From Cyclic Carbonate Bifunctional Couplers to Amphiphilic Poly(ethylene imine)s

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

vorgelegt von

Diplom-Chemikerin
YingChun He

aus Tianjin, V. R. China

Berichter: Universitätsprofessor Dr. rer. nat. Martin Möller
Universitätsprofessor Dr. rer. nat. Doris Klee

Tag der mündlichen Prüfung: 6. Februar 2012

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Acknowledgement

Die vorliegende Arbeit entstand in der Zeit von June 2006 bis Dezember 2010 am Institut für Technische und Makromolekulare Chemie der RWTH Aachen.

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Herr Phong Thahn Phan, und Herr Subrata Chattopadhyay von den ‚neuen Generation' bedanken, deren Diskussionsfreudigkeit, Hilfsbereitschaft und Kollegialität erheblich dazu beigetragen haben, viele Dinge gelassener zu betrachten.

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Curiosity changes attitudes, crosses borders and climbs to new horizons. It makes science the most exciting thing in the world. Be curious!

To My Parents

List of Publications

Parts of this thesis are published, submitted to be published and presented at conferences.

Articles:

1. *Synthesis, characterization, and selectivity of bifunctional couplers,* YingChun He, Vishal Goel, Helmut Keul, Martin Möller, *Macromol. Chem. Phys.* **2010**, 22, 2366-2381

2. *Synthesis, characterization, and application of a bifunctional coupler containing a five- and a six-membered ring carbonate*, YingChun He, Helmut Keul, Martin Möller, *React. Funct. Polym.* **2011**, 71 (2), 175-186

3. *Amphiphilic building blocks as head groups in linear polymers,* YingChun He, Helmut Keul, Martin Möller, *Eur. Polym. J.*, **2011**, 47 (8), 1607-1620

4. *Synthesis and characterization of amphiphilic monodispers compounds and poly(ethylene imine)s: influence of their microstructures on the antibacterial properties,* YingChun He, Elisabeth Heine, Nina Keusgen, Helmut Keul, Martin Möller, *Biomacromolecules,* **2012**, 13 (3), 612-623

5. *Synthesis and characterization of ionic/hydrophilic modified poly(ethylene imine)s and visualization of their single molecule absorption structures on Mica,* YingChun He, Ahmed Mourran, Helmut Keul, Martin Möller, *in preparation*

6. *Efficiency/Properties of antimicrobial polymers analogous with the same composition but different microstructures*, Mériem Er Rafik, YingChun He, Helmut Keul, Martin Möller, *in preparation*

Posters:

1. *From cyclic carbonates to mikto-arm star polymers*, YingChun He**,** Helmut Keul, Martin Möller, Aachen-Dresden International Textile Conference **2008**, Dresden

2. *Biomimetic amphiphilic branched poly(ethylene imines) (PEI) as antimicrobial agents*, Meriém Er Rafik, YingChun He, Nicolas Pasquier, Helmut Keul, Elisabethe Heine, Martin Moeller, Aachen-Dresden Interna-tional Textile Conference **2008**, Dresden

3. *New Carbonate Couplers*, Alexandra Mendrek, YingChun He, Nicolas Pasquier, Helmut Keul, Martin Möller, EPF European Polymer Congress, **2007**, Portorož (Slovenia).

Patents:

1. *Preparation of 4-[[(aminocarbonyl)oxy]methyl]-1,3-dioxolan-2-ones*, Andreas Job, Martin Moeller, Helmut Keul, Aleksandra Mendrek, Nicolas Pasquier, YingChun He; Ger. Offen. (**2008**), DE 102007023867 A1 20081127

2. *Preparation of 4-[[(aminocarbonyl)oxy]methyl]-1,3-dioxolan-2-ones*, Andreas Job, Martin Moeller, YingChun He, Aleksandra Mendrek, Nicolas Pasquier, Helmut Keul; PCT Int. Appl. (**2008**) WO 2008142097 A2 20081127

List of Abbreviations

°C	degree celsius
AcOEt	ethyl acetate
AFM	atomic force microscopy
ATRP	atom transfer radical polymerisation
br	broad signal (NMR)
CAC	critical aggregation concentration
CMC	critical micellization concentration
d	day(s)
d	doublet (NMR)
Da	Dalton
DCM	dichloromethane
DLS	dynamic light scattering
DMF	N,N-dimethylformamide
DP	degree of polymerisation
DSC	differential scanning calorimetry
eq.	equivalent(s)
Et_2O	diethyl ether
EtOH	ethanol
FT-IR	Fourier transform infrared spectroscopy
GPC	gel permeation chromatography
h	hour(s)
J	coupling constant (NMR)

k	rate constant
m	multiplet (NMR)
MeOH	methanol
MIC	minimal inhibitor concentration
Mn	number average molecular weight
Mw	weight average molecular weight
n.d.	not determined
NMP	N-methyl-pyrrolidone
NMR	nuclear magnetic resonance
PBS	phosphate buffer saline
PEI	poly(ethylene imine)
ppm	part per million
q	quartet (NMR)
r.t.	room temperature
R_h	hydrodynamic radius
RT	room temperature (21 °C)
s	singlet (NMR)
t	time
t	triplet (NMR)
TGA	thermogravimetric analysis
THF	tetrahydrofuran
TMS	tetrametylsilane
wt	weight
δ	chemical shift (NMR)
λ	wavenumber

Summary

This dissertation is concerned with two subjects: bifunctional couplers and multifunctional poly(ethylene imine)s (Figure 1). Bifunctional couplers discussed in this thesis are mostly bearing at least one five-membered ring carbonate. These bifunctional couplers were suitable for the selective combination of two functional primary amines. Via addition or substitution reactions of bifunctional couplers with functional amines, functional couplers with different properties bearing a five-membered ring carbonate were obtained. These functional couplers were used to prepare tailor made multifunctional poly(ethylene imine)s by ring-opening reaction of functional five-membered ring carbonates with poly(ethylene imine)s.

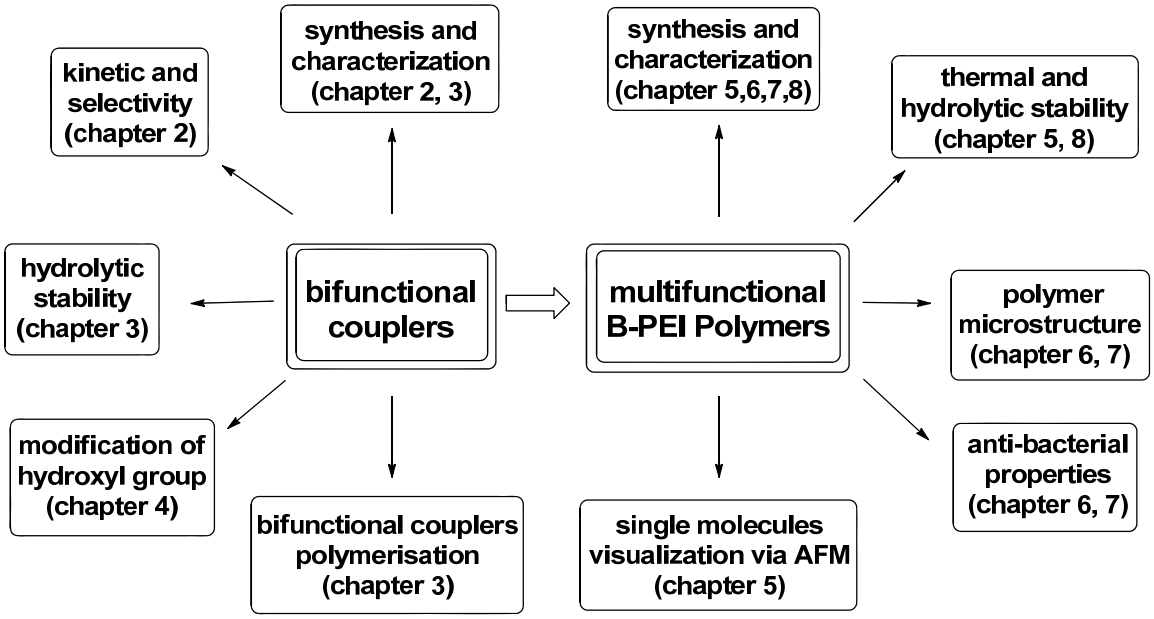

Figure 1: Graphic summary of the content of this thesis

Based on the chemistry of cyclic carbonates, phenyl carbonate, chloroformates, and epoxides, in total 10 bifunctional couplers (**A1-A5**, **B1-B3**, **C1-C2**, and **BC56**) with different coupling concepts – substitution/addition or addition/addition – were synthesized (Figure 2). Their reactivity and selectivity towards amines were studied. The hydrolytic stability of **BC56** and functional couplers synthesized from **BC56** were tested. In the ring opening reaction of cyclic carbonates with amines, urethane groups with an adjacent hydroxyl group were formed. The modification of these hydroxyl groups were studied in chapter 4. The application of bifunctional coupler **BC56** for the synthesis of hydroxyl functional polyurethanes was discussed in chapter 3.

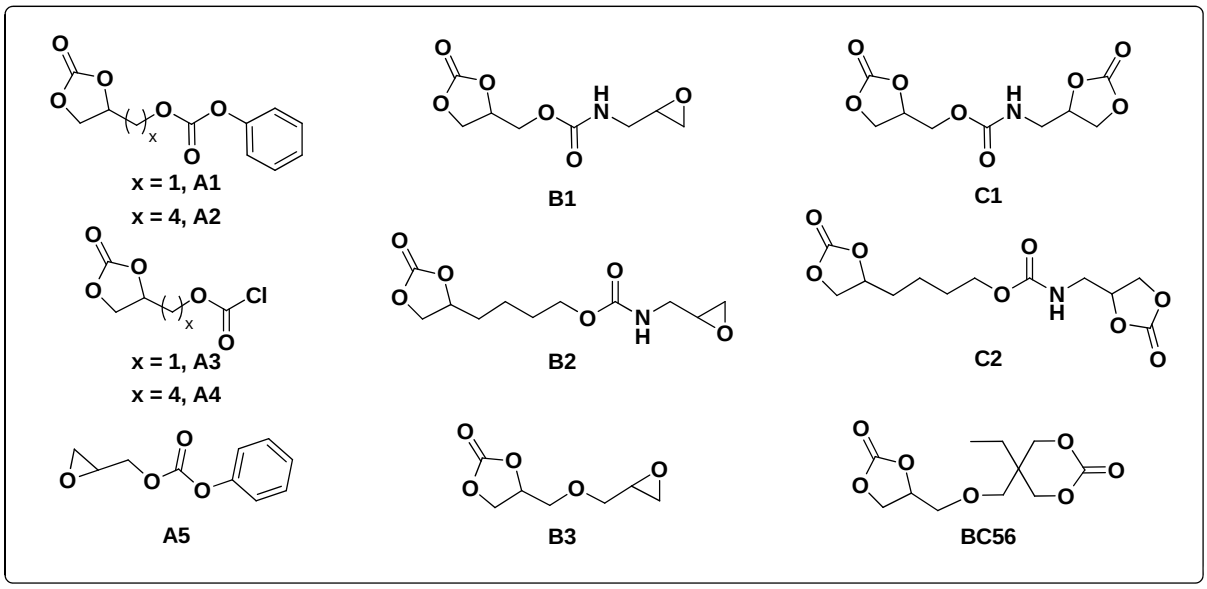

Figure 2: Bifunctional couplers synthesized and discussed in this thesis

Functional cyclic carbonates prepared from **A1 – A4** were used for the modification of poly(ethylene imine)s. Multifunctional poly(ethylene imine)s bearing functional groups such as hydrophobic chains of various lengths, hydrophilic groups, cationic (ammonium) groups, amphiphilic groups, and/or labeling groups (pyrene) were synthesized und characterized.

Multifunctional poly(ethylene imine)s can be obtained in one-step synthesis or in sequential addition synthesis by reaction with different kind of functional cyclic carbonates. The influence of the preparation process on their microstructure and antimicrobial properties was studied in chapter 6. In the antimicrobial process, cationic and hydrophobic functional groups interact both with the bacterial membrane. The way of cationic and hydrophobic groups linked to each other and linked on the poly(ethylene imine) molecules influenced the antimicrobial efficiency of the cationic/hydrophobic modified small compounds and poly(ethylene imine)s (chapter 7).

In order to study the absorption of multifunctional poly(ethylene imine)s on negatively charged surfaces, single molecules of multifunctional poly(ethylene imine)s were visualized on mica substrates via atomic force microscopy (AFM) (in chapter 5). The properties of multifunctional poly(ethylene imine)s in thin layers and in water solution and their hydrolytic and thermal stability were investigated in chapter 8.

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Chapter 1

Introduction

1.1 Bifunctional couplers – Chemistry of cyclic carbonates, chloroformates, and epoxides: reactions with amines

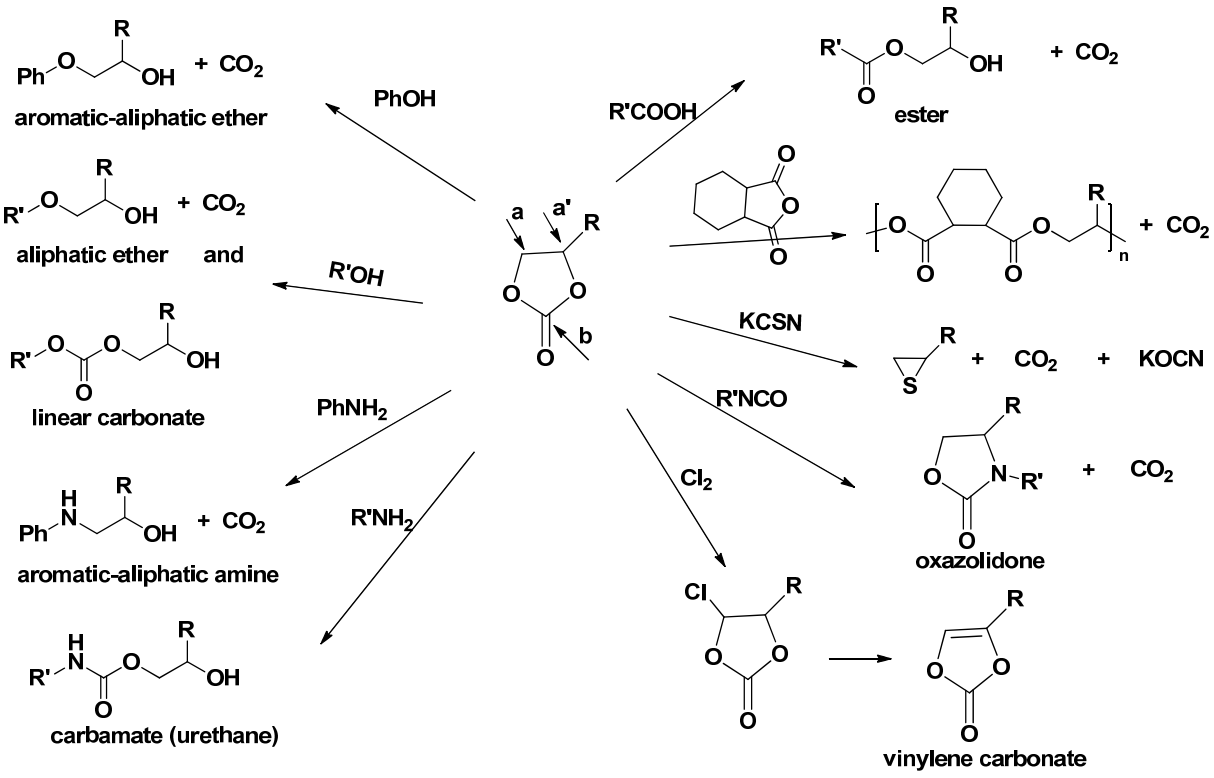
In the past, bifunctional couplers based on different chemistry were developed in several groups. In the group of Böhme[1], bifunctional couplers with one oxazoline group that reacts with carboxylic acid groups and one oxazinone group that reacts with hydroxyl or amino groups were developed. It was evidenced by highly selective model reactions. Moreover, these reactions can be controlled by temperature. In the groups of Bieniarz[2] and Reddy[3], a series of bifunctional coupler of various length were synthesized for covalent binding of proteins by active ester carbonyl-amine and maleimide-thiol bonds. In our group, the development of bifunctional couplers was based on five-membered ring carbonates and used for the selective conversion of primary amines [4-8]. In this work, bifunctional couplers with a combination of cyclic carbonate/cyclic carbonate, cyclic carbonate/phenyl carbonate, cyclic carbonate/chloroformate, and cyclic carbonate/epoxide were developed for the selective conversion of primary amine. In the following, the chemistry of these different functional groups with amines is discussed.

(1) Cyclic carbonates and their reactions with primary amine

The chemistry of cyclic carbonates has been explored since 1930s, which has come to be a rich area of research within the past 30 years[4, 5]. Carbon dioxide, aliphatic or aromatic esters of carbonic acid and phosgene derivatives are usually used as a source of carbonate groups in the synthesis of cyclic carbonates[6, 7]. Cyclic carbonates have been used in the preparation of polycarbonates[8], copolymers, composites[9], and also used as reagents and modification agents. Because of their biocompatibility, low toxicity, and biodegradability, homopolymers and copolymers of five-membered carbonates and six-membered carbonates with cyclic esters (lactones and lactides) have been found to be good materials for biomedical applications. The volume expansion effect of cyclic carbonates during the ring-opening polymerizations makes such polymers of particular interest in adhesive and mould filling applications[10].

Cyclic carbonates react with amines to afford the corresponding hydroxyurethanes[11-16]. The reactivity of cyclic carbonates toward amines is dependent on the ring-size and its strain as well as the kind and number of substituents[4]. Under the same reaction condition, the reaction of six-membered ring carbonates with amines is much faster than that of five-membered ring carbonates.

The reaction of five-membered cyclic carbonates with nucleophilic reagents proceeds in two manners[4]. Depending on the nucleophilicity of the reagent, the cyclic carbonate structure and the reaction temperature, a carbon atom neighbouring an oxygen atom (pathway **a** or **a**') or a carbonyl group (pathway **b**) of five-membered cyclic carbonate is attacked. In Scheme 1, the reactions of five-membered cyclic carbonates with different nucleophilic agents are summarized.

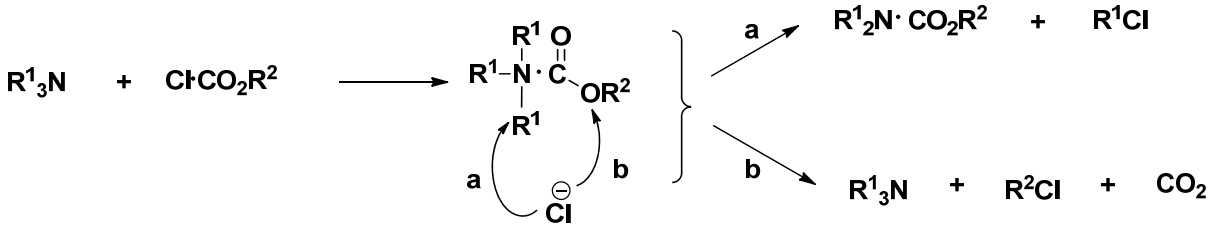


Scheme 1: Summary of the reactions of five-membered cyclic carbonates with different nucleophilic agents[4]

(2) Chloroformate and their reactions with amines[17-21]

Phosgene reacts with aliphatic hydroxyl compounds at room temperature or below to result in chloroformates in high yield. Low temperatures are preferred in many instances to minimize formation of dialkyl carbonates[18, 21]. This synthesis is usually carried out in the presence of a tertiary amine as the hydrogen chloride acceptor, especially when tertiary alcohols are used. Removal of the hydrogen chloride in this way minimizes acid-catalyzed side reactions in the case where acid-sensitive groups are present, and also increases the rate of conversion. Chloroformates can react with water, alkali metal hydroxides, alcohols and thiols, ammonia and amines, amino acid, amides, urethanes…under different conditions.

For the reactions of chloroformates with amines: (i) Primary and secondary amines react rapidly with chloroformates to yield the corresponding urethane compounds. Organic (excess amine) or inorganic bases can be used to remove the formed hydrogen chloride [19, 20]. Aromatic primary and secondary amines react with chloroformates under the same conditions as aliphatic amines. However, since aromatic amines are weaker bases (and weaker nucleophiles), the reactions are slower than with aliphatic amines. (ii) Toward tertiary amines there are two competitive ways of reactions (s. **Scheme 2**): **a)** dealkylation and **b)** decarboxylation[17, 18]. The reaction of chloroformates with tertiary amines in route a) often provides a convenient method for promoting dealkylation alternative to the better known von Braun cyanogen bromide cleavage. The ethyl and benzyl chloroformates have been most commonly used for this purpose, either in non-polar media, or under less generally useful Schotten-Baumann conditions. Phenyl chloroformate is much more effective than the alkyl chloroformate, partly due to the suppression of the competitive reaction ***b.***



Scheme 2: Two routes of reaction of chloroformate with tertiary amine[17].

(3) Epoxide and amines[22-26]

Epoxides are cyclic ethers with a highly strained equilateral triangle. Simple epoxides are named as derivatives of oxirane (ethylene oxide); when the epoxide is part of another ring system, it is shown by the prefix epoxy-; the common names are derived from the name of the alkene from which the epoxide is formally derived[24]. For the synthesis of epoxide there are

two different methods: alkylene epoxidation and ring closure. For the epoxidation method: ethylene oxide, one of the few epoxides manufactured on an industrial scale, is prepared by air oxidation of ethylene in the presence of catalysts; the most common laboratory method is oxidation of an alkene using a peroxycarboxylic acid (a peracid), like *meta*-chloroperoxybenzoic acid (*m*CPBA), peroxyacetic acid, or hydrogen peroxide, etc. The ring closure method for the synthesis of epoxide was carried out via halohydrins[27, 28].

Because of the strain associated with the three-membered ring, epoxides readily undergo various ring-opening reactions. The ring opening reaction of epoxide can be carried out in both acidic and basic condition. Depending on the reaction conditions, the reaction follows S_N1 (acidic condition) or S_N2 (basic condition) mechanism (Figure 1).

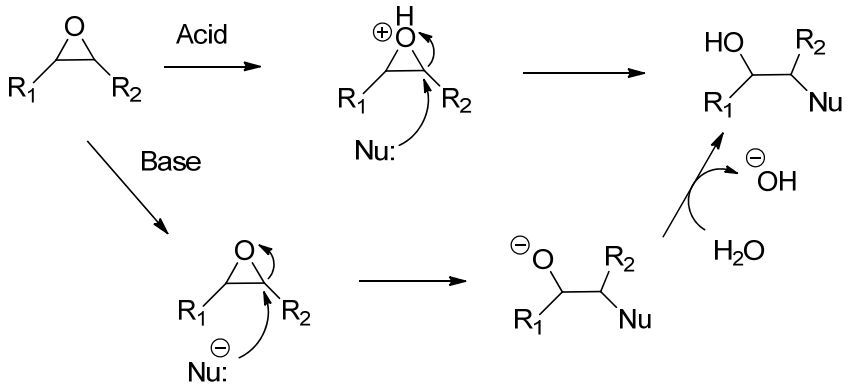


Figure 1: mechanism of epoxide ring opening reaction under different conditions[24]

The reactivity of the epoxide ring and amino groups depends on the reaction conditions (basic, neutral, or acidic), the basicity of the amine nucleophile and the steric hindrance due to substituents at the amine nitrogen atom. Epoxides react with primary, secondary, and tertiary amines under different conditions (Figure 2)[26, 29-31]. For the reaction of epoxide with primary amines, because of different following reactions, even at an equimolar ratio, mixtures of different products are obtained[19, 32].

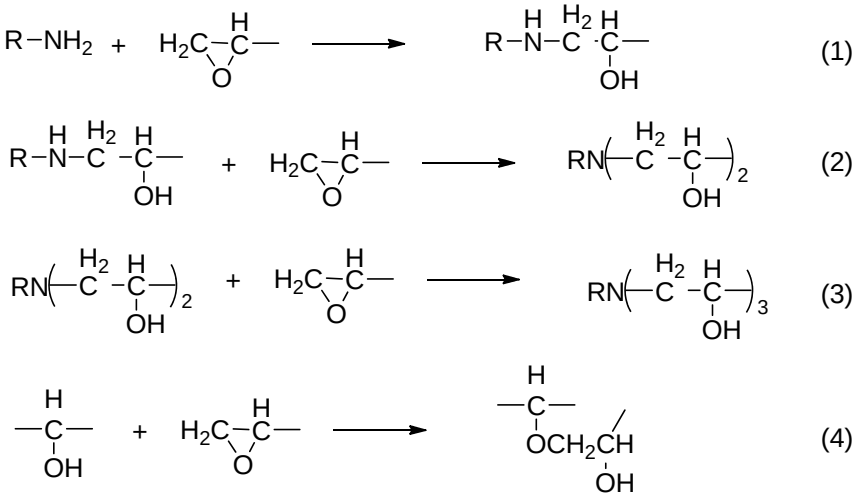


Figure 2: Epoxide/primary amine reaction scheme: (1) primary amine with epoxide; (2) formed secondary amine with epoxide; (3) formed tertiary amine with epoxide[30]; (4) formed hydroxyl group with epoxide

1.2 Single molecules study and atomic force microscopy (AFM)

A single-molecule study investigates the properties of an individual molecule and is contrasted with measurements on an ensemble or bulk collection of molecules, where the individual behaviour can not be distinguished and only average characteristics can be measured. Single molecule study of polymers opens new possibilities to collect important information for polymer and colloidal science[33-36]. Understanding the behaviour of single polymer chains in solution and in adsorbed state is crucial for adsorption processes, chromatography and other separation methods, design and applications of new advanced materials and technologies such as drug delivery systems, sensors, responsive materials, catalysts, miniaturized devices, lab-on-chip and micro fluidic technologies.

Since the 1990s, many techniques for probing single molecules were developed, such as atomic force microscopy (AFM)[37, 38], laser optical tweezers (LOTs)[39, 40], magnetic tweezers

(MTs)[41], biomembrane force probes (BFPs)[42] and single-molecule fluorescence (SMF)[43]. Nowadays, the most widespread and commercially available single-molecule technique in laboratories is the atomic force microscope (AFM).

Atomic force microscope (AFM) was invented by Binnig, Quate, and Gerber in 1985. The original AFM consisted of a diamond shard attached to a strip of gold foil. The diamond tip contacted the surface directly, with the interatomic van der Waals forces providing the interaction mechanism. Detection of the cantilever's vertical movement was done with a second tip – an STM placed above the cantilever. Today, most AFMs use a laser beam deflection system, introduced by Meyer and Amer, where a laser is reflected from the back of the reflective AFM lever and onto a position-sensitive detector. AFM tips and cantilevers are microfabricated from Si or Si_3N_4. Typical tip radius is from a few to 10s of nm.

The AFM can be used to study a wide variety of samples (i.e. plastic, metals, glasses, semiconductors, and biological samples such as the walls of cells and bacteria). Unlike STM or scanning electron microscopy, it does not require a conductive sample. AFM provides a 3D profile of the surface on a nanoscale, by measuring forces between a sharp probe (<10 nm) and surface at very short distance (0.2–10 nm probe-sample separation). The probe is supported on a flexible cantilever. The AFM tip "gently" touches the surface and records the small force between the probe and the surface.

1.3 Basics of bacteria, bacterial membrane, and antibacterial peptide

Bacteria are a large domain of single-celled, prokaryote microorganisms. Typically a few micrometers in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals (Figure 3)[44]. Bacteria are ubiquitous in every habitat on Earth, growing in soil, acidic hot springs, radioactive waste, water, and deep in the Earth's crust, as well as in organic

matter and the live bodies of plants and animals. Bacteria are of immense importance because of their extreme flexibility, capacity for rapid growth and reproduction, and great age — the oldest fossils known, nearly 3.5 billion years old, are fossils of bacteria-like organisms.

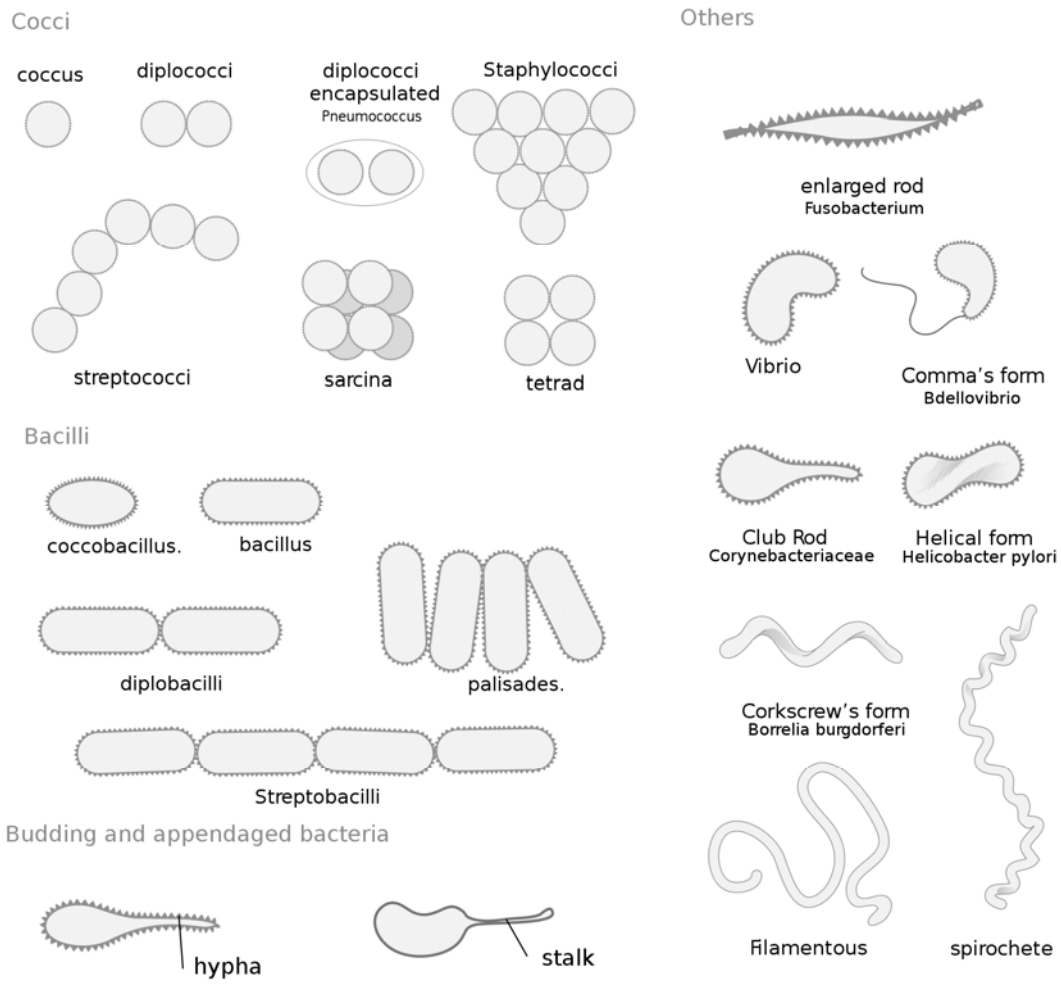


Figure 3: Bacteria morphology diagram[44]

Bacterial cells are about one tenth the sizes of eukaryotic cells and are typically 0.5–5.0 micrometers in length. The bacterial cell is surrounded by a lipid membrane, or cell membrane, which encloses the contents of the cell and acts as a barrier to hold nutrients, proteins and other essential components of the cytoplasm within the cell. Around the outside of the cell membrane is the bacterial cell wall. Bacterial cell walls are made of peptidoglycan, which is made from polysaccharide chains cross-linked by unusual peptides containing D-

amino acids. Bacterial cell walls are different from the cell walls of plants and fungi, which are made of cellulose and chitin, respectively. The cell wall is essential for the survival of many bacteria, and the antibiotic penicillin is able to kill bacteria by inhibiting a step in the synthesis of peptidoglycan.

Broadly speaking there are two different types of cell wall in bacteria, called Gram-positive and Gram-negative. The names originate from the reaction of cells with the Gram stain, a test long employed for the classification of bacterial species, named after the 19th century Danish bacteriologist (Hans Christian Joachim Gram**)** who developed it. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

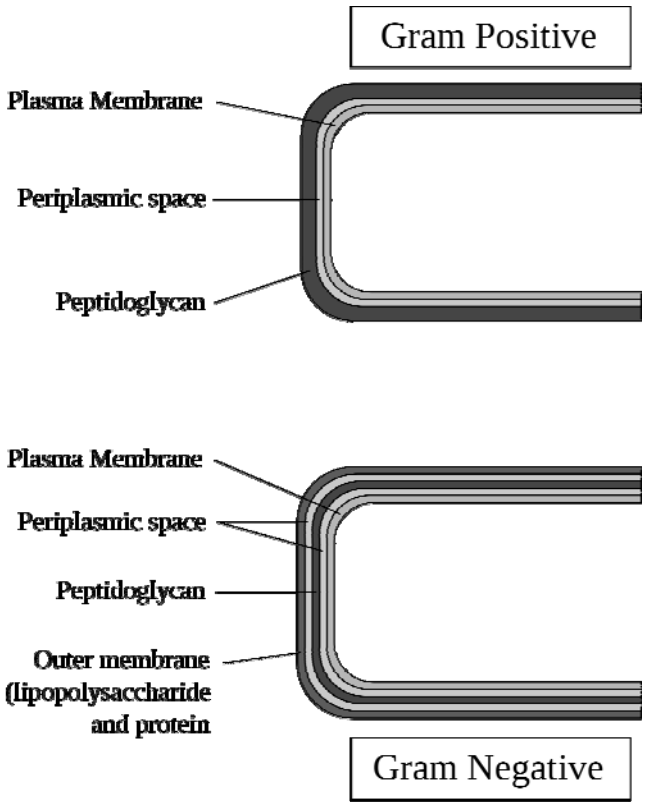


Figure 5: Gram positive and Gram negative cell wall structures

Antimicrobial peptides (AMPs)[45-47] are natural substances produced by many organisms to fight bacterial infection. These peptides have a variety of antimicrobial activities ranging from disrupting the bacteria cell membrane to disturbing biochemical processes inside the bacteria. AMPs may disrupt the membrane of their target cell in two ways: 1) By inserting into the membrane and undergoing polymerization inside it to form pores/channels and leads to depolarization of the membrane and to cell death. 2) By partially penetrating the membrane and compromising its structural integrity and leading to cellular death.

Both mechanisms of membrane disruption mentioned above rely on the AMP's physico-chemical properties. For example, many AMPs carry a positive charge, which allows them to bind preferentially to membranes carrying a negative electric charge on their external surface. This includes mainly bacterial membranes, but also those of mammalian cancer cells. As a result, AMPs are selective against both bacteria and cancer cells over normal mammalian cells. Moreover, many AMPs are α-helical and amphipathic, which means they have hydrophilic (usually basic) residues on one side, and hydrophobic residues on the other. Thus, these AMPs are positively charged only on one side. The amphipathic nature of AMPs is crucial to their mechanism of membrane disruption; it allows them to insert partly or completely into the lipid bilayer component of the membrane, where their hydrophobic side interacts with the lipid core of the bilayer, and their hydrophilic side interacts with the polar heads of membrane lipids.

1.4 Content of this thesis

This thesis is concerned with the synthesis and application of different types of cyclic carbonate based bifunctional couplers and the synthesis, characterization, and applications of amphiphlic poly(ethylene imine)s.

Chapter 1 (the present chapter) gives a short introduction on bifunctional couplers, atomic force microscopy, bacteria and antibacterial polymers, and presents the content of this work.

In **Chapter 2**, synthesis and characterization of bifunctional couplers based on carbonate chemistry is described. These bifunctional couplers combine molecules bearing amine groups via substitution and addition reaction (**Series A**) or only via addition (**Series B** and **C**). The reactivity and selectivity of the two functional groups of couplers toward primary amines in different conditions was studied. The reactivity of couplers of **Series B** towards secondary and tertiary amines was investigated. The effect of adjacent groups (α/δ position to O/N sides of urethane group or α position to ether groups) on the reactivity of the five-membered cyclic carbonates was studied using model compounds.

In **Chapter 3**, bifunctional coupler 5-ethyl-5-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)-1,3-dioxan-2-one (**BC56**) bearing a five- and six-membered ring carbonate was successfully synthesized and characterized. Model reactions of **BC56** with primary amine were carried out. Ionic, hydrophilic and hydrophobic functional couplers were synthesized. Polycondensation reaction of **BC56** with diamines yielded poly(hydroxyl urethane)s or its oligomers. The reaction conditions were optimized.

In **Chapter 4**, starting with asymmetric A,A´-carbonate bifunctional couplers via substitution reaction followed by addition reaction with primary amines, bis(functional) compounds with a free hydroxyl group were prepared. These compounds were used: (i) as initiators for ring-opening polymerization of ε-caprolactone; (ii) as substrate for the preparation of an ATRP initiator and application for MMA polymerization; (iii) as substrate for the preparation of an activated carbonate for grafting on an amine end-functionalized linear polymer (Jeffamine®

M1000). Via modifications (i) – (iii), amphiphilic end-functionalized poly(ε-caprolactone)s, poly(methyl methacrylate)s, and linear polymer Jeffamine® M1000 were prepared, respectively.

Chapter 5 describes the synthesis and characterization of ionic/hydrophilic modified poly(ethylene imine)s. Their single molecules absorption on mica surface from aqueous solution were studied via atomic force microscopy. The molecular weight and weight distribution was calculated via counting of single molecules.

In **Chapter 6**, the influence of the preparation processes on the microstructure of the obtained amphiphilic poly(ethylene imine)s were discussed. Via FT-IR and NMR analysis the influence of the preparation process on chemical structure was studied. Comparing the MIC value of polymers with the same composition but prepared by different procedures, the influence of preparation processes on the biological activity were discussed.

In **Chapter 7** two series of small amphiphilic compounds with cationic groups and alkyl chains with different microstructures were synthesized. Their MIC against *E .coli*, *B. subtilis*, and *S. aureus* were tested and compared. Starting from these amphiphilic compounds two amphiphilic poly(ethylene imine)s with different microstructures were synthesized and their antibacterial properties were tested and compared with the known amphiphilic poly(ethylene imine)s.

In **Chapter 8** the results on the BMBF project 'Easy Care', the use of amphiphilic polyethyleimine as laundry detergent ingredient for textile washing were summarized.

In **Appendixes** supplementary materials of Chapter 2 and Chapter 3 are provided.

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Chapter 2

Synthesis, Characterization, and Selectivity of Bifunctional Couplers

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2.1 Introduction

Functional and responsive polymers present a topic of increasing importance in polymer science since the end of the last century.[1] Functionalization of polymers is often used for compatibilisation of polymer blends, for modification of solubility properties, for the preparation of polymers for release systems and biomedical applications in general. Several strategies were developed for the preparation of functional polymers: (i) copolymerization of functional monomers; (ii) polymer analogous reactions on polymers bearing reactive groups; (iii) copolymerization of monomers with protected functional groups followed by selective deprotection and further functionalization. Polymer analogous reactions (post-synthetic modifications) are intensively studied in polymer chemistry. Thus, multifunctional polymers with tailored properties for specific applications were prepared. One of the procedures applied to prepare multifunctional polymers is based on couplers (**Figure 1**). In the past, an asymmetric A,A´-carbonate coupler ((2-oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate**, A1**) was developed in our group to selectively combine monodisperse functional amine building blocks and polyamines within a single molecule.[2-10] At low temperatures (0-25°C) the phenyl carbonate of the bifunctional coupler was first substituted selectively with hydrophilic, hydrophobic or ionic primary amines to result in functionalized couplers, or with polyamines to result in a polyamine-coupler-adduct. Then, at higher temperatures (60-80°C) the

functionalized couplers are reacted with a polyamine, respectively, the polyamine-coupler-adducts are reacted with amine building blocks to produce multifunctional polymers. During these reactions the cyclic carbonate ring is opened and a urethane group with an adjacent hydroxyl group is formed. Based on coupler **A1**, several functionalized couplers were prepared and successfully used for the modification of branched poly(ethylene imine) (B-PEI),[2-6, 10] chitosan, [8] and aminofunctional polysiloxane.[9]

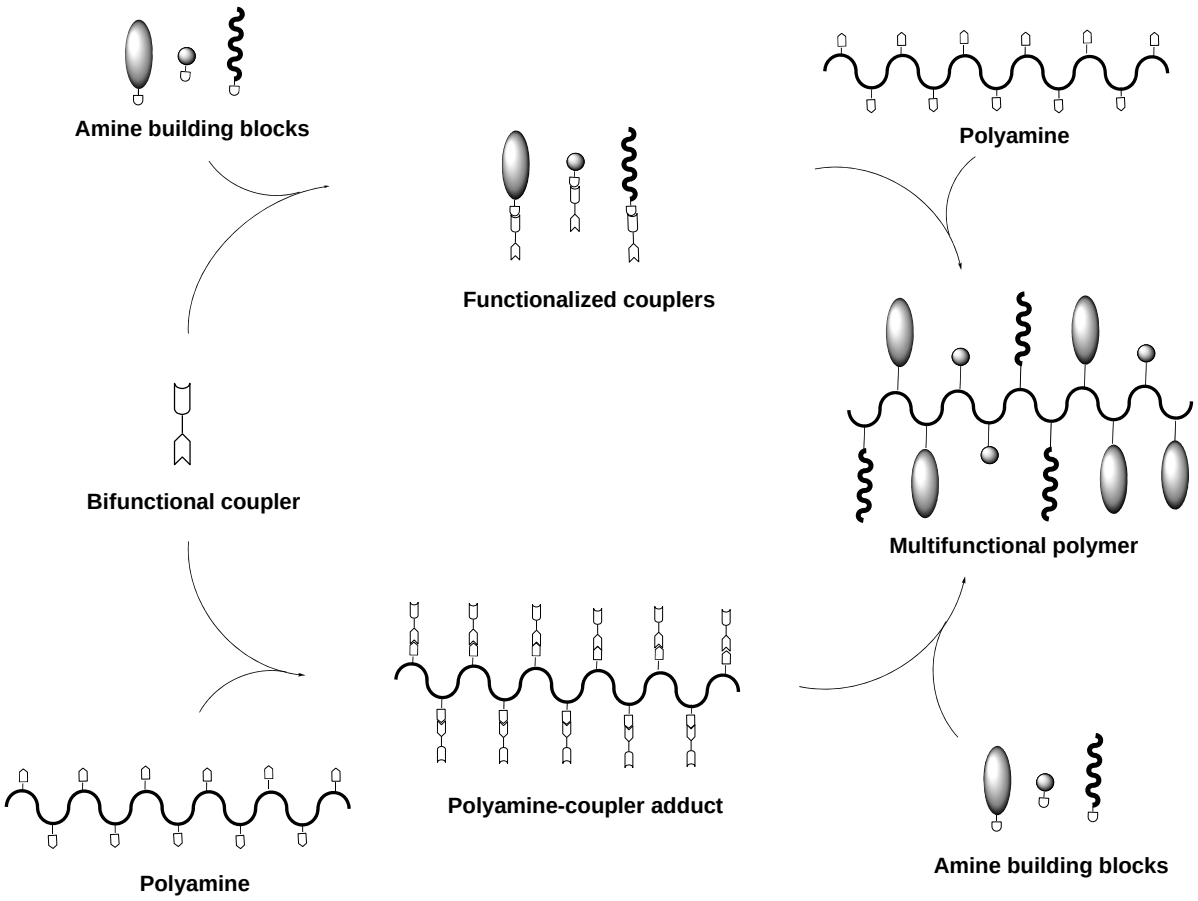
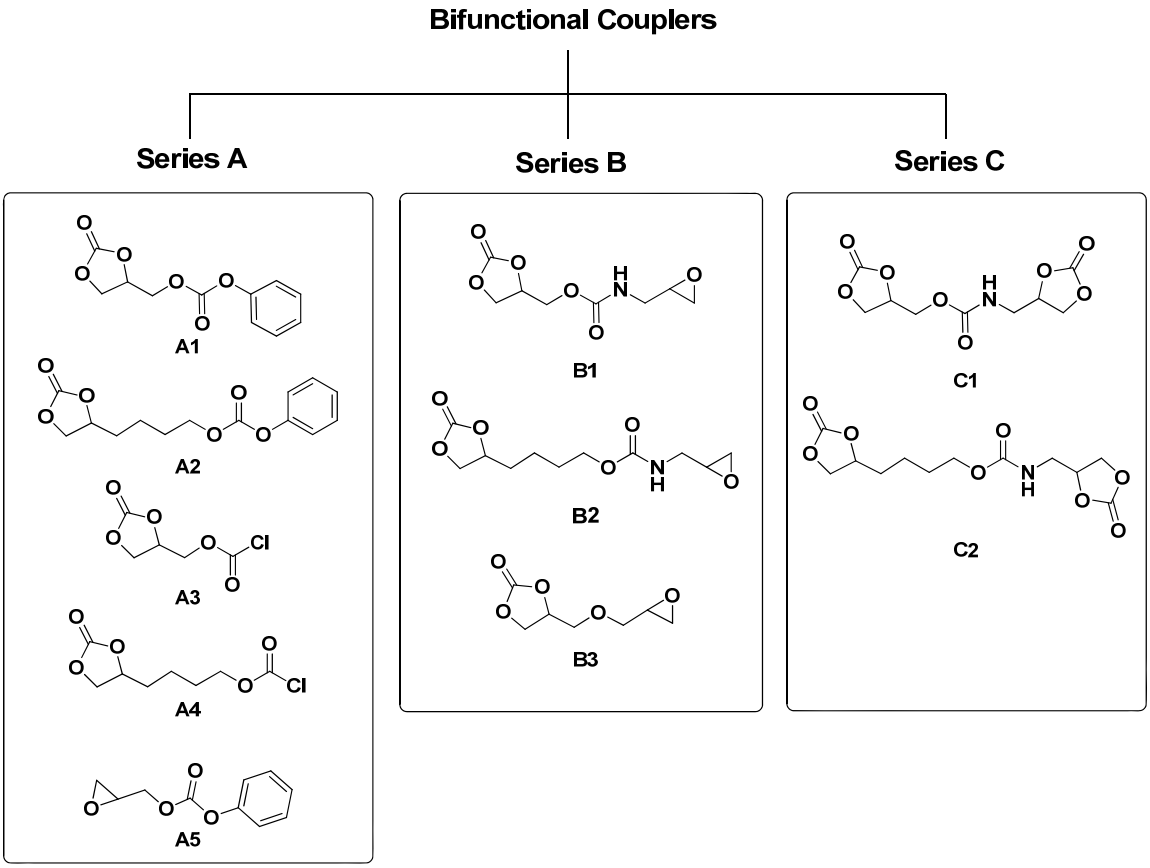


Figure 1: Synthesis of multifunctional polymers based on polyamines and amine building blocks using bifunctional couplers (e.g. (2-oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate, **A1**)

Polymers bearing amine groups so called polyamines are an interesting class of the polymers. Branched poly(ethylene imine) (B-PEI) is a polyamine containing primary, secondary and

tertiary amine groups[11] with a wide field of applications: paper production, dye fixation in textile processing, pigment dispersant, printing inks, lubricant in fiberglass production, laminated packaging films and so on.[12] In microbiological studies, B-PEI is also mentioned as membrane permeabilizer[13] and is one of the most efficient nonviral vectors for gene delivery.[14-16] Besides B-PEI different polyamines are commercially available: linear poly(ethylene imine) (L-PEI), polyvinylamine (PVAm), polyallylamine (PAAm), dentritic polypropyleneimine (PPI), chitosan and polylysine. These polyamines differ in their microstructure, their architecture and the content of primary, secondary and tertiary amine groups. L-PEI is a polymer containing only secondary amine groups, PVAm has only primary amino groups linked directly to the polymer backbone, PAAm has amino methyl groups attached to the main chain, and PPI dendrimers contain tertiary and primary amine groups. Chitosan is a polysaccharide, composed of ß-linked glucosamine and N-acetyl glucosamine units and is derived from chitin, the second most abundant polysaccharide in nature. Different structures of polylysine are available or can be synthesized: α-poly(L-lysine), ε-poly(L-lysine), branched poly(L-lysine).[17]

The carbonate coupler **A1** was successfully used in reactions with primary amine groups in B-PEI, some preliminary results concerning the modification of PVAm, PAAm, chitosan and polylysine were also reported,[6, 8] however, **A1** can not be used for the modification of L-PEI and the secondary amine groups of B-PEI, because both functional groups of this coupler are non reactive to secondary amines. Further, upon reaction of coupler **A1** with amines, phenol is produced as side-product. Even though the leaving phenol can be removed by crystallization of the functionalized couplers or precipitation of the multifunctional polymers, for some applications residual phenol might be a problem. As a consequence further bifunctional couplers for the modification of polyamines are needed.



Scheme 1: Structures of bifunctional couplers. **Series A**: coupling occurs via substitution and addition reaction; **Series B** and **C**: coupling occurs only via addition reaction.

In this work new couplers were synthesized and characterized that combine molecules bearing amine groups by substitution and addition reaction (**A-Series**) and that combine these molecules only via addition reaction (**Series B** and **C**) (**Scheme 1**). The reactivity of cyclic carbonate and epoxide groups towards primary, secondary and tertiary amine groups was studied and the influence of the spacer between the two functional groups on the reactivity of the couplers was examined.

2.2 Experimental Part

2.2.1 Materials

Glycerol (Aldrich, 99.5 %), glycidol (Fluka, 95 %), phenyl chloroformate (Acros Organics, 99 %), pyridine (Fluka, 99.8 %), dimethyl carbonate (Acros Organics, 99 %), 1,4-diazabicyclo[2,2,2]octane (DABCO, Aldrich, 98 %), allyl amine (Aldrich, 98 %), 3-chloroperoxybenzoic acid (*m*CPBA, Acros, 70-75 %), N,N-dimethyl-1,3-propanediamine (Aldrich, 99 %), iodomethane (Aldrich, 99 %), dihexylamine (Aldrich, 97 %), trihexylamine (Aldrich, 97 %), hexylamine (Aldrich, 97 %), triethylamine (Aldrich, 99.9 %), dodecylamine (Acros, 98 %), 3-(allyloxy)propane-1,2-diol (Aldrich, 99 %), allyl bromide (Fluka, 98 %), 1-dodecanol (Fluka, 98.5 %), silica gel 60 (AppliChem, 0.063 mm-200 mm) were used as received.

2.2.2 Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on a Brucker DPX-400 FT-NMR spectrometer at 400 and 101 MHz, respectively. Some of the spectra were recorded on a Brucker DPX-300 FT-NMR spectrometer at 300 and 75 MHz.

Carbon, hydrogen and nitrogen elemental analyses were performed on a Heraeus CHN-O Rapid Elementar Vario E1 instrument.

2.2.3 Syntheses

(2-Oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate (A1) [4] was prepared according to ref. 4. (NMR s. **SI-Fig1**) **(2-Oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (A3)**[18] and **4-(2-Oxo-1,3-dioxolan-4-yl)butyloxy chloroformate (A4)**[18] were prepared according to ref. 18. (NMR s. **SI-Fig3-4**)

Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl phenyl carbonate (A2)

Hexane-1,2,6-triol (6.7 g, 50 mmol), dimethyl carbonate (DMC, 36 g, 400 mmol) and DABCO (56 mg, 0.5 mmol) were mixed and heated to 90°C for 10 h. After distillation of methanol and excess DMC, 4-(4-hydroxybutyl)-1,3-dioxolan-2-one (7.93 g, 49.5 mmol) was used without further purification in the next step.

4-(4-Hydroxybutyl)-1,3-dioxolan-2-one (7.93 g, 49.5 mmol) and pyridine (3.92 g, 49.5 mmol) were dissolved in dry tetrahydrofuran (THF, 70 mL) and cooled to 0 °C; a solution of phenylchloroformate (7.75 g, 49.5 mmol) in THF (20 mL) was slowly added and the temperature was kept below 5 °C. The reaction was stirred for 16 h at RT. Pyridine hydrochloride was removed by filtration and the filtrate was condensed. The residue was purified by column chromatography (stationary phase: silica gel; eluent: ethyl acetate/*n*-hexane, 1/1, (v/v)) to obtain the product as slight yellow oil in 86 % (11.90 g).

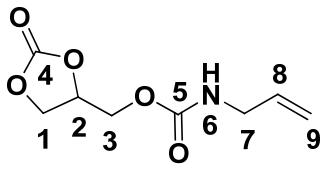
$C_{14}H_{16}O_6$ (280.27): Calcd. C 59.99, H 5.75; Found: C 60.29, H 5.87. (NMR s. **SI-Fig2**)

Synthesis of oxiran-2-ylmethyl phenyl carbonate (A5)

Glycidol (4.00 g, 54 mmol) and pyridine (4.27 g, 54 mmol) were dissolved in dry toluene (50 mL) and cooled to 0 °C. A solution of phenyl chloroformate (8.45 g, 54 mmol) in toluene (20 mL) was slowly added and the temperature was maintained at 0 °C. After 30 min at 0 °C pyridine hydrochloride was filtered. The filtrate was diluted with toluene (150 mL) and washed 2 times with brine, then dried over sodium sulfate. After removal of the solvent, the

residue was distillate (95 °C, 0.1 mbar) and the product was obtained as colourless oil. Yield: 6.30 g (60 %). (NMR s. **SI-Fig5**)

Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-allyl carbamate (1)



A3 (17.545 g, 94.26 mmol) was dissolved in THF (80 mL) and cooled to 0 °C. A solution of allylamine (5.382 g, 04.26 mmol) and triethylamine (9.538 g, 94.26 mmol) in tetrahydrofuran (70 mL) was slowly added under stirring and the temperature was maintained at 0 °C for 2 h and at room temperature for 20 h. Then triethylamine hydrochloride was filtered and the solvent was removed by destillation. The residue was purified by column chromatography (stationary phase: silica gel 60; eluent: ethyl acetate/pentane, 1/1 (v/v)) to obtain the product as colourless oil. Yield: 16.30 g (86 %).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.78 (t, 1 H, ^{3}J = 5.7 Hz, H^{7}), 4.22-4.40 (m, 3 H, $H^{1'}$ and H^{3}), 4.58 (dd, 1H, ^{3}J = 8.6 Hz, H1), 4.92-5.02 (m, 1 H, H^{2}), 5.16 (ddd, 2 H, ^{4}J =1.4 Hz, ^{2}J =13.8 Hz, ^{3}J =11.6 Hz, H^{9}), 5.62-5.75 (m, 1 H, H^{2}), 5.75-5.92 (m, 1 H, H^{8}) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 43.14 (C^{7}), 63.19 (C^{3}), 65.77 (C^{1}), 74.33 (C^{2}), 115.77 (C^{9}), 133.89 (C^{8}), 154.69 (C^{5}), 155.40 (C^{4}) ppm.

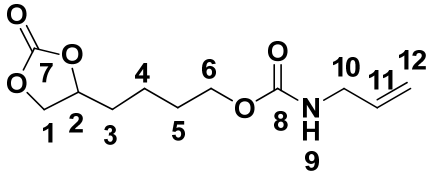
Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-(oxiran-2-yl)methyl carbamate (B1)

1 (3.43 g, 17.0 mmol) and *m*CPBA (5.13 g, 29.7 mmol) were dissolved in dichloromethane (CH_2Cl_2, 50 mL). The reaction mixture was stirred for 24 h at 45°C, cooled to 0 °C to precipitate 2-chloro benzoic acid and filtrated. The filtrate was diluted with CH_2Cl_2 (100 mL). The CH_2Cl_2 solution was washed 2 times with Na_2CO_3-10 % solution (50 mL) and 1 time with brine (50 mL), and then dried over sodium sulfate. After removal of the solvent, the row

product was obtained as slightly yellow oil. The row product was purified by column chromatography (stationary phase: silica gel 60; eluent: first ethyl acetate/*n*-pentane, 1/1(v/v), then ethyl acetate/diethyl ether, 1/1(v/v)) to obtain the product as colorless oil. Yield: 2.240 g (61 %).

$C_8H_{11}NO_6$ (217.18): Calcd. C 44.24, H 5.11, N 6.45; Found: C 44.08, H 5.16, N 6.19. (NMR s. **SI-Fig6**)

Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-allyl carbamate (2)



2 was prepared from **A4** according to the procedure described for **1**. Yield: 99 %.

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.30-1.60 (m, 2 H, H^4), 1.60-1.75 (m, 2 H, H^5), 1.75-1.95 (m, 2 H, H^3), 3.65-3.85 (m, 2 H, H^{10}), 3.95-4.20 (m, 3 H, H^6 and $H^{1'}$), 4.57 (t, 1 H, ^3J = 8.1 Hz, H^1), 4.68-4.84 (m, 1 H, H^2), 5.14 (ddd, 2 H, ^3J = 1.4 Hz, ^3J = 13.7 Hz, ^3J = 11.6 Hz, H^{12}), 5.46-5.64 (m, 1 H, H^9), 5.76-5.94 (tdd, 1 H, ^3J = 5.4 Hz, ^3J = 10.5 Hz, ^3J = 17.1 Hz, H^{11}) ppm

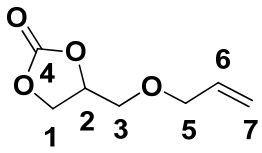
^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.8 (C^4), 28.4 (C^5), 33.1 (C^3), 43.1 (C^{10}), 64.0 (C^6), 69.3 (C^1), 77.0 (C^2), 115.3 (C^{12}), 134.8 (C^{11}), 155.1 (C^7), 156.6 (C^8) ppm

Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-(oxiran-2-yl)methyl carbamate (B2)

B2 was prepared from **2** according to the procedure described for **B1**. Yield: 97 %.

$C_{11}H_{17}NO_6$ (259.26): Calcd. C 50.96, H 6.61, N 5.40; Found: C 50.47, H 6.66, N 5.51. (NMR s. **SI-Fig7**)

Synthesis of 4-(allyloxymethyl)-1,3-dioxolan-2-one (3)



3-(Allyloxy)propane-1,2-diol (50 g, 378 mmol), dimethyl carbonate (DMC) (102 g, 1135 mmol) and DABCO (424 mg, 3.78 mmol) were mixed and heated at 90 °C for 10 h. After removal of MeOH and excess DMC in vacuum, 4-(allyloxymethyl)-1,3-dioxolan-2-one (**3**) was used without further purification. Yield: 59.20 g (99%).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 3.63 (dq, 2 H, ^{4}J = 3.2 Hz, ^{3}J = 11.4 Hz, H^3), 4.02 (dd, 2 H, ^{3}J = 1.1 Hz, ^{3}J = 5.2 Hz, H^5), 4.23-4.33 (m, 1 H, $H^{1'}$), 4.53 (t, 1 H, ^{3}J = 8.4 Hz, H^1), 4.85-5.00 (m, 1 H, H^2), 5.22 (dd, 2 H, ^{3}J = 13.9 Hz, ^{2}J = 28.6 Hz, H^7) and 5.80-5.96 (m, 1 H, H^6) ppm

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 66.36 (C^1), 69.40 (C^3), 71.95 (C^5), 75.81 (C^2), 116.62 (C^7), 134.76 (C^6) and 155.38 (C^4) ppm

Synthesis of (2-oxo-1,3-dioxolan-4yl)methyl (oxiran-2-yl)methyl ether (B3)

B3 was prepared from **3** according to the procedure described for **B1**. Yield: 78 %. (NMR s. **SI-Fig8**)

Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-(2-oxo-1,3-dioxolan-4-yl)methyl carbamate (C1)

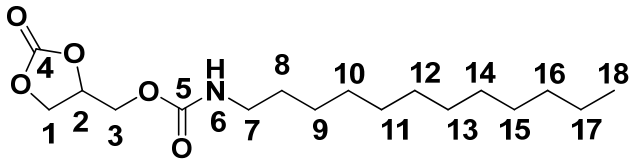
A solution of **B1** (4.700g, 21.64 mmol) and lithium bromide (94 mg, 1.08 mmol) in *N*-methylpyrrolidinone (NMP, 25 mL) was stirred for 24 h under atmospheric pressure of CO_2 at 90 °C. The solvent was removed in vacuum at 40 °C by destillation. The residue was purified by column chromatography (stationary phase: silica gel 60; eluent: ethyl acetate/*n*-hexane, 1/1 (v/v)) to obtain the product as colorless oil. Yield: 3.56 g (63 %). (NMR s. **SI-Fig9**)

Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-(2-oxo-1,3-dioxolan-4-yl)methyl carbamate (C2)

C2 was prepared from **B2** according to the procedure described for **C1**. Yield: 78 %.

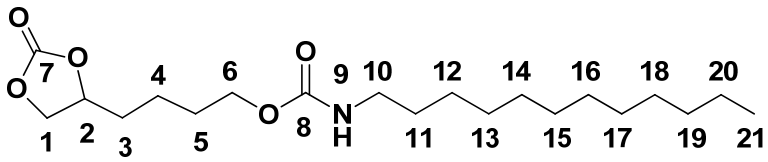
$C_{12}H_{17}NO_8$ (303.27): Calcd. C 47.53, H 5.65, N 4.62; found: C 47.95, H 5.57, N 4.85. (NMR s. **SI-Fig10**)

Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-dodecylcarbamate (4)



4 was prepared and characterized according to ref. 4

Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-dodecylcarbamate (5)

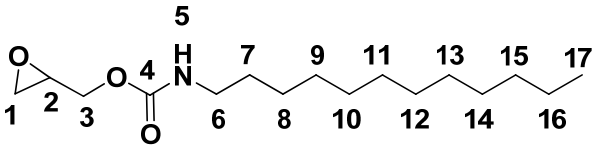


A2 (4.00 g, 14.3 mmol) was dissolved in THF (30 mL) and cooled to 0 °C. A solution of dodecylamine (2.65 g, 14.3 mmol) in tetrahydrofuran (30 mL) was slowly added under stirring and the temperature was maintained at 0 °C for 2 h and at room temperature for 24 h. After removal of the solvents, the residual was recrystallized with CH_2Cl_2/pentane (v/v = 1/1) at room temperature. The product was obtained as white powder. Yield: 4.25 g (80 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.7 Hz, 3 H, H^{21}), 1.15-1.38 (br., 18 H, $H^{12\text{-}20}$), 1.38-1.62 (m, 4 H, $H^{4,\,11}$), 1.62-1.93 (m, 4 H, $H^{3,5}$), 3.15 (dd, ^3J = 6.6 Hz, ^3J = 13.1 Hz, 2 H, H^{10}), 4.04-4.15 (br., 3 H, $H^{1,6}$), 4.54 (t, ^3J = 8.1 Hz, 1 H, $H^{1'}$), 4.65-4.80 (m, 2 H, $H^{2,\,9}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{21}), 21.0 (C^4), 22.7 (C^{20}), 26.8 (C^{11}), 28.6 (C^5), 29.30, 29.35, 29.56, 29.60, 29.63, 29.65, 29.99 (C^{12-18}), 31.9 (C^{19}), 33.5 (C^3), 41.1 (C^{10}), 63.9 (C^6), 69.3 (C^1), 76.9 (C^2), 155.0 (C^7), 156.6 (C^8) ppm

Synthesis of oxiran-2-ylmethyl N-dodecylcarbamate (6)



6 was prepared from **A5** according to the procedure described for **5**. The product was purified by column chromatography (stationary phase: silica gel 60; eluent: pentane) to obtain the product as white powder. Yield: 70 %.

^{1}H NMR ($DMSO-d_6$, 400 MHz): δ = 0.86 (t, 3 H, ^3J = 6.7 Hz, H^{17}), 1.15-1.35 (br., 18 H, H^{12-20}), 1.35-1.45 (m, 4 H, $H^{4, 11}$), 2.60 (dd, 1 H, ^2J = 2.6 Hz, ^3J = 5.0 Hz, H^1), 2.75 (t, 1 H, ^3J = 4.6 Hz, H^1), 2.96 (dd, 2 H, ^3J = 6.7 Hz, ^2J = 13.0 Hz, H^6), 3.08-3.20 (m, 1 H, H^2), 3.71 (dd, 1 H, ^3J = 6.6 Hz, ^2J = 12.3 Hz, H^3), 4.40 (dd, 1 H, ^3J = 6.0 Hz, ^2J = 8.4 Hz, H^3), 7.26 (t, 1 H, ^3J = 5.5 Hz, H^5) ppm

^{13}C NMR ($DMSO-d_6$, 101 MHz): δ = 13.8 (C^{17}), 22.1 (C^{16}), 26.4 (C^7), 28.6, 28.97, 28.98, 29.01, 29.06, 29.31 (C^{8-14}), 31.3 (C^{15}), 40.2 (C^6), 43.6 (C^1), 49.4 (C^2), 64.6 (C^3), 155.9 (C^{10}) ppm

Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-3-(dihexylamino)-2-hydroxypropyl carbamate (7)

B1 (0.652 g, 3.00 mmol) and dihexylamine (0.556 g, 3.00 mmol) were dissolved in THF (15 mL) and the mixture was stirred for 48 h under reflux at 70 °C. The solvent was removed

under vacuum at 40 °C. The product was obtained as slightly yellow oil with quantitative yield. (NMR s. **SI-Fig11**)

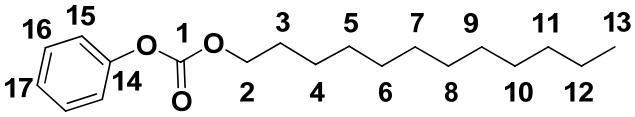
Synthesis of 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-3-(dihexylamino)-2-hydroxypropyl carbamate (8)

8 was prepared from **B2** according to the procedure described for **7** with quantitative yield. (NMR s. **SI-Fig12**)

Synthesis of 4-((3-(dihexylamino)-2-hydroxypropoxy)methyl)-1,3-dioxolan-2-one (9)

9 was prepared from **B3** according to the procedure described for **7** with quantitative yield. (NMR s. **SI-Fig13**)

Synthesis of dodecyl phenyl carbonate (10)

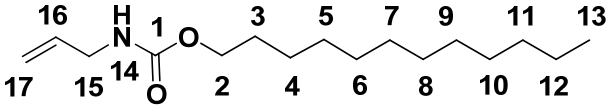


Dodecan-1-ol (20.09 g, 107.8 mmol) and pyridine (8.53 g, 107.8 mmol) were dissolved in dry tetrahydrofuran (THF, 150 mL) and cooled to 0 °C; a solution of phenylchloroformate (16.88 g, 107.8 mmol) in THF (50 mL) was slowly added and the temperature was kept below 5 °C. The reaction was stirred for 16 h at RT. Pyridine hydrochloride was removed by filtration. After removal of the solvents, the product was used for further reaction without purification. Yield: quantitative.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (t, ^3J = 6.50, 6.50 Hz, 3 H, H^{13}), 1.76-1.66 (m, 20 H, H^{3-12}), 4.22 (t, ^3J = 6.63, 6.63 Hz, 2 H, H^2), 7.14 (d, ^3J = 8.13 Hz, 2 H, H^{16}), 7.21 (t, ^3J = 7.35, 7.35 Hz, 2 H, H^{15}), 7.35 (t, ^3J = 7.55, 7.55 Hz, 1 H, H^{17}) ppm.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{13}), 22.7 (C^{12}), 25.7 (C^4), 28.6 (C^3), 29.24, 29.38, 29.52, 29.59, 29.67 (6 C, C^{5-10}), 31.9 (C^{11}), 69.0 (C^2), 121.1 (C^{15}), 125.9 (C^{17}), 129.5 (C^{16}), 151.2 (C^{14}), 153.8 (C^1) ppm

Synthesis of dodecyl N-allylcarbamate (11)

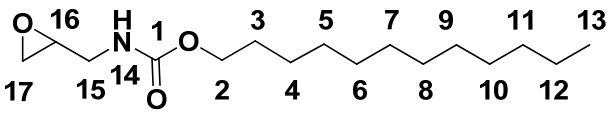


10 (11.50 g, 37.5 mmol) was dissolved in THF (80 mL) and cooled to 0 °C. A solution of allylamine (2.22 g, 37.5 mmol) in tetrahydrofuran (70 mL) was slowly added under stirring and the temperature was maintained at 0 °C for 2 h and at room temperature for 48 h. After removal of the solvents, the reaction mixture was recrystallized with CH_2Cl_2 at room temperature. The product was obtained as colorless needle-like crystals. Yield: 8.35 g (82 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.80 Hz, 1H, H^{13}), 1.15-1.45 (m, 18 H, H^{4-12}), 1.50-1.70 (m, 2 H, H^3), 3.65-3.90 (m, 2 H, H^{15}), 4.06 (t, ^3J = 6.61 Hz, 2 H, H^2), 4.50-5.00 (br., 1 H, H^{14}), 5.16 (dd, ^2J = 22.46 Hz, ^3J = 6.80 Hz, 2 H, H^{17}), 5.85 (ddd, ^3J = 22.46 Hz, ^3J = 10.58 Hz, ^3J = 5.46 Hz, 1 H, H^{16}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{13}), 22.7 (C^{12}), 25.9 (C^3), 29.03, 29.29, 29.34, 29.54, 29.58, 29.63, 29.65 (C^{4-10}), 31.9 (C^{11}), 43.4 (C^{15}), 65.2 (C^2), 115.9 (C^{17}), 134.7 (C^{16}), 156.7 (C^1) ppm.

Synthesis of dodecyl N-oxiran-2-ylmethylcarbamate (12)



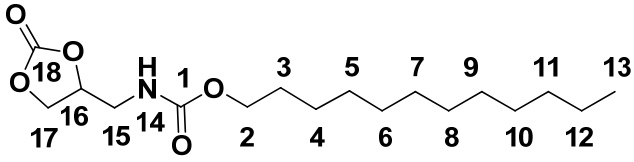
12 was prepared from **11** according to the procedure described for **B1**. Yield: 40 %.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3 H, ^3J = 6.7 Hz, H^{13}), 1.20-1.40 (m, 18 H, H^{4-12}), 1.55-1.65 (m, 2 H, H^3), 2.61 (dd, 1 H, ^3J = 2.6 Hz, ^3J = 4.4 Hz, H^{17}), 2.79 (t, 1 H, ^3J = 4.3 Hz,

$H^{17'}$), 3.05-3.15 (m, 1 H, H^{16}), 3.26 (td, 1 H, ^3J = 5.7 Hz, ^3J = 14.7 Hz, H^{15}), 3.55-3.67 (m, 1 H, $H^{15'}$), 4.06 (t, 2 H, ^3J = 6.4 Hz, H^2), 4.80-5.00 (br., 1 H, H^{14}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{13}), 22.7 (C^{12}), 25.9 (C^3), 29.00, 29.29, 29.36, 29.55, 29.59, 29.64, 29.66 ($C^{4\text{-}10}$), 31.9 (C^{11}), 42.1(C^{15}), 45.0 (C^{17}), 50.8 (C^{16}), 65.4 (C^2), 156.9 (C^1) ppm.

Synthesis of dodecyl N-(2-oxo-1,3-dioxolan-4-yl)methylcarbamate (13)

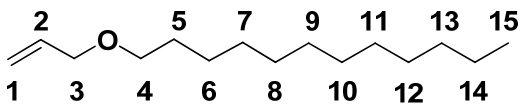


13 was prepared from **12** according to the procedure described for **C1**. Yield: 84 %.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3 H, ^3J = 6.7 Hz, H^{13}), 1.16-1.40 (m, 18 H, $H^{4\text{-}12}$), 1.54-1.68 (m, 2 H, H^3), 3.45-3.63 (m, 2 H, H^{15}), 4.07 (t, 2 H, ^3J = 6.6 Hz, H^2), 4.28 (dd, 2 H, ^3J = 7.3 Hz, $H^{17'}$), 4.53 (dd, 2 H, ^3J = 7.3 Hz, H^{17}), 4.78-4.90 (m, 1 H, H^{16}), 5.10-5.25 (br., 1 H, H^{14}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{13}), 22.7 (C^{12}), 25.8 (C^4), 28.9 (C^3), 29.26, 29.35, 29.53, 29.57, 29.63, 29.65 (C^1), 31.9 (C^{11}), 42.6 (C^{15}), 65.9 (C^{17}), 66.6 (C^2), 75.7 (C^{16}), 154.6 (C^{18}), 157.2 (C^1) ppm

Synthesis of 1-(allyloxy)dodecane (14)



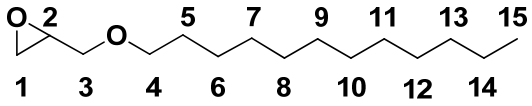
Dodecan-1-ol (6.55 g, 35.1 mmol) and allylbromid (6.38 g, 52.7 mmol) were dissolved in CH_2Cl_2 (40 mL), potassium hydroxid (powder, 3.95 g, 70.3 mmol) was added and the reaction mixture was stirred at room temperature for 24 hours. After filtration and removal of the

solvent, the row product was used for further reaction without purification. A small amount of the raw product was purified by column chromatography (eluent; pentane) to obtain the product as colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 1 H, ^{3}J = 6.7 Hz, H^{15}), 1.10-1.42 (br., 18 H, H^{6-14}), 1.48-1.66 (m, 2 H, H^{5}), 3.42 (t, 2 H, ^{3}J = 6.7 Hz, H^{4}), 3.96 (d, 2 H, ^{3}J = 5.6 Hz, H^{3}), 5.22 (ddd, 2 H, ^{3}J = 1.4 Hz, ^{3}J = 13.8 Hz, ^{3}J = 11.6 Hz, H^{2}), 5.92 (m, 1 H, H^{1}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{15}), 22.7 (C^{14}), 26.2 (C^{5}), 29.35, 29.50, 29.60, 29.66, 29.69, 29.77 (C^{6-12}), 31.9 (C^{13}), 70.5 (C^{4}), 71.8 (C^{3}), 116.6 (C^{2}), 135.1 (C^{1}) ppm

Synthesis of 2-(dodecyloxymethyl)oxirane (15)

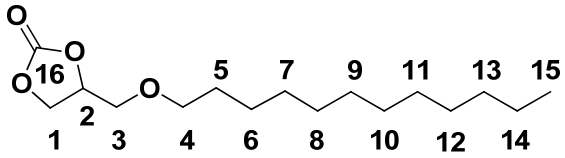


15 was prepared from **14** according to the procedure described for **B1**. Yield: 66 %.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3 H, ^{3}J = 6.7 Hz, H^{15}), 1.18-1.46 (br., 18 H, H^{6-14}), 1.50-1.65 (m, 2 H, H^{5}), 2.61 (dd, 1 H, ^{3}J = 2.7 Hz, J = 5.0 Hz, H^{1}), 2.80 (t, ^{3}J = 4.6 Hz, 1 H, $H^{1'}$), 3.12-3.18 (m, 1 H, H^{2}), 3.38 (dd, 1 H, ^{3}J = 5.8 Hz, ^{3}J = 11.5 Hz, H^{3}), 3.42-3.55 (m, 2 H, H^{4}), 3.71 (dd, 1 H, ^{3}J = 3.0 Hz, ^{3}J = 11.5 Hz, $H^{3'}$)ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{15}), 22.7 (C^{14}), 26.1 (C^{5}), 29.39, 29.51, 29.65, 29.68, 29.71 (C^{6-12}), 31.9 (C^{13}), 44.4 (C^{1}), 51.0 (C^{2}), 71.5 (C^{3}), 71.8 (C^{4}) ppm

Synthesis of 4-(dodecyloxymethyl)-1,3-dioxolan-2-one (16)



16 was prepared from **15** according to the procedure described for **C1**. Yield: 76 %.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, 3 H, ^{3}J = 6.7 Hz, H^{15}), 1.20-1.35 (br., 18 H, H^{6-14}), 1.50-1.64 (m, 2 H, H^{5}), 3.50 (t, 2 H, ^{3}J = 6.6 Hz, H^{4}), 3.57-3.70 (m, 2 H, H^{3}), 3.35-3.43 (m, 1 H, H^{1}), 4.5 (t, 1 H, ^{3}J = 8.3 Hz, $H^{1'}$), 4.77-4.87 (m, 1 H, H^{2}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{15}), 22.7 (C^{14}), 26.0 (C^{5}), 29.40, 29.50, 29.65, 29.68, 29.70 (C^{6-12}), 31.9 (C^{13}), 66.4 (C^{1}), 69.7 (C^{3}), 72.2 (C^{4}), 75.2 (C^{2}), 155.1 (C^{16}) ppm

2.3 Results and Discussion

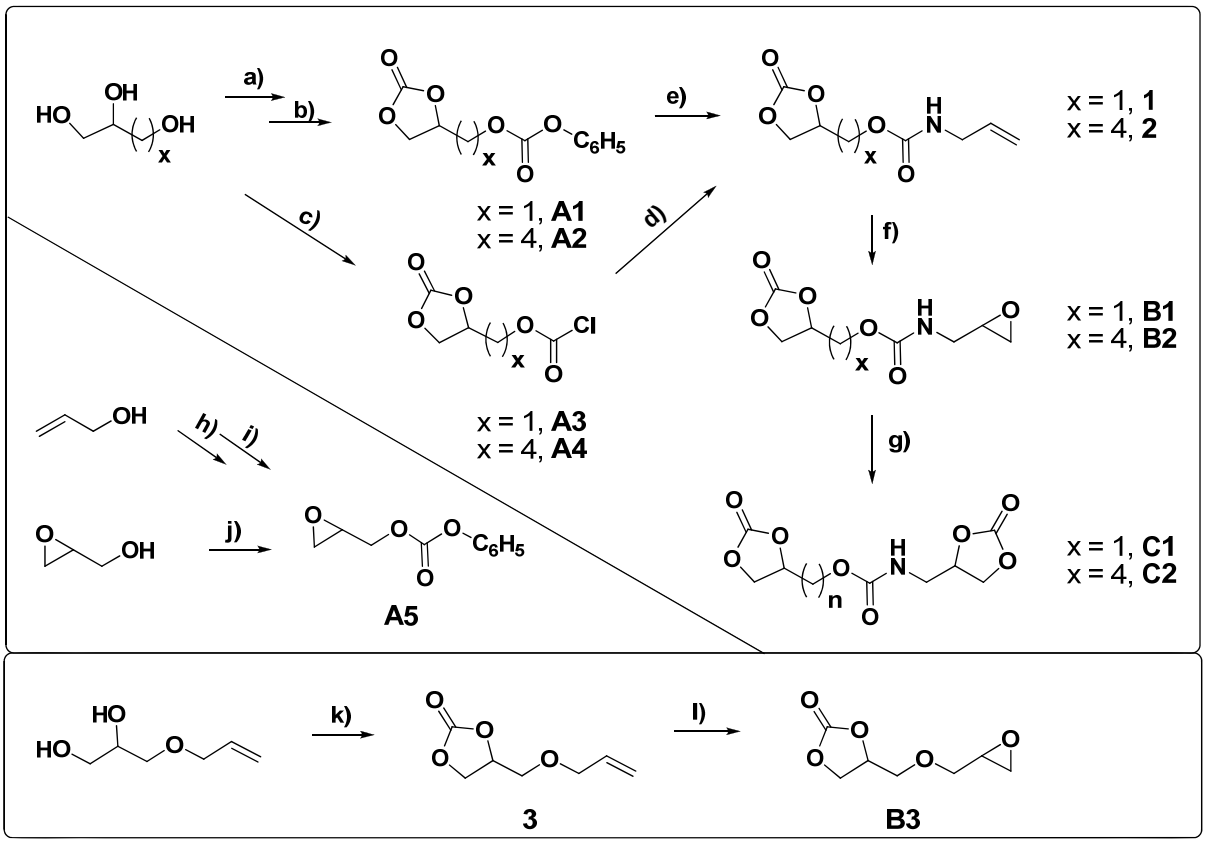
(2-Oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate – coupler **A1** was used for the preparation of multifunctional poly(ethylene imine)s suitable as antimicrobial agents.[4-6, 10] The preparation of these amphiphilic branched poly(ethylene imine)s comprises two steps: the first step – reaction of the couplers with a functional amine – occurs via a substitution reaction. The success of this step is linked to the selectivity of cleavage of the alkyl-phenyl carbonate group and to the efficient removal of phenol from the reaction mixture. For most application both requirements were achieved. Nevertheless, an improvement is possible by replacing the phenoxy leaving group by a chlorine leaving group. The consequences of this replacement are reported. In the second step the functionalized couplers were reacted with PEI.

Couplers of **Series B** and **C** were not reported previously. In both series coupling occurs by consecutive addition reactions with the consequence that no side product (condensate) is formed. The efficiency of the couplers was determined by the difference in selectivity/reactivity of the active group 1,3-dioxolan-2-one vs. oxirane, which was studied in **Series B** and the influence of the asymmetric spacer between the reactive groups addressed in all series with special emphasis in **Series C**.

2.3.1 Synthesis of the couplers

Starting with glycerol and 1,2,6-hexantriol the couplers **A1**, **A2** and **A3**, **A4** were obtained according to the literature by reaction with either dimethyl carbonate and phenyl chloroformate (for **A1** and **A2**) or reaction with phosgene (for **A3** and **A4**). All four couplers were obtained in high purity (^{1}H and ^{13}C NMR spectra, **SI-Fig1-4**).

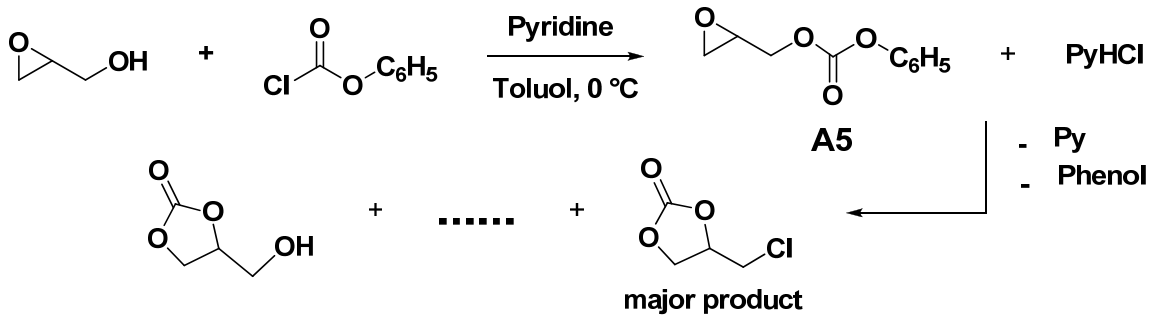
(2-Oxo-1,3-dioxolan-4-yl)methyl N-(oxiran-2-yl)methyl carbamate **(B1)** and 4-(2-oxo-1,3-dioxolan-4-yl)butyl N-(oxiran-2-yl)methyl carbamate **(B2)** were obtained either from **A1** and **A2** according to the literature[4, 5] or from **A3** and **A4** by a sequence of two reactions: condensation with allylamine followed by epoxidation. Finally, **B1** and **B2** were converted to **C1** and **C2** by reaction with carbon dioxide in the presence of lithium bromide as catalyst and N-methylpyrolidone as solvent at 90 °C according to literature.[19] All reactions used for the preparation of these couplers result in products of high purity. The ^{1}H and ^{13}C NMR spectra are shown in supplementary materials (**SI-Fig6-10**). **Scheme 2** summarizes the synthetic approaches employed for the synthesis of the couplers.



Scheme 2: Synthesis of bifunctional couplers: a) DMC, DABCO, 90 °C; b) phenyl chloroformate, pyridine, THF, 0 °C;[4, 5, 10] c) phosgene, 20-30 °C;[18] d) allylamine, triethylamine, THF, 0 °C; e) allylamine, THF; f) *m*CPBA, CH_2Cl_2, 45 °C; g) CO_2, NMP, LiBr, 90 °C;[19] h) phenyl chloroformate, pyridine, CH_2Cl_2, 0 °C;[20] i) *m*CPBA, CH_2Cl_2, RT;[20] j) phenyl chloroformate, pyridine, toluene, 0 °C, 1 h; k) DMC, DABCO, 90 °C; l) *m*CPBA, CH_2Cl_2, 45 °C.

