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Ready Access to Sialylated Oligosaccharide Donors

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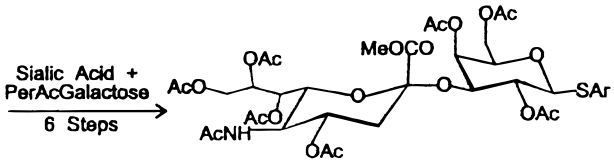
ABSTRACT



Numerous glycoconjugates contain the disaccharide Neu5Acα(2→3)DGal*p*. An efficient way to incorporate this disaccharide into synthetic glycoconjugates is to develop a disaccharide building block. This communication reports a chemoenzymatic route to such a building block which requires as few as four steps. Some examples using more chemical steps are also presented, which increase the flexibility. These disaccharide donors were used to prepare synthetic trisaccharides.

Pathogenic bacteria such as *Campylobacter jejuni*, *Neisseria meningitidis*, and Group B *Streptococcus* have sialylated oligosaccharides as integral parts of their outer surface. It is widely believed that these sialic acid residues mimic host cell surface oligosaccharides. This leads to reduced immunogenicity and, hence, evolutionary advantages for these pathogens.[1] As part of our Institute's program to develop protective vaccines against these organisms, we have initiated a program to synthesize some of these sialylated oligosaccharides.[2] Since we wish to make a large number of structural variants and derivatives, we envisaged a building block approach. This communication reports a highly efficient chemoenzymatic route to Neu5Acα(2→3)DGal*p* disaccharide donors.

Previously, we have accessed such donors by a chemical method involving a three-step preparation of a sialic acid glycosyl donor and a six-step synthesis of a DGal*p* acceptor. These monosaccharides are coupled in 60% yield, and three additional steps of functional group manipulations are required to make a disaccharide donor. The whole process requires nine chromatographic separations. A number of groups have employed similar strategies in their syntheses of sialylated oligosaccharides.[3] Some recent syntheses have improved the efficiency,[4] but such procedures still require too many synthetic steps and too many chromatographic separations.

In our synthesis, arylthio glycosides of galactose and lactose have been enzymatically sialylated to afford glycoside donors with minimal chemical steps and chromatography. Our new method involves a two-step preparation of an arylthio glycoside donor which requires one simple chromatographic separation (cf. **2b** and **3b** in Scheme 1). This is followed by enzymatic glycosylation using sialic acid, CTP, and glycosides as substrates and CMP-Neu5Ac synthetase and sialyl transferase as catalysts[5] to produce the Neu5Acα-(2→3)DGal*pβ*(1→)SAr disaccharides in >80% isolated yield after gel filtration or reverse phase purification; cf. **4a** and **4b**. We have scaled these reactions from 10 mg to 100 mg followed by 1 g with no diminution in yield.

(1) Jennings, H. J. *Curr. Top. Microbiol. Immunol.* **1990**, *150*, 97.
(2) Eichler, E.; Jennings, H. J.; Whitfield, D. M. *J. Carbohydr. Chem.* **1997**, *16*, 385.
(3) (a) Pozsgay, V.; Jennings, H. J.; Kasper, D. L. *J. Carbohydr. Chem.* **1987**, *6*, 41. (b) Paulsen, H.; Tietz, H. *Carbohydr. Res.* **1985**, *144*, 205. (c) Murase, T.; Kameyama, A.; Kartha, K. P. R.; Ishida, H.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **1989**, *8*, 265.
(4) (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) Demchenko, A. V.; Boons, G.-J. *Tetrahedron. Lett.* **1998**, *39*, 3065.
(5) The transferases and the synthetase have been cloned and expressed in the NRC laboratories; for experimental details see: (a) Gilbert, M.; Brisson, J.-R.; Karwaski, M.-F.; Michniewicz, J.; Cunningham, A.-M.; Wu, Y.; Young, N. M.; Wakarchuk, W. W. *J. Biol. Chem.* **2000**, *275*, 3896. (b) Gilbert, M.; Watson, D. C.; Wakarchuk, W. W. *Biotechnol. Lett.* **1997**, *19*, 417.

