Glycoconjugate J (1985) 2:11–15

Preliminary Communication

Stereoselective Synthesis of Asialo-G_{M1}- and Asialo-G_{M2}-Ganglioside*

MAMORU SUGIMOTO, TOSHIO HORISAKI and TOMOYA OGAWA**

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

Received January 3, 1985.

Key words: synthesis, gangliosides, asialo-G_{M1}, asialo-G_{M2}

Asialo-G_{M1}-ganglioside or gangliotetraosylceramide **1** was reported to be present as a rat erythrocyte antigen and as a mouse natural killer cell marker [2]. Antibodies directed to asialo-G_{M1}-ganglioside have been used to detect acute lymphatic leukemia cells [2]. The structure of **1** was determined by partial degradation and methylation analysis [3-5]. Asialo-G_{M2}-ganglioside or gangliotriaosylceramide **24** has been isolated from the brain of a Tay-Sachs patient [2], guinea pig erythrocytes [6], mouse Kirsten tumor [7], and rat hepatoma [8].

Due to their functions as tumor-associated markers, three independent approaches to the synthesis of the glycan part of asialo-G_{M1}- and asialo-G_{M2}-ganglioside have recently been reported [9-11]. We describe here the first total synthesis of asialo-G_{M1}- and asialo-G_{M2}-ganglioside **24** in a regio- and stereocontrolled way. A synthetic plan was designed (Fig. 1), according to a retrosynthetic analysis which gave rise to the two key intermediates **3** and **4** for the synthesis of asialo-G_{M1}-ganglioside. Since the glycosyl acceptor **4** was readily obtainable via **5** [12], the route to the glycosyl donor **3** (and**22**) was developed as follows.

Benzyl penta-*O*-benzyl-β-D-lactoside **6** [13] was selectively benzylated by the stannyl method [14-16] to give an 89% yield of benzyl hexa-*O*-benzyl-β-D-lactoside **7** (Fig. 2), $[\alpha]_D$

---

*Part 35 in the series "Synthetic Studies on Cell-surface Glycans", for part 34, see ref. 1.

**Author for correspondence

Nomenclature: Asialo-G_{M1}-ganglioside, gangliotetraosylceramide ($GgOse_4Cer$), Galβ1-3GalNAcβ1-4Galβ1-4 GlcCer; asialo-G_{M2}-ganglioside, gangliotriaosylceramide ($GgOse_3Cer$), GalNAcβ1-4Galβ1-4GlcCer.

