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ORIGINAL PAPER

Synthesis of heterobifunctional polyethylene glycols with azide functionality suitable for "click" chemistry

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Abstract A heterobifunctional polyethylene glycol (PEG) derivative possessing both "click" and electrophilic functionalities was prepared for use in bioconjugation applications. We utilized a dibenzyl-protected amine functional initiator to prepare high purity amino-PEG-alcohol by the polymerization of ethylene oxide. Subsequent chain-end modification of the heterobifunctional PEG afforded the desired N-hydroxy succinimidyl-PEG-azide derivative in 33% overall yield. This PEG derivative allows for versatile bioconjugation chemistry where activated ester chemistry and "click" chemistry can be selectively performed, an area of orthogonal bioconjugation that has not previously been accessible.

Keywords Heterobifunctional polyethylene glycol · Ethylene oxide polymerization · NHS-PEG-Azide · Bioconjugation

Abbreviations

PEG	Polyethylene glycol
NHS	N-hydroxy succinimidyl
SEC	Size exclusion chromatography
PDI	Polydispersity index
PAGE	Polyacrylamide gel electrophoresis

Introduction

Polyethylene glycol (PEG) is an inert, non-immuogenic polymer that is currently one of the polymers of choice for modifying the surface of biologically active molecules and nanoparticles [1–3]. PEG has been shown to enhance solubility, increase stability and prolong the circulation time for hydrophobic drugs, proteins, nucleic acids, liposomes and other polymer based delivery systems [4, 5]. In addition, PEG shields nanoparticles from serum proteins, preventing opsonization and subsequent detection from the immune system, thereby allowing for specific tumor targeting through the enhanced permeation and retention (EPR) effect [6–9]. In recent years, significant emphasis has been placed on identifying bioconjugation chemistries to attach two distinct biologically active moieties to a single PEG chain allowing, for example, the ability to join a targeting group and a therapeutic agent with a single PEG chain. As such, several diverse heterobifunctional PEG derivatives that allow for true orthogonal chemistries have been synthesized [10–14]. While some of these heterobifunctional PEG derivatives contain either azide [10] or succinimidyl ester functionalities [13], the synthesis of PEG polymers with both azide and succinimidyl ester functionalities has not been described.

"Click" chemistry, as coined by Sharpless and coworkers [15, 16], is an extremely versatile method for covalently linking molecular components. One of the most common click reaction is the Huisgen 1,3-dipolar cycloaddition between an azide and an alkyne [17]. It is possible to run this reaction using copper as a catalyst and this was first reported as a side reaction in 1984 by L'abbe [18], then later described by Meldal in 2002 [19]. The azide and alkyne functional groups are largely inert towards biological molecules and aqueous environments, which allows the use of the Huisgen 1,3-dipolar cycloaddition to yield triazoles that are also very stable, and almost impossible to oxidize or reduce [16, 20]. The copper(I)-catalyzed reaction, and its copper-free strained-alkyne variant, is mild and very

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efficient, can be performed in small volumes of aqueous solutions, and is robust to functional groups on peptides [21, 22]. As such, this reaction has allowed remarkable selectivity in conjugation reactions in biological samples and has been applied to oligonucleotides and proteins [22–26]. Furthermore, this reaction has been shown to be safe in vitro and in vivo, and has recently been safely applied to living organisms to create biomolecular probes for in vivo studies of live mice [27].

In this paper we describe the synthesis and characterization of heterobifunctional PEG containing both azide and N-hydroxy succinimidyl (NHS) moieties. The use of both "clickable" and electrophilic functional groups allows for a truly orthogonal synthetic pathway to heterobifunctional PEG derivatives. To this end, a highly pure azido-PEG-NHS ester was prepared in eight steps with an overall yield of 33%. The synthesis and use of dibenzyl amino ethanol, as a new protected initiator of ethylene oxide to give amine functionality, is also described.

Experimental section

Materials

Difluoroacetic and trifluoroacetic acid was obtained from Synquest (Alachua, FL) and distilled prior to use. Tetrahydrofuran (THF, pre-purified, water content <50 ppm), hexanes, molecular sieves (3 Å) and methanol (Technical Grade) were purchased from Fisher Scientific. Ethylene oxide was purchased from Balchem. $Pd(OH)_2$ was purchased from Acros. Argon (5.0 Ultra high purity, Praxair) was used as the inert gas. Dialysis membrane tubing Spectra/Por® Float-A-Lyzer (MWCO 1,000 and 3,500) was obtained from Spectrum Laboratories, Inc. All other reagents were purchased from Sigma-Aldrich Co. and were used as received unless stated otherwise. Naphthalene was purchased from Aldrich, recrystallized from ethanol and then sublimed prior to use. THF and hexanes were passed through molecular sieves before use. Methanesulfonyl chloride was distilled prior to use. Click-iT® Alexa Fluor® 488 DIBO alkyne and Novex 10–20% Tricine gels were purchased from Invitrogen (Carlsbad, CA).

