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Synthesis and Self-Assembly of Amphiphilic Chiral Poly(amino acid) Star Polymers

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ABSTRACT: Reversible addition−fragmentation chain transfer (RAFT) polymerization techniques were utilized to polymerize *N*-acryloyl-L-phenylalanine methyl ester (L-Phe-OMe) and tetrahydropyranyl acrylate to afford amphiphilic star polymers (with 4 and 6 arms). These copolymer stars have a chiral hydrophobic core domain and can undergo further solution self-assembly to form well-defined nanostructures, which have been characterized using DLS, TEM, and cryo-TEM analysis. The characterization and properties of these novel chiral assemblies and their potential in racemic resolution are reported.

Introduction

Branched polymers with architectures varying from stars and dendrimers to gels and hyperbranched systems have generated considerable interest over recent years. This is because, compared to linear polymers of the same molecular weight, they have considerably lower bulk and solution viscosities.[1,2] They also potentially show novel behavior in selective solvents leading to complex self-assembled morphologies. About a decade before the term "star" was used to describe branched polymers, Morton reported the synthesis of branched polystyrenes by quenching living styryllithium with silicon tetrachloride.[3] However, due to the available synthetic techniques there was always a compromise between control and functionality within the polymers. It was not until the advent of controlled radical polymerizations (CRP) that functional starlike architectures became readily accessible using considerably more facile and robust chemistries.[4−12]

Three different pathways are used to synthesize star polymers: (a) "arm first", (b) "coupling onto", and (c) "core first" approach. Using the "arm first" method, monofunctional living linear macromolecules are initially synthesized. Star formation then occurs by adding a difunctional comonomer, which provides cross-linking through propagation. In the "coupling onto" method, monofunctional living linear polymer chains are coupled onto a multifunctional coupling agent.[13,14] Using the "core first" method, the arms grow from multifunctional initiators or precursor molecules.[15−18] Unique to RAFT polymerization processes is the possibility of advancing the "core first" method using two different approaches: the Z-approach[19−23] and the R-approach,[24−27] where the chain transfer agent (CTA) is covalently bound via the Z-group and R-group to the core, respectively. It has to be mentioned that the choice of the approach has a significant impact on the outcome of the polymerization. Using the Z-approach CTA, if the number of arms attached to the core is high and the monomer is bulky, then bimolecular termination in the form of arm−arm coupling is often observed.[28,29] It is thought that this happens in most polymerizations using a Z-approach CTA, although to what extent depends on the number of arms, bulkiness of the monomer, monomer concentration, and conversion. An R-approach CTA also promotes bimolecular terminations although the products are different than in the Z group approach; what is seen predominantly are star−star coupled products where the propagating radicals residing on the end of arms attached to the core of the star have coupled. There are two advantages to using the Z-approach: first, the coupling products are not seen as early on in the polymerization as they are for the R-approach, and second, if a chain extension is to be undertaken, the linear arm−arm coupled termination products of the Z-approach are no longer active RAFT centers (unlike in the R-approach).[4]

In 1999, Chong and the CSIRO team reported the synthesis of two multifunctional dithioester initiators and their use as mediating CTAs in the synthesis of four- and six-armed polystyrene stars.[30] Since then, many reports have been made on the synthesis of multifunctional initiators and the use of these species for the polymerization of various monomers. The CAMD team in Australia has been largely instrumental in defining some of the fundamental aspects of star polymer synthesis using RAFT.[31−33] While studies into the synthesis and application of amphiphilic stars have been extensively reported by Tenhu and others,[34−43] the synthesis of star polymers (regardless of lyophilicity) containing chiral monomers has not been widely reported to date.[44] The application of CRP techniques for the synthesis of chiral polymers is of great recent interest, and of particular note is the work of Endo and Sanda in the area of poly(amino acids).[45,46] It has been shown that *N*-acryloyl amino acid monomers can be readily polymerized in a controlled manner to afford functional and responsive copolymers. Of further interest is the ability of amino acids, in particular phenylalanine microbeads, to facilitate the separation of racemates.[47] Indeed, we have recently demonstrated that a heterointeraction between the D- and L-isomers of leucine can occur within a micelle core to stabilize the resultant structure, thus indicating that chirally pure amino acid nanostructures may allow for selective entrapment of the opposite isomer.[48]

Hence, the preparations of chiral polymer stars, which are capable of undergoing self-assembly to form nanostructures, are of interest as they may find application in asymmetric organocatalysis, either directly or through providing confined chiral environments that could encapsulate achiral catalysts. They also

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have potential application in separation science as they may potentially behave in a similar fashion to cross-linked beads that are used to make stationary phases for column chromatography. In this work we explore the preparation of two amphiphilic star copolymers with a chiral core domain, their self-assembly, and potential in racemic separations.

Experimental Details

AIBN was recrystallized twice from methanol and stored in the dark at 4 °C. L-Phe-OMe, THPA, pentaerythritoltetrakis(3-(*S*-benzyltrithiocarbonyl)propionate), CTA (**1**), and pentaerythritolhexakis(3-(*S*-benzyltrithiocarbonyl)propionate), CTA (**2**), were synthesized using modified literature preparations.[19,49−52] All other reagents were purchased from Sigma-Aldrich and were used without further purification.