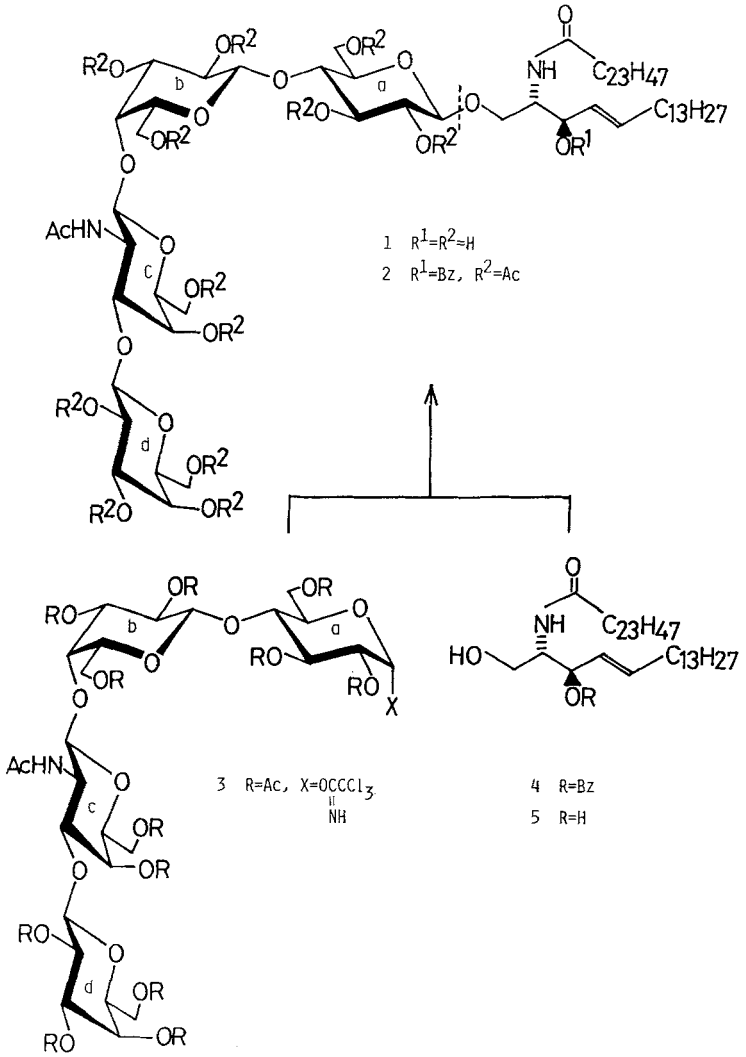

Figure 1. Key intermediates in the synthesis of asialo-G_{M1}-ganglioside. Abbreviation: Bz, benzoyl.

+22.1° (c 0.525), R_F 0.73 in toluene/EtOAc, 2/1 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. Glycosylation of **7** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl bromide **8** [17] in the presence of $AgOSO_2CF_3$ and molecular sieves 4 Å in $Cl(CH_2)_2Cl$ at 20°C afforded an 80% yield of the trisaccharide **9**, $[\alpha]_D$ +2.3° (c 0.70), R_F 0.48 in toluene/EtOAc, 3/1 by vol. The conversion of **9** into **10**, $[\alpha]_D$ +9.3° (c 0.15), R_F 0.50 in toluene/EtOAc, 1/1 by vol, was achieved in 3 steps in 91% overall yield; (i) NaOMe-MeOH, (ii) *n*-$BuNH_2$-MeOH under reflux, (iii) Ac_2O-pyridine. Deacetylation of **10** gave **11**, $[\alpha]_D$ +9.4° (c 0.15), R_F 0.47 in $CHCl_3$/MeOH, 19/1 by vol, which was converted in 94% yield into the benzylidene derivative **12**, $[\alpha]_D$ +28.2° (c 0.17), R_F 0.48

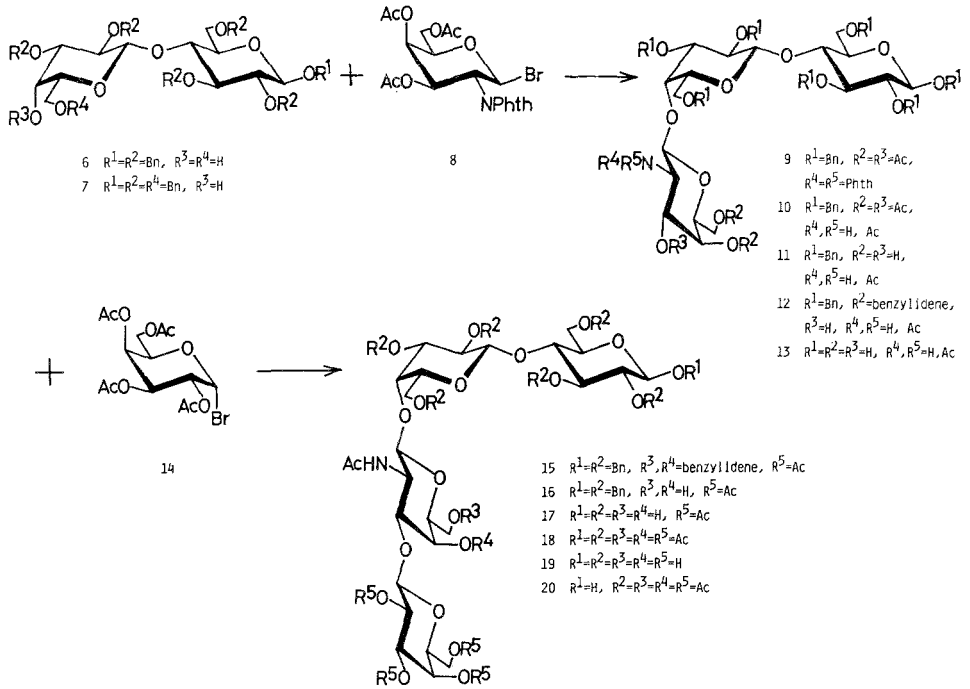

Figure 2. Synthesis of asialo-G_{M1}-ganglioside key intermediate. Abbreviations: Bn, benzyl; Phth, phthallyl.

