Synthesis of a Heterobifunctional PEG Spacer Terminated with Aminooxy and Bromide Functionality

Christopher W. Dicus, Michael H. Nantz[1]

Department of Chemistry, University of California Davis, Davis, CA 95616, USA
Fax +1(502)8527214; E-mail: michael.nantz@louisville.edu

Received 12 June 2006

Abstract: A simple and efficient synthesis of a novel heterobifunctional polyethylene glycol (PEG) spacer is described. The PEG spacer reagent is terminated with aminooxy and bromide functionality for ease of conjugation to a variety of electrophiles and/or nucleophiles.

Key words: heterobifunctional PEG, aminooxy, bioconjugation, molecular recognition, OEG, spacer

The modification of macromolecules by the covalent attachment of polyethylene glycol (PEG) often improves their transport to target tissues and, consequently, is a useful strategy in many biomedical applications. PEGylation of exogenous peptides or proteins provides hydrophilic bulk that reduces renal excretion, and the high mobility of PEG chains also suppress antigenic and immunogenic factors as well as the action of proteolytic enzymes.[2] Such single-chain, site-specific applications obviate the need for bifunctional PEG elements. Indeed, unreactive methyl ether groups generally terminate PEG chains opposite their reactive end.[3] A more recent area of interest examines the utility of PEG as a tether or spacer unit between a bioactive ligand and an anchor, such as a polymer.[4] In these applications, the PEG chain serves to improve molecular recognition between the ligand and its biological receptor by enhancing ligand presentation.[5] To facilitate the covalent attachment of PEG chains to unique functional groups present on either ligands or anchors, the availability of PEG reagents having chemoselectively differentiated termini becomes necessary.

Of specific interest to us is the use of PEG to tether the gene delivery polymer polyethylenimine (PEI) to ligands that are capable of enhancing gene transport in mammalian cells.[6] The efficacy of this technique has been demonstrated by a recent study wherein TAT, a membrane translocation peptide, was attached to PEI using a PEG spacer.[7] The administration of a derived DNA·PEI-$[PEG]_n$-TAT complex to lung tissue in vivo showed a significant increase in delivery efficiency when compared to either the corresponding DNA·PEI complex or the DNA·PEI-TAT complex. Similarly, the use of PEG to tether PEI to an integrin-targeted peptide also resulted in higher gene delivery efficiencies compared to experiments using unmodified PEI.[8]

Despite the importance of heterobifunctional PEG, the availability of PEG reagents having differentiated terminal functionality is limited.[9] Therefore, we pursued the synthesis of a heterobifunctional PEG spacer that would enable straightforward, chemoselective ligation of either end to corresponding electrophilic or nucleophilic functionality. We report herein a synthesis of PEG spacer reagents **1a** and **1b** (Figure 1). The PEG composition in these spacer reagents differs by one ethylene oxide unit and their use in tethering applications would provide a hydrophilic span of 10–13 atoms, a range deemed suitable for molecular presentation by a previous study on PEG linkers.[10]

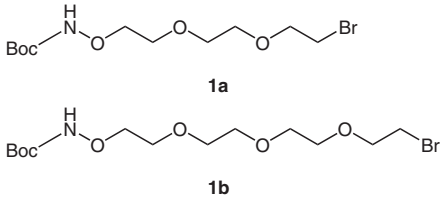


Figure 1 α-Aminooxy-ω-bromo PEG derivatives **1a** and **1b**

The alkyl bromide terminus was selected for its ability to facilitate simple alkylation of amines (e.g., as in PEI alkylation; path a, Figure 2), to make possible PEG dimerization or other PEG polymerization strategies,[11] and to permit end-group modification for attachment of other targeting functional groups. For example, chemoselective targeting of cysteine residues could be accomplished by conversion of the bromide to either a thiol reactive maleimide,[12] vinyl sulfone,[13] or an orthopyridyl disulfide[14] moiety (paths b–d, Figure 2).

