Synthesis and Characterization of a Well-Defined Amphiphilic Block Copolymer and Its Paclitaxel Prodrug from Methoxy Poly(ethylene glycol) and Oligomer of Glycolic Acid

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ABSTRACT: A well-defined amphiphilic block copolymer was synthesized by the coupling of carboxyl-terminated methoxy poly(ethylene glycol) (MPEG) with a hydroxyl-terminated octamer of glycolic acid, which was obtained by a stepwise synthetic procedure with the end-group protection and deprotection. The block copolymer had a polydispersity index (PDI) of 1.01, as determined by gel permeation chromatography. It was further coupled to paclitaxel to form a prodrug of paclitaxel. The paclitaxel content in the prodrug was about 10%, and its PDI was 1.02. The antitumor activity of the conjugate against human lung carcinoma A549 cells was evaluated by mitochondrial dehydrogenase (MTT) assay. The results show that paclitaxel could be released from the conjugate without losing cytotoxicity. Therefore, the well-defined amphiphilic block copolymer from MPEG and oligomer of glycolic acid could potentially provide novel opportunities to obtain reproducible pharmacokinetic behavior in the design of a drug-delivery system. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 758–766, 2011

Key words: biodegradable; block copolymers; conjugated polymers; drug delivery systems; oligomers

INTRODUCTION

The therapeutic effect of many anticancer drugs and other bioactive components with low molecular weights are often limited by their poor properties, such as low water solubility and rapid elimination from the body.[1] In addition, whereas the beneficial effects of anticancer drugs arise through their interactions with tumor cells, undesirable side effects and toxicity typically result from their exposure to other cell types.[2–4] Since Ringsdorf[5] first proposed the concept of conjugating low-molecular-weight drugs to water-soluble polymers and designed a rational model, considerable attention from polymer chemists, chemical engineers, and pharmaceutical scientists has been attracted to polymeric conjugates of conventional drugs (polymeric prodrugs). Polymer–drug conjugates have several advantages over corresponding low-molecular-weight drugs. For example, the water solubility can be improved for low-soluble or insoluble drugs, and the circulation time of the polymer drug conjugate in the plasma can be increased through polymeric attachment.[6] This enhancement in circulation time is the result of the decreased rate of renal filtration that correlates with the molecular size, as molecules with a larger hydrodynamic volume are generally eliminated more slowly. Furthermore, the ability of passive targeting to solid tumors can also be obtained upon conjugation of anticancer drugs to polymers.[7,8] This targeting is thought to be possible because of the increased permeability of tumor vasculature to macromolecules and limited lymphatic drainage, which results in the selective accumulation of macromolecules in the tumor tissue. This phenomenon is

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known as the *enhanced permeability and retention effect*.[9]

To achieve desirable pharmacokinetics and targeting results, three design principles for polymers are necessary: (1) the careful selection of carrier polymers with water solubility, special biocompatibility, low polydispersity, and the presence of a highly accessible functional handle for drug attachment; (2) the discreet choice of a polymer–drug linkage that should be relatively stable to prevent drug release during the body's blood circulation but sensitive to the conditions of the target site, so as to allow drug liberation and accumulation in cells; and (3) the introduction of homing devices that can alter the body distribution and cell uptake by active or passive targeting.[10–12] Great progress has been made in polymer–anticancer drug conjugates. Many of them have been in clinical trials, and a few of them have even progressed into phase III trials, with promising results in pharmacokinetics. In these trials, a significant number of constructs have been synthesized with random-coil linear polymers, such as poly(ethylene glycol),[13] poly[*N*-(2-hydroxypropyl) methacrylamide] copolymers,[14] poly(glutamic acid),[15,16] polyethyleneimine,[17] and dextrin (α-1,4-polyglucose).[18] It should be emphasized that both successful and failed cases indicate that all biological properties (activity and toxicity) are molecular-weight dependent and can change significantly once the respective conjugates are prepared. Furthermore, for clinical applications, the use of a material with a broad polydispersity index (PDI) is not desirable because it may lead to irreproducible or undesired pharmacokinetic behavior because of the presence of species with vastly different weight-average molecular weights (M_w's) within a given sample or because of variations in M_w distribution from preparation to preparation. Therefore, the task of obtaining a well-defined polymer as an ideal drug carrier seems to be very important. In general, drug conjugates of line polymers are prepared statistically by incorporation of a comonomer in the preparation; this makes the preparation of well-defined systems difficult. So the design and synthesis of biodegradable line polymers with well-defined M_w's and architecture in view of biological implications would broaden the scope of applicability of polymers in therapeutic applications and have been pursued extensively.

