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Synthesis of heterobifunctional poly(ethylene glycol) containing an acryloyl group at one end and an isocyanate group at the other end

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Summary

A New heterobifunctional poly(ethylene glycol) (PEG) having a polymerizable acryloyl group at one end and an isocyanate group at the other end was prepared in three efficient steps from commercially available t-Boc-PEG-NH_2. The end groups were characterized by ^1H NMR and MALDI-TOF-MS spectroscopy. Conjugation of the resulting PEG onto dextran *via* stable urethane linkage gave the PEG graft polymer with an acryloyl group at the free end of the graft chain.

Introduction

Poly(ethylene glycol) (PEG) has been frequently used as a biomaterial due to its biocompatibility, lack of toxicity and antigenicity/immunogenicity, and solubility in water or common solvents.[1,2] The utilization of PEG in the fields of biological and biomedical science includes drug delivery systems,[3,4] formation of biocompatible surfaces,[5] affinity partitioning,[6] and protein modification[7]. However, most PEG derivatives are limited to homobifunctional polymers and polymers with one reactive terminal and one unreactive terminal (mono-functional methoxy-PEG). For more functionalized conjugates or crosslinkers containing PEG chains, it is essential to develop heterobifunctional PEG derivatives. There are two major methods to prepare the heterobifuctional PEG derivatives. One is polymerization of ethylene oxide by an anionic initiator, containing a masked functional group,[8-10] the other is direct chemical modification of commercially available PEG chain end groups.[11]

The present paper describes the synthesis of heterobifunctional PEG derivatives starting with commercially available PEG. The resulting new derivatives possess a polymerizable acryloyl group at one end and isocyanate group at the other end. The isocyanate group is known to show a high reactivity with a primary amino group and hydroxy group. In addition, the coupling of this new PEG derivative onto dextrans was also investigated.

Experimental

Materials.

Acryloyl chloride, trifluoroacetic acid, N-hydroxy succinimide, and triethylamine (TEA) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Dextran (produced by leuconostoc mesenteroides, strain No. B-512, average mol. wt. 37,500) was obtained from Sigma Chemical Co. (St Louis, MO, USA). 20 % phosgene solution in toluene was purchased from Fluka (Ronkonokoma, NY, USA). t-Boc-PEG-NH_2 (average mol. wt. 3400) was obtained from Shearwater Polymers Inc. (Huntsville, AL, USA). All solvents used in this experiment were anhydrous grade and used without further purification.

*Preparation of t-Boc-PEG-acryloyl (**1**).*

t-Boc-PEG-NH_2 (3 g, 0.88 mmol) was dissolved in dry 40 mL of methylene chloride. Under a dry argon flow, the solution was cooled to 4 °C in an ice-water bath and 0.31 mL of anhydrous TEA (2.2 mmol) was added to the stirring solution. The flask was wrapped with aluminum foil to protect from light. Then acryloyl chloride (0.18 mL, 2.2 mmol) was slowly added over a five minute period. The reaction mixture was stirred overnight. The solvent was evaporated to dryness and 50 mL of isopropanol-diethyl ether (1:1 vol. ratio) was added and then stirred for 15 minutes. The precipitated product was filtered and washed with fresh ether several times. The polymer was dried *in vacuo* overnight. Yield of product was 98 %.

*Deprotection of t-Boc group from (**1**).*

Two grams of the polymer (1) was dissolved in 20 mL of methylene chloride-trifluoroacetic acid (v/v, 1/1). The reaction mixture was stirred for 1 h at room temperature. The solution was concentrated, precipitated in 50 mL of cold diethyl ether and the product was collected by filtration and dried *in vacuo* overnight (yield = 95 %).

*Preparation of α-isocyanate-ω-acryloyl PEG (**3**).*

Two grams of deprotected PEG (**2**) and 0.82 mL of dry TEA were dissolved in dry chloroform and transferred to a dropping funnel. In a round-bottom flask, 3 mL of phosgene solution was added to 30 mL of dry methylene chloride. The polymer solution from dropping funnel was added dropwise over a 2 h period in an ice-water bath temperature. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated to dryness and 100 mL of toluene was added to dissolve the product. The undissolved salt was filtered and toluene was evaporated to dryness. One hundred mL of dry diethyl ether was added to the flask to precipitate the product. The final polymer was dried *in vacuo* at room temperature for 24 h. Yield of the product was 96 %.