We selected the aminooxy functionality for the other terminus because of its ability to rapidly condense with aldehydes and ketones under a variety of conditions. The use of aminooxy groups in coupling reactions has emerged as a powerful tool for the labeling of modified proteins,[15] cell surfaces,[16] and carbohydrates.[17] These condensations often proceed in near quantitative yields under aqueous or organic conditions, providing a robust oxime ether linkage that is stable at physiological pH. The ease of oxime ether formation provides a superior method for targeting aldehyde and ketones compared to a reductive-amination approach, which is often fraught with difficulties.[18]

SYNLETT 2006, No. 17, pp 2821–2823
Advanced online publication: 09.10.2006
DOI: 10.1055/s-2006-950246; Art ID: S12706ST

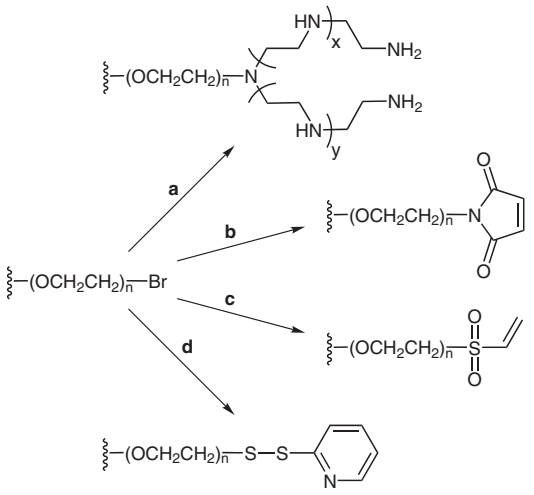
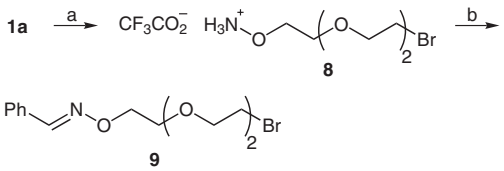


Figure 2 Reactions of PEG-bromide: (a) PEI alkylation; and transformations to thiol-reactive (b) maleimide, (c) vinyl sulfone, or (d) orthopyridyl disulfide groups.

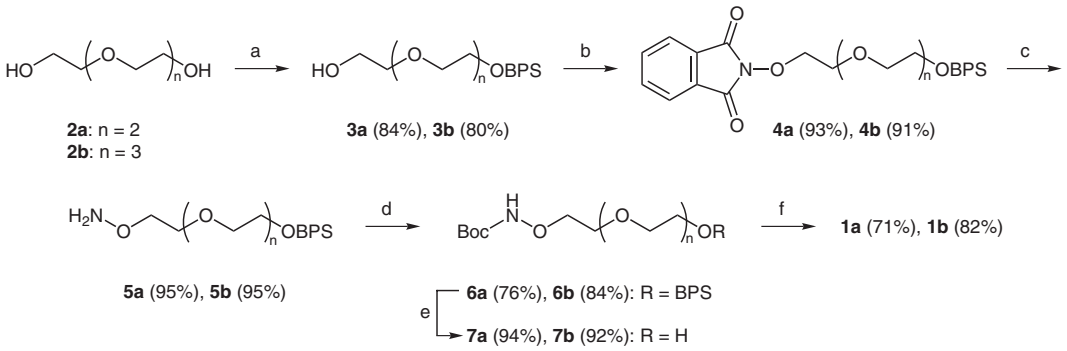
Our synthesis of α-aminooxy-ω-bromo PEG spacers **1a** and **1b** is summarized in Scheme 1.[19] Desymmetrization of commercially available tri- or tetra(ethylene glycol) (**2a** or **2b**) was accomplished by monosilylation of the glycol using *tert*-butyldiphenylsilyl chloride (BPSCl). The monosilyl ethers **3a** and **3b** were easily separated from unreacted PEG by simple aqueous washing, and were isolated from the corresponding bissilyl ether products by column chromatography (SiO_2). Incorporation of the aminooxy moiety followed a literature protocol[20] wherein **3a** or **3b** was treated with Ph_3P, *N*-hydroxyphthalimide and diisopropyl azodicarboxylate (DIAD) to afford phthalimido ethers **4a** and **4b**. Hydrazinolysis of the phthalimide group using hydrazine monohydrate in ethanol proceeded smoothly to give aminooxy compounds **5a** and **5b** in 88% and 86% overall yield from **3**, respectively. At this stage we felt it prudent to protect the aminooxy moiety to prevent PEG polymerization subsequent to bromide installation. Protection of compounds **5** as their *tert*-butyl carbamates was effected by reaction with di-*tert*-butyl dicarbonate (Boc_2O) and triethylamine in refluxing CH_2Cl_2. Boc-protected compounds **6a** and **6b** were obtained in 76% and 84% yields, respectively, after chromatography to remove small amounts of *N*,*N*-bis(Boc)aminoxy products. Introduction of the bromide terminus commenced by TBAF-mediated desilylation followed by the alcohol-to-bromide transformation using the Appel procedure.[21] Carbon tetrabromide was slowly added to a mixture of triphenylphosphine and **7a** or **7b** in CH_2Cl_2 at 0 °C to furnish α-(Boc)aminooxy-ω-bromo compounds **1a** and **1b** in 71% and 82% yield, respectively.[22]

