A Strategy for the Chemoselective Synthesis of *O*-Linked Glycopeptides with Native Sugar−Peptide Linkages

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The critical role of specific oligosaccharide structures in the biological function of many glycoproteins is now well appreciated.[1] The importance of protein-bound oligosaccharides in cell−cell recognition events[2] and in modulating protein folding and stability[3] has been highlighted in a number of recent landmark studies, inspiring the development of new synthetic methods for the construction of glycoproteins with defined, homogeneous glycoforms. Many of the difficulties inherent to the synthesis of such complex molecules, including the requirement for extensive protecting group manipulations and the chemical sensitivity of glycosidic linkages, have been elegantly addressed by several groups.[4,5] Yet, the convergent coupling of tailor-made oligosaccharides to a protein scaffold, an appealing strategy for the synthesis of complex glycoproteins, has been successful only in the construction of the amide sugar−peptide linkage found in *N*-linked glycopeptides. The extension of this approach to *O*-linked glycopeptides has been hindered by the difficulties endemic to the formation of a sugar−peptide glycosidic bond.

Here we report a strategy for the convergent synthesis of *O*-linked glycopeptide analogs with native sugar−peptide linkages using the principle of chemoselective ligation.[6] At the heart of this approach is the introduction of mutually and uniquely reactive functional groups (e.g., an aldehyde group and a hydroxylamino group) onto unprotected fragments and the coupling of these fragments in an aqueous environment. The general strategy is depicted in Figure 1. First, building block

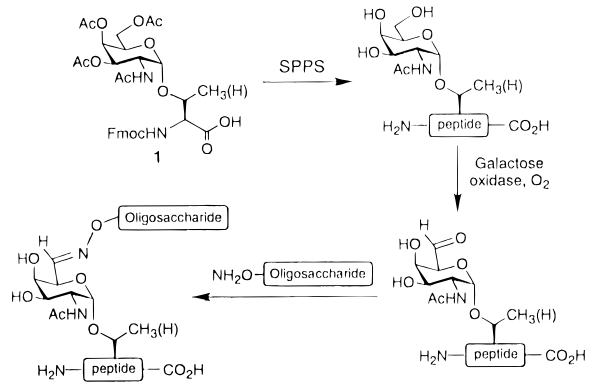

Figure 1. A general strategy for the chemoselective synthesis of *O*-linked glycopeptides with native sugar−peptide linkages.

1 [N^{α}-Fmoc-Thr/Ser(Ac_3-α-D-GalNAc)] is incorporated into a glycopeptide by solid-phase peptide synthesis (SPPS).[4c−j] Next, a chemically unique functional group for chemoselective ligation is introduced with use of the commercially available enzyme galactose oxidase,[7] which selectively converts galactose or GalNAc residues to the corresponding C-6 aldehydes. The aldehyde groups are reacted with an unprotected oligosaccharide bearing a hydroxylamino group at the reducing end, affording an oxime-linked analog of the β1→6 glycosidic linkage that is frequently observed in naturally occurring O-linked glycans.[8] This approach allows flexibility in the elaboration of outlying glycoforms while retaining the native proximal GalNAc-α-Ser/Thr linkage.

Our focus on preserving the sugar−peptide linkage was motivated by several studies suggesting a major role for the proximal GalNAc residue in modulating local peptide conformation.[9] In some glycopeptide targets, perturbation of this linkage might result in loss of native conformation and therefore function. Several methods are available for the covalent attachment of oligosaccharides to peptides through non-native linkages,[10] including chemoselective ligation of the reducing terminal aldehyde of an oligosaccharide to an N-terminal hydroxylamino group.[11] These methods may not be suitable, however, for the synthesis of glycoproteins with glycosylation-dependent active conformations.

To demonstrate this methodology we selected the insect-derived, antibacterial 19-amino acid glycopeptide drosocin, the biological activity of which is influenced by glycosylation.[12] Drosocin's potency in blocking bacterial growth is enhanced 2−8-fold (depending on the target bacterial strain) by a single *O*-linked disaccharide (Gal→GalNAc) at Thr11.[13] Threonine

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(1) (a) Bill, R. M.; Flitsch, S. L. *Chem. Biol.* **1996**, *3*, 145. (b) Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1. (c) Cumming, D. A. *Glycobiology* **1991**, *1*, 115.