Coupler **A5** was obtained previously from allyl alcohol by consecutive reaction with phenyl chloroformate - allyl phenyl carbamate being an intermediate - followed by epoxidation . In this work **A5** was prepared in a one step reaction from glycidol using phenyl chloroformate as reagent and pyridine as acid scavenger. This reaction gives according to ^{1}H NMR in the kinetic controlled regime the desired product **A5** in a yield in 97 %. However, on standing **A5** is converted in a consecutive reaction with pyridine hydrochloride to give (4-chloromethyl)-

1,3-dioxolan-2-one (**Scheme 3**) (^{1}H and ^{13}C NMR spectra after 30 % conversion of **A5** are shown in **SI-Fig5**).



Scheme 3: Synthesis of coupler **A5** from glycidol and phenyl chloroformate and the consecutive side reaction

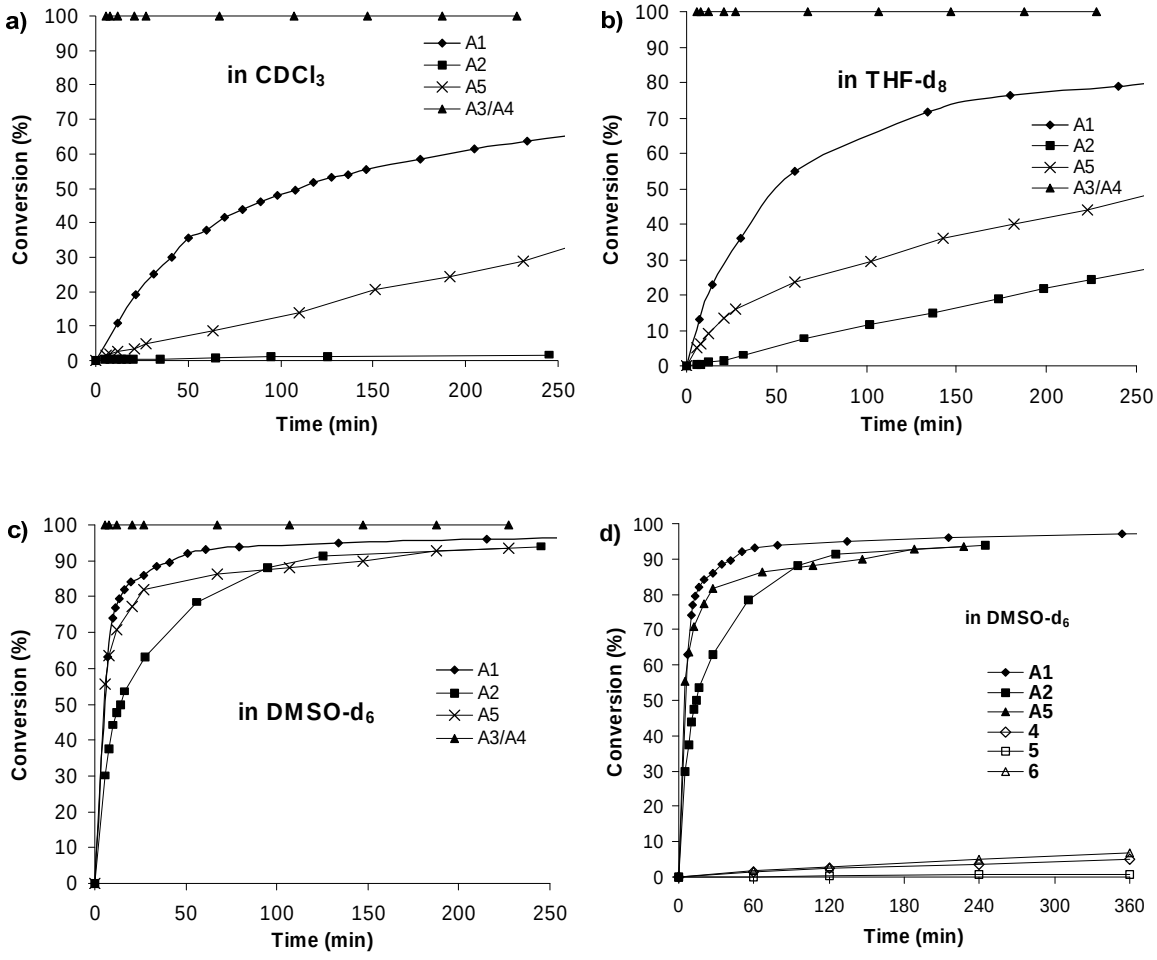
Coupler **B3** was synthesized in two steps starting with 3-(allyloxy)propane-1,2-diol. First by reaction with dimethyl carbonate in the presence of DABCO as catalyst at 90 °C, 4-(allyloxymethyl)-1,3-dioxolane-2-one (**3**) was obtained, which upon epoxidation results in **B3**. In the ^{1}H and ^{13}C NMR spectra of **B3** the formation of two diasteromers is observed. Protons of the methylene groups exhibit germinal and vicinal coupling resulting in a complex signal pattern at δ = 3.3 and 4.0 ppm (**SI-Fig8**).

2.3.2 Selectivity/reactivity of bifunctional couplers in Series A

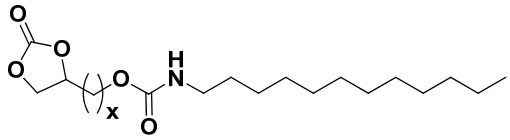
Couplers in **Series A** combine two amines the first via substitution and the second via an addition reaction. Reaction of the first amine with the couplers **A1**, **A2** and **A5** yields phenol as side product, which can be removed by recrystallization or precipitation of the product. Reaction of **A3** and **A4** with the first amine results in hydrochloric acid, which can be quenched by triethylamine or pyridine and the formed salts can be removed by filtration or

aqueous work-up. All couplers in this series have excellent selectivity to primary amine groups, many examples of functional couplers have been reported in literature.[3-6, 10, 21] Here, the reactivity of the substitution reaction of different couplers with primary amines was compared.

The reaction of different couplers with dodecylamine in 1:1 ratio was carried out in deuterated dimethylsulfoxid (DMSO-d_6), tetrahydrofuran (THF-d_8) or chloroform ($CDCl_3$) at an initial concentration of 0.21 M at room temperature, respectively. ^{1}H NMR measurements were performed at different time intervals. Time resolved conversion was calculated by the decrease of the integral of the NH_2CH_2 peak at δ = 2.65-2.80 ppm assigned to dodecylamine. The results were summarized in **Figure 2**.



e)



4 (x = 1)
5 (x = 4)

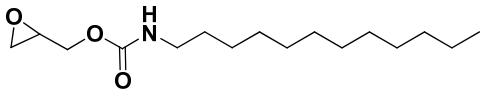


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Figure 2: Time-conversion curves in the substitution reaction of **A1 – A5** with dodecylamine at 0.21 M at room temperature: a) in $CDCl_3$; b) in THF-d_8 and c) in DMSO-d_6; d) Time-conversion curves in the substitution reaction of **A1**, **A2** and **A5** and in the addition reaction of **4**, **5** and **6** with dodecylamine in DMSO-d_6 at 0.21 M at room temperature; e) Structure of **4**, **5** and **6**.

As shown in **Figure 2a-2c**, couplers **A1** – **A5** show different reactivity in different solvents: (i) Couplers **A1** and **A2** with the same functional groups at different intervals are most similarly to each other. Although not large difference of their reactivity is expected, the experimental results reveal large difference in conversion depending on the solvents used. In $CDCl_3$ at given reaction condition, after 4 hours the conversion of coupler **A1** is about 60 %, but for coupler **A2** the conversion is only about 5 %; in THF-d_8, a faster conversion of both couplers is observed, after 4 hours, the conversion of both coupler is about 80 % (**A1**) and 25 % (**A2**), respectively; in DMSO-d_6, a further increase in the rate of conversion is observed; after 150 min, the conversion of both is close to 90 %. This means that for coupler **A1** and **A2**, because of the different distance/spacer between the two functional groups, they show different reactivity in all three solvents, coupler **A1** with shorter spacer reacts always faster than **A2**, but the difference in reactivity gets smaller in solvents with higher polarity. (ii) Coupler **A5** has the same functional group (phenyl carbonate) for substitution reaction as coupler **A1** and **A2**, but instead of cyclic carbonate it has an epoxide group for addition reaction. According to the experimental results, in all three solvents, the reactivity of **A5** was lower than that of **A1**, but higher than **A2**. (iii) Coupler **A3** and **A4** have a chloroformate

group for substitution reaction. The reaction of chloroformate with primary amine was too fast to be tested by proton NMR measurement. In all three solvents, quantitative conversion was observed after mixing the reactants. (iv) In all solvents the sequence of the reactivity of couplers is the same: **A2** < **A5** < **A1** < **A3** ≈ **A4**; the difference in reactivity of couplers in DMSO-d_6 was smaller than in THF-d_8 or in $CDCl_3$.

In **Figure 2d**, the reactivity of the couplers **A1**, **A2**, and **A5** is compared to the reactivity of the functional couplers **4**, **5,** and **6** (**Figure 2e**), in order to evaluate the probability of a sequential reaction. Therefore, above mentioned reactants were treated with equimolar amounts of dodecylamine under the reaction conditions used before. It was found that the reactivity of the addition reaction is much lower than that of the substitution reaction. After 4 hours, the conversion of substitution reactions for all couplers was higher than 95 %, and the conversion of the addition reaction was less than 5 %. The sequence of the reactivity of model compounds is **5 < 4 < 6 <<< A2 <A5 <A1**.

2.3.3 New bifunctional couplers in Series B

Bifunctional couplers in **Series A** are successfully used for the synthesis of different functional couplers. The by-products phenol (or hydrochloric acid) can be removed by aqueous work-up or crystallization of the functionalized couplers or by precipitation. However, for some applications residual phenol (or hydrochloride) might be a problem. As a consequence two type of new bifunctional couplers (**Series B** and **Series C**), which can combine amine functional building blocks and polyamines via addition reaction, were prepared. Couplers in **Series B** have a five-membered cyclic carbonate and an epoxy group. From previous studies it is known that epoxy ring can react with primary and secondary amines, [22] however with tertiary amines special reaction conditions are needed.[23] By contrast five-membered cyclic carbonates react only with primary amines, thus, couplers **B1** – **B3** are

expected to be able to selectively combine building blocks with primary and secondary amine groups.

(1) Selectivity of couplers B1 – B3 towards primary amines

To determine the selectivity, couplers **B1** – **B3**: were reacted with primary amines (molar ratio 1:1) in DMSO-d_6 with an initial concentration of 0.15 M; the reaction mixture was kept at room temperature and/or at 60 °C, respectively. At different reaction times the conversion was determined via ^{1}H NMR analysis (**Figure 3-5**).

With 1 equivalent hexylamine, both functional groups of the coupler **B1** were partially converted. After 7 days at room temperature 55 % of the five-membered carbonate ring and 45 % of the epoxide group was converted. After 2 days at 60 °C and full conversion of hexylamine, 40 % of the carbonate groups and 78 % of the epoxide groups were converted (part of the epoxide groups reacted with the secondary amine groups formed) (**Figure 3**).

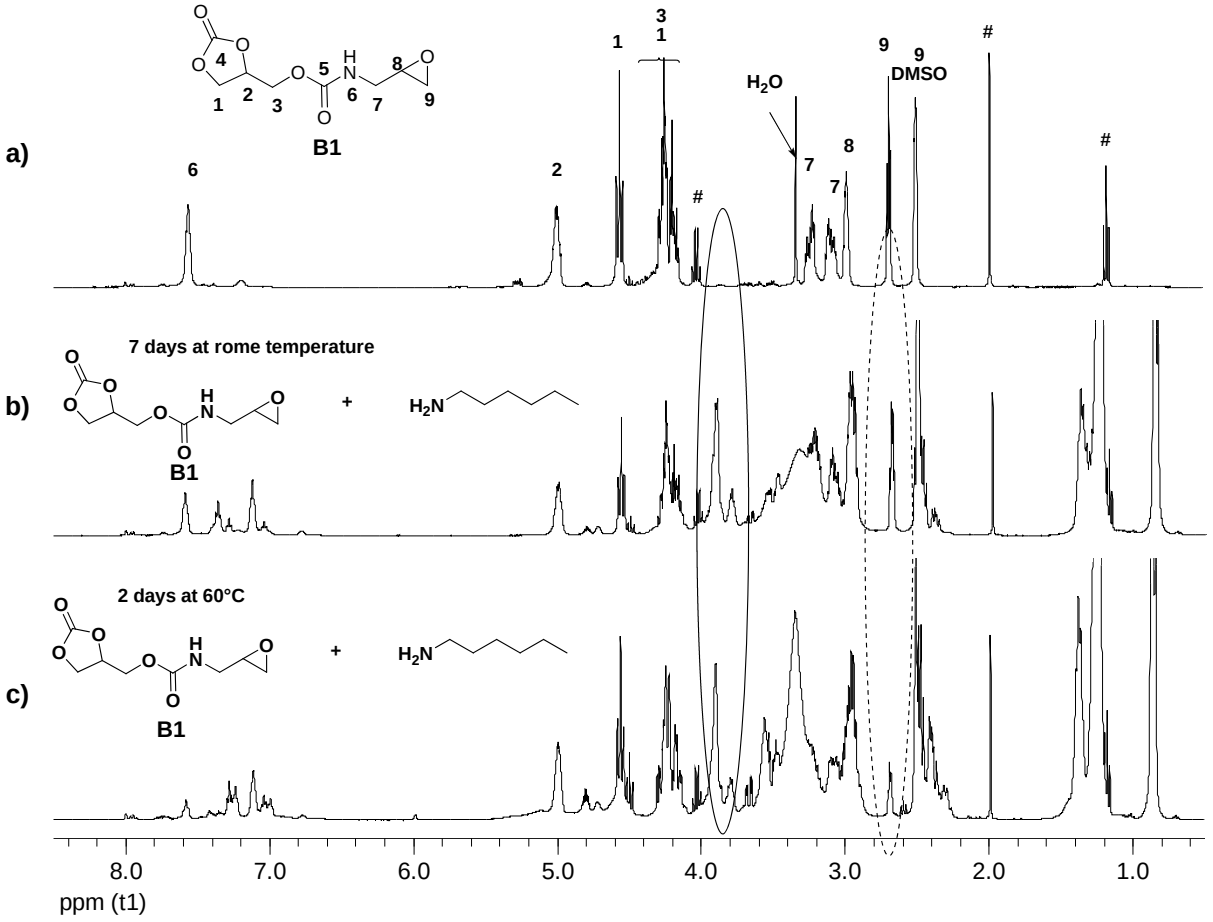


Figure 3: ^{1}H NMR (DMSO-d6, 400 MHz) analysis of the reaction of **B1** with hexylamine (1/1 molar ratio): a) **B1**; b) **B1** + hexylamine, 7 days at room temperature; c) **B1** + hexylamine, 2 days at 60 °C. (#) rest of ethyl acetate; (---) characteristic peak of protons attached to epoxide groups; (–) characteristic peak formed by ring-opening reaction of five-membered cyclic carbonate

The selectivity of coupler **B2** was tested with 1 equivalent N,N-dimethyl-1,3-propanediamine. After 7 days at room temperature the five-membered ring carbonate showed a conversion of 8 % and the epoxide ring of 74 %; after 2 days at 60 °C, the conversion of the five-membered ring carbonate is about 10 %, and the epoxide group was fully converted. Similar results were obtained with dodecylamine, after 2 days at 60 °C, the five-membered ring carbonate showed a conversion of 10 % and the epoxide of 80 % (**Figure 4**).

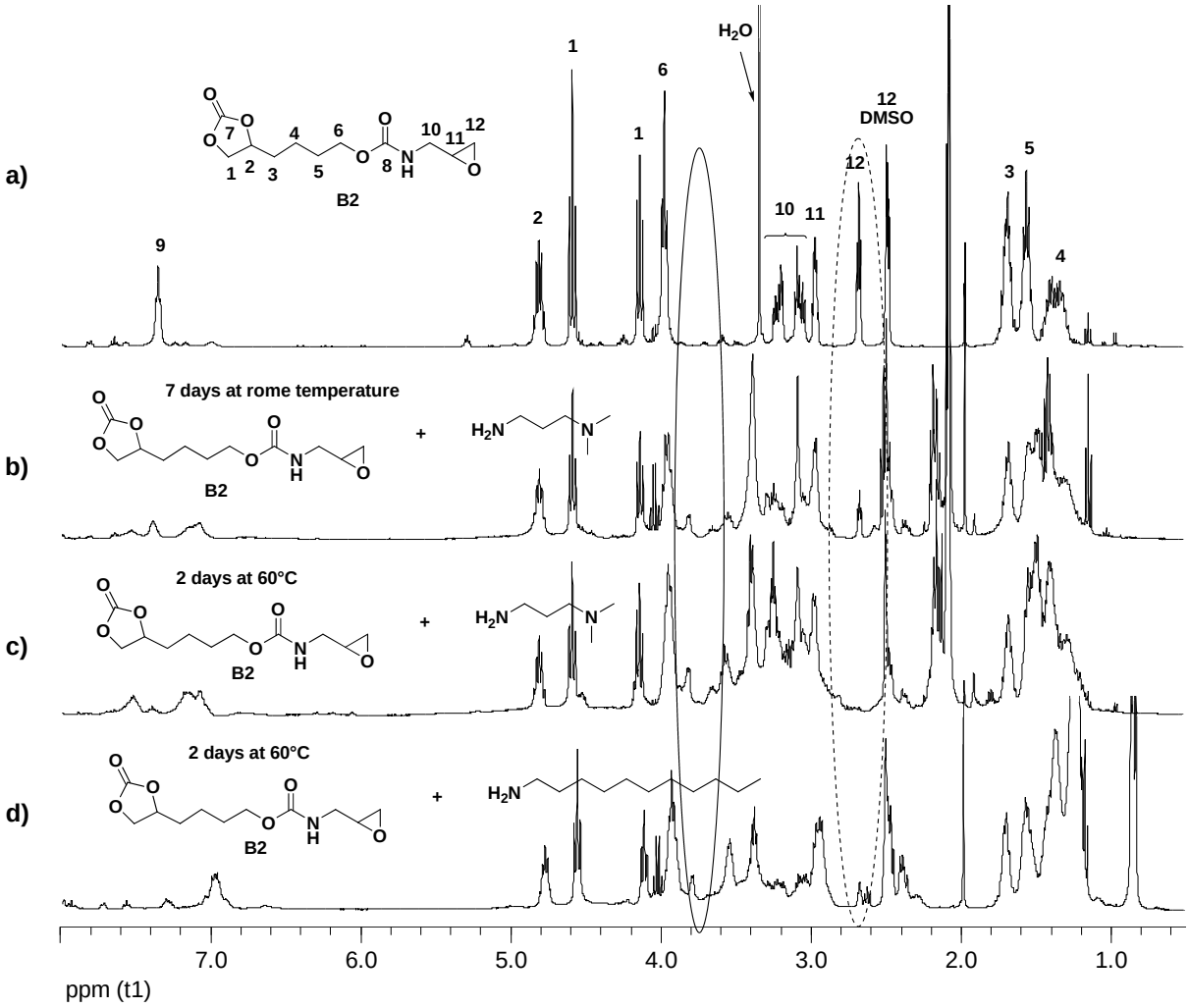


Figure 4: ^{1}H NMR (DMSO-d6, 400 MHz) analysis of the reaction of **B2** with primary amines in 1/1 ratio. a) **B2**; b) **B2** + N,N-dimethyl-1,3-propanediamine, 7 days at room temperature; c) **B2** + N,N-dimethyl-1,3-propanediamine, 2 days at 60 °C: d) **B2** + dodecylamine, 2 days at 60 °C. (---) characteristic peak of protons attached to epoxide groups; (–) characteristic peak formed by ring-opening reaction of five-membered cyclic carbonates

The selectivity of **B3** was also tested with 1 equivalent hexylamine. After 4 days at room temperature the five-membered cyclic carbonate was about 45 % converted and the conversion of the epoxide was 31 %; after 2 days at 60 °C, the conversion of five-membered cyclic carbonate is about 26 % and the conversion of the epoxide group was 80 % (**Figure 5**).

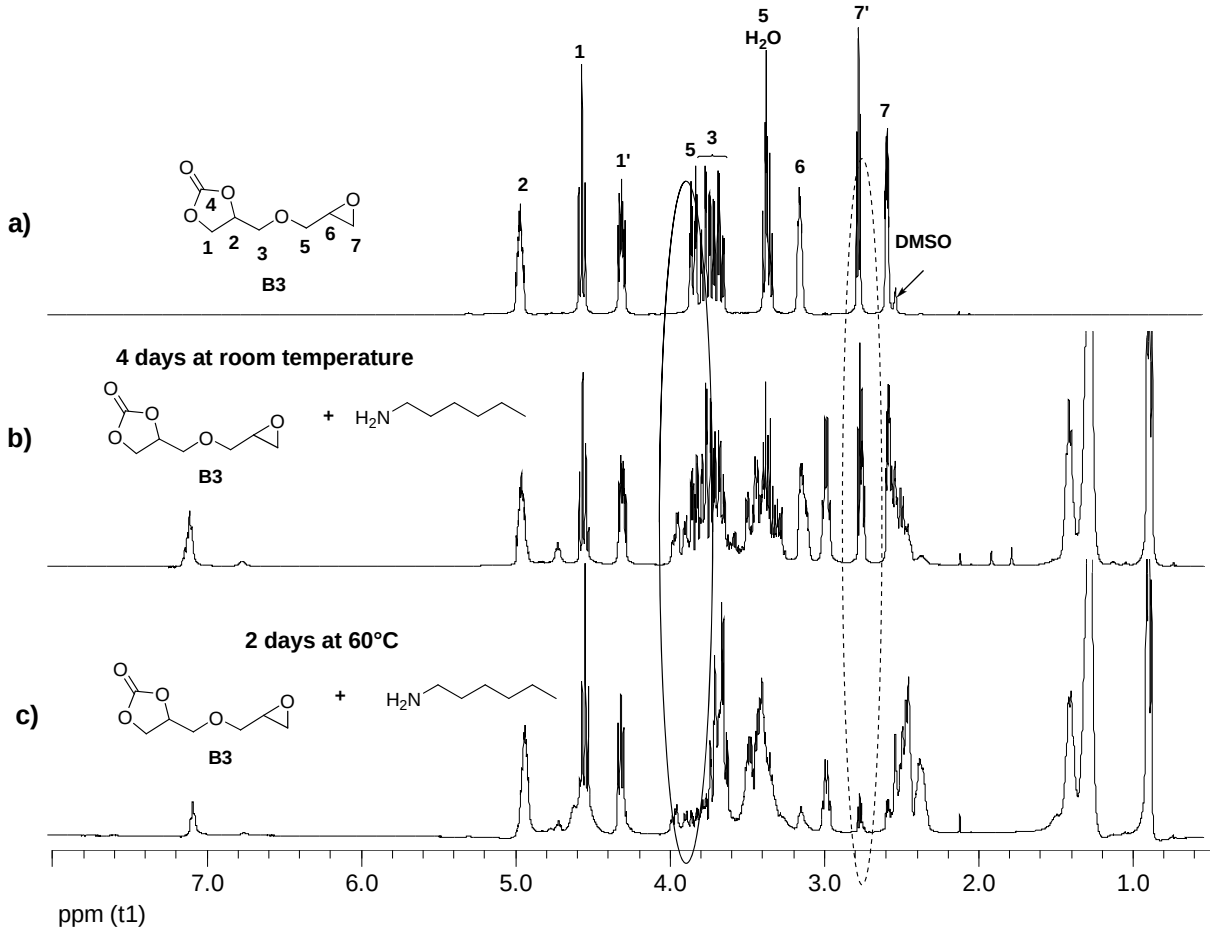
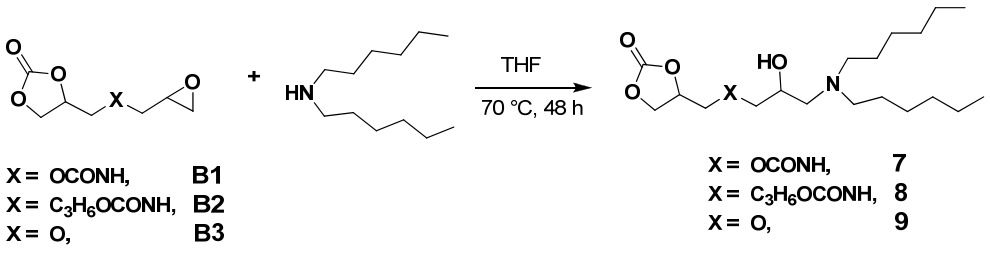


Figure 5: ^{1}H NMR (DMSO-d6, 400 MHz) analysis of the reaction of **B3** with hexylamine in 1/1 ratio. a) **B3**; b) **B3** + hexylamine, 4 days at room temperature; C) **B3** + hexylamine, 2 days at 60 °C. (---) characteristic peak of protons attached to epoxide groups; (–) characteristic peak formed by ring-opening reaction of five-membered cyclic carbonate

The selectivity of the couplers of **Series B** toward primary amines is not as good as for the couplers of **Series A**. In most cases, both functional groups – the cyclic carbonate and the epoxide - reacted with the primary amine; in addition the secondary amine formed during the reaction is able to react further with epoxides. The selectivity of **B2** is better than **B1** and **B3**, toward 1 equivalent primary amine, at room temperature and at 60 °C, only about 10 % of the cyclic carbonate group was reacted, while more than 80 % of the epoxide was converted.

(2) Model reactions of couplers B1 – B3 with secondary amine

Since the five-membered cyclic carbonate has no reactivity towards secondary amine groups even at 100 °C, however the epoxide ring can react with secondary amines, couplers **B1**, **B2** and **B3** were expected to have good selectivity toward secondary amines. Model reactions of **B1**, **B2** and **B3** with equimolar amounts of dihexylamine were carried out at 70 °C in tetrahydrofuran as solvents for 48 hours (**Scheme 4**).



Scheme 4: Model reaction of **B1**, **B2** and **B3** with dihexylamine.

As shown in **Figure 6**, the signal of cyclic carbonate are still present, well resolved in ^{1}H NMR spectra (proton 1, 1' and 2 in all cases) and in ^{13}C NMR spectra (carbon 1 and 2 for **7** in **Figure 6b**, for **8** and **9** in supplementary material **SI-Fig12-13**). The signal of protons attached to the epoxide ring disappeared in all cases, and the product signals could be identified in ^{1}H NMR spectra (proton 8 and 9 for **7** in **Figure 6a**, proton 11 and 12 for **8** in **Figure 6c**, and proton 6 and 7 for **9** in **Figure 6d**) and ^{13}C NMR spectra (carbon 8 and 9 for **7**, signal for **8** and **9** in supplementary material **SI-Fig12-13**).

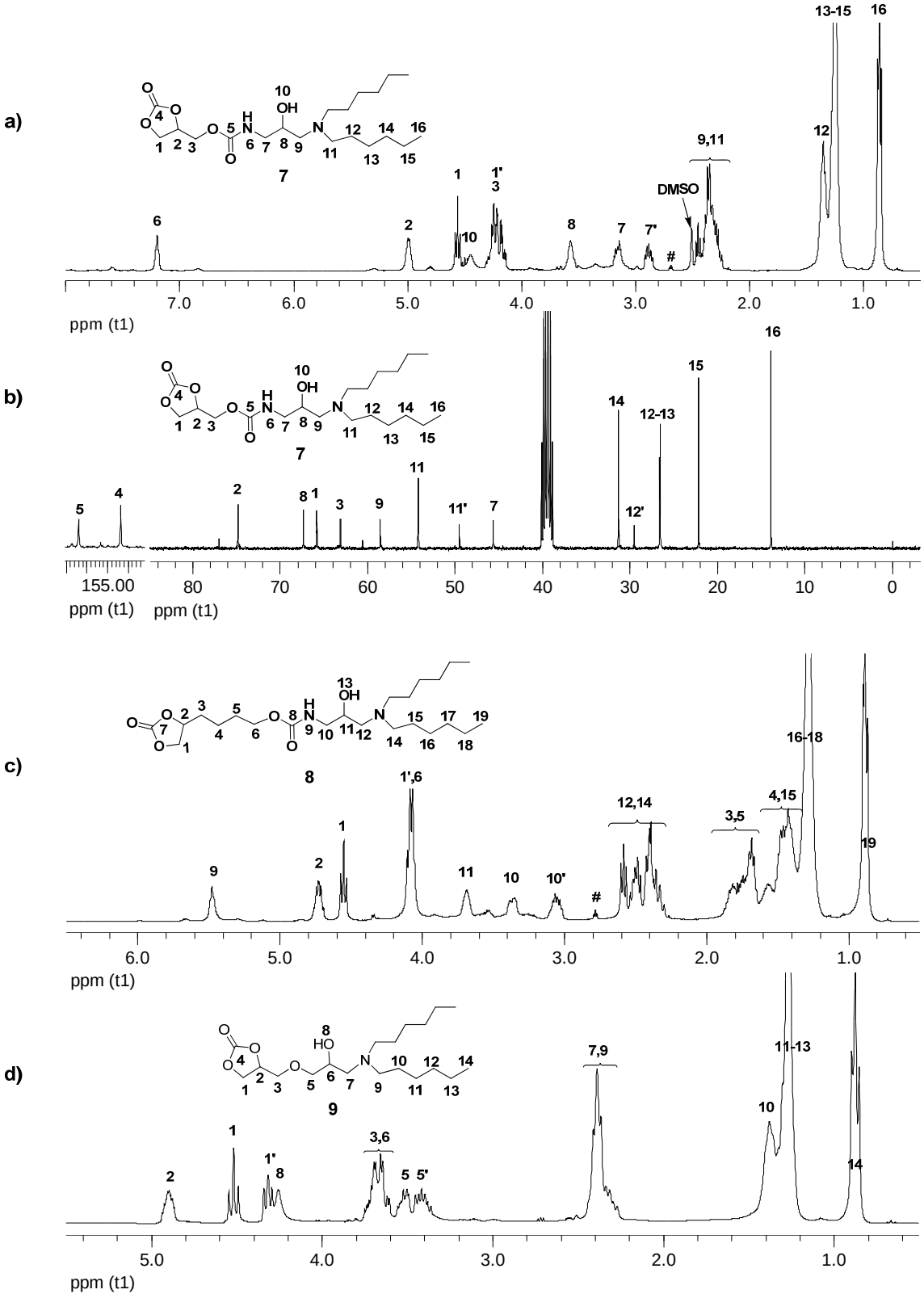


Figure 6: a) ^{1}H NMR (400MHz, DMSO-d_6) and b) ^{13}C NMR (101 MHz, DMSO-d_6) spectra of 7; c) ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of 8; d) ^{1}H NMR (300 MHz, DMSO-d_6) spectrum of 9. (#) Peak of the excess of dihexylamine

(3) Reaction of B1 – B3 with tertiary amine

It is known that for the conversion of epoxides with tertiary amines special reaction conditions are needed.[23] However in our experiments we used the same conditions as for primary and secondary amines, model reaction of couplers in **Series B** with tertiary amine was carried out using equimolar amounts of reactants in chloroform at 70 °C for 48 hours. Due to the low reactivity of epoxides towards tertiary amines, the reaction did not go to completion. Furthermore, ring opening of cyclic carbonate ring with trihexylamine was not observed. In comparison to the ^{1}H NMR spectra of the mixture of **B3** and trihexylamine at time zero (**Figure 7a**), in the spectra of the mixture after 48 hours at 70 °C (**Figure 7b**) the signals of epoxide ring (s. characteristic peak of proton 6 and 7) decreased significantly, but still could be seen. Reactions of **B1** and **B2** with trihexylamine under the same condition led to similar results. In this reaction chloroform might serve as catalyst, because under the same condition with tetrahydrofuran as solvent, no conversion of the epoxide group was observed.

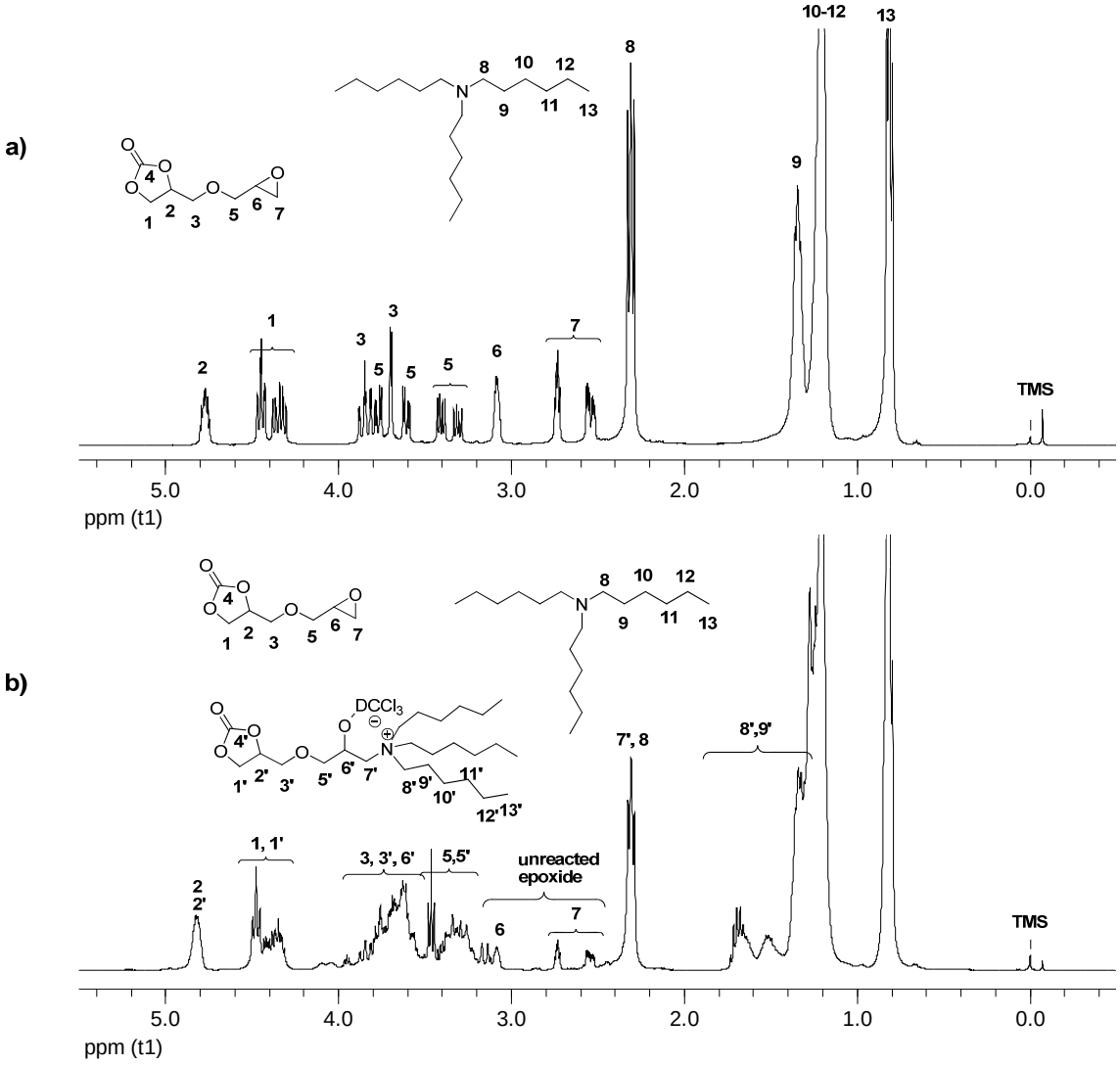


Figure 7: ^{1}H NMR spectrum of the reaction of **B3** and trihexylamine with 1/1 ratio in $CDCl_3$: a) time zero; b) after 48 hours under reflux, epoxide of **B3** was 70 % converted, five-membered carbonate ring remains.

2.3.4 New bifunctional couplers in Series C

The bifunctional couplers of **Series C** have two five-membered cyclic carbonate groups linked by a urethane group. In coupler **C1** both carbonate rings are at an interval of one carbon atom from the urethane group, in coupler **C2** the carbonate ring on the oxygen side is

at an interval of four carbon atoms, while the carbonate ring on the nitrogen side is still at an interval of one carbon atom. Their selectivity to primary amine was tested.

Coupler **C1** was reacted with one equivalent octylamine at an initial concentration of 0.15 M at room temperature. ^{1}H NMR analysis revealed that both cyclic carbonate groups reacted in the same extent. **Figure 8b** shows that the characteristic signals of the cyclic carbonates (signals 2 and 8) are still present, while a new urethane signal at δ = 7.0 ppm appeared. There is no selectivity between these two cyclic carbonates.

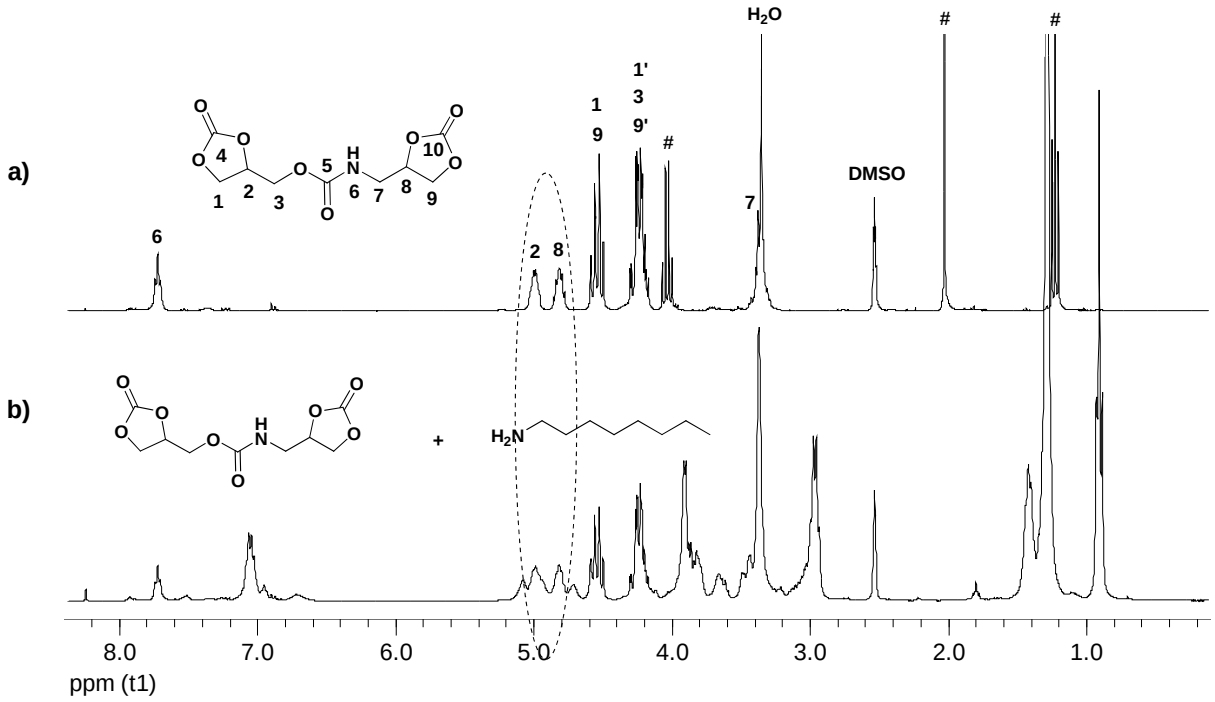


Figure 8: ^{1}H NMR (DMSO-d_6, 400 MHz) analysis of the reaction of **C1** with *n*-octylamine: a) **C1**; b) **C1** + octylamine. (---) comparison of the change of the characteristic peaks of the two cyclic carbonates

The selectivity of coupler **C2** was tested under similar conditions. ^{1}H NMR analysis in DMSO-d_6 revealed that after two days and seven days at room temperature, the carbonate ring on the nitrogen side of the urethane group was converted to 50 % and 65% respectively; the

carbonate ring on the oxygen side of the urethane group showed low reactivity (conversion < 5 %). New signals of urethane groups at δ = 7.1 ppm appeared. For the same reaction at 60 °C, after 2 days the carbonate ring on the nitrogen side of the urethane group was converted to 80 %; while the cyclic carbonate on the oxygen side of the urethane group was still below 5 %. With dodecylamine similar results were observed. The two carbonate rings in coupler **C2** show good selectivity to primary amines at room temperature and at 60 °C (**Figure 9**).

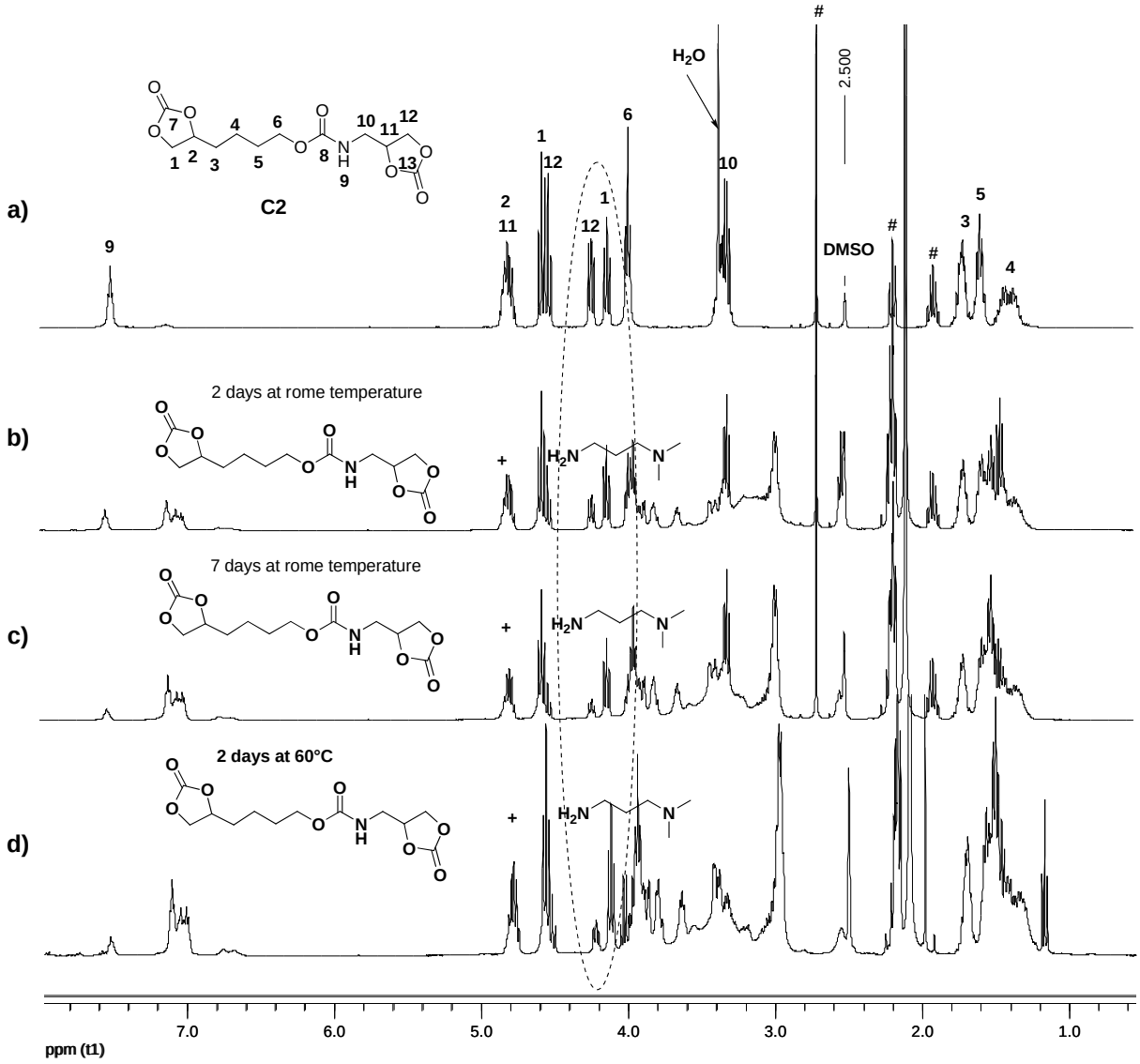


Figure 9: ^{1}H-NMR (DMSO-d_6, 400 MHz) analysis of the reaction of **C2** with primary amine. a) **C2**; b) **C2** + N,N-dimethyl-1,3-propanediamine, 2 days at room temperature; c) **C2** + N,N-dimethyl-1,3-propanediamine, 7 days at room temperature; d) **C2** + N,N-dimethyl-1,3-

propanediamine, 2 days at 60 °C. (**#)** peak of NMP; (---) comparison of the change of the characteristic peaks of the two cyclic carbonates

To get more precise information on the influence of adjacent functional groups on the reactivity of five-membered cyclic carbonates, four model compounds **4**, **5**, **13**, and **16** were synthesized (**Figure 10**) and analyzed with respect to the rate of conversion with hexylamine in DMSO-d_6 at room temperature at an initial concentration of 0.5 M. As expected the reactions follow second order kinetics. The lowest rate is observed for the carbonate group at a distance of four carbon atoms from the urethane group. For the carbonate groups in α-position to the functional groups higher relative rate are observed, as shown in **Table 1**.

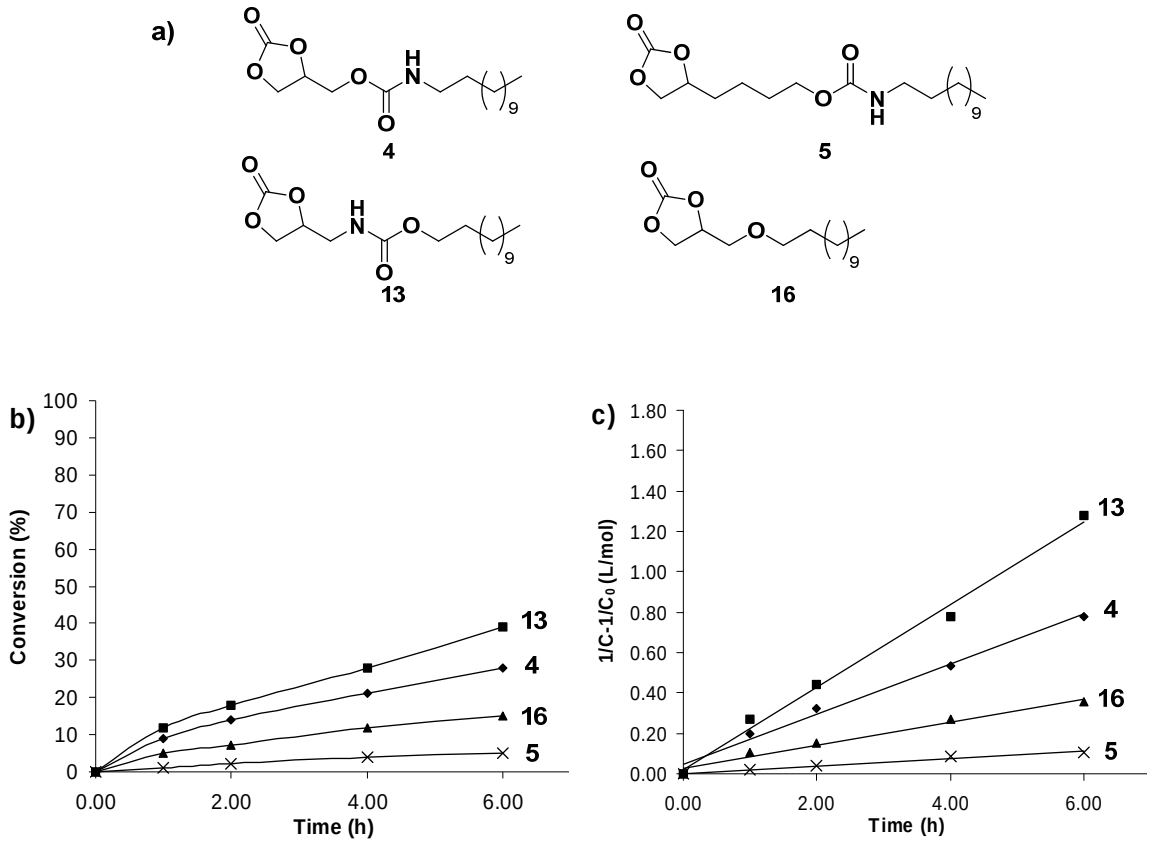


Figure 10: a) Structure of different mono five-membered cyclic carbonates **4**, **5**, **13** and **16**; b) Time-conversion curves of five-membered cyclic carbonates **4** (♦), **5** (×), **13** (■), and **16** (▲)

with hexylamine in DMSO-d_6 at room temperature at initial concentration of 0.5 M; c) Time-1/[C]-1/$[C]_0$ relationships of five-membered cyclic carbonates **4** (♦), **5** (×), **13** (■), and **16** (▲) with hexylamine in DMSO-d_6 at room temperature at initial concentration of 0.5 M

Table 1: Reaction of **4**, **5**, **13**, and **16** with hexylamine in DMSO-d_6 at room temperature

No.	k (L/mol·h)	k (relative)
4	0.124	6.8
5	0.018	1.0
13	0.204	11.3
16	0.057	3.1

2.4 Conclusion

Three series of bifunctional couplers were successfully synthesized and characterized via ^{1}H NMR and ^{13}C NMR spectroscopy.

Couplers of **Series A**, which combine two molecules with amine groups via substitution and addition reaction show high selectivity: the substitution reaction being much faster than the addition reaction. In addition the substitution reaction toward dodocylamine (as model amine) in all solvents used shows different reactivity: **A2** < **A5** < **A1** < **A3** ≈ **A4**. The rate of substitution reaction is higher the higher the polarity of the solvents is: DMSO-d_6 >> THF-d_8 ≈ $CDCl_3$.

Couplers in **Series B** have five-membered cyclic carbonate and an epoxide group show low selectivity toward primary amine and very high selectivity toward secondary amines. Coupler **B1** and **B3** with the functional groups in α-position to the urethane respectively ether group are much less selective than **B2**. In **B2** the reactivity of the carbonate group at a distance of

four carbon atoms from the urethane group is strongly decreased and therefore the epoxide group reacts preferentially with the amine (ca. 11 times faster).

Couplers of **Series C** with two five-membered cyclic carbonates groups show different reactivity if they are at different distance to the linking functional group (urethane group). The five-membered ring in α-position of coupler **C2** shows always a higher reactivity than the ring in δ position.

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Chapter 3

Synthesis, Characterization, and Application of a Bifunctional Coupler containing a Five- and a Six-membered Ring Carbonate

3.1 Introduction

The chemistry of cyclic carbonates, which has been studied since the 1930s, has come to be an interesting area of research within the past 30 years [1]. Polymers bearing carbonate groups either in the main or in the side chains attracted much attention because of their application as biocompatible, optical, high dielectric, and/or adhesive materials [2, 3]. Cyclic carbonates undergo both anionic and cationic ring-opening polymerizations. The polymerizability, however, depends on the ring-size. Six-membered cyclic carbonates result in the corresponding polycarbonates by anionic or cationic ring-opening polymerization [1, 4-12]. However, five-membered cyclic carbonates are thermodynamically stable and do not result in the corresponding polycarbonates; instead poly(ether carbonate)s are obtained by partial decarboxylation [13, 14]. Cyclic carbonates, even substituted five-membered cyclic carbonates, react efficiently with amines to afford the corresponding hydroxyl urethanes [15-19]. The reactivity of cyclic carbonates toward amines, however, is also dependent on the ring-size. Under the same reaction condition the rate constant (k) for the bimolecular reaction of six-membered ring carbonates with amines is ca. 29 – 62 times larger than that of five-membered ring carbonates depending on the reaction temperature [17-19].

Due to the different reactivity of substituted five- and six-membered ring carbonates these molecules are interesting candidates for bifunctional couplers. A coupler is a molecule, having two or more functional groups that can be addressed selectively so that different functionalities are combined within one molecule. With the help of couplers, the properties of a multifunctional polymer can be tailored. In the past, a series of asymmetric A,A´-carbonate couplers (**A1-A4**) were developed in our group to selectively combine monodisperse functional amine building blocks and polymers bearing amine groups within a single molecule (Figure 1) [20-24]. At low temperatures (0-25°C) the more reactive functional group (phenyl carbonate group or chloroformate group) was substituted selectively with hydrophilic, hydrophobic or ionic primary amines to result in functionalized couplers, or with a polyamine to result in a polyamine-coupler-adduct. Then, at higher temperatures (60-80°C) the resulting functionalized couplers are reacted with a polyamine respectively the polyamine-coupler-adduct is reacted with functional amine building blocks. During this reaction the carbonate ring is opened and a urethane group with an adjacent hydroxyl group is formed. These couplers were successfully used for modification of branched poly(ethylene imine) (B-PEI) [25-27] and chitosan [28]. In comparison to the couplers **A1** – **A4**, couplers bearing five- and six-membered cyclic carbonates have significant advantages. The couplers **A1 – A4** converted amines first via substitution reaction leading to the release of phenol or hydrochloric acid; this in some cases may be unacceptable. Couplers with two cyclic carbonate groups react with amines only by addition reactions, without the formation of side products.

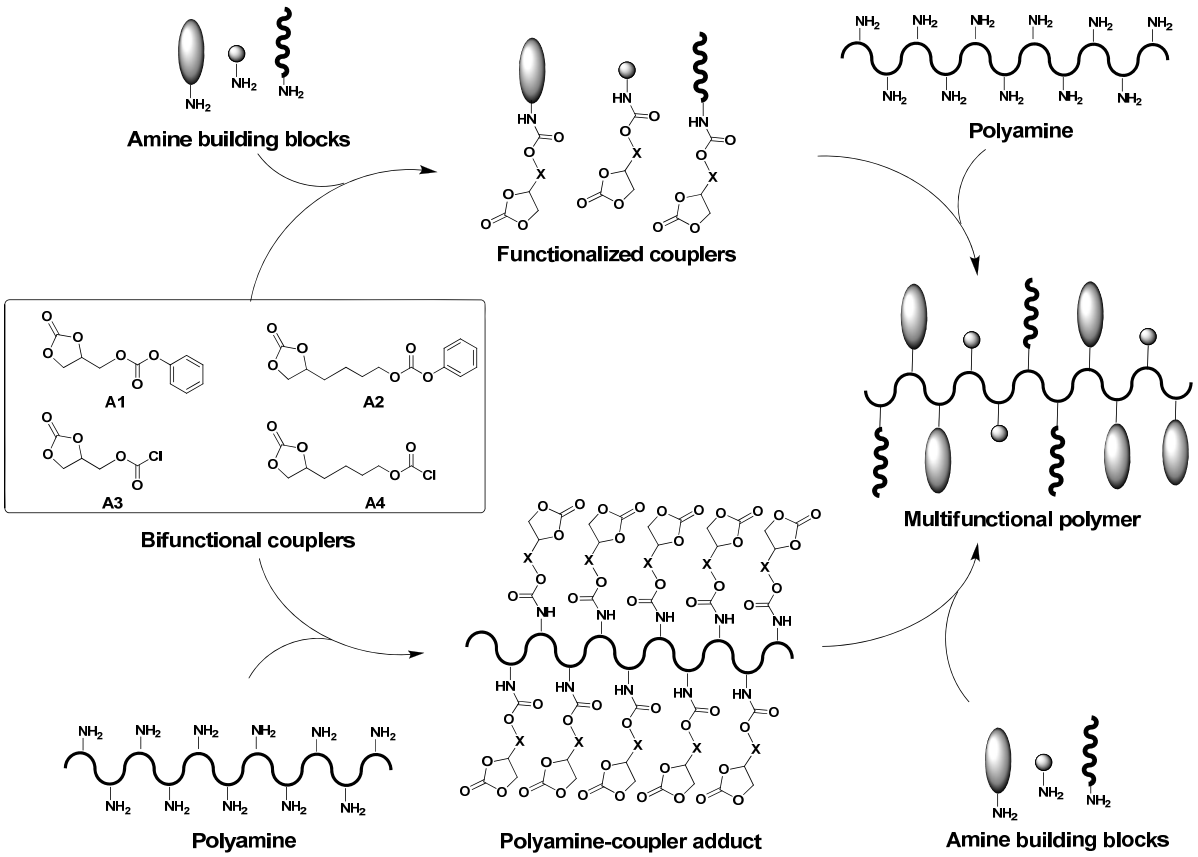


Figure 1: Concepts for the synthesis of multifunctional polymers via bifunctional couplers.

Monomers bearing five- and six-membered cyclic carbonate groups were polymerized via anionic ring-opening polymerization resulting in polymers bearing carbonate groups in the back bone and five-membered ring carbonates in the side chain [29]. Monomers bearing two six-membered cyclic carbonates and monomers bearing two five-membered cyclic carbonates were used for the preparation of poly(hydroxyl urethane)s [1, 16, 17, 19, 30-35]. However, the preparation of poly(hydroxyl urethane)s from monomers bearing five- and six-membered ring carbonates was not reported.

In this work a monomer bearing a five- and a six-membered ring carbonate (**BC56**) was synthesized, characterized and its potential for the preparation of multifunctional polymers was studied. From this monomer functional couplers with ionic, hydrophilic and hydrophobic groups were synthesized. Model reactions of the functional couplers with primary amine were

carried out. The use of this monomer for the synthesis of poly(hydroxyl urethane)s via polycondensation was tested. The influence of solvents, temperature, ratio of **BC56**/diamine, and the nature of the diamine on the molecular weight of the polymer were determined. The hydrolytic stability of **BC56** and of functional coupler prepared form **BC56** was determined. It is our goal to find suitable couplers/functional couplers, which can be used in a solvent promoting self-association during the synthesis of multifunctional polymers.

3.2 Experimental Part

2.2.1 Materials

Ethyl chloroformate (Acros Organics, 99 %), 2-allyl-2-propyl-1,3-propanediol (Aldrich, 98 %), triethylamine (Fluka, 99.8 %), 3-chloroperoxybenzoic acid (Acros, 70-75 %, *m*-CPBA), 3-dimethylamino-1-propylamine (Aldrich, 99 %), iodomethane (Aldrich, 99 %), dodecylamine (Acros, 98 %), Jeffamine® M1000 (Hunstmann), lithium bromide (LiBr), hexane-1,6-diamine (Aldrich, 98 %), 4,9-dioxadodecane-1,12-diamine (BASF, 99 %), triethylenetetramine (Aldrich, 98 %), *N*-methylpyrrolidinone (Acros, 99 %, NMP), dichlormethane (Merck, 99.5 %, CH_2Cl_2), tetrahydrofuran (Acros, 99.99 %, THF), chloroform (Aldrich, 99 %), ethyl acetate (Aldrich, 99.8 %), N,N-dimethylformamide (Aldrich, 99.8 %, DMF), N,N-dimethylacetamide (Aldrich, 99.8 %, DMAc), diethyl ether (tech.), silica gel 60 (AppliChem, 0.063 mm-200 mm), all chemicals are used as received.

3.2.2 Instruments

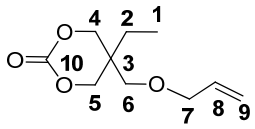
^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT NMR spectrometer at 400 and 101 MHz, respectively. Some of the spectra were recorded on a Bruker DPX-300 FT-NMR spectrometer at 300 and 75 MHz.

Carbon, hydrogen and nitrogen elemental analyses were performed on a Heraeus CHN-O Rapid Elementar Vario E1 instrument.

Size exclusion chromatography analyses (SEC) were carried out at 30 °C using a high pressure liquid chromatography pump (Bischoff 2250) and a refractive index detector (Jasco). The eluting solvent was dimethylformamide (DMF) with 1.00 $g \cdot L^{-1}$ LiBr and a flow rate of 1.0 $mL \cdot min^{-1}$. Four columns with PSS GRAM material were applied. The length of the pre-column was 50 mm and the diameter 8 mm. The remaining three columns had a length of 300 mm, diameter of 8 mm, particle size of 10 mm, and the nominal pore widths were 100, 1000 and 1000 Å. Calibration was achieved using narrow distributed polystyrene standards.

3.2.3 Syntheses

Synthesis of 5-(allyloxymethyl)-5-ethyl-1,3-dioxan-2-one (2)



(**2**)

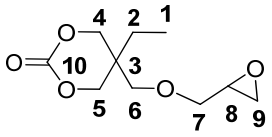
Ethyl chloroformate (2.289 g, 21.10 mmol) and triethylamine (2.136 g, 21.10 mmol) in THF (10 mL) were added drop wise to a solution of 2-allyl-2-propyl-1,3-propanediol (**1**, 1.839 g, 10.55 mmol) in THF (15 mL) at 0 °C. After stirring at room temperature for 12 h, the reaction mixture was filtered. The filtrate was concentrated in vacuum and then dissolved in ethyl acetate (50 mL). The solution was washed 2 times with aqueous hydrochloric acid (1 mol/L,

18 mL) and 2 times with water (30 mL). After removal of the solvents, the residue was purified by vacuum distillation to obtain **2** as colorless liquid in 78 % yield (1.648 g).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.92 (t, 3 H, ^{3}J = 7.62 Hz, H^{1}), 1.54 (q, 2 H, ^{3}J = 7.60 Hz, H^{2}), 3.41 (s, 2 H, H^{6}), 3.92-4.02 (m, 2 H, H^{7}), 4.10-4.20 (d, 2 H, $H^{4,5}$), 4.28-4.38 (d, 2 H, $H^{4',5'}$), 5.14-5.32 (m, 2 H, H^{9}), 5.78-5.94 (m, 1 H, H^{8}) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 7.0 (C^{1}), 22.9 (C^{2}), 35.0 (C^{3}), 67.9 (C^{6}), 72.0 (C^{7}), 72.4 (C^{4}, C^{5}), 116.9 (C^{9}), 133.7 (C^{8}), 148.2 (C^{10}) ppm.

Synthesis of 5-ethyl-5-((oxiran-2-ylmethoxy)methyl)-1,3-dioxan-2-one (3)



(3)

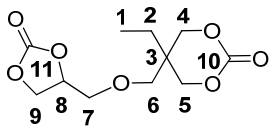
Cyclic carbonate **2** (5.610 g, 28.02 mmol) and 3-chloroperoxybenzoic acid (*m*-CPBA) (9.401 g, 39.22 mmol) were dissolved in CH_2Cl_2 (40 mL). The reaction mixture was stirred for 24 h at room temperature and then cooled to 0 °C and filtrated. The filtrate was diluted with CH_2Cl_2 (150 mL). Solid potassium carbonate (5 g) was added to neutralize the *m*-chlorobenzoic acid formed. The CH_2Cl_2 solution was washed 3 times with 50 mL Na_2CO_3-10% aqueous solution and 1 time with brine, and then dried by Na_2SO_4. After the removal of the solvent, the residue was used for further reaction. Yield of the row product: 5.710 g (94%). A pure sample was obtained by silica gel column chromatography (eluent: diethyl ether). Yield: 3.961 g (66 %).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.93 (t, 3 H, ^{3}J = 7.6 Hz, H^{1}), 1.54 (q, 2 H, ^{3}J = 7.6 Hz, H^{2}), 2.59 (dd, 1 H, ^{3}J = 2.7 Hz, ^{2}J = 4.9 Hz, $H^{9'}$), 2.80 (dd, 1 H, ^{3}J = 4.2 Hz, ^{2}J = 4.8Hz, H^{9}), 3.09-3.16 (m, 1 H, H^{8}), 3.35 (dd, 1 H, ^{2}J = 6.1 Hz, ^{3}J = 11.7 Hz, $H^{7'}$), 3.51 (dd, 2 H, ^{2}J = 9.7 Hz, H^{6}), 3.81 (td, 1 H, ^{3}J = 3.9 Hz, ^{2}J = 7.8 Hz, H^{7}), 4.17 (d, 2 H, ^{3}J = 10.7Hz, $H^{4,5}$), 4.34 (td, 2 H, ^{4}J = 2.0 Hz, ^{3}J = 4.8 Hz, $H^{4',5'}$) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 7.3 (C^1), 23.3 (C^2), 35.5 (C^3), 43.8 (C^9), 50.6 (C^8), 69.7 (C^6), 72.2 (C^7), 72.6 ($C^{4,5}$), 148.6 (C^{10}) ppm.

Anal. calcd. for $C_{10}H_{16}O_5$: C 55.55, H 7.46 %; found: C 55.27, H 7.61 %.

Synthesis of 5-ethyl-5-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)-1,3-dioxan-2-one (BC56)



(**BC56**)

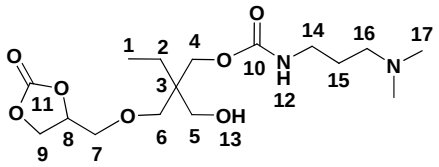
A solution of intermediate **3** (10.219g, 45.25 mmol) and lithium bromide (205 mg, 2.35 mmol) in *N*-methylpyrrolidinone (50 mL) was stirred for 24 h under atmospheric pressure of CO_2 at 90 °C. The solvent was removed in vacuum ($2 \cdot 10^{-2}$ mbar) at 40 °C. The row product was directly used for further reaction. Yield of the row product: 11.140 g (94%). A pure sample was obtained by silica gel column chromatography (eluent; ethyl acetate/*n*-hexane, 1/1, volume ratio). Yield: 7.465 g (63 %).

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.84 (t, 3 H, ^3J = 7.6 Hz, H^1), 1.39 (q, 2H, ^3J = 7.6 Hz, H^2), 3.40-3.52 (m, 2 H, H^6), 3.67 (dq, 2 H, ^3J = 3.1 Hz, ^3J = 11.4 Hz, H^7), 4.20-4.25 (br., 4 H, $H^{4,5}$), 4.28 (dd, 1 H, ^3J = 5.6 Hz, ^3J = 8.3 Hz, $H^{9'}$), 4.53 (t, 1 H, ^3J = 8.4 Hz, H^9), 4.85-5.00 (m, 1 H, H^8) ppm.

^{13}C NMR (DMSO-d_6, 75 MHz): δ = 7.1 (C^1), 22.4 (C^2), 34.8 (C^3), 66.1 (C^9), 69.2 (C^6), 70.4 (C^7), 72.0 (C^4 and C^5), 75.3 (C^8), 147.8 (C^{10}), 154.9 (C^{11}) ppm.

Anal. calcd. for $C_{11}H_{16}O_7$: C 50.77, H 6.20 %; found: C 50.47, H 6.48 %.

Synthesis of 2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)butyl 3-(dimethylamino)-propylcarbamate (4)



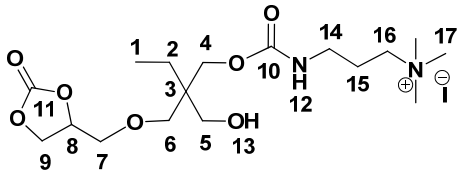
(**4**)

BC56 (2.584 g, 9.92 mmol) was dissolved in THF (30 mL), 3-dimethylamino-1-propylamine (1.015 g, 9.92 mmol) was added to the solution and the mixture was stirred 18 h at room temperature. After removal of the solvent, the product was obtained as slightly yellow oil with quantitative yield.

^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 0.81 (t, 3 H, ^{3}J = 7.4 Hz, H^{1}), 1.30 (q, 2 H, ^{3}J = 7.4 Hz, H^{2}), 1.53 (m, 2 H, H^{15}), 2.11 (s, 6 H, H^{17}), 2.19 (t, 2 H, ^{3}J = 7.1 Hz, H^{16}), 3.00 (dd, 2 H, ^{3}J = 6.7 Hz, ^{3}J = 13.0 Hz, H^{14}), 3.30 (m, 5 H, H $^{5\text{-}6,13}$), 3.50-2.70 (m, 2 H, H^{7}), 3.83 (q, 2 H, ^{3}J = 10.8 Hz, H^{4}), 4.24-4.36 (m, 1 H, H^{9}), 4.52 (t, 1 H, ^{3}J = 8.3Hz, H$^{9'}$), 4.92 (m, 1 H, H^{8}), 6.95-7.10 (m, 1 H, H^{12}) ppm

^{13}C NMR (DMSO-d$_6$, 75 MHz): δ = 7.19, 7.29 (C^{1}), 21.9 (C^{2}), 27.4 (C^{15}), 38.5 (C^{14}), 42.56, 42.67 (C^{3}), 45.0 (C^{17}), 56.6 (C^{16}), 60.72, 60.77 (C^{5}), 63.6 (C^{4}), 66.0 (C^{9}), 70.3 (C^{7}), 71.0 (C^{6}), 75.3 (C^{8}), 154.9 (C^{11}), 156.4 (C^{10}) ppm.

Synthesis of 3-((2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)butoxy carbonylamino)-N,N,N-trimethylpropan-1-aminium iodide (5)



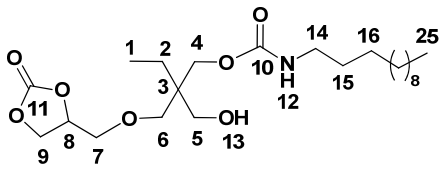
(**5**)

Compound **4** (1.807 g, 4.96 mmol) was dissolved in THF (20 mL), iodomethane (1.407 g, 9.92 mmol) was added to the solution. The mixture was stirred for 18 h at room temperature. After percipitation in ether/pentan (v/v = 1/1) the product was obtained as slightly yellow oil with a yield of 2.35 g (93 %).

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 0.81 (t, 3 H, ^{3}J = 7.4 Hz, H^{1}), 1.29 (dd, 2 H, ^{3}J = 7.1 Hz, ^{2}J = 14.5 Hz, H^{2}), 1.76-1.96 (m, 2 H, H^{15}), 3.00-3.18 (m, 11 H, $H^{14, 17}$), 3.22-3.45 (m, 6 H, $H^{5-6, 16}$), 3.50-3.70 (m, 2 H, H^{7}), 3.83 (q, 2 H, ^{3}J = 10.6 Hz, H^{4}), 4.29 (dd, 1 H, ^{2}J = 5.5 Hz, ^{3}J = 8.0 Hz, H^{9}), 4.32-4.48 (br., 1 H, H^{13}), 4.55 (t, 1 H, ^{3}J = 8.3 Hz, $H^{9'}$), 4.90-5.02 (m, 1 H, H^{8}), 6.80-7.30 (m, 1 H, H^{12}) ppm

^{13}C NMR (DMSO-d_6, 75 MHz): δ = 7.4 (C^{1}), 22.0 (C^{2}), 23.1 (C^{15}), 37.4 (C^{14}), 42.51, 42.61 (C^{3}), 52.2 (C^{17}), 60.65, 60.69 (C^{5}), 63.4 (C^{16}), 63.93, 64.13 (C^{4}), 66.1 (C^{9}), 70.3 (C^{7}), 70.95, 71.01 (C^{6}), 75.4 (C^{8}), 155.0 (C^{11}), 156.5 (C^{10}) ppm

Synthesis of 2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)butyl dodecylcarbamate (6)



(6)

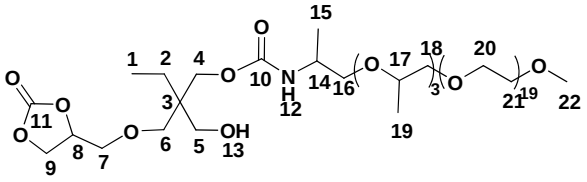
BC56 (1.490 g, 5.72 mmol) was dissolved in THF (20 mL), dodecylamine (1.061 g, 5.72 mmol) was added to the solution and the mixture was stirred for 18 h at room temperature. After removal of the solvent, the product was purified by silica gel column chromatography with ether as solvent. The product was obtained as slightly yellow oil. Yield: 1.47 g (76 %).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.80-0.95 (m, 6 H, H^{25} and H^{1}), 1.20-1.40 (m, 20 H, H^{2} and H^{16-24}), 1.43-1.55 (m, 2 H, H^{15}), 3.14 (dd, 2 H, ^{3}J = 6.6Hz, ^{3}J = 13.1Hz, H^{14}), 3.37 (d, 2 H, ^{3}J = 16.4 Hz, H^{6}), 3.42 (dd, 2 H, ^{3}J = 6.3 Hz, ^{3}J = 9.0 Hz, H^{5}), 3.65-3.80 (m, 2 H, H^{7}), 3.90-4.14 (m, 2 H, H^{4}), 4.35-4.48 (m, 1 H, H^{9}), 4.51 (t, 1 H, ^{3}J = 8.3 Hz, $H^{9'}$), 4.80-4.89 (m, 1 H, H^{8}), 5.18-5.36 (m, 1 H, H^{12}) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 7.33, 7.41 (C^{1}), 14.1 (C^{25}), 21.9, 22.3 (C^{2}), 22.7 (C^{24}), 26.8 (C^{15}), 29.32, 29.35, 29.58, 29.61, 29.64, 29.66, 29.9 (C^{16-22}), 31.9 (C^{23}), 41.2 (C^{14}), 43.6, 43.8

(C^3), 62.2, 62.7 (C^5), 64.3, 64.7 (C^4), 66.25, 66.35 (C^9), 70.1, 70.5 (C^7), 71.7, 72.3 (C^6), 75.3, 75.6 (C^8), 155.3, 155.4 (C^{11}), 157.40, 157.55 (C^{10}) ppm.

Synthesis of 2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)butyl 1-(1-(2-methoxy-ethoxy)propan-2-yloxy)propan-2-ylcarbamate (7)



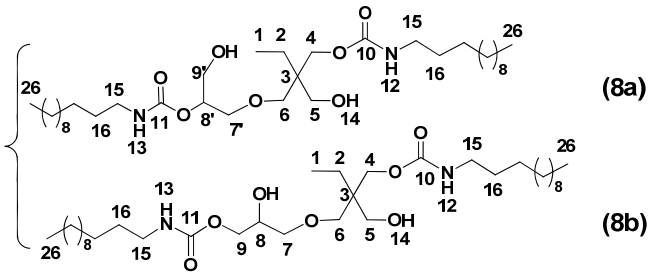
(**7**)

BC56 (1 g, 3.84 mmol) was dissolved in THF (30 mL), Jeffamine® M1000 (3.84 g, 3.84 mmol) was added to the solution and the mixture was stirred 48 h at room temperature. After removal of the solvent, the product was obtained as slightly yellow wax with quantitative yield.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.80 (t, 3 H, ^3J = 6.9 Hz, H^1), 0.95-1.10 (m, 9 H, H^{19}), 1.20-1.40 (m, 2 H, H^2), 3.24 (s, 3 H, H^{22}), 3.25-3.33 (br., 4 H, H^{5-6}), 3.33-3.70 (m, 110 H, $H^{7, 14-16, 17-18, 20-21}$), 3.80 (dd, 2 H, ^3J = 11.3 Hz, ^3J = 22.1 Hz, H^4), 4.24-4.31 (m, 1 H, H^9), 4.51 (t, 1 H, ^3J = 8.3 Hz, $H^{9'}$), 4.85-4.98 (m, 1 H, H^8), 6.50-7.00 (m, 1 H, H^{12}) ppm.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 7.05, 7.23, 7.34, 7.39 (C^1), 17.04, 17.13, 17.17, 17.46 (C^{19}), 21.59, 21.93, 22.42 (C^2), 34.8 (C^{14}), 42.56, 42.65, 43.43 (C^3), 45.90, 46.18, 46.43, 48.4 (C^{15}), 58.0 (C^{22}), 60.72, 60.76 (C^{16}), 61.21, 61.25 (C^5), 63.62 (C^4), 66.07 (C^9), 69.16, 69.56, 69.76, 70.02, 70.10, 70.23, 70.28, 70.44 (C^{20-21}), 70.98 (C^7), 71.25 (C^6), 71.53, 71.74, 71.98, 72.09, 72.13, 72.30, 72.35 (C^{18}), 74.02, 74.22, 74.29, 74.54, 75.65, 74.68 (C^{17}), 75.28, 75.36, 75.45 (C^8), 154.9 (C^{11}), 155.8 (C^{10}) ppm.

Synthesis of 2-(hydroxymethyl)-2-((2-hydroxymethyl)ethyl dodecylcarbamate) methoxyl)butyl dodecyl-carbamate (8a) and 2-(hydroxymethyl)-2-((2-hydroxyl)propyl dodecylcarbamate)methoxyl) butyl dodecylcarbamate (8b)



(8a)

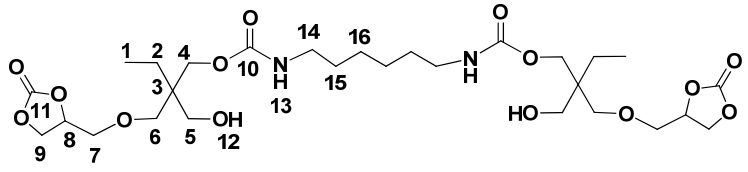
(8b)

Compound **6** (1.693 g, 3.80 mmol) was dissolved in ethyl acetate (20 mL), dodecylamine (0.774 g, 4.18 mmol) was added to the solution and the mixture was stirred for 18 h at 60 °C. After removal of the solvent, the product was purified via recrystallization in $CHCl_3$ and obtained as white powder with yield of 2.03 g (85 %).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 0.80-0.95 (m, 9 H, H^{26}, H^1), 1.20-1.40 (m, 38 H, H^{17-25}), 1.43-1.57 (m, 4 H, H^{16}), 3.15 (dd, 2 H, ^3J = 6.6Hz, ^3J = 13.1Hz, H^{15}), 3.36 (dd, 2 H, $^2J = {}^3J$ = 9.2 Hz, H^5), 3.41 (d, 2 H, ^2J = 21.2 Hz, H^6), 3.48 (d, 2 H, ^2J = 4.8 Hz, H^4), 3.90-4.30 (m, 5 H, H^{7-9}), 4.70-5.20 (m, 2 H, H^{13}) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 7.4 (C^1), 14.1 (C^{26}), 22.52 (C^2), 22.70 (C^{16}), 26.8 (C^{25}), 29.33, 29.36, 29.59, 29.62, 29.65, 29.70, 29.92 (C^{17-23}), 31.9 (C^{24}), 41.2 (C^{15}), 43.3 (C^3), 63.6, 63.7 (C^5), 64.6 (C^4), 66.2 (C^9), 69.0 (C^8), 69.2 (C^7), 72.28, 72.39 (C^6), 156.8 (C^{11}), 157.3 (C^{10}) ppm

Synthesis of bis(2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)- butyl) hexane-1,6-diyldicarbamate (9)



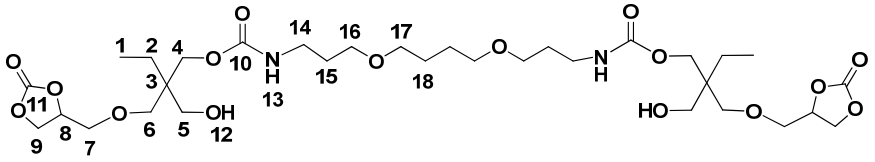
(**9**)

BC56 (331 mg, 1.28 mmol) was dissolved in THF (8 mL), hexane-1,6-diamine (74 mg, 0.64 mmol) was added to the solution and the mixture was stirred 48 h at room temperature. After removal of the solvent, the product was obtained as slightly yellow oil with quantitative yield.

^{1}H NMR (DMSO-d_6, 400 MHz): δ =0.70-0.90 (m, 6 H, H^{1}), 1.18-1.33 (m, 8 H, $H^{2,16}$), 1.33-1.46 (m, 4 H, H^{15}), 2.94 (dd, 4 H, ^{3}J = 6.5 Hz, ^{3}J = 12.8 Hz, H^{14}), 3.20-3.35 (m, 8 H, $H^{4,6}$), 3.45-3.70 (m, 4 H, H^{7}), 3.74-3.86 (m, 4 H, H^{5}), 4.20-4.32 (m, 2 H, H^{9}), 4.51 (t, 2 H, ^{3}J = 8.3 Hz, $H^{9'}$), 4.35-4.70 (br., 2 H, H^{12}), 4.86-4.96 (m, 2 H, H^{8}), 6.70-7.10 (m, 2 H, H^{13}) ppm.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 7.37 (C^{1}), 21.9 (C^{2}), 25.9 (C^{16}), 29.4 (C^{15}), 40.1 (C^{14}), 42.7 (C^{3}), 60.7 (C^{4}), 63.6 (C^{5}), 66.1 (C^{9}), 70.3 (C^{7}), 71.0 (C^{6}), 75.4 (C^{8}), 154.9 (C^{11}), 156.4 (C^{10}) ppm

Synthesis of bis(2-(hydroxymethyl)-2-(((2-oxo-1,3-dioxolan-4-yl)methoxy)methyl)-butyl) 3,3'-(butane-1,4-diylbis(oxy))bis(propane-3,1-diyl)dicarbamate (10)



(10)

BC56 (331 mg, 1.28 mmol) was dissolved in THF (8 mL), 4,9-dioxadodecane-1,12-diamine (131 mg, 0.64 mmol) was added to the solution and the mixture was stirred 48 h at room temperature. After removal of the solvent, the product was obtained as slightly yellow oil with quantitative yield.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.74-0.86 (m, 6 H, H^{1}), 1.15-1.35 (m, 4 H, H^{2}), 1.45-1.55 (m, 4 H, H^{15}), 1.55-1.68 (m, 4 H, H^{16}), 3.01 (dd, 4 H, ^{3}J = 6.5 Hz, ^{3}J = 12.9 Hz, H^{14}), 3.20-3.40 (m, 16 H, $H^{4,6,17,18}$), 3.50-3.70 (m, 4 H, H^{7}), 3.70-3.90 (m, 4 H, H^{5}), 4.20-4.30 (m, 2 H, H^{9}), 4.51 (t, 2 H, ^{3}J = 8.3 Hz, $H^{9'}$), 4.30-4.80 (br., 2 H, H^{12}), 4.85-4.98 (m, 2 H, H^{8}), 6.30-7.30 (m, 2 H, H^{13}) ppm.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 7.27, 7.37 (C^{1}), 21.9 (C^{2}), 26.0 (C^{18}), 29.7 (C^{15}), 37.6 (C^{14}), 43.5 (C^{3}), 60.70, 60.75 (C^{4}), 63.6 (C^{5}), 66.1 (C^{9}), 67.6 (C^{17}), 69.8 (C^{16}), 70.3 (C^{7}), 71.0 (C^{6}), 75.4 (C^{8}), 154.9 (C^{11}), 156.4 (C^{10}) ppm

Synthesis of polyhydroxyurethan – Polycondensation of BC56 with diamines

Procedure A: BC56 was dissolved in a suitable solvent, and 1 equivalent diamine was added to the solution. The mixture was heated for 3 days (or at RT for different days). The product was precipitated in pentane/diethyl ether (v/v = 1/1, 400 mL). After removal of solvent the product was dried under vacuum (10^{-2} mbar) at 50 °C. The detailed information on the reactants, reaction conditions and yields are summarized in Table 1**.** The molecular weight ($\overline{M}_n$ and $\overline{M}_w$) and molecular weight distribution ($\overline{M}_w / \overline{M}_n$) of the polymers prepared are given in Table 3 in section **3.4.1**.

Table 1: Reactants, reaction conditions and yields of poly(hydroxyl urethane)s prepared in Procedure A.

No.	BC56 (mg)	diamine (mg) [a)]	Solvent	T (°C)	Time	Yield [b)] mg (%)
P1	330	148 (**A_1**)	DMF	90	3 d	-
P2	330	260 (**A_2**)	DMF	90	3 d	-
P3	400	179 (**A_1**)	DMF	70	3 d	530 (92)
P4	400	179 (**A_1**)	DMAc	70	3 d	532 (92)
P5	372	209 (**A_3**)	DMAc	70	3 d	490 (85)
P6	500	223 (**A_1**)	THF	RT	60 d	-
P7	330	148 (**A_1**)	$CHCl_3$	60	3 d	440 (92)
P8	400	214 (**A_1**)	$CHCl_3$	60	3 d	600 (96)

[c)] A_1 = hexane-1,6-diamine, A_2 = 4,9-dioxadodecane-1,12-diamine, A_3 = triethylenetetramine (TETA).

[b)] The products of **P1, P2**, and **P6** were not precipitated; the GPC samples were taken directly from the reaction mixtures.

