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Short communication

Synthesis of heterobifunctional polyethylene glycols: Polymerization from functional initiators

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ABSTRACT

The large-scale synthesis of heterobifunctional polyethylene glycols (PEGs) with tightly controlled molecular weights ranging from 2 to 20 kDa is described. PEGs were prepared by the ring-opening polymerization of ethylene oxide (EO) initiated from a protected amine (*N*-dibenzyl), and a protected alcohol (*O*-trityl) initiator. Additionally, EO polymerizations from initiators bearing azide and alkyne, both prototypical "click" chemistry functionalities, are outlined. This demonstrates the first successful use of an initiator bearing either an azide or strained cycloalkyne functionality in an ethylene oxide polymerization. The key to the methodology proved to be the selection of base. The use of potassium naphthalenide or potassium hydride in paraffin wax was highly dependent on the initiator used.

1. Introduction

Polyethylene glycol (PEG) and its derivatives are biocompatible polymers that have found utility in medicine, cosmetics, and food additives [1]. Conjugation of biomolecules with PEG derivatives (PEGylation) can increase solubility, stability, and circulation half-life [2]. PEG derivatives have found considerable success in both liposome and micelle-based drug delivery systems [3]. Surface modification with PEG has been shown to insulate nanoparticles from serum proteins, thus restricting opsonization and consequential detection by the immune system, allowing for tumor targeting via the enhanced permeability and retention (EPR) effect [4]. The application of PEGs is dependent on the preparation of high quality polymers and the incorporation of the appropriate functional groups on the termini that facilitate their attachment to biomolecules. PEGs are commonly prepared by ring-opening polymerization (ROP) of ethylene oxide (EO) from a suitable initiator [5]. Functionalized PEGs arise from either the initiator used or through post-polymerization modification. As part of our own drug delivery system development, we required kilogram quantities of a heterobifunctional 12 kDa PEG capped with a methoxy group and a free amine (mPEG12K–NH_2). This hemitelechelic polymer serves as an initiator for a second set of polymerizations with a mixture of D and L *N*-carboxyanhydrides (NCA) to form triblock polymers whose formulations with daunorubicin [6], SN-38 (the active metabolite of irinotecan) [7], and epothilone D [8] extend circulation time, decrease toxicity, and improve efficacy over the free drugs.

A common method to incorporate amines into PEGs is through the post-polymerization route in which a terminal hydroxyl is converted to a sulfonyl intermediate, which is then displaced with an azide salt followed by a reduction step to produce the amine [9]. Alternatively, PEG amines have been made by a Mitsunobu-type reaction between a PEG hydroxyl and phthalimide, followed by treatment with hydrazine [10]. Any PEG amine produced via a hydroxyl can contain undesired contaminants; either unreacted starting materials, intermediates, or side reaction products. Furthermore, commercially available PEG amines are often prepared from stock PEGs that are functionalized *ad hoc*. This can lead to another possible contaminant: a diamine homofunctionalized PEG which would be especially problematic for the synthesis of our triblock polymers. Separation, and ultimately purification, of a mixture of polymer chains differing by only a single end group moiety has been a challenge for several decades [11]. The inherent difficulty in analyzing subtle functional group changes in the terminus of PEG macromolecules makes it arduous to quantify, let alone identify, any contaminates in a PEG amine sample. Thus, in order to reduce these issues we sought to prepare mPEG12K–NH_2 from an ethylene oxide polymerization with an amine containing initiator. Our hope was this strategy would provide us with the highest quality PEG amine for our subsequent NCA polymerizations. In addition to PEG amines, we also wanted to pursue EO polymerizations from initiators bearing "click" chemistry

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functionalities [12]. PEGs containing either alkyne or azide functionalities are highly useful polymers, having found extensive utility in bioconjugation research [13]. PEGs prepared from initiations containing alkynes or azides would reduce the need for extensive post-polymerization reactions to install such functionalities, helping to expand this area of research.

