Synthesis, Purification, and Characterization of ''Perfect'' Star Polymers via ''Click'' Coupling

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ABSTRACT: The copper (I)-catalyzed azide-alkyne cycloaddition "click" reaction was successfully applied to prepare well-defined 3, 6, and 12-arms polystyrene and polyethylene glycol stars. This study focused particularly on making "perfect" star polymers with an exact number of arms, as well as developing techniques for their purification. Various methods of characterization confirmed the star polymers high purity, and the structural uniformity of the generated star polymers. In particular, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry revealed the quantitative transformation of the end groups on the linear polymer precursors and confirmed their quantitative coupling to the dendritic cores to yield star polymers with an exact number of arms. In addition to preparing well-defined polystyrene and poly(ethylene glycol)homopolymer stars, this technique was also successfully applied to amphiphilic, PCL-*b*-PEG star polymers. © 2011 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 50: 1086–1101, 2012

KEYWORDS: atom transfer radical polymerization (ATRP); MALDI-TOF MS; separation of polymers; star polymers

INTRODUCTION Star polymers are branched polymers that consist of multiple linear chains connected to a central core.[1] Star polymers' multiarm structure, globular shape, and multiplicity of end groups imparts on them a unique set of properties (e.g., crystalline, mechanical, and viscoelastic properties), when compared with their linear analogs. Perhaps most significantly, the three-dimensional architecture of appropriately designed amphiphilic star polymers can be tailored to encapsulate guest molecules providing relatively rapid access to well-defined nanocarriers for applications in drug delivery.[2–4] There are three general strategies for the synthesis of star polymers, each of which exhibits particular advantages and disadvantages: the "core first" approach, the "arm first" approach, and the "graft onto" approach.[5–8] The "core first" approach initiates polymerization of the arms from a polyfunctional core, which likely results in nonuniform arms lengths because of potential differences in the reactivity of each initiating group. Likewise, when comparing a set of star polymers prepared from different cores, it is difficult to ensure uniformity of arm length from batch to batch. The "arm first" approach involves first the polymerization of the arms followed by their coupling, usually by continuation of the polymerization with addition of a crosslinking monomer. Although technically simple, this typically leads to materials with an inexact number of arms and broader polydispersities. The third major route, the "graft onto" approach involves the attachment of preformed arms onto core molecules via an efficient coupling reaction. This approach makes the conjugation of arms of uniform length onto different cores possible but requires highly efficient conjugation reactions to complete the coupling to the core. This route also requires an excess linear polymer arms to drive the coupling reaction to completion, and therefore involves tedious purification to remove the unreacted linear reactant. The "graft onto" method typically affords the most structurally uniform star polymers and provides access to comparable libraries with different cores yet identical arm lengths, however, truly well-defined structures can only be accessed with highly efficient coupling reactions and substantial purification efforts.

Much of the early work preparing well-defined star polymers used anionic polymerization methods owing to the exceptional control of this technique but suffered from limited monomer compatibility and extremely tedious experimental preparation due to the technique's sensitivity to trace impurities.[9] Recently, the development of controlled/living radical polymerization,[10] especially atom transfer radical polymerization (ATRP),[11,12] enabled the facile preparation of narrow dispersed polymers with broad monomer compatibility yet bearing uniformly end-functionalized polymer groups. Around the same time, the optimization of dendrimer chemistry afforded a number of efficient syntheses that provide access to well-defined dendritic cores, with exact numbers of functional groups.[13] The combination of these two

Additional Supporting Information may be found in the online version of this article.

approaches provides relatively straight forward routes to the well-defined precursors to make high purity star polymers via the "graft onto" approach.

The only remaining requirement for preparing well-defined star polymers, a highly efficient coupling reaction, is provided by the copper-catalyzed azide-alkyne cycloaddition (CuAAC) "click" coupling reaction.[14–16] This particular reaction is exceptionally powerful in overcoming the steric inhibition that frequently prevents the quantitative coupling of polymeric substrates. Its usage for polymer conjugations has been demonstrated widely[17–20] but is perhaps most apparent in the near quantitative coupling of hindered macromolecular components. Representative examples include the synthesis of dendronized polymers by coupling third generation dendrons onto each repeat unit of either a linear poly(-vinylacetylene) backbone[21] or a cyclic styrenic backbone;[22] the synthesis of polymer-peptide conjugates of bovine serum albumin with polystyrene (4.15 kDa)[23] or poly(*N*-isopropylacrylamide) (PNIPAM) (16.3 kDa);[24] and the synthesis of graft polymers via the coupling of 775 Da PEG chains onto a 27.5 k MW poly(hydroxyethyl methacrylate) backbone.[25] The CuAAC reaction has already been used in numerous examples to make star polymers, including poly(styrene) stars,[25] poly(*tert*-butyl acrylate) and poly(ethylene glycol) stars,[26] cyclodextrin-core seven-arm star poly(ε-caprolactone) (PCL),[27] cyclodextrin-core seven-arm and 21-arm star PNIPAM,[28] POSS-core block copolymer stars,[29,30] and so forth. In addition, other types of "click" reactions, such as thiol-ene reaction[31] and Diels-Alder[32] reactions have also been used to synthesize star polymers via the "graft onto" method. However, most of these materials were not analyzed in sufficient detail to judge their purity with regards to missing arms. Because of the interest in star polymers for biomedical applications such as transdermal drug carriers,[4,33,34] it is critical to develop synthetic, purification, and characterization methodologies, which can reproducibly yield "perfect" star polymers, those which exhibit the exact number of arms as were targeted in their synthesis. For these reasons, a detailed study of the coupling, purification, and characterization of star polymers was performed and described below.

EXPERIMENTAL

Materials

All reagents were purchased from Aldrich and used without further purification, unless otherwise noted. ε-CL was dried over calcium hydride at room temperature for at least 8 h and distilled under reduced pressure just before use. Solvents were purchased from Pharmaco Aaper, Aldrich, and Fisher Scientific. Tetrahydrofuran (THF) was dried over sodium refluxing overnight. Dichloromethane and hexane were dried over calcium hydride at room temperature overnight. All other solvents were reagent grade and used without further distillation or purification. Bio-Beads® S-X beads are porous crosslinked polystyrene polymers used for gel permeation separations of hydrophobic polymers in the presence of organic solvents. The dendrimers with hydroxyl group were synthesized by described procedure.[35] The detail can be found in the Supporting Information.

Characterization

All ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were obtained using a Varian Mercury spectrometer (Palo Alto, CA), using TMS = 0.00 ppm for ^{1}H calibration. Gel permeation chromatography (GPC) was performed on a Waters model 1515 series pump (Milford, MA) with three column series from Polymer Laboratories, consisting of PLgel 5 μm Mixed D (300 mm $\times$ 7.5 mm, molecular weight range 200–400,000), PLgel 5 μm 500 Å (300 mm $\times$ 7.5 mm, molecular weight range 500–30,000), and PLgel 5 μm 50 Å (300 mm $\times$ 7.5 mm, molecular weight range up to 2000) columns. The system was fitted with a Model 2487 differential refractometer detector and anhydrous THF was used as the mobile phase (1 mL min^{-1} flow rate). The resulting molecular weight was based on calibration using linear polystyrene standards. Data were collected and processed using Precision Acquire software. Mass spectral data was acquired using a Bruker Autoflex III matrix-assisted laser desorption ionization-time-of-flight mass spectrometer (MALDI-TOF MS) with delayed extraction using both positive ion and reflector detection modes. For all PCL polymers, THF stock solutions of 9-nitroanthracene as the matrix (20 mg mL^{-1}) and NaI as the counterion (10 mg mL^{-1}) were used. The polymer sample was prepared at a 2 mg mL^{-1} concentration in THF. MALDI samples were prepared by combining 50 μL of polymer solution, 100 μL of counterion solution, and 200 μL of matrix solution. For all PEG polymers, THF stock solutions of alpha-cyano 4-hydroxycinnamic acid (20 mg mL^{-1}) and sodium trifluoroacetate as the counterion (1 mg mL^{-1}) were used. The polymer sample was prepared at a 2 mg mL^{-1} concentration in THF. MALDI samples were prepared by combining 20 μL of polymer solution, 0.5 μL of counterion solution, and 10 μL of matrix solution. For all PSt polymers, THF stock solutions of dithranol (20 mg mL^{-1}) and silver trifluoroacetate as the counterion (1 mg mL^{-1}) were used. The polymer sample was prepared at a 2 mg mL^{-1} concentration in THF. MALDI samples were prepared by combining 20 μL of polymer solution, 0.5 μL of counterion solution, and 10 μL of matrix solution.

General Procedure for Synthesis of Alkynylated Dendrons

The alkynylated dendrons were synthesized according to a previously reported procedure.[36] Dendrimers with hydroxyl group on the periphery, pentynoic acid anhydride, 4-Dimethylaminopyridine (DMAP), and pyridine (1 hydroxide:1.67 pentynoic acid anhydride:0.33 DMAP:6 pyridine) were added to a round bottom flask with dichloromethane. After stirring for 12 h in room temperature under nitrogen, the mixture was quenched by DI water for about 2 h. Sequentially the reaction was washed three times with 1 M $NaHSO_4$ solution, 1 M $NaHCO_3$ solution and of brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated before dissolve in diethyl ether. After filtration, the filtrate was concentrated and precipitated into 40 mL of cold hexanes. The product was isolated as oil and dried *in vacuo*. The specific details are described in the Supporting Information.

Synthesis of Polystyrene Bromide

A 500 mL round bottomed flask containing 2.73 g (19.06 mmol) CuBr, 0.75 g (3.37mmol) $CuBr_2$, 4.66 g (26.9 mmol) PMDETA, and 300 g (2.88 mol) styrene was degassed using two freeze/pump/thaw cycles. After warming to room temperature, 4.15 g (22.4 mmol) of the initiator was added via syringe. The reaction mixture was placed in a preheated 75 °C oil bath and allowed to stir under nitrogen for 56 min. The reaction mixture was then cooled to room temperature and purified by extraction from water into dichloromethane followed by precipitation into methanol to yield the polymer as a white solid: 26.9 g (yield 8.86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9–1.1 (br, 3), 1.3–2.6 (br, 72), 4.4–4.6 (br, 1), 6.4–7.4 (br, 120). MS (MALDI-TOF): (*m/z*), M_n = 2450; PDI: 1.07. GPC: M_n = 2800; PDI = 1.12. FTIR: 3082, 3061, 3026, 2923, 2850, 1943, 1872, 1803, 1685, 1601, 1493, 1453, 1373, 1181, 1154, 1070, 1028, 907, 759, 698 cm^{-1}.