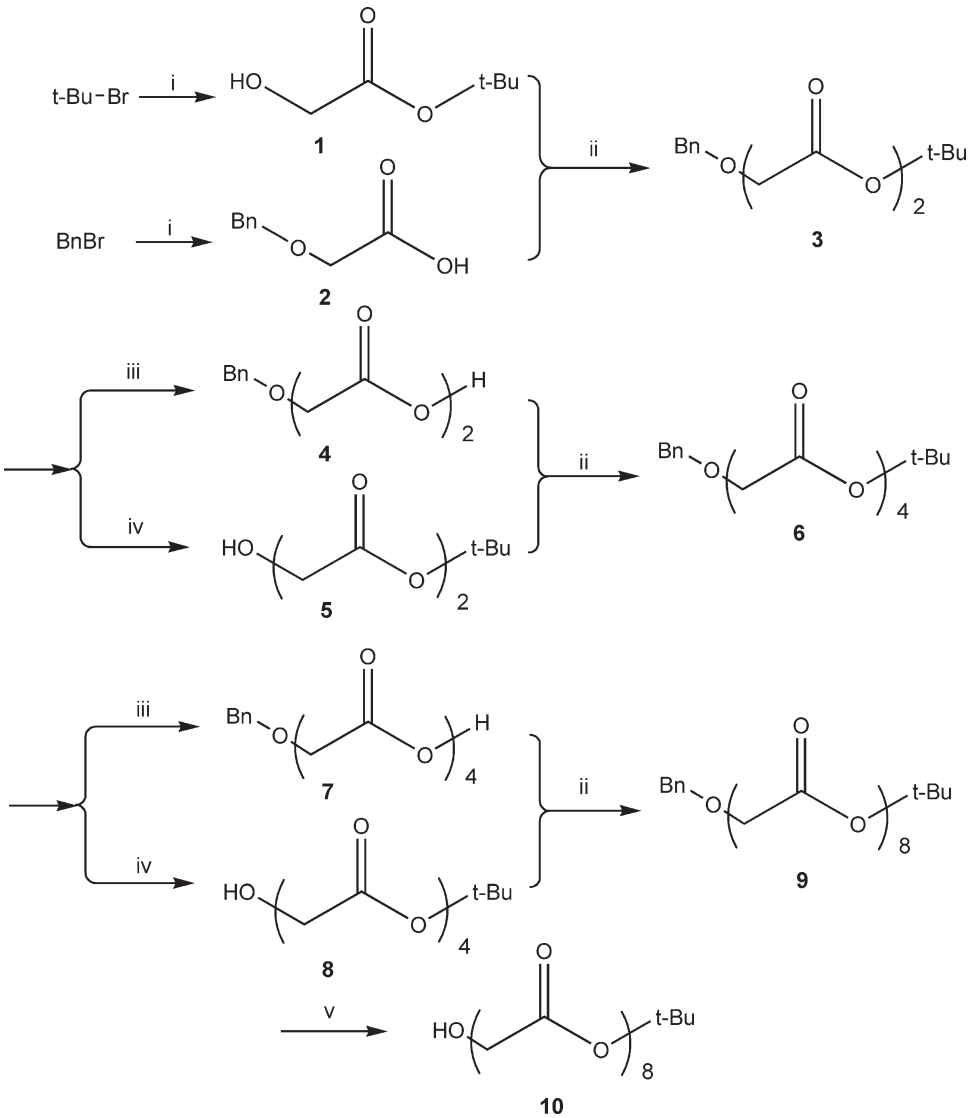
To obtain a kind of biodegradable and a well-defined amphiphilic block copolymer, glycolic acid oligomer was selected as the hydrophobic segment. It is well known that aliphatic polyesters, such as polyglycolide and poly(L-lactide), are of great interest as biomaterials because of their excellent biodegradability, bioresorbability, and mechanical properties.[16,19,20] They undergo hydrolysis upon implantation into the body, forming biologically compatible and metabolizable moieties (lactic acid and glycolic acid) that are eventually removed from the body by the citric acid cycle and do not affect the normal cell function.[21] They have been tested for toxicity and safety in extensive animal studies and have been exploited in biomedical applications, such as drug delivery and tissue engineering.[22] Poly(ethylene glycol) was chosen as the hydrophilic part for its water solubility, biocompatibility, and ability to prolong the plasma half-life and to lower immunogenicity.[23–26] In addition, it is available at a low polydispersity (PDI of 1.01); thereby, copolymers with similar PDI may be expected if the molecular length of the other block is controlled effectively. Here, paclitaxel was used as a prototype small-molecule anticancer drug for the preparation of a polymer prodrug, which exhibited significant activity against various solid tumors, including advanced ovarian carcinoma, metastatic breast cancer, nonsmall cell lung cancer, and head and neck carcinomas.[27–30] However, the clinical application of paclitaxel is often accompanied by its extremely low aqueous solubility and hypersensitivity reactions.[11,31–33] Furthermore, because the release of the drug is controlled solely by diffusion, conventional polymeric nanoencapsulates typically burst-release 60–90% of their payloads within a few to tens of hours.[34] Because the paclitaxel release kinetics of the prodrug is determined not only by diffusion but also by the hydrolysis of the ester linkage formed between paclitaxel and the oligomer of glycolic acid, the release of paclitaxel from the prodrug is more controllable and has a significantly reduced burst-release effect.[35]

In this study, therefore, a well-defined amphiphilic block copolymer was prepared from methoxy poly(ethylene glycol) (MPEG) and an oligomer of glycolic acid (octamer), which was obtained by a stepwise synthetic procedure with end-group protection and deprotection, and it was further conjugated with paclitaxel to obtain a prodrug of paclitaxel. The chemical structure and the cytotoxicity of the conjugate were experimentally investigated.

EXPERIMENTAL

Materials

MPEG, with a molecular weight of 5000, was obtained from Aldrich Co. (Pennsylvania, America). Before use, it was dried by an azeotropic distillation in toluene. Paclitaxel was purchased from Xi'an Baosai Biotechnology, Inc. (Shanxi, China). Dimethylaminopyridine (DMAP; 99%) and diglycolic anhydride were purchased from Acros (Belgium, America). Glycolic acid (99%) and *t*-butyl bromide were obtained from Shanghai Bangcheng Chemical Co., Ltd. (Shanghai, China). Benzyl bromide, potassium iodide, and silver oxide were purchased from



Scheme 1 Synthetic strategy of the well-defined oligomers of glycolic acid up to the octamer. Conditions: (i) glycolic acid, Ag_2O, KI, Et_2O; (ii) DCC, DMAP, DCM, 0°C; (iii) TFA, DCM, 0°C; (iv) Pd/C, formic acid, methanol; and (v) Pd/C, H_2, THF.

Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dicyclohexylcarbodiimide (DCC) and trifluoroacetic acid (TFA), supplied by GL Biochem, Ltd. (Shanghai, China), were used as received. Tetrahydrofuran (THF) and dichloromethane (DCM; CH_2Cl_2) were refluxed over CaH_2 and distilled under argon. Other reagents were commercially available and were used without further purification, except as otherwise stated.

Characterization

^{1}H-NMR spectra were recorded at room temperature on a Bruker (Bruker Instruments, Billerica, America) ARX-400 NMR spectrometer with CDCl3 as solvent. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were measured by gel permeation chromatography (Waters 1515 pump, Torrance, America) instrument with THF as the eluent (1.0 mL/min) at 25°C. Calibration was made with standard polystyrene (PSt). The ^{1}H-NMR spectrum was taken at 25°C on a Bruker ARX 400-MHz spectrometer with chloroform-d as a solvent and with tetramethylsilane as an internal reference.

Synthesis of the octamer of glycolic acid (Scheme 1)

t-Butyl glycolate (**1**)

Glycolic acid (3 g) was dissolved in diethyl ether (100 mL). Under stirring, 1.52 mL of *t*-butyl bromide, 4.6 g of silver oxide, and a catalytic amount of potassium iodide were added. This reaction was carried out in the dark and was essentially completed after 48 h. After the diethyl ether was evaporated under reduced pressure, the excessive glycolic acid was precipitated

by the addition of DCM and filtered. The solvent was evaporated, and then, the resulting mixture was chromatographed on a silica column (ethyl acetate (EtOAc)/petroleum ether 1 : 5 as the mobile phase). The product was obtained by the removal of the solvent.

Yield: 0.62 g (35%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 4.02 [s, 2H, $CH_2COOC(CH_3)_3$], 1.49 [s, 9H, $C(CH_3)_3$].

Benzyloxyacetic acid (**2**)

The same strategy was applied to compound **2**. Glycolic acid (3 g) was dissolved in diethyl ether (100 mL). Under stirring, 1.63 mL of benzyl bromide, 4.6 g of silver oxide, and a catalytic amount of potassium iodide were added. This reaction was carried out in the dark and was essentially completed after 48 h. After the diethyl ether was evaporated under reduced pressure, the excess glycolic acid was precipitated by the addition of DCM and filtered. After the solvent was evaporated, the reaction mixture was dissolved in saturated aqueous sodium bicarbonate and extracted with DCM two times. After the organic extracts were removed, the aqueous layer was adjusted to pH 3.0 with 0.1*M* HCl and extracted with DCM three times, and the extracts were dried over magnesium sulfate anhydrous. The product was obtained by removal of the solvent from the dried extracts.

Yield: 0.93 g (41%). ^{1}H-NMR (400 MHz, $CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.66 (s, 2H, $CH_2C_6H_5$), 4.24 (s, 2H, CH_2COOH).

Double-protected dimer, tetramer, and octamer of glycolic acids (**3, 6,** and **9,** respectively)

A typical procedure is given as follows with compound **3** as an example. Compound **1** (1 g) and compound **2** (1.26 g) were dissolved in 15 mL of anhydrous DCM at room temperature. After the solution was chilled to 0°C, 1.6 g of DCC and 50 mg of DMAP were added. The reaction was carried out at 0°C under stirring for 24 h. The dicyclohexylurea precipitate was filtered out and washed with CH_2Cl_2. After the solvent was evaporated, the resulting mixture was chromatographed on a silica column (EtOAc/petroleum ether 1 : 5 as the mobile phase).

Compound **3:** Yield: 1.88 g (89%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.66 (s, 2H, $CH_2C_6H_5$), 4.59 (s, $COOCH_2COO$), 4.22 (s, 2H, CH_2OCH_2COO), 1.48 [s, 9H, $C(CH_3)_3$].

Compound **6:** Yield: 86%. ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.81–4.83 (d, 4H, $2COOCH_2COO$), 4.66 (s, 2H, $CH_2C_6H_5$), 4.58 [s, 2H, $CH_2COOC(CH_3)_3$], 4.24 (s, 2H, CH_2OCH_2COO), 1.48 [s, 9H, $C(CH_3)_3$].

Compound **9:** Yield: 88%. ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.80–4.83 (m, 12H, $6COOCH_2COO$), 4.66 (s, 2H, $CH_2C_6H_5$), 4.58 [s, 2H, $CH_2COOC(CH_3)_3$], 4.23 (s, 2H, CH_2OCH_2COO), 1.48 [s, 9H, $C(CH_3)_3$].

Hydroxyl-protected dimer and tetramer of glycolic acid (**4** and **7,** respectively)

A typical procedure is given as follows with **4** as an example. Compound **3** (1 g) was dissolved in 10 mL of anhydrous CH_2Cl_2. The solution was chilled to 0°C and treated with 5 mL of TFA. After stirring for 2 h, TFA and CH_2Cl_2 were removed under reduced pressure. The residue was dissolved in saturated aqueous sodium bicarbonate and extracted with DCM two times. After the organic extracts were removed, the aqueous layer (adjusted to pH 3.0 with 0.1*M* HCl solution) was extracted with DCM three times, and the extracts were dried over $MgSO_4$. The product was obtained by removal of the solvent from the dried extracts.

Compound **4:** Yield: 0.73 g (91%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.65 (s, 2H, $CH_2C_6H_5$), 4.53 (s, 2H, CH_2COOH), 4.23 (s, 2H, CH_2OCH_2COO).

