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Note

A facile, large-scale preparation of the methyl 2-thioglycoside of *N*-acetylneuraminic acid, and its usefulness for the α-stereoselective synthesis of sialoglycosides*

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It is well known that naturally occurring sialo compounds such as glycoproteins and glycolipids in cell membranes, which play important roles in biological systems[2–5], contain sialic acids in α-glycosidic linkage. Therefore, a facile, α-selective synthesis of sialic-acid glycosides is very important.

Recently, new efforts[6–9] have been made towards the synthesis of α-glycosides of *N*-acetylneuraminic acid (Neu5Ac), but the procedure still remains to be improved with respect to yields, regio- and stereo-selectivity, and potential for scale-up.

We have developed[10–13] a procedure for regio- and α-stereo-selective glycosylation with sialosyl groups involving the reaction of the methyl α-thioglycosides of sialic acids as the glycosyl donors, suitably protected acceptors, and dimethyl(methylthio)sulfonium triflate[14] (DMTST) in acetonitrile under kinetically controlled conditions. In this manner, we have synthesized[10–12,15,16] a variety of gangliosides and their analogs. However, the preparation[11,17] of the important glycosyl donor, namely the methyl α-thioglycoside of Neu5Ac, requires several steps from Neu5Ac, and seems to be unsuitable for large-scale synthesis. For the efficient synthesis of various types of sialo-oligosaccharides and their analogs, required for studies of the functions of sialoglycoconjugates at the molecular level, it is critically important to circumvent this difficulty. We report here a facile, large-scale preparation of an alternative glycosyl donor, a 1:1 anomeric mixture of the methyl thioglycosides of Neu5Ac, and its usefuesness for the α-stereoselective synthesis of sialoglycosides.

Treatment of *N*-acetylneuraminic acid with Amberlite IR-120 (H^+) resin in methanol and subsequent acetylation with acetic anhydride in pyridine[18] gave methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**1**) in almost quantitative yield. The anomeric ratio (α:β) was estimated as 3:8 from the ^{1}H-n.m.r. spectrum. The replacement of the anomeric acetoxy group in **1** with methylthio by heating for 6 h at 50° with (methylthio)trimethylsilane (see General methods) in dry 1,2-dichloroethane in the presence of trimethylsilyl trifluoromethane-

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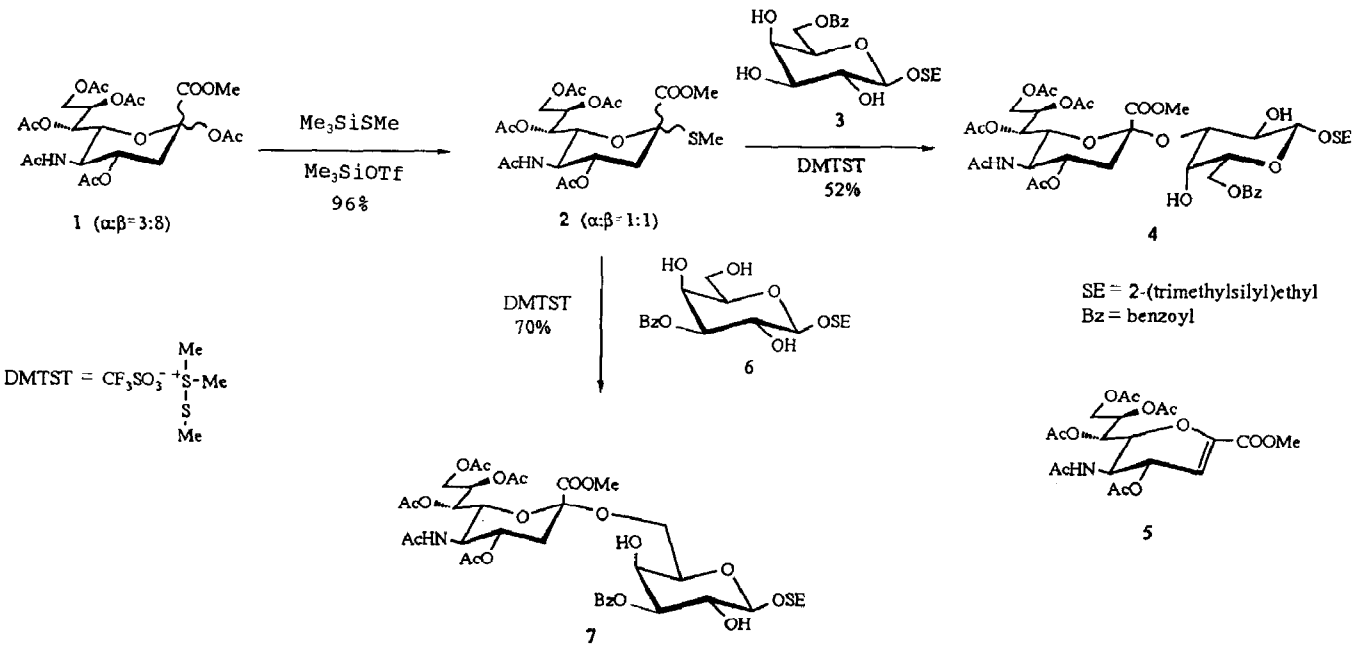
Me3SiSMe
Me3SiOTf
96%
1 (α:β=3:8)
2 (α:β=1:1)
3
DMTST
52%
4
SE = 2-(trimethylsilyl)ethyl
Bz = benzoyl
DMTST
70%
6
DMTST = CF3SO3- +S-Me
5
7

sulfonate (TMS-triflate) gave the methyl thioglycoside (**2**) of Neu5Ac in 96% yield as a 1:1 anomeric mixture. This exchange reaction is noteworthy, because no side reaction is observed in a large-scale preparation. However, when BF_3-etherate was used in the place of TMS-triflate in the reaction, almost none of the desired product was isolated. If the α,β mixture (**2**) obtained here can be applied in place of the previously used α anomer as the donor in syntheses of α glycosides of Neu5Ac, it will provide a great advantage for sialoglycoconjugate synthesis.

