JOC Note
pubs.acs.org/joc

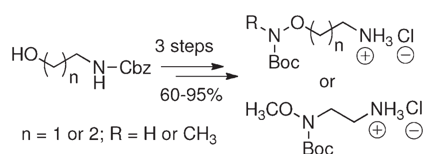
Synthesis of Aminooxy and N-Alkylaminooxy Amines for Use in Bioconjugation

Michael R. Carrasco,* Carolina I. Alvarado, Scott T. Dashner, Amanda J. Wong, and Michael A. Wong

Department of Chemistry and Biochemistry, Santa Clara University, 500 El Camino Real, Santa Clara, California 95053-0270

mcarrasco@scu.edu

Received June 2, 2010



Five Boc-protected aminooxy and N-alkylaminooxy amines have been synthesized in 60−95% overall yield using a common synthetic strategy from readily available two- and three-carbon Cbz-protected amino alcohols. The amines can be linked to biomolecules via amide formation and incorporated directly into peptoids via submonomer synthesis. Subsequent deprotection of the aminooxy and N-alkylaminooxy groups enables conjugation with desired target molecules via established chemoselective ligation methods. The range of derivatives synthesized allows different distances to be established between the conjugated molecules.

The reactions of aminooxy and N-alkylaminooxy groups have proven exceedingly useful for bioconjugate chemistry.[1] Aminooxy groups react chemoselectively with aldehydes and ketones to form oximes in mild aqueous solutions, and with appropriate additives the reactions can be performed at very low concentrations (Figure 1).[2] Oxime formation has been the basis of conjugation chemistry as varied as dendrimer synthesis; formation of peptide and protein assemblies; glycosylation of peptides, proteins, and cells; and labeling

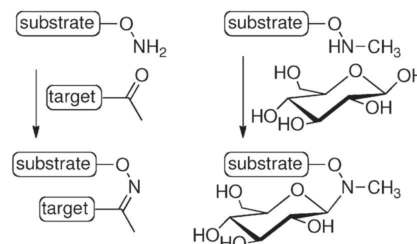


FIGURE 1. Aminooxy and N-alkylaminooxy strategies for bioconjugation chemistry. In mildly acidic aqueous buffers, aminooxy groups react with aldehydes and ketones to form oximes and N-alkylaminooxy groups react with reducing sugars to form glycoconjugates.

proteins for imaging applications. N-Alkylaminooxy groups have specific utility for glycosylation chemistry because they react chemoselectively with native reducing sugars to form glycoconjugates where the attached sugars maintain the biologically relevant cyclic conformations.[3] N-Alkylaminooxy strategies have been used for the glycosylation of peptides,[3,4] peptoids,[5] proteins,[6] carbohydrates,[7] microarrays,[8] and small molecules of pharmaceutical interest.[9] To effect these conjugation strategies, a wide array of special amino acids, sugars, and linkers have been reported. Rather than make different compounds for each context, however, it would be valuable to have a small set of derivatives that could be used to incorporate aminooxy and N-alkylaminooxy functionality into a wide array of desired molecules.

Because our own interest was in the synthesis of modified peptoid oligomers, we envisioned making the amines **1−5**. These amines could be used in the synthesis of peptoids by the submonomer method,[10] and they could also be used in the established bioconjugation methods for modification of carboxyl groups in molecules such as peptides and proteins. Removal of the Boc protection would then reveal the desired aminooxy (from **1** and **2**) or N-alkylaminooxy functionality (from **3−5**). As a result, **1−5** would allow for easy

