Chemoselective Elaboration of *O*-Linked Glycopeptide Mimetics by Alkylation of 3-ThioGalNAc

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Abstract: A critical branch point in mucin-type oligosaccharides is the $\beta 1 \rightarrow 3$ glycosidic linkage to the core α-*N*-acetylgalactosamine (GalNAc) residue. We report here a strategy for the synthesis of *O*-linked glycopeptide analogues that replaces this linkage with a thioether amenable to construction by chemoselective ligation. The key building block was a 2-azido-3-thiogalactose-Thr analogue that was incorporated into a peptide by fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis. Higher order oligosaccharides were readily generated by alkylation of the corresponding 3-thioGalNAc with *N*-bromoacetamido sugars. The rapid assembly of "core 1"and "core 3" *O*-linked glycopeptide mimetics was accomplished in this fashion.

Introduction

Mucin-type glycosylation, in which oligosaccharides are attached to proteins via the β-hydroxyl group of either serine or threonine, is a prevalent form of *O*-linked glycosylation found in mammals and other eukaryotes.[1] Mucin-type oligosaccharides may occur singly or in clusters, and may contain up to 20 monosaccharides per glycan. The initial step in the biosynthesis of this class of *O*-linked glycans is the addition of a single *N*-acetylgalactosamine (GalNAc) residue to the acceptor protein by one of several polypeptide GalNAc transferases. The resulting GalNAc(α1-*O*)Ser/Thr structure, commonly referred to as the T_N antigen (Figure 1), forms the foundation for the biosynthesis of some common "core" structures arising from branching at C-3 and/or C-6.[2] Of the eight core structures that have been identified, cores 1 and 2 are the most abundant and are widely distributed in both mucins and nonmucin glycoproteins. Other core structures, such as 3 and 4, occur less frequently and tend to be confined to mucins. Once in place, the core *O*-linked glycans may be elongated by the addition of sialic acid, fucose, and/or repeating units of galactose and *N*-acetylglucosamine (GlcNAc) to give poly-*N*-acetyllactosamine (LacNAc) chains. Together with further modifications, such as sulfation, these elaborations give rise to highly complex structures, often containing important recognition elements such as the Lewis and blood group antigens.[3]

Given the complex nature of their biosynthesis—relying on the availability of a variety of glycosyltransfersases and the corresponding sugar-nucleotide donors—it is not surprising that mucin-type glycoproteins exist as a heterogeneous mixture of glycoforms (i.e., proteins that differ only with respect to the positions and structures of the pendant glycans). As a result of this heterogeneity, it is difficult to isolate or generate (through traditional genetic methods) quantities of well-defined glycoproteins for structural and functional studies. Access to chemically defined material is especially desirable in the case of glycoprotein-based pharmaceuticals.[4] In light of this, much effort has been devoted to the synthesis of this important class of biomolecules.[5] Impressive mucin-related oligosaccharides have been synthesized by employing chemical and/or enzymatic methods, which allows their use in structural and functional studies.[6] Advances in the field of protein chemistry have also

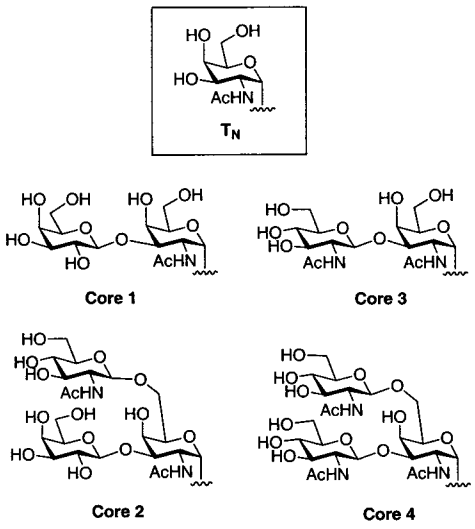

Figure 1.

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(1) (a) Carraway, K. L.; Hull, S. R. *Glycobiology* **1991**, *1*, 131. (b) Strous, G. J.; Dekker: J. *J. Crit. Rev. Biochem. Mol. Biol*. **1992**, *27*, 57. (c) Hilkens, J.; Ligtenberg, M. J. L.; Vos, H. L.; Litvinov, S. V. *Trends Biochem. Sci*. **1992**, *17*, 359. (d) Shimizu, Y.; Shaw, S. *Nature* **1993**, *366*, 630.

(2) For a review on the biosynthesis of *O*-linked glycans, see: (a) Bill, R. M.; Revers, L.; Wilson, I. B. H. *Protein Glycosylation*; Kluwer Academic: Boston, 1998; Chapter 6. (b) Brockhausen, I. In *Glycoproteins*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elvesier: Amsterdam, 1995; pp 201−259.

(3) Varki, A.; Cummings, R.; Esko, J.; Freeze, H.; Hart, G.; Marth, J. *Essentials of Glycobiology*; Cold Spring Harbor: New York, 1999; Chapter 16.

(4) Cumming, D. A. *Glycobiology* **1991**, *1*, 115.

(5) For a recent review, see: Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100*, 4495.

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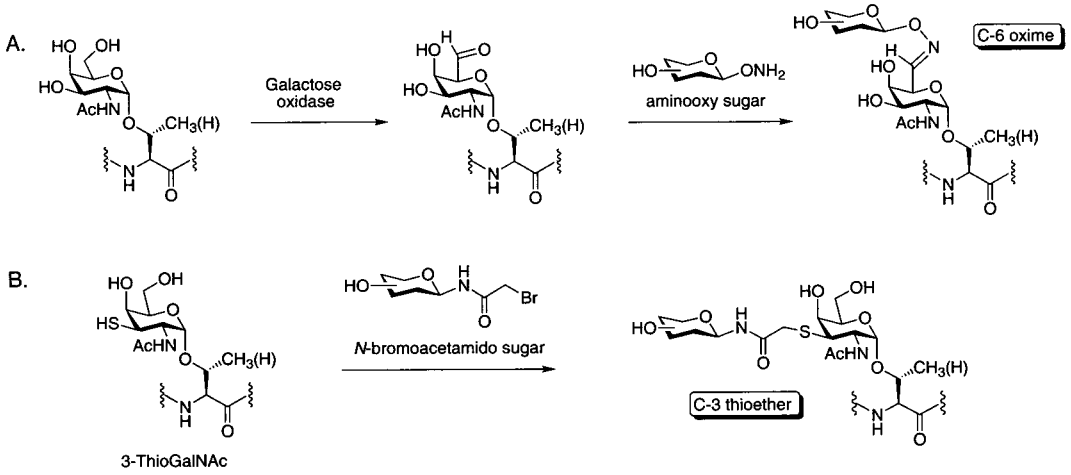


Figure 2.

made it possible to produce full-length glycoproteins using the technique of native chemical ligation.[7] Although great progress has been made in this area of research, the synthesis of mucin-type glycoproteins is still an arduous task, requiring a great deal of synthetic expertise or access to the necessary glycosyltransferases and sugar-nucleotide donors.

To address this need for homogeneous glycoproteins, we have been working to develop new methods for the synthesis of glycopeptide mimetics.[8,9] Ideally, the designed analogues should be more easily generated than the corresponding native structures yet possess the desired biological activity. Mindful of the importance of the core GalNAc(α1-*O*)Ser/Thr for establishing proper organization of the glycopeptide backbone,[10] we sought to preserve this structural element. Because it is often the peripheral sugars of *O*-linked glycans that contain the information required for molecular recognition,[3] we desired a means for presenting these "ligands" in the context of the native core structure. We reasoned that a viable route might involve replacement of glycosidic linkages to the core α-*O*-GalNAc with alternate, more facile linkages that are amenable to convergent fragment condensation. To this end, we chose to employ the principle of chemoselective ligation for the attachment of oligosaccharides to glycopeptides bearing the α-*O*-GalNAc epitope.

Analogous to enzymatic reactions, chemoselective ligation reactions are mild and selective and, thus, can be performed in the presence of richly functionalized peptides and carbohydrates.[11] The technique is based on the introduction of two mutually and uniquely reactive functional groups onto unprotected fragments and the convergent coupling of these fragments in an aqueous environment. One reaction that we have employed for the construction of glycopeptide mimetics is the coupling of ketones or aldehydes with aminooxy sugars.[8] Using this reaction, we synthesized glycopeptide analogues in which the $\beta 1 \rightarrow 6$ glycosidic linkage to α-*O*-GalNAc was replaced by an oxime ether.[8c] As shown in Figure 2A, a glycopeptide bearing the core α-*O*-GalNAc structure was subjected to oxidation by galactose oxidase to furnish the C-6 aldehyde. This uniquely functionalized peptide was then reacted with a variety of aminooxy sugars to give the corresponding oxime-linked products. By this method we were able to generate an analogue of the anti-microbial glycopeptide drosocin that retained its biological activity.

Because elongation of the core GalNAc residue of *O*-linked glycoproteins also occurs at C-3, we sought to introduce an orthogonal chemical handle at this position for coupling to appropriately functionalized sugars. The reaction of cysteine containing proteins with *N*-haloacetamido sugars has proven useful for the site-selective introduction of carbohydrates into a variety of neoglycoproteins.[12] This procedure has generally been exploited for the production of glycoproteins that mimic *N*-linked structures. Because thioether formation is compatible with and chemically orthogonal to oxime formation, we reasoned that the introduction of a thiol group at the 3-position of a peptide-bound GalNAc residue would allow for elaboration at both branch points. As an initial step toward this goal, we achieved the synthesis of *O*-linked glycopeptides bearing C-3 thioethers via alkylation of 3-thioGalNAc (Figure 2B) with *N*-bromoacetamido sugars. The syntheses of these analogues are described herein.

