table shows the ratio of oxime to linear copolymer Pol 7, along with other solvents, for 1,3-dipolarcycloaddition reactions:

Due to the nature of the polystyrene copolymers, it was not possible to interpret fully the ^{1}H NMR of polymer bound compounds. Peaks arising between the regions δ 2-6 only, are observable. The only plausible proof that the terminal alkene has been consumed in 1,3-dipolar reactions is the disappearance of the peaks around the regions of δ 3.9-4.2, 5.0 and 5.6

5.5.1: Poly (styrene-co-3-(4-*tert*-butylphenyl)-Δ^2-isoxazoline-5-yl-methyl ester) copolymer (Pol 12):

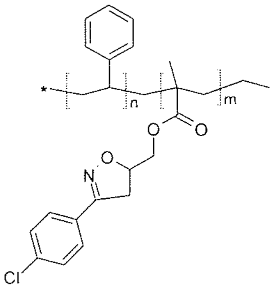


(Pol 12)

Yield: 8.36g, white powder,

^{1}H NMR: δ_H: 0.15-2.03(overlapping br m, polymer back bone CH_2, CH_3 and C(C$\underline{H}_3$)$_3$), 2.71-3.31(br m, 2H, C$\underline{H}_2$OC=O), 6.23-7.65 (overlapping br m, Ar-H from polymer bonded styrene units) ppm.

5.5.2: Poly (styrene-co-3-(4-chlorophenyl)-Δ^2-isoxazoline-5-yl-methyl ester) copolymer (Pol 13):

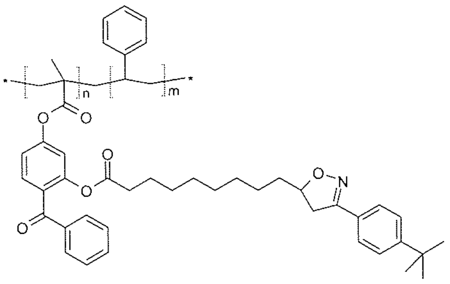


(Pol 13)

Yield: 5.12g, white powder.

^{1}H NMR: δ_H: 0.32-2.44 (overlapping br m, polymer back bone CH_2 and CH_3), 2.85-3.87(br m, 2H, CH_2OC=O), 6.25-7.74(overlapping br m, 1H, $OCH_2C\underline{H}$=CH_2), 6.5-7.60 (overlapping br m, Ar-H from polymer bonded styrene units) ppm.

5.5.3: Poly (syrene-co-9-[3-(4-*tert*-butylphenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid-5-acryloyloxy-2-benzoylphenoyl ester) copolymer (Pol 14):

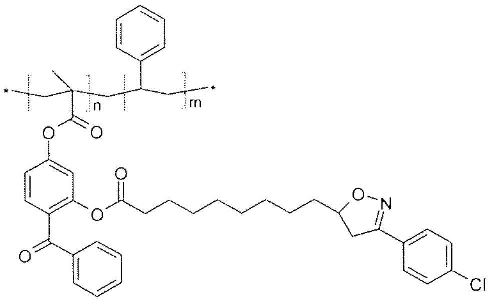


(Pol 14)

Yield: 1.07g, white powder.

^{1}H NMR δ_H: 0.34-1.05(br m, CH_3), 1.01-2.56(overlapping br m, polymer backbone CH_2, CH_2 from the aliphatic pendant chain and $C(CH_3)_3$), 4.51-4.85(m, 1H, isoxazoline chiral proton), 4.98-5.17(m, 1H, $C\underline{H}{=}CH_2$, un-reacted alkene), 5.39-5.67(overlapping m, 2H, $CH{=}C\underline{H_2}$, unreacted alkenes), 6.25-7.84(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.4: Poly (syrene-co-9-[3-(4-chlorolphenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid –5-acryloyloxy-2-benzoylphenoyl ester) copolymer (Pol 15):

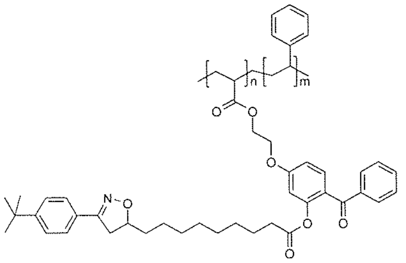


(Pol 15)

Yield: 3.87g white powder solid.

^{1}H NMR δ_H: 0.30-1.02(overlapping m, CH_3), 1.05-2.50(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 3.25(overlapping m, 1H, isoxazoline prochiral proton), 3.60-3.85(overlapping m, 1H, isoxazoline prochiral proton), 4.65-4.85(br m, 1H, isoxazoline chiral proton), 4.90-5.10(overlapping m, 2H, $CH{=}C\underline{H_2}$ unreacted alkenes), 5.40-5.65(overlapping br m, 1H $C\underline{H}{=}CH_2$), 6.30-97.8(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.5: Poly (styrene-co-9-[3-(4-*tert*-butylphenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 16):



(Pol 16)

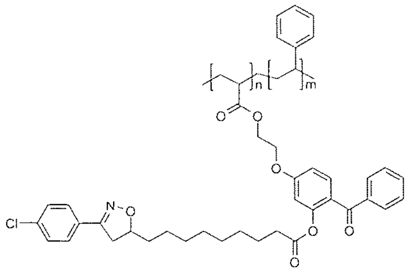
Yield: 1.27g, pale yellow solid.

^{1}H NMR δ_H: 0.99-2.35(overlapping br m, polymer backbone CH$_2$, CH$_2$ from the pendant aliphatic chain and C(CH$_3$)$_3$), 2.81-3.07(overlapping m, 1H, isoxazoline prochiral proton), 3.30-3.46(overlapping m, 1H, isoxazoline, prochiral proton), 3.53-4.24(overlapping br m, 4H, $\mathrm{OC\underline{H}_2C\underline{H}_2O}$-Ar) 4.60-4.80(br m, 1H, isoxazoline chiral proton), 6.13-7.81(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

^{13}C NMR δ_C: 24.1, 28.81, 33.72, 39.72-41.22(broad peaks, polymer back bone), 61.90-62.11($\mathrm{\underline{C}H_2OAr}$)65.5-66.0($\mathrm{COO\underline{C}H_2CH_2OAr}$), 77.0(CDCl3), 107.53, 113.24, 125.86, 126.36, 127.07-129.58((broad peaks, aromatic carbons), 132.42, 135.33, 138.15, 139.15, 144.56-145.77(broad peaks), 175.42 ppm.

IR ν = 3436, 3023, 2925, 1731 (C=O), 1606 (Ar ring stretch), 1274 cm^{-1}.

5.5.6: Poly (styrene-co-9-[3-(4-chlorophenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 17):

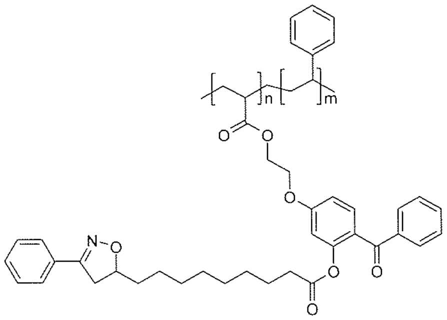


(Pol 17)

Yield: 3.56g, pale yellow solid,

^{1}H NMR δ_H: 0.67-2.31(overlapping br m, polymer backbone CH$_2$ and CH$_2$ from the pendant aliphatic chain and any new peaks from the new compound formed), 2.85-3.03(overlapping m, 1H isoxazoline prochiral proton), 3.28-3.42(overlapping m, 1H isoxazoline prochiral proton), 3.55-4.85(br m, 4H, $\mathrm{OC\underline{H}_2C\underline{H}_2O}$-Ar) 4.62-4.82(br m, 1H, isoxazoline chiral proton) 6.17-.7.96(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.7: Poly (styrene-co-9-(3-phenyl-Δ^2-isoxazoline-5-yl)-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 18):

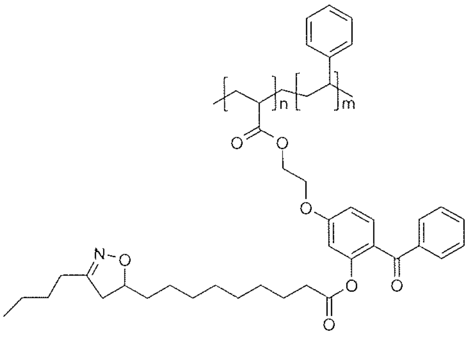


(Pol 18)

Yield: 3.35g, pale yellow solid.

^{1}H NMR δ_H: 0.80-2.28(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.75-3.10(overlapping m, 1H, isoxazoline prochiral proton), 3.15-4.33(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.71(m, 1H, isoxazoline chiral proton) 6.10-7.82(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant and aromatic group from the new compound formed) ppm.

5.5.8: Poly (styrene-co-9(3-butyl-Δ^2-isoxazoline-5-yl)-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 19):

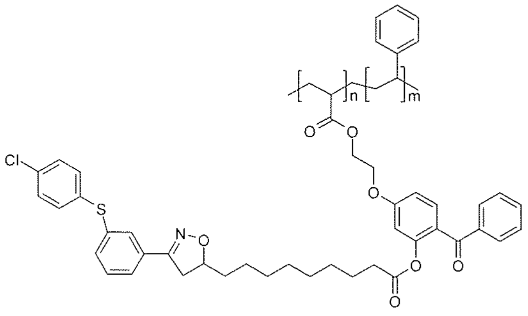


(Pol 19)

Yield: 3.43g, pale yellow solid.

^{1}H NMR δ_H: 1.02-2.42(overlapping br m, polymer backbone CH_2 and CH_2 from the aliphatic pendant chain, and any overlapping peaks coming from the aliphatic chain of the new compound formed), 2.43-2.63(m, 1H, isoxazoline prochiral proton), 2.87-3.05(m, 1H, isoxazoline prochiral proton), 3.14-4.28(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.51(m, 1H, isoxazoline chiral proton), 4.88-5.07(overlapping m, 2H, $CH{=}C\underline{H}_2$, unreacted alkenes), 5.71-5.90(m, 1H, $C\underline{H}{=}CH_2$, un-reacted alkene), 6.16-7.81(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant) ppm.

5.5.9: Poly (styrene-co-9-{-[3-(4-chloro-phenylsulphonyl) phenyl]-Δ^2-isoxazoline-5-yl}-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 20):

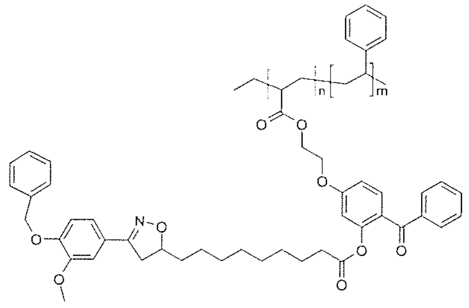


(Pol 20)

Yield: 3.86g, pale yellow solid.

^{1}H NMR δ_H: 0.83-2.45(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 3.02-3.17(overlapping m, 1H, isoxazoline prochiral proton), 3.37-3.55(overlapping m, 1H, isoxazoline prochiral proton), 3.55-4.30(br m, 4H, OC$\underline{H}_2$C$\underline{H}_2$O-Ar) 4.57-4.80(overlapping br m, 1H, isoxazoline chiral proton), 4.90-5.07(overlapping m, 2H, un-reacted alkene), 5.45-5.90(overlapping m, 1H, unreacted alkenes), 6.04-7.88(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant and aromatic groups from the new compound formed) ppm.

5.5.10: Poly (styrene-co-9-[3-(4-benzyloxy-3-methocyphenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 21):

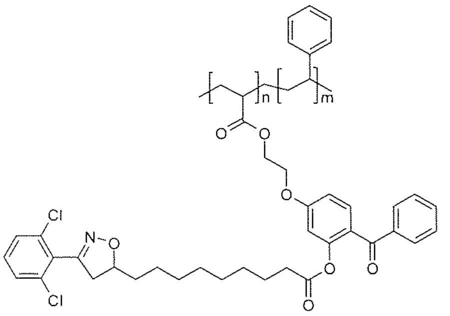


(Pol 21)

Yield: 5.48g, pale yellow solid.

^{1}H NMR δ_H: 0.69-2.48(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.81-3.03(overlapping m, 1H isoxazoline prochiral proton), 3.23-3.45(overlapping m, 1H isoxazoline prochiral proton), 3.50-4.28(overlapping br m, OC$\underline{H}_2$C$\underline{H}_2$O-Ar, and Ar-OCH_3) 4.62-4.78(m, 1H, isoxazoline chiral proton) 4.99-5.39(br m, O=C$\underline{H}_2$-Ar) 6.19-7.91(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant, and aromatic groups from the new compound formed) ppm.

5.5.11: Poly (styrene-co-9-[3-(2,6-dichlorophenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 22):

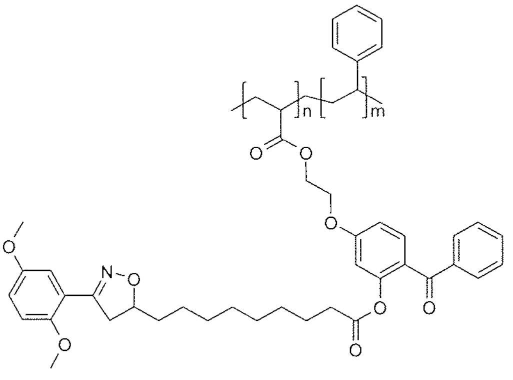


(Pol 22)

Yield: 5.23g, pale yellow solid.

^{1}H NMR δ_H: 0.83-2.33(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.83 –2.98(overlapping m, 1H, isoxazoline prochiral proton), 3.26-3.44(overlapping m, 1H, isoxazoline prochiral proton), 3.50-4.30(overlapping br m, $OC\underline{H}_2C\underline{H}_2O$-Ar) 4.73-4.89(m, 1H, isoxazoline chiral proton) 6.16-7.82(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant and aromatic groups from new compound formed) ppm.

5.5.12: Poly (styrene-co-9-[3-(2,5-dimethoxy-phenyl)-Δ2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 23):

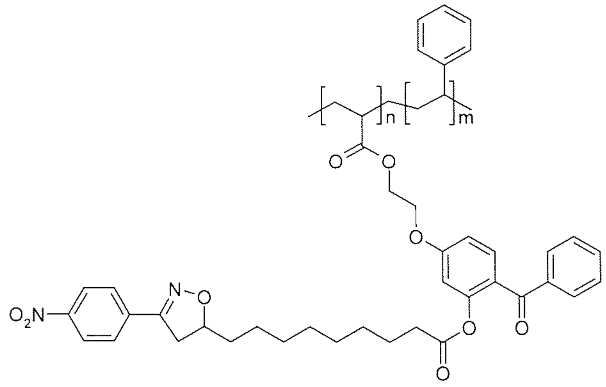


(Pol 23)

Yield: 4.6938g, pale yellow solid.

^{1}H NMR δ_H: 0.69-2.38(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.98–3.15(overlapping m, 1H, isoxazoline prochiral proton), 3.39-4.35(overlapping br m, 1H isoxazoline prochiral proton, $OC\underline{H}_2C\underline{H}_2O$-Ar and 2,5 ($O$-$CH_3)_2$), 4.56-4.80(m, 1H, isoxazoline chiral proton) 6.18-7.81(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant and aromatic group from new compound) ppm.

5.5.13: Poly (styrene-co-9-[3-(nitrophenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 24):

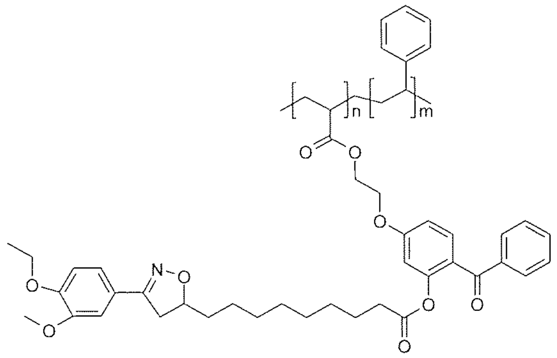


(Pol 24)

Yield: 2.22g, pale yellow solid.

^{1}H NMR δ_H: 0.80-2.51(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.89-3.09(overlapping m, 1H, isoxazoline prochiral proton), 3.30-3.50(overlapping m, 1H, isoxazoline prochiral proton), 3.55-4.25(overlapping br, m, $OC\underline{H}_2C\underline{H}_2O$-Ar) 4.72-4.93(m, 1H, isoxazoline chiral proton), 6.20-8.50(overlapping br m, Ar-H from the polymer bonded styrene, polymer pendant and aromatic groups from new compound formed) ppm.

5.5.14: Poly (styrene-co-9-[3-(4-ethoxy 3-methpxyphenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 25):

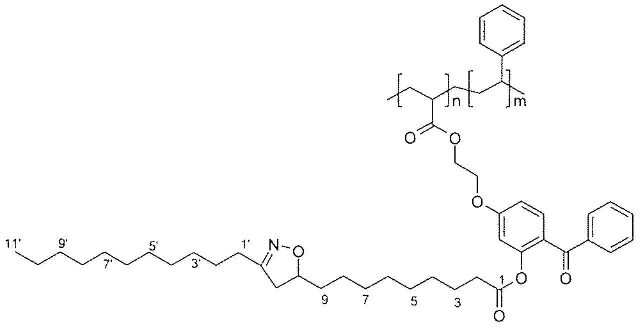


(Pol 25)

Yield: 3.81g, pale yellow solid.

^{1}H NMR δ_H: 0.84-2.41(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.86–3.04(overlapping m, 1H isoxazoline prochiral proton), 3.29-4.34(overlapping br m, isoxazoline prochiral proton, $OC\underline{H}_2C\underline{H}_2O$-Ar, and $3OC\underline{H}_3$, $4\text{-}OC\underline{H}_2C\underline{H}_3$) 4.59-4.80(m, 1H, isoxazoline chiral proton) 6.16-7.89(overlapping m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.15: Attempted preparation of poly (styrene-co-9-(3-undecyl)-Δ^2-isoxazoline-5-yl)-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 26):

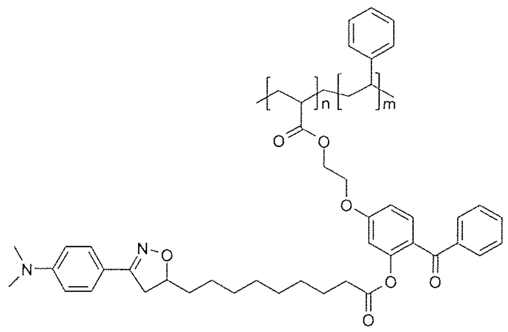


(Pol 26)

Yield: 2.25g, pale yellow solid.

^{1}H NMR δ_H: 1.08-2.28(overlapping br m, polymer backbone CH_2 and CH_2 from the aliphatic pendant chain, and any overlapping peaks coming from the aliphatic chain of the new compound formed), 2.33(t, J= 7.6 Hz, 2H $\underline{H}_2C1'$) 2.50(t, J= 7.2 Hz, 2H, $H_2CC{=}O$), 2.62(d,d, J= 15.3, 7.3 Hz, 1H, isoxazoline prochiral proton), 2.95(d,d, J= 16.0, 9.9 Hz, 1H, isoxazoline prochiral proton), 3.6-4.1(br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.42-4.58(br m, 1H isoxazoline chiral proton), 4.80-5.10(m, $CH{=}C\underline{H}_2$ un-reacted alkene), 5.72-5.87(m, $C\underline{H}{=}CH_2$ unreacted alkenes), 6.30-7.78(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.16: Attempted preparation of poly (styrene-co-9-[3-(4-dimethylaminophenyl)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 27):

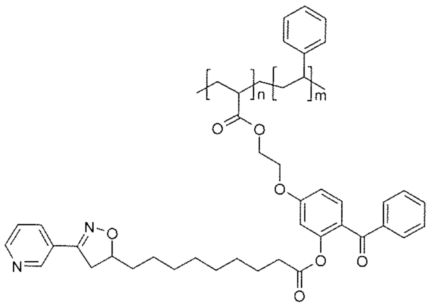


(Pol 27)

Yield: 4.58g, pale yellow solid.

^{1}H NMR same as **Pol 9** (starting material).

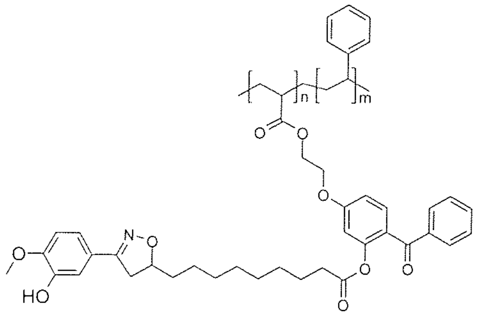
5.5.17: Attempted preparation of poly (styrene-co-9-(3-pyridine-3-yl-Δ^2-isoxazoline-5-yl)-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 28):



(Pol 28)

Yield:1.32g, Pale yellow solid.
^{1}H NMR same as **Pol 9** (starting material).

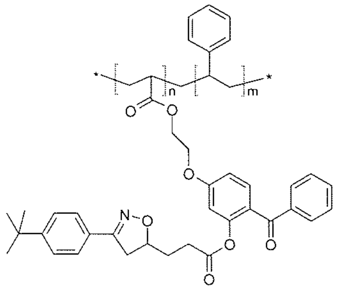
5.5.18: Attempted preparation of poly (styrene-co-9-[3-(4-methoxy-3-hydroxy)-Δ^2-isoxazoline-5-yl]-nonanoic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 29):



(Pol 29)

Yield: 3.58g, pale yellow solid
^{1}H NMR same as **Pol 9** (starting material).

