Aminooxy-, Hydrazide-, and Thiosemicarbazide-Functionalized Saccharides: Versatile Reagents for Glycoconjugate Synthesis

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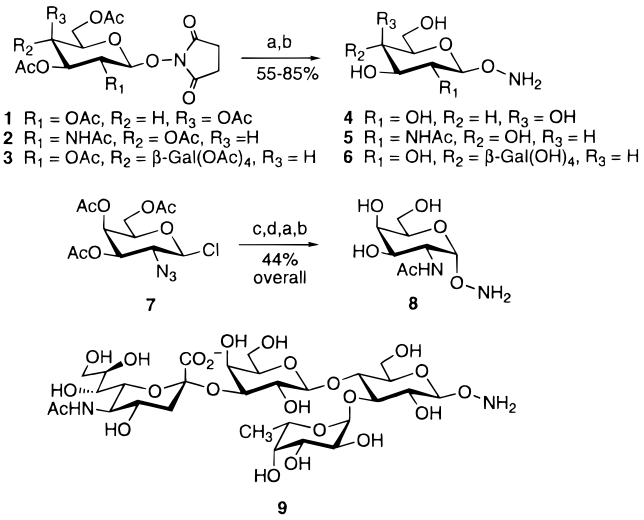
Organic chemists have exercised significant creativity in the construction of glycoconjugate assemblies as tools for studying carbohydrate recognition and as potential therapeutic agents.[1] These synthetic accomplishments, which include neoglycoproteins, glycodendrimers, glycoliposomes, and glycopolymers, have been sparked by the growing realization that glycoconjugates participate in a wide range of normal and pathophysiological processes.[2,3] Given the importance of glycoconjugates as tools for glycobiology and as emerging pharmaceutical reagents, new methods for attaching sugars to scaffolds are of significant current interest.

The highly selective condensation reactions of ketones or aldehydes with aminooxy, hydrazide, or thiosemicarbazide groups (forming oximes, hydrazones, and thiosemicarbazones, respectively) are popular for the conjugation of peptides and proteins.[4] The reactions proceed in aqueous solvent, and their high selectivity obviates the requirement for protection of other functional groups on the coupling partners. Despite the utility of these "chemoselective ligation" reactions[4] in the assembly of peptide-based macromolecules, their implementation in glycoconjugate synthesis is limited to a few examples.[5] The majority of current methods for attaching sugars to scaffolds involve the coupling of electrophilic carbohydrate derivatives with exposed thiol or amino groups. The electrophilic derivatives include α-haloacetamides, bromoethyl glycosides, maleimides, and isothiocyanates.[6] Here we report an alternate ligation strategy based on the coupling of *nucleophilic* carbohydrate derivatives to synthetic scaffolds. We synthesized carbohydrates bearing aminooxy, hydrazide, or thiosemicarbazide groups at their reducing termini and coupled these derivatives to ketone groups on a peptide scaffold. The novel neoglycopeptides produced in this fashion have structural motifs shared by native N-linked or O-linked glycopeptides.[7]

Scheme 1[a]



[a] Key: (a) $N_2H_4 \cdot H_2O$, 14 h; (b) HPLC on aminopropyl silica gel; (c) NHS, $Bu_4N(HSO_4)$, 1:1 CH_2Cl_2/1 M Na_2CO_3, 24 h; (d) H_2, 10% Pd/C, Ac_2O, 4 h.

The direct attachment of an aminooxy group to the reducing terminus of a mono- or disaccharide can be accomplished by formation of the *N*-hydroxysuccinimidoglycoside (*N*-hydroxysuccinimido = NHS)[8] followed by cleavage of the succinimide group with hydrazine, a strategy first reported by Roy and co-workers.[8b] Accordingly, we synthesized *β*-linked aminooxy analogues of galactose (**4**), *N*-acetylglucosamine (GlcNAc) (**5**), and lactose (**6**) from the corresponding protected NHS glycosides **1**, **2**, and **3**, respectively (Scheme 1). In addition, we prepared an α-aminooxy GalNAc derivative (**8**) to mimic the peptide-proximal α-GalNAc residue found in O-linked glycoproteins. This was achieved by first generating the α-NHS glycoside of peracetylated 2-azido-2-deoxygalactose by reaction of azido chloride **7**[9] with NHS. Subsequent reductive acetylation of the azide and deprotection with hydrazine furnished the desired analogue.[10]

