*J. Biomater. Sci. Polymer Edn*, Vol. 14, No. 10, pp. 1043–1056 (2003)

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# Penicillin V-conjugated PEG-PAMAM star polymers

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Received 19 August 2002; accepted 29 May 2003

**Abstract**—Starburst® PAMAM dendrimers are potential carriers for drug delivery due to their unique structure. Drug-delivery scaffolds were designed and built up based on the polyethylene glycol-polyamidoamine (PEG-PAMAM) star polymer. Penicillin V was used as a model carboxylic group containing drug to conjugate with full- and half-generation PAMAM dendrimers. G2.5 PAMAM (with 32 carboxylic groups on the surface) dendrimers and G3.0 (with 32 primary amine groups on the surface) were typically chosen. There are two strategies given in the paper where a drug carrying a carboxylic group (e.g. penicillin V) was coupled to star polymer via amide and ester bonds, respectively. FT-IR, UV-Vis and $^{1}$H-NMR were used to characterize the intermediates and drug–star polymer conjugates. A single-strain bacterium, *Staphylococcus aureus*, was grown up for penicillin-conjugated PEG-PAMAM (G3.0) star polymer activity test. The result verified the bioavailability of modified penicillin after the ester bond was cleaved.

*Key words*: Polyamidoamine dendrimers; polyethylene glycol; drug-delivery system; penicillin V; star polymer.

## INTRODUCTION

Small lipid vesicles known as liposomes have gained regulatory approval for passive targeted drug delivery [1] and are being widely explored for active targeting [1–3]. An advantage of the liposome-based carrier is that a relatively large cache of drug can be transported in the vesicle. Researchers have exploited the ability to control vesicle parameters including cholesterol content [4], surface charge [5] and vesicle size [6] to optimize liposome-based drug delivery. However, liposome-based drug delivery systems have problems, such as instability, low drug-loading efficiency and short circulation half-life of the vesicles. Recently, dendrimers have attracted much attention in drug delivery because of their unique structures [7, 8].

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Compared with linear polymers, dendrimers are well-defined globular highly-branched macromolecules [9]. Their many accessible reactive end-groups at the surface make it possible to tailor dendrimers' chemical and physical properties through proper chemical modification [10]. Their globular structure suggests they could play a similar role in drug delivery as liposomes do and overcome the problems liposome-based drug delivery systems have as well.

Starburst® polyamidoamine (PAMAM) dendrimers with a series of generations are commercially available (Dendritech, Midland, MI, USA). The biological studies on PAMAM dendrimers show they do have potential in drug delivery [8, 11]. Roberts *et al.* used V79 cells and male Swiss–Webster mice to study *in vitro* toxicity, *in vivo* toxicity, immunogenicity and biodistribution of PAMAM G3, G5 and G7 dendrimers [12]. It was found that PAMAM toxicity is dose- and generation-dependent *in vitro*. Low generations (G5 or lower) are not considered toxic. Moreover, in Roberts' study there is no evidence of immunogenicity for any tested generation. In a more recent study by Malik *et al.* [13], it was found that cationic dendrimers (with amine end groups) caused haemolysis and cytotoxicity. In contrast, 8-branched and 32-branched anionic carboxylate dendrimers, which were studied in their research, were neither lytic nor cytotoxic up to concentration of 2 mg/ml. Malic *et al.* pointed out that such unsuitable dendrimers as cationic PAMAM dendrimers may be accepted for biological application as long as the surface is attached to anionic or neutral groups. The positive evidence was obtained by using 12-branched and 36-branched poly(ethylene oxide) (PEO)-grafted carbosilane (CSi-PEO) dendrimer [13]. The modified dendrimers did not cause haemolysis up to 2 mg/ml. In the same range of concentration, they did not exhibit toxicity to CCRF and HepG2 cells. However, for B16F10 cells, viability was decreased at higher concentrations (over 0.1 mg/ml) of 12-branched or 36-branched Csi–PEO dendrimers. Interestingly, 36-branched dendrimers still gave the cells a higher viability than 12-branched dendrimers. 36-branched dendrimer showed more biocompatibility than 12-branched dendrimer because it has more surface sites to couple with PEG arms.

Size of particles is another factor regarding the transport of a dendrimer-based drug-delivery system. In Szentkuti's study [14], he investigated the transport of microspheres with smallest size (14 nm), medium-size (415 nm) and larger size (1.09 $\mu$m) through the mucous layer of the distal colon of rats. Only those larger-sized microspheres could not arrive at the apical surface. Norris *et al.* surveyed the permeability of polystyrene-modified nanoparticles through synthetic gastric mucin gels [15]. They found the particle size decreased the permeability sharply when the size increased from 0.1 $\mu$m to 0.3 $\mu$m. The diameter of G10, the largest-sized EDA core Starburst® PAMAM dendrimer, is just 13.5 nm. At this small size, there should not be any problem with the permeability for the transport of naked PAMAM dendrimer through membrane barriers in the body.