*Coupling of α–isocyanate-ω–acryloyl PEG (**3**) onto dextran.*

Dextran was dried *in vacuo* for a few days at 70-80 °C in the presence of phosphorous pentoxide. The dry dextran (0.014 g, 0.088 mmol) was dissolved in 5 mL of dry DMSO at 40 °C under Ar. Dry TEA (0.001 mL, 0.0088 mmol) was added at room temperature and 0.3 g of α–isocyanate-ω–acryloyl PEG (**3**) was added to the reaction flask. After stirring for 24 h, the resulting product was precipitated using a large excess of cold diethyl ether. The product was collected by filtration and dried *in vacuo* at room temperature overnight. The polymers were further purified by reprecipitation using DMSO as solvent and diethyl ether as non-solvent several times, filtered, and dried *in vacuo* overnight.

Characterization.

^{1}H NMR spectra were recorded with a Bruker 300 MHz spectrometer. All of the chemical shifts were reported in parts per million (ppm) using tetramethylsilane as an internal standard. The sample tube size was 5 mm with a sample concentration of 10 mg/mL in DMSO-d_6. Matrix-assisted laser desorption/ionization mass spectra (MALDI MS) were measured on a PerSeptive Biosystems' Voyager linear time-of-flight instrument. 3,5-Dimethoxy-4-hydroxycinnamic acid (sinapinic acid, Aldrich) was used without further purification as the matrix for the analysis. Approximately 2 pmol sample was deposited on the plate.

Results and discussion

The synthesis of heterobifunctional PEG derivative containing an acryloyl group at one end and an isocynate group at the other end involved the following three steps: (1) preparation of t-Boc-PEG-acryloyl; (2) deprotection of t-Boc group from t-Boc-PEG-acryloyl; (3) preparation of α–isocyanate-ω–acryloyl PEG. In the first step, acryloyl chloride was readily reacted with the amine terminal group of t-Boc-PEG-NH_2 yielding a polymerizable unsaturated double bond at one end. This double bond allowed further chemical modifications such as radical addition of thiols. The ^{1}H NMR spectrum of t-Boc-PEG-acryloyl (**1**) is shown in Figure 1-A. The spectrum exhibited the characteristic peaks at $\delta = 5.55–5.59$ (dd, 1H, H_a,H_bC=CH_c-CO-); 6.04-6.1 (q, 1H, H_a,H_bC=CH_c-CO-); 6.20-6.29 (dd, 1H, H_a,H_bC=CH_c-CO-), which correspond to the double bond of acryloyl chloride. There is also a peak at $\delta = 3.51$ due to the methylene protons in the main backbone chain of the PEG residue and peaks at $\delta = 8.16$ which are assigned to the amide proton (-CO-NH-). The incorporation ratios of acryloyl groups were evaluated to be 94 % as determined from the integration ratios of the signals from double bond protons to the methylene protons of PEG in the ^{1}H NMR spectrum.

Deprotection of t-Boc group in the second step was carried out in trifluoroacetic acid/methylene chloride (v/v, 1/1) for 1 h at room temperature. Disappearance of the *tert*-butyl peak at 1.37 ppm of the ^{1}H NMR (Figure 1-B) spectrum showed the complete removal of the Boc group. The double bond of acryloyl residue at the end of

t-Boc-NH-(CH_2CH_2O)-$CH_2CH_2NH_2$

↓ Acryloyl chloride, TEA

t-Boc-NH-(CH_2CH_2O)-CH_2CH_2NH-CO-CH=CH_2

(**1**)

