Multi-Arm Star Shaped Glycopolymers with Precisely Controlled Core Size and Arm Length

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Abstract: Star shaped glycopolymers provide very high binding activities towards lectins. However, straightforward synthesis method for the preparation of multi-arm glycopolymers in a one-pot approach has been challenging. Herein, we report a rapid synthesis of well-defined multi-arm glycopolymers via Cu(0)-mediated reversible deactivation radical polymerisation in aqueous media. *D*-Mannose acrylamide has been homo- and copolymerized with NIPAM to provide linear arms and then core crosslinked with a bisacrylamide monomer. Thus, the arm length and core size of multi-arm glycopolymers were tuned. Moreover, the stability of multi-arm glycopolymers was investigated and degradation reactions under acidic or basic conditions were observed. The binding activities of the obtained multi-arm glycopolymers with mannose-specific human lectins, DC-SIGN and MBL, were investigated via surface plasmon resonance spectroscopy. Finally, the encapsulation ability of multi-arm glycopolymers was examined using DHA and Saquinavir below and above the LCST of P(NIPAM).

Introduction

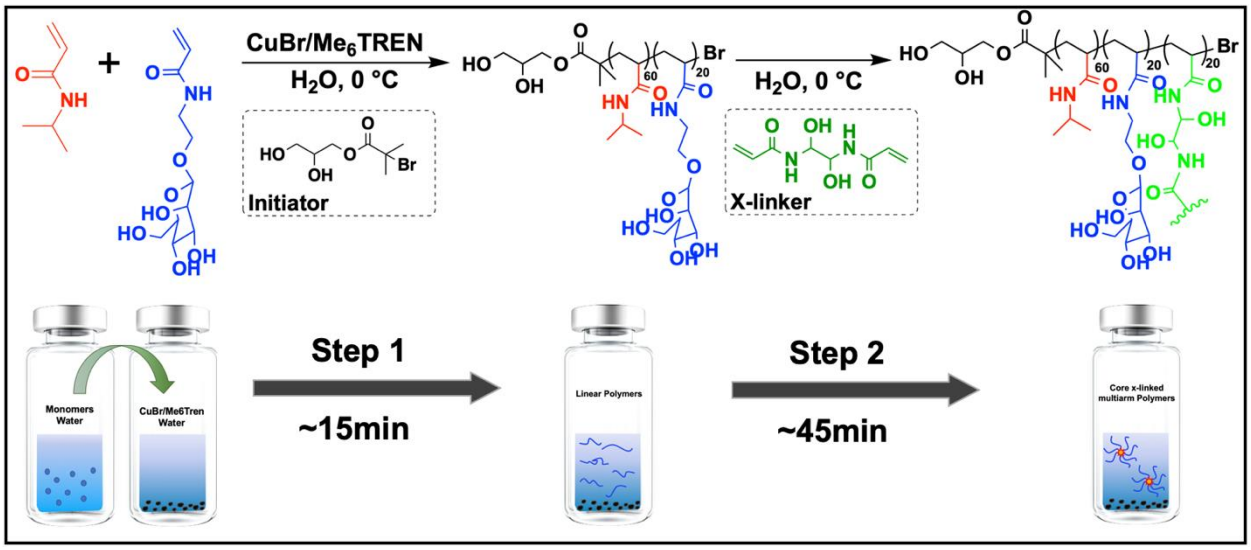
In nature, carbohydrates interact with lectins causing a series of biological responses which are vital to our existence. Lectins are a class of proteins that binds sugars specifically and are found on the cell surfaces in all living organisms. [1–4] However, plant and human lectins are believed to play different roles. For example, whilst plant lectins are thought to be part of defence mechanisms against parasites and predators, the interactions between carbohydrates and human lectins are responsible for a wider range of biological and recognition processes.[4,5] These include fertilization, immune response, cancer growth and the attachment of viruses and bacteria.[6] Although simple sugars bind to lectins specifically, their interactions are generally weak. Nevertheless, the use of larger sugar containing molecules, such as glycopolymers, has been found to improve the efficacy of binding due to what is known as the glyco-cluster effect.[7] Therefore, glycopolymers of different types and their interactions with lectins are now being widely explored for biological applications such as drug and gene delivery.[8–12]

Branched glycopolymers, in particular, have shown successful drug encapsulation as well as stronger interactions with lectins.[13,14] For this reason, the synthesis of various brush and star-shaped glycopolymers has been reported in the literature.[15–19] For example, Stenzel and co-workers have prepared star shaped glycopolymers based on 2- acryloylethyl-2′,3′,4′,6′-tetra-O-acetyl-α-*D*-mannopyranoside (MEA) and 2-hydroxyethyl acrylate (HEA) using RAFT polymerisation and a core-first approach. Stars with different tether lengths were synthesised and their binding with ConA was studied using turbidity assays. They found that the stars with medium tether lengths showed better binding than those that had long or short tethers. Their results demonstrated that both the shape and the length of glycopolymers can have a significant influence on lectin binding.[20] Furthermore, Chen *et al.* prepared star glycopolymers using the core-first

approach. However, their polymers were prepared from a tetrafunctional initiator and acryloyl-*D*-(+)-glucosamine via Cu(0) mediated reversible deactivation radical polymerization (Cu(0)-RDRP) in water. Turbidity measurements of the stars and other linear glycopolymers mixed with ConA were carried out and it was found that the stars bound more strongly than the linear counterparts.[19] Whilst this method allows the preparation of well-defined stars, the size of the core and the number of the propagating arms remain limited. On the other hand, the synthesis of star polymers via the arm-first approach presents fewer limitations on the core size and on the arm lengths, even though their analysis can be difficult to interpret. In the arm-first approach, linear polymers are prepared and then crosslinked using a difunctional vinyl monomer.[21] An example is that of Stenzel *et al*., who used RAFT polymerization to crosslink polymers based on a glucose methacrylamide monomer and styrene. The chosen crosslinker possessed a disulfide bond which was shown to undergo reduction in the presence of a thiol compound making it more suitable for biological applications.[22] Previously, the synthesis of star-shaped polymers *via* an arm-first approach, utilizing Cu(0)-RDRP in water and subsequent bisacrylamide induced crosslinking was reported. [23] This method allowed the synthesis of multi-arm star-shaped polymers with different core sizes from different acrylamides but not glycopolymer structures. The relatively fast polymerization rate achieved for Cu(0)-RDRP in water was harnessed for the preparation of both linear and branched polymers by Haddleton *et al.* as well as other research groups.[24–29]