5.5.19: Poly (styrene-co-9-[3-(4-*tert*-butylphenyl)-Δ^2-isoxazoline-5-yl]-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 30):

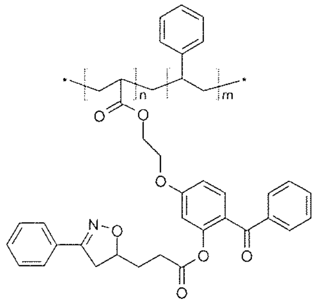


(Pol 30)

Yield: 6.92g, pale yellow solid.

^{1}H NMR δ_H: 0.75-2.25(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain, and any overlapping peaks from new compound formed), 3.10–4.14(overlapping m. isoxazoline prochiral protons and $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.62-4.79(overlapping m, isoxazoline chiral proton) 5.16-5.26(m, 2H, $CH{=}C\underline{H}_2$ unreacted alkenes), 5.48-5.57(m,, 1H, $C\underline{H}{=}CH_2$ unreacted alkenes), 6.17.8.25(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.20: Poly (styrene-co-9-(3-phenyl-Δ^2-isoxazoline-5-yl)-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 31):

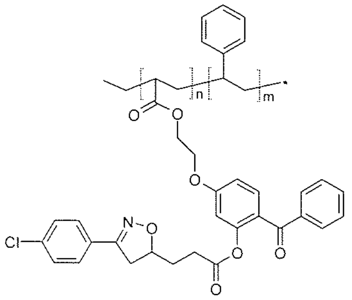


(Pol 31)

Yield: 5.22g, pale yellow solid.

^{1}H NMR δ_H: 0.73-2.52(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 3.05-3.15(overlapping br m, isoxazoline prochiral proton), 3.23-4.25(overlapping m, isoxazoline prochiral proton and $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.62-4.79(m, 1H, isoxazoline chiral proton), 4.93-5.09(m 2H, $CH{=}C\underline{H}_2$ unreacted alkenes), 5.66-5.85(m, $C\underline{H}{=}CH_2$ un-reacted alkenes), 6.19-8.03(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.21: Poly (styrene-co-9-[3-(4-chlorophenyl)-Δ^2-isoxazoline-5-yl]-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 32):

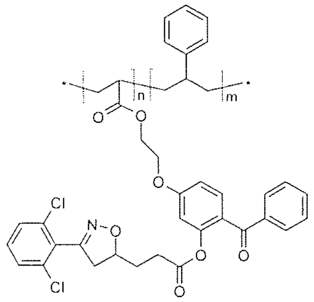


(Pol 32)

Yield: 4.98g, pale yellow solid.

^{1}H NMR δ_H: 0.80-2.60(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.45-2.55(br m, 2H, $H_2CC{=}O$), 2.82–3.05(overlapping br m, isoxazoline prochiral proton), 3.25-4.35(overlapping br m, isoxazoline prochiral proton, $OC\underline{H}_2C\underline{H}_2O$), 4.65-4.87(br m, 1H, isoxazoline chiral proton), 6.20-7.78(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.22: Poly (styrene-co-9-[3-(2,6-dichlorophenyl)-Δ^2-isoxazoline-5-yl]-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 33):

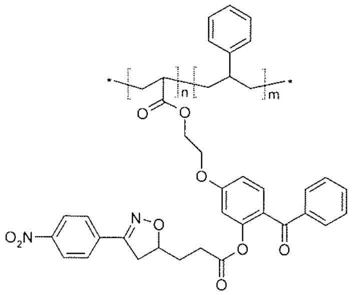


(Pol 33)

Yield: 1.70g, pale yellow solid.

^{1}H NMR δ_H: 0.85-2.60(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.80-2.95(overlapping br m, isoxazoline prochiral proton), 3.30-3.49(overlapping br m, isoxazoline prochiral proton), 3.55-4.30(overlapping br m, $OC\underline{H}_2C\underline{H}_2O$), 4.72-4.90(br m, 1H, isoxazoline chiral proton), 6.15-7.90(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.23: Poly (styrene-co-9-[3-(4-nitrophenyl)-Δ^2-isoxazoline-5-yl]-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 34):

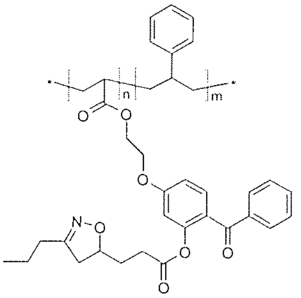


(Pol 34)

Yield: 1.31g, pale yellow solid.

^{1}H NMR δ_H: 0.80-2.75(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain), 2.57(t, J= 7.45 Hz, 2H, CH_2=O), 2.90-3.08(overlapping br m, Isoxazoline prochiral proton), 3.30-3.50(overlapping br m, isoxazoline prochiral proton) 3.55-4.25(overlapping br m, $OC\underline{H}_2C\underline{H}_2O$), 4.63-4.85(br m, 1H, isoxazoline chiral proton), 6.20-8.35(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.24: Attempted preparation of poly (styrene-co-9-(3-butylphenyl-Δ^2-isoxazoline-5-yl)-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 35):

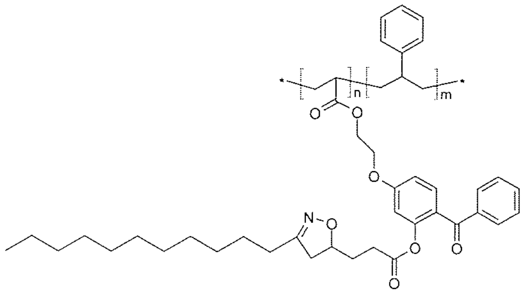


(Pol 35)

Yield: 4.10g, pale yellow solid.

^{1}H NMR δ_H: 0.61-2.73(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain and new compound), 2.85–3.14(m, 1H isoxazoline prochiral proton), 3.38-4.25(overlapping m, isoxazoline prochiral proton and $OC\underline{H}_2C\underline{H}_2O$-Ar), 4.42-4.59(m, 1H, isoxazoline chiral proton), 4.91-5.09(m 2H, CH=$C\underline{H}_2$ unreacted alkenes), 5.61-5.87(m, $C\underline{H}$=CH_2 un-reacted alkenes), 6.15-7.87(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.25: Attempted preparation of poly (styrene-co-9(3-undecylphenyl-Δ^2-isoxazoline-5-yl)-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 36):

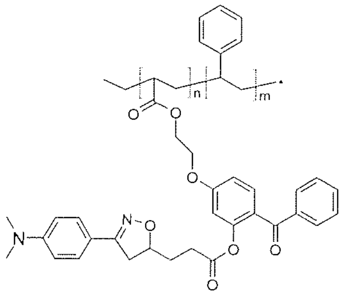


(**Pol 36**)

Yield: 1.56g, pale yellow solid.

^{1}H NMR δ_H: 0.80-2.80(overlapping br m, polymer backbone CH_2 and CH_2 from the pendant aliphatic chain and new compound), 2.84-3.15(br m, 1H isoxazoline prochiral proton), 3.30-4.20(overlapping br m, isoxazoline prochiral proton and $OC\underline{H}_2C\underline{H}_2O$), 4.40-4.60(br m, 1H, isoxazoline chiral proton) 4.90-5.05(br m, 2H, $CH{=}C\underline{H}_2$, unreacted alkenes), 5.70-5.90(br m, 1H, $C\underline{H}{=}CH_2$, unreacted alkenes), 6.20-7.90(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.26: Attempted preparation of poly (styrene-co-9-[3-(4-dimethylaminophenyl)-Δ2-isoxazoline-5-yl]-propionic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 37):

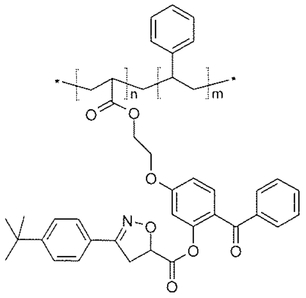


(**Pol 37**)

Yield: 3.29g, pale yellow solid.

^{1}H NMR same as **Pol 10**, the starting material.

5.5.27: Poly (Styrene-co-3(-4-*tert*-butylphenyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy-ethoxy)-2-benzoyl phenyl ester) copolymer (Pol 38):

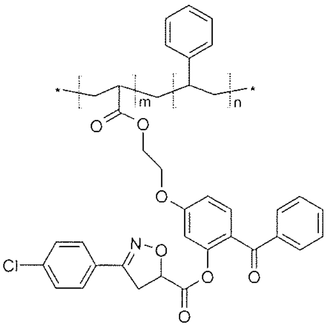


(Pol 38)

Yield: 4.58g, pale yellow solid.

^{1}H NMR δ_H: 0.84-2.39(overlapping br m, polymer backbone CH_2 and any overlapping peaks from the new compound formed), 3.47-4.29(br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar) 6.16-7.78(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.28: Poly (styrene-co-3(-4-chlorophenyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 39):

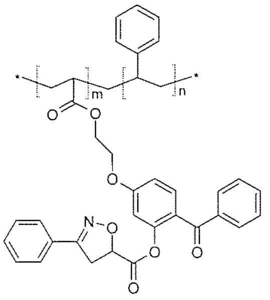


(Pol 39)

Yield: 3.62g, pale yellow solid.

^{1}H NMR δ_H: 0.81-2.45(overlapping br m, polymer backbone CH_2), 3.45-4.26(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 6.15-7.80(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.29: Poly (styrene-co-(3-phenyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 40):

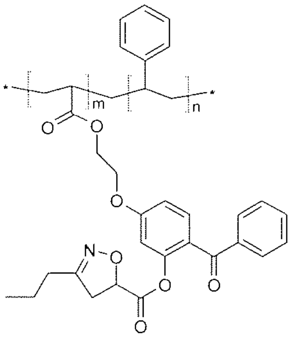


(Pol 40)

Yield: 3.35g, pale yellow solid.

^{1}H NMR δ_H: 0.85-2.55(overlapping br m, polymer backbone CH_2), 3.45-4.22(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar) 6.15-7.75(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.30: Poly (styrene-co-(3-butyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 41):

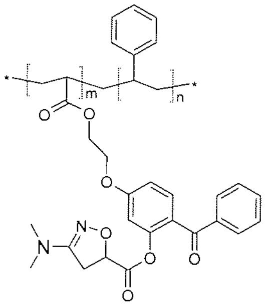


(Pol 41)

Yield: 3.91g, pale yellow solid.

^{1}H NMR δ_H: 0.76-2.50(overlapping br m, polymer backbone CH_2), 3.30-4.48(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 6.14-7.77(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.31: Poly (styrene-co-3(-4-dimethylaminophenyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy ethoxy)-2-benzoylphenyl ester) copolymer (Pol 42):

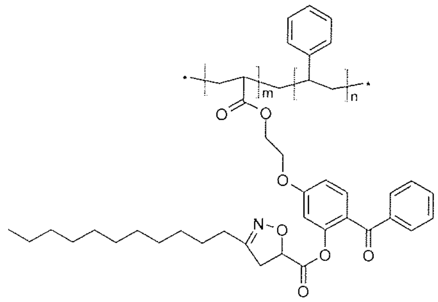


(Pol 42)

Yield: 1.17g, pale yellow solid.

^{1}H NMR δ_H: 0.75-2.50(overlapping br m, polymer backbone CH_2), 3.55-4.50(overlapping br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar) 6.15-7.75(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.5.32: Poly (styrene-co-(3-undecyl)-Δ^2-isoxazoline-5-carboxylic acid 5-(2-acryloyloxy ethoxy)-2-benzoyl phenyl ester) copolymer (Pol 43):



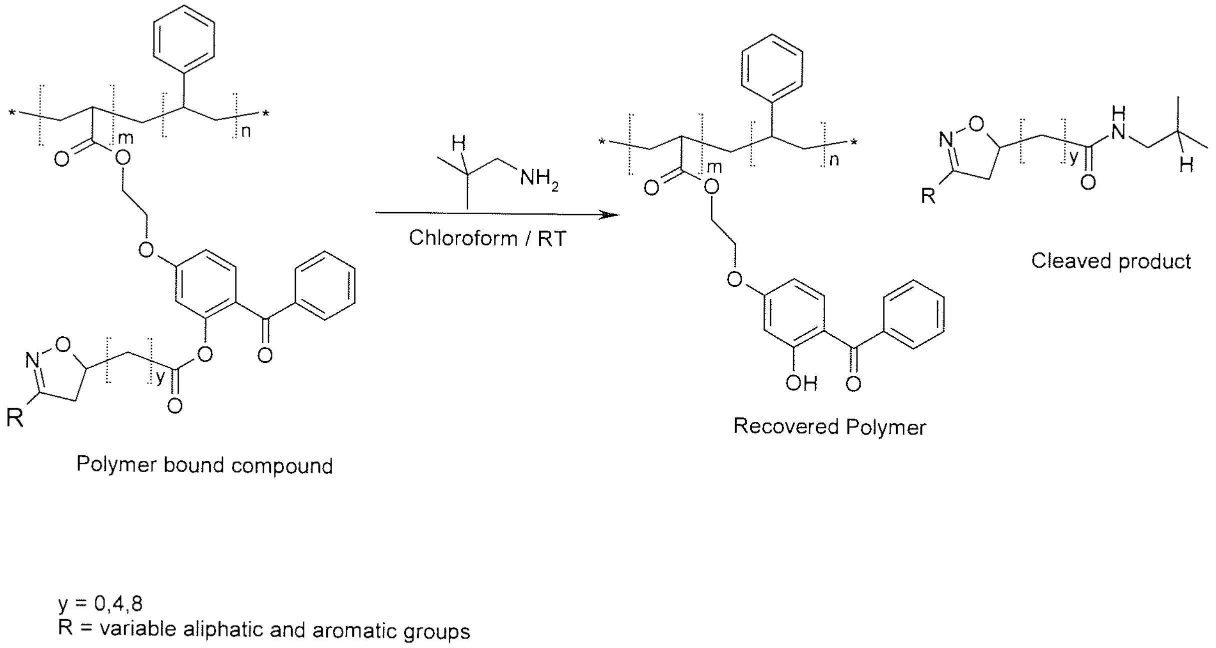
(Pol 43)

Yield: 1.58g, pale yellow solid.

^{1}H NMR δ_H: 0.73-2.43(overlapping br m, polymer backbone CH_2 and CH_2 from the aliphatic chain of new compound formed), 3.41-4.20(br m, 4H, $OC\underline{H}_2C\underline{H}_2O$-Ar), 6.04-7.79(overlapping br m, Ar-H from the polymer bonded styrene and polymer pendant aromatic groups) ppm.

5.6: Aminolysis of polymer bound compounds:

A general procedure for the cleavage of polymer bound compounds:



Scheme 34: Cleavage of polymer bound compounds.

The polymer was dissolved in chloroform and was treated with isobutylamine such that the concentration of isobutylamine in the reaction mixture was 20% v/v. After 18 hours the solution was concentrated under vacuum to ~ 5-7mL, and poured onto rapidly stirred methanol. The precipitated polymer was collected by filtration. The filtrate was collected and solvent was evaporated under vacuum to yield the crude cleaved product.

PAGE 191 - BLANK

Polymer No	Quantity (g)	Chloroform (mL)	Isobutyl amine (mL)	Recovered polymer (g/mg)	Cleaved product (g/mg)
Pol 12	5.0132	24	6	4.3014	742mg
Pol 13	5.0411	24	6	4.3919	636mg

Table 27: The table shows the quantities of reagents used to cleave off the polymer bound compounds, and the quantities of products obtained from Pol 12 and Pol 13.

Polymer	Quantity (g)	Chloroform (mL)	Isobutyl amine	Recovered polymer (g/mg)	Cleaved product (g/mg)
Pol 14	1.0421	4	1	841mg	204mg
Pol 15	3.0518	70	14	2.989	307mg

Table 28: The table shows the quantities of reagents used to cleave off the polymer bound compounds, and the quantities of products obtained from Pol 14 and Pol 15.

Polymer	Quantity (g)	Chloroform (mL)	Isobutylamine (mL)	Recovered polymer (g/mg)	Cleaved product (g/mg)
Pol 16	5.007	20	5	4.164	1.096
Pol 17	3.507	32	8	3.372	0.483
Pol 18	4.540	20	5	3.791	0.416
Pol 19	3.023	28	7	2.828	0.767
Pol 20	2.978	20	5	2.494	0.628
Pol 21	4.987	28	7	3.016	0.635
Pol 22	3.950	24	6	3.248	0.991
Pol 23	3.745	24	6	3.061	0.516
Pol 24	2.094	16	4	1.620	0.327
Pol 25	3.483	20	5	2.855	0.766
Pol 26	2.081	16	4	1.578	0.103

Table 29: **The table shows the quantities of reagents used to cleave off the polymer bound compounds, and the quantities of products obtained from Pol 16 - Pol 26.**

Polymer	Quantity (g)	Chloroform (mL)	Isobutylamine (mL)	Recovered polymer (g/mg)	Cleaved product (g/mg)
Pol 30	4.507	24	6	3.782	0.452
Pol 31	4.540	24	6	3.791	0.192
Pol 32	4.046	20	5	3.341	0.743
Pol 33	1.556	16	4	1.211	0.224
Pol 34	1.258	12	3	0.895	0.130

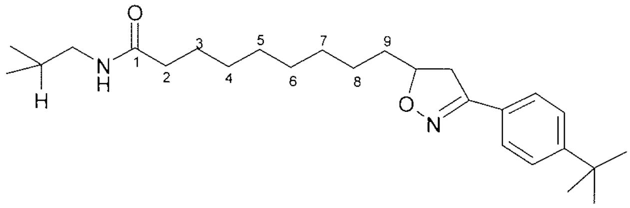
Table 30: The table shows the quantities of reagents used to cleave off the polymer bound compounds, and the quantities of products obtained from Pol 30 - Pol 34.

Polymer	Quantity (g)	Chloroform (mL)	Isobutylamine (mL)	Recovered polymer (g/mg)	Cleaved product (g/mg)
Pol 38	4.004	24	6	3.319	0.248
Pol 39	3.018	20	5	2.758	0.103
Pol 40	3.007	20	5	2.501	0.092
Pol 41	3.510	24	6	3.273	0.105

Table 31: The table shows the quantities of reagents used to cleave off the polymer bound products, and the quantities of products obtained from Pol 38 - Pol 41.

Most of the products cleaved from the polymers gave a crude mixture which was either very difficult to purify, or it showed very little evidence for the presence of the desired compound. Hence not all of the cleaved products were purified. Thus only the characterisation of the purified products, are presented below.

5.6.1: 9-[3-(4-*tert*-butylphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic isobutylamide: cleaved from the polymer (150):



150

The target compound was purified by flash chromatography eluting with ethyl acetate : petroleum ether (60-80 °C) 1:4., increasing to 11:9. The fractions corresponding to the R_f = 0.2 were combined and the solvent was evaporated under vacuum to yield a pale yellow coloured solid.

Yield: 37mg, 0.88mmol, pale yellow solid.

R_f = 0.2 (petroleum ether (60-80 °C) 55 : 45 ethyl acetate).

M p = 80.1 – 83.4 °C.

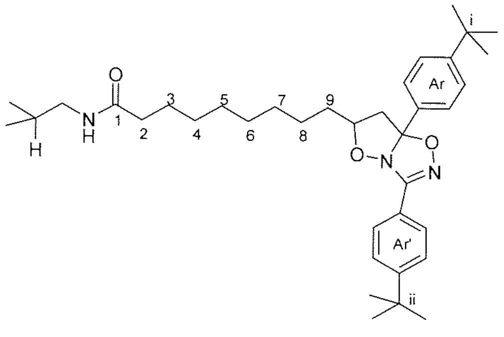
^{1}H NMR $\delta_{H:}$ 0.92(d, J= 6.67 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.30(overlapping br s, 12H, C^3-C^8 C$\underline{H}_2$s), 1.33(overlapping br s, 9H C(C$\underline{H}_3$)$_3$), 1.64(overlapping br m ,2H, C^9 aliphatic prochiral protons), 1.75(overlapping m, 1H, $(CH_3)_2C\underline{H}$), 2.20(t, J= 7.45 Hz, 2H, C^2 $\underline{H}_2$CC=O), 2.96(d,d, J= 16.5, 8.0 Hz, 1H isoxazoline prochiral proton), 3.09(t, J= 6.4 Hz, C$\underline{H}_2$NH), 3.39(d,d, J= 16.5, 10.25 Hz, 1H isoxazoline prochiral proton), 4.71(m, 1H isoxazoline chiral proton), 5.63(br s, 1H, NH), 7.43(d, J= 8.5 Hz, 2H, 3,5-ArH), 7.61(d, J= 8.6 Hz, 2H, 2,6-ArH) ppm.

MS (+APCI) [$C_{26}H_{42}N_2O_2$] m/z = 415 $(M+H)^+$

E(+) [$C_{26}H_{42}N_2NaO_2$] = Mass 437.3148, calculated mass = 437.3144 $[M+Na]^+$ [0.9 ppm].

IR ν = 3316(N–H), 2921, 2847, 1921, 1639(C=O), 1543, 1467, 1366, 1259 cm^{-1}.

5.6.2: 9-[3,7a-Bis-(4-*tert*-butylphenyl)-7,7a-dihydro-6H-isoxazolo[2,3-d][1,2,4]oxadiazol-6-yl] nonanoic acid isobutylamide (153):

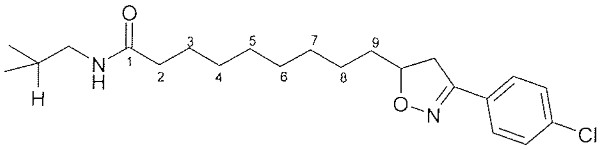


153

The compound **133** was isolated by flash column chromatography, in the same experiment and using the same solvent systems as for compound **132**. Fractions corresponding to Rf 0.23, eluting just before the compound **132** were collected and concentrated as above.

