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Functional Polylactide-*g*-Paclitaxel−Poly(ethylene glycol) by Azide−Alkyne Click Chemistry

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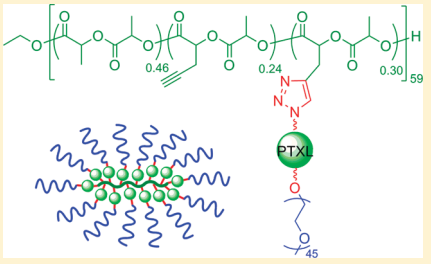
Supporting Information

ABSTRACT: Functional polylactide-*g*-paclitaxel−poly(ethylene glycol), a novel graft polymer−drug conjugate (GPDC) with paclitaxel (PTXL) as the divalent agent to bridge between the degradable polylactide (PLA)-based backbone and hydrophilic poly(ethylene glycol) (PEG) side chains, were prepared by the copper-catalyzed azide−alkyne cycloaddition reaction of acetylene-functionalized polylactide (PLA) with azide-functionalized PTXL−PEG conjugate. The acetylene-functionalized PLA was prepared by ring-opening copolymerization (ROCP) of acetylene-functionalized LA monomer with L-lactide (LA). The azide-functionalized PTXL−PEG conjugate was prepared by multistep organic synthesis. The well-controlled chemical structures of the GPDC and its precursors were verified by ^{1}H NMR and GPC characterizations. DLS analysis indicated that GPDC molecules assembled in water to form nanoparticles with sizes of 8−40 nm. GPC analysis of buffer solutions (pH = 5.5 and 7.4) of the GPDC suggested the occurrence of multiple hydrolysis reactions under the experimental conditions, which resulted in the release of PTXL moieties and the cleavage of PLA-based backbone.



INTRODUCTION

As a special class of functional polymeric materials, polymer−drug conjugates (PDCs) have labile linkages between drug moieties and polymers, and therefore, can allow drug molecules to be released through the cleavage of these linkages.[1−3] Relative to small molecule drugs that typically are insoluble in water, PDCs can have increased water solubility, reduced toxicity, prolonged circulation half-life, as well as improved accumulation in tumor tissue due to the enhanced permeability and retention (EPR) effect. As compared with polymeric encapsulation,[2] an alternative strategy for drug delivery via polymers, PDCs typically have well-controlled drug loadings and continuous release without burst effect. Generally, hydrophilicity of the base polymers is required to promote water solubility of the conjugated structures. If the molecular weights (MWs) of base polymers are higher than the renal clearance MW threshold (∼40 kDa), their degradability under bioactive environments is also needed.[1] Because peripheral hydrophobicity can affect solubility and induce nonspecific membrane binding,[4] it is desired that drug moieties are well-shielded within the macromolecular architectures of PDCs.

Copolymers having both PEG and aliphatic polyester components have become appealing base polymers for biomedical applications.[5−9] PEG is an important type of biocompatible and water-soluble polymer, and peglyation may increase the plasma clearance half-life of the resulting materials.[10,11] However, it is nondegradable and has only terminal functionalities available for conjugation.[10] On the other hand, aliphatic polyesters typically are biocompatible, biodegradable but hydrophobic. Multiple functionalities can be introduced to aliphatic polyesters via either polymerization using functionalized ester-based monomers or postpolymerization modification.[12−21] Therefore, combination of PEG and aliphatic polyesters may provide a variety of biodegradable, multivalent, and water-soluble or dispersible copolymers,[14−17,22−24] which may possess fabulous comprehensive properties for drug delivery applications via PDCs. For instance, as demonstrated by Fréchet and co-workers,[25] doxorubicin-functionalized biodegradable polyester dendrimer−PEG hybrids have been synthesized and they exhibited excellent antitumor effect in biomedical evaluation.

Both linear polymers and branched polymers have been used as the base polymer for PDCs.[1−3] Relative to their linear analogues with similar MWs, branched polymers not only have more terminal functionalities but also may possess better solubility and processability because of their low intermolecular interaction.[26] Besides other types of branched polymers, graft polymers have also been used as the base polymers for PDCs.[27−34] As compared with drug-conjugation with side-chains of graft polymers (via terminals,[27,28] Figure 1a; via side groups,[29,30] Figure 1b), drug-conjugation with backbones of grafted scaffolds (Figure 1c−e) may lead to well-shielded environments for drug moieties.[31−34] Because of their complicated architectures, it is challenging to synthesize GPDCs with well-controlled structures.

The recent developments of click chemistries have provided powerful tools in synthetic polymer chemistry.[35,36] With good tolerance of functionality, moderate reaction conditions, high yield and simple purification,[37] azide−alkyne click reaction has

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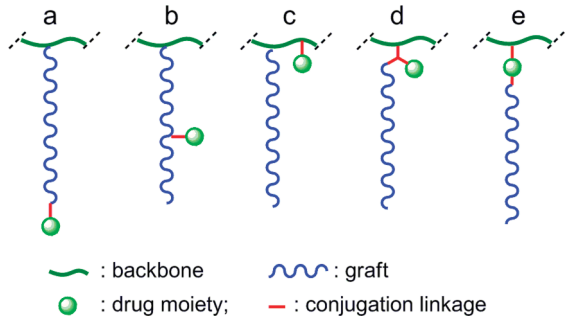


Figure 1. Schematic illustrations of structural designs of GPDCs.

been used for the preparation of a broad variety of well-defined functional polymeric architectures.[35,36,38−41] Several copolymers composed of PEG and aliphatic polyester components have been synthesized by using azide−alkyne click chemistry.[14−17,42−46] For examples, Baker and co-workers have synthesized functional PLA-*g*-PEG by the reaction of azide end-capped PEG with acetylene-functionalized PLA;[14] Jérôme and co-workers have prepared functional poly(ε-caprolactone)-*g*-PEG by the reaction of alkyne end-capped PEG with azide-functionalized PLA.[15] Besides other types of macromolecular conjugates,[47] GPDCs have also been synthesized through azide−alkyne reaction by several research teams. Emrick and co-worker have prepared GPDCs by using an alkyne-functionalized polylactone, along with monoazide-functionalized camptothecin and PEG, as reactants (Figure 1c).[31] Very recently, Grubbs and co-workers obtained novel GPDCs with divalent graft sites simultaneously linking a PEG chain and a doxorubicin moiety (Figure 1d) via azide−alkyne reactions,[32,33] and the brush-like densely grafted polymer scaffolds potentially may further allow their GPDCs to have controlled nanostructures.

