STUDY OF THE PHYSICO-CHEMICAL PROPERTIES OF MACROMOLECULAR ASSEMBLIES

by

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(Under the Direction of Geert-Jan Boons)

ABSTRACT

Block copolymers are a diverse group of macromolecules that have attracted a great deal of attention due to their ability to self-assemble into a variety of nanostructures. These nanostructures can be tuned over a wide range of morphologies and hence can be used in a plethora of applications ranging from delivery platforms for drugs and genes to nanosized reactors. Block copolymers, primarily used for drug delivery applications, consist of at least one hydrophobic and one hydrophilic polymer chain that are covalently linked to one another. There are many factors that play a role in determining the morphology of the self-assembled molecule for example, the nature and length of the polar hydrophilic group, the length of the hydrophobic polymer, temperature and the type of common solvent employed during the self-assembly.

The first project involved the synthesis and self-assembly of micelles using amphiphilic blocks copolymers like polyethylene glycol (PEG) and polycaprolactone (PCL) with different PEG and PCL lengths. These polymers showed good correlation between the molecular weights obtained from gel permeation chromatography (GPC) and nuclear magnetic resonance (NMR). We next demonstrated that by varying the individual components of the block copolymers, we could systematically change the micelles' physico-chemical property like size. The study of the

corresponding micelles showed that there was a slight increase in size with the increase of hydrophobic, PCL length, while a significant increase in the size was observed with the decrease of hydrophilic - PEG length. A single study on a selected PEG-PCL polymeric micelle using atomic force microscopy (AFM) analysis and click chemistry revealed the force required to destabilize the micelles by pulling the single polymer chain is 47pN.

The next study involved replacing the hydrophilic PEG with a sugar moiety and conducting similar study on the relationship of the block length and molecular weight on the morphology of the micelles. The synthesis of the polysaccharide based block copolymers was done by employing the copper-(I)-catalyzed azide-alkyne cycloaddition. It was observed that when lactose was used as the hydrophilic block, the micelles obtained were unstable. Synthesis of higher sugar analogues was proposed using Lewis acid mediated acetolysis of β-cyclodextrin. However, attempts to further modify the polysaccharide were futile and the synthesis of the higher analogues is still underway.

INDEX WORDS: Polyethylene glycol, Polycaprolactone, Micelle, Click reaction

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DEDICATION

I would like to dedicate this thesis to my parents Rajeev Sardar and Vandana Sardar for their unceasing love, support and encouragement.

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LIST OF ABBREVIATIONS

AFM ..... Atomic Force Microscopy

AgOTf ..... Silver trifluoromethane sulfonate

ATRP ..... Atom Transfer Radical Polymerization

BAIB ..... Bis (acetoxy) iodobenzene

BCl_3 ..... Boron trichloride

n-BuA ..... n-Butyl acrylate

CaH_2 ..... Calcium hydride

$CHCl_3$ ..... Chloroform

CMC ..... Critical Micelle Concentration

CuAAC ..... Copper-(I)-assisted azide alkyne cycloaddition

Cu(I)Br ..... Copper-(I)-bromide

Cu(I)I ..... Copper-(I)-Iodide

DBN ..... Di-*tert*-butyl-*N*-oxide

DCC ..... *N, N'*-Dicyclohexyl carbodiimide

DCM ..... Dichloromethane

DIBO ..... Dibenzylcyclooctynol

DLS ..... Dynamic Light Scattering

DMAc ..... Dimethylacetamide

DMF ..... Dimethyl formamide

EDC.HCl .....*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride

EtOAc .....Ethyl acetate

FT-IR .....Fourier transform infrared spectroscopy

GPC .....Gel permeation chromatography

GTP .....Group transfer polymerization

^{1}H-NMR..... Proton nuclear magnetic resonance

HBr. AcOH ..... Hydrobromic acid solution in acetic acid

HCl .....Hydrochloric acid

HG ..... Haplogroup

IB .....Isobutylene

LA ..... (±)-α-Lipoic acid

MALDI-TOF..... Matrix assisted laser desorption/ionization-time of flight

MeOH ..... Methanol

α-MeSt .....α-Methyl styrene

$MgSO_4$.....Magnesium sulfate

M_n ..... Number average molecular weight

M_w ..... Weight average molecular weight

MW ..... Molecular weight

$NaHCO_3$ ..... Sodium bicarbonate

NaOMe..... Sodium methoxide

NaN_3 .....Sodium azide

NHS .....*N*-hydroxy succiniimide

PAGlc .....Poly-glucosylacrylate

PCL .....Polycaprolactone

PDI .....Polydispersity index

PEG .....Polyethylene glycol

PGAs ..... Poly (glutamic acid) s

PGA ..... Polyglycolide

PHEA .....Poly (hydroxyethyl-L-asparagine)

PHEG .....Poly (hydroxyethyl-L-glutamine)

PICM .....Polyion complex micelle

PLA .....Poly-D, L-lactide

PLGA .....Poly (lactic-*co*-glycolic acid)

PMDETA .....*N, N, N', N", N"*-Pentamethyldiethylenetriamine

PαMeSt-PIB..... Poly-α-methylstyrene-polyisobutylene

PPh_3 ..... Triphenyl phosphine

PVP .....Poly (*N*-vinyl-2-pyrrolidine)

RAFT .....Reversible addition-fragmentation chain transfer

RES .....Reticuloendothelial system

RI .....Refractive index

ROMP .....Ring opening metathesis polymerization

ROP .....Ring opening polymerization

siRNA ..... Small interfering Ribonucleic acid

SLS .....Static Light Scattering

$Sn(Oct)_2$.....Stannous (II) octoate

SPAAC..... Strain promoted azide-alkyne cycloaddition

$TASHF_2$ ..... Tris (dimethylamino) sulfonium bifluoride

t-BMA ..... *tert*-Butyl methacrylate

TEM ..... Transmission electron microscopy

TEMPO ..... 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl

THF ..... Tetrahydrofuran

$TiCl_4$ ..... Titanium tetrachloride

TMS-HEA ..... 2-Trimethyl silyloxyethylacrylate

TNF-α ..... Tumor necrosis factor-α

TsCl ..... 4-Toluenesulfonyl chloride

UV-Vis ..... Ultraviolet-Visible spectroscopy

VEGF ..... Vascular endothelial growth factor

CHAPTER 1

INTRODUCTION AND LITERATURE OVERVIEW

Block copolymers are a fascinating group of polymeric materials belonging to a big family known as "soft materials."[1] This class of polymers is made by the covalent bonding of two or more polymeric chains that, in most cases, are chosen in such a way that they give rise to a rich variety of thermodynamically incompatible micro and nanostructures.[1] The physical and chemical characteristics of the constituent blocks play an important role in determining the morphology of the macromolecular structures.[2] With these remarkable materials, the molecular engineer can combine distinct polymers to give materials with defined physical properties. For example, a composite comprising glassy or crystalline domain in a rubbery matrix can be self-assembled by taking components with these characteristics and combining them in a block copolymer.[1] Block copolymers are widely used industrially. In the solid and rubbery states, they are used as thermoplastic elastomers. In solution, their surfactant properties are exploited in foams, oil additives, solubilizers, thickeners and dispersion agents to name a few. Recently, the use of block copolymer based micellar drug delivery systems to improve the therapeutic efficiency of many drugs has gained considerable attention.[3]

TYPES OF BLOCK COPOLYMERS

The architecture of block copolymers can be controlled by the method of synthesis and it is possible to prepare diblock, triblock, and starblock copolymers as shown in the Figure 1.1. The possibilities for molecular design are almost limitless and recently some complex architectures have also been synthesized as shown in Figure 1.1.[2]

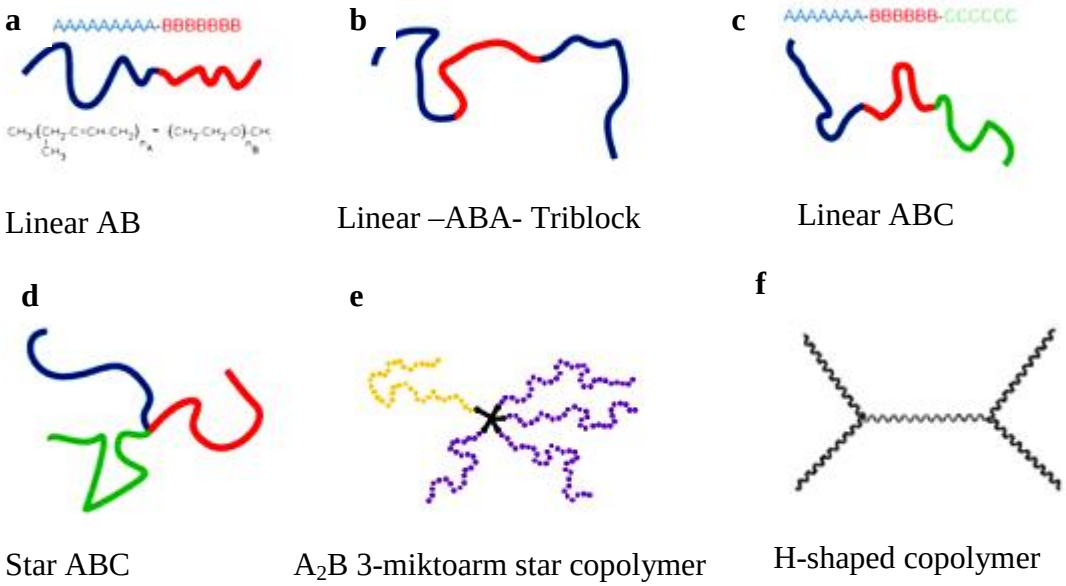


Figure 1.1: Block copolymer architectures (a) Linear AB diblock copolymer. (b) Linear ABA triblock copolymer. (c) Linear ABC triblock copolymer. (d) Star ABC triblock copolymer. (e)A_2B 3-miktoarm star copolymer, (f) H-shaped copolymer

BLOCK COPOLYMER SYNTHESIS

Polymerizations are multi-step synthetic processes involving the following steps:

- Initiation or oligomerization step
- Propagation step that involves successive addition of more monomers/ oligomers to these initial activated species.
- Termination step that terminates the chain growth to finally yield a stable polymeric product.

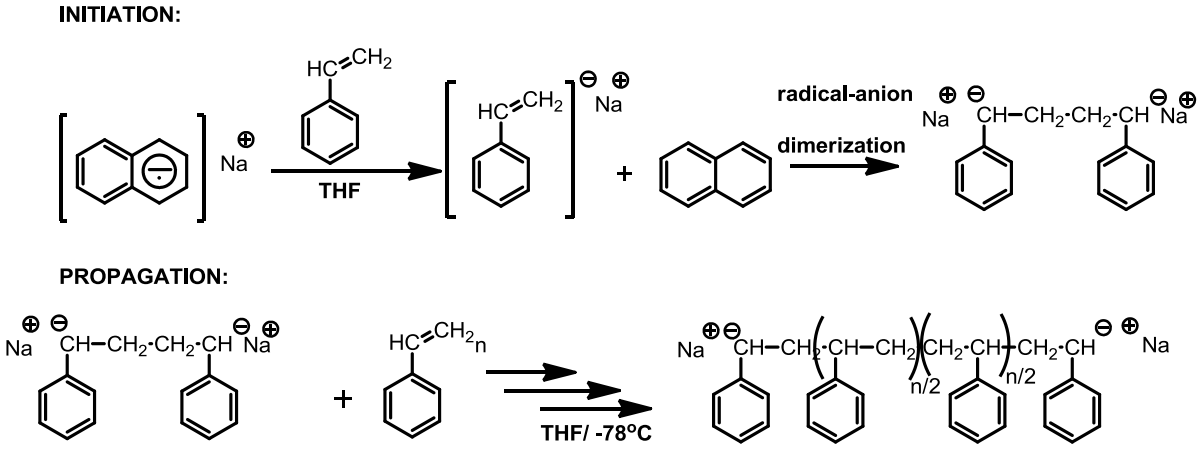
Most of the traditional polymer syntheses are chaotic processes in which all stages occur concurrently and growing chains can merge with or branch off from one another.[4] This might lead to formation of several side products, thereby reducing the yield of the overall reaction. Recently, several polymerization systems have been developed that offer much better control over the polymerization reaction.[1] For example, in living radical polymerization, each molecule

of the catalyst promotes rapid initiation and then stabilizes the growing chain to prevent branching or termination. Therefore, different types of monomer can be added to the reaction consecutively, leading to polymers with well-defined blocks that vary in structure and function.[1]Block copolymers can be synthesized via anionic, cationic, group transfer, radical and ring opening metathesis and ring opening polymerization. In the following section, each method will be briefly described along with its advantages and disadvantages. The application of the polymerization method for the synthesis of block copolymers will be discussed using examples from literature.

ANIONIC LIVING POLYMERIZATION

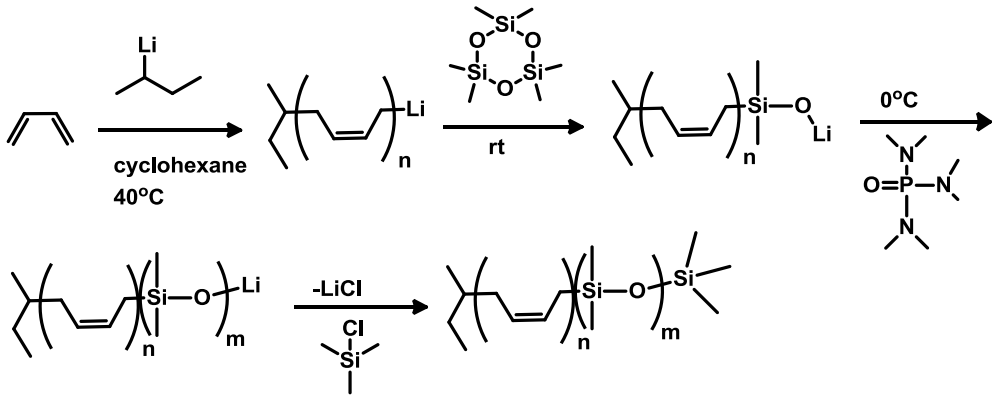
Anionic living polymerization has been known for almost sixty years. The concept of anionic polymerization was first developed by Ziegler and Schlenk in the early 1900s.[5,6] Their pioneering work on the polymerization of diene initiated with sodium metal set the stage for the use of alkali metal containing aromatic hydrocarbon complexes as initiators for various α-olefins. In 1956, Michael Szwarc[5,6] demonstrated the mechanism of anionic polymerization of styrene which drew significant and unprecedented attention to the field of anionic polymerization of vinyl monomers. Szwarc used sodium naphthalenide as an initiator for the polymerization of styrene in tetrahydrofuran (THF) **(**Scheme 1.1**)**. He characterized this polymerization technique as *living polymerization* and called the polymers as *living polymers.* Here, the term *living* refers to the ability of chain ends of these polymers retaining their reactivity for a sufficient time enabling continued propagation without termination and transfer reactions.[7] Since its discovery in the 1950s, it has emerged as the most powerful synthetic tool for the preparation of well-defined polymers, that is polymers having narrow molecular weight distribution and controlled molecular characteristics including molecular weight, composition, microstructure and

architecture. Its ability to form well-defined macromolecules stems from the absence of termination and chain transfer reactions under appropriate conditions.



Scheme 1.1: Anionic polymerization of styrene using sodium naphthalene[5,6] as an initiator in THF.

Anionic polymerization proceeds via organometallic sites, carbanions (or oxyanions) with metallic counterions.[1] Carbanions are nucleophiles and as a result, the monomers that can be polymerized by anionic polymerization are those bearing an electron attractive substituent on the polymerizable double bond. Like addition polymerization, it takes place in three steps: chain initiation, chain propagation and chain termination. For example, the anionic polymerization of cyclosiloxanes whereby the cyclosiloxanes have been used as second monomers in diblock copolymers' synthesis (Scheme 1.2). In the example shown in Scheme 1.2**,** butadiene was the first monomer to be polymerized. Initiators containing lithium as the counterion can be used in this case. Well-defined block copolymers can be synthesized if sufficient care is exercised in the purification of the siloxane monomer and in minimizing any reactions involving the Si-OLi active centers and the Si-O-Si bonds already formed by maintaining low polymerization temperatures and conversions below 80%.[8]



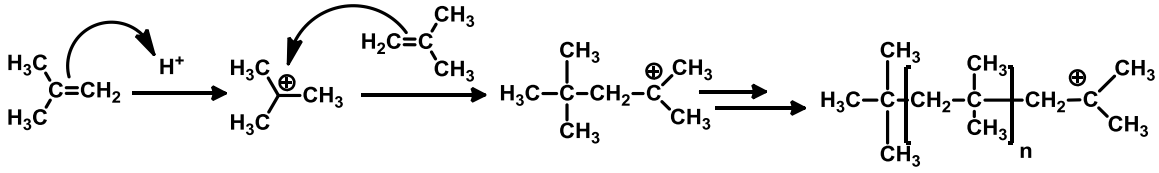
Scheme 1.2: Synthesis of a polybutadiene-polydimethylsiloxane diblock copolymer using anionic polymerization.

One of the remarkable features of living anionic polymerization is that the mechanism involves no formal termination step. In the absence of impurities, the carbanion would still be active and capable of adding another monomer. The chains will remain active indefinitely unless there is inadvertent or deliberate chain transfer or termination step. One great advantage of anionic polymerization is that it allows for a very straightforward synthesis of block copolymers. Since monomer batches are completely consumed, simple addition of co-monomer will ensure the synthesis of well-defined block copolymer. However, the sequential monomer addition technique has its own setbacks. One has to take into account the different reactivities of the monomers. Therefore, the sequence of addition of monomers is of great importance. Furthermore, the least stable anions are the strongest nucleophiles and may attack ester groups. For example, a block copolymerization starting with *tert*-butyl methacrylate (*t*BMA) followed by styrene polymerization will fail primarily because the cross over step will not be controlled. This is because the methacrylate anion is more stable than the styryl anion. Secondly the polystyryl anions will attack the ester function of *t*BMA, resulting in branching and termination. The main disadvantages of anionic polymerization are the limited choice of monomers and the extremely demanding reaction conditions.

CATIONIC POLYMERIZATION

Advances in cationic polymerization methodology, starting in the middle 1980s with the discovery of the true living cationic polymerization of vinyl ethers by Higashimura et al., have shown their real potential for the synthesis of tailor-made macromolecules.[9,10] In recent years, many investigations have demonstrated that almost all classes of cationically polymerizable vinyl and alkene-type monomers can be polymerized in a controllable way.[11-13] The formation of polymers having predictable molecular weight and narrow molecular weight distribution gives unambiguous experimental evidence for elimination or suppression of termination and chain transfer reactions in these systems. These studies opened the way for block copolymer synthesis using cationically polymerizable monomers, extending the range of block copolymers available for basic research and for possible technological applications. Many important monomers like isobutylene and alkyl vinyl ether can be polymerized only by cationic polymerization.

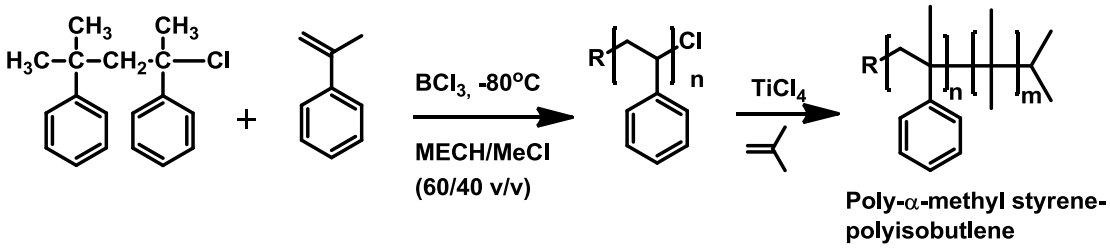
Cationic polymerization can be described as a polymerization reaction where chain propagation is achieved through a carbocation, which can be generated by a cationic initiator and a vinyl monomer (Scheme 1.3).[13,14]



Scheme 1.3: Mechanism of cationic polymerization of isobutylene[15,16]

Carbocations, in general are very reactive and unstable; consequently, they can participate in a number of side reactions like termination, chain transfer, and carbocation rearrangement. The major side reaction is chain transfer to monomer. Since the positive charge is present on the α-carbon of the double bond, the hydrogen atom on the β-carbon is acidic. The monomers used in cationic polymerization are nucleophilic; therefore, this kind of side reaction is intrinsic to most

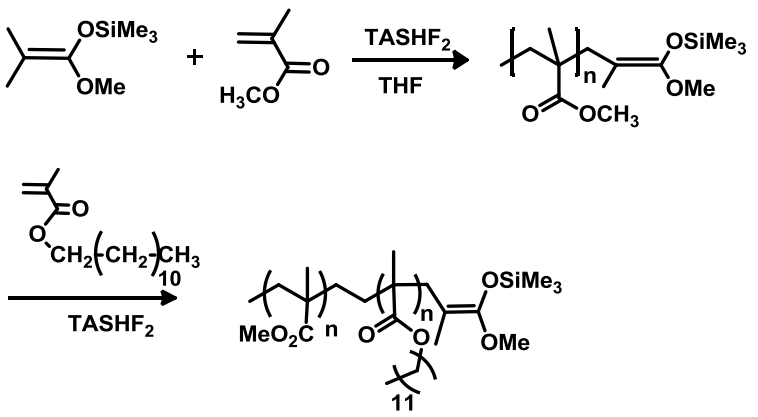
systems and difficult to eliminate. However, several methods have been proposed in order to overcome this difficulty. The most successful strategy is the stabilization of the carbocationic intermediate using either an appropriate counterion or carefully selecting a Lewis base. Both methods aim to decrease the positive charge on the α-C and as a result, decrease the β-H acidity.[17] For example; the pair α-methylstyrene (α-MeSt) and isobutylene (IB) have different reactivities. α-MeSt is more reactive than IB and therefore the synthesis of a polyαmethylstyrene-polyisobutylene (PαMeS-PIB) block copolymer must begin by the formation of the α-MeSt block. As a consequence, a different Lewis acid must be used for the polymerization of each monomer. Thus, α-MeSt is polymerized in the presence of a weaker Lewis acid like boron trichloride (BCl_3). This Lewis acid is inefficient to promote the living polymerization of IB. Thus secondary reactions can be avoided. After the formation of the α-MeSt block, the second monomer IB can be polymerized with the aid of a stronger Lewis acid for example $TiCL_4$ and thus a well-defined block copolymer can be formed (Scheme 1.4).Careful selection of a Lewis acid is a limiting parameter for making block copolymers via cationic polymerization in order to avoid side reactions or controlled polymerizations.



Scheme 1.4: Synthesis of poly-alpha-methylstyrene-block-polyisobutylene using a Lewis acid catalyst[18,19]

GROUP TRANSFER POLYMERIZATION

Group transfer polymerization (GTP) is a Michael-type catalyzed addition reaction. A silyl ketene acetal is generally used as an initiator.[20-22] The silane group is transferred to the growing chain end after the addition of each monomer unit (Scheme 1.5). Thus the chain end remains active until the complete consumption of the monomer. Due to the living nature of the polymerization reaction, the molecular weight of the polymer being synthesized can be predetermined by the amount of the initiator and the monomer used. This type of polymerization has been widely used for the polymerization of methacrylic monomers at room temperature, in the presence of wide variety of side groups, which are sensitive to ionic or radical polymerization reactions.[23] GTP is considerably tolerant to functionalities such as tertiary amines, epoxides, styrenic and allylic groups. The use of anionic or Lewis acid catalysts in addition to the initiator is advantageous to the progress of the polymerization reaction because these catalysts coordinate with the silicon atom (anionic catalysts) or the monomer (Lewis acids) facilitating group transfer. GTP is known to have several advantages for example; this type of polymerization can be carried out at room temperature and is air stable. It can be employed to synthesize telechelic polymers. However, it suffers from certain setbacks such as this type of polymerization is useful mainly for acrylate and methacrylate monomers. GTP requires water free conditions and hence use of dry reagents is necessary to ensure complete conversion.[1]



Scheme 1.5: Example of group transfer polymerization using silyl ketene acetal as the initiator and tris(dimethylamino) sulfonium bifluoride ($TASHF_2$) as the catalyst to synthesize block copolymers of methacrylates.[24]

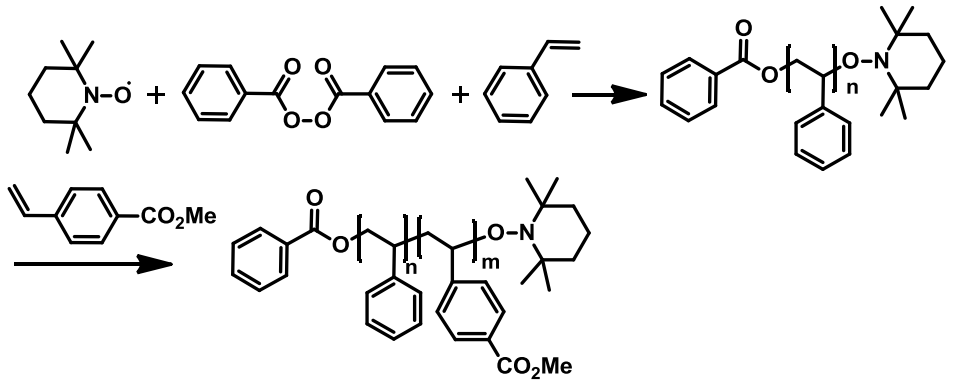
LIVING FREE RADICAL POLYMERIZATION

Free radical polymerization is the oldest type of polymerization of vinyl monomers.[25] This type of polymerization is widely used for the industrial preparation of a large number of polymeric materials. A wide range of monomers can be polymerized and copolymerized by free radical polymerization, under less rigorous experimental conditions compared to ionic polymerizations. Free radical polymerization processes are tolerant to protic and aqueous solvent media and certain functional groups. However the disadvantage of the free radical mechanism is that the polymerization leads to polydisperse polymers with little control over their molecular characteristics due to radical-radical combination thereby leading to termination and chain transfer reactions.

Recent advances in free radical polymerization have led to the development of synthetic methods for eliminating or suppressing the undesired termination and chain transfer reactions.[26] The three most important methods involve the use of stable free radicals such as nitroxide, as reversible terminating agents to control the polymerization method[27] and the use of transition metals

complexes which through a reversible catalytic action that involves atom transfer, stabilize the radical intermediates.[28] Another procedure includes the reversible addition- fragmentation whereby a chain transfer agent in the form of a thiocarbonylthio compound is used to afford control over the molecular weight and polydispersity. The exchange reactions in this system are very fast which lead to well-controlled systems.

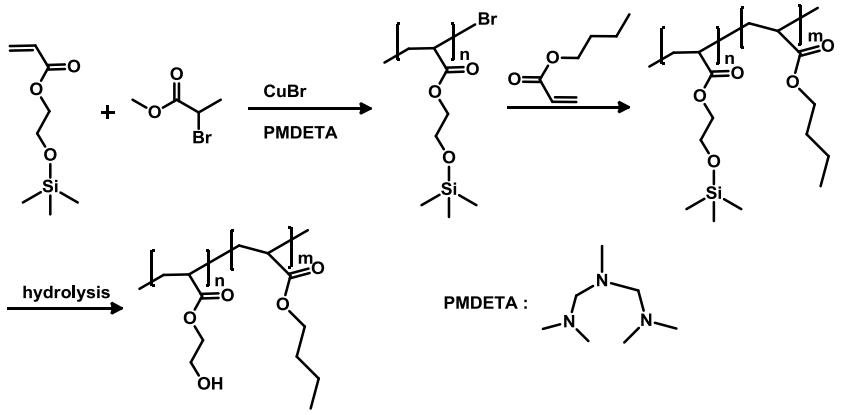
In nitroxide-mediated controlled free radical polymerization, covalent bond formation leads to deactivation of the growing chain.[29] The C-ON bond is stable at low temperatures, whereas at higher temperatures, homolytic cleavage of the bond is possible giving rise to the formation of the nitroxide radical. The macroradical can then grow through the addition of new monomer units. This cycle can be performed several times until the monomer is completely consumed. This ensures that the concentration of free radicals remains very low, decreasing the possibility for termination reactions. Nitroxide- mediated free radical polymerization has found success in the polymerization of mainly styrenic monomers, although the polymerization of dienic and acrylic monomers has also been reported.[30,31] For the synthesis of poly[(4-acetoxystyrene)-b-styrene] copolymers (Scheme 1.6), styrene was polymerized first using benzoylperoxide as initiator, 2, 2, 6, 6- tetramethylpiperidinoxy (TEMPO) as the nitroxide stabilizer and camphorsulphonic acid as the accelerator at 130^{o}C.The TEMPO end-capped polystyrene was used as a macromolecular initiator for the subsequent polymerization of 4-acetoxystyrene, resulting in well-defined block copolymer.



Scheme 1.6: Example of living free radical polymerization using benzoylperoxide as initiator and TEMPO as the nitroxide stabilizer.[32,33]

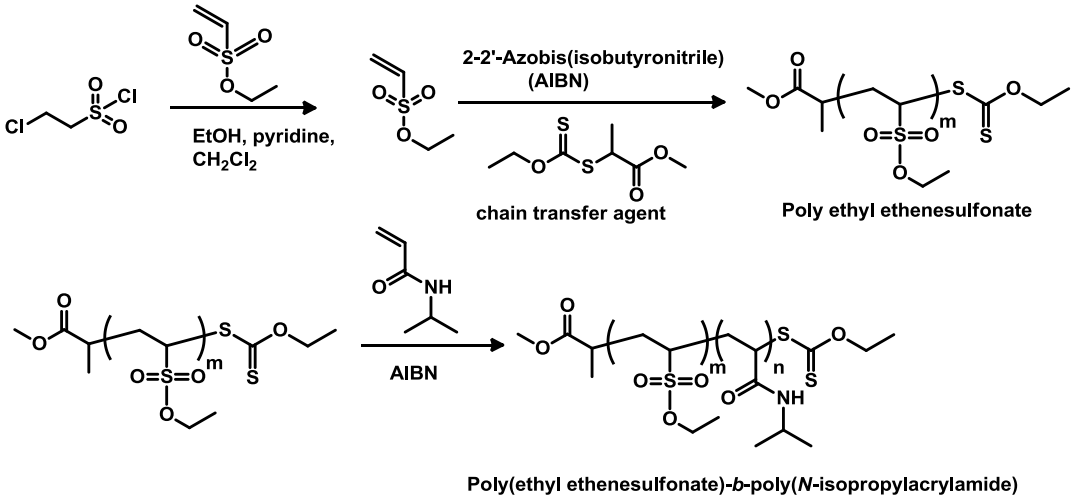
In the case of atom transfer free radical polymerization (ATRP), a transition metal is used as halogen atom carrier in a reversible redox reaction.[27] As the name implies, the atom transfer step is the main step in the reaction responsible for uniform chain growth. Transition metal atoms are usually complexed with an appropriate ligand that acts in various ways during the reaction. ATRP is among the most effective and widely used methods of controlled radical polymerization. This can be seen in the synthesis of 2-trimethylsilyloxyethylacrylate (TMS-HEA) and n-butyl acrylate (n-BuA) block copolymers using the CuBr/ N, N, N', N", N'-pentamethyldiethylenetriamine (PMDETA) and methyl 2-bromopropionate as initiator. Earlier when n-BuA was polymerized, polymers obtained had rather broad molecular weight distributions owing to the slow deactivation of the growing chains. Better control of the characteristics of the final polymer was observed when TMS-HEA was used as the first monomer, probably due to a more favorable ratio of cross-initiation and propagation mechanisms.The copolymers, thus obtained had predefined molecular weights and low polydispersities (Scheme 1.7). The ATRP allows scientists to easily form polymers in a controlled fashion thereby allowing creating a wide range of polymers with site specific tailored functionalities targeting specific properties for high value applications. For example, polymers

created using ATRP have been used for coatings and adhesives, and are currently under investigation for use in the medical and environmental fields.[34-38]



Scheme 1.7: Synthesis of block copolymers of 2-trimethylsilyloxyethylacrylate and n-butylacrylate using CuBr/ PMDETA and methyl 2-bromoproprionate as initiator.[39]

Another form of controlled radical polymerization is the reversible addition-fragmentation chain-transfer polymerization (RAFT) which makes use of a chain transfer agent in the form of a thiocarbonylthio compound to afford control over the molecular weight and polydispersity during a free-radical polymerization. As with other controlled radical polymerization techniques, RAFT polymerizations can be performed under conditions to favor low polydispersity and a pre-chosen molecular weight distribution. It is another popular method for designing polymers having complex architectures, such as linear block copolymers, comb-like, star and brush polymers.[40-42] Polymerization of vinyl sulfonate esters to give well-defined block copolymers having thermoresponsive property was possible via RAFT polymerization of ethyl ethane sulfonate to form poly ethyl ethanesulfonate by using a xanthate-type chain transfer agent. The RAFT polymerization was found to proceed in a controlled fashion under suitable conditions as was confirmed by the formation of narrow polydispersity products (Scheme 1.8).

