Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1505–1509

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Concise Synthesis of the 6-*O*- and 6′-*O*-Sulfated Analogues of the Sialyl Lewis X Tetrasaccharide

Anup Kumar Misra,[a] Yili Ding,[a] John B. Lowe[a,b] and Ole Hindsgaul[a,*]

[a]*The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA*
[b]*Howard Hughes Medical Institute, University of Michigan Medical School, 1150 West Medical Center Drive, Ann Arbor, MI 48109-0650, USA*

Received 16 February 2000; accepted 28 March 2000

Abstract—The octyl glycoside of the sialyl Lewis X tetrasaccharide and its 6-*O*-sulfated and 6′-*O*-sulfated analogues were chemically synthesized in a concise manner starting from readily accessible monosaccharide intermediates. The synthesis involved formation of an orthogonally protected tetrasaccharide intermediate from which all three materials were prepared. A selective catalytic hydrogenolysis of four *O*-benzyl ethers in presence of a 4,6-*O*-benzylidene group was the key step in the synthetic scheme.

During the inflammation process, the infiltration of leukocytes into tissues begins with carbohydrate mediated endothelial cell adhesion involving E-, P- and L-selectins.[1] Selectins are a family of carbohydrate binding membrane proteins, which play a vital role in leukocyte homing, platelet binding and neutrophil extravasation.[2] E-selectin appears on the vascular endothelial cells after stimulation by cytokines during the preliminary stage of inflammation and recruits neutrophils and monocytes to inflammatory sites leading to the extravasation of leukocytes.[3] P-selectins are temporarily stored in alpha and dense granules of platelets and Weibel–Palade bodies of endothelial cells and rapidly distributed after activation with thrombin, histamine, phorbol esters or the calcium ionophores.[4] L-selectin is the principal adhesion molecule present on leukocytes involved in the interaction with high endothelial venules (HEV) of peripheral lymph nodes. GlyCAM-1 and CD34 are two mucin like *O*-linked glycoproteins with sulfated, sialylated and fucosylated oligosaccharide sequences which were found to be the major ligands for L-selectin.[5,6]

The selectins recognize the sialyl Lewis X tetrasaccharide (sLeX) determinant (the sequence in **1**) which is found as the terminal carbohydrate structure in both glycolipids and glycoproteins, although these compounds bind with low affinity. The selectin-carbohydrate interaction plays a crucial role in a number of events including inflammation, reperfusion injury, metastasis, angiogenesis, etc.[7] It has been shown that the sialic acid and fucose moieties are essential for binding.[1c,d] sLeX has also been found on the surface of some tumor cells.[8] It has been emphasized that L-selectin prefers 6-*O*-sulfo-sLeX as a ligand over sLeX. Anti sLeX antibodies that bind 6-*O*-sulfo-sLeX react with HEV in lymph nodes inhibiting the binding of L-selectin to HEV.[9,10]

In order to study the function of sLeX and its sulfated analogues and to evaluate their potential as carbohydrate derived therapeutics, efficient and concise synthetic protocols are required. Several syntheses of these molecules have been reported,[11] and these require lengthy multistep sequences. Chemoenzymatic synthesis shows great promise but requires access to a panel of glycosyltransferases and sulfotransferases.

We report herein a concise chemical synthesis of the octyl glycosides of sLeX (**1**), its 6-*O*-sulfate (**2**) and 6′-*O*-sulfate (**3**) from the readily accessible protected monosaccharide precursors **4**–**7** (Fig. 1). The key step in the synthetic Scheme is the conversion of tetrasaccharide **12** to **16** which involves the selective hydrogenolysis of four *O*-benzyl ethers in the presence of a 4,6-*O*-benzylidene group.

