Multifunctional amphiphilic polymers, bearing azetidinium groups: synthesis and antimicrobial studies

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der RWTH Aachen University zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

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'Learn from yesterday, live for today, hope for tomorrow. The important thing is not to stop questioning'. - Albert Einstein

To my parents

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Chapter 1

Introduction

Synthetic polymers are one of the most essential parts of today's life. They are used in many fields like electronics, communication, pharmacy, medicine etc. Natural polymers are very well known and serve many vital roles in living organisms, like building blocks (proteins, carbohydrates etc.), information storage (deoxyribonucleic acids), catalysts (enzymes) etc. The properties of polymers depend mainly on the functional groups in the polymer backbone, the functional groups in the side chains, the ratio of hydrophobic/hydrophilic group, the shape of the polymers, the molecular weights etc. After the pioneering work of Hermann Staudinger, various new methods to prepare well defined polymers for various applications were reported in last years. The main challenge in today's research for synthetic polymer chemists is to synthesize polymeric materials that mimic the structure and function of natural polymers to attain similar properties as their natural models (prototypes).

Antimicrobial Polymers: One of the major problems in our day-to-day life of todays society - are infection related diseases. For example, in USA, the annual cost of medical care for treating infectious diseases is around 120 billion dollar[1]. Naturally occurring antimicrobial peptides (AMP) are very well known and are used as antimicrobial agents for a long time. The key structural parameters which influence the antimicrobial efficiency of AMPs are (a) the presence of cationic charges, (b) amphiphilicity - suitable hydrophobic/hydrophilic ratio. Cationic charges help the polymers to attach on the negatively charged bacterial cell membrane, whereas the hydrophobic parts help the polymer to insert into the bacterial membrane and destroy them[2]. Though AMPs are very efficient as antimicrobials, they have some limitations, such as: (a) they are affected by protease, serum etc.; (b) many of them are cytotoxic to mammalian cells, (c) relative high production cost [3]. To fulfill the need of today's

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society, synthesis of new synthetic antimicrobial polymers, mimicking the structural features of natural AMP is very challenging and an interesting domain of research.

To synthesize antimicrobial polymers, it is important to understand how polymers interact with microorganisms (bacteria). There are mainly two approaches for the uptake of active compounds by microorganisms[4, 5]:

(A) Molecular uptake of active compounds from solution.

(B) Molecular uptake of active compounds from direct contact to surfaces.

Natural antimicrobial peptides generally kill bacteria when they come in contact with bacteria mechanically like a sword or knife. These are amphiphilic polymers containing cationic hydrophilic parts and hydrophobic parts. The cationic parts help the polymers to attach on the negatively charged bacterial cell wall and the hydrophobic backbone acts as a knife or sword and disrupts the cell membrane and kills the bacteria[6] (Figure 1). The current thesis deals with the preparation and studies of polymers which can kill bacteria by this approach.
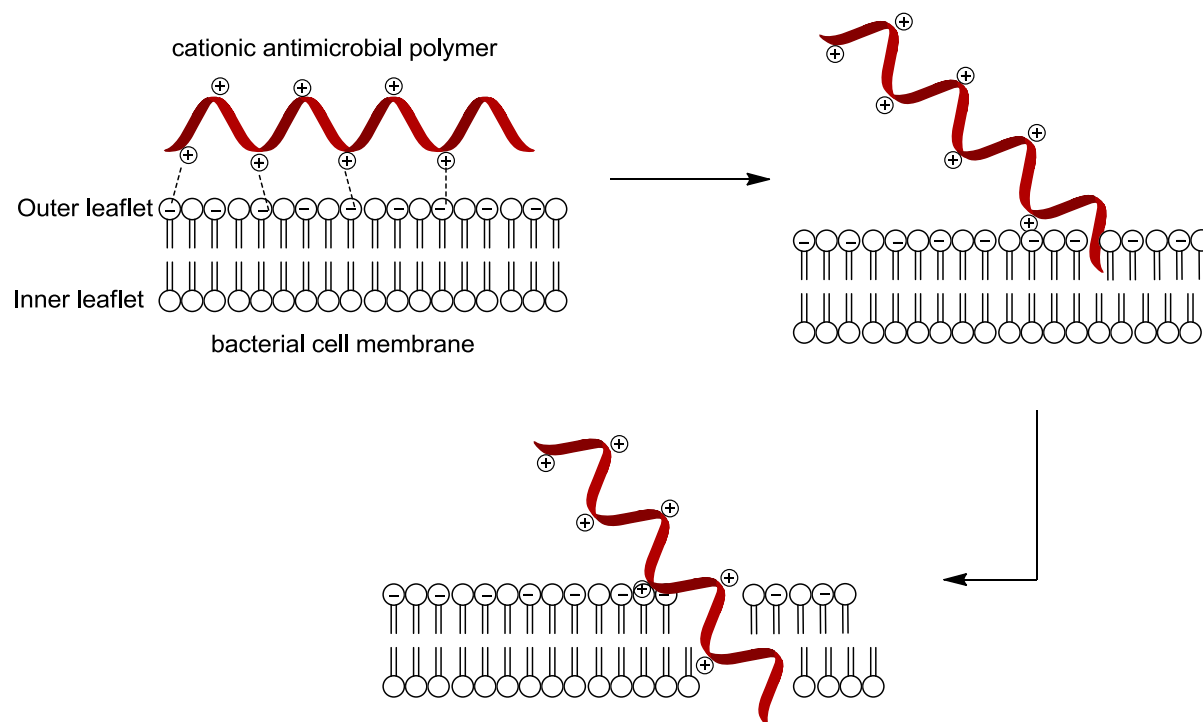

Figure 1: Contact active killing of bacteria by cationic amphiphilic polymers.

Aim and outline of the current thesis: The aim of the current thesis is to prepare new types of well defined antimicrobial polymers. Beside their antimicrobial properties these polymers should be useful for surface coating, to prepare antimicrobial surfaces. To achieve good adhesion of the polymer on surface, it is beneficial that the polymer contains reactive groups, which can help the polymer to covalently attach to the surface beside the physical interaction between polymer backbone and surface. To achieve these properties in the same polymer, in the current study we focus mainly on azetidinium functionalized polymers. Azetidinium functional groups are four membered heterocyclic rings containing a quaternary ammonium group. These types of functional groups are both cationic (due to the quaternary ammonium group) and reactive due to the Bayer strain (angel strain) in the four membered ring) (Scheme 1). It is expected that the presence of these cationic functional polymers help to improve the antimicrobial efficiency and adhesion properties[7] – some of the azetidinium groups can react with the nucleophilic groups of the surface and improve the adhesion via chemical bonding – where the remaining functional groups are still cationic to retain it's antimicrobial efficiency (Figure 2).
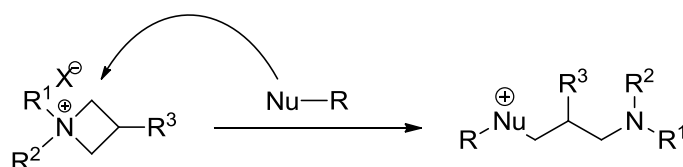
Scheme 1: Ring opening reaction of azetidinium compounds by nucleophiles.
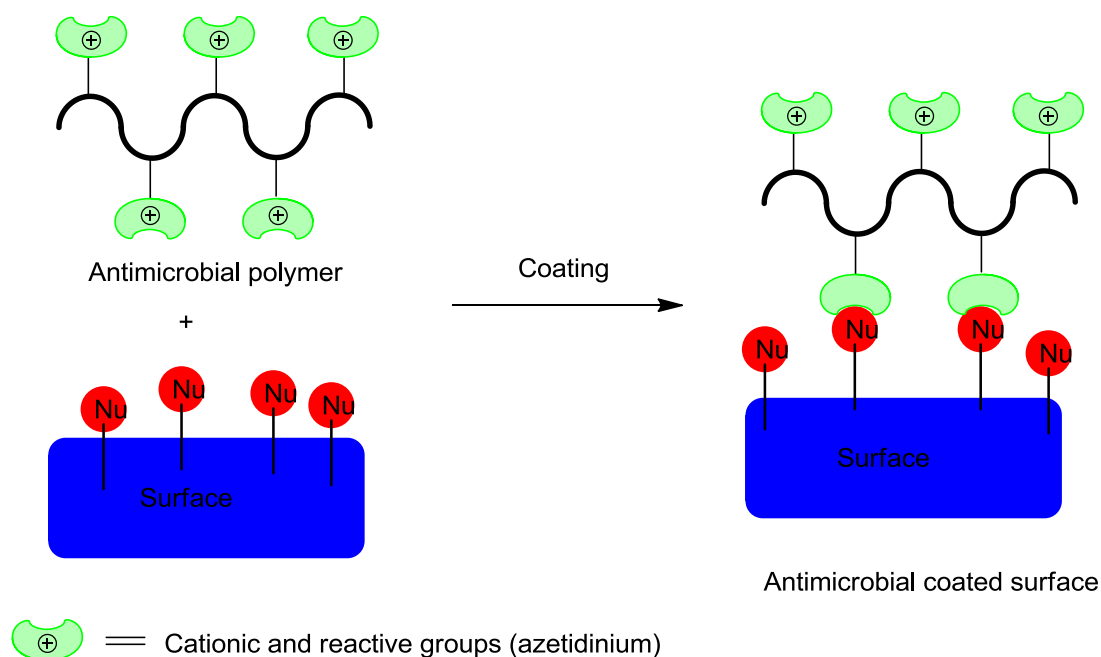
Figure 2: Concept for preparation of antimicrobial surface using azetidinium functionalized polymers

Though azetidinium functionalized polymers have high potential as antimicrobial polymers for different application, these groups are least reported. Only Qian et al. reported the antimicrobial studies of azetidinium functionalized guanidine based polymers[8]. The main problems to study these polymers are (a) the difficulty to synthesize these polymers and (b) the low stability of these polymers due to the high reactivity of the azetidinium groups[9, 10]. To overcome these problems, in the current thesis we studied and establish a new and easy synthetic approach for the preparation of azetidinium functionalized polymers and studied the antimicrobial efficiency of the newly prepared functional polymers.

In chapter 2, we present the state of the art of new azetidinium functionalized polymers with respect to synthesis, stability and applications.

Chapter 3 deals with the synthesis of functional polymers containing azetidinium groups in the backbone via the reaction of aminotelechelic polytetrahydrofurans, containing primary and secondary amino groups, with epichlorohydrin. During these reactions, the secondary

amino groups were transformed into azetidinium groups and the primary amino groups were transformed into amino-chlorohydrin, amino-epoxide, and azetidinium groups. The formation of the desired functional groups in the polymer was controlled by tuning the reaction conditions. To understand the influence of reaction conditions generating the desired functional groups, model reactions of primary and secondary amines with epichlorohydrin were studied in detail, including the influence of the mole ratio of the starting materials, effect of solvent, effect of pH, and reaction kinetics.

In chapter 4, we investigate a new approach for the preparation of azetidinium functionalized polymers from a piperazine based bifunctional coupler in water. A bifunctional coupler bearing an azetidinium and an aminochlorohydrin group was synthesized starting with piperazine and epichlorohydrin. Conversions of the bifunctional coupler with primary and secondary amines (hexylamine and diethylamine) were studied as a model reaction. Using the established reaction conditions, azetidinium functionalized polytetrahydrofurans were prepared in a controlled way by reacting the coupler in water as solvent with amine functionalized polytetrahydrofurans.

Chapter 5 presents antimicrobial studies of the newly prepared azetidinium functionalized polymers presented in chapter 4. The MIC values of these polymers were determined at different pH and a detailed study was performed to prepare antimicrobial coated textiles using the azetidinium functionalized polymers.

In Chapter 6, different functional polymers were prepared starting with the piperazine based bifunctional coupler via two different approaches: (i) by polycondensation - reacting the bifunctional coupler with a suitable diamine and (ii) by post-polymerization modification - reacting the coupler or functionalized couplers with a polyamine precursor. All reactions were performed using water as a solvent in a one pot synthesis approach.

Chapter 7 presents a simple, robust one pot procedure for the preparation of waterborne antimicrobial multifunctional poly(vinyl amine)s (**PVAm**s) by post-polymerization

modification of **PVAm** with the bifunctional piperazine coupler and functional couplers. By this procedure cationic, reactive azetidinium groups, and alkyl chains were introduced in the side chains of **PVAm**. The structure-activity relations (effect of hydrophobic modifications, effect of cationic modifications) of these antimicrobial polymers were studied; the minimum inhibitory concentration (MIC) against both - Gram positive and Gram negative bacteria - of the library of multifunctional poly(vinyl amine)s were determined to identify the candidates with highest efficiency. Furthermore, the hemolytic activity - the effective concentration at which 50% of red blood cells are killed (HC_{50}) - of selected polymers was determined. The ability of the polymers prepared to differentiate between microorganisms (Gram positive and Gram negative bacteria) and mammalian cells (red blood cells) was estimated by comparison of the HC_{50}/ MIC values.

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Chapter 2

Azetidinium functionalized polymers: a literature overview – state of the art

Azetidines are a class of heterocyclic compounds, containing a four membered ring with a nitrogen atom. When the nitrogen atom is positively charged, the corresponding functional group is called azetidinium (Figure 1). These types of compounds are very reactive due to the Bayer ring strain (angel strain) of the four membered ring.


Figure 1: General representation of azetidine and azetidinium groups.

Synthesis of these types of aza-heterocyclic compounds is of interest for synthetic chemists due to their remarkable biological activities. The two most common naturally occurring azetidine compounds are azetidine-2-ones (ß-lactams) and azetidine-2-carboxylic acid (Figure 2). Many of the derivatives of these compounds were synthesized and their biological activities were studied[1, 2].
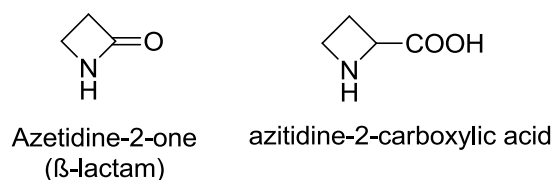
Figure 2: Common naturally occurring azetidine compounds.

Among various azetidine compounds, ß-lactams were most reported for their antibiotic activities. In the last century, many ß-lactam derivatives were synthesized, such as penicillins, carbapenems, cephalosporins, monobactams etc. which are well known as antibiotics (Figure 3). In today's market more than 50% commercial antibiotics (in term of sales) are ß-lactams[3].
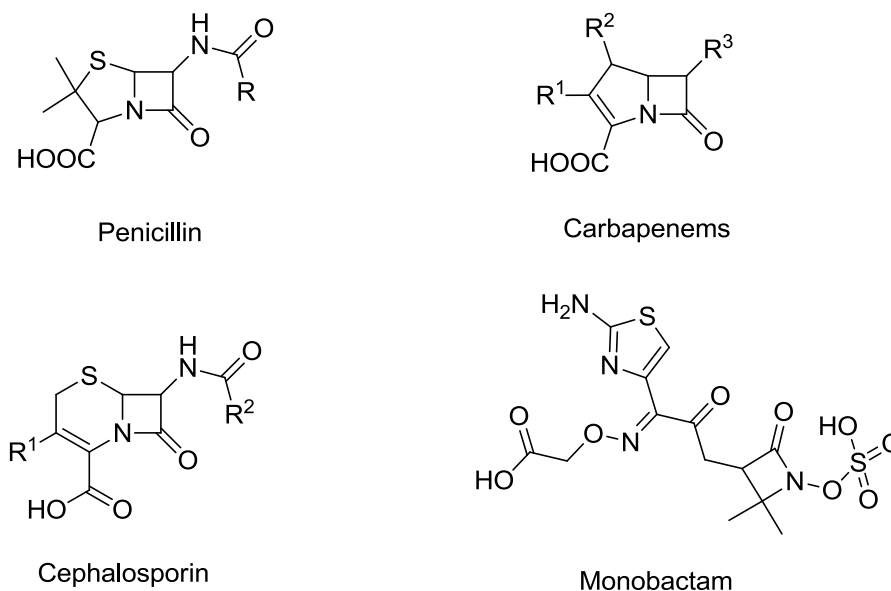

Figure 3: Core structure of different ß-lactam class antibiotics

In last years, a lot of synthetic approaches were reported to synthesize the low molecular weight azetidine compounds[4]. But these functional groups were least studied in polymer chemistry. The only well known polymer is Hercosett, which contains azetidinium functional groups[5]. It was proposed that the presence of azetidinium functional groups in the polymer backbone helps to improve the adhesion of these polymers to various surfaces, which makes them interesting candidate for industry. Though, Hercosett was the most inspiring, few further studies for azetidinium functionalized polymers were reported. The main problems are (i) the synthesis of well defined azetidinium functionalized polymers and (ii) the low stability of the polymers.

Synthetic approaches to prepare azetidine or azetidinium compounds:

It is important to understand, how azetidine/ azetidinium functional groups can be formed in a molecule before discussing the synthesis of azetidinium functionalized polymers. Here we first discuss some of the synthetic concepts for low molecular weight compounds which help us to understand the synthesis of azetidinium functionalized polymers.

(a) Via intra-molecular cyclization: Azetidine or azetidinium functionalized compounds were prepared via internal cyclization of amines, containing a leaving group at γ position with respect to amine group under suitable condition (Scheme 1)[6, 7]. Here the four membered rings were formed by nucleophilic displacement of the leaving group by the amine nucleophile.
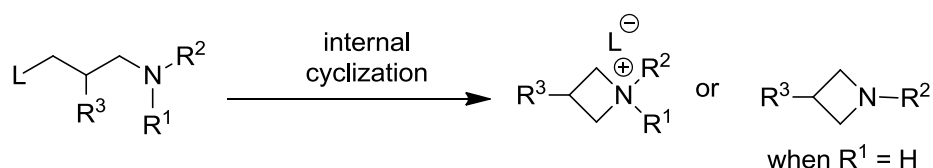
**Scheme 1**: Synthesis of azetidinium or azetidine heterocycles via internal cyclization reactions.

(b) Via reaction of primary and secondary amines with epihalohydrins:

In this approach, the amines react with epihalohydrins to prepare amino- halohydrins which cyclizes internally to form azetidine/azetidinium compounds. Ross et al. reported the first time the synthesis of azetidinium-3-ol by the reaction of diethyl amine and epichlorohydrin (Scheme 2)[8].
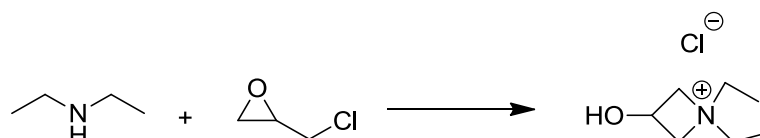
Scheme 2: Reaction of epichlorohydrin with diethyl amine.

Later Gartner et al. studied in detail the preparation of azetidines from amino-halohydrins[9]. The amino-chlorohydrins were prepared by the reaction of epichlorohydrin and primary amines. When amino-chlorohydrins were heated the azetidines were formed (Scheme 3). They observed that primary amines with bulky substituents form azetidines more easily. They proposed a planer transition state (T.S.) with a linear reacting triad, N…C…X.
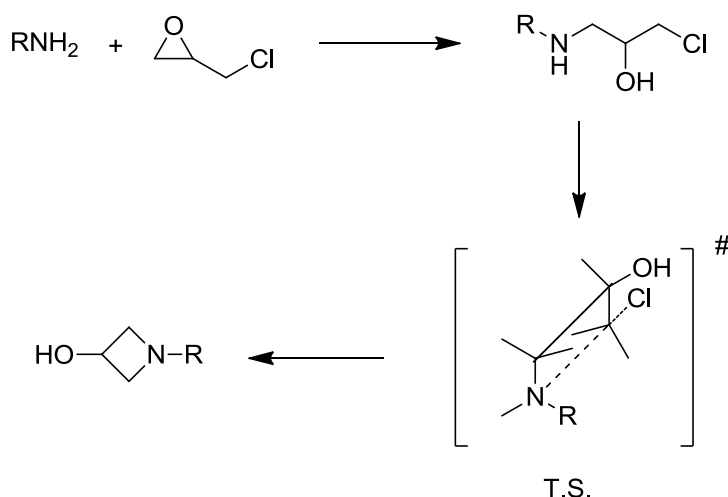
**Scheme 3**: Reaction of primary amine and epichlorohydrin to prepare azetidinium compounds.
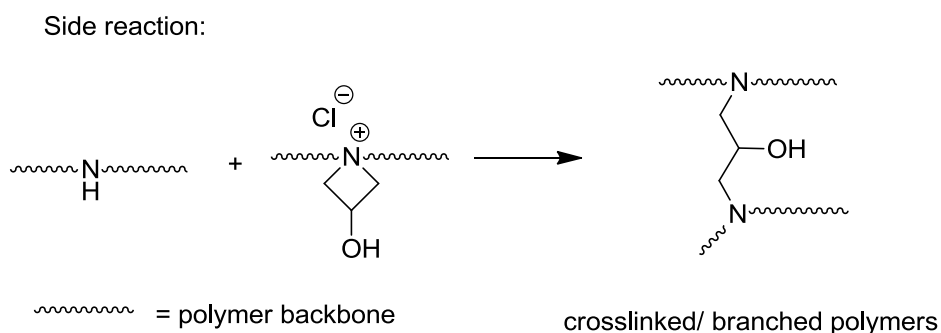
Azetidinium functionalized polymers:

(1) Hercosett:

Azetidinium functionalized polymers are mainly prepared based on the concept that the secondary amine group reacts with epichlorohydrin to form the azetidinium group. The best and most reported example of these types of polymers is Hercosett. Hercosett is a polyamino-amide epichlorohydrin resin prepared by the reaction of a poly(amino-amide) with epichlorohydrin (Scheme 4)[10]. During this reaction, the secondary amine groups of the poly(amino-amide) are transformed into azetidinium groups.
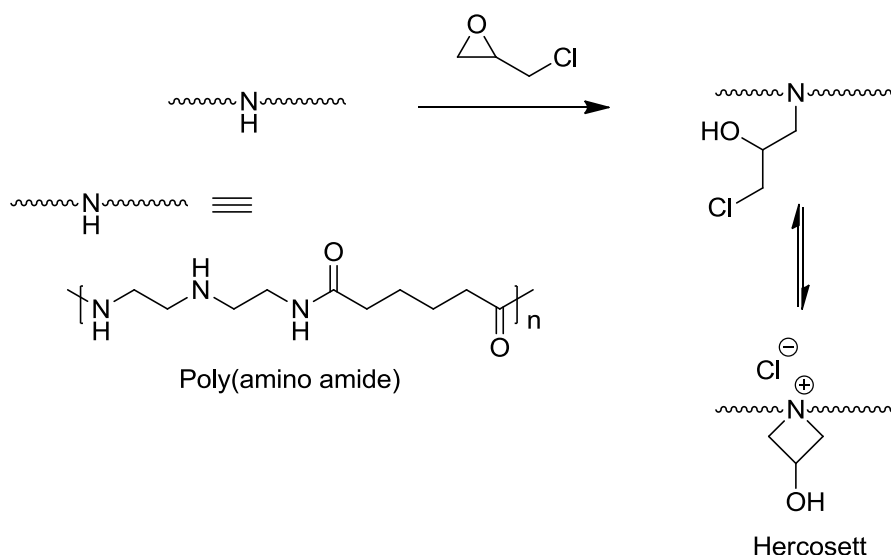
Scheme 4: Preparation of Hercosett

The main difficulty in the preparation and storage of these types of azetidinium functionalized polymers are the parallel side reaction between the azetidinium groups formed and the secondary amine groups already present in the backbone – which leads to crosslinking (Scheme 5). Due to these types of side reactions these polymers are less stable and can not be isolated in solid form. The aqueous solution of Hercosett can be stored but the percent of azetidinium groups present in the polymer backbone was not quantitatively determined.

Scheme 5: Proposed crosslinking reaction of azetidinium functionalized Hercosett resin.

The Hercosett is well known in industry for various applications, such as - to prepare shrink resist wool[11], wet strength paper[12] etc. The presence of reactive groups allows the polymer to crosslink on the surface, which is responsible for different properties. Beside this, the

presence of azetidinium groups improved the adhesion of the polymer on surface by (i) ionic interaction and (ii) by covalent bonds formed between the azetidinium groups and the functional groups on the surface such as amine (-NH$_2$), hydroxyl (-OH), carboxylate (-COO$^-$) etc.

Recently Wang et al. reported the preparation of thermo-responsive films using Hercosett/poly(N-isopropylacrylamide) [PNIPAM] composite films[13]. PNIPAM is a well known thermo-responsive polymer. Here the authors demonstrated that Hercosett as an ideal matrix for loading stimuli- responsive polymers. The composite film prepared from the well defined PNIPAM nanoparticle and Hercosett shows both: (a) thermo-responsive property (due to PNIPAM) and (b) swelling-shrinking behavior (due to Hercosett).

(2) Azetidinium functionalized guanidine based polymers:

Quin et al. reported the preparation of azetidinium functionalized guanidine based polymers via polycondensation, followed by post-polymerization modification (Scheme 6)[14]. During the reaction of the prepolymer (prepared by polycondensation of guanidine with different di-amines and tri-amines) with epichlorohydrin, the free secondary amine groups were converted into azetidinium groups. At the same time due to the parallel side reaction of azetidinium groups with the existing secondary amine groups of the polymer backbone, part of the polymer was cross-linked. These new modified guanidine based polymers with a higher molecular weight (due to crosslinking) and higher cationic charge density shows better antimicrobial efficacy than the prepolymer. The antimicrobial efficacy was tuned by verifying the ratio of guanidine and alkyl segments in the polymer backbone.
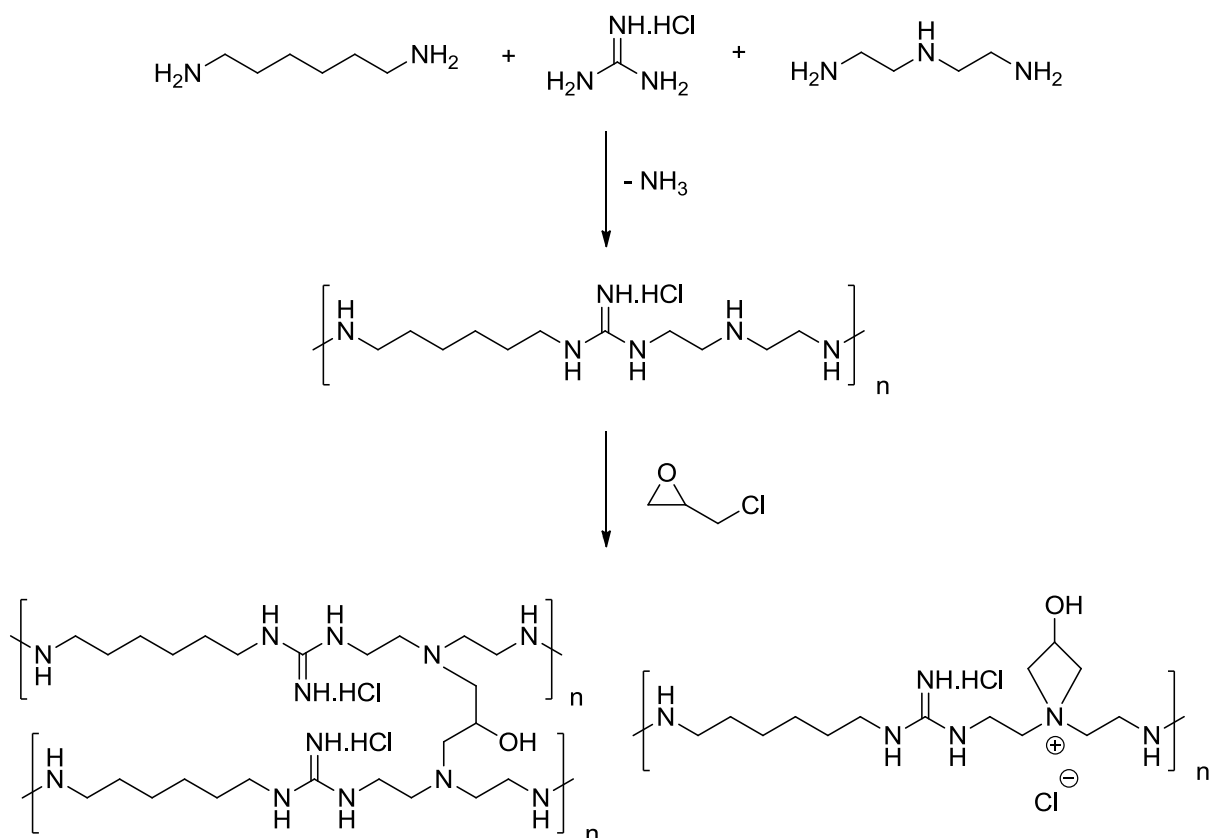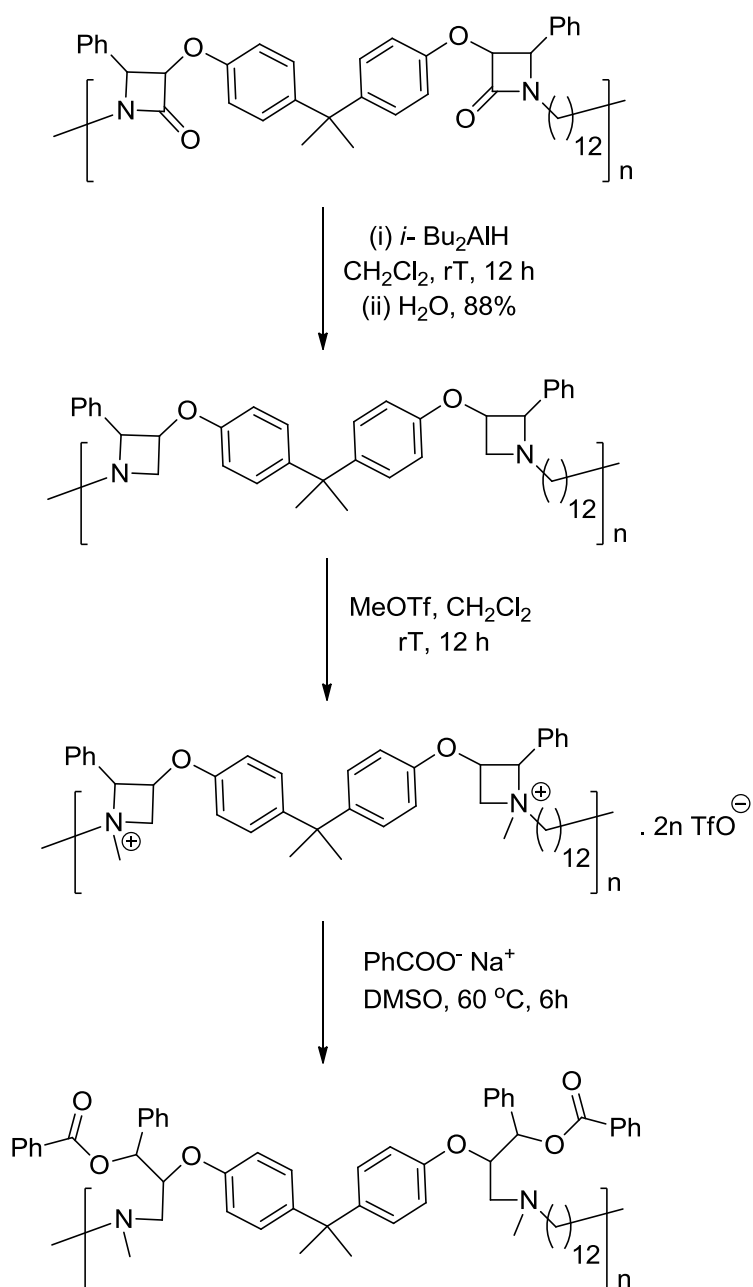
Scheme 6: Preparation of azetidinium functionalized guanidine based polymers.

(3) Preparation of azetidinium functionalized polymers via the selective reduction of 2-azetidinone moieties of the polymer backbone and post polymerization modification:
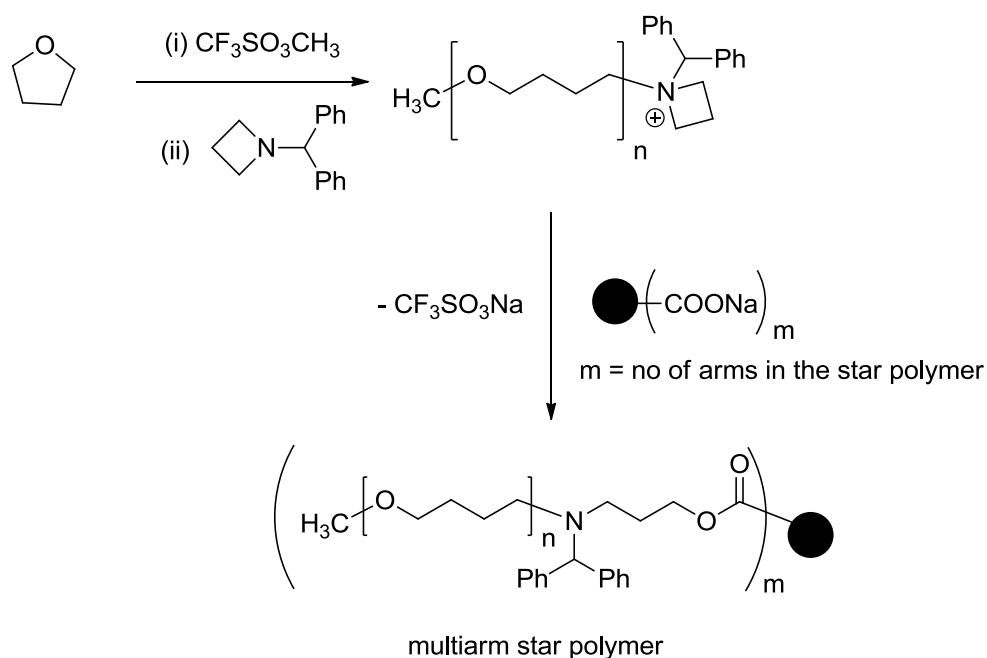
Sudo et al. prepared the azetidinium functionalized polymers via selective reduction of 2-azetidinone groups within the polymer backbone followed by quaternization (Scheme 7)[15]. First the 2-azetidinone moieties in the polymer backbone were transformed into azetidines using diisobutylaluminium hydride as reducing agent. In the next step, the azetidine functionalized polymer was reacted with methyl trifluoromethanesulfonate to prepare the corresponding azetidinium functionalized polymer. The azetidinium ring in the polymer backbone was opened by the reaction of the polymer with sodium benzoate.

(i) *i*-Bu$_2$AlH
CH$_2$Cl$_2$, rT, 12 h
(ii) H$_2$O, 88%

MeOTf, CH$_2$Cl$_2$
rT, 12 h

PhCOO$^-$ Na$^+$
DMSO, 60 °C, 6h

Scheme 7: Preparation of azetidinium functionalized compounds via selective reduction followed by quaternization.

(4) Preparation of polytetrahydrofurans, having an azetidinium salt end group:

Polytetrahydrofuran, having an azetidinium group at one of the chain ends, was prepared by polymerization of tetrahydrofuran with methyl triflate as initiator and 1-(diphenyl-methyl)-azetidine (Scheme 8)[16]. These end functionalized polymers were further used as end coupling

agent to prepare multi arm star shaped polymers via simple condensation reaction of the polymers with different types of carboxylates.

Scheme 8: Preparation of polytetrahydrofuran, containing azetidinium salt end group and their use to prepare multiarm star polymer.

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Chapter 3

Functional Polymers, Bearing Reactive Azetidinium Groups:

# Synthesis and Characterization[A]

Introduction:

Coating of textile surfaces is one of the most promising techniques, widely used to impart many benefits, such as improved wear resistance, pilling and wrinkling reduction, improved color definition, improved perfume longevity etc.

Hercosett 125[1, 2] is a well-known polymeric resin used in textile industry. It is a poly(amino-amide) epichlorohydrin resin, prepared via the reaction of poly(amino-amide)s with epichlorohydrin (Scheme 1).
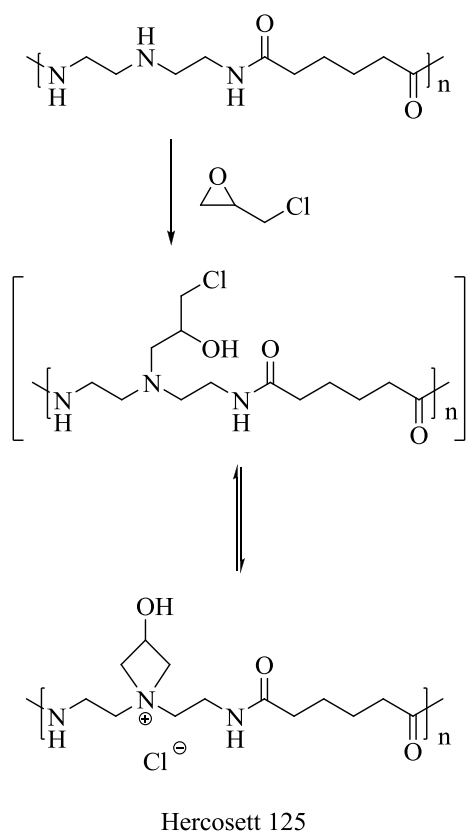
Hercosett 125

Scheme 1: Synthesis of Hercosett 125

It is assumed that azetidinium reactive groups are present in Hercosett 125. Due to their high reactivity direct evidence of the azetidinium groups and their quantification via spectroscopic techniques has not been reported. These functional groups are responsible for the high binding capacity of Hercosett 125 due to its ionic nature and cross linking ability, as the four membered strained rings can react with free secondary amino groups of the polymeric resin. The main disadvantage of Hercosett 125 is its low stability. The polymer can be handled only in solution; once the solvent is removed crosslinking occurs. This problem can be solved by synthesizing polymers, containing a lower concentration of azetidinium groups in the chain compared to Hercosett 125 and in addition hydrophobic segments. Research was started with a hydrophobic polymer containing two primary and a secondary amino groups (Scheme 2).
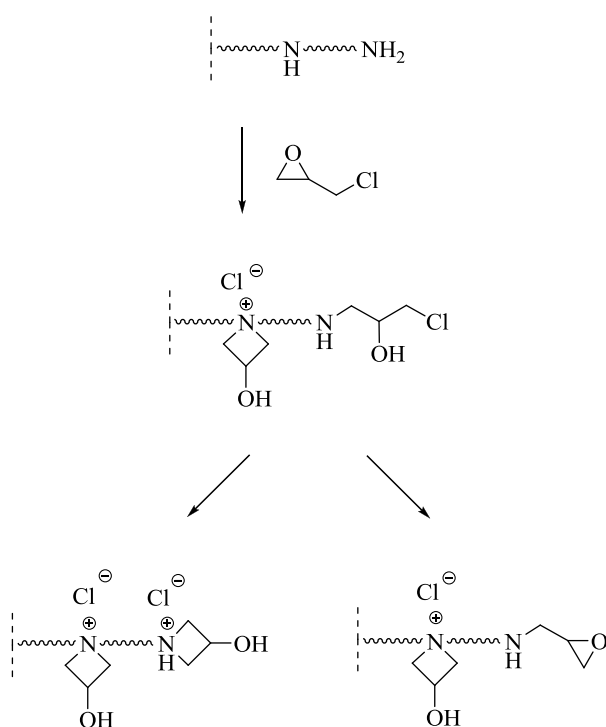

Scheme 2: Synthesis of azetidinium functionalized polymers.

These polymers are expected to absorb strongly on a wide range of substrates due to two type of interaction: (i) Ionic interaction: It is believed that the cationic azetidinium moiety, which is expected to be formed in the reaction of the secondary amino groups with epichlorohydrin,

interacts with the negatively charged surface of the substrate. (ii) Hydrophobic interaction: The hydrophobic polymer backbone is responsible for the interaction with hydrophobic domains of the substrate.

The main problem for the synthesis of the above designed polymers is the lack of selectivity for the reaction of primary amines with epichlorohydrin to give a desired product (Scheme 3). Since 1960[3], it is known that primary amines react with epichlorohydrin to give the corresponding chlorohydrin, which subsequently can be transformed to the respective epoxide via addition of base[4, 5]. On the other hand, this chlorohydrin can be spontaneously transformed into an azetidinium compound via internal cyclization[6, 7]. This cyclization is faster when the amine contains a bulky substituent (e.g.; tert. butyl, isopropyl group).
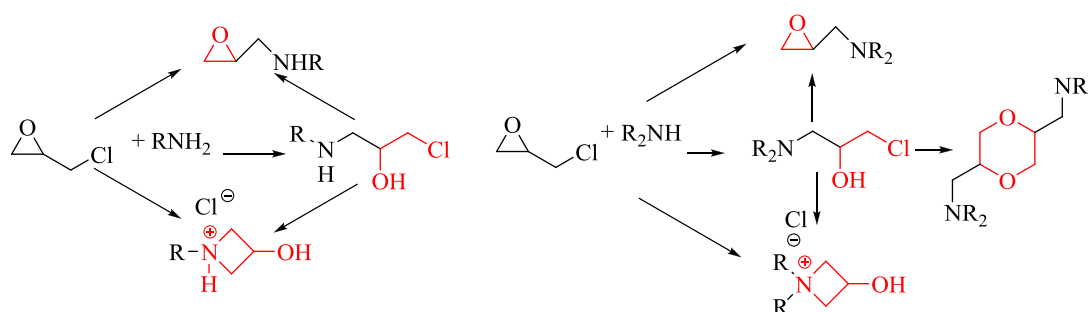
Scheme 3: Products obtained by the reaction of epichlorohydrin with amines.

The reaction of secondary amines with epichlorohydrin is more straightforward than that of primary amines. Coscia et. al. reported[8] the highly selective formation of an azetidinium compound, during the reaction of diethyl amine with epichlorohydrin. On the other hand, Heywood and Philipps[9] reported the formation of substituted dioxanes during the reaction of morpholine and piperidine with epichlorohydrin.

Due to the lack of selectivity in the reaction of epichlorohydrin with amines, it is difficult to obtain polymers with only one functional group. No systematic investigation on the effect of reaction condition on the product distribution was reported. To overcome this problem, it is

indispensable to study model reactions of primary and secondary amines with epichlorohydrin in detail. For the present study, hexylamine and diethyl amine were chosen as model amines. It is important to note that doing the reaction with low molecular weight amines, the products with different functional groups can be isolated using different purification procedures. However, doing this reaction with polymers different functional groups are linked to the same molecule. If a synergetic effect of the different functional groups, resulting from the conversion of epichlorohydrin with polyamines, is an advantage for the application as coating, has to be determined. The reaction conditions determined in model reactions, were successfully applied for the synthesis of functional polymers, containing azetidinium groups in the backbone beside other functional groups like chlorohydrin- or epoxide-groups, starting from commercially available aminotelechelic polytetrahydrofuran, called XTJ-548. The presence of the functional groups in the polymer backbone was proven by spectroscopic methods.

Experimental part:

Materials: Hexylamine (99%, Aldrich), diethylamine (99%, Aldrich), epichlorohydrin (99%, Merck), potassium iodide (99+%, Aldrich), sodium hydroxide (99%, Merck), phenol (99.5%, Merck), aminotelechelic polytetrahydrofuran (PTHF) [XTJ-548 (Huntsman)] were used without further purification. XTJ-548 is a mixture of NH_2-PTHF-NH_2 and NH_2-PTHF-NH-PTHF-NH_2 in a ratio of 1:4 and a number average molecular weight of 1200 g/mol. All the solvents (distilled water, diethyl ether, tetrahydrofuran, dichloromethane, dimethyl formamide, hexane, acetonitrile, dimethyl sulfoxide and ethanol) were used as received.

Measurements: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterated chloroform (CDCl$_3$) and deuterated dimethyl sulfoxide (DMSO-d$_6$) were used as solvents. Tetramethylsilane (TMS) was used as an internal standard. All the Raman spectra were recorded on a Buker RFS100/s

Raman spectrometer, fitted with a Nd: YAG laser (1064nm). The spectral resolution was 4 cm^{-1}. 1000 scans were collected for one spectrum at a laser power of 200mW.

Synthesis: The synthesis of the model reactions is described in the supplimentary information.

Effect of solvent on the reaction of hexylamine with epichlorohydrin.

Hexylamine (1.532 g, 15 mmol) was dissolved in the respective solvents (2mL water, ethanol, DMF or dichloromethane) and cooled down in an ice bath. Epichlorohydrin (1.417g, 15 mmol) was added drop wise to this solution maintaining the temperature below 5°C. The solutions were then allowed to stir at room temperature for 3 hours. The reaction mixture was analyzed by NMR. The yield was determined from the integral of H$_b$ (δ = 3.827 ppm) - as reference the methyl protons of hexylamine, δ = 0.88 ppm, was used (Figure SI 1).

Kinetics of the reaction between hexylamine and epichlorohydrin.

Hexylamine (0.15 g, 1.5 mmol) was dissolved in CDCl$_3$ (0.3 mL). Epichlorohydrin (0. 14 g, 1.5 mmol) was separately dissolved in CDCl$_3$ (0.3mL). These solutions were mixed at room temperature and analyzed by ^{1}H NMR spectroscopy at different time intervals.

Nomenclature of polymers: For the functional polymers - **PTHFAz$_1$Ch$_2$**, **PTHFAz$_3$**, **PTHFAz$_1$Ch$_4$**, and **PTHFAz$_1$E$_4$** - **PTHF** stands for the hydrophobic skeleton of THF repeating units, **Az$_1$** is the azetidinium group derived from the secondary amino groups, **Ch$_2$** are the chlorohydrin groups derived from a 1:1 conversion of –NH$_2$ with epichlorohydrin, **Az$_3$** refers to three azetidinium groups obtained from conversion of the secondary and the two primary amino groups, **Ch$_4$** are the four chlorohydrin groups derived from a 1:2 conversion of –NH$_2$ with epichlorohydrin, **E$_4$** stands for the four epoxy groups obtained from the conversion of **Ch$_4$**.

Synthesis of functional polymers: Synthesis of PTHFAz$_1$Ch$_2$: To a solution of aminotelechelic polytetrahydrofuran (PTHF) (XTJ-548) (4.5 g) in tetrahydrofuran (THF) (20

mL), was added at room temperature a solution of epichlorohydrin (0.71 g) in THF (2 mL). (The ratio of primary and secondary amine groups in XTJ-548 and epichlorohydrin (ech) is [NH_2: NH: ech = 3: 1: 4.5]). The reaction mixture was stirred at room temperature for 10 minutes and then at 60°C for 5 hours, before cooling down to room temperature. THF was evaporated in vacuum and a colorless viscous polymer was obtained. ^{1}H NMR (CDCl$_3$, 400MHz): δ = 4.71 (m, H^3), 4.36 (m, H^2, H^2), 3.71(m, H^1) ppm. Raman spectra: 700-750 cm$^-$^{1} (C-Cl bond)
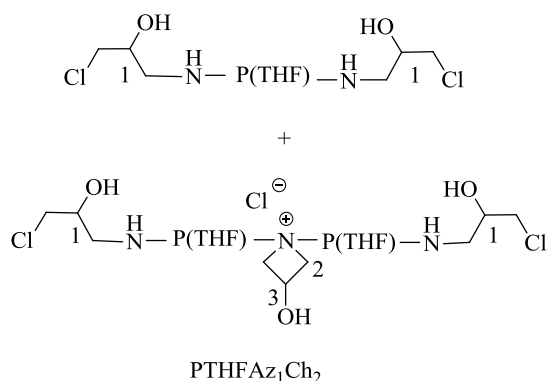
PTHFAz$_1$Ch$_2$

Synthesis of PTHFAz$_3$: To a THF solution of PTHFAz$_1$Ch$_2$ (prepared as described before), was added a solution of KI (1.3 g) in water (5 mL) and stirred for 4 hours at 40^0C. Then THF and water were removed in vacuum. ^{1}H NMR (CDCl$_3$, 400MHz): δ = 4.79, 4.58 (m, H^3), 4.44 (m, H^2, H^2), 3.90 (m, H^1) ppm.
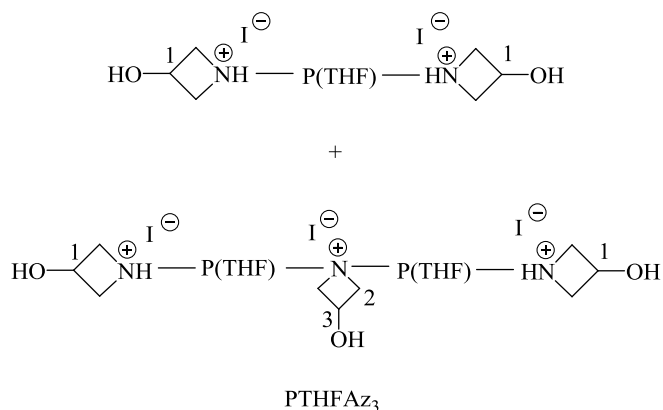

PTHFAz$_3$

Synthesis of PTHFAz$_1$Ch$_4$: To a solution of aminotelechelic polytetrahydrofuran (PTHF) (XTJ-548) (4.5 g) in THF (20 mL), was added at room temperature a solution of epichlorohydrin (1.4 g) in THF (2mL). (The ratio of primary and secondary amine groups in

XTJ-548 and epichlorohydrin (ech) is [NH_2: NH: ech=3:1:7.5]). The reaction mixture was stirred at room temperature for 10 minutes, then at $60^{\circ}C$ for 6 hours and cooled down to room temperature. THF was evaporated in vacuum and a colorless viscous polymer was obtained.