The simple aminooxy sugars **4**−**6** and **8** can be transformed into more complex oligosaccharides using established enzymatic methods.[11] As an example, we synthesized an aminooxy-functionalized analogue of the tetrasaccharide sialyl Lewis x (NeuAcα2→3Gal*β*1→4(Fucα1→3)GlcNAc), a motif recognized by the selectin family of adhesion molecules that has been explored as a selectin inhibitor in the form of many different conjugated assemblies.[12] Aminooxy lactose (**6**) was converted to the corresponding sialyllactose analogue

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(1) (a) Roy, R. *Curr. Opin. Struct. Biol.* **1996**, *6*, 696. (b) Roy, R. Sialoside Mimetics and Conjugates as Antiinflammatory Agents and Inhibitors of Flu Virus Infections. In *Carbohydrates in Drug Design*; Witczak, Z. J., Nieforth, K. A., Eds.; Marcel Dekker: New York, 1997; Chapter 3, pp 83−135.

(2) (a) Lee, Y. C.; Lee, R. T. *Methods Enzymol.* **1994**, *242*, 3 and references therein. (b) Palcic, M. M.; Hong, L.; Zanini, D.; Bhella, R. S.; Roy, R. *Carbohydr. Res.* **1998**, *305*, 433. (c) Zanini, D.; Roy, R. *J. Org. Chem.* **1998**, *63*, 3486 and references therein. (d) Reichert, A.; Nagy, J. O.; Spevak, W.; Charych, D. *J. Am. Chem. Soc.* **1995**, *117*, 829. (e) Spevak, D.; Foxall, C.; Charych, D. H.; Dasgupta, F.; Nagy, J. O. *J. Med. Chem.* **1996**, *39*, 1018. (f) Manning, D. D.; Strong, L. E.; Hu, X.; Beck, P. J.; Kiessling, L. L. *Tetrahedron* **1997**, *53*, 11937. (g) Roy, R.; Tropper, F. D.; Romanowska, A. *Bioconjugate Chem.* **1992**, *3*, 265.

(3) Varki, A. *Glycobiology* **1993**, *3*, 97 and references therein.

(4) (a) Muir, T. W. *Structure* **1995**, *3*, 649. (b) Lemieux, G. A.; Bertozzi, C. R. *Trends Biotechnol.* **1998**, in press.

(5) (a) Rodriguez, E. C.; Winans, K. A.; King, D. S.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 9905. (b) Cervigni, S. E.; Dumy, P.; Mutter, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1230. (c) Zhao, Y.; Kent, S. B. H.; Chait, B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1629.

(6) α-Haloacetamides: (a) Wong, S. Y. C.; Guile, G. R.; Dwek, R. A.; Arsequell, G. *Biochem. J.* **1994**, *300*, 843. (b) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1991**, 32, 6793. Bromoethyl glycosides: (c) Bengtsson, M.; Broddefalk, J.; Dahmen, J.; Henriksson, K.; Kihlberg, J.; Lönn, H.; Srinivasa, B. R.; Stenvall, K. *Glycoconjugate J.* **1998**, *15*, 223. Maleimides: (d) Hansen, P. R.; Olsen, C. E.; Holm, A. *Biooconjugate Chem.* **1998**, *9*, 126. Isothiocyanates: (e) Mullins, R. E.; Langdon, R. G. *Biochemistry* **1980**, *19*, 1199. (f) Witczak, Z. J. *Adv. Carbohydr. Chem. Biochem.* **1986**, *44*, 91.

(7) For a review on the synthesis of native glycopeptides, see: Large, D. G.; Warren, C. D. *Glycopeptides and Related Compounds: Synthesis, Analysis, and Applications*; Marcel Dekker: New York, 1997.