We have designed a drug-delivery scaffold based on ethylene diamine (EDA) core Starburst® PAMAM dendrimer (Fig. 1). We explored conjugation methods

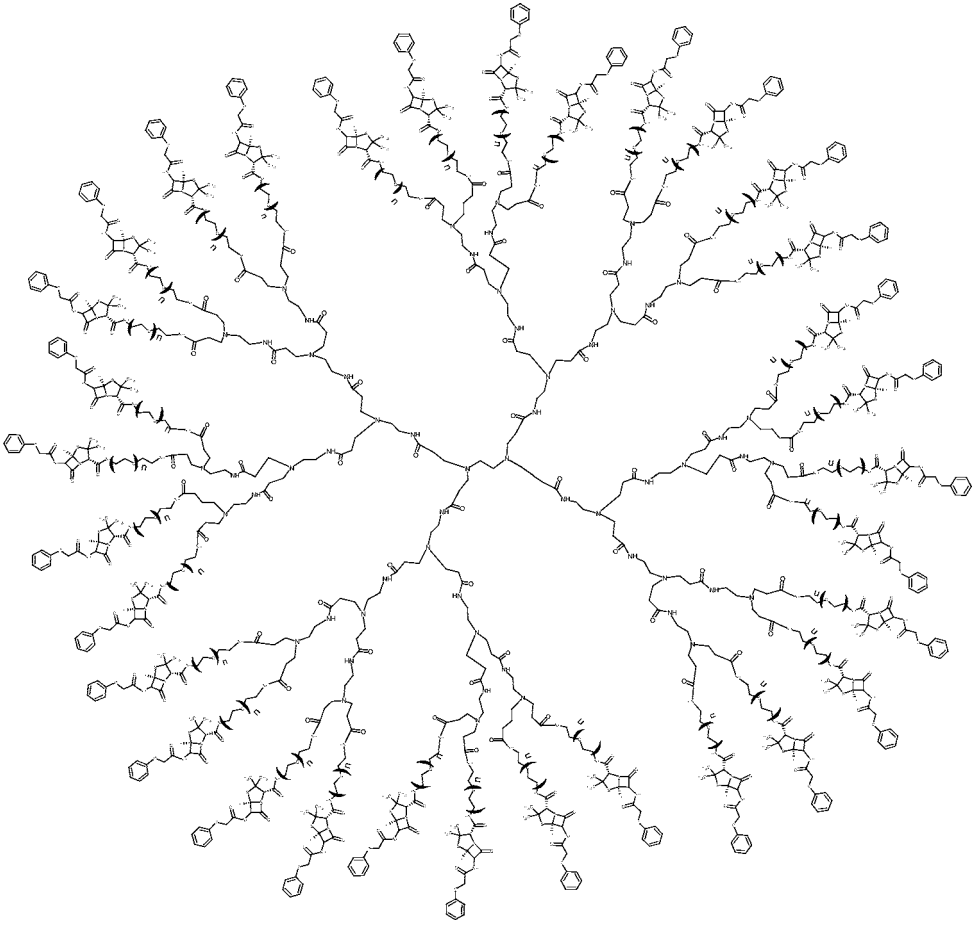
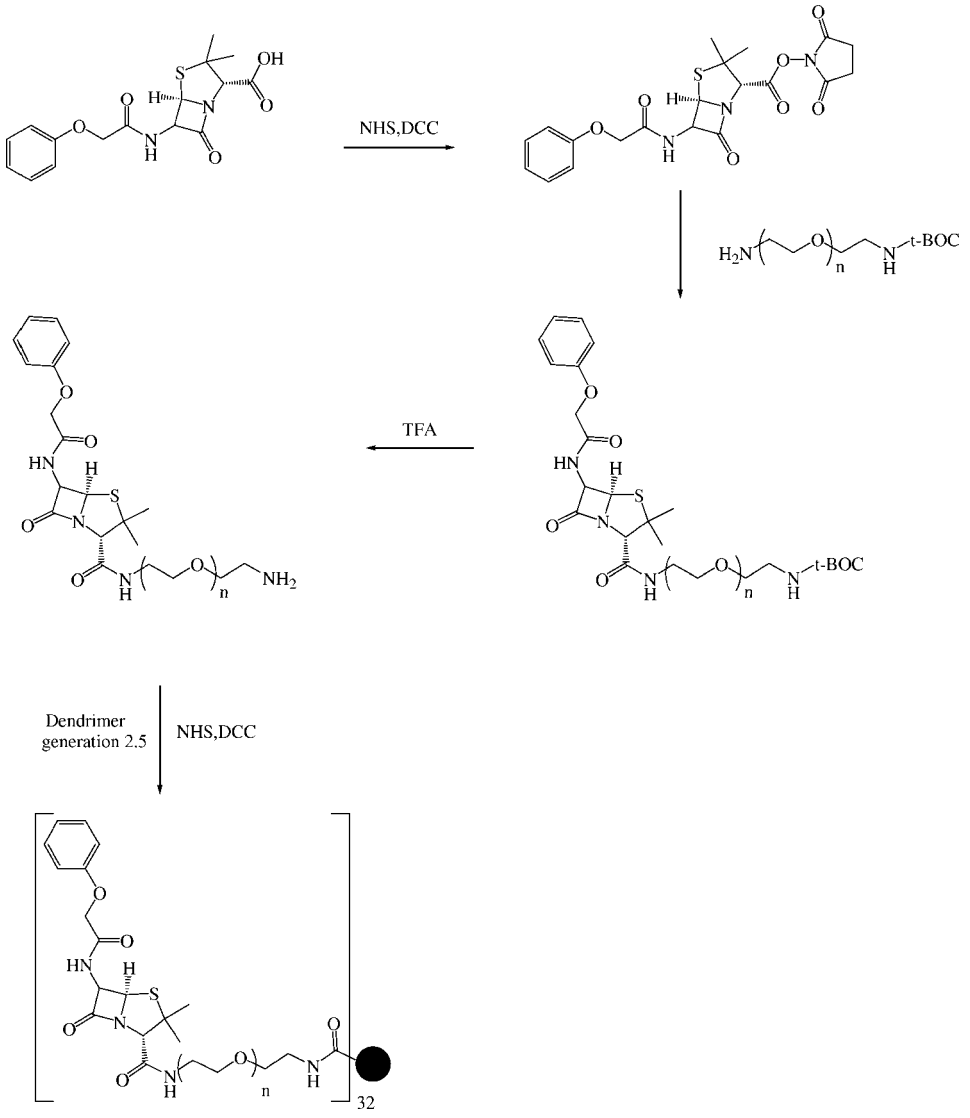


