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Preparation and evaluation of novel amphiphilic glycopeptide block copolymers as carriers for controlled drug release

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Abstract

In the present study we describe a synthesis and self-assembly and an *in vitro* evaluation of a kind of novel amphiphilic glycopeptide block copolymers as carriers for controlled drug release. Initially, an amphiphilic ABA triblock copolymer comprising polytetrahydrofuran (PTHF) as a central hydrophobic block flanked by poly(L-lysine)s (PLLs) as outer hydrophilic blocks was synthesized through the ring-opening polymerization of ε-benzyloxycarbonyl-L-lysine *N*-carboxyanhydride with a distal amine-terminated PTHF as a macroinitiator, followed by removal of the protecting group. Afterwards the resulting triblock copolymer was allowed to react with D-gluconolactone and lactobionolactone in the varying feeding ratios in the presence of diisopropylethylamine leading to the target glycopeptide block copolymers with high yields. They were found to easily self-assemble into nano-sized aggregates in water. The critical aggregation concentrations (CACs) were assessed by fluorescence measurement with *N*-phenyl-1-naphthylamine employed as a molecular probe. The particle sizes of the aggregates before and after doxorubicin loading were determined by dynamic light scattering (DLS) and the aggregate morphologies were evidenced by transmission electron microscopy (TEM) measurements. Finally, the *in vitro* doxorubicin loading capacity and release behavior were investigated with these glycopeptide copolymers as carriers for controlled release.

Keywords: Glycopeptide; Amphiphilic triblock copolymer; Self-assembly

1. Introduction

Self-assembly of block copolymers containing natural components, especially the peptides and saccharides, has drawn tremendous interests in recent years. For instance, polypeptide-derived block copolymers, by the ingenious design of their topological structures, can self-assemble into higher order structures, such as micelles, vesicles and tubules. They hold a great significance not only for their basic research, but also for their potential for use in different biomaterial applications, e.g. carriers for controlled drug release, vehicles for gene transfer therapy, scaffolds for tissue engineering, etc. [1–5]. Meanwhile, synthetic copolymers comprising pendant saccharide moieties, so called glycopolymers or sugar containing polymers, have also received wide attention, due to their important roles for biomedical purposes as reviewed by Miura [6]. Until now much effort has been devoted to the design and synthesis of glycopolymers with controlled architecture using different synthetic strategies, such as living ionic polymerization and atom transfer radical controlled/living polymerization [7–9].

The attachment of cell-specific saccharine groups to polymer backbone was reported as one promising way to enhance the efficacy of drug delivery and gene expression to target cells [10–13]. Evidently, incorporating saccharide moieties into synthetic polypeptide copolymers will give rise to a kind of novel polypeptide–carbohydrate conjugates or glycopeptide copolymers. Moreover, the research towards the self-assembly of amphiphilic glycopeptide block polymers will help highlighting their crucial roles in the cellular recognition and targeted drug and gene delivery processes. However, up to now there are only limited literatures focusing on the self-association of amphiphilic

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glycopeptide block copolymers in selective solvents and their applications as carriers for controlled drug release [14–19].

Therefore this paper reports the synthesis and self-assembly of a kind of novel amphiphilic glycopeptide block copolymers and their *in vitro* evaluation as carriers for controlled drug delivery. An amphiphilic ABA triblock copolymer was firstly prepared with polytetrahydrofuran (PTHF) as a central hydrophobic block flanked by poly(L-lysine)s (PLLs) as outer hydrophilic blocks. As is well known, due to the glassy state of hydrophobic domains, for instance polystyrene, direct dissolution of block copolymers in water is usually impossible at room temperature and organic solvents are always used in the process of micelle preparation. For the biomedical applications, however, most of the organic solvents are cytotoxic and hardly been removed from the micelles. Here PTHF was chosen as a low T_g hydrophobic middle segment which can be easily dissolved and stabilized by hydrophilic PLL segments at room temperature. Afterwards the resulting amphiphilic ABA triblock copolymer was allowed to react with either D-gluconolactone or lactobionolactone in a varying feeding ratio in the presence of diisopropylethylamine to yield the target glycopeptide copolymers. They were evidenced to self-assemble into the nano-sized aggregates in water. The *in vitro* loading capacity and release behavior of doxorubicin as a model drug were also examined with these glycopeptide-based nanoparticles as carriers for controlled release.

2. Experimental section

2.1. Materials and measurements

Bis(3-aminopropyl) terminated polytetrahydrofuran (CAS #72088-96-1, $M_n = 1100$) was purchased from Aldrich and was represented as AT-PTHF when used as a macroinitiator and as PTHF when used as a middle block of the copolymers. This prepolymer was dried under vacuum at 120 °C for 2 h before using. ε-Benzyloxycarbonyl-L-lysine (ZLLy) was available from Fluka and used without further purification. Tetrahydrofuran (THF) was refluxed with Na and then distilled. *N*, *N*-Dimethylformamide (DMF) was stirred with CaH_2 and distilled under high vacuum. ε-Benzyloxycarbonyl-L-lysine *N*-carboxyanhydride (ZLLy-NCA) was synthesized from ZLLy and triphosgene according to the Fuchs–Farthing method [20]. Ethyl acetate and *n*-hexane were distilled from CaH_2 under a nitrogen atmosphere. D-Gluconolactone and lactobionic acid were purchased from Sigma, and *N*-phenyl-1-naphthylamine (PNA) was available from Aldrich. Diisopropylethylamine (DIPEA) was purchased from Acros and used as received. Doxorubicin hydrochloride (DOX·HCl) was supplied by Beijing HuaFeng United Technology CO., Ltd. Sodium acetate buffered solution (pH 5.0) was prepared. All other solvents and reagents were obtained from commercial suppliers and used as received.

