Glycoconjugate J (1985) 2:5—9

Preliminary Communication

Synthesis of a Hematoside (G_{M3}-ganglioside) and a Stereoisomer*

MAMORU SUGIMOTO and TOMOYA OGAWA**

RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01 Japan

Received January 3, 1985.

Key words: hematoside synthesis, G_{M3}-ganglioside

The ganglioside G_{M3} had been isolated from brain, human spleen and dog erythrocytes, and the structure was determined to be **1** [2]. The biosynthesis of G_{M3}-ganglioside was correlated with intestinal epithelial differentiation [3, 4] and contact inhibition of cell growth [5, 6]. Baby hamster kidney fibroblast cell growth in the presence of fibroblast growth factor was specifically inhibited by the presence of G_{M3}-ganglioside added exogenously [7]. In spite of the important functions [8] carried by the gangliosides as components of biological membranes, total synthesis of G_{M3}-ganglioside and related gangliosides remains to be achieved.

In 1973, Shapiro [9] reported an approach to the synthesis of G_{M3}-ganglioside. The configurational assignment at C-2c of the synthetic G_{M3}-ganglioside, however, remains to be clarified. As part of our project on the synthesis of cell surface glycoconjugates, we describe here a first total synthesis of both a G_{M3}-ganglioside **1** and its stereoisomer, *epi*G_{M3}-ganglioside, **15** in a stereo- and regio-controlled manner.

The synthetic plan was designed based on the retrosynthetic analysis as shown in Fig. 1. The key glycotriosyl donor **2** may be synthesized starting either from **7** or **8** [10]. The glycosyl acceptor **3** could be prepared from D-*erythro*-ceramide **5** which was obtainable from D-glucose in an efficient way [11].

The glycosylation of the pentaacetylated lactose derivative **7** with the chloride **6** [12] (Fig. 2) in the presence of $HgBr_2$-$Hg(CN)_2$-molecular sieves 4 Å in $Cl(CH_2)_2Cl$ and chromato-

*Part 34 in the series "Synthetic Studies on Cell-surface Glycans", for part 33, see ref. 1.

**Author for correspondence.

Abbreviations: G_{M3}-ganglioside (hematoside), II^3NeuAc-LacCer; trityl, triphenylmethyl (Ph_3C).

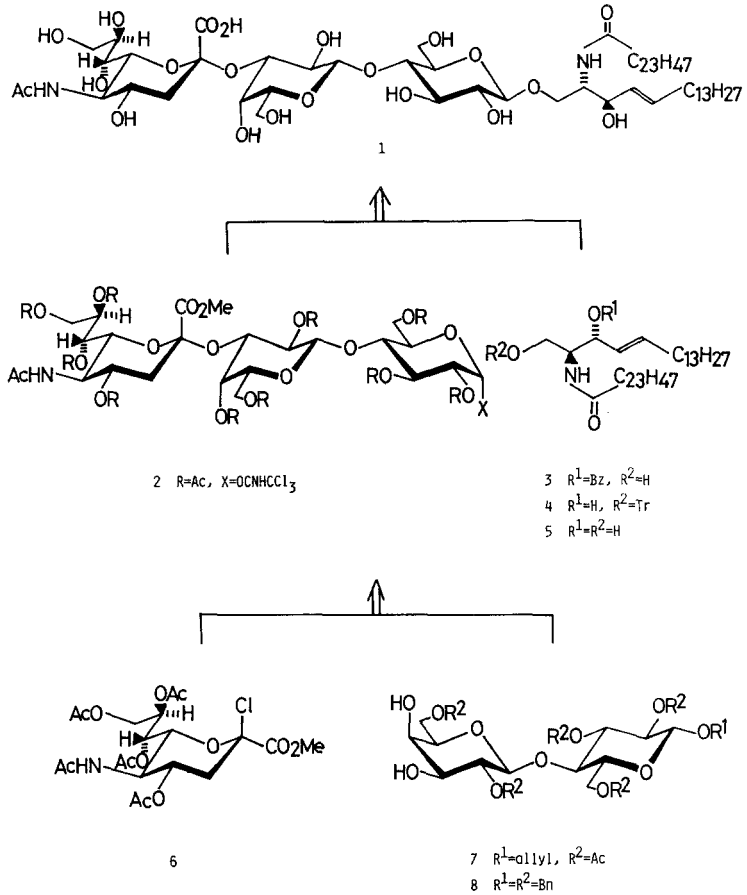

Figure 1. Proposed scheme for the synthesis of a G_{M3}-ganglioside.
Abbreviations: Tr, Trityl; Bn, benzyl; Bz, benzoyl.

