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2-(Dimethyloctylsilyl)ethyl lactoside: a versatile intermediate for chemical and enzymic ganglioside synthesis

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The discovery that glycoconjugates containing sialic acid fulfill a number of important biological functions [1] has led to an increased interest in the synthesis of sialosides. Yet despite significant advances in methodology, chemical synthesis of the naturally occurring α-sialosides remains a major challenge. Interest in antibodies that bind the carbohydrate epitopes of gangliosides such as GD_3 prompted the synthesis of an oligosaccharide that could be either deprotected or subjected to further reactions at the anomeric center. This led us to adopt a potentially general method for the combined chemical–enzymic preparation of gangliosides. A new 2-(dimethyloctylsilyl)ethyl [DMOSEt] protecting group for the anomeric center is introduced, and unlike its 2-(trimethylsilyl)ethyl [TMSEt] counterpart [2], DMOSEt can be used as a hydrophobic anchor to simplify oligosaccharide isolation from enzyme-catalyzed reactions, while maintaining the ease of subsequent synthetic transformation at the reducing terminus of complex oligosaccharides.

The versatility of TMSEt glycosides has been well demonstrated since they are readily converted under mild conditions to glycosyl donors, either directly [3,4] or via the anomeric hemiacetal [4,5]. The resulting glycosyl donor can then be coupled to a ceramide derivative to form the ganglioside [6]. In synthesizing the oligosaccharide portion, the use of activated derivatives of *N*-acetylneuraminic acid (Neu5Ac) as glycosyl donors under standard glycosylation conditions often gives both poor yields and anomeric mixtures of sialosides [7]. In order to promote the formation of natural α-sialosides and to increase the yields in glycosylation of secondary alcohols, more complex derivatives of Neu5Ac, such as xanthates, [8] thioglycosides [9], and glycosyl donors with a C-3 stereocontrolling auxiliary

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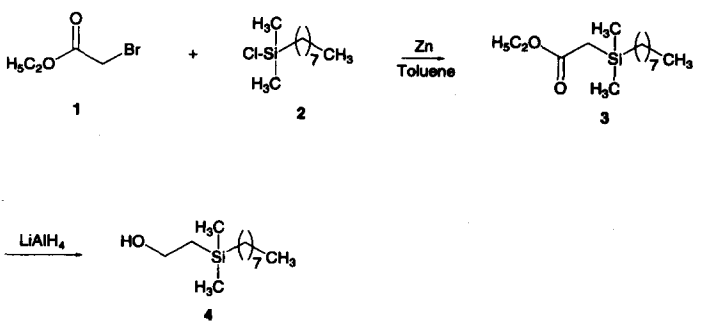
group [10] have been used in the chemical synthesis of sialyloligosaccharides [11].

