Modular synthesis of well-defined macromolecular architectures

Employment of “click” reactions in polymer chemistry

Een wetenschappelijke proeve op het gebied van de
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1 General introduction:
Synthesis of polymer bioconjugates
1.1. Introduction

In Nature many biopolymers are employed, which for their (biological) activity are dependent on a level of structural control that is unsurpassed by current synthetic materials. This level of control is a result of the well-defined order of arrangement of the biopolymer building blocks, e.g. nucleotides and amino acids, and the absolute control over molecular weight as observed in the case of DNA and proteins. However, this three-dimensional complexity also makes it often difficult to apply biopolymers in materials science, since they are quite susceptible to conformational changes, which can easily lead to loss of function.

Synthetic polymers have the advantage that they can be made in a wide range of different architectures and with a large variety of compositions. They can, therefore, be easily adapted to a specific application environment. However, since absolute control over composition and degree of polymerization cannot be achieved, the level of information and, hence, activity that can be incorporated in synthetic polymers remains limited when compared to biopolymers. A logical approach which has recently experienced much interest is to combine the natural structural control of biopolymers, leading to properties such as programmed assembly, recognition and bioactivity, with the versatility of synthetic polymers, in order to create a new class of hybrid macromolecules. Due to the synergistic effect of blending distinct properties in one single macromolecule, biohybrid polymers have many (potential) applications in medicine, nanotechnology and bioengineering.

Regarding medical applications, the usage of many potential pharmaceutical compounds is hampered by limitations such as harmful side-effects, nonspecific activity and short circulating half-life. Therefore, ideally, these compounds have to be modified in such a way that they can be transported to the site of action in an inactive form where they subsequently can be transformed into active species. In this respect, utilization of macromolecules as therapeutic agents has gained much interest in the last decade. An important class of such macromolecular therapeutics are cytostatics. As cancer cells in many aspects are similar to normal host cells which they are derived from, chemotherapeutic treatment lacks selectivity, thereby causing adverse toxicity, which limits the dose of administrable drugs. Furthermore, many chemotherapeutic agents have more drawbacks like low solubility, rapid degeneration and are sometimes rapidly excreted due to the presence of effective efflux pumps in tumor cells.\[1\] A possibility to overcome
these problems is the conjugation of drugs to polymer carriers. This concept of covalent linkage of polymers to drugs was first postulated by Ringsdorf and co-workers.[2,3]

An early example of such a macromolecular therapeutic is a conjugate of poly[styrene-co-(maleic anhydride)] (SMA) and neocarzinostatin (NCS), better known by the acronym SMANCS, which was developed by Maeda and co-workers (figure 1.1).[4-7] While studying the pharmacokinetics of SMANCS using an in vitro tumor model, a liver tumor/blood ratio over 2500 was observed, which surpassed any existing targeting system at that time and, consequently, it was approved in Japan in 1993 as a treatment for hepatocellular carcinoma. Maeda attributed this phenomenon to a combination of two factors, namely, the hyperpermeability of tumor tissue which enables the uptake of large polymers and the ineffective tumor lymphatic drainage which results in subsequent accumulation of these polymers. This passive form of drug targeting is nowadays referred to as the “Enhanced Permeation and Retention effect” (EPR effect).[8,9] This EPR effect only functions properly when the molecular weight of the polymers exceeds the limit of 40 kDa in order to evade renal clearance. Many macromolecular drugs taking advantage of the EPR effect are currently in clinical use, in clinical trials or in development. In some of these polymeric cytostatics pH-controlled[10,11] or enzymatic cleavable linkers[12-18] are incorporated to liberate the drugs at the tumor site by detachment from the polymer backbone.

In addition, a variety of novel peptides and proteins has emanated from the biotechnology revolution of the last two decades. Some of these peptides and proteins have become important new drugs for various diseases including cancer, infectious diseases, autoimmune diseases and AIDS/HIV. Unfortunately, the application of peptides and proteins is hampered by some limitations, like their susceptibility to denaturation by proteolytic enzymes, short circulating half life, short shelf life, low solubility, rapid kidney clearance and the tendency to generate negative immune responses.
The stabilization of proteins by the attachment of synthetic polymers was already recognized by Abuchowski, Davis and co-workers in the late 1970s\textsuperscript{[19,20]} They covalently linked PEG with molecular weights of 1.9 kDa and 5 kDa to the protein \textit{bovine liver catalase}. The obtained conjugates were injected in mice and exhibited a significantly enhanced circulating half life, reduced immunogenicity and antigenicity while retaining their bioactivity to a large extent. The rationale behind this stabilization effect is the sterical hindrance of the PEG shell which prevents reaction of immune cells with the protein and protects it from degrading proteases. Interactions of cells and proteins with PEG-protein conjugates would involve the energetically unfavorable displacement of hydrated water molecules, and the entropically unfavorable compression of the PEG chains.\textsuperscript{[21]}

The covalent attachment of PEG chains to peptides and proteins has since then been termed PEGylation and has been extensively reviewed.\textsuperscript{[22-29]} The PEGylation of peptides and proteins nowadays is widely used in the pharmaceutical industry. Although the PEGylation of proteins has proven to be very valuable, many of the first generation PEGylation products suffered from a severe loss in bioactivity, ranging from 20-95\%\textsuperscript{[30]} This decrease in activity mainly depends on the chain length of the attached polymers and the site of the protein they are coupled to.\textsuperscript{[31]} Moreover, the attachment of multiple PEG chains leads to mixtures of isomers with different molecular weights, which makes it very
difficult to reproduce drug properties from one batch to the next. For these reasons it is of the utmost importance to have control over the conjugation process.

Another field where polymer bioconjugates play an important role is nanotechnology, although this field is chiefly still in development. Self-assembly processes occur frequently in Nature to build up a plethora of functional structures, commencing from a limited amount of building blocks. One can think of the quaternary structure of proteins, the assembly of viruses or cell membranes. These well-defined three-dimensional structures are the result of a highly controlled hierarchical organization process, based on the information intrinsically stored in the biopolymer chain composition. By constructing biohybrid polymeric materials, a combination of the abovementioned assembly phenomena with the versatility of synthetic polymers could be envisaged, and therefore a class of materials with much potency in nanotechnology is to be expected.\cite{32-34}

An example of such a biohybrid polymer, as shown by Van Hest \textit{et al.}, which is able to assemble into micrometers long fibers is illustrated in figure 1.2.\cite{35} Utilizing protein engineering techniques, which will be discussed in somewhat more detail in section 1.3, a \(\beta\)-sheet forming peptide derived from \textit{Bombyx mori} silk fibroin was PEGylated which, as a result, circumvented macroscopic crystallization and, hence, the peptide was forced to assemble into fibrillar aggregates. An attractive feature of these fibers is the presence of glutamic acid residues on top allowing further modification.

![Figure 1.2](image.png)

\textit{Figure 1.2} ABA-type block copolymer containing a \(\beta\)-sheet segment flanked by two PEG chains, which is capable of forming well-defined fibrils\cite{35}
Another class of (potentially) interesting bioconjugate materials can be constituted by utilizing stimuli responsive synthetic polymers, which therefore can be regarded as “doubly smart”. The most extensively studied polymer in this respect is poly(N-isopropylacrylamide) (PNIPAAm). This polymer displays a lower critical solution temperature (LCST) at 32°C. Bioconjugates embracing PNIPAAm moieties exhibit thermally induced phase separation and this behavior has been exploited to precipitate enzymes from their reaction solutions, hence facilitating both product recovery from the supernatant and recycling of the enzyme. Moreover, poly(methacrylic acid) has been used to induce pH dependent phase separation for enzyme recovery.

Analogous to polymer-protein drug conjugates as discussed before, random conjugation of the abovementioned smart polymers can impede the activity of proteins. Therefore, Hoffman and Stayton have inserted specific reactive amino acids, like cysteine, in proteins via protein engineering to which they subsequently ligated PNIPAAm. Site specific introduction of PNIPAAm at a remote position from the active site of the protein hardly affected its activity.

Protein engineering techniques are feasible as well to deliberately attach responsive polymers in the vicinity of protein active sites. In this case, environmental changes result in reversible blocking or unblocking of the protein's active site, which also can lead to a triggered release of bound ligands from the protein binding site. This switching in enzyme activity is illustrated in scheme 1.1.
As stated throughout the examples given, control over the conjugation process is required in order to preserve the desired functionality. The recent increase in activities in the field of polymer bioconjugates has much to do with the availability of new synthetic techniques that allows us nowadays to create polymeric building blocks from synthetic and biological origin, and to construct well-defined hybrid architectures by coupling these building blocks at predetermined positions within these macromolecules. In the following three sections, an overview is given of the most important synthetic techniques that have surfaced in recent years in order to prepare such well-defined biohybrid polymer architectures, i.e. controlled/living polymerization techniques, polypeptide and oligonucleotide synthesis, and methodologies to conjugate synthetic polymers to biomolecules, respectively.

1.2. Controlled polymerization techniques

The discovery of living anionic polymerization in 1956 by Szwarc et al.\cite{51,52} enabled polymer chemists to gain control over the polymerization process, which means that the degree of polymerization (DP) can be predetermined and the polydispersity index (PDI) is low. Due to the exclusion of the termination process, every growing polymer chain remains active until all monomer is consumed.\cite{53,54} Until approximately 20 years ago, the
only methods for performing controlled polymerizations were based on ionic mechanisms. Because of the sensitivity of this technique, stringent reaction conditions have to be employed and the number of applicable monomers is limited. For this reason, polymer chemists have been striving for almost 40 years to introduce this high level of control in the free radical polymerization process. The reason for this is that radical processes tolerate numerous functional groups, such as present in biomolecules, and allow the polymerization of a myriad of distinct monomers. The advance of controlled or “living” radical polymerization (LRP) mechanisms in the past decade has led to methods that indeed combine the robustness of the radical process with the control first encountered only in anionic systems. It has to be noted that the word living is placed between quotation marks because these processes are quasi-living, due to the fact that termination reactions are suppressed to a great extent, but cannot be excluded completely. Therefore, strictly speaking, these processes are not truly living.

Owing to the living character of anionic polymerization and LRP, control over chain growth is achieved, which allows preparation of block copolymers by consecutive addition of different monomers. Inherently, this control over chain growth also implies control over the polymer chain ends, which subsequently can be modified into numerous functional groups such as amines, azides, thiols and carboxylic acids. These functional moieties can be exploited to specifically conjugate polymers to biomolecules via their end groups. Furthermore, owing to the control over the chain growth, polymers of various topologies, i.e. comb, star, dendritic and so forth, can also be synthesized in a controlled fashion.

The high level of control obtained in LRP is a result of reducing the free radical concentration, which, accordingly, leads to the suppression of termination reactions to a large extent. During free radical polymerizations, growing polymer chains terminate as a consequence of combination or disproportionation of two radicals. The bimolecular rate constants for coupling and disproportionation of most organic radicals are close to the diffusion-controlled limit (10^8 to 10^{10} M^{-1}s^{-1}). This implies that the apparent rates of these processes become relatively slow only at radical concentrations below 10^{-7} M. Thus, termination reactions in radical polymerizations can virtually be excluded only if the radical concentration is very low.

All known concepts of lowering the radical concentration in LRP are based on establishing a rapid dynamic equilibrium between a minute amount of growing radicals and a bulk of dormant species. The mechanisms of LRP can be subdivided in three
groups, depending on the mode of radical generation and deactivation, which is illustrated in scheme 1.2.

(a) \[ \overset{k_{act}}{\underset{k_{deact}}{\rightarrow}} \overset{k_{act}}{\underset{k_{deact}}{\leftarrow}} \]

(b) \[ \overset{k_{act}}{\underset{k_{deact}}{\rightarrow}} \overset{k_{act}}{\underset{k_{deact}}{\leftarrow}} \]

(c) \[ \overset{k_{exch}}{\underset{k_{exch}}{\rightarrow}} \]

Scheme 1.2 Representation of the three different methods to temporarily deactivate growing radical species in LRP, thereby suppressing termination reactions. The upper two mechanisms rely on the fact that the equilibrium is far to the dormant side \( (k_{deact} >> k_{act}) \), whereas the last mechanism is based on a degenerative transfer between two growing polymer chains. (a) Reversible addition and homolytic cleavage of a stable, persistent radical; (b) Reversible transfer of functional group X; (c) Reversible transfer of functional group X between two growing polymer chains.

The most frequently used LRP techniques are nitroxide mediated “living” radical polymerization (NMRP), atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization, which are based on the mechanisms illustrated in scheme 1.2.a, 1.2.b and 1.2.c, respectively. These techniques will be discussed in somewhat more detail in the ensuing three sections.

Another group of polymerization reactions that certainly is noteworthy are metathesis polymerizations. The development of improved catalysts for metathesis reactions has paved the way for controlled ring-opening metathesis polymerization (ROMP)[63] and acyclic diene metathesis polymerization (ADMET)[64,65] In ROMP, strained ring systems, such as norbornenes, can by polymerized by adopting a transition metal catalyst. Initially, ill-defined polymers were obtained using RuCl3; however, by utilizing other ruthenium-[66] and molybdenum-based[67] catalysts developed by the groups of Grubbs and Schrock, respectively, well-controlled polymerizations can be conducted. Analogous to LRP, ROMP is highly tolerant to functional groups and, therefore, monomers comprising biofunctionality can be polymerized in a controlled manner.[68-72]

1.2.1. Nitroxide mediated “living” radical polymerization

The mechanism of NMRP, as depicted in scheme 1.2.a, involves the reversible homolytic cleavage of a dormant chain to form a growing polymer and a stable, persistent
radical. The equilibrium of this cleavage is far to the dormant side \( (k_{\text{deact}} >> k_{\text{act}}) \) resulting in a low concentration of free radicals. In NMRP, of which an example is shown in scheme 1.3, alkoxyamine derivatives are used for the formation of the persistent radicals. These alkoxyamine radicals are very stable as a result of which they act as a radical trap, causing a shift of the equilibrium to the dormant side."[73] The dormant species is reactivated by a thermally induced cleavage of the carbon-oxygen bond.

Scheme 1.3 Example of the NMRP of styrene using a unimolecular initiator, i.e. the alkoxy moiety which is present at the growing polymer chain in the dormant state is incorporated in the initiator molecule. The advantage is that the initiating α-methylbenzyl radical and the mediating nitroxide radical are present in the correct 1:1 stoichiometry."[74]

The advantage of NMRP is that it is applicable in various different solvents and in the presence of many functional groups. Furthermore, it is possible to introduce end group functionality via the initiator and alkoxyamine moieties or on both sides of unimolecular initiators,"[75-77] although the synthesis can be quite challenging. Unfortunately, to date, the number of applicable monomers is slightly limited to styrene derivatives, acrylates, \( N,N \)-dimethylacrylamide, acrylonitrile and dienes.

1.2.2. Reversible addition-fragmentation chain transfer polymerization

The RAFT mechanism, which is illustrated in scheme 1.2.c, is based on the reversible transfer of functional groups between growing polymer chains. As can be seen in scheme 1.4 using the polymerization of styrene as an example, this group transfer is achieved by the addition of dithio compounds."[78] In an early stage after initiation, these dithio
compounds rapidly react with a growing polymer chain upon formation of a polymeric thiocarbonylthio species and a new propagating radical. Chain extension of the polymeric thiocarbonylthio compound proceeds in the same fashion. The reversible addition-fragmentation sequence of the dithio moiety ensures a low radical concentration, which results in a controlled polymerization process.

RAFT polymerizations can be conducted in a large variety of solvents and a wide range of monomers have already been polymerized.\cite{80-82} In analogy to other radical polymerization processes, RAFT is highly tolerant to functional groups. Furthermore, functional end groups can be introduced by incorporation in either the initiator moiety or in the RAFT agent. The latter methodology can have some limitations, since the nature of functional groups substantially influences the stability of the dithioester radical intermediate.\cite{83} Strong radical stabilizing groups will favor the formation of this dithioester radical intermediate, which enhances the reactivity of the S=C bond toward radical addition. However, the stability of the intermediate requires adjustment to promote fragmentation which liberates the reinitiation group.\cite{84}
1.2.3. Atom transfer radical polymerization

In 1995, ATRP was independently reported by Matyjaszewski[85] and Sawamoto[86]. Reduction of the free radical concentration during the ATRP process, as depicted in scheme 1.2.b, is based on the reversible transfer of functional groups between growing polymer chains and transition metal complexes. The equilibrium of this process is far to the side in favor of the polymer chains in the dormant state, i.e. with the functional groups present on the polymer termini.

The mechanism of ATRP is elucidated in scheme 1.5, using the polymerization of styrene as an example. During the initiation and propagation processes, radicals are generated via a reversible redox reaction catalyzed by a transition metal complex, which undergoes a one electron oxidation with concomitant abstraction of a halogen atom from a dormant species, with accompanying rate constant $k_{act}$. These formed radicals are able to propagate until a halogen atom is abstracted from the transition metal complex, causing the propagating polymer chains to return in the dormant state. This reaction proceeds with a rate constant $k_{deact}$.

\[
\text{initiation} \quad \begin{array}{c}
\text{Br} \\
\text{CuBr}_2/2 \text{dNbpy}
\end{array} 
\xrightarrow{K_{eq}'} 
\begin{array}{c}
\text{CuBr}_2/2 \text{dNbpy} \\
\text{CuBr}_2/2 \text{dNbpy}
\end{array}
\]

\[
\text{propagation} \quad \begin{array}{c}
\text{Br} \\
\text{n}
\end{array} 
\xrightarrow{k_p} 
\begin{array}{c}
\text{Br} \\
\text{n+1}
\end{array}
\]

\[
\begin{array}{c}
\text{CuBr}_2/2 \text{dNbpy} = \\
\text{CuBr}_2/2 \text{dNbpy}
\end{array} 
\xrightarrow{k_{eq}} 
\begin{array}{c}
\text{CuBr}_2/2 \text{dNbpy} \\
\text{CuBr}_2/2 \text{dNbpy}
\end{array}
\]

Scheme 1.5 Example of the copper-catalyzed ATRP of styrene[87]

As aforementioned, the equilibrium of this process is far to the dormant side, which implies that the rate of deactivation ($k_{deact}$) is much larger than the activation rate ($k_{act}$).
omitting the termination step in the ATRP process, combined with using a fast-equilibrium approximation, the rate law of ATRP can be derived (equation 1).

\[ R_p = k_{app}[M] = k_p[P\bullet][M] = k_pK_{eq}[In]\frac{[L_mM^{(z+1)}]}{[L_mM^{(z+2)}][X]} [M] \]  

(1)

\[ K_{eq} = \frac{k_{act}}{k_{deact}} = \frac{[P\bullet][L_mM^{(z+1)}X]}{[L_mM^{(z+2)}][PX]} \]  

(2)

The initiator concentration, [\textit{In}] (equation 1), is equal to the concentration of dormant polymer chains [\textit{PX}] (equation 2), assuming initiation is complete. Results from kinetic studies of ATRP using soluble catalytic systems, primarily performed by Matyjaszewski and co-workers,\cite{Matyjaszewski1998} indicate that the rate of polymerization is first order with respect to monomer ([\textit{M}]), initiator ([\textit{In}]) and transition metal ([\textit{LnM^{(z)}}]) concentration. These observations are consistent with the derived rate law, as depicted in equation 1. Furthermore, equation 3 illustrates, if initiation is complete and the degree of polymerization is sufficiently high, the dependence of the PDI (\textit{Mw}/\textit{Mn}) on the rate constants of propagation (\textit{k_p}) and deactivation (\textit{k_{deact}}).\cite{89}

\[ \frac{M_w}{M_n} = 1 + \left(\frac{[\textit{In}]k_p}{k_{deact}[L_mM^{(z+1)}]X} \right)\left(\frac{2}{p} - 1\right) \]  

(3)

As can be concluded from equation 3, the PDI drops with increasing conversion (\textit{p}), which \textit{e.g.} is confirmed with the polymerization of methyl acrylate.\cite{88} Since the rate constants of propagation of acrylates are relatively large, high PDI's were observed in the initial stage of the polymerization, due to the addition of several monomers during each activation step. As the polymerization progresses, growing polymer chains become more uniform owing to continuous exchange reactions, resulting in a decrease of the PDI. Furthermore, the PDI should decrease as well by utilizing catalysts that deactivate growing radical species more rapidly, \textit{i.e.} a smaller \textit{k_p}/\textit{k_{deact}}. Moreover, increasing the deactivator concentration ([\textit{LnM^{(z+1)}}]) also gives rise to a lower PDI. In case of copper-catalyzed ATRP, addition of small amounts of Cu(II)-halides eventuates in more control over the polymerization process, however, at the consequence of a decrease in the reaction rate.\cite{90}

A variety of different transition metal catalysts are employed in ATRP, such as molybdenum, rhenium, ruthenium, rhodium, palladium and iron, in combination with nitrogen- or phosphorus-based ligands.\cite{89,91} Most widely used in ATRP, however, are copper-based catalysts with complexed nitrogen-based ligands, due to their availability,
versatility and relatively low costs. There are several requirements these ATRP catalysts have to fulfill. First of all, they should be highly selective for atom transfer and not participate in side reactions, such as oxidative addition and reduction elimination. Furthermore, the catalyst should be a good halogenophile, thereby preventing \( \beta \)-hydrogen abstractions and attachment to carbon atoms. It should also be capable of deactivating growing polymer chains at nearly diffusion controlled rates, in order to shift the equilibrium far to the dormant side. The halogenophilicity and the deactivation rate of growing radical is governed by the ligands used. Namely, the \( \sigma \)-donating and \( \pi \)-accepting abilities of the ligands have a large influence on the redox-potential of the catalyst, which affects the equilibrium between dormant and active species. A more electron donating ligand stabilizes the higher oxidation state of the metal complex, which causes the equilibrium to shift to the active species. Another important role of the ligands is to improve the solubility of the catalyst.\(^{[92,93]}\)

The initiating system plays a crucial role in the ATRP process as well, e.g. the amount of added initiator with respect to monomer determines the final molecular weight of the polymers. Furthermore, suitable initiators have to fulfill several requirements. Initiation should be fast in comparison to propagation in order to obtain equal growth of polymer chains, which leads to low PDIs.\(^{[94]}\) Therefore, fluoride bearing initiators are not suitable owing to the strength of the carbon-fluoride bond which cannot be cleaved homolytically. A weak carbon-iodide bond, on the other hand, can also give rise to problems. Iodide comprising initiators are light sensitive, metal iodide complexes with unusual reactivity can be formed and the carbon-iodide bond can be readily cleaved heterolytically. Moreover, iodide is a good leaving group, which can result in an uncatalyzed iodide exchange. For these reasons, the most widely applied initiators in ATRP contain bromide functionality, such as benzylic bromides, \( \alpha \)-bromoesters, \( \alpha \)-bromoketones, \( \alpha \)-bromonitriles and sulfonyl bromides. Another feature of the initiating system is that it can determine the topology of the polymers. Utilizing multifunctional initiators provides chain growth in several directions resulting in telechelic or star polymer structures.\(^{[89,91,95]}\) Furthermore, the initiator can be used as a tool to introduce end-functionality in polymers (figure 1.3).\(^{[56]}\) This functionality can be exploited as a handle for conjugation, which will be discussed in section 1.3.

Another methodology to introduce end-functionality in polymers prepared by ATRP is via post-polymerization end group modifications. A consequence of the mechanism of ATRP is the incorporation of halogen groups at the growing terminus. This halogen
moeity can be exploited for the transformation into other functionalities by means of standard organic procedures. In polystyrene (PS) and polyacrylates, e.g. halogen end groups have been replaced by nucleophilic substitution reactions for azides, which subsequently can be reduced to afford amine end-functionality. However, this end group modification procedure requires that the halogen end group is stable throughout the polymerization. Termination reactions lead to loss of the halogen functionality, which subsequently entails incomplete introduction of functional groups. The employment of functional initiators, conversely, acquires quantitative introduction of functionality, on condition that no side-reactions occur, since every growing polymer chain contains an initiating moiety.

![Diagram](image)

**Figure 1.3** Examples of initiators used in ATRP to introduce terminal functionality. (a) Nitrile functionalized initiator; (b) Epoxy bearing initiator; (c) $\alpha$-bromo-tert-butylester; (d) 2-hydroxyethyl-$\alpha$-bromoisobutyrate; (e) Acetal $\alpha$-bromoester initiator which can readily be transformed into an aldehyde; (f) Protected amine functionalized initiator; (g) Initiator utilized to introduce maleimide end functionality via a retro Diels-Alder reaction; (h) Protected acetylene containing initiator; (i) Protected adenosine functionalized initiator.

ATRP reactions can be conducted in either bulk, in solution or in heterogeneous systems, such as emulsions and suspensions. In contrast to conventional free radical polymerizations, the Trommsdorf or gel effect does not occur when ATRP is carried out in the bulk. For solution phase polymerizations, a variety of aprotic solvents, such as toluene, anisole, diphenyl ether, ethyl acetate, acetone, $N,N$-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethylene carbonate and acetonitrile, protic solvents, such as alcohols and water, and special solvents, such as supercritical carbon dioxide, fluororous solvents and ionic liquids, have been employed.
The eligibility of various solvents, combined with the functional group tolerance of the ATRP process, has led to the controlled polymerization of a myriad of distinct monomers, such as styrenes, (meth)acrylates, (meth)acrylamides and acrylonitrile, also with tethered functional groups. The application of such functional monomers can be utilized to introduce pendant (biofunctional) moieties into the polymer backbone. In this fashion, polymers containing e.g. peptide, nucleobase, sugar and phosphorylcholine side groups have been prepared utilizing ATRP.

1.3. Polypeptide and oligonucleotide synthesis

The preparation of well-defined amino acid-based oligomers and polymers has always been a focal point of attention for synthetic chemists, since it allows the construction of bioactive moieties and molecules with a high level of control over the three-dimensional structure. Also in this field much progress has been made in recent years.

Pioneering work by Merrifield, which was extended by Bayer and Mutter, led to the synthesis of peptides via sequential coupling of amino acids on a solid-phase support. Solid-phase synthesis nowadays is still widely used to prepare a wide variety of peptides and oligonucleotides. This stepwise methodology, however, is limited to the preparation of peptides containing 40 to 50 amino acids. In order to obtain larger peptides with up to 200 amino acids, ligation methods can be used in which peptide sequences are “glued” together, as depicted in scheme 1.6.
General introduction: Synthesis of polymer bioconjugates

Native ligation

\[
\text{peptide 1} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \overset{O}{\underset{R'}{\text{SH}}} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \text{peptide 2}
\]

\[
\text{peptide 1} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \text{peptide 2}
\]

Staudinger ligation

\[
\text{peptide 1} \quad \overset{X}{\underset{P}{\text{Ph}}} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \overset{X}{\underset{P}{\text{Ph}}} \quad \text{peptide 2}
\]

\[
\text{peptide 1} \quad \overset{X}{\underset{P}{\text{Ph}}} \quad \overset{O}{\underset{R}{\text{SH}}} \quad \overset{X}{\underset{P}{\text{Ph}}} \quad \text{peptide 2}
\]

Scheme 1.6 Two examples of the chemical ligation process of peptides \[127-129\]

The most common way to prepare high molecular weight polypeptides is by polymerization of \(\alpha\)-amino acid-N-carboxyanhydrides (NCAs).\[130\] These NCA monomers, which are readily prepared in one step from commercially available amino acids, are capable of undergoing a ring-opening polymerization in the presence of nucleophiles or bases, yielding high molecular weight polypeptides with preservation of chirality at the \(\alpha\)-carbon center. By the development of nickel-based initiators, Deming succeeded in suppressing side-reactions, which resulted in the formation of polypeptides with controllable molecular weight and low PDI (scheme 1.7). Additionally, due to the “living” character of these nickel initiated NCA polymerizations, well-defined block copolypeptides can be synthesized as well.\[131,132\]
Scheme 1.7 Proposed mechanism of the nickel or cobalt mediated living NCA polymerization toward well-defined polypeptides

Albeit a very elegant technique to produce large quantities of block copolypeptides, no absolute control over the amino acid sequence in the peptides is possible. Control at the monomer level can be achieved by utilizing protein engineering. As in this case the protein production pathway of biological hosts, usually bacteria or yeast, is exploited for the synthesis of monodisperse polymers with predetermined chain lengths and primary amino acid sequences. The used microorganisms can be programmed to produce the protein of interest by introducing the corresponding genetic information. Although first developed for molecular biology purposes, in the last few years protein engineering has become an important tool in materials design.[133]

In comparison to other techniques, protein engineering seems to be limited to the ensemble of 20 amino acids. The introduction of functional groups in proteins that are absent in this series of proteinogenic amino acids, such as halides, alkynes and azides, would be useful in controlling properties or post-modification and conjugation reactions. As a consequence, much effort has been put into discovering methods to incorporate unnatural amino acids. Nowadays two approaches are available that allow the extension of amino acid building blocks to non-proteinogenic species. The first approach utilizes rarely used genetic codons for the site-specific incorporation of unnatural amino acids.[134] The second, so-called multi-site replacement approach replaces one of the 20 proteinogenic amino acids with a structural analogue, which contains the functionality of interest.[135-141] Some bacterial cell lines lack the ability to produce one of the amino acids. These so-called bacterial auxotrophs are dependent on external sources for obtaining this specific amino acid. If an unnatural analogue is added to a growth medium it can be incorporated in place of the natural substrate, on condition that it is recognized by the corresponding
aminoacyl-tRNA synthetase. Both approaches have shown to be highly versatile and have led to a large extension of available amino acid building blocks (figure 1.4).

![Figure 1.4 Examples of amino acid analogues shown to be incorporated in proteins via multi-site replacement](image)

In accordance with polypeptide synthesis, much research has been conducted with respect to oligonucleotide synthesis because, from a chemical perspective, the hydrogen bonding interactions between the nucleobase pairs in the DNA double helix can be regarded as the acme of molecular recognition.\[142\] Analogous to solid phase peptide synthesis, nucleotides can be coupled using a solid support, which allows facile removal of superfluous reactants and reagents.\[143\] Although in the past this synthesis was extremely laborious and time consuming, nowadays arbitrary sequences can be prepared automatically.\[144\]

In order to readily synthesize larger oligonucleotide strands, the recognition properties of the nucleobases can be exploited to preferentially associate multiple strands with complementary overhanging sequences, so-called “sticky ends”. Subsequently, these strands can be covalently linked using the enzyme DNA ligase, which catalyzes phosphoester bond formation in the backbone.\[145\] Additionally, other modifying enzymes are available, such as restriction enzymes that are able to cleave DNA at specific sequences or exonucleases that digest linear but not cyclic strands.\[146\] Furthermore, it is noteworthy to mention that oligonucleotide quantities can be scaled up by means of utilizing the polymerase chain reaction (PCR).\[147\]
The availability of the abovementioned synthetic toolbox, along with DNA being a chemically stable \[148\] and stiff polymer, it is possible to prepare various geometries composed of oligonucleotide strands, thereby opening up the possibility to utilize it as a material for nanotechnology purposes.\[146,149\]

### 1.4. Conjugation methodologies

All aforementioned techniques exhibit control on the molecular level in the synthesis of both polymers and biomolecules. Moreover, functional groups can be introduced at predetermined locations in these macromolecules, allowing the construction of conjugates of synthetic polymers and biomolecules in a defined fashion. Nowadays, many methodologies are available to couple synthetic and biological macromolecules specifically \textit{via} introduced functional groups.\[21,150,151\] Due to the reduced reactivity in these large macromolecules and the presence of many functional groups, coupling chemistry has to be used, which is both efficient and very specific. In addition to the previously shown ligation processes (scheme 1.6), various coupling reactions have been used to conjugate synthetic polymers to biomacromolecules, as depicted in scheme 1.8,\[22,23\]

\[\text{Reactivity towards amines}\]

\[\text{Reactivity towards thiols}\]

\[\text{"Click" chemistry}\]

\[\text{Scheme 1.8 Examples of conjugation methodologies}^{22,23,152}\]

As described in section 1.2, progress in polymer science has resulted in the controlled synthesis of a variety of polymers with numerous functional end groups.\[153\] These functional end groups can be exploited to conjugate them with biomolecules using coupling chemistry as shown in scheme 1.8, \textit{e.g.} by reaction with free amine and thiol
groups which are present in the lysine and cysteine residues of proteins, respectively. Some examples of the conjugation of polymers prepared by ATRP and proteins are depicted in scheme 1.9.

In order to simplify purification of the bioconjugate polymers obtained, solid phase synthesis can be adopted, as shown by Kiessling and co-workers.\textsuperscript{[154]} \(N\)-succinimidyl ester- and \(N\)-maleimido-functionalized polymers prepared by ROMP were anchored to a Rink-type PS resin via a Diels-Alder reaction, after which sugar moieties were tethered to the polymers. Subsequent cleavage of the desired product was accomplished using a retro Diels-Alder reaction with liberation of furan. Additionally, Klok, Duncan and co-workers applied solid phase peptide synthesis to specifically conjugate poly(ethylene glycol) to the \(N\)-termini of peptides\textsuperscript{[155]} and Nolte \textit{et al.} attached amine functionalized PS to an aldehyde bearing resin, from which subsequently peptides were grown utilizing standard peptide coupling techniques.\textsuperscript{[156]}

The combination of controlled polymerization techniques with conjugation methodologies implies that we nowadays have access to tailor-made polymer biohybrids with all sorts of compositions and architectures. In addition to control over the molecular weight and composition of the synthetic polymers, the chain topology can be altered as well, as already noted before. Branched polymer structures have an increased surface shielding effect, which has a positive contribution to the protection of conjugated proteins \textit{in vivo} against degenerative proteases and antibodies,\textsuperscript{[157]} which is of the utmost importance for pharmaceutical applications, as emphasized in the introductory section. Another advantage of branched polymers, graft polymers and star-shaped polymers is the presence of multiple sites which are available for bioconjugation. This is especially valid for three-dimensional structures such as dendrimers and dendronized linear polymers.\textsuperscript{[158,159]} Their surfaces are very suitable for high loading of biomoieties, which can be very useful for \textit{e.g.} drug design.\textsuperscript{[160-162]}
**Scheme 1.9** Terminal N-succinimidyl ester (a), aldehyde (b), N-maleimido (c) and pyridyl disulfide (d) functionalized polymers prepared by ATRP which are ligated to proteins

Instead of conjugating polymers chains, another methodology for preparing biohybrid polymers is to functionalize biomolecules with initiating systems and, subsequently, grow polymer chains directly from these biofunctional initiators. This “polymerizing from” methodology has been applied for ATRP, NMRP and RAFT polymerization.

Hitherto, the conjugation methodologies described are based on the coupling of synthetic polymers to amino and thiol groups present in lysine and cysteine residues or at the N-termini of proteins. Especially the amino acid lysine often is ubiquitously present in proteins, which usually results in statistical multiple site additions. To limit the number of conjugation sites, an approach is to replace lysine residues by other amino acids. Free cysteines, on the other hand, have been specifically introduced at surfaces of proteins.
either through reduction of disulfide bridges or by introduction of cysteine residues by protein engineering.[173-176] However, if several cysteines are present in a protein, this results in multiple conjugation as well.

Therefore, methods have been developed to target other proteinogenic amino acids. Specific conjugation to the amide group of glutamine and the hydroxyl functionality of serine and threonine can be accomplished under mild conditions using enzymes. Amine functionalized polymers can be coupled to glutamine units using the enzyme transglutaminase.[177] For the site-specific attachment of poly(ethylene glycol) (PEG) to serine and threonine residues a different methodology has been applied.[178] First, N-acetylgalactosamine (GalNAc) was specifically attached to serine and threonine residues using the recombinant enzyme O-GalNAc. Subsequently, to these glycosylated sites sialic acid functionalized PEG was coupled enzymatically, which was accomplished by a sialyltransferase. Recently, a method was developed to specifically alkylate tyrosine residues using $\pi$-allylpalladium complexes.[179]

As discussed in section 1.3, protein engineering can be exploited to introduce additional functionality in proteins via the incorporation of unnatural amino acids. This methodology makes it possible to apply coupling strategies which are orthogonal with respect to other functional groups present. In this respect, so-called “click” reactions,[180] in particular the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition between azides and terminal acetylenes[181] is a perfect candidate, owing to the inertness to other functional groups, the applicability in a wide range of solvents, including aqueous solutions, temperatures and pH values and the efficiency of the reaction. The power of this “click” reaction is recognized by many researchers in different fields of chemistry and, accordingly, numerous articles appeared in recent years where “click” chemistry is applied in e.g. organic synthesis and combinatorial chemistry.[182,183]

As aforementioned, advances in controlled polymerization techniques enabled the introduction of functional groups at both the termini and side-chains of polymers. Consequently, “click” chemistry provoked a real revolution in polymer chemistry and materials science where effective coupling strategies are required owing to the reduced reactivity in macromolecules.[184,185] By employing this copper-catalyzed reaction between azides and terminal acetylenes, main-chain triazole polymers,[186,187] dendrimers,[188-191] dendronized linear polymers,[192,193] hydrogels,[194,195] block copolymers,[196,197] graft copolymers,[198,199] star polymers[200-203] and H-shaped triblock copolymers[204] have been
synthesized. Moreover, it also resulted in the synthesis of a new class of triazole monomers which can be readily provided with functional groups.[205] Since this “click” chemistry is applicable to several classes of macromolecules of both biological and synthetic origin it, therefore, has been recognized recently as a perfect tool to prepare well-defined bioconjugated polymers.[206-210] Two examples of the employment of “click” chemistry in the synthesis of biohybrid polymers are depicted in scheme 1.10. Furthermore, it has been demonstrated to be possible to first prepare aggregates comprising synthetic polymers and accommodate them afterwards with biofunctionality.[211]

![Scheme 1.10](image)

**Scheme 1.10** Examples of the use of “click” chemistry for the synthesis of well-defined biohybrid polymers. **(a)** Coupling of ω-azide functionalized PS to an acetylene functionalized protein Bovine Serum Albumin[206]; **(b)** Site specific PEGylation of azide functionalized Superoxide Dismutase-1[207]

### 1.5. Aim of research and outline of thesis

Polymer biohybrids have been recognized for many years as versatile materials for application in especially the field of drug delivery. Other applications, such as diagnostics and bioactive surfaces, are developing rapidly. The well-defined self-assembly properties of certain types of bioconjugates are potentially useful for nanotechnology purposes, albeit that this field of research is still more on a fundamental level than the other examples given.

Most of the currently applied biohybrid polymers are synthesized employing traditional chemistry. Although impressive results have already been obtained with these macromolecules, it is to be expected that the recent advantages in controlled
polymerization techniques and methodologies to introduce functionality at predetermined locations in biomolecules, as discussed in this chapter, will lead to many opportunities for improvement of bioconjugate structure and, hence, functionality. Therefore, bioconjugate research will have as important theme within the coming years to implement new synthetic schemes, leading to new and improved materials applications.

As stated in the introductory section, one of the main goals in biohybrid synthesis is to gain control over the conjugation process in order to obtain the desired properties introduced into the biohybrid. Hence, site specific coupling of synthetic polymers to biomolecules is demanded. Owing to the presence of multiple functional groups in these biomolecules, this requires chemistry that is very specific, i.e. other functionalities are not allowed to interfere in the reaction pathway. Moreover, the chemistry used has to be efficient as well, considering the reduced reactivity that occurs between macromolecules. In this respect, the improvement of the Huisgen’s 1,3-dipolar cycloaddition between azides and terminal acetylenes employing copper(I)-catalysis by Sharpless et al., which is the most pronounced example of a class of reactions defined as “click” chemistry, is an exceedingly suitable reaction type.

Given that this “click” reaction satisfies all conditions required to specifically conjugate synthetic polymers and biomolecules, the research described in this thesis is directed towards the application of “click” chemistry in the synthesis of well-defined (biohybrid) polymer architectures. Initially, hardly anything was known about the employment of this type of chemistry in polymer synthesis. Therefore, a toolbox had to be developed to introduce azide and acetylene end functionality in polymers, and to, subsequently, couple these macromolecules via their end groups. Chapter 2 deals with the introduction of end functionality and testing the scope of the “click” reaction with respect to the coupling of polymer modules, thereby forming AB- and ABA-type block copolymers.

This modular formation of block copolymers was extended to the preparation of an ABC-type triblock copolymer, which is the subject of chapter 3. Both azide and acetylene end groups were introduced in polymer chains, which by applying a protective group strategy for the acetylene moiety, allowed the performance of two successive “click” reactions onto a central polymer chain.

The introduction of azide and acetylene functionality into single polymer chains opens up the possibility of utilizing them as precursors to prepare cyclic polymers. The application of “click” chemistry in the synthesis of cyclic polymers is described in chapter 4.
Chapter 5 deals with the synthesis of biohybrid block copolymers using “click” chemistry. Synthetic polymers were conjugated with the fibril forming peptide KTVIIE, the peptide (VPGVG)$_3$ which exhibits lower critical solution temperature (LCST) behavior, and to polymers comprising both thymine and adenine pendant groups.

Another approach in polymer bioconjugate synthesis is outlined in chapter 6. Amphiphilic polystyrene-block-poly(acrylic acid) (PS-b-PAA) was self-assembled into vesicular aggregates, so-called polymersomes, with the periphery being covered with either azide or acetylene groups. These polymersomes were used as scaffolds for further functionalization using “click” chemistry. By applying this strategy, a fluorescent probe, a biotin-streptavidin complex and enhanced green fluorescent protein (EGFP) were conjugated to the exterior of the vesicles.

1.6. References

General introduction: Synthesis of polymer bioconjugates


Spijker, H.J.; Dirks, A.J.; van Hest, J.C.M. *Polymer* 2005, 46, 8528-8535.


General introduction: Synthesis of polymer bioconjugates


The copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition, which is the most eminent type of “click” reaction, was used to modularly synthesize synthetic block copolymers. Therefore, first of all, terminal acetylene and azide moieties were introduced into polymer building blocks. For polymers prepared via atom transfer radical polymerization (ATRP), this was accomplished utilizing two different methodologies, viz. employing functionalized initiators and by post-polymerization end group modification procedures. Terminally acetylene functionalized polystyrene (PS) and poly(methyl methacrylate) (PMMA) were synthesized using an acetylene functionalized initiator. Azide functionalized mono- and bifunctionalized PSs were prepared via the latter method by substitution of bromide end groups, present after polymerization, for azides. Furthermore, both azide and acetylene monofunctionalized poly(ethylene glycol)s (PEG) were synthesized by modification of the hydroxyl terminus. The thus obtained polymer building blocks were subsequently coupled by means of 1,3-dipolar cycloaddition reactions by applying a copper catalyst. Quantitative formation of block copolymers was confirmed by size exclusion chromatography (SEC) measurements. Moreover, the excess of polymeric precursor was removed successfully either by a washing step or by using an azide functionalized scavenger resin, depending on the polymers used.
2.1. Introduction

Block copolymers are macromolecules composed of either linear or non-linear arrangements of chemically distinct polymers (blocks).\cite{1} In general, the different blocks are incompatible, giving rise to multiple, highly regular, self-assembled structures in bulk and films as well as in solution. In the bulk state and in thin films, microphase separation may occur, owing to segregation of the distinct blocks, which induces the formation of a variety of patterns on a mesoscopic length scale, ranging from spheres and cylinders to lamellae.\cite{2-5} As a consequence, block copolymers have received much attention for nanotechnology applications in a “bottom-up” approach.\cite{6} Likewise, in solution amphiphilic block copolymers have the tendency to self-assemble into multiple distinct morphologies, varying from micelles and micellar rods to vesicular structures.\cite{7-10} Accordingly, these materials are perfect candidates for utilization as drug delivery vehicles\cite{11,12} or nanoreactors.\cite{13} Furthermore, in general, block copolymers embrace exceptional stability which, conjoined with the possibility of readily tailoring the chemical, physical or biological properties, makes them easily adaptable to different application environments.

The most straightforward method for the synthesis of block copolymers is by consecutive polymerization of distinct monomers using a living or controlled polymerization technique.\cite{14} That means, after consumption of monomer A, the polymer terminus remains active and will continue to propagate by addition of monomer B or by a re-initiation step. Nowadays, a wide range of living or controlled polymerization techniques is available in order to prepare block copolymers of various architectures, functionality and solubility. These polymerization methods comprise living ionic\cite{15,16} and cationic\cite{17} polymerization, group-transfer polymerization,\cite{18} coordination polymerization,\cite{19} ring opening metathesis polymerization (ROMP),\cite{20} and controlled radical polymerization techniques, like atom transfer radical polymerization (ATRP),\cite{21,22} reversible addition-fragmentation chain transfer (RAFT) polymerization,\cite{23} and nitroxide mediated radical polymerization (NMRP).\cite{24} Each polymerization system provides a limited range of polymers and, therefore, the various techniques can also be combined to prepare a myriad of block copolymer structures.\cite{25-33}

Another approach for block copolymer synthesis is the coupling of terminally functionalized polymers.\cite{34-36} An advantage of this methodology can be that complete formation of block copolymers is easy to assess, because a difference in mass of
unreacted homopolymer and formed block copolymer can be observed. This may allow purification of the block copolymers by size exclusion chromatography (SEC). An additional advantage is the possibility of complete analysis of the polymer building blocks prior to formation of the block copolymer. However, owing to the reduced reactivity of end groups in polymers, extremely efficient coupling strategies have to be used which involve the utilization of highly reactive moieties that, accordingly, are prone to undergo side reactions. For this reason “click” chemistry processes are exceptionally suitable since these types of reactions are very efficient and specific.

“Click” chemistry, a term proposed by Sharpless, is defined as a reaction process that is modular, wide in scope, high yielding, generating only inoffensive byproducts that can be removed by nonchromatographic methods, and stereospecific (but not necessarily enantioselective). Moreover, characteristic for the “click” chemistry process is that it encompasses simple reaction conditions, readily available starting materials and reagents, the use of no solvent or a solvent that is mild (like water) or that can be easily removed, and it requires facile product isolation.

