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Preliminary Communication

Stereoselective Total Synthesis of Ceramide Di-, Tri- and Tetrahexosides of Wheat Flour*

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The first total synthesis of glycosphingolipids isolated from wheat flour has been achieved in a regio- and stereo-controlled manner.

Unique D-mannose-containing glycosphingolipids have been isolated [1-4] from wheat flour and their structures were proposed [5-7] to be **1**, **2** and **3** (Fig. 1). Anomeric configurations in the structures were analysed through chromic acid oxidation of fully acetylated derivatives of the glycosphingolipids [5-7].

Although the biological functions of these plant glycosphingolipids remain to be elucidated, the potential importance of this class of compounds as biomembrane constituents is rather evident. As part of our synthetic project on glycosphingolipids, we describe here a first total synthesis of this class of plant glycolipid.

Retrosynthetic analysis of the target structures **4** (n = 0-2) which contain 4-hydroxy-*N*-tetracosanoyl-(*2S,3S,4R*)-sphinganine as a ceramide, led us to design a mannosyl donor **5**, a D-glucose derivative **6** as a glycosyl acceptor, and a glycosyl acceptor **7** corresponding to a ceramide part (Fig. 2). The stereoselective synthesis of 4-hydroxy-(*2S,3S,4R*)-sphinganine was reported by Gigg and Gigg in 1966 starting from either D-galactose [8] or 2-benzamidodeoxy-D-glucose [9]. Our approach to the compound **7** followed essentially the route developed by Gigg and Gigg with some modification, which has made the synthetic sequence quite efficient and practical. Thus, a 12 step conversion of the

Abbreviations: THF, tetrahydrofuran; DMF, dimethylformamide.

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Man β 1 → 4Glcβ 1 → 1Cer 1

Man β 1 → 4Manβ 1 → 4Glcβ 1 → 1Cer 2

Man β 1 → 4Manβ 1 → 4Manβ 1 → 4Glcβ 1 → 1Cer 3

Figure 1. Mannose-containing glycosphingolipids from wheat flour.

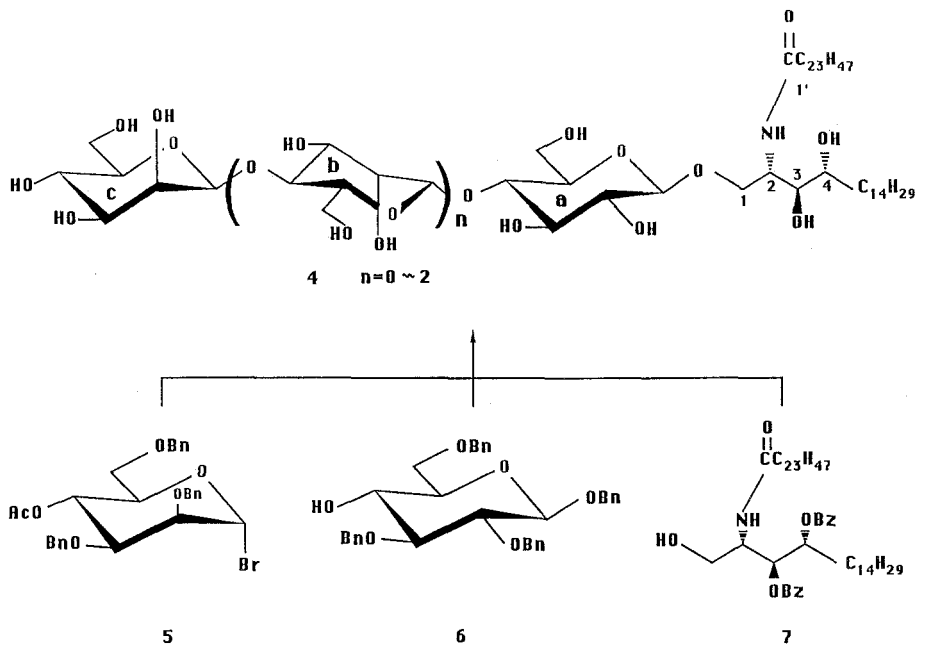


Figure 2. Retrosynthetic analysis of the target structures.