Procedure B: BC56 was dissolved in a suitable solvent, and 0.5 equivalents diamine was added to the solution. The mixture was stirred at RT for 3 days until the six-membered cyclic

carbonate was fully converted. To the reaction mixture or isolated product (dissolved in solvent again) the second portion of diamine (0.5 equivalents or 0.7 equivalents) was added. The reaction mixture was heated for 3 days. Then the product was precipitated in pentane/diethyl ether (v/v = 1/1, 400 mL). After removal of solvent the product was dried under vacuum (10^{-2} mbar) at 50 °C. The detailed information on the reactants, reaction conditions and yields are summarized in Table 2. The molecular weight ($\overline{M}_n$ and $\overline{M}_w$) and molecular weight distribution ($\overline{M}_w / \overline{M}_n$) of the polymer prepared are given in Table 4 in section **3.4.2**.

Table 2: Reactants, reaction conditions and yields of poly(hydroxyl urethane)s prepared in Procedure B.

No.	Bis(carbonate) (mg) [a)]	1. diamine (mg)[b)]	2. diamine (mg) [b)]	Solvent	T (°C)[c)]	Time[d)]	Yield [e)] mg (%)
P9	330 (**BC56**)	74 (**A$_1$**)	74 (**A$_1$**)	DMF	90	3 d	-
P10	330 (**BC56**)	180 (**A$_2$**)	180 (**A$_2$**)	DMF	90	3 d	-
P11	384 (**9**)	- (**A$_1$**)	148 (**A$_2$**)	THF	60	3 d	505 (95)
P12	438 (**10**)	- (**A$_2$**)	85 (**A$_1$**)	THF	60	3 d	490 (94)

[a)] For polymers **P9** and **P10** the second 0.5 equivalent diamines were added to the reaction mixtures after the first steps; for polymers **P11** and **P12**, the second 0.5 equivalent diamines were added to the solution of the isolated intermediates after the first steps (compounds **9** and **10**, respectively).

[b)] **A$_1$** = hexane-1,6-diamine, **A$_2$** = 4,9-dioxadodecane-1,12-diamine.

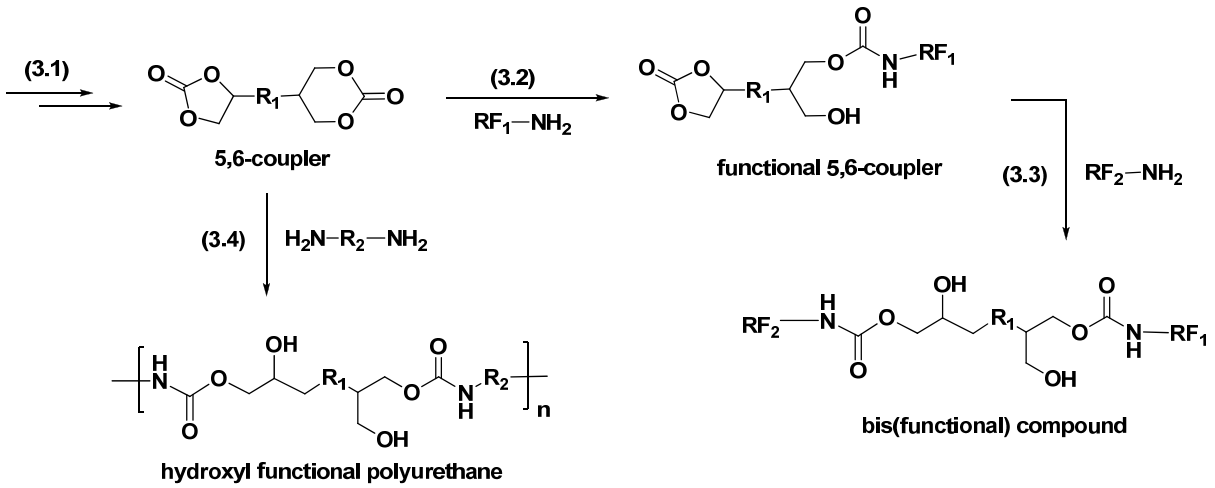
[c)] Reaction temperature in the second step.

[d)] Reaction time in the second step.

[e)] The products of **P9** and **P10** were not precipitated; the GPC samples were taken directly from the reaction mixtures.

3.3 Results and Discussion

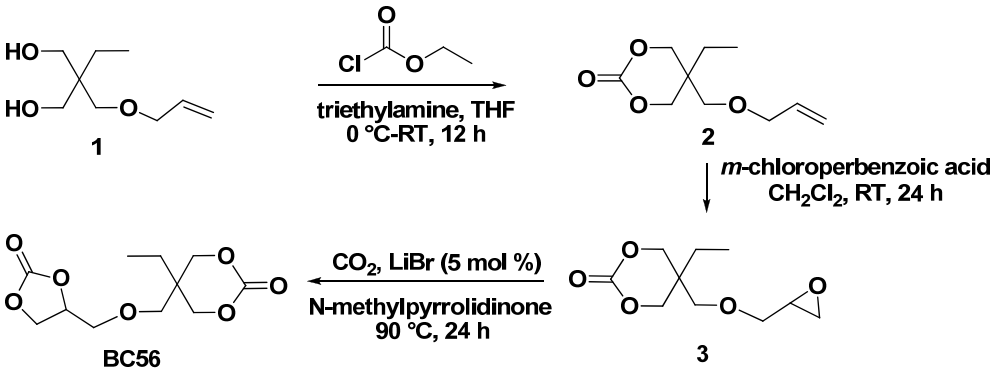
Five-membered cyclic carbonates and six-membered cyclic carbonates have different reactivity toward primary amines. This special property enables a coupler containing both cycles – 5,6-coupler (Scheme 1) – to be used for a selective linking of two functional amines (RF_1-NH_2 and RF_2-NH_2) within one molecule and to form a bis(functional) compound. On the other hand, both five- and six-membered cyclic carbonates react with primary amines. Reacting with a diamine, hydroxyl functional polyurethanes can be obtained. In the following, the synthesis of a coupler with a five and a six-membered ring (**BC56**) and its application for combining two functional amines and as monomer for the synthesis of poly(hydroxyl urethane)s are discussed.



Scheme 1: Possible use of a bifunctional coupler with a five- and a six-membered carbonate ring (5,6-coupler).

3.3.1 Synthesis of 5,6-coupler BC56

Starting with the commercially available 2-allyl-2-propyl-1,3-propanediol (**1**) the coupler **BC56** was synthesized by a sequence of three reactions (Scheme 2). First, the starting material **1** was converted to the six membered ring carbonate **2** using ethyl chloroformate as reagent and triethylamine as acid scavenger [36]. Removal of basic impurities immediatly after synthesis is important since traces of base may induce ring-opening polymerization during purification via distillation. In the next step the C,C-double bond was epoxidized using 3-chloroperoxybenzoic acid (*m*CPBA) as reagent. Epoxidation was nearly quantitative, however, upon purification via silica gel column chromatography the six-membered ring carbonate was partially converted, decreasing the yield of pure product **3** to ca. 66 %. The conversion of the epoxide ring to the five membered ring carbonate is a known process [3, 37-39]: treatment of **3** in N-methylpyrrolidone with carbon dioxide in the presence of lithium bromide as catalyst at 90 °C lead to the end product **BC56**.



Scheme 2: Synthesis of the coupler **BC56** comprising a five- and a six-membered ring carbonate

The successful preparation of the coupler **BC56** results from the ^{1}H and ^{13}C NMR analysis. In the ^{1}H NMR spectrum (Figure 2a) the protons attached to the five- and six-membered rings at δ = 4.85-5.00 ppm and δ = 4.28, 4.53 ppm (signal 8 and 9 resp. 9') and at δ = 4.22 ppm (signal 4 and 5) are characteristic. In the ^{13}C NMR spectrum the carbonyl carbon atom signals

11 and 10 at δ = 154.9 ppm and δ = 147.8 ppm prove the presence of the two rings in the coupler **BC56** (Figure 2b).

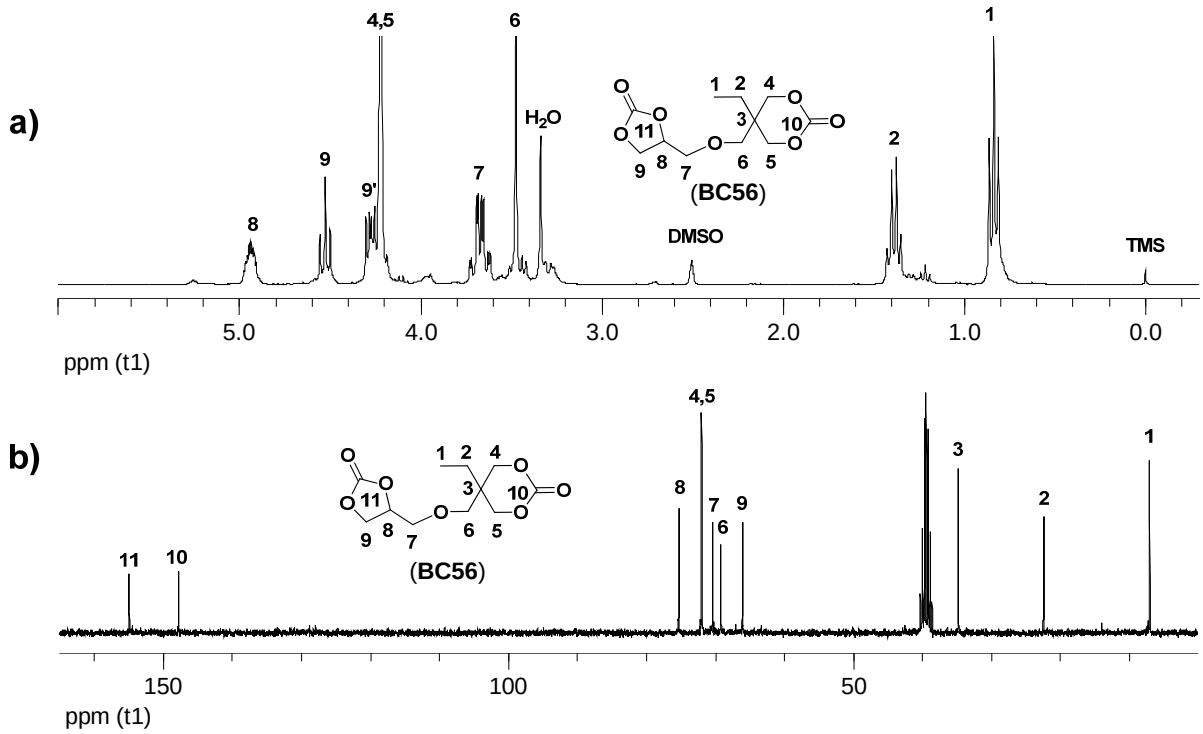
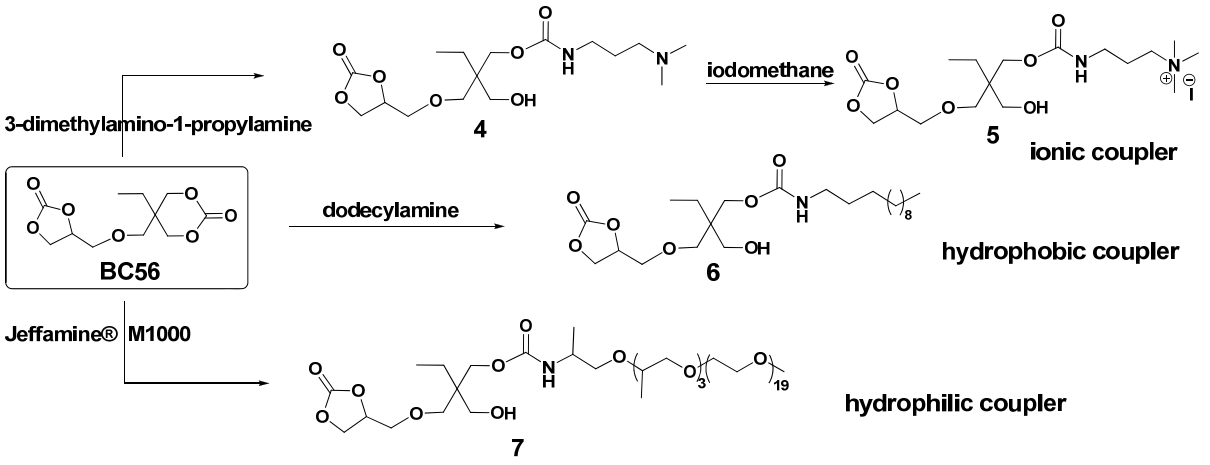


Figure 2: (a) ^{1}H NMR and (b) ^{13}C NMR spectra of **BC56** in DMSO-d_6.

3.3.2 Synthesis of functional couplers based on coupler BC56

As already known the reactivity of six-membered ring carbonates is higher than that of five membered ring carbonates due to the larger ring-strain in six-membered cyclic carbonate rings [18]. Therefore coupler **BC56** is expected to be able to react selectively with primary amines. Model reactions with different primary amines were carried out. Ionic (**5**), hydrophobic (**6**), and hydrophilic (**7**) functional couplers were synthesized starting with 3-dimethylamino-1-propylamine (followed by quarternization with iodomethane), dodecylamine, and Jeffamine® M1000 (Scheme 3).



Scheme 3: Synthesis of functional couplers: ionic coupler **5**, hydrophobic coupler **6** and hydrophilic coupler **7**.

In the reaction of **BC56** with one equivalent of 3-dimethylamino-1-propylamine or Jeffamine® M1000, only ring opening of the six-membered ring carbonate was observed, the five-membered ring carbonate did not react. As example for this selective conversion the ^{1}H and ^{13}C NMR spectra of compound **4** - the reaction product of **BC56** with 3-dimethylamino-1-propylamine – are shown (Figure 3). In the ^{1}H NMR spectrum (Figure 3a) the characteristic signals at δ = 4.30, 4.52, and 4.92 ppm (protons 8 and 9) indicate the presence of the five-membered ring carbonate; the signal at δ = 7.00 ppm is the characteristic signal for urethane proton (signal 12). Furthermore the signal characteristic for the CH_2 groups within the six-membered cyclic carbonate at δ = 4.22 ppm (Figure 2a) disappeared and new signals 4 and 5 characteristic for CH_2OCONH and CH_2OH appeared at δ = 3.83 and δ = 3.30 ppm (Figure 3a). In the ^{13}C NMR spectrum (Figure 3b) the characteristic signal of six-membered cyclic carbonate at δ = 147.8 ppm disappeared and the signal at δ = 154.9 ppm characteristic for the five-membered ring carbonate remained. The new signal at δ = 156.4 ppm is typical for a urethane carbonyl group. For the reaction of **BC56** with dodecylamine, the ratio of ring-opening of the five/six membered ring carbonate in this reaction is 8/92. The product mixture

can be separated by silica gel column chromatography. The possible explanation of this experimental result is that different microenvironment induced by the different amines causes differences in the reactivity of the amines.

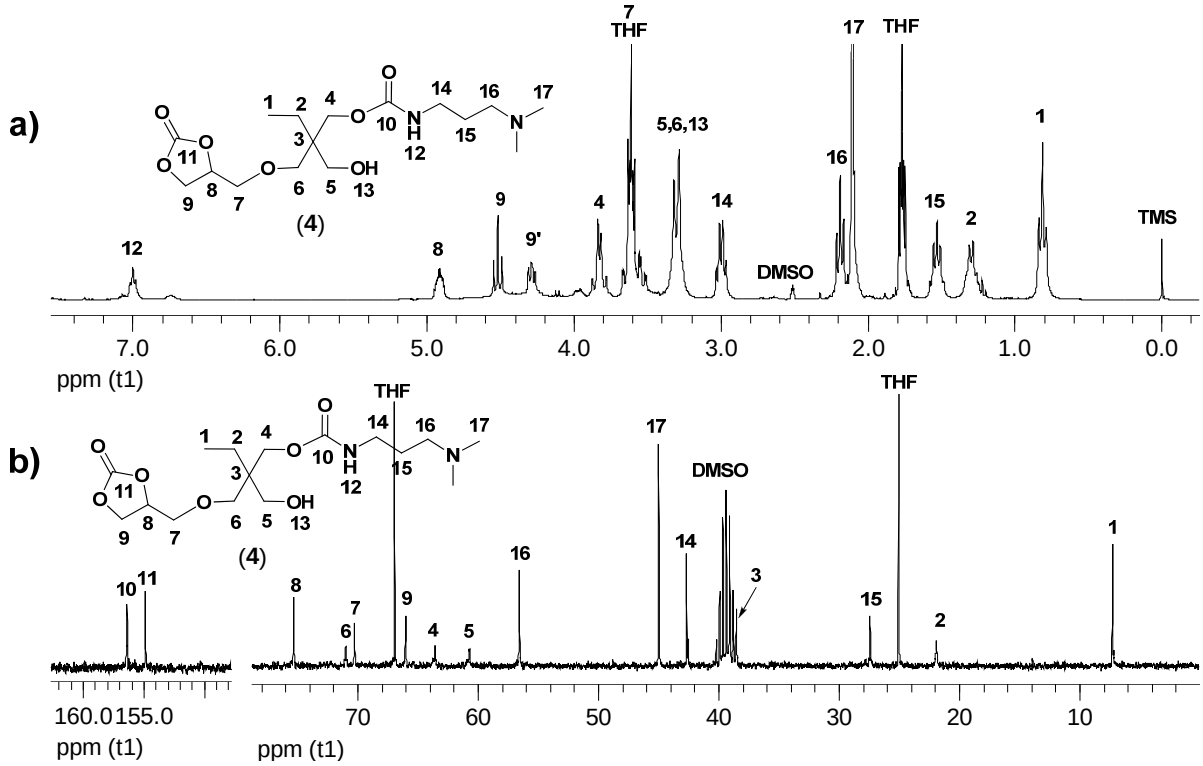
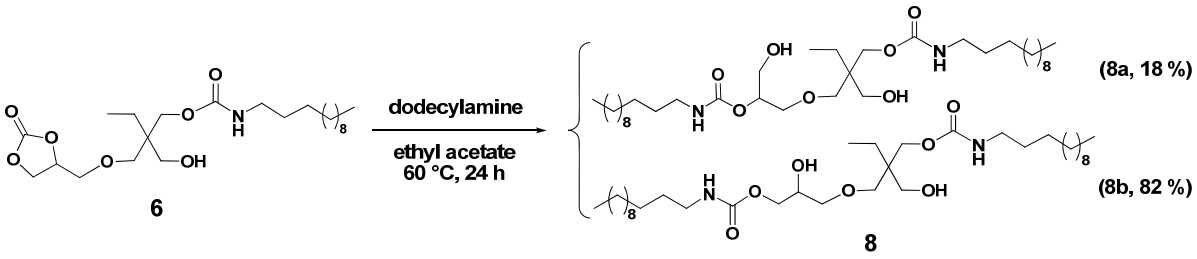


Figure 3: a) ^{1}H NMR and b) ^{13}C NMR Spectra of **4** in DMSO-d_6.

3.3.3 Model reaction of the functional coupler with primary amines.

As a model reaction of the functional coupler with primary amines the reaction of **6** with 1.1 equivalents dodecylamine was carried out. After 24 h at 60 °C in ethyl acetate as solvent, the five-membered cyclic carbonate was fully converted. The excess of dodecylamine was removed by washing the product solution with 1 % aqueous HCl. Due to the asymmetry of the substituted five-membered ring carbonate two regioisomers are expected (Scheme 4).



Scheme 4: Model reaction of the functional coupler **6** with *n*-dodecylamine

The ^{1}H NMR spectra of the functional coupler **6** and the coupling product **8** are shown in Figure 4. The characteristic signals of the five-membered ring carbonate in coupler **6** at δ = 4.40 – 4.80 ppm (signals 8 and 9, in Figure 4a) disappeared after reaction with dodecylamine (Figure 4b). The signal at δ = 3.85 ppm (proton 9', in Figure 4b) is characteristic for isomer **8a**. The signal of proton 14 is the same for both isomers. From the ratio of the integrals of proton 9' and 14, the ratio of **8a**/**8b** was determined to be 18/82.

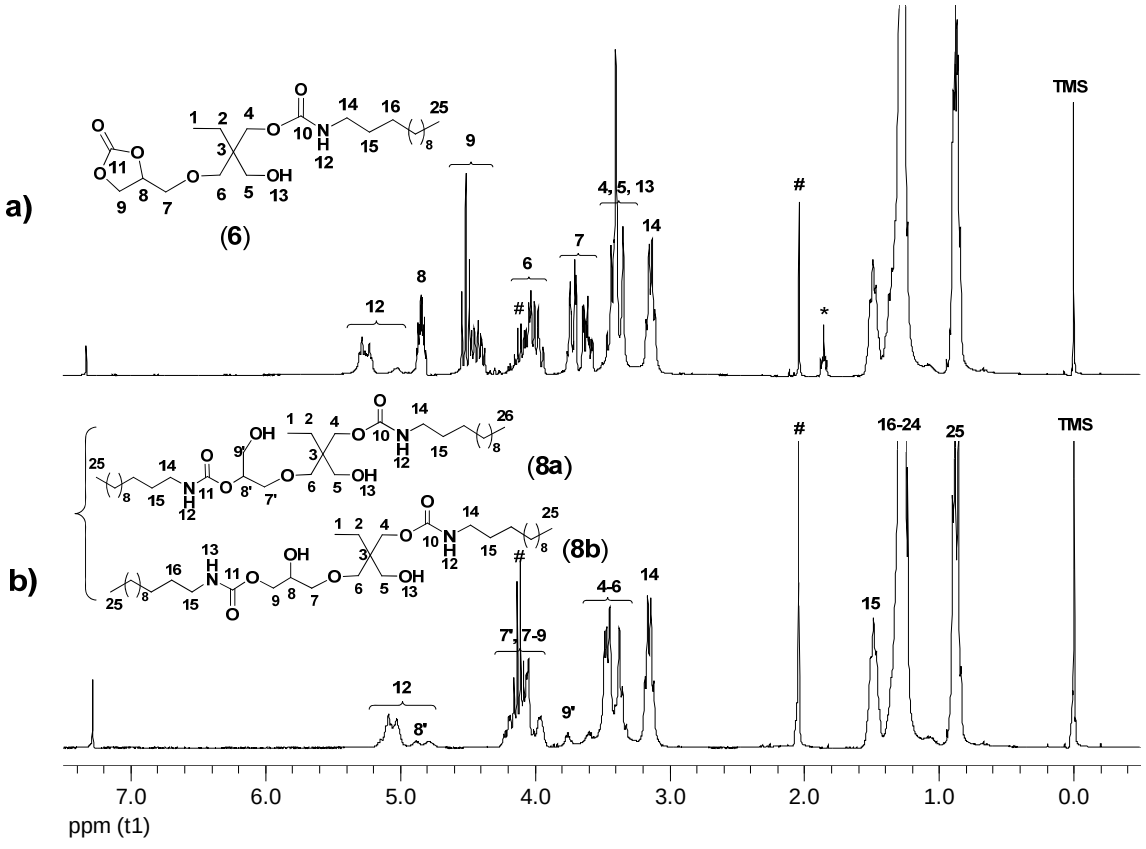
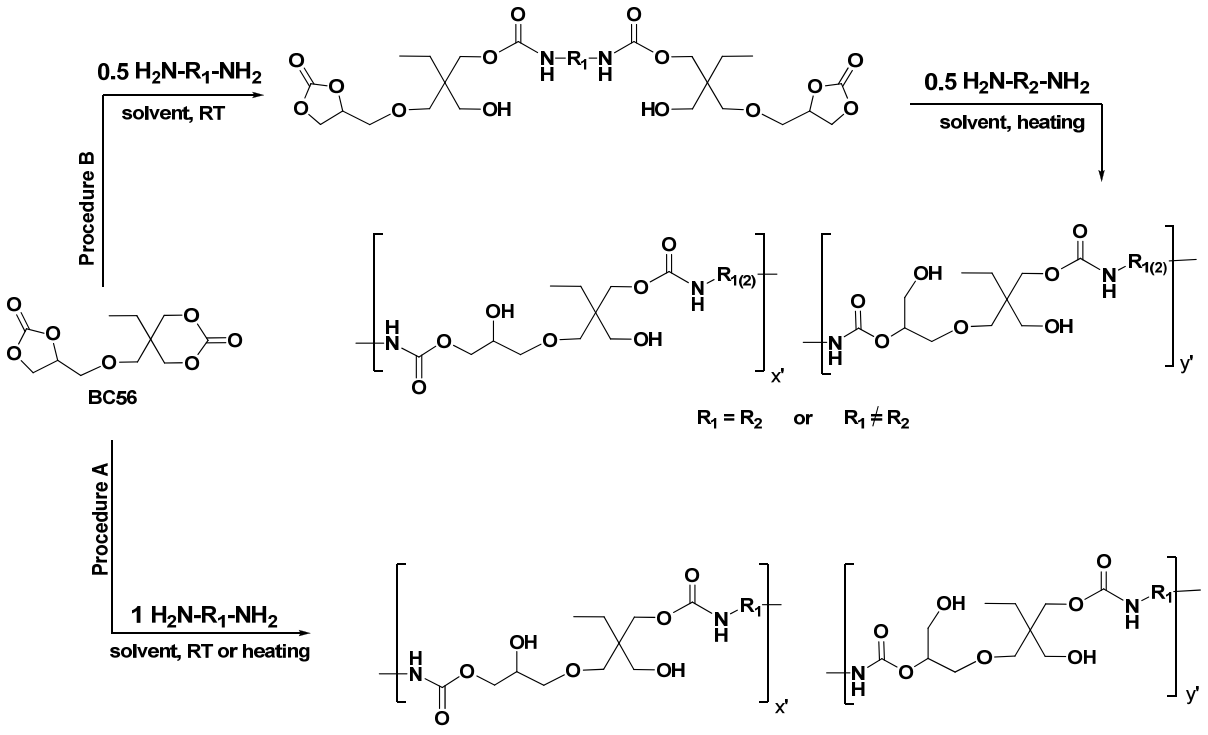


Figure 4: a) ^{1}H NMR spectrum of **6** in $CDCl_3$ and b) ^{1}H NMR spectrum of **8** in $CDCl_3$ (# = ethyl acetate; * = THF).

3.3.4 Polycondensation of BC56 with diamines

Poly(hydroxyl urethane)s attracted the notice of polymer chemists because of their hydrophilicity, their potential to absorb water, and their possibility for further modification. From bis(five-membered cyclic carbonates), bis(six-membered cyclic carbonates) or asymmetric A,A´-carbonate couplers **A1** and **A2**, poly(hydroxyl urethane)s were synthesized by reaction with diamines [1, 15, 16, 31, 40]. In general, poly(hydroxyl urethane)s prepared from cyclic carbonates have lower molecular weights than conventional polyurethanes prepared from diisocyanates and diols. In literature, the synthesis of poly(hydroxyl urethane)s was carried out by reaction of the bis(cyclic carbonate)s and diamines (molar ratio 1/1) in a one-pot reaction using solvents of high polarity (e.g. DMSO, DMAc, and NMP) at 50 °C ≤ T ≤ 100 °C.



Scheme 5: Polycondensation of **BC56** and diamines in Procedure A or Procedure B

Considering the different reactivity of the five- and six-membered cyclic carbonates, for **BC56** different synthetic procedures were applied: in procedure A **BC56** is reacted with one equivalent of diamine in a one-pot reaction, while in procedure B **BC56** is reacted sequentially first with 0.5 equivalent of diamine and after full conversion addition of another 0.5 equivalent of the same or a different diamine (Scheme 5).

3.3.4.1 Synthesis of poly(hydroxyl urethane)s according to procedure A

The polycondensation of **BC56** and diamines according to procedure A was carried out with hydrophobic and hydrophilic diamines: hexane-1,6-diamine (A_1), 4,9-dioxadodecane-1,12-diamine (A_2), and triethylenetetramine (A_3). By changing the reaction medium (polarity of the solvent) and the temperature polymers **P1 – P8** were prepared (Table 3). For purification the crude reaction mixture was precipitated in a large excess of diethyl ether at 0 °C. After decantation and drying in vacuum (10^{-2} mbar) at room temperature the polymers were obtained as highly viscous oils. The molecular weight ($\overline{M}_n$ and $\overline{M}_w$) and molecular weight distribution ($\overline{M}_w / \overline{M}_n$) of the poly(hydroxyl urethane)s were determined via SEC with DMF as eluent (Table 3). The most important parameter with influence on the molecular weight is the solvent polarity. (i) Polymers prepared in DMF (**P1 – P3)** have relative low molecular weights (2000 g/mol $< \overline{M}_w <$ 3000 g/mol), mainly oligomers are obtained. (ii) The molecular weights of polymers prepared in DMAc (**P4**, **P5**) (4500 g/mol $< \overline{M}_w <$ 6100 g/mol) are similar, a small increase is observed. (iii) Polymers (**P6, P7**) prepared in solvents with low polarity (THF and $CHCl_3$) have relative higher molecular weight ($\overline{M}_w \approx$ 10000 g/mol).

Table 3: Reaction conditions, molecular weight ($\overline{M}_n$ and $\overline{M}_w$) and molecular weight distribution ($\overline{M}_w / \overline{M}_n$) of poly(hydroxy urethane)s prepared under different conditions.

No.	Diamine[a)]	BC56/ diamine	Solvent	T(°C)	Time	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w / \overline{M}_n$ [b)]
P1	$\mathbf{A_1}$	1/1	DMF	90	3 d	1340	2240	1.70
P2	$\mathbf{A_2}$	1/1	DMF	90	3 d	1340	2500	1.85
P3	$\mathbf{A_1}$	1/1	DMF	70	3 d	1700	2600	1.56
P4	$\mathbf{A_1}$	1/1	DMAc	70	3 d	3850	6090	1.58
P5	$\mathbf{A_3}$	1/1	DMAc	70	3 d	3200	4500	1.45
P6	$\mathbf{A_1}$	1/1	THF	RT	60 d	4820	10200	2.11
P7	$\mathbf{A_1}$	1/1	$CHCl_3$	60	3 d	7140	10800	1.55
P8	$\mathbf{A_1}$	1/1.2	$CHCl_3$	60	3 d	3280	5060	1.54

[a)] A_1 = hexane-1,6-diamine, A_2 = 4,9-dioxadodecane-1,12-diamine, A_3 = triethylenetetramine;

[b)] SEC of **P1, P2** and **P6** were measured from the reaction mixture without any purification of the products.

For the polymers **P1 - P7**, a 1/1 molar ratio of **BC56**/diamine was used. It is expected that polymers with different chain end functionality - cyclic carbonate or primary amine – are obtained. For polymer **P8**, using an excess of diamine (BC56/diamine = 1/1.2), amino telechelic poly(hydroxyl urethane) oligomers ($\overline{M}_w \approx$ 5000 g/mol) are expected, which can be used as building blocks for the synthesis of multi-block copolymers or as a crosslinking agents.

The successful coupling of primary amine groups and cyclic carbonates was proven by NMR analysis. The assignment of the ^{1}H and ^{13}C NMR spectra is exemplarily shown for the poly(hydroxyl urethane) **P8** (Figure 5). In the ^{1}H NMR spectrum the coupling between the cyclic carbonate and the primary amine is proven by the urethane signals 12 and 13 at at δ = 6.60-7.20 ppm. Signals at δ = 2.90 ppm and 2.55 ppm belong to *CH_2*-NH groups in the

backbone (signal 14) and the CH_2-NH_2 groups at chain end (signal 17), respectively. The formation of primary and secondary hydroxyl groups is proven by the presence of the characteristic signals 8' and 9' at δ = 4.70, 3.45 ppm (primary hydroxyl group) and signals 7-9 at δ = 3.60-4.40 ppm (secondary hydroxyl group). In the ^{13}C NMR spectrum the characteristic peaks for the five- and six-membered cyclic carbonate (δ = 154.9 and 147.8 ppm) disappeared, and characteristic peaks for urethane carbonyl groups can be seen (signals 11 and 10 at δ = 155.9, 156.2 and 156.5 ppm). Signals of the diamine in the backbone and at the chain ends can be well distinguished (signals 14-16 vs. signals 17-19).

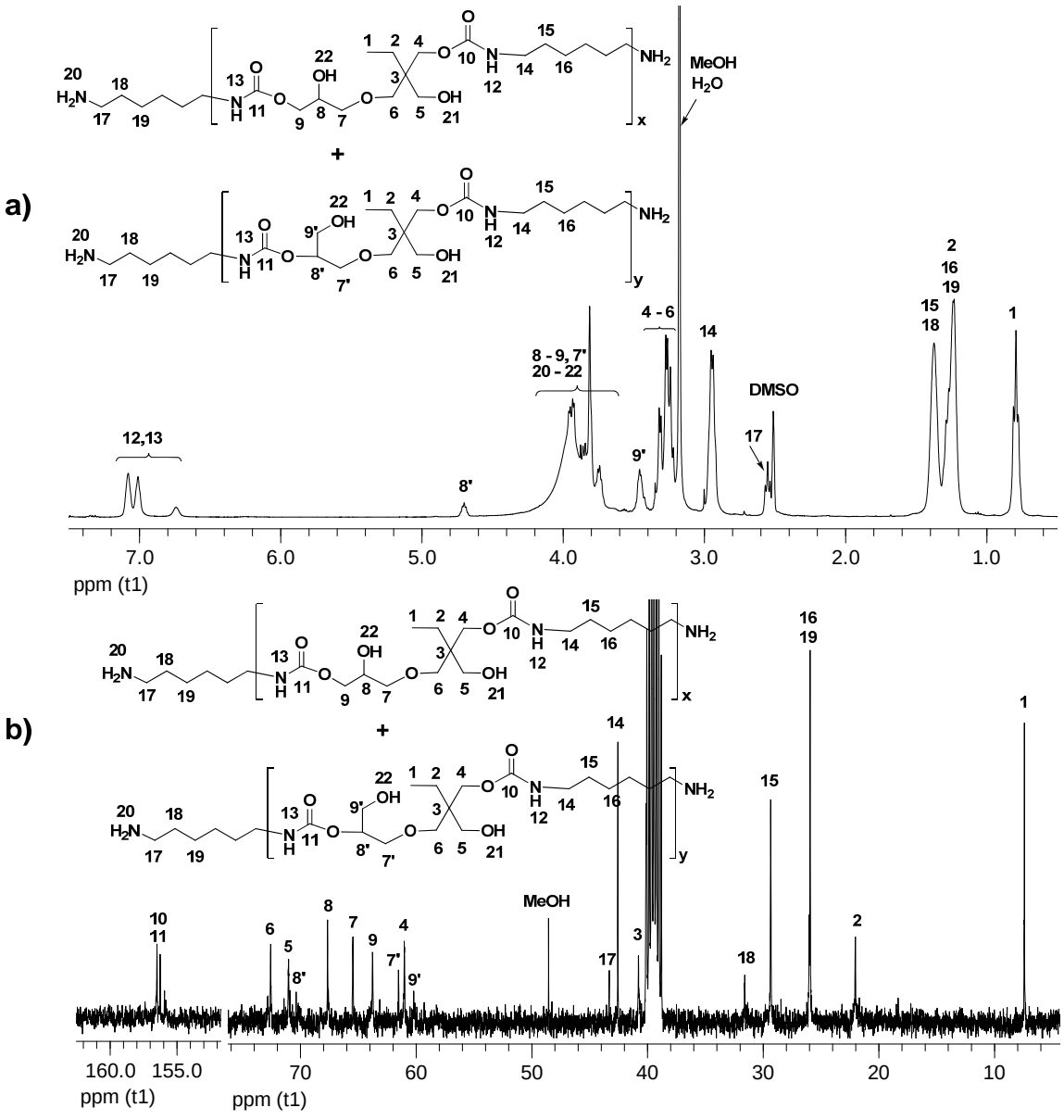
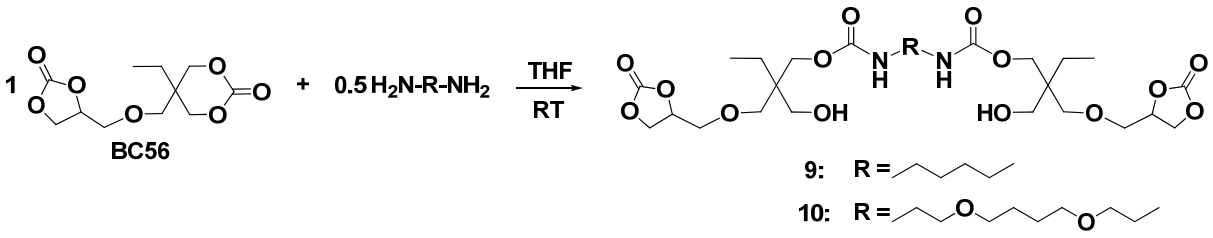


Figure 5: a) ^{1}H NMR and b) ^{13}C NMR spectra of **P8** in DMSO-d_6.

3.3.4.2 Synthesis of poly(hydroxyl urethane)s according to procedure B

As already discussed before (section **3.2**), the six-membered ring in **BC56** is much more reactive than the five-membered ring. Addition of 0.5 eq. diamine to **BC56** at room temperature was expected to convert only (or mainly) the six-membered ring carbonates. Model reactions of **BC56** with 0.5 eq. diamine were carried out with hexane-1,6-diamine and 4,9-dioxadodecane-1,12-diamine, respectively (Scheme 6). **BC56** was dissolved in THF and reacted at room temperature with 0.5 eq. diamine. After 3 days, the six-membered carbonate ring was fully converted. After removal of the solvents and drying in vacuum, compounds **9** and **10** were obtained in quantitative yield. These two compounds were further used in the synthesis of poly(hydroxyl urethane)s.



Scheme 6: Model reactions of **BC56** with 0.5 eq. diamines: synthesis of **9** and **10**.

For the synthesis of poly(hydroxyl urethane)s according to procedure B (polymers **P9** and **P10**) the same diamine was added to the reaction mixture after conversion of the first 0.5 equivalents. For the polymers **P11** and **P12**, a different diamine was reacted with the isolated intermediates **9** and **10**, respectively. The influence of the solvent polarity on the molecular weight of the polymers was similar to procedure A: in DMF oligomers are obtained ($\overline{M}_w$ = 2000-3000 g/mol); in THF molecular weights higher than 10000 g/mol were obtained (Table

4). By using different diamines in the two steps, polymers with hydrophilic and hydrophobic alternating diamine building blocks in backbone (**P11** and **P12**) were obtained.

Table 4: reaction conditions, molecular weight ($\overline{M}_n$ and $\overline{M}_w$) and molecular weight distribution ($\overline{M}_w / \overline{M}_n$) of poly(hydroxyl urethane)s prepared in different conditions

No.	First [a)] diamine	Second diamine	BC56/ diamine	solv.	T (°C)	t	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_w / \overline{M}_n$
P9	**A_1**	**A_1**	1/1	DMF	90	3 d	1500	2550	1.70
P10	**A_2**	**A_2**	1/1	DMF	90	3 d	1650	2980	1.80
P11	**A_1**	**A_2**	1/1	THF	60	3 d	7500	11400	1.55
P12	**A_2**	**A_1**	1/1	THF	60	3 d	8800	13900	1.58

[a)] A_1 = hexane-1,6-diamine, A_2 = 4,9-dioxadodecane-1,12-diamine;

3.3.5 Hydrolytic stability test

Nowadays, the use of water as solvent in polymer chemistry attracts more and more attention. (i) Water as a solvent has obvious environmental and economic advantages relative to organic solvents, being both inexpensive and non-toxic. (ii) In some cases highly polar solvents increase reaction rates and enhance reaction selectivity. (iii) Most important for polymer chemistry is the fact that water promotes self-association of amphiphilic compounds generating selectively secondary structures. Using water as solvent for the synthesis of multifunctional polymers by application of the coupler chemistry, in situ self-association of amphiphilic grafts is expected. However, for a successful application of water as solvent, the hydrolytic stability of the reactants has to be determined. In this work, the hydrolytic stability of **BC56** and of the functional coupler **7** was determined exemplarily.

BC56 is insoluble in water, however, in aqueous DMSO (DMSO/water = 3/2 v/v) **BC56** forms a homogeneous solution. The hydrolytic stability of **BC56** was determined in DMSO-

d_6/D_2O (v/v = 3/2) at room temperature, at 60 °C and at 90 °C in a concentration of 20 g/L. (Table 5). After 7 days at 90 °C, the six-membered cyclic carbonate ring was fully hydrolyzed. At room temperature and 60 °C, the conversion of the six-membered ring is 15 % and 40 %, respectively. The five-membered ring is hydrolytically stable under the test conditions. From this result it can be concluded, that conversion of **BC56** with amines in aqueous media should be possible due to the higher nucleophilicity of the amine.

Table 5: Hydrolytic stability of the five- and six-membered carbonate rings of **BC56** in DMSO-d_6/D_2O (v/v = 3/2; concentration 20 mg/mL)

No.	Temperature	Time	Hydrolysis of five-ring	Hydrolysis of six-ring
1	RT	20 d	0 %	15 %
2	60 °C	7 d	0 %	40 %
3	90 °C	7 d	0 %	100 %

Functional coupler **7** is soluble in water. Its hydrolytic stability was determined in D_2O at room temperature and at 60 °C using ^{1}H NMR spectroscopy. Comparing the spectrum of functional coupler **7** in D_2O at time zero (Figure 6a) and after 14 days at room temperature no conversion of any functional group could be observed (Figure 6b). However, after 14 days at 60 °C, the characteristic signals of the five-membered cyclic carbonate at δ = 4.40, 4.55, and 4.97 ppm (proton 8 and 9) decreased significantly. From the ^{1}H NMR spectrum it was determined that 55% of the carbonate ring was hydrolyzed (Figure 6c). The characteristic signals of protons 15 and 19 at δ = 1.00-1.18 ppm, however remained unchanged, indicating the stability of the urethane group under the test conditions.

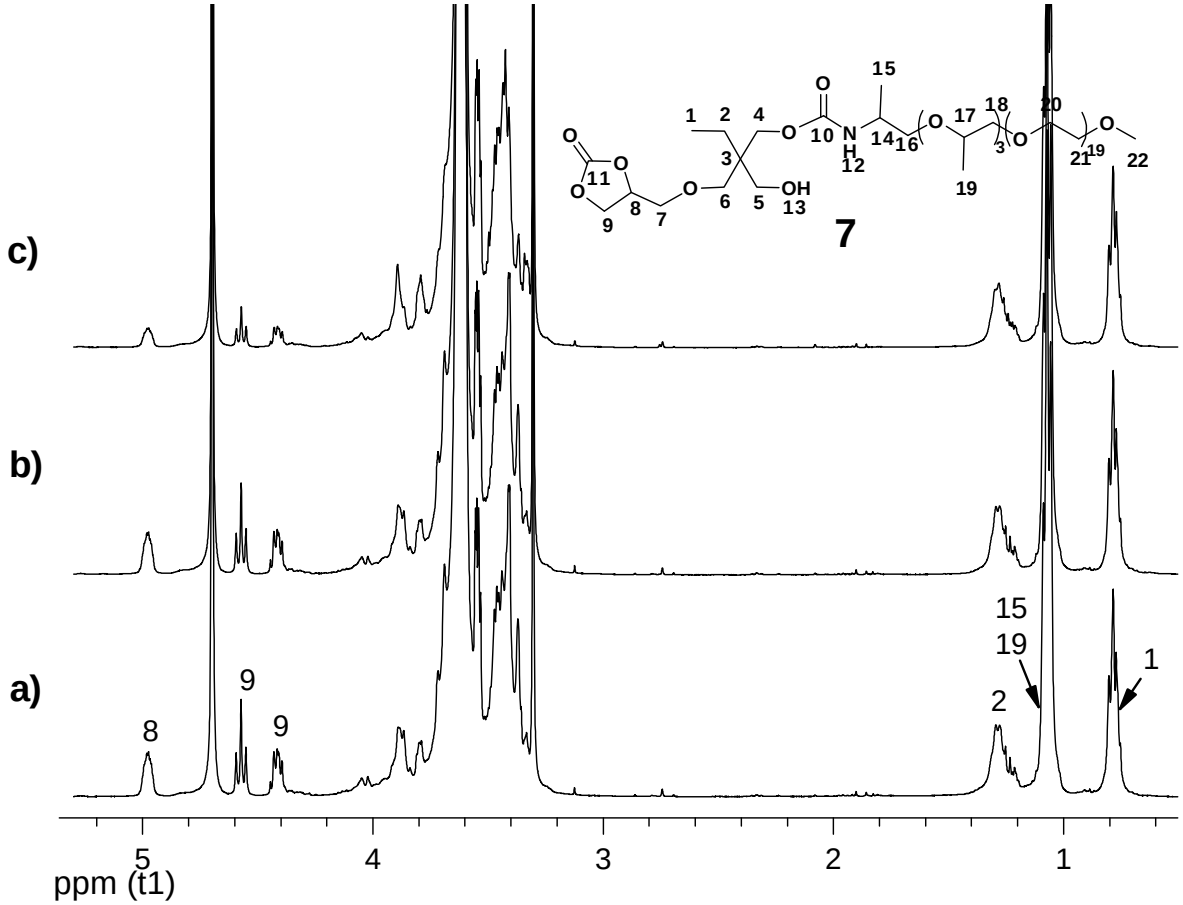


Figure 6: Hydrolysis of functional coupler **7** in D_2O monitored by ^1H NMR spectroscopy: a) sample at time zero; b) sample after 14 days at RT; c) sample after 14 days at 60 °C.

3.4 Conclusion

Bis(cyclic carbonate) coupler **BC56** bearing five- and six-membered carbonate rings was successfully synthesized and characterized. It was evidenced by means of model reactions that the reaction of **BC56** with one equivalent primary amine proceeds highly selective, only/mainly the six-membered cyclic carbonate was converted. Functional couplers with hydrophobic, ionic, and hydrophilic groups were prepared starting with **BC56** and corresponding primary amines. In a later step the five-membered carbonate ring in the

functional couplers can be opened by primary amines, too. If the second amine group is part of a polyamine, multifunctional polymers are obtained.

Poly(hydroxyl urethane)s were prepared from **BC56** and diamines in a one-pot procedure (procedure A**)** or in a two-step reaction (procedure B). By adding different diamines in the two step procedure, polymers with hydrophilic and hydrophobic alternating diamine building blocks in backbone were prepared. In both procedures, similar influences of solvents on the molecular weight are observed. Solvents with low polarity (e.g. THF and $CHCl_3$) result in polymers with relative high molecular weight ($\overline{M}_w \approx$ 10000 g/mol) whereas in solvents with high polarity (e.g. DMAc, DMF) oligomers (2000 g/mol $< \overline{M}_w <$ 6000 g/mol) were obtained.

In the future, starting with **BC56** functional couplers will be prepared and used for the synthesis of multifunctional polyamines (e.g. poly(ethylene imine), polyvinylamine….) in water as solvent. For example for the reaction using hydrophobic functional couplers, these couplers can form micelles in water or aqueous solution, with alkyl chains well organized in the micellar core and the five-membered ring carbonate groups in the periphery. These micelles then react with polyamines, their structure being preserved during and after the reaction. Thus a secondary structure stabilized by hydrophobic interaction in aqueous medium is generated. It is expected that by this procedure polymers with predesigned secondary/tertiary structure can be obtained. From hydrolysis tests of the functional couplers prepared from **BC56** it was concluded that these substrates are stable in water and might be used as substrates in polymer analogous reaction in water.

The poly(hydroxyl urethane)s obtained in the condensation of **BC56** will be used as starting materials for comb shaped polymers, as segments for block copolymers, or as cross-linkers.

The physical properties and potential for applications of poly(hydroxyl urethane)s with hydrophilic and hydrophobic alternating diamine building blocks will be studied.

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Chapter 4

Amphiphilic Building Blocks for Head Groups in Linear Polymers

4.1 Introduction

End-functionalized polymers are of great interest due to their potential application in many important areas; thus a variety of end-functionalized polymers were successfully prepared [1-4]. Lipid-end-functionalized poly(ethylene glycol)s were used for stabilization of organized macromolecular assemblies composed of a polymer core and a lipid shell. These composites combine the advantages of particles (mechanical stability) with attributes of lipids (biocompatibility) [5]. A poly(vinyl alcohol) end-functionalized with long alkyl chains was used for the stabilization of liposomes. The efficiency of the stabilization of such a modified poly(vinyl alcohol) is much higher than that of a non-modified poly(vinyl alcohol) [6]. Fullerene-end-capped polymers (C_{60}-end capped polymers) are of particular interest because of the potential use for electro optic materials. The hydrophobic C_{60} moieties self-assemble in polar solvents and form micelle or vesicle-like aggregates in a nanometer scale; self-assembly in bulk was observed, too [7-10]. Poly(methyl methacrylate) (PMMA) and PMMA containing polyhedral oligomeric silsesquioxane (PMMA-POSS) homopolymer blends are totally miscible in the amorphous phase over the entire composition. These organic-inorganic hybrid materials attracted increasing attention in recent years due to the enhancements in a variety of physical and mechanical properties [11].

Polymers with pendant functional groups are of interest as starting materials for complex polymer architectures, e.g., comb, brush, dendron jacketed linear, and hypergrafted polymers. In the literature, different polymers with pendant hydroxyl groups were used for polymer analogues and grafting reactions [12], such as polyglycidol [13, 14], polyvinylalkohol [15], poly(hydroxyl acrylate), poly(hydroxyl methacrylate) [16, 17], cellulose [18], chitosan [19], etc. Starting from bis(cyclic carbonates) and diamines, poly(hydroxyl urethane)s were prepared via polycondensation. Because of their high hydrophilicity compared to conventional polyurethane and their possibility for further modification, poly(hydroxyl urethane)s have been paid much attention in the past [20-22]. However, the modification of the hydroxyl groups in poly(hydroxy urethane)s has not been reported.

Polymers with pendant hydroxyl groups are suitable for different modifications. They can be used (i) as initiators for ring-opening polymerization (ROP) [23]; (ii) to prepare initiators for controlled radical polymerization (nitroxide-mediated radical polymerization (NMRP) [24], and atom transfer radical polymerization (ATRP) [25, 26]; and (iii) to prepare activated carbonates for grafting reactions [27].

In the past, starting with asymmetric A,A´-carbonate couplers **A1** - **A4** (see later, **Scheme 1**) via substitution reaction with primary amines at low temperature (0 °C - 25 °C), different functional couplers were prepared [28-30]. These functional couplers were further reacted with primary amine via addition reaction at high temperature (60 °C - 80 °C). Thus, bis(functional) compounds with a hydroxyl group in α or β-position to the formed urethane groups were obtained. Via modification of the formed hydroxyl groups, these bis(functional) compounds can be introduced as head groups to polymer chains.

In this work, starting from asymmetric A,A´-carbonate couplers **A1** – **A4**, several model bis(functional) alcohols were prepared and used as initiators for ring opening polymerization and were converted to an ATRP initiator. Poly(ε-caprolactone) and poly(methyl methacrylate) with tailored amphiphilic head group were synthesized. The modification of the hydroxyl groups to phenyl carbonate groups was performed and used for the functionalization of Jeffamine® M1000.

4.2 Experiment part

4.2.1 Nomencluture

Products are named as described in the following: The first unit gives the applied bifunctional coupler skeleton, which is either glycerol (**C_3**) from bifunctional coupler **A1** and **A3** or hexane-1,2,6-triol (**C_6**) from bifunctional coupler **A2** and **A4**. The next unit assigns the primary amine used for the substitution reaction (first step): **A8** and **A12** presents the octylamine and dodecylamine, respectively; **Q** presents the N,N-dimethylpropane-1,3-diamine (**QI** the corresponding trimethylammonium compounds, **QHCl** the corresponding hydrochloride); **J1000** presents Jeffamine ®M1000. The third unit indicates the second primary amine used for the ring-opening reaction of the five-membered cyclic carbonate; the abbreviations of amines are the same as in the second unit; **a** and **b** refer to the isomer with secondary hydroxyl group (**a**) and the one with primary hydroxyl group (**b**). The forth unit present the modification of the hydroxyl group formed upon ring-opening of the five-membered cyclic carbonate with amine: **PCL** presents the modification via ring-opening polymerization of ε-caprolactone; **Br** presents the modification with 2-bromo-2-methyl propionyl bromide to initiator; **PMMA** presents the modification of the **Br**-initiator via ATRP

of methyl methacrylate (**Br** was changed to **PMMA**); **Ph** presents the modification with phenyl chloroformate; after reacting with linear Jeffamine® M1000 (**J1000**), **Ph** was changed to **J1000**.

The following compounds were prepared: **C_6A8-Q-PCL**, **C_6A8-A8-PMMA**, **C_3J1000-Q-PMMA**, **C_6A8-Q-PMMA**, **C_3A12-Q-PMMA** and **C_3A12-QI-PEI**.

4.2.2 Materials

Glycidol (Fluka, 95 %), phenyl chloroformate (Acros Organics, 99 %), pyridine (Fluka, 99.8 %), dimethyl carbonate (Acros Organics, 99%), 1,4-diazabicyclo[2,2,2]octane (DABCO, Aldrich, 98 %), hexane-1,2,6-triol (Aldrich, 96%), octylamine (Aldrich, 99 %), dodecylamine (Acros, 98 %), triethylamine (Fluka, 99.8 %), N,N-dimethylpropane-1,3-diamine (Aldrich, 99 %), iodomethane (Aldrich, 99 %), 2-bromo-2-methylpropionyl bromide (Aldrich, 99.8 %), dichlormethane (Merck, 99.5 %, CH_2Cl_2), tetrahydrofuran (Acros, 99.99 %, THF), 2,2'-bipyridine (bipy), *N*,*N*,*N*,*N*,*N*-pentamethyldiethylenetriamine (PMDETA), all chemicals are used as received. Methyl methacrylate (MMA, 99%) was purchased from Aldrich and purified by passing through a column filled with basic alumina to remove the inhibitors and antioxidants.

4.2.3 Measurements

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 and 101 MHz, respectively. Some of the spectra were recorded on a Bruker DPX-300 FT-NMR spectrometer at 300 and 75 MHz.

Carbon, hydrogen and nitrogen elemental analyses were performed on a Heraeus CHN-O Rapid Elementar Vario E1 instrument.

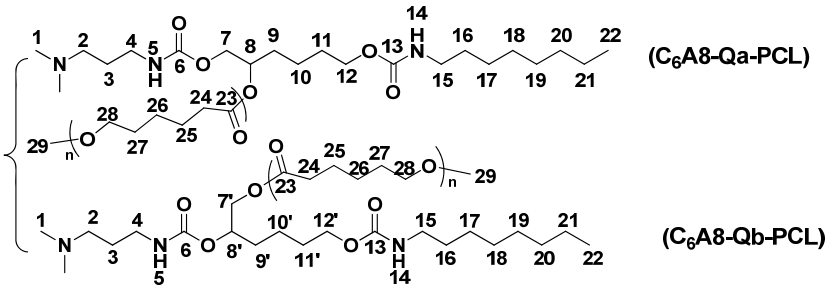
Size exclusion chromatography (SEC) analyses were carried out at 35 °C using a high performance liquid chromatography pump (ERC HPLC 64200) and a refractive index detector (ERC-7215 a). The eluting solvent was tetrahydrofuran (HPLC grade) with 250 mg · L^{-1} 2,6-*di-tert*-butyl-4-methylphenol and a flow rate of 1 mL · min^{-1}. Five columns with MZ gel were applied. The length of the first column was 50 mm and of the four other columns it was 300 mm. The diameter of each column was 8 mm, the diameter of the gel particle 5 mm, and the nominal pore widths were 50, 50, 100, 1000 and 10000 Å, respectively. Calibration was achieved using narrow distributed poly(methyl methacrylates) standards.

Differential Scanning Calorimetry analysis (DSC) was carried out on Netzsch DSC 204 'Phoenix' differential scanning calorimeter in nitrogen atmosphere with heating and cooling rates of 10 K/min.

4.2.4 Synthesis

(2-Oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate (A1) was prepared according to ref. [29], **4-(2-oxo-1,3-dioxolan-4-yl)butyl phenyl carbonate (A2)** was prepared according to ref. [28], (**2-oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (A3)** and **4-(2-oxo-1,3-dioxolan-4-yl)butyloxy chloroformate (A4)** were prepared according to ref. [31]. **C_3A12** and **C_3A12-Q** was prepared according to ref [30]. The synthesis and characterization of functional couplers **C_3J1000** and **C_6A8**, bis(functional) compounds **C_3J1000-Q**, **C_6A8-Q**, and **C_6A8-A8**, ATRP initiators **C_3A12-Q-Br**, **C_6A8-Q-Br**, **C_3J1000-Q-Br** and ATRP polymerization products **C_3A12-Q-PMMA**, **C_6A8-Q-PMMA**, **C_3J1000-Q-PMMA** is described in supporting information document.

Synthesis of C_6A8-Q-PCL



In a Schlenk tube, **C_6A8-Q** (0.183 g, 0.44 mmol) was dissolved in ε-caprolactone (2.00 g, 17.5 mmol) and heated to 130 °C. Then $Zn(oct)_2$ (50 mg) were added and the mixture was stirred for 18 h at 130 °C under nitrogen. The polymerization was stopped by cooling, exposing the solution to air and diluting it with CH_2Cl_2. The solution was precipitated in pentane. A waxy solid was obtained. Yield: 66 %. From SEC (THF): $\overline{M}_n$ = 4800, $\overline{M}_w$ = 12 900, $\overline{M}_w / \overline{M}_n$ = 2.45.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (t, ^3J = 6.5 Hz, 3 H, H^{22}), 1.20-1.32 (br., 10 H, $H^{17\text{-}21}$), 1.32-1.54 (m, $H^{9\text{-}11,\ 9'\text{-}11'}$ and H^{26}), 1.55-1.75 (m, $H^{3,25,27}$), 2.05-2.40 (t, ^3J = 7.5 Hz, $H^{1,24}$), 2.40-2.60 (m, H^2), 3.15 (dd, ^3J = 6.6 Hz, ^3J = 13.1 Hz, 2 H, H^{15}), 3.20-3.40 (m, 2 H, H^4), 3.95-4.15 (t. ^3J = 6.6 Hz, H^{28}), 4.20-4.35 (m, $H^{7,7'9,9'}$), 4.70-5.30 (m, NH, $H^{5,14}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{22}), 22.6 (C^{21}), 24.5 (C^{27}), 25.5 (C^{26}), 27.4 (C^3), 28.3 (C^{25}), 28.7, 29.2, 31.8, 32.3 ($C^{16\text{-}20}$), 34.1 (C^{24}), 35.2 (C^1), 40.0 (C^{15}), 41.0 (C^4), 45.0 (C^1), 56.5 (C^2), 62.5 (C^{12}), 64.1 (C^{28}), 68.1 ($C^{7,\ 7'}$), 70.1 ($C^{8,\ 8'}$), 155.7, 156.0 ($C^{6,\ 13}$), 173.5 (C^{23}) ppm

Synthesis of C6A8-A8-PCL and C3A12-Q-PCL - Optimization of the reaction conditions

The reactions were performed using a 1-step and a 2-step procedure using different amounts of Zn(oct)2 catalyst.

1-step procedure: **C6A8-A8** (194 mg, 0.44 mmol) or **C3A12-Q** (188 mg, 0.44 mmol), ε-caprolactone (2.00 g, 17.5 mmol) and Zn(oct)2 (20 mg, 50 mg, 80 mg, 120 mg) were reacted 18 h at 130 °C under nitrogen. The products were characterized via GPC and NMR.

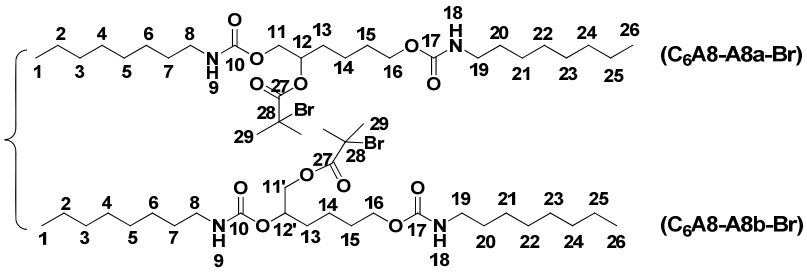
2-Step procedure: **C6A8-A8** (194 mg, 0.44 mmol), ε-caprolactone (0.50 g, 4.4 mmol) and $Zn(oct)_2$ were reacted under nitrogen atmosphere at 130 °C.

2-Step-A: After 10 hours, another portion of ε-caprolactone (1.50 g, 13.2 mmol) was added and heated at 130 °C for another 12 hours (**2-step procedure A**). Alternatively, the polymer was isolated by precipitated in pentane, dried under vacuum, before reacting with a second portion of ε-caprolactone.

2-Step-B: The product obtained in the first step was dissolved ε-caprolactone (1.50 g, 13.2 mmol), treated with 50 mg of $Zn(oct)_2$ and then heated at 130 °C for another 12 hours under nitrogen.

The products was isolated as described before and characterized via GPC and NMR (**Table 1** in section **4.3.2)**.

Synthesis of C_6A8-A8-Br



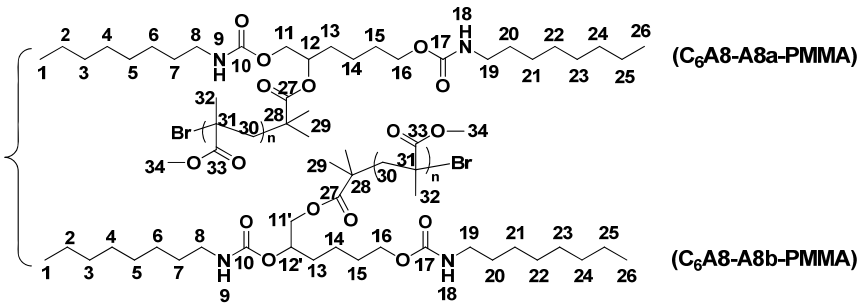
C_6A8-A8 (**a/b**) (920 mg, 2.07 mmol) and pyridine (213 mg, 2.69 mmol) were dissolved in dry THF (20 mL) under nitrogen atmosphere and cooled to 0 °C. A solution of 2-bromo-2-methylpropionyl bromide (618 mg, 2.69 mmol) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 24 h. The reaction mixture was filtrated to remove the formed pyridine hydrochloride. After removal of the solvent, the residue was diluted with $CHCl_3$ (100 mL) and washed with $NaHCO_3$-

saturated aqueous solution three times and the organic phase was dried over sodium sulfate. After removal of solvent and drying in vacuum (10^{-2} mbar, 3 h, room temperature) the product was obtained as white powder. Yield: 980 mg (80 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.6 Hz, 6 H, $H^{1,26}$), 1.20-1.35 (br., 20 H, H^{2-6} and H^{21-25}), 1.38-1.55 (m, 6 H, $H^{7,14,20}$), 1.55-1.75 (m, 4 H, $H^{13,15}$), 1.92 (s, 6 H, H^{29}), 3.15 (dd, ^3J = 6.5 Hz, ^3J = 12.8 Hz, 2 H, H^{15}), 4.04 (t, ^3J = 6.3 Hz, 2 H, H^{16}), 4.08-4.34 (m, 2 H, $H^{11,11'}$), 4.70-4.90 (br., 2 H, $H^{9,18}$), 4.95-5.20 (m, 1 H, $H^{12,12'}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (2 C, $C^{1,26}$), 21.45, 21.61 ($C^{14,14'}$), 22.6 (2 C, $C^{2,25}$), 26.7 (2 C, $C^{7,20}$), 28.56, 28.70 ($C^{15,\ 15'}$), 29.19, 29.22, 29.92, 29.98 (6 C, $C^{4-6,\ 21-23}$), 30.29, 30.48 ($C^{13,\ 13'}$), 30.63, 30.68 (C^{29}), 31.8 (2 C, $C^{3,24}$), 40.99, 41.05 ($C^{8,19}$), 55.6 (C^{28}), 64.20, 64.33 (C^{16}), 66.7 ($C^{11,11'}$), 71.3 ($C^{12'}$), 73.4 (C^{12}), 155.95, 156.01, 156.66 ($C^{10,17}$), 171.15, 171.36 (C^{27}) ppm

Synthesis of C_6A8-A8-PMMA



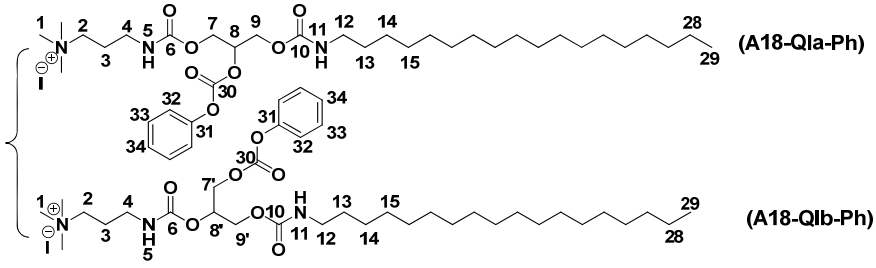
A typical ATRP procedure was performed as follows: **C_6A8-A8-Br** (178 mg, 0.299 mmol), MMA (3 g, 29.9 mmol), CuBr (43 mg, 0.299 mmol), 2,2′-bipyridine (bpy, 94 mg, 0.598 mmol), and butyl acetate (3 mL) were placed in a Schlenk tube. After deoxygenation, the Schlenk tube was placed into an oil bath at 60 °C under nitrogen atmosphere. At time intervals, samples were withdrawn via a syringe for ^{1}H NMR and SEC analysis. The polymerization was stopped by cooling, exposure to air, and dilution with CH_2Cl_2. After work-up using 5 %-HCl aqueous solution, the polymer was purified by precipitations in

pentane from CH_2Cl_2 solution. Yield: 2.27 g (72 %). From SEC (THF): $\overline{M}_n$ = 20 800 g/mol, $\overline{M}_w$ = 26000 g/mol, $\overline{M}_w / \overline{M}_n$ = 1.25.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.60-0.95 (br., $H^{1, 26, 29, 32}$), 1.01 (s, $H^{2-7, 21-25, 29, 32}$), 1.13 (s, H^{20}), 1.15-1.50 (m, $H^{13-15, 29,32}$), 1.80-2.30 (m, H^{30}), 3.15 (dd, ^3J = 6.8 Hz, ^3J = 13.2 Hz, 4 H, $H^{8, 19}$), 3.60 (s, H^{34}), 3.96-4.26 (m, $H^{12, 12', 16, 16'}$), 4.70-5.10 (m, $H^{11, 11'}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 13.9 ($C^{1, 26}$), 16.0, 18.4 ($C^{26, 29}$), 21.0, 21.3 ($C^{14, 14'}$), 22.1 ($C^{2, 25}$), 26.4 ($C^{7, 20}$), 28.64, 28.67, 28.70 ($C^{15, 15'}$), 29.0, 29.2, 29.4, 30.2 ($C^{4-7, 21-24}$), 31.2 ($C^{13, 13'}$), 31.7 ($C^{3, 24}$), 40.3 ($C^{8, 19}$), 43.9, 44.2 ($C^{25, 28}$), 51.7 (C^{31}), 53.6 (C^{27}), 61.9, 62.3 ($C^{11, 11'}$), 65.7 ($C^{16, 16'}$), 70.2, 71.7 ($C^{12, 12'}$), 155.8, 156.2 ($C^{6, 10}$), 176.1, 176.3, 177.1, 177.3 ($C^{24, 30}$) ppm

Synthesis of A18-QI-Ph



Hydrophobic coupler **A18** and 3-dimethylamino-1-propylamine (1.2 eq.) were dissolved in THF and the mixture was stirred for 24 hours at 70 °C. After removal of the solvents and the excess of 3-dimethylamino-1-propylamine in vacuum, the residue was dissolved in dichloromethane. Pyridine (237 mg, 3 mmol) was added and the mixture was cooled to 0 °C. A solution of phenyl chloroformate (5.62 g, 35.8 mmol) in CH_2Cl_2 (20 mL) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 18 h. After removal of the solvents the reaction mixture was dissolved in CH_2Cl_2 (7 mL) and precipitated in pentane/ Et_2O (v/v = 2/1, 600 mL) for two times and then dissolved in CH_2Cl_2 (200 mL) and washed three times with 10 %-Na_2CO_3 aqueous solution (3 x 50 mL)

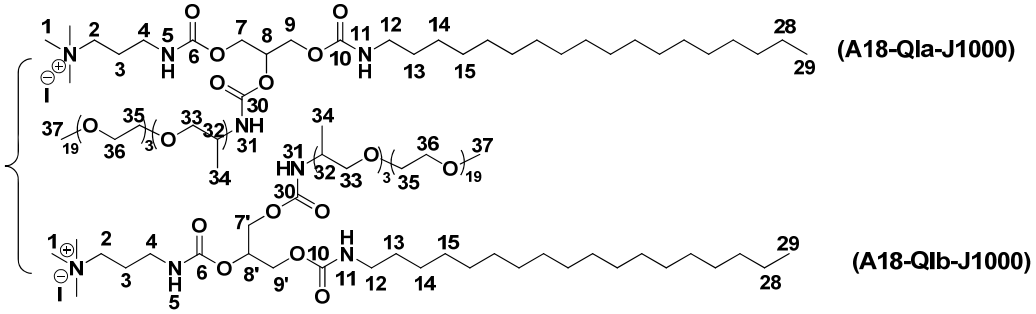
and one time with brine (50 mL). The organic phase was dried over sodium sulfate. After that, at room temperature and under vigorous stirring, iodomethane (2.58 g, 18.19 mmol) was added and the mixture was stirred at room temperature for 20 hours. After removal of the solvents the reaction mixture was dissolved in CH_2Cl_2 (7 mL) and precipitated in pentane/ Et_2O (v/v = 2/1, 600 mL) for two times. The product was dried in vacuum (10^{-2} mbar, 3 h, room temperature). A slightly yellow powder was obtained. Yield: 9.64 g (84 %).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, ^3J = 6.6 Hz, 3 H, H^{29}), 1.22-1.23 (m, 30 H, H^{14-28}), 1.36-1.50 (br., 2 H, H^{13}), 1.88-2.00 (br., 2 H, H^3), 2.90-3.04 (m, 2 H, H^4), 3.04-3.30 (m, 11 H, $H^{1,12}$), 3.38 (t, ^3J = 8.0 Hz, 2 H, H^2), 3.80-4.60 (m, 4 H, $H^{7,7',9,9'}$), 4.90-5.40 (m, 1 H, $H^{8,8'}$), 6.80-7.60 (m ,7 H, $H^{5,11,32-34}$) ppm.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 13.9 (C^{29}), 22.0 (C^{28}), 26.2 (C^3), 27.2, 28.7, 28.8, 29.0 (C^{14-20}), 29.6 (C^{13}), 31.4 (C^{21}), 38.5 (C^4), 40.2 (C^{12}), 52.1 (C^1), 61.5, 62.0 ($C^{7,9}$), 63.3 (C^2), 66.8 ($C^{7',9'}$), 69.4 ($C^{8'}$), 75.3 (C^8), 121.1 (C^{33}), 127.5 (C^{34}), 129.7 (C^{32}), 149.5, 150.6 (C^{30}), 152.5, 152.8 (C^{31}) 155.2, 155.6 ($C^{6,10}$) ppm.

Synthesis of A18-QI-J1000

A18-QI-Ph (4.03 g, 5.85 mmol) and Jeffamine® M1000 (1.00 g, 23.2 mmol) were dissolved in DMF (20 mL) and the reaction mixture was heated at 70 °C for 24 h. After removal of the solvents the product was dissolved in $CHCl_3$ (5 mL) and precipitated in pentane/Et_2O (v/v = 2/1, 600 mL) for three times. The product was dried in vacuum at room temperature. A yellow waxy solid was obtained. Yield: 3.53 g (73 %).



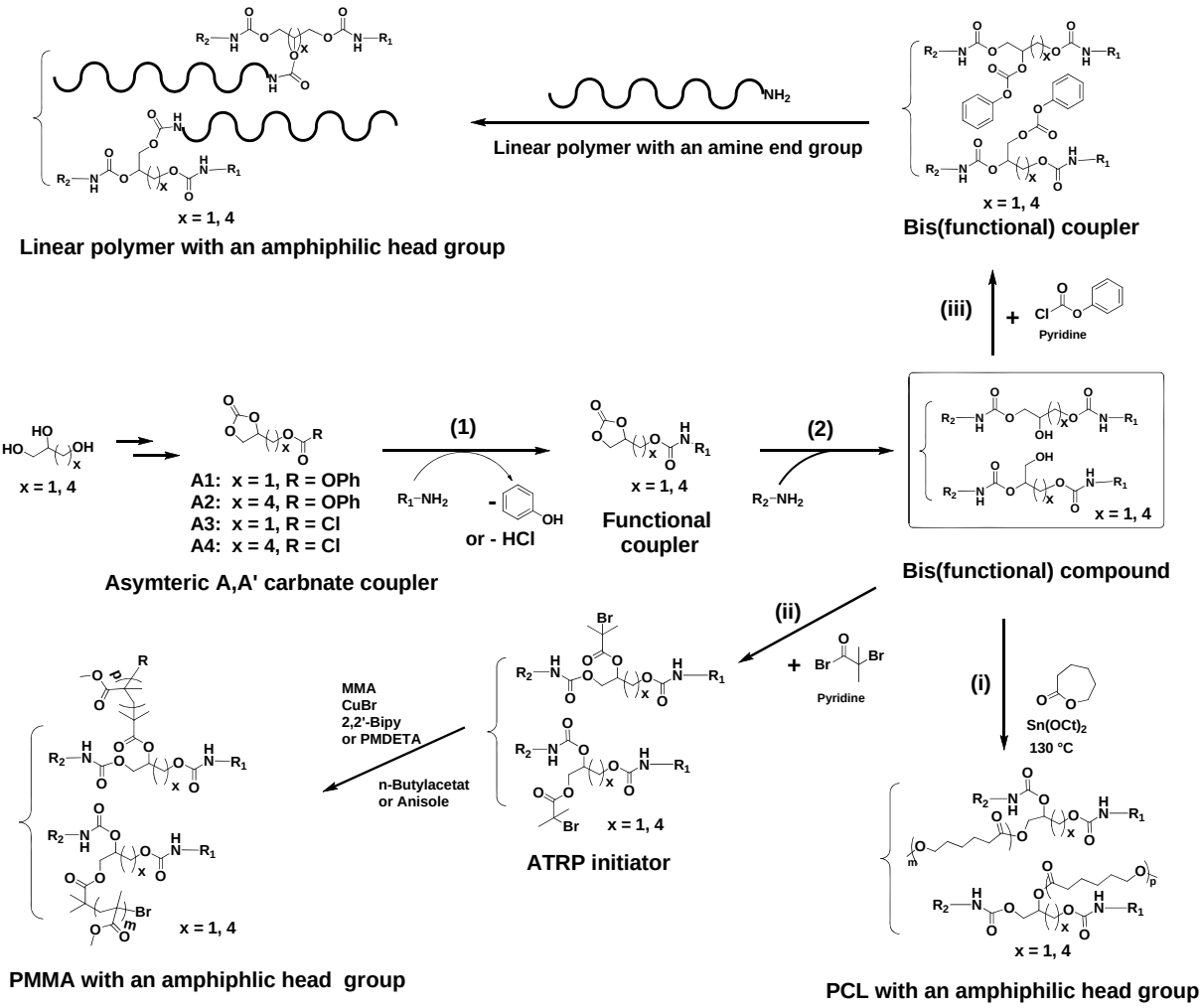
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.85 (t, ^{3}J = 6.6 Hz, 3 H, H^{29}), 0.96-1.12 (br., 9 H, H^{34}), 1.16-1.32 (m, 30 H, H^{14-28}), 1.32-1.44 (m, 2 H, H^{13}), 1.72-1.94 (m, 2 H, H^{3}), 2.94 (dd, 2 H, ^{3}J = 5.8 Hz, ^{2}J = 12.0 Hz, H^{12}), 3.00-3.12 (s, 13 H, $H^{1,2,4}$), 3.24 (s, 3 H, H^{37}), 3.26-3.72 (m, jeffamine® backbone), 3.82-4.30 (m, 4 H, $H^{7,7',9,9'}$), 4.69-5.20 (m, 1 H, $H^{8,8'}$), 6.70-7.50 (m ,3 H, $H^{5,11,31}$) ppm.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 13.9 (C^{29}), 16.98, 17.04 (3 C, C^{34}), 22.0 (C^{28}), 23.0, 26.2 (C^{3}), 27.2, 28.6, 28.7, 28.8, 28.9, 29.0 (C^{14-20}), 29.3 (C^{13}), 31.2 (C^{27}), 37.3 (C^{4}), 40.2 (C^{12}), 46.3, 46.6 (C^{32}), 52.1 (C^{1}), 58.0 (C^{37}), 62.2, 62.4, 62.7 ($C^{7,9}$), 63.3 (C^{2}), 64.9 ($C^{7',9'}$), 69.5, 69.7 ($C^{35,36}$), 70.0 (C^{8}), 71.2, 72.0 ($C^{32'}$), 74.1 (C^{33}), 152.5, 152.8 (C^{31}), 154.7, 155.4, 155.6, 155.7 ($C^{6,10,30}$) ppm.

4.3 Results and Discussion

As already reported, asymmetric A,A´-carbonate couplers **A1** and **A2** have two functional groups, which can selectively react with primary amines. At low temperature (0-25°C) the phenyl carbonate group of **A1** or **A2** was first substituted selectively with one equivalent hydrophilic, hydrophobic, or ionic primary amines to result in functional couplers. Then, at higher temperatures (60-80°C) the functional couplers were converted with a second primary amine to result in a bis(functional) compound. During this reaction the five-membered cyclic carbonate ring is opened and a urethane group with an adjacent hydroxyl group (in α or β position to the urethane group) is formed. The use of this hydroxyl group as initiator for polymerization reaction and modification of polyamines will be discussed. Via grafting-from modification by ring-opening polymerization or ATRP, linear polymers with amphiphilic head groups were synthesized. Via polymer analogues reaction, amphiphilic building blocks

were attached to an amine-end functionalized polymer, forming linear polymers with amphiphilic head groups (Scheme 1).



Scheme 1: Concepts for the use of the hydroxyl groups of bis(functional) compounds for grafting-from reaction and modification of polyamine: (1) synthesis of functional couplers; (2) synthesis of bis(functional) compounds; (i) ring-opening polymerization of ε-caprolactone; (ii) synthesis of an ATRP initiator and polymerization of MMA; (iii) synthesis of a phenyl carbonate and polymer analogous reactions with an amine end-capped linear polymer.

4.3.1 Synthesis of the bis(functional) hydroxyl compounds

Bis(functional) hydroxyl compounds were prepared by sequential reaction of functional amines with A,A´-carbonate couplers. Starting with glycerol and 1,2,6-hexantriol the asymmetric carbonate couplers **A1**, **A2** and **A3**, **A4** were obtained according to the literature by reaction with either dimethyl carbonate and phenyl chloroformate (for **A1** and **A2**) or reaction with phosgene (for **A3** and **A4**). The synthesis of functional coupler – reaction of the R_1-NH_2 with the coupler – was carried out as described in literature. The products were purified via crystallization (**C_3A12**, **C_6A8**) or by precipitation (**C_3J1000**). The second amine (R_2-NH_2) was subsequently reacted with the functional coupler.

The reactivity of five-membered cyclic carbonates with primary amines (R–NH_2) depends very much on the substituent next to the carbonate ring [32]. For a substituent with a high electron-withdrawing effect, the conversion of the five-membered cyclic carbonate can reach 100 % very fast, even at room temperature; for a substituent with low electron-withdrawing effect, the conversion of five-membered cyclic carbonate does not reach 100 % even after 50 days at 70 °C if equimolar amounts of reagents are used. Kinetics of the ring-opening reaction in functional couplers with primary amines has been studied [28, 33]. In practical work, a slight excess of amine was used for nearly quantitative conversion of the cyclic carbonate. The excess amines were removed in vacuum (for N,N-dimethylpropane-1,3-diamine) or by aqueous work-up (for octylamine). Using this procedure the following bis(functional) hydroxyl componds were prepared: combining hydrophilic/ionic, hydrophobic/ionic, and hydrophobic/hydrophobic building blocks: **C_3J1000-Q**, **C_3A12-Q, C_6A8-Q**, and **C_6A8-A8**.

In **Figure 1** exemplarily the ^{1}H NMR spectra of **C_6A8-Q** (**Figure 1a**) and **C_3J1000-Q** (**Figure 1b**) are shown. Prepared from **C_6A8** with 1.1 equivalent N,N-dimethylpropane-1,3-

diamine in $CHCl_3$ at 70 °C for 24 h, the product was obtained in quantitative yield. The ^1H NMR shows two isomers **C_6A8-Qa**/**C_6A8-Qb**; chemical shifts of the protons of the regioisomers were assigned; and a ratio of **C_6A8-Qa**/**C_6A8-Qb** = 38/62 was obtained. Signals at δ = 3.03-3.15 are characteristic peaks for the CH_2 group next to the nitrogen (signal 4 and 15). Using proton correlation spectroscopy, the signal at δ = 3.03-3.10 was definitively assigned to proton 15, and the signal at δ = 3.10-3.15 to proton 4. **C_3J1000-Q** was prepared from **C_3J1000** in a similar way. As in **C_6A8-Q** two regioisomers were assigned in **C_3J1000-Q**. Signals for N,N-dimethylpropane-1,3-diamine segment in **C_3J1000-Q** (Proton 1 - 4) are similar as in **C_6A8-Q**. The Signal at δ = 4.88 ppm is characteristic for the isomer with a secondary hydroxyl group (proton 8'). The ratio of **C_3J1000-Qa**/**C_3J1000-Qb** was calculated to be 82/18. The difference between the ratio of **C_6A8-Qa**/**C_6A8-Qb** and **C_3J1000-Qa**/**C_3J1000-Qb** is because of the different substitution groups next to the five-membered cyclic carbonate group [34]: with a more electron-donor group next to the cyclic carbonate (C_6-carbonte compared to C_3-carbonate) a higher amount of the regioisomer with a primary alcohol group is obtained. The detail about this result will be discussed in a next paper.