We have long-term research interests on grafted polymeric systems, including GPDCs. Recently, we reported the synthesis of well-defined brush-like GPDCs by ring-opening metathesis copolymerization of norbornene-functionalized paclitaxel (PTXL) monomer and PEG macromonomer (Figure 1c).[34] With cycloacetal-based conjugated linkages, these GPDCs exhibited acid-triggered release of PTXL moieties. However, their biomedical applications are restricted by their nondegradable polynorbornene backbones. In this paper, we report our most recent research work on a PTXL-containing GPDC with degradable PLA-based backbone and PEG grafts by azide−alkyne click chemistry. Representing a new structural design, this GPDC has drug moieties serving as the divalent units covalently bridging between backbones and grafts (Figure 1e).

EXPERIMENTAL SECTION

Materials. 4-Dimethylaminopyridine (DMAP; 99+%), pyridinium chlorochromate (PCC; 98%), *N*,*N*′-dicyclohexyl-carbodiimide (DCC, 99%), *p*-toluenesulfonic acid (PTSA, 98.5+%), zinc powder (99+%), L-LA (98%), glutatic anhydride (95%), sodium carbonate (>99.9%) and magnesium sulfate anhydrate were purchased from Sigma-Aldrich. L-Malic acid (L-MA, 99%), *N*,*N*′-diisopropylcarbodiimide (DIC; 99%), sodium azide (99%), propargyl bromide (80 wt % solution in toluene), 2-bromopropionyl bromide (97%), copper(I) bromide (98+%), *N*,*N*,*N*′,*N*′,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), and boron trifluoride diethyl etherate ($BF_3 \cdot OEt_2$, 98%) were purchased from Acros. Diethyl ether (HPLC), tetrahydrofuran (THF; HPLC), dichloromethane (DCM; HPLC), ethyl acetate (HPLC), hexane (HPLC), triethylamine (99+%), ethyl alcohol (99.5+%), and *N*,*N*′-dimethylformamide (DMF; HPLC) were purchased from Fisher Chemical. PTXL (99%) was purchased from AvaChem Scientific. 6-Bromo-1-hexanol was purchased from TCI. α-Methyloxy PEG (MW: 2000) was purchased from RAPP Polymere GmbH. Ethyl glyoxylate (50 wt % in toluene) was purchased from Alfa Aesar, and was distilled before use. DCM, DMF, THF and EtOAc were dried by distillation over CaH_2. Zinc powder was treated with 2 M HCl, washed sequentially with distilled water and absolute ethanol, and dried under vacuum at 60 °C. LA was recrystallized from dry EtOAc twice prior to use. α-Methyloxy-ω-carboxyl PEG was prepared by the esterification reaction of α-methyloxy PEG with an excess of glutatic anhydride in the presence of triethylamine in DCM.[48] 2-Hydroxy-4-pentynoic acid was synthesized following a reference method.[14] 4-(Dimethyleamino)pyridinium 4-toluenesulfonate (DPTS) was prepared from DMAP and PTSA based on a literature approach.[49] All chemicals were used without further purification unless stated otherwise.

Synthesis of 2-(2-Bromopropanoyloxy)-4-pentynoic Acid (1). The synthetic reaction was conducted based on a literature method.[19] A DCM solution (∼170 mL) of 2-hydroxy-4-pentynoic acid (8.0 g, 70 mmol) and triethylamine (7.1 g, 70 mmol) was added dropwise over 30 min into an ice-cold DCM solution (∼170 mL) of 2-bromopropionyl bromide (15.6 g, 70 mmol) and DMAP (0.86 g, 7.0 mmol) under nitrogen atmosphere. After being stirred for 18 h at room temperature, the reaction mixture was concentrated and precipitated by addition of diethyl ether (200 mL) to remove salts. The filtrate was further concentrated by rotary evaporation. The resulting yellow oil was purified by flash column chromatography on silica gel using hexane/EtOAc (50/50) as eluent to give **1** as a light yellow oil (16.5 g, yield: 95%). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 11.44 (s, 1H, COO*H*), 5.27−5.37 (m, 1H, O*CH*COO), 4.39−4.53 (m, 1H, Br*CH*COO), 2.86 (m, 2H, CH≡CCH_2), 2.12 (m, 1H, C*H*≡CCH_2), 1.89 (m, 3H, CH_3). FT-IR (cm^{-1}): 3292, 3080, 2983, 2920, 1727, 1447, 1421, 1208, 1158, 1093, 983, 842, 772, 642.

Synthesis of Acetylene-Functionalized LA (2). The synthetic reaction was conducted based on a literature method.[19] A DMF solution (∼160 mL) of **1** (10 g, 40 mmol) was added dropwise over 10 h to a suspension of Na_2CO_3 (2.1 g, 20 mmol) in 750 mL of DMF under rapid stirring at room temperature under nitrogen atmosphere. After the reaction mixture was stirred for 36 h, DMF was removed in vacuo. The residue was diluted with acetone (200 mL), and the white precipitate formed was removed by filtration. Concentration in vacuo of the filtrate yielded a light brown oil which was purified by flash column chromatography on silica gel using hexane/EtOAc gradient as eluent (until hexane/EtOAc = 30/70) to give **2** (2.9 g, yield: 43%; a mixture of stereoisomers) as a colorless crystal. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.33 and 5.08 (m, 2H, 2 × OCHCOO; major resonances at 5.08), 2.88−3.07 (m, 2H, CH_2C≡CH), 2.13 and 2.20 (m, 1H, C≡C*H*), 1.70 (m, 3H, CH_3). FT-IR (cm^{-1}): 3279, 2998, 2936, 1753, 1453, 1411, 1361, 1293, 1241, 1128, 1093, 1040, 981, 961, 910, 881, 791, 762, 656. HRMS: calculated for $C_8H_8O_4$, 168.0417; found, 168.04143.

