MAX-PLANCK-INSTITUT FÜR POLYMERFORSCHUNG
JOHANNES GUTENBERG-UNIVERSITÄT MAINZ

Anionic Polymerisation of Aziridines

Diplomarbeit zur Erlangung des Grades
eines Diplom-Chemikers
am Institut für Organische Chemie
des Fachbereiches Chemie, Pharmazie und Geowissenschaften
der Johannes Gutenberg-Universität Mainz

vorgelegt von
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geboren in Frankfurt am Main

Mainz 2013

Diese Arbeit wurde in der Zeit von November 2012 bis Juli 2013 am Institut für Organische Chemie der Johannes Gutenberg-Universität Mainz und am Max-Planck-Institut für Polymerforschung in Mainz unter der Betreuung von Herrn Prof. Dr. Holger Frey und Frau Prof. Dr. Katharina Landfester durchgeführt.

für meine Eltern

“We live on an island surrounded by a sea of ignorance. As our island of knowledge grows, so does the shore of our ignorance.”

- John Archibald Wheeler

Danksagung

Mein Dank gilt Herrn Prof. Dr. Holger Frey und Frau Prof. Dr. Katharina Landfester für die Bereitstellung des Themas und die ausgezeichneten Arbeitsbedingungen. Zudem danke ich Herrn Dr. Frederik Wurm für die freundliche Aufnahme in seine Gruppe und die hervorragende Betreuung der Arbeit.

Bei allen Mitgliedern der Arbeitsgruppe Frey bedanke ich mich für die wunderbare Arbeitsatmosphäre und die Unterstützung bei dieser Arbeit.
Herrn Christian Moers und Herrn Jan Seiwert danke ich, dass sie mir die anionische Polymerisation von Styrol und Ethylenoxid näher gebracht haben.
Bei Frau Anna Hesse und Herrn Christian Moers bedanke ich mich für das Korrekturlesen dieser Arbeit.
Frau Katja Weber danke ich für die Bereitstellung des 2-Methyl-*N*-tosylaziridins.

Für die GPC Messungen danke ich Frau Christine Rosenauer und insbesondere Frau Monika Schmelzer.
Bei Frau Dr. Elena Berger-Nicoletti möchte ich mich für zahlreiche MALDI Messungen bedanken.
Frau Maria Müller danke ich für die Messungen der Elementaranalyse und Frau Magarete Deptolla für ihre Hilfe bei der Aufreinigung der Monomere.
Bei Frau Ines Wollmer möchte ich mich für die freundliche und bereitwillige Aufnahme in ihren Abzug bedanken.

Mein tiefster Dank gilt meiner Familie, insbesondere meinen Eltern, ohne deren Unterstützung mir mein Studium nicht möglich gewesen wäre.

Contents

Abstract

Within the field of polymer science, anionic polymerisation stands out as one of the most important techniques to synthesize well defined polymers with a low polydispersity. Well-established are carb-anionic and oxy-anionic polymerisation techniques. What is almost unexplored, however, is the aza-anionic polymerisation. This work focuses on the polymerisation of activated aziridines to yield poly(ethylene imine)-like structures with the possibility to generate a great variety of novel materials and architectures. A switch from carb- to aza-anionic polymerisation and from aza- to oxy-anionic polymerisation is performed, providing a facile synthesis of block copolymers or the specific introduction of end groups.

1. Introduction

This work focuses on the anionic ring-opening polymerisation of aziridines. By this, poly(aziridine)s (PAz) can be obtained, a novel polymer class that is similar to poly(ethylene imine) (PEI).

1.1. Poly(ethylene imine)

Poly(ethylene imine) (see Figure 1), the most prominent representative of the polyimines, is a well-known and important material to scientists. Despite its toxicity it has found applications in many different areas, e.g. the paper industry,[1] as an additive for printing ink,[2] as a chelating agent for metal ions,[3] in waste water treatment,[1] carbon dioxide capture[4] and especially as a non-viral vector system.[5] Its most important structural feature lies in its backbone, every third atom of which is a nitrogen. The free electron pair of this nitrogen can either form complexes with metal ions, which leads to the application in waste water treatment or it can be protonated, making PEI a polycation with a very high charge density. PEI exhibits a large buffering capacity, as the different structural elements result in different pK_a values and the protonation of one amine group may suppress protonation of the neighbouring groups due to electrostatic repulsion. As a polycation, PEI can form complexes with polyanions such as DNA.

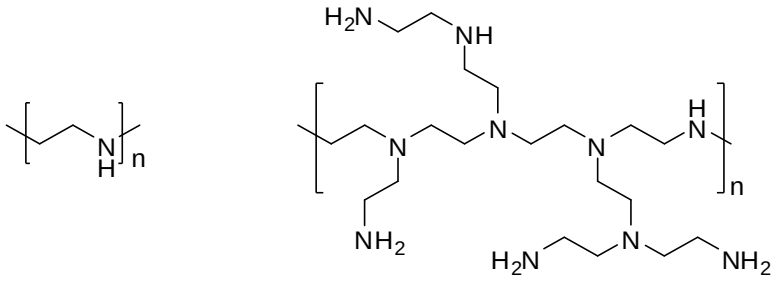

Figure 1: Linear and branched poly(ethylene imine)

PEI is available in a branched or a linear structure. Branched PEI (*br*PEI) is commonly synthesized via cationic polymerisation of aziridine, which was investigated by Zomlefer *et al.* in 1944.[6] They proposed the mechanism shown in Figure 2, which proceeds via cationic ring-opening of the aziridine. An electrophile, e.g. a Lewis acid or a proton, initiates the polymerisation; the growing chain carries an aziridinium ion as its active chain end. Propagation occurs when the lone ion pair of a nitrogen atom attacks this aziridinium ion. If the electron pair belongs to an aziridine, a linear polymer unit is added. If it belongs to a nitrogen atom, which is already incorporated into a polymer chain, branching occurs. This leads to a polymer consisting of primary, secondary and tertiary amines, the latter being a branching point in the polymer. The ratio of the different amine groups was

calculated to be 1:2:1 (primary : secondary : tertiary), however, experimental data showed that the ratios were closer to 1:1:1, meaning that the degree of branching is higher than assumed.[7] As shown in Figure 2, termination can occur either by the addition of a nucleophile or the elimination of a proton. This elimination can occur at random points throughout the polymerisation, which leads to broad molecular weight distributions. The eliminated proton itself can initiate a new chain, thus further broadening the distribution. Polymer textbooks are limited to this uncontrolled cationic polymerisation of aziridines, an anionic mechanism has not been established.

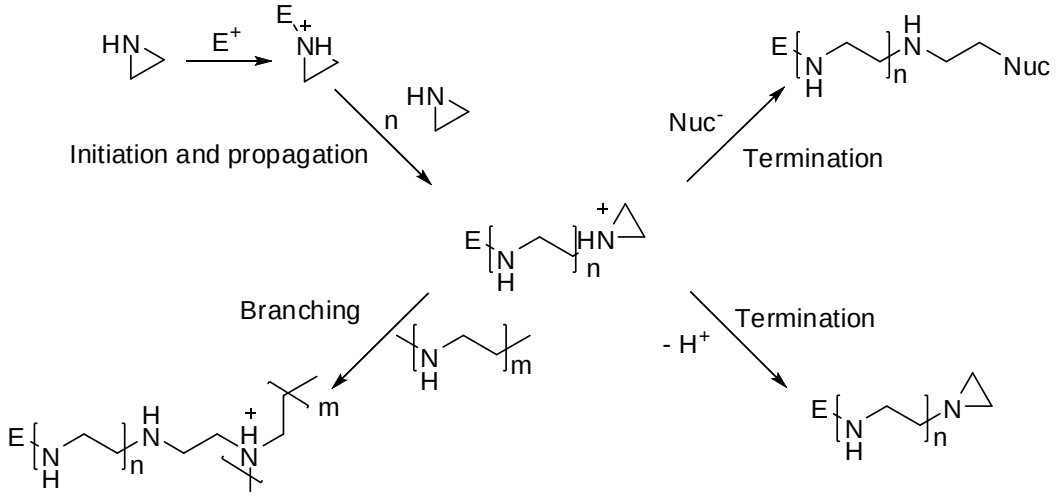

Figure 2: Cationic polymerisation of aziridine

Another polymerisation route offers the possibility of synthesizing linear PEI (*l*PEI). As shown in Figure 3, the cationic polymerisation of 2-substituted oxazolines yields acylated *l*PEI. The amide bond can be cleaved using either acidic[8] or basic hydrolysis.[9] However, harsh conditions are required and full conversion is not easily achieved, especially since the polymer hydrochloride precipitates during the reaction.

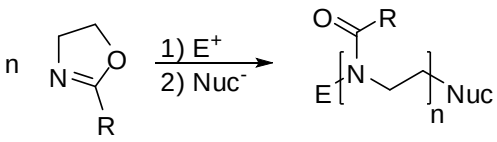

Figure 3: Cationic polymerisation of oxazolines

1.2. Anionic polymerisation

Since Szwarc first described the living anionic polymerisation of styrene in 1956,[10] anionic polymerisation has become one of the most significant methods for the preparation of polymers with narrow polydispersities and well-defined structures. Anionic polymerisation follows a chain growth

mechanism carrying an anion at the active chain end. As long as the polymerisation is carried out under the absence of water, oxygen and carbon dioxide no termination reactions occur. Therefore, the polymerisation is often referred to as 'living', meaning that the chain ends remain active even after depletion of the monomer. If further monomer is added subsequently, the polymerisation continues. Hence, block copolymers or specific end groups are easily attained by this method. Narrow molecular weight distributions are obtained when the rate constant of the initiation k_i is larger than the rate constant of the propagation k_p, which can be visualised by all chains starting their growth almost simultaneously. Therefore, all chains are subjected to the same amount of time and monomer, i.e. they experience equal conditions for propagation. The structure of the active chain end is amongst other factors dependent on the solvent, the temperature and the affinity of the counter ion. The influence of the solvent is illustrated in Figure 4. With increasing polarity of the solvent, the association of the ions decreases. The polymerisation can proceed faster, if the counter ion is not closely bound to the active chain end. Consequently, polymerisations carried out in apolar solvents often need to be heated, e.g. poly(styrene) (PS) in cyclohexane, while those in polar solvents have to be kept at low temperatures to suppress exothermal reactions, e.g. poly(styrene) in tetrahydrofuran (THF). Suitable solvents for anionic polymerisations include ethers and hydrocarbons (cyclohexane, THF, bis(2-methoxyethyl) ether). Halogenated or protic solvents must not be used, as they would react with the anion. The reactivity in apolar solvents can be enhanced by adding a complexation agent in order to further separate the counter ion from the active chain end. Crown ethers or *N*,*N*,*N'*,*N'*-tetramethylethylendiamine are commonly used.[11]

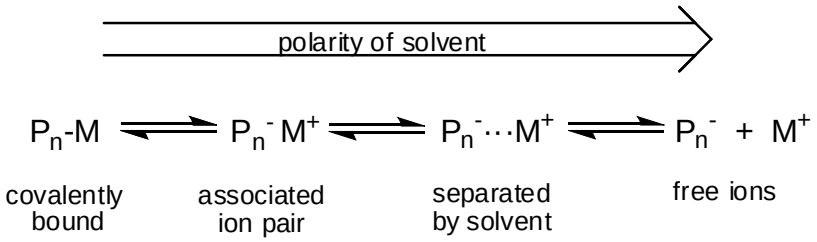

Figure 4: Active chain end in dependence of solvent polarity

Well established in literature are carb-anionic and oxy-anionic polymerisations. Almost unexplored, however, is the aza-anionic polymerisation.

1.2.1. Anionic polymerisation of aziridines

When it comes to polymerising aziridines, the prevailing opinion is that it can only be done cationically (see 1.1), because of the acidic proton and the instability of the resulting amine anion.[11] However, substituting the proton at the aziridine nitrogen enables anionic polymerisation, as

discovered by Toste *et al.* in 2005.[12] The tosyl (Ts) or mesyl (Ms) group, also referred to as activating groups (see section 1.5), serve three purposes. Firstly, they substitute the acidic proton, making sure that it does not terminate the anionic polymerisation. Secondly, they stabilise the aza-anion (a sulfonamide anion) at the active chain end as the charge is delocalised over the activating group. Thirdly, they increase the reactivity of the monomer. By withdrawing electron density from the ring they render the aziridine ring electron deficient. This facilitates a nucleophilic attack of an initiator or the chain end. Figure 5 shows the structure of activated aziridines.

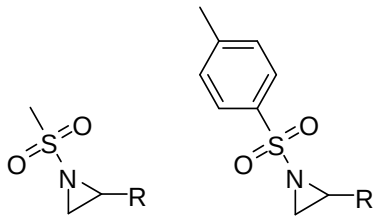

Figure 5: Aziridines activated by a mesyl (left) or a tosyl (right) group

It is unknown whether an electron withdrawing substituent is needed to enable anionic polymerisation or whether an alkyl substituent would serve the same purpose. Considering the instability of the aza-anion, it seems likely that the substituent has to stabilise the negative charge. Taking this into account, the anionic polymerisation of acylated aziridines should work as well. Anionic polymerisation of aziridines yields a novel polymer structure, PAz. The basic structure is depicted in Figure 6. The backbone resembles PEI, since nitrogen alternates with two carbon atoms. The activating group at the nitrogen is retained from the polymerisation. A side chain is easily introduced at the C-1 position, which cannot be done by the classic cationic synthesis. This potentially allows tuning the polymer properties, e.g. the glass transition temperature, and introducing new functionalities into the polymer.

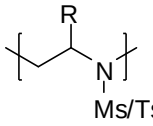

Figure 6: Poly(aziridine)

PAz with a defined tacticity can be synthesized from aziridines bearing a stereo-center (Figure 7). However, the resulting polymers, namely poly((2*S*)-*N*-tosyl-2-benzylaziridine) and poly((2*R*)-*N*-tosyl-2-methylaziridine), are reported to be hardly soluble, hindering chain growth and analysis.[12] Therefore the preparation of atactic PAz is to be preferred.

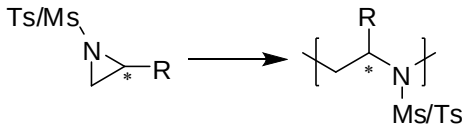

Figure 7: Preparation of poly(aziridine) with a defined tacticity

1.3. State-of-the-art poly(ethylene imine) structures

This chapter presents the current possibilities to modify the structure of PEI. Since it was first tested for its potential in gene delivery in 1995,[5] PEI has become one of the most important non-viral transfection agents. 25 kDa branched PEI and 22 kDa linear PEI are most commonly used. However, novel structures are being developed in an attempt to achieve better transfection efficiency while lowering the toxicity. Specific cell targeting, enhanced blood circulation times, controlled drug release and storage stability are other properties to be improved.[13]

Figure 8 shows the most common modification possibilities for PEI. Isocyanate addition leads to carbamates (Figure 8a).[14] The amino groups can be alkylated by a nucleophilic substitution with alkylhalides (Figure 8b).[15] An Eschweiler-Clarke reaction methylates the amine groups (Figure 8c).[16] Acetylation is possible using an activated carboxylic acid (Figure 8d), e.g. a *N*-hydroxysuccinimide (NHS) ester.[17] Acrylates react in a Michael addition (Figure 8e).[18] As often with post-polymerisation reactions, the yield is hardly quantitative. Depending on the desired degree of functionalisation, harsher reaction conditions and longer reaction times have to be applied to obtain the product in good yields.

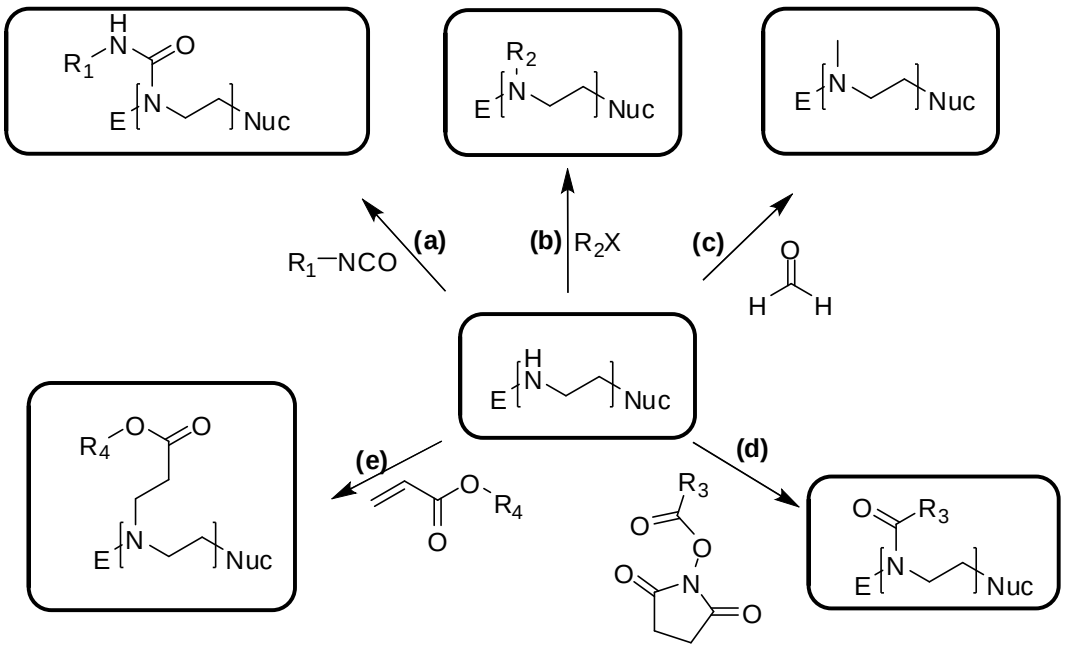

Figure 8: Possible modifications of poly(ethylene imine)[14, 15, 16, 17, 18]

These methods allow the modification of the basic PEI structure by targeting the amine groups. However, adjusting the toxicity while maintaining a high transfection efficiency is a challenging endeavour, as there is a reciprocal connection between those two properties. The prevailing opinion in literature is that the toxicity of PEI is caused by electrostatic interactions with cell membranes and the extracellular matrix.[19] However, decreasing the positive charge density of PEI decreases its ability to form complexes with polyanions like DNA as well and consequently reduces the transfection efficiency. In the following a few examples of modified PEI structures shall be discussed.

1.3.1. Copolymers with poly(ethylene glycol)

In an attempt to decrease the toxicity of PEI, copolymers with biocompatible polymers were investigated. Examples include pluronics®,[20] poly-*N*-(2-hydroxypropyl)methacrylamide (PHPMA)[21] and poly(ethylene glycol) (PEG).[21] The latter is the most thoroughly investigated, as PEG is a well-known polymer in bioconjugation science. PEGylation, i.e. the covalent attachment of PEG, of pharmaceuticals enhances blood circulation time and reduces opsonisation, recognition by parts of the immune system as well as unspecific cellular uptake.[22] This is due to the 'stealth effect' of the PEG chain, caused by the hydrophilicity of PEG. The polymer has a hydrate shell, therefore endogenous substances do not perceive the polymer and cannot interact with it. PEI-PEG copolymers were reported to show decreased interactions with blood components, such as antibodies and proteins of the immune system, as well as an enhanced blood circulation time.[23] On the other hand, the PEGylation seems to interfere with the DNA binding efficiency of PEI.[24]

PEG-PEI copolymers can be achieved through different techniques (Figure 9). Petersen *et al.* added a 'macrostopper' to the cationic polymerisation of aziridine (Figure 9a).[25] The amino functional PEG terminates ethylene imine polymerisation, resulting in a *br*PEI-PEG copolymer. A different approach uses PEG as a macroinitiator for the polymerisation of 2-methyloxazoline (Figure 9b).[26] The resulting PEG-*block*-PMeOx can be hydrolysed under alkaline conditions to PEG-*block*-*l*PEI. Using a bifunctional macroinitiator for the polymerisation of 2-methyloxazoline, Zhong *et al.* synthesized a triblock copolymer (Figure 9c).[27] The acetyl group can be removed by acidic hydrolysis.

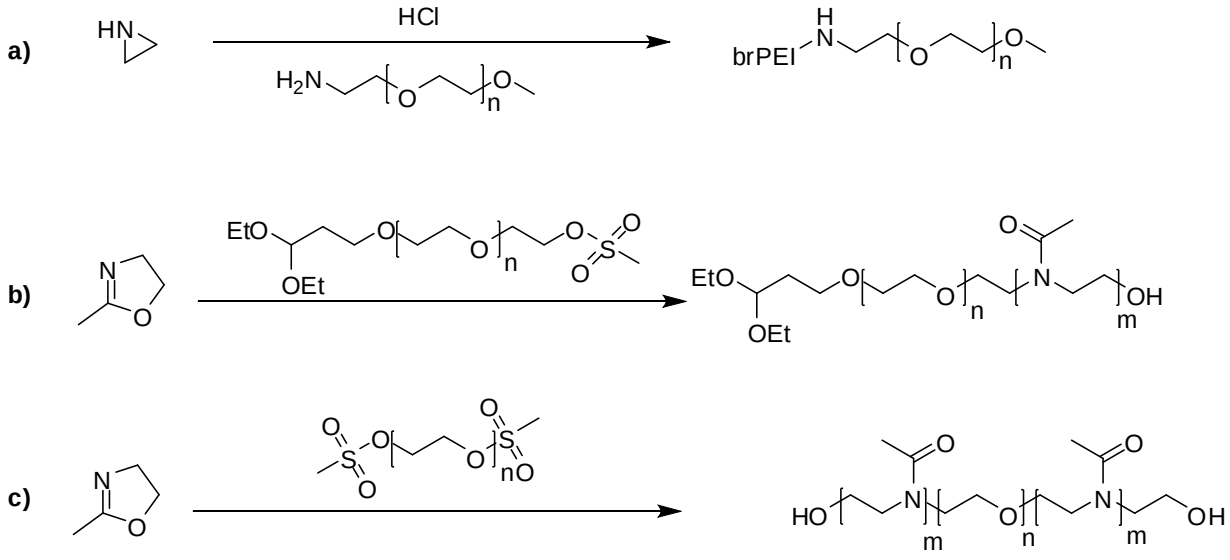

Figure 9: Approaches for PEI-PEG block copolymers[25, 26, 27]

These methods show a controlled approach to PEGylation, which is needed as uncontrolled PEGylation leads to a decrease in the DNA binding abilities of PEI.

1.3.2. Conjugates with bioactive molecules

Conjugating PEI to bioactive molecules, e.g. proteins, enables the PEI-DNA complex to target specific cells. This ensures that the DNA is not distributed in the whole body, but is directed to a certain cell type. Thereby, side effects are reduced and the dose that has to be applied is lowered. The preferred method is modifying PEI with an active ester first and linking it to the protein in a second step. Figure 10 shows exemplary procedures. Activating PEI with *N*-succinimidyl-3-(2-pyridyldithio)-propionate (SPDP) allows coupling to the thiol group of a protein (Figure 10a).[28] By activating the protein with SPDP as well, coupling to an amine group is possible. A drawback for this method is the lability of the disulfide group. It was shown that the S-S-bond is unstable in the presence of physiological concentration of thiols.[29] Another method uses *N*-(maleimidoundecanoyloxy)sulfosuccinimide ester (sulfo-KMUS) for connecting the polymer to thiol groups (Figure 10b). The protocol can be modified to allow coupling to amine groups as well by thiolating PEI with 2-iminothiolane and activating the protein with sulfo-KMUS.[30] However, the *N*-hydroxysuccinimid moiety is sensitive to hydrolysis. Coupling PEI to dithiobis(succinimidylpropionate) (DSP) offers another possibility to link PEI to amine groups (Figure 10c).[31] Again the disulfide bond may be cleaved in the presence of thiols.

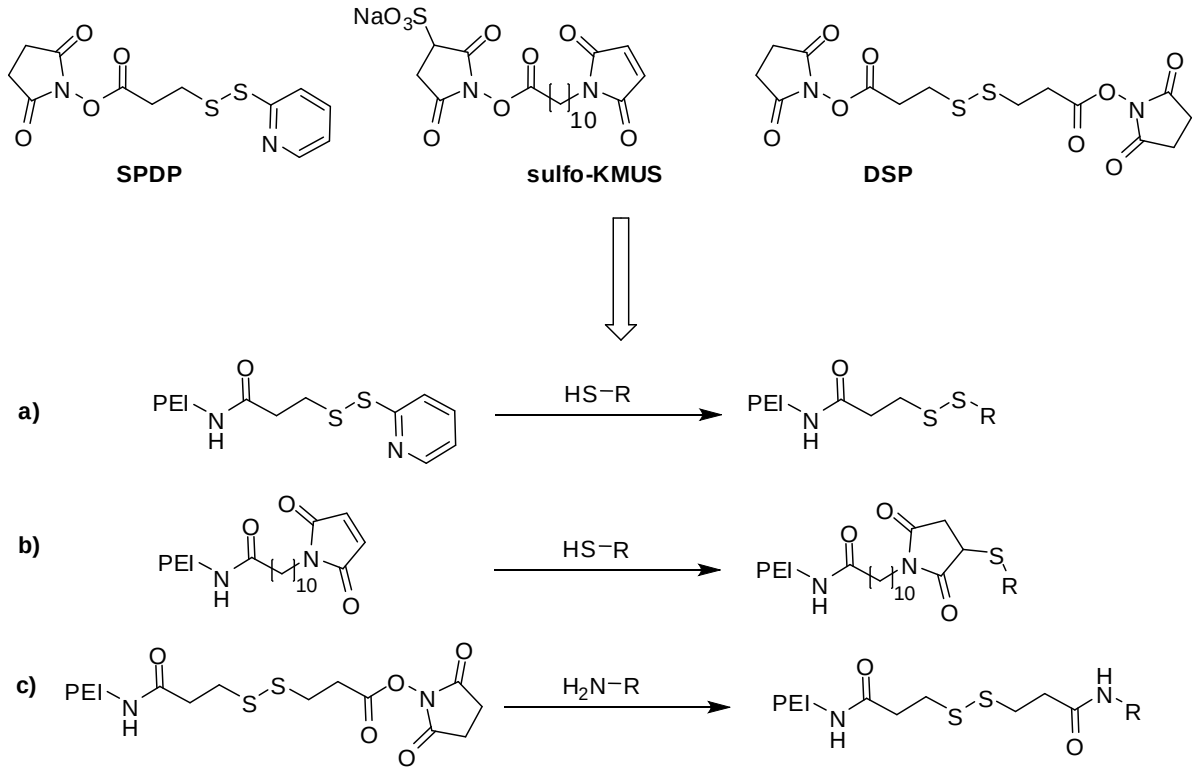

Figure 10: Strategies for poly(ethylene imine)-conjugates[28, 30, 31]

PEI conjugates to carboxylic acids may also be synthesized using *N*,*N*'-dicyclohexylcarbodiimide (DCC) or 1-ethyl-(3-dimethylaminopropyl 1-ethyl-3)carbodiimide (EDC).[32] This allows coupling to bioactive molecules bearing a carboxyl group, e.g. folate.

1.3.3. PEI-PEG conjugates with bioactive molecules

Simple conjugates of PEI with bioactive molecules were often found to show little or no specific targeting activity. This is thought to be caused by the strong electrostatic interactions of PEI with the negatively charged cell membranes that predominates any specific ligand-receptor interaction. To improve upon that, the conjugates are further coupled to PEG as a spacer unit. Due to the 'stealth effect' of the PEG chain the unspecific interactions of PEI with membranes are reduced and the blood circulation time is enhanced. In addition, this shielding effect can impede specific targeting as well. There are several approaches to PEI-PEG conjugates (see Figure 11), all of which use bifunctional PEG to provide a link between PEI and the protein. Using α-vinylsulfone-ω-*N*-hydroxysuccinimide ester poly(ethylene glycol), PEI was coupled using the activated NHS ester while thiol groups of bioactive molecules react with the vinylsulfone moiety (Figure 11a).[17] However, the authors reported that introducing a PEG spacer decreased targeting efficiency compared to PEI conjugates without a spacer, possibly because the PEG spacer shielded the targeting unit as well. Another bifunctional

PEG, pyridyl-disulfide poly(ethylene glycol)-succinimidyl propionic acid (NHS-PEG-OPSS) allows to link PEI to bioactive molecules via disulfide bonds (Figure 11b).[33] Bioactive molecules containing a carboxylic acid, such as folate, may be activated using NHS and coupled to an amino functional PEG (Figure 11c). Activating the carboxy terminus of the bifunctional PEG with NHS enables the coupling to PEI as well.[34] All these methods allow only stoichiometric control, the side of the reaction cannot be influenced. For that the methods described in section 1.3.1 have to be employed.