Polymer analysis

Gel permeation chromatography (GPC) measurements were carried out using a Waters 515 isocratic pump connected in series to a PSS GRAM columns bank [Polymer Standard Service, one analytical Guard Column (8×50 mm, 10 μM) and two analytical columns (8×300 mm, 10 μM, 100 Å pore size)] and a Wyatt rEX refractive index detector. A 50/50 *v/v* DMF/THF mixture was used as the eluent at a flow rate of 1 mL/min at 50 °C. Molecular weight calibration was done using a series of standard PEGs (1.9, 3.3, 4.6, 8.8 and 11.9 kg/mol), and toluene was used as an internal standard (appears in chromatogram as a monodisperse peak at around 27 min). The ^1H and ^{13}C NMR spectra were measured with a VARIAN VNRMS 400 MHz spectrometer, and chemical shifts are reported in parts per million using DMSO d6 as an internal standard. MALDI-TOF MS was performed on a Bruker Autoflex II series in linear mode. PEG samples were dissolved in 50:50 acetonitrile: H_2O: 0.1% THF and combined with a saturated solution of ferrulic acid (FA) in acetonitrile/THF solution and cesium chloride (0.1 M) in acetonitrile THF. Sample:FA:salt ratio was 10:10:1 μL, with 0.5 μL of the resulting solution was spotted onto the plate. A total of 500 acquisitions were used to acquire the signal. High resolution mass spectrometry (HRMS) was done using a 6540 Q-TOF LC/MS System and data was processed using Agilent MassHunter software.

Synthesis of 2-(dibenzylamino)ethanol (1)

Benzyl chloride (278.5 g, 2.2 mol), ethanol amine (60 mL, 1 mol), potassium carbonate (283.1 g, 2.05 mol) and ethanol (2 L) were mixed together in a 3 L 3-neck flask, fitted with an overhead stirrer, a condenser and a glass plug. The solution was heated to reflux for 36 h, followed by filtration to remove accumulated solids. The filtrate was recovered and ethanol was removed by evaporation under reduced pressure. The viscous liquid was re-dissolved in ether, again filtered to remove salts, then washed twice with water. The ether solution was retained and the aqueous layer was washed twice with dichloromethane (2×400 mL). The organic fractions were combined, dried over $MgSO_4$, stirred over carbon black for 15 min and filtered through a celite pad. The solvent was evaporated under reduced pressure and the solid was re-dissolved into a minimal amount of ether (300 mL). Hexanes (1,700 mL) was added and the solution was heated gently until complete dissolution of the product. The solution was slowly cooled, placed at 4 °C overnight yielding colorless crystals. The re-crystallization was repeated a second time and the product was recovered by filtration giving 166.63 g (69% yield) of colorless crystals. ^1H NMR (d_6-DMSO) δ 7.39–7.24 (10H), 4.42 (1H), 3.60 (4H), 3.52 (2H), 2.52 (2H). ^{13}C NMR ($CDCl_3$) δ 138.93, 129.08, 128.53, 127.32, 58.77, 58.35, 54.95. HRMS: calculated for $C_{16}H_{19}NO$ (MH)+, 242.1544; found 242.1541

Synthesis of Dibenzylamino-PEG-OH (2)

2-(dibenzylamino)ethanol **1** (3.71 g, 15.4 mmol) and dry THF (1.2 L) were introduced into a round bottom flask under an argon overpressure. The alcohol **1** was deprotonated with

the addition of potassium naphthalenide (0.2 M solution into THF, 0.9 equivalent). The flask was then cooled to 10 °C and ethylene oxide (184 mL, 4 mol) was condensed under vacuum at −30 °C into a jacketed addition funnel. Once the appropriate amount of ethylene oxide was condensed, the liquid ethylene oxide was added directly to the cooled alkoxide solution. After complete ethylene oxide addition, the reaction flask was backfilled with argon. While stirring, the following temperature ramp was applied to the reaction: 12 h at 20 °C, 1 h from 20 °C to 40 °C and 3 days at 40 °C. The reaction was terminated by the addition of excess methanol. The solution was concentrated by rotary evaporation and used without further purification. ^{1}H NMR (d_6-DMSO) δ 7.4–7.2 (10 H), 4.55 (1 H), 3.83–3.21 (1,040 H) ppm. GPC M_n = 12.3 kg/mol, Polydispersity index (PDI) = 1.04.

Synthesis of H_2N-PEG-OH (3)

Dibenzylamino-PEG-OH **2** (186 g, 14.7 mmol), $Pd(OH)_2/C$ (32 g, 45.6 mmol), ammonium formate (80 g, 1.27 mol) and ethanol (1.2 L) were combined in a 2 L flask. The reaction was heated to 80 °C and stirred overnight. The reaction was cooled to room temperature and filtered through a bed of Isolute HM sorbent (Biotage), concentrated by rotary evaporation, whereupon the product precipitated, was re-dissolved into 800 mL of a 50/50 brine/saturated potassium carbonate mixture and extracted three times with dichloromethane (3 × 700 mL). The organic fractions were combined, dried over $MgSO_4$, filtered, concentrated to 800 mL by rotary evaporation and used as-is for the next step. ^{1} H NMR (d_6-DMSO) 4.55 (1 H), 3.83–3.21 (1,040 H), 2.96 (2 H) ppm. (Conversion is assumed to be quantitative as no starting material or aromatic resonances are present in the ^{1} H NMR spectrum.)

Synthesis of BOC-NH-PEG-OH (4)

Di-*tert*-butyl dicarbonate (32 g, 0.147 mol) was added to the dichloromethane solution of H_2N-PEG-OH **3** (176 g, 14.7 mmol) and allowed to stir at room temperature overnight. The solution was concentrated by rotary evaporation, precipitated, re-dissolved into 600 mL of water and washed three times with 500 mL of ether. The water was then extracted three times with 800 mL of dichloromethane. The organic fractions were combined, dried over $MgSO_4$ filtered, concentrated to ~600 mL and poured into 7 L of ether, whereupon the product precipitated. The white powder was filtered and dried overnight in a vacuum oven. ^{1}H NMR (d_6-DMSO) δ 6.75 (1 H), 4.55 (1 H), 3.83–3.21 (1,040 H), 3.06 (2 H), 1.37 (9 H) ppm.

