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Functional block copolymer nanoparticles: toward the next generation of delivery vehicles†

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Abstract

The self-assembly of functional block copolymers (BCPs) into dispersed nanoparticles is a powerful technique for the preparation of novel delivery vehicles with precise control of morphology and architecture. Well-defined BCPs containing an alkyne-functional, biodegradable polylactide (PLA) block were synthesized and conjugated with azide-functional coumarin dyes *via* copper catalyzed azide alkyne cycloaddition ‘click’ chemistry. Self-assembled nanoparticles with internal nanophase-separated morphologies could then be accessed by carefully controlling the composition of the BCPs and release of the covalently attached model payload was shown to occur under physiological conditions *via* the degradation of the PLA scaffold. These results demonstrate the potential of self-assembled nanoparticles as modular delivery vehicles with multiple functionalities, nanostructures, and compartmentalized internal morphology.

Introduction

The design of efficient drug delivery vehicles has been a long standing challenge in polymer and materials science. A variety of polymeric platforms have been developed in recent years based on an array of different structures including micelles,^{1–6} dendrimers,^{7–10} hydrogels,^{11–13} and encapsulant particles.^{14–20} While drug carriers based on biodegradable poly(lactic acid-*co*-glycolic acid) (PLGA) nanoparticles developed by Langer and coworkers^{21–23} represent one of the most promising platforms to date, further progress will require a more engineered, multifunctional vehicle. Addressing this challenge will require the convergence of chemical design with precise control of morphology and architecture leading to vehicles with discreet nanostructures and compartmentalization functionality.^{24,25}

Traditionally, the bulk self-assembly of block copolymers (BCPs)^{26,27} has been exploited in a variety of interesting applications including tunable photonic crystals,²⁸ nanolithography,^{29,30} and nanoporous membranes;^{31–34} however, recent studies have shown that the solution self-assembly of BCPs can produce highly structured nanoparticles in a

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simple and reproducible manner. One approach relies on the aqueous emulsification of a BCP with a surfactant in which BCP nanoparticles are formed within the emulsion droplets.^{35–39} Alternatively, Shimomura and coworkers have developed a versatile strategy where a solution of a BCP in water-miscible organic media (*e.g.* THF) is simply titrated with water to a predetermined concentration and well-defined BCP nanoparticles are obtained after evaporation of the organic solvent.^{40,41} Nanoparticles containing a variety of unique internal morphologies have been investigated^{42–14} and this technique has also been extended to polymer blends⁴⁵ and metal-polymer composite nanoparticles.⁴⁶ Importantly, this solvent exchange method negates the use of any harmful additives like surfactants.

Exploiting the concept of dispersion self-assembly of soft materials in the drug delivery arena requires the incorporation of multiple functionalities into a judiciously designed BCP platform. In order to accurately target the desired nanoparticle morphology, both polymer blocks must be relatively hydrophobic to avoid the formation of structures such as micelles and vesicles that result from the self-assembly of amphiphilic BCPs in solution. Poly(allyl glycidyl ether) (PAGE) is a hydrophobic derivative of poly(ethylene glycol) that possesses pendent alkene functionality along the polymer backbone and has garnered interest for a variety of diverse applications.^{47–50} Building on these recent advances, an orthogonally functional BCP based on PAGE-*b*-polylactide (PAGE-*b*-PLA)^{51,52} represents a promising material for accessing well-defined, modular, internally structured nanoparticles. By integrating a controlled amount of alkyne functionality into the PLA block, the inclusion of a payload can be accurately modulated using copper catalyzed azide alkyne cycloaddition (CuAAC) ‘click’ chemistry⁵³ and its release from the nanoparticle accomplished through biodegradation of the polymer scaffold. Importantly, covalent attachment provides for greater control and diversity when compared to alternative methods such as physical encapsulation.⁵⁴ The orthogonality of the CuAAC process also allows the alkene groups on the PAGE to remain available for subsequent modification by thiol-ene coupling^{55,56} facilitating further development and refinement through the attachment of ancillary components like targeting moieties and auxiliary drugs.

Experimental

Materials

Chemicals were purchased from Sigma Aldrich or TCI America and used as received unless otherwise noted. (Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBop) was purchased from Fluka. *rac*-Lactide was recrystallized three times from toluene, ethyl acetate, and toluene (sequentially) prior to use. Tetrahydrofuran (THF) was collected directly from a dry solvent system. Benzyl alcohol was dried over calcium hydride and distilled prior to use. Allyl glycidyl ether was degassed through four freeze-pump-thaw cycles and distilled from butyl magnesium chloride to a pre-weighed and flame-dried buret immediately prior to use. Potassium naphthalenide was prepared from potassium metal and naphthalene (recrystallized from diethyl ether) in dry THF and allowed to stir with a glass-coated stir-bar for 24 h at room temperature prior to use. Deuterated solvents were obtained from Cambridge Isotope Laboratories, Inc. Water with a pH of 6.7 was used directly from a Millipore purification system.

Instrumentation

^1H and ^{13}C NMR spectra were recorded using a Varian 400, 500, or 600 MHz spectrometer with the solvent signal as internal reference. Gel permeation chromatography (GPC) was performed on a Waters 2690 separation module equipped with Waters 2414 refractive index and 2996 photodiode array detectors using CHCl_3 containing 0.25% triethylamine as eluent at a flow rate of 1 mL min^{-1} . Molecular weight distributions (PDIs) were calculated relative to linear polystyrene standards. Transmission electron microscopy (TEM) was conducted on a FEI-T20 instrument, operating at 200 kV. Samples were prepared by casting a concentrated dispersion of particles onto copper grids (Electron Microscopy Sciences) and the droplet was wicked through the grid using tissue paper. Dynamic light scattering measurements were performed on a Wyatt DynaPro NanoStar instrument using Dynamics 7.0 software. Data were collected at $25\text{ }^\circ\text{C}$ with an acquisition time of 5 s and the hydrodynamic radii were averaged over 20 acquisitions. ESI mass spectrometry was performed on a Micromass QTOF2 quadrupole/time-of-flight tandem mass spectrometer. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrometer using pol-y(methyl methacrylate) cuvettes with an optical path length of 10 mm. The excitation wavelength was set at 410 nm and the emission was recorded from 420 nm to 700 nm with the use of 5 nm excitation and emission slits.