(2) (a) Varki, A. *Glycobiology* **1993**, 3, 97. (b) Snell, W. J.; White, J. M. *Cell* **1996**, *85*, 629. (c) Lasky, L. A. *Annu. Rev. Biochem.* **1995**, *64*, 113. (d) Sears, P.; Wong, C.-H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12086.

(3) (a) O'Connor, S. E.; Imperiali, B. *Chem. Biol.* **1996**, *3*, 803. (b) Rickert, K. W.; Imperiali, B. *Chem. Biol.* **1995**, *2*, 751. (c) Wyss, D. F.; Choi, J. S.; Li, J.; Knoppers, M. H.; Willis, K. J.; Arulanandam, A. R. N.; Smolyar, A.; Reinherz, E. L.; Wagner, G. *Science* **1995**, *269*, 1273. (d) Gowda, D. C.; Jackson, C. M.; Kurzban, G. P.; McPhie, P.; Davidson, E. A. *Biochemistry* **1996**, *35*, 5833. (e) Garone, L.; Edmunds, T.; Hanson, E.; Bernasconi, R.; Huntington, J. A.; Meagher, J. L.; Fan, B.; Gettins, P. G. W. *Biochemistry* **1996**, *35*, 8881. (f) Live, D. H.; Kumar, R. A.; Beebe, X.; Danishefsky, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 12759.

(4) (a) Roberge, J. Y.; Beebe, X.; Danishefsky, S. J. *Science* **1995**, *269*, 202. (b) Cohen-Anisfeld, S. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 10531. (c) Meldal, M.; Bock, K. *Glycoconjugate J.* **1994**, *11*, 59. (d) Meldal, M. *Curr. Opin. Struct. Biol.* **1994**, *4*, 710. (e) Paulsen, H.; Schleyer, A.; Mathieux, N.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 281. (f) Bielfeldt, T.; Peters, S.; Meldal, M.; Bock, K.; Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 857. (g) Meinjohanns, E.; Meldal, M.; Schleyer, A.; Paulsen, H.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1996**, 985. (h) Ciommer, M.; Kunz, H. *Synlett* **1991**, 593. (i) Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *292*, 71. (j) Polt, R.; Szabó, L.; Treiberg, J.; Li, Y.; Hruby, V. J. *J. Am. Chem. Soc.* **1992**, *114*, 10249. (k) Anisfeld, S. T.; Lansbury, P. T., Jr. *J. Org. Chem.* **1990**, *55*, 5560. (l) Vetter, D.; Gallop, M. A. *Bioconjugate Chem.* **1995**, *6*, 316.

(5) (a) Witte, K.; Sears, P.; Martin, R.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 2114. (b) Schuster, M.; Wang, P.; Paulson, J. C.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1135. (c) Wong, C.-H.; Schuster, M.; Wang, P.; Sears, P. *J. Am. Chem. Soc.* **1993**, *115*, 5893. (d) Wang, L.-X.; Fan, J.-Q.; Lee, Y. C. *Tetrahedron Lett.* **1996**, *37*, 1975. (e) Imperiali, B.; Rickert, K. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 97.

(6) (a) Muir, T. W. *Structure* **1995**, *3*, 649. (b) Hilvert, D. *Chem. Biol.* **1994**, *1*, 201. (c) Canne, L. E.; Ferré-D'Amaré, A. R.; Burley, S. K.; Kent, S. B. H. *J. Am. Chem. Soc.* **1995**, *117*, 2998. (d) Lu, W.; Qasim, M. A.; Kent, S. B. H. *J. Am. Chem. Soc.* **1996**, *118*, 8518. (e) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893. (f) Liu, C.-F.; Rao, C.; Tam, J. P. *J. Am. Chem. Soc.* **1996**, *118*, 307. (g) Rose, K. *J. Am. Chem. Soc.* **1994**, *116*, 30. (h) Rose, K.; Zeng, W.; Regamey, P.-O.; Chernushevich, I. V.; Standing, K. G.; Gaertner, H. F. *Bioconjugate Chem.* **1996**, *7*, 552.

(7) (a) Amaral, D.; Bernstein, L.; Morse, D.; Horecker, B. L. *J. Biol. Chem.* **1963**, *238*, 2281. (b) Gahmberg, C. G.; Tolvanen, M. *Meth. Enzymol.* **1994**, *230*, 32.

(8) *Molecular Glycobiology*; Fukuda, M., Hindsgaul, O., Eds.; Oxford University: Oxford, 1994.