(8) (a) Andersson, M.; Oscarson, S. *Glycocnjugate J.* **1992**, *9*, 122. (b) Cao, S.; Tropper, F. D.; Roy, R. *Tetrahedron* **1995**, *51*, 6679.

(9) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57*, 1244.

(10) Glycosylhydrazines have been reported. They react with aldehydes to form hydrazones, but the glycosidic linkage hydrolyzes in water after several hours. By contrast, aminooxy sugars are stable in aqueous solution. (a) Takasaki, S.; Mizuochi, T.; Kobata, A. *Methods Enzymol.* **1982**, *83*, 263. (b) Tolvanen, M.; Gahmberg, C. G. *J. Biol. Chem.* **1986**, *261*, 9546.

(11) (a) Palcic, M. M. *Methods Enzymol.* **1994**, *230*, 300. (b) McGarvey, G. J.; Glenn, J.; Wong, C.-H. *Liebigs Ann.* **1997**, 1059.

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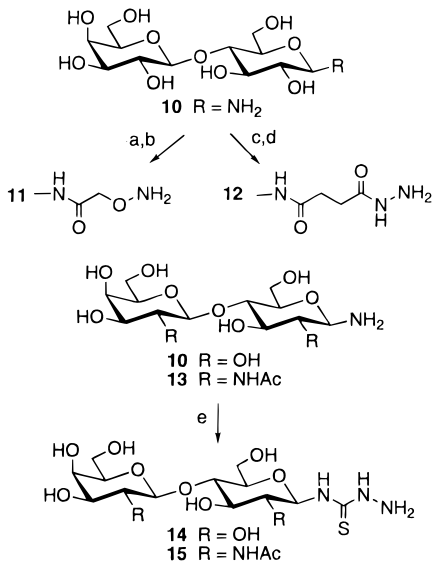
Scheme 2[a]



[a] Key: (a) $BocNHOCH_2CO_2H$, DIEA, BOP, 2 h, 41%; (b) TFA, 1.5 h, 100%; (c) monomethyl succinate, DIEA, BOP, 2 h, 48%; (d) $N_2H_4 \cdot H_2O$, 18 h, 59%; (e) (i) Cl_2CS, 0.3 M $NaHCO_3$, 20 min, (ii) $N_2H_4 \cdot H_2O$, 10 min, 50−70%. Yields are isolated after HPLC on aminopropyl silica gel.

using an α(2,3)-sialyltransferase and the glycosyl donor CMP-sialic acid. Without isolation, aminooxy sialyllactose was fucosylated using an α(1,3)-fucosyltransferase with GDP-fucose as the glycosyl donor, affording sialyl Lewis x analogue **9** (Scheme 1).[13,14] The presence of the aminooxy group did not adversely affect the course of the enzymatic reactions.

To generate complex oligosaccharide coupling partners without the use of glycosyltransferases, we required a strategy for functionalizing the reducing terminus with minimal protecting group manipulations. We therefore developed methods for the synthesis of aminooxy-, hydrazide-, and thiosemicarbazide-functionalized sugars that employ the well-known Kochetkov procedure[15] for generating glycosylamines from unprotected free-reducing sugars. β-Amino lactose (**10**, Scheme 2) was prepared as previously described.[15b] Aminooxy and hydrazide groups were then introduced in two-step processes as depicted in Scheme 2.

The most efficiently prepared saccharide derivatives for chemoselective ligation reactions were glycosyl thiosemicarbazides, prepared from the corresponding isothiocyanates[16] as shown in Scheme 2. β-Amino lactose (**10**) was reacted with thiophosgene in aqueous solution to give the corresponding isothiocyanate, which was immediately converted to thiosemicarbazide **14** by treatment with hydrazine. The same procedure when applied to β-amino chitobiose (**13**)[15b] furnished thiosemicarbazide **15**. The overall yields of the isolated thiosemicarbazides ranged from 50 to 70%. The synthesis of compound **15** highlights the utility of this procedure for transforming small amounts of precious oligosaccharides into suitable coupling partners.