In this study, we report the synthesis of well-defined multi-arm star-shaped glycopolymers and their degradation behaviour when subjected to pH changes in their environment. The star-shaped macromolecules were rapidly synthesized within 60 minutes via Cu(0)-RDRP in aqueous media (**Scheme 1**) and their recognition and binding kinetics with two different human lectins that are Dendritic Cell-Specific Intercellular Adhesion Molecule-3-Grabbing Nonintegrin (DC-SIGN)

and Mannose-Binding Lectin (MBL) were investigated. Furthermore, their thermo-responsiveness as well as their ability to encapsulate hydrophobic molecules are discussed.



Scheme 1: Synthesis of multi-arm star-shaped glycopolymers via Cu(0)-RDRP in water. **Step 1:** monomer addition to an aqueous solution of the disproportionated catalyst/ligand complex to yield linear polymers. **Step 2**: Addition of a bisacrylamide crosslinker to the reaction mixture, leading to the formation of core-crosslinked multi-arm star polymers.

Experimental Section

Materials. 1,2-Dihydroxypropane-3-oxy-(2-bromo-2-methylpropionyl) (initiator), Me_6TREN (ligand) and the mannose-derived acrylamide (ManAc) were synthesised following literature procedures and the details are also provided in the supporting information (**Fig S1-S4**).[30,31] *N*-Isopropylacrylamide (NIPAM, 98%), *N*-hydroxylethyl acrylamide (97%, contains 1000 ppm MEHQ as inhibitor), D-(+)-mannose (≥99%), sodium methoxide (CH_3ONa, 25 wt % in methanol), boron trifluoride diethyl etherate (≥46.5% BF_3 basis), acetic anhydride (≥99.5%), (R)-(−)-2,2-Dimethyl-1,3-dioxolane-4-methanol (98%), α-Bromoisobutyryl bromide (98%) and glacial acetic

acid were purchased from Sigma-Aldrich. CuBr was purified with glacial acetic acid, washed with ethanol, dried, and stored under inert gas prior to use.

Instruments and Analysis.

Proton nuclear magnetic resonance (^{1}H NMR) was measured on a Bruker DPX-300. The NMR samples were prepared using a 5 mg/mL concentration in $(CD_3)_2SO$. For DMSO-*d*6, the resonance signal of residual DMSO at 2.50 ppm (^{1}H) served as reference peaks for chemical shifts. ***Gel Permeation Chromatography (GPC)*** measurements of polymers were carried out on an Agilent 1260 Infinity II-MDS instrument with two PLgel Mixed-D columns operating in DMF with 5mM NH_4BF_4 equipped with the following detectors: refractive index (RI), viscometer, light scattering (LS) and variable wavelength detector (VWD). The instrument was calibrated with linear poly(methyl methacrylate) standards. All samples were passed through 0.2 micron nylon filters prior to GPC measurements. ***Dynamic light scattering (DLS)*** measurements were carried out on a Malvern Nano-series DLS instrument. The measurements were carried out in distilled water at 25-40 °C and were repeated three times. ***UV-vis measurements*** were performed on an Agilent Cary Series UV-vis Spectrophotometer. The polymer samples were dissolved in distilled water at a concentration of 1 mg/mL, whereas the model drug was added in excess at a concentration of 5 mg/mL. ***Surface Plasmon Resonance (SPR)*** measurements were performed on a T200 BIAcore system (GE Healthcare). The lectins DC-SIGN and MBL (5 μg/mL) were immobilised on a gold CM5 chip via amino coupling reactions with *N*-hydroxysuccinimide and *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide at a flow rate of 5 μL/min for 5 minutes at ambient temperature. The measurements were carried out in a buffer solution (10 mM HEPES, 150 mM NaCl, 5 mM $CaCl_2$) of which pH= 7.4. The polymers were dissolved in the buffer and their concentration varied between 10 μM and 0.63 μM. The regeneration was performed with the

injection of a solution of g 10 mM HEPES pH 7.4, 150 mM NaCl, 10 mM EDTA, and 0.01% Tween20. The kinetic data of the bindings were determined from BIAevalulation 3.1 software.

General Synthetic Method of the Linear Statistical Copolymers. A vial containing CuBr (3.46 mg, 0.02 mmol) was degassed using N_2 at 0 °C for 20 min before transferring an aqueous solution of Me_6TREN (2.77 mg, 0.01 mmol) to the vial. The catalyst was allowed to disproportionate for 30 min in an ice-bath whilst the solution was kept free of oxygen. Thereafter, a degassed solution of NIPAM (200 mg, 1.8 mmol), ManAc (163 mg, 0.6 mmol) and the initiator (7.32 mg, 0.03 mmol) was added to start the reaction. The reactions were performed at 0°C and full conversion of the polymeric arms was obtained within 10-60 min. Finally, the reaction mixture was filtered over a short column of neutral alumina to remove residual copper species and the polymers were purified by dialysis against water for 2-3 days. The purified polymers were obtained as white powders after lyophilization.

Synthesis Procedure of Linear Block Copolymers. Block copolymers were prepared by following the method above, using the same amounts of Me_6TREN (2.77 mg, 0.01 mmol), initiator (7.32 mg, 0.03 mmol) and CuBr (3.46 mg, 0.02 mmol). Upon complete disproportionation of CuBr with Me_6TREN at 0 °C, a degassed solution containing NIPAM (200 mg, 1.8 mmol) and the initiator was added to the reaction vial. Meanwhile, a solution of ManAc (163 mg, 0.6 mmol) was degassed in a separate vial. After 15 min, PNIPAM was obtained with full conversion and ManAc was added to the reaction mixture. Therefore, block polymers were achieved after 45 min. Finally, the CuBr was filtered off and the products were dialysed against water. In addition, this procedure was followed to prepare polymers in which the first block consisted of ManAc and the second block consisted of NIPAM.