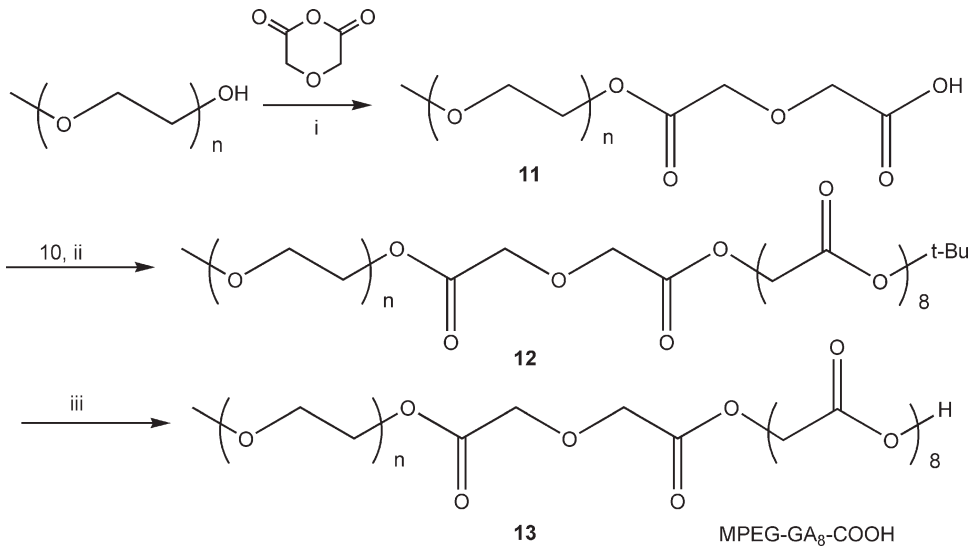
Compound **7:** Yield: 89%. ^{1}H-NMR ($CDCl_3$, δ, ppm): 7.33–7.37 (m, 5H, C_6H_5), 4.79 (d, 4H, $2COOCH_2COOCH_2$), 4.72 (s, 2H, CH_2COOH), 4.65 (s, 2H, $CH_2C_6H_5$), 4.23 (s, 2H, CH_2OCH_2COO).

Carboxyl-protected dimer and tetramer of glycolic acid (**5** and **8,** respectively)

A typical procedure is given as follows with **5** as an example. The double-protected dimer of glycolic acid (0.5 g) dissolved in 10 mL of 4.4% formic acid–methanol was added to a 50-mL, round-bottom flask containing approximately 1.0 g of freshly prepared palladium black catalyst and 10 mL of 4.4% formic acid–methanol. The mixture was continuously stirred under a nitrogen atmosphere. The reaction was completed within 1 h, as monitored by thin-layer chromatographic analysis of samples taken at various intervals. After completion, we isolated the products by filtering off the catalyst and washing with an additional 10 mL of methanol. The filtrate was then concentrated by evaporation under reduced pressure at room temperature. The product was obtained by chromatography on a silica column (EtOAc/petroleum ether 1 : 5 as the mobile phase).

Compound **5:** Yield: 0.31 g (91%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.56 [s, 2H, $CH_2COOC(CH_3)_3$], 4.39 (s, 2H, $HOCH_2$), 1.48 [s, 9H, $C(CH_3)_3$].

Compound **8:** Yield: 88%. ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.82 (d, 4H, $2COOCH_2COO$), 4.56 [s, 2H, $CH_2COOC(CH_3)_3$], 4.39 (s, 2H, $HOCH_2$), 1.47 [s, 9H, $C(CH_3)_3$].



Scheme 2 Synthetic routes to MPEG–GA_8–COOH. Conditions: (i) $CHCl_3$, DMAP, reflux; (ii) DCC, DMAP, DCM, 0°C; and (iii) TFA, DCM.

Carboxyl-protected octamer of glycolic acid (**10**)

Pd/C catalyst (20 wt %, 1 g) was introduced into a reactor, together with THF (10 mL) and 0.5 g of the starting material compound **9** under a hydrogen atmosphere (60 psi) at room temperature. The reaction mixture was shaken for 5 h at room temperature. The suspended solid was then collected by filtration, and the solvent was removed *in vacuo*. The resulting residue was chromatographed on a silica column (EtOAc/petroleum ether 1 : 2 as the mobile phase).

Yield: 0.35 g (82%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.82 (d, 12H, $6COOCH_2COO$), 4.56 [s, 2H, $CH_2COOC(CH_3)_3$], 4.39 (s, 2H, $HOCH_2$), 1.47 [s, 9H, $C(CH_3)_3$].

Synthesis of carboxyl-terminated MPEG (Scheme 2)

A 100-mL flask with three necks and equipped with a magnetic stirrer and a condenser was flame-dried and argon-purged three times. Diglycolic anhydride (0.2 g), MPEG–OH (1 g), DMAP (0.21 g), and 50 mL of chloroform were added under an argon stream. The solution was stirred at reflux temperature for 24 h. We precipitated the product by pouring the concentrated solution into ice-cooled diethyl ether. The resultant precipitate was filtered and dried at room temperature *in vacuo*.

Yield: 0.91 g (89%). ^{1}H-NMR ($CDCl_3$, δ, ppm): 4.35 (s, 2H, CH_2O CH_2COOH), 4.25 (s, 2H, OCH_2COOH), 3.64 (s, nH, OCH_2CH_2O of MPEG), 3.36 (s, 3H, CH_3O of MPEG).