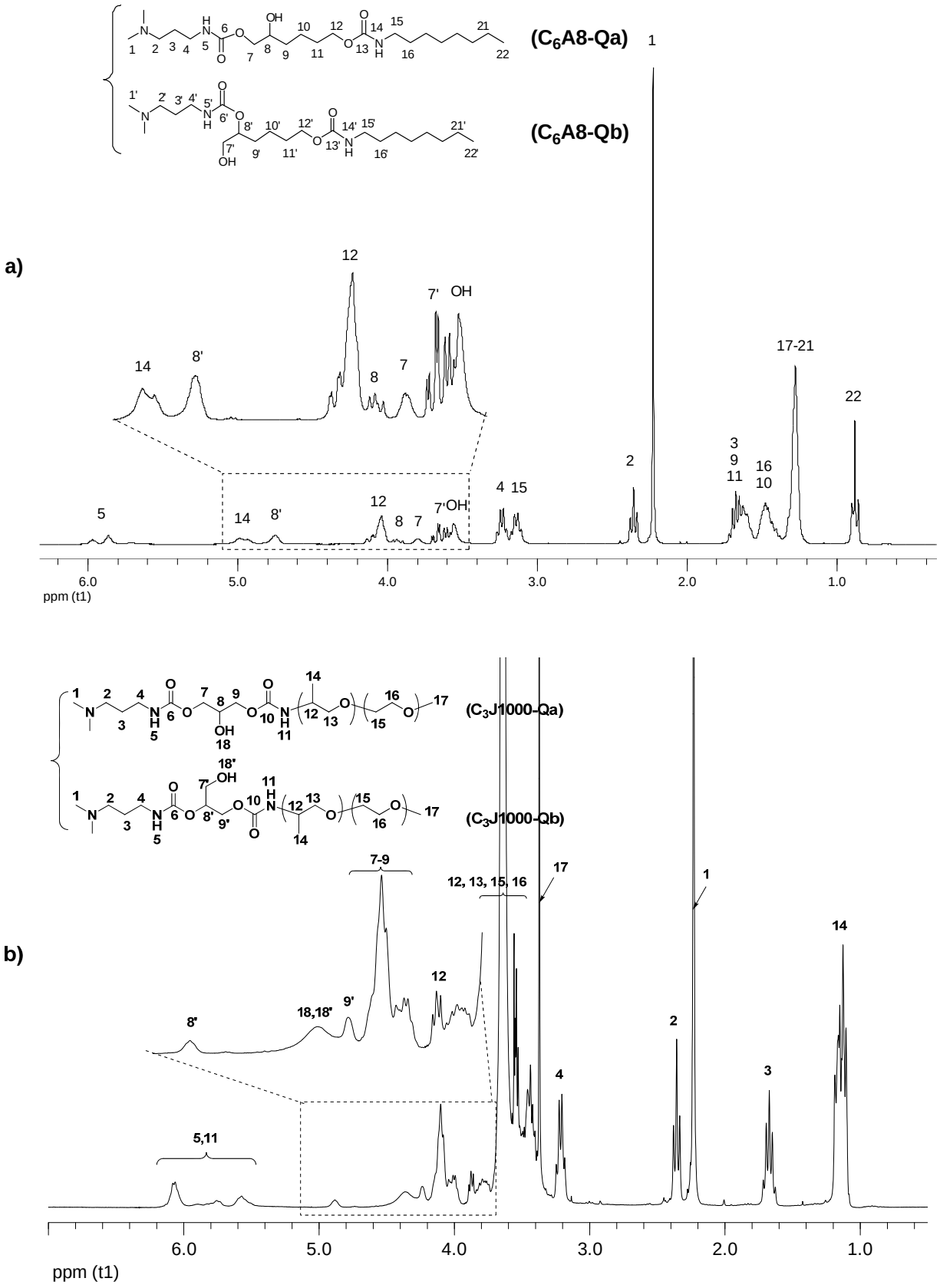


Figure 1: a) ^{1}H NMR of bis(functional) compound **C_6A8-Q** in $CDCl_3$; b) ^{1}H NMR of bis(functional) compound **C_3J1000-Q** in $CDCl_3$

4.3.2 Use of the bis(functional) hydroxyl compounds as initiator for ring-opening polymerization of ε-caprolactone

The ring-opening polymerization of ε-caprolactone, with **C_6A8-Q** as initiator, was used as a model reaction. The reaction was carried out in bulk at 130 °C using $Zn(oct)_2$ as catalyst. The initiator **C_6A8-Q** and ε-caprolactone were heated to form a homogeneous solution, before the catalyst was added. After purification, the polymers are obtained as waxy solids. The ^{1}H NMR spectrum of the product **C_6A8-Q-PCL** is shown in **Figure 2**. The signals of the initiator and of the poly(ε-caprolatone) are well resolved. The characteristic quartet of the CH_2 group next to the nitrogen of the urethane groups – proton 4 and 15 – can be clearly assigned. This proves the successful incorporation of the initiator as head group of the poly(ε-caprolatone).

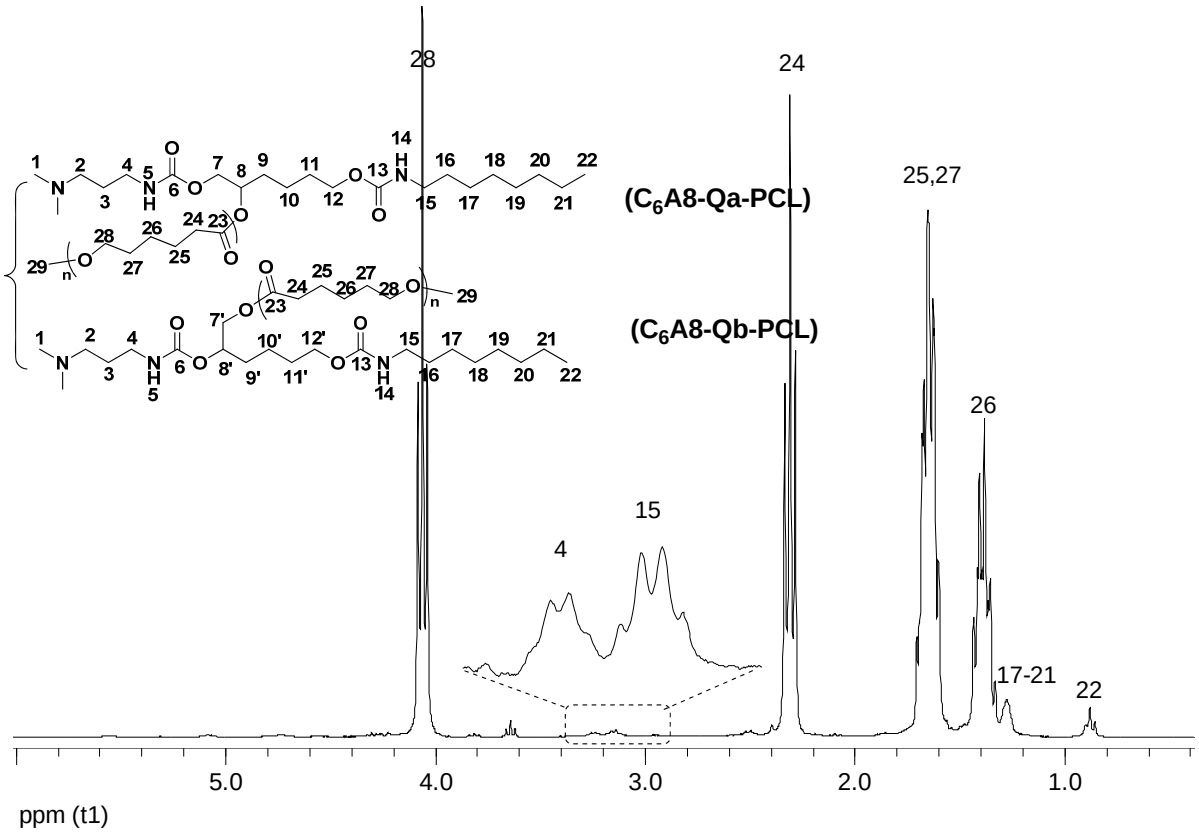


Figure 2: ^{1}H NMR spectrum of **C_6A8-Q-PCL** in $CDCl_3$.

Due to the broad molecular weight distribution of the model reaction (s. **Table 1**, $\overline{M}_w / \overline{M}_n$ = 2.45), further reactions were carried out, to find out the possible reasons for this result and for optimization of the reaction. In **Table 1** the results of these experiments are listed: (i) By using another amphiphilic initiator - **C3A12-Q** (experiment no.1) - instead of **C6A8-Q** (model experiment), very similar results were obtained, the polydispersity of the product being slightly lower. However, when a rather hydrophobic initiator was used - **C6A8-A8** (experiment no. 2-7) – in different reaction procedure the polydispersity of the product was between ca. 1.4 - 1.5. (a) In experiment no. 2 – 5, when the amount of catalyst was changed from 20 mg to 120 mg, keeping the monomer to initiator ratio and the reaction conditions identical, slightly different values for the polydispersity were observed ($1.42 \leq \overline{M}_w / \overline{M}_n \leq 1.54$). (b) In experiment 6 and 7, the reaction was carried out in a 2-step procedure. The initiator was firstly reacted with 25 % of the total amount of ε-caprolactone (M/I = 10/1) to form a macroinitiator and to overcome the problem of different initiation rates of the primary and secondary hydroxyl groups. Then to this intermediate the rest of ε-caprolactone was added directly (variant A) and reacted to full conversion (experiment no. 6) or the intermediate was isolated and then used as macroinitiator for polymerization of the rest of ε-caprolactone (experiment no. 7). The $\overline{M}_w / \overline{M}_n$ value of experiment no. 6 ($\overline{M}_w / \overline{M}_n$ = 1.39) is smaller than that of experiment no. 7 ($\overline{M}_w / \overline{M}_n$ = 1.45), but the difference is quite small. Comparing the polydispersity index of experiments 2 – 7 using a non polar initiator **C6A8-A8** with the model experiment and experiment 1 in which amphiphilic initiators (**C6A8-Q** or **C3A12-Q**) are used large differences in the $\overline{M}_w / \overline{M}_n$ values are observed. The possible reason for this result is that amphiphilic head groups in polymers with low degree of polymerization influences stronger the hydrodynamic volume of the macromolecules than in polymers with higher degree of polymerization, leading to considerably higher values of the polydispersity index.

Table 1: Molecular weight ($\overline{M}_n$, $\overline{M}_w$) and polydisperisty ($\overline{M}_w / \overline{M}_n$) from SEC (THF) of linear PCL samples with different head groups prepared in different conditions.

No.	Initiator	Catalyst (mg)	Procedure[a)]	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w / \overline{M}_n$
Model	**C_6A8-Q**	50	1-step	12900	4800	2.45
1	**C_6A8-A8**	20	1-step	18600	13100	1.42
2	**C_6A8-A8**	50	1-step	20400	14200	1.44
3	**C_6A8-A8**	80	1-step	16700	11000	1.52
4	**C_6A8-A8**	120	1-step	19800	12900	1.54
5	**C_6A8-A8**	50	2-step-A	30800	22000	1.39
6	**C_6A8-A8**	50	2-step-B	30300	20800	1.46
7	**C_3A12-Q**	50	1-step	11600	5200	2.25

4.3.3 Conversion of the bis(functional) hydroxyl compounds to an ATRP initiator and polymerization of MMA

The modification of the bis(functional) hydroxyl compounds **C_3J1000-Q**, **C_3A12-Q, C_6A8-Q**, and **C_6A8-A8** via ATRP comprises two steps: first the hydroxyl groups of the bis(functional) compounds were reacted with 2-bromo-2-methylpropionyl bromide to form an ATRP initiator; then this initiator was applied for the ATRP of MMA using CuBr/2,2'-bipyridine or CuBr/PMDETA as catalyst.

As a model reaction, **C_6A8-Q** was first transformed into the initiator **C_6A8-Q-Br** by reaction with 2-bromo-2-methylpropionyl bromide. This initiator was used for the polymerization of methyl methacrylate (MMA) (M/I = 100/1). In **Figure 3** the first-order kinetic plot (**Figure 3a**) and the dependence of M_n vs. conversion (**Figure 3b**) are shown. A linear correlation of $\ln([M]_0/[M]_t)$ vs. time up to 75 % conversion is observed indicating that the concentration of

growing radicals is constant during this period. For M_n vs. converison a linear dependence is found, too. Due to the relative high molecular weight of the initiator (M (initiator) = 567 g/mol), extrapolation of the line does not pass the origin.

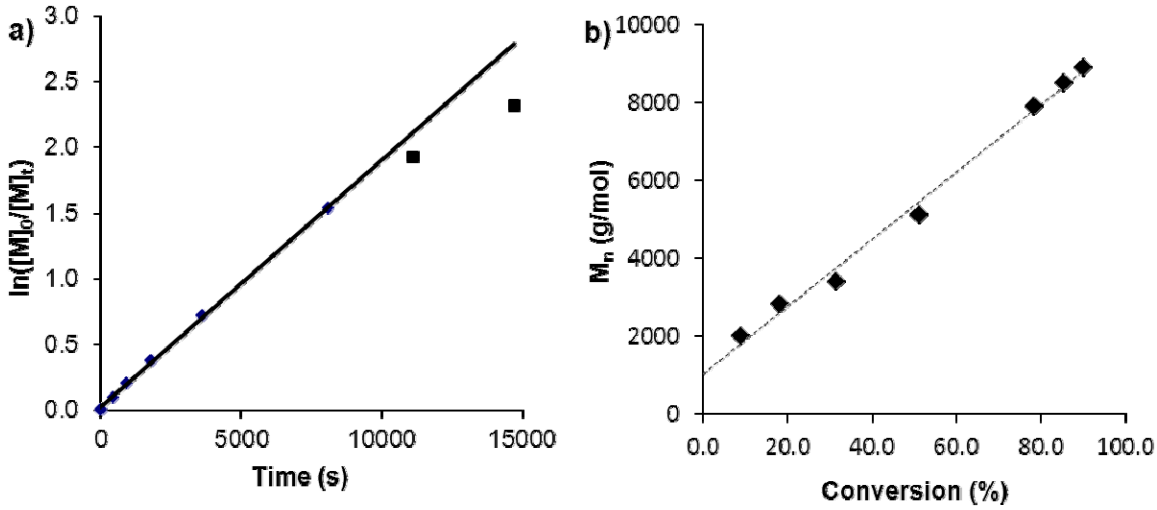


Figure 3: Atom transfer radical polymerization (ATRP) of methyl methacrylate (MMA) with [MMA]:[I]:[CuBr]:[Bipy] = 100:1:1:2 (I = **C_6A8-Q-Br**): a) first-order plot; b) Number-average molecular weight (M_n) (determined by SEC) vs. conversion (determined by NMR).

The ^{1}H NMR spectrum of the product **C_6A8-Q-PMMA** is shown in **Figure 4**. The characteristic quartet peaks of proton 4 and 15 from the initiator can clearly be assigned. The signals of proton 1 and 2 are seen, too. This indicates the successful polymerization of MMA using an amphiphilic ATRP initiator and the successful synthesis of **C_6A8-Q-PMMA** via ATRP. From the integrals of initiator and the polymer signals the molecular weight of **C_6A8-Q-PMMA** was calculated and listed in **Table 2.**

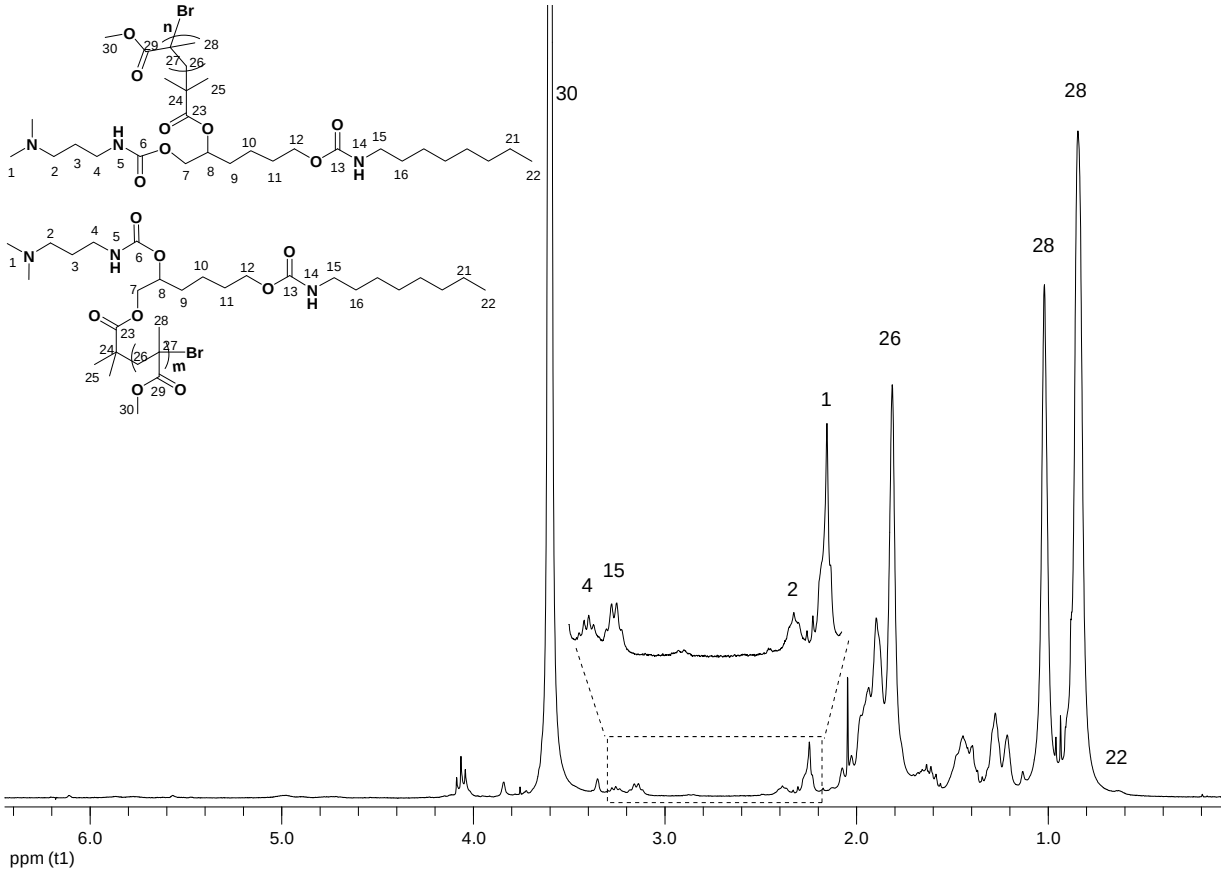


Figure 4: ^{1}H NMR of linear polymer with amphiphilic head group **C_6A8-Q-PMMA** in $CDCl_3$.

Table 2: Molecular weight ($\overline{M}_n$, $\overline{M}_w$) and polydisperisty ($\overline{M}_w / \overline{M}_n$) from SEC (THF) and molecular weight ($\overline{M}_n$) determined via ^{1}H NMR spectroscopy (300 MHz, $CDCl_3$) of linear PMMA with different head groups.

End-Functionalized polymers	$\overline{M}_w$ **(SEC)**	$\overline{M}_n$ **(SEC)**	$\overline{M}_w / \overline{M}_n$ **(SEC)**	$\overline{M}_n$ **(NMR)**
C_3J1000-Q-PMMA	15800	12300	1.29	14600
C_3A12-Q-PMMA	27600	19100	1.44	13400
C_6A8-A8-PMMA	26000	20800	1.25	17900
C_6A8-Q-PMMA	14600	11000	1.34	10800

Using this method, different bis(functional) compounds with other functionalities were used as initiators for the polymerization of MMA (**Table 2**). The modification of **C_6A8-A8** and

C_3A12-Q was performed exactly like **C_6A8-Q**, using 2,2'-bipyridine as ligand and butyl acetate as solvent. For bis(functional) compound **C_3J1000-Q**, the ligand was changed to PMDETA and anisole was used as solvent to get a higher conversion of MMA and a linear first-order plot.

4.3.4 Summary of the direct initiating routes

Because of the two directions of the ring-opening reaction of the five-membered cyclic carbonate ring in the functional couplers with primary amines, regioisomers with primary and secondary hydroxyl groups were always formed in the preparation of bis(functional) compounds. The primary hydroxyl group was known to be more efficient as initiator for ring-opening polymerization of ε-caprolactone than the secondary hydroxyl group. For this reason the final product – poly(ε-caprolactone) with an amphiphilic head group – has a relative broad molecular weight distribution **C6A8-Q** and **C3A12-Q** (cf. Table 1, No. 1 and 7). In addition the influence of amphiphilic head groups changes the properties of short chain poly(ε-caprolactone) more than those of higher chain length polymers. Due to this fact it is expected that the hydrodynamic radius of the molecules with low and higher degree of polymerization change non-linearly, leading to higher values of $\overline{M}_w / \overline{M}_n$. With different less polar initiators (**C6A8-A8**) the molecular weight distribution is lower proving our hypothesis on the influence of the head group on the molecular weight distribution.

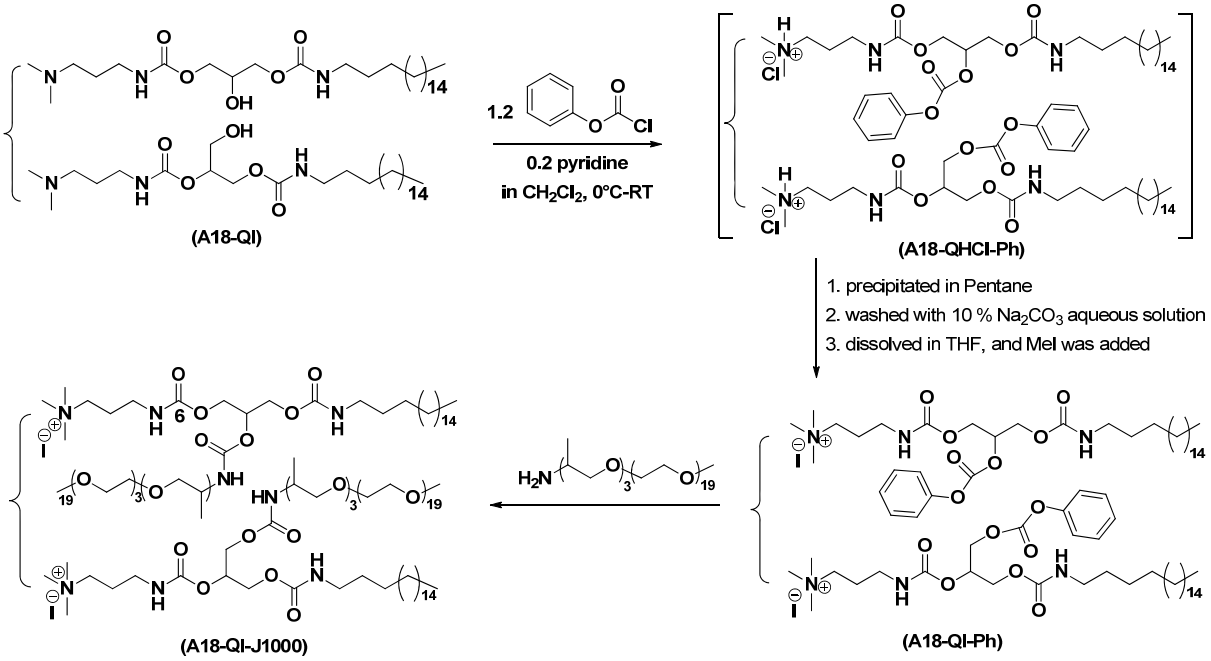
For ATRP of MMA, the hydroxyl groups of bis(functional) compounds were functionalized with 2-bromo-2-methylpropionyl bromide. The influence of the different kind of hydroxyl groups to which the initiating function is linked, on ATRP was much smaller than on ring-opening polymerization. From kinetic investigation it was observed that the ATRP proceeds well controlled. The polydispersity of the product was in the typical range for atom transfer

radical polymerizations. The influence of different types of initiators in ATRP is not as significant as in ring-opening polymerization reactions. It could be concluded, that modification via ATRP is more controlled than ring-opening polymerization.

4.3.5 Use of the bis(functional) hydroxyl compounds for polymer analogous reaction

As shown in **Scheme 2**, reacting the bis(functional) hydroxyl compounds with phenyl chloroformate in the presence of pyridine, bis(functional) couplers are obtained. The phenyl carbonate groups in these bis(functional) coupler can react with primary amines to form urethane groups. Reacting these bis(functional) couplers with amino-telechelic polymers bis(functional) head- or end-groups are introduced.

As a model reaction, the conversion of the bis(functional) compound **C_3A18-Q** to produce **C_3A18-QI-Ph** and further modified to **C_3A18-QI-J1000** is described (**Scheme 2**). **C_3A18-Q** was reacted with 1.2 equivalent phenyl chloroformate in the presence of 0.2 equivalents pyridine in CH_2Cl_2 to produce the intermediate **C_3A18-QHCl-Ph**. A slight excess of phenyl chloroformate is needed for the full conversion of the hydroxyl groups. Because there is a tertiary amine group present in **C_3A18-Q** for quenching the hydrochloric acid formed, only 0.2 equivalents of pyridine are needed. By precipitation in pentane followed by washing with Na_2CO_3-10% aqueous solution, the excess of phenyl chloroformate, the pyridine hydrochloride and the hydrochloric acid attached to the tertiary amine group were completely removed and the bis(functional) coupler **C_3A18-Q-Ph** was obtained. After quaternization with iodomethane, the bis(functional) coupler **C_3A18-QI-Ph** with an alkyl chain, a cationic group and a reactive phenyl carbonate group was obtained.



Scheme 2: Synthesis of linear polymer with amphiphilic head group **A18-QI-J1000** via **A18-QI-Ph**

In **Figure 5** the ^{1}H NMR spectrum of **C_3A18-QI-Ph** is shown. After modification of the hydroxyl groups, the proton signals of the glycerol skeleton in the two isomers – protons 7-9 and 7'-9' – are similar; there are only differences between CH_2 and CH groups, the signals at δ = 5.00-5.08 were assigned to the CH groups (protons 8 and 8'), and the signals at δ = 4.00 - 4.60 belong to the protons of the CH_2 groups (protons 7, 7' and 9, 9'). The signals at δ = 7.20 -7.60 evidenced the existing of active phenyl carbonate group.

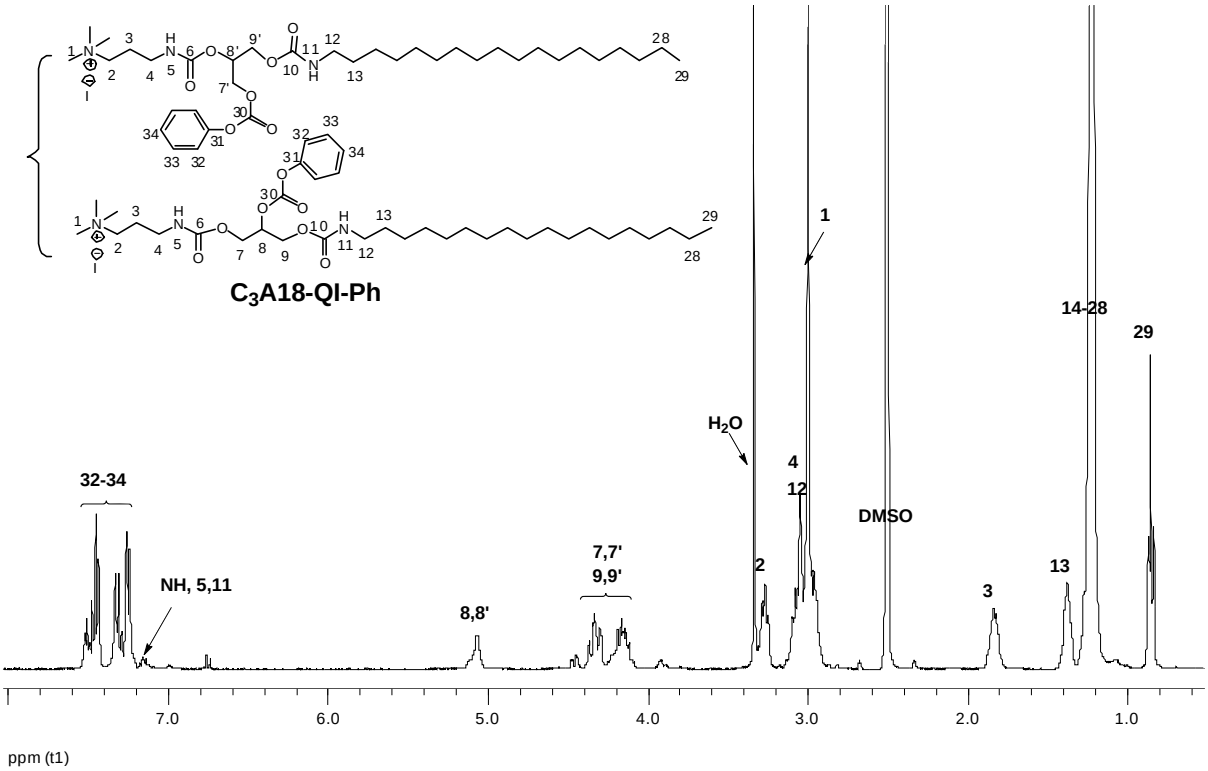


Figure 5: ^{1}H NMR of bis(functional) coupler **C_3A18-QI-Ph** in DMSO-d_6.

Using this bis(functional) coupler, Jeffamine® M1000 with one primary amine group at one chain end was modified at 70 °C to form a linear polymer with an amphiphilic head group (**Scheme 3**). The formed phenol was removed by precipitation in a mixture of Et_2O/pentane. The product **C3A18-QI-J1000** was obtained as yellow highly viscose oil. The ^{1}H NMR spectrum of the product was shown in **Figure 6**. After 3 times precipitation, the characteristic signal for the side product – phenol – at δ = 6.80 and 7.18 ppm disappeared. The signals at δ = 0.85, 1.23, 1.37 ppm indicate the existence of the alkyl chain; the signals at δ = 1.85, 3.05 ppm are characteristic for the quaternized ammonium group; and the signals at δ = 1.04, 3.51 ppm for the Jeffamine® M1000 segment.

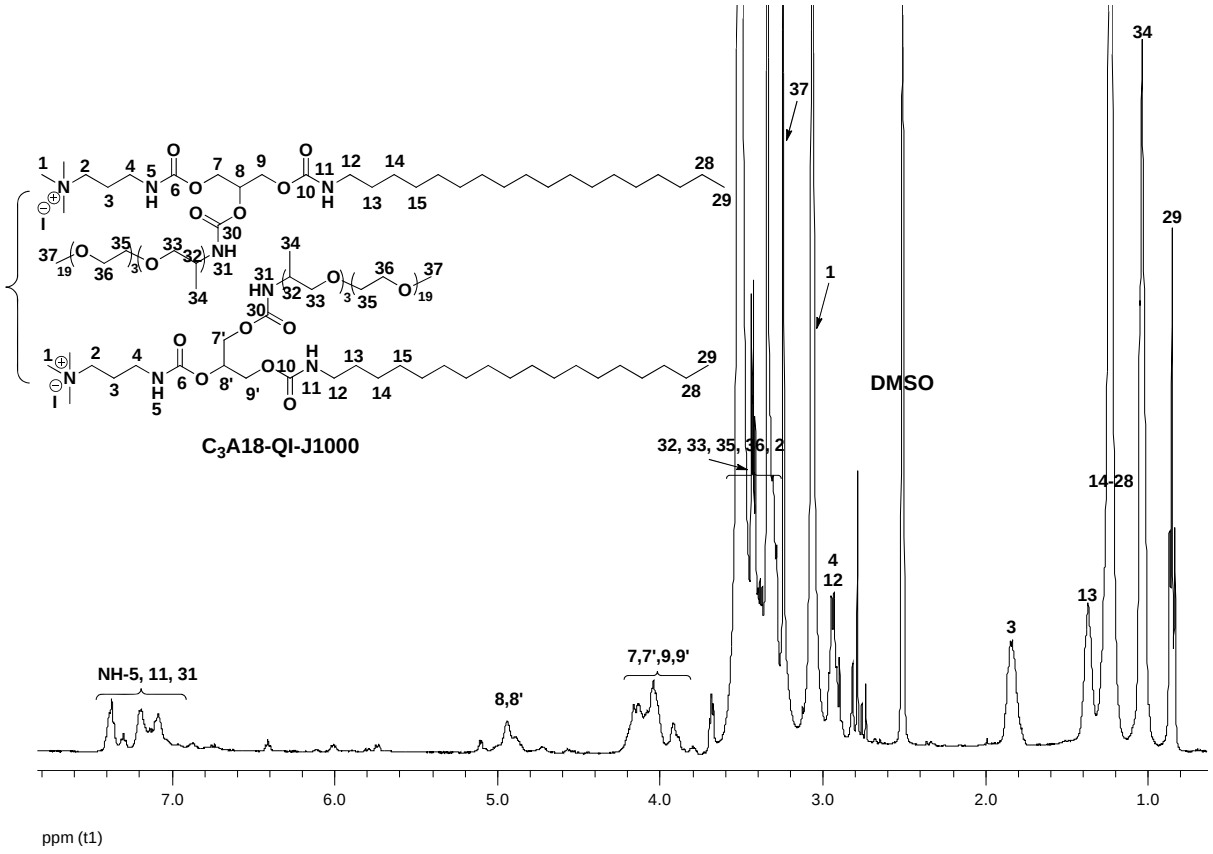


Figure 6: ^{1}H NMR of linear Jeffamine® M1000 with amphiphilic head **C_3A18-QI-J1000** in DMSO-d_6.

In Figure 7 the DSC traces of **C3A18-QI** and **C3A18-QI-J1000** between -20 °C and 150 °C and between -60 °C and 150 °C are shown, with a heating and cooling rate of 10 K/min, respectively.

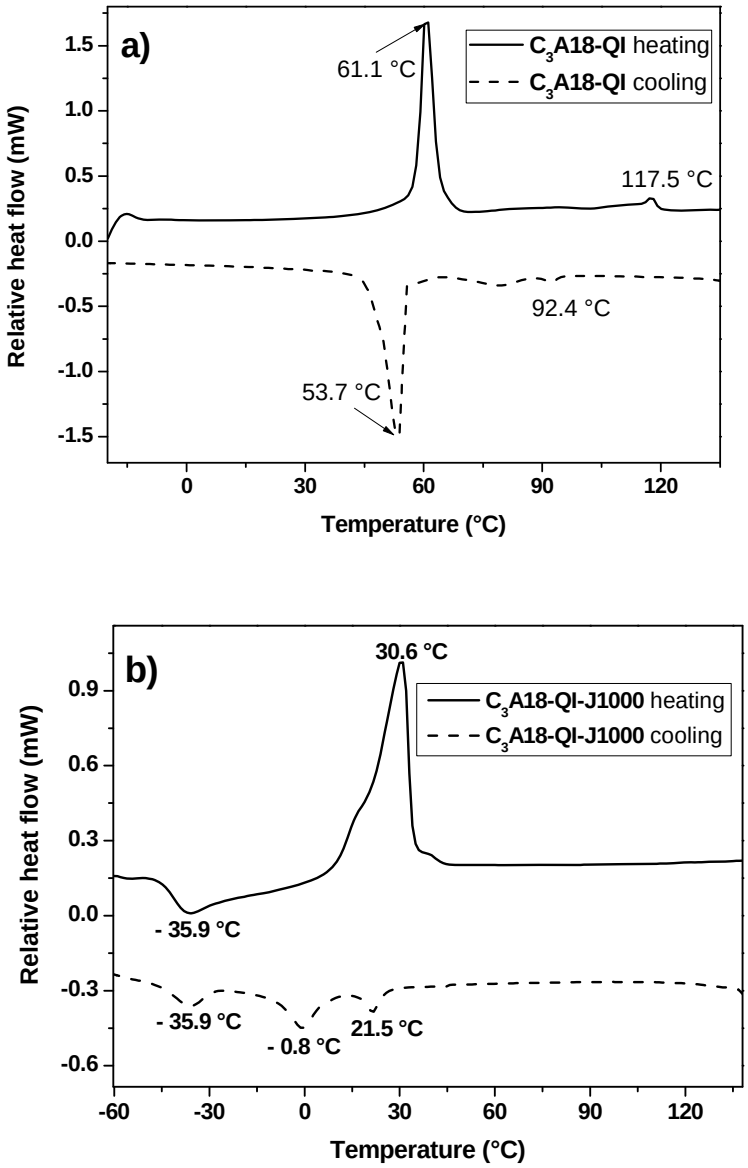


Figure 7: DSC traces of a) **C_3A18-QI** and b) **C_3A18-QI-J1000**: (—) heating; (---) cooling

For the bifunctional compound **C3A18-QI** (Figure 7a), during heating two peaks were observed: the melting peak of the C18 – alkyl chains (T_p = 61.1 °C) and the disordering of hydrogen bridge within the urethane groups (T_p = 117.5 °C); in the cooling process, the recrystallization of alkyl chains (T_p = 53.7 °C) and the ordering of the hydrogen bridges between urethane groups (T_p = 92.4 °C) is observed [35]. After coupling with Jeffamine® M1000, in the heating process of compound **C3A18-QI-J1000** (Figure 7b) an exothermic crystallization peak and an endothermic melting peak attributed to the PEO/PPO chains of

Jeffamine® M1000 is the dominating he thermal transition, the melting peak of alkyl chain is observed around 45 °C, however, the disordering of hydrogen bridge within the urethane groups can not be observed anymore. In the cooling process of **C3A18-QI-J1000** three crystallization peaks are observed, whch belong to the PEO/PPO chains of Jeffamine® M1000 (T_p = -35.9 °C and -0.8 °C) and alkyl chain (T_p = -21.5 °C), respectively.

4.4 Conclusion

From asymmetric A,A´-carbonate couplers **A1 - A4** and primary amines by a selective substitution at low temperature (**step 1**) followed by an addition reaction to the five-membered cyclic carbonate at higher temperature (**step 2**), functional building blocks with two different functionalities – ionic/hydrophilic, ionic/hydrophobic, and hydrophobic/ hydrophobic – bearing primary or secondary hydroxyl groups are produced.

The use of these bis(functional) compounds with a hydroxyl group as initiator for ring opening polymerization of ε-caprolactone (i), were transformed to ATRP initiators (ii), and to phenyl carbonates (iii) were successfully carried out. The bis(functional) ATRP initiators were used for the preparation of PMMA with amphiphilic head groups and the phenyl carbonates were used for the modification of amino telechelic linear polymers. Preliminary thermal result of linear polymers with amphiphilic head groups, show the strong influence of the head group on the thermal properties.

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Chapter 5

Synthesis and Characterization of Ionic/Hydrophilic Modified Poly(ethylene imine)s and Visualization of Their Single Molecule Absorption Structures on Mica

5.1 Introduction

For decades, polymers and especially polyelectrolytes have received a lot of attention because of their vast number of possible applications. Poly(ethylene imine) (PEI), a commercially available, branched, and polydisperse polyelectrolyte, was widely used as additives in the paper industry, for waste water treatment, in dye fixation in textile processing, pigment dispersant, and so on[1-3]. In microbiological studies[4-6], PEI is one of the most efficient nonviral vectors for gene delivery[7-9]. Due to its relatively high gene delivery efficiency and ready availability, branched, 25-kDa PEI has become a benchmark to which other polymers, especially newly designed and synthesized materials, are often compared[10]. As a weak polyelectrolyte PEI is also well known as an efficient nucleotide carrier due to formation of PEI-nucleotide complexes that show high stability, controllable size, adjustable unpackaging properties in cells, and flexibility for addition of moieties that target specific entities on cell membranes and intracellular structures[11].

Although PEI itself has been very efficient in microbiological applications, its functionalities can be improved via modification[12]. A lot of efforts have been made for the modification of PEI to improve its biological activities, or to minimize its toxic properties[10]. In the past, a convenient method for the modification of poly(ethylene imine) was developed in our group. Using carbonate bifunctional couplers, different functionalities as hydrophilic, hydrophobic, ionic…., can be tailored introduced to PEI molecules via covalent bonds. This method allows easy entrance for the systematic study of microbiological effects[13].

As already known in literature, the functionality of PEI as a nucleotide carrier is significantly improved by incorporating hydrophilic poly(ethylene glycol) (PEG) into PEG-PEI copolymers[11]. Complexation of PEG-PEI copolymers with nucleotides produces particles with a core-shell structure, whereby PEI-nucleotide is sequestered toward the particle core and the PEG chains form a brush like corona, although the precise nature of this arrangement is still an open question. In general, PEG provides polyplexes with improved solubility, lower surface charge, diminished aggregation, lower cytotoxicity, and possibly improved stealthiness in the bloodstream. However, these desirable properties may come at a cost of lower transfection efficiency due to both reduced membrane interaction and less efficient endosomal escape.

To get a better understand of the microbiological properties, the adsorption of PEI and modified PEI to a surface and the alteration of the surface properties are important issues and of high scientific interest. It is therefore necessary to understand the process of adsorption on the scale of single molecules. Scanning probe techniques offer the unique possibility to look at the adsorption on the nanometer scale[14-18]. The quantification of for example sizes of single molecules allows for following the adsorption process in detail. These data make it possible to draw conclusions on the relevant interactions of the molecules.

In this work, modification of PEI with PEG (PEO segment) was accomplished. To increase the membrane interaction, cationic functionality was introduced to the PEI molecules. The synthesized polymers were characterized via NMR, FT-IR, GPC and light scattering. To compare the membrane absorption effect of the ionic, hydrophilic, hydrophilic/ionic modified PEI with the unmodified PEI, single molecules visualization of these polymers were carried out on mica substrate.

5.2 Experiment part

5.2.1 Chemicals

Glycerol (Aldrich, 99.5 %), 3-dimethylamino-1-propylamine (Aldrich, 99 %), Jeffamine® M1000 (Mw = 1000, Hunstman), iodomethane (Aldrich, 99 %), triethylamine (Aldrich, 99.9 %), N,N-dimethylformamide (Merck, 99%, DMF), branched poly(ethylene imine) (Mw = 750 kDa, 50 wt. % in aqueous solution, BASF). Branched poly(ethylene imine) with Mw = 750 kDa was freeze dried before use, all other chemicals are used as received. The branched poly(ethylene imine)s feature three different types of amino groups: primary, secondary, and tertiary amino groups. The ratio of primary-to-secondary-to-tertiary amino groups is 1:2:1. In addition, the poly(ethylene imine) molecules show a polydisperse character (Mw/Mn ≈ 12.5 for PEI with Mw = 750 kDa).

5.2.2 Instruments

Atomic Force Microscopy (AFM): A scanning force microscope (Nanoscope Multimode IIIa, Digital Instruments, Santa Barbara, CA), silicon cantilevers (Nanosensors, Wetzlar,

Germany) with typical resonance frequencies around 300 kHz and spring constants around 40 N/m were applied. Structure and particle size analysis were done with the DI software. Statistical calculations were performed with a stochastic freeware program from Prof. Stoyan (Freiberg University, Germany).

The AFM images were taken with a Nanoscope III operated in tapping mode. The measurements were performed at ambient conditions using Si cantilevers with a spring constant of ca. 42 N/m and a resonance frequency of about 320 kHz.

Sample preparation: Drops of the polyelectrolyte aqueous solution with different concentration (from $1 \cdot 10^{-6}$ g/L to $5 \cdot 10^{-6}$ g/L) were put onto freshly cleaved mica surfaces and directly spin coated. In all cases, deionized and filtered water, further purified with a Milli-Q Synthesis A10 system, was used. The muscovite mica used was purchased from Balzers Union (Lichtenstein). All measurements were performed at ambient conditions in air.

Infrared spectroscopy (FT-IR) was measured on a Thermo Nicolet Nexus 470 spectrometer with a resolution of 4 cm^{-1}. The samples were prepared as KBr pellets for the measurement in transmission mode.

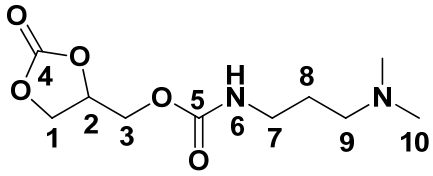
Differential scanning calorimetry analyses (DSC) were performed on a Netzsch DSC 204 under nitrogen using a 10 $K \cdot min^{-1}$ scan rate. The samples (3-12 mg) were measured in a temperature range from -100 °C to 200 °C.

Thermogravimetric analyses (TGA) were performed on a Netzsch TG 209C thermobalance with a TA System Controller TASC 414/4. The measurements were performed under nitrogen atmosphere with a heating rate of 10 $K \cdot min^{-1}$. The sample mass was kept ca. 30 mg.

5.2.3 Synthesis

(2-Oxo-1,3-dioxolan-4-yl)methyloxy chloroformate was prepared according to Ref[19].

Synthesis of (2-Oxo-1,3-dioxolan-4-yl)methyl 3-(dimethylamino)propylcarbamate

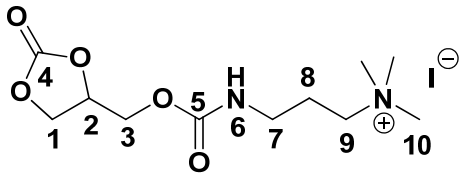


(2-Oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (**1**, 7.00 g, 37.6 mmol) was dissolved in chloroform (150 mL) and cooled to 0 °C. A solution of 3-dimethylamino-1-propylamine (3.844 g, 37.6 mmol) in chloroform (70 mL) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 18 h. The reaction mixture was diluted with chloroform (250 mL), and then washed three times with 10%-Na_2CO_3-solution (60 mL), the organic phase was dried with sodium sulfate, and the solvent was removed by distillation (rotary evaporator, 50 °C, 20 mbar). Yield: 7.067 g (82.4%). The product was used for next step without further purification.

^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 1.52 (p, 2 H, $^3J = {}^3J = 7.1$ Hz, H^8), 2.09 (s, 6 H, H^{10}), 2.17 (t, 2 H, $^3J = 7.1$ Hz, H^9), 3.00 (dd, 2 H, $^3J = 6.6$ Hz, $^3J = 13.0$ Hz, H^7), 4.10-4.30 (m, 3 H, H^1 and H^3), 4.55 (t, 1 H, $^3J = 8.6$ Hz, $H^{1'}$), 4.93-5.03 (m, 1 H, H^2), 7.32 (t. 1 H, $^3J = 5.4$ Hz, H^6) ppm.

^{13}C NMR (DMSO-d$_6$, 75 MHz): δ = 27.3 (C^8), 38.7 (C^7), 45.1 (C^{10}), 56.5 (C^9), 63.0 (C^3), 65.8 (C^1), 74.8 (C^2), 154.6 (C^4), 155.5 (C^5) ppm

Synthesis of *N*,*N*,*N*-Trimethyl-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonylamino) propan-1-ammonium iodide (QI)



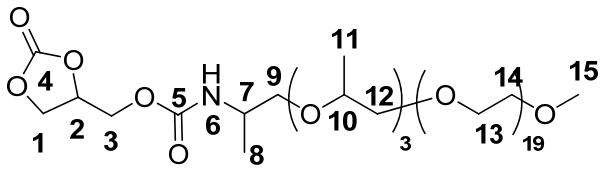
(2-Oxo-1,3-dioxolan-4-yl)methyl 3-(dimethylamino)propylcarbamate (6.00 g, 24.3 mmol) was dissolved in THF (40 mL), a solution of iodmethane (3.62 g, 25.6 mmol) in THF (20 mL) was added at 80 °C under stirring within 30 min. After stirring for an additional hour under

reflux, the solid was filtered and dried at 60 °C in vacuum (20 mbar) to obtain slightly yellow powder. Yield: 8.50 g (90 %)

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.75-1.92 (m, 2 H, H^8), 2.95-3.15 (br., 11 H, H^7 and H^{10}), 3.25-3.35 (m, 2 H, H^9), 4.10-4.35 (m, 3 H, H^1 and H^3), 4.58 (t, 1 H, ^3J = 8.6 Hz, $H^{1'}$), 4.95-5.10 (m, 1 H, H^2), 7.47 (t, 1 H, ^3J = 5.6 Hz, H^6) ppm.

^{13}C NMR (DMSO-d_6, 75 MHz): δ = 23.0 (C^8), 35.3 (C^7), 52.2 (3C, C^{10}), 63.2 (2C, C^9 and C^3), 65.9 (C^1), 74.8 (C^2), 154.7 (C^4), 155.7 (C^5) ppm

Synthesis of (2-Oxo-1,3-dioxolan-4-yl)methyl 1-(1-(2-methoxyethoxy)propan-2-yloxy) propan-2-ylcarbamate (J)



(2-Oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (**1**, 7.95 g, 42.7 mmol) was dissolved in tetrahydrofuran (100 mL) and cooled to 0 °C. A solution of Jeffamine® M1000 (46.90 g, 42.7 mmol) and triethylamine (4.32 g, 42.7 mmol) in tetrahydrofuran (150 mL) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 18 h. Triethylamine hydrochloride was removed by filtration and the filtrate was condensed. The residue was purified by precipitation in pentane/diethyl ether. Yield: 45.07 g (85 %).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.05-1.25 (m, 9 H, H^{11}), 3.37 (s, 3 H, H^{15}), 3.40-3.90 (m, backbone of PEO and PPO, H^{7-10} and H^{12-14}), 4.20-4.42 (m, 3 H, H^1 and H^3), 4.58 (t, 1 H, ^3J = 8.6 Hz, $H^{1'}$), 4.90-5.02 (m, 1 H, H^2), 5.50-6.0 (br.,1 H, H^6) ppm.

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 17.0-17.8 (C^{11}), 47.4 (C^8), 58.9 (C^{15}), 63.2 (C^3), 65.9 (C^1), 70.5 (backbone), 71.9 (C^9), 74.4 (C^2), 75.0 (C^7), 154.59, 154.63 (C^4), 155.0 (C^5) ppm

General Procedure for the modification of b-PEI's with various functional cyclic carbonates

As mentioned in section **5.2.1**, branched poly(ethylene imine) is a commercial hyperbranched polymer with primary, secondary, and tertiary amines in the molar ratio of 25:50:25 respectively. The primary amino groups present in PEI were modified with functional couplers (**QI** and/or **J**) by ring-opening reaction of five-membered cyclic carbonate with formation of a stable urethane and a hydroxyl group. The secondary and tertiary amino groups remained unmodified. At 60 °C the respective amounts of cyclic carbonates (**Table 1**) were dissolved in DMF (10 mL) and added simultaneously to a solution of freeze dried PEI in 15 mL DMF. After stirring at 60 °C for 72 hours the polymer was precipitated in a mixture of pentane and diethylether (v/v = 2/1). DMF was nearly quantitatively removed by multiple precipitations. At the end the residue was concentrated and dried over vacuum (room temperature, $2 \cdot 10^{-2}$ mbar, and 48 hours) to yield a slightly yellow solid.

Table 1. Details of the reactions of PEI with cyclic carbonate couplers

No.	Polymer Note	$\overline{M_w}$ [a] (kDa)	$\overline{M_n}$ [a] (kDa)	b-PEI (g)	Cyclic Carbonate Coupler		Yield (g (%))
					QI (g)	J (g)	
mPEI-1	**PEI_{750k}-QI_{25}**	2440	195	2.000	4.507	----	5.68 (87)
mPEI-2	**PEI_{750k}-$QI_{12.5}$-$J_{12,5}$**	4300	344	1.000	1.127	3.605	4.86 (85)
mPEI-3	**PEI_{750k}-J_{25}**	6150	492	1.000	-----	7.209	6.33 (77)

[a] The weight average molecular weight ($\overline{M_w}$) and number average molecular weight ($\overline{M_n}$) for **mPEI-1 – 3** was calculated from the molecular weight of unmodified **b-PEI** (PDI = 12.5 for PEI with Mw = 750 kDa) with the estimation that the molecular weight distribution (PDI) have not changed after modification.

The absence of characteristic peaks of the cyclic carbonate in ^{1}H NMR and ^{13}C NMR indicates that all the cyclic carbonate has reacted with PEI.

In the subsequent text the index of PEI indicates its molecular weight in kDa, while the extension **QI_{25}**, **$QI_{12.5}$**, **J_{25}**, and **$J_{12.5}$** denotes a PEI where 25 mol-% or 12.5 mol-% of the amino groups have been reacted with quaternary ammonium coupler (**QI**) or Jeffamine® M1000 coupler (**J**), respectively.

5.3 Results and Discussion

The goal of this study was to prepare and characterize a series of cationic and/or PEO segments coated PEI materials for the potential use as a nucleotide carrier. Starting with carbonate bifunctional coupler (**1**), functional coupler with quaternary ammonium groups (**QI**) and functional coupler with PEO segments (**J**) were prepared via substitution reactions. These functional couplers were introduced to highly branched poly(ethylene imine) (PEI) via addition reaction. By these coupling reactions only the primary amine groups were converted, leaving the secondary and tertiary amine groups unchanged (**Figure 1**). The ammonium groups were used for the interaction with the substrate surface; due to the cooperative effect of the ammonium groups a strong interaction between substrate and the polymer was obtained. The PEO segments are introduced for better solubility, lower surface charge, diminished aggregation, and lower cytotoxicity. The ratio between ammonium groups and PEO segments are tailored in order to obtain a good compromise.

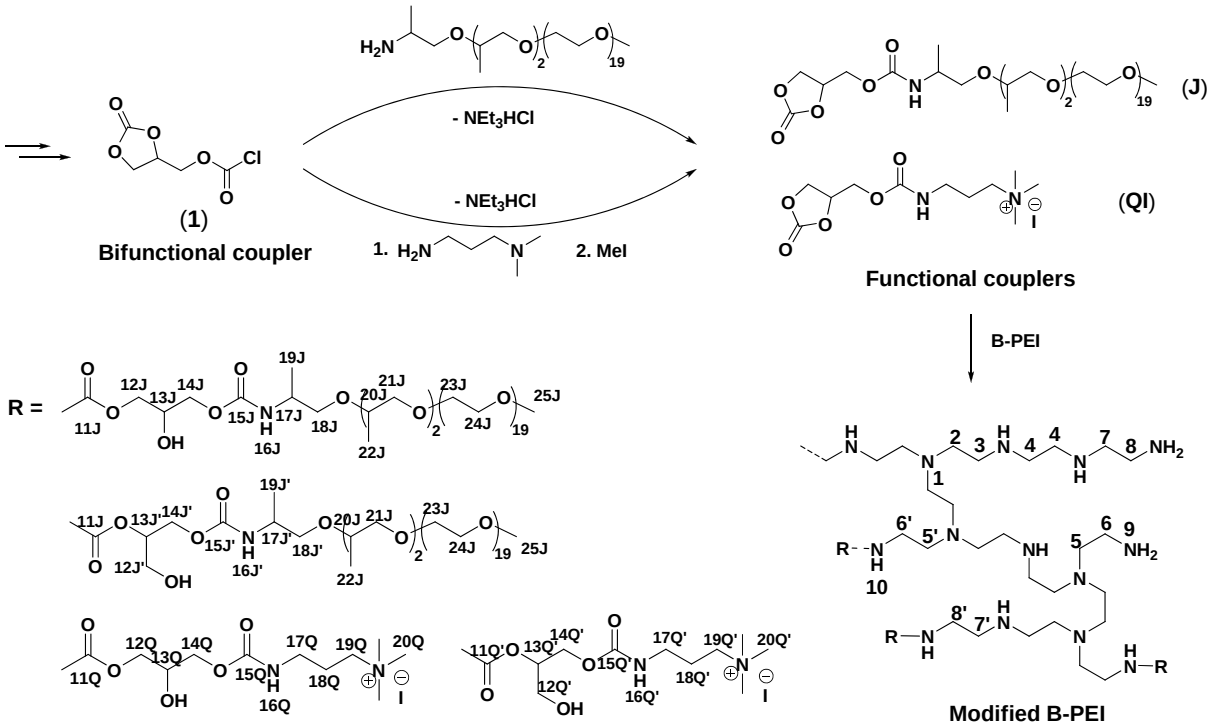


Figure 1: Synthesis of a PEI bearing PEO segments and/or quaternary ammonium groups using functional carbonate couplers **J** and/or **QI**.

5.3.1 Characterization of the modified poly(ethylene imine)s

The structure of the polymers was investigated by means of ^{1}H and ^{13}C NMR spectroscopy. The NMR spectra of **mPEI-2** were discussed as an example (**Figure 2**). In the ^{1}H NMR spectrum the coupling between PEI and the functional carbonates is proven by the urethane signals 10 which overlap with those of signal 16J/16J'/16Q/16Q'. The signals at δ = 3.55-4.00 and 4.40-4.60 ppm are characteristic for the ring-opening reaction product of the five-membered cyclic carbonates with primary amines. In addition the CH_3 and CH_2 groups of the alkane and the CH_3 groups of the ammonium moiety are clearly observed (signals 26A, 16A, 17A-25A and 13Q). In the ^{13}C NMR spectrum the characteristic signals for the functional building blocks – the propyl (signal 10Q-12Q) and glycerol (signal 10A-12A and 10'A-12'A)

– are observed. In addition the NMR spectra show no characteristic signals of the functional cyclic carbonates proving full conversion.

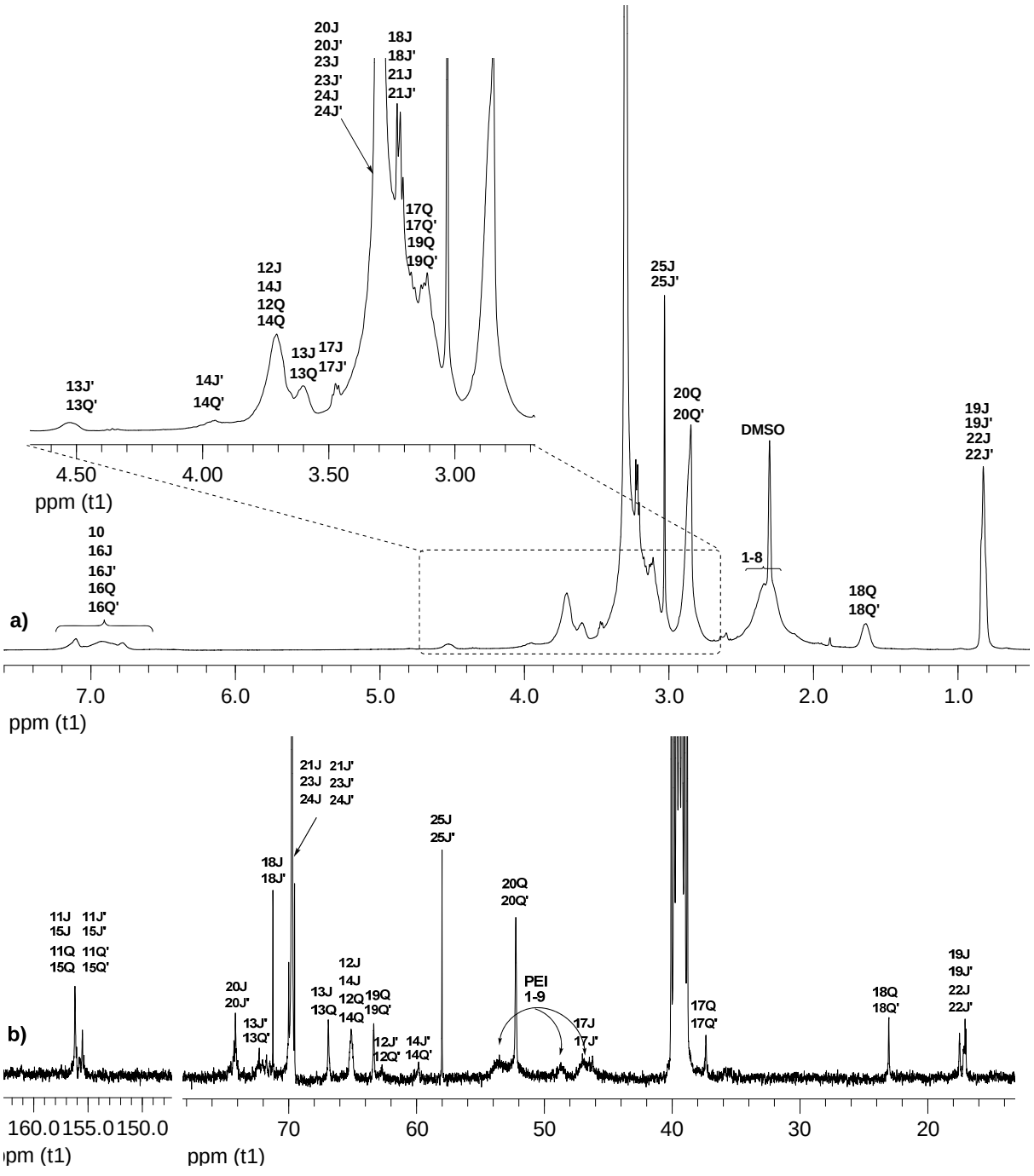


Figure 2: a) ^{1}H NMR and b) ^{13}C NMR spectra of **mPEI-2** in DMSO-d_6

For the FT-IR measurement, PEI (750k) was freezed dried. Comparing the FT-IR spectra of **PEI (750k), mPEI-1**, **mPEI-2**, and **mPEI-3** (**Figure 3**), the presence of urethane groups

(**mPEI-1**, **mPEI-2**, and **mPEI-3**) in the macromolecules was confirmed. The bands at 1248 cm^{-1} (OC-N - stretch), 1530 cm^{-1} (Amide-II) and 1712 cm^{-1} (Amide-I) are characteristic for -CO-NH- groups, while the band at 1108 cm^{-1} that appeared only in **mPEI-2**, and **mPEI-3** was attributed to the C-O-C stretching vibration of the PEO segments of the Jeffamine® M1000 tail.

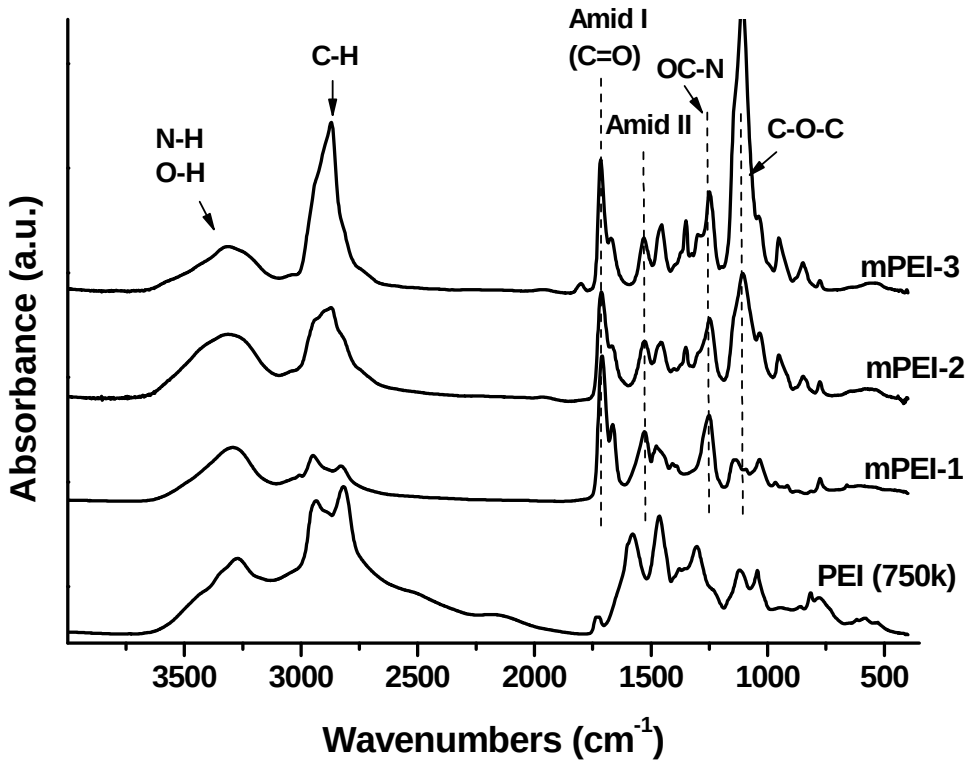


Figure 3: FT-IR spectra of **PEI**, **mPEI-1**, **mPEI-2**, and **mPEI-3** ($\nu_{Amid\text{-}I}$ = 1712 cm^{-1}, $\nu_{Amid\text{-}II}$ = 1530 cm^{-1}, $\nu_{OC\text{-}N}$ = 1248 cm^{-1}, $\nu_{O\text{-}C\text{-}O}$ = 1108 cm^{-1}).

5.3.2 Thermal properties of PEI and mPEIs

The DSC of these polymers was measured between -100 °C and 180 °C with heating and cooling rate of 10 K/min (**Figure 4**). Because of the high mass fraction of JEFFAMINE® (PEO/PPO) in **mPEI-3**, DSC trace of **mPEI-3** shows the characteristic melting (T_p = 31.6 °C) and crystallization (T_p = - 26.4 °C) peaks of JEFFAMINE® as the dominating thermal

transition[20]. Because of the lower content of JEFFAMINE® (PEO/PPO) segment in **mPEI-2** compared to **mPEI-3**, in the heating curve of **mPEI-2**, the melting peak of the JEFFAMINE® (PEO/PPO) segment is much smaller than the peak in **mPEI-3**, and the peak value changed to 25.4 °C; in the cooling curve the crystallization peak cannot be seen in the second cooling process due to the relative low crystallization rate, and instead there is a crystallization process in the next heating curve, which are not appeared here. The DSC traces of **PEI** and **mPEI-1** show that these two polymers are amorphous polymer, the glass transition temperature of **mPEI-1** is higher than **PEI** due to the modification.

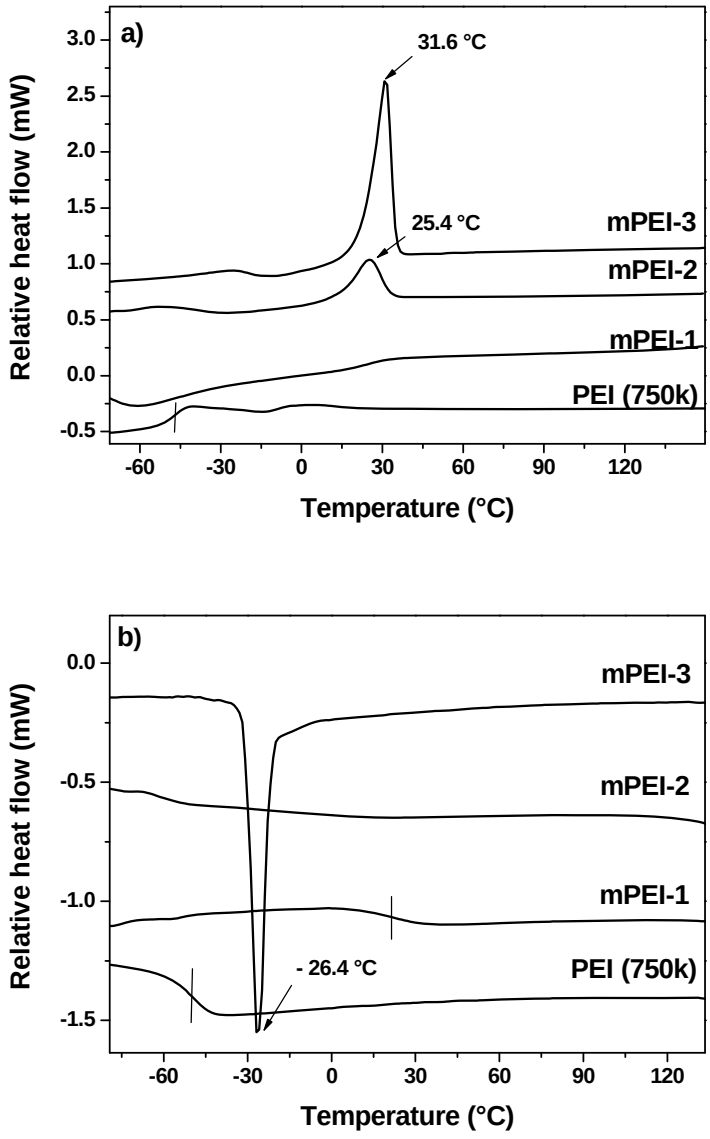


Figure 4: DSC traces of **PEI** (Mw = 750 k) and **mPEI-1** – **mPEI-3**: a) heating; b) cooling

5.3.3 Visualization of the single molecule absorption structures of PEI and mPEIs on mica surface

To study the adsorption behavior and get a detail view of the structures of **PEI** and **mPEIs**, these polymers were spin coated onto fresh cleaved mica surface from a dilute aqueous solution (in Milli-Q water) and the surface topography was studied by AFM.

5.3.3.1 Single molecules absorption structures of PEI on mica surface at different concentrations

Commercial poly(ethylene imine)s are obtained by cationic ring-opening polymerization of aziridine (ethylene imine) in water or water-alcohol mixture with protonic acid as catalyst. The branching processes occur already in the early stages of the polymerization. In the latter stages, the main contribution of chain growth is the combination of lower oligomers, i.e. the macromolecules grow by reaction of an activated (protonated) aziridine attached to an oligomer with a primary or preferentially secondary amine of another oligomer[2, 3].

Single molecules absorption structures of branched poly(ethylene imine) have been studied in different groups on graphite, on polystyrene latexes, and on mica substrate[16-18]. In order to get a better understand of the absorption structures of the modified PEI, the measurement of single molecules absorption structures of **PEI (750k)** was repeated in this work.

The study was carried out at different aqueous solution concentrations: from $1 \cdot 10^{-6}$ g/L to $5 \cdot 10^{-6}$ g/L. In **Figure 7** the absorption structures of **PEI (750k)** at a concentration of $1 \cdot 10^{-6}$ g/L was shown. From the height image and the phase image (**Figure 7a** and **7b**) the fully

stretched structures of the single molecules can be well seen. From the section analysis (**Figure 7c**) of the magnified image (**Figure 7b**), the surface of the molecules is not smooth, which evidenced the fully stretched structures. As known in literature, in the synthesis procedure of branched poly(ethylene imine), small amounts of linear poly(ethylene imine) was obtained at the same time. In **Figure 7b** a molecule with quasi linear shape is labeled. The difference of the molecule shapes and diameters may indicate the apparent polydispersity of the polymer.

In the image of **PEI (750k)** at the concentration of $2 \cdot 10^{-6}$ g/L (**Figure 8a-c**), molecules with islands shape are obtained. In **Figure 8a** there are more than 13 molecules with islands shape in 1 μm^2 area. From the magnification image (**Figure 8b**) and its section analysis (**Figure 8c**) the islands structures can be well defined: the sphere has a height about 0.2 nm and the peaks in the middle with a height of 0.7 nm to 1.2 nm. In the image of **PEI (750k)** at the concentration of $3 \cdot 10^{-6}$ g/L (**Figure 8d-f**), as described in literatures, molecules with pancake like shapes are obtained.

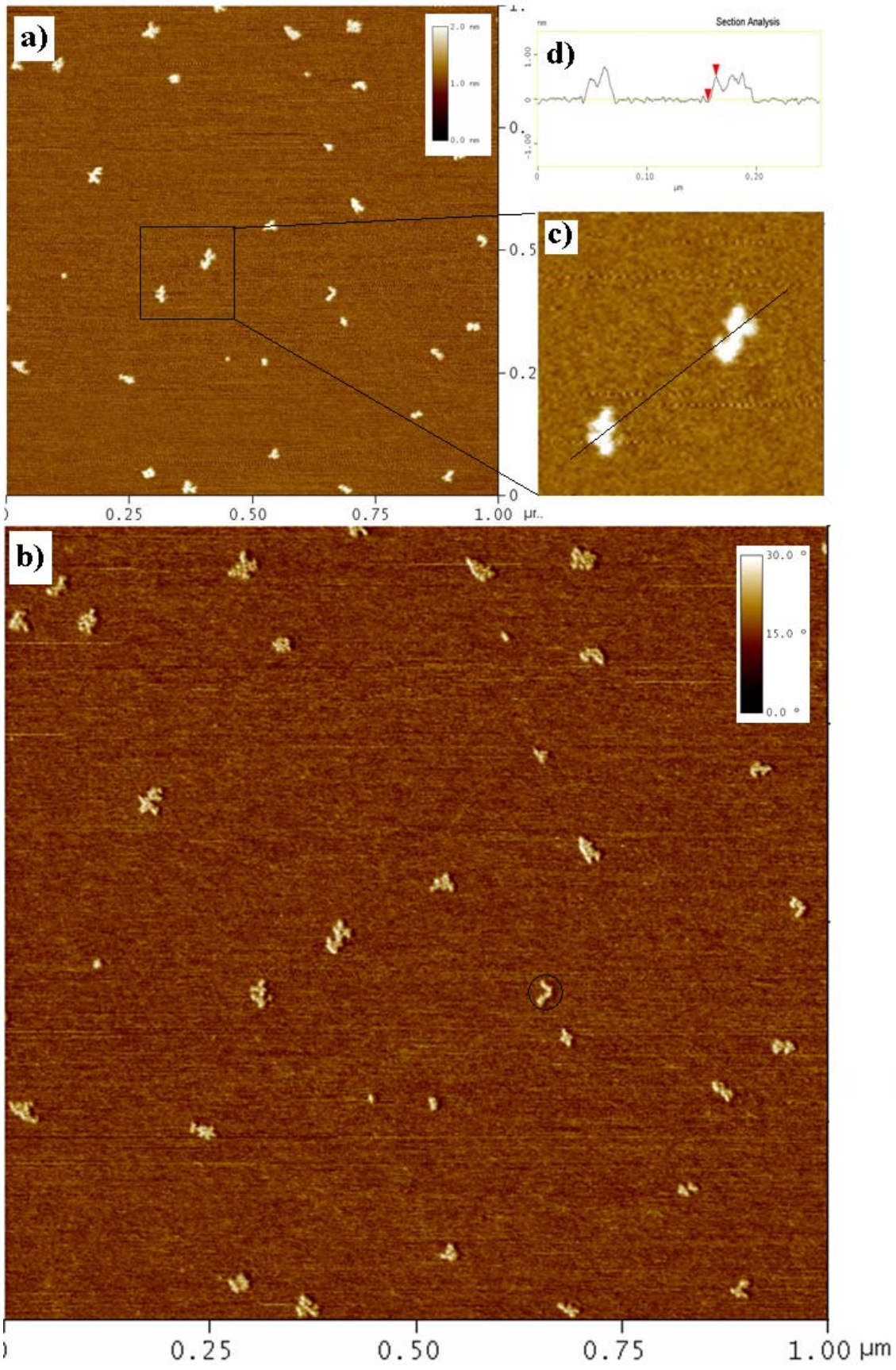


Figure 7: AFM images of **PEI (750k)** at a concentration of $1 \cdot 10^{-6}$ g/L: a) as cast height images; b) phase image; c) high magnification image of a); d) section analysis of c).

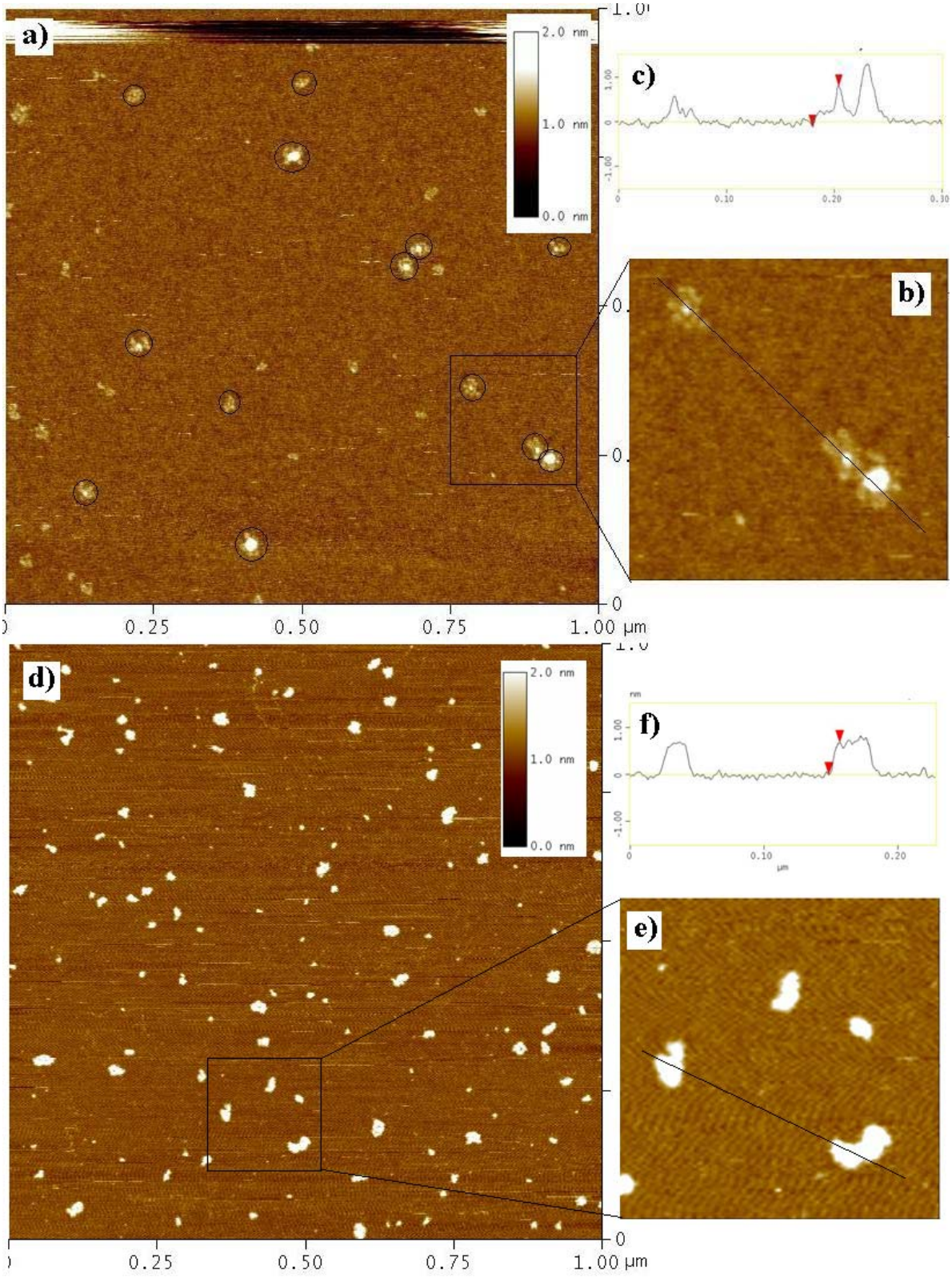


Figure 8: a) as cast height images of **PEI (750k)** from aqueous solution at a concentration of $2 \cdot 10^{-6}$ g/L (13 molecules with islands shape were labeled); b) high magnification image of a); c) section analysis of b). d) as cast height images of **PEI (750k)** from aqueous solution at concentration of $3 \cdot 10^{-6}$ g/L; e) high magnification image of d); f) section analysis of e).

In **Figure 9a**, the relationship of the absorption amounts (surface coverage area · mean height · density) to polymer solution concentrations is shown. Even though the single molecules absorption structures are different at different concentrations, a linear plot of the absorption amounts to concentrations is obtained. The average molecular weight of **PEI (750k)** was calculated from the mean surface area and mean height of each molecule. To ensure accurate counting of visualized molecules, several images were collected from the same sample but in different area, using different scan sizes and scan directions[15]. **Figure 9b** displays the mass distribution of 156 molecules counted from the images measured at the concentration of $1 \cdot 10^{-6}$ g/L, assuming that the density of the polymer is 1.08 g·cm^{-3} (value of the branched poly(ethylene imine) in bulk given in literature). The number average mass is calculated to be ca. 127 KDa, which is comparable to the molecular weight of the polymer (M_n = 60 KDa). This result confirmed that the fully stretched molecules at the concentration of $1 \cdot 10^{-6}$ g/L are single absorbed molecules.

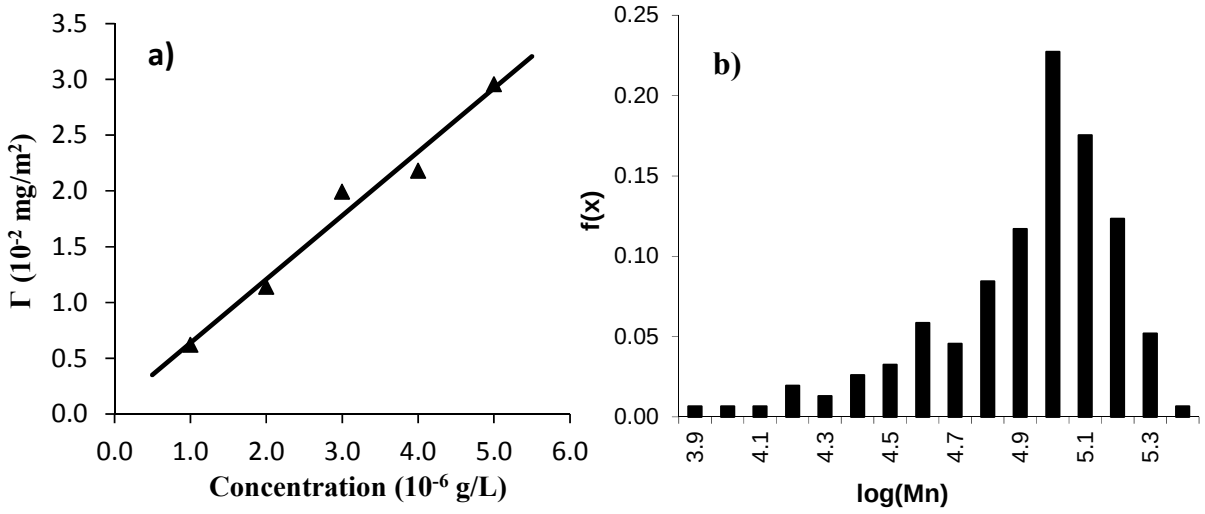


Figure 9: a) Variation of surface density (**Γ**, absorption amount) upon mica depending on the concentration of **PEI$_{750K}$** in aqueous solution; b) molecular weight distribution of **PEI$_{750k}$** estimated over 156 molecules measured at a concentration of $1 \cdot 10^{-6}$ g/L.

5.3.3.2 Single molecules absorption structures of modified PEI (mPEIs) on mica surface

The single molecules absorption structures of modified PEI (**mPEI-1** - **mPEI-3**) were studied in a similar way as that of **PEI (750k)**. The polymers were dissolved in Milli-Q water to prepare a series of much diluted aqueous solutions. And then the solutions were spin-coated on fresh cleaved mica surface and measured directly after the coating (as cast). From the AFM study of **PEI (750k)**, the fully stretched structures can only be obtained at much diluted regime. For the measurements of modified PEIs, a starting concentration of $1 \cdot 10^{-6}$ g/L was used for each polymer.

For **mPEI-1** (PEI_{750k}-QI_{25}) the fully stretched single molecules absorption structures were obtained at the concentration of $1 \cdot 10^{-6}$ g/L (**Figure 10**). Assuming that the modification will not change the polydispersity of the polymer, M_n of **mPEI-1** was calculated to be ca. 195 kDa, which is 3.2 times higher than that of **PEI (750k)**. At the same concentration, average number of **mPEI-1** molecules in 1 μm^2 area on mica is expected to be 3.2 times less than that of **PEI (750k).** In **Figure 10a**, as expected in 1 μm^2 area only 10 molecules were detected, while in **Figure 7a**, in 1 μm^2 area 34 molecules were observed. The same sample was measured again after 1 day maintained in ambient environment. The fully stretched absorption structures are preserved, but the average height of the molecules increases from 0.40 nm to 0.80 nm because of the absorption of humidity.

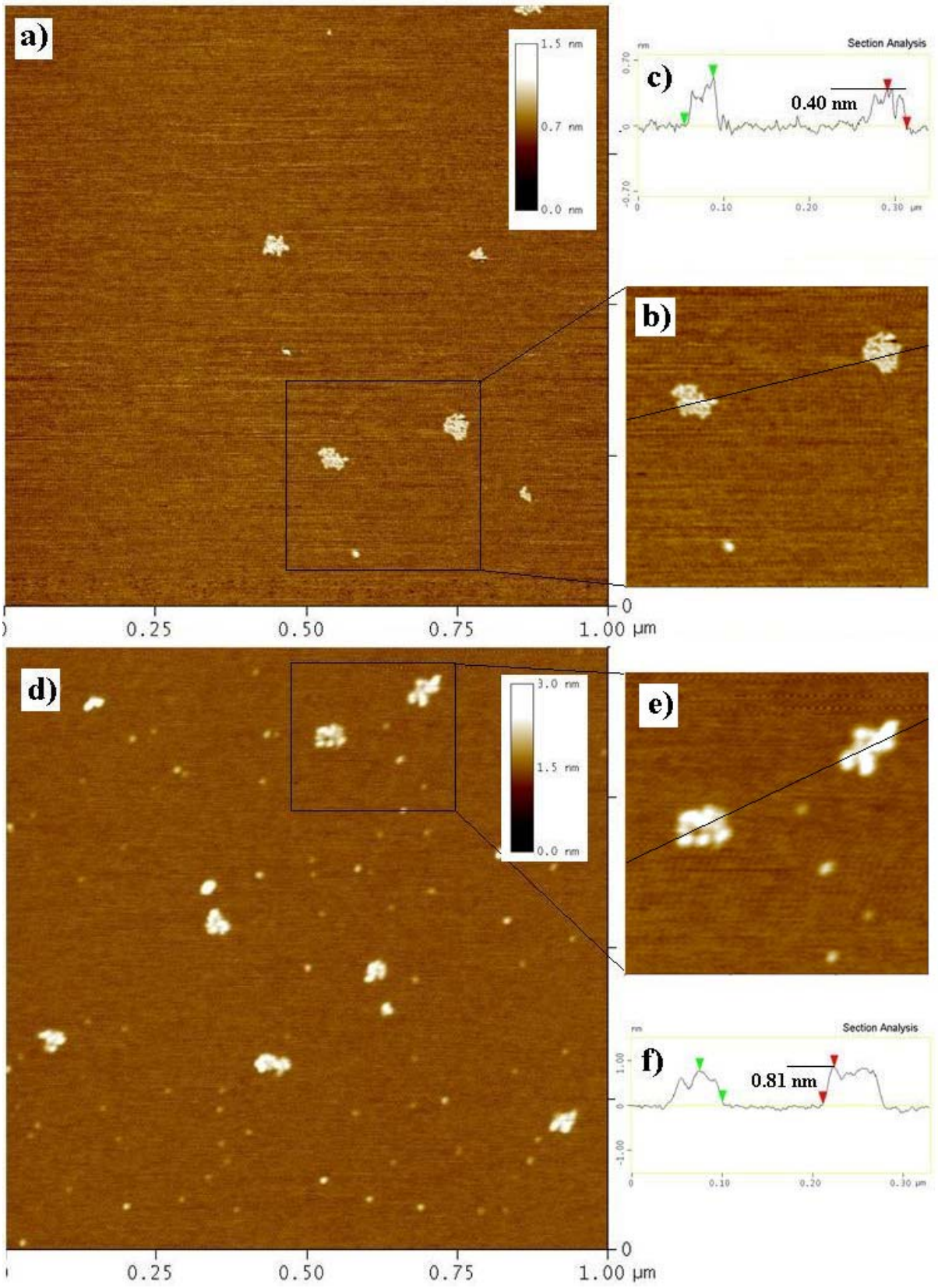


Figure 10: a) As cast height images of **mPEI-1** (PEI_{750K}-QI_{25}) from aqueous solution at concentration of $1 \cdot 10^{-6}$ g/L; b) high magnification image of a); c) section analysis of b). d)

Height images of the same sample after 1 day in ambient environment; e) high magnification image of d); f) section analysis of e).