Synthesis Procedure of Multi-Arm Star Copolymers. Multi-arm star-shaped copolymer synthesis was achieved by following the general procedure for the synthesis linear statistical and block copolymers and the subsequent addition of *N,N'*-(1,2-dihydroxyethylene)bisacrylamide (DHEBA) crosslinking agent (120 mg, 0.6 mmol) to the polymerization mixture. After full monomer conversion was observed, a degassed solution of the bisacrylamide DHEBA was added to the vial containing the linear polymers. The consumption of the crosslinker was monitored by ^{1}H NMR and was typically observed after 30-45 min. Finally, the crosslinked polymers were purified by dialysis against water and obtained after lyophilization.

Degradation Assays of Multi-Arm Star Copolymers. Multi-arm star-shaped copolymers (10 mg/mL) were dissolved in either acidic ($3.3x10^{-5}$ M, $5.6x10^{-6}$ M, $1x10^{-6}$ M HCl) or basic ($1x10x^{-6}$ M NaOH) aqueous solutions at 37 °C (body temperature), prepared from the addition of HCl and NaOH in distilled water. Generally, a sample was taken after 4h from the start of the reaction which showed successful degradation. The hydrolysis of the acrylamides was monitor via GPC in which a shift of the star polymer peak towards lower molecular weights was observed.

Encapsulation of Hydrophobic Compounds. Multi-arm star-shaped copolymers (1 mg/mL) were dissolved in distilled water (3 mL). Thereafter, an excess of 1, 4-dihydroxyanthraquinone (DHA) (15 mg) or Saquinavir (15 mg) was added to the polymer solutions, which were allowed to stir overnight at room temperature. Alternately, some of the solutions were allowed to stir at 37 °C in a vacuum for the same period of time. Finally, the insoluble molecule (DHA or Saquinavir) was filtered off the solutions and UV-vis measurements were carried out on the filtrate to determine the encapsulation capability of the polymers for the hydrophobic, otherwise insoluble compounds.

Results and Discussion

Synthesis of the Multi-Arm Star-Shaped Copolymers.

The preparation of crosslinked star-shaped glycopolymers by the arm-first approach has been studied in detail. Utilising Cu(0)-RDRP conditions allowed a rapid synthesis of linear glycopolymers, which were achieved within 10-40 min. The polymerisations were allowed to reach monomer conversions of 95-98 % before proceeding with Step 2, which is crosslinking with a bisacrylamide. The general conditions used for the synthesis of linear polymers were selected as: [Monomer]/[Initiator]/[CuBr]/[Me_6TREN] = [60]/[1]/[0.8]/[0.4]. By varying the monomer ratio and the copolymerization order of NIPAM and ManAc, a small library of homopolymers and statistical or block copolymers was successfully synthesized (**Table 1**). **L1, L2, L3** and **L4** are the linear homo, statistical and block copolymers and they are used in the preparation of **S1, S2, S3** and **S4** which are the homo, statistical, and block multi-arm star-shaped copolymers, respectively. ^{1}H NMR of **L2, L3** and **L4** are provided in the ESI (**Figure S5-S7**). **S3** has a reverse order in the block copolymer in comparison to **S4**, and the effect of block order was investigated in terms of lectin recognition. In addition, **S2** and **S5** have the same arm composition and length but different core size, obtained by varying the amount of crosslinker.

Table 1: List of polymer compositions obtained in this study with varying arm length and core size. Number average molar mass ($M_{n,GPC}$) and dispersity values ($Đ$) were obtained by GPC and the diameter (D) of the particles measured by DLS.

Code	Type	Composition	$M_{n,GPC}$ (kDa)	$Đ$	D (nm)
L1	Linear Homo	$P(NIPAM)_{60}$	16.8	1.07	4
L2	Linear Statistical	$P(NIPAM)_{60}$-*r*-$(ManAc)_{20}$	18.1	1.06	5
L3	Linear Block	$P(NIPAM)_{60}$-*b*-$(ManAc)_{20}$	21.7	1.09	6
L4	Linear Block	$P(ManAc)_{20}$-b-$(NIPAM)_{60}$	17.3	1.12	5
S1	Star Homo	$P(NIPAM)_{60}$-*b*-$(DHEBA)_{20}$	86.7	1.39	17
S2	Star Statistical	$P(NIPAM)_{60}$-*r*-$(ManAc_{20})$-*b*-$(DHEBA)_{20}$	160.0	1.47	16
S3	Star Block	$P(ManAc)_{20}$-*b*-$(NIPAM)_{60}$-*b*-$(DHEBA)_{20}$	252.3	1.32	24
S4	Star Block	$P(NIPAM)_{60}$-*b*-$(ManAc_{20})$-*b*-$(DHEBA)_{20}$	130.4	1.24	14
S5	Star Statistical	$P(NIPAM)_{60}$-*r*-$(ManAc_{20})$-*b*-$(DHEBA)_{30}$	178.2	1.28	68

NIPAM: *N*-isopropyl acrylamide; ManAC: mannose acrylamide; DHEBA: *N*,*N*'-(1,2-dihydroxyethylene)bisacrylamide

As it can be seen from the Table 1, the molar mass values of the linear polymers show a significant increase of the Mn values when multi-arm star-shaped polymers are formed. Moreover, the increase of the particle size in the DLS also indicated successful formation of multi-arm star-shaped polymers. After the addition of the bisacrylamide crosslinker, the reaction mixture was left in the ice bath for 30-40 min, until ^{1}H NMR analysis showed full consumption of DHEBA, indicated by the disappearance of the vinyl peaks at 6.3-5.5 ppm region (**Figure 1**). Furthermore, it should be emphasized that, even though our previous studies showed NIPAM has a faster polymerization rate than ManAc, the different behaviours of our polymers observed from SPR,

UV-vis and GPC measurements show that NIPAM and ManAc adapt a casual allocation within the statistical copolymers when prepared in a non-block manner.[32]