Synthesis of MPEG–Octamer of Glycolic $Acid_8$–paclitaxel (Scheme 3)

MPEG–GA_8–COOH (**13**)

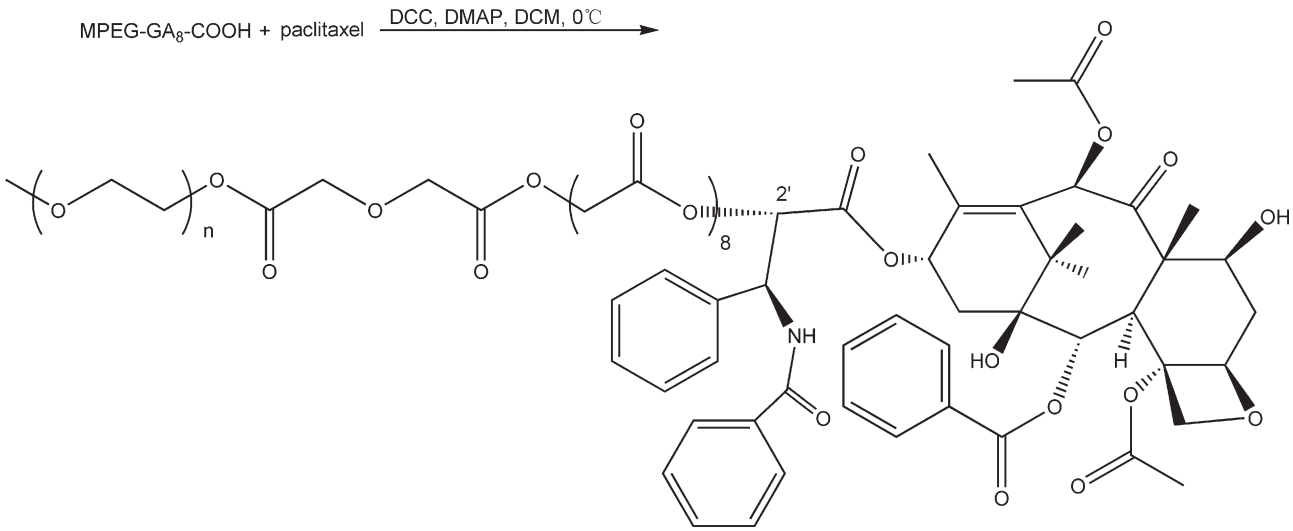
Carboxyl-terminated MPEG (1 g) and excess carboxyl-protected octamer of glycolic acid were dissolved in 25 mL of anhydrous DCM at room temperature. After the solution was chilled to 0°C, 50 mg of DCC and 50 mg of DMAP were added. The reaction was carried out at 0°C under stirring for 24 h. The dicyclohexylurea precipitate was filtered and washed with CH_2Cl_2. The filtrate was concentrated to 5 mL and then poured into ice-cooled diethyl ether. After being dried *in vacuo*, the precipitate was dissolved in 15 mL of anhydrous CH_2Cl_2. The solution was chilled to 0°C and treated with 10 mL of TFA. After stirring for 2 h, TFA and CH_2Cl_2 were removed under reduced pressure. We precipitated the product by pouring the concentrated solution into ice-cooled diethyl ether and drying it under reduced pressure (yield: 0.90 g, 82%).

MPEG–GA_8–paclitaxel

In a dried flask, 0.1 g of MPEG–GA_8–COOH and 30 mg of paclitaxel were dissolved in 10 mL of anhydrous DCM. DCC (5 mg) and DMAP (3 mg) were added at 0°C. The reaction was continued under stirring for 24 h at 0°C. The precipitate was filtered out, and the filtrate was washed with 0.1*M* HCl and water. The organic phase was dried with anhydrous $MgSO_4$, condensed, and poured into ice-cooled diethyl ether to precipitate the final product (yield: 0.087 g, 78%).

In vitro cytotoxicity assay

The cytotoxicity of the synthesized MPEG–GA_8–paclitaxel was evaluated by mitochondrial dehydrogenase (MTT) assay.[34] Human lung carcinoma A549 cells were chosen as target cells. They were cultured in the growth medium RPMI-1640 containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin, and the cell density of the cell



Scheme 3 Synthesis of MPEG–GA_8–paclitaxel.

suspension obtained was adjusted to 5×10^4 cells/mL. One hundred and fifty microliters of this suspension was added to the wells in a 96-well plate and incubated for 4 h in a humidified atmosphere containing 5% CO_2 at 37°C. The conjugate used was made of MPEG (M_n = 5000)–GA_8 and its weight content of paclitaxel was 10%. It was dissolved in dimethyl sulfoxide (DMSO) at a proper concentration, diluted 100-fold with phosphate buffer saline (PBS) buffer solution, and added to the wells. After 72 h of incubation, a 20 mL MTT 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazoli-um bromide solution (5 mg/mL) was added to each well of the plate. The incubation was continued for another 4 h. Then, the MTT derivative was dissolved with DMSO, and the optical density of the solution was determined by a microplat reader (Bio-Rad model 680, California, America) at 490 nm. The relative cell viability was calculated and averaged.

RESULTS AND DISCUSSION

A lot of work has been done to prepare paclitaxel prodrugs. In this study, our objective was to obtain a polymeric prodrug of paclitaxel with a low PDI to achieve reproducible pharmacokinetic behavior. To synthesize the paclitaxel prodrug based on a well-defined amphiphilic block copolymer from MPEG and oligomer of glycolic acid (octamer), the oligomer of glycolic acid was prepared by a stepwise synthetic procedure involving the protection or deprotection of the groups, the *t*-butyl ester and the benzyl ether as protecting groups for the carboxyl and hydroxyl groups of glycolic acid, respectively. The hydroxyl group of MPEG was converted into a carboxyl group and reacted with the oligomer, and then, the amphiphilic block polymer was further conjugated with paclitaxel. The detailed synthesis process is discussed as follows.