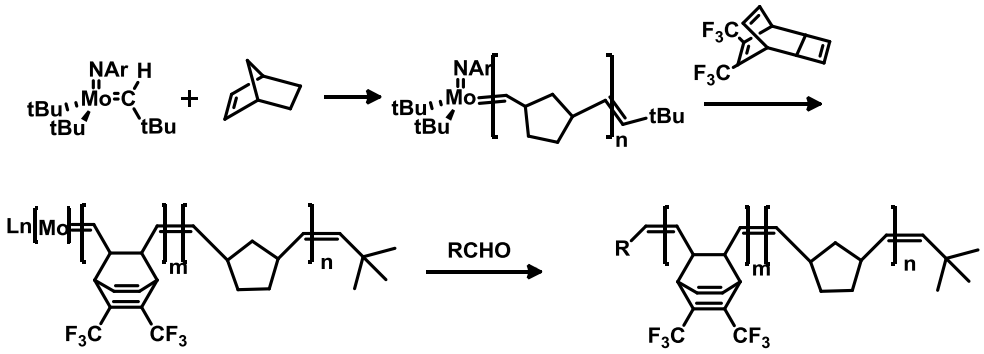


Scheme 1.8: Synthesis of block copolymers of ethyl ethenesulfonate and *N*-isopropylacrylamide using RAFT.[43]

RING OPENING METATHESIS POLYMERIZATION

Ring opening metathesis polymerization (ROMP) has emerged in recent years as a valuable tool for the polymerization of a wide variety of strained cyclic alkene monomers.[44] ROMP is a transition metal mediated polymerization technique and has been shown to proceed in a living manner if the transition metal initiator, coinitiator and other experimental conditions are properly chosen. The driving force for the reaction is the relief of ring strain of cyclic olefins. The wide utility of ROMP has led to the discovery of a variety of catalysts. A characteristic example is the polymerization of norborene with titanacyclobutane complexes.[45,46] The metalcyclobutane is in equilibrium with its ring opened carbene (Scheme 1.9) which is the actual polymerizing form of the initiator. Propagation proceeds in the absence of any deleterious side reactions until the monomer is completely consumed. Polymerization can be terminated by adding a ketone or aldehyde in order to deactivate the metal site. In terms of homogeneous catalysts, most tungsten and molybdenum catalysts[47-49] have rapid initiation rates and can produce "living" polymerizations with excellent control of polydispersity and chain tacticity, but the low

functional group tolerance limits the usable monomers. Ruthenium metathesis catalysts (Grubbs catalysts) tend to have lower initiation rates, often leading to higher polydispersities, but their air stability and greater tolerance for functional groups makes them user friendly and enables the use of wide range of functional monomers and additives.

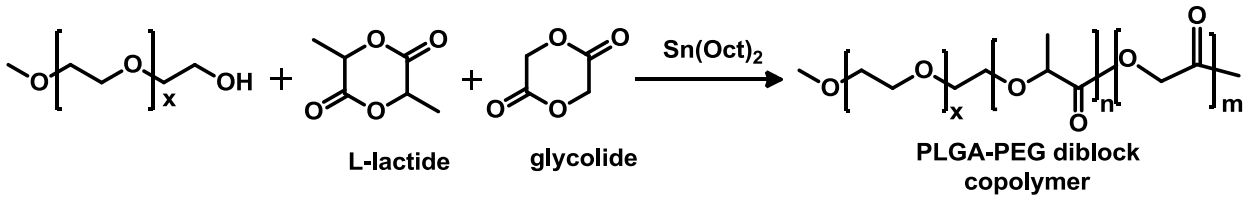


Scheme 1.9: Synthesis of polynorbornene-poly [7, 8-bis (trifluoromethyl)tricyclo-[4.2.2.0] deca-3, 7, 9-triene] block copolymers using ROMP.[50]

RING OPENING POLYMERIZATION

Ring opening polymerization (ROP) is a form of chain-growth polymerization in which the terminal end of the polymer acts as a reactive center, where further cyclic monomers join to form a larger polymer chain through ionic propagation. A wide variety of aliphatic cyclic monomers have been successfully polymerized by the ring opening polymerization. This includes cyclic lactones (esters), amines, sulfides, olefins, cyclotriphosphazenes etc. Poly (lactic-co-glycolic acid) (PLGA) can be synthesized by means of ring-opening copolymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. During polymerization, successive monomers of glycolic or lactic acid are linked together in PLGA by ester linkages, thus yielding linear, aliphatic polyester as the product (Scheme 1.10). The polymerizability of a cyclic monomer depends on both kinetic and thermodynamic factors. Kinetically, polymerization needs a mechanism through which a ring can open and undergo

reaction. Presence of a heteroatom provides a site for nucleophilic or electrophilic attack by an initiator species, resulting in initiation and subsequent propagation by ring opening. The most important factor that is often encountered, however, is the thermodynamic factors, which are the relative stabilities of the cyclic monomer. Small rings, such as 3- and 4- membered rings, are highly strained, and accordingly have a large exothermic energy associated with the ring opening.[51]



Scheme 1.10: Synthesis of poly (lactic-co-glycolic acid)-polyethylene glycol diblock copolymer using ring opening polymerization.[51]

The development of reproducible and effective drug delivery systems requires the fine tailoring of the properties of the used synthetic polymers. Aliphatic polyesters were the most useful and commonly used polymers for such applications. As far as the aliphatic polyesters are concerned, the control of their biodegradability, hydrophilicity and crystallinity are of utmost importance and relies on the availability of suitable synthetic procedures. Aliphatic polyesters such as poly-ε-caprolactones (PCL), polylactides and polyglycolides can be prepared by two distinct mechanisms:

(i) Step-growth polymerization or condensation

(ii) Ring opening polymerization (chain polymerization)

The major drawbacks of the step-growth polymerization are the required high temperatures and long reaction times thereby favoring side reactions, together with the detrimental effect on the

molecular weight. Water must also be removed from the polymerization medium to increase the conversion and molecular weight.[53]

The ring opening polymerization of lactides and lactones is free of these limitations and is therefore preferred as the synthetic route for the making polymers with tailor-made properties. High molecular weight polyesters can be easily prepared under mild conditions from lactones of different ring sizes, substituted or unsubstituted.[54,55] A broad range of cationic, anionic and coordination catalysts have been reported for ring opening polymerization. Generally speaking, ionic initiators are much reactive, and in case of polyesters, are responsible for detrimental inter- and intra-molecular transesterifications lowering the molecular weight and broadening the molecular weight distribution of the polymer. Many organometallic derivatives of the metals such as Al, Sn, Zr, Ca, are imparting control to the polymerization in contrast to their anionic counterparts. As a result, ring opening polymerization of lactones and lactides is a living controlled process that leads to the formation of polyesters with narrow molecular weight distribution with molecular weights that are predetermined by the monomer-to-initiator molar ratio.[56,57]Currently, tin octoate and tin/aluminum alkoxides are the most widely used organometallic initiators for the ring opening polymerization of lactones. Therefore, in the current thesis, for the synthesis of polycaprolactone polymers, ring opening polymerization mediated by tin (II) octoate will be used as this is the best known method for the synthesis of polyesters.

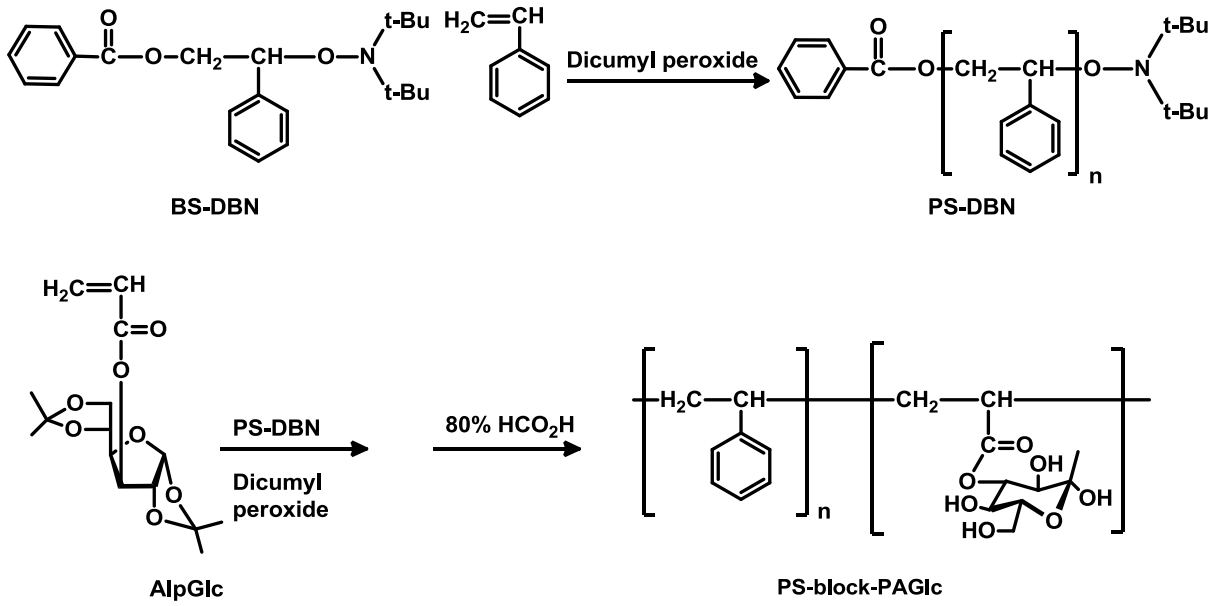
SYNTHESIS OF GLYCOPOLYMERS

Synthetic carbohydrate containing macromolecules or glycopolymers have attracted increasing attention owing to important roles played by carbohydrates in biological systems.[58] With an ability to code biological information, carbohydrates are an essential part of every mammalian cell in the form of polysaccharides, glycoproteins or glycolipids. Carbohydrates play an

important role in the cell-cell recognition events which are vital to a variety of biological processes.[58] Therefore, carbohydrate based polymers are emerging as an important well-defined tool for studying the biological roles of sugars as well as advanced materials for the investigation on multivalent interactions aiming at biomedical, pharmaceutical and medical applications. The synthesis of glycopolymers gained popularity in the early 1990s and most of the attention was focused on the polymerization of monomers containing carbohydrate moieties.[60]

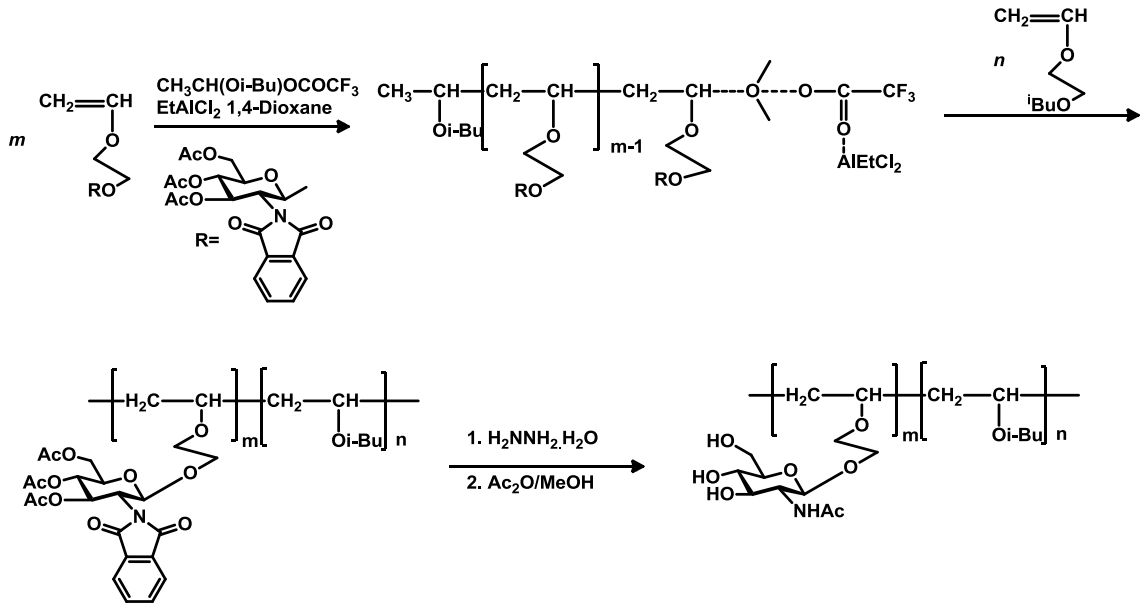
Synthesis of glycopolymers using vinyl polymerization has been the popular route for synthesis using sugar containing vinyl monomers.[60] However, it suffers from some disadvantages, for example the molecular weights and molecular weight distribution were not sufficiently controlled until living polymerization was successfully applied to glycopolymer synthesis.[60]

Radical Polymerization: Use of controlled living radical polymerizations such as nitroxide controlled polymerization[27b] and atom transfer radical polymerization[34] have paved the way for the synthesis of well-defined glycopolymers. Fukada and co-workers were able to successfully polymerize styrene and methacrylate derivatives with pendant saccharide residues by nitroxide controlled[61] and ATRP[62] techniques respectively. Using di-tert-butyl-N oxide (DBN) capped polystyrene as an initiator; Fukada and co-workers were able to successfully synthesize block copolymers polystyrene-*block*-polyglucosyl acrylate (PAGlc) as shown in Scheme 1.11.



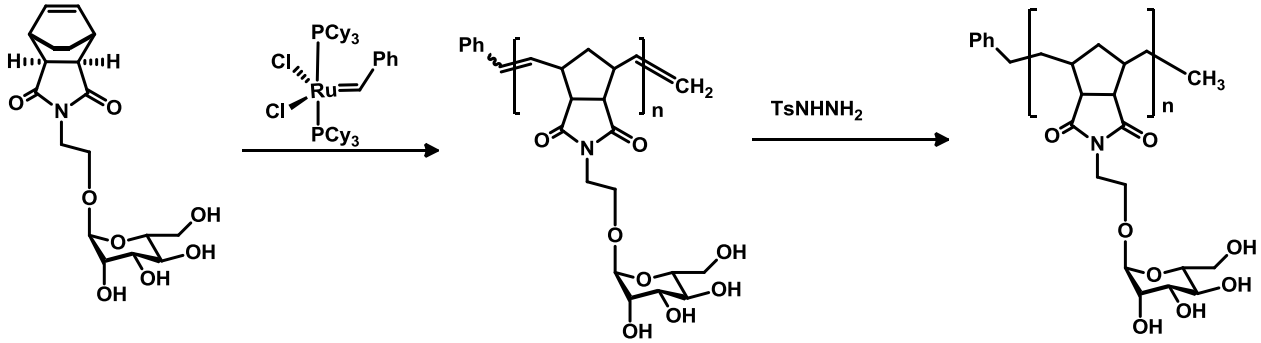
Scheme 1.11: Synthesis of polystyrene-poly (glucosyl acrylate) block copolymer using polystyrene N, N-di-tert-butyl-N-oxide using radical polymerization[62]

Cationic Polymerization: Conventional cationic polymerization of vinyl ethers and styrenes are frequently accompanied with chain transfer reactions and therefore polymers with controlled molecular weights cannot be obtained. Higashimura et al. developed useful techniques to achieve living polymerizations of vinyl ethers and styrenes by using either hydrogen iodide and iodine or hydrogen iodide and weak Lewis acid initiator systems to stabilize the growing chain end.[63] Miyamoto et al. succeeded in cationically polymerizing carbohydrate carrying vinyl ethers in a living manner to synthesize the corresponding glycopolymers with controlled molecular weights.[65] They were able to successfully synthesize amphiphilic block copolymers of vinyl ethers having pendant *N*-acetyl-D-glucosamine residues by living cationic polymerization of isobutyl vinyl ether and vinyl ether carrying 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D glucose. (Scheme 1.12)



Scheme 1.12: Synthesis of sugar-containing vinyl ether block copolymer by cationic living polymerization[65c]

Ring Opening Metathesis Polymerization: This is a very attractive route for the synthesis of a variety of glycopolymers for several reasons.[66] First ROMP catalysts have been developed that tolerate monomers with unprotected polar functionalities. This is very important feature as many biologically relevant saccharide determinants contain sulfated groups which cannot be easily masked. Second, well-defined catalysts can afford polymers with specific chain lengths with narrow polydispersities. Third, the polymerization process can also be used to generate block copolymers which can be useful to modulate the immuongeneticity of a particular material or to target it to a particular cell type. Kiessling et al. designed monomer to display a single saccharide residue per unit and to serve as a highly reactive substrate for ROMP (synthesis of α-D-mannose carrying polymer by ROMP is shown in Scheme 1.13)[67]



Scheme 1.13: Synthesis of α-D-mannose carrying polymer by ROMP

CLICK CHEMISTRY AND ITS APPLICATIONS IN POLYMER SYNTHESIS

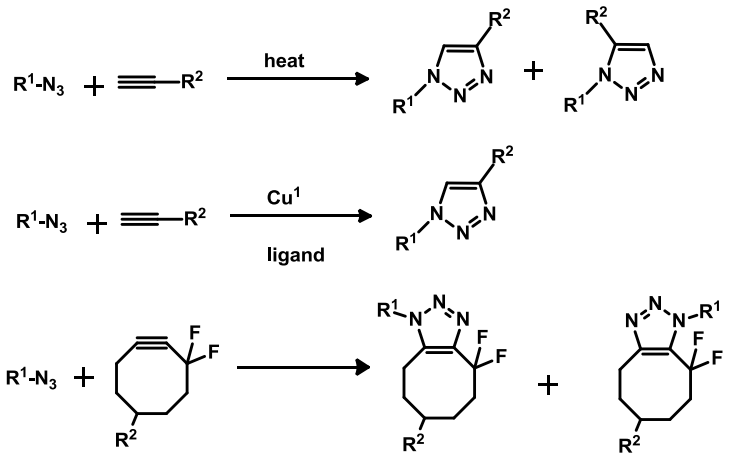
In order for an organic reaction to be applicable and useful in polymer synthesis, the reaction must proceed in high yield with little or no by-product. Both of these requirements are fulfilled by the recent development of the click chemistry concept by Sharpless. Sharpless and coworkers established a set of stringent rules for a reaction to comply[68]:

1. Reaction must be modular and wide in scope
2. Reaction must have very high yields, generating only inoffensive by products that can be removed by non-chromatographic methods
3. Reaction must be stereospecific
4. Reaction conditions must be simple and the reaction should be insensitive to oxygen and water
5. Starting material and reagents should be benign.

Only those reactions which satisfy all of the above conditions would be classified as click reactions.

The Huisgen 1,3- dipolar azide-alkyne cycloaddition (Scheme 1.14) is characterized by high reliability and broad tolerance to a wide variety of functional groups.[69-71] In addition as neither the azide nor alkynes naturally occur, the cycloaddition reaction is characterized by a unique orthogonality. Meldal[70] and Sharpless discovered the Cu(I) catalyzed variant of the click reaction

that led to the realization of the actual potential of this reaction. They introduced a transition metal catalyst to drastically increase the rate of the reaction. They called the copper mediated azide alkyne cycloaddition reaction as "click reaction."



Scheme 1.14: Different types of azide- alkyne cycloaddition: (A) Standard thermal cycloaddition[69], (B) copper-(I)-catalyzed cycloaddition[70], (C) strain promoted and fluorine activated cycloaddition.[71]

As a result of this discovery, applications of the azide-alkyne cycloadditions now extend further beyond organic synthesis to further challenging goals in chemistry, polymer science and biology. A variety of click reactions exist, however the Huisgen 1,3-dipolar cycloaddition of azides and alkynes plays a particularly important role in organic synthesis and has evolved into a reaction used quite often for assembling diverse structures. The foray of copper assisted azide-alkyne cycloaddition (CuAAC) reactions into biochemical systems raises a major concern of copper toxicity and thus limits its applications in such systems. The contribution of copper to oxidative stress in biomacromolecules is well known. Cu ions promote the formation of reactive oxygen species which is responsible for biological damage. In the past couple of years there has been significant interest in developing click reactions that do not require any metal catalyst while exhibiting all the beneficial properties of CuAAC reactions. One elegant approach involving the

use of strained cyclooctynes was reported by Bertozzi and coworkers (Figure 1.2).[72] This strain promoted azide- alkyne [3+2] cycloaddition (SPAAC) reaction was developed from the initial work of Witting and Krebs.[73] However, the rate of the SPAAC reactions with the first generation of cyclooctynes was relatively slow compared to the CuAAC reactions. Boons and coworkers[74] reported the synthesis of active cyclooctynes by introducing benzyl groups to increase the ring strain. They used derivatives of 4-dibenzocyclooctynol (DIBO) (Figure 1.2) to label glycoconjugates of living cells metabolically for visualization. The compound possesses several advantageous features such as ease of synthesis and possibility of further functionalization of the cyclooctyne ring and for example derivatives **3** and **4** which were shown to exhibit even higher rates of reaction than the parent compound. In order to explore the scope of this reactions, Boons and coworkers reported the metal free [3+2] cycloadditions of the cyclooctynes with a variety of 1,3- dipoles having different reactivities.[75] They found that 1,3- dipolar cycloadditions with nitrile oxides exhibited faster reaction kinetics than the corresponding reactions with azides. They demonstrated, for the first time, that strain promoted click reactions can be performed in a sequential manner by tuning the reactivity of 1,3- dipoles or by employing a latent dipole such as oxime which after oxidation to the corresponding nitrile oxide using bis(acetoxy)iodobenzene (BAIB) as the oxidant gives an active 1,3-dipole for cycloaddition (Scheme 1.15).[75]

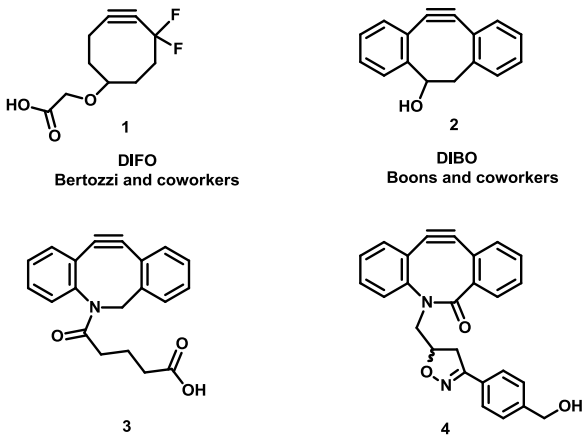
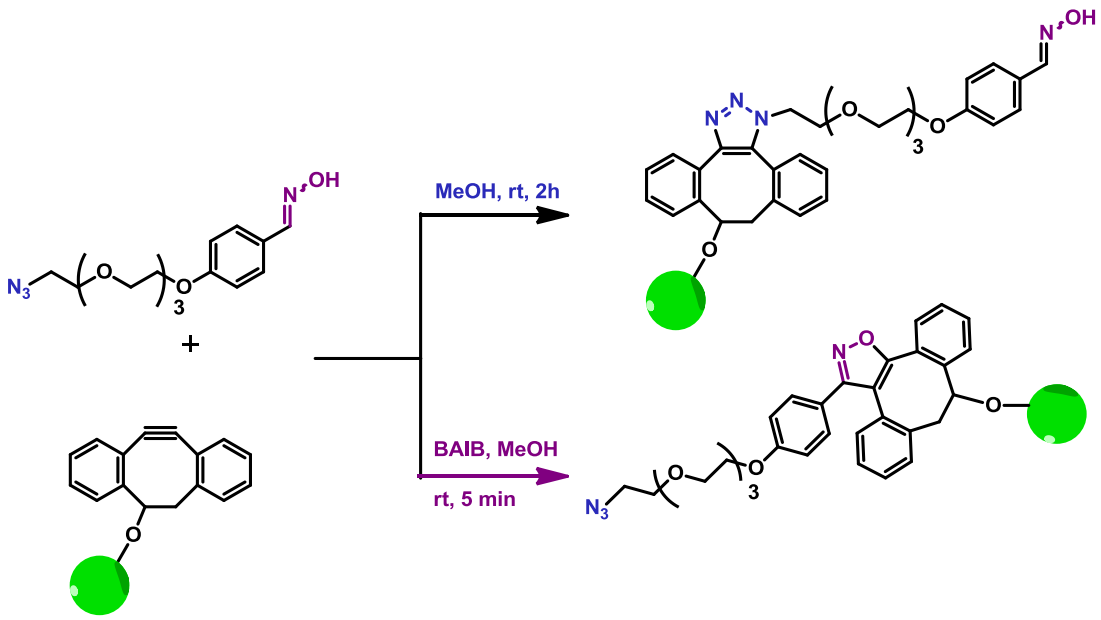


Figure 1.2: Cyclooctynes for metal- free reactions



Scheme 1.15: Orthogonal [3 +2] cycloaddition with cyclooctynes and various 1, 3- dipoles

While the main application of the click reaction had been envisioned by Sharpless to be useful for the synthesis of biologically active molecules[76], the click concept has found tremendous applications in polymer chemistry. Click chemistry affords several advantages in the field of polymer synthesis:

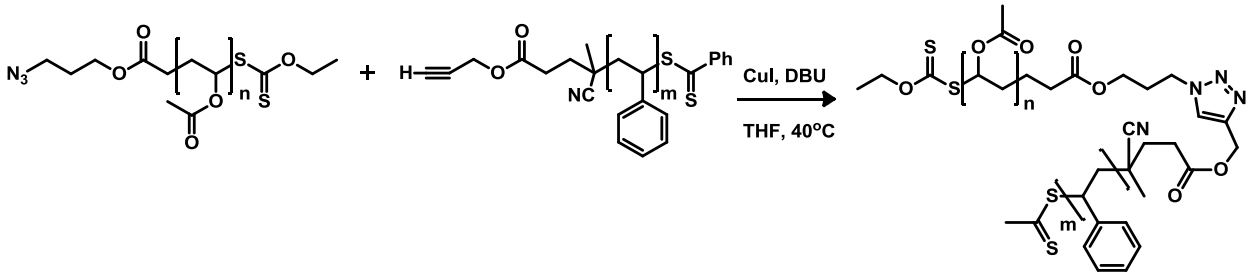
(i) Efficient coupling conditions

(ii) Lack of side products

(iii)Facile purification[77]

Click chemistry has enabled the synthesis of macromolecular assemblies that would not have been possible before. For example, block copolymer synthesis can be achieved via a range of polymerization techniques with living characteristics. These processes require the execution of two consecutive polymerizations, where the initially prepared polymer strand is chain extended with a second monomer. The material design problem that has to be overcome in select cases is the fact that some monomer combinations cannot be employed to prepare well-defined polymer

owing to their disparate reactivities. In other cases, only impure and homopolymer containing block copolymer mixtures are obtained through an inefficient chain extension. A typical example is the target structure of a block copolymer of styrene and vinyl acetate or ethylene. To overcome this problem, an efficient reaction can be conceived that links two separately prepared polymer chains together. Although this method is applicable to a large variety of monomers, the presence of residual homopolymers is difficult to avoid. Also, coupling of polymers is a thermodynamically unfavorable.[78] The steric hindrance of the polymer chains acts as a shield preventing the molecular reaction between polymer end groups. This problem can be overcome with the aid of click chemistry. Stenzel et al. used the click chemistry strategy for assembling block copolymers from monomers with disparate reactivities (Scheme 1.16).[79]



Scheme 1.16: Use of click chemistry for assembling polystyrene-*b*- polyvinylacetate block copolymer[79]

The utility of the click reaction has been amply demonstrated for the synthesis and modification of polymers with a wide range of composition, functionality, architecture and intended purpose.[80] In the current work, click chemistry was used to assemble block copolymers comprising of the hydrophobic polycaprolactone and the hydrophilic sugar molecules.

APPLICATIONS OF BLOCK COPOLYMERS

Block copolymers have a long history as industrial surfactants. The major types of block copolymers such as those made from ethylene oxide and propylene oxide or ethylene oxide and

styrene are cheap and easy to tailor-make for specific applications. In the manufacturing of an amphiphilic block copolymer for a specific application, there are several degrees of freedom as compared with the synthesis of conventional, low-molecular weight surfactants:

1. The size of both the hydrophilic and the hydrophobic part can be varied at will,
2. The molecular weight can be varied within wide ranges while maintaining constant hydrophilic-lipophilic balance, and
3. The properties and function of a block copolymer at an interface can be governed by the molecular architecture.

Some of the most prevalent applications of block copolymers in the industry have been enumerated below:

Emulsifiers: Ethylene glycol- propylene oxide block copolymers are used both as emulsifiers and as emulsion stabilizer. The emulsifier application generally requires low molecular weight polymers, usually below 2000, while post-stabilization of a ready-made emulsion works best with high molecular weight.

Demulsifiers: Ethylene glycol- propylene oxide block copolymers have an established position as demulsifier in oil production.

Defoamers and low-foaming surfactants: Block copolymers based on ethylene glycol and propylene oxide are efficient defoamers. Foam control agents in general should be hydrophobic; the best anti-foam agents have very limited water solubility.

Over the past few decades, block copolymers have been extensively developed industrially. With the occurrence of a wide range of new block copolymers, also the usefulness of these in practical applications has increased. As with other industrial applications, many uses of these copolymers in the development of pharmaceutical formulations are largely related in one way or another to

the amphiphilic nature of these substances. The amphiphilic nature of block copolymers makes them ideal as stabilizers of pharmaceutical colloidal dispersions, e.g., emulsions, liposomes, or nanoparticles, since they typically contain one block which experiences poor solvency and thus is capable of achieving a firm anchoring of the copolymer at the drug carrier surface, and one or several blocks experiencing good solvency, resulting in an efficient steric repulsion, and thus in good colloidal stability. Apart from controlling the colloidal stability of pharmaceutical dispersions, adsorbed block copolymers may also affect the biological response to these dispersions.