(1) (a) Peri, F.; Nicotra, F. *Chem. Commun.* **2004**, 623. (b) Zatsepin, T. S.; Stetsenko, D. A.; Gait, M. J.; Oretskaya, T. S. *Bioconjugate Chem.* **2005**, *16*, 471. (c) Langenhan, J. M.; Griffith, B. R.; Thorson, J. S. *J. Nat. Prod.* **2005**, *68*, 1696. (d) Langenhan, J. M.; Thorson, J. S. *Curr. Org. Synth.* **2005**, *2*, 59. (e) Lees, A.; Sen, G.; LopezAcosta, A. *Vaccine* **2006**, *24*, 716. (f) Heredia, K. L.; Maynard, H. D. *Org. Biomol. Chem.* **2007**, *5*, 45. (g) Nicotra, F.; Cipolla, L.; Peri, F.; La Ferla, B.; Redaelli, C. In *Advances in Carbohydrate Chemistry and Biochemistry*; Derek, H., Ed.; Academic Press: New York, 2007; Vol. 61, p 353. (h) Iha, R. K.; Wooley, K. L.; Nyström, A. M.; Burke, D. J.; Kade, M. J.; Hawker, C. *J. Chem. Rev.* **2009**, *109*, 5620. (i) Kalia, J.; Raines, R. T. *Curr. Org. Chem.* **2010**, *14*, 138.

(2) (a) Dirksen, A.; Hackeng, T. M.; Dawson, P. E. *Angew. Chem., Int. Ed.* **2006**, *45*, 7581. (b) Thygesen, M. B.; Munch, H.; Sauer, J.; Cló, E.; Jørgensen, M. R.; Hindsgaul, O.; Jensen, K. J. *J. Org. Chem.* **2010**, *75*, 1752.

(3) Peri, F.; Dumy, P.; Mutter, M. *Tetrahedron* **1998**, *54*, 12269.

(4) (a) Carrasco, M. R.; Nguyen, M. J.; Burnell, D. R.; MacLaren, M. D.; Hengel, S. M. *Tetrahedron Lett.* **2002**, *43*, 5727. (b) Carrasco, M. R.; Brown, R. T. *J. Org. Chem.* **2003**, *68*, 8853.

(5) Seo, J.; Michaelian, N.; Owens, S. C.; Dashner, S. T.; Wong, A. J.; Barron, A. E.; Carrasco, M. R. *Org. Lett.* **2009**, *11*, 5210.

(6) (a) Matsubara, N.; Oiwa, K.; Hohsaka, T.; Sadamoto, R.; Niikura, K.; Fukuhara, N.; Takimoto, A.; Kondo, H.; Nishimura, S.-I. *Chem.—Eur. J.* **2005**, *11*, 6974. (b) Leung, C.; Chibba, A.; Gómez-Biagi, R. F.; Nitz, M. *Carbohydr. Res.* **2009**, *344*, 570.

(7) (a) Peri, F.; Deutman, A.; La Ferla, B.; Nicotra, F. *Chem. Commun.* **2002**, 1504. (b) Peri, F.; Jiménez-Barbero, J.; García-Aparicio, V.; Tvaroška, I.; Nicotra, F. *Chem.—Eur. J.* **2004**, *10*, 1433.

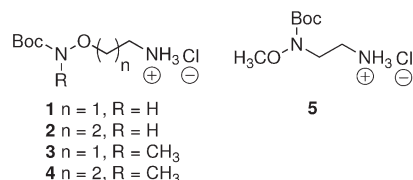
(8) (a) Bohorov, O.; Andersson-Sand, H.; Hoffmann, J.; Blixt, O. *Glycobiology* **2006**, *16*, 21C. (b) Cló, E.; Blixt, O.; Jensen, K. J. *Eur. J. Org. Chem.* **2010**, 540.

(9) (a) Langenhan, J. M.; Peters, N. R.; Guzei, I. A.; Hoffmann, F. M.; Thorson, J. S. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12305. (b) Ahmed, A.; Peters, N. R.; Fitzgerald, M. K.; Watson, J. A., Jr.; Hoffmann, F. M.; Thorson, J. S. *J. Am. Chem. Soc.* **2006**, *128*, 14224. (c) Griffith, B. R.; Krepel, C.; Fu, X.; Blanchard, S.; Ahmed, A.; Edmiston, C. E.; Thorson, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 8150. (d) Langenhan, J. M.; Engle, J. M.; Slevin, L. K.; Fay, L. R.; Lucker, R. W.; Smith, K. R.; Endo, M. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 670. (e) Goff, R. D.; Thorson, J. S. *Org. Lett.* **2009**, *11*, 461.

