Synthesis and Self-Assembly of Amphiphilic Polymers as Artificial Cell Prototypes

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Bachelorarbeit im Studiengang Chemie an der Johannes Gutenberg-Universität Mainz

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List of Abbreviations

AROP ..... anionic ring opening polymerization
BDP 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
BDP 630/650 ..... red fluorescence $\lambda_{excitation}/\lambda_{emission}$ 630/650 nm
BDP FL ..... green fluorescence $\lambda_{excitation}/\lambda_{emission}$ 503/512 nm
DAD ..... diode array detector
DBCO-acid ..... dibenzocyclooctyne-acid
DBU ..... 1,8-diazabicyclo[5.4.0]undec-7-ene
DCC ..... *N,N'*-dicyclohexylcarbodiimide
DCM ..... dichloromethylene
DHU 1,3-dicyclohexylurea
DI water ..... deionized water
DMAP 4-dimethylaminopyridine
DMF ..... *N,N*-dimethylformamide
EEP ..... ethyl ethylene phosphate
GPC ..... gel permeation chromatography
GUV ..... giant unilamellar vesicle
HPLC ..... high performance liquid chromatography
LUV ..... large unilamellar vesicle
MLV ..... multilamellar vesicle
MVV ..... multivesicular vesicle
MWCO ..... molecular weight cut-off
PB ..... poly(butadiene)
PDI ..... polydispersity index
PDMS ..... poly(dimethyl siloxane)
PEO ..... poly(ethylene oxide)
Poly-OH ..... hydroxyl-terminated polymer
Poly-OTos ..... tosylated polymer
SUV ..... small unilamellar vesicle
TosCl ..... *p*-toluenesulfonyl chloride
TU ..... *N*-[3,5-bis(trifluoromethyl)phenyl]-*N'*-cyclohexyl-thiourea

1 Summary

A library of amphiphilic block copolymers was synthesized. The hydrophilic block used was poly(ethyl ethylene phosphate), a biodegradable polymer,[1] the hydrophobic block was either poly(butadiene) (PB_{72}-*b*-$PEEP_p$) or poly(dimethyl siloxane) ($PDMS_{56}$-*b*-$PEEP_p$). Both combinations have been shown to self-assemble into giant unilamellar vesicles (GUVs) under non-assisted film hydration.[2,3] In a second step, the polymers that yielded GUVs, were functionalized with alkyne and azide moieties. Dibenzocyclooctyne (DBCO) was chosen as the alkyne as it can be used for Cu-free azide-alkyne coupling, which is a bioorthogonal reaction.[4] The amphiphilic alkyne-functionalized polymer could form vesicles on its own. Amphiphilic block copolymers were doped with 10 wt% of azide-functionalized polymers to form vesicles. The functionalized vesicles were combined in a vial after formation and observed for the duration of one week.

2 Zusammenfassung

Eine Bibliothek amphiphiler Blockcopolymere wurde synthetisiert. Der verwendete hydrophile Block war Poly(ethylethylenphosphat), ein biologisch abbaubares Polymer.[1] Als hydrophober Block wurde entweder Poly(butadien) (PB_{72}-*b*-$PEEP_p$) oder Poly(dimethylsiloxan) ($PDMS_{56}$-*b*-$PEEP_p$) eingesetzt. Beide Varianten eignen sich zur Bildung von sogenannten *giant unilamellar vesicles* (GUVs) unter einfacher Filmhydratation.[2,3] Die Polymere, die zu GUVs führten, wurden in einem zweiten Schritt mit Alkin- und Azidgruppen funktionalisiert. Dibenzocyclooctin (DBCO) wurde als Alkin gewählt, da es für kupferfreie Azid-Alkin-Kopplung verwendet werden kann, die eine bioorthogonale Reaktion[4] ist. Das amphiphile alkinfunktionalisierte Polymer konnte alleine Vesikel bilden. Zur Bildung von Vesikeln aus azidfunktionalisierten Polymeren wurden amphiphile Blockcopolymere mit 10 Gew.-% des funktionalisierten Polymers dotiert. Die funktionalisierten Vesikel wurden nach der Bildung in einem Gefäß kombiniert und für die Dauer von einer Woche beobachtet.

3 Introduction

All cells in living organisms have one thing in common: a cell membrane. The outer membrane of a cell protects the chemical and biological processes inside the cell from the extracellular environment.[5] The membranes of compartments inside the cell make pH gradients possible, which in turn are used in energy generation. The semi permeability of the cell membrane, a phospholipid bilayer, enables communication between these compartments. To mimic membranes and study the complex interplay of their physical and chemical characteristics, scientists have studied lipid-based membrane mimics called liposomes (from the Greek "λίπος (*lípos*)" fat and *some* meaning "body of") for decades,[5] and since 1999[6] they have also been looking at polymersomes. As the name implies, polymersomes are the polymeric counterpart of liposomes, which brings significant advantages in areas such as chemical tunability and added stability (higher longevity).[5,7,8] Due to the higher molecular weight of the building blocks and resulting lower lateral fluidity in polymersomes, they are better at encapsulation.[5] Another advantage of the polymersomes compared to liposomes is that their component amphiphilic block copolymers are widely available.

Amphiphilic molecules, like lipids and like those block copolymers that form vesicles, contain a hydrophilic and a hydrophobic entity. To minimize the surface energies of the hydrophobic block with water, they arrange in double layer structures when put into an aqueous medium. If they have the proper hydrophilic ratio, the bilayers can form hollow spherical structures that are called vesicles.[7] It is generally accepted that the preparation procedure is of high importance and a particular polymer only forms vesicles when careful preparation steps are taken.[9] The vesicles formed are not energetically stable but inert.[9] In fact, research suggests that giant unilamellar vesicles (GUVs, 1 µm to 200 µm in diameter[5]) are more energetically stable than their smaller equivalents, large or small unilamellar vesicles (LUVs, 100 nm – 1000 nm and SUVs, 20 nm – 100 nm), even though they are entropically less favored.[8]

Predominantly, research into polymersomes has been focused on small or large unilamellar vesicles[2] (SUVs or LUVs) with diameters between 20 nm and 1,000 nm.[5] In comparison, typical eukaryotic cells have diameters in a range of 10 µm to 100 µm.[5] While there are multiple methods to form small unilamellar vesicles, bigger, so called giant unilamellar vesicles (GUV) are less easily obtainable[2] but they mimic cells more closely.[9] The larger diameter of GUVs, compared to SUVs and LUVs, and comparable membrane thickness of around 4 nm[9] results in a higher cargo-to-polymer-ratio which can be advantageous for the up-take of hydrophilic cargo. The polymersomes can encapsulate hydrophilic cargo in the volume of the sphere and hydrophobic cargo in the membrane and were even shown to encapsulate nanoparticles using a mechanism similar to endocytosis.[10] Polymersomes have various useful applications in fields such as chemistry, materials science and biology.[8,11]

In this thesis diblock copolymers of poly(butadiene)-*block*-poly(ethyl ethylene phosphate) (PB-*b*-PEEP), as well as of poly(dimethyl siloxane)-*block*-poly(ethyl ethylene phosphate) (PDMS-*b*-PEEP) were used as building blocks for GUVs. The PB-*b*-PEEP polymers were first introduced for vesicle formation by Rideau *et al*.[2] The PDMS-*b*-PEEP polymers were shown to form GUVs by the same method by Rideau *et al.*[3] and could be mixed with PB-*b*-PEEP in GUVs to form domains. The hydrophilic phosphate block bears a close resemblance to the phosphate group in the phospholipids of the cell membranes of living

cells. Therefore PEEP block copolymers are superior cell mimics, especially compared to polymers using the poly(ethylene oxide) (PEO) as a hydrophilic block that are currently dominating the field of vesicles for drug delivery.[2,11,12] PEEP not only outperforms the PEO based amphiphilic block copolymers in respect to the vesicle formation process by film hydration,[2,13] it is also completely biodegradable to water, carbon dioxide and phosphate.[1,5] While GUVs in the past have usually been assembled through electroformation[2] the novel PB-*b*-PEEP self-assemble using a simple yet effective non-assisted film hydration method with high-yielding results.[13]

Additionally the polymersomes show great variability as chemists are able to functionalize them without significantly reducing their ability to form vesicles.[5] Liposomes on the other hand are smaller molecules and chemical functionalization is significantly influencing their ability to self-assemble.[5] This tuneability of the polymersomes opens up new opportunities to use the vesicles.

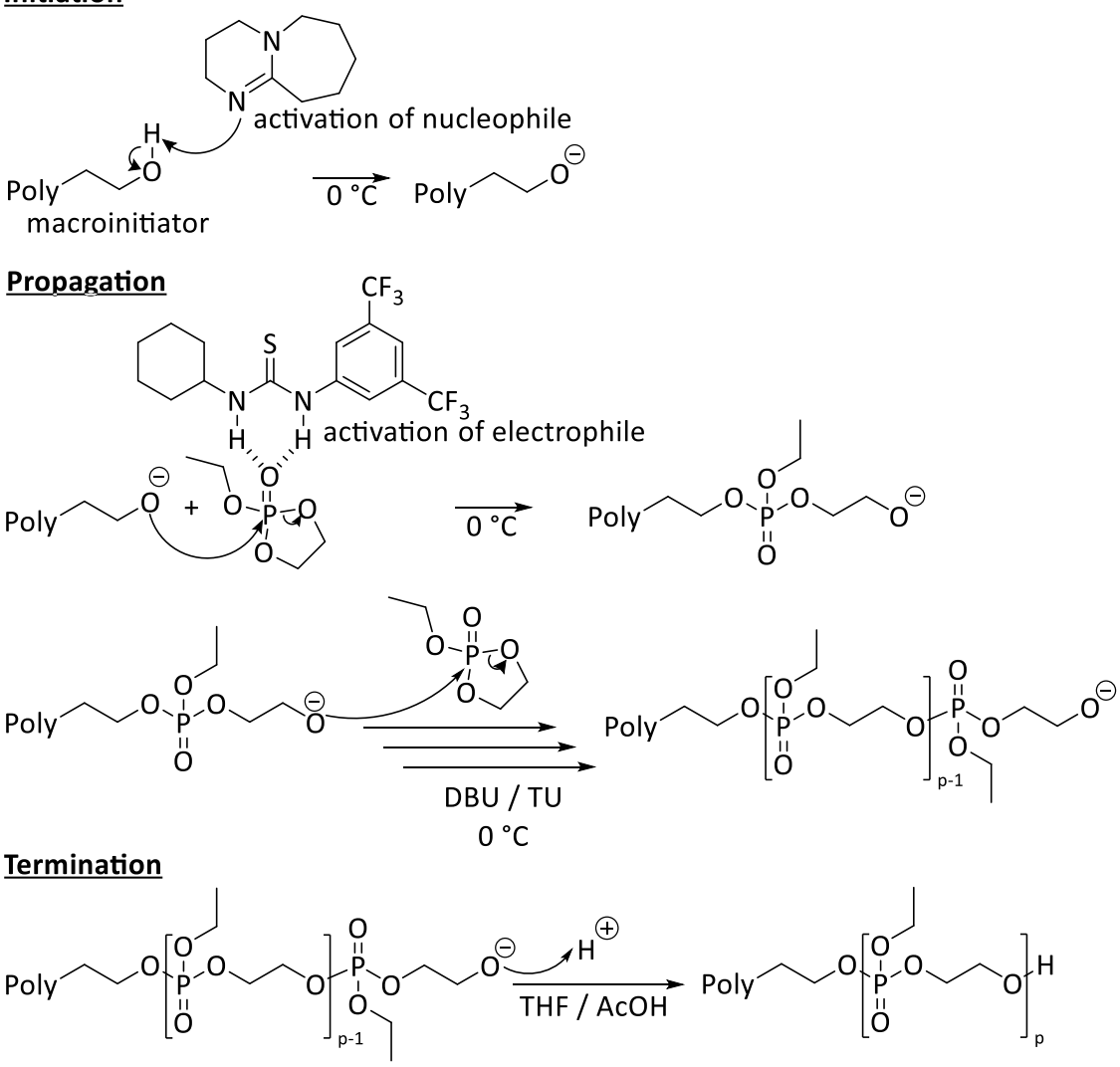
In this thesis, PB-*b*-PEEP polymers were functionalized to contain alkyne and azide moieties. The purpose of this functionalization was to combine the vesicles via azide-alkyne cycloaddition, through a metal-free method for modular covalent coupling, so called *"click reactions"*.[14] The term click chemistry was coined by Sharpless *et al.* describing reactions that are efficient, regioselective and have a fast reaction rate, all while being performed under mild reaction conditions.[15] Cu(I)-catalyzed Huisgen-Sharpless click reaction was employed by Loosli *et al.*[16] for liposome (LUVs, 100 nm diameter) aggregation and fusion. Jin *et al.* reported similar results for their coupling of branched polymersomes (5-10 µm) using Cu.[15] Azide-alkyne click reactions without Cu are especially interesting since strain promoted azide-alkyne coupling is a bioorthogonal reaction.[4] Although Cu is a necessary trace element, excess can be life-threatening even in small doses.[17] Therefore Cu-free synthesis is advantageous and it has also been proven to have higher *in vitro* stealth.[14]

In the past many efforts were made to mimic cell-cell aggregation by looking at vesicle-vesicle aggregation.[15] Cell-cell aggregation is one of the most fundamental processes in living organisms. It occurs during inflammation, immune responses and the development of neuronal tissue.[15] Vesicle-vesicle aggregation chemistry is called cytomimetic as it is the artificial analogue of cellular morphology change.[18] Many different groups have been studying vesicle-vesicle aggregation induced by hydrogen bonding, electrostatic interactions, hydrophobic interactions, host-guest interactions and metal coordination, but covalent bonds were only seldomly used.[15] This thesis applies the alkyne- and azide-functionalized polymers as mimics of desmosomes, protein complexes that link cells in epithelial tissues.[19] In many cases the vesicles linked for aggregation were shown to fuse with time. Vesicle fusion has drawn just as much scientific attention with many trying to induce it by methods such as complementary DNA strands, peptides, metal-complex formation and also complementary functional groups.[4]

4 Results and Discussion

4.1 Polymer Synthesis

The PEEP blocks were obtained by metal-free anionic ring opening polymerization (AROP).[1,20] The mechanism is depicted below (Scheme 1) for any macroinitiator *Poly-OH*. 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) initiates the polymerization by deprotonating the hydroxyl-terminated macroinitiator. *N*-[3,5-bis(trifluoromethyl)phenyl]-*N'*-cyclohexyl-thiourea (TU) is used to activate the monomer by complexation[20] of the oxygen at the electrophilic phosphorus, thereby increasing the polarity of the bond. The anionic macroinitiator attacks the activated ethyl ethylene phosphate (EEP) thus opening the ring for polymerization. Transesterification is a possible side reaction as depicted on page 7 (Scheme 2). The activation of EEP by TU reduces the chances of transesterification by increasing AROP kinetics. The polymerization is terminated by acetic acid which protonates the oxide.



Scheme 1. Reaction mechanism for the anionic ring opening polymerization of ethyl ethylene phosphate (EEP) using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and *N*-[3,5-bis(trifluoromethyl)phenyl]-*N'*-cyclohexyl-thiourea (TU) as cocatalysts.

We chose to polymerize EEP from PB and PDMS macroinitiators as those block copolymers have been shown to form GUV by film hydration.[2,3] The characteristics of the polymers synthesized and the macroinitiators used are summarized in Table 1. The number of repeating units of the macroinitiator and PEEP block was calculated by ^{1}H NMR using signals for PB(1,4) at 5.5 – 5.3 ppm, for PDMS at 0 ppm and (**H$_2$**C)$_3$-O-P signals of the PEEP block at 4.3 – 4.1 ppm. This number was used to determine the number-average molecular weight M_n and the hydrophilic mass ratio *f(hydrophilic)* calculated according to section 4.3 using equation 1. Achieving a precise polymerization of EEP is challenging as the numbers of repeating units of EEP that were desired are small (below 20), making it difficult to be on target.

Table 1. Library of synthesized polymers and macroinitiators.

Entry	Polymer[a]	*f*[b]	M_n / kDa[a]	*Đ*[c]	Vesicles[d]
1	PB_{72}-OH	-	3.9	1.03	-
2	PB_{72}-*b*-$PEEP_6$	0.19	4.8	1.12	×
3	PB_{72}-*b*-$PEEP_{11}$	0.30	5.6	1.09	✓
4	PB_{72}-*b*-$PEEP_{13}$	0.34	5.9	1.08	✓
5	$PDMS_{56}$-OH	-	4.2	1.13	-
6	$PDMS_{56}$-*b*-$PEEP_9$	0.25	5.5	1.13	×
7	$PDMS_{56}$-*b*-$PEEP_{13}$	0.30	6.1	1.18	×

[a]The degree of polymerization and the number-average molecular weight M_n were determined using ^{1}H NMR. [b]*f* is the hydrophilic mass ratio *f(hydrophilic)* calculated using equation 1. [c]The molar mass dispersity *Đ* was determined using GPC. [d]The polymers either did (✓) or did not (×) form vesicles by non-assisted film hydration.

Amphiphilic block copolymers form vesicles in aqueous media to minimize the surface energy and separate the hydrophobic block from surrounding water solution. The hydrophilic block on both sides of the thin film shields the PDMS or PB block from water while mixing with itself and water.

The ratio *f(hydrophilic)* of the mass of hydrophilic polymer compared to the mass of the block copolymer is important for self-assembly of the polymers into polymersomes. Different hydrophilic ratios yield different shapes in the self-assembly.[21] Micelles, cylindrical micelles or reverse micelles could be formed instead of vesicles. To calculate the mass ratio *f(hydrophilic)* the following equation was used.

$$f(hydrophilic) = \frac{M_n(hydrophilic\ block)}{M_n(polymer)} \qquad (1)$$

To generate amphiphilic polymers that self-assemble into vesicles the hydrophilic ratio *f(hydrophilic)* has to be within a certain range, which is thought to be between 25 % and 45 % of the polymers weight.[2,5] These outer limits were empirically identified[5] and also confirmed for PB-*b*-PEEP.[2] In our synthesized library of block copolymers (Table 1), most of our block copolymers (entries 3-4 and 6-7) are within these empirical boundaries. Only PB_{72}-b-$PEEP_6$ (*f* = 0.19) (entry 2) is below.

As seen in Figure 1, with growing degree of polymerization of PEEP the intensity of the peak at 4.3-4.1 ppm increases. This peak belongs to the (**H$_2$**C-O)$_3$P=O and corresponds to 6 **H** in each phosphate repeat unit.

The PB-peaks used to assess the hydrophilic ratio at 5.5 ppm - 5.3 ppm belong to the 1,4 PB repeat units. The 1,2 PB units have significantly smaller peaks with approximately 10 mol% of the PB. Diffusion ordered ^{1}H NMR spectroscopy (DOSY) was used to confirm that EEP polymerized onto the macroinitiator (see Experimental, section 5.3). PB_{72}-*b*-$PEEP_p$ polymers with a degree of polymerization *p* = 6, 11 and 13 were synthesized. The PB based amphiphilic block copolymers have molecular weights between 4.8 kDa and 5.9 kDa with the macroinitiator weighing 3.9 kDa. $PDMS_{56}$-*b*-$PEEP_p$ copolymers were synthesized with a degree of polymerization *p* of 9 and 13. These PDMS based block copolymers have a number-average molecular weight of 5.5 kDa and 6.1 kDa, with the PDMS-macroinitiator weighing 4.2 kDa.

