The First Total Synthesis of 6-Sulfo-de-*N*-acetylsialyl Lewis^x Ganglioside: A Superior Ligand for Human L-Selectin**

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Selectins (E-, P-, and L-)[1, 2] are a family of carbohydrate-binding proteins found on leukocytes, vascular endothelium, and platelets, which mediate cell adhesion through recognition and binding of cell-specific oligosaccharide ligands on a variety of glycoconjugates such as glycoproteins, glycolipids, and glycosaminoglycans. There is now a general agreement that all three selectins can bind sialyl Lewis^x (sLe^x), sialyl Lewis^a (sLe^a), and structurally related Lewis blood group oligosaccharides found on many cancer cells,[3] and it has been suggested that the selectin–carbohydrate interactions might be involved in many physiological events such as leukocyte trafficking, inflammation, thrombosis, and cancer metastasis. This has stimulated research activities aimed at the development of novel synthetic carbohydrate compounds and their mimetics as therapeutic agents against reperfusion injury, chronic inflammatory diseases, allergy, autoimmunity, and cancer.[4] It is also known that L- and P-selectin can efficiently bind to sulfated carbohydrates such as fucoidan, sulfatide, sulfated glucuronyl oligosaccharides, heparin, sulfo Le^x, and sulfo Le^a structures.[1, 2, 5–7]

L-selectin (leukocyte endothelial cell adhesion molecule 1) is a lymphocyte homing receptor involved in the binding of lymphocytes to the high endothelial venules (HEV) in peripheral lymph nodes. One of the endothelium-derived counter-receptors for L-selectin is GlyCAM-1, a mucin-like glycoprotein with sulfated, sialylated, and fucosylated oligosaccharide sequences.[8, 9] We have demonstrated with chemically synthesized gangliosides[10a] that the sLe^x sequence with 6-O-sulfation at *N*-acetylglucosamine (6-sulfo sLe^x) is the preferred ligand for human L-selectin.[11] In addition, anti-sialyl Lewis^x antibodies that bind to 6-sulfo sLe^x reacted to HEV in lymph nodes and inhibited the binding of L-selectin to HEV.[10b] The 6-sulfo sLe^x sequence has also been identified as a major carbohydrate capping group of the L-selectin ligand on HEV in human lymph nodes by using newly established monoclonal antibodies directed against the chemically synthesized sulfo sLe^x gangliosides and their derivatives.[12] In the course of work with the chemically synthesized gangliosides, a novel de-*N*-acetylated form of 6-sulfo sLe^x has been identified as a superior L-selectin ligand to the *N*-acetyl form of 6-sulfo sLe^x. This novel molecule was originally discovered[11] as a minor by-product of the parent 6-sulfo sLe^x hexasaccharide ganglioside. To corroborate the superior reactivity of this novel molecule we undertook, selectively, for the first time, the total synthesis of the 6-sulfo de-*N*-acetylsialyl Le^x ganglioside **1** (see Table 1 for spectroscopic and physical data), and examined its binding by human L-selectin.

The most important problems in the total synthesis of the title compound **1** are a) selective protection of the amino group in neuraminic acid, b) regio- and α-stereoselective glycosylation of sialic acid, c) selective protections of the 3- and 6-hydroxyl groups of *N*-acetylglucosamine (GlcNAc) that undergo fucosylation and sulfation, respectively, and d) efficient construction of the glycolipid structure. The first problem was solved by protecting the amino group of neuraminic acid with the trifluoroacetyl (TFAc) group, which

Table 1. Selected physical data of **4**, **7**, **10**, **12**, **14**, and **1**.

4: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.04$ (m, 2H, CH_2SiMe$_3$), 1.99, 2.01, 2.12, 2.17 (4 × s, 12H, 4AcO), 2.74 (dd, 1H, $J_{gem} = 13$, $J_{3eq,4} = 4.6$ Hz, H-3b$_{eq}$), 3.67 (s, 3H, COOMe), 4.44 (dd, 1H, $J_{1,2} = 7.8$ Hz, H-1a), 4.97 (m, 1H, $J_{3ax,4} = J_{4,5} = 10.5$ Hz, H-4b), 5.28 (dd, 1H, $J_{6,7} = 2.1$, $J_{7,8} = 8.9$ Hz, H-7b), 5.35 (s, 1H, *CH*Ph), 5.42 (m, 1H, H-8b), 7.25–7.48 (m, 5H, Ph); ^{19}F NMR (476.5 MHz, $CDCl_3$) δ (relative to fluorobenzene) = 37.03 (s, CF_3CO); $[\alpha]_D^{25} = -3.9^\circ$ ($c = 1.3$ in $CHCl_3$).

