Carbohydrate Research, 101 (1982) 271–277
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF 3-*O*-(2-ACETAMIDO-2-DEOXY-3-*O*-β-D-GALACTOPYRANOSYL-β-D-GALACTOPYRANOSYL)-1,2-DI-*O*-TETRADECYL-*sn*-GLYCEROL*

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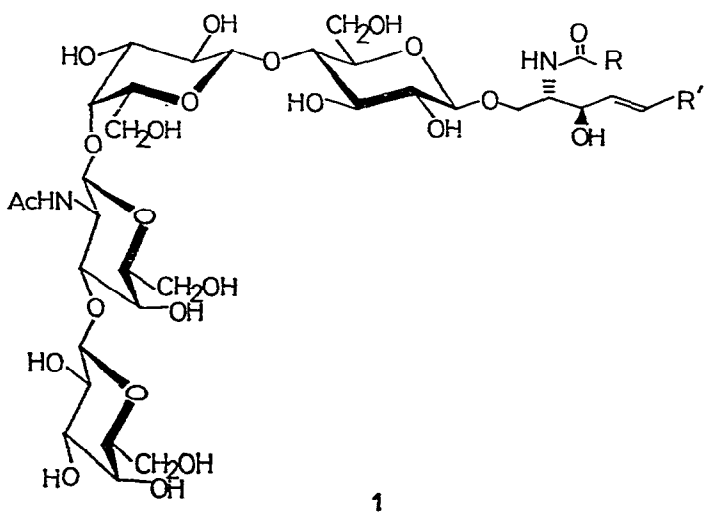
(Received September 24th, 1981; accepted for publication, October 19th, 1981)

ABSTRACT

Stereo- and regio-selective synthesis of 3-*O*-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)-1,2-di-*O*-tetradecyl-*sn*-glycerol by use of 1,2-di-*O*-tetradecyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl)-*sn*-glycerol as a key intermediate is described.

INTRODUCTION

In 1980, Habu *et al.*[2] showed that the glycosphingolipid asialo-GM1 (**1**) is present on very early thymocytes, is lost as the mature, murine T-cell protein-antigens develop on these cells, and may be regarded as a differentiation antigen of fetal thymocytes. Asialo-GM1 (**1**) was also reported[3] to be expressed on mouse natural



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*Part 16 in the series "Synthetic Studies on Cell-Surface Glycans". For part 15, see ref. 1.
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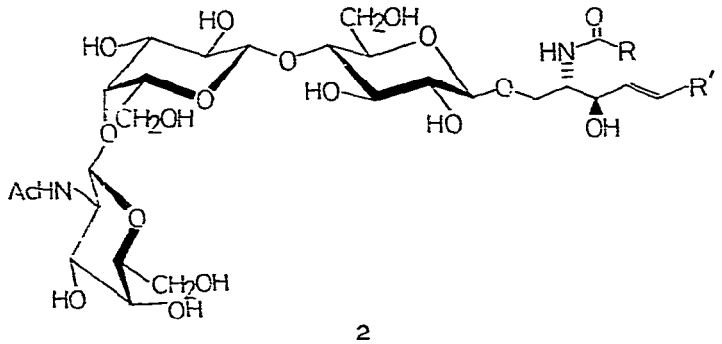

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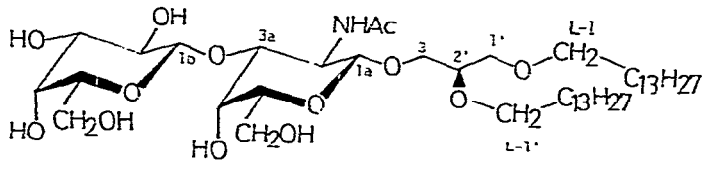