Synthesis of BOC-NH-PEG-Mesyl (5)

BOC-NH-PEG-OH **4** (~160 g, 13.3 mmol) was dried by azeotropic distillation from toluene (~500 mL) and dissolved into 600 mL of dry dichloromethane under inert atmosphere. The solution was cooled to 0 °C, then methanesulfonyl chloride (3.48 mL, 45 mmol) was added followed by triethylamine (4.18 mL, 30 mmol). The reaction was allowed to warm up to room temperature and stirred overnight under inert atmosphere. The solution was evaporated to dryness by rotary evaporation and used as-is for the next step. ^{1}H NMR (d_6-DMSO) δ 6.75 (1 H), 4.36 (2 H), 3.83–3.21 (1,040 H), 3.06 (2 H), 1.37 (9 H) ppm.

Synthesis of Boc-NH-PEG-N_3 (6)

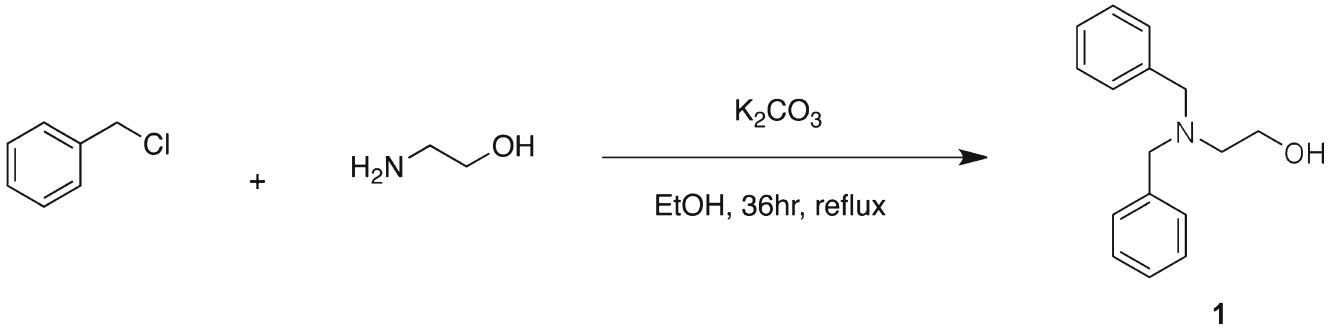
BOC-NH-PEG-Mesyl **5** (~124 g, 10.4 mmol) and NaN_3 (6.76 g, 104 mmol) were dissolved in 800 mL of ethanol in a 2 L round-bottom flask. The solution was heated to 80° C and allowed to stir overnight. The solution was then evaporated to dryness by rotary evaporation and dissolved in 250 mL of dichloromethane. The product was purified by silica gel flash column chromatography using CH_2Cl_2/MeOH as the eluent. The elution profile was the following: 3:97 MeOH/CH_2Cl_2 for 1 column volume (CV), 10:90 MeOH/CH_2Cl_2 for 3 CV and 15:85 MeOH/CH_2Cl_2 for 4 CV. One CV was used as a transition between different solvent compositions. The polymer-containing fractions were combined, concentrated by rotary evaporation and precipitated into a 10-fold excess of diethyl ether. The polymer was recovered by filtration and dried *in vacuo* giving 105 g (8.75 mmol, 60% overall yield over 5 steps) of white powder. ^{1}H NMR (d_6-DMSO) δ 6.75 (1 H), 3.83–3.21 (1,040 H), 3.06 (2 H), 1.37 (9 H) ppm.

Synthesis of H_2N-PEG-N_3 (7)

Boc-NH-PEG-N_3 **6** (30 g, 2.5 mmol) was dissolved in dichloromethane (100 mL) and trifluoracetic acid (100 mL). The reaction was stirred at room temperature for 2 h and the polymer was then precipitated in diethyl ether (1.5 L). The product was recovered by filtration and was re-dissolved in 200 mL of a 50/50 brine/saturated potassium carbonate mixture. The aqueous solution was extracted three times with dichloromethane (3 × 500 mL). The organic fractions were combined, dried over $MgSO_4$, filtered, concentrated to ~200 mL by rotary evaporation then precipitated into diethyl ether (1.5 L). The product was recovered by filtration and dried *in vacuo* to give 24.5 g (2.04 mmol, 82% yield) of a white powder. ^{1}H NMR (d_6-DMSO) δ 4.25 (2 H), 3.83–3.21 (1,040 H) ppm.

Synthesis of succinic-NH-PEG-N_3 (8)

H_2N-PEG-N_3 **7** (10 g, 0.83 mmol) and succinic anhydride (0.83 g, 8.3 mmol) were dissolved in a saturated solution of K_2CO_3 in water (100 mL) and stirred for 16 h. This solution



Scheme 1 Synthesis of 2-(dibenzylamino)ethanol 1

was extracted three times with dichloromethane (3× 200 mL). The organic fractions were combined, dried over $MgSO_4$, filtered, concentrated to ~100 mL by rotary evaporation then precipitated into diethyl ether (500 mL). The product was recovered by filtration and dried *in vacuo* to give 8.77 g (0.73 mmol, 88% yield) of a white powder. ^{1}H NMR (d_6-DMSO) δ 7.15 (3 H), 3.68 (2 H), 3.60 (2 H), 3.83–3.21 (1,040 H) ppm.