Glycosylation of octyl 2-acetamido-4,6-*O*-benzylidine-2-deoxy-β-D-glucopyranoside (**4**), prepared from N-acetyl-D-glucosamine in four steps, and ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[12] (**5**) using $CuBr_2$-Bu_4NBr[13]

*Corresponding author. Fax: +1-858-646-3193.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00207-9

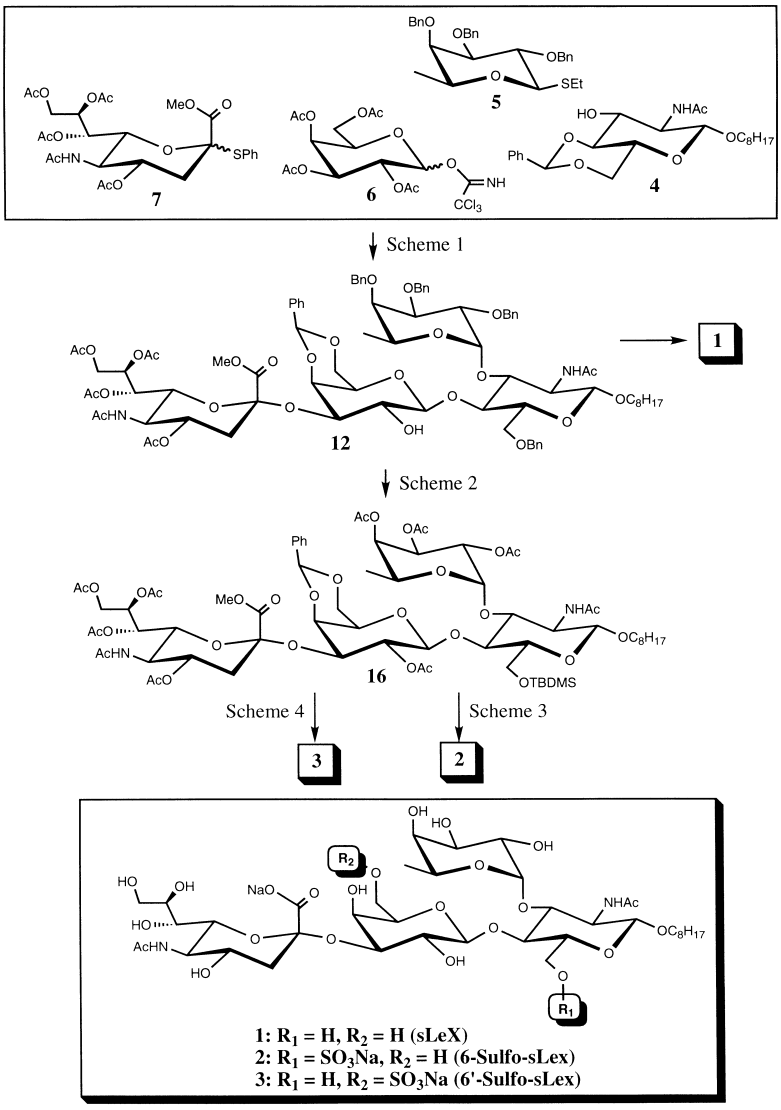


Figure 1. Key features of the synthetic approach to the sLeX analogues **1**–**3**.