^{1}H NMR (CDCl$_3$, 400MHz): δ = 4.54 (m, H^3), 4.38 (m, H^2, H^2), 3.86(m, H^1) ppm. Raman spectra: 700-750cm^{-1} (C-Cl bond)
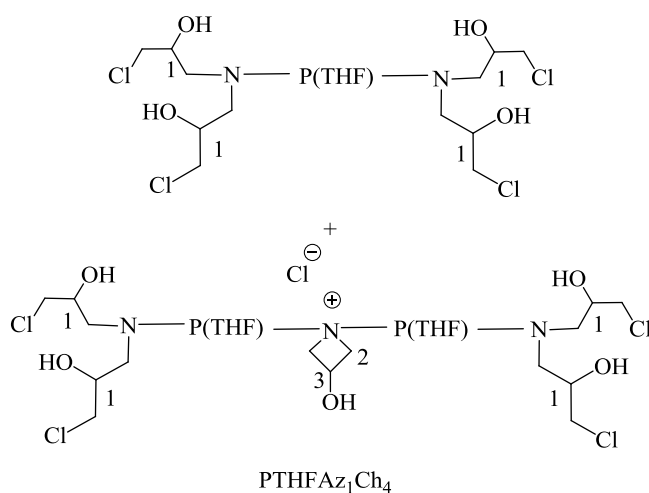

PTHFAz$_1$Ch$_4$

Synthesis of PTHFAz$_1$E$_4$: To a THF solution of PTHFAz$_1$Ch$_4$ (prepared as described before) was added dropwise an aqueous solution of NaOH until pH 14. The reaction mixture was stirred for 2 hours. Finally, the solvent was evaporated in vacuum. ^{1}H NMR (CDCl$_3$, 400MHz): δ = 4.67 (m, H^3), 4.32 (m, H^2, H^2), 3.03, 2.85(m, H^1, H$^{1'}$) ppm. Raman spectra: 1259 cm^{-1} (Epoxide)

PTHFAz$_1$E$_4$
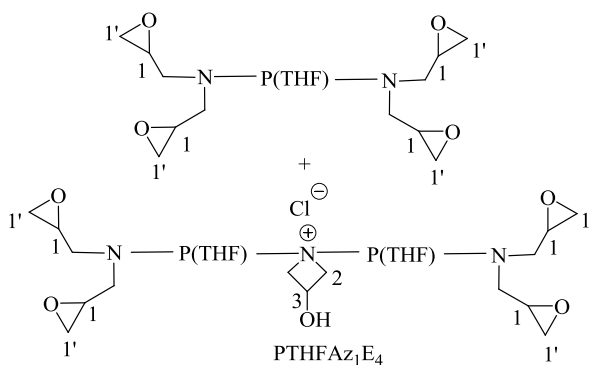
Results and Discussions:

Starting from an aminotelechelic polytetrahydrofuran with a secondary amine group in its backbone, different functional polymers, containing an azetidinium group in the backbone, and chlorohydrin, epoxide or azetidinium groups at the chain end were prepared (Scheme 4).
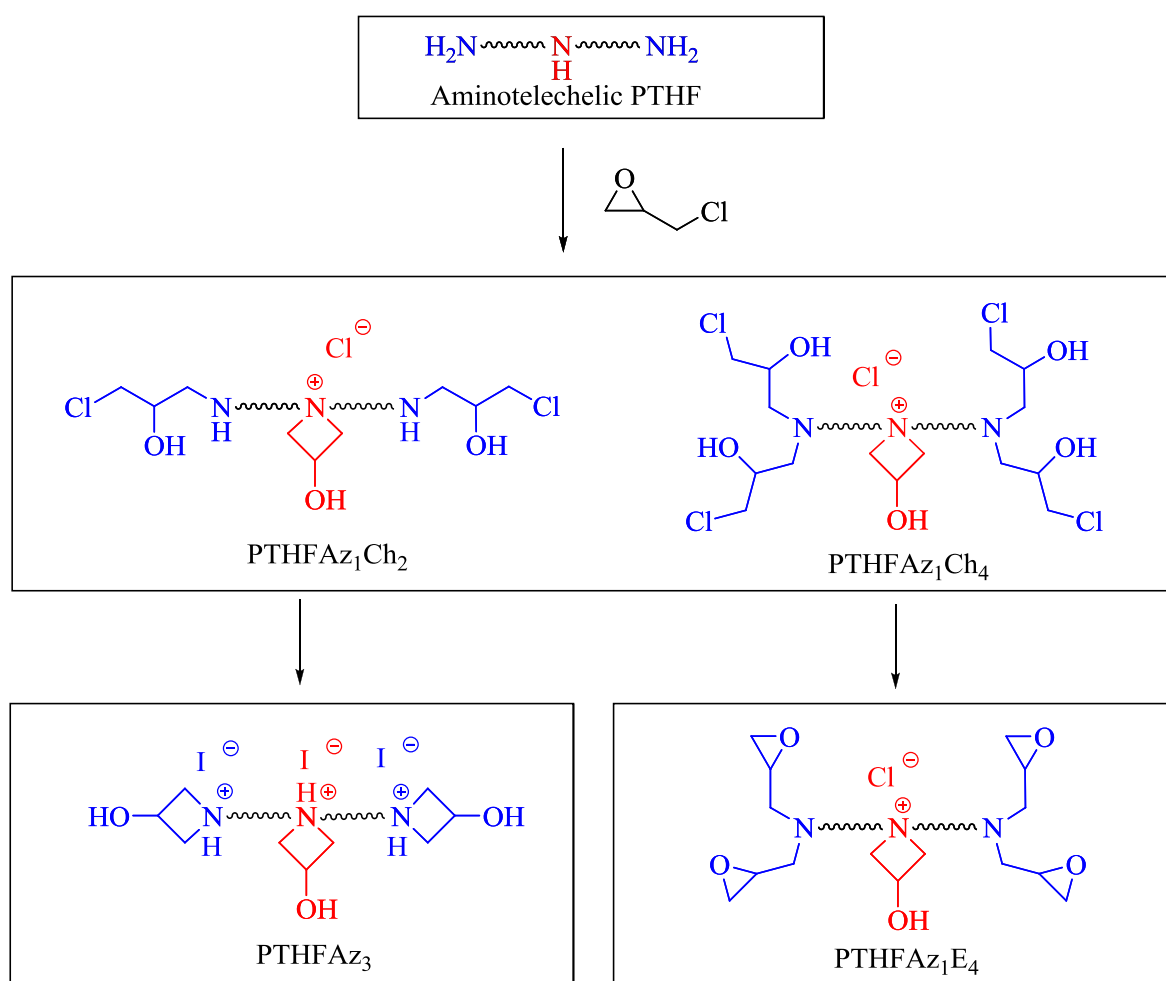
Scheme 4: Schematic presentation of different functional polytetrahydrofurans (for clarity reasons only the major component of the aminotelechelic polymer (XTJ-548) is considered in this Scheme)

To prepare the polymers having the desired functional groups in their backbone, it is indispensable to determine the reaction condition which generate a particular functional group via the reaction of amines and epichlorohydrin. Therefore, model reactions of a primary and secondary amine with epichlorohydrin were performed under different reaction conditions. The main aim behind this study was: (i) to understand the influence of reaction parameters on product distribution; (ii) analysis of the side products generated during the reactions; (iii) detailed kinetic study of the reaction of a primary amine and epichlorohydrin.

The knowledge gained from the model reactions were successfully used to synthesize functional polymers having the desired functional groups.

Model reactions:

Reactions of primary and secondary amines with epichlorohydrin have been reported in the literature. However, no systematic variation of the reaction conditions combined with an analysis of the reaction products has been reported. Here the model reactions were performed to understand the influence of reaction parameters on the formation of products having specific functional group. Later side products generated during these reactions were determined.

Reactions of hexylamine with epichlorohydrin.

The reaction products obtained from epichlorohydrin (A) and hexylamine (B) in a molar ratio of A/B = 1/1, 2/1 and 1/2 are shown in scheme 5. In the following, the reaction conditions applied to obtain these products, having different functional groups, are reported.

Chlorohydrin C: The reaction between epichlorohydrin (A) and hexylamine (B) (molar ratio 1/1) was performed in different solvents (water, ethanol, dimethylsulfoxide, triethylamine, dichloromethane, chloroform, diethylether, chloroform, THF, hexane etc.). The main product was always the chlorohydrin C with side products in low concentration. The reaction rate

however, is strongly dependent on the solvent used: the higher the polarity of the solvent, the higher the reaction rate. The side products in this reaction were a consequence of the fact that the primary reaction product, which contains a secondary amino group and an activated carbon/halogen bond can further react with epichlorohydrin and with hexylamine.
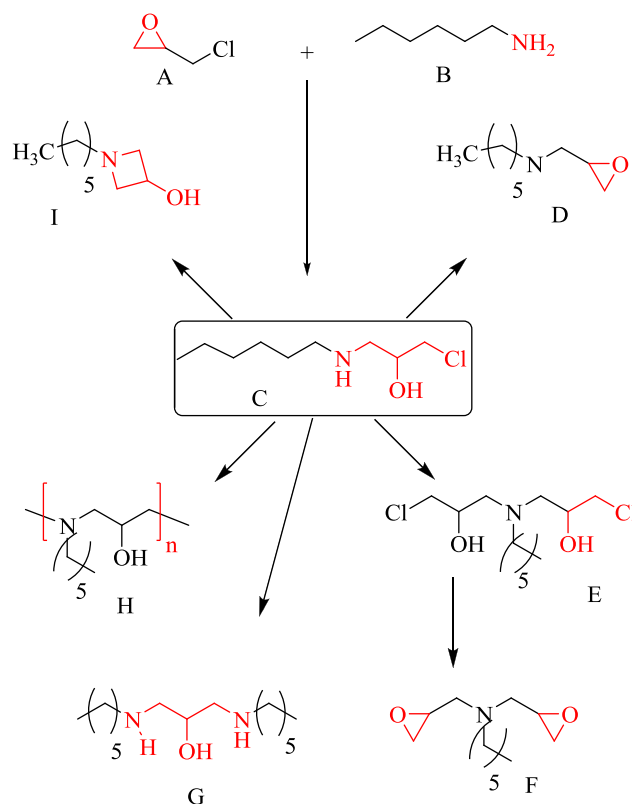
Scheme 5: Reactions between hexylamine and epichlorohydrin under different reaction conditions.

Epoxide D: Treatment of the chlorohydrin C with aqueous sodium or potassium hydroxide leads to the formation of an epoxide group. Analysis of the reaction product shows the presence of side products. (The explanation given above is still valid) *Bis-chlorohydrin E*: If epichlorohydrin (A) and hexylamine (B) are reacted in a molar ratio 2/1 the product of the reaction is as expected b*is*-chlorohydrin *E*. It could be proven that one of the side products during synthesis of product C is product E. **Bis-epoxide F**: Reacting product E with excess of aqueous sodium hydroxide yielded *bis*-epoxide *F*. **Diamine G**: Reaction of epichlorohydrin
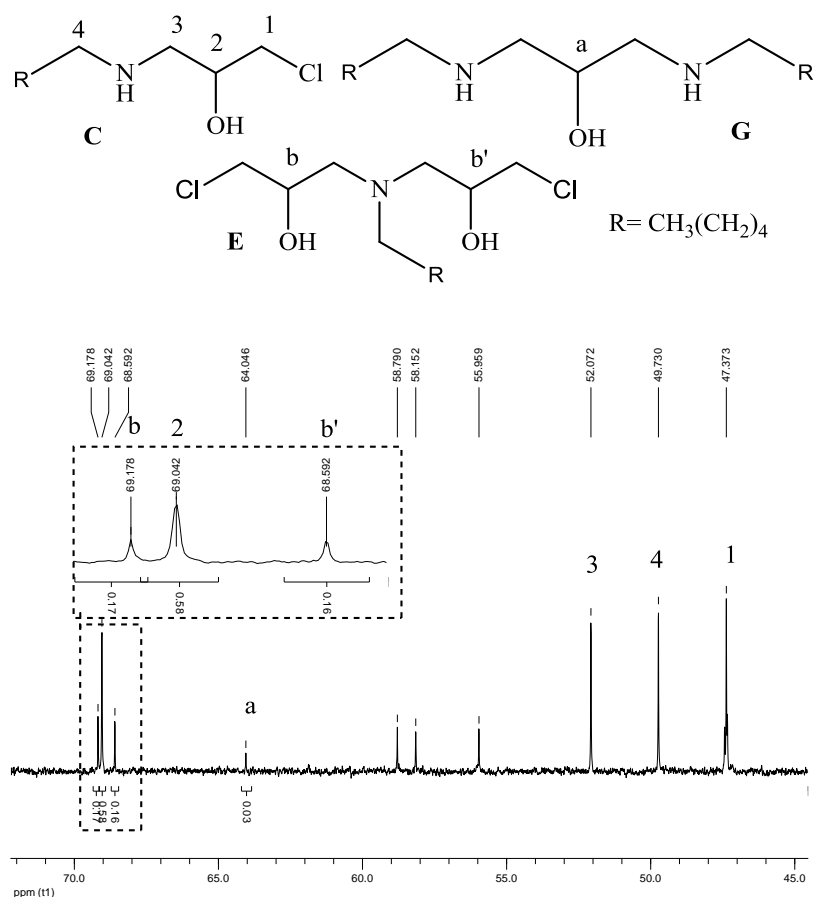
(A) and hexylamine (B) in a molar ratio 1/2 results the formation of diamine G. This product is another side products observed in the preparation of product C. **Polymer H**: By heating product C to 90°C resulted in the formation of a polymeric product which was not fully characterized. We assume that this polymer is formed by polycondensation with elimination of hydrochloric acid. **Azetidine I**: Conversion of product C into product I, containing a reactive azetidine functional group, was performed by a Finkelstein reaction (addition of potassium iodide) or by heating chlorohydrin C in acetonitrile under reflux followed by neutralization with aqueous sodium hydroxide.

The model reactions proved that by application of certain reaction conditions for the conversion of hexylamine with epichlorohydrin, a particular functional group - chlorohydrin, epoxide, azetidine - can be generated. Side reactions can be reduced to a minimum.

In the following the NMR spectroscopic characterization of the products and side products generated in the reaction of hexylamine and epichlorohydrin and the reaction kinetics is reported.

Reaction of Hexylamine with epichlorohydrine (1:1 mole ratio): NMR analysis

Quantitative ^{13}C NMR spectra (Figure 1) of the raw product obtained in the reaction of hexylamine and epichlorohydrin in water showed that beside the main signals, which can be assigned to product C, signals of lower intensity which can be assigned to the side product E and G are observed. The side products are a consequence of further reaction of the functional group in the product C with starting material: C and B results in G and C and A results in E.

Figure 1: Quantitative ^{13}C spectrum showing the main and two side products in the reaction

of hexylamin and epichlorohydrin (molar ratio 1:1).

The signals of side product E were further verified by comparing the ^{13}C NMR spectra of the

raw product (containing C as the major product) with the ^{13}C NMR spectrum of E, prepared

by reaction of hexylamine and epichlorohydrine in a molar ratio of 1:2 (Figure 2).
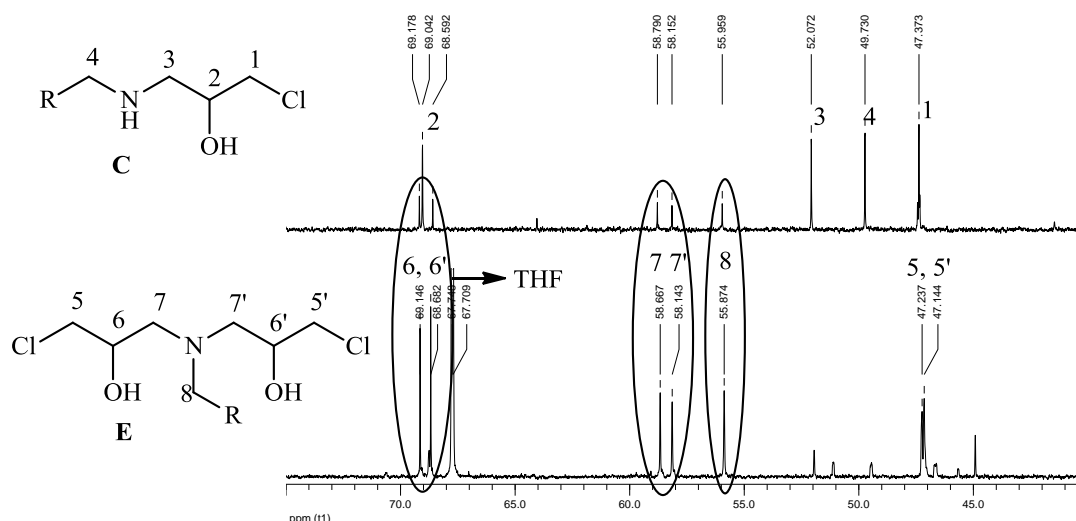
Figure 2: Comparison of the ^{13}C NMR spectrums of C and E (in CDCl$_3$).

The other side product G was isolated by dissolving the product mixture in acetonitrile, G was found to be insoluble in acetonitrile.

When the raw product C was converted into the corresponding epoxide (D) by treatment with aqueous sodium hydroxide, the signals of low intensity found in the ^{13}C NMR spectrum around $\delta = 57, 56, 51, 50$ and 44 ppm could be assigned to bis-epoxide F beside the major product D (Figure 3). This is an indirect prove for the presence of E as a side product in the raw product mixture, containing C as major product.
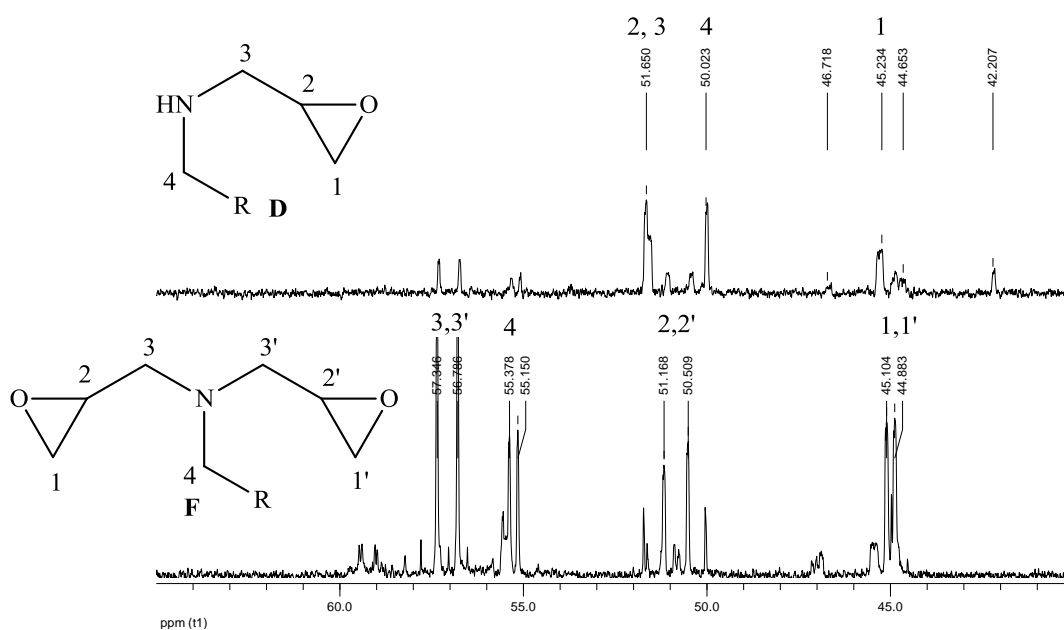
Figure 3: Comparison of the ^{13}C NMR spectra (in CDCl$_3$) obtained during the synthesis of D, showing the formation of F as a side product.

The aminochlorohydrin C was converted to corresponding azetidinium compound (I) by two different procedures: (i) by heating chlorohydrin C in acetonitrile under reflux[7]. The formation of the azetidinium compound under these conditions can be explained by the enhanced nucleophilicity of the secondary amine in acetonitrile leading to the intramolecular substitution of the chlorine atom and formation of the four membered rings. Quantitative conversion of the chlorohydrin was achieved (Figure 4B). (ii) By treating chlorohydrin C with potassium iodide. In the Finkelstein reaction the C-Cl bond is partially converted into a C-I bond, followed by spontaneous internal cyclization and formation of the azetidinium compound. For this reaction only partial conversion of the chlorohydrin was achieved as is shown in Figure 4A. The formation of azetidinium compound was proven by ^{13}C NMR analysis: the appearance of new peaks around ☐= 64, 60 and 59 ppm are characteristic for azetidinium compounds (Figure 4).

R = CH$_3$ (CH$_2$)$_4$
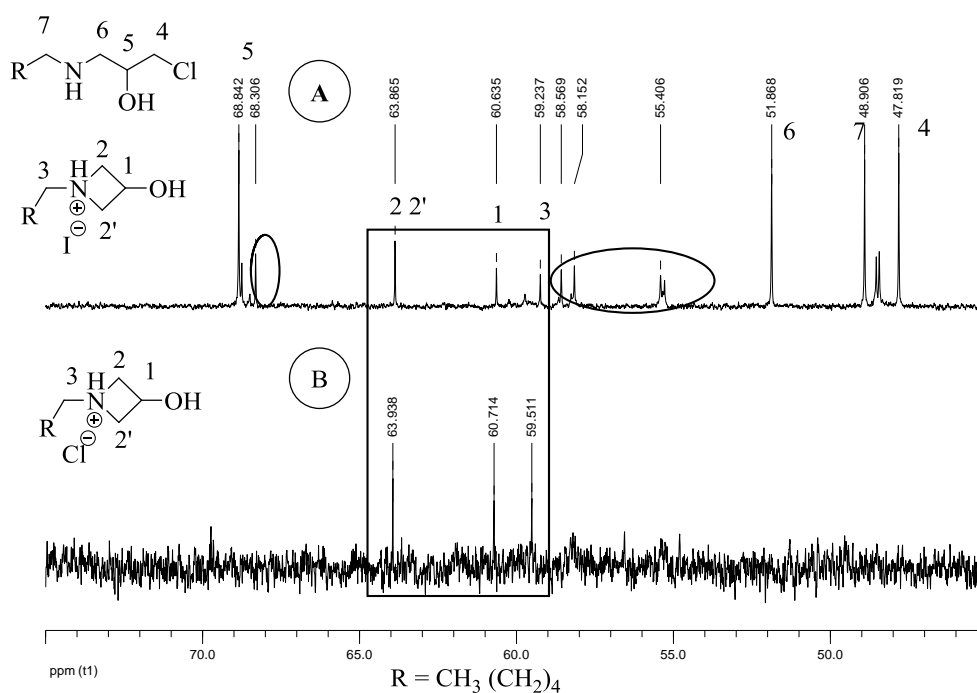
Figure 4: Comparison of the ^{13}C NMR spectra of the reaction product obtained by conversion of amino-chlorohydrin C: (A) with KI (in this reaction only partial conversion was obtained) (B) by refluxing in acetonitrile (full conversion of C)

Kinetics and effect of solvents for the reaction of hexylamine with epichlorohydrin

It is known that the solvent has a large effect on the rate and selectivity of substitution reactions. Protic, and aprotic solvents of different polarity were chosen to perform the reaction between hexylamine and epichlorohydrin (mole ratio = 1:1): water (dielectric constant 80), dimethylformamide (dielectric constant 38), ethanol (dielectric constant 30), dichloromethane (dielectric constant 9.1) and chloroform (dielectric constant 4.8). It turned out that within 3.5 hours, the highest yield of the chlorohydrin was obtained in water (80%), followed by ethanol (62%), dimethylformamide (31%) and dichloromethane (24%) (Figure 5).
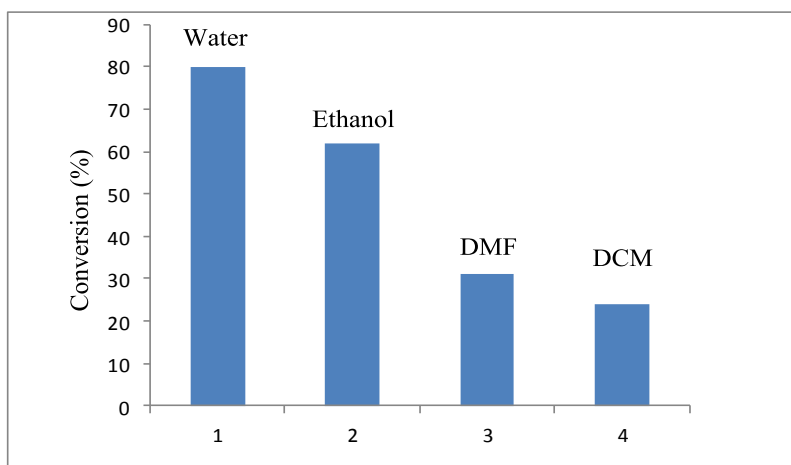
Figure 5: Reaction of hexylamine (7.5 mol/L) and epichlorohydrin in different solvents: conversions obtained after 3.5 hours at 5°C.

From these results, it was concluded that polar protic solvents show the highest reaction rate. This result can be understood, since the reaction proceeds via a second order nucleophilic addition reaction, in which the transition state is stabilized by polar protic solvents (Scheme 6).
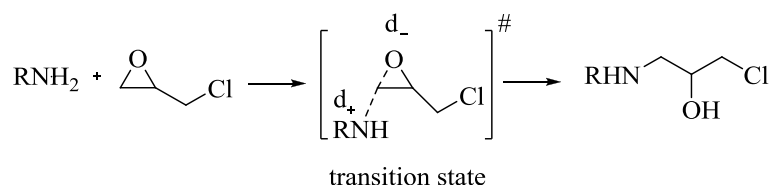
Scheme 6: Mechanistic approach for the nucleophilic addition reaction of amines to epoxides

From ^{13}C NMR analysis (Figure SI 2), it was concluded that side reactions could be minimized in non polar solvents – meaning that at lower reaction rates higher selectivity can be obtained.

To better understand the course of the reaction, the conversion of epichlorohydrin and the formation of amino-chloro-hydrine was followed directly via ^{1}H NMR analysis using deutero chloroform as solvent (Figure SI 1). The concentration of epichlorohydrin was determined from the integral of H_a (δ = 3.2 ppm) while the concentration of the product was determined from the integration of H_b (δ = 3.827 ppm) (as reference the methyl protons of hexyl amine, δ = 0.88 ppm, were used). The reaction was found to follow a second order kinetics with a change in the slope (AB and BC) after ca. 90 minutes (Figure 6).
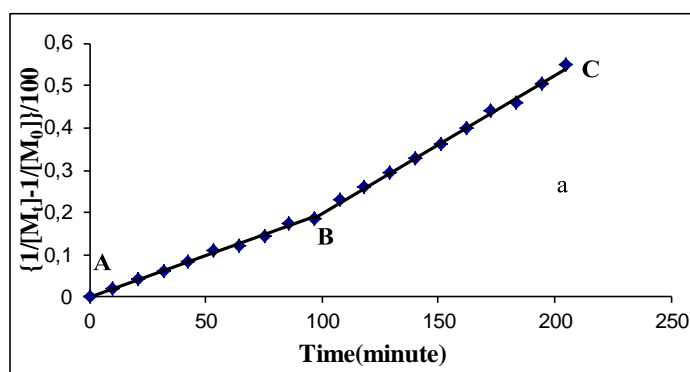

Figure 6: Kinetics of the reaction between hexylamine and epichlorohydrin (mole ratio = 1:1) at room temperature in CDCl$_3$: (a) kinetics for the first 210 minutes of reaction; (b) kinetics for the first 90 minutes of the reaction.

This change of the slope can be explained by the side reaction, which is a consequence of the reaction of the secondary amine group of the product C with epichlorohydrin (A). For the first 90 minutes the rate constant of the reaction was calculated to be 0.033×10^2 L mol^{-1} sec^{-1}.

Reaction of secondary amines with epichlorohydrin:

When diethyl amine and epichlorohydrin were reacted in a 1: 1 ratio, 1, 1'-diethyl-3-hydroxy azetidinium chloride was obtained as the only product in high purity (Scheme 7).
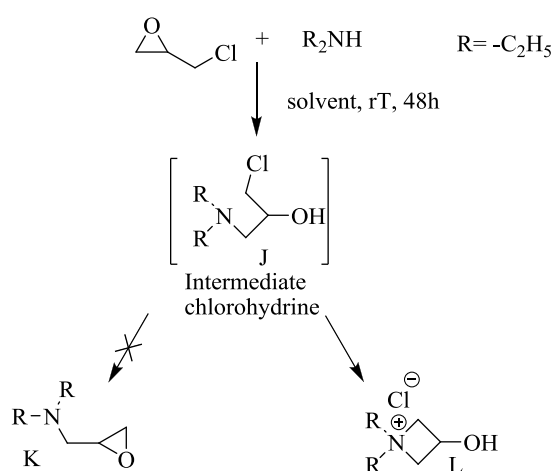
**Scheme 7**: Reaction of diethyl amine with epichlorohydrin.

Although, 1-chloro-3(diethyl amino) propan-2-ol (compound J) was found as an intermediate in the reaction, this was completely transformed to the azetidinium compound L. This is explained by the higher thermodynamic stability of the azetidinium ring compared to its linear precursor and the isomeric oxirane K.

3-Hydroxy azetidinium salts contain a four membered ring, which can exist in two conformations (Figure 7). Due to the rigidity of this ring, the two conformations should have different NMR-spectra at room temperature.
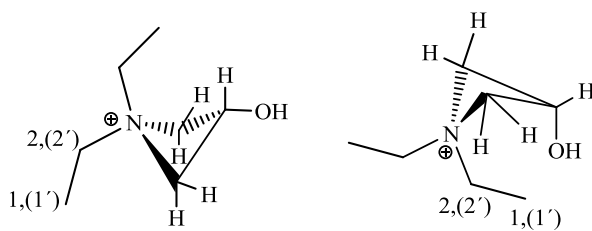
Figure 7: possible conformers of 1, 1'-diethyl-3-hydroxy azetidinium chloride

The ^{13}C spectrum (Figure 8) shows two different peaks for the carbon atoms of the ethyl groups (signals 1 and 2) and one peak for the two equivalent endocyclic CH_2 protons indicating that only one conformer was formed.
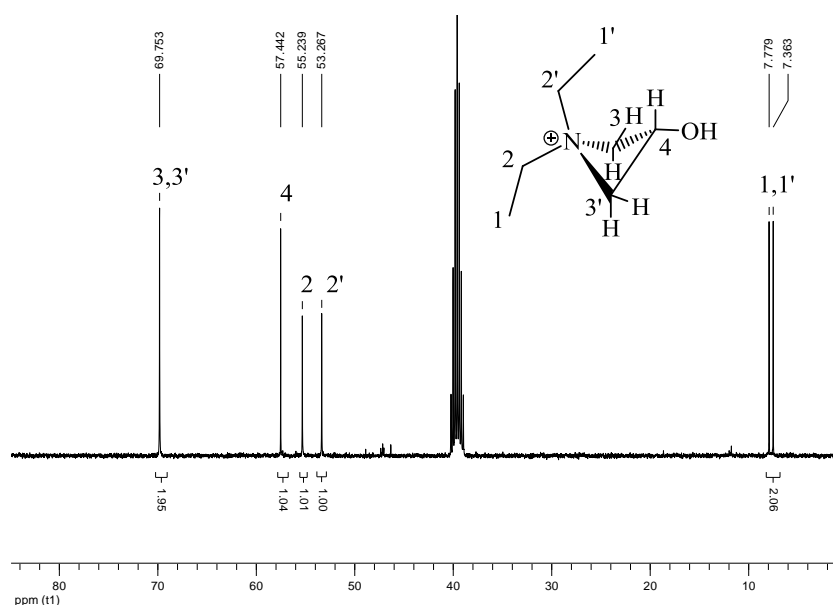

Figure 8: ^{13}C NMR spectra of 1, 1'-diethyl-3-hydroxy azetidinium chloride.

Ab initio calculation indicate that there is no ring inversion possible in this molecule and as known from substituted cyclobutane derivatives, only equatorial isomers are observed.[10]

**Reaction of the 1, 1'-diethylazetidinium chloride with nucleophiles:**

The azetidinium group has a large ring strain, and as a consequence it is expected that this group shows a high reactivity towards nucleophiles. Since this behaviour may play a certain role in the adsorption properties of polymers bearing this group, we have studied the

nucleophilic ring-opening reaction of 1, 1'-diethylazetidinium chloride using different nucleophiles. The positive charge centered on the nitrogen atom makes the 2 and 4 position of the ring suitable for a nucleophilic attack (Figure 9), leading to a ring opened product.
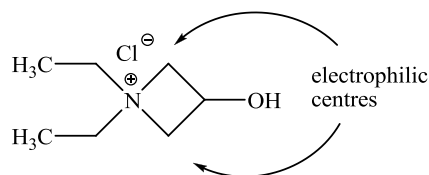
Figure 9: Reactive centers for nucleophiles in 1, 1'-diethylazetidinium chloride

It was found that phenol does not react with 1, 1'-diethylazetidinium chloride, however phenoxide can easily open the ring leading to product M. In addition, both primary and secondary amines were found to be sufficiently nucleophilic to open the ring at room temperature, leading to product N and O (Scheme 8).
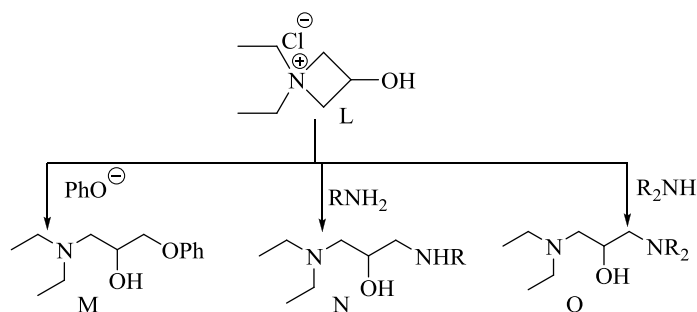
Scheme 8: Ring opening reaction of the azetidinium ring using nucleophiles

The NMR spectra of the products clearly show that full conversion of the substrate was achieved and only one main product is obtained (SI Fig 3, 4, 5).

Synthesis of functional polytetrahydrofurans with different substitution pattern:

Based on the results obtained in the model reactions, reaction conditions were defined under which the primary and secondary amine groups in the aminotelechelic polytetrahydrofuran can be selectively converted. Four different multifunctional polymers with the same polymer

backbone, an azetidinium group in the center of the polytetrahydrofuran dimer and different functional end groups were prepared from XTJ-548, by applying the reaction condition selected for the model reactions (Scheme 9 and 10).

Polytetrahydrofuran with one azetidinium groups and two chlorohydrin groups(PTHFAz$_1$Ch$_2$) and polytetrahydrofurans with three azetidinium groups (PTHFAz$_3$) were prepared from aminotelechelic polytetrahydrofuran XTJ-548 and epichlorohydrin using one equivalent of epichlorohydrin per nitrogen atom.

PTHFAz$_1$Ch$_2$ contains the amino chlorohydrin moiety at the chain ends and an azetidinium ring in the polymer backbone and was obtained by simply mixing XTJ-548 with epichlorohydrin.

For the preparation of **PTHFAz$_3$** PTHFAz$_1$Ch$_2$ was dissolved in acetonitrile and the solution was heated to reflux. Instead of PTHFAz$_3$ a crosslinked rubberlike polymer was found to be formed. This can be explained by the enhanced nucleophilicity of the secondary amine groups in the amino-chlorohydrin moiety of the polymer in acetonitrile which reacts with nascent azetidinium groups leading to crosslinking. However, by reacting PTHFAz$_1$Ch$_2$ with potassium iodide at 40°C for 4 hours (Finkelstein reaction), transformation of the amino-chlorohydrin moiety into azetidinium rings could be achieved.
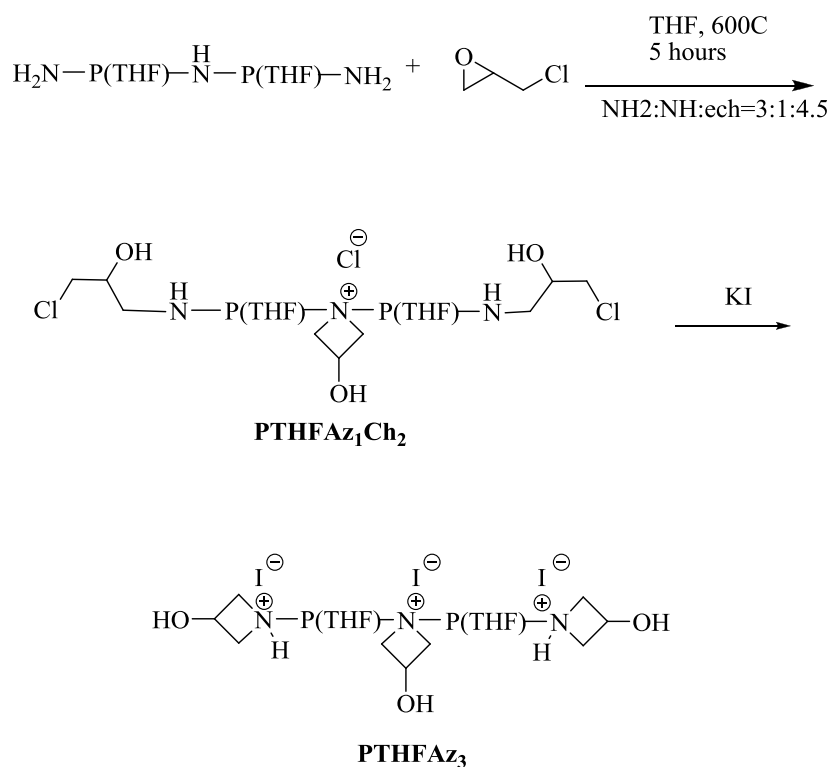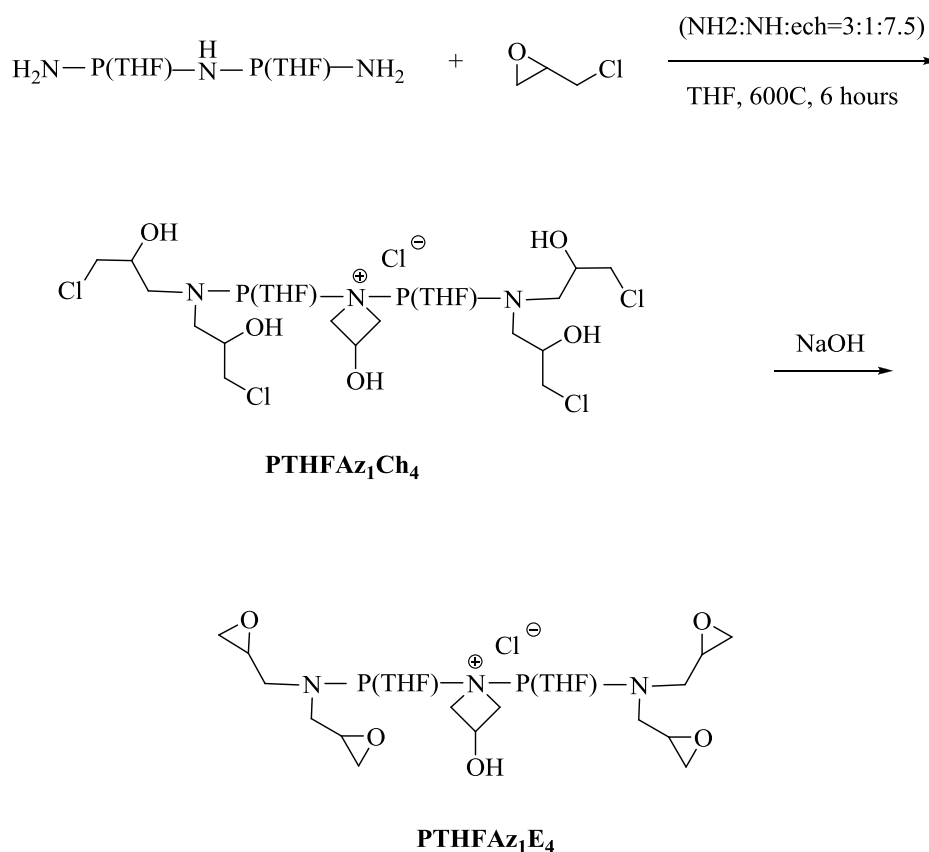
Scheme 9: Synthesis of the PTHFAz$_1$Ch$_2$ and PTHFAz$_3$.

The third sample **PTHFAz$_1$Ch$_4$** was prepared from aminotelechelic polytetrahydrofuran XTJ-548 and epichlorohydrin using one equivalent of epichlorohydrin per nitrogen-hydrogen bond. The product contains an azetidinium ring and four amino chlorohydrin groups (thus the concentration of the chlorohydrin groups is twice as high as in PTHFAz$_1$Ch$_2$).

The fourth sample **PTHFAz$_1$E$_4$** was obtained by reaction of PTHFAz$_1$Ch$_4$ with aqueous sodium hydroxide. This treatment converts all chlorohydrin groups into epoxy functional groups.

$$\text{H}_2\text{N}\!-\!\text{P(THF)}\!-\!\overset{\text{H}}{\underset{}{\text{N}}}\!-\!\text{P(THF)}\!-\!\text{NH}_2 \quad + \quad \overset{\text{O}}{\triangle}\!\!\diagdown\text{Cl} \xrightarrow[\text{THF, 600C, 6 hours}]{\text{(NH2:NH:ech=3:1:7.5)}}$$

PTHFAz₁Ch₄

PTHFAz₁E₄

Scheme 10: Synthesis of the PTHFAz$_1$Ch$_4$ and PTHFAz$_1$E$_4$.

The structure of the functional groups in these four polymer samples was proven by ^{1}H NMR – and Raman spectroscopy. The signals of the characteristic protons in the NMR spectra of functional polymers were identified by comparison with the corresponding spectrum of the model compounds. The formation of the azetidinium group during the reaction of the secondary amine of XTJ-548 and epichlorohydrin was proven by the characteristic peaks at δ = 4.3-4.8 ppm for all four functional polymers as shown in the Figure 10.

The synthesis of PTHFAz$_1$Ch$_2$ from XTJ-548 was proven by the appearance of the signal at δ = 3.71 ppm, characteristic chiral proton of the chlorohydrin group, which in case of the model compound (C), was found at δ = 3.87 ppm. When the model compound C was transformed to I, the same proton was shifted to δ = 4.13 ppm (Δ δ = 0.26 ppm). When similar reaction condition were used for the synthesis of PTHFAz$_3$, the proton was found to be shifted at δ =

3.91 ppm ($\Delta \delta = 0.2$ ppm), which proves the transformation of the chlorohydrin groups to azetidinium groups.
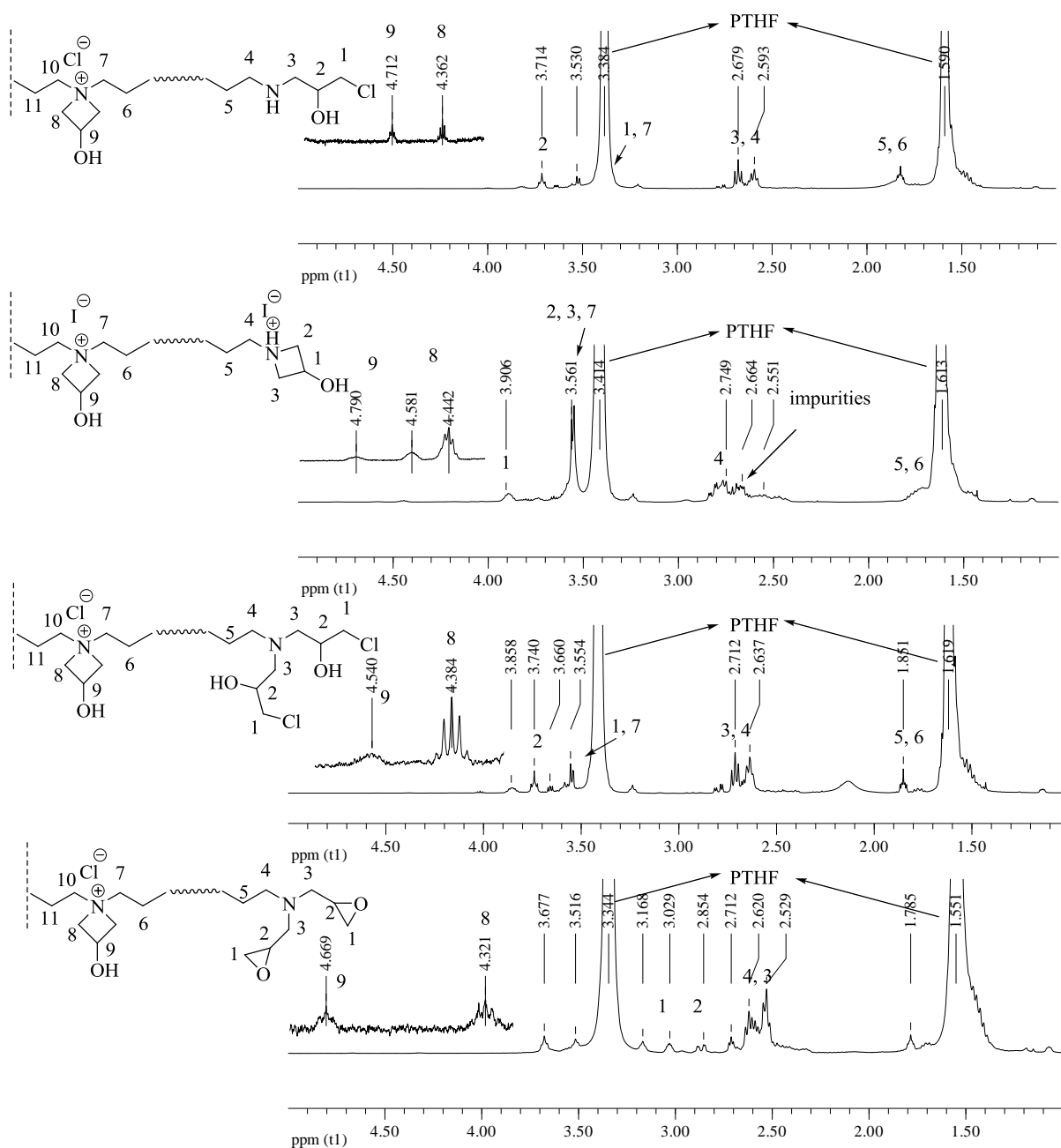
Figure 10: ^{1}H NMR spectrum of different functional polymers: (A) PTHFAz$_1$Ch$_2$ (B) PTHFAz$_1$Ch$_4$ (C) PTHFAz$_1$E$_4$ (D) PTHFAz$_3$.

The synthesis of PTHFAz$_1$Ch$_4$ from XTJ-548 was proven by the appearance of the peak, at $\delta = 3.74$ which is characteristic for the proton attached to the chiral carbon atom of the

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chlorohydrin group. The formation of PTHFAz$_1$E$_4$ was proven by the appearance of new peaks at $\delta = 3.03$ and $\delta = 2.85$ ppm, which are characteristic for the epoxide ring protons.

The synthesis of these functional polymers was also proven by Raman spectroscopy, which is considered an excellent spectroscopic method to prove the presence of different functional groups like epoxide, amine and alkyl chloride. Raman spectra were chosen due to the fact that, peaks for amino groups are stronger than those for alcohol groups. The conversion of amino groups in presence of an alcohol groups can be proven by this spectroscopic tool. However, there is no established spectroscopic method to prove the formation of azetidinium groups. Thus the formation of azetidinium groups cannot be directly proven by any spectroscopic technique.
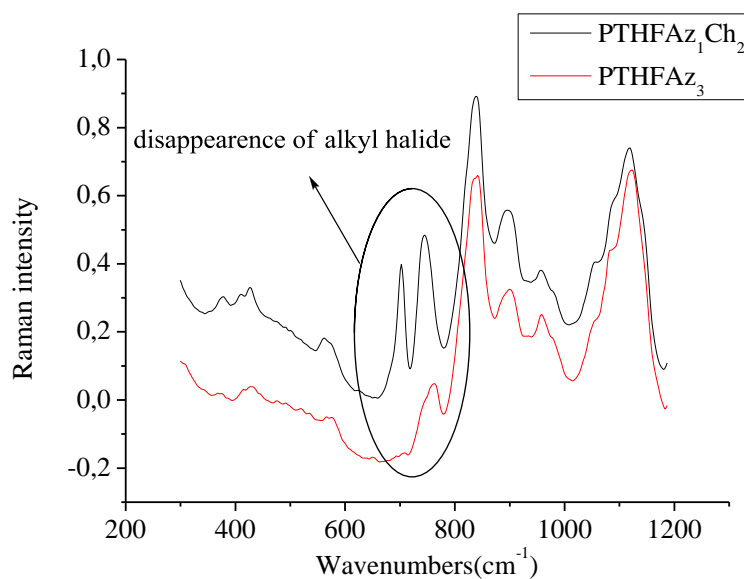
During the synthesis of PTHFAz$_1$Ch$_2$ from XTJ-548, the primary amino groups were transformed to an amino chlorohydrin groups.

