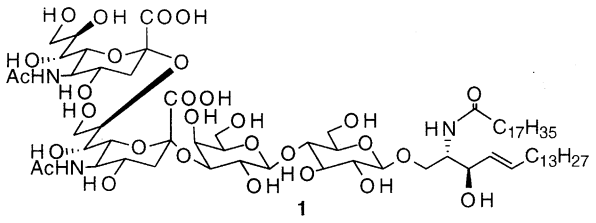
Total Synthesis of GD_3, A Ganglioside

Tadao Kondo,* Toshiyuki Tomoo,† Hiroyuki Abe,† Minoru Isobe,† and Toshio Goto†
Chemical Instrument Center, Nagoya University, Chikusa, Nagoya 464-01
†*Laboratory of Organic Chemistry, School of Agriculture, Nagoya University, Chikusa, Nagoya 464-01*

(Received January 16, 1996)

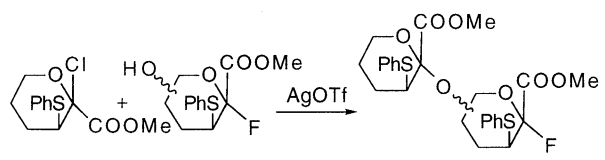
On the basis of new methodology involving α-stereocontrolled sialylation using our 2-halo-3β-phenylthio-Neu5Ac and construction of Neu5Ac(α2-8)Neu5Ac due to differential reactivity between 2-chloro and 2-fluoro-Neu5Ac, we realized an efficient total synthesis of GD_3.

The disialyl ganglioside, GD_3 **1**, is a very attractive target molecule for organic synthesis due to its various biological activities, especially as a human melanoma associated antigen.[1]



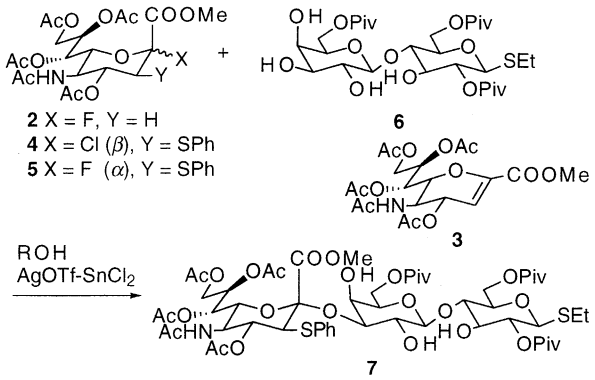
1

The active center of GD_3 has been presumed to be the Neu5Ac(α2-8)Neu5Ac portion. The key problems concerning synthetic work on the ganglioside are the formation of Neu5Ac(α2-8)Neu5Ac and efficient condensation of the disialic acid with a lactoside. Previous syntheses of GD_3 were reported by Ogawa[2] and Hasegawa.[3] In the former case benzylsialyl-(α2-8)-Δ^2-sialate was used as a key intermediate, needed for the multiple transformation steps to the donor form, with the latter a naturally occurring disialic acid was used as starting material avoiding the difficulty of α-glycosylation. In this paper we describe an efficient total synthetic method for GD_3 involving regio- and α-stereocontrolled sialylation on the basis of differential reactivity between sialyl chloride and its fluoride and neighbouring group participation of 3β-phenylthio substituent on Neu5Ac.



Though the sialyl fluoride **2** proved stable not only under normal halide promoting conditions such as in the presence of silver triflate (AgOTf) but also under basic and weakly acidic conditions, the fluoride in an anomeric position could be activated with AgOTf-$SnCl_2$ or the AgOTf-Hf-complex by the Mukaiyama-Suzuki method.[4,5] Therefore, the sialyl fluoride is useful as the corresponding elongatable sialyl donor. However, condensation of **2** with stereochemically hindered acceptor oligosaccharide resulted predominantly in an elimination reaction to provide the Δ^2-Neu5Ac derivative **3** as a major product, and no production of the sialoside.[6]

