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Note

A novel stereoselective synthesis of *N*-acetyl-α-neuraminosyl-galactose disaccharide derivatives, using anomeric *S*-glycosyl xanthates

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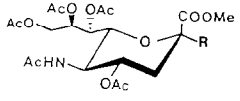
N-Acetyl-α-neuraminic acid (Neu5Ac) frequently terminates oligosaccharide chains of glycoproteins and glycolipids of cell membranes and plays a vital role in their biological activities. Therefore, there is a need for efficient and selective methods of synthesis of α-glycosides of Neu5Ac. Such glycosyl donors as methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosyl chloride)onate[1] or the 2-bromo analogue[2] usually result in poor yields and low selectivities[3]. Various indirect solutions to this problem have been developed[4–6]. Hasegawa *et al.*[7] have demonstrated that the methyl 2-thio-α-glycoside of Neu5Ac was an efficient and stereoselective glycosyl donor. We now report a novel donor of Neu5Ac, namely, the crystalline *S*-glycosyl xanthate[8] **1**. As Neu5Ac in glycoconjugates is frequently α-linked to either HO-6 or HO-3 of a D-galactopyranose residue, we have considered only these two situations.

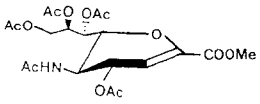
Treatment of methyl 3-*O*-benzoyl-β-D-galactopyranoside[9] (**5**) with 0.5 equiv. of **1** in acetonitrile for 1.5 h at −15° in the presence of 1 equiv. of dimethyl(methylthio)sulfonium trifluoromethanesulfonate[10] (DMTST) gave 48% (based on **1**) of methyl 3-*O*-benzoyl-6-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-β-D-galactopyranoside (**7**) and



1 R = S-C(=S)-OEt

2 R = SEt

3 R = S-C(=O)-OEt



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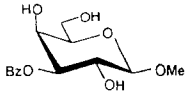

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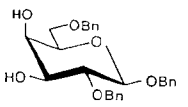

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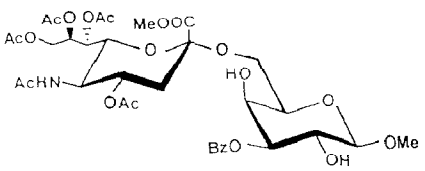
16% of the β-glycoside **8**, so that 64% of **1** was converted into the disaccharide derivatives **7** and **8** (54% of **5** was recovered). Lowering of the temperature of reaction to −25° did not affect the αβ-ratio. ^{1}H-N.m.r. spectra of **7** and **8** contained the expected signals (2 d) for HO-2,4, and the chemical shift data and *J* values for the Neu5Ac moiety are characteristic of α and β linkages[2,11], respectively.



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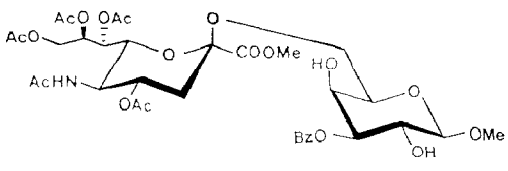

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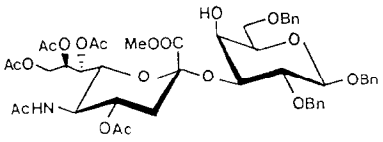
In order to compare directly the above glycosylation method with the thioglycoside procedure[7,12], **5** was treated with 0.5 equiv. of the ethyl 2-thio-α-glycoside **2**[8] under the prescribed conditions. The derivatives **7** and **8** were obtained in yields of 24 and 8% (based on **2**), respectively. Glycosylation of **5** with either **1** or **2** (1.3 equiv.) in acetonitrile for 14 h at −25° in the presence of DMTST (4 equiv.) gave **7** and **8** in yields of ~50 and 15%, respectively. Thus, under similar conditions, **1** appears to react more rapidly than **2**. Whether the α-selectivity observed by Hasegawa *et al.*[7] under similar conditions was due to the use of the methyl 2-thio-α-glycoside donor[12] or to the structure of the acceptor remains to be established.