From a chemistry standpoint, one simple way to design self-assembling drug delivery systems is to use micelles. The spontaneous assembly in supramolecular complexes with strictly controlled composition and structure is characteristic of most micelle systems. Several micelle-based drug delivery systems have been investigated. Among these approaches, methods using block copolymer micelles as a basic element of a delivery system are experiencing rapid development. The use of amphiphilic block copolymers in experimental medicine and pharmaceutical sciences has a long history. For example, intensive studies have been performed on gels and emulsions of poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (Pluronic) as components of artificial blood formulations, drug release systems, immunoadjuvant, anti-tumour and anti-inflammatory agents. The *in vivo* studies demonstrated that Pluronic copolymers possess reduced toxicity compared to the low molecular mass surfactants which permit their administration in man.The decade of studies on block copolymer micelles as drug carriers has revealed the great potential of this approach and its importance for targeted drug delivery. It is expected that studies in this area will intensify in the near future, and new and important results will occur.[1,2]

BLOCK COPOLYMERS IN DILUTE SOLUTION

Micelles are assemblies of amphiphilic molecules in which the hydrophobic ends of the amphiphilic molecules make the center of the micelles (core) while the hydrophilic ends make the corona (shell). Due to the unique structure of amphiphilic compounds, they have a tendency to accumulate at the boundary of two phases and thus are termed surfactants. In aqueous solutions, amphiphilic compounds orient themselves so that the hydrophobic blocks are removed from the aqueous environment in order to achieve a state of minimum free energy. As the concentration of amphiphiles in solution is increased, the free energy of the system begins to rise because of the unfavourable interactions between the water molecules and the hydrophobic region of the amphiphile resulting in structuring of the surrounding water and a subsequent decrease in entropy. Micelles form when sufficient concentration of free molecules is reached and each micelle contains a defined number of amphiphiles (Figure 1.3). The concentration of amphiphilic molecules at which the micelles are formed is called a critical micelle concentration (CMC).[81] The formation of micelles effectively removes hydrophobic portion of the amphiphiles from solution minimizing the unfavourable interactions. If the amphiphile concentration in solution remains above the CMC, micelles are thermodynamically stabilized against disassembly. The higher the CMC, the less stable the micelles are towards dilution, which leads to their disassembly. The rate of the disassembly is largely dependent on the structure of the amphiphiles and the interactions between the chains. Micelles are a thermodynamically stable system at equilibrium. In this respect, amphiphilic copolymer micelles have a distinct advantage over those formed from conventional surfactants such as Cremophor EL ™ or polysorbates, since they typically not only display lower CMCs, but also in some cases resist disassembly upon dilution due to the physical interactions among chains in the micelle core.[81]

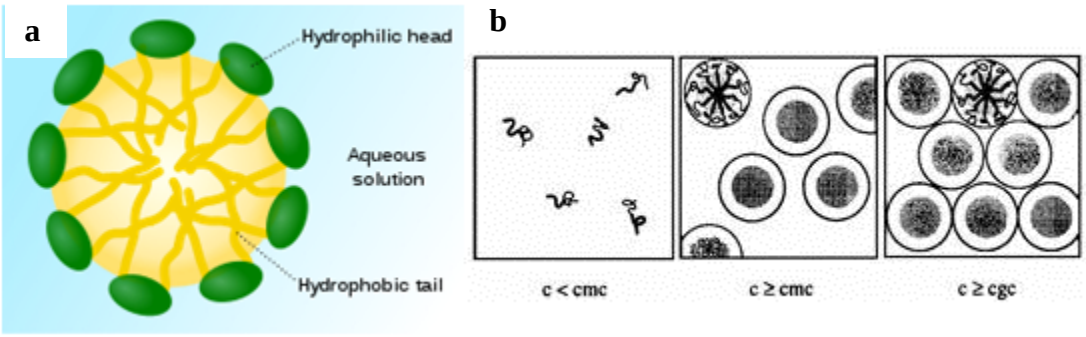


Figure 1.3: a) Schematic representation of a micelle b) Schematic representation of a micelle forming above the critical micelle concentration (CMC) [82]

The hydrophilic blocks forming the corona region become highly water bound and adopt a "splayed" appearance, giving rise to different conformations such as a polymer "brush". These conformations sterically suppress opsonization by blood components, thus resisting phagocytosis by macrophages and decreasing clearance by the reticuloendothetial system (RES), resulting in prolonged circulation times.[83]

POLYMERIC MICELLES: COMPOSITION AND STRUCTURE

Block copolymer micelles can be classified according to the type of intermolecular forces driving the segregation of the core segment from the aqueous milieu. In the past few decades, at least three main categories were identified, mainly

a) Amphiphilic micelles formed by hydrophobic interactions,
b) Polyion complex micelles (PICM) resulting from electrostatic interactions and
c) Micelles stemming from metal complexation.[84]

METHODS OF MICELLE PREPARATION

There are two principal methods for the preparation of block copolymer micelles, the direct dissolution method and the dialysis (Figure 1.4). The choice of which method to use depends mostly on the solubility of the block copolymer in water. To this point, mostly star-type micelles

have been investigated as drug carriers. Star-type micelles are formed from block copolymers which have corona-forming blocks that are longer than the core forming blocks. If the copolymer is marginally soluble in water, the direct dissolution method is employed, whereas if the copolymer is poorly soluble in water, the dialysis method is usually employed.[81]

The direct dissolution simply involves adding the copolymer to water or another aqueous medium such as phosphate buffer saline. The micelles formed from the Pluronic copolymers are routinely formed by direct dissolution, but in some cases the copolymer and water are mixed at elevated temperatures to ensure micellization.[85]

The dialysis method is often used when micelles are to be formed from a copolymer that is not easily soluble in water.[97] In this case; the copolymer is first dissolved in a common organic solvent that is miscible with water such as dimethylformamide (DMF), tetrahydrofuran (THF) or dimethylacetamide (DMAc). The copolymer solvent mixture is stirred and then dialyzed against double distilled water. During the process of dialysis, micelle formation is induced and the organic solvent is removed.

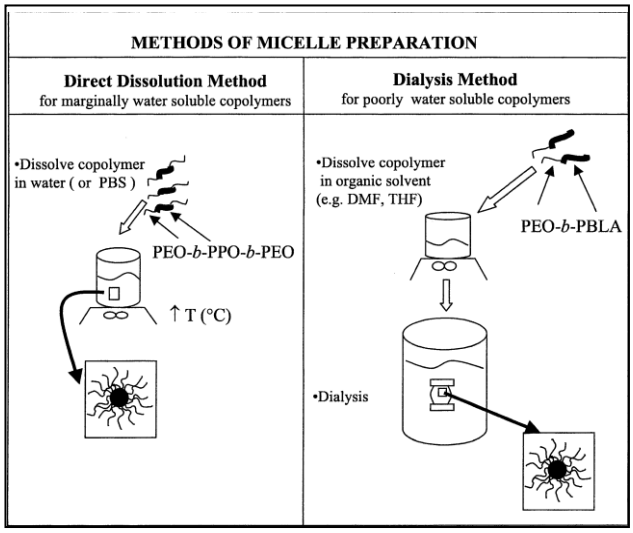


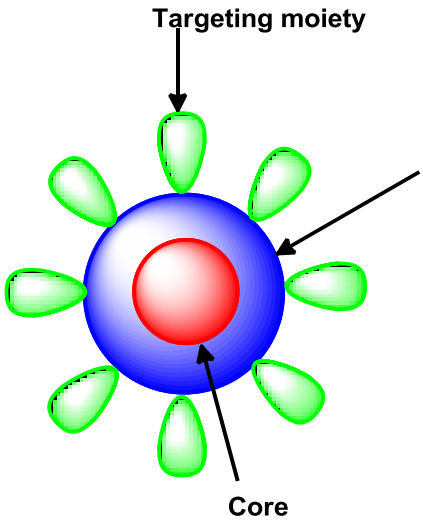
Figure 1.4: A schematic of the two principal methods employed for the preparation of block copolymer micelles

While micelles are often pictured as spheres,[86] it is critical to recognize that the micelles are not always spherical and not solid particles. The individual polymer chains that form a micelle are in dynamic equilibrium with the chains that remain in the bulk solution, at the solvent interface and incorporated into adjacent micelles.

THE MICELLE CORONA

The micelle shell acts as a stabilizing interface between the hydrophobic micelle core and the external medium.[81] The properties of the outer shell will predominantly affect the biodistribution of the micelle and thereby that of the incorporated drug as well as its pharmacokinetic parameters (Figure 1.5).

PEG as the corona forming block: In most cases, the hydrophilic shell forming block is polyethylene glycol (PEG) with a molecular weight which is usually between 1000 and 12,000 g mol^{-1} In the bulk, PEG is a non-ionic crystalline, thermoplastic water soluble polymer.[87] The molar mass as well as the polydispersity of the polymer has been shown in many applications to be important for biocompatibility and stealth behavior. The molar mass of polyethylene glycol used in different pharmaceutical and medical applications ranges from 400Da to about 50kDa. Polyethylene glycol with a molar mass of 20kDa to 50kDa is mostly used for the conjugation of low-molar mass drugs such as small molecules, oligonucleotides and siRNA. This results in increasing the size of the conjugates which is above the renal clearance threshold. From a theoretical point of view, a biodegradable polymer would be more beneficial in drug delivery applications, since difficulties in achieving complete excretion would be avoided.[88] However, a point to be noted is that the excretion of the polymer is not directly dependent on the molar mass of the polymer, but rather on the hydrodynamic volume, which in turn is affected by the architecture of the polymer. For example, star-shaped polymers and dendrimers show lower hydrodynamic volumes than linear polymers with similar molar masses.[89]



Key Physical Properties

1. Surface density of hydrophilic chains
2. Charge
3. Hydrophilicity
4. Block length
5. Derivatization (for example ligand attachment)

Figure 1.5: Key physical properties of the micelle corona

In general, a low polydispersity index (PDI) is a basic prerequisite for the polymer to have pharmaceutical applications. A PDI value below 1.1 provides a polymer with an acceptable homogeneity to ensure reproducibility in terms of body-residence time and immunogenicity of the carrier system. This demand is readily fulfilled by polyethylene glycol, since very well-defined polymers with PDIs around 1.01 are readily accessible by the anionic polymerization of ethylene oxide.[90] Furthermore, polyethylene glycol shows a high solubility in organic solvents and, therefore end-group modifications are relatively easy. At the same time, polyethylene glycol is soluble in water and has a low intrinsic toxicity that renders the polymer ideally suited for biological applications. It provides drugs with a greater physical and thermal stability as well as preventing or reducing aggregation of the drugs in vivo, as well as during storage, as a result of the steric hindrance and/ or masking of charges provided through formation of a conformational cloud. This cloud is generated by the highly flexible polymer chains, which have a large total number of possible conformations. The higher the rate of transition from one conformation to another, the more the polymer exists statistically as a conformational cloud which prevents

interactions with blood components as well as interactions such as enzymatic degradation or opsonization followed by uptake by the RES.[91]

Polyethylene glycol has a unique ability to influence the pharmacokinetic properties of drugs and drug carriers and hence there has been a surge in its applications for medicinal purposes. The change in the pharmacokinetic of administered drugs by being shielded by or being bound to PEG results in prolonged blood circulation times. This consequently increases the probability that the drug reaches its site of action before being recognized as foreign and cleared from the body. Therefore, a vast majority of the conjugated drugs as well as liposomal and micellar formulations currently in the market or in advanced clinical trials are PEG-containing products. Some of the drug delivery systems stabilized by PEG that have received regulatory appeal in the US/ EU are shown in Table 1.1.In fact, all polymer-based stealth drug-delivery systems that have been brought to the market up to now contain PEG-functionalized products and no other synthetic polymer has yet reached this status.[89]

Table 1.1: Drug delivery systems stabilized with PEG that have received regulatory appeal in the US and/ EU.

PEG drug description	**Company**	**Indication**	**Year of approval**
Adagen (11-17x5kDa mPEG per adenosine deaminase)	Enzon Inc. (USA and Europe)	Severe combined immunodeficiency	1990 (USA)
PEG-Intron (2x20kDa mPEG-interferon-α-2a)	Schering-Plough Corp. (USA and EU)	Chronic Hepatitis C	2000 (EU) 2001(USA)
Cimzia (2x40kDa	UCB S.A. (USA and	Crohn's disease,	2008(USA)

mPEG-anti-TNFα)	EU)	rheumatoid arthritis	2009 (USA)
Macugen (2x20kDa mPEG-anti-VEGF-aptamer)	Pfizer	Age-related macular degeneration	2004 (USA) 2006 (EU)
Somavert (4-6x5 kDa mPEG per structurally modified HG receptor antagonist)	Pfizer	Acromegaly	2002(EU) 2003(USA)

Use of micelle-forming amphiphilic polymers as drug-delivery vehicles has been reported by Ringsdorf et al. in the 1970s. However, Kabanov and co-workers were the first to report the use of PEG as the hydrophilic portion of linear block copolymers for micellization in 1989. Kwon and Kataoka helped developing PEG-containing block copolymers as potential drug delivery vehicles. This has opened the doors for the synthesis of dendritic and star-shaped amphiphilic polymers which exhibit control over architecture, shape, size and surface functionality of the micelles at the cost of higher complexity compared to linear block copolymers.[92]

With the increasing attention being paid to the potential applications of PEG and PEGylated products in pharmaceutical research, attention was also being paid to the likelihood of potential side effects of using such compounds. Some of the major drawbacks associated with PEG are enumerated below:

1. Early studies have shown that PEG has a tendency to induce blood clotting and clumping of cells which may lead to embolism. This indicates that PEG may have non-specific interactions with blood.[7] Since then, it has been shown that PEG may induce specific and non-specific recognition by the immune system, thus leading to rejection by the body to intravenously

administered PEG formulations such as liposomal and micellar carrier systems. However, a conclusive statement cannot be reached as to whether PEG alone or a combination of several factors causes hypersensitivity.

2. PEGs of low molar mass (below 400 Da) are known to possess some toxicity in humans as a result of sequential oxidation into diacid and hydroxyl acid metabolites by alcohol and aldehyde dehydrogenase. The oxidative degradation significantly decreases with increasing molar mass and therefore, a molar mass well above 400Da should be used to avoid toxicity issues.[93]

3. The most prominent side product formed during the synthesis of PEG is the cyclic dimer of ethylene oxide, 1,4-dioxane. Dioxane has been classified by the International Agency for Research on Cancer to be a potential carcinogen for humans. PEG can contain residual ethylene oxide from polymerization. Another by-product of PEG is formaldehyde, which is also a known carcinogen.[7]

PEG is a heavily employed polymer with an overwhelming number of positive properties, as is easily confirmed by the literature. The huge number of advantageous properties has made it possible for PEG to be used in a wide variety of everyday products, industrial applications and in many biomedical drug-delivery systems. Although, there have been several reports on the non-biodegradability of PEG, the positive properties of PEG definitely outweigh the negative effects discussed. As a result, PEG remains a highly used polymer and the gold standard in biomedical applications. However, the search for alternative biopolymers has begun and carbohydrates are one of the most promising candidates.

Corona forming blocks other than PEG: There has been very little investigation into the use of hydrophilic blocks other than PEG for design of block copolymers to be used in drug delivery

applications. Inoue et.al.[94] reported the formation of micelles from the amphiphilic copolymer oligo(methyl methacrylate) and poly(acrylic acid) which self assembles in aqueous environment to produce micelles with a negative charge on the surface. Poly (*N*-vinyl-2- pyrrolidine) (PVP) is a non-ionic, biocompatible and water soluble synthetic polymer. Owing to its lyoprotectant and cryoprotectant properties, PVP is often preferred to PEG for preparation methods that involve freeze drying. Also, PVP has been shown to interact with a variety of hydrophilic and hydrophobic pharmaceutical agents, thus potentially increasing the solubilizing capacity of the micelles.[84] Use of poly (amino acids) such as poly (glutamic acid)s (PGA), poly (hydroxyethyl- L- asparagine)s (PHEA) and poly (hydroxyethyl- L-glutamine)s (PHEG) as alternatives to PEG is also being investigated. They combine various advantages for drug- delivery systems such as prolonged blood circulation and biodegradability. Several alternatives to PEG have been shown in Figure 1.6.

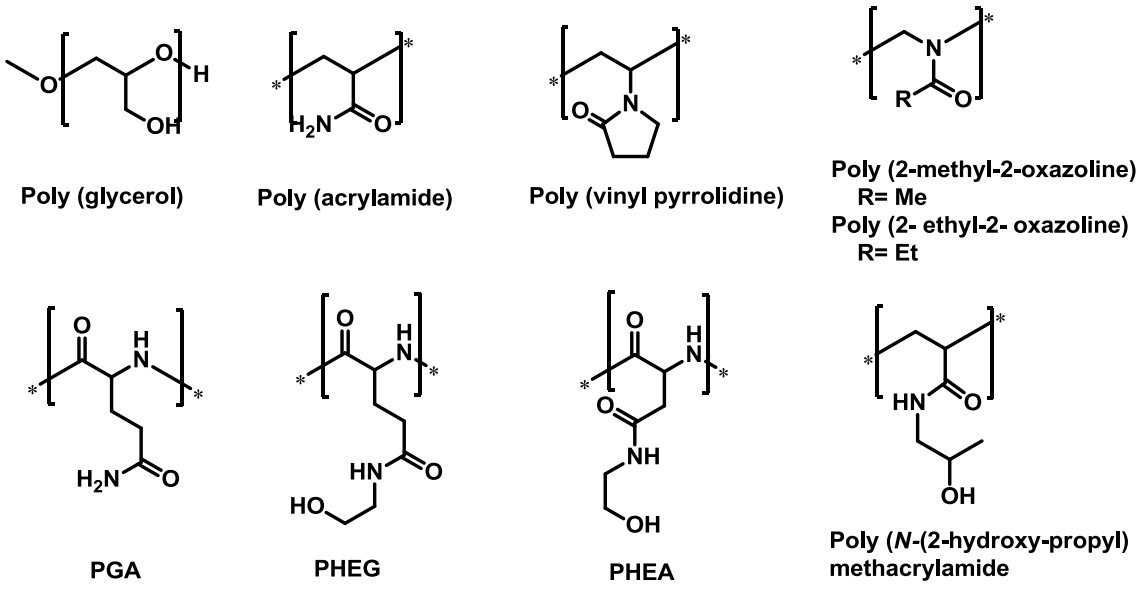


Figure 1.6: Examples of different hydrophilic polymers as alternatives to PEG.

THE MICELLE CORE

The micelle core serves as the cargo space for various lipophilic drugs. For a copolymer with a constant hydrophilic block length, an increase in the length of the core-forming block has been found to increase the partition coefficient of the solubilization between the micelles and the external medium. Yu et.al measured the partition coefficient for pyrene between water and PCL-*b*-PEG micelles formed form copolymers with constant PEG block length and different PCL block lengths. The partition coefficient was found to increase from 240, 760 and 1450 for micelles formed from PCL-*b*-PEG with the same PEG block length and 14, 21 and 40 units of caprolactone respectively.

An increase in the length of the core forming block has been found to decrease the critical micelle concentration and also cause an increase in the core size per micelle, which in turn, results in an increased loading capacity per micelle. For example, for any one block copolymer system, as one increases the length of the core forming block, the aggregation number increases thereby resulting in a larger core size. However due to an increase in the aggregation number per micelle, the total number of micelles in solution will decrease per unit mass of polymer. In addition, Kabanov's group has found a clear reciprocal relationship between the CMC and the length of the core-forming block.[81]

PCL was one of the earliest polymers synthesized by Carothers group in the 1930s. It became commercially available following efforts to identify synthetic polymers that could be degraded by microorganisms. PCL can be prepared by either ring opening polymerization of ε-caprolactone using a variety of anionic, cationic and coordination catalysts or via free radical ring opening polymerization of 2-methylene-1-3-dioxepane. PCL is a hydrophobic, semicrystalline polymer; its crystallinity tends to decrease with increasing molecular weight. The good solubility of PCL and low melting point (59-64^{o}C) has stimulated extensive research into

its potential application in the biomedical field. Consequently, during the resorbable- polymer-boom of the 1970s and 1980s, PCL and its copolymers were used in a number of drug- delivery devices. Attention was drawn to these biopolymers owing to their numerous advantages over other biopolymers in use at that time. These included tailorable degradation kinetics and mechanical properties, their ease of manufacture and the controlled release of the drugs encapsulated within their matrix. Functional groups could also be added to make the polymer more hydrophilic or biocompatible to facilitate favorable cell responses. Due to the fact that PCL degrades at a slower rate than polyglycolide (PGA), poly-D, L-lactide (PLA), it was originally used in drug delivery devices that remain active for over a year and in slow degrading suture materials.[95]

Apart from PCL, several other polymers such as D, L-lactide, glycolide and δ-valerolactone are often employed as the hydrophobic block because of their safety profiles and solubilizing capabilities. In addition to the above, vinylic polymers are also widely used as they can be easily tailored for hydrophobic interactions and ionic association. However, one major shortcoming of vinylic polymers is that they are not biodegradable.

TECHNIQUES FOR THE STUDY OF SELF-ASSEMBLED STRUCTURES

GEL PERMEATION CHROMATOGRAPHY: Size exclusion chromatography, often referred to as gel permeation chromatography (GPC) is one of the methods for determining the molecular weights and molecular weight distribution of synthesized polymers. The method involves the permeation of a polymer through tightly packed column of porous material like microporous beads or crosslinked polymer gel. A dilute solution of the polymer is injected into the gel column and the solvent (eluent) is forced through the column at a controlled flow rate. The flow-through times of different molar mass depends on their hydrodynamic volume. Larger

molecules having no access to the pores and therefore move along with the eluent flow and elute first. Smaller molecules can penetrate into a much larger volume of the porous gel, and therefore they stay on the column for a longer time. The concentration of the eluted polymers is recorded continuously as a function of time or elution volume by detectors such as refractive index (RI) and/ or ultraviolet-visible (UV-Vis) detector. Using the RI detector, the difference in the refractive index between the eluted solution and the pure solvent, which is proportional to the concentration of the polymer chains, is measured. The UV-Vis detector, on the other hand, detects only the polymers containing UV-active units at specific wavelengths.

GPC is a relative method and therefore the elution volume has to be calibrated with polymer standards of known molecular weights and narrow molecular weight distribution. A calibration curve is then obtained by plotting the molecular weight of the standards *vs.* their elution volume. The corresponding molecular weight of every fraction can be determined according to the calibration curve. The concentration of eluted molecules, c_i, in each fraction, i, is measured. The number average and weight average molecular weights, M_n and M_w, as well as the polydispersity index ($PDI = M_w/M_n$) can be calculated according to the following equation[1,2]

$$M_n = \frac{\sum ci}{\sum ci/Mi}$$

$$M_w = \frac{\sum ci/Mi}{\sum ci}$$

LIGHT SCATTERING is a widely used technique for characterizing polymer and colloidal particles in dilute solutions. A beam of monochromatic light is made to pass through a system and the molecules therefore interact with the electromagnetic radiation and scatter light. The scattered light emits light in all directions and has almost the same wavelength as the incident light. In 1869, John Tyndall discovered that the intensity of the scattered light depends on the

wavelength of the incident light and the detection angle. The first theoretical description of light scattering was developed by Lord Rayleigh based on the theory of electromagnetic waves.

a. Static Light scattering (SLS) is used for the determination of size, shape and weight average molecular weight of the analyzed particles as well as information on the interactions between the particles in solution.

b. Dynamic Light scattering (DLS) is a well-established technique for determining the size and polydispersity of polymer or aggregates suspended in solution. This method is based on the fact that the intensity of the light scattered from a solution of aggregates is a result of the interactions of the scattered radiation by each of them with the ones from the others.[1,2]

TRANSMISSION ELECTRON MICROSCOPY: (TEM) is an imaging technique using a beam of electrons to examine objects with high resolution. It was first developed by Max Knoll and Ernst Ruska in the early 1930s. A modern TEM consists of an illumination system, a specimen stage, an objective lens system, the magnification system, the date recording system and the chemical analysis system. Like other electron microscopes, TEM has to be operated under high vacuum to avoid scattering of electrons by any particle. A schematic diagram of TEM is shown in Figure 7. The electron gun produces a stream of electrons. The stream is then focused to a small beam using two condenser lenses. The beam strikes the specimen and parts of the beam are transmitted. The objective lens focuses the transmitted beam onto an image. The objective

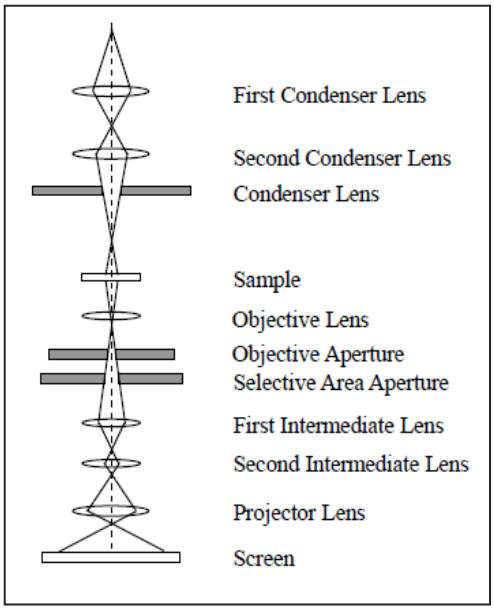


Figure 1.7: TEM instrumentation

aperture enhances the contrast while the magnification system enlarges the image. Specimens for TEM examination have to be extremely thin (50-100nm) so that the electron can be transmitted through it and create an image. Normally the sample can be spread on a support grid. Details of the sample can be enhanced by the use of stains. Compounds of heavy metals such as osmium or uranium can be used to selectively deposit heavy atoms in the sample and enhance structural detail.

TEM has been utilized for the characterization of the size, shape, size distribution and internal structure of block copolymer micelles. The technique relies on the production of phase contrast between the micellar core and corona by selectively staining one of the micelle's parts. Two methodologies are used for sample preparation. In the first method, a drop of a dilute solution of micelles is spread on a carbon film, and the solvent is allowed to evaporate. Then the dry isolated micelles can be stained and observed under the microscope. In a second, more recent, method called cryo-TEM, the solution is rapidly frozen, by liquid nitrogen, stained and observed under appropriate low-tempearture conditions. In both cases, the final specimen for examination is a representative collection of micelles similar to their state in solution.However, in the first case, the dry micelles are in a collapsed state. This has some limitations in the correct determination of the micellar size in solution, but valuable information about the size distribution, shape and internal structure of the micelles can be obtained. Ultracryomicroscopy method on the other hand, allows freezing of the solution maintaining the micelles in their unperturbed solution state, and the information obtained gives a better idea about their true size, structure, and shape in solution.[1,2]

ATOMIC FORCE MICROSCOPY (AFM): AFM is unique in its ability to image single molecules in their native environment. AFM offers a means to visualize surface structures at

high resolution and in physiological conditions. AFM is a very high resolution scanning probe microscopy

The AFM consists of a cantilever with a sharp tip. The general principle of AFM is to scan the tip over the surface of a sample, while sensing the physical interactions between the tip and the sample. This provides a three-dimensional image that can be generated directly in the aqueous solution. The cantilever is generally made of silicon or silicon nitride. When the tip is brought into close proximity with the sample surface, interactions between the tip and the sample causes a deflection in the cantilever according to Hooke's law. The forces most commonly studied using AFM are van der Waals forces, chemical bonding and electrostatic interactions to name a few.

A number of different AFM imaging modes are available which differ primarily in the way the tip is moving over the sample. In the contact- mode, the AFM tip is makes soft physical contact with the surface of the sample and therefore it is necessary to have a cantilever which is soft enough to be deflected by very small forces and has a high enough resonant frequency to not be susceptible to vibrational instabilities. In the non-contact mode, the tip of the cantilever does not make contact with the sample surface. Here the probe operates in the attractive force region and the tip-sample interaction is minimized. The non-contact mode allows for multiple scanning without influencing the shape of the sample by the tip-sample forces. In the tapping mode, the cantilever is made to oscillate at a frequency nearly equal to its resonance frequency. The main advantage of using the tapping mode is the damage that can be avoided to the surface of the sample as well as to the tip. Thus AFM can be used to image and manipulate atoms and structures on a variety of different scales.[96]

HYPOTHESIS

The hypothesis of the work is to synthesize a library of block copolymers based on PEG and well defined PCL to study the effect on morphology by varying the length and molecular weight of both the hydrophobic and hydrophilic block. Eisenberg and co-workers[97] synthesized a library of block copolymers based on polystyrene and polyacrylic acid. They showed that by varying the hydrophobic block length, an increase in the size of the micelles can be achieved. On the other hand, an increase in the length and molecular weight of the hydrophilic block leads to a decrease in the size of the micelle. They also found that by increasing the hydrophobic block length changes the morphology of the nanostructures from rod-like micelles to spherical micelles.[98] The aim of the hypothesis is to compare the results obtained from our research with those published by Eisenberg and co-workers. The well-defined polycaprolactone is to be synthesized by step-growth polymerization of ε-caprolactone.[99] PEG is to be coupled to the preformed polymer using either the amide or ester coupling.

Block copolymeric micelles have gained a lot of impetus due to their potential use in a variety of applications ranging from drug delivery to nanosized reactors.[100-101] Extensive research has been done on the bulk compositions and stability of these micelles using X-ray photoelectron spectroscopy, TEM and small angle X-ray Scattering. However, it is difficult to examine chemo-physical or chemi-mechanical properties of individual micelles in a native environment and a comprehensive study is imperative in understanding the mechanical properties of individual macromolecules in these assemblies. Towards this goal, the current study aims at synthesis of a library of micelles with varied sizes from a series of block copolymers upon self-assembly followed by studying the force required to pull a polymer chain from the micelle using AFM. In this thesis we focus on using AFM technique and a novel copper free click approach[73,102] to

construct chemi-mechanical maps by studying the force-displacement curves obtained by pulling apart a single polymer chain from the micelles.

Use of polysaccharides as alternatives to PEG in biomedical applications is a very attractive field owing to the known role played by polysaccharides in the cellular machinery.[58] Apart from that, being polyhydroxy compounds, polysaccharides offer the advantage of being highly hydrophilic. There are polymerization methods known which can be useful to assemble a library of block copolymers based on monomers containing pendant saccharide moieties. However, use of polymerization methods requires tedious multi-step synthesis of the monomers. Use of the copper catalyzed azide alkyne cycloaddition to couple preformed polymers with polysaccharides is an attractive alternative strategy. A library of block copolymers will be assembled using saccharides such as lactose and maltoheptaose and PCL as the hydrophobic block. The synthesized block copolymers will be self-assembled into nansotructures and morphology of the structures would be studied.

REFERENCES

1. Hadjichristidis, N.; Pispas. S.; Floudas, G. A. (Ed) (**2003**) Block Copolymers: Synthetic Strategies, Physical Properties, and Applications, John Wiley & Sons, Inc., New Jersey.
2. Hamley, I. W. (Ed) (**1998**) The Physics of Block Copolymers, Oxford University Press, Inc., New York.
3. Luo, L.; Tam, J.; Maysinger, D.; Eisenberg, A. *Bioconjugate Chem.* **2002**, *13*, 1259-1265.
4. Hawker, C. J.; Wooley, K. L. *Science* **2005**, *309*, 1200-1205.
5. Szwarc, M.; Levy, M.; Milkovich, R.; *J. Am. Chem. Soc.* **1956**, *78*, 2656-2657.
6. Szwarc, M. *Nature* **1956**, *178*, 1168-1169.

7. Muller, A. H. E.; Matyjaszewski, K. (Ed) (**2009**) Controlled and Living Polymerizations, Wiley-VCH Verlag Gmbh & Co. Weinheim.
8. Bellas, V.; Iatrou, H.; Hadjichristidis, N. *Macromolecules* **2000**, *33*, 6993-6997.
9. Miyamoto, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1984**, *17*, 265-268.
10. Miyamoto, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1984**, *17*, 2228-2230.
11. Faust, R.; Shaffer, T. D. (Ed) (**1997**) Cationic Polymerization: Fundamentals & Applications, *ACS Symposium Series*, *665*, Washington D.C.
12. Matyjaszewski, K. (Ed) (**1996**) Cationic Polymerization: Mechanism, Synthesis and Application, Marcel and Dekker, New York.
13. Sawamoto, M. *Prog. Polym. Sci*. **1991**, *16*, 111-172.
14. Kennedy, J. P.; Ivan, B. (**1992**) Designed Polymers by Carbocationic Macromolecular Engineering: Theory and Practice, Hanser Publishers, New York.
15. Faust, R.; Kennedy, J. P. *Polymer Bulletin* **1986**, *15*, 317-323.
16. Faust, R.; Kennedy, J. P. *J. Poly. Sci. Part A: Polym. Chem.* **1987**, *25*, 1847-1869.
17. Higashimura, T.; Aoshima, S.; Sawamoto, M. *Makromol. Chem. Macromol. Symp.* **1988**, *13/14*, 457.
18. Li, D.; Faust, R. *Macromolecules* **1995**, *28*, 1383-1389.
19. Li, D.; Faust, R. *Macromolecules* **1995**, *28*, 4893- 4898.
20. Webster, O. W.; Hertler, W. R.; Sogah, D. Y.; Farnham, W. B.; Rajanbabu, T. V. *J. Am. Chem. Soc.* **1983**, *105*, 5706-5708.
21. Webster, O. W.; Anderson (**1992**) New Methods for Polymer Synthesis, Mijs W. J. (Ed) Plenum Press, New York, Chapter 1.
22. Brittain, W. J. *Rubber Chem. Technol.* **1992**, *65*, 588-595.

23. Webster, O. W. *Makromol. Chem. Macromol. Symp.* **1990**, *33*, 133.

24. Sogah, D. Y.; Hertler, W. R.; Webster, O. W. *Macromolecules* **1987**, *20*, 1473-1488.

25. Odian, G. (**1981**) Principles of Polymerization, John Wiley & Sons, Inc. USA.

26. Matyjaszewski, K. (**1998**) Controlled Radical Polymerization, *ACS Symposium Series*, Washington DC, Vol 685.

27. a) Moad, G.; Rizzardo, E.; Solomon, D. H. *Macromolecules* **1982**, *15*, 909-914. b) Georges, M. K.; Veregrin, R. P. N.; Kazmaier, P. M.; Hamer, G. K. *Macromolecules* **1993**, *26*, 2987-2988.

28. Matyjaszewski, K.; Gaynor, S.; Wang, J. S. *Macromolecules* **1995**, *28*, 2093-2095.

29. Malmstrom, E. E.; Hawker, C. J. *Macromol. Chem. Phys.* **1998**, *199*, 923-935.

30. Keoshkerian, B.; Georges, M.; Quinlan, M.; Veregin, R.; Goodbrand, B. *Macromolecules* **1998**, *31*, 7559-7561.

31. Benoit, D.; Harth, E.; Fox, P.; Waymouth, R. M.; Hawker, C. J. *Macromolecules* **2000**, *33*, 363-370.

32. Keoshkerian, B.; Georges, M. K.; Boils-Boissier, D. *Macromolecules* **1995**, *28*, 6381-6382.

33. Listigovers, N. A.; Georges, M. K.; Honeyman, C. H. *Polym. Prepr.* **1997**, *38*, 410.

34. a) Wang, J.; Matyjasweski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614-5615. b) Wang, J.; Matyjasweski, K. *Macromolecules* **1995**, *28*, 7901-7910.