Undisputedly, the best known and most widely used “click” reaction is the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition between azides and terminal alkynes to give 1,2,3-triazoles (scheme 2.1). The fact that azide and alkyne moieties are inert to most other functional groups, combined with their stability in a wide range of solvents, temperatures and pH values, and the efficiency of the reaction between the two functionalities, has paved the way for applications in organic synthesis, bioconjugation, combinatorial chemistry and materials science.

Due to the abovementioned advantages, “click” chemistry also has taken a tremendous flight in polymer chemistry recently. This has eventuated in the synthesis of main-chain triazole polymers, dendrimers, dendronized linear polymers, hydrogels, block copolymers, graft copolymers, star polymers and a new class of functionalized triazole monomers. Furthermore, it has also been used to functionalize both end groups and side chains of polymers. Moreover, the reaction between acetylenes and azides is orthogonal with respect to other functional groups and, therefore, it appears to be a perfect tool for the preparation of polymer bioconjugates.
Scheme 2.1  

(a) Schematic representation of the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition;  (b) Proposed catalytic cycle. First, copper(I) acetylide I is formed which, according to density theory calculations, proceeds via a stepwise ligation sequence (B-1 → B-2 → B-3) in contrast to the concerted [2+3] cycloaddition mechanism (B-direct).\(^{[38,76]}\) Additionally, there is empirical evidence concerning the existence of copper(I) triazolide IV\(^{[77]}\).

As stated in the first chapter, the research described in this thesis is directed towards the specific conjugation of synthetic polymers and biomolecules utilizing “click” chemistry. At the start of the research described in this thesis, hardly anything was known about the application of “click” chemistry in polymer synthesis, especially not into the area of conjugation of macromolecules via their end groups. Therefore, to obtain a proof of principle, first a toolbox had to be developed for the introduction of azide and acetylene end-functionality in macromolecules, prior to testing the scope of the “click” reaction with respect to the connection of (bio)macromolecules. To demonstrate proof of principle it was chosen to introduce azide and acetylene end groups in synthetic polymer chains which were linked in a subsequent “click” coupling step, thereby forming block copolymers in a modular fashion, as depicted in figure 2.1.
Figure 2.1 Schematic illustration of the modular formation of a block copolymer by the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction between azide and terminal acetylene functionalized polymers.

Although this reaction is very efficient, the used azide and acetylene reactive groups are inert to other functionalities and merely react with each other in the presence of a copper(I)-catalyst or at elevated temperatures. Therefore, polymers containing azide and acetylene end groups can be prepared and safely stored, and, subsequently, block copolymers can be synthesized by subjecting the building blocks to a copper(I)-catalyst. In order to modularly synthesize block copolymers employing “click” chemistry, polymers bearing terminal acetylene and azide functionality are required. The preparation of these end-functional polymers is described in the next two sections. The subsequent modular formation of block copolymers using these polymer building blocks is discussed in section 2.4.

2.2. Acetylene end functionalized polymers prepared by atom transfer radical polymerization

The necessary acetylene and azide functionalized polymeric precursors are synthesized using the controlled radical polymerization technique atom transfer radical polymerization (ATRP). Applying ATRP allows the introduction of end-functionality into polymer chains by utilizing functional initiators or by post-polymerization end group modification procedures.

The most straightforward strategy for the introduction of acetylene termini in polymers prepared by ATRP is by adopting functional initiators. In this case, provided that no side reactions occur, it is ensured that every growing polymer chain contains the desired functionality. Since α-bromoesters are suitable as initiating system and frequently...
employed in ATRP, 2-propynyl-2-bromo-2-methylpropanoate (I) was synthesized by an esterification reaction of propargyl alcohol and 2-bromoisobutyryl bromide in tetrahydrofuran (THF) with triethyl amine (Et₃N) as a base, as depicted in scheme 2.2.

\[
\text{HOCH}_2 + \text{BrCH}_2\text{CH}_2\text{Br} \xrightarrow{\text{Et}_3\text{N}} \text{CH}_2\text{CH}_2\text{Br}
\]

**Scheme 2.2** Synthesis of acetylene functionalized ATRP initiator 1. Reagents and conditions: i. Et₃N, THF, 0°C→rt., 1.5 h, 97%

Acetylene functionalized initiator 1 was used to polymerize methyl methacrylate (MMA), thus introducing an acetylene end group (scheme 2.3). As a catalytic system, a 1:2 complex of CuBr and N-(n-propyl)-2-pyridymethanimine (2) was employed. The polymerization was conducted in xylene at 90°C. Unfortunately, several attempts to polymerize MMA failed. The reaction kinetics deviated from first order behavior, which is an indication that the polymerization process proceeded in an uncontrolled fashion.

\[
\text{O} + \text{BrCH}_2\text{CH}_2\text{Br} + \text{CuBr} \xrightarrow{2 \text{N-C}_3\text{H}_7} \text{O} + \text{nCuCl}
\]

**Scheme 2.3** Schematic representation of the ATRP of MMA using acetylene functionalized initiator 1

A conceivable explanation for the deviation from first order reaction kinetics of the polymerizations performed with initiator 1 can be complexation of this initiator with the copper(I) species used as a polymerization catalyst. Consequently, a copper(I)-acetylide may be formed, which results in catalyst deactivation. After all, contemplating figure 2.1, such copper(I)-acetylides are thought to play an important role in the “click” reaction between acetylenes and azides. In order to circumvent the problem of complexation, a new initiator containing a trimethylsilyl (TMS)-protected acetylene moiety was prepared, as depicted in scheme 2.4. 3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methyl propanoate (3) was synthesized using a similar procedure to initiator 1, starting with commercially available 3-trimethylsilyl-2-propyn-1-ol.
TMS-protected initiator 3 was exploited to polymerize MMA and styrene (St) (scheme 2.5). The polymerization of MMA was conducted using equal conditions as used before, *viz.* with a complex of CuBr and two equivalents of N-(n-propyl)-2-pyridylmethanimine (2), using xylene as a solvent.\[^{[83]}\] Styrene was polymerized in bulk using a 1:1 complex of CuBr and N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA) as the catalytic system.\[^{[84]}\] To this reaction mixture anisole was added as an internal standard to be able to monitor the reaction. Therefore, samples were taken periodically during the polymerization reactions for analysis by gas chromatography (GC). As can be seen in figure 2.2, in this case, all conducted polymerizations proceeded *via* first order reaction kinetics, indicating good control over the polymerization process. Consequently, this resulted in polymers with reasonably low polydispersity indices (PDIs) (M\(_w\)/M\(_n\) \leq 1.23), as measured with size exclusion chromatography (SEC) (table 2.1). Additionally, the initiator efficiency (I\(_{\text{eff}}\)) was calculated for the polymerization of MMA from the ratio of the degree of polymerization (DP) obtained from GC measurements and the DP as calculated from the \(^1\)H NMR spectrum of the polymer, which amounted to 0.91. This implies that 91 percent of the initiator molecules actually initiated a polymerization reaction. Although the polymerization reactions developed in a controlled fashion, it has to be noted that partial loss of the TMS protective group occurred during polymerization, as observed in the \(^1\)H NMR and matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) spectra of the obtained polymers. In case of the polymerization of styrene using CuBr/PMDETA as a catalyst even 70 percent of the TMS groups disappeared. A feasible side reaction that may have occurred can be nucleophilic attack of one of the nitrogen groups of PMDETA on the TMS group. This conclusion was drawn because in case of the polymerization of MMA, using the less nucleophilic ligand 2, approximately 85 percent of the TMS groups was still present in the acquired polymer, according to \(^1\)H NMR spectroscopy measurements.
Scheme 2.5 Introduction of acetylene end-functionality in polymers by ATRP using TMS protected initiator 3 and subsequent removal of the TMS group by treatment with TBAF.

The last step in preparing acetylene terminated polymers was removal of the residual TMS protecting group. Deprotection was readily realized by subjecting the polymers to a solution of tetrabutylammonium fluoride (TBAF) in THF (scheme 2.5). Complete removal of the TMS groups was determined by disappearance of the signals of the methyl protons adjacent to the silicon atoms ($\delta=0.18$ ppm) in $^1$H NMR spectra.
2.3. Introduction of acetylene and azide end groups in polymers by post-polymerization end group modification

2.3.1. Functionalizing the hydroxyl terminus of poly(ethylene glycol)

As mentioned in the introductory section, end functionality in polymers can also be introduced by modification of the end groups after polymerization. This methodology was applied to incorporate acetylene functionality in poly(ethylene glycol) (PEG), as shown in scheme 2.6. 4-Pentynoic acid was attached to the hydroxy end group via a carbodiimide mediated coupling in dichloromethane (CH₂Cl₂). As carbodiimide 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was used on account of its hydrophilic character, as well as of the formed urea, which allowed easy removal by an aqueous extraction step. Formation of the desired product was ascertained by a downfield shift of the methine protons adjacent to the end groups (from δ 3.54 to 4.14 ppm), combined with the appearance of an acetylene proton signal (δ = 1.99 ppm) in the ¹H NMR spectrum and the presence of an acetylene signal (3261 cm⁻¹) in the FTIR spectrum of the product. Furthermore, end group analysis of the MALDI-ToF MS spectrum of 6 demonstrated the attachment of 4-pentynoic acid as well.
Likewise, the hydroxyl terminus of PEG was replaced by an azide by a two step procedure, comprising tosylation of the hydroxy group, followed by a nucleophilic substitution reaction applying sodium azide (NaN₃) (scheme 2.7). The tosylation reaction first was performed in THF/H₂O (1:1) using NaOH as a base. However, no higher conversions than approximately 50 percent could be obtained. Therefore, the reaction was conducted in pyridine which acts both as a solvent and as a base. In this case, complete tosylation of the hydroxyl functionality was attained, according to ¹H NMR measurements. Moreover, from the MALDI-ToF MS spectrum the presence of tosylate end groups could be calculated. Subsequently, the tosylate groups were successfully substituted for azides using sodium azide in N,N-dimethylformamide (DMF), as confirmed by an upfield shift of the end group protons in ¹H NMR spectra (from 4.14 to 3.86 ppm), the appearance of an azide stretch vibration in the FTIR spectrum (2098 cm⁻¹) and the correct masses, as obtained by MALDI-ToF MS measurements.

**Scheme 2.6** Synthesis of acetylene functionalized PEG 6 by esterification of the hydroxyl terminus. Reagents and conditions: i. EDCI, DMAP, CH₂Cl₂, -20°C → rt., 20 h, 83%

**Scheme 2.7** Preparation of azide terminated PEG 8 by tosylation and substitution of the hydroxyl end group. Reagents and conditions: i. TsCl, pyridine, rt., 20 h, 85%; ii. NaN₃, DMF, rt., 22 h, 82%

### 2.3.2. Introduction of azide end-functionality in polystyrene

As aforementioned, polymers prepared by ATRP are appropriate for the introduction of functionality via end group modification procedures. As can be seen in chapter 1, after the ATRP process, polymers are terminated with halide atoms, which are susceptible for substitution reactions.⁸⁵-⁸⁸ Therefore, azide functionality can be readily introduced by nucleophilic substitution reactions.⁸⁹,⁹⁰

Additionally, initiating polymerization reactions with multifunctional initiators provides chain growth in multiple directions. Therefore, the application of a difunctional initiator results in telechelic polymers from which both end groups can be replaced by azides, thereby opening up possibilities to synthesize ABA triblock copolymers via “click” chemistry. For that reason, difunctional initiator 9 was synthesized by a reaction of
Modular synthesis of AB and ABA type block copolymers

ethylene glycol with the acid bromide, 2-bromopropionyl bromide, as depicted in scheme 2.8. In this case, 2-bromopropionyl bromide was chosen instead of 2-bromoisobutyryl bromide, since the formed initiator 9 generates secondary radicals in contrast to tertiary radicals, which leads to a slower initiation process due to less stabilization of the secondary radicals. This initiator, hence, may be more suitable for the polymerization of styrene owing to similar stability of formed radicals.

Scheme 2.8 Synthesis of bifunctional ATRP initiator 9. Reagents and conditions: i. Et3N, THF, 0°C → rt., 2 h, 94%

Subsequently, mono- and difunctional polystyrene (PS) were prepared by ATRP utilizing 1-bromoethyl benzene and 2-[(2-bromopropanoyl)oxy]ethyl-2-bromopropanoate (9), respectively, to initiate the polymerization reactions, as depicted in scheme 2.9. For the preparation of ω-bromo-PS, a stoichiometric complex of CuBr and PMDETA was used as the polymerization catalyst, and telechelic α,ω-dibromo-PS was synthesized employing a 1:2 complex of CuBr and 2,2'-bipyridine (bpy). In both cases, anisole was added as an internal standard in order to be able to monitor the reactions by GC. As illustrated in figure 2.3, both polymerization reactions properly proceeded in accordance with first order reaction kinetics. This implied that the ATRP processes developed in a controlled fashion. Correspondingly, the PDIs were low (1.15 and 1.14 for the mono- and difunctional polymer, respectively) as measured by SEC, which is denoted in table 2.1 as well. As a matter of fact, using well-defined polymers for post-polymerization end group modifications is a prerequisite as termination reactions during polymerization result in loss of bromide functionality and, therefore, eventually in incomplete introduction of azides.
Scheme 2.9 Preparation of mono- and difunctionalized PS ((a) and (b), respectively) by ATRP and the subsequent introduction of azide functionality by nucleophilic substitution of the bromide end groups applying Me₃Si-N₃ and TBAF.

The bromide end groups present after executing the ATRP experiments were facilely transformed into azides by nucleophilic substitution reactions, using azidotrimethylsilane (Me₃Si-N₃) and TBAF in THF as a solvent (scheme 2.9), yielding ω-azido-PS (10) and α,ω-diazido-PS (11). First, a nucleophilic attack of the fluoride ion of TBAF on the silicon atom of Me₃Si-N₃ occurs which liberates an azide ion that enforces a substitution of the bromide end groups. This reaction was chosen because it can be performed in THF, thereby avoiding DMF as a solvent when NaN₃ would have been used. Successful formation of azide end-functionalized polymers was determined by a complete upfield shift of the end group protons (from δ 4.46 to 3.91 ppm for 10 and from δ 4.42 to 3.93 ppm for 11) in ¹H NMR spectra, along with the appearance of azide signals (2090 cm⁻¹ for 10 and 2094 cm⁻¹ for 11) in FTIR spectra.
In these first two sections the possibilities have been shown to successfully introduce acetylene as well as azide end groups in a controlled fashion by adopting ATRP as a polymerization technique. These polymeric precursors can be used to examine the opportunities of utilizing the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition as a tool for connecting macromolecules via their end groups.

2.4. Modular formation of block copolymers by “click” chemistry

Most “click” reactions are performed using a copper(II)-source, usually CuSO₄•5H₂O, which is reduced in situ to copper(I) using a reducing agent, for instance sodium ascorbate or copper(0). Additionally, ligands, such as oligotriazoles, can be added to stabilize the copper(I)-species under aerobic conditions. However, these reactions are generally conducted in an aqueous environment, in which most synthetic polymers do not dissolve. Nevertheless, it was attempted to work with dispersions of PS in tert-butanol/H₂O (1:1), because it is conceivable that the polymer substrates are capable of participating in “click” reactions being suspended in the solvent system. Unfortunately, all conducted “click” experiments using PS in an aqueous environment failed.

Another possibility is to utilize copper(I)-salts directly without application of a reducing agent. A major advantage is that, in this case, organic solvents can be employed. Conversely, the reactions have to be carried out under an inert atmosphere by the exclusion of oxygen, in order to prevent oxidation of the copper(I)-catalyst and to
suppress side reactions to occur. For example, in the presence of oxygen, Glaser couplings are known to take place, which lead to the unwanted formation of bis-acetylenes.

Various copper(I)-salts, for example CuI, CuBr, CuOTf•C₆H₆, CuI•P(OEt)₃, CuBr•(PPh₃)₃ and Cu(NCCH₃)₄][PF₆], have been exploited in numerous solvents, such as acetonitrile (CH₃CN), CH₂Cl₂, THF, toluene, DMF and neat N-ethyldiisopropylamine (DiPEA). Furthermore, in combination with a copper(I)-salt, at least one equivalent of a nitrogen base is required, which is thought to play a role in formation of the copper-acetylide complex, in analogy with the Sonogashira reaction. Frequently used bases are Et₃N, DiPEA, pyridine, 2,6-lutidine and 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU).

The first “click” reaction was performed utilizing α-acetylene-ω-bromo-poly(methyl methacrylate) (4a) and α-methoxy-ω-azido-poly(ethylene glycol) (8), thereby forming the diblock copolymer PMMA-b-PEG (4a-b-8), as shown in scheme 2.10.a. The reaction was conducted in THF at 35°C, using CuI and DBU as the copper(I)-source and the nitrogen base, respectively. Since polymers possess a distribution of distinct molecular weights, it is difficult to work exactly with equimolar amounts. Therefore, in order to drive the reaction to completion, a slight excess of PEG (1.2 equivalents with respect to PMMA 4a) was utilized. After reaction, this excess of PEG was readily removed by a washing step with methanol (MeOH), because this polymer remained soluble in this polar solvent, as opposed to the formed PMMA-b-PEG block copolymer. Completion of the reaction was determined with SEC, by means of a complete shift towards higher molecular weight owing to the formation of the diblock copolymer product, as illustrated in figure 2.4.a. Furthermore, as can be seen in table 2.1, no increase of the PDI was observed, implying quantitative block copolymer formation, accompanied by the fact that there was no or only minute amounts residual PEG present. Otherwise, a bimodal distribution, or at least a broadening of the signal, would be observed as a consequence of remaining starting materials, which would have resulted in an increase of the PDI with respect to the precursors. Moreover, the side reaction which led to partial loss of the TMS groups during polymerization, as discussed in section 2.2, did not affect the acetylene moiety because it was still completely available for reaction.
Modular synthesis of AB and ABA type block copolymers

Scheme 2.10 Modular synthesis of the diblock copolymers PMMA-b-PEG (a), PS-b-PEG (b), PMMA-b-PS (d) and the triblock copolymer PEG-b-PS-b-PEG (c) exploiting “click” chemistry. Reagents and conditions:
i. CuI, DBU, THF, 35°C, overnight; ii. azidomethyl PS resin 12, THF, rt., 25 h
As a comparison, the same reaction was carried out once more, yet utilizing α-acetylene-ω-bromo-poly(methyl methacrylate) (4b), which had a higher molecular weight (18.1 kg/mol in contrast to 7.1 kg/mol) (table 2.1). Equal reaction conditions were applied and, likewise, completion of the reaction was assessed by SEC (figure 2.4.b). As can be seen in the SEC chromatogram of the PMMA 4b building block, a small shoulder peak of higher molecular weight was present, which indicates the occurrence of termination reactions during the polymerization process. After the “click” coupling with PEG 8, the SEC data provided inconclusive information concerning the fact if these terminated polymer chains actually participated in the reaction. A possible side reaction during the ATRP process could have been termination of growing polymer chains by addition to the acetylene moiety, which, as a result, is not accessible anymore for further reaction with azide groups in the “click” reaction. This can be due to the partial loss of the TMS protecting group during preparation of the polymer, as noted in section 2.2. Nevertheless, the product contained only traces of this terminated material and the majority of the PMMA chains were coupled to PEG, indicated by a shift towards higher molecular weight (table 2.1).
Table 2.1 SEC data of the performed polymerizations and subsequent “click” coupling reactions

<table>
<thead>
<tr>
<th>polymer</th>
<th>( M_n ), calc</th>
<th>( M_n ), SEC</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )-acetylene-( \omega )-bromo-poly(methyl methacrylate)</td>
<td>( 4a )</td>
<td>7.1</td>
<td>7.9</td>
</tr>
<tr>
<td>( \alpha )-acetylene-( \omega )-bromo-poly(methyl methacrylate)</td>
<td>( 4b )</td>
<td>18.1</td>
<td>21.3</td>
</tr>
<tr>
<td>( \alpha )-acetylene-( \omega )-bromo-poly(methyl methacrylate)</td>
<td>( 4c )</td>
<td>15.4</td>
<td>13.5</td>
</tr>
<tr>
<td>( \alpha )-methoxy-( \omega )-(4-pentynoyl) poly(ethylene glycol)</td>
<td>6</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>( \alpha )-methoxy-( \omega )-azido-poly(ethylene glycol)</td>
<td>8</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>( \omega )-azido-polystyrene</td>
<td>10</td>
<td>4.2</td>
<td>3.5</td>
</tr>
<tr>
<td>( \alpha,\omega )-diazido-polystyrene</td>
<td>11</td>
<td>12.9</td>
<td>11.7</td>
</tr>
<tr>
<td>PMMA-b-PEG</td>
<td>( 4a-b-8 )</td>
<td>9.1</td>
<td>11.8</td>
</tr>
<tr>
<td>PMMA-b-PEG</td>
<td>( 4b-b-8 )</td>
<td>20.2</td>
<td>25.3</td>
</tr>
<tr>
<td>PS-b-PEG</td>
<td>( 5-b-8 )</td>
<td>7.2</td>
<td>7.5</td>
</tr>
<tr>
<td>PEG-b-PS-b-PEG</td>
<td>( 6-b-11-b-6 )</td>
<td>17.0</td>
<td>17.7</td>
</tr>
<tr>
<td>PMMA-b-PS</td>
<td>( 4c-b-10 )</td>
<td>19.6</td>
<td>17.8</td>
</tr>
</tbody>
</table>

[a] \( M_n \) calculations based on conversion measured by gas chromatography. The calculated \( M_n \) of the block copolymers formed is based on the \( M_n \)'s of the individual blocks.

[b] \( M_n \) calculation based on the \( M_n \) given for commercially available PEG.

PS-b-PEG (\( 5-b-8 \)) was synthesized by coupling of the same PEG 8 as stated above and \( \alpha \)-acetylene-\( \omega \)-bromo-polystyrene (5), as depicted in scheme 2.10.b. This reaction was conducted in THF at 35°C using CuI/DBU as well. Once again, completion of the reaction was determined by SEC (figure 2.5). Analogous to the higher molecular weight PMMA 4b, in case of the preparation of PS 5, probably termination reactions have occurred during the polymerization process, as was visualized by the shoulder peak at shorter retention time in the SEC chromatogram, illustrated in figure 2.5. Here, the terminated polymer chains appeared to have participated in the “click” reaction with PEG, as the SEC chromatogram of the product exhibited a shift of the shoulder peak towards higher molecular weight as well. This can be explained by the fact that, in this case, termination of growing polymer chains took place by combination of two active centers, which led to PS chains of twice the molecular weight that possess two acetylene end groups, stemming from the utilized initiator. This lead to the assumption that, owing to the still active acetylene functionality present, these polymers were coupled to PEG as well, yielding some PEG-b-PS-b-PEG impurities. The major product, nonetheless, was the desired PS-b-PEG diblock copolymer, as illustrated in figure 2.5 by the increase of the molecular weight in comparison to the starting materials (table 2.1).

It has to be emphasized that the small amounts of impurities found in the last two formed block copolymers were caused by side reactions which have occurred during the ATRP process and not by incomplete coupling reactions. As long as azide and acetylene
functionalities were present, the “click” coupling reactions appeared to proceed nearly quantitatively.

![Figure 2.5 SEC traces of the PS 5 and PEG 8 polymer modules and the subsequently formed PS-b-PEG diblock copolymer 5-b-8](image)

As described in the previous section, telechelic $\alpha,\omega$-diazido-polystyrene (11) was synthesized as well. Since both termini of the polymer embraced an azide moiety, performing a “click” coupling using an acetylene functional polymer should lead to the formation of an ABA type triblock copolymer. To create this structure, $\alpha$-methoxy-$\omega$-(4-pentynoyl)-poly(ethylene glycol) (6) was “clicked” to PS 11, applying the same conditions as used in the preceding coupling reactions, because it was proven that these “click” conditions worked well (scheme 2.10.c). To drive the reaction to completion, an excess of PEG 6 (1.23 equivalents) was used. Completion of the reaction, in this case, was established by measuring the disappearance of azide groups with FTIR (2094 cm$^{-1}$), in combination with a complete shift of the SEC trace to higher molecular weight (figure 2.6). As pointed out in table 2.1, no increase of the PDI was observed for the PEG-$b$-PS-$b$-PEG triblock copolymer compared to the telechelic PS building block. This means that the “click” reaction was complete, excluding the presence of PS and incomplete PS-$b$-PEG diblock copolymer, and the residual PEG chains were successfully removed by a washing step with MeOH.
Hitherto, the modular formation of block copolymers via “click” chemistry has been described in which the excess of one of the building blocks could be readily removed due to differences in solubility with respect to the formed block copolymers. Obviously, this is only an exception and, in most cases, purification of the block copolymer products cannot be fulfilled simply by a washing procedure. As aforementioned, the utilized acetylene and azide moieties are extremely stable and inert to most other functional groups, which means that after reaction the residual polymeric precursors still have reactive groups present that are available for a subsequent “click” reaction.

This remaining reactivity allows removal of the excess of polymer by a coupling reaction to a scavenger resin that, afterwards, can be removed easily by filtration. Therefore, an azide functionalized PS resin 12 was synthesized by substitution of the chloride functionality present in commercially available Merrifield resin using NaN\textsubscript{3} in DMSO at 60°C (scheme 2.11). After an extensive washing procedure, the presence of azide groups in the resin was confirmed by an azide stretch vibration (2094 cm\textsuperscript{-1}) present in the FTIR spectrum.

![Scheme 2.11](image)

**Scheme 2.11** Synthesis of azide functionalized PS scavenger resin 12. Reagents and conditions: i. NaN\textsubscript{3}, DMSO, 60°C, 48 h
In order to test the possibilities of exploiting this scavenger resin, α-acetylene-ω-bromo-poly(methyl methacrylate) (4c) and ω-azido-polystyrene (10) were coupled by a “click” reaction using 20 mol percent CuI/DBU in THF at 35°C (scheme 2.10.d). An excess of PMMA (1.2 equivalents) was used and completion of the reaction was assessed by the disappearance of the azide signal (2090 cm\(^{-1}\)) with FTIR. Afterwards, azidomethyl PS resin 12 was added (10 to 15 equivalents, based on the theoretical loading of the resin, with respect to residual PMMA) and the reaction proceeded for an additional 25 hours. After reaction, the scavenger resin was removed by a filtration step. As can be seen in the SEC chromatograms depicted in figure 2.7, the PMMA-\(b\)-PS block copolymer (4c-\(b\)-10) was formed and, moreover, no or minute amounts of residual PMMA were observed, from which the conclusion can be drawn that the purification methodology, concerning the “click” reaction onto a scavenger resin, was successful.

![SEC chromatograms](image)

**Figure 2.7 SEC chromatograms of the polymeric precursors PMMA 4c and PS 10 and the formed PMMA-\(b\)-PS (4c-\(b\)-10) diblock copolymer**

### 2.5. Conclusions

In this chapter, the successful application of “click” chemistry in the modular synthesis of AB and ABA type block copolymers has been demonstrated. Utilizing ATRP as the polymerization technique allowed the introduction of acetylene end-functionality by employing a functionalized initiator. The protection of the acetylene functionality, nonetheless, was required in order to circumvent interaction with the copper-catalyst during polymerization reactions, which, consequently led to quenching of the polymerization. Despite the fact that the polymerizations proceeded in a controlled...
fashion, partial loss of TMS protecting groups was observed possibly due to a nucleophilic attack of the ligand of the catalyst on the TMS group.

Another methodology for introducing functionality in polymers prepared by ATRP is via modification of the halogen end groups present after polymerization. Azide end groups were successfully introduced in mono- and bifunctional PS. Furthermore, this post-polymerization end group modification procedure was used to prepare acetylene and azide functionalized PEG in a controlled fashion.

The thus obtained building blocks were coupled by subjection to CuI and DBU, using THF as a solvent at 35°C. However, when polymers are being used, one always has to deal with a distribution of distinct molecular weights, which makes it difficult to work with equimolar ratios. Therefore, a small excess of one polymer module was used, which ensured complete reaction of the other polymeric precursor. For the block copolymers containing a PEG unit, an excess of this building block was used which could be readily removed by a washing step with MeOH. On the other hand, for the synthesis of the PMMA-\textit{b}-PS block copolymer, this method was not applicable, due to fact that both building blocks are insoluble in the polar solvent MeOH. Therefore, the acetylene functionality present in the residual PMMA was successfully exploited to couple these polymer chains to an azide functionalized scavenger resin, which subsequently was removed easily from the reaction mixture by a filtration step.

2.6. Experimental

2.6.1. Materials

Propargyl alcohol (Acros, 99%), 3-trimethylsilyl-2-propyn-1-ol (Acros, 99%), 2-bromoisobutyril bromide (Aldrich, 98%), 2-bromopropionyl bromide (Aldrich, 97%), 1-bromoethyl benzene (Acros, 97%), methyl methacrylate (MMA) (Aldrich, 99%), xylene (mixture of isomers) (Acros, >98%), tetrabutylammonium fluoride (TBAF) (Janssen Chimica, 1 M solution in THF), N,N,N’,N”,N”-pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), 2,2’-bipyridine (Aldrich, >99%), azidotrimethylsilane (Janssen Chimica, 97%), ethylene glycol (Acros, >99%), poly(ethylene glycol) methyl ether (PEG) (Aldrich, M=2000 g/mol), 4-pentynoic acid (Aldrich, 95%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (Aldrich, >98%), 4-dimethylaminopyridine (DMAP) (Acros, 99%), sodium azide (NaN₃) (Merck, >99%), CuI (Aldrich, 98%), 2-pyrindinecarboxaldehyde (Acros, 99%), \textit{n}-propylamine (Aldrich, >99%), 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU) (Acros, 98%), 2,5-dihydroxybenzoic acid (DHB) (Fluka, >99%) and Merrifield resin (Fluka, crosslinked with 2% DVB, 200-400 mesh, loading 1.0-1.5 mmol/g) were used as received. CuBr was purified by washing with glacial acetic acid three times and twice with diethyl ether.\textsuperscript{[95]} Toluene-4-sulfonyl chloride (TsCl) was dissolved in diethyl ether (Et₂O), washed with aqueous 10% NaOH and dried using Na₂SO₄ and, subsequently, crystallized by cooling in powdered dry ice. Triethyl amine (Et₃N) was distilled
under nitrogen from potassium hydroxide. Styrene and methyl methacrylate were distilled under reduced pressure. Tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone. Dichloromethane (CH₂Cl₂), ethyl acetate (EtOAc) and Et₂O were distilled under nitrogen from calcium hydride. Pyridine was distilled under reduced pressure from potassium hydroxide. N,N-dimethylformamide (DMF) was dried with magnesium sulfate and distilled under reduced pressure.

2.6.2. Instrumentation

Proton and carbon-13 nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker DPX300 spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane (δ = 0.00 ppm) for ¹H NMR spectra and relative to the solvent for ¹³C NMR spectra (δ = 77.16 ppm).

Infrared (IR) spectra were obtained using an ATI Matson Genesis Series FTIR spectrophotometer fitted with an ATR cell. Data are presented as the frequency of absorption (cm⁻¹).

Molecular weight distributions were measured using size exclusion chromatography (SEC) on a system equipped with a guard column and a PL gel 5 µm mixed D column (Polymer Laboratories) with differential refractive index and UV (254 nm) detection, using THF as an eluent at 1 mL/min and T = 35°C. Poly(methyl methacrylate) (PMMA) standards in the range 620 to 254,100 g/mol and polystyrene (PS) standards in the range of 580 to 377,400 g/mol were used to calibrate the SEC.

Gas chromatography (GC) measurements were conducted on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary column (HP1701, 25m x 0.32mm x 0.25 µm), using flame ionization detection.

Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV or permanganate reagent.

Column chromatography was performed using silica gel, Acros (0.035-0.070 mm, pore diameter ca. 6 nm), unless otherwise stated.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectra were measured on a Bruker Biflex III machine. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix. Samples were prepared by mixing 10 µL of a 40 mg/mL matrix solution, 10 µL of a 1 mg/mL polymer solution and 1 µL of a 5 mg/mL AgOTf solution. From this mixture 1 µL was spotted on a MALDI plate.

2.6.3. N-(n-propyl)-2-pyridylmethanimine[83] (2)

A solution of n-propylamine (3.91 g, 66.1 mmol) in Et₂O (2 ml) was added dropwise to a solution of pyridine-2-carboxaldehyde (3.24 g, 30.2 mmol) at 0°C. After complete addition of the amine, anhydrous magnesium sulfate was added and the formed slurry was stirred for two hours at room temperature. Completion of the reaction was determined using GC. The reaction mixture was passed through a filter to remove magnesium sulfate. The excess of n-propylamine and the solvent were removed in vacuo to give a gold colored yellow oil.

Yield: 4.32 g (96%); ¹H NMR (300 MHz, CDCl₃) δ 8.65 (m, 1H, pyridyl H6), 8.38 (m, 1H, pyridyl-C(=N-Pr)H), 8.05 (m, 1H, pyridyl H4), 7.71 (m, 1H, pyridyl H3), 7.30 (m, 1H, pyridyl H5), 6.35 (dt, 2H, J = 6.91 Hz, J = 1.50 Hz, CH=N-C₃H₇), 1.76 (sextet, 2H, J = 7.21 Hz, CH₂-CH₂-CH₃), 0.97 (t, 3H, J = 7.21 Hz, CH₂-CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 161.83 (pyridyl-C(=N-Pr)H), 154.78 (pyridyl C2), 149.50 (pyridyl C6), 139.81 (pyridyl C5), 121.27 (pyridyl C3), 63.40 (CH=N-C₃H₇), 23.96 (CH₂-CH₂-CH₃), 11.93 (CH₂-
Modular synthesis of AB and ABA type block copolymers

C

\^H_3); FTIR-ATR 3308, 3278, 3058, 3006, 2958, 2928, 2868, 1645 \(\nu\text{C=O, ester})\), 1584, 1563, 1467, 1437, 1381, 1333, 1290, 1225, 1139, 1044 cm\(^{-1}\)

2.6.4. \(\text{3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methylpropanoate (3)}\)

A solution of 2-bromoisobutyryl bromide (2.05 g, 8.93 mmol) in THF (20 mL) was added dropwise to a solution of 3-trimethylsilyl-2-propyn-1-ol (777 mg, 6.06 mmol) and Et\(_3\)N (905 mg, 8.95 mmol) in THF (40 mL) at 0°C. After complete addition, the reaction mixture was allowed to stir for one hour at room temperature. The excess of the acid bromide was quenched by addition of methanol (5 mL). The formed triethylammonium bromide was filtered off and the solvent was removed in vacuo. The crude product was dissolved in CH\(_2\)Cl\(_2\) and washed two times with a saturated ammonium chloride solution and two times with distilled water. The organic layer was dried with anhydrous magnesium sulfate and the solvent was removed in vacuo, yielding a yellow oil which was purified using flash chromatography (\(n\)-heptane/EtOAc 19:1). The product was isolated as a colorless oil which was dried under vacuum.

Yield: 1.56 g (93%); TLC: R\(_f\) (\(n\)-heptane/EtOAc 19:1) = 0.38; \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.75 (s, 2H, \(\equiv\text{C}-\text{H}_2\text{-O}\)), 1.95 (s, 6H, O\(_2\text{C-CH(H)}_3\)Br), 0.18 (s, 9H, \((\text{H}_3\text{C})_3\text{Si})\); \(^1\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 171.00 (O-\text{C}(=\text{O}))

2.6.5. \(\alpha\)-(trimethylsilyl acetylene)-\(\omega\)-bromo-poly(methyl methacrylate) (4a)

Typical polymerization procedure:

\(\text{CuBr (57.6 mg, 0.40 mmol)}\) was placed in a Schlenk tube which was fitted with a stopper, evacuated and back-filled with dry nitrogen. This procedure was repeated three times. After the evacuating cycles the stopper was replaced by a septum. Xylene (7 mL), \(N\)-(\(n\)-propyl)-2-pyridylmethanimine (2) (119 mg, 0.80 mmol) and \(\text{MMA (2.03 g, 20.3 mmol)}\) were added and the reaction mixture was cooled in an ice bath. Subsequently, 3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methylpropanoate (3) (111 mg, 0.40 mmol) was added and the reaction mixture was purged with dry nitrogen for five minutes and then placed in a statically controlled oil bath at 90°C. Samples were taken periodically for conversion analysis by GC. The polymerization was stopped after 270 minutes (81% conversion) by cooling and dilution with EtOAc. The catalyst was removed by column chromatography over a basic alumina column, using EtOAc as an eluent. The polymer was isolated by precipitation in \(n\)-heptane as a white solid, which was dried under vacuum.

Yield: 1.33 g (76%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.61 (br. s, \(\equiv\text{C}-\text{H}_2\text{-CO}_2\)), 3.63-3.48 (br. s, backbone \(\text{H}_3\text{C-O}_2\text{C}, \text{H}_3\text{C-O}_2\text{H}\)), 0.18 (s, \((\text{H}_3\text{C})_3\text{Si}-\equiv\))

2.6.6. \(\alpha\)-acetylene-\(\omega\)-bromo-poly(methyl methacrylate) (4a)

Typical procedure:

\(\alpha\)-(trimethylsilyl acetylene)-\(\omega\)-bromo-poly(methyl methacrylate) (1.27 g, 0.18 mmol) was dissolved in THF (15 mL) and TBAF (1.8 mL) was added. The reaction mixture was stirred for 18 hours at room
Chapter 2

In vacuo

The reaction mixture was concentrated in vacuo and the polymer was purified over a basic alumina column using EtOAc as the eluent. Subsequently, the polymer was precipitated in heptane, yielding a white solid which was dried under vacuum.

Yield: 1.05 g (83%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 4.63 (br. m, \(-\equiv\mathrm{C}-\mathrm{H}-\mathrm{O}_2\mathrm{C}\)), 3.63-3.48 (br. s, backbone \(\mathrm{H}_2\mathrm{C}_2\mathrm{O}_2\mathrm{C}\)), 2.45 (br. m, \(-\equiv\mathrm{C}\)), 2.11-0.73 (br. m, backbone \(\mathrm{CH}_3\), \(\mathrm{CH}_2\)); SEC (PMMA standards): \(M_\text{n} = 7.85 \text{ kg/mol}; M_\text{w}/M_\text{n} = 1.14\)

2.6.7. \(\alpha\)-(trimethylsilyl acetylene)-\(\omega\)-bromo-polystyrene

A Schlenk tube which was fitted with a stopper was loaded with CuBr (71.5 mg, 0.50 mmol), evacuated and back-filled with dry nitrogen. This procedure was repeated three times. Afterwards, the stopper was replaced by a septum. Anisole (0.6 mL), styrene (3.19 g, 30.6 mmol) and PMDETA (86.3 mg, 0.50 mmol) were added and the reaction mixture was stirred for 15 minutes to allow complex formation. Subsequently, the reaction mixture was cooled in an ice bath and purged with dry nitrogen for five minutes. The reaction mixture was placed in a statically controlled oil bath at 90\(^\circ\)C and 3-(1,1,1-trimethylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (3) (137 mg, 0.49 mmol) was added. Samples were taken periodically for conversion analysis by GC. The polymerization was stopped after 420 minutes (78% conversion) by cooling and dilution with CH\(_2\)Cl\(_2\). The reaction mixture was washed four times with a 0.055 M EDTA solution. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in MeOH, yielding a white solid which was dried under vacuum.

Yield: 2.14 g (82%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.37-6.34 (br. m, arom. H), 4.61 (br. m, \(-\equiv\mathrm{C}-\mathrm{H}-\mathrm{O}_2\mathrm{C}\)), 4.48 (br. m, CH\(_2\)-CH(Ph)-Br), 2.25-1.18 (br. m, backbone \(\mathrm{CH}_3\), \(\mathrm{CH}_2\)), 0.85 (br. m, \(\mathrm{O}_2\mathrm{C}-\mathrm{CH}(\mathrm{CH}_3)-\mathrm{CH}_2\)); SEC (PS standards): \(M_\text{n} = 4.82 \text{ kg/mol}; M_\text{w}/M_\text{n} = 1.23\)

2.6.8. \(\alpha\)-acetylene-\(\omega\)-bromo-polystyrene (5)

\(\alpha\)-(trimethylsilyl acetylene)-\(\omega\)-bromo-polystyrene (2.00 g, 0.38 mmol) was dissolved in THF (8.5 mL). Subsequently, TBAF (3.5 mL, 3.5 mmol) was added and the reaction mixture was stirred for 20 hours at room temperature. The reaction mixture was concentrated in vacuo and the polymer was precipitated in methanol. The product was isolated as a white solid which was dried under vacuum.

Yield: 0.83 g (87%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.37-6.34 (br. m, arom. H), 4.61 (br. m, \(-\equiv\mathrm{CH}_2\mathrm{O}_2\mathrm{C}\)), 4.48 (br. m, CH\(_2\)-CH(Ph)-Br), 2.25-1.18 (br. m, backbone \(\mathrm{CH}_3\), \(\mathrm{CH}_2\)), 0.85 (br. m, \(\mathrm{O}_2\mathrm{C}-\mathrm{CH}(\mathrm{CH}_3)-\mathrm{CH}_2\)); SEC (PS standards): \(M_\text{n} = 4.82 \text{ kg/mol}; M_\text{w}/M_\text{n} = 1.23\)

2.6.9. \(\alpha\)-methoxy-\(\omega\)-(4-pentynoyl)-poly(ethylene glycol) (6)

A solution of EDCI (92.4 mg, 0.48 mmol) in CH\(_2\)Cl\(_2\) (2 mL) was added dropwise to a solution of poly(ethylene glycol) methyl ether (800 mg, 0.40 mmol), 4-pentynoic acid (47.4 mg, 0.49 mmol) and DMAP (6.4 mg, 0.052 mmol) in CH\(_2\)Cl\(_2\) (4 mL) at -20\(^\circ\)C. After complete addition, the reaction mixture was allowed to stir for 20 hours at room temperature. The reaction mixture was washed twice with a 1 M sodium hydroxide solution and two times with distilled water. The organic layer
was dried with anhydrous magnesium sulfate and solvent was removed in vacuo. The product was isolated as a white solid which was dried under vacuum.

Yield: 0.69 g (83%); 1H NMR (300 MHz, CDCl3) δ 4.25 (t, J = 4.81 Hz, O-CH2-CH2-CO2), 3.69 (t, J = 4.81 Hz, O-CH2-CH2-CO2), 3.63 (s, O-(CH2)2-O), 3.37 (s, CH3-O), 2.61-2.46 (m, O-C-(CH2)2-≡), 1.99 (t, J = 2.41 Hz, H-≡); FTIR-ATR 3261 (νC≡H), 2880, 1960, 1727, 1467, 1338, 1282, 1238, 1143 cm⁻¹; SEC (PS standards): Mn = 2.72 kg/mol; Mw/Mn = 1.04; MALDI-ToF MS: matrix: DHB; m/z = 1835 ± 44.04 (38 repeating units + end groups + Na⁺)

2.6.10. α-methoxy-ω-tosyl-poly(ethylene glycol) (7)

Poly(ethylene glycol) methyl ether (2.00 g, 1.00 mmol) was dissolved in pyridine (10 mL) and, subsequently, TsCl (1.91 g, 10.0 mmol) was added. The reaction mixture was stirred for 20 hours at room temperature and then poured into cold distilled water. The product was extracted with CH2Cl2. The organic layer was washed twice with a cold 6 M hydrochloric acid solution and three times with cold distilled water. Afterwards, the organic layer was dried with anhydrous magnesium sulfate and the solvent was removed in vacuo. The product was recovered as a white solid.

Yield: 1.82 g (85%); 1H NMR (300 MHz, CDCl3) δ 7.77 (m, arom. H), 7.31 (m, arom. H), 4.14 (t, J = 4.80 Hz, CH2-CH2-OTs), 3.63 (s, O-(CH2)2-O), 3.37 (s, CH3-O); FTIR-ATR 2881, 2738, 2695, 1960, 1714, 1645, 1467, 1342, 1282, 1243, 1143, 1100, 1057 cm⁻¹; SEC (PS standards): 3.23 kg/mol; Mw/Mn = 1.04; MALDI-ToF MS: matrix: DHB; m/z = 1850 ± 44.04 (36 repeating units + end groups + Na⁺)

2.6.11. α-methoxy-ω-azido-poly(ethylene glycol) (8)

α-methoxy-ω-tosyl-poly(ethylene glycol) (7) (1.72 g, 0.80 mmol) was dissolved in DMF (10 mL) and sodium azide (650.3 mg, 10.00 mmol) was added. The reaction mixture was stirred for 22 hours at room temperature. CH2Cl2 (25 mL) was added and the reaction mixture was washed three times with cold distilled water, twice with cold 6 M hydrochloric acid solution and again two times with cold distilled water. The organic layer was dried with anhydrous magnesium sulfate and the solvent was removed in vacuo. The product was recovered as a white solid.