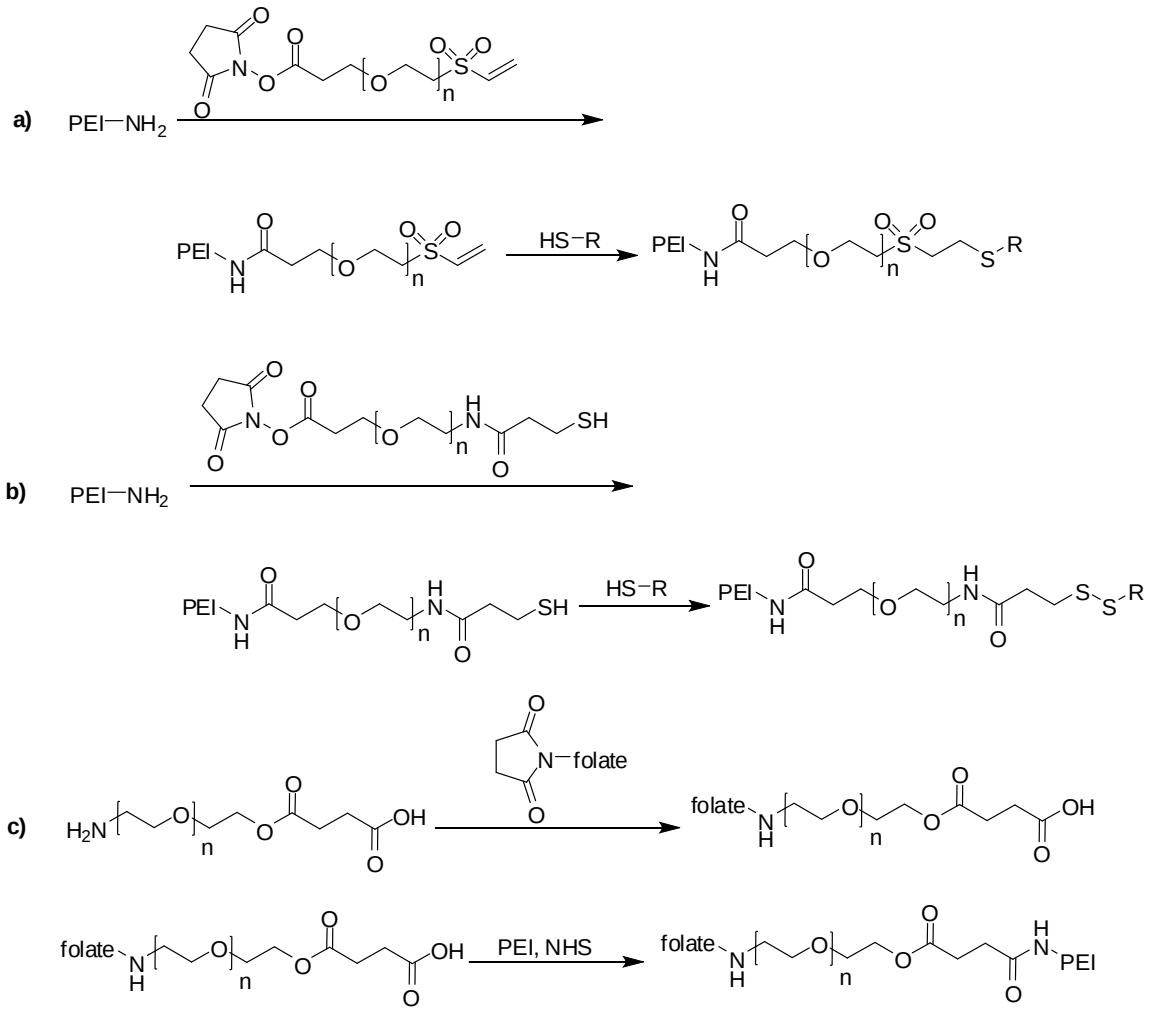

Figure 11: PEG-PEI conjugates with bioactive molecules[17, 33, 34]

1.3.4. Biodegradable poly(ethylene imine)

Low molecular weight PEI has been reported to have a lower toxicity and transfection efficiency than high molecular weight material. Therefore, biodegradable PEI structures are of interest, as they combine the reduced toxicity of low molecular weight PEI with the transfection rate of high molecular weight PEI. Lee *et al.* synthesized linear poly(ethylene imine sulfide) (Figure 12a).[35] The disulfide linkers were chosen because disulfides are stable under the oxidative conditions in the

extracellular environment and are rapidly reduced once they enter a cell. He *et al.* used enzymatic ring-opening polymerisation to synthesize a copolymer of 5-methyl-5-allyloxy carbonyl-trimethylenecarbonate (MAC) and 5,5-dimethyl-trimethylene carbonate (DTC). By epoxidation of the allyl group, PEI could be linked to the backbone, forming a graft copolymer (Figure 12b).[36] Studies on elimination of the polymer fragments from the body have not been made, as the novel polymer structures have not been tested *in vivo*.

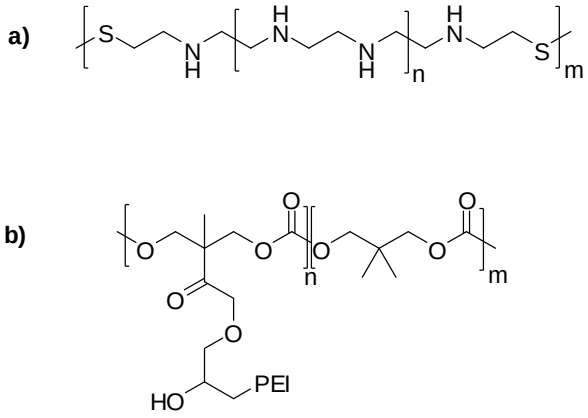

Figure 12: Biodegradable PEI[35, 36]

1.4. Coupling to other anionic polymerisations

Different anions at the active chain end of polymers possess different reactivities. This offers the possibility to form block copolymers of diverse materials by gradually descending the reactivity. The basicity of an azaanion ($pK_a \approx 30\text{-}40$) lies between those of a carbanion ($pK_a \approx 40\text{-}50$) and an oxyanion ($pK_a \approx 15\text{-}20$). However, the activating group lowers the electron density at the azaanion, decreasing its reactivity. Theoretically, a switch from carb-anionic to aza-anionic and subsequently to oxy-anionic polymerisation should be possible. This chapter describes the polymerisation techniques relevant for this switch and lists switching techniques known in literature.

1.4.1. Anionic polymerisation of styrene

The anionic polymerisation of styrene is regarded in respect to the possibility of forming block copolymers with aziridines. Initiating the styrene polymerisation requires strong nucleophiles, such as *sec*-butyllithium (*sec*-BuLi) or potassium naphthalenide. A carbanion like the poly(styryl) anion is nucleophilic enough to open the aziridine ring. However, it is not known whether the chain propagates further after the addition of the first aziridine (Figure 13a) or stops after this step (Figure 13b), thereby introducing a sulfonamide end group. Factors influencing the reaction pathway include temperature, solvent and the counter ion. The nature of the aziridine monomer is a potential tuning point as well. A certain way to introduce a single aziridine unit is to use one that cannot polymerise

on its own, either due to steric hindrance of the monomer or because the aza-anion is not reactive enough. The poly(styryl) anion could still open the ring, the resulting aza-anion, however, would be not able to polymerise any further.

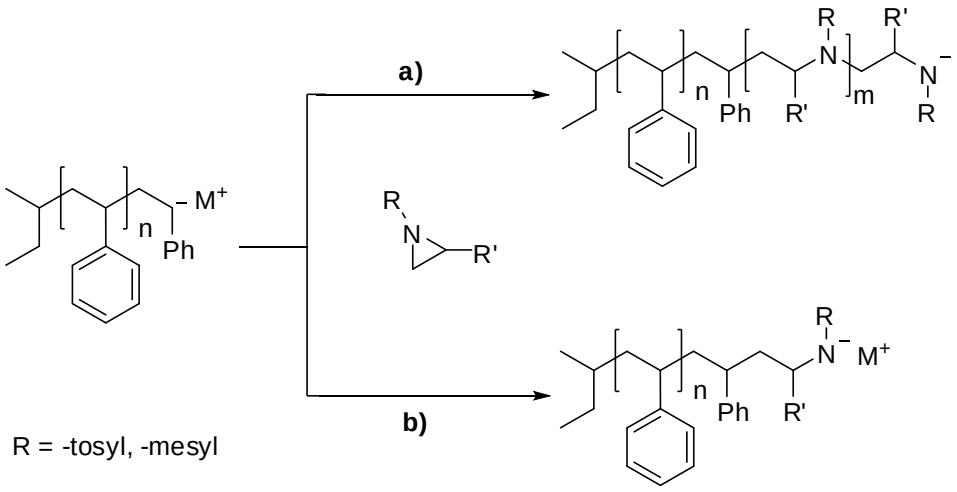

Figure 13: Switching from carb-anionic to aza-anionic polymerisation

Switching from PS to PAz provides an easy way to introduce sulfonamide groups or amine functionalities upon the removal of the activating group.

1.4.2. Anionic polymerisation of ethylene oxide

As described in section 1.4, an azaanion is generally more nucleophilic than an oxyanion, meaning that only a switch from aza- to oxy-anionic polymerisation is possible (Figure 14a). However, the electron withdrawing substituent could shift the order of reactivity, enabling a switch from oxy- to aza-anionic polymerisation (Figure 14b). Both directions are conceivable, depending on how much the activating group lowers the electron density of the aza-anion. As with PS the chain might not propagate upon addition of the new monomer if the reaction conditions for the two monomers are not compatible.

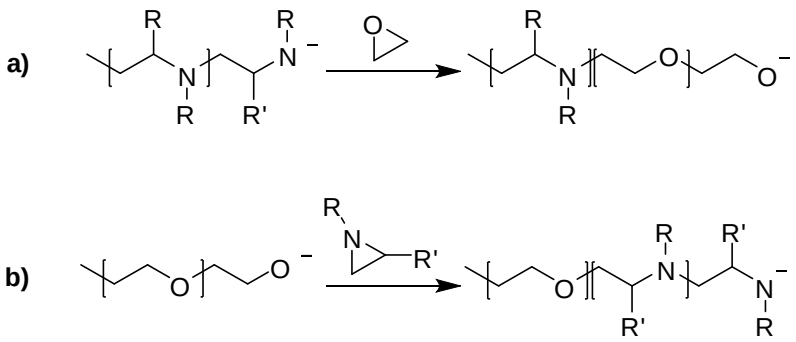

Figure 14: Switch between aza-anionic and oxy-anionic polmerisation

1.4.3. Reported switches between anionic polymerisations

Switching between two types of anionic polymerisation is not new to literature. This section presents several examples where the technique has been used before.

Employing the concept of a 'carbanion pump' Sheik *et al.* were able to initiate carb-anionic polymerisation using alkoxides (Figure 15).[37] Usually an alkoxide is not nucleophilic enough to form a carbanion from styrene. It can, however, open a highly strained ring such as dimethylsilacyclobutane. The resulting carbanion was shown to initiate the polymerisation of styrene and methyl methacrylate.

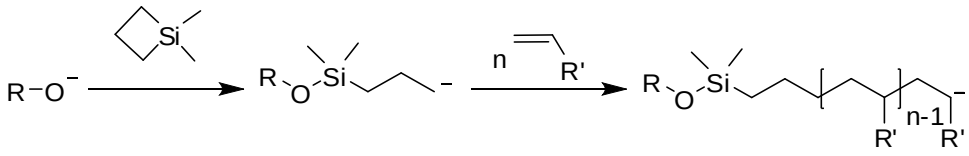

Figure 15: Principle of a carbanion pump[37]

Das *et al.* used 1,1-diphenylethylene-terminated PEG as a macroinitiator to form ABA triblock copolymers with styrene.[38] The carbanionic end groups generated by the addition of *sec*-BuLi are nucleophilic enough to initiate the styrene polymerisation. While this method adeptly utilises different anion reactivities, no real switch between living polymerisations is performed, as the PEG precursor was purchased and modified accordingly. 1,1-Diphenylethylene (DPE) is an important tool in polymer chemistry to adjust the reactivity of the active chain end and achieve well defined architectures.[39, 40]

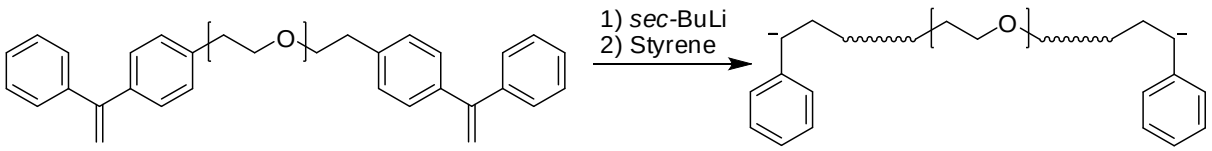

Figure 16: Synthesis of ABA triblock copolymers[38]

Tonhauser *et al.* employed the affinity of the counter ion to the active chain end, in order to introduce specific end groups to poly(styrene).[41] Poly(styrene), initiated with *sec*-BuLi is terminated using different epoxide derivatives. The high affinity of lithium to the oxyanion prohibits the propagation of the chain.

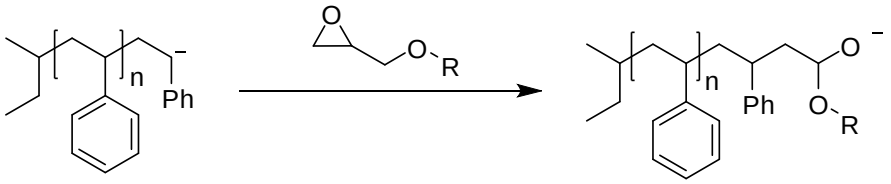

Figure 17: Synthesis of end-functionalised poly(styrene)[41]

1.5. Aziridines

Aziridine, also known as ethylene imine or azacyclopropane, is the nitrogenous analogue to ethylene oxide. It is a colourless liquid (bp = 57 °C) and a highly toxic material, as the strained ring is a powerful alkylating agent. The ring strain of aziridine is estimated to be comparable to ethylene oxide (ca. 111 kJ/mol).[42] In both ethylene oxide and aziridine, the electronegativity of the heteroatom further facilitates nucleophilic attacks on the ring. In contrast to ethylene oxide, aziridine possesses an additional valency on the heteroatom. Consequently, the nitrogen's proton is often substituted in order to avoid deprotonation as a side reaction. Aziridine derivatives are classified as 'activated' or 'non-activated' depending on the nature of the substituent. Activating substituents are able to stabilise the transition state during a nucleophilic attack to the ring, e.g. *N*-acetylaziridine.[43] Further activating substituents include sulfonyl, sulfinyl, phosphoryl, phosphinyl and other carbonyl groups. The activation is primarily caused by an inductive effect, resonance plays just a small role, as the resonance isomer greatly increases the ring strain (Figure 18).

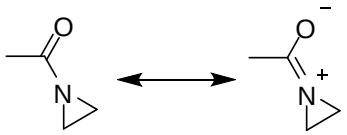

Figure 18: Resonance in *N*-acetylaziridine

Sulfonyl substituents are the most commonly used, due to their easy preparation. However, removal of the sulfonyl group requires strong reductive conditions (see section 3.3.3). Some modified sulfonyl groups are known, that could maintain the advantages of the sulfonyl moiety while being easier to remove, but they have not been investigated as activating substituents for aziridine yet. Figure 19 shows the β-(trimethylsilyl)ethylsulfonyl (SES) group[44] and the 4-nitrobenzene sulfonyl (nosyl) group.[45] The SES group can be cleaved by fluoride ions. The nosyl group is removed by thiolate ions, presumably via formation of Meisenheimer complexes.

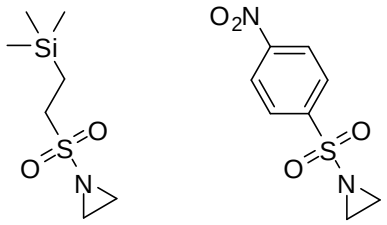

Figure 19: *N*-(β-(trimethylsilyl)ethylsulfonylaziridine (left) and *N*-(4-nitrobenzenesulfonyl)aziridine (right)[44, 45]

There are several routes employed to synthesize aziridine derivatives, e.g. addition of nitrenes to alkenes (Figure 20a). Figure 20 depicts the reaction of a singlet nitrene, which reacts stereospecifically, while triplet nitrenes (not shown) form a mixture of stereoisomers.[42] Nitrenes are usually generated *in situ* from the corresponding azide as they are a short-lived and reactive species. In a complementary reaction the aziridine is formed by an imine and an ylid (Figure 20b). The ylid is interchangeable with a carbene.[46] Figure 20c shows the Wenker synthesis, the intramolecular reaction of an aminoalcohol to an aziridine.[47] The alcohol has to be converted to a leaving group by sulfuric acids. Other leaving groups, i.e. halogenides or tosylates may be used as well. The Gabriel-Cromwell reaction (Figure 20d) uses α-bromoacrylates and amines to form aziridines.[48] The reaction proceeds by an initial Michael addition of the amine to the vinylogous system and subsequent ring formation by an intramolecular substitution. Aziridine synthesis from epoxides can be achieved by using metal azides (Figure 20e).[12]

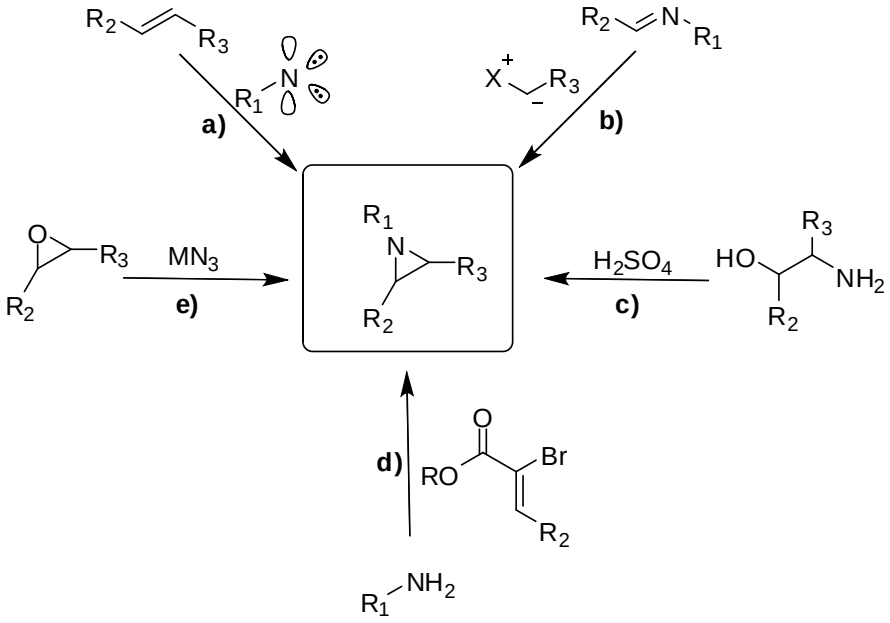

Figure 20: Aziridine synthesis[12, 42, 46-48]

2. Motivation

As explained in section 1.1, the prevailing opinion is that aziridines cannot be polymerised via an anionic mechanism. Even though the structure of activated aziridines is well-known in literature, they have only been discovered to undergo living anionic ring-opening polymerisation in 2005.[12] As the topic has been unnoticed ever since, this work aims to establish the aza-anionic polymerisation of aziridines as a way to yield PEI-like structures.

As described in section 1.1, PEI is an important material that is used in many different areas. Especially as a transfection agent though, there is need and room for improvement. For medical applications in particular, demands on polymers in terms of uniformity and purity are extraordinary. It is noticeable that the vast majority of novel PEI-like structures (see section 1.3) are achieved by modifying commercially purchased PEI. The anionic polymerisation of aziridines allows tuning the structure of the polymer on step further ahead in a controlled and modular way. New possibilities in diversifying the structure of linear PEI by introducing side chains or forming copolymers offer potential tuning points for designing polymers with customised properties. Naturally, the applications for PAz, as they are a novel polymer class, remain to be examined. They could potentially be used in the same fields as PEI as the two polymer classes share many similarities. However, their differences could lead to PAz not being suitable as an alternative or improvement to PEI, but for completely different applications.

This thesis aims to further explore the field of the aza-anionic polymerisation. Several points were to be investigated:

- Aziridine monomers were to be synthesized by two different reaction pathways. In three steps activated aziridines were to be obtained either from amino acids or epoxides.
- Reproducing the work of Toste *et al.* on the anionic ring-opening polymerisation of aziridines by using the same initiator system and monomers as well as testing new monomers for their capability for anionic polymerisation.
- The introduction of functional side chains and addressing them in a polymer modification reaction.
- The possibility to switch between different anionic polymerisation techniques, i.e. carb-, aza- and oxy-anionic. The prospect of varying the reaction conditions, i.e. solvent, counter ion and temperature, to influence whether termination or propagation occurred was to be investigated. For switching between aza- and oxy-anionic polymerisation the order of reactivity was to be examined.

- Finding a protocol for the removal of the activating groups from the polymer.
- All polymers were to be characterised using NMR, SEC and MALDI-Tof MS analysis regarding degree of polymerisation, polydispersity and composition.

3. Results and discussion

3.1. Monomers

This chapter focuses on the synthesis and characterisation of several different monomers from which poly(aziridine)s were obtained. Two synthetic routes were employed to prepare activated aziridines (Figure 21). Although a detailed investigation of possible monomers was beyond the scope of this work, monomer synthesis is possible from a wide array of substances.

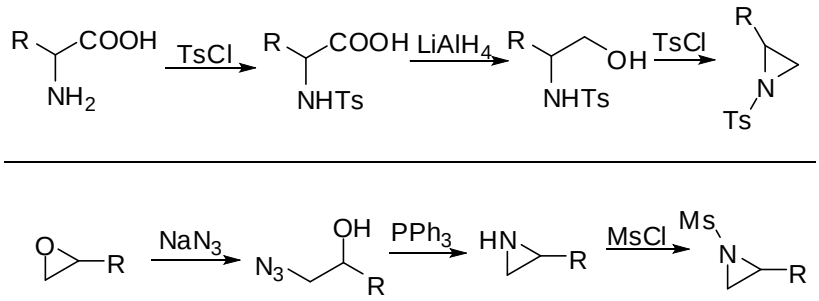

Figure 21: Routes for monomer synthesis

As shown in Figure 21, the activated aziridine monomers can be prepared via two different pathways, either from amino acids or from epoxides. Five monomer structures were chosen, whose substituents were thought to be stable under the conditions of anionic polymerisation and not interfere with the polymerisation mechanism. Figure 22 depicts all synthesized monomers.

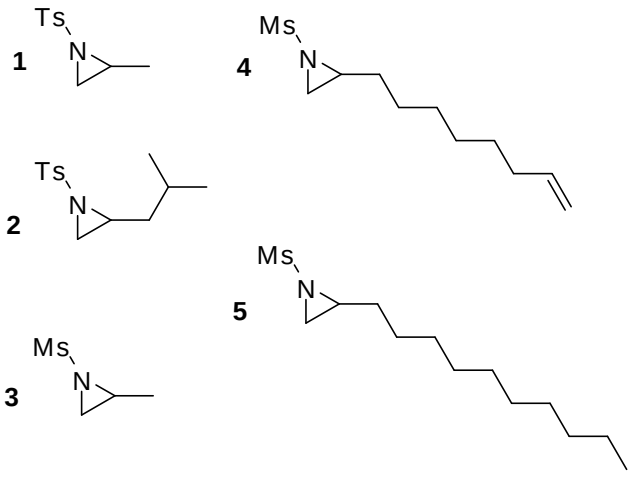

Figure 22: Monomers prepared and polymerised in this thesis

1 is derived from the amino acid alanine, **2** from leucine, **3** from the commercially available 2-methylaziridine, **4** from 1,2-epoxydec-9-ene and **5** from 1,2-epoxydodecane. **4** is particularly interesting as the double bond offers the possibility of polymer modification reactions.

3.1.1. Synthesis

First the synthetic route starting from an amino acid shall be examined. In a first step the amino group is tosylated once (Figure 23), following a S_N2 mechanism. Amino acids with aliphatic side chains (such as leucine, alanine, etc.) lie well within the scope of this reaction, tosylation of amino acids with a reactive side chain, e.g. serine, is possible as well, albeit with lower yields.[49] *N*-Tosyl-leucine was obtained in good yields (81 %). Tosylation of the amino group in the first step does not seem necessary at first, but it circumvents a 2-aminoalcohol. These compounds rapidly form chelate complexes with metal cations, which would further complicate purification. The synthesis of 2-methyl-*N*-tosylaziridine is not described, as it was kindly provided by Katja Weber.

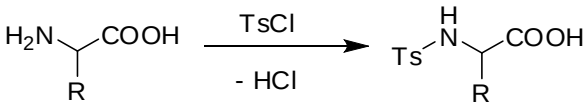

Figure 23: Tosylation of the amino group

Lithium aluminum hydride is used to reduce the carboxylic acid to the respective alcohol (Figure 24). Isolation of the product proved difficult, as it had to be extracted from a viscous mixture of amphoteric compounds such as aluminum hydroxide. The literature suggests filtration through celite®,[49] but it was found that this step complicated the extraction unnecessarily. Thus, all solid components were simply removed by filtration. By thorough extraction with ethyl acetate, *N*-tosyl-leucinol was obtained with 78 % yield.

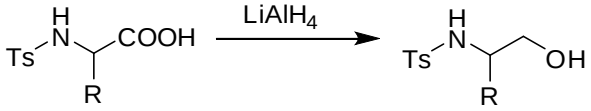

Figure 24: Reduction with lithium aluminum hydride

The resulting primary alcohol can be transformed into an excellent leaving group by a second tosylation step (Figure 25). This initiates an intramolecular nucleophilic substitution, leading to ring closure and the desired aziridine.[49] 2-Isobutyl-*N*-tosylaziridine was obtained with only 38 % yield, as much of the product was lost during chromatography on silica gel.

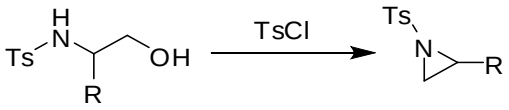

Figure 25: Aziridine formation

The second synthetic route starts from an epoxide. Sodium azide opens the ring in a nucleophilic attack, thus forming the azide (Figure 26). The attack at the unsubstituted carbon atom of the ring is favoured, because it is sterically less hindered. Furthermore, as long as the substituent is an aliphatic group, i.e., not electron withdrawing but rather with a positive inductive effect, the unsubstituted carbon is also slightly more electrophilic. Both epoxides were converted with high yields, i.e. 93 % of 1-azido-2-hydroxydecane and 90 % of 1-azido-2-hydroxydec-9-ene were obtained.

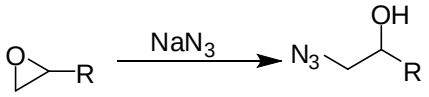

Figure 26: Ring opening of the epoxide and azide formation

Ring closure is initiated by triphenylphosphine, which first forms an iminophosphorane (Figure 27). The driving force for this reaction is the evolution of nitrogen. The ring is closed under elimination of triphenylphosphine oxide, which is energetically favored due to the high affinity between oxygen and phosphorus. Purification of the products proved to be difficult. Neither distillation nor chromatography worked at first, so that at first the crude product was used for the next reaction step, which worked, but lowered the yield tremendously. 2-*n*-Decylaziridine was distilled with 79 % yield, although the compound was found to have a higher boiling point than reported in literature (63-85 °C at $6*10^{-2}$ mbar as opposed to 95 °C at 133 mbar[12]). Taking this into account, the distillation of 2-*n*-(7-octenyl-)aziridine should be possible as well in future synthesis.

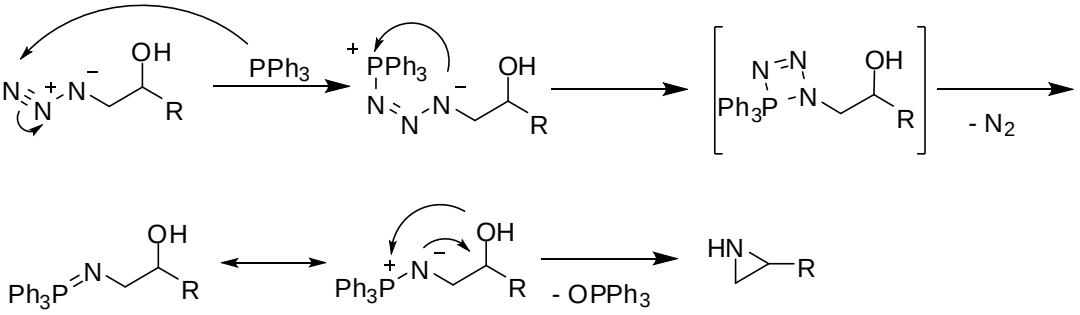

Figure 27: Proposed mechanism of ring closure

Complete consumption of the starting material could easily be verified by IR spectroscopy, where a disappearance of the azide and hydroxy bands at 3360 and 2100 cm^{-1} was observed (Figure 28).