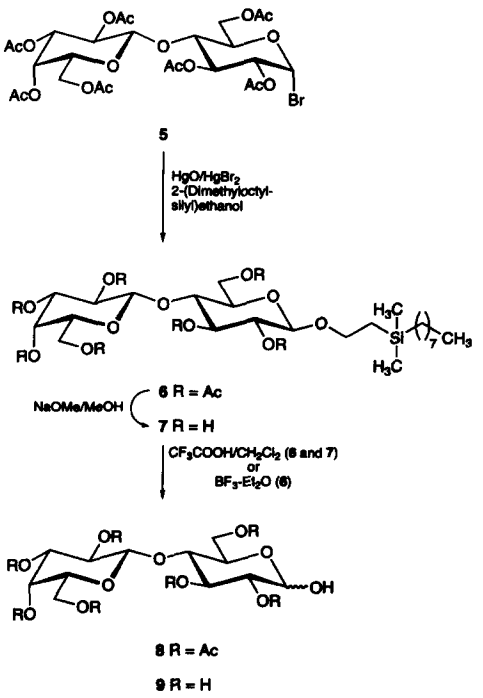
An alternate approach to the preparation of such oligosaccharides uses enzyme-catalyzed syntheses that show a high degree of stereo- and regio-selectivity in product formation. In particular, by using galactosyl- [12] and sialyl-transferases [13], the synthesis of a number of complex oligosaccharides have been reported [1,14]. By combining the chemical flexibility of the TMSEt protecting group with hydrophobic characteristics that permit glycosides formed in an enzyme incubation to be readily separated from unreacted sugar-nucleotide and byproducts after adsorption on a reversed-phase C_{18} cartridge, we sought to utilize the convenience and efficiency of sialyltransferase enzymes for the synthesis of ganglioside precursors. This reversed-phase recovery protocol is well documented with glycosides of hydrophobic alcohols such as octanol and 8-methoxycarbonyloctanol [15]. Not surprizingly, initial tests with 2-(trimethylsilyl)ethyl lactoside showed that only 30% of the material in aqueous solution could be recovered using Sep-Pak recovery [15], presumably due to the unfavourable balance between the hydrophobicity of the aglycon and polarity of the disaccharide. Since the unfavourable partitioning of product glycosides could only deteriorate by addition of polar Neu5Ac residues, a strategy was developed to synthesize DMOSEt glycosides. In principle, the products from the enzymic reaction with such glycosides should be converted typically in >90% yield into the corresponding 1-*O*-acyl [4], hemiacetal [4], or 1-chloro-sugars [16], en route to glycoconjugates. Sialylated oligosaccharides usually are activated as 1-trichloroacetimidates [17] which yield glycoconjugates under mild conditions.



The DMOSEt alcohol **4** was prepared by a Reformatsky-type reaction [18]. Ethyl bromoacetate (**1**) reacted smoothly with chlorodimethyloctylsilane (**2**) and zinc. After the standard workup procedure, involving treatment of the mixture with M hydrochloric acid to remove the zinc salts and washing with sodium bicarbonate solution, subsequent reduction of ethyl dimethyloctylsilyl acetate (**3**) with lithium–aluminium hydride in ether [19] gave 2-(dimethyloctylsilyl)ethanol (**4**, 55% yield from **2**).

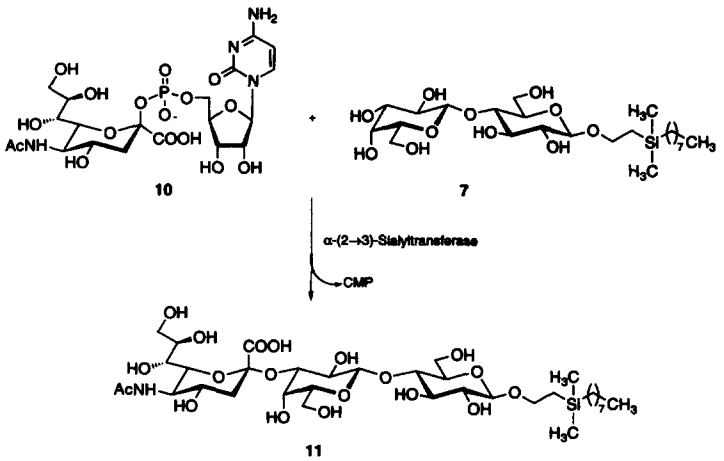
The DMOSEt glycoside was obtained using a procedure by Lipshutz and co-workers [20], who used Königs–Knorr conditions to produce 2-(trimethylsilyl)ethyl glycosides. Starting from the lactosyl bromide **5**, mercury-salt catalyzed glycosylation gave the β-DMOSEt glycoside **6** in 73% yield. Deacetylation under Zemplén conditions afforded **7** in 91% yield. Before attempting the enzymic reaction, selective anomeric deblocking was

carried out under the mild conditions described for TMSEt glycosides [4] and applied to sialylated oligosaccharides [21]. Therefore, **6** or **7** were dissolved in a dichloromethane–trifluoroacetic acid mixture at room temperature, and the hemiacetals **8** and **9** could be isolated in >90% yield. The reaction time (1.5 h) recorded was three times longer than that reported for the TMSEt group [4,21]. An alternate procedure, treatment with $BF_3 \cdot Et_2O$, was also shown to yield **8** from **6** under mild conditions [3].