in toluene/EtOAc, 1/2 by vol, by treatment with $C_6H_5CH(OMe)_2$ and p-toluenesulfonic acid in CH_3CN at 20°C. The glycosylation of **12** with acetobromogalactose **14** in the presence of $Hg(CN)_2$ and molecular sieves 4 Å in benzene/nitromethane, 1/1 by vol, at 60°C gave the tetrasaccharide derivative **15**, $[\alpha]_D$ +47.7° (c 0.17), R_F 0.54 in toluene/EtOAc, 1/1 by vol, in 97% yield. The structure of **15** was deduced from the reaction sequence and confirmed by the transformation into free tetrasaccharide **19** in 3 steps. Debenzylidenation of **15** in 80% aqueous AcOH at 80°C gave **16**, $[\alpha]_D$ +22.7° (c 0.75), R_F 0.29 in toluene/EtOAc, 1/2 by vol. Deacetylation of **16** with NaOMe in MeOH and subsequent hydrogenolysis with 10% Pd-C in AcOH afforded a 60% yield of **19**, $[\alpha]_D$ +21.4° (c 0.28 in H_2O), R_F 0.20 in *n*-BuOH/EtOH/H_2O, 2/2/1 by vol. The 400 MHz ^{1}H-NMR (in ^2H_2O at 25°C) of **19** contained the signals at δ 5.217 (d, *J* 2.9 Hz, H-1aα), 4.693 (d, *J* 8.5 Hz, H-1aβ), 4.665 (d, *J* 8.5 Hz, H-1c), 4.444 (d, *J* 7.6 Hz, H-1b and H-1d), 4.152 (bs, H-4c), and 4.118 (bs, H-4b), in good agreement with the assigned stereochemistry.

Catalytic hydrogenolysis of **16** over 10% Pd-C in AcOH at 80°C gave **17**, R_F 0.38 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol, which was acetylated with Ac_2O and pyridine to give peracetylated tetrasaccharide **18**, R_F 0.38 in EtOAc. Chemoselective deacetylation at the anomeric position of **18** to give **20**, $[\alpha]_D$ +24.5° (c 0.67), R_F 0.34 in EtOAc, was effected in 75% yield by treatment with NH_2NH_2-AcOH [18]. Treatment of **20** with NaH and Cl_3CCN as described by Schmidt *et al.* [19, 20] afforded a 52% yield of the desired glycosyl donor **3** R_F 0.50 in EtOAc, δH (C^2HCl_3): 6.48 (d, *J* 4.8 Hz, H-1a), and 8.64 (s, C=NH).

The glycotriosyl donor **22**, R_F 0.45 in toluene/EtOAc, 1/2 by vol, δH (C^2HCl_3): 6.47 (d, *J* 5.0 Hz, H-1a) and 8.64 (bs, C=NH), δC (C^2HCl_3): 93.0 (C-1a), 98.6 (C-1b) and 101.0 (C-1c), for the synthesis of asialo-GM_2-ganglioside was also prepared via **21** in a straightforward way from **10** in 4 steps, (i) 10% Pd-C, H_2, (ii) Ac_2O-pyridine, (iii) NH_2NH_2-AcOH, and (iv) NaH-Cl_3CCN (Fig. 3).

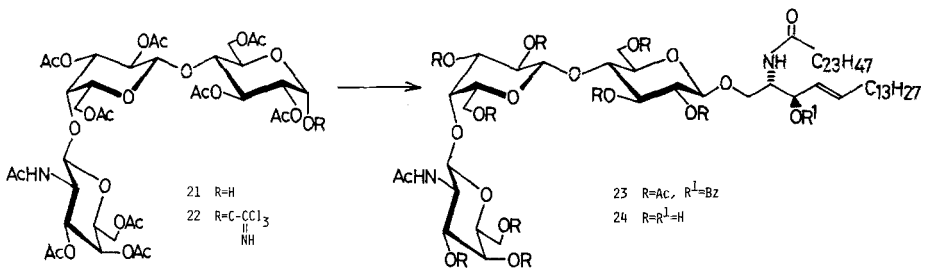

Figure 3. Final stages in the synthesis of asialo-G_{M2}-ganglioside. Abbreviation: Bz, benzoyl.