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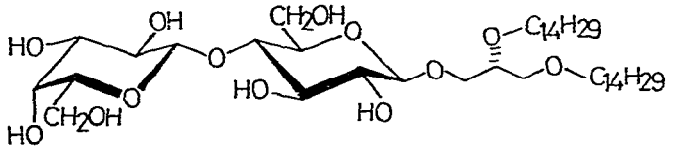
killer cells in high concentration. A related glycosphingolipid, namely, ganglio-*N*-triosylceramide (**2**), was recently established[4] as a tumor-specific, cell-surface marker for the tumor, in mice, derived from Kirsten virus-transformed 3T3 cells. In view of such crucial importance of these glycosphingolipids, we describe here a synthetic study of the model glycoglycerolipid (**3**), which carries the terminal disaccharide sequence of asialo-GM1 (**1**), and which may be used to raise specific antibody for immunological study[3,5]. In close connection to the present study, we have reported[6] the synthesis of the model glycoglycerolipid **4**, corresponding to lactosylceramide, which carries the internal disaccharide sequence of asialo-GM1.

RESULTS AND DISCUSSION

Synthetic target **3** may be retrosynthesized into three synthons, namely, one glycosyl acceptor[7] (**5**) and two glycosyl donors, **6** and **7**. 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-galactopyranose (**6**) was expected to be an efficient glycosyl donor in the presence of trimethylsilyl triflate, according to previous observations[8], and was prepared similarly to 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-*β*-D-glucopyranose[9].

2-Amino-2-deoxy-D-galactose hydrochloride (**8**) was rapidly treated with NaOMe in MeOH, to neutrality, and the amine was allowed to react with phthalic anhydride and Et_3N; the product was then treated with Ac_2O in pyridine, to give crystalline **6** in 44.3% overall yield. The ^{1}H-n.m.r. spectrum of **6** confirmed the assigned structure, as it contained signals for H-1, H-2, H-3, and H-4 at δ 6.45 (doublet, J 8 Hz), 4.66 (quartet, J 8, 10 Hz), 5.94 (quartet, J 2.5, 10 Hz), and 5.51 (doublet, J 2.5 Hz).



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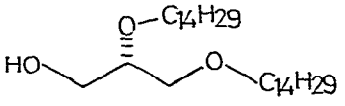

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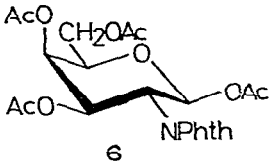

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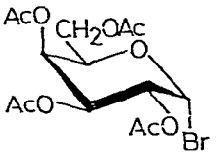

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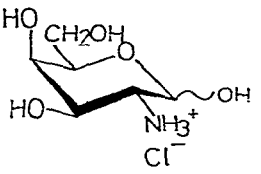

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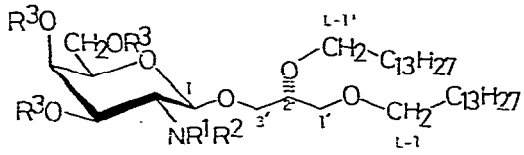
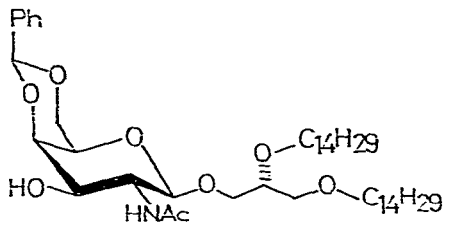
The reaction of **6** with the glycosyl acceptor **5** in the presence of added[8] $Me_3SiOSO_2CF_3$ afforded an 81.5% yield of the β-glycoside **9**. The β-anomeric configuration of **9** was assigned from ^{1}H-n.m.r. data: a signal for H-1 at δ 5.28 as a doublet with J 8 Hz. Deacylation of **9** with[10] $BuNH_2$, to give **10**, and *N*-acetylation of **10**, afforded crystalline **11** in 73.8% yield. The ^{13}C-n.m.r. spectrum of **11** has a signal for C-1 at δ 101.5, with $^1J_{CH}$ 159.7 Hz, confirming the β configuration of C-1. Treatment of **11** with benzaldehyde and $ZnCl_2$ afforded a 72% yield of crystalline **12**. Glycosylation of HO-3 of **12** with the glycosyl donor **7** was performed by using $Hg(CN)_2$ in benzene–nitromethane[11], to give the expected product **13** in 78.7% yield. Hydrolysis of the benzylidene group of **13** in aqueous AcOH, to give **14**, and deacylation of **14** with Et_3N–MeOH–H_2O, afforded an 83.3% yield of the target molecule **3**. The anomeric configurations in **3** were both assigned as β from the ^{13}C-n.m.r. spectrum, which showed two signals, at δ 101.0 with $^1J_{CH}$ 159.7 Hz, and at δ 103.9 with $^1J_{CH}$ 156.8 Hz, for C-1a and C-1b, respectively. Introduction of a β-D-galactopyranosyl group at O-3a of **11** was confirmed by the presence of a deshielded signal for C-3a at δ 78.8, due to the α-effect of glycosylation.