This transformation was proven (Figure 11) (a) by the disappearance of the peak for primary amines around 3300 cm^{-1} in the spectrum of PTHFAz$_1$Ch$_2$, which was present in the spectrum of XTJ-548 and (b) by the appearance of characteristic peaks for alkyl chloride bands around 700-750 cm^{-1}, in the spectrum of PTHFAz$_1$Ch$_2$, which were absent in the spectrum of XTJ-548.
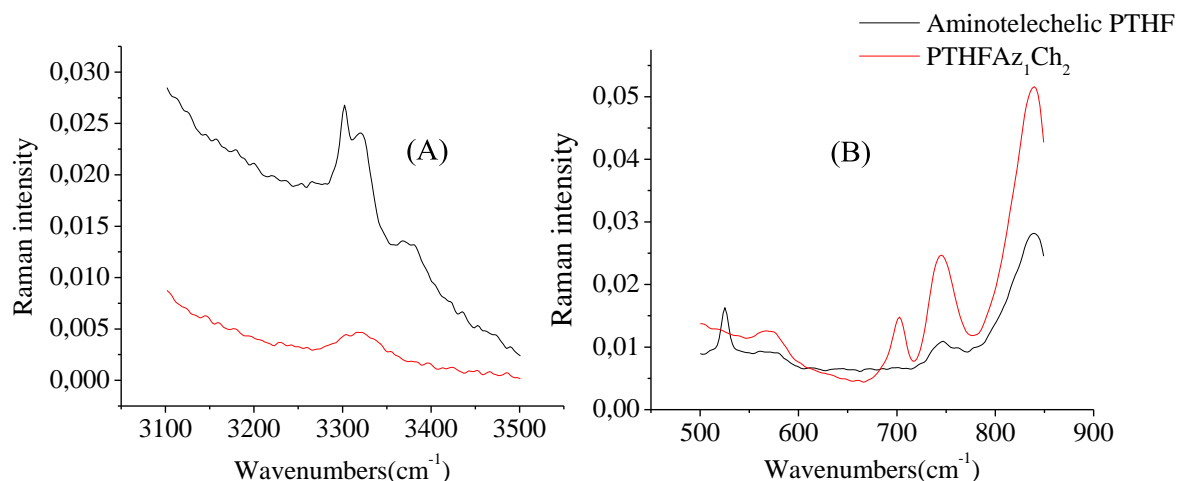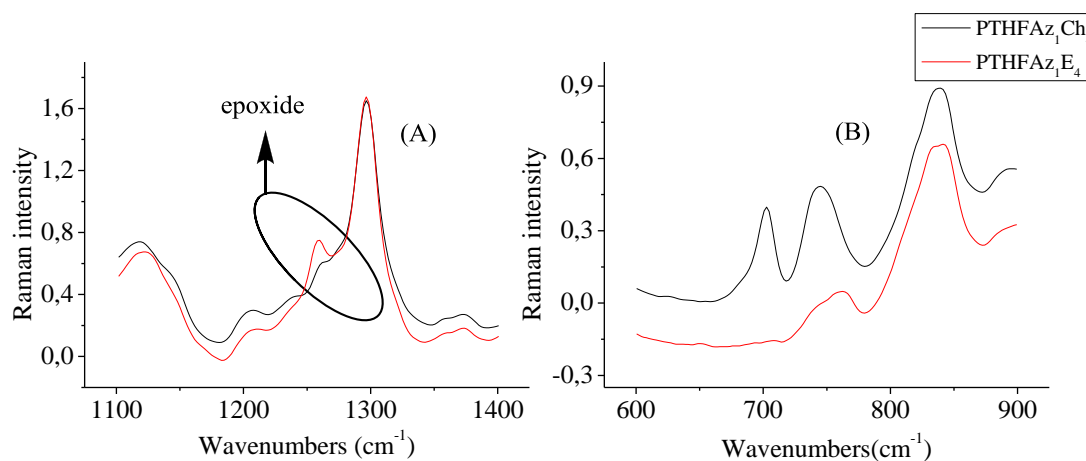
Figure 11: Raman spectra showing the formation of $PTHFAz_1Ch_2$: (A) disappearance of primary amine groups, (B) appearance of alkyl halide groups.

The formation of azetidinium groups cannot be directly proven during the synthesis of $PTHFAz_3$ from $PTHFAz_1Ch_2$. However, this transformation can be supported by Raman spectroscopy due to the disappearance of characteristic alkyl chloride peaks around 700-750 cm^{-1}, confirming the transformation of aminochlorohydrin to an azetidinium group (Figure 12).

Figure 12: Raman spectra showing the disappearance of alkyl chloride during the transformation of $PTHFAz_1Ch_2$ to $PTHFAz_3$

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The transformation of $PTHFAz_1Ch_4$ to $PTHFAz_1E_4$ was also proven by Raman spectroscopy (Figure-13). Here the chlorohydrin reactive groups were transformed into epoxide groups. This transformation was proven by the appearance of the characteristic peaks for the epoxide groups at 1259 cm^{-1} and disappearance of the characteristic peaks of alkyl halide around 750-700 cm^{-1}.

Figure 13: Raman spectra showing the synthesis of $PTHFAz_1E_4$ from $PTHFAz_1Ch_4$: (A) formation of epoxide groups, (B) disappearance of alkyl halide groups.

Conclusion: The reactions of amines with epichlorohydrin were studied in detail. It was proven that: (i) compound with one and two chlorohydrin groups can be synthesized starting with primary amines and epichlorohydrin using the mole ratio 1:1 and 1:2 respectively. (ii) The chlorohydrin groups could be transformed to epoxy groups via reaction with aqueous NaOH. (iii) The aminochlorohydrin group was transformed to azetidinium group via Finkelstein reaction. (iv) The reaction between secondary amines and epichlorohydrin results in the formation of azetidinium salts. (v) The azetidinium ring reacts with nucleophiles leading to ring opening.

These reactions were successfully used to synthesize functional polymers ($PTHFAz_1Ch_2$, $PTHFAz_3$, $PTHFAz_1Ch_4$ & $PTHFAz_1E_4$) from aminotelechelic polytetrahydrofuran. The

generation of the functional groups was controlled by fine tuning the reaction conditions. The formation of these functional groups was proven by ^{1}H NMR and Raman spectroscopy.

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Supplementary information for Chapter 3

Model reactions: Reaction of hexylamine and epichlorohydrin:

Synthesis of 1-chloro-3-(hexylamino) propan-2-ol:

To a solution of hexylamine (1.532 g, 15 mmol) in distilled water (2 mL), epichlorohydrin (1.417g, 15 mmol) was added drop wise maintaining the temperature below 5°C. The solution was allowed to stir for 5 hours. Then the solvent was evaporated in vacuum.
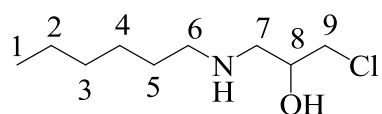

^{1}H NMR (CDCl$_3$, 400MHz): δ = 3.87 (m, H^8), 3.67 (NH, OH), 3.54 (m, H^9), 2.83-2.51(m, H^7, H^6), 1.48 (m, H^5), 1.27 (m, H^2, H^3, H^4), 0.86 (t, H^1) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 69.04 (C^8), 52.07 (C^7), 49.73 (C^6), 47.37 (C^9), 31.64 (C^5), 29.52 (C^4), 26.83 (C^3), 22.57 (C^2), 14.01(C^1) ppm.

Synthesis of N-glycidyl, N-hexyl amine :

To a solution of hexylamine (1.532 g, 15 mmol) in diethyl ether (5mL), epichlorohydrin (1.417g, 15 mmol) was added drop wise. This solution was stirred for 24 hours at room temperature and for 3 hours at reflux. Aqueous NaOH solution (25 wt. %) was added to adjust the pH of the solution to 14 and stirred for 1.5 hour. The ether layer was separated from the water layer dried (at T=2°C) using sodium hydroxide pallets. The solution was decanted from the drying agent and the diethyl ether was removed in vacuum. A colorless liquid was obtained.
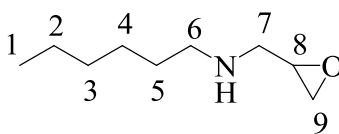
^{1}H NMR (CDCl$_3$, 400MHz): δ = 3.02 (m, H^8), 2.87 (m, H^9), 2.72-2.43(m, H^7, H^6), 1.40 (m, H^5), 1.22 (m, H^2, H^3, H^4), 0.81 (t, H^1) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 51.65 (C^8, C^7), 50.02 (C^6), 45.23 (C^9), 31.65 (C^5), 30.04 (C^4), 26.89 (C^3), 22.51 (C^2), 13.89(C^1) ppm.

Synthesis of N, N'-bis (1-chloropropan-2-ol) - hexylamine:

To a solution of hexylamine (1.532 g, 15 mmol) in tetrahydrofuran (5mL), epichlorohydrin (2.83g, 30 mmol) was added drop wise. The solution was allowed to stir for 48 hours at room temperature. Then THF was evaporated in vacuum. A white solid was obtained.
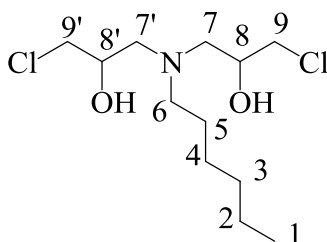

^{1}H NMR (CDCl$_3$, 400MHz): δ = 4.27 (OH), 3.90 (m, H^8, H$^{8'}$), 3.43 (m, H^9, H$^{9'}$), 2.77-2.44(m, H^7, H7', H6), 1.33 (m, H^5), 1.16 (m, H^2, H^3, H^4), 0.77(t, H^1) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 69.15 (C^8), 68.68 (C$^{8'}$), 58.67 (C^7), 58.14 (C$^{7'}$), 55.87 (C^6), 47.24 (C^9), 47.14 (C$^{9'}$), 31.61 (C^5), 28.87 (C^4), 26.82 (C^3), 22.46 (C^2), 13.83(C^1) ppm.

Synthesis of N, N' bis (glycidyl) - hexylamine:

To a solution of hexylamine (1.532 g, 15 mmol) in diethyl ether (5mL), epichlorohydrin (2.83 g, 30 mmol) was added drop wise. This solution was allowed to stir for 24 hours at room temperature and 3 hours at reflux. Aqueous NaOH solution (25 wt. %) was added to adjust the

pH of the solution to 14. The suspension was stirred for another 2 hour. The ether layer was separated and dried using sodium hydroxide pallets (at T=2°C). The solution was decanted from the drying agent and the diethyl ether was removed in vacuum. A colorless liquid was obtained.
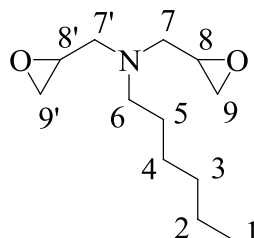
^{1}H NMR (CDCl$_3$, 400MHz): δ = 3.07-2.37(m, H^8, H8', H^9, H$^{9'}$, H^7, H$^{7'}$, H^6), 1.45 (m, H^5), 1.28 (m, H^2, H^3, H^4), 0.87 (t, H^1) ppm. ^{13}C NMR (CDCl$_3$, 100 MHz): δ = 57.34 (C^7), 55.78 (C$^{7'}$), 55.37 (C^8), 55.15 (C$^{8'}$), 51.17 (C^9), 50.51 (C$^{9'}$), 44.88(C^6), 31.73 (C^5), 30.04 (C^4), 27.02 (C^3), 22.60 (C^2), 14.02(C^1) ppm.

Synthesis of 1, 3-bis (hexylamino) propan-2-ol:

To a solution of hexylamine (3.1 g, 30 mmol) in distilled water (2mL), epichlorohydrin (1.43g, 15 mmol) was added drop wise maintaining the temperature below 5°C. This solution was allowed to stir for 1 hour in an ice bath. The solution was kept at room temperature and allowed to stir for additional 24 hours. Later water was evaporated in vacuum. A white solid was obtained, which was further purified by crystallization from DMSO.
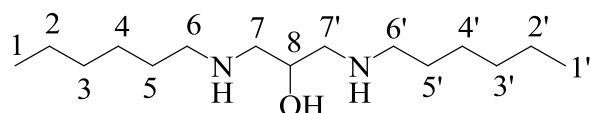

^{1}H NMR (DMSO, 400MHz): δ = 3.74(m, H^8), 2.65-2.50(m, H^7, H7', H^6, H$^{6'}$), 1.44 (m, H^5, H$^{5'}$), 1.25(m, H^2, H$^{2'}$, H3, H$^{3'}$, H^4, H$^{4'}$), 0.86(t, H^1, H$^{1'}$) ppm. ^{13}C NMR (DMSO, 100 MHz): δ

= 66.65 (C^8, $C^{8'}$), 53.18 (C^7, $C^{7'}$), 48.75 (C^6, $C^{6'}$), 31.09 (C^5, $C^{5'}$), 28.28 (C^4, $C^{4'}$), 26.23 (C^3, $C^{3'}$), 22.01 (C^2, $C^{2'}$), 13.87 (C^1, $C^{1'}$) ppm.

Synthesis of 1-hexylazetidine-3-ol:

Method 1: To a solution of hexylamine (1.532 g, 15 mmol) in hexane (2mL), epichlorohydrin (1.417g, 15 mmol) was added drop wise. The solution was allowed to stir for 48 hours at room temperature, before hexane was removed in vacuum. The residue was dissolved in acetonitrile (10 mL) and heated under reflux for 5 hours. A colorless oily residue was obtained by evaporating acetonitrile in vacuum.
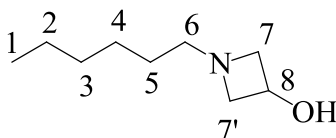
^{1}H NMR (DMSO, 400MHz): δ = 3.44 (m, H^8), 2.76-2.17 (m, H^7, H7', H6), 1.37 (m, H^5), 1.25 (m, H^2, H^3, H^4), 0.85 (t, H^1) ppm. ^{13}C NMR (DMSO, 100 MHz): δ = 63.94 (C^7, $C^{7'}$), 60.71 (C^8), 59.51 (C^6), 31.25 (C^5), 27.37 (C^4), 26.52 (C^3), 22.12 (C^2), 13.84(C^1) ppm.

Method 2: To a solution of hexylamine (1.532 g, 15 mmol) in distilled water (2mL), epichlorohydrin (1.417g, 15 mmol) was added drop wise maintaining the temperature below 5°C. This solution was allowed to stir in an ice bath for 5 hours. After that, aqueous solution of potassium iodide (50 wt. %) (5 mL) was added and allowed to stir for another 4 hours. The chlorohydrin groups were partially converted to azetidinium groups via this method.

^{1}H NMR (DMSO, 400MHz): δ = 4.13(m, H^8), 3.50, 2.67 (m, H^7, H7'), 2.4(m, H^6) ppm.

Reaction of epichlorohydrin and diethyl amine:
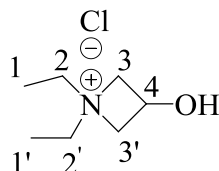
Synthesis of 1, 1'-diethyl-3-hydroxyazetidinium chloride:

To a solution of diethyl amine (2.67 g, 36 mmol) in distilled water (2 mL), epichlorohydrin (3.38 g, 36 mmol) was added drop wise maintaining the temperature below 5°C. The reaction mixture was stirred in an ice bath for 1 hour and for 30 hours at room temperature. Then water was removed in vacuum.


^{1}H NMR (DMSO, 400MHz): δ =4.613 (m, H^4), 4.44, 4.089 (m, H^3, H$^{3'}$), 3.47, 3.35 (m, H^2, H$^{2'}$), 1.084 (m, H^1, H$^{1'}$), 6.69 (d, OH) ppm. ^{13}C NMR (DMSO, 100 MHz): δ = 69.73 (C^3, C$^{3'}$), 57.45 (C^4), 55.30, 53.34 (C^2, C$^{2'}$), 7.816, 7.39 (C^1, C$^{1'}$) ppm.

Nucleophilic ring opening reactions of 1, 1'-diethyl-3-hydroxyazetidinium chloride:

Synthesis of 1-(diethylamino)-3-(hexylamino) propan-2-ol:

To a solution of 1, 1'-diethyl-3-hydroxyazetidinium chloride (0.355 g, 2.1 mmol) in distilled water (3 mL), hexylamine (0.214 g, 2.1 mmol) was added drop wise. The reaction mixture was stirred for 24 hours at 80°C, cooled down to room temperature and extracted with dichloromethane. The pure product was obtained by evaporating dichloromethane in vacuum.
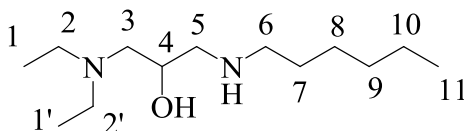
^{1}H NMR (DMSO, 400MHz): δ =3.582 (m, H^4), 2.57-2.30 (m, H^2, H$^{2'}$, H^3, H^5, H^6), 1.39 (m, H^7), 1.26 (m, H^8, H^9, H^{10}), 0.94 (m, H^1, H$^{1'}$), 0.87 (m, H^{11}) ppm. ^{13}C NMR (DMSO, 100 MHz): δ = 67.37(C^4), 57.92 (C^3), 54.47(C^5), 49.53 (C^6), 47.18 (C^2, C2'), 31.31 (C^8), 29.67 (C^7), 26.55 (C^9), 22.12 (C^{10}), 13.77 (C^{11}), 11.76 (C^1, C$^{1'}$) ppm.

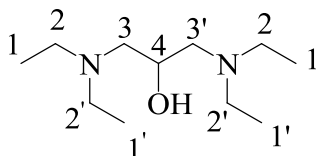**Synthesis of 1, 3-bis (diethyl amino) propan-2-ol:**

To a solution of 1, 1'-diethyl-3-hydroxyazetidinium chloride (0.355g, 2.1 mmol) in distilled water (3 mL), diethyl amine (0.155 g, 2.1 mmol) was added drop wise. The reaction mixture was stirred for 30 hours at 80°C, cooled down to room temperature and extracted with dichloromethane. The pure product was obtained by evaporating dichloromethane in vacuum.

^{1}H NMR (DMSO, 400MHz): δ = 3.605(m, H^4), 2.5 (m, H^2, H$^{2'}$), 2.4, 2.28 (m, H^3, H$^{3'}$), 0.96 (m, H^1, H$^{1'}$), 3.94 (d, OH) ppm. ^{13}C NMR (DMSO, 100 MHz): δ = 66.43 (C^4), 57.76 (C^3, C$^{3'}$), 47.08 (C^2, C$^{2'}$), 11.75 (C^1, C$^{1'}$) ppm.

Synthesis of 1-(diethyl amino)-3-phenoxypropan-2-ol:

To a solution of 1, 1'-diethyl-3-hydroxyazetidinium chloride (0.355g, 2.1 mmol) in distilled water (2 mL), was added drop wise a solution of phenol (0.2 g, 2.1 mmol) in 1(N) aqueous KOH (4 mL). The reaction mixture was stirred for 12 hours at 80°C, cooled down to room temperature and extracted with dichloromethane. The pure product was obtained by evaporating dichloromethane in vacuum.
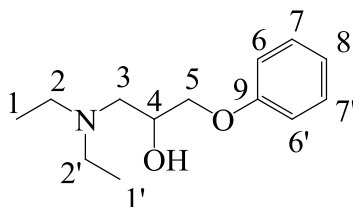
^{1}H NMR (DMSO, 400MHz): δ = 7.27(m, H^6, H$^{6'}$), 6.93(m, H^7, H$^{7'}$, H^8), 3.98 (m, H^4), 3.85 (m, H^5), 2.48(m, H^2, H$^{2'}$, H3), 0.94(m, H^1, H$^{1'}$) ppm. ^{13}C NMR (DMSO, 100 MHz): δ = 158.75(C^9), 129.39 (C^7, C$^{7'}$), 120.29(C^8), 114.36(C^6, C6'), 70.65 (C^5), 67.35 (C^4), 55.90 (C^3), 47.32(C^{2,} C$^{2'}$), 11.89 (C^1, C$^{1'}$) ppm.

Reaction kinetics and effects of solvents:

^{1}H NMR analysis to determine the conversion for the reaction of hexylamine and epichlorohydrin (mole ratio = 1:1):
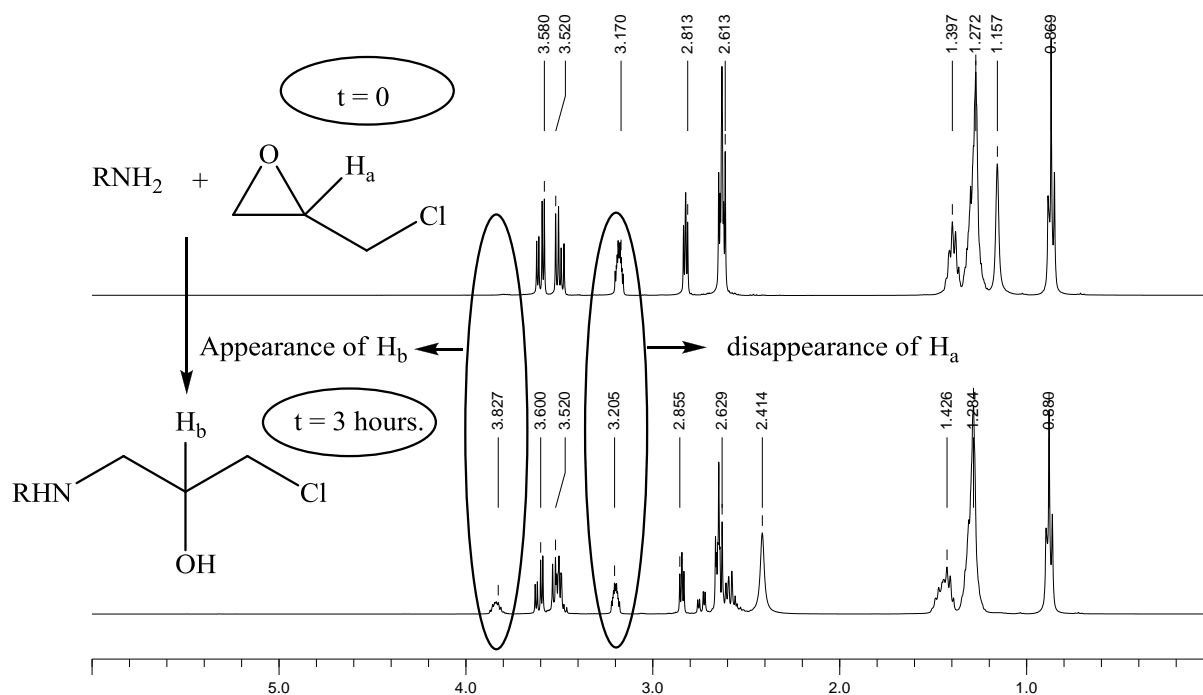
Figure SI 1: ^{1}H NMR analysis (in $CDCl_3$) of the reaction between hexylamine and epichlorohydrin: conversion of epichlorohydrin (disappearance of H_a) and formation of the amino-chloro-hydrine (appearance of H_b).

^{13}C NMR spectrums to understand the effect of solvents. These NMR spectrums proves that the side products are minimized in case of polar aprotic to nonpolar solvent.
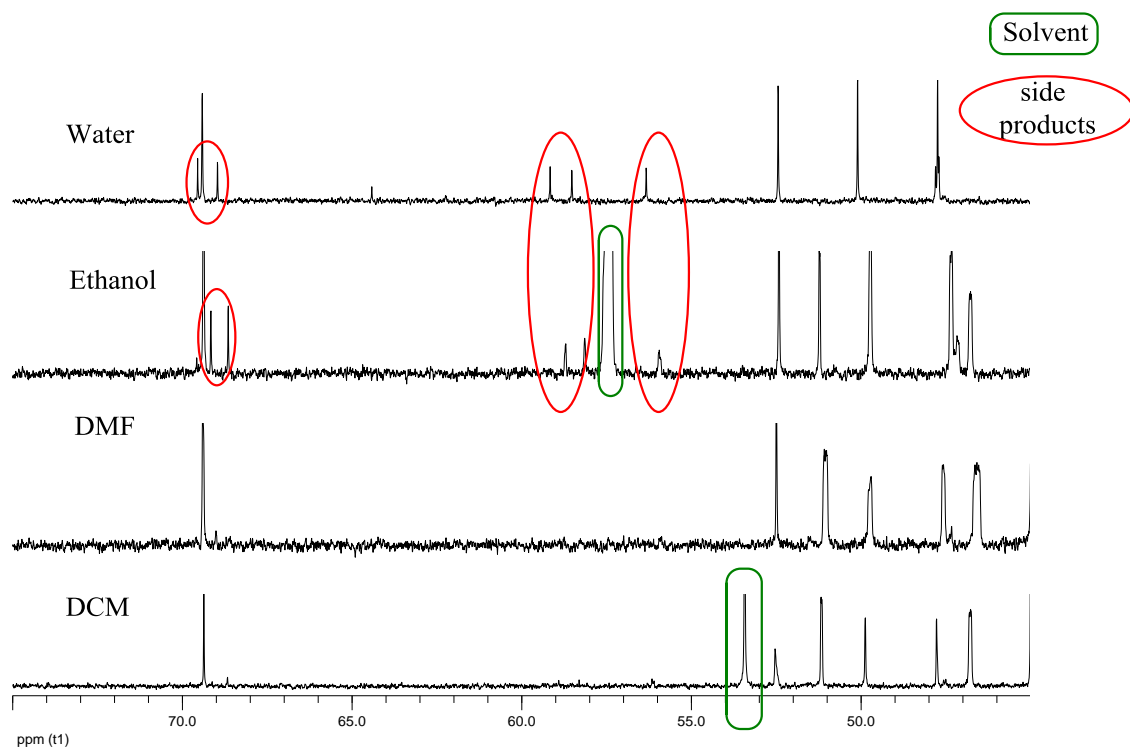
Figure SI 2: ^{13}C NMR spectra (in $CDCl_3$) for the reaction of hexylamine and epichlorohydrin (1:1 mole ratio) using different solvent. (After 3 hours of reaction below 5°C)

NMR spectrums for the products obtained during the ring opening reaction of azetidinium compound:
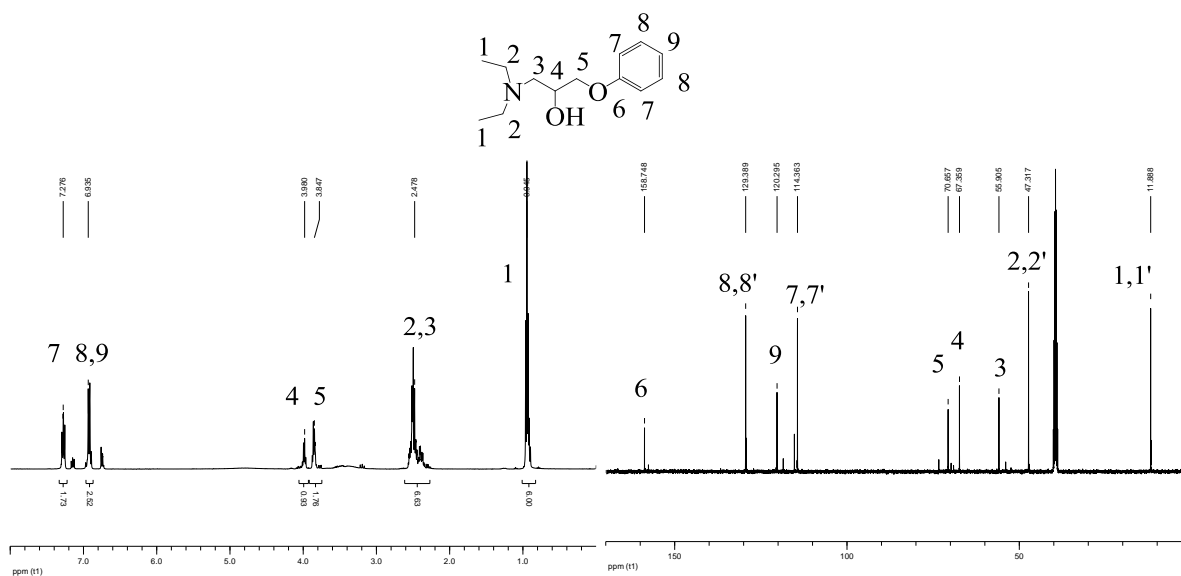
Figure SI 3: ^1H and ^{13}C NMR spectra for 1-(diethyl amino)-3-phenoxypropan-2-ol
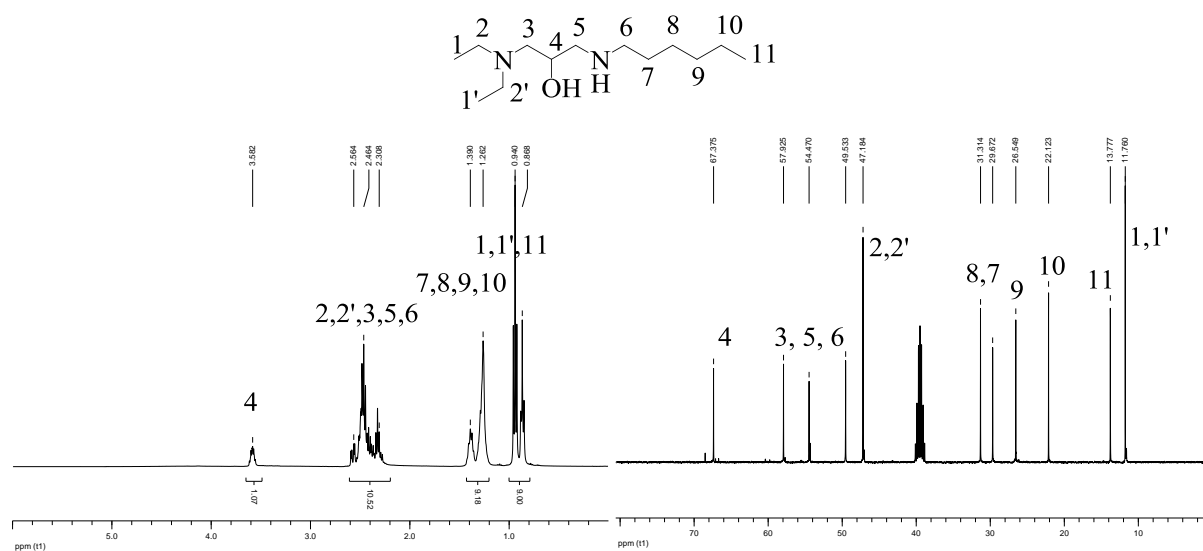
Figure SI 4: ^{1}H and ^{13}C NMR spectra for 1-(diethylamino)-3-(hexylamino) propan-2-ol
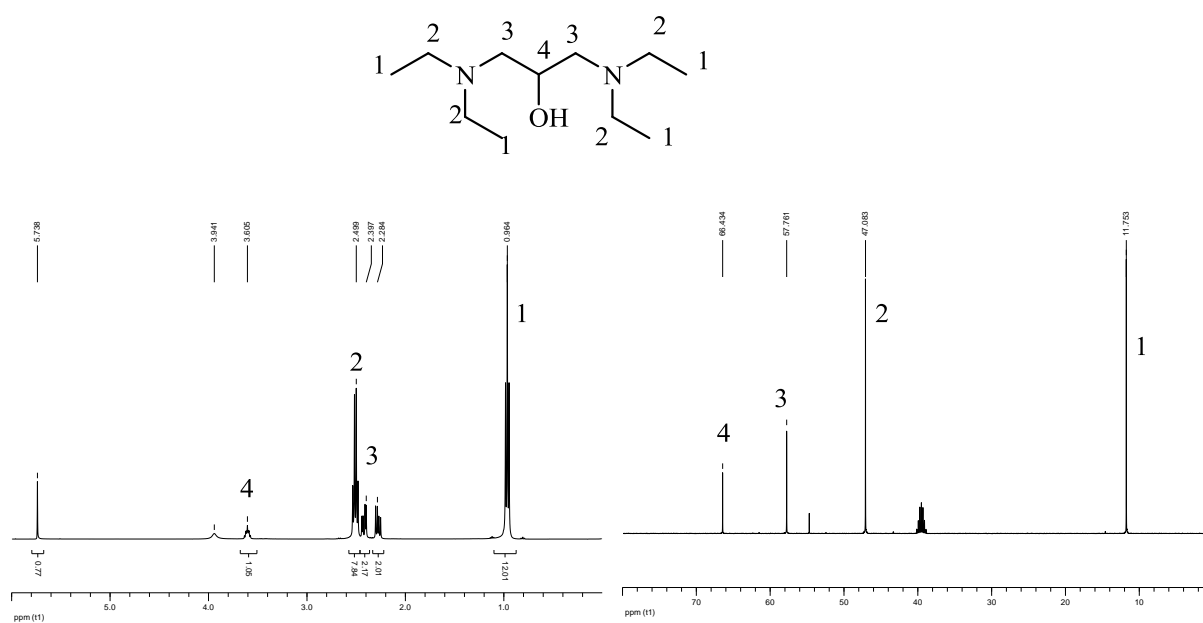
Figure SI 5: ^{1}H and ^{13}C NMR spectra for 1, 3-bis (diethyl amino) propan-2-ol

Raman Spectroscopy of the Polytetrahydrofurans:
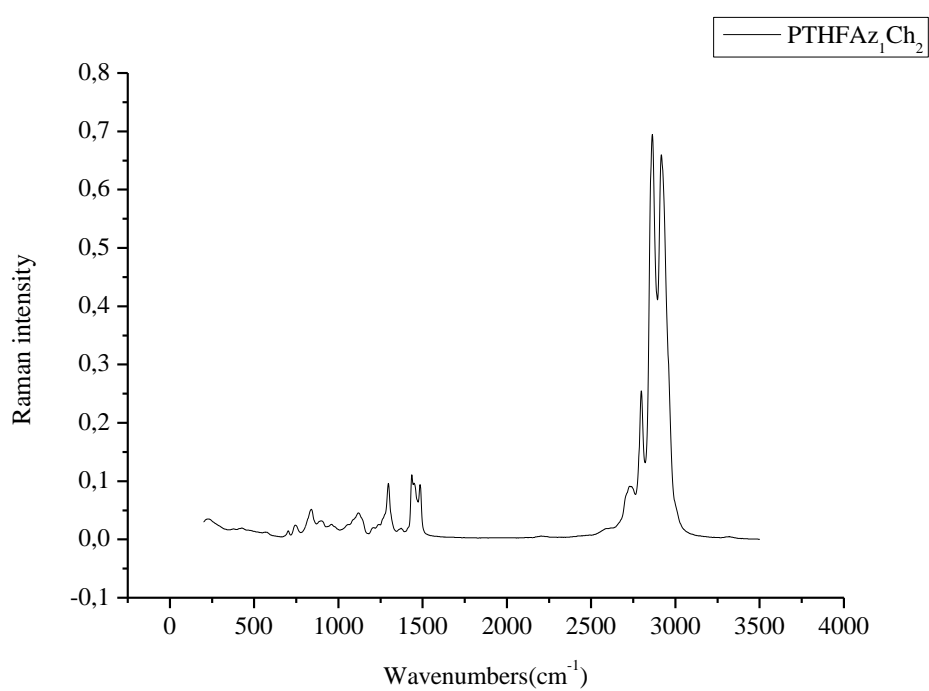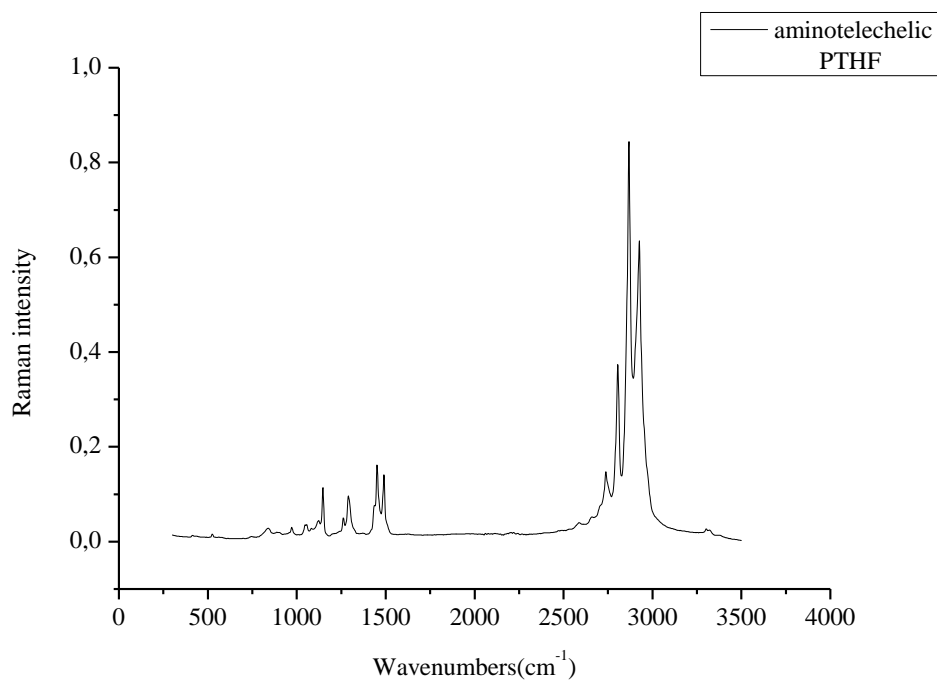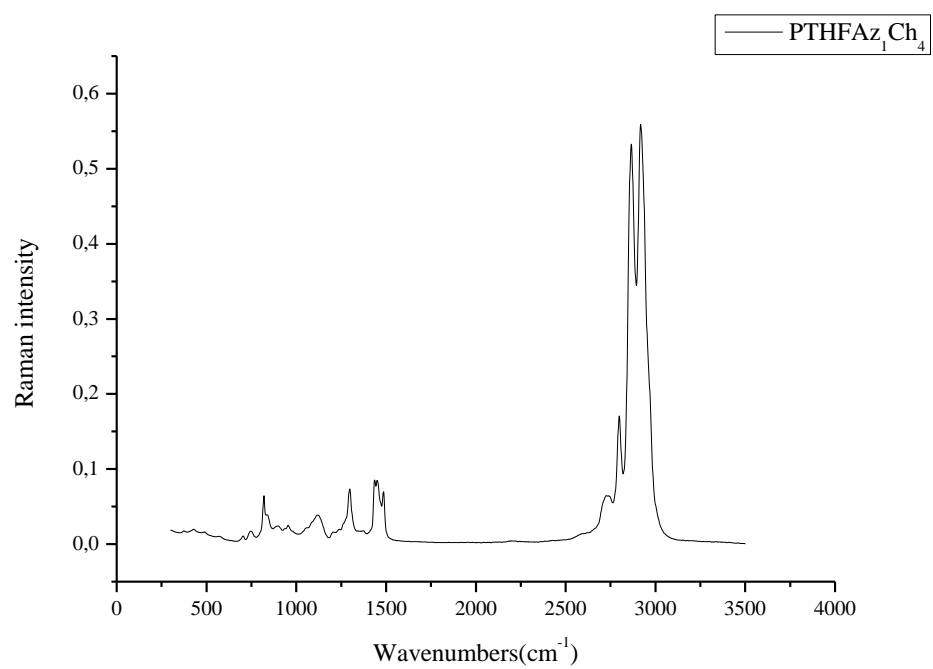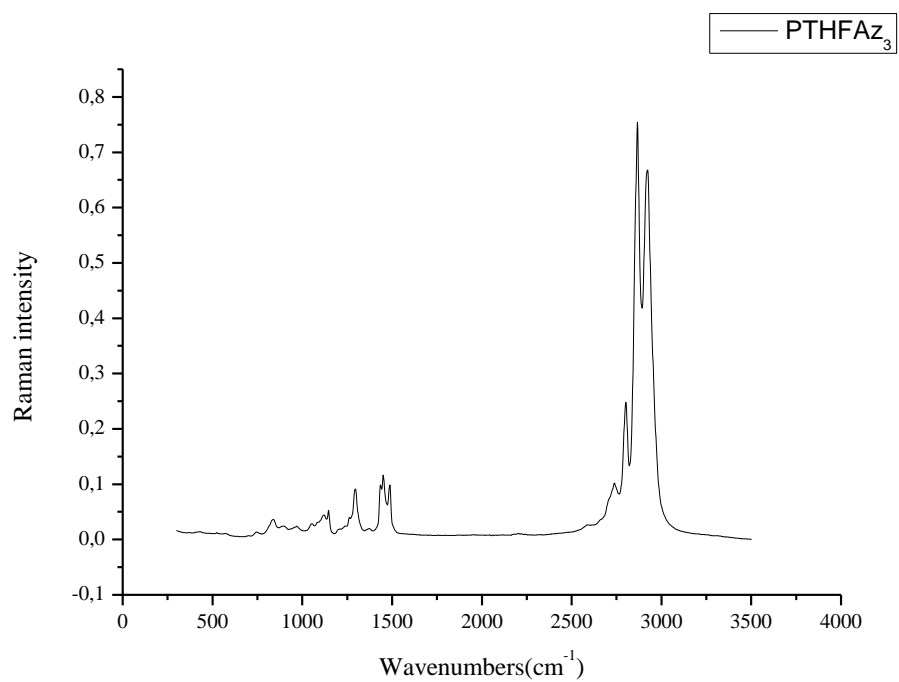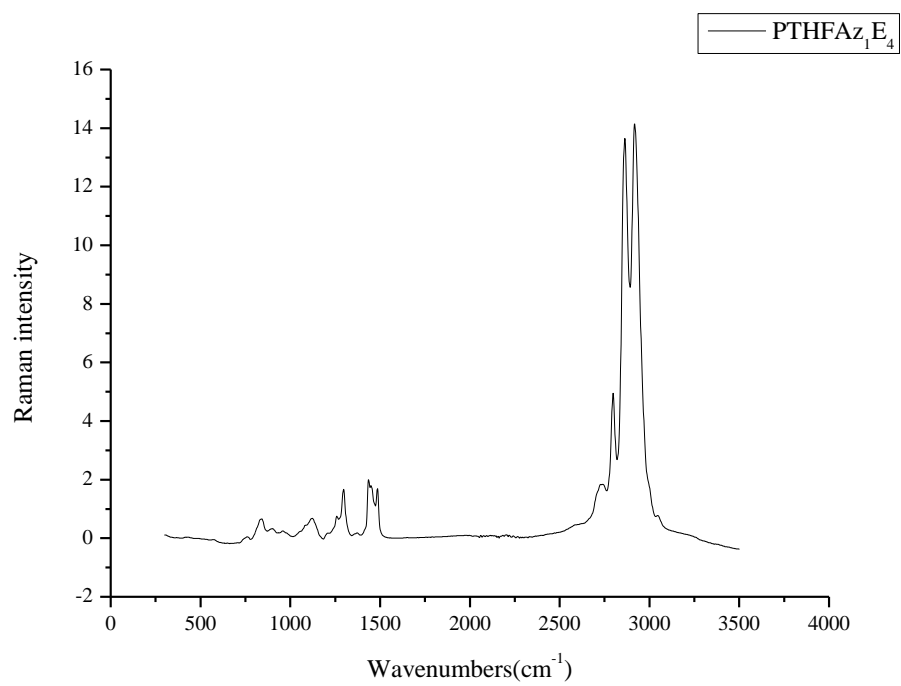
Chapter 4

Synthesis of azetidinium functionalized polymers using a piperazine based coupler[A].

Introduction:

Polymers, bearing reactive and charged functional groups, are of increasing interest in today's research due to their potential application in various fields[1-4]. In this context, azetidinium functionalized polymers are important because they serve this purpose; they have a quaternary ammonium groups and are highly reactive[5-7].

A few azetidinium functionalized polymers are known in literature and were found to improve various properties like adhesion to surfaces[8], and in addition showing antimicrobial activity[9] due to the presence of cationic azetidinium groups. These four membered functional groups are reactive due to the angle strain of these four membered rings and can react with a number of nucleophile (like: amines, phenol, sulphides etc). Due to the reactivity of these groups, azetidinium functionalized polymers can also be modified for various applications[10]. Although this type of polymers is important, only few research articles are known on their synthesis. The main problem is the difficulty to prepare and stabilize the reactive azetidinium groups within these polymers.

First results regarding the selective synthesis of azetidinium functionalized compounds were reported by Coscia et al. The reaction of a secondary amine (diethyl amine) with epichlorohydrin yields the corresponding azetidinium compound in high yield and high purity[11]. Later, based on this concept, azetidinium functionalized polymers were prepared by conversion of secondary amine groups within the polymer backbone with epichlorohydrin. For example, Hercosett 125 was synthesized via the reaction of a poly(amino-amide) with

56

epichlorohydrin[12]. Azetidinium functionalized guanidine based polymers were also prepared via the reaction of guanidine based prepolymers, such as polyhexamethylene guanidine hydrochloride, polyhexamethylene diethylene triamine guanidine hydrochloride, with epichlorohydrin[13]. In our previous work[14], different azetidinium functionalized polymers were prepared starting with an aminotelechelic polytetrahydrofuran, bearing primary and secondary amine groups. While the secondary amine group in the polymer leads solely to azetidinium groups the primary amine groups can be converted to amino-chlorohydrin and amino-epoxy propane groups depending on the reaction conditions. Conversion of the amino-chlorohydrin groups to azetidinium groups is rather difficult; the azetidinium groups being obtained with low ($\approx 50\%$) conversion.

The main disadvantage for the synthesis of azetidinium functionalized polymers from a starting material with primary and secondary amine groups is the occurrence of parallel or consecutive side reactions leading to a cross-linked polymer (Scheme 1) [15].
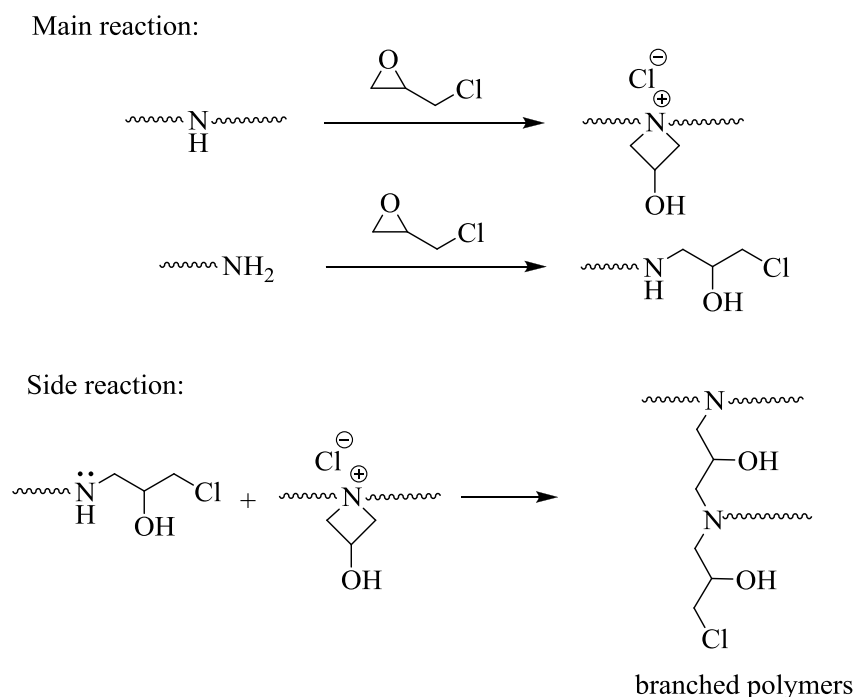
Scheme 1: Main reaction and side reaction during conversion of an amino-functional

substrate with epichlorohydrin.

The side reactions occur due to interaction of the azetidinium group with amino-chlorohydrin or not converted secondary amine groups. This reaction occurs upon storage of the solution or during evaporation of the solvent leading to branched polymers and finally to cross-linking.

To solve these problems, it is indispensable to find a more suitable method by which azetidinium functionalized polymers can be prepared in a controlled way. Quantification of these groups in the polymer backbone and elimination of side reactions are also important.

In the current work, a bifunctional coupler was used to prepare azetidinium functionalized polymers. Bifunctional couplers[16] are molecules containing two functional groups which due to their different reactivity can be addressed selectively. One of the active groups of these couplers reacts with a suitable group of the polymer. As a result, a new functionalized polymer is obtained (Figure 1). Couplers were used to bring different functionalities in the polymer backbone or side chains which were otherwise difficult to introduce[17, 18].
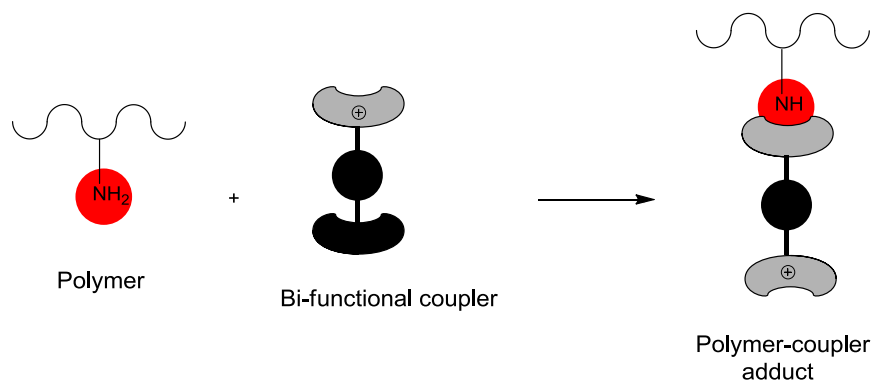
Figure 1: Synthesis of functionalized polymers using a bifunctional coupler.