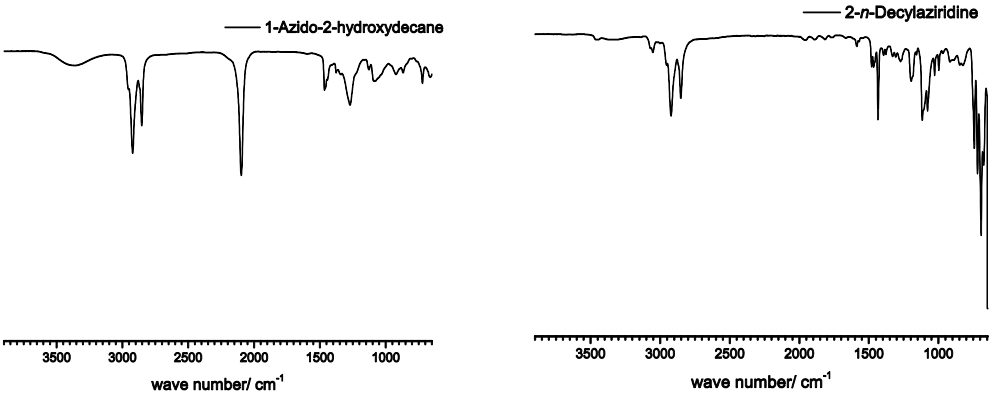

Figure 28: IR spectra of 1-azido-2-hydroxydecane and 2-*n*-decylaziridine

The final step is the activation of the aziridine by mesylation (Figure 29), as explained in section 1.2.1. The reaction follows a S_N2 mechanism. 2-*n*-Decyl-*N*-mesylaziridine was obtained with 70 % yield, 2-*n*-(7-octenyl-)*N*-mesylaziridine with only 34 %, as the starting material was still impure. Future syntheses using the purified product are expected to result in higher yields. 2-Methyl-*N*-mesylaziridine was obtained with 54 % yield.

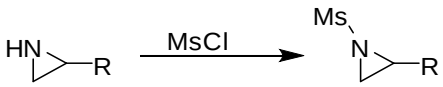

Figure 29: Mesylation of the aziridine

It should be noted that mesylation and tosylation in these procedures are interchangeable, mesylated monomers can be obtained from amino acids and tosylated monomers are accessible by the epoxide route.

3.1.2. Characterisation

The monomers were characterised by ^{1}H NMR spectroscopy. Figure 30 shows the spectrum of 2-methyl-*N*-tosylaziridine in $CDCl_3$. The sample still contains ethyl acetate as indicated by the quartet at 4.11 ppm. Before any polymerisation all starting materials were dried under vacuum, therefore traces of solvents in the monomers can be disregarded. The spectrum shows the characteristic signals of a substituted aziridine ring: a multiplet at 2.86-2.78 ppm (**d**) and two dublets at 2.61 and 2.02 ppm (**c**). **c** splits into two different dublets because the protons couple with a stereo-center (**d**). Comparison of signals **b** and **e** shows that tosylation is quantitative.

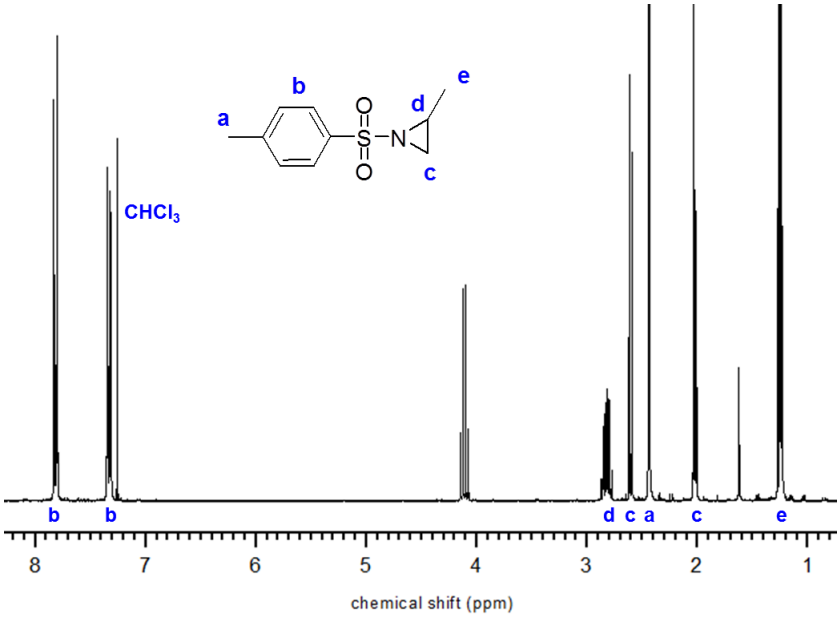

Figure 30: ^{1}H NMR spectrum of 2-methyl-*N*-tosylaziridine in $CDCl_3$

The ^{1}H NMR spectrum of 2-isobutyl-*N*-toslaziridine in $CDCl_3$ displays the characteristic aziridine signals **c** and **d** (Figure 31). Comparison of the integrals **b** and **g** confirms quantitative tosylation.

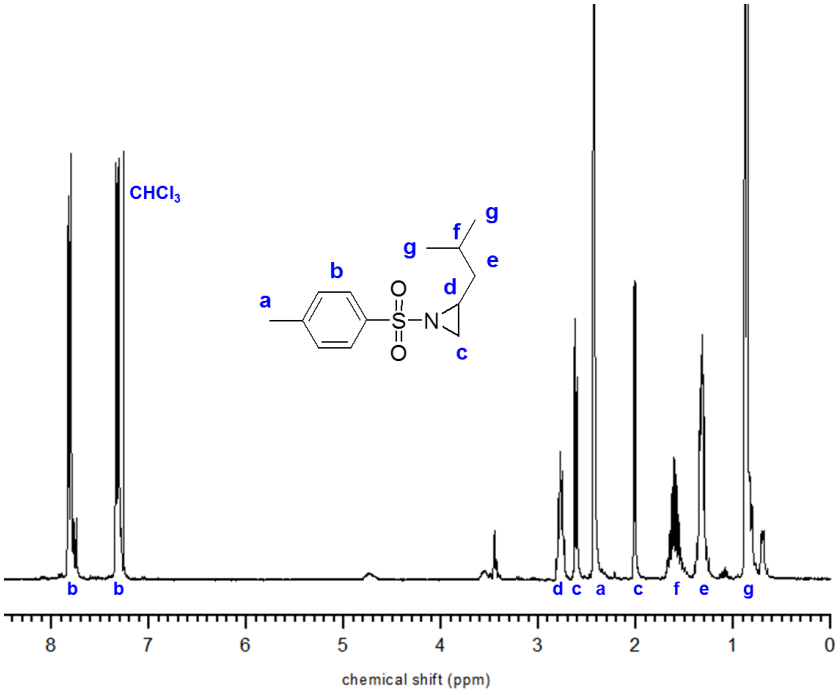

Figure 31: ^{1}H NMR spectrum of 2-isobutyl-*N*-tosylaziridine in $CDCl_3$

Figure 32 depicts the spectrum of 2-*n*-decyl-*N*-mesylaziridine in $CDCl_3$. **b** and **a** represent the aziridine ring protons. Quantitative mesylation can be verified by comparing the integrals of **e** and **d**.

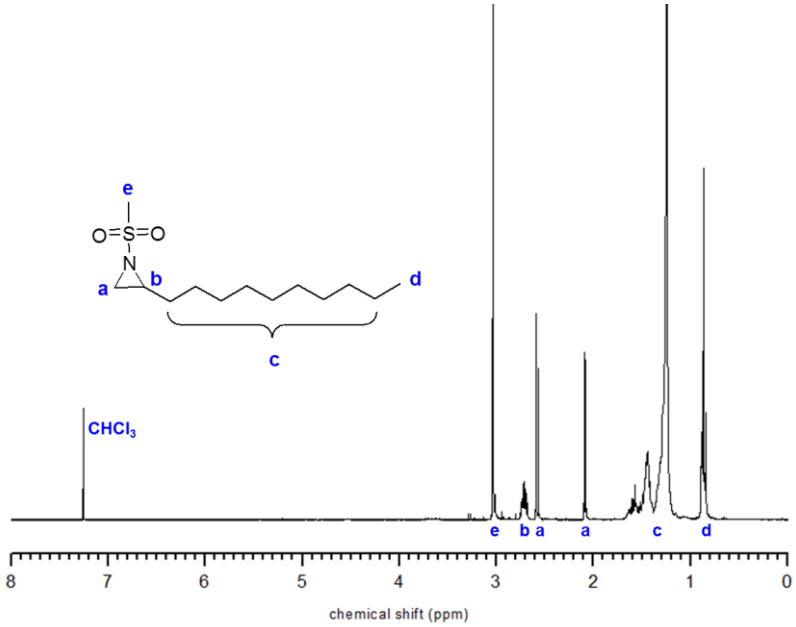

Figure 32: ^{1}H NMR spectrum of 2-*n*-decyl-*N*-mesylaziridine in $CDCl_3$

The spectrum of 2-*n*-(7-octenyl-)*N*-mesylaziridine in $CDCl_3$ displays the characteristic aziridine signals **a** and **b** (Figure 33). Comparison of the integrals of **e** and **g** confirms quantitative mesylation. An isomerisation of the double bond would be indicated by a peak from the resulting methyl group at 1.30 ppm. However, this cannot be discerned from the respective signal of the alkyl chain (**c**). As the integration of **c** is in accordance with the remaining integrals, it can be assumed that no significant isomerisation of the double bond occurs.

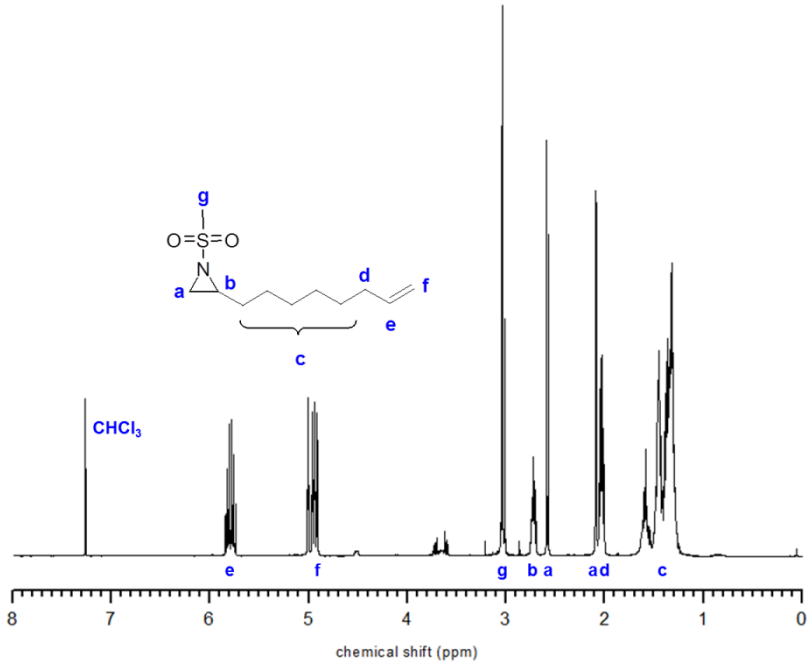

Figure 33: ^{1}H NMR spectrum of 2-*n*-(7-octenyl-)*N*-mesylaziridine in $CDCl_3$

Figure 34 shows the spectrum of 2-methyl-*N*-mesylaziridine. Peaks **a** and **b** confirm the presence of the aziridine ring, while comparing the integrals of **c** and **e** shows quantitative mesylation. The peak at 1.63 ppm indicates the presence of water. This can be disregarded, since all monomers were dried from benzene prior to polymerisation.

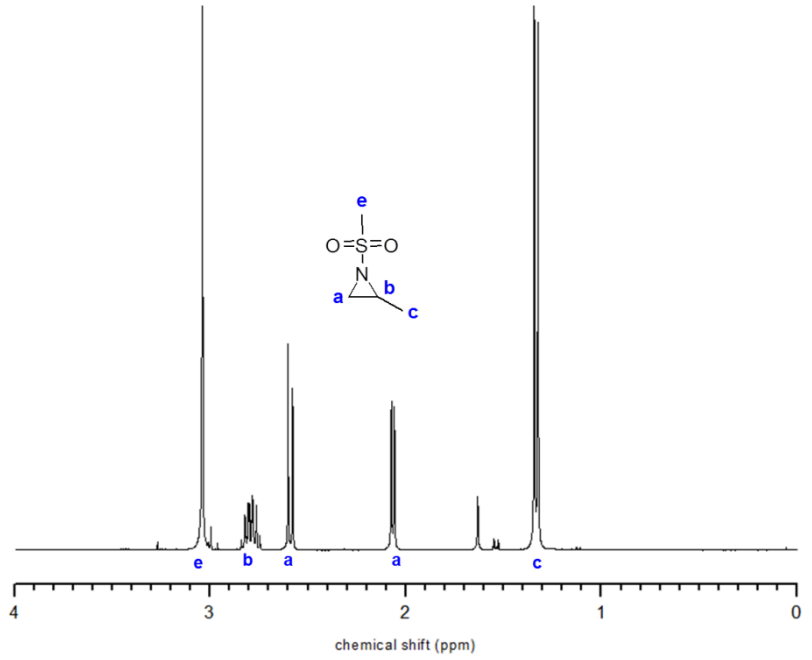

Figure 34: ^{1}H NMR spectrum of 2-methyl-*N*-mesylaziridine in $CDCl_3$

3.2. Poly(aziridine)s

This chapter describes the synthesis and characterisation of poly(aziridine)s. Polymers of a molecular weight between 3000 and 19000 g/mol were synthesized (which is probably not the molecular weight limit). Block copolymers synthesis was targeted to show the living character of this polymerisation technique and to enhance the potential of the method. The double bond functionality in poly(aziridine) polymerised from 2-*n*-(7-octenyl-)*N*-mesylaziridine was to be addressed using a thiol-ene reaction.

3.2.1. Nomenclature

First the nomenclature used for poly(aziridine)s will be explained using the example of poly(TMAz)$_{20}$. The first letter, in this case T, indicates whether a tosyl (T) or mesyl (M) group was used to activate the aziridine. The second letter characterises the substituent at the second position, in this case a methyl group (M). Other possibilities include a decyl group (D) or an octenyl group (O). Az stands for aziridine. The index indicates the degree of polymerisation.

3.2.2. Homopolymers

Anionic ring-opening polymerisation of activated aziridines was performed using a system of two initiators (Figure 35), *N*-benzyl-mesylamide (BnNHMs) and potassium bis(trimethylsilyl)amide (KHMDS). The latter is a strong, sterically hindered base (pK_a = 26),[50] which can also be used to initiate the polymerisation of ethylene oxide.[51] In this case it deprotonates BnNHMs, which in turn initiates polymerisation. The aromatic protons of BnNHMs are required as a reference signal for the ^{1}H NMR characterisation of the polymers.

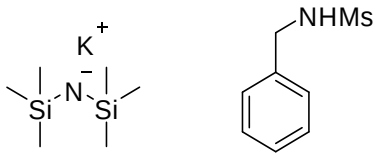

Figure 35: Potassium bis(trimethylsilyl)amide and *N*-benzyl-mesylamide

KHMDS is commercially available, while BnNHMs was synthesized in a one-step procedure from benzylamine (Figure 36) with 30 % yield.[52]

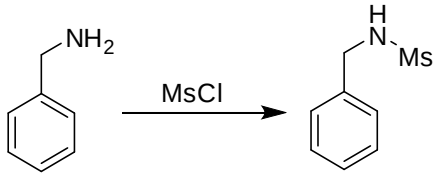

Figure 36: Synthesis of *N*-benzyl-mesylamide

The compound was characterised using ^{1}H NMR spectroscopy (Figure 37). Comparing the integrals of the mesyl group (**d**) and the aromatic protons (**a**) confirms that the amine was only mesylated once.

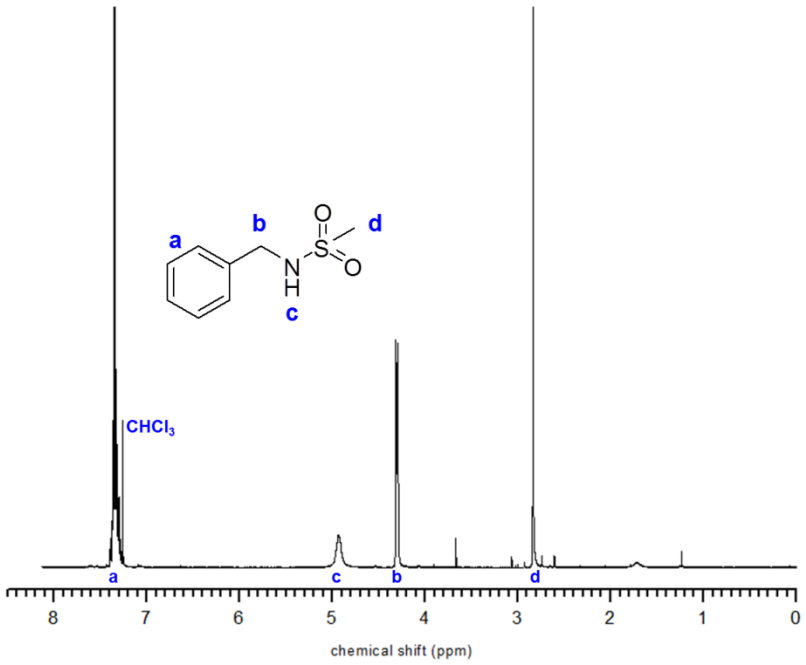

Figure 37: ^{1}H NMR spectrum of *N*-benzyl-mesylamide in $CDCl_3$

For the anionic polymerisation it is vital that any traces of water were completely removed (see section 1.2). Water acts as a chain transfer agent, broadening the distribution and lowering the degree of polymerisation. Therefore all educts were dried under vacuum to remove all volatiles. Benzene was added to remove water azeotropically. Since KHMDS decomposes under aqueous conditions to hexamethyldisilazane and potassium hydroxide, it does not necessarily need to be dried. The evacuated flasks containing the educts were brought into an argon filled glovebox and the initiation was carried out under argon atmosphere in a water-free environment. Although the experimental setup shown in Figure 79 permits in a very convenient way to work under vacuum or argon atmosphere, in this case it is less advantageous than a glovebox. The polymerisation of activated aziridines requires many different flasks and syringes, which would weaken the integrity of

the septa. In the glovebox the educts were dissolved in anhydrous *N*,*N* dimethylformamide (DMF). In some cases the resulting solution of KHMDS was tinged yellow, indicating the presence of hexamethyldisilazane and therefore potassium hydroxide as well. In these cases the solution was discarded and a fresh one was prepared, as hydroxide ions interfere with the anionic polymerisation. The initiator solutions were mixed first, making sure BnNHMs was fully deprotonated before adding the initiators to the monomer. Polymerisation was carried out outside the glovebox in a sealed vial for at least 18 hours. Polymerisation mixtures were often tinged yellow. This can be attributed to the aza-anion whose charge is delocalised over the tosyl or mesyl group. The polymers were precipitated in methanol and obtained as colourless or slightly brown solids. The brown colour probably originates from residual DMF in the samples.

Figure 38 shows the proposed mechanism for the aza-anionic polymerisation of activated aziridines. The general principle is analogous to the anionic ring opening polymerisation of ethylene oxide. The mesylate withdraws electrons from the aziridine ring, rendering it more susceptible to the nucleophilic attack of the initiator.

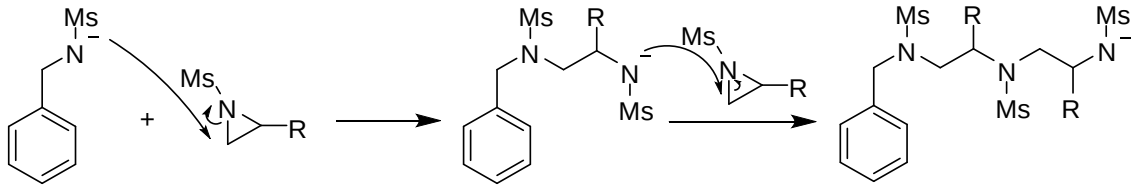

Figure 38: Mechanism for the aza-anionic polymerisation

Figure 39 shows the possible monomer configurations in poly(MMAz). The main structure should be the head to tail attachment, resulting from two successive attacks at the unsubstituted carbon of the aziridine ring. It is unlikely that only the unsubstituted position is attacked, but it is intuitive that this should be the favoured position, as it is sterically less hindered. This would be in accordance with the findings of Quirk *et al.* on a related issue, i.e. the termination of polystyrene with propylene oxide, which resulted in 97 % product of the attack at the least hindered carbon of the ring, while 3 % were the product of the substituted carbon.[53] Attacks at the substituted carbon result in different building blocks as shown in Figure 39.

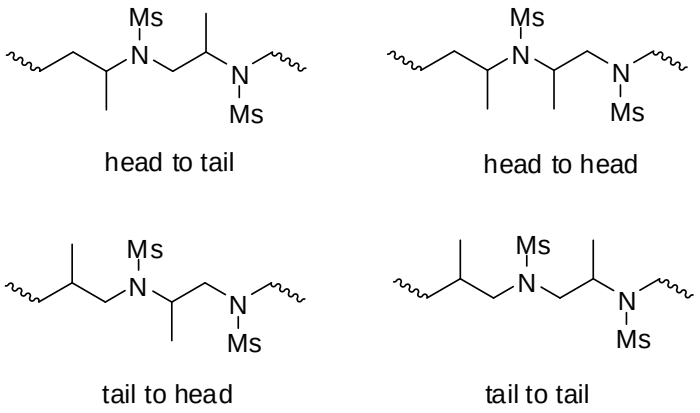

Figure 39: Possible configurations in poly(MMAz)

All resulting poly(aziridine)s were characterised by ^{1}H NMR spectroscopy. Figure 40 shows the spectrum of poly(MDAz)$_{72}$. The resonances of the poly(aziridine) backbone (**c**) can be found between 3.93 ppm and 3.12 ppm. Neither the signal of the methylene group of the initiator (**c**) nor the signal of the mesyl groups (**b**) at 3.45 ppm can be clearly distinguished from the backbone. The aromatic protons of the initiator (**a**) are set to 5. By comparing this integral with the signal of the methyl group (**d**) the number of repeat units is calculable. Thereby the number average molar mass M_n can be determined. If tosylated aziridines are polymerised, this is not possible, as the aromatic protons of the tosyl group overlay those of the initiator. In this case, only a different initiator would make determination of M_n possible.

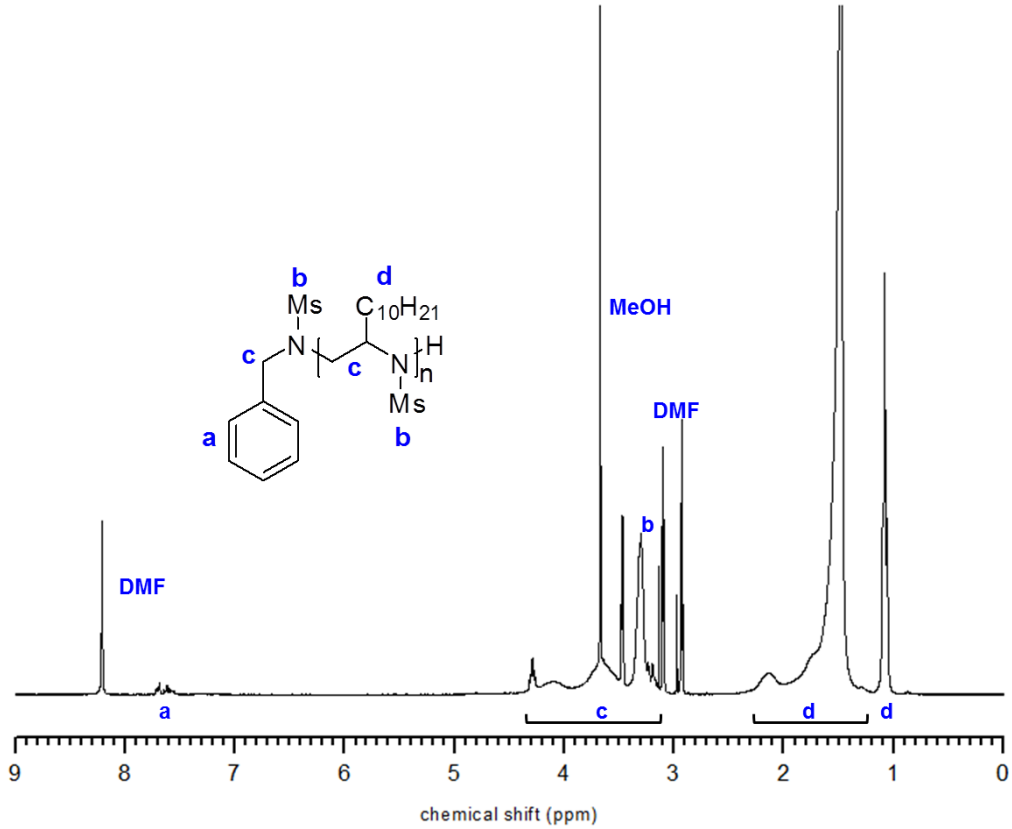

Figure 40: ^{1}H NMR spectrum of poly(MDAz)$_{72}$ in DMF-d_7

To gain a better understanding of the ^{1}H NMR spectra of poly(aziridine)s, a COSY spectrum was measured (Figure 41). The horizontal axis shows the ^{1}H NMR spectrum, the vertical axis shows the ^{13}C NMR. The ^{1}H-^{13}C COSY spectrum was measured using the DEPT (Distortionless Enhancement by Polarization Transfer) method. Normally ^{13}C spectra are measured in a hydrogen-decoupled mode to avoid signal splitting due to coupling between ^{13}C and hydrogen. The resulting signals would overlap, making identification very difficult. Decoupling results in sharp signals, but it means that information regarding spin-spin coupling between ^{13}C and ^{1}H is lost. The DEPT method provides this information. By variation of the pulse angle, this method allows differentiating between CH_2 groups on the one hand and CH and CH_3 groups on the other hand. The spectrum reveals that the signal at 4.40-4.04 ppm belongs to the substituted carbon atom (**b**) of the backbone, while the signal at 3.64-3.04 ppm can be attributed to the methylene group (**a**).

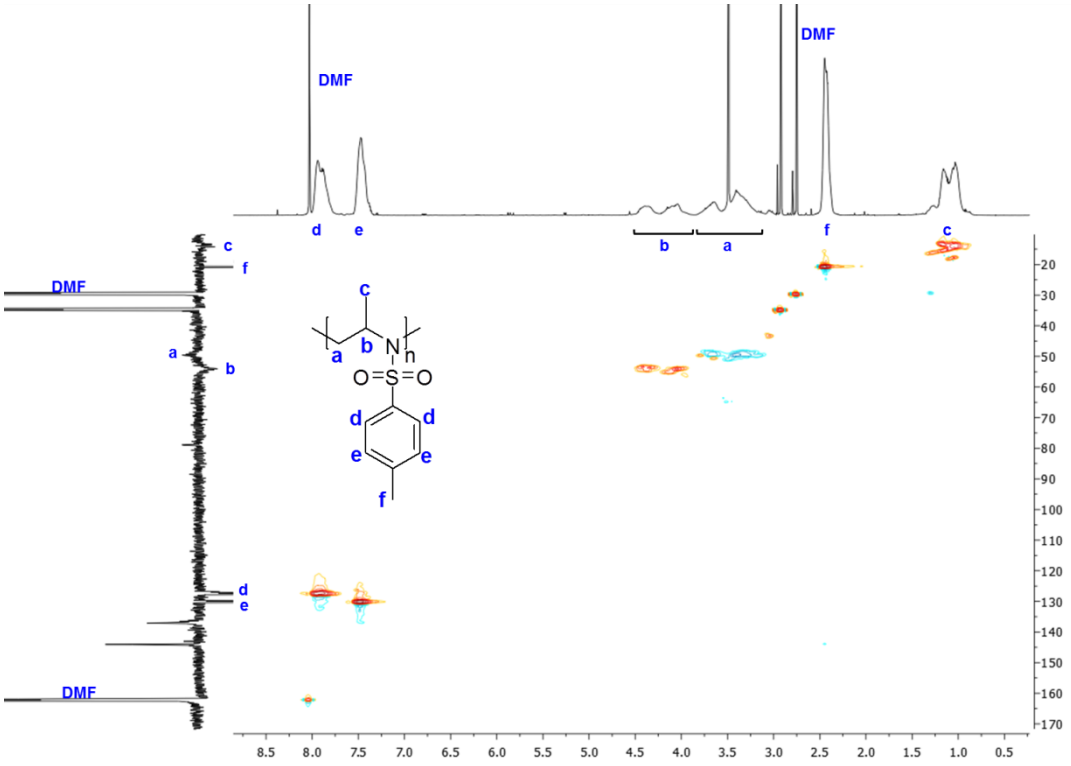

Figure 41: ^{1}H-^{13}C COSY spectrum of poly(TMAz)

The polymers were further characterised by SEC in DMF (see section 5.1.2). Since no poly(aziridine) standard is available for calibration, the molar mass distributions obtained from this method are unreliable. Samples were measured with a PEG standard and it can be assumed that the hydrodynamic radius of PEG and PAz differ. Nevertheless SEC is useful for evaluating the molecular weight distribution of a given polymer, the PDI and whether it is a monomodal distribution or not. When the sample is analysed prior to precipitation, SEC can indicate whether turnover was complete. Figure 42 shows the SEC trace of poly(MDAz)$_{72}$. V_e is the elution volume. Analysis resulted in a narrow, monomodal distribution, which was generally the case for poly(aziridine)s. In some cases SEC traces showed a small peak that could be attributed to residual monomer, indicating incomplete consumption of the starting material. This surplus monomer was removed by precipitation and was not visible in following SEC traces.