Synthesis of succinic-succinimidyl-NH-PEG-N_3 (9)

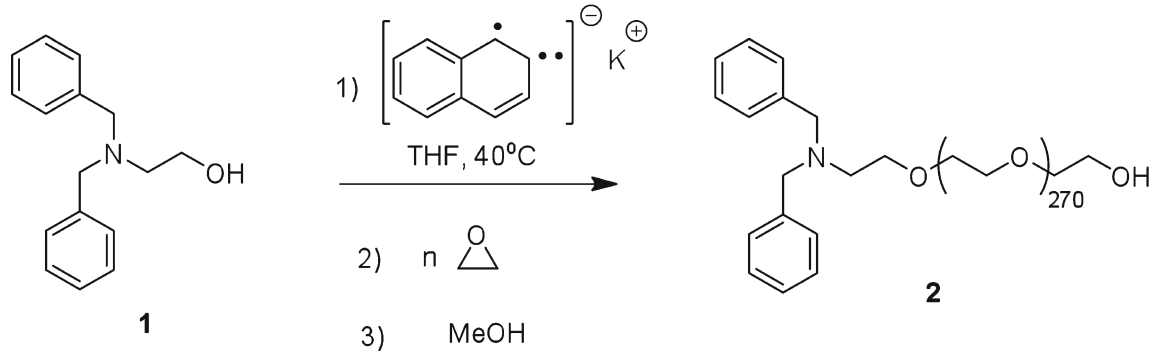
Succinimidyl-NH-PEG-N_3 **8** (2 g, 0.17 mmol) was dried by azeotropic distillation from toluene (~30 mL) and dissolved into 25 mL of dry dichloromethane under inert atmosphere. N-hydroxy succinimide (96 mg, 0.84 mmol) and carbodimide resin (625 mg, 0.83 mequiv, Biotage) were added and the resulting solution stirred for 16 h at room temperature. The resulting solution was filtered, with the filtrate precipitated into diethyl ether (100 mL). The precipitate was recovered by filtration under an argon blanket to reduce atmospheric water condensation. The solid was redissolved in dry dichloromethane and stirred over isocyanate resin (0.5 g, Biotage). The solution was filtered to remove the resin then precipitated into diethyl ether (100 mL). The product was recovered by filtration under argon and dried *in vacuo* to give 1.54 g (0.13 mmol, 77% yield) of a white powder. ^{1}H NMR (d_6-DMSO) δ 3.68 (2 H), 3.60 (2 H), 3.83–3.21 (1,040 H), 2.83 (4 H) ppm. GPC M_n= 12.6 kg/mol, PDI=1.03.

End group analysis of succinic-succinimidyl-NH-PEG-N_3 (9)

To verify end group functionality, a 50 μL solution of Succinic-Succinimidyl-NH-PEG-N_3 **9** (60 mg/mL in H_2O) was added to a 5 μL solution of holo-transferrin (10 mg/mL in H_2O, Sigma) and incubated for 3 h at room temperature. Two and a half μL of 0.5 mg/mL Click-iT® Alexa Fluor® 488 DIBO alkyne was then added to the solution, the solution diluted with dH_2O to a final volume of 250 μL, mixed and incubated for an additional 60 min. Experimental and control samples (12.5 μL of each) were electrophoresed on Novex 10–20% Tricine gels. Gels were visualized for Alexa Fluor® 488 fluorescence on a Kodak Imager using the UV excitation and a 535 nm emission filter.

Results and discussion

In order to achieve the highest possible polymer end-group purity, heterobifunctional PEG was prepared by the polymerization of ethylene oxide. While a protected amine functionalized initiator has been reported in the literature by Kataoka [28], we chose to utilize 2-(dibenzylamino) ethanol **1** for use as our functional initiator. This benzyl-