^{1}H and ^{13}C NMR spectra were obtained with a Bruker DPX-300/DPX-400 spectrometer using $CDCl_3$ or d_6-DMSO. Chemical shifts are reported in ppm (δ) relative to $CHCl_3$ (7.26 ppm for ^{1}H and 77.2 ppm for ^{13}C) or d_6-DMSO (2.50 ppm for ^{1}H) as an internal reference. Gel permeation chromatography (GPC) data were obtained on a PL GPC 50 integrated GPC system with a PL-AS RT autosampler and a Varian 390-LC detector suite with DRI and viscosimetry. Three mixed D, 5 μm columns (one guard and two main) were used at 50 °C with a dimethylformamide (DMF) flow rate of 1.0 mL min^{-1}. All molecular weights are relative to PMMA standards. Specific rotation measurements for the polymers were recorded in THF or $CHCl_3$ on a Perkin-Elmer 241 polarimeter using a sodium source (λ = 589 nm). Infrared (IR) spectra were obtained on Perkin-Elmer Spectrum 100 ATR FT-IR spectrometer. Critical aggregation concentration determinations were performed using fluorescence spectroscopy on a Cary Eclipse single-beam fluorimeter. Elemental analysis was performed in duplicate by Warwick Analytical services. Hydrodynamic diameters (D_h) and size distributions of the stars in aqueous solution were determined by dynamic light scattering (DLS). HPLCs were performed on a Varian 920-LC integrated liquid chromatography system. The DLS measurements were taken on a Malvern Nano S Zetasizer Nano Series instrument. All determinations were made in triplicate (with 12 runs recorded). Transmission electron microscopy (TEM) samples were prepared by drop deposition onto copper/carbon grids that had been treated with oxygen plasma to increase the surface hydrophilicity. The particles were stained using a dilute 5% solution of uranyl acetate. Micrographs were collected at magnifications varying from 12K to 25K and calibrated digitally. Histograms of number-average particle diameters (D_{av}) and standard deviations were generated from the analysis of a minimum of 100 particles from at least three different micrographs. Cryo-TEM was performed on a JEOL 2100 TEM with GATAN 924 cryoholder, and a CP3 cryoplunge from GATAN, with the imaging performed on a GATAN 4K camera.

The homopolymerizations detailed in the Experimental Details section were explored using both CTA (**1**) and CTA (**2**); for reasons of clarity each polymerization will only be detailed using CTA (**1**). It can be assumed that molar equivalents of reagents per arm on the CTA, reaction conditions, and work-up are the same for both CTAs. Likewise, only the preparation of CTA (**1**) is outlined; however, an identical procedure was carried out for CTA (**2**) with the same equivalents of base, CS_2, and benzyl bromide per thiol functionality in the starting material.

Synthesis of CTA (1) and CTA (2). Pentaerythritoltetrakis-(3-(*S*-benzyltrithiocarbonyl)propionate), CTA (**1**), was prepared by adding pentaerythritol (3-mercaptopropionate) (1 g, 2.05 mmol) to a stirred suspension of K_3PO_4 (2.60 g, 12.27 mmol) in CS_2 (1.87 g, 24.60 mmol) and THF (10 mL). Stirring was maintained for 30 min, after which time benzyl bromide (2.10 g, 12.27 mmol) was added, and stirring continued for a further 24 h. After filtration to remove the inorganic salts, the solvent was removed in vacuo. The resulting yellow oil was purified using column chromatography on silica eluting with 20% EtOAc in hexane to yield a viscous yellow oil.

CTA (**1**): Yield = 62%. $C_{49}H_{52}O_8S_{12}$ required C: 51.01%, H: 4.54%, found C: 51.52%, H: 4.98%. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.24 (5 × Ar−*H*, m), 4.51 (CH_2−Ph, s), 4.09 (O−CH_2, s), 3.53 (S−CH_2−CH_2, t), 2.70 (S−CH_2−CH_2, t); δ_C (100 MHz, $CDCl_3$): 222.2, 178.3, 136.0, 129.7, 129.4, 128.6, 66.2, 42.4, 34.8, 32.9. IR ν (cm^{-1}): 2968, 2909, 1736, 1496, 1456, 1394, 1351, 1233, 1151, 1059, 803, 698.

CTA (**2**): Yield = 72%. $C_{74}H_{78}O_{13}S_{18}$ required C: 50.71%, H: 4.49%, found C: 50.83%, H: 4.62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H = 7.24 (5 × Ar−*H*, m), 4.18 (CH_2−Ph, s), 4.09 (O−CH_2, s), 3.53 (S−CH_2−CH_2, t), 2.70 (S−CH_2−CH_2, t); δ_C (100 MHz, $CDCl_3$): 221.5, 179.8, 135.7.0, 129.9, 128.8, 128.2, 66.9, 42.2, 35.1, 33.2. IR ν (cm^{-1}): 2969, 2909, 1736, 1497, 1455, 1394, 1233, 1151, 1059, 803, 698.

