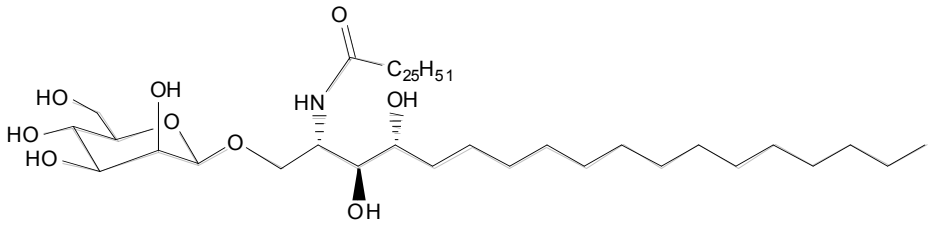
The synthesis of beta-mannosylceramide may be carried out using conventional methods including those described herein for exemplary compound 1. In general, compound 1 may be obtained from the reaction between compound 2 and a desired elctrophile (acid chloride).



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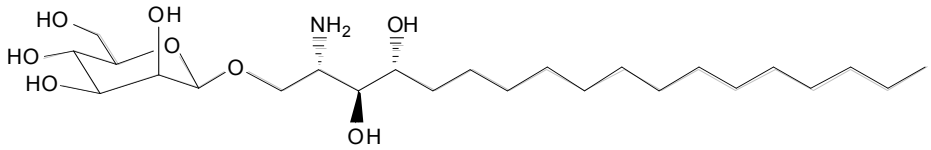


2

The synthesis of compound 2 can be carried out as follows.

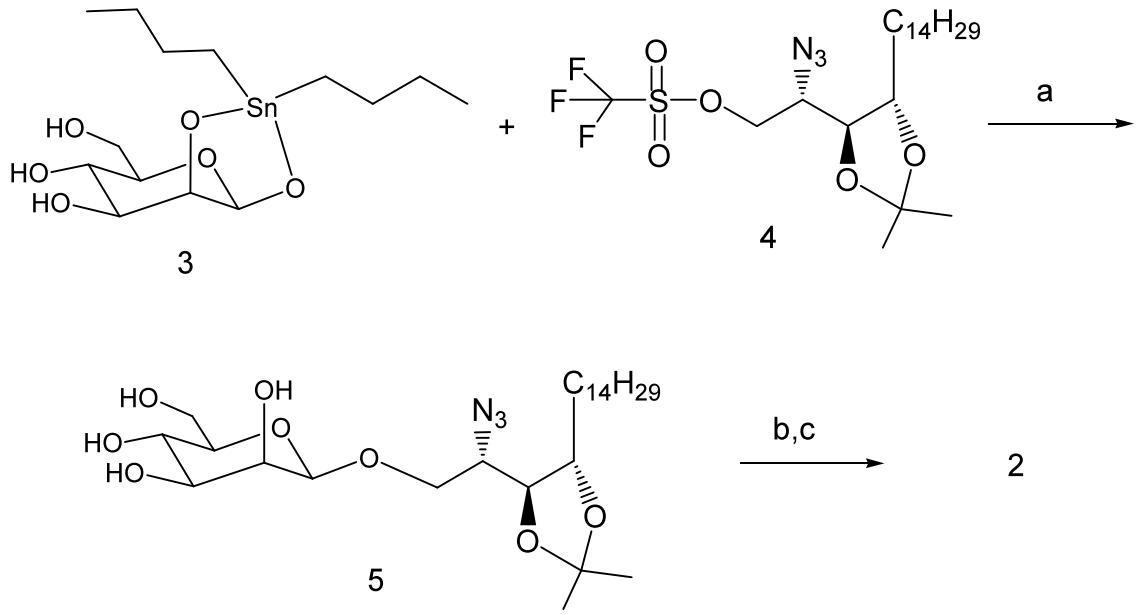
The 1,2-O-stannylene acetal of D-mannose 3 was prepared using the procedure of Hodosi et al.(1). Glycosyl bond formation with compound 4 gave beta-mannoside 5.

Compound 4, 2-azido-3,4-isopropylidene-D-ribo-1-*O*-trifluoromethanesulfonyl 1,3,4-octadecanetriol was prepared from phytosphingosine (Avanti Polar Lipids). Following the procedure of van der Berg et al. (2), the phytosphingosine was converted into azide 6. The isopropylidene group was introduced by reaction with 2,2-dimethoxypropane giving 7. Compound 7 was converted to 4 by reaction with trifluoromethanesulfonyl anhydride.