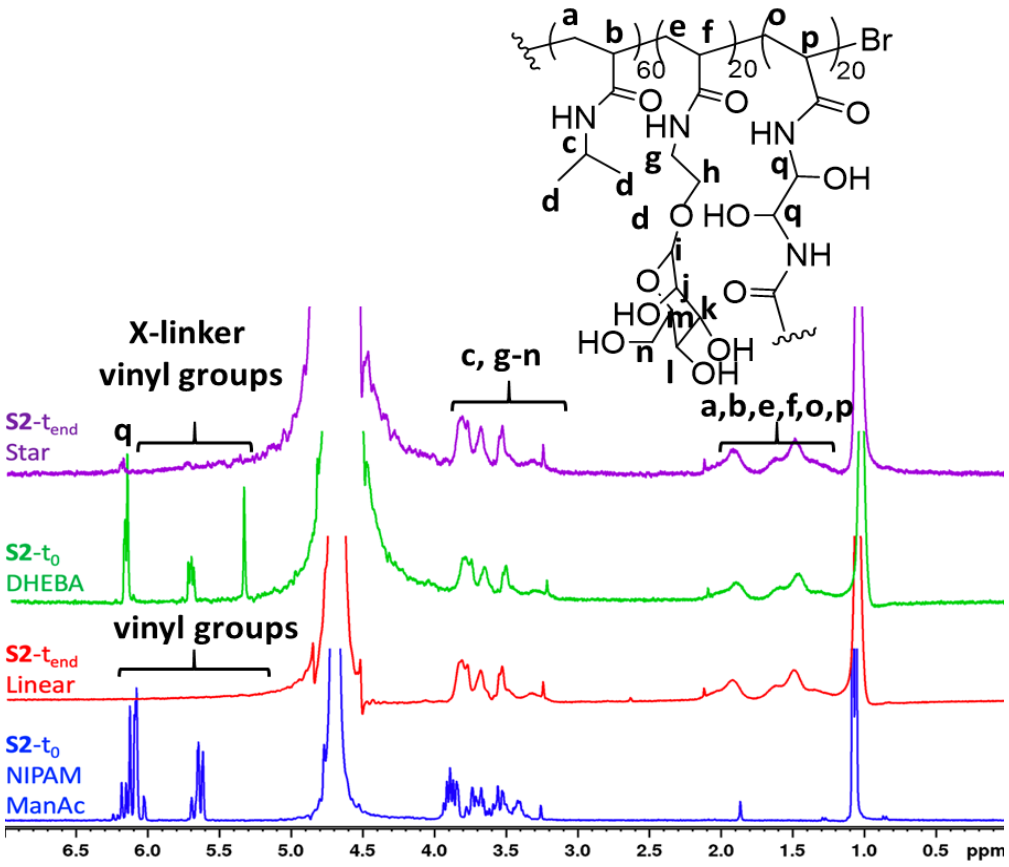


Figure 1: ^{1}H NMR traces of different synthesis steps of **S2** star statistical glycopolymer P(NIPAM)$_{60}$-*r*-(ManAc$_{20}$)-*b*-(DHEBA)$_{20}$: (**A**) The vinyl peaks of the monomers (NIPAM and ManAc) are visible in the spectrum before the polymerization (5.5-6.3 ppm, blue spectrum); (**B**) the disappearance of vinyl resonances upon quantitative monomer consumption (red spectrum); (**C**) double bonds of the crosslinker (DHEBA) are visible in the NMR upon its addition to the reaction vial (green trace); (**D**) consumption of crosslinker (DHEBA) indicated by the disappearance of vinyl resonances in the spectrum at the end of the reaction (purple trace).

Thus, multi-arm star-shaped copolymers of narrow dispersity (Đ = 1.2-1.4) were obtained with only minor linear polymer impurities (10-25% left over arm), which was calculated by deconvolution of the GPC traces. The formation of the multi-arm star-shaped polymers was investigated by GPC, showing a clear shift to higher molecular weights upon the addition of crosslinker, while retaining low polymer dispersity values (**Figure 2**). The presence of leftover linear polymer chains in the GPC trace indicated an incomplete coupling of linear precursors into the star polymer core. Individual GPC traces of all polymers are provided in **Figure S13**. Dialysis against water was carried out in order to remove the small amount of low molecular weight species. However, after dialyzing the solution for 3 days, linear species were still detected in the GPC traces. Model star compounds prepared from the crosslinking of P(NIPAM$_{80}$) were used to determine the arm number of the star glycopolymers (**Figure S11**). Given that the model compounds were prepared using the same conditions and obtained with similar conversions, we assume that the average number of arms of the star polymers is between 45 and 50. In addition, our studies have shown that increasing amounts of crosslinker (DHEBA) generally results in an increase in arm number as well as branching (**Figure S12**). Thus, we believe that S5, prepared with 30 equiv. of DHEBA, has a higher number of arms than the other star polymers.

The degree of polymerization (DP) for the synthesized star polymer library was kept identical while varying the monomer composition. The variation in the obtained number average molecular weights (M_n) for the star polymers was therefore thought to result from the changing hydrodynamic volumes of the different polymer species (**Table 1**).