Synthesis of Acetylene-Functionalized PLA (3). In a 10 mL Schlenk flask, **2** (0.80 g, 4.76 mmol), L-LA (0.68 g, 4.76 mmol) and DMAP (77 mg, 0.63 mmol) were dissolved in 5 mL of DCM under nitrogen atmosphere. Then ethanol (7.3 mg, 0.158 mmol) was added using a syringe. The flask was placed in an oil bath at 35 °C for 48 h under stirring. About 90% conversion of comonomers was estimated by ^{1}H NMR analysis of an aliquot of polymerization solution, based on the resonance intensities of the CH protons of remaining comonomers at 5.08 ppm relative to the CH protons of the resulting polymer at 5.14−5.26 ppm. Then the polymerization solution was precipitated in ice-cold methanol to give **3** as a colorless polymer (0.97 g, isolated yield: 66%). $M_{n,NMR}$ = 9.31 kDa, $M_{n,GPC}$ = 11.7 kDa, and PDI_{GPC} = 1.23. Mole fractions of **2** and LA units were 54% and 46% as determined by ^{1}H NMR spectroscopy, based on the resonance intensities of the acetylene protons from **2** at 2.09 ppm and the CH protons from both comonomers

at 5.14−5.42 ppm. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 5.14−5.26 (br m, 2H from **2** and LA, 2 × OC*H*COO), 4.21 (m, 2H from EtOH, CH_3CH_2O), 2.84−2.92 (br m, 2H from **2**, CH≡CCH_2), 2.08 (s, 1H from **2**, *CH*≡CCH_2), 1.57−1.61 (br m, 3H from **2** and 6H from LA, CH_3 of **2** and 2 × CH_3 of LA). FT-IR (cm^{-1}): 3285, 2995, 2946, 1749, 1452, 1380, 1262, 1180, 1129, 1085, 1045, 867, 753, 656.

Synthesis of PTXL−PEG (4). In a 50 mL round flask, α-methyloxy-ω-carboxyl−PEG (1.05 g, 0.50 mmol), PTXL (448 mg, 0.525 mmol), DCC (113 mg, 0.55 mmol), DMAP (12 mg, 0.1 mmol), DPTS (29 mg, 0.1 mmol) were added in 15 mL of DCM at room temperature under nitrogen atmosphere. After being stirred for 48 h, the reaction mixture was filtered. The filtrate was concentrated in vacuo, followed by precipitation in 200 mL of diethyl ether to give **4** (1.34 g, yield: 90%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.14 (d, 2H, J = 7.5 Hz, *H*−Ph), 7.79 (2H, d, J = 7.5 Hz, *H*−Ph), 7.61 (t, 1H, J = 7.5 Hz, *H*−Ph), 7.19−7.53 (m, 11H, *H*−Ph and N*H*), 6.23−6.29 (m, 2H, 2 × *CH*OCO), 5.97 (d, 1H, J = 8.5 Hz, PhC*H*), 5.68 (d, 1H, J = 6.5 Hz, C*H*OCOPh), 5.48 (m, 1H, OC*H*CO (H2′)), 4.97 (d, 1H, J = 8.0, C*H*OCH$_2$), 4.44 (m, 1H, C*H*OH (H7)), 4.31 (d, 1H, J = 9.0 Hz, C*H*), 4.21 (m, 3H, C*H* and mPEG−CH_2OCOCH$_2$), 3.45−3.80 (br m, 179H, 1 × C*H* and 89 × CH_2 from PEG), 3.37 (s, 3H, CH_3O−PEG), 2.30−2.59 (m, 10H, O*H* from C7, 2 × C*H*, OOCCH_3 and 2 × CH$_2$CH_2COO), 2.23 (s, 3H, OOCCH_3), 2.18 (m, 1H, C*H*), 1.88−1.94 (m, 6H, C*H*, CH_3, and CH_2CH$_2$COO), 1.68 (s, 3H, CH_3), 1.21 (s, 3H, CH_3), 1.13 (s, 3H, CH_3).

Synthesis of 6-Bromo-1-hexanal (5). PCC (3.3 g, 75 mmol) was suspended in 200 mL of DCM in a 500 mL round flask, and then 6-bromo-1-hexanol (9.05 g, 50 mmol) in 50 mL of DCM was added into the reaction mixture. After being stirred at room temperature for 16 h, the reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel using hexane/EtOAc gradient as eluent (until hexane/EtOAc = 70/30) to give **5** as a colorless liquid (yield: 55%). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 9.77 (t, 1H, J = 1.5 Hz, CH$_2$C*H*O), 3.42 (t, 2H, J = 7.0 Hz, BrCH_2), 2.39−2.48 (m, 2H, CH_2CHO), 1.89 (m, 2H, BrCH$_2$CH_2), 1.67 (m, 2H, CH_2CH$_2$CHO), 1.50 (m, 2H, BrCH$_2$CH$_2$CH_2). FT-IR (cm^{-1}): 2938, 2863, 2727, 1721, 1458, 1431, 1409, 1390, 1264, 1116, 946, 853, 733, 641. HRMS: calculated for $C_6H_{10}OBr$, 176.9910; found, 176.99109.

Synthesis of 6-Azido-1-hexanal (6). Sodium azide (0.65 g, 10 mmol) and 6-bromohexanal **5** (0.88 g, 5.0 mmol) were added and dissolved in 20 mL of DMF in a 100 mL round flask. After being stirred under room temperature for 36 h, the reaction mixture was added with 30 mL of DCM and then extracted with water (30 mL), brine (30 mL) and saturated $NaHCO_3$ aqueous solution (30 mL) respectively. The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and the resulting mixture was purified by column chromatography on silica gel using hexane/EtOAc gradient as eluent (until hexane/EtOAc = 50/50) and gave **6** as a pale yellow liquid (yield: 79%). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 9.77 (t, 1H, J = 1.5 Hz, CH$_2$C*H*O), 3.29 (t, 2H, J = 7.0 Hz, N_3CH_2), 2.39−2.48 (m, 2H, CH_2CHO), 1.67 (m, 4H, $N_3CH_2CH_2CH_2CH_2$), 1.44 (m, 2H, $N_3CH_2CH_2CH_2$). FT-IR (cm^{-1}): 2941, 2864, 2092, 1724, 1455, 1352, 1258, 1182, 1128, 1089, 1044, 868, 806, 738, 660.

Synthesis of 2-(2-(5-Azidopentyl)-5-oxo-1,3-dioxol-4-anyl)acetic Acid (7). 6-Azidohexanal **6** (200 mg, 1.42 mmol) and L-MA (285 mg, 2.12 mmol) were dissolved in 30 mL of diethyl ether at room temperature in a 100 mL round flask. Then $BF_3 \cdot OEt_2$ (1.0 g, 7.1 mmol) was added directly to the reaction mixture. The reaction was conducted in room temperature for 68 h under N_2 atmosphere. The reaction mixture was purified by column chromatography on silica gel using hexane/EtOAc gradient as eluent (until hexane/EtOAc = 50/50) and gave **7** as a colorless liquid (yield: 74%). ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 10.45 (br, 1H, COO*H*), 5.73 (t, 1H *trans*, J = 4.5 Hz, OC*H*(CH$_2$)O), 5.57 (t, 1H *cis*, J = 4.5 Hz, OC*H*(CH$_2$)O), 4.65 (t, 1H *trans*, J = 4.5 Hz, C*H*CH$_2$COOH), 4.61 (t, 1H *cis*, J = 4.5 Hz, C*H*CH$_2$COOH), 3.30 (m, 2H, N_3CH_2), 2.99 (m, 2H, CHCH_2COOH), 1.84 (m, 2H, CH$_2$CH_2COO), 1.62 (m, 2H, CH_2), 1.47 (m, 4H, 2 × CH_2). FT-IR (cm^{-1}): 3045, 2932, 2865, 2095, 1795, 1716, 1400, 1353, 1194, 1125, 1037, 968, 818, 736, 633. HRMS: $[M + Na]^+$ calculated for $C_{10}H_{15}O_5N_3$, 280.0904; found, 280.09085.

