Dendron–Polymer Conjugates via the Diels–Alder “Click” Reaction of Novel Anthracene-Based Dendrons

Ozgul Gok, Sezin Yigit, Meliha Merve Kose, Rana Sanyal, Amitav Sanyal

Department of Chemistry, Bogazici University, Bebek, Istanbul 34342, Turkey

Correspondence to: A. Sanyal (E-mail: amitav.sanyal@boun.edu.tr) or R. Sanyal (E-mail: rana.sanyal@boun.edu.tr)

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ABSTRACT: Diblock and triblock dendron–polymer conjugates containing biodegradable polyester dendron blocks and polyethylene glycol (PEG) polymer were synthesized using the Diels–Alder “click” cycloaddition reaction. PEG polymers with furan-protected maleimide functionality were synthesized and reacted with biodegradable polyester dendrons containing an anthracene moiety at their focal point. First through third generations of biodegradable polyester dendrons containing an anthracene unit at their focal point were synthesized using a divergent strategy. Efficient conjugation of the dendrons to polymers was demonstrated using ^{1}HNMR and size exclusion chromatography. This modular approach provides an easy access to the design of multivalent PEG conjugates. © 2013 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 3191–3201

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INTRODUCTION Attachment of proteins, peptides, and small drug molecules to appropriate polymeric supports has demonstrated increase in their therapeutic value by addressing challenges such as poor solubility, rapid excretion, and nontargeted delivery.[1–4] Over the past decades, owing to major advancements in the area of drug delivery via conjugation of therapeutic materials to polymers, “polymer therapeutics” has emerged as a discipline.[5–9] Increased *in vivo* efficacy of polymer conjugates observed owing to enhanced permeation and retention effect, which is preferential uptake and accumulation of macromolecular entities in tumor owing to leaky vasculature, is one of the key advantages of polymer drug conjugates.[10–13] One of the most widely explored polymeric supports in this regard has been polyethylene glycol (PEG).[14,15] Although the attachment to PEG or PEGylation offers several advantages such as increased bioavailability, reduced toxicity, and prolonged residence time in the body,[16–18] one of the major limitations of using linear PEG polymer as a support is its low loading capacity. One alternative approach to address this problem is to employ branched PEG, that is multiarmed PEGs.[19–21] In this regard, recent years have witnessed an increasing interest in the synthesis of well-defined biocompatible and preferably biodegradable polymeric supports owing to their potential drug delivery agents.[22–26] As opposed to their linear counterparts, these are more expensive and usually have higher polydispersities. Another approach that has been evaluated is to increase the multivalency of PEG by introduction of branching at the side chain or chain ends.[27–32] This can be achieved by introduction of well-defined dendritic end-groups.[33–36] Usually, the method employed for the synthesis of dendron polymer conjugates involves growth of the dendrons by using the PEG diol-based macroinitiator.[37,38] Novel modular approaches toward both polymer synthesis and modification have attracted great attention owing to the advantages offered by such processes. Recently, dendron–polymer–dendron conjugates were synthesized via Huisgen-type click cycloaddition[39–43] between telechelic azide groups containing PEG with polyester dendrons containing alkyne group at their focal point.[44] It was shown that such dendron–polymer conjugates can be utilized to fabricate functionalizable hydrogels.[45,46] In recent years, the Diels–Alder reaction has attracted considerable attention as a powerful metal-free conjugation reaction and to obtain a wide variety of discrete macromolecular constructs efficiently.[47–53]

Herein, we report the synthesis of novel dendron–polymer conjugates that allow facile access to multivalent PEG polymers using the Diels–Alder cycloaddition reaction. Novel biodegradable polyester dendrons containing an electron-rich anthracene group at their focal point and hydroxyl groups at their periphery were synthesized. These dendrons were reacted with either mono-functional or telechelic linear PEG polymers containing furan-protected maleimide group at

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chain ends to furnish diblock and triblock copolymers, respectively. Thus, reagent-free thermal Diels–Alder cycloaddition provides facile access to linear-PEG polymers containing several hydroxyl functional groups at chain ends for further functionalization (Fig. 1).

EXPERIMENTAL

Materials and Instrumentation

All reagents were obtained from commercial sources (Merck, Aldrich, and Alfa Aesar) and were used as received unless otherwise stated. Dry solvents (CH_2Cl_2, tetrahydrofuran [THF], and toluene) were obtained from ScimatCo Purification System and other dry solvents were dried over molecular sieves. Column chromatography was performed using silicagel-60 (43–60 nm). Thin-layer chromatography (TLC) was performed using silica gel plates (Kiesel gel 60 F254, 0.2 mm, Merck). Plates were viewed under 254 nm UV lamp otherwise plates were developed by $KMnO_4$ stain. Acetonide-2,2-*bis*(methoxy)-propionic anhydride (**1**) was prepared according to the previously published literature procedures.[55,56] PEG diol (2K) and monomethoxy PEG (2K) were dried under vacuum after azeotropic distillation with toluene. The dendron and polymer characterizations involved ^{1}HNMR spectroscopy (Varian, 400 MHz) at the Advanced Technologies Research and Development Center at Bogazici University, FlashEA^R 1112 Series Elemental Analyzer (CHNS Separation Column, PTFE; 2 m; 6 × 5 mm) and Fourier transform infrared (ATR-FTIR) spectroscopy (Thermo Fisher Scientific, Nicolet 380). The molecular weights were estimated by a gel permeation chromatography (GPC) analysis using a Viscotek GPCmax VE-2001 analysis system. PLgel (length/ID 300 mm × 7.5 mm, 5 μm particle size) Mixed-C column was calibrated with polystyrene standards (1–150K), using refractive index detector. THF was used as the eluent at a flow rate of 1 mL/min at 30 °C. A size exclusion chromatography system equipped with a Shimadzu RID-10A refractive index detector and PLgel (length/ID 8 × 300 mm, 10 μm particle size) Mixed-B columns was also used to obtain some of the molecular weights and molecular weight distributions. THF was used as mobile phase (1 mL/min, 30 °C) using a Shimadzu LC20AD pump.

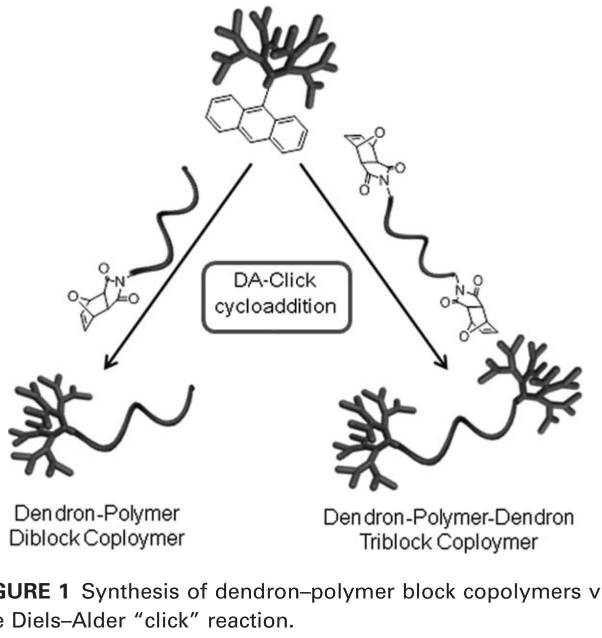


FIGURE 1 Synthesis of dendron–polymer block copolymers via the Diels–Alder "click" reaction.