The goal of the present study is to establish a novel procedure for synthesis of azetidinium functionalized polymers using a bifunctional coupler. To achieve this, a suitable bifunctional coupler was designed, synthesized and treated with low molecular weight primary and secondary amines as model reaction to obtain azetidinium functionalized compounds. Later using these reaction conditions, azetidinium functionalized polytetrahydrofurans were

prepared by reacting amine functionalized polytetrahydrofurans with the coupler. Aminotelechelic polytetrahydrofurans [XTJ-548 (Huntsman Corporation)] was used as models for amine functionalized polymers in general.

Experimental part:

Materials: Hexylamine (99%, Aldrich), diethylamine (99%, Aldrich), epichlorohydrin (99%, Merck), piperazine (99+%, Aldrich), aminotelechelic polytetrahydrofuran (PTHF) [XTJ-548 (Huntsman Corporation)], were used without further purification. XTJ-548 is a mixture of NH_2-PTHF-NH_2 and NH_2-PTHF-NH-PTHF-NH_2 in a ratio of 1:4 and a number average molecular weight of 1700 g/mol. Distilled water was used as solvent for all the reactions.

Measurements: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterium oxide (D_2O) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as solvents. Tetramethylsilane (TMS) was used as an internal standard. All Raman spectra were recorded on a Buker RFS100/s Raman spectrometer, fitted with a Nd: YAG laser (1064nm). The spectral resolution was 4 cm^{-1}. For one spectrum 1000 scans were collected at a laser power of 200mW.

Nomenclature of polymers: For the functional polymers - **PTHFAz$^{pz}_3$, PTHFAz$^{pz}_5$** - PTHF stands for the hydrophobic skeleton of polytetrahydrofuran, Az^{pz}_n (n = 3, 5) stands for the azetidinium group (Az) attached to piparazine (pz) and n shows the number of azetidinium groups in a single polymer chain.

Synthesis:

Synthesis of the bifunctional coupler [7-(3-chloro-2-hydroxypropyl)-2-hydroxy-7-aza-4-azoniaspiro [3.5] nonane chloride] (3):

To a solution of piperazine (**1**) (1.65 g, 0.019 mol) in water (15 mL), epichlorohydrin (**2**) (3 mL, 0.038 mol) was added. The mixture was stirred for 2 days at 25°C. Then water was

removed in vacuum. The bifunctional coupler **3** was obtained as a white solid (> 95% purity). This compound was further purified via extracting the aqueous solution of the coupler with dichloromethane and then simply removed the water using vacuum.

3
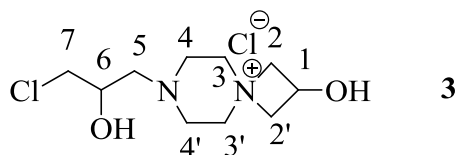
1H NMR (DMSO-d^6, 400MHz): δ = 6.70 & 5.36 (d, OH), 4.65 (m, H^1), 4.49 & 4.17 (m, H^2, H$^{2'}$), 3.84 (m, H^6), 3.7-3.4 (m, H^3, H$^{3'}$, H^7), 2.8-2.4 (m, H^4, H$^{4'}$, H^5) ppm. **13C NMR** (DMSO-d^6, 100MHz): δ = 70.25 (C^2, C$^{2'}$), 67.83 (C^6), 60.58 (C^3), 59.59 (C$^{3'}$), 58.84 (C^5), 57.72 (C^1), 48.33 (C^7), 47.88 (C^4), 47.54 (C$^{4'}$) ppm.

Elemental analysis (C$_{10}$H$_{20}$Cl$_2$N$_2$O$_2$): calculated C: 44.29%, H: 7.43%, N: 10.33%, found: C: 44.15%, H: 7.82%, N: 10.35%

Reaction of the coupler 3 with hexyl amine (1:1 mole ratio); Synthesis of the functional coupler 4.

To a solution of coupler **3** (1.42 g, 5.25 mmol) in distilled water (5 mL), hexyl amine (0.53 g, 5.25 mmol) was added. The solution was allowed to stir for 5 hours at 90°C and then cooled down to room temperature. Removal of water yielded the pure functional coupler **4**.

4
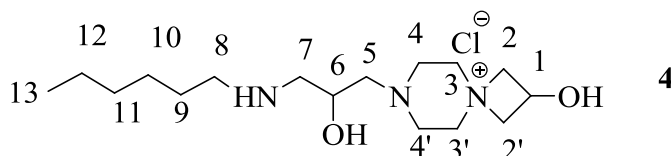
1H NMR (DMSO-d^6, 400MHz): δ = 6.70 & 5.6 (d, OH), 4.64 (m, H^1), 4.52 & 4.17 (m, H^2, H$^{2'}$), 3.7-3.3 (m, H^3, H$^{3'}$, H^6), 2.8-2.4 (m, H^4, H$^{4'}$, H^5), 1.8-1.2 (m, H^9, H^{10}, H^{11}, H^{12}), 0.87 (m, H^{13}) ppm. **13C NMR** (DMSO-d^6, 100MHz): δ = 70.25 (C^2, C$^{2'}$), 63.78 (C^6), 60.52 & 60.047

$(C^3, C^{3'})$, 58.76 (C^5), 57.65 (C^1), 51.04 (C^7), 47.85-47.35 $(C^4, C^{4'})$, 47.03 (C^8), 30.67 (C^9), 26.72 (C^{10}), 25.48 (C^{11}), 21.80 (C^{12}), 13.77 (C^{13}) ppm.

Elemental analysis ($C_{16}H_{34}ClN_3O_2$, HCl, ½ H_2O): calculated C: 50.39%, H: 9.51%, N: 11.02%, found: C: 50.81%, H: 9.85%, N: 10.81%

Reaction of the coupler 3 with hexyl amine (2:1 mole ratio); Synthesis of compound 5.

To a solution of coupler **3** (1.42 g, 5.25 mmol) in distilled water (5 mL), hexyl amine (0.27 g, 2.65 mmol) was added. The solution was allowed to stir for 9 hours at 90°C and then cooled down to room temperature. Removal of water yielded the pure compound **5**.
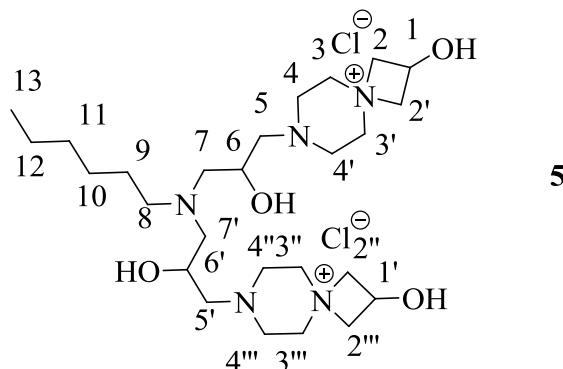
¹H NMR (DMSO-d⁶, 400MHz): δ = 6.70 & 5.6 (d, OH), 4.65 (m, H^1), 4.49 & 4.16 (m, H^2, $H^{2'}$), 3.7-3.3 (m,), 3.8-2.4 (m, H^3, $H^{3'}$, $H^{3''}$, $H^{3'''}$, H^4, $H^{4'}$, $H^{4''}$, $H^{4'''}$, H^5, $H^{5'}$, H^6, $H^{6'}$, H^7, $H^{7'}$, H^8), 1.8-1.2 (m, H^9, H^{10}, H^{11}, H^{12}), 0.87 (m, H^{13}) ppm. **¹³C NMR** (DMSO-d⁶, 100MHz): δ = 70.44 (C^2, $C^{2'}$, $C^{2''}$, $C^{2'''}$), 64.27 & 63.83 (C^6, $C^{6'}$), 60.58- 59.85 (C^3, $C^{3'}$, $C^{3''}$, $C^{3'''}$), 58.81 (C^5, $C^{5'}$), 57.72 (C^1, $C^{1'}$), 51.06 (C^7, $C^{7'}$), 47.92- 47.39 (C^4, $C^{4'}$, $C^{4''}$, $C^{4'''}$), 47.08 (C^8), 30.69 (C^9), 26.77 (C^{10}), 25.52 (C^{11}), 21.84 (C^{12}), 13.82 (C^{13}) ppm.

Elemental analysis ($C_{26}H_{53}Cl_2N_5O_4$, 2HCl): calculated C: 47.17%, H: 8.22%, N: 10.58%, found: C: 47.6%, H: 8.22%, N: 10.30%

Reaction of the coupler with diethyl amine (1:1 mole ratio); synthesis of the functional coupler 6.

To a solution of coupler **3** (0.363 g, 1.34 mmol) in distilled water (2 mL), diethyl amine (0.098 g, 1.34 mmol) was added. The solution was allowed to stir for 7 hours at 90°C and then cooled down to room temperature. Removal of water yielded the pure functional coupler **6**.
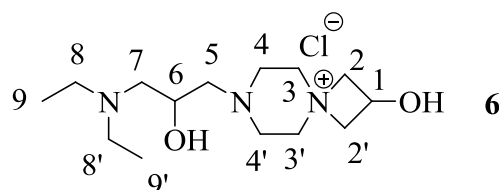
^{1}**H NMR** (DMSO-d^6, 400MHz): δ = 6.70 & 5.6 (d, OH), 4.64 (m, H^1), 4.50 & 4.15 (m, H^2, H$^{2'}$), 3.8-3.3 (m, H^3, H$^{3'}$, H^6), 3.15 (m, H^8, H$^{8'}$), 3.00-2.3 (m, H^4, H$^{4'}$, H^5, H^7), 1.21 (m, H^9, H$^{9'}$) ppm. ^{13}**C NMR** (DMSO-d^6, 100MHz): δ = 70.39 (C^2, C$^{2'}$), 62.99 (C^6), 60.34 & 60.26 (C^3, C$^{3'}$), 58.88 (C^5), 57.70 (C^1), 55.03 (C^7), 47.36 & 47.21 (C^4, C$^{4'}$), 47.05 & 41.16 (C^8, C$^{8'}$), 10.86 & 8.51 (C^9, C$^{9'}$) ppm.

Synthesis of PTHFAz$^{pz}_3$ (7):

To a solution of aminotelechelic PTHF (XTJ-548) (2.25 g) in water (5 mL), was added at 60°C a solution of the coupler **3** (1.003 g, 0.0037mol) in water (5.5 mL). (The ratio of primary and secondary amine groups in XTJ-548 and coupler is [NH$_2$: NH: coupler =3:1:4.4]). The reaction mixture was stirred at 90°C for 5 hours and then cooled down to room temperature. Removal of water in vacuum yielded polymer **7** as a white solid.

7
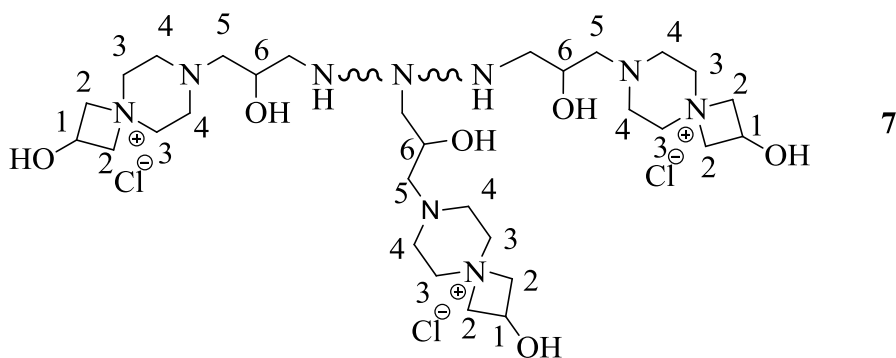
^{1}**H NMR** (DMSO-d^6, 400MHz): δ = 4.64 (m, H^1), 4.48 & 4.15 (m, H^2), 3.6 (m, H^3, H^6), 2.8-2.2 (m, H^4, H^5, H^7) ppm. ^{13}**C NMR** (DMSO-d^6, 100MHz): δ = 70.25 (C^2), 64.30 (C^6), 60.71 & 59.93 (C^3), 58.94 (C^5), 57.74 (C^1), 47.97 & 47.62 (C^4) ppm.

Synthesis of PTHFAz$^{pz}_5$ (8):

To a solution of aminotelechelic PTHF XTJ-548 (2.25 g) in water (5 mL), was added at 60°C a solution of the coupler **3** (1.708 g, 0.0063mol) in water (8 mL). (The ratio of primary and secondary amine groups in XTJ-548 and coupler is [NH$_2$: NH: coupler =3:1:7.4]). The reaction mixture was stirred at 90°C for 8 hours and then cooled down to room temperature. Removal of water in vacuum yielded polymer **8** as a white solid.

8
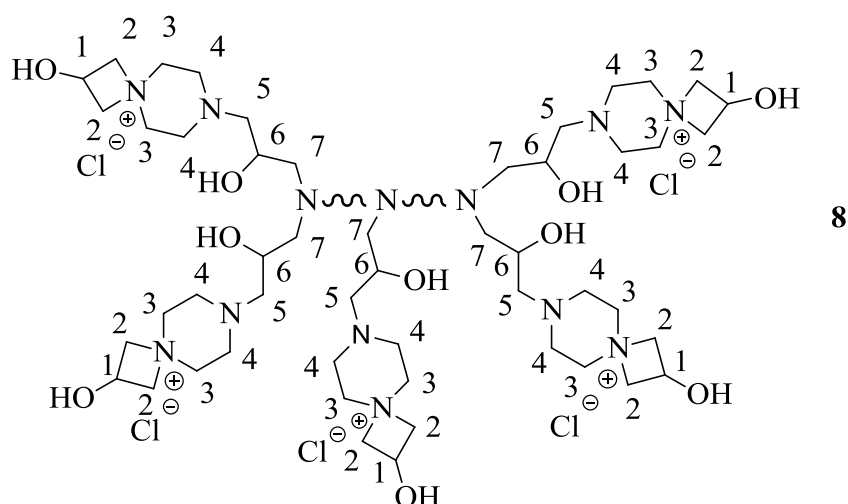
^{1}H NMR (DMSO-d^6, 400MHz): δ = 4.65 (m, H^1), 4.49 & 4.15 (m, H^2), 3.6-3.4 (m, H^3, H^6), 2.85-2.2 (m, H^4, H^5, H^7) ppm. **^{13}C NMR** (DMSO-d^6, 100MHz): δ = 70.25 (C^2), 64.30 (C^6), 60.65 & 59.91 (C^3), 58.89 (C^5), 57.73 (C^1), 48.30-47.55 (C^4) ppm.

Results and discussions:

Multifunctional polymers are prepared either by copolymerization of functional monomers (Figure 2, route 1), mainly used in controlled vinyl- and ring-opening polymerization reactions or by post polymerization modification reaction (Figure 2, route 2 and 3). The preparation of functional monomers is often a multistep process associated with laborious purification procedures; in addition due to their high reactivity precaution measures must be taken for the storage of these monomers.

In post polymerization modification two approaches have to be considered: generation of the desired functionality by direct conversion of functional groups in the polymer with a suitable reagent (Figure 2, route 2) and by using a coupler (Figure 2, route 3). In the second approach, first functional couplers are prepared with the desired functionalities, and then these functional couplers are attached to the polymer.

(1) Co-polymerization of functional monomers:

functional monomers

multifunctional polymer
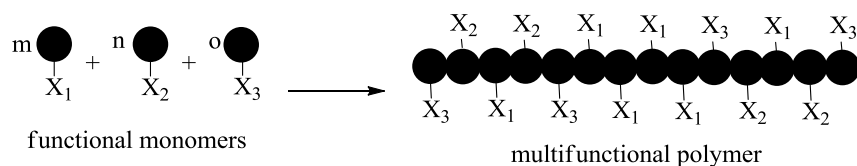
(2) Post-polymerization modification using reagents to generate the functionality:

$m\,b_1 + n\,b_2 + o\,b_3$

reagent

functional polymer

multifunctional polymer

$(a + b_{1\text{-}3} = X_{1\text{-}3})$
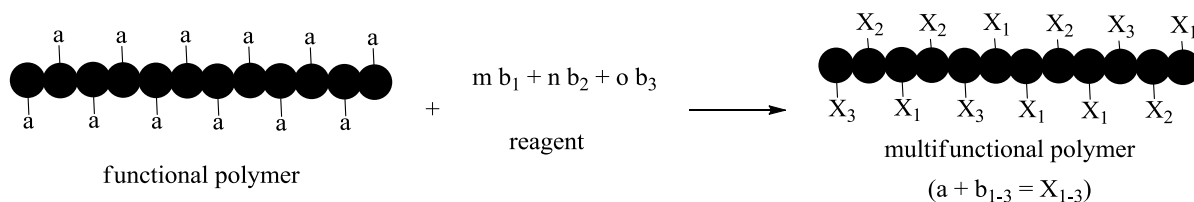
(3) Post-polymerization modification using a mixture of functional couplers:

functional polymer

functional couplers

multifunctional polymer

$(a + c = d)$
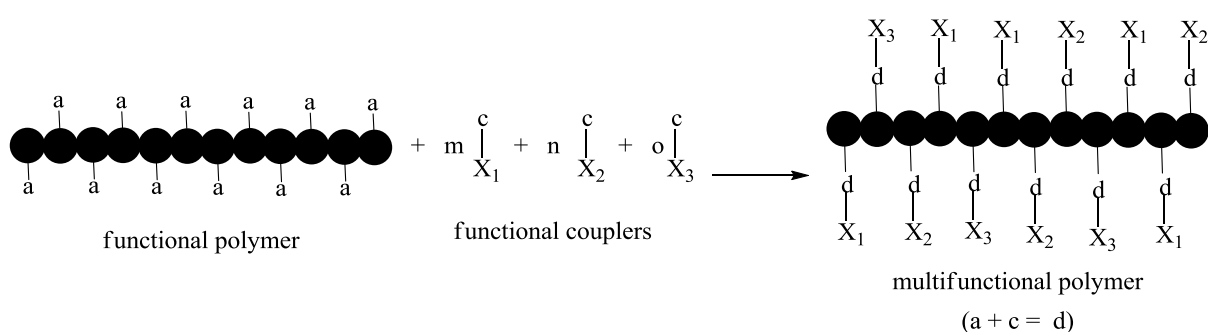
Figure 2: Different approaches to synthesize multifunctional polymers.

In the previous paper we have shown that, polymers bearing primary or secondary amine groups can be converted under suitable conditions to polymers with azetidinium groups by reaction of these polymers with epichlorohydrin. In the current work a new synthetic strategy was developed to prepare azetidinium functionalized polymers from amine functionalized polymers, using a piperazine based bifunctional coupler (Scheme 2).
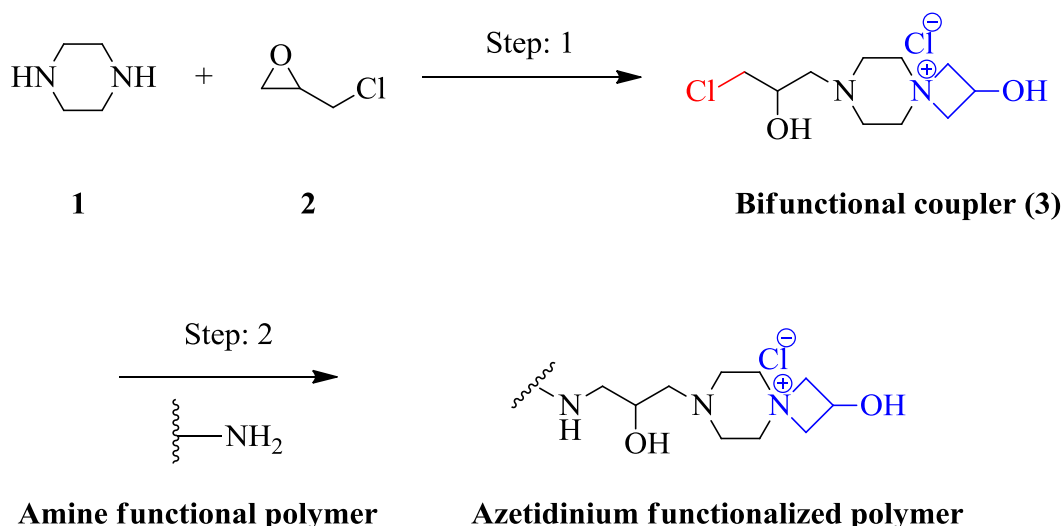
Scheme 2: Schematic presentation for the synthesis of azetidinium functionalized polymers using a bifunctional coupler.

Reactions of piperazine with epichlorohydrine were reported in literature[19]. By varying the reaction conditions different products were obtained (Scheme 3). In dry ethanol at ca. 35°C the reaction of piperazine (1equivalent) with epichlorohydrin (2 equivalents) resulted in N,N'-bis-(3-chloro-2-hydroxypropyl)piperazine (**A**), which upon treatment with aqueous sodium hydroxide gave N, N'-bis(2,3-epoxy-n-propyl)piperazine (**B**); the chlorohydrin groups being converted to epoxide groups[20]. Alkylation of the bis-chlorohydrin **A** with methyl iodide at 100°C resulted in the quaternary ammonium compound N-methyl-N,N'-bis-(3-chloro-2-hydroxypropyl) piperazinium iodide (**C**), while heating with methanol yielded N-(3-chloro-2-hydroxypropyl)-N-(2-hydroxy-1,3'-trimethylene)- piperazinium chloride (**D**) in 42% yield, in which one of the amino- chlorohydrin groups was converted into an azetidinium group. All these reactions show that, formation of different products was possible by adjusting the reaction parameters (solvents, pH, temperature, reaction time etc.).
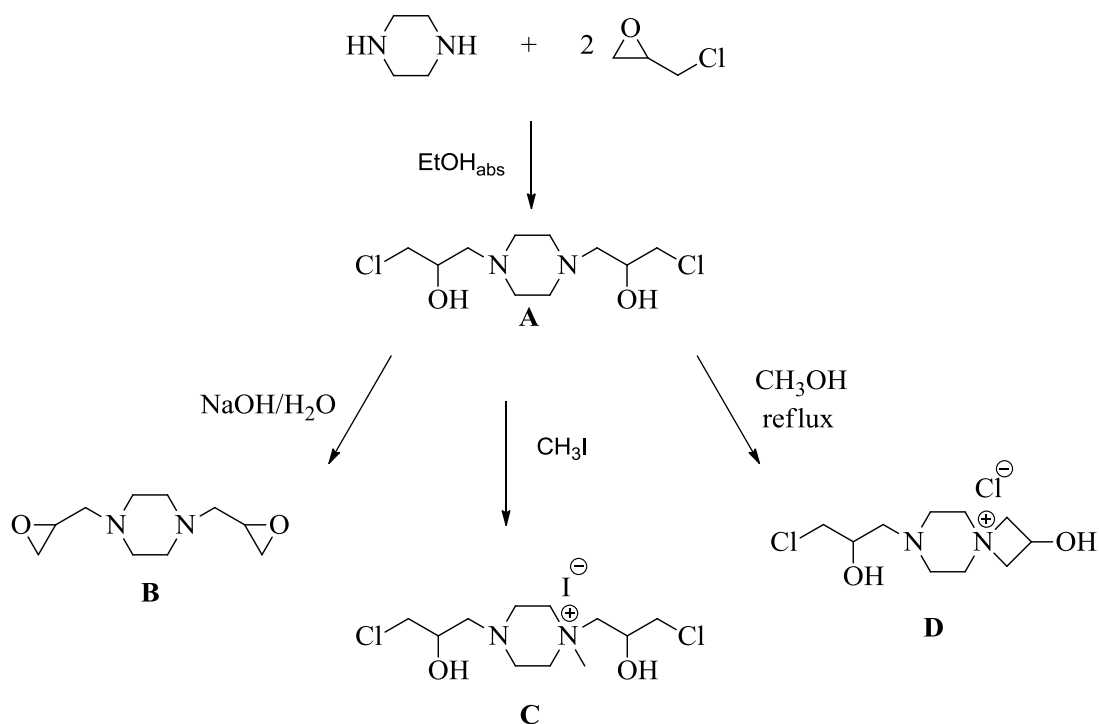
Scheme 3: Piperazine / epichlorohydrin based products.

In the current work, one of the piperazine derivatives (**D**) was synthesized in a more simple and quantitative way (> 95% yield) via the reaction of piperazine with epichlorohydrin (mole ratio 1:2) in water at ambient temperature. Obviously in the high polar solvent water the ammonium salt (the azetidinium chloride) is more stable than its covalent isomer (the amino-chlorhydrin). The product has two isomeric functional groups with different reactivity (azetidinium chloride and amino-chlorhydrin) and thus can potentially act as a bifunctional coupler. To establish the reaction conditions for preparing functional couplers model reactions with low molecular weight primary (hexylamine) and secondary amines (diethylamine) were performed (Scheme 4): (i) Model reactions with hexyl amine were performed using molar ratios of coupler : amine of 1:1 and 1:2. (ii) Reaction of the coupler with diethyl amine was performed using 1: 1 molar ratio.

Scheme 4: Model reactions of the coupler with low molecular weight amines.

The bifunctional coupler (**3**) and all the coupler derived products (**4**, **5**, **6**) obtained by reactions of the coupler with amines were characterized by spectroscopic methods.

Spectroscopic analysis:

The ^{1}H NMR spectrum of coupler **3** (Figure 3A) shows the characteristic peaks for the azetidinium ring protons at $\delta = 4.1\text{-}4.7$ ppm, the protons associated with the piperazine group were split into two region- the protons adjacent to the azetidinium group at $\delta = 3.5$ ppm and the protons adjacent to the chlorohydrin group at $\delta = 2.6$ ppm. The characteristic peaks of the chlorohydrin groups were found at $\delta = 3.84$ (H^6), 3.46 (H^7) and 2.4 (H^5) ppm. The secondary alcohol groups were found at $\delta = 5.36$ ppm (OH associated with chlorohydrin group) and at $\delta = 6.70$ ppm (OH associated with azetidinium group).

Figure 3: ^{1}H NMR spectra (in DMSO) of (A) the bifunctional coupler **3**, (B) the product **4** prepared by reacting the coupler with hexylamine in a 1:1 mole ratio and (C) the product **5** prepared by reacting the coupler with hexylamine in a 2:1 mole ratio. (* DMSO)

The ^{13}C NMR spectrum (Figure 4A) shows the characteristic peaks for carbon atoms associated with the azetidinium ring at δ = 70.24 (C^2, C$^{2'}$) and 57.72 (C^1) ppm cm^{-1} (Figure SI 1 shows the CH-HSQC of coupler **3**). The signals for the carbon atoms associated with the piperazine group were found at δ = 60.58 and 59.59 (C^3, C$^{3'}$), 47.88 and 47.54 (C^4, C$^{4'}$) ppm. The characteristic peaks of the carbon atoms associated with chlorohydrin groups were found at δ = 67.83 (C^6), 58.84 (C^5) and 48.33 (C^7) ppm. In the Raman spectrum of coupler **3** the characteristic signals for the alkyl chloride bond was found at 750-650 cm^{-1} (Figure SI 2).

Reactions of the coupler with hexyl amine:

The reaction products of the coupler with hexylamine were analyzed by NMR spectroscopy (Figure 3 and 4). The substitution pattern of the primary amine group was determined/proven by ^{1}H NMR spectral analysis; comparison of the integration ratio of the protons associated with the methyl group (H^{13}) with the characteristic proton (H^1) of the azetidinium group (Figure 3B, 3C). For the mono substituted product **4** the integration ratio H^{13}: H^1 is ca. 3:1 and for the bis substituted product **5** the integration ratio H^{13}: (H^1 +H$^{1'}$) is ca. 3:2.

In addition, formation of the two different products was proven by the appearance of characteristic signals for the carbon atoms marked as C^6, C^7 at δ = 63.78 and 51.04 ppm (for the mono substituted product **4**) (Figure 4B) and at δ = 64.27, 63.82 and 51.06 ppm (for the bis substituted product, **5**) (Figure 4C), respectively.
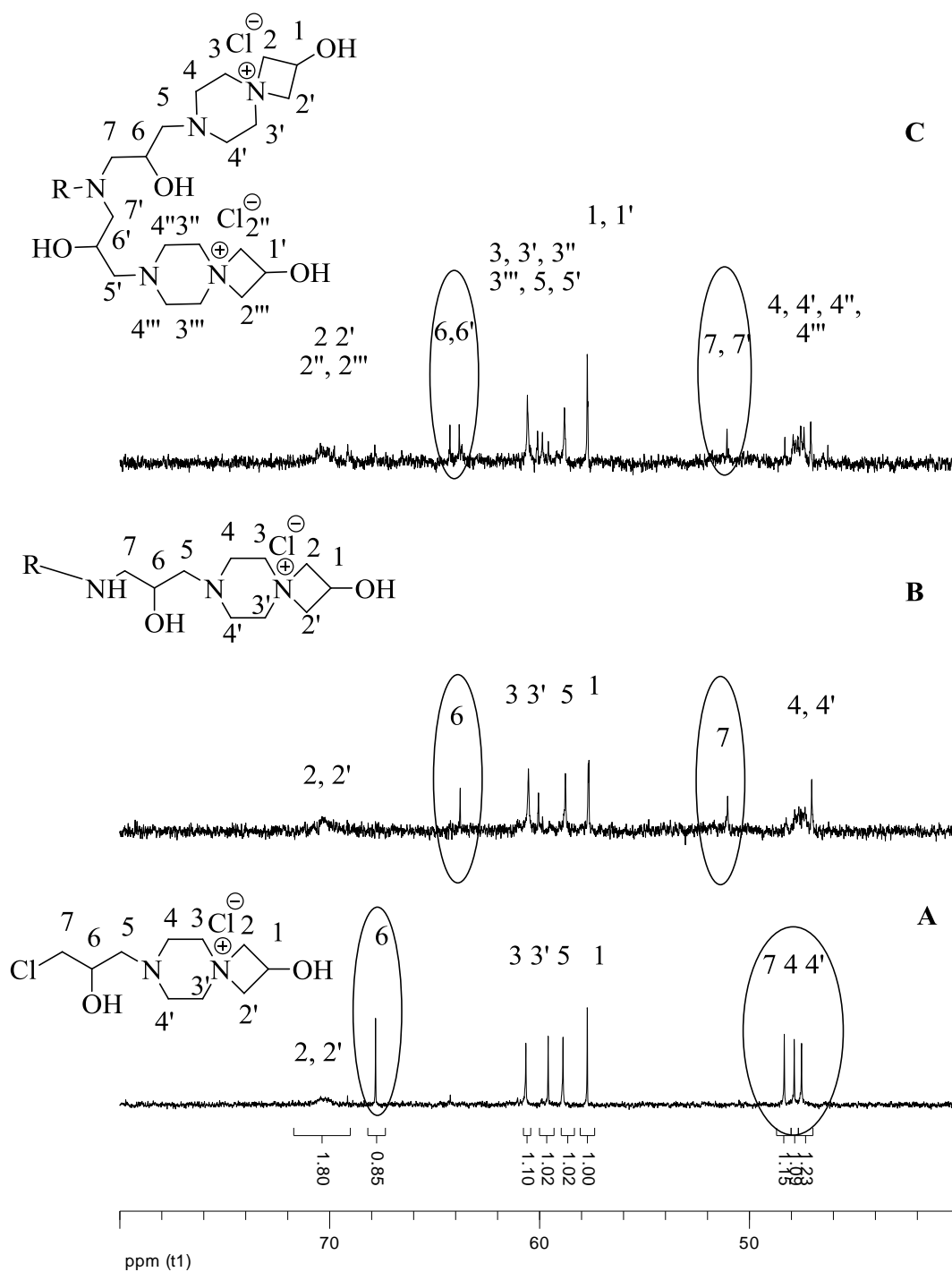
Figure 4: ^{13}C NMR spectra (in DMSO, R= C_6H_{13}) : (A) Bifunctional coupler **3**, (B) mono substituted product **4**: prepared via the reaction of coupler with hexylamine in 1: 1 mole ratio, (C) bis substituted product **5**: prepared via the reaction of coupler with hexylamine in 2: 1 mole ratio

Reaction of the coupler with diethyl amine (1:1 mole ratio):

Reaction of the coupler with diethyl amine (molar ratio 1:1) leads to the corresponding azetidinium functionalized amine as the only product. The ^{1}H NMR spectrum of product 6 (Figure 5A) shows characteristic signals for the protons associated with the azetidinium group
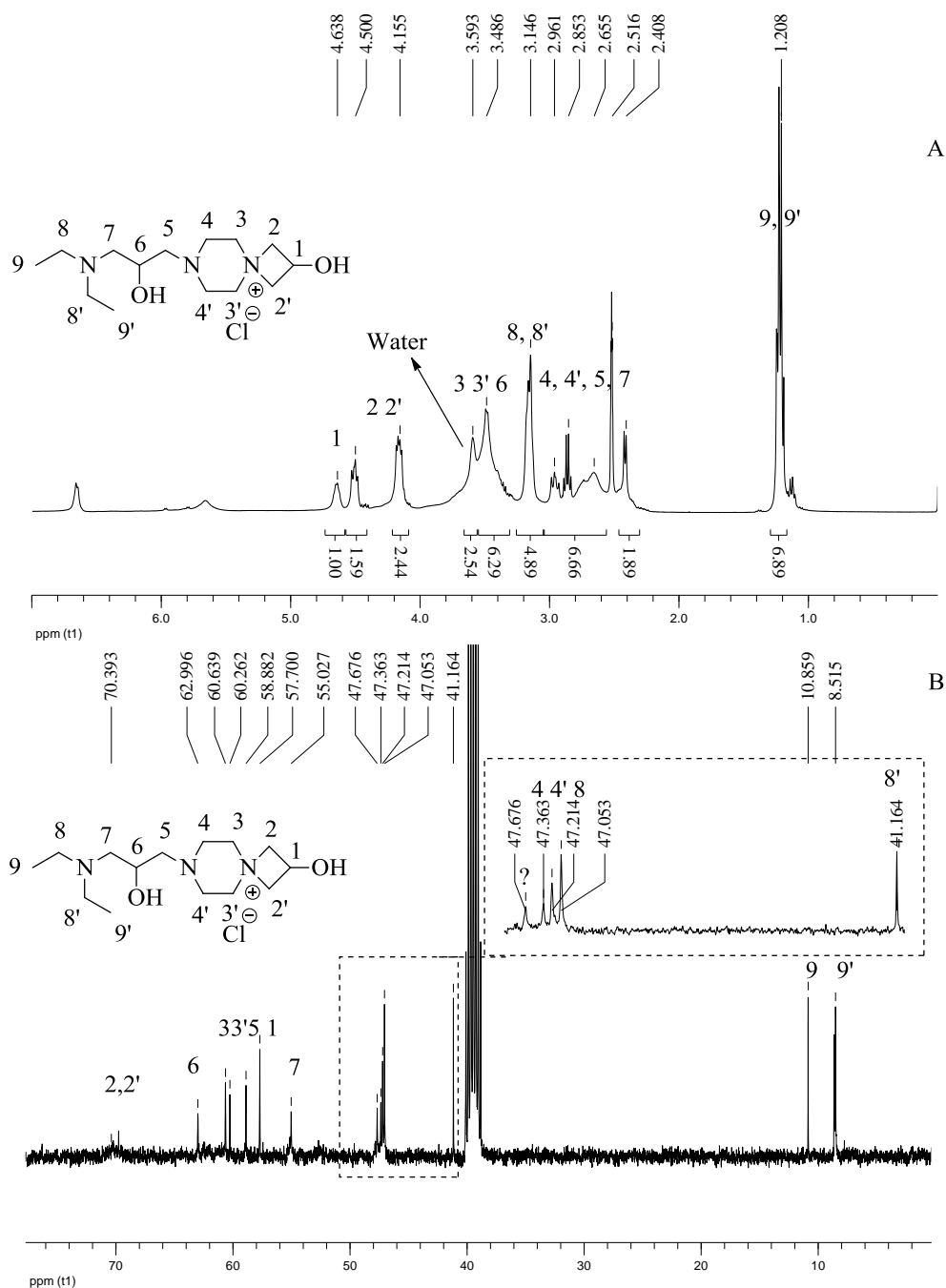
Figure 5: ^{1}H NMR- (A) and ^{13}C NMR spectra (B) of the product **6** obtained by the reaction of the coupler **3** with diethyl amine (coupler : diethyl amine = 1:1)

at δ = 4.10- 4.65 ppm. The signals characteristic for the carbon atoms marked as C^6 and C^7 (Figure 5B) are found at δ = 62.99 and 55.03 ppm.

Synthesis of azetidinium functionalized polymers using the bifunctional coupler:

Based on the model reactions using low molecular weight amines, two different azetidinium functionalized polytetrahydrofurans, containing the same backbone and different concentration of azetidinium groups were prepared from aminotelechelic polytetrahydrofuran XTJ-548. (Scheme 5).
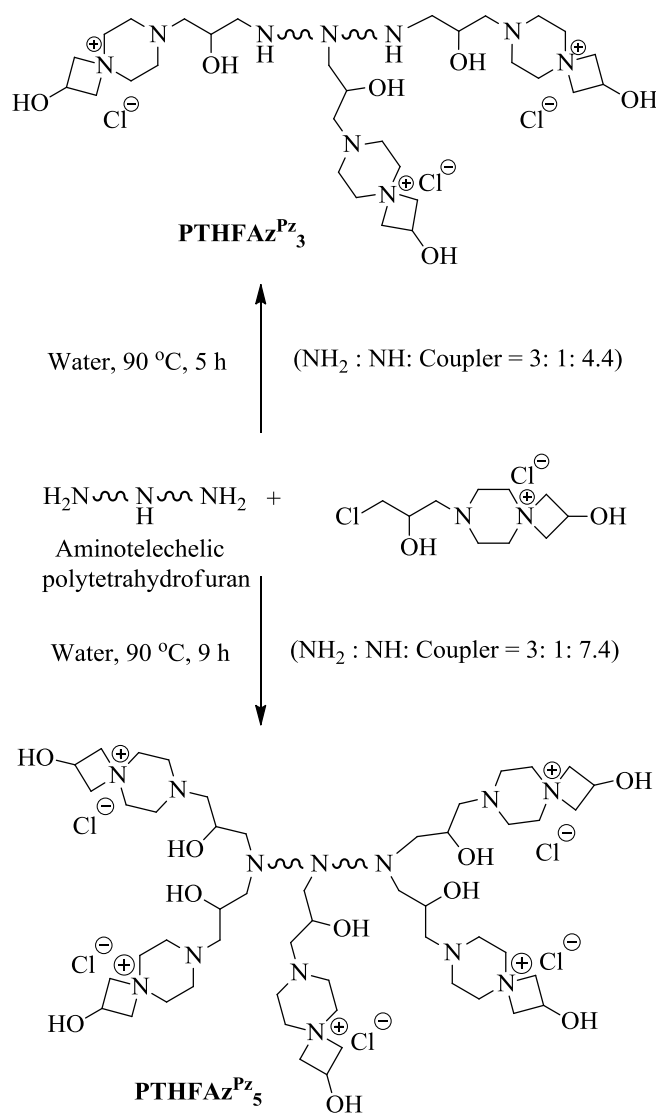
Scheme 5: Synthesis of azetidinium functionalized polymers using the bifunctional coupler.

(a) **PTHFAz$^{pz}_3$**, was synthesized from aminotelechelic polytetrahydrofuran and bifunctional coupler, using one equivalent of coupler per nitrogen atom.

(b) **PTHFAz$^{pz}_5$**, was synthesized from aminotelechelic polytetrahydrofuran and bifunctional coupler, using one equivalent of coupler per nitrogen-hydrogen (N-H) bond.

Formation of the azetidinium functionalized polytetrahydrofurans was proven by ^{1}H NMR spectral analysis (Figure 6). The disappearance of the characteristic peak for the proton (H^6) at δ = 3.84 ppm of the chlorohydrin group and the presence of the characteristic peaks of protons associated with azetidinium group (H^1 and H^2) at δ = 4.1- 4.7 ppm confirm the formation of azetidinium functionalized polytetrahydrofuran.
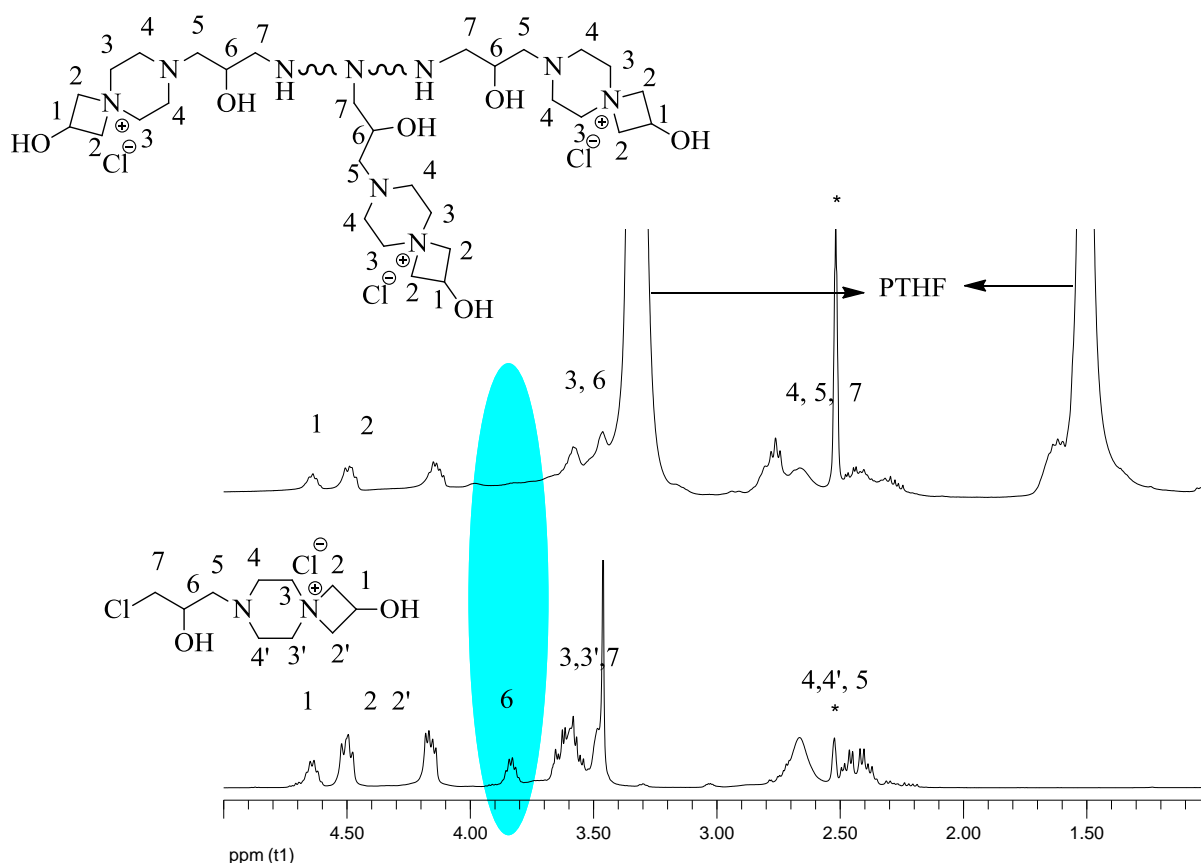
Figure 6: Reaction of aminotelechelic PTHF with the coupler (-NH$_2$: -NH: coupler =3: 1: 4.4 mole ratio): ^{1}H NMR spectral analysis (* DMSO).

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This result was confirmed by Raman spectroscopy. Here the disappearance of the characteristic peaks at 750-650 cm^{-1} of the alkyl halide bond proves the formation of functionalized polytetrahydrofurans (Figure 7).
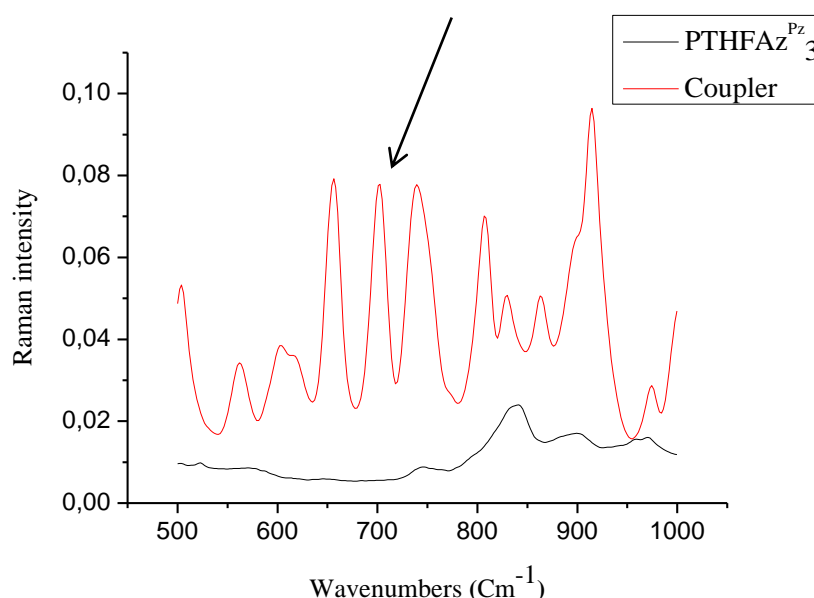
Figure 7: Reaction of aminotelechelic PTHF with the coupler (-NH$_2$: -NH: coupler =3: 1: 4.4 mole ratio): Raman spectroscopic analysis.

Another important aspect during the preparation of azetidinium functionalized polymers is the stability of the highly reactive azetidinium groups in the polymer backbone, in the presence of free secondary amine groups.

For Hercosett [poly(amino-amide) / epichlorohydrin adduct], it is known that the unreacted secondary amine groups of the polymer backbone react with the formed azetidinium groups leading to chain coupling. This reaction occurs as parallel or consecutive reaction to the desired conversion of the secondary amine groups with epichlorohydrin.

The current synthetic strategy in which a bifunctional coupler was used to introduce azetidinium groups within an amino functional polymer was found to avoid further reaction of these reactive cationic groups with the newly formed secondary amine groups.

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The ^{1}H NMR spectra for the azetidinium functionalized polymers (PTHFAz$^{pz}_3$, PTHFAz$^{pz}_5$) are given below (Figure 8).
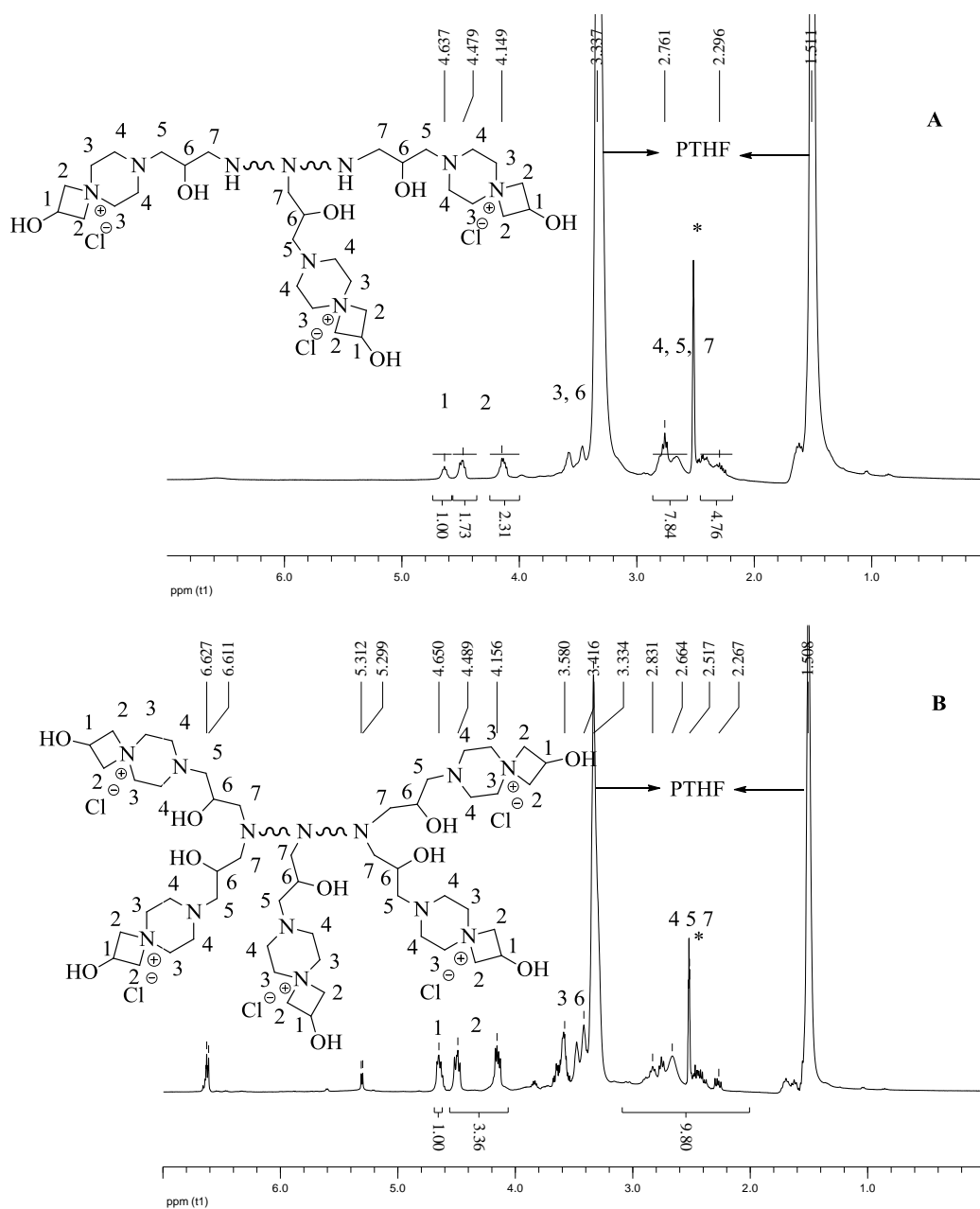
Figure 8: ^{1}H NMR spectra of azetidinium functionalized PTHFs: (A) PTHFAz$^{pz}_3$, (B) PTHFAz$^{pz}_5$. (* DMSO)

The integration ratio I (3- 2.5 ppm): I (4.6 ppm) is 12: 1 (for PTHFAz$^{pz}_3$) and 10: 1 (for PTHFAz$^{pz}_5$) proving that the azetidinium groups remained intact under the reaction conditions. This was further confirmed by the GPC data (Table SI 1)

Conclusion: In the current work a piperazine based bifunctional coupler was designed, synthesized and characterized by NMR and Raman spectroscopy. Reaction of the bifunctional coupler – with an amino chlorohydrine and an isomeric azetidinium functional group - with a primary amine (hexyl amine) and a secondary amine (diethyl amine) in water – a highly polar solvent - were studied to determine the relative reactivity of the two isomeric functional groups. It was shown that reaction of the coupler with amines results in opening of the azetidinium ring and formation of an 3-alkyl- (dialkyl-) amino-2-hydroxypropyl-piperazine unit and isomerization of the existing 3-chlor-2-hydroxypropyl-piperazine unit in a new azetidinium group.