(10) Zuckermann, R. N.; Kerr, J. M.; Kent, S. B. H.; Moos, W. H. *J. Am. Chem. Soc.* **1992**, *114*, 10646.

DOI: 10.1021/jo101066c
Published on Web 07/15/2010
© 2010 American Chemical Society

incorporation of aminooxy and *N*-alkylaminooxy moieties into a wide range of desired substrates, and their variation allows for four-, five-, or six-atom distances to be established between the conjugated molecules. This distance variation is particularly important in the glycopeptoid context, where it allows control of the distances between attached sugars and the peptoid backbone.



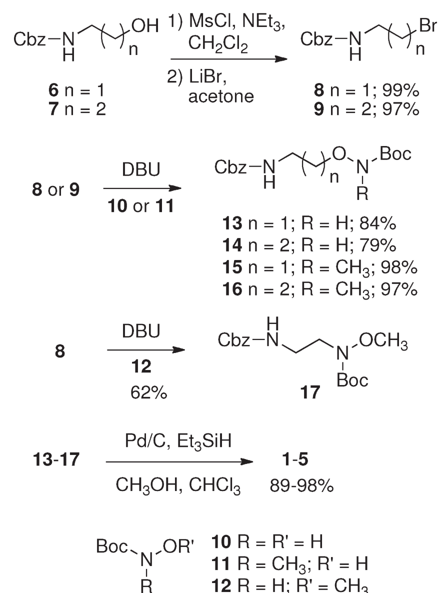
Perusal of the literature revealed that syntheses of **1**[11] and **2**[12] as free amines had been reported, and we recently disclosed a synthesis of the free amine of **4**.[5] However, to enable their practical and general use, especially by practitioners interested in bioconjugation but only minimally trained in organic synthesis, it would be desirable to have a common route to **1**−**5**. Moreover, that route would comprise few steps, be high-yielding, require no advanced techniques, and be amenable to large scale synthesis. The published syntheses lack common strategies and intermediates, and several suffer from low overall yields and troublesome purifications.

We now report a general, practical synthesis of **1**−**5** in three steps and 60−95% overall yield. All reactions proceed at room temperature and without particular sensitivity to moisture or air. Further, the majority of the purifications may be accomplished solely with extractive workup procedures or triturations.

Our sequence is indicated in Scheme 1. Although bromides **8** and **9** are known,[13] we found it most convenient to prepare them from the corresponding, readily available Cbz-protected amino alcohols **6** and **7**. Under a slight modification of the conditions of Albrecht et al.,[14] reaction of **8** or **9** with *N*-Boc-hydroxylamine, **10**, or *N*-methyl-*N*-Boc-hydroxylamine, **11**,[15] yielded the four bis-protected amino hydroxylamines **13**−**16**; reaction of **8** with *N*-Boc-*O*-methylhydroxylamine, **12**,[16] yielded **17**. Notably, the alkylation reactions with **11** required solely extractive workup procedures to provide excellent yields of analytically pure **15** and **16**; column chromatography was required for the purification of **13**, **14**, and **17**, but each was still obtained in good yield. Use of the bromides **8** and **9** rather than the corresponding mesylates of **6** and **7** resulted in higher yields for the alkylation reactions as well as easier reaction monitoring