Synthesis of First-Generation Anthracene-Functionalized Dendron (2)

Compound **1** was synthesized according to the previously reported literature procedures.[57,58] To a solution of anhydride **1** (4.25 g, 12.86 mmol) in CH_2Cl_2 (40 mL), 9-anthracene methanol (1.79 g, 8.58 mmol), 4-dimethylaminopyridine (DMAP) (0.42 g, 3.43 mmol), and pyridine (2.50 mL) were added. The mixture was stirred at room temperature for 20 h followed by quenching of excess anhydride with (1:1) mixture of pyridine and water (8.20 mL) for 5 h. Reaction mixture was extracted with 1 M of $NaHSO_4$ (3 × 60 mL), 10% of Na_2CO_3 (3 × 60 mL), and then with brine (1 × 60 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. The product was obtained as a yellow solid (5.70 g, 90% yield). The obtained solid (2.00 g, 5.30 mmol) was dissolved in MeOH (30 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of starting material was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_3$. The product was obtained by filtration to give **2** as a yellow solid (1.78 g, 99% yield).

^{1}HNMR ($CDCl_3$, δ, ppm) 8.51 (s, 1H, ArH), 8.30 (d, 2H, $J = 8.4$ Hz, ArH), 8.03 (d, 2H, $J = 8.4$ Hz, ArH), 7.57 (dd, 2H, $J = 8{,}4$, 6.4 Hz, ArH), 7.49 (dd, 2H, $J = 8{,}4$, 6.4 Hz, ArH), 6.21 (s, 2H, CH_2—Ar), 3.85 (d, 2H, $J = 11.2$ Hz, OCH_2), 3.66 (d, 2H, $J = 11.2$ Hz, OCH_2), 2.68 (br s, 2H, OH), 0.97 (s, 3H, $C(CH_3)$). ^{13}CNMR ($CDCl_3$, δ, ppm) 176.1, 131.3, 131.0, 129.4, 129.2, 126.8, 125.7, 125.2, 123.7, 68.4, 59.7, 49.4, 17.1. Anal. calcd. for G1-anthracene dendron [$C_{20}H_{20}O_4$]: C, 74.06; H, 6.21; O, 19.73. Found: C, 74.25; H, 6.44; O, 19.31.

Synthesis of Second-Generation Anthracene-Functionalized Dendron (3)

Compound **2** (0.32 g, 0.99 mmol) was added to a solution of DMAP (0.06 g, 0.49 mmol), pyridine (0.72 mL), and compound **1** (1.14 g, 3.45 mmol) in CH_2Cl_2 (10 mL). The mixture was then stirred at room temperature for 20 h followed by quenching of excess anhydride with (1:1) mixture of pyridine and water (2.40 mL) for 5 h. Reaction mixture was diluted with CH_2Cl_2 (30 mL) and extracted with 1 M of $NaHSO_4$ (3 × 40 mL), 10% of Na_2CO_3 (3 × 40 mL), and then with brine (1 × 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. Crude product was purified by column chromatography to give a yellow solid (0.48 g, 76% yield). Obtained compound (0.48 g, 0.76 mmol) was dissolved in MeOH (18 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of starting material was observed via TLC. The resin was then filtered off and washed with MeOH. Crude product was purified by column chromatography to give **3** as a yellow solid (0.38 g, 91% yield).

^{1}HNMR ($CDCl_3$, δ, ppm) 8.51 (s, 1H, ArH), 8.32 (d, 2H, J = 8.8 Hz, ArH), 8.02 (d, 2H, J = 8.8 Hz, ArH), 7.57 (dd, 2H, J = 8.8, 7.6 Hz, ArH), 7.49 (dd, 2H, J = 8.8, 7.6 Hz, ArH), 6.23 (s, 2H, CH_2—Ar), 4.34 (d, 2H, J = 11.2 Hz, CH_2 ester protons), 4.22 (d, 2H, J = 11.2 Hz, CH_2 ester protons), 3.70–3.51 (m, 8H, OCH_2), 2.90–2.86 (m, 4H, OH), 1.25 (s, 3H, $C(CH_3)$), 0.80 (s, 6H, $C(CH_3)$). ^{13}CNMR (CD_3COD, δ, ppm) 175.9, 175.6, 132.9, 132.4, 130.5, 131.2, 127.9, 127.2, 126.3, 125.1, 66.5, 65.8, 64.4, 60.7, 51.7, 18.2, 17.2. Anal. calcd. for G2-anthracene dendron [$C_{30}H_{36}O_{10}$]: C, 63.44; H, 6.61; O, 29.95. Found: C, 63.52; H, 6.82; O, 29.66.

Synthesis of Third-Generation Anthracene-Functionalized Dendron (4)

Compound **3** (0.40 g, 0.72 mmol) was added to a solution of DMAP (0.19 g, 1.55 mmol), pyridine (2.70 mL), and compound **1** (1.90 g, 5.75 mmol) in CH_2Cl_2 (18 mL). The mixture was then stirred at room temperature for 20 h. Excess anhydride was quenched with (1:1) mixture of pyridine and water (9 mL) for 5 h. Reaction mixture was diluted with CH_2Cl_2 (20 mL) and then extracted with 1 M of $NaHSO_4$ (3 × 40 mL), 10% of Na_2CO_3 (3 × 40 mL), and then with brine (1 40 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The residue was concentrated in *vacuo*. The product was obtained as a pure light yellow solid (0.83 g, 98% yield). Thus obtained compound (0.83 g, 0.71 mmol) was dissolved in MeOH (20 mL) and to this solution Dowex H^+ resin was added with a tip of spatula. The resulting mixture was stirred at room temperature until the consumption of **4** was observed via TLC. The resin was then filtered off and washed with MeOH. The crude product was purified by recrystallization with $CHCl_3$. The product was obtained by filtration to give **7** as a yellow solid (0.68 g, 95% yield).

^{1}HNMR (CD_3COD, δ, ppm) 8.56 (s, 1H, ArH), 8.42 (d, 2H, J = 8.8 Hz, ArH), 8.06 (d, 2H, J = 8.4 Hz, ArH), 7.60 (dd, 2H, J = 8.8, 6.8 Hz, ArH), 7.50 (dd, 2H, J = 8.8, 6.8 Hz, ArH), 6.26 (s, 2H, CH_2—Ar), 4.58 (br s, 8H, OH), 4.28–4.05 (m, 16H, CH_2 ester protons), 3.65 (d, 8H, J = 10.8 Hz, OCH_2 protons), 3.58 (d, 8H, J = 10.8 Hz, OCH_2 protons),1.23 (s, 3H, $C(CH_3)$), 1.12 (s, 12H, $C(CH_3)$), 0.99 (s, 6H, $C(CH_3)$). ^{13}CNMR (CD_3COD, δ, ppm) 183.6, 181.7, 181.4, 140.5, 140.1, 139.0, 138.3, 136.8, 135.4, 134.6, 133.8, 74.2, 73.9, 73.6, 73.2, 72.8, 59.9, 56.0, 55.8, 26.7, 25.9. Anal. calcd. for G3-anthracene dendron [$C_{50}H_{68}O_{22}$]: C, 57.64; H, 6.80; O, 35.56. Found: C, 57.65; H, 6.55; O, 35.80.