Synthesis of Polystyrene Azide

To a 500 mL round bottomed flask was added 26.9 g (10.4 mmol) PS-Br. The polymer was then dissolved into 60 mL dimethylformamide (DMF), and 3.37 g (0.51.8 mmol) sodium azide was added as a solid. The solution was allowed to stir overnight at room temperature before purification by precipitation into methanol to give a white solid: 22.3 g (yield 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9–1.1 (br, 3), 1.3–2.6 (br, 72), 3.8–4.1 (br, 1), 6.4–7.4 (br, 120). MS (MALDI-TOF): (*m/z*), M_n = 2500; PDI: 1.04. GPC: M_n = 2800; PDI = 1.12. FTIR: 3082, 3060, 3026, 2923, 2849, 2094, 1943, 1872, 1803, 1745, 1601, 1493, 1453, 1374, 1181, 1154, 1069, 1028, 907, 760, 698 cm^{-1}.

Synthesis of PEG-OMs

A typical procedure of synthesis of PEG-OMs was previously reported.[37] PEG-OH (M_n = 2000, 4 g, 2 mmol) was heated to 50 °C in oil bath and dried *in vacuo* for 12 h. It was cooled to room temperature before dissolved in 100 mL of dried dichloromethane. The solution was cooled to 0 °C in ice-water bath, and distilled triethylamine (1.53 mL, 11.0 mmol) and methanesulfonyl anhydride (1.74 g, 10 mmol) were sequentially slowly added. After stirring for 12 h in room temperature under nitrogen, the mixture was filtered and sequentially washed three times with 100 mL of 1 M $NaHSO_4$ solution, 100 mL of 1 M $NaHCO_3$ solution and 50 mL of brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated before precipitation from dichloromethane into 40 mL of cold ethyl ether. The product was isolated via filtration and dried *in vacuo*; 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.06 (s, 3H), 3.35 (s, 3H), 3.51–3.53 (m, 2H), 3.61–3.63 (br, 189H), 4.36–4.37 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 37.91, 59.23, 61.80, 69.20, 69.52, 70.47, 72.08, 72.84. MS (MALDI-TOF): (*m/z*), M_n = 2260; PDI: 1.01. GPC: M_n = 2800; PDI = 1.02.

Synthesis of PEG-N_3

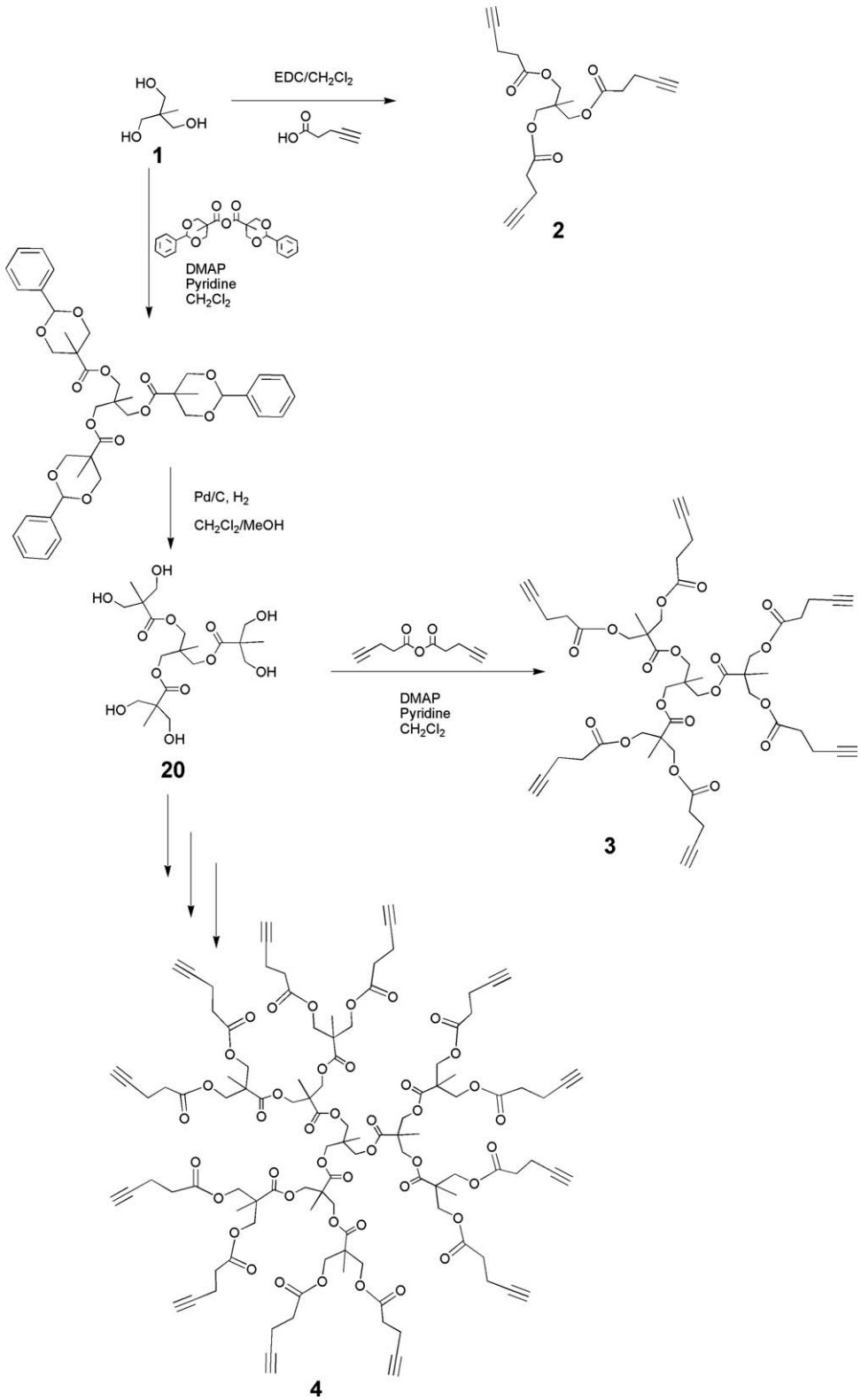
A typical procedure of synthesis of PEG-N_3 was previously reported.[20] PEG-OMs (2.00 g, 1.00 mmol) and sodium azide (0.33 g, 5.00 mmol) were separately heated to 50 °C under vacuum for 12 h, and then cooled to room temperature. The PEG-OMs was dissolved in 25 mL of dried DMF, followed by the addition of sodium azide. The reaction was heated to 50 °C and stirred under nitrogen for 12 h. The reaction was dissolved in 50 mL of DCM, and then washed three times with 100 mL of DI H_2O, and three times with 100 mL of brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated before precipitation from dichloromethane into 20 mL of cold ethyl ether. The product was isolated via filtration and dried *in vacuo*; 78% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.99 (s, 2H), 3.35 (s, 3H), 3.35–3.37 (m, 4H), 3.62–3.64 (br, 189H). ^{13}C NMR (100 MHz, $CDCl_3$,) δ 50.87, 59.27, 70.25, 70.78, 72.13. MS (MALDI-TOF): (*m/z*), M_n = 2100; PDI = 1.01. GPC: M_n = 2700; PDI = 1.02.

Synthesis of Three Arm PCL-OH

1,1,1-tris(hydroxymethyl)ethane (0.421g, 3.5 mmol) was placed in a previously dried 25 mL two neck round bottom flask equipped with a stir bar and dried under vacuum for about 1 h. ε-Caprolactone (10 g, 87.6 mmol) was added through the rubber septum via syringe to the initiator. After all initiator was dissolved in the monomer, tin(II) ethylhexanoate (0.177g, 0.4 mmol) was added via syringe, and the reaction flask was immediately submerged in an oil bath at 130 °C. After 3 h 10 min, the flask was removed from the oil bath, and the contents were diluted with dichloromethane, precipitated twice into a 1:1 mixture of hexanes and diethyl ether, recovered by filtration, and dried *in vacuo* before characterization. Characterization data: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (m, 2n), 1.57 (m, 4n), 1.88 (m, 2, J = 6.3 Hz), 2.27 (t, 2n, J = 7.6), 3.37 (t, 2, J = 6.3), 3.60 (t, 2, J = 6.6 Hz), 4.03 (t, 2n, J = 6.3 Hz), 4.14 (t, 2, J = 6.2 Hz). Calcd M_n = 4600. ^{13}C NMR ($CDCl_3$, δ, ppm) 24.78, 25.73, 28.54, 34.32, 64.36, 173.79. MS (MALDI-TOF): (*m/z*), M_n = 2220, PDI = 1.06. GPC: M_n = 4040, PDI = 1.08.

Synthesis of Six Arm PCL-OH

G1$(OH)_6$ (0.295g, 0.63 mmol) was placed in a previously dried 25 mL two neck round bottom flask equipped with a stir bar and dried under vacuum at 110 °C for 24 h. The temperature of the flask was then raised to 130 °C under vacuum and backfilled with nitrogen. ε-Caprolactone (2.67 g, 25.2 mmol) was added through the rubber septum via syringe to the initiator. After all initiator was dissolved in monomer, tin(II) ethylhexanoate (0.025 g, 0.07 mmol) was added via syringe. After 40 min after the addition of the catalyst, the flask was removed from the oil bath, and the contents were diluted with dichloromethane, precipitated twice into a 1:1 mixture of hexanes and diethyl ether, recovered by filtration, and dried *in vacuo* before characterization. Characterization data: ^{1}H NMR ($CDCl_3$, δ, ppm) 1.31 (m, 2n), 1.57 (m, 4n), 1.88 (m, 2, J = 6.3 Hz), 2.27 (t, 2n, J = 7.6), 3.37 (t, 2, J = 6.3), 3.60 (t, 2, J = 6.6 Hz), 4.03 (t, 2n, J = 6.3 Hz), 4.14 (t, 2, J = 6.2 Hz). calcd M_n = 4600. ^{13}C NMR ($CDCl_3$, δ, ppm) 24.78, 25.73, 28.54, 34.32, 64.36, 173.79. MS (MALDI-TOF): (*m/z*), M_n = 5800, PDI = 1.06. GPC: M_n = 7760, PDI = 1.14.