↓ Deprotection of t-Boc, CF_3COOH/MC

$CF_3COO^{\ominus}\ ^{\oplus}NH_3$-($CH_2CH_2O$)-$CH_2CH_2$NH-CO-CH=CH

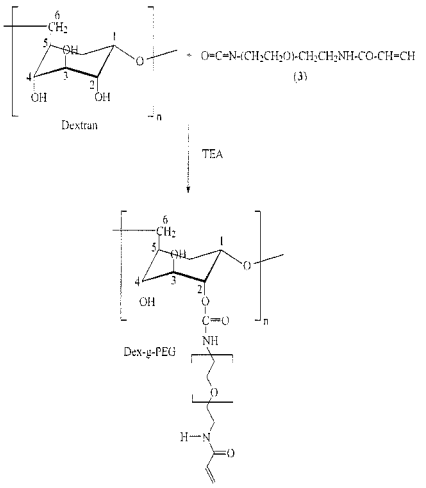
(**2**)

↓ TEA/Phosgene

O=C=N-(CH_2CH_2O)-CH_2CH_2NH-CO-CH=CH_2

(**3**)

Scheme 1. Synthesis of α–isocyanate-ω–acryloyl PEG



Scheme 2. Coupling of α–isocyanate-ω–acryloyl PEG onto dextran

polymer was intact during the deprotection reaction. It is well known that a primary amine group can be easily converted to an isocyanate group by phosgenation method.[12] Hence, phosgenation of the amine end group was carried out by dissolving it in an inert solvent (chloroform) before treatment with phosgene solution. As can be seen (Figure 1-C), the methylene protons next to isocyanate group overlapped with the backbone chain of PEG. In order to evaluate the degree of conversion of PEG amine to isocyanate group, α–isocyanate-ω–acryloyl PEG (3) was further reacted with N-hydroxysuccinimide (NHS) in the presence of triethylamine (TEA) as a base catalyst at room temperature overnight. The degree of substitution was evaluated to be 75 % by taking the ratio of integrated intensity of the NHS protons (δ = 2.80) to the integrated intensity of the PEG backbone protons (δ = 3.51).

Figure 2 shows the MALDI-TOF-MS spectra of t-Boc-PEG-NH_2 (Figure 2-A) and α–isocyanate-ω–acryloyl PEG (**3**) (Figure 2-B). As can be seen, the molecular weight of the final product, α–isocyanate-ω–acryloyl PEG (**3**) (M_n = 3230), approximates that of the starting PEG (M_n = 3110). This means no dimerization occurred during preparation as a result of reaction of PEG amine with the newly formed isocyanate.

The obtained heterobifunctional PEG can be utilized in biomedical applications. As an example, grafting of the α–isocyanate-ω–acryloyl PEG (**3**) onto dextran was carried out in the presence of triethylamine (TEA) as a base catalyst. In general, the reactions between isocyanates and polymers containing hydroxyl groups in the presence of catalyst are efficient methods for the formation of stable urethane linkages. Dextran has been used as a plasma expander and has been frequently chosen as drug carrier due to its biocompatibility, biodegradability, and water solubility characteristics. However, conjugation of dextran with hydrophobic drugs can result in water insolubility. It is expected that the incorporation of PEG side groups will enhance the solubility of the drug carrier. The ^{1}H NMR spectrum of the dextran-g-PEG (Figure 1-