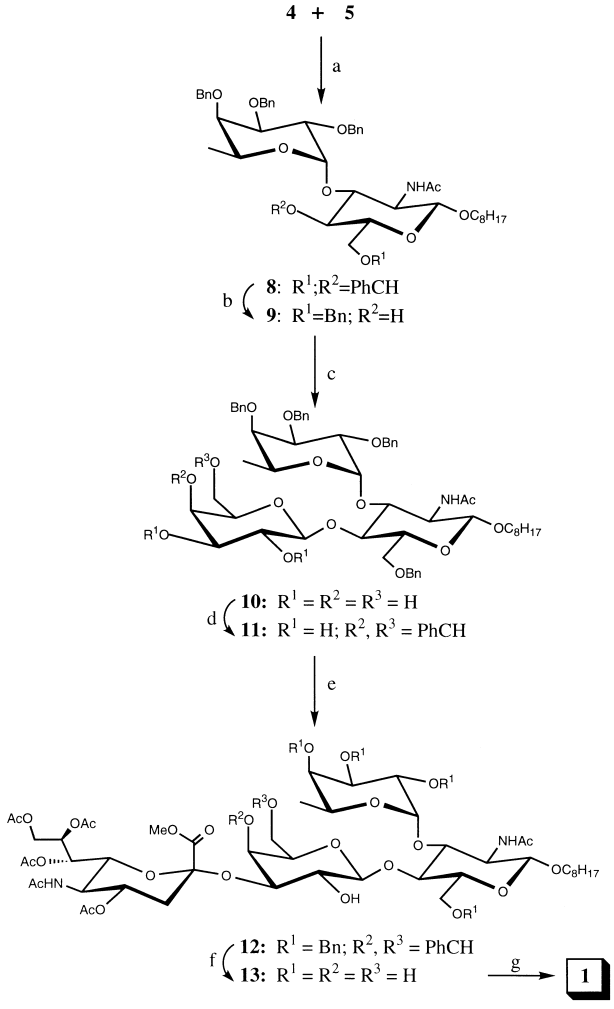
afforded the α(1→3) linked disaccharide (**8**) in 88% yield. Regioselective reductive ring opening[14] of the benzylidene acetal in **8** using $NaBH_3CN$, furnished the disaccharide acceptor (**9**) in 79% yield (Scheme 1). Doublets at δ 4.88 ($J_{1,2}=9.0$ Hz; GlcNAc H-1) and at δ 4.61 ($J_{1,2}=3.6$ Hz; Fuc H-1) in the ^1H NMR spectrum confirmed the stereoselective glycosylations. The β-D-Gal-trichloroacetimidate (**6**) proved effective as donor with $BF_3{\cdot}Et_2O$ as promoter (Et_2O:CH_2Cl_2, 2:1). On deacetylation (NaOMe/MeOH), trisaccharide tetraol **10** was obtained in 77% yield. Careful benzylidenation of **10** using benzaldehyde dimethylacetal and *p*-TsOH afforded the trisaccharide acceptor **11** in 74% yield (longer reaction times lead to defucosylation). The observed chemical shifts and coupling constants (δ 5.07 (d, $J_{1,2}=8.1$ Hz, GlcNAc H-1), 4.93 (d, $J_{1,2}=2.7$ Hz, Fuc H-1), 4.41 (d, $J_{1,2}=7.8$ Hz, Gal H-1)) in the ^1H NMR spectra unambiguously established the expected stereochemistry.

Sialylation of the trisaccharide diol acceptor **11** using the sialyl donor **7**[15] and NIS/TfOH as promoter[16] afforded tetrasaccharide **12** in 46% yield. Characteristic proton and carbon signals in the ^1H and ^{13}C NMR spectra (δ 5.43 (s, PhC*H*), 5.05 (d, $J=8.1$ Hz, GlcNAc H-1), 4.94 (d, $J=2.6$ Hz, Fuc H-1), 4.60 (d, $J=7.8$ Hz, Gal H-1), 2.76 (dd, $J=12.9$ Hz, 4.5 Hz, H-$3_e'''$); ^{13}C NMR: δ 101.5, 99.5, 99.2, 97.8, 97.5) confirmed the structure of **12**. Several others sialyl donors and various promoters[17] were examined but the yields were similar.