Synthesis of glycolic acid oligomers (octamer) and carboxyl-protected octamer of glycolic acid (mono-*t*-butyl ester of octamer)

The octamer of glycolic acid (GA) was synthesized from glycolic acid in a stepwise manner, which could be divided into three parts: (1) synthesis of the carboxyl-protected derivatives, (2) synthesis of hydroxyl-protected ones, and (3) conjugation of them. We focused on protecting groups that could be removed selectively in the presence of the other protecting group under mild conditions and without any effect on the ester bond between repeating units of glycolic acid as well. The protection or deprotection of the groups proved to be most challenging but was accomplished with the combination of the benzyl group and *t*-butyl group. The benzyl ether could be removed by either hydrogenation or mild acidolysis in very good yield without any detectable loss of the *t*-butyl group. The *t*-butyl group, which is used as a carboxyl protecting group, is stable under the cleavage conditions of the benzyl group and is usually removed with TFA under anhydrous conditions to give isobutylene as the byproduct. The coupling of these two kinds of monoprotected substrates was accomplished with DCC/DMAP. The synthetic procedures are presented in Scheme 1. Successful synthesis was confirmed by the ^{1}H-NMR spectra of the resulting octamer (Fig. 1) and mono-*t*-butyl ester of the octamer of glycolic acid (Fig. 2). There appeared the characteristic peak at 1.48 ppm (f) belonging to the *t*-butyl group, the peaks at 4.30–4.86 ppm (b, d, and e) corresponding to the methylene group protons of glycolic acid, and the peaks at 4.66 ppm (c) corresponding to the methylene group in benzyl group, respectively (Fig. 1). Furthermore the integrated-area ratios of these peaks were close to 9 : 16 : 2; this indicated that glycolic acid

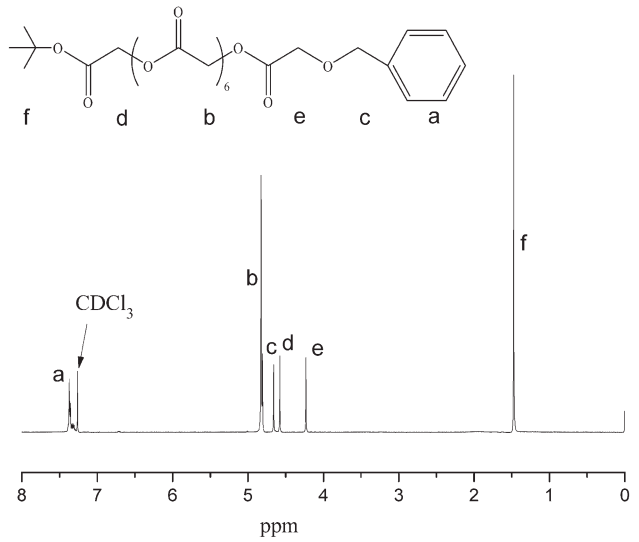


Figure 1 ^{1}H-NMR spectra of the octamer of glycolic acid.

oligomers (octamer) were obtained. In Figure 2, the characteristic peak corresponding to benzyl (7.33–7.37, 4.66 ppm) disappeared completely; this demonstrated complete elimination of the benzyl group. The peaks related to the methylene group protons of glycolic acid and the *t*-butyl group still existed; furthermore, the integrated-area ratios of these peaks were close to 9 : 16. This indicated that the carboxyl-protected octamer of glycolic acid (mono-*t*-butyl ester of octamer of glycolic acid) was prepared successfully.

Synthesis of MPEG–GA$_8$–paclitaxel

MPEG–GA$_8$–COOH was obtained by the reaction of MPEG–COOH with the carboxyl-protected octamer of glycolic acid (mono-*t*-butyl ester of the octamer of glycolic acid) in the presence of DCC and, subse-

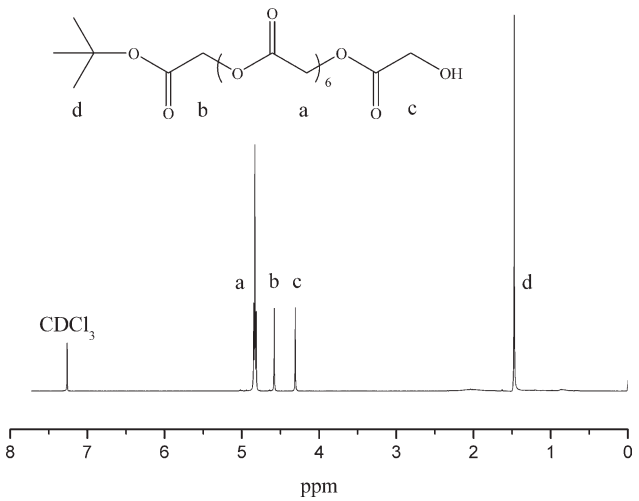


Figure 2 ^{1}H-NMR spectra of the carboxyl-protected octamer of glycolic acid.

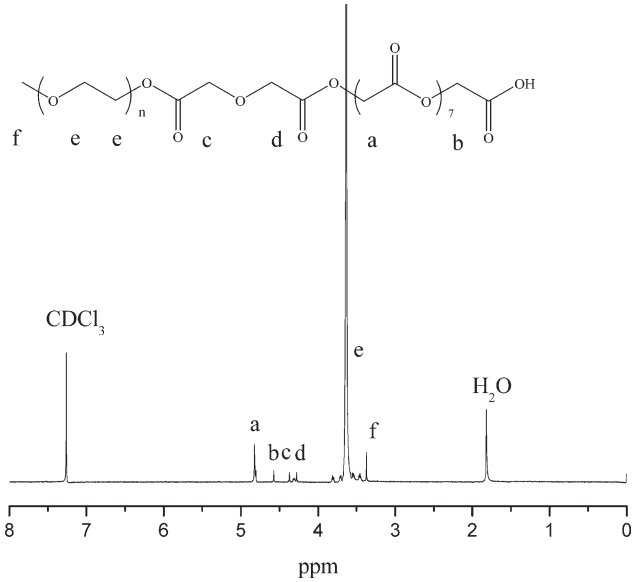


Figure 3 ^{1}H-NMR spectra of MPEG–GA$_8$–COOH.