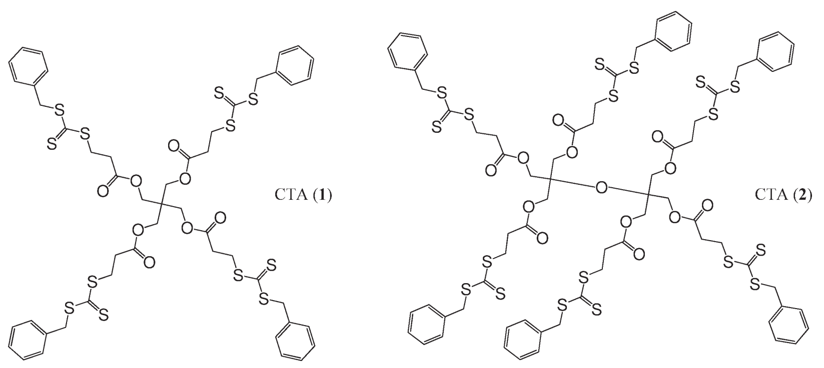
Polymerization of Tetrahydropyran Acrylate (THPA) Using CTA 1 and 2. A typical polymerization procedure is described as follows: CTA (**1**), (0.0132 g, 0.012 mmol, THPA (1.498 g, 9.57 mmol), AIBN (0.0008 g, 0.0048 mmol), and dioxane (1:1 v/v with THPA) (2:1 v/v with THPA used with CTA (**2**)) were placed in a dry glass ampule equipped with magnetic stirrer bar. After degassing the solution using three freeze−evacuate−thaw cycles, the vessel was sealed and held at 60 °C for 2 h. Conversions were calculated using ^{1}H NMR spectroscopy by comparing the vinyl signals of the monomer (δ = 6.45) with the backbone signals and heterocycle signals from the monomer and polymer signals (δ ca. 2.52−1.32). The reaction was quenched with liquid N_2, diluted with THF, and precipitated twice into cold diethyl ether. Polymer **3** was isolated as a glassy yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.92−5.74 (O−C*H*−O, br), 3.88−3.43 (O−CH_2, br; O−CH_3, br), 2.43−2.19 (C*H* backbone, br), 2.13−1.23 (CH_2 backbone, br; O−CH_2(CH_2)$_3$−CH). IR ν (cm^{-1}): 2927, 2854, 1733, 1445, 1252, 1159, 1115, 1020, 897, 863, 820. M_n^{NMR} ($CDCl_3$) = 52.9 kDa, M_n^{GPC} ($CHCl_3$) = 37.2 kDa, M_w/M_n = 1.12.

Polymer **4** was isolated as a glassy yellow solid. M_n^{NMR} ($CDCl_3$) = 79.4 kDa, M_n^{GPC} ($CHCl_3$) = 65.6 kDa, M_w/M_n = 1.14.

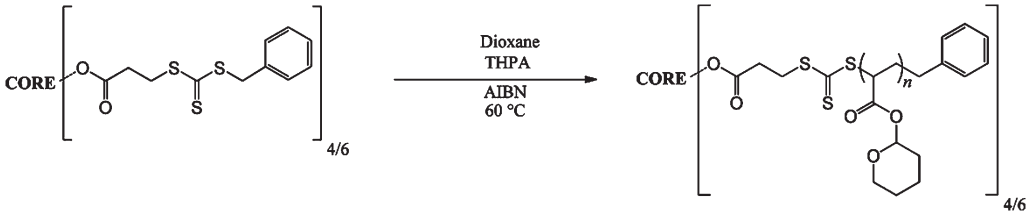
Chain Extension of 3 with L-Phe-OMe To Afford $[P(THPA)_{85}$-*b*-$P(L\text{-}Phe\text{-}OMe)_{47}]_4$, 5. A typical polymerization procedure is described as follows: macro-CTA (**3**) [0.2 g, 3.69 × 10^{-3} mmol, synthesized from THPA using CTA (**1**), (0.0132 g, 0.012 mmol, THPA (1.498 g, 9.57 mmol), AIBN (0.0008 g, 0.0048 mmol) and dioxane (1:1 v/v with THPA)], L-Phe-OMe (0.689 g, 2.96 mmol), AIBN (2.4 × 10^{-4} g, 1.47 × 10^{-3} mmol), and dioxane ([M] = 0.82 mol dm^{-3}) were placed in a dry glass ampule equipped with a magnetic stirrer. The solution was degassed using three freeze−evacuate−thaw cycles, and the vessel was then sealed and held at 70 °C for 0.5 h. After this time the ampule was cooled in liquid N_2, and the reaction mixture was diluted with THF. The polymer was precipitated into stirred, cold diethyl ether and allowed to stand, and then the solvent was decanted off. The resulting polymer, **5**, was freeze-dried from dioxane and isolated as a very pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.91−7.33 (N−*H*, br), 7.29−6.85 (5 × Ar−*H*), 5.92−5.74 (O−C*H*−O, br), 4.79−4.27 (C*H*−CO_2, br), 3.88−3.43 (O−CH_2, br; CO_2CH_3, br), 3.2−2.7 (Ar−CH_2, br), 2.5−2.2 (C*H* backbone, br), 2.2−1.3 (CH_2 backbone, br). IR ν_{max} (cm^{-1}): 3358, 2952, 2884, 1735, 1678, 1531, 1444, 1208, 1160, 1119, 1022, 898, 870, 747, 702. M_n^{NMR} ($CDCl_3$) = 97.9 kDa, M_n^{GPC} (THF) = 72.7 kDa, M_w/M_n = 1.38.