(9) (a) Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 3352. (b) The second sugar attached to a glycopeptide may also play an important role in defining the local conformation. Liang, R.; Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1995**, *117*, 10395. (c) Gerken, T. A.; Butenhof, K. J.; Shogren, R. *Biochemistry* **1989**, *28*, 5536. (d) Shogren, R.; Gerken, T. A.; Jentoft, N. *Biochemistry* **1989**, *28*, 5525.

(10) (a) Stowell, C. P.; Lee, Y. C. *Adv. Carbohydr. Chem. Biochem.* **1980**, *37*, 225. (b) Wong, S. Y. C.; Guile, G. R.; Dwek, R. A.; Arsequell, G. *Biochem. J.* **1994**, *300*, 843.

(11) (a) Cervigni, S. E.; Dumy, P.; Mutter, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1230. (b) Zhao, Y.; Kent, S. B. H.; Chait, B. T. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1629.

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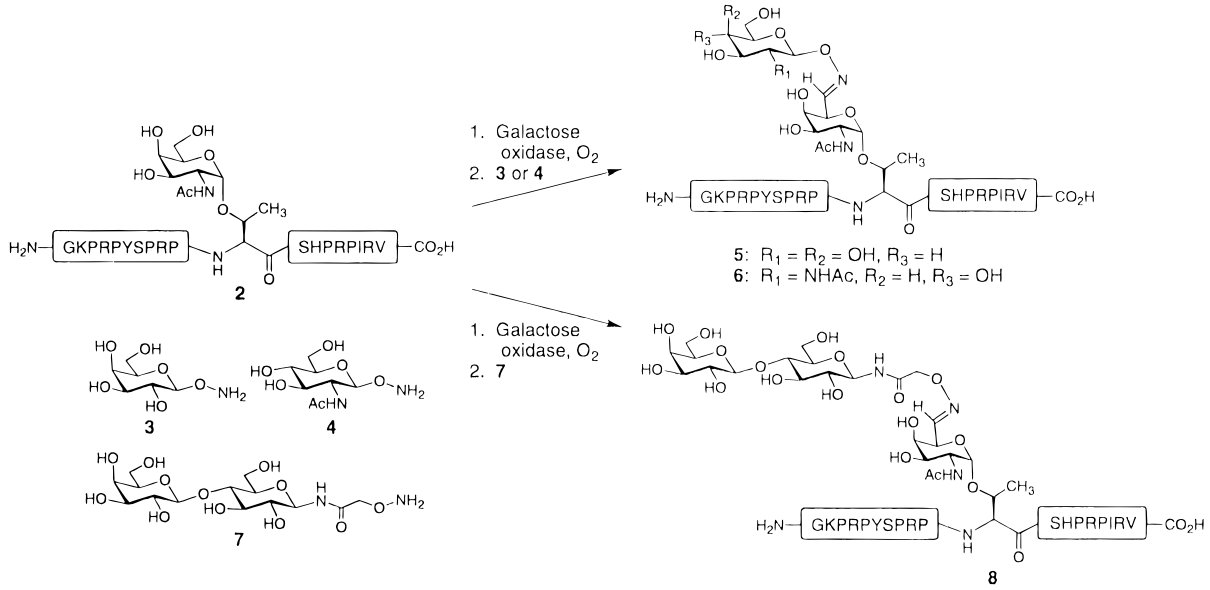
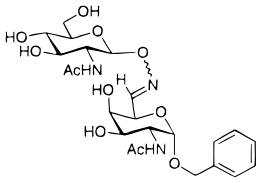
Scheme 1



derivative **1**[4h] (Figure 1) was incorporated into drosocin with use of Fmoc-based solid-phase methods to give GalNAc-drosocin **2** (Scheme 1), which was oxidized with galactose oxidase to the corresponding C-6 aldehyde (isolated yields were >70%). Hydroxylamino derivatives of galactose (**3**) and GlcNAc (**4**) were prepared from the corresponding *N*-hydroxysuccinimidoglycosides with the method of Roy and coworkers.[14] Compounds **3** and **4** were coupled with the glycopeptide aldehyde to give chemoselectively ligated products **5** and **6**, respectively (isolated yields were >80%). The glycosylation sequence of glycopeptide **5** mimics that of native drosocin. The glycan in glycopeptide **6** mimics the naturally occurring GlcNAcβ1→6GalNAc ("core 6") structure; an oxime group substitutes for the natural glycosidic bond and the linkage is extended by one atom.