This piperazine based coupler was successfully used to prepare azetidinium functionalized polytetrahydrofurans in a controlled way starting with aminotelechelic polytetrahydrofuran. It is expected that other amine functional polymers can be transformed in a similar way to azetidinium functionalized polymers.

References:

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Supplimentary information for chapter 4

Heteronuclear single quantum coherence (C-H HSQC) spectrum of the coupler:
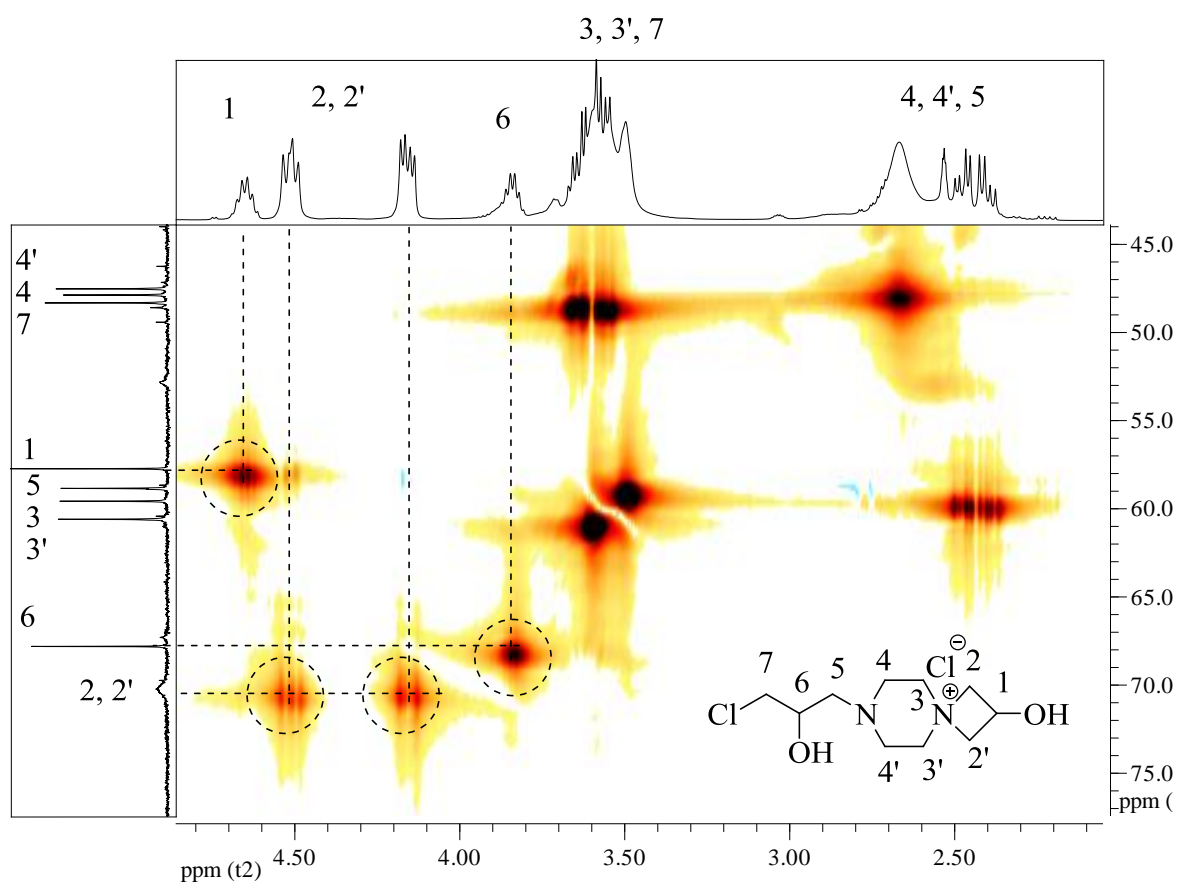
Figure SI 1: CH HSQC of the coupler **3**.

Raman spectrum of the coupler 3:
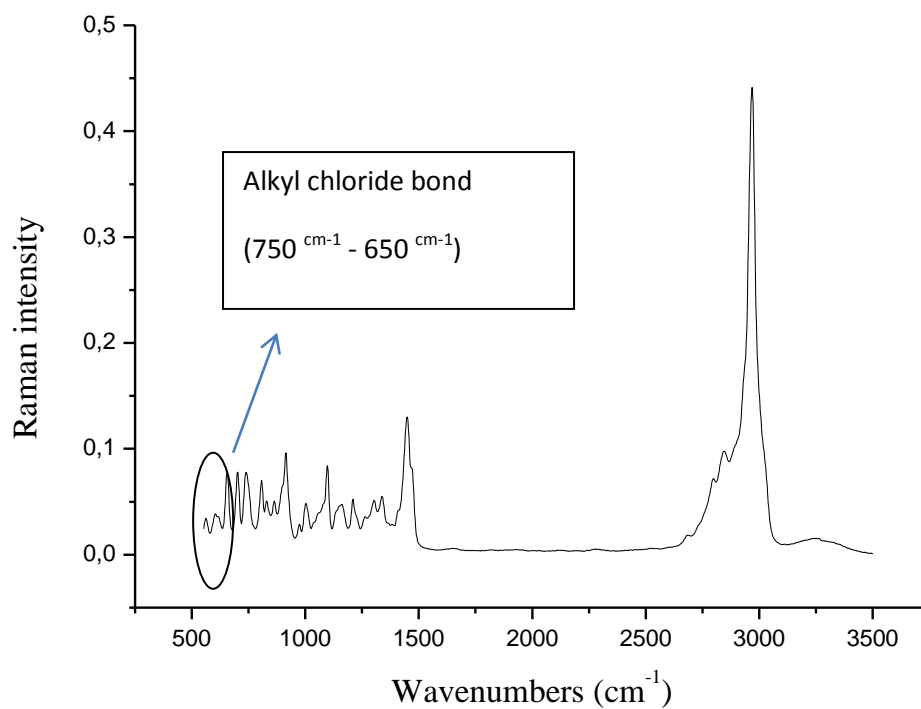
Figure SI 2: Raman spectrum of coupler **3** showing the characteristic band of the alkyl

chloride bond.

Table SI 1: GPC data for the new polymers:

Polymer	Molecular weight(GPC)	Molecular weight(calculated)
XTJ-548	1,700 (reported by Huntsman)	
PTHFAz$^{pz}_3$	$M_n = 3,300$, $M_w = 3,800$	2,400
PTHFAz$^{pz}_5$	$M_n = 3,300$, $M_w = 3,800$	2,870

C-H HSQC spectra of the product obtained via the reaction of coupler with hexyl amine in 2:1 mole ratio:
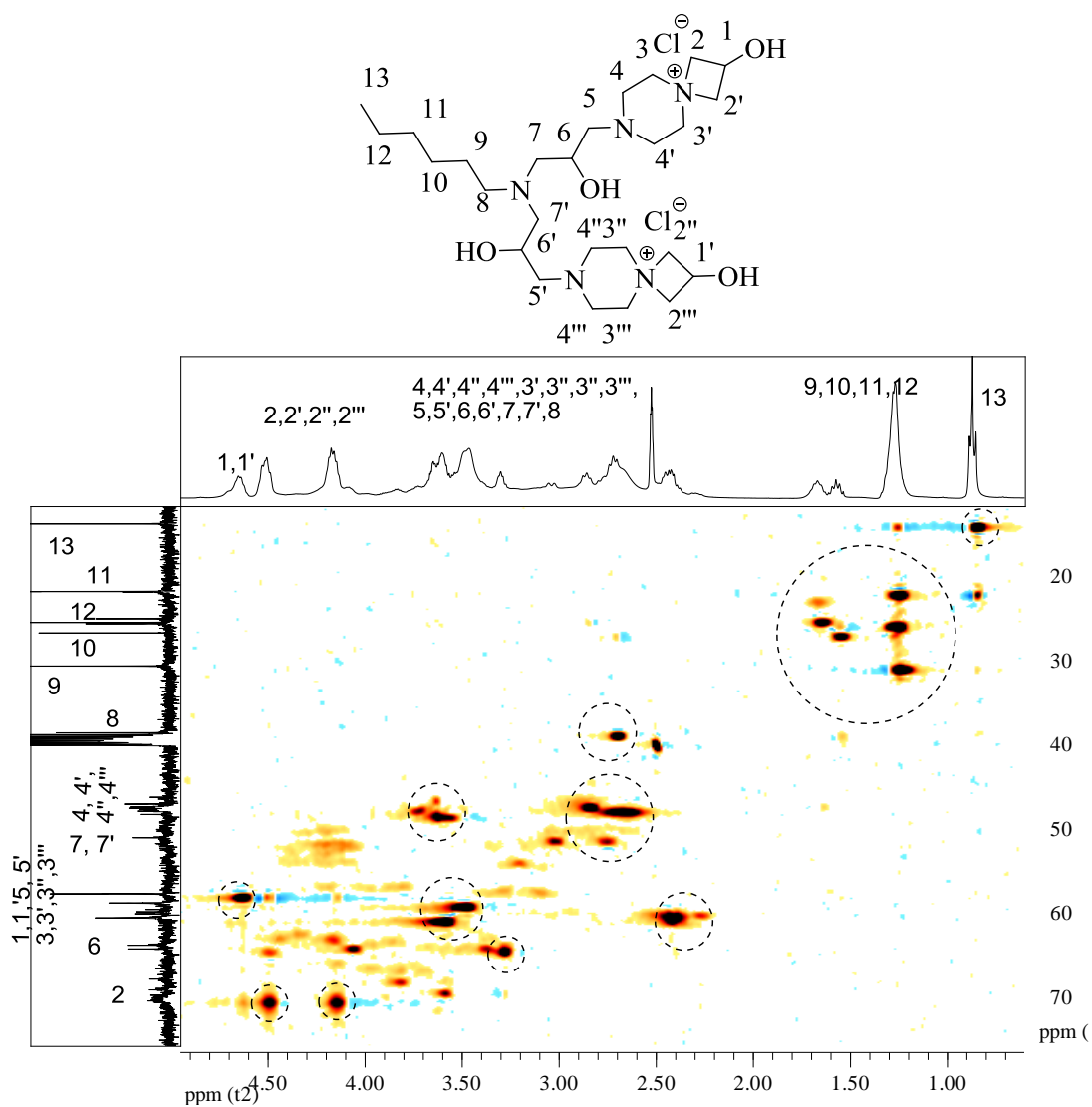
Figure SI 3: CH HSQC of the product obtained via the reaction of coupler with hexyl amine in 1:2 mole ratios

Chapter 5

Azetidinium functionalized polytetrahydrofurans: antimicrobial properties in solution and application to prepare antimicrobial surface

Introduction:

Microbial infections are one of the most challenging problems in today's life in many areas like hospitals (nosocomial infection), food packaging, textile products, biomedical devices etc[1, 2, 3, 4]. To solve the problem, preparation of new antimicrobial agents, studying their properties and application to prepare antimicrobial surfaces is an interesting domain in current research[5]. There are three main strategies developed for designing antimicrobial surfaces[6]: (i) adhesion resistance, (ii) biocide leaching, and (iii) contact killing. The first approach is focused on preparation of surfaces, that resist the adhesion of microbes via different physical repulsion techniques, such as preparation of negatively charged surfaces (here negatively charged surfaces repulse the negatively charged bacterial cell wall) or super hydrophobic surfaces (here aqueous suspensions of bacteria have restricted contact with the surface due to very high contact angle [> 150 ^{o}]). The second approach is based on the release and diffusion of cytotoxic compounds from the material surface. These cytotoxic compounds cause the death of bacteria in the nearby surroundings. The third approach is contact killing; where antimicrobial polymers are generally amphiphilic polycations, which interact with the cell wall components via different mechanisms, like, e.g., cation exchange and membrane disruption and induce cell lysis etc.

Cationic amphiphilic polymers were found to be most interesting as antimicrobials, due to their ability to resist bacterial growth in solution and as well as at the surface. In the last

decade many reports were published on antimicrobial polymers containing cationic groups like quaternary ammonium, pyridinium etc[7, 8, 9, 10]. For example, Tiller et al. prepared antimicrobial glass surface by attaching poly(4-vinyl-N-alkylpyridinium bromide) to glass slides via covalent linkage[11]. Fuchs and Tiller reported the preparation of contact active antimicrobial coatings from an aqueous suspension of polystyrene-co-poly(N-vinyl-N-methylpyridinium iodide)[12]. Pasquier et al. reported the one step preparation of multifunctional poly(ethylene imine)s bearing quaternary ammonium groups, alkyl chains and allyl groups and showed their antimicrobial efficacy in solution as well as at the surface[13].

In spite of the large developments on the preparation and structure-property relationship of antimicrobial polymers, very few of them are practically suitable for preparing polymer coated antimicrobial surfaces required to solve hygiene related problems in our life[14]. For example, to solve the hospital related infections, one challenging way is to prepare antimicrobial textiles (antimicrobial polymer coated textile). The main challenges in the related area are: (i) easy synthetic procedure of the antimicrobial polymers – reactions in water (without any organic solvent) are strongly recommended, (ii) simple and practically applicable procedure of textile coating, (iii) 99.99% (log 4 reduction) of bacterial growth inhibition by the antimicrobial textiles, (iv) excellent durability (wash fastness).

For the preparation of the polymer coated antimicrobial surfaces, it is advantageous, if the functional groups present in the polymer can be covalently linked with the active groups on the surface – resulting in a high adhesion of the polymer to the surface. To prepare polymer coated antimicrobial surfaces in the current work, we focused on the application of azetidinium functionalized polymers. The advantages of azetidinium groups in this application are: (a) inhibition of bacterial growth via interaction with cell components due to the cationic nature of this group[15] and (b) improving the adhesion via both (i) ionic interaction

and (ii) covalent linkage. The four membered rings attached to the polymers react with the functional groups on the surface forming covalent bonds[16].

Here, we report the antimicrobial efficacy of different azetidinium functionalized polytetrahydrofurans and their applications to prepare antimicrobial surfaces (Figure 1). For our current study we choose textiles as model surfaces: cellulose based surface- cotton and polyester- poly(ethylene terephthalate) (PET), as the preparation of antimicrobial textiles is one of the prime requirements in our society to prevent hospital-acquired infections and also to solve day-to-day hygiene related problems. The advantage of the current approach is that: (i) antimicrobial polymers used in the current work were prepared using simple one pot reactions in water without using any catalyst, starting from cheap and commercially available starting materials, (ii) the textiles were coated with the polymers following a padding procedure - used in industry, (iii) presence of 0.5 % owf - 1 % owf (on weight-of-fabric (owf)) polymers on the textile surface makes the whole textile very efficient (99.99% bacterial growth inhibition before and after repeated washings).
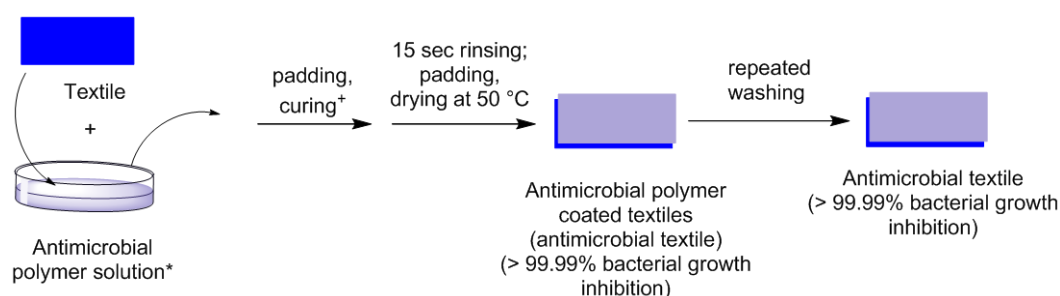
Figure 1: Scheme showing the concept for the preparation of antimicrobial textiles. * the amount of polymer in the solution was calculated on weight-of-fabric (owf) concerning the liquor uptake after padding; + curing was done at 50 °C or at 100/150 °C.

Nomenclature: For PTHFAz$^{pz}_3$ (**3**), PTHFAz$^{pz}_5$ (**4**), PTHF – stands for polytetrahydrofuran - Az^{Pz} represents that azetidinium groups, attached with a piperazine coupler and the numbers represents the no of groups in the polymer backbone.

Experimental section:

Materials: Epichlorohydrin (99%, Merck), piperazine (99+%, Aldrich), aminotelechelicpolytetrahydrofuran (PTHF) [XTJ-548 (Huntsman)] were used without further purification. XTJ-548 is a mixture of NH_2-PTHF-NH_2 and NH_2-PTHF-NH-PTHF-NH_2 in a ratio of 1:4 and a number average molecular weight of 1700 g/mol. Distilled water and tetrahydrofuran were used as solvents. For antimicrobial activity, the tests were performed against the Gram-negative bacterium *Escherichia coli* (*E. coli*, ATCC 23716) and the Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*, ATCC 6538).

Measurements: ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform (CDCl$_3$) were used as solvent. Tetramethylsilane (TMS) was used as an internal standard. Raman spectra were recorded on a Bruker RFS100/s Raman spectrometer, fitted with aNd: YAG laser (1064nm). The spectral resolution was 4 cm^{-1}. For one spectrum 1000 scans were collected at a laser power of 200mW. Size exclusion chromatography analysis (SEC) were carried out using water (with addition of 0.1 M NaCl, 0.1% TFA, 0.01% NaN$_3$) as eluting solvents. For water as eluting solvent high pressure liquid chromatography pump (Agilent 1100) and refractive index detector (Wyatt, Optilab DSP) were used at 30°C with a flow rate of 1 mL/ min. Three columns with PSS Novema gel were applied. The length of each column was 300 mm, the diameter was 8 mm, the diameter of the gel particles were 10 μm and the nominal pore widths were 30, 3000 and 3000 Å. Calibration was achieved using Pullulan standards. Padding machine was from Ernst Benz AG, Zuerich/CH used at a contact pressure of 1.5 bar. Colorimetry of the dyed fabrics was performed using the Datacolor system (Spectraflash SF600 plus CT UV, Datacolor, Marl/D). Proliferation of bacteria was monitored using the multi well plate readers / incubators Genios Pro and Infinite 200 Pro (TECAN).

Synthesis of polymers: Synthesis of polymers was given of supplementary information.

Preparation of polymer formulations:

1 g of the polymer was dissolved in 100 mL ultrapure water, containing 0.01 % DOW. pH value was adjusted to pH 5.5, 8.5 and 12 as required. Formulations were also tested to understand antimicrobial efficacy.

Method for the preparation of polymer coated textiles:

First, the polymers solutions were shaken for 10 min at 30 °C. Textile fabric samples [cotton and polyester (poly(ethylene terephthalate) (PET), standard fabrics from TESTEX, Bad Muenstereifel/D free from chemical residues, dyes and optical brighteners] with a size of 40 cm x 5 cm were prepared and weighed.

Padding was performed in a glass beaker equipped with glass rods that function as deflection rollers. 0.01 % 3-(polyoxyethylene) propylheptamethyltrisiloxane (DOW Chemicals) in distilled water at pH 5.5 (using diluted acetic acid) (for some experiments pH = 8.5 and 12) was used as wetting agent. The liquor ratio was adjusted to 1:10. Padding was performed at 25 °C, fabric samples were passed through the pad bath once (bath and padding machine), and then dried and cured (thermal oven at 100 °C, temperature was enhanced to 150 °C over 15 min, at 150 °C additional curing for 2 min; alternatively drying was performed at 50 °C). For the pad method, pick-up (liquor uptake) of the fabric by using this method was calculated initially.

Textiles were finished using PTHFAZ$^{Pz}_3$, PTHFAZ$^{Pz}_5$ in 0.35 and 0.5 % owf, at 25 °C and a liquor ratio of 1:10. After that they were prone to 15 sec rinsing and drying at 100 / 150 °C – the finished textiles were prone to staining tests, to analyze the homogeneity of the finishing (Lanasol® Blue 3R staining test), to washing tests, and to the test on antimicrobial efficacy.

Washing test: Washing test was performed in a Labomat (Mathis) using the nonionic surfactant Uniperol O Microperl, BASF (0.1 %) at 40 °C and 60 °C for 30 min at a liquor ratio of 1:100 (modified according to DIN EN ISO 105/C06).

Washed fabrics were also prone to the analysis as mentioned above.

Staining tests

Dyestuff: Lanasol® Blue 3 R (Huntsman, Basel/CH), Uniperol O Micropearl (BASF, Ludwigshafen/D)

The homogeneity of the uptake of cationic polymers on surfaces can be visualized by staining. The staining test is based on a test used for the visualisation of the Hercosett resin (which also contains cationic azetidinium groups in the backbone) on wool. For this, the reactive α-bromoacrylamide dyestuff Lanasol Blue 3R (C. I. Reactive Blue 50, CAS-No.: 12225-61-5; Ciba Specialty Chemicals, now: Huntsman Textile Effects, Basel/CH) was applied. Treated textile fabrics (1 g fabric and 100 mL staining solution) were incubated in an aqueous solution (1 g/L Lanasol Blue 3R, 1 mL/L Uniperol O, 5 mL/L acetic acid, pH = 3.5) for 5 min at RT and under gentle shaking, then thoroughly rinsed with cold water and dried at ambient conditions. Reference samples that were blind treated without polymer were also prone to the staining test.

Antibacterial studies:

The antibacterial activity of the amphiphilic polymers **in solution** was determined by measuring the minimum inhibitory concentration (MIC) using different test bacteria. The testing organisms used were *Escherichia coli* as a Gram negative and *Staphylococcus aureus* as Gram positive bacteria. Suspensions of strains with known colony forming units (CFU; *E. coli*, 2 x10^6 CFU/mL; *S. aureus,* 2 x10^6 CFU/mL) were incubated at 37°C in nutrient solutions with different concentrations of the test samples together with a wetting agent

(0.001% 3-(Polyoxyethylene)propylheptamethyltrisiloxane (DOW)). The growth of the bacteria was followed during the incubation over 20 h by measuring the optical density at 612 nm every 30 min and 1000 s shaking at 100 rpm per cycle of 30 min by using a microplate reader/incubator. The minimal inhibitory concentration (MIC) corresponds to the concentration of the test substance at which a log 4 reduction of the growth of the inoculated bacteria was observed by comparison with control samples without test substance. Experiments were triplicated.

The **efficacy of textile surfaces finished with the antimicrobial polymers** was assessed in a two-step testing procedure (EXPOSE test, DWI). The antimicrobial effect was tested under growth (bacteria suspension in nutrient solution) conditions. All tests were carried out at least three times (in order to reduce statistical artifacts). Samples sized 2 cm x 2 cm taken from textile substrates finished with functionalized polymers were placed into Petri dishes (Ø = 3 cm) and 50 μL bacteria suspension of *E. coli* (2 x 10^6 colony forming units per mL (cfu/mL)) containing 0.01 wt-% DOW were inoculated onto each surface. As reference blind finished textile substrate was exposed to 50 μL bacteria suspension of *E. coli* (2 x 10^6 cfu/mL) by pipetting 20-22 drops of the inoculum to separate sites of the sample. As sterility control, a finished textile substrate was exposed to 50 μL of nutrient solution by pipetting 20-22 drops of the inoculum to separate sites of the sample (sample c). The exposure was performed in a climate chamber at 25 °C and 97 % rH (relative humidity) for 2.5 h or 23 h. Thereafter, 2 mL of nutrient solution was pipetted (dilution 1:50) in every Petri dish (a-c) and the samples were shaken at RT for 30 min with 150 revolutions per minute (rpm). Then, from each Petri dish 200 μL solution were transferred to a well plate and the proliferation potency was monitored for 20 h at 37 °C in the multi well plate incubator/reader under the conditions given above.

Leaching test: 180 μL of the shake solution of sample c (sterility control) were transferred to a well plate and inoculated with 20 μL bacteria suspension of *E. coli* (2 x 10^7 cfu/mL). This leaching test served as a proof that during the growth test no inhibition is caused due to an

amount of polymer transferred from the coated surface to the well plate of the growth test, i.e., as a proof that the growth test is valid and the growth inhibition is only due to the influence of the polymer on the bacteria during the exposure on the surface.

Antimicrobial assessment according to modified ASTM 2149 method:

Four glass beakers were filled with 5 mL of 3 mM KH_2PO_4 solution pH 7.1 and three of them were inoculated with *E. coli* ($1x10^5$ cfu/mL), the fourth was kept for sterility and leaching test without inoculation. Per finished textile fabric sample four pieces each with a size of 3 cm x 3 cm were taken and sterilized at 120 °C for 30 min under dry conditions. Thereafter, samples were placed into the beakers and shortly shaken by hand. Directly after that, a 20 µL liquor sample was taken from the beakers (0 h exposure) and transferred to a well with 180 mL nutrient solution in a 96 well plate. The beakers were transferred to a thermal shaker and incubated at 25°C and 300 rpm. Liquor samples were also taken after 1 h, 2h and 20 h and transferred to a well plate for monitoring proliferation potency of the bacteria exposed to the textile samples (growth test overnight at 37 °C and 1000 s shaking per cycle of 30 min).

To monitor the **leaching** from the finished textiles into the exposure liquor 2 samples of 180 µL were pipetted from the exposed but non-inoculated beaker into a well plate. Samples were inoculated with *E. coli* and proliferation curves were monitored.

Measurement of hemolytic activity

Human erythrocytes (red blood cells (RBC), 0, Rh positive; citrate blood) were obtained by centrifugation (3000 rpm, 10 min) to remove plasma, washed 3 times in PBS and diluted in PBS to obtain a stock solution $2.6x10^8$/mL RBC. 250 µL of the stock solution was pipetted into solutions of defined polymer concentration in PBS up to 500 µL; the final amount of RBC being $1,3x10^8$ RBC/mL. The RBC were exposed for 60 min at 37 °C, thereafter centrifuged (4000 rpm, 10 min) and the absorption of the supernatant was determined at 414 nm in a 96 well plate. As reference solutions (i) PBS for determining spontaneous hemolysis

and (ii) 0.5 % Triton X-100 for 100 % hemolysis (positive control) were used. Hemolysis was plotted as a function of polymer concentration and the hemolytic activity was defined as the polymer concentration that causes 50 % hemolysis of human RBC relative to the positive control (EC_{50}).

Durability test: The polymer coated textiles were washed for 10 times at 60°C repeatedly as described earlier and then the antimicrobial efficacy of the washed textiles against *E. coli* was studied.

Results and discussions:

In the current report we discuss the antimicrobial efficacy of azetidinium functionalized polytetrahydrofurans and their applications to prepare antimicrobial surfaces. In literature, azetidinium functionalized polymers (example: Hercosett) are known for surface coating in various applications like: preparing wet-strength paper[17], shrink resist wool[18], thermo-responsive films for optically responsive coatings[19] etc. Though these polymers are potentially important for many applications, in the last years very few articles were reported on azetidinium functionalized polymers (most studies were reported on Hercosett). The main problem is the difficulty to synthesize and stabilize the four membered aza-heterocyclic rings in the polymer[20]. To solve this problem in the recent years we reported easy one pot synthetic approaches to prepare well-defined azetidinium functionalized polymers[21, 22]. In the current work, the polymers were prepared via post polymer modification as reported in literature (Scheme 1). PTHFAZ$^{Pz}_3$ **3** and PTHFAZ$^{Pz}_5$ **4** were prepared by reaction of the aminotelechelic tetrahydrofuran (XTJ-548) (**1**) with a bifunctional coupler (**2**), using one equivalent of the coupler per nitrogen atom or one equivalent of the coupler per N-H bond[23]. For the preparation of the polymers in our current work, we used XTJ-548 as precursor polymer, as it is well-known to be used as coating material for different applications in industries[24, 25, 26].
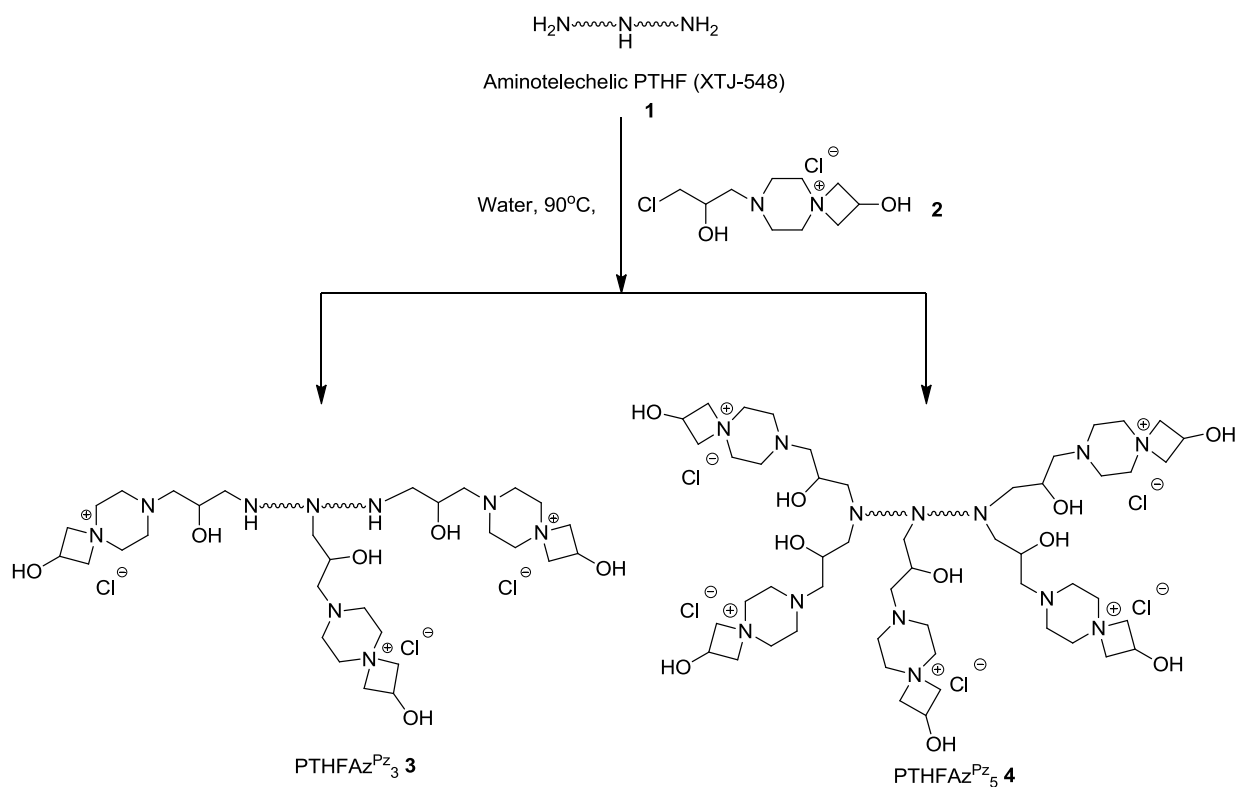
Scheme 1: Preparation of azetidinium functionalized polytetrahydrofurans.

Antimicrobial studies:

The antimicrobial efficacy of the azetidinium functionalized polytetrahydofurans **3** and **4** was studied first in solution. Minimum inhibitory concentration (MIC) of these polymers was determined to understand their antimicrobial efficacy in solution. These polymers are expected to have antimicrobial properties mainly due to the presence of cationic azetidinium groups in the polymer backbone and different hydrophobic / hydrophilic ratio. It was found that the MIC (minimum concentration of the polymers for 99.99% bacterial growth inhibition) values of both the polymers were same: the MIC values were 500 µg/ mL and 100 µg/ mL against gram positive (*S. aureus*) and gram- negative (*E. coli*) bacteria respectively (Figure 2).
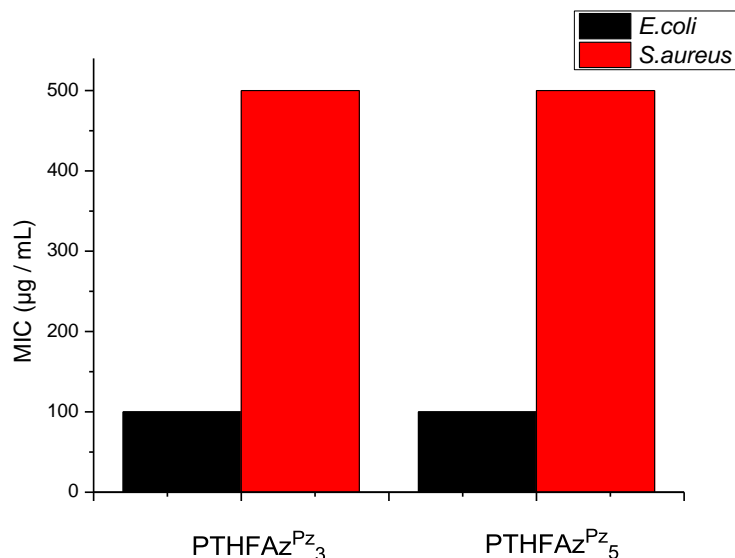
Figure 2: MIC values of the azetidinium functionalized PTHFs in solution.

As the polymers showed a good antimicrobial efficacy in solution, they were used to coat surfaces [cotton – a cellulose based textile and PET: Poly(ethylene terephthalate)]. Textile fabrics were finished with these polymers in defined concentration – 0.35% owf, 0.5% owf and 1.0 % owf [on weight-of-fabric (owf)]. The presence of the cationic polymer on the surface was confirmed by staining the polymer coated surface using a staining test (Figure 3) and measuring the color values (Table SI 1). The staining of the polymers on the fabrics was performed by Lanasol® Blue 3R staining test which is specific for polymers which contain cationic groups. If cationic polymers are present on the fabrics, a blue staining results due to electrostatic interactions of the sulfonic acid residues in the dye molecule and cationic groups in the polymer. Furthermore, the staining test provides information on the homogeneity of the polymer distribution on the fabric surface. The blue staining remains after washing in a liquor containing nonionic surfactants at 60 °C.
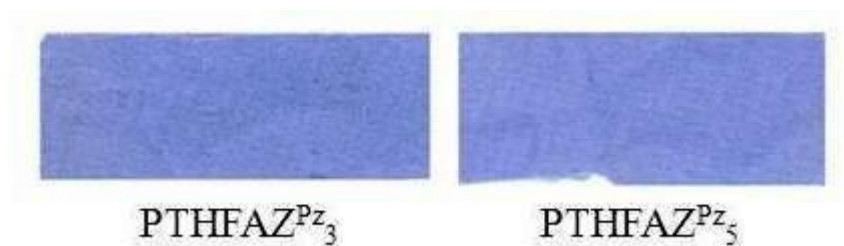
Figure 3: Stained (using Lanasol® Blue 3G dye) polymer coated cotton surfaces proving the presence of cationic azetidinium groups. (0.5 % owf) (Reference samples which were blind treated without polymer did not show any blue staining).

The polymer attach on a surface by the combination of physical interaction of the polymers with the surface (hydrophobic interaction, ionic interaction and hydrogen bonding) and chemical interaction – chemical bonds formed via the reaction of functional groups at the surface (- OH, -COO⁻) with the azetidinium groups of the polymer backbone (Figure 4). One can expect that these types of interaction should vary when the surface was coated at different conditions, e.g., using different pH of the polymer solution for coating, different drying temperatures, which can influence the antimicrobial efficacy of polymer coated textile.
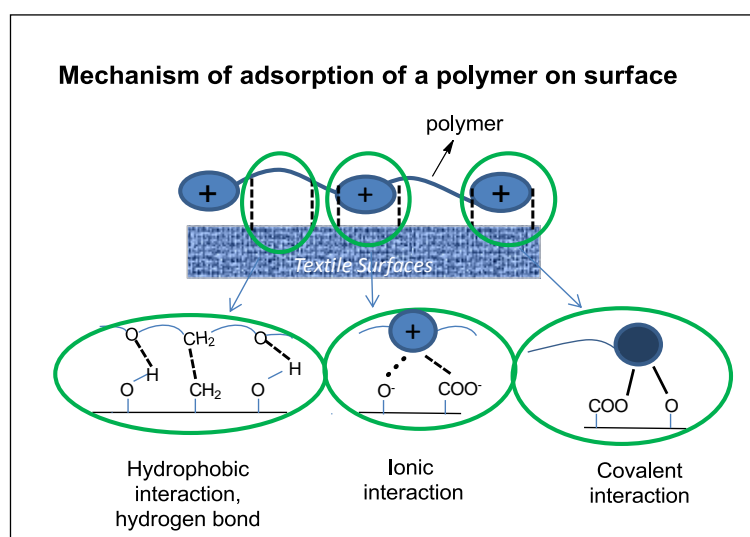
Figure 4: Interaction of the polymer coating with functional groups at the surface of the substrate.

To prove the concept, the pH of the polymer solution was adjusted to three different values (pH = 5.5, pH = 8.5 and pH = 12) before coating of the surface and after coating the surface was cured at 50°C and 150°C respectively and then rinsed to remove the unbound polymer. Both cotton and PET fabrics were used for coating and the antimicrobial efficacies of the polymer coated textiles were studied. To determine if part of the polymer was transferred from the coating into the exposure solution the growth inhibition of the exposure solution was also monitored (inhibition by leaching). Results are given in Table 1 and Table 2:

Table 1: Antimicrobial efficacies of polymer coated cotton (0.5 % owf) at different drying temperatures (50°C and 150°C) against *E. coli* (CFU: $2.6* 10^{6}$)

pH of the polymer solution used for padding	Inhibition (%)		Inhibition by leaching (%)	
	When dried at 50°C	when dried at 150°C	When dried at 50°C	when dried at 150°C
5.5	99.999	99.99	0	0
8.5	99.99999 - 100	100	~ 80	~ 50
12	99.99999 -100	--	0	--

Table 2: Antimicrobial efficacies of polymer coated PET (0.5 % owf and 1 % owf, pH = 8.9) at different drying temperatures (50°C and 150°C) against *E. coli* (CFU: $2.6* 10^{6}$) (pH of the polymer solution for coating was pH 8.9)

Amount of polymer on coton surface (% owf)	Inhibition (%)		Inhibition by leaching (%)	
	When dried at 50°C	when dried at 150°C	When dried at 50°C	when dried at 150°C
0.5	> 99	99.9999	0	0
1.0	99.99999	100	0	0

It was found that after 22 hours exposure, cotton fabrics coated with polymer solutions at pH 5.5 show a 99.999% (log 5) reduction of the bacterial count (*E. coli*) compared to an untreated reference surface. When the coating was performed with polymer solutions at pH = 8.5 and 12, even higher reduction rates of the bacterial count (99.99999 – 100%) were observed. The higher antimicrobial efficacy at higher pH indicates higher adhesion of the polymer on the surface since at higher pH as the covalent interaction should increase due to enhanced nucleophilicity of the reactive groups at the surface. However for PET the temparature plays a significant role. At higher temparature the efficacy is higher. It is due to the higher degree of covalents bonding of the polymer on surface at higher temparature – leads to crosslinking and grafting of the polymer on surface and improves the adhesion of the polymer on surface. The higher adhesion leads to better antimicrobial efficacy. For PET the polymer was only coated with only the solution at pH 8.9,the maximum suitable pH for PET finishing. Coating of PET at pH 5.5 did not show high antimicrobial efficacy (50 % - 90 %).

After having proved that all the polymer coated textiles showed excellent efficacies (>99.99 – 100%), when applied under slightly alkaline conditions, both cotton and polyester fabric (poly(ethylene terephthalate) (PET)) were coated at pH = 8.5, and the antimicrobial efficacy of the surfaces against *S. aureus* was also studied (Table SI 2). The results indicate high

efficacies for both types of polymer coated textiles against both gram positive and gram negative bacteria.

Finally, to understand a time dependent growth inhibition of *E. coli* when exposed to cotton fabrics finished with polymers, experiments were performed using **PTHFAZ$^{Pz}_5$** following modified standard ASTM 2149 method (Table 3). In the ASTM test the exposure of *E. coli* on the cotton samples is performed under non-growth conditions in buffer for defined times. Thus, a very good impression is given how fast the antimicrobial finishing on cotton with **PTHFAZ$^{Pz}_5$**, works. Under the testing conditions applied cotton finished with 0.5 % owf **PTHFAZ$^{Pz}_5$** dried at 150 °C gives better antimicrobial effect after 20 h exposure compared with samples dried at 50 °C. After 1, 2 and 20 hours under dynamic shaking the bacterial count is reduced by a factor of 10, 100 and 10^4 to 10^5 respectively. Cotton finished with 0.25 % owf **PTHFAZ$^{Pz}_5$** does not lead to 99.99 % growth inhibition of *E. coli* even after 20 h exposure on the fabrics, the amount applied to the fabrics being too low.

Table 3: Antimicrobial assessment of cotton fabrics finished with PTHFAZ$^{Pz}_5$ at pH 5.5 according to the ASTM 2149-10 standard testing method; Growth Inhibition is given in % after exposure in 3 mM KH$_2$PO$_4$ (Non-Growth) of *E. coli*, (4,6x10^5 CFU/mL)

	Bacterial growth inhibition by polymer coated cotton fabric			
Exposure time	**untreated**	**0.5% owf PTHFAZ$^{Pz}_5$ dried at 150°C**	**0.5% owf PTHFAZ$^{Pz}_5$ dried at 50°C**	**0.25% owf PTHFAZ$^{Pz}_5$ dried at 150°C**
1 h	-	50-90 %	80-99 %	0 %
2 h	-	99 %	99-99.9 %	< 50 %
20 h	-	99.99 – 100 %	99-99.9 %	0-90 %

Under these conditions of testing no leaching of the polymer into the exposure solution was observed.

Durability test: To understand the durability of the coating, the antimicrobial efficacy against *E. coli* were analyzed after 10 times of repeated washing of the polymer coated textiles. In all the cases, the antimicrobial coated textiles showed excellent efficacies (> 99.9% growth inhibition) after 10 times washing (Table 4). It is important to note that this method does not replicate the durability test method used in industry, however the results obtained after 10 times of washing shows that the textiles remains antimicrobially active − which indicates the durability of the coating in a laboratory based procedure.

Table 4: Antimicrobial studies of the polymer coated textiles after 10 times washing

Padding condition	Bacterial growth inhibition (%) against *E.coli* (CFU = 3.0 * 10^6/ mL)
Cotton padded with PTHFAz$^{pz}_3$ (0.5% owf) at pH 8.5, dried at 100/150 °C	100
PET padded with PTHFAz$^{pz}_3$ (1.0% owf) at pH 8.9, dried at 100/150 °C	99.99

Hemolytic activity tests: In many cases, antimicrobial polymers active against mammalian cells, e.g. red blood cells (erythrocytes) as well. To understand the antimicrobial selectivity, the polymer PTHFAZ$^{Pz}_3$ and PTHFAZ$^{Pz}_5$ were tested for their hemolytic activity. The antimicrobial selectivity was calculated as a ratio of HC$_{50}$ / MIC$_{99}$ as reported in literature[27]. (HC$_{50}$ = effective concentration of active compound lysing 50% of red blood cells and MIC$_{99}$

= minimum inhibitory concentration for 99% bacterial growth inhibition). The results are given in Table 5:

Table 5: Hemolytic activity and selectivity of the polymers

Polymers	MIC$_{99}$ (µg / mL)		HC$_{50}$ (µg/mL)	Selectivity (HC$_{50}$/MIC$_{99}$)	
	E.coli	*S.aureus*		*E. coli*	*S. aureus*
PTHFAZ$^{Pz}_3$	50	250	260	5.2	1.04
PTHFAZ$^{Pz}_5$	50	250	230	4.6	0.92

The results indicate that though both polymers show good selectivity against *E.coli* ($4-5$ times), they do not show any selectivity against *S.aureu*s.

Conclusion: In the current work, the antimicrobial efficacy of water soluble azetidinium functionalized polytetrahydrofurans in solution and on surfaces was studied. The different types of textile fabrics, coated with polymers at different well defined conditions, showed excellent antimicrobial efficacy (> 99.99 % growth inhibition). Furthermore, the polymer coated cotton showed excellent wash fastness and durability as understood by the similar antimicrobial efficacy (> 99.99% bacterial growth inhibition) before and after 10 times washing. Though these polymers did not show high selectivity between microbial and mammalian cells, they can be used to prepare antimicrobial textiles used in hospitals due to their excellent adhesion (non leaching) on the surface.