Before starting a preparative sialylation, acceptor **7** was first tested in an α-(2→3)-sialyltransferase assay [22], using ^{14}C-labeled CMP-Neu5Ac (**10**). After incubation of an enzyme extract and donor **10** (100 μM) with acceptor **7** (1 mM), the mixture was diluted with water (5 mL), then applied to a freshly conditioned Sep-Pak cartridge. The cartridge was first washed with water, and then the trisaccharide α-Neu5Ac-(2→3)-β-D-Gal-(1→4)-β-D-Glc-(1→O)-DMOSEt (**11**) was eluted with methanol. The turnover rate for the lactose derivative **7** was ca. 2% of that reported for a β-D-Gal-(1→3)-D-GlcNAc acceptor (concentration 0.4 mM with 100 μM donor **10**) [23].

Sialylation on a larger scale was accomplished in a similar manner. Due to the longer reaction time (6 days), the labile donor CMP-Neu5Ac (**10**) was added in small portions over the reaction period. After initial recovery on the Sep-Pak cartridge, product **11** could be isolated in 39% yield by silica gel column chromatography on Iatrobeads.



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In the foregoing example we have demonstrated that the DMOSEt group combines the known versatility of the TMSEt protection group for the anomeric center with the hydrophobicity necessary to use reversed-phase adsorbents for simple and rapid product workup from enzyme reactions. This result permits the facile incorporation of an enzyme-catalyzed glycosylation step into a chemical sequence for the preparation of oligosaccharides, including subsequent manipulations at the reducing terminus of labile oligosaccharides.

1. Experimental

General methods.—CMP-Neu5Ac:β-D-galactosideα-(2→3)-*N*-acetylneuraminyltransferase (EC 2.4.99.6), a soluble form of α-(2→3)-sialyltransferase, cloned and expressed in insect cells, was used for preparative synthesis [24]. Calf intestinal mucosa orthophosphoric monoester phosphohydrolase (EC 3.1.3.1), specific activity 1500 U/mg protein, was by Sigma Chemical Co. (St. Louis, MO, USA). ^{1}H NMR spectra were recorded with Bruker AM 360 (360 MHz) or Varian Unity 500 (500 MHz) instruments. Chemical shifts are referenced to residual $CHCl_3$ (δ_H 7.25) for $CDCl_3$ solutions and for compound **11** in D_2O–CD_3OD solution relative to internal 1% v/v acetone-d_6 (δ 2.05). All reactions were monitored by TLC on Silica Gel FG_{254} (E. Merck, Darmstadt, Germany) and with detection by UV light or by charring with H_2SO_4. Column chromatography was performed on Silica Gel 60 (230–400 mesh, E. Merck, Darmstadt, Germany) or Iatrobeads 6RS-8060 (Iatron, Tokyo, Japan). Sep-Pak C_{18} reversed-phase cartridges were obtained from Waters Associates (Mississauga, ON, Canada), and were conditioned before use by washing with 10 mL of MeOH and 20 mL of water. Elemental analyses were performed by the Analytical Service Laboratories of the University of Alberta, Edmonton. Optical rotations were determined on a Perkin–Elmer 241 polarimeter in a 1 dm cell at ambient temperature (23 ± 1°C).

