Synthesis and Encapsulation of Uniform Star-Shaped Block-Macromolecules

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Linear uniform oligomers synthesized via a two-step iterative cycle are postmodified with uniform octaethylene glycol monomethyl ether and finally coupled via azide-alkyne cycloaddition to yield uniform star-shaped block macromolecules with a mass ranging from 10 to 14 kDa. Each of the molecules is carefully characterized by NMR, electrospray ionization mass spectrometry (ESI-MS), and size exclusion chromatography (SEC) to underline their purity as well as their uniformity. The obtained star-shaped macromolecules are investigated in their ability to encapsulate dye molecules by carrying out qualitative solid–liquid phase transfer experiments.

Over the course of time, the synthesis and the corresponding industrial processes of polymers has significantly advanced, allowing control over the polymerization process itself.[1,2] However, sophisticated polymerization techniques, such as metal coordinated insertion polymerization, anionic/cationic or controlled radical polymerizations, such as atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), or reversible addition-fragmentation chain-transfer (RAFT) polymerization, cannot achieve 100% control over the polymerization process, leading to at least a certain degree of dispersity in all cases.[3,4] On the other hand, nature has evolved to synthesize perfectly defined macromolecules. The complexity of life largely derives from the sequence-definition of these biomacromolecules, i.e., DNA and proteins. Only recently, research in polymer chemistry focused on synthesizing monodisperse macromolecules with defined sequence and structure using iterative synthesis approaches. This subgroup of "sequence-controlled polymers" was defined as "sequence-defined polymers",[4–7] which exhibit no dispersity at all (i.e., $Đ = 1$) and are thus perfectly uniform.[8–12]

The synthesis of such sophisticated structures involves either the utilization of protecting groups or orthogonal reactions, which are then carried out in an iterative synthesis cycle to build the desired uniform structures. Often, a linear approach is chosen as it offers the highest possible control over the sequence as well as architecture, but also bidirectional routes, or the so-called iterative exponential growth (often termed divergent/convergent approach), are commonly applied.[11,13,14]

As the synthesis of uniform macromolecules always involves a multistep procedure, purification, high conversion, absolute control over the reaction/side reactions as well as high yields are a necessity. Therefore, multicomponent reactions, such as the Passerini-3-component reaction (P-3CR), or "click chemistry" like in the azide-alkyne cycloaddition, are often considered for this task.[15–21] The P-3CR, which was already used by our working group to synthesize diverse linear and cyclic macromolecules,[14,22] was herein combined with the azide-alkyne cycloaddition for the synthesis of uniform star-shaped block co-macromolecules.[23,24]

Sequence-defined macromolecules can help to understand structure-property or structure-activity relationships, yet their synthesis mainly focused on linear architectures so far, which are, for instance, applied in data storage or the establishment of structure–property relationships.[22,25–32] However, multidirectional approaches, as well as more sophisticated structures like monodisperse star-shaped macromolecules, have not been reported yet, probably due to the complexity of their synthesis. Closely related are dendrimers, which also come with a very narrow dispersity, but are often not completely uniform.[33–35]

Generally, star-shaped structures provide unique properties, for instance regarding their rheology or self-assembly.[36–39] The latter is of special interest, as star-shaped polymers have a higher critical micelle concentration than their linear counterparts.[36] Especially heteroarm stars with a water-soluble shell have shown fascinating supramolecular formations, which can be further influenced by change of external conditions like solvent, pH value and temperature.[36,40] Furthermore, the ability of phase-transfer and drug encapsulation have been shown.[38,41,42] The sophisticated core and shell structures of such polymers can be tuned to fit different guest molecules and often solvatochromic dyes are applied to quantify encapsulation.[42,43]

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However, until now, such star-shaped block copolymers have only been synthesized with a dispersity higher than one.[44]

Herein, we thus report an efficient synthesis toward monodisperse star-shaped block co-macromolecules with molar masses ranging from 9.8 to 14.1 kDa via the arm-first approach. The arms were synthesized by using a two-step cycle approach of a Passerini-three-component reaction and reductive hydrogenation followed by postmodification with monodisperse octaethylene glycol monomethyl ether (Me-8EG-OH) thus resulting in monodisperse ($Đ$ = 1.00) oligomers. Upon tethering of the core by an azide-alkyne cycloaddition, the stars bear arms with flexible lipophilic backbones and customizable sidechains as well as a hydrophilic shell.