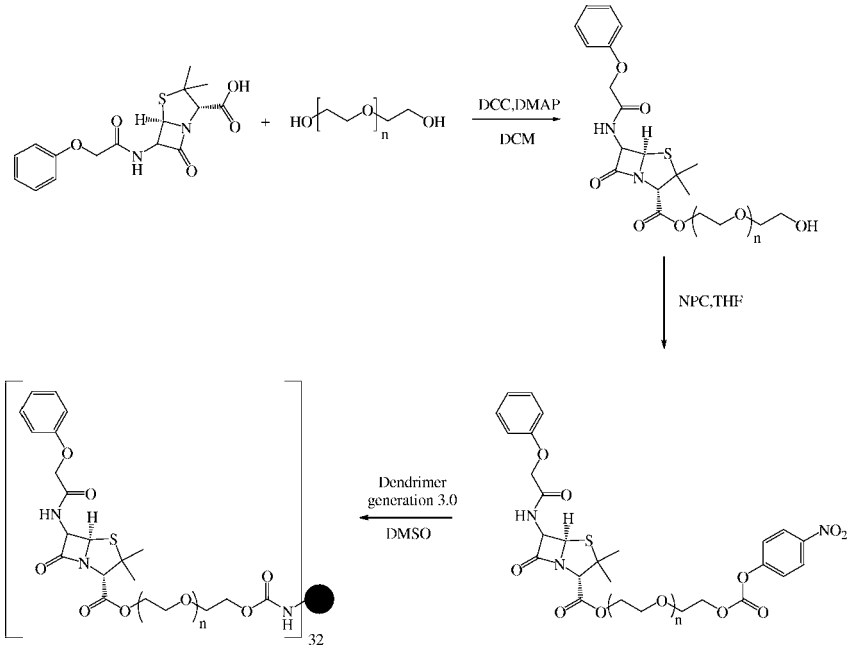
**Figure 1.** Idealized schematic view of drug contained poly ethylene glycol star polymer. G2.5 PAMAM dendimer contains 32 carboxylate groups which connect MW 3400 polyethylene glycol; penicillin V is attached to the end of the PEG.

which could be used to attach carboxylic-group-carrying drugs to the PEG-PAMAM star polymers. For Starburst® PAMAM dendrimers, half and full generations have carboxylic and primary amine groups on the surface, respectively. Accordingly, two synthesis routes to produce drug-PEG-dendrimer conjugates due to the variation of the surface functional groups are described (Scheme 1 and Scheme 2). In our study, the carboxylic-group-containing antimicrobial agent penicillin V was chosen as a model drug, G2.5 Starburst® PAMAM dendrimer with 32 surface carboxylate groups and G3.0 Starburst® PAMAM dendrimer with 32 surface primary amine groups were used. The linkage between penicillin V and PEG is formed by amide and ester bonds in Scheme 1 and Scheme 2, respectively. Since it is believed that a carboxylic acid group is essential for the antibacterial activity of penicillin V, hydrolysis release for penicillin V coupling with star polymer *via* ester bonds




**Scheme 1.** Synthesis of penicillin V-PEG-PAMAM (G2.5) conjugates.

was studied. In contrast, penicillin V bound through an amide bond (Scheme 2) was not expected to release from the matrix because the amide bond is stable *in vivo* [16]. However, the strategy we presented (Scheme 2) would be referred to a general procedure for the formation of amide linkage between carboxylic group containing drug and star polymer, which could be applied to those drugs whose bonding stability is highly required and biological activity is less or not dependent on the carboxylic group. The star-based drug-delivery system could perform in a fashion to increase drug circulation time in the body and may provide more stability than liposome-based drug delivery systems.