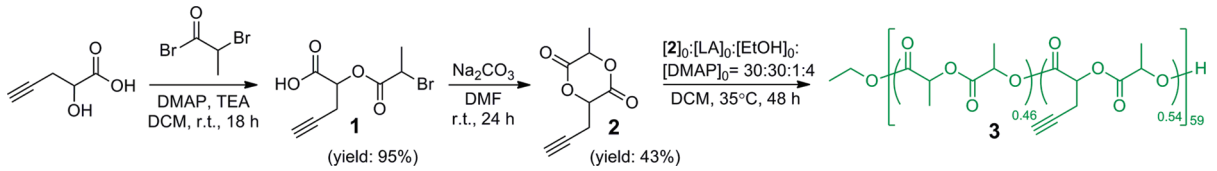
Synthesis of Azide−PTXL−PEG (8). In a 25 mL round flask, **7** (0.455 g, 1.77 mmol) and **4** (1.3 g, 0.44 mmol) were dissolved in 10 mL of DCM, followed by adding DIC (0.444 g, 3.52 mmol) and DMAP (53 mg, 0.44 mmol). After being stirred about 30 min at room temperature, the reaction mixture became dark. Then the reaction mixture was heated to reflux under stirring for 3 days. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated and precipitated in ice-cold diethyl ether. The brown precipitate was further purified by column chromatography on silica gel with DCM/methanol gradient as eluent (until DCM/methanol = 90/10) and gave **8** as a brown solid (813 mg, yield: 58%). $M_{n,NMR}$ = 3.20 kDa, $M_{n,GPC}$ = 10.6 kDa, PDI_{GPC} = 1.02. ^{1}H NMR (500 MHz, $CDCl_3$, ppm): 8.13 (d, 2H, J = 7.5, *H*−Ph), 7.78 (2H, d, J = 7.5 Hz, *H*−Ph), 7.62 (t, 1H, J = 7.5 Hz, *H*−Ph), 7.34−7.54 (m, 10H, *H*−Ph), 7.20 (d, 1H, J = 9.0 Hz, N*H*), 6.24 (m, 2H, 2 × *CH*OCO), 5.98 (d, 1H, J = 8.5 Hz, PhC*H*), 5.61−5.71 (m, 2H, 2 × C*H*, C*H*OCO (H7) and C*H*OCOPh), 5.48−5.55 (m, 2H, 2 × C*H*, OC*H*CO (H2′) and CH$_2$C*H*OO from cycloacetal), 4.96 (d, 1H, J = 8.5, C*H*OCH$_2$), 4.73 (m, 1H, OC*H*COO from cycloacetal), 4.33 (d, 1H, J = 8.5 Hz, C*H*), 4.20 (m, 3H, C*H* and mPEG−CH_2OCOCH$_2$), 3.45−3.94 (br m, 179H, 1 × C*H* and 89 × CH_2 from PEG), 3.37 (s, 3H, CH_3O−PEG), 3.28(m, 2H, N_3CH_2), 2.96−3.09 (m, 2H, CHCH_2COO), 2.16−2.74 (m, 15H, 3 × C*H*, 2 × OOCCH_3, 1 × CH$_2$CHOO and 2 × CH$_2$CH_2COO), 1.81−2.00 (m, 8H, CH_2, C*H*, CH_3, and CH_2CH$_2$COO), 1.13−1.68 (m, 15H, 3 × CH_3 and 3 × CH_2). FT-IR (cm^{-1}): 2882, 2096, 1796, 1742, 1661, 1524, 1466, 1359, 1342, 1278, 1239, 1145, 1103, 1060, 962, 841, 710.

Synthesis of Functional PLA-*g*−PTXL−PEG (9). To a 10 mL reaction flask, **3** (45 mg, 155 μmol of acetylene group), **8** (249 mg, 78.2 μmol), and PMDETA (16.3 mg, 93.8 μmol) were added and dissolved with 2 mL of DMF. The mixture was degassed by three freeze−pump−thaw cycles followed by the addition of copper(I) bromide (13.5 mg, 93.8 μmol).[50] The reaction mixture was stirred for 24 h at 35 °C under nitrogen atmosphere. Reaction extent of 95% was estimated by GPC analysis of an aliquot of reaction mixture. Then the reaction mixture was precipitated in diethyl ether and residual copper bromide was removed by passing through a short alumina column using DCM as eluent to yield **9** as a brown solid (162 mg, yield: 54%). $M_{n,NMR}$ = 66.0 kDa, $M_{n,GPC}$ = 46.7 kDa, PDI_{GPC} = 1.32. Grafting density, as the number ratio of grafts to all of the repeat units on backbone, was 30% as determined by ^{1}H NMR spectroscopy. ^{1}H NMR(500 MHz, $CDCl_3$): 7.20−8.14 (br m, 16H, C*H*, *H*−Ph and N*H*), 6.24 (m, 2H, 2 × *CH*OCO), 5.98 (d, 1H, J = 8.5 Hz, PhC*H*), 5.49−5.69 (m, 4H, 4 × C*H*), 5.17 (br, C*H*, OC*H*COO from PLA), 4.95 (br, 1H, C*H*OCH$_2$), 4.73 (br, 1H, OC*H*COO from cycloacetal), 4.33 (d, 1H, J = 8.5 Hz, C*H*), 4.20 (m, 3H, C*H* and mPEG−CH_2OCOCH$_2$), 3.45−3.94 (br m, 179H, 1 × C*H* and 89 × CH_2 from PEG), 3.37 (s, 3H, CH_3O−PEG), 1.15−2.48 (br m, 4× C*H*, 10 × CH_2, 6 × CH_3 and CH_2, CH_3 from PLA). FT-IR (cm^{-1}): 2867, 1745, 1661, 1526, 1452, 1349, 1239, 1180, 1091, 982, 947, 847, 711.