Preparation of polymers

General procedure for the synthesis of poly(allyl glycidyl ether) (PAGE)—

Anionic polymerizations were carried out on a Schlenk line in custom glass reactors under an argon atmosphere. The potassium alkoxide initiator was formed by titration of benzyl alcohol with potassium naphthalenide under argon until a persistent green color was observed in solution indicating the quantitative deprotonation of the benzyl alcohol. The bulk polymerization of allyl glycidyl ether was carried out at $30\text{ }^\circ\text{C}$ for 20 h followed by termination with methanol.

A detailed procedure for the synthesis of PAGE (9.1 kg mol^{-1}) is provided. Benzyl alcohol (104 mg, 0.966 mmol) was titrated with potassium naphthalenide followed by the addition of allyl glycidyl ether (10.0 g, 87.6 mmol). After termination with methanol the polymer was precipitated into hexane, the hexane was decanted, and the polymer was dried under vacuum and obtained in near quantitative yield. Further purification by filtration through a short silica plug with ethyl acetate as the eluent resulted in a clear, colorless, viscous polymer liquid. ^1H NMR (500 MHz, CDCl_3): δ 3.42–3.68 (m, 403H, $-\text{CH}_2-\text{CH}(\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2)-\text{O}-$), 3.98 (d, 161H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), 4.53 (s, 2H, $\text{Ph}-\text{CH}_2-\text{O}-$), 5.11–5.29 (m, 155H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), 5.83–5.93 (m, 73H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), and 7.27–7.35 (m, 5H, $\text{Ph}-\text{CH}_2-\text{O}-$) ppm. ^{13}C NMR (600 MHz, CDCl_3): δ 69.97, 70.34, 72.42, 78.94, 116.88, 127.74, 128.51, and 135.09 ppm. M_n (^1H NMR) = 9.1 kg mol^{-1} ; PDI (GPC) = 1.10.

General procedure for the synthesis of poly(allyl glycidyl ether)-*b*-

poly(lactide) (PAGE-*b*-PLA)—PAGE macroinitiator was weighed into an oven-dried, 10 mL round bottom flask equipped with a stir bar and septum and dried over night at $45\text{ }^\circ\text{C}$ under vacuum ($\sim 20\text{ mmTorr}$). The flask was charged with lactide and a 0.02 M solution of

tin(n) 2-ethylhexanoate ($\text{Sn}(\text{Oct})_2$) in THF corresponding to a molar ratio of 5 : 1 initiator: $\text{Sn}(\text{Oct})_2$. The contents were mixed briefly and THF was subsequently removed under vacuum for 3 h. The flask was backfilled with dry argon and placed in an oil bath maintained at 130 °C where it was left to react for 1–4 h, depending on the targeted PLA molecular weight. After the allowed reaction time the flask was removed from heat, cooled quickly under a stream of water, and the contents dissolved in a small amount of dichloromethane (DCM). The reaction solution was precipitated into 20 mL of hexane to remove the catalyst, the hexane was decanted, and the polymer was dried under vacuum. Monomer conversion was determined using ^1H NMR spectroscopy and residual lactide monomer was readily removed by vacuum sublimation⁵⁷ at 70 °C (~15 mTorr) for 2–3 h.

A detailed procedure for the synthesis of **6** is provided. PAGE (0.5548 g, 0.03511 mmol) was dried overnight followed by the addition of lactide (0.5063 g, 3.513 mmol) and $\text{Sn}(\text{Oct})_2$ (350 μL , 0.02 M in THF). After THF removal, the reaction was allowed to proceed for 3 h at 130 °C. A monomer conversion of 72% was calculated from the crude ^1H NMR spectrum after which residual lactide monomer was removed to yield 0.864 g of a clear, viscous polymer. ^1H NMR (500 MHz, CDCl_3): δ 1.44–1.61 (m, 376H, $-\text{C}(\text{O})-\text{CH}-$ (CH_3)-O-), 3.42–3.69 (m, 663H, $-\text{CH}_2-\text{CH}(\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2)-\text{O}-$), 3.98 (d, 257H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), 4.53 (s, 2H, $\text{Ph}-\text{CH}_2-\text{O}-$), 5.10–5.30 (m, 374H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$ and $-\text{C}(\text{O})-\text{CH}-$ (CH_3)-O-), 5.82–5.93 (m, 120H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), and 7.27–7.35 (m, 5H, $\text{Ph}-\text{CH}_2-\text{O}-$) ppm. ^{13}C NMR (600 MHz, CDCl_3): δ 16.79, 20.65, 66.81, 69.20, 69.94, 70.31, 72.38, 78.93, 116.83, 127.70, 128.47, 135.05, and 169.52 ppm. $M_{n, \text{Total}}$ (^1H NMR) = 24.6 kg mol⁻¹; $M_{n, \text{PLA}}$ (^1H NMR) = 8.8 kg mol⁻¹; PDI (GPC) = 1.15.

General procedure for the synthesis of poly(allyl glycidyl ether)-*b*-poly(lactide-co-propargyl glycolide) (PAGE-*b*-P(LA-co-PG))—A similar procedure

to the synthesis of PAGE-*b*-PLA was followed with propargyl glycolide⁵⁸ added in varying stoichiometry as a comonomer. The conversion of propargyl glycolide was quantitative in each polymerization as indicated by ^1H NMR measurements.