Yield: 1.32 g (82%); 1H NMR (300 MHz, CDCl3) δ 3.86 (t, J = 4.80 Hz, CH2-CH2-N3), 3.63 (s, O-(CH2)2-O), 3.37 (s, CH3-C); FTIR-ATR 2881, 2098 (νN3), 1960, 1463, 1342, 1100 cm⁻¹; SEC (PS standards): 3.18 kg/mol; Mw/Mn = 1.04; MALDI-ToF MS: matrix: DHB; m/z = 1824 ± 44.04 (38 repeating units + end groups + Na⁺)

2.6.12. 2-[(2-bromopropanoyl)oxy]ethyl-2-bromopropanoate (9)

A solution of 2-bromopropionyl bromide (6.0 mL, 55.0 mmol) in THF (25 mL) was added to a solution of ethylene glycol (1.58 g, 25.4 mmol) and Et3N (5.63 g, 55.7 mmol) in THF (75 mL) at 0°C. After complete addition of the acid bromide, the reaction mixture was allowed to stir for two hours at room temperature. MeOH (10 mL) was added to quench the excess of 2-bromopropionyl bromide. The formed triethylammonium bromide was filtered off and the solvent was removed in vacuo. The crude product was dissolved in CH2Cl2 and washed two times with a saturated ammonium chloride solution and two times with distilled water. The organic layer was dried with magnesium sulfate and solvent was removed in vacuo, yielding a
yellow oil which was purified using column chromatography (n-heptane/EtOAc 4:1). The product was isolated as a slightly yellow colored oil, which was dried under vacuum. Yield: 7.96 g (94%); TLC: R$_f$ (n-heptane/EtOAc 4:1) = 0.20; $^1$H NMR (300 MHz, CDCl$_3$) δ 4.42 (s, 4H, O-(CH$_2$)$_2$O), 4.39 (q, 2H, $^3$J = 6.99 Hz, 2x O$_2$C-CHBr-CH$_3$), 1.84 (d, 6H, $^3$J = 6.99 Hz, 2x O$_2$C-CHBr-CH$_3$); $^1$C NMR (75 MHz, CDCl$_3$) δ 170.11 (O-C(=O)), 63.18 (O-(CH$_2$)$_2$O), 39.69 (O$_2$C-CHBr-CH$_3$), 21.68 (O$_2$C-CHBr-CH$_3$); FTIR-ATR 2963, 2928, 1735 ($\nu$C=O, ester), 1446, 1377, 1333, 1212, 1148, 1066 cm$^{-1}$

2.6.13. $\omega$-bromo-polystyrene

CuBr (72.1 mg, 0.50 mmol) was placed in a Schlenk tube, which was evacuated and back-filled with dry nitrogen. The evacuating cycles were repeated three times. Anisole (0.6 mL), styrene (3.11 g, 29.9 mmol) and PMDETA (87.5 mg, 0.50 mmol) were added and the reaction mixture was stirred for 15 minutes and a homogeneous solution was obtained. The reaction mixture was cooled in an ice bath, 1-bromoethyl benzene (93.1 mg, 0.50 mmol) was added, and the mixture was purged with dry nitrogen for five minutes. The polymerization was started by placing the reaction mixture in a statically controlled oil bath at 90°C. The polymerization was monitored by periodical samples analysis by GC. The polymerization was stopped after 450 minutes (67% conversion) by cooling and dilution with CH$_2$Cl$_2$. The reaction mixture was washed with a 0.055 M EDTA solution for three times. The organic layer was dried using anhydrous magnesium sulfate and was concentrated in vacuo. The polymer was precipitated in MeOH and isolated as a white solid, which was dried under vacuum. Yield: 2.07 g (95%); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.36-6.38 (br. m, arom. H), 4.46 (br. m, CH$_2$-CH(Ph)-Br), 2.17-1.24 (br. m, backbone CH$_2$, CH$_3$), 1.11-1.02 (br, s, H$_3$C-CH(Ph)-CH$_2$); FTIR-ATR 3019, 2925, 2837, 2008, 1943, 1865, 1796, 1597, 1493, 1450 cm$^{-1}$; SEC (PS standards): $M_n$ = 3.66 kg/mol; $M_w/M_n = 1.15$

2.6.14. $\omega$-azido-polystyrene (10)

$\omega$-bromo-polystyrene (1.83 g, 0.50 mmol) was dissolved in THF (14 mL). Subsequently, azidotrimethylsilane (576 mg, 5.00 mmol) and TBAF (5.0 mL, 5.0 mmol) were added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the polymer was purified by precipitation in methanol. The product was isolated as a white solid which was dried under vacuum. Yield: 1.35 g (91%); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.36-6.38 (br. m, arom. H), 3.91 (br. m, CH$_2$-CH(Ph)-N$_3$), 2.17-1.24 (br. m, backbone CH$_2$, CH$_3$), 1.11-1.02 (br, s, H$_3$C-CH(Ph)-CH$_2$); FTIR-ATR 3019, 2925, 2837, 2090 ($\nu$N$_3$), 2008, 1943, 1865, 1796, 1597, 1493, 1450 cm$^{-1}$; SEC (PS standards): $M_n$ = 3.66 kg/mol; $M_w/M_n = 1.15$

2.6.15. $\alpha,\omega$-dibromo-polystyrene

A Schlenk tube was loaded with CuBr (143 mg, 1.00 mmol) and 2,2’-bipyridine (313 mg, 2.00 mmol), evacuated and back-filled with dry nitrogen. This evaporating cycle was repeated four times. Subsequently, anisole (5 mL) and styrene (10.35 g, 99.4 mmol) were added and the reaction mixture was placed in an ice bath.
2-[(2-bromopropanoyl)oxy]ethyl-2-bromopropanoate (9) (168 mg, 0.51 mmol) was added and the reaction mixture was purged with dry nitrogen for five minutes. The polymerization was started by placing the reaction mixture in a statically controlled oil bath at 90°C. Samples were taken periodically for conversion analysis by GC. The polymerization was stopped after 1380 minutes (64% conversion) by cooling and dilution with CH₂Cl₂. The reaction mixture was washed with a 0.055 M EDTA solution three times. The organic layer was dried with anhydrous magnesium sulfate and concentrated by rotary evaporation. The polymer was precipitated in MeOH, yielding a white solid which was dried under vacuum.

Yield: 6.11 g (90%); ¹H NMR (300 MHz, CDCl₃) δ 7.38-6.35 (br. m, arom. H), 4.42 (br. m, CH₂-C₆H₄-(Ph)-Br), 3.36 (br. m, O₂C-(CH₂)₂-CO₂), 2.25-1.18 (br. m, backbone CH₃, CH₂), 0.85 (br. m, O₂C-CH(C₆H₅)-CH₂); FTIR-ATR 3023, 2919, 2021, 1939, 1873, 1805, 1731, 1597, 1493, 1450 cm⁻¹; SEC (PS standards): Mₙ = 11.69 kg/mol; M_w/Mₙ = 1.14

2.6.16. α,ω-diazido-polystyrene (11)

α,ω-dibromo-polystyrene (1.03 g, 0.080 mmol) was dissolved in THF (4 mL). Subsequently, azidotrimethylsilane (39.1 mg, 0.34 mmol) and TBAF (0.35 mL, 0.35 mmol) were added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo and the polymer was precipitated in MeOH, yielding a white solid which was dried under vacuum.

Yield: 1.65 g (85%); ¹H NMR (300 MHz, CDCl₃) δ 7.38-6.35 (br. m, arom. H), 3.93 (br. m, CH₂-C₆H₄-(Ph)-N₃), 3.36 (br. m, O₂C-(CH₂)₂-CO₂), 2.25-1.18 (br. m, backbone CH₃, CH₂), 0.85 (br. m, O₂C-CH(C₆H₅)-CH₂); FTIR-ATR 3023, 2919, 2094 (νₕ), 2021, 1939, 1873, 1805, 1731, 1597, 1493, 1450 cm⁻¹; SEC (PS standards): Mₙ = 11.69 kg/mol; M_w/Mₙ = 1.14

2.6.17. azidomethyl polystyrene resin (12)

Merrifield resin (1.01 g, loading 1.0-1.5 mmol/g) was reacted with sodium azide (974 mg, 15.0 mmol) in DMSO at 60°C for 48 hours. The suspension was cooled to room temperature and filtered. Subsequently, the resin was washed extensively with methanol and CH₂Cl₂ to give azidomethyl polystyrene resin. FTIR-ATR 3053, 3022, 2915, 2846, 2094 (νₕ), 1940, 1867, 1798, 1715, 1598 1493, 1453 1262 cm⁻¹

2.6.18. poly(methyl methacrylate)-block-poly(ethylene glycol) (4a-b-8)

Typical procedure:
A Schlenk tube which was fitted with a stopper was loaded with α-acetylene-ω-bromo-poly(methyl methacrylate) (4a) (33.3 mg, 4.70 μmol) and α-methoxy-ω-azido-poly(ethylene glycol) (8) (11.5 mg, 5.70 μmol). Subsequently, the Schlenk tube was evacuated and back-filled with dry nitrogen. After repeating this evacuation cycle three times, the stopper was replaced by a septum, and THF (1.0 mL) was added. Next, 4.7 μL of a stock solution of CuI (1.0 M) and DBU (1.0 M) in THF was added. The reaction mixture was placed in a statically controlled oil bath at 35°C and stirred for 19 hours. The polymer was precipitated in MeOH, yielding a slightly green colored solid,
which was washed afterwards extensively with MeOH, in order to remove the excess of α-azido-ω-methoxy-poly(ethylene glycol). The product was dissolved in CH₂Cl₂ and washed with a 0.055 M EDTA solution. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated again in MeOH and isolated as a white solid, which was dried under vacuum.

Yield: 30.2 mg (71%); SEC (PMMA standards): \( M_n = 11.77 \text{ kg/mol; } M_w/M_n = 1.12 \)

2.6.19. polystyrene-block-poly(ethylene glycol) (5-b-8)

\[
\begin{align*}
\text{α-acetylene-ω-bromo-polystyrene (5)} \quad (31.2 \text{ mg, 6.03 } \mu\text{mol}) & \quad \text{and} \\
\text{α-methoxy-ω-azido-poly (ethylene glycol) (8)} \quad (14.8 \text{ mg, 7.12 } \mu\text{mol})
\end{align*}
\]

were placed in a Schlenk tube fitted with a stopper. The Schlenk tube was evacuated and back-filled with dry nitrogen. This evacuation cycle was repeated three times and the stopper was replaced by a septum and THF (1.5 mL) was added. Subsequently, 6 \( \mu\)L of a stock solution of CuI (1.0 M) and DBU (1.0 M) in THF was added and the reaction mixture was placed in a statically controlled oil bath at 35°C. The reaction mixture was stirred for 18 hours. The polymer was precipitated in MeOH and, subsequently, washed extensively with MeOH. The product was dissolved in CH₂Cl₂ and washed with a 0.055 M EDTA solution. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in MeOH, yielding a white solid which was dried under vacuum.

Yield: 34.3 mg (79%); SEC (PS standards): \( M_n = 7.53 \text{ kg/mol; } M_w/M_n = 1.17 \)

2.6.20. poly(ethylene glycol)-block-polystyrene-block-poly(ethylene glycol) (6-b-11-b-6)

\[
\begin{align*}
\text{α,ω-diazido-polystyrene (11)} \quad (44.6 \text{ mg, 3.47 } \mu\text{mol}) & \quad \text{and} \\
\text{α-methoxy-ω-(4-pentynoyl)-poly(ethylene glycol) (6)} \quad (17.7 \text{ mg, 8.51 } \mu\text{mol})
\end{align*}
\]

were loaded into a Schlenk tube which was fitted with a stopper. The Schlenk tube was evacuated and back-filled with dry nitrogen. After repeating this evacuation cycle three times, the stopper was replaced by a septum, and THF (1.0 mL) was added. Next, 3.5 \( \mu\)L of a stock solution of CuI (1.0 M) and DBU (1.0 M) in THF was added. The reaction mixture was placed in a statically controlled oil bath at 35°C and stirred for 18 hours. The product was precipitated in MeOH and washed extensively afterwards with MeOH. The polymer was dissolved in CH₂Cl₂ and washed with a 0.055 M EDTA solution. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. Subsequently, the polymer was precipitated in MeOH, yielding a white solid which was dried under vacuum.

Yield: 50.2 mg (82%); SEC (PS standards): \( M_n = 17.69 \text{ kg/mol; } M_w/M_n = 1.13 \)
2.6.21. **polystyrene-block-poly(methyl methacrylate) (4c-b-10)**

A Schlenk tube which was fitted with a stopper was loaded with α-acetylene-ω-bromo-poly(methyl methacrylate) (4c) (2.41 g, 0.16 mmol), ω-azido-polystyrene (10) (541 mg, 0.13 mmol) and CuI (7.8 mg, 0.041 mmol), and subsequently evacuated and back-filled with dry nitrogen. This procedure was repeated three times. After the evacuating cycles, THF (10 mL) and DBU (6.1 mg, 0.040 mmol) were added and the reaction mixture was placed in a statically controlled oil bath at 35°C for 18 hours. Next, azidomethyl polystyrene resin 12 (302 mg, theoretical loading 0.3-0.5 mmol) was added and the reaction mixture was stirred for 25 hours at room temperature. The resin was filtered off and the reaction mixture was dissolved in CH₂Cl₂ and washed with a 0.055 M EDTA solution. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in methanol yielding a slightly blue colored solid. The polymer was recovered as a white solid which was dried under vacuum.

Yield: 2.25 g (89%); SEC (PS standards): $M_n = 17.78$ kg/mol; $M_w/M_n = 1.13$

### 2.7. References

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The “click” reaction between azides and terminal acetylenes was exploited to synthesize ABC type triblock copolymers. In order to prepare a polymer composed of three different polymer building blocks, a strategy was required in which a heterotelechelic B block was employed, of which both azide and acetylene end groups could react independently of each other in two consecutive block copolymer “click” reactions. During the first “click” reaction, the acetylene moiety therefore had to be fully protected, to prevent reaction with the azide groups already present in the B block which would lead to linear chain extended or cyclic polymers. The synthesis of heterotelechelic polymers containing both azide and protected acetylene termini was accomplished by adopting atom transfer radical polymerization (ATRP), which allows the introduction of end-functionality by employing functional initiators as well as by modification of the halogen end groups present after polymerization. Polystyrene (PS), poly(tert-butyl acrylate) (PtBA) and poly(methyl acrylate) (PMA) were prepared by ATRP, using an acetylene functionalized initiator which was protected with a triisopropyl silyl (TIPS) group. After polymerization, azide moieties were introduced at the other termini by substitution of the halogen end groups present after polymerization. A PMA-b-PS-b-PtBA triblock copolymer was synthesized in a modular fashion by conducting two successive “click” reactions. Successful formation of this ABC type triblock copolymer was determined by size exclusion chromatography (SEC). Furthermore, the excess of polymers used was removed by reduction of residual azide moieties, which allowed facile purification by column chromatography.
3.1. Introduction

In the preceding chapter, the modular synthesis of AB and ABA type block copolymers is described.[1] As a coupling strategy the copper(I)-catalyzed 1,3-dipolar cycloaddition was employed.[2] This most pronounced example of a “click” reaction[3] proved to be suitable for the connection of polymer modules via their end groups, owing to the high efficiency of the reaction. Moreover, employing atom transfer radical polymerization (ATRP) allowed the quantitative introduction of the required azide and terminal acetylene moieties by adopting a post-polymerization end group modification procedure and the use of a functional initiator, respectively.

In extension, by applying both methodologies onto one single polymer chain, heterotelechelic polymers bearing two distinct end-functionalities can be prepared. Such heterotelechelic polymers containing both terminal acetylene and azide moieties have been utilized to linearly extend polymers[4,5] and for the synthesis of cyclic polystyrene (PS),[6] by the application of “click” chemistry, as illustrated in scheme 3.1.

Scheme 3.1 Representation of the employment of α-acetylene-ω-azido-polystyrene to perform either a linear chain extension[4,5] (a) or a cyclization reaction[6] (b) using “click” chemistry

In this chapter the modular synthesis of block copolymers via “click” coupling of azide and acetylene precursor blocks will be extended by exploiting α-acetylene-ω-azide functionalized polymers. In this case, heterotelechelic polymers containing both protected acetylene and azide end-functionalities will be used, thereby opening up possibilities to functionalize polymer chains in an asymmetrical fashion, as depicted in figure 3.1. Therefore, first a “click” reaction will be performed on the azide terminus of the polymer. The presence of a protective group on the acetylene functionality will prevent it from participating in this reaction. After removal of the protective group, a second “click”
coupling can be enforced on the acetylene moiety. This methodology of conducting two successive “click” reactions onto a single polymer enables the modular synthesis of ABC-type triblock copolymers. It has to be emphasized that complete protection of the acetylene moiety is demanded, otherwise side reactions as linear chain extension and cyclization (scheme 3.1) are likely to occur. Moreover, these linearly chain extended and cyclic polymers are difficult to separate from the desired block copolymers.

**Figure 3.1** Schematic illustration of the modular synthesis of an ABC-type triblock copolymer via consecutive “click” couplings, starting with a heterotelechelic polymer module bearing both an acetylene functionality with a protective group (PG) attached and an azide moiety. After removal of the protective group, the acetylene group is available for a second “click” reaction.

Recently, Tunca *et al.* prepared ABC-type triblock copolymers in a modular fashion as well by simultaneous attachment of two polymer chains to a core B-block, performing both a Diels-Alder and a “click” reaction (scheme 3.2). The advantage is that, in this case, triblock copolymers can be synthesized in one pot. Executing the Diels-Alder reaction, however, required elevated temperatures, which may increase the probability of side-reactions to occur. The in this chapter described strategy of utilizing solely “click” chemistry, on the other hand, generally can be carried out at room temperature thereby reducing the likelihood of side-reactions. Moreover, when this one-pot methodology is extended to the preparation of polymer biohybrids, these elevated temperatures are not applicable, since most biomolecules cannot withstand high temperatures.
The following section deals with the synthesis of the required heterotelechelic polymer building blocks, terminated with both a protected acetylene and an azide group. This will be followed by a discussion of the usage of these heterotelechelic building blocks in the modular formation of ABC-type block copolymers utilizing “click” coupling reactions. First, the optimization of “click” chemistry conditions is described, which subsequently were adopted for the asymmetric functionalization of heterotelechelic polymers, eventuating in the synthesis of triblock copolymers.

3.2. Preparation of heterotelechelic polymers containing protected acetylene and azide end-functionality

The introduction of acetylene end-functionality in polymers was previously discussed in section 2.2. There, well-defined polymers were synthesized using ATRP, allowing the introduction of end group functionality via the initiator from which the polymer chains were grown. In order to circumvent complexation with the copper-catalyst during polymerization, trimethylsilyl (TMS) protected acetylene functionalized initiator 1 was used (scheme 3.3). However, although the polymerization reactions proceeded in a controlled fashion, $^1$H NMR and matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) spectra of the obtained polymers revealed the loss of the TMS protective group up to 70 percent in case of employing a 1:1 complex of CuBr and $N,N,N',N',N''$-pentamethyldiethylenetriamine (PMDETA) as the ATRP catalyst. Conversely, polymerizations performed with a complex of CuBr and two equivalents of...
N-(n-propyl)-2-pyrindylmethanimine displayed only an approximate 15 percent loss of the TMS group (scheme 3.3). A conceivable side reaction, therefore, can be nucleophilic attack of a nitrogen atom from the complexed ligand on the TMS group, since PMDETA is more nucleophilic in comparison to N-(n-propyl)-2-pyrindylmethanimine. In order to see if this side reaction could be suppressed completely, the even less nucleophilic ligand 2,2'-bipyridine (bpy) was tested as well in a polymerization of styrene, utilizing the TMS protected acetylene functionalized initiator 1, as depicted in scheme 3.3. Despite the fact that the employment of bpy suppressed the deprotection reaction substantially, yet 10 percent of the TMS groups was still removed.

Scheme 3.3 ATRP reactions performed with TMS protected acetylene functionalized initiator 1 applying different catalyst systems. The loss of the TMS protective groups was estimated from 1H NMR spectra of the obtained polymers by comparison of the TMS signals with those stemming from the initiator moiety.

As noted before, the complete protection of acetylene end groups is a prerequisite. Therefore, as a next step in eliminating this undesirable side reaction, the TMS protective group was replaced by a more stable triisopropyl silyl (TIPS) group. TIPS protected
acetylene functionalized initiator 2 was synthesized starting from commercially available propargyl alcohol (scheme 3.4). First, the acetylene moiety was deprotonated using the strongly basic Grignard reagent EtMgBr, after which the thus formed acetylide anion was reacted with TIPS-Cl.[8,9] This reaction required at least two equivalents of EtMgBr because the hydroxy group reacted with the Grignard reagent as well. The hydroxyl function, nevertheless, was recovered during an acidic work up procedure. Although the TIPS protection step did not go to completion, as determined by TLC and 1H NMR, the subsequent esterification step was conducted without further work-up due to purification difficulties. However, after reaction with 2-bromoisobutyryl bromide, the unprotected side product was readily removed by column chromatography, yielding the pure TIPS-protected acetylene functionalized initiator 2.

Scheme 3.4 Synthesis of TIPS protected acetylene functionalized initiator 2. Reagents and conditions: i. EtMgBr, THF; rt.→reflux, 18 h; ii. TIPS-Cl, THF, rt.→reflux, 5 h; iii. 2-bromoisobutyryl bromide, Et3N, THF, 0°C→rt., 2 h, 60% (overall)

Subsequently, the TIPS-acetylene initiator 2 was utilized for the ATRP of styrene (St), tert-butyl acrylate (tBA) and methyl acrylate (MA), as represented in scheme 3.5. All polymerizations were performed using a stoichiometric complex of CuBr and PMDETA as a catalyst. The ATRP reactions of styrene and methyl acrylate were conducted in bulk, whereas tert-butyl acrylate was polymerized employing acetone as a solvent. In all cases, anisole was added as an internal standard to enable monitoring of the reaction. This was done by taking samples regularly for analysis by gas chromatography (GC) or by 1H NMR, regarding the polymerization of tert-butyl acrylate. As shown in figure 3.2, the performed polymerizations of styrene and tert-butyl acrylate exhibited first order reaction kinetics, which is a feature of a controlled polymerization. Accordingly, the PDIs, as measured with size exclusion chromatography (SEC), were reasonably low (Mw/Mn ≤ 1.24), as depicted in table 3.1. The kinetics of the ATRP of methyl acrylate, however, deviated slightly from first order behavior. Nevertheless, the obtained PDI was low (Mw/Mn = 1.18). Furthermore, for the polymerization of styrene, the initiator efficiency (Ieff) was determined to be 0.99, which implies that virtually all initiator molecules actually started a polymerization reaction. This Ieff was calculated from the ratio of the degree of polymerization (DP) obtained by GC compared to the value obtained by 1H NMR. More
importantly, the acquired MALDI-ToF MS and \(^1\)H NMR spectra of the polymers displayed no loss of the TIPS protecting group during polymerization. In the \(^1\)H NMR spectra, the correct ratio of the signals originating from the end group protons and the initiating moiety in comparison to those stemming from the TIPS group was observed, as can be seen for PS in figure 3.3. From these results it can be concluded that the TIPS group is significantly more stable under the applied ATRP conditions compared to the TMS group. Most likely, this can be attributed to the more bulky character of the TIPS group.

**Scheme 3.5** Introducing TIPS protected acetylene functionality in polymers via ATRP adopting functionalized initiator 2
Now that fully protected acetylene end-functionalized polymers were prepared, the halogen terminus, which is present after ATRP, was replaced for an azide functionality via a nucleophilic substitution reaction. In the previous chapter, azide functionality was introduced in PS by substitution of the bromide end groups using azidotrimethylsilane (Me₃Si-N₃) and tetrabutylammonium fluoride (TBAF) in THF as a solvent (section 2.3.2).[10-12] The advantage of applying these conditions was that the use of DMF as a solvent can be circumvented, whereas for the use of sodium azide (NaN₃) DMF as a solvent is required. However, the previous used conditions were not suitable now, because subjecting the TIPS-acetylene functionalized polymers to TBAF would lead to deprotection of the acetylene moiety, since these are generally employed conditions for the removal of silyl protecting groups. For this reason, azide end-functionality was introduced using NaN₃ in DMF, as depicted in scheme 3.6.
Modular synthesis of ABC type block copolymers by asymmetric functionalization of polymers

Scheme 3.6 Preparation of α-(triisopropyl acetylene)-ω-azide functionalized polymers by nucleophilic substitution of bromide end groups present after performing ATRP. Reagents and conditions: i. NaN₃, DMF, rt., overnight, 96% (6a), 89% (6b), 78% (7)

Formation of the heterotelechelic polymers α-(triisopropyl acetylene)-ω-azido-polystyrene (6a, b), and α-(triisopropyl acetylene)-ω-azido-poly(methyl acrylate) (7) was confirmed by a complete upfield shift of the protons adjacent to the end groups (from δ 4.48 to 3.92 ppm for 6a and 6b, and from δ 4.32 to 3.96 ppm for 7) in ¹H NMR spectra (figure 3.3), and the presence of azide signals in FTIR spectra (2094 cm⁻¹ for 6a and 6b, and 2111 cm⁻¹ for 7). Additionally, end group analysis of the MALDI-ToF MS spectra confirmed the presence of azide end groups as well as the TIPS protected acetylene units.

Figure 3.3 300 MHz ¹H NMR spectrum of α-(triisopropylsilyl acetylene)-ω-azido-polystyrene (6a). The ratio of the protons indicate complete protection of the acetylene moiety. Moreover, the proton adjacent to the end group has shifted upfield, implying successful introduction of azide end-functionality
In order to form block copolymers in a modular fashion applying “click” chemistry, obviously polymer building blocks bearing free acetylene groups are required as well. Therefore, the TIPS group of \( \alpha \)-(triisopropylsilyl acetylene)-\( \omega \)-bromo-poly(tert-butyl acrylate) (4) was removed using equal conditions as applied for the TMS protected polymers (see section 2.2), viz. by treatment with TBAF using THF as a solvent (scheme 3.7). Successful removal of the TIPS group was determined by the complete disappearance of the resonance signals stemming from the isopropyl protons in the \(^1\)H NMR spectrum, along with an observed minuscule difference in elution behavior on TLC compared to the protected polymer 4 (\(R_f = 0.92\) for 8 and \(R_f = 0.95\) for 4, using CH\(_2\)Cl\(_2\)/MeOH 95:5 as an eluent).

![Scheme 3.7 Deprotection of the acetylene end group of \( \alpha \)-(triisopropylsilyl acetylene)-\( \omega \)-bromo-poly(tert-butyl acrylate) (4). Reagents and conditions: i. TBAF, THF, rt., 18 h, 80%](image)

The thus synthesized heterotelechelic polymer building blocks containing both azide and TIPS protected were utilized in order to prepare ABC-type triblock copolymers in a modular fashion. This will be discussed in the following sections.

### 3.3. Modular synthesis of ABC-type triblock copolymers using “click” chemistry

Since it was found that the “click” conditions used in the previous chapter did not always give optimal results, better functioning reaction conditions had to be found for performing “click” reactions in organic media. The most logical strategy to adopt is varying the copper(I)-catalyst required to execute “click” chemistry. The research carried out with respect to finding better copper(I)-catalysts is discussed in the following section.

#### 3.3.1. Dual catalyst systems for ATRP and “click” reaction

As aforementioned, the “click” reaction between azides and terminal acetylene actually is a Huisgen 1,3-dipolar cycloaddition, which is enhanced by utilizing copper(I)-catalysis. The addition of a copper(I)-source tremendously increases the reaction rate and quantitative yields can be obtained. Moreover, the reaction becomes regiospecific, i.e. the 1,4-regioisomer is obtained exclusively, whereas the thermal 1,3-dipolar cycloaddition
yields the 1,5-regiosomer as well.\textsuperscript{[2]} In previous research it was shown that for instance polytriazoles\textsuperscript{[13]} and bipyridines\textsuperscript{[14]} are potent ligands to be used in copper-catalyzed “click” reactions.

Analogous to “click” chemistry, in ATRP copper(I)-catalysis is utilized as well. As can be seen in chapter 1 (scheme 1.5), during polymerization the catalyst is in dynamic equilibrium between a copper(I) and copper(II) oxidation state. However, in order to obtain a controlled polymerization process, it mainly has to be present in the copper(I) oxidation state.\textsuperscript{[15-17]} As a consequence, a vast amount of research in the field of ATRP has been directed towards the discovery of new active ligands which stabilize copper(I) species. One class of active ATRP copper(I)-catalysts are based on linear, branched or cyclic polyamines.\textsuperscript{[18-20]}

The fact that both in ATRP and “click” chemistry copper(I)-catalysis is employed, gave rise to the idea to explore the possibilities of using ATRP catalyst systems in “click” reactions between acetylenes and azides. The ligands PMDETA and Me\textsubscript{6}TREN (12), which from ATRP are known to be active copper(I)-catalysts, were adopted to perform test “click” reactions between equimolar amounts of 3-azido-1-propanol (9) and methyl propiolate (scheme 3.8). First, complexes of CuI and CuBr with PMDETA were utilized as catalysts in “click” reactions conducted in THF and DMF at room temperature. The reactions all were performed under an argon atmosphere with the exclusion of oxygen, in order to prevent oxidation of the copper(I)-species. In all four cases, after 18 hours of reaction, complete disappearance of both substrates was observed with thin layer chromatography using CH\textsubscript{2}Cl\textsubscript{2}/MeOH (95:5) as an eluent. The applied CuBr/PMDETA catalyst yielded exclusively the 1,4-regiosomer 10 in both THF and DMF as solvents, as observed with \textsuperscript{1}H NMR. However, utilizing CuI/PMDETA as a catalyst, led to the formation of 1,4-regiosomer 10 accompanied by 1,5 regiosomer 11. The presence of the 1,5-regiosomer 11 was determined with \textsuperscript{1}H NMR spectroscopy, by the presence of two signals stemming from both distinct 1,2,3-triazole protons ($\delta = 8.18$ for 10 and $\delta = 8.26$ for 11). From the \textsuperscript{1}H NMR spectra, the amount of 11 was calculated to be approximately 15 percent for the reactions conducted in both solvents. A possible explanation can be that CuI is labile and is oxidized readily by homolytic cleavage of the Cu-I bond, especially when a strong electron donating ligand as PMDETA is used, which stabilizes the copper(II)-species. This oxidation may have led to loss of regiospecificity in the “click” reaction.
Next to PMDETA, the tetradentate ligand Me₆TREN (12) was used in the series of test reactions. When THF was used as a solvent, a precipitate was formed at the moment of addition of methyl propiolate to a solution containing the catalyst. This may indicate that the formed copper-acetylide was insoluble, even at elevated temperatures. As a result, no reaction using CuBr/Me₆TREN was observed in THF. Using DMF as a solvent, on the other hand, complete formation of solely the 1,4-regioisomer 10 was accomplished at room temperature, as confirmed with TLC and ¹H NMR (scheme 3.8).

From the results described above, the conclusion can be drawn that complexes of CuBr with PMDETA and Me₆TREN are suitable for performing “click” reactions. Since from the field of ATRP is known that the two polyamine ligands stabilize the copper(I)-species, these complexes are potentially, highly active catalyst. Hence, these catalysts were used for the “click” functionalization of heterotelechelic polymers, as discussed in the next sections. Meanwhile, other ATRP catalyst have proven to be active catalysts in the “click” reaction between azides and terminal acetylenes.[4,5,21] An accessory advantage of this type of dual catalysts is that they can be utilized for performing ATRP and subsequent “click” reactions in one-pot.[22]
3.3.2. Asymmetric functionalization of polystyrene using “click” chemistry

The catalyst systems found in the previous section now were applied for the asymmetric functionalization of polymers, i.e. first performing a “click” reaction on the azide terminus and, after deprotection of the acetylene moiety, conducting a second “click” coupling. As discussed in section 3.1, this methodology of enforcing two consecutive “click” reactions allows the modular build-up of ABC-type triblock copolymers (see figure 3.1).

Prior to the synthesis of these triblock copolymers, the scope of this sequential method for functionalizing both polymer end groups with distinct substrates was examined with small organic substrates. Therefore, propargyl alcohol and 2-azidoacetic acid (13) were used to functionalize the azide and acetylene termini, respectively, of α-(triisopropyl acetylene)-ω-azido-polystyrene (6b), as depicted in scheme 3.9. In the first step, a “click” reaction was performed between 6b and propargyl alcohol using CuBr/PMDETA as a catalyst. To drive the reaction to completion a 10-fold excess of propargyl alcohol was used which could be removed by precipitation of the polymer in methanol and extensive washing afterwards. Subsequently, in order to liberate the acetylene functionality, hence making it available for reaction, the polymer TIPS-6b-OH was subjected to a solution of TBAF in THF which removed the TIPS protective group. Complete disappearance of the signal of the triisopropyl protons in the 1H NMR spectrum confirmed completion of this deprotection step. To the thus formed polymer bearing a free acetylene moiety (H-6b-OH), 2-azidoacetic acid (13) was coupled using equal conditions pertaining to the first “click” reaction. Likewise, a 10-fold excess of 2-azidoacetic acid (13) was used to ensure complete functionalization, which could be removed by a washing step with methanol as well. It has to be noted that the “click” reactions were performed under an argon atmosphere with the exclusion of oxygen, thereby preventing oxidation of the copper(I) species.
Scheme 3.9 Asymmetric functionalization of PS 6b by performing successive “click” reactions on both end groups of the polymer; Reagents and conditions: i. CuBr, PMDETA, propargyl alcohol, THF, rt., 18 h, 93%; ii. TBAF, THF, rt., 17 h, 95%; iii. CuBr, PMDETA, 2-azidoacetic acid (13), THF, rt., 18 h, 82%

Formation of the hydroxy functionalized PS TIPS-6b-OH was visualized by thin layer chromatography (TLC), accompanied by the disappearance of the azide signal in the FTIR spectrum and the presence of signals stemming from the newly formed end group in the $^1$H NMR spectrum. The subsequent removal of the TIPS protective group was quantitative according to the complete disappearance of the signals of the isopropyl protons in the $^1$H NMR spectrum of H-6b-OH. Completion of the second “click” reaction was also visualized by TLC, owing to the increased polarity of HOOC-6b-OH. Furthermore, additional signals of the carboxylic end group were observed in the $^1$H NMR spectrum. Moreover, as can be seen in table 3.1, no increase of the polydispersity index (PDI) was observed in the SEC traces after the performed “click” reactions, implying that no linear chain extension of PS has occurred. Therefore, the conclusion can be drawn that the TIPS protecting group fully prevented reaction of the acetylene moiety under the applied “click” conditions. Additionally, as illustrated in figure 3.4, the MALDI-ToF MS spectra displayed formation of the desired products. The difference in mass exactly corresponded to the loss of the TIPS protective group in combination with the attachment of azidoacetic acid. The observation of a second distribution in the MALDI-ToF MS spectrum of HOOC-6b-OH was in agreement with the complexation of potassium ions in stead of silver ions, which were added as an ionizing agent during sample preparation. By examination of the MALDI-ToF MS spectrum of TIPS-6b-OH, however, the presence of a small second distribution can be observed (figure 3.4). The mass corresponds to the loss of hydrogen bromide during the polymerization process. By kinetic modeling studies$^{[23]}$ and experimentally with $^1$H NMR$^{[24]}$ and two-dimensional
liquid chromatography, it has been shown that for the ATRP of styrene a minute amount of elimination of hydrogen bromide can occur, especially at high conversions, which probably is catalyzed by the Cu(II)-species of the ATRP catalyst. This loss of bromide end groups eventually entails incomplete introduction of azide functionality. With this in mind, the ATRP of styrene was stopped at relatively low conversion (62%). Nonetheless, although the polymerization process was stopped in an early stage, still a small signal of terminated PS was present in the MALDI-ToF MS spectrum. As can be seen, the peaks of this impurity seem to be higher at low molecular weight and are hardly present at high molecular weight. Probably, at low conversions some termination reactions occurred. The amount of impurities could not be calculated, since they were not observed neither with TLC nor in the 1H NMR spectrum. At least this implies that only trace amounts of terminated PS were present. Nevertheless, this loss of functionality is a drawback of the post-polymerization end group functionalization method and inherent to the ATRP process. This problem of elimination reactions has no influence on the other methodology of introduction of end group functionality, i.e. by applying functional initiators. This method leads to quantitative introduction of functionality, given that no other side reactions occur.

![Figure 3.4 MALDI-ToF MS spectra of α-(triisopropyl acetylene)-ω-((1,2,3-triazol-4-yl)methanol)-polystyrene TIPS-6b-OH and α-(2-(1,2,3-triazol-4-yl)acetic acid)-ω-((1,2,3-triazol-4-yl)methanol)-polystyrene HOOC-6b-OH](image)

3.3.3. Modular synthesis of ABC-type triblock copolymers

The experiments described in the previous section showed the possibilities of conducting two consecutive “click” reactions onto one single polymer chain by exploiting
a TIPS protecting strategy. This paved the way to modularly synthesize ABC triblock copolymers utilizing a similar strategy. In order to achieve this, the prepared heterotelechelic building blocks, as discussed in section 3.2, were used.

Initial attempts to couple \( \alpha \)-(triisopropyl acetylene)-\( \omega \)-azido-polystyrene (6a) and \( \alpha \)-acetylene-\( \omega \)-bromo-poly(tert-butyl acrylate) (8) using CuBr/PMDETA as the catalyst in THF, however, did not result in complete conversion, even though a 1.5-fold excess of the azide functionalized PS 6a was utilized. After a reaction time of 20 hours, the SEC chromatogram of the product still displayed the presence of unreacted acetylene functionalized P\(_{\text{tBA}}\) 8. Since the \( ^1 \)H NMR spectrum of 8 pointed out complete deprotection of the acetylene moiety, apparently the reaction conditions were not optimal. Using elevated temperatures even up to reflux and DMF as a solvent did not have the desired effect. Therefore, the catalyst system was replaced for CuBr/Me\(_6\)TREN, as discussed in section 3.3.1.

Accordingly, a “click” reaction between the azide functionalized PS 6a and the acetylene functionalized P\(_{\text{tBA}}\) 8 was conducted in DMF with the CuBr/Me\(_6\)TREN
catalyst system, as depicted in scheme 3.10. In order to drive the reaction to completion, a slight excess (1.13 equivalents) of PS 6a was used. In order to remove this excess, first the residual azide groups were transformed into amine functionalities via a Staudinger reduction by the addition of triphenylphosphine (PPh₃). Due to this end group transformation, the residual PS had a completely different elution behavior on silica. Complete reduction of all residual azide groups was visualized using TLC by the disappearance of PS 6a in combination with the presence of a ninhydrin positive spot at the baseline, indicating that amine groups were present. This difference in elution behavior allowed facile purification of the formed diblock copolymer by column chromatography. CH₂Cl₂/MeOH (95:5) was used as an eluent, which caused the block copolymer to elute with an Rf value of 0.98, whereas the amine functionalized PS did not elute at all. Formation of the TIPS protected acetylene functionalized PS-b-PtBA diblock copolymer TIPS-6a-b-8 was observed by a complete shift towards higher molecular weight in the SEC chromatogram (figure 3.5). Moreover, as depicted in table 3.1, no increase in the PDI was observed for the formed block copolymer with respect to the individual polymer modules, indicating that the “click” reaction went to completion, along with the successful removal of the excess of PS 6a. In addition, this implies as well that the protected acetylene group did not participate in the “click” reaction, thereby forming linearly extended polymers. Furthermore, a comparison of the benzylic protons to those stemming from the complete backbone and the tert-butyl esters in the ¹H NMR spectrum roughly indicated that both building blocks were equally present. By keeping in mind that the signal of the benzylic protons of PS was caused by five protons of every repeating unit, the contribution of the PS backbone protons on the signal stemming from the complete backbone of the block copolymer combined with the pendant tert-butyl groups could be calculated. Hence, the total amount of protons stemming from PtBA was known as well. This PtBA signal was caused by 12 protons for every repeating unit and, therefore, the DP could be calculated. This amounted to approximately 19, which is in good agreement with the DP of the individual PtBA block, calculated from the ¹H NMR spectrum as well.
Figure 3.5 SEC traces of the PS 6a and PtBA 8 polymer precursors and the subsequently formed TIPS protected acetylene functionalized PS-b-PtBA diblock copolymer TIPS-6a-b-8

Subsequently, the acetylene end-functionality of the block copolymer was liberated in order to allow attachment of a third block. This was established by treatment with TBAF, as depicted in scheme 3.10. Successful formation of the unprotected acetylene functionalized PS-b-PtBA diblock copolymer H-6a-b-8 was determined by the complete disappearance of the TIPS signal in the $^1$H NMR spectrum of the product.

Table 3.1 SEC data of used polymer building blocks and block copolymers formed by “click” chemistry

<table>
<thead>
<tr>
<th>polymer</th>
<th>$M_n$, SEC (kg/mol)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-bromo-polystyrene</td>
<td>3a</td>
<td>5.8</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-bromo-polystyrene</td>
<td>3b</td>
<td>7.0</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-bromo-poly(tert-butyl acrylate)</td>
<td>4</td>
<td>3.6</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-bromo-poly(methyl acrylate)</td>
<td>5</td>
<td>7.2</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-azido-polystyrene</td>
<td>6a</td>
<td>5.8</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-azido-polystyrene</td>
<td>6b</td>
<td>7.0</td>
</tr>
<tr>
<td>$\alpha$-acetylene-$\omega$-bromo-poly(tert-butyl acrylate)</td>
<td>8</td>
<td>3.6</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-azido-poly(methyl acrylate)</td>
<td>7</td>
<td>7.2</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-$\omega$-((1,2,3-triazol-4-yl)methanol)-polystyrene</td>
<td>TIPS-6b-OH</td>
<td>7.3</td>
</tr>
<tr>
<td>$\alpha$-(2-(1,2,3-triazol-4-yl)acetic acid)-$\omega$-((1,2,3-triazol-4-yl)methanol)-polystyrene</td>
<td>HOOC-6b-OH</td>
<td>7.5</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-polystyrene-block-poly(tert-butyl acrylate)</td>
<td>TIPS-6a-b-8</td>
<td>8.3</td>
</tr>
<tr>
<td>$\alpha$-(triisopropyl acetylene)-poly(methyl acrylate)-block-polystyrene-block-poly(tert-butyl acrylate)</td>
<td>7-b-6a-b-8</td>
<td>15.3</td>
</tr>
</tbody>
</table>

The last step in the formation of an ABC-type triblock copolymer was coupling of azide functionalized PMA 7 to the unprotected acetylene functionalized diblock
copolymer H-6a-b-8 (scheme 3.10). For this “click” reaction the same conditions were applied as for the synthesis of the diblock copolymer, viz. using CuBr/Me₆TREN as the catalyst system in DMF with the azide functionalized PMA 7 in excess (1.50 equivalents). Nevertheless, in this case the reaction did not go to completion over night, as determined by the presence of residual diblock copolymer H-6a-b-8 in the SEC trace of the reaction mixture. The reaction, therefore, was performed at 50°C, which resulted in complete disappearance of the diblock copolymer, and thus formation of the PMA-b-PS-b-PtBA triblock copolymer (7-b-6a-b-8) according to SEC measurements. The azide end groups of the residual PMA were transformed into amines by addition of PPh₃. Completion of this Staudinger reduction was determined by TLC, according to the disappearance of the PMA precursor, associated with the presence of a ninhydrin positive spot on the baseline. The thus formed amine end-functionalized PMA was removed from the triblock copolymer over a silica column with CH₂Cl₂/MeOH (95:5) as an eluent. The successful formation of PMA-b-PS-b-PtBA is illustrated in the SEC chromatogram (figure 3.6) by a complete shift of the molecular weight distribution towards higher molecular weight. As can be seen in table 3.1, no increase in the PDI after reaction was observed in the SEC chromatogram of the product. This means that the purification of the triblock copolymer was successful and, moreover, the protected acetylene functionality of PMA was not involved in the reaction, otherwise a shoulder peak at the high molecular weight side, i.e. shorter retention time, would have been observed.

![Figure 3.6 SEC trace of the PMA-b-PS-b-PtBA triblock copolymer (7-b-6a-b-8) accompanied by the chromatograms of the PS-b-PtBA (TIPS-6a-b-8) and PMA (7) building blocks](image)

**Figure 3.6** SEC trace of the PMA-b-PS-b-PtBA triblock copolymer (7-b-6a-b-8) accompanied by the chromatograms of the PS-b-PtBA (TIPS-6a-b-8) and PMA (7) building blocks.
As can be seen in scheme 3.10, the modularly synthesized triblock copolymer $7-b-6a-b-8$ still contained both a TIPS protected acetylene and a bromide terminus which could be used for further functionalization. In order to prepare an ABCABC-type hexablock copolymer, the triblock copolymer batch was divided. From one portion, the TIPS group was removed by treatment with TBAF and the bromide end groups of the remainder were replaced by azides using NaN₃ in DMF. Unfortunately, the subsequent “click” reaction in order to form the hexablock copolymer failed. Furthermore, it was attempted to couple acetylene functionalized poly(methyl methacrylate), which was used in experiments described in the previous chapter, to the azide functionalized triblock copolymer. Once more, no reaction was observed as concluded from SEC measurements. This could be caused by a too low concentration of functional groups due to the high molecular weight of the polymers. A second explanation can be that the end groups were inaccessible for further reaction, owing to the folding behavior of the triblock copolymer in solution. Due to the incompatibility of the distinct blocks phase separation may have occurred which prevented the reactive groups of coming in vicinity of each other. Possibly, changing the solvent system or increasing the temperature may circumvent this problem and eventuate in more positive results. Another, less presumable, explanation is that chain entanglements may have caused a significant reduction in reactivity, owing to a reduced mobility of the reactive end groups. However, the molecular weight of the triblock copolymer was not that high ($M_n = 15.29$ kg/mol) and, therefore, chain entanglements were probably not present. Since for the synthesis of the triblock copolymer was proven that the strategy of performing successive “click” reactions works, most likely the reactive end groups were still present in the formed triblock copolymer. Therefore, more research has to be conducted in optimizing the reaction conditions, particularly because this route appears to be suitable for the synthesis of multiblock copolymers, e.g. ABCABC hexablock copolymers, which are very difficult to synthesize in another fashion.

3.4. Conclusions

The successful modular preparation of an ABC-type triblock copolymer has been established by conducting successive “click” reactions on both end groups of a polymer building block. This methodology required the use of heterotelechelic polymer precursors, containing both an acetylene and azide terminus. Moreover, complete
protection of the acetylene moiety was a prerequisite in order to circumvent interference in the first “click” coupling, thereby forming linear chain extended or cyclic polymers.

Employment of a TIPS protected acetylene functionalized ATRP initiator yielded well-defined polymers of which the acetylene end-functionalities were still completely protected. Furthermore, the use of ATRP provided bromide functionality on the other termini which, subsequently, were substituted for azides.