Chain Extension of 4 with L-Phe-OMe To Afford $[P(THPA)_{83}$-*b*-$P(L\text{-}Phe\text{-}OMe)_{27}]_6$, 6. A typical polymerization procedure is described as follows: macro-CTA (**4**) [0.191 g, 2.41 × 10^{-3} mmol, synthesized from THPA using CTA (**2**) (0.0132 g, 0.012 mmol), THPA (1.498 g, 9.57 mmol), AIBN (0.0008 g, 0.0048 mmol), and dioxane (1:1 v/v with THPA)], L-Phe-OMe (0.673 g, 2.89 mmol), AIBN (1.57 × 10^{-4} g, 9.62 × 10^{-3} mmol), and dioxane ([M] = 0.82 mol dm^{-3}) were placed in a dry glass ampule equipped with a magnetic stirrer. The solution was

Scheme 1. Structures of 4- and 6-Armed Star CTAs 1 and 2



Scheme 2. Polymerization of THPA Using CTA 1 and 2



degassed using three freeze−evacuate−thaw cycles, and the vessel was then sealed and held at 70 °C for 0.5 h. After this time the ampule was cooled in liquid N_2, and the reaction mixture was diluted with THF as necessary. The polymer was precipitated into stirred, cold diethyl ether and allowed to stand, and then the solvent was decanted off. The resulting polymer, **6**, was freeze-dried from dioxane and isolated as a very pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.91−7.33 (N−*H*, br), 7.29−6.85 (5 × Ar−*H*), 5.92−5.74 (O−C*H*−O, br), 4.79−4.27 (C*H*−CO_2, br), 3.88−3.43 (O−CH_2, br; CO_2CH_3, br), 3.2−2.7 (Ar−CH_2, br), 2.5−2.2 (C*H* backbone, br), 2.2−1.3 (CH_2 backbone, br). IR ν_{max} (cm^{-1}): 3482 (broad), 2963, 2861, 1731, 1669, 1455, 1255, 1176, 1119, 1082, 1048, 870, 832. M_n^{NMR}($CDCl_3$) = 117.1 kDa, M_n^{GPC} (THF) = 66.1 kDa, M_w/M_n = 1.48.

Deprotection of 5 and 6. P(THPA)-*b*-P(L-Phe-OMe) (**5**) or (**6**) was stirred in dioxane (0.1 g mL^{-1}) for 24 h at 100 °C. The solution was freeze-dried to remove solvent and dihydropyran to yield an off-white solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ = 12.45−12.19 (COO*H*, br), 7.99−7.51 (N−*H*, br), 7.38−7.03 (5 × Ar−*H*), 4.76−4.24 (C*H*−CO_2, br), 3.88−3.43 (CO_2CH_3, br), 3.22−2.79 (Ar−CH_2, br), 2.51−2.00 (C*H* backbone, br), 2.00−0.76 (CH_2 backbone, br). IR ν(cm^{-1}): 3500, 2966, 2861, 1730, 1712, 1668, 1455, 1255, 1118, 1083, 890, 870. T_g = 84 and 95 °C. (**7**) [P(AA)$_{85}$-*b*-P(L-Phe-OMe)$_{47}$]$_4$: M_n^{NMR} (d_6-DMSO) = 69.4 kDa. (**8**) [P(AA)$_{83}$-*b*-P(L-Phe-OMe)$_{27}$]$_6$: M_n^{NMR} (d_6-DMSO) = 75.3 kDa.

Micellization of Star Copolymers 7 and 8. An example is as follows: A round-bottom flask equipped with a stirrer bar was charged with polymer (**7** or **8**) (0.1 g), DMSO (100 mL) was added, and the solution was allowed to stir at RT for 30 min to ensure the mixture was homogeneous. Nanopure water (100 mL) was added to the rapidly agitated solution via a peristaltic pump at the rate of 15 mL h^{-1}. After all of the water had been added, the bluish/opaque micelle solution was transferred to dialysis tubing (MWCO ca. 12−14 kDa) and dialyzed exhaustively against nanopure water to remove all of the residual DMSO. (**9**): $D_h^{(DLS)}$ = 83 nm (PDI = 0.06); $D_{av}^{(TEM)}$: 50 ± 10 nm. (**10**): $D_h^{(DLS)}$ = 122 nm (PDI = 0.04); $D_{av}^{(TEM)}$: 84 ± 6 nm.

Results and Discussion

Two CTAs were used in this study: one with four arms, CTA (**1**), the other with six, CTA (**2**) (Scheme 1). They were both synthesized from modified literature preparations using THF as solvent and K_3PO_4 as base.[50] At the core of both of these CTAs is a thiol-functionalized pentaerythritol or bispentaerythritol building block.[19] Both CTAs were bright yellow and very viscous and were characterized using infrared, elemental analysis, and ^{13}C and ^{1}H NMR spectroscopy.