A detailed procedure for the synthesis of **10** is provided. PAGE (0.1706 g, 0.01875 mmol) was dried overnight followed by the addition of lactide (0.1900 g, 1.318 mmol), propargyl glycolide (0.0293 g, 0.152 mmol), and $\text{Sn}(\text{Oct})_2$ (380 μL , 0.01 M in THF). After THF removal, the reaction was allowed to proceed for 3 h at 130 °C. The reaction mixture was cooled, dissolved in a small amount of DCM, and precipitated into 20 mL of hexane. The conversion of lactide was calculated to be >95% from the crude ^1H NMR spectrum after which residual lactide monomer was removed by vacuum sublimation to yield 0.359 g (93%) of a clear, viscous polymer. ^1H NMR (500 MHz, CDCl_3): δ 1.51–1.63 (m, 432H, $-\text{C}(\text{O})-\text{CH}-$ (CH_3)-O-), 2.04–2.11 (m, 18H, $-\text{C}(\text{O})-\text{CH}-$ ($\text{CH}_2-\text{C}\equiv\text{CH}$)-O-), 2.77–3.00 (m, 37H, $-\text{C}(\text{O})-\text{CH}-$ ($\text{CH}_2-\text{C}\equiv\text{CH}$)-O-), 3.42–3.69 (m, 412H, $-\text{CH}_2-\text{CH}(\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2)-\text{O}-$), 3.98 (d, 164H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), 4.54 (s, 2H, $\text{Ph}-\text{CH}_2-\text{O}-$), 5.12–5.28 (m, 302H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$ and $-\text{C}(\text{O})-\text{CH}-$ (CH_3)-O-), 5.28–5.43 (m, 18H, $-\text{C}(\text{O})-\text{CH}-$ ($\text{CH}_2-\text{C}\equiv\text{CH}$)-O-), 5.83–5.93 (m, 76H, $-\text{O}-\text{CH}_2-\text{CH}=\text{CH}_2$), and 7.27–7.35 (m, 5H, $\text{Ph}-\text{CH}_2-\text{O}-$) ppm. ^{13}C NMR (500 MHz, CDCl_3): δ 16.82, 20.65, 21.74, 66.81, 69.22, 69.65, 69.94, 70.38, 71.00, 71.86, 72.38, 78.94, 116.86, 127.71, 128.48, 135.06,

166.90, and 169.48 ppm. $M_{n, \text{Total}}$ (^1H NMR) = 20.9 kg mol $^{-1}$; $M_{n, \text{PLA}}$ (^1H NMR) = 10.1 kg mol $^{-1}$; $M_{n, \text{PPG}}$ (^1H NMR) = 1.7 kg mol $^{-1}$; PDI (GPC) = 1.34.

Preparation of coumarin derivatives

Synthesis of 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid, Et₂N-Coumarin-COOH

Et₂N-Coumarin-COOH was synthesized according to the literature method.⁵⁹ Diethylmalonate (13.0 mL, 85.5 mmol), 4-dimethylaminosalicylaldehyde (8.236 g, 42.62 mmol), and piperidine (4.2 mL, 42 mmol) were combined with 120 mL absolute ethanol in a 250 mL round bottom flask equipped with a stir bar and condenser. After refluxing for 5.5 h, the solution was cooled to room temperature followed by the addition of NaOH (60 mL, 6 M). The solution was returned to reflux for 25 min, cooled in an ice bath, and acidified to pH 2 with concentrated HCl. The solution was filtered and the solid washed with water followed by recrystallization from ethanol to yield 9.47 g (85%) of orange needles, mp 229–230 °C.

Synthesis of 3-chloropropyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate, Et₂N-Coumarin-ester-Alkyl-Chloride

Et₂N-Coumarin-COOH (1.189 g, 4.550 mmol), 3-chloropropanol (0.46 mL, 5.5 mmol), and 4-dimethylaminopyridine (DMAP, 63 mg) were dissolved in 17 mL DCM in a 50 mL round bottom flask equipped with a stir bar and septum. Dicyclohexylcarbodiimide (DCC) (1.146 g, 5.555 mmol) was dissolved in 3 mL of DCM and added to the reaction mixture slowly *via* syringe. After stirring at room temperature for 2 days, the solution was filtered and the filtrate was concentrated. The resulting yellow solid was recrystallized from 100 mL of a hexanes/ethanol (50/50) mixture and dried under vacuum to yield 1.36 g (88%) of a yellow crystalline solid, mp 143–144 °C. ^1H NMR (500 MHz, CDCl₃): δ 1.21 (t, 6H, J = 7.1 Hz, $-\text{CH}_2\text{CH}_3$), 2.20 (p, 2H, J = 6.2 Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 3.42 (q, 4H, J = 7.1 Hz, $-\text{CH}_2\text{CH}_3$), 3.71 (t, 2H, J = 6.4 Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 4.43 (t, 2H, J = 6.0 Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{Cl}$), 6.43 (d, 1H, J = 2.4 Hz), 6.59 (dd, 1H, J = 9.0, 2.5 Hz), 7.34 (d, 1H, J = 9.0 Hz), and 8.39 (s, 1H) ppm. ^{13}C NMR (500 MHz, CDCl₃): 5 12.56, 31.85, 41.64, 45.31, 61.90, 96.88, 107.84, 108.59, 109.81, 131.27, 149.49, 153.07, 158.24, 158.66, and 164.36 ppm. MS (ESI): m/z [M + Na] $^+$ calcd for [C₁₇H₂₀ClNO₄ + Na] $^+$, 360.098; found, 360.098.

Synthesis of 3-azidopropyl 7-(diethylamino)-2-oxo-2H-chromene-3-carboxylate, Et₂N-Coumarin-ester-Alkyl-Azide (13)

Et₂N-Coumarin-ester-Alkyl-Chloride (0.5878 g, 1.740 mmol) and sodium azide (0.2010 g, 3.092 g) were combined with 5 mL dimethylsulfoxide (DMSO) in a 50 mL round bottom flask equipped with a stir bar and septum. The mixture was heated overnight with stirring in an oil bath maintained at 75 °C. The reaction was removed from heat, 50 mL H₂O added and the solution extracted with ethyl acetate (2 $\times$ 100 mL). Combined organic fractions were washed with H₂O (2 $\times$ 100 mL), brine (50 mL), dried over MgSO₄, and concentrated. The crude product was purified by column chromatography on silica using a gradient of 15 to 50% ethyl acetate in hexanes resulting in a red liquid that crystallized slowly under vacuum to afford 0.553 g (92%) of a yellow-orange crystalline solid. ^1H NMR (500 MHz, CDCl₃): δ 1.20 (t, 6H, J = 7.1 Hz, $-\text{CH}_2\text{CH}_3$), 2.01 (p, 2H, J = 6.4 Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 3.42 (q, 4H, J = 7.1 Hz, $-\text{CH}_2\text{CH}_3$), 3.49 (t, 2H, J = 6.7 Hz, $-\text{OCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 4.36 (t, 2H, J = 6.1 Hz,

–OCH₂CH₂CH₂N₃), 6.42 (d, 1H, $J = 2.1$ Hz), 6.59 (dd, 1H, $J = 8.9, 2.2$ Hz), 7.33 (d, 1H, $J = 8.9$ Hz), and 8.38 (s, 1H) ppm. ¹³C NMR (500 MHz, CDCl₃): 5 12.52, 28.36, 45.28, 48.40, 62.00, 96.84, 107.81, 108.52, 109.80, 131.26, 149.48, 153.06, 158.22, 158.64, and 164.34 ppm. MS (ESI): m/z [M + Na]⁺ calcd for [C₁₇H₂₀N₄O₄ + Na]⁺, 367.138; found, 367.137.