Polymer **mPEI-2** (PEI_{750k}-$QI_{12.5}$-$J_{12.5}$) has cationic groups and hydrophilic jeffamine® M1000 chains (19 PEO and 3 PPO) in periphery. The cationic groups help to absorbed on the mica surface by electrostatic force, and the hydrophilic groups form a brush like corona. The single molecules absorption structures of **mPEI-2** were obtained only at the concentration of $2 \cdot 10^{-6}$ g/L (**Figure 11**), because of the higher molecular weight of **mPEI-2** (M_n = 344 kDa) and less electrostatic force between the polymer and the substrate in comparison with **mPEI-1**. In the as cast height image (**Figure 11a**), molecules with core-shell structures are obtained. The same sample was measured after 1 day maintained at ambient environment. The height of the molecules increases from ca. 0.40 nm to ca. 0.77 nm because of the absorption of humidity. The fully stretched structures cannot be seen any more, and pancake like structures are obtained (**Figure 11d**). A possible explanation is that the rearrangement and crystallization of the jeffamine®M1000 chains at the environment humidity changes the absorption structures.

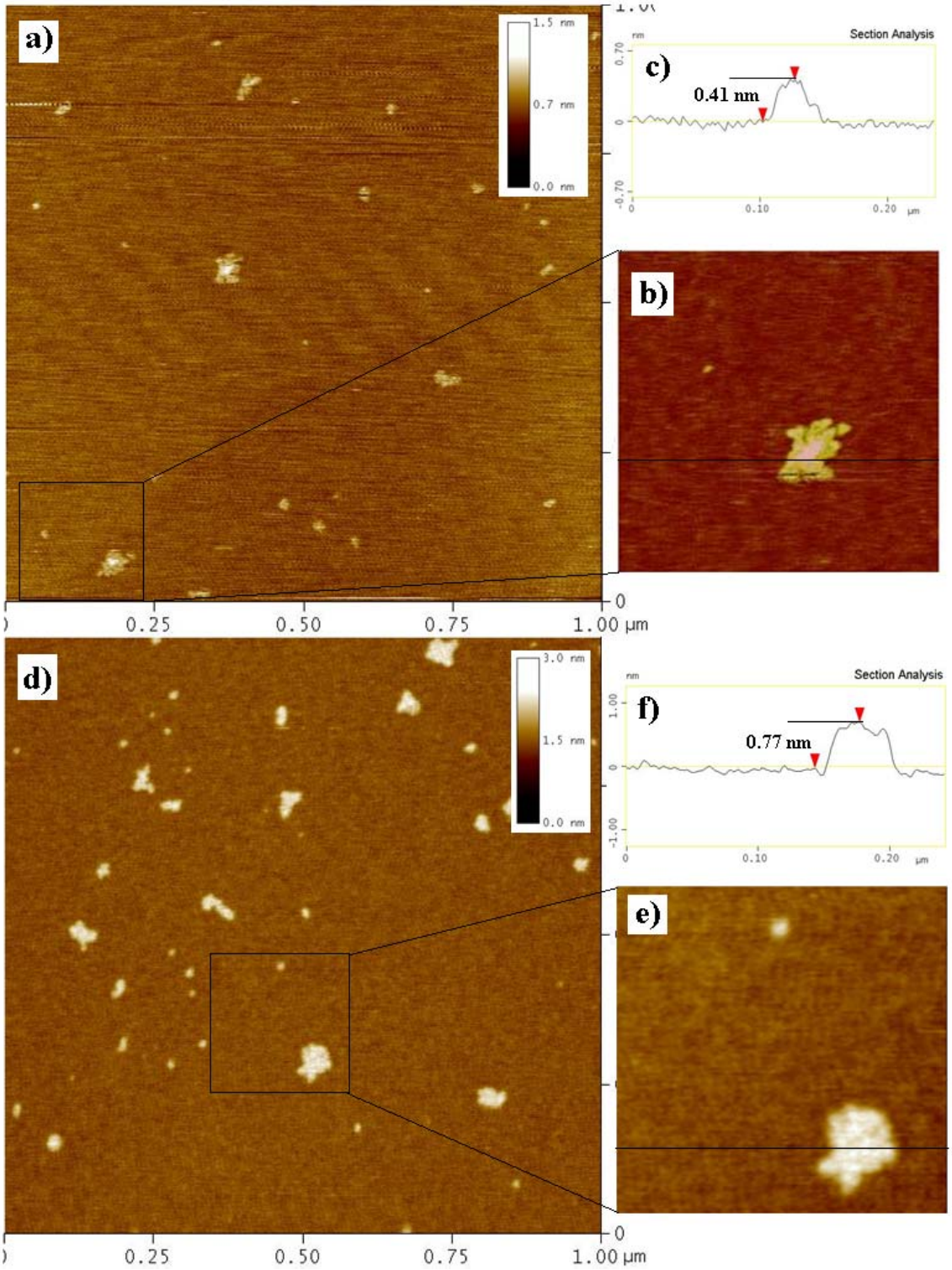


Figure 11: a) As cast height images of **mPEI-2** (PEI_{750K}-$QI_{12.5}$-$J_{12.5}$) from aqueous solution at concentration of $1 \cdot 10^{-6}$ g/L; b) high magnification and resolution image of a); c) section analysis of b). d) Height images of the same sample after 1 day in ambient environment; e) high magnification image of d); f) section analysis of e).

Polymer **mPEI-3** (PEI_{750k}-J_{25}) has no cationic groups in the periphery, and the Jeffamine® M1000 chains form a brush like corona around the poly(ethylene imine) backbone, therefore, there is no electrostatic force between the polymer and the mica substrate. On the other hand, polymer **mPEI-3** has a very high molecular weight (M_n = 492 kDa). As a result, the single molecules absorption structures of **mPEI-3** were obtained not until at the concentration of $4 \cdot 10^{-6}$ g/L (**Figure 12**).

From the DSC measurement (**Figure 4**), the Jeffamine® chains in **mPEI-3** crystallize at very low temperature (Tp = -26.4 °C) and melt at relative higher temperature (Tp = 31.6 °C). At room temperature (T = 25 °C), the Jeffamine® chains are expected to stay in crystalline form. From the high resolution height image (**Figure 12b**) of the big molecules in **Figure 12a**, the molecule has a core in the middle with a height of 0.53 nm, and the side chains with a height of ca. 0.33 nm crystallize and stretch to different directions. From the single molecule absorption image of **PEI (750k)** in much diluted regime (**Figure 7**) the unmodified **PEI (750k)** has different backbone structures, therefore, the modified **PEI (750k)** should have different single molecules absorption structures, too. As expected, in **Figure 12a, 12d, and 12e** molecules with different sizes and absorption structures were obtained.

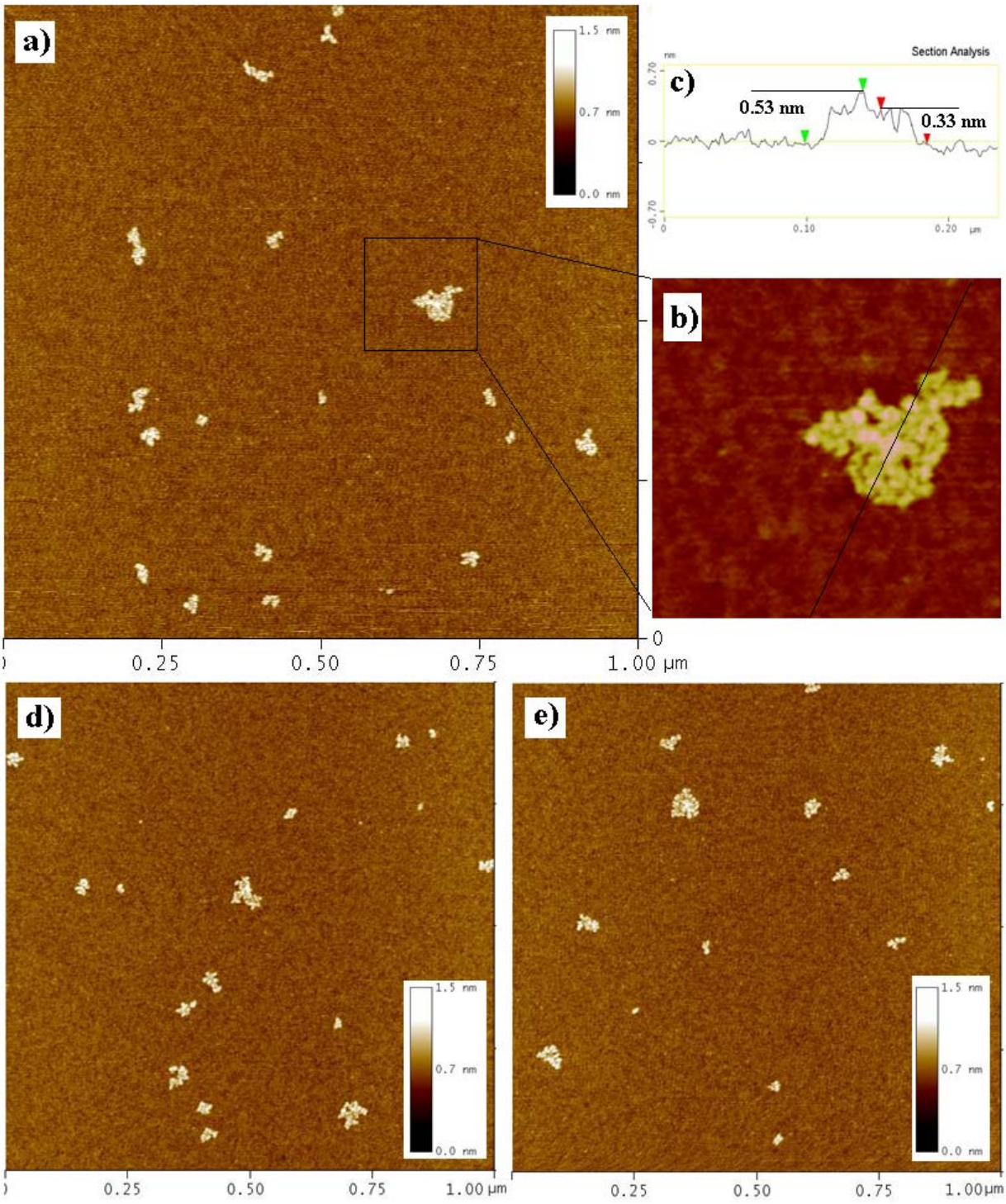


Figure 12: a) As cast height image of **mPEI-3** (PEI_{750K}-J_{25}) from aqueous solution at concentration of $4 \cdot 10^{-6}$ g/L; b) high magnification and resolution image of a); c) section analysis of b); d) and e) as cast height images of **mPEI-3** from aqueous solution at concentration of $4 \cdot 10^{-6}$ g/L with different single molecules absorption structures.

The average molecular weights of **mPEI-1 – mPEI-3** were calculated in the same way as **PEI (750k)**, from the as cast images measured at the concentration of $1 \cdot 10^{-6}$ g/L (**mPEI-1**), $2 \cdot 10^{-6}$ g/L (**mPEI-2**), and $4 \cdot 10^{-6}$ g/L (**mPEI-3**), respectively. For the density the same value as before (d = 1.08 $g \cdot cm^{-3}$) was used. For polymer **mPEI-1**, because of the strong electrostatic force between the cationic modified polymers and the mica substrate, polymers with different sizes can be absorbed on the surface, a broad distribution is obtained. For polymer **mPEI-3**, there are no cationic grafts on the periphery; the absorption was driven by the van de Waals force. A relative narrow distribution is obtained. Polymer **mPEI-2** was 50 % modified with cationic grafts, its molecular weight distribution stands in-between.

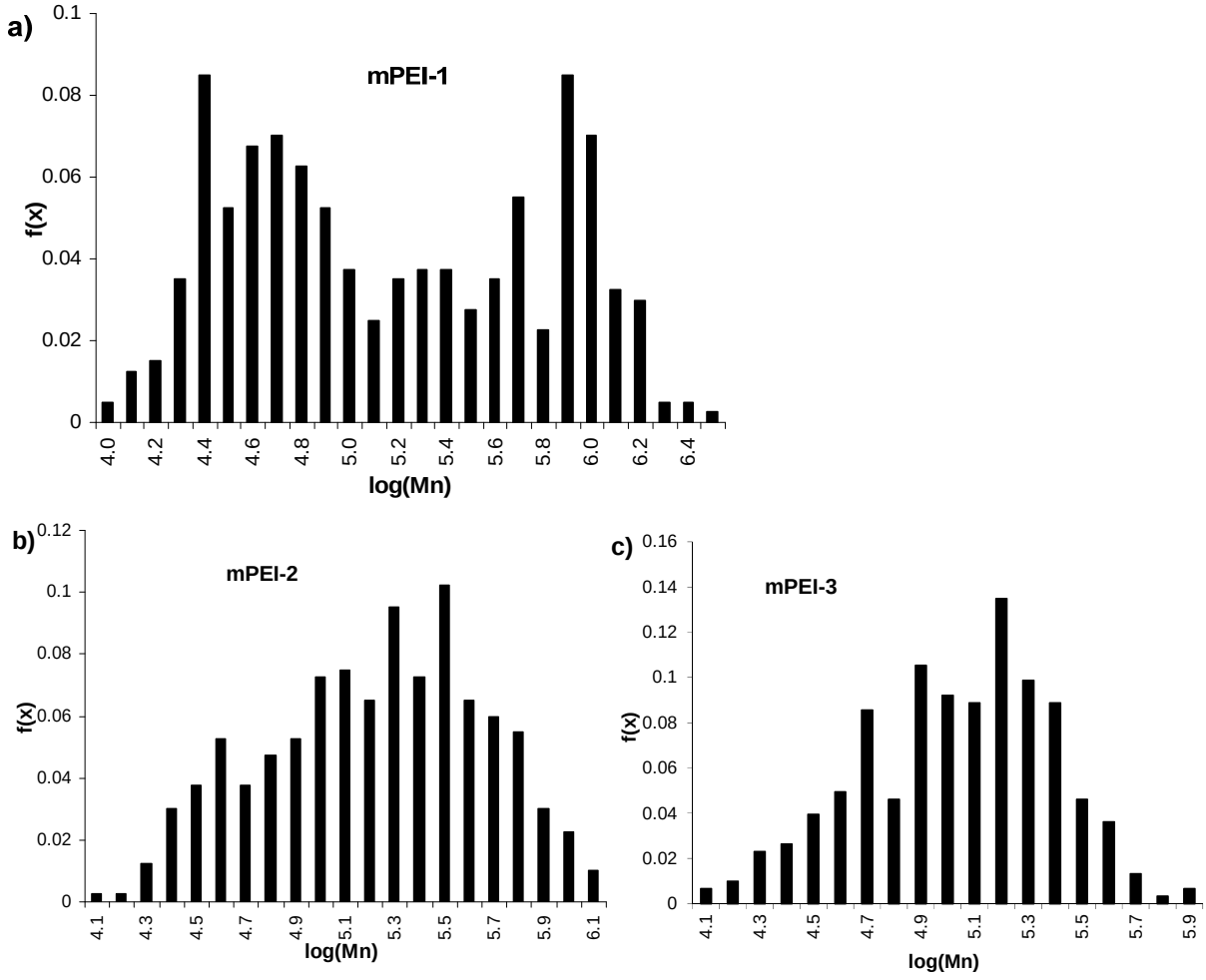


Figure 13: a) molecular weight distribution of **mPEI-1** estimated over 440 molecules measured from concentration of $1 \cdot 10^{-6}$ g/L; b) molecular weight distribution of **mPEI-2**

estimated over 400 molecules measured from concentration of $2 \cdot 10^{-6}$ g/L; c) molecular weight distribution of **mPEI-3** estimated over 250 molecules measured from concentration of $4 \cdot 10^{-6}$ g/L;

5.4 Conclusion

In this work, a series of ionic, ionic/hydrophilic, and hydrophilic modified PEI (**mPEIs**) were successfully synthesized and characterized via NMR, FT-IR, and DSC. From the DSC results, polymers bearing hydrophilic grafts – PEO/PPO segments – have semi-crystalline properties; polymer modified only with cationic groups and unmodified polymer are amorphous.

The single molecule absorption structures of **PEI (750k)** and **mPEI-1 – mPEI-3** on mica substrate were studied by means of atomic force microscopy. In much diluted regime ($1 \cdot 10^{-6}$ g/L), single molecules of **PEI (750k)** with fully stretched structures were detected. Molecules with different shapes were observed. In the images of **PEI (750k)** measured at higher concentrations, island structures ($2 \cdot 10^{-6}$ g/L) and pancake structures ($3 \cdot 10^{-6}$ g/L) were observed. The fully stretched single molecule absorption structures of **mPEIs** were obtained at a concentration of $1 \cdot 10^{-6}$ g/L (cationic), $2 \cdot 10^{-6}$ g/L (cationic/hydrophilic), and $4 \cdot 10^{-6}$ g/L (hydrophilic), respectively.

5.5 Reference

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Chapter 6

Influence of Preparation Processes to the Microstructures of the Obtained Amphiphilic Poly(ethylene imine)s

6.1 Introduction

Self-assembly of soft matter is one of the most effective approaches for constructing nanostructures with controllable order[1-3]. Soft matter has been considered as a key element for producing the newest generation of smart and functional materials. The fundamental basis for engineering its self-assembly behavior is an essential step toward nanoscale regulation of structure and functioning. Block copolymers composing of two or more covalently connected chains per molecule constitute an important class of soft material capable of self-assembling into a rich variety of nanostructures; a lot of study about this type of material has been done. Self-assembly of amphiphilic modified polymers, like amphiphilic poly(ethylene imine)[4-8], amphiphilic poly(propylene imine)[9], were also reported in some papers.

Self-assembly is also essential for the biological function of cells and the fabrication of nanomaterials for drug delivery[10], diagnostic, environmental cleanup applications[11]. However, the origin of the shape of various self-assemblies, such as the shape of cells, is not altogether clear; although it is known that some form of membrane amphiphile heterogeneity is responsible for the variability seen in synthetic membrane curvature[12].

In previous work a convenient method for the modification of poly(ethylene imine) using cyclic carbonate couplers was developed in our group[13-15]. By feeding different functional cyclic carbonate couplers – ionic, hydrophobic, hydrophilic… – simultaneously, amphiphilic poly(ethylene imine)s with statistically distributed surface properties were obtained. This kind of amphiphilic polymer was evidenced to have antibacterial property[14].

The interest of this work is to study the influence of the different preparation processes – the sequence of addition of the functional couplers – to the microstructure and antibacterial properties of amphiphilic poly(ethylene imine) polymers. In this work, amphiphilic poly(ethylene imine)s were prepared via functional couplers mentioned above using different addition processes: simultaneously, hydrophobic couplers firstly, and hydrophilic couplers firstly. The influence of the addition processes to the microstructures of polymers was investigated in one hand under the scope of chemical structures by the comparison of the FT-IR spectra and the ^{1}H- and ^{13}C - NMR spectra of the obtained polymers. On the other hand, the antibacterial properties of these polymers against Gram-negative bacteria were tested to study the influence of preparation processes to their biological activity.

6.2 Experimental Part

6.2.1 Materials and Methods

6.2.1.1 Material

(2-Oxo-1,3-dioxolan-4-yl)methyl chloroformate (Saltigo, 97 %), dodecylamine (Acros, 98 %), triethylamine (Fluka, 99.8 %), 3-dimethylamino-1-propylamine (Aldrich, 99 %), iodomethane (Aldrich, 99 %), Jeffamine®M1000 (Hunstman), *N*,*N*-dimethylformamide (Acros Organics,

99.9 %, DMF), poly(ethylene imine) (water-free, M_w(LS) = 2.5 × 10^4 g/mol, Aldrich, PEI25000), all chemicals are used as received. For antimicrobial activity amphiphilic polymers were tested against the Gram-negative bacterium *Escherichia coli* (DSMZ 498).

6.2.1.2 Bacterial culture

The nutrient solution pH 7 contained 5 g peptone, 3 g meat extract per L bidistilled water. Phosphate-buffered saline (PBS) contained 9.0 g NaCl per L 0.1 M disodium hydrogenophosphate/sodium dihydrogenophosphate buffer solution adjusted to pH 6.5. Soft agar was prepared from 10.0 g peptone, 3.0 g meat extract, 6.0 g NaCl and 7.0 g agar-agar per L bidistilled water. All solutions were autoclaved for 15 min at 120 °C prior to use.

6.2.1.3 Antibacterial assessments of polymer solutions: Growth test

A suspension of strains with known colony forming units (CFU; *E. coli*: 5 × 10^7 cfu/mL) was incubated at 37 °C in nutrient solution to which the respective antimicrobial polymer was added in different concentration. The growth of the bacteria was followed over 20 h by measuring every 30 min the optical density at 612 nm using a microplate reader/incubator. From these data a minimal inhibitory concentration (MIC) was as obtained as the concentration of the test substance at which a log 4 reduction of the growth of the inoculated bacteria was observed relative to a control sample. This test does not discriminate whether the substance is bactericidal or bacteriostatic. Experiments were triplicated.

6.2.1.4 Sample preparation for fluorescence and laser light scattering

Stock solutions of 10.0 g/L in Milli-Q water were prepared.

For laser light scattering, the stoppered flasks were allowed to equilibrate overnight at room temperature. To get sample solutions for fluorescence, a known amount of pyrene in acetone

was added to each series of 25.0 mL volumetric flasks and the acetone was evaporated. The amount was chosen to give a pyrene concentration of in the final solution of 1.2×10^{-7} M. To the first flask of the series, a measured amount of stock solution was added, followed by Milli-Q water. A polymer dilution series was prepared with constant pyrene concentration varying in concentration from 1.5×10^{-5} to 1.0 g/L. The stoppered flasks were allowed to equilibrate overnight at room temperature.

6.2.1.5 Fluorescence measurements

For fluorescence measurements 2 mL of solution was placed in 1.0 × 1.0 cm square cuvette. All spectra were run on air-saturated solutions using a LS50 Luminescence Spectrophotometer (Perkin Elmer) with 90° geometry using slit openings of 2.5 nm. Excitation spectra were registered at emission wavelength (λ_{em}) of 390 nm and were accumulated with an integration time of 0.5 s/0.5 nm. A critical aggregation concentration (CAC) for the solubilization of pyrene was determined based on the shift of the (0,0) bands in the pyrene excitation spectrum as described elsewhere[16]

6.2.1.6 Laser light scattering

Dynamic light scattering was measured (polymer concentration: 10 mg/ml in Milli-Q water) at a constant temperature of 25°C at an angle of 173° on a Nano-ZS, Model ZEN3600, zetasizer (Malvern). For evaluation of data, the DTS (Nano) program was used. The mean of the intensity-hydrodynamic radius (R_h) distribution was taken as values for data comparison in the charts and tables.

6.2.2 Synthesis

(2-Oxo-1,3-dioxolan-4-yl)methyl, N-dodecylcarbamate (**A12**), 3-*N*,*N*,*N*-trimethyl-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonylamino)propan-1-ammonium iodide (**QI**), and (2-oxo-1,3-dioxolan-4-yl)methyl N-1-(1-(2-methoxyethoxy)propan-2-yloxy) propan-2-ylcarbamate (**J**) were synthesized and characterized according to literature. The polymers used in this work were synthesized in different procedures (**Procedure A - C**), the detail information was listed in Table 1. The obtained amphiphilic poly(ethylene imine)s was characterized according to Ref[14].

Procedure A (simultaneous synthesis): to a solution of PEI (M_w = 25 kDa) in DMF (20 mL) at 60 °C a mixture of functional couplers (**QI/A12** or **J/A12**) were added simultaneously. The reaction mixture was stirred at 60°C for 3 days. After removal of the solvent in vacuum, the residual was dissolved in MeOH (5 mL) and precipitated into Et_2O/pentane (600 mL, v/v = 1/2). The procedure was repeated for another two times. After drying in vacuum ($2 \cdot 10^{-2}$ mbar) at RT for 2 days, a slightly yellow highly hygroscopic material was obtained.

Procedure B (hydrophilic first sythesis): to a solution of PEI (M_w = 25 kDa) in DMF (20 mL) at 60 °C a first functional coupler – hydrophilic functional couplers (**QI** or **J**) – were added and the solution was stirred at 60 °C for 2 days. After that the second functional coupler – hydrophobic functional coupler (**A12**) – was added to the solution and the reaction mixture was stirred at 60 °C for another 2 days. The reaction was monitored by ^{1}H NMR. The polymer was purified by precipitation, as described in procedure A.

Procedure C (hydrophobic first synthesis): to a solution of PEI (M_w = 25 kDa) in DMF (20 mL) at 60 °C a first functional coupler – hydrophobic functional coupler (**A12**) – were added and the solution was stirred at 60 °C for 2 days. After that the second functional coupler – hydrophilic functional couplers (**QI** or **J**) – was added to the solution and the reaction mixture

was stirred at 60 °C for another 2 days. The reaction was monitored by ^{1}H NMR. The polymer was purified by precipitation, as described in procedure A.

Table 1: Details of the reactions of **PEI** (M_w = 25 kDa) with functional couplers

Nr.	Polymer feeding	Functional couplers [a]		Yield (%)	QI (J) /A12 [b]	Polymer obtained
		QI (J)	A12			
NRP283	**PEI_F-$QI_{12.5}$-$A12_{12.5}$**	1127 (QI)	956	86	12.5/12.5	**PEI_O-$QI_{12.5}$-$A12_{12.5}$**
NRP287	**PEI_F-1.$QI_{12.5}$-2.$A12_{12.5}$**	1127 (QI)	956	75	15/10	**PEI_O-1.QI_{15}-2.$A12_{10}$**
NRP288	**PEI_F-1.$A12_{12.5}$-2.$QI_{12.5}$**	1127 (QI)	956	84	12.9/12.1	**PEI_O-1.$A12_{12.1}$-2.$QI_{12.9}$**
H2H	**PEI_F-$J_{12.5}$-$A12_{12.5}$**	3605 (J)	956	83	12.5/12.5	**PEI_O-$J_{12.5}$-$A12_{12.5}$**
H33	**PEI_F-1.$J_{12.5}$-2.$A12_{12.5}$**	3605 (J)	956	73	15.1/9.9	**PEI_O-1.$J_{15.1}$-2.$A12_{9.9}$**
H34	**PEI_F-1.$A12_{12.5}$-2.$J_{12.5}$**	3605 (J)	956	82	12.7/12.3	**PEI_O-1.$A12_{12.3}$-2.$J_{12.7}$**
NRP279	**PEI_F-QI_{15}-$A12_{10}$**	1352 (QI)	765	83	15.3/9.7	**PEI_O-QI_{15}-$A12_{10}$**
HYC483	**PEI_F-1.$QI_{10.5}$-2.$A12_{14.5}$**	946 (QI)	1109	81	12.4/12.6	**PEI_O-1.$QI_{12.4}$-2.$A12_{12.6}$**
HYC484	**PEI_F-1.$A12_{10}$-2.QI_{15}**	1352 (QI)	765	89	15.5/9.5	**PEI_O-1.$A12_{9.5}$-2.$QI_{15.5}$**

[a] for 1000 mg PEI 25 000 in the feed; [b] results obtained via quantitative analysis of ^{1}H NMR

6.3 Results and Discussion

In order to examine the influence of the preparation processes to the microstructures of obtained polymers, two series of polymers **NRP283/NRP287/NRP288** and **H2/H33/H34** were prepared by addition of the hydrophilic (**QI** or **J**) and hydrophobic (**A12**) functional couplers with the same feeding amount in different feeding sequences: simultaneously for **NRP283** and **H2** (Procedure A); hydrophilic first for **NRP287** and **H33** (Procedure B); and

hydrophobic first for **NRP288** and **H34** (Procedure C). The detail of preparation information was described in experiment part and illustrated in Figure 1.

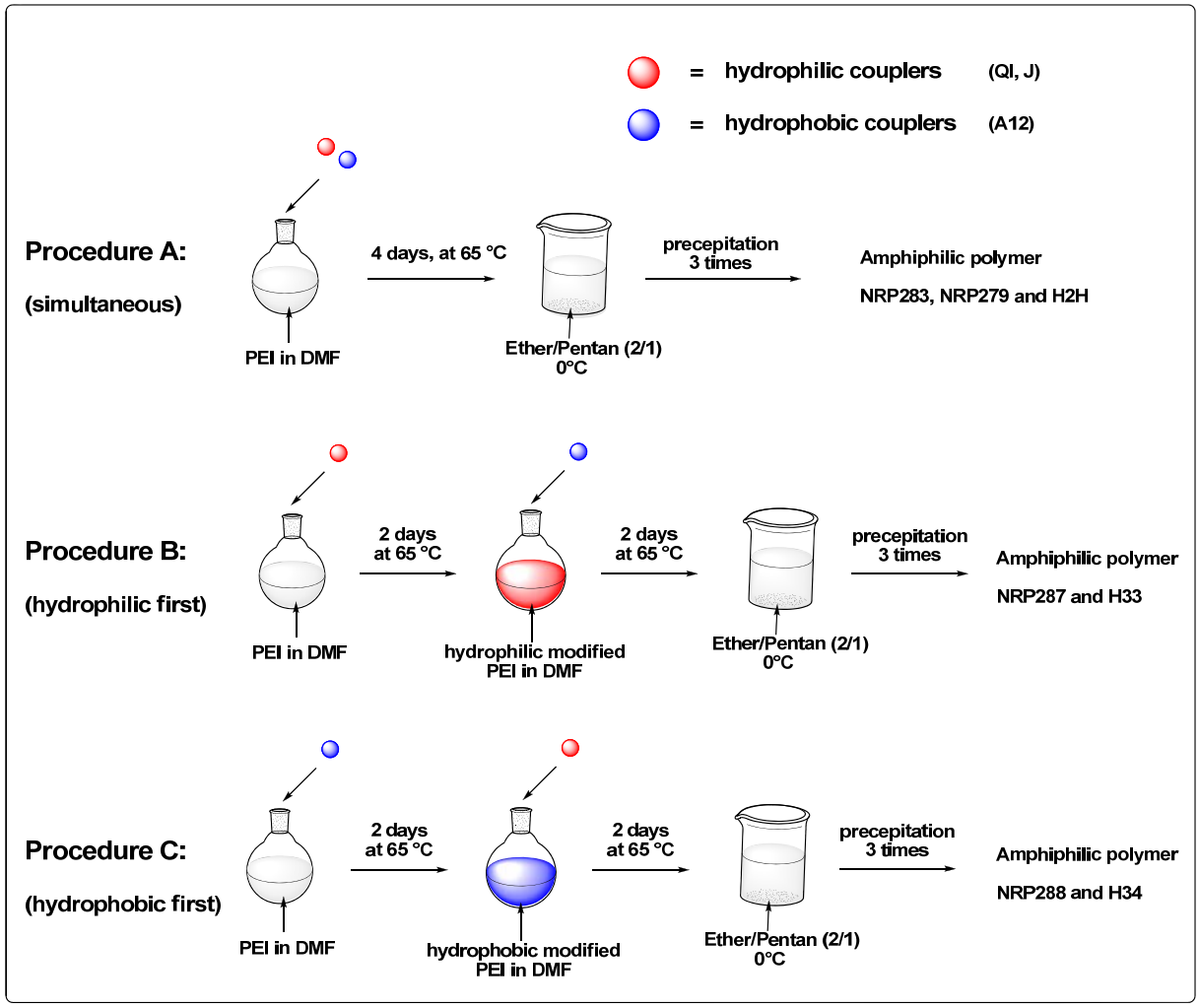


Figure 1: Schematic representation of the polymer preparation procedures A – C.

6.3.1 The influence of preparation processes to polymer chemical structures – FT-IR and NMR analysis

The structure of the polymers prepared from different preparation processes was investigated by means of FT-IR and NMR analysis. Comparing the FT-IR spectra of **H2H, H33,** and **H34** (Figure 2a), the presence of urethane groups in the polymers was confirmed. The bands at

1248 cm^{-1} (OC-N - stretch), 1530 cm^{-1} (Amide-II) and 1720 cm^{-1} (Amide-I) are characteristic for-CO-NH- groups, while the band at 1108 cm^{-1} was attributed to the C-O-C stretching vibration of the PEO segments of the Jeffamine® M1000 tail. The most significant difference was observed in the range of 1600-1800 cm^{-1}. In the spectra of **H33** and **H34** there is an obvious shoulder peak (1668 cm^{-1}) next to the main peak at 1720 cm^{-1}. In the FT-IR spectra of **NRP283**, **NRP287**, and **NRP288** similar differences in range of 1600-1800 cm^{-1} were observed (Figure 2b). The peak at 1668 cm^{-1} appeared only in polymers prepared via sequential addition procedures (Procedure B and C). But the sequence of the addition of couplers (hydrophobic first or hydrophilic first) shows no difference.

In Figure 3, the literarily given FT-IR values of the C = O bond in urethane and in urea were summarized[17-19]. In free urethane situation, the C=O stretching peak is at 1730 cm^{-1}, and in H-bonded urethane the peak is at 1700-1705 cm^{-1}. In case of urea, the C=O stretching peak is in free urea at 1715 cm^{-1}, in H-bonded urea at 1640 cm^{-1}, and in monodentate urea at 1650-1700 cm^{-1}. Therefore, the extra shoulder peak at 1668 cm^{-1} in sequential addition systems is in typical range of a monodentate urea bond. It means that, urea groups are formed in polymers prepared in sequential addition procedures.

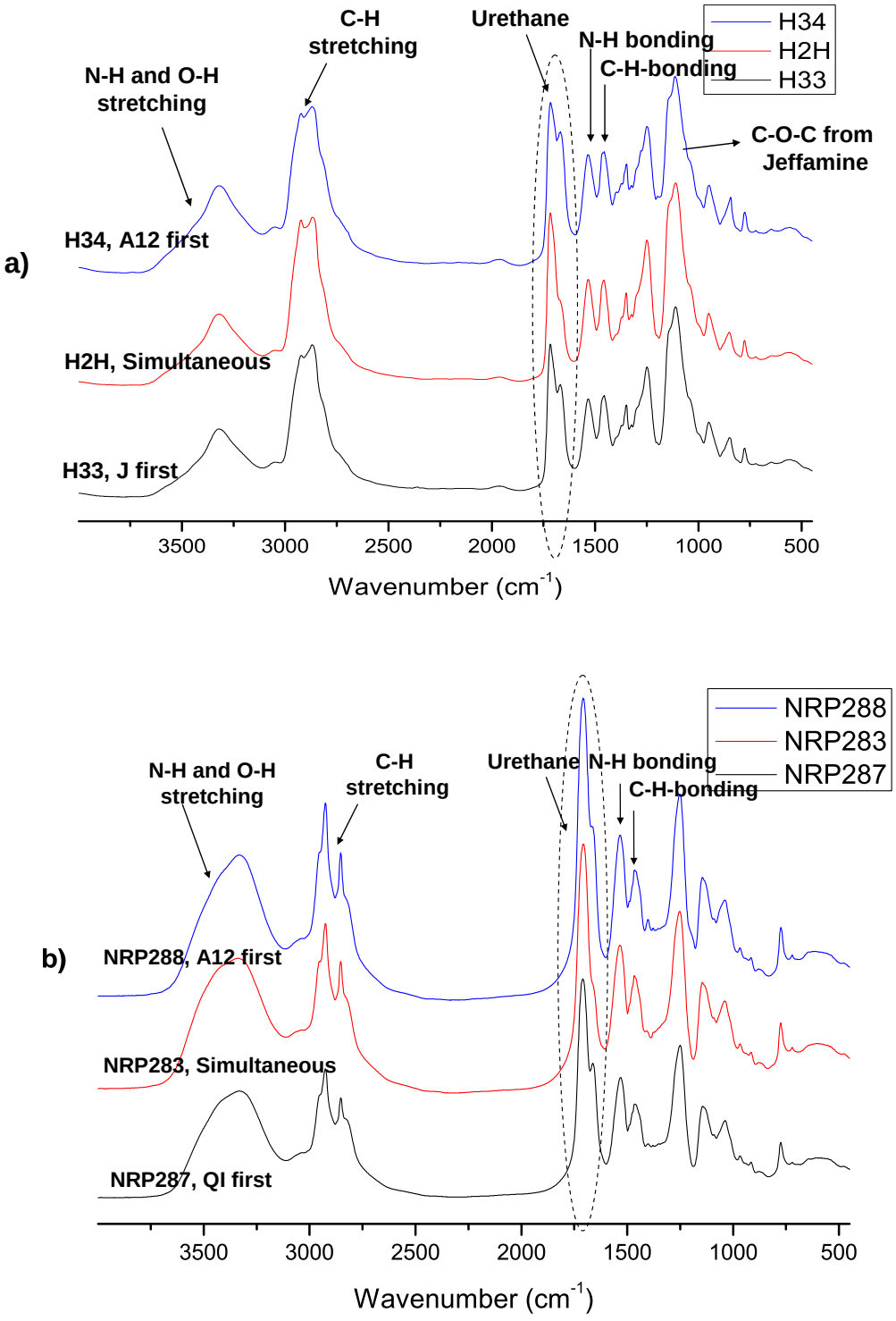


Figure 2: FT-IR of polymers prepared in different procedures: a) **H33/H2H/H34**; b) **NRP287/NRP283/NRP288**

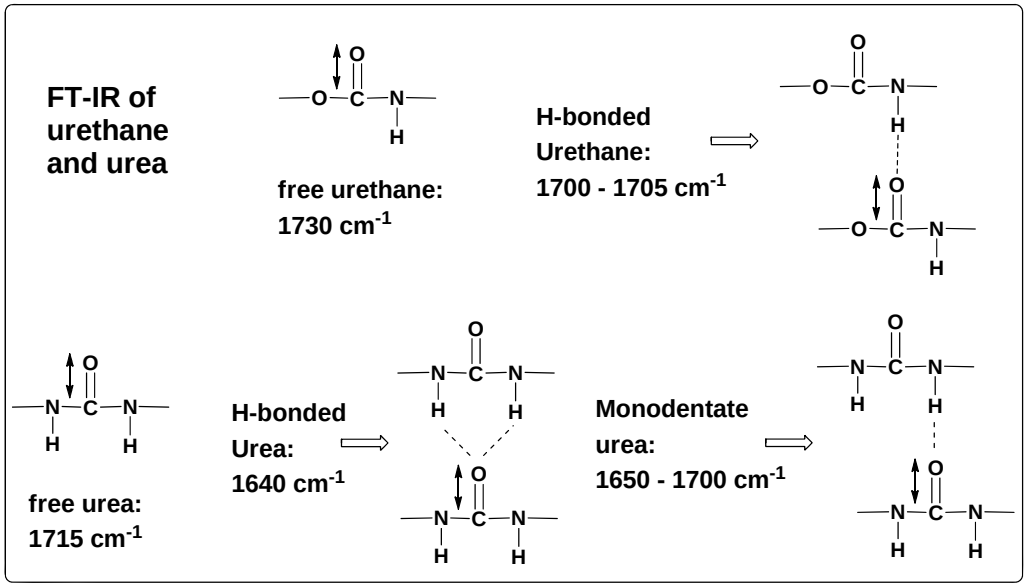


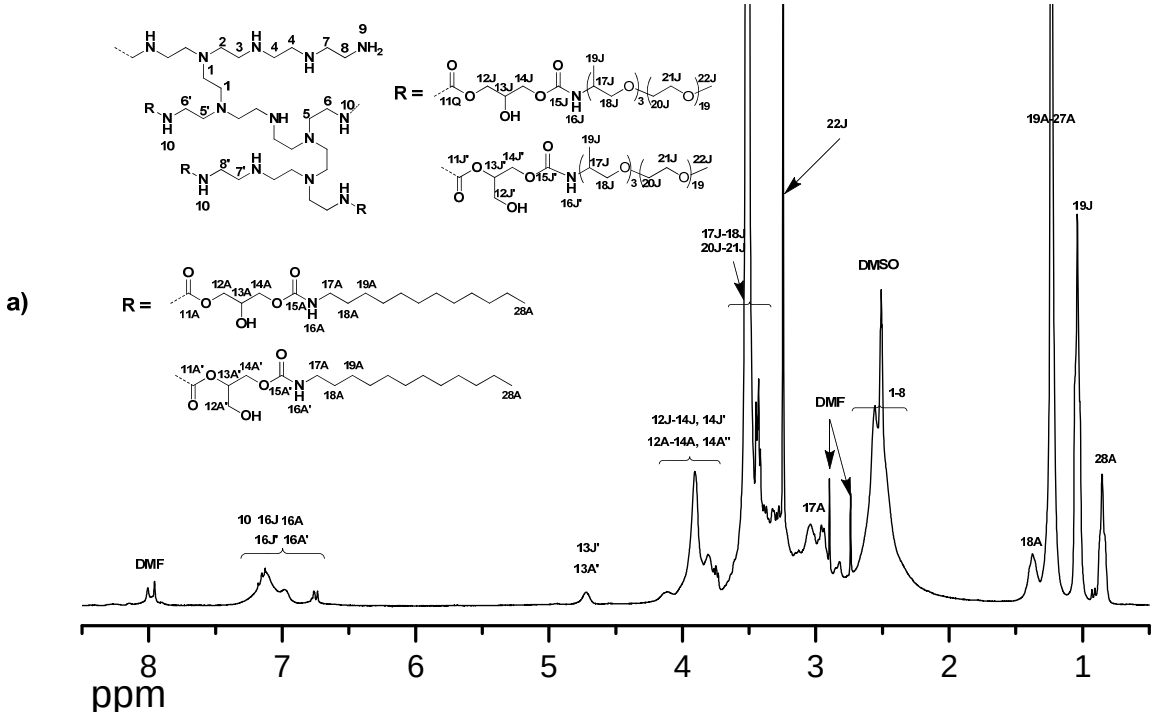
Figure 3: FT-IR value of urethane and urea bond from the literatures[17-19].

The chemical microstructure of these polymers was further studied via ^{1}H and ^{13}C NMR. For the polymers in Series **H2H/H33/H34**, the ^{1}H NMR of **H2H** was assigned exemplarily (Figure 4a). The comparison of **H33** and **H34** with **H2H** was shown in Figure 4b and 4c. The quantitative analyses of the ^{1}H NMR spectra indicate that the functional composition of **H34** and **H2H** are very similar (Table 1, ratio hydrophilic/alkyl of 12.5/12.5 and 12.7/12.3, respectively). **H33** did not fulfill the expectation (the ratio of hydrophilic/alkyl of the obtained polymer equal to 15.1/9.9 instead of 12.5/12.5) and was obtained with a yield ca. 10% lower than the two others. At the end of the reaction, all cyclic carbonates have reacted and the ratio of hydrophilic/alkyl was in agreement with the expected values. It is only after the purification of **H33**, that the ratio of composition changed.

In Figure 4b significant differences of the three spectra were observed in range R1 – R3. (i) In range R1 the signals in the spectra of **H33** and **H34** are much bigger than the one in the spectrum of **H2H**, even though there is still rest of DMF peak in spectrum of **H2H** (because

of the existence of DMF peaks in range R1, the difference in this region can only be detected after the purification). These signals are possibly belong to the N-H of urea groups. (ii) In range R2, the coupler skeleton signals (12-14 J/A12) in spectrum of **H2H** distinguished with the signals of the Jeffamine® tail, however, the coupler skeleton signals in the spectra of **H33** and **H34** overlapped with the signals of the Jeffamine® tail, which indicated a higher amount of Jeffamine® grafts and confirmed the quantitative analysis results. (iii) In range R3 there is an extra signal in the spectrum of **H34**. From the correlation spectroscopy this signal does not belong to functional coupler but belong to the poly(ethylene imine) backbone and has correlation with the signal in R1.

In Figure 4c the ^{13}C signal of urea can be well seen in the spectra of H33 and H34, which confirmed the results of FT-IR. In the ^{1}H NMR spectra of series **NRP283/NRP287/NRP288** (Figure 4d) similar results were obtained. The ratio of hydrophilic/alkyl is 12.5/12.5 for **NRP283**, 15.0/10 for **NRP287**, and 12.9/12.1 for **NRP288**, respectively.



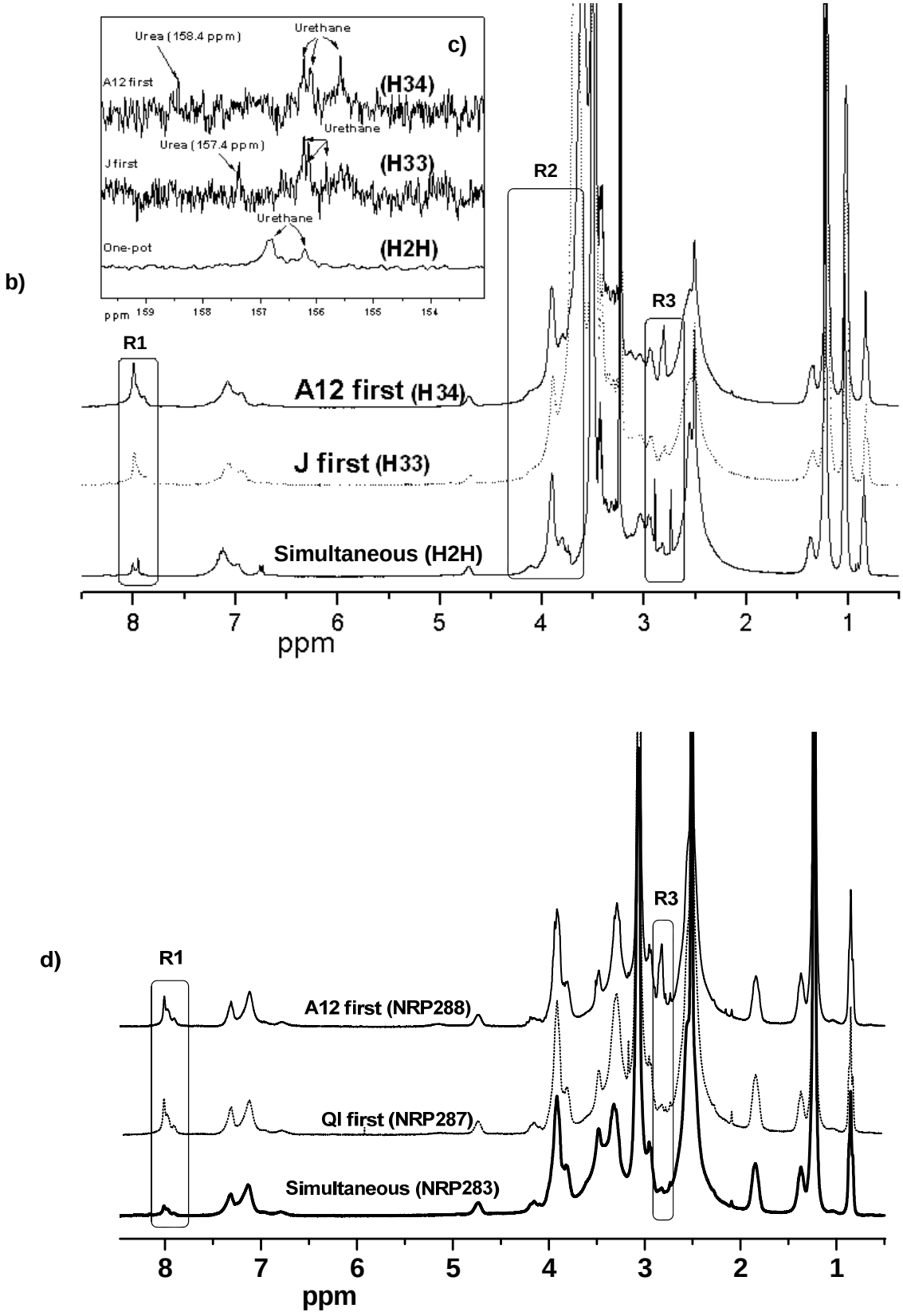


Figure 4: a) ^{1}H NMR spectrum (DMSO-d_6, 400 kHz) of **H2H** with signal assignment; b) Comparison of the ^{1}H NMR spectra (DMSO-d_6, 400 kHz) of **H2H**, **H33**, and **H34**; c)

Comparison of the ^{13}C NMR spectra (DMSO-d_6, 400 kHz) of **H2H**, **H33**, and **H34** in range δ = 153-160 ppm; d) Comparison of the ^{1}H NMR spectra (DMSO-d_6, 400 kHz) of **NRP283**, **NRP287**, and **NRP288**

It is known in literature, that primary amines can react with urethane groups to form urea groups[20, 21]. The possible mechanism of the formation of urea was illustrated in Figure 5. By sequential addition of functional couplers, there are still a large excess of primary amine after the fully conversion of the first added functional couplers. These primary amines can further react with urethane groups via substitution reaction to form urea group[20, 21]. Considering about the substitution positions, three types of reactions (Type I – Type III) are possible. (i) In reaction Type I, the primary amine attacks the urethane group near to the PEI backbone, and a urea group between two backbone primary amine groups is formed (Urea I). In this case the functional moieties fall off from the functional polymer molecules and the amount of functional composites in the final product will be less. (ii) In reaction Type II and Type III, the primary amine groups attack the urethane groups near to functional moieties and urea groups between backbone and functional moieties are formed (Urea II).

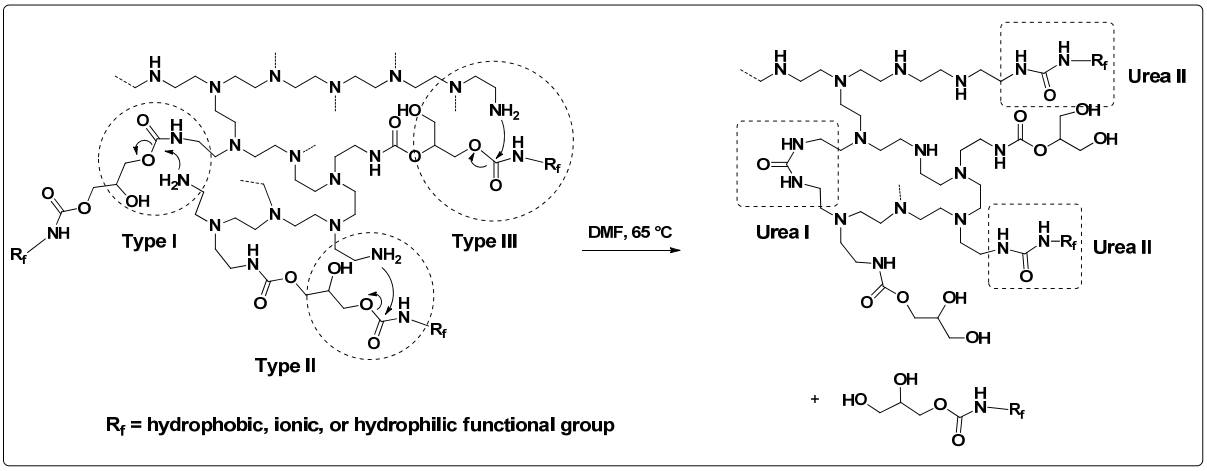


Figure 5: Possible mechanism of the formation of urea groups.

Using this hypothesis model, the phenomena observed can be explained. In hydrophilic first procedure (Procedure B), the hydrophilic functional couplers were added first and linked to PEI backbone. The functional moieties have similar polarity as the PEI backbone and can stay near to the PEI molecules surface. In this case the substitution in Type II and Type III are dominated because of the higher access possibility, and urea groups like Urea II are formed. The lost of functional moieties are suppressed. However, in hydrophobic first procedure (Procedure C) alkyl chain functional moieties were firstly added and brought to PEI molecules. Because of the immiscibility of the functional moieties and the PEI backbone, the alkyl chains tend to stay away from the PEI molecules surface. Thus, the attack of primary amines to urethane groups in Type I is dominated, and urea groups like Urea I are formed. A lost of alkyl chain moieties are obtained as expected, which is coincident with the NMR results.

6.3.2 The influence of preparation process to biological activity

From previous study, cationic and hydrophobic modified PEI has antibacterial properties against Gram-positive and Gram-negative bacteria[14, 15]. The influence of microstructure changes to the antibacterial activity is quite interesting. Since the ratio of cationic/alkyl has a high influence of the MIC value, three polymers (**NRP279**, **HYC483**, and **HYC484**) were synthesized in concerning about the ratio of cationic/alkyl. Thus, two series of polymers with the same obtained ratio of cationic/alky but prepared in different procedures were obtained. Their obtained composition, CAC, R_h, and MIC against *E.coli* were summarized in Table 2. Because of the lacking of cationic groups, hydrophobic and Jeffamine® coupler modified PEI

has no activity against bacteria. For these reasons, in this part of work, the series of **H2H/H33/H34** was not studied.

In table 2, polymers were grouped in two series depending on the obtained cationic/alkyl ratio – Series **S-I** (Q/A12 = 12.5/12.5) and Series **S-II** (Q/A12 = 15/10). (i) The CAC values of polymers in Series **S-I** are similar (CAC = 0.014 ~ 0.019 mg/mL), but the values in Series **S-II** are more different (CAC = 0.028 ~ 0.068 mg/mL). (ii) In both Series polymers prepared in simultaneous procedure have higher R_h values than polymers prepared in sequential addition procedures. (iii) Comparing the MIC against *E.coli* in the two series, different trends are obtained. In Series **S-I** with relatively low cationic content (cationic/alkyl ratio = 12.5/12.5), polymer prepared in hydrophilic first process (**HYC483**) has the lowest MIC; however, in Series S-II with relatively high cationic content (cationic/alkyl ratio = 15/10), polymer prepared in hydrophobic first process (**HYC484**) has the lowest MIC.

Table 2: Samples compared with the obtained compositions

Series	No.	QI/A12	Proc.	Polymer obtained	CAC [mg/mL]	R_h (nm)	MIC (*E.coli*) [mg/mL]
S-I	**NRP283**	12.5/12.5	A	PEI_O-$QI_{12.5}$-$A12_{12.5}$	0.019	6.7	0.3
	HYC483		B	PEI_O-1.$QI_{12.4}$-2.$A12_{12.6}$	0.014	5.2	0.05
	NRP288		C	PEI_O-1.$A12_{12.1}$-2.$QI_{12.9}$	0.017	3.6	0.3
S-II	**NRP279**	15/10	A	PEI_O-QI_{15}-$A12_{10}$	0.028	6.7	$0.2 \leq$ MIC ≤ 0.3
	NRP287		B	PEI_O-1.QI_{15}-2.$A12_{10}$	0.052	5.4	0.2
	HYC484		C	PEI_O-1.$A12_{9.5}$-2.$QI_{15.5}$	0.068	6.0	0.04

From literatures[22] the interaction between peptides or peptide likes polymers with bacterial membrane follows two basic steps: the first step is the preferential electrostatic interaction of positively-charged part of peptides or peptide like polymers with negatively-charged phospholipids; the second step is that the hydrophobic part interacts with non-polar lipid alkyl chains. From this basic principle, the MIC results of the two series can be better understood: (i) In Series **S-I** with relatively low cationic content (cationic/alkyl ratio = 12.5/12.5), the step of polymer molecules attaching to the bacterial membrane surface is the decisive step. Polymer **HYC483** prepared via hydrophilic first (QI first) process has more cationic functional groups on the outer peripheries, because of the easy access of QI functional couplers to the primary amines on the outer peripheries of the PEI molecules. This kind of structure favors the attaching of the polymer molecules on the bacterial membrane. Therefore, comparing with **NRP283** (MIC = 0.3 mg/mL) and **NRP288** (MIC = 0.3 mg/mL), a relatively low MIC for polymer **HYC483** (MIC = 0.05 mg/mL) is obtained. For polymer **NRP288** and polymer **NRP283**, because there is already enough amount of alkyl chain, the A12 first process shows no obvious advantage. No difference of MIC values of these two polymers was observed. (ii) In Series **S-II** with relatively high cationic content (cationic/alkyl ratio = 15/10), the electrostatic interaction with the bacterial membrane is sufficient because of the high amount of cationic functional group. The step of interaction of hydrophobic alkyl chain with non-polar lipid alkyl chains is the decisive step. Polymer **HYC484** prepared via hydrophobic first (A12 first) process has more alkyl chain functional groups on the outer peripheries. This kind of structure helps the interaction of alkyl chain of the polymer molecules with the non-polar lipid alkyl chains. Therefore, a relatively low MIC for **HYC484** (MIC = 0.04 mg/mL) is obtained. For polymer **NRP287** and **NRP279**, because there is already enough amounts of cationic groups, the QI first process doesn't present obvious advantage. The MIC values of

polymer **NRP287** (MIC = 0.2 mg/mL) is just a little lower than that of polymer **NRP279** (0.2 mg/mL < MIC < 0.3 mg/mL).

6.4 Conclusion

Nine amphiphilic poly(ethylene imine) polymers were synthesized in different preparation processes – simultaneous (Procedure A), hydrophilic first (Procedure B), or hydrophobic first (Procedure C) – and characterized. The influence of the preparation processes to polymer microstructures and to their antibacterial properties was studied.

Firstly, two series of polymers prepared with the same feeding amounts of functional couplers were compared. From FT-IR and NMR analysis the formation of urea groups in the polymers prepared in Procedure B and C are detected. The possible mechanism is discussed. The obtained ratio of hydrophilic/alkyl of polymers prepared in Procedure A and C was similar as the feeding ratio. However, the obtained ratio of hydrophilic/alkyl of polymers prepared in Procedure B changed (e.g. feeding ratio equal to 12.5/12.5, obtained ratio equal to 15/10). Using different hydrophilic couplers (QI or J) similar results were obtained.

Secondly, the antibacterial properties of polymers with the same obtained ratio of hydrophilic/alkyl were compared. In Series **S-I** with hydrophilic/alkyl equal to 12.5/12.5, polymer prepared in hydrophilic first procedure (Procedure B) has the lowest MIC; In Series **S-II** with hydrophilic/alkyl equal to 15/10, polymer prepared in hydrophobic first procedure (Procedure C) has the lowest MIC. This result can be explained by the basic function principle of peptide or peptide like polymer with bacterial membrane.

6.5 Reference

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Chapter 7

Synthesis and Characterization of Amphiphilic Monodisperse Compounds and Poly(ethylene imine)s: Influence of their Microstructures on the Antibacterial Properties

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7.1 Introduction

Over the last decade, an increasing resistance against currently available antibiotics was reported worldwide[1-4]. For the seven major classes of known antibiotics resistance has developed within 1-4 years from the time of their clinical introduction[1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming a community-based as well as a hospital based problem[5]. Hospital and community-acquired *Escherichia coli* infection will pose an increasing challenge to health care systems in the years to come[6]. Since many developmental antibiotics are based on existing classes of antimicrobial agents, it has been of great concern that antibiotic resistance will develop quickly and notably through cross-resistance with existing agents. Finding new classes of therapeutic agents with novel mechanisms of action to prevent society from entering a "post-antibiotic age" is an urgent need.

Antimicrobial peptides represent ancient host defense effector molecules present in organisms and are a major component of innate immunity that is lethal to a broad spectrum of pathogens, such as Gram-positive and Gram-negative bacteria, fungi and protozoa, and the development

of bacterial resistance to peptides is difficult[7-9]. They are in most cases non-toxic to mammalian cells, because of the fundamental difference between microbial and mammalian cells[10, 11]. Certainly, there are also exceptions, for example melittin, a very nonspecific cytolytic peptide that attacks all lipid membranes, here the amount of hydrophobic groups is decisive[12, 13]. A high level of amphipathicity and with this a high hydrophobic moment, is correlated with high hemolytic activity (destruction of mammalian red blood cells)[14]. Related to this, recently it was shown that in the case of block and random amphiphilic copolymers with similar monomer compositions the random copolymers exhibit a higher hemolytic activity than block copolymers[15, 16].

Bacterial membranes are organized in such a way that they contain relatively large amounts of exposed anionic lipids, while the outer leaflet of the membranes of plants and animals is composed mainly of lipids with no net charge. According to literature the majority of the antibacterial peptides kill bacteria by causing an impact on components of the cell wall, e.g. by permeabilizing the cell membrane and forming pores. As a result, vital ion gradients are dissipated and small essential molecules (e.g., ATP) leak out of the cell[17]. Currently, there are three main models of peptide-induced pore formation or disruption of phospholipid bilayers: (i) the barrel-stave model; (ii) the toroid (or wormhole) model; (iii) the carpet-mechanism[18, 19]. In all models, the interference of this class of membrane active molecules with the cell envelope of microbes is based on rather general structural principles such as amphipathic properties and cationic charges. This mechanism is driven first by electrostatic forces between the cationic groups of the antimicrobial molecules and the negatively charged sites of the lipid bilayer membrane, and then by Van der Waals interactions between the hydrophobic moieties of both antimicrobial molecules and phospholipids[10, 20-24].

Mimicking the physicochemical principles of antimicrobial peptides, a lot of antimicrobial synthetic polymers have been developed in the past[25-34]. These antimicrobial synthetic

polymers are in demand for industrial applications like disinfection and antimicrobial coatings[35]. Ultimately, they may be useful as antibiotics even in pharmaceutical applications, depending on the range of targeted organisms, and the absence of toxicity, e.g., measured via the degree of hemolytic activity. However, the general mechanism mentioned above will be influenced by many parameters – membrane composition, nature of the antimicrobial molecules and environmental parameters (temperature, salt concentration). Small changes in natural host-defense peptides, as well as in cationic amphiphilic polymers, can have a dramatic influence on their antibacterial and hemolytic activities[15, 21, 22, 36-38]. In our previous work, a novel approach for the preparation of amphiphilic antimicrobial polymers based on a one-step multifunctionalization of poly(ethylene imine) (PEI) with functionalized cyclic carbonates has been reported and the influence of the hydrophobic/hydrophilic balance (the ratio of alkyl to cationic groups), the length of the hydrophobic groups and the molecular weight of the functional PEIs to the polymer antibacterial properties has been systematically studied[39, 40].

The goal of this study was to prepare amphiphilic compounds and polymers with different microstructures. Two series of amphiphilic compounds were prepared: one with alkyl chains directly linked to cationic groups (Series **B-I**) and the other with alkyl chains and cationic groups linked by a spacer (Series **B-II**). In addition three kind of amphiphilic poly(ethylene imine)s were prepared: in the first - **PEI-I** - cationic and hydrophobic grafts are randomly linked to PEI; in the second - **PEI-II** - amphiphilic grafts of **B-I** type are linked to PEI; and in the third - **PEI-III** - amphiphilic grafts of **B-II** type are linked to PEI (Figure 1).

The antibacterial properties of these amphiphilic compounds and polymers were tested against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) and the hemolytic activity of selected compounds was determined. The influence of the distribution of cationic/hydrophobic grafts, the alkyl chain length, and

the bacteria inoculum sizes on their minimum inhibitory concentration (MIC) was compared and discussed.

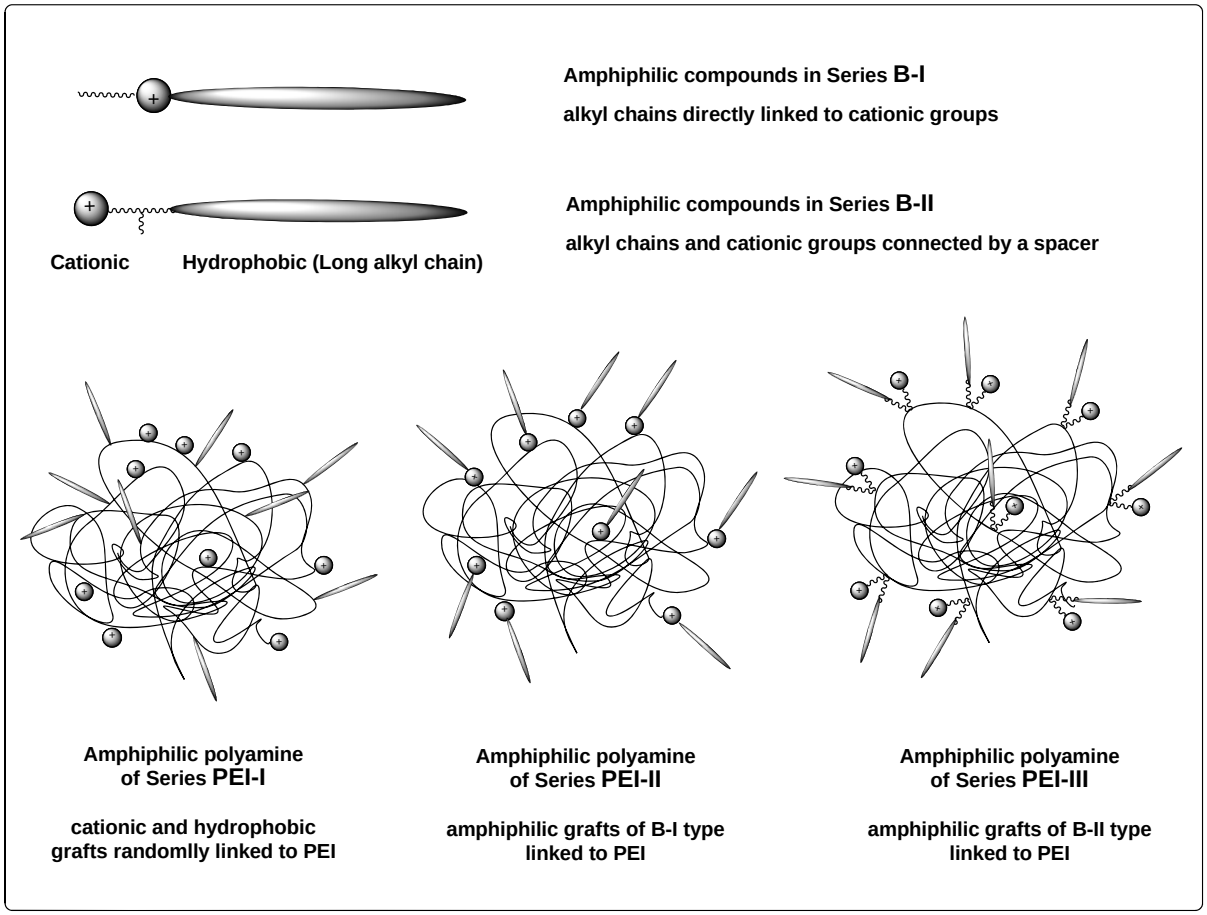


Figure 1: Schematic representation of the microstructure of the two kinds of amphiphilic compounds in series **B-I** and **B-II** and the three kinds of amphiphilic poly(ethylene imine)s in series **PEI-I**, **PEI-II**, and **PEI-III**.

7.2 Experimental Part

7.2.1 Materials

Starting materials were used as received: 1-octylamine (Aldrich, 99 %), 1-decylamine (Fluka, 98 %), 1-dodecylamine (Acros Organics, 98 %), 1-tetradecylamin (Fluka, 98.5 %), 1-

hexadecylamine (Fluka, 99 %), 1-octadecylamine (Aldrich, 97 %), triethylamine (Aldrich, 99.9 %), 3-dimethylamino-1-propylamine (Aldrich, 99 %), iodomethane (Aldrich, 99 %), 1-bromooctane (Aldrich, 99 %), 1-bromodecane (Aldrich, 98 %), 1-bromododecane (Aldrich, 97 %), 1-bromotetradecane (Aldrich, 97 %), 1-bromohexadecane (Aldrich, 97 %), 1-bromooctadecane (Aldrich, 97 %), phenyl chloroformate (Acros Organics, 99 %), pyridine (Fluka, 99.8 %), (2-oxo-1,3-dioxolan-4-yl)methyl chloroformate (Aldrich, 90 %), *N*,*N*-dimethylformamide (DMF, Acros Organics, 99 %), poly(ethylene imine) (PEI, M_w~25 000 by light scattering (LS) M_n~10 000 by gel permeation chromatography (GPC), water-free, Aldrich), tetrahydrofuran (Acros, 99.99 %, THF), and dichlormethane (Merck, 99.5 %, CH_2Cl_2). For antimicrobial activity amphiphilic compounds and amphiphilic polymers were tested against the Gram-negative bacterium *Escherichia coli* (DSMZ 498) and the Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (DSMZ 347) bacteria.

7.2.2 Bacterial culture

The nutrient solution pH 7 contained 5 g peptone, 3 g meat extract per L bidistilled water. Phosphate-buffered saline (PBS) contained 9.0 g NaCl per L 0.1 M disodium hydrogenophosphate/sodium dihydrogenophosphate buffer solution adjusted to pH 6.5. All solutions were autoclaved for 15 min at 120 °C prior to use.

7.2.3 Instruments

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-400 FT-NMR spectrometer at 400 MHz and 101 MHz, respectively. Chloroform-*d* ($CDCl_3$) and dimethyl sulfoxide-d_6 (DMSO-d_6) were used as solvents and tetramethylsilane (TMS) served as an internal standard. Thermal shaker (Heidolph), Microplate Incubator/Reader Genios Pro (Tecan), Photometer

Cary 100 (Varian), Drying oven and Clean Bench (Kendro) were used for the antimicrobial assay.

7.2.4 Measurements

7.2.4.1 Sample preparation for fluorescence measurements

Stock solutions of amphiphilic compounds or polymers in Milli-Q water with concentration of 10.0 g/L were prepared.

For laser light scattering, the stoppered flasks were allowed to equilibrate overnight at room temperature.

To get sample solutions for fluorescence, a known amount of pyrene in acetone was added to each series of 25.0 mL volumetric flasks and the acetone was evaporated. The amount was chosen to give a pyrene concentration in the final solution of 1.2×10^{-7} M. To the first flask of the series, a measured amount of stock solution was added, followed by Milli-Q water. A polymer dilution series from 1.5×10^{-5} to 1.0 g/L was prepared with constant pyrene concentration. The stoppered flasks were allowed to equilibrate overnight at room temperature.

7.2.4.2 Fluorescence measurements

For fluorescence measurements 2 mL of stock solution was placed in 1.0 cm × 1.0 cm cuvette. All spectra were run on air-saturated solutions using a LS50 Luminescence Spectrophotometer (Perkin Elmer) with 90° geometry using slit openings of 2.5 nm. Excitation spectra were registered at emission wavelength (λ_{em}) of 390 nm and were accumulated with an integration time of 0.5 s/0.5 nm. A critical micelle concentration (CMC) or critical aggregation concentration (CAC) for the solubilization of pyrene was determined

based on the shift of the (0,0) bands in the pyrene excitation spectrum as described elsewhere[41].

7.2.4.3 Antibacterial assessments of polymer solutions: Growth test

A suspension of strains with known amount of colony forming units (CFU; *E. coli*: 6 × 10^7 CFU/mL) was incubated at 37 °C in nutrient solution to which the respective antimicrobial polymer was added in different concentrations. The growth of the bacteria was followed over 20 h by measuring every 30 min the optical density at 612 nm using a microplate reader/incubator. From these data a minimal inhibitory concentration (MIC) was ascertained as the concentration of the test substance at which a log 4 reduction of the growth of the inoculated bacteria was observed relative to a control sample. This test does not discriminate whether the substance is bactericidal or bacteriostatic. Experiments were triplicated.

7.2.4.4 Measurement of hemolytic activity

Hemolytic activity was assessed according to literature[16]. Human erythrocytes (red blood cells (RBC), 0, Rh positive) 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 $5x10^8$/mL RBC. Solutions of defined polymer concentration were pipetted into 500 µL of the stock solution; the final amount of RBC being $1,2x10^6$ RBC/mL. The RBC were exposed for 60 min at 37 °C; centrifuged thereafter (4000 rpm, 10 min) and the absorption of the supernatant was determined at 414 nm. As reference solutions (i) PBS for determining spontaneous hemolysis and (ii) 1 % 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}).

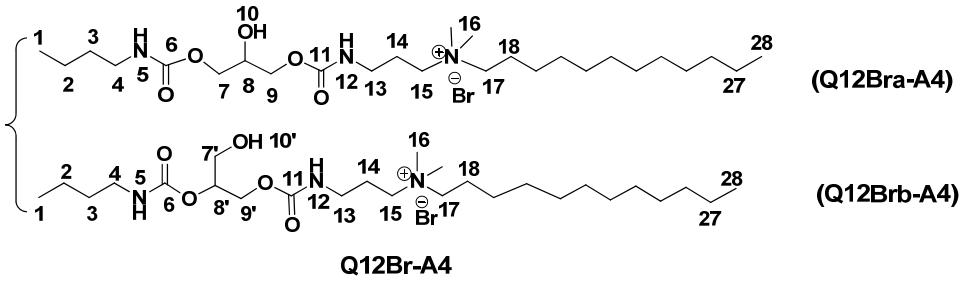
7.2.5 Synthesis

(2-Oxo-1,3-dioxolan-4-yl)methyl, N-octylcarbamate (**A8**), (2-oxo-1,3-dioxolan-4-yl)methyl, N-decylcarbamate (**A10**), (2-oxo-1,3-dioxolan-4-yl)methyl, N-dodecylcarbamate (**A12**), (2-oxo-1,3-dioxolan-4-yl)methyl, N-tetradecylcarbamate (**A14**), (2-oxo-1,3-dioxolan-4-yl)methyl, N-hexadecylcarbamate (**A16**), and (2-oxo-1,3-dioxolan-4-yl)methyl, N-octadecylcarbamate (**A18**), 3-*N*,*N*,*N*-trimethyl-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonyl-amino)propan-1-ammonium iodide (**QI**), *N*,*N*-dimethyl-*N*-(3-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonylamino)propyl)octan-1-ammonium bromide (**Q8Br**), *N*,*N*-dimethyl-*N*-(3-(((2-oxo-1,3-dioxolan-4-yl)methoxy)carbonylamino)propyl)dodecan-1-ammonium bromide (**Q12Br**) were prepared and characterized according to literature[39, 40].

General procedure for the synthesis of amphiphilic compounds in Series B-I

Amphiphilic coupler **QYBr** (Y= 10, 14, 16, 18, in Series **B-I**) was prepared in a similar way as **Q8Br** and **Q12Br** as described in literature[40]. After purification, **QYBr** was dissolved in chloroform. Butylamine (1.2 eq.) was added to the solution and the mixture was heated at 70 °C for 18 h. Then part of the solvent was evaporated and the residue was precipitated in diethyl ether/pentane mixture (v/v = 1/1). After removal of the solvent by decantation and drying in vacuum, viscous oil (**Q8Br-A4**) or slightly yellow gel (**Q18Br-A4**) was obtained.

^{1}H and ^{13}C NMR signal assignment of **Q12Br-A4** is presented exemplarily. The signal assignment of the other compounds in this series was performed accordingly.



Q12Br-A4

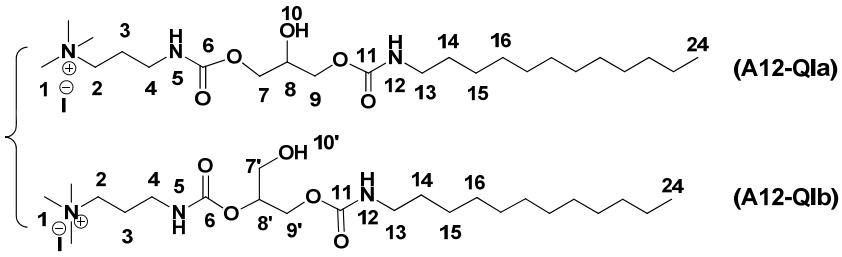
^{1}H NMR of **Q12Br-A4** ($CDCl_3$, 400 MHz): δ = 0.84-0.95 (m, 6 H, $H^{1, 28}$), 1.18-1.42 (m, 20 H, $H^{2, 19-27}$), 1.44-1.53 (m, 2 H, H^{3}), 1.67-1.80 (m, 2 H, H^{18}), 2.02-2.16 (m, 2 H, H^{14}), 3.13 (dd, 2 H, ^{3}J = 6.7 Hz, ^{2}J = 13.2 Hz, H^{4}), 3.20-3.37 (m, 8 H, $H^{13, 16}$), 3.37-3.47 (m, 2 H, H^{15}), 3.64-3.80 (m, 2 H, H^{17}), 4.00-4.30 (m, 5 H, $H^{7-9, 9'}$), 4.48 (br., 1 H, $H^{10'}$), 4.80 (s, 1 H, $H^{10'}$), 4.90 (m, 1 H, $H^{8'}$), 5.62-5.77 (m, 1 H, H^{5}), 6.60-6.90 (m, 1 H, H^{12}) ppm

^{13}C NMR of **Q12Br-A4** ($CDCl_3$, 101 MHz): δ = 13.7, 14.1 (2 C, $C^{1, 24}$), 19.9 (C^{2}), 22.6 (C^{27}), 22.8, 23.1 (C^{14}), 26.3 (C^{3}), 29.2, 29.3, 29.4, 29.5, 29.6, 31.8 (C^{18-24}), 31.9 (C^{25}), 34.1 (C^{26}), 37.8 (C^{13}), 40.8 (C^{4}), 51.0 (C^{16}), 53.4 (C^{17}), 62.5 ($C^{7'}$), 64.5 ($C^{9'}$), 65.8 (C^{15}), 66.1 ($C^{7,9}$), 68.1 (C^{8}), 73.5 ($C^{8'}$), 156.5, 156.6, 156.7, 157.1 ($C^{6, 11}$) ppm

General procedure for the synthesis of amphiphilic compounds in Series B-II

Hydrophobic coupler **AY** (Y = 8, 10, 12, 14, 16, or 18) and 3-dimethylamino-1-propylamine with a ratio of 1/1.2 were dissolved in THF and the mixture was stirred for 24 hours at 70 °C. After removal of the solvents and excess of 3-dimethylamino-1-propylamine in vacuum, the residue was dissolved in dichloromethane. Iodomethane (1.1 eq.) was added to the solution under vigorous stirring. The mixture was stirred for 18 hours at room temperature, and then, diethyl ether and pentane were added to the solution (dichlormethane/diethyl ether/pentane = 1/1/1). The mixture was stirred for additional 24 hours; during this time the product precipitated. After filtration and drying in vacuum (~10^{-2} mbar), the ammonium iodide salt **AY-QI** (Y = 8, 10, 12, 14, 16, or 18) was obtained as a slightly yellow solid.

^{1}H and ^{13}C NMR signals assignment of **A12-QI** is presented exemplarily. The signal assignment of the other compounds in this series was performed accordingly.

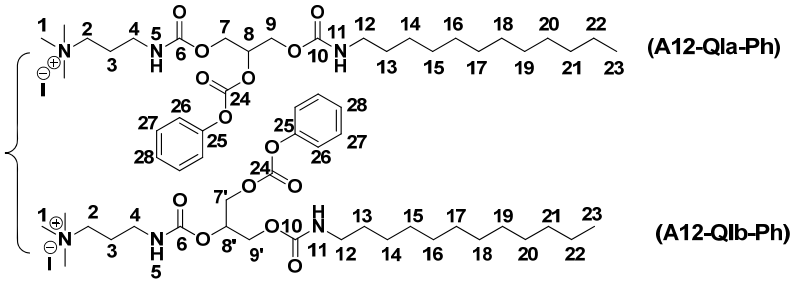


A12-QI

^{1}H NMR of **A12-QI** (DMSO-*d*6, 400 MHz): δ = 0.83 (t, ^{3}J = 6.7 Hz, 3H, H^{24}),1.10-1.30 (m, 18 H, H^{15-23}), 1.30-1.40 (m, 2 H, H^{14}), 1.75-1.90 (br, 2 H, H^{3}), 2.92 (dd, ^{3}J = 6.4 Hz, ^{3}J = 12.7 Hz, 2 H, H^{12}), 2.97-3.18 (m, 11 H, $H^{1,4}$), 3.25-3.35 (m, 2 H, H^{2}), 3.47 (m, 2 H, $H^{7'}$), 3.70-4.05 (m, 6 H, $H^{7-9,9'}$), 4.14 (dd, ^{2}J = 11.7 Hz, ^{3}J =3.3Hz, 1 H, $H^{9'}$), 4.50-4.80 (m, 1 H, $H^{8'}$), 4.80-4.95 (m, 1 H, $H^{10'}$), 5.00-5.20 (m,1 H, H^{10}), 6.70-7.40 (t, ^{3}J = 5.6 Hz, 2 H, $H^{5,11}$) ppm.

^{13}C NMR of **A12-QI** (DMSO-d_6, 101 MHz): δ = 13.9 (C^{24}), 22.1 (C^{23}), 23.1 (C^{3}), 26.2 (C^{14}), 28.70, 28.73, 28.99, 29.04, 29.3 (C^{15-21}), 31.3 (C^{22}), 37.3 (C^{4}), 40.2 (C^{13}), 52.16, 52.19, 52.23 (C^{1}), 59.8 ($C^{7'}$), 62.6 ($C^{9'}$), 63.3 (C^{2}), 64.9 (C^{7}), 65.1 (C^{9}), 66.9 (C^{8}), 72.7 ($C^{8'}$), 155.9, 156.1, 156.2 ($C^{6,11}$) ppm

Synthesis and NMR assignment of A12-QI-Ph



A12-QI-Ph

A12-Q (a/b) (12.90 g, 29.9 mmol) and pyridine (237 mg, 3 mmol) were dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C. A solution of phenyl chloroformate (5.62 g, 35.8 mmol) in CH_2Cl_2 (20 mL) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 18 h. After removal of the solvents the reaction

mixture was dissolved in CH_2Cl_2 (7 mL) and precipitated in pentane/ Et_2O (v/v = 2/1, 600 mL). The precipitation procedure was repeated for another two times to remove the excess of phenyl chloroformate. The product **A12-QHCl-Ph** was then dissolved in CH_2Cl_2 (200 mL) and washed three times with 10 %-Na_2CO_3 aqueous solution (3 x 50 mL) and one time with brine (50 mL). The organic phase was dried over sodium sulfate. After removal of the solvent the residue was dried in vacuum (10^{-2} mbar, 3 h, room temperature). The intermediate product **A12-Q-Ph** was obtained as a slightly yellow powder. Yield: 13.61 g (82 %).

A12-Q-Ph (a/b) (9.12 g, 16.54 mmol) was dissolved in THF (35 mL). At room temperature and under vigorous stirring, a solution of iodomethane (2.58 g, 18.19 mmol) in THF (15 mL) was added within 30 min. The mixture was stirred at room temperature for 20 hours. After removal of the solvents the reaction mixture was dissolved in CH_2Cl_2 (7 mL) and precipitated in pentane/ Et_2O (v/v = 2/1, 600 mL). The purification procedure was repeated for another two times to remove the excess iodomethane. The product was dried in vacuum (10^{-2} mbar, 3 h, room temperature). A slightly yellow powder was obtained. Yield: 9.64 g (84 %).