These well-defined $\alpha$-(triisopropylsilyl acetylene)-$\omega$-azide functionalized heterotelechelic polymer building blocks were used to modularly synthesize ABC-type triblock copolymers adopting “click” chemistry. In order to test the scope of the asymmetric functionalization methodology, propargyl alcohol and 2-azidoacetic acid were coupled to the azide and acetylene end groups of polystyrene, respectively, using CuBr/PMDETA as a catalyst. Subsequently, a triblock copolymer was synthesized. In the first step, $\alpha$-acetylene-$\omega$-bromo-poly(tert-butyl acrylate) was successfully “clicked” to $\alpha$-(triisopropyl acetylene)-$\omega$-azido-polystyrene, applying CuBr/Me$_6$TREN as a catalyst using DMF as a solvent. Prior to coupling of the third block, the TIPS protective group was removed entirely by subjecting the diblock copolymer to a solution of TBAF in THF. Afterwards, $\alpha$-(triisopropyl acetylene)-$\omega$-azido-poly(methyl acrylate) was coupled to the free acetylene end group of the PS-b-PtBA block copolymer by employing CuBr/Me$_6$TREN as a catalyst in DMF as a solvent. It has to be noted that, in order to drive the “click” coupling reactions between the polymer building blocks to completion, in both cases an excess of azide functionalized polymer was added. After the “click” reactions, residual polymer was completely removed from the reaction mixture by reduction of the azide moieties and subsequent application of column chromatography.

3.5. Experimental

3.5.1. Materials

Chlorotriisopropylsilane (TIPS-Cl) (Acros, 97%), ethylmagnesium bromide (Aldrich, 3.0 M solution in Et$_2$O), propargyl alcohol (Acros, 99%), 2-bromoisobutyryl bromide (Aldrich, 98%), 3-bromo-1-propanol (Aldrich, 97%), methyl propiolate (Aldrich, 99%), 2-bromoacetic acid (Aldrich, 97%), tetrabutylammonium fluoride (TBAF) (Janssen Chimica, 1 M solution in THF), N$_2$N,N',N',N"-pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), 2,2'-bipyridine (bpy) (Aldrich, >99%), sodium azide (NaN$_3$) (Acros, 99%), tris-(2-aminoethyl)amine (Aldrich, 96%), silver trifluoromethanesulfonate (AgOTf) (Aldrich, >99%), 3-indoleacrylic acid (IAA) (Aldrich, ≥ 99%) and anisole (Aldrich, >99%) were used as received. Copper(I)bromide (CuBr) was purified by washing with glacial acetic acid three times and twice with diethyl ether. Triphenylphosphine (PPh$_3$) was recrystallized from isopropanol. Triethylamine (Et$_3$N) was distilled under nitrogen.
from potassium hydroxide. Styrene, methyl acrylate and tert-butyl acrylate were distilled under reduced pressure. Tetrahydrofuran (THF) was distilled under nitrogen from sodium/benzophenone. Dichloromethane (CH₂Cl₂), ethyl acetate (EtOAc) and diethyl ether (Et₂O) were distilled under nitrogen from calcium hydride. N,N-dimethylformamide (DMF) was dried with magnesium sulfate and distilled under reduced pressure.

3.5.2. Instrumentation

Proton and carbon-13 nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker DPX300 spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane (δ = 0.00 ppm) for ¹H NMR spectra and relative to CDCl₃ (δ = 77.16 ppm) for ¹³C NMR spectra. Infrared (IR) spectra were obtained using an ATI Matson Genesis Series FTIR spectrophotometer fitted with an ATR cell. Data are presented as the frequency of absorption (cm⁻¹).

Molecular weight distributions were measured using size exclusion chromatography (SEC) on a system equipped with a guard column and a PL gel 5 μm mixed D column (Polymer Laboratories) with differential refractive index and UV (254 nm) detection, using THF as an eluent at 1 mL/min and T = 35°C. Polystyrene (PS) standards in the range of 162 to 6,035,000 g/mol were used to calibrate the SEC.

Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV, permanganate or ninhydrin reagent.

Column chromatography was performed using silica gel, Acros (0.035-0.070 mm, pore diameter ca. 6 nm), unless otherwise stated.

Gas chromatography (GC) measurements were conducted on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary column (HP1701, 25m x 0.32mm x 0.25μm), using flame ionization detection.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectra were measured on a Bruker Biflex III machine. 3-Indoleacrylic acid (IAA) was used as a matrix. If necessary, silver trifluoroacetate (AgOTf) was added as an ionizing agent. Samples were prepared by mixing 10 μL of a 40 mg/mL matrix solution, 10 μL of a 1 mg/mL polymer solution and 1 μL of a 5 mg/mL AgOTf solution. From this mixture 1 μL was spotted on a MALDI plate.

3.5.3. 3-(1,1,1-triisopropylsilyl)-2-propyn-1-ol

A solution of propargyl alcohol (1.13 g, 20.2 mmol) in THF (20 mL) was added dropwise at room temperature to a 3.0 M solution of ethylmagnesium bromide (20.0 mL, 60.0 mmol) which was diluted with THF (50 mL). After complete addition the reaction mixture was refluxed for 18 hours. The reaction mixture was allowed to cool to room temperature and a solution of TIPS-Cl (5.57 g, 28.9 mmol) in THF (20 mL) was added dropwise and, subsequently, refluxed for five hours. Formation of the product was confirmed by TLC (n-heptane/EtOAc 3:2). The reaction mixture was cooled to room temperature and poured into a 10% (m/m) HCl solution (30 mL). The aqueous layer was separated and the product was extracted with Et₂O. The combined organic layers were washed with brine, dried with anhydrous magnesium sulfate and the solvent was removed in vacuo. The crude product was isolated as a yellow oil and used without further purification.

TLC: Rᵣₜ (n-heptane/EtOAc 3:2) = 0.54; ¹H NMR (300 MHz, CDCl₃) δ 4.30 (s, 2H, ≡−CH₂-OH), 1.06-1.05 (m, 21H, ((C₆H₁₃)₂C₆H₃-Si); ¹³C NMR (75 MHz, CDCl₃) δ 103.42 (Si-C≡C(CH₃)), 87.52
Modular synthesis of ABC type block copolymers by asymmetric functionalization of polymers

$$\text{Si} \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2$$

$$\equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2 \equiv \text{C} \equiv \text{C} - \text{CH}_2$$

3.5.4. 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (2)

A solution of 2-bromoisobutyryl bromide (3.75 mL, 30.4 mmol) in THF (40 mL) was added dropwise to a solution of 3-(1,1,1-triisopropylsilyl)-2-propyn-1-ol (20.2 mmol) and Et$_3$N (4.23 mL, 30.4 mmol) in THF (60 mL) at 0°C. Afterwards, the reaction mixture was allowed to stir for two hours at room temperature. Completion of the reaction was determined by TLC ($n$-heptane/EtOAc 95:5). The formed triethylammonium salts were removed by filtration and the solvent was removed by rotary evaporation. The crude product was dissolved in CH$_2$Cl$_2$ and washed twice with a saturated NH$_4$Cl solution and twice with distilled water. The organic layer was dried using anhydrous magnesium sulfate and the solvent was removed in vacuo, yielding a dark brown oil which was purified using column chromatography ($n$-heptane/EtOAc 95:5). The product was isolated as a colorless oil which was dried under vacuum.

Yield: 4.37 g (60%, 2 steps); TLC: R$_f$ ($n$-heptane/EtOAc 95:5) = 0.29; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.80 (s, 2H, $\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 1.95 (s, 6H, $\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 1.07 (m, 21H, ((CH$_3$)$_2$CH)$_3$Si); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 170.91 ($\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 100.27 (((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 89.25 (((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 55.35 ((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 54.38 (((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 18.65 (((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 11.21 (((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), FTIR-ATR 2941, 2859, 2185 ($\equiv \text{C} \equiv \text{C}$), 1748 ($\equiv \text{C} \equiv \text{C}$), 1463, 1385, 1368, 1273, 1152, 1109, 1070, 1027 cm$^{-1}$

3.5.5. $\alpha$-(triisopropyl acetylene)-$\omega$-bromo-polystyrene (3a)

Typical procedure: CuBr (359 mg, 2.50 mmol) was placed in a Schlenk flask which was fitted with a stopper. The Schlenk flask was evacuated and back-filled with argon. This procedure was repeated three times. Subsequently, the stopper was replaced by a septum. Degassed styrene (16.65 g, 159.9 mmol), anisole (2 mL) and PMDETA (437 mg, 2.52 mmol) were added and the reaction mixture was stirred for 20 minutes to allow copper complex formation. The reaction mixture was placed in a statically controlled oil bath at 90°C. 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (2) (904 mg, 2.50 mmol) was added and the polymerization was monitored by analyzing samples by $^1$H NMR. The polymerization was stopped after 155 minutes (62% conversion) by cooling and dilution with CHCl$_3$. The reaction mixture was washed three times with a 0.055 M EDTA solution and twice with distilled water. The organic layer was dried using magnesium sulfate and concentrated in vacuo. The polymer was purified further by column chromatography using CHCl$_3$ as the eluent. The polymer was isolated by precipitation in MeOH as a white solid, which was dried over night in a vacuum oven at 60°C.

Yield: 10.56 g (94%); TLC: R$_f$ (CH$_2$Cl$_2$) = 0.91, R$_f$ ($n$-hexane/Et$_2$O/CH$_2$Cl$_2$ 14:3:3) = 0.42; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.35-6.27 (br. m, arom. H), 4.48 (br. m, CH$_2$-CH(Ph)-Br), 4.05 (br. m, $\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), 2.58-1.17 (br. m, backbone CH$_2$, CH$_3$), 1.12-0.83 (br. m, ((CH$_3$)$_2$CH)$_3$Si-$\equiv \text{C} \equiv \text{C} - \text{CH}_2$-$\text{O}_2\text{C}$), O$_2$C-C(CH$_3$)$_2$-CH$_3$); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 ($\equiv \text{C} \equiv \text{C}$), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm$^{-1}$; SEC: $M_n$ = 5.82 kg/mol; $M_n/M_w$ = 1.13; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 6121 ± 104.06 (55 repeating units + end groups + K$^+$)
3.5.6. \( \alpha \)-(triisopropyl acetylene)-\( \omega \)-bromo-poly(\textit{tert}-butyl acrylate) (4)

**Typical procedure:**

A Schlenk flask fitted with a stopper was loaded with CuBr (144 mg, 1.00 mmol) and was evacuated and back-filled with argon. The evacuating cycle was repeated three times and, afterwards, the stopper was replaced by a septum. Subsequently, degassed tert-butyl acrylate (10.38 g, 81.0 mmol), anisole (0.4 mL), acetone (2.4 mL) and PMDETA (178 mg, 1.03 mmol) were added and the reaction mixture was purged with argon for 5 minutes. The reaction mixture was stirred for 15 minutes in order to allow formation of the copper complex. Afterwards, the reaction mixture was placed in a statically controlled oil bath at 50 °C and 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (2) (340 mg, 0.94 mmol) was added. Upon polymerization, samples were periodically taken for conversion analysis by GC. The polymerization was stopped after 180 minutes (51% conversion) by cooling and dilution with CHCl₃. The reaction mixture was washed three times with a 0.055 M EDTA solution. The organic layer was dried with anhydrous magnesium sulfate and concentrated in vacuo. The crude polymer was purified using column chromatography (Acros, aluminum oxide, activated basic, 60-200 \( \mu \)m) with CHCl₃ as an eluent. The polymer was isolated as a colorless viscous oil by precipitation in MeOH/H₂O 1:1. The product was dried over night in a vacuum oven at 60°C.

Yield: 4.67 g (83%); TLC: \( R_f \) (CH₂Cl₂/MeOH 95:5) = 0.95; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 4.68 (br. m, \( \equiv-CH_2O_2C \)), 4.12 (br. s, CH₂-CH(C(O₂Bu)-Br), 2.36-1.21 (br. m, backbone CH₂, CH), 1.44 (br. s, CO₂-C(CH₃)₃), 1.13-0.85 (br. m, ((CH₃)₂CH₃)Si-\( \equiv \), O₂C-C(CH₃)₂-CH₂); FTIR-ATR 2976, 2924, 2863, 1722, 1767, 1450, 1390, 1368, 1256, 1139 cm\(^{-1}\); SEC: \( M_n \) = 3.58 kg/mol; \( M_w/M_n \) = 1.24; MALDI-ToF MS: matrix: IAA+AgOTf⁺; \( m/z \) = 2381 ± 128.17 (15 repeating units + end groups + Ag⁺)

3.5.7. \( \alpha \)-(triisopropyl acetylene)-\( \omega \)-bromo-poly(methyl acrylate) (5)

**Typical procedure:**

A Schlenk tube which was fitted with a stopper was equipped with CuBr (43.0 mg, 0.30 mmol), evacuated and back-filled with argon. This evacuating cycle was repeated three times prior to replacement of the stopper by a septum. Afterwards, degassed methyl acrylate (1.88 g, 21.8 mmol), anisole (0.2 mL) and PMDETA (51.7 mg, 0.30 mmol) were added and the reaction mixture was purged with argon for 5 minutes. To allow complex formation, the reaction mixture was stirred for an additional 15 minutes. The reaction mixture was placed in a statically controlled oil bath at 90°C and 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (2) (106 mg, 0.29 mmol) was added. Samples were taken periodically for conversion analysis by GC. The polymerization was stopped after 150 minutes (88% conversion) by cooling down and dilution with CHCl₃. The reaction mixture was washed three times with a 0.055 M EDTA solution. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was purified further by column chromatography (Acros, aluminum oxide, activated basic, 60-200 \( \mu \)m) using CHCl₃ as an eluent and precipitated in \( n \)-heptane. The polymer was isolated as a colorless sticky solid which was dried over night in a vacuum oven at 60°C.

Yield: 1.18 g (67%); TLC: \( R_f \) (CH₂Cl₂/MeOH 95:5) = 0.31; \(^1\)H NMR (300 MHz, CDCl₃) \( \delta \) 4.68 (br. m, \( \equiv-CH_2O_2C \)), 4.32 (br. m, CH₂-CH(C(O₂CH₃)-Br), 3.97-3.29 (br. s, H₃C-O-C(=O)), 2.51-
1.37 (br. m, backbone CH₂, CH₃), 1.23-0.95 (br. m, (C₃H₇)₂CHSi−≡, O₂C-C(CH₃)₂CH₂); FTIR-ATR 3447, 3054, 2951, 2306, 1727, 1433, 1381, 1324, 1264, 1193, 1159, 1048 cm⁻¹; SEC: Mₙ = 7.17 kg/mol; Mₘ/Mₙ = 1.18; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 5803 ± 86.09 (62 repeating units + end groups + Ag⁺)

3.5.8. α-(triisopropyl acetylene)-ω-azido-polystyrene (6a) Typical procedure: α-(triisopropyl acetylene)-ω-bromo-polystyrene (3a) (3.02 g, 0.67 mmol) was dissolved in DMF (20 mL). Subsequently, NaN₃ (439 mg, 6.75 mmol) was added and the reaction mixture was stirred for 25 hours at room temperature. CHCl₃ (40 mL) was added and the reaction mixture was washed three times with distilled water. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in MeOH and isolated as a white solid which was dried overnight under vacuum.

Yield: 2.86 g (96%); TLC: Rᵣ (CH₂Cl₂) = 0.84, Rᵣ (n-hexane/Et₂O/CH₂Cl₂ 14:3:3) = 0.40; ¹H NMR (300 MHz, CDCl₃) δ 7.35-6.27 (br. m, arom. H), 4.18-3.77 (br. m, ≡−C₇H₅-O₂C, CH₂-C(CH₃)₂(N₃)), 2.58-1.17 (br. m, backbone CH₂, CH₃), 1.12-0.83 (br. m, ((C₃H₇)₂CHSi−≡, O₂C-C(CH₃)₂CH₂); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 (νC≡C), 2094 (νN₃), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm⁻¹; SEC: Mₙ = 5.82 kg/mol; Mₘ/Mₙ = 1.13; MALDI-ToF MS: matrix: IAA+AgOTf; m/z 6164 ± 104.06 (55 repeating units + end groups + Ag⁺)

3.5.9. α-(triisopropyl acetylene)-ω-azido-poly(methyl acrylate) (7) Typical procedure: α-(triisopropyl acetylene)-ω-bromo-poly(methyl acrylate) (5) (3.02 g, 0.67 mmol) was dissolved in DMF (20 mL). Subsequently, NaN₃ (638 mg, 9.81 mmol) was added and the reaction mixture was stirred for 19 hours at room temperature. CHCl₃ (40 mL) was added and the reaction mixture was washed three times with distilled water. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in n-heptane and isolated as a colorless sticky solid which was dried overnight under vacuum.

Yield: 2.34 g (78%); TLC: Rᵣ (CH₂Cl₂/MeOH 95:5) = 0.26; ¹H NMR (300 MHz, CDCl₃) δ 4.67 (br. m, =C=H-O,C, CH₂-C(Ph)-N₃), 3.98-3.29 (br. m, CH₂-CO₂CH₃-N₃), 2.49-1.36 (br. m, backbone CH₂, CH₃), 1.22-0.96 (br. m, ((C₃H₇)₂CHSi−≡, O₂C-C(CH₃)₂CH₂); FTIR-ATR 3447, 3054, 2950, 2306, 2111 (νN₃), 1727, 1433, 1381, 1325, 1264, 1191, 1161, 1048 cm⁻¹; SEC: Mₙ = 7.15 kg/mol; Mₘ/Mₙ = 1.18; MALDI-ToF MS: matrix: IAA+AgOTf; m/z 5767 ± 86.09 (62 repeating units + end groups + Ag⁺)

3.5.10. α-acetylene-ω-bromo-poly(tert-butyl acrylate) (8) Typical procedure: α-(triisopropyl acetylene)-ω-bromo-poly(tert-butyl acrylate) (4) (449 mg, 0.075 mmol) was dissolved in THF (4 mL). Subsequently, TBAF (0.75 mL, 0.75 mmol) was added and the reaction mixture was stirred for 18 hours at room temperature. In order to remove the liberated triisopropyvl fluoride, the polymer was purified by
column chromatography (Acros, aluminum oxide, activated basic, 60-200 µm). Afterwards, the polymer solution was concentrated in vacuo. The polymer was precipitated in MeOH/H₂O (1:1) and isolated as a white sticky solid which was dried over night in a vacuum oven at 60°C.

Yield: 350.6 mg (80%); TLC: R_f (CH₂Cl₂/MeOH 95:5) = 0.92; ¹H NMR (300 MHz, CDCl₃) δ 4.63 (br. m, CH₂-C₃H₂-O₂C), 4.11 (br. s, CH₂-C₃H₂-(CO₂tBu)-Br), 2.44 (br. m, CH₂=CH₂), 2.36-1.21 (br. m, backbone CH₂, CH₃), 1.44 (br. s, CO₂-C(CH₃)₃), 1.04-0.94 (br. s, O₂C-C(CH₃)₂-CH₂); FTIR-ATR 2976, 2925, 2862, 1724, 1476, 1450, 1389, 1368, 1256, 1141 cm⁻¹; SEC: Mₙ = 3.56 kg/mol; M_w/Mₙ = 1.24; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 2227 ± 128.17 (15 repeating units + end groups + Ag⁺)

3.5.11. 3-azido-1-propanol[27] (9)

3-bromo-1-propanol (10.00 g, 72.0 mmol) was dissolved in DMF (70 mL). Subsequently, NaN₃ (18.97 g, 291.9 mmol) was added and the reaction mixture was stirred for 16 hours at room temperature. Et₂O (200 mL) was added and the organic layer was washed two times with distilled water and twice with brine. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The crude product was purified by column chromatography (n-pentane/Et₂O 1:1→1:3) and 9 was isolated as a colorless oil.

Yield: 6.58 g (90%); ¹H NMR (300 MHz, CDCl₃) δ 3.69 (t, 2H, 3J = 5.9 Hz, CH₂-C₃H₂-OH), 3.39 (t, 2H, 3J = 5.9 Hz, CH₂-C₃H₂-N₃), 2.32 (br. s, 1H, CH₂-OH), 1.77 (dt, 2H, 3J = 5.9 Hz, CH₂-C₃H₂-CH₃); ¹³C NMR (75 MHz, CDCl₃) δ 59.84 (CH₂-C₃H₂-OH), 48.93 (CH₂-C₃H₂-CH₃), 31.96 (CH₂-C₃H₂-CH₂)

3.5.12. N₁,N₁-di[2-(dimethylamino)ethyl]-N₂,N₂-dimethyl-1,2-ethanediamine (Me₆TREN)[28] (12)

A solution of tris-(2-aminoethyl)amine (4.60 g, 31.4 mmol) was added dropwise over 30 minutes to a stirred solution of formaldehyde (50 mL) and formic acid (50 mL) at 0°C. After stirring for one hour at 0°C, the reaction mixture was allowed to warm up to room temperature. The color of the reaction mixture slowly changed from yellow to orange/brown. Furthermore, CO₂ gas evolved from the reaction mixture. Subsequently, the reaction mixture was refluxed over night and no more CO₂ evolved from the reaction mixture. All volatile fractions were removed by rotary evaporation, yielding a viscous orange/brown oil. 15 mL of a concentrated NaOH solution was added and the color of the reaction mixture changed from orange/brown to yellow. The product was extracted with CH₂Cl₂. The solvent was removed in vacuo and the product was isolated as a brown oil which was dried under vacuum.

Yield: 6.60 g (91%); ¹H NMR (300 MHz, CDCl₃) δ 2.62 (t, 6H, 3J = 7.8 Hz, ((CH₃)₂N-CH₂-C₃H₂)₃N), 2.39 (t, 6H, 3J = 7.8 Hz, ((CH₃)₂N-CH₂-CH₂)₃N), 2.24 (s, 18H, ((CH₃)₂N-CH₂-CH₂)₃N); ¹³C NMR (75 MHz, CDCl₃) δ 57.51 ((CH₃)₂N-CH₂-C₃H₂)₃N), 52.91 ((CH₃)₂N-CH₂-CH₂)₃N), 45.89 ((CH₃)₂N-CH₂-CH₂)₃N); FTIR-ATR 2941, 2855, 2812, 2760 (νtertiary amine), 1675, 1455, 1364, 1333, 1264, 1152, 1122, 1096, 1031 cm⁻¹

3.5.13. 2-azidoacetic acid[29] (13)

Sodium azide (1.48 g, 22.8 mmol) was suspended in DMSO (50 mL) and stirred for an hour to give a yellow solution. Next, a solution of 2-bromoacetic acid (1.52 g, 11.0 mmol) in DMSO (30 mL) was added dropwise to the reaction mixture, which imparted an orange color. After complete addition, the reaction mixture was
stirred for 18 hours at room temperature and afterwards was diluted with distilled water (100 mL). The aqueous solution was acidified using concentrated hydrochloric acid and extracted three times with EtOAc. The combined organic layers were washed with brine and the solvent was removed in vacuo. The product was isolated as a white solid which was dried under vacuum.

Yield: 732.8 mg (66%); 1H NMR (300 MHz, CDCl3) δ 3.75 (s, 2H, HO2C-C\(\text{H}_2\)-N3); 13C NMR (75 MHz, CDCl3) δ 170.14 (HO-C(=O)-CH2), 49.87 (HO2C-C\(\text{H}_2\)-N3); FTIR-ATR 3546, 3464, 3340, 2245, 2129 (\(\nu\)N3), 1610, 1407, 1303, 1225, 962 cm\(^{-1}\).

3.5.14. \(\alpha\)-(triisopropyl acetylene)-\(\omega\)-((1,2,3-triazol-4-yl)methanol)-polystyrene (TIPS-6b-OH)

\(\alpha\)-(triisopropyl acetylene)-\(\omega\)-azido-polystyrene (6b) (206 mg, 0.047 mmol) was placed in a Schlenk tube fitted with a stopper, evacuated and back-filled with argon. This evacuating cycle was repeated three times and, afterwards, the stopper was replaced by a septum. THF (5 mL) and propargyl alcohol (27.3, 0.49 mmol) were added. Subsequently, 0.2 mL of a stock solution containing CuBr (0.45 M) and PMDETA (0.45 M) in THF, which was prepared under Schlenk conditions, was added. The reaction mixture was stirred for 18 hours at room temperature. Completion of the reaction was determined by disappearance of the azide signal in FTIR-ATR, and TLC (CH\(_2\)Cl\(_2\)). CH\(_2\)Cl\(_2\) (4 mL) was added and the reaction mixture was washed three times with a 0.055 M aqueous EDTA solution and twice with distilled H\(_2\)O. The organic layer was dried using anhydrous magnesium sulfate and concentrated by rotary evaporation. The polymer was precipitated in MeOH and isolated as a white solid which was dried under vacuum.

Yield: 194.3 mg (93%); TLC: R\(_f\) (CH\(_2\)Cl\(_2\)) = 0.12; R\(_f\) (CH\(_2\)Cl\(_2\)/MeOH 95:5) = 0.96; 1H NMR (300 MHz, CDCl3) δ 7.60 (br. s, triazole H), 7.41-6.25 (br. m, arom. H), 5.08 (br. m, CH\(_2\)-C(Ph)-N), 4.66 (br. m, CH\(_2\)-OH), 4.08 (br. m, \(\equiv\text{CH}_{2}\)-O2C), 2.33-1.12 (br. m, backbone CH\(_3\), CH\(_2\)), 1.12-0.78 (br. m, O\(_2\)C-C(CH\(_3\))=CH\(_2\)), \((\text{CH}_{3})_2\text{C}=\equiv\); FTIR-ATR 3084, 3054, 3023, 2919, 2846, 1943, 1865, 1799, 1493, 1450, 1359, 1260, 1182, 1025 cm\(^{-1}\); SEC: Mn = 5.98 kg/mol; M\(_w\)/M\(_n\) = 1.14; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 7247 ± 104.06 (65 repeating units + end groups + Ag\(^+\)).

3.5.15. \(\alpha\)-acetylene-\(\omega\)-((1,2,3-triazol-4-yl)methanol)-polystyrene (H-6b-OH)

\(\alpha\)-(triisopropyl acetylene)-\(\omega\)-azido-polystyrene (TIPS-6b-OH) (58.5 mg, 0.013 mmol) was dissolved in THF (1 mL). TBAF (0.13 mL, 0.13 mmol) was added and the reaction mixture was stirred for 17 hours at room temperature. Completion of the reaction was ascertained by TLC (CH\(_2\)Cl\(_2\)). The reaction mixture was passed through a basic alumina column in order to remove triisopropylsilyl fluoride. The reaction mixture was concentrated in vacuo and the polymer was isolated as a white solid by precipitation in MeOH. The polymer was dried under vacuum.

Yield: 51.0 mg (95%); TLC: R\(_f\) (CH\(_2\)Cl\(_2\)) = 0.09; 1H NMR (300 MHz, CDCl3) δ 7.41-6.24 (br. m, arom. H), 5.08 (br. m, CH\(_2\)-C(Ph)-N), 4.65 (br. m, CH\(_2\)-OH), 4.06 (br. m, \(\equiv\text{C}_{2}\)-O2C), 2.33-1.12 (br. m, backbone CH\(_3\), CH\(_2\)), 1.07-0.92 (br. m, O\(_2\)C-C(CH\(_3\))=CH\(_2\)), FTIR-ATR 3084, 3054, 3023, 2919, 2846, 1943, 1865, 1799, 1731, 1601, 1493, 1450, 1359, 1260, 1182, 1025 cm\(^{-1}\); MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 7093 ± 104.06 (65 repeating units + end groups + Ag\(^+\)).
3.5.16. \(\alpha-(2-(1,2,3\text{-triazol-4-yl})\text{acetic acid)}-\omega-((1,2,3\text{-triazol-4-yl})\text{methanol})\text{-polystyrene (HOO-C-6b-OH)}\)

A Schlenk tube fitted with a stopper was loaded with \(\alpha\text{-}acetylene-\omega-(\text{1,2,3\text{-triazol-4-yl}})\text{methanol}\text{-polystyrene (H-6b-OH)}\) (47.0 mg, 0.011 mmol) and azidoacetic acid (11.2 mg, 0.11 mmol). The Schlenk tube was evacuated and back-filled with argon. After repeating this procedure three times, the stopper was replaced by a septum and THF (1 mL) was added. Subsequently, 0.1 mL of a stock solution containing CuBr (0.11 M) and PMDETA (0.11 M) in THF, which was prepared under Schlenk condition, was added. The reaction mixture was stirred for 18 hours at room temperature. Completion of the reaction was determined by TLC (CH\(_2\)Cl\(_2\)/MeOH 95:5). CH\(_2\)Cl\(_2\) (5 mL) was added and the reaction mixture was washed three times with a 0.055 M aqueous EDTA solution and twice with distilled H\(_2\)O. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in MeOH and isolated as a white solid which was dried under vacuum.

Yield: 39.6 mg (82%); TLC: \(R_f\) (CH\(_2\)Cl\(_2\)/MeOH 95:5) = 0.04; \(^1\text{H NMR (300 MHz, CDCl}_3\)) \(\delta 7.41-6.24\) (br. m, arom. \(\text{H}\)), 5.20-4.92 (br. m, HOOC-\(\text{CH}_2\)-N, \(\text{CH}_2\)-C(Ph)-N, N(C-)=C-CH\(_2\)-O2C), 4.65 (br. m, C\(_2\)-OH), 2.33-1.12 (br. m, backbone C\(_2\), C\(_6\)), 1.07-0.92 (br. m, O2C-C(C\(_3\))2CH\(_2\)); FTIR-ATR 3084, 3054, 3023, 2920, 2846, 1943, 1865, 1796, 1731, 1728, 1601, 1493, 1450, 1359, 1260, 1178, 1083, 1078, 1027 cm\(^{-1}\); SEC: \(M_n = 6.02\) kg/mol; \(M_w/M_n = 1.14\); MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 7192 ± 104.06 (65 repeating units + end groups + Ag\(^+\))

3.5.17. \(\alpha-(\text{triisopropyl acetylene})\text{-polystyrene-block-poly(tert-butyl acrylate)}\) (TIPS-6a-b-8)

\(\alpha-(\text{triisopropyl acetylene})\text{-}\omega\text{-azido-polystyrene (6a)}\) (533 mg, 0.085 mmol) and \(\alpha\text{-acetylene-}\omega\text{-bromo-poly(tert-butyl acrylate) (8)}\) (436 mg, 0.075 mmol) were placed in a Schlenk tube. The Schlenk tube was evacuated and back-filled with argon three times and, subsequently, DMF (4 mL) was added. After complete dissolution of the polymers, 0.1 mL of a stock solution containing CuBr (0.75 M) and Me\(_6\)TREN (12) (0.75 M) in DMF, which was prepared under Schlenk conditions, was added. The reaction mixture was stirred for 18 hours at room temperature. Subsequently, PPh\(_3\) (25.8 mg, 0.098 mmol) was added and the reaction mixture was stirred for another 22 hours in order to reduce all residual azide groups. Successful reduction of residual azides was determined by TLC (CH\(_2\)Cl\(_2\)/MeOH 95:5) by disappearance of the azide functionalized PS in combination with the presence of a ninhydrin positive spot at the baseline. The reaction mixture was concentrated by rotary evaporation and the excess of polystyrene was removed by column chromatography (CH\(_2\)Cl\(_2\)/MeOH 95:5). Afterwards, the product was precipitated in MeOH and isolated as a white solid, which was dried under vacuum.

Yield: 824.7 mg (91%); TLC: \(R_f\) (CH\(_2\)Cl\(_2\)/MeOH 95:5) = 0.98; \(^1\text{H NMR (300 MHz, CDCl}_3\)) \(\delta 7.43-6.19\) (br. m, arom. \(\text{H}\)), 5.05 (br. m, N(C-)=C-CH\(_2\)-O2C), 4.78 (br. m, CH\(_2\)-C(Ph)-N), 4.18-3.59 (br. m, \(\equiv\text{-C}\text{H}_2\)-O2C, CH\(_2\)-C(O\(_2\)tBu)-Br), 2.49-1.13 (br. m, CO\(_2\)-C(CH\(_3\))\(_3\)), backbone CH\(_2\), CH\(_3\)), 1.12-0.76 (br. m, O\(_2\)C-C(CH\(_3\))\(_3\)-CH\(_2\)), ((CH\(_3\))\(_3\)Si=); FTIR-ATR 3447, 3062, 3023, 2976, 2920, 2850, 1947, 1874, 1796, 1727, 1679, 1597, 1493, 1446, 1394, 1368, 1251, 1124, 1022 cm\(^{-1}\); SEC: \(M_n = 8.30\) kg/mol; \(M_w/M_n = 1.22\)
3.5.18. α-acetylene-polystyrene-block-poly(tert-butyl acrylate) (H-6a-b-8)

![Structure of H-6a-b-8]

α-(triisopropyl acetylene)-polystyrene-block-poly(tert-butyl acrylate) (TIPS-6a-b-8) (385 mg, 0.046 mmol) was dissolved in THF (2 mL). Subsequently, TBAF (0.46 mL, 0.46 mmol) was added and the reaction mixture was stirred for 20 hours at room temperature. Afterwards, the polymer was purified using a basic alumina column. The mixture was concentrated by rotary evaporation and the polymer was precipitated in MeOH. The deprotected diblock copolymer was isolated as a white solid which was dried under vacuum.

Yield: 353.0 mg (92%); TLC: Rf (CH$_2$Cl$_2$/MeOH 95:5) = 0.94; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.60 (triazole $H$), 7.41-6.25 (br. m, arom. $H$), 5.06 (br. m, N(C=)C-CH$_2$-O$_2$C), 4.76 (br. m, CH$_2$-C(CH$_3$)$_2$-N), 4.20-3.85 (br. m, $\equiv$-CH$_2$-O$_2$C, CH$_2$-C(H$_3$)$_3$-N), 2.48-1.12 (br. m, O$_2$C-C(CH$_3$)$_2$-CH$_2$), 1.06-0.92 (br. m, O$_2$C-C(CH$_3$)$_3$-CH$_2$); FTIR-ATR 3063, 3019, 2967, 2915, 2853, 1939, 1860, 1722, 1679, 1601, 1491, 1455, 1390, 1364, 1256, 1143 cm$^{-1}$; SEC: $M_n = 8.32$ kg/mol; $M_w/M_n = 1.22$

3.5.19. α-(triisopropyl acetylene)-poly(methyl acrylate)-block-polystyrene-block-poly(tert-butyl acrylate) (7-b-6a-b-8)

![Structure of 7-b-6a-b-8]

α-acetylene-polystyrene-block-poly(tert-butyl acrylate) (H-6a-b-8) (64.9 mg, 5.40 μmol) and α-(triisopropylacetylene)-ω-azido-poly(methyl acrylate) (7) (46.2 mg, 8.13 μmol) were placed in a Schlenk tube, which was evacuated and back-filled with argon three times. DMF (1 mL) was added and the reaction mixture was stirred for 15 minutes in order to dissolve all polymers. Subsequently, 0.1 mL of a stock solution containing CuBr (0.054 M) and Me$_6$TREN (12) (0.054 M) in DMF, which was prepared under Schlenk conditions, was added. The reaction mixture was stirred for 20 hours at 50°C. The reaction mixture was allowed to cool to room temperature, PPh$_3$ (7.4 mg, 28.2 μmol) was added and the reaction mixture was stirred for another 19 hours in order to reduce the azide functionalized PMA which was still present. Successful reduction of azide groups was confirmed with TLC (CH$_2$Cl$_2$/MeOH 95:5) by disappearance of azide bearing PMA and the presence of a ninhydrin positive spot at the baseline. The reaction mixture was concentrated by rotary evaporation and the formed amine functionalized poly(methyl acrylate) was removed by column chromatography (CH$_2$Cl$_2$/MeOH 95:5). Subsequently, the triblock copolymer was precipitated in MeOH and isolated as a white solid, which was dried under vacuum.

Yield: 53.8 mg (72%); TLC: Rf (CH$_2$Cl$_2$/MeOH 95:5) = 0.89; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.48-6.17 (br. m, arom. $H$), 5.09-4.98 (br. m, N(C=)C-CH$_2$-O$_2$C), 4.83-4.69 (br. m, CH$_2$-C(H$_3$)$_2$-N, backbone CH$_2$, CH$_2$), 4.20-3.57 (br. m, $\equiv$-CH$_2$-O$_2$C, CH$_2$-C(H$_3$)$_3$-N, backbone CH$_2$, CH$_2$), 3.99-3.28 (br. s, H$_3$C-O-C(=O)), 2.53-0.73 (br. m, CO$_2$-C(CH$_3$)$_3$-O$_2$C-C(CH$_3$)$_3$-N, CO$_2$-C(CH$_3$)$_3$-C(H$_3$)$_3$-N, backbone CH$_2$, CH$_2$); SEC: $M_n = 15.29$; $M_w/M_n = 1.20$
3.6. References


The formation of cyclic polymers was accomplished by ring closure of linear polymers by performing “click” reactions. To be able to perform these cyclizations, heterotelechelic precursor polymers bearing both terminal acetylene and azide functionality were prepared by atom transfer radical polymerization (ATRP). By employing an acetylene bearing initiator and substitution of the bromide end groups for azides after polymerization, \(\alpha\)-acetylene-\(\omega\)-azide functionalized polystyrene (PS), poly(tert-butyl acrylate) (PtBA) and a polystyrene-block-poly(tert-butyl acrylate) (PS-b-PtBA) diblock copolymer were synthesized. Under dilute conditions the linear homopolymer precursors were cyclized by subjecting them to copper-catalysts. To prevent linear chain extension of unreacted polymer during concentration of the reaction mixture and to be able to separate the linear precursors from the formed cyclic polymers, Staudinger reduction conditions were applied to convert the possibly present terminal azide groups into amine functionalities. Subsequently, the cyclic polymers were purified by column chromatography. Successful formation of the cyclic analogues was established using size exclusion chromatography (SEC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS).Owing to the reduced hydrodynamic volume of the cyclic polymers in reference to the linear precursors, a complete shift towards lower molecular weight was observed in the SEC chromatograms, whereas MALDI-ToF MS displayed no change in molecular weight. Cyclic PS-b-PtBA, unfortunately, was not formed, most likely due to incompatibility of both blocks which circumvented the reactive groups to approximate each other.
4.1. Introduction

The vast majority of polymers have a chain topology. Only in some specific cases, such as with certain ring opening polymerization mechanisms, the formation of cyclic structures is well conceivable. Since many of the characteristic polymer properties are determined by their chain ends,[1] there has been a growing interest in the polymer community to obtain generic methods for the formation of cyclic polymers to study more methodologically their topology, the stringent restrictions on their backbone conformations and, obviously, the absence of chain ends.[2]

The two most applied strategies for the synthesis of cyclic polymers are based on anionic polymerization processes and are depicted in scheme 4.1.[3] The linear polymer precursors obtained by anionic polymerization have either two identical or different end groups. The former group of precursors comprising equal end groups (X-P-X) can be cyclized utilizing electrophilic coupling agents (Y-A-Y), whereas the latter class of polymers, containing two distinct end groups (X-P-Y), can be directly linked, often with the aid of an activating agent (K).

(a)\[X-P-X + Y-A-Y \rightarrow X-P-A-Y + X-Y \rightarrow P_A + 2 X-Y\]

possible side reactions

(b)\[X-P-Y + K \rightarrow \left( X-P-Y-K \right) \rightarrow P + X-Y + K\]

Scheme 4.1 Schematic illustration of the two most frequently used methodologies for the synthesis of cyclic polymers. The first strategy is based on the reaction of a living α,ω-dicarbanionic polymer (X-P-X) with a difunctional electrophilic reagent (Y-A-Y) (a). The second method involves an intramolecular reaction of an α,ω-heterotelechelic polymer (X-P-Y) which often requires application of an activator (K) (b).

The strategy depicted in scheme 4.1.a has been used to prepare cyclic polystyrenes (PS),[4-8] polydienes,[9-11] poly(vinyl pyridine),[12,13] and block copolymers.[14,15] An example of the synthesis of cyclic PS is shown in scheme 4.2. A considerable drawback of this method is the necessity of an exact stoichiometry between the two reactants in order to
avoid intermolecular coupling reactions, which lead to linear chain extension or the formation of larger ring structures, as depicted in the box in scheme 4.1.a.

![Scheme 4.2 Example of the synthesis of cyclic PS via coupling of dimethyldichlorosilane and α,ω-difunctional living polystyrylsodium](image)

The advantage of the second strategy, as schematically illustrated in scheme 4.1.b, is that no exact stoichiometry is required since both reactive groups are present in the same polymer chain and, generally, only a catalytic amount of activator (K) is demanded for the reaction to occur. This methodology has been employed for the synthesis of cyclic PS,[16-20] polyethers,[21,22] poly(methyl methacrylate)[23,24], and block copolymers.[25,26] An example of this strategy as adopted for the preparation of cyclic PS, is depicted in scheme 4.3.

![Scheme 4.3 Example of the cyclization of a heterotelechelic PS precursor by direct connection of both end groups](image)

A criterion both methods have to fulfill is that cyclization reactions have to be conducted in extreme dilute solution in order to circumvent intermolecular reactions, which cause linear chain extension. The probability of intramolecular reaction ($P_i$), i.e. of finding the $\omega$-end within a small volume $\nu$, in proximity of the $\alpha$-terminus is given by
equation 1, where $<r^2>^{1/2}$ is the mean square end-to-end distance of the polymer precursor.$^{[3,27,28]}$

$$P_c = \left(\frac{3}{2\pi}\right)^{3/2} \frac{1}{r^2} \frac{\nu_e}{\langle r^2 \rangle^{3/2}}$$  \hspace{1cm} (1)

The probability of intermolecular reaction ($P_l$), which means finding the end of another molecule is stated in equation 2, where $N_A$ is Avogadro’s number and $c$ is the polymer concentration.

$$P_l = \left(\frac{N_A c}{M}\right) \nu_e$$ \hspace{1cm} (2)

By combination of the two equations shown above, the ratio of cyclization versus linear chain extension can be deduced, as depicted in equation 3.

$$\frac{P_c}{P_l} = \left[\left(\frac{3}{2\pi}\right) <r^2>\right]^{3/2} \frac{M}{N_A c}$$ \hspace{1cm} (3)

As can be seen in equation 3, the higher the dilution of the reaction mixture, the less probable it is for linear chain extension to occur.

An elegant strategy to prepare cyclic polymers, developed by Grubbs et al., that does not require this high dilution restriction and the use of linear polymeric precursors, utilizes ring-opening metathesis polymerization (ROMP) (scheme 4.4).$^{[29,30]}$ Due to the fact that the ends of the growing polymer chains remain attached to the metal center, only cyclic polymers can be formed and, consequently, linear chain extension reactions are excluded completely. By means of an intramolecular chain transfer reaction, the cyclic polymer is separated from the ruthenium catalyst. Likewise, cyclic poly(methyl acrylate) has been prepared via a radical mechanism by insertion to a cyclic initiator.$^{[31]}$ This process, however, required the use of $^{60}$Co $\gamma$-rays to induce the polymerization reaction.

Scheme 4.4 Synthesis of cyclic polymers via ring-opening metathesis polymerization (ROMP)$^{[29]}$. Application of the cyclic ruthenium catalyst yielded, after intramolecular chain transfer, cyclic polyoctenamer and regeneration of the catalyst, whereas the non-cyclic catalyst provided linear polyoctenamer.
Although the previous strategies using anionic polymerization and ROMP are very well suited for the synthesis of cyclic polymers, the application of anionic polymerization requires stringent reaction conditions and, moreover, in both cases the number of applicable monomers is limited. Controlled radical polymerization techniques, on the other hand, combine functional group tolerance of the radical polymerization process with control over molecular weight and the polydispersity index (PDI). More significantly, end-functionality of the obtained polymers is governed during the polymerization process which, therefore, allows further modification and makes these polymers very suitable to be used as precursors for the synthesis of cyclic polymers. Albeit a promising and versatile strategy to prepare a variety of cyclic polymers, up till now it has not been used frequently. One example, which is depicted in scheme 4.5, is based on the synthesis of $\alpha$-carboxy-$\omega$-hydroxy-PS utilizing nitroxide mediated “living” radical polymerization (NMRP), which in a next step could be ring-closed via an esterification reaction. Recently, cyclic PS was prepared by coupling of the end groups of $\alpha,\omega$-dithiol-PS upon formation of a disulfide linkage. This dithiol precursor was synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization adopting a difunctional RAFT agent. In this case, the cyclic polymer can be reopened readily by breaking the disulfide bridge.

As aforementioned, inevitable for these cyclization reactions is the use of extreme low polymer concentrations in order to circumvent intermolecular reactions leading to linear chain extension. Therefore, it is a prerequisite to employ very efficient coupling reactions,
because incomplete reaction leads to the presence of residual linear polymer precursors, which could be very difficult to separate from their cyclic counterparts. In the previous two chapters, the use of the highly efficient “click” reaction between azides and terminal acetylenes for the coupling of polymer modules is described. Furthermore, in chapter three, heterotelechelic polymers bearing both azide and acetylene end groups were prepared. This gave rise to the idea to exploit these $\alpha$-acetylene-$\omega$-azide functionalized polymers as precursors for the synthesis of cyclic polymers, as illustrated in figure 4.1. The efficiency of the “click” reaction is a clear improvement when compared to the abovementioned coupling chemistries and therefore could allow the preparation of a variety of cyclic (block co)polymers which are difficult to prepare by other means. Moreover, if the reaction proceeds quantitatively, no residual linear precursor is present after reaction, which facilitates purification of the cyclic product. At the start of the here described research, there was nothing reported concerning the application of “click” chemistry for the preparation of cyclic polymers. However, meanwhile an article appeared in which the viability of this approach was demonstrated by the synthesis of cyclic PS via “click” chemistry.[39]

![Figure 4.1 Schematic illustration of the cyclization of an $\alpha$-acetylene-$\omega$-azide functionalized polymer precursor using “click” chemistry](image)

In the following section the synthesis of $\alpha$-acetylene-$\omega$-azido linear polymer precursors is discussed concisely, since the synthesis is analogous to the preparation of the polymer modules, as described in the previous chapter. The subsequent ring closure of these precursors is discussed in section 4.3.

### 4.2. $\alpha$-Acetylene-$\omega$-azido linear polymer precursor synthesis

It may be evident that, prior to the preparation of cyclic polymers, linear precursors have to be synthesized containing both acetylene and azide termini. For the modular synthesis of ABC-type triblock copolymers, as discussed in the preceding chapter, such
polymers were required as well, except for the fact that in that case full protection of the acetylene moiety was necessary in order to circumvent interference in the first “click” coupling reaction.