Synthesis of N-(3-chloropropyl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxaniide, Et₂N-Coumarin-*amide*-Alkyl-Chloride—Et₂N-Coumarin–COOH

(0.9793 g, 3.748 mmol), 3-chloro-propylamine hydrochloride (0.5440 g, 4.184 mmol), and PyBop (1.958 g, 3.762 mmol) were combined with 20 mL of DCM in a 100 mL round bottom flask equipped with a stir bar and septum. N,N-diisopropylethylamine (DIEA) (2.0 mL, 12 mmol) was added *via* syringe at which point the contents became soluble. After stirring overnight at room temperature the DCM was evaporated and the solid was dissolved in ethyl acetate, washed with water, dried over MgSO₄, and concentrated. The resulting solid was recrystallized from a hexanes/ethanol mixture and dried under vacuum to yield 1.07 g (85%) of bright yellow crystalline fibers, mp 165–166 °C. ¹H NMR (500 MHz, CDCl₃): δ 1.21 (t, 6H, $J = 7.1$ Hz, –CH₂CH₃), 2.08 (p, 2H, $J = 6.6$ Hz, –NHCH₂CH₂CH₂Cl), 3.43 (q, 4H, $J = 7.1$ Hz, –CH₂CH₃), 3.56 (q, 2H, $J = 6.4$ Hz, –NHCH₂CH₂CH₂Cl), 3.60 (t, 2H, $J = 6.6$ Hz, –NHCH₂CH₂CH₂Cl), 6.48 (d, 1H, $J = 2.5$ Hz), 6.63 (dd, 1H, $J = 9.0, 2.5$ Hz), 7.40 (d, 1H, $J = 8.9$ Hz), 8.66 (s, 1H), and 8.86 (t, 1H, $J = 6.0$ Hz, –NHCH₂CH₂CH₂Cl) ppm. ¹³C NMR (500 MHz, CDCl₃): δ 12.54, 32.57, 37.09, 42.63, 45.32, 96.85, 108.60, 110.24, 110.29, 131.28, 148.22, 152.60, 157.74, 162.87, and 163.54 ppm. MS (ESI): m/z [M + Na]⁺ calcd for [C₁₇H₂₁ClN₂O₃ + Na]⁺, 359.114; found, 359.112.

Synthesis of N-(3-azidopropyl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxaniide, Et₂N-Coumarin-*amide*-Alkyl-Azide (14)—Et₂N-Coumarin-*amide*-Alkyl-Chloride

(0.5016 g, 1.489 mmol) and sodium azide (0.1658 g, 2.550 mmol) were combined with 4 mL DMSO in a 10 mL round bottom flask equipped with a stir bar and septum. The mixture was heated overnight with stirring in an oil bath maintained at 75 °C. The reaction was removed from heat and upon cooling the product crystallized from solution. The solid was washed with water and dried under vacuum to afford 0.462 g (90%) of a dull yellow crystalline material. ¹H NMR (500 MHz, CDCl₃): δ 1.22 (t, 6H, $J = 7.1$ Hz, –CH₂CH₃), 1–91 (p, 2H, $J = 6.8$ Hz, –NHCH₂CH₂CH₂Cl), 3.39 (t, 2H, $J = 6.7$ Hz, –NHCH₂CH₂CH₂Cl), 3.44 (q, 4H, $J = 7.1$ Hz, –CH₂CH₃), 3.51 (q, 2H, $J = 6.5$ Hz, –NHCH₂CH₂CH₂Cl), 6.51 (d, 1H, $J = 2.5$ Hz), 6.66 (dd, 1H, $J = 9.0, 2.5$ Hz), 7.42 (d, 1H, $J = 8.9$ Hz), 8.68 (s, 1H), and 8.87 (t, 1H, $J = 5.9$ Hz, –NHCH₂CH₂CH₂Cl) ppm. ¹³C NMR (500 MHz, CDCl₃): 5 12.54, 29.19, 37.09, 45.46, 49.39, 97.08, 108.80, 110.40, 110.48, 131.33, 148.27, 152.51, 157.75, 162.90, and 163.49 ppm. MS (ESI): m/z [M + Na]⁺ calcd for [C₁₇H₂₁ClN₂O₃ + Na]⁺, 366.154; found, 366.153.

CuAAC ‘click’ attachment of coumarin dyes

Synthesis of PAGE-*b*-P(LA-*co*-PG)-*click*-Coumarin_{ester} (15)—PAGE-*b*-P(LA-*co*-PG) **10** (43.3 mg, 0.0331 mmol of alkynes) and **13** (6.3 mg, 0.018 mmol) were dissolved in 2 mL THF in a 10 mL Schlenk flask equipped with a stir bar and septum. A 0.10 M stock