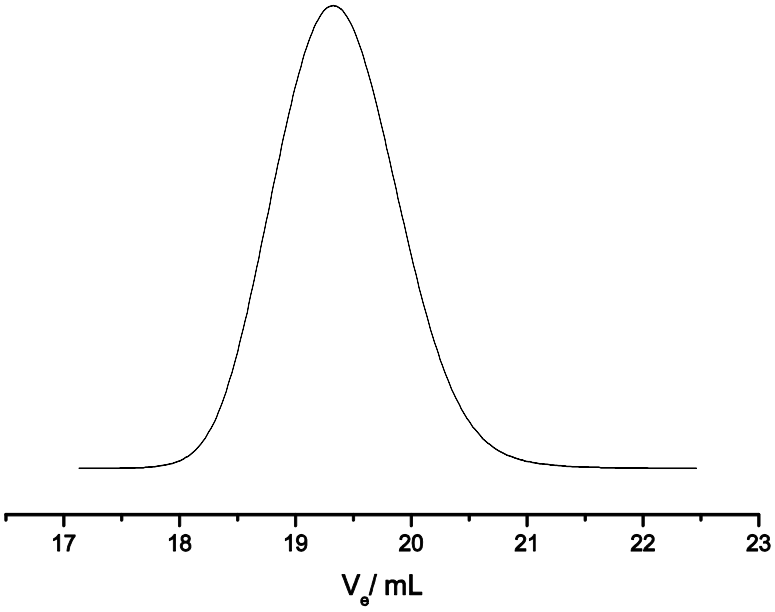

Figure 42: SEC trace of poly(MDAz)$_{72}$ in DMF (RI signal)

Table 1 summarises the NMR and SEC data of poly(aziridine)s. Poly(aziridine)s ranging from 3600 to 19200 g/mol were synthesized. Generally, very narrow molecular weight distributions were obtained with PDIs below 1.10. The only exception is poly(TMAZ)$_{40}$, which can be attributed to insufficient stirring of this sample. Since the aromatic protons of the tosyl group mask the aromatic protons of the initiator BnNHMs, it was not possible to determine M_n and the number of repeat units from NMR for tosylated poly(aziridine)s. It was shown that initiation is also possible with only KHMDS, but the methyl groups of the initiator could not be discerned in the NMR spectrum, which made integration impossible (usually KHMDS gives a signal at 0.04 ppm in DMF-d_7). However, the combination of both initiators and mesylated monomers allowed calculation of M_n and the repeat units from NMR data. As expected, molecular weights from NMR and SEC data differ, usually by a factor of 2-3. SEC analysis results in an underestimate of the actual molar mass. As explained in section 5.1.2, the determining factor for SEC analysis is the hydrodynamic radius of a given polymer. This decides how long the polymer remains in the separation column of the SEC. Poly(aziridine)s appear to be able to form a more compact structure than poly(ethylene glycol), which was used as a standard, i.e. their hydrodynamic radius is generally smaller. This is reflected by a lower apparent molar mass. However, this does not mean that M_n calculated by NMR is perfectly accurate. The larger the polymer signals become, the less accurate is integration over the initiator signal. This leads to an increasing error when polymers of a higher molar mass are analysed.

Table 1: Overview of the NMR and SEC data of poly(aziridine)s, M_n is given in g/mol, a PEG standard was used for SEC analysis

Sample	Monomer (activating group/ substituent)	Initiator	M_n (theo)	M_n (SEC)	PDI	M_n (NMR)	Repeat units$_{(NMR)}$
PTMAz	Ts/Methyl	KHMDS/BnNHMs	4400	2100	1.06	-*	-*
PTMAz	Ts/Methyl	KHMDS	8600	3200	1.10	-*	-*
PTMAz	Ts/Methyl	KHMDS	8600	7400	1.20	-*	-*
$PMDAz_{72}$	Ms/Decyl	KHMDS/BnNHMs	21100	6200	1.06	19200	72
$PMDAz_{30}$	Ms/Decyl	KHMDS/BnNHMs	13200	4000	1.05	8000	30
$PMOAz_{20}$	Ms/Octenyl	KHMDS/BnNHMs	11700	2800	1.09	4800	20
$PMMAz_{25}$	Ms/Methyl	KHMDS/BnNHMs	2900	1900	1.06	3600	25

* Determination of M_n not possible, due to overlay of the aromatic protons of the tosyl-group and the initator.

The polymers were further characterised by MALDI-ToF MS. This method confirms that the monomers employed are actually incorporated in the polymer chain. The method is not well-suited to assess the distribution of a polymer, since some species may be more difficult to ionise than others and are therefore underrepresented in the spectrum (see section 5.1.3). The MALDI-ToF MS spectrum of $Poly(MDAz)_{30}$ can be seen in Figure 43. The main distribution matches the calculated mass of Poly(MDAz) initiated by BnNHMs with potassium as a counter ion. The smaller subdistribution could not be attributed to a specific counter ion or a polymer initiated with KHMDS, but the distance between two peaks matches the mass of the repeat unit.

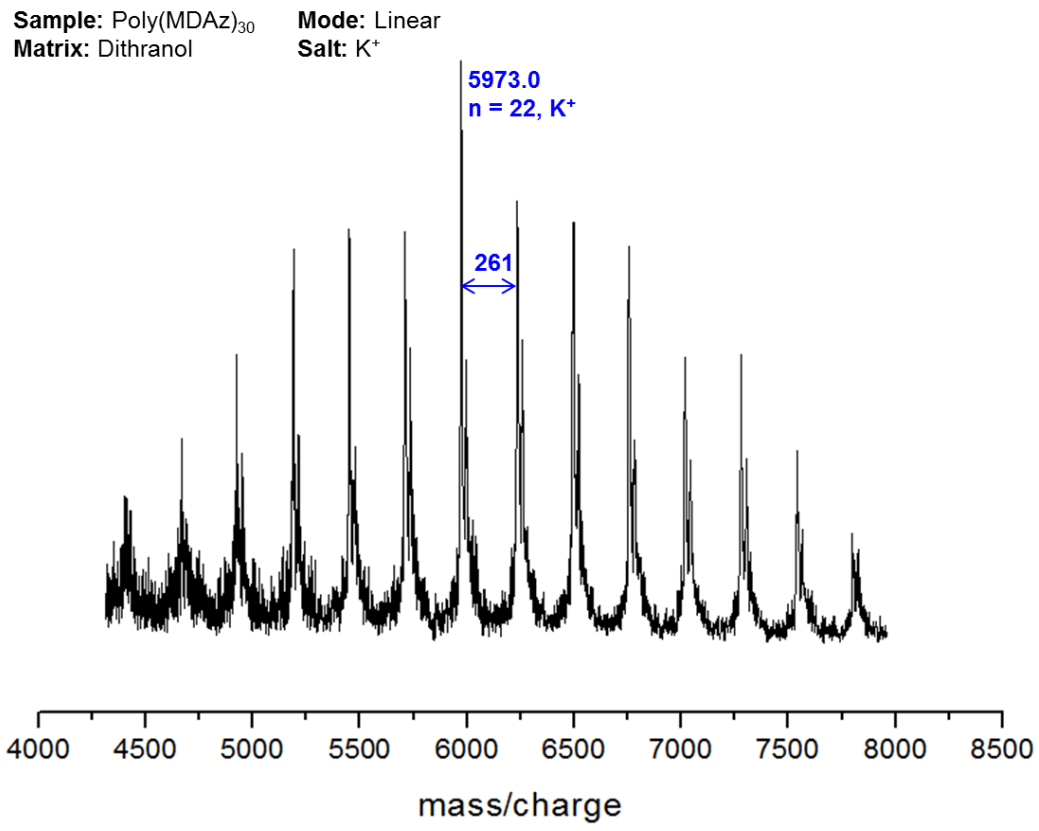

Figure 43: MALDI-ToF MS spectrum of poly(MDAz)$_{30}$

3.2.3. Copolymers and block copolymers

In this section the synthesis of copolymers and block copolymers consisting of different aziridine monomers is described. All copolymers and block copolymers were initiated using the system of KHMDS and BnNHMs described in section 3.2.2. For copolymers a solution of both monomers was prepared inside a glovebox to which the initator solution was added. For block copolymers the second monomer had to be added inside the glovebox. Simple addition via a gas tight syringe and resealing the puncture point resulted in termination of the chain, no further monomer was added. However, successful synthesis of block copolymers is important to show the living character of this polymerisation technique. The resulting copolymers and block copolymers were characterised by ^{1}H NMR spectroscopy, SEC and MALDI-ToF MS. Figure 44 shows the ^{1}H NMR spectrum of poly(MDAz)$_{41}$-*block*-poly(MOAz)$_{2}$. The initiator signal (**a**) is set to 5, which allows determination of the repeat units of the monomer blocks. Dividing the signal of the methyl group (**e**) by three yields the repeating units of 2-*n*-decyl-*N*-mesylaziridine. By subtracting the remaining protons of the decyl chain from signal **d** and dividing the result by twelve, the repeat units of 2-*n*-octen-7-yl-*N*-mesylaziridine are obtained. No isomerisation of the double bond could be observed. Therefore determination of the repeat units of 2-*n*-octen-7-yl-*N*-mesylaziridine is also possible by dividing signal **g** by one or signal **h** by two.

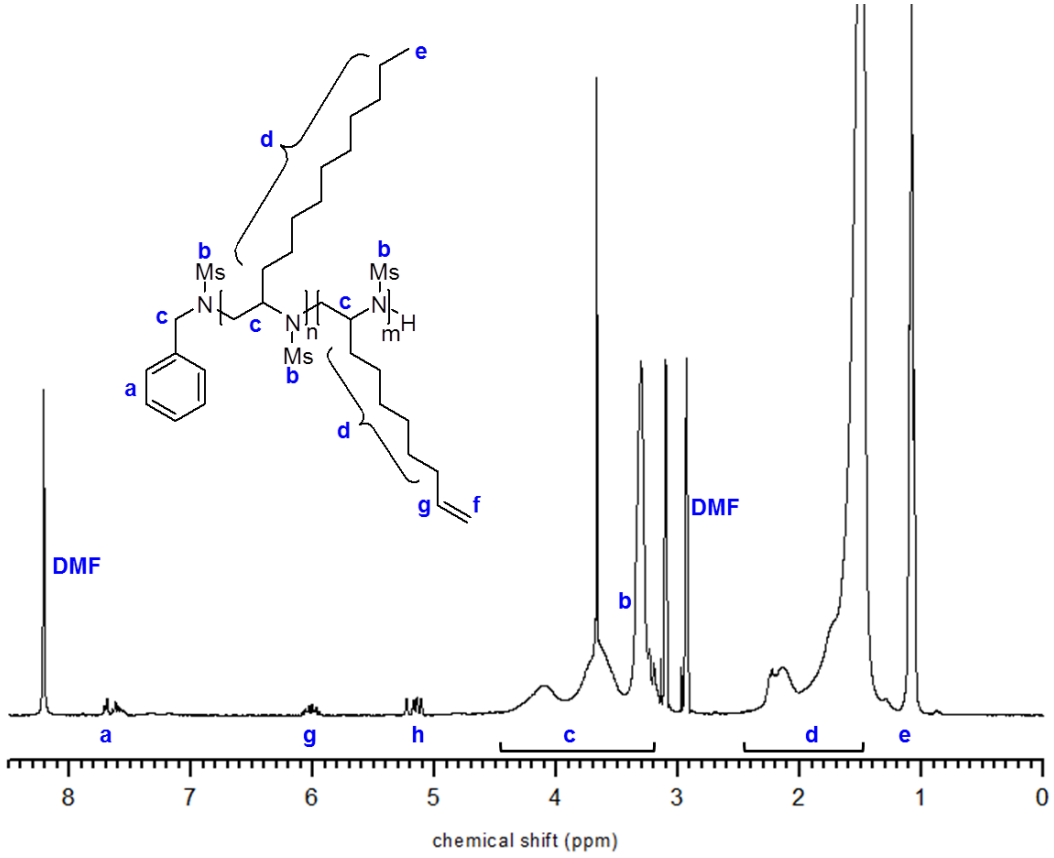

Figure 44: ^{1}H NMR spectrum of Poly-(MDAz)$_{41}$-*block*-poly(MOAz)$_{2}$ in DMF-d_7

SEC analysis of poly(aziridine) copolymers and block copolymers results in a monomodal, narrow molecular weight distribution for all samples (Figure 45). This is clear evidence that the monomers copolymerised, otherwise several distribution modes should have been detected by SEC.

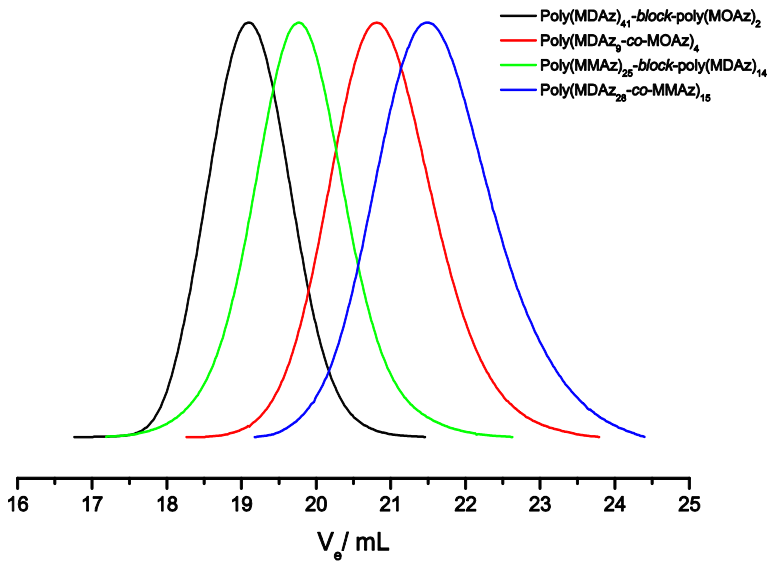

Figure 45: SEC traces of poly(aziridine) copolymers and block copolymers in DMF (RI signal)

Table 2 summarises the SEC and NMR analysis of poly(aziridine) copolymers and block copolymers. Usually PDIs below 1.10 were obtained. Again there is a discrepancy between $M_{n\ (SEC)}$ and $M_{n\ (NMR)}$, as explained in section 3.2.2. Poly(MDAz)$_{41}$-*block*-poly(MOAz)$_2$ and poly(MDAZ$_9$-*co*-MOAz)$_4$ show noticeable results. NMR characterisation of poly(MDAz)$_{41}$-*block*-poly(MOAz)$_2$ shows that polymerisation of the first block worked very well, while only two units of 2-*n*-octen-7-yl-N-mesylaziridine were added to the polymer chain. The simplest explanation for this is that during the addition of the second monomer most polymer chains were terminated and only a few actually propagated with 2-*n*-octen-7-yl-*N*-mesylaziridine as the second monomer. However, in this case SEC should be able to separate these two polymer species and show a bimodal distribution. Furthermore SEC analysis of the crude polymerisation mixture showed a residual monomer peak, meaning that the consumption was not complete and indicating that the polymerisation of 2-*n*-octen-7-yl-*N*-mesylylaziridine is slower than that of the remaining polymer. This is further confirmed by analysis of poly(MDAZ$_9$-*co*-MOAz)$_4$, where 20 and 10 repeating units were aimed for, but only 9 and 4 were achieved. Addition of 2-*n*-octen-7-yl-*N*-mesylaziridine seems to lower the reactivity of the whole system. A similar trend was observed with poly(MOAz)$_{20}$, where only 41 % of the targeted molar mass was achieved.

Table 2: SEC and NMR data of aziridine copolymers and block copolymers, M_n is given in g/mol, a PEG standard was used for SEC analysis

Sample	Repeat units (theo)	$M_{n\ (theo)}$	$M_{n\ (SEC)}$	PDI	$M_{n\ (NMR)}$	Repeat units (NMR)
Poly(MDAz)$_{41}$-*block*-poly(MOAz)$_2$	40/20	15200	4500	1.06	11300	41/2
Poly(MDAZ$_9$-*co*-MOAz)$_4$	20/10	7700	2100	1.09	3500	9/4
Poly(MMAz)$_{25}$-*block*-poly(MDAz)$_{14}$	20/10	8100	3300	1.09	7200	25/14
Poly(MDAz$_{28}$-*co*-MMAz)$_{15}$	40/20	13300	1500	1.11	9500	28/15

It is unknown whether aziridine copolymers form statistical copolymers, blocks or gradient structures, as kinetic aspects have not yet been investigated. It can however be concluded, that 2-*n*-octen-7-yl-*N*-mesylaziridine lowers the polymerisation rate, even if another monomer is present. This might be due to electronic interaction between the electron rich double bond and the electron deficient aziridine ring. Coordination of the double bonds to the aziridine rings inter- or intramolecularly would increase the electron density in the ring. Consequently, the probability of a nucleophilic attack by the initiator or a propagating polymer chain would be less likely. Steric hindrance of the monomers could impede an attack of the initiator as well. This hypothesis might be verified by adding an inert molecule with a double bond, e.g. hexene, to a polymerisation that would otherwise proceed normally.

Figure 46 shows the MALDI-ToF MS spectrum of poly(MDAz)$_{41}$-*block*-poly(MOAz)$_2$. Both monomer units are observed in the spectrum, which proves that both were incorporated into the polymer chain. The maximum of the main distribution corresponds to a polymer with 27 repeat units, which is much less than the amount calculated from the NMR. This could be due to the mass discrimination effect, often observable in MALDI-ToF MS.

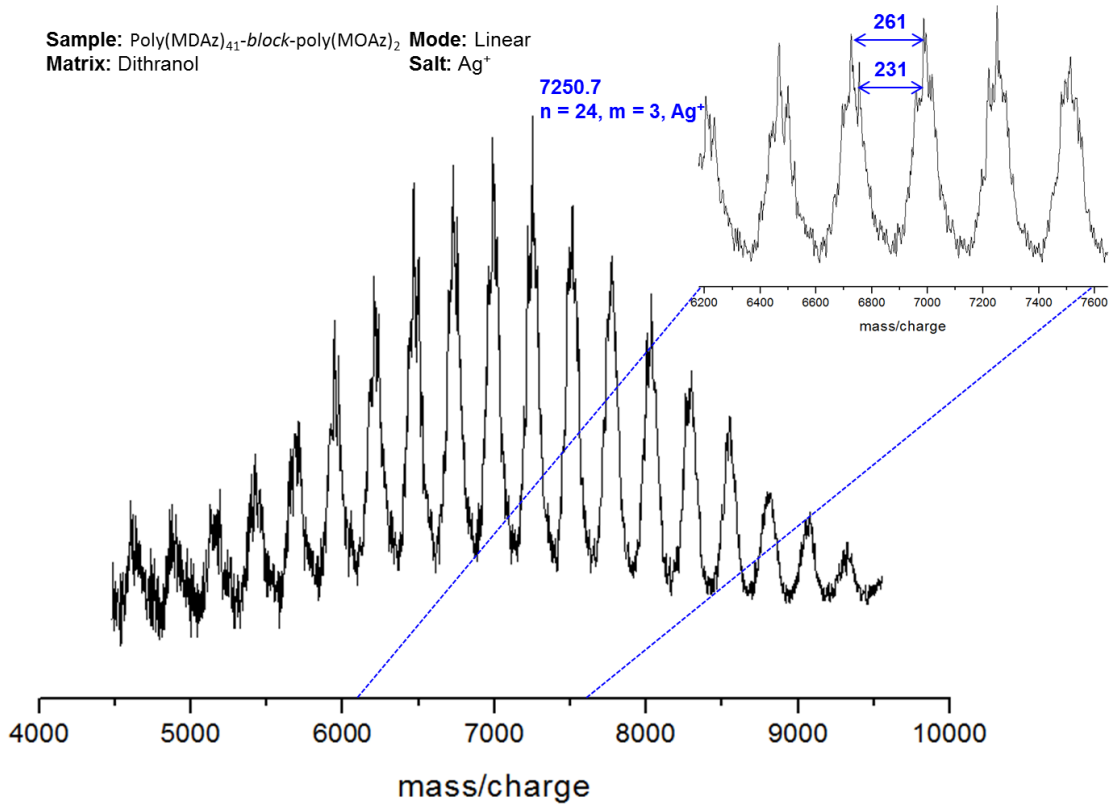

Figure 46: MALDI-ToF MS spectrum of poly(MDAz)$_{41}$-*block*-poly(MOAz$_2$)

3.2.3.1. Thiol-ene modification

2-*n*-Octen-7-yl-N-mesylaziridine is a monomer that offers the possibility of further modification on the polymer chain. To address the double bond, a thiol-ene reaction was performed. A protocol known in literature was used.[54] Poly(MDAZ$_9$-*co*-MOAz)$_4$ was chosen as a polymer. *N*-Acetyl-L-cysteine methyl ester was chosen as the thiol compound, as it is detectable by NMR spectroscopy and is a model compound for future peptide attachment, as the coupling of poly(aziridine)s to biological compounds could offer potential for targeted transfection. Testing the transfection ability of poly(aziridine)s was beyond the scope of this work, but coupling the polymers to specific ligands, could offer the possibility to direct DNA to specific cells with the respective receptor (see section 1.3.2).

Figure 47 shows the ^{1}H NMR spectrum of the product. The signals of the double bond at 5.91-5.77 and 5.05-4.93 ppm have disappeared, confirming complete conversion. All new peaks can be attributed to *N*-Acetyl-L-cysteine methyl ester, as shown in the spectrum. Integration of the *N*-Acetyl-L-cysteine methyl ester signals matches the number of double bonds in the starting material. The integrals of the aziridine backbone and the mesyl group are unchanged, as expected the reaction did not affect them.

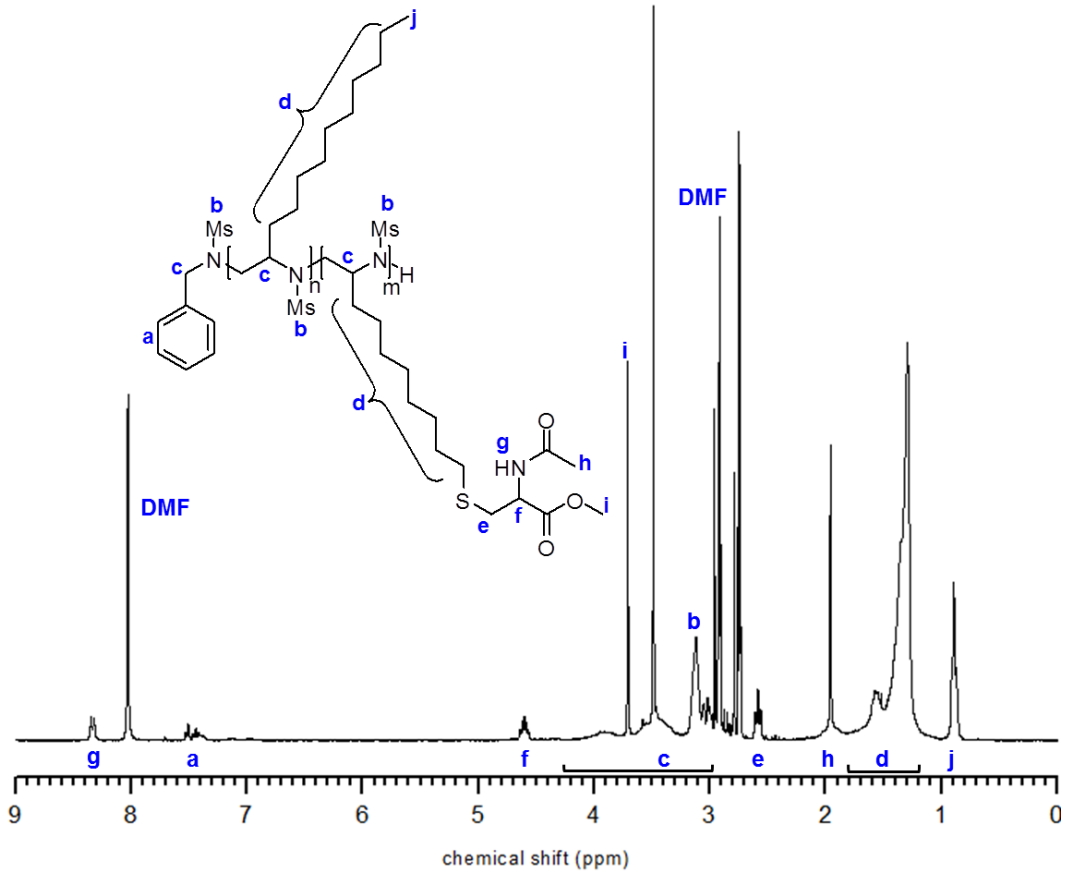

Figure 47: ^{1}H NMR spectrum of poly(MDAz$_9$-*co*-MOAz)$_4$ conjugate to *N*-Acetyl-L-cysteine methyl ester in DMF-d_7

3.2.4. 2-Isobutyl-*N*-tosylaziridine

This section describes the polymerisation of 2-isobutyl-*N*-tosylaziridine. The results are summarised in Table 3. At first, polymerisation was initiated with stock solutions of the initiators. When this did not yield any polymer, the idea of using stock solutions was discarded and fresh solutions were prepared every time. Longer reaction times did not result in any polymerisation either, therefore BnNHMs was removed from the system to eliminate a potential source for contaminations. However, no polymerisation of 2-isobutyl-*N*-tosylaziridine could be observed and BnNHMs continued to work when other monomers were employed. Polymerisation was tried again at elevated temperatures for a longer time, which still failed to yield polymer. As not even oligomerisation was observed, it was concluded that the isobutyl group is sterically hindering the nucleophilic attack of the initiator on the aziridine ring. Contaminations in the monomer could prevent polymerisation as well, but this is unlikely as the NMR spectrum was pure and 2-methyl-*N*-tosylaziridine, which polymerised well, was prepared by the same chemical route. It is possible that polymerisation could be forced by changing the reaction conditions further, e.g. using a smaller initiator, which could more easily attack the bulky monomer. Adding 18-crown-6 could change matters as well as it forms a complex with the counter

ion, in this case potassium. This may leave more room at the active chain end and increase its reactivity, so it could potentially open the aziridine ring.

Table 3: Results of polymerisation attempts with 2-isobutyl-*N*-tosyl-aziridine

Monomer	Initiator	Solvent	Temperature / °C	Reaction time/ h	Repeat units$_{(theo)}$	result
2-^{i}B-*N*-tosyl-aziridine	KHMDS/BnNHMs (stock solution)	DMF	50	20	40	no polymer
2-^{i}B-*N*-tosyl-aziridine	KHMDS/BnNHMs (stock solution)	DMF	50	20	30	no polymer
2-^{i}B-*N*-tosyl-aziridine	KHMDS/BnNHMs	DMF	50	70	40	no polymer
2-^{i}B-*N*-tosyl-aziridine	KHMDS	DMF	50	20	40	no polymer
2-^{i}B-*N*-tosyl-aziridine	KHMDS	DMF	90	70	40	no polymer

3.3. Coupling to poly(styrene)

This chapter describes the coupling of the carb-anionic polymerisation of styrene to the aza-anionic polymerisation of aziridines. It stands to reason that a carbanion such as the living chain end of poly(styrene) should be sufficiently nucleophilic to open the ring of an activated aziridine. The question is though, does the chain propagate and form a block copolymer or does the reaction stop after the addition of one aziridine? It is intuitive that the counter ion should play a significant role in this. If the affinity of the counter ion to the active chain end is too high, which is the case for lithium and an oxyanion, the chain will not propagate any further. As shown in section 3.2, potassium serves perfectly well as a counter ion for poly(aziridine). Therefore, poly(styrene) initiated with potassium naphthalenide should be able to form block copolymers, in this case tri-blocks, as initiation with potassium naphthalenide leads to two active chain ends. Another common initiator for polystyrene is *sec*-BuLi. How lithium behaves as a counter ion for aza-anionic polymerisation is unknown and an interesting question. Its affinity for nitrogen is not as high as for oxygen, as oxygen is classified as the harder lewis base according to the HSAB theory and therefore shows a high affinity to lithium as a hard lewis acid. But it could still be high enough to terminate the propagation. The influence of the solvent should be considered as well. As shown in Figure 4, polar solvents lead to dissociated ions, leaving a more reactive chain end. Common solvents for the polymerisation of styrene are cyclohexane and THF. If the chain propagates in cyclohexane, it should do the same in THF, since it is more polar than cyclohexane.