7: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 1.00$ (m, 2H, CH_2SiMe$_3$), 1.41, 1.48, 1.94, 2.00, 2.04, 2.06, 2.10, 2.18 (8 × s, 24H, 6AcO, AcN, and *Ac*CH$_2$CH$_2$), 2.51 (m, 4H, Ac*CH$_2$CH$_2$*), 2.53 (dd, 1H, $J_{gem} = 13$, $J_{3eq,4} = 4.8$ Hz, H-3e$_{eq}$), 3.75 (s, 3H, *Me*OPh), 3.84 (s, 3H, COOMe), 5.30 (dd, 1H, $J_{1,2} = 8.1$, $J_{2,3} = 10$ Hz, H-2d), 6.76–8.21 (m, 40H, 8Ph); $[\alpha]_D^{25} = +11.3^\circ$ ($c = 1.1$ in $CHCl_3$).

10: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.99$ (m, 2H, CH_2SiMe$_3$), 1.21 (3H, $J_{5,6} = 6.4$ Hz, H-6f), 1.48, 1.90, 1.93, 2.02, 2.03, 2.04, 2.05, 2.08, 2.15, 2.18 (10 × s, 30H, 8AcO, AcN, and *Ac*CH$_2$CH$_2$), 2.46–2.54 (m, 4H, Ac*CH$_2$CH$_2$*), 2.57 (dd, 1H, $J_{gem} = 12.4$, $J_{3eq,4} = 4.1$ Hz, H-3e$_{eq}$), 3.85 (s, 3H, COOMe), 5.07 (d, 1H, $J_{1,2} = 3.0$ Hz, H-1f), 7.06–8.15 (m, 40H, 8Ph); $[\alpha]_D^{25} = -9.4^\circ$ ($c = 1.7$ in $CHCl_3$).

12: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.24$ (d, 3H, $J_{5,6} = 6.4$ Hz, H-6f), 1.48 (s, 3H, AcN), 1.94–2.20 (16 × s, 48H, 15AcO and *Ac*CH$_2$CH$_2$), 2.56 (dd, 1H, $J_{gem} = 12.6$, $J_{3eq,4} = 4.4$ Hz, H-3e$_{eq}$), 2.61–2.80 (m, 4H, Ac*CH$_2$CH$_2$*), 3.87 (s, 3H, COOMe), 6.47 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1a), 7.52–8.17 (m, 5H, Ph), 8.66 (s, 1H, C=NH); $[\alpha]_D^{25} = +5.8^\circ$ ($c = 1.3$ in $CHCl_3$).

14: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H, $J_{vic} = 6.6$ Hz, 2*Me*CH$_2$), 1.46 (s, 3H, AcN), 1.63–2.22 (15 × s, 45H, 15AcO), 2.57 (dd, 1H, $J_{gem} = 12$, $J_{3eq,4} = 4.4$ Hz, H-3e$_{eq}$), 3.89 (s, 3H, COOMe), 5.86 (dt, 1H, $J_{4,5} = 13.5$, $J_{5,6} = J_{5,6'} = 7.0$ Hz, H-5 of sphingosine), 7.42–8.18 (m, 10H, 2Ph); ^{19}F NMR (476.5 MHz, $CDCl_3$) δ (relative to fluorobenzene) = 36.82 (s, CF_3CO); $[\alpha]_D^{25} = -17.6^\circ$ ($c = 1.2$ in $CHCl_3$).

1: FAB-MS (negative ion mode, triethanolamine matrix): *m*/*z*: 1795.6 [*M* − H+Na]$^-$, 1773.0 [*M* − H]$^-$, 1750.6 [*M* − Na]$^-$, 1500.8 [*M* − H − neuraminic acid sodium salt]$^-$, 1479.8 [*M* − neuraminic acid sodium salt − Na]$^-$, 1339 [1500.8 − Gal]$^-$, 1317.8 [1479.8 − Gal], 888.7 [lactosylceramide]$^-$, 726.7 [glucosylceramide]$^-$, 564.5 [ceramide]$^-$; calcd for $C_{77}H_{136}N_3Na_2O_{37}S$ (*M* − H): 1772.8369; found: 1773.05.