(6) (a) Schwarz, J. B.; Kuduk, S. D.; Chen, X.-T.; Sames, D.; Glunz, P. W.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 2662. (b) Glunz, P. W.; Hintermann, S.; Schwarz, J. B.; Kuduk, S. D.; Chen, X.-T.; Williams, L. J.; Sames, D.; Danishefsky, S. J.; Kudryashov, V.; Lloyd, K. O. *J. Am. Chem. Soc*. **1999**, *121*, 10636. (c) Glunz, P. W.; Hintermann, S.; Williams, L. J.; Schwarz, J. B.; Kuduk, S. D.; Kudryashov, V.; Lloyd, K. O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 7273. (d) Mathieux, N.; Paulsen, H.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2359. (e) Wang, Z.-G.; Zhang, X.-F.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *295*, 25; (f) Singh, L.; Nakahara, Y.; Ito, Y.; Nakahara, Y. *Tetrahedron Lett.* **1999**, *40*, 3769. (g) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2000**, *6*, 3442. (h) Leppanen, A.; Mehta, P.; Ouyang. Y.-B.; Ju, T.; Helin, J.; Moore, K. L.; van Die, I.; Canfield, W. M.; McEver, R. P.; Cummings, R. D. *J. Biol. Chem.* **1999**, *274*, 24838. (i) Koeller, K. M.; Smith, M. E.; Huang, R.-F.; Wong, C.-H. *J. Am. Chem. Soc.* **2000**, *122*, 4244. (j) Koeller, K. M.; Wong, C.-H. *Chem. Eur. J.* **2000**, *6*, 1243.

(7) (a) Marcaurelle, L. A.; Mizoue, L. S.; Wilken, J.; Oldham, L.; Kent, S. B. H.; Handel, T. M.; Bertozzi, C. R. *Chem. Eur. J.* in press. (b) Shin, Y.; Backes, B. J.; Kent, S. B. H.; Ellman, J. A.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1999**, *121*, 11684. (c) MacMillan, D.; Bertozzi, C. R. *Tetrahedron* **2000**, *56*, 9515.

(8) (a) Marcaurelle, L. A.; Bertozzi, C. R. *Chem. Eur. J.* **1999**, *5*, 1384. (b) Marcaurelle, L. A.; Rodriguez, E. C.; Bertozzi, C. R. *Tetrahedron Lett.* **1998**, *39*, 8417. (c) Rodriguez, E. C.; Winans, K. A.; King, D. S.; Bertozzi, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 9905. (d) Rodriguez, E. C.; Marcaurelle, L. A.; Bertozzi, C. R. *J. Org. Chem.* **1998**, *63*, 7134.

(9) See also: (a) Davis, B. G.; Jones, J. B. *Synlett* **1999**, *9*, 1495. (b) Davis, B. G. *J. Chem. Soc., Perkin Trans. 1* **1999**, *22*, 3215.

(10) (a) Live, D. H.; Williams, L. J.; Kuduk, S. D.; Schwarz, J. B.; Glunz, P. W.; Chen, X.-T.; Sames, D.; Kumar, R. A.; Danishefsky, S. J. *Proc. Natl. Acad. Sci.* **1999**, *96*, 3489. (b) Huang, X.; Barchi, J. J. Jr.; Lung, F.-D. T.; Roller, P. P.; Nara, P. L.; Muschik, J.; Garrity, R. R. *Biochemistry* **1997**, *36*, 10846. (c) Gerken, T.; Butenhof, K. J.; Shogren, R. *Biochemistry* **1989**, *28*, 8, 5536. (d) Liang, R.; Andreotti, A. H.; Kahne, D. *J. Am. Chem. Soc.* **1995**, *117*, 10395.

(11) For a review, see: Lemieux, G. A.; Bertozzi, C. R. *Trends Biotech.* **1998**, *16*, 506.

(12) (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.

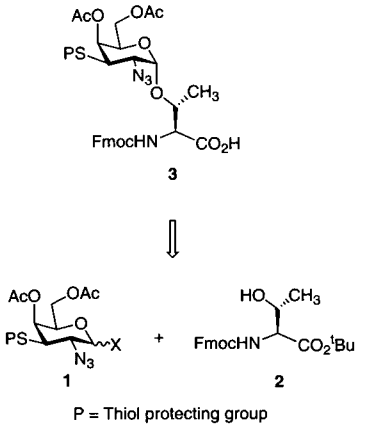
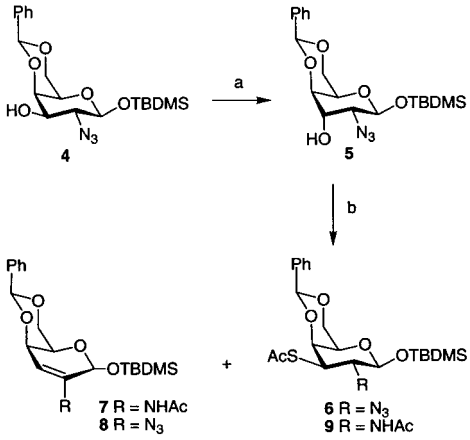


Figure 3.

Scheme 1[a]



[a] Reagents: (a) (i) Tf_2O, pyr, CH_3Cl_2, −15 °C; (ii) $TBANO_2$, CH_3CN, rt, overnight, 70%; (b) (i) KSAc, DMF, 60 °C, 5 h, 68% **7**, 8% **6**.

Results and Discussion

1. Synthesis of 2-Azido-3-ThioGal-Thr. To gain access to glycopeptides bearing the 3-thioGalNAc epitope, we required a suitably protected glycosyl amino acid for use in peptide synthesis. A key intermediate would be a glycosyl donor containing the desired thiol functionality at C-3 and an azide at C-2 (**1**, Figure 3). Glycosylation of this donor with an appropriately protected serine or threonine, such as the known threonine derivative **2**[13] (Figure 3) would yield the desired amino acid building block (**3**).

The first approach that we explored for the synthesis of the target glycosyl donor involved a double inversion at C-3 of the known benzylidene derivative **4**[14] (Scheme 1). Treatment of **4** with trifluoromethanesulfonic anhydride (Tf_2O) in the presence of pyridine, followed by reaction with tetrabutylammonium nitrite ($TBANO_2$),[15] afforded 2-azido gulose **5** in 70% yield. Subsequent 3-*O*-triflate formation and treatment with potassium thioacetate (KSAc) in DMF at 60 °C yielded the desired 3-thiogalactose derivative **6** but in very poor yield (∼8%). Instead, the major product of this reaction was the 2,3-unsaturated 2-acetamido compound **7**, which was obtained in 68% yield. Given that thioacetic acid is often used for the reductive acetylation of azides, this result was not entirely unexpected.[6a,16] Furthermore, elimination products are often obtained during displacement reactions at C-3.[17] Interestingly, the formation of neither **8** nor **9** was observed. Because an azide (or another nonparticipating amine equivalent) at C-2 was required to achieve α-selectivity in the intended glycosylation with threonine **2**, we decided to seek an alternate method for introducing the 3-thiol.

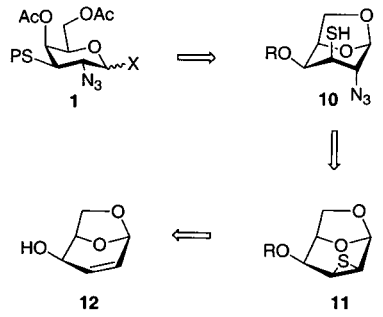


Figure 4.

As depicted in Figure 4, we envisioned that the glycosyl donor **1** could be obtained from a 1,6-anhydrosugar (**10**) via an episulfide intermediate (**11**). The rigid, bicyclic framework of 1,6-anhydrosugars renders them useful for the generation of modified carbohydrates. The constrained internal acetal serves to protect the 1- and 6-hydroxyl groups and allows for modification at positions 2, 3, and 4 in a regio- and stereoselective manner.[18] On the basis of analogous reactions with 2,3-epoxysugars,[19] we anticipated that opening of the 2,3-episulfide with azide ion would yield the desired trans diaxial product (**10**).

The precursor to episulfide **11** (the 1,6-anhydro pseudo-glycal, **12**[20]) has been prepared by a number of methods, including acid-catalyzed intramolecular Ferrier-rearrangements of D-galactal[21] and reduction of isolevoglucosenone (**13**, Scheme 2).[22] Although our attempts to employ Ferrier-type reactions were of limited success, reduction of isolevoglucosenone (**13**) proved to be a viable route. Isolevoglucosenone (**13**) was most easily prepared from tri-*O*-acetyl-D-glucal (**15**) as described by Honda et al,[23] with the exception that tetrapropylammoniumperruthenate (TPAP)/*N*-methylmorpholine *N*-oxide (NMO)[24] was used in place of MnO_2 for the oxidation of allylic alcohol **16**.[20] Owing to the instability of enone **13**, TPAP was found to be a superior oxidant, allowing for the direct conversion of **16** to **12** by a

(13) (a) Kunz, H. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed.; Marcel Dekker: New York, 1997; pp 274−275. (b) Paulsen, H.; Adermann, K. *Liebigs Ann. Chem.* **1989**, 751.

(14) Grundler, G.; Schmidt, R. R. *Liebigs Ann. Chem.* **1984**, 1826−1847.

(15) (a) Lattrell, R.; Lohaus, G. *Liebigs Ann. Chem.* **1974**, 901. (b) Eisle, T.; Schmidt, R. R. *Liebigs Ann.* **1997**, 865.

(16) (a) Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580. (b) Bieldfeldt, T.; Peters, S.; Meldal, M.; Bock, K.; Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 857.

(17) (a) Aguilera, B.; Fernández-Mayoralas, A.; Jaramillo, C. *Tetrahedron* **1997**, *53*, 5863. (b) Aguilera, B.; Fernández-Mayoralas, A. *Chem. Commun.* **1996**, 127.

(18) Cerny, M.; Stanek, *J. Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 23.

(19) (a) Paulsen, H.; Kolár, C.; Stenzel, W. *Chem. Ber.* **1978**, *111*, 2358. (b) Boons, G.-J.; Heskamp, B. In *Carbohydrate Chemistry*; Boons, G.-J., Ed.; Thomson Science: New York, 1998; pp 50−53.

(20) Ranganayakulu, K.; Singh, U. P.; Murray, T. P.; Brown, R. K. *Can. J. Chem.* **1974**, *52*, 988.