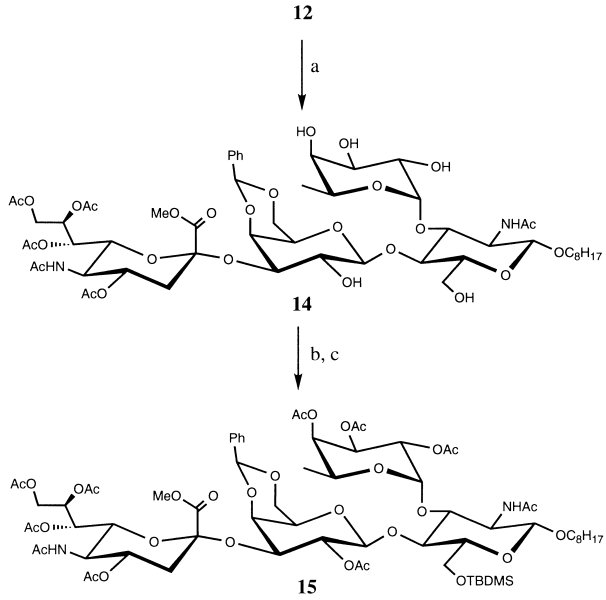
The key step in the use of the single intermediate **12** to furnish both the 6-*O* and 6′-*O*-sulfo-sLeX was the controlled removal of the *O*-benzyl groups in **12** employing catalytic hydrogenation using Pearlman's catalyst[18] (20% $Pd(OH)_2$-C) (Scheme 2). In order to remove benzyl ethers in presence of the benzylidene acetal several trials for selective hydrogenolysis of the benzyl ethers were carried out. Using 10% Pd-C in 2-propanol, 20% $Pd(OH)_2$-C in cyclohexene or 10% Pd-C and ammonium formate in refluxing methanol as the indirect hydrogen sources gave only low yields of product. Use of Pd-C in ethanol and H_2 resulted in the slow complete removal of the benzyl ethers and benzylidene acetal. Use of acetic acid:ethanol (1:2) as the hydrogenation solvent resulted

in some cleavage of sialic acid and defucosylation. Finally, using 20% $Pd(OH)_2$-C (1:1 by wt) in MeOH with a reaction time for 5 h at room temperature furnished the debenzylated product in 77% yield with the benzylidene acetal intact which was confirmed by its proton signal δ 5.43 (s, PhC*H*) in the ^1H NMR spectrum. This reaction condition has been applied for several other selective hydrogenolysis of Lewis X trisaccharide derivative and for some other disaccharides. In every case, a satisfactory yields and reproducibility was achieved. Prolonged (36 h) hydrogenation of **12** under the same reaction conditions gave tetrasaccharide heptaol **13** (87%), which on saponification gave **1** in 68% yield. Selective silylation[19] (TBDMS) of the primary hydroxyl group in **14** followed by conventional acetylation of the secondary hydroxyl groups afforded the orthogonally protected key tetrasaccharide intermediate **15** in 70% yield (Scheme 2).

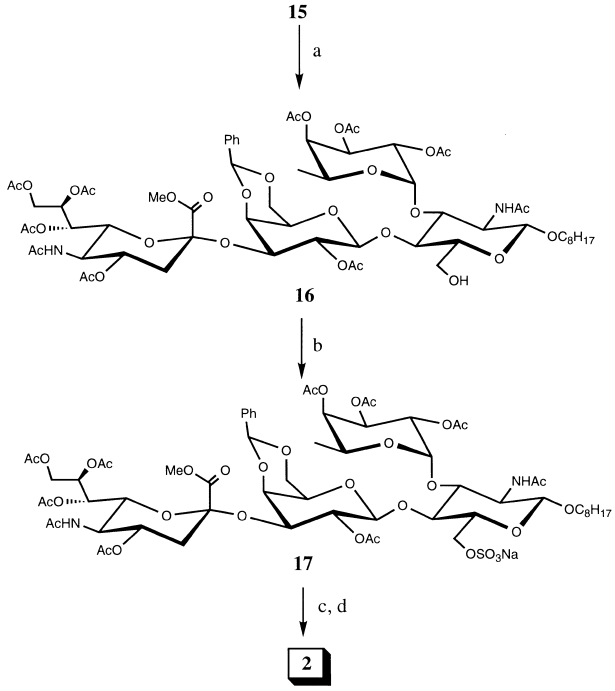
Tetrasaccharide **15** was treated with excess HF-Pyr[20] in THF to produce the 6-OH derivative **16** in 68% yield, which on sulfation (SO_3·Pyr) gave the 6-*O*-sulfate **17** in 73% yield. Removal of the benzylidene acetal through catalytic hydrogenolysis using Pearlman's catalyst followed by saponification gave **2** in 61% yield (Scheme 3). Extended hydrogenolysis of **15** gave the 4′, 6′-diol **18** in 74% yield. Selective sulfation of the 6′-hydroxyl group of **18** followed by saponification of **19** then afforded the 6′-*O*-sulfate **3** in 64% yield (Scheme 4). Selected ^1H