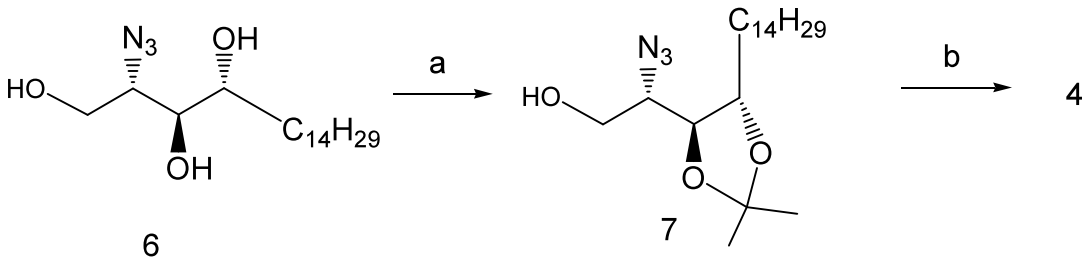
Isopropylidene protection group from 5 was removed using method reported by Dalpozzo et al., (3), followed by reduction of the azide giving 2.

Reaction of 2 with acid chlorides via Schmidt et al., (4) can provide reasonable yields of beta-mannosylceramides. For example, compound 1 was prepared from 2 and hexacosanoyl chloride in 70 % yield.

The synthesized beta-mannosylceramides can be separated from a reaction mixture and further purified by a method such as column chromatography, high pressure liquid chromatography.



Reagents (yields in parentheses): a) CsF, DMSO, MS 4 Å, (60% yield). b) $Ce(OTf)_3$, CH_2Cl_2, CH_3NO_2 (quant. yield). c) H_2S, pyridine, H_2O (80% yield).



Reagents (yields in parentheses): a) 2,2-dimethoxypropane, TsOH (69% yield). b) Tf_2O, Et_3N, CH_2Cl_2 (quant. yield).

Preparation of 2-azido-3,4-isopropylidene-D-ribo-1,3,4-octadecanetriol (7).

Compound 6 (1.0 g, 2.91 mmol) and 2,2-dimethoxypropane (40 ml) were treated with toluene-*p*-sulfonic acid (20 mg) for 14 h at room temperature. The mixture was then neutralised with saturated aqueous sodium hydrogencarbonate (10 ml) and the product was extracted with EtOAc (3x20 ml). The combined extracts were washed with water (2x20 ml), dried over Na_2SO_4 and concentrated under reduced pressure. The desired product 7 (770 mg, 69% yield) was obtained as a clear oil after chromatography (SiO_2, hexane:EtOAc 5:1). NMR (^1H, $CDCl_3$) δ 4.21 (m, 1H), 4.05-3.88 (m, 2H), 3.90 (dd, J=11.0, 4,2 Hz, 1H), 3.44 (m, 1H), 2.09 (bs, 1H, HO-1), 1.52 (m, 2H), 1.48 (s, 3H), 1.22 (s, 3H), 1.26-1.19 (m, 24H), 0.85 (t, J=6.2 Hz, 3H); NMR (^{13}C, $CDCl_3$) δ 107.4, 77.0, 75.4, 62.9, 60.2, 30.9, 28.7, 28.6, 28.4, 28.3, 27.0, 25.5, 24.6, 21.7, 12.1. ES-MS m/e ($[M+Na]^+$) 406.2

Preparation of 2-azido-3,4-isopropylidene-D-ribo-1-*O*-trifluoromethanesulfonyl-1,3,4-octadecanetriol (4).

Et_3N (0.243 mL, 2.0 mmol) and triflic anhydride (0.323 mL, 1.92 mmol) were added to a solution of lipid 7 (700 mg, 1.82 mmol) in CH_2Cl_2 (8 mL) at -20 °C. The reaction mixture was stirred for 1h and saturated aqueous sodium hydrogencarbonate (1 mL) was added. The product was extracted with CH_2Cl_2 (3x10ml), and the combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound 4 was employed in the next step without further purification.

NMR (^1H, $CDCl_3$) δ 4.84 (dd, J= 11.0, 2.4 Hz, 1H), 4.76 (dd, J=11.0, 4,2 Hz, 1H), 4.21 (m, 1H), 4.05 (m, 1H), 3.74 (m, 1H), 1.52 (m, 2H), 1.48 (s, 3H), 1.22 (s, 3H), 1.26-1.19 (m, 24H), 0.85 (t, J=7.0Hz, 3H).

Preparation of 2-azido-3,4-isopropylidene-D-ribo-1-*O*-β-mannopyranosyl-1,3,4-octadecanetriol 5.

Mannose donor 3 (3.0 g, 7.3mmol) was dissolved in anhydrous DMSO (12 mL), molecular sieves (4 Å, 1g) and CsF (1.09g, 7.2 mmol) were added. After addition of lipid 4 (930 mg, 1.82 mmol), the mixture was stirred vigorously at 24 °C for 36 h, and concentrated. The residue was triturated with acetonitrile (20 mL), the resulting suspension was filtered through a pad of Celite, solids were washed with acetonitrile (3x10 mL), and the combined filtrate was concentrated. The residue was purified chromatographically (SiO_2, CH_2Cl_2:MeOH, 14:1) to give compound 5 (580 mg, 60% yield) as a solid foam.

