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TOTAL SYNTHESIS OF AN EXTENDED *GANGLIO*-GANGLIOSIDE, IV^{4}GalNAcβGM1b[1)]

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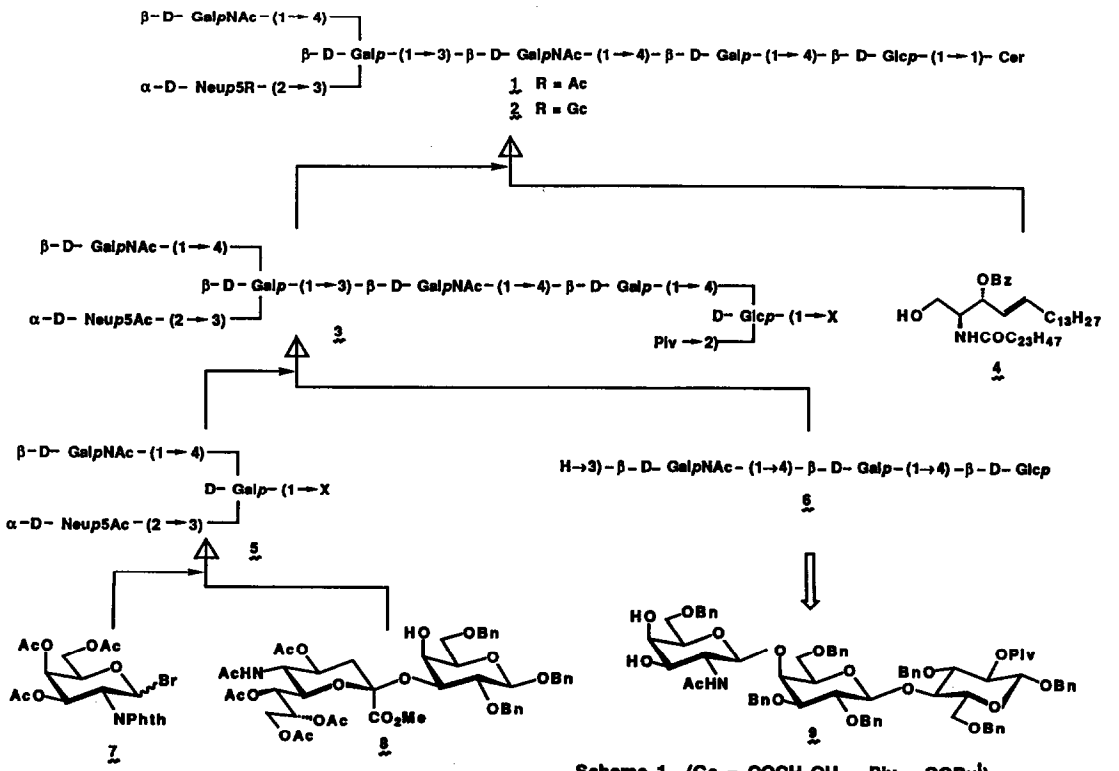
Abstract: First total synthesis of extended *ganglio*-ganglioside IV^{4}GalNAcβGM1b, β-D-Gal*p*NAc-(1→4)-[α-D-Neu*p*5Ac-(2→3)]-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→1)-Cer was achieved in a stereocontrolled manner.

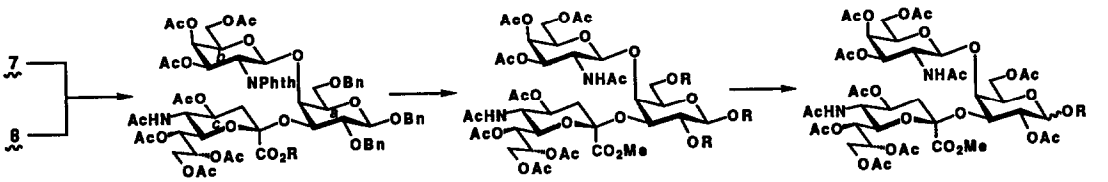
In 1981, a new *ganglio*-monosialylpentaosylceramide, was isolated from Tay-Sachs brains and the structure was assigned as IV^{4}GalNAcGM1b, **1** from enzymic degradation[2]. In 1987, this extended *ganglio*-series glycosphingolipids **1** and the glycolyl analogue **2** were isolated from mouse spleen[3,4] and ^{1}H-n.m.r. data of **2** was in reasonable agreement with the proposed structure[3]. Since **1** and **2** were isolated only from mouse spleen T lymphocytes but not from B lymphocytes, these extended *ganglio*-gangliosides may be speculated to play a role in either immune cell circulation or localization in lymphoid organs[5]. In 1988, **1** was also identified as an antigen for monoclonal IgM in a patient with neuropathy[6]. We now describe first total synthesis of **1** in a stereocontrolled manner.



