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Synthesis of Ganglioside M5 from Sea Urchin Egg

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ABSTRACT: A main component of ganglioside M5 from egg of the sea urchin, *Anthocidaris crassispina*, Neu5Gcα2→6Glcβ1→1Cer (**1**), which has *N*-glycolyl group in neuraminic acid part, as well as phytosphingosine and saturated nonhydroxy fatty acid in ceramide part was first synthesized starting from *N*-acetylneuraminic acid.

The ganglioside M5 was isolated from egg of the sea urchin, *Anthocidaris crassispina*, as a mixture of fatty acid congeners in ceramide moiety. The structure of ganglioside M5 elucidated by Hoshi *et al.*,[1] is characterized by the presence of *N*-glycolylneuraminic acid as a sialic acid moiety, and of C_{18}-phytosphingosine with one extra hydroxyl group at C4 position in ceramide part, in comparison with those of usual gangliosides such as GM_3 (Neu5Acα2→3Galβ1→4Glcβ1→1Cer). The fatty acid part in ganglioside M5 has a variety depending on presence or absence of hydroxyl group at C2' position (R^1) as well as chain length and number of double bonds (R^2).

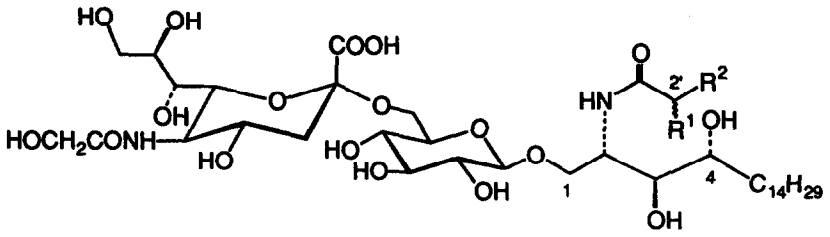

Fig. 1. The Structure of Ganglioside M5.
(R^1 = H or OH, R^2 = C_nH_{2n+1}, C_nH_{2n-1}, C_nH_{2n-3}, C_nH_{2n-5} (n = 12~25))

In spite of important biological activity such as neuritogenic or growth-inhibitory activity of *N*-glycolylneuraminic acid containing ganglioside,[2] only few synthetic works for this particular ganglioside have been reported.[3] One of the reason of this situation seems to be due to less availability of *N*-glycolylneuraminic acid including its cost.[4] Starting from *N*-acetylneuraminic acid[5] we attempted to synthesize ganglioside M5 (22:0) (**1**) [R^1 = H, R^2 = *n*-$C_{20}H_{41}$] by using *n*-docosanoic acid (behenic acid) as a fatty acid in the ceramide part.