2-(Dimethyloctylsilyl)ethanol (**4**).—In a three-necked, round-bottomed flask equipped with a stirrer, dropping funnel, and condenser arranged for distillation, were placed toluene (200 mL) and freshly sandpapered zinc strips (9.78 g, 150 mmol). To ensure dryness,

toluene (20 mL) was distilled, and the condenser was replaced by a reflux condenser with a $CaCl_2$ drying tube. A solution of chlorodimethyloctylsilane (19.5 mL, 81 mmol) and ethyl bromoacetate (16.6 mL, 150 mmol) in dry toluene (30 mL) and anhyd ether (30 mL) was added over a 30-min period to the gently refluxing mixture. During the addition, a crystal of iodine was added to help initiate the reaction. After the addition, the mixture was refluxed until the zinc was dissolved (2–4 h). The mixture was cooled in an ice bath, and M HCl (100 mL) was added over a 15-min period with stirring. The mixture was stirred for 5 min and the phases were separated. The organic phase was washed with M HCl (100 mL) and the combined aqueous layers were extracted with ether. The combined organic layers were washed with water, satd $NaHCO_3$, water, and dried ($CaSO_4$). To reduce the ester function, the solution of ethyl dimethyloctylsilylacetate in ether was added directly to a suspension of $LiAlH_4$ (3.6 g, 100 mmol) in dry ether (150 mL). After heating (1 h) under reflux, excess $LiAlH_4$ was destroyed by addition of satd K_2CO_3 solution (50 mL). After evaporation of the ether phase, the products were separated on silica gel (50:1 hexane–EtOAc) to yield **4** (9.77 g, 55%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.04 (s, 6 H, $SiCH_3$), 0.56 (m, 2 H, Si $CH_2C_7H_{15}$), 0.93 (t, 3 H, $SiC_7H_{14}CH_3$), 0.99 (m, 2 H, $SiCH_2CH_2OH$), 1.27–1.48 (m, 12 H, $SiCH_2C_6H_{12}CH_3$), 3.73 (m, 2 H, $HOCH_2CH_2Si$). Anal. Calcd for $C_{12}H_{28}OSi$: C, 66.59; H, 13.04. Found: C, 66.46; H 13.03.

*2-(Dimethyloctylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galacto-pyranosyl)-β-*D*-glucopyranoside* (**6**).—2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-glucopyranosyl bromide **5** (1.3 g, 1.87 mmol) was added to a stirred suspension of HgO (406 mg, 1.87 mmol), $HgBr_2$ (50 mg, 0.14 mmol), $CaSO_4$ (503 mg, 3.74 mmol), and 2-(dimethyloctylsilyl)ethanol **4** (370 mg, 1.70 mmol) in dry CH_2Cl_2 (100 mL). The mixture was stirred (15 h) with protection from light, then filtered through Celite, and washed with satd aq $NaHCO_3$ and water. The organic phase was dried and concentrated, and the residue was dissolved in 1:1 toluene–nitromethane (60 mL), and $HgBr_2$ (50 mg) was added. The mixture was stirred for 24 h at 50°C [in order to transform the 2-(dimethyloctylsilyl)ethyl orthoacetate into **6**], passed through a column of activity grade 2 alumina, concentrated and chromatographed (8:2 toluene–acetone) to yield **6** (727 mg, 52%) as a colorless syrup; $[\alpha]_D^{20}$ −21° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 0.11 (s, 6 H, $SiCH_3$), 0.64 (m, 2 H, Si $CH_2C_7H_{15}$), 1.00–1.08 (m, 5 H, $SiC_7H_{14}CH_3$, $SiCH_2CH_2OH$), 1.38–1.44 (m, 12 H, $SiCH_2C_6H_{12}CH_3$), 2.09, 2.15, 2.16, 2.17, 2.18, 2.24, 2.27, 2.37 (8 s, 24 H, OAc), 3.64 (m, 2 H, OCH_2CH_2Si), 3.69 (ddd, 1 H, $J_{4,5}$ 8.9, $J_{5,6a}$ 1.9, $J_{5,6b}$ 4.9 Hz, H-5), 3.87 (dd ~ t, 1 H, $J_{3,4}$ 8.9 Hz, H-4), 3.95 (ddd ~ t, 1 H, $J_{4',5'} < 1$, $J_{5',6a'} = J_{5',6b'} = 6$ Hz, H-5′), 3.99 (m, 2 H, OCH_2CH_2Si), 4.11–4.24 (m, 4 H, H-6a,6b,6a′,6b′), 4.54, 4.55 (2 d, 2 H, $J_{1,2} = J_{1',2'} = 7.1$ Hz, H-1,1′), 4.94 (dd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 5.03 (dd, 1 H, $J_{2',3'}$ 9.6, $J_{3',4'}$ 3.1 Hz, H-3′), 5.17 (dd, 1 H, H-2′), 5.26 (dd, 1 H, H-3), 5.40 (dd, 1 H, H-4′). Anal. Calcd for $C_{38}H_{62}O_{18}Si$: C, 54.66; H, 7.48. Found: C, 54.60; H 7.23.