**Scheme 2.** Synthesis of penicillin V-PEG-PAMAM (G3.0) conjugates.

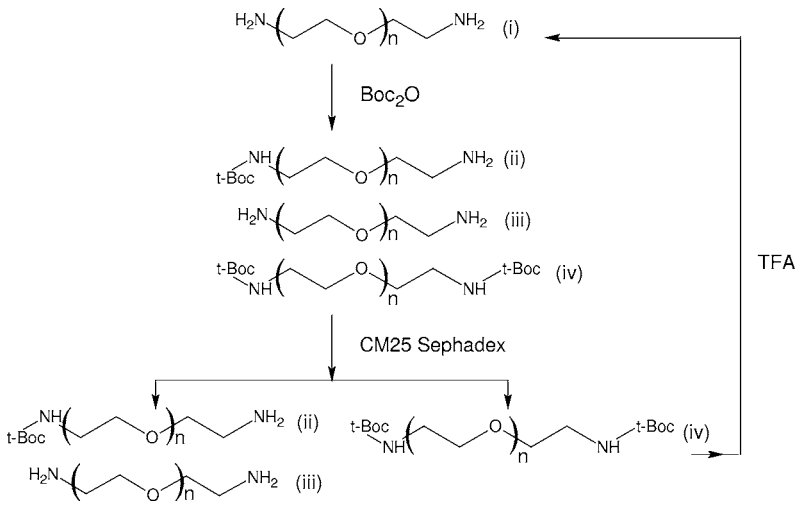
## MATERIALS AND METHODS

### *Materials*

O,O-Bis(2-aminoethyl)polyethylene glycol (PEG-diamine, MW 3400, 1.8 mol amine/mol PEG), poly(ethylene glycol) (PEG, MW 2000), phenoxymethylpenicillinic acid (penicillin V), Starbust® Polyamidoamine (PAMAM) dendrimer Generation 2.5 (10 wt% solution in methyl alcohol, containing 32 surface carboxylate groups, FW 6267) and Generation 3.0 (20 wt% solution in methyl alcohol, containing 32 surface primary amine groups, FW 6909) were obtained from Sigma. Di-*tert*-butylpyrocarbonate ($Boc_2O$), N-hydroxysuccinimide (NHS), dicyclohexyl-carbodiimide (DCC), 4-dimethylaminopyridine (DMAP) and p-nitrophenyl chloroformate (NPC) were purchased from Aldrich. Trifluoroacetic acid (TFA), dichloromethane (DCM), dimethylformamide (DMF), tetrohydrofuran (THF) and ethyl acetate were obtained from Sigma. Distilled water was used through all experiments. All starting materials and organic solvents were directly used without further purification. A single strain of microorganism, *Staphylococcus aureus* (#6538P), was purchased from the American Tissue Culture Collection.

### *Characterization*

FT-IR spectra were obtained on a Nicolet Nexus 670 spectrometer. UV/Visible absorbance data were gained on a Beckman DU® 640 Spectrophotometer. $^1$H-NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. The internal




**Scheme 3.** The route for preparation of BOC-protected polyethylene glycol-diamine.

standard was tetramethylsilane. Deuterium oxide ($D_2O$, 99.9%), chloroform-d ($CDCl_3$, 99.8%) and dimethyl-$d_6$ sulfoxide (DMSO-$d_6$, 99.9%) were obtained from Cambridge Isotope Laboratories. 5 mg of each sample was dissolved in deuterated solvent, filtered and degassed before measurement. The chemical shifts of $D_2O$, CDCl3 and DMSO-$d_6$ are 4.8 ppm, 7.27 ppm and 2.50 ppm, respectively. Analytical thin-layer chromatography (TLC) was operated on Merck precoated aluminum-backed silica gel plates with an $F_{254}$ indicator. The plate was visualized by exposure to ultraviolet radiation.

*Synthesis of penicillin V-conjugated PEG-PAMAM (G2.5) star polymer*

*Preparation of penicillin-conjugated poly (ethylene glycol) via amide bonds.* Both 710.8 mg penicillin V (2 mmol) and 230.2 mg NHS (2 mmol) were dissolved and mixed in 5 ml THF. To the solution of penicillin V and NHS was added 5 ml THF containing 412.7 mg dicyclohexyl-carbodiimide (DCC, 2 mmol) while stirring. The reaction mixture was stirred 48 h at room temperature. The completeness of the reaction was confirmed by thin-layer chromatography. The mixture was placed at $-20^{\circ}$C for 1 h for further precipitation of dicyclohexyl urea (DCU). DCU was filtered off and the solvent was evaporated under vacuum. The product was purified by recrystallization with ethyl acetate.

PEG-diamine was used as a starting material. Before the attachment of penicillin V to the polymer, PEG-diamine was converted to mono-protected homo-bifunctional PEG following a similar procedure as reported previously [17] (Scheme 3). Briefly, 340 mg PEG-diamine (0.1 mmol) was dried in vacuum and dissolved in 600 $\mu$l DCM. 21.8 mg $Boc_2O$ (0.1 mmol) was dissolved in 200 $\mu$l DCM and then added dropwise to the PEG-diamine solution. The reaction system was stirred

overnight. The major product $NH_2$-PEG-NH-t-Boc was obtained together with minor product t-Boc-NH-PEG-NH-t-Boc and unreacted $NH_2$-PEG-$NH_2$. The mixture was dried by rotary evaporation and dissolved in distilled water. Unreacted $Boc_2O$ was extracted by ether. The water layer passed through a column with CM-25-Sephadex ion exchanger. t-Boc-NH-PEG-NH-t-Boc was eluted out by water. The eluent was changed to 10% ammonia solution when no signal could be detected by an UV-Vis spectrometer at 233 nm. The retained materials were eluted by ammonia solution, collected and dried. No further effort for separation of $NH_2$-PEG-NH-t-Boc and $NH_2$-PEG-$NH_2$ was made.

To 252 mg polymer mixture product (Boc-NH-PEG-$NH_2$ dominated, 74 $\mu$mol) that was in 2 ml 0.1 M sodium bicarbonate was added 148 $\mu$mol penicillin V-NHS ester (66.4 mg) which was dissolved in 1 ml dimethylformamide (DMF). The reaction system was stirred for 4 h, after which the reaction mixture was evaporated to dryness. The residue was extracted by acetone and the polymer was precipitated by ether. The crude product was characterized by $^1$H-NMR to confirm the presence of penicillin V bound to the polymer. The polymer was then treated with 90% TFA for 10 min to remove BOC groups. NaOH solution was added to the solution to neutralize the excess acid. The product was ready for further reaction.

FT-IR for mono-BOC protected PEG diamine: 3610 $cm^{-1}$ and 3550 $cm^{-1}$ (—NH stretching of $-NH_2$); 3370 $cm^{-1}$ (—NH stretching of —CONH); 1720 $cm^{-1}$ (—C=O stretching of amide); 1120 $cm^{-1}$ ($-CH_2-O-CH_2-$ stretching).