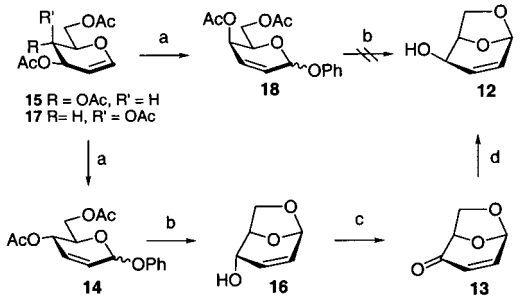
(21) (a) Oberdorfer, F.; Haeckel, R.; Lauer, G. *Synthesis* **1998**, 201. (b) Haekel, R.; Lauer, G.; Oberdorfer, F. *Synlett* **1996**, 21. (c) Sharma, G. V. M.; Ramanaiah, K. C. V.; Krishnudu, K. *Tetrahedron: Asymmetry* **1994**, *5*, 1905. (d) Mereyala, H. B.; Venkataramanaiah, K. C.; Dalvoy, V. S. *Carbohydr. Res.* **1992**, *225*, 151.

(22) (a) Köll, P.; Schultek, T.; Rennecke, R.-W. *Chem. Ber.* **1976**, *109*, 7. (b) Horton, D.; Roski, J. P.; Norris, P. *J. Org. Chem.* **1996**, *61*, 3783. (c) Taniguchi, T.; Nakamura, K.; Ogasawara, K. *Synlett*, **1996**, 971.

(23) Honda, I.; Shibagaki, M.; Kimino, H.; Takahashi, K.; Matsushita, H. (Nippon Tobacco Sangyo, Japan). Preparation of Isolevoglucosenone. Jpn. Kokai Tokyo Koho JP 05125080, 1993.

(24) For a pertinent review, see: Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, 639.

Scheme 2[a]



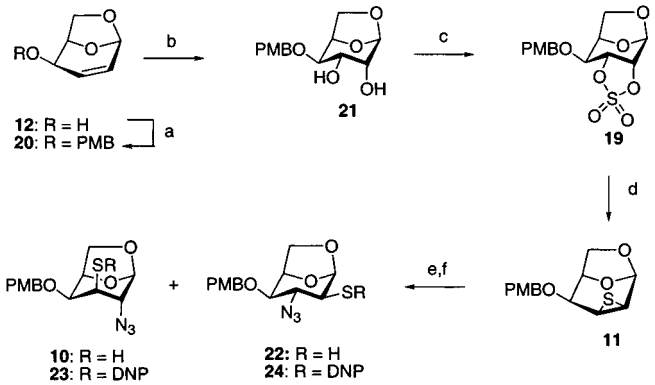
[a] Reagents: (a) PhOH, PhCl, reflux, 4 h; (b) NaOH, H_2O, reflux, 3 h, 76%; (c) TPAP, NMO, CH_2Cl_2, 0 °C to rt, 30 min; (d) $NaBH_4$, $CeCl_3$, CH_2Cl_2, −78 °C, 1 h, 70% (2 steps).

one-pot oxidation/reduction sequence. Thus, without isolation, enone **13** was immediately reduced under Luche conditions ($NaBH_4/CeCl_3$, −78 °C)[25] to give the 1,6-anhydro compound (**12**) in 70% yield. It should be noted that attempts to generate **12** directly from tri-*O*-acetyl-D-galactal (**17**) via intermediate **18** were not successful, necessitating this oxidation/reduction sequence.

With ample quantities of **12** in hand, we turned our attention to the synthesis of episulfide **11**. The most common method for the preparation of episulfides involves the opening of epoxides with thiourea or thiocyanate ions.[26] Unfortunately, such reactions are not feasible for strained cyclic substrates,[26g] such as 2,3-epoxides derived from 1,6-anhydro sugars. Thus, to gain access to episulfide **11,** we chose to use an alternate strategy based on the double inversion of cyclic sulfates.[27] Analogous to epoxides, cyclic sulfates can be opened by nucleophilic attack at either carbon center. Opening of the cyclic sulfate generates a sulfate monoester, which allows for further transformations, such as intramolecular attack by an internal nucleophile.[28] In this manner the one-pot synthesis of episulfides can be achieved via reaction with KSAc or KSCN, followed by treatment with NaOMe.[27] By this approach, episulfide **11** was generated from cyclic sulfate **19** as depicted in Scheme 3. First, allylic alcohol **12** was protected as the *p*-methoxybenzyl (PMB) ether (**20**) and subsequently treated with OsO_4/NMO at 0 °C to give diol **21** in 90% yield. Reaction of **21** with $SOCl_2$ and Et_3N produced a diastereomeric mixture of cyclic sulfites, which was directly converted to cyclic sulfate **19** by oxidation with $NaIO_4-RuCl_3\cdot 3H_2O$.[29] Finally, opening of the cyclic sulfate (**19**) was achieved by reaction with KSAc at 60 °C, followed by treatment with NaOMe to give the desired 2,3-episulfide (**11**) in high yield (91%).

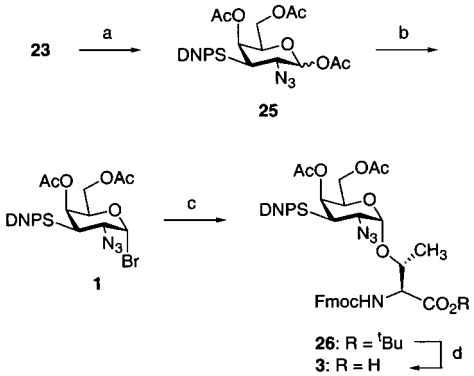
To our surprise, nucleophilic ring opening of episulfide **11** with azide ion proved to be somewhat difficult. Attempts to open the episulfide with LiN_3, $TMS-N_3$ or $TBAN_3$ under a variety of conditions did not produce the desired product,

Scheme 3[a]



[a] Reagents: (a) NaH, PMBCl, TBAI, DMF, 62%; (b) OsO_4, NMO, acetone/H_2O, 0 °C, 90%; (c) (i) $SOCl_2$, Et_3N, CH_2Cl_2; (ii) $RuCl_3\cdot H_2O$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$, 66%; (d) (i) KSAc, DMF, 60 °C, 2 h; (ii) NaOMe, MeOH, rt, 30 min, 91%; (e) $TBAN_3$, $Hg(OAc)_2$, DMF, 0 °C to rt overnight, 68% **10**, 17% **22**; (f) dinitrofluorobenzene, DIEA, CH_2Cl_2, rt, 12 h, 65% **23**, 10% **24**.

Scheme 4[a]



[a] Reagents: (a) 9:1 Ac_2O/TFA, 60 °C, 18 h, 5:1 α/β, 100%; (b) $TiBr_4$, CH_2Cl_2, rt, 14 h, 92%; (c) **2**, $AgClO_4$, collidine, 4 Å MS, CH_2Cl_2, rt, 4 h, 55%; (d) 95% aq TFA, 100%.

yielding only unreacted starting material. In the end, we found it necessary to activate the episulfide with a thiophilic promoter. Thus, treatment of **11** with $TBAN_3$ in the presence of $Hg(OAc)_2$ afforded the desired trans diaxial product **10** in 68% yield. While a small amount of the undesired diequatorial product (**22**) was also obtained in the reaction (17%), the diaxial compound could be isolated in pure form after conversion to the corresponding 2,4-dinitrophenyl (DNP)-derivative **23**. Formation of the DNP thioether served not only to aid in the purification of the desired isomer, which crystallized from CH_2Cl_2, but also to protect the 3-thiol during subsequent reactions, such as glycosylation and peptide synthesis (vide infra).

Having installed the desired thiol functionality at C-3, we then completed the synthesis of the glycosyl donor. Opening of the 1,6-anhydro ring was achieved by dissolving **23** in Ac_2O/trifluoroacetic acid (TFA) and stirring overnight at 60 °C (Scheme 4). In the event, acetolysis of the PMB ether also occurred, yielding the peracetylated compound **25** in excellent yield. Based on previous positive experience[30] with glycosyl bromides for constructing α-*O*-linked GalNAc−Thr/Ser building blocks, we chose to use bromide **1** as the glycosyl donor. Although attempts to generate the α-bromide with HBr/AcOH lead to a mixture of products, **1** could be obtained in high yield when $TiBr_4$ was employed (91%). Glycosylation of **1** with

(25) Luche, J.-L. *J. Am. Chem. Soc.* **1978**, *110*, 2226.

(26) (a) Dittmer, D. C. In *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Reese, C. W., Lowski, W., Eds.; Pergamon Press: New York, 1984; Vol. 7, p 171. (b) van Tamelen, E. E. *J. Am. Chem. Soc.* **1951**, *73*, 3444. (c) Ettinger, M. G. *J. Am. Chem. Soc.* **1950**, *72*, 4792. (d) Culvenor, C. C. J.; Davies, W.; Savige, W. E. *J. Chem. Soc.* **1952**, 4480. (e) Bordwell, F. G.; Andersen, H. M. *J. Am. Chem. Soc.* **1953**, *75*, 4959. (f) Gao, Y.; Sharpless, K. B.; *J. Org. Chem.* **1988**, *53*, 4114. (g) Fokin, A. V.; Kolomiets, A. F. *Russ. Chem. Rev.* **1975**, *44*, 138 and references therein.

(27) Calvo-Flores, F. G.; García-Mendoza, P.; Hernández-Mateo, F.; Isac García, J.; Santoyo-González, F. *J. Org. Chem.* **1997**, *62*, 3944.

(28) For recent reviews on cyclic sulfates, see: (a) Byun, H.-S.; He, L.; Bittman, R. *Tetrahedron* **2000**, *56*, 7051. (b) Kolb, H. C.; VanNieuwenhze, M. S.; Sharpless, K. B. *Chem. Rev.* **1994**, *94*, 2483. (c) Lohray, B. B. *Synthesis*, **1992**, 1035.

(29) Gao, Y.; Sharpless, K. B. *J. Am. Chem. Soc.* **1988**, *110*, 7538.

(30) Winans, K. A.; King, D. S.; Rao, V. R.; Bertozzi, C. R. *Biochemistry* **1999**, *38*, 11701.

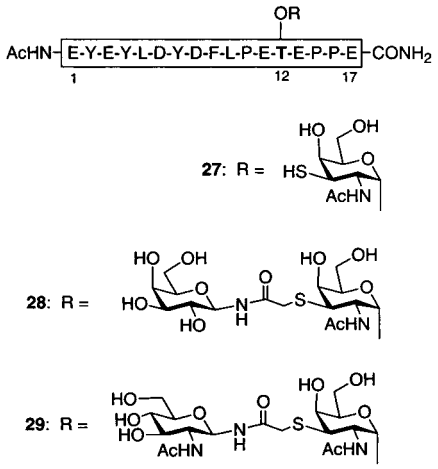


Figure 5.