^{1}H NMR $\delta_{H:}$ 0.89(d, J= 6.5 Hz, 6H $CH(CH_3)_2$), 1.25(overlapping br s, 12H, C^3-C^8 of aliphatic chain), 1.30(br s, 9H, $^i(CH_3)_3$), 1.32(br s, 9H, $^{ii}(CH_3)_3$), 1.59(overlapping m, 2H, C^9 aliphatic prochiral protons), 1.73(overlapping m, 1H, $CH(CH_3)_2$), 2.15(t, J= 7.50 Hz, 2H $^2CH_2$), 2.42(d,d, J= 11.5, 12.75 Hz, 1H, isoxazol prochiral proton), 2.97(d,d J= 13.25, 4.25 Hz, 1H, isoxazol prochiral proton), 3.07(t, 6.25 Hz, 2H, $NHC\underline{H}_2CH(CH_3)_2$), 4.13(m, 1H, isoxazol chiral proton), 5.51(br s, 1H NH), 7.42(m, 4H, 3,5-ArH and 3,5-Ar'H), 7.59(d, J= 8.5 Hz, 2,6-ArH), 7.88(d, J= 8.75 Hz, 2H, 2,6-Ar'H) ppm.
MS (+APCI) [$C_{37}H_{55}N_3O_3$] m/z = 590 $(M+H)^+$.

5.6.3: 9-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (158):



158

The target compound was purified by flash column chromatography, eluting with petroleum ether (60–80 °C) and ethyl acetate (7:3) increasing to 1:9 respectively. The fractions corresponding to the Rf = 0.32 were collected, concentrated under reduced pressure and dried under vacuum to yield the target compound.

Yield: 0.14g (142 mg), 362mmol, pale yellow solid.
Rf: 0.21 (petroleum ether (60-80 °C) 1 : 3 ethyl acetate).
M p = 113.3 – 117.4 °C,

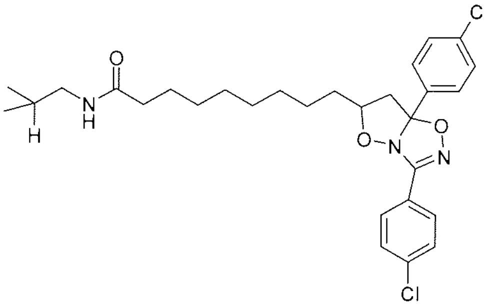
^{1}H NMR $\delta_{H:}$ 0.90(d, J= 6.8 Hz, 6H, CH(C$\underline{H}_3$)$_2$), 1.31(br s, 12H, C^3-C^8 aliphatic chain), 1.60(overlapping br m, 2H C^9 aliphatic prochiral protons), 1.75(overlapping m, 1H (CH$_3$)$_2$C$\underline{H}$). 2.17(t, J= 7.8 Hz, 2H, $\underline{H}_2$CC=O), 2.93(d,d, J= 16.5, 8.3 Hz, 1H isoxazoline prochiral proton), 3.08(t, J= 6.7 Hz, 2H C$\underline{H}_2$NH), 3.37(d,d, J= 16.4, 10.4 Hz, 1H isoxazoline prochiral proton), 4.74(m, 1H isoxazoline chiral proton), 5.50(br s, 1H, NH), 737.(d, J= 8.7 Hz, 2H, 2,6-ArH), 7.60(d, J= 8.7 Hz, 2H, 3,5-ArH) ppm.

MS (±APCI) [$C_{22}H_{33}N_2O_2$] m/z = 393 (M+H)$^+$, 391 (M−H)$^-$.

E(+) [$C_{22}H_{33}N_2NaO_2$] = Mass, 415.2126, calculated mass = 415.2128 [M+Na]$^+$ [−0.5. ppm].

IR ν = 3305(N–H), 3090(Ar-H), 2921, 2849, 1642(C=O), 1559, 1464, 1382, 1254, 1097 cm^{-1}.

5.6.4: 9-[3,7a-Bis-(4-chlorophenyl)-7,7a-dihydro-6H-isoxazolo[2,3-d][1,2,4]oxadiazol-6-yl] nonanoic acid isobutylamide (155):

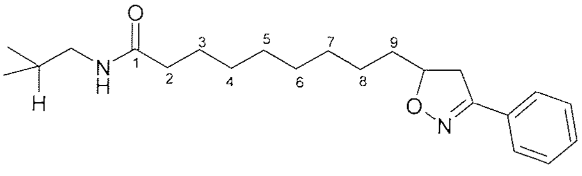


155

The compound **136** was isolated by flash column chromatography, in the same experiment and using the same solvent systems as for compound **140**. Fractions corresponding to the Rf = 0.36 were collected as above to yield this bis-adduct.

MS (+APCI) [$C_{29}H_{37}Cl_2N_3O_3$] m/z = 546(M+H)$^+$.

5.6.5: 9-[3-(1-Propenylbuta-1,3-dienyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (159):



159

The target compound was purified by flash column chromatography. The column was eluted with petroleum ether (60 – 80 °C) : ethyl acetate 1:1 increasing the polarity to neat ethyl acetate. The fractions corresponding to Rf = 0.24 were collected. The solvent was removed under reduced pressure and the target compound was dried under vacuum.

Yield: 101mg, 0.28mmol, pale yellow solid.

Rf: 0.34 (petroleum ether (60-80 °C) 1 : 2 ethyl acetate.

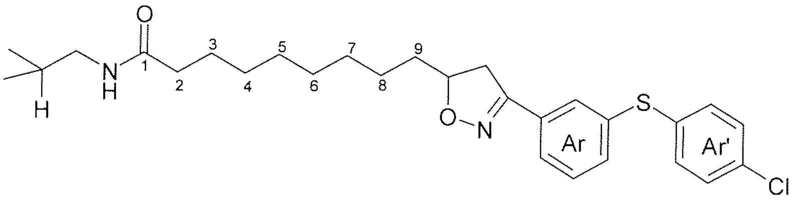
m.p = 90 – 91.8 °C,

^{1}H NMR $\delta_{H:}$ 0.91(d, J= 6.7 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.31(s, 12H C^3-C^8 aliphatic chain), 1.62(overlapping br m, 2H C^9 aliphatic prochiral protons), 1.76(overlapping m, 1H (CH$_3$)$_2$C$\underline{H}$), 2.17(t, J= 7.5 Hz, 2H, $\underline{H_2}$CC=O), 2.960(d,d, J= 16.5, 8.2 Hz, 1H isoxazoline prochiral proton), 3.08(t, J= 6.5 Hz, 2H C$\underline{H_2}$NH), 3.39(d,d, J= 16.5, 10.3 Hz, 1H isoxazoline prochiral proton), 4.73(m, 1H isoxazoline chiral proton), 5.56(br s, 1H, NH), 7.39(m, 3H, 3,4,5-ArH), 7.66(m, 2H, 2,6-ArH) ppm.

MS (±APCI) [$C_{22}H_{34}N_2O_2$] m/z = 359 (M+H)$^+$, 357(M−H)$^-$.

IR ν = 3318(N–H), 3073(Ar-H), 2925, 2852, 1644(C=O), 1544, 1467, 1450, 1357, 1263 cm^{-1}.

5.6.6: 9-{3-[3-(4-Chlorophenylsulfanyl) phenyl]-4,5-dihydroisoxazol-5-yl} nonanoic acid isobutylamide (160):



160

The target compound was purified by flash chromatography, eluting with petroleum ether (60- 80 °C) : ethyl acetate (1:1), increasing the polarity to (1:9) respectively. The fractions corresponding to Rf = 0.36 were collected and concentrated under reduced pressure and dried under vacuum to yield the product.

Yield: 314mg, 0.62 mmol, pale yellow solid.

Rf: 0.45, petroleum ether (60-80 °C) 1 : 2 ethyl acetate

m p = 52.7-54.1 °C

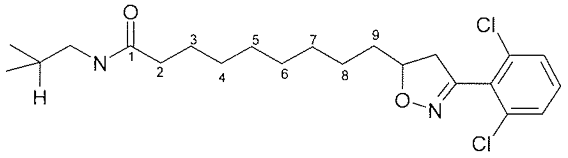
^{1}H NMR $\delta_{H:}$ 0.91(d, J= 6.7 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.30(s, 12H, C^3-C^8 aliphatic chain), 1.63(overlapping br m, 2H, C^9 aliphatic prochiral protons), 1.7(overlapping m, 1H (CH$_3$)$_2$C$\underline{H}$), 2.17(t, J= 7.5 Hz, 2H, $\underline{H_2}$CC=O), 3.04-3.14(overlapping m, 3H, isoxazoline prochiral proton C$\underline{H_2}$NH), 3.48(d,d, J= 16.5, 10.2 Hz, 1H isoxazoline prochiral proton), 4.70(m, 1H isoxazoline chiral proton), 5.58(br s, 1H, NH), 7.10(m, 1H, 5-Ar), 7.23-7.31(overlapping m, 6H, 4,6-Ar, Ar'H), 7.45(m, 1H, 2-Ar) ppm.

MS (+APCI) [$C_{28}H_{37}ClN_2O_2S$] m/z = 501(M+H)$^+$

E(+) [$C_{28}H_{37}ClN_2NaO_2S$] = Mass, 523.2166, calculated mass = 523.2162 [M+Na]$^+$ [0.4. ppm].

IR ν = 3313(N–H), 3085(Ar-H), 2925, 2849, 1640(C=O), 1550, 1475(Ar ring stretch), 1430, 1390, 1347, 1266 cm^{-1}.

5.6.7: 9-[3-(2,6-Dichlorophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (161):



(161)

The target compound was isolated by flash chromatography. The column was eluted with petroleum ether (60 – 80 °C): ethyl acetate 3:2 respectively, increasing to neat ethyl acetate. The fractions corresponding to Rf = 0.17 were collected, concentrated under reduced pressure and dried under vacuum to yield the target compound.

Yield: 409mg, 0.95mmol, pale yellow solid.

Rf: 0.40 (petroleum ether (60-80 °C) 1 : 2 ethyl acetate.

m p = 47.1 – 48.3 °C,

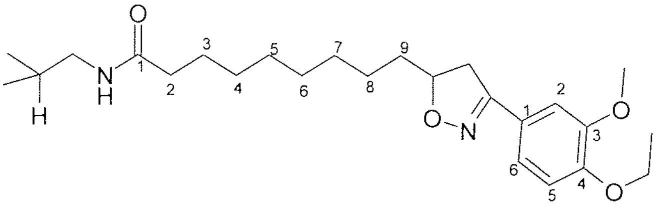
^{1}H NMR $\delta_{H:}$ 0.90(d, J= 6.7 Hz, 6H, $HC(C\underline{H}_3)_2$), 1.31(s, 12H C^3-C^8 aliphatic chain), 1.61(overlapping br m, 2H, C^9 aliphatic prochiral protons), 1.78(overlapping m, 1H $(CH_3)_2C\underline{H}$), 2.17(t, J= 7.5 Hz, 2H, $\underline{H_2}CC{=}O$), 2.91(d,d, J= 16.9, 7.8 Hz, 1H isoxazoline prochiral proton), 3.06(t, J= 6.5 Hz, 2H $C\underline{H_2}NH$), 3.35(d,d, J= 16.9. 10.4 Hz, 1H isoxazoline prochiral proton), 4.81(m, 1H isoxazoline chiral proton), 5.89(br s, 1H, NH), 7.25-7.39(overlapping br m, 3H, ArH) ppm.

MS (+APCI) [$C_{22}H_{32}Cl_2N_2O_2$] m/z = 427 $(M+H)^+$

E(+) [$C_{22}H_{32}Cl_2N_2NaO_2$] = Mass, 449.1762, calculated mass 449.1739 $[M+Na]^+$ [5.1ppm].

IR ν = (NaCl) 3311(N–H), 2928, 2855, 1645(C=O), 1557, 1430, 1324, 1267 cm^{-1}.

5.6.8: 9-[3-(4-Ethoxy-3-methoxyphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (154a):



154a

The target compound was purified by flash chromatography, eluting with ethyl acetate : petroleum ether (60 – 80 °C) (7:3), increasing the polarity to neat ethyl acetate. The fractions corresponding to Rf = 0.24 were collected and concentrated under reduced pressure. The product was dried under vacuum.

Yield: 215mg, 0.498mmol, pale yellow,

Rf = 0.32 ethyl acetate :petroleum ether (60-80 °C) 2:1

m p = 103.8 – 107.2 °C,

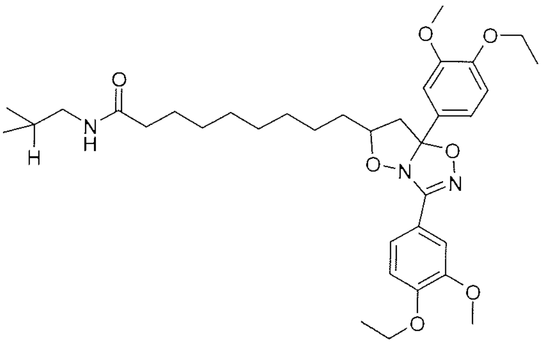
^{1}H NMR $\delta_{H:}$ 0.91(d, J= 6.7 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.31(br s, 12H C3-C8 aliphatic chain), 1.49(t, J= 7.0 Hz, 3H, Ar-OCH$_2$C$\underline{H}_3$), 1.69(overlapping br m, 2H, C^9 aliphatic prochiral protons) 1.76(overlapping m, 1H (CH$_3$)$_2$C$\underline{H}$), 2.17(t, J= 7.5 Hz, 2H, $\underline{H}_2$CC=O), 2.94(d,d, J= 16.5, 8 Hz, 1H isoxazoline prochiral proton), 3.08(d,d. 6.8, 6 Hz, 2H C$\underline{H}_2$NH), 3.38(d,d, J= 16.25, 10.25 Hz, 1H isoxazoline prochiral proton), 3.91(s, 3H, Ar-OC$\underline{H}_3$), 4.13(q, J= 7 Hz, 2H, Ar-OC$\underline{H}_2$CH$_3$), 4.71(m, 1H isoxazoline chiral proton), 5.50(br s, 1H, NH), 6.85(d, J= 8.3 Hz, 1H, 5-ArH), 7.01(d,d, J= 8.5, 2.0 Hz, 1H, 6-ArH), 7.39(d, J= 2.0 Hz, 1H, 2-ArH), ppm.

MS (±APCI) [$C_{25}H_{40}N_2O_4$] m/z = 433 (M+H)$^+$, 431 (m-H)$^-$.

E(+) [$C_{25}H_{40}N_2NaO_4$] = Mass, 455.2852, calculated mass 455.2886 [M+Na]$^+$ [–7.5 ppm].

IR ν = 3321(N–H), 3085(Ar-H), 2926, 1640(C=O), 1601, 1551, 1521, 1469, 1425, 1369, 1263 cm^{-1}.

5.6.9: Bicyclic adduct (154b)



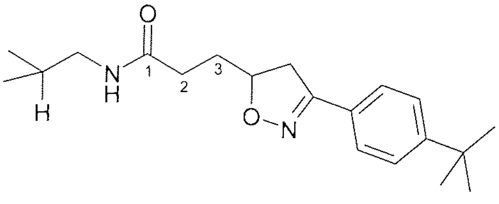
(54b)

^{1}H NMR $\delta_{H:}$

MS (+APCI) [$C_{35}H_{51}N_3O_7$] m/z =626(M+H)$^+$.

The crude compounds that are cleaved from the polymeric support are reported in the section below. Only the peaks corresponding to the target compounds are characterised.

5.6.10: 3-[3-(4-*tert*-Butylphenyl)-4,5-dihydroisoxazol-5-yl]-N-isobutylpropionamide (162):



162

This compound was purified by flash chromatography eluting with ethyl acetate : petroleum ether (60-80 °C) 1:9, increasing the polarity to 3:2. The fractions corresponding to the R_f of 0.2 were combined and the solvent was evaporated under vacuum to yield a pale yellow coloured solid.

Yield: 123mg, 0.37mmol, pale yellow solid.

R_f = 0.23 (petroleum ether (60-80 °C) 1:1 ethyl acetate).

M p = 109-112 °C

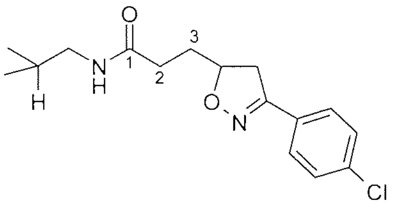
* ^{1}H NMR $\delta_{H:}$ 0.88(d, J= 6.75 Hz, 6H $CH(CH_3)_2$), 1.32(s, 9H, $C(CH_3)_3$), 1.74(m, 1H, prochiral aliphatic proton), 1.74(overlapping m, 1H, $O{=}CCH_2CH_2$ prochiral aliphatic proton), 1.94(overlapping m, 1H, $O{=}CCH_2CH_2$ prochiral aliphatic proton), 2.07(overlapping m, 1H, $(CH_3)_2CH$), 2.40(t, J= 7.25 Hz, 2H, $O{=}CCH_2CH_2$), 3.00(overlapping m, 1H, isoxazoline prochiral proton), 3.05(overlapping t, J= 6.5 Hz, $NHCH_2$), 3.42(d,d, J= 16.5, 10.25 Hz, 1H, isoxazoline prochiral proton), 4.75(m, 1H, Isoxazoline chiral proton), 6.15(br s, NH), 7.41(d, J= 8.5 Hz, 2H, 3,5-ArH), 7.58(d, J= 8.5 Hz, 2H, 2,6-ArH) ppm.

MS (±APCI) [$C_{20}H_{30}N_2O_2$] m/z = 331 $(M+H)^+$, 329 $(M-H)^-$

IR ν = 3356(N–H), 2954, 2868, 1649(C=O), 1556, 1463, 1414, 1361, 1268 cm^{-1}.

*= Even a flash chromatography couldn't yield a very clean product and additional peaks not corresponding to the target compound were observed. Hence only peaks corresponding to the product are presented above. Other peaks observed included δ1.25(s), 7.42(overlapping m), 7.78(d, J= 8.25 Hz) ppm.

5.6.11: 3-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl]-N isobutylpropionamide (163):



(163)

The target compound was purified by flash column, eluting with petroleum ether 60- 80 °C : ethyl acetate (3 : 7) increasing to neat ethyl acetate to 20 % methanol. The fractions corresponding to Rf = 0.41 were collected and concentrated under vacuum. The product was dried under vacuum.

Yield: 263mg, 0.85mmol, pale yellow solid.

Rf: 0.38 (dominating spot), 0.23(faint spot) (petroleum ether (60-80 °C) 1 : 3 ethyl acetate).

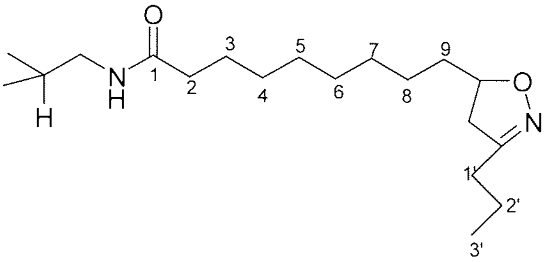
m.p = 135.7-150.9 (softened), 159.6-164.1°C (melted completely).

^{1}H NMR $\delta_{H:}$ 0.90(d, 6H, J= 6.7 Hz, $HC(CH_3)_2$), 1.76(m, 1H C^3, aliphatic prochiral proton), 1.98(overlapping m, 1H C^3, aliphatic prochiral proton), 2.11(overlapping m, 1H $(CH_3)_2CH$), 2.39(t, J=8.25 Hz, 2H $H_2C^2C{=}O$), 2.99(overlapping m, 1H isoxazoline prochiral proton), 3.07(t, J= 6.25 Hz, 2H C$\underline{H}_2$NH), 3.42(d,d, J= 16.6, 10.4 Hz, 1H isoxazoline prochiral proton), 4.78(m, 1H isoxazoline chiral proton), 5.81(br s, 1H, NH), 7.37(d, J= 8.8 Hz, 2H 2,6-ArH), 7.58(d, J= 8.7 Hz, 2H 3,5-ArH), ppm.

MS (-APCI) [$C_{16}H_{21}N_2O_2$] m/z = 307 (M−H)$^-$.

IR ν = 3311(N–H), 2928, 2855, 1645(C=O), 1557, 1430, 1324, 1267 cm^{-1}.

5.6.12: 9-(3-Propyl-4,5-dihydroisoxazol-5-yl)-nonanoic acid isobutylamide (164)



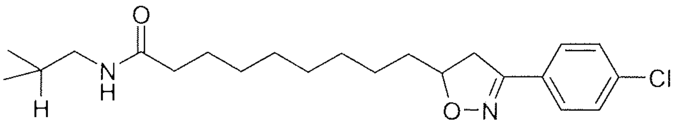
164

Yield: 212mg crude product, dark brown oil.

^{1}H NMR δ_H: Peaks corresponding to the target compound are: 0.82(d, J= 6.7 Hz, 6H CH(C$\underline{H}_3$)$_2$), 0.96(overlapping m, 3H $^{1'}\mathrm{CH_2}{}^{2'}\mathrm{CH_2}{}^{3'}\mathrm{C\underline{H}_3}$), 1.20(br s, 12H, C3-C8 aliphatic protons), 1.52(overlapping m, 2H, C9 aliphatic prochiral protons), 1.69(overlapping m, C$\underline{H}$(CH$_3$)$_2$), 1.94(overlapping m 2H, $^{1'}\mathrm{CH_2}{}^{2'}\mathrm{C\underline{H}_2}{}^{3'}\mathrm{CH_3}$), 2.11(t, J= 7.5 Hz, 2H, O=CCH$_2$), 2.23(t, J= 7.3 Hz, 2H, $^{1'}\mathrm{C\underline{H}_2}{}^{2'}\mathrm{CH_2}{}^{3'}\mathrm{CH_3}$), 2.45(d,d, J= 16.8, 8.3 Hz, 1H, isoxazoline prochiral proton), 2.85(overlapping m, 1H, isoxazoline prochiral proton), 2.98(t, J= 6.5 Hz, 2H, CH$_2$NH), 4.42(m, 1H, isoxazoline chiral proton), 6.17(br s, NH) ppm. Other peaks include δ4.86(t, J= 10.0 Hz, CH=C$\underline{H}_2$ un-reacted alkene), 5.71(m, 1H, C$\underline{H}$=CH$_2$ un-reacted alkene) ppm.