2. Experimental section

2.1. Materials and methods

Ethylene oxide (EO) was obtained from Balchem via Praxair; *CAUTION – ethylene oxide is extremely flammable and highly toxic. All the necessary precautions and safeguards should be in place before any operations begin. Failure to follow the required safety measures can result in fire, explosion, and physical harm. Please consult the EO Product Stewardship Manual* [14] *for details prior to starting any use*. Anhydrous tetrahydrofuran (THF) used for all polymerizations was purchased from Fisher (OptiDry®, FisherPak®, ≤50 ppm H_2O) and passed through an Mbraun MB-SPS encapsulated solvent purification system (dried to ≤10 ppm H_2O as measured by Karl Fischer titration) directly into the reaction vessel. Anhydrous THF for all other uses was purchased from Sigma Aldrich. Heptane, hexanes, cyclohexane, ethyl acetate, dimethyl sulfoxide (DMSO), and acetonitrile were purchased from Fisher Scientific. Absolute ethanol was obtained from Decon Laboratories. Naphthalene was purchased from Sigma Aldrich, sublimed, and recrystallized from methanol and then cyclohexane before use. 1,5-Cyclooctadiene was purchased from Sigma Aldrich and distilled from CaH_2 prior to use. Potassium ingot, potassium hydride in mineral oil, paraffin wax (mp ≥ 65 °C), ethanolamine, Rhodium(II) acetate dimer ($Rh_2(OAc)_4$), ethyl diazoacetate (87% in DCM), lithium aluminum hydride ($LiAlH_4$), potassium *tert*-butoxide solution (1 M in THF), 3-chloro-2,2-dimethyl-1-propanol, and triphenylmethyl chloride were purchased from Sigma Aldrich and used as received. Benzyl chloride and methyl iodide were acquired from Acros and used as received. Sodium azide, sodium thiosulfate, and ethylene glycol were purchased from Fisher Scientific and used as received.

2.2. Equipment

The ethylene oxide transfer manifold was constructed from Swagelok stainless steel fittings, tubing, and valves. The polymerization flask was purchased from Chemglass Life Sciences and was cooled with a Thermo Scientific Phoenix II recirculating chiller containing Syltherm XLT Heat Transfer Fluid (*Warning - ethylene oxide polymerizations are exothermic and require careful temperature control; the recirculating chiller should always be connected to an emergency power source*). Gel permeation chromatography (GPC) measurements were carried out using a Waters 515 isocratic pump connected in series to a Waters guard column (200 Å, 6 × 40 mm, 6 μm), two Ultrahydrogel 250 columns (7.8 × 300 mm, 6 μm), an Ultrahydrogel 500 column (7.8 × 300 mm, 10 μm), a Waters 2487 UV detector, a Wyatt Dawn Heleos light scattering detector, and a Wyatt Optilab rEX refractive index detector. A H_2O:CH_3CN (60:40, v/v, with 0.1% TFA) mixture used as the eluent at a flow rate of 0.7 mL/min at 35.5 °C. GPC molecular weights were calibrated using polyethylene glycol standards ranging from 1000 to 40,000 Da. ^{1}H NMR spectra were measured with a Varian VNMRS 400 MHz spectrometer; chemical shifts are reported in parts per million.

2.3. Potassium naphthalenide (0.35 M)

A dry 1 L Schlenk flask was charged with naphthalene (19.5 g, 152 mmol, 1 eq.), followed by potassium metal (6.0 g, 154 mmol, 1 eq.) that was freshly cut into small pieces (~0.3 × 0.3 × 2 cm) under mineral oil and rinsed with heptane under argon. The flask was evacuated below 0.03 Torr and then back filled with argon. Anhydrous THF (400 mL) was added, the flask was sealed, and then stirred at room temperature for ~14 h. The resulting deep malachite green solution was left to settle without stirring for 20 min before use. The solution was used on the same day; transferred as needed by cannula under a slight positive pressure of argon. The solution was drawn carefully from the top as to avoid disturbing the precipitated impurities at the bottom of the flask.

2.4. Potassium hydride in paraffin wax (25% w/w)

Potassium hydride (100 g, 25–35 wt%, dispersion in mineral oil) in a sealable bottle was allowed to settle undisturbed for 7 days. The mineral oil was carefully decanted off, quenched with isopropanol, and discarded. Paraffin wax (160 g) was melted in a beaker and left to cool to ~85 °C before pouring 156 g into the KH sediment. The bottle was flushed with argon, sealed, and vigorously shaken until the mixture homogenized and began to cool to ~30 °C; at which point it began to take on a putty like consistency. The material was allowed to cool and dry *in vacuo*. This yielded 258 g of a light grey, crumbly mixture. Standard titration techniques [21] were used to quantify the KH content as 25% by weight.