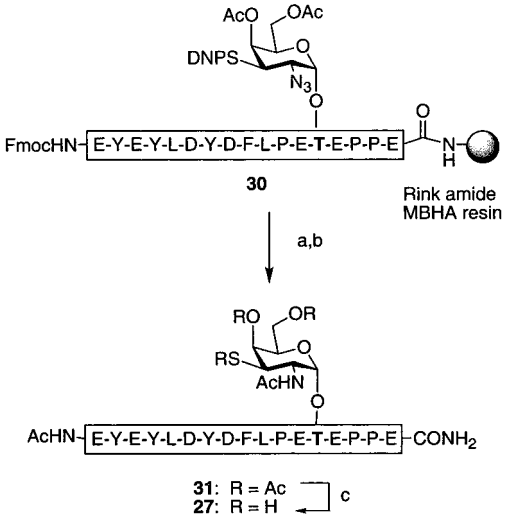
N-fluorenylmethoxycarbonyl (Fmoc) threonine *tert*-butyl ester (**2**)[13] using $AgClO_4$ as the promoter furnished the desired α-linked glycosyl amino acid (2**6**) in 55% yield. The only side product of this reaction was the hydrolyzed donor, which could be recovered and acetylated to give **25**. The DNP thioether proved to be stable in the glycosylation reaction, and formation of the undesired *β*-anomer was not observed. Finally, cleavage of the *tert*-butyl ester was accomplished by treatment with TFA to yield building block **3**, primed for use in peptide synthesis.

2. Glycopeptide Synthesis. To evaluate the stability of **3** during Fmoc-based peptide synthesis, we chose to synthesize a 17-amino acid fragment of P-selectin glycoprotein ligand-1 (PSGL-1), a dimeric membrane-bound mucin found on leukocytes that binds to the selectins and initiates the inflammation-adhesion cascade.[31] The target glycopeptide (**27**, Figure 5) corresponds to the N-terminal region of the glycoprotein, which contains a core 2 *O*-linked glycan at Thr12. The presence of this *O*-linked glycan, along with tyrosine sulfation, is essential for high-affinity binding to P-selectin.[6h,31] The target thioether analogues (**28** and **29**) correspond to core 1 and core 3 structures.

The synthesis of glycopeptide **27** was carried out on MBHA Rink amide resin on a 0.1 mmol scale using N^{α}-Fmoc-amino acids. Chain assembly to produce glycopeptide **30** (Scheme 5) was accomplished on an automated peptide synthesizer using *N*,*N*′-dicyclohexylcarbodiimide (DCC)/*N*-hydroxybenzotriazole (HOBt)-mediated couplings, except for the coupling of **3**, which was performed manually using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)/HOBt. Following deprotection and acetylation of the N-terminus, reduction of the C-2 azide in **30** was performed on resin by reaction with dithiothreitol (DTT)/DBU in DMF as described by Meldal and co-workers.[32] As anticipated, DNP removal was also accomplished in this step. Following acetylation of the resulting amine and thiol groups with Ac_2O/pyridine, cleavage from the resin was achieved by using treatment with TFA to yield acetylated glycopeptide **31** as the major product (Figure 6). After purification by RP-HPLC, **31** was treated with 10% aqueous hydrazine in the presence of DTT to afford the deacetylated target glycopeptide **27**.

3. Synthesis of *N*-Bromoacetamido Sugars. Having successfully achieved the synthesis of glycopeptide **27**, we turned our attention to the *N*-bromoacetamido sugars (**32**-**33**, Figure 7) required to generate analogues **28** and **30**. The synthesis of *N*-bromoacetamido sugars has been described previously by several groups.[12,33] Accordingly, **32** and **33** were generated as reported by Thomas[33a] via reaction of the commercially available glycosylamines of galactose and GlcNAc, respectively, with 1 equiv of bromoacetic anhydride in DMF. The resulting *N*-bromoacetamido sugars, **32** and **33**, were easily purified by crystallization from MeOH/Et_2O. Although we chose to use only simple monosaccharides for our initial thiol alkylation experiments, it should be noted that *N*-haloacetamido sugars bearing up to 10 monosaccharides have been prepared from the corresponding free sugars via their glycosylamines.[33b,c]

Scheme 5[a]



[a] Reagents: (a) (i) 20% piperidine in DMF, rt, 20 min; (ii) Ac_2O/DMF, rt, 20 min; (b) (i) DTT, DBU, DMF, rt, 30 min; (ii) Ac_2O/pyridine, rt, 20 min; (c) 10% aq N_2H_4, excess DTT, rt, 30 min.

4. Alkylation of 3-ThioGalNAc. Formation of the target thioether-linked glycopeptides was achieved by treatment of **27** with an excess of the *N*-bromoacetamido sugar (**32** or **33**) in sodium phosphate buffer (pH 7.0) at 37 °C (Figure 8). The reactions were monitored by RP-HPLC and judged to be complete after 6 h. In each case, the only product obtained was the desired thioether-linked glycopeptide. No formation of the disulfide-bound homodimer was observed. Purification of **28** and **29** was accomplished by RP-HPLC, and their identities were confirmed by ESI-MS.

One concern we had with the general approach was the potential for competitive alkylation of free cysteine residues. Indeed, in model reactions with cysteine-containing peptides, alkylation with compound **33** was found to occur at a comparable rate as that observed with 3-thioGalNAc peptide **27** (not shown). Thus, to achieve site-selectivity in glycan elongation, free cysteine residues should be avoided. Their participation in disulfide bonds or orthogonal protection would circumvent this problem.

Summary

The synthesis of thioether-linked glycopeptides was accomplished via the incorporation of a novel glycosyl amino acid (**3**) bearing a thiol group at C-3 into solid-phase peptide

(31) (a) McEver, R. P.; Cummings, R. D. *J. Clin. Invest.* **1997**, *100*, 485. (b) Epperson, T. K.; Patel, K. D.; McEver, R. P.; Cumming, R. D. *J. Biol. Chem.* **2000**, *275*, 7839. (c) Li, F.; Erickson, H. P.; James, J. A.; Moore, K. L.; Cummings, R. D.; McEver, R. P. *J. Biol. Chem.* **1996**, *271*, 6342.

(32) St. Hilaire, P. M.; Cipolla, L.; Franco, A.; Tedebark, U.; Tilly, D. A.; Meldal, M. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3559.

(33) (a) Thomas, E. W. *Methods Enzymol.* **1997**, *46*, 362. (b) Manger, I. D.; Rademacher, T. W.; Dwek, R. A. *Biochemistry* **1992**, *31*, 10742. (c) Wong, S. Y. C.; Manger, I. D.; Guile, G. R.; Rademacher, T. W.; Dwek, R. A. *Biochem. J.* **1993**, *296*, 817. (d) Black, T. S.; Kiss, L.; Tull, D.; Withers, S. G. *Carbohydr. Res.* **1993**, *250*, 195.

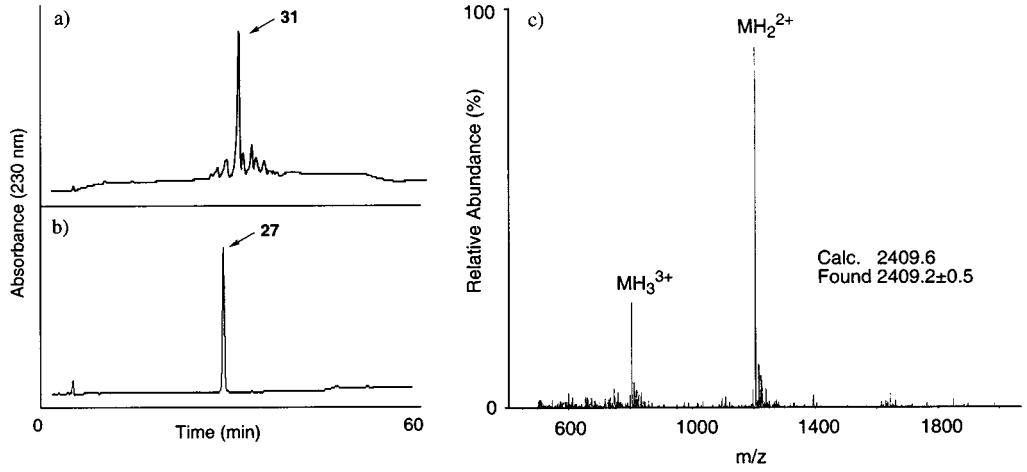


Figure 6. (a) Analytical RP-HPLC of crude glycopeptide **31** after cleavage from resin (10 → 60% CH_3CN in H_2O, 0.1% TFA, 50 min); (b) analytical RP-HPLC of purified, deacetylated glycopeptide **27** (same conditions as above); (c) electrospray mass spectrum of glycopeptide **27**.

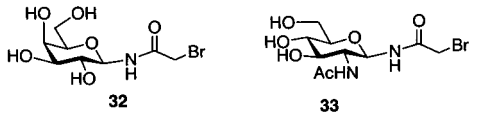


Figure 7.

synthesis. Alkylation of the 3-thiol with derivatives **32** and **33** proceeded smoothly to yield the target glycopeptides (**29** and **30**) as the only products. Building block **3** was generated in 12 steps from readily available tri-*O*-acetyl-D-glucal and FmocThrO^{t}Bu **2**.[13] A key feature of this method is that it circumvents the requirement of higher order glycosylated amino acid building blocks, the synthetic complexity of which should not be underestimated. Rather, a modest synthetic effort produces the 3-thioGalNAc building block (**3**), enabling the convergent assembly of numerous complex structures from a single precursor. It should be noted that the coupling partners, *N*-haloacetamido sugars, can be generated in two steps from unprotected oligosaccharides. Moreover, these derivatives have been prepared from large glycans (i.e., decasaccharides) that have been isolated in small quantities from natural sources.[33b,c]

Having demonstrated that amino acid **3** is compatible with Fmoc-based SPPS, we are now in position to gain access to biantennary *O*-linked glycopeptides (elaborated at both C-6 and C-3) via oxime/thioether formation. The synthesis and evaluation of these analogues will be reported in due course.