Stability and Degradation Study of GPDC 9. With pH of 5.5 and 7.4, phosphate buffer solutions of **9** (10 mg per 100 mL) were prepared by the aid of ultrasonification for 5 min. Then both solutions were incubated in oil bath at 37 °C under slow stirring. After 2, 3, 5, and 7 days, 10 mL of solution was withdrawn and dried in vacuo. Finally, the residues were dissolved in DMF for GPC measurement.

Characterization. ^{1}H NMR (500 MHz) spectra were acquired in $CDCl_3$ using a Varian INOVA-500 spectrometer at room temperature. Tetramethylsilane (TMS) was used as an internal standard for ^{1}H NMR

Scheme 1



spectroscopy. FT-IR spectra were obtained on a Bruker Tensor 27 system using attenuated total reflectance (ATR) sampling accessories. High-resolution mass spectrometry (HRMS) data were recorded on a VG 70-SE mass spectrometer with electron ionization mode.

GPC data were obtained from Viscotek GPC system equipped with a VE-3580 refractive index (RI) detector, a VE 1122 pump, and two mixed-bed organic columns (PAS-103 M and PAS-105M). DMF (HPLC grade) with 0.1 M LiBr was used as the solvent for polymers and eluent for GPC with a flow rate of 0.5 mL/min at 55 °C. The GPC instrument was calibrated with narrowly dispersed linear polystyrene standards purchased from Varian.

Dynamic light scattering measurements were performed using a Nano ZS90 Zetasizer (Malvern Instruments). A 4 mW 633 nm HeNe laser was used as the light source and all experiments were performed at a temperature of 25.0 °C at a measuring angle of 90° to the incident laser beam. The correlation decay functions were analyzed by cumulants method coupled with Mie theory to obtain volume distribution. Dilute solutions of GPDC **9** (≤4.0 mg/mL) were prepared by using water and DMF as solvents. For the preparation of water solution of **9**, 5 min of ultrasonication was performed to promote the dissolving process. The ultrasonication was conducted by using a 150 Series Digital Sonic Dismembrator (Fisher Scientific) equipped with a SLP Microtip (0.08 in. in diameter, Brason Ultrasonics), and the continuous mode with the amplitude of 70% was selected. The solutions were passed through 450 nm low protein binding hydrophilic LCR (PTFE) membrane filter (Millex-LCR, Millipore) for dust free process before DLS measurements. All determinations were repeated five times.

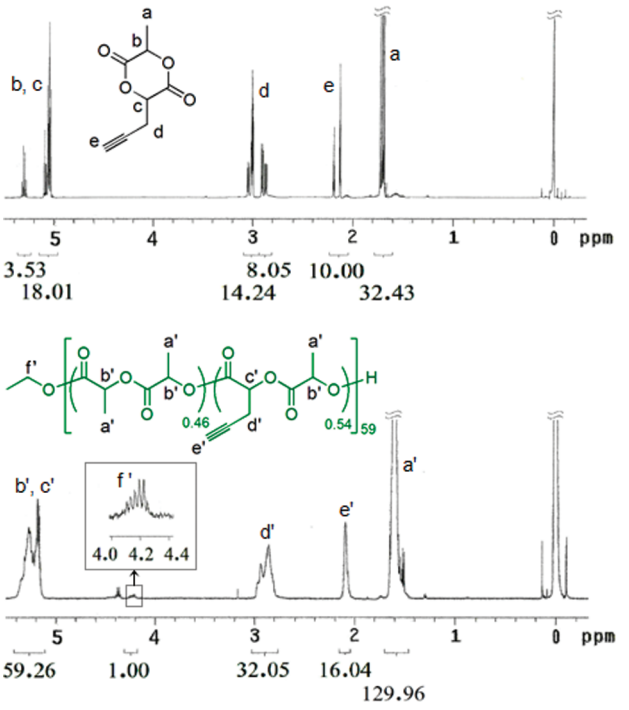


Figure 2. ^{1}H NMR (500 MHz) spectra of acetylene-functionalized LA **2** and PLA **3**.

RESULTS AND DISCUSSION

The structural design of GPDC incorporated with considerations on structures of backbone, graft, drug unit, and conjugation linkage. First, because PLA is a degradable polymer with broad biomedical applications,[51] a PLA-based backbone was chosen for GPDC. Second, with useful properties for drug delivery applications,[10] water-soluble PEG was employed as the polymeric component of graft. Third, PTXL, a potent anticancer drug with two reactive hydroxyl groups,[52] was selected as the divalent drug for this study. Fourth, hydrolyzable linkages, including ester linkages and an acid-sensitive cycloacetal-based conjugation linkage,[34] were used to link PTXL with PLA-based backbone and PEG chain. To achieve the GPDC with the targeted structures via azide−alkyne "grafting-onto" strategy, the corresponding precursor polymers having either azide or alkyne functionality need to be prepared.

As the precursor polymer of the backbone of GPDC, functional PLA **3** with pendent acetylene functionalities was prepared by the synthesis of monoacetylene-functionalized LA monomer **2**, followed by ROCP of **2** with L-LA (Scheme 1). Using 2-hydroxy-4-pentynoic acid as a starting chemical, **2** was obtained in two-step organic synthesis with 41% overall yield. The chemical structure of **2** (as a mixture of stereoisomers) was verified by the quantitative resonance intensities of its acetylene proton at 2.2 ppm (Figure 2). Besides **2**, diacetylene-functionalized LA monomer has also been reported.[14] Because the high density of alkyne groups in the backbone precursor polymer is not required due to the bulky size of the drug-containing azide-functionalized grafting-onto agent in this work, we chose **2** as the functional LA monomer and its copolymerization with L-LA for the preparation of **3** ($[\mathbf{2}]_0$:$[LA]_0$:$[EtOH]_0$:$[DMAP]_0$ = 30:30:1:4; 35 °C, 48 h, in DCM; 90% overall conversion of comonomers). Following the literature conditions,[53] ethanol was used as the initiator and DMAP was used as the organocatalyst for the copolymerization. The well-controlled chemical structure of the resulting **3** was verified by ^{1}H NMR (Figure 2) and GPC (Figure 3) characterizations. On the basis of the resonance intensities of the acetylene protons from **2** at 2.09 ppm and the CH protons from both comonomers at 5.14−5.42 ppm, the molar fraction of 54% for **2** in copolymer **3** was determined, and it was close to the 50 mol % feed of **2** for copolymerization. Number-average degree of polymerization (DP_n) of 59 for **2** was further estimated by comparing the resonance intensities of the CH protons from comonomer units at 5.14−5.42 ppm with these of the terminal CH_2 protons from ethanol at 4.21 ppm,[53] and this experimental DP_n value was in good agreement with the theoretical DP_n value of 54. Calculated from DP_n and composition of **3**, the number-average molecular weight (M_n) of **3** was 9.31 kDa. GPC analysis indicated that **3** had a M_n of 11.7 kDa and a PDI of 1.23 relative to linear polystyrenes.