In order to overcome the difficulty we conducted a novel stereochemical sialylation by neighbouring group participation and suppression of the elimination reaction using an auxiliary substituent at C-3 of Neu5Ac.[7] Using methyl 2β-chloro-3β-phenylthio-*N*-acetyl-pentaacetylneuraminate **4** complete α-sialylation was earlier realized.[7e,8] The 2α-fluoro-3β-phenylthio-Neu5Ac **5** was therefore considered a suitable reducing terminal sialic acid. The chloride **4** was treated with H_2O-AgOTf in CH_3CN followed by DAST in CH_2Cl_2 at -78 °C to afford the α-fluoride (**5**, $J_{F,3Hax}$ = 15.5 Hz) with a total 98% yield.[9] In order to evaluate the glycosylation ability of the fluoride, the **5** was condensed to ethyl 2,6,6'-tri-*O*-pivaloyl-thiolactoside[8] **6** with $SnCl_2$-AgOTf to afford the corresponding α-glycoside **7** at a 66% yield.



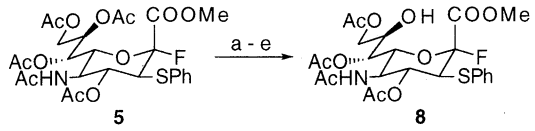
2 X = F, Y = H
4 X = Cl (β), Y = SPh
5 X = F (α), Y = SPh

6

3

7

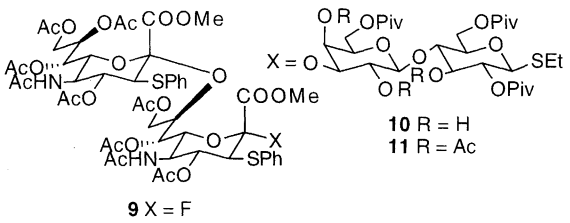
Transformation of **5** to a sialyl acceptor was carried out as follows. The **5** was deacetylated with *tert*-BuOK-MeOH, and treated with acetone in the presence of Dowex 50 (H^+) to form the 8,9-*O*-isopropylidene at 77%, subsequently acetylated with Ac_2O-pyridine. Removal of the acetonide group with 80% aq. acetic acid followed by selective acetylation with 1 eq. AcCl at -30 °C gave the desired 8-unprotected Neu5Ac acceptor **8** (δ 4.1, H-8) at a 81% yield.



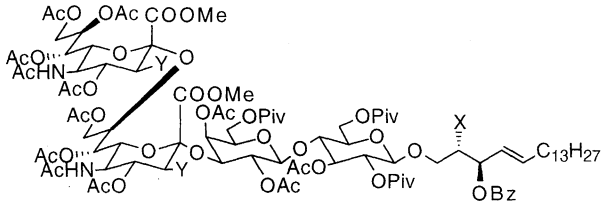
5 **8**

a) *t*-BuOK / MeOH, 91%; b) H^+/ acetone, 77%; c) Ac_2O / Pyr., 96%; d) 80% aq. AcOH, 82%; e) AcCl / pyr., -30 °C, 81%.

Glycosylation of the sialyl fluoride acceptor **8** with the sialyl chloride donor **4** (a mole ratio of **8**:**4**, 1:2) promoted by AgOTf in the presence of MS-4A in CH_2Cl_2 gave exclusively the corresponding Neu5Ac(α2-8)Neu5Ac **9**, amorphous mass, mp 118 °C; $[\alpha]_D^{20}$ +30.9° (c = 0.11, $CHCl_3$), the yield being 49%. Glycosylation of **6** with the α2-8 linked disialyl fluoride **9**



(**6**:**9** ratio, 1.2:1) in the presence of AgOTf, $SnCl_2$ and MS-AW300 in CH_3CN for 2 days at room temperature only gave the α-glycoside[10] **10** at a 39% yield; **10**, amorphous mass, mp 116 °C; $[\alpha]_D^{20}$ +22.5° (c = 0.12, $CHCl_3$). The newly formed anomer configuration should be α due to the neighbouring group participation of the 3β-substituted phenylthio group. Acetylation of **10** gave the acetate **11**. The glycosylation position was determined to be at the 3'-OH of the thiolactoside by HMBC because of observation of correlation between the anomer ^{13}C of the reducing terminal sialic acid and the H-3 proton (δ 5.04 ppm) of the galactose residue of **11**.