FAB-MS (negative ion mode, [18]crown-6 in 3-nitrobenzyl alcohol matrix): *m*/*z*: 1750.9 [*M* − Na]$^-$, 1728.90 [*M* − 2Na+H]$^-$, 1479.2 [1728.9 − neuraminic acid]$^-$; calcd for $C_{77}H_{137}N_3NaO_{37}S$ (*M* − Na): 1750.8549; found: 1750.64.

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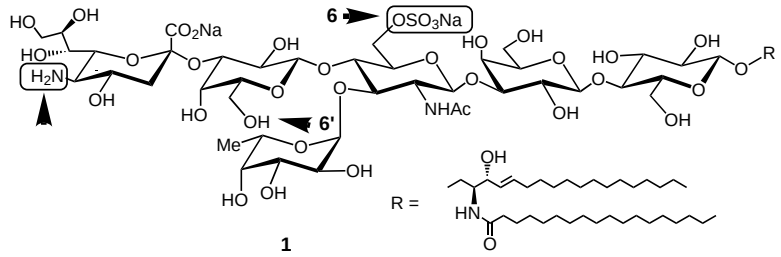

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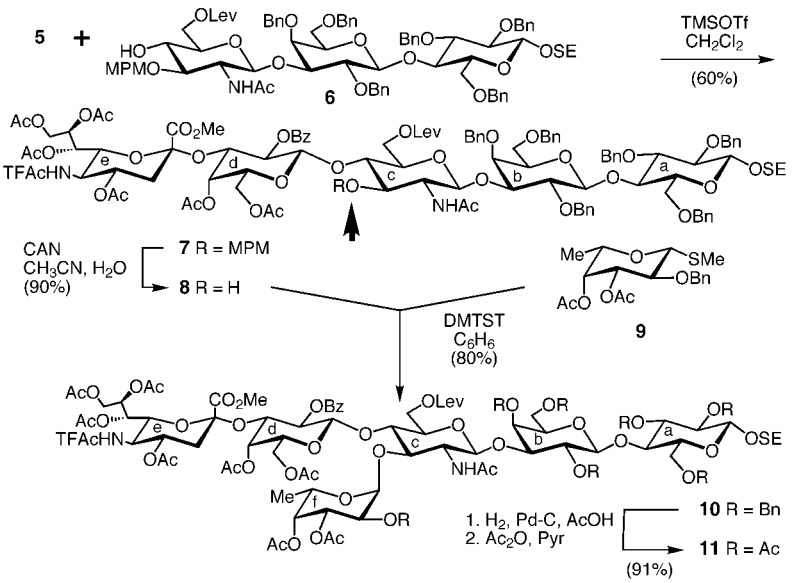
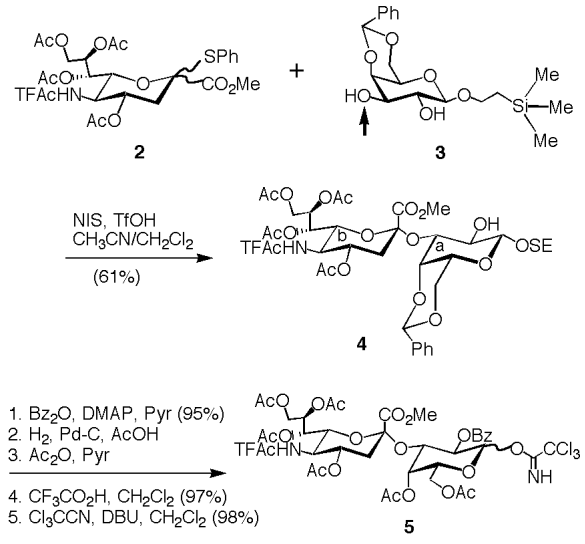
is stable in acidic conditions but can be readily removed by alkaline treatment. For the second problem, we have already developed[13] a highly efficient, regio- and α-stereoselective glycosylation of sialic acid by using the 2-thioglycoside derivatives of sialic acid as glycosyl donors. The third problem could be solved by constructing the trisaccharide building block **6**[10a] in which the 3- and 6-hydroxyl groups of GlcNAc are suitably protected by the *p*-methoxybenzyl (MPM) and levulinoyl (Lev) groups, respectively.