MS = (+APCI) [$C_{19}H_{36}N_2O_2$] m/z = 325(M+H)$^+$

5.6.13: 9-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl]-nonanoic acid isobutylamide (158):



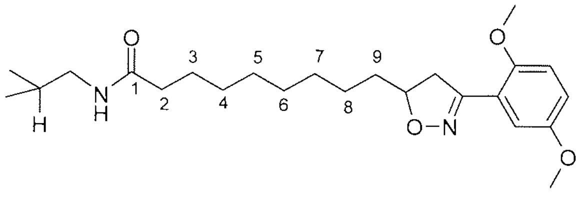
158

Yield: 307mg crude product, dark brown semi solid.

^{1}H NMR δ_H:.0.9(d, 6H, (CH2)2, 1.28(br s, H from the aliphatic chain of the target compound), 1.6(br complex m, 2H, aliphatic prochiral protons), 1.74(overlapping m, 1H HC(CH$_2$)$_2$), 2.19(t, 2H O=CCH$_2$), 2.9(d,d, 1H, isoxazoline prochiral proton), 3.08(t, 2H, CH2NH), 3.32(d,d, 1H, isoxazoline prochiral proton), 4.73(complex m, 1H, isoxazoline chiral proton), 4.93(t, un-reacted alkene), 5.6(br s, 1H NH), 5.7-5.85(m, 1H, un-reacted alkene), 7.34(d, 2H, 2,6-Ar-H), 7.59(d, 2H, 3,5Ar-H) ppm.

MS = (+APCI) [$C_{22}H_{33}N_2O_2Cl$] m/z = 393 (M+H)$^+$.

5.6.14: 9-[3-(2,5-Dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (165):



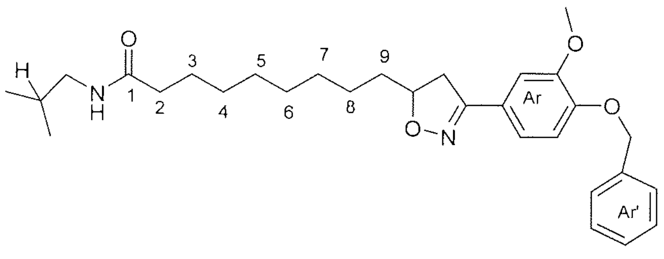
165

Yield: 516 mg crude product, dark brown oil.

^1H NMR δ_H: Peaks corresponding to the product, 0.99(d, 6.6 Hz,6H, $HC(CH_2)_2$), 1.40(s, 12H, C^3-C^8 aliphatic proton), 1.70(overlapping complex m, 2H, aliphatic prochiral protons), 1.85(overlapping m, 1H $HC(CH_2)_2$), 2.29(t, J= 8.5 Hz, 2H, $O{=}CCH_2$), 3.09(m, 1H isoxazoline prochiral proton), 3.45(overlapping m, isoxazoline prochiral proton, $C\underline{H}^2NH$), 3.84(overlapping s, 3H, 5 O-CH_3), 3.88(overlapping s, 3H, 2 O-CH_3), 4.45-5.0(overlapping br m, isoxazoline chiral proton and NH), 7.00(m, 2H 3,4-ArH), 7.69(br s, 1H, 6-ArH) ppm.

MS = (+APCI) [$C_{24}H_{38}N_2O_2$] m/z = 419$(M+H)^+$.

5.6.15: 9-[3-(3-Ethyl-4-phenethylphenyl)-4,5-dihydroisoxazol-5-yl]-nonanoic acid isobutylamide (164)



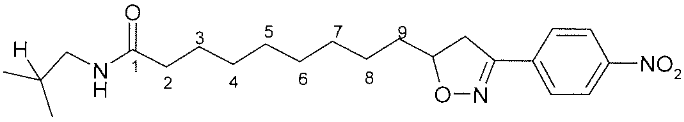
164

Yield: 635 mg crude product, dark brown oil,

^1H NMR δ_H: Peaks corresponding to the product, 0.89(br d, J= 6.7 Hz, $HC(CH_2)_2$), 1.28(br s, 12H, C^3-C^8 aliphatic proton), 1.59(overlapping br m, 2H, C^9 aliphatic prochiral protons), 1.70(overlapping m, 1H, $\underline{H}(CH_2)_2$), 2.15(m, 2H, $H_2CC{=}O$), 2.89(overlapping m, 1H, isoxazoline prochiral proton), 3.04(m, 2H, $\underline{H_2}CNH$), 3.24(overlapping m, 1H, isoxazoline prochiral proton) 3.88(s, 3H, O–$C\underline{H_3}$), 4.69(complex m, 1H, isoxazoline chiral proton), 5.15(s, 2H, $OC\underline{H_2}Ph$) 6.15 (br s 1H, NH), 6.75–7.55(overlapping m, Ar and Ar' protons) ppm.

MS = (+APCI) [$C_{30}H_{42}N_2O_2$] m/z = 495$(M+H)^+$.

5.6.16: 9[3-(4-Nitrophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid isobutylamide (167):

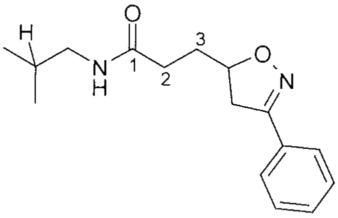


(167)

Yield: 469mg crude product, dark brown oil.

^{1}H NMR δ_H: Peaks corresponding to the product, 0.92(d, J= 6.6 Hz, 6H, $CH(C\underline{H}_3)_2$), 1.31(s, 12H, C^3-C^8 aliphatic protons), 1.65(overlapping br m, 2H, C^9 aliphatic prochiral protons), 1.77(overlapping m, 1H, $C\underline{H}(CH_3)_2$), 2.27(br m, $\underline{H}_2CC{=}O$), 2.94(d,d, J= 16.4, 8.3 Hz, 1H, isoxazoline prochiral proton), 3.10(br s, CH_2NH), 3.26-3.42(m, 1H, isoxazoline prochiral proton), 4.74(m, 1H, isoxazoline chiral proton), 6.43(s, 1H NH), 7.30-7.89(overlapping m, ArH) ppm.

5.6.17: *N*-isobutyl-3-(3-phenyl-4,5-dihydroisoxazol-5-yl)-propionamide (168):



168

Yield: 192mg crude product, dark brown oil.

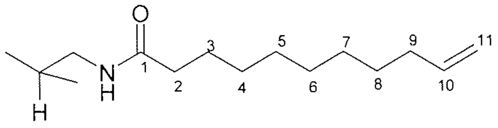
^{1}H NMR δ_H: Peaks corresponding to the product, 0.87(d, J= 6.3 Hz, 6H, $HC(C\underline{H}_3)_2$), 1.75(overlapping m, 2H, $C^2H_2C^3\underline{H}_2$ prochiral protons), 1.96(overlapping m, 1H, $\underline{H}C(CH_3)_2$), 2.36(overlapping m, 2H, $C^2\underline{H}_2C^3H_2$), 2.80(br m, 1H, isoxazoline prochiral proton), 2.99(m, 2H, CH_2NH), 3.32(overlapping m, isoxazoline prochiral proton), 4.73(m, 1H, isoxazoline chiral proton), 4.99(t, 2H, $CH{=}CH_2$ un-reacted alkene), 5.79(m, 1H, CH=CH2, un-reacted alkene), 6.76(br s, 1H NH), 7.36-7.61(overlapping m, 5H, ArH) ppm.

MS = (±APCI) [$C_{16}H_{22}N_2O_2$] m/z = no peak at M±H.

5.7: Solution phase synthesis of Isoxazoline compounds:

5.7.1: Preparation of undec-10-enoic acid isobutylamide (199)

Methyl-10-undecenoate (4.51g, 22.24mmol) was mixed in isobutylamine (14.71g, 201.12mmol) and heated under reflux at 80°C for nine days. The reaction was followed up with a TLC. The isobutylamine was removed under high vacuum to yield a crude product. The crude product was washed with ether achieved a yellow solid as a clean product.



199

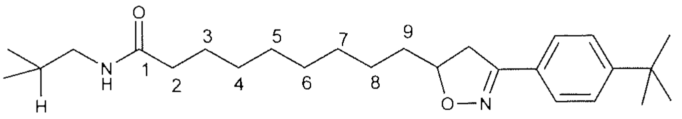
Yield: 5.012g, 94%, 21.0mmol,

R_f = 0.27 (petroleum ether (60-80 °C) 70 : 30 ethyl acetate).

^{1}H NMR (250MHz, $CDCl_3$, δ 7.27) δ_H 0.92(d, J= 6.7 Hz, 6H, $CH(C\underline{H_3})_2$), 1.29(br s, 10H, C^3-C^7 protons), 1.63(br m, 2H, -$O{=}CCH_2C\underline{H_2}$-), 1.81(m, 1H $C\underline{H}(CH_3)_2$), 2.02(m, 2H, $C\underline{H_2}CH{=}CH_2$), 2.31(m, 2H, $O{=}CC\underline{H_2}$), 3.01(t, J= 6.3 Hz, 2H, $C\underline{H_2}NH$), 4.95(m, 2H, $CH{=}C\underline{H_2}$), 5.81(m, 1H, $C\underline{H}{=}CH_2$), 6.38(br, s, 1H NH).

MS = (+APCI) [$C_{15}H_{29}NO$] m/z = 240 $(M+H)^+$

5.7.2: Solution-phase synthesis of 9-[3-(4-*tert*-butylphenyl)-4,5-dihydroisoxazol-5-yl]-nonanoic acid isobutylamide (150):



150

Undec-10-enoic acid isobutylamide (4.00g, 16.74mmol) was dissolved in DCM (350mL). Bleach (160mL) was added to the reaction and stirred rapidly. A solution of 4-*tert*-butylbenzaldehyde oxime (8.89g, 50.21mmol) in DCM (50mL) was added drop-wise over a period of 20 minutes. The reaction was stirred overnight at 25°C. The two layers were separated and the aqueous layer was washed with DCM, organic layers were combined, and dried over magnesium sulphate. The DCM was removed under vacuum to yield a yellow colour crude product.

The compound was purified by flash chromatography eluting with petroleum ether (60-80 °C) and ethyl acetate 1/1 increasing the polarity to 9/11. The fractions corresponding to R_f 0.22 were combined and the solvent was evaporated to give the target compound as a pale yellow crystalline solid.

Yield: 1.35g, 3.26 mmol, 19.5 % pale yellow solid.

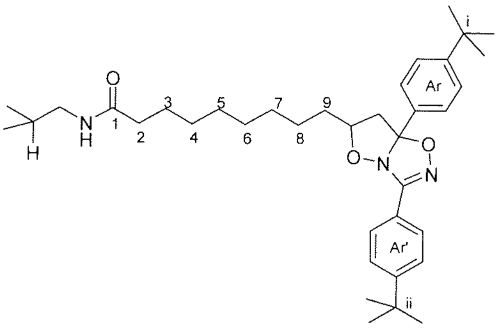
R_f = 0.22 (petroleum ether (60-80 °C) : ethyl acetate) 45 : 55

^{1}H NMR δ_H 0.89(d, J= 6.8 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.29(overlapping br s, 12H, C3-C8 aliphatic protons), 1.31(overlapping br s, 9H C(CH$_3$)$_3$), 1.62(overlapping br m, 2H C9 aliphatic prochiral protons), 1.76(overlapping m, 1H, (CH$_3$)$_2$C$\underline{H}$), 2.19(t, J= 7.6 Hz, 2H, $\underline{H_2}$CC=O), 2.94(d,d, J= 16.5, 8.1 Hz, 1H isoxazoline prochiral proton), 3.07(t, J= 6.4 Hz, C$\underline{H_2}$NH), 3.38(d,d, J= 16.4, 10.3 Hz, 1H isoxazoline prochiral proton), 4.70(m, 1H isoxazoline chiral proton), 5.88(br s, 1H, NH), 7.4(d, J= 8.6 Hz, 2H, 3,5-ArH), 7.6(d, J= 8.6 Hz, 2H, 2,6-ArH) ppm.

MS (+APCI) [$C_{26}H_{42}N_2O_2$] m/z = 415(M+H)$^+$.

IR ν = 3310(N–H), 2960, 2847, 1922, 1644(C=O), 1550, 1465, 1363, 1256 cm^{-1}.

5.7.3: Solution phase synthesis of 9-[3,7a-bis-(4-*tert*-butylphenyl)-7,7a-dihydro-6H-isoxazol-[2,3-d][1,2,4]oxadiazol-6-yl]-nonanoic acid isobutylamide (153):

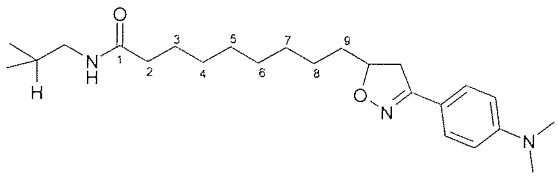


153

^{1}H NMR $\delta_{H:}$ 0.91(d, J= 6.7 Hz, 6H CH(CH$_3$)$_2$), 1.25(overlapping br s, 12H, C^3-C^8 of aliphatic chain), 1.30(br s, 9H, ^{i}(CH$_3$)$_3$), 1.33(br s, 9H, ^{ii}(CH$_3$)$_3$), 1.60(overlapping m, 2H, C^9 aliphatic prochiral protons), 1.77(overlapping m, 1H, CH(CH$_3$)$_2$), 2.16(t, J= 7.57 Hz, 2H ^{2}CH$_2$), 2.41(d,d, J= 11.5, 13 Hz, 1H, isoxazoline prochiral proton), 2.97(d,d J= 13, 4 Hz, 1H, isoxazoline prochiral proton), 3.07(t, 6.25 Hz, 2H, NHC$\underline{H}_2$CH(CH$_3$)$_2$), 4.11(m, 1H, isoxazoline chiral proton), 5.52(br s, 1H NH), 7.42(m, 4H, 3,5-ArH and 3,5-Ar'H), 7.59(d, J= 8.5 Hz, 2,6-ArH), 7.88(d, J= 8.75 Hz, 2H, 2,6-Ar'H) ppm.

MS (+APCI) [$C_{37}H_{55}N_3O_3$] m/z = 590(M+H)$^+$.

5.7.4: 9-[3-(4-Dimethylaminophenyl)-4,5-dihydroisoxazol-5-yl]-nonanoic acid isobutylamide (200):



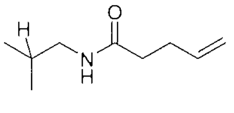
(200)

Undec-10-enoic acid isobutylamide (4.00g, 16.74mmol) was dissolved in DCM (350mL). Bleach (160mL) was added to the reaction and stirred rapidly. A solution of N,N-dimethylamine benzaldehyde oxime (10.98g, 66.96mmol) in DCM (20mL) was added drop-wise over a period of 20 minutes. The reaction was stirred overnight at 25°C. The two layers were separated and the aqueous layer was washed with DCM, organic layers were combined, and dried over magnesium sulphate. The DCM was removed under vacuum to yield a yellow colour crude product.

The target compound was isolated by flash chromatography, eluting with neat ethyl acetate, increasing the polarity to ethyl acetate:methanol (17:3) respectively. The fractions corresponding to Rf = were collected and concentrated under vacuum and the product was dried under vacuum.

Yield: 78mg, 0.2mmol, dark brown solid,
Rf:
M p = 76.8 – 79.6 °C
^{1}H NMR $\delta_{H:}$ 0.91(d, J= 6.7 Hz, 6H, HC(C$\underline{H}_3$)$_2$), 1.30(s, 12H, C^3-C^8 aliphatic chain), 1.63(overlapping br m, 2H, C^9 aliphatic prochiral protons), 1.77(overlapping m, 1H (CH$_3$)$_2$C$\underline{H}$), 2.19(t, J= 8.0 Hz, 2H, $\underline{H}_2$C$_2$C=O), 2.86-3.11(overlapping m, 9H isoxazoline prochiral proton, C$\underline{H}_2$NH and Ar-N(C$\underline{H}_3$)$_2$), 3.36(d,d, J= 16.3, 10.1 Hz, 1H isoxazoline prochiral proton), 4.65(m, 1H isoxazoline chiral proton), 5.61(br s, 1H, NH), 6.69(d, J= 9.1 Hz, 2H, 3,5-ArH), 7.54(d, J= 9.0 Hz, 2H, 2,6-ArH) ppm.
MS (+APCI) [$C_{24}H_{40}N_3O_2$] m/z = 402 (M+H)$^+$
E(+) [$C_{24}H_{40}N_3NaO_2$].= Mass = 4242940, calculated mass = 424.2940 [M+Na]$^+$ [6.4.ppm].
IR ν = 3440(N–H), 2919, 2847, 2344, 1636(C=O), 1607, 1532, 1463, 1364, 1258, 1158 cm^{-1}.

5.7.5: Preparation of pent-4-enoic acid isobutylamide:

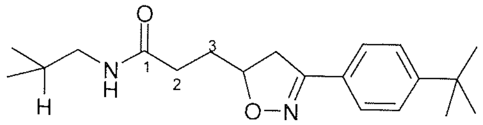


201

Isobutylamine (1.09g, 14.96mmol) and Hüng base (2.15g, 16.46mmol) was mixed with anhydrous THF (15mL). 4-pentonyl chloride (12.68 mmol) was added over a period of 5 min and the reaction was cooled in an ice bath. The reaction was stirred at room temperature (23 °C) for ~ 40 hours. The reaction was poured onto sodium bicarbonate solution (30mL) and crude product was extracted with DCM (30mL).

Yield: 1.706g, 85.86%, 11.008mmol, yellow solid.
R$_f$:
^{1}H NMR δ_H: 0.85(d, J= 6.8 Hz, 6H, (C$\underline{H}_2$)$_2$CH, 1.71(m, 1H, (CH$_2$)$_2$C$\underline{H}$), 2.28(m, 2H C$\underline{H}_2$CHC=H$_2$), 3.01(t, 2H, J= 6.5 Hz C$\underline{H}_2$C=O), 4.98(m, 2H, C$\underline{H}_2$CH), 5.78(m 1H, CH$_2$=C$\underline{H}$), 6.02(br s, 1H NH) ppm.
MS (+APCI) [$C_9H_{17}NO$] m/z = 331 (M+H)$^+$

5.7.6: Solution phase synthesis of 3-[3-(4-*tert*-butylphenyl)-4,5-dihydroisoxazol-5-yl]-*N*-isobutylpropionamide (162):



162

Pent-4-enoic acid isobutylamide (1.00g, 6.45mmol) was dissolved in DCM (150mL). Bleach (62mL) was added to the reaction and stirred rapidly. A solution of 4-*tert*-Butylbenzaldehyde oxime (3.42g, 19.35mmol) in DCM (20mL) was added drop-wise over a period of 15 minutes. The reaction was stirred overnight at room temperature (5 - 10 °C). The two layers were separated and the aqueous layer was washed with DCM (50mL X 3), organic layers were combined, and dried over magnesium sulphate. The DCM was removed under vacuum to yield a yellow colour crude product.

The compound was purified by flash chromatography eluting with petroleum ether (60-80 °C) and ethyl acetate 4:1 rising to 1:1. The fractions corresponding to R_f 0.24 were combined and the solvent was evaporated to give the target compound as a pale yellow crystalline solid.

Yield: 531mg, 7.26 %, 1.75mmol, white solid.

Rf = 0.43 neat ethyl acetate,

M p = 109 -112

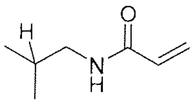
^{1}H NMR δ_H: 0.89(d, J= J= 6.7 Hz, 6H $HC(C\underline{H}_3)_2$), 1.31(s, 9H, $C(CH_3)_3$), 1.74(m, 1H, C^3 prochiral aliphatic proton), 1.94(m, 1H, prochiral aliphatic proton), 2.08(m, 1H, $(CH_3)_2C\underline{H}$), 2.24(t, J= 7.2 Hz, 2H, $\underline{H_2}CC{=}O$), 2.99(overlapping m, 1H, isoxazoline prochiral proton), 3.06(overlapping m, $NHC\underline{H_2}$), 3.34(d,d, J= 16.7, 10.4 Hz, 1H, isoxazoline prochiral proton), 4.74(m, 1H, isoxazoline chiral proton), 6.07(br s, NH), 7.41(d, J= 8.6 Hz, 2H, 3,5-ArH), 7.58(d, J= 8.6 Hz, 2H, 2,6-ArH) ppm.

MS (±APCI) [$C_{20}H_{30}N_2O_2$] m/z = 331 $(M+H)^+$, 329 $(M-H)^-$.

E(+) = [$C_{20}H_{30}N_2NaO_2$] = Mass = 331. 2383, calculated mass = 331.2386, $[M+Na]^+$–[0.9 ppm].

IR ν = 3329(N–H), 2958, 1653(C=O), 1548, 1465, 1428, 1373, 1263 cm^{-1}.

5.7.7: Preparation of *N*-isobutyl acrylamide (202):



202

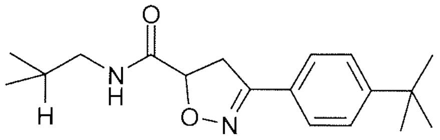
Isobutylamine (2.58g, 35.31mmol) and Hüng base (4.65g, 36.02mmol) was mixed with anhydrous THF (20 mL). Acryloyl chloride (30.0 mmol) was added over a period of 10 min and the reaction was cooled in an ice bath. The reaction vessel was wrapped around with aluminium foil. The reaction was stirred at room temperature (23 °C) for overnight. The reaction was poured onto sodium bicarbonate solution (50 mL) and crude product was extracted with DCM (50 mL). The DCM was evaporated under vacuum to yield a.