2.5. N,N-dibenzylethanolamine (**1**)

A 3 L 2-neck round bottom flask fitted with a mechanical stirrer and a reflux condenser was charged with ethanolamine (61 mL, 61.7 g, 1.01 mol, 1 eq.), benzyl chloride (255 mL, 281 g, 2.22 mol, 2.2 eq.), potassium carbonate (286 g, 2.07 mol, 2.05 eq.), and absolute ethanol (1.75 L). The mixture was heated to reflux with vigorous stirring for 60 h. The reaction was cooled to 30 °C and activated charcoal (50 g) was added. After stirring for 45 min, the mixture was filtered through celite and the filter cake was rinsed with additional ethanol (1.5 L). The filtrate was concentrated on a rotary evaporator (50 °C, 10 Torr) to remove the ethanol. The resulting oil was partitioned between dichloromethane (0.5 L) and water (1 L, pH adjusted to ~11 with NaOH). The aqueous phase was separated and extracted with dichloromethane (2 × 0.5 L). The combined organic layers were dried over Na_3PO_4, filtered, and concentrated on a rotary evaporator. The crude oil was dissolved in hexanes (~0.5 L) before the addition of cyclohexane (0.75 L). The mixture was stored at 4 °C overnight to facilitate crystallization. The crystals were collected and washed with additional hexanes. The product was recrystallization two additional times from 3:2 cyclohexane: hexanes and dried under reduced pressure to yield *N,N*-dibenzylethanolamine **(1)** (216 g, 88.6%) as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.39–7.24 (m, 10H), 4.42 (br s, 1H), 3.60 (s, 4H), 3.52 (m, 2H), 2.52 (m, 2H).

2.6. Bn_2N-PEG-OMe (**2**)

A jacketed 3-neck round-bottom flask fitted with a double-jacketed addition funnel was charged with *N,N*-dibenzylethanolamine **(1)** (1 eq.) under argon. Anhydrous THF (~8 mL/mL of EO) was added and the mixture was stirred at ambient temperature until dissolution (~5 min). A potassium naphthalenide solution (0.35 M in THF) was added via cannula until a deep green color persisted for over 5 min. The reaction mixture was cooled to 10 °C and ethylene oxide (# eq. to reach desired degree of polymerization) was condensed under vacuum at −30 °C into the addition funnel. The liquid ethylene oxide was added in one portion to the cooled alkoxide solution and the reaction flask was backfilled with argon. The reaction was sealed and stirred at 10 °C for 14 h, at which

point the reaction mixture became slightly turbid. The jacket temperature was increased to 20 °C (over 90 min) and held for 4 h. Then the following temperature ramp was applied: 25 °C for 24 h, 30 °C for 24 h, and finally 35 °C for 24 h. The polymerization reaction mixture was cooled to 23 °C and additional potassium naphthalenide solution was added via cannula until a deep green color persisted for over 5 min. Methyl iodide (neat, 1.6 eq.) was added dropwise via a syringe pump over 30 min ^{1}H NMR analysis (DMSO-d_6) after ~22 h showed complete disappearance of the R-OH signal (δ 4.6 ppm). The reaction mixture was transferred to a large bucket and precipitated with vigorous mechanical stirring and the gradual addition of diethyl ether (~3–4 vol/total reaction volume). The product was collected by vacuum filtration, washed with additional diethyl ether (~0.5 vol/total reaction volume), and partial dried on the filter under a nitrogen stream. Further drying *in vacuo* for 48 h provided Bn_2N-PEG-OMe **(2)** (typically >95% yield) as a voluminous white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.37–7.19 (m, 10H), 3.65–3.40 (m, ~n × 4H), 3.24 (s, 3H), 2.55 (t, J = 6.1 Hz, 2H).