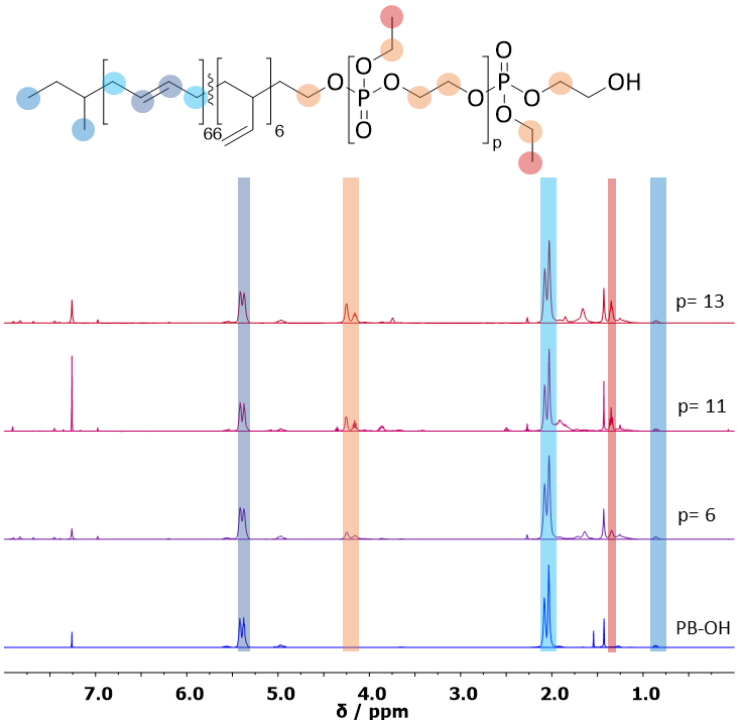


Figure 1. Stacked spectra of the PB_{72}-OH macroinitiator, and the PB_{72}-*b*-$PEEP_p$ block copolymers with *p* = 6 / 11 /13 respectively.

The polymers' molar mass dispersity *Đ* was determined using gel permeation chromatography (GPC). All synthesized block copolymers had a low polydispersity (PDI) with *Đ* < 1.2. These values were similar to the macroinitiator used *Đ* = 1.03 for PB (Entry 1, Table 1) and 1.13 for PDMS (Entry 5, Table 1). Despite their low PDI, the GPC traces show that the polymerization of EEP onto the macroinitiator resulted in bimodality. A small shoulder at lower elution volume can be observed (Figure 2). For PB based block copolymers (Figure 2a), the shoulder is more apparent than for the PDMS block copolymers (Figure 2b). These polymers with a higher hydrodynamic radius could be a product of transesterification during the EEP polymerization, despite the combination of TU and DBU minimizing these side-reactions[20]. The transesterification happens through the following reaction scheme (Scheme 2).

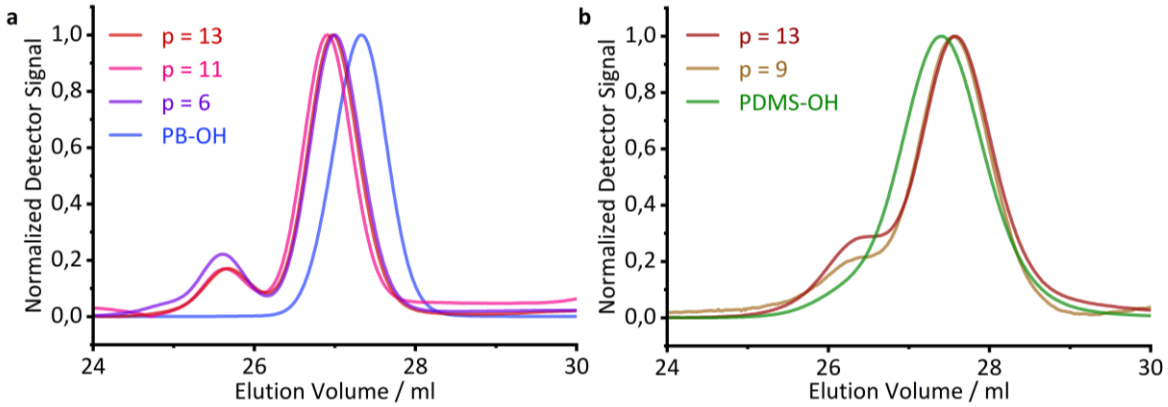
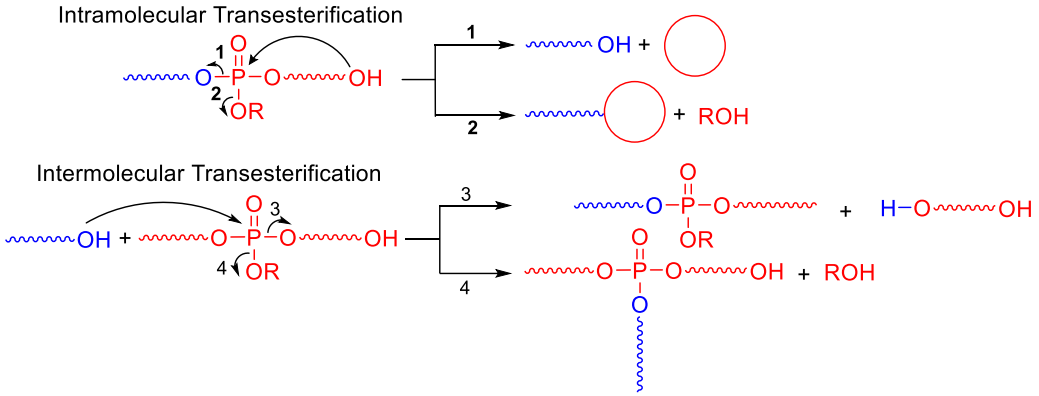


Figure 2. GPC traces of a) poly(butadiene) block copolymers PB_{72}-*b*-$PEEP_p$ (*p* = 6 / 11 / 13) and the hydroxyl-functionalized initiator PB_{72}-OH. b) Poly(dimethyl siloxane) block copolymers $PDMS_{56}$-*b*-$PEEP_p$ (*p* = 9 / 13) and the hydroxyl-functionalized initiator $PDMS_{56}$-OH. The detector signal is normalized to 1 for each trace.

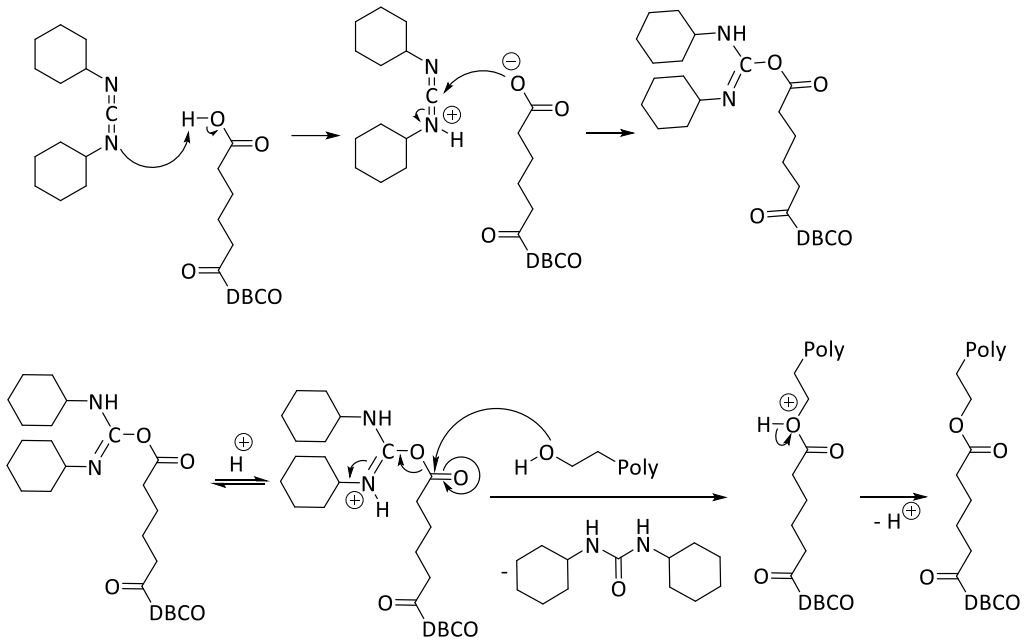


Scheme 2. Reaction scheme of the transesterification side reaction. Modified from [21].

4.2 Chemical Functionalization of the Polymers

Following their synthesis, we wanted to modify our block copolymers in order to express functionality on the surface of the vesicles. We chose to add a dibenzocyclooctyne (DBCO) moiety and an azide moiety, respectively, at the terminal hydroxyl group of the polymers to carry out Cu-free click chemistry between the GUVs.

For the functionalization with DBCO, Steglich esterification[22] was employed in a similar fashion to the attachment of BDP dyes to PB-*b*-PEEP block copolymers by Rideau *et al.*[3] (Scheme 3). First the DCC reacts with the carboxylic acid to form an *O*-acyl urea, making the carbonyl carbon more electropositive (Scheme 5). This increased polarity allows the alcohol to attack fast, making the enthalpically stable 1,3-dicyclohexylurea (DHU) a leaving group. This second, rate determining step, allows for a rearrangement reaction. To limit the side reaction, the DMAP is used as an acyl transfer agent. Its reaction with the DCC is faster, as it is the better nucleophile compared to the alcohol. The active ester that forms through this reaction is attacked by the alcohol faster than the *O*-acylisourea, yielding DMAP and the desired ester.



Scheme 3. Reaction mechanism of the Steglich esterification of the hydroxyl-terminated polymer *Poly-OH*.

For the azidation of hydroxyl terminated polymer, we first activated the hydroxyl group with the tosylate group, and, in a second step, functionalized the polymer with the azide moiety (see experimental section 5.5 for detailed procedure). The tosylation provides a superior leaving group for the subsequent azidation. The *p*-toluenesulfonyl chloride is attacked by the hydroxyl group on the polymer at the partial positive sulfur. The nucleophilic substitution cleaves the sulfur-chlorine bond, making chloride the leaving group. The molecule is deprotonated after. The azidation is another nucleophilic substitution reaction with the tosylate as a leaving group. The anionic azide ion attacks the *alpha*-carbon on the polymer.

The DBCO terminal functionalization, tosylation and azidation of the polymers were determined by NMR (see Experimental section, 5.4 and 5.5). Only small peaks were observed, and it is not clear whether these stem from attachment or the free molecules. As the intensity of peaks of the end group is only a fraction of the intensity of peaks stemming from protons in the polymeric repeat units. DOSY NMR spectroscopy was carried out as well, to prove the attachment (5.4 and 5.5**Fehler! Verweisquelle konnte nicht gefunden werden.**). The DOSY spectra show, that the functionalization diffused at the time length of the polymer, indicating attachment. Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) measurements were attempted, but a favorable matrix could not be determined in limited time frame of this thesis. GPC data was compared for the functionalized polymers (Figure 3). In GPC the polymers are separated by their hydrodynamic radius, which in turn is determined by the extent of solvation in the chosen eluent (THF). This impact of chemical composition enables us to see even small changes in respect to molecular weight like the tosylation using GPC traces. The functionalized polymers show shifts in the GPC traces that are indicative of a change in the end group (Figure 3a). HPLC results support the conclusions drawn from GPC (see experimental sections 5.4 and 5.5).

We first tested the functionalization of DBCO and tosylate on the homopolymer PB_{72}-OH that was used as a macroinitiator for the PEEP polymerization. PB_{72}-DBCO showed signs of crosslinking as the GPC and HPLC data for this polymer is bimodal. The

GPC trace (Figure 3a) shows a new shoulder at lower elution volume (bigger hydrodynamic radius). Subsequent synthesis of DBCO- and azide-functionalized polymers were stored at low temperature (-20 °C) and never placed under reduced pressure to minimize degradation. The PB_{72}-*b*-$PEEP_{13}$-DBCO showed no signs of crosslinking, which suggests that the careful handling was successful or the DBCO moiety was not attached at all. The GPC traces of PB_{72}-*b*-$PEEP_{13}$ and PB_{72}-*b*-$PEEP_{13}$-DBCO (Figure 3b) look very similar. The tosylation and azidation of the block copolymer seem to correlate with faster degradation of the PEEP, resulting in a smaller shoulder compared to the unfunctionalized polymer (Figure 3c). The assumption of faster degradation is also supported by the NMR spectra, in which the peaks of the PEEP units decrease relative to the PB-block.

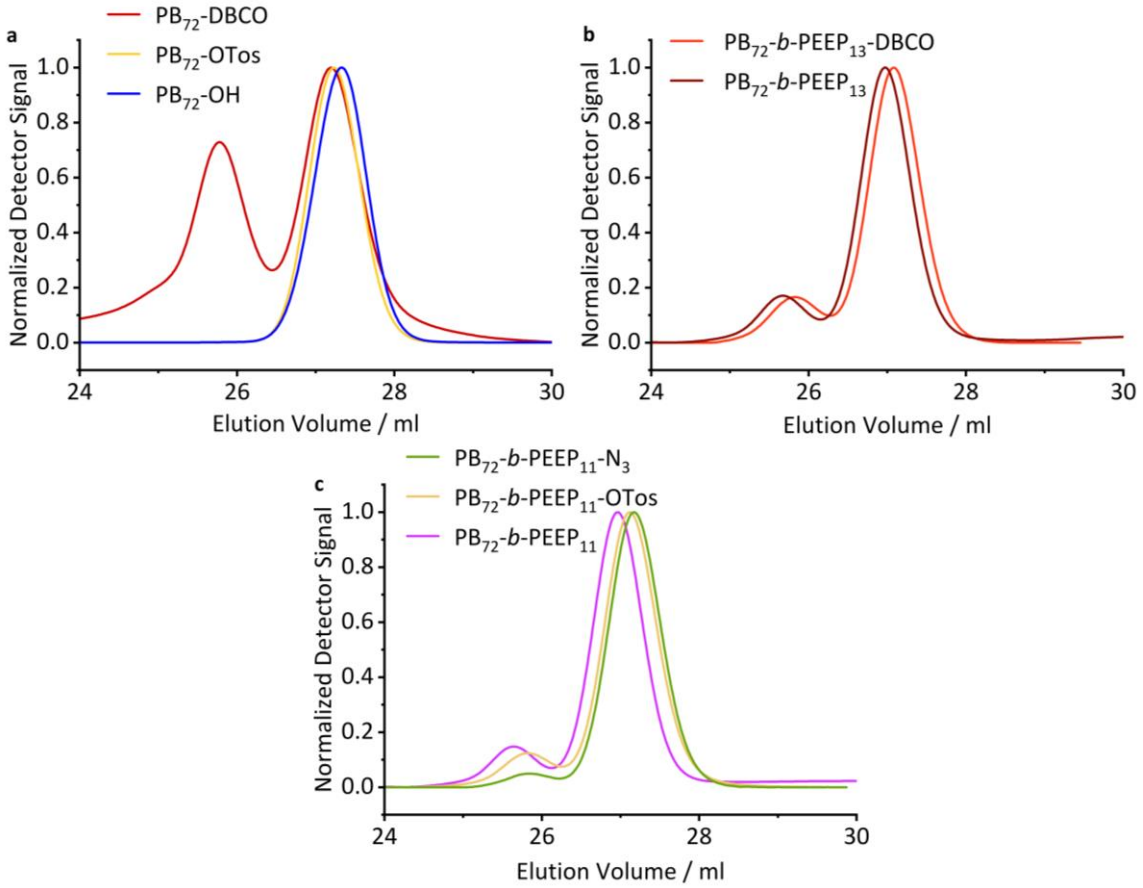


Figure 3. The GPC traces for the functionalized polymers a) the macroinitiator PB_{72}-OH, the DBCO-functionalized polymer PB_{72}-DBCO and the tosylated polymer PB_{72}-OTos b) PB_{72}-*b*-$PEEP_{13}$ and DBCO-functionalized PB_{72}-*b*-$PEEP_{13}$-DBCO c) PB_{72}-*b*-$PEEP_{11}$, tosylated PB_{72}-*b*-$PEEP_{11}$-OTos and azide-functionalized PB_{72}-*b*-$PEEP_{11}$-Azide.

4.3 Formation of Vesicles

To observe whether any vesicle-vesicle aggregation or vesicle fusion would happen, the above-mentioned amphiphilic block copolymers were used to form GUVs by spontaneous film hydration. All synthesized block copolymers with a hydrophilic mass fraction between 25 % and 45 % of the polymer were expected to form vesicles. However, only PB_{85}-*b*-$PEEP_{11}$ (entry 3) and PB_{85}-*b*-$PEEP_{13}$ (entry 4) yielded GUVs. Despite evidence that PDMS-*b*-PEEP can form GUVs,[3] the two synthesized PDMS-*b*-PEEPs (entries 6 and 7) did not, even though they were within the recommended regime for the hydrophilic ratio.

This unexpected result is another example of how we do not yet fully comprehend the mechanism of self-assembly of the polymer. The PB-*b*-PEEP polymers readily self-assembled to GUVs from the dry film in the course of 12 hours, which was deemed impossible

by the scientific community before.[2,9] Our PDMS-*b*-PEEP polymers on the other hand did not form vesicles from non-assisted film hydration at all. It seems that the PDMS based amphiphiles need more defined conditions for self-assembly into GUVs.

This lack of PDMS-*b*-PEEP vesicles is unfortunate as they could have been functionalized and tested for any kind of fusion with PB-b-PEEP. The immiscibility of PDMS and PB based block copolymers can be overcome through click reactions forming PB-*b*-PEEP-*b*-PDMS triblock copolymers. As PDMS and PB show only slight miscibility,[23] different regimes within the fused vesicles could have been distinguished under the optical microscope for longer timescales than the solely PB based systems enable us to do, as they are miscible and have higher diffusivity between the different regimes.

The dyes used to make the polymeric structures visible were fluorescent BDP dyes. In these the fluorophores are covalently linked to block copolymers, similar to the ones used for vesicle formation.[3] Previously synthesized PDMS-BDP FL was used to label PDMS-*b*-PEEP polymersomes and PB-BDP 630/650, PB-*b*-PEEP-BDP 630/650 or PB-*b*-PEEP-BDP FL for PB polymersomes. The fluorescently tagged polymers were mixed in 1 mol% to the unlabeled polymers prior to film formation. Then the film was dried, usually under high vacuum, and hydrated with a 1 mmol/l sucrose solution in water (for a more detailed description see section 5.6 Film Hydration). Overnight, vesicles formed as depicted in Figure 4a and b. While the vesicles are not monodisperse, in terms of size they are clearly in the realm of giant unilamellar vesicles.

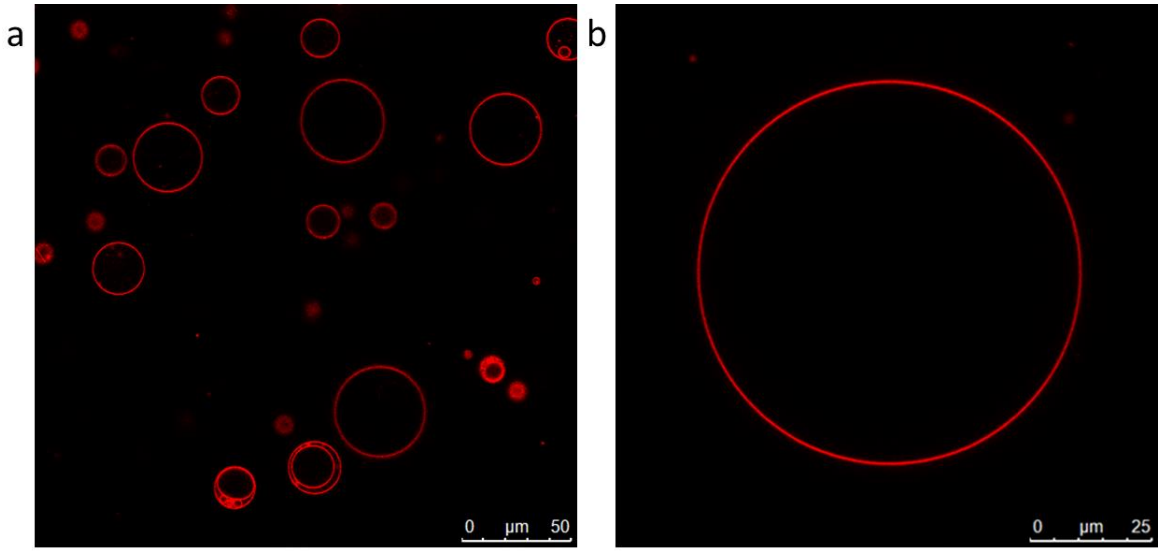


Figure 4. a) Typical image of the GUVs formed by PB_{72}-*b*-$PEEP_{13}$. b) Zoomed in image of a GUV from the same polymer.