Glycosylation of benzyl 2,6-di-*O*-benzyl-β-D-galactopyranoside[13] (**6**) with 0.5 equiv. of **1** in acetonitrile for 2 h at −15° in the presence of 1.0 equiv. of DMTST gave 26% (based on **1**) of the known[14] benzyl 2,6-di-*O*-benzyl-3-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α-D-*glycero*-D-*galacto*-2-nonulopyranosylonate)-β-D-galactopyranoside (**9**) and 4% of the known[14] β-anomer **10**; 78% of **6** was recovered. When this glycosylation was performed in dichloromethane, **10** was the only product isolated (25% based on xanthate **1**) and 76% of **6** was recovered. Although not obtained in pure form, the known lactone **11**[14] was identified as a product. In each of the glycosylations reported, the glycal derivative **4** was formed as major by-product.

In dichloromethane, the glycosylations probably involve a reactive anomeric oxycarbenium ion which is subject to axial attack to give **10** and a small amount of lactone **11**. In acetonitrile, the reaction may involve a β-nitrilium ion, which, presumably, is more reactive than the α isomer as a consequence of the anti-anomeric effect displayed by a positively charged leaving group. This remarkable α-directing effect of acetonitrile has already been observed[7,15].

The thiocarbonate **3**[8] and methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-*S*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-*galacto*-2-nonulopyranosonate[16] were not glycosylating agents in either acetonitrile or dichloromethane.

Crystalline **1** is a stable compound which can be prepared easily from



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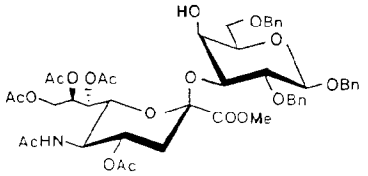

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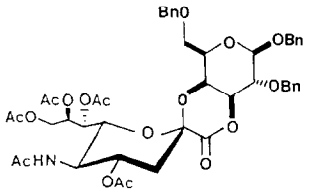

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Neu5Ac[8]. It is a rapid (1.5 h) and α-stereoselective glycosylating agent with DMTST in acetonitrile under kinetically controlled conditions. The yields (based on **1**) are among the best reported so far. The use of anomeric *S*-glycosyl xanthates as glycosylating agents is being studied further.

EXPERIMENTAL

General methods. — Melting points were determined with a Büchi Model 510 capillary apparatus and are uncorrected. Optical rotations were measured at 20 ±2° with a Perkin–Elmer Model 241 polarimeter. Elemental analyses were performed at the Service Central d'Analyse (C.N.R.S., Vernaison). ^{1}H-N.m.r. spectra were recorded for solutions in C_6D_6 (internal Me_4Si) with a Bruker AM-400 spectrometer. Reactions were monitored by t.l.c. on Silica Gel 60 F_{254} (Merck), using either ethyl acetate, 6:1 toluene–methanol, or 20:1 chloroform–methanol, with detection by charring with sulfuric acid. Flash column chromatography[17] was performed on Silica Gel 60 (230–400 mesh, Merck). All products isolated by column chromatography with toluene–methanol were eluted from a short column of silica gel with ethyl acetate in order to eliminate contaminating colloidal silica. Preparative t.l.c. was performed on Silica Gel 60 F_{254} (1-mm layer, Merck) with ethyl acetate as eluant.

Methyl 3-*O*-benzoyl-β-D-galactopyranoside (**5**) was prepared according to Das *et al.*[9]. Flash column chromatography (ethyl acetate) of the crude product gave **5** (15%), m.p. 120–121° (from ethyl acetate–hexane), $[\alpha]_D$ +57° (*c* 1, ethanol); lit.[9] m.p. 106–109°, $[\alpha]_D$ +56.7° (*c* 0.55, ethanol); lit.[18] m.p. 127–128°. The structure was confirmed by ^{1}H-n.m.r. analysis.

The xanthate **1**[8] had m.p. 102–104° (from benzene–hexane).