orthoester **8** into **7** could be performed in 15% overall yield (Fig. 3). Sequential treatment of orthoester **8** with 1) aqueous acetic acid, and 2) K_2CO_3-MeOH gave an 80% yield of hemiacetal **9**, $[\alpha]_D$ -45.4° (c 1.4), R_F 0.39 in toluene/EtOAc, 1/2 by vol. The values of $[\alpha]_D$ were recorded for solutions in $CHCl_3$ unless otherwise noted. Synthetic samples described with $[\alpha]_D$ gave reasonable data for elemental analysis. Hemiacetal **9** has been synthesized by Gigg and Gigg [8] using a different route. Conversion of compound **9** into mesylate **10** $[\alpha]_D$ +8.6° (c 0.6), R_F 0.22 in hexane/THF, 9/1 by vol, δH 2.910 (s, 3H, SO_2Me) was performed in 47% yield by the sequential operation; 1) oxidation with sodium metaperiodate, 2) Wittig olefination of the resulting aldehyde, 3) methylsulfonylation of the alcohol, and 4) selective reduction of the double bond with tosyl hydrazine and sodium acetate [10].

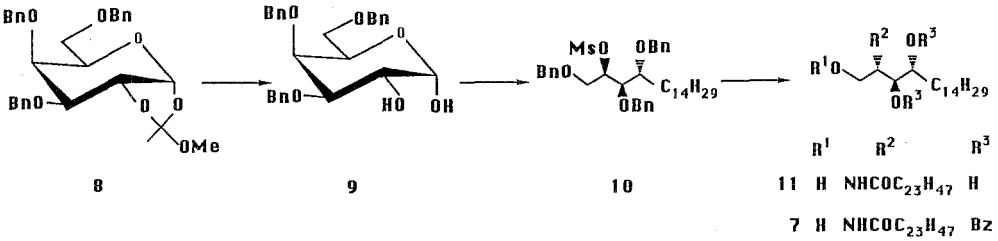
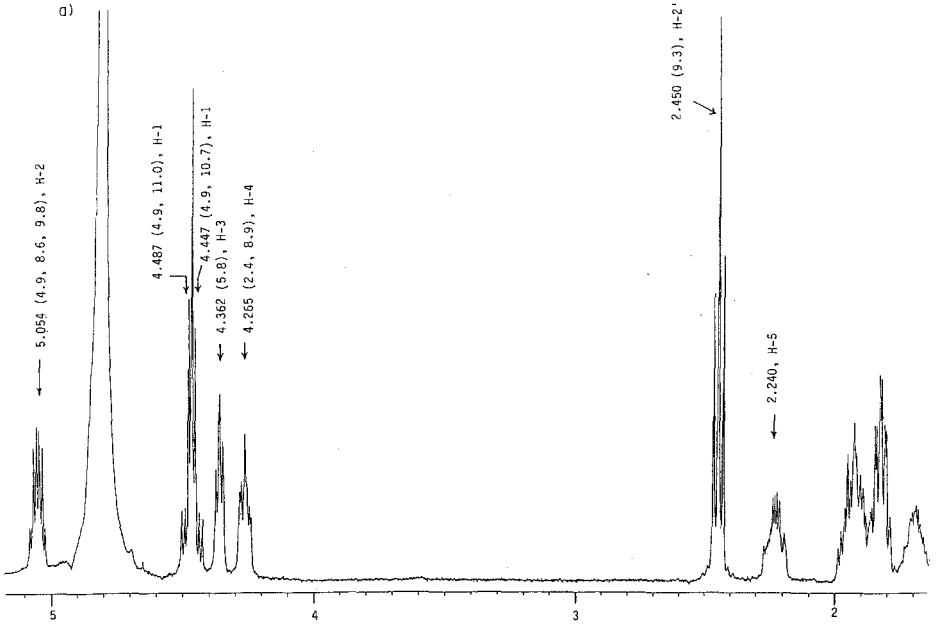
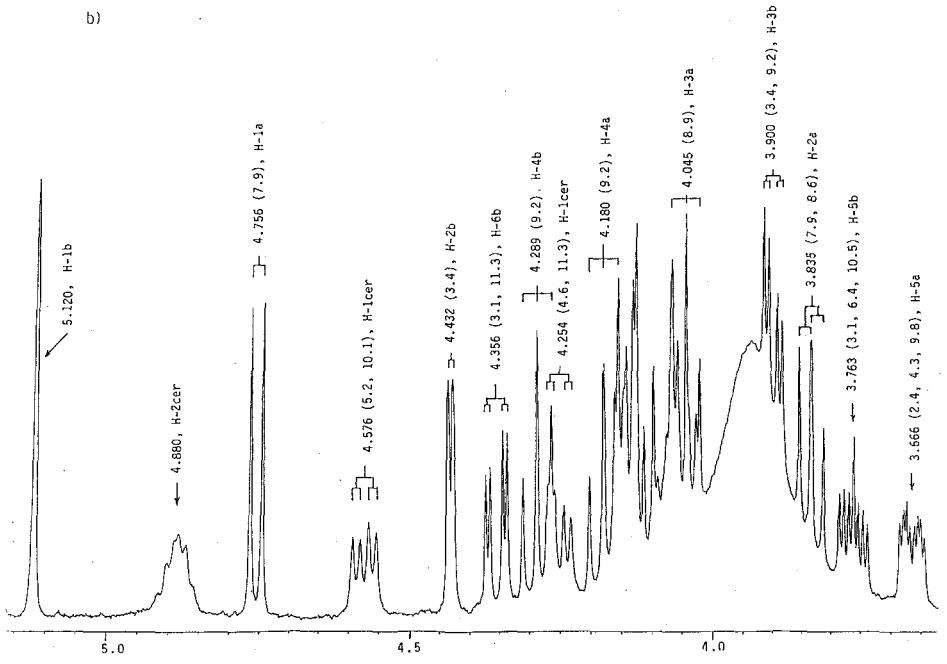