35. a) Kato, M.; Kamigaito, M.; Sawamoto, M.; Higashimura, T. *Macromolecules* **1995**, *28*, 1721-1723. b) Endo, T.; Kato, M.; Kamigaito, M.; Sawamoto, M. *Macromolecules* **1996**, *29*, 1070-1072.

36. a) Patten, T. E.; Xia, J. H.; Abernathy, T.; Matyjasweski, K. *Science* **1996**, *272*, 866-868. b) Xia, J. H.; Matyjasweski, K. *Macromolecules* **1997**, *30*, 7697-7700.
37. Percec, V.; Barboiu, B.; Neumann, A.; Ronda, J. C.; Zhao, M. Y. *Macromolecules* **1996**, *29*, 3665-3668.
38. Pan, C. Y.; Lou, X. D.; Wang, Y. L.; Wu, C. P. *Acta Polym. Sinica* **1998**, *3*, 311.
39. Muhlebach, A.; Gaynor, S. G.; Matyjasweski, K. *Macromolecules* **1998**, *31*, 6046-6052.
40. Chiefari, J.; Chong, Y. K.; Ercole, F.; Krstina, J.; Jeffrey, J.; Le, T. P. T.; Mayadunne, R. T. A.; Meijs, G. F.; Moad, C. L.; Moad, G.; Rizzardo, E.; Thang, S. H. *Macromolecules* **1998**, *31*, 5559-5562.
41. Le, T. P.; Moad, G.; Rizzardo, E.; Thang, S. H. *PCT Int. Appl.* WO9801478A1 980115.
42. Chiefari, J.; Mayadunne, R. T. A.; Moad, G.; Rizzardo, E.; Thang, S. H. *PCT Int. Appl.* WO9931144A1 990624.
43. Mori, H.; Saito, Y.; Takahashi, E.; Nakabayashi, K.; Onuma, A.; Morishima, M. *Polymer* **2012**, *53*, 3861-3877.
44. Feast, W. J.; Khosravi, E. (**1995**) in New Methods in Polymer Synthesis, Chapman & Hall, Vol 2, Chapter 3.
45. Gilliom, L. R.; Grubbs, R. H. *J. Am. Chem. Soc.* **1986**, *108*, 733-742.
46. Grubbs, R. H.; Tumas, W. *Science* **1989**, *243*, 907-915.
47. Kress, J.; Osborn, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 6346-6347.
48. Schrock, R. R. *Acc. Chem. Res.* **1990**, *23*, 158-165.
49. Schrock, R. R.; DePue, R. T.; Feldman, J.; Schaverien, C. J.; Dewan, J. C.; Liu, A. H. *J. Am. Chem. Soc.* **1988**, *110*, 1423-1435.
50. Krouse, S. A.; Schrock, R. R. *Macromolecules* **1988**, *21*, 1885-1888.

51. a) Ivin, K. J.; Saegusa, T. (Ed) (**1984**) Ring- Opening Polymerization, Elsevier Applied Science, London,Vol. 1-3. b) McGrath, J. E. (Ed) (**1985**) Ring-Opening Polymerization: Kinetics, Mechanisms and Synthesis, American Chemical Society, Washington DC. c) Brunelle, D. J. (Ed) (**1993**) Ring-Opening Polymerization/ Mechanisms, Catalysis, Structure, Utility, Hanser Publisher.
52. Albertsson, A. C.; Varma, I. K. *Biomacromolecules* **2003**, *4*, 1466-1486.
53. Enomoto, K.; Ajioka, M.; Yamaguchi, A. **1994**, *US Patent 5310865*.
54. Lou, X.; Detrembleur, C.; Jerome, R. *Macromol. Rapid Comm.* **2003**, *24*, 161-172.
55. Williams, C. K. *Chem. Soc. Rev.* **2007**, *36*, 1573-1580.
56. Penczek, S.; Cypryk, M.; Duda, A.; Kubisa, P.; Slomkowski, S. *Prog. Polym. Sci.* **2007**, *32*, 247-282.
57. Lecomte, P.; Jerome, R. (Ed) (**2005**) Biodegradable Polymers for Polymer Industrial Applications, Woodhead Publishing Ltd.
58. Coullerez, G.; Seeberger, P. H.; Textor, M. *Macromolecular Bioscience* **2006**, *6*, 634-637.
59. Bertozzi, C. R.; Kiessling, L. L. *Science* **2001**, *291*, 2357- 2364.
60. Okada, M. *Prog. Polym. Sci.* **2001**, *26*, 67-104.
61. Ohno, K.; Tsuji, Y.; Miyamoto, T.; Fukuda, T.; Goto, M.; Kobayashi, K.; Akaike, T. *Macromolecules* **1998**, *31*, 1064-1069.
62. Ohno, K.; Tsuji, Y.; Fukuda, T. *J Polym Sci, A: Polym Chem* **1998**, *36*, 2473-2481.
63. Ohno, K.; Izu, Y.; Yamamoto, S.; Miyamoto, T.; Fukuda, T. *Macromol Chem Phys* **1999**, *200*, 1619-1625.

64. Higashimura, T.; Sawamoto, M.; Eastmond, G. C.; Ledwith, A.; Russo, S.; Sigwalt, P. editors. *Comprehensive Polymer Science*, vol. 3. Oxford: Pergamon Press, **1989**. p. 673-696.

65. a) Yamada, K.; Yamaoka, K.; Minoda, M.; Miyamoto, T. *J Polym Sci, A: Polym. Chem.* **1997**, *35*, 255-261. b) Yamada, K.; Minoda, M.; Miyamoto, T. *J Polym Sci, A: Polym. Chem*. **1997**, *35*, 751-757. c) Yamada, K.; Minoda, M.; Miyamoto, T. *Macromolecules* **1999,** *32*, 3553-3558.

66. Manning, D. D.; Strong, L. E.; Hu, X.; Beck, P. J.; Kiessling, L. L. *Tetrahedron* **1997**,*53*,11 937-11952.

67. a) Fournier, D.; Hoogenboom, R.; Schubert, U. S. *Chem. Soc. Rev.* **2007**, *36*, 1369-1380. b) Binder, W. H.; Sachsenhofer, R. *Macromol. Rapid Commun.* **2007**, *28*, 15-54. c) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128-1137. d) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.***2006**, 51-68.

68. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021.

69. Huisgen, R. *Angew. Chem. Int. Ed.* **1963**, *75*, 604-637, *Angew. Chem. Int. Ed.* **1963**, *2*, 565-598.

70. a) Tornoe, C. W.; Christsen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064. b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *114*, 2708-2711, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599. c) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem. Int. Ed.* **2005**, *117*, 2250-2255, *Angew. Chem. Int. Ed.* **2005**, *44*, 2210-2215.

71. Baskin, J. M.; Prescher, J. A.; Laughlin, S. T.; Agard, N. J.; Chang, P. V.; Miller, T. A.; Lo, A.; Codelli, J. A.; Bertozzi, C. R. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16793-16797.

72. Laughlin, S. T.; Baskin, J. M.; Amacher, S. L.; Bertozzi, C. R. *Science* **2008**, *320*, 664-667.

73. Wittig, G.; Krebs, A. *Chem. Ber. Recl.* **1961**, *94*, 3260-3275.

74. Ning, X. H.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255.

75. Sanders, B. C.; Friscourt, F.; Ledin, P. A.; Mbua, N. E.; Arumugam, S.; Guo, J.; Boltje, T. J.; Popik, V. V.; Boons, G. J. *J. Am. Chem. Soc.* **2011**, *133*, 949-957.

76. Hawker, C. J.; Fokin, V. V.; Finn, M. G.; Sharpless, K. B. *Aust. J. Chem.* **2007**, *60*, 381-383.

77. Barner-Kowollik, C.; Inglis, A. J. *Macromol. Chem. Phys.***2009**, *201*, 987-992.

78. a) Ladmiral, V.; Mantovani, G.; Clarkson, G. J.; Cauet, S.; Irwin, J. L.; Haddleton, D. M. *J. Am. Chem. Soc.* **2006**, *128*, 4823-4830. b) Mantovani, G.; Ladmiral, V.; Tao, L.; Haddleton, D. M. *Chem. Comm.* **2005**, 2089-2091. c) Johnson, J. A.; Lewis, D. R.; Diaz, D. D.; Finn, M. G.; Koberstein, J. T.; Turro, N. J. *J. Am. Chem. Soc.***2006**, *128*, 6564-6565. d) Luxenhofer, R.; Jordan, R. *Macromolecules* **2006**, *39*, 1709-1718.

79. Quemener, D.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Chem. Comm.***2006**, 5051-5053.

80. Golas, P. L.; Matyjaszewski, K. *Chem. Soc. Rev.* **2010**, *39*, 1338-1354.

81. Allen, C.; Maysinger, D.; Eisenberg, A. *Colloids Surf. B* **1999**, *16*, 3-27.

82. Letchford, K.; Burt, H. *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259-269.

83. a) Vonarbourg, A.; Passirani, C.; Saulnier, P.; Benoit, J. P. *Biomaterials* **2006**, *27*, 4356-4373. b) Kataoka, K.; Matsumoto, T.; Yokoyama, M.; Okano, T.; Sakurai, Y.; Fukushima, S.; Okamoto, K.; Kwon, G. S. *J. Controlled Release* **2000**, *64*, 143-153.

84. Gaucher, G.; Dufresne, M. H.; Sant, V. P.; Kang, N.; Maysinger, D.; Leroux, J. C. *J. Controlled Release* **2005**, *109*, 169-188.

85. Kabanov, A. V.; Nazarov, I. R.; Astafieva, I. V.; Batrakova, E. V.; Alakhov, V. Y.; Yaroslavov, A. A.; Kabanov, V. A. *Macromolecules* **1995**, *28*, 2303-2314.

86. Owen, S. C.; Chan, D. P. Y.; Shoichet, M. S. *Nano Today* **2012**, *7*, 53-65.

87. a) Elbert, D. L.; Hubbell, J. A. *Ann. Rev. Mater. Sci.***1996**, *26*, 365-394. b) Lee, J. H.; Lee, H. B.; Andrade, J. D. *Prog. Polym. Sci.* **1995**, *20*, 1043-1079.

88. a) Grayson, S. M.; Godbey, W. T. *J. Drug Targeting* **2008**, *16*, 329-356. b) Luxenhofer, R.; Bezen, M.; Jordan, R. *Macromol. Rapid Commun.* **2008**, *29*, 1509-1513.

89. Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. *Angew. Chem. Int. Ed.* **2010**, *49*, 6288-6308.

90. a) Pasut, G.; Veronese, F. M. *Prog. Polym. Sci.* **2007**, *32*, 933-961. b) Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10*, 1451-1458.

91. a) Torchilin, V. P. *J. Microencapsulation* **1998**, *15*, 1-19. b) Petersen, H.; Fechner, P. M.; Fischer, D.; Kissel, T. *Macromolecules* **2002**, *35*, 6867-6874.

92. a) Kabanov, A. V.; Chekhonin, V. P.; Alakhov, V. Y.; Batrakov, E. V.; Lebedev, A. S.; Melik-Nabarov, N. S.; Arzakhov, S. A.; Levashov, A. V.; Morozov, G. V. *Adv. Drug Delivery Rev.* **1995**, *16*, 295-309.

93. Herold, D. A.; Keil, K.; Bruns, D. E. *Biochem. Pharmacol.* **1989**, *38*, 73-76.

94. Inoue, T.; Chen, G.; Nakamae, K.; Hoffman, A. S. *J. Controlled Release* **1998**, *51*, 221-229.

95. a) van Natta, F. J.; Hill, J. W.; Carruthers, W. H. *J. Am. Chem. Soc.* **1934**, *56*, 455-459. b) Huang, S. *Biodegradable Polymers*. In: Mark F, Bikales N, Overberger C, Menges G, Kroshwitz J, editors. *Encyclopedia of Polymer Science & Engineering*. New York: John Wiley and Sons; **1985**, p. 220-243. c) Chandra, R.; Rustgi, R. *Prog. Polym. Sci.* **1998**, *23*, 1273-1275. d) Okada, M. *Prog. Polym. Sci.* **2002**, *27*, 87-133. E) Nair, L. S.; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32*, 762-798.

96. a) Hinterdorfer, P.; Dufrene, Y. F. *Nature Methods* **2006**, *3*, 347-355. b) Dufrene, P. Y.; Hinterdorfer, P. *Eur. J. Physiol.* **2008**, *256*, 237-245. c) Stroh, C.; Wang, H.; Bash, R.; Ashcroft, B.; Nelson, J.; Gruber, H.; Lohr, D.; Lindsay, S. M.; Hinterdorfer, P. *Proc. Natl. Acad. Sci.* **2004**, *34*, 12503-12507.

97. Azzam, T.; Eisenberg, A. *Angew. Chem. Int. Ed.* **2006**, *45*, 7443-7447.

98. Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728-1731.

99. Takizawa, K.; Tang, C.; Hawker, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 1718-1726.

100. Smart, T.; Lomas, H.; Massignani, M.; Flores-Merino, M. V.; Perez, L. R.; Battaglia, G. *Nanotoday* **2008,** *3*, 38-46.

101. Savic, R.; Luo, L.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615-618.

102. Guo, J.; Chen, G.; Ning, X.; Li, X.; Zhou, G.; Jagielski, J.; Xu, B.; Boons, G. J. *Chem. Eur. J.* **2012**, *18*, 4568-4574.

CHAPTER 2

STUDY OF PHYSICO-CHEMICAL PROPERTIES OF POLY (ETHYLENE GLYCOL)-*b*-POLYCAPROLACTONE BLOCK COPOLYMERS

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ABSTRACT

Block copolymers represent a subject of current research with emphasis ranging from the development of new synthetic strategies and molecular architectures to application of advanced theoretical methods. Almost sixty years after development of the first anionic polymerization method, scientific interests in these materials continues to grow, as does the market for block copolymer materials. Block copolymers are composed of two or more chemically distinct polymer chains linked together at one or more junction points through covalent bonds. In solution, block copolymers will form micelles, when the solvent is selective for one of the blocks. A wide variety of micelle morphologies are known and these depend on the polymer composition and the methods used to form micelles. One of the most attractive features of block copolymers is their ability to self- assemble into a variety of well-defined nanostructures with varied morphologies.[1] The focus of current research was to understand the role by the different block lengths of the block copolymer in defining the various sizes of the nanostructures. The differences in the sizes were studied with the aid of various instrumentation techniques such as dynamic light scattering (DLS) and transmission electron microscope (TEM). It was seen that, increasing the length of the hydrophobic block while maintaining the length and molecular weight of the hydrophilic block led to an increase in the size of the micelles obtained in solution. Study of the micelles using TEM confirmed that micelles formed were spherical and the sizes were consistent with those obtained by DLS. Use of atomic force microscopy (AFM) and strain promoted azide-alkyne cycloaddition revealed that the force required to pull apart a single polymer chain from the micelle was 47pN.

INTRODUCTION

Block copolymers are useful in many applications, where a variety of polymers are joined together to yield a material with hybrid properties. A block copolymer molecule contains two or more polymer chains attached at their ends. Linear block copolymers comprise two or more polymer chains in sequence, whereas a star-block copolymer comprises more than two linear block copolymers attached at a common branching point. Polymers containing at least three homopolymers attached at a common branch point have been termed multi-graft copolymers. Amphiphilic block copolymers are a class of block copolymers containing molecules which have an affinity for two different kinds of environment.[2] There has been a lot of interest in the development of degradable self-assembled compounds that will be suitable for drug delivery applications. Of the commonly employed copolymers, polyethylene glycol (PEG) is often chosen as the hydrophilic component and polycaprolactone (PCL) as the hydrophobic one. PEG is known to be biocompatible and impart "stealth" like character to the micelle, thus preventing its uptake by the reticuloendothelial system and enabling longer circulation times in the body. PCL undergoes degradation via ester hydrolysis under physiological conditions and is able to maintain neutral pH environment upon degradation. It also has high permeability to small drug molecules thus enabling its use for drug delivery.[3] As PEG is the commonly employed hydrophilic polymer for the synthesis of block copolymers to be employed in drug delivery, extensive clinical studies have been done on PEGylated polymers and that has helped in understanding both the strengths and weaknesses of the polymer.[1]

To better predict and control the properties of a micellar system, it is imperative that the copolymers utilized be well-defined. The nature of the polymer to be synthesized will determine the most appropriate polymerization technique to be employed for example anionic, cationic, ring opening or radical polymerization.[4] For the synthesis of block copolymers containing PEG

and PCL blocks, the most common method employed is the ring opening polymerization catalyzed by a transition metal for example stannous (II) octoate.[5] There are several advantages of using ring opening polymerization. One of the major advantages is the use of lower temperatures and shorter reaction times as compared to polycondensation techniques. A broad range of higher molecular weight polymers can be obtained under mild reaction conditions. Probably the most popular polymerization initiator for ROP of aliphatic polyester is tin (II) bis-(2-ethylhexanoate) also referred as tin octoate, ($Sn(Oct)_2$).Tin octoate is also efficient in the copolymerization of various lactones.[5]

Extensive reviews on experimental techniques suitable for block copolymer micelle characterization have been provided by Tuzar[6a], Zana[6b], and Hamley[6c]. The most commonly employed techniques include AFM, DLS, GPC, NMR and TEM to name a few. AFM has been demonstrated to be an invaluable technique for characterization of nanoscale structures at the surface of block copolymers. DLS has been exploited to study diffusion in polymer solutions. GPC works to separate the particles according to their effective size in solution. In the current project, polymers synthesized were characterized by GPC to determine the molecular weight distribution of the polymers. The amphiphilic block copolymers were self-assembled and dynamic light scattering was employed to study the sizes of the corresponding micelles, while transmission electron microscopy was used to visualize the morphology of the micelles. AFM was conducted on one of the micellar systems to study the morphology of the micelle in its native state as well as study the forces employed in maintaining the stability of the micelle.

RESULTS AND DISCUSSION

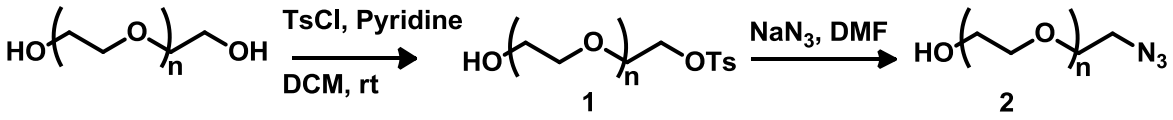
PART A: SYNTHESIS OF PEG-*block*-PCL COPOLYMERS

SYNTHESIS OF PEG DERIVATIVES

Controlled polymerizations result in polymers with well-defined end-groups, which can be subsequently converted into terminal azide functionality. The reaction shown in Scheme 2.1 demonstrates the conversion of the hydroxyl terminus of PEG into an azide terminal polymer. The introduction of the azide serves several purposes:

a. The azide can serve as a reactive center that can be used to click on several groups, thereby allowing for surface modification of the block copolymers
b. Reduction of the azide to amine followed by reaction with α-lipoic acid allows for the introduction of di-sulfide linkage that will help in the adhering the micelles to the gold surface during the AFM studies.

The azide functionality can be introduced by treating the alcohol with pyridine and 1.12 equivalents of *p*-toluenesulfonyl chloride (TsCl) to afford the easily substitutable tosyl-activated alcohol (**1**), which is subsequently reacted with sodium azide (NaN_3) to provide the terminal azide polymer (**2**). The activation of the alcohol via formation of the tosylate did give rise to a small amount of di-tosylated product (~ 10-15%), which when reacted with the sodium azide gave the diazido derivative as well (~ 10- 20%). The di azido derivative could be separated from the desired product via silica gel chromatography to afford the mono-azido derivative **2** in fairly good yields of 60-70% (Table 2.1). The presence of the terminal azide was proved conclusively by IR (Figure 2.1) which showed the characteristic *N=N=N* stretching vibration at 2100-2270 cm^{-1}. Furthermore, the ^{1}H-NMR spectrum showed a shift in the methylene protons adjacent to the azido group relative to the hydroxyl group in the starting material. The MALDI also showed characteristic (M-28) peak due to the loss of nitrogen molecule.



Scheme 2.1: Synthesis of Azido-PEG

Number average molecular weights, M_n ~ 1k, 2k and 3.5k were used in the current study.

Table 2.1: Synthesis of azido- PEG derivatives

No.	M_n of PEG	Tosyl activated alcohol	Terminal azide polymer	% Yield over 2 steps
1	1k	**1a**	**2a**	65%
2	2k	**1b**	**2b**	58%
3	3.5k	**1c**	**2c**	71%

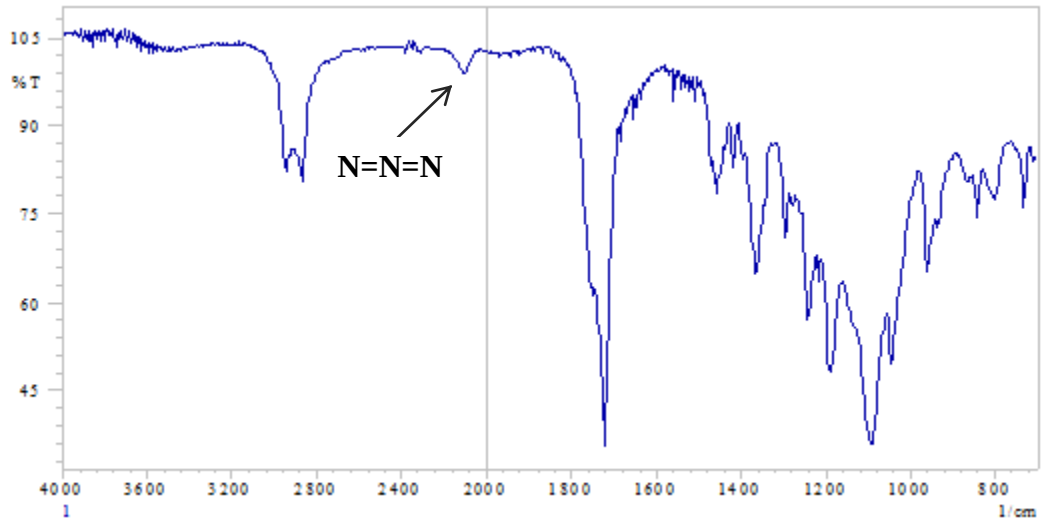
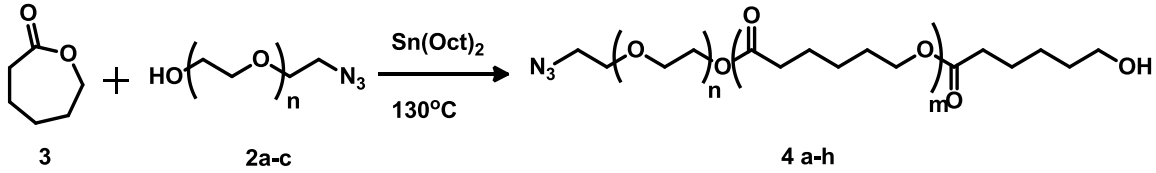


Figure 2.1: IR spectra of N_3-PEG_{1k}-OH

SYNTHESIS OF BLOCK COPOLYMERS OF PEG AND PCL

The copolymerization of PEG and polydisperse PCL was brought about by the use of catalytic amount of stannous octoate[7,8] at a temperature of 130^{o}C (Scheme 2.2). Molecular weights of

PCL ranging from 2k-6k were targeted. In this polymerization, desired molecular weights of the PCL were obtained by tuning the initiator/ monomer ratio. The corresponding block copolymers were characterized by NMR and GPC.



Scheme 2.2: Synthesis of PEG-*b*-PCL block copolymer

The molecular weights of the PEG and PCL that were targeted in this study are shown in Table 2.2 below.

Table 2.2: Library of PEG-*b*-PCL block copolymers

MW of PEG	MWs of PCL
1k	2k, 4k, 6k
2k	2k, 4k, 6k
3.5k	4k, 6k

The polymers obtained by ring opening polymerization were purified by precipitation from cold diethyl ether. The library of block copolymers of PEG-*b*-PCL with azido terminal moieties on the PEG was characterized by ^{1}H-NMR, FT-IR and GPC. The ^{1}H-NMR showed the presence of the newly formed ester linkages and the integration of the methylene protons adjacent to the ester linkages with respect to the PEG peaks confirmed that the reaction had gone to completion and the targeted molecular weights of the block copolymers had been achieved. These polymers showed good correlation between the molecular weights obtained from GPC and NMR as can be seen from Table 2.3. GPC analysis was performed on a Viscotek Gel Permeation chromatograph at 25^{o}C using narrow polystyrene standards and chloroform as the eluent at a flow rate of

1mL/minute. The GPC showed fairly narrow distribution of PDI for the block copolymers except for the polymers made from PEG of 1kDa molecular weight. We speculated that the intrinsic higher PDI of commercial PEG_{1k} might be responsible for such distribution. Also the chromatograph for the PEG_{2k}-*b*-PCL_{2k} showed a bimodal distribution and therefore accurate determination of the polydispersity could not be taken. The bimodal distribution most probably indicates the presence of homopolymer.

Table 2.3: GPC and NMR data correlation for library of PEG-*b*-PCL block copolymers

No.	**MW of PEG**	**Targeted MW of PCL**	**MW of PCL from NMR**	**MW of PCL from GPC**	**Polydispersity M_w/M_n**
4a	1kDa	2kDa	2.8kDa	5kDa	1.56
4b	1kDa	4kDa	4.4kDa	5.4kDa	1.43
4c	1kDa	6kDa	12.5kDa	10.4kDa	1.21
4d	2kDa	2kDa	1.4kDa	2.7kDa	N.D.
4e	2kDa	4kDa	3.5kDa	3.7kDa	1.30
4f	2kDa	6kDa	5.5kDa	6kDa	1.22
4g	3.5kDa	4kDa	5.5kDa	6.2kDa	1.20
4h	3.5kDa	6kDa	8kDa	8.7kDa	1.17

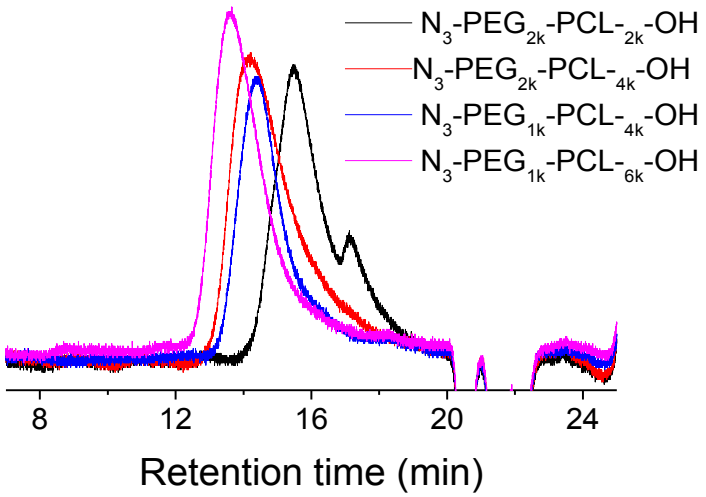


Figure 2.2: GPC of the different molecular weight of block copolymers.

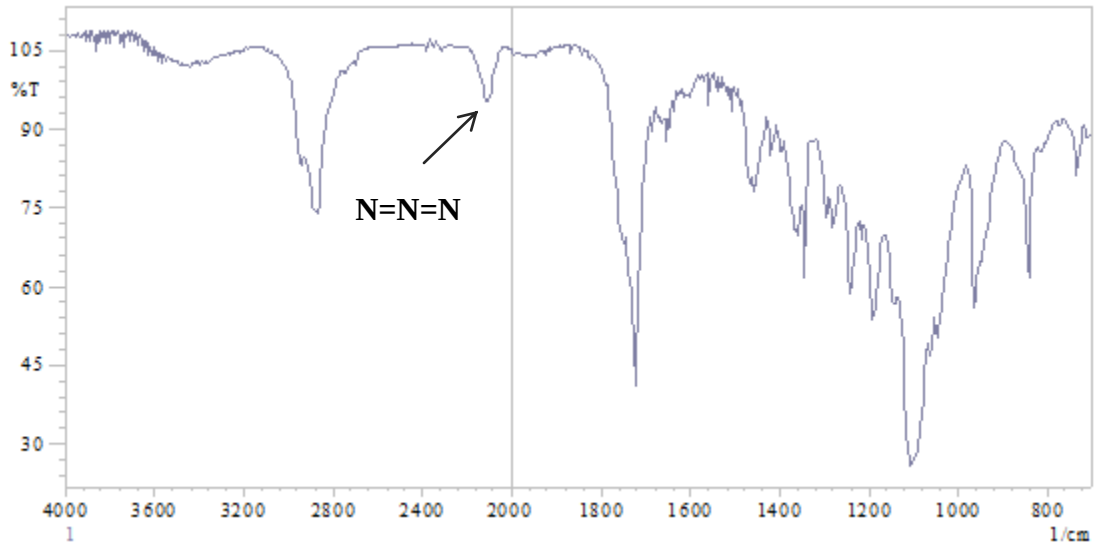
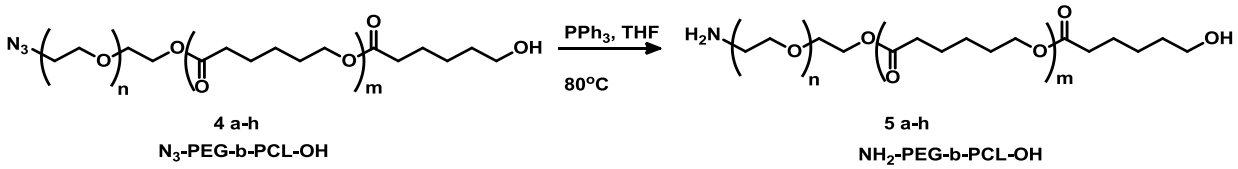


Figure 2.3: IR spectrum of N_3-$PEG_{3.5k}$-*b*-PCL_{4k} block copolymer

SYNTHESIS OF NH_2-PEG-*b*-PCL BLOCK COPOLYMERS

The azido group of the block copolymers **4a-h** was reduced to an amine using triphenylphosphine as the reducing agent to give **5a-h** as shown in Scheme 2.3. The amine group will provide a handle for further modifications and applications.



Scheme 2.3: Synthesis of Amine-terminated PEG-*b*-PCL block copolymer

The reduction of azide to amine was first attempted by transfer hydrogenation using Pd/ C and 1, 4-cyclohexadiene. However, the reduction was unsuccessful. The reaction never went to completion and only starting material remained. Therefore, reduction was performed using triphenylphosphine in water and tetrahydrofuran as solvent and heating the reaction mixture at 80^{o}C. The solvent was removed under vacuum and the product was dissolved in dichloromethane and co-prepicipated using diethyl ether. The reduction was successful and was confirmed by the disappearance of the azide stretching vibration at 2280^{-1} in the IR. The characteristic absorption for the amine occurs in the region 3300-3500cm^{-1}. However the OH stretching frequency is also in the same region and therefore the disappearance of the azide peak gave the confirmation of the reduction. The ninhydrin test was also positive for the amine, thereby confirming the conversion of the azide to the amine. The yields for the various copolymers are shown in Table 2.4.

Table 2.4: Reaction yields for the reduction of azide.