^1H and ^{13}C NMR spectra of the samples were measured with Bruker ARX 400 using DMSO-d_6 or D_2O as solvent containing TMS as internal standard at 25 °C. 2-D COSY NMR measurements were performed on Bruker AV400 at 25 °C. Elemental analyses were performed on Elementar Vario EL (Germany). The Size Exclusion Chromatography (SEC) measurements for protected ABA triblock copolymers were conducted at 50 °C with a Waters 1515 pump, three Waters Styragel columns (HT3, HT4, HT5) and a Waters 2414 differential refractive index detector. DMF containing 0.5 M LiBr was used as eluent at a flow rate of 1.0 ml/min. Polystyrene standards were utilized for calibration. The content of bromide ions was titrated by a solution of $Hg(NO_3)_2$ at 0.01 mol/l. Fluorescence experiments were performed on Varian Cary Eclipse fluorescence spectrophotometer at 25 °C with a slit of 5 nm for both excitation and emission. The excitation wavelength was 340 nm for emission spectra. The dynamic light scattering (DLS) measurements were performed on Brookhaven Instruments BI-200SM Goniometer with a BI-TurboCorr Digital Correlator. The vertical polarized laser was supplied by a solid-state laser source (Mini L-30, Brookhaven, 30 mW) operating at 636 nm. All samples were kept at constant temperature (25.0 °C), measured at 90° and filtered through 0.45 μm filters before the examination. The time autocorrelation function of the scattered light intensity was measured in the self-beating mode. Brookhaven Instrument software (9KLDSW) was used. Time correlation functions were analyzed with a Laplace inversion program CONTIN. The transmission electron microscopy (TEM) graphs were taken with a Hitachi-700 transmission electron microscope operated at 100 kV accelerating voltage. Negative stained specimens were prepared as follows. A drop of solution was spread on a carbon-coated copper grid and the extra droplet was instantly removed by filter paper. After being naturally desiccated, a drop of 1.5 wt% uranyl acetate in ethanol solution was dripped on the copper grid for about 60 s and the extra droplets were also removed. Then the grid was dried in air for about 3 h before TEM observation. UV spectra were performed on a Hitachi U-2800 spectrophotometer.

2.2. Synthesis of poly(ε-benzyloxycarbonyl-L-lysine)-b-poly(tetrahydrofuran)-b-poly(ε-benzyloxycarbonyl-L-lysine) triblock copolymers ($PZLL_{30}$-b-PTHF-b-$PZLL_{30}$)

A typical synthesis of $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ was carried out as follows. ZLLy-NCA (2.38 g, 7.80 mmol) was dissolved in 40 ml of dried DMF. Then, a solution of 0.14 g (0.13 mmol) AT-PTHF in 20 ml dried DMF was injected. The total reactant concentration was 3–5 g/100 ml. The ring-opening polymerization was allowed to proceed for five days under stirring at room temperature. All operations and reactions were done under a N_2 atmosphere. The reaction was discontinued and the mixture was added dropwise to a large excess of diethyl ether to yield $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ as white precipitates. The products were washed by diethyl ether several times and then dried in vacuum (yield: 86%). The degree of polymerization (DP) was calculated based on the intensity ratio of methylene groups adjacent to the –NH–CO– group on side chain to methylene groups adjacent to oxygen atoms on PTHF segments whose M_n was indicated as 1100 by supplier.

2.3. Synthesis of poly(L-lysine)-b-poly(tetrahydrofuran)-b-poly(L-lysine) triblock copolymers (PLL_{30}-b-PTHF-b-PLL_{30})

$PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ (0.20 g) was dissolved in 2.0 ml trifluoroacetic acid (TFA), to which a four-fold excess (per lysine per unit) of a 33% solution of HBr in AcOH was added. After 1 h, the mixture was added dropwise into diethyl ether to terminate the reaction. The precipitates were re-dissolved in methanol and then precipitated by diethyl ether. The crude deprotected triblock copolymer was dialyzed against bidistilled water for three days and the water was changed at intervals of 5–6 h. Finally, the amphiphilic PLL_{30}-*b*-PTHF-*b*-PLL_{30} was obtained as white powders after freeze-drying (yield: 68%).

2.4. Synthesis of Glu-g-PLL_{30}-b-PTHF-b-PLL_{30}-g-Glu ($PLTL_{30}$-Glu)

The partial gluconoylation of PLL_{30}-*b*-PTHF-*b*-PLL_{30} was conducted as follows. D-Gluconolactone (26.2 mg) was added to 30.0 mg PLL_{30}-*b*-PTHF-*b*-PLL_{30} in 3 ml DMSO in the presence of 25 μl DIPEA and 300 μl H_2O. The solution was stirred for 24 h at 20 °C. Then the amphiphilic glycopeptide copolymer was obtained by freeze-drying after dialyzing against bidistilled water for three days with the substitution degree (SD) of 59%.

2.5. Synthesis of Lac-g-PLL_{30}-b-PTHF-b-PLL_{30}-g-Lac ($PLTL_{30}$-Lac)

Initially, lactobionic acid was converted to lactobionolactone according to literature [14]. The solution of lactobionic acid in methanol was evaporated and repeated in vacuum many times in the presence of catalytic amount of TFA. Afterwards 65.0 mg lactobionolactone was added to 39.0 mg PLL_{30}-*b*-PTHF-*b*-PLL_{30} in 3 ml DMSO in the presence of 33 μl DIPEA and 300 μl H_2O. The solution was stirred for 24 h at 20 °C. Lac-*g*-PLL_{30}-*b*-PTHF-*b*-PLL_{30}-*g*-Lac was obtained by freeze-drying after dialyzing against bidistilled water for three days with the SD of 47%.

2.6. Sample preparation for fluorescence measurements

The stock solution of the glycopeptide block copolymers at 1.0 mg/ml was prepared by dissolving in bidistilled water with vigorous stirring for 48 h at room temperature. To get sample solutions, a given amount of PNA as a fluorescence probe in acetone was added to each of a series of 10 ml volumetric flasks and then acetone was evaporated. The concentration of PNA in the final solution is 2.0×10^{-6} mol/l. The sample was kept for 48 h to equilibrate the PNA and aggregates.