Condensation of the azidosphingosine[11] with the thiotetrasaccharide **11** activated by DMTST was carried out to give the corresponding glycosylazidosphingosine **12** at 84%; **12**, amorphous mass, mp 100 °C; $[\alpha]_D^{20}$ +32.5° (c = 0.16, $CHCl_3$). The anomeric configuration was β (δ 4.55, $J_{1,2}$ = 7.9 Hz). Transformation of the azide **12** to the ceramide was performed according to our previous report with the phosphine reduction-acylation method.[8] Reduction of the azide **12** with tri-*n*-butylphosphine (1.3 eq.) in the presence of octadecanoic acid (2 eq.) in CH_2Cl_2 followed by addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride for completion of the reaction afforded the ceramide **13**. Removal of the two phenylthio groups of **13** by radical reduction using *n*-Bu_3SnH[7e] afforded the protected GD_3 **14** at a 66% yield without any damage to the olefin. Finally, *O*-deacylation of **14** with *tert*-BuOK in MeOH, with subsequent saponification of the resulting methyl ester, yielded quantitatively the ganglioside GD_3 **1**; $[\alpha]_D^{20}$ -2.5° (c = 0.1, 1:1 $CHCl_3$-MeOH), for which the ^1H-NMR data were completely consistent with those of the naturally occuring one.[12]

This synthesis revealed that the fluoride group on the reducing terminal functioned in protection and sialyl donation, and also the 3β-phenylthio substituent of neuraminic acid contributes to α-stereoselective sialylation. Employment of this simple methodology provides a promising approach for syntheses of a series of α-polysialyl gangliosides and their analogs.

This work was supported by the Grant-in-Aid for Scientific Research on Priority Areas No. 07259207 and 07229223 from the Ministry of Education, Science and Culture, Japan.

References and Notes

1 a) C. S. Pukel, K. O. Lloyd, L. R. Travassos, W. G. Dippold, H. F. Oettgen, and L. J. Old, *J. Exp. Med.*, **155**, 1133 (1982); b) E. Nudelman, S. Hakomori, R. Kannagi, S. Levery, M.-Y. Yeh, K. E. Hellström, and I. Hellström, *J. Biol. Chem.*, **257**, 12752 (1982).
2 Y. Ito, M. Numata, M. Sugimoto, and T. Ogawa, *J. Am. Chem. Soc.*, **111**, 8508 (1989).
3 a) A. Hasegawa, H. Ishida, and M. Kiso, *J. Carbohydr. Chem.*, **12**, 371 (1993); b) H. Ishida, Y. Ohta, Y. Tsukada, M. Kiso, and A. Hasegawa, *Carbohydr. Res.*, **246**, 75 (1993).
4 T. Mukaiyama, Y. Murai, and S. Shoda, *Chem. Lett.*, **1981**, 431.
5 T. Matsumoto, H. Maeta, K. Suzuki, and G. Tsuchihashi, *Tetrahedron Lett.*, **29**, 3567 (1988).
6 H. Kunz and H. Waldmann, *J. Chem. Soc., Chem. Commun.*, **1985**, 638.
7 a) K. Okamoto, T. Kondo and T. Goto, *Bull. Chem. Soc. Jpn.*, **60**, 637 (1987); b) K. Okamoto, T. Kondo and T. Goto, *Tetrahedron*, **43**, 5909 (1987); c) K. Okamoto, T. Kondo and T. Goto, *Tetrahedron*, **43**, 5919 (1987); d) K. Okamoto, T. Kondo and T. Goto, *Tetrahedron*, **44**, 1291 (1988); e) T. Kondo, H. Abe and T. Goto, *Chem. Lett.*, **1988**, 1657; f) Y. Ito and T. Ogawa, *Tetrahedron Lett.*, **29**, 3987 (1988); g) T. Ercégovic and G. Magnusson, *J. Org. Chem.*, **60**, 3378 (1995).
8 T. Tomoo, T. Kondo, H. Abe, S. Tsukamoto, M. Isobe, and T. Goto, *Carbohydr. Res.*, in press.
9 Direct transformation of **4** to the fluoride **5** using AgF gave only a 40% yield.
10 T. Kondo, H. Abe, S. Tsukamoto, K. Esaka, and T. Goto, Abstracts of papers, the 1989 International Chemical Congress of Pacific Basin Societies, Honolulu, Bios 411 (1990).
11 P. Zimmermann and R.R. Schmidt, *Liebigs Ann. Chem.*, **1988**, 663.
12 R.K. Yu, T.A.W. Koerner, J.N. Scarsdale, and J. H. Prestegard, *Chem. Phys. Lipids*, **42**, 27 (1986).