Experimental Section

General Methods N^{α}-Fmoc-amino acids, Rink amide MBHA resin, HBTU, HOBt, and DCC were purchased from Novabiochem. All other chemical reagents were obtained from commercial suppliers and used without further purification. The following solvents were distilled under a nitrogen atmosphere prior to use: THF was dried and deoxygenated over Na and benzophenone, CH_2Cl_2 and CH_3CN were dried over CaH_2, and methanol was dried over Mg and I_2. Unless otherwise noted, all air and moisture sensitive reactions were performed under an argon or nitrogen atmosphere. Analytical thin-layer chromatography (TLC) was conducted on Analtech Uniplate silica gel plates with detection by ceric ammonium molybdate (CAM), PPh_3/ninhydrin or UV light. For flash chromatography, 60-Å silical gel (Bodman) was employed. Reversed-phase high-pressure liquid chromatography (RP-HPLC) was performed on a Rainin Dynamax SD-200 HPLC system using Microsorb and Dynamax C_{18} reversed-phase columns (analytical: 4.6 × 250 mm, 1 mL/min; preparative: 25 × 250 mm, 20 mL/min) and UV detection (230 nm) was performed with a Rainin Dynamax UV-1 detector.

Infared spectra were recorded on a Perkin-Elmer FT-IR 1600 series spectrometer. ^{1}H NMR spectra were obtained at either 300 or 500 MHz with Bruker AMX 300 and DRX 500 spectrometers. Chemical shifts are reported in parts per million (δ) relative to $CHCl_3$ (7.26 ppm) for spectra run in $CDCl_3$. ^{13}C NMR spectra were obtained at 100 MHz on a Bruker DRX 500 and are reported in δ relative to $CDCl_3$ (77.00 ppm) as an internal reference. High-resolution FAB mass spectra were recorded at the Mass Spectrometry Facility at the University of California at Berkeley. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Hewlett-Packard 1100 mass spectrometer.

***Tert*-butyldimethylsilyl 2-azido-2-deoxy-4,6-*O*-benzylidene-β-D-gulose (5).** A solution of compound **4**[14] (217 mg, 0.53 mmol) in dry CH_2Cl_2 (5 mL) was cooled to −15 °C and treated with pyridine (77 μL, 0.95 mmol) and Tf_2O (143 μL, 0.85 mmol). After stirring for ∼45 min at −15 °C, the reaction was diluted with CH_2Cl_2 (20 mL) and washed with saturated (satd) aq $NaHCO_3$ (2 × 10 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude residue was redissolved in CH_3CN (5 mL) and treated with tetra-*n*-butylammonium nitrite (442 mg, 1.6 mmol). After stirring for 24 h at room temperature the solvent was evaporated in vacuo, and the resultant residue was dissolved in EtOAc (40 mL), washed with water (3 × 10 mL), and dried ($MgSO_4$). Removal of the solvent and purification of the residue by flash chromatography (hexanes:EtOAc, 6:1) gave **5** (152 mg, 70%) as a white solid: IR (film) 3469, 2928, 2856, 2110, 1724, 1393 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (m, 2H), 7.38 (m, 3H), 5.54 (s, 1H), 5.06 (d, 8.0 Hz), 4.25 (m, 1H), 4.05 (m, 2H), 3.99 (dd, 1H, J = 3.0, 1.3 Hz), 3.75 (d, 1H, J = 1.4 Hz), 3.69 (dd, 1H, J = 8.0, 3.0 Hz), 2.41 (s, 1H), 0.96 (s, 9H), 0.21 (s, 3H), 0.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 129.4, 128.6, 126.5, 126.3, 101.4, 94.9, 75.1, 70.1, 69.6, 65.9, 63.9, 25.9, 18.2, −3.8, −4.6; HRMS (FAB) $[M + Li]^+$ calcd for $C_{19}H_{29}N_3O_5SiLi$, 414.2037; found, 414.2040.

Compounds 6 and 7. A solution of compound **5** (55 mg, 0.14 mmol) in dry CH_2Cl_2 (1 mL) and pyridine (25 μL) was treated at 0 °C with Tf_2O (45 μL, 0.28 mmol) and stirred for 30 min at 0 °C and for 1 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with satd. aq $NaHCO_3$ solution (5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was redissolved in anhydrous DMF (1 mL) and treated for 5 h with KSAc (46 mg, 0.40 mmol) at 60 °C. The reaction mixture was concentrated in vacuo, redissolved in EtOAc (10 mL), washed with water (3 × 5 mL), and dried ($MgSO_4$). After removal of the solvent in vacuo, the residue was purified by flash chromatography (hexanes: EtOAc, 6:1) to yield **6** (43 mg, 68%) and **7** (5 mg, 8%). (**6**)**:** IR (film) 2926, 2111, 1694, 1362, 1254, 1164, 1084 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (dd, 2H, J = 7.5, 1.5 Hz), 7.39 (m, 3H), 5.52 (s, 1H), 4.71 (d, 1H, J = 7.5 Hz), 4.26 (dd, 1H, J = 12.5, 1.5 Hz), 4.03 (dd, 1H, J = 12.5, 2.0 Hz), 3.99 (d, 1H, J = 2.0 Hz), 3.74 (dd, 1H, J = 12.0, 3.5 Hz), 3.57 (m, 2H), 2.41 (s, 3H), 0.94 (s, 9H), 0.20 (s, 3H), 0.19 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.5, 129.0, 128.2, 126.1, 101.1, 98.9, 74.9, 69.1, 63.1, 46.5, 30.5, 25.6, 18.0, −4.1, −5.0; HRMS (FAB) $[M + Li]^+$ calcd for $C_{21}H_{31}N_3O_5SSiLi$, 472.1914; found, 472.1922. (**7**): IR (film) 3373, 2928, 2855, 2357, 1666, 1516, 1400,

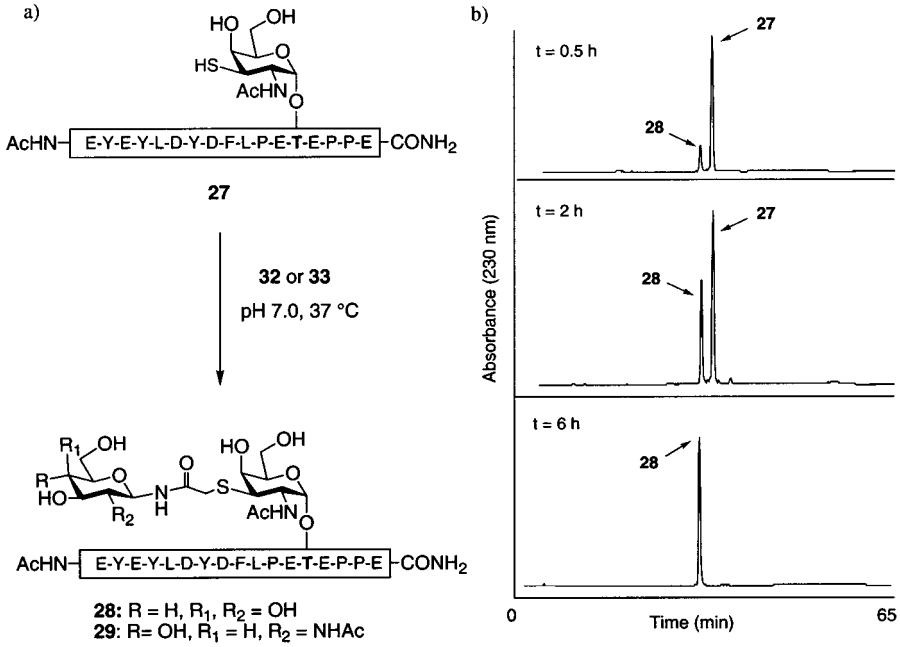


Figure 8. (a) Alkylation of **27** with **32** or **33** to give analogs **28** and **29**; (b) RP-HPLC analysis of ligation reaction between **27** and **32** at various time points (10 → 60% CH_3CN in H_2O, 0.1% TFA, 50 min). Similar results were obtained for the alkylation of **27** with **33** to give **29**.

1367, 1254, 1158, 1093 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, 2H, J = 9.5, 2.0 Hz), 7.34 (m, 3H), 7.07 (s, 1H), 6.60 (d, 1H, J = 5.5 Hz), 5.56 (s, 1H), 5.35 (s, 1H), 4.39 (app dt, 1H, J = 5.5, 2.0 Hz), 4.32 (d, 1H, J = 12.5 Hz), 4.17 (dd, 1H, J = 12.5, 2.5 Hz), 3.53 (s, 1H), 2.06 (s, 3H), 0.95 (s, 9H), 0.25 (s, 3H), 0.24 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.1, 128.9, 128.2, 126.4, 125.8, 107.5, 100.9, 92.8, 69.9, 69.0, 66.9, 25.9, 25.6, 24.5, 18.0, −3.7, −4.2; HRMS (FAB) $[M + Li]^+$ calcd for $C_{21}H_{31}NO_5SiLi$, 412.2132; found, 412.2140.