solution of pentamethyldiethylenetriamine (PMDETA) in THF (0.17 mL, 0.017 mmol) was added to the flask *via* syringe followed with degassing by three freeze-pump-thaw cycles. CuBr (2.4 mg, 0.017 mmol) was added to the solution under a flow of argon and the flask was resealed. After stirring at room temperature for 30 min the reaction solution was partially concentrated under vacuum and immediately precipitated into 20 mL of hexane, the hexane was decanted, and the green solid was dried briefly under vacuum. The polymer was redissolved in 8 mL of DCM and washed with a 0.1 M ethylenediaminetetra-acetic acid (EDTA) solution, brine, dried over MgSO₄, partially concentrated and precipitated again into 20 mL hexane. The solution was centrifuged, the hexane decanted, and the solid dried under vacuum to yield 34 mg (69%) of a yellow polymer. ¹H NMR (500 MHz, CDCl₃): δ 1.18–1.27 (m, 76H, –CH₂CH₃), 1.47–1.66 (m, 676H, –C(O)–CH–(CH₃)–O–), 1.97–2.11 (m, 6H, –C(O)–CH–(CH₂–C≡CH)–O–), 2.27–2.40 (m, 23H, –OCH₂CH₂CH₂–), 2.74–2.95 (m, 12H, –C(O)–CH–(CH₂–C≡CH)–O–), 3.21–3.80 (m, 489H, –CH₂–CH(CH₂–O–CH₂–CH=CH₂)–O– and –CH₂CH₃), 3.98 (d, 164H, –O–CH₂–CH=CH₂), 4.21–4.31 (m, 22H, –OCH₂CH₂CH₂–), 4.50–61 (m, 23H, –OCH₂CH₂CH₂–), 5.07–5.28 (m, 303H, –O–CH₂–CH=CH₂ and –C(O)–CH–(CH₃)–O–), 5.29–5.36 (m, 6H, –C(O)–CH–(CH₂–C≡CH)–O–), 5.37–5.52 (m, 11H, –C(O)–CH–(CH₂–tri-azole)–O–), 5.83–5.93 (m, 75H, –O–CH₂–CH=CH₂), 6.38–6.48 (m, 11H), 6.57–5.65 (m, 11H), 7.34–7.42 (m, 12H), 7.27–7.34 (m, 5H, Ph–CH₂–O–), 7.62–7.83 (m, 10H, triazole), and 8.32–8.44 (m, 10H) ppm. PDI (GPC) = 1.40.

Synthesis of PAGE-*b*-P(LA-*co*-PG)-click-Coumarin_{amide} (16)—PAGE-*b*-P(LA-*co*-PG) **10** (43.4 mg, 0.0332 mmol of alkynes) and **14** (6.3 mg, 0.018 mmol) were dissolved in 2 mL THF in a 10 mL Schlenk flask equipped with a stir bar and septum. A 0.36 M stock solution of PMDETA in THF (0.060 mL, 0.022 mmol) was added to the flask *via* syringe followed with degassing by three freeze-pump-thaw cycles. CuBr (2.7 mg, 0.019 mmol) was added to the solution under a flow of argon and the flask was resealed. TLC identified the quantitative conversion of coumarin-azide after stirring at room temperature for 30 min. The reaction solution was partially concentrated under vacuum and immediately precipitated into 20 mL of hexane, the hexane was decanted, and the green solid was dried briefly under vacuum. The polymer was redissolved in 10 mL of DCM and washed with a 0.1 M EDTA solution, brine, dried over MgSO₄, partially concentrated and precipitated again into 20 mL hexane. The solution was centrifuged, the hexane decanted, and the solid dried under vacuum to yield 44 mg (88%) of a yellow, glassy polymer. ¹H NMR (500 MHz, CDCl₃): δ 1.19–1.27 (m, 49H, –CH₂CH₃), 1.47–1.66 (m, 472H, –C(O)–CH–(CH₃)–O–), 1.98–2.12 (m, 10H, –C(O)–CH–(CH₂–C≡CH)–O–), 2.14–2.26 (m, 15H, –NHCH₂CH₂CH₂–), 2.74–2.98 (m, 19H, –C(O)–CH–(CH₂–C≡CH)–O–), 3.26–3.80 (m, 477H, –CH₂–CH(CH₂–O–CH₂–CH=CH₂)–O– and –CH₂CH₃ and –NHCH₂CH₂CH₂–), 3.98 (d, 164H, –O–CH₂–CH=CH₂), 4.37–4.46 (m, 14H, –NHCH₂CH₂CH₂–), 4.54 (s, 2H, Ph–CH₂–O–), 5.09–5.29 (m, 306H, –O–CH₂–CH=CH₂ and –C(O)–CH–(CH₃)–O–), 5.29–5.38 (m, 9H, –C(O)–CH–(CH₂–C≡CH)–O–), 5.39–5.54 (m, 7H, –C(O)–CH–(CH₂–tri-azole)–O–), 5.83–5.93 (m, 78H, –O–CH₂–CH=CH₂), 6.44–6.52 (m, 7H), 6.60–5.69 (m, 7H), 7.28–7.36 (m, 5H, Ph–CH₂–O–), 7.40–7.46 (m, 7H), 7.59–7.81 (m, 6H, triazole), 8.61–8.72 (m, 7H), and 8.83–8.97 (m, 7H, –NHCH₂CH₂CH₂–) ppm. PDI (GPC) = 1.39.

Block copolymer nanoparticle formation and self-assembly

Water (9.0 mL) was added *via* a syringe pump at a rate of 1.0 mL min^{-1} to a continuously stirred solution of the BCP dissolved in THF (4.5 mL, 0.10 mg mL^{-1}). THF was then allowed to evaporate under ambient conditions (typically 1 week), resulting in an aqueous dispersion of BCP nanoparticles. All nanoparticle assembly was conducted in 20 mL glass vials with an open 12 mm diameter top.

Fluorescence spectroscopy

A pH 5 buffer solution was prepared by mixing 12.9 mL and 7.1 mL of 0.10 M aqueous solutions of sodium acetate and acetic acid, respectively. Nanoparticle samples were prepared by diluting 0.50 mL of the nanoparticle dispersions with pure water or pH 5 buffer to a final solution volume of 5.0 mL. Samples were incubated at 37°C and their fluorescence spectra measured at different time intervals over a 20 day period. The fluorescence signals at the peak maxima were recorded, corrected by subtracting the background fluorescence signal at time zero, and the data for each sample normalized against the fluorescence signal at 20 days.