At the start of our investigations, a core-first approach was investigated, as it is generally considered the simpler and more efficient approach. For this purpose, we adapted an iterative cycle, which was already used by our group to synthesize a monodisperse icosamer,[14] as well as precursors for monodisperse macrocycles up to 152 ring atoms.[22] Yet, the procedure consisting of a P-3CR (starting with a tetracarboxylic acid as the core scaffold) and subsequent hydrogenation of a benzylic ester, did not transfer well from mono/bidirectional to the new tetradirectional approach towards star-shaped macromolecules. The P-3CR worked in reliable manner, but the deprotection process was accompanied by an unknown coupling reaction, leading to molecules of higher mass (typically 0.2–1% of this side-reaction took place, as determined by size exclusion chromatography (SEC)), which could not be separated from the main product. Thus, an arm-first approach was sought, combining the aforementioned iterative synthesis cycle (**Scheme 1**), yielding linear monodisperse oligomers in very good yields. These oligomers were subsequently coupled to a core molecule via azide-alkyne cycloaddition (Scheme 1).[14] The synthesis of building block A1 (three step synthesis starting from 11-aminoundecanoic acid, overall yield 94%, see the Supporting Information) was recently improved in terms of sustainability and efficiency,[45] also

allowed the synthesis of a second building block, A2 (five step synthesis starting from 11-aminoundecanoic acid, overall yield 79%, Scheme 1a)), effectively reducing the reaction steps toward the desired linear oligomers (for instance eight steps to the deprotected heptamer C4$_b$ instead of 14 compared to previous publications).[14] Theoretically, side-chain variation in the P-3CR step is possible by applying different aldehyde moieties during the P-3CR, yet 2-ethylbutyraldehyde was herein used throughout to establish the synthesis protocol. Thus, linear tri-, penta- and heptamers were successfully synthesized starting from 11-bromoundecanoic acid in a 4-/6-/8-step procedure with overall yields of 87%, 79%, and 70%, respectively (Scheme 1b)). These oligomers were subsequently functionalized with two Me-8EG-OH to introduce the hydrophilic shell of the desired star-shaped block macromolecules. The building blocks B1 and B2 were synthesized starting from the commercially available monomethyl and monobenzyl tetraethylene glycol, of which the first was activated with para-toluenesulfonyl chloride (p-TsCl) and subsequently coupled with the monobenzyl tetraethylene glycol and deprotected to yield uniform Me-8EG-OH.[46]

Subsequently, Me-8EG-OH was converted to building block B1 by attachment to 6-formamidohexanoic acid followed by subsequent dehydration to an isocyanide. B2 was obtained by attachment to 4-formylbenzoic acid (see the Supporting Information). Both building blocks were obtained in good yields (64%, 5 steps for B1, 67%, 4 steps for B2, both starting from monomethyl glycol) and were purified by column chromatography. The Me-8EG-bearing isocyanide as well as the Me-8EG-bearing aldehyde allowed the introduction of two 8EG chains to the presynthesized oligomer-chains C$_{2b-4b}$ in one reaction step (Scheme 1c)).