Synthesis of Mono-Maleimide PEG (7)

To a solution of dried monomethoxy PEG (2.00 g, 1 mmol) dissolved in THF (5 mL), triethylamine (0.21 mL, 1.50 mmol) was added at 0 °C. In a separate flask, succinic anhydride (0.17 mg, 1.70 mmol) and DMAP (0.024 mg, 0.20 mmol) were dissolved in THF (5 mL) and transferred drop wise onto the PEG solution at 0 °C for more than 30 min. The clear solution was stirred for 20 h at room temperature under N_2. The crude was concentrated and precipitated in cold diethyl ether. The product was filtered and dried under vacuum. The precipitation procedure was repeated twice to give pure mono-acid PEG as white solid **5** (1.95 g, 93% yield). Then, polymer **5** (1.50 g, 0.71 mmol) was dried under vacuum after azeotropic distillation with toluene. To a solution of **5** dissolved in CH_2Cl_2 (10 mL), the furan-protected maleimide group containing alcohol (**6**) (0.49 g, 2.14 mmol), DMAP (0.09 g, 0.07 mmol), and EDCI (0.15 g, 0.78 mmol) were added. The solution was stirred for 20 h at room temperature under N_2. To the reaction mixture, CH_2Cl_2 (20 mL) was added and the mixture was washed with saturated $NaHCO_3$ (2 × 15 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and all volatiles were evaporated. After precipitating twice in cold diethyl ether, pure mono-maleimide PEG was filtered and dried under vacuum as a white solid **7** (1.22 g, 75% yield).

^{1}HNMR ($CDCl_3$, δ, ppm) 6.48 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.22 (t, 2H, J = 4.8 Hz, OCH_2), 4.03 (t, 2H, J = 6.4 Hz, NCH_2), 3.82–3.41 (m, 4H, OCH_2CH_2 of PEG), 3.35 (s, 3H, OCH_3 of PEG), 2.82 (s, 2H, CH-CH bridge protons), 2.69–2.60 (m, 4H, CH_2C=O), 1.89 (tt, 2H, J = 6.0, 4.8 Hz, $NCH_2CH_2CH_2O$).

Synthesis of Bis-Maleimide PEG (9)

To a solution of dried PEG (2 g, 1 mmol) dissolved in THF (5 mL), triethylamine (0.42 mL, 2.99 mmol) was added at 0 °C. In a separate flask, succinic anhydride (0.34 g, 3.42 mmol) and DMAP (0.05 g, 0.40 mmol) were dissolved in THF (5 mL) and transferred drop wise onto the PEG solution at 0 °C for more than 30 min. The clear solution was stirred for 20 h at room temperature under N_2. The crude was concentrated and precipitated in cold diethyl ether. The product was filtered and dried under vacuum. The precipitation procedure was repeated twice to give pure bis-acid PEG as white solid **8** (2.13 g, 97% yield). Then, polymer **8** (1.50 g, 0.68 mmol) was dried under vacuum after azeotropic distillation with toluene. To a solution of 1a dissolved in CH_2Cl_2 (15 mL), the furan-protected maleimide group containing alcohol (**6**) (0.90 g, 4.10 mmol), DMAP (0.016 g, 0.14 mmol), and EDCI (0.29 g, 1.49 mmol) were added. The solution was stirred for 20 h at room temperature under N_2. To the reaction mixture, CH_2Cl_2 (30 mL) was added and the mixture was washed with saturated $NaHCO_3$ (2 × 30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and all volatiles were evaporated. After precipitating twice in cold diethyl ether, pure bis-maleimide PEG was filtered and dried under vacuum as a white solid **9** (1.40 g, 79% yield).

^{1}HNMR ($CDCl_3$, δ, ppm) 6.48 (s, 2H, CH=CH), 5.23 (s, 2H, CH bridgehead protons), 4.22 (t, 2H, J = 4.8 Hz, OCH_2), 4.03 (t, 2H, J = 6.0 Hz, NCH_2), 3.80–3.42 (m, 4H, OCH_2CH_2 of PEG), 2.82 (s, 2H, CH—CH bridge protons), 2.67–2.59 (m, 4H, CH_2C=O), 1.90 (tt, 2H, J = 6.0, 4.8 Hz, $NCH_2CH_2CH_2O$).

General Procedure for Diblock Copolymer Synthesis

Mono-maleimide PEG **(7)** (0.20 g, 0.09 mmol) and G3-anthracene dendron **(4)** (0.092 g, 0.09 mmol) were dissolved in toluene (4 mL) and the mixture was heated under nitrogen at 110 °C for 6 h. Then, all volatiles were removed *in vacuo* and after precipitating twice in cold diethyl ether, the product was filtered and dried under vacuum to give **P3** as a light yellow solid (0.22 g, 77% yield).

^{1}HNMR data for copolymer **P1:** ($CDCl_3$, δ, ppm) 7.39–7.29 (m, 4H, ArH), 7.19–7.16 (m, 4H, ArH), 5.54–5.42 (m, 2H, $CH_2OC{=}O$), 4.77 (s, 1H, CH bridgehead proton), 4.22 (t, 2H, J = 4.4 Hz, OCH_2), 3.90–3.83 (m, 4H, CH_2 ester protons), 3.79 (t, 2H, J = 4.8 Hz, NCH_2), 3.70–3.58 (m, 4H, OCH_2CH_2 of PEG), 3.49–3.42 (m, 2H, $NCH_2CH_2CH_2O$), 3.35 (s, 3H, OCH_3 of PEG), 3.28 (br s, 1H, CH—CH bridge proton), 3.18 (t, 1H, J = 6.8 Hz, CH—CH bridge proton), 2.97 (br s, 2H, OH), 2.64–2.55 (m, 4H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.12 (s, 3H, $C(CH_3)$).

^{1}HNMR data for copolymer **P2**: ($CDCl_3$, δ, ppm) 7.34–7.10 (m, 8H, ArH), 5.50–5.40 (m, 2H, $CH_2OC{=}O$), 4.71 (s, 1H, CH bridgehead proton), 4.44–4.36 (m, 2H, OCH_2), 4.25–4.16 (m, 4H, CH_2 ester protons), 3.96–3.90 (m, 2H, NCH_2), 3.74–3.39 (m, 18H, OCH_2CH_2 of PEG, CH_2 ester protons, $NCH_2CH_2CH_2O$, and OH), 3.30 (s, 3H, OCH_3 of PEG), 3.24–3.11 (m, 2H, CH—CH bridge protons), 2.57–2.52 (m, 4H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.12 (d, 6H, J = 6.0 Hz, $C(CH_3)$), 1.02 (d, 3H, J = 4.8 Hz, $C(CH_3)$).

^{1}HNMR data for copolymer **P3**: ($CDCl_3$, δ, ppm) 7.41–7.14 (m, 8H, ArH), 5.62–5.39 (m, 2H, $CH_2OC{=}O$), 4.76 (d, 1H, J = 3.2 Hz, CH bridgehead proton), 4.40–4.20 (m, 16H, OCH_2, NCH_2, and CH_2 ester protons), 3.80–3.13 (m, 35H, OCH_2CH_2 of PEG, CH_2 ester protons, OCH_3 of PEG, $NCH_2CH_2CH_2O$, CH—CH bridge protons, and OH), 2.63–2.54 (m, 4H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.31 (s, 3H, $C(CH_3)$), 1.28 (s, 3H, $C(CH_3)$), 1.26 (s, 3H, $C(CH_3)$), 1.05 (s, 6H, $C(CH_3)$).

General Procedure for Triblock Copolymer Synthesis

Bis-maleimide PEG **(9)** (0.20 g, 0.08 mmol) and G3-anthracene dendron **(4)** (0.16 g, 0.15 mmol) were dissolved in toluene (5 mL) and the mixture was heated under nitrogen at 110 °C for 6 h. Then, all volatiles were removed *in vacuo* and after precipitating twice in cold diethyl ether, the product was filtered and dried under vacuum to give **P6** as a light yellow solid (0.29 g, 84% yield).