$^1$H-NMR for mono-BOC protected PEG diamine (300 MHz, $D_2O$): $\delta$ 1.43 ppm (s, 9H, $-(CH_3)_3$ in BOC); 3.59 ppm (m, 4n, $-(O-CH_2-CH_2)_n-$).

$^1$H-NMR for penicillin-NHS ester (300 MHz, $CDCl_3$): $\delta$ 7.50 ppm (2H, dd, $J = 7.2$ Hz), 7.06 ppm (1H, dd, $J = 6.9$ Hz), 6.95 ppm (2H, d, $J = 8.1$ Hz), 5.80 ppm (1H, q, $J = 4.5$ Hz), 5.60 ppm (1H, d, $J = 4.5$ Hz), 4.57 ppm (1H, s), 4.13 ppm(2H, q, $J = 7.2$ Hz), 2.76 ppm (4H, s), 1.71 ppm (6H, d, $J = 14.1$ Hz).

$^1$H-NMR of penicillin-conjugated PEG (300 MHz, $D_2O$): $\delta$ 1.45 ppm (s, 9H, $-(CH_3)_3$ in BOC); 1.73 ppm (s, 6H, $-(CH_3)_2$ in penicillin V); 3.59 ppm (m, 4n, $-(O-CH_2-CH_2)_n-$); 6.93–7.36 ppm (m, 5H, $C_6H_5$).

*Preparation of penicillin-conjugated PEG-PAMAM (G2.5) star polymer.*
Starburst® dendrimer was dried to remove methanol using a rotary evaporator. Then 10.0 mg (1.6 $\mu$mol) G2.5 Starburst® dendrimer was dissolved in water and then converted to the acid form by acidification with 5 M HCl to pH 1.0. The acidified solution was evaporated to dryness under vacuum. The dendrimer in free acid form was dissolved in 1 ml DMF. It reacted with 5.9 mg NHS (51.2 $\mu$mol) and 10.5 mg DCC (51.2 $\mu$mol). The reaction system was stirred 14 h. DCU precipitated during the reaction and was removed by centrifugation.

The activated dendrimer in DMF was added to bicarbonate solution of 250 mg penicillin V prodrug. The mixture was continuously stirred for 3 h. The mixture was dried under vacuum. It was dissolved in acetone followed by precipitation in ether/hexane. For further separation of star polymers from unreacted PEG, ultra-

filtration was conducted against water using an Amicon® stirred cell with YM10 membrane.
$^1$H-NMR for penicillin-conjugated PEG-PAMAM (G2.5) star polymer (300 MHz, $D_2O$): $\delta$ 1.73 ppm (s, $-(CH_3)_2$ in penicillin V); 2.44–3.44 ppm (m, protons in dendrimer); 2.65 ppm (s, protons in NHS); 3.59 ppm (m, 4n, $-(O-CH_2-CH_2)_n-$); 6.93–7.36 ppm (m, $C_6H_5$ in penicillin V).

*Synthesis of penicillin V-conjugated PEG-PAMAM (G3.0) star polymer*

*Preparation of penicillin-conjugated poly (ethylene glycol) via ester bonds.* The synthesis followed a general procedure given by Zalipsky *et al.* [18]. 4 g PEG (2 mmol) and 158.8 mg DMAP (1.2 mmol) were dissolved in 200 ml DCM. 455.5 mg penicillin V (1.2 mmol) was added to the solution. The solution was stirred and cooled in an ice-water bath. To the solution was added 268.2 mg DCC (1.3 mmol). The mixture was continuously stirred for 4 h. The precipitant dicyclohexyl urea (DCU) was removed by filtration. The residue was dried using rotary evaporation and then extracted by acetone. Centrifugation was employed for a further clearance of DCU. The product was precipitated in diethyl ether.
$^1$H-NMR of penicillin-conjugated PEG (300 MHz, $D_2O$): $\delta$ 1.73 ppm (s, 6H, $-(CH_3)_2$ in penicillin V); 3.59 ppm (m, 4n, $-(O-CH_2-CH_2)_n-$); 6.93–7.36 ppm (m, 5H, $C_6H_5$).

*Preparation of penicillin-conjugated PEG-PAMAM (G3.0) star polymer.* 0.94 g PEG–penicillin V (approx. 0.4 mmol) was dissolved in THF (40 ml). To the solution were added 80.6 mg NPC (0.4 mmol) and 56 $\mu$l triethylamine (0.4 mmol). The mixture was stirred for 24 h at room temperature. The salt was filtered off. The filtrate was concentrated and dropwise added to diethyl ether. The precipitant was purified by reprecipitation from THF into diethyl ether. PEG–p-nitrophenyl chloroformate was recovered by evaporation under reduced pressure. To a solution of 27.6 mg G3.0 PAMAM dendrimer (4 $\mu$mol) in dimethyl sulfoxide (10 ml), PEG p-nitrophenyl carbonate was added and the solution was stirred for 72 h at room temperature. The reaction system was evaporated to dryness using rotary evaporation. The unreacted PEG and low-molecular-weight reagents were removed via ultrafiltration using an Amicon® stirred cell with YM10 membrane.
$^1$H-NMR for penicillin-conjugated PEG–PAMAM (G3.0) star polymer (300 MHz, $D_2O$): $\delta$ 1.73 ppm (s, $-(CH_3)_2$ in penicillin V); 2.44–3.44 ppm (m, protons in dendrimer); 3.59 ppm (m, 4n, $-(O-CH_2-CH_2)_n-$); 4.30 ppm (d, $-CH_2$ next to carbonate bond from PEG) 6.93–7.36 ppm (m, $C_6H_5$ in penicillin V).