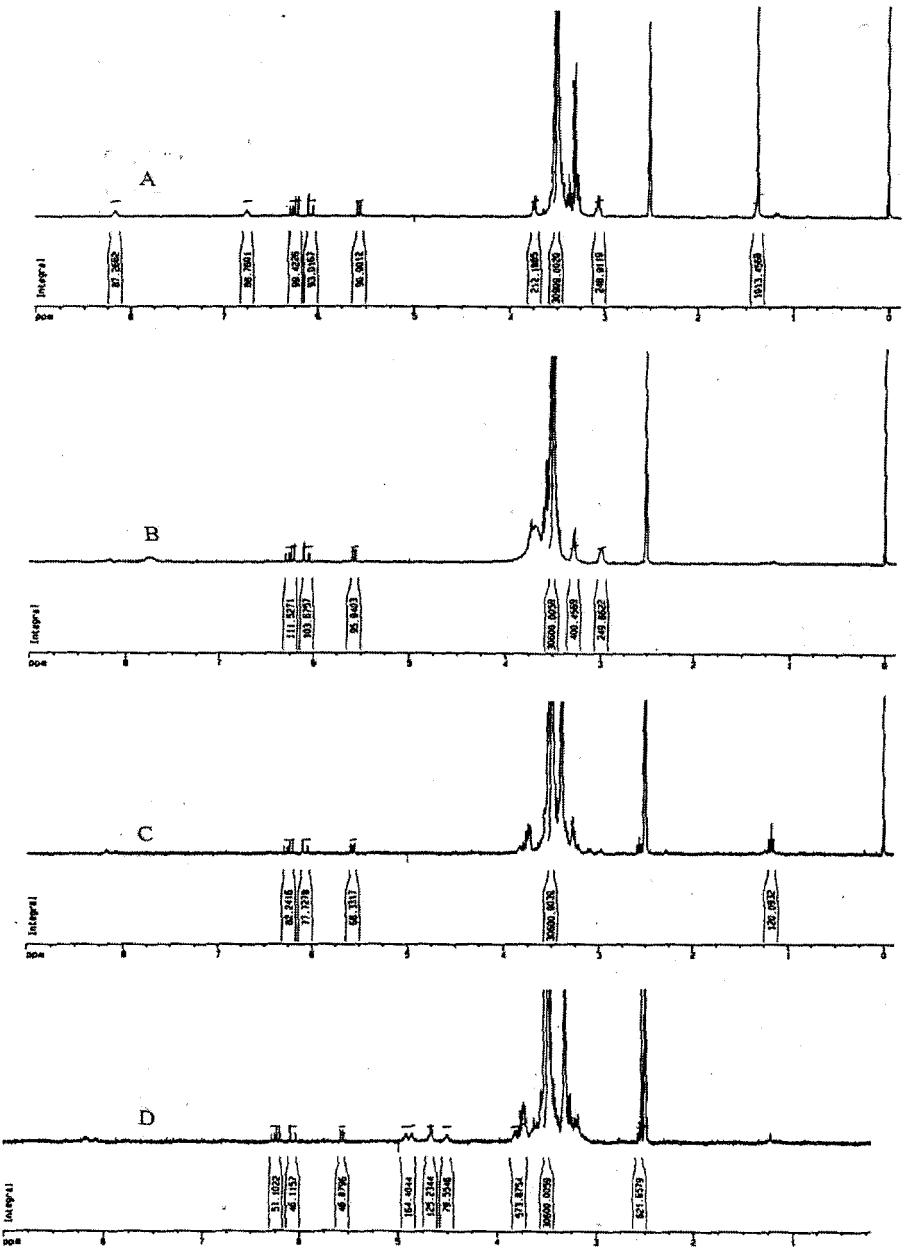


Figure 1. 1H NMR spectra of (A) t-Boc-PEG-acryloyl, (B) NH_2-PEG-acryloyl, (C) α–isocyanate-ω–acryloyl PEG, and (D) Dextran-g-PEG-acryloyl

D) shows a band at $\delta = 3.51$, which corresponds to the backbone chain of PEG, and multiplets in the region $\delta = 5.54$ to 6.29 due to the protons of double bond of the

pendant PEG. There are also several peaks between $\delta = 4.52$ and 4.93 which are due to anomeric protons linked to the dextran backbone carbon atoms.