^{1}HNMR data for copolymer **P4**: ($CDCl_3$, δ, ppm) 7.38–7.18 (m, 8H, ArH), 5.54–5.40 (m, 2H, $CH_2OC{=}O$), 4.76 (s, 1H, CH bridgehead proton), 4.22 (br s, 2H, OCH_2), 3.90–3.79 (m, 6H, CH_2 ester protons, and NCH_2), 3.70–3.16 (m, 10 H, CH—CH bridge protons, OCH_2CH_2 of PEG, $NCH_2CH_2CH_2O$, and OH), 2.62–2.57 (m, 8H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.12 (s, 6H, $C(CH_3)$).

^{1}HNMR data for copolymer **P5**: ($CDCl_3$, δ, ppm) 7.48–7.25 (m, 8H, ArH), 5.64–5.50 (m, 2H, $CH_2OC{=}O$), 4.84 (d, 1H, J = 2.8 Hz, CH bridgehead proton), 4.58–4.50 (m, 2H, OCH_2), 4.39–4.30 (m, 4H, CH_2 ester protons), 3.90–3.81 (m, 2H, NCH_2), 3.79–3.60 (m, 18H, OCH_2CH_2 of PEG, CH_2 ester protons, $NCH_2CH_2CH_2O$, and OH), 3.56–3.51 (m, 1H, CH—CH bridge protons), 3.39–3.23 (m, 1H, CH—CH bridge protons), 2.71–2.66 (m, 4H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.42 (s, 3H, $C(CH_3)$), 1.15 (d, 6H, J = 5.6 Hz, $C(CH_3)$). FTIR (cm^{-1}): 3437, 2882, 1732, 1698.

^{1}HNMR data for copolymer **P6**: ($CDCl_3$, δ, ppm) 7.36–7.10 (m, 8H, ArH), 5.56–5.35 (m, 2H, $CH_2OC{=}O$), 4.72 (s, 1H, CH bridgehead protons), 4.32–4.18 (m, 16H, OCH_2, NCH_2, and CH_2 ester protons), 3.75–3.52 (m, 28H, OCH_2CH_2 of PEG, CH_2 ester protons, and OH), 3.44–3.40 (m, 4H, $NCH_2CH_2CH_2O$), 3.28–3.10 (m, 2H, CH—CH bridge protons), 2.58–2.52 (m, 4H, $O{=}C{-}CH_2CH_2{-}C{=}O$), 1.26 (s, 3H, $C(CH_3)$), 1.22 (d, 6H, J = 10.4 Hz, $C(CH_3)$), 1.01 (d, 12H, J = 2.8 Hz, $C(CH_3)$).

Alkyne Functionalization of P5

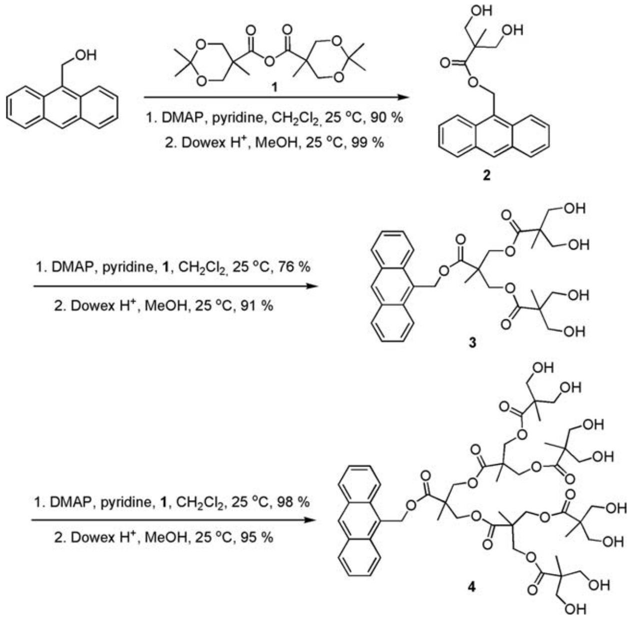
Copolymer G2-PEG-G2 **(P5)** (0.30 g, 0.08 mmol), pyridine (0.27 mL), and DMAP (0.016 g, 0.13 mmol) were dissolved in dry CH_2Cl_2 (5 mL) in a 10-mL round-bottom flask. To the stirring reaction mixture, 5-pentyonic acid anhydride (0.18 g, 1.01 mmol) was added and the solution was stirred for 20 h at room temperature under N_2. Then, pyridine:water solution (0.6 mL, 1:1) was added to the reaction mixture and stirred at room temperature for 1 h. Reaction mixture was diluted with CH_2Cl_2 (20 mL) and then extracted with 1 M of $NaHSO_4$ (3 × 20 mL), 10% of Na_2CO_3 (3 × 20 mL), and then with brine (1 × 20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the residue was concentrated *in vacuo*. Crude product was purified by precipitation in diethyl ether to give **P7** as a white solid (0.21 g, 60% yield). $M_{n,theo} = 4219$, $M_{n,GPC} = 4703$, $M_w/M_n = 1.15$, relative to PS.

^{1}H NMR ($CDCl_3$, δ, ppm) 7.40–7.14 (m, 8H, ArH), 5.66–5.27 (m, 2H, $CH_2OC{=}O$), 4.76 (d, 1H, J = 1 Hz, CH bridgehead proton), 4.35–4.11 (m, 6H, OCH_2, and CH_2 ester protons), 3.79 (m, 2H, J = 4.8 Hz, NCH_2), 3.72–3.42 (m, 12H, OCH_2CH_2 of PEG, CH_2 ester protons, and $NCH_2CH_2CH_2O$), 3.38–3.26 (m, 1H, CH—CH bridge protons), 3.21–3.10 (m, 1H, CH—CH bridge protons), 2.62–2.46 (m, 20H, $O{=}C{-}CH_2CH_2{-}C{=}O$ and $O{=}C{-}CH_2CH2{-}$C[tbond]CH), 1.89 (bs, 4H, C[tbond]H), 1.29–1.22 (m, 9H, $C(CH_3)$). FTIR (cm^{-1}): 3276, 2885, 1735, 1700.

Conjugation of Thioglycerol to P7

Alkyne-functionalized G2-PEG-G2 (**P7**) (10 mg, 2.37×10^{-3} mmol), 5% weight 2,2′-dimethoxy-2-phenylacetophonone, and thioglycerol (20.50 mg, 0.19 mmol) in 0.1 mL of DMF were placed in a vial. Then, the vial was purged with N_2 for 10 min and irradiated for 1 h at 365 nm at room temperature. Subsequently, the reaction content was precipitated in diethyl ether to give **P8** as a viscous solid (11.5 mg, 82% yield). $M_{n,theo} = 5950$, $M_{n,GPC} = 4600$, and $M_w/M_n = 1.23$, relative to PS.

^{1}H NMR ($CDCl_3$, δ, ppm) 7.38–7.14 (m, 8H, ArH), 5.62–5.17 (m, 2H, $CH_2OC{=}O$), 4.74 (s, 1H, CH bridgehead proton), 4.53–4.03 (m, 6H, OCH_2, and CH_2 ester protons), 3.78 (m, 2H, J = 4.6 Hz, NCH_2), 3.67–3.29 (m, 14H, OCH_2CH_2 of PEG, CH_2 ester protons, and $NCH_2CH_2CH_2O$), 3.32–3.27 (m, 2H, CH—CH bridge protons), 3.24–3.09 (m, 4H, CHS), 3.01–2.83 (m, 8H, CH_2S), 2.71–2.44 (m, 20H, $O{=}C{-}CH_2CH_2{-}C{=}O$, and $O{=}C{-}CH_2CH2$-C[tbond]CH), 1.29–1.07 (m, 9H, $C(CH_3)$). FTIR (cm^{-1}): 3398, 2868, 1734, 1670.



SCHEME 1 Divergent synthesis of anthracene-functionalized polyester dendrons.