Yield: 2.583g, 67.79%, 20.33 mmol, yellow solid.

^{1}H NMR δ_H: 0.92(s, J= 6.7 Hz, 6H, $HC(C\underline{H}_3)_2$), 1.81(m, 1H $(CH_2)_2C\underline{H}$), 3.16(t, J= 6.5 Hz, 2H, $CH_2C{=}O$), 5.63(d,d, 1H, J= 1.8, 9.6 Hz, $CH{=}CH_2$), 5.87(br s, 1H, NH), 6.12(d,d, 1H, J= 9.9, 16.9 Hz, $CH{=}CH_2$), 6.28(d,d, 1H, J= 1.8, 16.9 Hz, $CH{=}CH_2$) ppm.

MS (+APCI) [$C_7H_{13}NO$] m/z = 331 $(M+H)^+$

5.7.8: Preparation of 3-(4-*tert*-butylphenyl)-4,5-dihydro-isoxazole-5-carboxylic acid isobutylamide (203):



203

N-isobutylacrylamide (2.50g, 19.68 mmol) was dissolved in DCM (350 mL). Bleach (190 mL) was added to the reaction and stirred rapidly. A solution of 4-*tert*-Butylbenzaldehyde oxime (10.45g, 59.05 mmol) in DCM (50 mL) was added drop-wise over a period of 20 minutes. The reaction was stirred overnight at room temperature (5 - 10 °C). The two layers were separated and the aqueous layer was washed with DCM (75 mL x 3), organic layers were combined, and dried over magnesium sulphate. The DCM was removed under vacuum to yield a solid crude product.

A flash chromatography was performed on the crude product to purify it, eluting with petroleum ether (60-80 °C) and ethyl acetate 9:1 rising to 1:9. The fractions corresponding to R_f 0.48 were combined and the solvent was evaporated to give the target compound as a yellow solid.

Yield: 1.33g, 22.52%, 4.40 mmol,

R_f = 0.61, neat ethyl acetate,

M p = 87 – 90 °C

^{1}H NMR δ_H: 0.90(d, J= 6.75 Hz, 6H, $HC(\underline{H}_3)_2$), 1.34(s, 9H, $CH(C\underline{H}_3)_3$), 1.70(overlapping m, 1H, $C\underline{H}(CH_3)_2$), 3.03(overlapping m, 2H, isoxazoline prochiral protons), 3.66(m, 2H CH_2NH), 5.13(d,d, J= 9.5, 7.7 Hz, 1H, isoxazoline chiral proton), 6.87(br s, 1H, NH), 7.45(d, J= 8.8 Hz, 2H, 3,5-ArH), 7.61(d, J= 8.8 Hz, 2H, 2,6-ArH) ppm.

MS (+APCI) [$C_{18}H_{26}N_2O_2$] m/z = 303 $(M+H)^+$.

IR ν = 3364(N– H), 2951, 1652(C=O), 1527, 1467, 1346, 1258 cm^{-1}.

5.8: Synthesis of aldoximes:

A general procedure for the synthesis of aldoximes:

A mixture of aldehyde and hydroxylamine hydrochloride (1.2 M equivalent) in ethanol was stirred at room temperature. An aqueous solution of sodium hydroxide (1.1 M equivalent) was added drop wise to the reaction mixture over a period of 15 minutes and cooled in an ice bath. The reaction was heated under reflux for 2 hours. The reaction was allowed to cool to room temperature and poured onto rapidly stirred water. The precipitate formed was collected by filtration and extracted with ether. A solid product was achieved and was dried under vacuum. The reagents used for preparation of aldoximes are shown in **Table 32** below.

Aldehyde (mmol)			Hydroxylamine hydrochloride		Sodium hydroxide			Ethanol (mL)	Water (mL)
Name of the aldehyde	(g)	(mmol)	(g)	(mmol)	(g)	(mmol)	water		
4-tert-butylbenzaldehyde	28.57	176.35	14.79	214.35	7.92	198.00	35	100	500
4-Chlorobenzaldehyde	7.01	50.07	4.14	60.00	2.24	56.00	20	70	400
2,6-Dichlorobenzaldehyde	8.75	50.00	4.15	60.14	2.20	55.00	20	70	400
4-Dimethylamine-benzaldehyde	7.47	50.13	4.13	59.86	2.21	55.25	20	70	400
4-Benzyloxy-3-methoxy-benzaldehyde	12.15	50.21	4.26	61.74	2.23	55.75	20	70	400
3-Benzyloxy-4-methoxy-benzaldehyde	12.24	50.68	4.20	60.87	2.21	55.25	20	70	400
2,5-Dimethoxy-benzaldehyde	8.31	50.06	4.09	59.28	2.27	56.75	20	70	400
2-(4-Chloro-phenylsulfanyl)-benzaldehyde	4.03	16.21	1.63	23.62	0.71	17.82	10	50	350
4-Ethoxy-3-methoxy-benzaldehyde	9.02	50.11	4.14	60.00	2.20	55.00	20	70	400
3-Hydroxy-4-methoxy-benzaldehyde	7.61	50.07	4.15	60.14	2.20	55.00	20	70	400
4-Hydroxy-3-methoxy-benzaldehyde	7.61	50.07	4.15	60.14	2.21	55.25	20	70	400
Dodecanal	26.06	141.07	14.00	202.00	7.92	198	35	100	500
Pyridine-3-carbaldehyde	13.99	194.78	28.02	261.68	8.40	210.00	35	100	500

Table 32: The table above shows the molar ratio of aldehyde to hydroxylamine, used to prepare the aldoximes from their corresponding aldehyde precursors.

5.8.1: 4-*tert*-butylbenzaldehyde oxime:

Yield: 31.07g, 99%, 175.54mmol white crystalline solid.

m.p = 85.9–90.4 °C[127]

R_f = two spots 0.83 (dominant), 0.70 (faint) (petroleum ether (60-80 °C) 3:7 ethyl acetate).

^{1}H NMR $\delta_{H:}$ 1.36(s, 9H, C(C$\underline{H}_3$)$_3$), 7.43 (d, J= 8.4 Hz, 2H, 3,5 Ar-H), 7.54 (d, J= 8.4 Hz, 2H 2,6 Ar-H), 8.16(s, 1H, $\underline{H}$C=N) ppm.

MS (+APCI) [$C_{11}H_{15}NO$] m/z = 178 (M+H).

IR ν = 3278, 2965, 1918, 1686(C=N), 1607, 1460, 1306, 1105 cm^{-1}.

5.8.2: 4-Chlorobenzaldehyde oxime:

Yield: 7.20g, 93%, 46.45mmol pale yellow solid.

m.p = 82.6–90 °C[128]

R_f = 0.76, (petroleum ether (60-80 °C) 3 : 7 ethyl acetate)

^{1}H NMR $\delta_{H:}$ 7.37(d, J= 8.6 Hz, 2H, 2,6 Ar-H), 7.52(d, J= 8.5 Hz, 2H, 3,5 Ar-H), 8.14(s, 1H, $\underline{H}$C=N) ppm.

MS (+APCI) [C_7H_6ClNO] m/z = 156 $(M+H)^+$.

IR ν = 3308, 2773, 1920, 1793, 1654(C=N), 1594, 1493, 1316, 1086 cm^{-1}

5.8.3: 2,6-Dichlorobenzaldehyde oxime:

Yield: 24.93g, 82%, 132 mmol white crystalline solid.

m p = 118–131 °C[129]

R_f = 0.78 (petroleum ether (60-80 (petroleum ether (60-80 °C) 1 : 9 ethyl acetate)

^{1}H NMR (δ_{DMSO}= 2.5) δ_H : 7.40(d,d, J= 9.3, 7.0 Hz, 1H, 4-ArH), 7.51-7.55(overlapping t, J= 7.3, 1.5 Hz, 2H, 3,5-ArH), 8.21(s, 1H, $\underline{H}$C=N) ppm.

MS (+APCI) [$C_7H_5Cl_2NO$] m/z = 190 $(M+H)^+$.

IR ν = 3290 (O-H stretch), 1943, 1574, 1560, 1438, 1292 cm^{-1}.

5.8.4: *N.N*-4-Dimethylamine benzaldehyde oxime:

Yield: 27.86g, 90.5%, 170mmol brown solid.

M p = 143.9–146.6 °C

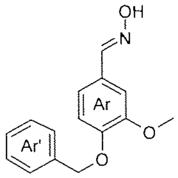
R_f = 0.61, (petroleum ether (60-80 °C) 1 : 4 ethyl acetate)

^{1}H NMR $\delta_{H:}$ 3.01(s, 6H, HC(CH$_3$)$_2$), 6.72(d, J= 8.9 Hz, 2H 3,5-Ar-H), 7.47(d, J= 8.9 Hz, 2H 2,6-Ar-H), 8.08(s, 1H HC=N) ppm.

MS (+APCI) [$C_9H_{12}N_2O$] m/z = 165 $(M+H)^+$, 148 $(M-OH)^+$.

IR ν =3273(O-H stretch) 2912, 1609, 1526, 1445, 1362, 1302, 1225, 1186 cm^{-1}.

5.8.5: 4-Benzyloxy-3-methoxybenzaldehyde oxime:



204

Yield: 12.68g, 98.67%, 49.33mmol. cream colour powdery solid

M p =107.3–110.5°C

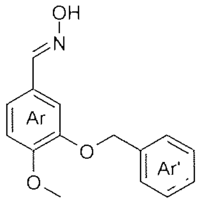
R_f = 0.73 ethylacetate : petroleum ether (2:1).

^{1}H NMR δ_H (peaks corresponding to the product) 3.95(s, 3H, O-CH_3), 5.29(s, 2H, C$\underline{H_2}$-Ar'), 6.91(d, J= 8.3 Hz, 1H, 5-ArH), 7.04(d,d J= 11.0, 2.3 Hz, 1H, 6-ArH), 7.34 – 7.48(overlapping m, within m an overlapping d, at 7.25 1H, J= 1.75 Hz, 2-ArH), and 5H, Ar'H), 8.32(s, HC=N) ppm.

MS (+APCI) [$C_{15}H_{15}NO_3$] m/z = 258 $(M+H)^+$, 241 $(M-OH)^+$.

IR ν = 3304(O-H stretch), 2932, 1598, 1511, 1464, 1418, 1376, 1337, 1260 cm^{-1}.

5.8.6: 3-Benzyloxy-4-methoxybenzaldehyde oxime:



205

Yield : 12.41g, 97%, 48.30mmol, cream colour powdery solid (crude product).

M p =89.5 – 90.5 °C

R_f = 0.70 ethyl acetate : petroleum ether (60 –80 °C) (2:1)

^{1}H NMR δ_H : 3.91(s, 3H, O-C$\underline{H_3}$), 5.16(s, 2H, C$\underline{H_2}$-Ar'), 6.88(d, J= 8.3 Hz, 1H, 5-ArH), 7.06(d,d 1H, J= 8.3, 1.9 Hz, 6-ArH), 7.25(d, 1H, J= 1.9 Hz, 2-ArH), 7.29-7.47(m, 5H, Ar'H), 8.05(s, HC=N) ppm.

MS (+APCI) [$C_{15}H_{15}NO_3$] m/z = 258 $(M+H)^+$, 241 $(M-OH)^+$.

IR ν = 3453, 2925, 2593, 1598, 1513, 1440, 1386, 1332, 1260, 1133 cm^{-1}.

5.8.7: 2,5-Dimethoxybenzaldehyde oxime:

Yield: 7.71g, 85%, 42.6mmol, off white solid.

M p = 98.6 – 100.7 °C

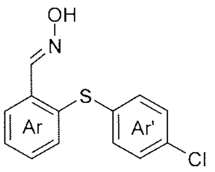
R_f = 0.71 ethylacetate : petroleum ether (2:1),

^{1}H NMR δ_H : 3.79(s, 3H, 5,O-C$\underline{H}_3$), 3.83(s, 3H, 2,O-CH_3), 6.91(overlapping m, 2H, 3,4-ArH), 7.26 (fine d, J= 3.0 Hz, 1H, 6-Ar), 8.48(s, 1H, HC=N) ppm.

MS (+APCI) [$C_9H_{11}O_3N$] m/z = 182 $(M+H)^+$, 165 $(M-OH)^+$, 148.

IR ν = 3432, 3246, 2951, 2837, 1577, 1502, 1462, 1389, 1324, cm^{-1}.

5.8.8: 2-(4-Chlorophenylsulfanyl) benzaldehyde oxime:



206

Yield: 4.00g, 93%, 15.17mmol.

M p =

R_f = 0.74, (petroleum ether (60-80 °C) 3 : 7 ethyl acetate)

^{1}H NMR δ_H :7.12(overlapping m, 2H, 2,6-Ar'), 7.26(overlapping m, 2H, 3,5-Ar'), 7.37(m, 3H, 3,5,6-Ar), 7.85(m, 1H, 4-Ar), 8.63(s, 1H, HC=N) ppm.

MS (+APCI) [$C_{13}H_{10}ClNOS$] m/z = 264 $(M+H)^+$.

5.8.9: 4-Ethoxy-3-methoxybenzaldehyde oxime:

Yield : 6.89g. 70%, 35.32mmol, cream colour solid.

M p =100.5 – 102.2°C

R_f = two spots, 0.61 (dominant), 0.5,(petroleum ether (60-80 °C) 3 : 7 ethyl acetate)

^{1}H NMR $\delta_{H:}$ 1.49(t, J= 7.0 Hz, 3H, O-$CH_2C\underline{H}_3$), 3.90(s, 3H, O-C$\underline{H}_3$), 4.13(q, J= 7.0 Hz, 2H, O-C$\underline{H}_2CH_3$), 6.85(d, J= 8.27 Hz, 1H, 5Ar-H), 7.02(d,d, J= 8.27, 1.9 Hz, 1H, 6-Ar-H), 7.22(d, J= 1.9 Hz, 1H, 2Ar-H), 8.09(s, 1H, HC=N) ppm.

MS (+APCI) [$C_{10}H_{13}NO_3$] m/z = 196 $(M+H)^+$, 179 $(M-OH)^+$, 168, 150.

IR ν = 3470, 2979, 1605, 1512, 1464, 1420, 1337, 1263, 1229, cm^{-1}.

5.8.10: 3-Hydroxy-4-methoxybenzaldehyde oxime:

Yield : 4.73g, 56.58%, 28.29 mmol brown solid.

M p= 205 – 211.7 °C

R_f = two spots, 0.56 (dominant), 0.41,(ethyl acetate : petroleum ether (60-80 °C)) 2 : 1

^1H NMR δ_H : 3.93(s, 3H, O-C$\underline{H}_3$), 6.89(d, J= 8.3 Hz,. 1H 5-ArH), 7.04(d,d, J= 8.3, 2.0 Hz, 1H 6-ArH), 7.22(d, J= 2.0 Hz, 1H, 2-Ar), 8.05(s, 1H, HC=N) ppm.

MS (+APCI) [$C_8H_9NO_3$] m/z = 168 $(M+H)^+$, 151 $(M-OH)^+$.

IR ν = 3350, 2454, 1582, 1514, 1325, 1265, 1168, 1127, 1024 cm^{-1}.

5.8.11: 4-Hydroxy-3-methoxybenzaldehyde oxime:

Yield: 6.17g, 74%, 36.94mmol. yellow solid (crude product).

M p = 88.1 – 90.3 °C

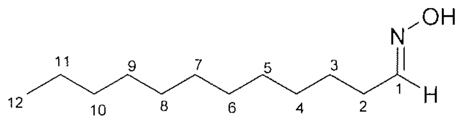
R_f = Two spots, 0.54 (dominant), 0.37(faint), (ethylacetate : petroleum ether) 2:1,

^1H NMR δ_H : peaks corresponding to the product, 3.93(s, 3H, O-CH_3), 6.92(d, J=8.2 Hz, 1H.5-Ar), 7.00(d,d, J= 8.1, 1.8 Hz, 1H 6-Ar), 7.21(d, J= 1.8 Hz, 1H 2-Ar), 8.10(s, 1H, HC=N) ppm.

MS (+APCI) [$C_8H_9NO_3$] m/z = 168 $(M+H)^+$, 151 $(M-OH)^+$.

IR ν = 3449, 3222, 3007, 2941, 1599, 1516, 1466, 1248, 1374, 1324, 1269, cm^{-1}

5.8.12: Dodecanal oxime:



207

Yield : 15.36g, 54.63%, 88.18mmol white crystalline solid

M p = 74.3 – 76.6 °C

R_f = 0.71 ethyl acetate : petroleum ether (60 – 80 °C) (2:1),

^1H NMR δ_H : 0.89(t, J= 6.6 Hz, CH_3), 1.3(s, 16H, ^{4}C-^{11}C CH_2), 1.5(br m, 2H, $^3CH_2$), 2.2(m, 1H, $^2CH_2$), 2.4(br m, 1H, $^2CH_2$) 7.44(t, J= 6.1 Hz, 1H, 1HC=N) ppm.

MS (+APCI) [$C_{12}H_{25}NO$] m/z = 200 (M+H)+, 101

IR ν = 3089, 3202, 2919, 2846, 1665(C=N), 1467, 1446, 1324 cm^{-1}.

5.8.13: Pyridine-3-carbaldehyde oxime:

Yield: 11.59g, 48.77%, 95mmol white powdery solid.

M p = 131.5 – 136.6 °C

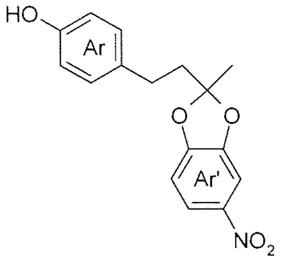
R_f = 0.29, ethyl acetate : petroleum ether (2:1),

^1H NMR (DMSO = δ 2.5) δ_H :7.41(d,d, J= 7.9, 4.7 Hz, 1H, 5-ArH), 7.98(d, 1H,J= 7.9 Hz, 6-ArH), 8.55(d,d, 1H, J= 4.8, 1.6Hz, 4-ArH), 8.75(d, J= 2.0 Hz, 1H, 2-ArH), 11.52(s, 1H, HC=N) ppm.

MS = (+APCI) [$C_6H_6N_2O$] m/z = 123$(M+H)^+$, 105$(O-H)^+$

IR ν = 3262, 3165, 3060, 2949, 2704, 1954, 1637(C=N), 1582, 1518, 1411, 1312, 1256 cm^{-1}.

5.9: Preparation of 4-[2-(5-nitro-2-methylbenzo[1,3]dioxol-2-yl)ethyl] phenol (181):



181

A mixture of 1-(4-hydroxyphenyl)-3-butanon (2.17g, 13.3mmol), 4-nitrocatechol (2.06g, 13.3mmol) and *p*TSA (0.34g, 1.79mmol) was mixed with Toluene (80mL) and heated under reflux, with stirring in conjunction with a Dean-Stark apparatus for 96 hours. The toluene was removed under vacuum. The crude product was dissolved in ethyl acetate (50mL) and washed with sodium hydroxide x 3 (5.00g, 0.125 mol/dm^3) (50mL). The ethyl acetate layer was dried with magnesium sulphate, which was removed by filtration. The ethyl acetate was removed under vacuum to yield dark brown oil as a crude product. A TLC analysis of the crude product revealed the presence of starting materials, and the product was purified by flash chromatography eluting with petroleum ether (60-80 °C) and ethyl acetate 3:1. The fractions corresponding to R_f 0.27 were combined and the solvent was evaporated to give the target compound as a yellow coloured solid.

Yield: 3.015g, 75%, yellow solid.

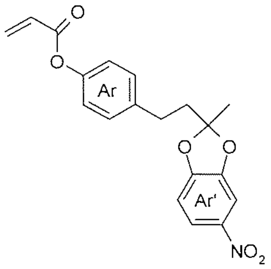
R_f = 0.27 (petroleum ether (60-80 °C) : ethyl acetate) 3:1

^{1}H NMR δ_H: 1.72(s, 3H, $C\underline{H}_3$), 2.26(m, 2H, Ar- $CH_2C\underline{H}_2COOCH_3$), 2.71(m, 2H, Ar-$C\underline{H}_2CH_2COOCH_3$), 6.76(overlapping d,d J= 8.6, 2.1 Hz, 1H, 3-Ar'H), 6.80(overlapping d, J= 8.6 Hz, 2H, 2,6 Ar-H), 7.04(d, J= 8.5Hz, 2H, 3,5-ArH), 7.61(d, 1H, J= 2.3 Hz, 6-Ar'H), 7.87(d,d, 1H, J= 8.6, 2.3Hz, 4-Ar'H) ppm.

MS = (+APCI) [$C_{16}H_{15}NO_5$] m/z = 302 $(M+H)^+$'208 $(M-ArOH)^+$, 107.

IR ν = ($CHCl_3$), 3600(O-H stretch), 3023, 2362, 1606, 1517, 1492, 1337, 1264

5.9.1: Preparation of Acrylic acid 4-[2-(5-nitro-2-methylbenzo[1,3]dioxol-2-yl)-ethyl] phenyl ester (182):



(182)

The 4-[2-(5-nitro-2-methylbenzo[1,3]dioxol-2-yl) ethyl]-phenol (5.00g, 16.61mmol) was dissolved in anhydrous chloroform (100mL). The triethylamine (2.18g, 21.60mmol) was added, followed by drop-wise addition of acryloyl chloride (1.79g. 19.93mmol) and reaction was cooled in an ice-bath. The reaction was stirred at room temperature (10-15 °C) overnight. The reaction mixture was poured onto rapidly stirred ice-water (150 mL). A saturated solution of sodium hydrogen carbonate (40mL) was added. The organic layer was separated and aqueous layer was washed with dichloromethane (2 X 100 mL). The organic layers were combined and dried over magnesium sulphate. Magnesium sulphate was removed by filtration and chloroform and dichloromethane were removed under vacuum to obtain a crude product. The target compound was purified by flash chromatography eluting with ethyl acetate : petroleum ether (60-80 °C) 1:5. The fractions corresponding to the R_f of 0.38 were combined and the solvent was evaporated under vacuum to yield a pale yellow coloured solid.