Modified Procedure[23] for the Synthesis of 1,6-Anhydro-2,3-dideoxy-*β-threo*-hex-2-enopyranose (12).[20] Tri-*O*-acetyl-D-glucal (15 g, 55 mmol) and phenol (40 g) were dissolved in chlorobenzene (300 mL) and heated at reflux for 4 h. The solvent and excess phenol were removed under high vacuum, and the resulting residue was dissolved in CH_2Cl_2, washed with 1 M aq NaOH and brine, and dried ($MgSO_4$) to give **14**[23] as a mixture of anomers. Without purification, **14** was combined with 10 g of NaOH and heated at reflux for 3 h in H_2O (200 mL). After cooling to room temperature, the mixture was extracted several times with EtOAc, dried ($MgSO_4$), and concentrated. Purification of the resulting residue by flash chromatography (hexanes:EtOAc, 2:1 → 1:2) gave **12**[20] (5.2 g, 76%) as a colorless oil. Compound **12** (4.7 g, 36.7 mmol) was dissolved in dry CH_2Cl_2 (75 mL) in the presence of 4-Å molecular sieves (9 g) and NMO (6.45 g, 55.1 mmol). After cooling to 0 °C, TPAP (645 mg, 1.80 mmol) was slowly added. The mixture was stirred at room temperature for 1 h and then filtered through a short plug of silica gel (CH_2Cl_2). Fractions containing the desired enone (**13**)[22] were combined, concentrated to a total volume of 80 mL, and cooled to −78 °C. A solution of $CeCl_3$ in MeOH (96 mL, 0.4 M) was added, and the mixture was stirred at −78 °C for 30 min. $NaBH_4$ (1.4 g, 37.0 mmol) was added portionwise over a period of 10 min and stirring was continued until TLC (hexanes:EtOAc, 1:1) showed complete consumption of **13**. The reaction was quenched with acetone and concentrated in vacuo. The residue was purified by flash chromatography (hexanes:EtOAc, 1:1) to give **12** (3.3 g, 70%) as a colorless oil: ^1H NMR (300 MHz, $CDCl_3$) δ 5.85 (ddd, 1H, J = 9.7, 3.0, 1.7 Hz), 5.67 (ddt, 1H, J = 9.7, 1.9, 1.4 Hz), 5.47 (d, 1H, J = 3.0 Hz), 4.74 (m, 1H), 4.46 (m, 1H), 4.15 (dd, 1H, J = 8.1, 2.0 Hz), 3.87 (app t, 1H, J = 6.8 Hz), 2.04 (br s, 1H). All data are in agreement with literature reports.[19]

1,6-Anhydro-4-*O*-*p*-methoxybenzyl-2,3-dideoxy-*β-threo*-hex-2-enopyranose (20). To a solution of **12** in anhydrous DMF (150 mL) was added NaH (1.23 g, 51.2 mmol) and TBAI (473 mg, 1.28 mmol) at 0 °C. After stirring for 10 min, *p*-methoxylbenzyl chloride (7.0 mL, 51.2 mmol) was added. After stirring at room temperature for 12 h, water (50 mL) was added, and the mixture was concentrated in vacuo. Purification of the residue by flash chromatography (hexanes:EtOAc, 5:1) yielded compound **20** (4.2 g, 62%) as a colorless oil: IR (film) 3041, 2970, 2902, 2862, 2836, 1730, 1612, 1513, 1465, 1383, 1249, 1174, 1122, 1087, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.25 (d, 2H, J = 8.7 Hz), 6.89 (d, 2H, J = 8.7 Hz), 5.86 (ddd, 1H, J = 9.8, 3.0, 1.3 Hz), 5.77 (ddt, 1H, J = 9.8, 1.9, 0.7 Hz), 5.48 (d, 1H, J = 3.1 Hz), 4.59 (d, 1H, J = 11.6 Hz), 4.51 (m, 2H), 4.48 (d, 1H, J = 11.6 Hz), 4.23 (m, 1H), 3.84 (m, 1H), 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 130.0, 129.3, 128.2, 127.5, 113.9, 96.0, 74.6, 73.7, 71.0, 62.8, 55.3; HRMS (FAB) $[M^+]$ calcd for $C_{14}H_{16}O_4$, 248.1049; found, 248.1049.

1,6-Anhydro-4-*O*-*p*-methoxybenzyl-*β*-D-gulopyranose (21). To 4.2 g (20 mmol) of **20** in acetone (80 mL) was added 3.96 g of NMO (33.8 mmol) followed by water (10 mL). The mixture was cooled to 0 °C, and a solution of OsO_4 in *t*-BuOH (7 mL, 2.5wt %/v) was added. After stirring for 3 h, saturated Na_2SO_3 (50 mL) was added. The reaction mixture was then extracted with Et_2O (3 × 100 mL), washed with brine (100 mL), dried ($MgSO_4$), and concentrated in vacuo. Purification of the residue by flash chromatography (hexanes:EtOAc, 1:1) yielded diol **21** (4.3 g, 90%) as a white solid: TLC (EtOAc:hexanes, 2:1) R_f = 0.39; IR (film) 3424, 2895, 1611, 1514, 1302, 1249, 1181, 1130, 1089, 1025 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, 2H, J = 8.5 Hz), 6.88 (d, 2H, J = 8.5 Hz), 5.41 (d, 1H, J = 2.0 Hz), 4.67 (d, 1H, J = 11.6 Hz), 4.59 (d, 1H, J = 11.6 Hz), 4.39 (app t, 1H, J = 4.4 Hz), 4.00 (d, 1H, J = 7.6 Hz), 3.77 (m, 2H), 3.80 (s, 3H), 3.60 (m, 2H), 2.41 (br s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 130.0, 129.4, 114.0, 101.0, 77.6, 72.5, 72.4, 70.7, 69.5, 63.9, 55.3; HRMS (FAB) $[M + Li]^+$ calcd for $C_{14}H_{18}O_6Li$, 289.1263; found, 289.1260.

1,6-Anhydro-4-*O*-*p*-methoxybenzyl-*β*-D-gulopyranose 2,3-cyclic sulfate (19). An ice-cooled and magnetically stirred solution of diol **21** (4.28 g, 15.1 mmol) and Et_3N (8.4 mL, 60 mmol) in dry CH_2Cl_2 (60 mL) was added to a solution of $SOCl_2$ (1.66 mL, 22.7 mmol) in CH_2Cl_2 (40 mL) over a period of 10 min. Stirring was continued at 0 °C until TLC (hexanes:EtOAc, 1:1) indicated complete disappearance of the starting material. The mixture was diluted with CH_2Cl_2 (200 mL) and washed with water (2 × 100 mL) and brine (100 mL). The organic solution was dried (Na_2SO_4) and concentrated in vacuo to afford a mixture of the cyclic sulfites, which were purified by a short column of silica gel (hexanes:EtOAc, 4:1) to ensure removal of any residual Et_3N. To a solution of the cyclic sulfites in a 1:1 mixture of CH_3CN/CCl_4 (100 mL) was added $NaIO_4$ (6.46 g, 30.2 mmol), followed by a catalytic amount of $RuCl_3 \bullet 3H_2O$ (150 mg) and water (50 mL). The

reaction mixture was stirred vigorously for 15 min at room temperature and then diluted with ether (800 mL). The organic layer was washed with water (2 × 200 mL) and brine (200 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification of the crude product by column chromatography (hexanes:EtOAc, 4:1) gave cyclic sulfate **19** (3.6 g, 66%) as a white solid: TLC (hexanes:EtOAc, 2:1) $R_f = 0.62$; IR (film) 2909, 2839, 1612, 1514, 1390, 1251, 1212, 1136, 1101, 1040 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26 (d, 2H, $J = 8.7$ Hz), 6.90 (d, 2H, $J = 8.7$ Hz), 5.67 (d, 1H, $J = 1.5$ Hz), 4.92 (app t, 1H, $J = 6.3$ Hz), 4.84 (dd, 1H, $J = 6.4$, 1.6 Hz), 4.72 (d, 1H, $J = 11.4$ Hz), 4.54 (d, 1H, $J = 11.3$ Hz), 4.52 (app t, 1H, $J = 4.6$ Hz), 4.18 (app t, 1H, $J = 6.1$ Hz), 4.04 (dd, 1H, $J = 8.2$, 0.8 Hz), 3.82 (s, 3H), 3.69 (dd, 1H, $J = 8.2$, 5.3 Hz); ^{13}C NMR (100 Hz, $CDCl_3$) δ 159.8, 129.8, 128.5, 114.0, 96.6, 83.2, 77.5, 74.5, 72.6, 72.5, 63.8, 55.3; HRMS (FAB) [M^+] calcd for $C_{14}H_{16}O_8S$, 344.0566; found, 344.0563.

1,6:2,3-Dianhydro-2,3-epithio-4-*O*-*p*-methoxybenzyl-β-D-talopyranose (11). To a solution of cyclic sulfate **19** (3.57 g, 9.96 mmol) in anhydrous DMF (50 mL) was added 1.7 g (15 mmol) of KSAc. The resultant mixture was stirred at 60 °C until TLC analysis (hexanes:EtOAc, 2:1) showed complete conversion of the cyclic sulfate into baseline material (2 h). The mixture was concentrated and dissolved in anhydrous MeOH (180 mL). A solution of NaOMe (30 mL, 1 M in MeOH) was added, and the mixture was stirred at room temperature until TLC (hexanes:EtOAc, 2:1) showed complete disappearance of the starting material (30 min). A saturated solution of NH_4Cl (100 mL) was added, and the MeOH was removed in vacuo. Water (100 mL) was added, and the resulting solution was extracted with EtOAc (3 × 300 mL). The organic layers were collected, dried ($MgSO_4$), and evaporated. Purifcation of the crude product by flash chromatogaphy (hexanes:EtOAc, 5:1) yielded episulfide **11** (2.5 g, 91%) as a yellow syrup: TLC (hexanes:EtOAc, 2:1) $R_f = 0.59$; IR (film) 2959, 2899, 2840, 2356, 1608, 1508, 1462, 1302, 1243, 1137, 1110, 1091 cm^{-1};^1H NMR (300 MHz, $CDCl_3$) δ 7.31 (d, 2H, $J = 8.3$ Hz), 6.90 (d, 2H, $J = 7.8$ Hz), 5.87 (d, 1H, $J = 3.9$ Hz), 4.80 (d, 1H, $J = 11.3$ Hz), 4.56 (d, 1H, $J = 11.3$ Hz), 4.33 (m, 2H), 4.12 (d, 1H, $J = 7.4$ Hz), 3.81 (s, 3H), 3.58 (dd, 1H, $J = 6.7$, 4.0 Hz), 3.45 (app t, 1H, $J = 6.7$ Hz), 3.09 (app t, 1H, $J = 6.2$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.5, 129.6, 129.5, 113.9, 97.9, 72.0, 69.8, 69.9, 62.8, 55.3, 43.1, 30.0; HRMS (FAB) [M^+] calcd for $C_{14}H_{16}O_4S$, 280.0769; found, 280.0764.