3.3.1.1. Poly(aziridine) initiated with *sec*-butyllithium

For switching from the carb-anionic polymerisation of styrene to the aza-anionic polymerisation of aziridines, it is important to know whether an aziridine monomer can be initiated using *sec*-BuLi. It is unknown how well the aza-anionic polymerisation works, when the counter ion is lithium instead of potassium. Two model reactions were conducted, which differed only in their temperature. The first was kept at 55 °C, which is the temperature at which polymerisation of aziridine were usually carried out. The second was kept at -100 °C, which is the temperature at which the aziridine was added to the living poly(styrene) chain.

Table 4 summarises the SEC data of the experiments. The data shows that *N*-tosylaziridine can be polymerised with lithium as a counter ion. This means that, theoretically, adding *N*-tosylaziridine to a living poly(styrene) chain should result in addition of a second block of poly(aziridine). It should be noted that poly(*N*-tosylaziridine)s proved to be hardly soluble in common laboratory solvents. Insoluble residual material was removed via filtration prior to characterisation.

Table 4: SEC data of poly(aziridine) initiated with *sec*-BuLi, M_n is given in g/mol, a PEG standard was used for SEC analysis

Sample	Temperature/ °C	Solvent	$M_{n\ (SEC)}$	PDI
Poly(TAz)	55	THF	1300	1.21
Poly(TAz)	-100	THF	1300	1.54

Figure 48 shows the MALDI spectrum of the experiment conducted at -100 °C. Only one, very distinct distribution can be detected, which contradicts the PDI of 1.54 obtained by SEC characterisation. It can be assumed that the MALDI spectrum is not representative of the whole sample, as parts were insoluble. In this experiment the molar mass obtained from SEC seems to be in good accordance with the MALDI-ToF spectrum. This is an indicator that only a portion of the sample is represented in the latter, as SEC using a PEG standard results in an underestimation of the molecular weight of PAz.

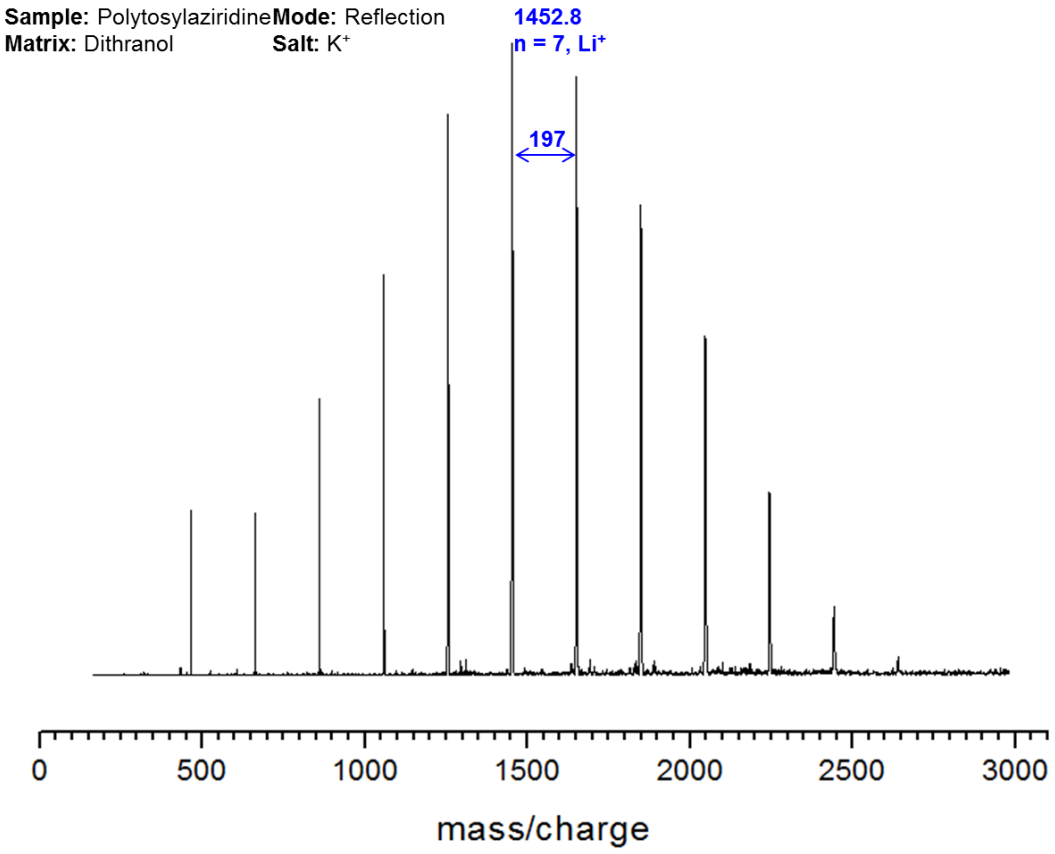

Figure 48: MALDI-ToF MS spectrum of Poly(TAz)

3.3.2. Synthesis and characterisation

The aim was to synthesize poly(styrene) initiated by *sec*-BuLi and add >1 equivalent of *N*-tosylaziridine to the living chain end. *N*-tosylaziridine was chosen because it is the simplest possible aziridine monomer. Any substituents at the ring, such as a methyl group, could potentially transfer a proton to the living poly(styrene) chain and lead to a normal termination, as Quirk *et al.* proposed in 1998 for propylene oxide.[53] The polymerisation of poly(styrene) was conducted in the experimental setup shown in Figure 79 to ensure a water and oxygen free environment. For a more detailed description of the setup see section 5.3. For any anionic polymerisation it is vital that traces of water are removed, as water acts as a chain termination agent and thereby broadens the distribution. The setup was heated under reduced pressure and flooded with argon repeatedly prior to use. After purification of the styrene (see section 5.3), the polymerisation is initiated by adding *sec*-BuLi via a gas tight syringe. The bright red colour confirms the formation of a poly(styryl) anion (see Figure 49). Upon addition of the aziridine the colour abruptly changes to yellow. This is due to the presence of the aza-anion in the mixture. The negative charge at the nitrogen is delocalised over the tosyl group. Addition of methanol terminates the living chain and results in a colourless solution.

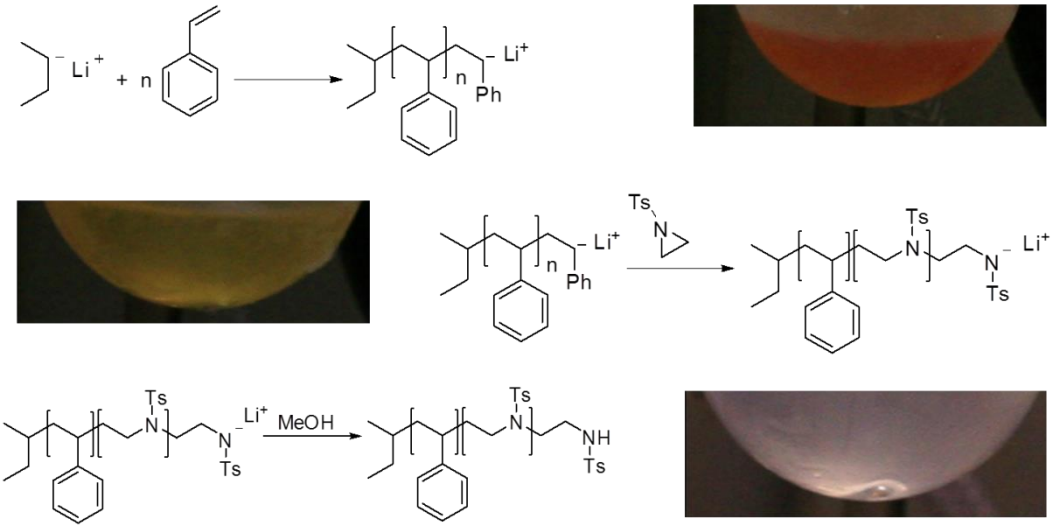

Figure 49: Separate reaction steps of coupling styrene and *N*-tosylaziridine

The exact reaction conditions varied as a suitable protocol was developed and are summarised in Table 5. The first four attempts were not terminated with methanol but stirred until the solution was colourless, which usually took 18 hours. $t_{(PS)}$ and $t_{(PAz)}$ are the polymerisation times for styrene and *N*-tosylaziridine respectively. $T_{(PS)}$ and $T_{(PAz)}$ are the temperatures at which the polymerisation was carried out.

Table 5: Experimental conditions for coupling *N*-tosylaziridine with styrene

Sample	solvent	repeat units (theo., styrene)	repeat units (theo., aziridine)	$t_{(PS)}$/ h	$t_{(PAz)}$/ h	$T_{(PS)}$/ °C	$T_{(PAz)}$/ °C
PS-*block*-TAz-1	THF	20	2	0.5	18.0	-80	-80
PS-*block*-TAz-2	Cyclohexane	20	2	18.0	18.0	r.t.	r.t.
PS-*block*-TAz-3	Cyclohexane	20	2	18.0	18.0	r.t.	-30
PS-*block*-TAz-4	Cyclohexane	20	5	18.0	44.0	r.t.	-50
PS-*block*-TAz-5	THF	20	3	0.5	2.5	-80	-80
PS-*block*-TAz-6	THF	20	6	1.5	1.5	-100	-100
PS-*block*-TAz-7	THF	20	11	1.5	2.0	-100	-100
PS-*block*-TAz-8	THF	10	30	1.5	2.0	-100	-100

As explained in section 3.3, there was the possibility that propagation would occur only in THF, as cyclohexane could be too apolar. In the first two attempts, PS-*block*-TAz-1 and PS-*block*-TAz-2, both solvents were tested. ^{1}H NMR characterisation (see Figure 50) showed aziridine signals, corresponding to one aziridine unit per chain, indicating termination of the polymer chain. However the NMR solution was found to scatter light, suggesting aggregation of the polymer. This could not be circumvented by use of other common laboratory solvents. As poly(TAz) is hardly soluble, it is likely that the poly(styrene) chain wraps itself around and masks the aziridine units. This leads to poor detection by NMR. MALDI spectra of these samples could not be obtained. Thin layer chromatography (TLC) with toluene as the mobile phase showed two spots, the first moved with the mobile phase, while the second did not move at all. Both were UV active, the second one responded to treatment with ninhydrin. It was concluded that the first spot was non-functionalised poly(styrene), terminated by proton transfer, while the second spot consisted of the desired copolymer.

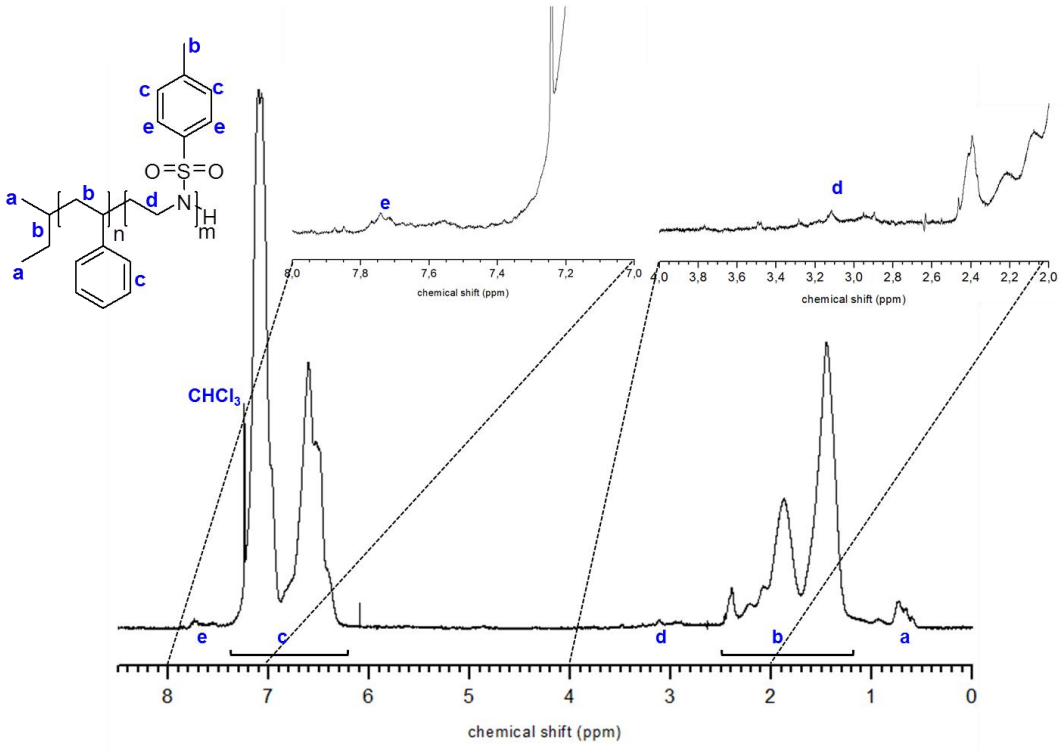

Figure 50: ^{1}H NMR spectrum of PS-*block*-TAz-1 in $CDCl_3$

The protocol was modified to suppress proton transfer to the poly(styrene) chain. For PS-*block*-TAz-3 the temperature was lowered upon aziridine addition, in order to favour the main reaction. Yet TLC analysis still resulted in two spots. For PS-*block*-TAz-4 the temperature was lowered even further and more equivalents of aziridine were used, but TLC still showed two spots. Interestingly, the NMR

spectrum of PS-*block*-TAz-4 displayed very distinct aziridine signals for the first time (see Figure 51). They correspond to three aziridine units per chain. Considering that parts of the spectrum consist of non-functionalised poly(styrene), the actual poly(aziridine) chain length should be even higher.

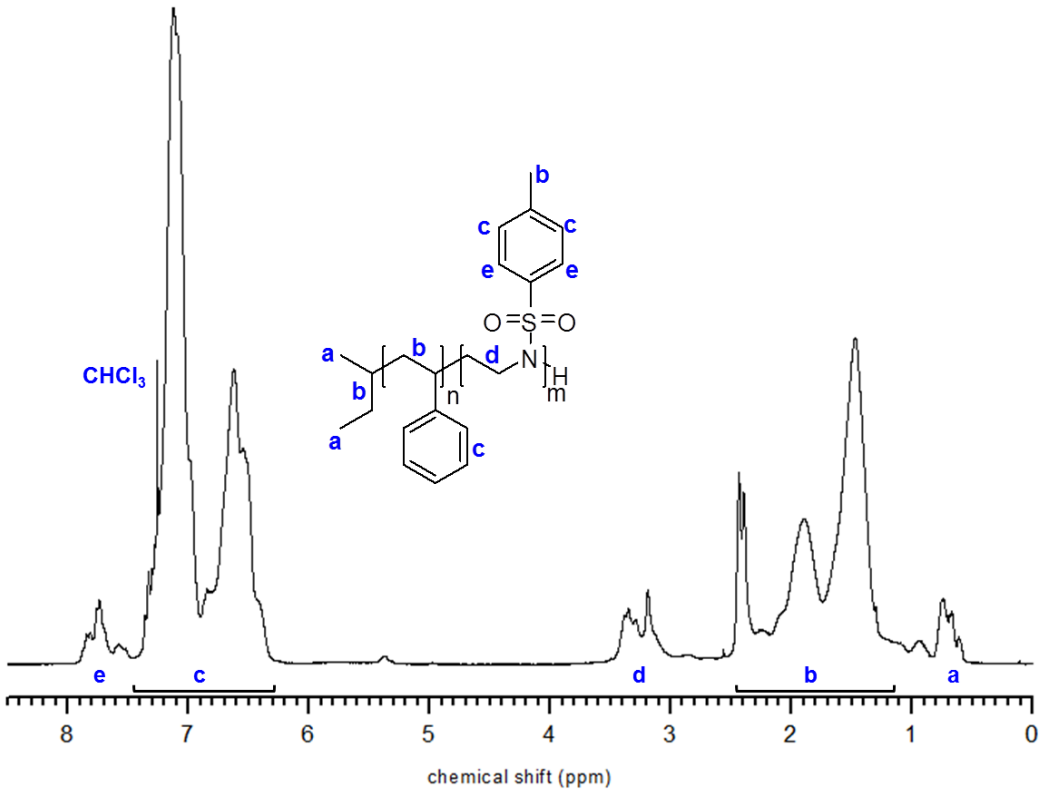

Figure 51: ^{1}H NMR spectrum of PS-*block*-TAz-4 in $CDCl_3$

Figure 51 suggests that cyclohexane allows propagation of the aziridine chain, but the procedure still resulted in partly unmodified poly(styrene). Since the experimental setup for polymerising in cyclohexane is more complicated, as *N*-tosylaziridine is insoluble in cyclohexane, further experiments were carried out in THF. *N*-Tosylaziridine was purchased at 98 % purity, which could easily be not pure enough for an anionic polymerisation. Consequently, it was recrystallised from petrol ether (PE)/ethyl acetate (EA) (6:4), the purified compound was used for all further experiments. The temperature for aziridine addition was lowered even further and addition was conducted with a gas tight syringe, to reduce the possibility of oxygen and water contamination. PS-*block*-TAz-5 to -8 were carried out under these new conditions. TLC analysis of all samples showed only one spot, which did not move with the mobile phase. Figure 52 shows the superimposed ^{1}H NMR spectra of PS-*block*-TAz-5 to -8. Spectra were standarised on the initiator signal. All samples show aziridine signals, for a detailed correlation of signals to chemical structure see Figure 51. As explained before, integration of

the aziridine signals was not reliable, therefore determination of aziridine repeat units by NMR spectroscopy was not possible.

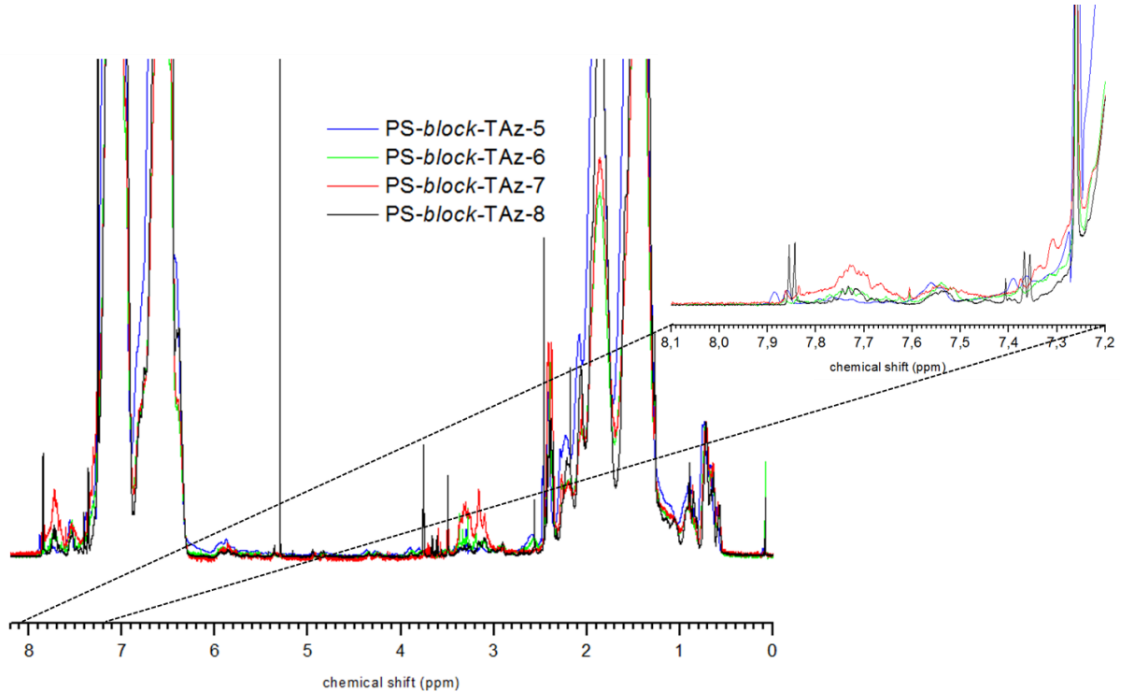

Figure 52: ^{1}H NMR spectrum of PS-*block*-TAz-5 to -8 in $CDCl_3$

To confirm that the aziridine units were covalently attached to the polystyrene backbone, DOSY spectra were taken from all samples. Figure 53 shows an exemplary spectrum of PS-*block*-TAz-7. All spectra contained only one species, proving covalent linkage of styrene and aziridine units.

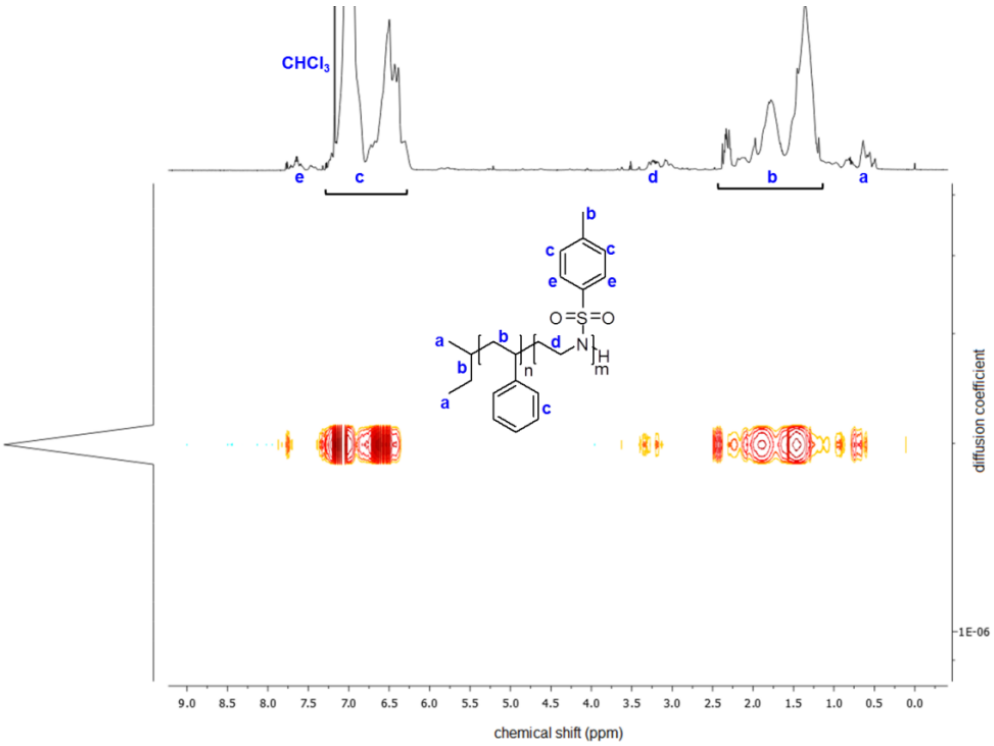

Figure 53: DOSY spectrum of PS-*block*-TAz-7

SEC characterisation of PS-*block*-TAz-5 to -8 showed monomodal distributions for all samples (Figure 54). SEC traces of the crude polymerisation mixture of PS-*block*-TAz-7 and PS-*block*-TAz-8 revealed residual monomer in the solution.

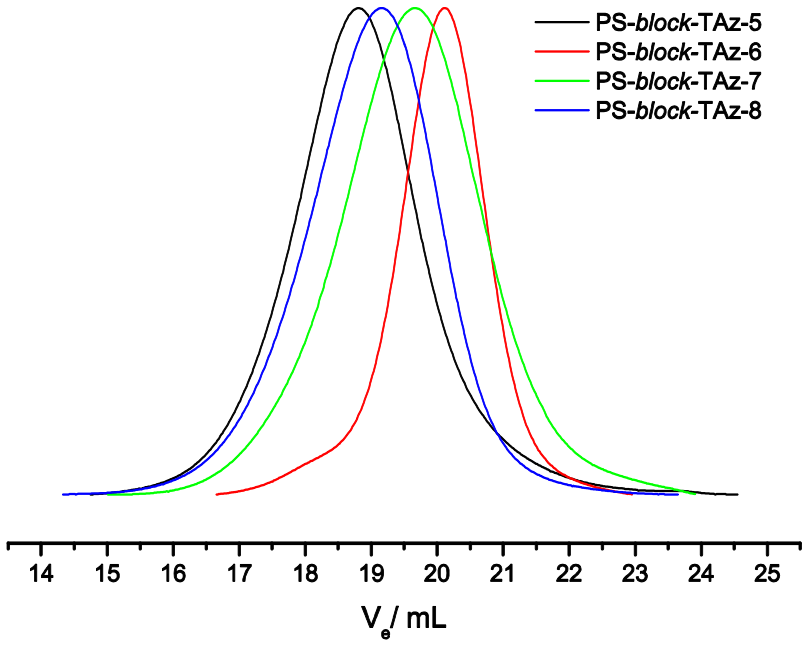

Figure 54: SEC traces of PS-*block*-TAz-5/6/7/8 in DMF (RI signal)

The polymers were further analysed by MALDI-ToF MS. It is noticeable that MALDI-ToF MS analysis of PS-*block*-TAz-1 to -4 was not possible, although these samples contained non-functionalised poly(styrene). This should have been detectable, as poly(styrene) usually ionises very well in MALDI analysis when a silver salt is added. However it is possible that the functionalised polymer chains alter the behaviour of the mixture entirely, so that poly(styrene) cannot be ionised anymore.

MALDI characterisation of PS-*block*-TAz-5 to -8 was inconclusive. A styrene repeat unit weighs 104 g/mol, a *N*-tosylaziridine repeat unit 197 g/mol, i.e. almost twice as much. This makes interpretation of a copolymer spectrum very difficult, as one aziridine repeat unit can hardly be distinguished from two styrene repeat units. Therefore MALDI-ToF MS is not suitable for determining the exact number of aziridine units and only serves to show that both monomers were incorporated into the polymer.

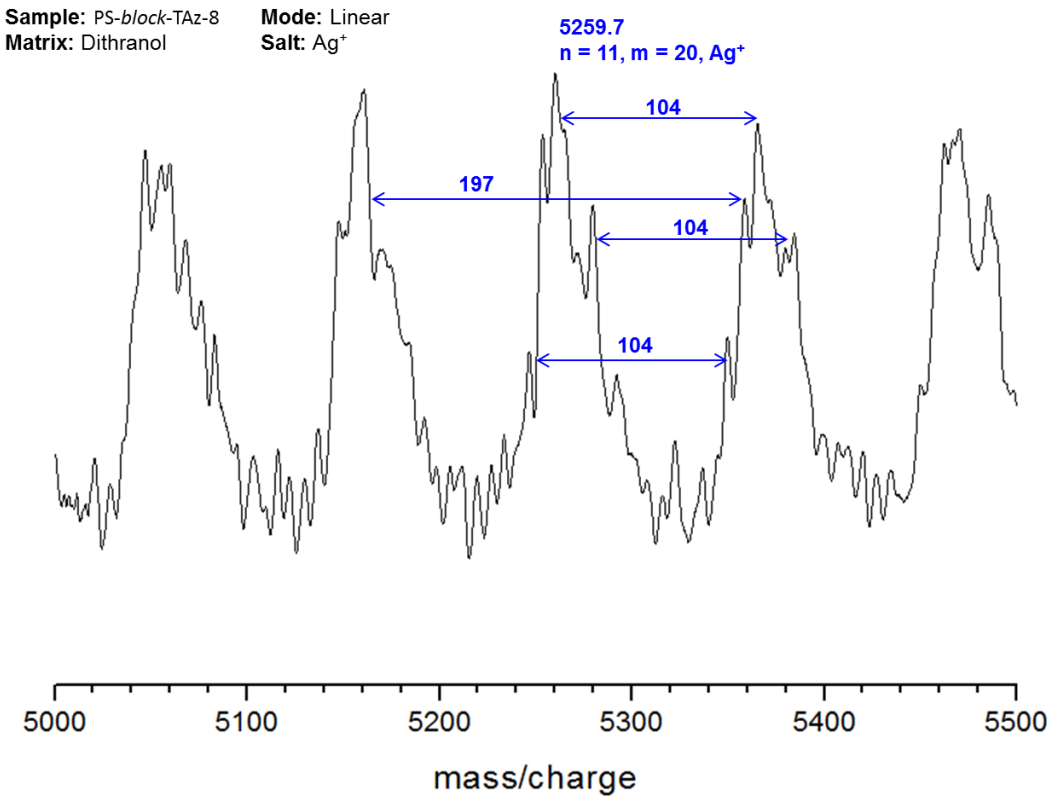

Figure 55: MALDI-ToF MS spectrum of PS-*block*-TAz-8

To estimate how much nitrogen a sample contained, elemental analysis of PS-*block*-TAz-5 to -8 was carried out. The results are shown in Table 7. Elemental analysis, however, only allows determination of a molecular formula. From that an approximate ratio of the two repeat units is calculable. The molecular formula of the copolymer is $C_4H_9(C_8H_8)_n(C_9H_{11}NSO_2)_mH$, where *n* is the number of styrene units and *m* is the number of aziridine units. The calculation is shown using the example of PS-*block*-TAz-5, where the molecular formula obtained from elemental analysis is $C_{270x}H_{195x}N_{1x}S_{1x}$

$$270 = 8n + 9m \quad (1)$$

$$196 = 8n + 11m \quad (2)$$

Using these equations, the ratio of styrene to aziridine units, *n*/*m* can be determined. In this case *m* equals one. *n* can only be calculated for *m* = 1, therefore the ratio *n*/*m* is obtained. An approximation is made, because the initiator is not accounted for, but as this affects all samples equally, the overall trend should not be altered. As the number of carbon (or hydrogen) atoms is in relation to one nitrogen atom, it would be incorrect to subtract four carbons for the initiator, as it is unknown whether one initiator unit equals one aziridine unit. Table 6 shows that the ratio decreases from PS-*block*-TAz-5 to PS-*block*-TAz-7, while PS-*block*-TAz-8 does not fit into the pattern. However, a

difference in ratios can be caused by variation of the poly(styrene) chain length alone. Therefore the ratio does not allow concluding whether propagation or mere termination occurs.