In this case, of course, the acetylene function needed to be deprotected to allow the polymers to ring close. The introduction of acetylene and azide end groups was similar to the strategy used in chapter 3, i.e. the polymerizations were induced utilizing acetylene comprising initiator 1 and after polymerization the bromide end groups, which were present owing to the ATRP process, were converted into azides, as depicted in scheme 4.6.

Scheme 4.6 Synthesis of α-acetylene-ω-bromide functionalized linear precursor polymers by ATRP, followed by introduction of azide end groups and deprotection of acetylene moieties
Because the possibilities regarding the introduction of acetylene and azide end functionality in PS, poly(tert-butyl acrylate) (PtBA) have been demonstrated already in the previous chapter, here the same polymers were chosen to perform the cyclization experiments. Additionally, PS-b-PtBA diblock copolymer 4 was synthesized by consecutive polymerization of styrene and tert-butyl acrylate (scheme 4.6). An interesting feature of this block copolymer is that the pendant tert-butyl esters can be hydrolyzed readily, yielding an amphiphilic polystyrene-block-poly(acrylic acid) (PS-b-PAA) block copolymer. Direct cyclization of such an amphiphilic block copolymer is difficult to realize due to incompatibility of both blocks, which decreases the likelihood of the end groups to approach each other.

All ATRP reactions were conducted using a 1:1 complex of CuBr and N,N,N',N',N"-pentamethyldiethylenetriamine (PMDETA) with triisopropylsilyl (TIPS) protected acetylene bearing initiator 1 to introduce α-acetylene functionality (see chapter 3). The conditions used for the polymerizations were equal to those used in chapter 3. All polymerizations proceeded with first order kinetics, indicating good control over the ATRP process. Accordingly, the PDIs were low (Mn/Mw ≤1.17, see table 4.1 page 104), as determined with size exclusion chromatography (SEC). The polymerization reactions were stopped at relative low conversion to sufficiently preserve bromide end groups.

Afterwards, these bromide end groups of the homopolymers were replaced for azides via nucleophilic substitution using sodium azide in DMF. Completion of this reaction was confirmed by 1H NMR spectroscopy by a complete shift of the signal stemming from the protons adjoining the end groups (from δ 4.48 to 3.92 ppm for 2, and from δ 4.32 to 3.96 ppm for 3). Moreover, in the FTIR spectra, azide groups were present at 2094 cm⁻¹, and 2112 cm⁻¹ for 2, and 3, respectively. In the last step, the acetylene moieties were liberated by treatment of the polymers with a solution of tetrabutylammonium fluoride (TBAF) in THF. Quantitative deprotection of the polymers was ascertained by the absence of TIPS signals in the 1H NMR spectra of the products.

For the PS-b-PtBA block copolymer, the introduction of an azide end group and the deprotection of the acetylene moiety was executed in one-pot by application of 20 equivalents of TBAF and 10 equivalents of azidotrimethylsilane (Me3Si-N₃), as depicted in scheme 4.6. Formation of block copolymer 4 was determined by 1H NMR and FTIR as well.
4.3. Cyclic polymer synthesis by “click” chemistry

The thus prepared α-acetylene-ω-azido linear precursors were exploited to synthesize cyclic polymers by subjection to a copper(I)-catalyst. As aforementioned, these ring closure reactions have to be performed under extreme dilute conditions in order to circumvent intermolecular coupling of polymer chains. To obtain this low concentration, a solution containing the linear precursor was added slowly to a dilute solution of the copper(I)-complex.

As illustrated in scheme 4.7, α-acetylene-ω-azido-polystyrene (2) was cyclized utilizing CuBr/PMDETA as the copper(I)-source. Therefore, 100 mL of a solution of 0.042 mmol of heterotelechelic PS 2 in 150 mL THF was added using a syringe pump with a rate of 0.1 mL/min to a solution containing 0.41 mmol of the copper-catalyst in 1.0 L THF. After complete addition, the end concentration of polymer amounted to 0.2 mM in the presence of 10 equivalents of catalyst. The reaction mixture was allowed to stir for an additional hour at room temperature and, subsequently, the polymer was concentrated in vacuo, prior to precipitation in MeOH. SEC analysis of the obtained product, however, revealed the presence of a broad signal at higher molecular weight, indicating that linear chain extended polymers (6) were present. Nevertheless, the SEC chromatogram also displayed a shoulder peak at lower molecular weight in comparison to the PS precursor, which could be attributed to cyclic PS 5. This conclusion can be drawn because cyclic polymers possess a lower hydrodynamic value in comparison to their linear analogues,[6,40] which implies that they have longer retention times in SEC, thus appear at lower molecular weight in the chromatogram. Supposedly, the linear precursor was not completely converted and subsequent increase in concentration of the polymer solution led to the occurrence of chain extension reactions (6).

![Scheme 4.7](image)

Scheme 4.7 Cyclization of α-acetylene-ω-azido-polystyrene (2). Reagents and conditions: i. CuBr, PMDETA, THF, rt., 17.5 h
Hence, in a following experiment the reaction mixture was diluted to a greater extent and the reaction time was extended because still much starting material was present in the reaction mixture. Furthermore, the Staudinger reduction strategy as applied for the modular formation of the ABC-type triblock copolymer (section 3.3.3) was adopted. With this modification procedure residual azide groups were transformed into amines, which would eliminate the possibility of linear chain extension reactions to occur during concentration of the reaction mixture (scheme 4.8). Another advantage of this reduction step was that the formed amine functionalized linear PS could be removed facilely using column chromatography owing to the difference in elution behavior. In general, separation of linear precursors from cyclic polymers is quite difficult and it normally requires the application of e.g. ultracentrifugation sedimentation[6] or liquid chromatography at critical conditions (LCCC).[41-43]

The cyclization was performed twice as diluted as the previous reaction, i.e. the polymer end concentration was 0.1 mM in the presence of 10 equivalents of catalyst. The reaction mixture was allowed to stir for two days prior to the addition of 10 equivalents of triphenyl phosphine (PPh₃). Subsequently, the reaction mixture was stirred for an additional day. After removing the solvent, the polymer was purified using column chromatography with n-heptane/CH₂Cl₂ 3:2 as an eluent. As can be seen in the SEC trace of the crude reaction mixture in figure 4.2, after performing the cyclization reaction, the majority of linear precursor 2 did not react, presumably owing to the low concentration of functional groups. However, in addition to the formation of chain extended polymer 7, a broadening at the lower molecular weight side of the chromatogram, i.e. longer retention times, was observed indicating the presence of cyclic PS 5. Because of the applied reduction strategy, this cyclic product was successfully purified by applying column chromatography.

Because the reaction did not go to completion, a following reaction was performed at elevated temperature in order to increase the reaction rate. Furthermore, to exclude intermolecular reaction to a higher degree, the reaction was conducted at an even lower concentration. In this case, a 0.28 mM solution of PS 2 in THF was added via a syringe pump with 0.1 mL/min to a refluxing solution of THF containing a 15-fold excess of CuBr/PMDETA (0.42 mM) (scheme 4.8). After 16 hours, a total of 0.027 mmol of linear precursor 2 was added which resulted in a polymer concentration of 24.6 μM. Subsequently, the reaction mixture was allowed to reflux for 3 days after which PPh₃ was added to reduce residual azide functionality, if present. After refluxing for an additional
day, the reaction mixture was concentrated and purified using column chromatography. Finally, the polymer was isolated by precipitation in MeOH.

Scheme 4.8 Representation of the cyclization of precursor PS 2. Because of the applied Staudinger reduction conditions, linear chain extended polymers 7 and residual linear precursor 8 which might be present could be removed readily by applying column chromatography. Reagents and conditions: i. CuBr, PMDETA, THF, reflux, 3 d; ii. PPh3, THF, reflux, 1 d, 40% (overall)

Figure 4.2 SEC chromatograms of precursor PS 2 and the obtained product before and after purification by column chromatography

As depicted in figure 4.3, the isolated polymer was analyzed with SEC and the peak in the chromatogram displayed a shift towards lower molecular weight in comparison to its linear counterpart ((M_n,ring)_{app}=0.82M_n, linear), which is characteristic for cyclic polymers. Furthermore, no peaks at higher molecular weight could be observed in the SEC chromatogram, indicating that either the cyclization reaction went to completion or the purification methodology was successful. Additional proof of the exclusive presence of cyclic PS 5 in the final product could be derived from the fact that no increase in the PDI
was observed after purification, as rendered in table 4.1. In case of the presence of linear precursors or chain extended polymers, the PDI would have shown an increase due to the presence of shoulder peaks.

**Table 4.1 SEC data of the applied linear precursors and the subsequently formed cyclic polymers**

<table>
<thead>
<tr>
<th>polymer</th>
<th>$M_n,_{SEC}$ (kg/mol)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-acetylene-ω-azido-polystyrene</td>
<td>2</td>
<td>7.1</td>
</tr>
<tr>
<td>α-acetylene-ω-azido-poly(tert-butyl acrylate)</td>
<td>3</td>
<td>5.7</td>
</tr>
<tr>
<td>α-acetylene-ω-azido-polystyrene-block-poly(tert-butyl acrylate)</td>
<td>4</td>
<td>8.7</td>
</tr>
<tr>
<td>cyclic polystyrene</td>
<td>5</td>
<td>5.9</td>
</tr>
<tr>
<td>cyclic poly(tert-butyl acrylate)</td>
<td>9</td>
<td>5.2</td>
</tr>
<tr>
<td>cyclic polystyrene-block-poly(tert-butyl acrylate)</td>
<td>13</td>
<td>x</td>
</tr>
</tbody>
</table>

**Figure 4.3 SEC traces of linear precursor 2 and cyclic PS 5**

Although the apparent molecular weight of the cyclic polymer measured with SEC was lower than from the linear precursor it was derived from, logically the absolute molecular weight had to remain equal. Therefore, by performing matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectrometry on both precursor polymer 2 and cyclic PS 5, it was verified that no change in molecular weight occurred due to the applied “click” conditions, as illustrated in figure 4.4. As can be seen in the MALDI-ToF MS spectrum of heterotelechelic PS 2, a second larger distribution is visible with a difference in mass of 26, which exactly corresponds to the reduction of azide end groups into amines. This reduction of azide moieties under applied MALDI-ToF MS conditions has been observed more often.[44] The fact that this second
distribution is not observed in the MALDI-ToF MS spectrum of cyclic PS 5, when measured under equal conditions, implies that no residual azide moieties were present in the isolated polymer. Furthermore, it also shows that if amine functionalized PS 7 and 8 were formed by the applied Staudinger reduction conditions, it was successfully removed by column chromatography. In addition, the absence of azide residues in the cyclic polymer was also determined with FTIR by a disappearance of the azide stretch vibration at 2094 cm⁻¹. The combined SEC and MALDI-ToF MS data demonstrate the successful formation of cyclic PS 2, along with the absence of remainder precursors and linear chain extended polymers.

The reaction conditions probably can be optimized further because 40% yield was obtained. This implies that still residual linear PS and/or linear chain extended polymers were present or cyclic product was lost during the application of column chromatography. Laurent and Grayson claimed nearly quantitative yields for the “click” cyclization of PS. However, in their case no purification of the cyclic polymer was carried out.

In order to explore the possibilities regarding the “click” cyclization of linear polymers further, cyclic poly(tert-butyl acrylate) (PtBA) 9 was prepared by ring closure of linear α-acetylene-ω-azido-PtBA 3, as depicted in scheme 4.9. In this case, a complex of CuBr and Me₆TREN (12) was employed since previous couplings with PtBA using PMDETA as a ligand did not go to completion (see section 3.3.3). A drawback, however, was that DMF
had to be adopted as a solvent owing to solubility problems in THF. Apart from that, similar reaction conditions were applied compared to the last cyclization performed with PS$_2$, i.e. a 0.21 mM solution of linear precursor 3 in DMF was added with a rate of 0.1 mL/min to a DMF solution comprising 15 equivalents of CuBr/Me$_6$TREN (12) (0.63 mM) which was heated to 60°C. After 16 hours, 0.020 mmol of P$_{BA}$ was added which amounted to a polymer concentration of 22.3 μM. After complete addition of the linear precursor, the reaction mixture was stirred for 2 days at 60°C after which PPh$_3$ was added in order to reduce the azide end groups of the possibly present residual linear precursor and chain extended linear polymers. The reaction mixture was stirred for an additional day at 60°C prior to removal of the solvent. Finally, cyclic P$_{BA}$ was purified by column chromatography and precipitated in MeOH/H$_2$O (1:1).

Scheme 4.9 Formation of cyclic PtBA by ring closure of α-acetylene-ω-azide functionalized precursor 3. Reagents and conditions: i. CuBr, Me$_6$TREN (12), DMF, 60°C, 2 d; ii. PPh$_3$, DMF, 60°C, 1 d, 64% (overall)

Formation of cyclic polymer 9 was determined by SEC and MALDI-ToF. As can be seen in figure 4.5, the SEC trace of cyclic P$_{BA}$ emerged at longer retention times in relation to linear analogue 3. This was caused by the smaller hydrodynamic volume of the cyclic polymer and is in agreement with the previous experiments with PS. Moreover, the PDI remained the same after cyclization (see table 4.1), which implied successful removal of possibly present linear polymers by consecutively reduction of azide moieties and column chromatography. Otherwise, the PDI would have shown an increase upon cyclization due to coexisting molecular weight distributions. Conversely, MALDI-ToF measurements demonstrated that the molecular weight of cyclic polymer 9 was equal to that of linear counterpart 3. Therefore, from these results the conclusion was drawn that cyclic P$_{BA}$ was formed and successfully isolated in 64% yield.
The last part of the research described in this chapter was directed towards the synthesis of cyclic block copolymers. The synthesis of several cyclic block copolymers has been described in literature, e.g. polydimethylsiloxane-block-polystyrene (PDMS-\textit{b}-PS),\textsuperscript{45,46} poly(ethylene oxide)-block-poly(propylene oxide) (PEO-\textit{b}-PPO),\textsuperscript{47} polystyrene-block-polybutadiene (PS-\textit{b}-PBD),\textsuperscript{48} polystyrene-block-polysoprene (PS-\textit{b}-PI),\textsuperscript{49-51} polystyrene-block-poly(2-tert-butylbutadiene) (PS-\textit{b}-P/BBD),\textsuperscript{52} polystyrene-block-poly(2-vinylpyridine) (PS-\textit{b}-P2VP),\textsuperscript{14} polystyrene-block-polysoprene-block-poly(methyl methacrylate) (PS-\textit{b}-PI-\textit{b}-PMMA),\textsuperscript{26} and polystyrene-block-poly(ethylene oxide) (PS-\textit{b}-PEO).\textsuperscript{25} Nearly all of the cyclic AB diblock copolymers were prepared by ring closure of linear ABA triblock copolymers, because incompatibility of the distinct blocks makes it difficult to directly cyclize AB diblock copolymer precursors.

In consideration of the efficient “click” reaction that was used throughout the research described here, it was thought to be possible to directly cyclize heterotelechelic AB diblock copolymers. As such a diblock copolymer precursor was chosen for \textit{\(\alpha\)}-acetylene-\textit{\(\omega\)}-azido-PS-\textit{b}-P\textsubscript{t}BA (4) (scheme 4.10), since the pendant tert-butyl ester groups can be readily hydrolyzed, yielding cyclic amphiphilic polystyrene-block-poly(acrylic acid) (PS-\textit{b}-PAA) 14, as discussed already in the introductory section. Up till now, there are not many examples of cyclic amphiphilic block copolymers.

Unfortunately, ring closure reactions performed on precursor 4 utilizing equal reaction conditions as for the cyclizations of PS and PtBA, \textit{i.e.} employing both catalyst systems CuBr/PMDETA and CuBr/Me\textsubscript{6}TREN in THF and DMF, respectively, did not yield the desired cyclic diblock copolymer 13. After application of the “click” conditions, the SEC
trace was identical to that of precursor 4. Combined with the fact that still azide signals were present in the FTIR spectrum, this implies that no reaction occurred and, accordingly, neither cyclic block copolymer nor linear chain extended polymers were formed. Supposedly, the reactive end groups were not in vicinity of each other due to incompatibility of both blocks, as a result of which no reaction occurring leading to cyclic product 13. Perhaps some other solvents suitable for both blocks can be found which circumvents microphase separation that may occur. Otherwise, linear ABA triblock copolymers have to be prepared which allow both end groups to approach one another.

Scheme 4.10 Representation of the cyclization of α-acetylene-ω-azido-polystyrene-block-poly(tert-butyl acrylate) (4). Upon hydrolysis of the pendant tert-butyl ester groups, cyclic amphiphilic polystyrene-block-poly(acrylic acid) diblock copolymer (14) is formed

4.4. Conclusions

α-Acetylene-ω-azide functionalized heterotelechelic polymers have been successfully applied as linear precursors for the synthesis of cyclic polymers utilizing “click” chemistry. Acetylene moieties were introduced in polystyrene (PS), poly(tert-butyl acrylate) (PtBA) and a polystyrene-block-poly(tert-butyl acrylate) (PS-b-PtBA) diblock copolymer using an acetylene comprising initiator. After performing the ATRP reactions, the bromide functionalities present at the other termini were converted into azides by means of nucleophilic substitution reactions, yielding the desired linear precursors.

The homopolymer precursors subsequently were cyclized by exposing them to copper-catalysts. Conducting these cyclization reaction in a dilute environment was a prerequisite in order to circumvent the formation of linear polycondensates. Therefore, solutions of the heterotelechelic polymers were added to dilute solutions containing an excess of the copper-catalyst with a rate of 0.1 mL/min using a syringe pump. In order to exclude linear chain extension of possibly present unreacted linear precursors upon work up of the polymer mixture, PPh3 was added which reduced the azide moieties. An additional
advantage of this reduction strategy is that the linear precursors could be readily removed from the cyclic analogous by applying column chromatography.

Formation of the cyclic polymers was determined with size exclusion chromatography (SEC), as a result of a reduced hydrodynamic volume on account of the cyclization. Along with the thus resulting shift towards lower molecular weight of the cyclic polymers in the SEC chromatograms, no peak at higher molecular weight was observed which would indicate the formation of linear chain extended polymers. Furthermore, with matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectrometry it was established that the molecular weights of the cyclic polymers were equal to those of their linear counterparts.

The cyclic block copolymer PS-β-PtBA, unfortunately, was not formed. A plausible explanation can be that no reaction occurred due to incompatibility of the distinct blocks as a result of which the reactive end groups were unable to approach each other.

4.5. Acknowledgements

Marieke Reijnders is gratefully acknowledged for her contribution to this chapter.

4.6. Experimental

4.6.1. Materials

Tetrabutylammonium fluoride (TBAF) (Aldrich, 1 M solution in THF), N,N,N',N',N"-pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), sodium azide (NaN₃) (Acros, 99%), silver trifluoromethanesulfonate (AgOTf) (Aldrich, >99%), 3-indoleacrylic acid (IAA) (Aldrich, ≥ 99%), 2,5-dihydroxybenzoic acid (DHB) (Fluka, >99%) and anisole (Aldrich, >99%) were used as received. Copper(II)bromide (CuBr) was purified by washing with glacial acetic acid three times and twice with diethyl ether. Triphenylphosphine (PPh₃) was recrystallized from isopropanol. Styrene and tert-butyl acrylate were distilled under reduced pressure. Tetrahydrofuran (THF) was distilled under nitrogen atmosphere from sodium/benzophenone. Dichloromethane (CH₂Cl₂) was distilled under nitrogen atmosphere from calcium hydride. N,N-dimethylformamide (DMF) was dried with magnesium sulfate and distilled under reduced pressure.

4.6.2. Instrumentation

Proton and carbon-13 nuclear magnetic resonance (¹H NMR and ¹³C NMR) spectra were recorded on a Bruker DPX300 spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane (δ = 0.00 ppm) for ¹H NMR spectra and relative to CDCl₃ (δ = 77.16 ppm) for ¹³C NMR spectra.

Infrared (IR) spectra were obtained using an AIT Matson Genesis Series FTIR spectrophotometer fitted with an ATR cell. Data are presented as the frequency of absorption (cm⁻¹).
Molecular weight distributions were measured using size exclusion chromatography (SEC) on a system equipped with a guard column and a PL gel 5 μm mixed D column (Polymer Laboratories) with differential refractive index and UV (254 nm) detection, using CHCl₃ as an eluent at 1 mL/min and T = 30°C. Polystyrene (PS) standards in the range of 162 to 6,035,000 g/mol were used to calibrate the SEC.

Thin layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV, permanganate or ninhydrin reagent.

Column chromatography was performed using silica gel, Acros (0.035-0.070 mm, pore diameter ca. 6 nm), unless otherwise stated.

Gas chromatography (GC) measurements were conducted on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary column (HP1701, 25m x 0.32mm x 0.25 μm), using flame ionization detection.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectra were measured on a Bruker Biflex III machine. 3-Indoleacrylic acid (IAA) and 2,5-dihydroxybenzoic acid (DHB) were used as matrices. If necessary, silver trifluoroacetate (AgOTf) was added as an ionizing agent. Samples were prepared by mixing 10 μL of a 40 mg/mL matrix solution, 10 μL of a 1 mg/mL polymer solution and 1 μL of a 5 mg/mL AgOTf solution. From this mixture 1 μL was spotted on a MALDI plate.

4.6.3. 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (1)

The synthesis of 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate is described in chapter 3, sections 3.5.3 and 3.5.4.

4.6.4. α-acetylene-ω-azido-polystyrene (2)

CuBr (33.8 mg, 0.24 mmol) was placed in a Schlenk flask which was fitted with a stopper. The Schlenk flask was evacuated and back-filled with argon. This procedure was repeated three times. Subsequently, the stopper was replaced by a septum. Styrene (2.10 g, 20.16 mmol), anisole (0.2 mL) and PMDETA (55.9 mg, 0.32 mmol) were added and the reaction mixture was purged for 30 minutes with argon. The reaction mixture was placed in a statically controlled oil bath at 90°C and 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (1) (904 mg, 2.50 mmol) was added. Samples were taken periodically for conversion analysis by 1H NMR. The polymerization was stopped after 150 minutes (30% conversion) by cooling and dilution with CHCl₃. The catalyst was removed by column chromatography (Acros, aluminum oxide, activated basic, 60-200 μm) using CH₂Cl₂ as an eluent. The polymer was isolated by precipitation in MeOH and vacuum dried to yield a white solid.

Yield: 1.43 g (97%); TLC: Rᵣ(CH₂Cl₂) = 0.91, Rᵣ(n-hexane/Et₂O/CH₂Cl₂ 14:3:3) = 0.42, 1H NMR (300 MHz, CDCl₃) δ 7.35-6.27 (br. m, arom. H), 4.48 (br. m, CH₂-CH(Ph)-Br), 4.05 (br. m, ≡−CH₂-O₂C), 2.58-1.17 (br. m, backbone CH₂, CH₃), 1.12-0.83 (br. m, ((CH₃)₂C)Si=≡, O₂C-C(CH₃)₂-CH₂); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 (ν≡C), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm⁻¹; SEC: Mₙ = 7.24 kg/mol; Mₘ/Mₙ = 1.09
Macrocyclic polymers

$\alpha$-(triisopropylsilyl acetylene)-ω-bromo-polystyrene (10.0 g, 2.25 mmol) was dissolved in DMF (50 mL) and, subsequently, NaN$_3$ (1.46 g, 22.5 mmol) was added. The reaction mixture was allowed to stir for 2 days at room temperature. CH$_2$Cl$_2$ was added and the reaction mixture was washed three times with distilled water. The organic layer was dried using anhydrous magnesium sulfate and concentrated in vacuo. The polymer was precipitated in MeOH to yield a white solid, which was dried under vacuum. TLC: $R_f$ (CH$_2$Cl$_2$) = 0.84, $R_f$ ($n$-hexane/Et$_2$O/CH$_2$Cl$_2$ 14:3:3) = 0.40; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.35-6.27 (br. m, arom. H), 4.18-3.77 (br. m, $\equiv-C\equiv$H$_2$-$O_2$C, CH$_2$-$C$H(Ph)-N$_3$), 2.58-1.17 (br. m, backbone CH$_2$, CH$_3$), 1.12-0.83 (br. m, ((C$_3$H$_3$)$_2$CH)$_3$Si$\equiv$O$\equiv$-$O_2$C-C(C$_3$H$_3$)$_2$-CH$_2$); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 ($\nu$C=C), 2094 ($\nu$N$_3$), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm$^{-1}$; SEC: $M_n$ = 7.23 kg/mol; $M_w$/M$_n$ = 1.08

$\alpha$-(triisopropylsilyl acetylene)-ω-azido-polystyrene (0.50 g, 0.077 mmol) was dissolved in THF (10 mL) prior to the addition of TBAF (0.8 mL, 0.8 mmol). The reaction mixture was stirred for 12 hours at room temperature. The polymer was purified using column chromatography (Acros, aluminum oxide, activated basic, 60-200 μm) using CH$_2$Cl$_2$ as an eluent and subsequent precipitation in MeOH to yield a white solid which was dried under vacuum. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.35-6.27 (br. m, arom. H), 4.18-3.77 (br. m, $\equiv-C\equiv$H$_2$-$O_2$C, CH$_2$-$C$H(Ph)-N$_3$), 2.58-1.17 (br. m, backbone CH$_2$, CH$_3$), 1.07-0.92 (br. m, O$_2$C-C(C$_3$H$_3$)$_3$), 1.13-0.85 (br. m, ((C$_3$H$_3$)$_2$CH)$_3$Si$\equiv$O$,O_2$C-C(C$_3$H$_3$)$_2$-CH$_2$); FTIR-ATR 2978, 2923, 2863, 1719, 1475, 1451, 1390, 1368, 1182, 1122, 1070, 1027 cm$^{-1}$; SEC: $M_n$ = 7.12 kg/mol; $M_w$/M$_n$ = 1.09; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 6940 ± 104.06 (64 repeating units + end groups + Ag$^+$)

4.6.5. $\alpha$-acetylene-ω-azido-poly(tert-butyl acrylate) (3)

A Schlenk tube fitted with a stopper which was loaded with CuBr (144 mg, 1.00 mmol) was evacuated and back-filled with argon. This evacuating cycle was repeated three times prior to the addition of degassed tert-butyl acrylate (10.38 g, 80.99 mmol), anisole (0.4 mL), acetone (2.4 mL) and PMDETA (178 mg, 1.03 mmol). The reaction mixture was purged with argon for five minutes and, subsequently, 3-(1,1,1-trisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (1) (340 mg, 0.94 mmol) was added. The reaction mixture was placed in an oil bath at 50°C and during polymerization samples were taken at periodic intervals for conversion analysis with GC. The polymerization was stopped after 180 minutes (51% conversion) by cooling and dilution with CHCl$_3$. The catalyst was removed by column chromatography (Acros, aluminum oxide, activated basic, 60-200 μm), using CHCl$_3$ as an eluent and subsequent precipitation in MeOH/H$_2$O (1:1) and was dried overnight in a vacuum oven at 60°C. Yield: 4.48 g (78%); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.67 (br. m, $\equiv-C\equiv$H$_2$-$O_2$C), 4.32 (br. s, CH$_2$-CH(CO$_2$tBu)-Br), 2.36-1.22 (br. m, backbone CH$_2$, CH$_3$), 1.44 (br. s, CO$_2$-C(CH$_3$)$_3$), 1.13-0.85 (br. m, ((CH$_3$)$_2$CH)$_3$Si$\equiv$O, O$_2$C-C(CH$_3$)$_3$-CH$_2$); FTIR-ATR 2978, 2923, 2863, 1719, 1475, 1451, 1390, 1368, 1254, 1139 cm$^{-1}$; SEC: $M_n$ = 5.87 kg/mol; $M_w$/M$_n$ = 1.15

$\alpha$-(triisopropylsilyl acetylene)-ω-bromo-polym(tert-butyl acrylate) (1.53 g, 0.26 mmol) was dissolved in DMF (15 mL) and NaN$_3$ (171 mg, 2.62 mmol) was added. The reaction mixture was stirred for 20 hours at room temperature. Afterwards, the
solvent was removed in vacuo. The crude product was dissolved in Et₂O and was washed five times with H₂O. The organic layer was dried using anhydrous magnesium sulfate. The reaction mixture was concentrated by rotary evaporation and the polymer was precipitated in MeOH/H₂O (1:1). The product was isolated as a sticky white solid and dried overnight in a vacuum oven at 60°C.

Yield: 0.98 g (65%); ¹H NMR (300 MHz, CDCl₃) δ 4.67 (br. m, ≡−CH₂−O₂C), 3.96 (br. s, CH₂−C₄H₉(CO₂tBu)-N₃), 2.36-1.22 (br. m, backbone CH₃, CH₂), 1.44 (br. s, CO₂-C(CH₃)₃), 1.13-0.85 (br. m, ((C₃H₉)₂C₃H)₃Si≡−O₂C-C(CH₃)₂-CH₂); FTIR-ATR 2978, 2923, 2863, 2112 (νN₃), 1719, 1475, 1451, 1390, 1368, 1254, 1139 cm⁻¹; SEC: Mn = 5.89 kg/mol; Mw/Mn = 1.15

α-((triisopropylsilyl acetylene)-ω-azido-poly(tert-butyl acrylate) (1.02 g, 0.17 mmol) was dissolved in THF (10 mL). Subsequently, TBAF (1.7 mL, 1.7 mmol) was added and the reaction mixture was stirred for 18 hours at room temperature. The polymer was purified by column chromatography (Acros, aluminum oxide, activated basic, 60-200 μm), concentrated in vacuo and was precipitated in MeOH/H₂O (1:1). The product was isolated as a white sticky solid which was dried overnight in a vacuum oven at 60°C. 

Yield: 0.70 g (71%); ¹H NMR (300 MHz, CDCl₃) δ 4.63 (br. m, ≡−CH₂−O₂C), 3.96 (br. m, CH₂−C₄H₉(CO₂tBu)-N₃), 2.43 (br. m, H−≡−CH₂), 2.36-1.22 (br. m, backbone CH₃, CH₂), 1.44 (br. s, CO₂-C(CH₃)₃), 1.05-0.94 (br. m, O₂C-C(CH₃)₂-CH₂); FTIR-ATR 2978, 2923, 2863, 2112 (νN₃), 1719, 1475, 1451, 1390, 1368, 1254, 1139 cm⁻¹; SEC: Mn = 5.71 kg/mol; Mw/Mn = 1.15; MALDI-ToF MS: matrix: DHB; m/z = 4420 ± 128.17 (33 repeating units + end groups + Na⁺)

4.6.6. α-acetylene-ω-azido-polystyrene-block-poly(tert-butyl acrylate) (4)

α-(triisopropylsilyl acetylene)-ω-bromo-polystyrene was prepared as described in section 4.6.4. 

Yield: 11.93 g (93%); ¹H NMR (300 MHz, CDCl₃) δ 7.35-6.27 (br. m, arom. H), 4.48 (br. m, CH₂−C₄H₉(Ph)-Br), 4.05 (br. m, ≡−CH₂−O₂C), 2.58-1.17 (br. m, backbone CH₃, CH₂), 1.12-0.83 (br. m, ((CH₃)₂CH)₃Si≡−O₂C-C(CH₃)₂-CH₂); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 (νC≡C), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm⁻¹; SEC: Mn = 5.88 kg/mol; Mw/Mn = 1.19

A Schlenk tube which was fitted with a stopper was loaded with CuBr (8.5 mg, 0.06 mmol) and α-(triisopropylsilyl acetylene)-ω-bromo-polystyrene (384 mg, 0.06 mmol). The Schlenk tube was evacuated and back-filled with argon. This procedure was repeated three times. Degassed tert-butyl acrylate (456 mg, 3.56 mmol) and acetone (0.12 mL) were added and the reaction mixture was stirred until all polymer was dissolved. Anisole (10 μL) as internal standard and PMDETA (11.3 mg, 0.06 mmol) were added and the reaction mixture was purged with argon for 5 minutes. Subsequently, the reaction mixture was placed in an oil bath at 60°C. During the polymerization, samples were taken periodically for conversion analysis with GC. The polymerization was stopped after 300 minutes by cooling and dilution with acetone. The polymer was purified by column chromatography (Acros, aluminum oxide, activated basic, 60-200 μm), concentrated in vacuo and precipitated in MeOH/H₂O (1:1). The product was isolated as a white solid which was dried overnight in a vacuum oven at 60°C.
Yield: 564 mg (87%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.35-6.27 (br. m, arom. H), 4.34-4.01 (br. m, \(\equiv\)-C\(_2\)H\(_2\)-O\(_2\)C), 2.45-1.15 (br. m, backbone \(\equiv\)-C\(_2\)H\(_2\), backbone C\(_2\)H\(_2\)), 1.12-0.83 (br. m, ((C\(_\equiv\)H\(_2\))\(_3\)C\(_3\)H\(_2\))\(_3\)Si\(-\equiv\), O\(_2\)C-C(C\(_3\)H\(_3\))\(_2\)-CH\(_2\)); SEC: \(M_n = 8.74\) kg/mol; \(M_w/M_n = 1.17\)

\(\alpha\)-(triisopropylsilyl acetylene)-\(\omega\)-bromo-polystyrene-block-poly(tert-butyl acrylate) (446 mg, 0.05 mmol) was dissolved in THF (5 mL). Subsequently, azidotrimethylsilane (115 mg, 1.00 mmol) and TBAF (0.5 mL, 0.5 mmol) were added and the reaction mixture was stirred for 19 hours at room temperature. Subsequently, the polymer was purified by column chromatography, concentrated in vacuo and the polymer was precipitated in MeOH/H\(_2\)O (1:1). The product was recovered as a white solid which was dried overnight in a vacuum oven at 60°C.

Yield: 346 mg (81%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.35-6.27 (br. m, arom. H), 4.36 (br. m, \(\equiv\)-C\(_2\)H\(_2\)-O\(_2\)C), 3.96 (br. s, CH\(_2\)-C\(_2\)H\(_2\)(Ph)-N\(_3\)) 2.45-1.15 (br. m, backbone \(\equiv\)-C\(_2\)H\(_2\), backbone C\(_2\)H\(_2\)), 1.06-0.95 (br. m, O\(_2\)C-C(C\(_3\)H\(_3\))\(_2\)-CH\(_2\)); FTIR-ATR 3084, 3058, 3024, 2978, 2922, 2859, 2844, 2114 (\(\nu\)N\(_3\)), 1719, 1475, 1603, 1493, 1451, 1390, 1368, 1254, 1182, 1122, 1070, 1028 cm\(^{-1}\); SEC: \(M_n = 8.69\) kg/mol; \(M_w/M_n = 1.17\)

4.6.7. cyclic polystyrene (5)

A mixture of CuBr (59.1 mg, 0.41 mmol) and PMDETA (72.4 mg, 0.42 mmol) was dissolved in THF (1 L) and heated to reflux. \(\alpha\)-Acetylene-\(\omega\)-azido-polystyrene (2) (0.26 g, 0.042 mmol) was dissolved in THF (150 mL) and added to the reaction mixture using a syringe pump with a speed of 0.1 mL/min. After 16 hours, 0.027 mmol of 2 was added. The brownish solution was refluxed for three days. PPh\(_3\) was added and the reaction mixture was refluxed for an additional day. The reaction mixture was concentrated in vacuo and the crude product was precipitated in MeOH to yield a yellow colored solid. The product was purified using column chromatography (n-heptane/CH\(_2\)Cl\(_2\) 3:2) and subsequently precipitated in MeOH. The polymer was isolated as a white solid which was dried under vacuum.

Yield: 0.10 g (40%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.35-6.27 (br. m, arom. H), 4.16-3.78 (br. m, triazole-C\(_2\)H\(_2\)-O\(_2\)C, CH\(_2\)-C\(_2\)H\(_2\)(Ph)-N\(_3\)), 2.58-1.17 (backbone \(\equiv\)-C\(_2\)H\(_2\), backbone \(\equiv\)-C\(_2\)H\(_2\)), 1.07-0.92 (br. m, O\(_2\)C-C(C\(_2\)H\(_2\))\(_2\)-CH\(_2\)); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm\(^{-1}\); SEC: \(M_n = 5.86\) kg/mol; \(M_w/M_n = 1.08\); MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 6940 ± 104.06 (64 repeating units + rest groups + Ag\(^+\))

4.6.8. cyclic poly(tert-butyl acrylate) (9)

CuBr (71.4 mg, 0.50 mmol) and Me\(_6\)TREN (12) (115 mg, 0.50 mmol) were dissolved in DMF (800 mL) and heated in an oil bath to 60°C. A solution of \(\alpha\)-acetylene-\(\omega\)-azido-poly(tert-butyl acrylate) (3) (182 mg, 0.032 mmol) in DMF (150 mL) was added to the copper complex solution using a syringe pump with a rate of 0.1 mL/min. After 16 hours, a total of 0.020 mmol of linear precursor polymer 3 was added to the reaction mixture. The reaction mixture was stirred for 2 days prior to
the addition of PPh₃. Subsequently, the reaction mixture was stirred for an additional day and the solvent was removed in vacuo. The crude product was dissolved in CH₂Cl₂ and purified by column chromatography. The polymer was isolated by precipitation in MeOH/H₂O (1:1) as a sticky white solid which was dried under vacuum.

Yield: 116 mg (64%); ¹H NMR (300 MHz, CDCl₃) δ 4.82 (br. m, triazole-C₂H₃-O₂C), 4.12 (br. s, CH₂-C(CO₂-Bu)-N), 2.36-1.22 (br. m, backbone C₂H₅, CH₂), 1.44 (br. s, CO₂-C(C₂H₅), 1.05-0.94 (br. m, O₂C-C(C₂H₅)-CH₂); FTIR-ATR 2978, 2923, 2863, 1719, 1475, 1451, 1390, 1368, 1254, 1139 cm⁻¹; SEC: Mn = 5.19 kg/mol; Mₘ/Mₙ = 1.15; MALDI-ToF MS: matrix: DHB; m/z = 4420 ± 128.17 (33 repeating units + rest groups + Na⁺)

4.7. References


Biohybrid block copolymer synthesis

The synthesis of biohybrid block copolymers was established utilizing “click” chemistry as a conjugation methodology. In the first line of research, the goal was to modularly synthesize ABA-type triblock copolymers of which the A-blocks comprised nucleobase functionality, in order to obtain telechelic precursors for the formation of supramolecular (block) copolymers. With atom transfer radical polymerization (ATRP), acetylene end functionalized oligomers containing either thymine or adenine were prepared with good control. Subsequent attachment of the thymine oligomer to \( \alpha, \omega \)-diazido-poly(ethylene glycol) (PEG) was established using CuBr/PMDETA as a catalyst. However, this “click” coupling did not succeed for the adenine oligomer, most likely owing to complexation of the adenine residues to the copper-catalyst. In the second line of research, the peptides KTVIIE and (VPGVG)\(_3\) were chosen for coupling to synthetic polymers because of their interesting properties, viz. KTVIIE is capable of forming amyloid like fibrils in an aqueous environment, whereas the latter elastin mimetic peptide displays a lower critical solution temperature (LCST). The peptides were synthesized by a solid-phase procedure and equipped with acetylene handles by reaction of pentynoic acid with the \(N\)-termini. Azide functionalized polymers were coupled to the peptides which were still attached to the resin, thereby allowing the use of an excess of polymer in order to drive the reactions to completion. These excesses could be removed afterwards by a washing step. By employing this protocol, KTVIIE was accommodated with PEG and to (VPGVG)\(_3\) polystyrene (PS) and poly(tert-butyl acrylate) (PtBA) were coupled successively. For the synthesis of this latter biohybrid triblock copolymer a triisopropylsilyl (TIPS) protecting group strategy, analogously to the methodology described in chapter 3, was applied to be able to functionalize both termini of the center PS block. Unfortunately, the yields for both biohybrid block copolymers were low due to difficulties with cleaving the products from the resin. Therefore, the properties of these biohybrids still have to be investigated.
5.1. Introduction

As pointed out in the introductory chapter, the translation of the structural control and functionality of biomolecules into biohybrid polymer structures has eventuated in the use of such conjugates in applications in the fields of medicine, nanotechnology and bioengineering. Some of these applications require the coupling of synthetic polymers to biomolecules at specific locations in order to preserve the biological properties of these biomolecules. However, this specific conjugation of synthetic- and biopolymers can be quite a difficult synthetic task since in most biomolecules, e.g. proteins and DNA, many functional groups are present. Therefore, coupling chemistry is demanded which is orthogonal with respect to these other functional groups. As stated throughout this thesis, the “click” reaction between terminal acetylenes and azides is such a highly specific coupling process. \[1\] The utilized acetylene and azide moieties are inert to most other functionalities and merely react in the presence of a copper-catalyst or at elevated temperatures. In addition to this functional group tolerance, “click” reactions can be performed in aqueous environment which opens up possibilities for bioconjugation.

On that account, “click” chemistry has been exploited for the labeling of cowpea mosaic virus (CPMV) with fluorescent dyes (scheme 5.1.a).\[2,3\] This virus consists of 60 identical copies of a two-protein asymmetric unit from which either the lysine or cysteine residues were functionalized with azide or acetylene moieties, which subsequently were employed to perform “click” reactions. In another example, as depicted in scheme 5.1.b, the cell surface of *Escherichia coli* was functionalized utilizing “click” chemistry.\[4,5\] By employing protein engineering techniques, which were discussed briefly in section 1.3, azidohomoalanine was incorporated in a protein present at the bacterium’s outer surface which acted as a handle for biotinylation via “click” chemistry. This allowed staining of the labeled bacteria with fluorescent streptavidin which, therefore, could be segregated from the unlabeled ones using flow cytometric separation.
As shown in the two examples above, adopting the copper-catalyzed cycloaddition of azides and acetylenes enables the functionalization of complex biomolecules, such as viruses and even bacteria. Along with the possibilities regarding the introduction of azide and acetylene functionality in synthetic polymers,\(^{[6,7]}\) as demonstrated in the previous three chapters, a synthetic toolbox is available for the preparation of well-defined biohybrid polymers. Recently, some examples regarding the synthesis of biohybrid polymers employing “click” chemistry have been described in literature.\(^{[8-12]}\) In scheme 5.2, an example is outlined of the preparation of a conjugate between azide bearing polystyrene (PS) and the protein bovine serum albumin (BSA) embracing an acetylene functionality.\(^{[8]}\) This acetylene moiety was introduced by reaction of a cysteine residue (Cys-34) exposed at the outside of BSA with \(N\)-propargyl maleimide, yielding a singly alkynated protein. With transmission electron microscopy (TEM) it was visualized that this biohybrid polymer self-assembled into micellar aggregates in an aqueous environment.
Chapter 5

Scheme 5.2 Synthesis of biopolymer block copolymer consisting of polystyrene and the protein bovine serum albumin (BSA). This bioconjugate displayed micelle formation in aqueous solution as illustrated in the TEM image.

In this chapter, preliminary results with respect to the preparation of biohybrid polymer architectures applying “click” chemistry are discussed. The following section deals with the synthesis of block copolymers containing nucleobase moieties. In sections 5.3 and 5.4, the synthesis of block copolymers comprising peptide blocks is described.

5.2. Nucleobase bearing block copolymers

In all the polymers discussed thus far, the distinct monomers were linked in a covalent fashion. However, when the polymer backbone is formed through secondary interactions such as hydrogen bonding or ionic interactions, a supramolecular polymer is obtained which can be readily switched between single monomeric species and polymeric state due to the reversible character of the non-covalent bonds (scheme 5.3).

Although hydrogen bonding between two neutral organic molecules is not amongst the strongest non-covalent interactions, it holds a prominent place in supramolecular chemistry because of its versatility and directionality. Lehn and co-workers were the first to build-up such a supramolecular main-chain polymer via linkage of the monomers by triple hydrogen bonding.
Scheme 5.3 Schematic representation of the supra-polymerization of bifunctional associating monomers

The strength of the interaction between the monomers has a strong influence on the degree of polymerization (DP). Therefore, in the group of Meijer the ureido-pyrimidinone (UPy) synthon was developed which is capable of forming quadruple hydrogen bonds and, as a result, has a high association constant ($K_a = 6 \cdot 10^7$ M$^{-1}$ in chloroform).\cite{15,16} As can be seen in scheme 5.4, due to the sufficiently strong and directional interaction between the UPy moieties, association of low molecular weight telechelic building blocks ($M_w < 10^3$) led to materials in which chain entanglements gave rise to polymer-like properties.\cite{17,18}

Although much progress has been made in the construction of supramolecular polymers, the present day techniques are hampered by some limitations. Most of the applied hydrogen bonding motifs are self-complementary and the association constants cannot be adjusted. In Nature, on the other hand, the cornerstone of the recognition properties of DNA is based on the complementary hydrogen bonding interaction between the pyrimidine nucleobases thymine (T) and cytosine (C), and the purine nucleobases adenine (A) and guanine (G), respectively.\cite{19} This so-called Watson-Crick base pairing, therefore, seems to be a logical choice to build up supramolecular polymer materials. Rowan and co-workers attached single nucleobase moieties to telechelic polymers which provided sufficiently strong interaction in order to induce supramolecular polymeric character in the solid state.\cite{20,21,22} Nevertheless, to be able to regulate the material properties the strength of the hydrogen bonding interaction is required to be adjustable. Craig et al. utilized natural DNA with different chain lengths in order to gain control over this interaction strength.\cite{23-25} However, because of the application of natural DNA the quantity at which these supramolecular polymers can be prepared is limited.
The functionalization of hydroxy functionalized telechelic poly(ethylene/butylene), which is a viscous oil, with quadruple hydrogen bond forming ureidopirimidinone synthons leads to the formation of a supramolecular chain extended polymer which is a rubber-like material with a Young’s modulus of 5 MPa\[^{17,18}\].