Scheme 2 Synthesis of Dibenzylamino PEG-OH 2

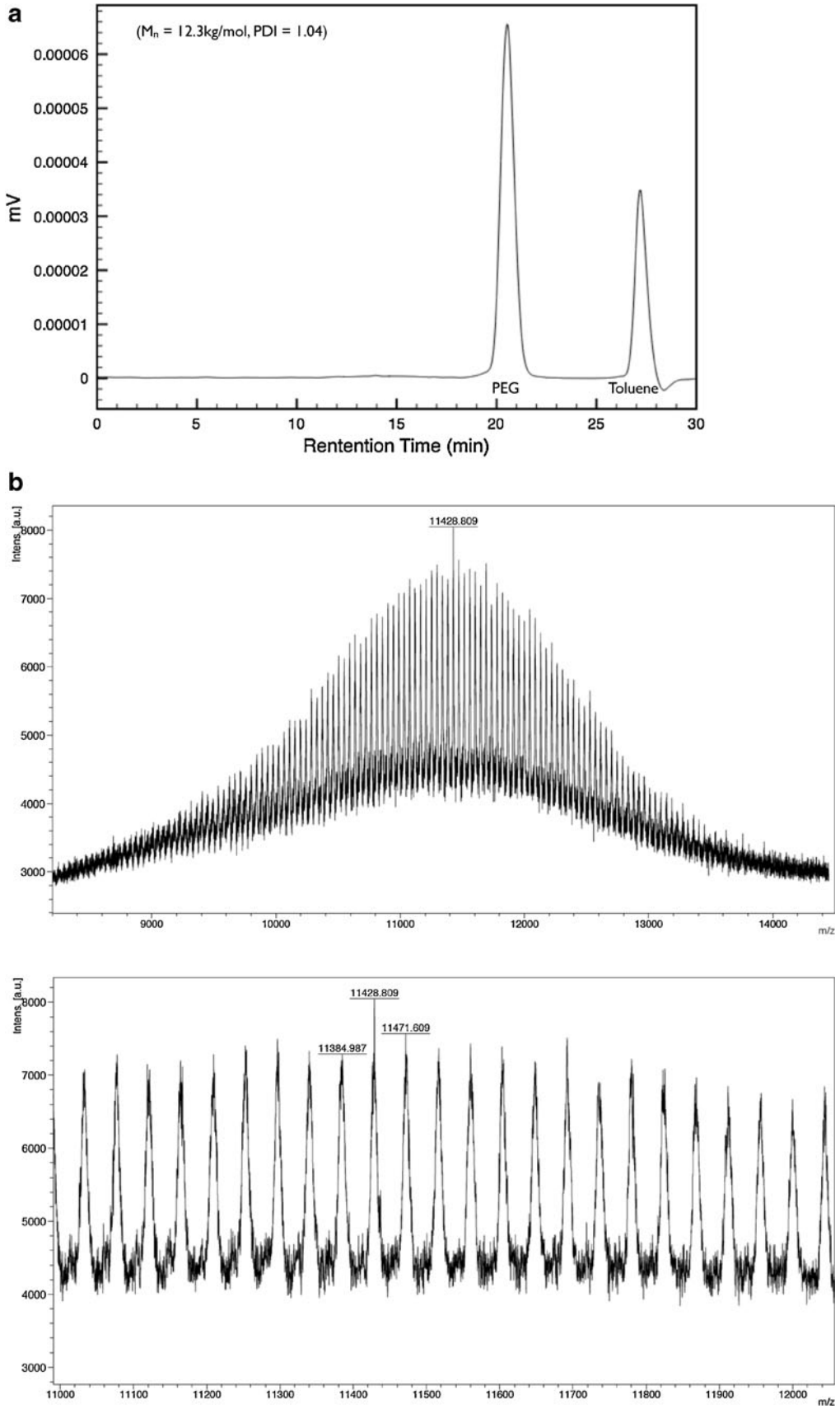
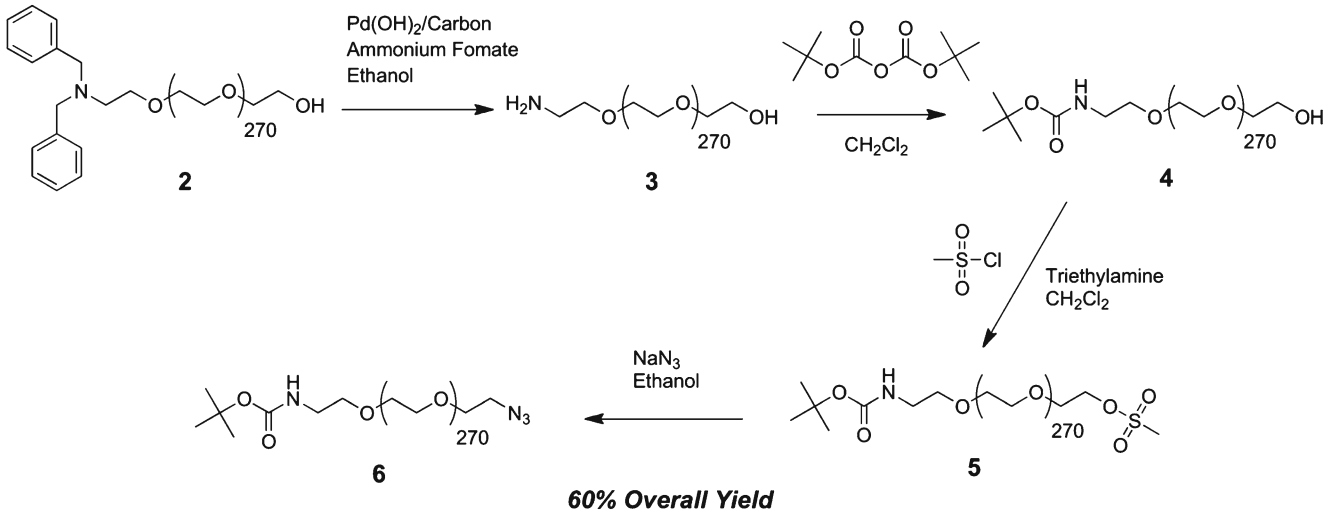


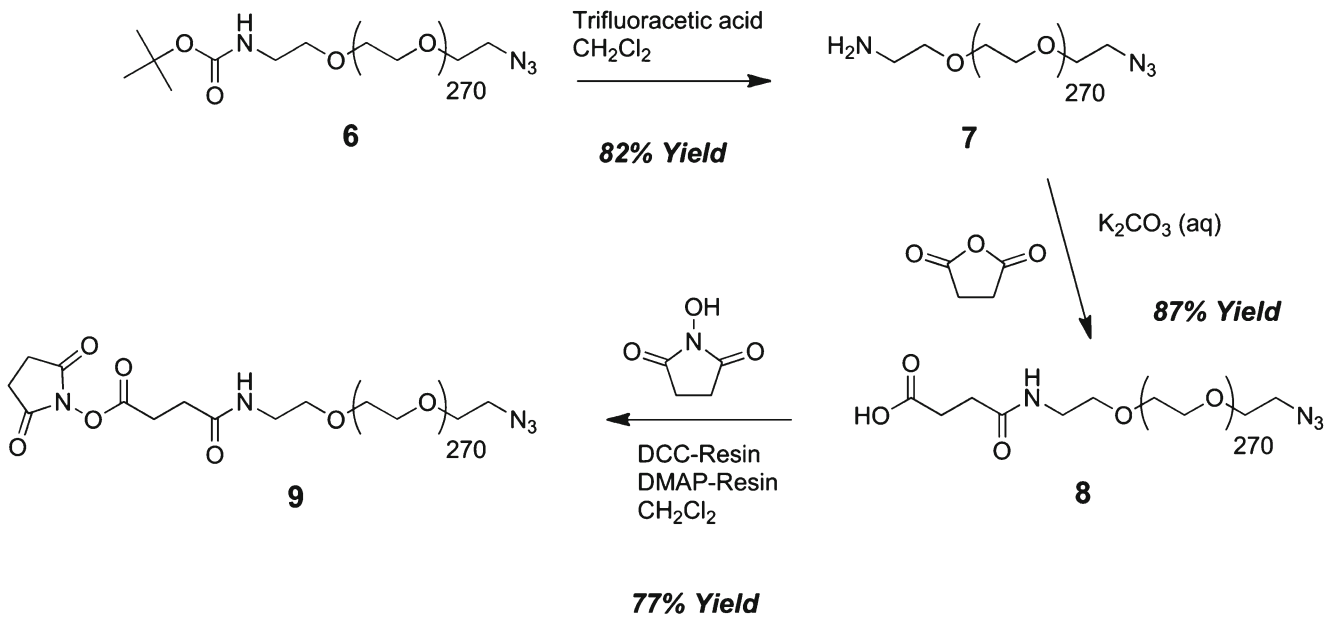
Fig. 1 SEC analysis (**a**) and MALDI-TOF spectrum (**b**) of Dibenzylamino PEG-OH 2