Yield : 3.778g, 64 %, 10.64mmol.

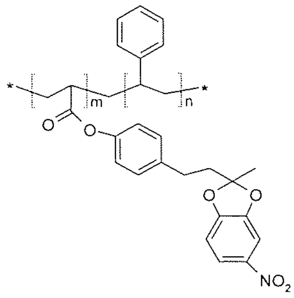
R_f: (petroleum ether (60-80 °C) : ethyl acetate) 4:1

0.3 8 ^{1}H NMR δ_H: 1.72(s, 3H, methyl group), 2.30(m, 2H, Ar-$CH_2C\underline{H_2}COOCH_3$), 2.79(m, 2H, Ar-$C\underline{H_2}CH_2COOCH_3$), 6.01(d,d, J=10.4, 1.4 Hz, 1H CH=$C\underline{H_2}$), 6.31(d,d, J=17.3, 10.4 Hz, 1H $C\underline{H}$=CH_2), 6.60(d,d, J= 17.2, 1.4 Hz, 1H CH=CH_2), 6.79(d, 1H, J=8.6, 2.0 Hz, 1H, 3-Ar'H) 7.04(d, J= 6.6 Hz, 2H, 2,6-ArH), 7.20(m, 2H, 3,5-ArH), 7.61(d, J= 1H, J=2.3 Hz, 6-Ar'H), 7.87(d,d, 1H, J=8.6, 2.3 Hz, 1H, 4-Ar'H) ppm.

MS (+APCI) [$C_{19}H_{17}NO_6$] m/z = 355 (M+H)+.

IR ν = ($CHCl_3$), 3031, 2362, 1741(C=O), 1517, 1486, 1337

5.9.2: Poly (styrene-co-4-[2-(5-nitro-2-methylbenzo[1,3]dioxol-2-yl) ethyl] phenyl ester) copolymer (Pol 44):



(Pol 44)

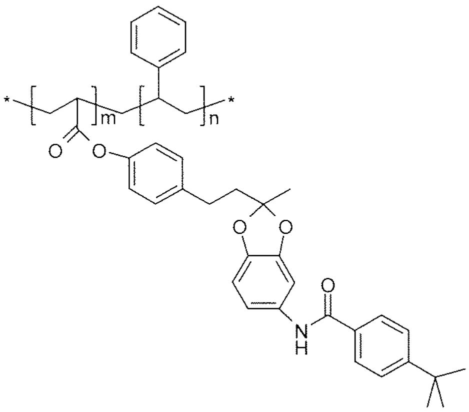
A solution of acrylic acid 4-[2-(5-nitro-2-methylbenzo [1,3]dioxol-2-yl)-ethyl]phenyl ester (0.73g, 2.059 mmol), AIBN (36 mg), and styrene (1.67g, 10.295 mmol) in toluene (20mL) was de-oxygenated by alternate applications of vacuum and argon (x 5) with rapid stirring. The solution was heated under a reflux condenser, for 4 days under an argon atmosphere. The toluene was removed under the vacuum until there was just enough toluene to keep everything in solution. The solution was poured onto rapidly stirred methanol. The resulting precipitate was collected by filtration, re-dissolved in a small amount of dichloromethane and re-precipitated onto methanol. The final precipitate was collected by filtration and dried under vacuum to give the product as a solid.

Yield: 0.49g, 27%, 1.14mmol/g loading of the functional group.

^1H NMR δ_H: 0.9-2.15(overlapping broad m containing, $C\underline{H}_2$ from polymer back bone and Ar-$CH_2CH_2COOC\underline{H}_3$), 2.27(overlapping br m, 2H Ar-$CH_2C\underline{H}_2COOC\underline{H}_3$), 2.74(br m, Ar-$C\underline{H}_2CH_2COOC\underline{H}_3$), 6.10-7.59(overlapping br m, protons from Ar, Ar' and styrene from polymer back bone), 7.63(overlapping s, 1H, 6-Ar'H), 7.88(overlapping d, J= 8.8 Hz, 1H, 4-Ar'H) ppm.

IR ν = 3417, 3023, 2921, 1949, 1752(C=O), 1604, 1492

5.9.3: Attempted preparation of poly (styrene-co-4-{3-[5-(4-*tert*-butylbenzoylamino)-2-methylphenoxy]-3-hydroxybutyl} phenyl ester) copolymer (Pol 45):



(Pol 45)

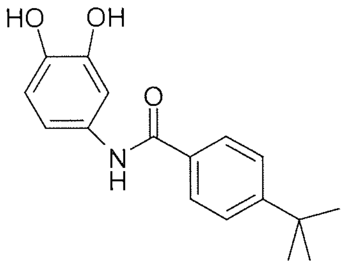
Polymer **Pol 44** (150mg, 0.13mmol), THF (8mL) and 10% Pd-C (10mg) were mixed together in a hydrogenator. The reaction was purged with hydrogen gas (x 5) while stirring rapidly. The reaction was heated at 30°C while it was exposed to a pressure of 100 psi (8 bar) for about 9 hours.

The system was purged with argon (x 5) replacing the H_2 (g) with argon. A solution of Hung base (0.24g, 0.33 mL, 1.88mmol) in TFA (0.7 mL) was injected into the reaction mixture followed by *p*-tert-butyl benzoyl chloride solution (0.43g, 0.25 mL, 1.25mmol) in TFA (0.75 mL). The reaction was stirred under argon at RT for ~20 hours.

The reaction mixture was filtered through celite 545 plug, washed with THF (10mL) and the filtrated was collected, solvent volume was reduced to about 2 mL under vacuum and the solution was poured on rapidly stirred methanol (100mL). The resulting precipitate was collected by filtration and dried under vacuum.

Yield: 0.0892g (89.2 mg),
The ^{1}H NMR spectrum was same as the starting material **Pol 45**.

5.9.4: Attempted cleavage of 4-*tert*-butyl-*N*-(3,4-dihydroxyphenyl) benzamide from Pol 45 (183):



183

1st attempt:[130]

Polymer **Pol 45** (51 mg)was stirred with a solution of 5M HCl (1 mL con HCl in 1 mL DME, = 5M HCl), in DME (2mL), Water (20 µL) was added to the reaction mixture. The reaction was heated under reflux for 3 hours. The reaction was cooled down, concentrated under vacuum and poured onto rapidly stirred methanol (5 mL).The precipitate was collected by filtration and filtrate was collected and solvent was evaporated under vacuum to give a crude product.

The ^{1}H NMR and MS analysis of the crude product didn't show any evidence for the presence of the product in question. Therefore a second attempt was made using the reminder of the polymer **Pol 45.**

2nd attempt:

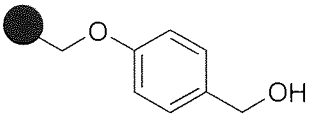
Polymer **Pol 45** (30mg) was treated with 20% TFA in THF (2 mL solution, 400 µL TFA and 1.6mL THF). The reaction was stirred at RT (23 °C) for 3 hours. The reaction was followed up with TLC. No new spot was observed on the TLC plate.

5.10: Solid-Phase Synthesis:

5.10.1: Preparation of isoxazoline compounds using cross-linked polystyrene support (Wang resin):

5.10.1.1: Wang Resins

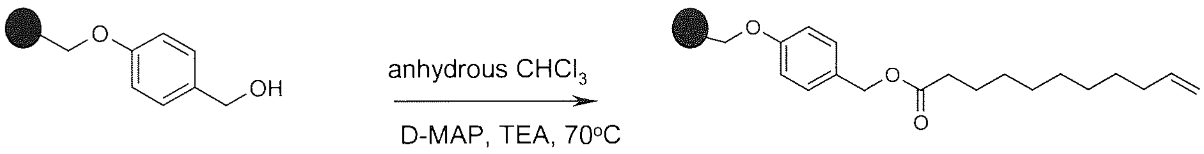
IR for Wang-resins (Alkoxybenzyl Alcohol resins.



W 1

IR ν = 3384 (O-H), 3019, 2906 (C-H), 2340, 1947 cm^{-1}.

5.10.2: The acylation of the Wang-resins (alkoxybenzyl alcohol resins:)



W 1

W 2

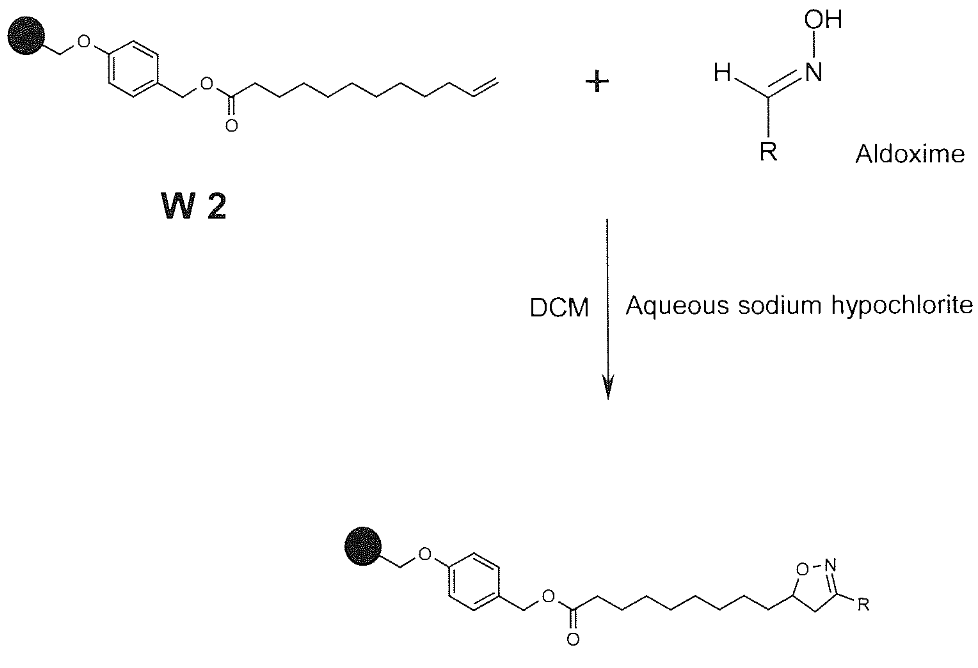
The **W 1** (1.7mmol/g of functional loading)(20.08g, 34.136mmol), and D-MAP (300mg) were mixed in anhydrous chloroform (200mL) and stirred very gently for 30 min, or until the resins were fully swelled. Triethylamine (13.79g, 18.5 mL, 136.54 mmol) was added drop-wise and the resins were stirred for another 5 minutes. 10-undecenoyl chloride (23.75 mL, 111 mmol) was added drop-wise over a period of 10 minutes. The reaction was heated at reflux under argon for 48 hours.

The reaction was cooled to room temperature and was filtered. The resins were washed with chloroform (100 mL, x3), water (100 mL, x3), methanol (100 mL, x3) and THF (100 mL, x3) respectively. The resins were dried under vacuum.

Infra red analysis of acylated Wang Resins (W 2):

IR ν = 3446 (O-H), 3024, 2924 (C-H), 1939, 1876 1736(C=O), 1611, 1510, 1450 cm^{-1}.

5.10.3: General procedure for the 1,3-dipolar cycloaddition reactions between nitrile oxides and resins-bound alkenes:



W 2

W 3

R = aromatic group with variable substituents.

The **W 2** was allowed to swell in DCM for 30 minutes. Aqueous sodium hypochlorite was added and a solution of aldoxime in DCM was added drop-wise over a period of 20 minutes while suspension was agitated gently. The suspension was shaken for 24 hours at room temperature. The resins were collected by filtration, washed with DCM (300 mL, x3), water (300 mL, x3), methanol (300 mL, x3) and THF (300 mL, x3). The resins were washed in chloroform in a soxhlet apparatus for 24 hours and dried under vacuum. The quantities of various reagents used are given in **Table 33**. **Table 34** shows the cleaved products and the yields obtained.

Wang linker 3 (g)	Wang linker achieved	Aldoxime			DCM (mL)	Sodium hypochlorite (mL)
		Name of oxime	(g)	(mmol)		
0.70	**W4**	4-*tert*-Butyl benzaldehyde oxime	0.84	4.75	40	12.5
1.10	**W5**	4-Chloro benzaldehyde oxime	0.79	5.1	50	18
1.01	**W6**	2,6-Dichloro benzaldehyde oxime	1.21	6.42	50	21.5
1.03	**W7**	2,5-Dimethoxy benzaldehyde oxime	1.25	6.88	50	21.5
1.01	**W8**	4-Dimethylamine-benzaldehyde oxime	1.12	6.83	70	20
1.01	**W9**	4-Ethoxy-3-methoxy -benzaldehyde oxime	1.34	6.87	50	23
1.00	**W10**	3-(4-Chloro-phenylsulfanyl)-benzaldehyde oxime	1.70	6.45	65	30

Table 33: The table shows the quantities of reagents used for the 1,3-dipolar cycloaddition reaction of the resin bound terminal alkene with various aldoximes.

5.10.4: Resin bound isoxazoline compounds:

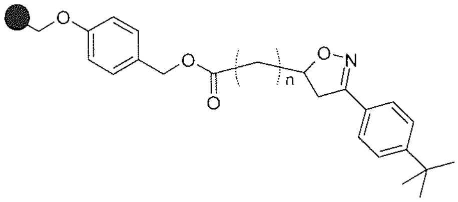
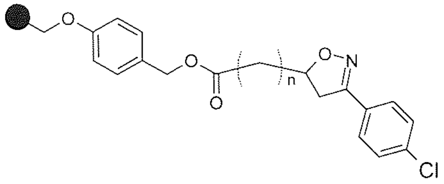
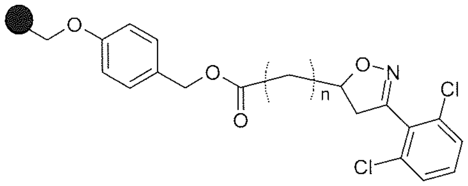
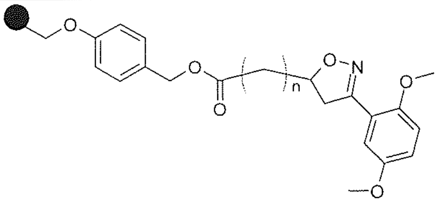
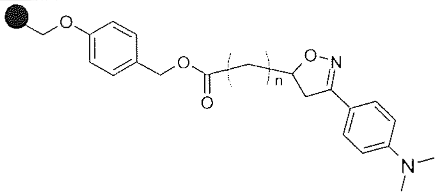
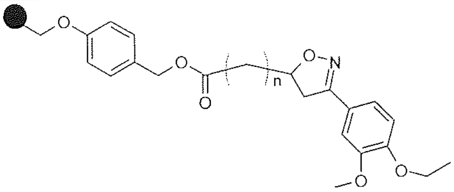
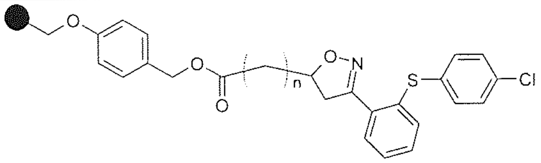
Resin No	Structure (with IR analysis, where necessary)	Yield (g)
W4	 IR ν = 3445, 3025, 2924, 1944, 1879, 1733(C=O), 1609, 1511, 1451 cm^{-1}.	0.82
W5	 	1.11
W6	 	1.19
W7	 	1.40
W8	 	0.84
W9	 	1.14
W10	 	1.10

Table 34: Table shows the yield obtained for various resin bound compounds.

5.10.5: The cleavage of the resin bound compounds:

The Wang resins were treated with 20% trifluoroacetic acid (TFA) in DCM. The resins were shaken for 24 hours at RT. The resins were removed by filtration. The filtrate was collected and solvent was evaporated under high vacuum to give crude product. The reagents used to cleave the products from solid support are shown in **Table 35** below.

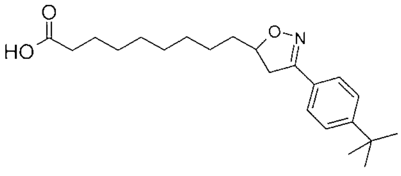
PAGE 226 BLANK

Wang resins		DCM (mL)	TFA (mL)	Resins recovered (mg)	Crude cleaved product obtained (mg)
Number	Quantity (g)				
W4	0.403	12	3	307	55
W5	1.00	24	6	695	157
W6	1.00	24	6	785	123
W7	1.00	24	6	693	425
W8	0.750	20	5	650	35
W9	1.00	24	6	717	456
W10	0.950	24	6	662	403

Table 35: **The table shows the amount of TFA used to cleave off the resin bound compounds, the amount of crude product obtained and the resins recovered.**

The crude products cleaved from the Wang Resins were not purified. Hence, for the ^{1}H NMR analysis of following cleaved compounds, only the peaks corresponding to the compounds are given.

5.10.5.1: 9-[3-(4-*tert*-butylphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid (169):



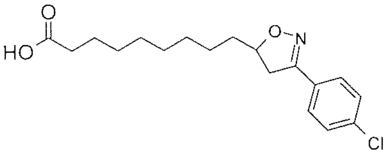
169

Yield: 55mg crude product, dark brown oil.

^{1}H NMR δ_H : 1.32(overlapping br s, 12H, $(CH_2)_6$ aliphatic chain), 1.62(overlapping s, 9H, $(CH_3)_3$), 1.52(overlapping m, 2H, CH_2, prochiral aliphatic protons), 2.34(t, J= 7.5 Hz, 2H COOHC$\underline{H}_2$), 2.96(d,d, J= 16.5, 8.0 Hz, 1H, isoxazoline prochiral proton), 3.39(d,d, J= 16.3, 10.3 Hz, 1H, isoxazoline prochiral proton), 4.71(m, 1H, isoxazoline chiral proton), 7.43(d, J= 8.6 Hz, 2H, 3,5-ArH), 7.61(d, J= 8.5 Hz, 2H, 2,6-ArH) ppm.

MS (+APCI) $[C_{22}H_{33}NO_3]$ m/z = 360 $(M+H)^+$,

5.10.5.2: 9-[3-(4-Chlorophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid (170):



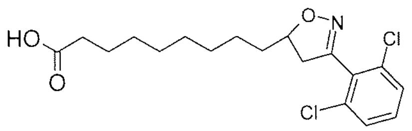
(170)

Yield: 157 mg crude product as a dark brown solid.

^{1}H NMR δ_H : 1.35(s, 12H CH_2 aliphatic chain), 1.42(overlapping m, 2H, CH_2 prochiral aliphatic protons), 2.35(t, J= 7.5 Hz, 2H, COOHC$\underline{H}_2$), 2.96(d,d, 16.8, 8.1 Hz, 1H, isoxazoline prochiral proton), 3.39(d,d, J= 16.8, 10.3 Hz, 1H, isoxazoline prochiral proton), 4.71(m, 1H, isoxazoline chiral proton), 7.43(d, J= 8.6 Hz, 2H, 2,6-ArH), 7.61(d, J= 8.5 Hz, 2H, 3,5-ArH) ppm.

MS (+APCI) $[C_{18}H_{24}NO_3Cl]$ m/z =

5.10.5.3: 9-[3-(2,6-Dichlorophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid (171):

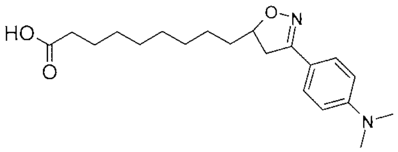


(171)

Yield: 123mg, dark brown solid,

^{1}H NMR δ_H: 1.15(br overlapping s, 12H $(CH_2)_6$ aliphatic chain), 1.37(overlapping m, 1H, prochiral aliphatic proton), 1.60(overlapping m, 1H, prochiral aliphatic proton), 2.05(t, J= 7.4 Hz, 2H, COOHC$\underline{H_2}$), 2. (d,d, J= , 1H, isoxazoline prochiral proton), 3. (d,d, J= 1H, isoxazoline prochiral proton), 4.70(overlapping m, 1h, isoxazoline chiral proton), 7. (m, 3H, ArH) ppm.

5.10.5.4: 9-[3-(4-Dimethylaminophenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid (172):



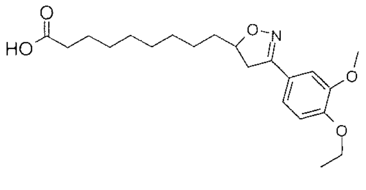
(172)

^{1}H NMR δ_H : 35 mg crude product.

The ^{1}H NMR Spectrum gave no evidence for the presence of this compound. There was, however, a peak at M+H in the MS APCI+ (374). Due to the small amount of sample obtained no further purification was done on this crude product.

MS (+APCI) [$C_{20}H_{30}N_2O_3$] m/z = 347 $(M+H)^+$.

5.10.5.5: 9-[3-(4-Ethoxy-3-methoxyphenyl)-4,5-dihydroisoxazol-5 yl]-nonanoic acid (173):



(173)

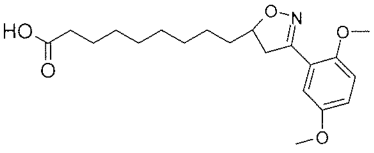
Yield : 456mg crude product, brown solid.

It was attempted to purify the product using the flash chromatography eluting with a ethyl acetate and petroleum ether .The pure product was not obtained. Here is the provisional ^{1}H NMR of the one of the fraction that gave the better looking ^{1}H NMR from the others.