NMR (^1H, CD_3OD:$CDCl_3$ 3:1) δ 4.59 (bs, 1H), 4.15 (m, 1H), 4.08 (dd, J=11.0, 7.4Hz, 1H), 4.00 (J=11.0, 2.5 Hz, 1H), 3.95 (dd, J=9.6, 5.2 Hz, 1H), 3.91 (dd, J=2.9 Hz, 1H), 3.87 (dd, J=11.6, 2.4 Hz, 1H), 3.74 (dd,

J=11.8, 5.4 Hz, 1H), 3.63-3.58 (m, 2H), 3.45 (dd, J=9.4, 2.9 Hz, 1H), 3.22 (m, 1H), 1.66-1.51 (m, 4H), 1.38 (s, 3H), 1.29 (s, 3H), 1.29-1.25 (m, 22H), 0.87 (t, J=6.9 Hz, 3H).

NMR (^{13}C, CD_3OD:$CDCl_3$ 3:1) δ 109.3, 101.2 ($J_{C1,H}$=157Hz, C1 mannose), 78.4, 76.6, 74.9, 72.4, 71.2, 70.8, 68.1, 62.5, 60.6, 32.9,30.2, 28.4,27.8 24.6, 15.0.

ES-MS m/e ($[M+Na]^+$) 568.4

Preparation of 2-amino -1-*O*-β-mannopyranosyl-D-ribo-1,3,4-octadecanetriol 2.

To a solution of 5 (500 mg, 0.92 mmol) in wet nitromethane (2 mL) and CH_2Cl_2 (1 mL) was added $Ce(OTf)_3$ (83 mg, 0.3 mmol) with vigorous stirring. The reaction mixture was stirred at 40 °C for 2h and saturated aqueous sodium hydrogencarbonate (3 mL) was added. The product was extracted with CH_2Cl_2 (3x8 ml), and the combined extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The crude compound was employed in the next step without further purification. ES-MS m/e ($[M+Na]^+$) 528.2.

A solution of compound in pyridine-water (5:1, 3 mL) was saturated with H_2S and stirred for 24 h at 24°C under H_2S. The solution was concentrated under reduced pressure. Reaction mixture was dissolved in $CHCl_3$-MeOH (6:1) and passed through silica pad ($CHCl_3$-MeOH 6:1, as eluent). The solvents were concentrated under reduced pressure to give 2 as a white solid (350 mg, 0.73 mmol, 80% yield).

NMR (^1H, CD_3OD:$CDCl_3$ 2:1) δ 4.50 (bs, 1H), 4.05 (dd, J=11.0, 3.8Hz, 1H), 3.86 (dd, J=11.0, 2.5 Hz, 2H), 3.69 (m, 2H), 3.62-3.44 (m, 4H), 3.41 (dd, J=9.4, 2.9 Hz, 1H), 3.19 (m, 1H), 1.64-1.55 (m, 2H), 1.29-1.25 (m, 24H), 0.92 (t, J=6.9 Hz, 3H). ES-MS m/e ($[M+H]^+$) 480.3, ($[M+Na]^+$) 502.3.

Preparation of 2-hexacosanoylamino -1-*O*-β-mannopyranosyl-D-ribo-1,3,4-octadecanetriol 1.

To a solution of lipid 2 (100 mg, 0.21 mmol) in tetrahydrofuran-50% NaOAc in water (2:1.5) was added hexacosanoyl chloride (130 mg, 0.32 mmol) with vigorous stirring. The reaction mixture was stirred at 24 °C for 4h. The organic phase was separated and the water phase was extracted with tetrahydrofuran (3x4 ml), and the combined extracts and organic phase were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified chromatographically (SiO_2, CH_2Cl_2:MeOH, 11:1) to give compound 1 (127mg, 70% yield) as a white solid.

NMR (^1H, CD_3OD:$CDCl_3$ 1:2) δ 4.50 (bs, 1H), 4.16 (m, 1H), 4.05 (dd, J=11.0, 3.8Hz, 1H), 3.86 (dd, J=11.0, 2.5 Hz, 2H), 3.69 (m, 2H), 3.62-3.44 (m, 3H), 3.41 (dd, J=9.4, 2.9 Hz, 1H), 3.19 (m, 1H), 2.19 (t, J=6.7Hz,

2H), 1.61 (m, 4H), 1.29-1.25 (m, 68H), 0.92 (t, J=6.9 Hz, 6H). High resolution ES-MS m/e ($[M+Na]^+$) 880.7218.

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