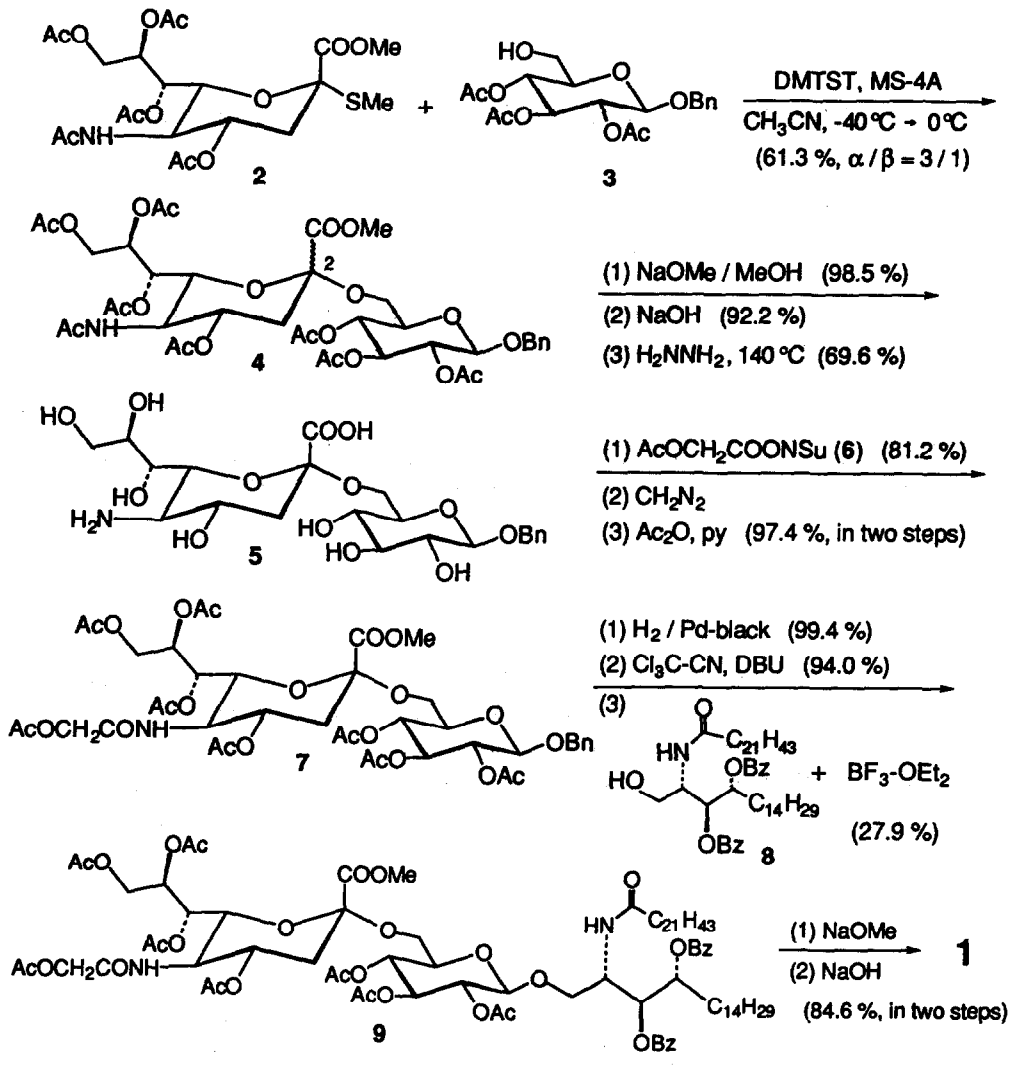

Fig. 2. Synthesis of Ganglioside M5 (22:0) (1).

N-Acetylneuraminic acid was transformed into 2-methylthio derivative **2** by six steps according to Hasegawa's procedure.[6] On the other hand, the protected glucose with a free 6-hydroxyl group **3** was obtained from D-glucose through eight steps.[7] The donor molecule **2** was coupled with the acceptor one **3** *in the presence of DMTST (dimethyl(methylthio)sulfonium triflate)* [8] and molecular sieves (MS)-4A [9] to give a disaccharide **4**. A ratio of anomeric isomers at C2 position of sialic acid moiety (α to β) was 3 to 1.[10] After isolation of α anomer, deprotections of *O*-acetyl groups and methyl ester were carried out for this anomer. *N*-Acetyl group of disaccharide benzyl glycoside was cleaved by hydrazine treatment at 140

°C to give a free amino compound **5** without cleavage of benzyl group.[11] The amine **5** thus obtained was then *N*-glycolylated with acetylglycolic acid *N*-hydroxysuccinimide ester (**6**), followed by methyl esterification and *O*-acetylation to give a compound **7** as shown in Fig. 2.

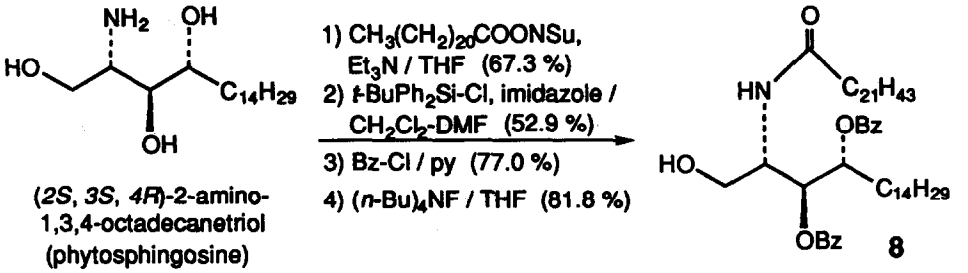

Fig. 3. Preparation of Protected Ceramide **8**.