A Boc-protected aminooxy moiety can be deprotected by stirring in trifluoroacetic acid (TFA), and the resultant, crude trifluoroacetoxy salt generally can be reacted with an aldehyde or ketone carbonyl group to obtain the oxime ether conjugate in a good yield.[23] To demonstrate this application using the title PEG compounds, and, more importantly, to illustrate that the presence of the primary bromide does not react with the aminooxy group as it deprotects, we linked spacer reagent **1a** to benzaldehyde as a representative coupling reaction (Scheme 2). The aminiumoxy trifluoroacetoxy salt **8** was isolated by simple vacuum removal of TFA after **1a** was stirred in TFA at room temperature. Dissolution of the crude salt in a solution of benzaldehyde in pyridine (ca. 0.2 M) followed by aqueous work-up after 2 hours gave oxime ether conjugate **9** in 90% yield.[24]



Scheme 2 *Reagents and conditions*: (a) CF_3CO_2H, r.t., 0.5 h; (b) PhCHO, pyridine, r.t., 2 h.

In summary, we have developed a reliable synthesis of a heterobifunctional PEG spacer that features aminooxy functionality at one terminus. The high reactivity of the aminooxy moiety toward aldehydes and ketones allows for chemoselective attachment of PEG to a wide range of compounds. The synthetic route is amenable to other PEG lengths and thus should find utility in bioconjugation applications.



Scheme 1 *Reagents and conditions*: (a) BPS-Cl (0.30 equiv), imidazole (2.0 equiv), DMAP (cat.), CH_2Cl_2, r.t., 4 h; (b) DIAD (1.1 equiv), Ph_3P (1.1 equiv), *N*-hydroxyphthalimide (1.1 equiv), CH_2Cl_2, –40 °C to r.t., 4 h; (c) $H_2NNH_2{\cdot}H_2O$ (2.0 equiv), EtOH, r.t., 18 h; (d) Boc_2O (1.0 equiv), Et_3N (2.0 equiv), CH_2Cl_2, reflux, 18 h; (e) TBAF (1.2 equiv), THF, r.t., 3 h; (f) CBr_4 (2.0 equiv), Ph_3P (1.2 equiv), CH_2Cl_2, 0 °C, 30 min.

Acknowledgment

This work was supported by the National Institutes of Health (NS-046591).