*Antimicrobial activity test of modified penicillin*

In order to prove the activity of modified penicillin for the prohibition of bacterial growth, the biotest was conducted as follows. A pure strain of microorganism, *Staphylococcus aureus* (#6538P), was purchased from the American Type Culture

Collection (ATCC, Rockville, MD, USA) and revived via ATCC medium 3. The bacteria were allowed to grow for one week, followed by transfer to new nutrient medium. After one day growth in the new nutrient medium (to ensure an adequate population density) the appropriate amount (1 ml of $10^5$ U/l for penicillin or approximately equivalent for modified penicillin) was added to each 10 ml microbes. One set of flasks did not receive penicillin/modified penicillin and served as the control. The resulting solutions were allowed to grow for another 24 h. Afterwards the microbial activity was determined by the most probable number (MPN) plate counts. Serial dilutions ($10^{-2}$ to $10^{-7}$) were conducted in triplicate for reproducibility and reliability purposes.

## RESULTS AND DISCUSSION

### *Synthesis of penicillin V-conjugated PEG-PAMAM star polymer*

PEG is considered non-toxic and non-immunogenic. In addition, the ethylene oxide repeating units have excellent solubility in both aqueous and most organic solutions. So PEG is one of the most widely used biocompatible polymers. In our study, PEG was used to link drug and dendrimer core. PEG was first hetero-functionalized to eliminate the chances of forming loops and crosslinks. Here, homo-bifunctional polyethylene glycol diamine (MW 3400) was used as starting material. Primary amine groups are more reactive than hydroxyl groups typically capping the PEG. We modified the elegant procedure of Ehteshami *et al.* [17] to obtain herero-functionalized PEG. We employed $Boc_2O$ as protecting agent to block amine groups. The ratio of $Boc_2O$ to PEG was still 1 : 1. When reaction was complete, unreacted $Boc_2O$ was removed by extraction. The mixture of $NH_2$-PEG-NH-t-Boc (ii), $NH_2$-PEG-$NH_2$ (iii) and t-Boc-NH-PEG-NH-t-Boc (iv) was passed through an ion-exchange column packed with Sephadex CM-25 ion exchanger. Ion-exchange chromatography was used only once to separate t-Boc-NH-PEG-NH-t-Boc from the other two components, $NH_2$-PEG-NH-t-Boc and $NH_2$-PEG-$NH_2$. Since BOC is acid sensitive, $NH_2$-PEG-NH-t-Boc and $NH_2$-PEG-$NH_2$ were eluted by 10% ammonium oxide. The eluent fraction was monitored by UV-Vis spectrophotometer. The amine groups in the polymer could be assayed by either ninhydrin test [19] or trinitrobenzenesulfonic acid (TNBS) test [20]. In our experiment, $^1$H-NMR was used not only to confirm the presence of BOC groups but also to give a quantitative estimation of amount of BOC groups in the polymer (spectrum not shown). The singlet peak at 1.43 ppm shows the presence of methyl groups from BOC. There is one single peak observed at 1.79 ppm in $CDCl_3$ which disappeared in $D_2O$, since protons in $-NH_2$ rapidly exchange with $D_2O$. It is more reliable to calculate the percentage of BOC in the backbone using the proton peak from BOC. Statistically, the fraction of $NH_2$-PEG-NH-t-Boc in the amine contained PEG ($NH_2$-PEG-NH-t-Boc and $NH_2$-PEG-$NH_2$) is 0.67. The actual mol fraction of BOC-contained PEG from the spectrum was 0.54. The total yield was 45%. That

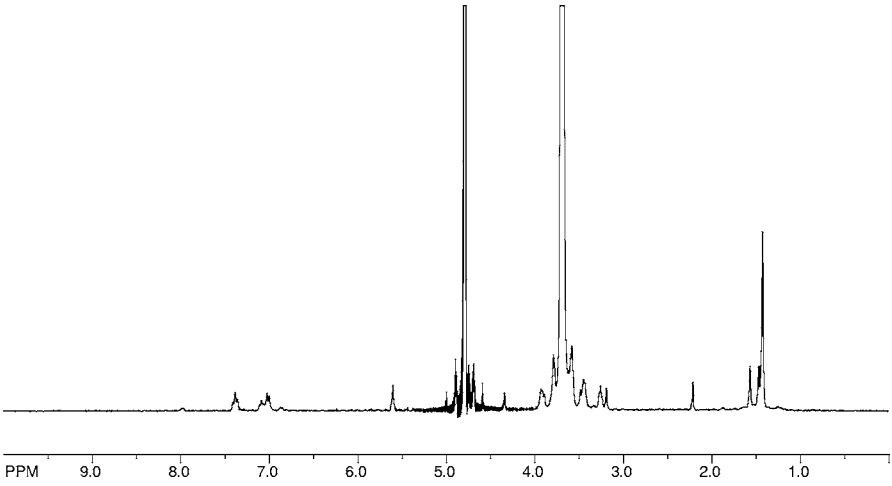



**Figure 2.** 300 MHz $^1$H-NMR spectrum of t-BOC-PEG-Penicillin conjugate in $D_2O$.

gave relatively pure mono-Boc-blocked PEG. The later coupling reaction between amine-contained PEG and excess activated ester consumed free amine groups and those BOC-protected amine groups were recovered by treatment with 50% TFA. Then, only $NH_2$-PEG-Penicillin was available for further reaction. Through this step, the formations of loops, dimers, trimers, etc., were decreased significantly.