^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 0.86 (t, ^3J = 6.5 Hz, 3 H, H^{23}), 1.22-1.23 (m, 18 H, H^{14-22}), 1.36-1.50 (br., 2 H, H^{13}), 1.88-2.00 (br., 2 H, H^3), 2.90-3.04 (m, 2 H, H^4), 3.04-3.30 (m, 11 H, $H^{1,12}$), 3.38 (t, ^3J = 8.0 Hz, 2 H, H^2), 3.80-4.60 (m, 4 H, $H^{7,7',9,9'}$), 4.90-5.40 (m, 1 H, $H^{8,8'}$), 6.80-7.60 (m ,7 H, $H^{5,11,26-28}$) ppm.

^{13}C-NMR (DMSO-d_6, 101 MHz): δ = 13.9 (C^{23}), 22.0 (C^{22}), 26.2 (C^3), 27.2, 28.7, 28.8, 29.0 (C^{14-20}), 29.6 (C^{13}), 31.4 (C^{21}), 38.5 (C^4), 40.2 (C^{12}), 52.1 (C^1), 61.5, 62.0 ($C^{7,9}$), 63.3 (C^2), 66.8 ($C^{7',9'}$), 69.4 ($C^{8'}$), 75.3 (C^8), 121.1 (C^{27}), 127.5 (C^{28}), 129.7 (C^{26}), 149.5, 150.6 (C^{24}), 152.5, 152.8 (C^{25}) 155.2, 155.6 ($C^{6,10}$) ppm.

General procedure for synthesis of amphiphilic poly(ethyleneimine)s PEI-I/II/III

To a solution of PEI (M_w = 25 kDa) in DMF (20 mL) functional couplers were added (s.Table 1). The solution was stirred at 60°C or at room temperature for 20h or 3d. After removal of

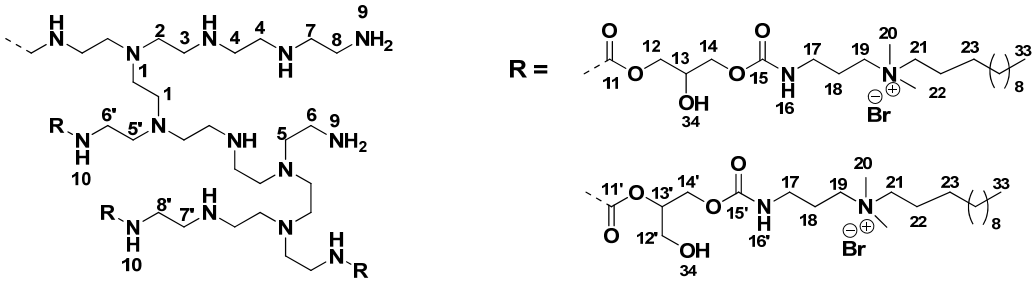
the solvent the residue was dissolved in MeOH (5 mL) and precipitated into Et_2O/pentane (600 mL, v/v = 1/2). The procedure was repeated for another two times. After drying in vacuum ($2 \cdot 10^{-2}$ mbar) at RT, a slightly yellow highly viscous material was obtained.

Table 1: Details of the reactions of PEI (M_w = 25 kDa) with functional couplers

No.	PEI (mg)	Functional couplers				Temperature	time	Yield (mg (%))
		QI (mg)	A12 (mg)	Q12Br (mg)	A12-QI-Ph (mg)			
PEI-I	**1000**	**1127**	**956**	**-----**	**-----**	**60 °C**	**3 d**	**2816 (91)**
PEI-II	**1000**	**-----**	**-----**	**1438**	**-----**	**60 °C**	**20 h**	**2140 (88)**
PEI-III	**1000**	**-----**	**-----**	**-----**	**2013**	**RT**	**20 h**	**2550 (92)**

PEI-I was characterized according to Ref[39, 42].

^{1}H and ^{13}C NMR assignment of polymer PEI-II

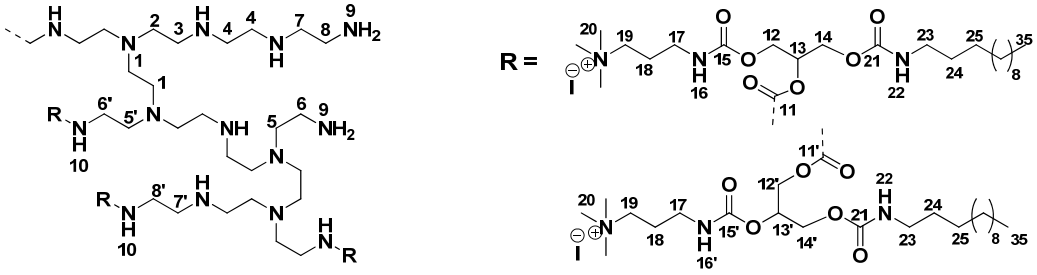


^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.83 (t, 3 H, ^3J = 6.6 Hz, H^{33}), 1.18-1.30 (br., 18 H, H^{23-32}), 1.62 (s, 2 H, H^{22}), 1.80 (s, 2 H, H^{18}), 2.20-2.80 (PEI backbone), 3.00 (s, 8 H, $H^{17, 20}$), 3.20-3.32 (br., 4 H, $H^{19, 21}$), 3.32-3.56 (br., H^{34} and PEI backbone), 3.74-4.04 (m, $H^{12-14, 14'}$), 4.10-4.20 (s, $H^{14'}$), 4.68-4.80 (s, $H^{13'}$), 6.80-8.70 (m, H^{16}) ppm

^{13}C NMR ($CDCl_3$, 4101 MHz): δ = 13.9 (C^{33}), 21.7 (C^{22}), 22.1 (C^{32}), 22.6 (C^{18}), 25.7 (C^{23}), 28.50, 28.69, 28.84, 28.93. 29.00 (C^{24-30}), 31.3 (C^{31}), 37.4 (C^{17}), 40.8, 47.0, 48.8 (PEI

backbone), 50.0 (C^{20}), 51.4, 52.3, 53.9 (PEI backbone), 59.8 ($C^{12'}$), 60.8 (C^{21}), 62.9 ($C^{14'}$), 63.1 (C^{19}), 65.1 ($C^{12, 14}$), 66.8 (C^{13}), 72.4 ($C^{13'}$), 155.7, 156.1, 156.2 ($C^{11, 15}$) ppm

^{1}H and ^{13}C NMR assignment of polymer PEI-III



^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.83 (t, 3 H, ^{3}J = 6.3 Hz, H^{35}), 1.14-1.30 (s, 18 H, H^{25-34}), 1.30-1.42 (m, 2 H, H^{24}), 1.76-1.90 (m, 2 H, H^{18}), 2.20-2.80 (PEI backbone), 2.80-2.98 (m, 2 H, H^{23}), 2.98-3.20 (s, 11 H, $H^{17, 20}$), 3.26-3.40 (m, 2 H, H^{19}), 3.70-4.30 (m, $H^{12, 12', 14, 14'}$), 4.68-4.80 (s, $H^{13, 13'}$), 6.80-7.90 (m, $H^{16, 16'}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 13.9 (C^{35}), 22.1 (C^{34}), 23.0 (C^{18}), 26.3 (C^{24}), 28.72 (C^{25}), 29.02, 29.31 (C^{26-32}), 31.3 (C^{33}), 37.4 (C^{17}), 40.2 (C^{23}), 47.2, 48.8 (PEI backbone), 52.2 (C^{20}), 54.1 (PEI backbone), 62.3, 62.6 ($C^{12, 14, 12', 14'}$), 63.3 (C^{19}), 69.7, 70.0 ($C^{13, 13'}$), 155.4, 155.7, 155.8 ($C^{11, 15, 21, 11', 15'}$) ppm

7.3 Results and discussion

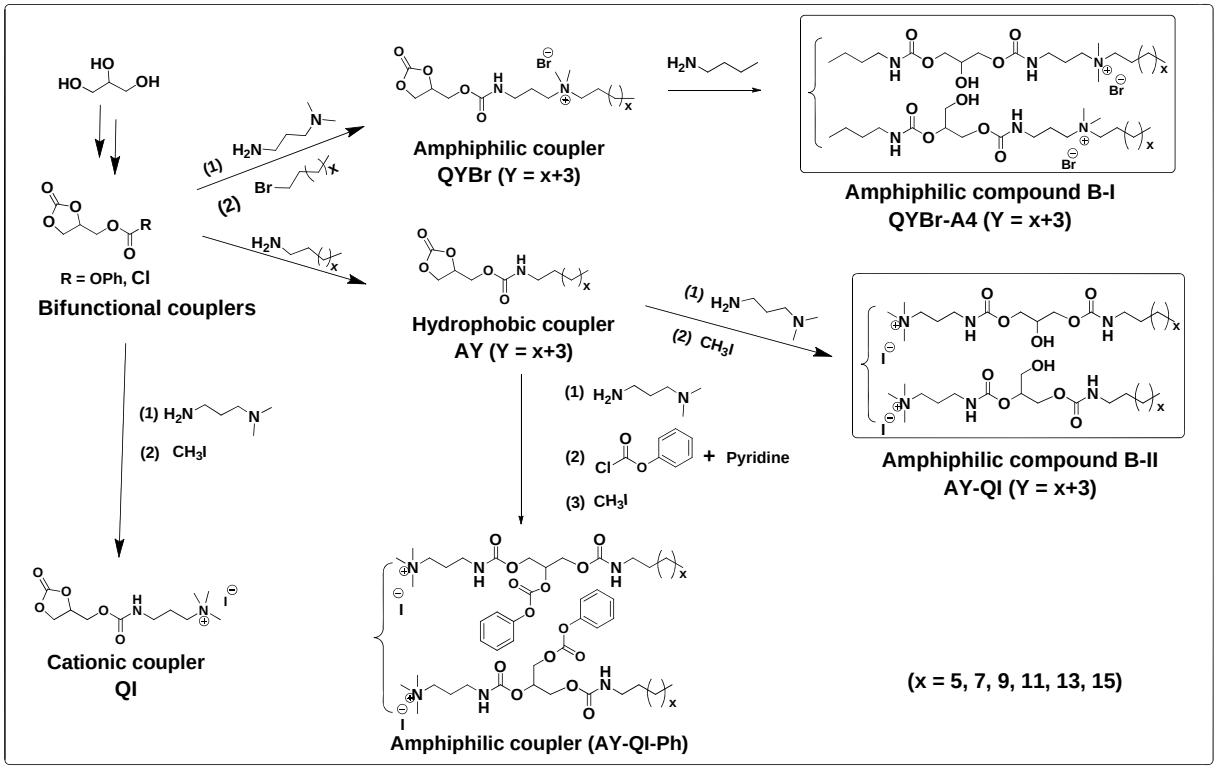
As already known from literature, amphiphilic monodisperse compounds or amphiphilic polymers with cationic groups and long alkyl chains are active against microorganisms. The cationic groups help to attach to the negatively charged bacterial membrane, and the long alkyl chains help to penetrate the lipid bilayer of the bacterial membranes. Using asymmetric bifunctional carbonate couplers[42-44] cationic and hydrophobic functional molecules with a

primary amine functional group can be selectively combined to amphiphilic compounds or amphiphilic polymers.

Synthesis of amphiphilic compounds in series B-I and B-II

Starting with glycerol, A,A'-asymmetrical bifunctional couplers (2-oxo-1,3-dioxolan-4-yl)methylphenyl carbonate (R = OPh) or (2-oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (R = Cl) were synthesized according to the literature[40, 42, 45] (Scheme 1). From these bifunctional couplers a cationic coupler **QI**, hydrophobic couplers **AY** (**Y = 8**, **10**, **12**, **14**, **16**, or **18**), and amphiphilic couplers **QYBr** (**Y = 8**, **10**, **12**, **14**, **16**, or **18**) were synthesized by reacting the couplers with the corresponding primary amines at lower temperature (T = 0 °C – RT), followed by quarternization with iodomethane for **QI** or an alkyl bromide for **QYBr**. The details on the synthesis and characterization of these couplers have been described in previous work[40]. The synthesis of **AY-QI-Ph** (Y = 12) was carried out in a similar way as for the amphiphilic coupler **A18-QI-Ph**, which was described in Ref[46]. Amphiphilic compounds in Series **B-I** (**QYBr-A4**) were obtained from **QYBr** by reaction with butylamine. Butylamine was used here to open the five-membered carbonate ring with formation of a urethane and adjacent hydroxyl group. Amphiphilic compounds in Series **B-II** (**AY-QI**) were prepared via ring opening reactions of hydrophobic couplers (**AY**) with 3-dimethylamino-1-propylamine, followed by quarternization with iodomethane. In the amphiphilic compounds **QYBr-A4**, the long alkyl chain is directly linked to the ammonium group and consequently they present a unity (entity). In amphiphilic compounds **AY-QI**, there is a spacer between the ammonium groups and the long alkyl chains – the coupler molecule - which facilitates a reorientation/movement of hydrophobic and ionic groups relative to each other (the two groups are not fixed, they can move along the spacer axis and rearrange).

In the ring-opening reaction of the five-membered carbonate ring of the functional couplers with buthylamine or 3-dimethylamino-1-propylamine, two regioisomers are obtained. The ratio of the two isomers in all cases is similar, secondary hydroxyl groups/ primary hydroxyl groups = 80/20 ~76/24.



Scheme 1: Synthesis of amphiphilic (**QYBr** and **AY-QI-Ph**), hydrophobic (**AY**), and cationic (**QI**) couplers and t synthesis of amphiphilic compounds in Series **B-I** (**A4-QYBr**) and **B-II** (**AY-QI**).

The ^{1}H and ^{13}C NMR spectra of **Q12Br-A4** and **A12-QI** (as an example for amphiphilic compounds in Series **B-I** and **B-II**, respectively) were shown in Figure 2 and Figure 3, respectively. In Figure 2a, the successful coupling of the 3-dimethylamino-1-propylamine and butylamine with the bifunctional coupler was confirmed by the signals at δ = 2.90-3.10 ppm for the CH_2 group next to the nitrogen (proton 4 and 13) and signals at δ = 6.70-7.30

ppm for the NH group of the urethanes (proton 5 and 12). The signals at δ = 0.70-1.40 ppm are characteristic for the alkyl chains. The signal at δ = 3.00 ppm is characteristic for the methyl ammonium group and the signals at δ = 3.20-3.30 ppm for methylene groups attached to the ammonium group. The formation of two isomers was proved with the signal at δ = 4.70 ppm characteristic for the isomer with a primary hydroxyl group (proton 8'). In Figure 2b, the signals at δ = 13-33, 50, 58-74, and 156 ppm are characteristic for alkyl chains, methyl ammonium groups, glycerol segments, and urethane groups, respectively. In Figure 3, the assignments were made in a similar way.

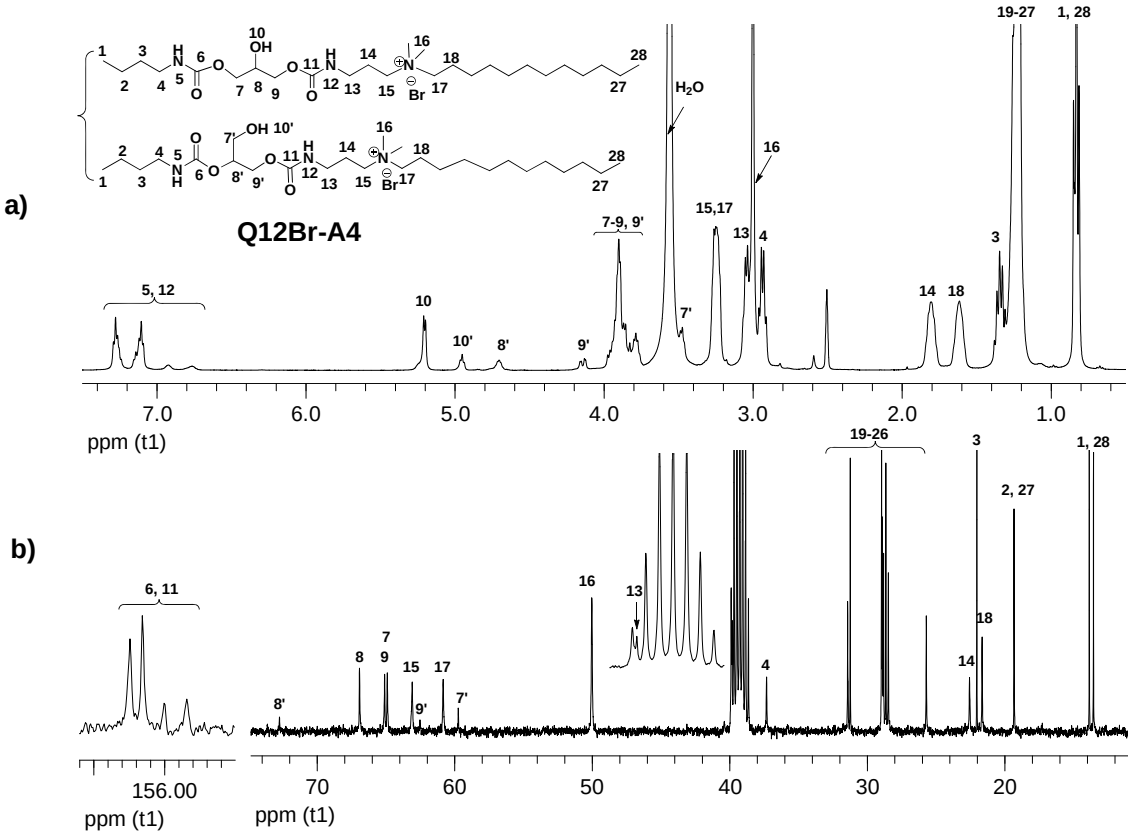


Figure 2: a) ^{1}H and b) ^{13}C NMR spectra of **Q12Br-A4** in DMSO-d_6; an example for **B-I**.

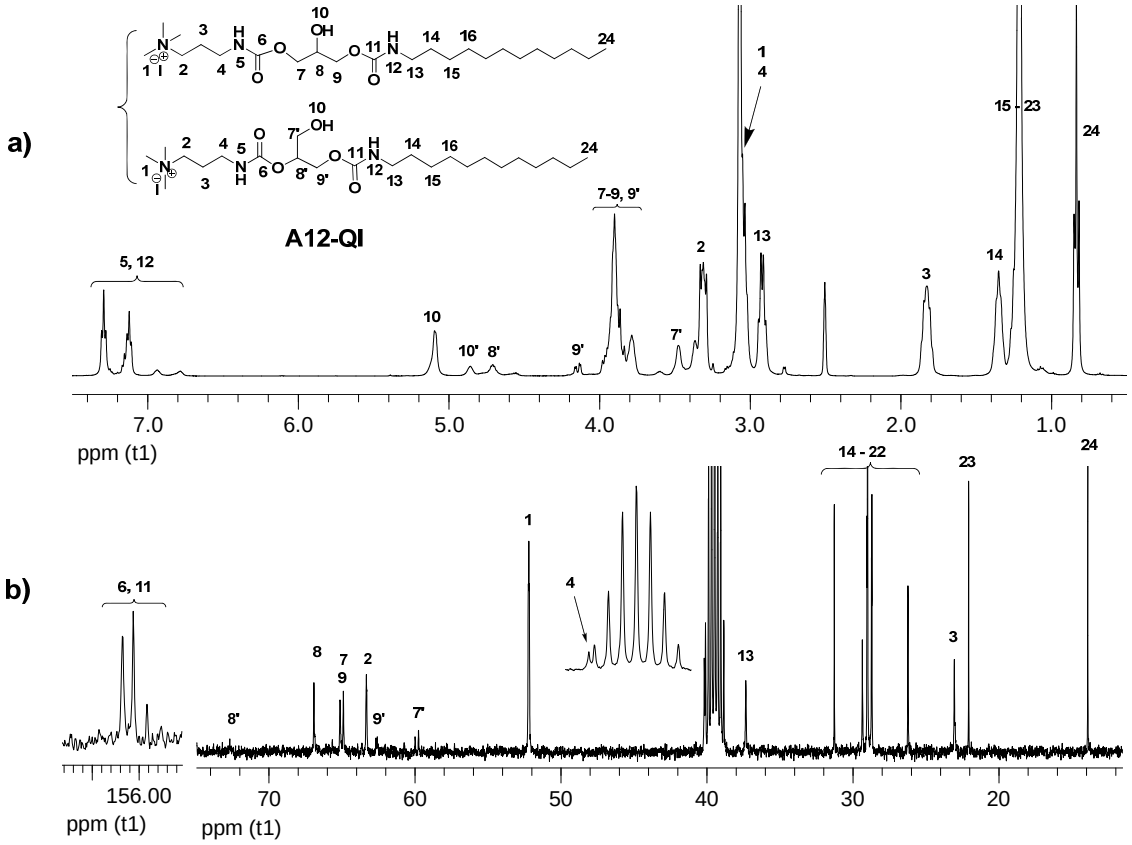
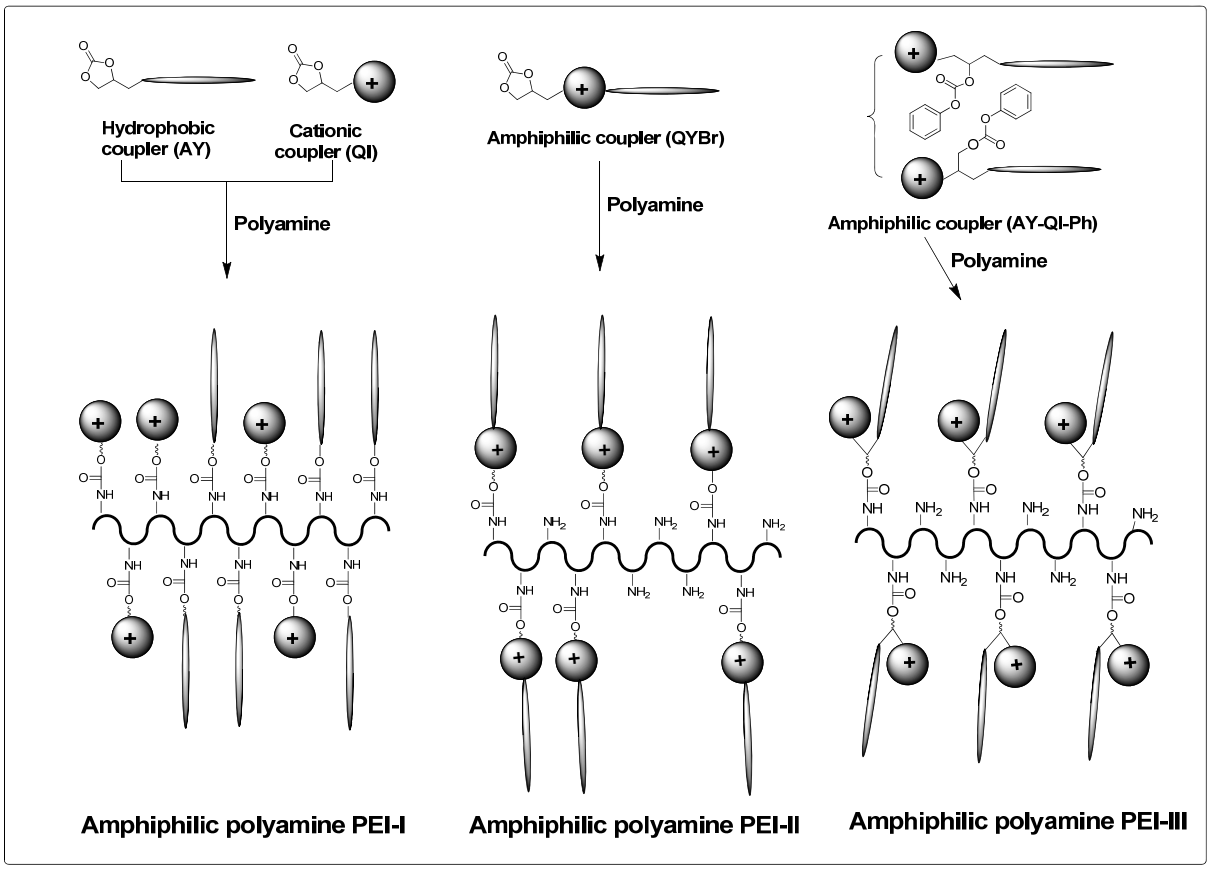


Figure 3: a) ^{1}H and b) ^{13}C NMR spectra of **A12-QI** in DMSO-d_6; an example for **B-II**.

Synthesis of amphiphilic poly(ethylene imine)s in series PEI-I, PEI-II and PEI-III

The synthesis of amphiphilic poly(ethyleneimine)s in series **PEI-I** with cationic and hydrophobic grafts randomly linked to the polymer backbone was achieved via one-pot addition of cationic (**QI**) and hydrophobic couplers (**AY**) to the branched poly(ethylene imine)[39, 42]. Amphiphilic poly(ethyleneimine)s in Series **PEI-II** with amphiphilic grafts of B-I type linked to the polymer backbone was obtained via adding amphiphilic couplers **QYBr** to poly(ethylene imine). The synthesis of amphiphilic poly(ethylene imine)s in Series **PEI-III** was achieved via addition of amphiphilic couplers **A12-QI-Ph** to poly(ethylene imine). In Scheme 2, the concept for the synthesis of these three kinds of amphiphilic polyamines is summarized. To be comparable with respect to their antibacterial properties with **PEI-I**,

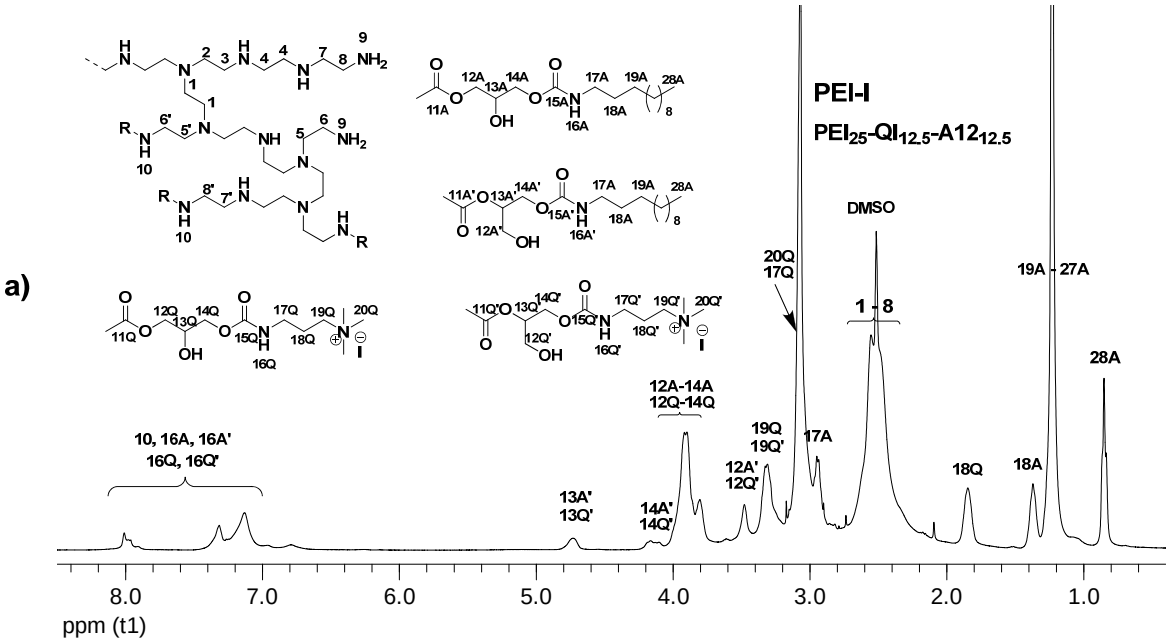
polymers **PEI-II** and **PEI-III** are only partially functionalized (50 % of the primary amine groups were converted). In all polymers the cationic, hydrophobic, or amphiphilic grafts are linked via urethane groups to the polymer backbone.



Scheme 2: Schematic representation of the structure of amphiphilic polyamines **PEI-I**, **PEI-II**, and **PEI-III** (The chemical structures of **AY**, **QI**, **QYBr** and **AY-QI-Ph** were shown in **Scheme 1**).

In this work, one example of each Series was synthesized and analysed with respect to the antibacterial properties. For hydrophobic grafts, dodecylamine was used. The branched PEI used had a molecular weight (M_w) of 25 kDa. The ratio of primary, secondary and tertiary amine groups is ca. 31:39:30, as determined by quantitative ^{13}C NMR spectroscopy. Using functional couplers bearing five-membered carbonate ring (**QI**, **A12**, and **Q12Br**) or phenyl

carbonate active groups (**A12-QI-Ph**) only the primary amine groups (ca. 30 % of the reapeating units) can be modified. It is known from previous study that full conversion of primary amine groups is difficult to be reached or will need longer reaction time. For the synthesis of **PEI-I**, a desired degree of functionalization of 25% was aimed for. In this case, 12.5 % of the primary amine groups were modified with hydrophobic grafts (**A12**), and 12.5 % with cationic grafts (**QI**). For the synthesis of **PEI-II** and **PEI-III**, amphiphilic couplers **Q12Br** and **A12-QI-Ph** were used, respectively. To be comparable with **PEI-I** with respect to the antibacterial properties, in the preparation of polymers **PEI-II** and **PEI-III** only 12.5 % of the primary amines of PEI were modified. The structure of the obtained polymers was characterized with ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectra of these three polymers with signal assignment are shown in Figure 4. The characteristic signals formed by ring-opening of the cyclic carbonate in polymers **PEI-I** and **PEI-II** (protons 12-14(A/Q) and 12'-14'(A/Q)) indicated the successful modification. In polymer **PEI-III**, the shape of these signals (protons 12-14 and 12'-14') changed because of the absence of hydroxyl groups. The assignments of signals were confirmed by 2D NMR.



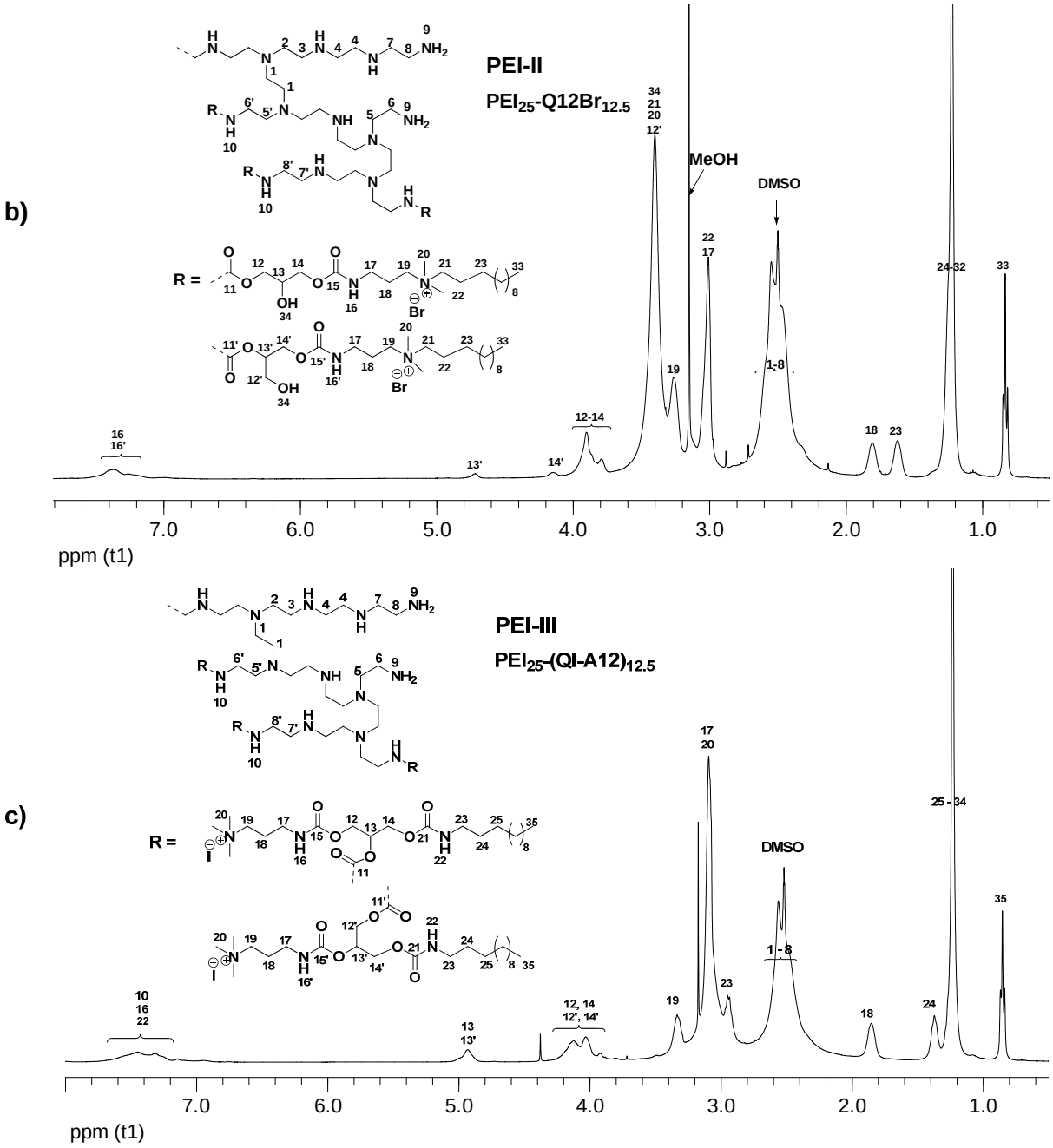


Figure 4: ^{1}H NMR spectra (DMSO-d_6, 400 MHz) of: a) **PEI-I** (**PEI_{25}-$QI_{12.5}$-$A12_{12.5}$**); b) **PEI-II** (**PEI_{25}-Q12Br$_{12.5}$**); and c) **PEI-III** (**PEI_{25}-(QI-A12)$_{12.5}$**).

Antimicrobial test with *Staphylococcus aureus* – the influence of the inoculum size on the MIC value

Staphylococcus aureus (*S. aureus*) is a facultatively anaerobic, Gram-positive coccus, being responsible for food poisoning, occurring as a commensal on skin, hosting phages, infecting

other tissues when barriers have been breached, and which can cause a severe disease staphylococcal scalded skin syndrome (SSSS). This type of bacteria can survive for hours to days, weeks, or even months on dry environmental surfaces depending on the respective strain. In this work, the antibacterial efficacy of compounds in Series **B-I**, **B-II**, and polymer **PEI-I**, **PEI-II**, and **PEI-III** against *S. aureus* was tested.

The experiment was carried out in a similar way as the reported procedures for *E. coli* and *B. subtilis* developed in our institute[47]. To establish a standard measurement condition, compounds in Series **B-I** were used as examples. The test to determine the minimum inhibitory concentration (MIC) was carried out with different inoculum sizes (from $6 \cdot 10^4$ cfu/mL to $6 \cdot 10^7$ cfu/mL). The results are summarized in Table 2.

Table 2: MIC of **B-I** against ***S. aureus*** obtained at different inoculum sizes.

Exp.	Molecules	MIC against *Staphylococcus aureus* [mg/mL]				
		$1 \cdot 10^8$ [cfu/mL]	**$6 \cdot 10^7$ [cfu/mL]**	**$6 \cdot 10^6$ [cfu/mL]**	**$6 \cdot 10^5$ [cfu/mL]**	**$6 \cdot 10^4$ [cfu/mL]**
HYC431	**Q8Br-A4**	n.d	0.2	0.1	0.1	0.1
HYC432	**Q10Br-A4**	n.d	0.05	0.03	0.03	0.03
HYC433	**Q12Br-A4**	0.02	0.01	0.01	0.01	0.01
HYC434	**Q14Br-A4**	n.d	0.003	0.002	0.002	0.002
HYC435	**Q16Br-A4**	n.d	0.003	0.002	0.002	0.001
HYC436	**Q18Br-A4**	n.d	0.003	0.002	0.002	0.001

From the results in Table 2, the amphiphilic compounds in Series **B-I** are all effective against *S. aureus*. Compounds with a longer alkyl chain have lower MIC values. For compounds with C8 to C14 alkyl chains, the MIC value decreased 3 ~ 5 times for the increasing of each 2 carbon atoms in all bacteria concentration. For compounds with C14 to C18 alkyl chain, the MIC value is the same except for the inoculum size of $6 \cdot 10^4$ cfu/mL. The MIC values of one

compound for all inoculum sizes tested are similar. Slight difference was noticed between the inoculum sizes of $6 \cdot 10^7$ cfu/mL and $6 \cdot 10^6$ cfu/mL for all compounds and between the inoculum sizes of $6 \cdot 10^5$ cfu/mL and $6 \cdot 10^4$ cfu/mL for compounds with C16 and C18 chains. As a conclusion, for the compounds **B-I** tested against *S. aureus* a minimum chain length of 14 carbon atoms in the hydrophobic residue is required and the compounds are highly effective also at larger inoculum sizes. To be comparable with the previously reported results on *E. coli* and *B. subtilis,* the MIC of **Q8Br-A4** – **Q18Br-A4** measured at an inoculum size of *S. aureus* of $6 \cdot 10^7$ cfu/mL, was used in the following sections.

Antimicrobial efficiency of B-I and B-II against Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative bacteria (*E. coli*)

According to the successful bifunctional coupler strategy, cationic amphiphilic compounds in Series **B-I** and **B-II** with different alkyl chain lengths were prepared in order to study their microstructure-property relationship against different bacteria. These compounds were grouped in two series by their microstructures: (i) Series **B-I**, alkyl chain directly linked to the cationic group; (ii) Series **B-II**, alkyl chain and cationic group connected by a spacer. The comparison was carried out in the same series with different alkyl chain length and between the series.

To better understand their self-assembly and solubility properties in the test environment, their critical micellization concentration (CMC) was determined by the fluorescence method in pure water, in PBS (pH = 6.8), and in nutrient liquor, respectively. Their MIC value against *E. coli*, *B. subtilis*, and *S. aureus* was determined by measuring the increase of optical density of inoculated nutrient solutions in the presence of the compounds at various concentrations.

The test bacteria inoculum size was 5 ~ $6 \cdot 10^7$ cfu/mL. The hemolytic activity was measured. The results are summarized in Table 3.

Table 3: Composition and properties of the amphiphilic compounds **B-I** and **B-II**

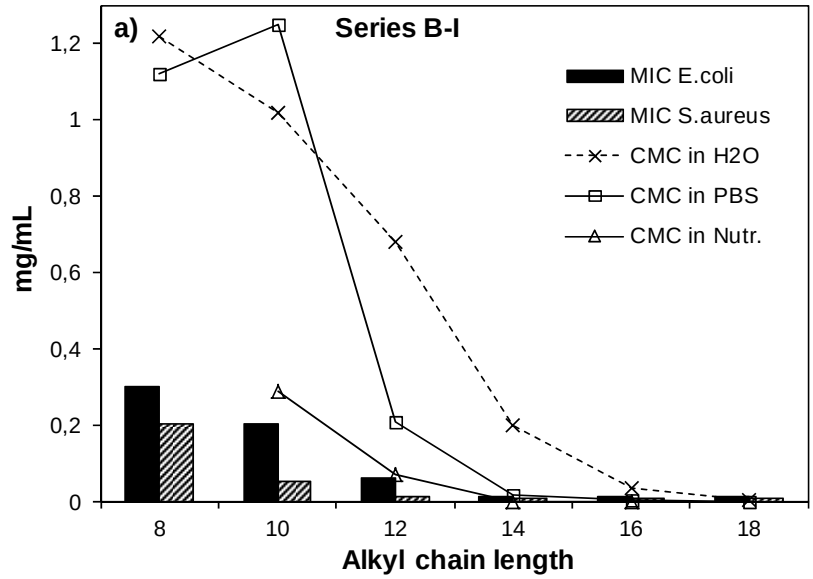
Series	Exp. No.	Molecules	CMC (H_2O) [mg/mL]	CMC (PBS)[a)] [mg/ml]	CMC (Nutr.)[b)] [mg/mL]	MIC [c)] *E. coli* [mg/ml]	MIC [d)] *B. subtilis* [mg/ml]	MIC [e)] *S. aureus* [mg/ml]	EC50 [f)] [mg/mL]
B-I	**HYC431**	**Q8Br-A4**	1.22	1.12	n.d	0.3	> 0.05	0.2	n.d.
	HYC432	**Q10Br-A4**	1.02	1.25	0.29	0.2	0.05	0.05	n.d.
	HYC433	**Q12Br-A4**	0.68	0.21	0.073	0.06	n.d	0.01	>0.05
	HYC434	**Q14Br-A4**	0.20	0.019	0.0031	0.008	n.d	0.003	0.022
	HYC435	**Q16Br-A4**	0.038	0.0063	0.0015	0.01	n.d	0.003	0.004
	HYC436	**Q18Br-A4**	0.0068	0.0041	n.d	> 0.01	n.d	0.003	n.d.
B-II	**HYC406**	**A8-QI**	0.66	n.d	n.d	> 0.5	> 0.1	> 0.5	n.d.
	HYC407	**A10-QI**	0.84	0.224	0.24	0.2	0.08	0.2	n.d.
	HYC408	**A12-QI**	0.45	0.035	0.031	0.02	0.02	0.02	0.028
	HYC409	**A14-QI**	0.1	0.0046	0.0045	0.06	0.004	0.006-0.008	0.007
	HYC410	**A16-QI**	0.05	0.0022	0.0033	> 0.5	0.005	0.006-0.008	0.004
	HYC411	**A18-QI**	n.d	n.d	n.d	> 0.5	0.02	0.05	n.d.

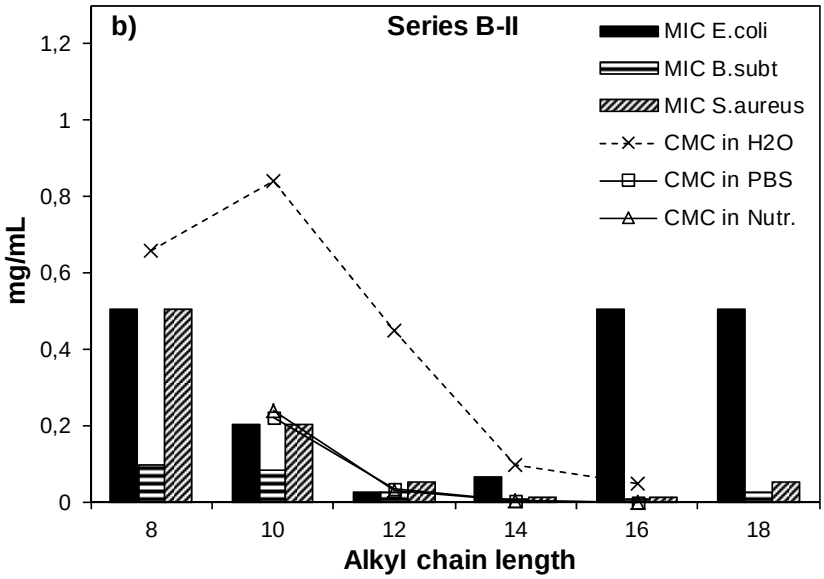
[a)] pH = 7; [b)] polymer in PBS solution diluted in 1/10 in nutrient; [c)] inoculum size = $5 \cdot 10^7$ cfu/mL; [d)] inoculum size = $5 \cdot 10^7$ cfu/mL; [e)] inoculum size = $5 \cdot 10^7$ cfu/mL; [f)] $1.2 \cdot 10^6$ RBC/mL

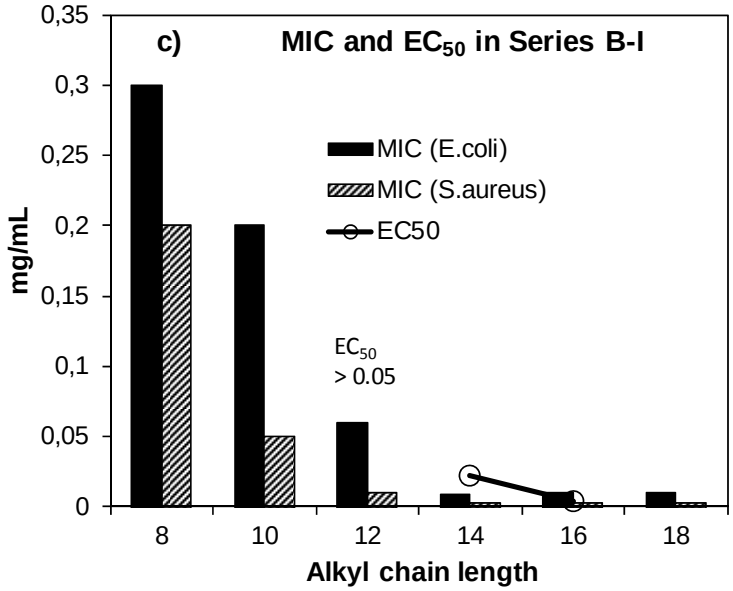
In Figure 5, the results of Table 3 are compared: i) For compounds of both series, in the short alkyl chain region (C8 and C10), the CMC values of the compounds in all media are higher than the MIC values; in the long alkyl chain region (C12 to C18), the values do not show the same trend (Figure 5a and Figure 5b). The compounds with lower CMC have lower MIC for the Gram-positive and Gram-negative bacteria, with exception of **A16-QI** (C16) and **A18-QI** (C18) against *E.coli* in series **B-II** (Figure 5b). ii) In Figure 5c, the MIC for *E.coli* of

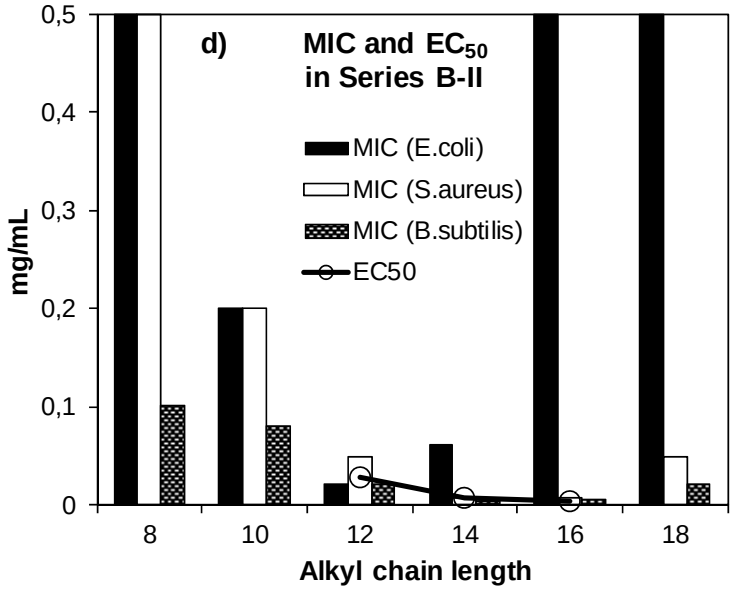
compounds in series **B-I** decreased from C8 to C14, and slightly increased from C14 to C18; the MIC value for *S. aureus* of compounds in series **B-I** decreased from C8 to C14, and stays the same from C14 to C18; these compounds are more efficient against *S. aureus* than against *E. coli*. For C12 to C16 the hemolytic potential (distruction of RBC membrane and liberation of haemoglobin) increases with increasing alkyl chain length. iii) In Figure 5d, the MIC for *E.coli* against compounds in Series **B-II** decreased from C8 to C12 and increased from C12 to C18; the MIC for *S. aureus* and for *B. subtilis* against compounds in series **B-II** decreased from C8 to C14, and slightly increased from C14 to C18. The compounds are more efficient against *B. subtilis* than against *S. aureus*. The compounds with C16 and C18 alkyl chain have similar efficacy as compounds with C12 and C14 alkyl chain against *B. subtilis* and *S. aureus,* while their efficacy against *E. coli* is much lower than compounds with C12 and C14 chains (much higher MIC). Like in Series **B-I** the hemolytic potential of compounds with C12 to C16 chains of Series **B-II** increases (similar efficacy at lower concentration) with increasing alkyl chain length; the hemolytic potential of compounds of Series **B-II** being higher compared with compounds of Series **B-I** as shown in Figure 5c. iv) The comparison of MIC of compounds in two series against *E. coli* is shown in Figure 5e. For compounds of Series **B-I** the MIC decreased with increasing alkyl chain length (from C8 to C14) and then slightly increased (from C14 to C18). The MIC of compound with C14 is the lowest. For the compounds in series **B-II**, the lowest MIC was obtained for the compound with C12 alkyl chain. The compounds with C16 and C18 alkyl chain are not effective. v) The comparison of MIC of compounds in two series against *S. aureus* is shown in Figure 5f. At the given inoculum size, the MIC of compounds in Series **B-I** decreased with the increasing alkyl chain length, the MIC values of compounds with C14 to C18 alkyl chain are the same; the MIC of compounds in series **B-II** decreased first and then increased slightly with increasing alkyl chain length. Compounds with C14 and C16 chain exhibit the lowest MIC. vi) For compounds with long alkyl chain (C14-C18), compounds in series **B-I** are efficient against

both *E. coli* and *S. aureus*, while for compounds in series **B-II**, they are efficient against Gram-positive bacteria, but not efficient against Gram-negative bacteria.






c)
MIC and EC50 in Series B-I
MIC (E.coli)
MIC (S.aureus)
EC50
EC50
> 0.05
mg/mL
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
8
10
12
14
16
18
Alkyl chain length


d)
MIC and EC50
in Series B-II
MIC (E.coli)
MIC (S.aureus)
MIC (B.subtilis)
EC50
mg/mL
0,5
0,4
0,3
0,2
0,1
0
8
10
12
14
16
18
Alkyl chain length

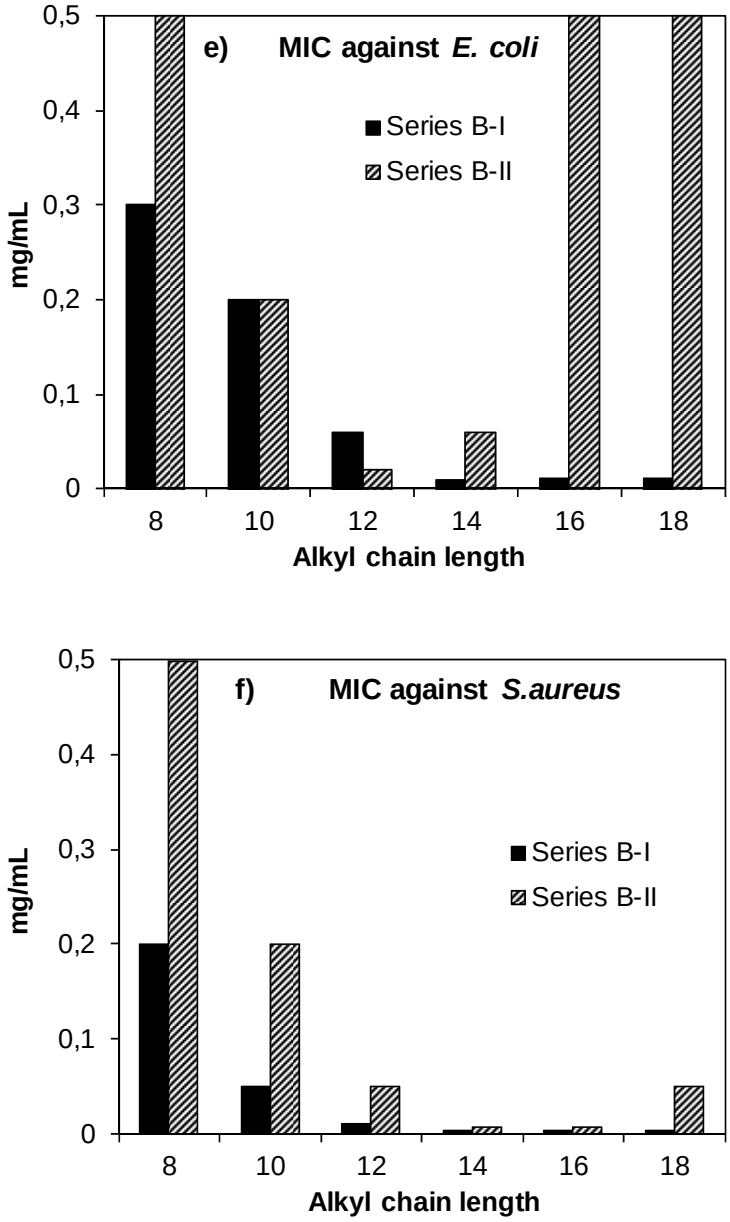


Figure 5: CMC, MIC and EC_{50} of compounds in Series **B-I** and Series **B-II**: a) comparison of CMC and MIC in Series **B-I**; b) comparison of CMC and MIC in Series **B-II**; c) MIC of Series **B-I** against *E. coli* and *S. aureus* and EC_{50}; d) MIC of Series **B-II** against *E. coli*, *S. aureus*, and *B. subtilis* and EC_{50}; e) MIC of Series **B-I** and **B-II** against *E. coli*; f) MIC of Series **B-I** and **B-II** against *S. aureus*

For compounds with C12 to C16 chains in Series **B-I** and **B-II** CMC values in PBS directly correlate with EC_{50} (Figure 6). For the compounds with C12 and C14 chains of Series **B-I**

MIC against *E. coli* and *S. aureus* is lower than the CMC and than EC_{50} for the compound with C16 chain it is in the same order of magnitude. For the compounds with C12 and C14 chains of Series **B-II** MIC against *E. coli* and *S. aureus* is equal or higher than CMC or EC_{50}. Thus, it seems likely that in Series **B-II** aggregates of the compounds contribute to the antibacterial effect and in Series **B-I** the single molecules.

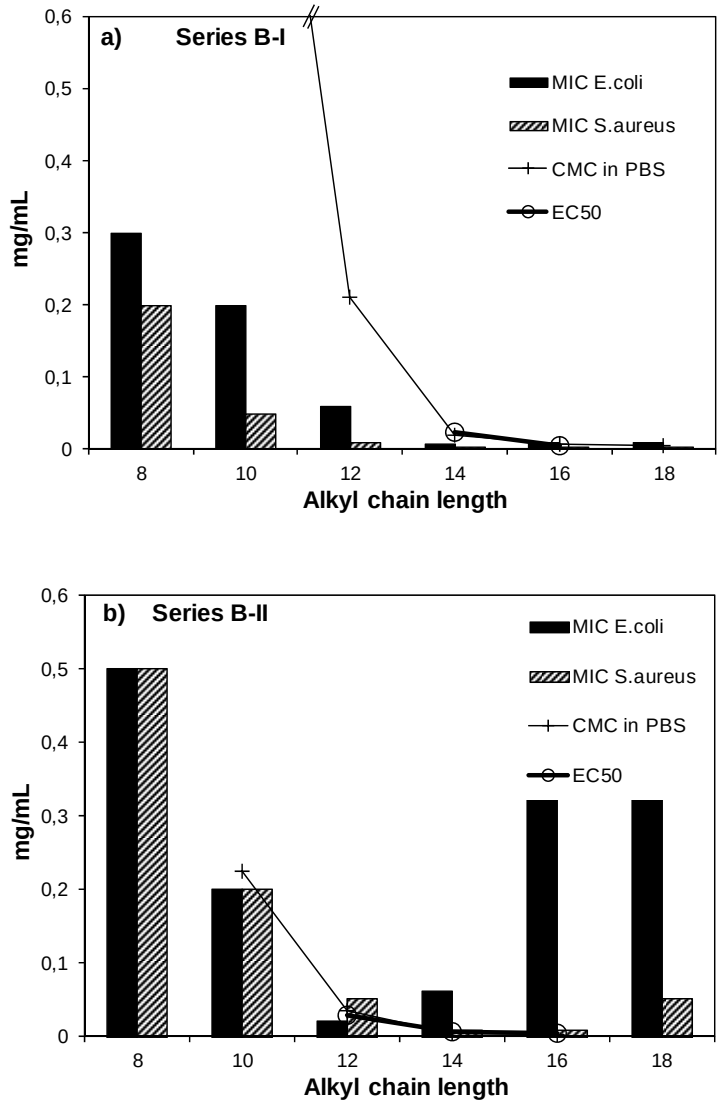


Figure 6: CMC, MIC and EC_{50} of compounds in Series **B-I** and Series **B-II**: a) comparison of CMC in PBS, EC_{50} and MIC against *E. coli* and *S. aureus* in Series **B-I**; b) comparison of CMC in PBS, EC_{50} and MIC against *E. coli* and *S. aureus* in Series **B-II**

Antimicrobial efficiency of PEI-I, PEI-II, and PEI-III against *E. coli* and *B. subtilis*

As schematically shown in Figure 1 and Scheme 2, polymers **PEI-I**, **PEI-II**, and **PEI-III** have the same amount of cationic and hydrophobic groups but different microstructure. For polymer **PEI-I**, 25 % of amine groups (the primary amine groups) were modified, for polymer **PEI-II** and **PEI-III**, 12.5 % of primary amine groups were modified. Polymers similar to **PEI-II** and **PEI-III** but with 25 % of modified amine groups were also prepared. However, they are not soluble in PBS buffer solutions. For this reason, only polymers **PEI-II** and **PEI-III** with 12.5% of modified primary amine groups were used in this study. Polymer **PEI-I** does not exhibit solubility problems in water, in PBS buffer solution, and in nutrient solutions.

The MIC values of polymers **PEI-I**, **PEI-II**, and **PEI-III** against *E. coli* and *S. aureus* were measured under the same conditions with the same bacteria inoculum size. The results are summarized and listed in Table 4. For the MIC measurement, a low start concentration was used for both cases. **PEI-II** polymers showed the highest efficacy against both Gram-positive and Gram-negative bacteria. They have the lowest MIC values against *E. coli* and *S. aureus*. Polymer **PEI-III** has slightly better antibacterial efficiency against *S. aureus* than polymer **PEI-I**. The MIC value of **PEI-III** against *E. coli* is much higher than the MIC value of **PEI-II** against *E. coli*. The hemolytic effect of **PEI-I** is lower compared with that of PEI-II and PEI-III. EC_{50} values of all 3 polymers are much lower than MIC values against *E. coli* and *S. aureus* and one order of magnitude lower than the critical association concentration in H_2O. Because of the solubility difficulty of the polymers **PEI-II** and **PEI-III**, their critical association concentrations in PBS were not measured. However, the latter indicates that aggregation of **PEI-II** and **PEI-III** is more likely to occur in PBS.

Table 4: Composition and properties of the functionalized samples obtained by reaction of **PEI** with the functional carbonate couplers.

Series	Exp.	Molecules	CMC (H_2O) [mg/mL]	CMC [a] (PBS) [mg/mL]	MIC *E. coli* [mg/mL]	MIC *B. subt.* [mg/mL]	MIC *S. aureus* [mg/mL]	EC50 [b] [mg/mL]
PEI-I	**NRP283**	**PEI_{25}-$QI_{12.5}$-$A12_{12.5}$**	0.019	0.010	0.3	0.04	0.1	0.0026
PEI-II	**HYC627**	**PEI_{25}-$Q12Br_{12.5}$**	0.104	n.d	0.06-0.1	n.d	0.03	<<0.001
PEI-III	**HYC628**	**PEI_{25}-$(QI-A12)_{12.5}$**	0.113	n.d	> 0.2	n.d	0.08	<0.001

[a] concentration onset of association, [b] $1.2 \cdot 10^6$ RBC/mL

Hemolytic activity of B-I, B-II and PEI-I, PEI-II, and PEI-III

The results of the concentration related hemolytic activity of selected monodisperse compounds of series **B-I** and **B-II** and of polymers **PEI-I**, **PEI-II**, and **PEI-III** are summarized in Table 5.

Up to a concentration of 0.01 mg/mL hemolysis is similar for compounds with C12 to C16 chain length of both Series **B-I** and Series **B-II**, compounds of Series **B-I** being slightly less hemolytic. However, at 0.05 mg/mL all selected compounds exhibit 100 % hemolysis except the compound with a C12 chain length of Series B-I which only shows 12 % hydrolysis.

In contrast to the anionic character of bacterial membranes the erythrocyte membrane is neutral, thus, interaction with amphiphilic compounds in the first line will be performed via hydrophobic residues. Interpreting the concentration related results of the hemolytic test for the functionalized polymers implies that the degree of amphipathicity and thus the hydrophobic moment[48] of the polymer aggregates of **PEI-I** is lower compared to **PEI-II**

and to **PEI-III**. This is also confirmed by the fact that the critical association concentration of **PEI-I** in H_2O is lower compared to that of **PEI-II** and **PEI-III**. It is assumed that the hydrophobic residues of **PEI-I** in water are buried to a higher extent than those of **PEI-II** and **PEI-III**.

Table 5: Hemolytic activity of different concentrations of functionalized **PEI** polymers and of selected compounds of Series **B-I** and Series **B-II**.

		Hemolysis in %		
	mg/mL	**PEI-I** PEI_{25}-$QI_{12.5}$-$A12_{12.5}$	**PEI-II** PEI_{25}-$Q12Br_{12.5}$	**PEI-III** PEI_{25}-$(QI\text{-}A12)_{12.5}$
polymers	**0,001**	0	68.9 ±4.2	59.2 ±6.0
	0,003	61.7 ±1.0	78.2 ±1.0	100 ±2.5
	0,005	69.3 ±0.6	96.9 ±0.2	100 ±0.4
	0,01	100 ±4.0	100 ±0.2	100 ±0.3
	0,05	100 ±0.2	100 ±0.7	100 ±0.03
	mg/mL	**HYC433** **Q12Br-A4**	**HYC434** **Q14Br-A4**	**HYC435** **Q16Br-A4**
B-I	**0,001**	1.4 ±0.5	5.2 ±2.6	4.3 ±0.2
	0,003	4.8 ±0.7	5,8 ±0.4	17,4 ±1.6
	0,005	5.5 ±2.3	7.2 ±0.6	100 ±0.3
	0,01	6.2 ±0.3	29.9 ±1.7	100 ±0.1
	0,05	12.3 ±0.8	100 ±0.5	100 ±0.1
	mg/mL	**HYC408** **A12-QI**	**HYC409** **A14-QI**	**HYC410** **A16-QI**
B-II	**0,001**	2.8 ±1.9	3.9 ±1.3	3.1 ±0.6
	0,003	2.3 ±1.3	10.2 ±1.1	21.5 ±1.2
	0,005	5.0 ±0.8	18.8 ±0.9	72.9 ±0.9
	0,01	7.4 ±1.5	99.7 ±0.4	100 ±0.7
	0,05	99.9 ±0.3	99.4 ±0.3	100 ±0.5

7.4 Conclusion

In this work, two Series of amphiphilic compounds (Series **B-I** and Series **B-II**) and three types of amphiphilic polymers (polymers **PEI-I**, **PEI-II**, and **PEI-III**) with different microstructure were successfully synthesized and characterized. Their antibacterial properties were tested against both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli*) bacteria.

For amphiphilic compounds in Series **B-I** and Series **B-II** against Gram-positive bacteria *S. aureus* or *B. subtilis*, compounds with longer alkyl chain (C14-C18) in both series are more efficient than compounds with short alkyl chain (C8-C12). For amphiphilic compounds in both series against Gram-negative bacteria *E. coli*, compounds with longer alkyl chain (C14-C18) in Series **B-I** are more efficient than compounds in this series with short alkyl chain (C8-C12), however, compounds with longer alkyl chain (C14-C18) in Series **B-II** are much less efficient than compounds in this series with short alkyl chain (C12).

For the three types of amphiphilic polymers, **PEI-II** and **PEI-III** with amphiphilic grafts have better antibacterial properties against *S. aureus* than **PEI-I** with randomly linked cationic and hydrophobic grafts. The MIC value of **PEI-III** against *E. coli* is also lower than that of **PEI-I,** the MIC value of **PEI-II** against *E. coli* being lowest. However the hemolytic activity of **PEI-II** and **PEI-III** is higher compared with **PEI-I**.

From all the results obtained it can be concluded that the microstructure of amphiphilic monodisperse compounds and of polymers have a significant influence on their antibacterial properties and hemolytic activity. Compounds with alkyl chains directly linked to cationic groups have a high antibacterial efficiency for long alkyl chain (C14-C18). Similar results were observed with amphiphilic poly(ethylene imine)s.

Because of their low solubility, polymers with a high degree of functionalization or longer alkyl chains could not be evaluated.

7.5 References

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Chapter 8

Multifunctional Poly(ethylene imine)

– New Laundry Detergent Ingredient

8.1 Introduction

Doing laundry is a common human activity with very long history. The symbol used by the ancient Egyptians to represent launderer was a pair of legs immersed in water. A detergent is a material used for cleaning, the earliest detergent substance was undoubtedly water. Mechanical agitation facilitates the removal of solid soil, and water dissolves the hydrophilic stains composed of, for example, sugar, salt, and certain dyes. It has long been known, however, that the washing power of water can be increased in various ways. Rainwater, for example, was found to be more suitable for washing than well water. Hot water also was found to have more washing power than cold, and certain additives seemed to improve any water's effectiveness. Even the ancient Egyptians used soda ash as a wash additive. Soda ash with sodium silicate, used to make the water softer, formed the basis of the first commercial detergent brand and appeared on the German market in 1878, Henkel's “Bleichsoda”. Used along with soap, as the oldest surfactants known to the Sumerians by ca. 2500 B.C., this product prevented the formation of inactive material known as “lime soaps”, and the laundry no longer suffered from a buildup of insoluble soap residues[1].

With the using of machine washing, there is a need for appropriate changes in the formulation of laundry detergents. Soap, notorious for its sensitivity to water hardness, was gradually

replaced by so-called synthetic surfactants with their more favorable characteristics. Synthetic surfactants with their reduced sensitivity to water hardness relative to soap have been generally accepted worldwide since 1940s. Another important step in the development of laundry detergents was replacement of builders such as sodium carbonate by complexing agents. The first complexing agents that were used were of the sodium diphosphate type, but these were replaced after World War II by the more effective sodium triphosphate. Nowadays the introduction of other ingredients for improving detergency performance, and their presence in formulations has been state of the art for modern laundry detergents.

However, along with the development also problems appeared. User of modern laundry machines sometimes experience accidental shrinkage of garments, especially when applying heat. For wool garments, this is due to scales on the fibers which on heat and stirring/mixing stick together. Other fabrics are stretched by mechanical forces during production, and shrink slightly when heated. Some clothes are "pre-shrunk" to avoid this problem. The high temperature of machine washing, which is necessary for hygiene (to kill bacteria) and for an improved washing performance, causes high energy consumption/high costs. Another common problem is color bleeding. For example, washing a red shirt with white underwear can result in pink underwear. Beside of this, the discovery that many surfactants could emerge unchanged even from a modern sewage treatment plant and thus enter surface waters led to the adoption of the first German Detergent Law in 1961, whose provisions took effect in 1964[2]. Manufacturers were subsequently enjoined from marketing any detergent or cleansing agent whose biodegradability fell below 80 % in a test devised by the Detergent Commission. The using of sodium triphosphates was also legislated in some countries. Therefore, detergents with good washing performance, color care effect (dye transfer inhibition), antibacterial activity and biodegradability at low temperature are very promising for energy saving and consumer friendliness.

One way to accomplish the needs is the use of multifunctional amphiphilic polymers. Functional polymers present a topic of increasing importance in polymer science since the end of the last century[3]. Functionalization of polymers is often used for compatibilisation of polymers blends, for modification of solubility properties, for the preparation of polymers for release systems and biomedical applications in general. One of the most important procedures applied to prepare multifunctional polymers is based on couplers. In the past, asymmetric A,A´-carbonate couplers were developed in our group (**Figure 1** and **2**, **A1** and **A3**)[4-7] to selectively combine monodisperse functional amine building blocks and polyamines within a single molecule, which are successfully used for the modification of branched poly(ethylene imine) (B-PEI)[8-11]. B-PEI is a commercial product containing primary, secondary and tertiary amine groups with a wide field of applications: paper production, dye fixation in textile processing, pigment dispersant, printing inks, lubricant in fiberglass production, primer for coatings, water treatment, metalworking, mining industry and pressure sensitive adhesives[12]. In microbiological studies, B-PEI is also mentioned as membrane permeabilizer[13] and is one of the most efficient nonviral vectors for gene delivery[14-16]. By introducing different amount of hydrophobic, hydrophilic and ionic functional groups, modified B-PEI can serve as polymeric surfactant. The special properties of B-PEI itself and the property of tailored modification make it a very attractive candidate as laundry detergent ingredient.

In this past, coupler (or linker) based multifunctional poly(ethylene imine)s (P) were synthesized and their properties as laundry detergent additives were determined: primary washing performance against oily stains, soil release effect, color care effect, fixation of aromas, anti-graying effect and shape retention effect were tested at Henkel[17-27] and best candidates toward different performances were selected. For a better understanding of the results obtained and for a better design of new properties, in this work, structure-property

relationships were established toward one series of tested polymers. Using TGA and DSC their self-assembling property in bulk was investigated. The thin film properties of multifunctional B-PEIs on a silicon wafer were evaluated by ellipsometry, contact angle measurement and atomic force microscopy (AFM). The aggregation properties in water were determined by dynamic light scattering and cryo-SEM measurement. The antibacterial effects toward Gram positive and Gram negative bacteria were also tested. To meet the German Detergent Law, the biodegradability of these compounds was studied.

8.2 Experimental parts

Materials: Functional couplers and multifunctional B-PEI used (**Figure 1**) were synthesized as described in the literature[7-9, 11, 28]. It should be mentioned that substituted cyclic five-membered carbonate, i.e. functional couplers, react selectively with primary amine groups; secondary and tertiary amine groups may act as catalyst, but are not converted.

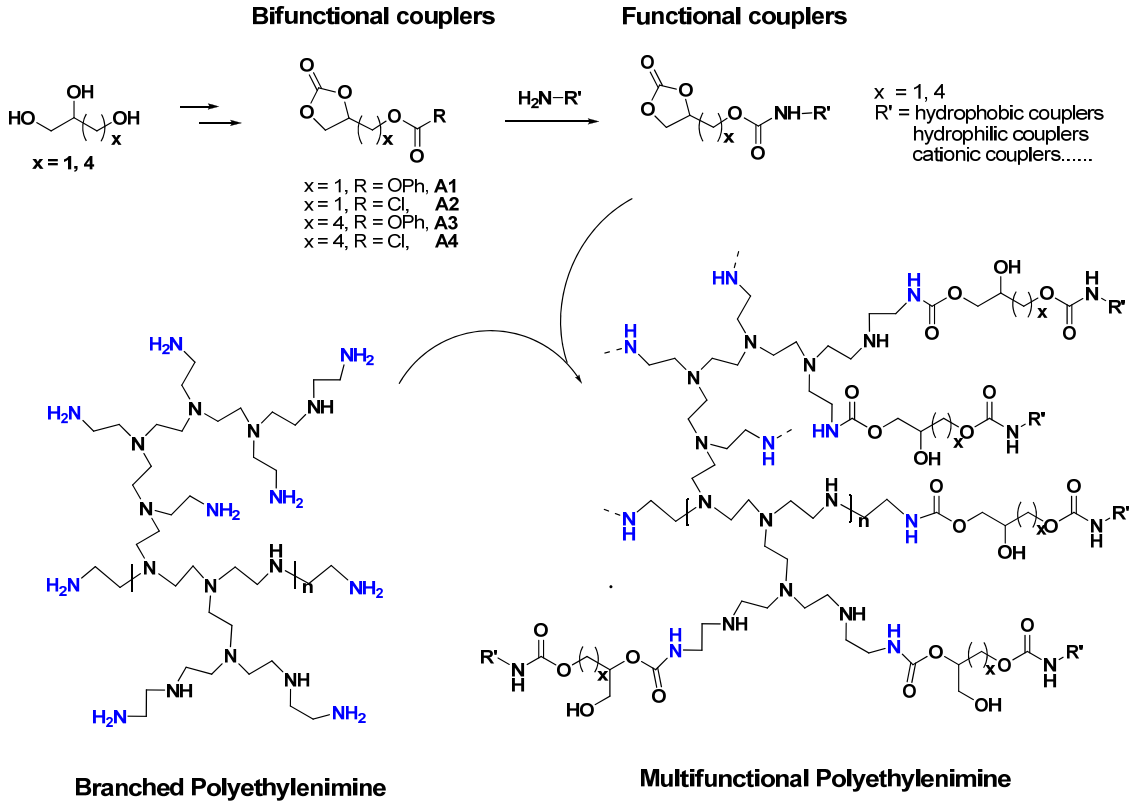


Figure 1: Principle of the synthesis of multifunctional B-PEI.

Starting with glycerol and 1,2,6-hexantriol the couplers **A1**, **A2**, **A3**, and **A4** were obtained according to the literature by reaction with either dimethyl carbonate and phenyl chloroformate (for **A1** and **A3**)[4-7] or with phosgene (for **A2** and **A4**)[29]. The preparation of multifunctional B-PEI comprises two steps: the first step – reaction of the bifunctional coupler with a functional amine – occurs via a substitution reaction to obtain the functional coupler. In the second step the functional coupler is reacted with B-PEI via a ring-opening reaction of the five-membered cyclic carbonate group by the primary amine groups of B-PEI.

To generate a wide range for modifications, different kinds of functional couplers were synthesized based on the bifunctional couplers (**A1** – **A4**) as summarized in **Figure 2** and **3** with their structures and abbreviations used in the later text[28].

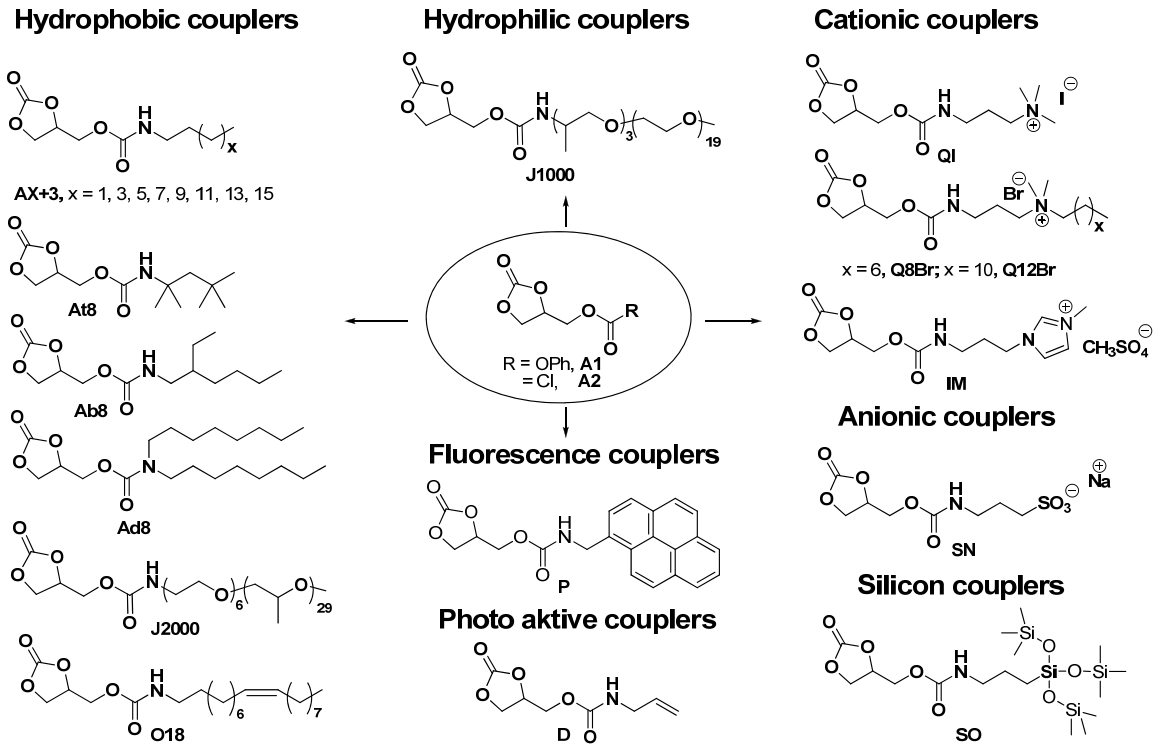


Figure 2: Summary of functional couplers based on (2-oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate (**A1**) or (2-oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (**A2**).

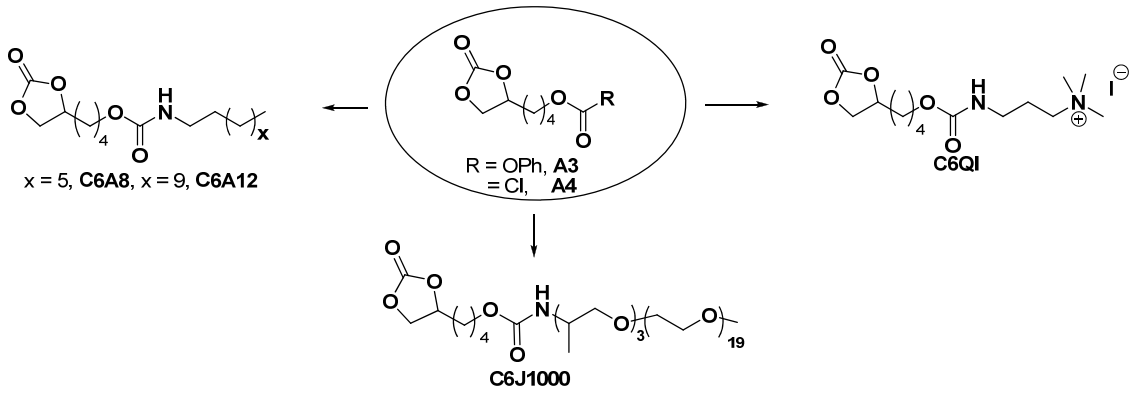


Figure 3: Summary of functional couplers based on 4-(2-oxo-1,3-dioxolan-4-yl)butyl phenyl carbonate (**A3**) or 4-(2-oxo-1,3-dioxolan-4-yl)butyloxy chloroformate (**A4**).

A wide range of modified polymers were synthesized and tested toward washing tests[27]. In this article a series of the tested polymer will be discussed (**Table 1**). In the following text, the index of **PEI** indicates the molecular weight of B-PEI in kDa, while the extension, for

example **QI_5**, denotes that 5 mol-% of the amino groups of B-PEI have been reacted with quaternary ammonium coupler (**QI**).

Table 1: Summary of multifunctional B-PEIs discussed here.

No.	Notation
P1	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$
P2	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$Ad8_{14.9}$-$P_{0.2}$
P3	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A12_{14.9}$-$P_{0.2}$
P4	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A18_{14.9}$-$P_{0.2}$
P5	$PEI_{1.3}$-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$
P6	PEI_{750}-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$
P7	PEI_{25}-$C_6QI_{4.95}$-$C_6J1000_{4.95}$-$C_6A8_{14.9}$-$P_{0.2}$

Microorganisms: For antibacterial activity the polymers were tested against the Gram negative bacterium *Escherichia coli* (DSMZ 498) and the Gram positive bacterium *Bacillus subtilis* (DSMZ 347).

Bacterial culture: The nutrient solution pH 7 contained 5 g peptone, 3 g meat extract per L bidistilled water. Phosphate-buffered saline (PBS) contained 8.0 g NaCl per L 0.1 M disodium hydrogenophosphate/sodium dihydrogenophosphate buffer solution adjusted to pH 6.5. Soft agar was prepared from 10.0 g peptone, 3.0 g meat extract, 6.0 g NaCl and 7.0 g agar-agar per L bidistilled water. All solutions were autoclaved for 15 min at 120 °C prior to use.

Antibacterial assessments of polymer solutions: Thermal shaker (Heidolph), Microplate Incubator/Reader Genios Pro (Tecan), Photometer Cary 100 (Varian), Drying oven and Clean Bench (Kendro) were used for the anti-bacterial assay. The minimal inhibitory concentration (MIC) value for antibacterial assessments were determined as described elsewhere[8, 11, 30].

Atomic Force Microscopy (AFM): A scanning force microscope (Nanoscope Multimode IIIa, Digital Instruments, Santa Barbara, CA), silicon cantilevers (Nanosensors, Wetzlar, Germany) with typical resonance frequencies around 300 kHz and spring constants around 40 N/m were applied. Structure analysis was done with the Nanoscope 1.10. The samples for measurements were prepared by dip coating from aqueous solution of polymers with a weight concentration of 5 g/L on silicon wafer. The AFM images were taken in tapping mode.

Contact angle: G40 contact angle measuring instrument (Krüss GmbH) using the sessile drop method. The temperature was maintained at 20 ± 0.1°C by means of a digital thermostat.

Cryo scanning electron microscopy (cryo-SEM): Hitachi S-4800

Differential Scanning Calorimetry (DSC): Netzsch DSC 204 'Phoenix' differential scanning calorimeter in nitrogen atmosphere with heating and cooling rates of 10 K/min.

Dynamic light scattering: Nano-ZS, Model ZEN3600, zetasizer (Malvern). Polymer was dissolved in Milli-Q water with a concentration of 10 mg/mL at a constant temperature of 25°C at an angle of 173°.

NMR: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DPX-300 FT NMR spectrometer at 300 MHz and 75 MHz, respectively.

Thermogravimetric analyses (TGA): TG 209 with a TA System Controller TASC 414/2 from Netzsch. The measurements were performed in nitrogen atmosphere with a heating rate of 10 K/min.

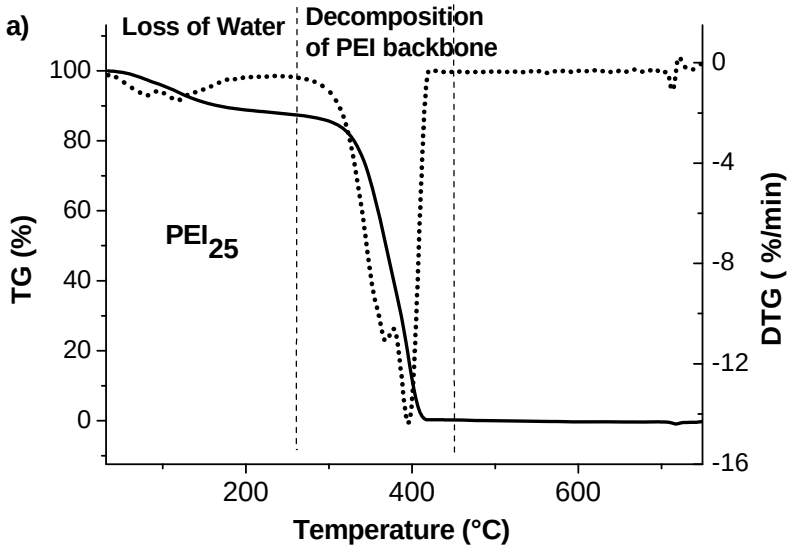
8.3 Results and disscusion

8.3.1 Structure – property relationship

In the following the properties of different polymers in bulk, in water solution and in thin films will be presented. Structure properties relationships are important for understanding and designing of new polymers.