Table 6: Elemental analysis results of copolymers with styrene

Sample	N%	ΔN%	Molecular formula*	n/*m*
PS-*block*-TAz-5	0.41	0.09	$C_{270x}H_{195x}N_{1x}S_{1x}$	27.82
PS-*block*-TAz-6	0.61	0.04	$C_{172x}H_{151x}N_{1x}S_{1x}$	18.90
PS-*block*-TAz-7	0.85	0.00	$C_{120x}H_{123x}N_{1x}S_{1x}$	13.95
PS-*block*-TAz-8	0.49	0.04	$C_{215x}H_{221x}N_{1x}S_{1x}$	26.02

*oxygen was not determined and is therefore not represented in the molecular formula

A molar mass is needed to determine more than the ratio. SEC with light scattering detection was run to obtain an absolute molar mass, but yielded no results. This could be because the polymers were too small to be detected properly. The only available molar mass distribution was M_n from SEC analysis using RI detection. These were obtained using a poly(styrene) standard and aziridine end groups or blocks can be expected to change the hydrodynamic radius of the polymer chain, as indicated by NMR spectroscopy. A different approach was taken and the number of styrene units *n* from the NMR spectrum was used for further calculations. Insertion of *n* in *n*/*m* results in *m*. Table 7 shows the final results. Several approximations were made during the calculation, i.e. the initiator was not considered and n_{NMR} is subject to several sources of error as well. $m_{calc.}$ does not follow a clear trend. Presumably deviations lie within the margin of error, therefore it has to be concluded that a clear propagation of the chain does not occur. PS-*block*-TAz-8 in particular showed a large amount of residual monomer in the SEC trace of the polymerisation mixture, which supports this result.

It was shown in section 3.3.1.1 that *N*-tosylaziridine can be initiated with *sec*-BuLi in THF. However, only termination or oligomerisation occurs when a poly(styryl) anion is treated with *N*-tosylaziridine under the same conditions. This is unexpected, because the reaction conditions were shown to work for the polymerisation of *N*-tosylaziridine and the addition of aziridines to the poly(styrene) chains shows that the poly(styryl) anion is nucleophilic enough to attack the aziridine ring. An explanation could be that upon addition of the first aziridine unit, the poly(styrene) chain wraps around the active chain end to shield it from the solvent. It was shown before that poly(*N*-tosylaziridine) is very difficult to dissolve, which supports this hypothesis. This would mean that propagation can only occur very slowly, as new aziridine monomers can hardly reach the active chain end, resulting in oligomerisation at best. This theory is in accordance with the findings that PS-*block*-TAz-4 showed the clearest aziridine signals (see Figure 51) as in this sample the reaction time for the aziridine was the longest. The theory could be more closely examined by using a different aziridine, which shows

better solubility, like 2-methyl-*N*-mesylaziridine. Of course, in that case there would be the risk that the poly(styryl) anion abstracts a proton from the methyl group, as it happens with propylene oxide.[53] Again the reaction conditions would have to be optimised to suppress side reactions.

Table 7: Analysis of copolymers with styrene, M_n is given in g/mol, a PS standard was used for SEC analysis

Sample	$n_{theo.}$	$m_{theo.}$	$M_{n\ (SEC)}$	PDI	n_{NMR}	$m_{calc.}$
PS-*block*-TAz-5	20	3	6800	1.35	61	2.19
PS-*block*-TAz-6	20	6	4200	1.18	32	1.69
PS-*block*-TAz-7	20	11	4900	1.33	37	2.65
PS-*block*-TAz-8	10	30	6400	1.29	11	0.42

3.3.3. Removal of the activating group

To show that the developed procedure was not only a strategy to introduce sulfonamides but also amine functionalities to a polystyrene chain, the sulfonamide bond had to be cleaved. Several protocols are known in literature, e.g. sodium in liquid ammonia,[55] magnesium and methanol under ultrasonication conditions,[56] samarium iodide[57] or lithium and naphthalene.[12] All these procedures employ reducing conditions, as *p*-toluenesulfonamides are not easily cleaved by hydrolysis. The first protocol tested used magnesium and methanol.[56] PS-*block*-TAz-5 was dispersed in methanol and benzene. Magnesium was added and the mixture was sonicated for three days, the mixture was analysed by ^{1}H NMR spectroscopy after each day. Figure 56 shows the ^{1}H NMR spectra of the educt and the crude mixture after three days. The spectra are normalised on the initiator signal. No difference in the tosyl signals (**a**) was observable. Therefore, it has to be concluded that the procedure did not affect the tosyl groups. The ^{1}H NMR of PS-*block*-Az-5 contains several signals corresponding to solvents, as the crude reaction mixture was analysed. Solvents like benzene (7.37 ppm) and methanol (3.49 ppm) are to be expected, but there is also water present (1.54 ppm). It is possible that the solvents were not sufficiently dry for the reaction to work, although the sample still contained enough magnesium after three days. As the procedure has only been applied to low molecular weight substances before, it might be not suitable for macromolecular structures due to solubility effects. Especially ultrasonication is often not suited for polymers, as they can fragment due to the strong shear forces.

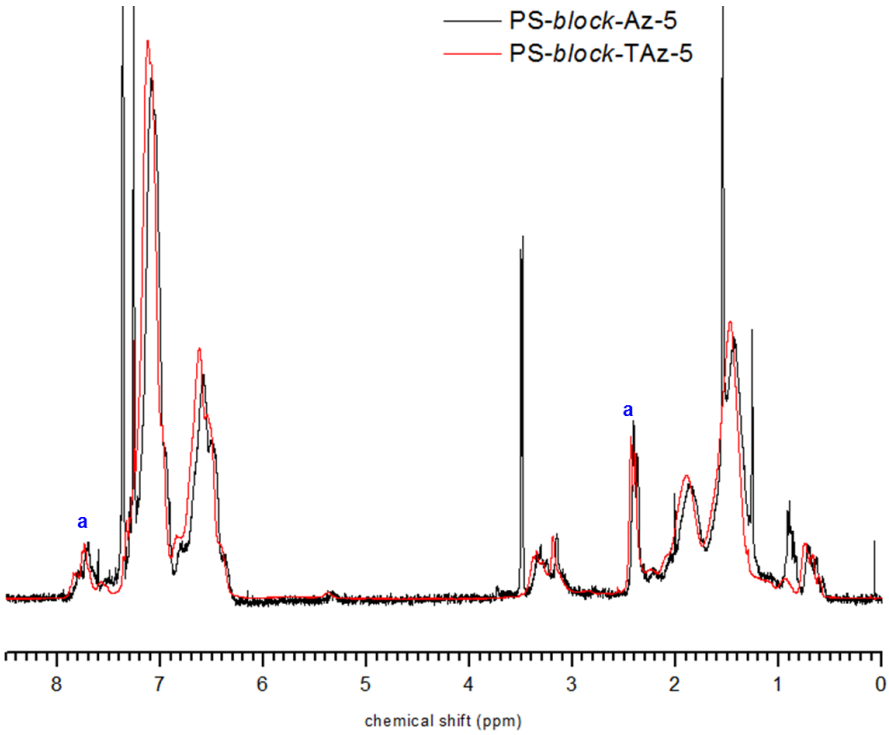

Figure 56: ^{1}H NMR spectrum of PS-*block*-TAz-5 and PS-*block*-TAz-5 in $CDCl_3$

A procedure using hydrobromic acid in acetic acid and phenol, that had been applied to polymers before, was tested next.[35] The proposed reaction mechanism is shown in Figure 57.[58] The sulfonamide is cleaved to the amine hydrobromide and the sulfonylbromide. The hydrobromic acid reduces the sulfonylbromide to the disulfide. Phenol is needed to capture the bromine in order to get unsubstituted amines.

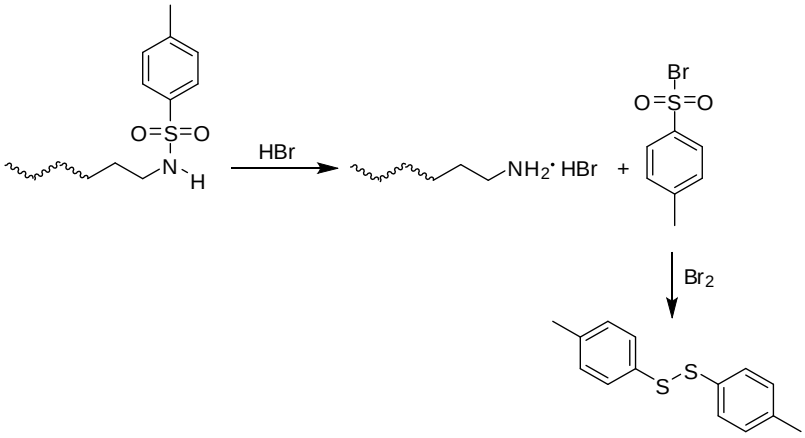

Figure 57: Cleavage of sulfonamides using hydrobromic acid[58]

The reaction was carried out for seven days, as analysis after three days showed incomplete conversion of the starting material. Figure 58 shows the ^{1}H NMR spectrum of the deprotected PS-

block-PAz-7 after seven days reaction time and the spectrum of the starting material PS-*block*-PTAz-7 for reference. The spectra are normalised on the initiator signal. The aromatic signals of the tosyl group (**a**) and the signals of the methyl group (**b**) have disappeared. It is noticeable that the signals of the aziridine backbone (**c**) have vanished as well, confirming the masking effect by the polystyrene chain.

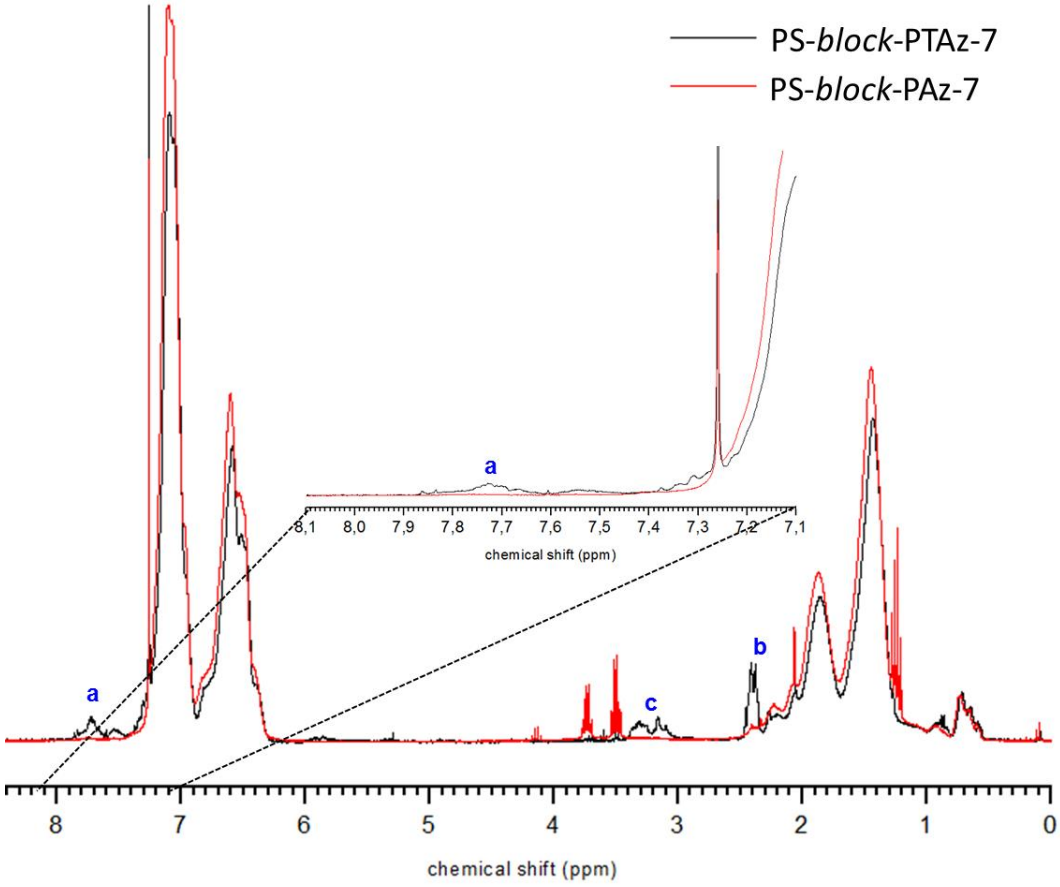

Figure 58: ^{1}H NMR spectrum of PS-*block*-PTAz-7 and PS-*block*-PAz-7 in $CDCl_3$

In order to ensure that the removal of the tosyl group was successful, the amine functionality was addressed with acryloyl chloride as shown in Figure 59. Triethylamine was added as a base to prevent protonation of the amine group. The reaction was stirred for three days, longer than normally needed, to account for the lower reactivity of the macromolecular starting material. As a control the same reaction was carried out for the protected starting material.

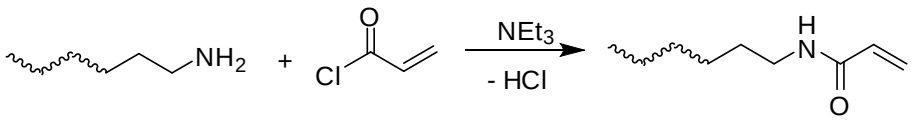

Figure 59: Reaction of an amine and an acid chloride

The products were characterised by ^{1}H NMR spectroscopy. The spectra are shown in Figure 60. The suffix AA refers to the introduction of acrylic acid as an end group. Both products show signals at 6.23-5.84 ppm, which can be attributed to the double bond of the acrylic acid. The integrals of PS-*block*-PTAz-AA equal one acrylic acid. This proves that the sulfonamide end group is still nucleophilic enough to react with an acid chloride, even though the sulfonyl group lowers the reactivity of the nitrogen. PS-*block*-PAz-AA shows broader peaks, indicating addition of more than one acrylic acid. This is confirmed by the integrals that equal just over two units of acrylic acid. This is in accordance with the calculated amount of 2.65 aziridine units per chain (see Table 7).

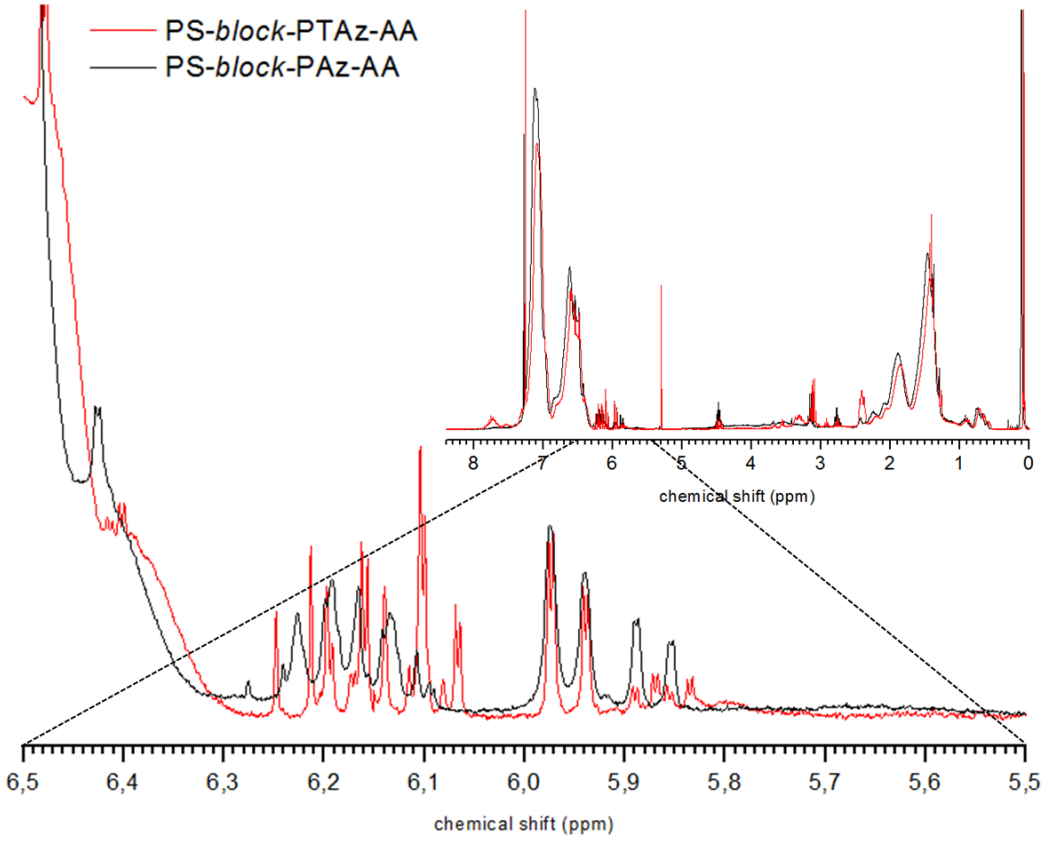

Figure 60: ^{1}H NMR spectrum of PS-*block*-PTAz-AA and PS-*block*-PAz-AA in $CDCl_3$

3.4. Coupling to poly(ethylene oxide)

This chapter describes the switch from the aza-anionic polymerisation of aziridine to the oxy-anionic polymerisation of ethylene oxide. In theory, an azaanion (pK_a ≈ 30-40) is much more basic than an oxyanion (pK_a ≈ 15-20), however, the tosyl or mesyl group at the nitrogen lowers the electron density and consequently a sulfonamide anion should be expected to have a lower reactivity. In this case the differences in reactivity are not as clear as in chapter 3.3, where the reactants were a carbanion and

an azaanion. Both directions, the switch from aza-anionic to oxy-anionic and the other way around, were tested for their potential success.

3.4.1. Switch from aza-anionic to oxy-anionic polymerisation

First the switch from aza-anionic to oxy-anionic polymerisation will be described. The reaction scheme can be seen in Figure 61. As it is known that potassium works as a counter ion for the polymerisation of ethylene oxide,[59] the initiator system of KHMDS and BnNHMs was used. 2-Methyl-*N*-mesylaziridine was used as a monomer.

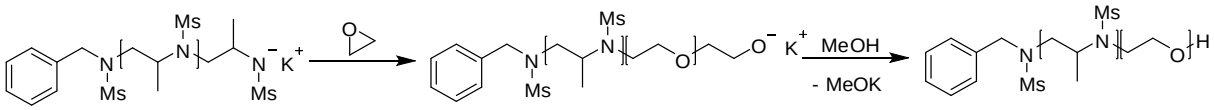

Figure 61: Reaction scheme for the coupling of poly(MMAz) to ethylene oxide

An experimental setup similar to the one depicted in Figure 79 was used. Flask **e_1** was exchanged for a graduated ampoule to measure the volume of ethylene oxide used and stopcock **b** was connected to a steel cylinder of ethylene oxide. The results are shown in Table 8. PMMAz-*block*-PEG-1 was carried out using only the apparatus for anionic polymerisation. THF was used as a solvent, as it was incorporated into the setup and could be added to the experiment via distillation into the cold, reducing the possibility of water or oxygen contamination. KHMDS and BnNHMs were dried separately and mixed inside an argon filled glovebox. A syringe filled with the initiator mixture was removed from the glovebox and added to the monomer solution. The puncture point was sealed immediately. Aziridine polymerisation was carried out overnight, to ensure that monomer consumption was complete. Ethylene oxide was brought to the polymerisation flask via distillation into the cold with a small detour through the graduated ampoule to determine the condensed amount. The polymerisation of ethylene oxide was carried out overnight as well. ^{1}H NMR characterisation showed no PEG backbone and the PAz backbone consisted only of seven repeat units. Presumably, the poly(aziridine) chain was terminated, meaning that there was no active chain end and therefore no possibility for propagation upon addition of ethylene oxide. The protocol was changed to stay as close as possible to the procedure described in 3.2.2, which was shown to work well for PAz. The initiators were added to the monomer solution inside a glovebox, the sealed flask was added to the experimental setup. The next day the whole setup was dried under reduced pressure repeatedly to remove any traces of water before addition of ethylene oxide. It was observable that the distillation into the cold took longer than for PMMAz-*block*-PEG-1, as the static vacuum is compromised by the argon atmosphere that is still present in the polymerisation flask

upon opening it. Analysis of PMMAz-*block*-PEG-2 showed that while polymerisation of 2-methyl-*N*-mesylaziridine worked well, there was still no PEG backbone. Apparently the active chain end of the poly(aziridine) chain is not reactive enough to open the ethylene oxide ring. In a third attempt, 18-crown-6 was added form a complex with the potassium counter ion and result in a more reactive chain end. This increased the nucleophilicity of the sulfonamide anion enough to successfully open the epoxide ring as PMMAz-*block*-PEG-3 shows.

Table 8: Analysis of PMMAz-*block*-PEG, M_n is given in g/mol, a PEG standard was used for SEC analysis

Sample	Repeat units$_{(theo, Az)}$	Repeat units$_{(theo, EO)}$	Solvent	M_n $_{(SEC)}$	PDI	Repeat units$_{(NMR, Az)}$	Repeat units$_{(NMR, EO)}$
PMMAz-*block*-PEG-1	20	60	THF	7800	1.09	7	-
PMMAz-*block*-PEG-2	20	230	DMF	2000	1.08	23	-
PMMAz-*block*-PEG-3	20	270	DMF	3200	1.13	35	41

Figure 62 shows the ^{1}H NMR spectrum of PMMAz-*block*-PEG-3. The repeat units of 2-methyl-*N*-mesylaziridine and ethylene oxide were determined relative to the initiator signal **a**. The PEG backbone (**e**) is visible at 3.6 ppm. The sample still contains 18-crown-6 and DMF.

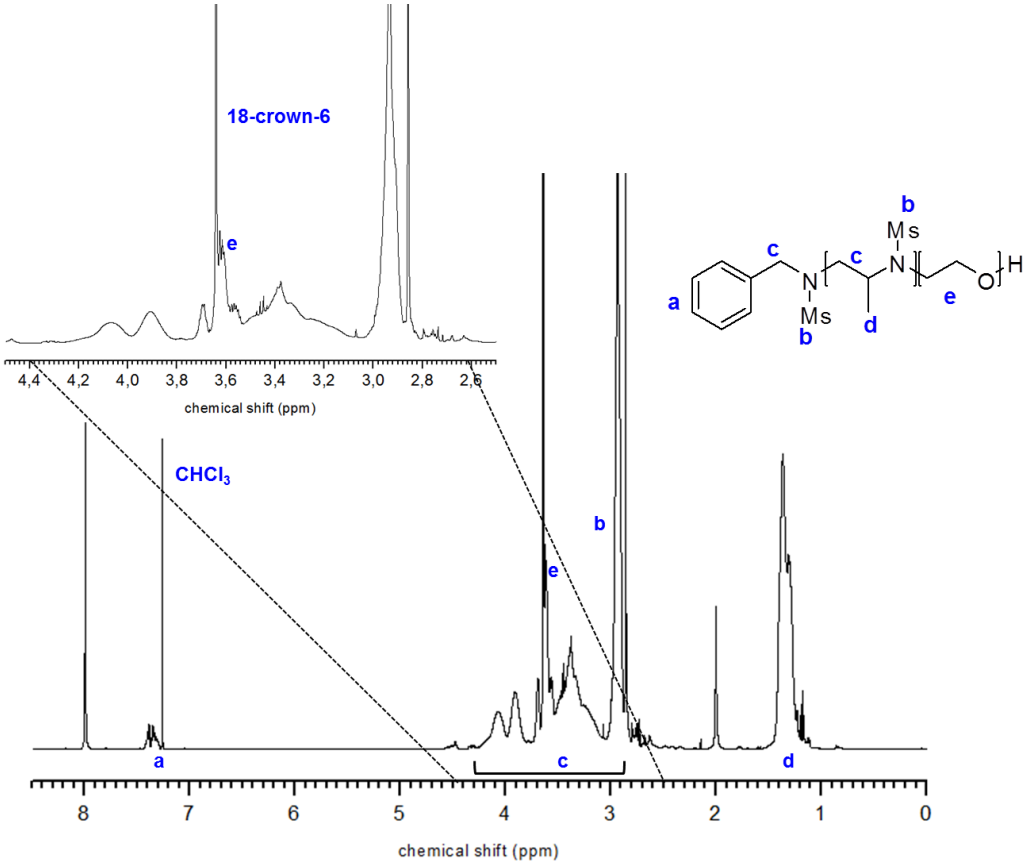

Figure 62: ^{1}H NMR spectrum of PMMAz-*block*-PEG-3 in $CDCl_3$

DOSY measurements and MALDI-ToF MS were used to prove the covalent attachment of the two polymer backbones. The DOSY spectrum (see Figure 63) shows that only one polymer species was obtained. For a detailed correlation of peaks to chemical structure see Figure 62. The sample still contains crown ether and residual DMF.

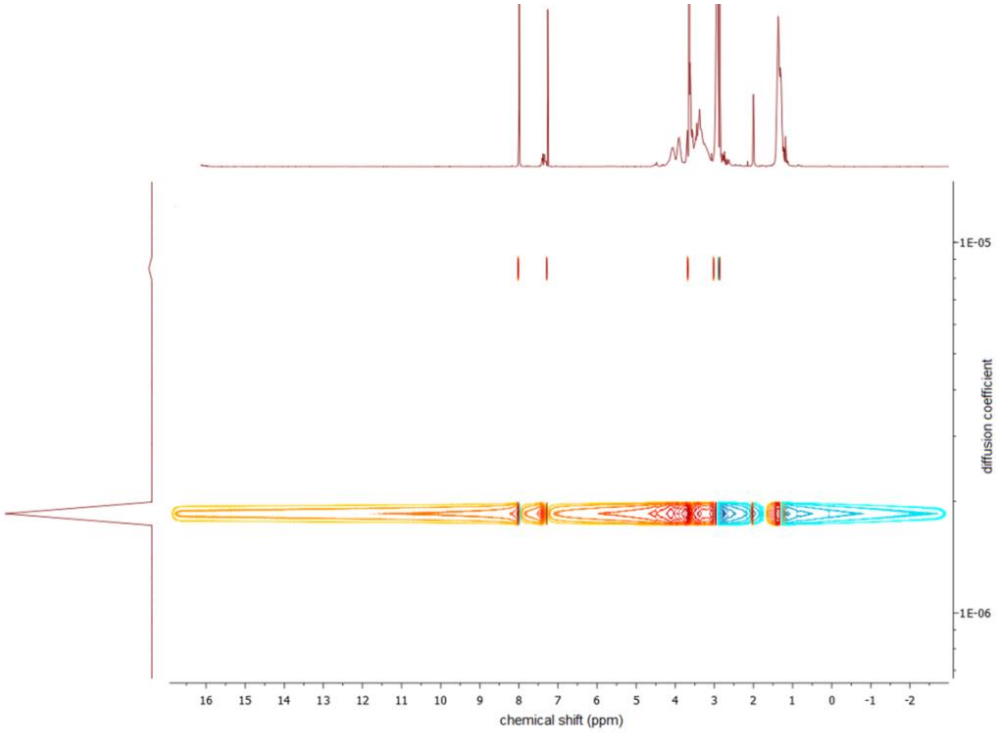

Figure 63: DOSY spectrum of PMMAz-*block*-PEG-3

Figure 64 shows the MALDI-ToF MS spectrum of PMMAz-*block*-PEG-3. Both monomer intervals can be found, proving the formation of a copolymer.

