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Preliminary communication

Total synthesis of gangliosides GM_1 and GM_2*

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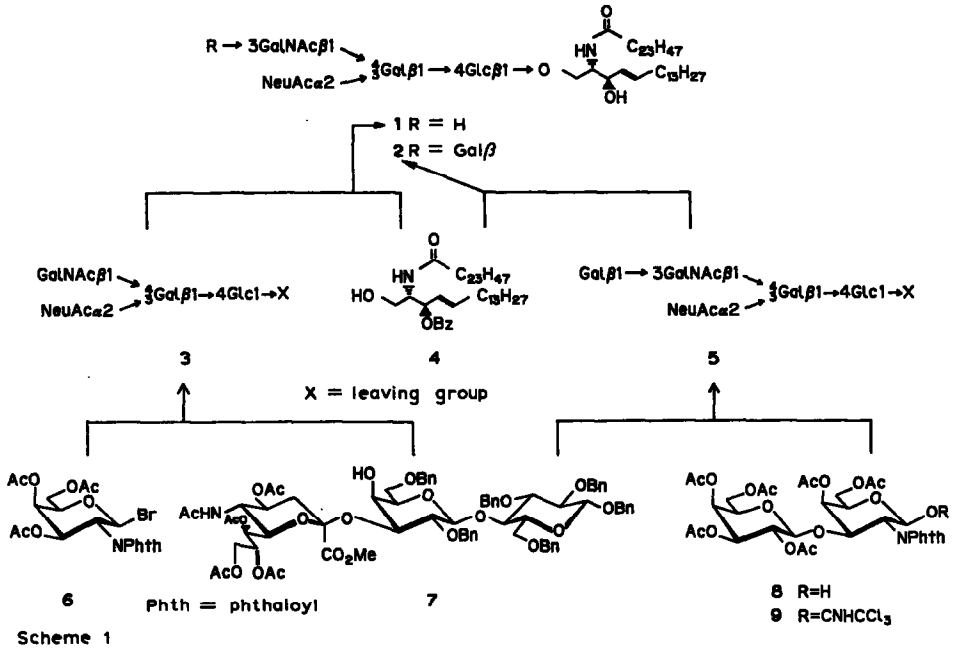
Both GM_2 (**1**) and GM_1 (**2**) are known as components of brain, as well as of various extraneural tissues[2]. Their structures were proposed from the data obtained through chemical and enzymic degradations[2]. Due to significant biological functions ascribed to gangliosides, such as membrane receptors[3], cell-growth modulators[4], and neurotrophic factors[5], we started a project on synthesis of gangliosides[6]. We now describe a total synthesis of GM_2 and GM_1 in a stereo- and regio-controlled way.



Scheme 1

*Part 50 in the series "Synthetic Studies on Cell-Surface Glycans". For Part 49, see ref. 1.
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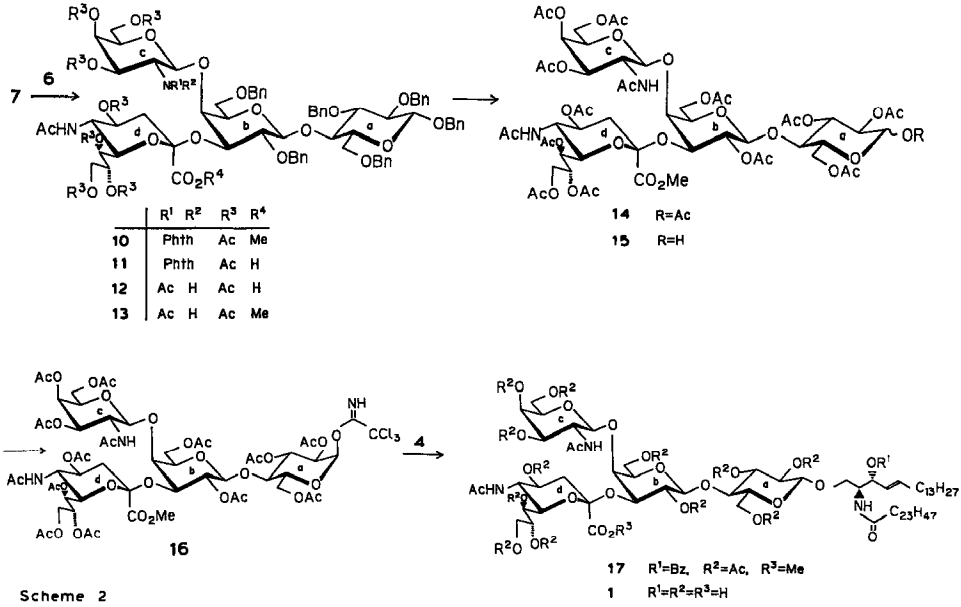
Retrosynthetic analysis for GM_2 and GM_1 led us to design two oligoglycosyl donors, **3** and **5**, respectively, to be used in combination with a known[7] glycosyl acceptor **4**. The glycosyl donors **3** and **5** were then disconnected into a common glycosyl acceptor[8] **7** and two glycosyl donors **6** (ref. 9) and **9**, respectively.