Scheme 1 (Gc = $COCH_2OH$, Piv = $COBu^t$)

Based on a retrosynthetic analysis of the target molecule **1** (Scheme 1), carbohydrate donor **3** should be armed with a pivaloyl auxiliary[7] at O-2a to achieve an efficient coupling with ceramide derivative **4**. The glycohexaosyl sequence of **3** may be devided into two glycotriosyl sequences **5** and **6**. The glycosyl donor **5** may be obtainable by glycosylation of compound **8**[8] with the bromide **7**[9], while the known diol **9**[10] will be employed as an equivalent to the glycosyl acceptor **6**.

Silver triflate and powdered molecular sieves 4A promoted coupling[11] of **8** with 1.5 equivalents of **7** in CH_2Cl_2 gave **10**[12] (86%), which was converted into **12**[12] in 4 steps via **11**, (i LiI in Py[13], 115°, 3h, ii $NH_2NH_2 \cdot H_2O$ in EtOH, reflux, 7h, iii Ac_2O in Py, iv CH_2N_2 in Et_2O-MeOH, 78% overall). Compound **12** was smoothly converted into α-trichloracetimidate **15**[12] via **13**[12] and **14**[12] in 4 steps (i 10% Pd-C, H_2 in MeOH, ii Ac_2O in Py, iii $NH_2NH_2 \cdot AcOH$ in DMF, 50°, 5 min[14], iv Cl_3CCN, DBU in $(CH_2Cl)_2$[15], 83% overall).



Scheme 2

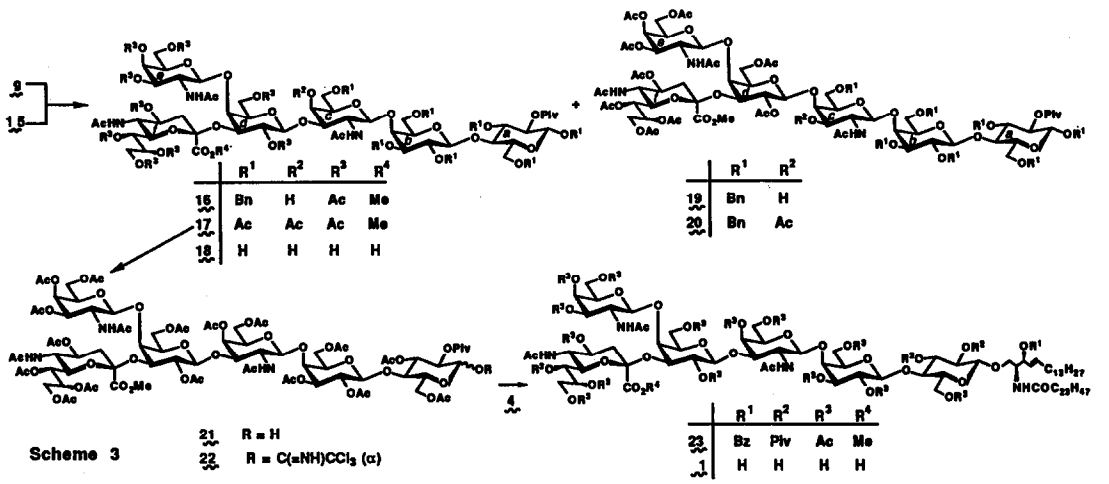
10 R = Me
11 R = H
12 R = Bn
13 R = Ac
14 R = H
15 R = C(=NH)CCl3 (α)