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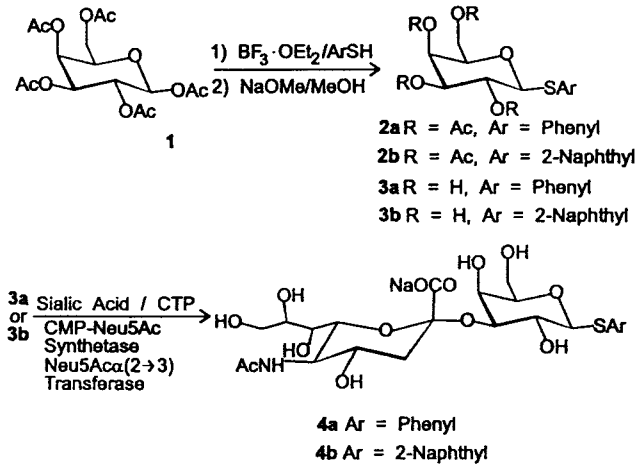
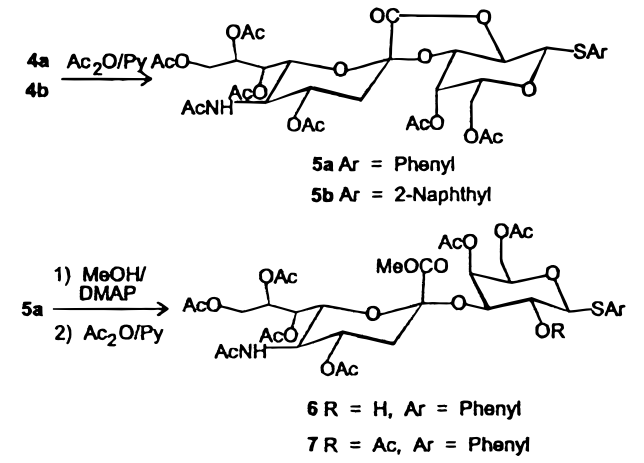
Scheme 1



A simple acetylation produces the lactones **5a** and **5b** in 75% and 90% yields with the Neu5Ac carboxyl esterified with DGal*p* O-2 (see Scheme 2).[6] The lactone either can be

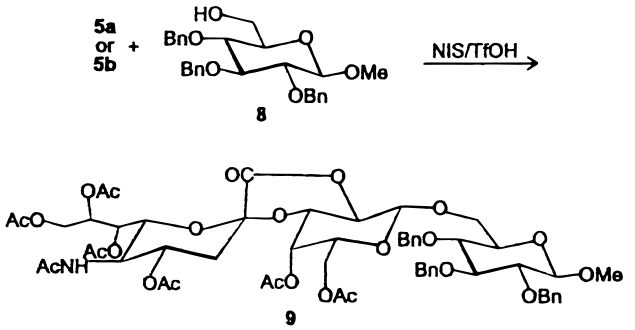
Scheme 2



used directly as a glycosyl donor or can be opened to the methyl ester **6**[7] and the DGal*p* O-2 acetylated to give **7** in 71% yield from **5a**. It is readily apparent that other esters and other acyl groups could be prepared (see below). These arylthio glycosides can be used directly as glycosyl donors. In certain cases other activating groups may be desirable and thioglycosides can be converted into bromides, fluorides, or trichloroacetimidates as required.[8] This procedure is sufficiently simple that these donors could be produced commercially, thereby allowing for the synthesis of bacterial antigens and other sialylated oligosaccharides.

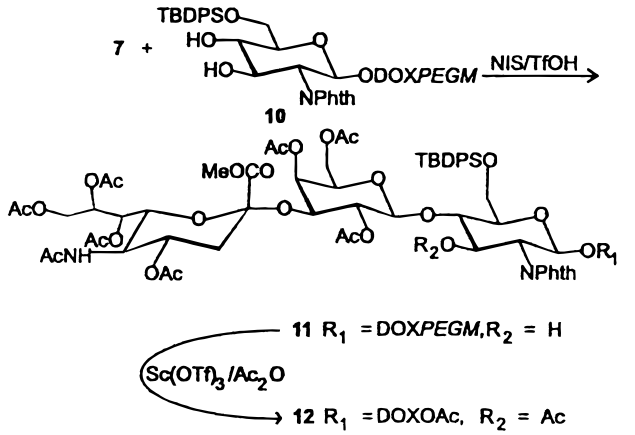
As proof of principle, disaccharides **5a** and **5b** have been used as donors to glycosylate the O-6 of DGlc*p* acceptor **8**

Scheme 3



to yield trisaccharide **9** in 90% and 86% yields, respectively (see Scheme 3). In a more demanding application the polymer-bound diol **10** has been glycosylated with donor **7** to yield **11**. The polymer is the soluble monomethyl ether of poly(ethylene glycol) ($MeO(CH_2CH_2O)_nOH$, MPEG),[9] and dioxyxylene (p-$OCH_2C_6H_4CH_2O-$, DOX) is the linker.[10] Routine cleavage of **11** from the polymer using $Sc(OTf)_3$/Ac_2O afforded the trisaccharide **12** in 36% overall yield (see Scheme 4).[11] This trisaccharide is a synthon for the Neu5Acα-