*Methyl 3-O-benzoyl-6-O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α- and -β-*D*-glycero-*D*-galacto-2-nonulopyranosylonate)-β-*D*-galactopyranoside* (**7** *and* **8**). — (*a*) To a mixture of **5** (90 mg, 0.3 mmol), dimethyl(methylthio)-sulfonium trifluoromethanesulfonate (150 mg, 0.3 mmol, of a 1:1 mixture of DMTST and 3 Å molecular sieve), activated 3 Å powdered molecular sieve (150 mg), and anhydrous acetonitrile (1 mL), stirred for 15 min at −15°, was added a solution of **1** (90 mg, 0.15 mmol) in anhydrous acetonitrile (1 mL) during 30 min. Stirring was continued for 60 min at −15°, di-isopropylamine (~0.2 mL) and dichloromethane were added, and the mixture was filtered through Celite and concentrated. Column chromatography (toluene–methanol, 30:1→15:1) of the residue gave, first, **8** (18.6 mg, 16% based on **1**), $[\alpha]_D$ −5° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 8.22–8.19 and 7.13–7.02 (2 m, 5 H, Ph), 6.02 (d, 1 H, $J_{5,NH}$ 9.8 Hz, NH), 5.97–5.92 (m, 2 H, H-7′,8′), 5.70 (ddd, 1 H, $J_{3'a,4'}$ 11.3, $J_{3'e,4'}$ 5.0, $J_{4',5'}$ 10.5 Hz, H-4′), 5.46 (dd, 1 H, $J_{2,3}$ 9.8, $J_{3,4}$ 3.3 Hz, H-3), 5.34 (m, 1 H, H-9′a), 4.80 (dd, 1 H, $J_{5',6'}$ 10.8, $J_{6',7'}$ 1.7 Hz, H-6′), 4.69 (ddd, 1 H, $J_{4,5}$ ~0.4, $J_{4,OH}$ 4.0 Hz, H-4), 4.53 (dd, 1 H, $J_{8',9'b}$ 7.7, $J_{9'a,9'b}$ 12.4 Hz, H-9′b), 4.39–4.33 (m, 2 H, H-5,5′), 4.29 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.19 (ddd, 1 H, $J_{2,OH}$ 4.0 Hz, H-2), 4.12 (d, 1 H, HO-4), 3.84 (dd, 1 H, $J_{5,6a}$ 8.4, $J_{6a,6b}$ 4.7 Hz, H-6a), 3.67 (dd, 1 H, $J_{5,6b}$ 8.7 Hz, H-6b), 3.35 and 3.33 (2 s, 6 H, 2 MeO), 2.78 (dd, 1 H, $J_{3'a,3'e}$ 13.0 Hz, H-3′*e*), 2.55 (d, 1 H, HO-2), 1.93, 1.91, 1.64, 1.61, and 1.45 (5 s, 15 H, 5 Ac), 1.81 (dd, 1 H, H-3′*a*).

Anal. Calc. for $C_{34}H_{45}NO_{19}$: C, 52.92; H, 5.88; N, 1.81. Found: C, 52.87; H, 5.92; N, 1.75.

Eluted second was a mixture of **7** and **5**, which was submitted to preparative t.l.c. to give **5** (49 mg, 54%) and **7** (56 mg, 48% based on **1**), $[\alpha]_D$ −7° (*c* 1, chloroform). ^{1}H-n.m.r. data: δ 8.27–8.22 and 7.13–7.02 (2 m, 5 H, Ph), 5.79 (ddd, 1 H, $J_{7',8'}$ 7.4, $J_{8',9'a}$ 2.6, $J_{8',9'b}$ 6.8 Hz, H-8′), 5.50 (dd, 1 H, $J_{6',7'}$ 2.3 Hz, H-7′), 5.31 (dd, 1 H, $J_{2,3}$ 10.0, $J_{3,4}$ 3.3 Hz, H-3), 4.82 (ddd, 1 H, $J_{3'a,4'}$ 12.2, $J_{3'e,4'}$ 4.6, $J_{4',5'}$ 10.4 Hz, H-4′), 4.75 (dd, 1 H, $J_{9'a,9'b}$ 12.4 Hz, H-9′a), 4.40 (ddd, 1 H, $J_{5',6'}$ 10.8, $J_{5,NH}$ 10.3 Hz, H-5′), 4.37 (ddd, 1 H, $J_{4,5}$ ~0.5, $J_{4,OH}$ 6.5 Hz, H-4), 4.30 (dd, 1 H, H-9′b), 4.24 (ddd, 1 H, $J_{1,2}$ 7.8, $J_{2,OH}$ 3.7 Hz, H-2), 4.22 (d, 1 H, NH), 4.17 (d, 1 H, H-1), 4.12 (dd, 1 H, H-6′), 4.03 (dd, 1 H, $J_{5,6a}$ 5.8, $J_{6a,6b}$ 10.0 Hz, H-6a), 3.99 (dd, 1 H, $J_{5,6b}$ 7.4 Hz, H-6b), 3.60 (ddd, 1 H, H-5), 3.40 and 3.35 (2 s, 6 H, 2 MeO), 2.91 (d, 1 H, HO-4), 2.66 (dd, 1 H, $J_{3'a,3'e}$ 12.8 Hz, H-3′*e*), 2.44 (d, 1 H, HO-2), 2.06, 2.03, 1.68, 1.61, and 1.59 (5 s, 15 H, 5 Ac), 1.99 (dd, 1 H, H-3′*a*).