Scheme 3 Synthesis of BOC-amine-PEG-N3 6

protected amine initiator is synthesized from aminoethanol and benzyl chloride and can be readily prepared on the hundreds of gram scale and is sufficiently pure for use as a polymerization initiator following purification by crystallization from ethanol [29]. The peak molecular weight from high resolution mass spectrometry (HRMS) was 242.1541, which corresponds well with the targeted MW of 242.1544. Furthermore, the benzyl protecting group is a robust functional group which translates to long term storage as well as minimal, if any, side product generation during the deprotonation and polymerization reaction. Indeed, this molecule can be used as an initiator to ultimately produce various amine-terminated PEG polymers of discrete sizes [30] (Scheme 1).

The polymerization of ethylene oxide was completed by first deprotonating the alcohol **1** with potassium naphthalenide in THF as shown in Scheme 2. This alkoxide solution was then cooled to 10 °C followed by condensation of ethylene oxide in a jacketed addition funnel at −30 °C. Once the appropriate volume of ethylene oxide was collected, the liquid was added directly to the alkoxide solution. The temperature of the reaction solution was raised to 20 °C over 4 h, then raised to 40 °C over 20 h, then held at 40 °C for an additional 48 h. PEG derivative **2** was recovered following precipitation into ether. This intermediate was then characterized by ^{1}H NMR and by size exclusion chromatography (SEC) in DMF/THF to determine the purity of the material. Figure 1a shows the narrow, monomodal peak