In conclusion, the model glycoglycerolipid **3**, which carries the terminal disaccharide sequence of asialo-GM1 **1**, was synthesized in a regio- and stereo-controlled way.



9 R^1, R^2 = phthaloyl, R^3 = Ac
10 R^1 = R^2 = R^3 = H
11 R^1 = R^3 = H, R^2 = Ac



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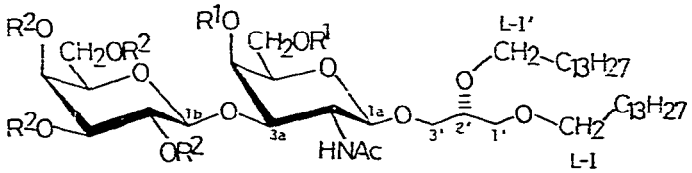

13 R^1, R^1 = benzylidene, R^2 = Ac
14 R^1 = H, R^2 = Ac

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter for solutions in $CHCl_3$ at 25°, unless otherwise noted. I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, as KBr pellets for the crystalline samples, and as neat films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with a Varian HA-100 n.m.r. spectrometer, using tetramethylsilane as the internal standard. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT NMR spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downward from the internal standard for solutions in $CDCl_3$, unless otherwise noted. Powdered molecular sieve 4A, employed in glycosylations, was activated before use by heating *in vacuo* for 10–15 h at 180–200°. Column chromatography was performed in columns of Silica Gel Merck (70–230

mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography was performed on precoated plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) of Silica Gel 60 F_{254}.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose (**6**). — To a solution of NaOMe in MeOH, prepared from Na (2.1 g, 0.09 mol) and MeOH (90 mL), was added **8** (19.8 g, 0.09 mol) in one portion at 15–20°, with stirring. The mixture was further stirred for 5 min, and filtered. To the filtrate were added powdered phthalic anhydride (6.6 g, 446 mmol) and Et_3N (9 g), the mixture was stirred at 15–20° until it became a clear solution, and then further phthalic anhydride (7.2 g, 486 mmol) was added. The mixture was stirred for 30 min at 15–20° and then for 20 min at 50°, cooled to room temperature, and diluted with Et_2O (300 mL), to give crystals which were collected, dried, and dissolved in pyridine (200 mL) and Ac_2O (100 mL). The mixture was stirred for 15 h at 15–20°, and evaporated *in vacuo*, to give an oil which was chromatographed on 2:1 SiO_2–Hyflo Super-Cel (500 g) in 3:1 toluene–EtOAc, to afford **6** (19.4 g, 44.3%); m.p. 99–101° (from i-Pr_2O), $[\alpha]_D$ +31.1° (*c* 0.83); R_F 0.45 in 2:1 toluene–EtOAc; δ_H: 7.9–7.7 (m, 4 H, phthaloyl), 6.45 (d, 1 H, *J* 8 Hz, H-1), 5.94 (q, 1 H, *J* 2.5, 10 Hz, H-3), 5.51 (d, 1 H, *J* 2.5 Hz, H-4), 4.66 (q, 1 H, *J* 8, 10 Hz, H-2), 4.21 (bs, 3 H, H-5,6,6'), and 2.21, 2.06, 2.01, and 1.86 (s, four 3 H, 4 OAc).