The protected ceramide **8** was prepared from (*2S, 3S, 4R*)-2-amino-1,3,4-octadecanetriol (phytosphingosine)[12] through four reaction steps as shown in Fig. 3. Thus, phytosphingosine was *N*-acylated with *n*-docosanoic acid *N*-hydroxysuccinimide ester. After protection of a primary alcohol of the triol by *t*-butyldiphenylsilyl group, the other secondary alcohols were benzoylated. Finally, deprotection of silyl group was carried out to give the protected ceramide **8** with a free primary hydroxyl group.

The benzyl glycoside **7** was converted to trichloroacetimidate by catalytic hydrogenation and then treatment with trichloroacetonitrile and DBU (1,8-diazabicyclo[5,4,0]undec-7-ene). In the presence of boron trifluoride etherate, the activated donor was coupled with compound **8** to give a protected ganglioside **9**. Finally, deprotection of all the protecting groups was performed in two steps. Acetyl and benzoyl groups were cleaved with sodium methoxide, and then the methyl ester was hydrolyzed with sodium hydroxide to give a compound **1**.

Data of plasma desorption mass spectrum[13] and ^{1}H-NMR spectrum[14] for the synthetic ganglioside M5 (22:0) (**1**) thus obtained, support the expected structure as shown above unequivocally. The synthetic ganglioside **1** was identical with one of the components in the natural ganglioside M5 in respects of TLC and immunoreactivity.[15] Thus, the structure of the natural ganglioside M5 could be confirmed by this chemical synthesis.

This synthetic strategy for ganglioside M5 could be useful for the synthesis of other biologically important ganglioside with *N*-glycolylneuraminic acid as well as phytosphingosine, such as neuritogenically active GAA-7.[2d]

ACKNOWLEDGMENT

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5. In our study on the enzymatic glycosidation of sialic acid in the presence of the sialidase from *Arthrobacter ureafaciens*, sialyl-D-glucose was obtained in the reaction with D-glucose. This enzymatic product can be also useful for preparation of ganglioside M5. (cf. T. Yamamoto, T. Teshima, and T. Shiba, *ABSTRACTS OF 15th JAPANESE CARBOHYDRATE SYMPOSIUM*, 1993, pp27-28.)
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7. a)Ac_2O/py, b)H_2NNH_2+AcOH/DMF, c)Cl_3C-CN+DBU/CH_2Cl_2 (68.8 %, in three steps), d)BnOH+BF_3-OEt_2/CH_2Cl_2 (61.4 %), e)NaOMe/MeOH (99.7 %), f)Ph_3C-Cl/py, g)Ac_2O/py (72.4 %, in two steps), h)80% AcOH (94.0 %).
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10. An assignment of configurations in compound **4** was based on the ^{1}H-NMR chemical shifts of the equatorial proton at C3 position. After deprotection and separation of α and β-isomers by using HPLC, the stereochemistry at C2 position was confirmed enzymatically. Namely, the assigned α-isomer could be only hydrolyzed by sialidase to give *N*-acetylneuraminic acid.
11. Besides the free amine **5** as a predominant product, the starting material with an acetylamino group was recovered in 17.9 % yield .
12. Phytosphingosine is commercially available in SIGMA chemical company.
13. In positive and negative-ion plasma desorption mass spectrum of the synthetic ganglioside M5 (22:0) (**1**), molecular ion ($[M+H]^+$) peak (positive) and ($[M-H]^-$) peak (negative) were clearly observed at m/z of 1111 and 1109, respectively. (The calculated molecular weight is 1110.)
14. ^{1}H-NMR spectral data of synthetic ganglioside M5 (22:0) (**1**) (270 MHz, in DMSO-d_6): δ0.85 (6H, t, J = 6.6 Hz, $(CH_2)_n$-$\underline{CH_3}$), δ1.10 - δ1.50 (64H, m, $32CH_2$), δ 2.06 (2H, m, -HNCO-$\underline{CH_2}$), δ2.69 (1H, m, H_{3e} of Neu5Gc), δ2.94 - δ4.12 (20H, m), δ4.46 - δ4.88 and δ5.50 (10H, m, OH), δ6.55 and δ7.67 (2H, m, NH). The signals of protons in OH and NH groups disappeared in addition of D_2O.
15. Synthetic ganglioside M5 (22:0) (**1**) was found to be bound for the antibody which recognizes the natural ganglioside M5. (cf. H. Kubo and M. Hoshi, *J. Biochem.*, **1990**, *108*, 193-199.)

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