RESULTS AND DISCUSSION

Multivalent PEG-based diblock (AB) and triblock (ABA) dendron–polymer conjugates were obtained by conjugation of anthracene-containing dendrons with linear PEG polymers bearing furan-protected maleimide groups at the chain ends. Three generations of polyester dendrons bearing an anthracene moiety at the focal point were synthesized as shown in Scheme 1. Dendrons were synthesized using a divergent methodology, that is the growth of the repeating units of the dendron started from the anthracene core toward the periphery. Reaction of the anhydride **1** with 9-anthracenemethanol yields the first generation acetal-protected dendron bearing the anthracene group at its core. The acetonide groups of this dendron were deprotected by treatment with an acid resin DOWEXH$^+$ to yield the first-generation hydroxyl containing dendron (**2**). Dendron **2** was treated with the anhydride **1** to yield an acetal-protected dendron that was deprotected under acidic conditions to yield the second-generation dendron **3**. Similar steps were followed to obtain third-generation dendron **4**.

Polyester dendrons containing peripheral hydroxyl functional groups were obtained in their pure form via column chromatography and recrystallization. These dendrons were characterized by ^{1}HNMR, ^{13}CNMR, and elemental analysis. In a typical ^{1}HNMR of a G3 dendron **4**, the proton resonances from the anthracene moiety, the protons adjacent to the ester group and three methyl groups in the dendron can be clearly seen around 7.5–9.0, 4.0, and 1.0 ppm, respectively (Fig. 2).

For the synthesis of diblock and triblock copolymers, PEG was end-functionalized by furan-protected maleimide groups (Scheme 2). End-groups of commercially available linear-PEG polymers were converted to carboxylic acid by treatment with succinic anhydride in the presence of triethyl amine.[54] The carboxylic acid end-groups on the polymers were reacted with an alcohol bearing a furan-protected maleimide group. These resultant polymers are referred to as mono-maleimide PEG (**7**) and bis-maleimide PEG (**9**).

The desired telechelic PEG polymers were obtained in high purity as inferred from their ^{1}HNMR spectra. Peaks at 6.48 and 5.23 ppm corresponding to the oxabicyclic moiety observed in ^{1}HNMR analysis proved the presence of the maleimide group in the structure of bis-maleimide PEG (Fig. 3, peaks h and g, respectively).

Diblock and triblock copolymers were synthesized by the Diels–Alder [4 + 2] cycloaddition reaction between the PEG polymers bearing the masked dienophile and the anthracene-containing polyester dendrons at 110 °C in toluene. At this temperature, unmasking of the maleimide groups at the

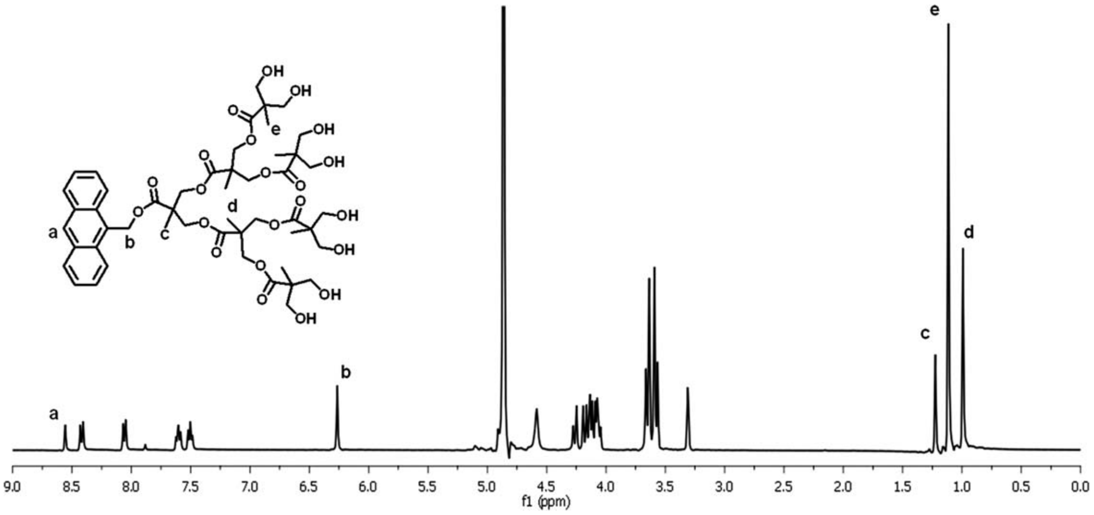
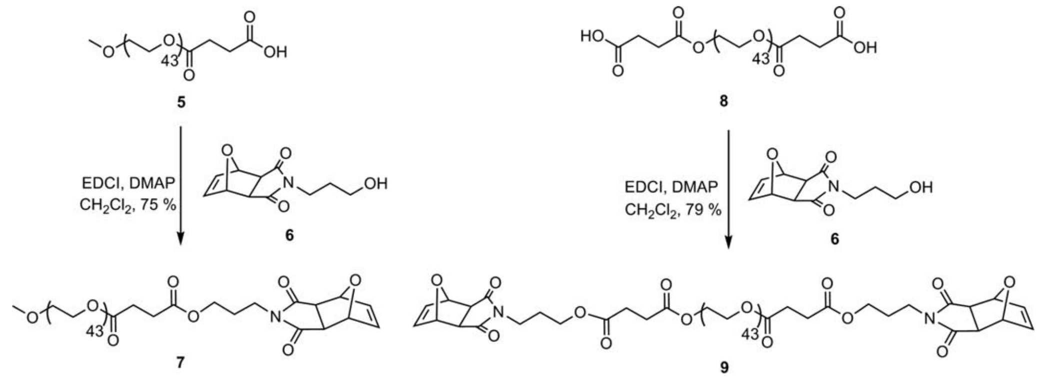


FIGURE 2 ^{1}HNMR spectrum of G3-anthracene dendron (4) in CD_3OD.



SCHEME 2 Synthesis of furan-protected maleimide-containing PEG polymers.

polymer chain ends occurs by the removal of furan via the retro Diels–Alder cycloreversion. The *in situ*-generated maleimide moiety reacts with the anthracene group on the dendron (Scheme 3). Reaction of the dendrons with monofunctional linear-PEG polymer **7** yields diblock copolymers (Scheme 4), whereas the utilization of telechelic PEG polymer **9** provides the triblock dendron–polymer conjugates (Scheme 5).

Synthesized diblock and triblock copolymers were purified by precipitation in diethyl ether and characterized by ^{1}HNMR. The Diels–Alder reaction between the maleimide PEG polymers and the dendrons resulted in the disappearance of peaks at 6.48 and 5.23 ppm belonging to bicyclic furan–maleimide cycloadduct at the PEG chain termini. Proton resonances from the aromatic protons owing to the anthracene unit can be seen between 7.5 and 7.0 ppm, whereas the bridgehead proton appears at 4.7 ppm (s, 1H, and CH bridgehead proton) and the protons on the methylene unit adjacent to the parent anthracene ring appears around 5.5–5.3 ppm (m, 2H, $CH_2OC{=}O$) (Figs. 4 and 5, peak f).