quently, by the deprotection of the *t*-butyl with TFA under anhydrous conditions (Scheme 2). In the ^{1}H-NMR spectrum of MPEG–GA$_8$–COOH (Fig. 3), the characteristic peak corresponding to *t*-butyl (1.48 ppm) disappeared completely; this demonstrated the complete elimination of the *t*-butyl group. The peaks related to the methylene group protons of glycolic acid still existed; furthermore, their integrated-area ratio to the end group of MPEG (CH_3O) was approximately 16 : 3. This implied that their coupling was successful. At the same time, the molecular weight of MPEG–GA$_8$–COOH was determined by GPC (Fig. 4 and Table I); a single and sharp peak was shown with the PDI of 1.01, which was equal to the PDI of MPEG we used. This indicated that a well-defined amphiphilic block copolymer from MPEG and the oligomer of glycolic acid (octamer) with a low PDI was synthesized successfully.

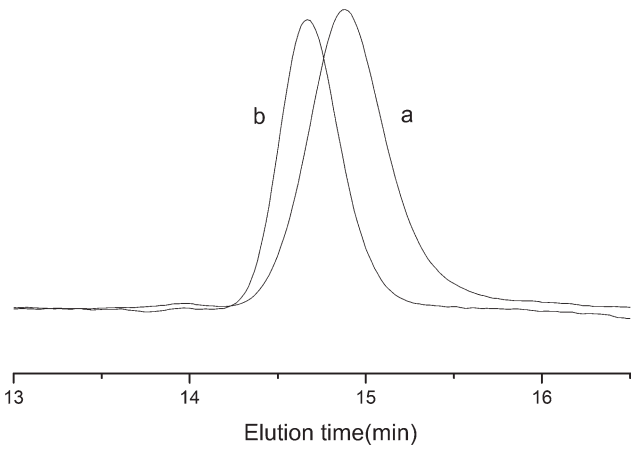


Figure 4 GPC traces of (a) MPEG–GA$_8$–COOH and (b) MPEG–GA$_8$–paclitaxel.

TABLE I
Molecular Weight and Distribution Values of MPEG–GA_8–COOH and MPEG–GA_8–Paclitaxel

Sample[a]	$M_{n,GPC}$ $\times 10^4$	M_w/M_n by GPC
MPEG–GA_8–COOH	1.75	1.01
MPEG–GA_8–paclitaxel	2.03	1.02

[a] The molecular weight of MPEG was 5000.

Usually, the 2′-hydroxyl of paclitaxel is more active than the 7-hydroxyl because of space hindrance, and thus, esterification often takes place preferentially with the 2′-hydroxyl (Scheme 3).[36] Therefore, MPEG–GA_8–paclitaxel was prepared by the reaction of paclitaxel with MPEG–GA_8–COOH in the presence of DCC and DMAP at 0°C (Scheme 3). The ^{1}H-NMR spectra of MPEG–GA_8–paclitaxel and pure paclitaxel are shown in Figure 5. Obviously, the characteristic peaks of paclitaxel could all be found in the ^{1}H-NMR spectra of MPEG–GA_8–paclitaxel; this indicated successful preparation of the conjugate. The integrated-area ratio of the peak at 8.16 ppm, which was assigned to the two protons on the phenyl ring of paclitaxel at the C2 position,[37] to that of the end group (CH_3O, 3.38 ppm) was approximately 1 : 1.75, and theoretically, it should have been 2 : 3. This implied that the molar ratio of paclitaxel conjugated to the MPEG–GA_8–COOH chain was about 85.6%. Furthermore, the GPC curve of MPEG–GA_8–paclitaxel [Fig. 4(b)] exhibited a single and sharp peak with the PDI of 1.02 (Table I) and was left-shifted compared to that of MPEG–GA_8–COOH [Fig. 4(a)]. All of these results support the conclusion that the paclitaxel was conjugated with MPEG–GA_8–COOH successfully.

In vitro cytotoxicity assay of the paclitaxel conjugate

The antitumor activity of the paclitaxel prodrug against human lung carcinoma A549 cells was evaluated with MTT assay. Figure 6(A) shows that the cell viabilities after 72 h of incubation for the pure paclitaxel, the prodrug, and the pure copolymer were 70, 73, and 96%, respectively. This implies that pure copolymer was biocompatible, and the prodrug exhibited obvious cytotoxicity, close to that of pure paclitaxel, against A549 cells. That is, the paclitaxel was released from MPEG–GA_8–paclitaxel without losing cytotoxicity. In Figure 6(B), the cell viability is plotted against the paclitaxel concentration used. It can be seen that the cytotoxicity was dependent on the concentration of the prodrug. The value of 25 ng/mL was a proper concentration under the test conditions.

CONCLUSIONS

We reported that a well-defined amphiphilic block copolymer from MPEG and the oligomer of glycolic acid and its paclitaxel prodrug were synthesized by a stepwise synthetic procedure. Its synthesis consisted of three steps: (1) the oligomer of glycolic acid (octamer) was synthesized by group protections and deprotections, with the *t*-butyl ester and the benzyl ether as protecting groups for the carboxyl and hydroxyl groups of glycolic acid, respectively; (2) the group of MPEG was converted into a carboxyl group and reacted with the oligomer; and (3) the latter was also reacted with paclitaxel in the presence of DCC and DMAP. The antitumor activity of the conjugate against human lung carcinoma A549 cells was evaluated by MTT assay. The results indicated that