As for PEGylation of drugs, the methods for the coupling reactions between functional groups in the drug molecules and polymeric moiety are given in Refs [16, 21, 22]. Such drugs containing carboxyl groups as penicillin V are commonly activated into succinimide esters using N-hydroxy-succinimide facilitated by condensation agent, dicyclohexyl carbodiimide (DCC). Scheme 1 was the route we employed to synthesize drug-containing star polymer via amide linkage. $^1$H-NMR verified the structure in the penicillin V-PEG conjugate (Fig. 2); the typical peaks from penicillin V are two methyl groups at 1.73 ppm and hydrogen atoms in the aromatic ring between 6.93 ppm and 7.36 ppm. The repeat units of PEG have multiple peaks at 3.59 ppm.

The strategies for the activation of the carboxyl surface groups of Starburst® dendrimer are similar to those of low-molecular-weight molecules. One of the methods which proved effective is to prepare NHS esters [23] as well. In our study, the mole ratio of PEG to dendrimer core was over 1.0 to make sure the surface of the dendrimer was saturated with PEG arms. $D_2O$ was used as the deuterated solvent for star polymers when obtaining $^1$H-NMR spectra, as the signals of the protons at different positions within dendrimers give more details in $D_2O$ than in DMSO (spectrum not shown). The ideal conformation would have a star polymer containing penicillin V in each arm (Fig. 1). In $^1$H-NMR (Fig. 3), we evaluated the conjugation by examining the signals from penicillin V, PEG and dendrimer. The signals from penicillin V and PEG in the star polymer are identical to those showing up in the penicillin V-PEG conjugate. The protons

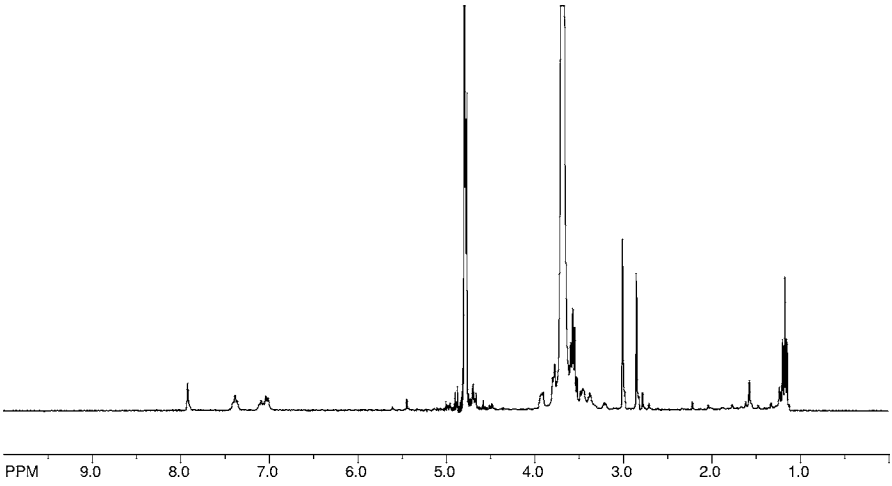



**Figure 3.** 300 MHz $^1$H-NMR spectrum of Penicillin V-PEG-PAMAM (G2.5) conjugate in $D_2O$.

from the dendrimer were also found between 2.44 and 3.44 ppm. Studies on the fundamental parameters of dendrimer [24, 25] show that the loading of PEG to dendrimer is PAMAM generation and PEG arm length dependent. In the study of modification of dendrimer surface, $^1$H-NMR was one of techniques used to estimate surface occupation efficiency and was proved feasible by comparing the result with MALDI-TOF MS [26, 27]. We synthesized a Boc-blocked PEG-PAMAM star polymer previously using the same method described above. Its $^1$H-NMR spectra shows (Fig. 4) that the ratio of methyl at 1.43 ppm from BOCs in PEG to methylenes at 2.43 ppm next to carbonyl groups in the dendrimer is 54 : 1 which means that 18 PEG arms occupy 56% of dendrimer surface sites. The estimation of the PEG-loading percentage on the surface for penicillin-PEG-PAMAM (G2.5) is 41% which is lower than that of PEG-PAMAM star polymer due to stronger steric hindrance.

Since it is believed that the carboxylic acid group is essential for the antibacterial activity of penicillin V, another route was presented (Scheme 2) using hydrolysable ester bonds to connect drug and star polymer, and PEG was used as a starting material. Using terminal hydroxyl groups of PEG to couple with carboxylic group containing drug is straightforward way for esterification. An efficient way to realize esterification between penicillin V and PEG was demonstrated by Zalipsky *et al.* [18]. In order to obtain a high percentage of mono-functionalized PEG, a large reaction volume, high ratio of free PEG to penicillin V and low temperature were employed. The polymer was activated by NPC into the active carbonate form which readily reacts with dendrimer surface primary amine groups. In this route, dendrimer is not necessary to be activated before coupling. A new peak appears at 4.3 ppm in the $^1$H-NMR spectrum which is considered evidence of the formation of carbonate bonds between PEG and dendrimer (Fig. 5). A similar estimate according to the integration of this peak and the methylene groups at 2.43 ppm shows there are 15 PEG arms with penicillin covering the dendrimer surface.