SCHEME 1 Synthesis of alkynylated dendrimer cores **2, 3**, and **4**.

The Preparation of Three Arms PCL-Alkynes

The polymer three arms PCL-OH (1.122 g, 0.51 mmol) was dissolve in 10 mL of dichloromethane. 4-pentynoic anhydride (1.000 g, 5.61mmol), pyridine (0.755 g, 9.51mmol), and 4-(dimethylamino)pyridine (0.069 g, 0.561mmol) were added to the reaction flask. The reaction was stirred at room

temperature for 10 h. Then the crude reaction mixture was extracted three times from saturated aqueous $NaHSO_4$ and three times from saturated aqueous $NaHCO_3$ into dichloromethane. The organic layer was dried over anhydrous Na_2SO_4. After removing most of the solvent, the product was isolated by precipitation from dichloromethane into cold methanol. Yield (89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (m, 2n), 1.57 (m, 4n), 1.88 (m, 2), 1.98 (s, 1), 2.27 (t, 2n,), 2.51 (t, 4,), 4.03 (t, 2n). MS (MALDI-TOF) (*m/z*), M_n = 2660, PDI = 1.04). GPC: M_n = 4600, PDI = 1.08.

Preparation of Six Arms PCL-Alkynes

The polymer six arms PCL-OH (0.200g) was dissolve in 10 mL of dichloromethane. 4-pentynoic anhydride (0.114 g), pyridine (0.098 g), and 4-(dimethylamino)pyridine (0.008 g) were added to the reaction flask. The reaction was stirred at room temperature for 10 h. Then, the crude reaction mixture was extracted three times from saturated aqueous $NaHSO_4$ and three times from saturated aqueous $NaHCO_3$ into dichloromethane. The organic layer was dried over anhydrous Na_2SO_4. After removing most of the solvent, the product was got by precipitation from dichloromethane into cold methanol. Yield (87%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (m, 2n), 1.57 (m, 4n), 1.98 (s, 1), 2.27 (t, 2n), 2.51 (t, 4), 4.03 (t, 2n), 4.14 (t, 2). MS (MALDI-TOF) (*m/z*), M_n = 6330, PDI = 1.04). GPC: M_n = 8600, PDI = 1.08.

General Procedure of Cu(I)-Catalyzed "Click" Assembly of Star Polymers

Alkyne functionalized star cores, azide functionalized macromolecule, and PMDETA (1 alkyne:1.05 azido-polymer:2 PMDETA) were added to a round bottom flask with dichloromethane. After two freeze-pump-thaw cycles, Cu(I)Br (1:1 with PMDETA) was added, and the third freeze-pump-thaw cycle was performed. The reaction was stirred for 16 h at room temperature, then was washed with de-ionized water and brine, and finally was dried with Na_2SO_4. Following concentration *in vacuo*, the products were run through a short silica gel column to remove residual copper. The detail procedure for synthesis of star polymers is described in the Supporting Information.

RESULTS AND DISCUSSION

To prepare "perfect" star polymers via the "graft onto" approach, well-defined polymer arms and dendritic cores must first be prepared with complimentary functionalities.

The dendritic cores were prepared via the highly efficient divergent synthetic approach, using benzylidene protected bis-MPA.[38] After a series of monodisperse dendrimers with 3, 6, and 12 hydroxyl groups were obtained ("generations" G0, G1, and G2, respectively), they were reacted with pentynoic acid anhydride to quantitatively introduce alkyne groups onto their periphery to yield core **2, 3, 4** (Scheme 1).

The acquired ^{1}H and ^{13}C NMR spectra verified the purity of the products, which exhibiting exceptional symmetry. The quantitative esterification of the peripheral hydroxyls could be confirmed by comparing the integration of the resonances of the terminal alkyne (1.98 ppm) and those of the methylene adjacent to the ester oxygen (4.05 ppm) to those of the methyl groups at the core (G0: 1.03 ppm; G1: 1.05 ppm; G2: 1.12 ppm) (Supporting Information Fig. S1 and Table S1). Elemental analysis was also used to confirm the purity of

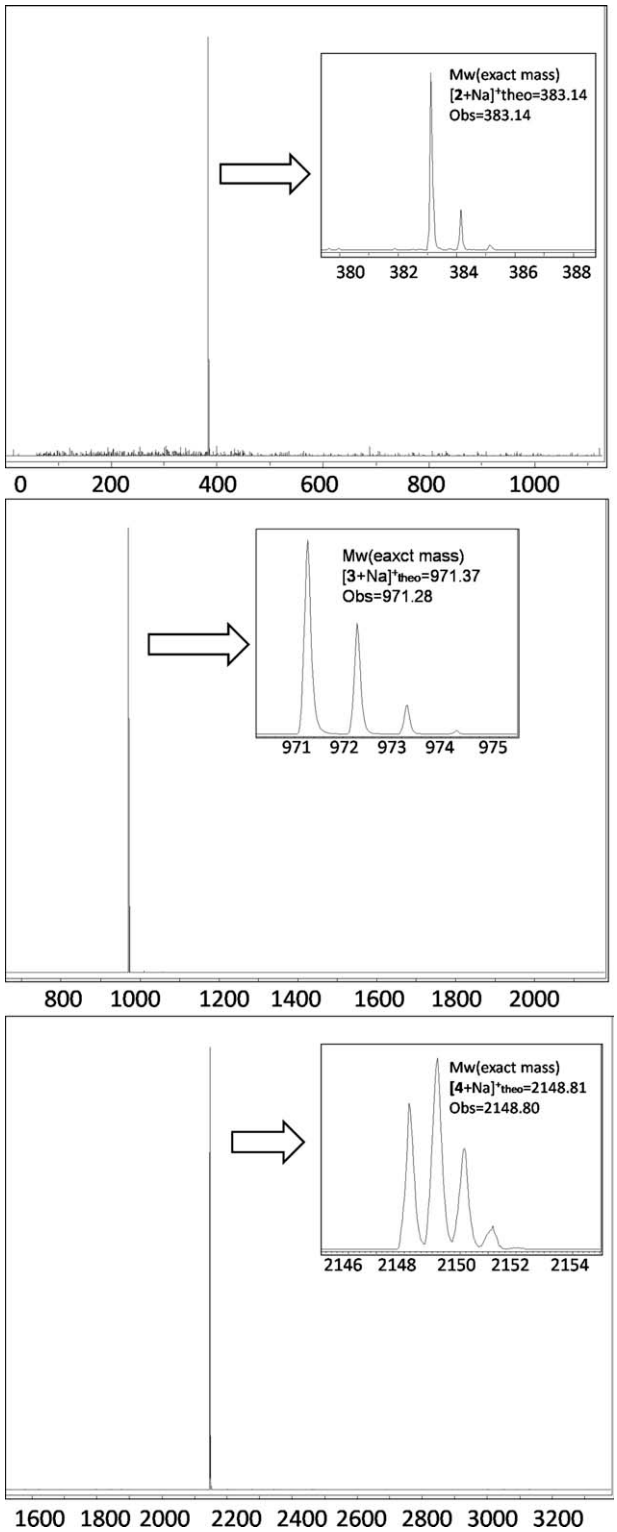
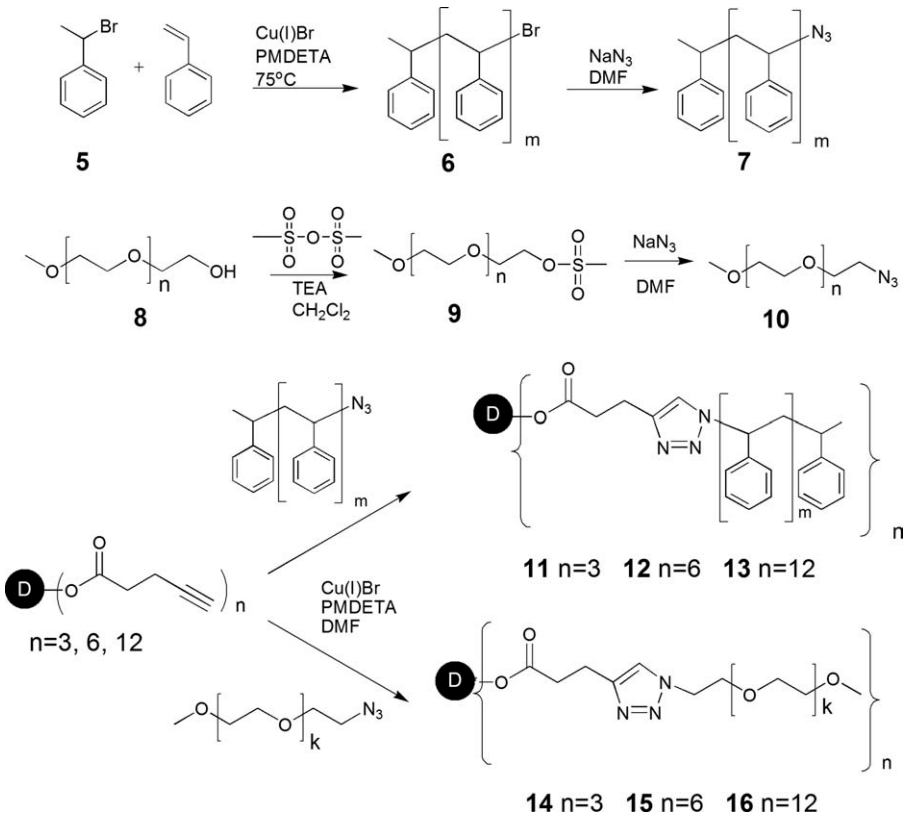


FIGURE 1 MALDI-TOF mass spectrum of dendritic core **2, 3**, and **4**.