(11) Gaertner, H.; Offord, R.; Botti, P.; Kuenzi, G.; Hartley, O. *Bioconjugate Chem.* **2008**, *19*, 480.
(12) (a) Lemieux, G. A.; Yarema, K. J.; Jacobs, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 4278. (b) Salisbury, C. M.; Maly, D. J.; Ellman, J. A. *J. Am. Chem. Soc.* **2002**, *124*, 14868.
(13) (a) Katchalski, E.; Ishai, D. B. *J. Org. Chem.* **1950**, *15*, 1067. (b) Keller, M.; Jorgensen, M. R.; Perouzel, E.; Miller, A. D. *Biochemistry* **2003**, *42*, 6067. (c) Gao, F.; Yan, X.; Shakya, T.; Baettig, O. M.; Ait-Mohand-Brunet, S.; Berghuis, A. M.; Wright, G. D.; Auclair, K. *J. Med. Chem.* **2006**, *49*, 5273.
(14) Albrecht, S.; Defoin, A.; Tarnus, C. *Synthesis* **2006**, 1635.
(15) (a) House, H. O.; Richey, F. A., Jr. *J. Org. Chem.* **1969**, *34*, 1430. (b) Freeman, J. P.; Lillwitz, L. D. *J. Org. Chem.* **1970**, *35*, 3107. (c) Carrasco, M. R.; Brown, R. T.; Serafimova, I. M.; Silva, O. *J. Org. Chem.* **2003**, *68*, 195.
(16) Kawase, M.; Kitamura, T.; Kikugawa, Y. *J. Org. Chem.* **1989**, *54*, 3394.

SCHEME 1. Synthesis of 1−5



and purifications because the mesylates had very similar R_f's by TLC to the desired products.

Removal of the Cbz protecting group from **13**−**17** was accomplished using the method of Mandal and McMurray,[17] and the addition of chloroform to the reactions allowed **1**−**5** to be isolated directly as their HCl salts in excellent yields. In the case of **1**, **3**, and **5**, simple trituration of the solids with hexane was sufficient to obtain analytically pure material in nearly quantitative yield; the HCl salts of **2** and **4** were not as crystalline, but both could be purified with a simple filtration through a short plug of silica gel. We found the nature of the Pd/C catalyst and the amount of chloroform added to be very important for successful deprotection. Less active catalysts, lesser amounts of catalyst, and greater amounts of chloroform led to sluggish reactions where additional portions of Pd/C and triethylsilane had to be added over time to effect complete conversion. In the end, we settled on using a relatively large amount (40 wt %) of Degussa type E101 NE/W 10% Pd/C and only 125 mol % of chloroform as conditions that gave complete reaction in short (< 20 min) reaction times. Importantly, there was no evidence of cleavage of the hydroxylamine N−O bond or loss of the Boc protecting group, which could have been a concern with the generation of HCl during the reaction.

In the end, we established a highly efficient synthesis of a suite of aminooxy and *N*-alkylaminooxy amines. We are currently using them for the synthesis of arrays of glycopeptoids, and we expect that they will find broad application in bioconjugation strategies.

Experimental Section

***N*-(2-Bromoethyl)carbamic Acid, Phenylmethyl Ester (8).** A flask was charged with benzyl *N*-(2-hydroxyethyl)carbamate, **6** (9.76 g, 50.0 mmol, 100 mol %), methanesulfonyl chloride (4.65 mL, 60.0 mmol, 120 mol %), and CH_2Cl_2 (150 mL). To this stirring solution was added NEt_3 (9.01 mL, 65.0 mmol, 130 mol %). Stirring was continued for 45 min, and then LiBr (43.5 g, 500 mmol, 1000 mol %) and acetone (150 mL) were added. The reaction mixture was stirred for an additional 21.5 h,

(17) Mandal, P. K.; McMurray, J. S. *J. Org. Chem.* **2007**, *72*, 6599.

and then the solvents were removed by rotary evaporation. The contents were partitioned between Et_2O (300 mL) and H_2O (200 mL), and the Et_2O layer was washed with 0.1 M $KHSO_4$ (3 × 100 mL) and brine (1×), dried (Na_2SO_4), filtered, evaporated to dryness, and placed under vacuum to yield 12.77 g (49.5 mmol, 99%) of **8** as a white solid. Mp: 47−48 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 5H), 5.27 (br s, 1H), 5.11 (s, 2H), 3.59 (q, J = 5.9 Hz, 2H), 3.45 (t, J = 5.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.3, 136.3, 128.7, 128.4, 128.3, 67.1, 42.8, 32.6. Anal. Calcd for $C_{10}H_{12}BrNO_2$: C, 46.53; H, 4.69; N, 5.43. Found: C, 46.92; H, 4.62; N, 5.53.