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As the precursor polymer of the drug-conjugated graft of GPDC, azide-functionalized PTXL−PEG conjugate **8** was synthesized (Scheme 2). Because the 2′-hydroxyl group of PTXL is significantly more reactive than the 7-hydroxyl group (the third hydroxyl group of PTXL has no considerable reactivity),[52] the DCC/DMAP/DPTS-catalyzed esterification reaction of acid-functionalized PEG_{2000} (prepared from MeO−PEG_{2000}−OH with a M_n of 2.00 kDa) with a slightly excess amount of PTXL (1.05 equiv) gave 2′-PTXL PEG ester **4** as the predominant product in 90% yield. A cycloacetal-based azide-functionalized carboxylic acid **7** was prepared using 6-bromo-1-hexanol as a starting compound via three-step organic synthesis with 32% overall yield. Finally, the targeted conjugate **8**, which has an PTXL moiety connected with PEG and cycloacetal-based azide through ester linkages on 2′- and 7-positions respectively, was obtained in 58% yield by the DMAP/DIC-catalyzed esterification reaction of an excess amount of **7** (4.0 equiv) with **4**. The chemical structure of **8** was verified by ^{1}H NMR analysis (Figure 4). The disappearance of the characteristic resonances the 2′-CH and 7-CH of PTXL at 4.78 and 4.40 ppm respectively suggested the quantitative functionalizations of PTXL through its two hydroxyl groups. The 2′-CH and 7-CH protons of the PTXL moiety of **8** resonated at 5.49 and 5.70 ppm, respectively, with considerable overlaps with the resonances of other protons. Therefore, the resonance intensities of the 3′-CH and 5-CH protons of PTXL at 5.99 and 4.97 ppm were used to compare with the intensities of the characteristic resonances of all protons from PEG and the $-CH_2N_3$ protons at 3.20−3.76 ppm (no resonances from PTXL protons at this region).[54] As a result, the DP_n of 45 for the PEG chain of **8** was determined, and this DP value agreed exactly with the DP_n of the initial PEG_{2000} reactant, verifying the quantitative one PEG chain per molecule of **8**. The intensities of the characteristic resonances of the cycloacetal CH proton at 4.73 ppm essentially were the same as these for the PTXL proton at 5.99 or 4.97 ppm, further indicating the quantitative presence of cycloacetal-based azide structure in **8**. On the basis of the structural information on **8** revealed by ^{1}H NMR spectroscopy, **8** has a M_n of 3.20 kDa. According to GPC analysis, **8** had a M_n of 10.6 kDa and a PDI of 1.02 relative to linear polystyrenes (Figure 3). The significant difference between the $M_{n,NMR}$ and $M_{n,GPC}$ of **8** was ascribed to the different MW-hydrodynamic volume relationship for **8** and polystyrene in DMF.

Following Scheme 3, functional PLA-*g*-PTXL−PEG, i.e., GPDC **9**, was prepared by copper(I)-mediated azide−alkyne click reaction of azide−PTXL−PEG **8** with acetylene-functionalized PLA **3** using PMDETA as ligand in DMF at room temperature for 24 h ($[azide]_0:[alkyne]_0:[CuBr]_0:[PMDETA]_0 = 1:2:1.2:1.2$).[50] The reaction mixture was analyzed by GPC. Along with the disappearance of GPC peak of **3** and the appearance of new GPC peak for the formation of **9**, the area of the GPC peak of **8** decreased by about 20 times during the reaction, suggesting ~95% conversion of **8**. After the work-up procedure, GPDC **9** was obtained in 66% isolated yield. The composition and graft density of **9** were determined by ^{1}H NMR spectroscopy (Figure 5).

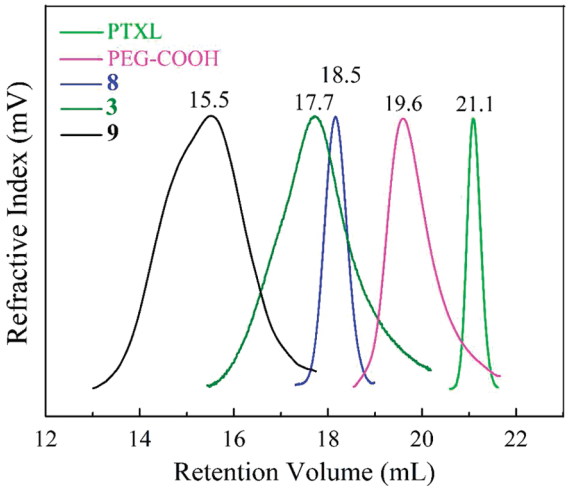


Figure 3. GPC curves of acetylene-functionalized PLA **3**, PTXL, PEG−COOH, azide−PTXL−PEG **8**, and GPDC **9**.

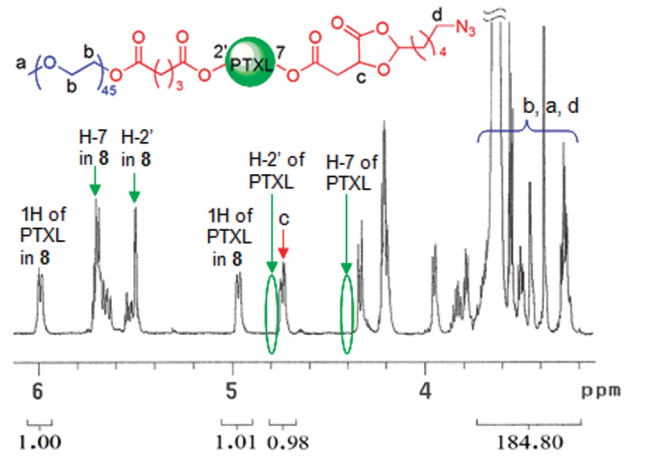
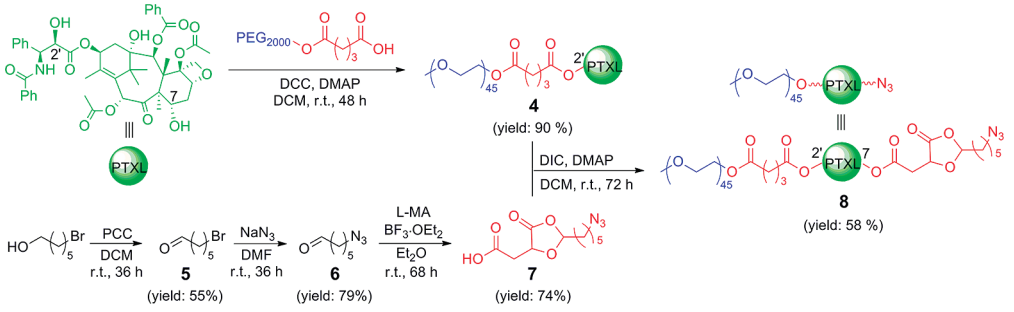


Figure 4. ^{1}H NMR (500 MHz) spectrum of azide−PTXL−PEG **8**.