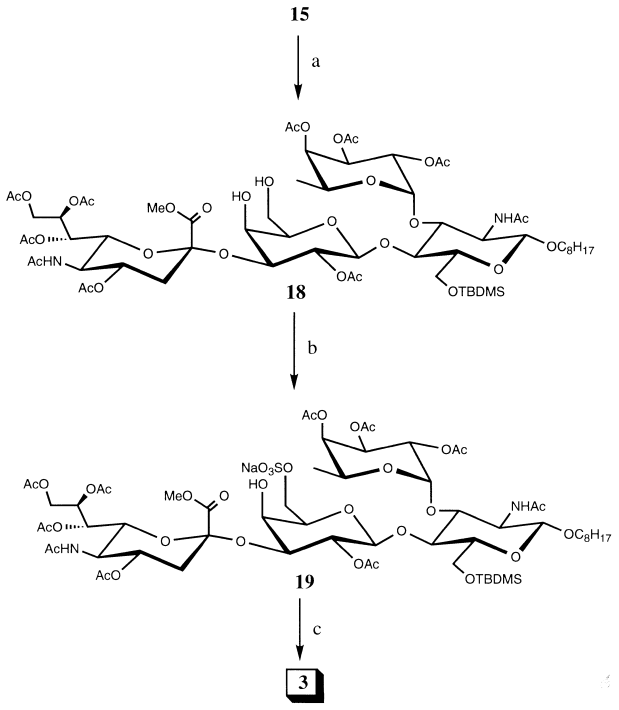
Scheme 1. (a) $CuBr_2$, Bu_4NBr, 1,2-DCE:DMF (5:1), MS-4 Å, rt, 24 h (88%); (b) $NaBH_3CN$, HCl-Et_2O, MS-3 Å, THF, 0–5 °C, 3 h (79%); (c) (i) **6**, BF_3·Et_2O:CH_2Cl_2 (2:1), −10 °C–rt, 3 h; (ii) solid $NaHCO_3$, 1 M MeONa, MeOH (77%); (d) $PhCH(OMe)_2$, *p*-TsOH, CH_3CN, rt, 2 h (74%); (e) NIS, TfOH, CH_3CN:CH_2Cl_2 (5:1), MS-3 Å, −20 °C, 16 h (43%); (f) H_2, $Pd(OH)_2$-C (20%), MeOH, rt, 36 h (87%); (g) 0.1 M MeONa, MeOH, rt, 12 h, then H_2O was added and stirred at rt for 12 h (68%).



Scheme 2. (a) H_2, $Pd(OH)_2$-C (20%), MeOH, rt, 5 h (77%); (b) TBDMS-Cl, imidazole, DMF, rt, 2 h; (c) Ac_2O, pyridine, rt, 12 h (70% in two steps).



Scheme 3. (a) HF-pyridine, THF, 0–5 °C, 4 h (68%); (b) SO_3·Pyr complex, pyridine, 0 °C–rt, 7 h, then Dowex 50W X8 (Na^+) (73%); (c) H_2, $Pd(OH)_2$-C (20%), MeOH, rt, 32 h (72%); (d) 0.1 M MeONa, MeOH, rt, 12 h, then H_2O was added and stirred at rt for 12 h (61%).