Because film hydration is not a controlled method it can result in the formation of vesicles of various sizes, of agglomerated vesicles as well as of vesicles inside other bigger vesicles, so called multivesicular vesicles (MVVs) (Figure 5). Some vesicles also show a double membrane structure, they are then called multilamellar vesicles (MLVs). Most of the vesicles obtained are giant unilamellar vesicles also known as GUVs the term giant is used for vesicles in the range of 1 µm to 200 µm.[5]

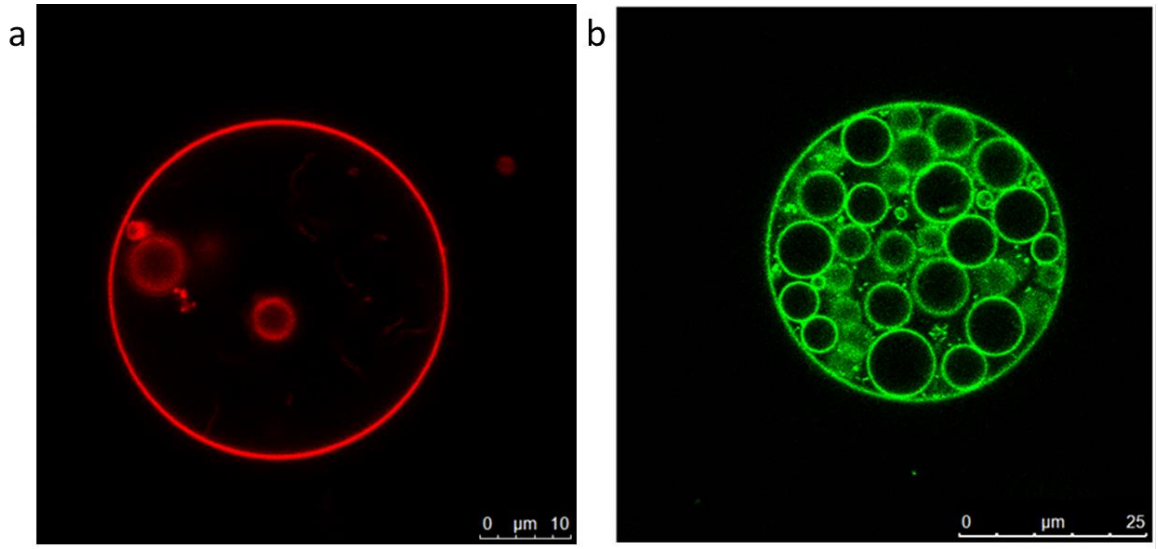


Figure 5. Polymersome made from a) PB_{72}-*b*-$PEEP_{13}$ b) PB_{72}-*b*-$PEEP_{11}$-N_3 (10 wt% in PB_{73}-*b*-$PEEP_{21}$) with smaller polymersomes inside, so called multivesicular vesicles (MVVs).

While the PB-*b*-PEEP copolymers formed vesicles, the azide functionalized copolymer PB_{72}-*b*-$PEEP_{11}$-Azide did not form vesicles on its own. The DBCO functionalized polymer PB_{72}-*b*-$PEEP_{13}$-DBCO, on the other hand, did form vesicles. However, its yield seemed somewhat lower than the yield of vesicles from the unfunctionalized block copolymer PB_{72}-*b*-$PEEP_{13}$. Figure 6 shows an image of the vesicles formed solely with the copolymer PB_{72}-*b*-$PEEP_{13}$-DBCO.

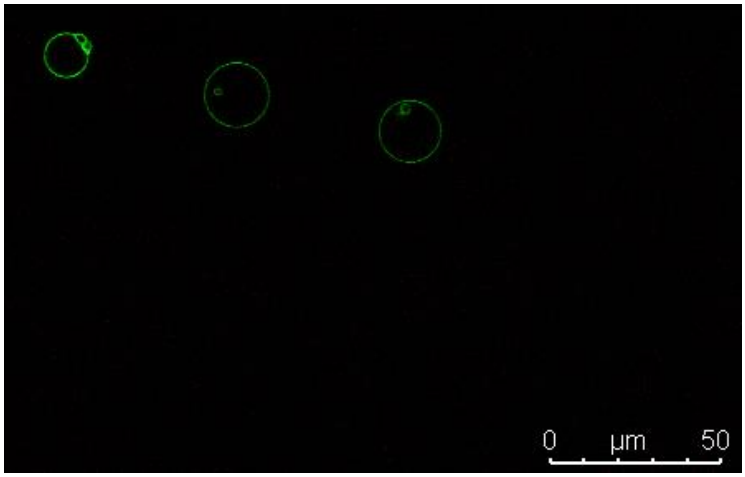


Figure 6. Vesicle formed with the pure PB_{72}-*b*-$PEEP_{13}$-DBCO block copolymer, dyed with 1 mol% PB_{73}-*b*-$PEEP_{12}$-BDP FL.

To test vesicle-vesicle aggregation, vesicles formed from previously synthesized block copolymers PB_{73}-*b*-$PEEP_{21}$[3] were combined with functionalized polymers PB_{72}-*b*-$PEEP_{11}$-Azide (PB_{72}-*b*-$PEEP_{13}$-DBCO) (10 wt%) and green fluorescent PB-*b*-PEEP-BDP FL (red fluorescent PB-*b*-PEEP-BDP 630/650) (1 mol%). The solution in chloroform was subsequently dried. Two different drying methods were employed for comparison. For each polymer one vial was set up and dried under high vacuum and another vial was dried under argon flow. Contrary to the common belief in the field,[9] the drying process did not affect the yield of polymersomes. The polymers formed vesicles with both methods, as depicted in Figure 7. One should, however, be cautious in drawing conclusions from the scans depicted, as they show only a small fraction of the vesicles formed. Hence the yield and dispersity cannot be assessed by looking at these images, nevertheless they show qualitatively that GUVs were formed in large numbers.

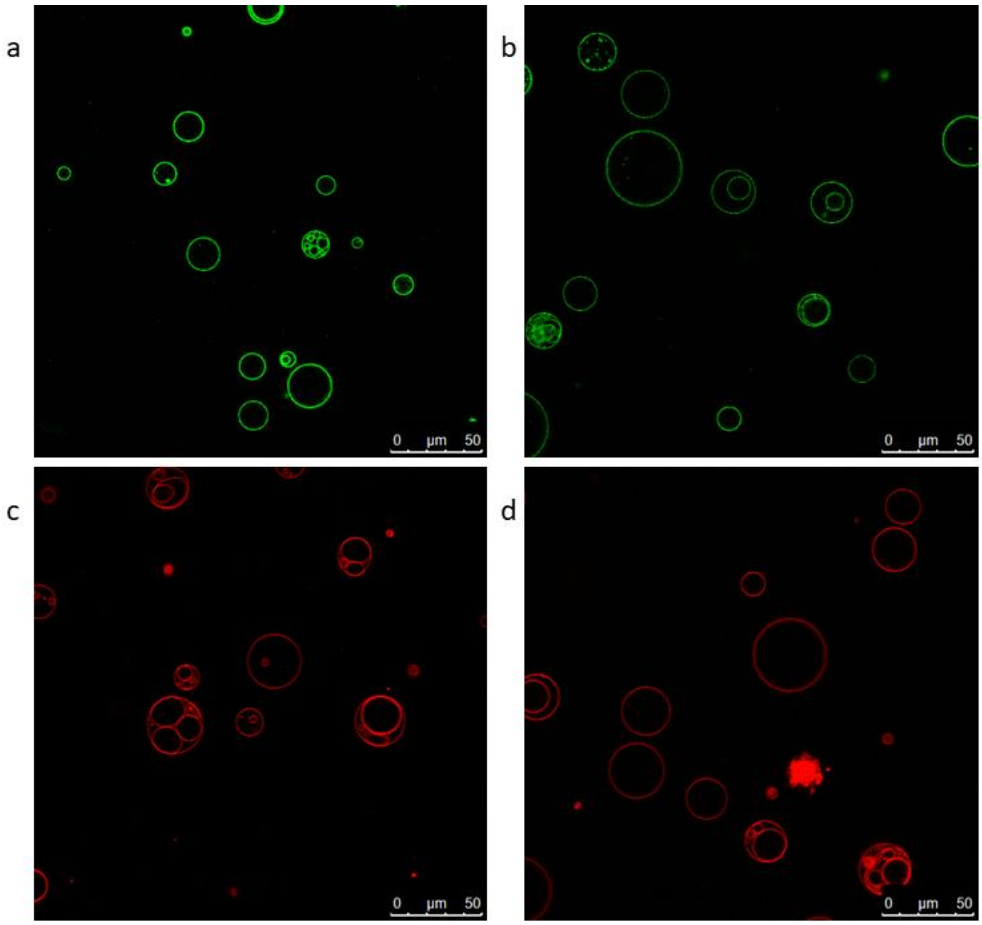


Figure 7. Vesicles formed with PB_{73}-*b*-$PEEP_{21}$ block copolymers combined with functionalized polymers (green PB_{72}-*b*-$PEEP_{11}$-Azide, red PB_{72}-*b*-$PEEP_{13}$-DBCO) (10 wt%) and 1 mol% fluorescent dyes (green PB_{73}-*b*-$PEEP_{12}$-BDP FL, red PB_{73}-*b*-$PEEP_{21}$-BDP 630/650). The vesicles on the left, a) and c), were formed from films dried by Argon flow, the vesicles on the right, b) and d), were formed after evaporation in high vacuum.

These separately formed GUVs were also combined in a well on the plate for optical microscopy. They were analyzed after a few hours, a day and a week. Figure 8 shows the corresponding scans. Despite very close proximity in many cases (see Figure 9) no evidence of the vesicles aggregating by covalent click chemistry could be found as seen in Figure 8 below. This absence of aggregates could be due to various reasons, e.g. the functionalized moiety could be hidden within the membrane of the polymer, instead of reaching out to the aqueous phase, as both the azide and DBCO are not soluble in water, thus the click reaction could not take place. Alternatively the shear forces within the polymersome might not be high enough, so that the polymers after click reaction might get pulled out of the membrane rather than hold both membranes together. Another reason could be that the functionalized polymers might have degraded too much before the formation of vesicles or the functionalization reaction might not have worked altogether.

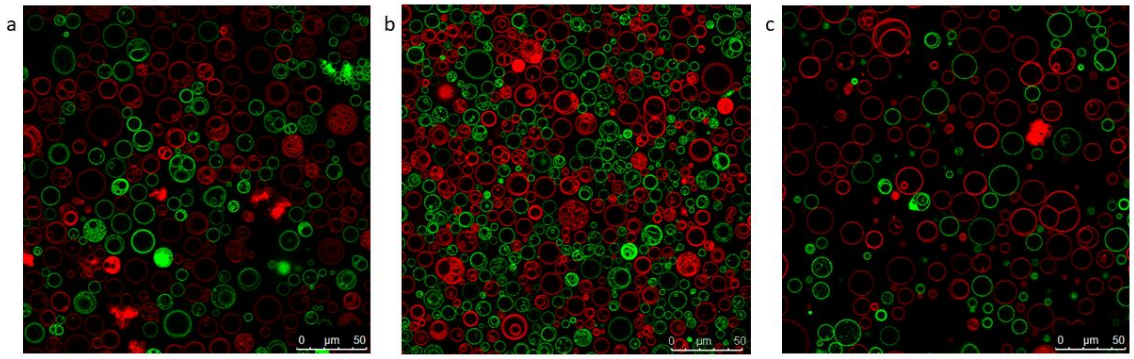


Figure 8. 10 wt% Azide- (green) and DBCO-functionalized vesicles (red) after a) a few hours, b) one day and c) one week from the flow dried films.

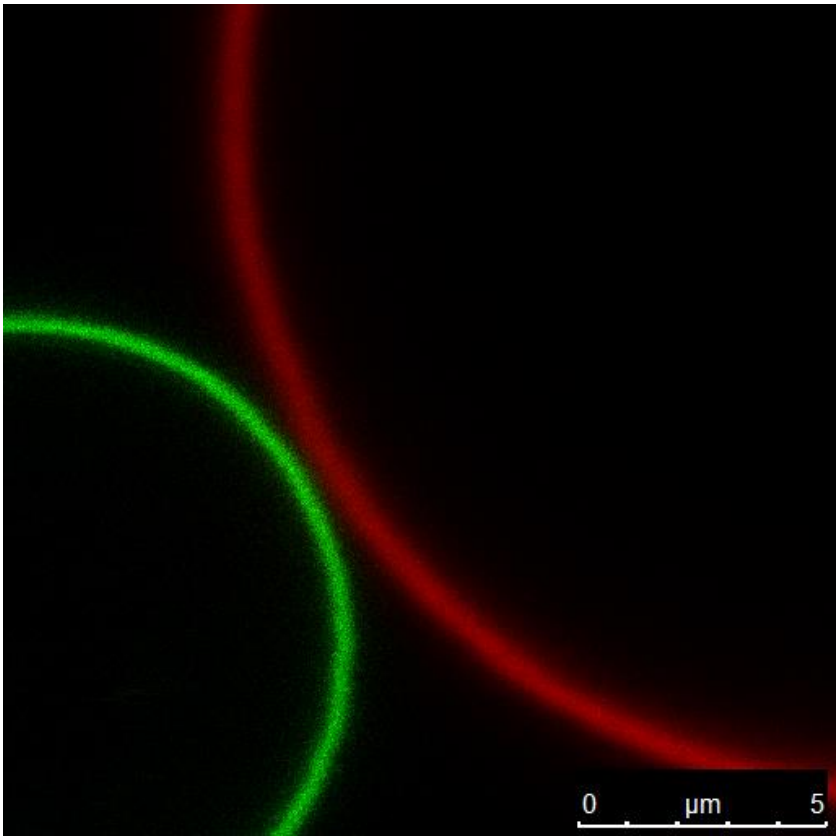


Figure 9. 10 wt% Azide- (green) and DBCO-functionalized vesicles (red) vesicles after one week from vacuum dried film.

5 Experimental

5.1 Materials and Instrumentation

Polymer analysis

^{1}H NMR, ^{31}P NMR and ^{1}H DOSY spectra were measured on a Bruker 500 AMX NMR. All spectra were recorded at room temperature in $CDCl_3$ and were processed using MestReNova Version 6.1.0-6224 © 2010 Mestrelab Research S.L.. Gel Permeation chromatography (GPC) measurements were performed in THF with a PSS SecCurity system (Agilent Technologies 1260 Infinity). Sample injection was performed by a 1260-ALS autosampler (Agilent) at 30 °C with a flow rate of 1 ml/min. SDV(PSS) columns with dimensions of 0.8*30 cm, 10 µm particle size, and pore sizes of 10^6, 10^4, and 500 Å were employed. Calibration was achieved using PI standards provided by Polymer Standards Service. The 1260-RID and UV 1260-VWD detectors (Agilent) were used for detection. High performance liquid chromatography (HPLC) measurements were performed in THF/water gradient with an Agilent Technologies Series 1200 system equipped with a degasser, quaternary gradient pump, column oven and a diode array detector (DAD) and the Varian ELSD-detector 385-LC. For injection the Rheodyne 7725i injection valve was used with a 20 µl loop. Dialysis was performed using a Pre-wetted Spectra/Por® 6 Standard Regenerated Cellulose tubing (45*29 mm, MWCO 1000).

Microscopy imaging

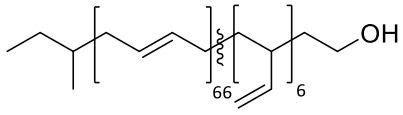
Optical fluorescence microscopy was carried out on confocal microscope Leica TCS-SP5, Wetzlar, Germany using a Leica HC PL APO CS2 63x/1.2 (11506346) water immersion objective in µ-slide 8 wells with glass bottom (Ibidi® 80827). The images were recorded at a 512x512 or 1024x1024 resolution at a 400 Hz scan speed. The 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene BDP FL fluorescence was excited at 496 nm (20% laser power) and the emission was detected by a photomultiplier using a bandwidth of 520 nm-540 nm. The BDP-630/650 fluorescence was excited at 633 nm (20% laser power) and the emission was detected by a photomultiplier using a bandwidth of 650 nm-700 nm. The vesicles were analyzed using Leica LAS X.

Chemicals

Chemicals and reagents were obtained from Sigma Aldrich, Acros Organics, ThermoFisher, Invitrogen™, Polymer Source™ and were used as received unless otherwise stated. Dry tetrahydrofuran and toluene were purchased from Sigma Aldrich or Acros Organics with an AcroSeal®, a seal advertised for dry solvent, stored under inert atmosphere over molecular sieves and was used as such. All reactions involving oxygen/moisture sensitive reagents were performed with anhydrous solvents under a positive pressure of anhydrous argon, using standard Schlenk techniques. Cooling of reaction mixtures to 0 °C was achieved using an ice/water bath.

5.2 Starting Materials

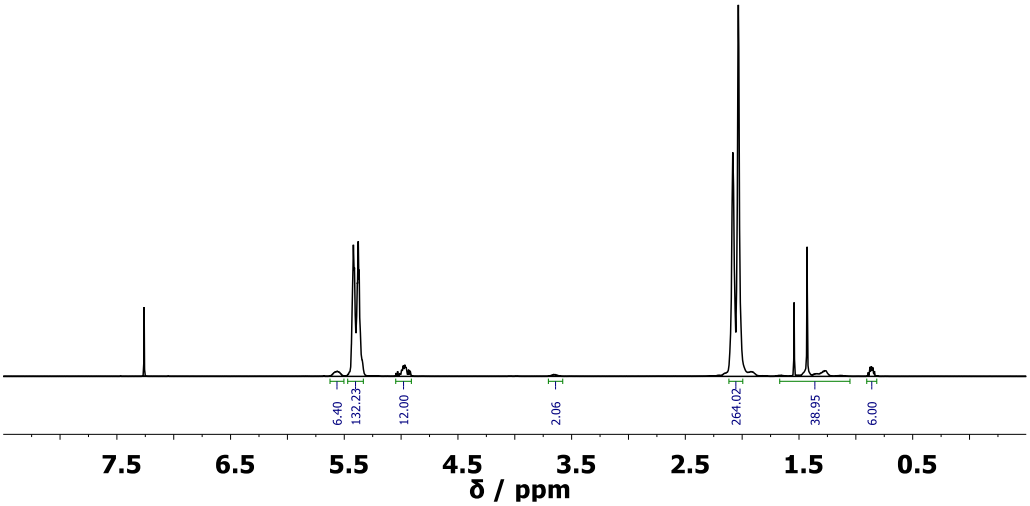
PB_{72}-OH



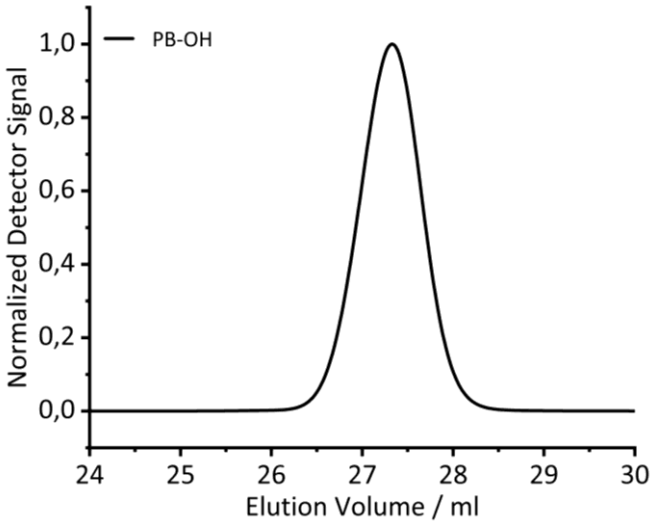
^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 5.63 – 5.50 (m, 6H, 6* C**H**=CH_2), 5.47 – 5.33 (m, 132H, 66*C**H**=C**H**), 4.98 (tdd, J = 22.3, 18.4, 6.8 Hz, 12H, 6* CH=C**H$_2$**), 3.65 (dd, J = 12.6, 5.9 Hz, 2H, C**H$_2$**-O), 2.06 (d, J = 24.6 Hz, 264H, C**H$_2$** and C**H**), 1.67 – 1.05 (m, 39H, C**H$_2$** and C**H**), 0.90 – 0.82 (m, 6H, 2*C**H$_3$**). **Đ** = 1.03.