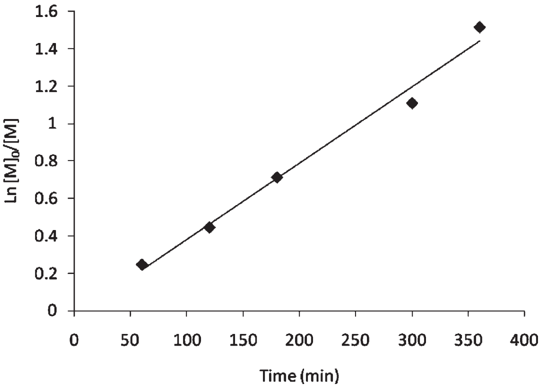


Figure 1. Pseudo-first-order rate plot for the solution polymerization of THPA in 2:1 v/v dioxane (with respect to THPA) at 60 °C using CTA (**2**). M:CTA (**2**):AIBN = 1200:1:0.6. k_p = 0.0041 min^{-1}.

As the end goal was to synthesize chiral amphiphilic star block copolymers that would undergo self-assembly to form core−shell nanostructures, the hydrophilic block needs to be furthest away from the core of the star, which impacts the polymer synthesis. To synthesize a blocky star polymer that will self-assemble in aqueous solution from a Z-approach CTA, it is necessary to form the hydrophilic shell precursor block first; the opposite is true for an R-approach CTA. We chose tetrahydropyranyl acrylate given its readily deprotectable nature to afford hydrophilic poly(acrylic acid) (PAA) by maintaining a solution of the polymer in dioxane (0.1 g mL^{-1}) at 100 °C for 24 h. This strategy could only be employed if the trithiocarbonate was stable to the deprotection conditions. Indeed, if it was degraded during the deprotection, the arms could be cleaved from the central core of the star. In order to assess this, two solutions, each containing one of the CTAs, were stirred at 100 °C for 24 h. After removal of the dioxane, ^{1}H NMR spectroscopic analysis of the resulting material showed that no degradation of the CTA occurred.

Hence, the polymerization of THPA using CTA **1** and **2** was explored using well-established polymerization conditions for the trithiocarbonate-mediated polymerization of acrylates (Scheme 2). Under these conditions (60 °C, dioxane, AIBN 0.1 equiv relative to trithiocarbonate group, THPA 200 equiv relative to trithiocarbonate group) well-defined polymers (M_w/M_n ca. 1.2) were obtained in 2 h using both CTAs. The polymerization kinetics of both CTAs were explored and were found to display a linear pseudo-first-order rate as expected for a controlled radical polymerization (Figure 1 and also see Supporting Information). In addition, the molecular weight (M_n^{GPC}) was observed to increase linearly with conversion (Figure 2) but was always lower that the theoretical M_n. This is often observed in the GPC molecular weight analysis of star polymers given their smaller hydrodynamic radius compared to the linear analogue. We also used a UV detector on the GPC (at 309 nm) to confirm the presence of the trithiocarbonate functionality in both the 4- and 6-armed star copolymers (see SI).

PTHPA macroinitiators were synthesized using the optimized polymerization conditions as detailed above for CTA (**1**) and CTA (**2**). These were synthesized to be chain extended with L-Phe-OMe in order to make amphiphilic star block precursors. All polymerizations proceeded in a controlled fashion and yielded polymers with good control over the molecular weight and polydispersity. The characterization data are summarized in Table 1. Given the difficulty in obtaining accurate molecular weight data from GPC analysis, ^{1}H NMR spectroscopy was used to calculate the molecular weights by comparing the relative integrals of the signals in the aromatic region (Figure 3), corresponding to five protons from the CTA, with the signal (δ = 5.92−5.81) corresponding to one proton from PTHPA. Inspection of the ^{1}H NMR spectra as well as IR spectroscopy confirmed the absence of any deprotected PAA signals.

The polymerization of *N*-acryloylphenylalanine methyl ester has been reported previously using RAFT polymerization techniques and has been shown to proceed in a controlled manner when the polymerizations were run at relatively high dilution and stopped at relatively low conversion. Using multifunctional CTA **1**, the polymerization of *N*-acryloylphenylalanine methyl ester was initially explored under a range of conditions (see Table S1 from 60 to 90 °C) and as previously reported for the monofunctional CTAs the monomer concentration played a key role in ensuring polymerization control.[53] At low concentrations reasonable polymerization control (M_w/M_n ca. 1.4) could be obtained in around 3 h at 70 °C.

Using the macroinitiators **3** and **4**, the preparation of chiral diblock copolymers stars was explored (Scheme 3). During the star chain extension process some deprotection of the PTHPA was observed (ca. 5% in the 4 arm and 10% in the 6 arm) as confirmed by the presence of a signal (ca. δ = 12.2) in the ^{1}H NMR spectrum relating to the PAA proton. It appears that that the conditions required for the chain extension lead to some thermal cleavage of the protecting group; however, the THPA group of the stars is stable after preparation of the diblock. This

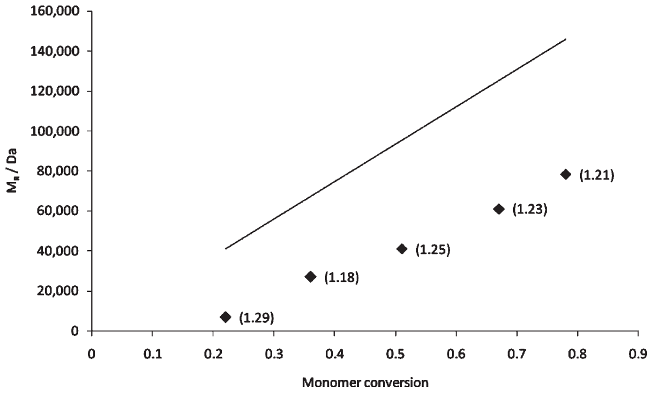


Figure 2. Evolution of molecular weight and polydispersity with conversion for the polymerization of THPA in 1:1 v/v dioxane with CTA (**2**) at 60 °C. M:CTA (**1**):I 800:1:0.4 with the trend line $M_{n,th}$.