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Supplementary information for chapter 5

Experimental Section:

Synthesis of Polymers:

Bifunctional coupler **(2)**, PTHFAz$^{pz}_3$ **(3)**, PTHFAz$^{pz}_5$ **(4)** were synthesized as described in chapter 4[1,]

Table SI 1: Colour values (CIELAB) for cotton samples after staining test (CIELAB colour scale, Datacolor measurement after Lanasol test): (differences in the colour space correspond to visual differences;CIELAB colour space is organized in a cube form: L* axis from top to bottom, maximum is 100 which is a perfect white and Zero which represents black. a* and b* axes have no numerical limits; positive a* is red, negative a* is green; positive b* is yellow, negative b* is blue. Delta values indicate how much a standard and a sample differ from one another (Delta L* = L*$_{sample}$ − L*$_{standard}$). Negative Delta L* means: sample is darker than the standard (untreated standard cotton); negative Delta b* means: sample is darker blue compared to the standard http://www.hunterlab.com/appnotes/an07_96a.pdf

Polymer used for coating (condition)	L*	b*	Delta L*	Delta b*
Standard Cotton	89.39	0.53		
PTHFAz$^{pz}_3$ (0.5% owf, pH 5.5, 50 °C)	60.92	-27.33	-28.46	-27.86
PTHFAz$^{pz}_3$ (0.5% owf, pH 5.5, 150°C)	62.30	-25.19	-27.09	-25.72

PTHFAz^{pz}$_3$ (0.35%, pH 5.5, 50°C)	66.84	-21.66	-22.55	-22.19
PTHFAz^{pz}$_3$ (0.35%, pH 5.5, 150°C)	63.82	-23.15	-25.57	-23.68
PTHFAz^{pz}$_3$ (0.5% owf, pH 8.5, 50 °C)	61.04	-28.01	-28.34	-28.54
PTHFAz^{pz}$_3$ (0.5% owf, pH 8.5, 150°C)	68.15	-21.53	-21.24	-22.06
PTHFAz^{pz}$_3$ (0.35%, pH 8.5, 50°C)	62.51	-25.88	-26.88	-26.41
PTHFAz^{pz}$_3$ (0.35%, pH 8.5, 150°C)	62.85	-25.87	-26.53	-26.40
Standard PET	89.16	0.82		
PTHFAz^{pz}$_3$ (0.5% owf, pH 8.9, 50 °C)	64.88	-22.05	-24.27	-22.86
PTHFAz^{pz}$_3$ (0.5% owf, pH 8.9, 150°C)	66.32	-20.27	-22.84	-21.09
PTHFAz^{pz}$_3$ (1.0%, pH 8.9, 50°C)	70.70	-16.83	-18.46	-17.65
PTHFAz^{pz}$_3$ (1.0%, pH 8.9, 150°C)	54.87	-27.04	-34.29	-27.86

Figure SI 1: Lanasol dye test of polymer coated cotton fabrics: before and after washing (polymers in 0.5 % owf) .
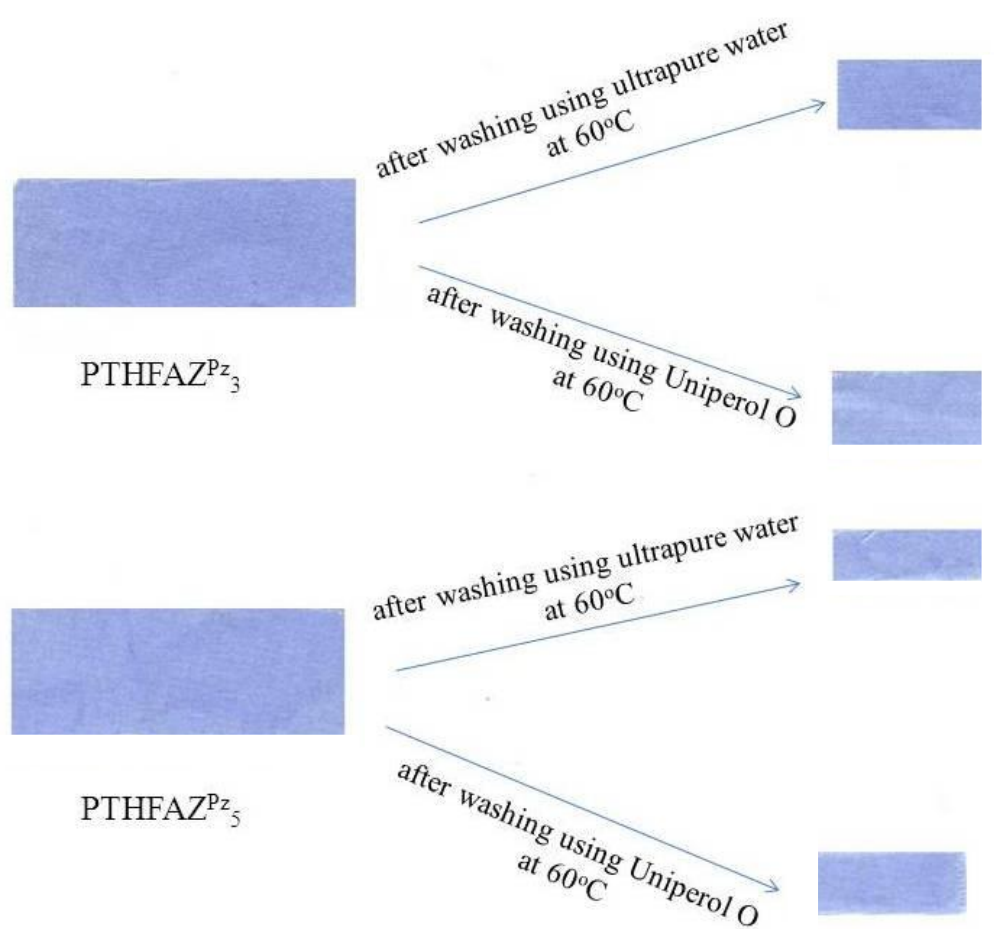
Table SI 2: Antimicrobial efficacy of cotton and polyester fabrics finished with PTHFAZ$^{Pz}_3$ at pH 8.5 (*E. coli* 2.6 x 10^6 and *S. aureus* 2.4 x 10^6 cfu/mL; a) due to exposure on the polymer coated surface and b) due to polymer leached into the exposure solution it is both: as said above in "Method for the preparation of polymer coated textiles" (thermal oven at 100 °C, temperature was enhanced to 150 °C over 15 min, at 150 °C additional curing for 2 min; alternatively drying was performed at 50 °C).

a)	Drying temperature after padding	Bacterial growth inhibition (%) of polymer coated cotton containing different amounts of polymer on the surface		Bacterial growth inhibition (%) of polymer coated PET containing different amounts of polymer on the surface	
		0.35 % owf	**0.50 % owf**	**0.50% owf**	**1.0 % owf**
E. coli					
	50 °C	99.99	100	99	99.999
	100 / 150 °C	99.9	100	99.9999	99.9999 – 99.99999
S. aureus					
	50 °C	0	90 - 99	0	99.9
	100 / 150 °C	0	90	0	99.999

b)	Drying temperature after padding	Bacterial growth inhibition (%) of polymer coated cotton containing different amounts of polymer on the surface by polymers LEACHING to the exposure solution		Bacterial growth inhibition (%) of polymer coated PET containing different amounts of polymer on the surface by polymers LEACHING to the exposure solution	
		0.35 % owf	**0.50 % owf**	**0.50% owf**	**1.0 % owf**
E. coli					
	50 °C	<50	~ 80	0	0
	100 / 150 °C	0	~ 50	0	0
S. aureus					
	50 °C	0	<50	0	0
	100 / 150 °C	0	<50	0	0

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Chapter 6

Preparation of waterborne functional polymers using a

bifunctional coupler[A]

Introduction: Preparation of functional polymers is of great interest in today's research, due to the wide range of applications of these polymers[1, 2]. Functional polymers can be prepared via several approaches (Figure 1): (a) controlled radical polymerization of functional vinyl monomers[3], (b) selective condensation reaction of trifunctional monomers, (c) condensation reaction of AA' type bicyclic monomers, (d) post polymerization modification of polymers with reactive side groups[4, 5].

Different methods to prepare functional polymers
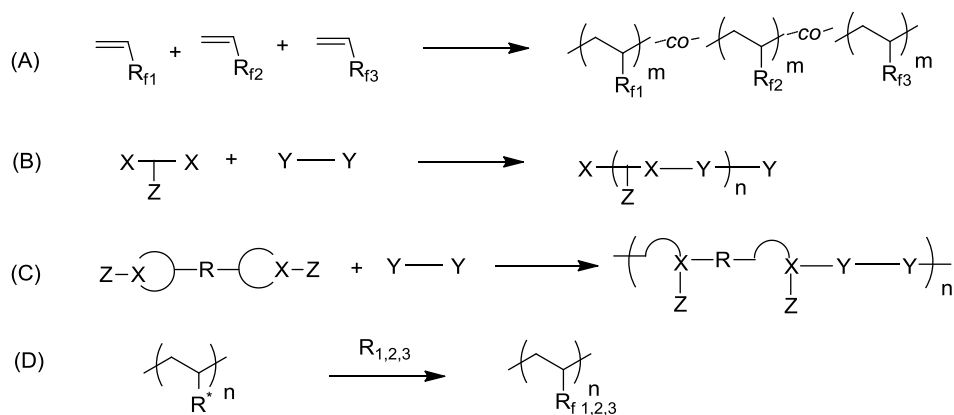
Figure 1: Preparation of functional polymers: (A) controlled radical polymerization of functional vinyl monomers, (B) selective condensation reaction of trifunctional monomers, (C) condensation reaction of AA' type bicyclic monomers, (D) post polymerization modification of polymers with suitable side groups

Direct polymerization of the functional monomers is an attractive way to prepare functional polymers. With the developments of different controlled radical polymerization techniques

the functional group tolerance during polymerization has improved. In current days, development of selective condensation reaction allows to prepare functional polymers from different multifunctional monomers by polycondensation reaction. However, in spite of these developments, there is still a broad range of functional groups which is not possible to introduce in the polymer chain via direct polymerization. Post polymerization modification can overcome the limitation of functional group tolerance and hence is a very attractive approach for the synthesis of functional polymers. The success of this method depends on the efficient conversion of reactive groups under mild condition and high functional group tolerance. This approach is useful for the determination of structure property relationships, as a library of functional polymers having identical molecular weight and molecular weight distribution can be prepared by this approach starting from a single polymer precursor. This strategy is also attractive for the preparation of combinatorial materials. In literature, most reported reactions used for post-polymerization modification are Diels-Alder reaction[6], click chemistry[7], Michael-type addition reaction etc. Most of these reactions were performed in non-aqueous medium and involve complex and time consuming purification processes. Nowadays post polymerization modification using suitable couplers is gaining a lot of attention due to the simplicity and selectivity of this approach[8].

In spite of the large development in polymer synthesis over the past decades, one of the most important challenges in today's research is to find out new method's to prepare functional polymers with precise functionalities which make use of simple, robust and bio-friendly 'green' reaction conditions[9, 10]. Here, we report simple and efficient reactions in water to prepare different types of functional polymers. First, the synthesis of a bifunctional coupler and of functionalized couplers is presented. Then the reaction of these couplers with reactive polymers leading to multifunctional polymers is described. The main advantages of the current approach for the preparation of functional polymers are: (a) use of water as the only

solvent, (b) one – pot syntheses, (c) high conversion of these reactions (more than 95 %), (d) no need of any catalyst, and (e) no need for purification.

In this work, we describe the polycondensation of the bifunctional coupler with diamines resulting in functional polymers with tertiary and secondary amine groups in the main chain and hydroxyl groups in the side chains; and post polymerization modification of polyamines by the bifunctional coupler and functionalized couplers.

Experimental part: Experimental details are described in Supplementary Information.

Results and discussions:

Starting point for the preparation of multifunctional polymers is the bifunctional coupler **3** synthesized by the reaction of piperazine and epichlorohydrin in water (Scheme 1). It should be noted that reaction of the two secondary amine groups in piperazine with epichlorohydrin results in two different functional groups within the same molecule, (i) a highly reactive azetidinium group and (ii) an amino chlorohydrin group. These two different functional groups can be addressed selectively by nucleophiles such as primary and secondary amines for example[11]. Reaction of the bifunctional coupler **3** with decylamine in a molar ratio of 1:1 in water at 90°C yielded the functionalized coupler **4** in high yield (> 95%). The pure functional coupler **4** was obtained simply via removing the water from the reaction mixture. It should be mentioned that during this reaction the highly reactive azetidinium ring is converted, with formation of a tertiary, a secondary amino group in the main chain and a hydroxyl side group. In the same time the amino chlorohydrin group is converted to a highly reactive azetidinium group. (The changes in the ^{1}H NMR spectra are shown in the SI.) In the next step to establish the reaction of the functionalized coupler **4** with amines, **4** was reacted with hexylamine in water at 90°C. This reaction was analyzed via ^{1}HNMR spectroscopy, too (Figure SI 1); the disappearance of the characteristic peaks of the protons associated with the

azetidinium ring of **4** at δ = 4.1-4.7 (H[1], H[2]) in the ^{1}HNMR spectrum of the product confirms the formation of the amine-coupler adduct **5**.
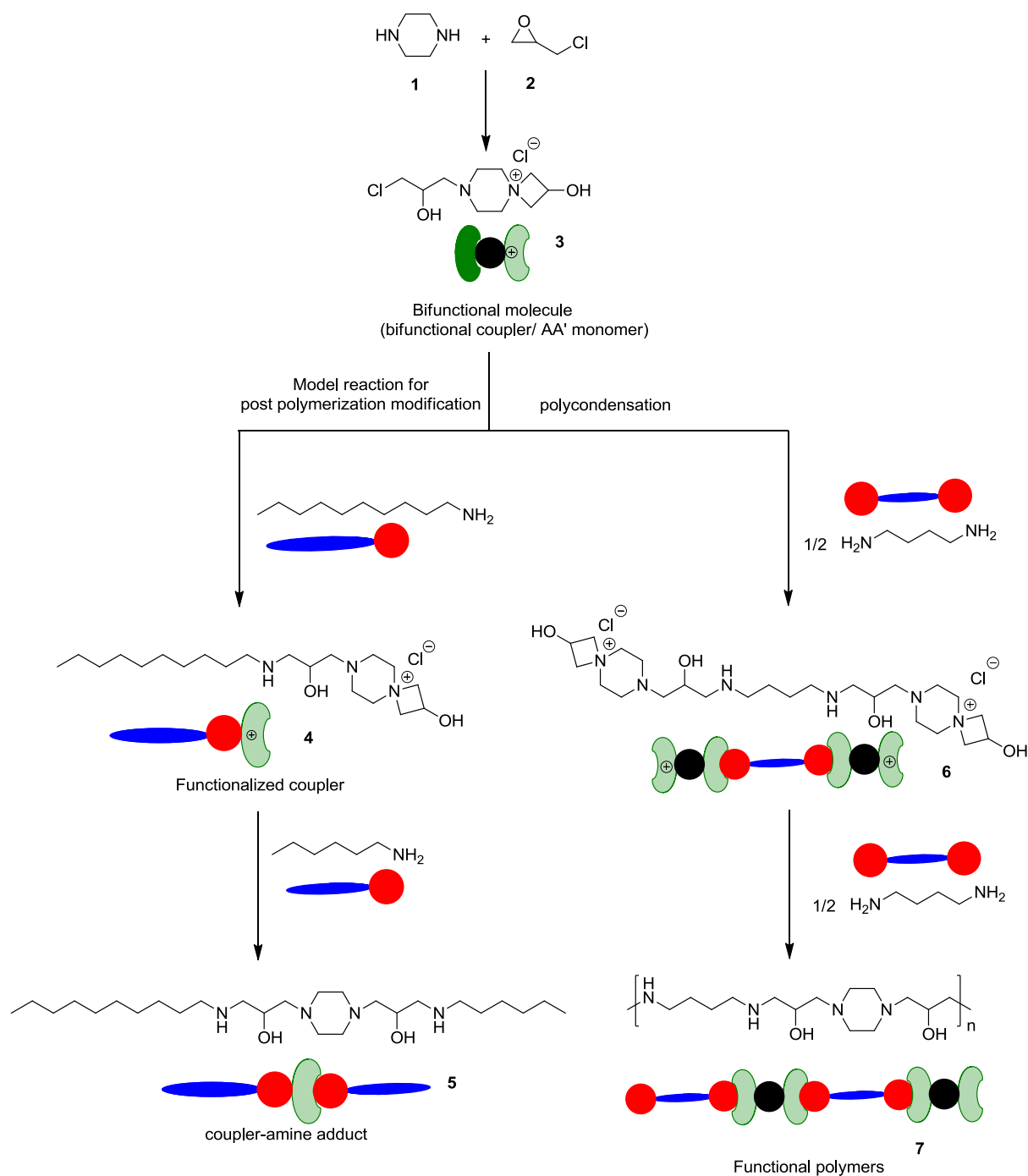

Scheme 1: Model reaction of the coupler with amines to proof the coupling concept for the preparation of functional polymers.

Based on these results the bifunctional coupler 3 was used as a AA' monomer in the reaction with 1,4 diaminobutane as BB monomer (Scheme 1). Starting with a 1:1 molar ratio of the monomers resulted in an alternating copolymer 7. The linear polymer **7**, containing secondary and tertiary amine groups in the backbone and secondary hydroxyl groups in the side chain was characterized by ^{1}H NMR. The appearance of protons at δ = 2.3-3.2 ppm (CH_2 protons attached with secondary and tertiary amine groups), δ = 4.0 ppm (protons on the C – atom with secondary hydroxyl groups attached) and δ = 1.4-1.8 ppm (central CH_2 groups of the 1,4-diaminobutane building block) (Figure 2D) prove the formation of the multifunctional polymer. The prepared polymer **7** had a molecular weight 1600 with a molecular weight distribution 1.1 as analyzed via GPC. This type of linear polymers containing secondary and tertiary amine groups in the backbone are difficult to prepare by known procedures due to the problem of crosslinking. To prove the alternating structure of the polymer the intermediate building block **6** was prepared (yield > 95 %) by reacting the bifunctional coupler **3** with 1,4-diaminobutane in a 2:1 molar ratio. The bis-azetidinium product **6** was characterized by ^{1}H NMR spectroscopy (Figure SI 1); characteristic peaks of the protons associated with azetidinium groups were found at δ = 4.7-4.1 ppm (H^1 H^2 and $H^{2'}$), those for the 1,4-diaminobutane unite at δ = 2.6 ppm (H^8) and at δ = 1.6 ppm (H^9).

Post polymerization modification of poly(vinyl amine) (M_n = 3,60,000 and PDI = 2.07) was achieved using the bifunctional coupler **3** and the functionalized coupler **4**. Reacting the bifunctional coupler **3** with poly(vinyl amine) in a ratio of (amine repeating unites) / (bifunctional coupler) = 5/1 at 90^oC and a substrate concentration of 1wt% (Scheme 2) resulted in an azetidinium functionalized polymer **8**. The selectivity of the reaction was high; the value of the degree of polymerization set by the ratio of reactants in the feed and the degree of functionalization determined by ^{1}H NMR spectroscopy was the same ca. 20%. The advantage of this reaction is that at well defined reaction conditions 5 to 30 % of amine

groups can selectively be converted to azetidinium functional groups; thus two functional groups with the prerequisite to react with each other can coexist in one molecule. By increasing the reaction time or increasing the substrate concentration a cross linked material was obtained.
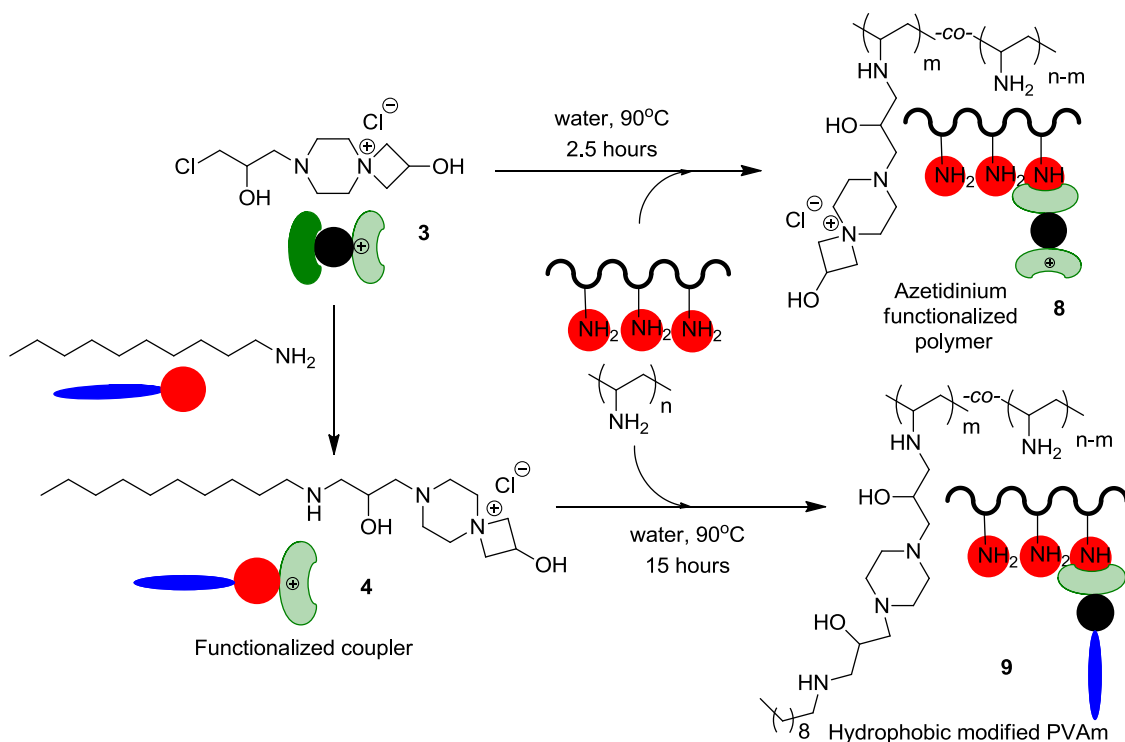

Scheme 2: Post polymerization modification of poly(vinyl amine) using the bifunctional coupler and a functionalized coupler.

An explanation for this experimental result supported by NMR analyses could be the elecrostatic repulsion of the macromolecules with azetidinium groups along the polymer back bone in addition to an unfavaroble molecular conformation which do not permit or retard a reaction between amine and azetidinium groups.

In a 2nd approach a functional building block (decylamine) is attached to poly(vinyl amine) using the bifunctional coupler **3**. In a first step by the reaction of the bifunctional coupler with decylamine the functionalized coupler **4** is prepared. In a second step intermediate **4** was

reacted with poly(vinyl amine) to yield the hydrophobically modified poly(vinyl amine) **9** after a prolonged reaction time (15h at 90°C).

All these functional polymers were characterized via ^{1}H NMR spectroscopy (Figure 2). The azetidinium functionalized polymer **8** was characterized mainly by the presence of signals characteristic for the azetidinium ring - protons (H^1, H^2) at δ = 4.2-4.8 ppm (Figure 2B). The functional polymer **9**, containing the long alkyl chains (C-10) was characterized via the appearance of the characteristic peaks of the alkyl group at δ = 0.89 ppm, 1.31 ppm and 1.65 ppm (Figure 2C).
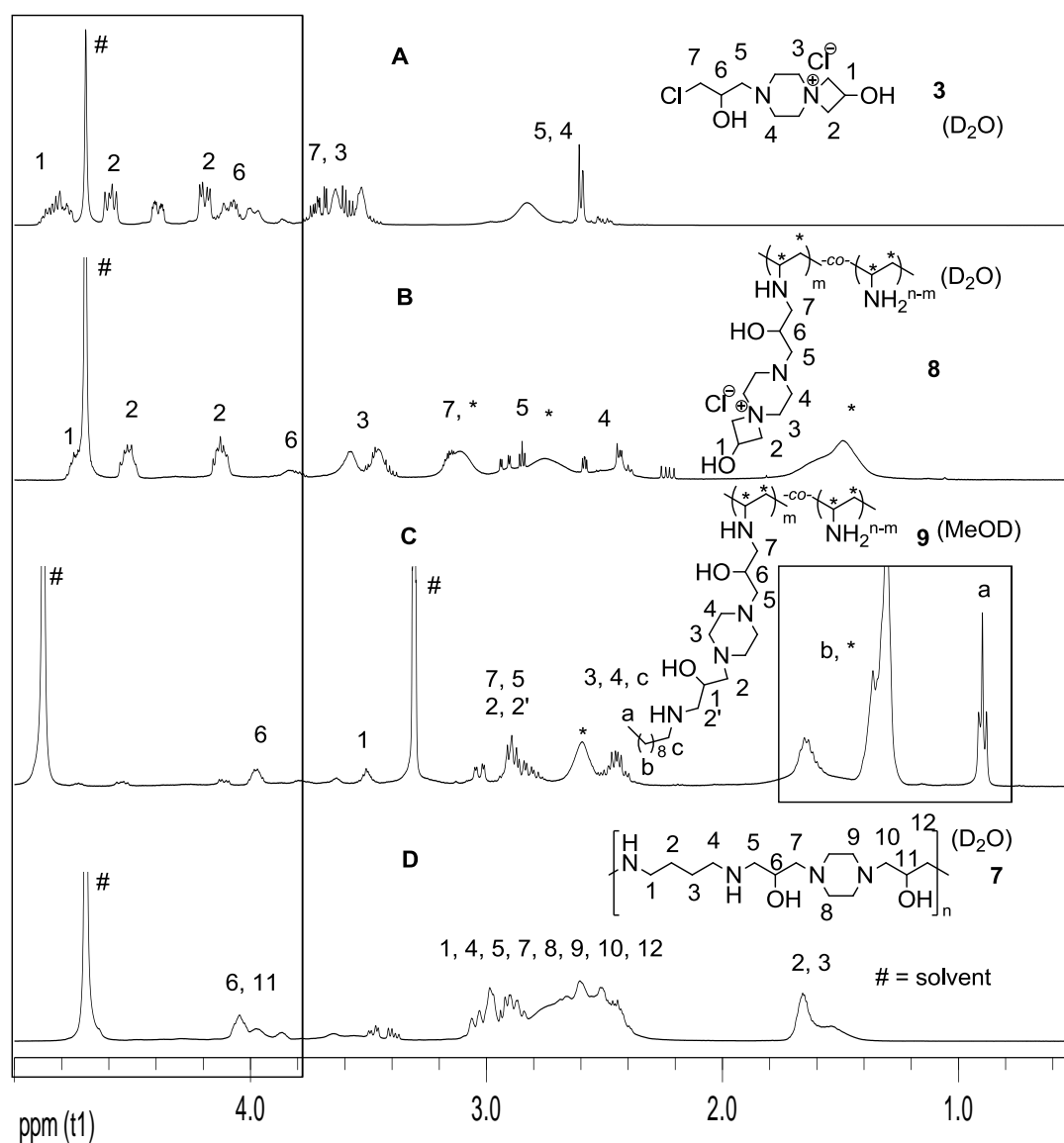
Figure 2: ^{1}H NMR of different functional polymers prepared from bifunctional molecule

Conclusion: In this work, functional polymers were prepared in aqueous solution starting with a bifunctional piperazine based coupler **3** using two different approaches. In the 1st approach, an alternating copolymer **7** was prepared by reaction of the bifunctional coupler (AA' monomer) **3** with 1, 4-diaminobutane. In the 2nd approach, cationic and hydrophobic functional polymers (**8, 9**) were prepared from poly(vinyl amine) via post polymerization modification using suitable azetidinium functionalized couplers.

References:

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Supplementary information for chapter 6

Experimental section:

Materials: Hexylamine (99%, Aldrich), decylamine (99%, Aldrich), epichlorohydrin (99%, Merck), piperazine (99+%, Aldrich), 1, 4-diaminobutane (99%, Aldrich), triethylamine (99%, VWR) were used as received. Salt free poly(vinyl amine) was obtained from BASF as aqueous solution. This aqueous solution was freeze dried and the solid poly(vinyl amine) was used as starting material. Distilled water was used as solvent for all the reactions.

Measurements:

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterium oxide (D_2O), deuterated methanol (CD_3OD) and deuterated dimethyl sulfoxide (DMSO-d_6) were used as solvents. Tetramethylsilane (TMS) was used as an internal standard. All Raman spectra were recorded on a Buker RFS100/s Raman spectrometer, fitted with a Nd: YAG laser (1064nm). The spectral resolution was 4 cm^{-1}. For one spectrum 1000 scans were collected at a laser power of 200mW. Size exclusion chromatography analysis (SEC) were carried out with water (with addition of 0.1 M NaCl, 0.1% TFA, 0.01% NaN$_3$) as eluting solvent at 30°C with a flow rate of 1 mL/ min using a high pressure liquid chromatography pump (Agilent 1100) and refractive index detector (Wyatt, Optilab DSP). Three columns with PSS Novema gel were applied. The length of each column was 300 mm, the diameter was 8 mm, the diameter of the gel particles were 10 μm and the nominal pore widths were 30, 3000 and 3000 Å. Calibration was achieved using Pullulan standards.

Synthesis:

Preparation of the bifunctional coupler 3:
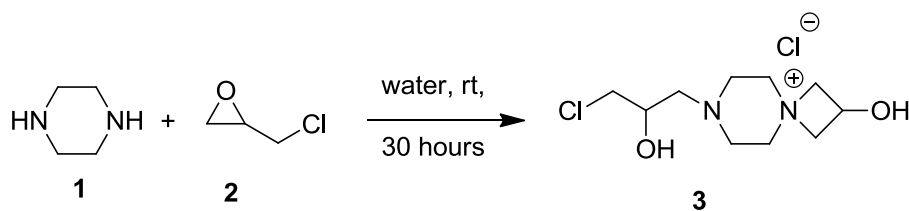
The bi-functional coupler (**3**) was synthesized as described in chapter 4.

Model reaction of the bifunctional coupler with decyl amine (preparation of the functionalized coupler 4):
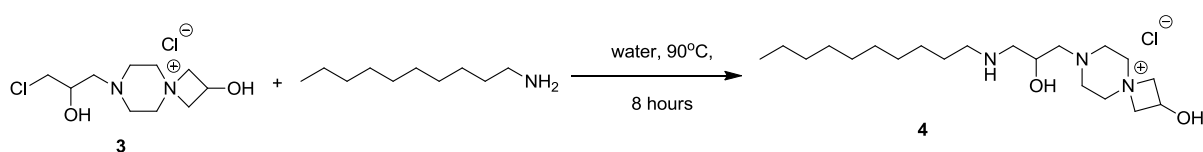
To a solution of coupler **3** (1.36 g, 5.00 mmol) in distilled water (5 mL), decyl amine (0.787 g, 5.00 mmol) was added. The solution was stirred for 8 hours at 90°C and then cooled down to room temperature. Removal of water yielded the pure functionalized coupler **4**.
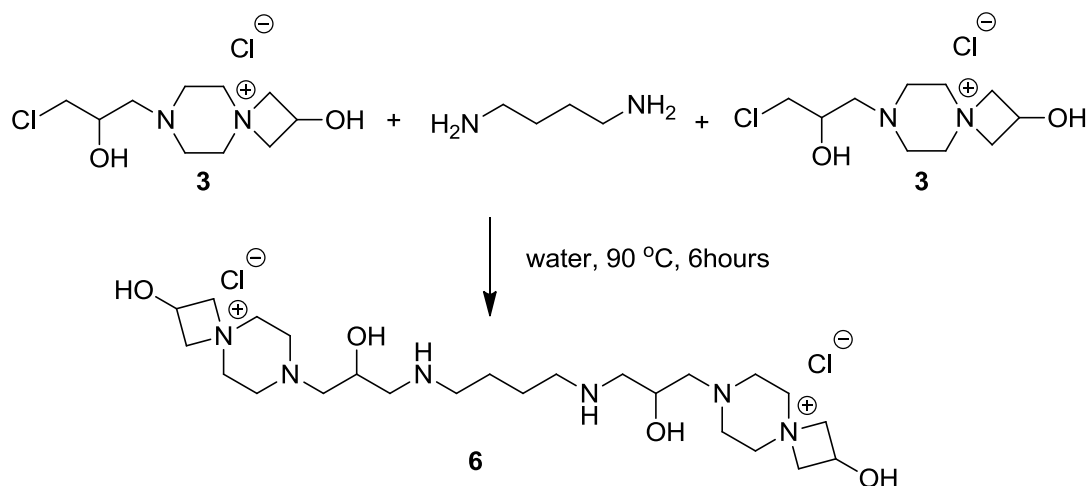
Yield: > 95%
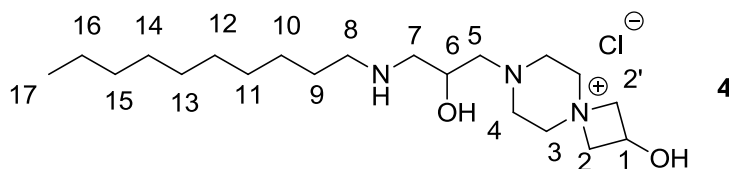
^{1}H NMR (DMSO-d^6, 400MHz): δ = 4.63 (m, H^1), 4.48 & 4.12 (m, H^2, H$^{2'}$), 3.7-3.2 (m, H^3, H^6), 2.9-2.1 (m, H^4, H^5, H^7, H^8), 1.7-1.1 (m, H^9, H^{10}, H^{11}, H^{12}, H^{13}, H^{14}, H^{15}, H^{16}), 0.84 (m, H^{17}) ppm. **^{13}C NMR** (DMSO-d^6, 100MHz): δ = 69.16 (C^2, C$^{2'}$), 64.26 (C^6), 60.68 & 59.89 (C^3, C$^{3'}$), 58.91 (C^5), 57.74 (C^1), 53.39 (C^7), 47.94-47.59 (C^4, C$^{4'}$), 38.67 (C^8), 31.25 (C^9), 28.87 (C^{10}), 28.81 (C^{11}), 28.65(C^{12}), 28.53 (C^{13}), 26.89 (C^{14}), 25.86 (C^{15}), 22.05(C^{16}), 13.91 (C^{17}) ppm.

Elemental analysis ($C_{20}H_{42}ClN_3O_2$, HCl, ½ H_2O): calculated C: 54.91%, H: 10.14%, N: 9.60%, found: C: 54.96%, H: 10.12%, N: 9.14%

Model reaction of the functionalized coupler with primary amines (preparation of the coupling product 5):
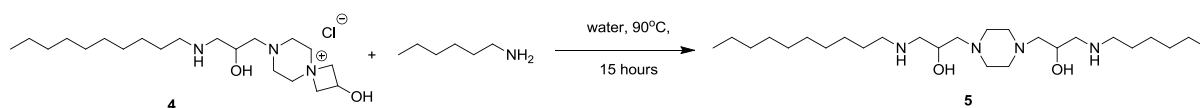
To a solution of functionalized coupler **4** (1.96 g, 5.00 mmol) in distilled water (5 mL), hexyl amine (0.506 g, 5.00 mmol) was added. The solution was stirred for 15 hours at 90°C and then cooled down to room temperature. Removal of water yielded the coupling product **5**.

Yield: > 90%
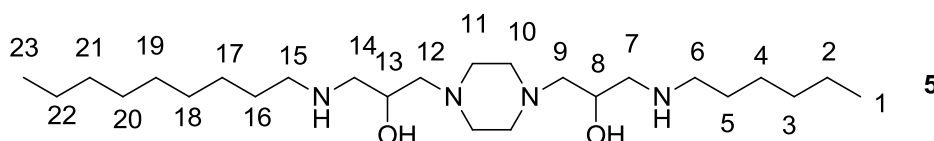
^{1}H NMR (DMSO-d^6, 400MHz): δ = 3.9 (m, H^8, H^{13}), 3-2.4 (m, H^6, H^7, H^9, H^{10}, H^{11}, H^{12}, H^{14}, H^{15}), 1.8-1.2 (m, H^2, H^3, H^4, H^5, H^{16}, H^{17}, H^{18}, H^{19}, H^{20}, H^{21}), 0.856 (m, H^1, H^{23}) ppm.

^{13}C NMR (DMSO-d^6, 100MHz): δ = 63.61 & 63.38 (C^8, C^{13}), 53.26 (C^9, C^{12}), 51.49 (C^7, C^{14}), 47.12 (C^{10}, C^{11}), 31.26 (C^{16}), 30.7 (C^5), 28.5-28.8 (C^4, C^{17}), 26.86 (C^{18}), 26.02 (C^{19}), 25.7 (C^{20}), 25.49 (C^{21}), 25.49 (C^3), 22.069 (C^{22}), 21.86 (C^2), 13.93 (C^{23}), 13.82 (C^1) ppm.

Reaction of the bifunctional coupler with 1, 4-diaminobutane 2:1 moler ratio (Selective conversion of one functional group of the coupler, preparation of compound 6):

To a solution of coupler **3** (6.15 g, 22.68 mmol) in distilled water (24 mL), 1, 4-diaminobutane (1.0 g, 11.3 mmol) was added. The solution was stirred for 6 hours at 90°C and then cooled down to room temperature. Removal of water yielded the pure product **6**.

Yield: > 95%
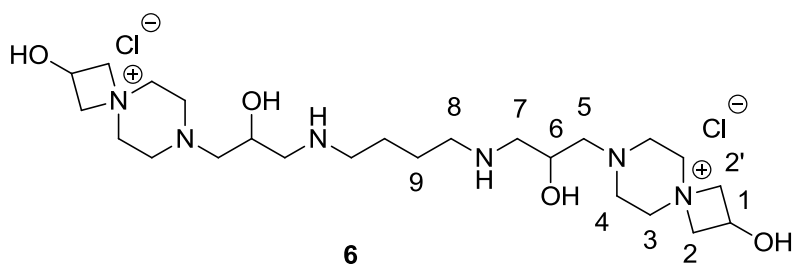
¹H NMR (DMSO-d^6, 400MHz): δ = 6.64 & 5.62 (OH), 4.66 (m, H^1), 4.53 & 4.17 (m, H^2, H$^{2'}$), 3.7-3.4 (m, H^3, H$^{3'}$, H^6), 2.8-2.4 (m, H^4, H$^{4'}$, H^5, H^7), 2.44 (m, H^8), 1.7 (m, H^9) ppm. **¹³C NMR** (DMSO-d^6, 100MHz): δ = 70.36 (C^2, C$^{2'}$), 63.7 (C^6), 60.59 (C^3), 58.86 (C^5), 57.72 (C^1), 50.97 (C^7), 47.85-47.35 (C^4, C$^{4'}$), 37.92 (C^8), 23.84 (C^9) ppm.

Elemental analysis (C$_{24}$H$_{50}$Cl$_2$N$_6$O$_4$, 2HCl, 2½ H$_2$O): calculated C: 42.67%, H: 8.5%, N: 12.44%, found: C: 42.99%, H: 8.9%, N: 12.15%

Use of the bifunctional coupler as a monomer to prepare multifunctional polymers via condensation polymerization:

Preparation of copolymers containing secondary hydroxyl groups, secondary and tertiary amine groups in the backbone 7:
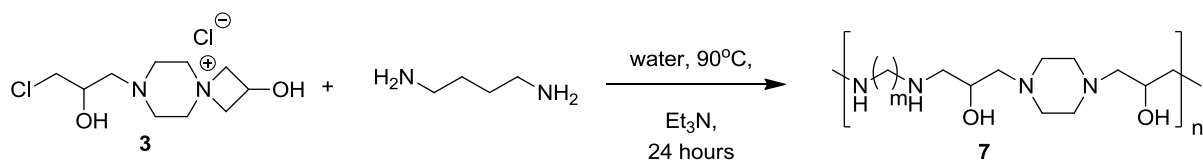

To a solution of bifunctional coupler **3** (1.49 g, 0.0055 mol) in distilled water (10 mL), 1,4-diaminobutane (0.487 g, 0.0055 mol) and triethylamine (0.55 g, 0.0055 mol) was added. The solution was allowed to stir for 24 hours at reflux and then cooled down to room temperature. The polymer **7** was purified by dialysis.
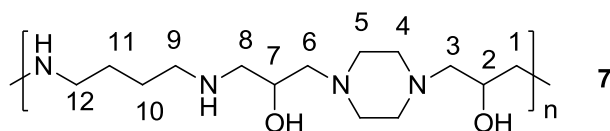
^{1}**H NMR** (D$_2$O, 400MHz): δ = 4.05 (H^2, H^7), 3.6-3.3 (H$^{chain\ end}$), 3.1-2.2 (H^1, H^3, H^4, H^5, H^6, H^8, H^9, H^{12}), 1.8-1.4 (m, H^{10}, H^{11}) ppm.

Elemental analysis: Calculated considering one repeating unit as following
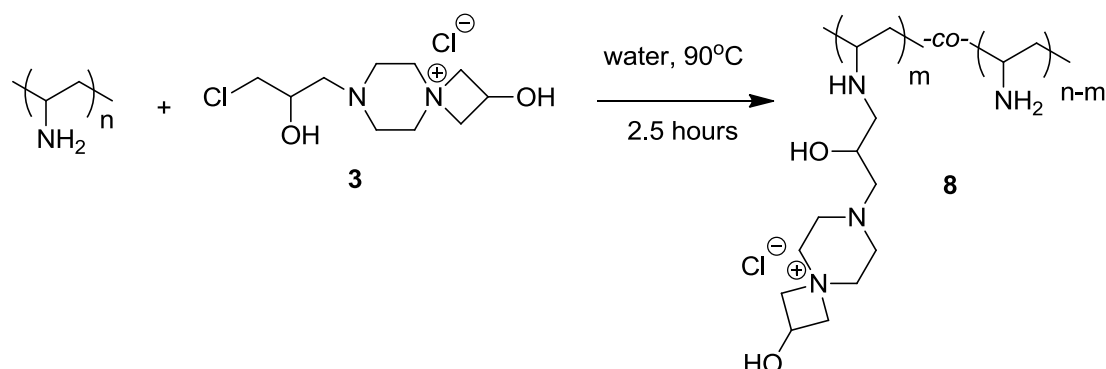

, 2 HCl, 3 H$_2$O
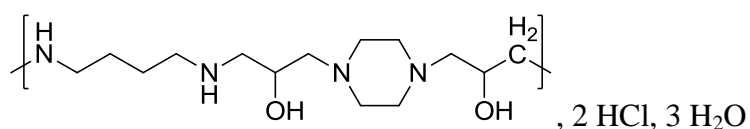
Calculated: C: 40.48%, H: 9.27%, N: 13.55%, found: C: 40.97%, H: 9.92%, N: 12.96%

GPC analysis:

M$_n$ = 1400, M$_w$ = 1600, PDI = 1.1

Preparation of functional polymers:

Preparation of azetidinium functionalized poly(vinyl amine) 8 (procedure for 20% functionalization of the amine groups using the bifunctional coupler 3):

To a solution of poly(vinyl amine) (0.09 g, 0.00209 mol repeating unit) in distilled water (10 mL), the bifunctional coupler **3** (0.114 g, 0.00042 mol) was added. The solution was stirred for 2.5 hours at 90°C and then cooled down to room temperature.
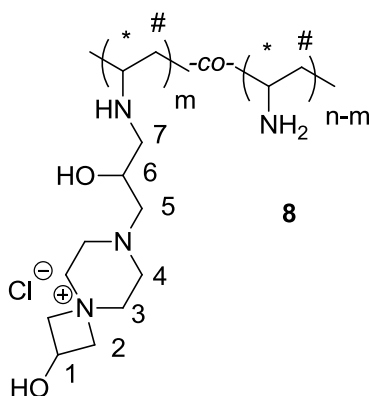

^{1}**H NMR** (D$_2$O, 400MHz): δ = 4.74 (m, H^1), 4.52 & 4.13 (m, H^2, H$^{2'}$), 3.84 (m, H^6), 3.6-3.4 (m, H^3), 3.2-3.0 (H^7, H*), 2.8-2.6 (m, H^4, H5, H^7), 1.7-1.3 (H$^\#$) ppm.

Calculation for the degree of functionalization by ^{1}H NMR spectroscopy:

The degree of functionalization was calculated from the integration related to the protons H^2 (δ = 4.52 ppm) and the protons attached to the polymer backbone H$^\#$ (δ = 1.48 ppm)

From the current NMR spectrum, $I(H^2)/I(H^\#) = 4m/2n$

$2m/n = I(H^2)/I(H^\#) = 2.11/4.98$

$m/n = 1.055/4.98$

$m = 21.18\%$ of n, (The expected functionalization was 20%)

Preparation of alkyl functionalized poly(vinyl amine) 9, (procedure for 20% functionalization of the amine groups using the functionalized coupler 4):

To a solution of poly(vinyl amine) (0.1 g, 0.002 mol repeating unit) in distilled water (8 mL), the functionalized coupler **4** (0.157 g, 0.0004 mol) was added. The solution was stirred for 15 hours at 90°C and then cooled down to room temperature.
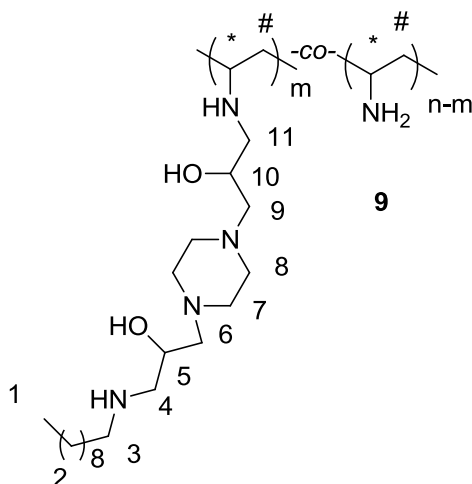

^{1}**H NMR** (CD$_3$OD, 400MHz): δ = 3.81 (m, H^5), 3.33 (m, H^{10}), 3.1 (m, H^6, H^7), 3-2.1 (m, H^3, H^4, H^6, H^9, H^{11}, H*), 1.6-1.0 (m, H^2, H$^\#$), 0.92 (m, H^1) ppm.

GPC analysis of the prepared PVAm polymers: For GPC analysis the above described polymers were prepared using HPLC grade water using the above mentioned procedure. The results are given below:

| Polymer	M_n	M_w	PDI
Poly(vinyl amine) (PVAm)	$3.60 * 10^5$	$7.45 * 10^5$	2.07
Azetidinium functionalized PVAm **8**	$3.63 * 10^5$	$1.03 * 10^6$	2.83

Figure SI 1: ^{1}H NMR analysis for model reactions (DMSO-d^6):
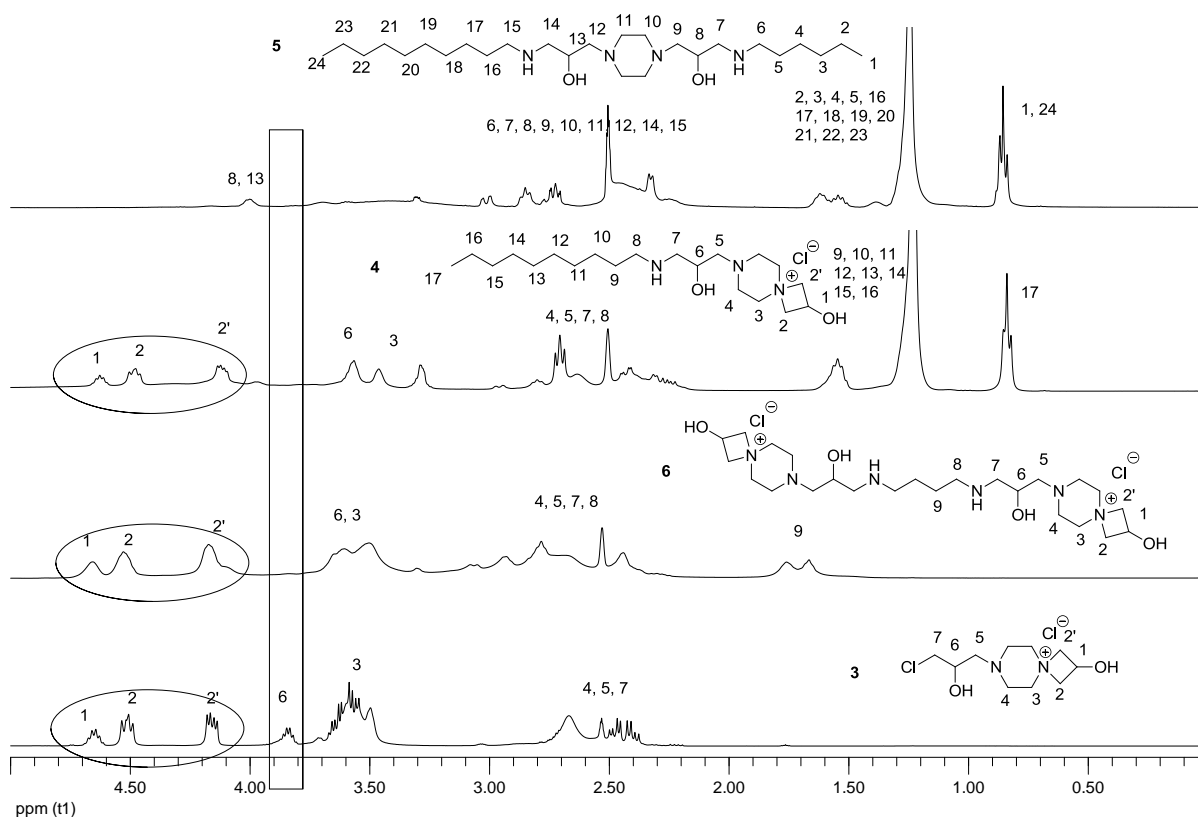
Proposed mechanism for the reaction of the bifunctional coupler with primary amines:
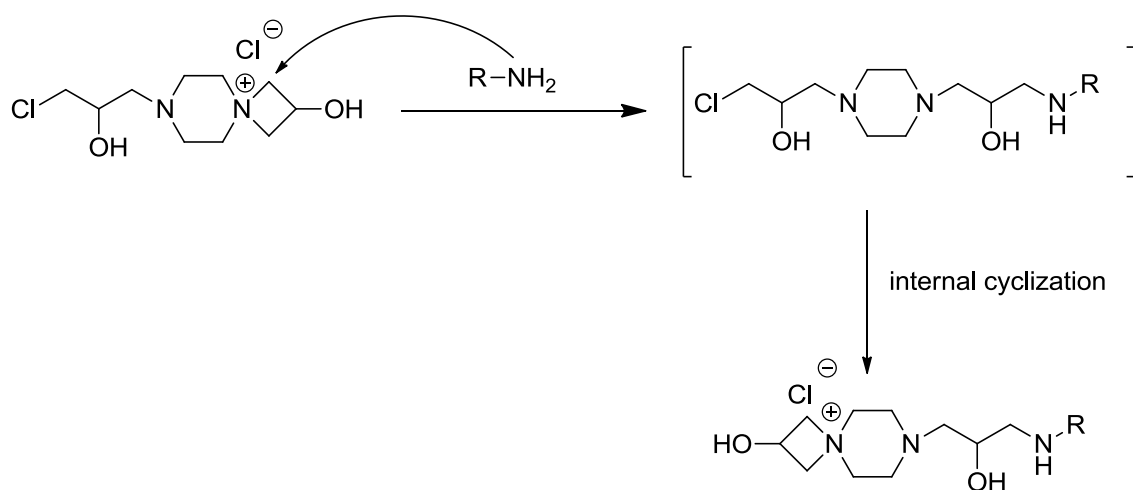
Proposed crosslinking network formed during the prolonged heating of azetidinium

functionalized PVAm 8 :

crosslinked polymer
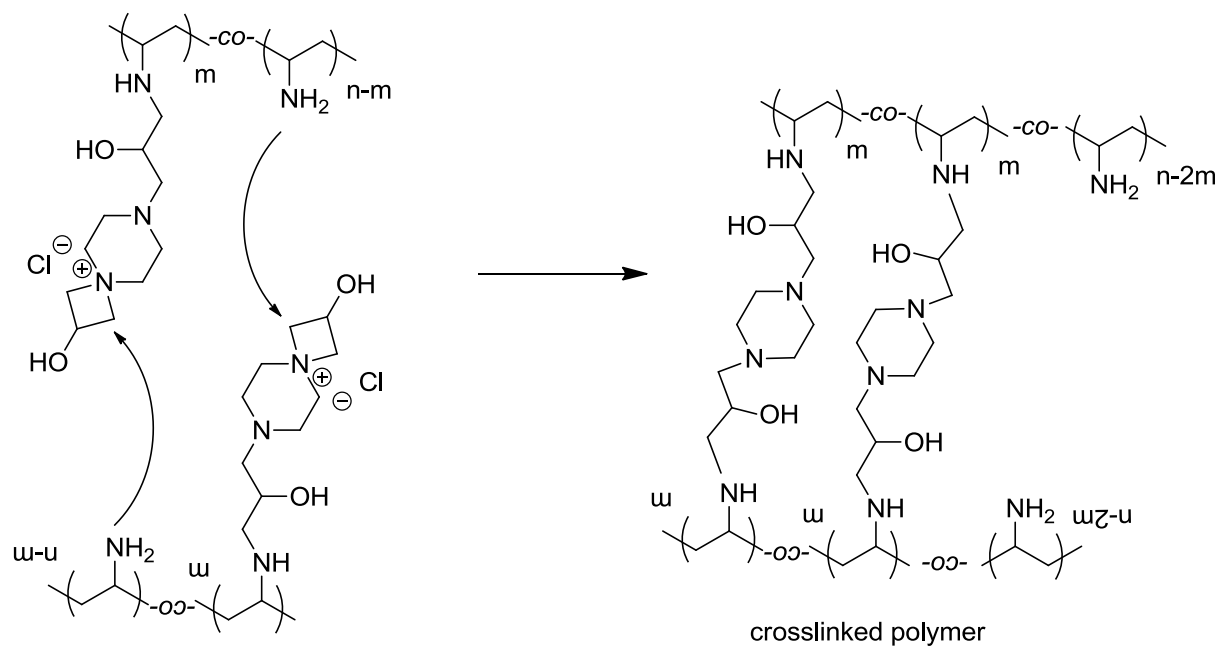
Chapter 7

Antimicrobial Poly(vinyl amine)s bearing azetidinium functional groups: one pot preparation in water and structure-activity relation.