1,6-Anhydro-2-azido-2-deoxy-4-*O*-*p*-methoxybenzyl-3-thio-β-D-galactopyranose (10) and 1,6-Anhydro-3-azido-3-deoxy-4-*O*-*p*-methoxybenzyl-2-thio-β-D-idopyranose (22). Episulfide **11** (2.53 g, 9.0 mmol) was dissolved in anhydrous DMF (45 mL) and cooled to 0 °C. $TBAN_3$ (14.4 g, 45.0 mmol) was added, followed by $Hg(OAc)_2$ (2.4 g, 9.0 mmol). The reaction mixure was allowed to warm slowly to room temperature. After stirring fo 24 h, the reaction was quenched with an excess of β-mercaptoethanol (2.0 mL) and concentrated in vacuo. The residue was dissolved in EtOAc (400 mL) and washed with water (2 × 100 mL) and brine (100 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification of the crude product by flash chromatography (hexanes:EtOAc, 7:1) gave **10** (1.96 g, 68%) and **22** (0.49 g, 17%) as colorless oils. (In practice, compounds **10** and **22** can be used as a mixture in the next reaction. The DNP derivative of the diaxial isomer (**23**) can be isolated in pure form by precipitation from CH_2Cl_2, while the undesired isomer (**24**) remains in solution.) **(10):** TLC (hexanes:EtOAc, 3:1) $R_f = 0.59$; IR (film) 2957, 2901, 2869, 2836, 2568, 2102, 1611, 1514, 1250, 1130, 1033 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, 2H, $J = 8.7$ Hz), 6.90 (d, 2H, $J = 8.7$ Hz), 5.42 (app s, 1H), 4.64 (d, 1H, $J = 11.2$ Hz), 4.63 (d, 1H, $J = 7.8$ Hz), 4.46 (d, 1H, $J = 11.2$ Hz), 4.43 (app t, 1H, $J = 4.6$ Hz), 3.96 (dd, 1H, $J = 6.4$, 4.5 Hz), 3.81 (s, 3H), 3.68 (app s, 1H), 3.58 (m, 2H), 2.24 (d, 1H, $J = 7.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 129.6, 129.1, 114.0, 100.9, 73.8, 71.6, 70.5, 66.0, 64.1, 55.3, 38.9; HRMS (FAB) [M + Li]$^+$ calcd for $C_{14}H_{17}N_3O_4SLi$, 330.1099; found, 330.1098. **(22):** $R_f = 0.50$**;** ^1H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, 2H, $J = 8.7$ Hz), 6.90 (d, 2H, $J = 8.7$ Hz), 5.33 (d, 1H, $J = 1.3$ Hz), 4.67 (d, 1H, $J = 11.3$ Hz), 4.55 (d, 1H, $J = 11.3$ Hz), 4.40 (app t, 1H, $J = 4.5$ Hz), 4.07 (d, 1H, $J = 7.8$ Hz), 3.81 (s, 3H), 3.69 (dd, 1H, $J = 7.2$, 5.2 Hz), 3.54 (ddd, 1H, $J = 8.7$, 4.0, 1.0 Hz), 3.41 (dd 1H, $J = 9.8$, 8.8 Hz), 2.57 (app t, 1H, $J = 10.4$ Hz), 1.76 (d, 1H, $J = 11.0$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 129.6, 129.3, 114.0, 103.2, 78.0, 73.2, 72.8, 68.3, 65.3, 55.3, 46.4; HRMS (FAB) [M + Li]$^+$ calcd for $C_{14}H_{17}N_3O_4SLi$, 330.1099; found, 330.1098.

1,6-Anhydro-2-azido-2-deoxy-3-*S*-(2,4-dinitrophenyl)-4-*O*-*p*-methoxybenzyl-3-thio-β-D-galactopyranose (23) and 1,6-Anhydro-3-azido-3-deoxy-2-*S*-(2,4-dinitrophenyl)-4-*O*-*p*-methoxybenzyl-2-thio-β-D-idopyranose (24). A mixture of compounds **10** and **22** (1.24 g, 3.80 mmol) was dissolved in dry CH_2Cl_2 (40 mL) and treated with 2,4-dinitrofluorobenzene (784 mg, 4.20 mmol) in the presence of DIEA (1.0 mL, 5.8 mmol) at room temperature. After stirring for 18 h, the solution was concentrated in vacuo. Compound **23** (1.2 g, 65%) was isolated as a bright yellow solid by trituration with ice-cold CH_2Cl_2. The undesired diequatorial product (**24)** was obtained by evaporation of the filtrate and purification of the resulting residue by flash chromatography (hexanes:EtOAc, 6:1) (180 mg, 10%). **(23):** IR (film) 2962, 2358, 2103, 1592, 1514, 1340, 1250, 1133 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 8.93 (d, 1H, $J = 2.5$ Hz), 8.22 (dd, 1H, $J = 9.0$, 2.6 Hz), 7.55 (d, 1H, $J = 9.2$ Hz), 7.14 (d, 1H, $J = 8.7$ Hz), 6.82 (d, 1H, $J = 8.7$ Hz), 5.52 (s, 1H), 4.62 (d, 1H, $J = 8.1$ Hz), 4.53 (m, 3H), 4.29 (dd, 1H, $J = 6.9$, 4.0 Hz), 3.95 (d, 1H, $J = 6.9$ Hz), 3.80 (s 3H), 3.73 (dd, 1H, $J = 7.5$, 4.0 Hz), 3.68 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.8, 146.4, 145.7, 144.2, 129.7, 128.6, 128.4, 126.6, 121.2, 114.0, 100.1, 73.1, 72.2, 70.9, 64.5, 63.7, 55.2, 47.6; LRMS (FAB) [M + Li]$^+$ $C_{20}H_{19}N_5O_8SLi$, 496.2. **(24):** ^1H NMR (300 MHz, $CDCl_3$) δ 9.01 (s, 1H, $J = 2.5$ Hz), 8.35 (dd, 1H, $J = 9.0$, 2.5 Hz), 7.79 (d, 1H, $J = 9.1$ Hz), 7.29 (d, 1H, $J = 8.7$ Hz), 6.91 (d, 1H, $J = 8.7$ Hz), 5.45 (s, 1H), 4.69 (d, 1H, $J = 11.4$ Hz), 4.61 (d, 1H, $J = 11.5$ Hz), 4.48 (m, 1H), 4.18 (d, 1H, $J = 8.0$ Hz), 3.83 (s, 3H), 3.76 (m, 3H), 3.28 (d, 1H, $J = 9.5$ Hz); LRMS (FAB) [M + Li]$^+$ $C_{20}H_{19}N_5O_8SLi$, 496.2.

1,4,6-Tri-*O*-acetyl-2-azido-2-deoxy-3-*S*-(2−4-dinitrophenyl)-3-thio-α/β-D-galactopyranose (25). Compound **23** (938 mg, 1.92 mmol) was dissolved in 100 mL of Ac_2O/TFA (9:1) and stirred at 60 °C for 22 h. The reaction mixture was coevaporated with toluene (3 × 20 mL), and the resulting residue was purified by flash chromatography (hexanes:EtOAc, 5:1 → 3:1) to give **25α** (822 mg, 83%) and **25β** (164 mg, 17%). **(25α):** IR (film) 3098, 2111, 1747, 1588, 1521, 1362, 1343, 1210, 1137, 1044, 1011 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 9.00 (d, 1H, $J = 2.5$ Hz), 8.44 (dd, 1H, $J = 8.9$, 2.5 Hz), 7.79 (d, 1H, $J = 8.9$ Hz), 6.44 (d, 1H, $J = 3.3$ Hz), 5.44 (app s, 1H), 4.35 (app t, 1H, $J = 6.5$ Hz), 4.08 (dd, 1H, $J = 11.5$, 6.3 Hz), 4.00 (m, 2H), 3.95 (dd, 1H, $J = 11.9$, 3.2 Hz), 2.24 (s, 3H), 2.16 (s, 3H), 2.02 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.6, 168.4, 147.3, 145.1, 141.9, 129.0, 127.1, 121.6, 90.0, 69.3, 66.7, 61.7, 58.1, 47.5, 21.0, 20.6, 20.3; HRMS (FAB) [M + Li]$^+$ calcd for $C_{18}H_{19}N_5O_{11}SLi$, 520.0962; found, 520.0966. **(25β):** ^1H NMR (500 MHz, $CDCl_3$) δ 9.00 (d, 1H, $J = 2.5$ Hz), 8.43 (dd, 1H, $J = 8.9$, 2.5 Hz), 7.80 (d, 1H, $J = 8.9$ Hz), 5.72 (d, 1H, $J = 8.0$ Hz), 5.40 (d, 1H, $J = 2.5$ Hz), 4.11 (m, 3H), 3.91 (dd, 1H, $J = 12.0$, 8.5 Hz), 3.57 (dd, 1H, $J = 11.5$, 3.0 Hz), 2.26 (s, 3H), 2.22 (s, 3H), 2.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.6, 168.6, 147.0, 145.1, 142.1, 129.3, 127.1, 121.5, 94.4, 66.6, 61.5, 60.8, 50.9, 20.9, 20.6, 20.3; HRMS (FAB) [M + Li]$^+$ calcd for $C_{18}H_{19}N_5O_{11}SLi$, 520.0962; found, 520.0966.