Scheme 4. (a) H_2, $Pd(OH)_2$-C (20%), MeOH, rt, 48 h (74%); (b) SO_3·Pyr complex, Pyridine, 0°−10 °C, 7 h, then Dowex 50W X8 (Na^+) (70%); (c) 0.1 M MeONa, MeOH, rt, 12 h, then H_2O was added and stirred at rt for 12 h (64%).

NMR and MS data for key compounds are presented below.[21] Compounds **1**–**3** are under evaluation as selectin inhibitors and as sulfotransferase acceptors.

In conclusion, the 6- and 6′-*O*-sulfated analogues of sLeX were synthesized in a concise and practical way.

Acknowledgements

The authors would like to thank Dr. O. P. Srivastava, Alberta Research Council for providing conditions for the synthesis of **9**. This work was supported by National Institutes of Health Grant PO1 CA 71932.

References and Notes

1. (a) Lawrence, M. B.; Springer, T. A. *Cell* **1991**, *65*, 859. (b) Watson, S. R.; Fennie, C.; Lasky, L. A. *Nature* **1991**, *349*, 164. (c) Lowe, J. B.; Stoolman, L. M.; Nair, R. P.; Larsen, R. D.; Berhend, T. L.; Marks, R. M. *Cell* **1990**, *63*, 475. (d) Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I., Paulsen, J. C. *Science* **1990**, *250*, 1130.
2. (a) Drickamer, K. *J. Biol. Chem.* **1988**, *263*, 9557. (b) Varki, A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390. (c) Crocker, P. R.; Feizi, T. *Curr. Opin. Struct. Biol.* **1996**, *6*, 679. (d) Kansas, G. S. *Blood* **1996**, *88*, 3259.
3. (a) Bevilacque, M. P.; Pober, J. S.; Mendrick, D. L.; Cotran, R. S.; Gimbrone, M. A. *Proc. Natl. Acad. Sci, USA* **1987**, *84*, 9238. (b) Lasky, L. A. *Annu. Rev. Biochem.* **1995**, *64*, 113. (c) Pigott, R.; Dillon, L. P.; Hemingway, L. H.; Gearing, A. J. H. *Biochem. Biophys. Res. Commun.* **1992**, *187*, 584.
4. Johnston, G. I.; Kurosky, A.; McEver, R. P. *J. Biol. Chem.* **1989**, *264*, 1816.
5. (a) Rosen, S. D. *Semin, Immunol.* **1993**, *5*, 237. (b) Imai, Y.; Lasky, L. A.; Rosen, S. D. *Nature* **1993**, *361*, 555.
6. (a) Hemmerich, S.; Bertozzi, C. R.; Leffler, H.; Rosen, S. D. *Biochemistry* **1994**, *33*, 4820. (b) Hemmerich, S.; Leffler, H.; Rosen, S. D. *J. Biol. Chem.* **1995**, *270*, 12035.
7. (a) Simanek, E. E.; McGarver, G. J.; Jablonowski, J. A.; Wong, C.-H. *Chem. Rev.* **1998**, *98*, 833. (b) Varki, A. *Curr. Opin. Cell. Biol.* **1992**, *4*, 257.
8. Fukushima, K.; Hirota, M.; Terasaki, P. I.; Wakisaka, A.; Togashi, H.; Chia, D.; Suyama, N.; Fukushi, Y.; Nudelman, E.; Hakomori, S. *Cancer Res.* **1984**, *44*, 5279.
9. Galustian, C.; Lawson, A. M.; Komba, S.; Ishida, H.; Kiso, M.; Feizi, T. *Biochem. Biophys. Res. Commun.* **1997**, *240*, 748.
10. (a) Mitsuoka, C.; Kawakami-Kimura, N.; Kasugai-Sawada, M.; Hiraiwa, N.; Toda, K.; Ishida, H.; Kiso, M.; Hasegawa, A.; Kannagi, R. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 546. (b) Mitsuoka, C.; Sawada-Kasugai, M.; Ando-Furui, K.; Izawa, M.; Nakanishi, H.; Nakamura, S.; Ishida, H.; Kiso, M.; Kannagi, R. *J. Biol. Chem.* **1998**, *273*, 11225.