Compound No.	**MW of PEG-*b*-PCL**	**Yield**
5a	PEG_{1k}-*b*-PCL_{5k}	68%
5b	PEG_{1k}-*b*-$PCL_{5.4k}$	72%
5c	PEG_{1k}-*b*-$PCL_{10.4k}$	78%
5d	PEG_{2k}-*b*-$PCL_{2.7k}$	52%

5e	PEG_{2k}-*b*-$PCL_{3.7k}$	71%
5f	PEG_{2k}-*b*-PCL_{6k}	73%
5g	$PEG_{3.5k}$-*b*-$PCL_{6.2k}$	72%
5h	$PEG_{3.5k}$-*b*-$PCL_{8.7k}$	76%

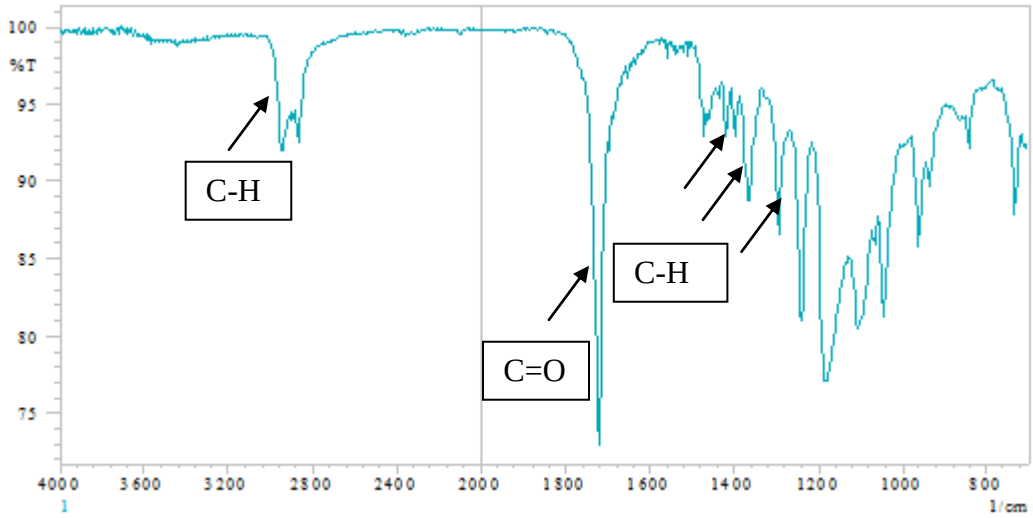
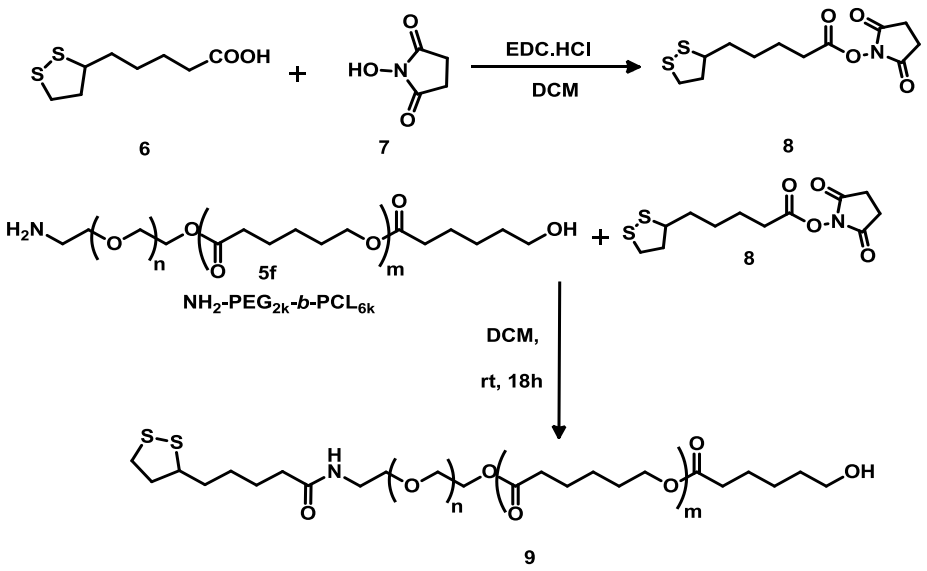


Figure 2.4: IR spectra of NH_2-$PEG_{3.5k}$-*b*-PCL_{4k} block copolymer**.**

SYNTHESIS OF α-LIPOIC ACID TERMINATED PEG-*b*-PCL BLOCK COPOLYMER

To study the micelle morphology and stability of the micelles, AFM technique was employed. In this study, we used AFM technique in conjunction with a novel copper free click approach[9] to construct chemi-mechanical maps by studying the force-displacement curves obtained by pulling apart a single polymer chain from the micelle. In order to perform high resolution AFM studies in native environment, we adhered the micelles to a gold substrate in solution using thiol-gold linkage and performed the scanning and force-displacement measurements. For this purpose, compound **9** was synthesized which had the lipoic acid at the polar PEG terminal for attachment to the gold surface (Scheme 2.4)**.** The synthesis began by the activation of the lipoic acid by

reaction with *N*-hydroxy succiniimide. The corresponding activated ester was then treated with the amine functionalized block copolymer **5f** under standard coupling conditions to furnish the lipoic acid terminated block copolymer in moderate yields.



Scheme 2.4: Synthesis of lipoic acid terminated PEG_{2k}-*b*-PCL_6

PART B: CHARACTERIZATION OF MICELLES

MICELLE FORMATION AND CHARACTERIZATION BY DYNAMIC LIGHT SCATTERING, TRANSMISSION ELECTRON MICROSCOPY

There are several reports in literature regarding the formation and purification of micelles from block copolymers.[7] Dialysis is a common technique where the poorly water soluble copolymer is dissolved in an organic solvent like THF. Initial study was conducted to determine the ideal organic solvent for dissolving the block copolymers. The procedure for micelle formation was as follows: 5mg of the block copolymer **4d** was dissolved in 1mL of organic solvent (acetone, acetonitrile, tetrahydrofuran, dimethlformamide and dimethyl sulfoxide) followed by the dropwise addition of 2mL nanopure water and stirring the solution for 5-10 minutes. This was followed by the addition of 8ml nanopure water and the solution was stirred further for 2-3minutes and the organic solvent was removed under vacuum. The solutions were next

centrifuged using a Millipore filter with a cutoff of 30kDa at 3000rpm at 4^{o}C and the sizes of the micelles were determined using dynamic light scattering (DLS). The results are shown in Figure 2.5

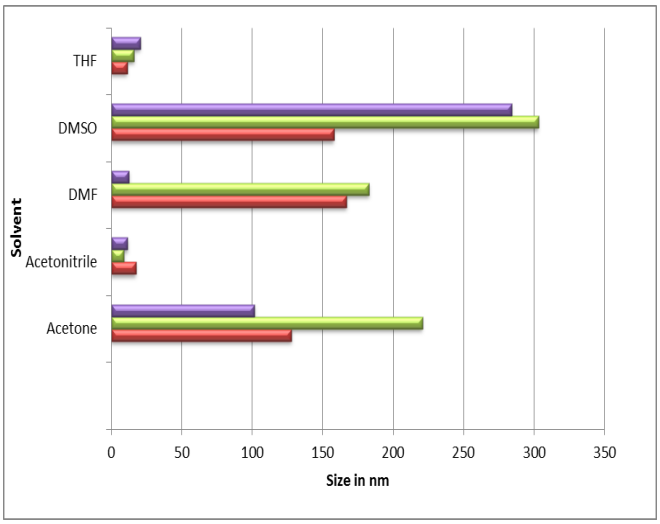


Figure 2.5: Sizes of the micelles obtained using different organic solvents

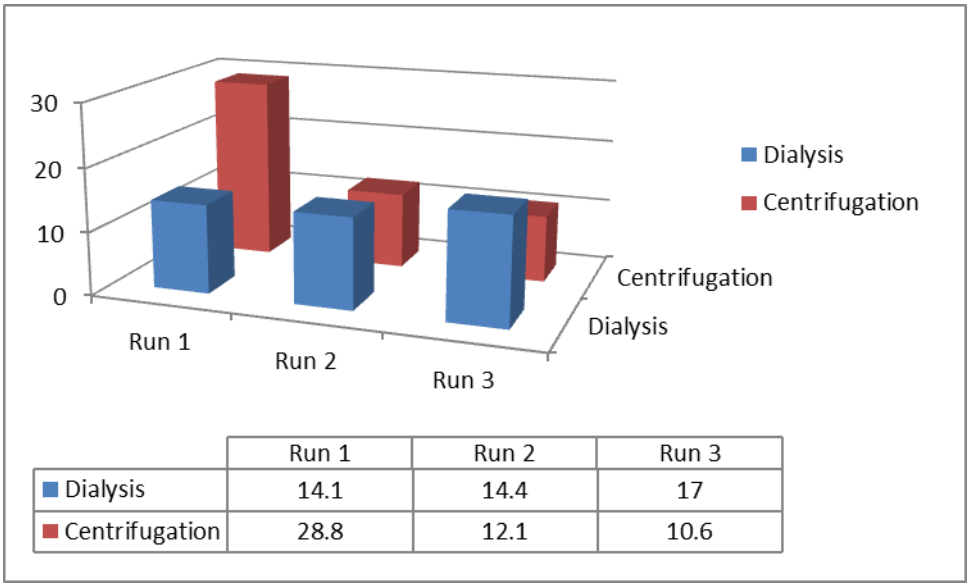
Table 2.5: Sizes of micelles obtained after DLS

Solvent	Run 1	Run 2	Run 3
THF	11.1	16	25
DMSO	157.8	303	283.9
DMF	23.6	167.4	6.4
Acetonitrile	17.3	8.9	11.5
Acetone	93.4	13.5	9.1

From the initial study conducted, it was seen that THF was the best organic solvent to induce micellization and therefore THF was used as the solvent for further studies.

The next study was conducted block copolymer **4d** to identify the best method for inducing micellization. The block copolymer solution required for centrifugation was prepared as described before. After centrifugation, the solution was filtered through 0.45µ filter. For the study by dialysis, 2.5mg of **4d** was dissolved in 0.5mL in THF followed by drop wise addition of

2mL of nanopure water. The solution was stirred overnight and then dialyzed against nanopure water using a pre-swollen semipermeable membrane (cut off 12-14kDa), filtered through 0.45µ filter. The resulting micelles were characterized by dynamic light scattering; the results are shown in Figure 2.6.



	Run 1	Run 2	Run 3
Dialysis	14.1	14.4	17
Centrifugation	28.8	12.1	10.6

Figure 2.6: Comparison of methods: dialysis and centrifugation

Based on the study conducted, it was observed that the sizes of the micelles obtained via dialysis were more consistent in all the three runs and therefore, dialysis was chosen as the method of choice.

The micelles from the corresponding block copolymers were prepared by drop-wise addition of THF solution of block copolymer in to nanopure water (1 % v/v). The final mixture was exposed to air to allow evaporation of THF and formation of micelles. The micellar solution was dialyzed against nanopure water using a pre-swollen semipermeable membrane (cut off 12-14 kDa), filtered through a 0.45µ syringe filter. The resulting micelles were characterized by various methods, including DLS and TEM. When the sizes of the micelles obtained from the DLS were analyzed, there was a slight increase in size with the increase of hydrophobic, PCL length, while

a significant increase in the size was observed with the decrease of hydrophilic - PEG length, as seen in Figure 2.7a. A good correlation between the sizes obtained from DLS to that of TEM was observed as shown in Figure 2.7b using micelles made from PEG_{2k}-*b*-$PCL_{7.5k.}$ It was seen that most of the micelles were stable when stored at 4^{o}C. However, after two weeks of storage, the micelles were no longer stable and the polymers started to precipitate out.

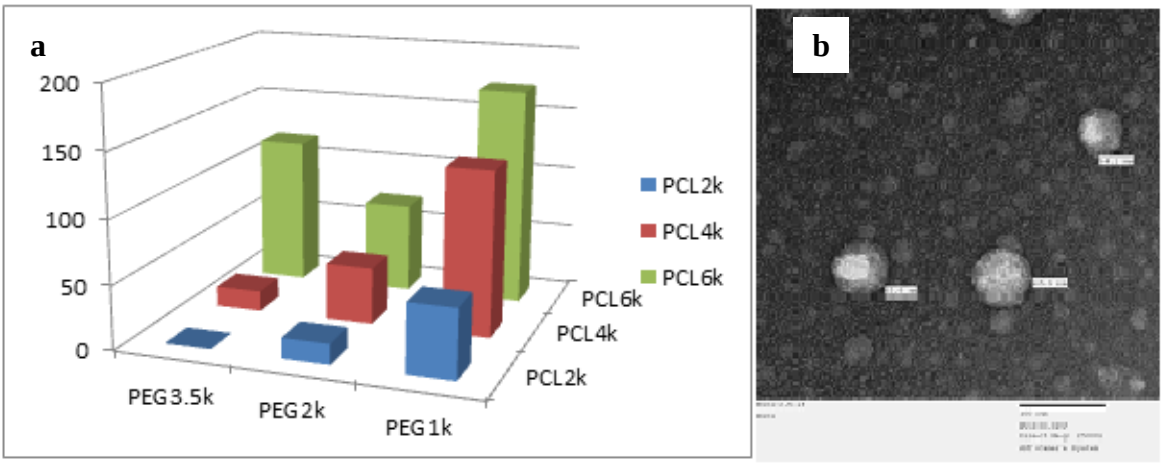


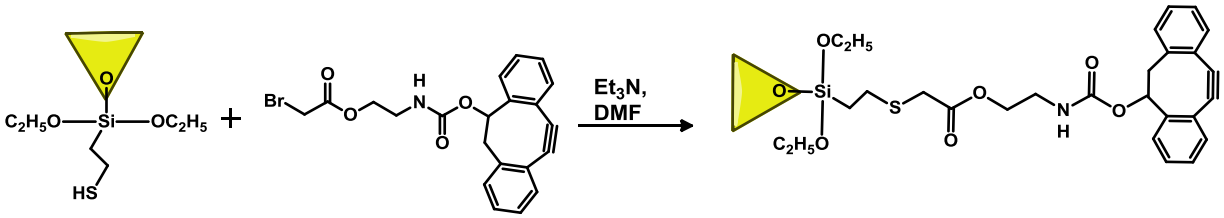
Figure 2.7: Results of the characterization of micelles using DLS and TEM a) Sizes of the micelles obtained in water using DLS measurements. Effect on size with the variation of PEG and PCL molecular weights, b) TEM image of PEG_{2k}-$PCL_{7.5k}$.

Table 2.6: Sizes of the micelles obtained by DLS

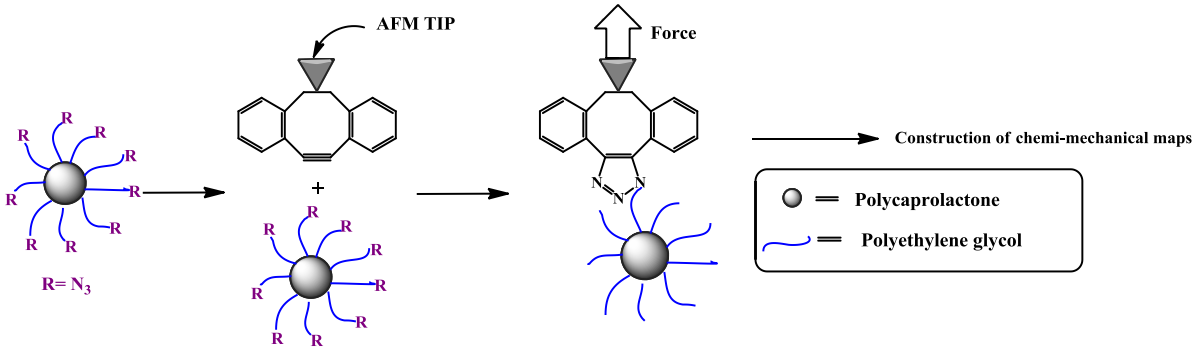
Number	PEG-*b*-PCL block copolymer	Micelle size from DLS
4a	PEG_{1k}-*b*-PCL_{5k}	68.1 $\pm$ 10
4b	PEG_{1k}-*b*-$PCL_{5.4k}$	130.7 $\pm$ 5
4c	PEG_{1k}-*b*-$PCL_{10.4k}$	232.5 $\pm$ 12
4d	PEG_{2k}-*b*-$PCL_{2.7k}$	15.5 $\pm$ 2
4e	PEG_{2k}-*b*-$PCL_{3.7k}$	44.7 $\pm$ 10
4f	PEG_{2k}-*b*-PCL_{6k}	71.3 $\pm$ 6
4g	$PEG_{3.5k}$-*b*-$PCL_{6.2k}$	15.7 $\pm$ 2
4h	$PEG_{3.5k}$-*b*-$PCL_{8.7k}$	43.9 $\pm$ 12

CHARACTERIZATION OF MICELLES BY AFM

The AFM studies were done in collaboration with Dr. Bingqian Xu's laboratory. For the AFM experiments, the AFM tips had to be modified with DIBO derivative (Scheme 2.6) in order to detect the surface azido groups of the block copolymer. In order to modify the tips, they were first cleaned by UV for 30 min and then coated with a magnetic film by the e-beam deposition. The tips were then immediately placed in a small container in a glass desiccator filled with argon. Next, (3-mercaptopropyl)triethoxysilane (20 mL) and N,N-diisopropylethylamine (10 mL) (Scheme 2.5) were added to the small containers; and then the desiccator was placed under a reduced pressure at 1 torr for 60 min.[9,11] The organosilicon coated tips were washed with hexane for 15 min using sonication and then immersed in the solution of the DIBO derivative (8.8 mg, 7.15 mmol) in DMF (400 mL) and triethylamine (5 mL) for 5 h. Finally, the tips were rinsed several times with water and kept in pure water at 48°C.



Scheme 2.5: Modification of AFM Tip with DIBO moiety



Scheme 2.6: Schematic illustration of the DIBO modified tip and its use in AFM studies

The micelles were made in the similar fashion as before except a mixture of 5% lipoic acid-PEG_{2k}-*b*-PCL_{6K} and 95% N_3-PEG_{2k} -*b*-PCL_{6k} was used. A fresh thermal evaporated gold surface was annealed by hydrogen flame, then immediately covered with a nanoparticle solution (1 mg/mL) for 2 h at 4 ºC. The surface was rinsed three times with 18 MΩ DI water and then examined by the AFM. As shown in Figure 2.6, the micelles were evenly distributed on gold surface. Individual micelle was clearly resolved from both topography (Figure 2.8a) and phase image (Figure 2.8b). Through measuring the diameters of multiple micelles, the histogram was built and its Gaussian fitting gave out the most probable value of micelle diameter of 61.40 ± 2.27 nm which were very close to the sizes obtained by DLS and TEM. For the force pulling experiment, 10-fold diluted solution of the micelles was prepared and the AFM cantilevers were stretched under several different pulling rates, ranging from 300-400nms^{-1}.It was seen that the force required to pull out a block copolymer molecule from the micelle required a force of 47$\pm$1pN at the nominal rate of 40nN/s (Figure 2.8d).

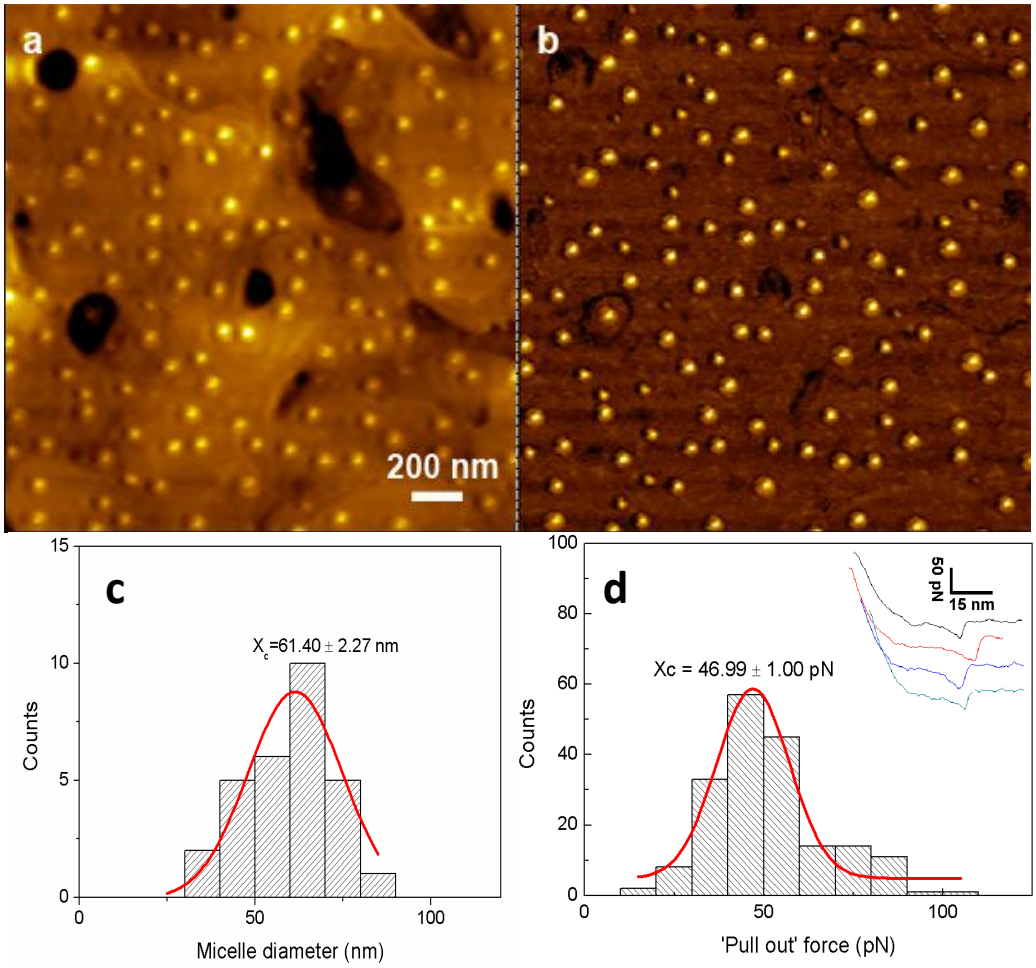


Figure 2.8: AFM measurements on micelles adhered on a gold surface: a) topography image, b) phase image, c) the distribution of the micelle diameter and most probable value from Gaussian fitting, d) force histogram and most probable 'pull out' force, the typical force distance curves were exampled in the inset.

SUMMARY AND FUTURE OUTLOOK

The synthesis of block copolymers of PEG and PCL was successfully performed by ring opening polymerization using stannous (II) octoate as the initiator. The polymerization proceeded well and gave the library of well-defined block copolymers in quantitative yield. The characterization of the block copolymers by NMR and GPC showed that the molecular weights achieved were in good correlation. The self-assembly of micelles was done using dialysis against

nanopure water and by dissolving the block copolymers in THF. The shapes of the micelles were mostly spherical as confirmed by the TEM and AFM studies. The study conducted on the PEG-*b*-PCL copolymers has shown a direct correlation of molecular weight of PCL to size and an inverse correlation with the PEG molecular weight. The use of the force displacement experiment helped in providing insights about the hydrophobic forces that play an important role in the stability of the micelles.

Future studies will focus on modification of hydrophilic PEG with a polysaccharide to explore the effect on morphology and stability of the micelles. Substitution of azide with oxime will be carried out to explore oxime-DIBO click chemistry for such applications.

EXPERIMENTAL SECTION

MATERIALS:

All reagents such as *p*-toluenesulfonyl chloride, sodium azide and solvents such as pyridine, dimethyl formamide, dichloromethane, toluene and methanol were purchased from Sigma Aldrich Chemical Co. α- lipoic acid was purchased from Alfa Aesar. Dialysis membrane Spectra /Pro 2 (molecular weight cutoff 12-14kDa) was purchased from Spectrum laboratories. PEGs (MW 1000, 2000, and 3500) were co-evaporated with toluene before use. ε-caprolactone was vacuum distilled over CaH_2 before use. Stannous octoate (Sn $(Oct)_2$, 95%) was used without further purification.

INSTRUMENTATION: Gel Permeation Chromatography (GPC) was performed on Viscotek Gel Permeation chromatography equipment and the molecular weights and the polydispersity index (PDI), M_w/M_n of the polymer samples was determined with respect to polystyrene standards (Viscotek, Malvern Inc.). ^{1}H and ^{13}C-Nuclear Magnetic Resonance was obtained on a Varian Inova-300 (300MHz) and Varian Inova- 500 (500MHz) at $300^{o}K$ equipped with Sun

workstations. Mass spectra were obtained on an Applied Biosystems Voyager DE-Pro Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF). Fourier Transform – Infrared (FT-IR) spectra were obtained on a Shimadzu IR Prestige-21 spectrophotometer. Transmission Emission Microscopy studies were done on Philips/FEI Tecnai-20 instrument at an accelerating voltage of 200kV. Dilute solutions of the micelles in water were deposited on copper grids coated with carbon (Electron Microscopy Science, Hatfield, PA), followed by staining with 4% aqueous uranyl acetate for 3 minutes. Dynamic Light Scattering (DLS) experiments were performed on a Zeta Potential and Particle Size Analyzer (ZetaPALS, Brookhaven Instruments Corp., US). Measurements were made at 25^{o}C at a scattering angle of 90^{o}. An Agilent 5500 Atomic Force Microscopy (AFM) system equipped with an inverted light microscope system (Agilent, Chandler, AZ) was used for the AFM experiments. An Agilent multipurpose AFM scanner was applied for scanning an area of $10\mu m^2$. CS-10 silicon AFM probe was purchased from NanoScience Instruments.Silicon cantilever tips with a spring constant of 0.1 Nm^{-1} were used for all the experiments, which were performed in aqueous solution under magnetic AC mode.

General procedure for preparation of micelles:

Centrifugation: A mixture of the block copolymer (5mg) in organic solvent (1mL) was slowly added to the nanopure water (2mL) and stirred for 5- 10 minutes. To the solution, 8mL nanopure water was added and the solution was stirred for 2-3 minutes. The organic solvent was removed under vacuum and the solution was centrifuged using a Millipore filter having a cut off of 30kDa at 3000 rpm and 4^{o}C.

Dialysis: A mixture of the block copolymer (2.5mg) in THF (0.5mL) was slowly added to the nanopure water (2mL). The final mixture was opened to air overnight, allowing slow evaporation

of THF and formation of micelles, then dialyzed against 2L of nanopure water (pre-swollen semi-permeable membrane: cut off 12-14kDa) for 5h, with the water being changed every hour.

Dynamic Light Scattering (DLS). DLS measurements were performed on a Zeta Potential and Particle Size Analyzer (ZetaPALS, Brookhaven Instruments Corp., US). Dust-free vials were used for the aqueous solutions. Measurements were made at 25°C with a scattering angle of 90°. For each sample, five replicates were obtained to determine the average size and size distribution. 500μL of the sample solution was added to 1.5mL of distilled water for DLS study.

TEM Characterization of the Micelles. TEM observations were made using a Philips/FEI Tecnai 20 instrument operating at an accelerating voltage of 200 kV. Dilute solutions of the micellar solution (as prepared for DLS) were deposited on copper grids coated with carbon (Electron Microscopy Science (EMS), Hatfield, PA). Excess solvent was removed by touching the edge of the grids with a small piece of filter paper (Whatman-1). For staining, a drop of 4% uranyl acetate solution (freshly prepared in nanopure water and filtered through 0.45μ filter membrane) was added to the dry samples on the grid. After staining for 2min, the excess reagent was removed using filter paper.

Preparation of micelles for AFM study: A mixture of **4e** and **9** (9:1 w/w) (10mg) in THF (0.8mL) was slowly added to nanopure water (15mL) The final mixture was exposed to air overnight to allow evaporation of THF and formation of micelles. The resulting solution was then dialyzed against nanopure water (2.0 L) using a pre-swollen semipermeable membrane (cutoff 12000–14 000 Da) for 4 h, and the water was replaced every hour.

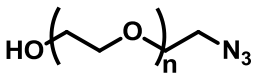
AFM sample preparation: A fresh thermal evaporated gold surface was annealed by a hydrogen flame for 2 minutes. The gold surface was immediately covered by a drop of the

micelle solution (1mg/mL) and stored at 4^{o}C for 4 h. The surface was rinsed with 18 MΩ distilled water (3 times) and then examined by AFM.

AFM experiemental procedure: An Agilent 5500 AFM system equipped with an inverted light microscope (ILM) system (Agilent, Chandler, AZ) was employed for scanning an area of 10mm. Silicon cantilever tips with a nominal spring constant of 0.1Nm^{-1} were used throughout the experiments. All images were collected in water using recognition imaging module based on Agilent magnetic AC (MAC) mode with a magnetically coated lever.

SYNTHETIC PROCEDURES:

Synthesis of N_3-PEG-OH (2a)

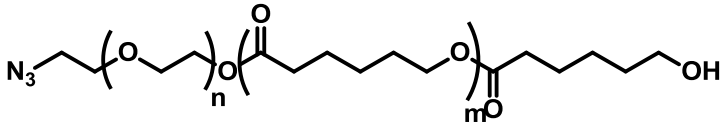


Tosyl chloride (2.1g, 11mmol) was added to a solution of polyethylene glycol (10g, number average molecular weight, M_n ~ 1000Da, 10 mmol) dissolved in dichloromethane (100 mL) and pyridine (4 mL). The reaction mixture was stirred at room temperature under an atmosphere of argon. The solvents were evaporated and the residue (**1a)** was dissolved in DMF (30 mL) and sodium azide (2 g) was added and the resulting mixture was stirred at 80^{o}C for 12h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 10/1, v/v) to give **2a** as a yellow solid (10.4g, 65% yield over two steps). FT-IR: 2880 (C-H), 2120 (N=N=N), 1455, 1341, 1283 (C-H), 1100 (C-O) cm^{-1}. H^{1}NMR ($CDCl_3$, 300 MHz) δ 3.69 (2H, t, CH_2OH), 3.65-3.68 (180H, m, CH_2O), 3.38 (2H, m, CH_2N_3); ^{13}CNMR ($CDCl_3$, 300MHz) δ 72.43 (CH_2), 70.56 (CH_2), 70.52 (CH_2), 70.46 (CH_2), 70.21 (CH_2), 69.91 (CH_2), 61.51 (CH_2OH), 50.54 (CH_2N_3).

Synthesis of N_3-PEG-OH (2b) Tosyl chloride (1.05g, 5.5mmol) was added to a solution of polyethylene glycol (10g, number average molecular weight, M_n ~ 2000Da, 5mmol) dissolved in dichloromethane (100 mL) and pyridine (2 mL). The reaction mixture was stirred at room temperature under an atmosphere of argon. The solvents were evaporated and the residue was dissolved in DMF (80 mL) and sodium azide (17 g) was added and the resulting mixture **1b** was stirred at 80^{o}C for 12h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 10/1, v/v) to give **2b** as a yellow solid (3.0g, 58% yield over two steps). FT-IR: 2880 (C-H), 2120 (N=N=N), 1455, 1341, 1283 (C-H), 1100 (C-O) cm^{-1}. H^1NMR ($CDCl_3$, 300 MHz) δ 3.69 (2H, t, CH_2OH), 3.65-3.68 (180H, m, CH_2O), 3.38 (2H, m, CH_2N_3); ^{13}CNMR ($CDCl_3$, 300MHz) δ 72.43 (CH_2), 70.56 (CH_2), 70.52 (CH_2), 70.46 (CH_2), 70.21 (CH_2), 69.91 (CH_2), 61.51 (CH$_2$OH), 50.54 (CH$_2$N$_3$).(**Appendix A, Figure 4.1)**

Synthesis of N_3-PEG-OH (2c) Tosyl chloride (1.5g, 7.9mmol) was added to a solution of polyethylene glycol (25g, number average molecular weight, M_n ~ 3500Da) dissolved in dichloromethane (100 mL) and pyridine (3 mL). The reaction mixture was stirred at room temperature under an atmosphere of argon. The solvents were evaporated and the residue was dissolved in DMF (100 mL) and sodium azide (0.409g) was added and the resulting mixture (**1c**) was stirred at 80^{o}C for 12h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH, 10/1, v/v) to give **2c** as a yellow solid (18.13g, 71% yield over two steps). FT-IR: 2880 (C-H), 2120 (N=N=N), 1455, 1341, 1283 (C-H), 1100 (C-O) cm^{-1}. H^1NMR ($CDCl_3$, 300 MHz) δ 3.69 (2H, t, CH_2OH), 3.65-3.68 (180H, m, CH_2O), 3.38 (2H, m, CH_2N_3); ^{13}CNMR ($CDCl_3$, 300MHz) δ 72.43 (CH_2), 70.56 (CH_2), 70.52 (CH_2), 70.46 (CH_2), 70.21 (CH_2), 69.91 (CH_2), 61.51 (CH$_2$OH), 50.54 (CH$_2$N$_3$).

Synthesis of N_3-PEG-*b*-PCL copolymer (4a)



ε-caprolactone monomer (0.44 mL, 4.0mmol) was added to a flask containing dried N_3-PEG-OH (**2a**) (0.250 g, 0.25 mmol) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.162g, 0.4 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, to the resulting viscous colorless oil MeOH (3mL) was added while cooling to room temperature to stop the polymerization. The polymer was then precipitated from cold ether (7 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.32g, ~ 48% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4a**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (21H, m, $CH_2CH_2CH_2O$), 3.80-3.58(281H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (20H, m, CH_2C=O), 1.65-1.55 (51H, m, CH_2), 1.30-1.22 (18H, m, CH_2). Molecular Weight (MW) (NMR) = 5400Da. M_n (GPC): 6000Da and PDI = 1.43.

Synthesis of N_3-PEG-*b*-PCL copolymer (4b) ε-caprolactone monomer (0.89 mL, 8.0mmol) was added to a flask containing dried N_3-PEO-OH (**2a**) (0.250 g, 0. 25 mmol) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.324g, 0.8 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, 5mL MeOH was added to the resulting viscous colorless oil and cooled to room temperature. The polymer

was then precipitated from cold ether (12 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (1.2g, ~ 99% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4b**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (40H, m, $CH_2CH_2CH_2O$), 3.80-3.58(177H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (33H, m, CH_2C=O), 1.65-1.55 (94H, m, CH_2), 1.30-1.22 (39H, m, CH_2). MW (NMR) = 3800Da. Mn (GPC): 6000Da and PDI = 1.56.