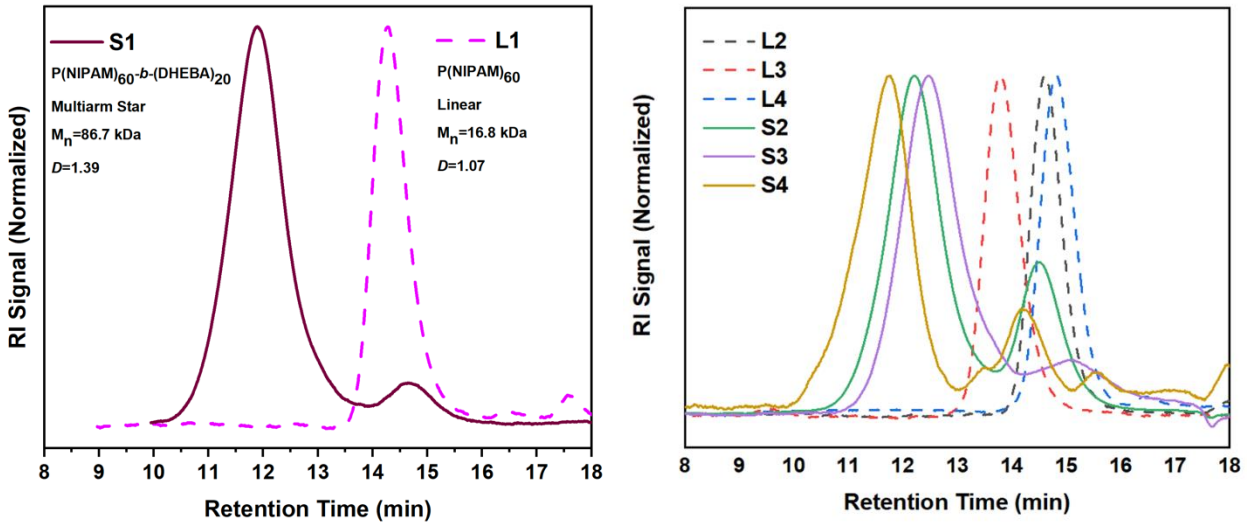


Figure 2: GPC traces of linear (**L1**) and multi-arm star shaped P(NIPAM) (**S1**) (*left*) and of linear (**L2, L3** and **L4**) and multi-arm star glycopolymers (**S2**, **S3**, and **S4)** (*right*). The dashed lines correspond to the linear polymers whereas the solid lines correspond to the multi-arm star-shaped copolymers.

Thermoresponsive Solubility Behaviour and Degradation of Star Polymers in Aqueous Media.

DLS measurements of the linear (**L1-L3**) and the multi-arm star-shaped copolymers (**S1-S5**) were carried out in distilled water (1 mg/mL) at 25 °C (**Figure 3A-3C**). DLS traces revealed a significant difference in size between the linear polymers and the stars, indicating the successful conversion of the polymers into multi-arm star-shaped copolymers. On average, the star particles seemed to be three to four times larger (14.3-24.3 nm) than their linear counterparts (4-6 nm). Moreover, DLS measurements of multi-arm star-shaped copolymers with different core sizes (**S2** and **S5**) were carried out (**Figure 3D**). The measurements showed that by varying the amount of crosslinker from 20 eq. (**S2**) to 30 eq. (**S5**) multi-arm star-shaped copolymers with a larger core were created.

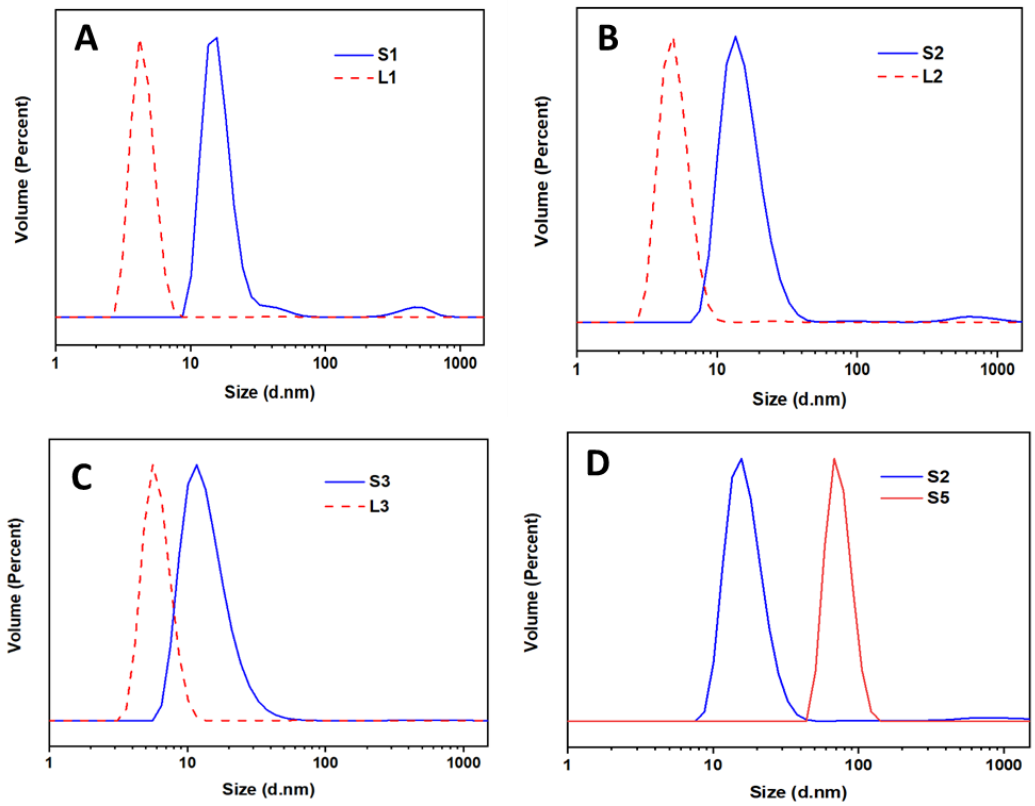
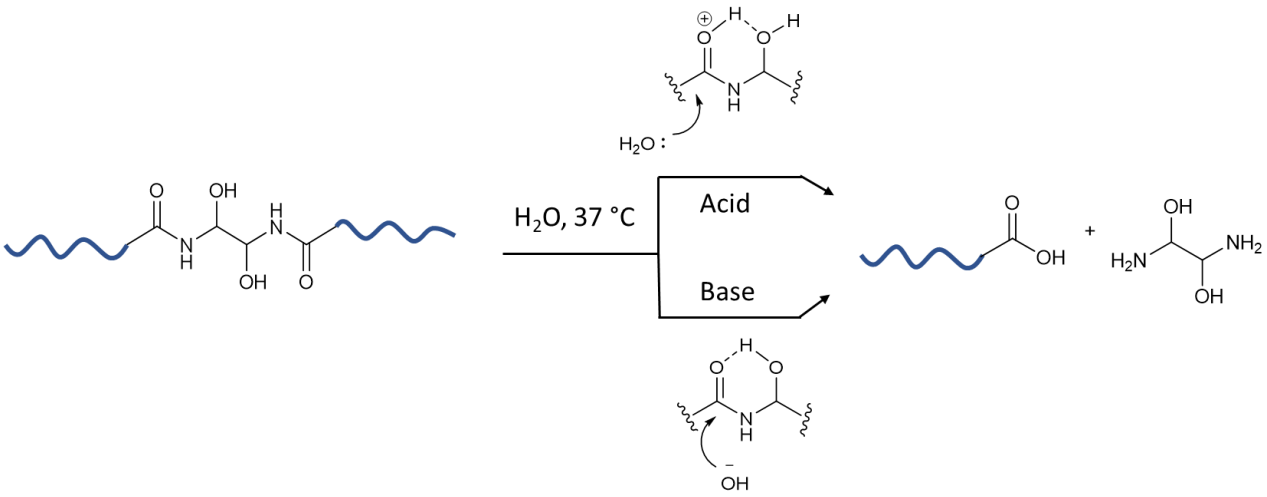