graphy of the product over SiO_2 afforded a 6% yield of the glycotrioside **9**, $[\alpha]_D$ -8.9° (c 0.94), R_F 0.40 in $CHCl_3$/MeOH, 25/1 by vol. Values of $[\alpha]_D$ were measured for $CHCl_3$ solutions at 25°C, unless noted otherwise. Compounds having $[\alpha]_D$ recorded gave satisfactory data for elemental analyses. The α-epimer of **9** at C-2c could not be detected by TLC examination of the reaction mixture. The regiochemistry of the new glycosidic linkage of **9** was assigned as 2-3 by ^{13}C NMR (^2H_2O) of the deacetylated product **10** which revealed a deshielded signal for C-3b at δ 78.1. The configuration at C-2c of **9** was tentatively assigned as β-D- from the 400 MHz ^1H NMR data (in ^2H_2O) of **10** which showed the signals for H-$3c_{ax}$ and H-$3c_{eq}$ at δ 1.678 (t, J 12.0 Hz) and 2.454 (dd, J 4.6 and 12.9 Hz), respectively [10, 13] and further confirmed by its transformation into **15**, the β-epimer at C-2c of G_{M3}-ganglioside, in the following way.

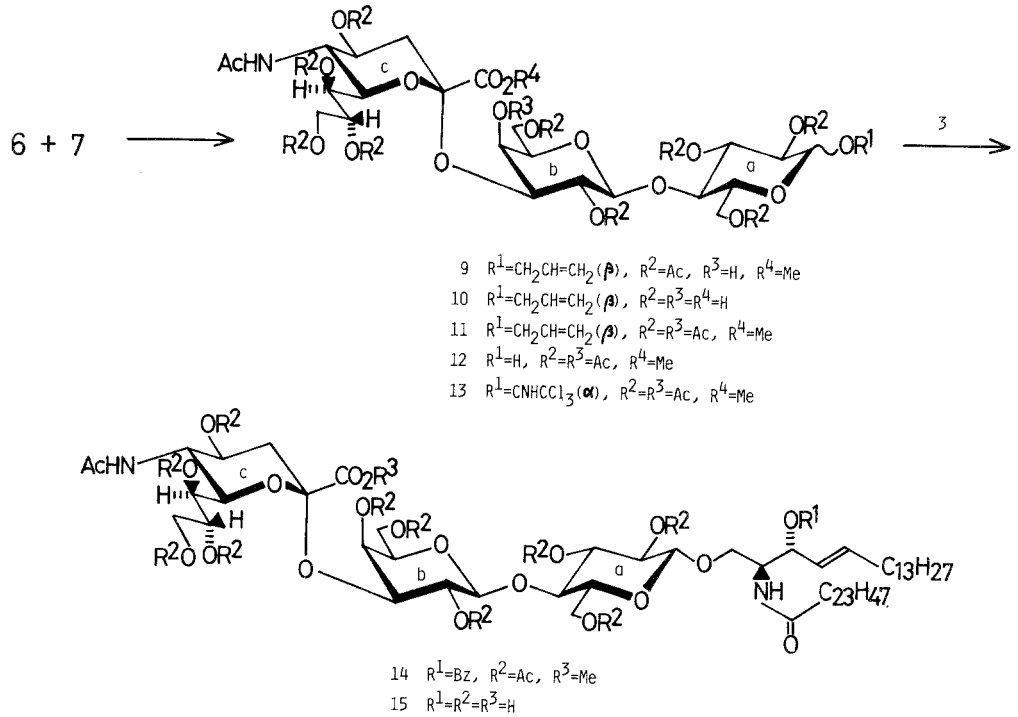

Figure 2: Synthesis of an *epi*G_{M3}-ganglioside.