Scheme 2



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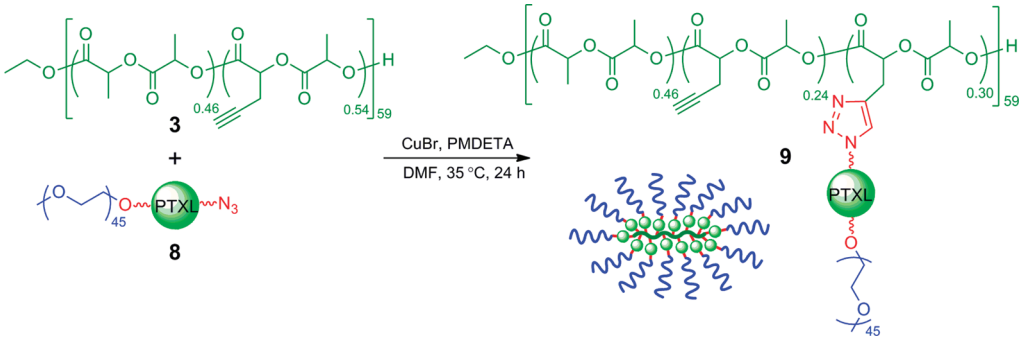
Scheme 3



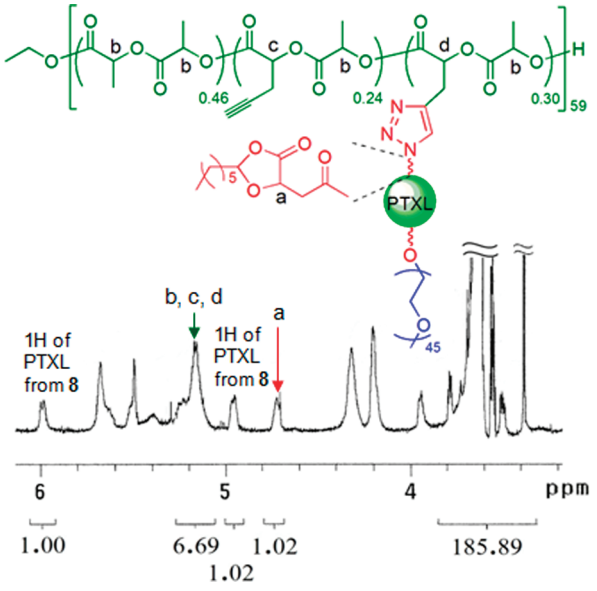


Figure 5. ^{1}H NMR spectrum of GPDC **9**.

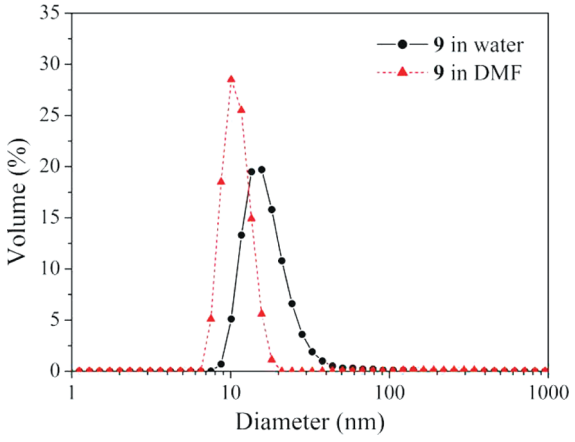


Figure 6. DLS size distribution profiles of GPDC **9** in water and in DMF.

The characteristic resonances of protons of side chains of **9** (including the 3′-CH and 5-CH protons from PTXL at 5.99 and 4.96 ppm,[54] a cycloacetal CH proton at 4.72 ppm, all PEG protons and the methylene protons of $-CH_2NH_2$ at 3.14−3.88 ppm) exhibited essentially the same intensity ratio as the resonances of the corresponding protons of **8**, indicating that all conjugation linkages were stable under the reaction and work-up conditions for the preparation of **9**. Because the resonances of the proton of 1,2,3-triazole ring overlapped with these of phenyl protons from PTXL at 7.20−7.84 ppm, the resonance intensity of the cycloacetal CH proton at 4.72 ppm was selected to compare with the resonance intensity of the backbone CH proton at 5.05−5.29 ppm. The resulting experimental grafting density, as the number ratio of grafts to all of the repeat units on backbone, was 30% which was close to the grafting density of 26% calculated from the feed ratio and the conversion of **8**. On the basis of the structural information on **9** revealed by ^{1}H NMR spectroscopy, **9** had a M_n of 66.0 kDa, with 23 wt % of PTXL and 54 wt % of PEG. By GPC analysis, **9** had a M_n of 46.7 kDa and a PDI of 1.32 related to linear polystyrenes. Because 86 wt % of **9** was from precursor **8**, according to the large $M_{n,GPC}$ relative to $M_{n,NMR}$ for **8**, the small $M_{n,GPC}$ relative to $M_{n,NMR}$ for **9** indicated its compact macromolecular architecture due to grafting.[55] By DLS analysis (Figure 6), **9** showed hydrodynamic sizes of 8−40 nm, with volume-average hydrodynamic diameter ($D_{h,v}$) of 18.4 nm, in water that is a selective solvent for PEG chains of **9**. On the other hand, **9** exhibited hydrodynamic sizes of 6−20 nm, with $D_{h,v}$ of 10.6 nm, in DMF that is a good solvent for all types of structural components of **9**. The larger hydrodynamic sizes of **9** in water relative to DMF indicated that molecules of **9** assembled or aggregated into multimolecular structures in water.