6,9,9-Trimethyl-7-oxo-5,8-dioxa-2,6-diazadecanoic Acid, Phenylmethyl Ester (15). A flask was charged with *N*-Boc-*N*-methylhydroxylamine, **11** (2.06 g, 14.0 mmol, 140 mol %), DBU (2.24 mL, 15.0 mmol, 150 mol %), and Et_2O (8 mL). To this stirring solution was added **8** (2.58 g, 10.0 mmol, 100 mol %), and the walls of the flask were rinsed with additional Et_2O (2 mL). Stirring was continued for 15 min, and then the Et_2O was removed by rotary evaporation. The concentrated mixture was stirred for an additional 13 h and then partitioned between EtOAc (200 mL) and water (150 mL). The EtOAc layer was washed with 0.1 M $KHSO_4$ (3 × 50 mL), 0.1 M NaOH (5 × 50 mL), and brine (1×) and then dried (Na_2SO_4) and filtered. Evaporation of the solvents and drying under vacuum yielded 3.18 g (9.80 mmol, 98%) of **15** as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (m, 5H), 5.83 (br s, 1H), 5.11 (s, 2H), 3.87 (t, J = 4.8 Hz, 2H), 3.40 (m, 2H), 3.07 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.5, 156.6, 136.7, 128.5, 128.10, 128.07, 82.0, 73.0, 66.7, 39.5, 36.7, 28.3. Anal. Calcd for $C_{16}H_{24}N_2O_5$: C, 59.24; H, 7.46; N, 8.64. Found: C, 58.86; H, 7.72; N, 8.66.

***N*-(2-Aminoethoxy)-*N*-methylcarbamic Acid, 1,1-Dimethylethyl Ester Hydrochloride (3).** A flask was charged with **15** (0.352 g, 1.09 mmol, 100 mol %), $CHCl_3$ (0.109 mL, 1.36 mmol, 125 mol %), and CH_3OH (3 mL). The flask was alternately evacuated and flushed with Ar (3×), and then Degussa type E101 NE/W 10% Pd/C (0.282 g, 40 wt % on dry basis) was added. Triethylsilane (1.74 mL, 10.9 mmol, 1000 mol %) was added dropwise, and the reaction mixture was stirred for 50 min. The solution was filtered through Celite and then evaporated to dryness. The solids were triturated with hexane, filtered, and dissolved in CH_2Cl_2. Evaporation of the solvent and drying under vacuum yielded 0.242 g (1.07 mmol, 98%) of **3** as a white solid. Mp: 148−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (br s, 3H), 4.20 (br s, 2H), 3.28 (br t, J = 4.4 Hz, 2H), 3.10 (s, 3H), 1.48 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.8, 83.4, 69.9, 38.2, 37.2, 28.1. Anal. Calcd for $C_8H_{19}ClN_2O_3$: C, 42.38; H, 8.45; N, 12.36. Found: C, 42.15; H, 8.69; N, 12.10.

Acknowledgment. We thank Brian J. McNelis for many helpful discussions and Joseph M. Langenhan for acquiring NMR spectra. We are grateful to the National Institutes of Health (R15 GM081851-01) and the Camille and Henry Dreyfus Foundation for their support of this work.

Supporting Information Available: Synthetic procedures for **1**, **2**, **4**, **5**, **9**, **13**, **14**, **16**, and **17** and ^{1}H and ^{13}C NMR spectra for **1**−**5**, **8**, **9**, and **13**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.