Anal. Calc. for $C_{22}H_{23}NO_{11}$: C, 55.35; H, 4.86; N, 2.93. Found: C, 55.84; H, 4.93; N, 2.85.

1,2-Di-O-tetradecyl-3-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-galacto-pyranosyl)-sn-glycerol (**9**). — To a solution of **6** (3.7 g, 7.75 mmol) and **5** (5.6 g, 11.6 mmol) in $Cl(CH_2)Cl$ (30 mL) was added $Me_3SiOSO_2CF_3$ (1.7 mL). The mixture was stirred for 3 h at 15–20°, poured into ice–water, and the mixture extracted with $CHCl_3$. The extract was successively washed with aqueous $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on 2:1 SiO_2–Hyflo Super-Cel (500 g) in 7:1 toluene–EtOAc, to give **9** (5.8 g, 81.5%); $[\alpha]_D$ −10.9° (*c* 0.53); R_F 0.60 in 3:1 toluene–EtOAc; δ_H 7.9–7.7 (m, 4 H, phthaloyl), 5.85 (q, 1 H, *J* 4, 11 Hz, H-3), 5.48 (d, 1 H, *J* 4 Hz, H-4), 5.28 (d, 1 H, *J* 8 Hz, H-1), 4.52 (q, 1 H, *J* 8, 11 Hz, H-2), 2.36, 2.19, 2.06, 1.85 (s, four 3 H, 4 OAc), 1.28 (bs, 48 H, CH_2), and 0.88 (bt, 6 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{51}H_{83}NO_{13} \cdot C_6H_5CH_3$: C, 68.95; H, 9.08; N, 1.39. Found: C, 69.07; H, 9.84; N, 1.42.

3-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-1,2-di-O-tetradecyl-sn-glycerol (**11**). — A mixture of **9** (5.8 g, 6.3 mmol), $BuNH_2$ (30 mL), and MeOH (150 mL) was boiled and stirred under reflux for 19 h, and evaporated *in vacuo*. To the residue in MeOH (60 mL)–oxolane (30 mL) was added Ac_2O (3 mL) dropwise at −5 to 0°, and the mixture was stirred for 2 h at 5°, evaporated *in vacuo*, and the residue triturated with Et_2O (100 mL), to give crude, solid **11** (3.2 g, 73.8%); m.p. 163–165° (from MeOH), $[\alpha]_D$ +1.5° (*c* 0.26, pyridine); R_F 0.27 in 10:1 $CHCl_3$–MeOH; δ_C (Me_2SO-d_6, 80°): 101.5 ($^1J_{CH}$ 159.7 Hz, C-1), 77.1 (C-2'), 75.0 (C-5), 71.3 (C-3),

70.5 and 70.3 (L-1, L-1'), 69.0 (C-1'), 67.8 (C-3'), 67.3 (C-4), 60.2 (C-6), and 51.9 (C-2).

Anal. Calc. for $C_{39}H_{77}NO_8$: C, 68.08; H, 11.28; N, 2.04. Found: C, 67.72; H, 11.38; N, 2.23.

3-O-(2-Acetamido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranosyl)-1,2-di-O-tetradecyl-sn-*glycerol* (**12**). — A mixture of **11** (3.2 g, 4.7 mmol), $ZnCl_2$ (3.5 g), and benzaldehyde (70 mL) was stirred for 18 h at 15–20°. To this mixture were added i-Pr_2O (300 mL) and saturated aqueous NH_4Cl (10 mL), and the mixture was stirred for 1 h; the precipitated crystals were collected, to give **12** (2.6 g, 72.0%); m.p. 111–114° (from i-Pr_2O), $[\alpha]_D$ −4.6° (*c* 0.26; R_F 0.56 in 10:1 $CHCl_3$–MeOH; δ_H: 7.6–7.2 (m, 5 H, aromatic), 6.86 (d, 1 H, *J* 5 Hz, NH), 5.56 (s, 1 H, C*H*Ph), 2.02 (s, 3 H, NAc), 1.28 (bs, 48 H, CH_2), and 0.88 (bt, 6 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{46}H_{81}NO_8$: C, 71.19; H, 10.51; N, 1.80. Found: C, 71.18; H, 10.56; N, 1.80.