Having prepared the glycosyl donor **15** designed to be equivalent with **5**, crucial glycosylation of diol **9** with 0.7 molar equivalents[16] of **15** was performed in the presence of $BF_3 \cdot OEt_2$[15] and powdered molecular sieves 4A in $(CH_2Cl)_2$ to give, after separation by silica gel chromatography in 25:1 $CHCl_3$-MeOH, two compounds **16**[12] and **19**[12] in 43 and 7% yield, respectively. The structure of the minor product **19** was assigned from the ^{1}H-n.m.r. data (HOHAHA) of the acetylated derivative **20**[12] which showed a deshielded signal for H-3c at δ 4.672 (dd, 3.0 and 11.2 Hz) as well as a signal for H-1d at δ 4.498 (d, 7.9 Hz). The structure of the major product **16** was assigned after partial deprotection into **18**[12] in two steps (i NaOMe in MeOH then in H_2O-MeOH, ii H_2/Pd-C, 47% overall). ^{1}H-N.m.r. data taken in D_2O at 24° was in reasonable agreement with the data[17] for related oligosaccharides and contained a signal for H-1d at δ 4.528 (d, 7.5 Hz), supporting that configuration of a newly introduced glycosidic linkage at C-1d was β-D. The key intermediate **16** was further converted into the glycohexaosyl trichloracetimidate **22**[12] via **17**[12] and **21**[12] in 4 steps (i 10% Pd-C, H_2 in MeOH, ii Ac_2O in Py, iii $NH_2NH_2 \cdot AcOH$ in DMF, 50°, 5 min, iv Cl_3CCN, DBU in $(CH_2Cl)_2$, 28% overall).

Finally, glycosylation of **4** with 0.7 molar equivalents of the imidate **22** in the presence of $BF_3 \cdot OEt_2$ and powdered molecular sieves 4A in $CHCl_3$ afforded a 15% yield of the desired compound **23**. The structure of **23** was assignable from its ^{1}H-n.m.r. data (HOHAHA) which showed a signal for H-1a at δ 4.417 (d, 7.7 Hz) as well as a signal for both H-4c and H-4e at δ 5.349 (d, 2.9 Hz), confirming both the β-D configuration at newly introduced glycosidic linkage at C-1a and the regiochemistry (1d→3c) for the previously introduced glycosidic linkage at C-1d. Deprotection of **23** into **1** was achieved in 74% yield by successive treatment with 0.06*M* NaOMe in

1:1 MeOH-THF and then with 0.03*M* NaOH in 1:1:1 H_2O-MeOH-THF. The ^{1}H-n.m.r. data of synthetic **1** and those[3] of natural **2** were found to be in reasonable agreement.

In summary first stereocontrolled total synthesis of IV^{4}GalNAcGM1b **1** was achieved by employing a glycotriosyl trichloroacetimidate **15** and a glycohexaosyl trichloroacetimidate **22** as the key glycosyl donors for the crucial stereoselective glycosylations.



Scheme 3

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Reference and Notes

1 Part 73 in the series "Synthetic Studies on Cell-Surface Glycans", For Part 72, see M. Numata, M. Sugimoto, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, in press.

2 T. Itoh, Y.-T. Li, S.-C. Li, and R. K. Yu, *J. Biol. Chem.*, **256**, 165 (1981).

3 K. Nakamura, M. Suzuki, F. Inagaki, T. Yamakawa and A. Suzuki, *J. Biochem.*, **101**, 825 (1987).

4 J. Müthing, H. Egge, B. Kniep, and P. F. Mühlradt, *Eur. J. Biochem.*, **163**, 407 (1987).

5 K. Nakamura, Y. Hashimoto, T. Yamakawa, and A. Suzuki, *J. Biochem.*, **103**, 201 (1988); J. Müthing, B. Schwinzer, J. Peter-Katalinic, H. Egge, and P. Mühlradt, *Biochemistry*, **28**, 2923 (1989).

6 A. A. Ilyas, S.-C. Li, D. K. H. Chou, Y.-T. Li, F. B. Jungalwala, M. C. Dalakas, and R. H. Quarles, *J. Biol. Chem.*, **263**, 4369 (1988).

7 S. Sato, S. Nunomura, T. Nakano, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, **29**, 4097 (1988).

8 T. Ogawa and M. Sugimoto, *Carbohydr. Res.*, **135**, C5 (1985); M. Numata, M. Sugimoto, K. Koike, and T. Ogawa, *Carbohydr. Res.*, **163**, 209 (1987).