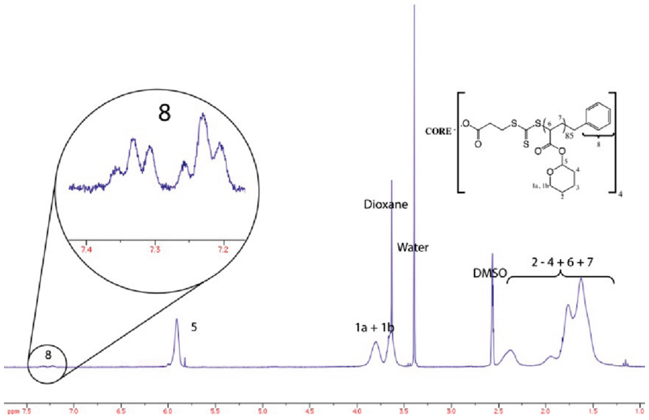


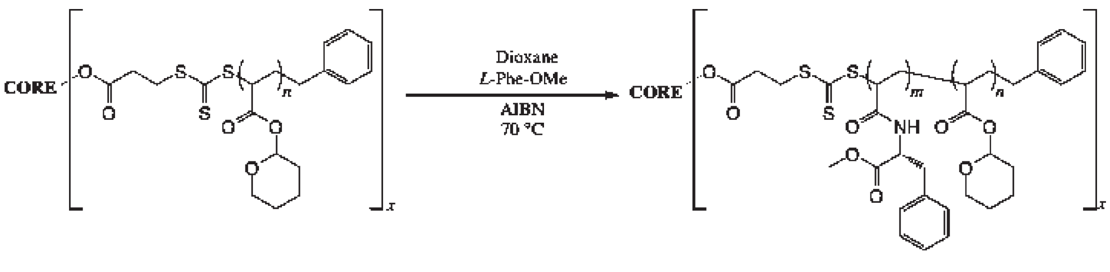
Figure 3. ^{1}H NMR spectrum in d_6-DMSO of 4-armed THPA macro-CTA, **3** (M_n^{NMR} = 42.3 kDa, M_w/M_n = 1.19). The expanded region of the spectrum shows the end-group signals used to calculate the DP and M_n of all the macroinitiators used in this study.

Table 1. Polymerization Data for THPA Macroinitiators Synthesized from either CTA (1) or (2)

polymer	CTA	conv (%)	time (h)	$M_{n,th}$ (kDa)[a]	M_n^{NMR} (kDa)[b]	M_n^{GPC} (kDa)[c]	M_w/M_n[c]
(**3**)	(**1**)	44	2.75	56.0	52.9	37.2	1.12
(**4**)	(**2**)	45	3.5	85.9	79.4	65.6	1.14

[a] Calculated from ^{1}H NMR spectroscopy. [b] Calculated from end-group analysis by ^{1}H NMR spectroscopy. [c] Determined using DMF GPC calibrated with PMMA.

Scheme 3. Conditions for Chain Extension of 4- and 6-Armed PTHPA Stars (3 and 4) with L-Phe-OMe



along with the interaction of the phenylalanine block with the column material leads to a broadening of the molecular weight trace by GPC analysis. Hence, given the difficulty in obtaining accurate molecular weight data by GPC analysis, ^{1}H NMR analysis was used (see Supporting Information). For both polymers **5** and **6**, the relative integral of the characteristic THPA signal at 5.8 ppm was compared to that for the signal at 3.0 ppm for the P(L-Phe-OMe). The M_n values obtained by NMR analysis compare well with those calculated based on the conversion.

Table 2. Polymerization Data for the Chain Extension of 3 and 4 with L-Phe-OMe in Dioxane ([M] = 0.82 mol dm^{-3}) for 2 h at 70 °C (M:macroCTA:I for 3: 800:1:0.4; for 4: 1200:1:0.6)

	conv (%)	$M_{n,th}$ (kDa)[a]	M_n^{NMR} (kDa)[b]	M_n^{GPC} (kDa)[c]	M_w/M_n[c]
(**5**)	28	106.3	97.9	72.7	1.38
(**6**)	15	121.3	117.1	66.1	1.48

[a] Calculated from ^{1}H NMR spectroscopy. [b] Calculated from end-group analysis by ^{1}H NMR spectroscopy. [c] Determined using DMF GPC calibrated with PMMA.

Table 3. TEM and DLS Characterization Data Obtained after Sample Filtration (0.45 μm Nylon Syringe Filter) for the Nanostructures 9 and 10

		DLS: D_h[a] (nm)				
	conc (g L^{-1})	vol	num	int	PDI	TEM: D_{av}[b] (nm)
(**9**)	0.42	95	83	105	0.06	50 ± 10
(**10**)	0.33	135	122	139	0.04	84 ± 6

[a] Hydrodynamic diameters in aqueous solution by dynamic light scattering. [b] Average diameters were measured by TEM, calculated from the values for 100 particles.