First, we describe a synthetic sequence for GM_2 (**1**). Glycosylation of trisaccharide **7** with the glycosyl bromide **6** in the presence of $AgOSO_2CF_3$–molecular sieves 4Å in CH_2Cl_2 afforded a 60% yield of tetrasaccharide **10**, R_F 0.57 in EtOAc, δ_H ($CDCl_3$) 5.431 (d, 1 H, *J* 8.5 Hz, H-1c). Dealkylative cleavage of the methyl ester was achieved in 92% yield by treatment of compound **10** with LiI in pyridine[10] for 6 h at reflux, to give acid **11**, $[\alpha]_D$ +0.5° (*c* 0.8)*, R_F 0.49 in 9:1 $CHCl_3$–MeOH, which was further converted into methyl ester **13**, $[\alpha]_D$ −11.3° (*c* 0.9, $CHCl_3$), R_F 0.41 in 25:1 $CHCl_3$–MeOH, in 68% overall yield in 4 steps *via* compound **12**, R_F 0.33 in 20:3 $CHCl_3$–MeOH: (*1*) $H_2NNH_2 \cdot H_2O$–EtOH, (*2*) Ac_2O–MeOH, (*3*) Ac_2O–pyridine, and (*4*) CH_2N_2 in CH_3OH–Et_2O.



Scheme 2

Hydrogenolysis of compound **13** in the presence of 10% Pd–C in MeOH, and subsequent acetylation with Ac_2O–pyridine afforded an 84% yield of peracetylated tetrasaccharide **14**, R_F 0.36 in 20:1 $CHCl_3$–MeOH, δ_H 3.817 (s, 3 H, OCH_3). Treatment of compound **14** with $H_2NNH_2 \cdot AcOH$ in DMF, and then CCl_3CN (ref. 11) and DBU gave a 44% yield of a key glycosyl donor, the trichloroacetimidate **16**, $[\alpha]_D$ +9.8° (*c* 0.6), R_F 0.54 in 4:1 EtOAc–acetone, a synthetic equivalent to the glycosyl donor **3** depicted in Scheme 1. The structure of compound **16** was

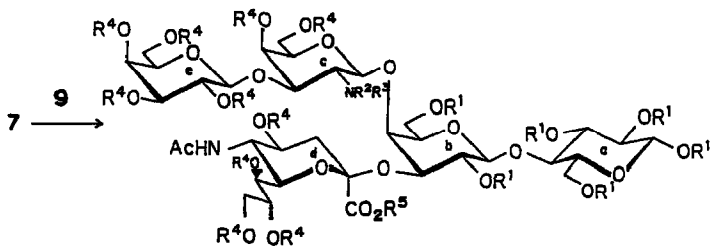
*Values of $[\alpha]_D$ were recorded for solutions in $CHCl_3$ at 25°, unless noted otherwise.

supported by ^{1}H-n.m.r. data, which revealed signals at δ_H 8.650 (s, 1 H, C=N*H*), 6.493 (d, 1 H, *J* 3.9 Hz, H-1a), 5.905 (dd, 1 H, *J* 3.4 and 11.2 Hz, H-3c), 3.842 (s, 3 H, OMe), 2.847 (dd, 1 H, *J* 4.4 and 13.1 Hz, H-3d-eq), and 1.745 (t, 1 H, *J* 12.7 Hz, H-3d-ax). A crucial glycosylation of ceramide derivative **4** in the presence of $BF_3 \cdot Et_2O$–powdered molecular sieves AW-300 in $CHCl_3$ afforded an 11% yield of peracetylated GM_2 **17**, $[\alpha]_D$ −12.2° (*c* 0.1), R_F 0.39 in 20:1 $CHCl_3$–MeOH. Compound **17** was deacylated with NaOMe in 1:1 MeOH–THF, and the product saponified with NaOH in 1:1 MeOH–THF, to give the target GM_2 (**1**), R_F 0.54 in 2:1:1 BuOH–EtOH–H_2O. The structure of synthetic GM_2 (**1**) was assigned from the reaction sequence, and established by its ^{1}H-n.m.r. data (49:1 $SOMe_2$-d_6–D_2O at 30°): δ_H 5.528 (td, 1 H, *J* 6.7 and 15.0 Hz, H-5cer), 5.345 (dd, 1 H, *J* 7.5 and 15.4 Hz, H-4cer), 4.805 (d, 1 H, *J* 9.0 Hz, H-1c), 4.269 (d, 1 H, *J* 7.8 Hz, H-1b), 4.148 (d, 1 H, *J* 8.3 Hz, H-1a), 3.034 (t, 1 H, *J* 7.8 Hz, H-2a), 1.871 and 1.773 (s, 2 × 3 H, 2 NAc). These data for synthetic **1** were found to be in agreement with those of the natural sample[12].