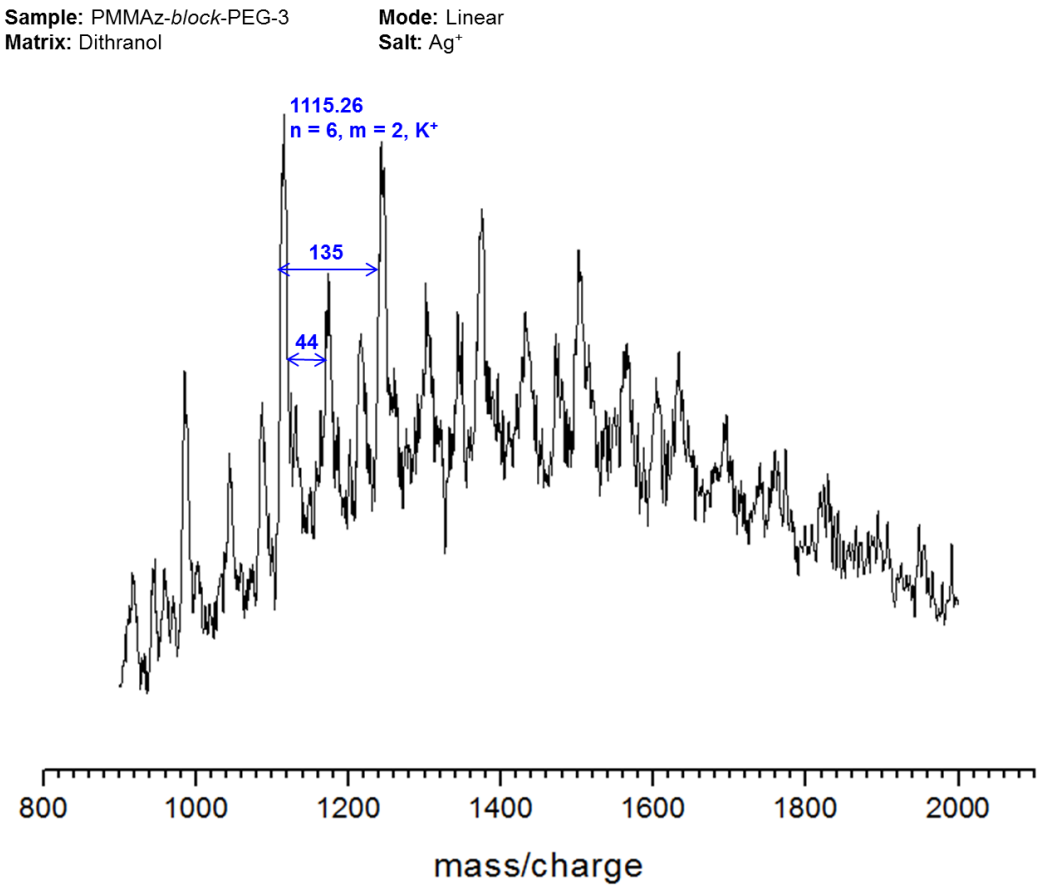

Figure 64: MALDI-ToF MS spectrum of PMMAz-*block*-PEG-3

3.4.2. Poly(aziridine) initiated with potassium methoxide

To test whether a switch from the oxy-anionic polymerisation of ethylene oxide to the aza-anionic polymerisation of sulfonamides is possible, 2-methyl-*N*-mesylaziridine was initiated using potassium methoxide. Potassium methoxide decomposes to methanol and potassium hydroxide under aqueous conditions and it is to be expected that an unknown amount of potassium methoxide has decomposed due to air humidity. Therefore, the initiator solution would contain hydroxide ions, which could initiate as well and lead to polymer chains with two growing chain ends. To circumvent this and to obtain only one polymer species, the potassium methoxide was dried from benzene and methanol. Potentially present hydroxide ions deprotonate the methanol, forming potassium methoxide and water. The former is used as an initiator anyway and the latter is removed as an azeotrop with benzene, as well as surplus methanol. This leads to a uniform initiation. The disadvantage is that the exact amount of initiator is unknown, as an unknown mixture of potassium methoxide and potassium hydroxide is weighed. Table 9 shows the SEC data of the resulting polymer, proving that potassium methoxide is able to initiate the polymerisation of 2-methyl-N-mesylaziridine.

Table 9: SEC analysis of MeO-PMMAz, M_n is given in g/mol, a PEG standard was used for SEC analysis

Sample	$M_{n\ (SEC)}$	PDI
MeO-PMMAz	3200	1.10

Figure 65 shows the MALDI-ToF MS spectrum of MeO-PMMAz. Two distributions carrying the methoxy end group can be identified, one ionised with silver and one with potassium as a counter ion. Interestingly, $M_{n\ (SEC)}$ is bigger than the masses observable in the MALDI spectrum, while poly(aziridine) initiated with BnNHMs was found to give smaller values in SEC analysis compared to MALDI or ^{1}H NMR. The sloping curve seen in Figure 65 is typical for samples showing a mass discrimination effect, so the difference in weight can partly be attributed to this. But it should also be noted, that the differences in SEC analysis can be due to the difference in initiators, as end groups have an enormous effect on the properties of a polymer. ^{1}H NMR data is not included, as the initiator signal of the methyl group cannot be discerned from the aziridine backbone.

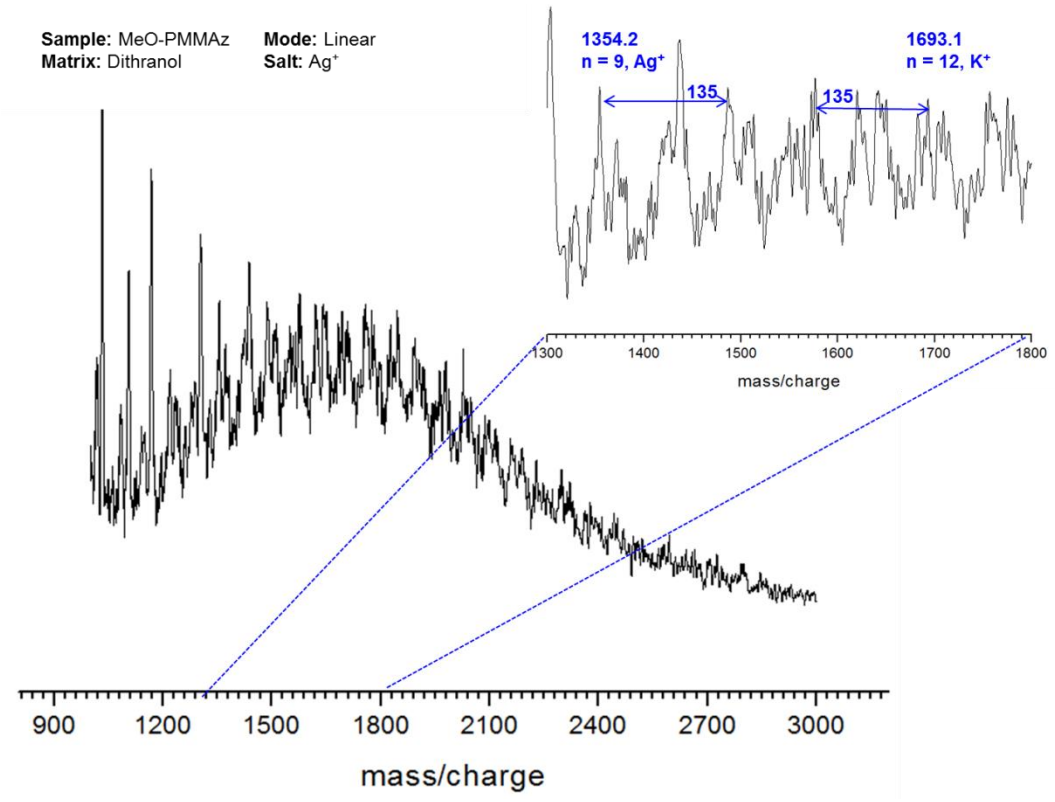

Figure 65: MALDI-ToF MS spectrum of MeOH-PMMAz

4. Summary and outlook

4.1. Summary

This work focuses on the anionic ring opening polymerisation of activated aziridines.

Section 3.1 describes the synthesis of activated aziridines. Two reaction pathways were investigated, using amino acids or epoxides as starting materials. Protected aziridines derived from leucine, alanine, 2-methylaziridine, 1, 2-epoxydec-9-ene and 1, 2-epoxydodecane were synthesized in overall good to moderate yields.

In section 3.2 the synthesis of poly(aziridine)s is discussed. Poly(aziridine)s ranging from a molecular weight of 3000 to 19000 g/mol were successfully synthesized. This is not thought to be the molecular weight limit. Copolymers and block copolymers were successfully synthesized. Poly(aziridine)s were characterised using NMR, SEC and MALDI-ToF MS analysis. 2-*n*-(7-Octenyl)-*N*-methansulfonylaziridine was found to lower the polymerisation rate in both homopolymerisation and copolymerisation. The double bond functionality was addressed in a thiol-ene reaction with *N*-acetyl-L-cysteine methyl ester. 2-Isobutyl-*N*-tosylaziridine did not polymerise under the investigated reaction conditions, presumably because the isobutyl group is sterically hindering the nucleophilic attack of the initiator. Possibilities to circumvent this were discussed.

In section 3.3 the reaction of activated aziridines with a poly(styryl) anion was examined. It was shown that the reaction conditions used for the polymerisation of styrene can be applied to *N*-tosylaziridine, i.e. the activated aziridine polymerises at -100 °C in THF with lithium as a counter ion. However, no distinct block copolymers could be detected when *N*-tosylaziridine was added to a poly(styryl) anion. The repeat units of *N*-tosylaziridine could be calculated using data obtained by elemental and ^{1}H NMR analysis. It was concluded that fast propagation cannot occur due to solubility effects. Propagation is hindered as the living chain end is masked by the poly(styrene) chain. While *N*-tosylaziridine is soluble in THF, poly(*N*-tosylaziridine) is not. This is reminiscent of poly(aziridine)s with a defined tacticity.[12]

Section 3.4 describes the switch between oxy- and aza-anionic polymerisation. Block copolymers of 2-methyl-*N*-mesylaziridine and ethylene oxide were synthesized as confirmed by ^{1}H NMR, DOSY and MALID-ToF MS analysis. It was found that activated aziridines can be initiated by alkoxides such as potassium methoxide.

4.2. Outlook

Many questions remain to be answered concerning the field of aza-anionic polymerisation. This section will first describe smaller points raised during the experiments conducted for this thesis and subsequently list broader ideas and new concepts for further research.

As described in section 3.2.3, 2-*n*-(7-octenyl)-*N*-methansulfonylaziridine was found to lower the polymerisation rate of the whole system. It was proposed that this is due to electrostatic interactions between the electron rich double bond and the electron deficient aziridine monomers. This could be verified by examining a system where an inert compound with a double bond, e.g. hexene, is added to an otherwise normal polymerisation.

Another interesting point are the kinetic aspects of the copolymerisation (see section 3.2.3). By following a copolymerisation with ^{1}H NMR analysis, the incorporation rate of the monomers can be assessed. Doing this for different activated aziridines would allow estimates on how much the activating group and the substituent at the aziridine ring influence the reactivity.

In section 3.3 it was found that activated aziridines can theoretically form block copolymers when added to a poly(styryl) anion. However, the examined *N*-tosylaziridine showed poor solubility and clearer results could be achieved using a protected aziridine with a substituent at the ring. The problem of proton abstraction from the substituent might occur as a side reaction to the copolymerisation.[53] Addition of 2-isobutyl-*N*-tosylaziridine to the poly(styryl) anion could be a way to terminate the polymer, as 2-isobutyl-*N*-tosylaziridine does not propagate.

In section 3.4 it was shown that the switch between oxy- and aza-anionic polymerisation can occur in either direction. The reaction conditions still need to be adjusted, as PMMAz-*block*-PEG-3 showed only a small PEG backbone. The similar reactivity of the oxyanion and the azaanion of an activated aziridine could enable a direct copolymerisation of the two compounds.

In section 1.2.1 the necessity of an electron withdrawing group at the nitrogen was discussed. By polymerising an *N*-alkylaziridine this could be investigated. Both the aziridine monomer as well as the active chain end would bear an enhanced electron density. These effects could balance each other out. However, the electron withdrawing substituent could be needed to stabilise the azaanion.[11]

In order to increase the versatility of poly(aziridine)s it is important that the activating group can be removed from the polymer without affecting the chain. First attempts were described in section 3.3.3. While the protocol worked, the reaction conditions used were quite harsh. The chapter lists several methods, all of them could be screened for their potential. Alternative activating groups for aziridines (see section 1.5), that are more easily removed can be tested as well.

In general, the anionic polymerisation of aziridines offers countless new possibilities to modify polymer properties and architectures, comparable to the anionic polymerisation of epoxides. By using multifunctional initiators, more complicated architectures, like star polymers, become accessible.

Deprotonating PAz, from which the activating group has already been removed, could allow grafting new side chains onto the polymer. However, the number and length of these side chains is likely to be impeded by steric hindrance of the polymer.

Introducing a cross linking agent to the aza-anionic polymerisation should be possible as well. This compound would have to carry at least two aziridine rings. These substances are usually referred to as polyaziridines as well and are used as cross linkers in coatings and adhesives.[60] An alternative is offered by telechelic poly(phosphoester)s. Bifunctional phosphoesters can be polymerised using ADMET, acyclic diene metathesis.[61] Olefins act as a chain termination agent in the polymerisation, as shown by Steinmann *et al.*[62] Adding 2-*n*-(7-octenyl)-*N*-methansulfonylaziridine to an ADMET reaction would yield a poly(phosphoester) with two aziridine functional groups at the chain ends. This compound could be used as a macromolecular cross linking agent in the polymerisation of activated aziridines.

Further studies aim to investigate applications for PAz as a transfection agent. Poly(aziridine)s without activating groups are very similar to PEI. It could be argued that consequently, they must possess a similar level of transfection efficiency. However, a transfection agent obtained from a polymer modification reaction is much less convenient than PEI. Therefore, the transfection efficiency of unmodified PAz is more interesting. Its DNA binding abilities can be expected to be lowered compared to PEI, as the lone electron pair of the nitrogen is delocalised over the activating group and is harder to protonate. But this does not have to lower the transfection ability. Gabrielson *et al.* found that partly acetylated PEI showed an enhanced transfection efficiency.[63] The reduced binding capability results in an improved release of the DNA from the polycation, overall increasing the transfection efficiency. The decreased charge density can be expected to lower the toxicity as well.

5. Experimental section

All chemicals were purchased from common suppliers (*Acros, Fisher Scientific, Fluka, Roth, Sigma-Aldrich, TCI Europe*) and used without further purification unless stated otherwise. Deuterated solvents were supplied by *Deutero GmbH* (Kastellaun, Germany) and stored over molecular sieves. Dehydration of common organic solvents was performed according to literature procedures.[64]

^{1}H NMR spectra were obtained at a Bruker AC300 with a frequency of 300 MHz or at a Bruker AC700 with a frequency of 700 MHz. ^{13}C NMR spectra were obtained at a Bruker AC400 with a frequency of 400 MHz Chemical shifts are given in ppm. The deuterated solvent was used as a reference signal.

SEC measurements were carried out in DMF containing 0.25 g/L of lithium bromide. An Agilent 1100 Series SEC Setup was used as an integrated instrument, including a PSS HEMA column (10^6/10^5/10^4 g/mol), a UV- (254 nm) and RI-detector. Calibration was achieved using poly(ethylene glycol) and poly(styrene) standards provided by Polymer Standards Service. The eluent was used at 50 °C and at a flow rate of 1 mL/min.

IR spectra were recorded on a Thermo Scientific Nicolet iS10 with an ATR-unit and are reported as wavenumbers $\tilde{\nu}$ in cm^{-1}.

MALDI-ToF measurements were obtained using an Axima CFR MALDI-ToF mass spectrometer in either linear or reflective mode. The matrix material was dithranol, potassium or silver was used as a counter ion. The components were mixed in a ratio of 10:10:1 (matrix:sample:salt).

Elemental analysis was run on an Elementar Vario EL cube.

5.1. Methods of characterisation

5.1.1. 2D nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is the most important tool in organic chemistry for structural elucidation. 2D NMR techniques in particular offer versatile ways to examine the structure of a compound. Diffusion based NMR spectroscopy (DOSY – diffusion ordered spectroscopy) separates different compounds in a mixture based on their diffusion coefficients. A ^{1}H NMR spectrum is plotted versus the diffusion coefficient. DOSY is especially useful after polymer modification reactions or to characterise block copolymers, as it verifies that all structural parts of a polymer are covalently linked. Correlation spectroscopy (COSY) examines which spins are coupled to each other. Usually coupling between ^{1}H and ^{1}H or ^{1}H and ^{13}C is observed.

5.1.2. Size-exclusion chromatography

Size-exclusion chromatography (SEC) is the most important method to assess the molar mass distribution and polydispersity index (PDI) of a polymer. The principle of separation is shown in Figure 66. The sample passes through a column filled with a porous material of defined pore sizes. Small molecules are able to diffuse inside this stationary phase, which means they take longer to pass the column, while larger molecules bypass the column material and elute first. This means that SEC has an upper and lower exclusion limit: Molecules that are too large to enter any of the pores all elute at the same time, as do molecules that are small enough to enter all pores. Consequently, SEC separates molecules by their hydrodynamic radius.

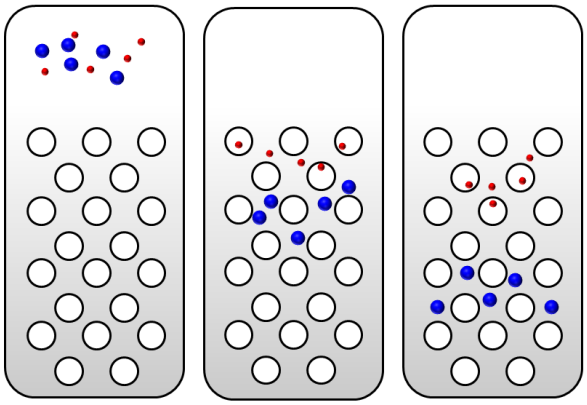

Figure 66: Principle of SEC

Since SEC is a relative method, a calibration is needed. Well defined polymer standards are used to link a given elution volume to a certain molecular weight. However, once the structure or composition of a sample differs from the used standard, e.g. poly(styrene) is measured against a poly(ethylene glycol) standard, the hydrodynamic radii are not comparable and the molar mass distributions obtained from the SEC become an indicator at best.

5.1.3. Matrix-assisted-laser-desorption/ionisation time-of-flight mass spectrometry

Matrix-assisted-laser-desorption/ionisation time-of-flight mass spectrometry (MALDI-ToF MS) is a special ionisation technique in mass spectrometry, which is particularly suited for analysing macromolecules, such as polymers, DNA or proteins, which tend to fragment when ionised by other methods. The sample is embedded in a matrix and salts are added to enhance ionisation. A laser pulse is directed at the sample, which ablates the upper layer. The matrix is chosen with an absorption maximum matching the wavelength of the laser, so that it absorbs the larger part of the energy and the analyte is not fragmented. The analyte is ionised and accelerated in an electric field. The time of flight, the time it takes an analyte to reach the detector, depends on the mass to charge

ratio (m/z). Lighter molecules are faster and reach the detector first. MALDI-ToF MS is a valuable method for determining the composition of a sample and can be used for end group characterisation. It is however not always representative of the complete sample. Some species may be harder to ionise than others and will be underrepresented or not show up in the spectrum at all. Especially the higher molecular weight range tends to be underrepresented, which is known as the mass discrimination effect.

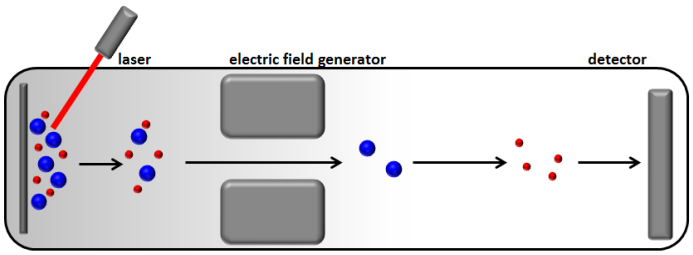

Figure 67: Matrix-assisted-laser-desorption/ionisation time-of-flight mass spectrometry

5.2. Small molecules

5.2.1. Monomers derived from amino acids

2-Methyl-N-tosyl-aziridine

The compound was synthesized according to literature procedures.[49]

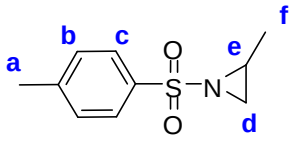

Figure 68: 2-Methyl-N-tosyl-aziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83-7.80 (m, 2, **c**), 7.35-7.32 (m, 2, **b**), 2.86-2.78 (m, 1, **e**), 2.61 (d, 1, J = 7.0 Hz, **d**), 2.44 (s, 3, **a**), 2.02 (d, 1, J = 4.6 Hz, **d**), 1.25 (d, 3, J = 5.6 Hz, **f**).

N-Tosyl-leucine

The compound was synthesized according to literature procedures.[49] Briefly, a solution of leucine (10.12 g, 77 mmol) in 2 M sodium hydroxide (39 mL) was cooled to 0 °C. 4-Toluenesulfonyl chloride (15.35 g, 81 mmol), *N*-ethyl-*N*,-*N*-diisopropylamine (15 mL, 85 mmol) and acetone (39 mL) were added. The mixture was stirred overnight and then washed with diethylether. The organic phase was

washed with 2 M sodium hydroxide. The combined aqueous phases were cooled to 0 °C and acidified (pH 1) by addition of conc. hydrochloric acid. The product was extracted with ethyl acetate. The combined extracts were washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was triturated with 40°-60° petrol, sonicated and filtrated to yield the product as a colourless solid (17.78 g, 62 mmol, 81 %).

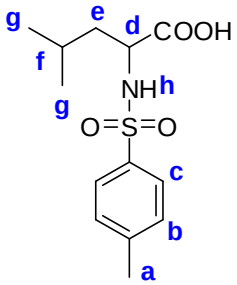

Figure 69: *N*-Tosyl-leucine

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74-7.71 (m, 2, **c**), 7.29-7.26 (m, 2, **b**), 5.23 (d, 1, J = 9.7 Hz, **h**), 3.95-3.87 (m, 1, **d**), 2.41 (s, 3, **a**), 1.77-1.72 (m, 1, **f**), 1.53-1.47 (m, 2, **e**), 0.85 (dd, 6, J_1 = 24.1 Hz, J_2 = 6.6 Hz, **g**).

N-Tosyl-leucinol

The compound was synthesized according to literature procedures.[49] To a stirred suspension of lithium aluminum hydride (3.42 g, 90 mmol) in dry diethylether (190 mL) under argon atmosphere, a solution of *N*-tosyl-leucine (8.55 g, 30 mmol) in a mixture of diethylether (95 mL) and THF (95 mL), both dried, was added dropwise. The mixture was heated under reflux for 30 minutes and then cooled to – 10 °C. 1 M sodium hydroxide (40 mL) was added, the resulting white slurry was filtrated and washed thoroughly with ethyl acetate. The filtrate was acidified (pH 5) with 2 M hydrochloric acid and saturated with sodium chloride. The aqueous phases were extracted with ethyl acetate, dried over magnesium sulfate and concentrated under reduced pressure to give the product as a yellowish solid (6.58 g, 24 mmol. 78 %).

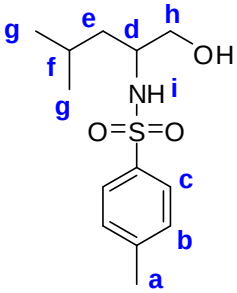

Figure 70: *N*-Tosyl-leucinol

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80-7.76 (m, 2, **c**), 7.31-7.26 (m, 2, **b**), 5.08 (d, 1, J = 6.0 Hz, **i**), 3.57 (dd, 1, J_1 = 11.3 Hz, J_2 = 3.7 Hz, **h**), 3.44 (dd, 1, J_1 = 11.3 Hz, J_2 = 5.0 Hz, **h**) 3.28 (m, 1, **d**), 2.42 (s, 3, **a**), 1.49-1.49-1.40 (m, 1, **f**), 1.27-1.18 (m, 2, **e**), 0.76 (d, 3, J = 6.6 Hz, **g**), 0.62 (d, 3, J = 6.5 Hz, **g**).

2-Isobutyl-N-tosyl-aziridine

The compound was synthesized according to literature procedures.[49] To a solution of *N*-tosyl-leucinol (6.58 g, 24 mmol) in dry dichloromethane (DCM) under argon atmosphere, 4-toluenesulfonyl chloride (5.68 g, 29 mmol), 4-dimethylaminopyridine (592.8 mg, 4.9 mmol) and triethylamine (10.1 mL, 73 mmol) were added. The reaction mixture was stirred overnight at room temperature. The solution was poured into a mixture of aqueous citric acid (2 % w/v, 250 mL) and ethyl acetate (250 mL). The aqueous phase was saturated with sodium chloride and washed with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium bicarbonate and brine, dried over magnesium sulfate and concentrated under reduced pressure. The crude product was chromatographed on silica gel (PE/EA 4:1, R_f = 0.53) to give the product as a yellowish liquid (2.78 g, 11 mmol, 38 %).

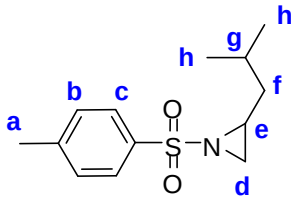

Figure 71: 2-Isobutyl-*N*-tosylaziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.82 (d, 2, J = 7.1 Hz, **c**), 7.33 (d, 2, J = 7.9 Hz, **b**), 2.82-2.73 (m, 1, **e**), 2.61 (d, 1, J= 7.0 Hz, **d**), 2.43 (s, 1, **a**), 2.02 (d, 1, J = 4.6 Hz, **d**), 1.67-1.54 (m, 1, **g**), 1.38-1.25 (m, 2, **f**), 0.87 (d, 6, J = 6.6 Hz, **h**).

5.2.2. Monomers derived from epoxides

1-Azido-2-hydroxydodecane

The compound was synthesized according to literature procedures.[12] To a solution of 1,2-epoxydodecane (12.58 g, 68 mmol) in ethanol (120 mL) and water (30 mL), ammonium chloride (4.73 g, 89 mmol) and sodium azide (5.78 g, 89 mmol) were added. The solution was heated to reflux overnight. The reaction mixture was cooled to room temperature and extracted with DCM. The combined organic phases were washed with brine, dried over magnesium sulfate and concentrated under reduced pressure to yield the product as a yellow liquid (14.49 g, 63 mmol, 93 %).

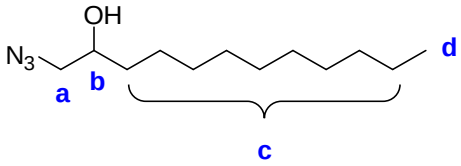

Figure 72: 1-Azido-2-hydroxydodecane

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79-3.73 (m, 1, **b**), 3.38 (dd, 1, J_1 = 12.4 Hz, J_2 = 3.3 Hz, **a**), 3.25 (dd, 1, J_1 = 12.4 Hz, J_2 = 7.4 Hz, **a**), 1.51-1.20 (m, 18, **c**), 0.88 (t, 3, J = 6.6 Hz, **d**).

IR: $\tilde{\nu}$ = 3367 (w), 2922 (m), 2853 (m), 2101 (s), 1465 (m), 1271 (m), 1086 (m), 1048 (m), 922 (m), 878 (m), 721 (m) cm^{-1}.

2-n-Decylaziridine

The compound was synthesized according to literature procedures.[12] To a solution of 1-azido-2-hydroxydodecane (12.39 g, 55 mmol) in dry THF (130 mL), triphenylphosphine (17.30 g, 66 mmol) was added. The solution was stirred at room temperature until gas evolution ceased (about two hours) and heated to reflux overnight. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The product was obtained as a colourless liquid by distillation (63 – 85 °C at 6.1*10^{-2} mbar, 7.84 g, 43 mmol, 79 %).

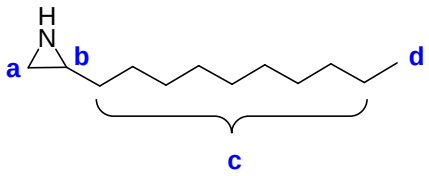

Figure 73: 2-*n*-Decylaziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.93-1.87 (m, 1, **b**), 1.71 (d, 1, J = 5.8 Hz, **a**), 1.39-1.18 (m, 19, **a**, **c**), 0.85 (t, 3, J = 6.7 Hz, **d**).

2-n-Decyl-N-mesylaziridine

The compound was synthesized according to literature procedures.[12] 2-*n*-Decylaziridine (7.84 g, 43 mmol) and triethylamine (9.10 mL, 66 mmol) were dissolved in dry DCM (170 mL) under argon atmosphere. The solution was cooled to -50 °C and mesylchloride (3.71 mL, 48 mmol) was added dropwise over a period of 20 minutes, the reaction mixture was then stirred at -50 °C for an hour. Saturated aqueous sodium bicarbonate (170 mL) was added. The mixture was brought to room temperature and washed with brine. The organic phase was dried over magnesium sulfate and

concentrated under reduced pressure. Chromatography over silica gel (PE/EA 4:1, R_f = 0.49) yielded the product as a yellowish liquid (7.78 g, 30 mmol, 70 %)

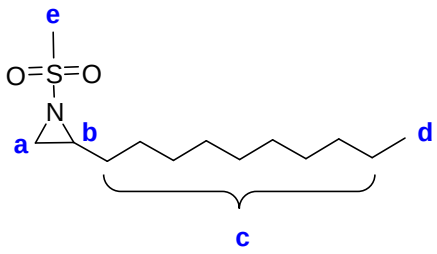

Figure 74: 2-*n*-Decyl-*N*-mesylaziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.04 (s, 3, **e**), 2.74-2.68 (m, 1, **b**), 2.58 (d, 1, J = 7.1 Hz, **a**), 2.09 (d, 1, J = 4.6 Hz, **a**), 1.60-1.25 (m, 18, **c**), 0.87 (t, 3, J = 6.7 Hz, **d**).