Therefore, in the research discussed in this section, a more versatile approach was chosen where natural DNA was replaced by oligomers comprising nucleobase functionality. This implies that in fact ABA-type triblock copolymers had to be synthesized from which the outer A-blocks contained nucleobase functionality. The most straightforward methodology to prepare such block copolymers is by utilizing a controlled radical polymerization technique in combination with nucleobase functionalized monomers. Unfortunately, in previous research was shown that using atom transfer radical polymerization (ATRP) along with the application of nucleobase functionalized monomers gave rise to problems during the preparation of block copolymers\[^{26}\]. For that reason, in this line of research was chosen to employ the modular synthesis methodology using “click” chemistry, as described in chapter 2.

In analogy to the research described in that chapter, first polymer precursors bearing terminal azide and acetylene functionality had to be prepared. Since to both ends of the central B-block nucleobase comprising A-blocks had to be coupled, this telechelic B-block had to be provided with two functional end groups. The most convenient way was to introduce azide end functionality to this central block. Therefore, acetylene functionality was introduced to the outer nucleobase blocks.

The first step towards these terminal acetylene functionalized nucleobase oligomers was to accommodate nucleobases with polymerizable handles. As depicted in scheme 5.5, first...
3-bromopropylmethacrylate (1) was prepared by treatment of commercially available 3-bromo-1-propanol with methacryloyl chloride.\[^{[27]}\] Subsequently, thymine and adenine were alkylated in moderate yields using 3-bromopropylmethacrylate (1) and K\(_2\)CO\(_3\) and NaH as bases, respectively (scheme 5.5).\[^{[28]}\] These moderate yields were caused by the occurrence of side reactions such as dialkylation at both the \(N1\) and \(N3\) positions in case of thymine monomer 2, this side product was isolated and confirmed by \(^1\)H NMR, and possible Michael addition of the formed nucleobase anions to the methacrylate moiety of 1. Nonetheless, both monomers could be purified by column chromatography.

![Scheme 5.5 Synthesis of thymine 2 and adenine 3 bearing methacrylate monomers. Reagents and conditions: i. 3-bromo-1-propanol, Et\(_3\)N, CH\(_2\)Cl\(_2\), 0\(^\circ\)C→rt., 1.5 h, 75%; ii. 1, K\(_2\)CO\(_3\), TBAI, DMF, 5 d, rt., 40%; iii. 1, NaH, DMF, rt.→40\(^\circ\)C, 50%](image)

Thymine monomer 2 as well as adenine monomer 3 were polymerized under ATRP conditions using CuCl/2,2'-bipyridine (bpy) as the catalyst (scheme 5.6). In this case CuCl was chosen, whereas in all previous polymerizations CuBr was utilized, to improve control over the polymerization process. The copper-chloride bond being more stable than the corresponding copper-bromide bond, using a bromide functionalized initiator in combination with CuCl as the catalyst system leads to a fast deactivation of the polymerization, resulting in a decrease in propagation rate (\(k_p\)).\[^{[29]}\] This halogen exchange process had to be applied because of the large \(k_p\) of both monomers, even at ambient temperature for 2, which probably was caused by stacking of the nucleobase monomers along with the application of the polar solvent dimethyl sulfoxide (DMSO), which was necessary for solubility.
Scheme 5.6 ATRP of nucleobase functionalized methacrylate monomers 2 and 3 utilizing acetylene bearing initiator 4

For both polymerizations deuterated DMSO was adopted to allow monitoring of the reaction kinetics with $^1$H NMR by comparison of the methacrylate proton at 5.99 ppm with the signal at 7.51 ppm stemming from the thymine H6 proton (N-CH=) for thymine monomer 2, and for adenine monomer 3 by comparing the methacrylate proton at 5.91 ppm with the combined signals of purine H2 and H8 at 8.11 and 8.14 ppm, respectively. As can be seen in figure 5.1, both ATRP reactions proceeded according to first order kinetics. As a consequence, the polydispersity indices were reasonably low ($M_w/M_n = 1.23$ for 5 and $M_w/M_n = 1.19$ for 6). After polymerization, the trimethylsilyl (TMS) was removed quantitatively using tetrabutylammonium fluoride (TBAF), as determined with $^1$H NMR spectroscopy.
Biohybrid block copolymer synthesis

Figure 5.1 First order kinetic plots for the polymerization of 3-(thymin-1-yl)propyl methacrylate (2) (△) and 3-(adenin-9-yl)propyl methacrylate (3) (●); (△) \([2]_0=0.40 \text{ M}, [\text{CuCl}]_0=[\text{bpy}]_0/2=[4]_0=0.040 \text{ M}; (●) \[3]_0=0.40 \text{ M}, [\text{CuCl}]_0=[\text{bpy}]_0/2=[4]_0=0.040 \text{ M}\)

As the center B-block was chosen for azide bifunctional poly(ethylene glycol) (PEG) (\(M_n = 10.0 \text{ kg/mol}\) (7), which was synthesized by tosylation of the hydroxyl termini and subsequent substitution with sodium azide, as discussed in chapter 2 (scheme 5.7).

\[\begin{align*}
\text{HO-(-O-)}_n\text{-OH} & \xrightarrow{i, ii} \text{HO-(-O-)}_n\text{-O-} \xrightarrow{\text{NaN}_3, \text{DMF, rt.}} \text{HO-(-O-)}_n\text{-N}_3
\end{align*}\]

Scheme 5.7 Preparation of telechelic azide functionalized PEG (7). Reagents and conditions: i. TsCl, pyridine, rt., 18 h, 81%; ii. NaN_3, DMF, rt., 21 h, 79%

The last step in order to modularly prepare the ABA triblock copolymers containing nucleobase functionality was to couple the formed blocks via “click” reactions. The first attempt to couple oligo-thymine 5 and α,ω-diazo-PEG (7) utilizing CuI and 1,8-diaza[5.4.0]bicycloundec-7-ene (DBU) as a catalyst in N,N-dimethylformamide (DMF) as a solvent failed. After 20 hours of reaction, no formed product could be detected with size exclusion chromatography (SEC). A probable cause could be complexation of the thymine moieties to the copper catalyst, which accordingly was deactivated. Therefore, this coupling was performed utilizing the more stable CuBr/N,N,N',N',N”-pentamethyldiethylenetriamine (PMDETA) catalyst complex (scheme 5.8) at 35°C. As can be seen in the SEC traces in figure 5.2, in this case a product was formed at higher molecular weight. It has to be noted that an excess of the thymine oligomer was used (2.4 equivalents) which was not removed. Because of this residual starting material, the PDI of formed triblock copolymer 5-b-7-b-5 was higher than from the individual precursors
However, in the FTIR spectrum of the reaction mixture no azide peak was observed which, in combination with the SEC results, led to the conclusion that triblock copolymer 5-b-7-b-5 was formed. Unfortunately, due to the small scale on which this reaction was executed it was not possible to purify the product.

Scheme 5.8 Formation of poly[3-(thymin-1-yl)propyl methacrylate]-block-poly(ethylene glycol)-block-poly[3-(thymin-1-yl)propyl methacrylate] (5-b-7-b-5). Reagents and conditions: i. CuBr, PMDETA, DMF, 22 h, 35°C

A reaction between oligo-adenine 6 and α,ω-diazido-PEG (7) was carried out under the same conditions, viz. using CuBr/PMDETA as a catalyst in DMF as a solvent at 35°C, yet in this case no reaction was observed in the SEC chromatogram of the reaction mixture. Presumably, the adenine residues associated even stronger to the copper(I)-species than thymine, thereby inactivating the catalyst. Subsequent attempts at elevated temperature, unfortunately, also did not yield the desired triblock copolymer. Therefore, it is worthwhile to perform this reaction utilizing Me6TREN as a ligand for the copper species since it is known to form a stable complex. The fact that in section 3.3.1 it was shown that this complex is a suitable catalyst to enforce “click” reactions supports this assumption. Subsequently, the precursor triblock copolymers comprising complementary nucleobase moieties can be utilized to construct supramolecular block copolymers by assembly of stoichiometric amounts of these building blocks. Ultimately, supramolecular multiblock copolymers can be built up by employment of both complementary pairs of nucleobases.
Figure 5.2 SEC traces of acetylene functionalized thymine oligomer 5 and the formed ABA-type triblock copolymer 5-b-7-b-5 measured in DMSO using UV detection

5.3. Amyloid peptide modified biohybrid block copolymer

Under certain circumstances, soluble proteins or protein fragments have the ability to spontaneously aggregate into β-amyloid fibrillar structures. This fibril formation is the major cause of several disorders, such as Alzheimer’s disease[30,31] and diabetes type II.[32] Apart from hydrogen bonding, hydrophobic interactions play an important role in this fibril formation process as well, as it occurs in an aqueous environment. Based on this phenomenon, it has been shown that fibrils can be stabilized by increasing the hydrophobicity by means of the attachment of alkyl tails to fibrillar peptides.[33] It is interesting to examine what will happen to the fibril formation process when the hydrophobicity is drastically altered by conjugation of hydrophobic and hydrophilic polymer chains.

In order to investigate this effect, polymer chains have to be attached to peptides which are capable of forming fibrils in an aqueous environment. The conjugation of polymer and peptide will be established utilizing the “click” reaction between azides and acetylenes.[1] As a model fibrillar peptide was chosen for the hexapeptide H-KTVIIE-NH₂ (K = Lys = lysine, T = Thr = threonine, V = Val = valine, I = Ile = isoleucine, E = Glu = glutamic acid).[34] As illustrated in scheme 5.9, peptide 8 was synthesized on the solid phase using 9-fluorenylmethoxy carbamate (Fmoc) protecting chemistry and a Breipohl resin. Subsequent to coupling of all amino acids, the N-terminus was functionalized with pentynoic acid, providing terminal acetylene functionality.
In order to functionalize KTVIIIE (8), α-methoxy-ω-azido-poly(ethylene glycol) (9) (Mₙ = 2.0 kg/mol) was used, which was prepared by tosylation of the hydroxyl terminus followed by substitution using sodium azide (scheme 5.10).

Azide terminated PEG 9 was coupled via a “click” reaction to peptide 8 which was still attached to the resin (scheme 5.11), which enabled the application of an excess of polymer, thereby assuring complete conversion of all acetylenes present. As a copper-catalyst, CuI in combination with N,N-diisopropylethyl amine (DiPEA) was employed. After reaction, biohybrid product 10 was readily purified by filtration and an extensive washing procedure of the resin. Subsequently, the product was deprotected and cleaved from the resin by acidolysis via treatment with trifluoroacetic acid (TFA), and was isolated by precipitation in diethyl ether (Et₂O).
Scheme 5.11 Preparation of peptide-polymer hybrid 10 by solid-phase “click” coupling. Reagents and conditions: i. CuI, DiPEA, THF, rt., 18 h; ii. TFA/H₂O (95:5), 4 h, 43%

Formation of PEG-b-KTVIIE (10) was confirmed by thin layer chromatography (TLC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS). TLC analysis (CHCl₃/MeOH/H₂O 65:25:4) displayed the formation of a new product in the form of a stripe on the TLC plate, with the center of the stripe having an Rₜ value of 0.3. Furthermore, this spot colored readily with iodine, which is characteristic for PEG, and with ninhydrin, indicating the presence of the peptide block. Additionally, the bare peptide 8 did not elute at all using this eluent, whereas PEG 9 eluted with an Rₜ value of approximately 1. As can be seen in the MALDI-ToF MS spectra in figure 5.3, the molecular weight distribution shifted towards higher molecular weight upon attachment of peptide 8. This difference in mass corresponds to the molecular weight of the peptide. The second distribution which was present could be attributed to the complexation of two sodium ions instead of one (see inset figure 5.3).
Unfortunately, the amount of material isolated was insufficient to investigate the properties of the PEG-\textit{b}-KTVIIE (10) biohybrid diblock copolymer. In following research, however, was found that attachment of PEG tails to the fibril forming peptide prevented it from forming fibrils.\cite{35} By performing circular dichroism (CD) spectroscopy measurements it was established that PEG-\textit{b}-KTVIIE was constituted in a random coil conformation, whereas the bare peptide exhibited $\beta$-sheet character which indicated fibril formation. Additionally, transmission electron microscopy (TEM) images of the diblock copolymer displayed no fibril formation.

**5.4. Tropoelastin comprising biohybrid triblock copolymer**

A second topic of investigation concerned a peptide-polymer hybrid which was based on one of the most important types of naturally occurring structural proteins, namely elastin.\cite{36,37} Out of many distinct types of elastin, tropoelastin, which is the precursor protein of mammalian elastin, is one of the best studied species. Tropoelastin mainly consists of repeats of the amino acid sequence VPGVG ($V = Val =$ valine, $P = Pro = $ proline, $G = Gly = $ glycine).\cite{38-40} One of the most pronounced properties of poly(VPGVG) is the exhibition of a lower critical solution temperature (LCST).\cite{41} This implies that the polypeptide is water-soluble at room temperature and upon temperature increase it precipitates abruptly due to an increased hydrophobicity. The sudden change in polarity is caused by expelling water molecules which are bound to the hydrophobic side chains of the polypeptide. It has been shown that incorporation of VPGVG fragments
into synthetic polymers leads to materials that have the ability to reversibly precipitate out of solution.\cite{42,43}

The rationale behind utilizing this remarkable peptide here is to explore the possibilities of preparing temperature responsive block copolymer aggregates that are capable of reversibly changing morphology by exploiting the LCST behavior of VPGVG. Therefore, the amphiphilic block copolymer poly(acrylic acid)-block-polystyrene-block-(VPGVG)\textsubscript{3} was synthesized according to the strategy described in chapter 3, \textit{viz.} by “click” coupling of the three distinct blocks. The hydrophilic/hydrophobic ratio of this biohybrid block copolymer is temperature dependent owing to the presence of the (VPGVG)\textsubscript{3} block and, presumably, will lead to different aggregate morphologies above and under its LCST.

Peptide \textit{11}, comprising three repeats of VPGVG and an acetylene moiety on its N-terminus, was synthesized on a Wang resin utilizing a standard Fmoc strategy (scheme 5.12). Subsequent to coupling of the 15 amino acids, pentynoic acid was attached to the outermost positioned valine.

\begin{center}
\begin{figure}
\includegraphics[width=\textwidth]{scheme_5_12.png}
\caption{Scheme 5.12 Solid-phase synthesis of acetylene functionalized (VPGVG)\textsubscript{3} (\textit{11}). Reagents and conditions: i. Fmoc-Xxx, DIPCDI, HOBt, DMF; ii. piperidine, DMF; iii. pentynoic acid, DIPCDI, HOBt, DMF, 18 h}
\end{figure}
\end{center}

\textit{α}-(Triisopropylsilyl acetylene)-\textit{ω}-azide functionalized polymers were prepared according to the methodology described in chapters 3 and 4. Heterotelechelic polystyrene (PS) \textit{13} and poly(\textit{tert}-butyl acrylate) (P\textit{t}BA) \textit{14} were prepared employing protected acetylene functionalized initiator \textit{12}, followed by substitution of the bromide end groups for azides (scheme 5.13). As opposed to direct synthesis of poly(acrylic acid) (PAA) it was chosen to initially prepare P\textit{t}BA, of which the pendant \textit{tert}-butyl ester moieties can be hydrolyzed readily upon formation of the desired carboxylic acid functionalities. The reason for this indirect strategy is that using acrylic acid as a monomer in ATRP leads to inactivation of the copper catalyst due to protonation of the nitrogen based ligands, thereby disrupting their coordination to the copper center.\cite{44,45} Analogous to the polymers prepared in chapter 3, the polymerizations proceeded in a controlled fashion in accordance with first order kinetics. Consequently, the polydispersities (PDI\textsubscript{S}) were low (M\textsubscript{w}/M\textsubscript{n} = 1.11 for \textit{13} and M\textsubscript{w}/M\textsubscript{n} = 1.12 for \textit{14}). Complete conversion of the bromide end functionality into azides was confirmed by upfield shifts of the protons adjacent to the end groups in \textit{1H} NMR spectra and the presence of azide signals in the FTIR spectra.
Scheme 5.13 Preparation of α-(TIPS-acetylene)-ω-azide functionalized PS (13) and PtBA (14)

In order to prepare the biohybrid block copolymer PAA-b-PS-b-(VPGVG)₃ (15), first α-(TIPS-acetylene)-ω-azido-PS (13) was coupled to acetylene functionalized (VPGVG)₃ utilizing a complex of CuBr and N,N,N',N',N''-pentamethyldiethylenetriamine (PMDETA) as the copper(I)-catalyst. This catalyst was chosen because it has been proven to be an active catalyst, as found in chapter 3. As illustrated in scheme 5.14, the peptide was still attached to the resin, which allowed the application of an excess of PS 13 to assure complete conversion and, subsequently, facile purification of the formed PS-b-(VPGVG)₃ (15). A small portion of the product was cleaved from the resin for analysis by treatment with TFA/CH₂Cl₂/TIS/H₂O (45:45:5:5). With SEC it was found that the apparent molecular weight of biohybrid product 15 decreased in comparison to precursor PS 13, as depicted in figure 5.4. Furthermore, the PDI increased from 1.11 to 1.47, as a result of tailing on the low molecular weight side, i.e. high retention times in the chromatogram. This tailing probably was caused by interaction of hybrid diblock copolymer 15 with the column during the measurement, which perturbs the size exclusion process. In contrast to the SEC results, with MALDI-ToF MS an increase in molecular weight was observed as compared to PS 13 corresponding to the molecular weight of the peptide (figure 5.5). In the MALDI-ToF MS spectrum of biohybrid 15, two more
distributions were present stemming from the corresponding sodium and potassium salts, as can be seen in the inset of figure 5.5.

Scheme 5.14 Synthesis of biohybrid triblock copolymer 17. Reagents and conditions: i. CuBr, PMDETA, DMF, 20 h; ii. TBAF, DMF, 19 h; iii. TFA/CH2Cl2/TIS/H2O (45:45:5:5), 15 h, 7% (overall)

Figure 5.4 SEC traces of precursor PS 13, and the biohybrid di- (15) and triblock copolymers (17) bearing the peptide (VPGVG)3

Figure 5.4 SEC traces of precursor PS 13, and the biohybrid di- (15) and triblock copolymers (17) bearing the peptide (VPGVG)3.
In a second step, the hydrophilic PAA block was introduced by “clicking” terminally azide functionalized PtBA 14 to PS-\-b-(VPGVG)\_3 15. Prior to this “click” reaction, the acetylene functionality of the PS block was deprotected by treatment with tetrabutylammonium fluoride (TBAF) using DMF as a solvent. After conjugation of the PtBA block, the product was cleaved from the resin by applying a mixture of TFA/CH\_2Cl\_2/TIS/H\_2O (45:45:5:5). Owing to these acidic conditions, the pendant tert-butyl esters were hydrolyzed, yielding the PAA-\-b-PS-\-b-(VPGVG)\_3 biohybrid triblock copolymer 17. Unfortunately, the yield of the isolated product was very low (7\%), which was caused by difficulties with cleaving the product from the resin. Even after extensive washing of the resin, no more material was obtained. A plausible explanation can be that the attached polymers shielded the cleavage site by back-folding into the resin, as a result of which the product remained attached to the solid phase. Therefore, other cleaving conditions have to be developed to improve the yield. Otherwise the reactions have to be conducted in solution phase. Perhaps the LCST behavior of the peptide can be exploited to purify the product.

An indication of the formation of PAA-\-b-PS-\-b-(VPGVG)\_3 (17) was acquired by performing SEC. As can be seen in the chromatogram depicted in figure 5.4, the molecular weight of the triblock copolymer now increased in comparison to the PS-\-b-(VPGVG)\_3 diblock copolymer (15). Yet tailing of the peak was still observed due to interaction of the biohybrid material with the column. Unfortunately, no MALDI-ToF
MS results were obtained to provide additional information concerning formation of the triblock copolymer.

5.5. Conclusions

Initial research regarding the synthesis of biohybrid block copolymers employing “click” chemistry has been conducted. In order to prepare supramolecular (block co)polymers based on nucleobase interaction, both thymine and adenine oligomers bearing acetylene end functionality were synthesized in good control employing atom transfer radical polymerization (ATRP). In a next step, the thymine oligomer was coupled to diazide functionalized telechelic poly(ethylene glycol) (PEG) using CuBr/PMDETA as a catalyst. Formation of the resulting ABA-type triblock copolymer was confirmed by the appearance of a higher molecular weight polymer in the size exclusion chromatogram along with the absence of a residual azide signal in the FTIR spectrum. Unfortunately, applying equal conditions did not lead to the formation of the adenine containing triblock copolymer. Probably, this “click” coupling failed due to inactivation of the copper-catalyst by complexation to the adenine residues.

In a second line of research, the fibril forming peptide KTVIIE and the peptide (VPGVG)₃, which possesses a lower critical solution temperature (LCST), were synthesized applying a solid-phase strategy and provided with acetylene handles by reaction of pentynoic acid with the N-termini of these peptides.

Hereafter, azide terminated polymers were conjugated to these acetylene functionalized peptides by applying a copper(I)-catalyst, while the peptides were still attached to the resin. This “click” reaction on the solid phase was adopted to enable the use of an excess of polymer, which was removed readily by a washing step. In this way, PEG-ₖ-KTVIIE and poly(acrylic acid)-block-polystyrene-block-(VPGVG)₃ (PAA-ₖ-PS-ₖ-(VPGVG)₃) were prepared. Unfortunately, owing to difficulties by cleaving the products from the resin, the yields of both biohybrid block copolymers were low, in particular of the latter one. Further experiments have to be performed in order to investigate the properties of these materials.
5.6. Acknowledgements

Dr. Henri Spijker is acknowledged for his contribution to the synthesis of the nucleobase monomers. Marjolijn Roeters and Hans Adams are acknowledged for the synthesis of the peptides KTVIIE and (VPGVG)_3, respectively.

5.7. Experimental

5.7.1. Materials

Wang resin (Bachem, 200-400 mesh, loading 0.9-1.2 mmol/g), 9-fluorenlymethoxy carbamate (Fmoc) and tert-butyl carbamate (Boc) protected lysine (Fmoc-Lys(Boc)-OH) (Bachem, >99%), Fmoc-threonine (Fmoc-Thr-OH) (Bachem, >99%), Fmoc-valine (Fmoc-Val-OH) (Bachem, >99%), Fmoc-isoleucine (Fmoc-Ile-OH) (Bachem, >99%), Fmoc and tert-butylester (O

5.7.2. Instrumentation

Proton and carbon-13 nuclear magnetic resonance (1H NMR and 13C NMR) spectra were recorded on a Bruker DPX300 spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane (δ = 0.00 ppm) for CDCl₃ or to the solvent signal for DMSO-d₆ (δ = 2.50 ppm). Infrared (IR) spectra were obtained using an ATI Matson Genesis Series FTIR spectrophotometer fitted with an ATR cell. Data are presented as the frequency of absorption (cm⁻¹). Molecular weight distributions were measured using size exclusion chromatography (SEC) on a system equipped with a guard column and a PL gel 5 μm mixed D column (Polymer Laboratories) with differential refractive index and UV (254 nm) detection, using THF as an eluent at 1 mL/min and T = 35°C. Polystyrene (PS) standards in the range of 162 to 6,035,000 g/mol were used to calibrate the SEC.
Thin layer chromatography (TLC) was carried out on *Merck* precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV, iodine, permanganate or ninhydrin reagent.

Column chromatography was performed using silica gel, *Acros* (0.035-0.070 mm, pore diameter ca. 6 nm), unless otherwise stated.

Gas chromatography (GC) measurements were conducted on a *Hewlett-Packard 5890 Series II* gas chromatograph equipped with a capillary column (*HP1701, 25m x 0.32mm x 0.25μm*), using flame ionization detection.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF MS) mass spectra were measured on a *Bruker Biflex III* machine. 3-Indoleacrylic acid (IAA), 2,5-dihydroxybenzoic acid (DHB) and dithranol were used as matrices. If necessary, silver trifluoroacetate (AgOTf) was added as an ionizing agent. Samples were prepared by mixing 10 μL of a 40 mg/mL matrix solution, 10 μL of a 1 mg/mL polymer or peptide solution and 1 μL of a 5 mg/mL AgOTf solution. From this mixture 1 μL was spotted on a MALDI plate.

The peptides were synthesized on a *Labortec SP4000* and a *Labortec SP640* semiautomatic peptide synthesizer.

### 5.7.3. 3-bromopropyl methacrylate\(^{[27]}\) (1)

![3-bromopropyl methacrylate](image)

A solution of methacryloyl chloride (21.5 mL, 0.22 mol) in CH\(_2\)Cl\(_2\) (50 mL) was added dropwise to a solution of 3-bromo-1-propanol (27.3 g, 0.20 mol) and Et\(_3\)N (30.5 mL, 0.22 mol) in CH\(_2\)Cl\(_2\) (250 mL) at 0°C. After complete addition, the reaction mixture was allowed to stir for 1.5 hours at room temperature. Completion of the reaction was determined by TLC (heptane/EtOAc 9:1).

The unreacted methacryloyl chloride was quenched by addition of MeOH. The reaction mixture was washed three times with a saturated sodium bicarbonate solution and twice with distilled water. The organic layer was dried using anhydrous magnesium sulfate and, subsequently, the solvents were removed *in vacuo*. The product was purified by vacuum distillation (30°C, 131 mTorr) and isolated as a colorless oil.

Yield: 31.2 g (75%); TLC: Rf = 0.34 (heptane/EtOAc 9:1); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 6.17\) (dt, 1H, \(\textit{J} = 1.64\) Hz, \(\textit{J'} = 0.96\) Hz, O\(_2\)C-C(CH\(_3\))=C\(_2\)H\(_2\)), 5.58 (dt, 1H, \(\textit{J} = 1.64\) Hz, \(\textit{J'} = 1.51\) Hz, O\(_2\)C-C(CH\(_3\))=C\(_2\)H\(_2\)), 4.29 (t, 2H, \(\textit{J} = 6.17\) Hz, CH\(_2\)-C\(_2\)H\(_4\)), 3.51 (t, 2H, \(\textit{J} = 6.58\) Hz, Br-C\(_2\)H\(_5\)-CH\(_2\)), 2.24 (m, 2H, CH\(_2\)-C\(_2\)H\(_4\)-CH\(_2\)), 1.95 (m, 3H, O\(_2\)C-C(CH\(_3\))=CH\(_2\)); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 166.96\) (O-\(\text{C} (=\text{O})\)-C(CH\(_3\))=CH\(_2\)), 136.05 (O\(_2\)C-C(CH\(_3\))=CH\(_2\)), 125.63 (O\(_2\)C-C(CH\(_3\))=CH\(_2\)), 62.55 (CH\(_2\)-C\(_2\)H\(_4\)-O\(_2\)C), 32.04 (Br-C\(_2\)H\(_4\)-CH\(_2\)), 29.70 (CH\(_2\)-CH\(_2\)-CH\(_2\)), 18.63 (O\(_2\)C-C(CH\(_3\))=CH\(_2\)); FTIR-ATR 1716 (\(\nu_{\text{o-c, ester}}\), cm\(^{-1}\))

### 5.7.4. 3-(thymin-1-yl)propyl methacrylate\(^{[28]}\) (2)

![3-(thymin-1-yl)propyl methacrylate](image)

Thymine (3.79 g, 30.0 mmol) was dissolved in DMF. Subsequently, K\(_2\)CO\(_3\) (4.15 g, 30.0 mmol) and TBAI (0.74 g, 2.01 mmol) were dispersed in the reaction mixture. 3-bromopropyl methacrylate (1) (5.10 g, 24.6 mmol) was slowly added *via* a syringe. The reaction mixture was stirred for five days at room temperature. Completion of the reaction was determined by TLC (EtOAc/heptane 4:1). Saturated NH\(_4\)Cl solution and distilled water were added to the reaction mixture and the product was extracted with EtOAc. The organic layer was dried using anhydrous magnesium sulfate. Solvents were removed *in vacuo*. Last traces of DMF were removed by azeotropic distillation with toluene. The crude product was
purified by column chromatography (EtOAc/heptane 4:1) and isolated as a white solid which was dried under vacuum.

Yield: 2.46 g (40%); TLC: Rf (n-heptane/EtOAc 1:4) = 0.30; 1H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 11.20 (br. s, 1H, C(=O)-N\(\text{H}-\)C(=O)), 7.51 (s, 1H, C=C(CH\(_3\)=C)), 5.99 (dt, 1H, \(J = 1.64\) Hz, \(J = 0.96\) Hz, O\(\text{C}-\)C(CH\(_3\)=CH\(_2\))=CH\(_2\)), 5.66 (dt, 1H, \(J = 1.64\) Hz, \(J = 1.51\) Hz, O\(\text{C}-\)C(CH\(_3\)=C)), 4.11 (t, 2H, \(J = 6.80\) Hz, N-C(CH\(_3\)=C-H), 1.95 (m, 2H, CH\(_2\)-C(CH\(_3\)=C-H), 1.86 (dd, 3H, \(J = 1.51\) Hz, \(J = 0.96\) Hz, O\(\text{C}-\)C(CH\(_3\)=C-H), 1.73 (d, 3H, \(J = 0.68\) Hz, C(CH\(_3\)=C-H), 1.58 (s, 3H, O\(\text{C}-\)C(CH\(_3\)=C-H)), 1.46 (d, 3H, \(J = 0.68\) Hz, CH\(_3\)-C=CH-N); 13C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 166.42 (O\(\text{C}-\)C(CH\(_3\)=C-H)), 164.26 (purine C\(_5\)), 150.90 (purine C\(_8\)), 141.37 (purine C\(_6\)), 135.76 (purine C\(_3\)), 125.67 (purine C\(_1\)), 108.50 (purine C\(_2\)), 62.06 (CH\(_2\)-C(CH\(_3\)=C-H)), 44.83 (N-C(CH\(_3\)=C-H)), 27.47 (CH\(_2\)-C(CH\(_3\)=C-H)), 17.90 (O\(\text{C}-\)C(CH\(_3\)=C-H)), 11.90 (H(C-H)=CH-N); FTIR-ATR 1716, 1685, 1664 cm\(^{-1}\)

5.7.5. 3-(adenin-9-yl)propyl methacrylate\(^{[28]}\) (3)

Adenine (4.05 g, 30.0 mmol) was dispersed in DMF. Subsequently, sodium hydride (0.72 g, 29.9 mmol) was added and the reaction mixture was stirred for one hour at room temperature. 3-bromopropyl methacrylate (1) (4.13 g, 20.0 mmol) was added slowly via a syringe and the reaction mixture was stirred for 20 hours at room temperature and, additionally, for two hours at 40°C. Completion of the reaction was determined by TLC (CH\(_2\)Cl\(_2\)/MeOH 9:1). The excess of adenine was filtered off and DMF was removed in vacuo. The crude product was dissolved in CH\(_2\)Cl\(_2\) and washed once with saturated NH\(_4\)Cl solution and three times with distilled water. The organic layer was dried using anhydrous magnesium sulfate and solvent removed by rotary evaporation. The crude product was purified by column chromatography (CH\(_2\)Cl\(_2\)/MeOH 9:1), yielding a white solid which was dried under vacuum.

Yield: 2.61 g (50%); TLC: Rf (CH\(_2\)Cl\(_2\)/MeOH 9:1) = 0.37; m.p. = 133.6°C; 1H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 8.14 (s, 1H, purine H\(_2\)), 8.11 (s, 1H, purine H\(_8\)), 7.20 (br. s, 2H, N\(\text{H}-\)C(CH\(_3\)=CH\(_2\))), 5.91 (dt, 1H, \(J = 6.03\) Hz, N-C(CH\(_3\)=C(H\(_2\))=CH\(_2\))), 4.25 (t, 2H, \(J = 6.66\) Hz, CH\(_2\)-C(CH\(_3\)=C-H)), 4.08 (t, 2H, \(J = 6.03\) Hz, N-C(CH\(_3\)=C(H\(_2\))=CH\(_2\))), 1.83 (s, 3H, O\(\text{C}-\)C(CH\(_3\)=C-H)), 1.46 (d, 3H, \(J = 0.68\) Hz, CH\(_3\)-C=CH-N); 13C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 155.93 (purine C\(_5\)), 152.36 (purine C\(_8\)), 149.58 (purine C\(_6\)), 148.70 (purine C\(_1\)), 135.76 (purine C\(_3\)), 125.67 (purine C\(_2\)), 118.80 (purine C\(_4\)), 61.86 (CH\(_2\)-C(CH\(_3\)=C-H)), 40.33 (N-C(CH\(_3\)=C-H)), 28.39 (CH\(_2\)-C(CH\(_3\)=C-H)), 17.86 (O\(\text{C}-\)C(CH\(_3\)=C-H)); FTIR-ATR 1697, 1651, 1639, 1593, 1574 cm\(^{-1}\)

5.7.6. 3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methylpropanoate (4)

The synthesis of 3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methylpropanoate is described in chapter 2, section 2.6.4.

5.7.7. poly[3-(thymin-1-yl)propyl methacrylate (5)

A Schlenk tube was loaded with CuCl (14.8 mg, 0.15 mmol), bpy (46.9 mg, 0.30 mmol) and thymine monomer 2 (379 mg, 1.50 mmol). This was followed by performing three cycles of evacuating and back-filling with argon. After these cycles, DMSO-\(d_6\) (3.75
mL) was added and the reaction mixture was purged with argon for 5 minutes. Subsequently, acetylene functionalized initiator 4 (41.7 mg, 0.15 mmol) was added using a syringe. Samples were taken periodically for conversion analysis by $^1$H NMR. The polymerization was stopped after 190 minutes (79% conversion) by cooling and dilution with DMSO. The TMS protected acetylene functionalized polymer was precipitated in a 0.055 M EDTA solution, filtrated and extensively washed with a 0.055 M EDTA solution. The polymer was isolated as a white solid which was dried under vacuum.

Yield: 283.4 mg (83%); SEC (DMSO): $M_n = 2.5$ kg/mol; $M_w/M_n = 1.23$ ($M_{\text{theo}} = 2.2$ kg/mol); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.21 (br. s, C(=O)-N=H-C(=O)), 7.52 (br. s, C=CH-N), 4.82 (br. s, $\equiv$-CH$_3$O,C), 4.32-3.60 (br. m, CH$_2$-C=CH$_2$, N-C=CH$_3$, CH$_2$-CH$_2$-CH$_2$, O$_2$C-C(C(CH$_3$)$_2$)), 0.15 (br. s, (CH$_3$)$_3$Si).

The TMS protected acetylene functionalized polymer (103 mg, 0.046 mmol) was dissolved in DMF (4 mL) and, subsequently, TBAF (0.4 mL, 0.4 mmol) was added. The reaction mixture was stirred for 19 hours at room temperature. Polymer 5 was isolated by precipitation in Et$_2$O and drying under vacuum.

Yield: 90.1 mg (91%); SEC (DMSO): $M_n = 2.4$ kg/mol; $M_w/M_n = 1.23$; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.21 (br. s, C(=O)-N=H-C(=O)), 7.52 (br. s, C=CH-N), 4.66 (br. m, $\equiv$-CH$_3$O,C), 4.32-3.60 (br. m, CH$_2$-CH$_2$O, N-C=CH$_2$), 2.27 (br. m, H-$\equiv$), 2.20-0.58 (br. m, backbone CH$_2$, CH$_2$C=CH-N, CH$_2$-CH$_2$-CH$_2$, O$_2$C-C(C(CH$_3$)$_2$)).

5.7.8. poly[3-(adenin-9-yl)propyl methacrylate] (6)

CuCl (14.9 mg, 0.15 mmol), bpy (47.0 mg, 0.30 mmol) and adenine monomer 3 (392 mg, 1.50 mmol) were placed in a Schlenk tube, evacuated and back-filled with argon. This cycle was repeated three times. Subsequently, DMSO-$d_6$ (3.75 mL) was added and the reaction mixture was purged with argon for 5 minutes. The reaction mixture was placed in an oil bath at 35°C and acetylene functionalized initiator 4 (41.6 mg, 0.15 mmol) was added via a syringe. Samples were taken periodically for conversion analysis by $^1$H NMR. The polymerization was stopped after 240 minutes (73% conversion) by cooling and dilution with DMSO. The TMS protected acetylene functionalized adenine polymer was precipitated in a 0.055 M EDTA solution, filtrated and washed extensively. The polymer was isolated as a white solid and dried under vacuum.

Yield: 325.3 mg (88%); SEC (DMSO): $M_n = 2.4$ kg/mol; $M_w/M_n = 1.19$ ($M_{\text{theo}} = 2.2$ kg/mol); $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.22-7.96 (br. m, purine H$_2$, H$_8$), 7.21 (br. s, NH$_2$), 4.74 (br. s, $\equiv$-CH$_3$O,C), 4.48-3.63 (br. m, CH$_2$-CH$_2$O, N-C=CH$_2$), 2.24-0.47 (br. m, backbone CH$_3$, CH$_2$-CH$_2$-CH$_2$, O$_2$C-C(C(CH$_3$)$_2$)), 0.16 (br. s, (CH$_3$)$_3$Si).

The TMS protected acetylene functionalized adenine polymer (308, 0.14 mmol) was dissolved in DMF (5 mL) by sonication. Subsequently, TBAF (1.4 mL, 1.4 mmol) was added and the reaction mixture was stirred for 18 hours at room temperature. Polymer 6 was isolated as a white solid by precipitation in Et$_2$O and drying under vacuum.
Yield: 172.6 mg (59%); SEC (DMSO): $M_n = 2.3$ kg/mol; $M_w/M_n = 1.19$; $^1$H NMR (300 MHz, DMSO-$d_6$) δ 8.22-7.96 (br. m, purine $H_2$, $H_8$), 7.21 (br. s, $NH_2$), 4.63 (br. m, $\equiv-CH_2-O_2C$), 4.48-3.63 (br. m, $CH_2-C\equiv-H_2$, $N-CH_2-CH_3$), 2.27 (br. m, $H-\equiv$), 2.24-0.47 (br. m, backbone $CH_2$, $CH_3$, $CH_2-C\equiv-CH_2$, $O_2C-C(CH_3)_2$)

5.7.9. $\alpha,\omega$-diazido-poly(ethylene glycol) (7)

For the preparation of $\alpha,\omega$-diazido-poly(ethylene glycol) (7) see chapter 2, sections 2.6.10 and 2.6.11, with the exception that here 2.4 equivalents of TsCl and NaN₃ were used.

5.7.10. poly[3-(thymin-1-yl)propyl methacrylate]-block-poly(ethylene glycol)-block-poly[3-(thymin-1-yl)propyl methacrylate] (5-b-7-b-5)

A Schlenk tube was loaded with $\alpha,\omega$-diazido-PEG (7) (7.9 mg, 0.79 μmol) and $\alpha$-acetylene-poly[3-(thymin-1-yl)propyl methacrylate] (5) (4.2 mg, 1.95 μmol), evacuated and back-filled with argon. This procedure was repeated three times. DMF (1.0 mL) was added and the reaction mixture was stirred for 30 minutes in order to dissolve the polymers. Subsequently, 2.0 μL of a stock solution of CuBr (1.0 M) and PMDETA (1.0 M) in DMF was added. The reaction mixture was placed in an oil bath at 35°C and stirred for 22 hours. The solvent was removed in vacuo and the crude product was dissolved in DMSO for SEC analysis.

SEC (DMSO): $M_n = 10.6$ kg/mol; $M_w/M_n = 1.37$

5.7.11. pentynoyl-Lys-Thr-Val-Ile-Ile-Glu-NH₂[33] (8)

The peptide was synthesized using a Fmoc peptide coupling strategy on a Breipohl resin.[47,48] All amino acids couplings were performed utilizing 3 equivalents of Fmoc protected amino acid, 3.3 equivalents of DIPCDI and 3.6 equivalents of HOBT in DMF. Completion of the reactions was determined using the Kaiser test.[49]

The dry Breipohl resin was swollen in DMF for 20 minutes prior to removal of the Fmoc group by treatment with piperidine in DMF (20% v/v) three times for six minutes. After coupling of the six amino acids, the resin was thoroughly washed with DMF, CH₂Cl₂, MeOH, and Et₂O. Subsequently, the resin was dried under vacuum. The dry resin was swollen for 20 minutes in DMF. Subsequently, the Fmoc protecting group was removed from the N-terminus of the lysine residue by subjecting the resin to piperidine in DMF (20% v/v) three times for six minutes. After deprotection, a solution of pentynoic acid (3.0 equivalents) in DMF, DIPCDI (3.3 equivalents) and HOBT (3.6 equivalents) were added and the resin was agitated for 20 hours. Completion of the reaction was confirmed by a negative Kaiser
test. The resin was thoroughly washed with DMF, CH₂Cl₂, MeOH, and Et₂O, and dried under vacuum. A small portion of the peptide was cleaved from the resin by treatment with TFA/H₂O (95:5) for analysis with MALDI-ToF.

MALDI-ToF: matrix DHB; m/z 803.2 (M+Na⁺), 819.1 (M+K⁺) (calc. masses of 8+Na⁺ (C₃₇H₆₄N₈O₁₀Na) = 803.46, 8+K⁺ (C₃₇H₆₄N₈O₁₀K) = 819.44)

5.7.12. α-methoxy-ω-azido-poly(ethylene glycol) (9)

For the preparation of α-methoxy-ω-azido-poly(ethylene glycol) see chapter 2, sections 2.6.10 and 2.6.11.

5.7.13. poly(ethylene glycol)-block-(Lys-Thr-Val-Ile-Ile-Glu-NH₂) (10)

The Breipohl resin with attached pentynoyl-Lys-Thr-Val-Ile-Ile-Glu-NH₂ (8) was swollen in DMF for 30 minutes. Subsequently, α-methoxy-ω-azido-poly(ethylene glycol) (9) (5.0 equivalents) was added and the mixture was shaken for 15 minutes to allow complete dissolution of the polymer. A solution of CuI (10 equivalents) and DiPEA (10 equivalents) was added and the reaction mixture was shaken for 18 hours. Afterwards, the resin was thoroughly washed with DMF, CH₂Cl₂, MeOH, acetic acid and Et₂O, and dried under vacuum. The peptide-polymer hybrid was cleaved from the resin by treatment with TFA/H₂O (95:5) for four hours. The product was isolated as a white solid by precipitation in Et₂O and drying under vacuum.

Yield: 34.6 mg (43%); MALDI-ToF MS: matrix DHB; m/z 2484 ± 44.04 (36 repeating units + end groups + Na⁺)

5.7.14. pentynoyl-(Val-Pro-Gly-Val-Gly)₃-OH (11)

The peptide was synthesized using a Fmoc peptide coupling strategy on a Wang resin. Glycine was immobilized on the resin using 3 equivalents of Fmoc-Gly-OH, DIPCDI (3.3 equivalents) and HOBt (3.6 equivalents). The Fmoc group was removed by treatment with piperidine in DMF (20% v/v) three times for six minutes. Deprotection of the amino group was observed by a positive Kaiser test. By determining the quantity of liberated 9-methylenefluorene using UV spectroscopy, the loading was established to amount to 0.43 mmol/g. Subsequently, valine was coupled to the N-terminus of glycine using 3 equivalents of Fmoc-Val-OH, DIPCDI (3.3 equivalents) and HOBt (3.6 equivalents). The efficacy of the reaction was controlled using a Kaiser test. After removal of the Fmoc protecting group by subjection to piperidine in DMF (20% v/v) three times for six minutes, this coupling procedure cycle was repeated 13 times. After the synthesis of the peptide, the resin was thoroughly washed with DMF, CH₂Cl₂, MeOH, and Et₂O, and dried under vacuum.

The dry resin was swollen for 30 minutes in DMF. Subsequently, the Fmoc protecting group was removed from the N-terminus of valine by treatment of the resin with piperidine in DMF (20% v/v) three times for six minutes. After this deprotection procedure, a solution of pentynoic acid (3.0 equivalents) in DMF, DIPCDI (3.3 equivalents) and HOBt (3.6 equivalents) was added and the resin was shaken for 18 hours. Completion of the acylation reaction was confirmed by a
negative Kaiser test. The resin was thoroughly washed with DMF, CH₂Cl₂, MeOH, and Et₂O, and dried under vacuum.

MALDI-ToF MS: matrix: DHB; m/z 1348.3 (M+Na⁺), 1364.2 (M+K⁺), 1371.2 (10%) (M+2Na⁺) (calcld. masses of 11+Na⁺ (C₆₂H₉₉N₁₅O₁₇Na) = 1348.72, 11+K⁺ (C₆₂H₉₉N₁₅O₁₇K) = 1364.70, 11+2Na⁺ (C₆₂H₉₉N₁₅O₁₇Na₂) = 1371.71)

5.7.15. 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate (12)

The synthesis of 3-(1,1,1-triisopropylsilyl)-2-propynyl 2-bromo-2-methylpropanoate is described in chapter 3, sections 3.5.3 and 3.5.4.