Figure 3: DLS traces showing the difference in size between the linear and the crosslinked polymers (A-C) and between star polymers with different core sizes (D).

The thermoresponsive behaviour of P(NIPAM) has been widely known and studied in the literature. The cloud point of P(NIPAM) is usually around 32 °C. Therefore, we performed DLS measurements of **S1** at 25, 30 and 40 °C to examine the expected thermoresponsiveness (**Figure S14**). It is clearly seen that the diameter of **S1** is around 17 nm below the cloud point of P(NIPAM) and around 190 nm above its cloud point.

As mentioned previously, one of the advantages of multi-arm star-shaped copolymers is that they can encapsulate and protect small medicinal drugs from rapid excretion from the body. Moreover, their characteristics can be tuned in a way that allows the delivery of these drugs to a specific site, thus reducing potential side effects caused by non-targeted delivery. However, even

though the use of branched polymers is advantageous, the body struggles to eliminate large polymers.[33] Therefore, polymeric vehicles should ideally degrade when they reach their targets in order to release the passenger compounds and be excreted from the body. In this study, multi-arm star-shaped copolymers were dissolved in acidic environments of different pH values between 4-6 as well as a basic environment of which pH= 8 and the degradation of their core was investigated (**Scheme 2**). Amides are known to be extraordinarily stable compounds and normally require harsh conditions to undergo hydrolysis. However, hydrolysis of amides having neighbouring hydroxyl groups has previously been reported under very mild conditions.[34] This is only possible because the hydroxyl groups allow for the formation of intermediates which facilitate the reaction.



Scheme 2: Acid and base catalyzed mechanism for the degradation of the star polymers' core.

Thus, the polymer solutions were stirred at body temperature (37 °C) in acidic and basic environments and their degradation was monitored by GPC. It was observed that the core of the polymers underwent complete degradation within 4 h in these conditions and that the GPC traces of the resulting products overlapped with those of linear precursors, suggesting complete

degradation of the crosslinked core (**Figure 4**). On the contrary, the linear precursors, also consisting of amides, were found to remain intact under such mild conditions even after 24 h.

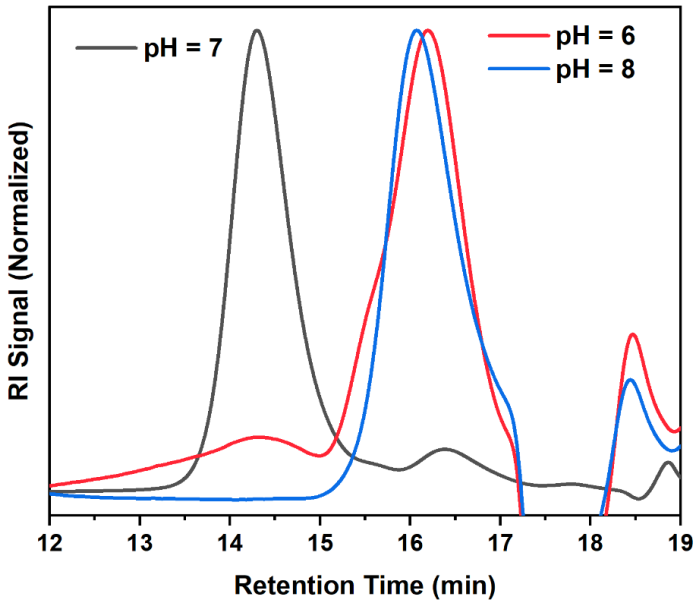


Figure 4: GPC traces of star polymer (**S2**) degradation in acidic and basic conditions.

Surface Plasmon Resonance Analysis using DC-SIGN, MBL and Multi-Arm Star-Shaped Glycopolymers.

The interactions between lectins and carbohydrates are exploited in a large range of biomedical applications leading to improved treatments of popular diseases including influenza, HIV and cancer.[30,35–38] SPR is an effective technique for measuring these interactions using minimal amounts of label-free samples and lectins. For this study, SPR was employed to measure the binding activity between the star glycopolymers (5 uM) and two human-derived lectins, which are DC-SIGN and MBL. Both DC-SIGN and MBL are C-type mannose-specific lectins and are responsible for important biological mechanisms. More specifically, DC-SIGN is found on the surface of dendritic cells (DC) and binds to microorganisms including viruses such as HIV-1 and hepatitis C.[37,39] MBL is one of the main lectins of the immune system and has been shown to bind

to numerous pathogens in the body such as fungi, bacteria and viruses.[40,41] Consequently, preparing glycopolymers that can bind to these lectins and inhibit their interactions with such pathogens provides enormous potential in both the treatment, prevention and study of widely spread diseases. The architecture, sugar density, and the molecular weight of glycopolymers have all been demonstrated to influence the efficacy of the interactions of carbohydrates towards lectins. Therefore, the two lectins MBL and DC-SIGN were immobilised on a gold chip and their interactions with the star polymers were investigated (**Figure 5**).