2.7. Evaluation of DOX encapsulation

To investigate the encapsulation ability of the self-assemblies of glycopeptide block copolymers as carriers for controlled drug release, DOX was selected as a model drug. The drug-loaded nanoparticles were prepared as follows. Corresponding polymer (10 mg) was directly dissolved into 10 ml solution of DOX·HCl in water at 100 μg/ml. The resulting solution was stirred for 48 h at room temperature. The drug-loaded particles were separated from the free drug by dialysis method. Solution (2 ml) was dialyzed against 50 ml distilled water for 3 h and the release medium (dialyzate) was replaced by fresh water per hour. The concentration of DOX in dialyzate was detected by UV at 485 nm. The encapsulation efficiency (EE) and loading capacity (LC) were calculated by the following Eq. (1) and Eq. (2), respectively:

$$EE = (a - b)/a \tag{1}$$

$$LC = (a - b)/c \tag{2}$$

where a is the total mass of drug in the solution, b is the mass of drug in the dialyzate and c is the mass of blank glycopeptide copolymer.

2.8. Evaluation of DOX release in vitro

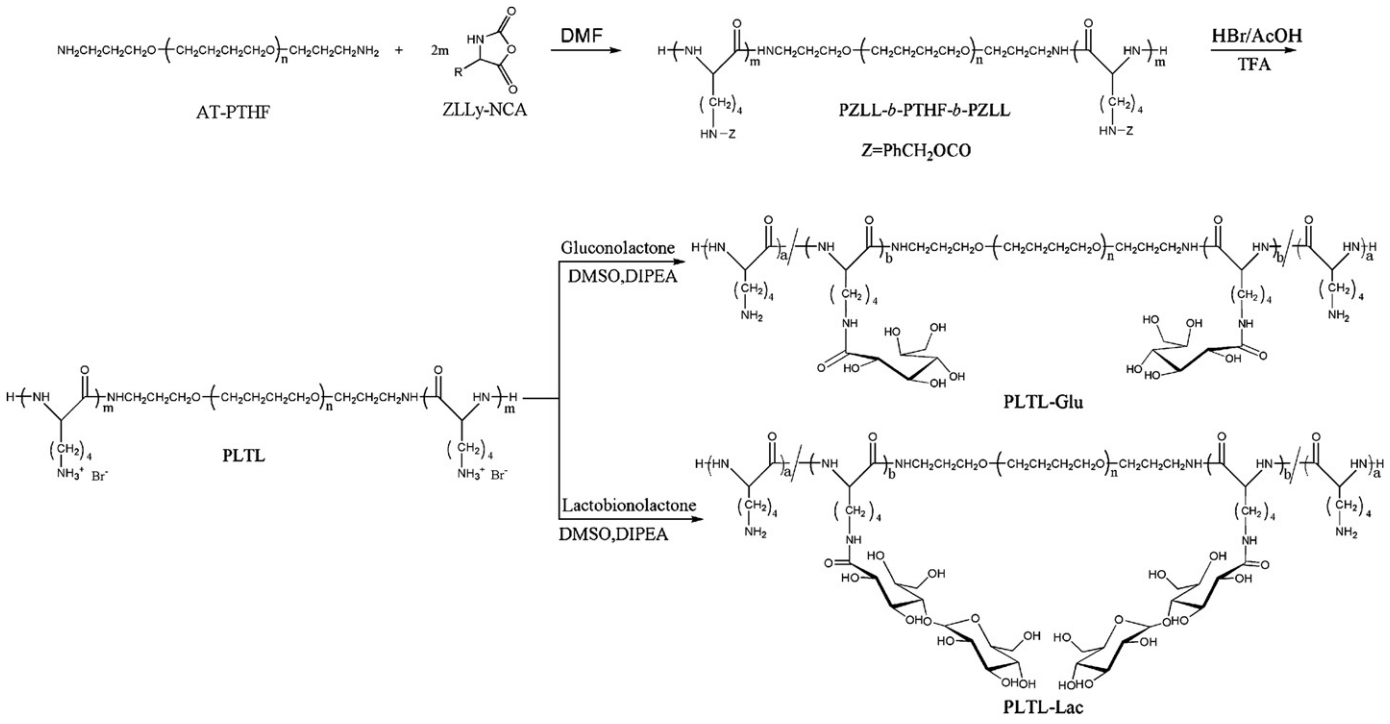
According to Chinese Pharmacopoeia (2005 Published by Chemical Industry Press) recommendation, distilled water was used as the dialyzate in this study. Dialysis bag (MWCO 3500) containing 2 ml DOX-loaded nanoparticle solution was placed into a flask containing 20 ml distilled water. The flask was shaken at 100 rpm at 37 ± 0.5 °C. Three milliliter of the dialyzate was taken at predetermined time to measure the content of drug in dialyzate and the same volume of fresh water was replenished immediately. The concentration of released DOX was analyzed by UV spectrophotometer at 485 nm. The release behavior in acidic condition (pH 5.0) was also examined.

3. Results and discussion

3.1. Synthesis and characterization of amphiphilic glycopeptide block copolymers

The synthetic pathway of the target amphiphilic glycopeptide copolymers is outlined in Scheme 1. Firstly, an ABA triblock polypeptide copolymer $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ was prepared via the ring-opening polymerization of ZLLy-NCA initiated with a nucleophilic attack of the distal amino groups of AT-PTHF. The degree of polymerization (DP) of each PZLL block was found by ^{1}H NMR analysis to be 30, well consistent with the NCA to macroinitiator feeding ratio. Since the calibrated standard used for SEC analysis possesses the different hydrodynamic behavior from the target copolymers in the solvent, the molecular weight derived from SEC ($M_n = 57{,}230$) was substantially higher than that calculated by ^{1}H NMR ($M_n = 16{,}820$). However, this triblock copolymer exhibited a nearly symmetrical narrow molecular weight distribution ($M_w/M_n = 1.10$) as evidenced in Fig. 1 by SEC.