2.7. H_2N-PEG-OMe (**3**)

Bn_2N-PEG-OMe **(2)** (1 eq.) was dissolved in H_2O (5 mL/g of PEG) before the addition of $Pd(OH)_2$ (5% w/w). The flask was placed under reduced pressure until gas could no longer be seen evolving from the reaction mixture. The flask was backfilled with H_2 and then re-evacuated under reduced pressure. The process was repeated two additional times. The reaction was left to stir at room temperature under H_2 (1 atm, balloon pressure). After 24 h, an aliquot (~0.7 mL) was taken and filtered through a 0.45 μm syringe-driven filter into a small vial in order to remove as much catalyst as possible. The majority of the water was removed on a rotary evaporator and then the vial was left to dry *in vacuo*. Reaction progress was monitored via ^{1}H NMR (DMSO-d_6) for the disappearance of the starting material R-C$\underline{H_2}$NBn$_2$ methylene (δ 2.55) and aromatic (δ ~7.4–7.2) signals and appearance of the fully deprotected R–C$\underline{H_2}$NH$_2$ methylene (δ 2.63) signals. When the reaction was complete, NaCl (to give ~0.36 g/mL) was added and mixture was stirred until dissolution. The reaction mixture was then filtered through a Millipore Durapore™ 0.45 μm membrane to remove the catalyst. The flask and filter were rinsed with brine. The pH of the clear filtrate was adjusted to ~12–13 with 1 M NaOH and then extracted with DCM (3 × 5 mL/g of PEG). The combined organic layers were stirred with a generous amount of Na_3PO_4 and $MgSO_4$ for 30 min before filtering. The clear filtrate was concentrated on a rotary evaporator to a volume of ~4 mL/g of PEG and precipitated with the addition of Et_2O (10 vol). The precipitation was cooled to 4 °C for ~12 h before the solids were collected by vacuum filtration and washed with additional Et_2O. The product was dried under reduced pressure to yield H_2N-PEG-OMe **(3)** as a white solid (typically >90% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.55–3.46 (m, ~n × 4H), 3.24 (s, 3H), 2.63 (t, J = 5.8 Hz, 2H).

2.8. (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol) (**4**)

Prepared following a literature procedure [19].

2.9. BCN-PEG-OH (**5**)

A jacketed 3-neck round-bottom flask fitted with a double-jacketed addition funnel was charged with 3-methyl-3-pentanol (0.85 eq.) and anhydrous THF (~8 mL/mL of EO) under argon. A potassium naphthalenide solution (0.35 M in THF) was added via cannula until a deep green color persisted for over 5 min. To the resulting alkoxide solution was added (1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethanol) **(4)** (1 eq.) under argon. The reaction mixture was cooled to 10 °C and ethylene oxide (# eq. to reach desired degree of polymerization) was condensed under vacuum at −30 °C into the addition funnel. The liquid ethylene oxide was added in one portion to the cooled alkoxide solution and the reaction flask was backfilled with argon. The reaction was sealed and stirred at 10 °C for 20 h, at which point the reaction mixture became slightly turbid. The jacket temperature was slowly increased step-wise to 40 °C and held for 72 h. The polymerization reaction mixture was cooled to ambient temperature and sparged with argon for 10 min. Methanol (5 eq.) was added and the mixture was stirred for 30 min. The reaction mixture was transferred to a large beaker and precipitated with vigorous mechanical stirring and the gradual addition of diethyl ether (~3–4 vol/total reaction volume). The product was collected by vacuum filtration, washed with additional diethyl ether (~0.5 vol/total reaction volume), and partial dried on the filter under a nitrogen stream. Further drying *in vacuo* for 48 h provided BCN-PEG-OH **(5)** (typically >70% yield) as a voluminous white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.72–3.53 (~n × 4H), 3.51 (d, J = 7.6 Hz, 2H), 2.41 (br s, 1H), 2.31–2.12 (m, 4H), 1.59–1.45 (m, 2H), 1.35–1.25 (m, 1H), 0.94–0.83 (m, 2H).

2.10. 3-Azido-2,2-dimethyl-1-propanol (**8**)

To a mixture of NaI (86.0 g, 574 mmol, 0.98 eq.) and NaN_3 (74.8 g, 1.15 mol, 1.96 eq.) in DMSO (210 mL) was added 3-chloro-2,2-dimethyl-1-propanol (71.8 g, 586 mmol, 1 eq.). The mixture was heated on oil bath at 140°C for 38 hours behind a blast shield, under air-cooled reflux condenser, while the condenser top was being swept with a gentle stream of nitrogen in order to prevent any accumulation of hydrazoic acid. The reaction was cooled, diluted with water (~600 mL), and extracted with Et_2O (2 × 500 mL). The organic layer was washed with water (500 mL) and then dried with $MgSO_4$ and concentrated *in vacuo*. The resulting crude material was fractionally distilled with the product isolated at 30-35°C/0.1 Torr. This yielded 3-azido-2,2-dimethyl-1-propanol **(8)** (60.9 g, 80.5%) as a colorless slightly viscous liquid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38 (d, J = 5.1 Hz, 2H), 3.22 (s, 2H), 1.85 (t, J = 5.2 Hz, 1 H), 0.92 (s, 6H) ppm.