9 R. U. Lemieux and R. M. Ratcliffe, *Can. J. Chem.*, **57**, 1244 (1979).

10 M. Sugimoto, K. Fujikura, S. Nunomura, T. Horisaki, Y. Ito, and T. Ogawa, *Tetrahedron Lett.*, in press.

11 M. Sugimoto, M. Numata, K. Koike, Y. Nakahara, and T. Ogawa, *Carbohydr. Res.*, **156**, C1 (1986).

12 Physical data for key compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were recorded for

solutions in $CHCl_3$ and $CDCl_3$, respectively, at 23±3°, unless noted otherwise. **1**: R_F 0.44 in 2:1:1 Bu^nOH-EtOH-H_2O; δ_H (49:1 $(CD_3)_2SO$-D_2O, 60°) 5.557 (dt, 15.0 and 6.1 Hz, 5Cer), 5.352 (dd, 15.0 and 6.8 Hz, 4Cer), 4.781 (d, 8.3 Hz, 1e), 4.586 (d, 8.3 Hz, 1c), 4.320 (d, 7.9 Hz, 1d), 4.234 (d, 7.7 Hz, 1b), 4.193 (d, 7.9 Hz, 1a), 2.049 (m, $COCH_2CH_2$), 1.898, 1.867 and 1.841 (3s, 3Ac), 0.859 (t, 6.8 Hz, $2CH_2CH_3$). **10**: $[\alpha]_D$ -29.0° (c 2.4); δ_H 6.085 (dd, 3.3 and 11.7 Hz, 3b), 5.485 (d, 3.3 Hz, 4b), 5.374 (d, 8.4 Hz, 1b), 5.357 (m, 8c), 5.240 (dd, 2.2 and 8.0 Hz, 7c), 4.783 (m, 4c), 4.488 (d, 7.3 Hz, 1a), 3.893 (s, OMe), 2.876 (dd, 7.7 and 9.8 Hz, 2b), 2.766 (dd, 4.4 and 13.2 Hz, 3c*eq*); δ_C 102.1 (1a), 98.7 and 98.3 (2c and 1b), 37.2 (3c). **11**: $[\alpha]_D$ -20.2° (c 2.0); δ_H ($CDCl_3$-CD_3OD) 6.113 (dd, 3.7 and 11.7 Hz, 3b), 5.493 (d, 3.7 Hz, 4b), 5.428 (m, 8c), 2.946 (dd, 7.7, 9.5 Hz, 2b), 2.888 (dd, 3.9 and 12.3 Hz, 3c*eq*), 1.475 (t, 11.4 Hz, 3c*ax*); δ_C 102.3 (1ab), 99.5 (2c). **12**: $[\alpha]_D$ -26.5° (c 1.2); δ_H 5.317 (d, 2.9 Hz, 4c), 4.860 (d, 8.8 Hz, 1b), 4.486 (d, 7.4 Hz, 1a), 3.883 (s, OMe); δ_C 102.4, 102.2, 99.2 (1ab and 2c), 35.1 (3c). **13**: δ_H (α:β=1:1) 6.269 (d, 4.0 Hz, 1aα), 5.745 (d, 8.0 Hz, 1aβ), 3.897 and 3.887 (2s, OMe), 2.202 and 2.536 (2dd, 4.1 and 12.7 Hz, 3c*eq*). **14**: $[\alpha]_D$ -1.5° (c 1.2); δ_H (α:β=1:1) 3.939 and 3.929 (2 s, OMe), 2.660 and 2.598 (2dd, 4.6 and 11.2 Hz, 3c*eq*); δ_C 101.3 and 101.1 (1b), 98.7 and 98.5 (2c), 95.6 (1aβ) and 91.1 (1aα). **15**: $[\alpha]_D$ +18.5° (c 0.9); δ_H 8.600 (s, C=NH), 6.569 (d, 3.3 Hz, 1a), 4.865 (d, 8.8 Hz, 1b), 3.925 (s, OMe), 2.286 (dd, 5.3 and 13.7 Hz, 3c*eq*), 1.858 (t, 12.1 Hz, 3c*ax*). **16**: R_F 0.36 (19:1 $CHCl_3$-MeOH) δ_H 7.40-7.16 (m, 7Ph), 3.851 (s, OMe), 2.751 (dd, 4.1 and 12.7 Hz, 3f*eq*). **17**: α:β=1:1; δ_H 6.305 (d, 4.0 Hz, 1aα), 5.703 (d, 8.4 Ha, 1aβ), 5.733 (dd, 3.7 and 11.4 Hz, 2aα), 5.267 (t, 9.2 Hz, 2aβ), 2.715 (dd, 4.8 and 13.2 Hz, 3f*eq*), 