The procedure can be found in reference [2,24].

^{1}H-NMR (500 MHz, $CDCl_3$)



Molar Mass Dispersity (THF, PB calibration)



M_n	M_W	Đ
4908.15	5061.88	1.03

PDMS$_{56}$-OH

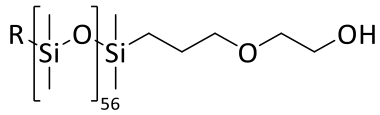
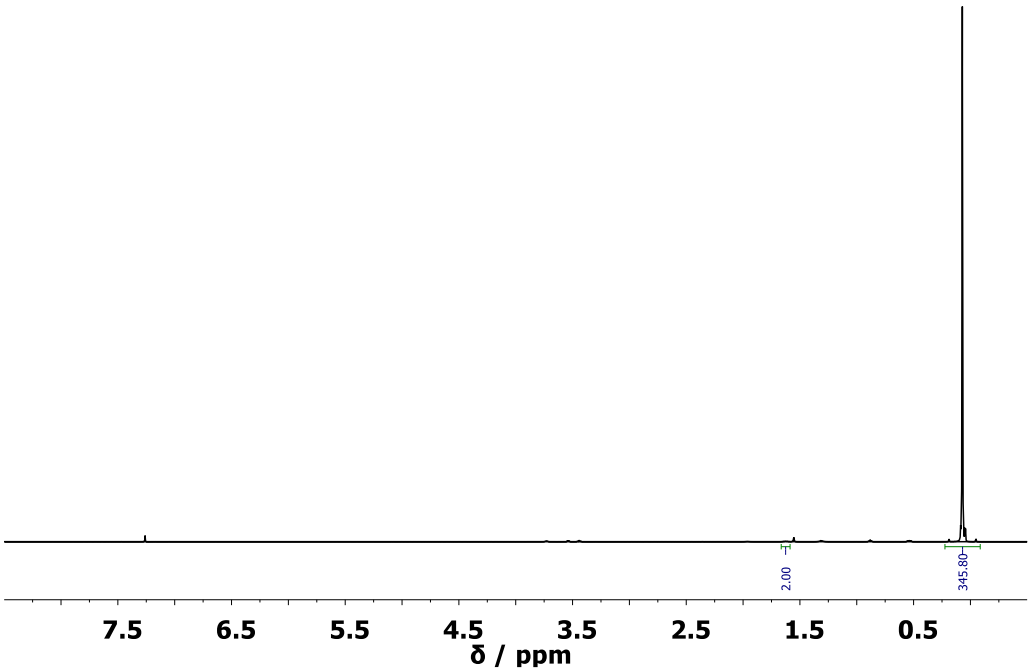


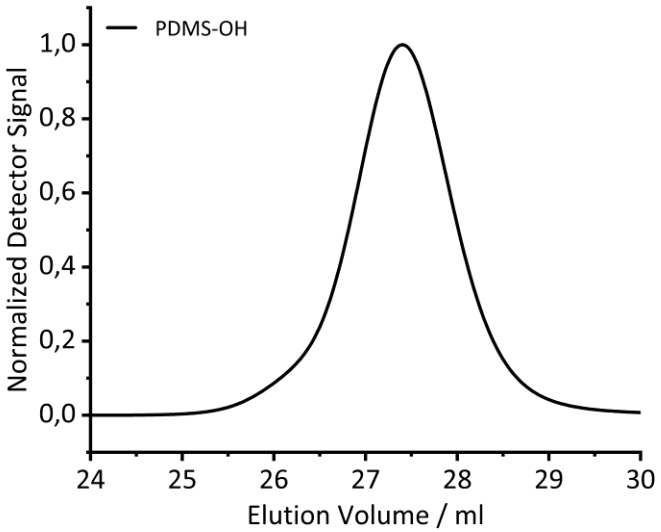
Figure 10. Chemical structure of the PDMS macroinitiator.

^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 1.62 (dt, *J* = 14.7, 7.0 Hz, 2H, C**H$_2$**), 0.22 – -0.09 (m, 172H, 56*Si(C**H$_3$**)$_2$). **Đ** = 1.13.

^{1}H NMR (500 MHz, $CDCl_3$)



Molar Mass Dispersity (THF, PB calibration)



M_n	M_W	Đ
6038.85	6805.35	1.13

Ethyl Ethylene Phosphate (EEP)

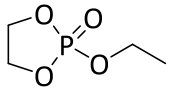


Figure 11. Chemical structure of EEP.

Synthetic procedure can be found in references [2,20].

N-[3,5-bis(trifluoromethyl)phenyl]-*N'*-cyclohexyl-thiourea (TU)

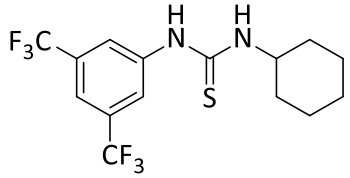
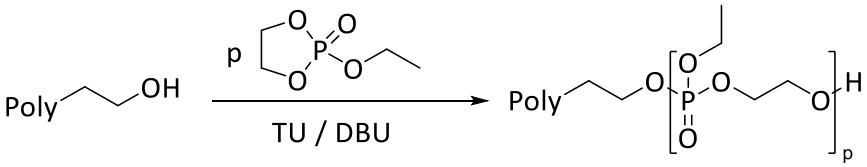


Figure 12. Chemical Structure of TU

Synthetic procedure can be found in references [2,25].

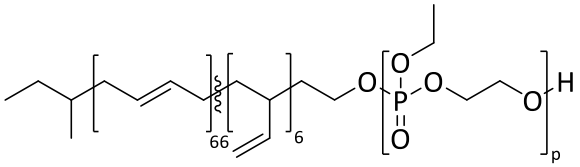
5.3 Synthetic Procedure and Characterization

Poly-*b*-PEEP$_p$



Based on a modified literature procedure[2], in a round bottom flask, the hydroxyl-terminated macroinitiator *Poly-OH* (0.1 mmol, 1 eq) was dissolved in anhydrous toluene (3 ml) and dried under reduced pressure by azeotropic distillation of toluene and water (x 3). *N*-[3,5-bis(trifluoromethyl)phenyl]-*N'*-cyclohexyl-thiourea (TU) (185 mg, 0.500 mmol, 5.00 eq) was added to *Poly-OH* and dissolved in a mixture of anhydrous toluene (5 ml) and anhydrous THF (5 ml) and dried under reduced pressure (x 3). The resulting white residue was then dried under high vacuum overnight. After dissolving the *Poly-OH*/TU residue in anhydrous THF (3 ml) under inert atmosphere, ethyl ethylene phosphate (EEP) (0.*p* mmol, *p* eq) was added. After cooling the solution to 0 °C, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (76 mg, 0.50 mmol, 5.0 eq) was added and the reaction was stirred at 0 °C for 1 h. Then the reaction was quenched using acetic acid in THF (10 ml, 1.0 mol/l) and stirred for another 30 min at 0 °C. The solution was then transferred to a regenerated cellulose dialysis tubing (molecular weight cut-off (MWCO) = 1 kDa for dialysis against deionized water (DI water). To secure a clean polymer, the product was dialyzed at least 48 h, while the dialysis water (~2 l) was changed twice a day.

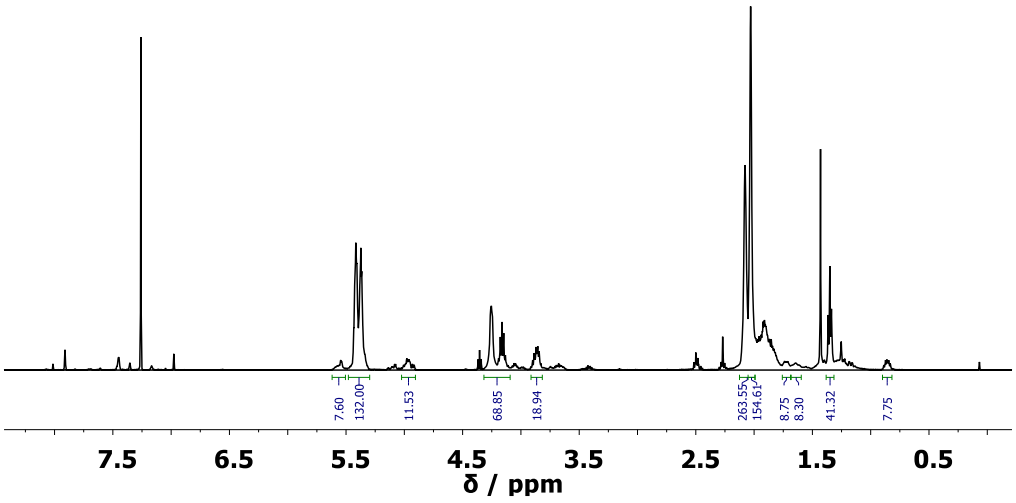
PB_{72}-b-$PEEP_p$



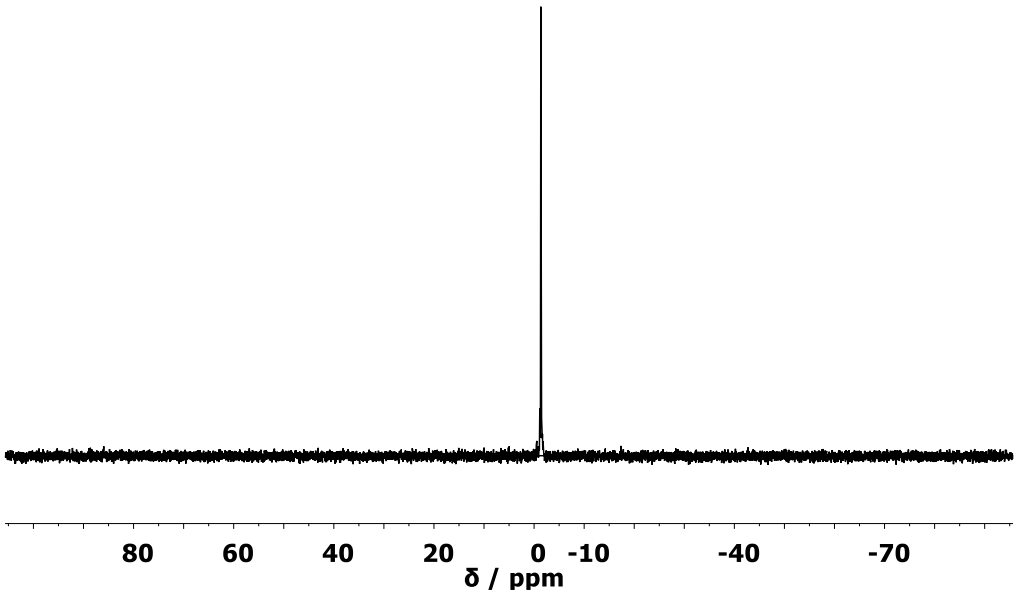
^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 5.56 (dd, *J* = 13.3, 6.2 Hz, 6H, 6*C**H**=CH_2), 5.48 – 5.29 (m, 132H, 66*C**H**=C**H**), 4.96 (ddd, *J* = 22.5, 17.2, 8.2 Hz, 12H, 6*CH=C**H$_2$**), 4.33 – 4.08 (m, p*6H, , p*(C**H**$_2$-O)$_3$P=O), 3.91 – 3.80 (m, 2H, C**H$_2$**-OH), 2.05 (d, *J* = 24.6 Hz, 264H, C**H$_2$**), 2.03 (s, 155H, C**H$_2$**), 1.72 (dd, *J* = 10.0, 4.0 Hz, 3H, C**H$_2$** and C**H**), 1.64 (s, 2H, C**H$_2$**), 1.35 (t, *J* = 7.0 Hz, p*3H, p*C**H$_3$**-CH_2-O), 0.90 – 0.82 (m, 6H, (C**H$_3$**)$_2$-CH).
^{31}P NMR (202 MHz, $CDCl_3$) δ_P/ppm -1.2 (s, 1P)

A) PB_{72}-*b*-$PEEP_{11}$

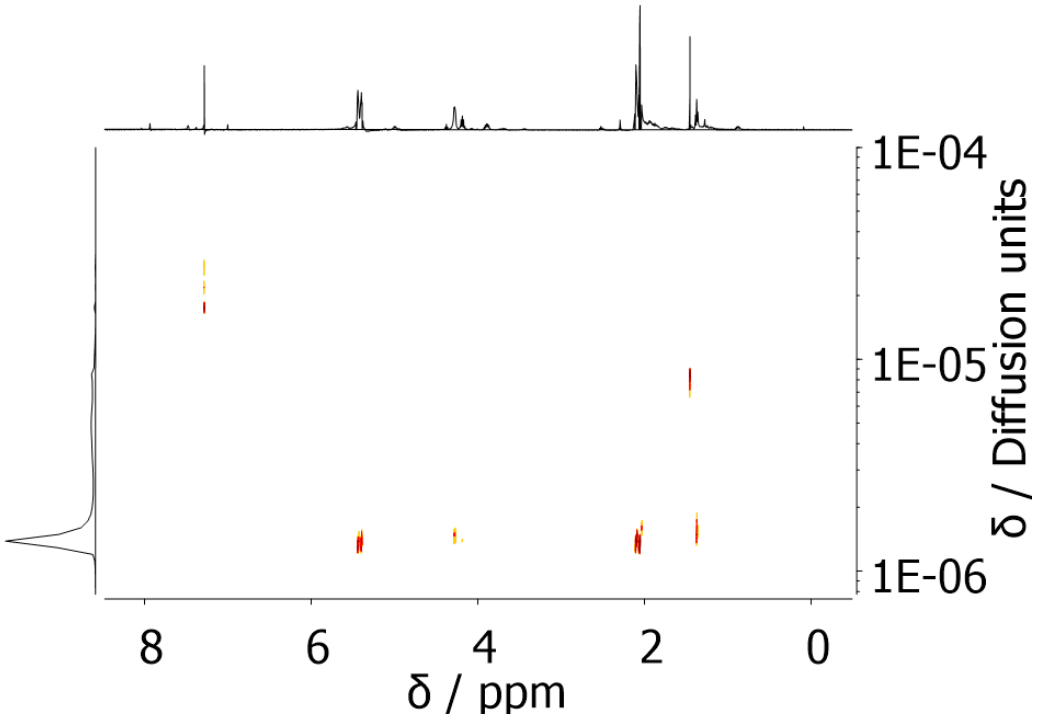
^{1}H NMR (500 MHz, $CDCl_3$)



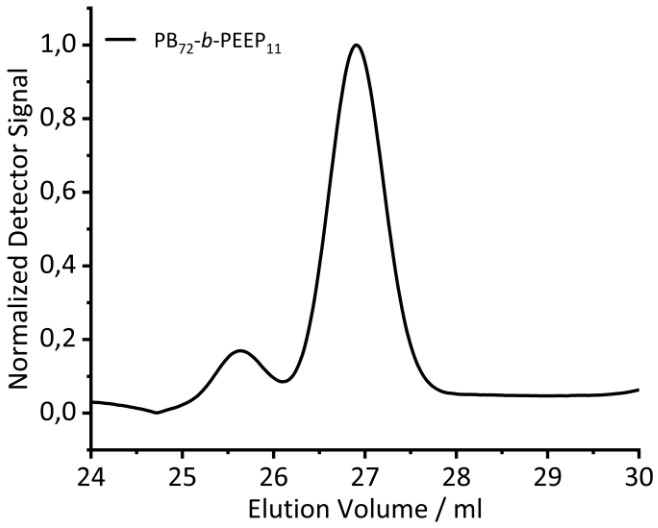
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



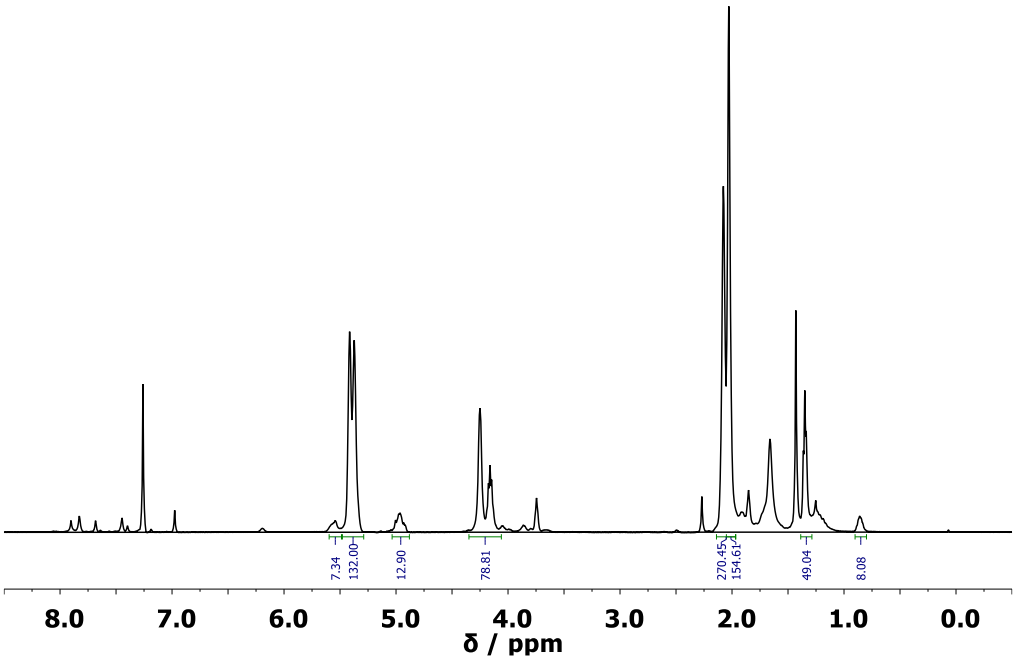
Molar Mass Dispersity (THF, PB calibration)



