

Total Synthesis of GD₃, A GangliosideTadao Kondo,* Toshiyuki Tomoo,[†] Hiroyuki Abe,[†] Minoru Isobe,[†] and Toshio Goto[†]

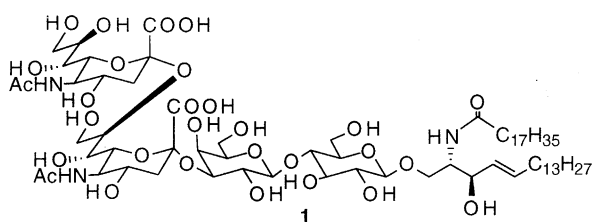
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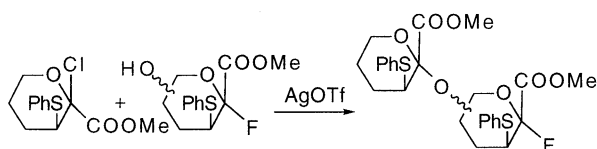
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On the basis of new methodology involving α -stereo-controlled sialylation using our 2-halo-3 β -phenylthio-Neu5Ac and construction of Neu5Ac(α 2-8)Neu5Ac due to differential reactivity between 2-chloro and 2-fluoro-Neu5Ac, we realized an efficient total synthesis of GD₃.

The disialyl ganglioside, GD₃ **1**, is a very attractive target molecule for organic synthesis due to its various biological activities, especially as a human melanoma associated antigen.¹

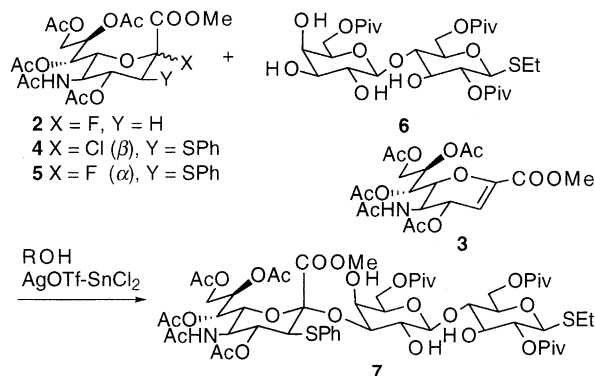


The active center of GD₃ has been presumed to be the Neu5Ac(α 2-8)Neu5Ac portion. The key problems concerning synthetic work on the ganglioside are the formation of Neu5Ac(α 2-8)Neu5Ac and efficient condensation of the disialic acid with a lactoside. Previous syntheses of GD₃ were reported by Ogawa² and Hasegawa.³ In the former case benzylsialyl-(α 2-8)- 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 GD₃ involving regio- and α -stereocontrolled sialylation on the basis of differential reactivity between sialyl chloride and its fluoride and neighbouring group participation of 3 β -phenylthio substituent on Neu5Ac.

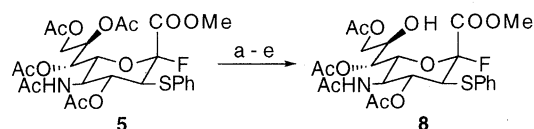


Though the sialyl fluoride **2** proved stable not only under normal halide promoting conditions such as in the presence of silver triflate (AgOTf) but also under basic and weakly acidic conditions, the fluoride in an anomeric position could be activated with AgOTf-SnCl₂ or the AgOTf-Hf-complex by the 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 2 β -chloro-3 β -phenylthio-*N*-acetyl-pentaacetylneuraminate **4** complete α -sialylation was earlier realized.^{7e,8} The 2 α -fluoro-3 β -phenylthio-Neu5Ac **5** was therefore considered a suitable reducing terminal sialic acid. The chloride **4** was treated with H₂O-AgOTf in CH₃CN followed by DAST in CH₂Cl₂ at -78 °C to afford the α -fluoride (**5**, $J_{F,3H_{ax}} = 15.5$ 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-thiolactoside⁸ **6** with SnCl₂-AgOTf to afford the corresponding α -glycoside **7** at a 66% yield.

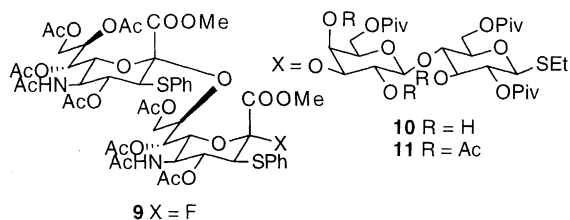


Transformation of **5** to a sialyl acceptor was carried out as follows. The **5** was deacetylated with *tert*-BuOK-MeOH, and treated with acetone in the presence of Dowex 50 (H⁺) to form the 8,9-*O*-isopropylidene at 77%, subsequently acetylated with Ac₂O-pyridine. Removal of the acetonide group with 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.

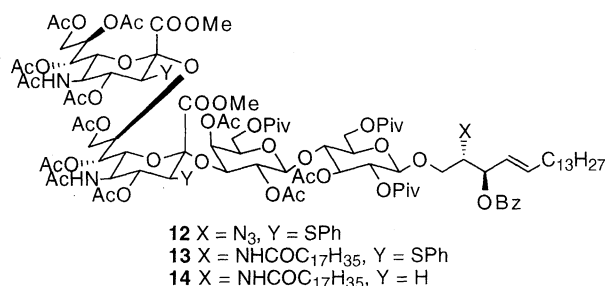


a) *t*-BuOK / MeOH, 91%; b) H⁺ / acetone, 77%; c) Ac₂O / Pyr., 96%;
d) 80% aq. AcOH, 82%; e) AcCl / pyr., -30 °C, 81%.

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 CH₂Cl₂ gave exclusively the corresponding Neu5Ac(α 2-8)Neu5Ac **9**, amorphous mass, mp 118 °C: $[\alpha]_D^{20} +30.9^\circ$ ($c = 0.11$, CHCl₃), the yield being 49%. Glycosylation of **6** with the α 2-8 linked disialyl fluoride **9**



(6:9 ratio, 1.2:1) in the presence of AgOTf, SnCl₂ and MS-AW300 in CH₃CN for 2 days at room temperature only gave the α -glycoside¹⁰ **10** at a 39% yield; **10**, amorphous mass, mp 116 °C; [α]_D²⁰ +22.5° (c = 0.12, CHCl₃). The newly formed anomer configuration should be α due to the neighbouring group participation of the 3 β -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 ¹³C of the reducing terminal sialic acid and the H-3 proton (δ 5.04 ppm) of the galactose residue of **11**.



Condensation of the azidosphingosine¹¹ with the thiotetra-saccharide **11** activated by DMTST was carried out to give the corresponding glycosylazidosphingosine **12** at 84%; **12**, amorphous mass, mp 100 °C; [α]_D²⁰ +32.5° (c = 0.16, CHCl₃). 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 CH₂Cl₂ 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*-Bu₃SnH^{7e} afforded the protected GD₃ **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 GD₃ **1**; [α]_D²⁰ -2.5° (c = 0.1, 1:1 CHCl₃-MeOH), for which the ¹H-NMR data were completely consistent with those of the naturally occurring one.¹²

This synthesis revealed that the fluoride group on the reducing terminal functioned in protection and sialyl donation, and also the 3 β -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.

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