After introduction of the 8EG units, the bromine moiety was converted to an azide, enabling a copper(I)-catalyzed azide alkyne cycloaddition (CuAAC) to attach the oligomers to the core scaffold (Scheme 1c,d)) and thus to obtain the desired star-shaped block macromolecules. Butane-1,2,3,4-tetrapropargylcarboxylate E1 was therefore synthesized and the final star-shaped
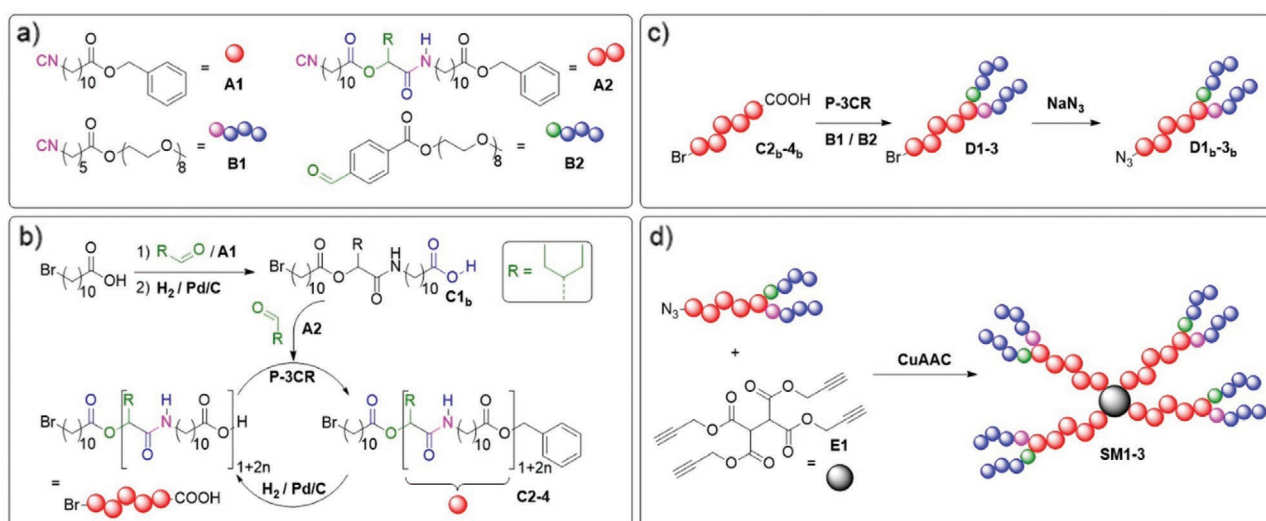
Scheme 1. a) Building blocks, which were used for the synthesis of the oligomers (**A1** and **A2**) and the Me-8EG introduction (**B1** and **B2**). b) Iterative cycle toward uniform oligomers. c) Postmodification of the oligomers with uniform Me-8EG and sodium azide. d) Synthesis of uniform star-shaped block-copolymers via CuAAC.

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macromolecules SM1-3 were obtained in an overall yield of 63%, 48%, and 44%, in a 7-/9-/11-step synthesis, respectively.

In previous publications, the combination of the P-3CR and subsequent hydrogenation had shown its value as a reliable tool for the synthesis of sequence-defined macromolecules, due to virtually absent side reactions, easy purification, and generally high yields (mostly >90%). Here, the necessary bromide-bearing oligomers (Scheme 1b)) introduced some limitations, as nucleophilic substitution reactions with the free carboxylic acid moiety were observed, both inter- and intramolecularly. Thus, macrocycles or oligomers with doubled chain lengths were observed by SEC, decreasing yield and hindering purification. The cyclization reaction was negligible, as the P-3CR was carried at high concentration (0.22–1.5 mol L^{-1} reactants in dichloromethane) and the cycles were inert to the following reactions, allowing easy separation via column chromatography at a later stage. The chain-doubling, however, proved to be more troublesome, as the polarity of product and side-product were very similar, leading to yield losses during column chromatography due to mixed fractions. This reduced the effective yield, especially in the PEGylating step, as, for the trimer D1 and pentamer D2, heating had to be applied for sufficient conversion, which also increased the amount of chain-doubled side products. Therefore, heating was not applied for the heptamer synthesis, which allowed for simpler purification, but resulted in lower conversion (trimer D1 (81%), pentamer D2 (67%), heptamer D3 (69%)). The chain-doubling side-products were not isolated but were verified via electrospray ionization mass spectrometry (ESI-MS) measurements and their yields ranged between 0.25% and 1.5%, as determined by crude SEC measurements of the reaction mixtures.

Despite these problems, the oligomers were isolated in good yields and high purity and were characterized by ^{1}H, ^{13}C NMR, and IR spectroscopy as well as mass spectrometry and SEC. The chromatograms (**Figure 1**) confirm monodispersity (99% purity determined by SEC). All other analytic data also confirms the expected oligomer structures and is provided in the Supporting Information. The thus obtained PEGylated oligomers were then converted to their corresponding azides by refluxing them in acetonitrile with an excess of sodium azide (3.00 eq.) for 12 h (Scheme 1c)).

The azide moieties were exploited in subsequent CuAAC to form the star-shaped block macromolecules. The couplings were conducted in chloroform at 65 °C for 24–40 h with Cu(I) and diisopropylethylamine (DIPEA) as ligand, followed by column chromatography, yielding the trimer/pentamer/heptamer stars in very good yields (90–91%).