SCHEME 2 Synthesis of 3, 6, and 12-arm star PS and PEG.

the dendritic cores used in this study. However, the cores' MALDI-TOF mass spectra were most valuable for confirming the truly monodisperse nature of alkynylated dendrimers as only one signal was observed in each one of their mass spectra (Fig. 1).

Because the linear arms must also exhibit narrow polydispersity and precise end group control, "living" polymerization techniques were used to prepare the azido-functional arms. The linear polystyrene precursors were synthesized via ATRP from a 2-ethylbromo benzene initiator **5**, using $Cu(I)Br/Cu(II)Br_2$ as catalyst and PMDETA as a ligand (Scheme 2).

To ensure near-quantitative functionalization with a bromide group at the end of each polymer chain, the reaction was stopped at a low conversion of the monomer (20%) yielding polystyrene **6** with a M_n of 2800, and PDI = 1.12 by GPC. Their MALDI-TOF mass spectra exhibited monomodal distributions and the observed signals matched the expected distribution caused by complexation to Ag^+ in addition to the previously reported elimination of HBr [$PSBr+Ag^+$-HBr].[39] Calculations from the MALDI-TOF mass spectra confirmed the well-defined character of the polymers, and agreed closely with the GPC data (M_n = 2500, PDI = 1.12; Fig. 2 and Table 1).

To confirm the end group identity, a previously reported procedure was used to calculate from mass spectral data the "residual mass (M_{res})" and the "end group mass (M_{end})." With a polymer of unknown end groups, it is impossible to calculate its end group mass exactly, exclusively from the mass spectra, because it is unknown how many monomer units contribute to a given *n*-mers mass, and how much is contributed by the end groups. A technique has been developed, however, to define the set of possible end group masses. This is performed by determining the smallest possible end group mass, M_{res}, by extrapolation from every *n*-mer in the mass spectra. From this value of M_{res}, the series of possible end group masses can be identified (by adding multiples of the monomer mass). Because the hypothetical end group structure is known for these linear and star polymers, the theoretical end group mass (M_{end}) can be easily

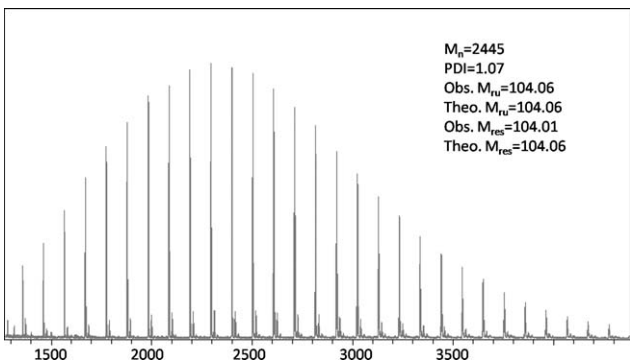


FIGURE 2 MALDI-TOF mass spectrum of polystyrene **6**.

TABLE 1 Repeat Unit (M_{ru}) and End Group Mass (M_{res}) Data for Polystyrene, **6**

N	Theoretical (**6**+Ag-HBr)$^+$	Observed (**2.1a**+Ag-HBr)$^+$	M_{ru} ($\mathbf{6}_n$-$\mathbf{6}_{n-1}$)	M_{res} for **6**
12	1459.72	1459.67		104.02
13	1563.78	1563.79	104.12	104.07
14	1667.84	1667.81	104.03	104.04
15	1771.91	1771.88	104.07	104.05
16	1875.97	1875.93	104.05	104.03
17	1980.03	1979.99	104.05	104.03
18	2084.09	2084.08	104.09	104.06
19	2188.16	2188.12	104.04	104.04
20	2292.22	2292.17	104.05	104.02
21	2396.28	2396.21	104.04	104.00
22	2500.34	2500.27	104.07	104.00
23	2604.41	2604.32	104.05	103.99
24	2708.47	2708.39	104.07	104.00
25	2812.53	2812.44	104.05	103.99
26	2916.60	2916.49	104.05	103.97
27	3020.66	3020.54	104.05	103.96
28	3124.72	3124.59	104.05	103.95
29	3228.78	3228.68	104.09	103.97
		Average	104.06	104.01
		Theoretical	104.06	104.06

determined and compared with the calculated mass. For example, in the case of the bromide terminated polymer, which was known to ionize via elimination of HBr and complexation with Ag^+, the mass of each monoisotopic peak was determined, and the trend extrapolated to the smallest possible positive value, which yielded and M_{res} of 104.01, and a repeat unit mass of 104.06. The repeat unit mass agrees with the expected value (104.0626), while the end group mass could be one of the series 104.01 + n(104.06). In this case $n = 0$ yields 104.01, which agrees closely with the theoretical value of 104.06. For the larger 12-arm star polymers, the substantial mass of the core yields solutions where $n >> 0$.

To enable their click conjugation, the bromine end groups were then converted to the azide by reaction with ~5 equivalents of sodium azide in DMF[40] The product retained the narrow polydispersity of the precursor, as judged by GPC ($M_n = 2800$, PDI = 1.12). The MALDI-TOF mass spectra provided additional strong evidence for the near-quantitative conversion to the azide end group, as the previously described bromide resonances were completely lost, and a new set of resonances, dominated by the unique metastable fragmentation of the azide functionality $[PSN_3+Ag\text{-}N_2]^+$ (metastable ion observed as $[PSN_3\text{-}23]^+$) were observed in reflector mode. Further confirmation of the metastable nature of these signals was gained by acquiring the MALDI-MS spectra in linear mode (which does not exhibit the effects of metastable fragmentation), yielding a strong set of signals corresponding to $[PSN_3+Na]$ in addition to other in-source decay fragments (e.g., $[PSN_3+Ag\text{-}N_2(\text{in source})]^+$ observed as $[PSN_3\text{-}28.0]^+$ (Supporting Information Fig. S2 and Table S2). The unusual behavior has been observed for multiple azide containing polymers and examined in detail elsewhere.[41]

The azido-PEG was prepared following the previously reported procedure (Scheme 2).[42] By reacting with methanesulfonyl anhydride with monomethyl polyethylene glycol (PEG-OH) ($M_n = 2090$), the hydroxyl end group of PEG-OH **8** could be quantitatively converted to be mesylate group. This result was verified by their MALDI-TOF spectra, which exhibited a mass increase of 78.07 Da ($\Delta_{theo.}$= 77.98 Da) for each peak (Fig. 3).

Subsequently, the mesylated PEG **9** was reacted with 5 equivalent of sodium azide[40] to afford PEG with a single azide end group. Again, MALDI-TOF revealed that the azido-PEG **10** was formed near quantitatively with the expect shift of molecular weight ($\Delta_{obs.} = -52.03$ Da, $\Delta_{theo.} = -52.97$

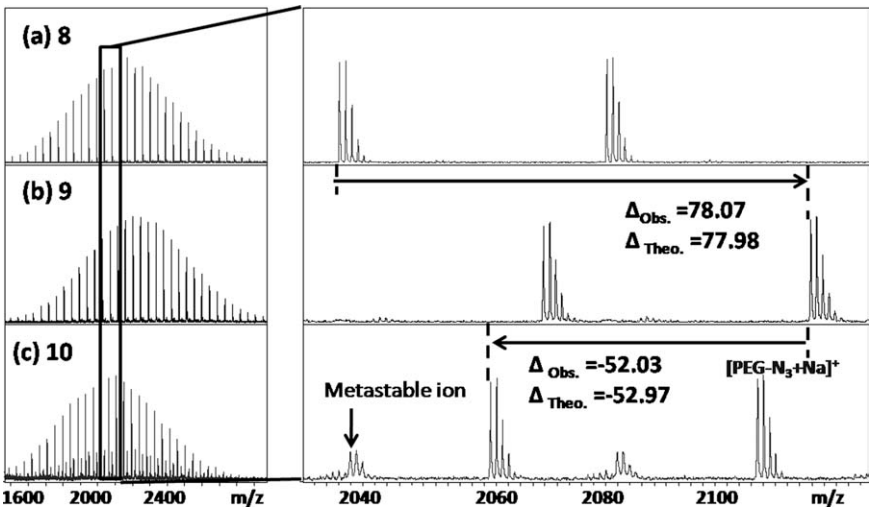


FIGURE 3 MALDI-TOF mass spectra of the PEG methyl ether 2000, **8**, mesylated PEG, **9**, and azido-PEG, **10**.

TABLE 2 MALDI-TOF and GPC Characterization of Purified Polystyrene Stars with Calculated Value in Parenthesis

Compound	PS Stars	M_n by MALDI	PDI	M_n by GPC	PDI	M_{ru}	M_{res}
7	PS-N_3	2,500	1.04	2,800	1.12	104.1 (104.1)	−0.1 (0)
11	3-arm	8,110 (7,860)	1.02	8,100	1.07	104.2 (104.1)	72.9 (72.9)
12	6-arm	15,000 (15,900)	1.01	13,600	1.04	104.2 (104.1)	60.9 (61.5)
13	12-arm	30,000 (32,100)	1.02	18,300	1.05	N/A	N/A

Da) and the appearance of the characteristic azide metastable ion using reflector mode. These end group transformation were also confirmed by the ^{13}C NMR spectrum, where the signal of CH_2OH (59 ppm) disappeared in mesylated PEG **9** spectrum and the signal of $-OSO_2CH_3$ (38 ppm) disappeared in **10** spectrum (Supporting Information Fig. S3).