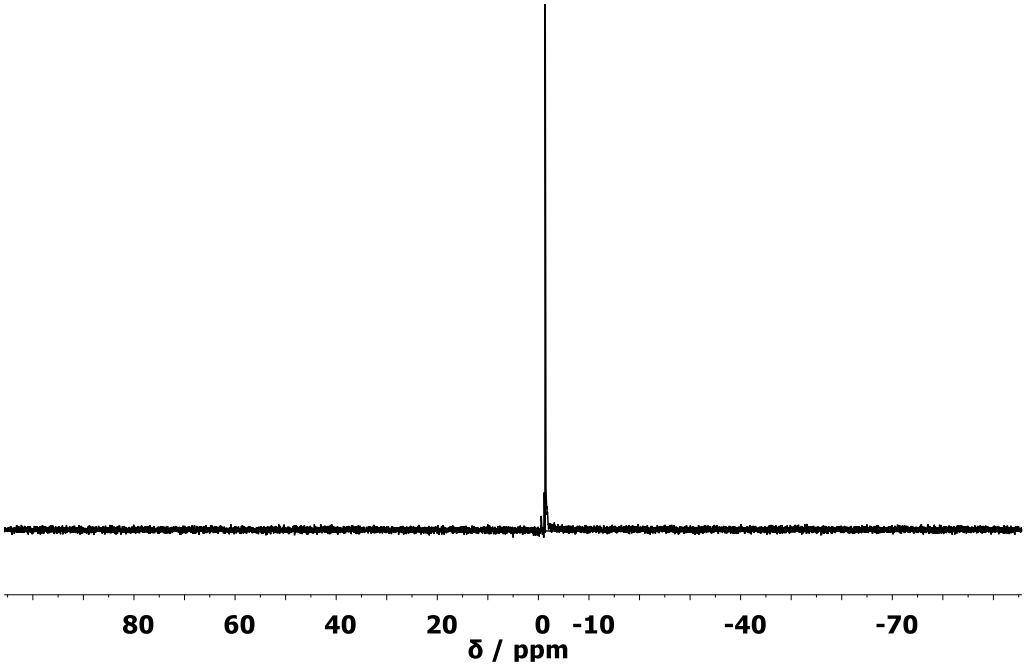
M_n	M_W	$Đ$
6185.93	6692.5	1.08

B) PB_{72}-*b*-$PEEP_{13}$

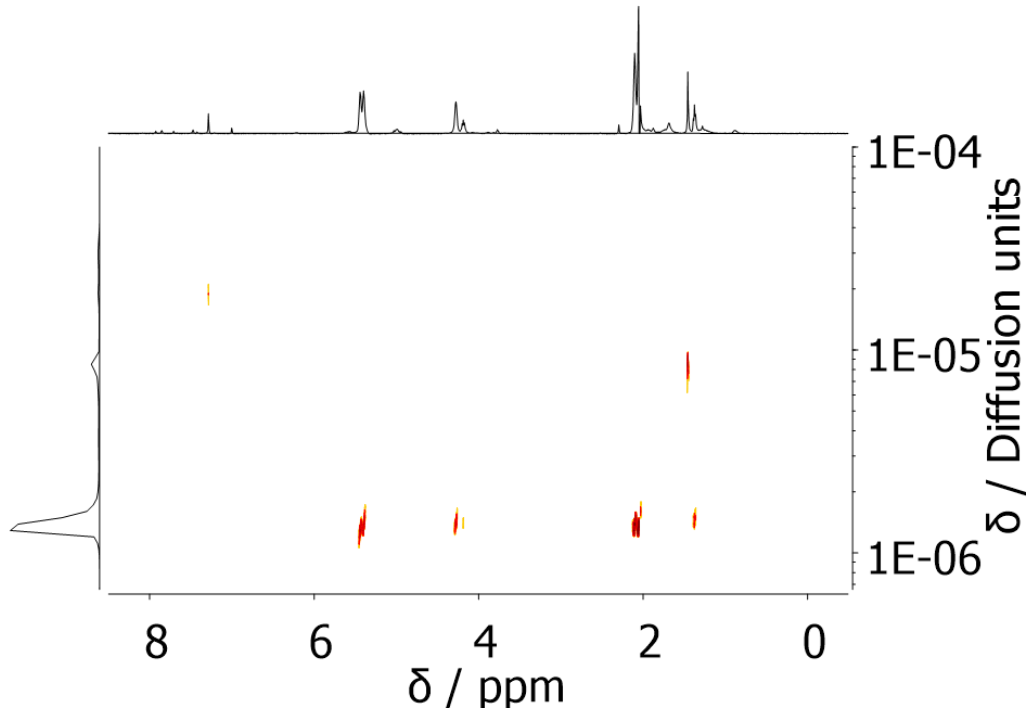
^{1}H NMR (500 MHz, $CDCl_3$)



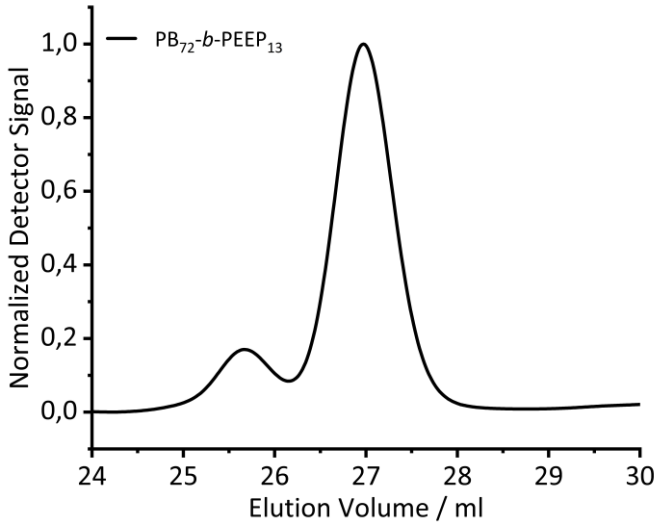
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



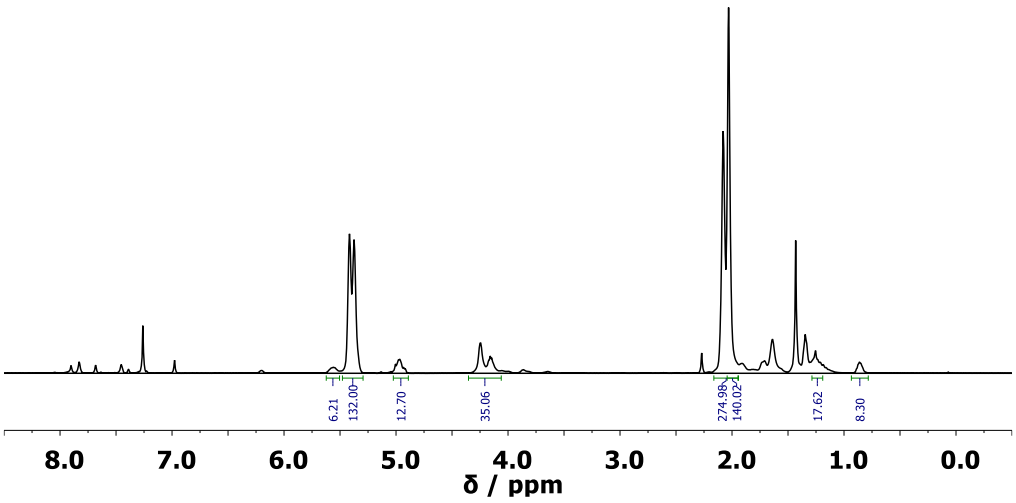
Molar Mass Dispersity (THF, PB calibration)



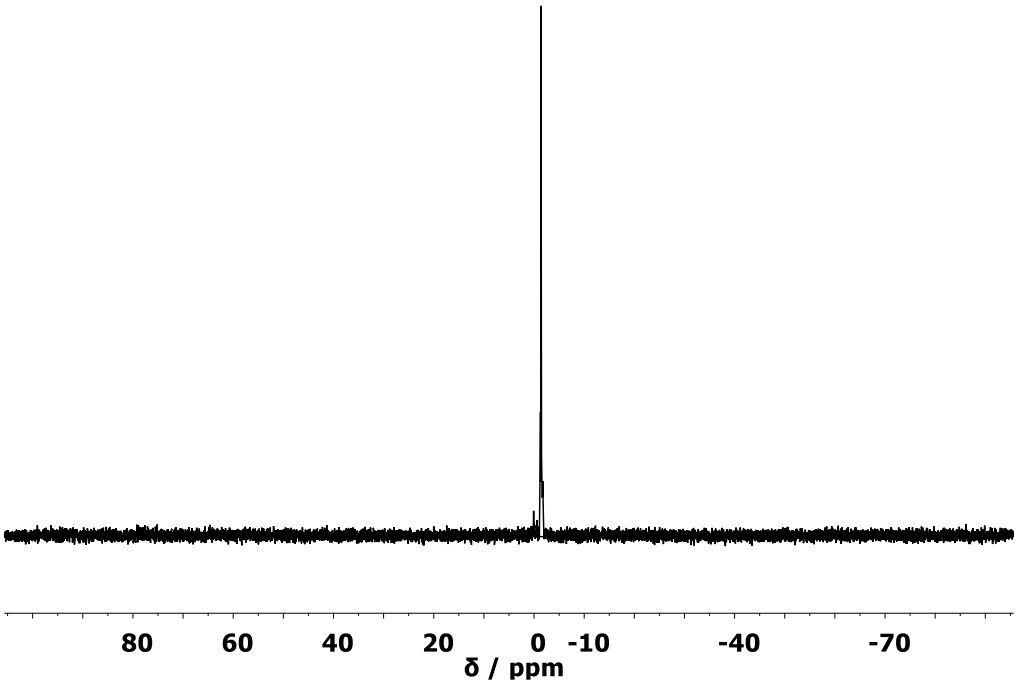
M_n	M_W	$Đ$
6216.23	6750.55	1.09

C) PB_{72}-*b*-$PEEP_6$

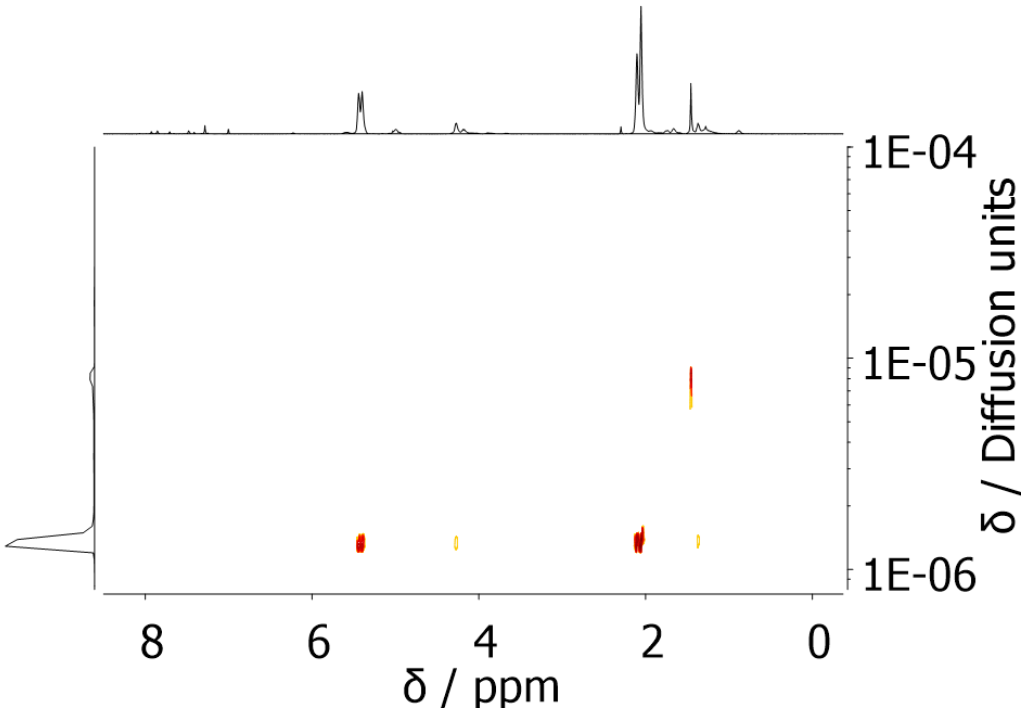
^{1}H NMR (500 MHz, $CDCl_3$)



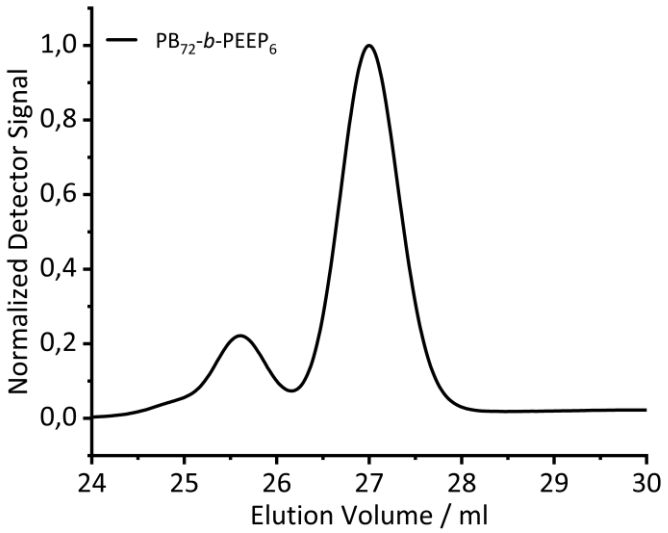
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



Molar Mass Dispersity (THF, PB calibration)



M_n	M_W	$Đ$
6307.64	7042.39	1.12

$PDMS_{56}$-b-$PEEP_p$

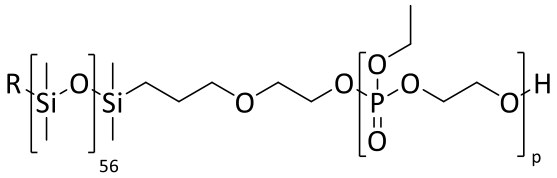
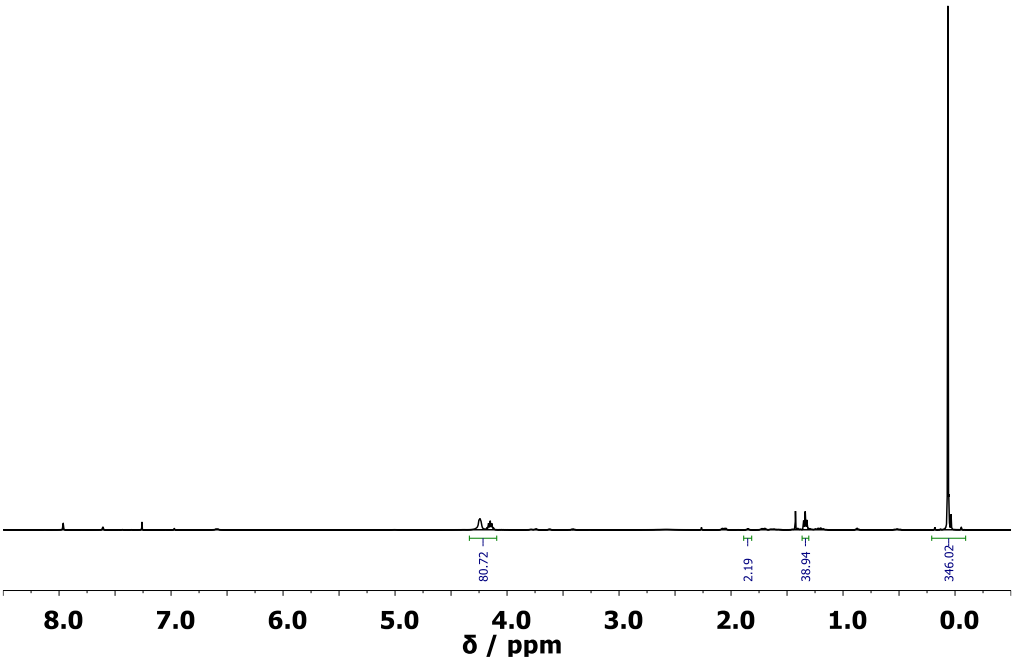


Figure 13. Chemical structure of the $PDMS_{56}$-*b*-$PEEP_p$ copolymers. R is an unknown initiator.

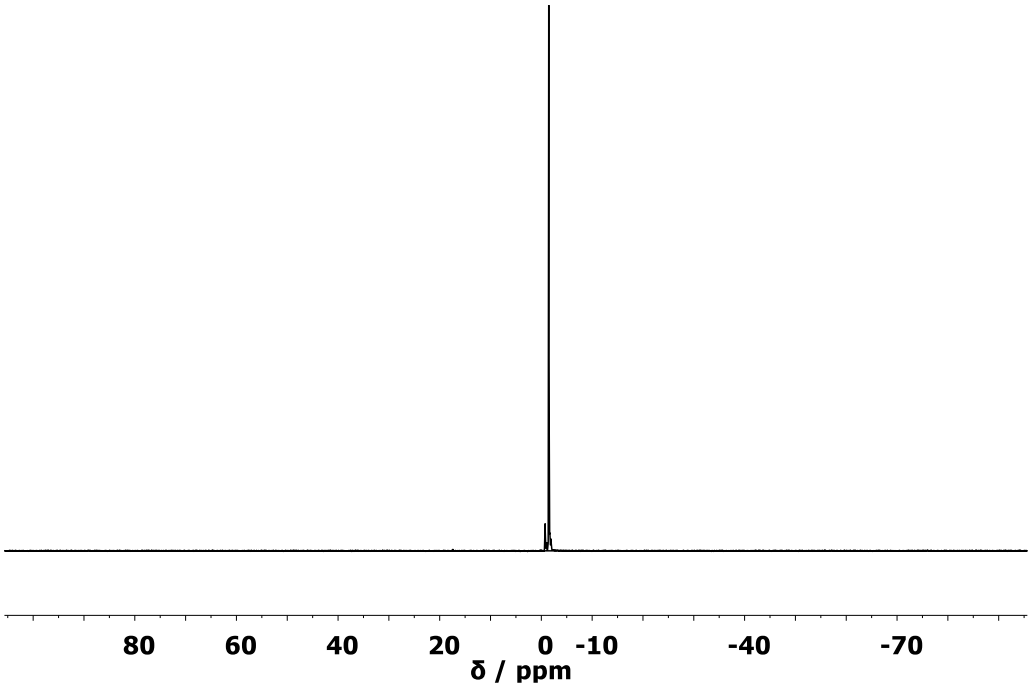
^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 4.33 – 4.08 (m, p*6H, p*(C**H$_2$**-O)$_3$P=O), 1.87 – 1.83 (m, 2H, C**H$_2$**), 1.34 (t, *J* = 7.1 Hz, p*3H, p*C**H$_3$**-CH_2-O), 0.06 (s, 57*6H, 57*Si(C**H$_3$**)$_2$).
^{31}P NMR (202 MHz, $CDCl_3$) δ_P/ppm -1.5 (s, 1**P**)

A) $PDMS_{56}$-*b*-$PEEP_{13}$

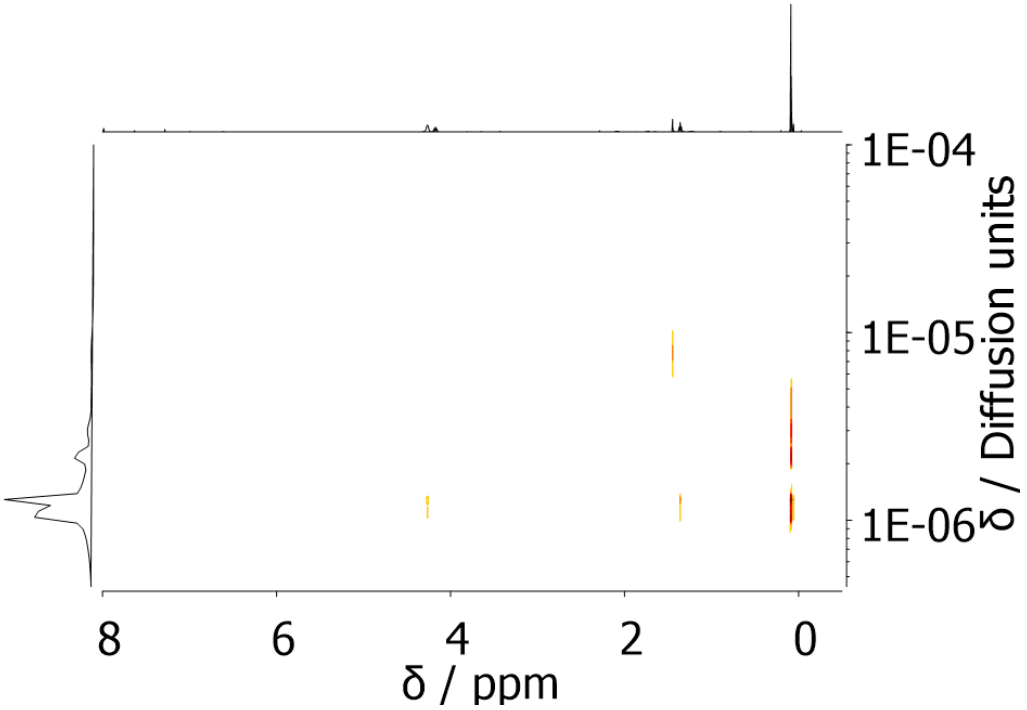
^{1}H NMR (500 MHz, $CDCl_3$)