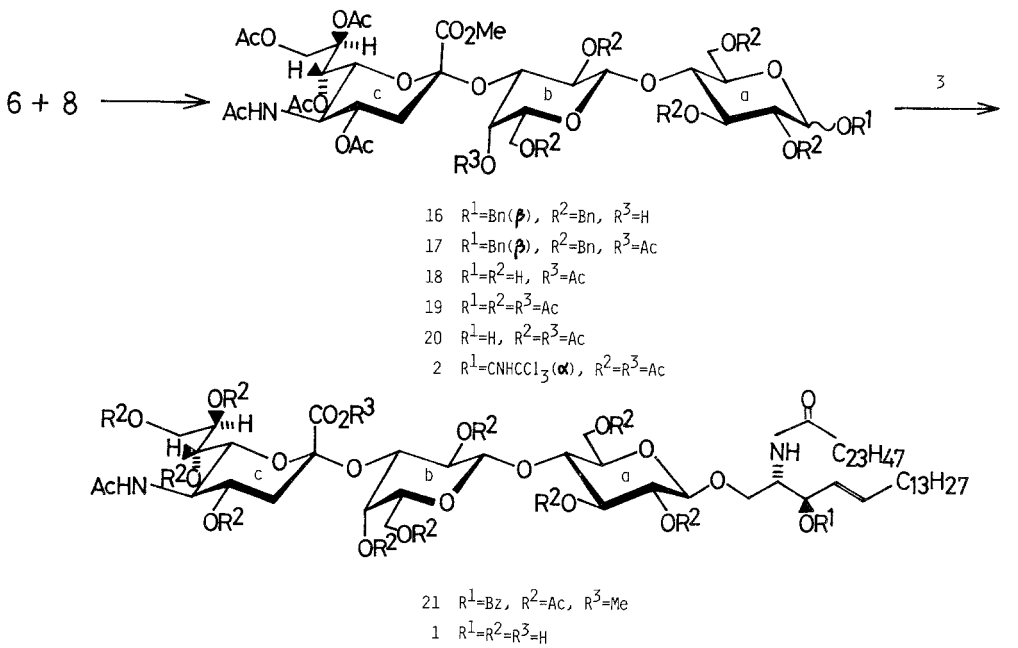

Figure 3: Synthesis of a G_{M3}-ganglioside.

Acetylation of **9** with Ac_2O and pyridine gave **11** in 93% yield, $[\alpha]_D$ -6.9° (c 0.72), R_F 0.46 in $CHCl_3$/MeOH, 25/1 by vol, δH (C^2HCl_3): 1.795 (t, *J* 12.2 Hz, H-3c_{ax}), 2.429 (dd, *J* 4.6 and 13.4 Hz, H-3c_{eq}). Deallylation of **11** with $PdCl_2$-AcONa in aqueous AcOH [14, 15] under ultrasonic stirring for 5 h at 20°C afforded a 56% yield of **12**, which was treated with NaH and Cl_3CCN according to the method of Schmidt *et al.* [16, 17] to give the α-trichloroacetimidate **13** in 70% yield, R_F 0.33 in EtOAc, δH (C^2HCl_3): 6.46 (d, *J* 3.6 Hz, H-1a), 8.68 (s, C=NH). The glycosyl acceptor **3**, $[\alpha]_D$ +16.5° (c 1.10), R_F 0.31 in $CHCl_3$/MeOH, 33/1 by vol, was prepared in 71% overall yield from D-*erythro*-ceramide **5** [11] via the trityl derivative **4**, $[\alpha]_D$ -0.94° (c 0.96), R_F 0.34 in hexane/EtOAc, 4/1 by vol, according to the method of Shapiro and Flowers [18] (see Fig. 1).

The glycosylation of **3** with the donor **13** in the presence of BF_3-Et_2O and molecular sieves 4 Å in $CHCl_3$ for 20 h at 20°C afforded a 36% yield of **14** $[\alpha]_D$ +4.2° (c 0.65), R_F 0.33 in $CHCl_3$/MeOH, 25/1 by vol, δH (C^2HCl_3): 1.90, 1.96, 1.98, 2.02, 2.03, 2.04, 2.05, 2.07, 2.08 and 2.29 (10 singlets for $COCH_3$). Deacetylation and saponification of **14** afforded **15**, the C-2c epimer of G_{M3}-ganglioside, $[\alpha]_D$ -7.7° (c 0.55 in $CHCl_3$/MeOH, 1/1 by vol), R_F 0.57 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol. The configuration of the newly introduced glycosidic linkage at C-1a of **14** and **15** was assigned as β-D- according to the ^1H NMR data of **15** in $^2H_6$-dimethyl-sulfoxide/^2H_2O, 98/2 by vol, which contained a doublet for two anomeric protons at δ 4.17 (*J* 7.6 Hz) for both H-1a and H-1b and was not identical with that of natural G_{M3}-ganglioside reported by Koerner Jr *et al.* [19], as is expected from the stereochemistry assigned to **9**.

The synthesis of G_{M3}-ganglioside was achieved by use of a stereochemically defined glycotriosyl intermediate **16** with the α-D-configuration at C-2c [10] as follows (Fig. 3). Acetylation of **16** gave a 93% yield of **17**, $[\alpha]_D$ -3.33° (c 1.17), R_F 0.30 in MeOH/toluene, 1/9 by vol, δH (C^2HCl_3): 2.598 (dd, *J* 4.6 and 12.7 Hz, H-3c_{eq}). Catalytic hydrogenolysis of **17** gave **18**, R_F 0.55 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol, which was acetylated to give **19** in 70% overall yield, R_F 0.28 in EtOAc. Chemoselective deacetylation of **19** was achieved by treatment with NH_2NH_2-AcOH in dimethylformamide [20] to give an 81% yield of the hemiacetal **20**, R_F 0.29 in EtOAc/MeOH, 99/1 by vol. Treatment of **20** with NaH and Cl_3CCN afforded the glycosyl donor **2**, R_F 0.38 in EtOAc, δH (C^2HCl_3): 6.49 (d, *J* 3.7 Hz, H-1a), 8.66 (s, C=NH).