References and Notes

(1) Present address: Department of Chemistry, University of Louisville, Louisville, KY 40292, USA
(2) (a) Bailon, P.; Berthold, W. *Pharm. Sci. Technol. Today* **1998**, 352. (b) Veronese, F. M. *Biomaterials* **2001**, 405. (c) Pasut, G.; Veronese, F. M. *Adv. Polym. Sci.* **2006**, 95.
(3) Dreborg, S.; Akerblom, E. B. *Crit. Rev. Ther. Drug Carrier Syst.* **1990**, 315.
(4) Smaller PEG units (MW <500), also referred to as oligoethylene glycol (OEG), often are employed as spacers; see, for example: Engel, A.; Chatterjee, S. K.; Al-Arifi, A.; Riemann, D.; Langner, J.; Nuhn, P. *Pharm. Res.* **2003**, 51.
(5) For recent examples, see: (a) Chen, H.; Chen, Y.; Sheardown, H.; Brook, M. A. *Biomaterials* **2005**, 7418. (b) Otsuka, H.; Nagasaki, Y.; Kataoka, A. *Langmuir* **2004**, 11285. (c) Manta, C.; Ferraz, N.; Betancor, L.; Antunes, G.; Batista-Viera, F.; Carlsson, J.; Caldwell, K. *Enzyme Microb. Technol.* **2003**, 890.
(6) For recent reviews, see: (a) Kichler, A. *J. Gene Med.* **2004**, S3. (b) Kircheis, R.; Wightman, L.; Wagner, E. *Adv. Drug Delivery Rev.* **2001**, 341.
(7) Kleemann, E.; Neu, M.; Jekel, N.; Fink, L.; Schmehl, T.; Gessler, T.; Seeger, W.; Kissel, T. *J. Controlled Release* **2005**, 299.
(8) Kim, W. J.; Yockman, J. W.; Lee, M.; Jeong, L. H.; Kim, Y.-H.; Kim, S. W. *J. Controlled Release* **2005**, 224.
(9) Roberts, M. J.; Bentley, M. D.; Harris, J. M. *Adv. Drug Delivery Rev.* **2002**, 459.
(10) Engel, A.; Chatterjee, S. K.; Al-Arifi, A.; Nuhn, P. *J. Pharm. Sci.* **2003**, 2229.
(11) Loiseau, F. A.; Hii, K. K.; Hill, A. M. *J. Org. Chem.* **2004**, 639.
(12) Goodson, R. J.; Katre, N. V. *Biotechnology (N.Y.)* **1990**, *8*, 343.
(13) Morpurgo, M.; Veronese, F. M.; Kachensky, D.; Harris, J. M. *Bioconjugate Chem.* **1996**, *7*, 363.
(14) Woghiren, C.; Sharma, B.; Stein, S. *Bioconjugate Chem.* **1993**, *4*, 314.
(15) Tumelty, D.; Carnevali, M.; Miranda, L. P. *J. Am. Chem. Soc.* **2003**, 14238.
(16) (a) Lemieux, G. A.; Yarema, K. J.; Jacobs, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, 4278. (b) Sadamoto, R.; Niikura, K.; Ueda, T.; Monde, K.; Fukuhara, N.; Nishimura, S.-I. *J. Am. Chem. Soc.* **2004**, 3755.
(17) Perouzel, E.; Jorgensen, M. R.; Keller, M.; Miller, A. D. *Bioconjugate Chem.* **2003**, *14*, 884.
(18) Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem.* **1991**, 4326.
(19) For moisture-sensitive reactions, optimized yields were obtained only after drying the PEG intermediate by toluene azeotropic distillation immediately prior to use.
(20) (a) Grochowski, E.; Jurczak, J. *Synthesis* **1976**, 682. (b) Nicolaou, K. C.; Groneberg, R. D. *J. Am. Chem. Soc.* **1990**, 4085. (c) Su, S.; Giguere, J. R.; Schaus, S. E. Jr.; Porco, J. A. Jr. *Tetrahedron* **2004**, 8645.
(21) Appel, R. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 801.
(22) ^{1}H NMR and ^{13}C NMR data (300 MHz and 75 MHz, respectively; in $CDCl_3$) for all compounds: Compound **1a**: ^{1}H NMR: δ = 7.23 (br s, 1 H), 4.03 (m, 2 H), 3.82 (t, *J* = 6.3 Hz, 2 H), 3.74 (m, 2 H), 3.69 (app. s, 4 H), 3.48 (t, *J* = 6.3 Hz, 2 H), 1.48 (s, 9 H). ^{13}C NMR: δ = 156.7, 81.7, 75.4, 71.2, 70.5, 70.4, 69.4, 30.2, 28.2.
Compound **1b**: ^{1}H NMR: δ = 7.59 (br s, 1 H), 4.03 (m, 2 H), 3.82 (t, *J* = 6.3 Hz, 2 H), 3.70–3.66 (m, 8 H), 3.48 (t, *J* = 6.3 Hz, 2 H), 1.48 (s, 9 H). ^{13}C NMR: δ = 156.7, 81.5, 75.3, 71.2, 70.6 (3 C), 70.5, 69.3, 30.2, 28.2.