Synthesis of N_3-PEG-*b*-PCL copolymer (4c) ε-caprolactone monomer (1.78 mL, 16.0mmol) was added to a flask containing dried N_3-PEO-OH (**2a**) (0.250 g, 0. 25 mmol) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.648g, 1.6 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, 10mL MeOH was added to the resulting viscous colorless oil and it was cooled to room temperature. The polymer was then precipitated from cold ether (25 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (1.8g, ~ 90% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4c**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (104H, m, $CH_2CH_2CH_2O$), 3.80-3.58(76H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (104H, m, CH_2C=O), 1.65-1.55 (242H, m, CH_2), 1.30-1.22 (105H, m, CH_2). MW (NMR) = 13,500Da. Mn (GPC): 10,400Da and PDI = 1.21.

Synthesis of N_3-PEG-*b*-PCL copolymer (4d) The block polymers were synthesized by a ring opening polymerization at 130 °C under a stream of argon as previously reported[1] with some modifications. Briefly, ε-caprolactone monomer (0.22 mL, 2.0mmol) was added to a flask

containing dried N_3-PEG-OH (**2b**) (0.250 g, 0.125 mmol) and toluene (5mL) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.008g, 0.2 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, the resulting viscous colorless oil was dissolved in MeOH (2mL) and cooled to room temperature. The polymer was then precipitated from cold ether (4 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.46g, ~ 96% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4d**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (22H, m, $CH_2CH_2CH_2O$), 3.80-3.58(44H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (23H, m, CH_2C=O), 1.65-1.55 (43H, m, CH_2), 1.30-1.22 (22H, m, CH_2). MW (NMR) = 1348Da, Mw (GPC): 2700Da and PDI = 1.06.

Synthesis of N_3-PEG-*b*-PCL copolymer (4e) ε-caprolactone monomer (0.44 mL, 4.0mmol) was added to a flask containing dried N_3-PEG-OH (**2b**) (0.250 g, 0.125 mmol) and toluene (9mL) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.162g, 0.4 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, the resulting viscous colorless oil was dissolved in MeOH (4.3mL) and cooled to room temperature. The polymer was then precipitated from cold ether (5.3 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.79g, ~ 98% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4e**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (47H, m, $CH_2CH_2CH_2O$), 3.80-

3.58(257H, m, CH_2O), 3.36 (2H, m, CH_2N$_3$), 2.36-2.20 (48H, m, CH_2C=O), 1.65-1.55 (103H, m, CH_2), 1.30-1.22 (53H, m, CH_2). MW (NMR) =5500Da. M_n (GPC): 5700Da and PDI = 1.30.

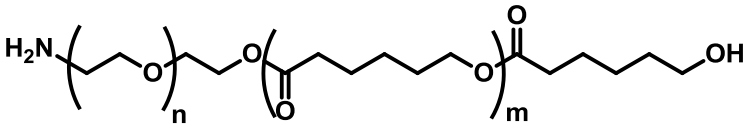
Synthesis of N_3-PEG-*b*-PCL copolymer (4f) ε-caprolactone monomer (0.89 mL, 8.0mmol) was added to a flask containing dried N_3-PEG-OH (**2b**) (0.250 g, 0.125 mmol) and toluene (9 mL) and the resulting mixture was placed under an argon atmosphere and then Sn(Oct)$_2$ (0.324g, 0.8 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, the resulting viscous colorless oil was dissolved in MeOH (6 mL) and cooled to room temperature. The polymer was then precipitated from cold ether (15 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.46g, ~ 97% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4f**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (66H, m, $CH_2CH_2CH_2O$), 3.80-3.58(178H, m, CH_2O), 3.36 (2H, m, CH_2N$_3$), 2.36-2.20 (78H, m, CH_2C=O), 1.65-1.55 (184H, m, CH_2), 1.30-1.22 (78H, m, CH_2). MW (NMR) = 7500Da. M_n (GPC): 8000Da and PDI = 1.22.

Synthesis of N_3-PEG-*b*-PCL copolymer (4g) ε-caprolactone monomer (0.253 mL, 2.29 mmol) was added to a flask containing dried N_3-PEG-OH (**2c**) (0.250 g, 0.07 mmol) and the resulting mixture was placed under an argon atmosphere and then Sn(Oct)$_2$ (0.081g, 0.06 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, the resulting viscous colorless oil was dissolved in MeOH (1mL) and cooled to room temperature. The polymer was then precipitated from cold ether (2.6 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.21g,

~ 85% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4g**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (48H, m, $CH_2CH_2CH_2O$), 3.80-3.58(375H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (50H, m, CH_2C=O), 1.65-1.55 (217H, m, CH_2), 1.30-1.22 (53H, m, CH_2). MW (NMR) = 5500Da. M_n (GPC): 6200Da and PDI = 1.20.

Synthesis of N_3-PEG-*b*-PCL copolymer (4h) ε-caprolactone monomer (0.51 mL, 4.6mmol) was added to a flask containing dried N_3-PEG-OH (**2c**) (0.250 g, 0.07 mmol) and the resulting mixture was placed under an argon atmosphere and then $Sn(Oct)_2$ (0.162g, 0.4 mmol) was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 135 °C with vigorous stirring. After 24 h, the resulting viscous colorless oil was dissolved in MeOH (1mL) and cooled to room temperature. The polymer was then precipitated from cold ether (5 mL). The white precipitate was collected by filtration and dried *in vacuo* at room temperature to give the products as a white solid (0.62g, ~ 82% yield). The degree of polymerization and the PDI was determined by GPC. Compound **4h**: FT-IR: 2880 (C-H), 2098 (N=N=N), 1728 (C=O), 1455, 1341, 1283 (C-H), 1100(C-O) cm-1. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 4.10-4.02 (70H, m, $CH_2CH_2CH_2O$), 3.80-3.58(467H, m, CH_2O), 3.36 (2H, m, CH_2N_3), 2.36-2.20 (76H, m, CH_2C=O), 1.65-1.55 (170H, m, CH_2), 1.30-1.22 (75H, m, CH_2). MW (NMR) = 8000Da. Mn (GPC): 8700Da and PDI = 1.17.

Synthesis of NH_2-PEG-*b*-PCL-OH (5a):



Triphenylphosphine (0.098 g, 0.37 mmol) was added to a solution of **4a** (1.00 g, ~0.33 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of

argon at 80 ^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5a** as a white amorphous solid (0.64 g, 63%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (21H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (281H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (20H, m, CH_2C=O), 1.75-1.55 (51H, m, CH_2), 1.50-1.30 (18H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5b): Triphenylphosphine (0.059 g, 0.224 mmol) was added to a solution of **4b** (1.00 g, ~0.2 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80 ^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5b** as a white amorphous solid (0.95 g, 95%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (40H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (177H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (33H, m, CH_2C=O), 1.75-1.55 (94H, m, CH_2), 1.50-1.30 (39H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5c): Triphenylphosphine (0.042 g, 0.16 mmol) was added to a solution of **4c** (1.00 g, ~0.143 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed

under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5c** as a white amorphous solid (0.84 g, 83%). FT-IR: 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (104H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (76H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (104H, m, CH_2C=O), 1.75-1.55 (242H, m, CH_2), 1.50-1.30 (015H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5d): Triphenylphosphine (0.07 g, 0.28 mmol) was added to a solution of **4d** (1.00 g, ~0.25mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5d** as a light-yellow amorphous solid (0.92 g, 91%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (22H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (44H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (23H, m, CH_2C=O), 1.75-1.55 (43H, m, CH_2), 1.50-1.30 (22H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5e): Triphenylphosphine (0.049 g, 0.19 mmol) was added to a solution of **4e** (1.00 g, ~0.167 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of

dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5e** as a light-yellow amorphous solid (0.82 g, 81%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (47H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (257H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (48H, m, CH_2C=O), 1.75-1.55 (103H, m, CH_2), 1.50-1.30 (53H, m, CH_2).

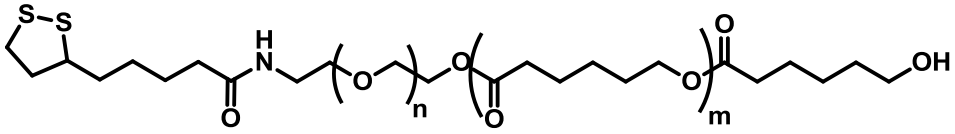
Synthesis of NH_2-PEG-*b*-PCL-OH (5f): Triphenylphosphine (0.037 g, 0.14 mmol) was added to a solution of **4f** (1.00 g, ~0.125 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5f** as a light-yellow amorphous solid (0.90 g, 89%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (66H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (178H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (78H, m, CH_2C=O), 1.75-1.55 (184H, m, CH_2), 1.50-1.30 (78H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5g): Triphenylphosphine (0.04 g, 0.15 mmol) was added to a solution of **4g** (1.00 g, ~0.133 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column

chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5g** as a yellow amorphous solid (0.82 g, 81%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (48H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (375H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (50H, m, CH_2C=O), 1.75-1.55 (217H, m, CH_2), 1.50-1.30 (53H, m, CH_2).

Synthesis of NH_2-PEG-*b*-PCL-OH (5h): Triphenylphosphine (0.0001 g, 0.19 mmol) was added to a solution of **4h** (1.00 g, ~0.105 mmol) in THF (40 mL). Next, water (2 mL) was added and the mixture stirred under an atmosphere of argon at 80^{o}C for 12 h. The solvent was removed under reduced pressure and the residue was redissolved in a small amount of dichloromethane/methanol (1:1, v/v) and purified by LH20 size exclusion column chromatography using dichloromethane/methanol (1:1, v/v) as eluent. Fractions containing pure polymer were collected and combined, and the solvent was removed under reduced pressure to obtain **5h** as a light-yellow amorphous solid (0.78g, 79%). FT-IR: 3300-3500 (NH_2), 2880 (C-H), 1722 (C=O), 1455, 1341, 1283 (C-H), 1100 (C-O). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (70H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (467H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (76H, m, CH_2C=O), 1.75-1.55 (170H, m, CH_2), 1.50-1.30 (75H, m, CH_2).

Synthesis of α-Lipoic acid terminated PEG-*b*-PCL block copolymer (9):



α-Lipoic acid (**6**) (1.3mg, 0.006 mmoles), *N*-hydroxy succinimide (**7**) (1.1 mg, 0.007 mmoles) and *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC.HCl) (2.4mg, 0.0125 mmol) were dissolved in DCM (1 mL) and stirred at room temperature under argon for

an hour. After one hour, add the reaction mixture to a solution of **5e (**50 mg, 0.00625 mmoles) under argon at 4^{o}C drop wise over a period of 35minutes. The reaction mixture was warmed to room temperature and stirred for 18 h. The product (**9**) was dissolved in MeOH (1mL)and coprecipitated using cold ether (2 mL) to obtain the product as yellow amorphous solid (42 mg, 85%). ^{1}H NMR ($CDCl_3$, 300MHz) δ 4.10-4.00 (66H, m, $CH_2CH_2CH_2O$), 3.70-3.58 (178H, m, CH_2O), 3.42-3.35 (2H, m, $H_2NCH_2CH_2O$), 2.40-2.20 (78H, m, $CH_2C{=}O$), 1.75-1.55 (184H, m, CH_2), 1.50-1.30 (78H, m, CH_2).

REFERENCES

1. Schacher, F. H.; Rupar, P. A.; Manners, I. *Angew. Chem. Int. Ed.* **2012**, *51*, 7898-7921.
2. Hadjichristidis, N.; Pispas. S.; Floudas, G. A. (Ed) (**2003**) Block Copolymers: Synthetic Strategies, Physical Properties, and Applications, John Wiley & Sons, Inc., New Jersey.
3. A) Ghoroghchian, P. P.; Li, G.; Levine, D. L.; Davis, K. P.; Bates, F. S.; Hammer, D. A.; Therien, M. J. *Macromolecules* **2006**, *39*, 1673-1675. B) Geng, Y.; Discher, D. E. *J. Am. Chem. Soc.* **2005**, *127*, 12780-12781.
4. Gaucher, G.; Dufresne, M. H.; Sant, V. P.; Kang, N.; Maysinger, D.; Leroux, J. C. *J. Controlled Release* **2005**, *109*, 169-188.
5. A) Jerome, C.; Lecomte, P. *Adv. Drug Deliv. Reviews* **2008**, *60*, 1056-1076. B) Stjerndahl, A.; Wistrand, A. F.; Albertsson, A. C. *Biomacromolecules* **2007**, *8*, 937-940. C) Majerska, K.; Duda, A.; Penczek, S. *MAcromol. Rapid Commun.* **2000**, *21*, 1327-1333. D) Qing, C.; Jianzhong, B.; Shenguo, W. *Polym. Adv. Technol.* **2002**, *13*, 105-111.
6. A) Tuzar Z (1996) Overview of polymer micelles. In: Webber SE, Munk P, Tuzar Z (eds) NATO ASI series, series E: Applied sciences, vol 327. Kluwer, Dordrecht. B) Zana R (2000) Fluorescence studies of amphiphilic block copolymers in solution. In:

Alexandridis P, Lindman B (eds) Amphiphilic block copolymers: self-assembly and applications. Elsevier, Amsterdam. C) Hamley IW (ed) (1998) The physics of block copolymers. Oxford Science Publications,Oxford.

7. Letchford, K.; Zastre, J.; Liggins, R.; Burt, H. *Colloids Surf. B* **2004**, *35*, 81-91.
8. Bogdanov, B.; Vidts, A.; van den Bulcke, A.; Verbeeck, R.; Schacht, E. *Polymer* **1998**, *39*, 1631-1636.
9. A) Guo, J.; Chen, G.; Ning, X.; Li, X.; Zhou, J.; Jagielski, A.; Xu, B.; Boons, G. J. Chem. Eur. J. 2012, 18, 4568 – 4574. B) Ning, X. H.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255.
10. Vangeyte, P.; Gautier, S.; Jerome, R. *Colloids Surf. A* **2004**, *242*, 203-211.
11. A) Stroh, C.; Wang, H.; Bash, R.; Ashcroft, B.; Nelson, J.; Gruber, H.; Lohr, D.; Lindsay, S. M.; Hinterdorfer, P. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12503-12507. B) Khatri, O. P.; Adachi, K.; Murase, K.; Okazaki, K.; Torimoto, T.; Tanaka, N.; Kuwabata, S.; Sugimura, H. *Langmuir* **2008**, *24*, 7785-7792.

CHAPTER 3

STUDY OF PHYSICO-CHEMICAL PROPERTIES OF SUGAR BASED BLOCK COPOLYMERS

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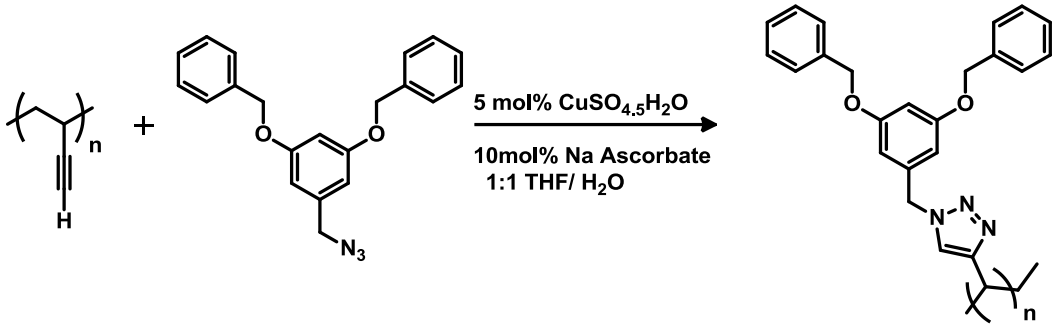
ABSTRACT

The use of polymers[1] for biomedical and pharmaceutical applications has gained a lot of importance over the past few decades. This has led to an increasing demand for the synthesis of well-defined polymers with tailorable properties. The development of 'click' chemistry has facilitated such synthesis. Click chemistry provides very attractive opportunities for bioconjugation reactions as it can be performed at ambient temperatures with readily available starting materials. Recently, click chemistry has also been applied for the synthesis of polymers with different architectures such as block and graft copolymers, and also polymers for various pharmaceutical and biomedical applications. In the current project, lactose was chosen as a model disaccharide for the hydrophilic block in block copolymer synthesis. In the first part, ε-caprolactone was modified with an alkyne handle, while lactose was synthesized with an azide linker. The block copolymer synthesis was facilitated using copper mediated azide- alkyne cycloaddition. The progress of the reaction was studied via matrix assisted laser desorption/ionization (MALDI-TOF). The self-assembly of the micelles revealed that the size of the hydrophilic component was not enough for the micelle stability and aggregates were observed. Therefore, it became necessary to synthesize higher analogues of sugars to ensure that stable micelles are obtained. Towards this end, lewis acid catalyzed acetolysis of β-cyclodextrin was performed to furnish a fully acetylated heptasaccharide in a single step. However, this reaction proceeds with relatively low yields and a large amount of fully acetylated lower oligomers were also obtained. The fractionation to collect the lower oligomers was also unsuccessful. Several attempts were made to install the bromide at the anomeric position of the peracetylated heptasaccharide. However, the bromide could not be installed and further manipulations of the heptasaccharide are currently underway.

INTRODUCTION

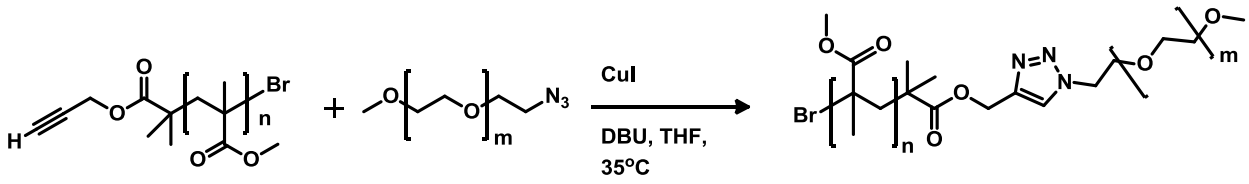
Biodegradable polymers are the youngest members of the materials family with ever increasing applications in pharmaceutical, medical and biomedical fields.[9] Biodegradable polymers are not limited to the release of drugs, peptides or proteins at specific rates or specific points in the body, but also extended to medical devices, wound devices and tissue engineering.[10] There are several traditional approaches for example, anionic polymerization, and ring opening polymerization for the synthesis of well-defined polymers. However, the most recent developments in polymer chemistry are based on the growing synergy between advanced organic synthesis and polymer chemistry.[11] To be applicable and useful in polymer synthesis, an organic reaction needs to proceed in high yield with little or no side products. Both of these criteria are aptly fulfilled by the development of the click chemistry by Sharpless et.al.[12]

In 2001, Sharpless and co-workers coined the concept of "click" chemistry to classify a particular set of nearly perfect reactions.[12] There are several well- known reactions that comply with the "click" conditions for example the hetero-Diels-Alder reaction, thio-ene coupling, Staudinger ligation amongst others and the most popular- copper (I) catalyzed azide- alkyne cycloaddition (CuAAC). The promise of click chemistry towards materials chemistry has been demonstrated by several examples. It has been used for the complete modification of the side-chain functionalities of a linear polymer by reaction with a sterically bulky dendritic unit (Scheme 3.1**)**.[13]



Scheme 3.1: Synthesis of first generation dendronized linear polymers using click chemistry.

Click chemistry has also been used as a highly efficient reaction to couple two distinct linear polymers to make block copolymers, a reaction known to be synthetically challenging mainly because of the reduced reactivity of the polymer chain ends. Linear homopolymers of methyl methacrylate and poly (ethylene glycol), for which α-alkynyl or ω-azido functionalities were incorporated, were found to undergo high- yielding Cu(I)-catalyzed dipolar cycloaddition reactions to produce block copolymers. (Scheme 3.2)[14]



Scheme 3.2: Modular synthesis of poly (methyl methacrylate)-*b*-poly (ethylene glycol) block copolymer using CuAAC.

The CuAAC reaction suffers from a few disadvantages:

1. Application in bioconjugation is hindered because of the presence of the toxic copper.
2. Reaction rates are slow.
3. The formation of diacetylenes through CuI-catalyzed Glaser homocoupling of terminal alkynes.

As a result, there has been quite a lot of research on the development of copper free strategies. Bertozzi and co-workers exploited the use of cyclooctynes as an effective method for lowering the activation barrier of azide-alkyne cycloaddition.[16] This strain promoted variant azide-alkyne cycloaddition (SPAAC) was used by Bertozzi and coworkers for the fluorogenic labeling of proteins and cell surface glycans in living cells and organisms, including mice and zebrafish.[16] Optimization of the reactivity of the cyclooctynes reagents and the development of more efficient synthetic routes for their preparation has led to the development of more variants of cyclooctynes (Figure 3.1). The versatility of SPAAC spreads beyond bioconjugation to applications in materials and polymer science.[15-18]

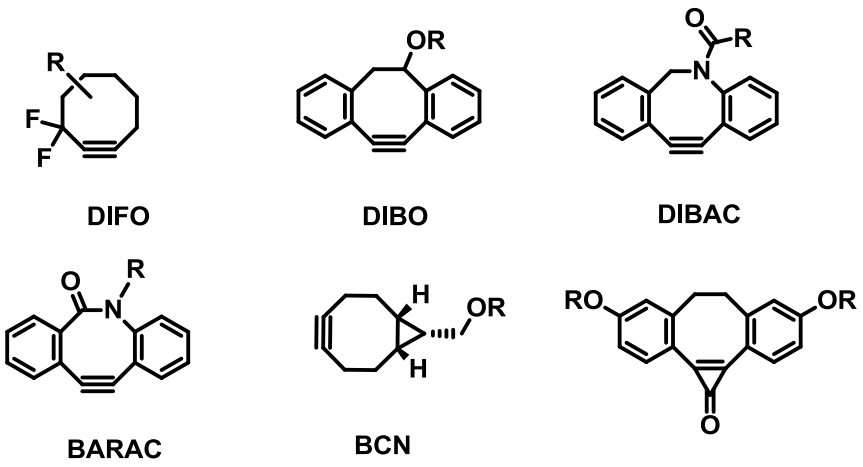


Figure 3.1: Activated cyclooctyne derivatives used in SPAAC bioconjugation

Polyethylene glycol (PEG) is currently the most used polymer in the biomedical field of drug delivery and the only polymer therapeutic that has market approval for different drugs. The success of PEG is based on the hydrophilicity and high biocompatibility. However, scientific results obtained in recent years show that it may also have possible drawbacks, such as interaction with the immune system, possible degradation under stress, and accumulation in the body above an uncertain excretion limit. If an alternative polymer to PEG has to be chosen, a wide range of chemically different synthetic polymers are available, although a limited number

of these are water soluble. The most attractive of these are carbohydrates. Polysaccharides represent the third main class of biomacromolecular components. Sugars are naturally occurring hydrophilic polymers that can be employed as alternatives to PEG in therapeutics. Sugars are important in nature because of the vital roles that they play in several biological recognition processes on the cell surface. The commonly employed sugars for this purpose are polysaccharides like dextran, hyalorunan and chitosan. Modification of biodegradable polyesters with saccharides, with an aim to improve the solubility in water and increase the functions of polyesters has been an active area of research.[19] The synthesis of saccharide end- capped polyesters can be achieved either by ring opening polymerization of lactones and lactides initiated by saccharides, or by chemical reaction between the saccharides and polyesters. The polymerization protocol, however, requires the use of several protection- deprotection steps as saccharides contain several hydroxyl groups that can initiate polymerization. Therefore, an ideal alternative to this would be coupling the saccharides after the polymerization steps.[19] With the surging interest in click chemistry, it provides a perfect tool for synthesizing a block copolymer consisting of sugar as the hydrophilic block and polycaprolactone as the hydrophobic block. In this project, we will be using the copper assisted azide- alkyne cycloaddition and strain promoted azide-alkyne cycloaddition to assemble the block copolymers.

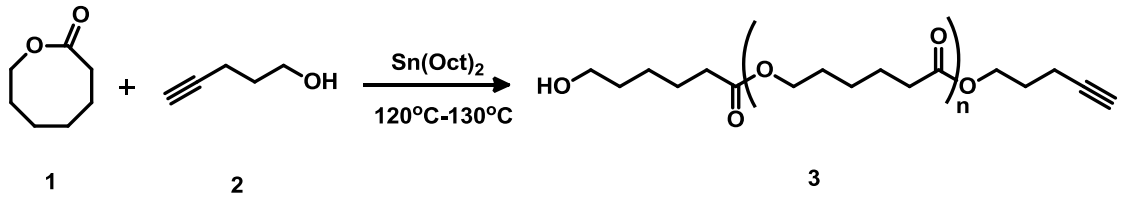
RESULTS AND DISCUSSION

PART A: SYNTHESIS OF POLYSACCHARIDE-block-PCL COPOLYMERS USING CuAAC

SYNTHESIS OF ALKYNE TERMINATED POLYCAPROLACTONE

The assembly of the block copolymer from polycaprolactone and sugars was achieved using copper (I) assisted azide- alkyne cycloaddition. To achieve this goal, ε-caprolactone was subjected to ring-opening polymerization using 4-pentyn-1-ol as the initiator using stannous (II)

octoate as the catalyst. The reaction was stirred at 120-130^{o}C. Earlier attempts were made to open the ε-caprolactone using benzyl alcohol as the initiator and then coupling the free alcohol with 4-pentynoic acid using standard *N, N'*-dicyclohexylcarbodiimide (DCC) coupling conditions. However, for the higher analogues of PCL, the attachment of the alkyne handle resulted in incomplete reactions. Therefore, alternative approach was employed where 4-pentyn-1-ol was used as the initiator (Scheme 3.3) for the polymerization of caprolactone. For the synthesis of the higher molecular weight analogues, reaction times of more than 12 hours were required to bring about the complete polymerization. The molecular weights of PCL targeted were 2k, 4k and 8k. They were on the same lines as the molecular weights of PCL from PEG-*b*-PCL block copolymers so that it would enable a better comparison between two libraries of polymers. The corresponding polymers obtained were characterized by NMR and GPC and the molecular weights were found to be in good correlation as seen in Table 3.1. Characterization of the polymers using MALDI-TOF did not prove helpful. The higher molecular weight PCLs (MW 4k and 8k) were difficult to characterize. Use of a different matrix like dithranol also did not help. Therefore, for the higher molecular weight polymers, techniques such as NMR and GPC were used.



Scheme 3.3: Synthesis of alkyne terminated polycaprolactone

The alkyne terminated polycaprolactone synthesized are shown in **Table 3.1.**

Table 3.1: Synthesis of alkyne terminated PCL analogues

No.	MW of PCL targeted	MW from NMR	MW from GPC	PDI
3a	2k	1600	2500	1.35
3b	4k	4800	5400	1.62
3c	8k	9300	10,400	1.63

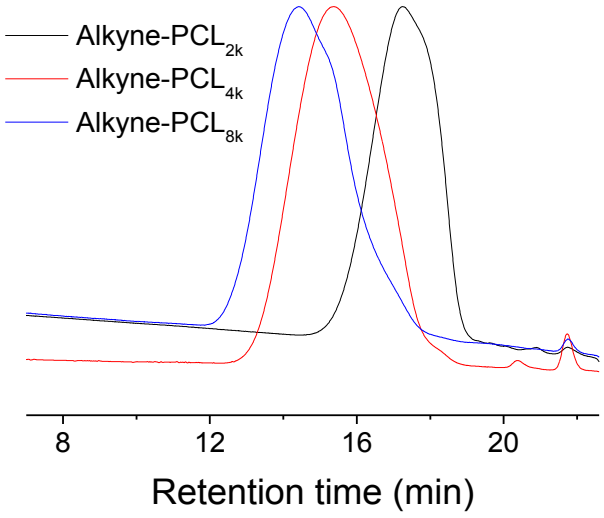
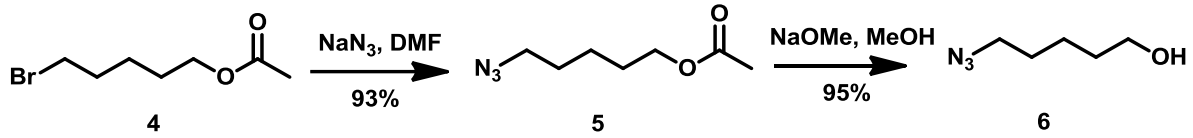


Figure 3.2: Gel permeation chromatograms for the PCL analogues

SYNTHESIS OF POLYSACCHARIDE BUILDING BLOCKS

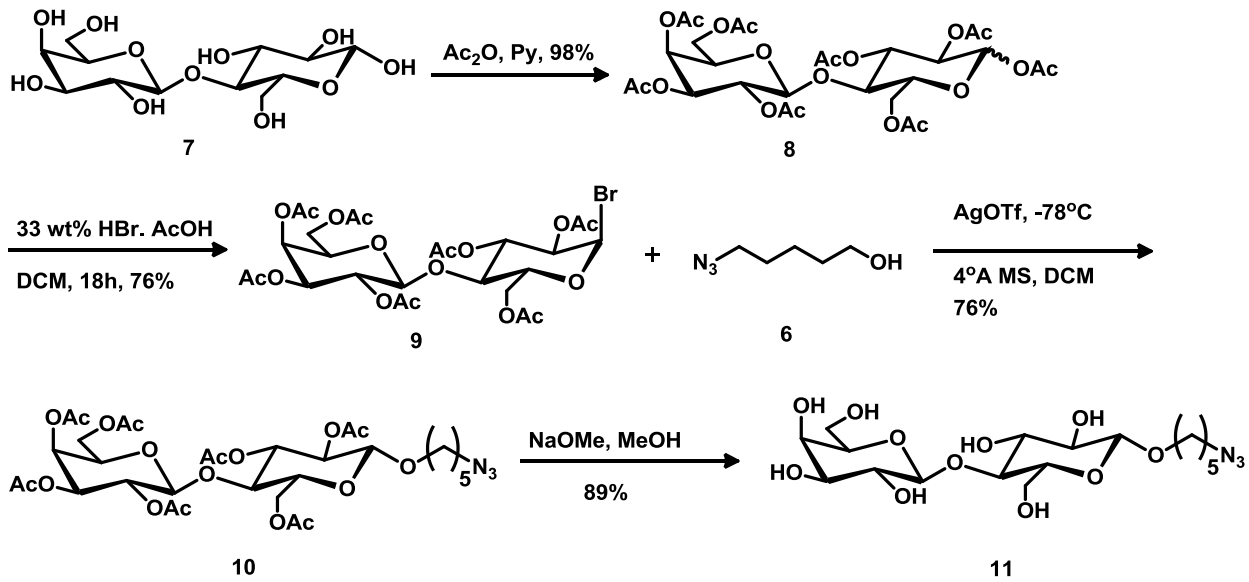
"Click" chemistry, and the copper (I)-catalyzed azide-alkyne cycloaddition (CuAAC) in particular, is a powerful new synthetic tool in polymer chemistry and material science. Success of the CuAAC in the engineering of (bio) polymer architectures stems, in part, from the possibility of introducing the required azide and alkyne functionalities at predetermined locations in macromolecular building blocks, is a result of advances in controlled polymerization techniques. The alkyne handle was established on the polycaprolactone via ring opening polymerization. The installation of the azide handle on the sugar block was done via Koenigs-

Knorr glycosylation of the sugar halide with a C5 azido linker. The synthesis of the linker began with the commercially available 5-bromo pentyl acetate. The bromide group was converted into the azido group by treating with sodium azide. Deacetylation of the 5-azido pentyl acetate using freshly prepared sodium methoxide in methanol gave the 5-azido pentanol in high yield (Scheme 3.4).