*2-(Dimethyloctylsilyl)ethyl 4-*O*-(β-*D*-galactopyranosyl)-β-*D*-glucopyranoside* (**7**).—The heptaacetate **6** (218 mg, 0.26 mmol) was dissolved in dry MeOH (50 mL), containing NaOMe (from sodium, 2 mg), and the solution was stirred at room temperature (12 h). After neutralization with dry ice and evaporation, the product was filtered through silica gel (1:1 CH_2Cl_2–MeOH) and subsequently through a cellulose acetate membrane (0.2-μm pore size). Yield: 126 mg (91%); colorless syrup; $[\alpha]_D^{20}$ −19° (*c* 0.9, MeOH); ^{1}H NMR

($CDCl_3$): δ −0.02 (s, 6 H, $SiCH_3$), 0.53 (m, 2 H, $SiCH_2C_7H_{15}$), 0.91 (m, 5 H, $SiC_7H_{14}CH_3$, $SiCH_2CH_2OH$), 1.29 (m, 12 H, $SiCH_2C_6H_{12}CH_3$), 3.22 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 8.0 Hz, H-2), 3.39 (ddd, 1 H, $J_{5',6a'}$ 7.1, $J_{5',6b'}$ 4.9 Hz, H-5'), 3.61 (m, 2 H, OCH_2CH_2Si), 3.68 (dd, 1 H, $J_{6a',6b'}$ 11.2 Hz, H-6b'), 3.77 (dd, 1 H, H-6a'), 3.81 (m, 1 H, H-4'), 3.83 (dd, 1 H, $J_{5,6a}$ 2.8, $J_{5,6b}$ 5.7, $J_{6a,6b}$ 14.2 Hz, H-6a), 3.89 (dd, 1 H, H-6b), 3.97 (m, 2 H, OCH_2CH_2Si), 4.29 (d, 1 H, H-1), 4.35 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1'). Anal. Calcd for $C_{24}H_{38}O_{11}Si$: C, 54.32; H, 7.22. Found: C, 53.91; H 7.41.

*2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-*D*-glucopyranose* (**8**).—*Method A*. The DMOSEt glycoside **6** (20 mg, 24 μmol), maintained under N_2, was dissolved in CH_2Cl_2 (2 mL), CF_3CO_2H (0.2 mL) was added, and the mixture stirred for 1.5 h at room temperature. Ethyl acetate (2 mL) and toluene (4 mL) were repeatedly added and evaporated under reduced pressure. A second portion of toluene (5 mL) was added, and the reducing sugar **8** was filtered through silica gel (1:1 toluene–acetone) to yield **8** (14 mg, 92%).

Method B. The DMOSEt glycoside **6** (11 mg, 13 μmol) was dissolved in dry CH_3CN (2 mL). $BF_3 \cdot Et_2O$ (1.4 μL, 11 μmol) was added, and the mixture stirred for 90 min at room temperature. Addition of water, extraction with CH_2Cl_2, removal of solvent, and filtration through silica gel (1:1 toluene–acetone) gave **8** (8 mg, 94%). The analytical data found for **8** were in agreement with the reported data [4].