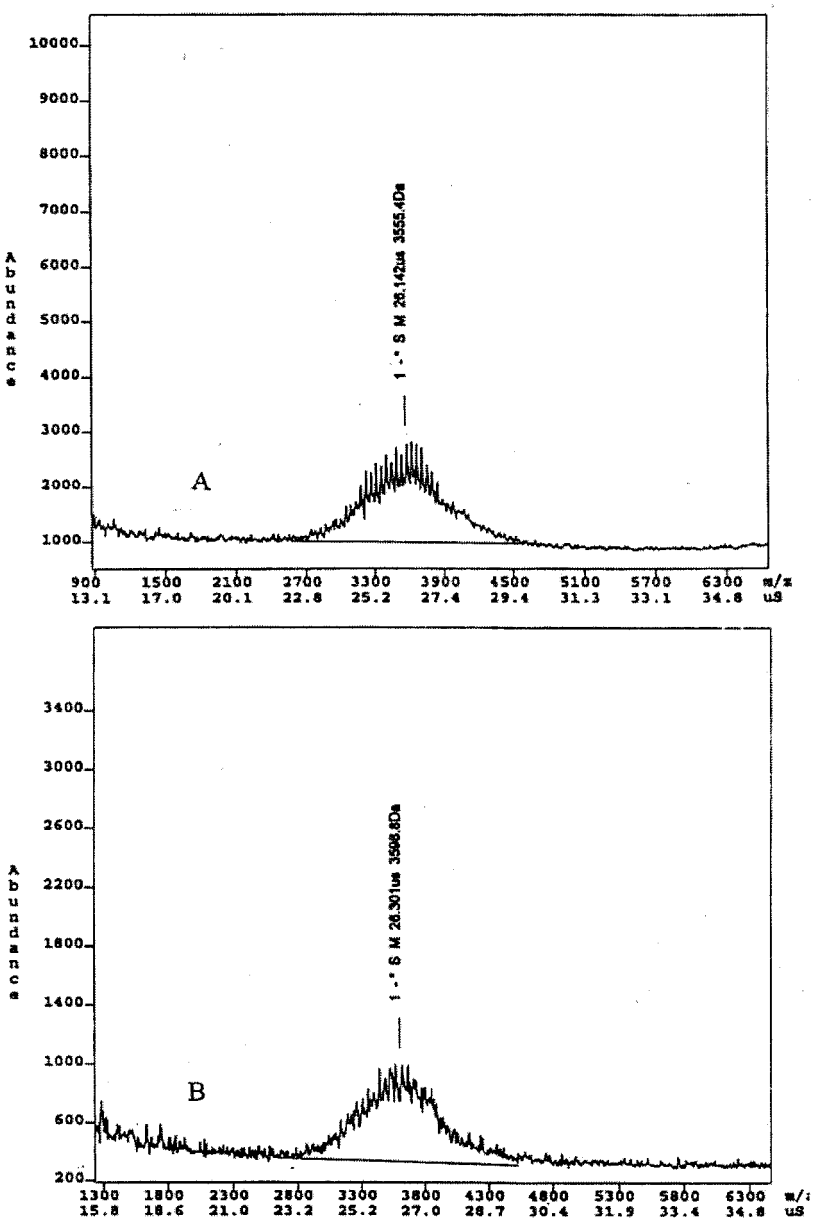


Figure 2. MALDI-TOF-MS spectra of (A) t-Boc-PEG-NH_2 and (B) α–isocyanate-ω–acryloyl PEG

The dextran molecule contains three primary hydroxyl groups per anhydroglucose (AHG) residue in the polymer chain. Each of these can react with the isocyanate of PEG in the same manner; however, their relative reactivity should vary considerably. Arranz et al.[14] reported that the relative reactivity of hydroxyl groups of dextran followed the order: $C_2>C_4>C_3$ based on the analysis of the ^{13}C NMR spectra of the ring carbons in the AHG units.

The degree of substitution (D.S.) was estimated from the ^1H NMR spectrum (Figure 1-D). In this study, the D.S. of 1.17 was obtained when a 3:1 molar ratio of α–isocyanate-ω–acryloyl PEG (**3**) to the hydroxyl group of dextran was used. If all of the three-hydroxyl groups per anhydroglucose unit of dextran were modified, D.S. would be 3.0.

The Dextran-g-PEG was soluble at room temperature in polar, aprotic solvents such as DMF, DMSO, DMAc, and NMP, whereas the unmodified dextran was only soluble in DMF/LiCl and DMSO/pyridine solvent systems at high temperature.

In conclusion, a well-defined α–isocyanate-ω–acryloyl PEG (**3**) was obtained by three steps starting with commercially available t-Boc-PEG-NH_2 in good yield. The incorporation ratios of acryloyl and isocyanate groups were evaluated to be 94, 75 %, respectively. Such heterobifunctional PEG possessing a polymerizable vinyl group at one end and a reactive isocyanate group at the other end has the potential to be used as a surface modifier, drug carrier, or crosslinking agent for hydrogels.

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