Figure 3. Twelve step conversion of orthoester **8** into **7**.

The mesylate **10** was converted into 4-hydroxy-*N*-tetracosanoyl-(*2S,3S,4R*)-sphinganine **11**, m.p. 113-114°C, $[\alpha]_D$ +1.5° (c 0.53, pyridine), R_F 0.22 in $CHCl_3$/propanol, 19/1 by vol, ^{1}H-NMR (see Fig. 4), in 49% yield in four steps; 1) sodium azide in DMF, 2) 1,3-propanedithiol/triethylamine in methanol [11], 3) tetracosanoic acid/2-chloro-methylpyridinium iodide [12] in CH_2Cl_2, and 4) H_2, 10% Pd-C in THF/propanol, 1/1 by vol. Conventional tritylation, benzoylation and hydrolysis transformed compound **11** in 78% yield into a glycosyl acceptor **7**, m.p. 59-60°C, $[\alpha]_D$ +50.2° (c 0.6), R_F 0.16 in hexane/EtOAc, 4/1 by vol.

Having prepared the lipid part **7** of the target compound **4**, a synthetic route to the glycan part was now examined by use of a glycosyl donor **5** and a glycosyl acceptor **6** as follows (Fig. 5). Trichloroethyl α-D-mannopyranoside **12**, m.p. 151-152°C, $[\alpha]_D$ +78.5° (c 1.1), R_F0.36 in chloroform/methanol, 4/1 by vol, δC 102.1 (*J* 170 Hz, C-1), was obtainable in 40% yield from 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl acetate by the sequential treatment with 1) trichloroethyl-,tributyl-tin oxide/tin tetrachloride in 1,2-dichloro-ethane and 2) sodium methoxide in methanol. Treatment of compound **12** with α,α-dimethoxytoluene and *p*-toluenesulfonic acid in DMF gave a 47% yield of benzylidene derivative **13**, m.p. 146-147°C, $[\alpha]_D$ +68.8° (c 1.0), R_F 0.39 in toluene/EtOAc, 1/1 by vol. Benzylation of compound **13** with α-bromotoluene and sodium hydride, and subsequent solvolysis in aqueous acetic acid afforded a 58% yield of diol **14**, m.p. 130-131°C, $[\alpha]_D$ +39.7° (c 1.0), R_F 0.24 in toluene/EtOAc, 3/2 by vol. Chemoselective monobenzylation of compound **14** through a stannylation-alkylation procedure [13-15] afforded a 95% yield of tribenzyl ether **15**, $[\alpha]_D$ +24.3°C (c 0.7), R_F 0.41 in hexane/EtOAc, 7/3 by vol, which was acetylated to give monoacetate **16**, δH 5.386 (t, *J* 9.8 Hz, H-4). Removal of the trichloroethyl group with zinc powder in acetic acid/THF, 1/2.5 by vol, gave a 93% yield of hemiacetal **17**, which was converted into *p*-nitrobenzoate **18** as a 3:2 mixture of β-D and α-D isomers, δH 6.426 (d, 0.4H, *J* 1.7 Hz, H-1α) and 4.463 (d, 0.6H, *J* 2.2 Hz, H-1β), which was used for the preparation of bromide **5** without separation. A glycosyl acceptor **6**, m.p. 59-60°C, $[\alpha]_D$ -40.3° (c 1.0) [16-19], was prepared from the benzylidene derivative **19** [20] *via* compound **20** in 68% yield by sequential treatment with aqueous acetic acid, and then bistributyl-tin oxide and α-bromotoluene [13-15]. Treatment of *p*-nitrobenzoate **18** with hydrogen bromide afforded an unstable bromide **5**, R_F 0.45 in hexane/EtOAc, 7/3 by vol, which was coupled with the glycosyl acceptor **6** in the presence of silver silicate according to the method of Paulsen and co-workers [21, 22] to give an 89% yield of a 1.9:1 mixture of a disaccharide **21**, $[\alpha]_D$ -26.3° (c 0.6), R_F 0.31 in hexane/EtOAc, 7/3 by vol, and an isomer **23**, $[\alpha]_D$ -3.6° (c 0.5), R_F 0.38 in hexane/EtOAc, 7/3 by vol. Newly introduced


a)
5.054 (4.9, 8.6, 9.8), H-2
4.487 (4.9, 11.0), H-1
4.447 (4.9, 10.7), H-1
4.362 (5.8), H-3
4.265 (2.4, 8.9), H-4
2.450 (9.3), H-2'
2.240, H-5
5
4
3
2


b)
5.120, H-1b
4.880, H-2cer
4.756 (7.9), H-1a
4.576 (5.2, 10.1), H-1cer
4.432 (3.4), H-2b
4.356 (3.1, 11.3), H-6b
4.289 (9.2), H-4b
4.254 (4.6, 11.3), H-1cer
4.180 (9.2), H-4a
4.045 (8.9), H-3a
3.900 (3.4, 9.2), H-3b
3.835 (7.9, 8.6), H-2a
3.763 (3.1, 6.4, 10.5), H-5b
3.666 (2.4, 4.3, 9.8), H-5a
5.0
4.5
4.0