1.825 (t, 13.2 Hz, 3f*ax*), and 1.144 and 1.135 (2s, tBu). **18**: δ_H (D_2O, 24° and 60°*) 5.335 (d, 3.2 Hz, 1aα), 4.845 (d, 7.8 Hz, 1aβ)*, 4.771 (d, 7.7 Hz, 1e)*, 4.744 and 4.728 (2d, 7.8 Hz, 1c)*, 4.528 (d, 7.5 Hz, 1d), 4.460 and 4.452 (2d, 7.9 Hz, 1b), 2.659 (dd, 3.9 and 11.9 Hz, 3f*eq*), 2.049 and 2.043 (2s, Ac), 2.040 (s, Ac), 2.017 (s, Ac), 1.913 (t, 11.9 Hz, 3f*ax*), 1.222 and 1.230 (2s, tBu). **19**: R_F 0.42 (19:1 $CHCl_3$-MeOH); δ_H 7.40-7.15 (m, 7Ph), 3.846 (s, OMe), and 1.145 (s, tBu). **20**: δ_H (HOHAHA) 5.902 (dd, 3.3 and 11.4 Hz, 3e), 5.391 (dd, 2.6 and 9.9 Hz, 7f), 5.362 (d, 3.7 Hz, 4e), 5.146 (d, 8.6 Hz, 1e), 4.672 (dd, 3.0 and 11.2 Hz, 3c), 4.620 (d, 7.6 Hz, 1d), 4.498 (d, 7.9 Hz, 1a), 4.443 (d, 8.1 Hz, 1c), 4.303 (d, 7.3 Hz, 1b). **21**: $[\alpha]_D$ +4.0° (c 2.4); δ_H 3.859 (s, OMe), 2.740 (dd, 4.4 and 13.1 Hz, 3f*eq*), 1.816 (t, 12.8 Hz, 3f*ax*), 1.187 (s, tBu); δ_C 100.3 and 100.2, 99.8 and 99.7, 99.6 and 98.5, 98.4, 97.9, and 95.8 and 90.1 (6 anomeric carbons). **22**: $[\alpha]_D$ +10.1° (c 1.6); δ_H 8.662 (s, C=NH), 6.514 (d, 4.0 Hz, 1a), 5.464 (ddd, 3.3, 5.5, and 8.8 Hz, 8f), 4.655 (d, 7.7 Hz, 1d), 2.713 (dd, 4.4 and 13.2 Hz, 3f*eq*), 1.820 (t, 12.5 Hz, 3f*ax*), 1.143 (s, tBu); δ_C 100.8, 99.8, 99.6, 98.7, 97.9, 92.9 and 90.8 (6 anomeric carbons and CCl_3). **23**: $[\alpha]_D$ -1.1° (c 0.3); δ_H (HOHAHA) 5.874 (dt, 15.0 and 7.7 Hz, 5Cer), 5.545 (t, 7.7 Hz, 3Cer), 5.459 (dd, 7.7 and 15.0 Hz, 4Cer), 5.349 (d, 2.9 Hz, 4ce), 5.184 (t, 9.2 Hz, 2a), 5.035 and 5.028 (2d, 8.2 Hz, 1ce), 4.646 (d, 7.7 Hz, 1d), 4.417 (d, 7.7 Hz, 1a), 4.360 (d, 7.7 Hz, 1b), 3.858 (s, OMe), 2.704 (dd, 4.6 and 12.7 Hz, 3f*eq*), 2.177, 2.161, 2.137, 2.109, 2.109, 2.102, 2.091, 2.077, 2.057, 2.051, 2.047, 2.035, 2.016, 2.010, 1.995, 1.991, 1.971, 1.909, and 1.874 (19s, 19Ac), 1.151 (s, tBu), 0.880 (t, 6.6Hz, $2CH_2CH_3$).

13 J. McMurry, *Org. React.*, **24**, 187 (1976).

14 G. Excoffier, D. Gagnaire, and J.-P. Utille, *Carbohydr. Res.*, **39**, 368 (1975).

15 R. R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, **25**, 212 (1986).

16 Use of either corresponding glycotriosyl fluoride (α+β) or methyl thioglycoside (β) in place of **15** gave inferior result in this particular step.

17 C. P. C. Soh, A. S. R. Donald, J. Feeney, W. T. J. Morgan, and W. M. Watkins, *Glycoconjugate J.*, **6**, 319 (1989).

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