4,6-Di-*O*-acetyl-2-azido-2-deoxy-3-*S*-(2,4-dinitrophenyl)-3-thio-α-D-galactopyranosyl bromide (1). A solution of **25** (768 mg, 1.50 mmol) in anhydrous CH_2Cl_2 (20 mL) was stirred for 12 h in the presence of $TiBr_4$ (1.37 g, 3.7 mmol), then diluted with CH_2Cl_2 (300 mL), washed with ice water (2 × 100 mL), dried (Na_2SO_4), and concentrated. The residue was promptly purified by flash chromatography (hexanes:EtOAc, 3:1) to give the bromide **1** (734 mg, 92%) as a yellow foam, which was dried under high vacuum and stored at −20 °C until use: ^1H NMR (300 MHz, $CDCl_3$) δ 9.00 (d, 1H, $J = 2.5$ Hz), 8.45 (dd, 1H, $J = 8.9$, 2.5 Hz), 7.87 (d, 1H, $J = 9.0$ Hz), 6.67 (d, 1H, $J = 3.0$ Hz), 5.50 (s, 1H), 4.50 (app t, 1H, $J = 6.5$ Hz), 4.18 (dd, 1H, $J = 11.7$, 5.6 Hz), 4.06 (m, 2H), 4.08 (dd, 1H, $J = 11.0$, 2.4 Hz), 2.19 (s, 3H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.3, 169.3, 146.8, 145.1, 142.1, 129.0, 127.4, 121.5, 90.5, 72.2, 67.2, 61.2, 60.7, 48.2, 20.6, 20.3.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-3-S−(2,4-dinitrophenyl)-3-thio-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (26).** To a flame-dried flask containing 4 Å molecular sieves (1.0 g) was added dry CH_2Cl_2 (10 mL), $AgClO_4$ (440 mg, 2.1 mmol), 2,4,6-collidine (290 μL, 2.1 mmol) and *N*-Fmoc-

threonine-O^{t}Bu (**2**)[13] (495 mg, 1.25 mmol). The mixture was stirred at room temperature for 10 min. A solution of bromide **1** (734 mg, 1.37 mmol) in dry CH_2Cl_2 (10 mL) was added slowly over 30 min via syringe. After stirring for 5 h at room temperature, the reaction was diluted with CH_2Cl_2 and filtered through Celite. The precipitate was thoroughly washed with CH_2Cl_2, and the filtrate was evaporated. Purification of the resulting residue by flash chromatography (hexanes: EtOAc, 4:1 → 3:1) afforded **25** (534 mg, 55%) as a yellow foam: IR (film) 2979, 2939, 2358, 2108, 1745, 1595, 1522, 1450, 1370, 1342, 1218, 1153, 1128, 1049 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) (major rotamer) δ 8.98 (d, 1H, J = 2.0 Hz), 8.43 (dd, 1H, J = 9.0, 2.0 Hz), 7.81 (d, 1H, J = 9.0 Hz), 7.75 (d, 2H, J = 7.5 Hz), 7.59 (m, 2H), 7.39 (m, 2H), 7.28 (m, 2H), 5.62 (d, 1H, J = 9.5 Hz), 5.39 (app s, 1H), 5.17 (d, 1H, J = 3.5 Hz), 4.50 (dd, 1H, J = 10.5, 7.0 Hz), 4.34 (m, 3H), 4.23 (m, 1H), 4.11 (m, 4H), 3.70 (dd, 1H, J = 12.0, 3.0 Hz), 2.14 (s, 3H), 2.03 (s, 3H), 1.52 (s, 9H), 1.40 (d, 3H, J = 6.5 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 171.1, 170.3, 169.7, 169.1, 156.7, 146.9, 144.9, 143.7, 143.5, 142.2, 141.3, 129.0, 128.9, 127.8, 127.3, 127.0, 126.9, 125.0, 124.9, 121.6, 120.0, 98.5, 98.3, 83.2, 68.1, 67.5, 67.4, 67.3, 67.2, 62.2, 62.1, 60.4, 59.2, 58.8, 58.5, 55.7, 47.1, 47.0, 46.5, 28.0, 27.3, 25.5, 21.0, 20.7, 20.6, 20.3, 18.7, 14.2; HRMS (FAB) [M + Li]$^+$ calcd for $C_{39}H_{42}N_6O_{14}SLi$, 857.2639; found, 857.2637.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(4,6-di-*O*-acetyl-2-azido-2-deoxy-3-*S*-(2,4-dinitrophenyl)-3-thio-α-D-galactopyranosyl)-L-threonine (3).** A solution of **26** (268 mg, 0.310 mmol) in 95% aq TFA (10 mL) was stirred at room temperature for 1 h. The solution was then coevaporated with toluene several times to give compound **3** (246 mg, 100%) as a yellow solid: IR (film) 2932, 2104, 1740, 1588, 1522, 1449, 1343, 1217, 1045 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, mixture of rotamers) (major rotamer) δ 8.94 (d, 1H, J = 2.0 Hz), 8.40 (dd, 1H, J = 9.0, 2.0 Hz), 7.76 (m, 3H), 7.57 (m, 2H), 7.38 (m, 2H), 7.28 (m, 2H), 5.67 (d, 1H, J = 9.0 Hz), 5.37 (app s, 1H), 5.16 (d, 1H, J = 3.0 Hz), 4.48 (m, 2H), 4.31 (m, 1H), 4.21 (m, 2H), 4.08 (m, 4H), 3.77 (dd, 1H, J = 12.0, 3.5 Hz), 2.15 (s, 3H), 2.00 (s, 3H), 1.35 (d, 1H, J = 5.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 173.7, 170.6, 169.8, 169.7, 156.6, 146.8, 144.9, 143.6, 143.4, 142.5, 142.1, 141.2, 129.1, 129.0, 128.9, 128.2, 127.8, 127.2, 127.1, 127.0, 124.9, 121.6, 121.5, 120.0, 98.5, 98.3, 68.1, 67.5, 67.4, 67.3, 62.3, 62.2, 59.2, 58.5, 58.4, 55.7, 47.1, 47.0, 46.8, 20.7, 20.6, 20.3, 18.0; HRMS (FAB) [M − H + 2 Li]$^+$ calcd for $C_{35}H_{33}N_6O_{14}SLi_2$, 807.2096; found, 807.2122.

Synthesis of Glycopeptide 31. Glycopeptide **31** was synthesized on Rink amide MBHA resin (0.1 mmol) using N^α-Fmoc-protected amino acids and DCC-mediated HOBt ester activation in NMP (Perkin-Elmer ABI 431A synthesizer, user-devised cycles). Glycosyl amino acid **3** was manually coupled, using 3.0 equivs of amino acid and activation with HBTU (3.0 equivs) in the presence of HOBt and DIEA (3.0 equivs each). After removal of the N-terminal Fmoc group, the resin-bound peptide was treated with 20% Ac_2O in DMF (v/v) for 20 min and then was washed successively with DMF (3×), CH_2Cl_2 (3 x) and DMF (3×). Azide reduction/DNP cleavage was achieved by treatment with DTT (3.0 equivs) and DBU (3.0 equivs) in DMF for 1 h. Following acetylation with Ac_2O:pyridine (1:2), the resin was washed thoroughly with DMF (3×) and CH_2Cl_2 (5×). Peptide cleavage/deprotection was accomplished by treatment with 95% aq TFA at room temperature for 4 h. The crude peptide was precipitated with *tert*-butyl methyl ether, dissolved in 50% aq CH_3CN, and lyophilized. The acetylated glycopeptide (**31**) was purified by preparative RP-HPLC with a gradient of 10−60% CH_3CN in water (0.1% TFA) over 50 min, and its identity confirmed by ESI-MS (calcd, 2535.7; found, 2535.5).

Deacetylation of Glycopeptide 27. Glycopeptide **31** was treated with 10% aq hydrazine hydrate in the presence of excess DTT at room temperature for 20 min. The resulting glycopeptide (**27**) was directly purified by preparative RP-HPLC with a gradient of 10−60% CH_3CN in water (0.1% TFA) over 50 min, and its identity confirmed by ESI-MS (calcd, 2409.6; found, 2409.3).

Synthesis of *N*-bromoacetamido Sugars 32 and 33.[33a] The corresponding glycosylamine (100 mg) (Sigma) was dissolved in anhydrous DMF (1 mL), and bromoacetic anhydride was added (1.2 equivs). After stirring for 4−6 h, the product was precipitated from ice-cold Et_2O and crystallized (MeOH/Et_2O) to give **32** and **33** as white solids (70−85%). **(32):** ^{1}H NMR (500 MHz, D_2O) δ 4.90 (d, 1H, J = 9.0 Hz), 3.95 (m, 2H), 3.77 (app t, 1H, J = 6.5 Hz), 3.69 (m, 3H), 3.63 (app t, 1H, J = 9.5 Hz). **(33):** ^{1}H NMR (500 MHz, D_2O) δ 5.04 (d, 1H, J = 9.5 Hz), 3.81 (m, 4H), 3.72, (1H, dd, J = 12.5, 5.0 Hz), 3.59 (app t, 1H, J = 9 Hz), 3.48 (m, 2H), 1.98 (s, 3H).

Thiol Alkylation: To peptide **27** (2 mg) in 250 μL of sodium phosphate buffer (0.1 M, pH 7.0) was added **32** or **33** (0.5 mg). The reaction mixture was incubated at 37 °C for 4 h, and the alkylated peptide (**28** or **29**) was isolated by RP-HPLC using a gradient of 10−60% CH_3CN in water (0.1% TFA) over 50 min. The product was lyophilized and analyzed by ESI-MS (pos). (**28**): calcd, 2628.8; found, 2629; (**29**): calcd, 2669.8; found, 2670.

Synthesis of Cysteine-Containing Peptide (34). A peptide with the sequence AcNH-EYELDYDYFLPE**C**EPPE-$CONH_2$ (**34**) was synthesized on Rink amide MBHA resin (0.1 mmol) using N^α-Fmoc-protected amino acids and DCC-mediated HOBt ester activation in NMP (Perkin-Elmer ABI 431A synthesizer, user-devised cycles). After removal of the N-terminal Fmoc, the resin-bound peptide was treated with 20% Ac_2O in DMF for 20 min and then washed successively with DMF (3×), CH_2Cl_2 (3×), and DMF (3×). Peptide cleavage/deprotection was accomplished by treatment with 94.5% TFA, 2.5% H_2O, 2.5% ethanedithiol, and 1% triethylsilane at room temperature for 4 h. The crude peptide was precipitated with *tert*-butyl methyl ether, purified by preparative RP-HPLC (10−60% CH_3CN in water with 0.1% TFA over 50 min), and analyzed by ESI-MS (calcd, 2192.3; found, 2192).

Selective Alkylation Experiment. Peptide **27** (2 mg) and **34** (2 mg) were dissolved in 300 μL of 0.1 M sodium phosphate buffer at pH 6.2, 6.6, and 7.0. Derivative **33** (1.1 equivs) was added, and the reaction mixtures were kept at room temperature or incubated at 37 °C. The reactions were monitored by RP-HPLC (10−60% CH_3CN in water with 0.1% TFA over 50 min). In all cases, no selective alkylation was observed. At pH 6.2, the disulfide-linked dimers were obtained in addition to the alkylated products.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for compounds **1**, **3**, **5**−**7**, **10**−**11**, **19**−**23**, **25**α, **25**β, and **26**. ^{1}H NMR spectra for compounds **24**, **32,** and **33** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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