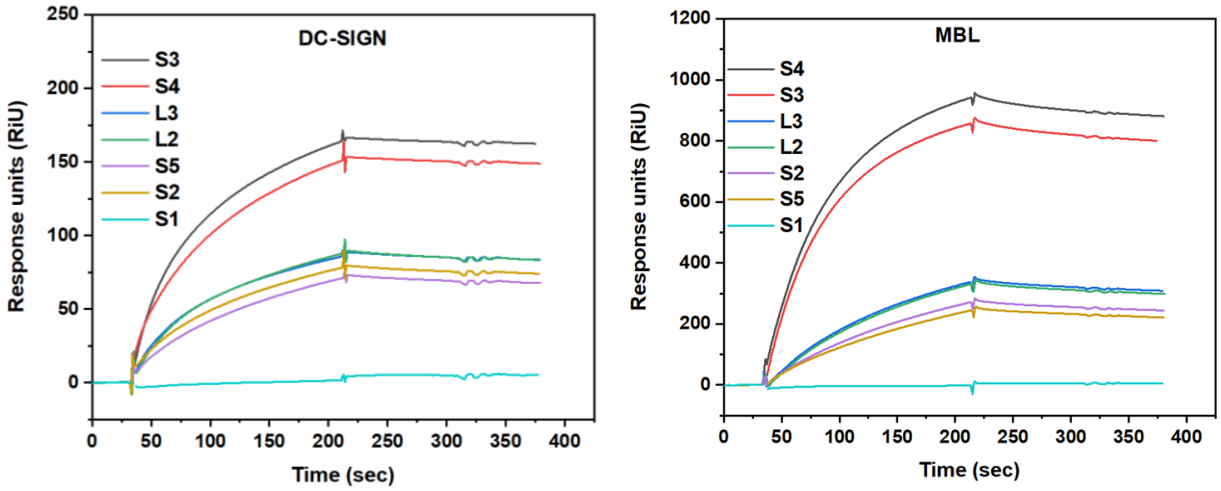


Figure 5: SPR measurements of glycopolymers with DC-SIGN (*left*) and MBL (*right*). The traces shown are all for glycopolymers at 5 uM.

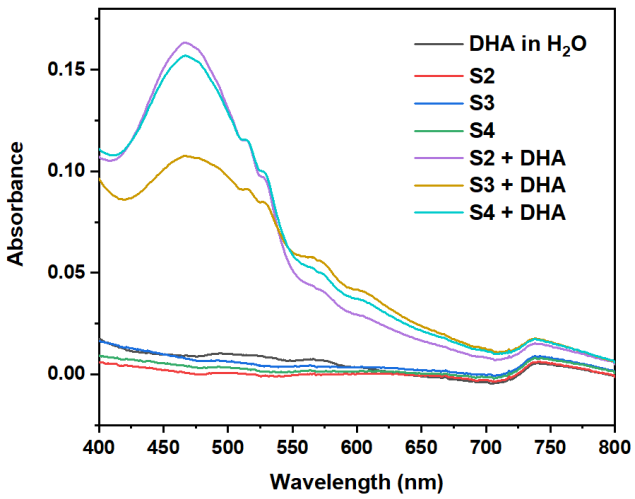
In general, the trends obtained for the two lectins were very similar. As expected, the majority of the glycopolymers showed binding affinity towards both MBL and DC-SIGN. The multi-arm star-shaped polymer P(NIPAM)$_{60}$-*b*-(DHEBA)$_{20}$ (**S1**) was used as the negative control and did not show any binding at the measured concentration (5 uM). The star polymer in which the mannose blocks were positioned in the outer corona (**S3**) showed very strong interactions with the two lectins. This result was expected due to the greater availability of the sugar units in **S3** to bind with the lectins. Surprisingly, the star polymer with the mannose blocks in the core and the

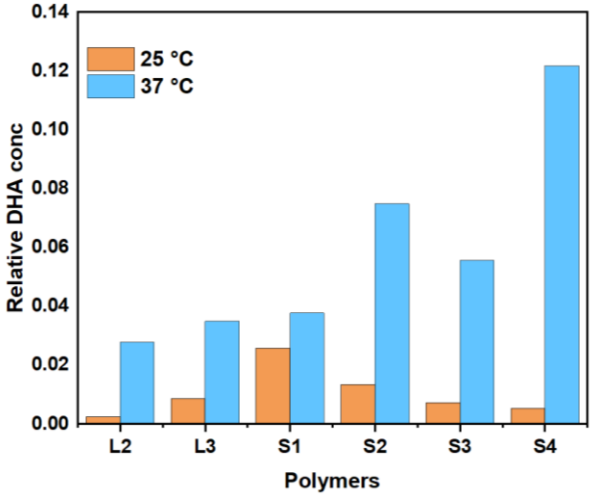
NIPAM blocks in the corona (**S4**) did not seem to show a decrease in the binding with both lectins in comparison, even though the mannose blocks are positioned further away. Thus, it is suggested that even these cross-linked polymer structures possess high level of flexibility. Moreover, **S3** and **S4**, the star polymers prepared from the crosslinking of block arms, bound to DC-SIGN and MBL significantly better than **S2** and **S5** which had statistical allocations of the mannose and NIPAM units. In fact, **S2** and **S5** did not show greater affinity than their linear counterparts **L2** and **L3**. However, these results are compliant with the literature as it has been previously reported that some lectins tend to prefer glycopolymers that have sugar units near one another over statistical allocation.[42] Furthermore, the preparation of a larger particle **S5** did not improve the biding with the lectins compared to **S2**, indicating that the core size and the greater branching level did not have an influence on the binding affinity. In general, the glycopolymers all showed high affinities towards DC-SIGN and MBL determined from the calculated kinetic values which showed fast binding (k_a) as well as slow dissociation (k_d) in the interactions with the two lectins (**Table S1**). Nevertheless, it seems that star polymers bind more strongly than their linear counterparts when prepared from the crosslinking of block polymers.