Results and discussion

Synthesis of model BCPs and the influence of composition on self-assembly

Exploiting the dispersion self-assembly of functional BCPs represents a novel strategy for the preparation of delivery vehicles with controlled and compartmentalized internal nanoscale morphology (Fig. 1). To investigate the effects of composition on the self-assembly process, a series of PAGE-*b*-PLA BCPs were initially synthesized prior to the incorporation of a functional monomer into the PLA block. Two different molecular weight PAGE homopolymers containing a single hydroxyl end group were used as macroinitiators for the ring opening polymerization of *rac*-lactide in the melt at 130°C catalyzed by $\text{Sn}(\text{Oct})_2$ (Scheme 1, path a). The polymerizations were well controlled as demonstrated by the close agreement between experimental molecular weights ($^1\text{H NMR}$) and those calculated on the basis of monomer feed ratios with PLA block fractions (f_{PLA}) ranging from 0.07 to 0.55 and overall molecular weights between 10.5 and 24.6 kg mol^{-1} (Table 1).

Nanoparticles were subsequently prepared by titrating solutions of the BCPs in THF with water at a rate of 1.0 mL min^{-1} followed by the slow evaporation of THF under ambient conditions (~ 1 week). The resulting nanoparticle dispersions were then analyzed by dynamic light scattering (DLS) and the morphologies of the nanoparticles investigated using transmission electron microscopy (TEM). The alkene functionality in the PAGE block proved to be a crucial component in characterizing the internal structure of the nanoparticles as it allowed for differentiation between polyether and polyester phases by preferential staining with OsO_4 . Interestingly, all of the nanoparticles contain a dimple-like feature which is thought to evolve during the self-assembly process and results from the nonuniform collapse of an initially formed glassy polymer shell on the exterior of the nanoparticle as the THF solvent is removed from the swollen core.^{60–62}

The internal morphology of the self-assembled PAGE-*b*-PLA nanoparticles was shown to be very sensitive to the BCP composition. Interestingly, only nanoparticles derived from materials **8** and **9** with f_{PLA} of 0.47 and 0.55, respectively, exhibited a clear internal nanophase-separated structure by TEM (Figure S2, supporting information). Nanoparticles prepared from polymers **1–7** with $0.07 < f_{\text{PLA}} < 0.37$ showed only a single mixed phase internal morphology as evidenced by the absence of contrast in the TEM images (Fig. 2). For **8** and **9**, the internal morphology of the phase-separated BCP nanoparticles appears to be a disordered bicontinuous structure that offers a route for enhancing the degradation of the nanoparticles by making the PLA phases more accessible.

Synthesis and self-assembly of alkyne-functional degradable BCPs

Based on the self-assembly results for the PAGE-*b*-PLA series, BCPs incorporating a functional polyester block were prepared keeping the polyester fraction around 0.5 to retain nanophase-separated structures in the self-assembled nanoparticles. Several strategies for the functionalization of PLA have been developed including ring opening polymerization of functional monomers⁶³ and post-polymerization modification using efficient ‘click’ transformations.^{58,64,65} Owing to the modularity of the latter strategy, lactide was copolymerized with the alkyne-functional lactide monomer, propargyl glycolide (PG), in order to introduce functionality into the PLA chain. Alkyne-functional PAGE-*b*-P(LA-*co*-PG) BCPs **10–12** were therefore synthesized in a similar fashion as PAGE-*b*-PLA with the incorporation of a controlled amount of PG in the monomer feed (Scheme 1, path b). Both the molecular weight as well as the fraction of poly(propargyl glycolide) (PPG) within the polyester block (f_{PPG}) could be accurately tailored while maintaining relatively low molecular weight distributions and overall molecular weights between 20.9 and 24.8 kg mol⁻¹ (Table 2). Specifically, f_{PPG} was modulated between 0.11 and 0.23 corresponding to overall polyester fractions ($f_{\text{PLA} + \text{PPG}}$) in the BCP composition of 0.50–0.57.

The BCPs were characterized by ¹H and ¹³C NMR spectroscopy and GPC which demonstrated their well-defined structure. GPC of the functional polymers displayed a significant shift to larger hydrodynamic volume relative to the starting PAGE macroinitiator while maintaining narrow and monomodal peak shapes (Fig. 3). Furthermore, ¹H NMR revealed the appearance of a new set of resonances corresponding to the terminal alkyne protons (2.04–2.11 ppm), the propargyl methylene protons (2.77–3.00 ppm), and the methine protons located on the polymer backbone (5.28–5.43 ppm) (Fig. 4). The signals corresponding to both the terminal alkyne and the propargyl methylene protons were integrated with respect to the two benzylic protons at the α chain-end to determine nominal degrees of polymerization for PPG which were then averaged to calculate the M_n of PPG within the polyester block.

The ability to accurately control the degree of incorporation of PPG into the polyester block directly translates to a high degree of control over guest attachment in the final nanoparticle vehicle. By relying on a covalent attachment strategy, the composition of the precursor BCP dictates the guest incorporation and allows for high payload loading to be achieved. In this study, we have demonstrated that BCPs with 23% functional group incorporation can be readily accessed and higher incorporations can be envisaged by simply increasing the feed

ratio of PG in the polymerization. Significantly, TEM micrographs of nano-particles prepared from alkyne functional BCPs **10–12** exhibit nanophase-separated internal morphologies similar to the parent PAGE-*b*-PLA nanoparticles which demonstrates that functionality can be installed on the backbone without major changes in the assembly properties of the BCP (*vide infra*, Figure S3 supporting information).

Synthesis and self-assembly of dye-conjugated degradable BCPs

Two different azide-functional coumarin fluorophores were synthesized and attached to the alkyne-functional PAGE-*b*-P(LA-*co*-PG) BCP scaffold to demonstrate the ability to specifically incorporate and then release small molecule cargos. Starting from 7-(diethylamino)coumarin-3-carboxylic acid, dyes bearing both an ester and amide linkage between the azide group and the coumarin skeleton were prepared (Scheme 2). Fluorophore **13** bearing a more labile ester linkage to the azide functional group was synthesized in two steps in 81% overall yield *via* a carbodiimide (DCC) coupling reaction with 3-chloropropanol followed by nucleophilic displacement of the chloride with sodium azide. The analogous dye containing a more robust amide linkage **14** was prepared in a similar manner by first coupling with 3-chloropropylamine hydrochloride using the peptide coupling reagent PyBop®⁶⁶ followed by substitution of the chloride with sodium azide in 77% overall yield. The dyes are highly crystalline, brightly colored materials that were thoroughly characterized by ¹H, ¹³C NMR and mass spectrometry.