The assembly of star polymers could then be performed using standard CuAAC "click" reaction condition (Scheme 2).[42] A slight excess of linear polymer was used to ensure the complete formation of star polymers, typically using 1:1.05 mole ratio of alkyne to azide. Because this approach requires very carefully measured ratios of linear polymer and dendritic core, the molecular weight of the polymer arms must be determined with high accuracy. For the below examples, the molecular weight used for stoichiometric calculations was determined from MALDI-TOF MS. All GPC and MALDI-TOF results suggest that the resultant crude star polymer sample exhibited quantitative coupling of polymer to each of the alkyne functionalities on the core molecules (Tables 2 and 3).

For the synthesis of the polystyrene three-arm star polymers, the GPC trace of the crude product exhibit a major distribution that exhibited a shift to a larger hydrodynamic volume, and only a small trace of residual "one-arm" linear polymer. The MALDI-TOF mass spectra of the product exhibited an approximate threefold increase in the molecular weight (Table 4).

In addition, end group analysis of the product agrees with the expected mass increase for three couplings to the tris-alkyne core, for example, the expected exact mass for a three arm polymer with 24 repeat unit per arm ($n = 72$) is 8096.09 (including silver counterion), and a signal is observed at 8096.08. Alternatively, using previously reported techniques, the "residual mass" of the "end groups" (all portions of the star that cannot be accounted for by styrene repeat units) can be calculated from the MALDI-TOF MS as 72.9 Da, which matches the theoretical value of 72.9 Da. Furthermore, the quantitative reaction of the azides can be confirmed by the fact that the products no longer exhibit any metastable fragmentation, consistent with previous observations, which confirm that the metastable decay characteristic of polymer azides is lost when they are converted to the relatively hearty triazole linkages.[43] Notably, no signal is observed in the molecular weight range of 3000 to 5000 that would correspond to the star with only two coupled arms.

Because of the difficulty in precisely determining the average molecular weight of the azide functionalized arms, a slight excess was required to ensure sufficient coupling to yield quantitatively coupled cores. As a result, a method of purification must be optimized to isolate the desired star polymer product from trace amounts (typically ~5%) of unreacted polymer arms. Many different technologies have been developed to purified polymers, such as fractionation,[37] dialysis,[44] GPC,[45] preparative GPC,[46] liquid chromatography at critical condition,[47] modified high performance liquid chromatography,[48] silica gel chromatography,[27] solid resin as scavenger,[49] and so forth. In this study, many of these have been investigated to ascertain the most effective and high-throughput technique for isolating "pure" star polymers. GPC and preparative GPC have demonstrated their ability to isolate the pure star polymers but are only amenable to purifications on small scales, therefore other techniques were sought. Dialysis is attractive for separating compounds of disparate sizes (e.g., removal of residual monomer from a polymer) but is less effective when there is a less substantial size difference. Even though the molecular weight increases substantially for the six-arm and 12-arm stars relative to the "one-arm" linear precursors, the more compact confirmation of the resulting star polymers reduces their size substantially in solution, complicating their separation by dialysis. In theory, scavenging resins functionalized with complimentary "click" functionalities offer a relatively simple and appealing method for removing unattached arms.[50,51] This method was investigated for a number of the crude "click" star-formation reaction mixtures, however, proved ineffective at removing all unreacted linear arms. For example during the synthesis

TABLE 3 MALDI-TOF and GPC Characterization of Purified PEG Stars with Calculated Value in Parenthesis

Compound	PEG Stars	M_n by MALDI	PDI	M_n by GPC	PDI	M_{ru}	M_{res}
10	PEG-N_3	2,110	1.01	2,700	1.02	44.0 (44.1)	13.0 (13.0)
14	3-arm	6,600 (6,690)	1.01	9,600	1.03	44.1 (44.1)	2.8 (2.8)
15	6-arm	13,600 (13,600)	1.01	14,100	1.04	44.0 (44.1)	13.7 (13.8)
16	12-arm	29,000 (27,400)	1.01	14,200	1.12	N/A	N/A

TABLE 4 Repeat Unit (M_{ru}) and Residual Mass (M_{res}) Data for Purified 3-Arm Star Polystyrene, **11**

n	Theoretical (**11**+Ag)$^+$	Observed (**11**+Ag)$^+$	M_{ru} (**11**$_n$-**11**$_{n-1}$)	M_{res} for **11**
66	7471.2	7470.9		72.6
67	7575.3	7575.1	104.1	72.6
68	7679.5	7679.2	104.2	72.6
69	7783.6	7783.5	104.3	72.8
70	7887.8	7887.6	104.1	72.7
71	7991.9	7992.0	104.4	72.9
72	8096.1	8096.1	104.1	72.9
73	8200.2	8200.3	104.2	73.0
74	8304.4	8304.6	104.3	73.1
75	8408.5	8408.8	104.2	73.2
76	8512.7	8513.1	104.2	73.3
		Average	104.2	72.9
		Theoretical	104.1	72.9

of the three-arm star polystyrene, a solid resin bearing alkyne functional groups was added to the crude star polymer products and the "click" conditions (Cu(I)Br/PMDETA) were reapplied (Scheme 3).

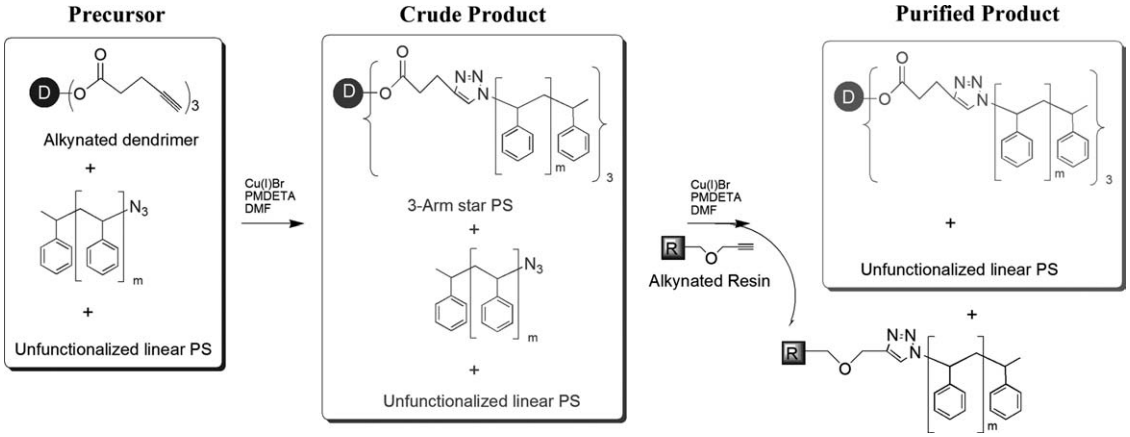
The GPC traces of the scavenger product showed only a slight reduction in the linear impurities (Fig. 4).

Additional scavenging attempts on the same sample did not seem to further reduce the amount of linear impurity. Similar results were observed for other PS and PEG stars, suggesting that a trace amount of the linear polymer had inactive end groups, thus preventing it from coupling to both the multiarm cores and the solid phase resin during subsequent attempts to scavenge. This was not unexpected considering the likelihood of losing a trace amount of end group functionality during ATRP, or in the case of PEG, the likelihood of a trace amount of a side reaction (e.g., hydrolysis), during the two-step end group transformation.

Purification by fractionation was an attractive alternative, as it should be insensitive to incomplete end group functionalizations, however, often requires multiple inefficient precipitations, and therefore a substantial reduction in yields. Using a separatory funnel, the crude-arm star PS reaction mixture was dissolved in toluene, and then methanol was added dropwise until the solvent turned cloudy. The solution was set overnight resulting in the formation of an oily layer in the bottom of the funnel. Characterization by GPC and MALDI TOF MS verified that the bottom layer was enriched with the high-molecular weight portions of the crude reaction mixture. After two fractionations, the GPC trace verified the removal of the small amount the linear starting material, and pure star polymers were obtained with low PDI (M_n = 8100; PDI = 1.07; Fig. 5).

The removal of the linear polymer distribution around m/z = 2600 in the MALDI-TOF mass spectra was particularly convincing, as it is well known that lower molecular weight analytes exhibit much stronger signal in MALDI-TOF MS analysis. Finally, although the well-defined one-arm distribution (MW ~2000–4000 with a monomer spacing of 104) has been removed, close examination of the baseline shows a weak, broad distribution (with unresolved monomer spacing) that is calculated to have a M_n of about 3100. These observations are consistent with triply charged polymer (Fig. 6).[52]

Using analogous synthetic and purification procedures, the six-arm and 12-arm polystyrene stars were also prepared. The crude products of both exhibited a large increase in size as judged by GPC and the expected increases in molecular weight as calculated by their MALDI-TOF mass spectra (Figs. 5 and 6). In the case of the six-arm polymer, the observed M_n was within 10% of the expected value ($M_{n\ theo}$ = 15,900, $M_{n\ obs}$ = 15,000) and a similar trend was noted for the 12-arm polymer ($M_{n\ theo}$ = 32,100, $M_{n\ obs}$ = 30,000). MS end group analysis again confirmed that the signal distributions agreed with those calculated, as further confirmed by the observed residual masses (Supporting Information Table S3). After purification by fractional precipitation, the six-arm and 12-arm star polymers exhibit a monomodal product with a



SCHEME 3 Purification of three-arm star PS by scavenging with alkyne functionalized solid phase resin.

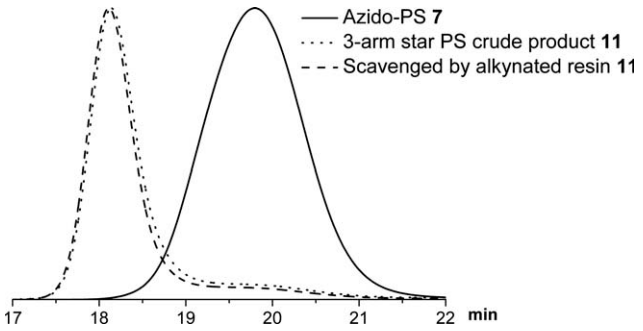


FIGURE 4 GPC spectrum of scavenged three-arm star polystyrene **7** and **11**.

very narrow polydispersity both by GPC (six-arm PDI = 1.04, 12-arm PDI = 1.05) and by MALDI-TOF MS (six-arm PDI = 1.01, 12-arm PDI = 1.02). MALDI-MS analysis confirms the loss of the "one-arm" PS precursor, (M_n ~2000–4000) but again shows trace amounts of triply charged species in a distinctly different mass range (e.g., for the six-arm star MW = 4500–6500 in Fig. 6). It should be noted that a slight increase in the M_n of the purified product was observed, most likely a result of the fractionation procedure favoring the precipitation of higher molecular weight star polymers. In addition, low MW analysis of the 12-arm product was not possible, because the laser power required for ionization of the high MW product resulted in substantial matrix noise below m/z = 15,000.