The details related to the synthesis and characterization of the dendron–polymer conjugates are summarized in Table 1. In all cycloaddition reactions, near-stoichiometric equivalents of the reactants were used. It was observed by ^{1}HNMR analysis that the cycloaddition reaction was fast enough for completion within 6 h. The yields of the copolymers listed in

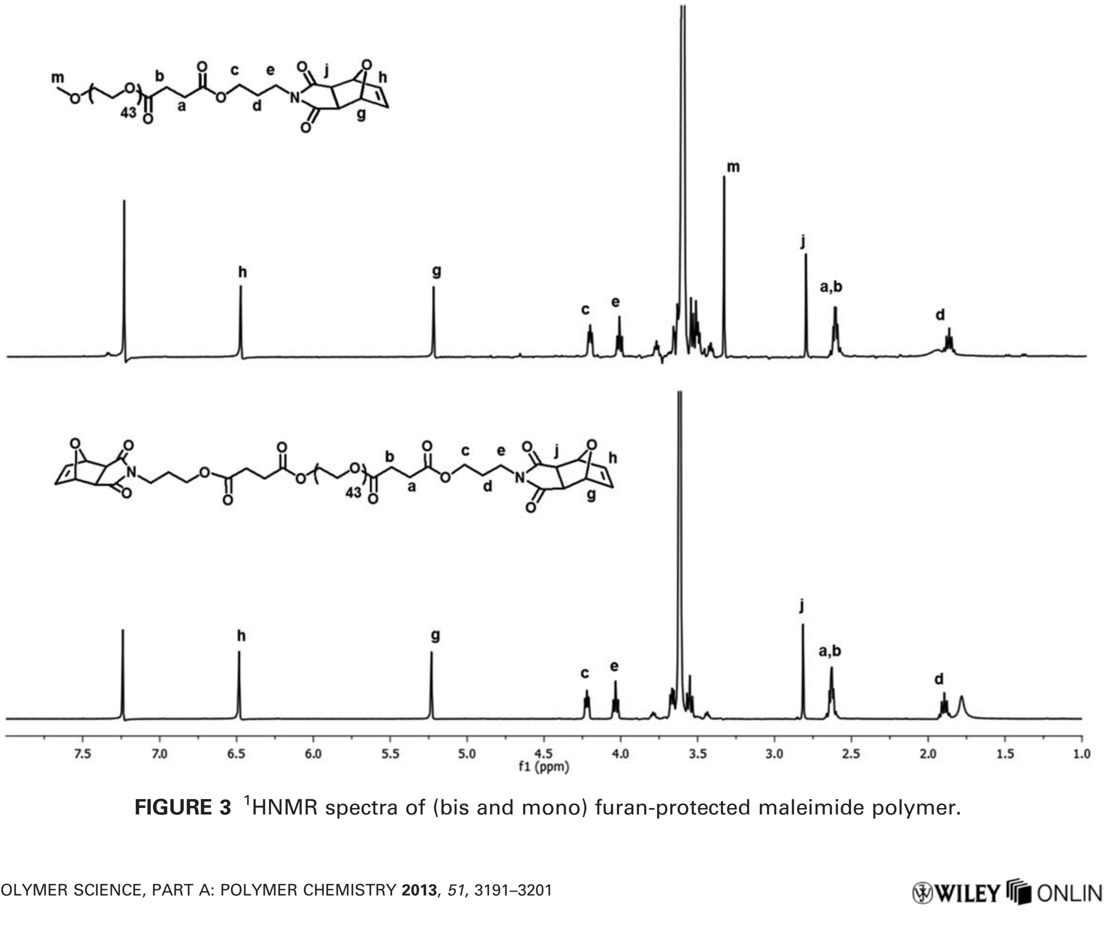
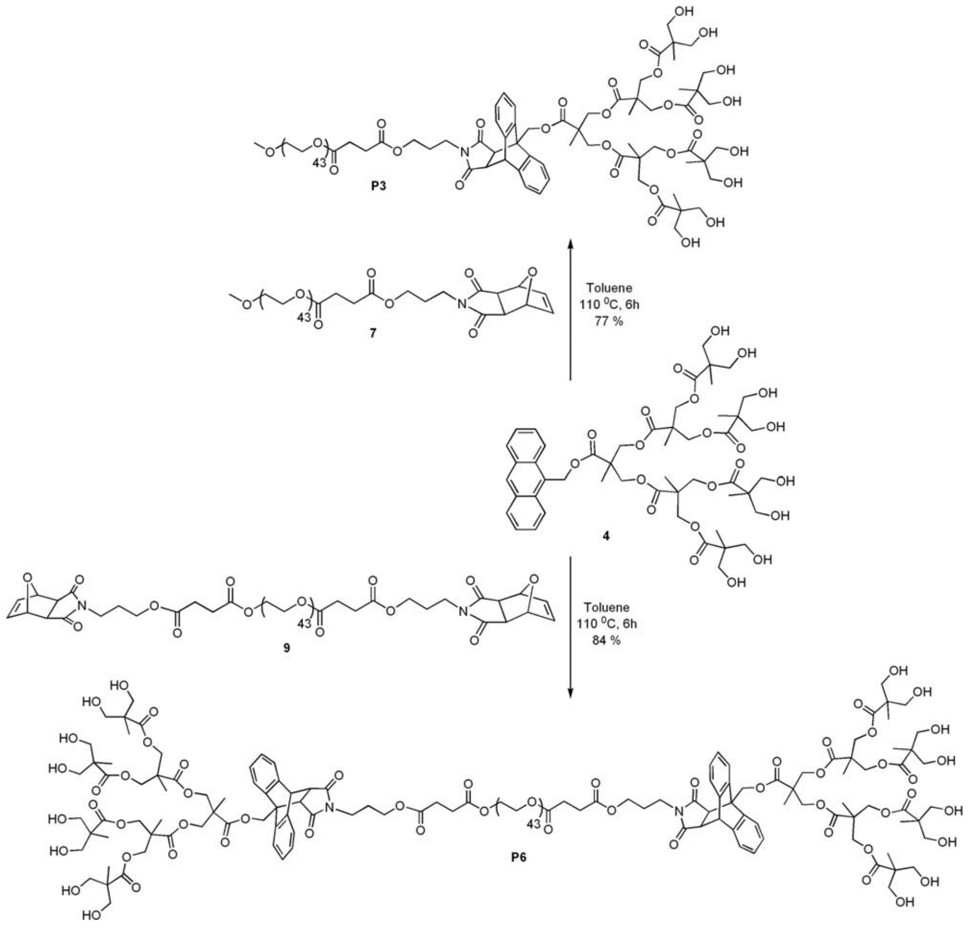
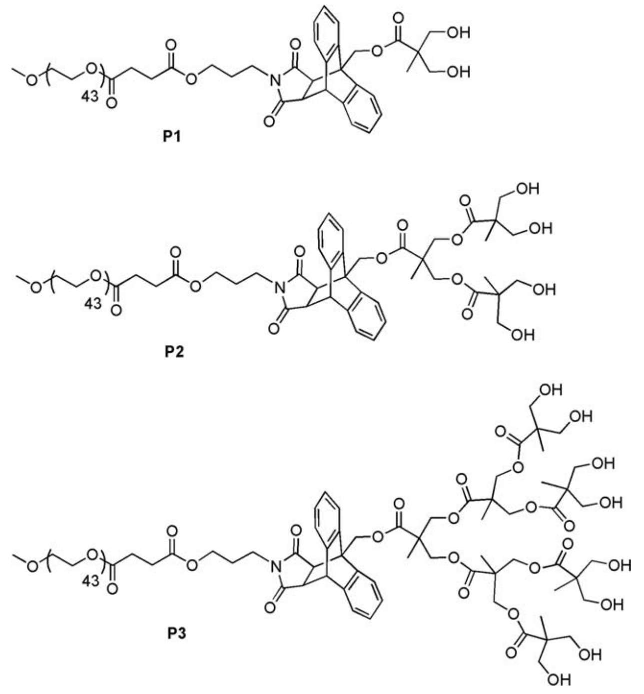


FIGURE 3 ^{1}HNMR spectra of (bis and mono) furan-protected maleimide polymer.