Compound **3a**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.44–7.35 (m, 6 H), 3.82 (t, *J* = 5.4 Hz, 2 H), 3.73–3.70 (m, 2 H), 3.66 (app. s, 4 H), 3.63 (m, 4 H), 2.17 (br s, 1 H), 1.05 (s. 9 H). ^{13}C NMR: δ = 135.6, 133.6, 129.6, 127.6, 72.5, 70.8, 70.5, 63.4, 61.8, 26.8, 19.2.
Compound **3b**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.44–7.35 (m, 6 H), 3.82 (t, *J* = 5.4 Hz, 2 H), 3.73–3.70 (m, 2 H), 3.66–3.58 (m, 12 H), 2.25 (br s, 1 H), 1.05 (s, 9 H). ^{13}C NMR: δ = 135.6, 133.6, 129.6, 127.6, 72.5, 72.4, 70.7, 70.7, 70.4, 63.4, 61.8, 26.8, 19.2.
Compound **4a**: ^{1}H NMR: δ = 7.83–7.80 (m, 2 H), 7.72–7.70 (m, 2 H), 7.70–7.65 (m, 4 H), 7.44–7.35 (m, 6 H), 4.36 (m, 2 H), 3.86 (m, 2 H), 3.74 (t, *J* = 5.4 Hz, 2 H), 3.65–3.62 (m, 2 H), 3.58–3.56 (m, 2 H), 3.52 (t, *J* = 5.4 Hz, 2 H), 1.03 (s, 9 H). ^{13}C NMR: δ = 163.4, 135.6, 134.4, 133.7, 129.6, 129.0, 127.6, 123.4, 77.2, 72.4, 70.8, 70.7, 69.4, 63.3, 26.8, 19.2.
Compound **4b**: ^{1}H NMR: δ = 7.84–7.81 (m, 2 H), 7.74–7.71 (m, 2 H), 7.70–7.66 (m, 4 H), 7.44–7.34 (m, 6 H), 4.36 (m, 2 H), 3.85 (m, 2 H), 3.79 (t, *J* = 5.4 Hz, 2 H), 3.67–3.63 (m, 2 H), 3.61–3.52 (m, 8 H), 1.04 (s, 9 H). ^{13}C NMR: δ = 163.4, 135.6, 134.4, 133.7, 129.6, 129.0, 127.6, 77.2, 72.4, 70.8, 70.7, 70.6, 70.5, 69.3, 63.4, 26.8, 19.2.
Compound **5a**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.44–7.35 (m, 6 H), 3.84 (m, 2 H), 3.81 (t, *J* = 5.4 Hz, 2 H), 3.69–3.59 (m, 8 H), 1.05 (s, 9 H). ^{13}C NMR: δ = 135.6, 133.6, 129.6, 127.6, 74.7, 72.4, 70.7, 70.6, 69.7, 63.4, 26.8, 19.2.
Compound **5b**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.44–7.35 (m, 6 H), 3.85 (m, 2 H), 3.81 (t, *J* = 5.4 Hz, 2 H), 3.69–3.63 (m, 10 H), 3.60 (t, *J* = 5.4 Hz, 2 H), 1.05 (s, 9 H). ^{13}C NMR: δ = 135.6, 133.6, 129.6, 127.6, 74.6, 72.4, 70.7, 70.6, 70.5, 70.5, 69.7, 63.4, 26.8, 19.2.
Compound **6a**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.51 (br s, 1 H), 7.44–7.34 (m, 6 H), 4.01 (m, 2 H), 3.82 (t, *J* = 5.4 Hz, 2 H), 3.72 (m, 2 H), 3.68–3.62 (m, 4 H), 3.61 (t, *J* = 5.4 Hz, 2 Hz), 1.46 (s, 9 H), 1.05 (s, 9 H). ^{13}C NMR: δ = 156.7, 135.6, 133.6, 129.6, 127.6, 81.5, 75.3, 72.4, 70.7, 70.6, 69.4, 63.4, 28.2, 26.8, 19.2.
Compound **6b**: ^{1}H NMR: δ = 7.70–7.67 (m, 4 H), 7.62 (br s, 1 H), 7.44–7.34 (m, 6 H), 4.01 (m, 2 H), 3.81 (t, *J* = 5.4 Hz, 2 H), 3.70 (m, 2 H), 3.67–3.62 (m, 8 H), 3.60 (t, *J* = 5.4 Hz, 2 H), 1.46 (s, 9 H), 1.05 (s, 9 H). ^{13}C NMR: δ = 156.7, 135.6, 133.6, 129.6, 127.6, 81.4, 75.3, 72.3, 70.7, 70.6, 70.5, 70.5, 69.2, 63.4, 28.2, 26.8, 19.1.
Compound **7a**: ^{1}H NMR: δ = 7.82 (br s, 1 H), 4.03 (m, 2 H), 3.76–3.72 (m, 4 H), 3.69 (s, 4 H), 3.64–3.61 (m, 4 H), 2.84 (br s, 1 H), 1.48 (s, 9 H). ^{13}C NMR: δ = 156.8, 81.6, 75.2, 72.6, 70.4, 70.2, 69.2, 61.6, 28.2.
Compound **7b**: ^{1}H NMR: δ = 8.08 (br s, 1 H), 4.02 (m, 2 H), 3.74–3.66 (m, 12 H), 3.63 (m, 2 H), 2.80 (br s, 1 H), 1.48 (s, 9 H). ^{13}C NMR: δ = 156.9, 81.4, 75.1, 72.6, 70.6, 70.4, 70.4, 70.3, 69.0, 61.7, 28.2.
(23) Gaertner, H. F.; Offord, R. E. *Bioconjugate Chem.* **1996**, *7*, 38.
(24) Compound **9** was obtained as a single isomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 8.13 (s, 1 H), 7.58–7.57 (m, 2 H), 7.38–7.36 (m, 3 H), 4.34 (t, *J* = 4.6 Hz, 2 H), 3.83–3.81 (m, 4 H), 3.70 (app. s, 4 H), 3.46 (t, *J* = 6.0 Hz, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.0, 132.2, 129.8, 128.7, 127.0, 73.5, 71.2, 70.7, 70.5, 69.7, 30.3.