Unprotected oligosaccharides, either synthetic or derived from natural sources, can be converted to the corresponding glycosylamines via Kochetkov amination.[4kl,15] Functionalization of these derivatives with a hydroxylamino group would provide access to a wide variety of oligosaccharide coupling partners. To demonstrate this approach we synthesized lactose hydroxylamine **7** by aminooxyacetylation[16] of a glycosylamine derivative. Compound **7** was coupled with the aldehyde derived from enzymatic oxidation of **2** to give chemoselectively ligated glycopeptide **8**.

We also applied the enzymatic oxidation and chemoselective ligation reactions to the simple monosaccharide α-benzyl GalNAc to obtain oxime-linked disaccharide **9** for spectroscopic comparison with oxime-linked glycopeptides (Figure 2). Both the *trans* and *cis* isomers of compound **9** were obtained in a ratio of 2.5:1 (*trans/cis*).[17] ^{1}H NMR analysis of drosocin analog **6**, bearing an identical oxime-linked disaccharide, revealed only a single isomer that was assigned the *trans* configuration based on the chemical shift of the oxime proton (*H*C═NOR, 7.69 ppm). Thus, the C-1 substituent of GalNAc appears to affect the *trans/cis* ratio of oximes formed at C-6. Conformational analysis of oligosaccharides possessing the native GlcNAcβ1→6GalNAcα1→OR structure has exposed interactions between the C-1 sustituent of GalNAc and the GlcNAc residue that affect the conformational preference of the β1→6 linkage.[18] The oxime-linked products reported here may experience similar interactions, a possibility warranting further investigation.



9 (2.5:1 *trans/cis*)

Figure 2.

Finally, we compared the relative potencies of unglycosylated drosocin and chemoselectively ligated analog **5** (the closest structural mimic to native Gal→GalNAc-drosocin) to determine the functional consequences of the unnatural oxime-linked glycan at Thr11. Glycopeptide **5** was found to be 3- to 4-fold more potent in blocking bacterial growth ($IC_{50} = 0.12 \pm 0.02$ μM) than unglycosylated drosocin ($IC_{50} = 0.40 \pm 0.05$ μM), similar to the trend observed with native glycosylated drosocin. This observation suggests that flexibility in the structure of the outlying glycoform is permitted as long as the native sugar−peptide linkage is maintained. Glycoproteins that follow this paradigm are predicted to be well suited synthetic targets for this chemoselective ligation approach.

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Supporting Information Available: Synthetic procedures and spectral data for compounds **5**−**8**, and experimental protocols and growth inhibition curves for antibacterial assays (8 pages). See any current masthead page for ordering and Internet access instructions.

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(12) (a) Bulet, P.; Dimarcq, J.-L.; Hetru, C.; Lagueux, M.; Charlet, M.; Hegy, G.; Van Dorsselaer, A.; Hoffman, J. A. *J. Biol. Chem.* **1993**, *268*, 14893. (b) Bulet, P.; Urge, L.; Ohresser, S.; Hetru, C.; Otvos, L., Jr. *Eur. J. Biochem*. **1996**, *238*, 64.

(13) The position of the linkage has not been directly determined but is suggested to be 1→3 (ref 12b).

(14) Cao, S.; Tropper, F. D.; Roy, R. *Tetrahedron* **1995**, *51*, 6679.

(15) Likhosherstov, L. M.; Novikova, O. S.; Derevitskaja, V. A.; Kochetkov, N. K. *Carbohydr. Res.* **1986**, *146*, C1.

(16) Kurth, M.; Pèlegrin, A.; Rose, K.; Offord, R. E.; Pochon, S.; Mach, J.-P.; Buchegger, F. *J. Med. Chem.* **1993**, *36*, 1255.

(17) The *trans* and *cis* isomers of compound **9** were assigned based on the chemical shift of the oxime proton (*H*C═NOR) in the ^{1}H NMR spectrum (400 MHz, D_2O) (Karabatsos, G. J.; Hsi, N. *Tetrahedron* **1967**, *23*, 1079). *Trans* isomer: 7.56 ppm (doublet, $J = 4.2$ Hz). *Cis* isomer: 6.95 ppm (doublet, $J = 4.4$ Hz).

(18) Pollex-Krüger, A.; Meyer, B.; Stuike-Prill, R.; Sinnwell, V.; Matta, K. L.; Brockhausen, I. *Glycoconjugate J.* **1993**, *10*, 365.