Scheme 4 Preparation of N3-PEG12k-NHS 9

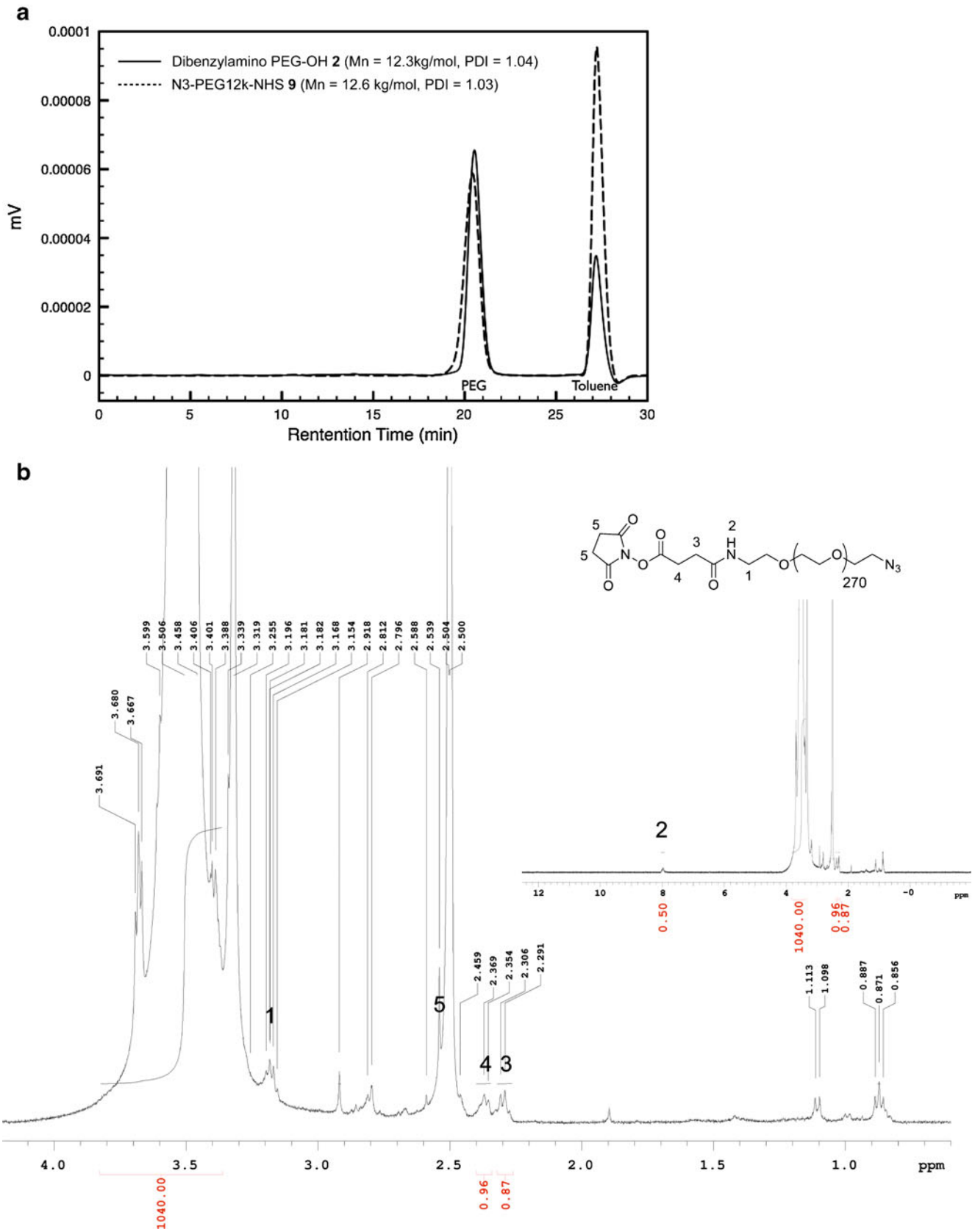


Fig. 2 SEC comparison of Dibenzylamino PEG-OH 2 and N3-PEG12k-NHS 9 (**a**). ^{1}H NMR analysis of N3-PEG12k-NHS 9 (**b**)

from the SEC analysis of PEG **2**. The absence of a dimer peak leads us to believe that little, if any, initiation from water has occurred, and is indicative of a highly pure polymer material. The peak molecular weight from matrix assisted laser desorption ionization mass spectrometry (MALDI-MS, Fig. 1b) corresponded with degree of polymerization (DP) of n=251, which corresponds well with the targeted DP of n=270. In addition, the MALDI spectra (Fig. 1b) showed an m/z peak spacing of ~44 corresponding to the mass of the repeat unit on the PEG. All peaks displayed in this spectrum are consistent with n repeating units of ethylene oxide (44.05 n) plus the mass of a cesium ion (Cs, M=132.91), and two end groups dibenzylamine ($C_{14}H_{14}N$, M=196.27 and ethanol C_4H_5O, M=45.06). For example, the mass of such a polymer chain containing 251 repeat units is $44.05\times251+132.91+196.27+45.06=11430.79$ which matches well with peak MW at 11428.809 shown in Fig. 1b.

Once dibenzyl amino-PEG-alcohol **2** had been prepared in high purity and yield, the terminal functional groups were manipulated to give the desired functionality. (Scheme 3) First, the dibenzyl groups of **2** were removed by hydrogenation to give the amino-PEG-alcohol derivative **3**. Benzyl protecting groups were then replaced by the acid-labile BOC protecting group following treatment of **3** with di-*tert*-butyldicarbonate to afford the BOC amine-PEG-alcohol **4**. The alcohol was then converted to the mesyl derivative **5** followed by nucleophilic attack of the mesyl