11. (a) Ellervik, U.; Magnusson, G. *J. Org. Chem.* **1998**, *63*, 9314; Ellervik, U.; Grundberg, H.; Magnusson, G. *J. Org. Chem.* 1998, 63, 9323 and the references cited therein. (b) Tsukida, T.; Yoshida, M.; Kurokawa, K.; Nakai, Y.; Achiha, T.; Kiyoi, T.; Kondo, H. *J. Org. Chem.* **1997**, *62*, 6876 and the references cited therein. (c) Kiyoi, T.; Inone, y.; Ohmoto, H.; Yoshida, M.; Kiso, M.; Kondo, H. *Bioorg. Med. Chem.* **1998**, *6*, 587. (d) Dumas, D. P.; Ichikawa, Y.; Wong, C.-H.; Lowe, J. B.; Nair, R. P. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 425. (e) Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8748. (f) Halcomb, R. L.; Huang, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1994**, *116*, 11315. (g) Seitz, O.; Wong, C.-H. *J. Am. Chem. Soc.* **1997**, *119*, 8766. (h) Blixt, O.; Norberg, T. *J. Org. Chem.* **1998**, *63*, 2705.
12. Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105.
13. Sato, S.; Mori, M.; Ito, Y.; Ogawa, T.. *Carbohydr. Res. 155, C6-C10*..
14. Garegg, P. J.; Hultberg, H.; Oscarson, S. *J. Chem. Soc., Perkin Trans. 1*, **1982**, 2395.
15. Kameyama, A.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1990**, *200*, 269.
16. (a) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313. (b) Veenemann, G. H.; van-Leeuwen, S. H.; vanBoom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.
17. (a) Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16. (b) Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1990**, *195*, 303. (c) Kanie, O.; Kiso, M.; Hasegawa, A. *J. Carbohydr. Chem.* **7**, 501.
18. Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663.
19. Nashed, E. M.; Glaudemans, C. P. J. *J. Org. Chem.* **1987**, *52*, 5255.
20. Nicolaou, K. C.; Randall, J. L.; Furst, G. T. *J. Am. Chem. Soc.* **1985**, *107*, 5556.
21. Partial NMR and MS data: **8**: δ 5.03 (d, $J_{1',2'}=3.6$ Hz, 1H, H-1′), 4.88 (d, $J_{1,2}=9.0$ Hz, 1H, H-1), 1.60 (s, 3H, NHAc), 0.78 (d, $J=6.6$ Hz, 3H, H-6′). **11**: δ 5.55 (s, 1H, PhC*H*), 5.07 (d, $J_{1,2}=8.1$ Hz, 1H, H-1), 4.93 (bd, $J_{1',2'}=2.7$ Hz, 1H, H-1′), 4.41 (d, $J_{1'',2''}=7.8$ Hz, 1H, H-1″), 1.58 (s, 3H, NHAc), 1.02 (d, $J=6.6$ Hz, 3H, H-6′). **12**: δ 5.43 (s, 1H, PhC*H*), 5.05 (d, $J_{1,2}=8.1$ Hz, 1H, H-1), 4.94 (d, $J_{1',2'}=2.1$ Hz, 1H, H-1′), 4.59 (d, $J_{1'',2''}=7.8$ Hz, 1H, H-1″), 3.63 (s, 3H, COO*Me*), 2.76 (dd, $J=12.9$ Hz, 4.5 Hz, H-3_e‴), 1.56–2.12 (6s, 18H, 4 OAc, and 2 NHAc), 1.01 (d, $J=6.6$ Hz, 3H, H-6′), **16**: δ 5.42 (m, 1H, H-8‴), 5.31 (s, 1H, PhC*H*), 5.12 (d, $J_{1',2'}=3.0$ Hz, 1H, H-1′), 4.73 (d, $J_{1,2}=8.1$ Hz, 1H, H-1), 4.44 (d, $J_{1'',2''}=7.5$ Hz, 1H, H-1″), 3.56 (s, 3H, COO*Me*), 2.72 (dd, 1H, H-3_e‴), 1.85–2.12 (10s, 30H, 8 OAc and 2 NHAc), 0.88 (s, 9H, C*Me*$_3$), 0.71 (d, $J=6.6$ Hz, 3H, H-6′), 0.06, 0.07 (2s, 3H each, 2 Me). **17** : δ 5.35 (s, 1H, PhC*H*), 5.21 (d, $J_{1',2'}=3.0$ Hz, 1H, H-1′), 4.73 (d, $J_{1,2}=8.1$ Hz, 1H, H-1), 4.53 (d, $J_{1'',2''}=8.1$ Hz, 1H, H-1″),