Secondly, an amphiphilic ABA triblock polypeptide copolymer PLL_{30}-*b*-PTHF-*b*-PLL_{30} was yielded by directly



Scheme 1. Synthetic strategy for partially sugar-modified block copolymers $PLTL_{30}$-Glu and $PLTL_{30}$-Lac ($m = a + b$).

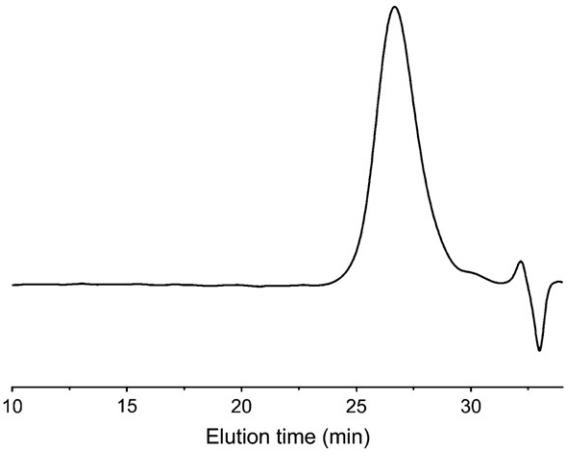


Fig. 1. SEC trace of $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ using 0.05 M LiBr in DMF as eluent at 50 °C.

removing the protecting benzyloxycarbonyl groups (Z-groups) from $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ using 33% HBr/AcOH (w/w) in CF_3COOH. According to ^1H NMR analysis, the remaining of Z-groups in PLL_{30}-*b*-PTHF-*b*-PLL_{30} remained below 2% as shown in Figs. 2 and 3.

Finally, a partial sugar modification of PLL_{30}-*b*-PTHF-*b*-PLL_{30} was carried out by the reaction with D-gluconolactone and lactobionolactone in a varying feed ratio in the presence of DIPEA under mild conditions. Here DIPEA was used as an acid-binding agent to capture HBr from the pendant-$NH_3^+Br^-$ group formed in the deprotection reaction as shown in Scheme 1. The SD value was calculated from the intensity ratio of the resonance peak at $\delta = 3.0$ ($-CH_2-NH_2$) to that at $\delta = 3.2$ ($-CH_2-NH-CO-$) as outlined in Figs. 4 and 5. To further clarify the structure of the amphiphilic glycopeptide block copolymers, 2-D COSY NMR analysis was carried out on $PLTL_{30}$-Glu-1. As shown in inset in Fig. 4, the resonance signals of h_1-h_4 protons were well-defined. The SD variation vs the feed ratio of reactants is summarized in Table 1. The SD data held only a slow increase, even the feed ratio increased by two or three times. This was probably due to the highly steric hindrance caused by the incorporation of bulky mono- or disaccharide groups into the side chains of amphiphilic PLL blocks. Interestingly, although a partial substitution was attained in PLL_{30}-*b*-PTHF-*b*-PLL_{30}, the elemental analytical data clearly showed that cytotoxic bromide ions were removed completely in the process of dialysis after sugar grafting reactions, which substantially benefits the biocompatibility of the resulting amphiphilic glycopeptide copolymers for use in the biomaterial applications.

3.2. The aggregation behavior of amphiphilic glycopeptide block copolymers in water

Both $PLTL_{30}$-Glu and $PLTL_{30}$-Lac are a type of amphiphilic glycopeptide block copolymers featured by the hydrophobic backbone and hydrophilic mono- or disaccharide side chains. The polypeptide blocks and the sugar moieties can act as either the donors or acceptors of hydrogen bonding. Hence the driving forces for their self-assembly are the result of the hydrophobic interaction and inter- and/or intra- molecular hydrogen bonding, which have also been addressed by other groups [16–18,21–24].

Two glycopeptide block copolymers were found to be easily dissolved in pure water leading to an optically transparent