The deprotection of the 4- and 6-armed stars, (**5**) and (**6**), was carried out at 100 °C in dioxane to yield two amphiphilic diblock star copolymers, (**7**) and (**8**). The deprotection was observed to have gone to >95% in both cases and was confirmed by ^{1}H NMR spectroscopy in d_6-DMSO by the loss of the signal (ca. δ = 5.8) associated with PTHPA and the appearance of the carboxylic acid signal (ca. δ = 12.2). The deprotection was further confirmed using IR by the presence of a large broad signal related to the acid stretch centered around 3416 cm^{-1}. The expected shift of the carbonyl stretching frequency from 1739 cm^{-1} (associated with the THPA ester) to ca. 1720 cm^{-1} (associated with the free carboxylic acid) is difficult to observe due to the presence of the ester signal from the L-Phe-OMe; however, a slight broadening of the signal was observed, and the presence of a small shoulder was also apparent. The amphiphilic stars **7** and **8** were explored by DLS analysis at low concentration in DMF and were found to have hydrodynamic diameters of 4.8 and 5.6 nm, respectively. This is in the range of sizes reported for related PS–PAA star copolymers.[38]

The self-assembly of amphiphilic diblock copolymers is widely studied and is often achieved by the dissolution of the polymer in a good organic solvent for both blocks, followed by a slow, controlled solvent swap and dialysis to yield an aqueous solution. The same methodology can be readily applied to the self-assembly of the chiral amphiphilic star-shaped block copolymers **7** and **8**. Hence, the polymers **7** and **8** were dissolved in DMSO at a concentration of 1 mg/mL, and an equal volume of nanopure water was added using a peristaltic pump. After the water addition, the solutions were transferred to membrane bags (MWCO = 3 kDa) and dialyzed exhaustively to remove all small molecule contaminants. The resulting structures **9** and **10**

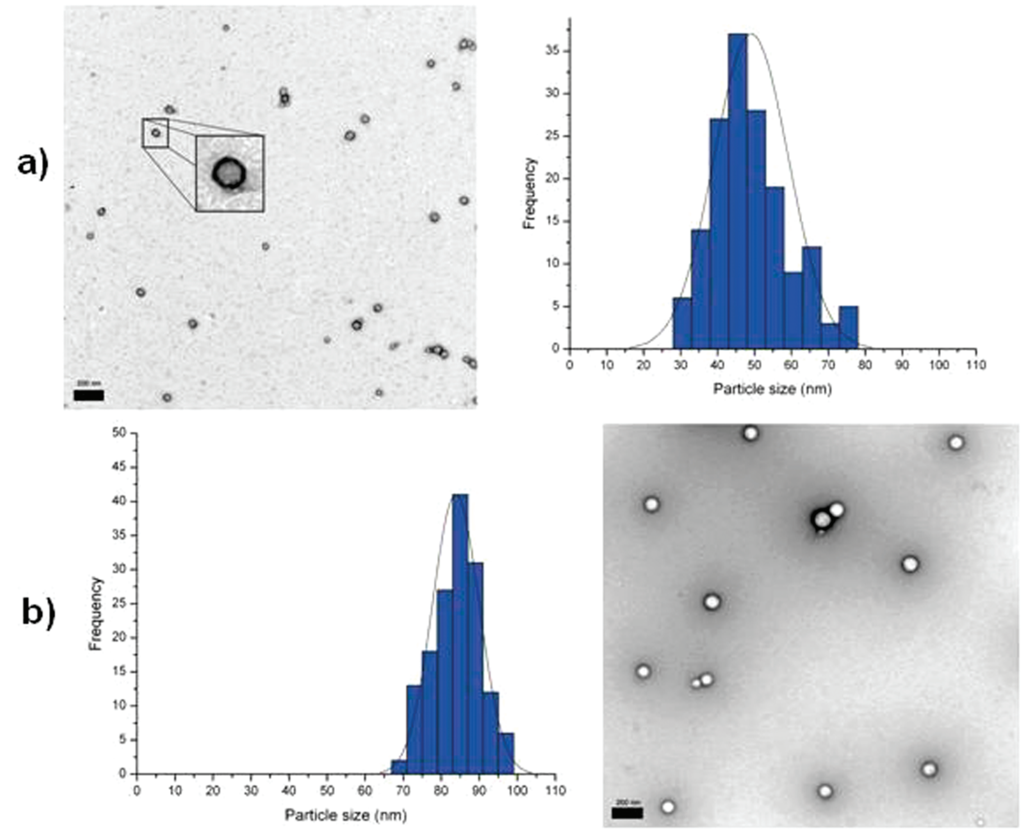


Figure 4. Representative TEM pictures obtained by drop-depositing solutions onto copper/carbon grids and staining with 5% uranyl acetate solution along with histograms to show size distribution of particles made from (a) **9** and (b) **10** containing amphiphilic diblock star copolymers. Scale bar = 200 nm. Inset image in TEM shows a magnified particle.