Introduction: Amphiphilic synthetic polymers that mimic the structure of natural antimicrobial peptides (AMP) show high biocide activity towards different microorganisms like: virus, bacteria etc.[1]. The main structural features of most natural **AMP**s are a combination of hydrophobic and cationic groups within a macromolecule. The nature and the ratio of hydrophobic/hydrophilic groups are important for the activity of antimicrobial peptides and polymers. Most natural **AMP**s destroy the bacteria by a contact active mechanism - the positive charge in the hydrophilic part helps to bind the polymers to the anionic outer membranes of bacteria, the hydrophobic part allows the polymer to insert into the bacterial cell membrane and destroy them[2].

Preparation of new synthetic polymers which mimic the structure of natural **AMP**s are of interest in today's research due to various types of applications[3,4]. It is important to prepare these polymers in a simple, robust, and ecologically friendly approach. In the last decades, many reports on the synthesis of antimicrobial polymers were published[5,6,7]. Kuroda et al. reported the preparation of amphiphilic polymethacrylate derivatives and showed that their antimicrobial efficiency can be tailored by adjusting the content of hydrophobic groups and by the molecular weight[8]. Lienkamp et al. reported the preparation of a library of homo- and copolymers by a molecular kit approach and showed that the antimicrobial efficiency can be tuned by variation of the hydrophobic/hydrophilic balance[9]. Sambhy et al. reported the antimicrobial efficiency of different pyridinium polymers varying the hydrophobic/hydrophilic balance[10]. Westman et al. prepared poly(vinyl amine) analogues

having alkyl chains of different chain length (C-6 chain, C-8 chain, C-12 chain) and determined their antimicrobial efficiency. They found that **PVAm**s modified with a C-6 chains were most efficient against *E. coli*, while **PVAm**s modified with a C-8 chains were most active against *B. subtilis*[11]. Palermo et al. showed that the antimicrobial efficiency of polymers also depends on the type of cationic and hydrophobic groups; due to the different pKa values and different hydrophobicity these groups influence the binding to and disruption of the bacterial membrane[12].

In the last decades many antimicrobial polymers containing quaternary ammonium-, pyridinium-, and phosphonium groups were presented, however, polymers with azetidinium functionalized groups were reported only by Qian et al.[13] On one hand, these polymers are difficult to prepare, the azetidinium groups are highly reactive and can be stabilized only at special conditions[14,15]. Recently, we reported a very simple and efficient method to prepare azetidinium functionalized polymers via a coupler approach[16, 17]. The advantage of azetidinium functionalized polymers is, that beside ionic interactions covalent bonds with suitable substrates and cross-linking within the polymer films are possible, increasing the stability of the film. As a consequence polymers with azetidinium groups are good candidates for surface coatings[18, 19, 20]. In the current work, we report the preparation and antimicrobial studies of multifunctional **PVAm** containing azetidinium functional groups. We are extending the synthetic approach of the coupler concept by the preparation of water borne multifunctional **PVAm**s - containing hydrophobic and cationic azetidinium building blocks, starting from a salt free **PVAm** precursor (Scheme 1). The hydrophobic and hydrophilic cationic building blocks are introduced by the reaction of **PVAm** with suitable hydrophobically functionalized couplers and a bifunctional coupler, respectively in a simple two step one pot synthesis.
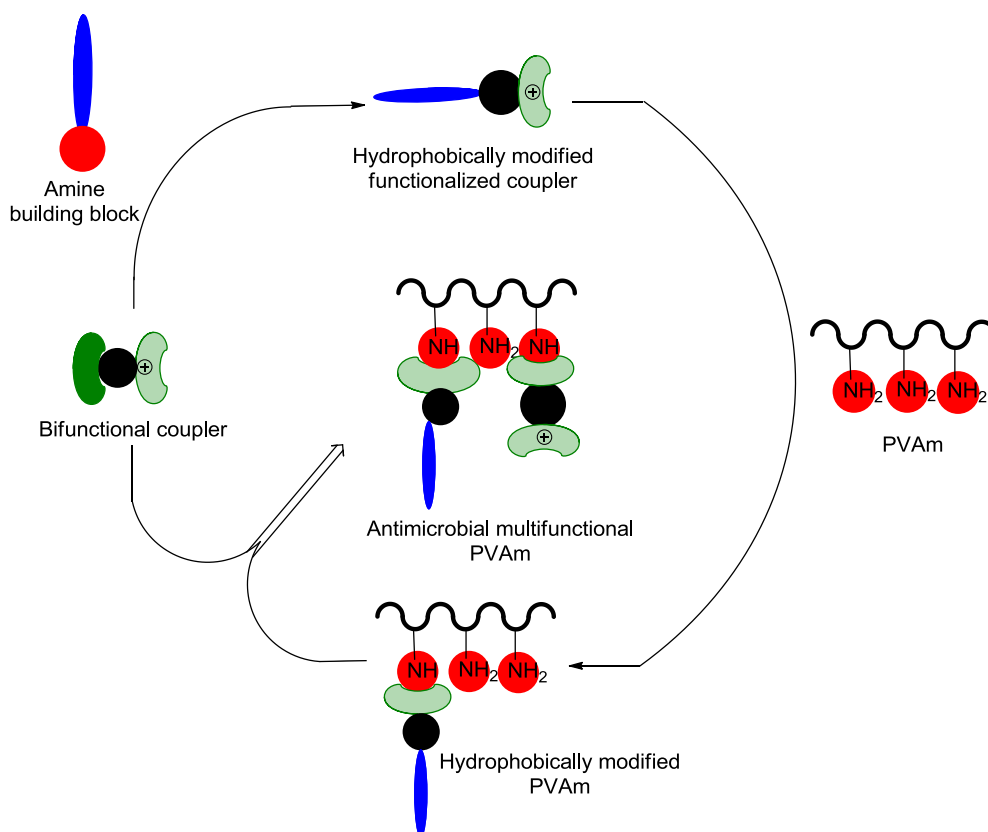
Figure 1: Concept for the preparation of antimicrobial multifunctional **PVAm**s using the coupler concept.

The structure – activity relationship of these polymers was determined to identify the best candidates – showing the highest antimicrobial efficiency (lowest minimum inhibitory concentration [MIC]). The hemolytic tests (HC_{50} value) of these polymers were performed to determine the antimicrobial selectivity (HC_{50}/MIC values) - the ability of these polymers to differentiate between microorganisms (Gram positive and Gram negative bacteria) and mammalian cells (red blood cells).

Nomenclature: For the functionalized couplers FCH^dC^{az} and FCH^hC^{az} – FC stands for functionalized coupler, H^d and H^h stands for hydrophobic modification (H) with a decyl (C-10, d) and a hexyl (C-6, h) chain respectively, C^{az} stands for cationic modification (C) with an azetidinium functional group (az). Similarly $PVAmH^d_xC^{az}_y$ and $PVAmH^h_xC^{az}_y$ stands for multifunctional poly(vinyl amine)s, having x% of vinyl amine repeating units modified with

FCH^{d}C^{az} (d) or with FCH^{h}C^{az} (h) coupler, respectively, and y% of vinyl amine repeating units modified with the bifunctional coupler (az stands for azetidinium groups).

**Experimental part:** The experimental details are given in the supplementary information.

Results and discussions: Starting point of the one-pot reaction approach for the preparation of multifunctional **PVAm**s was the synthesis of the bifunctional coupler by reaction of piperazine with epichlorohydrin in a 1:2 molar ratio in water as solvent (Scheme 1). The bifunctional coupler contains two isomeric functional groups – an amino-chlorohydrin and an azetidinium group – with different reactivity towards suitable nucleophiles like amines, phenols etc. The hydrophobically functionalized couplers were synthesized by reaction of the bifunctional coupler with decylamine (C- 10 amine) and hexylamine (C-6 amine) in a 1:1 molar ratio in water as solvent yielding the functionalized couplers (FCH^{h}C^{az}) and (FCH^{d}C^{az}) in high yield and high purity (yield > 95%)(Scheme 1). The structure of these hydrophobically modified couplers was confirmed by ^{1}H NMR spectroscopy (Figure SI 1) - from the ratio of the integration of the peaks assigned to the alkyl chains and azetidinium groups.
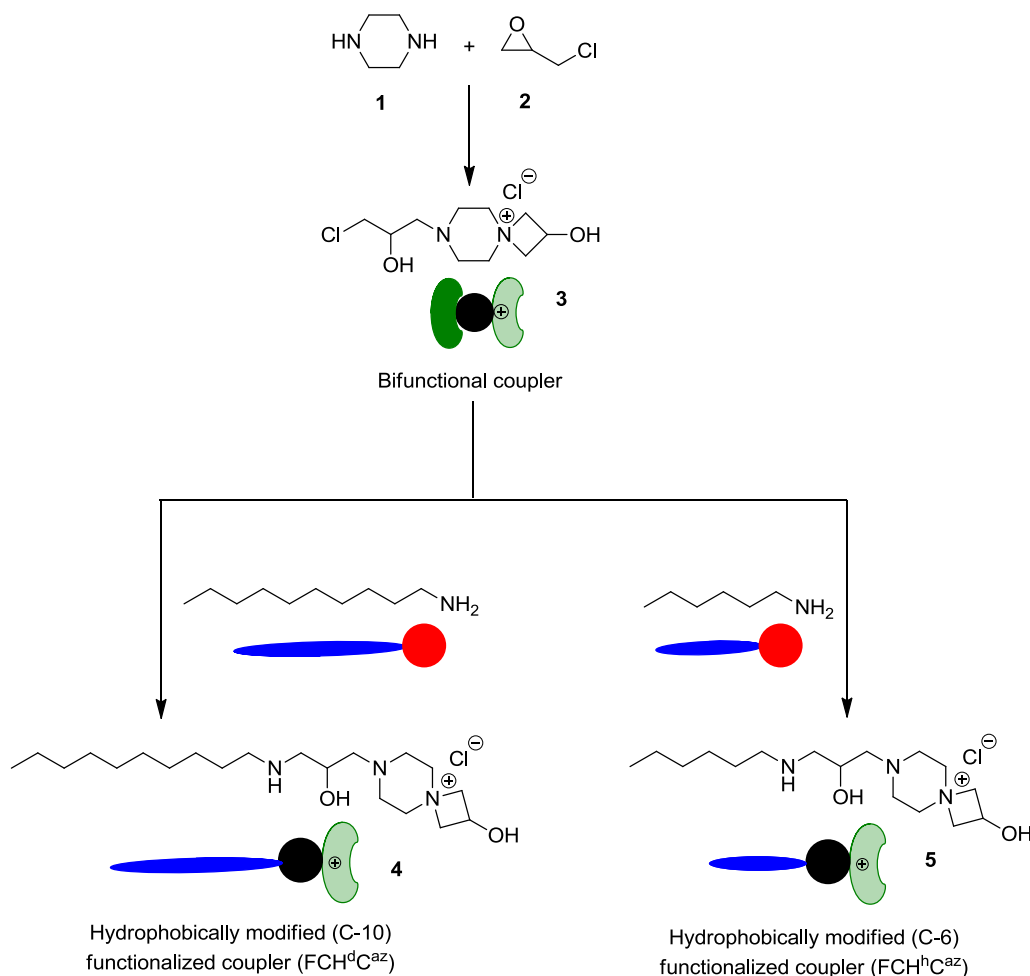
Scheme 1: Preperation of the bifunctional coupler and hydrophobically modified functional couplers.

Multifunctional PVAms (with x% hydrophobic and y% cationic groups, **PVAmH^h_xC$^{az}_y$** and **PVAmH^d_xC$^{az}_y$**) were prepared by the one-pot reaction approach starting from salt free **PVAm** (Scheme 2).

Salt free PVAm was reacted first with a certain amount of the hydrophobically modified functional coupler **4** or **5** to prepare the hydrophobically modified **PVAm**s **6**. Exemplarily the structure of the hydrophobically modified **PVAm 6** (**PVAmH$^d_{11}$**) was confirmed by ^{1}H NMR spectroscopy (Figure SI 2) – the characteristic peaks of the protons associated with alkyl groups appeared at δ = 0.87 ppm (H^a) and δ = 1.2 - 1.9 ppm (H^b) and the integration ratio of H^a: H^6: H^1 = 3: 0.9: 0.9 (Figure SI 2B). In the next step, the hydrophobically modified
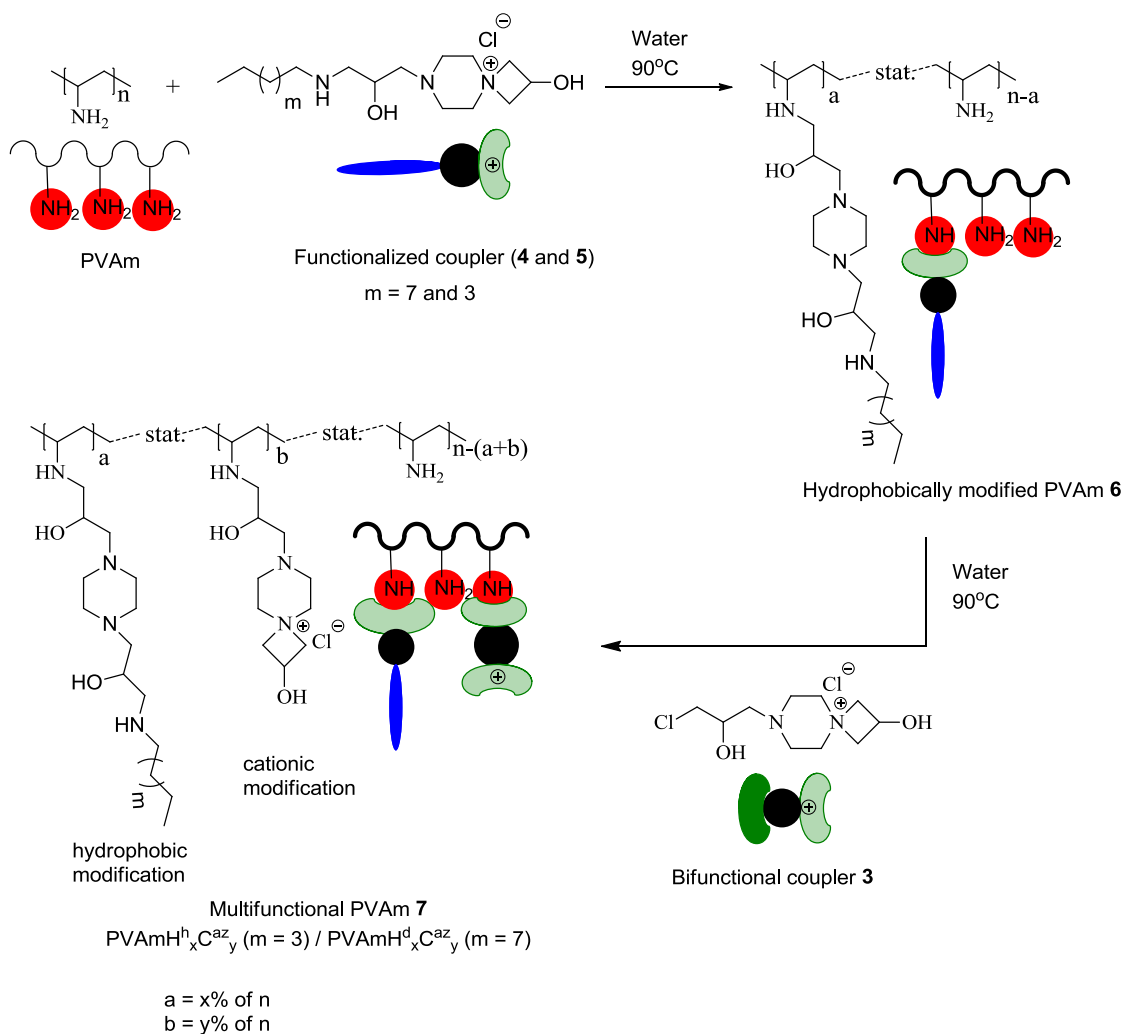
Scheme 2: One pot synthesis of multifunctional **PVAm**s by stepwise reaction of **PVAm** with the functionalized coupler (**4** or **5**) and the bifunctional coupler **3**.

PVAmH$^d_{11}$ 6 was reacted with the bifunctional coupler to prepare multifunctional **PVAm 7** - **PVAmH$^h_{11}$C$^{az}_{14}$**. The presence of azetidinium groups in the polymer side chain was confirmed by ^{1}H NMR spectroscopy – the characteristic peaks for the protons (H^8 and H^9) associated with the four membered azetidinium rings appeared at $\delta = 4.1 – 5.0$ ppm (Figure SI 2C). Using the above mentioned synthetic approach, a library of multifunctional **PVAms 7** – having different hydrophobic and different cationic modification was prepared and characterized by SEC (Table SI 1 and SI 2).

The antimicrobial activity of these multifunctional **PVAm**s – having different hydrophobic and cationic modification – were studied against Gram positive (*Staphylococcus aureus* [*S. aureus*]) and Gram negative bacteria (*Escherichia coli* [*E. coli*]) to determine the influence of different concentration of hydrophobic and cationic groups on the antimicrobial efficacy and find out the polymer with the highest antimicrobial efficacy.

To understand the effect of hydrophobic modification of **PVAm** on the antimicrobial efficacy three multifunctional **PVAm** samples having 14 % cationic repeating units and 5 %, 11 %, and 15 % hydrophobic repeating units (**PVAmH$^d_5$C$^{az}_{14}$**, **PVAmH$^d_{11}$C$^{az}_{14}$**, **PVAmH$^d_{15}$C$^{az}_{14}$**) were tested by comparison of their MIC (minimum inhibitory concentration preventing 99.99% bacterial growth) values. It was found that the MIC values against *S. aureus* decrease with increasing concentration of hydrophobic groups from 5% to 11% (**PVAmH$^d_5$C$^{az}_{14}$** = 20 µg/mL; **PVAmH$^d_{11}$C$^{az}_{14}$** = 8 µg / mL) and stays constant with further increase of hydrophobic groups to 15%. The same effect of hydrophobic modification is observed against *E. coli*. The MIC values decreases with increasing concentration of hydrophobic groups from 5% to 11% (**PVAmH$^d_5$C$^{az}_{14}$** = 20 µg/mL; **PVAmH$^d_{11}$C$^{az}_{14}$** = 10 µg / mL) and stays constant with further increase of hydrophobic groups to 15% (Figure 2, Table SI 3).

To understand the effect of cationic modification, the efficacy of three multifunctional **PVAm** samples having 11% hydrophobic C-10 repeating units and 5 %, 10 %, 14 % cationic repeating units (**PVAmH$^d_{11}$C$^{az}_5$**, **PVAmH$^d_{11}$C$^{az}_{10}$**, **PVAmH$^d_{11}$C$^{az}_{14}$**) were tested by comparison of their MIC values. It was found that MIC values against *S. aureus* increase from **PVAmH$^d_{11}$C$^{az}_5$** = 3 µg/mL, to **PVAmH$^d_{11}$C$^{az}_{10}$** = 5 µg/mL, further to **PVAmH$^d_{15}$C$^{az}_{14}$** = 8 µg/mL. For *E. coli* with increasing concentration of cationic groups no change in MIC values is observed (Figure 2, Table SI 2).

To understand the influence of the alkyl chain length used for hydrophobic modification, the MIC values of two multifunctional **PVAm**s - **PVAmH$^d_{11}$C$^{az}_{14}$** and **PVAmH$^h_{11}$C$^{az}_{14}$** – having the same content of hydrophobic (11 %) and cationic (14 %) groups was studied. It was found

that the MIC values for **PVAmH$^h_{11}$C$^{az}_{14}$** was 100 µg/mL against both *S. aureus* and *E. coli* and for **PVAmH$^d_{11}$C$^{az}_{14}$** was 8 µg/mL against *S. aureus* and 10 µg/mL against *E. coli* (Figure 3A). This result clearly shows that the antimicrobial activity increases significantly (10 times) for an increase of the chain length from C-6 to C-10 chains.
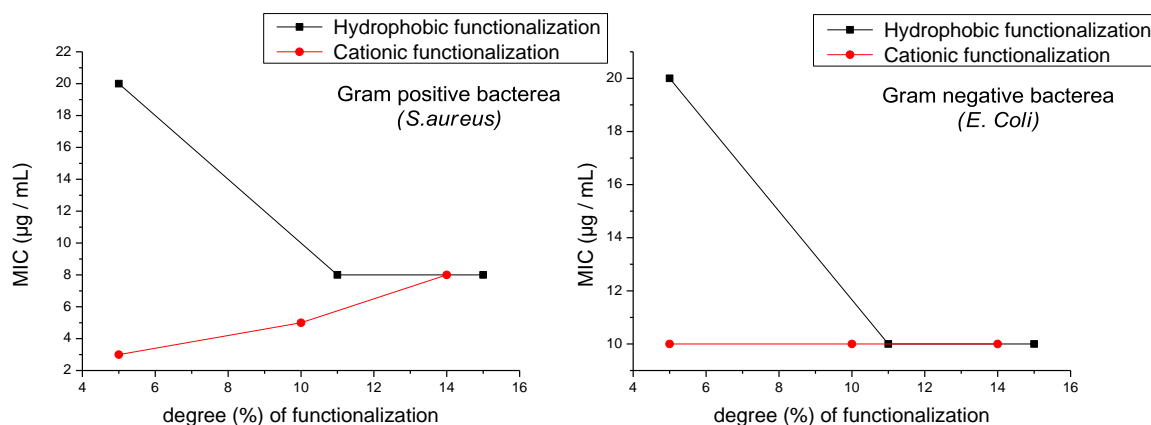
Figure 2: Antimicrobial efficiency (MIC) for different multifunctional **PVAm**s against Gram positive and Gram negative bacteria.

The most efficient antimicrobial **PVAm** samples were selected (samples with the lowest MIC values) to perform hemolytic tests and to determine the antimicrobial selectivity. The antimicrobial selectivity is defined as the ratio of the HC$_{50}$ (effective concentration of active compound lysing 50% of red blood cells) and the MIC (minimum inhibitory concentration preventing 99.99% bacterial growth) values. Two modified **PVAm**s - **PVAmH$^d_{11}$C$^{az}_{14}$** (11% hydrophobic and 14% cationic modification) and **PVAmH$^d_{11}$C$^{az}_5$** (11% hydrophobic and 5% cationic modification) – were selected to determine the hemolytic tests and to calculate the antimicrobial selectivity (Figure 3B and Table 1).
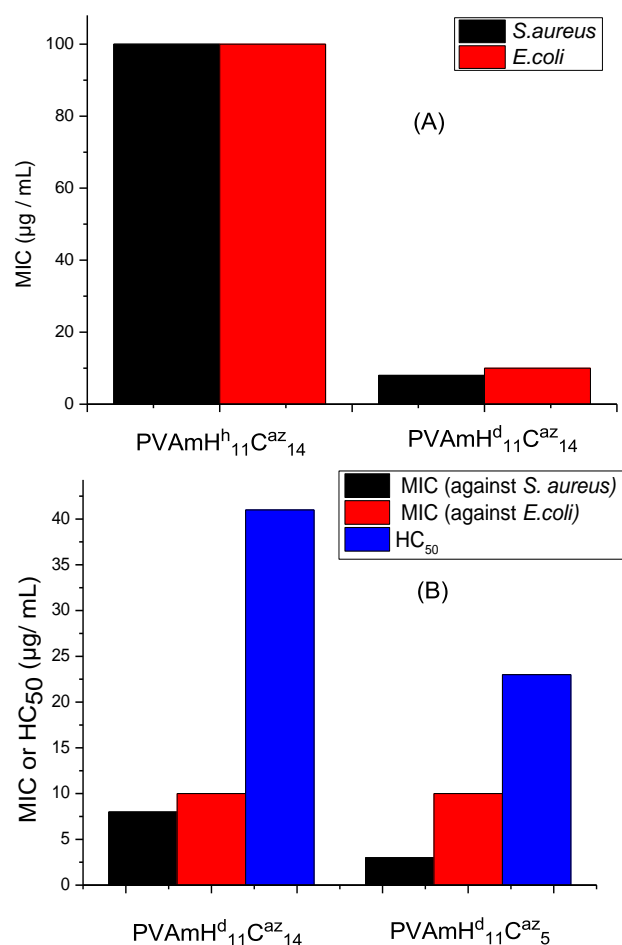
Figure 3: (A) Comparing MIC values of $\mathbf{PVAmH^{h}_{11}C^{az}_{14}}$ and $\mathbf{PVAmH^{d}_{11}C^{az}_{15}}$ (B)

Antimicrobial selectivity of $\mathbf{PVAmH^{d}_{11}C^{az}_{15}}$ and $\mathbf{PVAmH^{d}_{11}C^{az}_{5}}$

Table 1: Biological test results of antimicrobial **PVAm**s

Polymer	MIC (µg/ mL)		HC_{50} (µg/ mL)	antimicrobial selectivity	
	S.aureus	*E.coli*		*S.aureus*	*E.coli*
$PVAmH^{d}_{11}C^{az}_{14}$	8	10	41	5.125	4.1
$PVAmH^{d}_{11}C^{az}_{5}$	3	10	23	7.67	2.3

The results show that both polymers have a good antimicrobial selectivity against both types

of bacteria used in the current study – *S.aureus* and *E.coli*. For $\mathbf{PVAmH^{d}_{11}C^{az}_{14}}$ the selectivity

was more than 5 times higher against *S.aureus* and 4 times higher against *E.coli,* respectively.

For **PVAmH$^d_{11}$C$^{az}_5$** the selectivity against *S.aureus* was even better (7.6 times higher), however, lower against *E.coli*, respectively (2.3 times). These experimental observations can be rationalized by considering the different nature (different composition) of the cell membranes of *S.aureus*, *E.coli* and RBC.

Conclusion: Multifunctional azetidinium functionalized **PVAm**s, mimicking the structural parameters of natural **AMP**s - were prepared in a simple one pot synthesis approach using suitable functional couplers. The antimicrobial efficiencies of these polymers can be tuned by the ratio of the hydrophobic and hydrophilic modifications and the nature of the hydrophobic building block. Increasing the alkyl chain length of the hydrophobic moiety, the antimicrobial efficacy against *S. aureus* and *E.coli* increases significantly (10 times when the hexyl chain was replaced by a decyl chain). Furthermore the antimicrobial efficacy increases when the percentage of decyl groups was increased from 5% to 11%. However, further increase to 15 % does not improve the result. At the same time the antimicrobial efficiency against *S.aureus* of the polymers decreased slightly when the content of cationic groups increased from 5% to 14%. However, the efficiency against *E.coli* remains unchanged for different percentages of cationic modification. Finally, it was observed that **PVAm**, having 11% of hydrophobic and 5% to 14% of cationic groups are the best antimicrobial polymers. The antimicrobial selectivity - the ability of the polymers to differentiate between microorganisms (Gram positive and Gram negative bacteria) and mammalian cells (red blood cells) - of **PVAmH$^d_{11}$C$^{az}_{14}$** and **PVAmH$^d_{11}$C$^{az}_5$** was between 3-7 suggest that these polymers are excellent candidates for solving hygiene and health related problems in today's life.

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Supplementary information for Chapter7

Experimental section:

Materials: Decylamine (99%, Aldrich), hexylamine (99%, Aldrich), epichlorohydrin (99%, Merck) and piperazine (99+%, Aldrich) were used as received. Salt free poly(vinyl amine) was obtained from BASF as aqueous solution. This aqueous solution was freeze dried and the solid poly(vinyl amine) was used as starting material. Distilled water was used as solvent for all the reactions. For determination of the antimicrobial activity, amphiphilic compounds and amphiphilic polymers were tested against the Gram negative bacteria *Escherichia coli* (ATCC 23716) and the Gram positive bacteria *Staphylococcus aureus* (ATCC 6538).

Measurements:

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterated dimethyl sulfoxide (DMSO-d$_6$), deuterium oxide (D$_2$O) and deuterated methanol (CD$_3$OD) were used as solvents. Tetramethylsilane (TMS) was used as an internal standard. All Raman spectra were recorded on a Buker RFS100/s Raman spectrometer, fitted with a Nd:YAG laser (1064nm). The spectral resolution was 4 cm^{-1}. For one spectrum 1000 scans were collected at a laser power of 200mW. Size exclusion chromatography analysis (SEC) were carried out with water (containing 0.1 M NaCl, 0.1% TFA, 0.01% NaN$_3$) as eluting solvent at 30°C with a flow rate of 1 mL/min using a high pressure liquid chromatography pump (Agilent 1100) and refractive index detector (Wyatt, Optilab DSP). Three columns with PSS Novema gel were applied. The length of each column was 300 mm, the diameter was 8 mm, the diameter of the gel particles were 10 μm and the nominal pore widths were 30, 3000 and 3000 Å. Calibration was achieved using Pullulan standards. Thermal shaker (Heidolph), Microplate Incubator/Reader Genios Pro, Infinite 200Pro (Tecan), drying oven, and safety sterile hood (Kendro) were used for the antimicrobial assays.
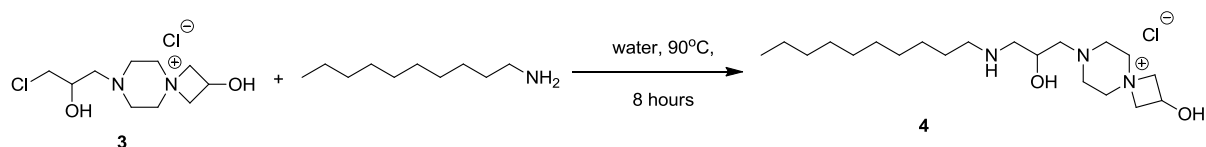
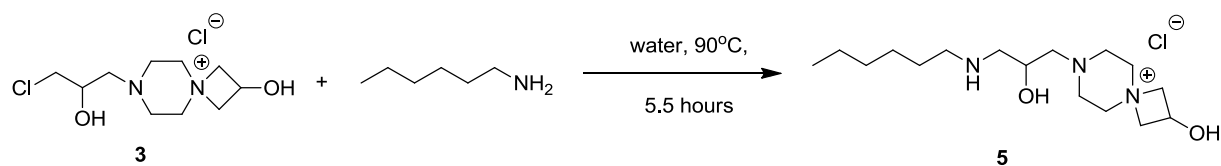## Synthesis:

Preparation of the bifunctional coupler 3:
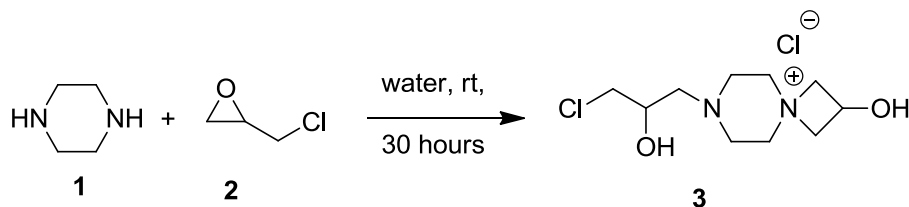
The bifunctional coupler **3** was synthesized as described in chapter 4.

## Synthesis of hydrophobically modified (C-10) functionalized coupler - FCH^{d}C^{az} 4 :

The hydrophobically modified (C-10) functionalized coupler - FCH^{d}C^{az} **4** was synthesized as described in chapter 6.

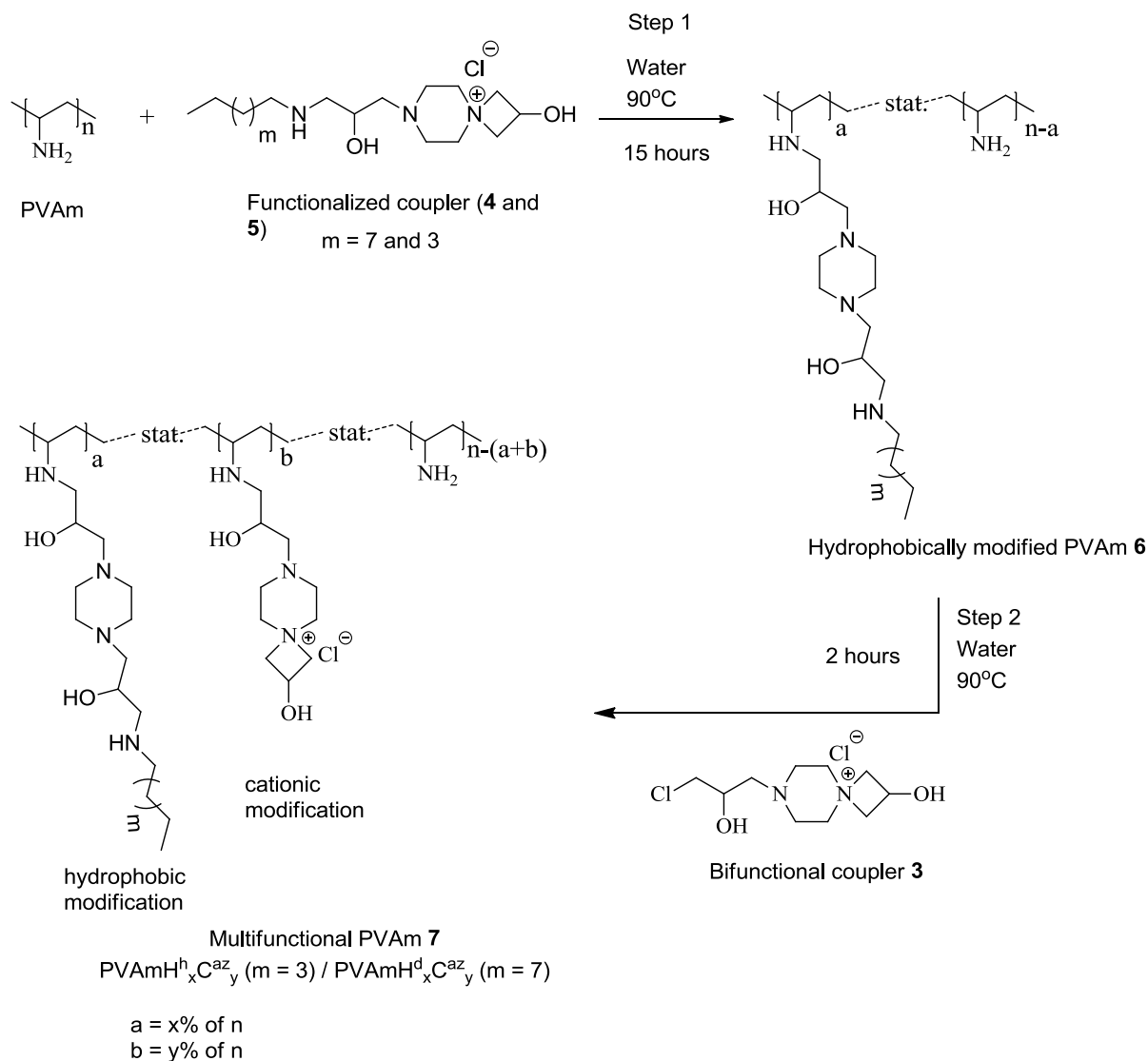## Synthesis of hydrophobically modified (C-6) functional coupler- FCH^{h}C^{az} 5 :

The hydrophobically modified (C-6) functional coupler- FCH^{h}C^{az} **5** was synthesized as described in chapter 4.

Figure SI 1: ^{1}H NMR spectra of hydrophobically modified functionalized couplers **4** and **5** in DMSO-*d*[6] *:
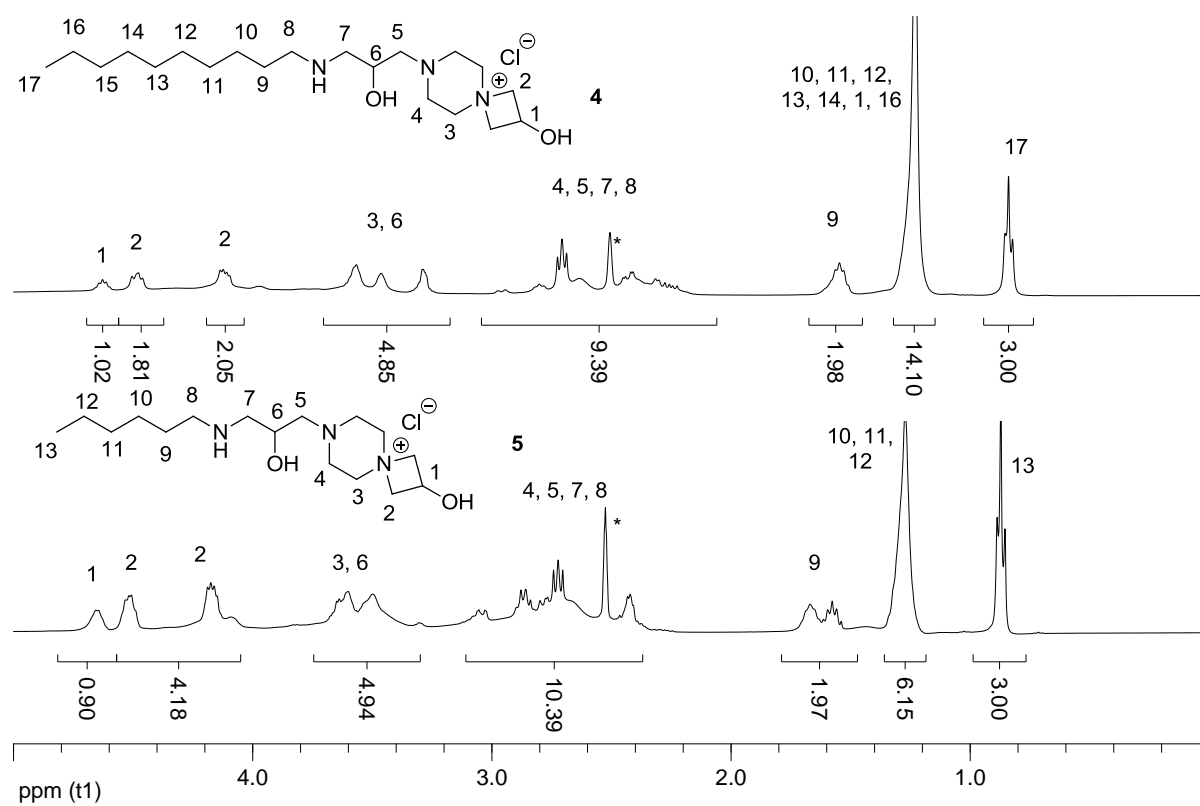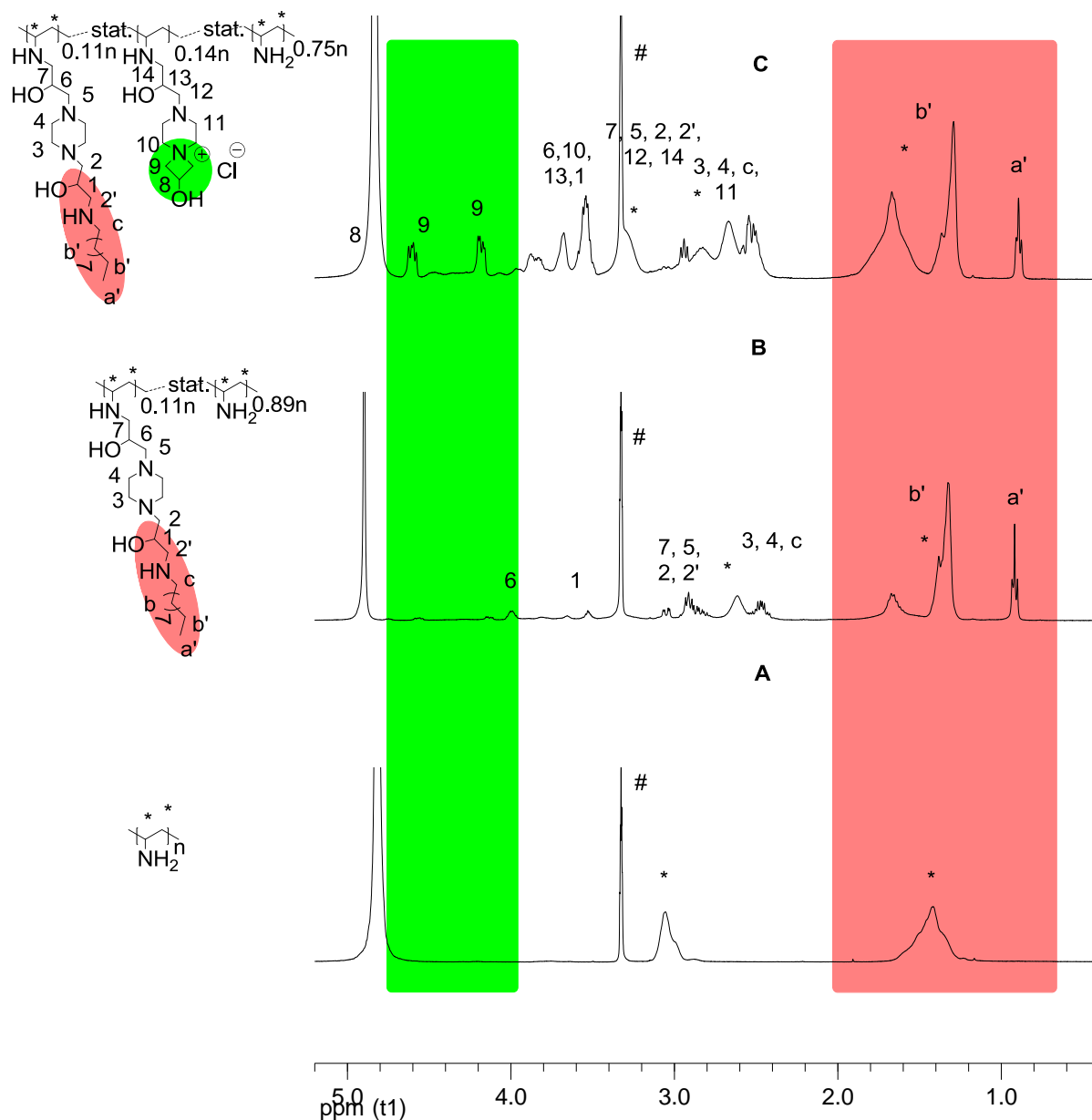
Preparation of multi functional poly(vinyl amine)s 7:

Step 1

Water
90°C
15 hours

PVAm

Functionalized coupler (**4** and **5**)
m = 7 and 3

Hydrophobically modified PVAm **6**

Step 2
Water
90°C
2 hours

Bifunctional coupler **3**

cationic modification

hydrophobic modification

Multifunctional PVAm 7
PVAmH^h_xC$^{az}_y$ (m = 3) / PVAmH^d_xC$^{az}_y$ (m = 7)

a = x% of n
b = y% of n

The multifunctional polymers were prepared in a two step procedure as following:

Step 1: To a solution of poly(vinyl amine) (0.18g, 0.0042 mol repeating unit) in distilled water (20 mL), the solution of functionalized coupler **4** (0.18 g, 0.00046 mol) in distilled water (5 mL) was added. The solution was stirred for 15 hours at 90°C and then cooled down to room temperature. The solution contains the hydrophobically modified poly(vinyl amine) **6**.

Step 2: To solution containing hydrophobic modified poly(vinyl amine) (as prepared in step 1), a solution of bifunctional coupler **3** (0.16 g, 0.00059 mol) in distilled water (3 mL) was

added. The solution was stirred for 2 hours at 90°C and then cooled down to room temperature. The resultant solution contains multifunctional poly(vinyl amine) **7**, **PVAmH$^d_{11}$C$^{az}_{14}$.**

Figure SI 2: ^{1}H NMR analysis of **PVAmH$^d_{11}$C$^{az}_{14}$** (MeOD + D$_2$O):

All the other multifunctional poly(vinyl amine)s were prepared using the same procedure. The starting materials are given in the following table:

Table SI 1: Starting materials for multifunctional PVAms:

Multifunctional PVAm	PVAm (g)	Functionalized coupler (g)	Bifunctional coupler (g)
PVAmH$^d_5$C$^{az}_{14}$	0.18	FCH$^d_1$C$^{az}_1$ (0.082)	0.16
PVAmH$^d_{11}$C$^{az}_{14}$	0.18	FCH$^d_1$C$^{az}_1$ (0.18)	0.16
PVAmH$^h_{11}$C$^{az}_{14}$	0.18	FCH$^h_1$C$^{az}_1$ (0.15)	0.16
PVAmH$^d_{15}$C$^{az}_{14}$	0.18	FCH$^d_1$C$^{az}_1$ (0.024)	0.16
PVAmH$^d_{11}$C$^{az}_5$	0.18	FCH$^d_1$C$^{az}_1$ (0.18)	0.057
PVAmH$^d_{11}$C$^{az}_{10}$	0.18	FCH$^d_1$C$^{az}_1$ (0.18)	0.114
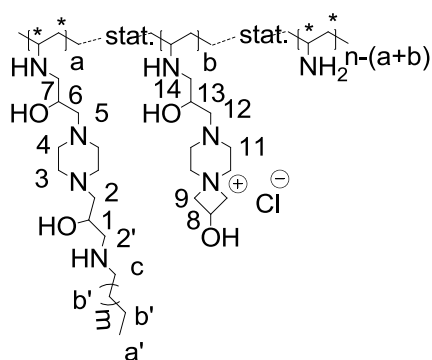
^{1}H NMR (D$_2$O + MeOD, 400MHz): δ = 4.82 (m, H^1), 4.65 & 4.13 (m, H^2, H$^{2'}$), 4.0 – 3.5 (H^1, H^6, H^{10}, H^{13}), 3.4 - 2.3 (H^c, H^2, H$^{2'}$, H^3, H^4, H^5, H^7, H^{11}, H^{12}, H^{14}, H*), 1.8 – 1.2 (H$^{b'}$, H*), 0.9 (H$^{a'}$) ppm.

Table SI 2: GPC analysis of multifunctional PVAms

Polymer	M_n	M_w	PDI
PVAm	$3.60 * 10^5$	$7.45 * 10^5$	2.07
PVAmH$^d_5$C$^{az}_{14}$	$3.67 * 10^5$	$8.49 * 10^5$	2.31
PVAmH$^d_{11}$C$^{az}_{14}$	$3.81 * 10^5$	$1.04 * 10^6$	2.75
PVAmH$^h_{11}$C$^{az}_{14}$	$3.59 * 10^5$	$6.20 * 10^5$	1.73
PVAmH$^d_{15}$C$^{az}_{14}$	$4.82 * 10^5$	$1.37 * 10^6$	2.84
PVAmH$^d_{11}$C$^{az}_5$	$3.75 * 10^5$	$8.80 * 10^5$	2.38
PVAmH$^d_{11}$C$^{az}_{10}$	$3.67 * 10^5$	$8.63 * 10^5$	2.34

Antimicrobial tests of polymer solutions:

The antibacterial activity of the amphiphilic polymers **in solution** was determined by measuring the minimum inhibitory concentration (MIC) using different test bacteria. The testing organisms used were Escherichia coli as a Gram negative and Staphylococcus aureus as Gram positive bacteria. Suspensions of strains with known colony forming units (CFU; E. coli, $2x10^6$ CFU/mL; S. aureus, $2x10^6$ CFU/mL) were incubated at 37°C in nutrient solutions with different concentrations of the test samples together with a wetting agent (0.001% 3-(Polyoxyethylene)propylheptamethyltrisiloxane (DOW)). The growth of the bacteria was followed during the incubation over 20 h by measuring the optical density at 612 nm every 30 min and 1000 s shaking at 100 rpm per cycle of 30 min by using a microplate reader/incubator. The minimal inhibitory concentration (MIC) corresponds to the concentration of the test substance at which a log 4 reduction (99.99%) of the growth of the

inoculated bacteria was observed by comparison with control samples without test substance. Experiments were triplicated.