Finally, glycosylation of **3** with the trichloroacetimidate **2** in the presence of BF_3-Et_2O and molecular sieves 4 Å in $CHCl_3$, gave a 37% yield of the fully protected G_{M3}-ganglioside **21**, $[\alpha]_D$ -4.51° (c 1.13), R_F 0.39 in EtOAc, δC (C^2HCl_3): 96.9 (C-2c), 100.6 (C-1a), 101.1 (C-1b); δH (C^2HCl_3): 2.57 (dd, *J* 4.7 and 12.7 Hz, H-3c_{eq}), 1.67 (t, *J* 12.5 Hz, H-3c_{ax}). Deacetylation and saponification of **21** afforded the desired **1** $[\alpha]_D$ +4.1° (c 0.24, $CHCl_3$/MeOH, 1/1 by vol), R_F 0.61 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol, which was completely identified with G_{M3}-ganglioside through comparison of its ^1H NMR data with that of the natural product [19]. It is to be noted that upon glycosylation with **6** a glycosyl acceptor **7** afforded stereoselectively only one product **9** with the β-D- configuration at C-2c, while the more reactive glycosyl acceptor **8** gave a mixture of **16** and the β-epimer at C-2c in a ratio of 1:2 [10]. This result strongly indicates that the glycosylation product reported by Shapiro [9] as the intermediate for the synthesis of G_{M3}-ganglioside should have the unnatural configuration β-D- at C-2c, since the glycosyl acceptor with all hydroxyl groups protected with the acetyl group had been employed as in the case of the glycosyl acceptor **7**.

In conclusion, by use of the glycosyl donor **2** and the glycosyl acceptor **3** as the key intermediates, G_{M3}-ganglioside was unambiguously synthesized for the first time. The synthetic G_{M3}-ganglioside **1**, but not the *epi*G_{M3}-ganglioside **15** was immunologically identical with natural G_{M3}-ganglioside by using a monoclonal antibody directed toward B-16 melanoma cells (Prof. Yoshio Hirabayashi, Dept. Biochem., Shizuoka College of Pharmacy).

Acknowledgements

We are indebted to Mr. Y. Shitori of Kantoishi Pharmaceutical Co., for the generous supply of *N*-acetylneuraminic acid. We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the NMR spectra and Dr. H. Honma and his staff for the elemental analyses. We also thank Ms. A. Takahashi for her technical assistance.

References

1 Ogawa T, Yamamoto H (1985) Agr Biol Chem, 49:475-82.
2 Hakomori S (1983) in Handbook of Lipid Research, Vol 3, Sphingolipid Biochemistry, eds. Kanfer JN, Hakomori S, Plenum Press, New York, p 89-166.
3 Glickman RM, Bouhours JF (1976) Biochim Biophys Acta 424:17-25.
4 Bouhours JF, Glickman RM (1976) Biochim Biophys Acta 441:123-33.
5 Sakiyama H, Gross SK, Robbins PN (1972) Proc Natl Acad Sci USA 69:872-76.
6 Critchey DR, Macpherson I (1973) Biochim Biophys Acta 296:145-59.
7 Bremer EG, Hakomori S, Bowen-Pope DR, Raines E, Ross R (1984) J Biol Chem 259: 6818-25.
8 Hakomori S (1983) in Handbook of Lipid Research, Vol 3, Sphingolipid Biochemistry, eds. Kanfer JN, Hakomori S, Plenum Press, New York, p 327-73.
9 Shapiro D (1974) 24th Int Congr Pure Appl Chem 2:153-66; (1976) Chem Abstr 85: 177 800.
10 Ogawa T, Sugimoto M (1984) Carbohydr Res, in press.
11 Koike K, Nakahara Y, Ogawa T (1984) Glycoconjugate J 1:107-9.
12 Kuhn R, Lutz P, MacDonald DL (1966) Chem Ber 99:611-17.
13 Haverkamp J, Van Halbeek H, Dorland L, Vliegenthart JFG, Pfeil R, Schauer R (1982) Eur J Biochem 122:305-11.
14 Bose R, Scheffold R (1976) Angew Chem 88:578-79.
15 Ogawa T, Nakabayashi S (1981) Carbohydr Res 93:C1-C5.
16 Schmidt RR, Michel J (1980) Angew Chem Int Ed Engl 19:731-32.
17 Schmidt RR, Michel J, Roos M (1984) Leibigs Ann Chem 1343-57.
18 Shapiro D, Flowers HM (1961) J Am Chem Soc 83:3327-32.
19 Koerner TAW Jr, Prestegard JH, Demou PC, Yu RK (1983) Biochemistry 22:2676-87.
20 Excoffier G, Gagnaire D, Utille J-P (1975) Carbohydr Res 39:368-73.