2.11. N_3–CH_2–$C(CH_3)_2$–CH_2-PEG-OH (**9**)

A jacketed 3-neck round-bottom flask fitted with a double-jacketed addition funnel was charged with potassium hydride (25% w/w in paraffin wax, 0.5 eq.) and anhydrous THF (~8 mL/mL of EO). The resulting suspension was stirred at room temperature for 30 min and then cooled to 10 °C. 3-azido-2,2-dimethyl-1-propanol (**8**, 1 eq.) was added dropwise and the mixture as allowed to slowly warm to room temperature over 14 h. The reaction was cooled to 10 °C and ethylene oxide (# eq. to reach desired degree of polymerization) was condensed under vacuum at −30 °C into the addition funnel. The liquid ethylene oxide was added in one portion to the cooled alkoxide solution and the reaction flask was backfilled with argon. The reaction was sealed and stirred at 10 °C for 2 h and then 15 °C for 20 h, at which point the reaction mixture became slightly turbid. Next, the following temperature ramp was applied: 20 °C for 8 h, 25 °C for 14 h, 30 °C for 9 h, 35 °C for 16 h, and finally 40 °C for 30 h. The polymerization reaction mixture was then cooled to 23 °C before the dropwise addition of acetic acid (1 eq.) followed by stirring for 15 min. 4 M HCl as a 2:1 v/v isopropanol: water solution (2.5 vol/vol of AcOH used) and silica gel (15 g/g of 3-azido-2,2-dimethyl-1-propanol) were added and the mixture was stirred for 30 min. The mixture was filtered and the silica cake was washed with DCM. The filtrate was transferred to a large plastic bucket and precipitated with vigorous mechanical stirring and the

gradual addition of methyl *tert*-butyl ether (~3–4 vol/total reaction volume). The product was collected by vacuum filtration, washed with additional methyl *tert*-butyl ether (~0.5 vol/total reaction volume), and partial dried on the filter under a nitrogen stream. Further drying *in vacuo* for 48 h provided N_3–CH_2–$C(CH_3)_2$–CH_2-PEG-OH **(9)** (typically yield >85%) as a voluminous white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.57 (t, J = 5.5 Hz, 1H), 3.63–3.38 (m, ~n × 4H), 3.20 (s, 2H), 3.15 (s, 2H), 0.86 (s, 6H).

2.12. 2-(trityloxy)ethanol (*10*)

In a 2 L round-bottom flask, solid triphenylmethyl chloride (70.0 g, 251 mmol, 0.2 eq.) was slowly added to a solution of ethylene glycol (70 mL, 77.9 g, 1.26 mol, 1 eq.) in CH_3CN (60 mL) and THF (350 mL). The mixture was stirred at ambient temperature until complete dissolution. Triethylamine (50 mL) was added and the mixture was stirred without cooling (mildly exothermic) for 14 h. The reaction was then diluted with water (4 L) and acidified (pH ~4) with conc. H_3PO_4 and stirred for 1 h. The product was collected by vacuum filtration and was washed with additional water (2 L). The crude product was recrystallized from cyclohexane twice to yield 2-(trityloxy)ethanol **(10)** (57.4 g, 75.1%) as white needles. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.23 (m, 15H), 3.78–3.74 (m, 2H), 3.28 (t, J = 4.7 Hz, 2H), 1.94 (t, J = 6.3 Hz, 1H).

2.13. Trityl-PEG-OH (*11*)

A jacketed 3-neck round-bottom flask fitted with a double-jacketed addition funnel was charged with 2-(trityloxy)ethanol **(10)** (1 eq.), potassium hydride (25% w/w in paraffin wax, 0.52 eq.), and anhydrous THF (8 mL/mL of EO) under argon. The resulting suspension was stirred at room temperature for 14 h before reducing the temperature to 10 °C. Ethylene oxide (# eq. to reach desired degree of polymerization) was condensed under vacuum at −30 °C into the addition funnel. The liquid ethylene oxide was added in one portion to the cooled alkoxide solution and the reaction flask was backfilled with argon. The reaction was sealed and stirred at 10 °C for 14 h, at which point the reaction mixture became slightly turbid. Next, the following temperature ramp was applied: 15 °C for 4 h, 20 °C for 24 h, 25 °C for 24 h, and finally 30 °C for 24 h. The polymerization reaction mixture was then cooled to 23 °C and quenched by the dropwise addition of acetic acid (1 eq.) in isopropanol (100 eq.) followed by stirring for 15 min. Silica (15 g/g of 2-(trityloxy)ethanol)) was added and stirred for 15 min before filtering, and washing the silica cake with DCM. The filtrate was transferred to a large plastic bucket and precipitated with vigorous mechanical stirring and the gradual addition of Et_2O (~3–4 vol/total reaction volume). The product was collected by vacuum filtration, washed with additional Et_2O (~0.5 vol/total reaction volume), and partial dried on the filter under a nitrogen stream. Further drying *in vacuo* for 48 h provided trityl-PEG-OH **(11)** (typically >90% yield) as a voluminous white powder. ^{1}H NMR (400 MHz, CD_3CN) δ 7.48–7.44 (m, 6H), 7.36–7.30 (m, 6H), 7.28–7.23 (m, 3H), 3.63–3.45 (m, ~n × 4H), 3.16–3.12 (m, 2H), 2.83–2.78 (m, 1H).