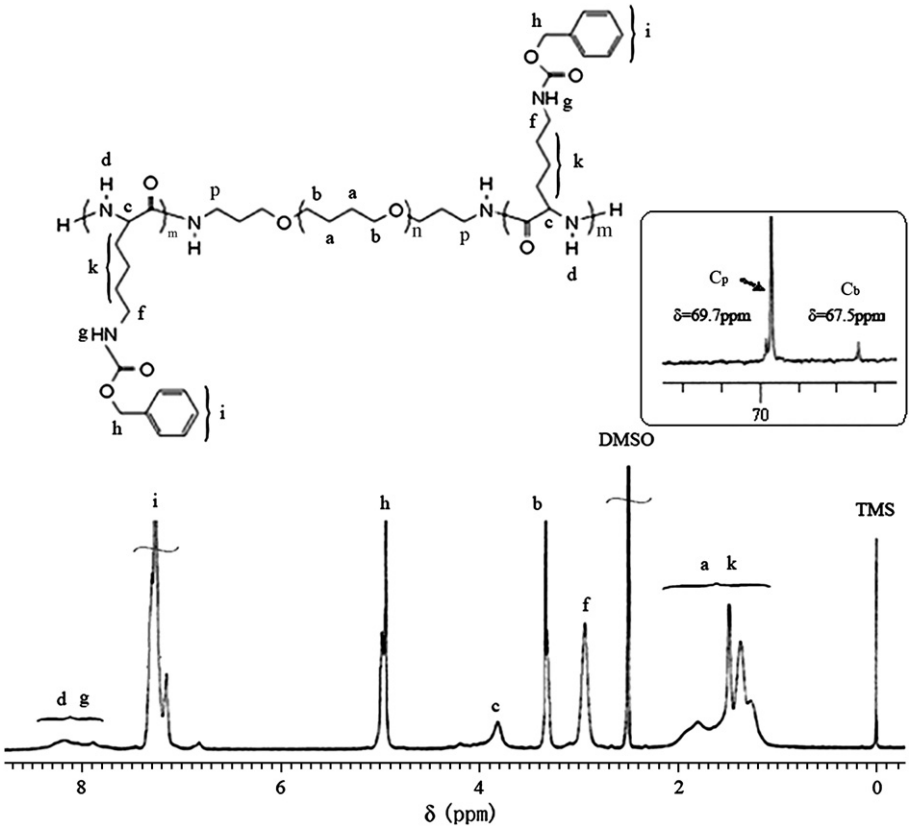


Fig. 2. ^{1}H NMR spectrum of $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$. The inset image is part of ^{13}C NMR spectrum of $PZLL_{30}$-*b*-PTHF-*b*-$PZLL_{30}$ (DMSO-d_6 at 25 °C).

stable solution. Their critical aggregation concentrations (CACs) were determined by using the fluorescence probe method. As PNA strongly emits fluorescence in a non-polar solvent or hydrophobic environment with regard to in polar media, it has been widely used as a molecular probe to highlight the aggregation behavior of surfactants and amphiphilic copolymers, including glycopolymers [18,25,26]. In the present study, PNA was also chosen, and its emission spectra at different concentrations of $PLTL_{30}$-Glu-3 are shown in Fig. 6. At a low copolymer concentration, the change of emission spectra was hardly seen as compared with in pure water. As the concentration of the glycopeptide block copolymer increased, the intensity of the emission spectra increased evidently in a small range of concentrations.

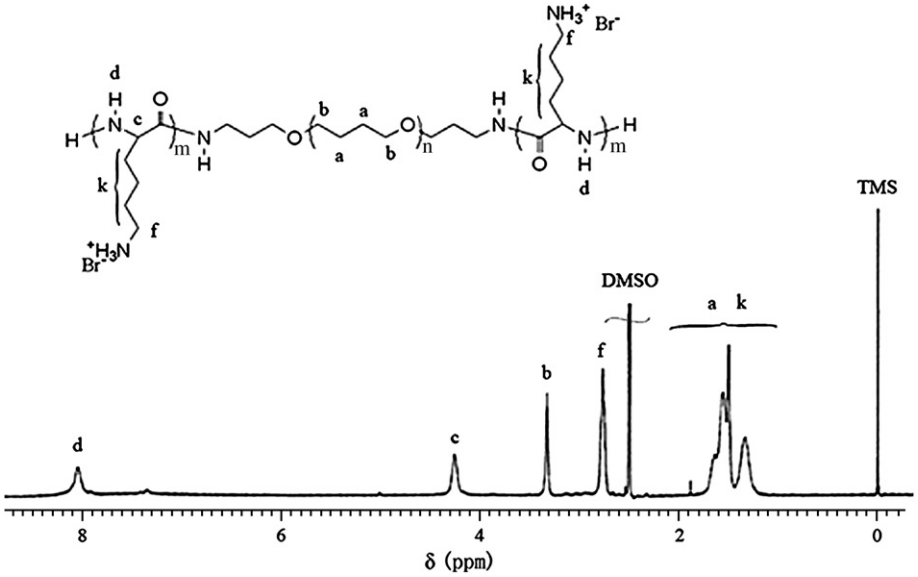


Fig. 3. ^{1}H NMR spectrum of PLL_{30}-*b*-PTHF-*b*-PLL_{30} (DMSO-d_6 at 25 °C).