To access well-defined hydrophilic stars, the attachment of mono-azide functionalized PEG to the same dendritic cores was also explored. Using analogous click coupling conditions, the PEG-N_3 linear arms and the trialkyne core, were coupled to generate three-arm star polymers. Before purification, the GPC trace revealed a nearly monomodal product with the expected increase in hydrodynamic volume, though the calculated M_n values were inaccurate because they were calibrated against linear polystyrene standards (M_n = 9660, PDI = 1.03; Fig. 7 and Table 3).

The MALDI-TOF mass spectra exhibited a single distribution with a M_n 6570, close to the calculated value of the expected three arm star, 6680. Again, rigorous calibration using dendritic standards enables the accurate verification of each signal within the observed mass spectra. For example, the calculated mass of the three-arm star polymer with exactly 138 ethylene glycol repeat units (corresponding to an average of 46 repeating units for each arm) when complexed to sodium is 6633.8 Da, while the observed signal was 6633.6 Da (Table 5).

Further verification across the entire distribution was achieved by comparing the calculated residual mass of 2.8 with the theoretical value of 2.8. Despite the increased signal strength for lower molecular weight analytes, no MS signals were observed that corresponded to star polymers with one or more failed click coupling reactions.

Although the fractionation approach enabled successful isolation of the pure polystyrene star polymers, the yields were generally low (around 20%) and the technique very time-consuming. For the PEG stars, preparative size exclusion chromatographic separation using a packed column of "Bio-Beads" proved to be a relatively fast and effective technique, also afforded substantially improved yields.[27] These were generally performed on a 20–100 mg scale using THF as the mobile phase, while collecting fractions in which the lower molecular weight linear impurities eluted last. Parallel analysis of each fraction by GPC and MALDI provide comprehensive characterization (Fig. 7). In the early fractions, Figure 7(c) oligomeric impurities were observed in the GPC, which were not observed in the crude precursor. It is presumed that trace amounts of higher molecular weight impurities only became visible because they were concentrated during chromatographic purification, though the possibility of contamination cannot be ruled out. The middle fractions exhibit a very pristine three-arm star PEG, as judged both MALDI-TOF MS and GPC, with the only trace amounts of low molecular weight signal in the mass spectra confidently assigned

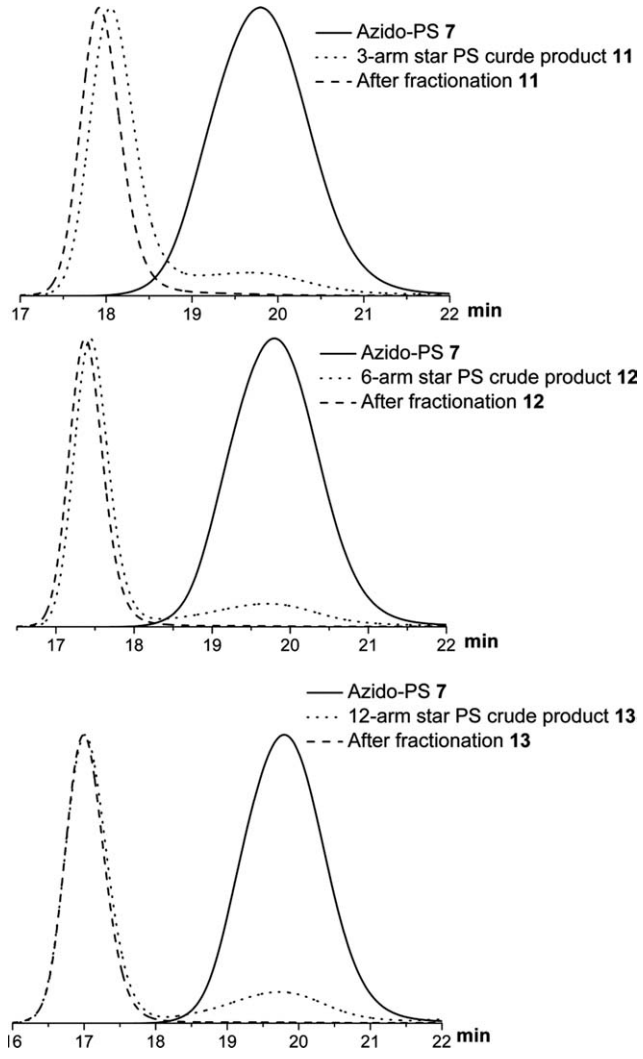


FIGURE 5 GPC spectra of three-arm star PS (**11**), six-arms star PS (**12**), and 12-arms star PS (**13**).

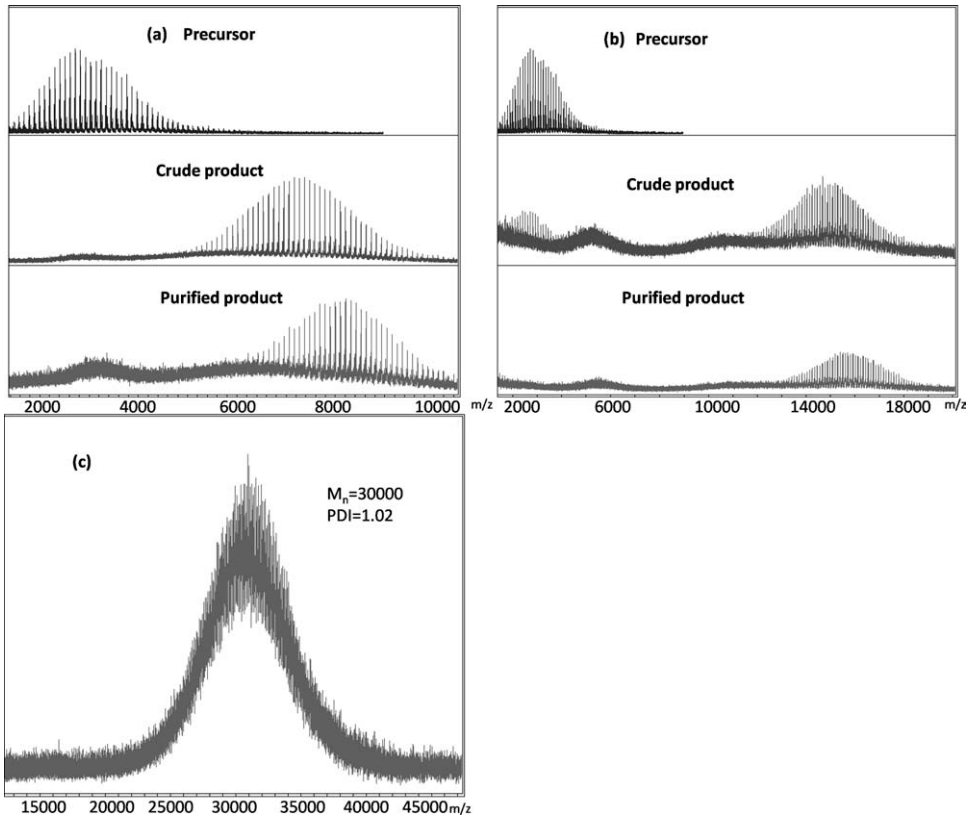


FIGURE 6 MALDI-TOF mass spectrum of three-arm PS **11** (a), six-arm star PS **12** (b), and 12-arm star PS **13** (c).

to double and triple charged species. As expected, the late fractions show a mixture of three-arm star and linear precursor, but again the linear precursor can be distinguished from multiple charged species because of the clear 44.0 Da (monomer mass) spacing between each signal.

Impurity of one, two-arm products was synthesized purposely using 2 equivalent of azido-PEG to tris-alkyne. MALDI-TOF MS showed the residual mass matched the expected by products. To confirm the ability to identify all likely impurities during the coupling reaction, a coupling

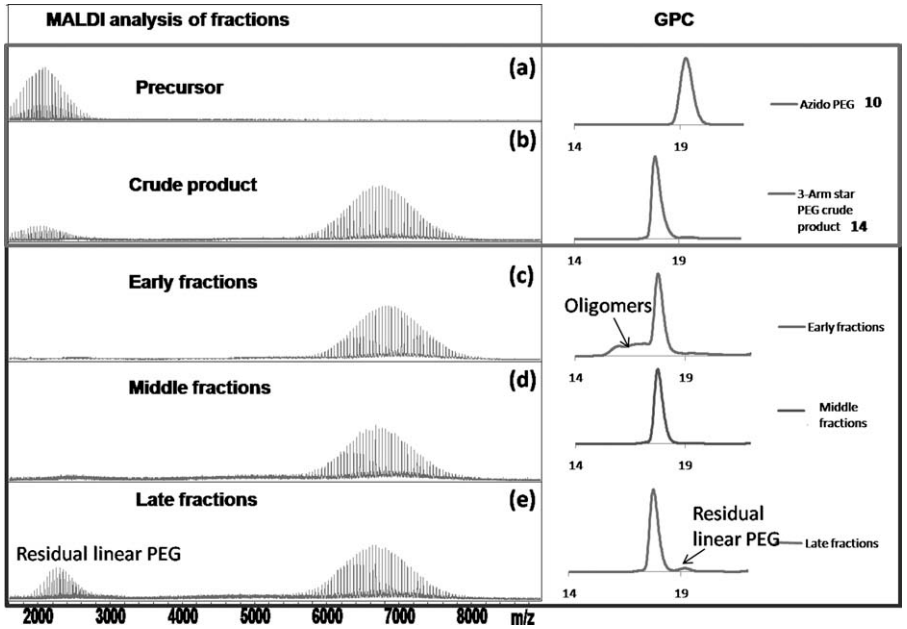


FIGURE 7 MALDI-TOF and GPC characterization of three-arm star PEG fractions from "Bio-Bead" chromatography.