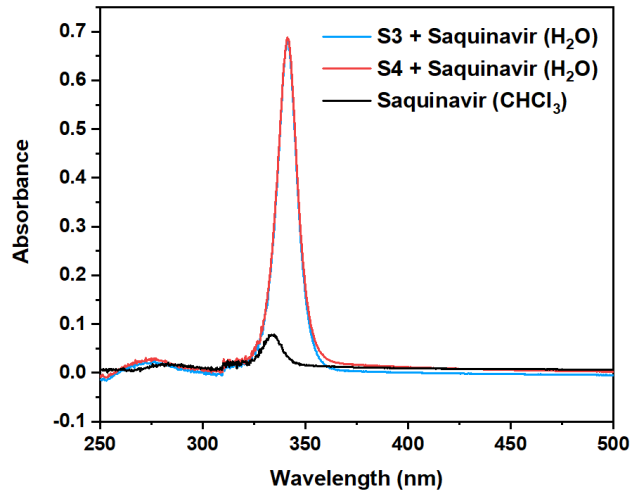
Drug Encapsulation

Polymers are being intensively explored for drug delivery applications. For example, polymeric carriers can encapsulate small hydrophobic or hydrophilic drugs and improve their bioavailability whilst reducing side effects of pharmaceuticals.[43] Furthermore, polymers can increase the circulation times of small drugs in the body by preventing rapid excretion. In particularly, glycopolymers act as promising drug carriers due to their ability to bind specific proteins (such as cell surface lectins), potentially allowing targeted delivery of active compounds.[44] UV-vis

measurements were carried out to test the efficacy of the star polymers to encapsulate a hydrophobic molecule (DHA). DHA was stirred in distilled water (5 mg/mL) with the star polymers (1 mg/mL) overnight. Thereafter, the insoluble DHA was removed and UV-vis measurements of the filtered solutions were carried out at 25 °C. Overall, efficient encapsulation was observed for both linear and star polymers at ambient temperature (Figure 7a-b), with **S1** showing the highest encapsulation activity because of its greater hydrophobic content. Furthermore, the difference in the encapsulation activity between the star-shaped (**S3**, **S3** and **S5**) and the linear (**L2** and **L3**) glycopolymers towards DHA was observed to be minimal at 25 °C.







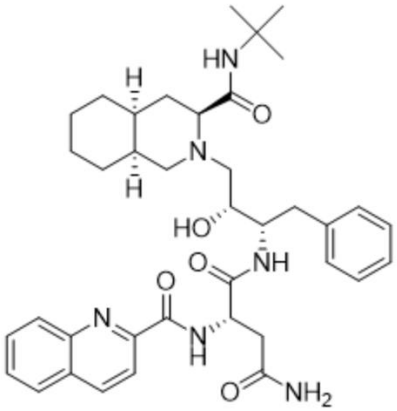


Figure 7: UV-vis spectra of the multi-arm star-shaped copolymers showing the encapsulation of DHA at 25 °C. (*top left*), relative DHA encapsulation using linear and star shaped polymers at 25 °C and 37 °C (*top right*), UV-vis measurements of Saquinavir in chloroform ($CHCl_3$) and Saquinavir and **S3** and **S4** in water (*bottom left*), the chemical structure of Saquinavir (*bottom right*).

Nevertheless, when the DHA/polymer solutions were subsequently stirred at 37 °C and UV-vis measurements were carried out, the obtained results were significantly different. Poly(NIPAM) is thermoresponsive and exhibits a LCST at around 32 °C.[45] Thus, a change in the behaviour of the polymers was expected upon temperature increase. In general, all polymers showed greater encapsulation at 37 °C. However, the control star polymer **S1,** which had the highest encapsulation value at ambient temperature, only showed a slight increase at 37 °C. Similar results were observed for **L2** and **L3** of which encapsulation activity did not increase drastically. Conversely, we observed that the absorption values obtained with the stars containing mannose were much greater after the temperature increase. This is because glycopolymers retain an amphiphilic character above the LCST of NIPAM, which is believed to be the cause of aggregations that can encapsulate greater amounts of DHA, whilst remaining soluble in solution. Conversely, the **S1** does not have an amphiphilic character and so it becomes less soluble at 37 °C. Hence, its ability to encapsulate molecules at higher temperature is believed to be limited.

Furthermore, **S2** and **S4** showed the greatest absorption values, probably due to the greater availability of the NIPAM units in these polymers to interact with DHA. This was particularly observed for **S4**, prepared from the crosslinking of block polymers which allowed all the NIPAM segments to be in the outer corona. On the contrary, the NIPAM segments in **S3** are positioned closer to the core and therefore, they are more hindered.

Finally, star polymers **S3** and **S4** (1 mg/ml) were mixed with the anti-HIV drug, Saquinavir (15 mg/mL), and stirred in water overnight. After 24 h, the insoluble particles were filtered off and UV-vis measurements of the polymer/drug mixture remaining in solution were carried out (**Figure 7c**). The UV-vis traces of the mixture showed a maximum at 350 nm, which corresponds to the absorption wavelength of Saquinavir in chloroform. Unfortunately, Saquinavir was only slightly soluble in chloroform or THF thus, the absorbance observed was low. However, considerable encapsulation of the drug by **S3** and **S4** is suggested by the high absorbance in water, despite the high hydrophobicity of Saquinavir.

Conclusion

This study demonstrated that the synthesis of well-defined star-shaped glycopolymers is easily achieved *via* SET-LRP in water. The star polymers ($Đ < 1.5$) were prepared using an arm-first approach with subsequent addition of bisacrylamide crosslinking agent in under 2 h. The polymers underwent degradation in an acidic and basic environment, which is promising for biomedical applications. SPR measurements were carried out, which showed that the star polymers generally have strong affinity towards the human lectins DC-SIGN and MBL, especially when the sugar units are near one another. Furthermore, the SPR measurements showed that star polymers possess higher affinities than their linear counterparts, when the sugar units are positioned together. On the contrary, star polymers with statistical allocations did not show higher affinities than the linear glycopolymers. It was demonstrated, that the star polymers are capable of efficiently encapsulating small hydrophobic compounds such as DHA and the anti-HIV drug Saquinavir, which allows their potential use as drug carriers. Finally, we observed that the absorption of DHA by star polymers increased dramatically at 37 °C, due to the thermoresponsive character of the incorporated NIPAM

segments into the polymer chains. Overall, the data suggest that multi-arm star polymers could be superb carriers for targeted drug delivery.

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Supporting Information

Synthetic procedures, NMR, GPC, DLS, UV-Vis, and SPR measurements are provided.

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