The stability of **9** in aqueous conditions would affect whether PTXL moieties can be effectively released and whether the PLA-based backbone can be eventually cleaved by hydrolysis. There are three hydrolyzable groups, including a cycloacetal group, 2′-ester and 7-ester groups, related to the release of small molecule PTXL moieties. The cycloacetal group generally is acid-sensitive and had reactivity in hydrolysis increasing with reaction acidity.[34] For derivatives of PTXL, 2′-ester typically is significantly more hydrolyzable than 7-ester.[52] Greenwald et al. also reported that the hydrolysis of C2′−PEG ester was slower in slightly acidic sterile water (pH = 5.7) than in physiological buffer (pH = 7.4).[56] The hydrolysis of PLA-based backbone is also due to its ester groups.[57] Because the hydrolysis of all these groups is expected to be pH-dependent, the stability of **9** was studied in aqueous buffer solutions with pH of 5.5 (a representative local pH within endosomal and lysosomal vesicles)[1] pH 7.4 (a typical pH of normal human blood) at 37 °C. The aliquots were withdrawn at 2, 3, 5, and 7 days, and analyzed by GPC (Figure 7). Multimode GPC curves were observed because of the occurrence of

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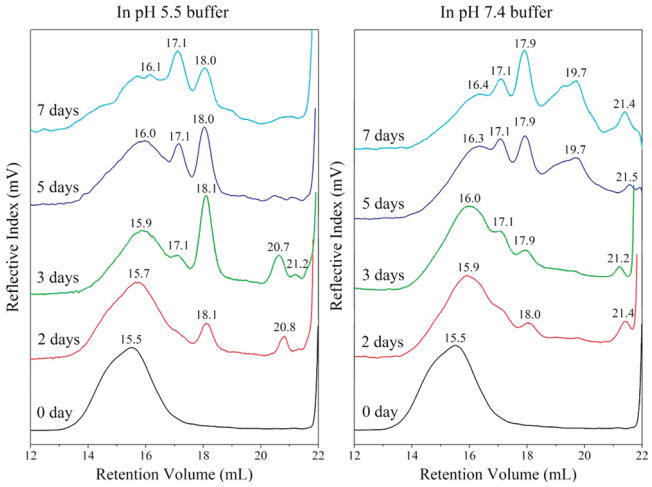

Figure 7. GPC curves of the buffer solutions (pH of 5.5 and 7.4) of GPDC **9** incubated at 37 °C for 0, 2, 3, 5, and 7 days.

hydrolysis of **9**. Using the GPC peak positions of GPDC **9** and its precursor polymers and structural components as references (Figure 3), these GPC curves were further interpreted and tentative assignments of their GPC peaks were made.

Because azide−PTXL−PEG **3**, as the precursor of the grafts of **9**, had elution peak at 18.2 mL, these GPC peaks at 17.9−18.1 mL observed in all trails were assigned to the detached PTXL−PEG species formed by hydrolysis of cycloacetal or 7-ester group (the elution peak positions were slightly affected by overlapping with other peaks). Because of the elution peak of pure acid-functionalized PEG at 19.6 mL, the strong GPC peaks at 19.7 mL observed for the trial of pH = 7.4 at 5 and 7 days, as well as GPC shoulders around this position in other trails, were assigned to the detached acid-functionalized PEG formed by hydrolysis of 2′-ester group. Because free PTXL showed an elution volume of 21.2 mL, the GPC peaks 21.2−21.6 mL, which were more evident for trials at pH of 7.4 than the trials at pH of 5.5, were assigned to the released PTXL formed by the hydrolysis of both 7-ester and 2′-ester groups. For trails at pH of 5.5, GPC peaks at 20.7−20.8 mL were also observed. Because the corresponding species were eluted out slightly before PTXL, they were tentatively assigned to the 7-ester derivative of PTXL that had a little larger hydrodynamic volume than PTXL and was formed by the hydrolysis of both cycloacetal and 2′-ester groups. On the basis of the GPC curves, the three hydrolyzable groups related to the release of PTXL indicated different reactivity in hydrolysis. In the buffer solution with pH of 5.5, the hydrolysis reactivity order was cycloacetal > 2′-ester > 7-ester. In the buffer solution with pH of 7.4, the hydrolysis reactivity order was 2′-ester > cycloactal or 7-ester. It should be noted that, because of the very low solubility of PTXL in aqueous solutions, the released PTXL moieties might not be quantitatively recovered under the experimental conditions. The polymeric species were eluted out before the detached PTXL−PEG species were assigned to the grafted residues from **9** that had elution volume of 15.5 mL. Because the decrease of hydrodynamic volumes of grafted polymers generally is less sensitive to graft detachment than backbone cleavage, the GPC peaks at 15.7−16.3 mL were assigned to the grafted residues without considerable occurrence of backbone cleavage, and the GPC peaks at 17.1 mL or bumps around the position were tentatively assigned to the grafted residues formed by backbone cleavage. Under both pH conditions, the heights of GPC peaks for backbone cleavage increased with time. These backbone cleavage signatures appeared with the GPC peaks of detached PTXL−PEG species, suggesting that the polar groups resulted from the graft detachment might promote the hydrolysis of backbone.[58,59] At the same times, these GPC peaks were higher at pH of 5.5 than pH of 7.4, presumably because the faster hydrolysis of cycloacetal groups at pH of 5.5 led to enhanced backbone hydrolysis.

CONCLUSIONS

The organocatalyzed-ROCP of monoacetylene-functionalized LA monomer with L-LA yielded well-defined functional PLA with pendent acetylene groups. The selective functionalizations of 2′- and 7-hydroxyl groups of PTXL gave a conjugate with the PTXL moiety that linked simultaneously with a PEG chain and a cycloacetal-based azide via ester linkages. Representing a new structural design of GPDC with drug as the divalent agent, the functional PLA-*g*-PTXL−PEG was synthesized by copper-catalyzed cycloaddition reaction of the acetylene-functionalized PLA with the azide-functionalized PTXL−PEG conjugate. The high extent (95%) of the grafting-onto reaction indicated that the azide−alkyne click chemistry is highly useful for the synthesis of GPDCs. With peripheral PEG chains and carrying 23 wt % of hydrophobic PTXL, the GPDC exhibited as multimolecular assembled or aggregated nanoparticles in water. GPC monitoring of the stability of the GPDC in aqueous buffer solutions (pH = 5.5 and 7.4) suggested that multiple hydrolysis reactions occurred under the experimental conditions. Relative to the ester linkage between PTXL and PEG, the cycloacetal group hydrolyzed faster under acidic conditions. The hydrolysis of 2′-ester group, on the other hand, showed higher hydrolysis reactivity at pH of 7.4 than pH of 5.5. The release of PTXL moieties and the hydrolysis of PLA-based backbone occurred under both pH conditions, suggesting the potential clinic application of the GPDC and the minimized long-term side-effects of the polymer scaffolds. The findings of this work provide useful information in our future research of drug-conjugated polymeric materials.

ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR spectra for compounds **1**, **4**−**7** and GPC curves of **3** before and after purification. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§These authors contributed equally to the present work

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