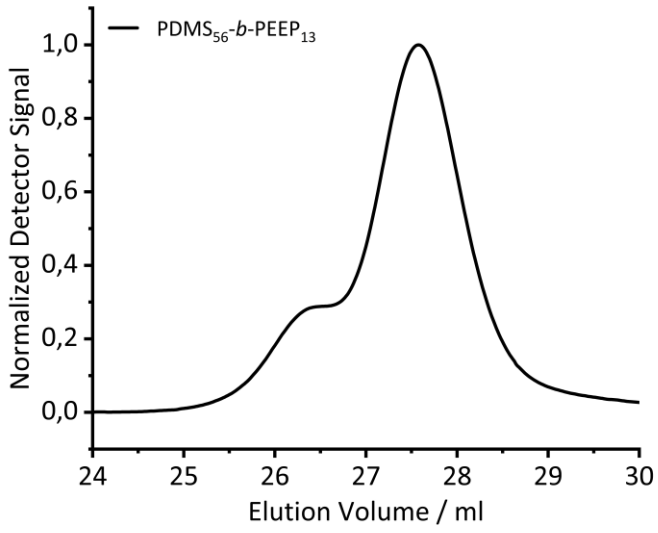
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



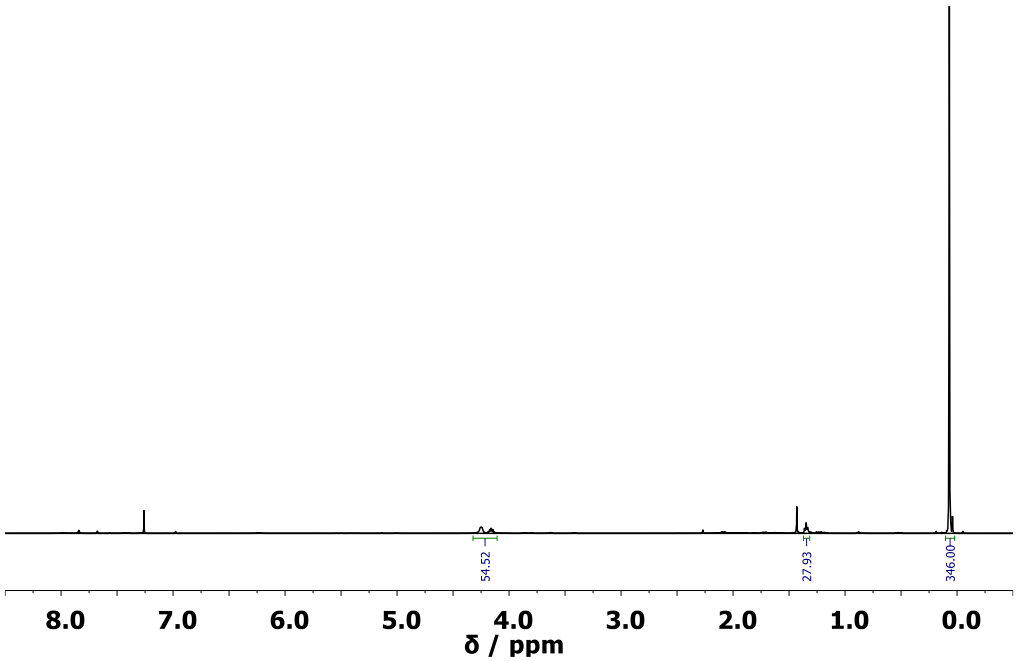
Molar Mass Dispersity (THF, PB calibration)



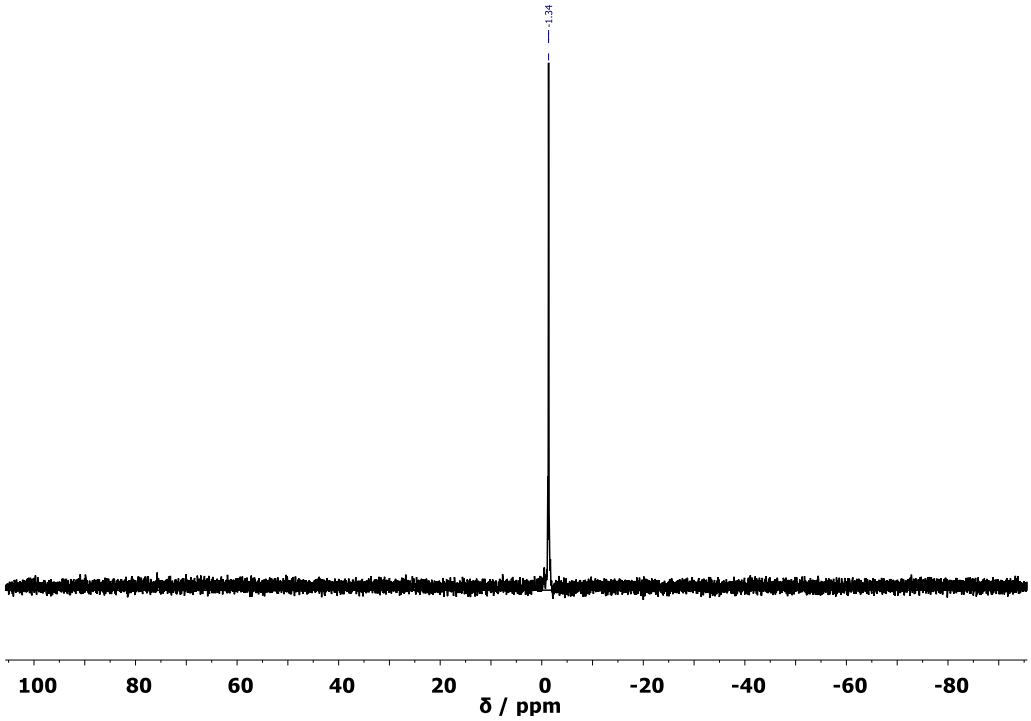
M_n	M_W	$Đ$
4276.94	5028.63	1.18

B) $PDMS_{56}$-*b*-$PEEP_9$

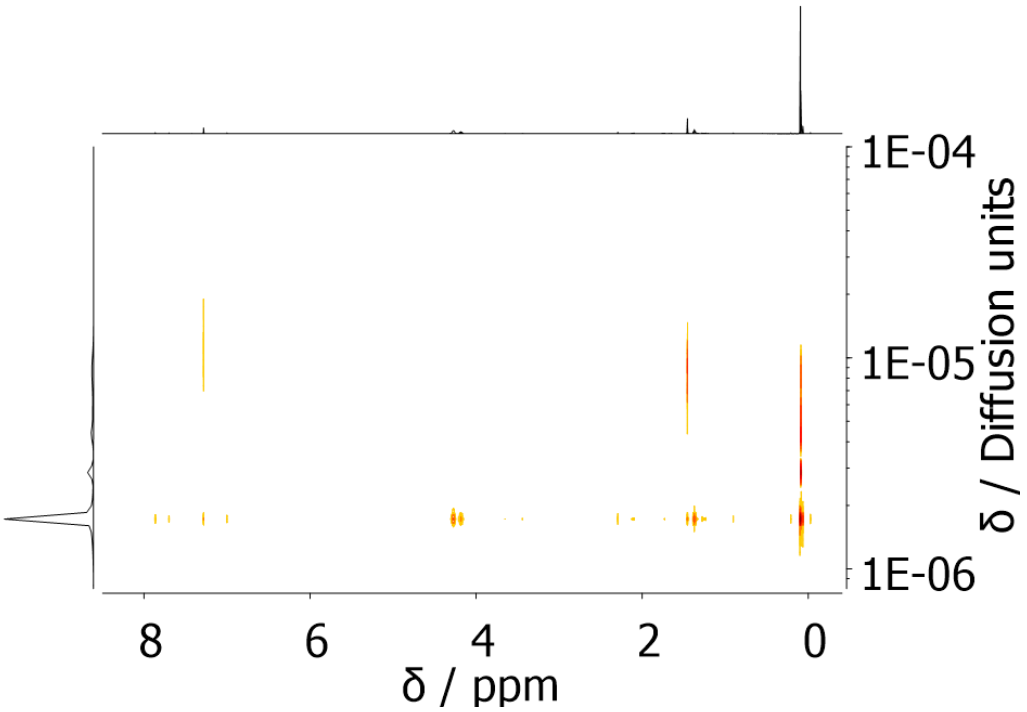
^{1}H NMR (500 MHz, $CDCl_3$)



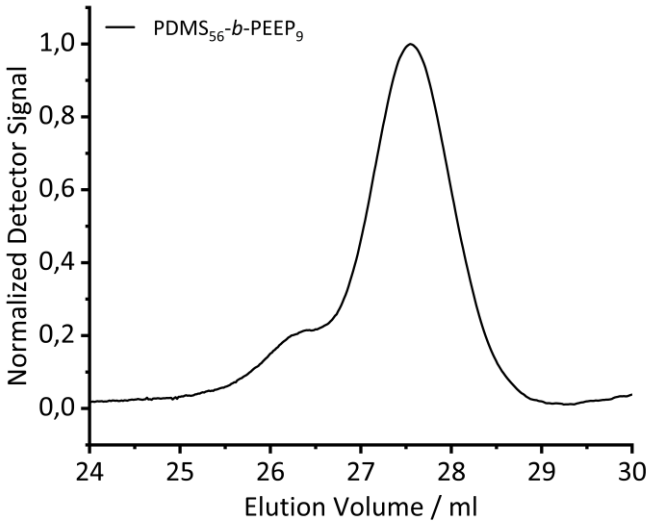
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)

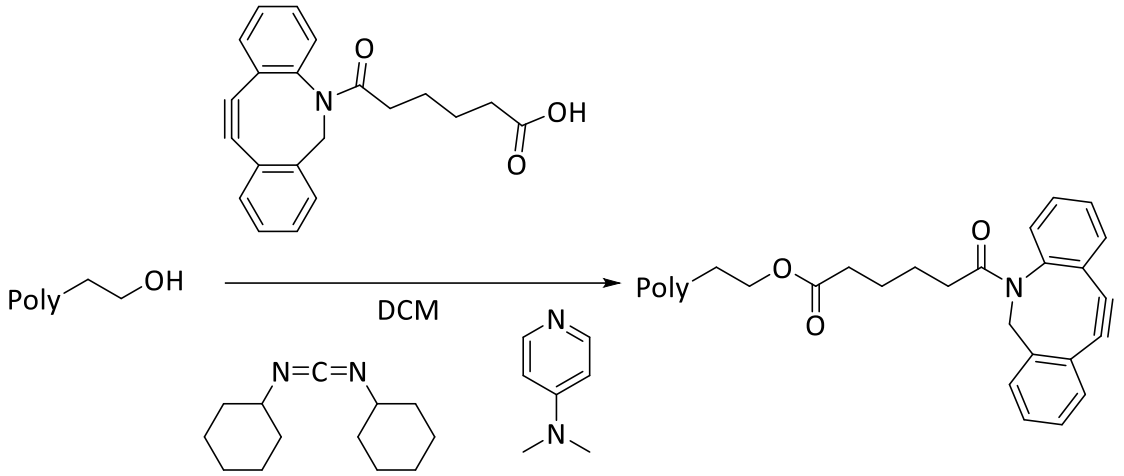


Molar Mass Dispersity (THF, PB calibration)



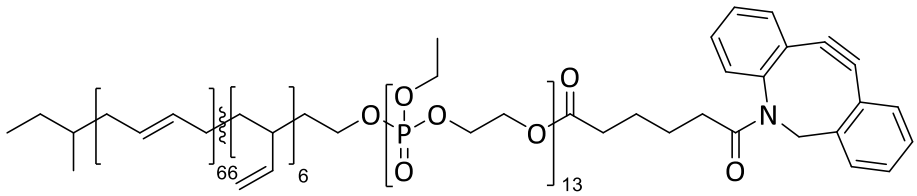
M_n	M_W	$Đ$
4569.44	5183.54	1.13

5.4 Dibenzocyclooctyne substituted polymer



Based on a modified literature procedure,[22] *Poly-OH* (10 µmol, 1.0 eq) was dissolved in anhydrous DCM (100 µl). Dibenzocyclooctyne-acid (DBCO-COOH) (4.0 mg, 12 µmol, 1.2 eq) was added to the polymeric solution as well as 4-dimethylaminopyridine (DMAP) (1.2 mg, 10 µmol, 1.0 eq) and washed with additional DCM (100 µl). After cooling the reaction at 0 °C, *N,N'*-dicyclohexylcarbodiimide (DCC) (4.1 mg, 20 µmol, 2.0 eq) was added. The reaction was then stirred overnight at room temperature. The reaction was quenched the next morning using acetic acid in THF (2 ml, 1 mol/l). The solution was then transferred to a regenerated cellulose dialysis tubing (MWCO = 1 kDa) for dialysis against ethanol and acetic acid for 2 days.

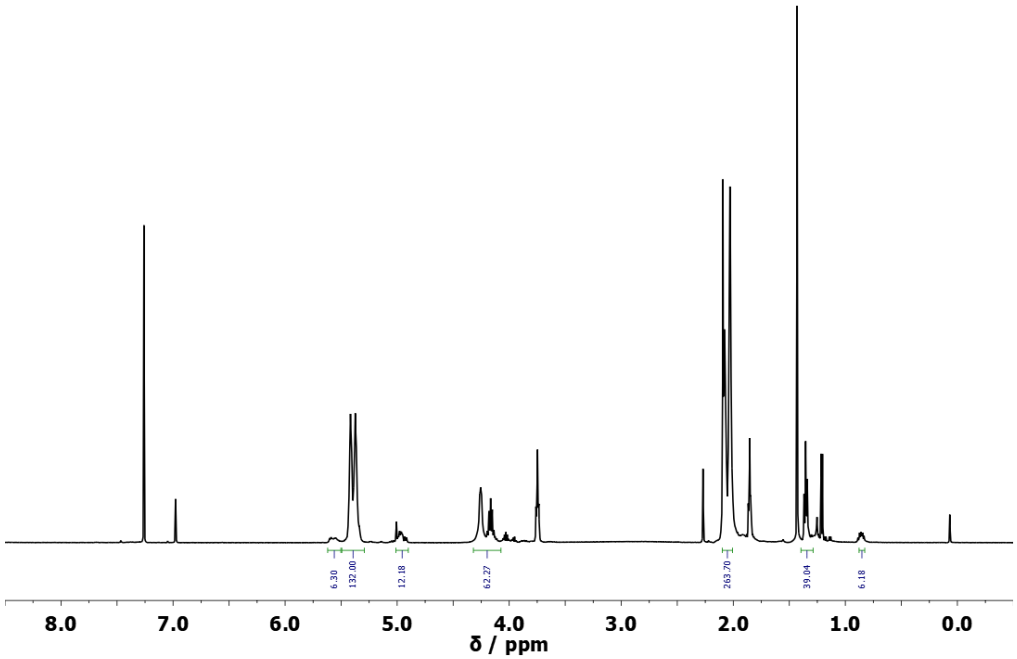
PB_{72}-b-$PEEP_{13}$-DBCO



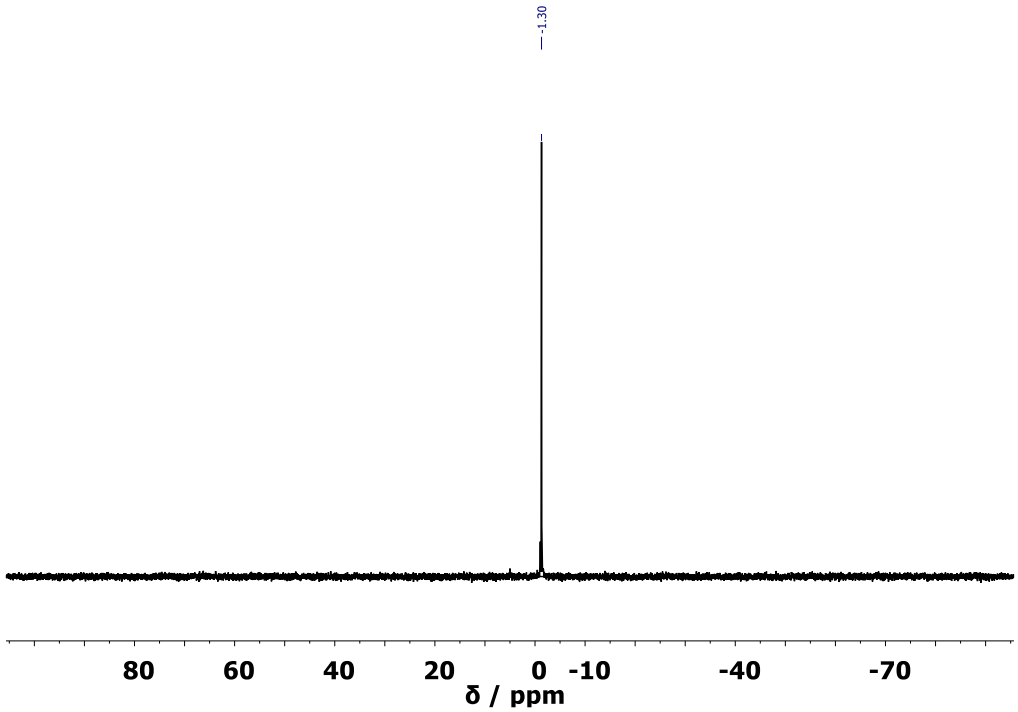
^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 5.62 – 5.50 (m, 6H, 6*C**H**=CH_2), 5.49 – 5.29 (m, 132H, 66*C**H**=C**H**), 5.01 – 4.89 (m, 12H, 6*CH=C**H$_2$**), 4.32 – 4.07 (m, p*6H, p*(C**H**$_2$- O)$_3$P=O), 2.10 – 2.01 (m, 264H, C**H$_2$**), 1.35 (q, *J* = 6.8 Hz, p*3H, p*C**H$_3$**-CH_2-O), 0.88 – 0.83 (m, 6H, (C**H$_3$**)$_2$-CH).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P/ppm -1.3 (s, 1P)

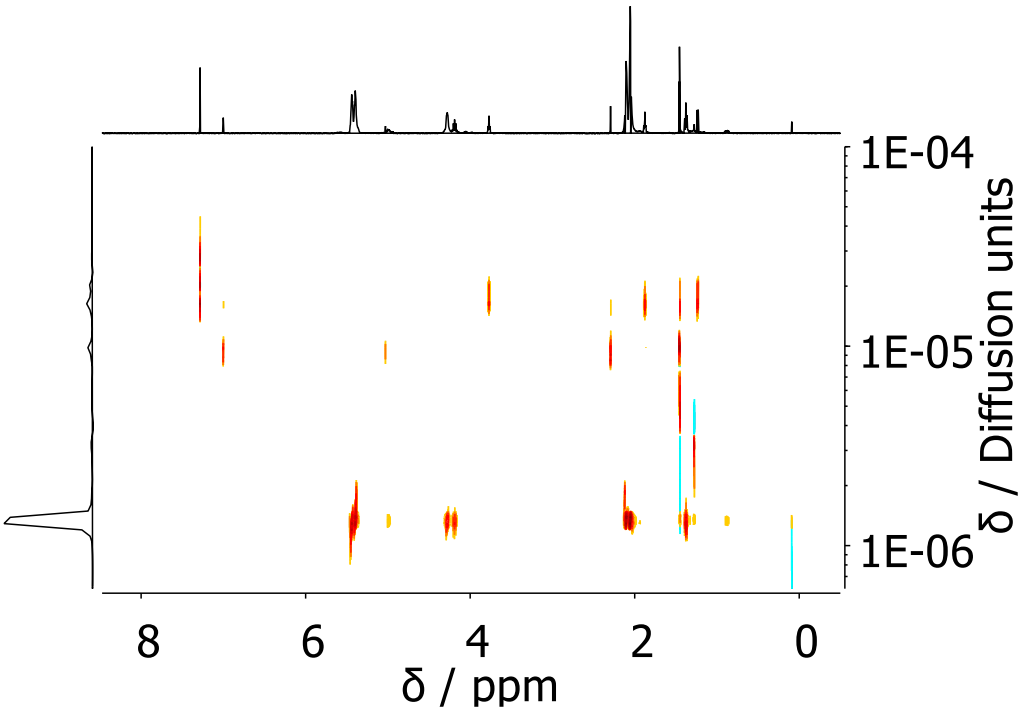
^{1}H NMR (500 MHz, $CDCl_3$)