3. Results and discussion

3.1. Synthesis of mPEG amine from N,N-dibenzylethanolamine

Among the options for protected amine initiators [15], we elected to use *N,N*-dibenzylethanolamine **(1)** as the initiator for our EO polymerization [16]. This allows for a simple hydrogenation deprotection with Pearlman's catalyst ($Pd(OH)_2$) to yield the desired PEG amine **3** (Scheme 1). *N,N*-dibenzylethanolamine **(1)** was prepared by treating ethanolamine with excess benzyl chloride followed by repeated recrystallizations from cyclohexane/hexanes before use. Our preferred base for this polymerization is a concentrated solution of freshly prepared potassium naphthalenide in THF. The deep malachite green color of the alkali metal naphthalenide salt allows for visual titration; base is added to a solution of *N,N*-dibenzylethanolamine **(1)** initiator in THF until the color persists. Liquid ethylene oxide was condensed in a double jacketed additional funnel and added to the reaction mixture at 10 °C. We found that initiating the polymerization at this temperature to be a suitable combination of reaction rate and safety. After approximately 14 h, the reaction mixture became turbid and magnetic stirring labored. Thus, the temperature was slowly increased to 35 °C stepwise over several days to help maintain suitable stirring and reaction rate of the living polymerization. Capping was achieved by first adding additional potassium naphthalenide to ensure complete oxyanion formation followed by methyl iodide. Alternatively, we have capped the polymer via the addition of acetic acid/isopropanol or methanol to generate a hydroxyl-terminated polymer [16a]. During the course of our work in micelle-based drug delivery, we prepared a range of mPEG-amines **2** from ~2 to 20 kDa (Fig. 1). In each instance, the target molecular was obtained and the polydispersity index (PDI) of the product was consistently below 1.1 (Table 1).

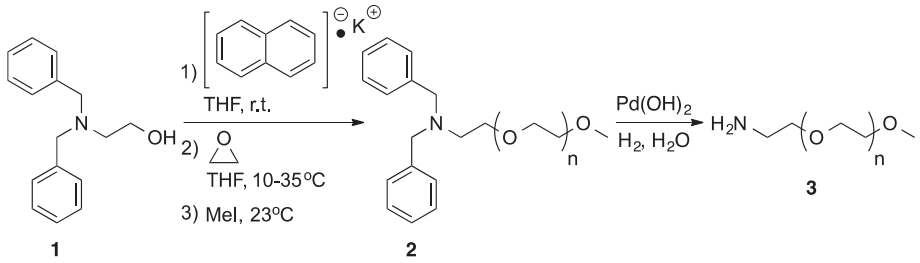
3.2. Ethylene oxide polymerization from BCN initiator

Alkyne functionality in PEGs is commonly installed via capping with propargyl bromide [17], although use of a trimethylsilyl protected alkyne initiator for EO polymerization has been demonstrated [18]. We elected to use a bicyclo[6.1.0]nonyne (BCN) initiator **4** for our polymerizations. BCN is a ring-strained alkyne that has found utility in metal-free cycloaddition reactions with azides and nitrones [19]. The BCN initiator **4** was prepared through a $Rh_2(OAc)_4$ catalyzed cycloaddition between 1,5-cyclooctadiene and ethyl diazoacetate followed by $LiAlH_4$ reduction, separation of isomers, and a bromination-elimination protocol [19]. Our initial ethylene oxide polymerization with the BCN initiator **4** used potassium naphthalenide as the base but suffered from large amounts of by-products. Using the anion of 3-methyl-3-pentanol as a sterically hindered base generated *in situ* with potassium naphthalenide proved to be more successful (Scheme 2). The PDI of the product was excellent, although the molecular weight was below target (Table 1). We identified a small amount of contamination by a polymer initiated from 3-methyl-3-pentanol, which helped explain the lower than expected molecular weight.