Table SI 3: MIC values of the antimicrobial PVAms:

Multifunctional PVAm	MIC (µg/ mL) (against *E. coli*)	MIC (µg/ mL) (against *S. aureus*)
$PVAmH^d_5C^{az}_{14}$	20	40
$PVAmH^d_{11}C^{az}_{14}$	10	8
$PVAmH^h_{11}C^{az}_{14}$	100	100
$PVAmH^d_{15}C^{az}_{14}$	10	8
$PVAmH^d_{11}C^{az}_5$	10	3
$PVAmH^d_{11}C^{az}_{10}$	10	5

Hemolytic activity test:

Hemolytic activity was assessed according to literature [1]. Human erythrocytes (red blood cells (RBC), 0, Rh positive; citrate blood) were obtained by centrifugation (3000 rpm, 10 min) to remove plasma, washed 3 times in PBS and diluted in PBS to obtain a stock solution 2.6×10^8/mL RBC. 250 µL of the stock solution was pipetted into solutions of defined polymer concentration in PBS up to 500 µL; the final amount of RBC being $1,3 \times 10^8$ RBC/mL. The RBC were exposed for 60 min at 37 °C, thereafter centrifuged (4000 rpm, 10 min) and the absorption of the supernatant was determined at 414 nm in a 96 well plate. As reference solutions (i) PBS for determining spontaneous hemolysis and (ii) 0.5 % Triton X-100 for

100 % hemolysis (positive control) were used. Hemolysis was plotted as a function of polymer concentration and the hemolytic activity was defined as the polymer concentration that causes 50 % hemolysis of human RBC relative to the positive control (HC_{50}).

Reference:

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Appendix 1

Preparation of glucose functionalized polymers and polymer nanoparticles: a synthetic approach using cascade reactions

Introduction:

Biocompatible polymers are of interest in today's research due to their applications in biomedical fields as they are used in contact with living cells, blood, proteins etc[1, 2]. Sugar containing polymers are now receiving special attention due to their biocompatibility and their role in various biological processes[3]. Sugar units or glycan chains fulfill an important role in biological recognition processes[4].

In recent years, different procedures for the preparation of sugar-containing polymers have been reported. For examples Smeds et al.[5] presented the preparation of hydrogels containing alginate and hyaluronan. These gels were found to be responsible for healing wounds and improve the reconstruction of soft tissues. Elvira et al.[6] prepared starch based hydrogels for drug delivery, Haddleton et al.[7] reported the preparation of a library of polymers containing mannose and galactose, that can be used as multivalent ligands for lectin binding studies. At the same time, with the developments in nanotechnology, nanoparticles containing sugar residues on the surface, received attention, due to their potential for different applications, e.g.; development of vaccines, targeted drug delivery, bioassays, imaging, immobilization of bioactive proteins etc[8, 9, 10].

In the last decades, many reports on the preparation of polymer nanoparticles were published. The most useful technique to prepare polymer nanoparticles was suspension-[11], emulsion-[12] and miniemulsion polymerization[13] of suitable functional monomers. Recently, preparation of polymer nanoparticles was reported using surfactant-free emulsion polymerization[14]. Here

oligoglycidol macromonomers were used as reactive surfactants (surfmers), which were polymerized using styrene to prepare core/shell nanoparticles, in which the oligoglycidol part forms the shell and the polystyrene forms the core. In this work, we extended this concept of preparing nanoparticles using glucose functionalized acrylates as reactive surfactants (surfmers) to prepare polystyrene and poly(methyl methacrylate) nanoparticles, with glucose units in the shell.

Recently, the synthesis of functional (meth)acrylates via enzyma-tic transacylation reactions were reported by our group[15, 16]. Methyl acrylate (or methyl methacrylate) was reacted with different primary alcohols using a *Lipase* as catalyst and tertiary alcohols as solvent to prepare functional (meth)acrylate monomers. These reactions were monitored by analyzing the methanol released in the reaction mixture. The crude reaction mixture was used for polymerization to prepare linear highly functional polymers in a cascade reaction. The advantage of this approach is that functional polymers are prepared in a one pot reaction without time consuming and difficult purification processes in the intermediate steps.

In the current work, using these one pot cascade reactions – enzyme catalyzed transacylation of glucose and methyl acrylate followed by free radical polymerization – the preparation of linear statistical copolyacrylates and core/shell nanoparticles – is reported (Figure 1). Glucose functionalized acrylate was prepared via transacylation of glucose with methyl acrylate using a *Lipase* as catalyst. To estimate the reactivity of glucose acrylate in the copolymerization reaction, model free radical copolymerizations of glucose acrylate and methyl acrylate were performed using AIBN as initiator. The kinetics of the polymerization reaction was studied and the relative rate of polymerization of glucose acrylate and methyl acrylate was determined. To prepare core/shell nanoparticles glucose acrylate monomer was used as reactive surfactant (surfmer) and polymerized in water with methyl methacrylate (MMA) and styrene.
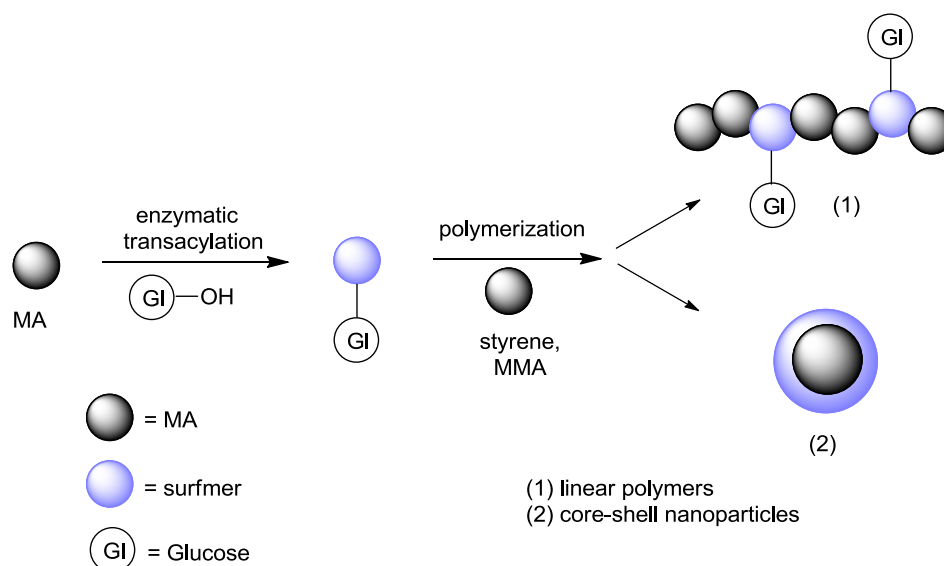
Figure 1: Concept for the preparation of glucose functionalized linear polymers and core/shell nanoparticles.

Experimental section:

Materials: Alpha-D-(+)-glucose, anhydrous (96%, Sigma Aldrich), D-(+)-galactose (98%, Sigma Aldrich), methyl acrylate (MA) (99%, Aldrich), methyl methacrylate (MMA) (99%, Aldrich) and styrene (99+%, Aldrich) were used without further purification. All solvents were used as received. A commercially available lipase, Novozyme 435 (*Lipase B* from *Candida Antarctica* immobilized on a macroporous acrylic resin, 10000 U*g^{-1} Novo Nordisk) was dried in vacuum at room temperature for 24 hours and stored under nitrogen before it was used as a biocatalyst for the transacylation reactions. All reactions were carried out in a nitrogen atmosphere. Nitrogen (Linde, 5.0) was passed over molecular sieves (4 Å) and finely distributed potassium on aluminum oxide.

Measurements:

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 100 MHz, respectively. Deuterated dimethyl sulfoxide (DMSO-d$_6$) was used as solvent and tetramethylsilane (TMS) as an internal standard. IR spectra were recorded on a

Thermo Nicolet Nexus 470 spectrometer. The spectra were measured using an ATR unit, SMART SPLITPEA fitted with Si crystal. For one spectrum 400 scans were collected.

Size exclusion chromatography analyses (SEC) were carried out using THF (with addition of 250 mg/L 2, 6-di-tert-butyl-4-methylphenol) and DMF (containing LiBr 1 mg/mL) as eluting solvents. For THF as the eluent (flow rate 1.0 mL/min) a high pressure liquid chromatography pump (ERC 6420) and a refractive index detector (WGE Dr. Bures ETA 2020) were used at 30°C. Four columns with MZ-DVB gel were applied: length of each column 300 mm, diameter 8 mm, diameter of gel particles 5 μm, nominal pore widths 50, 100, 1000, 10 000 Å. For DMF as solvent (flow rate 1.0 mL/min) a high pressure liquid chromatography pump (Bischoff HPLC compact pump) with a RI detector (Jasco RI 2031 plus) was used. Three columns with PSS GRAM gel were applied: length of each column 300 mm, diameter 8 mm, diameter of the gel particles 5μm, nominal pore widths 50, 100, 1000, 10 000 Å. Calibration was achieved using poly(methyl methacrylate) (PMMA) or polystyrene standards.

Transmission electron microscopy (TEM) was measured on a Zeiss LibraTM 120 (Carl Zeiss, Oberkochen, Germany). The electron beam accelerating voltage was set at 120 kV. A drop of the sample was trickled on a piece of Formvar and carbon-coated copper grid. Before being placed into the TEM specimen holder, the copper grid was air-dried under ambient conditions. At least 100 particles were measured and an average value was calculated.

Synthesis:

Lipase catalyzed transacylation reaction: To a suspension of glucose (**1**) (230 mg, 1.34 mmol) in *tert*-butanol (40 mL), methyl acrylate (**2**) (0.29g, 3.3 mmol) was added. The reaction was initiated by the addition of 25 mg/mL Novozyme 435 and the mixture was stirred for 24 hours in inert gas atmosphere at 55°C. The reaction was quenched by cooling the mixture to

ambient temperature and the enzyme was removed by filtration. The molar ratio of glucose

acrylate **3** and methyl acrylate **2** in the product was determined via ^{1}H NMR spectroscopy.

All other reactions were performed according to this procedure (Table 1).

Table 1: Lipase catalyzed transacylation reactions; starting materials and reaction condition:

No.	Hexose	MA: hexose mole/mole	Temp. (^{o}C)	Solvent
1	Glucose	1:1	35	*tert*-butanol
2	Glucose	1:1	55	*tert*-butanol
3	Glucose	1:1	70	*tert*-butanol
4	Glucose	2.5:1	55	*tert*-amyl alcohol
5	Glucose	2.5:1	55	*tert*-butanol
6	Galactose	2.5:1	55	*tert*-butanol
7	Galactose	2.5:1	55	*tert*-amyl alcohol

Glucose acrylate **3** (similar for galactose acrylate **3'**):
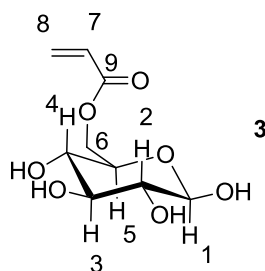
^{1}H NMR (DMSO-d^6): δ (ppm) = 6.22- 5.85 (H^7, H^8), 4.91 (H^1), 3.8- 3.5 (H^6, H^4), 3.4 (OH), 6.22- 5.85 (H^2, H^3, H^5).

^{13}C NMR (DMSO-d^6): δ (ppm) = 168 (C^9), 131.5 (C^8), 128.2 (C^7), 96.85 (C^1), 76.33(C^5), 74.63 (C^3), 73.33 (C^2), 72.12 (C^4), 64.16 (C^6).

IR (Si) (cm^{-1}): 3316 (OH), 1724 (ester), 1636 (alkene).

Kinetic studies:

Conversion/time dependence and effect of solvent: To a suspension of glucose (**1**) (230 mg, 1.34 mmol) in *tert*-butanol (40 mL), methyl acrylate (**2**) (0.29g, 3.3 mmol) was added. The reaction was initiated by the addition of 25 mg/ mL Novozyme 435 and the mixture was stirred for 24 hours in inert gas atmosphere at 55°C. Periodically samples were withdrawn from the reaction mixture and the consumption of substrate (MA) and formation of glucose acrylate (**3**) was followed via ^{1}H NMR spectroscopy.

For studying the conversion/time dependence and the effect of solvent on the transacylation reaction the experiments 4, 5, 6 and 7 of table 1 were analyzed.
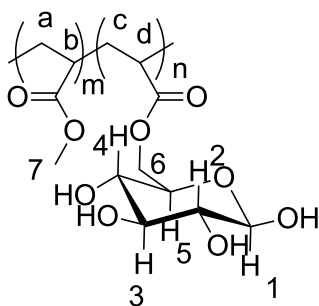
Polymerization reactions:

To a suspension of glucose (**1**) (230 mg, 1.34 mmol) in *tert*-butanol (40 mL), methyl acrylate (MA) (**2**) (0.29g, 3.3 mmol) was added. The reaction was initiated by the addition of 25 mg/

mL Novozyme 435 and the mixture was stirred for 24 hours in inert gas atmosphere at 55°C (Table 1, No. 5). After transacylation Novozyme was removed by filtration and the ratio of MA and glucose acrylate was determined – MA: glucose acrylate = 1.8: 1. To this solution methyl acrylate (0.22 g, 2.55 mmol) was added to adjust the mole ratio of MA : glucose acrylate = 3 : 1 (determined by ^{1}H NMR spectroscopy), AIBN (10 mg, 1.6 mole% of total monomer) was added and the mixture was stirred at 70°C for 24 hours. After this, the reaction mixture was cooled down to room temperature and the polymer **4** was purified by dialysis.

Poly(glucose acrylate-*co*-methyl acrylate) **4:**

GPC: M_n = 10,200 M_w = 16,700   PDI = 1.64

^{1}H NMR (DMSO-d^6): δ = 4.97 (H^1), 4.54- 4.34 (H^5, H^4), 3.71 (H^6), 3.64 (H^7), 3.14(H^2), 2.96 (H^3), 2.5- 1.8 (H^b, H^d), 1.8- 0.8 (H^a, H^c).

Kinetics of the polymerization reaction: To a suspension of glucose (**1**) (230 mg, 1.34 mmol) in *tert*-butanol (40 mL), methyl acrylate (MA) (**2**) (0.29g, 3.3 mmol) was added. The reaction was initiated by the addition of 25 mg/mL Novozyme 435 and the mixture was stirred for 24 hours in inert gas atmosphere at 55°C (Table 1, No. 5). After transacylation Novozyme was removed by filtration and the ratio of MA : glucose acrylate was determined to be 1.8 : 1. To this solution AIBN (10 mg, 1.8 mole% of total monomer) and DMF (1 mL) used as internal standard was added and stirred at 70°C for 50 hours. Periodically samples

were withdrawn from the reaction mixture and the conversion of both acrylates was followed by ^{1}H NMR spectroscopy.

To study the kinetics of the polymerization reaction, the solution obtained after the transacylation reaction of experiment 5 and 6 (Table 1) were used and the polymerizations were performed using the same procedure.
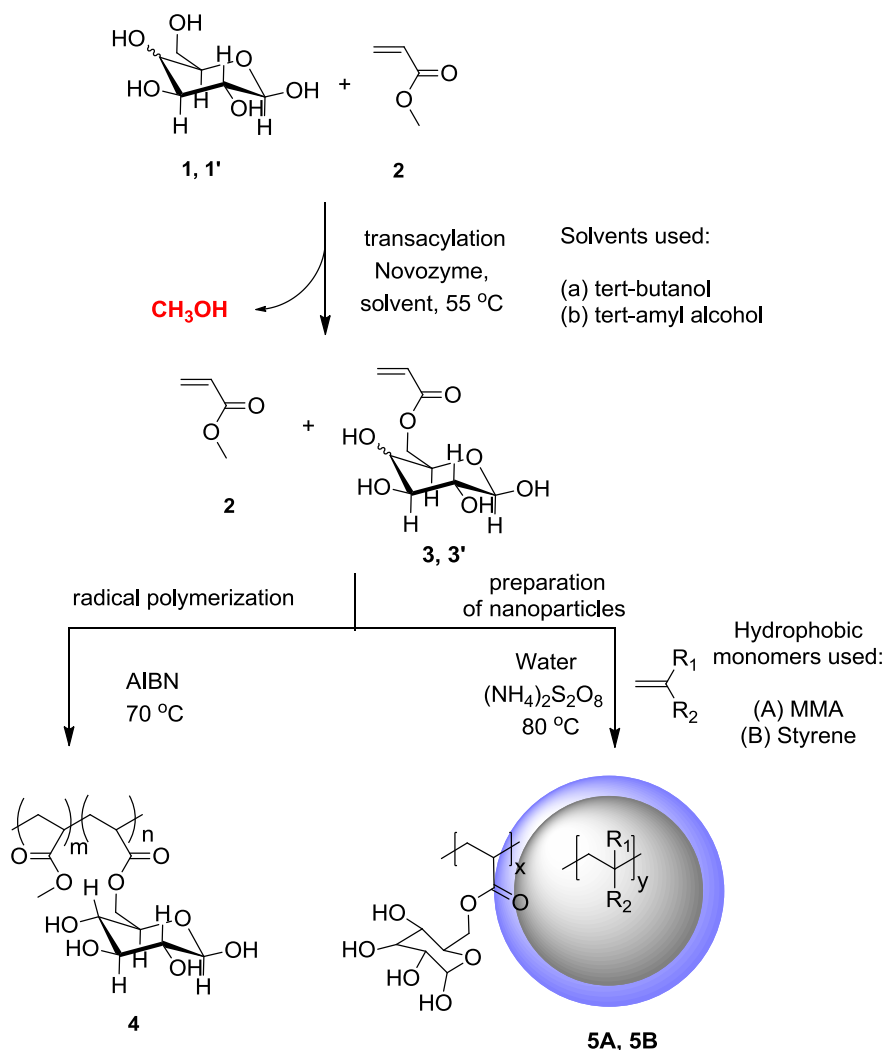
Preparation of polymer nanoparticles:

Glucose acrylate (**3**) (44 mg, 0.8 wt. % with respect to styrene) was dissolved in degased water (100 mL). Styrene (5.0 g) was added and the reaction mixture was heated to 80°C while stirring with a speed of 240 rpm. Ammonium persulfate (50 mg, 1.0 wt. %) was dissolved in degassed water (2 mL) and added to the reaction mixture. The polymerization was carried out at 80°C overnight.

Polymer nanoparticles based on MMA were prepared according to the same procedure. Starting materials are listed in table 2.

Particle	Glucose acrylate (mg)	Monomer (g)	$(NH_4)_2S_2O_8$ (mg)	Water (mL)
5A	44	Styrene (A) (5.0 g)	50	100
5B	44	MMA (B) (5.0 g)	50	100

Result and discussions:

Starting from commercially available glucose, we have developed a synthetic strategy for the preparation of polymers and nanoparticles containing monosaccharide building blocks. As already mentioned, enzyme catalyzed transacylation reactions are efficient for the preparation of functional acrylate monomers which then can be used to prepare functional polymers in a one pot reaction. In this work, in the 1st step glucose or galactose was reacted with methyl acrylate using Novozyme 435 as a catalyst and tertiary alcohols as solvents and subsequently in the next step, the newly formed glucose acrylate and MA were used to prepare copolymers **4** and core/shell particles **5** (Scheme 1). To estimate the reactivity of glucose and galactose acrylate, the solution obtained after the transacylation reaction, containing glucose and galactose acrylate and unreacted methyl acrylate, were used for free radical polymerization using AIBN as initiator. Kinetics of the polymerization reactions were studied to determine the relative rate of glucose and galactose acrylate (**3** and **3')** and methyl acrylate. Furthermore, polymer nanoparticles based on styrene (**5A)** and methyl methacrylate (**5B**) were prepared using the glucose acrylate **3** as reactive surfactant.

Scheme 1: Preparation of glucose containing copolymers and particles. [1: glucose (OH in 4 position equatorial), 1': galactose (OH in 4 position axial), 2: methyl acrylate (MA), 3: glucose acrylate (GluA), 3': galactose acrylate (GalA), 4: poly(MA-*co*-GluA), 5A: polystyrene core/shell nanoparticles, 5B: PMMA core/shell nanoparticles]

Synthesis of glucose and galactose acrylate:

Glucose or galactose was reacted with methyl acrylate using Novozyme 435 as catalyst to prepare the corresponding acrylates. The reaction was followed by ^{1}H NMR spectroscopy (Figure 2). The conversion of MA was calculated from the integration of the characteristic peaks associated with the protons of methanol formed during transacylation (δ = 3.17 ppm), and the newly formed characteristic peaks for the protons at the sp^2-hybridized carbon atoms

of the glucose acrylate (**3**) (δ = 5.8 – 6.4 ppm). It is important to mention that for ^{1}H NMR analysis, only the characteristic peaks of MA and glucose acrylate were considered and not the peaks for glucose since glucose is not completely soluble in *tert.*-butanol. After the reaction, methyl acrylate was removed in vacuum to obtain a solution of pure glucose acrylate **3**.
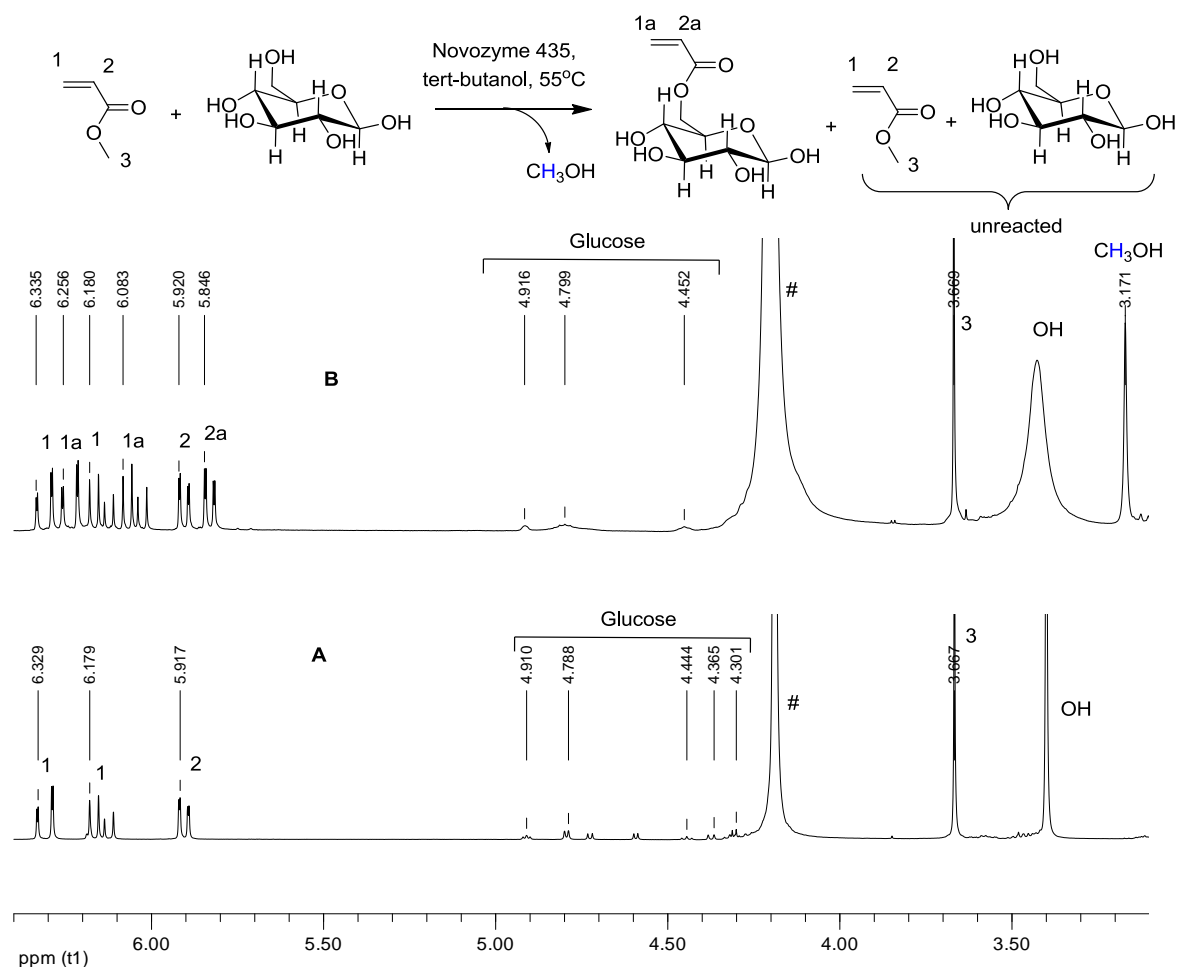
Figure 2: ^{1}H NMR spectra of (A) the mixture of methyl acrylate and glucose – starting materials (MA : glucose in the reaction feed 2.5 : 1) and (B) the mixture after transacylation (24 hours) (In the product mixture, MA : MeOH = 1.87: 1 and MA: glucose acrylate = 1.8: 1) (# = solvent peaks (*tert*-butanol).

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Kinetic study:

Conversion/time dependence and the effect of solvent: Glucose was reacted with MA (mole ratio glucose: MA = 1: 2.5) using Novozyme 435 as catalyst at $55^{o}C$ in *tert.*-butanol or in *tert.*-amyl alcohol. At different reaction times samples were analyzed via ^{1}H NMR spectroscopy and the conversion of MA was determined (Figure 3A). It was found that the conversion of MA was higher, when the reaction was performed in *tert.*-butanol. In all experiments the reaction reaches equilibrium after 6 hours and after this the conversion remains constant. All the reactions were performed also using galactose and similar results were obtained (Figure 3B). For both, glucose and galactose the maximum conversion was around 35% in *tert.*-butanol and 30 % in *tert.*-amyl alcohol.
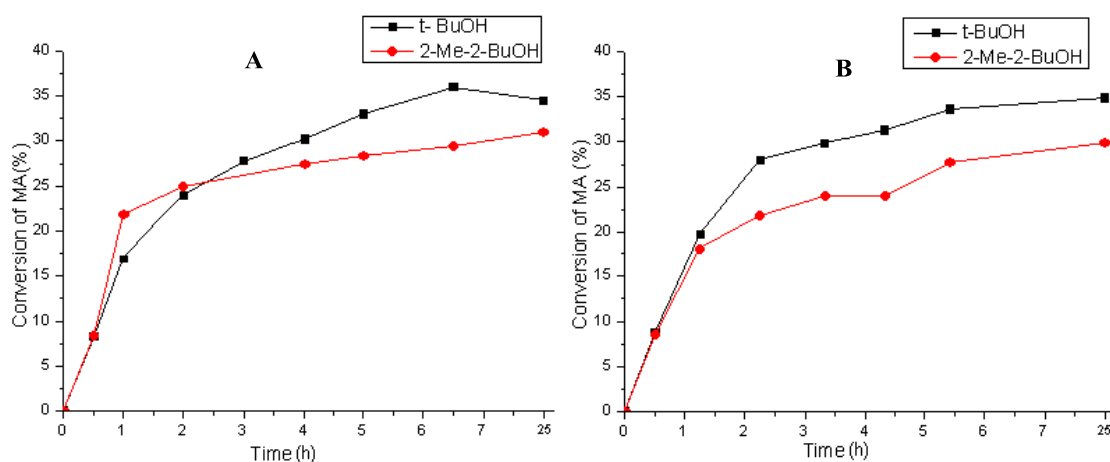
Figure 3: Transacylation of (A) glucose and (B) galactose using methyl acrylate as acyl donor and Novozyme 435 as catalyst. Conversion/time dependence in *tert.*-butanol and tert. amyl alcohol as solvents.

Free radical polymerization:

Glucose was reacted with methyl acrylate (mole ratio glucose : MA = 1 : 2.5) with Novozyme 435 as catalyst at $55^{o}C$ for 7 hours in *tert.*-butanol. After the transacylation reaction, the Novozyme was filtered off, and the mole ratio of glucose acrylate to MA was determined by

^{1}H NMR spectroscopy to be 1 : 1.8. The mole ratio of glucose acrylate and MA was then adjusted to 1:3 by addition of MA and the reaction mixture was polymerized for 48 hours at 70°C using AIBN as initiator (Scheme 1). After reaction the solution was cooled down to room temperature and the polymer was purified by dialysis and characterized by ^{1}H NMR spectroscopy and SEC.

In the ^{1}H NMR spectrum (Figure 4) of the polymer the glucose repeating units were identified by the characteristic protons at δ = 4.97 ppm (H^1), 4.54 ppm (H^4), 4.34 ppm (H^5), 3.71 ppm (H^6), 3.14 ppm (H^2), and 2.96 ppm (H^3). From the ^{1}H NMR spectrum, the ratio of methyl acrylate to glucose acrylate repeating units was calculated from the integration of the protons (H^1) of glucose, and the characteristic protons of the polymer backbone (H^a, H^b, H^c, and H^d); in the current case the ratio m : n was found to be 3.09 : 1.
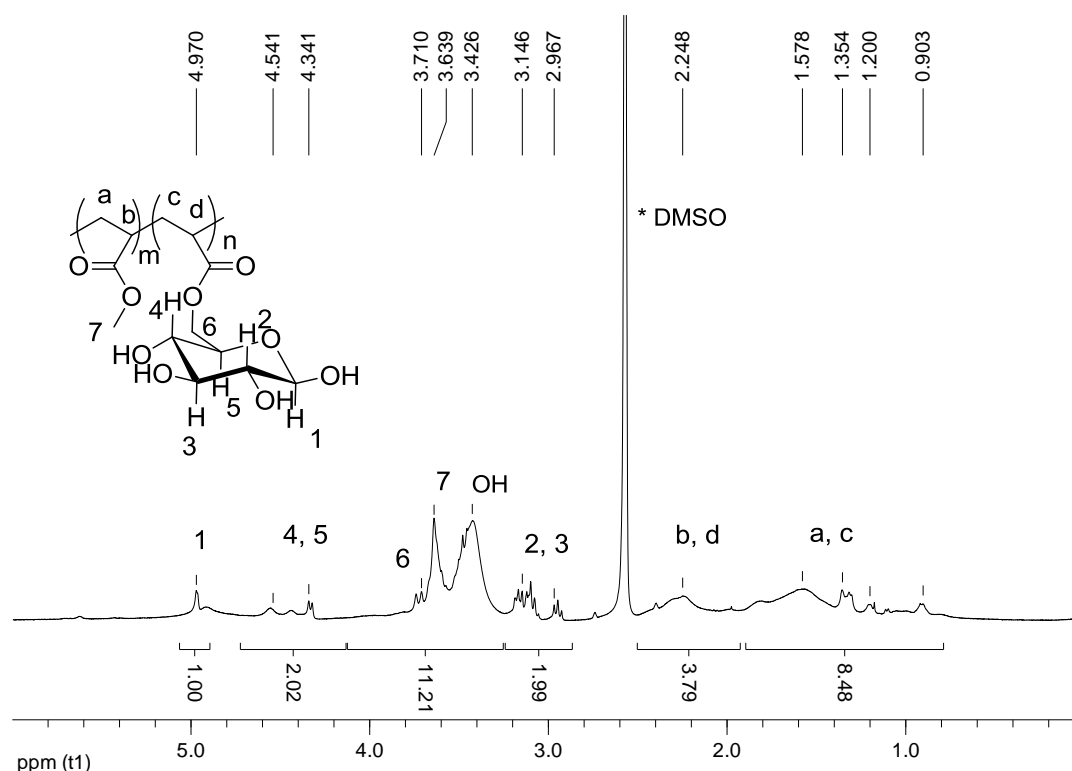
Figure 4: ^{1}H NMR spectrum (in DMSO D6) of poly(glucose acrylate-co-methyl acrylate) **4**

Kinetics of the free radical polymerization:

To determine the relative rate of polymerization for methyl acrylate and glucose acrylate monomer conversion vs. time dependence during polymerization were determined. For this glucose was reacted with methyl acrylate in a mole ratio 1:2.5 in presence of Novozyme 435 at 55°C for 24 hours in *tert.*-butanol. After that, Novozyme was filtered off and the resultant solution containing glucose acrylate **3** and methyl acrylate in a mole ratio 1: 1.78 (calculated from the ^{1}H NMR spectrum) was used for the polymerization reaction. A known amount of DMF was added to the reaction mixture and served as internal standard. At different reaction times samples were analyzed by ^{1}H NMR spectroscopy and the conversion of MA and glucose acrylate **3** was determined by comparing the integration of the alkene peaks of the two acrylates with the integral of the reference DMF (proton at $\delta = 8.0$ ppm). It was found that methyl acrylate was polymerized in higher rate compared to the glucose acrylate **3** (Figure 5A). For the first 6 hours, the overall polymerization rate was found to be close with the rate of conversion of the methyl acrylate; the conversion of methyl acrylate reached a value of 30% while that of glucose acrylate was found to be less than 5% (approximately 6 times less Similar results were obtained when using galactose in place of glucose in these experiments (Figure 5B).
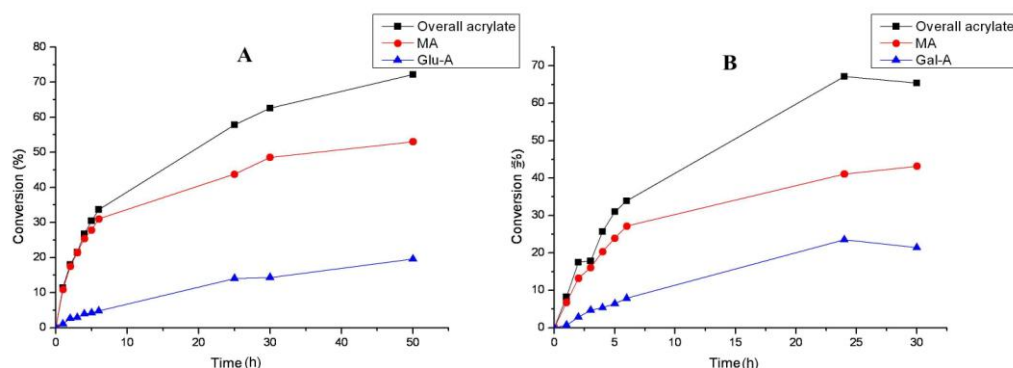
Figure 5: Kinetic study of the free radical polymerization of (A) glucose acrylate (GluA) and methyl acrylate (MA) and (B) galactose acrylate (GalA) and methyl acrylate (MA).

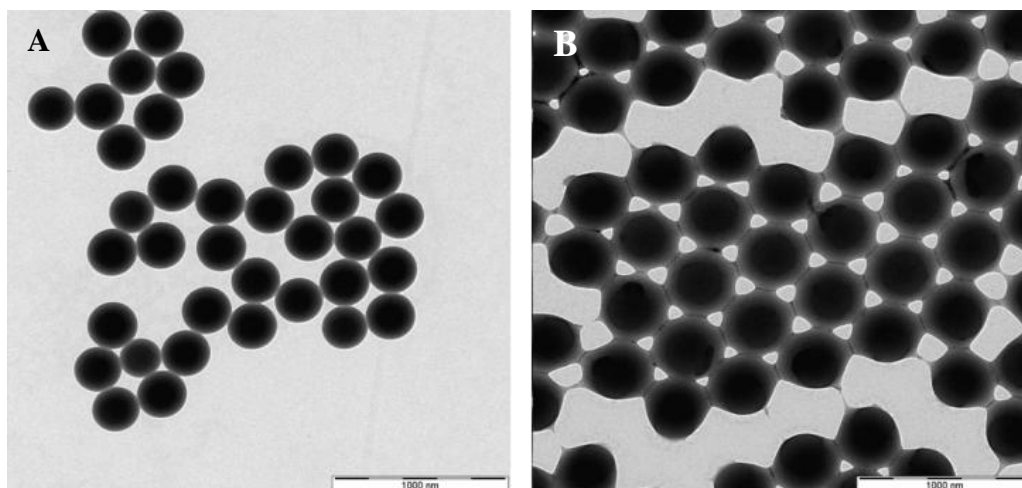**Preparation of polymer nanoparticles[*]:**

As mentioned earlier, the concept of using oligoglycidol macromonomers as reactive surfactant (surfmers) in the synthesis of polymeric nanoparticles by emulsion polymerization was reported by Pargen et al. With their hydroxyl shell these particles are potentially useful for biomedical applications. In this work, we expanded the concept of reactive surfactants using glucose functionalized acrylates **3** as reactive surfactants to prepare polymer nanoparticles based on polystyrene **5A** and poly(methyl methacrylate) **5B** with a monosaccharide enriched shell (Scheme 2).

Glucose acrylate **3**

A

B

Water
$(NH_4)_2S_2O_8$
80 °C

Water
$(NH_4)_2S_2O_8$
80 °C

Polymer nanoparticles **5A**

Polymer nanoparticles **5B**
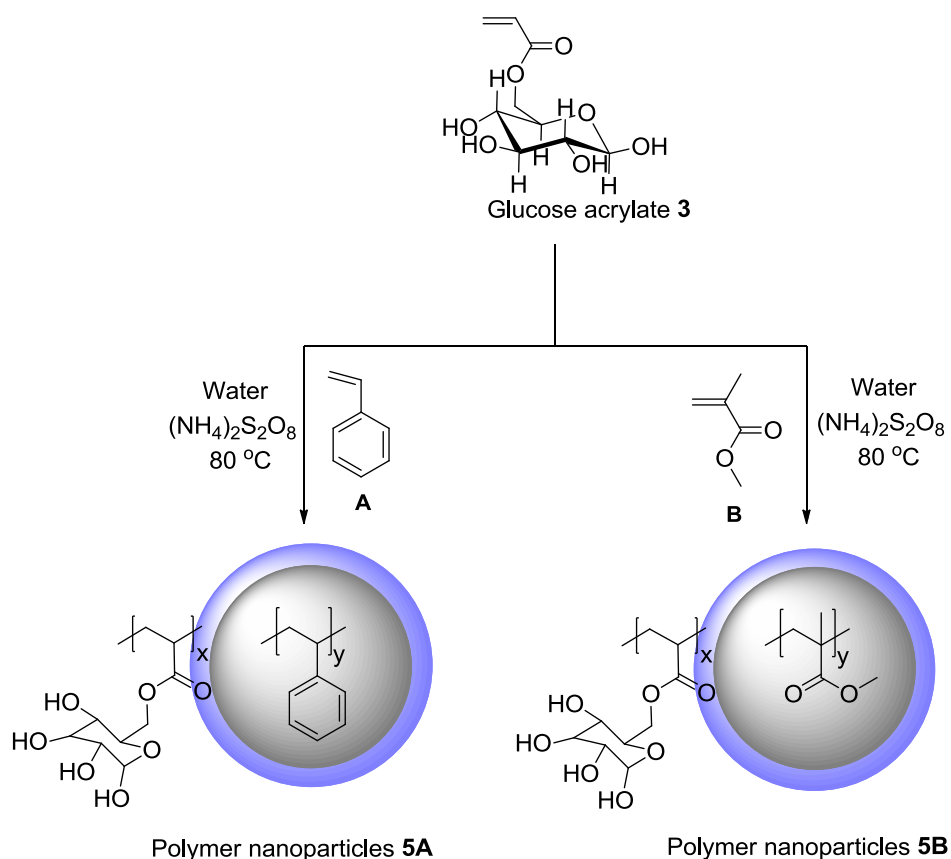
Scheme 2: Preparation of poly(methyl methacrylate) **5B** and polystyrene **5A** core/shell nanoparticles using glucose acrylate **3** as surfmer.

The synthesis of polymer nanoparticles was performed by emulsion polymerization using water soluble glucose acrylate **3** as reactive surfmer. No further emulsifier was added. Styrene

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and methyl methacrylate were used for the preparation of the nanoparticles. Nanoparticle **5A** and **5B** formed stable emulsions during polymerization. TEM images of the obtained polystyrene core/shell particles **5A** and PMMA core/shell particles **5B** are presented in Figure 6.

Figure 6: TEM images of A: polystyrene nanoparticles **5A** and B: PMMA nanoparticles **5B** prepared by emulsion polymerization with 0.8 wt.-% glucose acrylate.

Round shaped polystyrene/glucose-particles **5A** with diameters of about 270 nm and PMMA/glucose-particles **5B** with diameters of about 375 nm were obtained. Furthermore the PMMA particles **5B** appeared to be softer as they stick together compared to polystyrene particles **5A** which appear as non sticky spheres.

Particles **5A** and **5B** were dissolved in THF and analyzed by SEC using poly(methyl methacrylate) (PMMA) standards for calibration of sample **5B** and polystyrene standards for calibration of sample **5A** (Figure 7).
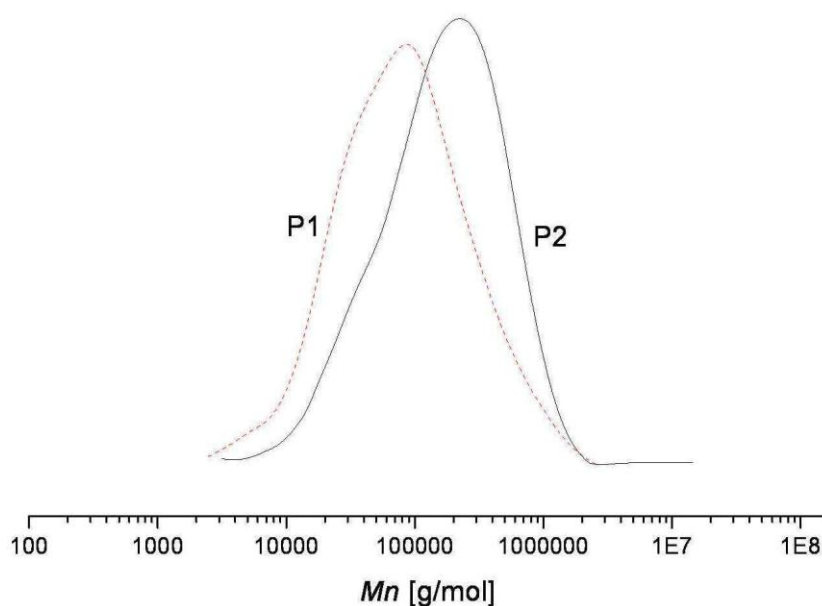
Figure 7: SEC traces of dissolved polystyrene (**P1**) and PMMA (**P2**) nanoparticles. Molecular weights were determined using THF as eluent and PMMA standards for calibration of sample **5B** and polystyrene standards for calibration of sample **5A**.

The dissolved polystyrene particles **5A** show a molecular weight of $Mn = 41000$ g/mol and the PMMA particles **5B** shows $Mn = 82000$ g/mol.

Conclusion:

In this work, glucose functionalized polymers **4** and polymer nanoparticles (**5A** and **5B**) were prepared via cascade reactions comprising enzymatically and chemically catalyzed steps. Transacylation of methyl acrylate as a substrate with glucose and galactose in the presence of Novozyme 435 lead to glucose functionalized acrylates **3** and galactose functionalized acrylates **3'** respectively, which later were copolymerized with methyl acrylate to prepare copolymers (**4** and **4'**) via free radical polymerization.

Furthermore, polymer nanoparticles (**5A** and **5B**) were prepared by emulsion polymerization using the glucose acrylates as reactive surfactants with styrene and methyl methacrylate as monomers without using a surfactant; stable nanoparticle emulsions were obtained.

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Summary

This thesis covers the synthesis and antimicrobial applications of azetidinium functionalized polymers. Synthesis of antimicrobial polymers is of great interest in current research due to their role to prevent infectious diseases and to solve hygiene related problems. Cationic azetidinium functionalized polymers are expected to be excellent candidates as antimicrobial polymers for preparing antimicrobial surface, as these polymers (e.g. Hercosett) are known as excellent candidates for surface coatings. However, the main challenge is to prepare well defined azetidinium functionalized polymers since the four membered rings are difficult to prepare and stabilize – and therefore only a few numbers of reports are found in literature.

In the current thesis we developed several approaches to prepare well defined multifunctional polymers, bearing azetidinium functional groups via post-polymerization modification of polyamines. As precursor polymers we used aminotelechelic polytetrahydrofuran (XTJ-548) and poly(vinyl amine). In the first part, different multifunctional polytetrahydrofurans, bearing azetidinium functional groups were prepared from aminotelechelic polytetrahydrofuran via two approaches:

(i)   Suitable and controlled reactions of the primary and secondary amine groups of the polytetrahydrofuran backbone with epichlorohydrin to form different well defined functional groups in the polymer backbone. The formation different functional groups was proven by the model reactions of hexyl amine and diethyl amine with epichlorohydrin.

(ii)   In the second approach, azetidinium functionalized polytetrahydrofurans were prepared using the coupler approach. A new bifunctional coupler was prepared by the reaction of piperazine and epichlorohydrin. Reactions of the bifunctional coupler with different low molecular weight primary and secondary amines used

as model reactions proved that azetidinium groups can be linked to amine building blocks. Using similar reactions well-defined azetidinium functionalized poltetrahydrofurans were prepared from aminotelechelic polytetrahydrofuran.

Next, the antimicrobial efficacy of these azetidinium functionalized polytetrahydrofurans was studied in solution and on surfaces. These polymers were found to be excellent candidates for antimicrobial textiles, as these polymer coated textiles (containing 0.5 wt. % of the polymer on the textile surface) showed 99.99 % -100 % bacterial growth inhibition against both *E.coli* and *S.aureus* before and after washings.

In the second part, different multifunctional PVAms, bearing azetidinium functional groups were prepared via the coupler approach. In the first part, the preparation of different multifunctional PVAm was demonstrated from simple one pot cascade reactions in water using the bifunctional coupler. Next, using the coupler concept, a library of antimicrobial multifunctional PVAms was prepared in water via cascade reactions. These modified PVAms have a well defined hydrophobically modified part, containing long alkyl chains and hydrophilic modified part, containing the cationic azetidinium groups. These polymers show excellent antimicrobial efficacy (MIC = 3-10 µg/mL, against both *S.aureus* and *E.coli*) with a good selectivity (HC_{50}/ $MIC_{99.99}$ = 5-7) to kill bacterial cells compared to human erythrocyte cells.

Beside this at the end of the thesis, a concept was demonstrated to prepare glucose functionalized core/shell nanoparticles via cascade reactions. In the first step, glucose functionalized methacrylate was prepared by the enzyme catalyzed transacylation of methacrylate with glucose. In the next step, glucose functionalized core/shell nanoparticles were prepared using glucose acrylate as reactive surfactant (surfmer) and methyl methacrylate or styrene as monomer, without using any external surfactants.

List of abbreviations:

AIBN   2,2' –azobisisobutyronitrile

ATRP   atom transfer radical polymerization

CDCl3   deuterated chloroform

D2O   deuterium oxide

DMF   N, N- dimethylformamide

DMSO   dimethylsulfoxide

FRP   free radical polymerization

GPC   gel permeation chromatography

h   hours

HC_{50} concentration of 50% cell lysis

IR   infra red

M_n number average molecular weight

M_w weight average molecular weight

MA   methyl acrylate

MeOD   deutereated methanol

MIC   minimum inhibitory concentration

MMA   methyl methacrylate

NMR   nuclear magnetic resonance

Owf   on weight of fabric

PET   poly(ethylene terephthalate)

PTHF   polytetrahydrofuran

PVAm   poly(vinyl amine)

r.T.   room temperature

SEC   size exclusion chromatography

St   styrene

TEM   transmission electron microscopy

THF   tetrahydrofuran

TMS   tetramethylsilane

Wt.   weight

δ   chemical shift

List of Publications:

Parts of this thesis are published, submitted for publication, patented or presented at conferences:

Patents:

- Chattopadhyay S., Keul H., Möller M., Durka M, Budzynski J.; Textile treatment compounds and Compositions, *UK patent application no. GB1216638.5*, **18.09.2012**

Publications:

- Chattopadhyay S., Keul H., Möller M, *Preparation of waterborne functional polymers using a bifunctional coupler,* <u>Green Chemistry</u> **2013**, DOI: 10.1039/c3gc41406j.

- Chattopadhyay S., Keul H., Möller M, *Synthesis of azetidinium functionalized polymers using a piperazine based couplers*, <u>Macromolecules</u> **2013**, 46, 638-646.

- Chattopadhyay S., Keul H., Möller M, *Functional polymers bearing reactive azetidinium groups: synthesis and characterization*, <u>Macromol. Chem. Phys</u>. **2012**, 213, 500-512.

- Chattopadhyay S., Heine E., Keul H., Möller M., *Antimicrobial Poly(vinyl amine)s bearing azetidinium functional groups: one pot preparation and structure-activity relation*, <u>Macromolecular Bioscience</u>, **2014,** DOI: 10.1002/mabi.201300576.

- Chattopadhyay S., Heine E., Keul H., Möller M., *Azetidinium functionalized polytetrahydrofurans: antimicrobial properties in solution and application to prepare antimicrobial surface*, <u>Polymers</u>, **2014**, 6(5), 1618- 1630 (**Special issue: Biomimetic Polymers**)

- Chattopadhyay S., Pargen S., Keul H., Möller M., *Preperation of glucose functionalized polymers and polymer nanoparticles: a synthetic approach using cascade reactions*, in preparation **2014.**

Conference Contributions:

- Subrata Chattopadhyay, Elisabeth Heine, Helmut Keul, Martin Moeller, *Synthesis of waterborne azetidinium functionalized multifunctional polymers via a coupler approach and their applications towards antimicrobial textile finishing,* EPF 2013, June 16-21 **2013**, Pisa, Italy (**Oral presentation**).

- Subrata Chattopadhyay, Sascha Pargen, Helmut Keul, Martin Moeller, *Synthesis of sugar-acrylates via enzymatic trans-acylation followed by the formation of sugar functionalized poly(meth)acrylates via cascade reaction*, POLYCHAR 20 World Forum on Advanced Materials, March 26-30 **2012,** Dubrovnik, Croatia (**Poster presentation**).

- Subrata Chattopadhyay, Elisabeth Heine, Helmut Keul, *Adhesion promoting polymers for surface activation*, Innovation convention 2011, December 5-6 **2011,** Brussels, Belgium **(Poster presentation)**.

- Subrata Chattopadhyay, Helmut Keul, Martin Moeller, *Multi-functional polymers, bearing reactive azetidinium groups: synthesis and characterization*, Aachen-Dresden International textile conference, November 24-25 **2011**, Aachen, Germany (**Poster presentation**).

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