Iodonium-ion promoted,[14] regio- and α-stereoselective glycosylation of **2**, which was readily prepared from the phenyl 2-thioglycoside of *N*-acetylneuraminic acid,[10a] with **3**[10a] was performed at $-30\,^{\circ}C$ in a solution of acetonitrile/dichloromethane (5/1) to give the desired sialyl $\alpha(2\rightarrow3)$Gal derivative **4** in 61% yield. Compound **4** was converted into the trichloroacetimidate derivative **5** in high yield by 2-O-benzoylation, successive debenzylidenation and acetylation, selective cleavage[15] of the 2-(trimethylsilyl)ethyl (SE) group, and treatment[16] with trichloroacetonitrile and DBU, (Scheme 1).

Coupling of **5** with the suitably protected trisaccharide acceptor **6** gave the sialyl $\alpha(2\rightarrow3)$neolactotetraose derivative **7**, which was treated with ceric ammonium nitrate (CAN) for 1 h at room temperature in a solution of acetonitrile/water (9/1) to give **8** in 90% yield. The 3-hydroxyl group of the GlcNAc residue in **8** was then fucosylated with **9** in the presence of dimethyl(methylthio)sulfonium triflate (DMTST)[17] in benzene (Scheme 2). The resulting sialyl Le^x hexasaccharide derivative **10** (80%) was hydrogenolized, followed by treatment with Ac$_2$O in pyridine, to afford **11** (91%), which was converted into the trichloroacetimidate derivative **12** in nearly quantitative yield as described for **5**.



Scheme 2. Synthesis of **10** and **11** by coupling of the building blocks **5**, **6**, and **9**. Bn = benzyl, MPM = *p*-methoxybenzyl, TMS = trimethylsilyl, Lev = levulinoyl, CAN = ceric ammonium nitrate, DMTST = dimethyl(methylthio)sulfonium triflate.



Scheme 1. Synthesis of **5**. Ac = acetyl, TFAc = trifluoroacetyl, NIS = *N*-iodosuccinimide, Tf = F_3CSO_2, DMAP = 4-dimethylaminopyridine, SE = 2-(trimethylsilyl)ethyl, Bz = benzoyl, Pyr = pyridine, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene.

Glycosylation of the azidosphingosine[18, 19] derivative **13** with **12**, and successive reduction of the azido group and N-acylation were carried out by the established method[20–22] (Scheme 3). The levulinoyl group was selectively removed by treatment with hydrazine monoacetate in ethanol for 2 h at room temperature to give **14** without cleavage of the TFAc group. The deprotected 6-hydroxyl group of the GlcNAc residue was then sulfated in an almost quantitative yield by treatment with a sulfur trioxide-pyridine complex in DMF. Finally, removal of all protective groups under the basic conditions furnished the target molecule **1**.

In binding experiments with the multivalent human L-selectin[11] for the immobilized synthetic ganglioside **1** and the 6-sulfo sLe^x we corroborated the superior binding to the former (Figure 1). The amounts of **1** and of 6-sulfo sLe^x required for 50% of maximal binding were 18 and 40 pmol per well, respectively. The structure of the synthetic byproduct (negative control) has been identified as a 6-sulfo sLe^x derivative containing "lactamized" neuraminic acid.[11] We observed previously[11] that 6-O-sulfation at the GlcNAc residue of the sLe^x (6-sulfo sLe^x) sequence results in substantial enhancement of the binding activity, while the 6′-sulfo sLe^x sequence (6-O-sulfation at the Gal residue) gives