TABLE 5 Repeat Unit (M_{ru}) and Residual Mass (M_{res}) Data for Purified 3-Arm PEG, **14**

n	Theoretical (**14**+Na)$^+$	Observed (**14**+Na)$^+$	M_{ru} (**14**$_n$-**14**$_{n-1}$)	M_{res} for **14**
131	6325.4	6325.3		2.8
132	6369.5	6369.2	43.9	2.6
133	6413.5	6413.3	44.1	2.7
134	6457.6	6457.3	44.0	2.6
135	6501.7	6501.4	44.1	2.6
136	6545.7	6545.4	44.1	2.7
137	6589.8	6589.5	44.1	2.7
138	6633.8	6633.6	44.1	2.7
139	6677.9	6677.7	44.1	2.8
140	6721.9	6721.7	44.0	2.7
141	6766.0	6765.6	43.9	2.6
142	6810.0	6810.0	44.4	2.9
143	6854.1	6853.9	43.8	2.7
144	6898.1	6897.9	44.1	2.8
145	6942.2	6941.9	43.9	2.6
146	6986.2	6986.1	44.2	2.8
147	7030.3	7030.0	43.9	2.7
148	7074.3	7074.2	44.1	2.8
		Average	44.1	2.7

with an insufficient amount of linear "arms" (~2 equivalents per three-arm core) was purposely performed. The resulting mixture of one-, two- and three-armed stars was characterized by MALDI TOF MS to confirm the unambiguous identification of each major component (Fig. 8 and Supporting Information Tables S4 and S5).

For one-arm impurity, the M_{res} was 20.9, close to the theoretical value of 21.0. The M_{res} of two-arm impurity was 33.9, while the expected value is 34.0.

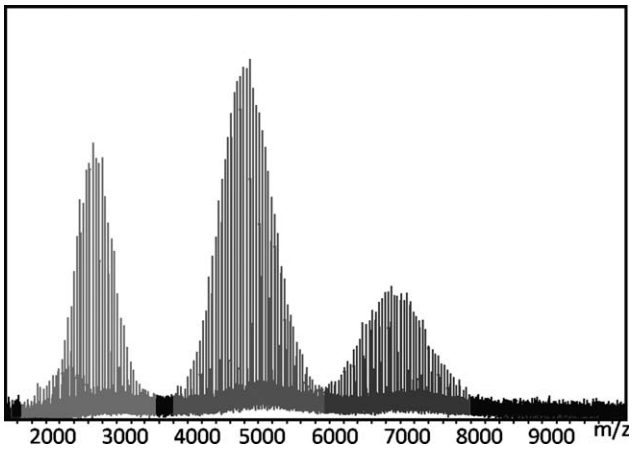


FIGURE 8 MALDI-TOF spectrum of incompletely coupled stars with only one arm (left), two arms (middle) and all three arms (right) of PEG on the trisalkyne core, **2**.

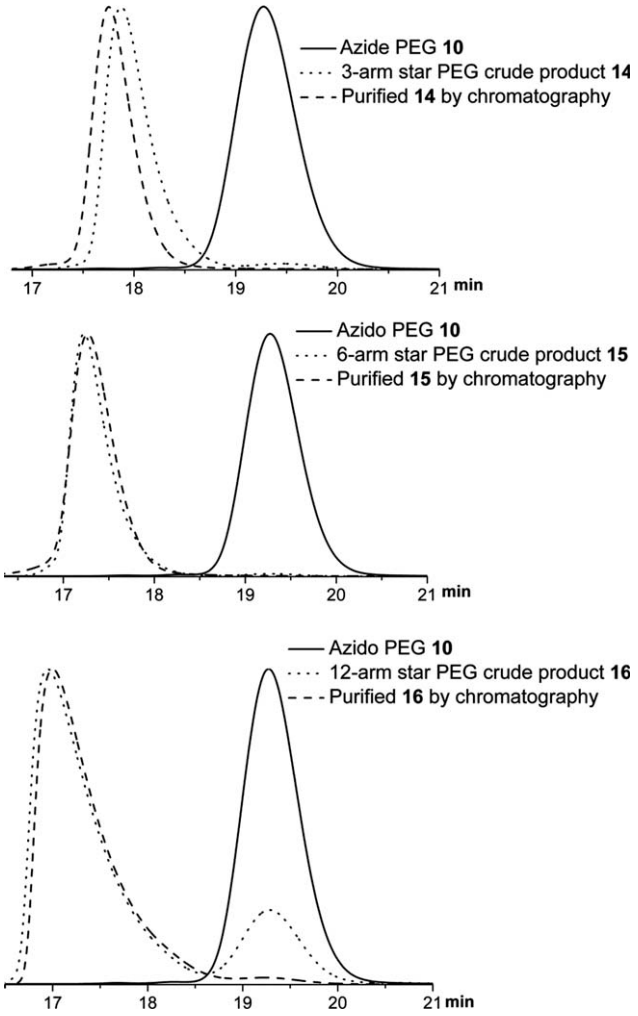


FIGURE 9 GPC of three-arm star PEG **14**, six-arms star PEG **15**, and 12 arms star PEG **16**.

Using analogous techniques, the PEG six-arm and 12-arm stars were prepared and purified. Characterization by GPC and MALDI-TOF provided strong evidence for a quantitative coupling of six-arm PEG, with the products after Biobead

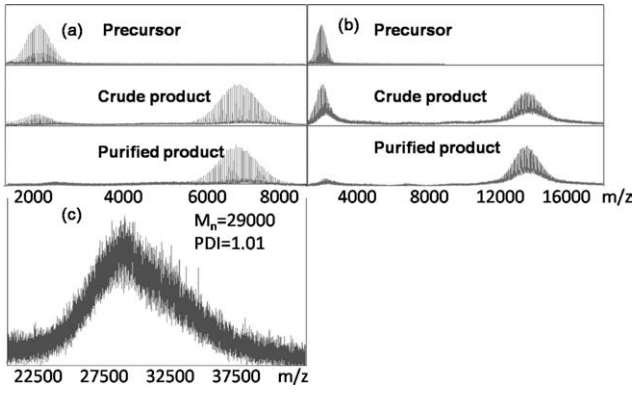
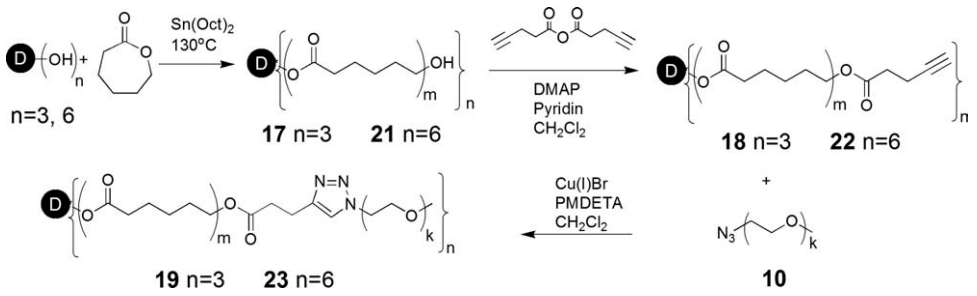


FIGURE 10 MALDI-TOF mass spectra of three-arm PEG **14** (a), six-arm star PEG **15** (b), and 12-arm star PEG **16** (c).



SCHEME 4 Synthesis of three- and six-arm star PCL-*b*-PEG **19** and **23**.

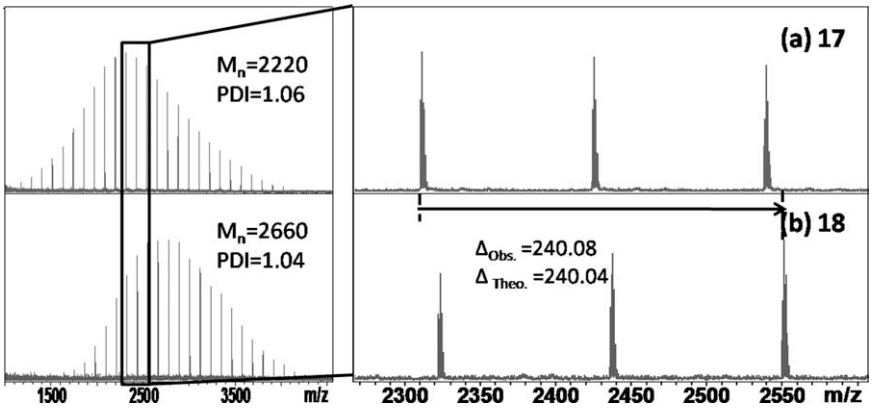


FIGURE 11 MALDI-TOF MS spectrum of three-arm star PCL **17** and its alkynylated product **18**.

purification exhibiting a narrow monomodal distribution in its GPC trace (PDI = 1.04) as well as its MALDI mass spectrum (PDI = 1.01) [Figs. 9 and 10(b) and Supporting Information Table S6].

As predicted, the M_n calculated from the MALDI-TOF mass spectra provides a more accurate value ($M_{n\ obs}$ = 13,600) than GPC ($M_{n\ obs}$ = 14,100) for the molecular weight of the PEG six-arm star ($M_{n\ theo}$ = 13,600). The purified 12-arm star PEG also exhibited monomodal products based on the GPC trace (M_n = 14,200, PDI = 1.12) [Figs. 9 and 10(c)]. The MALDI-TOF mass spectrum enabled the verification of the quantitative formation of pure 12-arm star with M_n of 29,100 ($M_{n\ theo}$ = 27400) and PDI of 1.01 without evidence of 11-arm or 10-arm impurities.[53] Similar with the 12-arm star PS, the MALDI-TOF mass spectra of 12-arm PEG stars could not enable visualization of low-molecular weight range due to matrix noise.