Anal. Found: C, 52.66; H, 5.98; N, 1.71.

Repetition of the reaction with **2** as the glycosyl donor gave **8** (8% based on **2**) and **7** (24%). A mixture of **4** and **2** was also isolated and not separated further.

(*b*) A mixture of **1** (230 mg, 0.39 mmol), **5** (90 mg, 0.3 mmol), activated 3 Å powdered molecular sieve (300 mg), and anhydrous acetonitrile (3 mL) was stirred for 15 min at room temperature, then cooled to −30°. DMTST (800 mg, 1.56 mmol, of a 1:1 mixture of DMTST and 3 Å molecular sieve) was added and the suspension was stirred for 24 h at −15°. Di-isopropylamine (~0.5 mL) then dichloromethane were added, the mixture was filtered through Celite, and concen-

trated. Column chromatography (toluene–methanol, 40:1→10:1) of the residue gave, first, **8** (39 mg, 17% based on **5**) and then **7** (119 mg, 51%).

When the glycosylation was performed for 14 h at −25°, **7** and **8** were obtained in yields of 48 and 18%, respectively.

Repetition of the reaction (−25°, 14 h) with **2** as the glycosyl donor gave **7** and **8** in yields of 45 and 15%, respectively.

*Benzyl 2,6-di-*O*-benzyl-3-*O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-α- and -β-*D*-glycero-*D*-galacto-2-nonulopyranosylonate)-β-*D*-galacto-pyranoside (**9** and **10**).* — To a mixture of **6** (180 mg, 0.4 mmol), DMTST (200 mg, 0.4 mmol, of a 1:1 mixture of DMTST and 3 Å molecular sieve), activated 3 Å powdered molecular sieve (300 mg), and anhydrous acetonitrile (3 mL), stirred for 15 min at −15°, was added a solution of **1** (120 mg, 0.2 mmol) in anhydrous acetonitrile (1 mL) during 30 min. Stirring was continued for 1.5 h at −15°, di-isopropylamine (~0.3 mL) and dichloromethane were added, and the mixture was filtered through Celite and concentrated. Column chromatography (ethyl acetate–toluene, 3:2→3:1) of the residue gave, first, **6** (140 mg, 78%), then a ~1:3 mixture (10 mg) of **11** and an uncharacterized monosaccharide derivative (^{1}H-n.m.r. analysis), and finally a mixture of **4, 9,** and **10**. Column chromatography (toluene–methanol, 70:1→30:1) of the last mixture gave, first, **10** (7.4 mg, 4% based on **1**) and then **9** (48 mg, 26%), which were identical with the authentic compounds[14].

When the glycosylation was performed in anhydrous dichloromethane, **10** was obtained (25% based on **1**) together with a ~1:1 mixture (20 mg) of **11** and an uncharacterized monosaccharide derivative.

Repetition of the reaction with **2** as the glycosyl donor (acetonitrile as solvent) gave **9** and **10** in yields of 17 and 3% (based on **2**), respectively. A mixture of **4** and **2** was also isolated and not separated further.

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