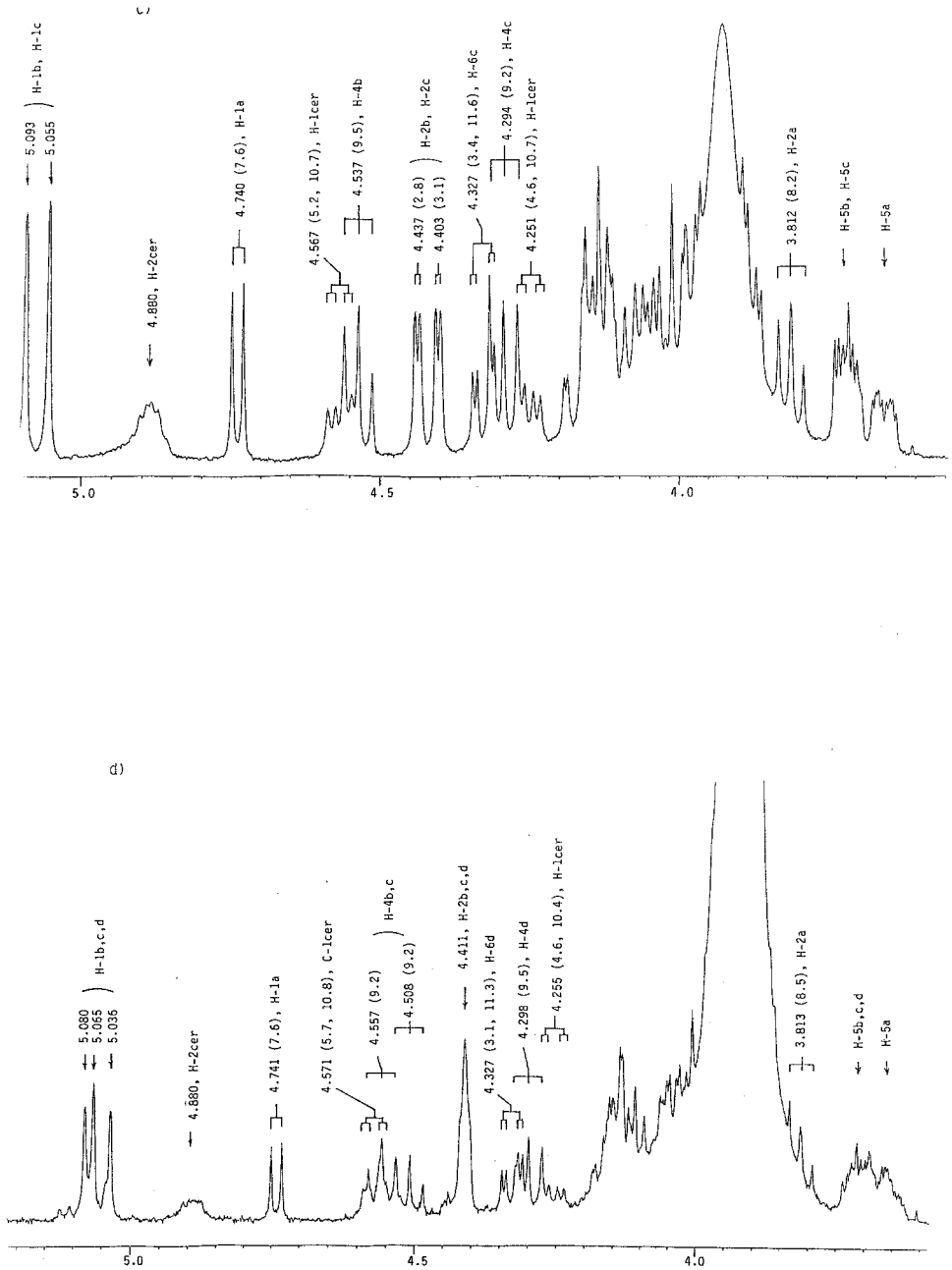


Figure 4. 400 MHz ^{1}H-NMR of synthetic sphingolipid and glycosphingolipids. The spectra were recorded in pyridine-d_5 for the sample after exchanging several times with pyridine-$^{2}H_2O$. The values in parenthesis are $^{3}J_{HH}$ values expressed in Hz. a) compound **11** at 35°C, b) compound **4** (n = 0) at 90°C, c) compound **4** (n = 1) at 90°C, and d) compound **4** (n = 2) at 90°C.