The glycosylation of 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside[10a,11] (**3**) with the anomeric mixture **2** (2.0 equiv. relative to the acceptor) in acetonitrile for 17h at −15° in the presence of DMTST (3.0 equiv. relative to the donor) gave the expected α-linked disaccharide of Neu5Ac, 2-(trimethylsilyl)ethyl *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonate)-(2→3)-6-*O*-benzoyl-β-D-galactopyranoside[10a,11] (**4**) in 52% yield based on the weight of acceptor employed, together with the 2,3-dehydro derivative (**5**) and some unreacted acceptor. Neither the β-glycoside nor any position isomers were isolated. In the same way, when treated with 2-(trimethylsilyl)ethyl 3-*O*-benzoyl-β-D-galactopyranoside[11] (**6**), the glycosyl donor **2** yielded the desired α glycoside[11] **7** of Neu5Ac in 70% yield; again no β-linked product was isolated. The results clearly indicate that the anomeric mixture **2** of the methyl thioglycosides of Neu5Ac, obtained easily on a large scale, is effective as a glycosyl donor affording the α glycosides of Neu5Ac regio- and stereo-selectively in good yields. The α-linked disaccharides described herein could find wide use as intermediates in ganglioside synthesis and also as building units for sialoglycoconjugate synthesis.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were determined with a Union PM-201 polarimeter at 25°, and i.r. spectra were recorded with a Jasco A-100 spectrometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a Jeol JNM-GX270 instrument. Preparative chromatography was performed on silica gel (Wako Chemical Co., 200 mesh) with the solvent systems specified. (Methylthio)-trimethylsilane was purchased from the Shin-Etsu Chemical Co., Tokyo. Concentrations and evaporations were conducted in vacuo.

*Methyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-*D*-glycero-*D*-galacto-2-nonulopyranosid)onate* (**2**). — To a stirred solution of methyl 5-acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**1**; 40 g, 61 mmol), prepared[18] in almost quantitative yield from Neu5Ac, in dry 1,2-dichloroethane (600 mL) were added (methylthio)trimethylsilane (27 g, 225 mmol), TMS-triflate (13.5 g, 61 mmol), and molecular sieves 4A (MS-4A; 10 g). The mixture was then heated, with stirring, for 6 h at 50°, the course of the reaction being monitored by t.l.c. One-molar sodium carbonate (500 mL) was added to the stirred, cooled mixture, and the organic layer was separated, washed with water, dried (Na_2SO_4), and concentrated to a syrup. This was chromatographed on a column of silica gel (700 mL)

by successive elution with (*a*) dichloromethane, (*b*) 200:1, and (*c*) 50:1 dichloromethane–methanol. Eluate *c* gave **2** (37.7 g, 96%) as an amorphous mass. The anomeric ratio (α:β) was estimated as ~1:1 from the ratio of intensities of the methyl ester group signals in the ^{1}H-n.m.r. spectrum.

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→3)-6*-O-*benozyl-β*-D-*galactopyranoside* (**4**). — To a solution of **2** (2.7 g, 5.2 mmol) and 2-(trimethylsilyl)ethyl 6-*O*-benzoyl-β-D-galactopyranoside[11] (**3**; 1.0 g, 2.6 mmol) in dry acetonitrile (20 mL) was added molecular sieves 3A (MS-3A; 3 g), and the mixture was stirred for 5 h at room temperature, then cooled to −40°. To the cooled suspension was added a mixture (6.53 g; 62% DMTST by weight) of DMTST (3.0 equiv. relative to the donor) and MS-3A, and this was stirred for 17 h at −15°. Methanol (1 mL) was added to the mixture, and it was neutralized with triethylamine. The solids were filtered off and washed thoroughly with dichloromethane, and the filtrate and washings were combined and concentrated. The residue was dissolved in dichloromethane and the solution was successively washed with M sodium carbonate and water, dried (Na_2SO_4), and evaporated to a syrup that was chromatographed on a column of silica gel (200 mL) with 1:1 ethyl acetate–hexane as eluent, to give compound **4** (1.16 g, 52%) as an amorphous mass. The i.r. and n.m.r. data, and $[\alpha]_D$ value (−6.0°, *c* 2.0, $CHCl_3$) were all identical with those of an authentic sample[10a,11].

2-(Trimethylsilyl)ethyl O-*(methyl 5-acetamido-4,7,8,9-tetra*-O-*acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosylonate)-(2→6)-3*-O-*benzoyl-β*-D-*galactopyranoside* (**7**). — To a solution of **2** (2.7 g, 5.2 mmol) and 2-(trimethylsilyl)ethyl 3-*O*-benzoyl-β-D-galactopyranoside[11] (**6**; 10 g, 2.6 mmol) in dry acetonitrile (20 mL) was added MS-3A (3 g), and the suspension was stirred for 6 h at room temperature, then cooled to −40°. A mixture (6.5 g; 62% DMTST by weight) of DMTST (3.0 equiv. relative to the donor) and MS-3A (3 g) was added, and stirring was continued for 17 h at −15°. Processing as described for **4**, and chromatography (silica gel; 300 mL) using 1:1 ethyl acetate–hexane as eluent afforded **7** (1.56 g, 70%) as an amorphous mass. The i.r. and n.m.r. data, and $[\alpha]_D$ value (−6.4°, *c* 0.4, $CHCl_3$) were all identical with those of an authentic sample[11].

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