1-Azido-2-hydroxydec-9-ene

The compound was synthesized according to literature procedures.[12] To a solution of 1,2-epoxydec-9-ene (7.01 g, 46 mmol) in ethanol (80 mL) and water (20 mL), ammonium chloride (3,15 g, 59 mmol) and sodium azide (3.85 g, 59 mmol) were added. The solution was heated to reflux overnight. The reaction mixture was cooled to room temperature and extracted with DCM. The combined organic phases were washed with brine, dried over magnesium sulfate and concentrated under reduced pressure to yield the product as a yellow liquid (8.08 g, 41 mmol, 90%).

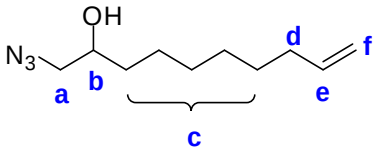

Figure 75: 1-Azido-2-hydroxydec-9-ene

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.79 (tt, 1, J_1 = 16.9 Hz, J_2 = 10.2 Hz, J_3 = 6.7 Hz, **e**), 5.00-4.89 (m, 2, **f**), 3.74-3.69 (m, 1, **b**), 3.36 (dd, 1, J_1 = 12.4 Hz, J_2 = 3.4 Hz, **a**), 3.23 (dd, 1, J_1 = 12.4 Hz, J_2 = 7.4 Hz, **a**), 2.03-1.99 (m, 2, **d**), 1.45-1-20 (m, 10, **c**).

IR: $\tilde{\nu}$ = 3361 (w), 2926 (m), 2855 (m), 2101 (s), 1640 (s), 1440 (m), 1269 (m), 1048 (m), 993 (m), 908 (m), 725 (m) cm^{-1}.

2-n-(7-Octenyl)-N-mesylaziridine

The compound was synthesized according to literature procedures.[12] To a solution of 1-azido-2-hydroxydec-9-ene (8.08 g, 41 mmol) in dry THF (90 mL), triphenylphosphine (13.03 g, 50 mmol) was added. The solution was stirred at room temperature until gas evolution ceased (about two hours), then heated to reflux overnight. The reaction mixture was cooled to room temperature and

concentrated under reduced pressure. The crude 2-n-(7-octenyl)-aziridine, still containing triphenylphosphine oxide, was used without further purification and dissolved in dry DCM (150 mL) under argon atmosphere. Triethylamine (8.71 mL, 61 mmol) was added and the solution was cooled to -50 °C. Mesyl chloride (3.53 mL, 45 mmol) was added dropwise over a period of 20 minutes, the reaction mixture was then stirred at -50 °C for an hour. Saturated aqueous sodium bicarbonate (150 mL) was added. The mixture was brought to room temperature and washed with brine. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. Chromatography over silica gel (PE/EA 2:1, R_f = 0.68) yielded the product as a yellowish liquid (1.58 g, 7 mmol, 17%).

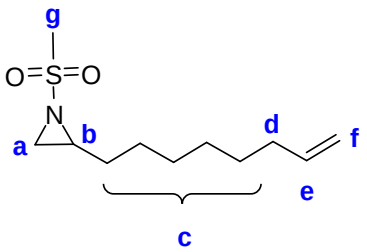

Figure 76: 2-*n*-octen-7-yl-*N*-mesylaziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 5.79 (tt, 1, J_1 = 16.9 Hz, J_2 = 10.2, J_3 = 6.7 Hz, **e**), 5.01-4.91 (m, 2, **f**), 3.04 (s, 3, **g**), 2.75-2.69 (m, 1, **b**), 2.58 (d, 1, J = 7.0 Hz, **a**), 2.09 (d, 1, J = 4.7 Hz, **a**), 2.06-2.00 (m, 2, **d**), 1.60-1.29 (m, 10, **c**).

2-Methyl-N-mesylaziridine

The compound was synthesized according to literature procedures.[12] 2-Methylaziridine was distilled into the cold prior to the reaction and stored under argon atmosphere until further use. 2-Methylaziridine (5.0 mL, 71 mmol) and triethylamine (14.7 mL, 106.2 mmol) were dissolved in dry DCM (140 mL) under argon atmosphere. The mixture was cooled to -30 °C and mesylchloride (6.0 mL, 78 mmol) was added dropwise over a period of 20 minutes. The mixture was stirred for an hour at -30 °C, saturated aqueous sodium bicarbonate (140 mL) was added and the mixture was brought to room temperature. The organic phase was washed with brine, dried over magnesium sulfate and concentrated under reduced pressure. Chromatography over silica gel (PE/EA 1:1, R_f = 0.51) yielded the product as a colourless solid (4.74 g, 35 mmol, 54 %).

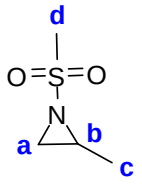

Figure 77: 2-Methyl-*N*-mesylaziridine

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.04 (s, 3, **d**). 2.84-2.75 (m, 1, **b**), 2.59 (d, 1, J = 7.0 Hz, **a**), 2.07 (d, 1, J = 4.6 Hz, **a**), 1.33 (d, 3, J = 5.6 Hz, **c**).

5.2.3. Initiator

N-benzyl-sulfonamide

The compound was synthesized according to literature procedures.[52] A solution of benzylamine (15.3 mL, 139 mmol) in dry diethylether (360 mL) was cooled to 0 °C. Mesylchloride (5.2 mL, 66 mmol) was added dropwise. The reaction mixture was stirred over night at room temperature. The precipitate was filtered and washed with diethylether. The organic phase was washed with 2 M hydrochloric acid and brine, dried over sodium sulfate and concentrated under reduced pressure. The resulting crude product was dissolved in THF (80 mL) and stirred for three hours at room temperature with a solution of 10% MeOH/ $NaHCO_3$ (40 mL). The mixture was concentrated under reduced pressure, the obtained syrup was dissolved in DCM. The organic phase was washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The product was obtained as a colourless solid (7.83 g, 42 mmol, 30 %).

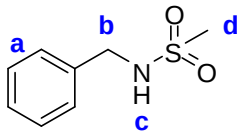

Figure 78: *N*-benzyl-mesylamide

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.26 (m, 5, **a**), 4.93 (s, 1, **c**), 4.30 (d, 2, J = 5.6 Hz, **b**), 2.84 (s, 3, **d**).

5.3. Polymerisation

All glassware was dried in an oven at 110 °C at least 12 hours prior to use.

Experimental setup for carb-anionic and oxy-anionic polymerisation

Figure 79 shows the experimental setup for the carb-anionic and oxy-anionic polymerisation. The whole system (**a**) is linked to a Schlenk line by a stopcock (**c**) and, if needed, to a steel cylinder of

ethylene oxide (**b**). Flasks (**d_1**, **e_1**, **f_1**) can be added to the setup, each one fitted with a stopcock (**d_2**, **e_2**, **f_2**) to allow individual evacuation or argon flooding. One of the flasks (**f_1**) may be exchanged for a graduated ampoule, to measure the used volume of ethylene oxide in the experiment. A fourth flask (**g_1**), which is also fitted with a stopcock (**g_2**), is filled with dry solvent, e.g. THF or cyclohexane. The setup was dried under reduced pressure three times before any polymerisation. The principle of working with this setup shall be explained using the example of transferring dry solvent from flask **g_1** to flask **e_1**. The connector **a** and flask **e_1** are evacuated, then all stopcocks are closed. Flask **e_1** is immersed into a cold bath (e.g. ethanol/$N_{2(l)}$) and stopcock **e_2** is opened. While stirring the solvent in flask **g_1**, stopcock **g_2** is gradually opened until the desired amount of solvent is condensed in flask **e_1**, then both stopcocks **g_2** and **e_2** are closed.

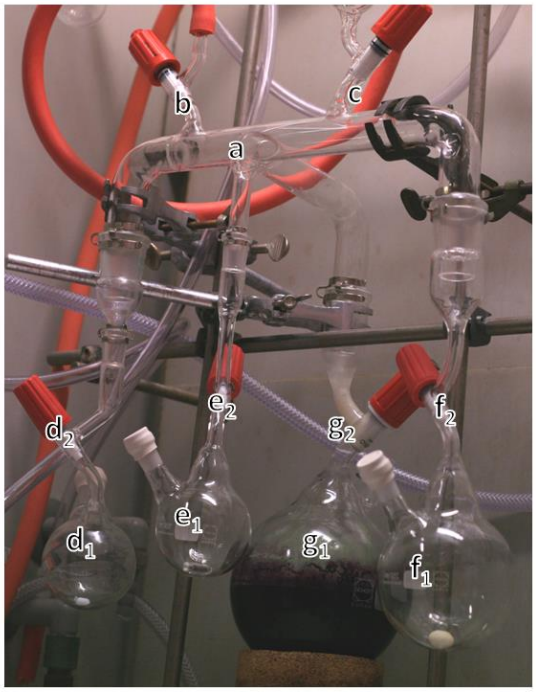

Figure 79: Setup for the anionic polymerisation of styrene and ethylene oxide

Poly(aziridine) – homopolymers

All educts were dried from benzene under vacuum overnight. In an argon filled glovebox a scintillation vial was charged with 2-*n*-decyl-*N*-mesylaziridine (0.20 g, 0.77 mmol) in dry DMF. KHMDS (19.1 mg, 0.096 mmol) and BnNHMs (17.7 mg, 0.096 mmol) were dissolved in dry DMF (1 mL respectively). A portion of each initiator solution (0.1 mL) was mixed and added to the monomer. The vial was sealed, removed from the glovebox and stirred at 55 °C for 18 hours. The polymer was precipitated in 10 mL degassed methanol as a colourless solid, collected by centrifugation and dried at 40 °C under vacuum (0.20 g, 99 %).

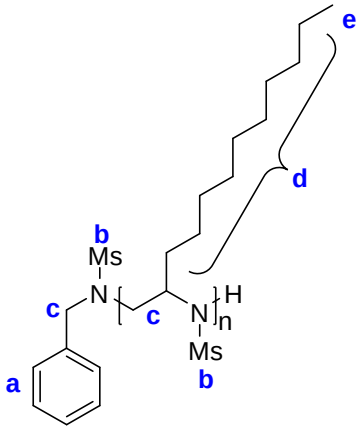

Figure 80: Poly(MDAz)

^{1}H NMR (300 MHz, DMF-d_7): δ 7.51-7.42 (m, 5, **a**), 3.93-3.12 (m, 445, **b**, **c**), 1.97-1.11 (m, 1314, **d**), 0.93-0.88 (m, 219, **e**).

^{13}C NMR (400 MHz, DMF-d_7): δ 40.13-39.29, 32.96, 30.95-30.45, 28.64, 23.64, 14.81.

Poly(aziridine) – copolymers

All educts were dried from benzene under vacuum overnight. In an argon filled glovebox a scintillation vial was charged with 2-*n*-decyl-*N*-mesylaziridine (0.20 g, 0.77 mmol) and 2-*n*-octen-7-yl-*N*-mesylaziridine (0.09 g, 0.38 mmol) in dry DMF. KHMDS (38.1 mg, 0.191 mmol) and BnNHMs (35.4 mg, 0.191 mmol) were dissolved in dry DMF (1 mL respectively). A portion of each initiator solution (0.2 mL) was mixed and added to the monomers. The vial was sealed, removed from the glovebox and stirred at 55 °C for 18 hours. The polymer was precipitated in 10 mL degassed methanol as a brown solid, collected by centrifugation and dried at 40 °C under vacuum (0.22 g, 74 %).

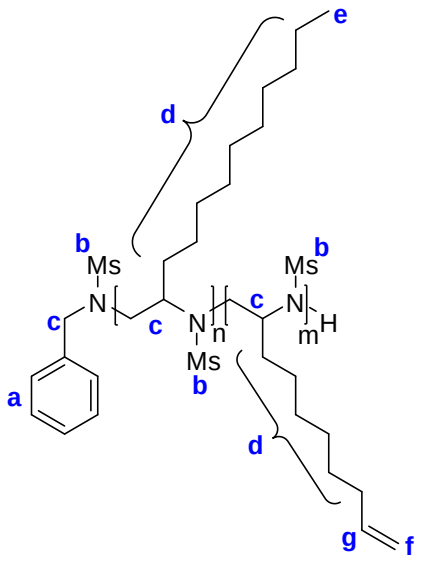

Figure 81: Poly(MDAz-*co*-MOAz)

^{1}H NMR (300 MHz, DMF-d_7): δ 7.53-7.37 (m, 5, **a**), 5.91-5.77 (m, 4, **g**), 5.05-4.93 (m, 8, **f**), 4.63-2.96 (m, 83, **b**, **c**), 2.06-1.29 (m, 210, **d**), 0.93- 0.87 (m, 27, **e**).

^{13}C NMR (400 MHz, DMF-d_7): δ 140.05, 129.78, 115.23, 40.46-39.30, 32.91, 30.95-30.12, 28.18, 24.78, 14.76.

Poly(aziridine) – block copolymers

All educts were dried from benzene under vacuum overnight. In an argon filled glovebox a taped scintillation vial was charged with a solution of 2-methyl-*N*-mesylaziridine (0.21 g, 1.55 mmol) in DMF. KHMDS (147.3 mg, 0.74 mmol) and BnNHMs (136.9 mg, 0.74 mmol) were dissolved in dry DMF (1 mL respectively). A portion of each initiator solution (0.1 mL) was mixed and added to the monomer. The vial was sealed, removed from the glovebox and stirred at 55 °C for 18 hours. Addition of 2-*n*-decyl-*N*-mesylaziridine (0.39 g, 1.48 mmol) in dry DMF was conducted in the glovebox. The vial was sealed, removed from the glovebox and stirred at 55 °C for 18 hours. The polymer was precipitated in 10 mL degassed methanol as a colourless solid, collected by centrifugation and dried at 40 °C under vacuum (0.4 g, 66 %).

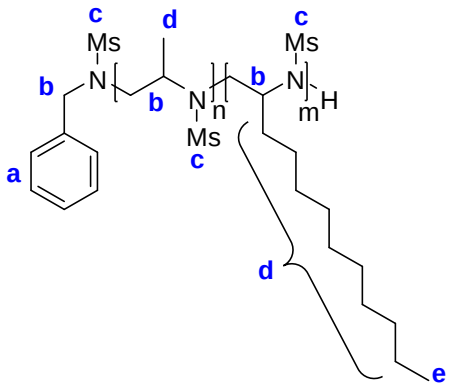

Figure 82: Poly(MMaz)-*block*-poly(MDAz)

^{1}H NMR (300 MHz, DMF-d_7): δ 7.52-7.37 (m, 5, **a**), 4.11-2.96 (m, 239, **b**, **c**), 1.96-1.19 (m, 327, **d**), 0.91-0.87 (m, 42, **e**).

^{13}C NMR (400 MHz, DMF-d_7): δ 128.72, 55.94-50.30, 40.29-39.72, 32.94, 31.38-30.42, 28.20, 22.59, 15.67, 13.74.

Coupling to poly(styrene) in tetrahydrofurane or cyclohexane

All glassware was dried under reduced pressure repeatedly prior to use. *N*-Tosyl-aziridine was recrystallized from PE/EE 6:4 and dried from benzene prior to use. For the experimental setup see Figure 79. One flask was charged with styrene (1.15 mL, 10 mmol) and calcium hydride. The suspension was stirred for one hour under argon atmosphere. The styrene was frozen with liquid nitrogen, exposed to dynamic vacuum for a few minutes and then brought to room temperature under static vacuum. This 'freeze-pump-thaw' procedure was repeated three times to remove all volatiles. Styrene was brought to the polymerisation flask via distillation into the cold, the solvent (about 40 mL) was added in the same way. *sec*-BuLi (0.38 mL, 0.5 mmol) was added via a gas tight syringe. The polymerisation was carried out for 30 minutes at – 100 °C in THF. *N*-tosylaziridine (0.59 g, 3.00 mmol) was dissolved in THF and added to the living polymer via a gas tight syringe. The mixture was stirred at -40 °C for two hours. The reaction was terminated by addition of degassed methanol. The polymer was precipitated in cold methanol as a colourless solid, collected by centrifugation and dried at 40 °C under vacuum (0.75 g, 56 %).

The polymerisation in cyclohexane was carried out overnight at room temperature. As the aziridine is insoluble in cyclohexane, it was dissolved in THF. To prevent precipitation upon addition to the polymerisation flask, THF was added to the living polymerisation via condensation. The flask was

cooled to – 30 °C before the aziridine was added. The mixture was stirred for 18 h or until it was colourless.

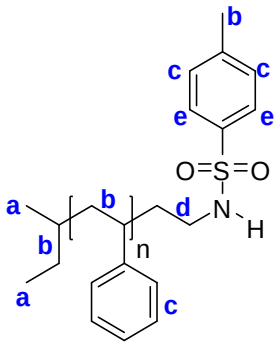

Figure 83: Poly(styrene)-*block*-poly(tosylaziridine)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89-7.36 (m, 2, **e**), 7.11-6.43 (m, 302, **c**), 4.37-2.60 (m, 4, **d**), 2.49-0.87 (m, 186, **b**), 0.76-0.61 (m, 6, **a**).

^{13}C NMR (400 MHz, $CDCl_3$): δ 146.17-145.20, 130.06-129.85, 128.37-25.61, 50.48-49.66, 46.51-40.47, 31.64, 26.92, 21.67, 11.34, 11.22.

Coupling with poly(ethylene glycol)

All glassware was dried under reduced pressure repeatedly prior to use. The experimental setup shown in Figure 79 with a graduated vial instead of one of the flasks was used. 2-Methyl-*N*-metsylaziridine (0.50 g, 3.70 mmol), KHMDS (73.70 mg, 0.37 mmol), BnNHMs (68.40 mg, 0.37 mmol) and 18-crown-6 (0.19 g, 0.74 mmol) were dried from benzene for three hours. In an argon filled glovebox a flask was charged with the monomer dissolved in dry DMF (3 mL). KHMDS and BnNHMs were dissolved in dry DMF (1 mL respectively). A portion of each initiator solution (0.5 mL) was mixed and added to the monomer. The flask was sealed, removed from the glovebox and added to the experimental setup (f_1 in Figure 79). Polymerisation was carried out for 23 hours at 55 °C. Dry DMF (1 mL) was added to the crown ether with a gas tight syringe. Ethylene oxide (2.35 mL, 50.74 mmol) was condensed in the graduated ampoule and then in the polymerisation flask. The crown ether was added with a gas tight syringe, polymerisation was carried out at 60 °C for 17 hours. The reaction was quenched by addition of degassed methanol. The polymer was precipitated in cold diethyl ether as a brown solid, collected by centrifugation and dried under vacuum (0.72 g, 26 %).

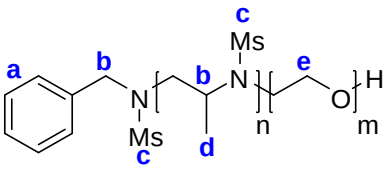

Figure 84: Poly(MMAz)-*block*-poly(EO)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41-7.33 (m, 5, **a**), 4.48-2.71 (m, 383, **b**, **c**, **e**), 1.49-1.27 (m, 106, **d**).

^{13}C NMR (400 MHz, $CDCl_3$): δ 129.26, 70.56-70.35, 54.22-49.37, 39.51, 15.70.

Poly(aziridine) initiated with potassium methoxide

2-Methyl-*N*-mesylaziridine (0.20 g, 1.48 mmol) was dried from benzene, potassium methoxide (5.19 mg, 0.074 mmol) was dried from benzene and methanol. In an argon filled glovebox the monomer was dissolved in dry DMF (3 mL). A solution of the initiator in dry DMF was added, the flask was sealed and removed from the glovebox. Polymerisation was carried out at 55 °C for 18 hours. The reaction was quenched by addition of degassed methanol. The polymer was precipitated in cold diethyl ether as a colourless solid, collected by centrifugation and dried under vacuum (0.2 g, 100 %).

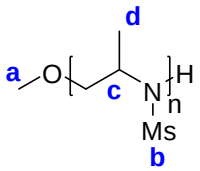

Figure 85: Poly(MMAz)

^{1}H NMR (300 MHz, $CDCl_3$): δ 4.08-3.09 (m, **a**, **c**, **b**), 1.37-1.31 (m, **d**).

Integration not possible, as the backbone superimposes the initiator signal.

^{13}C NMR (400 MHz, $CDCl_3$): δ 65.86, 56.93-53.34, 40.74-38.43, 16.06-15.48.

Poly(aziridine) initiated with sec-butyllithium

All glassware was dried repeatedly under reduced pressure prior to use. *N*-Tosyl-aziridine (1.55 g, 7.89 mmol) was recrystallized from PE/EE 6:4 and dried twice from benzene. *N*-Tosyl-aziridine was dissolved in dry THF (20 mL). The flask was cooled to – 100 °C and *sec*-BuLi (0.3 mL, 0.39 mmol) was added via a gas tight syringe. The reaction mixture was stirred overnight and quenched with degassed methanol. The polymer was precipitated in methanol as a colourless solid, collected via centrifugation and dried in a vacuum (0.90 g, .58 %)

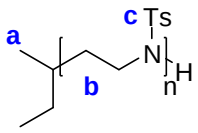

Figure 86: Poly(tosylaziridine)

^{1}H NMR (300 MHz, DMSO): δ 7.67-7.10 (m, 316, **c**), 3.51-3.05 (m, 316, **b**), 2.37-2.25 (m, 237, **c**), 1.23 (m, 6, **a**).

^{13}C NMR (400 MHz, THF-d_8): δ 130.93-128.02, 49.96, 23.65, 21.65, 14.62, 1.59.

5.4. Polymer modification reactions

Removal of the activating groups from poly(styrene)-block-poly(N-tosylaziridine)

The protocol was adapted from a literature procedure.[35] Poly(styrene)-*block*-poly(*N*-tosylaziridine) (0.2 g) and phenol (35.6 mg, 0.06 mmol) were added to a 33 % solution of HBr/AcOH (14 mL) and heated under reflux for seven days. The mixture was brought to room temperature. The liquid was decanted and the residual solid was washed with ethanol several times. After refluxing for one hour in ethanol the liquid was decanted. The product was washed with diethyl ether and dried under vacuum to yield the product as a brown solid (0.14 g, 77 %).

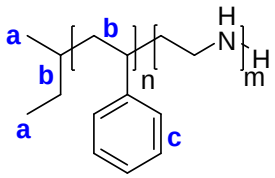

Figure 87: Poly(styrene)-*block*-poly(aziridine)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10-6.54 (m, 225, **c**), 2.41-0.92 (m, 152, **b**), 0.73-0.60 (m, 6, **a**).

^{13}C NMR (400 MHz, $CDCl_3$): δ 146.17-145.45, 128.18-125.78, 46.07-40.49.

The signals from the aziridine backbone are not accessible due to shielding by poly(styrene).

Reaction with acryloyl chloride

Poly(styrene)-*block*-*poly*(aziridine) (0.14 g, 0.036 mmol) or poly(styrene)-*block*-poly(*N*-tosylaziridine) (0.15 g, 0.036 mmol) and triethylamine (0.01 mL, 0.072 mmol) were dissolved in dry DCM (1.5 mL). Acryloyl chloride (0.03 mL, 0.37 mmol) was added. The reaction mixture was stirred at room temperature for three days, the reaction was quenched with ice water. The product was extracted with DCM. The organic phase was washed with saturated aqueous sodium bicarbonate and brine.

The organic phase was dried with sodium sulfate and concentrated under reduced pressure to yield the product as a colourless solid (010 g, 65 %).

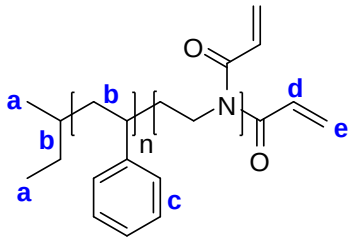

Figure 88: Poly(styrene)-*block*-poly(*N*-acrylamide-aziridine)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.10-6.40 (m, 257, **c**), 6.20-6.08 (m, 4, **e**), 5.95-5.83 (m, 2, **d**), 2.40-0.87 (m, 156, **b**), 0.72-0.59 (m, 6, **a**).

^{13}C NMR (400 MHz, $CDCl_3$): δ 131.38, 128.09-125.78, 45.99-40.47, 8.81.

Thiol-ene reaction

Poly(MDAz-*co*-MOAz) (0.09 g, 0.03 mmol, 0.10 mmol double bonds), AIBN (12.8 mg, 0.08 mmol) and *N*-acetyl-L-cysteine methyl ester (0.38 g, 2.08 mmol) were dissolved in DMF (0.4 mL). After three freeze-pump-thaw cycles, the mixture was stirred overnight at 75 °C. Dialysis against water yielded the product as a colourless solid (61 mg, 56 %).

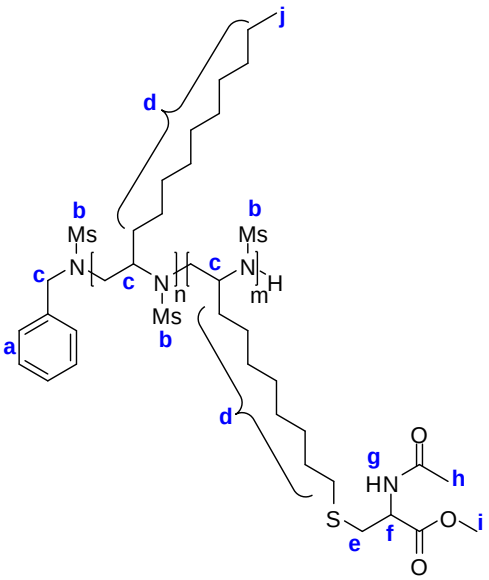

Figure 89: Poly(MDAz-*co*-MOAz) conjugate to *N*-acetyl-L-cysteine methyl ester

^{1}H NMR (300 MHz, DMF-d_7): δ 8.34 (d, 4, J = 7.8 Hz, **g**), 7.53-6.42 (m, 5, **a**), 4.60 (dd, 4, J_1 = 13.4 Hz, J_2 = 7.7 Hz, **f**), 3.95-2.96 (m, 83, **b**, **c**), 3.71 (s, 12, **i**), 2.59 (t, 8, J = 7.2 Hz, **e**), 1.96 (s, 12, **h**), 1.58-1.30 (m, 226, **d**), 0.89-0.87 (m, 27, **j**).

^{13}C NMR (400 MHz, DMF-d_7): δ 172.63, 53.65, 52.82, 32.94, 30.96-30.33, 23.63, 22.97, 14.79.

6. List of abbreviations

$\tilde{\nu}$	wavenumber
ADMET	acyclic diene metathesis
BnNHMs	*N*-benzyl-mesylamide
bp	boiling point
*br*PEI	branched poly(ethylene imine)
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DCM	dichloromethane
DEPT	distortionless enhancement by polarization transfer
DMF	*N*,*N* dimethylformamide
DMF-d_7	deuterated *N*,*N* dimethylformamide
DNA	deoxyribonucleic acid
DOSY	diffusion ordered spectroscopy
DPE	1,1-diphenylethylene
DSP	dithiobis(succinimidylpropionate)
DTC	5,5-dimethyl-trimethylene carbonate
EA	ethyl acetate
EDC	1-ethyl-(3-dimethylaminopropyl 1-ethyl-3)carbodiimide
et al.	and others
HSAB	hard and soft acids and bases
^{i}B	isobutyl
IR	infrared
KHMDS	potassium bis(trimethylsilyl)amide
k_i	rate constant of initiation
k_p	rate constant of propagation
*l*PEI	linear poly(ethylene imine)
m	repeat units N-tosylaziridine
MAC	5-methyl-5-allyloxy carbonyl-trimethylenecarbonate
MALDI-ToF MS	matrix-assisted-laser-desorption/ionisation time-of-flight mass spectrometry
M_n	number average molar mass
Ms	mesyl
m/z	mass to charge ratio
n	repeat units styrene
NHS	*N*-hydroxysuccinimide
NHS-PEG-OPSS	pyridyl-disulfide poly(ethylene glycol)-succinimidyl propionic acid
NMR	nuclear magnetic resonance
nosyl	4-nitrobenzene sulfonyl
Paz	poly(aziridine)
PDI	polydispersity index
PE	petrol ether
PEG	poly(ethylene glycol)
PEG-*block*-PMeOx	poly(ethylene glycol)-*block*-poly(methyloxazoline)
PEI	poly(ethylene imine)

PHPMA	poly-*N*-(2-hydroxypropyl)methacrylamide
pK_a	acid dissociation constant
ppm	parts per million
PS	poly(styrene)
R_f	retention factor
RI	refractive index
r.t.	room temperature
SEC	size exclusion chromatography
sec-BuLi	*sec*-butyllithium
SES	β-(trimethylsilyl)ethylsulfonyl
SPDP	*N*-succinimidyl-3-(2-pyridyldithio)-propionate
Sulfo-KMUS	*N*-(maleimidoundecanoyloxy)sulfosuccinimide ester
THF	tetrahydrofuran
t	time
T	temperature
TLC	thin layer chromatography
Ts	tosyl
UV	ultraviolet
V_e	elution volume

7. References

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