Scheme 3.4: Synthesis of azido linker

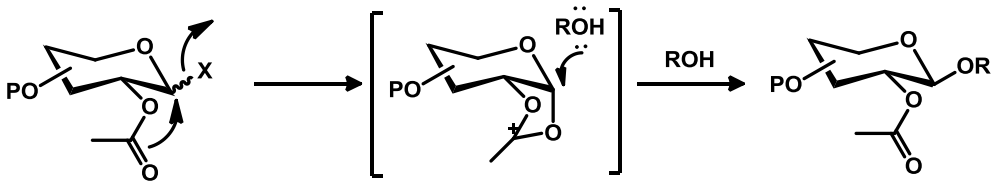
We used lactose and maltoheptaose as the sugar blocks for assembling the block copolymer. The synthesis route for the lactose (Scheme 3.5) is shown below:



Scheme 3.5: Synthesis of lactose building block

The synthesis of the disaccharide began with the acetylation of the commercially available lactose (**7**) using acetic anhydride and pyridine to give D- lactose octaacetate (**8**) as an α/β mixture of anomeric C1-acetate. Conversion of the anomeric acetyl into bromide (**9**) was achieved using 33 wt% HBr- AcOH, followed by the Koenigs-Knorr[19] glycosylation with the C5

linker (**6**) using silver trifluoromethanesulfonate (AgOTf) furnished the glycoside (**10**) in 76% yield. Because of the presence of the acetyl group at C2 position, only the β-glycoside was obtained. The acetyl group provides anchimeric assistance via neighboring group participation, assisting in the departure of the activated leaving group, thereby resulting in the formation of the more stable acetoxonium ion. Consequently, the glycosyl acceptor (5- azido pentanol) can only attack from the backside to form 1, 2-*trans* glycoside (Scheme 3.6). Deacetylation using freshly prepared sodium methoxide (NaOMe) in methanol (MeOH) gave 5- Azidopentyl-β-D-galactopyranosyl- (1→4)-β-D-glucopyranoside in 89% yield.

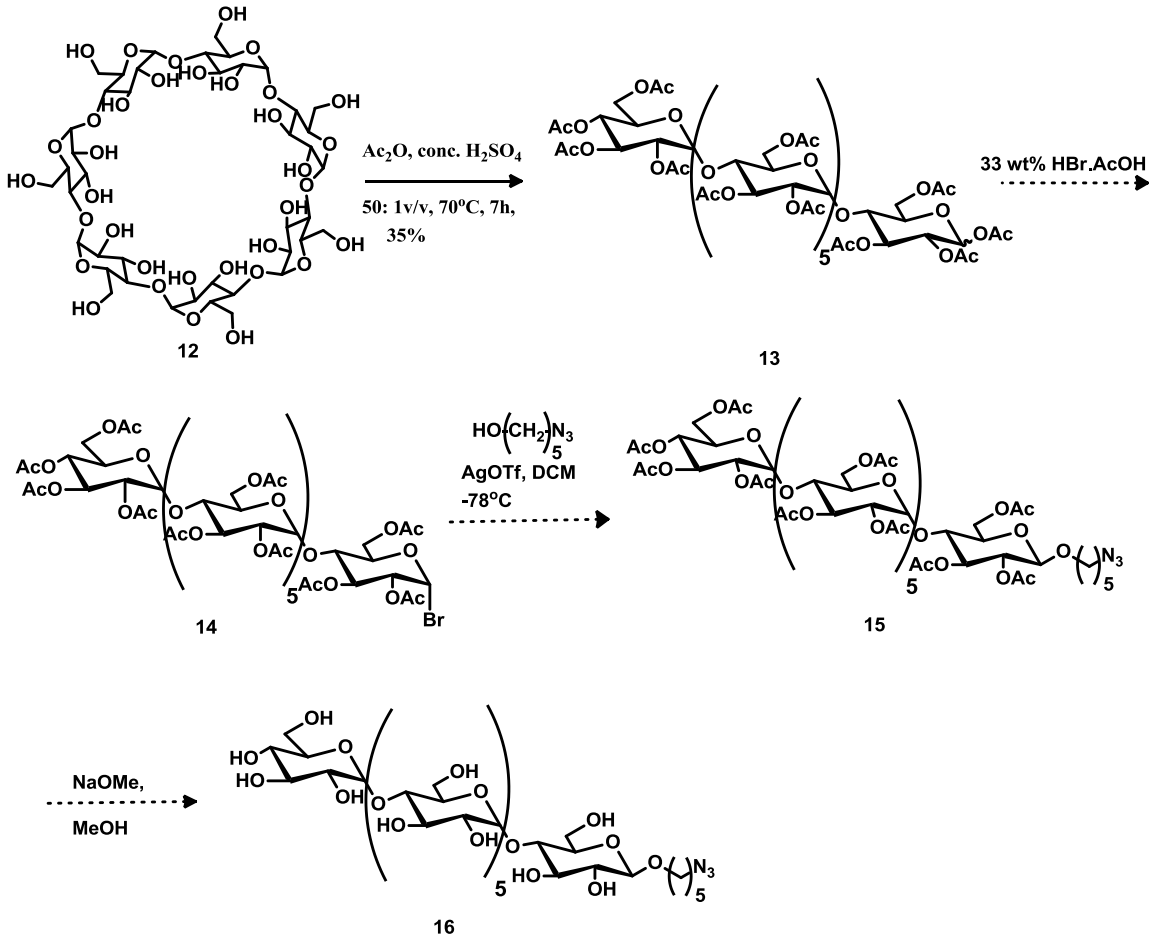


P = protecting group; X = leaving group; ROH = glycosyl acceptor

Scheme 3.6: The stereoselective formation of glycosidic bond by neighboring-group participation

The heptasaccharide and other oligosaccharide to be used for the current study were obtained by Lewis acid mediated acetolysis of the commercially available β- cyclodextrin (**Scheme 3.7**). β-cyclodextrin was dissolved in acetic anhydride and concentrated sulfuric acid (50:1 v/v) and heated for 7 h at 70^{o}C. The initial attempt to bring about the acetolysis of the β-cyclodextrin using iron (III) chloride hexahydrate ($FeCl_3.6H_2O$) was unsuccessful. The reaction failed to go to completion and always gave a 1:1 ratio of the starting material and product. The milder Lewis acid did not seem to bring about the acetolysis efficiently and therefore a stronger acid was used. The reaction went to completion resulting in a mixture of the lower oligosaccharides along with the heptasaccharide. The desired oligosaccharides (hepta to tetra) were accomplished using silica

gel chromatography to give the heptasaccharide in modest yield (35% yield). Several trials were made to install the anomeric bromide on the heptasaccharide and oligosacchride using the conventional 33 wt % HBr. AcOH (results shown in Table 3.3, Experimental Section). However, the reaction never proceeded. Attempts to convert the anomeric acetyl into bromide are still underway.

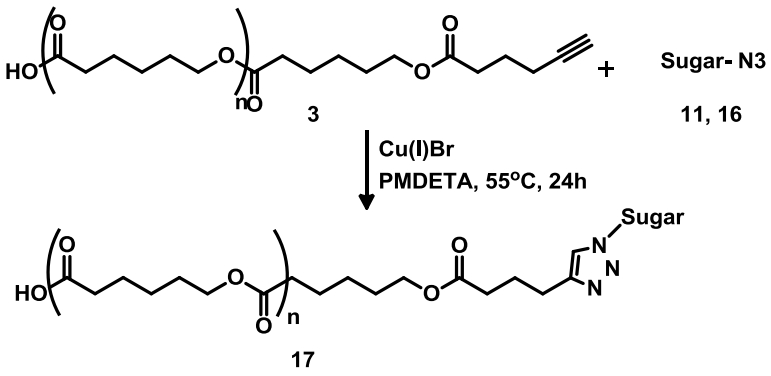


Scheme 3.7: Synthesis of heptasaccharide

SYNTHESIS OF POLYSACCHARIDE-block-PCL COPOLYMERS

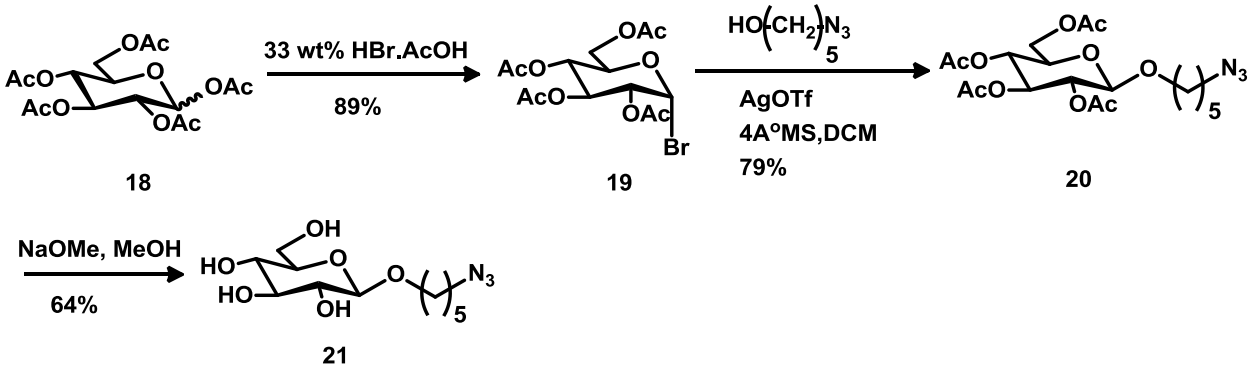
The assembly of the polysaccharide-*block*- PCL copolymer employed the traditional copper catalyzed azide-alkyne cycloaddition (CuAAC). The conditions used were copper (I) bromide

(CuBr) and *N,N,N',N',N''*-pentamethyldiethylenetriamine (PMDETA) in dimethyl formamide (DMF) at 55°C (Scheme 3.8).[20]



Scheme 3.8: Synthesis of polysaccharide- *block*- PCL copolymers

Inert conditions were maintained by triple freeze-thaw procedure. A trial click reaction was done by reacting 5-azidopentyl-β-D-glucopyranoside with alkyne terminated polycaprolactone (MW 1.5k). The synthesis of 5-azidopentyl-β-D-glucopyranoside is shown in Scheme 3.9.



Scheme 3.9: Synthesis of 5-azidopentyl-β-D- glucopyranoside

During the trial reaction, the progress of the reaction was monitored using MALDI. The MALDI showed the disappearance of the alkyne terminated polycaprolactone and appearance of new polymer plus sugar peaks. Several attempts were made to remove the copper from the reaction mixture once the reaction was complete. The best condition to remove the copper was passing the reaction mixture through a column of neutral alumina. The solvent was then removed under

vacuum from the column fractions and the product was precipitated from cold ether. However, even though different NMR solvents were tried, a clean NMR spectrum of the product was not obtained as the sugar signals were difficult to assign to aggregation and overlap with caprolactone peaks. However, there was a correlation between the *CH* of the triazole and the polycaprolactone peaks from the soluble portions of the polycaprolactone. The CuAAC reaction was then repeated with all the analogues to generate the library of block copolymers (Table 3.2).

Table 3.2: Observation on products obtained after click reactions of lactose with the PCL-alkynes.

No.	**Sugar**	**MW of PCL**	**Observations**	**NMR**
17a	Lactose	2k	The product is partially soluble in DMF and precipitates out overnight	NMR confirmed the presence of triazole
17b	Lactose	4k	The product is soluble in DMF and partially in $CHCl_3$ and precipitates slowly	NMR confirmed the presence of triazole
17c	Lactose	8k	Soluble in DMF and $CHCl_3$, barely precipitates	NMR confirmed presence of triazole

The NMR of the click reaction of lactose with PCL (M_n ~2k) (**17a**) initially showed excess of sugar peaks. The product was therefore, redissolved in DMF and precipitated into water to remove the excess sugar. The solution was stirred for 24 h. The solution was then centrifuged and the solid obtained was washed thrice with water, redissolved into 5mL DMF, diluted with 5mL water and then lyophilized. In case of **17b**, the product, obtained after passing the reaction mixture through neutral alumina, was redissolved in EtOAc and washed with water (2 x 1mL) and brine (2 x 1mL). The aqueous layer was further extracted with $CHCl_3$. The $CHCl_3$ layer was then washed with dilute HCl (2 x 1mL) and brine (2x 1mL). The organic layers were combined, dried with $MgSO_4$ and the solvents were removed under vacuum. The product was precipitated from ether.

The same protocol was followed for **17c** with small changes. The product obtained after passing the reaction mixture through neutral alumina was dissolved in $CHCl_3$, washed with water (1 x 2mL) and brine (1 x 2mL). The organic layer was dried with $MgSO_4$ and the solvent was removed under vacuum. The product obtained was then precipitated from ether.

The nanoparticles of lactose-PCL analogues were prepared by dissolving 2.5mg of the product in 0.5 mL DMF this solution was then added dropwishe to 2 mL of nanopure water. The resulting turbid solutions were stirred for 3-4 h. The solutions were then centrifuged using Amicon filter (MW cutoff ~ 30kDa). Only those solutions which were clear after centrifugation were subjected to DLS to study the micelle sizes.

SUMMARY AND FUTURE OUTLOOK

The synthesis of the polysaccharide based block copolymers was accomplished using the copper(I)-catalyzed azide alkyne cycloaddition. The use of neutral alumina to remove the traces of copper and use of MALDI alone proved quite useful for the characterization of the polymers.

However, the micelles formed using lactose-*b*-PCL block copolymers were unstable. This suggested that the lactose by itself was insufficient in length and molecular weight to induce micellization. Therefore, in the future, higher molecular weight analogues of the polysaccharide will be used to study the influence of molecular weight and block length on the morphology of the micelles. The synthesis of the higher molecular weight analogues of polysaccharides is currently underway.

The CuAAC reaction has been thoroughly exploited for applications ranging from bioconjugation to synthesis of macromolecular structures. The advent of SPAAC allowed for the use of azide-alkyne cycloaddition reaction for applications which were sensitive to the use of toxic metals. Boons and coworkers found that use of oxime as a dipole provides an orthogonal pair of functional group when teamed with an azide. The oxime serves as a latent dipole, which can be activated *in situ* by treatment with bis (acetoxy) iodo benzene to form the reactive dipole-nitrile oxide. Therefore, it would be desirable to synthesize a polymer having an azide at one end and an oxime at the other terminal, which can then be activated under different reaction conditions to undergo orthogonal click reactions. Towards this end, the synthesis of a lactose moiety having an azide at the reducing end and an oxime at the non-reducing end is currently underway.

EXPERIMENTAL PROCEDURE

MATERIALS:

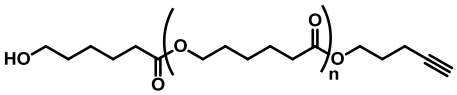
All reagents such as D-glucose, D-lactose, *p*-toluene sufonyl chloride, sodium azide, acetic anhydride, 33 wt % HBr- AcOH, silver trifluoromethanesulfonate, iron (III) chloride hexahydrate, copper (I) bromide, benzaldehyde dimethylacetal, *t*-butoxide and *N,N,N',N',N''*-pentamethyldiethylenetriamine and solvents such as pyridine, dimethyl formamide (DMF),

dichloromethane (DCM), toluene and methanol were purchased from Sigma Aldrich Chemical Co. ε-caprolactone was distilled over CaH_2 before use. Stannous octoate ($Sn(Oct)_2$, 95%) was used without further purification.

INSTRUMENTATION: ^{1}H-Nuclear Magnetic Resonance was obtained on a Varian Inova-300 (300MHz) and Varian Inova- 500(500MHz) at 300^{o}K equipped with Sun workstations. Mass spectra were obtained on an Applied Biosystems Voyager DE-Pro Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF). GPC was performed on Shimadzu LC-20AD coupled with a refractive index (RI) detector at 40^{o}C with a flow rate of 1mL/minute.

SYNTHETIC PROCEDURES

Synthesis of Alkyne terminated Polycaprolactone (3a):



ε-caprolactone monomer (**1**) (7.4g, 64.8mmol) was added to a flask containing 4-pentyn-1-ol (**2**) (0.6 g, 7.12 mmol) and the resulting mixture was placed under an argon atmosphere and then 0.2g of $Sn(Oct)_2$ was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 120°C with vigorous stirring. After 24 h, 5 mL MeOH was added to the resulting viscous colorless oil and cooled to room temperature. The solvent was removed under vacuum and the polymer was dissolved in DCM (3 mL) and precipitated from cold ether (5mL) to obtain **3a** as white powder. (5.1g, 63 %). The degree of polymerization and the PDI was determined by GPC. ^{1}H NMR ($CDCl_3$, 500MHz) δ 4.18-4.20 (t, 2H, $CH_2CH_2CH_2OCO$), 4.08-4.1 (28H, m, CH_2OCO), 3.65-3.70 (bs, 2H, CH_2OH), 2.28-2.35 (t, 32H, $CH_2C{=}O$), 1.95-1.99 (t, 1H, $CHC{\equiv}$), 1.85-1.90 (m, 2H, CH_2CH_2), 1.60- 1.80 (m, 62H, CH_2), 1.50-1.59 (m, 30H, CH_2). ^{13}CNMR (CDCl3, 500MHz) δ 173.1 (C=O), 84.7 ($C{=}CH_2$), 71.3 ($CH{=}CH_2$), 65.2 (CH_2OCO), 62.8 (CH_2OH), 62.3 (CH_2OCO), 33.9

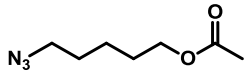
(CH_2CO), 28.2 (CH_2), 25.2 (CH_2), 24.7 (CH_2).MW (NMR) = 1600Da. Mn (GPC): 2500 Da and PDI = 1.35.

Synthesis of Alkyne terminated Polycaprolactone (3b): ε-caprolactone monomer (**1**) (6.5g, 57.3 mmol) was added to a flask containing 4-pentyn-1-ol (**2**) (0.15 g, 1.8 mmol) and the resulting mixture was placed under an argon atmosphere and then 0.08g of $Sn(Oct)_2$ was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 120°C with vigorous stirring. After 24 h, 7mL MeOH was added to the resulting viscous colorless oil and cooled to room temperature. The solvent was removed under vacuum and the polymer was dissolved in DCM (3mL) and precipitated from cold ether (5mL) to obtain **3b** as white powder. (5.5g, 78%). The degree of polymerization and the PDI was determined by GPC. ^{1}H NMR ($CDCl_3$, 500MHz) δ 4.18-4.20 (t, 2H, $CH_2CH_2CH_2OCO$), 4.08-4.1 (68H, m, CH_2OCO), 3.65-3.70 (bs, 2H, CH_2OH), 2.28-2.35 (t, 71H, $CH_2C{=}O$), 1.95-1.99 (t, 1H, $CHC{\equiv}$), 1.85-1.90 (m, 2H, CH_2CH_2), 1.60- 1.80 (m, 143H, CH_2), 1.50-1.59 (m, 70H, CH_2). ^{13}CNMR (CDCl3, 500MHz) δ 173.1 (C=O), 84.7 ($C{=}CH_2$), 71.3 ($CH{=}CH_2$), 65.2 (CH_2OCO), 62.8 (CH_2OH), 62.3 (CH_2OCO), 33.9 (CH_2CO), 28.2 (CH_2), 25.2 (CH_2), 24.7 (CH_2). MW (NMR) = 4700Da. Mn (GPC): 5400Da and PDI = 1.62.

Synthesis of Alkyne terminated Polycaprolactone (3c): ε-caprolactone monomer (**1**) (9.2g, 80.3mmol) was added to a flask containing 4-pentyn-1-ol (**2**) (0.1 g, 1.2 mmol) and the resulting mixture was placed under an argon atmosphere and then 0.1g of $Sn(Oct)_2$ was added and the mixture was stirred at room temperature for *ca*. 10 min until complete dissolution of the initiator. Next, the mixture was heated at 120°C with vigorous stirring. After 24 h, 10mL MeOH was added to the resulting viscous colorless oil and cooled to room temperature. The solvent was removed under vacuum and the polymer was dissolved in DCM (3 mL) and precipitated from

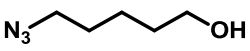
cold ether (5 mL) to obtain **3c** as white powder. (7.9g, 83%). The degree of polymerization and the PDI was determined by GPC. ^{1}H NMR ($CDCl_3$, 500MHz) δ 4.18-4.20 (t, 2H, $CH_2CH_2CH_2OCO$), 4.08-4.1 (102H, m, CH_2OCO), 3.65-3.70 (bs, 2H, CH_2OH), 2.28-2.35 (t, 106H, $CH_2C{=}O$), 1.95-1.99 (t, 1H, $CHC{\equiv}$), 1.85-1.90 (m, 2H, CH_2CH_2), 1.60- 1.80 (m, 222H, CH_2), 1.50-1.59 (m, 105H, CH_2). ^{13}CNMR (CDCl3, 500MHz) δ 173.1 (C=O), 84.7 ($C{=}CH_2$), 71.3 ($CH{=}CH_2$), 65.2 (CH_2OCO), 62.8 (CH_2OH), 62.3 (CH_2OCO), 33.9 (CH_2CO), 28.2 (CH_2), 25.2 (CH_2), 24.7 (CH_2). MW (NMR) = 9300Da, Mn (GPC): 10,400Da and PDI = 1.63.

Synthesis of 5-Azido pentyl acetate (5):



Dissolve sodium azide (15.49g, 238.31mmol) in tetrabutylammonium hydrogen sulfate (10 mL/g). To the solution add the solution of 5-bromo pentyl acetate **4** (10g, 47.66mmol) in DCM (10mL/ g of halide) and stir the solution at room temperature for 36 h. The reaction mixture was washed with 0.1M HCl solution (2 x 50mL) followed by water (25mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under vacuum. The crude product was purified using silica gel chromatography (Hexane/ EtOAc: 10: 1) to yield **5** as clear light yellow oil. (7.63g, 93%).^{1}H-NMR ($CDCl_3$, 300MHz) δ 3.90–3.98 (t, 2H, CH_2COCH_3), 3.15- 3.20 (t, 2H, CH_2N_3), 1.98- 2.0 (s, 3H, CH_3CO), 1.47-1.6 (m, 4H, CH_2), 1.27- 1.42 (m, 2H, CH_2). ^{13}CNMR ($CDCl_3$, 300MHz) δ 170.2 (C=O), 64.9 (CH_2OCO), 50.0 (CH_2N_3), 29.8 ($CH_2CH_2N_3$), 28.6 (CH_2CH_2O), 22.9 (CH_2), 20.0 (CH_3CO).

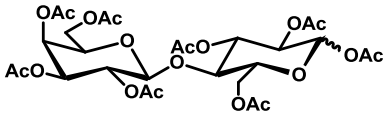
Synthesis of 5- azidopentanol (6):



5-Azido pentylacetate (**5**) (7.6g, 44.17mmol) was dissolved in anhydrous MeOH and freshly prepared sodium methoxide was added dropwise till the pH was 10-12. The reaction mixture was

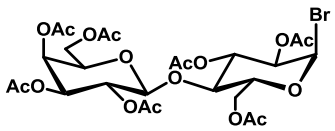
stirred for 2h and then filtered through celite. The filterate was removed under vacuum and the crude product was purified using silica gel chromatography (Hexane/ EtOAc: 4:1) to yield **6** as clear yellow oil (5.7g, 95%). ^{1}HNMR ($CDCl_3$, 300MHz) δ 3.85-3.91 (t, 2H, CH_2OH), 3.2- 3.36 (t, 2H, CH_2N3), 2.05- 2.15 (bs, 1H, *OH*), 1.5- 1.7 (m, 4H, CH_2), 1.39- 1.48 (m, 2H, CH_2). ^{13}CNMR ($CDCl_3$, 300MHz) δ 62.8 (CH_2OH), 50.0 (CH_2N_3), 31.9 (CH_2OH), 30.1 (CH_2N_3), 22.7 (CH_2).

Synthesis of D-lactose octaacetate (8):



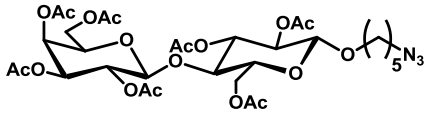
D-Lactose (**7**) (5.0 g, 13.877 mmol) was dissolved in pyridine (18 mL, 222.03 mmol) and acetic anhydride was added (11.3 g, 111.02 mmol) and the reaction mixture was stirred overnight. The solvent was removed under vaccum and the crude product was purified by silica gel chromatography (Hexane/ EtOAc 1:1) to yield **8** as white crystalline solid in quantitative yield (18.6 g, 100%). ^{1}H-NMR ($CDCl_3$, 500MHz) δ 6.25 (d, *J* = 3.55Hz, 1H, H-1), 5.46 (dd, *J*= 9.77 Hz, 9.74 Hz, 1H, H-3), 5.36 (d, *J* = 3.19 Hz, 1H, H'-4), 5.11 (dd, *J* = 10.18Hz, 8.08 Hz, 1H, H'-2), 5.00 (dd, *J* = 10.22 Hz, 3.58 Hz, 1H, H-2), 4.98 (dd, *J* = 10.33 Hz, 3.20 Hz, 1H, H'-3), 4.53 (d, *J* = 7.89 Hz, 1H, H'-1), 4.44 (d, *J* = 11.60 Hz, 1H, H-6a), 4.09- 4.17 (m, 3H, H-6b, H'6a, H'6b), 3.94 (dd, *J* = 6.77 Hz, 6.64 Hz, 1H, H'-5), 3.85 (dd, *J* = 9.73 Hz, 9.64 Hz, 1H, H-4), 1.97- 2.18 (s, 24H, CH_3CO). ^{13}CNMR (CDCl3, 500MHz) 169.0, 169.2, 169.7, 169.8, 170.2, 170.3, 170.48, 170.51(8s, 8*C*OCH_3), 101.0(d,C-1'), 91.6(d,C-1), 75.7 (d, C-4), 73.5 (d,C-5), 72.7 (d, C-3), 71.0 (d, C-3'), 70.8 (d, C-5'), 70.5 (d, C-2), 69.0 (d, C-2'), 66.7 (d, C-4'), 61.8 (t, C-6), 60.9 (t, C-6'), 20.60 (6 q, 8 CO*C*H_3).Calculated MW for $C_{28}H_{38}O_{19}Na$: 701.1906, found: 700.9989.

Synthesis of α-D- lactosyl bromide (9):



Lactose octaacetate **8** (2.3 g, 3.389 mmol) was dissolved in dry DCM (34 mL) and acetic anhydride (5 mL) added. The solution was cooled to 0 °C and HBr in AcOH (1.37g, 33 % solution) added. The mixture was stirred for 30 min at 0 °C and 18 h at r.t. The mixture was then diluted with DCM (20 mL) and washed with sodium bicarbonate (2 ×20 mL) followed by brine (20 mL). The organic layer was dried over MgSO4 and the solvent removed under vacuum to yield the crude product as light yellow oil (1.82 g, 76 %). ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 6.53 (d, *J* = 4.1 Hz, 1H, H-1), 5.56 (t, *J* = 9.7 Hz, 1H, H-3), 5.38 – 5.35 (m, 1H, H-4), 5.13 (dd, *J* = 10.3, 7.8 Hz, 1H, H'-4), 4.97 (dd, *J* = 10.4, 3.4 Hz, 1H, H'-3), 4.77 (dd, *J* = 9.8, 4.1 Hz, 1H, H-2), 4.55 – 4.47 (m, 2H, H'-1, H-5), 4.26 – 4.04 (m, 4H, H-6a, H-6b, H'-6a, H'-6b), 3.93 – 3.82 (m, 2H, H'-5), 2.19 – 2.03 (m, 24H). ^{13}C NMR (500 MHz, $CDCl_3$) δ169.0, 169.3, 170.0, 170.11, 170.17, 170.20, 170.4 (7 s, 7 *C*OCH_3), 100.8 (d, C-1'), 86.4 (d, C-1), 75.0 (d, C-4), 73.0 (d, C-5), 71.0 (d, C-3'), 70.9 (d, C-2), 70.8 (d, C-5'), 69.6 (d, C-3), 69.0 (d, C-2'), 66.6 (d, C-4'), 61.0 (t, C-6), 60.9 (t, C-6'), 20.5 (6 q, 6 CO*C*H_3).Calculated MW for $C_{26}H_{35}BrO_{17}Na$: 721.0955, found: 721.0968.

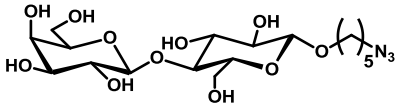
Synthesis of 5- azidopentyl- 2, 3, 4, 6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)- 2, 3, 6-tri-*O*-acetyl-β-D-glucopyranoside (10):



5-Azidopentyl- 2, 3, 4, 6-tetra-*O*-acetyl-β-D-galactopyranosyl- (1→4)- 2, 3, 6-tri-*O*- acetyl-β-D-glucopyranoside (**9**) (1.8 g, 2.58 mmol) and 5-azido pentanol (**6**)(0.66 g, 5.16 mmol) were

coevaporated with toluene (2 x 6mL), dissolved in freshly distilled DCM (0.1M), followed by the addition of activated molecular sieves 4^{o}A. The reaction mixture was cooled to -78^{o}C, followed by the addition of AgOTf (1.32 g, 5.16 mmol). The reaction was stirred at -78^{o}C for 2 h. The reaction mixture was filtered to remove the molecular sieves and silver (II) bromide precipitate. The filterate was washed with saturated $NaHCO_3$ (2 x 15 mL) followed by brine (2 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under vacuum. The crude product was purified by silica gel chromatography (Hex/ EtOAc: 1:1) to yield **10** as a yellow oil (1.21g, 63% pure β). ^{1}H NMR (500 MHz,) δ 5.35 (t, *J* = 3.6 Hz, 1H, H'-4), 5.25 – 5.16 (m, 1H, H-3), 5.10 (dd, *J* = 10.4, 8.2 Hz, 1H, H'-2), 4.99 – 4.94 (m, 1H, H'-3), 4.86 (dd, *J* = 14.6, 5.5 Hz, 1H, H-2), 4.64 (d, *J* = 8.8 Hz, 1H), 4.49 (ddd, *J* = 17.8, 13.1, 5.1 Hz, 1H, H'-1), 4.10 (dtd, *J* = 10.9, 9.5, 3.2 Hz, 5H), 3.92 – 3.75 (m, 4H), 3.72 (dd, *J* = 5.2, 2.0 Hz, 1H), 3.66 (t, *J* = 6.4 Hz, 6H), 3.28 (dd, *J* = 14.2, 7.2 Hz, 7H), 2.20 – 2.00 (m, 36H), 1.69 – 1.55 (m, 12H), 1.47 (td, *J* = 8.5, 4.5 Hz, 4H). ^{13}C NMR (500 MHz, $CDCl_3$) δ169.0, 169.3, 170.0, 170.11, 170.17, 170.20, 170.4 (7 s, 7 *C*OCH_3), 100.8 (d, C-1'), 86.4 (d, C-1), 75.0 (d, C-4), 73.0 (d, C-5), 71.0 (d, C-3'), 70.9 (d, C-2), 70.8 (d, C-5'), 70.1 (CH_2OH), 70 (CH_2N_3), 69.6 (d, C-3), 69.0 (d, C-2'), 66.6 (d, C-4'), 61.0 (t, C-6), 60.9 (t, C-6'), 30.2 (CH_2), 28 (2xCH_2), 20.5 (6 q, 6 CO*C*H_3). Calculated MW for $C_{31}H_{45}O_{18}N_3Na$: 770.6920, found: 769.9178.

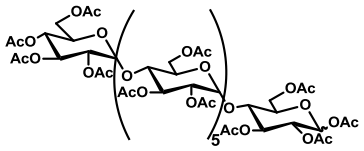
Synthesis of 5-azidopentyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (11):



Compound **10** (1.0 g, 1.34 mmol) was dissolved in anhydrous MeOH (15 mL) and freshly prepared sodium methoxide was added until the pH was between 10 and 11. The reaction mixture was stirred for 1 h. The reaction mixture was neutralized with Dowex 50, filtered

through celite and washed with EtOAc. The solvent was removed under vacuum to give the product as light yellow oil (0.5g, 89%). Calculated MW for $C_{17}H_{30}O_{11}N_3Na$: 475.4268, found: 476.0613.