5.7.16. α-(triisopropyl acetylene)-ω-azido-polystyrene (13)

Yield: 1.83 g (78%, 2 steps); TLC: Rf (CH₂Cl₂) = 0.84, Rf (n-hexane/Et₂O/CH₂Cl₂ 14:3:3) = 0.40; ¹H NMR (300 MHz, CDCl₃) δ 7.35-6.27 (br. m, arom. H), 4.18-3.77 (br. m, ≡−C₂H₆-O₂C, CH₂-C₂H₆(Ph)-N₃), 2.58-1.17 (br. m, backbone C₂H₂, C₂H), 1.12-0.83 (br. m, (C₆H₃)₂C₆H₃-Si−≡, O₂C-C(C₆H₃)₂-CH₂); FTIR-ATR 3084, 3058, 3024, 2920, 2846, 2181 (νC≡C), 2094 (νN₃), 1943, 1865, 1800, 1601, 1493, 1450, 1368, 1182, 1122, 1070, 1027 cm⁻¹; SEC: Mₙ = 3.58 kg/mol; Mₙ/Mₘ = 1.11; MALDI-ToF MS: matrix: IAA+AgOTf; m/z = 3863 ± 104.06 (33 repeating units + end groups + Ag⁺)

5.7.17. α-(triisopropyl acetylene)-ω-azido-poly(tert-butyl acrylate) (14)

Yield: 0.82 g (74%); TLC: Rf (CH₂Cl₂/MeOH 95:5) = 0.95; ¹H NMR (300 MHz, CDCl₃) δ 4.67 (br. m, ≡−C₂H₆-O₂C), 4.12 (br. s, CH₂-C₂H₆(CO₂tBu)-Br), 2.36-1.22 (br. m, backbone CH₃, CH₂), 1.44 (br. s, CO₂-C(CH₂)₄), 1.13-0.85 (br. m, ((C₆H₃)₂C₆H₃-Si−≡, O₂C-C(CH₂)₄-CH₂); FTIR-ATR 2978, 2923, 2863, 1719, 1475, 1451, 1390, 1368, 1254, 1139 cm⁻¹; SEC: Mₙ = 2.79 kg/mol; Mₙ/Mₘ = 1.12; MALDI-ToF MS: matrix: DHB; m/z = 2433 ± 128.17 (16 repeating units + end groups + Na⁺)

5.7.18. α-(triisopropyl acetylene)-polystyrene-block-(Val-Pro-Gly-Val-Gly)₃-OH (15)

Dry Wang resin with attached pentyynoyl-(Val-Pro-Gly-Val-Gly)₃-OH (11) (240 mg, 0.1 mmol, theoretical loading) was swollen in DMF for 45 minutes. The resin was filtered off and fresh DMF was added. Argon was bubbled through the reaction mixture continuously. α-(Triisopropyl acetylene)-ω-azido-polystyrene (13) (1.18 g, 0.35 mmol) was added and the reaction mixture was allow to stand for 20 minutes to completely dissolve the polymer. Subsequently, 0.5 mL of a stock solution
containing CuBr (1 M) and PMDETA (1 M), which was prepared under Schlenk conditions, was added and the reaction mixture stood for 20 hours. The resin was filtered off and thoroughly washed with DMF, acetic acid, CHCl₃, MeOH and Et₂O. Afterwards, the resin was dried under vacuum. A portion of the resin (77.1 mg) was taken to cleave off the product by treatment with TFA/CH₂Cl₂/TIS/H₂O (45:45:5:5) for four hours. The product was precipitated in Et₂O and isolated as a slightly yellow colored solid, which was dried under vacuum.

Yield: 28.9 mg (17%); TLC: Rₓ (CHCl₃/MeOH/H₂O 65:25:4) = 0.56; SEC: Mₓ = 1.35 kg/mol; Mₓ/Mₘ = 1.47; MALDI-ToF MS: matrix: dithranol; m/z = 5104 ± 104.06 (33 repeating units + end groups + Na⁺)

5.7.19. α-(triisopropyl acetylene)-poly(acrylic acid)-block-polystyrene-block-(Val-Pro-Gly-Val-Gly)₃-OH (17)

Dry Wang resin containing α-(triisopropyl acetylene)-polystyrene-block-(Val-Pro-Gly-Val-Gly)₃-OH (15) (163 mg, 0.070 mmol, theoretical loading) was swollen for 30 minutes in DMF. The resin was filtered off, fresh DMF and TBAF (0.7 mL, 0.7 mmol) were added and the reaction mixture was shaken for 19 hours. Afterwards, the resin was thoroughly washed with DMF, CHCl₃, MeOH and Et₂O and dried under vacuum.

The dry resin (70.0 mg, 0.030 mmol, theoretical loading) was swollen in DMF for 45 minutes. The resin was filtered off and fresh DMF was added. Argon was bubbled through the reaction mixture continuously. α-(Triisopropyl acetylene)-ω-azido-poly(tert-butyl acrylate) (14) (0.17 g, 0.056 mmol) was added. After 30 minutes, when all polymer was dissolved, 0.5 mL of a stock solution of CuBr 0.6 M and PMDETA 0.6 M, which was prepared under Schlenk conditions, was added and the reaction mixture was bubbled through with argon for 2 days. Subsequently, the resin was filtered off and washed thoroughly with DMF, CHCl₃, acetic acid, MeOH and Et₂O. The resin was dried under vacuum. The product was cleaved from the resin by treatment with TFA/CH₂Cl₂/TIS/H₂O (45:45:5:5) for 15 hours at room temperature. The product was isolated as a white solid by precipitation in Et₂O and drying under vacuum.

Yield: 16.1 mg (7%); SEC: Mₓ = 3.31 kg/mol; Mₓ/Mₘ = 1.21

5.8. References


“Clickable” polymersomes

“Click” chemistry was employed for the functionalization of polymeric vesicular aggregates, so-called polymersomes. In order to be able to functionalize these polymersomes, coverage of the periphery of the vesicles with either azide or acetylene moieties was required. Therefore, the amphiphilic block copolymer polystyrene-block-poly(acrylic acid) (PS-b-PAA), which is known for its capability to form aggregates in aqueous solution, was synthesized by consecutive ATRP polymerization of styrene and tert-butyl acrylate. Prior to hydrolysis of the tert-butyl esters, the bromide terminus was substituted for an azide functionality. The thus formed azide functionalized PS-b-PAA was allowed to self-assemble into vesicular aggregates, as visualized by transmission electron microscopy (TEM). Subsequently, acetylene functionalized dansyl probe, biotin and enhanced green fluorescent protein (EGFP) were coupled to the polymersomes by application of CuSO₄•5H₂O, in combination with sodium ascorbate which acts as a reductor to generate the catalytic copper(I)-species in situ. Moreover, to the resulting biotinylated polymersomes, the enzyme streptavidin, labeled with 6 nm colloidal gold particles, was complexed in a next step. According to the obtained TEM images after conducting the “click” reactions, no change in aggregate morphology was observed. Additionally, successful attachment of the different substrates was determined by size exclusion chromatography (SEC) and confocal laser-scanning microscopy (CLSM). The opposite strategy was performed as well. Therefore, acetylene functionalized PS-b-PAA was prepared by ATRP of tert-butyl acrylate and styrene, successively, using an acetylene functionalized initiator. This amphiphilic block copolymer formed vesicles in an aqueous environment as well, which could be post-functionalized with a dansyl dye by “click” chemistry.
6.1. Introduction

Block copolymers represent an interesting class of materials due to their ability to assemble on a mesoscopic length scale into multiple, highly regular morphologies in bulk as well as in solution.\cite{1} The aggregation behavior of amphiphilic block copolymers in solution has been extensively studied. Owing to the incompatibility of the distinct blocks, a variety of different morphologies, ranging from spherical micelles, rods, and vesicles to large compound micelles, can be obtained.\cite{2-4}

The main factor in controlling the aggregate morphology certainly is the length of the hydrophilic block.\cite{5} For a series of colloidal dispersions of polystyrene-\textit{block}-poly(acrylic acid) (PS-\textit{b}-PAA) in DMF/water mixtures, it was demonstrated that with decreasing corona-forming PAA block lengths, the morphology changes from spherical to rod-like micelles, to vesicles and to micrometer size spheres, respectively.\cite{6} These observations, regarding the influence of the block copolymer geometry on the aggregation behavior, were in qualitative agreement with the theory of Israelachvili \textit{et al.}, which relates the geometry of low molecular weight amphiphiles to the formed morphology.\cite{7} This implies that the formation of vesicles is favored upon decrease of the hydrophilic block length, \textit{i.e.} block copolymers comprised of long, hydrophobic core-forming blocks in combination with short, hydrophilic corona blocks prefer to form vesicular aggregates.\cite{5} Furthermore, vesicle formation is promoted by an increase in total molecular weight of the block copolymer due to an increase in the bending modulus of the block copolymer which lowers the curvature energy in the vesicles.\cite{8}

Polymersomes, which are hollow, spherical shell structures that are composed of block copolymers, embrace remarkable properties with respect to their low molecular weight counterparts.\cite{3-5} It has been shown that the diffusion of polymeric amphiphiles in these polymersomes is very low in contrast to low molecular weight phospholipids in liposomes, which for high molecular weight chain entangled polymers even results in reptation-type motions.\cite{9} Furthermore, the membrane thickness of polymersomes can exceed 200 nm, in comparison with an average thickness of 3-4 nm for liposomes.\cite{10}

The enhanced stability of polymersomes, conjoined with the remarkable membrane thickness makes them, consequently, very suitable as nanocontainers, which are ideal candidates to be employed as \textit{e.g.} drug delivery vehicles\cite{11} or nanoreactors.\cite{12} Furthermore, in contrast to conventional amphiphiles, block copolymer properties can be
readily tailored in order to adapt to specific applications, e.g. by altering the composition or the molecular weight.

In Nature, self-assembly processes are essential for generating biofunctionality. One can think of e.g. the hierarchical ordering of several proteins in order to form a working enzyme. This intricate three-dimensional self-assembly behavior of biomolecules is unsurpassed by current synthetic materials. For this reason, inspired by Nature, much research is focused on incorporating natural folding elements into synthetic materials in order to induce self-assembly processes. In virtue of their interesting properties, much effort has been put into the synthesis of well-defined biohybrid block copolymers. As a consequence, merging synthetic polymers and biomolecules into one single macromolecule, has led to interesting aggregates in solution. Nolte and co-workers synthesized block copolymers composed of polystyrene (PS) blocks combined with polyisocyanide blocks bearing pendant peptides, which formed a variety of aggregates, including helical ribbons and vesicles, depending on the size of the polyisocyanide corona-block. In the same group also so-called “giant amphiphiles” were prepared by the attachment of enzymes to synthetic polymers, both via coupling of PS to a cysteine residue exposed on the outside of the lipase B enzyme of candida antarctica (Cal B) and by co-factor reconstitution in the enzyme horse radish peroxidase (HRP), which formed micellar rods and vesicles in solution, respectively. Furthermore, Van Hest et al. showed the capability of vesicle formation of ABA triblock copolymers containing a peptide central block. Moreover, incorporation of peptides can result in vesicles that respond on external stimuli. This was demonstrated by Klok and co-workers by the preparation of vesicles comprising a pH responsive poly(glutamic acid) corona. They were able to alter the size of these vesicles upon pH variation.

Another strategy is to functionalize vesicular aggregates after they have been formed. Regarding this methodology, liposomal surfaces have been functionalized performing amidation and thiol-maleimide chemistry, as well as by accomplishing imine and hydrazone linkages. Recently, “click” chemistry has been adopted to functionalize the periphery of liposomes, as shown in figure 6.1.a. Successful development of the “click” reaction onto the surface of liposomes was visualized by the attachment of an azide containing dye which, in close proximity to the liposomes, gave a colorimetric response due to Förster resonance energy transfer (FRET).

Much research in the field of conjugation to the exterior of shell-crosslinked (SCK) nanoparticles has been conducted by Wooley and co-workers. Recently, they applied
“click” chemistry to crosslink micellar aggregates,\textsuperscript{[33]} and to functionalize both core and corona,\textsuperscript{[34]} as well as surfaces\textsuperscript{[35]} of SCK’s with fluorescent probes. An example of the latter research, regarding the peripheral conjugation of a fluorescent dye to an SCK is illustrated in figure 6.1.b.

![Figure 6.1](image)

**Figure 6.1** Schematic illustration of the functionalization of the surfaces of liposomal\textsuperscript{[29]} (a) and polymeric micellar\textsuperscript{[35]} (b) aggregates using “click” chemistry

Concerning polymersomes, Mirkin, Nguyen *et al.* have demonstrated the possibilities pertaining to the functionalization of their surfaces.\textsuperscript{[36]} Therefore, block copolymers bearing tosyl end groups were prepared. After self-assembly, these tosyl groups were present at the surface of the vesicles and, consequently, available for conjugation. A drawback of this tosylation strategy, however, is a lack of selectivity of the coupling reaction. In general, every nucleophile is able to substitute the tosyl group, which can lead to uncontrolled reaction, especially when substrates comprising multiple reactive groups, such as biomolecules, are used.

As discussed in the previous chapter, the “click” reaction between azides and terminal acetylenes is a powerful tool for the connection of biomolecules to synthetic polymers,
owing to the orthogonality and efficiency of the reaction.\cite{37-41} Moreover, this type of reaction can be performed in an aqueous environment, which is indispensable for most biomolecules.\cite{42}

Therefore, in the research outlined in this chapter, this orthogonal “click” reaction between azides and terminal acetylenes is adopted to functionalize the periphery of polymeric vesicles, which is compatible with biomolecular substrates. Moreover, this type of chemistry can be employed in an aqueous environment, which is an excellent medium for polymersome formation. Besides, an additional advantage is that a toolbox for introducing azide and acetylene end-functionalities in polymers has already been developed,\cite{43} as discussed in the chapters two and three, which can be utilized for preparing such polymersomal scaffolds.

In contrast to the research outlined in the previous chapter where the conjugations were performed in solution and on solid supports, here first stable vesicular aggregates are formed of which the exterior is covered with reactive groups that can be utilized as scaffolds for further functionalization, as illustrated in figure 6.2. Adopting this methodology, possibly large (biofunctional) moieties, such as targeting ligands or enzymes, can be introduced without disrupting the aggregate morphology. Owing to the reduced reactivity on the periphery of polymersomes, the highly efficient “click” reaction between azides and acetylenes is a logical choice.

![Figure 6.2 Schematic representation of the functionalization of the vesicular periphery using “click” chemistry](image)

Initially polymersomes have to be prepared which contain either an azide or an acetylene functional periphery to attain the possibility for subsequent modification. The
preparation of such vesicles is discussed in the following section. Section 6.3 deals with the subsequent functionalization of the periphery of these vesicles.

6.2. Preparation of polymersomes encompassing azide and acetylene functional coronas

In order to induce aggregate formation in solution, the applied block copolymers are required to have an amphiphilic character. A well-studied example of such an amphiphilic block copolymer is PS-\(b\)-PAA. Depending on the ratio between core and corona forming blocks, this block copolymer is able to form vesicles in an aqueous environment.\[^{6}\] Furthermore, PS-\(b\)-PAA diblock copolymers can be prepared by consecutive polymerization of the distinct monomers by adopting ATRP.\[^{44,45}\] Additionally, by employment of ATRP, azide and acetylene end-functionality can be introduced by utilizing functionalized initiators and post-polymerization end group modification procedures, as discussed in previous chapters.

6.2.1. Azide peripherally functionalized polystyrene-\(b\)-poly(acrylic acid) polymersomes

In order to prepare PS-\(b\)-PAA vesicles from which the exterior is functionalized with azide moieties, the terminus of the PAA polymer block should be functionalized, since this block will comprise the corona of the vesicles when self-assembly occurs in an aqueous environment.

Correspondingly, an \(\omega\)-azide functionalized PS-\(b\)-PAA block copolymer was synthesized by consecutive ATRP polymerization of styrene and \(\text{tert}\)-butyl acrylate, as depicted in scheme 6.1. As the second monomer \(\text{tert}\)-butyl acrylate was chosen instead of acrylic acid, because the latter monomer tends to inactivate the copper-catalyst during polymerization via protonation of the nitrogen based ligands, thereby disrupting its coordination to the metal center.\[^{44,46}\] Using \(\text{tert}\)-butyl acrylate, a polymer containing pendant \(\text{tert}\)-butyl ester groups is obtained, which can be hydrolyzed readily into the desired carboxylic acid moieties. Moreover, when this block is prepared in the last polymerization step, it is terminated with a halogen end group, which allows facile introduction of azide functionality.

Both polymerization reactions were accomplished using a 1:1 complex of CuBr and \(N,N,N',N',N''\)-pentamethyldiethylenetriamine (PMDETA) as a catalyst. This yielded a block copolymer with degrees of polymerization (DPs) amounting to 150 for the PS
block and 20 for the PtBA block, as determined with $^1$H NMR spectroscopy. The polydispersity indices (PDI) of the acquired PS homopolymer 1 and PS-$b$-PAA block copolymer 2 were low ($M_w/M_n = 1.09$ and 1.10, for 1 and 2, respectively). The size exclusion chromatography (SEC) traces are depicted in figure 6.3.

Subsequently, it was chosen to introduce the azide end-functionality prior to hydrolysis of the pendant tert-butyl esters. Therefore, in the next step, the bromide terminus was replaced for an azide group, adopting similar conditions as applied in chapter two, i.e. by a nucleophilic substitution reaction using azidotrimethylsilane (Me$_3$Si-N$_3$) and tetrabutylammonium fluoride (TBAF) in THF.[47] The presence of azide moieties in the block copolymer was confirmed by the appearance of an azide signal in the FTIR spectrum (2098 cm$^{-1}$). Quantification of this reaction, however, was difficult to assess due to the fact that the signal arising from protons adjacent to the end groups was not clearly visible in the $^1$H NMR spectrum of the obtained block copolymer. This was caused by the fact that this signal, stemming from one proton, disappeared in the noise of the baseline in contrast to the signal of the polymer backbone protons. For this reason, the integral value of this small signal is unreliable.

Scheme 6.1 Preparation of $\omega$-azido-polystyrene-block-poly(acrylic acid) (4) via consecutive ATRP polymerization of styrene and tert-butyl acrylate, end group substitution for azides and subsequent hydrolysis of the pendant tert-butyl esters. The thus obtained terminal azide functionalized amphiphilic block copolymer was allowed to self-assemble into vesicular aggregates (5) by slow addition of distilled water to a polymer solution in dioxane.

In order to induce amphiphilic character, the tert-butyl ester side groups were hydrolyzed under acidic conditions by the addition of a concentrated hydrochloric acid
solution, three equivalents with respect to the pendant ester groups present, to a solution of the PS-b-PtBA block copolymer (3) in dioxane and heating up to reflux temperature for four hours. Successful formation of the terminally azide functionalized amphiphilic block copolymer 4 was established by the disappearance of the signal stemming from the tert-butyl protons in the $^1$H NMR spectrum. Furthermore, the FTIR spectrum of the product displayed still the presence of azide end-functionality (2098 cm$^{-1}$). Unfortunately, analogous to the previous reaction to introduce the azide moiety, quantification of the amount of azide groups still present was difficult to ascertain due to the weak signal in the $^1$H NMR spectrum.

The corresponding $\omega$-azide functionalized PS-b-PtBA block copolymer was allowed to self-assemble in an aqueous environment. Therefore, a 10 mg/mL solution of the block copolymer in dioxane was prepared to which deionized water was added slowly until a cloudy suspension was obtained. Afterwards, additional deionized water was added at once and the suspension was dialyzed against deionized water to remove the still present dioxane. As beforehand predicted, the block copolymer assembled into vesicular aggregates (5), which were visualized by transmission electron microscopy (TEM). Figure 6.4 illustrates the feasibility to form vesicles which were fairly uniform in size. In this case, the polymersomes were quite small, yet the vesicle size can be altered e.g. by varying the water content of the solution.[48-50]
6.2.2. Exteriorly acetylene functionalized polystyrene-block-poly(acrylic acid) polymersomes

As noted in the previous section, when self-assembly of PS-\(b\)-PAA block copolymers occurs in an aqueous environment, the hydrophilic PAA block will constitute the corona of the vesicles and, thus, be present on the outside. Therefore, in this case, acetylene moieties have to be introduced at the terminus of the PAA block.

The most straightforward method for such acetylene functionality is by employing a functional initiator. For that reason tert-butyl acrylate was polymerized prior to preparation of the PS block. In case of consecutive ATRP reactions of styrene and acrylates, the order of the two polymerizations can be altered, due to the fact that both apparent rate constants are comparable. It has to be noted that efficient chain extension with a second monomer can merely be achieved when the apparent rate of crosspropagation is at least as fast as that of the subsequent propagation.\(^{[51]}\)

As depicted in scheme 6.2, acetylene end-functionality was introduced by utilizing a trimethylsilyl (TMS) protected acetylene functionalized initiator for the ATRP of tert-butyl acrylate. The ratio of initiator to monomer was chosen such that a DP of 20 was reached at 40\% conversion. Accordingly, the conversion during the polymerization process was monitored with \(^1\)H NMR and the reaction was stopped after 40\% conversion. After work-up of the polymer, a DP of 20 was confirmed by \(^1\)H NMR spectroscopy from the ratio of backbone protons with protons stemming from the initiator moiety.
Scheme 6.2. Schematic representation of the synthesis of ω-acetylene-polystyrene-block-poly(acrylic acid) (8) via successive ATRP polymerizations followed by hydrolysis of tert-butyl ester side groups. The accordingly formed amphiphilic block copolymer was allowed to self-assemble into vesicular aggregates, with the periphery being covered with acetylene functionality (9).

As illustrated in scheme 6.2, the obtained P_{tBA} was subsequently exploited as a macro-initiator for the polymerization of styrene, yielding P_{tBA}-b-PS diblock copolymer 6. This ATRP of styrene was conducted with CuBr/PMDETA as a catalyst, using anisole as a solvent at 100°C. During the polymerization, samples were taken at periodic intervals for analysis with 1H NMR and SEC. At the point that the SEC trace of P_{tBA}-b-PS 6 coincided with the trace of the previously synthesized PS-b-P_{tBA} 2, the polymerization was stopped by cooling and dilution with CHCl₃. The conversion of the polymerization reaction amounted to 50%, as determined by 1H NMR spectroscopy. As can be seen in figure 6.3, after work-up of block copolymer 6, the SEC trace still was corresponding to the chromatogram of 2, which means that the composition of both block copolymers was in good agreement since the P_{tBA} blocks possessed similar DPs. Additionally, the PDI of the TMS protected acetylene functionalized P_{tBA}-b-PS diblock copolymer 6 was low (M_w/M_n = 1.08), implying good control over the polymerization processes.
In succession, the TMS group was removed by treatment with TBAF and the tert-butyl esters were hydrolyzed by addition of a concentrated hydrochloric acid to a solution of block copolymer 7 in dioxane. Subsequently, using the same methodology as for ω-azido-PS-b-PAA 4, viz. by addition of deionized water to a solution of the block copolymer in dioxane, α-acetylene-PAA-b-PS 8 was allowed to self-assemble. Likewise, TEM images revealed the formation of vesicles out of the acetylene end-functionalized block copolymer 8.

6.3. “Click” functionalization of polymersomal surfaces

The thus prepared polymersomes embracing both azide and acetylene functionality on their surfaces, subsequently were exploited in conjugation reactions. First, in order to test the scope of using these polymersomes as scaffolds for further functionalization, “click” reactions of fluorescent probes in combination with these vesicles were executed. The choice for fluorescent dyes is obvious, since attachment of these probes can be readily visualized utilizing fluorescence microscopy techniques.

6.3.1. Conjugation of fluorescent dansyl probe to polymersomes

A dansyl group was chosen as fluorescent probe to be utilized for the conjugation experiments. The main arguments for selecting this dye were, first of all, that the commercially available dansyl chloride can be functionalized facilely. Secondly, the UV absorption is in a range suitable for measurements with available confocal microscopes (λ_{max} ≈ 350 nm), along with a significant bathochromic shift of the fluorescent emission which, therefore, is easily detectable, as illustrated in figure 6.5.
Acetylene functionalized dansyl probe 10 was synthesized according to a literature procedure in reasonable yield by coupling of propargyl amine to dansyl chloride using triethylamine (Et₃N) as a base, as depicted in scheme 6.3.

Scheme 6.3 Synthesis of acetylene functionalized dansyl probe 10. Reagents and conditions: i. Et₃N, CH₂Cl₂, 0°C→rt., 2 h, 78%

Azide comprising dansyl fluorescent dye 12 was prepared by a two step procedure from 3-chloro-1-aminopropane hydrochloride and dansyl chloride. First, the chloride group of 3-chloro-1-aminopropane hydrochloride was replaced for an azide via a nucleophilic substitution, yielding 3-azido-1-aminopropane (11) (scheme 6.4). This substrate was subsequently coupled to dansyl chloride, applying Et₃N as a base which led to the formation of azide functionalized dansyl probe 12.
Accordingly, the synthesized acetylene (10) and azide (12) bearing fluorescent dansyl probes were utilized to functionalize the peripheries of the azide (5) and acetylene (9) vesicles, respectively, by employing “click” chemistry conditions. In contrast to the “click” experiments described in the previous chapters, here, the reactions were conducted in an aqueous environment, since the employed block copolymers formed vesicular aggregates in water. As noted before, it is known that the copper(I)-catalyzed reaction between azides and terminal acetylenes works well in aqueous solutions. Furthermore, it has been shown that the general thermodynamic instability of the utilized Cu(I)-species, e.g. by oxidation to Cu(II) or disproportionation to Cu(0) and Cu(II), can be surmounted by applying ligands that stabilize Cu(I). The most applied ligand is tris-(benzyltriazolylmethyl)amine (TBTA), which contains 1,2,3-triazole rings itself that have been shown to complex strongly to Cu(I) and, consequently, accelerate “click” reactions.

In order to exploit this ligand in the polymersomal “click” reactions, it was prepared first via a triple “click” reaction between tripropargylamine and benzyl azide, as depicted in scheme 6.5. After reaction, TBTA (13) was isolated in moderate yield, probably due to the loss of material during a washing step of the formed crystals.

Scheme 6.4 Schematic representation of the preparation of azide functionalized dansyl probe 12. Reagents and conditions: i. NaN₃, H₂O, 80°C, 24 h, 50%; ii. Et₃N, CH₂Cl₂, 0°C→rt., 2 h, 89%

Scheme 6.5 Synthesis of tris-(benzyltriazolylmethyl)amine (TBTA). Reagents and conditions: 2,6-lutidine, Cu(MeCN)₄PF₆, acetonitrile, rt.→37°C, 4 days, 27%
In order to functionalize the periphery of azide functionalized vesicles with acetylene functionalized dansyl probe 10, first the polymersomes were freshly prepared using equal conditions as applied before, viz. by addition of deionized water to a block copolymer solution in dioxane. After dialysis of the polymersomes, aqueous solutions of the fluorescent probe 10, copper(II)sulfate pentahydrate (CuSO₄•5H₂O) and sodium ascorbate were added. The latter was added to reduce Cu(II), thereby generating in situ the catalytic Cu(I)-species. To stabilize Cu(I) in the reaction mixture, a solution of TBTA (13) was added to the vesicle solution. After gently stirring the reaction mixture for 24 hours at room temperature, the polymersomes were extensively dialyzed for two days against a 0.55 mM solution of ethylenediamine-tetraacetic acid tetrasodium salt tetrahydrate (EDTA) in deionized water. This dialysis methodology was applied in order to remove, beside all other reagents, the copper ions present by complexation to EDTA.

**Scheme 6.6** Schematic illustration of the functionalization of the exterior of polymersomes with a dansyl probe. Reagents and conditions: i. CuSO₄•5H₂O, sodium ascorbate, tris-(benzyltriazolylmethyl)amine (TBTA) (13), N1-(2-propynyl)-5-(dimethylamino)-4a,8a-dihydro-1-naphthalenesulfonamide (10), H₂O, 24 h

As illustrated in figure 6.6, with TEM it was visualized that no change in the aggregate morphology occurred due to the “click” functionalization with fluorescent dye 10. Both before and after the applied reaction conditions, vesicular aggregates were observed.

**Figure 6.6** TEM images of PS-b-PAA polymersomes before (a) and after (b) (5 and 14, respectively) attachment of acetylene functionalized dansyl probe 10
In case of successful attachment of fluorescent dye 10 to the periphery of the azide bearing polymersomes, these should exhibit fluorescent behavior as well. With confocal laser-scanning microscopy (CLSM), it was shown that the functionalized polymersomes 14 displayed significant fluorescence in contrast to the background, as can be seen in figure 6.7.b. In the apparatus set-up of the high-resolution CLSM used, only a laser with a wavelength of 411 nm was available. Albeit not the ideal wavelength, since the maximum absorption of the dansyl moiety is around 350 nm (figure 6.5), still sufficient absorption occurred to induce fluorescent emission. Additionally, in order to prove that the image depicted in figure 6.7.b certainly stemmed from the fluorescence of the functionalized polymersomes, a photobleaching experiment was executed (figure 6.7.c). By employing an intense photobleaching pulse of laser light, the dansyl dye was rendered non-fluorescent which caused a decay of the fluorescent emission. If the image was produced by an other phenomenon, such as light scattering, this decrease in emission would not have been observed. Combined with the fact that a performed control experiment with exclusion of CuSO₄•5H₂O did not yield fluorescent images, it was reasoned that the dansyl probe was attached to the periphery of the polymersomes.

Figure 6.7 Confocal laser-scanning microscopy (CLSM) images of PS-b-PAA vesicles with tethered dansyl probe (14) (transmission (a) and fluorescence excited at 411 nm (b)). Photobleaching of the dansyl moiety attached to the polymersomes (c)
From these performed CLSM experiments, however, covalent attachment of the dansyl dye to the vesicles cannot be deduced. Conceivably, the fluorescent behavior of the polymersomes could be caused by e.g. physical adsorption of the dye to the vesicles or incorporation due to diffusion through the bilayer. To establish the covalent linkage between the dansyl probe and the vesicles, they were dissolved in CHCl₃ and measured with SEC. As depicted in figure 6.8, the dissolved vesicles were detectable at 345 nm, whereas the bare ω-azido-PS-b-PAA (4) did not absorb light of this wavelength. A remark has to be made concerning the difference in retention times between PS-b-P/BA 3 (figure 6.3) and PS-b-PAA 14. This was caused by the lower hydrodynamic volume of PS-b-PAA, which probably was a result of internal hydrogen bonding between the acrylic acid repeating units. Furthermore, TLC analysis of the dissolved vesicles (14) using CHCl₃/MeOH (9:1) as an eluent clearly showed a fluorescent spot at the baseline under a UV lamp at 366 nm, as opposed to the unfunctionalized block copolymer 4, whereas dansyl probe 10 itself eluted with a Rf value of 0.68. From these SEC and TLC results, the conclusion can be drawn that the dansyl probe was covalently attached to the block copolymer.

![Figure 6.8](image-url)

**Figure 6.8** SEC traces of ω-azido-PS-b-PAA (4) measured at 254 nm and 345 nm, and the dissolved PS-b-PAA vesicles with pendant dansyl probe 14 measured at 345 nm

In order to determine the degree of functionalization of the polymersomes, a reference compound was synthesized by coupling acetylene bearing dansyl probe 10 and ω-azido-PS-b-PAA (4) via a “click” reaction in solution, utilizing CuBr/PMDETA as a catalyst and THF as a solvent. Comparison of the fluorescence of this model compound with the block copolymers functionalized in the polymersomes led to the estimation that 23
percent of the block copolymers present in the vesicles were accommodated with a dansyl moiety, assuming that the model compound was completely functionalized (figure 6.9). Since, most likely, the azide groups present in the interior of the vesicles were not available for conjugation, this implies that 40 to 50 percent of the block copolymers exposed to the environment were functionalized.

![Fluorescence emission measured at $\lambda = 540$ nm (excitation at $\lambda = 354$ nm) for a series of dansyl comprising PS-b-PAA block copolymers synthesized in solution (■) and conjugated to polymersomes (▲), measured in dioxane solution. From the ratio of the slopes of the regression lines, a relative amount of functionalization of the polymersomes of 23 percent in comparison to the degree of functionalization in solution was determined.](image)

**Figure 6.9** Fluorescence emission measured at $\lambda = 540$ nm (excitation at $\lambda = 354$ nm) for a series of dansyl comprising PS-b-PAA block copolymers synthesized in solution (■) and conjugated to polymersomes (▲), measured in dioxane solution. From the ratio of the slopes of the regression lines, a relative amount of functionalization of the polymersomes of 23 percent in comparison to the degree of functionalization in solution was determined.

To improve the degree of functionalization it was endeavored to optimize the “click” conditions. As depicted in table 6.1, four variables were altered, namely the temperature, the copper stabilizing ligand, the copper concentration and the pH-value of the reaction medium. As an alternative ligand, bathophenantrolinedisulfonic acid was chosen, which is known to be an excellent and very water-soluble catalyst. In all experiments, the concentrations of azide bearing vesicles (5) and acetylene functionalized dansyl probe 10 were kept constant. The sodium ascorbate concentration used was five times the copper concentration, and the concentration of the added ligand was twice this concentration. In the experiments conducted at pH 7.4, a phosphate buffer saline (PBS) solution was used. Additionally, the degree of functionalization was determined analogously to the previous experiment described, i.e. by comparison of the fluorescence of the dissolved PS-b-PAA after functionalization in the polymersomal aggregates with the fluorescence of the model compound prepared in solution.
Table 6.1 Reaction conditions used and the degrees of functionalization for the “click” functionalization of ω-azido-PS-b-PAA vesicles (5)

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<th>entry</th>
<th>temperature</th>
<th>ligand</th>
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<th>pH</th>
<th>degree of functionalization[a]</th>
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<td>7.4</td>
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</tr>
<tr>
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<td>ambient</td>
<td>bathophenantroline</td>
<td>0.5 mM</td>
<td>6.0</td>
<td>23.9%</td>
</tr>
</tbody>
</table>

[a] Degree of functionalization calculated from the ratio of fluorescence of dissolved block copolymers after functionalization in vesicular aggregate and model compound prepared in solution

[b] Bathophenantrolinedisulfonic acid sodium salt:

![Diagram](image)

As can be seen in table 6.1, not much improvement in the efficiency of the “click” functionalization was achieved. Although the system seemed to favor slightly higher temperature, higher copper concentration and pH 7.4, these effects were not significant. Furthermore, in this case, the choice of ligand appeared to have negligible influence. This leads to the conclusion that the obtained degree of functionalization was not affected by the employed “click” conditions, rather by the inaccessibility of the azide groups present at the surface of the vesicles. A probable explanation can be that these azide moieties in the vesicular aggregates are more densely packed than in solution, thereby preventing some of the azide groups from reacting. In conclusion, presumably the optimum degree of functionalization of the vesicles lies around 50 percent, owing to the fact that the reactive azide groups were not all sufficiently exposed to the outside of the vesicles, by which they were not available for reaction.

Furthermore, the polymersomes comprising acetylene moieties (9) were exploited to conjugate azide functionalized dansyl probe 12 using equal conditions as employed for the first “click” coupling of the azide vesicles, viz. utilizing CuSO₄•5H₂O, sodium ascorbate and TBTA (13) in aqueous solution. Attachment of the azide dansyl dye 12 was confirmed by TLC and the detection of the block copolymers at 345 nm with SEC, which was in agreement with the results obtained from the azide polymersomes.
6.3.2. Conjugation of enzymes to the periphery of azide bearing polymersomes

In the preceding section the possibilities regarding the utilization of “click” chemistry for the functionalization of the surface of polymersomes has been demonstrated by the attachment of a fluorescent dansyl dye. In the introductory section, however, it was hypothesized that “click” chemistry, owing to its specificity and efficiency, could allow decoration of the vesicular exterior with large (bio)macromolecules, such as enzymes or targeting ligands. This would be exceptionally useful for employment as drug delivery vehicles or nanoreactors.

Therefore, in order to test the possibilities of conjugation of large biomacromolecules to vesicular surfaces, first the attachment of the enzyme streptavidin was chosen. This enzyme is not directly linked to the vesicles, but in a non-covalent fashion using biotin, which is a high affinity binding ligand for streptavidin ($K_d \approx 10^{-14}$ M).[58]

In order to be able to decorate polymersomes with biotin via “click” chemistry, biotin was provided with an acetylene moiety. This was accomplished by coupling of propargyl amine to $N$-hydroxysuccinimidobiotin in CH$_2$Cl$_2$ (scheme 6.7). The reaction between amines and $N$-hydroxysuccinimide esters is a very neat and efficient reaction and, consequently, the yield was high.

Scheme 6.7 Preparation of acetylene containing biotin 15. Reagents and conditions: i, propargyl amine, CH$_2$Cl$_2$, rt., 2 h, 97%

Subsequently, acetylene bearing biotin 15 was utilized to functionalize azide vesicles (5) by “click” chemistry, as depicted in scheme 6.8. The same conditions were applied as used before, i.e. CuSO$_4$•5H$_2$O as the Cu(I)-source in combination with sodium ascorbate as the reductor for Cu(II), and TBTA (13) as the stabilizing ligand for Cu(I). After 24 hours of reaction at ambient temperature in deionized water, all reactants and unreacted acetylene biotin 15 were removed by extensive dialysis of the polymersomes against a 0.55 M EDTA solution. With TEM it was visualized that, also in this case, no change in the aggregate morphology occurred as a consequence of the applied reaction conditions. Unfortunately, after dissolving some of the vesicles, SEC and $^1$H NMR results were inconclusive concerning the covalent attachment of biotin to the vesicles. For SEC measurements the difference in molecular weight between the $\omega$-azido-PS-$b$-PAA block
copolymer (4) and the formed biotinylated block copolymer was too small to observe a significant shift in retention time. By keeping in mind the non-quantitative functionalization as observed for the dansyl coupling, the $^1$H NMR signals stemming from the biotin moiety were too low compared to the signal from the backbone protons to be visualized.

Scheme 6.8 Schematic illustration of the preparation of biotinylated polymersomes (16) and the subsequent complexation of streptavidin to the surface of these polymersomes. Reagents and conditions: i. CuSO$_4$•5H$_2$O, sodium ascorbate, tris-(benzyltriazolylmethyl)amine (TBTM) (13), 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N-(prop-2-ynyl)pentanamide (15), H$_2$O, rt., 24 h; ii. streptavidin/gold (6 nm), H$_2$O, 20 h

Hence, the best method to determine the attachment of biotin to the vesicles was to directly couple streptavidin to the polymersomes, since attachment of streptavidin indirectly proves the presence of biotin. Therefore, the biotinylated vesicles (16) were subjected for 24 hours to an aqueous solution of streptavidin labeled with colloidal gold particles of 6 nm (scheme 6.8). This labeled streptavidin was chosen because the colloidal gold particles should give a clear contrast in TEM owing to the release of secondary electrons, and could thus be utilized as a marker.$^{[59]}$ After 24 hours, the vesicles were extensively dialyzed against deionized water in order to remove all unbound streptavidin. TEM measurements displayed still the presence of polymersomal aggregates, however, no clear contrast due to the colloidal gold particles was observed. CLSM images, on the other hand, exhibited clear fluorescent behavior of the vesicles in contrast to the background when excited with a 411 nm laser (figure 6.10), while non-biotinylated vesicles subjected to streptavidin were not visible. From colloidal gold particles smaller than approximately 20 nm is known that they display fluorescence.$^{[60]}$ Moreover, the image shown in figure 6.10.b, cannot be caused by scattering, since the particles are too small to scatter light from 411 nm. Furthermore, scattering was filtered out during the measurements. From
the CLSM images can be concluded that the vesicles were functionalized successfully with the protein streptavidin.

![Figure 6.10 CLSM images of PS-b-PAA polymersomes with bound streptavidin/gold, 6 nm (17) (transmission (a) and fluorescence excited at 411 nm (b))](image)

In order to demonstrate direct conjugation of proteins to polymersomes, enhanced green fluorescent protein (EGFP), which was prepared by expression in *Escherichia Coli*, was accommodated with acetylene functionalities by reaction of pentyneic acid N-succinimidyl ester (7) with one or more of the 20 lysine residues exposed to the surface of the protein, as depicted in scheme 6.9.a. In a next step, acetylene comprising EGFP (19) was attached to azide functionalized PS-b-PAA vesicles 5 by applying similar conditions as utilized in previous “click” conjugations in a 0.1 M PBS buffer of pH 7.2 (scheme 6.9.b).
Scheme 6.9 Schematic representation of the functionalization of enhanced green fluorescent protein (EGFP) with acetylene moieties (a) and its subsequent coupling to azide polymersome 5 (b). Reagents and conditions: i. EGFP, 0.1 M PBS buffer pH=7.2, 2 days; ii. acetylene bearing EGFP (19), CuSO₄•5H₂O, sodium ascorbate, TBTA (13), 0.1 M PBS buffer pH=7.2, 22 h

The fluorescent behavior of the formed EGFP functionalized vesicles (20) was visualized by CLSM, as illustrated in figure 6.11. It has to be remarked that no crosslinking of the vesicles was observed, most likely due to the high dilution of the polymersomes. To determine that this fluorescence was caused by covalently coupled EGFP, a control experiment was performed utilizing equal reaction conditions with exception that the addition of CuSO₄•5 H₂O was omitted. In this case, no fluorescence was observed, implying all unreacted EGFP was removed from the reaction mixture. Therefore, the conclusion can be drawn that EGFP 19 was successfully conjugated to the azide functionalized polymersomes 5. Further evidence concerning the fact that the signal was caused by fluorescence of EGFP was provided by the ability to quench the fluorescence by photobleaching with an intense laser beam (figure 6.11.c and d) and by the absence of signal when the EGFP bearing vesicles 20 were excited at a wavelength of 561 nm, which is out of the absorption range of EGFP.
6.4. Conclusions

The possibilities concerning the use of polymersomes as scaffolds for further functionalization utilizing “click” chemistry have been demonstrated. Therefore, polystyrene-block-poly(acrylic acid) block copolymers containing both azide and acetylene functionality at the poly(acrylic acid) termini were prepared, which were capable of forming vesicles in aqueous solution.

By the attachment of dansyl probes to these vesicles it was shown with confocal laser-scanning microscopy (CLSM) that these vesicles were fluorescent after functionalization. Covalent linkage of the probes to the polymersomes was determined by size exclusion chromatography (SEC) measurements conducted using UV detection at 345 nm. The functionalized block copolymers were visualized at this wavelength, whereas the bare block copolymer was not detectable.

By comparison of the fluorescence signal of the functionalized block copolymers with a reference compound synthesized in solution, the degree of functionalization was determined to be 23%. Because, most likely, the azide functionality in the interior of the vesicles was not available for reaction, it was rationalized that 40 to 50% of the exterior was functionalized. Subsequent attempts to optimize the reaction conditions did not have a significant effect. Therefore, the conclusion can be drawn that this is approximately the
optimum degree of functionalization. Probably, the dense packing in the aggregates shielded the residual azide groups from reacting.

Next, this strategy of post-functionalization of polymersomes was extended by conjugation of proteins to their periphery. First, vesicles were biotinylated via “click” chemistry to introduce a binding ligand for the protein streptavidin. Complexation of streptavidin bearing 6 nm gold particles was visualized by CLSM. Direct linkage of proteins was established by attachment of acetylene functionalized enhanced green fluorescent protein (EGFP) to azide functionalized polymersomes. Due to the fluorescent behavior of EGFP, the vesicles displayed fluorescence after conjugation, as confirmed by CLSM.

6.5. Acknowledgements

René Brinkhuis is gratefully acknowledged for his contribution to this chapter. Dr. Dennis Löwik and Sanne Schoffelen are acknowledged for providing acetylene functionalized biotin and tris-(benzyltriazolylmethyl)amine (TBTA), respectively. Ton Dirks is acknowledged for supplying pentynoic acid N-succinimidyl ester and Rosalie Teeuwen is acknowledged for providing green fluorescent protein. Dr. Hans Engelkamp and the General Instrumentation department are acknowledged for performing confocal laser-scanning microscopy measurements.

6.6. Experimental

6.6.1. Materials

$N,N,N',N',N''$-pentamethyldiethylenetriamine (PMDETA) (Aldrich, 99%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (Aldrich, >98%), sodium azide (Merck, >99%), 2,2’-(ethylenedioxy)diethyl amine (Aldrich, 98%), copper(II)sulfate pentahydrate (Acros, 98%), sodium ascorbate (Aldrich, >98%), anisole (Acros, 99%), tetrabutylammonium fluoride (TBAF) (Aldrich, 1.0 M solution in THF), azidotrimethylsilane (Acros, 97%), streptavidin/gold, 6 nm (Electron Microscopy Sciences, PBS buffer (pH=7.6) with BSA (1%), and 15 mM NaN$_3$), dansyl chloride (Acros, 98%), propargyl amine (Acros, 99%), tripropargyl amine (Aldrich, 98%), benzyl azide (Alfa, 94%), N-hydroxysuccinimido-biotin (Bio-connect B.V.), 1-bromoethylbenzene (Acros, 97%), ethylenediamine-tetraacetic acid tetrasodium salt tetrahydrate (EDTA) (Fluka, ≥99%), N-hydroxysuccinimide (Aldrich, 98%), and bathophenantroline-disulfonic acid disodium salt hydrate (Acros, 98%) were used as received. Triethylamine (Et$_3$N) was distilled under a nitrogen atmosphere from potassium hydroxide. Styrene and tert-butyl acrylate were distilled under reduced pressure prior to use. CuBr was purified by washing three times with glacial acetic acid, twice with absolute ethanol and twice with diethyl ether. Tetrahydrofuran (THF) was distilled under a nitrogen atmosphere from
sodium/benzophenone. Dichloromethane (CH$_2$Cl$_2$) was distilled under a nitrogen atmosphere from calcium hydride.

6.6.2. Instrumentation

Proton and carbon-13 nuclear magnetic resonance ($^1$H NMR and $^{13}$C NMR) spectra were recorded on a Bruker DPX300 spectrometer. Chemical shifts are expressed in parts per million (δ scale) relative to the internal standard tetramethylsilane (δ = 0.00 ppm) for CDCl$_3$ or DMSO (δ = 2.50 ppm) for $^1$H NMR spectra and relative to CDCl$_3$ (δ = 77.16 ppm) and DMSO (δ = 39.52 ppm) for $^{13}$C NMR spectra.

Infrared (IR) spectra were obtained using an ATI Matson Genesis Series FTIR spectrophotometer fitted with an ATR cell. Data are presented as the frequency of absorption (cm$^{-1}$).

Molecular weight distributions were measured using size exclusion chromatography (SEC) on a system equipped with a guard column and a PL gel 5 μm mixed D column (Polymer Laboratories) with differential refractive index and UV (254 nm and 345 nm) detection, using CHCl$_3$ as an eluent at 1 mL/min and $T = 30^\circ$C. Polystyrene (PS) standards in the range of 580 to 377,400 g/mol were used to calibrate the SEC.

Gas chromatography (GC) measurements were conducted on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a capillary column (HP1701, 25m x 0.32mm x 0.25 μm), using flame ionization detection.

Thin layer chromatography (TLC) was performed on Merck precoated silica gel 60 F-254 plates (layer thickness 0.25 mm). Compounds were visualized by UV or permanganate reagent. Column chromatography (CC) was carried out using silica gel, Acros (0.035-0.070 mm, pore diameter ca. 6 nm).

Transmission electron microscopy (TEM) images were obtained using a JEOL JEM 1010 microscope (60 kV) equipped with a CCD camera. Samples were prepared by placing a carbon coated copper grid on top of a droplet of an aqueous aggregate solution. After one minute, the excess of water was removed using a filter-paper. The sample grids were dried under vacuum prior to use.