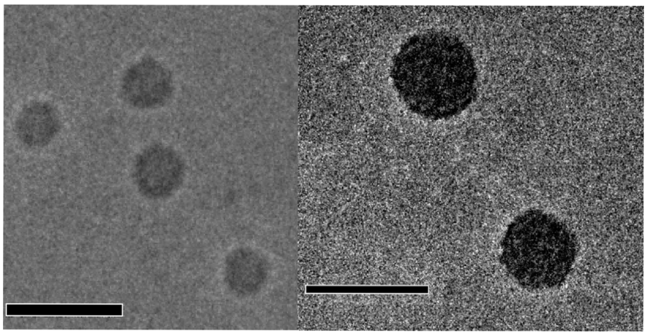


Figure 5. Representative cryo-TEM images of nanostructures **9** (LHS) and **10** (RHS), where the scale bar = 100 nm.

were characterized using DLS, TEM, and cryo-TEM techniques (Table 3).

DLS analysis of the assemblies **9** and **10** confirmed that well-defined nanostructures were obtained with narrow polydispersities and good correlation data. Interestingly, the size of the 6-armed star assembly is larger than the 4-armed despite their similar block lengths. This is in agreement with reports by Monteiro from a 4-armed PAA−PS star system that the larger the hydrophobic domain, the larger the resultant particle.[38] It is expected that the number of arms influences the interaction between the star polymers through the higher steric repulsion of the arms, and previous work has indicated that the greater number of arms lowers the aggregation number.[39,54] In these systems we were unable to obtain convincing static light scattering data due to changes in the micelle size upon changes in concentration; hence, the aggregation number of these particles was not determined. Further attempts to determine the size and shape of all the samples were made using TEM. The TEM images with uranyl acetate staining (Figure 4) of the filtered samples **9** and **10** showed them to be spherical, monodisperse, and, as expected, somewhat smaller than the size of the fully solvated particles observed in solution by DLS. The frequency distributions of the particles seen in each sample show the narrow polydispersity of the samples (Figure 4). From these stained TEM images the exact morphology of the particles was unclear. Upon examination of the hydrophobic:hydrophilic block ratio (by mass) polymer **7** has a ratio of around 1:1.8, whereas the ratio in **8** is around 1:1.3. This would suggest that spherical micelle morphologies may be formed (although the weight fraction for polymer **8**, in analogue to work by Bates et al. on poly(ethylene glycol)-*b*-polybutadiene, may suggest wormlike structures).[55,56] The formation of solely spherical micelles was confirmed upon further analysis of the particles by cryo-TEM for both **9** and **10** (Figure 5).

To further explore the self-assembly properties of these chiral star polymers, the critical aggregation concentration (cac) was determined using well-established pyrene excitation techniques (see Supporting Information).[57,58] Using this technique, the cac for **9** and **10** was found to be similar to that for the linear precursor reported previously; 4.8×10^{-3} and 2.5×10^{-3} respectively versus 9.1×10^{-3} M for the linear block.[53] The values of cac for **9** and **10** are similar to the values previously reported for 3- and 4-armed stars by Kim and also 6-armed stars by Tenhu.[35,59] In addition, the chiral properties of the assembled stars were confirmed by CD analysis (Figure S9).

Having demonstrated that the 4- and 6-armed amphiphilic star copolymers form well-defined micelle aggregates, we were interested in exploiting the chiral nature of the core domain. Hence, using the procedures described by Zhu in 2007, we explored the chiral recognition of our 4-armed star polymers.[60] Experimentally, the nanostructures **9** (2 mL of solution of concentration 0.42) were incubated overnight with D/L-phenylalanine. After this time the micelle solution was transferred to an ultrafiltration cell (MWCO 3.5 kDa), and ca. 0.5 mL of solute was collected. Alongside the experimental for **9** a control experiment was performed using a PMA micelle of similar size and block ratio ($[\mathrm{PAA}_{83}\text{-}b\text{-}\mathrm{PMA}_{20}]_4$, D_h = 93 nm). The resulting solutions were analyzed by polarimetry, and the control reaction had a specific rotation that was close to zero, which corresponds to the original racemic solution. However, the solution isolated from **9** had a specific rotation $[\alpha]_\mathrm{D}$ of −12.3, indicating that upon incubation with the chiral micelle **9** the surrounding solute was depleted in the D-isomer. This indicated the selective interaction and sequestration potential of the L-phenylalanine micelle core with the opposite enantiomer, thus allowing for racemic enhancement of the solution. Further work is currently exploring the extent and scope of this enhancement.

Conclusions

Using RAFT polymerization, two well-defined amphiphilic star block copolymers which have an L-phenylalanine core domain have been prepared. The self-assembly of these 4- and 6-armed amphiphilic copolymers has been explored using a range of characterization techniques. These polymers were found to form spherical micelle morphologies with relatively narrow dispersities. The solution properties of these structures were explored, and the critical aggregation concentration was determined. Overall, the micelle aggregates formed in this work behave in a similar manner to those previously reported. However, one unique feature of these nanostructures is the presence of chirality within the core domain which has been utilized in this work for enhancement of a racemic phenylalanine solution. This demonstrates the potential application of these star materials as two phase chiral separation media and also as selective nanoreactors. Current work is exploring further the potential of these materials and their enhancement ability compared to existing techniques.

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Supporting Information Available: Further characterization data for the star polymers along with cac data for the micelles. This material is available free of charge via the Internet at http://pubs.acs.org.

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