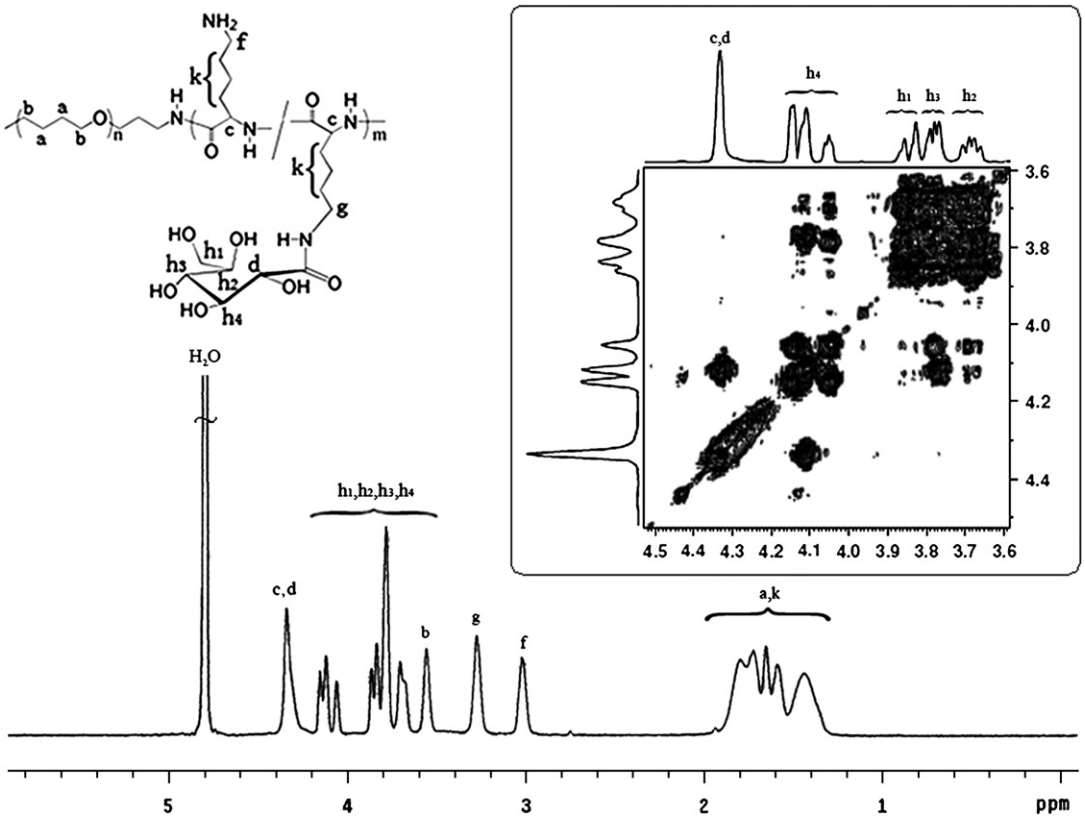


Fig. 4. ^{1}H NMR spectrum of $PLTL_{30}$-Glu-1 in D_2O at 25 °C. The inset image is part of 2-D COSY NMR spectrum of $PLTL_{30}$-Glu-1.

Meanwhile, the wavelength of maximum fluorescence emission of PNA was blue shifted in a large scale. Consequently, their CACs can be readily determined by intersecting the two straight lines as illustrated in Fig. 7, where I_0 represents the fluorescence intensity of PNA in pure water. The CAC data with varying SD are summarized in Table 1. Here, as data indicated, although the CACs of $PLTL_{30}$-Glu were slightly lower than that of $PLTL_{30}$-Lac, the tendency that the glycopeptide block copolymer with a higher SD in each series had a lower CAC remained obvious. This was likely arisen from the complicated interactions between hydroxyl groups, since the increasing hydroxyl group number not only makes the copolymer more hydrophilic but also strengthens the interaction of inter-/intramolecular hydrogen bonding.

The intensity-average hydrodynamic radius (R_h) of the aggregates were detected by DLS, and results are shown in Table 1. Compared with $PLTL_{30}$-Lac, $PLTL_{30}$-Glu showed a smaller particle size and a broader particle size distribution. It was found that R_h of $PLTL_{30}$-Glu decreased from ~65 nm to ~40 nm as the SD increased. However, for $PLTL_{30}$-Lac,

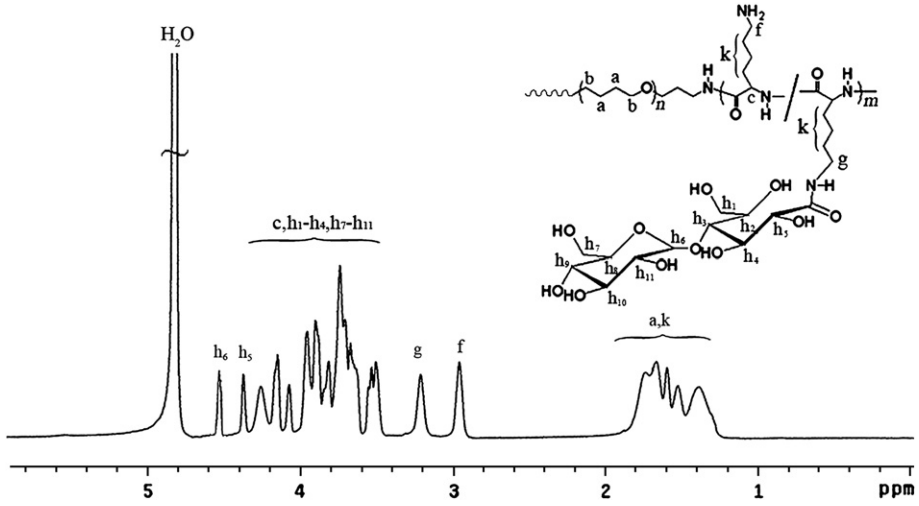


Fig. 5. ^{1}H NMR spectrum of $PLTL_{30}$-Lac-1 in D_2O at 25 °C.

Table 1
Characterization of sugar containing copolymers and their CAC and DLS data in water

Sample code	$PLTL_{30}$-Glu-1	$PLTL_{30}$-Glu-2	$PLTL_{30}$-Glu-3	$PLTL_{30}$-Lac-1	$PLTL_{30}$-Lac-2	$PLTL_{30}$-Lac-3
Ratio[a]	1:1	1:2	1:3	1:1	1:2	1:3
M_n[b] $\times 10^{-4}$	1.63	1.74	1.80	1.84	2.31	2.41
SD[b] (%)	59	68	72	47	70	75
CAC (g/l)	0.075	0.057	0.054	0.13	0.1	0.084
R_h[c] (nm)	64	57	38	85	84	92
PDI[d]	0.43	0.56	0.50	0.2	0.26	0.23

[a] The feeding ratio of $-NH_2$ to gluconolactone or lactobionolactone in the synthetic reaction of $PLTL_{30}$-Glu or $PLTL_{30}$-Lac.
[b] Calculated from ^1H NMR.
[c] Measured by DLS at 90°.
[d] Measured by DLS at 90° and calculated by CONTIN method.

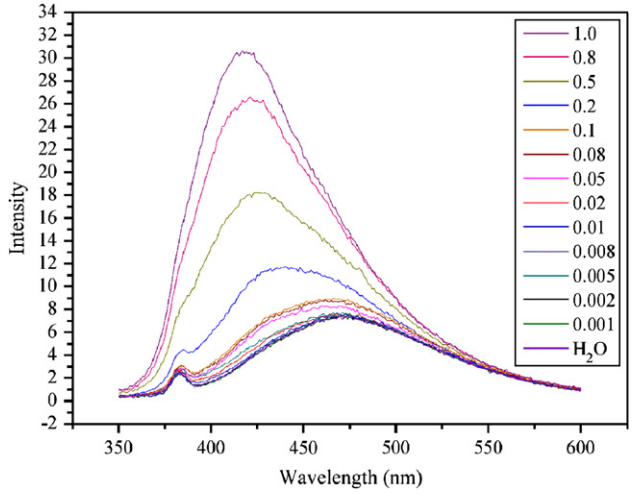


Fig. 6. Fluorescence emission spectra of PNA at different concentrations of $PLTL_{30}$-Glu-3 in water. [PNA] = 2.0×10^{-6} mol/l; C [mg/ml]; $\lambda_{ex} = 340$ nm.