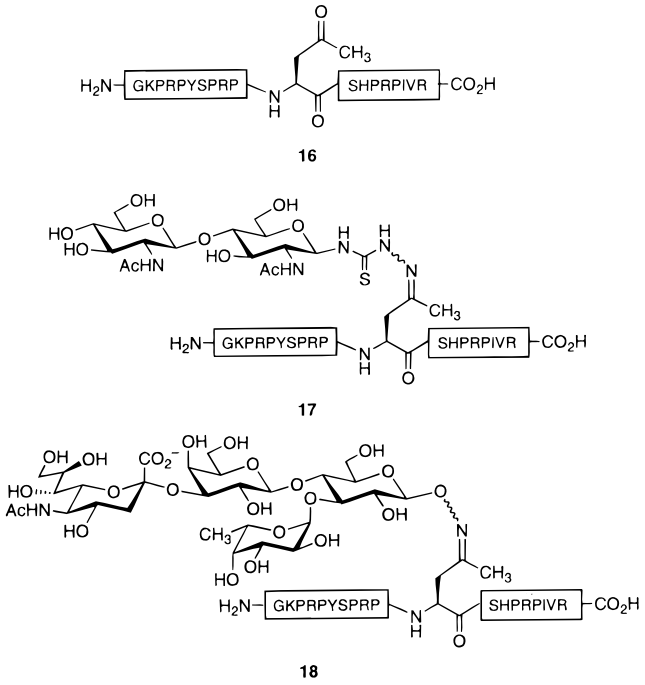


Figure 1.

Finally, we constructed neoglycopeptides containing motifs found in naturally occurring N- and O-linked glycopeptides by coupling the nucleophilic sugars to a synthetic peptide fashioned with a ketone group (**16**, Figure 1). Ketopeptide **16** was synthesized by the direct incorporation of (2*S*)-aminolevulinic acid into the peptide during Fmoc-based solid-phase synthesis, a procedure we have recently reported.[17] We reacted ketopeptide **16** with chitobiose thiosemicarbazide (**15**) to afford neoglycopeptide **17**, a structure that resembles the core chitobiosylasparagine motif shared by all N-linked glycoproteins (Figure 1).[18] The coupling reaction of ketopeptide **16** with aminooxy sialyl Lewis x analogue **9** produced neoglycopeptide **18**, which resembles the O-linked glycopeptides that function as native selectin ligands. It should be emphasized that these condensation reactions proceed in aqueous solvent without need for auxiliary reagents. In addition, subsequent purification is straightforward as the only other product of the reaction is water. The facile construction of neoglycopeptides related to native N- and O-linked structures underscores the utility of this approach for glycoconjugate synthesis.

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Supporting Information Available: Synthetic procedures and ^1H- and ^{13}C-NMR spectra for compounds **4**−**6**, **8**, **9**, **11**, **12**, **14**, and **15** (31 pages).

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(12) Simanek, E. E.; McGarvey, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833.
(13) Ichikawa, Y.; Lin, Y.-C.; Dumas, D. P.; Shen, G.-J.; Garcia-Junceda, E.; Williams, M. A.; Bayer, R.; Ketcham, C.; Walker, L. E.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 9283.
(14) The sialyltransferase and fucosyltransferase were purchased from Calbiochem.
(15) (a) Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1. (b) Vetter, D.; Gallop, M. A. *Bioconjugate Chem.* **1995**, *6*, 316.
(16) Unprotected glycosyl isothiocyanates have been used for protein modification (ref 6f), but their conversion to glycosyl thiosemicarbazides has not to our knowledge been reported.
(17) Marcaurelle, L. A.; Bertozzi, C. R. *Tetrahedron Lett.*, in press.
(18) Coupling reactions were performed in 1 M NaOAc buffer, pH 5.5, 37 °C. The neoglycopeptide products were purified by RP-HPLC and characterized by ES-MS. The yields of the coupling reactions ranged from 85 to 95%.