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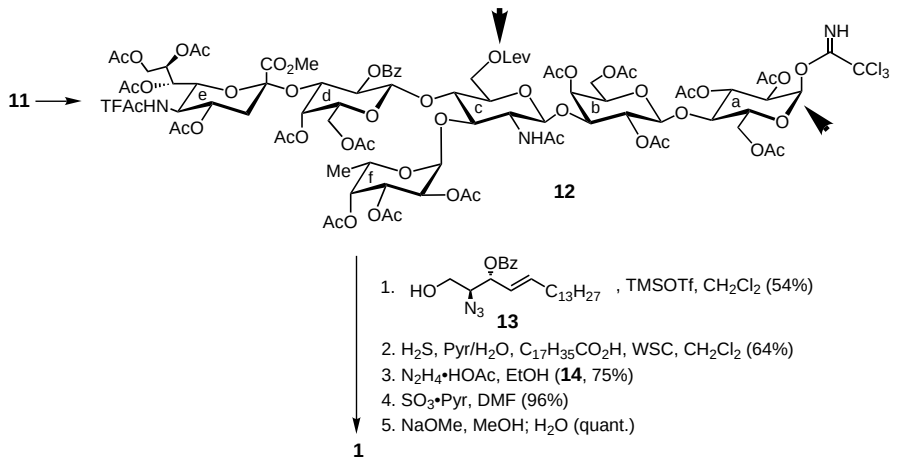

Scheme 3. Synthesis of 6-sulfo-de-*N*-acetylsialyl Lewis^x ganglioside **1**. WSC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride.

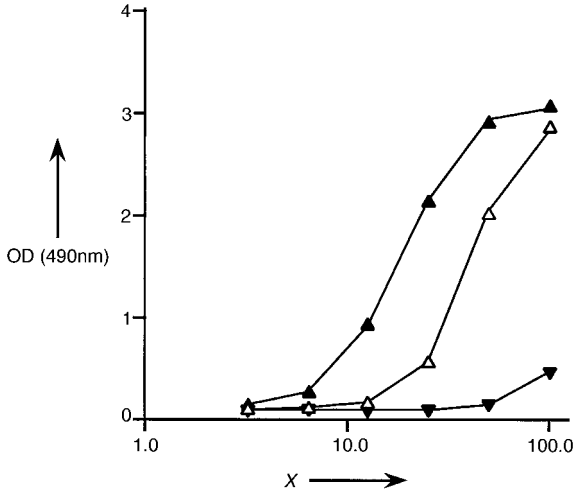

Figure 1. Binding of multivalent L-selectin (50 ng per well) to the chemically synthesized 6-sulfo-de-*N*-acetyl-sialyl Lewis^X (**1**) and related sLe^x structures. *X*: glycolipid added (pmol per well). ▲: **1**, △: 6-sulfo sLe^x, ▼: synthetic by-product (negative control) with "lactamized" neuraminic acid. For further information see the text.

little or no detectable binding. In addition, the presence of the additional sulfate as in 6,6′-bis-sulfo sLe^x results in impairment of the binding.[11] In conclusion, the order of binding ability of the sLe^x series with L-selectin was **1** > 6-sulfo sLe^x > (nonsulfated) sLe^x[20b] = 6,6′-bis-sulfo sLe^x ≫ 6′-sulfo sLe^x. We have raised the possibility that the de-*N*-acetylsialyl(neuraminyl) forms of 6-sulfo sLe^x and related structures may be among the high affinity endogenous ligands for L-selectin on HEV that are involved in the interaction of leukocytes with the vascular endothelium. This question will be solved by generating specific antibodies to 6-sulfo-de-*N*-acetyl sLe^x and related oligosaccharide structures by using the chemically synthesized gangliosides.