In situ reduction of copper(II)sulfate with L-ascorbic acid in THF/water was also tried but yielded inferior results. In order to counter the increasing viscosity of the oligomer solutions, the concentration of the reaction was slightly modified, albeit kept high. Therefore, the concentration was decreased with increasing arm length (i.e., 0.0350, 0.0258, and 0.0229 mol L^{-1} for the three star-shaped structures, respectively). The monodisperse macromolecules were obtained with purities above 99% (determined by SEC, Figure 1) and were characterized by ^{1}H, ^{13}C NMR, and IR spectroscopy as well as mass spectrometry and SEC to confirm their successful synthesis and verify their uniform character (see, e.g., **Figure 2** for spectra from SM1).
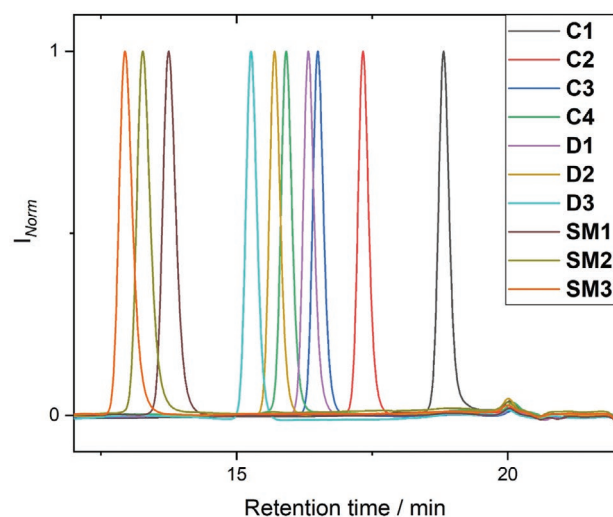

Figure 1. SEC traces of the benzyl-protected oligomers (**C1**, **C2**, **C3**, **C4**), block co-oligomers (**D1**, **D2**, **D3**) and star-shaped macromolecules (**SM1**, **SM2**, **SM3**) exhibiting high degree of definition. Dispersity for the oligomers **C1**–**C4** and **D1**–**D3** are 1.00. Dispersity for the respective star-shaped macromolecules are as follows: SM1: 1.01, SM2: 1.01, SM3: 1.01. The signal at 20 min is a system peak.

All data confirm a successful synthesis of the target compound in very high purity. Concluding, the herein reported arm-first approach, combined with an iterative two-step synthesis cycle of oligomers synthesis, is capable to prepare uniform star-shaped block co-macromolecules in sufficient yield and excellent purity.

To investigate possible applications, the obtained star-shaped macromolecules were tested in solid–liquid phase-transfer to evaluate their encapsulation of guest molecules, either as unimolecular micelles or as self-assembled copolymers. Two experiments were set up as depicted in **Figure 3**: the water-soluble SM1 was used to transfer water-insoluble Nile Red into an aqueous phase, while SM3 was used to transfer Orange II into an organic phase. First, solutions of different concentrations of SM1 were prepared and saturated with Nile Red. After 6 h of vigorous shaking, undissolved dye was removed via filtration and the colored solutions were measured by UV–vis spectroscopy to assess the already optically visible encapsulation (Figure 3a)). Likewise, SM3 was dissolved in dichloromethane and saturated with water soluble Orange II, filtrated and measured (Figure 3b)).