*4-*O*-(β-*D*-Galactopyranosyl)-*D*-glucopyranose* (**9**).—The DMOSEt glycoside **7** (10 mg, 18 μmol), maintained under N_2 was dissolved in CH_2Cl_2 (2 mL), CF_3CO_2H (0.2 mL) was added, and the mixture was stirred for 1.5 h at room temperature. Ethyl acetate (2 mL) and toluene (4 mL) were added and evaporated under reduced pressure. A second portion of toluene (5 mL) was added, and the reducing sugar **9** was filtered through Iatrobeads (60:40:3 CH_2Cl_2–MeOH–H_2O) to yield 6 mg (97%) **9**. The analytical data found for **9** were in agreement with the reported data[4].

*2-(Dimethyloctylsilyl)ethyl 4-*O*-[3-*O*-(5-acetamido-3,5-dideoxy-α-*D*-glycero-*D*-galacto-2-nonulopyranosylonate)-β-*D*-galactopyranosyl]-β-*D*-glucopyranoside* (**11**).—To a solution of DMOSEt glycoside **7** (5.0 mg, 9.3 μmol) and CMP-Neu5Ac (2.0 mg, 3.2 μmol) in sodium cacodylate buffer (2.1 mL, 0.16 M, pH 7.0) containing $MnCl_2$ (9 mM) and Triton CF_{54} (0.1%), α-(2→3)-sialyltransferase (300 mU) and alkaline phosphatase (4.0 U) were added. The mixture was gently stirred at 37°C for 6 days. Additional CMP-Neu5Ac (4×2.0 mg, 4×3.2 μmol) was added every day over the incubation period. After cooling to room temperature, water (5 mL) was added, the mixture passed through a Sep-Pak cartridge (C_{18} Plus), and the cartridge was washed with water (30 mL). The crude product was eluted with MeOH (30 mL), and after concentration by evaporation, **11** was purified on Iatrobeads (130:70:1 CH_2Cl_2–MeOH–H_2O); yield, 3.1 mg (39%) based on added acceptor **7**. ^1H NMR (500 MHz, 5:1 D_2O–CD_3OD): δ −0.10 (s, $SiCH_3$), 0.45 (m, $SiCH_2C_7H_{15}$), 0.81 (m, $SiC_7H_{14}CH_3$, $SiCH_2CH_2OH$), 1.22 (m, $SiCH_2C_6H_{12}CH_3$), 1.59 (m, $J_{3e'',3a''}$ 13.8 Hz, H''-3a), 2.06 (s, NHAc), 2.76 (dd, $J_{3e'',4''}$ 8.9 Hz, H''-3e), 4.20, 4.34 (2 d, $J_{1,2} = J_{1',2'} = 7.9$ Hz, H-1,1'). FABMS: m/z 855 $[M+Na]^+$ ($C_{35}H_{65}NO_{19}Si$ requires m/z 832). The configuration of the α-sialoside **11** was confirmed by the chemical shifts of both H-3 protons, according to data presented in the literature [25].