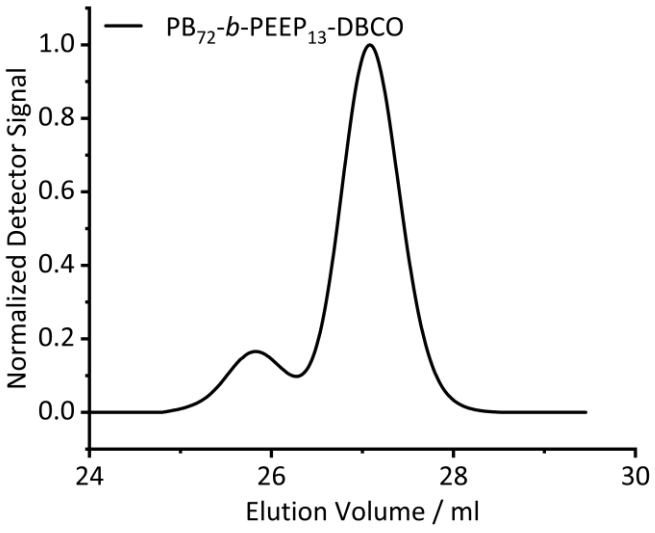
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)

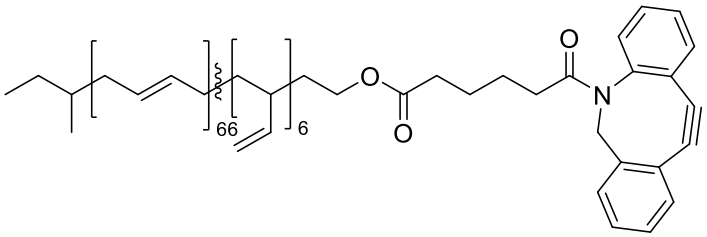


Molar Mass Dispersity (THF, PB calibration)



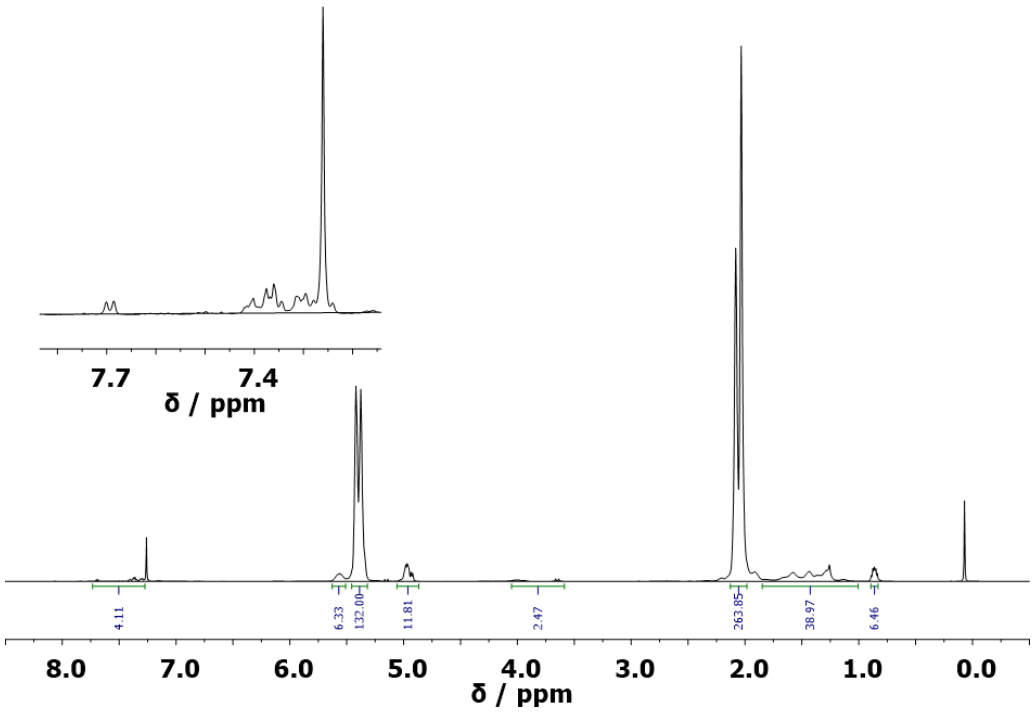
M_n	M_W	$Đ$
5869.73	6335.90	1.08

PB_{72}-DBCO

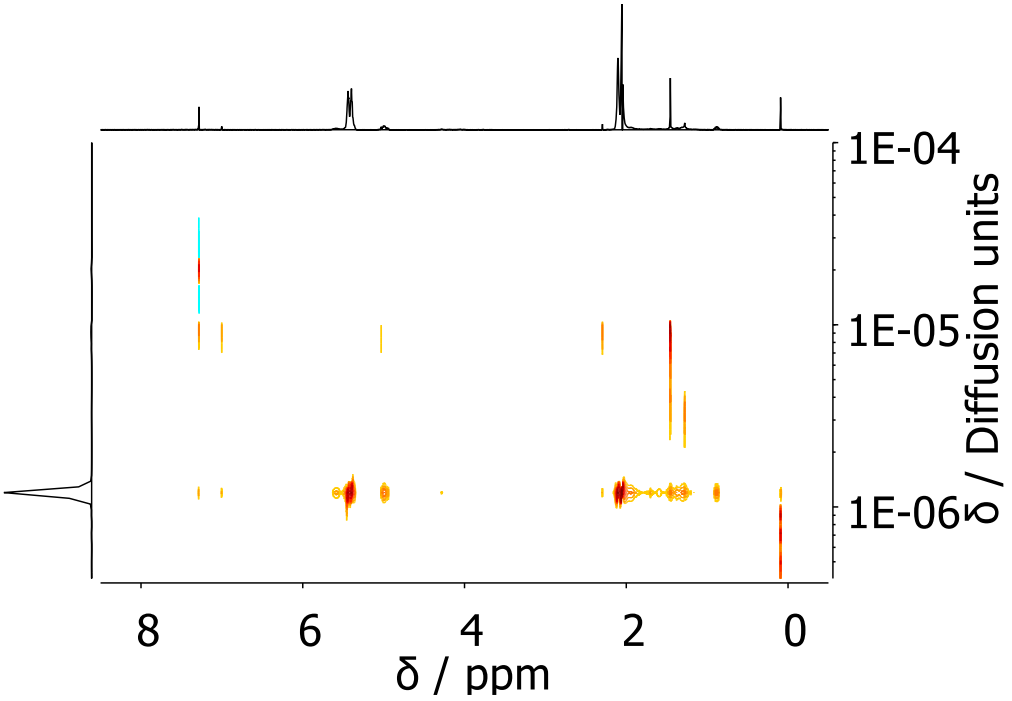


^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 7.73 – 7.27 (m, 4H, C**H** (DBCO)), 5.63 – 5.51 (m, 6H, 6*C**H**=CH_2), 5.40 (d, *J* = 21.3 Hz, 132H, 66*C**H**=C**H**), 5.06 – 4.87 (m, 12H, 6* CH=C**H_2**), 3.65 (d, *J* = 13.8 Hz, 2H, C**H_2**-O), 2.06 (d, *J* = 24.3 Hz, 264H, C**H_2** and C**H**), 1.86 – 1.00 (m, 39H, C**H_2** and C**H**), 0.86 (dt, *J* = 9.7, 6.8 Hz, 6H, 2*C**H_3**).

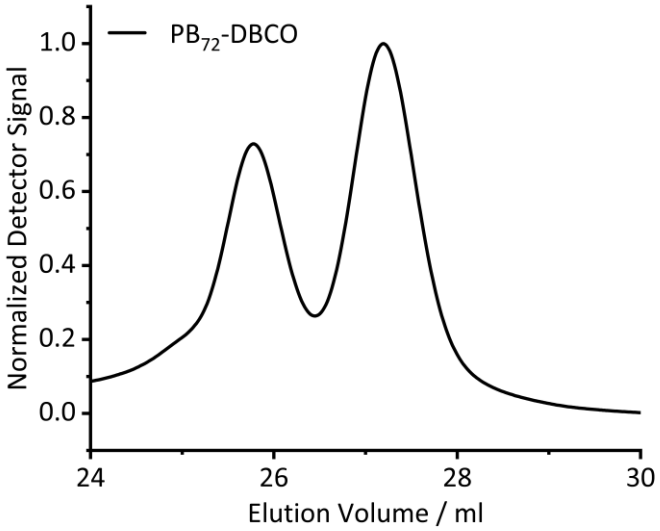
^{1}H NMR (500 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)

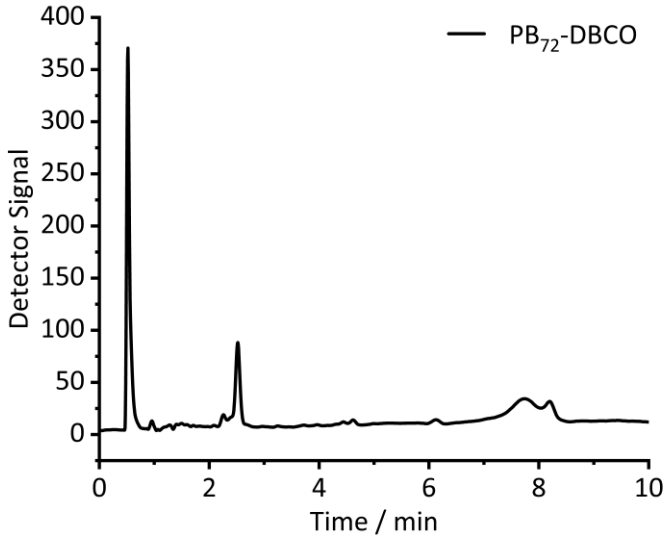


Molar Mass Dispersity (THF, PB calibration)



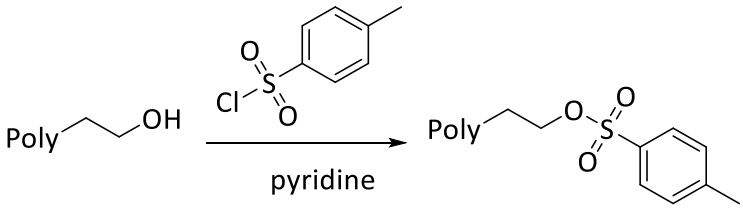
M_n	M_W	$Đ$
6653.16	9220.90	1.39

HPLC



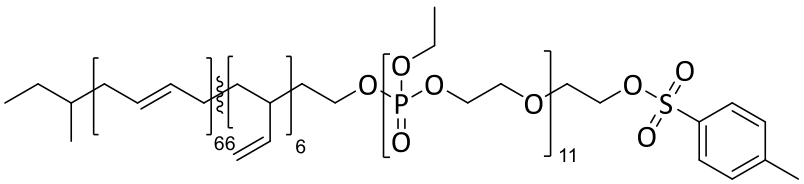
5.5 Azide substituted polymers

Tosylation



Based on a modified literature procedure,[26] *Poly-OH* (10 µmol, 1.0 eq) was dissolved in anhydrous pyridine (100 µl). *p*-Toluenesulfonyl chloride (TosCl) (1.9 mg,10 µmol, 1.0 eq) was added to the solution and the reaction was stirred at room temperature overnight. The reaction was then diluted in THF and transferred to a regenerated cellulose dialysis tubing (MWCO = 1 kDa) for dialysis against DI water and acetic acid for 2 days. Yielding the tosylated polymer *Poly-OTos.*

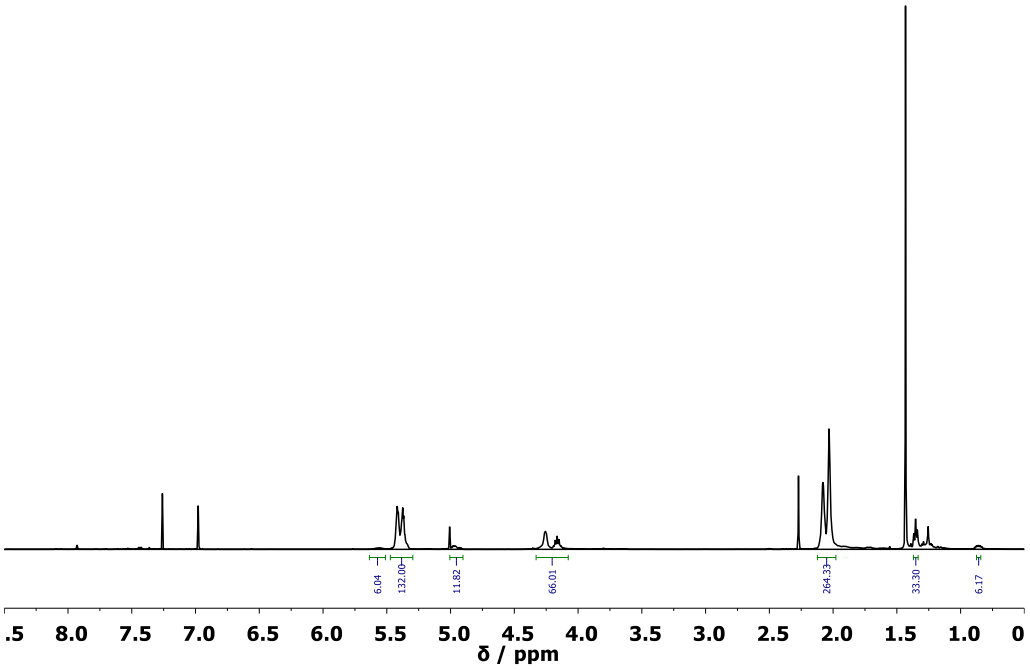
PB_{72}-b-$PEEP_{11}$-Tosylate



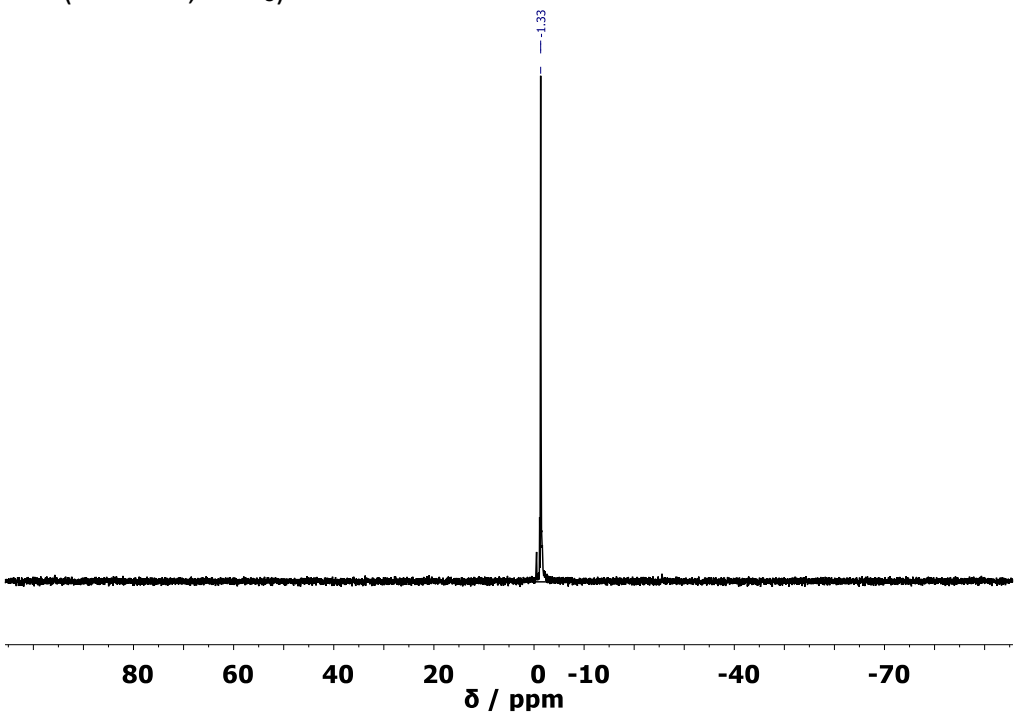
^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 5.64 – 5.51 (m, 6H, 6*C**H**=CH_2), 5.48 – 5.29 (m, 132H, 66*C**H**=C**H**), 4.96 (ddd, *J* = 22.4, 14.0, 4.7 Hz, 12H, 6*CH=C**H$_2$**), 4.33 – 4.08 (m, p*6H, p*(C**H**$_2$- O)$_3$P=O), 2.06 (d, *J* = 24.6 Hz, 264H, C**H$_2$**), 1.35 (t, *J* = 6.8 Hz, p*3H, p*C**H$_3$**-CH_2-O), 0.88 – 0.84 (m, 6H, (C**H$_3$**)$_2$-CH).

^{31}P NMR (202 MHz, $CDCl_3$) δ_P/ppm -1.3 (s, 1P)

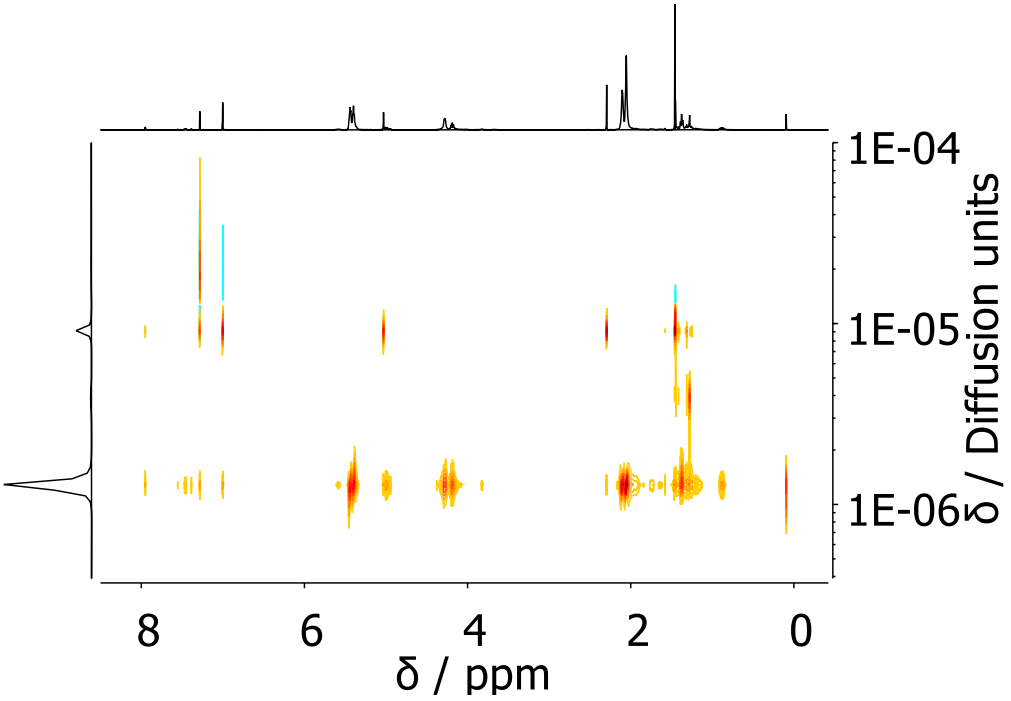
^{1}H NMR (500 MHz, $CDCl_3$)



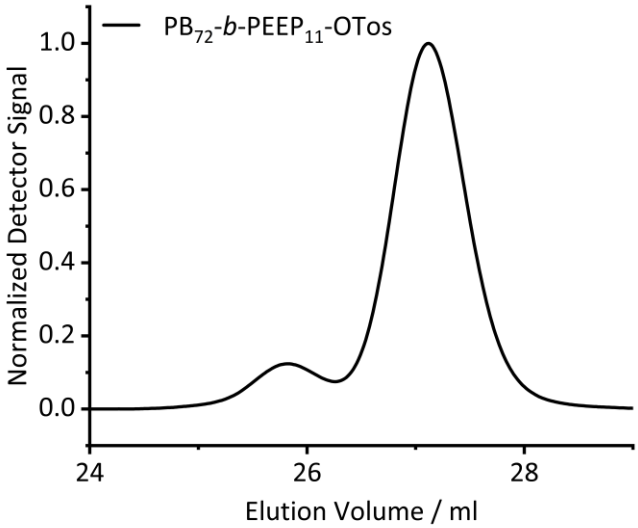
^{31}P NMR (202 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)