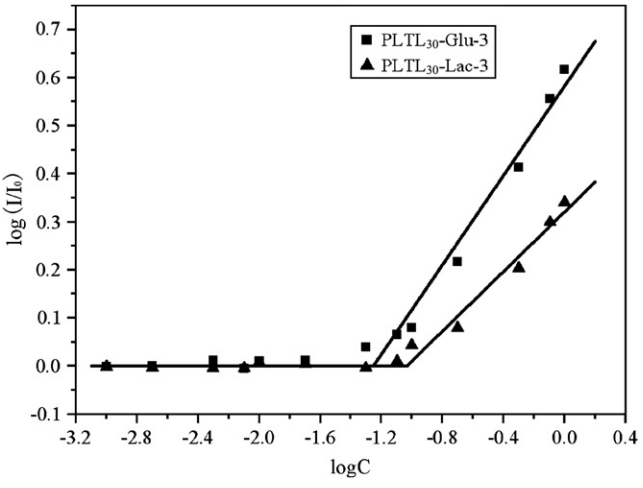


Fig. 7. Plot of the intensity ratio (I/I_0) as a function of the concentration of $PLTL_{30}$-Glu-3 and $PLTL_{30}$-Lac-3; C [mg/ml].

this value only changed from 85 nm to 92 nm with the SD variation. It seemed that the SD has no obvious effect on the R_h change of $PLTL_{30}$-Lac.

Verification of the aggregate morphologies was performed by TEM observations. Fig. 8 shows the typical TEM images of the aggregates made from $PLTL_{30}$-Glu-1 and $PLTL_{30}$-Lac-1 at a concentration of 1 mg/ml in pure water. Negative staining with uranyl acetate was used. The micrographs clearly confirmed the micelle-like structure of the particles. As can be seen, the aggregates of $PLTL_{30}$-Glu gave a broader particle size distribution with irregular shapes, consistent with the results summarized in Table 1.

3.3. Drug loading and release in vitro

$PLTL_{30}$-Glu-1 and $PLTL_{30}$-Lac-1 were taken as the examples for evaluating their loading capability and release behavior as carriers for doxorubicin controlled release. In general there are several methods to separate the non-encapsulated drugs from the micelles, such as column chromatography, ultracentrifugation and dialysis. Evidently, each method has its merit and defect. In this study, the resulting micelles are so small that they cannot be separated by ultracentrifugation. Furthermore, the solution may be diluted below CAC through the column chromatography which may provide inaccurate results. As for the dialysis method, if the dialysis time is too short, the free drug cannot be removed. Meanwhile, if the dialysis time is too long, most of the drug loaded in micelles may be released. The dialysis method was used here, and the optimization condition to separate the non-encapsulated drugs from drug-loaded micelles was adopted after lots of careful trials. A blank experiment of the dialysis of doxorubicin in the absence of micelles was also done. In the blank experiment, the mass of drug and experimental conditions were kept to be same as in the controlled drug release experiments in the presence of polymeric micelles. The process referred in the experimental section was chosen as the best condition for purification of the drug-loaded micelles. For the blank experiment, half of the free drug was remained in the first hour in the dialysis bag. After twice changing dialyzate and 3 h dialysis, there was nearly no drug left in the dialysis bag. Clearly, in the presence of micelles, all the drugs cannot be removed completely in 3 h compared to the blank experiment, and a longer time was needed.

As shown in Fig. 9, the drug-loaded particles were evidenced in round shape and around 100–150 nm in diameter by TEM observations. DLS analysis also gave the similar particle size and particle size distribution results as summarized in Table 2. However, in comparison with the blank copolymer

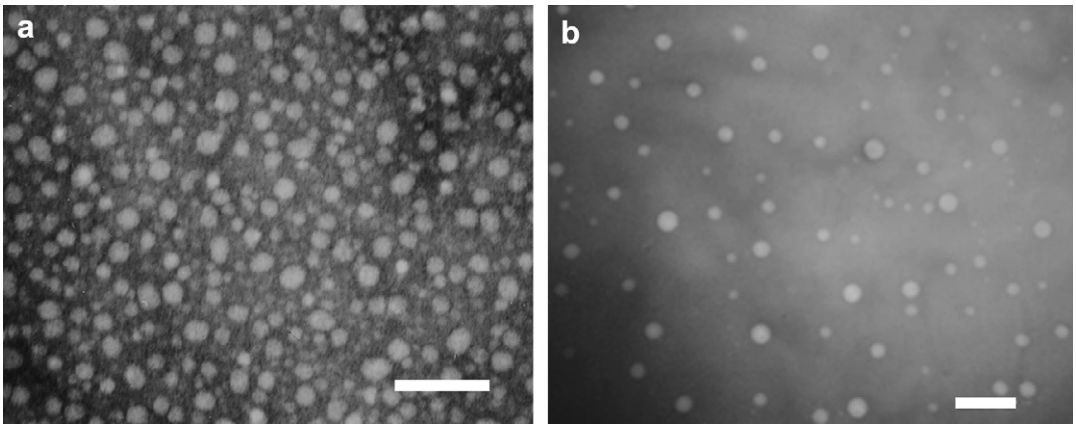


Fig. 8. Negative stain transmission electron micrographs of aggregates obtained from $PLTL_{30}$-Glu-1 (a) and $PLTL_{30}$-Lac-1 (b). The concentration of the copolymer sample is 1.0 mg/ml. Scale bar: 500 nm.