3-O-[2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-β-D-galactopyranosyl]-1,2-di-O-tetradecyl-sn-*glycerol* (**13**). — From a mixture of $Hg(CN)_2$ (100 mg) and 14:11 benzene–CH_3NO_2 (85 mL) was distilled off some of the solvent (70 mL). To the cooled mixture was added **12** (790 mg, 1.01 mmol) at room temperature, and to this stirred mixture was added, dropwise, a solution of **7** (410 mg, 1 mmol) in 1:1 benzene–CH_3NO_2 (2 mL) during 3 h at 60°. The mixture was stirred for 8 h at 60°, and then a solution of **7** (200 mg, 0.48 mmol) in 1:1 CH_3NO_2–benzene (1 mL) was added dropwise during 1 h. The mixture was stirred for a further 3 h, poured into ice–water, and extracted with CH_2Cl_2. The extract was successively washed with aqueous $NaHCO_3$, H_2O, and saturated aqueous NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on 2:1 SiO_2–Hyflo Super Cel (80 g) in 1:2 toluene–EtOAc, to give **13** (880 mg, 78.7%); m.p. 124–128° (from MeOH), $[\alpha]_D$ +25.3° (*c* 0.68); R_F 0.43 in 9:9:1 toluene–EtOAc–MeOH; δ_H: 7.7–7.2 (m, 5 H, aromatic), 5.58 (s, 1 H, C*H*Ph), 2.17 (s, 3 H, OAc), 2.05 (s, 6 H, 2 Ac), 1.99 (s, 6 H, 2 Ac), 1.29 (bs, 48 H, CH_2), and 0.88 (bt, 6 H, *J* 6 Hz, CH_3).

Anal. Calc. for $C_{60}H_{99}NO_{17} \cdot 2CH_3OH$: C, 63.62; H, 9.21; N, 1.20. Found: C, 63.02; H, 8.66; N, 1.22.

3-O-(2-Acetamido-2-deoxy-3-O-β-D-galactopyranosyl-β-D-galactopyranosyl)-1,2-di-O-tetradecyl-sn-*glycerol* (**3**). — A solution of **13** (711 mg, 0.64 mmol) in AcOH (30 mL)–H_2O (10 mL) was stirred for 3 h at 50°, and evaporated *in vacuo*, and toluene was twice added to, and evaporated from, the residue. A solution of the residue in Et_3N (5 mL)–H_2O (5 mL)–MeOH (30 mL) was stirred under reflux for 10 h, and cooled. The precipitated solid was collected, to give **3** (455 mg, 83.3%); m.p. 216–218° (from MeOH–H_2O), $[\alpha]_D$ +9.6° (*c* 0.24, pyridine); R_F 0.58 in 14:6:1 $CHCl_3$–MeOH–H_2O; δ_C (Me_2SO-d_6, 80°): 103.9 ($^1J_{CH}$ 156.8 Hz, C-1b), 101.0 ($^1J_{CH}$ 159.7 Hz, C-1a), 78.8 (C-3a), 77.1 (C-2'), 75.1 (C-5a or 5b), 74.8 (C-5b

or 5a), 73.0 (C-3b), 70.6 (L-1 or L-1'), 70.3 (L-1' or L-1,C-2a), 60.0 (C-1'), 67.9 (C-4b,3'), 66.9 (C-4a), 60.3 (C-6a,6b), and 51.0 (C-2a).

Anal. Calc. for $C_{45}H_{87}NO_{13}$: C, 63.57; H, 10.31; N, 1.65. Found: C, 63.59; H, 10.35; N, 1.56.

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