^{1}H NMR δ_H :1.30(overlapping br s, 12H, $(CH_2)_6$ aliphatic chain), 1.49(overlapping t, J= 7.0 Hz, 3H, Ar-$OCH_2C\underline{H}_3$), 1.60(overlapping m, 2H, aliphatic prochiral protons), 2.29(t, J= 7.4 Hz, 2H, $COOHC\underline{H}_2$), 2.94(d,d, J= 16.3, 8.0 Hz, 1H, isoxazoline prochiral proton), 3.37(d,d, J= 16.3, 10.2 Hz, 1H, isoxazoline prochiral proton), 3.90(overlapping s, 3H, Ar-$OC\underline{H}_3$), 4.13(q, J= 7.0 Hz, 2H, Ar-$OC\underline{H}_2CH_3$), 4.70(m, 1H, isoxazoline chiral proton), 6.84(d, J= 8.3 Hz, 1H, 5-ArH), 7.01(d,d, J= 8.3, 1.8 Hz, 1H, 6-ArH), 7.39(d, J= 1.8 Hz, 1H, 2-ArH) ppm.

MS (+APCI) [$C_{21}H_{31}NO_5$] m/z = 378 $(M+H)^+$.

5.10.5.6: 9-[3-(2,5-Dimethoxyphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic acid (174):



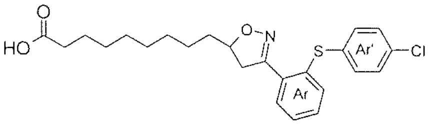
(174)

Yield: 425 mg, brown solid.

^{1}H NMR δ_H : 1.32(s, 12H, $(CH_2)_6$, aliphatic chain), 1.62(overlapping m, 2H, CH_2 prochiral aliphatic protons), 2.32(t, J= 7.5 Hz, 2H, $COOHC\underline{H}_2$), 3.07(d,d, J= 17.3, 8.3 Hz, 1H, isoxazoline prochiral proton), 3.50(d,d, 1H, J= 17.3, 10.1 Hz, isoxazoline prochiral proton), 3.79(overlapping s, 3H, 5-OCH_3), 3.81(overlapping s, 3H, 2-OCH_3), 4.67(m, 1H, isoxazoline chiral proton), 6.86(overlapping d, J= 9.0 Hz, 1H, 3-ArH), 6.93(overlapping d,d, J= 9.0, 3.0 Hz, 1H, 4-ArH), 7.30(d, J= 3.0 Hz, 1H, 6-ArH) ppm.

MS (+APCI) [$C_{20}H_{29}NO_5$] m/z = 364 $(M+H)^+$.

5.10.5.7: 9-{3-[2-(4-Chlorophenylsulfanyl) phenyl]-4,5-dihydroisoxazol-5-yl} nonanoic acid (175):



(175)

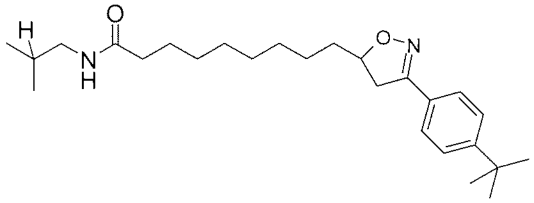
Yield: 403 mg crude product, brown solid.

The crude product mixture also contains the un-reacted alkenes (evident by the presence of a triplet at δ 4.9 and multiplets at δ 5.7-5.7 indicating the terminal double.

^{1}H NMR δ_H : 1.31(s, 12H, CH_2 aliphatic chain), 1,62(m, 2H, prochiral aliphatic protons), 2.31(t, J= 7.3 Hz, 2H, $COOHC\underline{H}_2$), 2.58(overlapping m, 1H, isoxazolibne prochiral proton), 3.48(overlapping d,d, J= 16.5, 10.2, Hz, 1H, isoxazoline prochiral proton), 4,73(m, 1H, isoxazoline chiral proton), 6.86-7.48(overlapping m, ArH and ArH') ppm.

Other peaks include δ4.95(t, 2H, $CH{=}C\underline{H}_2$ un-reacted alkene), and δ5.71(m, 1H, $C\underline{H}{=}CH_2$ un-reacted alkene).

5.10.5.8: 9-[3-(4-*tert*-Butylphenyl)-4,5-dihydroisoxazol-5-yl] nonanoic-isobutyl-amide (176):



(176)

Yield: 329mg crude product, brown oil.

Peaks corresponding to the product, ^{1}H NMR δ_H : 0.90(d, J= 6.67 Hz, 6H, $HC(C\underline{H}_3)_2$), 1.31(overlapping br s, 12H, C^3-C^8 $C\underline{H}_2$s), 1.32(overlapping br s, 9H $C(C\underline{H}_3)_3$), 1.63(overlapping br m ,2H, C^9 aliphatic prochiral protons), 1.75(overlapping m, 1H, $(CH_3)_2C\underline{H}$), 2.20(t, J= 7.45 Hz, 2H, C^2 $\underline{H}_2CC{=}O$), 2.96(m, 1H isoxazoline prochiral proton), 3.09(t, J= 6.4 Hz, $C\underline{H}_2NH$), 3.39(m, 1H isoxazoline prochiral proton), 4.71(m, 1H isoxazoline chiral proton), 5.64(br s, 1H, NH), 7.43(d, J= 8.5 Hz, 2H, 3,5-ArH), 7.61(d, J= 8.6 Hz, 2H, 2,6-ArH) ppm.

5.11: Experimental on antimicrobial study of polymers:

The polystyrene copolymers prepared were tested for antimicrobial activity. (for procedure and analysis of these polymers please refer to section 5.3 – 5.5.32). The procedure followed to test the antimicrobial activity was adapted from the literature [131],[132] Before the actual experiment was performed, all polymers were re-precipitated by dissolving in minimal amount of DCM and pouring onto rapidly stirred methanol. The precipitate was collected by filtration and dried under vacuum.

Glass slides used were frosted at one end and were purchased from . The nutrient agar and Middlebrook broth were purchased from Oxoid. The microbes were grown on a fresh agar plate and incubated at 37°C for 18 hours, prior to testing.

The antimicrobial activity was tested against four different microbes, which were,
Staphylococcus aureus (NCTC 6571),
Staphylococcus epidermidis, (NCTC 35984)
Micrococcus leuteus, (Aston University microbiology culture collection),
Micrococcus roseus, (Aston University microbiology culture collection),

5.11.1: Preparation of polymer coated slides:

A 5% w/v solution of each polymer was prepared by dissolving polymer (25mg) in chloroform (500µL). Five slides were coated on one side with 50µL of a polymer solution (the end of the pipette was used to spread the solution making sure that the slide is completely covered with the solution). The procedure was repeated for other polymer solutions so that five slides were prepared for each polymer solution. The coated slides were air dried for 2 hours at room temperature, under aseptic conditions, to evaporate the chloroform leaving behind a thin film of polymer.

Two sets of control slides were prepared.
1: uncoated slides, treated with chloroform only (control 1).
2: polystyrene polymer (**Pol 7**) (Control 2) coated slides, (polymer made from a single monomer styrene),

The coated slides were placed in a slide holder.

5.11.2: Preparation of cell suspension and exposing polymers to it:

A cell suspension of *S. aureus* (NCTC 6571) (5mL) in distilled water, was prepared giving an OD of ~1.0 (550 nm), containing ~ 10^9 cfc / mL. This was used to prepare a cell suspension (2-litres) in water, by adding 1.6mL / L, giving a cell concentration of ~ 10^6 cells / mL.

The uncoated control and the coated slides were placed into the *S. aureus* solution, making sure that slides were completely submerged in cell suspension solution. The slides were constantly agitated for 18 hours at RT.

The slide holder (with slides) was removed from the cell suspension and gently placed in fresh distilled water (x3, fresh water each time) to wash away any unbound / loosely bound cells from the polymer coated slides. The slides were gently pat dried with paper towels to remove any excess water and air dried under aseptic conditions for 30 minutes.

Under aseptic conditions, the coated side of the slides was used to make imprints onto the surface of nutrient agar plates (each slide was used to make one imprint). It was made sure that all air bubbles were removed when the imprints were made. The slides were removed from the agar surface once the imprints were made. The imprinted plates were incubated at 37°C for 18 hours. A colony count was performed.

All copolymers prepared, were subjected (*S. aureus* results in the table) to initial screening for biological activity. Based on the findings of these test a total number of 7 polymers were selected for further tests with other microbes. The polymers selected were, **polystyrene** (control 2), **Pol 16, Pol**

17, **Pol 29**, **Pol 31**, **Pol 38** and **Pol 39**. The above procedure was repeated for these copolymers against *S. epidermidis*, *M. leuteus*, and *M.roseus*.

5.11.3: Bacterial growth on glass slides coated with polymers:

The following **Tables 36, 37, 38** and **39** show the colony count for each type of copolymers that were used to test the antimicrobial activity against each type of microbes.

Polymer No	Number of colonies in each plate				
	1	2	3	4	5
Control 1	397	390	396	403	388
Control 2	164	150	172	168	177
Pol 3	400	412	408	449	398
Pol 8	410	403	425	429	447
Pol 9	369	355	309	371	383
Pol 10	398	410	411	403	388
Pol 11	415	423	407	411	418
Pol 12	462	412	458	463	444
Pol 13	423	429	439	431	424
Pol 14	466	501	656	491	507
Pol 15	405	392	387	394	398
Pol 16	506	566	565	535	501
Pol 17	515	501	507	521	513
Pol 18	342	350	336	339	357
Pol 19	372	339	358	374	368
Pol 20	352	389	376	371	398
Pol 21	386	399	412	451	428
Pol 23	350	375	319	380	372
Pol 24	410	419	433	447	460
Pol 25	300	311	323	358	297
Pol 26	303	313	315	297	309
Pol 28	451	432	409	411	425
Pol 29	194	201	198	187	203
Pol 30	412	415	427	420	406
Pol 31	218	222	199	207	214
Pol 34	375	351	382	378	370
Pol 35	271	287	296	298	391
Pol 37	392	358	399	380	387
Pol 38	300	299	306	311	302
Pol 39	227	222	219	230	225

Pol 40	380	409	386	410	418
Pol 43	460	452	455	468	472

Table 36: **The number of colonies of *S. aureus* in each plate for copolymers tested.**

Polymer No	Number of colonies in each plate				
	1	2	3	4	5
Control 1	157	163	133	137	117
Control 2	236	237	240	229	218
Pol 16	506	566	565	535	501
Pol 17	320	296	309	314	327
Pol 29	151	131	140	135	132
Pol 31	132	214	238	234	224
Pol 38	221	227	213	237	210
Pol 39	355	338	343	331	329

Table 37: **Table of number of colonies for *S. epidermidis* in each plate for selected copolymers.**

Polymer No	No of colonies in each plate				
	1	2	3	4	5
Control 1	527	401	441	477	508
Control 2	591	537	683	491	613
Pol 16	499	506	343	380	428
Pol 17	311	250	212	208	218
Pol 29	391	361	309	354	322
Pol 31	441	254	193	377	289
Pol 38	431	474	379	480	407
Pol 39	650	507	615	543	597

Table 38: **The number of colonies for *M. leuteus* in each plate for selected co-polymer.**

Polymer No	Number of colonies in each plate				
	1	2	3	4	5
Control 1	415	409	387	398	431
Control 2	295	287	310	319	301
Pol 16	419	440	489	465	471
Pol 17	410	397	447	421	408
Pol 29	369	381	389	395	405
Pol 39	410	417	425	419	428
Pol 38	328	317	345	311	356
Pol 31	293	299	341	301	308

Table 39: **Number of colonies of *M. roseus* in each plate for selected co-polymer.**

5.11.4: Screening of Δ^2-isoxazoline compounds for antimicrobial activity:

The purified isoxazoline compounds, synthesized both, on polymeric support and by traditional solution phase chemistry, were subjected to an initial biological screening (by gate testing) to show any antimicrobial activity against *M. fortuitum* (NCTC 10394). The initial testing was kindly done by, Shaina Chauhan a research student working with Professor P. A. Lamber. For the chemical analysis of these compounds please refer to the section 56.1 – 5.7.8.

5.11.4.1: Procedure to test cleaved compounds for antimicrobial activity:

The Mibblebrook medium was made according to the instructions given on the pack. The stock solution for each compound was prepared by dissolving 3.2mg of compound in 1mL DMSO.

For each test compound a test tube was prepared by adding together Middlebrook (950µL), stock solution (50µL) and *M. fortuitum* (NCTC 10394) inoculum (10µL). The tubes were incubated at 37 °C for 2 – 3 days.

The result for this test was simply recorded as YES (✓) for activity and a NO (✗) for no activity. **(Table 40).** If there is any biological activity the compounds are further test for MIC tests.

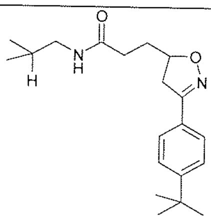
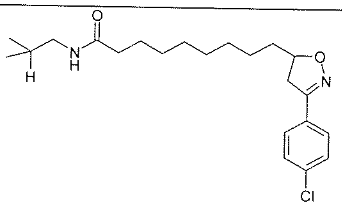
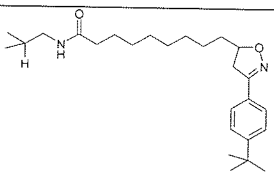
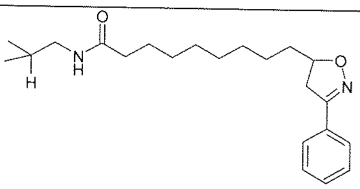
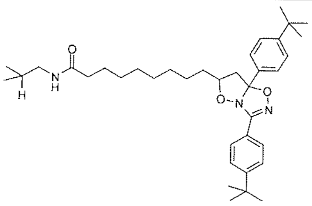
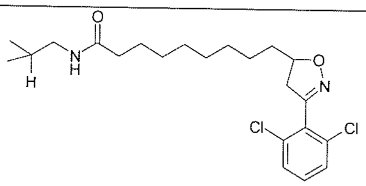
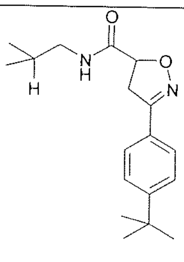
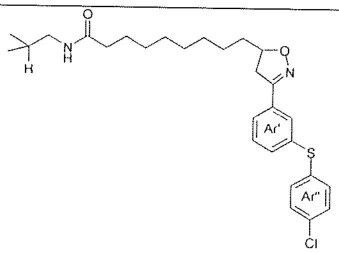
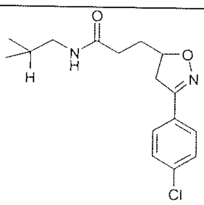
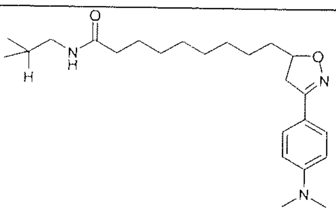
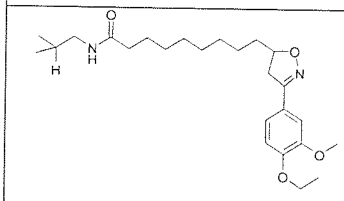
Compound	Activity obtained (activity ✓) (no activity ✗)	Compound	Activity obtained (activity ✓) (no activity ✗)
 **154**	✗	 **140**	✗
 **132**	✗	 **151**	✗
 **133**	✗	 **153**	✗
 **154**	✗	 **152**	✗
 **155**	✗	 **161**	✗
 **176**	✗		✗

Table 40: The results of initial screening of isoxazoline compounds for antimicrobial activity against *M. fortuitum* (NCTC 10394).

CHAPTER 6

References

References:

---

[1] Balkenhoh. F, Bussche-Hünnefeld, von dem C, Lanskey. A, Zechel. C, *Angew. Chem. Int. Ed. Engl.* **1996,** Vol. 35, pp. 2288-2337, Combinatorial Synthesis of Small Organic Molecules.

[2] Gravert. D. J, Datta. A, Wentworth Jr. P, Janda. K. D, *J. Am. Chem. Soc*, , **1998,** Vol. 120, No 37, pp. 9481-9495, Solubled supports Tailored for Organic Synthesis: Parallel Polymer Synthesis *Via* Sequential Normal/Living Free Radical Processes,

[3] Gravert. D.J, Janda. K. D, , *Drugs of the future,* **1997** Vol 22, No 10, pp. 1147-1150, Soluble polyethylene glycol for liquid-phase combinatorial synthesis

[4] Beebe. X, Schore. N. E, Kurth. M. J, *J. Org. Chem*, , **1995,** Vol. 60, pp. 4196-4203, Polymer Supported Synthesis of Cyclic Ethers: Electrophilic cyclization of Isoxazolines.

[5] Morphy. J. R, Rankovic. Z, York. M, *Tetrahedron*, **2001**, Vol. 42, pp. 7509-7511, Fluorocarbon Accelerated Supported Transformation (FAST) on REM Resin.

[6] Blackburn. C, *Biopolymers (Peptide Science),* **1998,** Polymer, Vol 47, pp. 311-351, Supports for Solid-Phase Organic Synthesis.

[7] Quan. C, Kurth. M, *J. Org. Chem*, **2004**, Vol 69, pp. 1470-1474**,** Solid-Phase Synthesis of 5-Isoxazol-4-yl-[1,2,4]oxadiazoles.

[8] Eggenmeiler. H. M, *DDT*, **1998**, Vol. 3, No. 12, Review, December, Linkers for Solid-Phase Synthesis of Small Molecules: coupling and cleavage techniques.

[9]. Crowley, I;Rappoport. J, H. *Acc. Chem. Res.* **1976**, 9, 135-144.

[10] Gravert. D. J, Janda. K. D, *Chem. Rev*, **1997**, Vol. 97, pp. 489-509, Organic Synthesis on Soluble Polymer Supports: Liquid-phase Methadologies.

[11] Braun. K. R, Freysoldt. T. H. E, Wierschem. F, *Chemical Society Reviews*, **2005,** 1, Vol. 34, pp. 507-516 ,3-Dipolar cycloaddition on solid support: nitrone approach towards isoxazolidines and isoxazolines and subsequent transformations.

[12] Cin M. D, Davalli. S, Marchioro. C, Passarini. M, Perini. O, Provera. S, Zaramella. A, *Il Farmaco,* **2002**, Vol. 57, No 6, pp. 497-510, Analytical Methods for the Monitoring of Solid Phase Organic Synthesis.

[13] James. I. W, *Tetrahedron*, **1999,**Vol 55, pp. 4855-4946, Linkers for Solid Phase Organic Synthesis.

[14] Merrifield. R. B, *J. Am. Chem. Soc,* **1963**, Vol. 85, pp. 2149-2154, July 20, Solid-Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide.

[15] Pei. Y, Moos. W. H, *Tet Lett*, **1994,** Vol. 35, No. 32, pp. 5825-5828, (references cited therein), Post-Modification of Peptoid Side Chains: [3+2] Cycloaddition of Nitrile Oxides with Alkenes and Alkynes on the Solid-Phase

[16] Cheng. J. F, Mjalli. A. M. M, *Tet Lett*, **1998,** Vol. 39, pp. 939-942, Solid-Phase Synthesis of Δ^2 Isoxazolines.

[17] Shankar. B. B, Yang. D. Y, Girton. S, Ganguly. A. K, *Tet Lett*, **1998**, Vol. 39, pp. 2447-2448, One Pot Solid-Phase Synthesis of Isoxazolines.

[18] Spanka. C, Wentworth Jr. P, Janda. K. D, *Combinatorial. Chemistry & High Throughput Screening*, **2002**, Vol. 5, pp. 233-240, Developing Soluble Polymers for High-Throughput Synthesis Chemistry.

[19] Gravert. D. J, Janda. K. D, *Current opinion in Chemical Biology*, **1997**, Vol. 1, pp. 107-113, Synthesis on Soluble Polymers: new reaction and the construction of small molecules.

[20] Chen. S, Janda. K. D, *Tet lett*, **1998**, Vol. 39, pp. 3943-3946, Total Synthesis of Naturally Occuring Prostaglandin $F_{2\alpha}$ on a Non-Cross-Linked Polystyrene Support.

[21] Kodera. Y, Tanaka. H, Matsushima. A, Inada. Y, *Biochemical and biophysical research communications,* **1992**, Vol. 184, No. 1, pp. 144-148, April 15, Chemical Modefication of L-Asparaginase with a Comb-Shaped Copolymer of Polyethylene Glycol Derivative and Maleic Anhydride.

[22] Fuke. I, Hayashi. T, Tabata. Y, Ikada. Y, *J. Controlled release*, **1994**, Vol. 30, pp. 27-34, Synthesis of Poly(ethylene glycol) Derivatives with Different Branchings and their Use for Protein Modification.

[23] Gravert. D. J, Janda. K. D, *Tet lett*, **1998**, Vol. 39, pp. 1513-1516, Bifunctional Initiators for Free Radical Polymerization of Non Crosslinked Block Copolymer.

[24] Benaglia. M, Annunziata. R, Cinquini. M, Cozzi. F, Russel. S, *J. Org. Chem*, **1998,** , Vol 63, pp. 8628-8629, Synthesis of New Poly(ethyleneglycol)s with a High Loading Capacity.

[25] Reed. N. N, Janda. K. D, *Org Lett*, **2000,** , Vol 2, No. 9, pp. 1311-1313, April 6, Stealth Star Polymers: A New High-Loading Scaffold for Liquid-Phase Organic Synthesis.

[26] Shang. Y. J, Wang. Y. G, *Synthesis*, **2002,** , No. 12, pp. 1663-1668, Synthesis of Isoxazolines and Isoxazoles Using Poly(ethylene glycol) as Support.

[27] Shang. Y, Jin. J, Wang. Y, *Reactive & Functional Polymers*, **2006,** , Vol. 66, pp. 447-453, Nitrile oxides Cycloadditions to PEG-bounded alkene dipolarophiles.

[28] Shemyakin. M. M, Ovchinnikov. Y. A, Kinyushkin. A. A, Kozhevnikova. I. V, *Tet Lett*, **1965,** , Vol. 27, pp. 2323-2327, Synthesis of Peptide

s in Solution on a Polymeric Support: I. Synthesis of Glycylglycyl-L-leucylglycine.