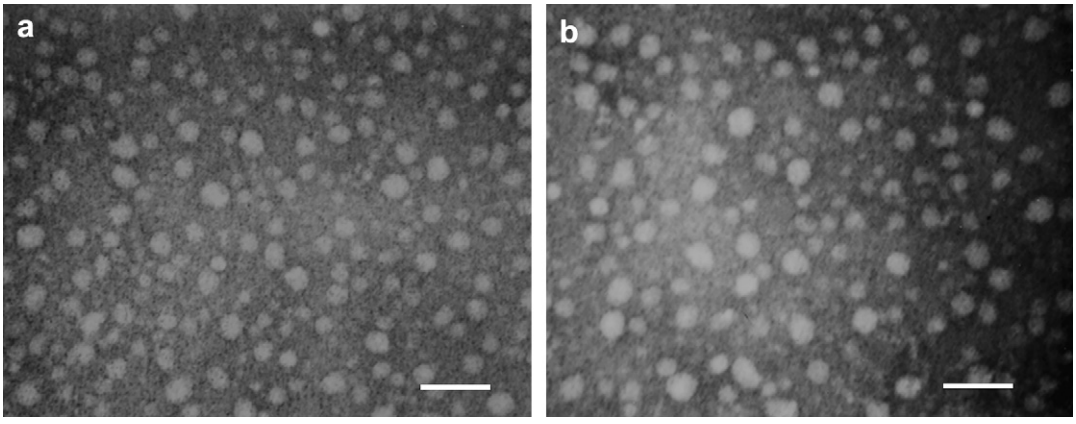


Fig. 9. Negative stain transmission electron micrographs of DOX-loaded particles obtained from $PLTL_{30}$-Glu-1 (a) and $PLTL_{30}$-Lac-1 (b). Scale bar: 500 nm.

Table 2
Characterization of DOX-loaded glycopeptide nanoparticles

Sample	EE[a] (%)	LC[b] (%)	R_h[c] (nm)	PDI[d]
$PLTL_{30}$-Glu-1	42	4.2	73	0.51
$PLTL_{30}$-Lac-1	27	2.7	87	0.45

[a] EE: encapsulation efficiency.
[b] LC: loading capacity.
[c] Measured by DLS at 90°.
[d] Measured by DLS at 90° and calculated by CONTIN method.

aggregates, the doxorubicin-loaded particles became a little larger in size. From the data shown in Table 2 and release curve in Fig. 10, it was found that $PLTL_{30}$-Glu-1 with a lower CAC has a higher drug loading capacity and a slower release rate with regard to $PLTL_{30}$-Lac-1. Since the local pH in solid tumor is lower than normal tissue, sodium acetate buffered solution at pH = 5.0 was also used to examine the doxorubicin release behavior in acidic environment. In contrast to the release at neutral condition, its release in acidic conditions was much faster. Initial rapid releases were found in all the samples in the initial 2 h. This phenomenon was attributed to the rapid diffusion of DOX from the particle to the dialyzate, since the concentration of DOX was very low in the initial dialyzate. After this period, the drug release rate was almost stable for about 10–15 h, and then the release rate was slow down again with time. It implied that the release process is mainly controlled by the diffusion mechanism.

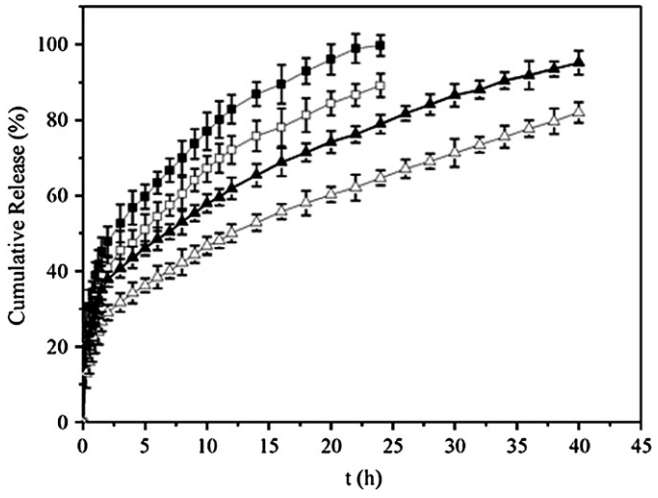


Fig. 10. Release of DOX from glycopeptides particles *in vitro*: (■) $PLTL_{30}$-Lac-1 at acidic condition (pH = 5.0); (□) $PLTL_{30}$-Lac-1 at neutral condition (water); (▲) $PLTL_{30}$-Glu-1 at acidic condition (pH = 5.0); (△) $PLTL_{30}$-Glu-1 at neutral condition (water). The standard deviation is shown by the error bars, $n = 4$.

Evidently, the biocompatibility of PLL segments was improved by introduction of saccharine groups in this paper, which effectively reduces their charge density. As carrier for controlled drug release, however, the biocompatibility and biodegradation of the central PTHF block always arouse general concern. As indicated in literatures [27,28], PTHF showed good biocompatibility with respect to tissue reactions and interactions not only as one part of copolyethers but also as the soft segment of polyurethanes for tissue engineering scaffolds. Therefore, it is promising for the as-prepared amphiphilic glycopeptide block copolymers to be used as biocompatible biomaterials for controlled drug release.

4. Conclusions

The synthesis and self-assembly of a kind of novel amphiphilic glycopeptide block copolymers and their *in vitro* evaluation as carriers for controlled drug delivery have been carried out in the present study. They were found to aggregate in the presence of doxorubicin into drug-loaded nanoparticles showing the characteristic controlled release behavior in water. Hence these copolymers, especially for lactobionolactone grafting ones in which the galactose moiety is attached, hold the potential to be used as the targetable site between carriers for controlled drug release and specific cell surfaces. From the viewpoint of the molecular recognition ability of saccharide grafting moieties, a study towards the *in vivo* application of this kind of amphiphilic glycopeptide block copolymers as target drug-loaded carriers is under way in our laboratory.

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