3.75 (s, 3H, COO*Me*), 2.75 (dd, 1H, H-$3_e'''$), 1.84–2.16 (10s, 30H, 8 OAc and 2 NHAc), 0.62 (d, $J=6.6$ Hz, 3H, H-6′). **20**: δ 5.20 (d, $J_{1',2'}=2.7$ Hz, 1H, H-1′), 4.75 (d, $J_{1,2}=8.1$ Hz, 1H, H-1), 4.45 (d, $J_{1'',2''}=8.1$ Hz, 1H, H-1″), 3.76 (s, 3H, COO*Me*), 2.80 (dd, 1H, H-3e‴), 1.84–2.11 (10s, 30H, 8 OAc and 2 NHAc), 1.15 (d, $J=6.6$ Hz, 3H, H-6′), 0.87 (s, 9H, CMe_3), 0.03 and 0.05 (2s, 6H, 2Me). **1**: δ 5.05 (d, $J_{1',2'}=3.9$ Hz, 1H, H-1′), 4.48 (2d, $J=7.5$ Hz, 2H, H-1 and H-1″), 4.04 (dd, 1H, H-3″), 2.72 (dd, 1H, H-$3_e'''$), 1.98, 1.99 (2s, 6H, 2 NHAc), 1.76 (t, 1H, H-$3_a'''$), 1.12 (d, $J=6.6$ Hz, 3H, H-6′); ^{13}C NMR: δ 102.2, 101.5, 100.2, 99.2. HRMS: calcd for $C_{39}H_{67}O_{23}N_2Na$ ($M+Na^+$) 977.9402; found 977.9397. **2**: δ 5.03 (d, $J_{1',2'}=3.9$ Hz, 1H, H-1′), 4.55 ($J_{1,2}=7.8$ Hz, 1H, H-1), 4.48 (d, $J_{''2''}=7.8$ Hz, 1H, H-1′, 2.67 (dd, 1H, H-$3_e'''$), 1.93, 1.96 (2s, 6H, 2NHAc), 1.73 (t, 1H, H-$3_a'''$), 1.11 (d, $J=6.6$ Hz, 3H, H-6′); ^{13}C NMR: δ 101.8, 101.6, 100.2, 99.2; HRMS: calcd For $C_{39}H_{66}O_{26}N_2Na_2S$ ($M+Na^+$) 1079.9862; found 1079.9855. **3**: δ 5.05 (d, $J_{1',2'}=3.9$ Hz, 1H, H-1′), 4.50 (d, $J_{1,\ 2}=7.8$ Hz, 1H, H-1), 4.47 (d, $J_{1'',2''}=8.1$ Hz, 1H, H-1″), 2.73 (dd, 1H, H-$3_e'''$), 1.98, 1.99 (2s, 6H, 2NHAc), 1.76 (t, 1H, H-$3_a'''$), 1.14 (d, $J=6.6$ Hz, 3H, H-6′); ^{13}C NMR: δ 102.2, 101.5, 100.3, 99.2; HRMS: calcd for $C_{39}H_{66}O_{26}N_2Na_2S$ ($M+Na^+$) 1079.9862; found 1079.9858.