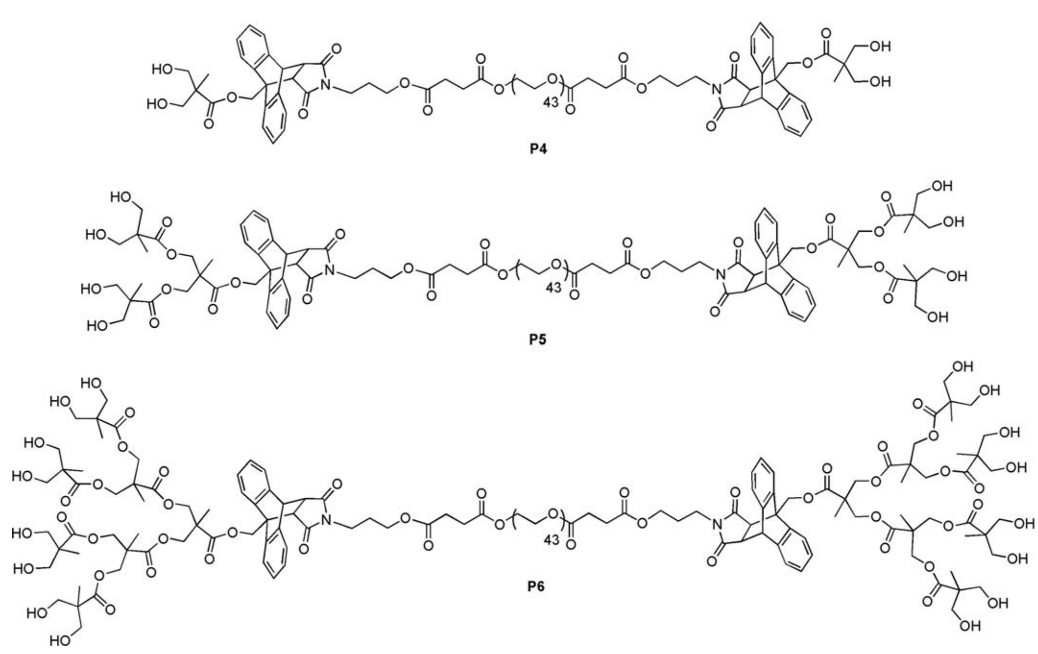
SCHEME 3 Diels–Alder reactions to synthesize diblock (**P3**) and triblock (**P6**) copolymers.



SCHEME 4 Structures of diblock dendron–polymer conjugates.

Table 1 are based on isolated products and any losses are occurring during precipitation and recovery.

Hydroxyl groups at the periphery of these dendron–polymer conjugates can be appended with "clickable" functional groups such as alkene or alkyne units to employ "thiol–ene" or "thiol–yne" chemistry. Both of these metal-free click reactions have been widely used to conjugate thiol-containing molecules to polymeric materials.[57–60] Here, we demonstrate that alkyne units can be easily appended and functionalized to decorate these dendron–polymer conjugates. Functionalization of the hydroxyl groups at the periphery of the triblock copolymers was obtained via the acylation reaction with pentyonic anhydride in the presence of pyridine (Scheme 6). This reaction yielded alkyne-functionalized triblock copolymer (**P7**). Appearance of a new proton resonances at 1.89 ppm proved the presence of alkyne units in the triblock copolymer (Supporting Information Fig. 15S). In the next step, thioglycerol conjugated these alkyne units via the thiol–yne reaction under UV-irradiation at 365 nm. The complete disappearance of the alkyne proton resonance at 1.89 ppm and appearance of new peaks belonging to the thioglycerol moieties around 3.15 and 2.93 ppm indicate near-quantitative conjugation (Supporting Information Fig. 16S).



SCHEME 5 Structures of triblock dendron–polymer–dendron conjugates.

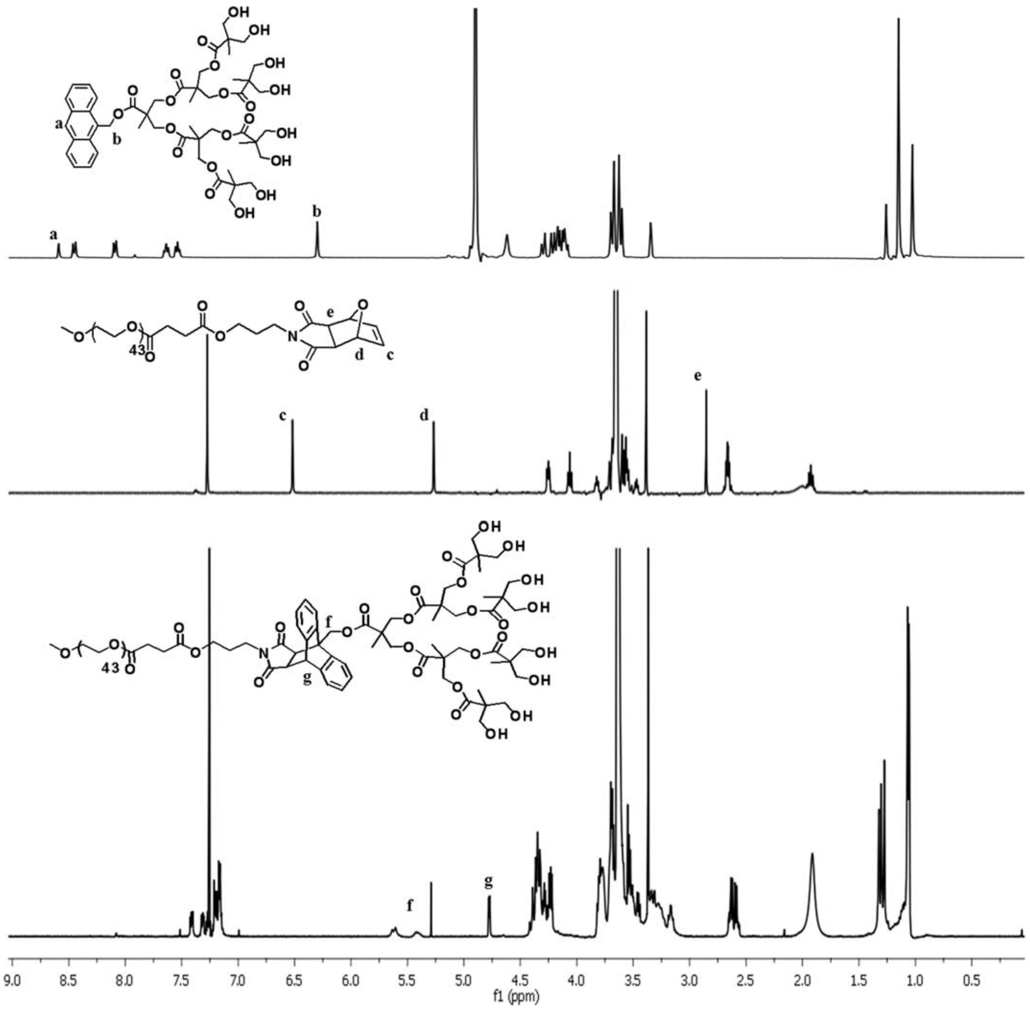


FIGURE 4 ^{1}HNMR spectra of G3-dendron (**4**) in CD_3OD, and polymer (**7**) and dendron–polymer conjugate (**P3**) in $CDCl_3$.