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[1] a) L. A. Lasky, *Science* **1992**, *258*, 964 – 969; b) R. D. Cummings, D. F. Smith, *BioEssays* **1992**, *14*, 849 – 856; c) M. P. Bevilacqua, R. M. Nelson, *J. Clin. Invest.* **1993**, *91*, 379 – 387; d) R. P. McEver, *Glycoconjugate J.* **1997**, *14*, 585 – 591.
[2] a) A. Varki, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7390 – 7397; b) P. R. Crocker, T. Feizi, *Curr. Opin. Struct. Biol.* **1996**, *6*, 679 – 691; c) G. S. Kansas, *Blood* **1996**, *88*, 3259 – 3287.
[3] a) A. Takada, K. Ohmori, T. Yoneda, K. Tsuyuoka, A. Hasegawa, M. Kiso, R. Kannagi, *Cancer Res.* **1993**, *53*, 354 – 361; b) R. Kannagi, *Glycoconjugate J.* **1997**, *14*, 577 – 584.
[4] E. E. Simanek, G. J. McGarver, J. A. Jablonowski, C.-H. Wong, *Chem. Rev.* **1998**, *98*, 833 – 862.
[5] S. D. Rosen, C. R. Bertozzi, *Curr. Biol.* **1996**, *6*, 261 – 264.
[6] a) P. J. Green, C.-T. Yuen, R. A. Childs, W. Chai, M. Miyasaka, R. Lemoine, A. Lubineau, B. Smith, H. Ueno, K. C. Nicolaou, T. Feizi, *Glycobiology* **1995**, *5*, 29 – 38; b) C.-T. Yuen, A. M. Lawson, W. Chai, M. Larkin, M. S. Stoll, A. C. Stuart, X. Sullivan, T. J. Ahern, T. Feizi, *Biochemistry* **1992**, *31*, 9126 – 9131.
[7] Y. Suzuki, Y. Toda, T. Tamatani, T. Watanabe, T. Suzuki, T. Nakao, K. Murase, M. Kiso, A. Hasegawa, K. Tadano-Aritomi, I. Ishizuka, M. Miyasaka, *Biochem. Biophys. Res. Commun.* **1993**, *190*, 426 – 434.
[8] Y. Imai, L. A. Lasky, S. D. Rosen, *Nature* **1993**, *361*, 555 – 557.
[9] S. Hemmerich, H. Leffler, S. D. Rosen, *J. Biol. Chem.* **1995**, *270*, 12035 – 12047.
[10] a) S. Komba, H. Ishida, M. Kiso, A. Hasegawa, *Bioorg. Med. Chem.* **1996**, *4*, 1833 – 1847; b) C. Mitsuoka, N. Kawakami-Kimura, M. Kasugai-Sawada, N. Hiraiwa, K. Toda, H. Ishida, M. Kiso, A. Hasegawa, R. Kannagi, *Biochem. Biophys. Res. Commun.* **1997**, *230*, 546 – 551.
[11] C. Galustian, A. M. Lawson, S. Komba, H. Ishida, M. Kiso, T. Feizi, *Biochem. Biophys. Res. Commun.* **1997**, *240*, 748 – 751.
[12] C. Mitsuoka, M. Sawada-Kasugai, K. Ando-Furui, M. Izawa, H. Nakanishi, S. Nakamura, H. Ishida, M. Kiso, R. Kannagi, *J. Biol. Chem.* **1998**, *273*, 11225 – 11233.
[13] a) T. Murase, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1988**, *184*, c1 – c4; b) A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1991**, *10*, 493 – 498; c) A. Hasegawa, M. Kiso in *Preparative Carbohydrate Chemistry* (Ed.: S. Hanessian), Dekker, New York, **1997**, pp. 357 – 379.
[14] a) G. H. Veeneman, S. H. van Leeuwen, J. H. van Boom, *Tetrahedron Lett.* **1990**, *31*, 1331 – 1334; b) P. Konradsson, U. E. Udodong, B. Fraser-Reid, *Tetrahedron Lett.* **1990**, *31*, 4313 – 4316.
[15] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, K. Stenvall, *J. Org. Chem.* **1988**, *53*, 5629 – 5647.
[16] R. R. Schmidt, G. Grundler, *Synthesis* **1981**, 885 – 887.
[17] P. Fügedi, P. J. Garegg, *Carbohydr. Res.* **1986**, *149*, c9 – c12.
[18] R. R. Schmidt, P. Zimmermann, *Tetrahedron Lett.* **1986**, *27*, 481 – 484.
[19] M. Kiso, A. Nakamura, Y. Tomita, A. Hasegawa, *Carbohydr. Res.* **1986**, *158*, 101 – 111.
[20] a) T. Murase, H. Ishida, M. Kiso, A. Hasegawa, *Carbohydr. Res.* **1989**, *188*, 71 – 80; b) A. Kameyama, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1991**, *10*, 549 – 560; c) A. Hasegawa, M. Kiso, *Methods Enzymol.* **1994**, *242*, 158 – 183.
[21] R. R. Schmidt, P. Zimmermann, *Angew. Chem.* **1986**, *98*, 722 – 723; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 725 – 726.
[22] A. Hasegawa, K. Ito, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1995**, *14*, 353 – 368.

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