Having prepared the key glycosyl donors **3** and **22**, the crucial glycosylations with the acceptor **4** were now examined. The treatment of **4** with the glycosyl donor **22** in the presence of BF_3-Et_2O and molecular sieves AW-300 afforded a 38% yield of the fully protected asialo-GM_2-ganglioside **23**, $[\alpha]_D$ +2.6° (c 1.41), R_F 0.66 in toluene/EtOAc, 1/2 by vol. Deacylation of **23** with MeONa in MeOH-tetrahydrofuran afforded a 70% yield of asialo-G_{M2}-ganglioside **24**, $[\alpha]_D$ -2.8° (c 0.50, $CHCl_3$/MeOH, 1/1 by vol), R_F 0.62 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol. The synthetic asialo-G_{M2}-ganglioside had ^{1}H-NMR data identical with the natural compound [21]. By employing the same reaction sequence, the glycosyl donor **3** afforded a 15% yield of the desired asialo-G_{M1}-ganglioside **1**, $[\alpha]_D$ +2.5° (c 0.25, $CHCl_3$/MeOH, 1/1 by vol), R_F 0.58 in *n*-BuOH/EtOH/H_2O, 2/1/1 by vol, via the fully protected asialo-G_{M1}-ganglioside intermediate **2**, $[\alpha]_D$ +7.9° (c 0.67), R_F 0.40 in EtOAc/toluene, 4/1 by vol. Again the identity between the synthetic asialo-G_{M1}-ganglioside and the natural sample was confirmed by the comparison of their ^{1}H-NMR data in $^2H_6$-dimethylsulfoxide-^2H_2O [22].

In conclusion, an unambiguous synthesis of both asialo-G_{M1}-ganglioside **1** and asialo-G_{M2}-ganglioside **24** was achieved by using the trichloracetimidates **3** and **22** as the key glycosyl donors.

Acknowledgements

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 Sugimoto M, Ogawa T (1985) Glycoconjugate J 2:5—9.
2 Hakomori S (1983) in Handbook of Lipid Research, Vol 3, Sphingolipid Biochemistry, eds. Kanfer JN, Hakomori S, Plenum Press, New York, p 121-29.
3 Svennerholm L (1962) Biochem Biophys Res Commun 9:436-41.
4 Kuhn R, Wiegandt H (1963) Chem Ber 96:866-80.
5 Kuhn R, Egge H (1963) Chem Ber 96:3338-48.
6 Seyama Y, Yamakawa T (1974) J Biochem (Tokyo) 75:837-42.
7 Rosenfelder G, Young WW Jr, Hakomori S (1977) Cancer Res 37:1333-39.
8 Hirabayashi Y, Taki T, Matsumoto M, Kojima K (1978) Biochim Biophys Acta 529: 96-105.
9 Wessel H-P, Iversen T, Bundle DR (1984) Carbohydr Res 130:5-21.
10 Sabesan S, Lemieux RU (1984) Can J Chem 62:644-54.
11 Paulsen H, Paal M, Hadamczyk D, Steiger K-M (1984) Carbohydr Res 131:C1-C5.
12 Koike K, Nakahara Y, Ogawa T (1984) Glycoconjugate J 1:107-9.
13 Liptak A, Jodal I, Nanasi P (1976) Carbohydr Res 52:17-22.
14 Ogawa T, Matsui M (1978) Carbohydr Res 62:C1-C4.
15 Ogawa T, Matsui M (1981) Tetrahedron 37:2363-69
16 Veyrieres A (1981) J Chem Soc Perkin Trans 1:1626-29.
17 Lemieux RU, Ratcliffe RM (1979) Can J Chem 57:1244-51.
18 Excoffier G, Gagnaire D, Utille J-P (1975) Carbohydr Res 39:368-73.
19 Schmidt RR, Michel J (1980) Angew Chem Int Ed Engl 19:731-32.
20 Schmidt RR, Michel J, Roos M (1984) Liebigs Ann Chem 1343-57.
21 Prestegard JH, Koerner TAW Jr, Demou PC, Yu RK (1982) J Am Chem Soc 104:4993-95.
22 Gasa S, Mitsuyama T, Makita A (1983) J Lipid Res 24:174-82.