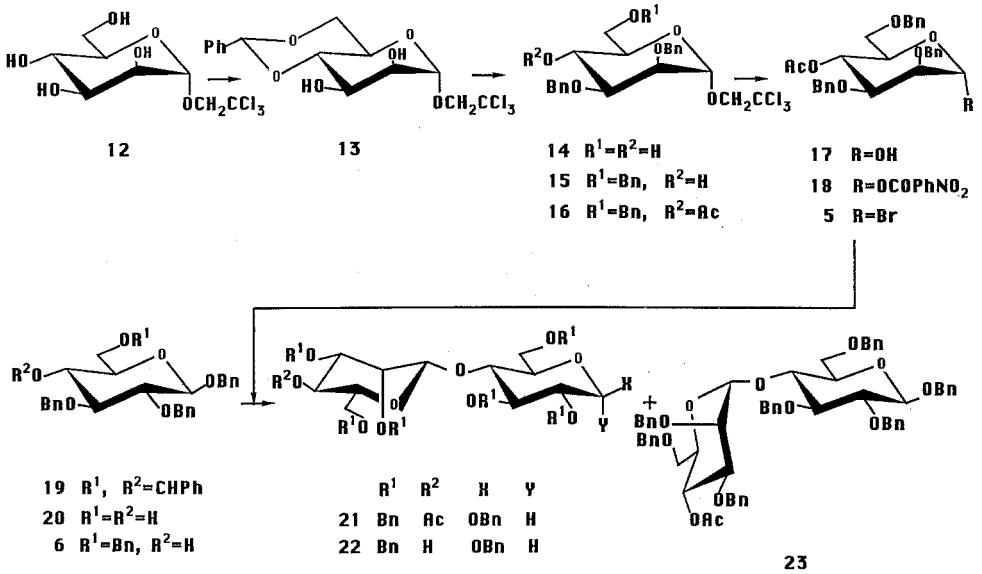


Figure 5. Synthetic scheme. Glycoside synthesis.

anomeric configurations of compounds **21** and **23** were assigned β-D and α-D, respectively, according to ^{13}C-NMR data which contained a signal for C-1b at δ 100.3 with *J* 156 Hz for compound **21** and at δ 100.2 with *J* 169 Hz for compound **23**.

Deacetylation of compound **21** afforded an 83% yield of a glycosyl acceptor **22** $[\alpha]_D$ -41.5° (c 0.5), which was coupled with the same glycosyl donor **5** under the same conditions to give a 69% yield of a 3.6:1 mixture of a trisaccharide **24**, $[\alpha]_D$ -38.0° (c 0.63), R_F 0.11 in toluene/EtOAc, 10/1 by vol, and an isomer **26**, $[\alpha]_D$ -18.5° (c 0.54), R_F 0.22 in toluene/EtOAc, 10/1 by vol (Fig. 6). ^{13}C-NMR of compound **24** contained three signals for β-D-anomeric carbon atoms at δ 102.5 (*J* 158 Hz), 101.3 (*J* 155 Hz), and 101.0 (*J* 155 Hz) for C-1a, C-1b and C-1c, while that of compound **26** contained two signals for β-D-anomeric carbon atoms at δ 102.5 (*J* 158 Hz) and 100.2 (*J* 155 Hz) for C-1a and C-1b, and a signal for an α-D anomeric carbon atom at δ 99.9 (*J* 171 Hz) for C-1c. Deacetylation of compound **24** gave alcohol **25** which was again glycosylated with the compound **5**, affording in 69% yield a mixture of the desired tetrasaccharide **27** $[\alpha]_D$ +2.9° (c 0.5), R_F 0.08 in toluene/EtOAc, 9/1 by vol, and an isomeric product **31** $[\alpha]_D$ -44.2° (c 0.7), R_F 0.17 in toluene/EtOAc, 9/1 by vol, in a ratio of 1:3.1. The stereochemistry of compounds **27** and **31** was again assigned from ^{13}C-NMR data. The compound **27** was hydrogenolysed over 10% Pd-C and acetylated to give peracetate **28**, which was chemoselectively deacetylated with hydrazinium acetate in DMF [23] to give hemiacetal **29**. Treatment of compound **29** with trichloroacetonitrile [24] and 1,8-diazabicyclo[5.4.0]undec-7-ene gave, in 49% overall yield from compound **27**, trichloroacetoimidate **30**, R_F 0.27 in hexane/THF, 1/1 by vol, δH 6.488 (d, *J* 3.7 Hz, H-1a).