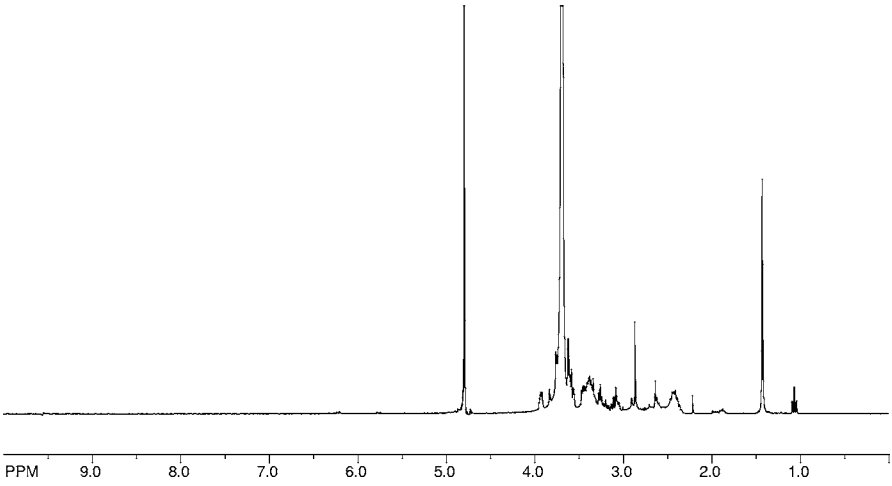



**Figure 4.** 300 MHz $^1$H-NMR spectrum of t-BOC-PEG-PAMAM (G2.5) conjugate in $D_2O$.

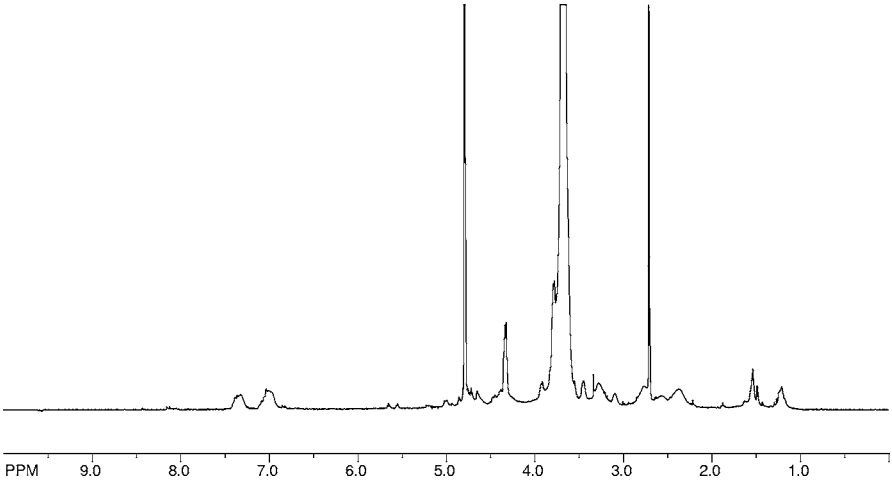



**Figure 5.** 300 MHz $^1$H-NMR spectrum of PAMAM -PEG-penicillin V conjugate (G3.0) in $D_2O$.

*Antimicrobial activity test of modified penicillin*

Penicillin V interferes with final stage of synthesis of the bacterial cell wall. After the modification (Scheme 2), carboxyl groups were converted to ester bonds which were used to couple penicillin to star polymers. The antibiotic activity of penicillin may have been deactivated by this coupling. In order to prove modified penicillin was still available to inhibit bacterial growth after hydrolysis, a penicillin-sensitive bacteria (*Staphylococcus aureus*) was cultured. Nathan *et al.* visually checked the suspension for turbidity which gave a qualitative indication of bacterial growth [28]. In our test, we provided a quantitative and more reliable method for the analysis of penicillin activity. A standard, most probable number (MPN) plate count was

conducted. The microbial activity after the addition of 1 ml of approximately $10^5$ U/l penicillin-star polymer to the *Staphylococcus* species was $2.58 \times 10^8$ colony forming units (CFU)/ml. The control test (no penicillin) had an activity of $7.8 \times 10^9$ CFU/ml over the same time period. Penicillin-conjugated PEG-PAMAM (G 3.0) star polymer (Scheme 2) decreased the *Staphylococcus aureus* cultures by more than an order of magnitude after ester hydrolysis. There was approximately the same order of magnitude decrease with the non-modified penicillin. Modified penicillin after hydrolysis reduced bacterial growth to 3% of that observed in the control. Therefore, the modification of penicillin through esterification did not alter the penicillin activity.

## CONCLUSIONS

In our study, penicillin V was coupled with PAMAM dendrimer through PEG spacer. The drug-delivery scaffold based on PEG-polyamidoamine star polymer proved feasible. The star polymers provide accessible ends which were used to chemically couple desired small molecules. The method described here could be extended to prepare star polymers with different PEG arm lengths and to couple different functional groups (drugs) of interest.

Comparing the two strategies proposed in this article, an advantage of Scheme 1, forming an amide linkage between carboxylic group containing drug and star polymer, is to give this drug-delivery system a bonding stability which may be highly desired. Those drugs whose biological activity is less or not dependent of carboxylic group could use this strategy to synthesize drug-star polymer conjugates. By contrast, Scheme 2 provides a way for the drug to release from the conjugate in a controlled manner via hydrolysis. The star-based drug delivery system could perform in a fashion to increase drug circulation time in the body and may provide more stability than liposome-based drug-delivery systems. Our result showed dendrimer could be a good alternative to liposomes. Further investigation of the transport and biodistribution of the drug-delivery system is another issue to be examined.

*Acknowledgements*

The authors gratefully acknowledge funding from an NSF CAREER award (BES-9984840), a University of Akron Faculty Research Grant (FRG-1484) and a Sigma Xi Grant-in-Aid of Research.

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