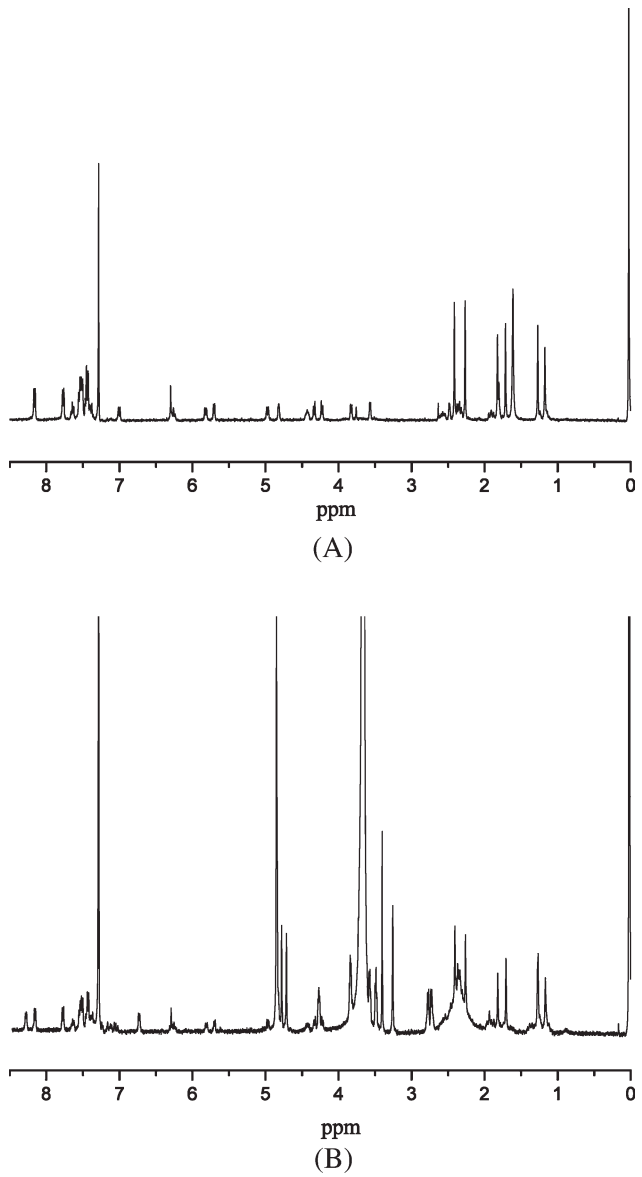


Figure 5 ^{1}H-NMR spectra of (a) paclitaxel and (b) MPEG–GA_8–paclitaxel.

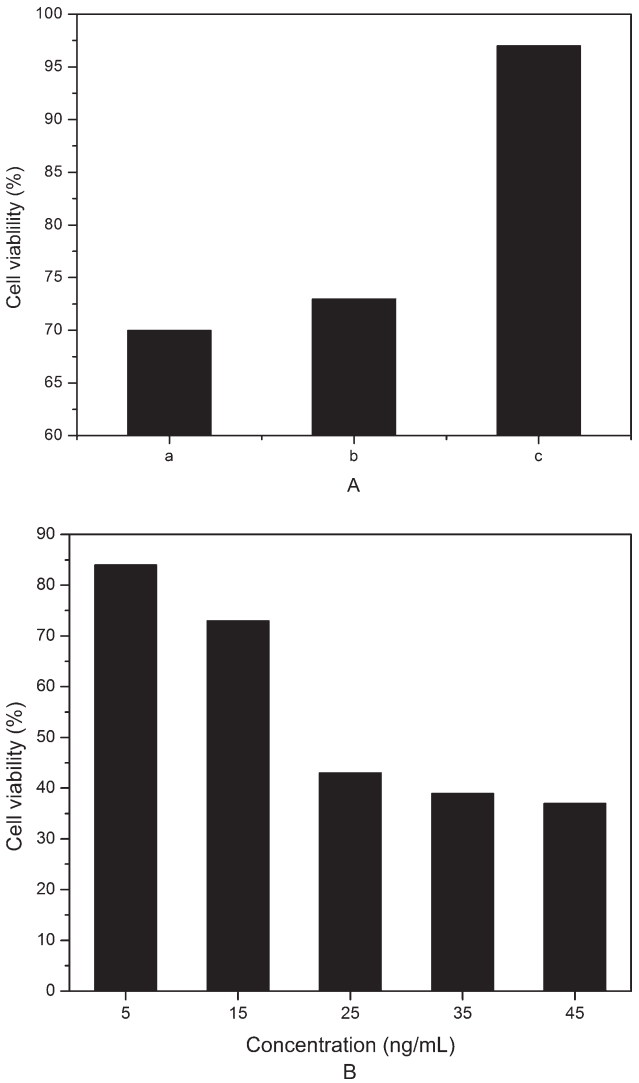


Figure 6 *In vitro* cytotoxicity of MPEG–GA_8–paclitaxel against human lung carcinoma A549 cells. The general test procedures are seen in the text. (A) Cell density = 1.5×10^5 cells/mL: (a) pure paclitaxel (25 ng/mL); (b) prodrug, paclitaxel concentration = 25 ng/mL; and (c) copolymer MPEG–GA_8–COOH, concentration = 200 ng/mL. The control was the 1 wt % solution of DMSO in PBS. Its cell viability was taken as unity. (B) Cell density = 5×10^4 cells/mL; the paclitaxel concentrations are as shown in the figure. The control was the 1 wt % solution of DMSO in PBS. Its cell viability was taken as unity.

paclitaxel could be released from the conjugate without losing cytotoxicity. These results confirmed that the well-defined amphiphilic block copolymer from MPEG and the oligomer of glycolic acid could potentially provide novel opportunities to obtain reproducible pharmacokinetic behavior in the design of drug-delivery systems and therapeutic applications.

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