The azide-functional coumarin dyes **13** and **14** were then attached to PAGE-*b*-P(LA-*co*-PG) **10** *via* a CuAAC reaction.^{67,68} However, it is important to note that even under these mild conditions, the functionalization of PLA is not straight-forward and degradation of the polyester backbone can occur. For example, Jérôme and coworkers reported the first successful ‘click’ derivatization of an azide-functional PLA copolymer using CuI and triethylamine in THF, but only after protection of the ω-hydroxyl end-group.⁶⁹ In an analogy to flash thermolysis of thermolabile compounds,⁷⁰ we anticipated that the degradation of PLA could be minimized during functionalization by limiting the reaction time, even when basic amines were employed and without protection of the terminal hydroxyl group. Fortunately, reactions carried out in THF at room temperature using CuBr and PMDETA as the ligand reached quantitative conversion of the azide-functional dye in only 30 min with little detectable degradation of the polyester backbone (Scheme 3). The GPC traces for the coumarin-functional BCPs demonstrate monomodal peaks with only slight broadening being observed. Furthermore, GPC monitored with a UV detector at 365 nm shows the coumarin absorption coincides with the refractive index peak of the BCP demonstrating the successful attachment of the dye onto the alkyne-functional BCP scaffold (Fig. 5, Figure S4 supporting information). The ¹H NMR spectra of the coumarin-functional BCPs **15** and **16** provide structural evidence for the successful ‘click’ reactions with the appearance of a series of unique resonances corresponding to the coumarin skeleton and the attenuation of signals belonging to the alkyne group of the BCP. Furthermore, a complete assignment of all peaks was possible with the aid of 2D COSY ¹H NMR spectroscopy (Figures S5–S7, supporting information). It should be noted that longer reaction times and different reaction conditions (*i.e.* THF/H₂O, CuSO₄, sodium ascorbate) result in significant cleavage of the PLA block as evidenced by the appearance of low molecular weight material.

Nanoparticles were prepared from the dye-conjugated BCPs using the same procedure described above with internal nano-phase-separated structures again being evident from TEM analysis. Critically, this demonstrates the ability to retain specific domains within the nanoparticle vehicles after the covalent incorporation of a guest (Fig. 6, S8 in supporting information). Importantly, the inclusion of the coumarin payloads in BCPs **15** and **16** does not show any significant influence on the morphology of the self-assembled nanoparticles. The major change observed is that the coumarin-functional BCP nanoparticles are slightly larger as measured by DLS with an average hydrodynamic diameter of 290 ± 9 nm compared to the PAGE-*b*-P(LA-*co*-PG) alkyne-functional nanoparticles at 240 ± 6 nm.

Release of guest from nanoparticle *via* degradation of PLA

The release of the coumarin fluorophores from the nanoparticles was then investigated using fluorescence spectroscopy. Importantly, the fluorescence of the nanoparticles prior to any degradation is quenched due to the high local concentration of coumarin dyes,^{71,72} a result that we have also seen recently in dendritic systems.⁷ However, an increase in fluorescence is observed upon release of the dye from the nanoparticle carrier into the surrounding media and this increase can be monitored directly without the need for elaborate experimental procedures.

Release of the model-drug from the nanoparticle vehicle was demonstrated to occur under physiologically relevant conditions *via* degradation of the PLA scaffold. Fluorescence measurements reveal similar profiles and release rates for nanoparticles prepared from BCPs **15** and **16** containing an ester and amide linkage, respectively, suggesting that the mechanism of guest release occurs through the degradation of the polyester backbone and not direct cleavage of the dye. In contrast, if direct cleavage of the model-payload from the polymer backbone within the carrier was occurring, significantly different release profiles between the two materials would be expected due to the variation in the rates of hydrolysis between the amide and ester bond linkages. The experiments were conducted with nanoparticle dispersions incubated at 37 °C for a period of 20 days in a pH 5 buffer and in water (pH = 6.7). The former condition mimics the acidic environment found in the endosome and, particularly, the lysosome of cells where enzymatic degradation occurs.⁷³ A pseudo logarithmic increase in the fluorescent signal was observed for both nanoparticles under acidic conditions that began to plateau after 20 days (Fig. 7a). In addition, fluorescence measurements demonstrate a more linear release profile for the coumarin fluorophore in water compared to the experiments performed in pH 5 buffer (Fig. 7b). Under these conditions, the fluorescent signals for nanoparticles of **15** and **16** increase steadily over 20 days corresponding to the consistent release of the model-drug over this time period.

Interestingly, intact nanoparticles result from the degradation of the PLA domains, presumably composed solely of PAGE. Nanoparticles absent of phase contrast are clearly observed in TEM images of the dispersions after degradation for 20 days in water at 37 °C and are similar in size to the non-degraded nanoparticles (Fig. 8). This result could have implications for alternative applications of functional self-assembled BCP dispersions in the preparation of unusually structured porous nanoparticles. In the present case, the internal structure of the nanoparticles is lost due to the low glass transition temperature of PAGE

which causes the particle to collapse; however, structural retention can be envisioned through the use of higher T_g or crosslinked materials.

Conclusion

We have demonstrated that the solution self-assembly of functional BCPs is a powerful technique for the preparation of novel delivery vehicles with control over the internal nanoscale morphology. Well-defined BCPs containing alkyne functionality within a biodegradable PLA block were synthesized by controlled ring opening polymerization and conjugated with azide-functional fluorescent model-drugs using CuAAC 'click' chemistry. Quantitative conversion of the azide group under mild conditions and short reaction times alleviates degradation of the polyester block during functionalization. Furthermore, by carefully controlling the composition of the BCP, self-assembled nanoparticles with internal nanophase-separated morphologies were prepared that release a covalently attached payload under physiological conditions *via* the degradation of the PLA scaffold. These results demonstrate the potential of this self-assembled nanoparticle platform for modular delivery vehicles with multiple functionalities and defined, three-dimensional internal morphologies. Further work will focus on exploiting the alkene functionality present in the poly(allyl glycidyl ether) block for orthogonal modifications and as platforms for alternative applications including architecturally diverse porous polymer nanoparticles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Schematic representation of the synthesis and self-assembly of multifunctional block copolymers containing a covalently attached payload released *via* the degradation of polylactide. Nanoparticles with compartmentalized internal morphologies can be accessed by carefully controlling the block copolymer composition.