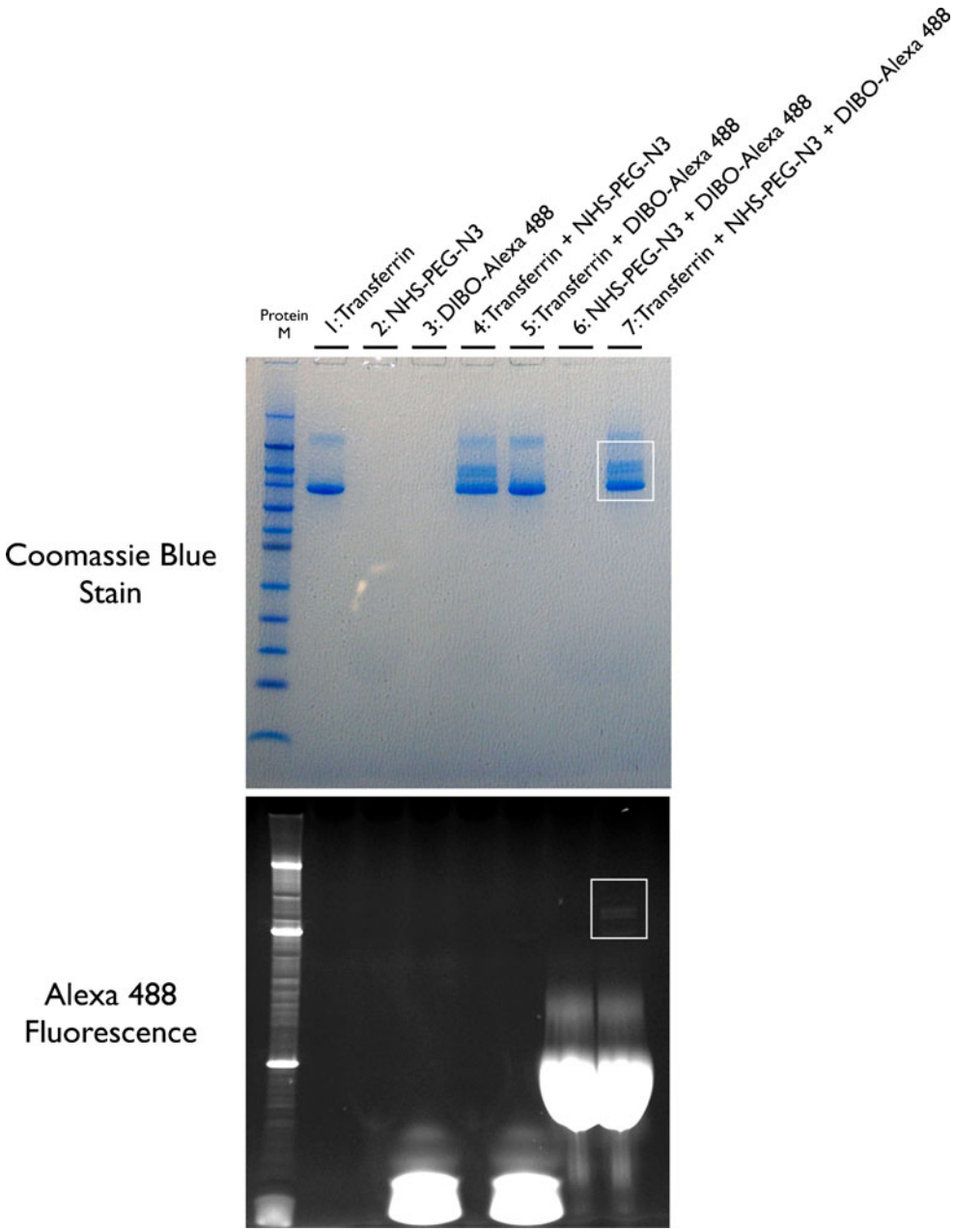


Fig. 3 Confirmation of end group functionality of N3-PEG12k-NHS 9

leaving group with NaN_3 to give the desired Boc-amine-PEG-Azide derivative **6**. While multiple transformations were necessary to achieve the desired product, purification was achieved by two extractions, one silica gel filtration, and three precipitations over the five steps. Furthermore, this reaction strategy is very amenable to scale up, as these reactions have been performed on the kilogram scale in our laboratory. Characterization of BOC-amine-PEG-Azide **6** by ^{1}H NMR confirmed the chemical functionality of the PEG derivative.

The BOC-amine-PEG-Azide **6** is an important, versatile intermediate for bioconjugation. The BOC protecting group or the azide can be independently and selectively converted to an amine for additional modification, or the azide can be utilized in a "click" cycloaddition reaction with an alkyne. In this instance, we chose to remove the BOC protecting group for the subsequent addition of an electrophilic moiety, as shown in Scheme 4. The BOC group of **6** was removed with trifluoroacetic acid in dichloromethane to give the amino-PEG-azide derivative **7**. The amine of **7** was utilized in the nucleophilic ring opening of succinic anhydride yielding the carboxylic acid-PEG-azide derivative **8**. The succinyl ester linkage was chosen for both its ease of synthesis of and reactivity of the NHS electrophilic moiety in aqueous solutions [31]. Finally, N-hydroxysuccinimide was coupled to the PEG derivative *via* carbodiimide chemistry to give the NHS-ester-PEG azide derivative **9**. As confirmed by SEC (Fig. 2a), the low polydispersity is maintained upon NHS functionalization of the PEG end group. Functionalization is also confirmed by ^{1}H NMR Fig. 2b.

Further confirmation of end-group functionality was achieved by reacting the heterobifunctional PEG with an 80 kDa transferrin protein (reaction with NHS) and an alkyne group-containing dye (DIBO-Alexa488, reaction with azide). Heterobifunctional PEG was initially incubated with the transferrin protein, then reacted with Click-iT® Alexa Fluor® 488 DIBO alkyne and subsequently analyzed by polyacrylamide gel electrophoresis (PAGE) Fig. 3. PAGE analysis of controls and experimental samples showed that indeed both azide and NHS groups were functional. Compared to transferrin control (Figure 4, Coomassie Blue stain, Lane 1), transferrin incubated with PEG produced additional bands above 80 kDa (Figure 4, Coomassie Blue stain, Lanes 4 and 7). Incubation with Click-iT® Alexa Fluor® 488 DIBO alkyne indicated that these additional bands corresponded to PEGylated transferrin (Figure 4, Alexa Fluor® 488 Fluorescence, Lane 7, white box).

We have recently used this heterobifunctional azido-PEG-NHS ester derivative for bioconjugation reactions onto Polyplex gene delivery nanoparticles [32]. Using a two-step method, Polyplex nanoparticles are made by initially complexing DNA with a Poly(D/L Asp-DET) cationic polymer. Subsequently, these Polyplexes are further modified by reacting the NHS ester groups on our heterobifunctional PEG derivate with deprotonated primary amines present on Poly(D/L Asp-DET) polymers to produce stable amide bonds [29]. This covalent attachment of PEG to the exterior of Polyplexes produces colloidally stable PEG-Polyplex nanoparticles that are below 100 nm in size and appear as spherical and rod structures, similar to Polyplexes. PEG-Polyplex nanoparticles are safe, extremely stable, resistant to salt and serum-induced aggregation and have more biocompatible characteristics [32]. Finally, the presence of functionalized azide groups on one end of the PEG chains allows additional surface modification of PEG-Polyplexes via "click" chemistry [21, 22]. As such, this heterobifunctional azido-PEG-NHS ester derivative is an ideal polymer candidate for use in the area of bioconjugation.

Conclusions

In summary, we have synthesized a new, highly pure heterobifunctional azido-PEG-NHS ester derivative, and have also described the synthesis of a new initiator for amine functionality. This PEG derivative will allow for the covalent attachment of PEG to primary amines of biologically active molecules for applications in nanomedicine. Since PEG is an inert, non-immunogenic polymer that offers both steric and colloidal stability, coating nanoparticles with this PEG derivative will help prevent opsonization from blood serum proteins and detection from immune system. Furthermore, the introduction of an azide group will also allow for the specific attachment of other moieties, such as targeting groups through highly biocompatible "click" chemistry, which will give nanoparticles tumor specific targeting properties.

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