8.3.1.1 Influence of the alkyl chains (hydrophobic building blocks) on the properties of multifunctional B-PEI

The thermal properties of the multifunctional B-PEI were determined using thermo gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The decomposition of **P1** (**Figure 4c**) shows three characteristic steps: (i) above 100 °C residual water is liberated; (ii) then at ca. 250 °C decomposition of the grafts is observed and (iii) at 350 °C the B-PEI backbone decomposes. This proposed mechanism of the decomposition is supported by analyzing $\mathbf{PEI_{25}}$ as one model (**Figure 4a**) and **C12-QI** (**Figure 4b**) as the second model. (**C12-QI** is a coupling product obtained by the reaction of the corresponding amines with coupler A1)[7]. In **Table 2** the onset for decomposition, the highest rate of the decomposition and the mass loss are summarized.


a)
Loss of Water
Decomposition
of PEI backbone
PEI25
TG (%)
DTG (%/min)
Temperature (°C)
100
80
60
40
20
0
0
-4
-8
-12
-16
200
400
600

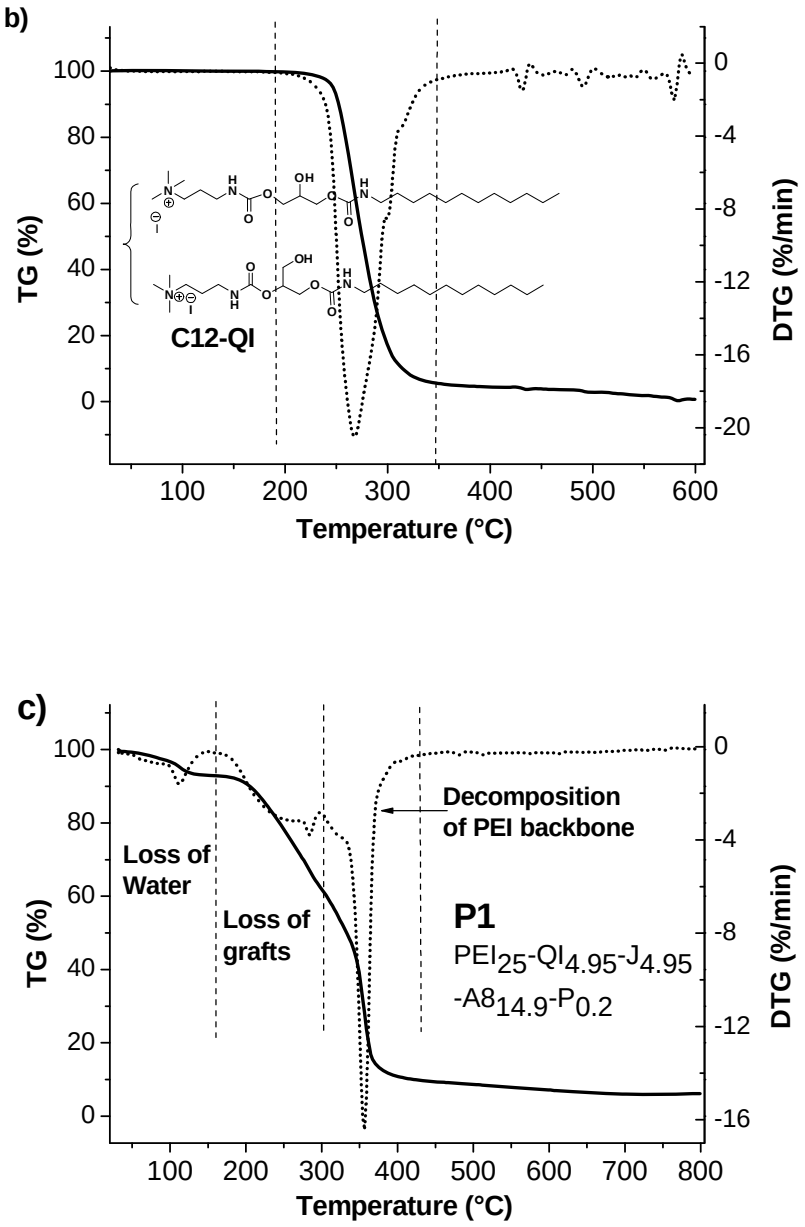


Figure 4: Amount (–) and rate (···) of loss in weight of: a) **PEI_{25}** (B-PEI, Mw = 25 kDa), b) **C12-Q**, and c) **P1**.

Table 2: Decomposition of **PEI_{25}** (B-PEI, Mw = 25 kDa), **C12-QI**, and **P1**.

No.	Loss of water			Loss of grafts			Decomposition of B-PEI backbone		
	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)
PEI_{25}	63.3	122.3	11.79	-	-	-	343.9	395.3	87.89
C12-QI	-	-	-	248.4	267.7	95.61	-	-	-
P1	93.7	112	7.18	213.4	283.9	30.24	345.7	356.2	52.51

Based on the results obtained for **P1**, the DSC of these polymers was measured between -100 °C and 190 °C with heating and cooling rate of 10 K/min (**Figure 5**). Because of the high mass fraction of JEFFAMINE® (PEO/PPO), all multifunctional B-PEIs show the characteristic melting point of JEFFAMINE® as the dominating thermal transition[31, 32]. In most of the DSC traces of the multifunctional B-PEIs, the Tg of the backbone cannot be seen, and the melting point of urethane groups which is expected at ca. 100 °C is also not visible, an exception is polymer **P4** with C18 alkyl chain. Here, besides the melting of the JEFFAMINE® segments at ca. 26 °C, the melting of the alkyl chains is observed up to 60 °C and the melting of the urethane group induced by hydrogen bond[33] is observed at ca. 110 °C. In the cooling traces of the **P4** all transition are observed: the crystallization of the urethane, the crystallization of the alkyl chains and the crystallization of the PEO chains. In all other samples, only parts of these transitions are observed.

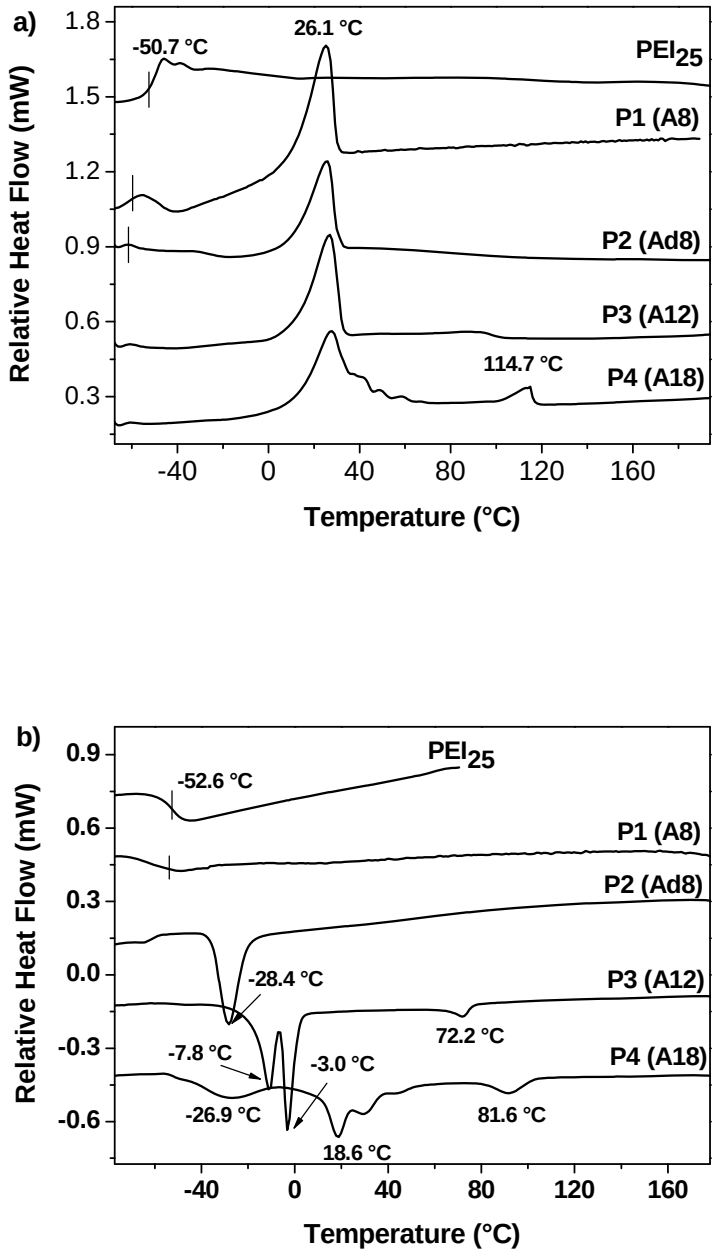


Figure 5: DSC traces of unmodified B-PEI (**PEI_{25}**) and multifunctional B-PEIs with different alkyl chains (**P1, P2, P3,** and **P4**): a) second heating; b) second cooling

Based on the thermal analysis, it can be concluded that the multifunctional B-PEIs prepared show self-organization in bulk, induced by the crystallizable side chain fragments.

The properties of thin films prepared on silicon wafers were studied next. Thin films were prepared from water solution of B-PEI and multifunctional B-PEIs in concentration of 10 g/L by dip coating. The hydrophilicity of the films was determined by contact angle measurements and the film thickness by ellipsometry (**Table 3**). All films are hydrophilic except those prepared from **P4**, which is hydrophobic. By annealing the films at 60°C for 30 min, no changing of hydrophilicity was observed except for **P4**, the hydrophobicity of which decreased. The thickness of the polymer films was quite similar, in a range of 13-14 nm.

Table 3: Contact angle and thickness of thin films prepared from B-PEI and modified B-PEI on silicon wafers.

	Treatment	PEI_{25}	P1	P2	P3	P4
Contact angle	Before annealing	< 20 °	< 20 °	< 20 °	23 °	94 °
	After annealing	< 20 °	< 20 °	25 °	< 20 °	54 °
Thickness (nm)	without annealing	14.4	13.4	14.4	13.5	13.1

Polymer thin films were further investigated via atomic force microscopy (AFM). Thin films were prepared in a similar way as for contact angle measurement from aqueous polymer solutions with a concentration of 5 g/L. Multifunctional B-PEIs with different alkyl chains show different morphologies as a result of microphase separation. The height images of different polymer films are given in **Figures 6-9**

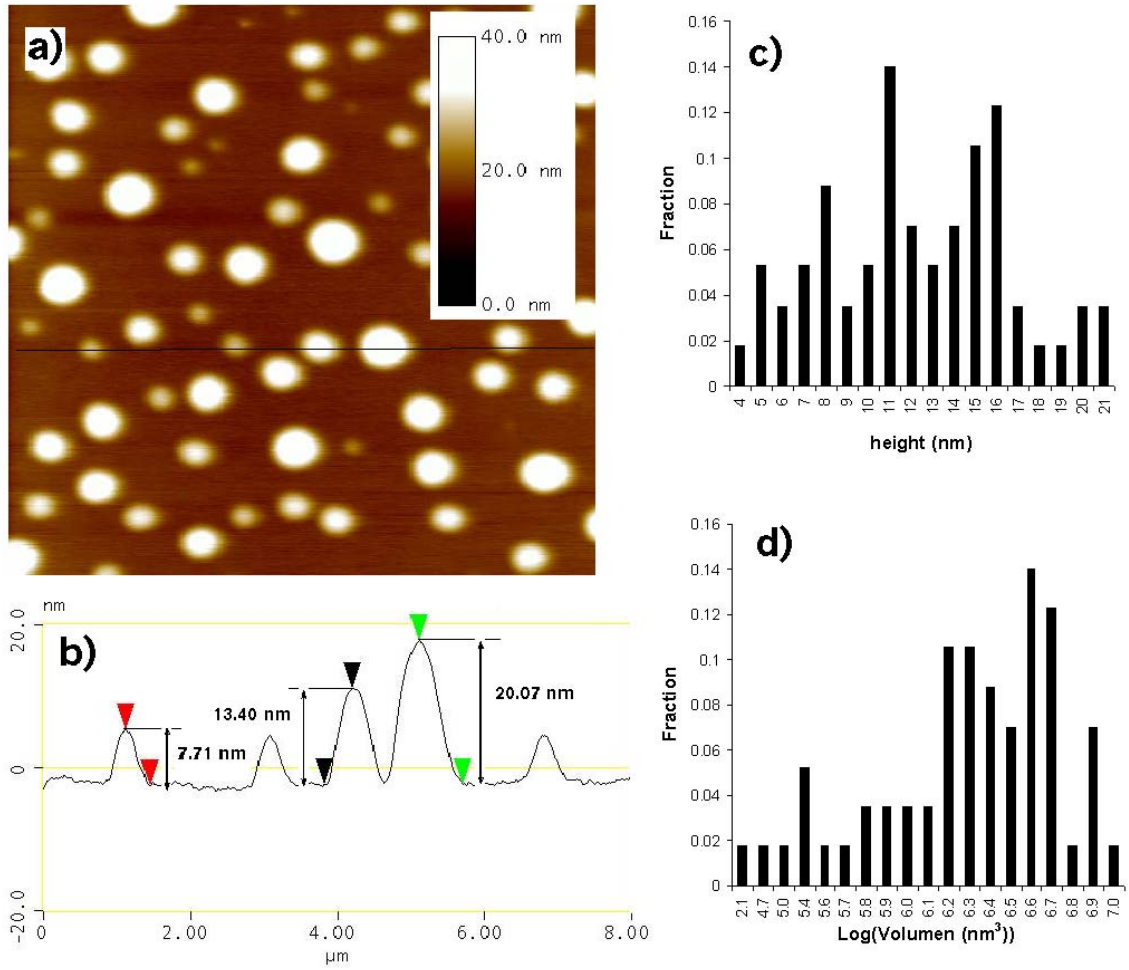


Figure 6: a) AFM height image (8 µm × 8 µm) of **P1** with height scale; b) section analysis: line scan on the surface; c) height distribution of the spherical aggregates measured by AFM for an ensemble of 60 aggregates; d) volume distribution of the spherical aggregates measured by AFM for an ensemble of 60 aggregates.

Polymer **P1** forms spherical aggregates of different size (**Figure 6**). The largest aggregate has a height of 20 nm and a diameter of 770 nm (**Figure 6b**). Their height distribution (**Figure 6c**) and volume distribution (**Figure 6d**) was calculated from 60 aggregates. Polymer **P2** forms also aggregates. However, because of the higher content of the alkyl groups in polymer

P2, aggregates of irregular shape are also observed (**Figure 7**). From the section analysis, the height of the largest aggregate is about 14 nm (**Figure 7b**).

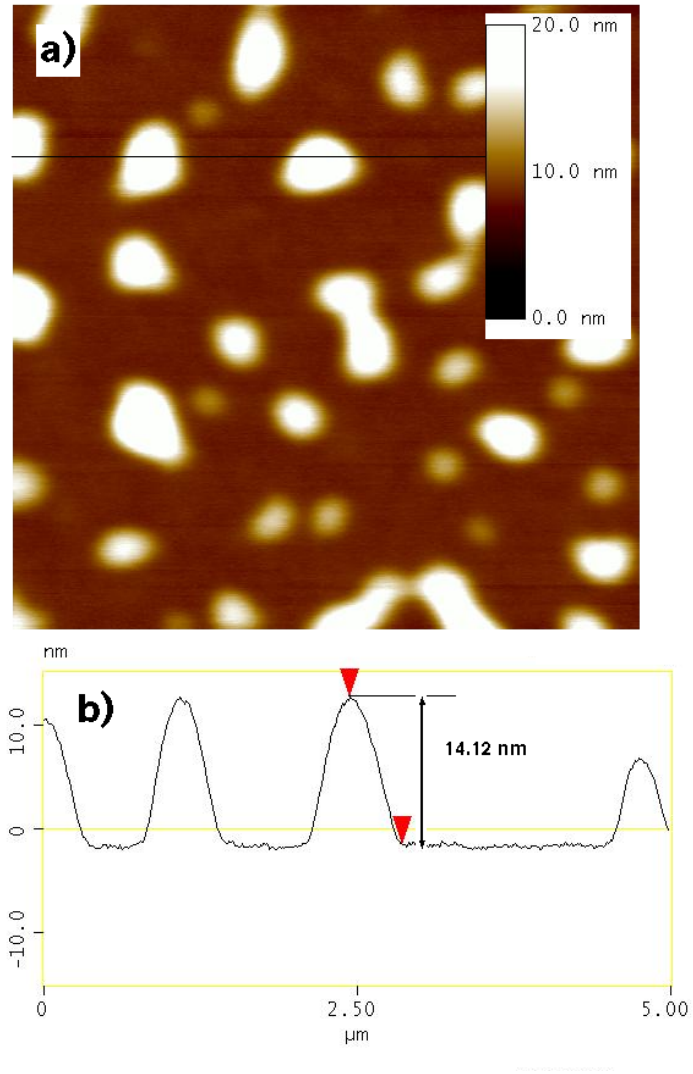


Figure 7: a) AFM height image (5 µm × 5 µm) of **P2** with height scale; b) the section analysis: line scan on surface

Polymer **P3** forms a layered structure (**Figure 8**). The section analysis (**Figure 8b**) shows that the height of a single layer is about 5.85 nm, and a double layer is twice as high ca. 12.2 nm. From the bearing analysis, the mean thickness of each layer was calculated to be ca. 5.89 nm

(**Figure 8c**). **Figure 8d** gives a schematic view on the ordering of alkyl chains and the hierarchical ordering of the layers[34].

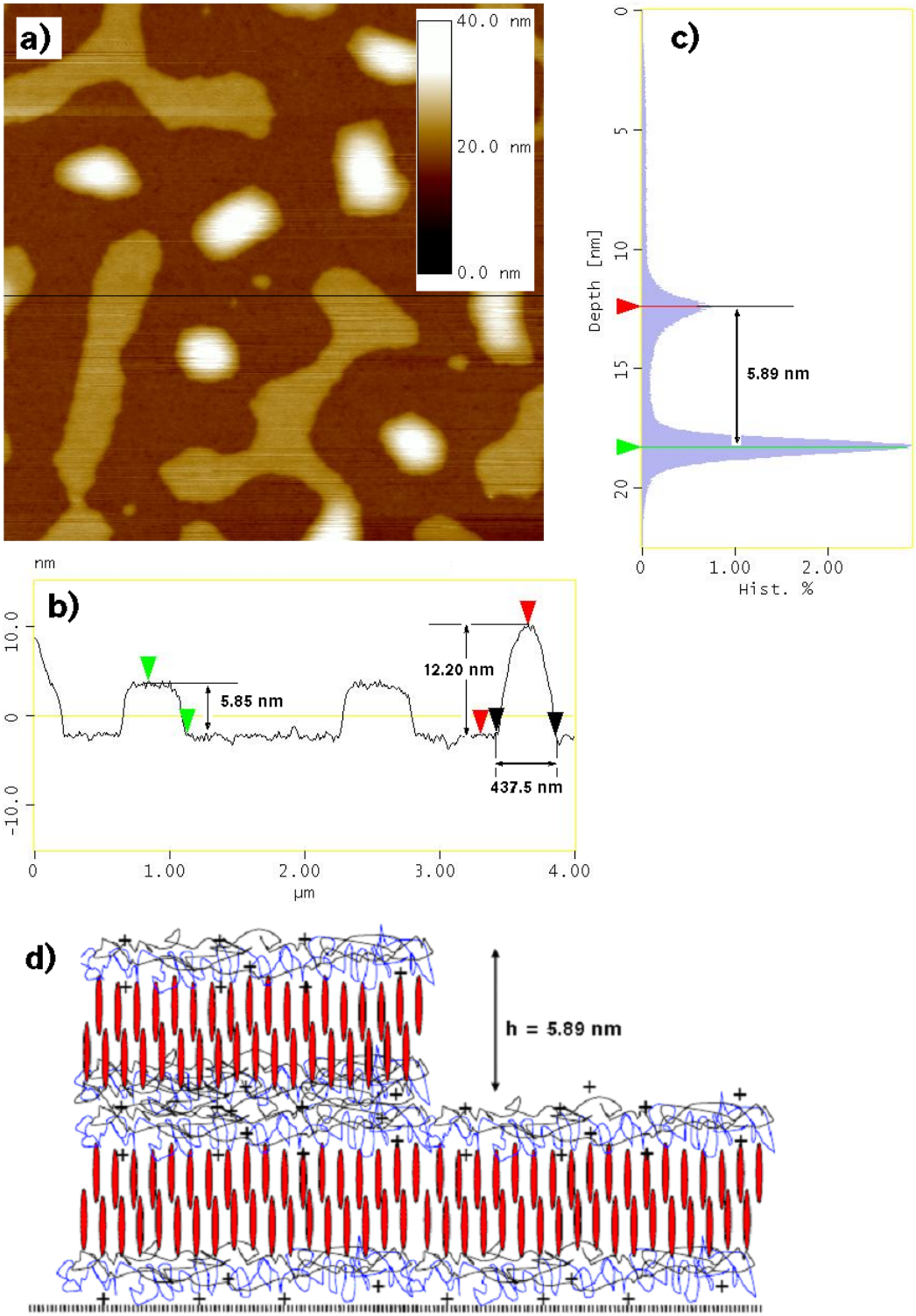


Figure 8: a) AFM height image (4 µm × 4 µm) of **P3** with height scale; b) section analysis: line scan on the surface; c) mean height of the surface calculated from bearing analysis; d) a schematic representation of the hierarchical ordering of the alkyl chains and of the layers.

In polymer **P4** the longest alkyl chains (C18) were incorporated in high concentration (60 % of all grafts). It is expected that crystallization of these chains will determine the morphology of the thin film. As can be seen from **Figure 9**, long needles are formed. From the section analysis, the diameter of the needles was determined as about 23.5 nm (**Figure 9b**). The mean height is calculated from bearing analysis to be around 3.91 nm (**Figure 9c**).

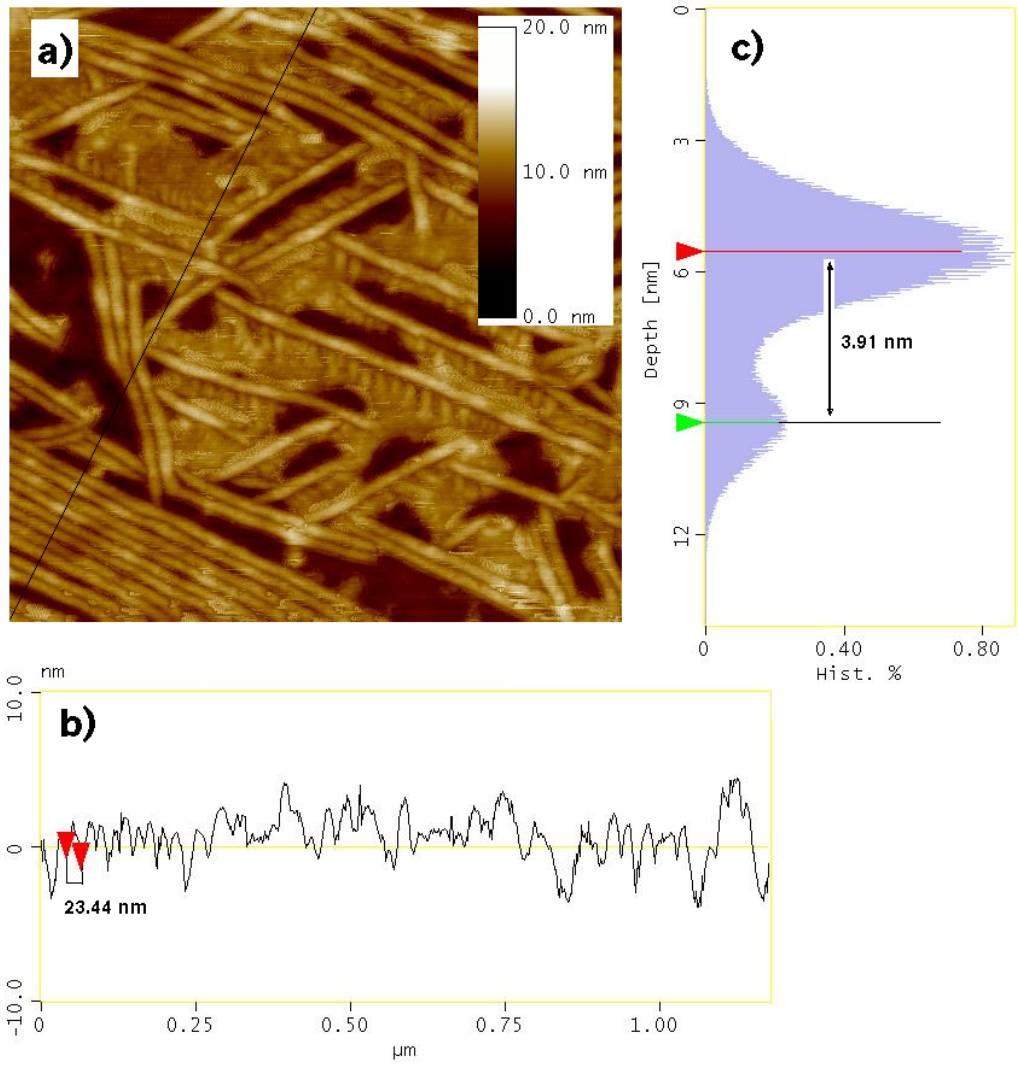


Figure 9: a) AFM height image (1 μm × 1 μm) of **P4** with height scale; b) the section analysis: line scan on the surface; c) the mean height of the surface.

8.3.1.2 Influence of the molecular weight on the properties of multifunctional B-PEI

In this part the influence of the molecular weight on the properties of multifunctional B-PEIs in bulk, in thin films and in aqueous solution is described. As a model for this analysis, multifunctional poly(ethylene imine)s with Mw = 1.3 kDa (**P5**), 25 kDa (**P1**) and 750 kDa (**P6**) were chosen; the composition of the grafts of all multifunctional B-PEIs was the same: 14.9 % **A8**, 4.95 % **QI**, 4.95 % **J** and 0.2 % **P**.

TGA analysis of these samples and for comparison reasons of B-PEI with molecular weight of 25 kDa show similar decomposition behavior (**Figure 10**). The first step is the loss of residual water; the second step is the decomposition of urethane groups of the linker and the removal of the grafts; and the final step is the decomposition of the poly(ethylene imine) backbone. In **Table 4** the onset of decomposition, the highest rate of the decomposition and the mass loss are summarized. The maximum rate of decomposition of the poly(ethylene imine) backbone is slightly different for samples of different molecular weight: $T_{dec,P6} > T_{dec,P5} > T_{dec,P1}$.

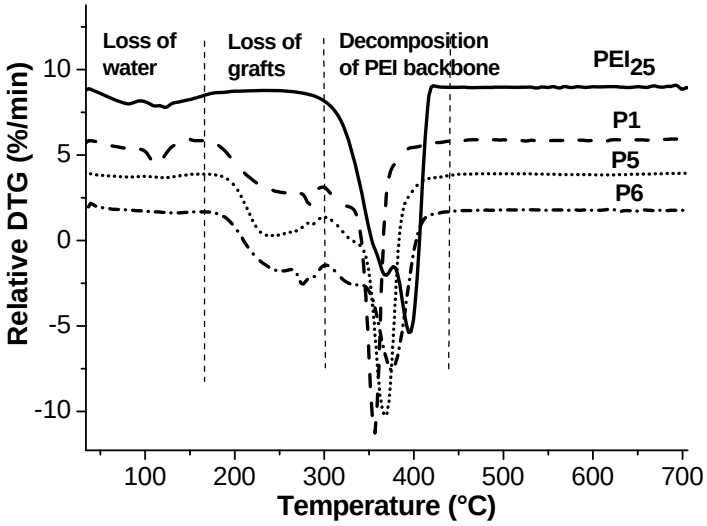


Figure 10: Rate of loss in weight of **PEI_{25}** (B-PEI, Mw = 25 kDa), **P1**, **P5** and **P6**.

Table 4: Decomposition of **PEI_{25}** (B-PEI, Mw = 25 kDa), **P1**, **P5** and **P6**.

No.	Loss of water			Loss of grafts			Decomposition of B-PEI backbone		
	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)
PEI	63.3	122.3	11.79	-	-	-	343.9	395.3	87.89
P1	93.7	112	7.18	213.4	283.9	30.24	345.7	356.2	52.51
P5	28.9	118.9	2.96	212.5	238.8	31.06	353	368.1	58.1
P6	-	-	3.43	220.4	275.9	36.68	357.6	375.1	57.55

DSC analysis of the polymers **P1**, **P5**, **P6** and for comparison reason of **PEI_{25}** was carried out between -100 °C and 190 °C. The second heating curves of all modified polymers look similar. They all have an exothermic crystallization peak before the endothermic melting peak, which can be attributed to the PEO/PPO chains of the JEFFAMINE® coupler in the polymers (**Figure 11**). From the DSC analysis, it can be concluded that the dominating factor for self-assembling is the JEFFAMINE® building block.

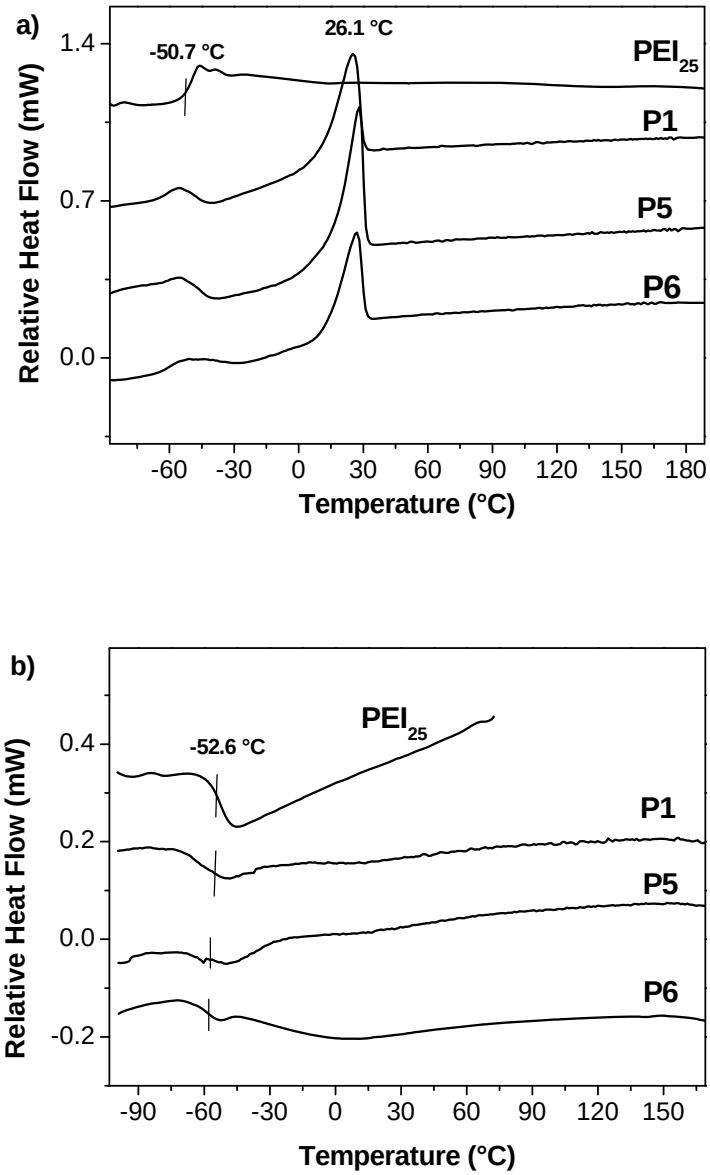


Figure 11: DSC traces of unmodified B-PEI (**PEI$_{25}$**) and multifunctional B-PEIs with different molecular weight of B-PEI skeleton (**P1**, **P5**, and **P6**): a) second heating; b) second cooling

For washing experiments the properties of thin films on model surfaces are more important, therefore thin films on silicon wafer were prepared from the water solution of PEI and modified B-PEI (polymer concentration 5 g/L) by dip coating. The thickness of all films is quite similar and in the range of 9 – 12 nm as determined by ellipsometry (**Table 5**).

Table 5: Thickness of PEI and modified PEI thin films on silicon wafer

	Treatment	PEI_{25}	P1	P5	P6
thinkness	**without annealing**	12.6 nm	8.6 nm	10.9 nm	10.3 nm

The morphology of the polymer films (**P1**, **P5**, and **P6**) were further investigated via atomic force microscopy (AFM). The height images (**Figure 12**) show that polymers **P1** and **P5** form large spherical aggregates of different sizes; aggregates of **P5** are even larger than those of **P1** (**Figure 12a and 12b**).

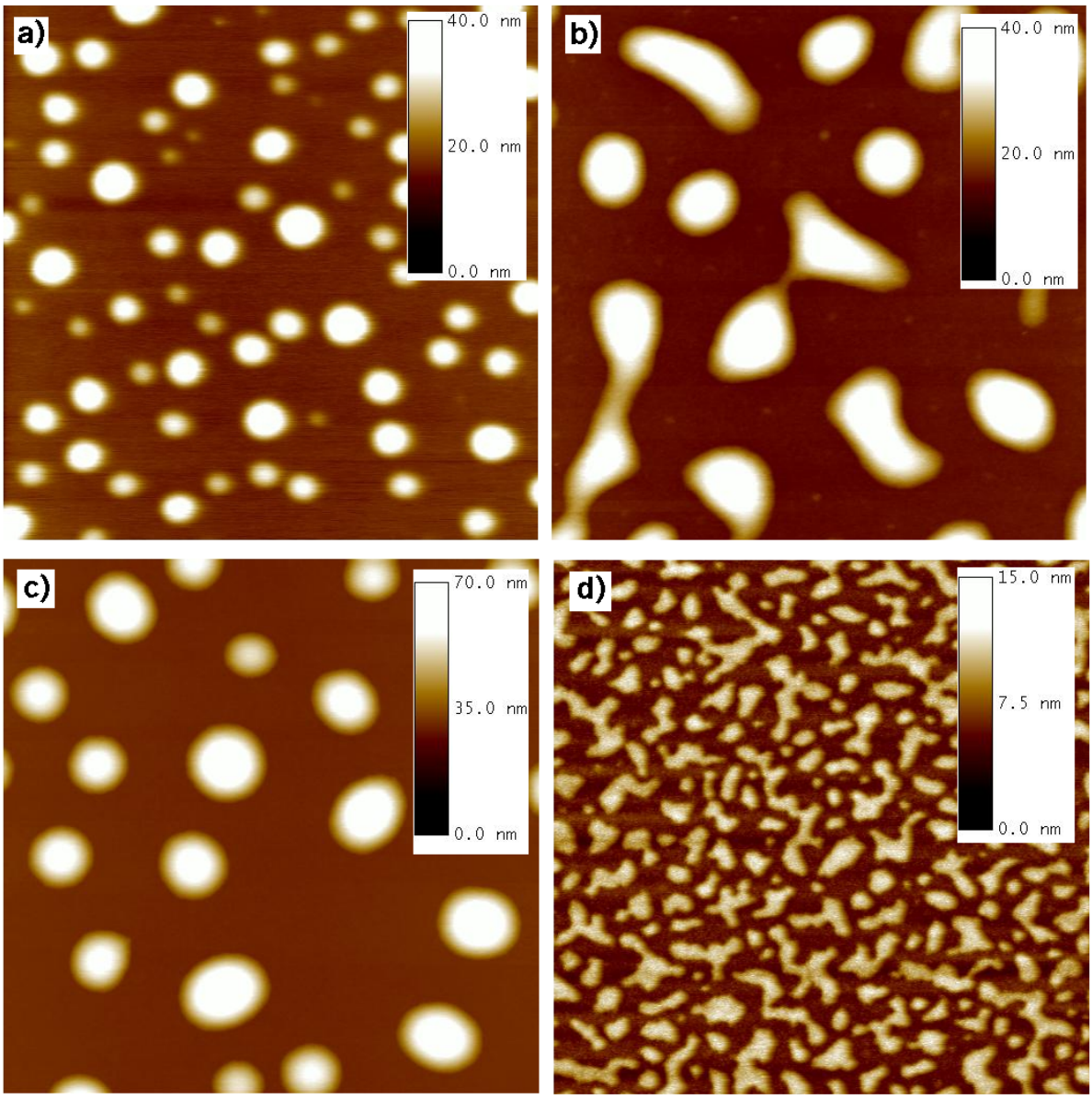


Figure 12: AFM height images (8 μm × 8 μm) with height scale of modified PEI from water solution with concentration of 5 g/L. a) **P1**, Mw (PEI) = 25 kDa. as cast; b) **P5**, Mw (PEI) = 1.3 kDa. as cast; c) **P5**, Mw (PEI) = 1.3 kDa. 7 days at room temperature; d) **P6**, Mw (PEI) = 750 kDa as cast.

The as-cast film of **P5** and the same film after 7 days are different. After 7 days only spherical aggregates are observed with more uniform size distribution. This is probably related to the higher diffusion rate of the small multifunctional B-PEI. The largest aggregate of **P5** has a height of 38 nm and a diameter of 1.2 μm. To understand why **P5** with lowest molecular

weight forms large aggregates, thin films of **P5** prepared from aqueous solution of different concentrations were further investigated via AFM (**Figure 13**).

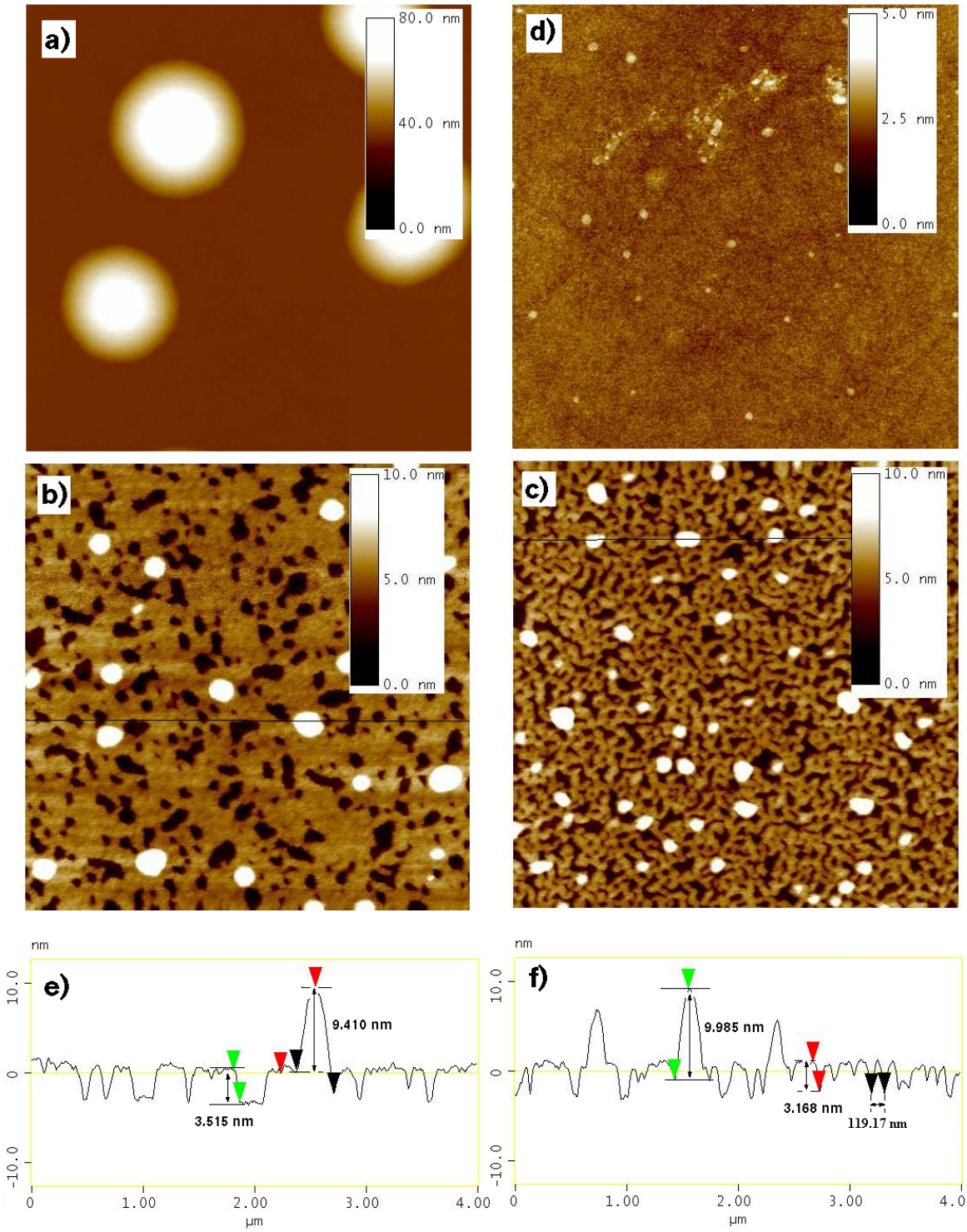


Figure 13: AFM height images (4 μm × 4 μm) of **P5** from aqueous solution with concentration of a) 5 g/L; b) 1 g/L; c) 0.5 g/L; d) 0.05 g/L; e) the section analysis of b); f) the section analysis of c).

From AFM height images, it can be seen that films prepared from a polymer solution of 5 g/L concentration show spherical aggregates structure (**Figure 13a**); at a concentration of 1 g/L, beside spherical aggregates a continuous layer with a thickness of ca. 3.5 nm is observed (**Figure 13b** and **13e**); at a concentration of 0.5 g/L, beside spherical aggregates more complex structures, similar to a network can be observed (**Figure 13c** and **13f**); and at a concentration of 0.05 g/L, spherical aggregates with much smaller size were observed (**Figure 13d**).

Depending on the concentration of a surfactant in aqueous solution, different structures, such as spherical micelles, rodlike micelles, bilayers, vesicles and multilayer vesicles are obtained by self-organization (**Figure 14**)[35, 36]. Polymer **P5** has a very small B-PEI backbone; it can be considered as a low molecular weight surfactant. From the AFM results presented before, it can be concluded that at higher concentration polymer **P5** forms onion like multilayer vesicles; at low concentration this polymer forms bilayers, rodlike micelles or spherical micelles.

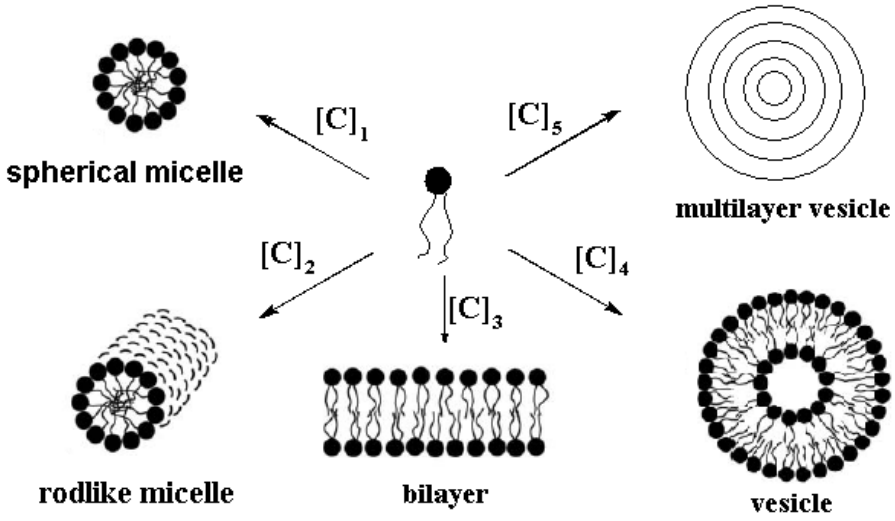


Figure 14: Structures formed by surfactant molecules at different concentrations: $[C]_1 < [C]_2 < [C]_3 < [C]_4 < [C]_5$

The aggregation properties of **P1**, **P5**, and **P6** in aqueous solution were investigated by Cryo-SEM at a concentration of 5 g/L (**Figure 16**). **P5** forms larger aggregates (**Figure 15a**) than **P1** and **P6** (**Figure 15b** and **15c**), and beside spherical aggregates, other shapes of aggregates (disc like shape. worm like shapes…) were observed. These results confirm the results already obtained via AFM analysis.

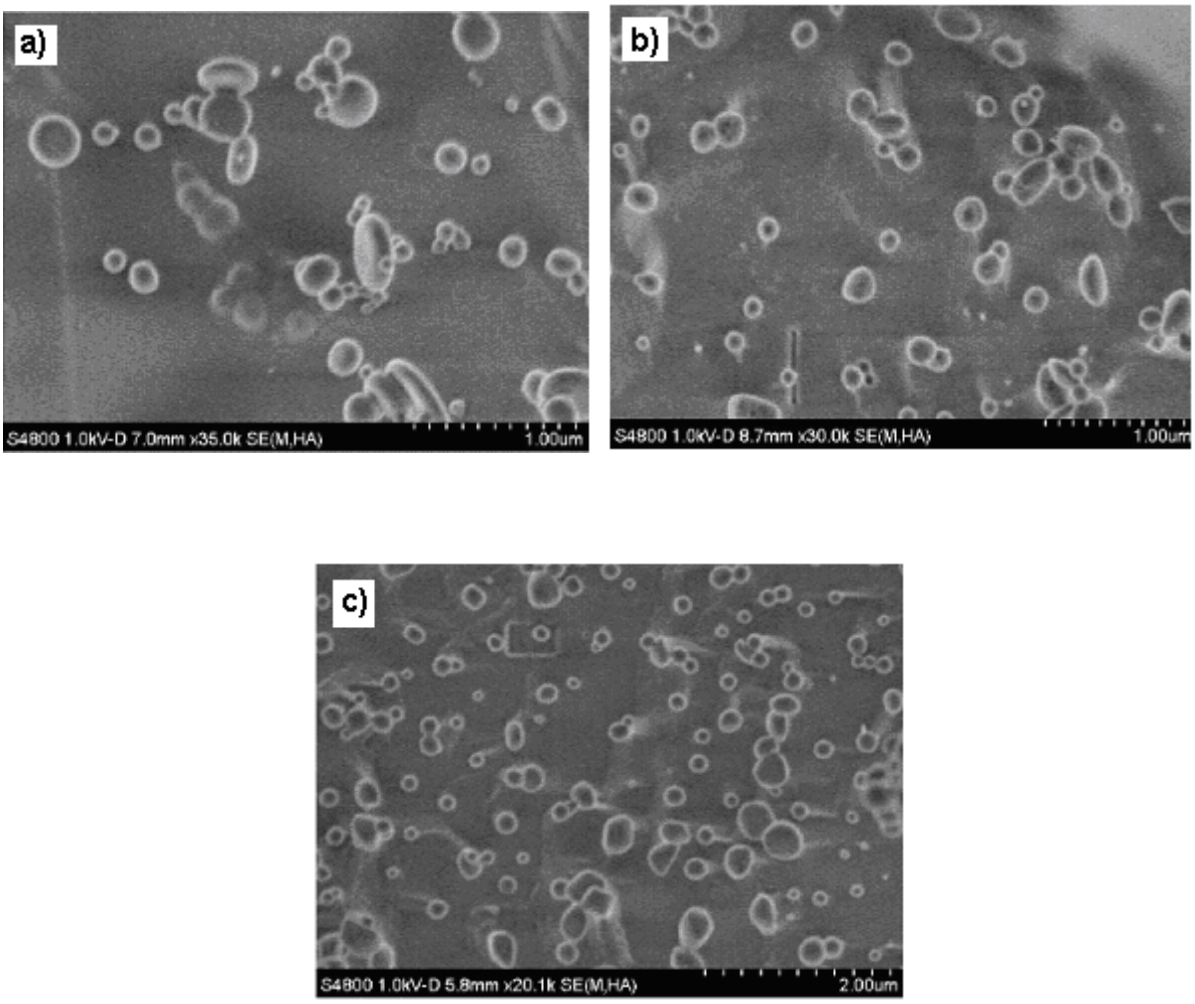


Figure 15: Cyro-SEM images of aqueous solutions of multifunctional B-PEIs (concentration 5 g/L): a) **P5**, Mw (PEI) = 1.3 kDa; b) **P1**, Mw (PEI) = 25 kDa; c) **P6**, Mw (PEI) = 750 kDa

8.3.1.3 Influence of the different couplers on the properties of multifunctional B-PEIs

It is observed that the size of the coupler (C3 or C6 coupler) or in other words, the spacer between the functional groups in the coupler (couplers **A1**, **A2** vs. **A3**, **A4 Figure 1**) has also an influence on the washing performances of the multifunctional B-PEI. In the following the properties of these polymers **P1** and **P7** (for comparison reason of $\mathbf{PEI_{25}}$) with the same hydrophilic/hydrophobic/ionic compositions are compared.

TGA analysis of these samples of **P1**, **P7**, and for comparison reasons of B-PEI with molecular weight of 25 kDa shows similar decomposition behavior (**Figure 16**). The first step is the loss of residual water; the second step is the decomposition of urethane groups of the linker and the removal of the grafts; and the final step is the decomposition of the poly(ethylene imine) backbone. One should notice that multifunctional B-PEIs prepared with the C6 coupler are more stable than those prepared with the C3 coupler (**Table 6**): the onset of the decomposition point of polymer **P7** is ca. 270 °C, that of the polymer **P1** is 210 °C; the highest decomposition rate for **P7** is at 382 °C, similar to the unmodified B-PEI, and is higher than that for polymer **P1**. It can be concluded that the polymers modified with C6 couplers are more stable than those modified with C3 coupler.

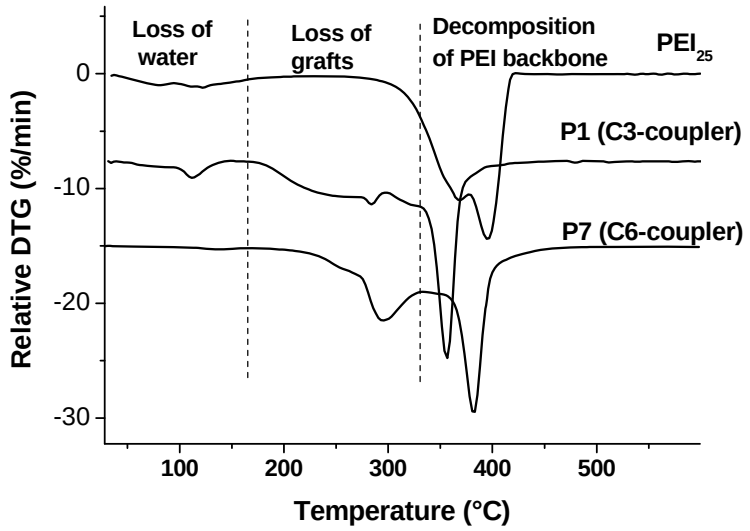


Figure 16: Rate of loss in weight of **PEI_{25}** (B-PEI, Mw = 25 kDa), **P1** and **P7**.

Table 6: Decomposition of **PEI_{25}** (B-PEI, Mw = 25 kDa), **P1** and **P7**.

No.	Loss of water			Loss of grafts			Decomposition of B-PEI backbone		
	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)	Onset (°C)	Peak (°C)	Mass Loss (%)
PEI	63.3	122.3	11.79	-	-	-	343.9	395.3	87.89
P1	93.7	112	7.18	213.4	283.9	30.24	345.7	356.2	52.51
P7	108.0	138.8	2.49	270.1	294.8	44.12	372.3	382.0	45.59

DSC analyses of the polymers **P1**, **P7** and for comparison reason of **PEI_{25}** was carried out between -100 °C and 190 °C. The second heating curves of polymers **P1** and **P7** both have an exothermic crystallization peak (broad peak for **P1** and sharp peak for **P7** at Tp = 2.6 °C) before the endothermic melting peak (Tp = 26.1 °C), which can be attributed to the PEO/PPO chains of the JEFFAMINE® coupler in the polymers (**Figure 17a**). This behavior indicates a slow crystallization of the crystalline PEO chains, which is not completed during the second cooling (**Figure 17b**)[31]. In heating and cooling curves of both polymers the glass transition of the B-PEI can be clearly seen (Tg is ca. -50 °C). In the cooling curve, PEO chains of **P7** have

crystallized partially, while those of **P1** do not crystallize; thus it can be concluded that polymer **P7** has a higher tendency for self-organization.

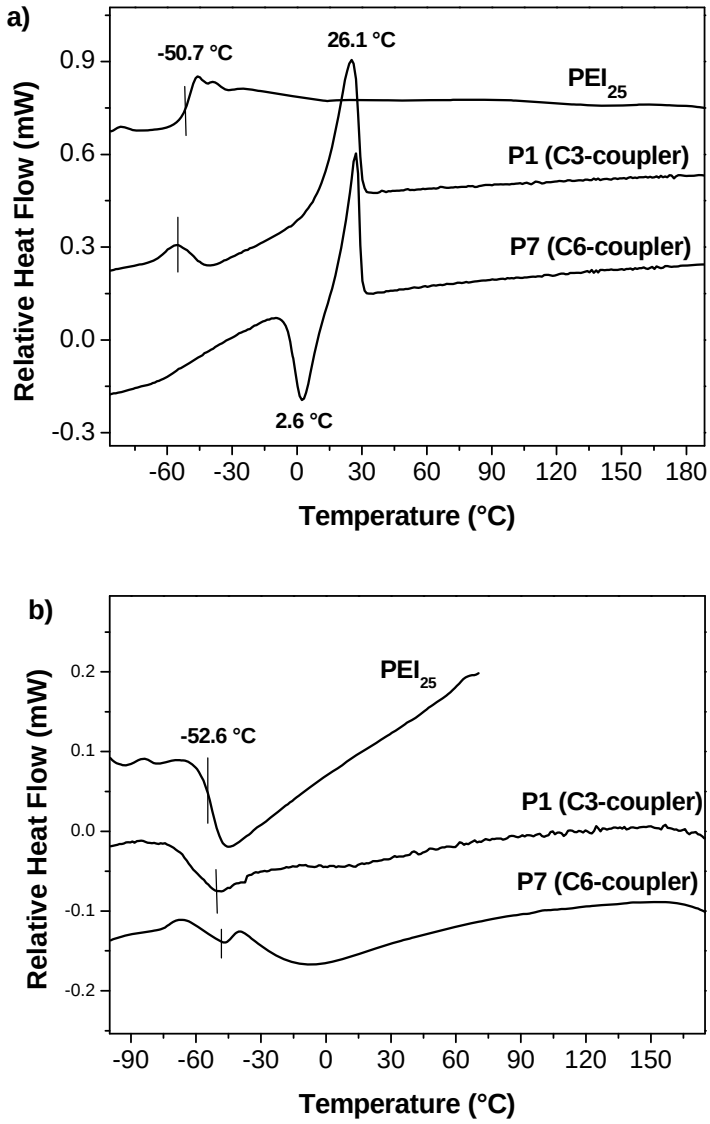


Figure 17: DSC traces of unmodified B-PEI (**PEI_{25}**) and multifunctional B-PEIs with different coupler (**P1** and **P7**): a) second heating; b) second cooling

Thin films of polymer **P1** and **P7** on a silicon wafer were prepared from aqueous polymer solutios (polymer concentration 5 g/L) by dip coating and investigated via AFM under taping mode. From the AFM height images (**Figure 18**), polymer **P1** with C3 coupler forms

spherical aggregates, while polymer **P7** with C6 coupler forms beside spherical aggregates other type of aggregates, indicating a higher self-assembly tendency.

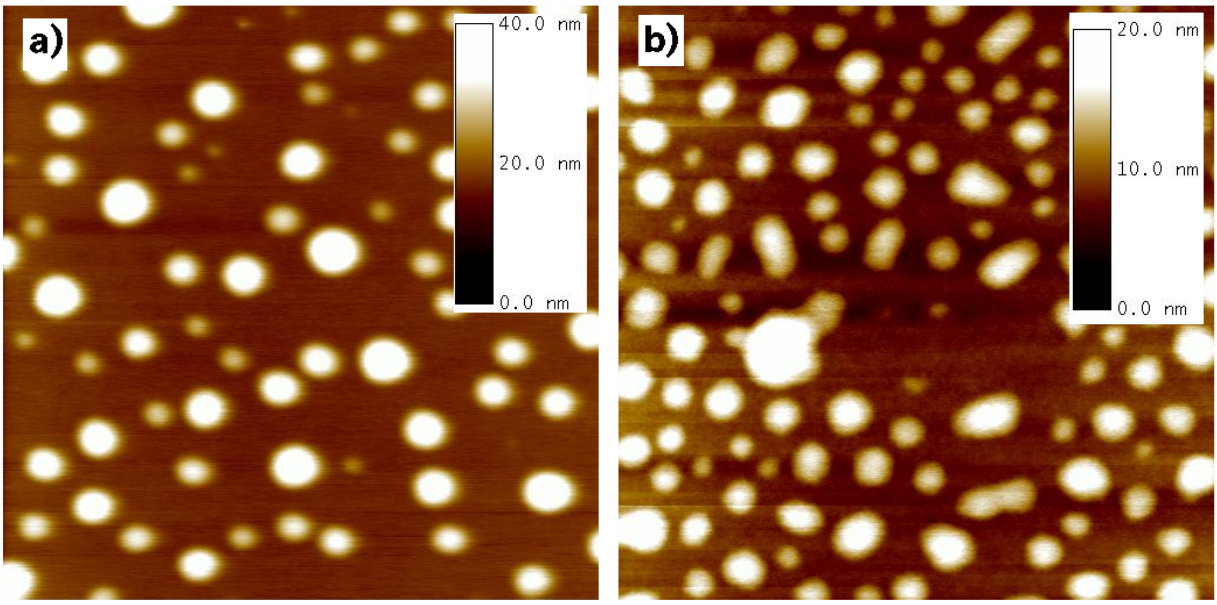


Figure 18: AFM height images (8 μm × 8 μm) with height scales of multifunctional B-PEI with different couplers: a) **P1**, C3 coupler; b) **P7**, C6 coupler.

In conclusion, multifunctional B-PEIs with the same amount of hydrophilic/ionic functional grafts but different alkyl chains show different self-organization (or aggregation) properties in bulk, in thin films and in water solution. Polymer **P1** has the lowest tendency for self-organization (or aggregation) compared with polymers with longer alkyl chains (**P3** with C12 alkyl chains and **P4** with C18 alkyl chains) or higher alkyl chains content (**P2** with Ad8). The self-organization (or aggregation) behavior of the polymers with the same functional units but different molecular weight shows that the polymer with low molecular weight has a better self-aggregation behavior and forms larger aggregates than the polymers with higher molecular weight. Polymer **P7** with C6 coupler has higher self-organization (or aggregation) tendency than polymer **P1** with C3 coupler.

8.3.2 Antibacterial effects of multifunctional B-PEIs

Amphiphilic multifunctional B-PEIs show an anti-bacterial effect against both Gram positive and Gram negative bacteria[7, 8, 11]. The antibacterial effect depends on the balance between hydrophobic and ionic units on one side, and the nature of the hydrophobic units on the other side[11]. The multifunctional B-PEIs studied in this work also composed of alkyl chains and cationic groups. Therefore these polymers were studied with respect to their anti-bacterial properties. Before presenting these results, an overview on the average number of functional groups within the amphiphlic B-PEIs of different molecular weight will be given (**Table 7**). In this table, the average number of nitrogen atoms is given; however, only 25 % of these N-atoms can be functionalized (primary amine groups, which can be functionalized). The low molecular weight B-PEI contains only 1.5 grafts of **QI** and **J1000** in average. As a consequence, all these groups will be attached to the bacterial surface. In the higher molecular weight B-PEI, due to sterical hindrance, not all grafts are effective.

Table 7: Statistic calculation of the number of functional groups of **P1**, **P5**, and **P6** and the radius of hydration (Rh) measured by DLS

	Mw of PEI (kDa)	**No. of N-atoms**	**No. of QI (4.95 mol%-N)**	**No. of J1000 (4.95 mol%-N)**	**No. of A8 (4.95 mol%-N)**	**R_h (nm)***
P1	25	580	28	28	83	8.34
P5	1.3	30	1.5	1.5	4.5	6.14
P6	750	17414	697	697	2092	26.62

* measured at a concentration of 5 g/L

The antibacterial properties of multifunctional B-PEIs were evaluated by determining the minimal inhibitory concentration (MIC) against Gram positive bacteria (*B. Subtilis*) and Gram negative bacteria (*E. Coli*) using a method developed in our laboratory[30]. All results are

summarized in **Table 8** and reveal that all polymers are more effective against Gram positive bacteria (showing lower MIC values).

Table 8: Minimal inhibitory concentration (MIC) of multifunctional B-PEIs against *E. coli* and *B. subtilis*

No.	Polymer Notation	MIC	
		***E.Coli* (mg/mL)**	***B.Subtilis* (mg/mL)**
P1	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$	>1	0.03
P2	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$Ad8_{14.9}$-$P_{0.2}$	>1	0.1
P3	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A12_{14.9}$-$P_{0.2}$	0.4	ca. 0.07 [a)]
P4	PEI_{25}-$QI_{4.95}$-$J1000_{4.95}$-$A18_{14.9}$-$P_{0.2}$	insoluble [b)]	Insoluble [b)]
P5	$PEI_{1.3}$-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$	>1	0.01
P6	PEI_{750}-$QI_{4.95}$-$J1000_{4.95}$-$A8_{14.9}$-$P_{0.2}$	>1	0.03
P7	PEI_{25}-$C_6QI_{4.95}$-$C_6J1000_{4.95}$-$C_6A8_{14.9}$-$P_{0.2}$	0.1	0.02

[a)] result is not reproduceable; [b)] insoluble in PBS-buffer

Except **P4** (insoluble in PBS-buffer), all multifunctional B-PEIs have an activity against Gram positive bacteria: (i) with increasing hydrophobicity the anti-bacterial properties decreased (MIC: **P1** < **P3** < **P2**); (ii) the polymer with the smallest molecular weight has a better anti-bacteria activity (MIC: **P5** < **P1** = **P6**); (iii) PEI modified with C6 coupler (**P7**) shows a better anti-bacterial activity than B-PEI modified with C3 coupler (**P1**).

In the literature, the efficiency of antibacterial properties has been systematically studied as a function of ionic/hydrophobic ratio[11]. It has been reported that multifunctional B-PEIs modified with high content of cationic groups, 20 % **QI** and 5 % **A12**, had the lowest MIC. For this ratio of **QI** to **A12**, the influence of the alkyl chain length was studied: B-PEI modified with C12 and C14 alkyl chain showed the lowest MIC. The multifunctional B-PEIs in this work all have the same molar amount of **QI**/**J1000**/alkyl chains (5/5/15 mol-%), but a

very high content of alkyl chains. This explains their lower efficiency against Gram negative bacteria. However, surprisingly the effect against Gram positive bacteria is less influenced (their MIC values are in the same range). With high alkyl chain content, B-PEI modified with C8 alkyl chain has the highest antibacterial activity (the higher alkyl chain concentration shifts the efficiency from C12 and C14 to C8). The effect of molecular weight on the antibacterial property of multifunctional B-PEI with high content of alkyl chains group is similar to multifunctional B-PEIs with high content of cationic groups; polymers with small molecular weight have lower MIC. Multifunctional B-PEIs modified with C6 coupler show a better antibacterial effect against both Gram negative and Gram positive bacteria than polymers modified with the C3 coupler.

8.3.3 Hydrolytic stability of multifunctional B-PEI

From the German Detergent Law in 1961[2], detergents or cleansing agents whose biodegradability fells below 80 % have to be tested by the Detergent Commission. For this reason, the hydrolytic stability of multifunctional B-PEIs was tested.

In **table 9** the composition of multifunctional B-PEIs (weight percent of the building blocks) was calculated for polymers **P1 – 7.** The weight percent of the B-PEI skeleton is from 23.1% to 26.1%. In different grafts, the coupler and the amine - the functional coupler - is considered: the weight percent of ionic grafts is from 10.3 % to 12.1 %, hydrophilic grafts (JEFFAMINE® coupler) from 33.0 % to 37.2 %, hydrophobic grafts from 24.6 % to 33.1 %, and pyrene grafts from 0.4 % to 0.5 %. The weight percent of all grafts is between 73.9 % and 76.9 %. The weight percent of the coupler within all grafts is between 18.5 % and 26.6 %. Since urethane groups are hydrolytically labile, it is expected that 73.9 – 76.9 wt-% of the multifunctional polymers will be biodegradable.

Table 9: Calculation of the weight percentages of the functional grafts of multifunctional B-PEIs

No.	B-PEI (wt-%)	Ionic grafts (wt-%)	Hydrophilic grafts (wt-%)	Hydrophobic grafts (wt-%)	Pyrene grafts (wt-%)	All grafts (wt-%)	Carbonate coupler (wt-%)
P1	26.1	11.6	37.2	24.6	0.5	73.9	21,9
P2	23.7	10.6	33.8	31.6	0.4	76.3	19,9
P3	24.8	11.1	35.4	28.3	0.4	75.2	20,9
P4	23.1	10.3	33.0	33.1	0.4	76.9	19,5
P5	26.1	11.6	37.2	24.6	0.5	73.9	21,9
P6	26.1	11.6	37.2	24.6	0.5	73.9	21,9
P7	24.5	12.1	36.2	26.8	0.4	75.5	26,6

For the hydrolysis test, polymer **P1** was chosen as a representative example. The test was carried out in D_2O at room temperature and at 60 °C, and monitored by means of ^1H NMR spectroscopy. To better understand the hydrolysis process, hydrolysis of a model compound synthesized from the coupler **A1** with propanolamine was studied under the same conditions as **P1**. The structural characteristic of the model compound is described in **Figure 19a**.

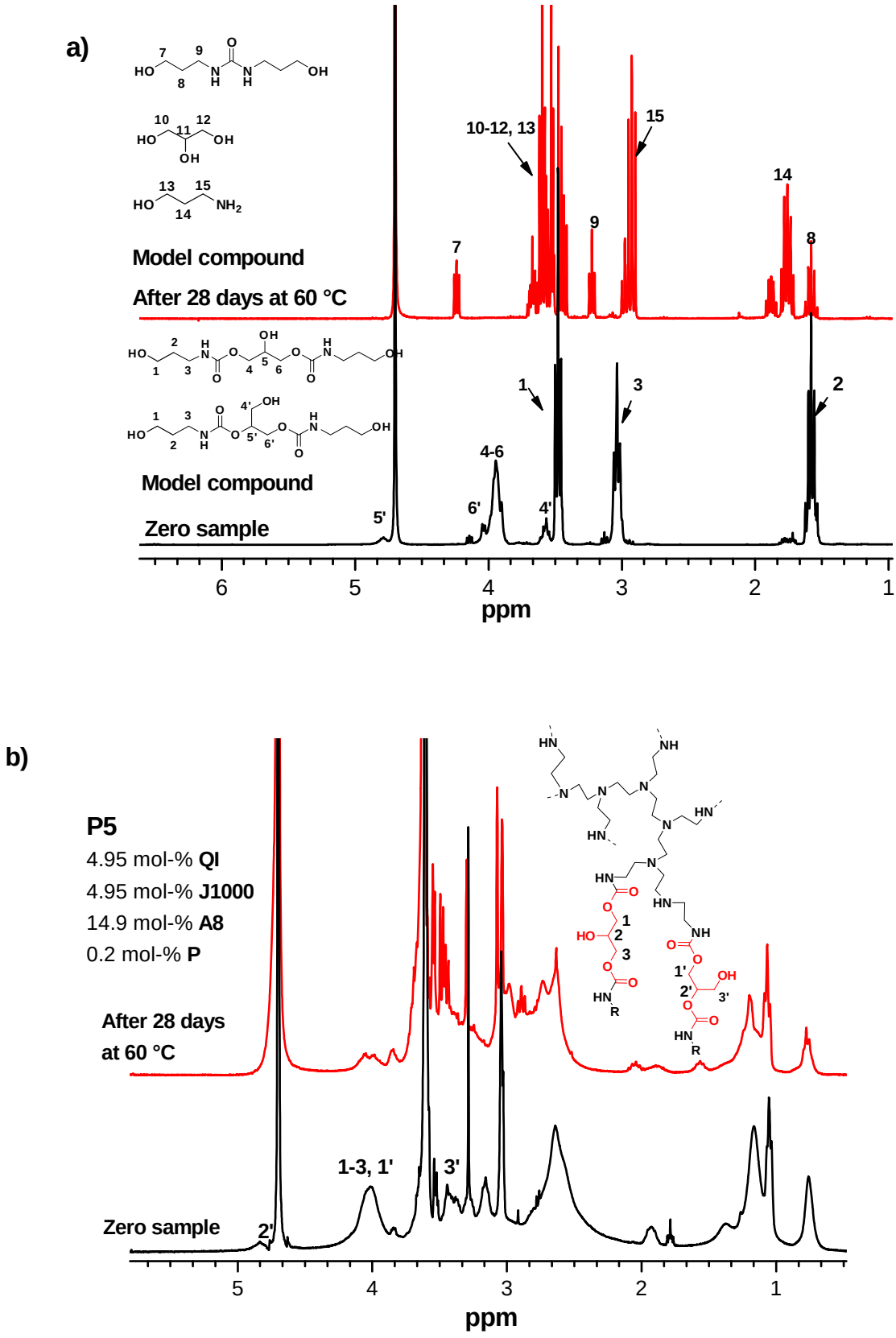


Figure 19: ^{1}H NMR spectra (300 MHz) of the hydrolysis of a) the model compound and b) **P1** in D_2O at 60 °C after 28 days.

After 28 days at 60 °C in D_2O, the model compound hydrolyzed completely, glycerol and propanolamine urea were formed (**Figure 19a**). In the ^1H NMR spectrum of **P1** after 28 days at 60 °C in D_2O (**Figure 19b**), the characteristic peaks (δ = 3.8-4.4. 4.8-5.0 ppm) formed upon attaching the functional coupler to PEI (ring-opening reaction of the cyclic carbonate groups of the functional coupler with primary amine groups of PEI) have almost totally disappeared, significant changes in range of δ = 2.6-3.0 and 3.1-3.6 ppm are observed.

The hydrolysis of polymer **P1** and of the model compound at room temperature is much slower than at 60 °C. The time and temperature dependent hydrolysis of **P1** and the model compound are shown in **Figure 20**. After 28 days at room temperature, about 20 % of polymer **P1** hydrolyzed; while about 5 % of the model compound is hydrolyzed. It should be noticed that the secondary and tertiary amine groups present in B-PEI accelerate (catalyze) the hydrolysis of urethane groups at room temperature. At higher temperature, this effect is not the dominating factor; hence the rate of hydrolysis of **P1** and the model compound is similar.

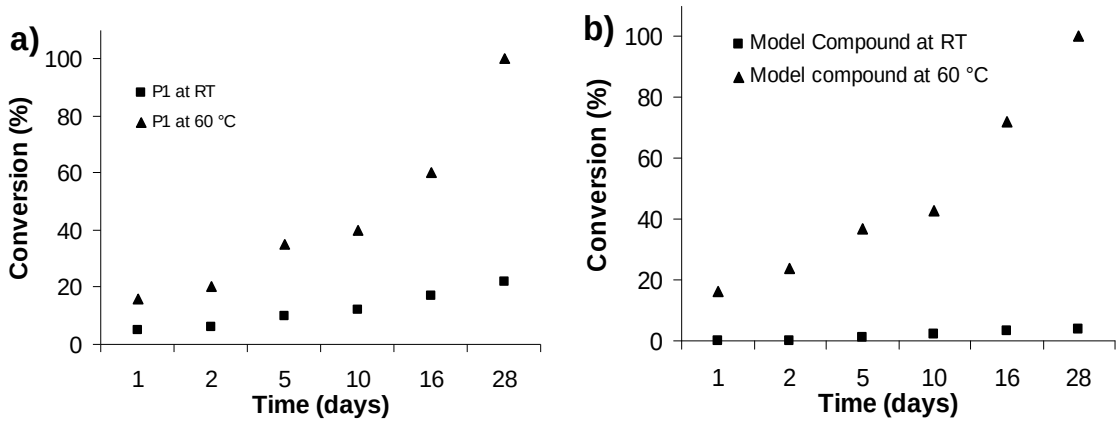


Figure 20: Time and temperature dependent hydrolysis of **P1** and of the model compound in D_2O.

8.4 Conclusion

In this work, firstly, the influences of the alkyl chains, the molecular weight of the B-PEI substrate and the structure of couplers on polymer self-organization (or aggregation) were investigated in bulk, in thin films and in aqueous solution.

From the TGA analysis it is concluded that multifunctional B-PEIs decomposed in three steps: the first step is the loss of residual water; the second step is the decomposition of urethane groups of the linker and the removal of the grafts; and the final step is the decomposition of the poly(ethylene imine) backbone. Multifunctional B-PEIs modified with the C6 coupler were more stable than the ones modified with the C3 coupler. DSC analyses reveal self-assembly of multifunctional B-PEI in bulk which is induced by the interaction of alkyl chains or PEO/PPO segments.

Thin films of multifunctional B-PEIs were prepared from aqueous solution. The films of polymer **P4** are hydrophobic (contact angle of 94°); after annealing the hydrophobicity decreased (contact angle 54 °). All other multifunctional B-PEIs give hydrophilic films. AFM images of polymer **P3** (C12 alkyl chains) show a well defined layer structure on silicon wafers with a layer thickness of 5.89 nm. For polymer **P4** with C18 alkyl chain grafts, crystallization of these grafts defines the surface topography (needles structure are observed on the surface). Polymer **P1** with C8 alkyl chain grafts and a molecular weight of PEI 25 kDa forms spherical aggregates of different size, the analog polymer **P5** derived from a PEI skeleton of 1.3 kDa forms more uniform and larger aggregates, while **P6** with a PEI skeleton of 750 kDa forms irregular structures. Because of the small molecular weight of B-PEI in polymer **P5**, this polymer resembles to a low molecular weight surfactant.

Secondly, the anti-bacterial activity of the multifunctional B-PEIs discussed was evaluated by determining the minimal inhibitory concentration (MIC) against Gram positive bacteria (*B. Subtilis*) and Gram negative bacteria (*E. Coli*) using a method developed in our laboratory. Because of the high content of hydrophobic grafts, multifunctional B-PEI here show lower activity against Gram negative bacteria than the multifunctional B-PEIs with high content of cationic grafts described in the literature, however, the activity against Gram positive bacteria is not affected and the MIC is in the same range as PEIs with high concentration of cationic grafts. Polymer **P1** with C8 alkyl chains shows the lowest MIC against Gram positive bacteria in comparison with polymer with the same B-PEI molecular weight and the same C3 coupler but different alkyl chains (the higher alkyl chain concentration shifts the efficiency from C12 and C14 to C8). Polymer **P5** with lowest molecular weight has the lowest MIC against Gram positive bacteria. Polymer **P7** with C6 coupler shows better activity against both kinds of bacteria than polymer **P1** with C3 coupler.

At the end, the hydrolytic stability of multifunctional B-PEIs was tested at room temperature and at 60 °C and monitored by means of ^{1}H NMR spectroscopy. Multifunctional B-PEIs have short-term stability in water and are fully hydrolyzed after longer time.

From the washing tests at Henkel[17-26], the anti-bacterial activity, hydrolytic stability and the nature of B-PEI, it can be concluded that multifunctional B-PEIs have several advantages to be used in laundry detergents applications: (i) with different modifications, they have different consumer comfort properties[17-26]; (ii) over 70 wt-% of the polymers is possible to be hydrolyzed in time; (iii) as an amphiphilic polymer, multifunctional B-PEIs have anti-bacterial activity at low temperature, as a consequence hygienic clean laundry can be obtained by washing at room temperature; (iv) the hydrophilic/ionic/hydrophobic balance of

multifunctional B-PEIs can be easily adjusted via carbonate coupler chemistry for special purpose and the manufacture in industrial scale is possible.

In the future, for a better understanding of the mechanism of different effects (different washing performances and anti-bacterial activity) and a better design of multifunctional B-PEIs for special purpose, the structure-properties relationships of other hydrophilic/ionic/hydrophobic balance will be studied, and the self-assembly property of multifunctional B-PEIs in bulk, in thin film and in aqueous solution will be further investigated via other techniques.

8.5 Reference

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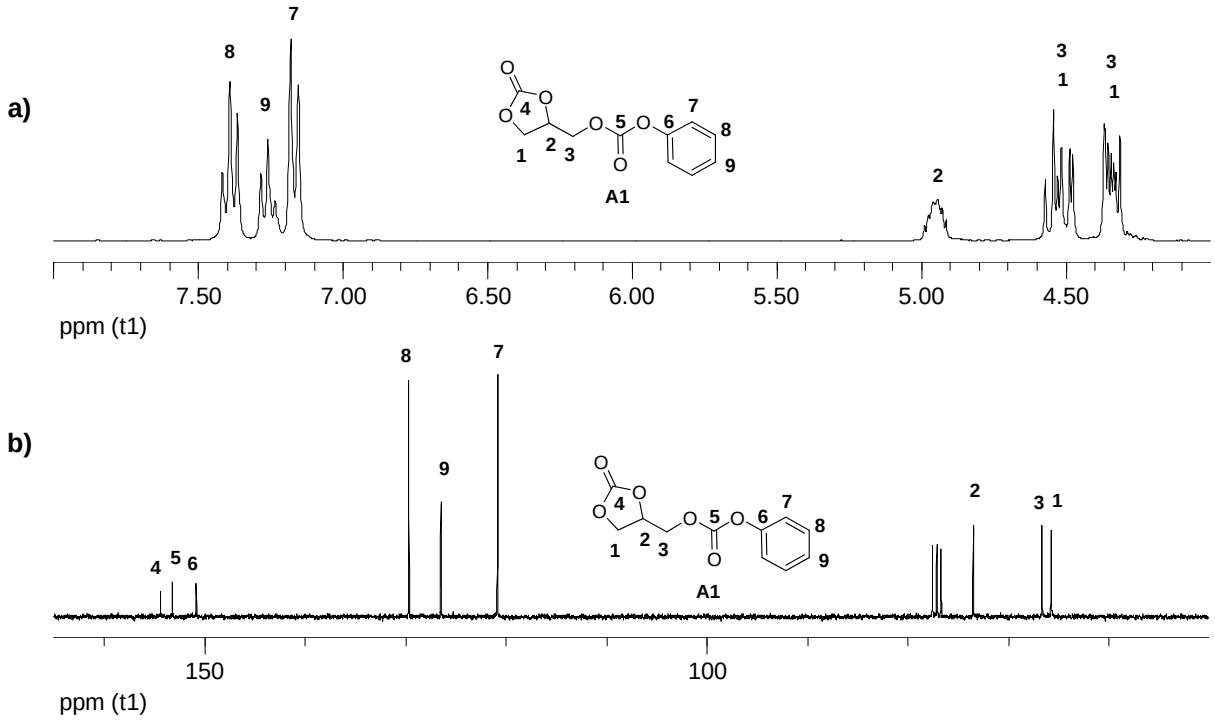
Appendix A

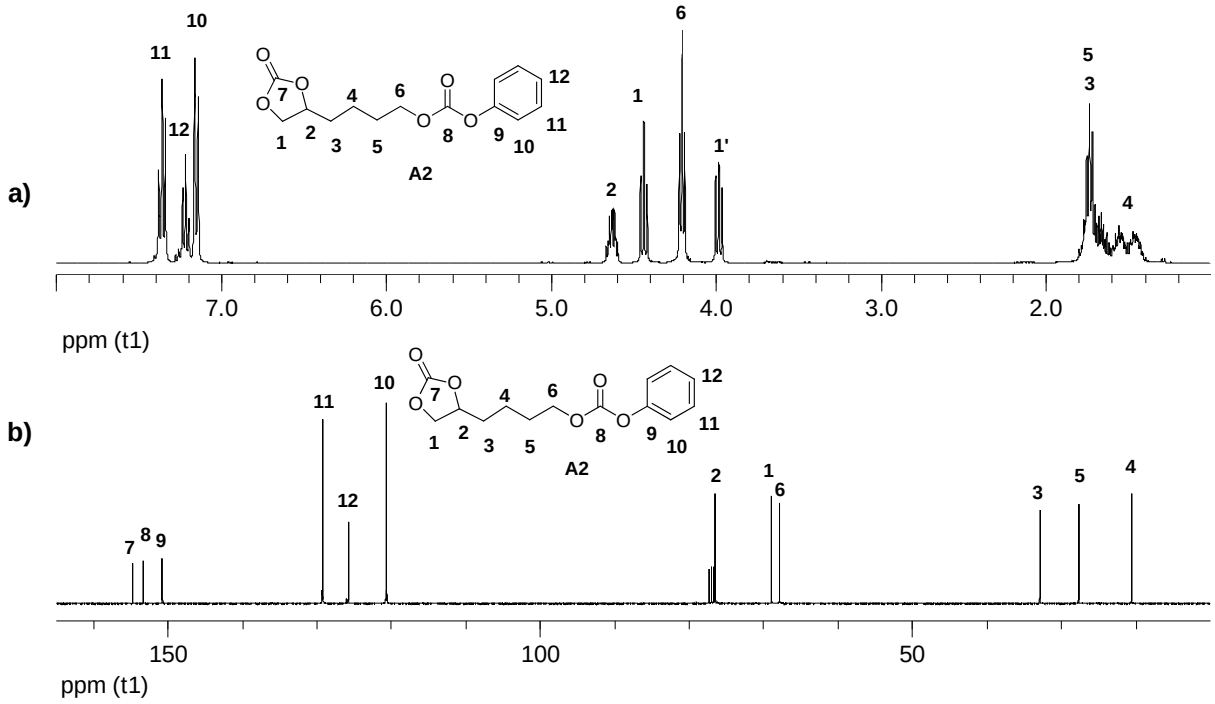
Synthesis, Characterization, and Selectivity of Bifunctional Couplers

(2-Oxo-1,3-dioxolan-4-yl)methyl phenyl carbonate (A1)



SI-Fig1: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **A1** in $CDCl_3$.

4-(2-Oxo-1,3-dioxolan-4-yl)butyl phenyl carbonate (A2)

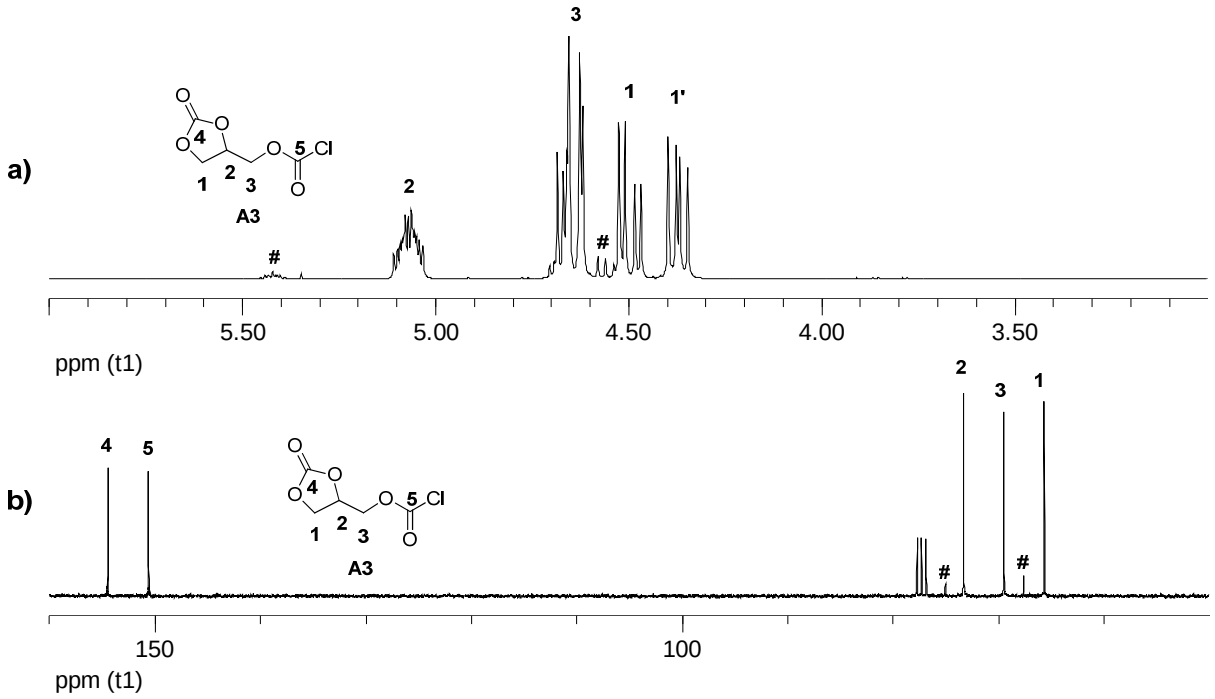


SI-Fig2: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **A2** in $CDCl_3$.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.39-1.60 (m, 1H, H^4), 1.60-1.84 (m, 2H, H^3 and H^5), 3.99 (dd, 1H, ^{2}J = 7.3 Hz, ^{3}J = 8.3 Hz, H^1), 4.21 (t, 1H, ^{3}J = 6.4 Hz, H^6), 4.44 (dd, 1H, ^{2}J = ^{3}J = 8.2 Hz, H^1), 4.63 (dq, 1H, ^{2}J = 4.9 Hz, ^{3}J = 7.7 Hz, H^2), 7.10-7.43 (m, 5H, phenyl) ppm.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 20.59 (C^4), 27.70 (C^5), 32.91(C^3), 67.85 (C^6), 68.96 (C^1), 76.50 (C^2), 120.70 (2C, C^{10}), 125.75 (C^{12}), 129.18 (2C, C^{11}) , 150.74 (C^9) , 159.29 (C^8) , 154.73 (C^7) ppm.