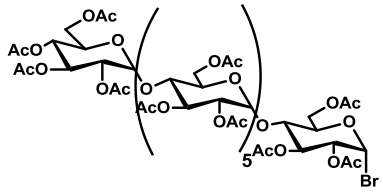
Synthesis of *O*-acetyl maltoheptaose (13):



β-cyclodextrin (**12**) (2.0 g, 1.8mmol) was dissolved in acetic anhydride (100mL) and concentrated sulfuric acid (2mL) was added to the same. The reaction mixture was stirred at 70^{o}C for 7 h under argon. The product was poured over ice and extracted with chloroform (2 x 15mL). The chloroform layer was then washed with saturated $NaHCO_3$ (2 x 15mL) followed by water (30mL), dried over $MgSO_4$ and purified using silica gel chromatography (0.71g, 35%). The lower yield was observed as other oligomers were also obtained during column seperations (disaccharide- hexasaccharide) ^{1}HNMR ($CDCl_3$, 600MHz) δ 6.24 (d, *J* = 3.6 Hz, 1H, H-1), 5.54 – 5.49 (m, 1H, H-3$_\alpha$), 5.45 – 5.34 (m, 6H, $H_{(B-F)}$-3, H_A-3), 5.30 (dt, *J* = 10.7, 3.2 Hz, 6H, $H_{(B-F)}$-1,H-3$_\beta$), 4.95 (dd, *J* = 10.0, 3.7 Hz, 1H, H-2$_{\alpha/\beta}$), 4.86 (dd, *J* = 10.5, 3.9 Hz, 1H), 4.74 (dt, *J* = 12.5, 4.7 Hz, 6H, $H_{(B-F)}$-2, H_A-4), 4.56 – 4.47 (m, 7H, $H_{(A-F)}$-6), 4.34 (d, *J* = 3.3 Hz, 7H, $H_{(A-F)}$-6), 4.33 – 4.29 (m, 1H, H-5), 4.26 (d, *J* = 3.1 Hz, 1H), 4.25 – 4.22 (m, 1H), 4.21 (d, *J* = 3.2 Hz, 1H), 4.18 (s, 1H), 4.05 (dd, *J* = 9.6, 3.3 Hz, 2H), 4.01 – 3.98 (m, 6H, $H_{(B-F)}$-5), 3.94 (ddd, *J* = 16.7, 8.6, 3.9 Hz, 6H), 2.27 – 1.95 (m, 69H). Calculated MW for $C_{88}H_{118}O_{59}Na$: 2142.8298, found: 2141.3987

Synthesis of 2, 3, 4, 6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2, 3, 6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)-2, 3, 6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)- 2, 3, 6-tri-*O*- acetyl-

α-D-glucopyranosyl-(1→4)- 2, 3, 6-tri-*O*- acetyl-α-D-glucopyranosyl-(1→4)- 2, 3, 6-tri-*O*-acetyl-α-D-glucopyranosyl-(1→4)- 2, 3, 6-tri-*O*-acetyl-α-D-glucopyranosylbromide (13):

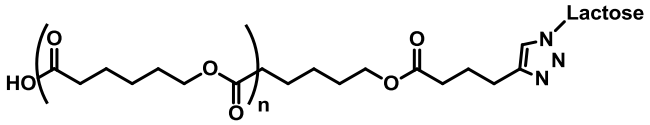


The various attempts to synthesize the molecule are shown in Table 3.3.

Table 3.3: Various attempts to install anomeric bromide.

No.	**Heptasaccharide**	**33 wt% HBr. AcOH**	**Observations**
1.	1 eq.	5eq	After 24h, no formation of product.
2.	1 eq.	2 eq.	After 18h, no formation of product.
3.	1 eq.	3 eq.	After 24h, no formation of product
4.	1 eq.	50 eq.	Starting material seemed to have decomposed
5.	1 eq.	20 eq.	After 24h, no formation of product

Synthesis of Lactose-*b*-PCL$_{2k}$ (17a):



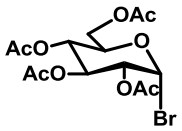
5-Azidopentyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (**11**) (0.07 g, 0.157 mmol), alkyne terminated PCL (**3a**) (0.13 g, 0.07 mmol) and *N, N, N', N'', N''*-Pentamethyldiethylenetriamine (0.03 g, 0.17 mmol) were dissolved in anhydrous DMF (7 mL) in a Schlenk flask. The reaction mixture was subjected to three freeze-thaw cycles. To the solution, then was added, Cu(I)Br (0.03 g, 0.23 mmol) and the reaction mixture was again subjected to three freeze-thaw cycles. The degassed reaction mixture was then heated to 50^{o}C for 24 h under argon. The solution turned dark green at the end of the 24 h period. The reaction mixture was then cooled to room temperature and then passed through neutral alumina twice to remove the residual copper. The solvent was removed under vacuum and the product was reprecipitated from ether and dried in vacuum. The product was redissolved in DMF and precipitated into water and the solution was stirred for 24h. The resulting solution was centrifuged and the solid was washed with water. The product was redissolved in 5 mL DMF and diluted with 5 mL water and lyophilized to give the final product in moderate yield (52%). MW (NMR) = 3600Da

Synthesis of Lactose-*b*-PCL$_{4k}$ (17b): 5-Azidopentyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (0.07g, 0.155mmol), alkyne terminated PCL (**3b**) (0.26 g, 0.07 mmol) and *N, N, N', N'', N''*-Pentamethyldiethylenetriamine (0.034 g, 0.20 mmol) were dissolved in anhydrous DMF (7 mL) in a Schlenk flask. The reaction mixture was subjected to three freeze-thaw cycles. To the solution, then was added, Cu(I)Br (0.03 g, 0.2 3mmol) and the reaction mixture was again subjected to three freeze-thaw cycles. The degassed reaction mixture was then heated to 50^{o}C for 24 h under argon. The solution turned dark green at the end of the 24 h period. The reaction

mixture was then cooled to room temperature and then passed through neutral alumina twice to remove the residual copper. The solvent was removed under vacuum and the product was redissolved in EtOAc and washed with water (2 x1mL) and brine (2x1mL). The aqueous layer was further extracted with $CHCl_3$ (2 x 1mL). The $CHCl_3$ layer was washed further with dilute HCl (2 x 1mL) and brine (2 x 1mL). The organic layers were combined, dried using $MgSO_4$ and solvent was removed under vacuum. The product was then precipitated from ether to yield the pure product in moderate yield. (58%). MW (NMR) = 5400Da

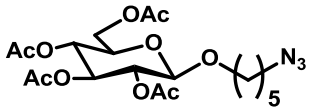
Synthesis of Lactose-*b*-PCL$_{8k}$ (17c): 5-Azidopentyl-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (0.13g, 0.28mmol), alkyne terminated PCL (**3c**) (0.77g, 0.10 mmol) and *N, N, N', N'', N''*-Pentamethyldiethylenetriamine (0.05g, 0.29mmol) were dissolved in anhydrous DMF (7mL) in a Schlenk flask. The reaction mixture was subjected to three freeze-thaw cycles. To the solution, then was added, Cu(I)Br (0.06g, 0.38mmol) and the reaction mixture was again subjected to three freeze-thaw cycles. The degassed reaction mixture was then heated to 50^{o}C for 24h under argon. The solution turned clear green at the end of the 24h period. The reaction mixture was then cooled to room temperature and then passed through neutral alumina twice to remove the residual copper. The solvent was removed under vacuum and the product was dissolved in $CHCl_3$ and washed with water (2 x 1mL) and brine (2 x 1mL). The organic layer was dried with $MgSO_4$, solvent was removed under vacuum and the product was precipitated from ether to give the final pure product in moderate yield. (52%). MW (NMR) = 13,000Da. Mn (GPC): 12,000Da and PDI = 1.72.

Synthesis of 2, 3, 4, 6-Tetra-*O*- acetyl-α-D-glucopyranosyl bromide (19):



1, 2, 3, 4, 6- Penta-*O*-acetyl-D-glucopyranose (**18**) (3.0g, 7.68mmol) was dissolved in dry DCM (15mL) and acetic anhydride (2mL) was added. The solution was cooled to 0^{o}C and HBr. AcOH (12.43g, 33 % solution) added. The mixture was stirred at 0^{o}C for 30 minutes and 18h at room temperature. The mixture was diluted with DCM (30mL) and washed with sodium bicarbonate (2 x 15mL) followed by brine (20mL). The organic layer was dried over MgSO4 and the solvent removed under vacuum to yield the crude product as a yellow oil (2.1g, 67%). ^{1}H NMR ($CDCl_3$, 500 MHz,) δ 6.61 (d, *J* = 4.0 Hz, 1H, H-1), 5.56 (t, *J* = 9.7 Hz, 1H, H-3), 5.16 (t, *J* = 9.8 Hz, 1H, H-4), 4.84 (dd, *J* = 10.0, 4.0 Hz, 1H, H-2), 4.32 (td, *J* = 12.6, 7.2 Hz, 2H, H-6a, H-5), 4.13 (dd, *J* = 12.4, 1.9 Hz, 1H, H-6a), 2.12 – 2.04 (m, 11H,C*H*$_3$COO).^{13}CNMR ($CDCl_3$, 500MHz) δ 86.2 (C_1), 71 (C_5), 70.1 (C_2), 69.8 (C_3), 66.0 (C_4), 60.8 (2x C_6), 22.0 (4q, 4 CH_3CO). Calculated MW for $C_{14}H_{19}O_9BrNa$: 434.1908, found: 434.0782

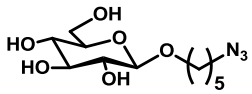
Synthesis of 5-Azidopentyl-2, 3, 4, 6, Tetra-*O*-acetyl-β-D-glucopyranoside (20):



2, 3, 4, 6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**19**) (2g, 4.87 mmol) and 5-azido pentanol (**6**) (1.26 g, 9.74 mmol) were coevaporated with toluene (2 x 6 mL), dissolved in freshly distilled DCM (0.1 M), followed by the addition of activated molecular sieves 4^{o}A. The reaction mixture was cooled to -78^{o}C, followed by the addition of AgOTf (2.5 g, 9.74 mmol). The reaction was stirred at -78^{o}C for 2 h. The reaction mixture was filtered to remove the molecular sieves and silver (II) bromide precipitate. The filterate was washed with saturated $NaHCO_3$ (2 x 15 mL) followed by brine (2 x 10 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under vacuum. The crude product was purified by silica gel chromatography (Hex/ EtOAc: 1:1) to yield **20** as a yellow oil (1.54 g, 70% pure β).^{1}H NMR ($CDCl_3$, 500 MHz,) δ 5.20

(t, *J* = 9.5 Hz, 1H, H-3), 5.09 (t, *J* = 9.6 Hz, 1H, H-4), 4.99 (dd, *J* = 9.6, 8.0 Hz, 1H, H-2), 4.50 (d, *J* = 8.0 Hz, 1H, H-1), 4.26 (dd, *J* = 12.3, 4.7 Hz, 1H, H-6a), 4.14 (dd, *J* = 12.3, 2.6 Hz, 1H, H-6b), 3.89 (dt, *J* = 9.6, 6.1 Hz, 1H, CH_2O), 3.71 – 3.64 (m, 1H, H-5), 3.49 (dt, *J* = 9.7, 6.6 Hz, 1H, CH_2O), 3.32 – 3.24 (m, 2H, CH_2N_3), 2.14 – 1.99 (m, 12H, CH_3CO), 1.68 – 1.57 (m, 6H, CH_2), 1.51 – 1.41 (m, 2H CH_2). ^{13}CNMR ($CDCl_3$, 500MHz) δ 100.1(C_1), 73.4 (C_3), 72.1 (C_5), 71.5 (C_2), 70.1 (CH_2OH), 70.0 (CH_2N3), 68.0 (C_4), 62 (2xC_6), 30.2 (CH_2), 28 (2xCH_2), 20.1 (4x CH_3). Calculated MW for $C_{19}H_{29}O_{10}N_3Na$: 482.4418, found: 481.9965

Synthesis of 5- Azidopentyl- β-D-Glucopyranoside (21):



Compound **20** (1.5 g, 3.28 mmol) was dissolved in anhydrous MeOH (15mL) and freshly prepared sodium methoxide was added until the pH was between 10 and 11. The reaction mixture was stirred for 1 hour. The reaction mixture was neutralized with Dowex 50, filtered through celite and washed with EtOAc. The solvent was removed under vacuum to give the product as light yellow oil (1.21 g, 99%). ^{1}H NMR (CD_3OD, 300 MHz,) δ 4.49 (d, *J* = 8.5 Hz, 1H, H-1), 4.25 (d, *J* = 7.9 Hz, 1H, H-3), 3.96 – 3.82 (m, 3H, H-6a,CH_2O), 3.72 – 3.62 (m, 1H, H-6b), 3.60 – 3.51 (m, 1H, H-5), 3.41 – 3.24 (m, 2H, CH_2N_3), 3.20 – 3.10 (m, 2H, H-4, H-2), 1.74 – 1.54 (m, 6H, CH_2), 1.55 – 1.38 (m, 2H,CH_2). ^{13}CNMR (CD_3OD, 300MHz) 103.18 (C_1), 70.1 (CH_2OH), 70.0 (CH_2N_3), 69.95 (C_3), 69.35 (C_5), 61.61(C_2), 61.53 (C_4), 61.39 (2x C_6), 31.94 (CH_2), 29.10 (2x CH_2). Calculated MW for $C_{11}H_{21}O_6N_3Na$: 314.2928, found: 313.9865

REFERENCES

1. van Dijk, M.; Rijkers, D. T. S.; Liskamp, R. M. J.; van Nostrum, C. F.; Hennink, W. E. *Bioconjugate Chem.***2009**, *20*, 2001-2016.

2. Knop, K.; Hoogenbaum, R.; Fischer, D.; Schubert, U. S. *Angew. Chem. Int. Ed.* **2010**, *49*, 6288-6308.

3. Li, C.; Wallace, S. *Adv. Drug Delivery Rev.* **2008**, *60*, 886-898.

4. Stiriba, S. E.; Kautz, H.; Frey, H. *J. Am. Chem. Soc.* **2002**, *124*, 9698-9699.

5. a) Kobayashi, S. *Prog. Polym. Sci*. **1990**, *15*, 751 – 823. b) Wiesbrock, F.; Hoogenboom, R.; Leenen, M. A. M.; Meier, M. A. R.; Schubert, U. S. *Macromolecules* **2005**, *38*, 5025 – 5034.

6. Lee, J. S.; Go, D. H.; Bae, J. W.; Le, S. J.; Park, K. D. *J. Controlled Rel.* **2007**, *117*, 204 – 209.

7. a) Larsen, C. *Adv. Drug Delivery Rev*. **1989**, *3*, 103 – 154. b) Sugahara, S. I.; Kajiki, M.; Kuriyama, H.; Kobayashi, T. R. *J. Controlled Rel.* **2007**, *117*, 40 – 50.

8. Janes, K. A.; Calvo, P.; Alonso, M. J. *Adv. Drug Delivery Rev.***2001**, *47*, 83 – 97.

9. a)Kumar, N.; Ravikumar, M. N. V.; Domb, A. J. *Adv. Drug Delivery Rev.* **2001**, *53*, 23-44. b) Nair, L. S.; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32*, 762-798.

10. a) Leenslag, J. W.; Pennings, A. J.; Bos, R. R.; Rozema, F. R.; Boering, G. *Biomaterials* **1987**, *8*, 70-73. b) Shi, F. Y.; Gross, R. A.; Rutherford, D. R. *Macromolecules* **1996**, *29*, 10-17. c) Martina, M.; Hutmacher, D. W. *Polymer International* **2007**, *56*, 145-157.

11. Hawker, C. J.; Wooley, K. L. *Science*, **2005**, *309*, 1200-1205.

12. A) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2002**, *114*, 2708-2711, *Angew. Chem. Int. Ed.* **2002**, *41*, 2596-2599. B) Kolb, H. C., Finn, M. G., and Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004-2021.

13. Helms, B.; Mynar, J. L.; Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **2004**, *126*, 15020-15021.

14. Opsteen, J. A.; van Hest, J. C. M. *Chem. Commun.* **2005**, 57-59.

15. a) Lallana, E.; Riquero, R.; Fernandez- Megia, E. *Angew. Chem. Int. Ed.* **2011**, *50*, 8794-8804. b) Lutz, J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2182-2184.

16. Agard, N. J.; Prescher, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2004**, *126*, 15046-15047.

17. Ning, X. H.; Guo, J.; Wolfert, M. A.; Boons, G. J. *Angew. Chem. Int. Ed.* **2008**, *47*, 2253-2255.

18. Sanders, B. C.; Friscourt, F.; Ledin, P. A.; Mbua, N. E.; Arumugam, S.; Guo, J.; Boltje, T. J.; Popik, V. V.; Boons, G. J. *J. Am. Chem. Soc.* **2011**, *133*, 949-957.

19. Xu, N.; Lu, F. Z.; Du, F. S.; Li, Z. C. *Macromol. Chem. Phy.* **2007**, *208*, 730-735.

20. A) Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, *8*, 497-510. B) Zhu, X.; Schmidt, R. R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900-1934.

21. Xu, N.; Lu, F. Z.; Du, F. S.; Li, Z. C. *Macromol. Chem. Phy.* **2007**, *208*, 730-735.

22. Maunier, V.; Boullanger, P.; Lafont, D.; Chevalier, Y. *Carbohydrate Research* **1997**, *29*, 49-57.

CHAPTER 4

CONCLUSIONS

The interest in block copolymers stems from the numerous applications of these systems. With the aid of ring opening polymerization, we have successfully synthesized a library of PEG-*b*-PCL block copolymers. The library consisted of eight polymers varying in the length as well as molecular weights of the hydrophobic and hydrophilic blocks. The block copolymers thus synthesized were well- defined as was confirmed from ^{1}H- nuclear magnetic resonance, mass spectroscopy and gel permeation chromatography. The block copolymers were self- assembled using both dialysis as well as centrifugation methods. Although centrifugation is a faster method, dialysis was used as the method of choice as the sizes of the micelles were more consistent. The polymers showed slow degradation over a period of two weeks, even when kept at -4^{o}C with the polymers precipitating out eventually. The micelles therefore, were always prepared fresh for analysis by TEM. The sizes of the micelles obtained from both DLS and TEM were in fairly good correlation. The study conducted on the PEG-*b*-PCL copolymers has shown a direct correlation of molecular weight of PCL to size and an inverse correlation with the PEG molecular weight. Use of AFM confirmed that the shape of the micelles was spherical as seen in the TEM. The force pulling experiment conducted with the aid of AFM showed that the force required to pull apart a polymer chain from the micelle is roughly 47pN.

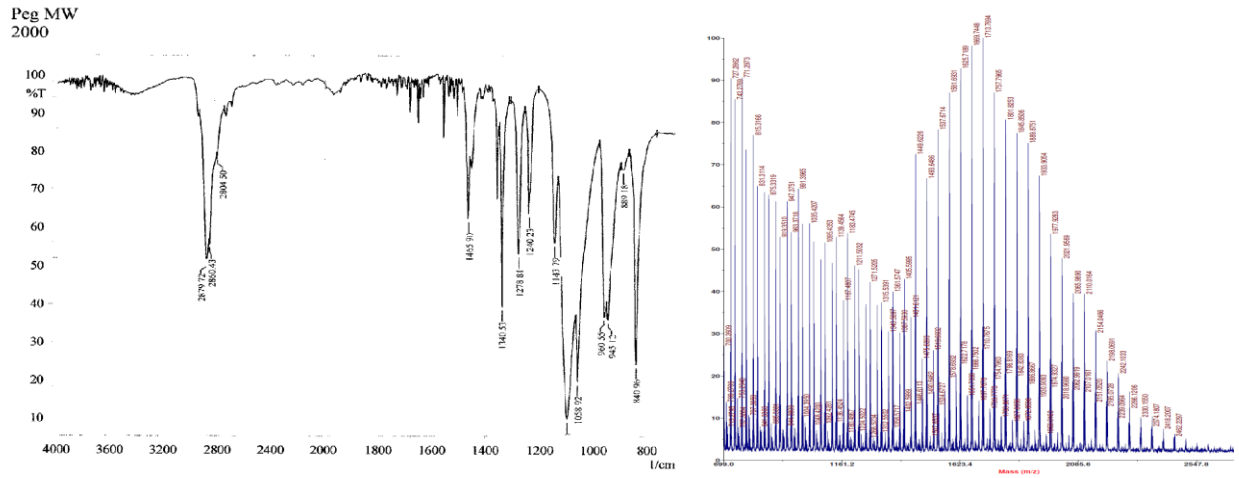
The aim of the second study was to synthesize a library of block copolymers using sugars as the hydrophilic block and PCL as the hydrophobic block. The block copolymers were to be assembled using the copper-(I)-assisted azide- alkyne cycloaddition reaction. The synthesis of block copolymers from lactose and three different molecular weight PCLs proceeded well.

However, the characterization of the polymers proved to be quite challenging. For the lower molecular weight PCL (M_w ~2k) the progress of the reaction could be tracked by matrix assisted laser desorption/ ionization (MALDI). However, as the molecular weight of the PCL increased, analysis of the starting material as well as the product using MALDI became difficult. The solubility of the block copolymers was very poor in solvents such as THF, CD_3OD and D_2O, making their study using NMR and GPC difficult. The products however showed better solubility in DMF and therefore DMF was chosen as a solvent for running NMR experiments. The corresponding micelles were self-assembled by dissolving the block copolymers in DMF (~ 1% v/v) and nanopure water followed by centrifugation. However, it was observed that the micelles were unstable and therefore it was concluded that higher analogues of sugar would be necessary to obtain stable and well-defined micelles. To this end, Lewis acid mediated acetolysis of β- cyclodextrin was performed which would thus furnish a fully acetylated maltopheptaose in a single step. The reaction yielded a mixture of the desired product along with the acetylated oligomers of lower molecular weights. Conversion of the anomeric acetyl group to bromide also proved unsuccessful despite several attempts. Further work on this part is currently underway.

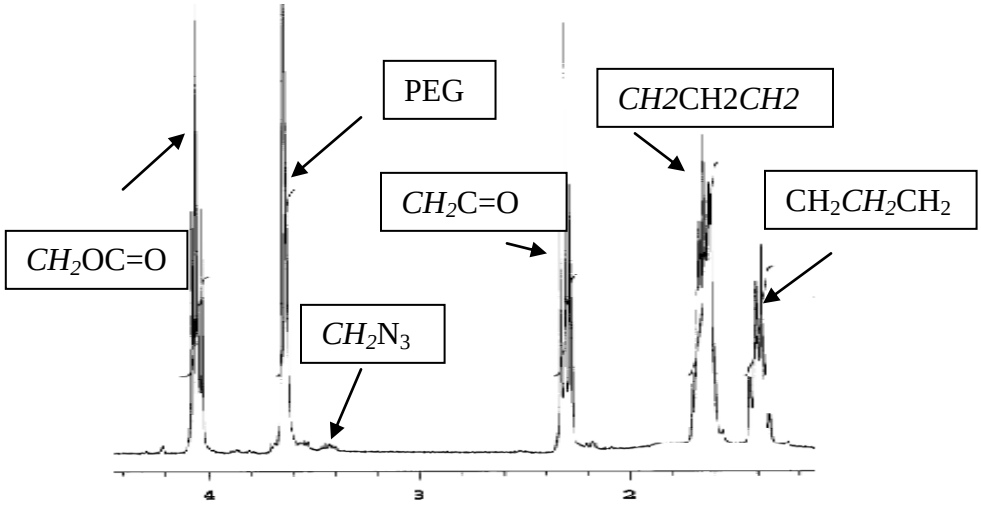
APPENDICES

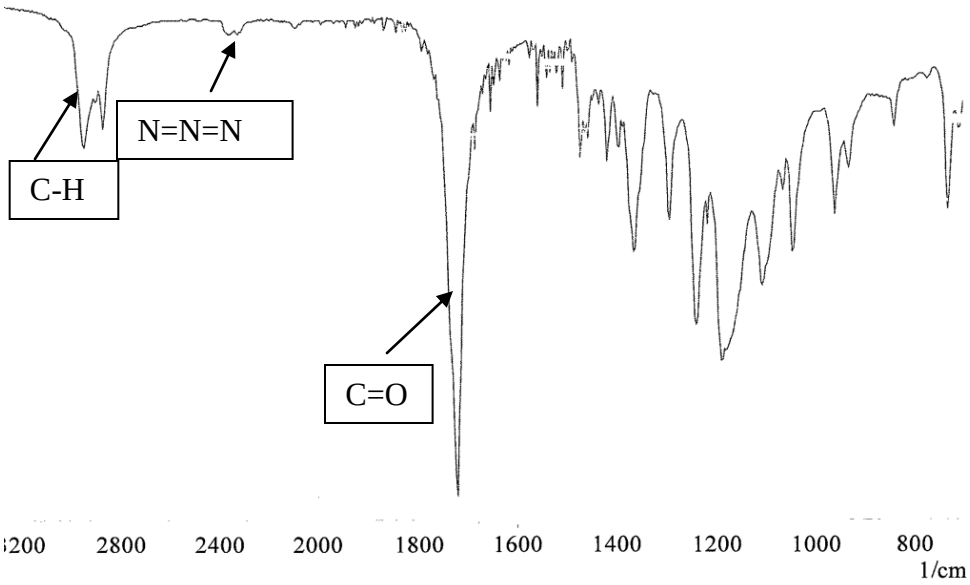
CHAPTER 2: Study of Physico-chemical properties of PEG-*b*-PCL block copolymers

1. N_3-PEG_{2k}-OH



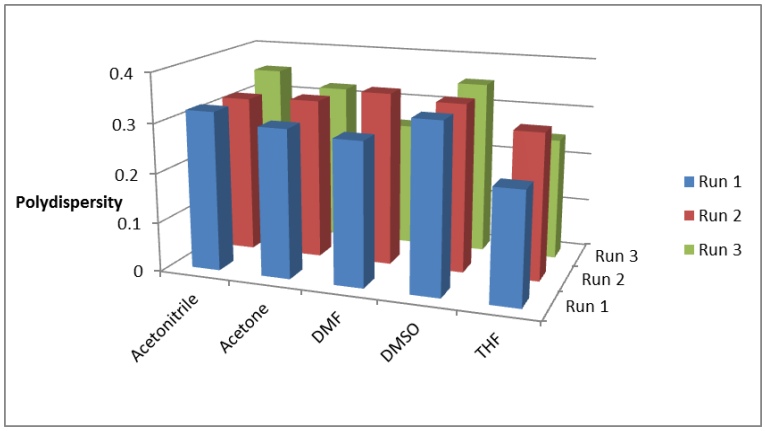
2. N_3-PEG_{2k}-*b*-PCL_{4k}-OH



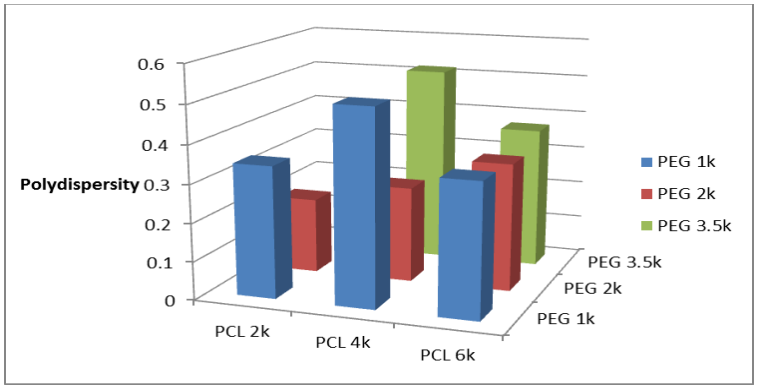


3. DLS data

a) Selection of solvent

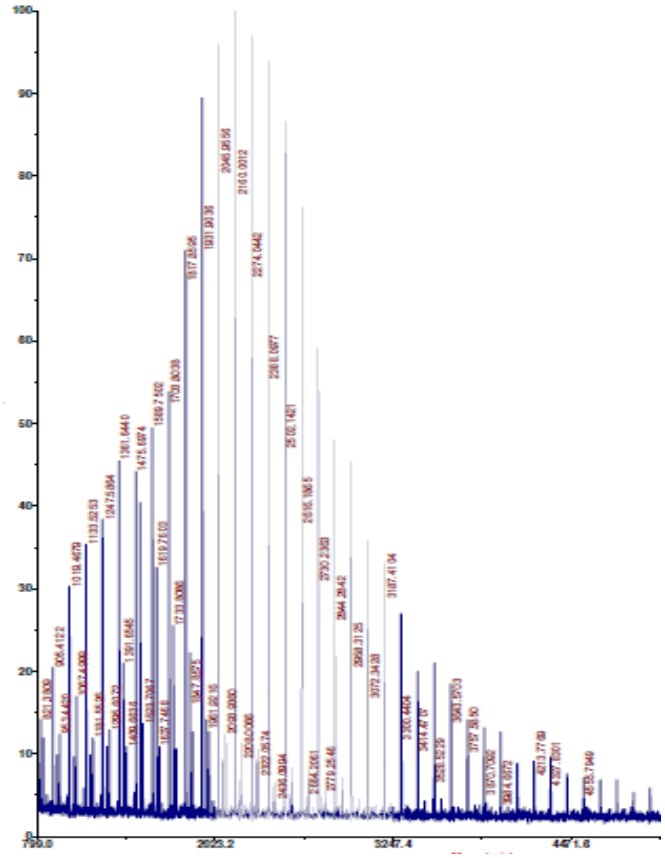


b) DLS data of library of block copolymers

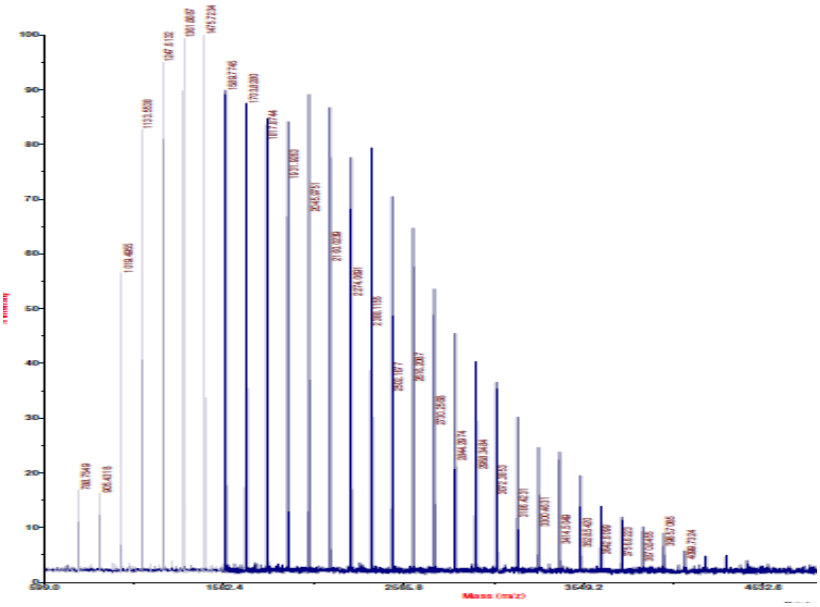


CHAPTER 3: Study of Physico-chemical properties of sugar-*b*-PCL block copolymers

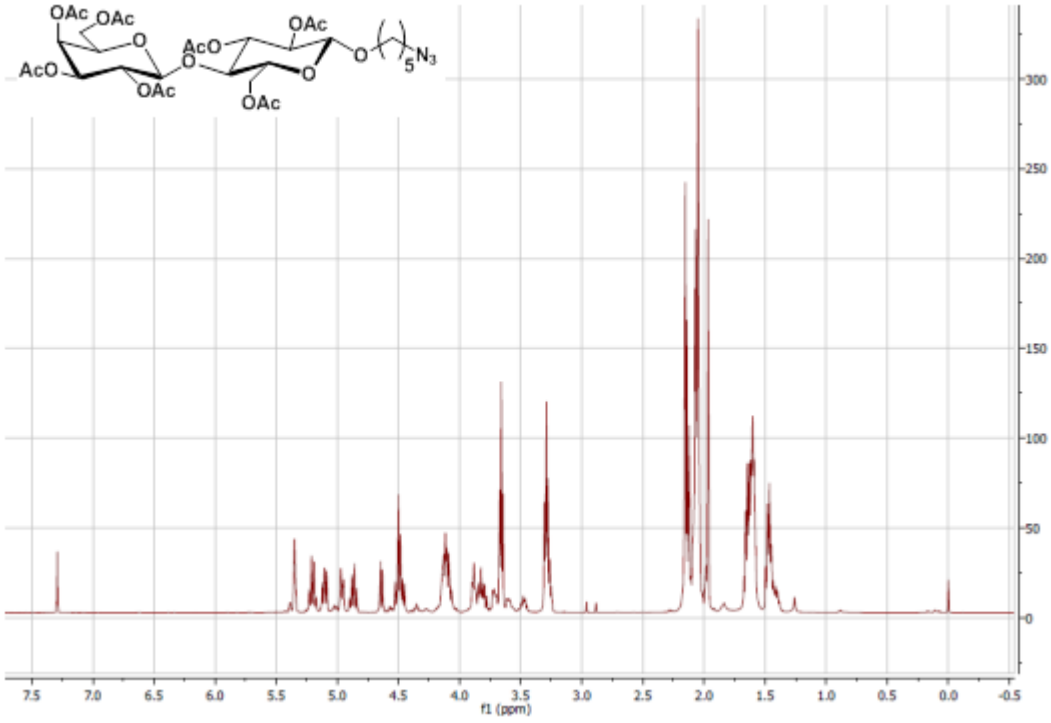
1. Alkyne terminated PCL (Mw ~ 2k)



2. Alkyne terminated polycaprolactone (M_w ~4k)



3. 5- azidopentyl- 2, 3, 4, 6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)- 2, 3, 6-tri-*O*-acetyl-β-D-glucopyranoside



4. Lactose-PCL$_{8k}$-OH