In both cases, the coloration of the solution increased with concentration of the amphiphilic macromolecules and the UV–vis measurements confirmed higher concentrations of the respective dye. The color as well as the UV–vis measurements confirmed the successful transfer of dye into the solvent phase, which is due to the amphiphilic character of the star-shaped-macromolecules and their ability to encapsulate guest molecules. An absolute determination of the encapsulated dye per macromolecule was not possible, as the observed nonlinear increase of the absorption maxima indicates the formation of self-assembled structures in solution. To probe this, we carried out dynamic light scattering (DLS) measurements of SM1 in mixtures of methanol and water (**Figure 4**), as methanol was determined to be a good solvent for SM1 and indicated unimolecular structures (see Figure S37 in the Supporting Information).
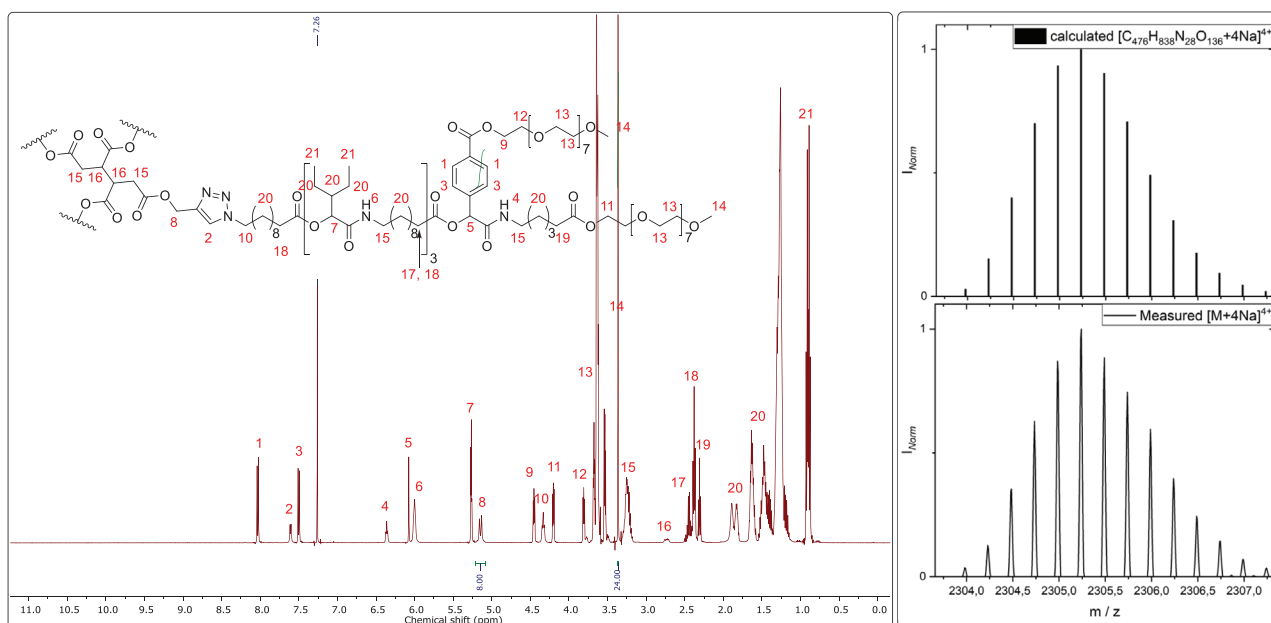
Figure 2. a) Structure and ¹H NMR spectrum and b) ESI-MS of the trimer-star-shaped macromolecule **SM1**. The mass pattern was calculated using the program MMass.
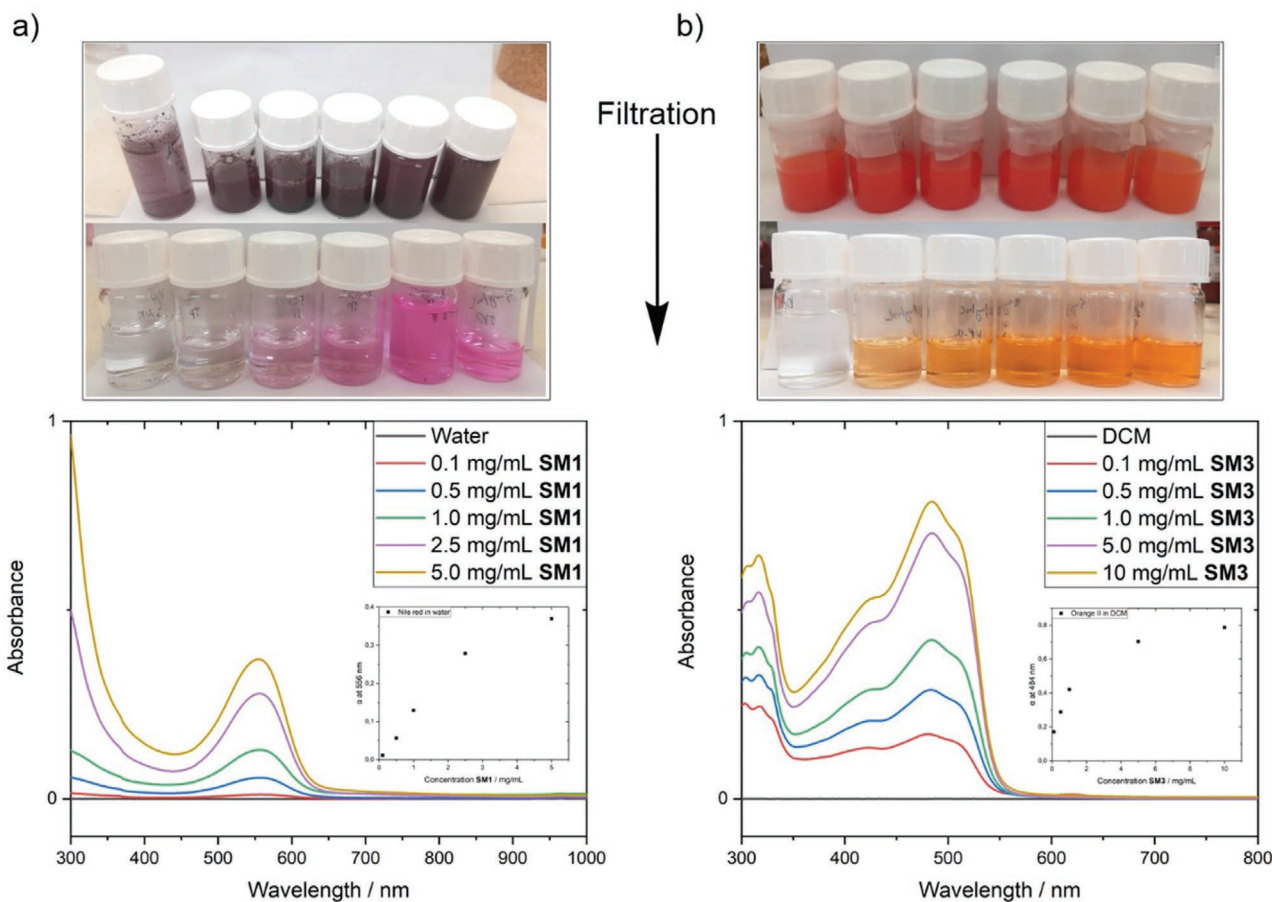