(2-Oxo-1,3-dioxolan-4-yl)methyloxy chloroformate (A3)

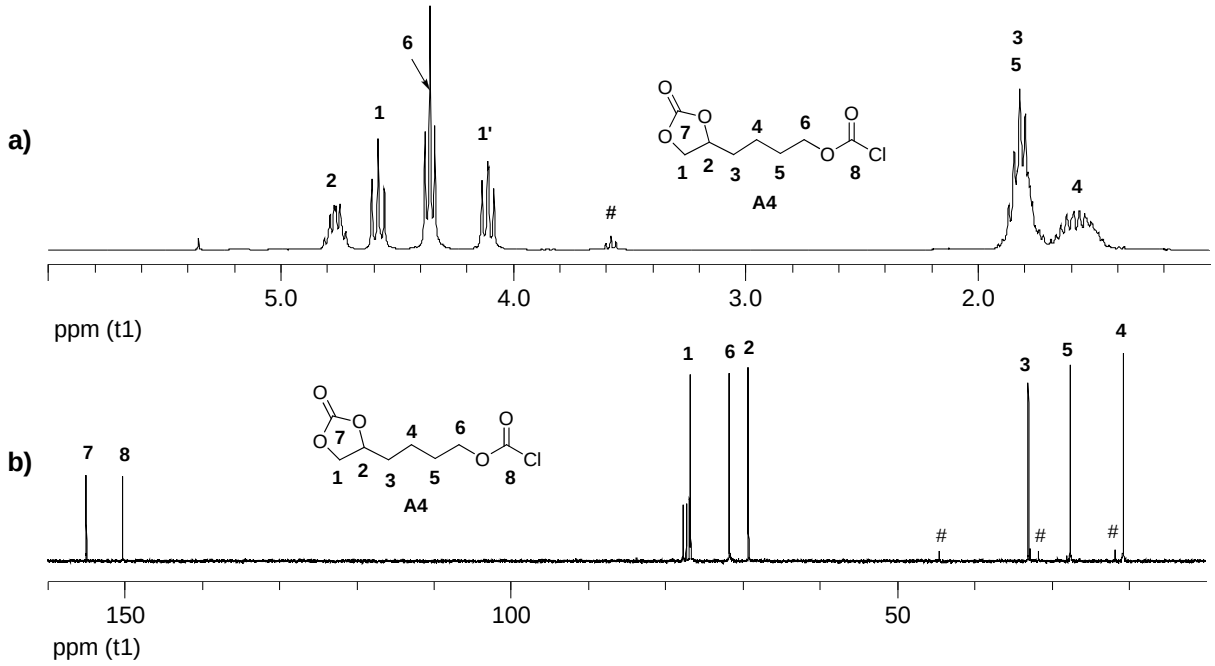


SI-Fig3: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **A3** in $CDCl_3$. (# peaks for impurities)

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.37 (dd, 1H, ^{2}J=6.2Hz, ^{3}J=9.0Hz, H^{1}), 4.50 (dd, 1H, ^{2}J = 4.8Hz, ^{3}J = 12.4Hz, H^{1}), 4.65 (m, 2H, H^{3}), 5.00-5.14 (m, 1H, H^{2}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 65.37 (C^{1}), 69.20 (C^{3}), 73.02 (C^{2}), 150.30 (C^{5}), 154.16 (C^{4}) ppm

4-(2-Oxo-1,3-dioxolan-4-yl)butyloxy chloroformate (A4)

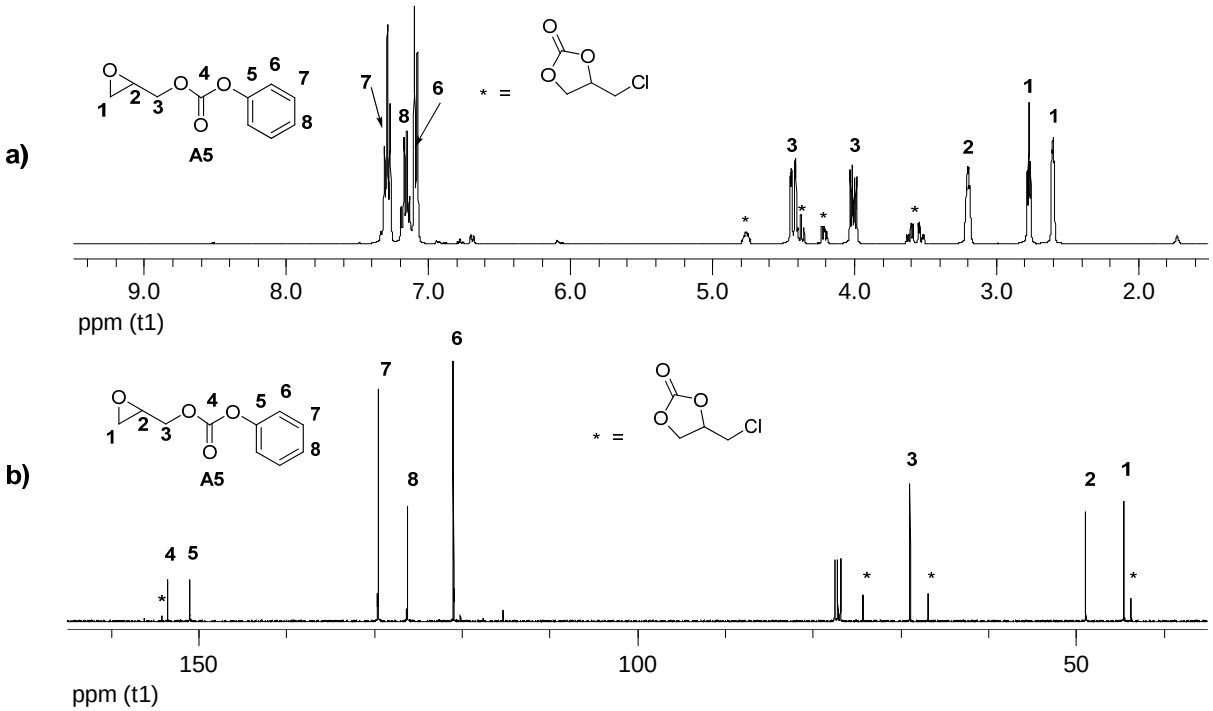


SI-Fig4: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **A4** in $CDCl_3$. (# peaks for impurities)

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.40-170 (m, 1 H, H^4), 1.70-1.94 (m, 2 H, H^3 and H^5), 4.14 (dd, 1 H, ^{2}J = 8.2 Hz, ^{3}J = 7.3 Hz, H^1), 4.36 (t, 1 H, ^{3}J = 6.4 Hz, H^6), 4.58 (dd, 1 H, $^{2}J = {}^{3}J$ = 8.1Hz, H^1), 4.70-4.84 (m, 1 H, H^2) ppm

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 20.89 (C^4), 27.78 (C^3), 33.19 (C^5), 69.43 (C^1), 71.91 (C^6), 76.93 (C^2), 150.43 (C^8), 155.09 (C^7) ppm

Oxiran-2-ylmethyl phenyl carbonate (A5)

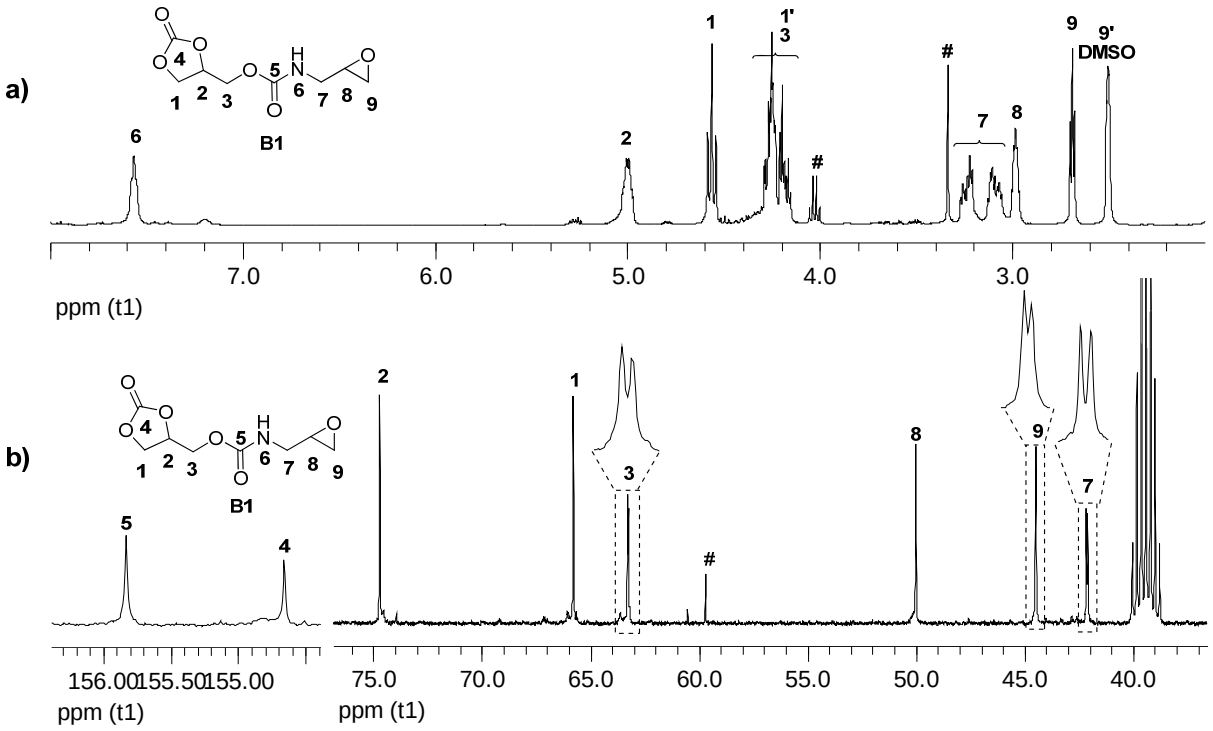


SI-Fig5: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **A5** in $CDCl_3$, which is 30 % converted to (4-chloromethyl)-1,3-dioxolan-2-one.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 2.61 (dd, 1H, ^{2}J = 2.6 Hz, ^{3}J = 4.8 Hz, H^{1}), 2.77 (t, 1H, ^{3}J = 4.5 Hz, H^{1}), 3.21 (m, 1H, H^{2}), 4.02 (dd, 1H, ^{3}J = 6.3 Hz, ^{2}J = 12.1 Hz, H^{3}), 4.46 (dd, 1H, ^{3}J = 3.0 Hz, ^{2}J = 12.1 Hz, H^{3}), 7.16-7.39 (m, 5H, phenyl) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 44.5 (C^{1}), 48.9 (C^{2}), 68.9 (C^{3}), 121.1 (2C, C^{6}), 126.2 (C^{8}), 129.5 (2C, C^{7}), 151.0 (C^{5}), 153.5 (C^{4}) ppm.

(2-Oxo-1,3-dioxolan-4-yl)methyl N-(oxiran-2-yl)methyl carbamate (B1)



SI-Fig6: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **B1** in DMSO-d_6. (# = ethylacetat)

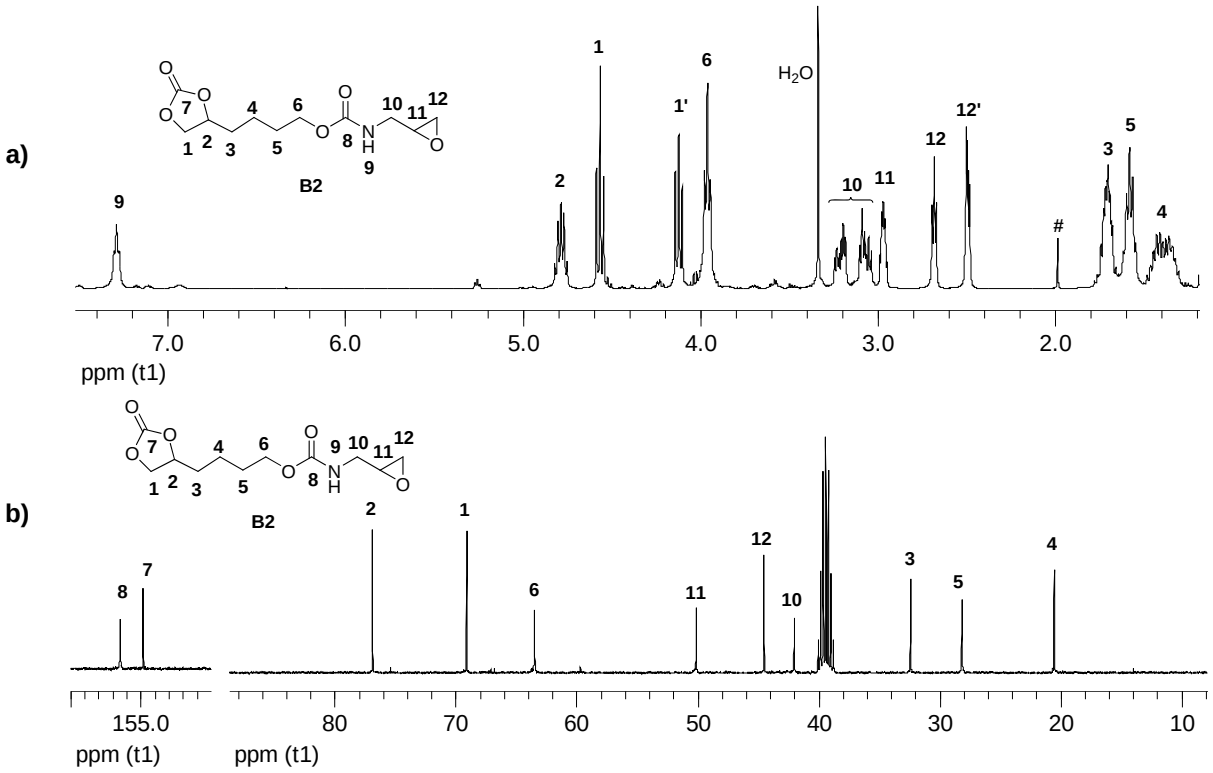
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.48-2.54 (m, 1H, H^{9}), 2.69 (t, 1H, ^{3}J=4.5 Hz; $H^{9'}$), 2.96-3.02 (m, 1H, H^{8}), 3.03-3.15 (m, 1H, H^{7}), 3.15-3.30 (m, 1H, $H^{7'}$), 4.10-4.30 (m, 3H, $H^{2'}$ and H^{3}), 4.56 (t, 1H, ^{3}J = 8.6 Hz, H^{2}), 4.94-5.10 (m, 1H, H^{2}), 7.54-7.64 (br, 1H, H^{6}) ppm

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 42.11 (C^{7}), 42.17 ($C^{7'}$), 44.48 (C^{9}), 44.52 ($C^{9'}$), 50.0 (C^{8}), 63.27 (C^{3}), 63.31 ($C^{3'}$), 65.8 (C^{1}), 74.7 (C^{2}), 154.7 (C^{4}), 155.8 (C^{5}) ppm

^{1}H NMR ($CDCl_3$): δ = 2.61 (dd, 1H, ^{2}J = 2.6 Hz, ^{3}J = 4.6 Hz, H^{9}), 2.80 (t, 1H, ^{3}J=4.3 Hz; $H^{9'}$), 3.05-3.15 (m, 1H, H^{8}), 3.15-3.30 (m, 1H, H^{7}), 3.57 (ddd, 1H, ^{2}J = 3.2 Hz, ^{3}J = 5.9 Hz, ^{2}J = 14.4 Hz, $H^{7'}$), 4.22-4.43 (m, 3H, $H^{2'}$ and H^{3}), 4.58 (t, 1H, ^{3}J = 8.6 Hz, H^{2}), 4.91-5.05 (m, 1H, H^{2}), 5.40-5.93 (br, 1H, H^{6}) ppm

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 42.52, 42.60 (C^{7}), 45.13 (C^{9}), 50.58 (C^{8}), 63.70 (C^{3}), 66.08 (C^{1}), 74.56 (C^{2}), 154.93 (C^{4}), 155.92 (C^{5}) ppm

4-(2-Oxo-1,3-dioxolan-4-yl)butyl N-(oxiran-2-yl)methyl carbamate (B2)



SI-Fig7: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **B2** in DMSO-d_6. (# = ethylacetat)

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 1.26-1.50 (m, 2 H, H^4), 1.50-1.65 (m, 2 H, H^5), 1.65-1.78 (m, 2 H, H^3), 2.49 (dd, 1 H, ^2J = 2.6 Hz, ^3J = 5.1 Hz, H^{12}), 2.68 (dd, 1 H, ^2J = ^3J = 4.5 Hz, H^{12}), 2.94-3.00 (m, 1 H, H^{11}), 3.07 (td, 1 H, ^3J = 2.6 Hz, ^2J = 5.1 Hz, H^{10}), 3.21 (ddd, 1 H, ^3J = 4.4 Hz, ^3J = 5.6 Hz, ^2J = 14.4 Hz, $H^{10'}$), 3.96 (dt, 1 H, ^3J = 6.4 Hz, H^6), 4.12 (dd, 1 H, ^3J = ^2J = 8.1 Hz, H^1), 4.57 (dd, 1 H, ^3J = ^2J = 8.1 Hz, $H^{1'}$), 4.73-4.84 (m, 1 H, H^2), 7.29 (t, 1 H, ^3J = ^2J = 5.5 Hz, H^9) ppm

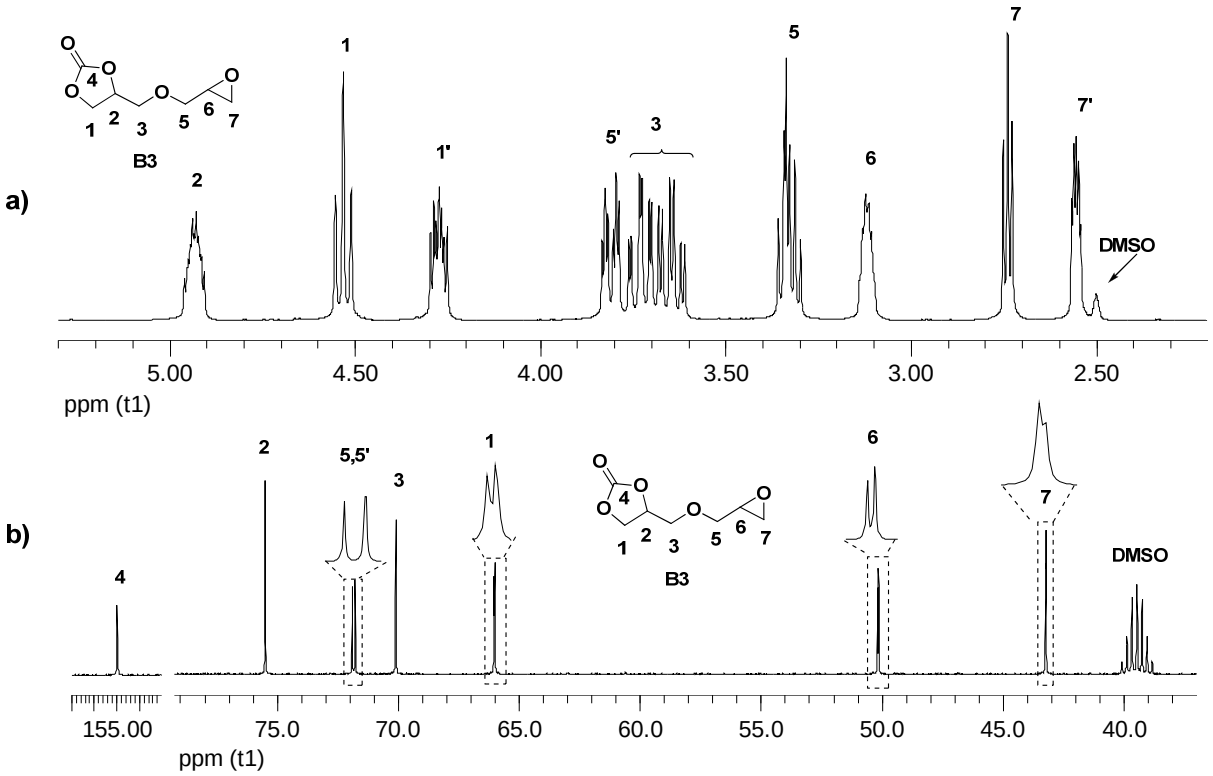
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 20.6 (C^4), 28.2 (C^5), 32.4 (C^3), 42.0 (C^{10}), 44.5 (C^{12}), 50.1 (C^{11}), 63.5 (C^6), 69.1 (C^1), 76.9 (C^2), 154.8 (C^7), 156.5 (C^8) ppm

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.34-1.62 (m, 2 H, H^4), 1.62-1.73 (m, 2 H, H^5), 1.73-1.92 (m, 2 H, H^3), 2.60 (dd, 1 H, ^2J = 2.6 Hz, ^3J = 4.7 Hz, H^{12}), 2.79 (dd, 1 H, ^2J = ^3J = 4.6 Hz, H^{12}), 3.05-3.15 (m, 1 H, H^{11}), 3.24 (td, 1 H, ^3J = 5.7 Hz, ^2J = 14.6 Hz, H^{10}), 3.40-3.66 (m, 1

H, H^{10}), 4.00-4.18 (m, 3 H, $H^{1'}$ and H^{6}), 4.57 (t, 1 H, ^{3}J = 8.1 Hz, H^{1}), 4.75 (m, 1 H, H^{2}) and 5.20-5.46 (br., 1 H, H^{9}) ppm

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.0 (C^{4}), 28.5 (C^{5}), 33.4 (C^{3}), 42.3 (C^{10}), 45.1 (C^{12}), 50.7 (C^{11}), 64.4 (C^{6}), 69.4 (C^{1}), 77.0 (C^{2}), 155.2 (C^{7}), 156.8 (C^{8}) ppm

(2-Oxo-1,3-dioxolan-4yl)methyl (oxiran-2-yl)methyl ether (B3)



SI-Fig8: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **B3** in DMSO-d_6.

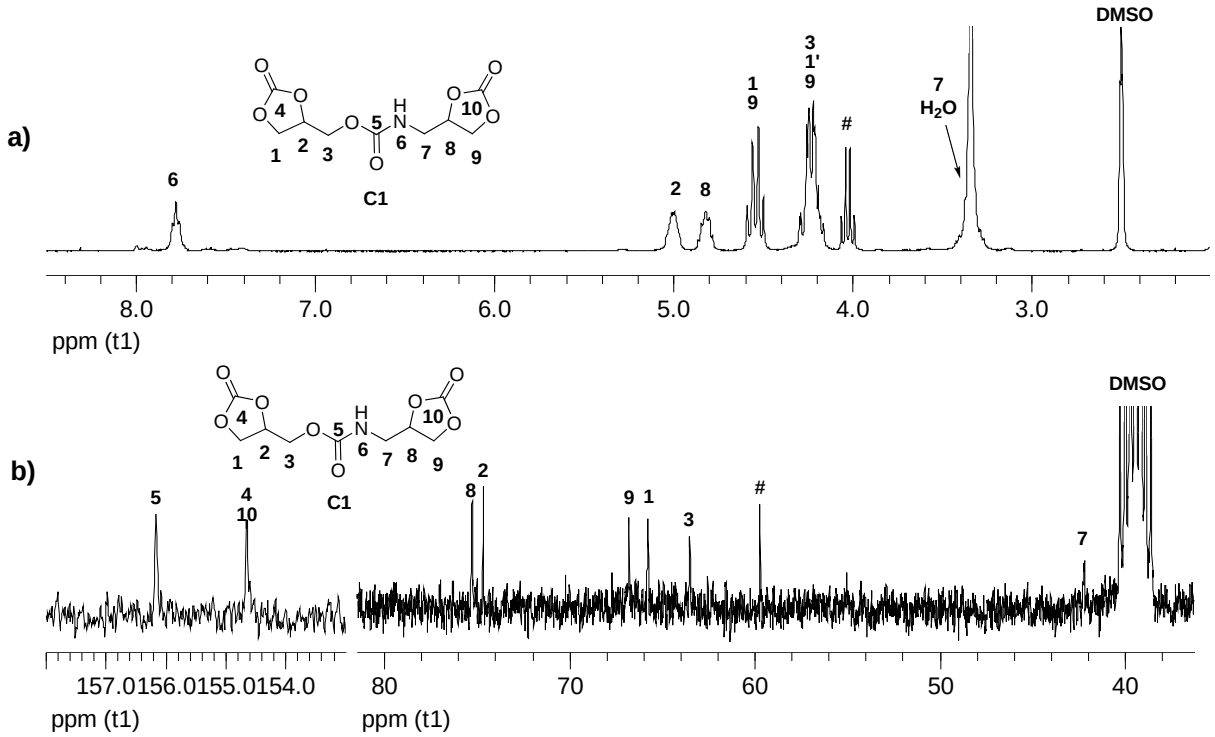
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 2.55 (td, 1H, ^{3}J = 2.2 Hz, ^{2}J = 4.7 Hz, H^{7}), 2.74 (t, 1H, ^{3}J = 4.7 Hz, $H^{7'}$), 3.08-3.15 (m, 1H, H^{6}), 3.33 (td, 1H, ^{3}J = 6.2 Hz, ^{2}J = 12.1 Hz, H^{5}), 3.65 (dd, 1H, ^{3}J = 4.3 Hz, ^{2}J = 11.5 Hz, H^{3}), 3.73 (dd, 1H, ^{3}J = 2.8 Hz, ^{2}J = 10.1 Hz, $H^{3'}$), 3.81 (dd, 1H, ^{3}J = 3.0 Hz, ^{2}J = 11.8 Hz, $H^{5'}$), 4.24-4.31 (m, 1H, H^{1}), 4.53 (t, 1H, ^{3}J = 8.5 Hz, $H^{1'}$), 4.89-4.96 (m, 1H, H^{2}) ppm

^{13}C NMR (DMSO-d$_6$, 101 MHz): δ = 43.2 (C^7), 50.14 and 50.18 (C^6), 66.0 (C^1), 70.1 (C^3), 71.78 and 71.90 (C^5), 75.5 (C^2), 155.0 (C^4) ppm

^{1}H NMR (CDCl$_3$, 300 MHz): δ = 2.57-2.66 (m, 1H, $H^{7'}$, H^7), 2.78-2.84 (m, 1H, H^7, $H^{7'}$), 3.12-3.20 (m, 1H, H^6, $H^{6'}$), 3.38 (dd, 1 H, ^2J =11.76 Hz, ^3J = 6.43 Hz, 0.40H, H^5), 3.48 (dd, ^2J = 11.94 Hz, ^3J = 5.70 Hz, 0.60H, $H^{5'}$), 3.68 (dd, ^2J = 11.12 Hz, ^3J = 3.65 Hz, 0.6H, $H^{3'}$), 3.77 (d, 2J = 3.65, 0.8H, H^3), 3.84 (dd, ^2J = 11.12 Hz, ^3J = 3.88 Hz, 0.60H, $H^{3'}$), 3.90 (dd, ^2J = 11.86 Hz, ^3J = 2.45 Hz, 0.60H, $H^{5'}$), 3.94 (dd, ^2J = 11.73 Hz, ^3J = 2.37 Hz, 0.40H, H^5), 4.35-4.57 (m, 2H, H^1, $H^{1'}$), 4.78-4.90 (m, 1H, H^2) ppm.

^{13}C NMR (CDCl$_3$, 75 MHz): δ = 43.8 and 43.8 (C^7), 50.6 and 50.7 (C^6), 66.1 (C^1), 70.2 and 70.3 (C^3), 71.9 and 72.6 (C^5), 75.0 (C^2), 154.9 (C^4) ppm.

(2-Oxo-1,3-dioxolan-4-yl)methyl N-(2-oxo-1,3-dioxolan-4-yl)methyl carbamate (C1)

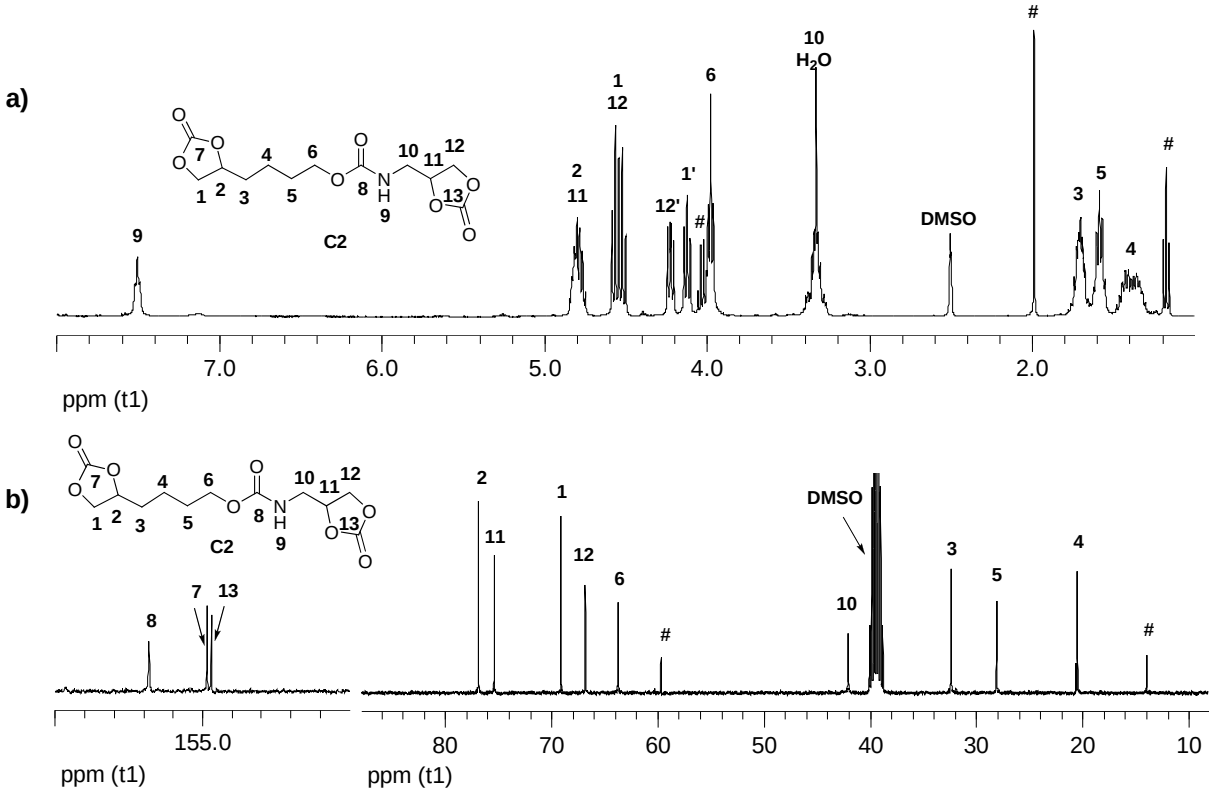


SI-Fig9: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **C1** in DMSO-d$_6$. (# = ethylacetat)

^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 3.59 (dd, 2 H, H^{1}, ^{3}J = 5.1 Hz, ^{3}J = 8.2 Hz, H^{7}), 4.15-4.33 (m, 4 H, H^{1}, H^{9} and H^{3}), 4.54 (dd, 1 H, ^{2}J = ^{3}J = 8.5 Hz, H$^{9'}$), 4.57 (dd, 1 H, ^{2}J = ^{3}J = 8.6 Hz, H$^{1'}$), 4.75-4.90 (m, 1 H, H^{8}), 4.95-5.08 (m, 1 H, H^{2}) and 7.79 (t, 1 H, ^{3}J = 5.8 Hz, H^{6}) ppm

^{13}C NMR (DMSO-d$_6$, 75 MHz): δ = 42.2 (C^{7}), 63.5 (C^{3}), 65.8 (C^{1}), 66.8 (C^{9}), 74.7 (C^{2}), 75.3 (C^{8}), 154.7 (2 C, C^{4} and C^{10}), 156.2 (C^{5}) ppm

4-(2-Oxo-1,3-dioxolan-4-yl)butyl N-(2-oxo-1,3-dioxolan-4-yl)methyl carbamate (C2)



SI-Fig10: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of coupler **C2** in DMSO-d$_6$.

^{1}H NMR (DMSO-d$_6$, 400 MHz): δ = 1.20-1.50 (m, 2 H, H^{4}), 1.50-1.65 (m, 2 H, H^{5}), 1.65-1.80 (m, 2 H, H^{3}), 3.32 (dd, 2 H, ^{3}J = 7.8 Hz, ^{3}J = 14.9 Hz, H^{10}), 3.98 (t, 2 H, ^{3}J = 6.4 Hz, H^{6}), 4.12 (dd, 1 H, ^{3}J = 7.3 Hz, ^{3}J = 8.1 Hz, H^{1}), 4.23 (dd, 1 H, ^{3}J = 6.3 Hz, ^{3}J = 8.5 Hz, H^{12}), 4.52

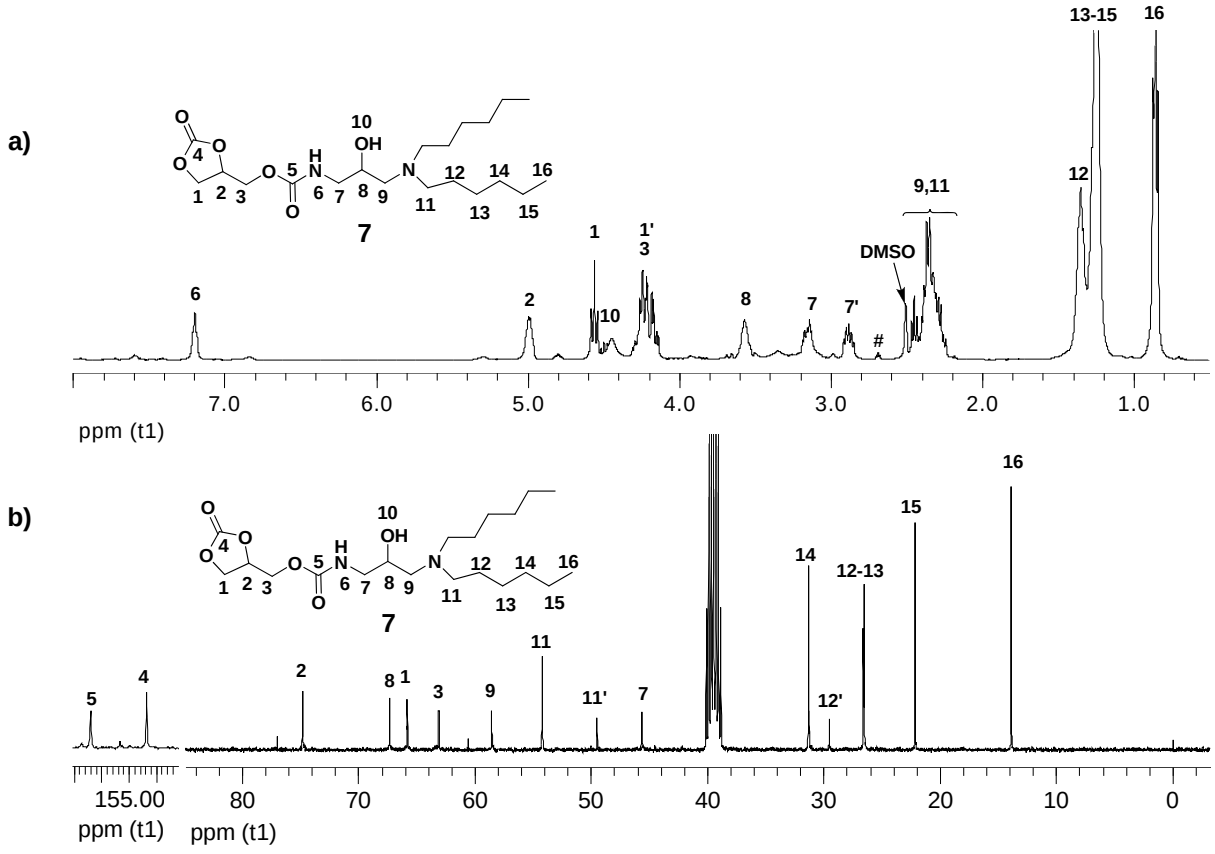
(t, 1 H, ^{3}J = 8.4 Hz, H^{12}), 4.57 (t, 1 H, ^{3}J = 8.1 Hz, H^{1}), 4.71-4.87 (m, 2 H, H^{2} and H^{11}), 7.50 (t, 1 H, ^{3}J = 5.8 Hz, H^{9}) ppm

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 20.5 (C^{4}), 28.1 (C^{5}), 32.4 (C^{3}), 42.1 (C^{10}), 63.7 (C^{6}), 66.8 (C^{12}), 69.1 (C^{1}), 75.9 (C^{2}), 76.9 (C^{11}), 154.7 (C^{13}), 154.8 (C^{7}), 156.8 (C^{8}) ppm

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.35-1.62 (m, 2 H, H^{4}), 1.62-1.74 (m, 2 H, H^{5}), 1.74-1.92 (m, 2 H, H^{3}), 3.52 (dd, 2 H, ^{3}J = 5.7 Hz, ^{3}J = 4.7 Hz, H^{10}), 4.03-4.18 (m, 3 H, H^{6} and H^{1}), 4.10 (dd, 1 H, ^{3}J = 6.9 Hz, ^{3}J = 13.7 Hz, H^{12}), 4.31 (dd, 1 H, ^{3}J = 6.6 Hz, ^{3}J = 8.6 Hz, H^{12}), 4.57 (dd, 1 H, ^{3}J = 8.1 Hz, H^{1}), 4.70-4.82 (m, 1 H, H^{2}), 4.82-4.93 (m, 1 H, H^{11}), 6.02 (t, 1 H, ^{3}J = 5.6 Hz, H^{9}) ppm

^{13}C NMR ($CDCl_3$, 75 MHz): δ = 21.0 (C^{4}), 28.4(C^{5}), 33.3 (C^{3}), 42.6 (C^{10}), 64.7 (C^{6}), 67.0 (C^{12}), 69.5 (C^{1}), 75.7 (C^{11}), 77.8 (C^{2}), 155.0 (C^{13}), 155.3 (C^{7}), 157.3 (C^{8}) ppm

(2-Oxo-1,3-dioxolan-4-yl)methyl 3-(dihexylamino)-2-hydroxypropylcarbamate (7)

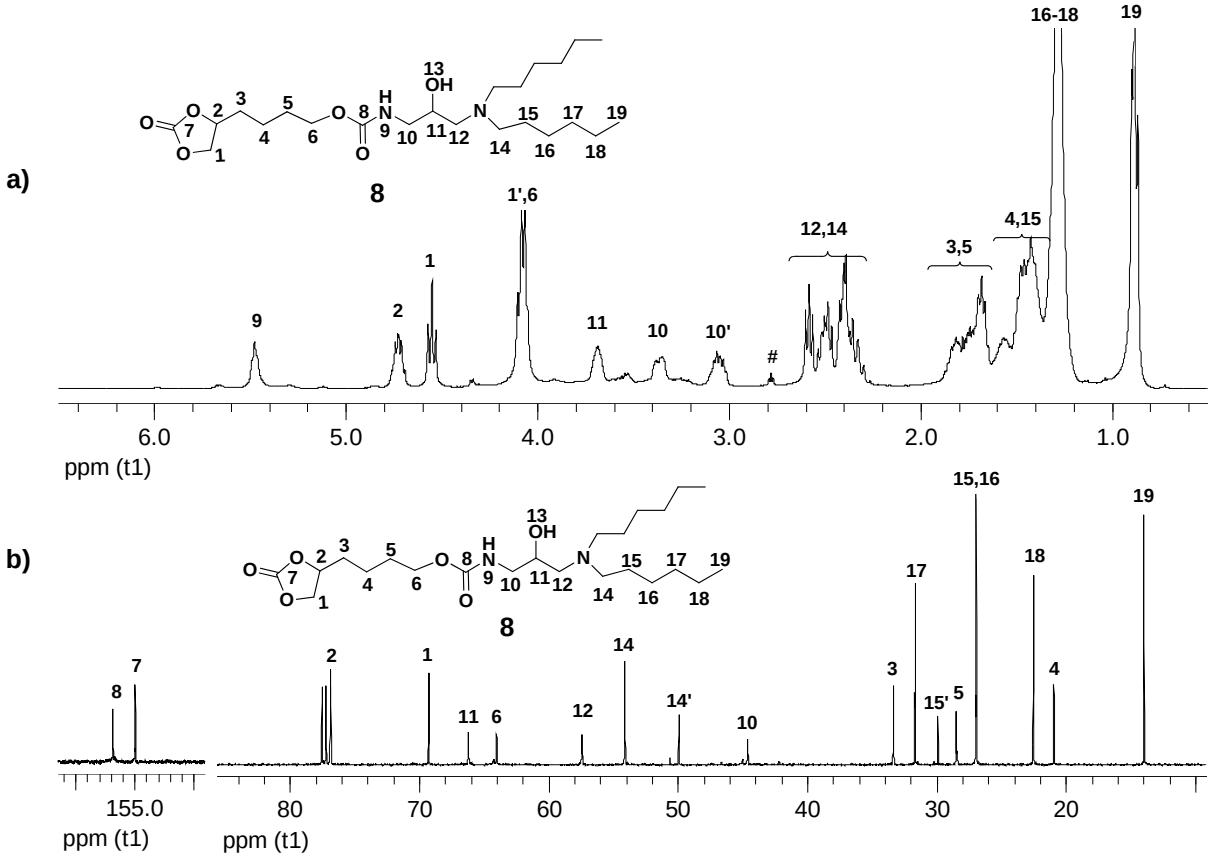


SI-Fig11: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of functional coupler **7** in DMSO-d_6. (# = excess of dihexylamine)

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.86 (t, 6 H, ^{3}J = 6.4 Hz, H^{16}), 1.15-1.30 (br., 12 H, H^{13-15}), 1.30-1.40 (br., 4 H, H^{12}), 2.20-2.45 (m, 6 H, H^{11} and H^{9}), 2.84-2.94 (m, 1 H, H^{7}), 3.05-3.20 (m, 1 H, $H^{7'}$), 3.30-3.60 (br., 2 H, H^{8} and H^{10}), 4.10-4.32 (m, 3 H, H^{1} and H^{3}), 4.57 (dd, 1 H, ^{3}J = 8.5 Hz, $H^{1'}$), 4.95-5.05 (m, 1 H, H^{2}), 7.15-5.25 (br., 1 H, H^{6}) ppm

^{13}C NMR (101 MHz, DMSO-d_6): δ = 13.8 (C^{16}), 22.1 (C^{15}), 26.45 (C^{13}), 26.51 (C^{12}), 31.2 (C^{14}), 45.6 (C^{7}), 54.2 (C^{11}), 58.5 (C^{9}), 63.1 (C^{3}), 65.8 (C^{1}), 67.3 (C^{8}), 74.8 (C^{2}), 154.6 (C^{4}), 155.7 (C^{5}) ppm

4-(2-Oxo-1,3-dioxolan-4-yl)butyl 3-(dihexylamino)-2-hydroxypropylcarbamate (8)



SI-Fig12: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of functional coupler **8** in $CDCl_3$. (# = excess of dihexylamine)

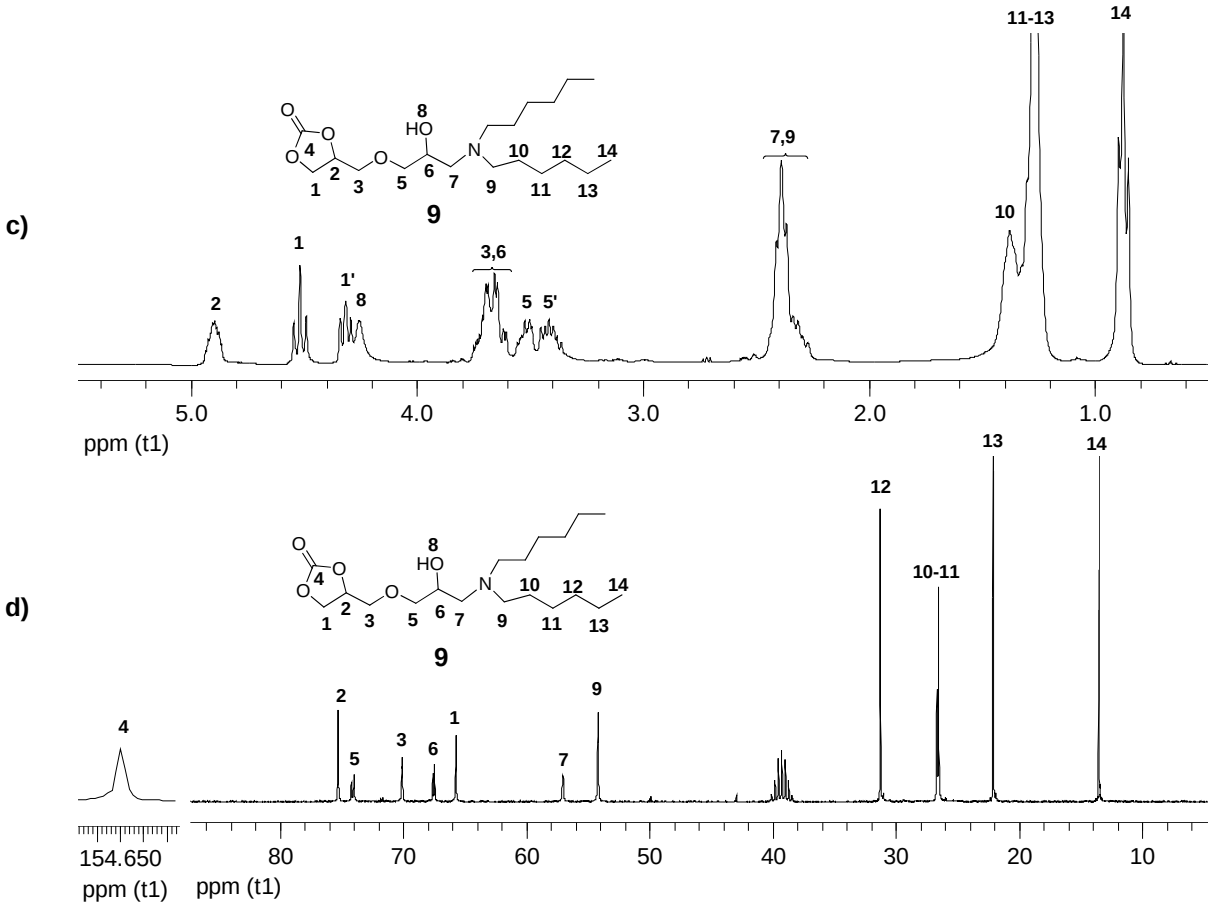
^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83-0.95 (m, 6 H, H^{19}), 1.20-1.36 (br., 12 H, H^{16-18}), 1.36-1.62 (m, 6 H, H^{4} and H^{15}), 1.62-1.90 (m, 4 H, H^{3} and H^{5}), 2.25-1.65 (m, 6 H, H^{12} and H^{14}), 2.95-3.15 (m, 1 H, H^{10}), 3.30-3.42 (m, 1 H, $H^{10'}$), 3.63-3.75 (m, 1 H, H^{11}), 4.00-4.20 (m, 3 H, $H^{1'}$ and H^{6}), 4.55 (dd, 1 H, ^{3}J = 8.1 Hz, H^{1}), 4.65-4.80 (m, 1 H, H^{2}), 5.40-5.60 (m, 1 H, H^{9}) ppm

^{13}C NMR (101 MHz, $CDCl_3$): δ = 13.9 (C^{19}), 20.9 (C^{4}), 22.5 (C^{18}), 26.9 (2 C, C^{15-16}), 28.5 (C^{5}), 31.7 (C^{17}), 33.4 (C^{3}), 44.6 (C^{10}), 54.1 (C^{14}), 57.4 (C^{12}), 64.0 (C^{6}), 66.2 (C^{11}), 69.3 (C^{1}), 76.8 (C^{2}), 155.0 (C^{7}), 156.9 (C^{8}) ppm

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.76-0.96 (m, 6 H, H^{19}), 1.15-1.30 (br., 12 H, H^{16-18}), 1.30-1.50 (br., 6 H, H^{4} and H^{15}), 1.52-1.63 (m, 2 H, H^{5}), 1.63-1.78 (m, 2 H, H^{3}), 2.20-2.45 (m, 6 H, H^{12} and H^{14}), 2.84-2.94 (m, 1 H, H^{10}), 3.05-3.20 (m, 1 H, $H^{10'}$), 3.30-3.60 (br., 2 H, H^{11} and H^{13}), 3.85-4.00 (m, 2 H, H^{6}), 4.05-4.17 (m, 1 H, H^{1}), 4.56 (dd, 1 H, ^{3}J = 8.1 Hz, $H^{1'}$), 4.70-4.85 (m, 1 H, H^{2}), 6.85-6.95 (t, 1 H, ^{3}J = 5.28 Hz, H^{9}) ppm

^{13}C NMR (101 MHz, DMSO-d_6): δ = 13.8 (C^{19}), 20.6 (C^{4}), 22.1 (C^{18}), 26.45 (C^{16}), 26.51 (C^{15}), 28.3 (C^{5}), 31.2 (C^{17}), 32.5 (C^{3}), 45.5 (C^{10}), 54.2 (C^{14}), 58.5 (C^{12}), 63.3 (C^{6}), 67.4 (C^{11}), 69.1 (C^{1}), 76.9 (C^{2}), 154.8 (C^{7}), 156.3 (C^{8}) ppm

4-((3-(Dihexylamino)-2-hydroxypropoxy)methyl)-1,3-dioxolan-2-one (9)



SI-Fig13: a) ^{1}H-NMR- (400 MHz) and b) ^{13}C-NMR (101 MHz) spectra with signal assignments of functional coupler **B3-Ad6** in DMSO-d_6.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 0.85-0.95 (t, 6 H, ^{3}J = 6.4 Hz, H^{14}), 1.15-1.30 (br., 12 H, $H^{11\text{-}13}$), 1.30-1.40 (br., 4 H, H^{10}), 2.20-2.50 (m, 6 H, H^{9} and H^{6}), 3.30-3.45 (m, 2 H, H^{4}), 3.45-3.60 (m, 1 H, $H^{4'}$), 3.60-3.80 (m, 3 H, H^{3} and H^{5}), 4.20-4.30 (br., 1 H, H^{8}), 4.32 (t, 1 H, ^{3}J = 8.1 Hz, H^{1}), 4.52 (t, 1 H, ^{3}J = 8.0 Hz, $H^{1'}$), 4.80-4.95 (m, 1 H, H^{2}) ppm

^{13}C NMR (101 MHz, DMSO-d_6): δ = 13.6 (C^{14}), 21.2 (C^{13}), 26.57 and 26.72 ($C^{10\text{-}11}$), 31.3 (C^{12}), 54.2 (C^{9}), 57.1 (C^{6}), 65.8 (C^{1}), 67.49 and 67.63 (C^{5}), 70.1 (C^{3}), 74.03 and 74.23 (C^{4}), 75.3 (C^{2}), 154.6 (C^{7}) ppm

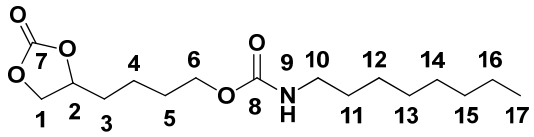
Appendix B

Amphiphilic Building Blocks

for Head Groups in Linear Polymers

Synthesis

Synthesis of 4-(2-Oxo-1,3-dioxolan-4-yl)butyl octylcarbamate (C_6A8)

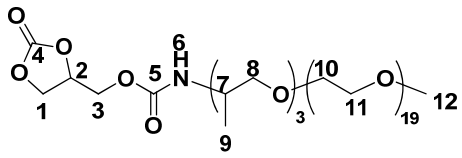


4-(2-Oxo-1,3-dioxolan-4-yl)butyl phenyl carbonate (4.00 g, 14.3 mmol) was dissolved in THF (30 mL) and cooled to 0 °C. A solution of octylamine (1.85 g, 14.3 mmol) in tetrahydrofuran (30 mL) was slowly added under stirring and the temperature was maintained at 0 °C for 2 h and at room temperature for 24 h. The product was recrystallized from CH_2Cl_2/pentane (v/v = 1/1) at -18 °C. A white powder was obtained. Yield: 3.61 g (80 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.7 Hz, 1 H, H^{17}), 1.15-1.38 (br., 10 H, $H^{12\text{-}16}$), 1.38-1.62 (m, 4 H, $H^{4,\,11}$), 1.62-1.90 (m, 4 H, $H^{3,5}$), 3.15 (dd, ^3J = 6.6 Hz, ^3J = 13.1 Hz, 2 H, H^{10}), 4.00-4.18 (br., 3 H, $H^{1,6}$), 4.54 (t, ^3J = 8.1 Hz, 1 H, $H^{1'}$), 4.65-4.80 (m, 2 H, $H^{2,\,9}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{17}), 21.0 (C^4), 22.6 (C^{16}), 26.8 (C^{11}), 28.6 (C^5), 29.21, 29.25, 29.98 ($C^{12\text{-}14}$), 31.8 (C^{15}), 33.5 (C^3), 41.1 (C^{10}), 63.9 (C^6), 69.3 (C^1), 76.9 (C^2), 155.0 (C^7), 156.6 (C^8) ppm

Synthesis of (2-oxo-1,3-dioxolan-4-yl)methyl N-1-(1-(2-methoxyethoxy)propan-2-yloxy) propan-2-ylcarbamate (C_3J1000)

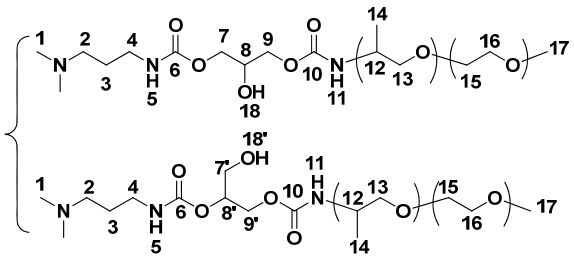


(2-Oxo-1,3-dioxolan-4-yl)methyl chloroformate (**A3**, 4.80 g, 26.1 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. A solution of JEFFAMINE® M1000 (28.65 g, 26.1 mmol) and triethylamine (2.64 g, 28.6 mmol) in THF (45 mL) was slowly added under stirring and and the temperature was kept below 5 °C for 2 h and at room temperature for another 18 h. The reaction mixture was filtrated and the formed triethylamine hydrochlorid salt was removed. After removal of solvent the product was purified by precipitation in pentane and dried in vacuum (10^{-2} mbar) at room temperature. A waxy solid was obtained. Yield: 27.45 g (85 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.05-1.30 (m, 9 H, H^9), 3.38 (s, 3 H, H^{12}), 3.40-3.58 (m, 8 H, $H^{7\text{-}8}$), 3.60-3.70 (br. 76 H, $H^{10\text{-}11}$), 3.85-3.95 (br. 1 H, $H^{7'}$, next to the urethane group), 4.22-4.40 (m, 3 H, $H^{1,3}$), 4.55 (t, ^3J = 8.6 Hz, 1 H, $H^{1'}$), 4.86-5.00 (m, 1 H, H^2), 5.40-6.00 (m, 1 H, H^6) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 16.92, 17.08, 17.46 (3 C, C^9), 47.20, 47.40, 47.46 (1 C, $C^{7'}$, next to the urethane group), 58.7 (C^{12}), 63.0 (C^3), 65.8 (C^1), 70.4 ($C^{10\text{-}11}$), 71.7 (C^8), 74.4 (C^2), 74.9 (2 C, C^7), 154.5 (C^4), 154.9 (C^5) ppm

Synthesis of C_3J1000-Q



(C_3J1000-Qa)

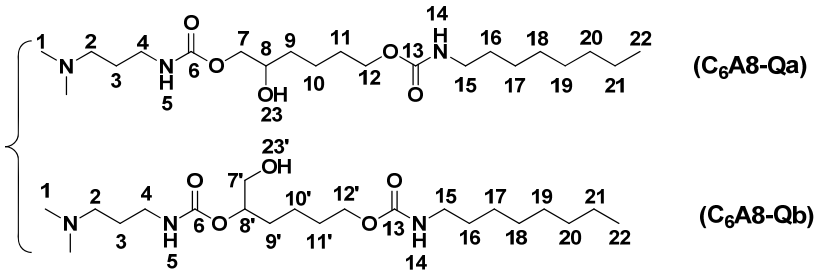
(C_3J1000-Qb)

C_3J1000 (12.53 g, 10.1 mmol) and N,N-dimethyl-1,3-propanediamine (1.54 g, 15.1 mmol) were dissolved in $CHCl_3$ (20 mL) and stirred at 70 °C for 24 h. Solvent and excess N,N-dimethyl-1,3-propanediamine were removed in vacuum (10^{-2} mbar, 4 h, 40 °C). A white powder was obtained. Yield: quantitative. Ratio of the two regioisomers: a/b = 73/27.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.05-1.25 (m, 9 H, H^{14}), 1.60-1.75 (m, 2 H, H^3), 2.23 (s, 6 H, H^1), 2.34 (t, ^3J = 6.9 Hz, 2 H, H^2), 3.22 (dd, ^3J = 6.3 Hz, ^3J = 12.4 Hz, 2 H, H^4), 3.37 (s, 3 H, H^{17}), 3.40-3.58 (m, 8 H, $H^{12,13}$), 3.58-3.74 (br., 76 H, $H^{15,16}$), 3.73-3.95 (br. 1.6 H, $H^{7'}$ and H^{12}, the one next to the urethane group), 3.95-4.20 (br., 2,66 H, $H^{7\text{-}9}$), 4.20-4.30 (m, 0.5 H, $H^{9'}$), 4.30-4.50 (br., 1 H, $H^{18,18'}$), 4.82-4.94 (m, 0.22 H, $H^{8'}$), 5.40-6.20 (m, 2 H, $H^{5,11}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 16.96, 17.68 (C^{14}), 26.9 (C^3), 39.7 (C^4), 45.1 (C^1), 57.4 (C^2), 58.8 (C^{17}), 60.2 ($C^{7'}$), 63.00 ($C^{9'}$), 65.6 ($C^{7,9}$), 68.16 (C^8), 70.4 ($C^{15,16}$), 71.7 (C^{13}), 74.85 (C^{12}), 75.54 ($C^{8'}$), 155.92, 156.59 ($C^{6,10}$) ppm

Synthesis of C_6A8-Q

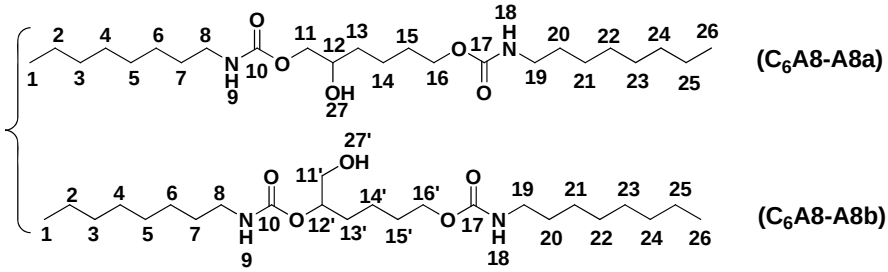


C_6A8 (4.50 g, 14.28 mmol) and N,N-dimethyl-1,3-propanediamine (2.04 g, 19.99 mmol) were dissolved in $CHCl_3$ (20 mL) and stirred at 70 °C for 24 h. Solvent and excess N,N-dimethyl-1,3-propanediamine were removed in vacuum (10^{-2} mbar, 4 h, 40 °C). A white powder was obtained. Yield: quantitative. Ratio of the two regioisomers: a/b = 34/66.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 0.88 (t, ^3J = 7.0 Hz, 3 H, H^{22}), 1.18-1.32 (br., 12 H, $H^{10,10'}$ and H^{17-21}), 1.33-1.48 (m, 4 H, $H^{11,11',16}$), 1.48-1.60 (m, 4 H, $H^{3,9,9'}$), 2.10 (s, 6 H, H^1), 2.18 (t, ^3J = 7.1 Hz, 2 H, H^2), 2.94 (dd, ^3J = 6.6 Hz, ^3J = 13.2 Hz, 2 H, H^{15}), 2.98 (dd, ^3J = 6.4 Hz, ^3J = 12.6 Hz, 2 H, H^4), 3.33-3.45 (m, 1.32 H, $H^{7'}$), 3.53-3.63 (m, 0.34 H, H^8), 3.76-3.84 (m, 0.68 H, H^7), 3.86-3.96 (m, 2 H, $H^{12,12'}$), 4.53-4.64 (m, 0.66 H, $H^{8'}$), 4.66-4.78 (m, 1 H, $H^{23,23'}$), 6.60-7.10 (m, 2 H, $H^{5,14}$) ppm

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 14.4 (C^{22}), 21.88 ($C^{10'}$), 21.98 (C^{10}), 22.6 (C^{21}), 26.7 (C^{16}), 27.9 (C^3), 28.21(C^9), 29.16, 29.20 ($C^{18,19}$), 29.33 ($C^{9'}$), 29.91 (C^{17}), 30.9 ($C^{10'}$), 31.7 (C^{20}), 33.7 (C^{10}), 39.0 (C^{15}), 40.6 (C^4), 45.6 (C^1), 57.1 (C^2), 63.1 ($C^{7'}$), 63.87, 63.97 ($C^{12,12'}$), 68.31 (C^7), 68.60 (C^8), 74.3 ($C^{8'}$), 156.70, 156.74, 156.77 ($C^{6,13}$) ppm

Synthesis of C_6A8-A8

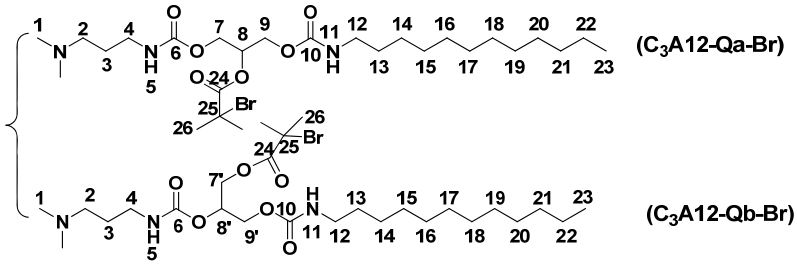


C_6A8 (2.01 g, 6.38 mmol) and *n*-octylamine (990 mg, 7.66 mmol) were dissolved in $CHCl_3$ (20 mL) and stirred at 70 °C for 24 h. After dilution with $CHCl_3$ (100 mL), the reaction mixture was washed two times with 5 %-HCl aqueous solution and two times with brine. The organic phase was dried over sodium sulfate. After removal of the solvent, the product was obtained as white powder. Yield: 2.62 g (92 %). Ratio of the two regioisomers: a/b = 34/66

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.6 Hz, 6 H, $H^{1,26}$), 1.20-1.38 (br., 20 H, $H^{1\text{-}6}$ and $H^{21\text{-}25}$), 1.40-1.70 (br., 10 H, $H^{13\text{-}15,\ 13'\text{-}15'}$ and $H^{7,20}$), 3.14 (dd, ^3J = 6.2 Hz, ^3J = 12.7 Hz, 4 H, $H^{8,19}$), 3.30-3.52 (br., 1 H, $H^{27,27'}$), 3.53-3.73 (m, 1.32 H, $H^{11'}$), 3.73-3.86 (m, 0.34 H, H^{12}), 3.87-3.98 (m, 0.34 H, H^{11}), 3.98-4.16 (m, 2.34 H, $H^{11,16}$), 4.65-4.85 (br., 0.66 H, $H^{12'}$), 4.90-5.40 (m, 2 H, $H^{9,18}$) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.0 (2 C, $C^{1,26}$), 21.73, 21.78 ($C^{14,\ 14'}$), 22.6 (2 C, $C^{2,25}$), 26.7 (2 C, $C^{7,20}$), 28.80 ($C^{15'}$), 28.88 (C^{15}), 29.16, 29.20, 29.83, 29.92 (6 C, $C^{4\text{-}6,\ 21\text{-}23}$), 30.34 ($C^{13'}$), 31.7 (2 C, $C^{3,24}$), 32.80 (C^{13}), 40.94 (C^8), 41.05 (C^{19}), 54.5 (C^{11}), 64.33, 64.41 ($C^{16,\ 16'}$), 64.64 ($C^{11'}$), 69.9 (C^{12}), 75.7 ($C^{12'}$), 156.83, 156.92, 157.08 ($C^{10,\ 17}$) ppm

Synthesis of C_3A12-Q-Br



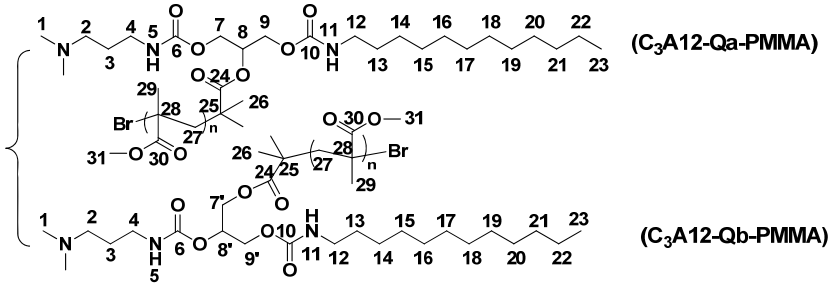
C_3A12-Q(a/b) (1.00 g, 2.31 mmol) was dissolved in dry THF (15 mL) under nitrogen atmosphere and cooled to 0 °C. A solution of 2-bromo-2-methylpropionyl bromide (692 mg, 3.01 mmol) was slowly added under stirring and the temperature was kept below 5 °C for 2 h and at room temperature for another 27 h. After removal of the solvent the residual was

diluted with CH_2Cl_2 (100 mL) and washed three times with $NaHCO_3$-saturated aqueous solution and the organic phase was dried over sodium sulfate. After removal of solvent and dried in vacuum (10^{-2} mbar, 3 h, room temperature), a white powder was obtained. Yield: 1.025 g (76 %).

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.85 (t, ^3J = 6.5 Hz, 3 H, H^{23}), 1.10-1.34 (br., 18 H, H^{14-22}), 1.35-1.55 (m, 2 H, H^{13}), 1.60-1.80 (m, 2 H, H^3), 1.90 (s, 6 H, H^{26}), 2.34 (s, 6 H, H^1), 2.52 (t, ^3J = 6.5 Hz, 3 H, H^2), 3.12 (dd, ^3J = 6.4 Hz, ^3J = 13.0 Hz, 2 H, H^{12}), 3.17-3.36 (m, 2 H, H^4), 3.90-4.50 (m, 4 H, $H^{7,7',9,9'}$), 4.60-5.00 (br., 1 H, H^5), 5.00-5.35 (br., 1 H, $H^{8,8'}$), 5.70-6.00 (br., 1 H, H^{11}) ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 14.1 (C^{23}), 22.7 (C^{22}), 26.2 (C^3), 26.7 (C^{13}), 29.28, 29.34, 29.54, 29.58, 29.62, 29.89, 30.32, 30.59, 30.65 (C^{14-19}), 31.9 (C^{26}), 32.1 (C^{20}), 39.7 (C^4), 41.2 (C^{12}), 44.6 (C^1), 55.7 (C^{25}), 57.1 (C^2), 62.3 (C^{7-9}), 71.5 (C^8), 155.47, 155.77, 155.86, 155.93 ($C^{6,10}$), 170.88 (C^{24}) ppm

Synthesis of C_3A12-Q-PMMA

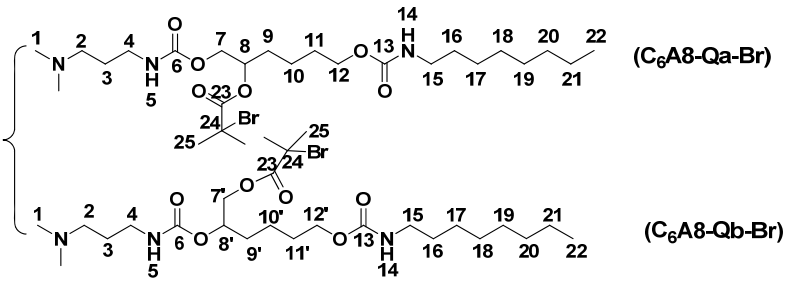


C_3A12-Q-PMMA was prepared from **C_3A12-Q-Br** according to the procedure described for **C_6A8-A8-PMMA**. Yield: 2.18 g (68 %). From GPC (THF): M_n = 19100 g/mol, M_w = 27600, M_w/M_n = 1.44.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.84 (s, $H^{23, 26, 29}$), 1.02 (s, $H^{14-21, 26, 29}$), 1.14 (s, H^{13}), 1.18-1.50 (m, $H^{26, 29}$), 1.70-2.30 (m, H^{27}), 2.75-2.94 (m, H^{12}), 3.06-3.24 (m, H^{4}), 3.60 (s, H^{31}), 4.00-4.40 (m, $H^{7, 9, 7', 9'}$), 4.80-5.20 (m, H8,8') ppm

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 13.9 (C^{23}), 16.1, 18.4 ($C^{26, 29}$), 21.9 (C^{3}), 22.6 (C^{22}), 26.2 (C^{13}), 28.2, 28.4, 29.0, 29.2, 29.4, 30.2, 31.2, 31.7 (C^{14-21}), 38.7 (C^{12}), 40.3 (C^{4}), 43.8, 44.2 ($C^{25, 28}$), 44.8 (C^{1}), 51.6 (C^{31}), 53.7 (C^{27}), 58.3 (C^{2}), 63.0, 63.8 ($C^{7, 7'}$), 66.2, 66.3 ($C^{9, 9'}$), 70.6, 71.2 ($C^{8, 8'}$), 155.8, 156.2 ($C^{6, 10}$), 176.1, 177.0, 177.3 ($C^{24, 30}$) ppm

Synthesis of C_6A8-Q-Br

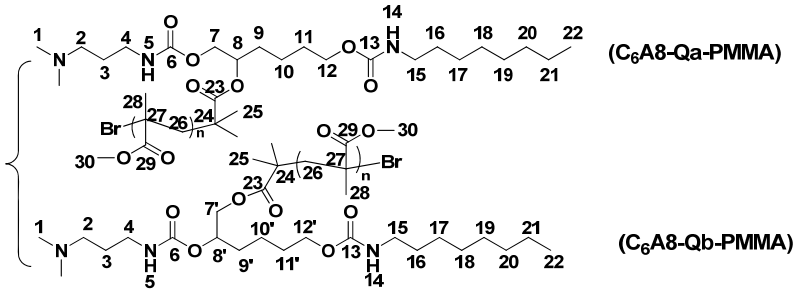


C_6A8-Q (**a/b**) (1.30 g, 3.11 mmol) was dissolved in dry THF (20 mL) under nitrogen atmosphere and cooled to 0 °C. A solution of 2-bromo-2-methylpropionyl bromide (618 mg, 2.69 mmol) was slowly added under stirring and and the temperature was kept below 5 °C for 2 h and at room temperature for another 27 h. After removal of the solvent the residue was diluted with CH_2Cl_2 (100 mL) and washed three times with $NaHCO_3$-saturated aqueous solution and the organic phase was dried over sodium sulfate. After removal of solvent the product was dried in vacuum (10^{-2} mbar, 3 h, room temperature). A white powder was obtained. Yield: 1.432 g (75 %).

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.88 (t, ^3J = 6.6 Hz, 3 H, H^{22}), 1.20-1.35 (br., 10 H, $H^{17\text{-}21}$), 1.40-1.55 (m, 5 H, $H^{9,10,16}$), 1.55-1.72 (m, 5 H, $H^{3,11,11',9'}$), 1.93 (s, 6 H, H^{25}), 2.20 (s, 6 H, H^1), 2.28-2.40 (m, 2 H, H^2), 3.14 (dd, ^3J = 6.6 Hz, ^3J = 13.2 Hz, 2 H, H^{15}), 3.18-3.30 (m, 2 H, H^4), 3.55-3.85 (m, 0.7 H, H^7), 3.88-4.10 (br., 2 H, $H^{12,\ 12'}$), 4.10-4.50 (m, 1.3 H, $H^{7'}$), 4.65-4.82 (br., 0.35 H, H^8), 4.85-4.97 (m, 0.65 H, H^5), 4.97-5.20 (m, 1 H, $H^{8'}$), 5.50-6.00 (m, 1 H, H^{14}) ppm

^{13}C-NMR ($CDCl_3$, 101 MHz): δ =14.1 (C^{22}), 21.62 ($C^{10'}$), 21.86 (C^{10}), 22.6 (C^{21}), 26.74 (C^{16}), 26.92 (C^{11}), 27.01 ($C^{11'}$), 28.74, 28.96 (C^3), 29.17, 29.22 ($C^{18,19}$), 29.98 (C^{17}), 30.49 ($C^{9'}$), 30.62, 30.68 (C^{25}), 31.75 (C^{20}), 32.92 (C^9), 40.23, 40.30 (C^4), 40.97 (C^{15}), 45.38 (C^1), 55.65 (C^{24}), 57.9 (C^2), 64.31 ($C^{12'}$), 64.46 (C^{12}), 66.68 (C^8), 69.12 (C^7), 69.98 ($C^{7'}$), 71.16 ($C^{8'}$), 156.04, 156.70, 156.76, 156.95 ($C^{6,13}$), 171.3 (C^{23}) ppm

Synthesis of C_6A8-Q-PMMA



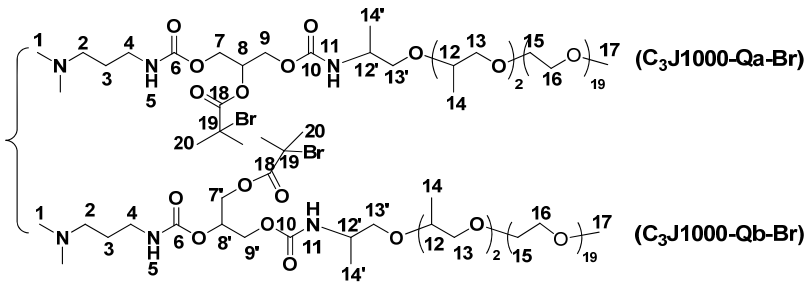
C_6A8-Q-PMMA was prepared from **C_6A8-Q-Br** according to the procedure described for **C_6A8-A8-PMMA**. Yield: 1.93 g (65 %). From SEC (THF): $\overline{M}_n$ = 11000 g/mol, $\overline{M}_w$ = 14600 g/mol, $\overline{M}_w / \overline{M}_n$ = 1.34.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.85 (s, $H^{22,\ 25,\ 28}$), 1.02 (s, $H^{17\text{-}21,\ 25,\ 28}$), 1.13 (s, H^{13}), 1.18-1.50 (m, $H^{9\text{-}11,\ 9'\text{-}11',\ 25,\ 28}$), 1.70-2.15 (m, H^{26}), 2.25 (s, H^1), 2.36-2.44 (m, H^2), 3.15 (dd, ^3J =

6.1 Hz, ^{3}J = 12.6 Hz, H^{15}), 3.27 (dd, ^{3}J = 6.0 Hz, ^{3}J = 11.8 Hz, H^{4}), 3.60 (s, H^{31}), 3.96-4.16 (m, $H^{7, 7', 12, 12'}$), 4.65-5.10 (m, $H^{8,8'}$) ppm

^{13}C-NMR ($CDCl_3$, 101 MHz): δ = 14.0 (C^{22}), 16.4, 18.7 ($C^{25, 28}$), 21.0 ($C^{10, 10'}$), 22.6 (C^{21}), 26.7 (C^{16}), 27.0 ($C^{11, 11'}$), 28.7 (C^{3}), 29.2, 30.0, 30.6, 31.8 (C^{17-20}), 32.5 ($C^{9, 9'}$), 39.1 (C^{15}), 40.0 (C^{4}), 44.5, 44.8 ($C^{24, 27}$), 45.5 (C^{1}), 51.8 (C^{30}), 54.3, 54.4 (C^{26}), 56.3 (C^{2}), 63.2, 64.3 (C^{12}), 65.6 ($C^{7, 7'}$), 69.0, 69.2 ($C^{8, 8'}$), 155.5, 155.8 ($C^{6, 13}$), 176.9, 177.8, 178.1 ($C^{23,29}$) ppm

Synthesis of C_3J1000-Q-Br

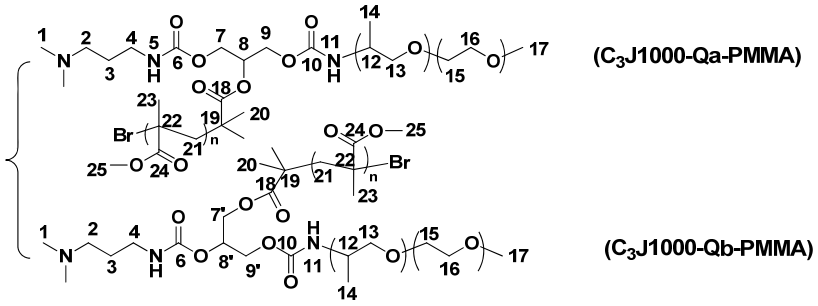


C_3J1000-Q (a/b) (2.27 g, 1.69 mmol) was dissolved in dry THF (15 mL) under nitrogen atmosphere and cooled to 0 °C. A solution of 2-bromo-2-methylpropionyl bromide (506 mg, 2.20 mmol) was slowly added under stirring and and the temperature was kept below 5 °C for 2 h and at room temperature for another 27 h. The reaction mixture was diluted with CH_2Cl_2, treated with acid ion exchange resin and filtrated. After removal of the solvent the residue was precipitated in pentane and dried in vacuum (10^{-2} mbar, 3 h, room temperature). A slightly yellow viscose oil was obtained. Yield: 1.51 g (60 %).

^{1}H-NMR (DMSO-d_6, 400 MHz): δ = 0.96-1.12 (m, 9 H, $H^{14, 14'}$), 1.74-1.84 (m, 2 H, H^{3}), 1.84-1.94 (m, 6 H, H^{20}), 2.70 (s, 6 H, H^{1}), 2.96-3.14 (m, 4 H, $H^{2, 4}$), 3.24 (s, 3 H, H^{17}), 3.25-3.45 (m, 8 H, $H^{12-13, 13'}$), 3.45-3.65 (br. 76 H, H^{15-16}), 3.65-3.73 (m, 1 H, $H^{12'}$), 3.80-4.50 (m, 4 H, $H^{7.7'9,9'}$), 4.95-5.30 (m, 2 H, $H^{8,8',5}$), 6.50-7.50 (m, 2 H, $H^{5, 11}$) ppm

^{13}C-NMR (DMSO-d_6, 101 MHz): δ = 17.00, 17.06, 17.41 ($C^{14,14'}$), 24.30, 24.40 (C^{3}), 30.16 30.24 (C^{20}), 37.5 (C^{4}), 42.1 (C^{1}), 46.4, 46.6 ($C^{12'}$), 54.4 (C^{2}), 56.8, 57.0 (C^{19}), 58.0 (C^{17}), 61.4, 61.6 ($C^{7,9}$), 62.0 ($C^{9'}$), 63.9 ($C^{7'}$), 67.8 ($C^{13'}$), 69.2 ($C^{8'}$), 69.55, 69.75, 70.01 ($C^{15,16}$), 71.24, 71.83 (C^{13}), 72.3 (C^{8}), 74.01, 74.2, 74.3 (C^{12}), 155.1, 155.7 ($C^{6,10}$), 169.4, 172.2 (C^{18}) ppm

Synthesis of C_3J1000-Q-PMMA



A typical ATRP was performed as follows: **C_3J-Q-Br** (746 mg, 0.299 mmol), MMA (3 g, 29.9 mmol), CuBr (43 mg, 0.299 mmol), PMDETA (62 mg, 0.359 mmol), and anisole (3 mL) were placed in a Schlenk tube. After deoxygenation, the Schlenk tube was placed in an oil bath at 70 °C under nitrogen atmosphere. An initial sample was taken and the solution was stirred at 70 °C for 4 hours. At time intervals, samples were analyzed by ^{1}H NMR and SEC, respectively. The polymerization was stopped by cooling, exposure to air and dilution with CH_2Cl_2. The CuBr salt was removed by passing through a column filled with dry silica gel 60. After removal of the solvent the polymer was purified by precipitations in pentane from a CH_2Cl_2 solution. Yield: 2.49 g (66 %). From SEC (THF): $\overline{M}_n$ = 12300 g/mol, $\overline{M}_w$ = 15800 g/mol, $\overline{M}_w / \overline{M}_n$ = 1.29.

^{1}H-NMR ($CDCl_3$, 400 MHz): δ = 0.84 (s, $H^{20, 23}$), 1.02 (s, $H^{14, 20, 23}$), 1.18-1.50 (m, $H^{20, 23}$), 1.60-1.68 (m, H^{3}), 1.70-2.30 (m, $H^{1, 2, 21}$), 2.35-2.60 (m, H^{2}), 3.20-3.30 (br., H^{4}), 3.38 (s, H^{17}),

3.40-3.58 (m, $H^{12\text{-}13}$), 3.60 (s, H^{25}), 3.64 (s, $H^{15,\ 16}$), 3.70-3.95 (m, $H^{12,13}$, next to the urethane group), 4.00-4.40 (m, $H^{7,\ 9,\ 7',\ 9'}$), 5.00-5.30 (m, $H^{8,8'}$) ppm

^{13}C-NMR ($CDCl_3$, 101 MHz): δ = 16.3 (C^3), 17.7 (C^{14}), 18.6 (C^3), 18.8 (C^3), 21.0 (C^3), 25.45 (C^3), 25.51 (C^3), 40.4 (C^4), 44.4, 44.7 ($C^{19,\ 22}$), 45.4 (C^1), 51.7 (C^{25}), 52.6, 53.4, 54.1, 54.3 (C^{21}), 55.4 (C^2), 58.9 (C^{17}), 65.3 ($C^{7,\ 7',\ 9,\ 9'}$), 68.9 ($C^{8,\ 8'}$), 70.4 ($C^{15,\ 16}$), 71.4 (C^{13}), 74.7 (C^{12}), 155.6, 155.9 ($C^{6,\ 10}$), 176.8, 177.7, 178.0 ($C^{18,\ 24}$) ppm

Curriculum Vitae

Name	YingChun He
Date of birth	January 06, 1979
Place of birth	Tianjin, China
Citizenship	Chinese

Education

06/2006-11/2010	PhD work under the supervision of Prof. Dr. Martin Möller at the institute of technical and macromolecular chemistry, RWTH Aachen University, Germany
	PhD thesis: 'From cyclic carbonate bifunctional couplers to amphiphilic poly(ethylene imine)s'
03/2006-06/2006	Intership under the supervision of Dr. Helmut Keul at DWI an der RWTH Aachen e.V., Germany
10/2002-02/2006	Studies in Chemistry (Diploma), University of Würzburg, Germany
05/2005-02/2006	Diploma thesis under the supervision of Prof. Dr. Reinhold Tacke: 'Synthesis, structure and properties of neutral pentacoordinate silicon (IV) complexes with *Si*FO_2C_2, *Si*$FONC_2$ and *Si*$SFOC_2$ skeletons'
09/1998-06/2002	Studies in Chemistry (Bachelor), Zhejiang University, China
10/2001-06/2002	Exchange study in Chemistry, University of Würzburg, Germany

Work Experience

12/2010 – now	Labor leader in BASF Polyurethanes, Lemförde, Germany
06/2006-11/2010	Scientific co-worker in the group of Prof. Dr. Martin Möller at the institute of technical and macromolecular chemistry, RWTH Aachen University, Germany