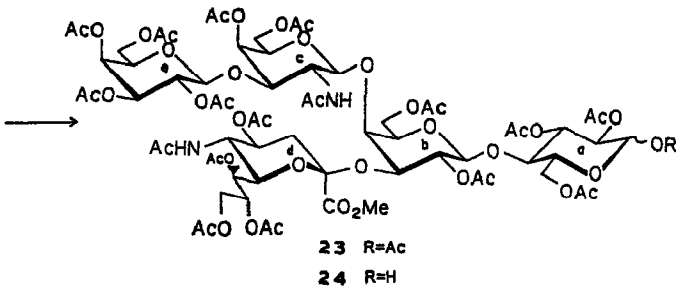
Having accomplished a total synthesis of GM_2, we now describe a total synthesis of GM_1 (**2**) according to a strategy shown in scheme 1. The disaccharide glycosyl donor **9**, $[\alpha]_D$ +58.1° (*c* 1.0), R_F 0.44 in 1:1 toluene–EtOAc, δ_H 6.436 (d, 1 H, *J* 8.6 Hz, H-1a), was readily obtainable from hemiacetal **8** (ref. 13), and reacted with trisaccharide **7** in the presence of $BF_3 \cdot Et_2O$–powdered molecular sieves AW-300, to give a 40% yield of pentasaccharide derivative **18**, $[\alpha]_D$ +6.6° (*c* 1.3), R_F 0.32 in 97:3 $CHCl_3$–MeOH, δ_H 5.283 (d, 1 H, *J* 8.5 Hz, H-1c), and 3.873 (s, 3 H, OMe), δ_C 102.5 (C-1a), 101.7 (C-1b), 100.8 (C-1e), 98.7 (C-2d), and 98.4 (C-1c). Cleavage of the methyl ester of compound **18** with LiI in pyridine gave a 65% yield of acid **19**, $[\alpha]_D$ +10.4° (*c* 1.6), R_F 0.18 in 50:1 EtOAc–HCO_2H, which was further transformed into compound **21**, R_F 0.31 in 3:2 THF–hexane, in 42% overall yield in 3 steps: (*1*) $H_2NNH_2 \cdot H_2O$–EtOH, (*2*) Ac_2O–pyridine, and (*3*) CH_2N_2 in MeOH–Et_2O. The structure of **21** was evident from the synthetic sequence, and was confirmed by transformation into the free pentasaccharide **22**, $[\alpha]_D$ +17.3° (*c* 0.2, H_2O), R_F 0.21 in 2:1:1 BuOH–EtOH–H_2O, in two steps, (*1*) NaOMe–MeOH, and (*2*) 10% Pd–C, H_2 in 4:1 MeOH–H_2O. ^{1}H-N.m.r. data of **22** were found to be in complete agreement with those reported for the natural product[14].

Compound **21** was further converted into pentasaccharide donor **25** (which was equivalent to the glycosyl donor **5** shown in scheme 1) as follows. Hydrogenolysis of compound **21** and subsequent acetylation gave a 78% yield of anomeric acetate **23**, R_F 0.45 in 20:1 $CHCl_3$–MeOH, which was selectively deacetylated with $H_2NNH_2 \cdot AcOH$, to afford hemiacetal **24**, $[\alpha]_D$ +4.2° (*c* 0.4), R_F 0.37 in 20:1 $CHCl_3$–MeOH in 74% yield. Treatment of compound **24** with Cl_3CCN and DBU gave the desired trichloroacetimidate **25**, $[\alpha]_D$ +6.8° (*c* 0.1), R_F 0.40 in 4:1 EtOAc–acetone, δ_H 8.655 (s, 1 H, C=N*H*), 6.491 (d, 1 H, *J* 3.9 Hz, H-1a), 3.819 (s, 3 H, OMe), and 2.858 (dd, 1 H, *J* 5.5 and 12.4 Hz, H-3d-eq).