Figure 3. a) Solid–liquid phase transfer of Nile Red into an aqueous phase utilizing the trimer star-shaped macromolecule **SM1**. b) Solid–liquid phase transfer of Orange II into DCM utilizing the heptamer star-shaped macromolecule **SM3**. Both experiments were verified by UV–vis measurements.
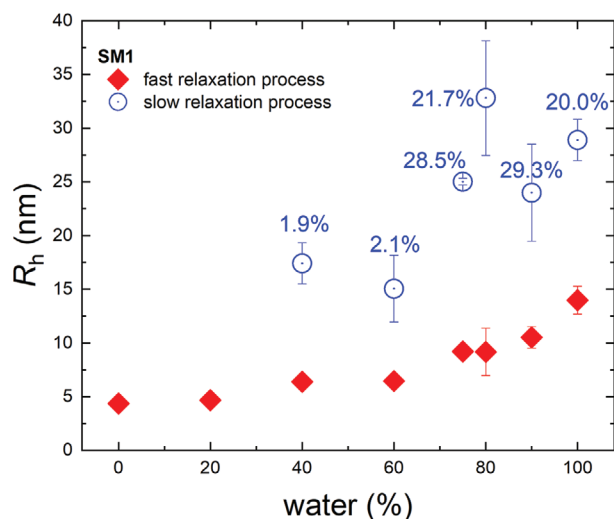
Figure 4. Hydrodynamic radii obtained from DLS measurements of **SM1** in mixtures of methanol and water: red diamonds are from the fast diffusing scatterers, blue circles from the slow ones, percentages indicate the relative concentration of the larger scatterers in the mixture.

At water content 0–20%, hydrodynamic radii (R_h) of 4.3–4.7 nm were measured, indicating the presence of unimers in methanol. With increasing water content, higher R_h values were observed with a more dramatic increase above 80% water content. This indicates that in the high water content regime, multiple entities of SM1 self-assemble, thus justifying the larger sizes observed (9.1–14.0 nm). It is also noted that at a water content above 40%, a prominent slower relaxation process corresponding to larger scatterers was observed ($R_h \approx$ 17.4–32.8 nm) with the relative concentration also increasing with water content (<3% relative concentration up to 60% water content, ≈20% at 100% water content). This was attributed to aggregation of SM1 molecules into larger structures.

It is noted that DLS measurements in DCM were not possible due to the low measured scattering intensity, which is probably a result of refractive index matching of macromolecule and solvent.

In conclusion, three amphiphilic star-shaped macromolecules of different, yet molecularly perfectly defined sizes were prepared utilizing the arm-first approach. The respective arms were synthesized applying an iterative synthesis cycle, postfunctionalized with monodisperse octaethylene glycol and subsequently coupled to a core scaffold. After careful analysis by NMR spectroscopy, ESI-MS, and SEC, their ability to carry water-insoluble hosts into an aqueous phase and water-soluble hosts into an organic phase were demonstrated by UV–vis spectroscopy, indicating their potential application in phase-transfer catalysis or drug delivery.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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