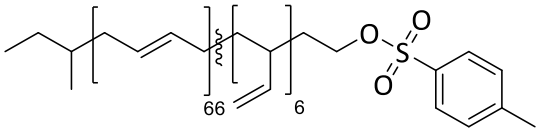


Molar Mass Dispersity (THF, PB calibration)



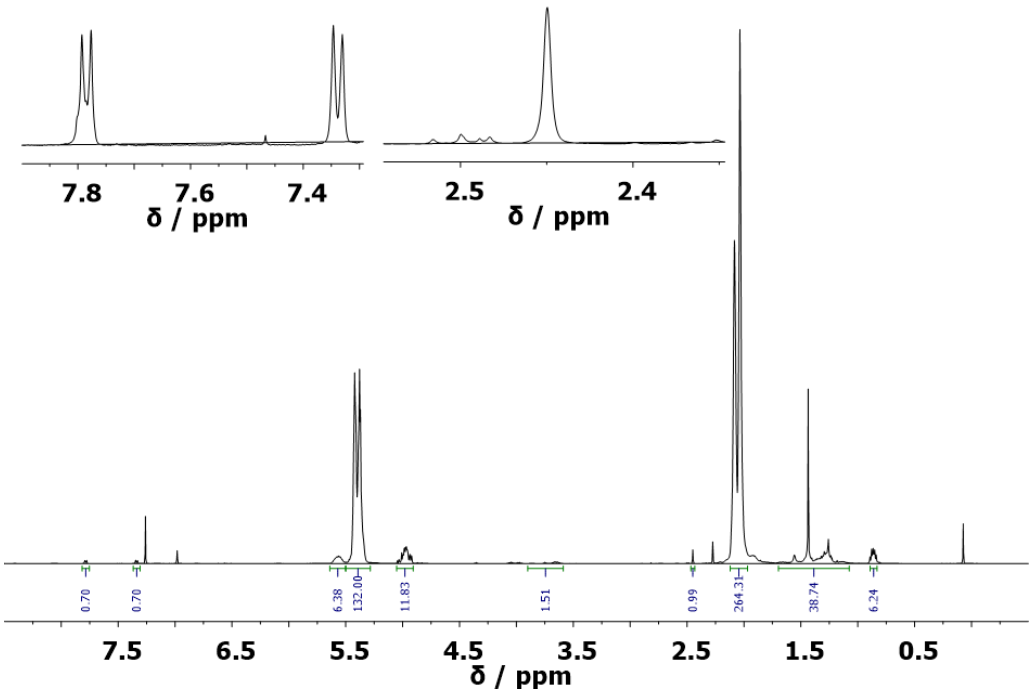
M_n	M_W	$Đ$
5610.98	6048.55	1.08

PB_{72}-Tosylate

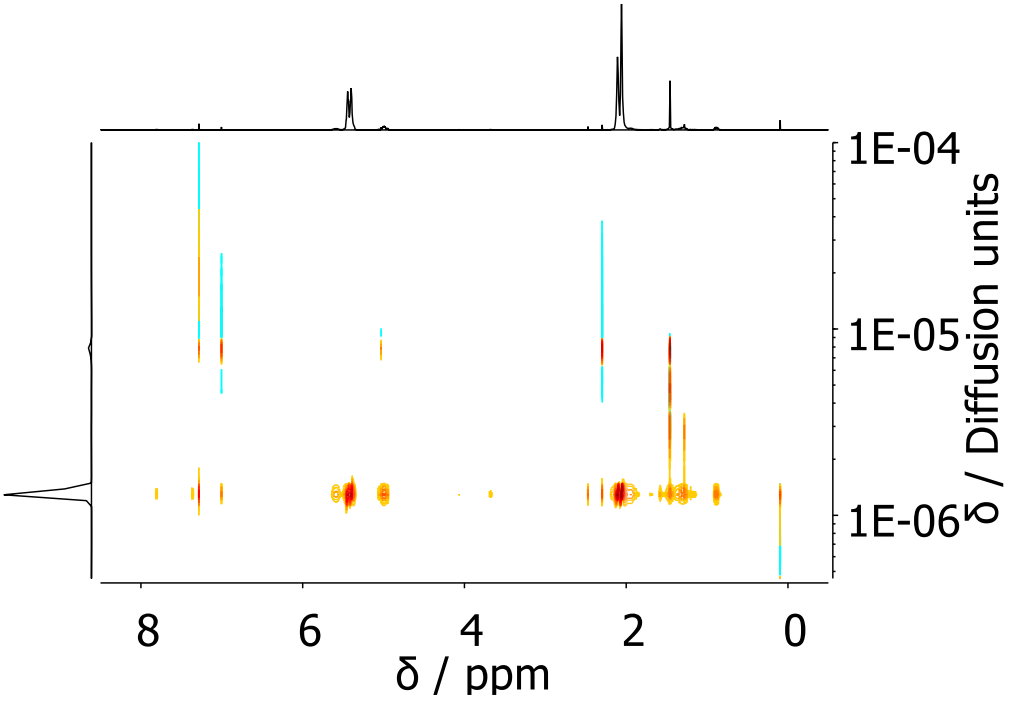


^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 7.78 (d, *J* = 8.1 Hz, 1H, C**H** (Tosyl)), 7.34 (d, *J* = 8.0 Hz, 1H, C**H** (Tosyl)), 5.63 – 5.50 (m, 6H, 6* C**H**=CH_2), 5.50 – 5.28 (m, 132H, 66*C**H**=C**H**), 5.04 – 4.91 (m, 12H, 6* CH=C**H_2**), 3.90 – 3.58 (m, 2H, CH_2-O-S), 2.45 (s, 1H, C**H_3**-arom), 2.06 (d, *J* = 24.6 Hz, 264H, C**H_2** and C**H**), 1.69 – 1.08 (m, 39H, C**H_2** and C**H**), 0.89 – 0.83 (m, 6H, 2*C**H_3**).

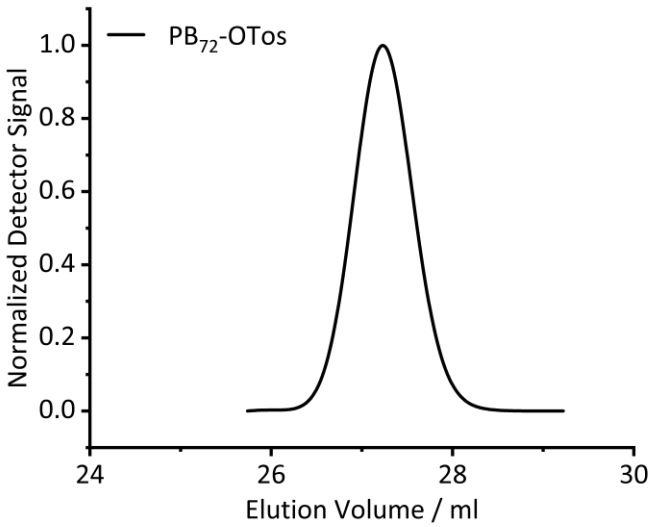
^{1}H NMR (500 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)

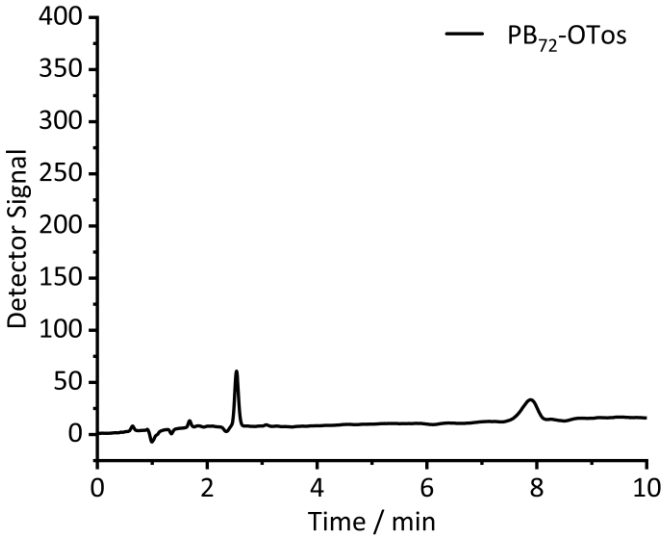


Molar Mass Dispersity (THF, PB calibration)

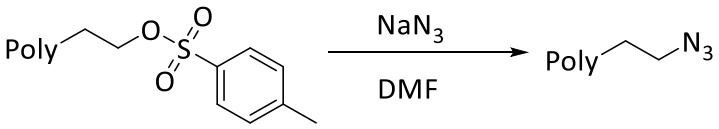


M_n	M_W	$Đ$
5085.27	5229.79	1.03

HPLC

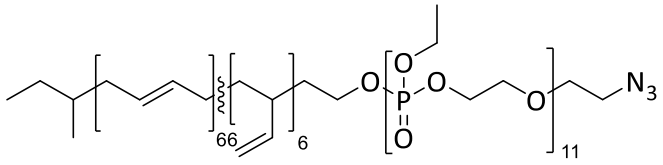


Azidation



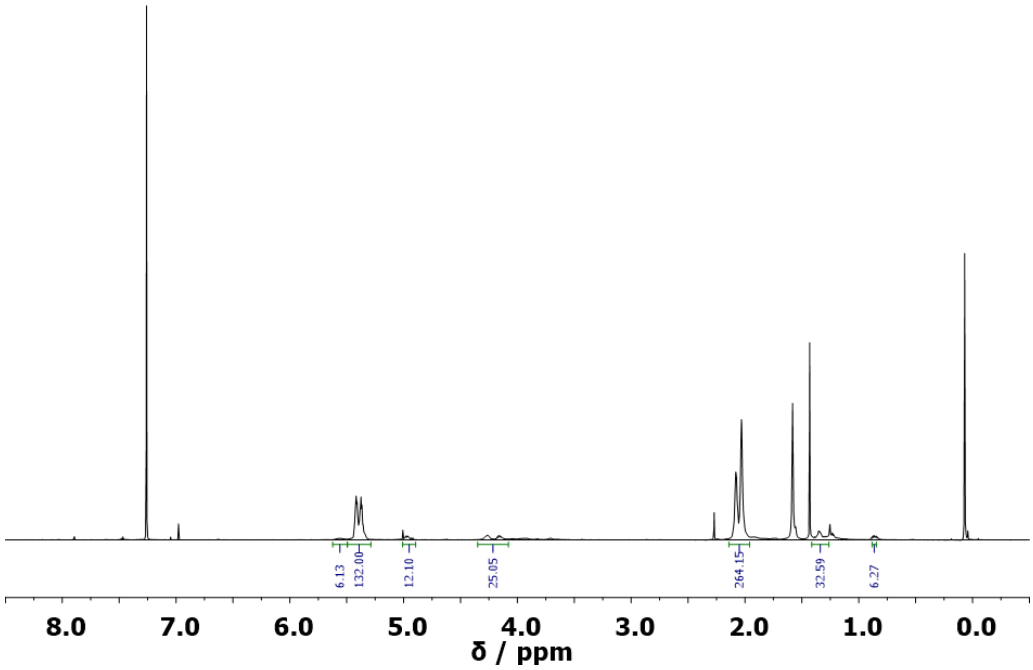
Based on a modified literature procedure,[26] the tosylated polymer *Poly-OTos* (20 µmol, 1.0 eq, see Tosylation) was dissolved in *N,N*-dimethylformamide (DMF) (0.5 ml). Sodium azide (81 mg, 1.25 mmol, 6.2 eq) was added and flushed with more DMF (0.5 ml). The reaction mixture was stirred at room temperature overnight. The reaction was then diluted in DCM and transferred to a regenerated cellulose dialysis tubing (MWCO = 1 kDa) for dialysis against ethanol and acetic acid for 2 days.

PB_{72}-b-$PEEP_{11}$-Azide

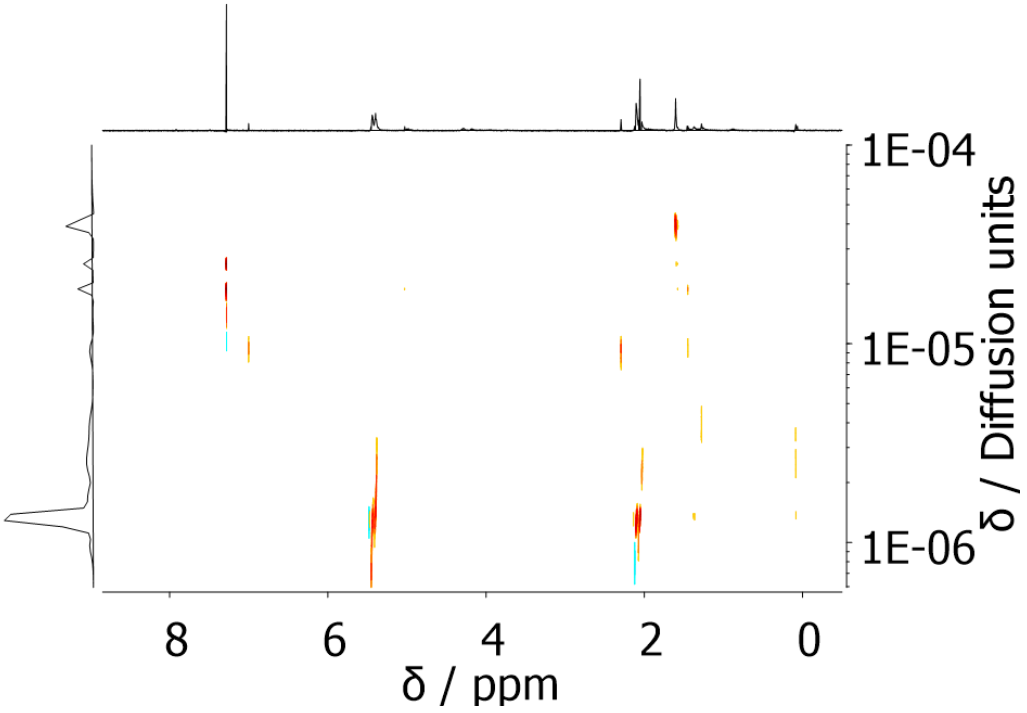


^{1}H NMR (500 MHz, $CDCl_3$) δ_H/ppm 5.56 (s, 6H, 6*C**H**=CH_2), 5.39 (dd, *J* = 21.3, 4.1 Hz, 132H, 66*C**H**=C**H**), 5.01 – 4.88 (m, 12H, 6*CH=C**H$_2$**), 4.19 (dd, *J* = 31.9, 24.8 Hz, p*6H, p*(C**H**$_2$-O)$_3$P=O), 2.06 (d, *J* = 24.8 Hz, 264H, , C**H$_2$**), 1.41 – 1.26 (m, p*3H, p*C**H$_3$**-CH_2-O), 0.88 – 0.85 (m, 6H, (C**H$_3$**)$_2$-CH).

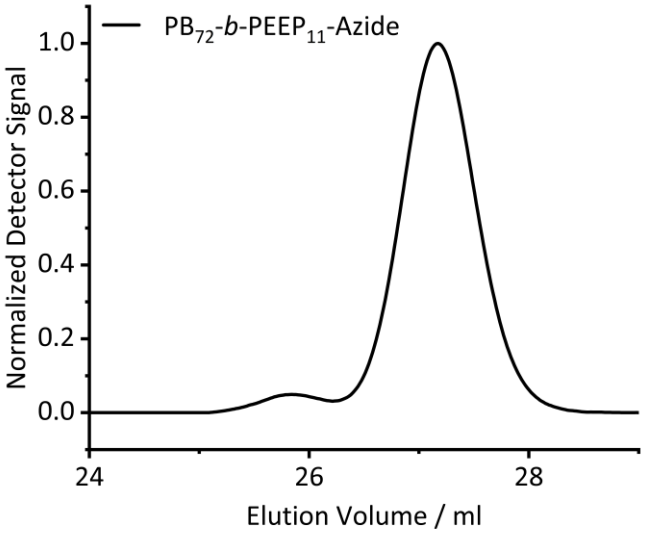
^{1}H NMR (500 MHz, $CDCl_3$)



DOSY (500 MHz, $CDCl_3$)



Molar Mass Dispersity (THF, PB calibration)



M_n	M_W	$Đ$
5329.92	5585.16	1.05

5.6 Film Hydration

In order to observe the formation of vesicles by confocal microscopy 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BDP) functionalized polymers were used as fluorescent markers. Two BDP dyes were used: BDP FL with green fluorescence $\lambda_{excitation}/\lambda_{emission}$ 503/512 nm and BDP 630/650 with red fluorescence $\lambda_{excitation}/\lambda_{emission}$ 630/650 nm. The functionalized polymers were PB_{73}-*b*-$PEEP_{12}$-BDP FL, PB_{73}-*b*-$PEEP_{21}$-BDP 630/650, $PDMS_{61}$-BDP FL and PB_{73}-BDP 630/650 synthesized previously by Rideau *et al*[3] . Based on a modified literature procedure,[22] a solution of amphiphilic block copolymers in chloroform (192 µl, 4 mg/ml) was doped with BDP FL and BDP 630/650 fluorescently labelled polymers solution in chloroform (8 µL, 1 mg/ml). The obtained solution (20 µl) was added in a GPC vial using the desiccator and high vacuum for 20 min. An aqueous sucrose solution (200 µl, 100 mmol/l) was added to the vial for hydration overnight. For visualization on the confocal microscope, an equiosmotic glucose stock solution (200 µl, 100 mmol/l) was added to the hydration medium (50 µl) in an 8 well glass slide. The higher molecular weight of sucrose vs. glucose ensured that the vesicles were found on the bottom of the well. The vesicles were observed using optical microscopy and images were taken in confocal mode.

6 Conclusion and Future Works

PB-*b*-PEEP and PDMS-*b*-PEEP diblock copolymers were synthesized within the desired hydrophilic mass ratio range (15 % < *f(hydrophilic)* < 45 %). The molecular weight of these block copolymers is between 4.2 kDa and 6.1 kDa with a low polydispersity ($Đ$ < 1.2).

Results published earlier by Rideau *et al.*[2] concerning the self-assembly of PB-*b*-PEEP copolymers with non-assisted film hydration could be reproduced. The PDMS-*b*-PEEP block copolymers did not form vesicles under the same circumstances, even though this type of block copolymer has been shown to form vesicles from non-assisted film hydration before.[3]

The polymers which formed vesicles were functionalized with DBCO- and azide-moieties for copper-free click reaction. The functionalization was first tested on PB-OH homopolymer, it proved difficult to confirm the attachment of DBCO- and azide-groups, however. The same procedure was then used on the PB-*b*-PEEP polymers that had formed vesicles. The DBCO-functionalized polymer formed vesicles on its own with a low yield. The azide-functionalized polymer did not form vesicles on its own. PB-*b*-PEEP polymers were doped with 10 wt% of the functionalized polymers and dyed with different colors. After drying the film with high vacuum as well as under argon flow, both doped polymers formed vesicles (Figure 7). These were subsequently combined in one well of the microscope plate to investigate any vesicle-vesicle aggregation happening from the click reaction. No vesicle-vesicle aggregation could be observed after a few hours, a day or a week, even though the vesicles were in close proximity to one another. There are many different possible reasons (see 4.3), among them the possibility of the functionalized end of the polymers being hidden in the hydrophobic part of the membrane instead of reaching out to the aqueous phase or insufficient yield of functionalized polymer. The degradation of the synthesized polymers could also be a reason for the absence of aggregation. For the future a less sensitive alkyne moiety for Cu-catalyzed click-reactions should be tried to achieve vesicle-vesicle aggregation.

7 Literature

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