3.3. Ethylene oxide polymerization from azide initiator

For the azide containing initiator, we envisioned 3-azidopropanol **(6)** and 2-(2-azidoethoxy)ethanol **(7)** as simple, commercially available options (Fig. 2) [20]. In our first experiments, we tested sterically hindered tertiary alkoxide bases from 3-methyl-3-pentanol and 2-ethylfenchol generated *in situ* with potassium naphthalenide. Neither azide initiator **6** nor **7** provided polymers of the requisite quality; all were plagued by small amounts of unidentified polymers of varying molecular weights. As a technical note, we found simple TLC analysis with Dragendorff's reagent for staining a highly effective way to visualize these impurities, which are often below GPC separation and NMR detection limits.

We suspected the selection of base was the underlying problem and decided to try potassium hydride. Traditionally, KH has been sold as a dispersion in mineral oil that is notoriously difficult to handle and measure. Taber developed KH as a 1:1 homogenate with paraffin wax (KH(P)) [21], an alternatively which has recently



Scheme 1. Synthesis of mPEG-NH_2 **(3)** Initiated from *N,N*-Dibenzylethanolamine (**1**).

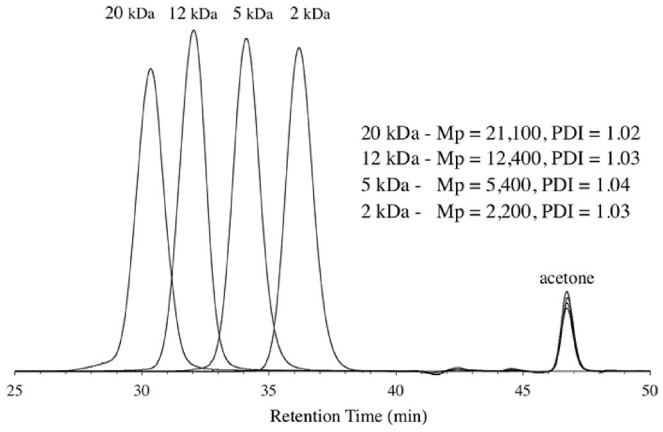
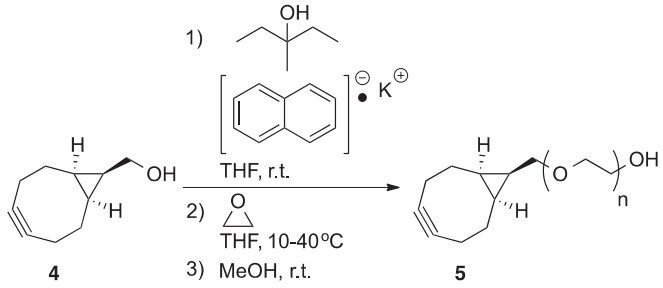


Fig. 1. GPC trace of mPEG-NH_2 polymers ranging from ~2 to 20 kDa with acetone as the flow marker.

Table 1
Ethylene oxide polymerizations with potassium naphthalenide.

Initiator	$MW_{theo.}$	$Mp_{obsd.}$	PDI	Amt (g)	Yield (%)
1	2,211	2,200	1.03	509	96
1	5,187	5,400	1.04	481	97
1	12,195	12,400	1.03	965	99
1	19,160	21,100	1.02	427	90
4	5,232	3,600	1.07	24.8	71



Scheme 2. Ethylene Oxide Polymerization from BCN initiator (**4**).

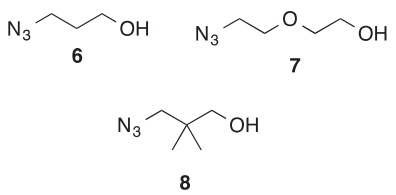
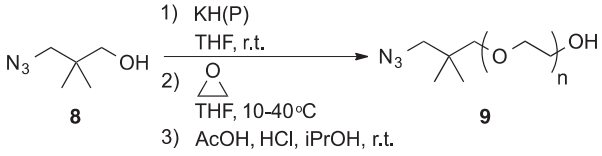


Fig. 2. Azide containing initiators tested for EO polymerization.