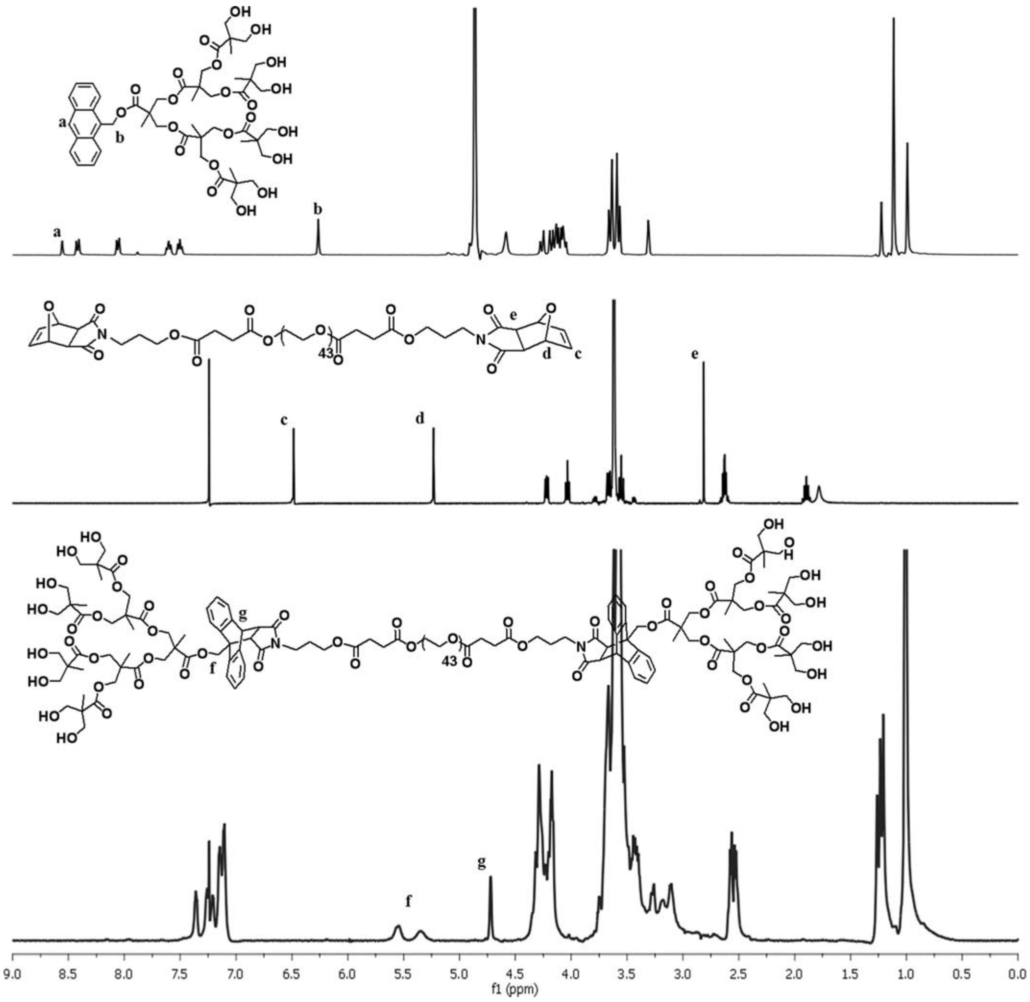


FIGURE 5 ^{1}HNMR spectra of G3-dendron (**4**) in CD_3OD, and polymer (**9**) and dendron–polymer conjugate (**P6**) in $CDCl_3$.

CONCLUSIONS

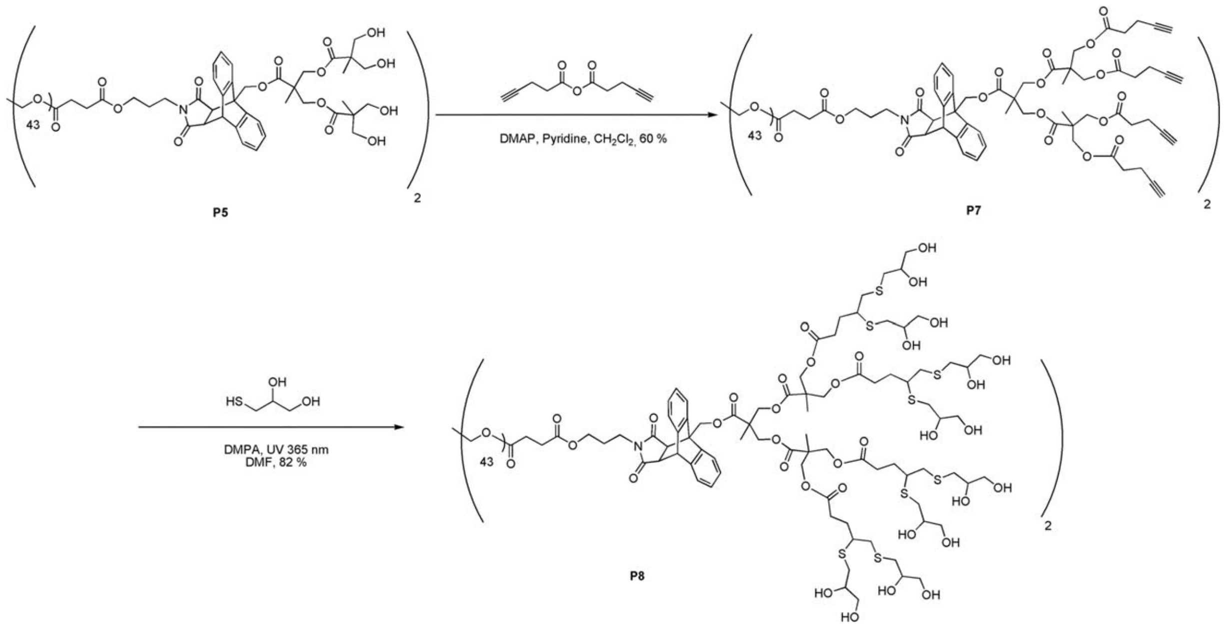
This study provides a new methodology toward ABA-type triblock dendron–polymer conjugate synthesis. Diels–Alder cycloaddition-based metal-free conjugation chemistry was utilized for the synthesis of dendron–polymer conjugates. Novel biodegradable dendrons containing an anthracene unit at their focal point were synthesized using a divergent growth strategy. Masked maleimide end-capped linear PEG polymers were used as the hydrophilic component of these triblock copolymers. The metal-free conjugation strategy using these novel

TABLE 1 Synthesis Details and Characterization Data of Dendron–Polymer Conjugates

Entry	Polymer	PEG[a]	Dendron[a]	Yield (%)	GPC[b] M_n (g/mol)	GPC[b] M_w/M_n	$M_{n,\ theo}$ (g/mol)
1	P1	7	**2** (G1)	84	3160	1.1	2560
2	P2	7	**3** (G2)	79	3440	1.1	2800
3	P3	7	**4** (G3)	77	3600	1.3	3260
4	P4	9	**2** (G1)	96	3660	1.2	3120
5	P5	9	**3** (G2)	83	4320	1.1	3590
6	P6	9	**4** (G3)	84	4980	1.3	4520

[a] $[\text{MI-PEG}]_0$:[Dendron] = 1:1.05 (P1–P3), 1:2.10 (P4–P6).

[b] Calibration with PS as standards. THF is the eluting solvent.



SCHEME 6 Alkyne-functionalized (**P7**) and thioglycerol-conjugated (**P8**) G2-PEG-G2 triblock copolymers.

dendrons provides a simple methodology to obtain multivalent linear PEG polymers. The multiple hydroxyl groups of these dendron–polymer conjugates could be easily decorated by alkyne groups to attach desired molecules via [3 + 2] Huisgen type or thiol–yne "click" reactions.[61–68]

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