Well-defined three-arm star block copolymers could be synthesized in at least three different ways: diblock arms could be grown divergently from a core molecule, preformed linear diblocks could be coupled to a functionalized core, or homopolymer arms could be coupled to the ends of a functionalized three-arm star homopolymer.[54] The last of these three approaches was tested by coupling linear PEG to a PCL three-arm star core (Scheme 4)

The PCL three-arm star was prepared via polymerization of ε-caprolactone in bulk from 1,1,1-tris(hydroxymethyl)ethane using $Sn(Oct)_2$ catalysts at 130 °C. Subsequently, the hydroxyl end groups of **17** were esterified with pentynoic acid anhydride to quantitatively functionalize each end with the requisite alkyne for click coupling. The GPC trace of the

TABLE 6 MALDI-TOF Data for 3-Arm PCL-OH (**17**) and 3-Arm PCL-Alkyne (**18**)

n	Theoretical (**17**+Na)$^+$	Observed (**17**+Na)$^+$	Theoretical (**18**+Na)$^+$	Observed (**18**+Na)$^+$
12	1511.885	1511.914	1751.964	1751.948
13	1625.953	1626.008	1866.032	1866.037
14	1740.022	1740.087	1980.1	1980.129
15	1854.09	1854.165	2094.168	2094.195
16	1968.158	1968.236	2208.236	2208.27
17	2082.226	2082.309	2322.304	2322.338
18	2196.294	2196.379	2436.373	2436.404
19	2310.362	2310.449	2550.441	2550.472
20	2424.43	2424.515	2664.509	2664.536
21	2538.498	2538.579	2778.577	2778.601
22	2652.566	2652.646	2892.645	2892.671
23	2766.634	2766.715	3006.713	3006.739
24	2880.702	2880.779	3120.781	3120.806
25	2994.77	2994.847	3234.849	3234.866
26	3108.838	3108.909	3348.917	3348.934
27	3222.907	3222.986	3462.985	3463.006
28	3336.975	3337.049	3577.053	3577.083
29	3451.043	3451.132	3691.121	3691.154

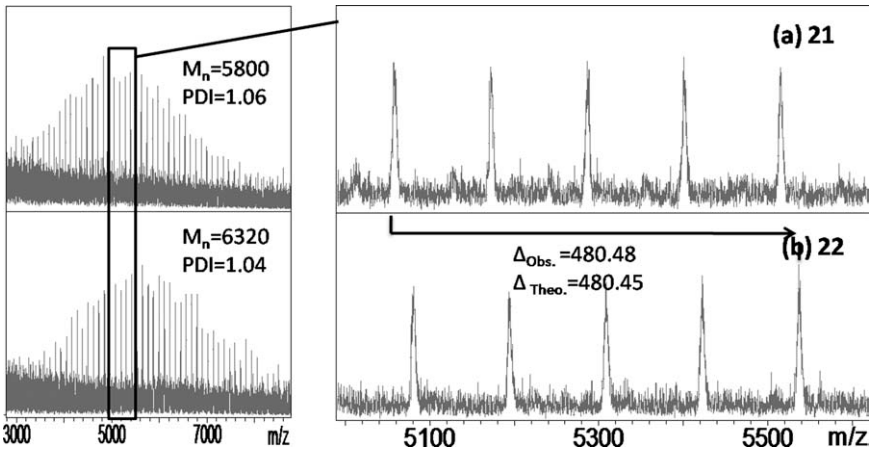


FIGURE 12 MALDI-TOF MS spectrum of six-arm star PCL **21** and its alkynylated product **22**.

hydroxyl terminated star **17**, showed a monomodal distribution with a narrow polydispersity and a M_n of 2230 when corrected for PCL (4650 when calibrated against PS standards) The MALDI-TOF mass spectra of the hydroxyl terminated polymer confirmed the synthesis of well-defined three-arm star PCL without any measurable amount of linear polymer byproducts that might occur from initiation from water or other small molecule impurities [Fig. 11(a)].

The observed repeat unit molecular weight (114.07) agreed closely with the theoretical value (114.07) and the M_n calculated by MALDI-TOF MS (2220) agreed closely with the GPC values. The identity of the core could also be confirmed by MS calculations of the residual mass (6.1 Da), which agrees closely with the theoretical value (6.0 Da). The attachment of the three pentynoate esters via esterification with 4-pentynoic acid anhydride could be confirmed by monitoring the mass shifts of a particular signal in the MALDI-TOF MS, for example, the 19-mer. The ion corresponding to the sodium adduct of the hydroxyl functionalized star with a total of 19 repeat units of caprolactone has a theoretical exact molecular mass of 2310.4 Da (observed 2310.4 Da). On functionalization with 3 pentynoate esters, the molecular weight for **18** is expected to increase by 240.04 Da (addition of 3 pentynoic groups each weighing 80.01 Da), which yields a theoretical molecular mass of 2550.4 (observed 2550.5) [Fig. 11(b) and Table 6].

The alkynylated PCL star **18** was then reacted with 2 kDa PEG-N_3 **10** under standard click conditions to yield the three-arm star PCL-*b*-PEG **19**. After purification, the GPC trace revealed the higher molecular weight star block copolymers were formed with narrow PDI (M_n = 13,600, PDI = 1.03) (Fig. 13). The MALDI-TOF mass spectra exhibited a M_n of 8970, close to calculated value of 9720. Detailed end group analysis of the block copolymer was not possible, however, because the non-equivalent masses of the different monomers lead to a complex distribution in which individual signals are no longer resolved (Fig. 14).

The six-arm PCL-PEG block copolymer was synthesized by following the same procedure but using **20** as the core molecule. The six-arm star PCL was prepared via polymerization of ε-caprolactone in bulk using $Sn(Oct)_2$ catalysts at 130 °C. From ^{1}H NMR data, we can see all peak of –C**H**$_2$OH group in core initiator shifted from 3.70 ppm double peak to 4.03 ppm confirming that every hydroxyl group from the core initiate the polymerization of a PCL arm (Supporting Information Figs. S5 and S9). The GPC shows a monomodal distribution with a narrow polydispersity (M_n = 7760, PDI = 1.11). The MALDI-TOF mass spectra also confirm the synthesis of well-defined six-arms PCL stars [Fig. 12(a)] and their quantitative functionalization with pentynoate esters (480.48 increase in mass; Fig. 12 and Supporting Information Table S7).

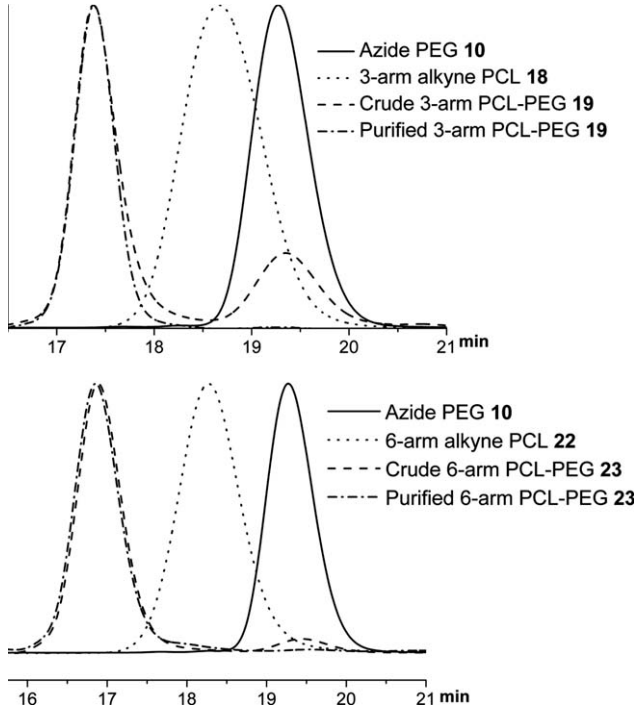


FIGURE 13 GPC of three-arm star and six-arm star PCL-*b*-PEG.

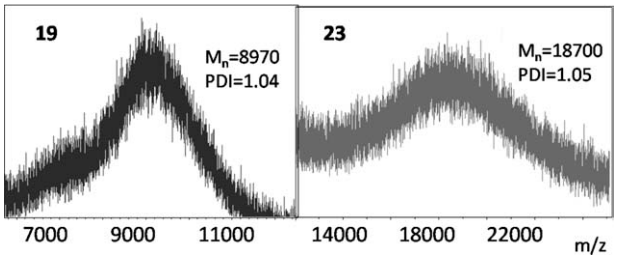


FIGURE 14 MALDI-TOF MS spectrum of three-arm (19) and six-arms (23) star PCL-*b*-PEG.

The alkynylated PCL star **22** was then reacted with 2 kDa PEG-N_3 **10** under standard click conditions to yield the six-arm star PCL-*b*-PEG. The GPC trace of the crude product confirmed a substantial increase in the molecular weight, as well as a trace of unreacted PEG. After using Bio-bead method to remove the unreacted PEG, a high pure six-arms star polymer with narrow PDI (GPC: M_n = 23,700, PDI = 1.07) was isolated (Fig. 13)

The MALDI-TOF mass spectra exhibited a M_n of 18,700, close to theoretical value of 18,980 (Fig. 14).

CONCLUSIONS

With the evolution of optimized dendronization methods and living polymerization techniques, well-defined polymeric components can be prepared with an exact number of functional groups. The recent focus on highly efficient couplings, chief among them the CuAAC coupling reaction, enables complex architectures to be assembled from these components yielding complex macromolecular architectures with unprecedented structural purity. In the case of star polymers, this has been demonstrated with both hydrophobic and hydrophilic arms attached via the "graft onto" method with dendritic cores. Although many purification methods were investigated, fractional precipitation and chromatographic purification seem the most general, scalable, and successful in removing residual linear starting materials. The exceptional purity of the stars were confirmed through detailed characterization by both GPC and MALDI-TOF MS. In addition, this approach to making multiarm star polymers shows promise toward making well-defined block copolymer stars via the same click route. The ability to synthesize well-defined star polymers and confirm their purity is critical to advancing their biomedical applications.

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