become commercially available. Our pilot EO polymerization with KH(P) was conducted using 3-azidopropanol **(6)** as the initiator. Unfortunately, this procedure provided the allyl ether-terminated PEG in a nearly pure form through elimination of the azide. To avoid this issue we envisioned an initiator with a gem-dimethyl moiety in place of the beta protons that lead to elimination of the azide. Hence we prepared 3-azido-2,2-dimethyl-1-propanol **(8)** from the corresponding chloro-compound via a Finkelstein reaction with NaI and NaN_3. We used this initiator with both potassium naphthalenide/3-methyl-3-pentanol and KH(P). Although the former did yield the desired PEG, we again found the product was contaminated with small amounts of 3-methyl-3-pentanol initiated polymers. Initially we used commercially available KH(P), but in some instances found higher than expected PEG dimer content which results from a competing hydroxide initiated ethylene oxide polymerization. We suspected the commercial KH(P) was variable from batch-to-batch, and in some instances contained trace amounts of KOH. For this reason we began waxing our own KH from a mineral oil slurry using a slightly modified version of Tabor's procedure [21]. We found freshly prepared in-house produced KH(P) a much more reliable base for EO polymerizations with 3-azido-2,2-dimethyl-1-propanol **(8)** (Scheme 3). We also noted that the standard 0.8–1.2 equivalents of 25% w/w KH(P) was yielding inconsistent molecular weights. We theorized that some of the initiator was physically sequestered in the solid paraffin wax in the reaction vessel, thus preventing it from participating in the reaction. We found that lowering the stoichiometry to ~0.5 equivalents, and thus reducing the total amount of paraffin wax, alleviated this problem. This subtle change to the procedure gave us consistently high quality PEGs with low PDI values, accurate molecular weights, at large production scales (Table 2).

3.4. Ethylene oxide polymerization from *O*-trityl initiator

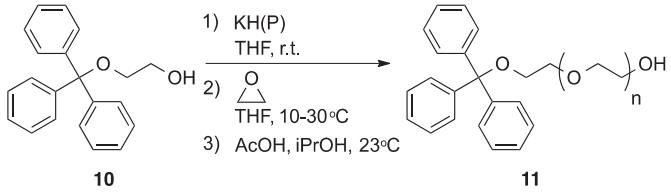
After our successful efforts with the dibenzylamine protected initiator **1**, and the alkyne and azide containing initiators **4** and **8**, we aspired to find a suitable protected alcohol initiator which could be used to prepare numerous heterobifunctional PEG derivatives. A tetrahydropyranyl (THP) protected ethylene glycol has been reported [17a], but we found that large-scale preparations yielded a mixture of the desired product and the corresponding acetal. This led us to explore the use of an *O*-triphenylmethyl (trityl) protected initiator **10**, which was prepared by treating ethylene glycol with



Scheme 3. Ethylene Oxide Polymerization Initiated from Azide **8**.

Table 2
Ethylene oxide polymerizations with potassium hydride in paraffin wax.

Initiator	$MW_{theo.}$	$Mp_{obsd.}$	PDI	Amt (g)	Yield (%)
8	2,133	1,800	1.31	187	75
8	5,130	5,000	1.20	214	89
8	10,049	10,600	1.08	223	94
8	19,555	22,300	1.01	363	96
10	2,264	1,900	1.12	462	85
10	5,238	5,200	1.11	462	93
10	9,841	11,100	1.01	475	98
10	19,128	19,300	1.11	437	92



Scheme 4. Ethylene Oxide Polymerization Initiated from *O*-trityl Ether (**10**).

trityl chloride and triethylamine [22]. Potassium naphthalenide is our preferred base and thus was tested first. To our surprise, this resulted in the clean reductive cleavage of the *O*-trityl group to yield the blood red trityl anion. Switching to KH(P) allowed us to successfully prepare *O*-trityl terminated PEGs **11** (Scheme 4) from ~2 to 20 kDa with low PDI values on half kilogram scale (Table 2). We found the *O*-trityl PEGs to be quite stable to base and heat, while labile in dilute acid [23].

4. Conclusion

In summary, large-scale ethylene oxide polymerizations from four initiators to produce high quality heterobifunctional PEGs have been described. Potassium naphthalenide was found to be the ideal base for the dibenzylamine and BCN initiators, while potassium hydride in paraffin wax performed best for the azide and *O*-trityl initiators. The polymerizations outlined provide protected alcohol and protected amine PEGs, in addition to azide and alkyne "click" chemistry reagents. This should increase the number of possible methods to incorporate PEGs via covalent attachment in order to take advantage of its unique biocompatible properties.

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