Crucial glycosylation of phytosphingosine derivative **7** with trichloroacetamidate **30** in the presence of boron trifluoride etherate gave an 18% yield of a completely protected glycosphingolipid **32**, $[\alpha]_D$ -13.6° (c 0.2), R_F 0.62 in hexane/THF, 1/1 by vol, which was

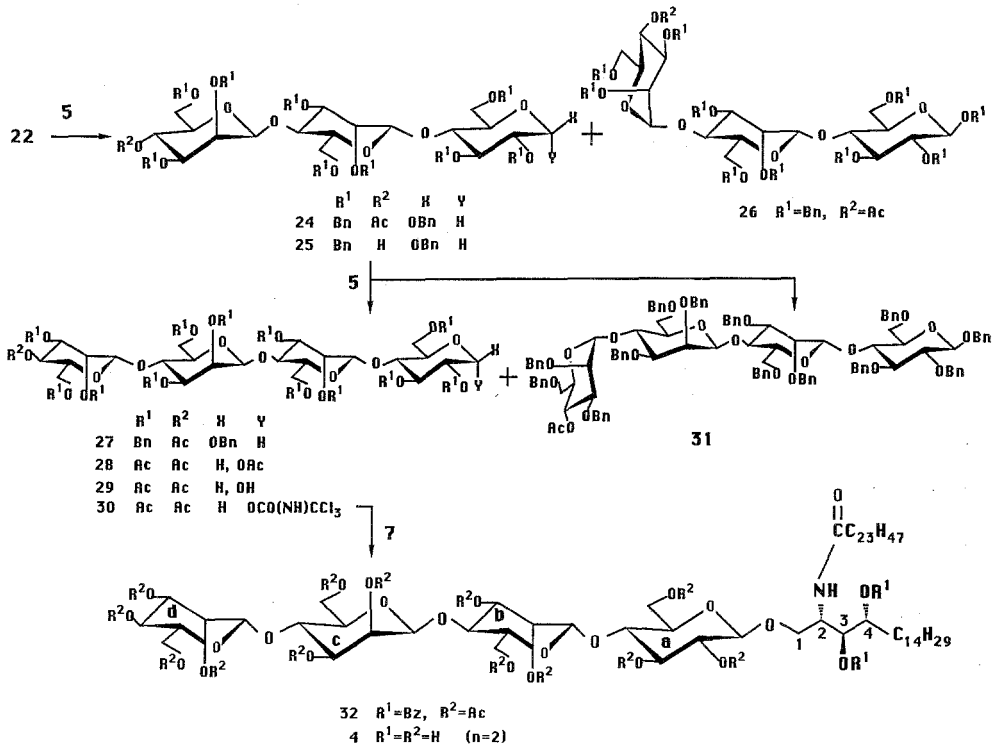


Figure 6. Synthetic scheme. Glycosphingolipid synthesis.

deacylated with sodium methoxide in THF/methanol, 1/1 by vol, to afford a 98% yield of target compound **4** (n = 2), $[\alpha]_D$ -18.2° (c 0.1, pyridine), R_F 0.23 in chloroform/methanol/water, 26/13/2 by vol. Homologous compounds **4** (n = 0 and 1) were also prepared in the same way by using a glycosyl acceptor **7** and the corresponding trichloroacetamidates, readily obtainable from compounds **21** and **24**. Crucial glycosylations between the trichloroacetimidates and compound **7** were achieved in 45 and 40% yields, respectively, for the compounds **4** (n = 0 and n = 1). Compound **4** (n = 0), $[\alpha]_D$ -15.8° (c 0.5, pyridine), R_F 0.72 in chloroform/methanol/water, 26/13/2 by vol. Compound **4** (n = 1), $[\alpha]_D$ -28.6° (c, 0.6, pyridine), R_F 0.45 in chloroform/methanol/water, 26/13/2 by vol.

The structures of synthetic compounds **4** (n = 0,1,2) were evident from the unambiguous synthetic sequence and confirmed by the ^{1}H-NMR data shown in Fig. 4. Thus, unique glycosphingolipids **4** (n = 0,1,2), which have previously been isolated from wheat flour, were synthesized in a stereo- and regio-controlled way.

Acknowledgements

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