Scheme 4

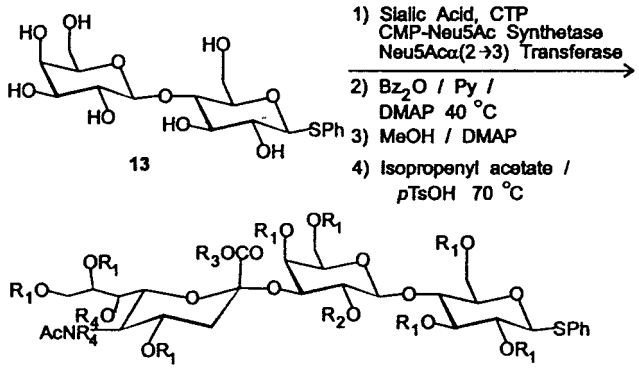


(2→3)DGal*pβ*(1→4)DGlcNAc*pβ*(1→) grouping found on many glycoconjugates.

In an extension of our idea, the phenylthio glycoside of lactose (DGal*pβ*(1→4)DGlc*p*, **13**) has been enzymatically sialylated to afford the trisaccharide **14** (see Scheme 5). Since glycosylations with O-2 acetylated lactose donors[12] are

(6) Yu, R. K.; Koerner, T. A. W.; Ando, S.; Yohe, H. C.; Prestegard, J. H. *J. Biochem.* **1985**, *98*, 1367.
(7) Hayashi, M.; Tanaka, M.; Itoh, M.; Miyauchi, H. *J. Org. Chem.* **1996**, *61*, 2938.
(8) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179.
(9) (a) Jiang, L.; Hartley, R. C.; Chan, T. H. *Chem. Commun.* **1996**, 2193. (b) Wang, Z. G.; Douglas, S. P.; Krepinsky, J. J. *Tetrahedron Lett.* **1996**, *39*, 6985. (c) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2510. (d) Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1997**, *119*, 5562. (e) Dreef-Tromp, C. M.; Willems, H. A. M.; Westerduin, P.; van Veelen, P.; van Boeckel, C. A. A. *Biorg. Med. Chem. Lett.* **1997**, *7*, 1175. (f) Eggenweiler, H. M.; Bayer, E. *Innovation Perspect. Solid-Phase Synth. Comb. Libr. Collect.* **1996**, *4*, 363.
(10) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1995**, *117*, 2116.
(11) Mehta, S.; Whitfield, D. M. *Tetrahedron Lett.* **1998**, *39*, 5907.

Scheme 5



14 R_1 = H, R_2 = H, R_3 = Na, R_4 = H

15 R_1 = Bz, R_2 = R_3 = lactone, R_4 = H

16 R_1 = Bz, R_2 = H, R_3 = Me, R_4 = H

17 R_1 = Bz, R_2 = Ac, R_3 = Me, R_4 = Ac

frequently complicated by ortho ester formation and acyl transfer, the trisaccharide **14** was benzoylated to give **15** in 44% yield.[13] A previous communication used a 2-O pivaloyl lactose derivative as glycosyl acceptor for enzymatic glycosylation and subsequently successfully used this to prepare synthetic GM3 glycolipid by chemical glycosylation and functional group manipulations.[14] This synthesis is a good precedent for our work, but our procedure is significantly simpler. Benzoylation of **14** produced the lactone between DGal*p* O-2 and the Neu5Ac carboxyl **15** as with acetylation of the Neu5Acα(2→3)DGal*p* disaccharides. The highly hindered Neu5Ac O-7 was not benzoylated under these conditions. Subsequent esterification with methanol **16** followed by N- and O-acetylation[15] led to the peracylated trisaccharide donor **17** in 51% overall yield. Preliminary experiments have shown that di-N-acetylated Neu5Ac containing oligosaccharide donors give higher glycosylation yields than mono-N-acetylated donors.[16] The regiochemistry of the acyl groups in **15** and **17** was confirmed by $^{13}C-^{1}H$ HMBC correlation experiments. Thus, a synthon for GM3 and derivatives has been efficiently prepared. Extrapolation of this procedure to larger oligosaccharides related to common gangliosides is in progress.

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Supporting Information Available: Full synthetic procedures for the oligosaccharides prepared in this communication, including spectral characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

OL990406K

(12) (a) Kunz, H.; Harreus, A. *Liebigs Ann. Chem.* **1982**, 41. (b) Sato, S.; Nunomura, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 4097. (c) Sato, S.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5267. (d) Nunomura, S.; Ogawa, T. *Tetrahedron Lett.* **1988**, *29*, 5681.

(13) Nukada, T.; Bérces, A.; Whitfield, D. M. *J. Org. Chem.* **1999**, *64*, 9030.

(14) Ito, Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 1603.

(15) Demchenko, A. V.; Boons, G.-J. *Chem. Eur. J.* **1999**, *5*, 1278.

(16) Eichler, E.; Whitfield, D. M. Unpublished observations.