Fig. 2.

TEM images of nanoparticles prepared from degradable block copolymers containing (a) a small fraction of polylactide, **1** ($f_{\text{PLA}} = 0.07$), with no observable internal nanophase-separation, and (b) a larger fraction of PLA, **9** ($f_{\text{PLA}} = 0.55$), comprising a nanophase-separated internal morphology. Dark regions correspond to PAGE domains stained with OsO_4 .

Fig. 3. GPC traces demonstrating the well-defined character of the alkyne-functional BCPs after chain extension of the poly(allyl glycidyl ether) macroinitiator (9.1 kg mol^{-1}) with polylactide.

Fig. 4. Representative ¹H NMR spectra for the poly(allyl glycidyl ether) macroinitiator (PAGE), PLA-based BCP **6**, and the orthogonal alkyne-functional BCP **10**. The structure of the alkyne-functional BCP is shown above with peak assignments.

Fig. 5. GPC traces for the alkyne-functional BCP **10** and dye-conjugated BCP **16** after CuAAC ‘click’ reaction recorded using a differential refractive index detector and a UV detector monitored at 365 nm. The coumarin UV absorption coincides with the refractive index response demonstrating successful attachment of the model-payload.

Fig. 6.

TEM images of nanoparticles prepared from the (a) alkyne-functional BCP **10**, and (b) dye-conjugated BCP **15**. An internal nano-phase-separated morphology is retained after guest attachment. Dark regions correspond to PAGE domains stained with OsO_4 .

Fig. 7.

Fluorescence plots demonstrating the release of the coumarin model-payload from the nanoparticles at 37 °C in (a) pH 5 buffer, and (b) pH 6.7 water. The fluorescence of the dye inside the nanoparticles is initially quenched ($t = 0$ days) and becomes restored upon release from the nanoparticle vehicle.

Fig. 8.

(a) Dynamic light scattering data for coumarin-functionalized BCP **15** demonstrate nanoparticles with an average diameter of 308 ± 26 nm remain after degradation of PLA. (b) TEM images of the nanoparticles as-prepared, and (c) after degradation in water (pH = 6.7) at 37 °C for 20 days. Absence of phase contrast post-degradation indicates PAGE nanoparticles remain after PLA removal. Dark regions correspond to PAGE domains stained with OsO₄.

Scheme 1.

Controlled synthesis of block copolymers (path a) without functionality in the degradable block for self-assembly modeling, and (path b) containing orthogonal alkyne functionality used for the covalent attachment of guest molecules.

Scheme 2.

Synthesis of azide-functional diethylamino-coumarin fluorophores, **13** and **14**. The alkyl azide groups are attached to the coumarin skeleton through either an ester or amide linkage providing two model-payloads with varying hydrolytic stability of the linking group.

Scheme 3.

Attachment of model payloads based on coumarins to the alkyne-functional BCP *via* CuAAC 'click' chemistry. Azide groups were maintained substoichiometric with respect to alkynes in the reaction.

Characterization of model PAGE-*b*-PLA BCPs with varying composition synthesized from two different poly(allyl glycidyl ether) macro-initiators ($M_n = 9.1$ and 15.8 kg mol^{-1})

Table 1

Polymer	[M]/[I] ^a	Conversion (%) ^b	$M_{n, \text{PLA}}^{\text{calc}}$ (kg mol ⁻¹) ^c	$M_{n, \text{PLA}}^{\text{expt}}$ (kg mol ⁻¹) ^b	f_{PLA}^d	$M_{n, \text{Total}}$ (kg mol ⁻¹) ^b	PDI ^e
1	10	93	1.3	1.4	0.07	17.2	1.14
2	10	97	1.4	1.4	0.11	10.5	1.10
3	40	90	5.2	5.0	0.20	20.8	1.17
4	65	87	8.2	7.5	0.27	23.3	1.16
5	35	96	4.9	5.0	0.30	14.1	1.12
6	100	72	10.4	8.8	0.31	24.6	1.15
7	50	97	7.0	6.6	0.37	15.7	1.18
8	75	95	10.3	10.1	0.47	19.2	1.19
9	150	81	17.4	14.3	0.55	23.4	1.18

^a Feed ratio of LA to PAGE macroinitiator.

^b Measured by ¹H NMR.

^c Calculated from the [M]/[I] feed ratio and adjusted for conversion.

^d $f_{\text{PLA}} = \text{NPLA} / (\text{NPLA} + \text{NPAGE})$.

^e Measured by GPC in chloroform relative to polystyrene standards.

Table 2

Characterization of multifunctional, degradable BCPs with varying alkyne incorporation synthesized from a 9.1 kg mol⁻¹ PAGE macroinitiator

Polymer	$M_{n, PPLA}^{calc}$ (kg mol ⁻¹) ^a	$M_{n, PLGA}^{exp1}$ (kg mol ⁻¹) ^a	$M_{n, PPG}^{calc}$ (kg mol ⁻¹) ^a	$M_{n, PPG}^{exp1}$ (kg mol ⁻¹) ^b	$M_{n, Total}$ (kg mol ⁻¹) ^b	f_{PPG}^c	PDI^d
10	10.1	10.1	1.5	1.7	20.9	0.11	1.34
11	12.4	13.5	1.9	2.2	24.8	0.11	1.38
12	10.9	11.2	4.2	4.4	24.7	0.23	1.43

^a Calculated from the [M]/[I] feed ratio and adjusted for conversion. The conversion of PG was essentially quantitative in each reaction. The conversion of LA was 97.3, 95.4, and 96.1% for polymers **10**, **11**, and **12**, respectively.

^b Measured by ¹H NMR.

^c $f_{PPG} = N_{PPG}/(N_{PPG} + N_{PLA})$.

^d Measured by GPC in chloroform relative to polystyrene standards.