Confocal laser-scanning microscopy: Laser light (Spectra Physics 2080 Ar$^+$ laser, 411 nm) was coupled into a single-mode optical fiber, reflected by a dichroic beam splitter (Chroma, 505dcxr) and focused on the sample by an oil immersion 100x objective (Zeiss, NA = 1.30), which was mounted on a Karl Zeiss Axiovert 200 inverted microscope. A power density at the sample of 1-2 kW cm$^{-2}$ was used. Fluorescence light emerging from the focal volume was collected through the same objective, passed through the beam-splitter, filtered (Chroma, HQ500lp), guided through a 50 μm pinhole and finally focused on an avalanche photodiode (Perkin Elmer SPCMAQR-14) coupled to a National Instruments PCI-6036E data acquisition card operating at 20 MHz. Samples were mounted onto a Physik Instrumente P-517.2 CL nanopositioner. Sample movement (scanning and precise positioning) and data collection were controlled by a LabView program.

6.6.3. ω-bromo-polystyrene (1)

Typical polymerization procedure:
A Schlenk tube was loaded with CuBr (143 mg, 1.00 mol), evacuated and back-filled with argon. This procedure was repeated three times. After the evacuating cycles the stopper was replaced by a septum. Styrene (30 mL, 250 mmol), anisole (6 mL) and PMDETA (173 mg, 1.00 mmol) were added and the reaction mixture was stirred until it imparted a light green color due to complex formation. 1-bromoethylbenzene (0.68 mL, 1.00 mmol) was added and the reaction mixture was placed in a
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statically controlled oil bath at 100°C. Samples were taken periodically for conversion analysis by 
\(^1\)H NMR. After reaching 50% conversion, the polymerization was stopped by cooling and 
dilution with CH\(_2\)Cl\(_2\). The organic layer was washed with a 0.055 M EDTA solution three times 
and dried using anhydrous magnesium sulfate. The reaction mixture was concentrated in vacuo 
and the polymer was precipitated in methanol, yielding a white solid which was dried in a vacuum 
oven at 60°C.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.23-6.24\) (br. m, arom. H), 4.48 (br. m, CH\(_2\)-CH(Ph)-Br), 2.25- 
1.18 (br. m, backbone CH\(_{11}\), CH\(_{11}\)), 1.11-0.97 (br. d, H-C-CH(Ph)-CH\(_2\)); FTIR-ATR 3023, 2915, 
1938, 1873, 1800, 1744, 1601, 1493, 1445, 905 cm\(^{-1}\); SEC: \(M_n = 16.2\) kg/mol, \(M_w/M_n = 1.09\)

### 6.6.4. \(\omega\)-bromo-polystyrene-\(\text{block}\)-poly(tert-butyl acrylate) (2)

![Diagram](image)

**Typical polymerization procedure:**

\(\omega\)-bromo-polystyrene (1) (1.01 g, 0.08 mmol) and CuBr (17.2 mg, 0.12 
mmol) were placed in a Schlenk tube which was fitted with a stopper, 
evacuated and back-filled with argon. This procedure was repeated three 
times. After the evacuating cycles the stopper was replaced by a septum. 

Subsequently, tert-butyl acrylate (461 mg, 3.60 mmol) and anisole (5 mL) were added. After 
complete dissolution of the macroinitiator, PMDETA (20.8 mg, 0.12 mmol) was added and the 
reaction mixture was placed in a statically controlled oil bath at 100°C. Samples were taken 
periodically for conversion analysis by GC. After reaching 40% conversion, the polymerization 
was stopped by cooling and dilution with CH\(_2\)Cl\(_2\). The organic layer was washed with a 0.055 M 
EDTA solution three times and dried using anhydrous magnesium sulfate. The organic layer was 
concentrated in vacuo and the polymer was precipitated in methanol/H\(_2\)O (1:1), yielding a white 
solid which was dried in a vacuum oven at 60°C.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.23-6.24\) (br. m, arom. H), 2.25-1.18 (br. m, backbone CH\(_{11}\), CH\(_{11}\)), 
1.44 (br. s, backbone \((\text{H}_3\text{C})_3\text{C}-\text{O}_2\text{C}\)), 1.11-0.97 (br. d, H-3C-CH(Ph)-CH\(_2\)); FTIR-ATR 3023, 2915, 
1938, 1873, 1800, 1744, 1727, 1601, 1493, 1445, 1148, 905 cm\(^{-1}\); SEC: \(M_n = 19.92\) kg/mol; 
\(M_w/M_n = 1.10\)

### 6.6.5. \(\omega\)-azido-polystyrene-\(\text{block}\)-poly(tert-butyl acrylate) (3)

![Diagram](image)

\(\omega\)-bromo-polystyrene-\(\text{block}\)-poly(tert-butyl acrylate) (2) (299 mg, 15 
\(\mu\)mol) was dissolved in THF (1.5 mL). Subsequently, Me\(_3\)Si-N\(_3\) (17.4 mg, 150 
\(\mu\)mol) and TBAF (0.15 mL, 150 \(\mu\)mol) were added via a syringe 
and the reaction mixture was stirred for 24 hours at room temperature. 

The reaction mixture was concentrated in vacuo and the polymer was 
precipitated in MeOH/H\(_2\)O (1:1). The product was isolated as a white solid which was dried 
under vacuum.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta 7.23-6.24\) (br. m, arom. H), 2.40-1.10 (br. m, backbone CH\(_{11}\), CH\(_{11}\)), 
1.44 (br. s, \((\text{H}_3\text{C})_3\text{C}-\text{O}_2\text{C}\)), 1.11-0.97 (br. d, H-C-CH(Ph)-CH\(_2\)); FTIR-ATR; 302 3, 2915, 2098 
(\(\nu\)Na), 1938, 1873, 1800, 1744, 1727, 1601, 1493, 1445, 1148, 905 cm\(^{-1}\); SEC: \(M_n = 19.51\) kg/mol; 
\(M_w/M_n = 1.10\)

### 6.6.6. \(\omega\)-azido-polystyrene-\(\text{block}\)-poly(acrylic acid) (4)

![Diagram](image)

\(\omega\)-azido-polystyrene-\(\text{block}\)-poly(tert-butyl acrylate) (3) (200 mg, 10.0 
\(\mu\)mol) was dissolved in dioxane (10 mL). Subsequently, concentrated 
HCl solution (3 equivalents with respect to \(\alpha\)BA groups) was added and
the reaction mixture was refluxed for 4 hours. Afterwards, the polymer was precipitated in H$_2$O and isolated as a white solid which was dried in a vacuum oven at 60°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.23-6.24 (br. m, arom. $H$), 2.40-1.10 (br. m, backbone $CH_2$, $CH$), 1.11-0.97 (br. d, $H_{3C}$CH(Ph)-CH$_2$); FTIR-ATR 3023, 2915, 2098 ($\nu_{N3}$), 1938, 1873, 1800, 1744, 1714, 1601, 1493, 1445, 905 cm$^{-1}$

6.6.7. Formation of $\omega$-azido-polystyrene-block-poly(acrylic acid) vesicles (5)

Deionized water (0.3 mL) was added dropwise over a period of 3 hours to a solution of $\omega$-azido-polystyrene-block-poly(acrylic acid) (4) (15 mg) in dioxane (1.5 mL). A cloudy solution was formed to which additional deionized water (8.2 mL) was added all at once. The vesicle solution was dialyzed against deionized water for 20 hours to remove dioxane.

6.6.8. $\omega$-(trimethylsilyl acetylene)-polystyrene-block-poly(tert-butyl acrylate) (6)

A Schlenk tube was loaded with CuBr (28.6 mg, 0.20 mmol), evacuated and back-filled with argon. This evacuation cycle was repeated three times prior to the addition of tert-butyl acrylate (773 mg, 6.03 mmol), anisole (0.65 mL) and PMDETA (34.6 mg, 0.20 mmol). Subsequently, the reaction mixture was placed in a statically controlled oil bath at 65°C and 3-(1,1,1-trimethylsilyl)-2-propynyl-2-bromo-2-methylpropanoate (30.5 mg, 0.11 mmol) (initiator) was added. During polymerization, samples were taken periodically for conversion analysis by $^1$H NMR. The polymerization was stopped at 40% conversion by cooling the reaction mixture and dilution with CHCl$_3$. The reaction mixture was washed three times with a 0.055 M EDTA solution. Subsequently, the organic layer was dried using anhydrous magnesium sulfate and the solvent was removed by rotary evaporation. The polymer was isolated as an off white sticky solid and dried under vacuum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 4.67 (br. m, $\equiv-CH_2-O_2C$), 4.10 (br. s, CH$_2$-C(H$_2$Br), 2.37-1.21 (br. m, CO$_2$-C(CH$_3$)$_3$, backbone CH$_2$, CH), 1.45 (br. s, CO$_2$-C(CH$_3$)$_3$, 0.18 (s, (CH$_3$)$_3$Si$\equiv$); FTIR-ATR 2982, 2927, 2862, 1724, 1478, 1449, 1388, 1369, 1256, 1140 cm$^{-1}$; SEC: $M_n$ = 2.89 kg/mol; $M_w/M_n = 1.11$

CuBr (143 mg, 1.00 mmol) was placed in a Schlenk tube which was evacuated and back-filled with argon. This procedure was repeated three times. Styrene (2.38 g, 22.85 mmol) and PMDETA (173 mg, 1.00 mmol) were added, and the reaction mixture was placed in a statically controlled oil bath at 100°C. Subsequently, 50 mL of a 0.092 M solution of $\alpha$-(trimethylsilyl acetylene)-poly(tert-butyl acrylate) (macroinitiator) in anisole (4.60 mmol) was added. Samples were taken periodically for conversion analysis by $^1$H NMR and SEC. After reaching 50% conversion, the polymerization was stopped by cooling the reaction mixture and dilution with CHCl$_3$. The reaction mixture was washed three times with a 0.055 M EDTA solution. The organic layer was dried using anhydrous magnesium sulfate and concentrated under vacuum. The polymer was isolated as a white solid by precipitation in MeOH/H$_2$O (9:1) and dried under vacuum.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.44-6.19 (br. m, arom. $H$), 4.13 (br. s, CH$_2$-CH(CO$_2$Bu)-Br), 2.51-1.14 (br. m, CO$_2$-C(CH$_3$)$_3$, backbone CH$_2$, CH), 1.43 (br. m, O$_2$-C-C(CH$_3$)$_3$-CH$_2$), 0.19 (s,
6.6.9. \( \omega \)-acetylene-polystyrene-block-poly(\( \text{tert} \)-butyl acrylate) (7)

\[
\text{(CH}_3\text{)}_3\text{Si-≡; FTIR-ATR 3023, 2915, 2859, 2362, 1938, 1873, 1800, 1744, 1727, 1601, 1493, 1445, 1253, 1142, 905 \text{ cm}^{-1} \); SEC: } M_n = 18.98 \text{ kg/mol; } M_w/M_n = 1.08
\]

\( \omega \)-(trimethylsilyl acetylene)-polystyrene-block-poly(\( \text{tert} \)-butyl acrylate) (6) (1.56 g, 0.082 mmol) was dissolved in THF (8 mL). Subsequently, TBAF (0.8 mL, 0.8 mmol) was added and the reaction mixture was stirred for 16 hours at room temperature. The polymer was purified by precipitation in MeOH/H$_2$O (1:1), yielding a white solid which was dried under vacuum.

\[^{1}H\] NMR (300 MHz, CDCl$_3$) \( \delta \) 7.43-6.19 (br. m, arom. H), 4.12 (br. s, CH$_2$-C(H$_2$)$_2$-Br), 2.53-1.14 (br. m, \( \equiv-\text{CH}_2 \), backbone CH$_2$, CH$_3$), 1.43 (br. m, O$_2$C-C(H$_2$)$_2$-CH$_2$); FTIR-ATR 3025, 2913, 2858, 2362, 1939, 1872, 1798, 1742, 1726, 1601, 1495, 1443, 1252, 1145, 908 cm$^{-1}$

6.6.10. \( \omega \)-acetylene-polystyrene-block-poly(acrylic acid) (8)

\( \omega \)-acetylene-polystyrene-block-poly(\( \text{tert} \)-butyl acrylate) (7) (0.63 g, 0.033 mmol) was dissolved in dioxane (5 mL). A concentrated HCl solution (3 equivalents with respect to BA groups) was added and, subsequently, the reaction mixture was refluxed for 4 hours. After reaction, the polymer was precipitated in H$_2$O and isolated as a white solid, which was dried under vacuum.

\[^{1}H\] NMR (300 MHz, CDCl$_3$) \( \delta \) 7.42-6.19 (br. m, arom. H), 4.22 (br. s, CH$_2$-C(H$_2$)$_2$-Br), 2.51-1.14 (br. m, \( \equiv-\text{CH}_2 \), backbone CH$_2$, CH$_3$), 1.43 (br. m, O$_2$C-C(H$_2$)$_2$-CH$_2$); FTIR-ATR 3024, 2913, 2858, 2362, 1939, 1872, 1798, 1742, 1726, 1601, 1495, 1443, 1252, 1145, 908 cm$^{-1}$

6.6.11. \( \text{N-(2-propynyl)-5-(dimethylamino)-naphthalene-1-sulfonamide}^{[52]} \) (10)

Propargyl amine (250 \( \mu \)L, 3.71 mmol) was added to a solution of dansyl chloride (500 mg, 1.85 mmol) and Et$_3$N (258 \( \mu \)L, 1.85 mmol) in CH$_2$Cl$_2$ (10 mL) at 0°C. After stirring for one hour, the reaction mixture was allowed to warm up to room temperature and stirred for an additional hour. The volatiles were removed in vacuo and the crude product was purified by column chromatography (\( n \)-hexane/Et$_2$O 1:1). The product was isolated as a yellow solid which was dried under vacuum.

Yield: 418 mg (78%); TLC: R$_f$ (CHCl$_3$/MeOH 9:1) = 0.68; \[^{1}H\] NMR (300 MHz, CDCl$_3$) \( \delta \) 8.56 (d, 1H, \( ^{3}J = 8.7 \text{ Hz, } C^7 \text{H} \)), 8.27 (m, 2H, \( C^3 \text{H}, C^4 \text{H} \)), 7.56 (m, 2H, \( C^6 \text{H}, C^5 \text{H} \)), 7.11 (d, 1H, \( ^{3}J = 7.5 \text{ Hz, } C^2 \text{H} \)), 4.81 (br. t, 1H, \( ^{3}J = 6.0 \text{ Hz, } N^8 \text{H} \)), 3.78 (dd, 2H, \( ^{2}J = 6.0 \text{ Hz, } ^{3}J = 2.4 \text{ Hz, } C^9 \text{H} \)), 2.89 (s, 6H, \( C^1 \text{H} \)), 1.92 (t, 1H, \( ^{2}J = 2.4 \text{ Hz, } C^10 \text{H} \)); \[^{13}C\] NMR (75 MHz, CDCl$_3$) \( \delta \) 152.1 ((CH$_3$)$_2$N-C), 134.2 (NH-SO$_2$-C), 130.8 (C-C-SO$_2$-NH), 129.7 (C$_{10}$H), 128.5 (C$_{10}$H), 123.2 (C$_{10}$H), 121.6 (C$_{10}$H), 119.5 (C$_{10}$H), 115.2 (C-C-N(CH$_3$)$_2$), 114.6 (C$_{10}$H), 77.6 (C$_{10}$H$_2$-C≡CH), 72.7 (C$_{10}$H$_2$-C≡CH), 45.4 (C$_{10}$H), 32.9 (C$_{10}$H)
6.6.12. 1-azido-3-aminopropane\(^{[53]}\) (11)

Sodium azide (9.75 g, 150.0 mmol) was added to a solution of 1-chloro-3-aminopropane hydrochloride (6.50 g, 50.0 mmol) in H\(_2\)O (150 mL). The reaction mixture was allowed to stir for 24 hours at 80°C. Subsequently, the reaction mixture was concentrated \textit{in vacuo} and Et\(_2\)O (50 mL) and potassium hydroxide (4.00 g, 71.3 mmol) were added. The organic layer was separated and the aqueous layer was extracted twice with Et\(_2\)O (50 mL). The combined organic layers were dried using anhydrous magnesium sulphate and the solvent was removed by rotary evaporation, yielding a yellow oil. The crude product was purified by distillation and isolated as a colorless oil.

Yield: 2.50 g (50%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 3.32 (t, 2H, \(\text{J} = 6.7\) Hz, H\(_2\)N-CH\(_2\)-CH\(_2\)), 2.76 (t, 2H, \(\text{J} = 6.8\) Hz, N\(_3\)-CH\(_2\)-CH\(_2\)), 1.68 (dt, 2H, CH\(_2\)-CH\(_2\)-CH\(_2\)); FTIR-ATR 3334 (\(\nu\)NH\(_2\)), 2958, 2098 (\(\nu\)N\(_3\)), 1562, 1480, 1298, 1255 cm\(^{-1}\)

6.6.13. N-(3-azidopropyl)-5-(dimethylamino)naphthalene-1-sulfonamide\(^{[54]}\) (12)

3-azido-1-aminopropane (11) (371 mg, 3.71 mmol) was added to a solution of dansyl chloride (500 mg, 1.85 mmol) and Et\(_3\)N (258 \(\mu\)L, 1.85 mmol) in CH\(_2\)Cl\(_2\) (10 mL) at 0°C. After stirring for one hour, the reaction mixture was allowed to warm up to room temperature and was stirred for an additional hour. The volatiles were removed \textit{in vacuo} and the crude product was purified by column chromatography (\(\eta\)-hexane/Et\(_2\)O 1:1). The product was isolated as a yellowish oil.

Yield: 548 mg (89%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 8.55 (d, 1H, \(\text{J} = 8.4\) Hz, C\(_7\)H\(_5\)), 8.26 (m, 2H, C\(_3\)H\(_2\) and C\(_4\)H\(_2\)), 7.56 (m, 2H, C\(_5\)H\(_2\) and C\(_6\)H\(_2\)), 7.12 (d, 1H, \(\text{J} = 7.6\) Hz, C\(_2\)H\(_2\)), 5.24 (br. s, 1H, N\(_8\)H), 3.21 (t, 2H, \(\text{J} = 6.4\) Hz, C\(_{11}\)H\(_2\)), 2.95 (dt, 2H, \(\text{J} = 6.4\) Hz, C\(_9\)H\(_2\)), 2.89 (s, 6H, C\(_1\)H\(_3\)), 1.62 (m, 2H, C\(_{10}\)H\(_2\))

6.6.14. Tris-(benzyltriazolylmethyl)amine (TBTA)\(^{[57]}\) (13)

A solution of tripropargylamine (10.0 g, 0.076 mol) in acetonitrile (150 mL) was treated sequentially with benzyl azide (45.3 g, 0.340 mol), 2,6-lutidine (12.2 g, 0.114 mol) and Cu(MeCN)\(_4\)PF\(_6\) (1.10 g, 1.3 mol-% with respect to alkyne moieties). Upon addition of the copper salt, the reaction mixture was cooled in an ice bath. The reaction mixture imparted a brown color and was stirred for three days at room temperature. Because no crystals were obtained, the reaction mixture was allowed to stir for an additional day at 37°C. Subsequently, crystallization was induced by blowing air over the surface of the reaction mixture. The reaction mixture was cooled to 4°C for an hour to accelerate the crystallization process. The crude off white crystals were filtered and washed with five portions of 150 mL of cold acetonitrile. The product was isolated as a white solid which was dried under vacuum.

Yield: 10.82 g (27%); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.67 (s, 3H, triazole H\(_3\)), 7.34 (m, 9H, arom. H\(_1\)), 7.26 (m, 6H, arom. H\(_2\)), 5.49 (s, 6H, Ph-CH\(_2\)-triazole), 3.70 (s, 6H, N-CH\(_2\)-triazole); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 134.7 (C=\(_2\)-arom.), 129.1 (N-CH\(_2\)-(=C)-N), 128.7 (arom. C\(_7\)), 123.8 (C=\(_5\)-N), 54.1 (N-CH\(_2\)-Ph), 47.0 (N-CH\(_2\)-triazole)
6.6.15. “Click” functionalization of vesicles with N1-(2-propynyl)-5-(dimethyl amino)-4a,8a-dihydro-1-naphthalenesulfonamide (14)

To 0.5 mL of a 0.75 mg/mL vesicle solution was added 460 μL deionized H₂O and 10 μL of aqueous solutions of dansyl probe 10 (50 mM), CuSO₄•5H₂O (50 mM), sodium ascorbate (250 mM) and 10 μL of a TBTA (13) solution in DMSO (100 mM). The reaction was allowed to proceed for 24 hours. Subsequently, the vesicle solution was dialyzed for 2 days against a 0.55 mM solution of EDTA in deionized H₂O.

6.6.16. Solution phase synthesis ω-biotinyl-polystyrene-block-poly(acrylic acid)

A Schlenk tube was loaded with ω-azido-polystyrene-block-poly(acrylic acid) (4) (10.0 mg, 0.72 μmol), N1-(2-propynyl)-5-(dimethylamino)-4a,8a-dihydro-1-naphthalenesulfonamide (10) (14.5 mg, 50 μmol) and CuBr (7.2 mg, 50 μmol). The Schlenk tube was evacuated and back-filled with argon. This procedure was repeated three times. Subsequently, THF (2 mL) and PMDETA (8.7 mg, 50 μmol) were added and the reaction mixture was stirred for 18 hours at room temperature. Afterwards, the dansyl functionalized block copolymer was purified by size exclusion chromatography using the analytical SEC setup.

6.6.17. 5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)-N-(prop-2-ynyl)pentanamide (15)

A suspension of N-hydroxysuccinimidobiotin (25.2 mg, 73.2 μmol) and propargyl amine (8.52 mg, 9.9 μL, 153.8 μmol) in CH₂Cl₂ (2 mL) was stirred for 2 hours at room temperature. Completion of the reaction was determined by TLC. The crude product was purified by column chromatography using CHCl₃/MeOH/H₂O (65:25:4) as an eluent. The product was isolated as a white solid.

Yield: 20.0 mg (97%); TLC: Rₜ (CHCl₃/MeOH/H₂O 65:25:4) = 0.53; ¹H NMR (300 MHz, DMSO-d₆) δ 8.26 (t, 1H, J = 6.0 Hz, N²H), 6.47 (s, 1H, N¹H), 6.41 (s, 1H, N¹H), 4.36 (t, 1H, J = 5.1 Hz, C³H), 4.19 (m, 1H, C¹H), 3.89 (dd, 2H, J = 6.0 Hz, J = 2.7 Hz, C¹³H), 3.09 (m, 2H, C²H, C¹⁴H), 2.82 (dd, 1H, J = 7.2 Hz, J = 5.1 Hz, C²¹H), 2.59 (m, 1H, C¹⁹H), 2.14 (t, 2H, J = 7.2 Hz, C²¹H), 1.57 (m, 2H C¹³H), 1.49 (m, 2H, C¹⁰H), 1.28 (m, 2H, C²¹H), 1.3C NMR (75 MHz, CD₃OD) δ 176.4 (H,C-C(=O)-NH), 166.9 (HN-C(=O)-NH), 81.5 (H,C-C≡CH), 72.9 (H,C-C≡CH), 64.2 (C³H), 62.4 (C⁶H), 57.8 (C⁵H), 41.8 (C⁸H), 37.3 (C¹¹H), 30.5 (C¹³H), 30.2 (C⁷H), 29.9 (C⁶⁰H), 27.5 (C³H)
6.6.18. “Click” functionalization of vesicles with propargylamidobiotin (16)  

To 0.5 mL of a 0.75 mg/mL vesicle solution was added 460 μL deionized H₂O and 10 μL of aqueous solutions of acetylene functionalized biotin (50 mM), CuSO₄•5H₂O (50 mM), sodium ascorbate (250 mM) and 10 μL of a TBTA solution in DMSO (100 mM). The reaction was allowed to proceed for 24 hours. Subsequently, the vesicle solution was dialyzed for 2 days against a 0.55 mM solution of EDTA in deionized H₂O.

6.6.19. Complexation of streptavidin to biotinylated vesicles (17)  

To 0.5 mL of a 0.75 mg/mL solution of biotinylated vesicles (16), 1.5 mL of an aqueous solution containing streptavidin bearing 6 nm gold particles was added. The reaction mixture was shaken for 24 hours. Subsequently, the vesicles were extensively dialyzed against deionized H₂O. The molecular weight cut-off of the used membrane was 100 kDa.

6.6.20. 4-pentynoic acid N-succinimidyl ester (18)  

To a flame dried round bottom flask 4-pentynoic acid (502 mg, 5.12 mmol), N-hydroxysuccinimide (631 mg, 5.48 mmol), and CH₂Cl₂ (20 mL) were added. After cooling the mixture to 0 °C, EDCI (1.09 g, 5.70 mmol) was added. The mixture was allowed to stir at 0 °C for 1 hour followed by 18 hours at room temperature. Completion of the reaction was determined by TLC (EtOAc/heptane 1:1). Upon dilution with EtOAc (100 mL), the mixture was washed with a saturated aqueous NH₄Cl solution (2 × 100 mL). The organic phase was dried over anhydrous Na₂SO₄ and concentrated in vacuo, yielding the desired product as a white solid.

Yield: 954 mg (95%); TLC: Rₓ (EtOAc/heptane 1:1) = 0.36; ¹H NMR (400 MHz, CDCl₃) δ 2.91 – 2.86 (m, 2H, CH₂-C₆H₄-CO₂), 2.84 (br. s, 4H, (O=)C-(C₆H₄)₂-C(=O)), 2.65 – 2.59 (m, 2H, ≡−C₆H₄-CH₂), 2.05 (t, 3J = 2.65 Hz, 1H, H−≡−CH₂)

6.6.21. Acetylene functionalized enhanced green fluorescent protein (EGFP) (19)  

To 0.5 mL of a 0.34 mg/mL solution of green fluorescent protein in 0.1 M PBS buffer pH=7.2, 10 μL of a 5.5 mM solution of pentyonoic acid N-succinimidyl ester was added. The reaction mixture was shaken for 25 hours at room temperature. Subsequently, the product was purified by extensive dialysis against 0.1 M PBS buffer pH=7.2. The molecular weight cut-off of the used membrane was 1 kDa.
6.6.22. “Click” conjugation of vesicles with EGFP (20)

To a 0.75 mg/mL vesicle solution was added 200 μL of a 0.17 mg/mL solution of acetylene functionalized EGFP in 0.1 M PBS buffer pH = 7.2. Subsequently, 10 μL of aqueous solutions of acetylene functionalized biotin (50 mM), CuSO₄·5H₂O (50 mM), sodium ascorbate (250 mM) and 10 μL of a TBTA solution in DMSO (100 mM) were added. The reaction was allowed to proceed for 22 hours. After reaction, the vesicle solution was extensively dialyzed against deionized H₂O. The molecular weight cut-off of the used membrane was 100 kDa.

6.7. References

"Clickable" polymersomes


Summary

In Nature, complex three-dimensionally ordered biopolymers, such as proteins and DNA, can be found which are dependent on a high level of structural control in order to perform their desired (biological) tasks. This, however, often also makes it difficult to apply biopolymers in material science due to their susceptibility to conformational changes. Synthetic polymers on the other hand, can be prepared in a wide range of architectures and compositions and are, therefore, readily adaptable to specific applications. However, although polymer chemistry clearly has advanced in the last decades, the level of control over the composition is still limited when compared to biopolymers. Accordingly, a logical approach is to combine the natural control of biopolymers with the versatility of synthetic polymers into a new class of hybrid macromolecules. Due to the currently available synthetic techniques to prepare well-defined polymer building blocks, both from synthetic and biological origin, this field of research has gained momentum in the last couple of years. In chapter 1, an overview is given regarding the preparation of such building blocks and the different methodologies to conjugate them in order to form biohybrid polymers.

Owing to the presence of many functional groups in biopolymers it is often difficult to couple them to synthetic polymers in a controlled fashion, which is a prerequisite to preserve (bio)functionality. Therefore, coupling chemistry is demanded which is both high yielding and selective. Recently, Sharpless et al. improved the Huisgen’s 1,3-dipolar cycloaddition reaction between azides and terminal acetylenes by means of copper(I)-catalysis. This type of “click” chemistry is very efficient and orthogonal with respect to other functional groups, therefore making it a perfect tool for bioconjugation. Because hardly anything was known about the application of “click” chemistry in polymer synthesis, first the possibility of connecting polymers via their end groups was tested, which is discussed in chapter 2. First and foremost, both azide and acetylene end groups had to be introduced in polymers, which was accomplished by utilizing atom transfer radical polymerization (ATRP) in combination with either functionalized initiators or post-polymerization end group modification procedures. Subsequently, these polymer building blocks were connected by applying a copper(I)-catalyst, as depicted in scheme 1. In this way, several AB and ABA-type block copolymers were prepared. In all cases, an excess of one of the two building blocks was utilized to drive the reaction to completion. These residual polymers were removed by precipitation or reaction of their acetylene
Summary

moieties with an azide functionalized scavenger resin, which enabled removal by a filtration step.

Scheme 1 Example of the modular synthesis of a poly(ethylene glycol)-block-poly(methyl methacrylate) diblock copolymer

Chapter 3 deals with the extension of this “click” coupling concept, in order to allow successive functionalization of both end groups of a single polymer. Therefore, polymer building blocks comprising both an azide as well as an acetylene end group were prepared by ATRP. In order to prevent interference in the first “click” reaction, the acetylene moiety was accommodated with a triisopropylsilyl (TIPS) protecting group, which could be readily removed afterwards. As a proof of concept, α-(TIPS acetylene)-ω-azido-polystyrene was consecutively functionalized with propargyl alcohol and azidoacetic acid. Next, a polymethylacrylate-block-polystyrene-block-poly(tert-butyl acrylate) ABC-type triblock copolymer was prepared in this modular fashion, as depicted in scheme 2. Analogous to the previous chapter, an excess of one of the building blocks was applied, which in this case was removed by reduction of the residual azide groups into the corresponding amines and subsequent application of column chromatography.

Scheme 2 Modular build up of an ABC-type triblock copolymer utilizing “click” chemistry in combination with a protecting group strategy

The introduction of both azide and acetylene moieties into one polymer chain gave rise to the idea to exploit these heterotelechelic polymers as linear precursors for the synthesis of cyclic polymers, which is the subject of chapter 4. Because of the absence of chain ends and the restrictions on the polymer backbone conformation, cyclic polymers have
some distinct properties in comparison to their linear counterparts. By subjecting the linear precursors to copper(I)-catalysts, cyclic-polystyrene and cyclic-poly(tert-butyl acrylate) were prepared (scheme 3). The cyclization reactions had to be conducted in a very dilute environment to prevent intermolecular reaction which leads to chain extension. In order to remove possibly present linearly chain extended polymers and residual linear precursors, their azide groups were reduced into amines which allowed removal by performing column chromatography. Attempts to synthesize the cyclic block copolymer polystyrene-\text{-}b\text{-}poly(tert-butyl acrylate) by cyclization of a heterotelechelic AB diblock copolymer precursor failed unfortunately, probably due to incompatibility of the distinct blocks, which may have prevented the reactive end groups to approach each other.

Scheme 3 Example of the synthesis of a cyclic polymer by executing an intramolecular “click” reaction on an α-acetylene-ω-azide functionalized linear precursor polymer

In chapter 5, initial research with respect to the synthesis of biohybrid block copolymers utilizing “click” chemistry is described. In the first line of research, ABA-type triblock copolymers of which the A-blocks embrace nucleobase functionality were synthesized, in order to obtain telechelic precursors for the formation of supramolecular (block) copolymers. Therefore, azide functionalized poly(ethylene glycol) and both acetylene functionalized thymine and adenine oligomers were prepared in good control. Subsequently, the azide and acetylene bearing polymers were coupled utilizing a Cu(I)-catalyst. The thymine comprising triblock copolymer was successfully isolated, whereas for the adenine analogue no reaction was observed. Probably, this was caused by inactivation of the Cu-catalyst by complexation of the adenine residues. When adenine containing triblock copolymers are prepared as well, supramolecular (block) copolymers based on nucleobase interactions can be formed.

In the second line of research, biohybrid block copolymers encompassing the peptides KTVIIE (K = lysine, T = threonine, V = valine, I = isoleucine, E = glutamic acid) and (VPGVG)\textsubscript{3} (V = valine, P = proline, G = glycine) were synthesized using “click” chemistry. These peptides were chosen because of their interesting properties, \textit{viz.}
KTVIIE is capable of forming amyloid like fibrils in an aqueous environment, while the latter elastin mimetic peptide displays lower critical solution temperature (LCST) behavior. Employment of the conjugation methodologies developed in chapter 2 and 3, i.e. using monofunctionalized and heterotelechelic bifunctionalized azide and acetylene precursors, resulted in the preparation of poly(ethylene glycol)-block-KTVIIE and poly(acrylic acid)-block-polystyrene-block-(VPGVG)_3 block copolymers. The synthesis of the latter biohybrid is depicted in scheme 4. It has to be emphasized that the coupling reactions were conducted with the peptides still attached to a resin in order to facilitate the purification process. However, owing to difficulties with cleaving the biohybrid block copolymers from the resin, the yields were very low. For that reason, no information regarding the properties of both materials was obtained.

Scheme 4 Modular synthesis of a poly(acrylic acid)-block-polystyrene-block-(VPGVG)_3 biohybrid triblock copolymer

Another approach in bioconjugated polymer synthesis is outlined in chapter 6. Here, amphiphilic polystyrene-block-poly(acrylic acid) containing either an azide or an acetylene end group on the poly(acrylic acid) terminus was self-assembled into vesicular aggregates, so-called polymersomes. These polymersomes of which the exterior was covered with reactive handles were utilized as scaffolds for further functionalization, as schematically illustrated in scheme 5.
Scheme 5 Schematic representation of the functionalization of the vesicular periphery using “click” chemistry

By application of a copper-catalyst, a fluorescent acetylene functionalized dansyl probe was attached to the vesicles, as visualized with confocal laser-scanning microscopy (CLSM). With UV spectroscopy was established that approximately 40 percent of the block copolymers exposed to the surface were functionalized. This yield could not be improved, which led to the conclusion that not all azide moieties were available for reaction owing to the dense packing of the block copolymers in the vesicles.

Subsequently, the azide bearing polymersomes were exteriorly functionalized with proteins. Initially, this was accomplished indirectly by labeling the vesicles with biotin, which were employed as ligands to bind gold labeled streptavidin. In a next experiment, acetylene functionalized enhanced green fluorescent protein (EGFP) was coupled in a direct fashion. In both cases, successful attachment of the proteins to the vesicular periphery was visualized by CLSM. These results open up possibilities to introduce (bio)active moieties such as targeting ligands or enzymes to these vesicular nanocontainers without disrupting the aggregate morphology. These active polymersomes can then be utilized as nanoreactors or drug delivery vehicles.
Samenvatting

In de natuur komen complexe, driedimensionaal geordende biopolymeren voor zoals eiwitten en DNA, die afhankelijk zijn van een hoge mate van structurele organisatie voor het uitvoeren van hun gewenste (biologische) taak. Deze eigenschap maakt het ook lastig om biopolymeren toe te passen in materialen, omdat ze gevoelig zijn voor conformationele veranderingen. Synthetische polymeren kunnen vervaardigd worden in een breed scala aan composites en architecturen, en zijn daardoor gemakkelijk aan te passen aan een gewenste toepassing. Hoewel op het gebied van de polymerchemie de laatste decennia veel vooruitgang is geboekt, is de mate van controle over de uiteindelijke samenstelling van polymeren nog altijd gelimiteerd in vergelijking met biopolymeren. Een logische aanpak derhalve is om de natuurlijke controle van biopolymeren met de veelzijdigheid van synthetische polymeren te combineren tot een nieuwe klasse van biohybride polymeren. Als gevolg van de synthesetechnieken die heden ten dage beschikbaar zijn om goed gedefinieerde, polymere bouwstenen te bereiden, zowel van synthetische als biologische origine, heeft dit onderzoeksgebied van biohybride macromoleculen de laatste jaren in een toenemende mate van belangstelling gestaan. In hoofdstuk 1 wordt een overzicht gegeven van de bereiding van polymere bouwstenen en van methodes om deze te koppelen, waardoor biohybride polymeren worden gevormd.

Doordat er in biopolymeren vele functionele groepen aanwezig zijn, is het vaak moeilijk om deze op een gecontroleerde wijze te koppelen aan synthetische polymeren, hetgeen heelal een vereiste is om (bio)functionaliteit te behouden. Dietenengevolge is koppelingchemie vereist met een hoog rendement en hoge selectiviteit. Sharpless and collega’s hebben onlangs de Huisgen 1,3-dipolaire cycloadditiereactie tussen azides en eindstandige alkynen geoptimaliseerd door middel van het toepassen van koper(I) katalyse. Deze vorm van “klik” chemie is uiterst efficiënt, waarbij andere functionaliteiten niet interfereren met deze koppelingreactie. Dit maakt deze reactie uitermate geschikt voor bioconjugatie.

Omdat zeer weinig bekend was over het toepassen van dit soort “klik” chemie in polymeersynthese, werd allereerst de mogelijkheid onderzocht om de uiteinden van polymeren aan elkaar te koppelen, hetgeen beschreven is in hoofdstuk 2. Daarvoor moesten polymeren gefunctionaliseerd worden met zowel azide als acetylen eindgroepen, wat gerealiseerd werd door het toepassen van atoom transfer radicaalpolymerisatie (ATRP) in combinatie met het gebruik van ofwel functionele initiatoren ofwel modificatie
van de polymeer eindgroepen. Vervolgens werden deze polymere bouwstenen aan elkaar gekoppeld door gebruik te maken van een koper(I) katalysator, zoals weergegeven in figuur 1. In alle gevallen werd een overmaat van één van de bouwstenen gebruikt om volledige omzetting te bewerkstelligen. De achtergebleven, ongereageerde polymeren werden verwijderd door precipitatie of reactie van de acetylene-eindgroepen met een azide gefunctionaliseerde hars, waardoor deze overmaat eenvoudig verwijderd kon worden door middel van filtratie.

**Figuur 1** Voorbeeld van de modulaire synthese van een polyethyleenglycol-blok-polymethylmethacrylaat diblok copolymer

Hoofdstuk 3 beschrijft de uitbreiding van dit “klik” koppelingsconcept naar onafhankelijke functionalisatie van beide eindgroepen van een polymeer. Daartoe werden polymere bouwstenen, die zowel azide als acetylene eindgroepen bevatten, gemaakt door middel van ATRP. Om participatie in de eerste “klik” reactie te voorkomen, werd de acetylene functionaliteit voorzien van een triisopropylsilyl (TIPS) beschermgroep, welke achteraf gemakkelijk verwijderd kon worden. Dit concept werd bewezen door α-(TIPS acetylene)-ω-azido-polystyreen achtereenvolgens te functionaliseren met propargyl alcohol en azidoazijnzuur. Vervolgens werd een polymethylacrylaat-blok-polystyreen-blok-poly(tert-butylacrylaat) ABC-type triblok copolymer op modulaire wijze bereid (zie figuur 2). Analoog aan de modulaire koppeling zoals beschreven in het voorgaande hoofdstuk werd ook in dit geval een overmaat van één van de bouwstenen gebruikt, welke verwijderd werd door reductie van de overgebleven azide groepen in de corresponderende amines, waardoor zuivering met behulp van kolomchromatografie mogelijk was.
De invoering van zowel een azide- als een acetyleneengroep in één polymeerketen gaf aanleiding tot het idee om deze polymeren te gebruiken als lineaire uitgangsmaterialen voor het synthetiseren van cyclische polymeren, hetgeen het onderwerp is van hoofdstuk 4. Door de afwezigheid van ketuimiteinden en de conformationele restricties van de polymeerketen hebben cyclische polymeren andere eigenschappen in vergelijking tot hun lineaire tegenhangers. Door middel van het toepassen van koper(I) katalyse werden cyclisch-polystyreen en cyclisch-poly(tert-butylacrylaat) bereid (zie figuur 3). Deze cyclisatieacties moesten in een uiterst verdund milieu uitgevoerd worden om intermoleculaire reacties, wat lineaire ketenverlening tot gevolg heeft, te voorkomen. Om mogelijk aanwezige lineaire polymeerketten te verwijderen, werden de azide groepen gereduceerd tot amines, waarna de cyclische polymeren gezuiverd werden door middel van kolomchromatografie. Pogingen om het cyclische blok copolymer polystyreen-blok-poly(tert-butylacrylaat) te synthetiseren zijn helaas mislukt. De oorzaak is waarschijnlijk incompatibiliteit van beide blokken, met als gevolg dat de reactieve eindgroepen elkaar niet benaderen konden.

Figuur 3 Voorbeeld van de synthese van een cyclisch polymer door het uitvoeren van een intramoleculaire “klik” reactie aan een α-acetylen-o-azide gefunctionaliseerd lineair polymere bouwsteen
Samenvatting

In hoofdstuk 5 staat initieel onderzoek beschreven met betrekking tot de synthese van biohybride blok copolymeren, gebruik makende van “klik” chemie. In de eerste onderzoekslijn werden ABA-type triblok copolymeren bereid waarvan de A-blokken nucleobase functionaliteit bevatten. Deze triblok copolymeren zouden moeten dienen als uitgangsmateriaal voor de opbouw van supramoleculaire (blok) copolymeren. Allereerst werden azide gefunctionaliseerde telecheel polyethyleenglycol en oligomeren voorzien van zowel thymine als adenine functionaliteit op gecontroleerde wijze gesynthetiseerd. Daarna werden deze azide en acetylen bevattende polymeren gekoppeld door het toepassen van een koper(I) katalysator. Het thymine bevattende triblok copolymeer werd geïsoleerd, terwijl voor de adenine analogo in het gehele geen reactie waargenomen werd. Waarschijnlijk werd dit veroorzaakt door inactivering van de koper katalysator als gevolg van complexering aan de adenine residuen. Wanneer ook adenine triblok copolymeren bereid kunnen worden, is het mogelijk om supramoleculaire (blok) copolymeren te vormen welke gebaseerd zijn op nucleobase interacties.

In de tweede onderzoekslijn werden door middel van “klik” chemie biohybride blok copolymeren gesynthetiseerd met de peptiden KTVIIE (K = lysine, T = threonine, V = valine, I = isoleucine, E = glutaminezuur) en (VPGVG)$_3$ (V = valine, P = proline, G = glycine). Deze peptiden zijn gekozen vanwege hun interessante eigenschappen. KTVIIE is namelijk in staat tot het vormen van amyloïde vezels in een waterig milieu en het tweede peptide, dat een elastine mimeticum is, heeft als eigenschap dat het zogenaamd “lower critical solution temperature” (LCST) gedrag vertoond. In analogie met de conjugatiemethodologieën beschreven in de hoofdstukken 2 en 3, d.w.z. door gebruik te maken van monofunctionele en heterotelechele, bifunctionele bouwstenen, voorzien van azide en acetylen groepen, zijn polyethyleenglycol-blok-KTVIIE en polyacrylzuur-blok-polystyreen-blok-(VPGVG)$_3$ gesynthetiseerd. De synthese van het laatste biohybride blok copolymeer is afgebeeld in figuur 4. Het moet benadrukt worden dat de koppelingsreacties werden uitgevoerd met de peptiden nog verankerd aan een hars, om het opzuiveren te vergemakkelijken. Echter, als gevolg van problemen met het afplitsen van de producten van het hars, waren de opbrengsten erg laag. Om die reden is geen informatie met betrekking tot materiaaleigenschappen verkregen.
Figuur 4 Modulaire synthese van een polyacrylzuur-blok-polystyreen-blok-(VPGVG)₃ biolybride triblok copolymer

In hoofdstuk 6 is een andere benadering om bio geconjugeerde polymeer te synthetiseren beschreven. Het amfisfe polystyreen-blok-polyacrylzuur, gefunctionaliseerd met een azide- of acetylenengroep aan het uiteinde van het polyacrylzuur blok, werd geassembleerd tot vesiculaire aggregaten, ook wel polymersomen genoemd. Deze polymersomen, bedekt met reactieve groepen, werden gebruikt voor verdere functionalisering, zoals schematisch weergegeven in figuur 5.

Figuur 5 Schematische illustratie van de functionalisering van een vesiculaire periferie middels “klik” chemie
Door middel van het toepassen van een koper katalysator werd een fluorescent, acetylene gefunctionaliseerd dansyl molecuul vastgekoppeld, hetgeen gevisualiseerd werd met confocale laser-scanning microscopie (CLSM). Met behulp van UV spectroscopie werd vastgesteld dat ongeveer 40 procent van de blok copolymeren in de buitenlaag van de vesicles was voorzien van een fluorescent label. Deze opbrengst kon niet worden verhoogd, hetgeen leidde tot de conclusie dat niet alle azide groepen beschikbaar waren voor reactie, als gevolg van de dichte pakking van de blok copolymeren in de vesicles.

Vervolgens werd de buitenzijde van de azide bevattende polymersomen gefunctionaliseerd met eiwitten. Allereerst werd dit gedaan op een indirecte manier door de vesicles te voorzien van biotine, dat diende als ligand om goud gelabeld streptavidine te koppelen. In een vervolgexperiment werd acetylene gefunctionaliseerd “enhanced green fluorescent protein” (EGFP) direct aan de vesicles gekoppeld. In beide gevallen werd de verankering van de eiwitten aan de buitenzijde van de vesicles gevisualiseerd met behulp van CLSM. Deze resultaten bieden nu de mogelijkheid om (bio)actieve groepen, zoals liganden of enzymen te introduceren in vesiculaire nanocontainers, zonder de aggregaat morfologie te verstoren. Deze actieve polymersomen zouden toegepast kunnen worden als nanoreactoren of voor het transport van medicijnen in het lichaam.
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Joost
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- J. Opsteen, J. Smeenk, L. Ayres, D. Löwik, J.C.M. van Hest; Polymer peptide hybrid architectures, *PMSE* 2006, 94, 181
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