Finally, glycosylation of benzoyl ceramide **4** with the glycosyl donor **25** in

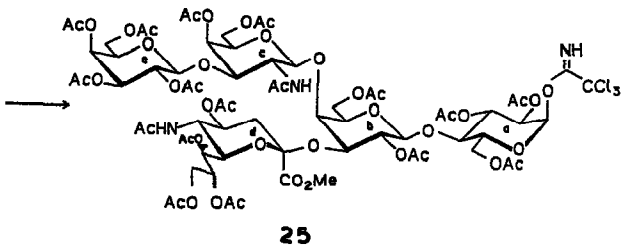


	R^1	R^2	R^3	R^4	R^5
18	Bn	Phth	Ac	Me	
19	Bn	Phth	Ac	H	
20	Bn	Ac	H	Ac	H
21	Bn	Ac	H	Ac	Me
22	H	Ac	H	H	H



23 R=Ac

24 R=H



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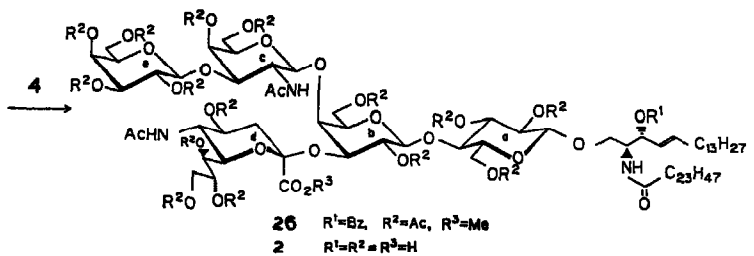

26 R^1=Bz, R^2=Ac, R^3=Me

2 R^1=R^2=R^3=H

Scheme 3

$CHCl_3$ in the presence of $BF_3 \cdot Et_2O$ and powdered molecular sieves AW-300 afforded a 33% yield of peracetylated GM_1 (**26**), R_F 0.45 in 20:1 $CHCl_3$–MeOH, which was treated with NaOMe–MeOH and then NaOH in MeOH, to give an 84% yield of GM_1 (**2**), $[\alpha]_D$ +7.3° (*c* 0.2, pyridine), R_F 0.53 in 2:1:1 BuOH–EtOH–H_2O. ^{1}H-N.m.r. data of synthetic GM_1 were found to be identical with those reported for the natural product[10].

In conclusion, a regio- and stereo-controlled, total synthesis of GM_1 and GM_2 was achieved for the first time by use of the key glycosyl donors **16** and **25**, and the glycosyl acceptor **4** for the crucial glycosylations.

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REFERENCES

1 S. Sato, Y. Ito, T. Nukada, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, in press.
2 S. Hakomori, in J. N. Kanfer and S. Hakomori (Eds.), *Handbook of Lipid Research, 3, Sphingolipid Biochemistry*, Plenum, New York, 1983, pp. 117–129.
3 S. Hakomori, *Annu. Rev. Biochem.*, 50 (1981) 733–764.
4 E. G. Bremer, S. Hakomori, D. F. Bowen-Pope, E. Raines, and R. Ross, *J. Biol. Chem.*, 259 (1984) 6818–6825; S. Tsuji, J. Nakajima, T. Sasaki, and Y. Nagai, *J. Biochem. (Tokyo)*, 97 (1985) 969–972.
5 R. Ledeen, in A. Lajtha (Ed.), *Handbook of Neurochemistry*, Vol. 3, Plenum, New York, 1983, pp. 41–90; B. A. Sabel, M. D. Slavin, and D. G. Stein, *Science*, 225 (1984) 340–342; R. Ledeen, *Trends NeuroSci.*, 8 (1985) 169–174.
6 M. Sugimoto, T. Horisaki, and T. Ogawa, *Glycoconjugate J.*, 2 (1985) 11–15.
7 K. Koike, Y. Nakahara, and T. Ogawa, *Glycoconjugate J.*, 1 (1984) 107–109.
8 T. Ogawa and M. Sugimoto, *Carbohydr. Res.*, 135 (1985) c5–c9; H. Paulsen and U. von Deessen, *ibid.*, 146 (1986) 147–153.
9 R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, 57 (1979) 1244–1251.
10 E. Taschner and B. Liberek, *Rocz. Chem.*, 30 (1956) 323; *Chem. Abstr.*, 51 (1957) 1039*d*; F. Elsinger, J. Schreiker, and A. Eschenmoser, *Helv. Chim. Acta*, 43 (1960) 113–118.
11 R. R. Schmidt and J. Michel, *Angew. Chem., Int. Ed. Engl.*, 19 (1980) 731–732; R. R. Schmidt, J. Michel, and M. Roos, *Justus Liebigs Ann. Chem.*, (1984) 1343–1357.
12 T. A. W. Koerner, Jr., J. H. Prestegard, P. C. Demou, and R. K. Yu, *Biochemistry*, 22 (1983) 2676–2687; S. Gasa, T. Mitsuyama, and A. Makita, *J. Lipid Res.*, 24 (1983) 174–182.
13 S. Sabesan and R. U. Lemieux, *Can. J. Chem.*, 62 (1984) 644–653.
14 S. Sabesan, K. Bock, and R. U. Lemieux, *Can. J. Chem.*, 62 (1984) 1034–1045.
15 G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.