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References

[1] (a) R. Schauer, *Adv. Chem. Biochem.*, 40 (1982) 131–143; (b) S. Sabesan, J.O. Duus, P. Domaille, S. Klem, and J.C. Paulson, *J. Am. Chem. Soc.*, 113 (1991) 5865–5866; (c) L.A. Lasky, *Science*, 258 (1992) 964–969; (d) Y. Ito, J.J. Gaudino, and J.C. Paulson, *Pure Appl. Chem.*, 65 (1993) 753–762.
[2] G. Magnusson, *Trends Glycosci. Glycotechnol.* 4 (1992) 358–367.
[3] K. Jansson, T. Frejd, J. Kihlberg, and G. Magnusson, *Tetrahedron Lett.*, 29 (1988) 361–362.
[4] K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmén, G. Noori, and K. Stenvall, *J. Org. Chem.*, 53 (1988) 5629–5647.
[5] K. Toshima and K. Tatsuta, *Chem. Rev.*, 93 (1993) 1503–1531.
[6] A. Hasegawa, H. Ishida, T. Nagahama, and M. Kiso, *J. Carbohydr. Chem.*, 12 (1993) 703–718.
[7] H. Iijima and T. Ogawa, *Carbohydr. Res.*, 186 (1989) 95–106.
[8] (a) A. Marra and P. Sinaÿ, *Carbohydr. Res.*, 187 (1989) 35–42; (b) W. Birberg and H. Lönn, *Tetrahedron Lett.*, 32 (1991) 7453–7456.
[9] (a) H. Ishida, Y. Ohta, Y. Tsukada, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, 246 (1993) 75–88; (b) T. Murase, A. Kameyama, K.P.R. Kartha, I. Ishida, M. Kiso, and A. Hasegawa, *J. Carbohydr. Chem.*, 8 (1989) 265–283.
[10] (a) Y. Itoh and T. Ogawa, *Tetrahedron Lett.*, 29 (1988) 3987–3990; (b) T. Ercegovic and G. Magnusson, *J. Chem. Soc., Chem. Commun.* 1994 831–832.
[11] (a) K. Okamoto and T. Goto, *Tetrahedron*, 46 (1990) 5835–5857; (b) Y. Ito, M. Numata, M. Sugimoto, and T. Ogawa, *J. Am. Chem. Soc.*, 111 (1989) 8508–8510.
[12] C.-H. Wong, Y. Ichikawa, T. Krach, C. Gautheron-Le Navor, D.P. Dumas, and G.C. Look, *J. Am. Chem. Soc.*, 113 (1991) 8137–8145.
[13] S. Sabesan and J.C. Paulson, *J. Am. Chem. Soc.*, 108 (1986) 2068–2072.
[14] (a) E.J. Toone, E.S. Simon, M.D. Bednarski, and G.M. Whitesides, *Tetrahedron*, 45 (1989) 5365–5422; (b) Y. Ichikawa, Y.-C. Lin, D.P. Dumas, G.-J. Shen, E. Garcia-Junceda, M.A. Williams, R. Bayer, C. Ketcham, L.E. Walker, J.C. Paulson, and C.-H. Wong, *J. Am. Chem. Soc.*, 114 (1992) 9283–9298.
[15] M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconj. J.*, 5 (1988) 49–63.
[16] K. Jansson, G. Noori, and G. Magnusson, *J. Org. Chem.*, 55 (1990) 3181–3185.
[17] R.R. Schmidt and G. Grundler, *Synthesis*, (1981) 885–887.
[18] R.J. Fessenden and J.S. Fessenden, *J. Org. Chem.*, 32 (1967) 3535–3537.
[19] H. Gerlach, *Helv. Chim. Acta*, 60 (1977) 3039–3044.
[20] B.H. Lipshutz, J.J. Pegram, and M.C. Morey, *Tetrahedron Lett.*, 22 (1981) 4603–4606.
[21] (a) H. Ishida, Y. Ohta, Y. Tsukada, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, 246 (1993) 75–88; (b) A. Kameyama, H. Ishida, M. Kiso, and A. Hasegawa, *ibid.*, 200 (1990) 269–285.
[22] M.M. Palcic, A.P. Venot, R.M. Ratcliffe, and O. Hindsgaul, *Carbohydr. Res.*, 190 (1989) 1–11.
[23] K.B. Wlasichuk, M.A. Kashem, P.V. Nikrad, P. Bird, C. Jiang, and A.P. Venot, *J. Biol. Chem.*, 268 (1993) 13971–13977.
[24] S. Gosselin, M. Alhussain, M. Streiff, K. Talakayasi, and M.M. Palcic, *Anal. Biochem.*, 220 (1994) 92–97.
[25] S. Sabesan, K. Bock, and J.C. Paulson, *Carbohydr. Res.*, 218 (1991) 27–54.