[29] Prof. Cramer. F, Helbig. R, Hettler. H, Scheit. K. H, Seliger. H, *Angew. Chem. Internat. Edit.* **1966,** Vol. 5, No. 6, pp. 601, Oligonucleotide Synthesis with a Soluble Polymer as Carrier.

[30] Hayatsu. H, Khorana. H. G, *J. Am. Chem. Soc*, **1966,** , Vol. 88, pp. 3182-3183, Dioxyribooligonucletide Synthesis on a Polymer Support.

[31] Toy. P. H, Janda. K. D, *Acc. Chem. Res*, **2000,** Vol. 33, pp. 546-554, Soluble Polymer-Supported Organic Synthesis.

[32] Chen. S, Janda. K. D, *J. Am. Chem. Soc*, **1997**, Vol. 119, pp. 8724-8725, Synthesis of Prostaglandin E_2 Methyl Ester on a Soluble Polymer Support for the Construction of Prostanoid Libraries.

[33] Maher. J. J, Furey. M. E, Greenburg. L. J, *Tetrahedron Lett*, **1971,** Vol. 1, pp. 27-28, Improved Solid-Phase Peptide Synthesis on Non-Crosslinked Resins.

[34] Hayatsu. H, Khorana. H. G, *J. Am. Chem. Soc*, **1967**, Vol 89, pp. 3880-3887, Studies on Polynucleotides: Deoxyribonucleotides Synthesis on a Polymer Suport.

[35] Guthrie. R. D, Jenkins. A. D, Stehicek. J, *J. Chem. Soc. (C)*, **1971,** pp. 2690-2696, Synthesis of Oligosaccharides on Polymer Supports. Part I. 6-*O*-(p-Vinylbenzoyl) Derivatives of Glucopyranose and Their Copolymers with Styrene.

[36] Manzotti. R, Tang. S. Y, Janda. K. D, *Tetrahedron*, **2000**, Vol. 56, pp. 7885-7892, Improved Synthesis of Prostanoids on a Non-Cross-Linked Polystyrene Soluble Support.

[37] Malagu. K, Guérin. P, Guillemin. J. C, *Synlett*, **2000**, No. 2, pp. 316-318, Preparation of Soluble Polymeric Supports with a Functional Group for Liquid-Phase Organic Synthesis.

[38] Dublanchet. A. C, Lusinchi. M, Zard. S. Z, *Tetrahedron*, **2000**, Vol. 58, pp. 5715-5721, Xanthates and Solid-Phase Chemistry. A new Soluble Polymer analogue of Wang Resin.

[39] Heatley. F, Lovell. P. A, McDonald. J, *Eur. Polym. J*, **1993,** Vol. 29, No. 2/3, pp. 255-268.

[40] Mennicken. M, Nagelsdiek. R, Keul. H, Hocker. H, Macromol. *Chem. Phys.* **2004,** Vol. 205, No. 18, pp. 2429-2437.

[41] Nagelsdiek. R, Mennicken. M, Maier. B, Keul. H, Hocker. H, *Macromolecules*, **2004,** Vol. 37, pp. 8923-8932.

[42] Liu. Y, Mao. R, Huglin. M. B, Holmes. P. A, *Polymer*, **1996,** Vol. 37, No. 8, pp. 1437-1441.

[43] Matsumoto. A, Asai. S, Shimizu. S, Aota. H, *Eur. Polymer. J*, **2002,** Vol. 38, pp. 363-368.

[44] Higgins. J. P. J, Weale. K. E, *J. Poly. Sci, Part A*, **1968,** Vol 6, pp. 3007-3013, Soluble High Polymers from Allyl Methacrylate.

[45] Nagelsdiek. R, Mennicken. M, Maier. B, Keul. H, Hocker. H, *Macromolecules*, **2004,** Vol. 37, pp. 8923-8932.

[46] Williams. D. H, Fleming. I, *Spectroscopic methods in organic chemistry*, Fourth edition revised

[47] Sanders. J. K. M., Hunter. B. K, Modern NRM spectroscopy*: A guide for chemists*, **1987** oxford press.

[48] Kemp. W, Organic spectroscopy first edition, **1983**.

[49] Wang. L, Dong. W, Xu, Y, *Carbohydrate Polymers*, **2007**, V. 68, No. 4, p.626-636. Synthesis and characterization of hydroxypropyl methylcellulose and ethyl acrylate graft copolymers

[50] Arseniyadis. S, subhash. P. V, Valleix. A, Wagner. A, Mioskowski. C, *Chem. Commun.* **2005**, pp. 3310-3312, Unprecedented, fully recyclable, solid-supported reagent for the kinetic resolution of racemic amines through enantioselective N-acetylation.

[51] Booth. R. J, Hodges. J. C, *Acc. Chem. Res.* **1999**, Vol. 32, pp. 18-26, Solis supported reagents for rapid purification of combinatorial synthesis products.

[52] Kaldor. S. W, Siegel. M. G, Fritz. J. E, Dressman. B. A., Hahn. P. J, *Tetrahedron Lett.* **1996**, Vol. 37, No. 40, pp. 7193-7196, Use of solid supported nucleophiles and electrophiles for the purification of non-peptide small molecule libraries.

[53] Gayo. L. M, Suto. M. J, *Tetrahedron Lett.* **1997**, V. 38, No. 4, pp. 513-516, Ion-exchange resins for solution phase parallel synthesis of chemical libraries.

[54] Synthesis, **1997**, pp. 1217-1239

[55] Coppola. G. M, *Tetrahedron Lett.* **1998**, V. 39, pp. 8233-8236, A new scavenger resin for amines.

[56] Hughes. I, *Tetrahedron Lett.* **1996**, Vol. 37, No. 42, pp. 7595-7598, Application of polymer bound phosphonium salts as traceless support for solid phase synthesis.

[57] Brenard. M, Ford. W. T, *J. Org. Chem*, **1983**, Vol. 48, No.3, pp. 326-332, Wittig reagents bound to cross linked polystyrene.

[58] Marsh. A, Carlisle. S. J, Smith. S. C, *Tetrahedron. Lett*, **2001**, Vol. 42, pp. 493-496, High-loading scavenger resins for combinatorial chemistry.

[59] Guino. M, Hii. K. K. (Mimi), *Tetrahedron. Lett*, **2005**, Vol. 46, No 40, pp. 6911-6913, Phosphine functionalised polymer resins as Pd scavengers

[60] Gayo. L. M, Suto. M. J, *Tetrahedron Lett.* **1997**, Vol 38, No. 4, pp. 513-516, Ion-exchange resins for solution phase parallel synthesis of chemical libraries.

[61] Gothelf K. V, Jorgensen K. A, *Chem. Rev.* **1998**, Vol. 98, pp. 863-909, Asymmetric 1,3-Dipolar Cycloaddition Reactions.

[62] Carratuthers W, *Cycloaddition Reactions in Organic Synthesis – a Tetrahedron Organic Chemistry series,* **1990,** Volume 8.

[63] Rastelli. A, Gandolfi. R, Amade. M. S, *J. Org. Chem*, **1998**, Vol 63, No 21, pp. 7425-7436, Ab initio study of the regiochemistry of 1,3-dipolar cycloadditions. Reactions of diazomethane and formonitrile oxides with ethane, propene, acrylonitrile and methyl vinyl ether.

[64] Volume 36, Organic reactions, published by John Wiley & sons, inc, Chapter 1, pp.9-13.

[65] Vullo. V, Danks. T. N, Wagner. G, *Eur. J. Org. Chem*, **2004**, pp. 2046-2052, Cycloaddition of benzonitrile oxide to acetonitrile, propyne and propene. A comparative theoretical study of the reaction mechanism and regioselectivity.

[66] Corsaro. A, Chiacchio. U, Pistara. V, rescifina. A, buemi. G, Romeo. G, *J. Chem. Soc., Perkin Trans. 2*, **2000**, pp. 1761-1766, Cycloadditions of mesitonitrile oxide with amino- and nitrostilbenes.

[67] Eicher T, Hauptmann S, *The Chemistry of Heterocycles*, second edition, Wiley-VCH.

[68] Medicinal chemistry dictionary,

[69] Kobayashi S, Akiyama R, *Tetrahedron Lett*, **1998**, Vol. 39, pp. 9211-9214, Lanthanide triflate-catalyzed 1,3-dipolar cycoladdition reactions of polymer-supported nitrones with alkenes for the preparation of diverse 2-isoxazoline derivatives.

[70] Soucy C, Lacoste J. E, Breau L, *Tetrahedron Lett*, **1998**, Vol. 39, pp. 9117-9120, Synthesis of 2-isoxazolines From Olefins Derived from Norephdrin And Pulegone.

---

[71] Kiegiel J, Poplawska M, Jozwik J, Kosior M, Jurczak J, *Tet. Lett*, **1999**, Vol. 40, pp. 5605-5608, New methods of *is situ* generation of nitrile oxides by MnO_2 oxidation of aldoxines.

[72] Matt C, Gissot A, Wagner A, Mioskowski C, *Tet. Lett*, **2000**, Vol. 41, pp. 1191-1194, Nitrolic acids: efficient precursors of nitrile oxides under neutral conditions.

[73] Berlin, DeGruyter, **1994**, *Concise Encyclopaedia Chemistry.* Translated and revised by Mary Eagleson.

[74] Jose. B, Antoci. V, Zeiger. A. R, Wickstrom. E, Hickok. N. J, *Chem. & Bio.* **2005**, Vol. 12, pp. 1041-1048, Vancomycin Covalently Bonded to Titanium Beads Kills Staphylococcus aureus.

[75] Woo. G. L. Y, Mittelman. M. W, Santerre. J. P, *Biomaterials*, **2000**, Vol. 21, pp. 1235-1246, Synthesis and Characterization of a Novel Biodegradable Antimicrobial Polymer.

[76] Woo. G. L. Y, Yang. M. L, Yin. H. Q, Jaffer. F, Mittelman. M. W, Santerre. J. P, *John Wiley and Sons, Inc*, **2001,** Biological characterization of a novel biodegradable antimicrobial polymer synthesized with fluoroquinolones.

[77] Tiller. J. C, Liao. C. J, Lewis. K, Kilbanov. A. M, *PNAS*, **2001**, Vol. 98, No. 11, pp. 5981-5985, may 22, Designing Surfaces that Kill Bacteria on Contact.

[78] Elliott. T. S. J, *Journal of Antimicrobial Chemotherapy*, **1999**, Vol. 43, pp. 441-446, Role of Antimicrobial central venous Catheters for the Prevention of Associated Infections.

[79] Mandeville, III et al, United state patent, Patent No.; US 6,395,777,B2

[80] Kenawy. E. R, Abdel-Hay. F. I, Shanshoury. A. E. R. E, Newehy. M. H. E, *Journal of Controlled release*, **1998**, Vol. 50, pp. 145-152, Biologically Active Polymers: Synthesis and Antimicrobial Activity of Modified Methacrylate Polymers having a Quaternary Ammonium and Phosphonium groups.

[81] Kenawy. E. R, Abdel-Hay. F. I, Magd. A. A. E, Mahmoud. Y, *Reactive and functional polymers*, **2006**, Vol. 66, pp. 419-429, Biologically Active Polymers: VII. Synthesis and Antimicrobial Activity of Some Crosslinked Copolymers with Quaternary Ammonium and Phosphonium groups.

[82] Park. D, Wang. J, Klibanov. A. M, *Biotechnol. Prog.* **2006,** Vol. 22, pp. 584-589, One-Step, Painting-Like Coating Procedure to Make Surfaces Highly and Permanently Bactericidal.

[83] Wilson. M, McNab. R, Henderson. B, *Bacterial Disease Mechanisms – an introduction to cellular microbiology*, Chapter 1.

[84] Evens. A. S, Brachman. P. S, *Bacterial Infections of Humans – epidemiology and control*, Second Edition, Chapter 36.

[85] A. Story MPH RGN, *The Pharmaceutical Journal*, **2004**, Vol. 273, No 7314, pp. 289–291, 28 Aug Tuberculosis- a general introduction.

[86] Grosso. A, Jani. Y, Minton. J, *The Pharmaceutical Journal*, **2004**, Vol 273, No 7317, pp 386–388, 18 Sep, Tuberculosis chemotherapy in the UK.

[87] Plotkin & Mortimer, *Vaccines*, second edition.

[88] Duncan. K, *Tuberculosis*, **2003**, Vol. 83, pp. 201-207, Progress in TB drug development and what is still needed.

[89] Duncan K. *Chemistry & Industry*, **1997,** pp. 861-865.

[90] World Health Organisation, TUBERCULOSIS report fact, **2006.**

[91] Hugo. W. G, Russell. A. D, *Pharmaceutical Microbiology*, sixth edition, Chapter 16, pp 332 – 333.

[92] Foye. W. O, Lemke. T. L, Williams. D. A, *principles of MEDICINAL CHEMISTRY*, fourth edition, chapter 33, pp 747.

[93] Chatterjee. D, *Current Opinion in Chemical Biology*, **1997**, Vol. 1, pp. 579–588, The mycobacterial cell wall: structure, biosynthesis and sites of drug action.

[94] Crick. D. C, Mahapatra. S, Brennan. P. J, *Glycobiology*, **2001**, Vol. 11, No. 9 pp. 107-118, Biosynthesis of the arabinogalactan-peptidoglycan complex of *Mycobacterium tuberculosis.*

[95] Yagi. T, Mahapatra. S, Mikusˇova. K, Crick. D. C, Brennan. P. J, *The journal of biological chemistry*, **2003,** Polymerization, Vol. 278, No. 29, pp. 26497–26504, July 18, of Mycobacterial Arabinogalactan and Ligation to Peptidoglycan.

[96] Lambert. P. A, mechanism of action of antibiotics and synthetic anti-infective agents, Chapter 12.

[97] Wilson. M, McNab. R, Henderson. B, *Bacterial Disease Mechanisms, an introduction to cellular microbiology*, **2002,** chapter 2.

[98] Singleton. P, Sainsbury. D, *Dictionary of microbiology and molecular biology*, 3rd Edition, Wiley.

[99] Yuan. Y, Barry. C. E, *Biochemistry*, **1996**, Vol. 93, pp. 12828–12833, A common mechanism for the biosynthesis of methoxy and cyclopropyl mycolic acids in Mycobacterium tuberculosis.

[100] Barry III, C.E. *et al*, US Patent **1997**, 5,610,198, Antimycobacterial composition and their use for the treatment of TB and related disease.

[101] Sacchetin J. *et al*, US Patent 1998, 5,837,732, Antimycobacterial compounds and methosa of using same.

[102] Sacchetin J. *et al*, US Patent 1997, 5,648,392, Antimycobacterial compounds and methosa of using same.

[103] Coxon. G. D, Dulayymi. J. R. Al, Morehouse. C, Brennan. P. J, Besra. G. S, Baird. M. S, Minnikin. D. E, *Chemistry and Physics of Lipids*, **2004**, Vol 127, pp. 35-46, (and references cited therein), Synthesis and properties of methyl 5-(1'R,2'S)-(2-octadecylcycloprop-1-yl)pentanoate and other ω-19 chiral cyclopropane fatty acids and esters related to mycobacterial mycolic acids.

[104] K. Johnsson, King. D. S, Schultz. P.G, , *J. Am. Chem, Soc*, **1995**, Vol. 117, pp. 5009-5010, Studies on the Mechanism of action of Isoniazid and Ethionamide in The Chemotherapy of Tuberculosis.

[105] Johnsson. K, Schultz. P.G, *J. Am. Chem. Soc,* **1994**, Vol. 116, pp. 7425–7426, Mechanistic studies of oxidation of isoniazid by the Catalase peroxidase from the *Mycobacterium tuberculosis.*

[106] Delgado. J. N, Remers. W. A, *TEXTBOOK of ORGANIC MEDICINAL and PHARMACEUTICAL CHEMISTRY,* Chapter 7, tenth edition.

[107] Zhao. X, Yu. H, Yu. S, Wang. F, Sacchettini. J. C, Magliozzo. R. S, *Biochemistry*, **2006**, Vol. 45, pp. 4131–4140, Hydrogen peroxide-Mediated Isoniazid Activation Catalyzed by Mycobacterium tuberculosis Catalase-Peroxidase (KatG) and its S315T mutant.

[108] Chen. B, Li. J.H, Xu. Y. M, Wang. J, Cao. X. M, *Clinica Chimica Acta*, **2006,** Vol. 365, pp. 104–108, The influence of NAT2 genotype on the plasma concentration of isoniazid and acetylisoniazid in Chinese pulmonary tuberculosis patients.

[109] Katzung. B.G*, Basic & Chlinical Pharmacology*, Chapter 47, eighth edition.

[110] Rang. H. P, Dale. M. M, Ritter. J. M, *Pharmacology*, Chapter 37, third edition.

[111] Häusler. H, Kawakami. R. P, Mlaker. E, Severn. W. B, Stütz. A. E, *Biooerg & Med. Chem. Let*, **2001**, Vol. 11, pp. 1679–1681, Ethambutol Analogues as Potential Antimycobacterial Agents.

[112] Zhang. Y, Wade. M. M, Scorpio A, Zhang. H, Sun. Z, *J. Antimicrobial Chemotherapy*, **2003,** Vol 52, pp. 790–795, Mode of action of pyrazinamide: disruption of Mycobacterium *tuberculosis* membrance transport and energetics by pyrazinoic acid.

[113] Chopra. I, Brennan. P, *Tubercle and Lung Disease*, **1998**, Vol. 78, No. 2, pp 89–98, Molecular action of antimycobacterial agents.

[114] Zhu. M, Namdar. R, Stambaugh. J. J, Starke. J. R, Bulpitt. A. E, Berning. S. E, Peloquin. C. A, *Tuberculosis*, **2002**, Vol. 82, issue (2/3), pp 91– 96, Population pharmacokinetics of ethionamide in patients with tuberculosis.

[115] Cheng. J. F, Mujalli. A. M. M, *Tetrahedron Letters*, **1998,** Vol 39, pp 939-942, Solid-phase synthesis of Δ^2-isoxazolines.

[116] Pie. Y, Moos. W. H, *Tetraherdon Letters*, **1994,** Vol. 32, No. 32, pp. 5825-5828, Post-Modification of peptoid side chains: [3+2] Cycloaddition of nitrile oxides with alkenes and alkynes on the solid-phase.

[117] Katorowski. E. J, Kurth. M. J, *Molecular diversity*, **1996,** Vol 2, pp. 207-216, Dipolar cycloadditions in solid-phase organic synthesis (SPOS).

[118] Grundmann. C, Datta. S. K, *The J. Org. Chem,* **1969,** Vol. 34, No 6, June, Nitrile oxides. XII. Cycloaliphatic and Aliphatic stable Nitrile Oxides.

[119] Miller. D. J, Scrowston. R. M, Kennewell. P. D, Westwood. R, *Tetrahedron*, **1992,** Vol 48, No. 36, pp- 7703-7712, 1,3-Dipolar cycloaddition reactions of nitrile oxides with 4,5-Dihydrooxazole and 4,5-Dihydrothiazole derivatives.

[120] Simpson. J, PhD Thesis, **2000**, Aston University, UK.

[121] Simpson J, Forrester R, Tisdale M. J, Billington, D.C, Rathbone D.L, *Bioorg. Med. Chem. Lett*, **2003**, Vol. 13, pp. 2435-2439, Effects of catechol Derivatives on Cell Growth and Lipoxygnase Activity.

[122] Maki. D. G, Weise C. E, Sarafin H. M, *New England Journal of medicine,* **1977**, Vol. 296, pp. 1305-1309, A semiquantitative culture method for identifying intravenous catheter related infections.

[123] Sherertz. R. J, Heard. S. O, Raad. I. I, *Journal of clinical microbiology*, **1997**, Vol. 35, No. 3, pp. 641-646, Diagnosis of triple-lumen catheter infection: Comparasion of roll plate, sonication and flushing methodologies.

[124] Lamber. P, unpublished work, Aston university, England.

[125] Woo. G. L.Y, Mittelman. M. W, Santerre. J. P, *Biomaterials*, **2000**, Vol. 21, pp. 1235–1246. Synthesis and Characterization of a novel biodegradable antimicrobial polymer.

---

[126] Woo. G. L.Y, Yang. M. L, Yin. H. Q, Jaffer. F, Mittelman. M. W, Santerre. J. P, pp 35 – 45, Biological characterization of a novel biodegradable antimicrobial polymer synthesized with fluoroquinolones.

[127] Shiino. M, Watanabe. Y, Umezawa. K, *Bioorg Chem*, EN (31), 2, **2003**, pp 129-135, Synthesis and tyrosinase inhibitory activity of novel N-hydroxybenzyl-N-nitrosohydroxylamines.

[128] Singh. A., Gupta. V.D, Srivastava. G, Mehrotra. R.C, *J. Org met. Chem*, Vol 64 (**1974**), pp 145-169, O-Organometal hydroxylamines and oximes.

[129] Sarvari. H, Mona, *Synthesis*, EN (5), **2005**, pp 787-790, ZnO/CH_3COCl: A New and Highly Efficient Catalyst for Dehydration of Aldoximes into Nitriles Under Solvent-Free Condition.

[130] Deninno. M. P, Richard. J. P, Howard. E, Stanley. DiDomenico, *J. Org. Chem*, **1992**, 57, 7115-7118, The enantioselective synthesis of the potent dopamine D1 agonist (1R,3S)-3-(1'-adamanty.l)-1-(aminomethyl)-3,4-dihydro-5,6-dihydroxy-1H-2-benzopyran.

[131] Woo. G. L.Y, Mittelman. M. W, Santerre. J. P, *Biomaterials*, **2000**, Vol. 21, pp. 1235–1246, Synthesis and Characterization of a novel biodegradable antimicrobial polymer.

[132] Woo. G. L.Y, Yang. M. L, Yin. H. Q, Jaffer. F, Mittelman. M. W, Santerre. J. P, pp 35 – 45, Biological characterization of a novel biodegradable antimicrobial polymer synthesized with fluoroquinolones.