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Note

Synthesis of the α-Neu5Ac-(2→6)-Gal structure. Facile 6-*O*-sialylation following stannylene activation of an unprotected D-galactopyranoside*†

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The α-Neu5Ac-(2→6)-Gal unit structure occurs[2] widely in the glycan parts of glycoproteins and neolacto-series glycolipids. With a view to investigating the biological significance of sialic acid-containing glycans, many attempts to synthesize such structures have been made[3–7].

We now describe the facile preparation of the α- and β-Neu5Ac-(2→6)-Gal disaccharides by coupling the chloride **1** with 2-(trimethylsilyl)ethyl β-D-galactopyranoside (**2**), activated by stannylation with dibutyltin oxide. We expected that this procedure would yield a glycoside of α-Neu5Ac-(2→3)-Gal, because the reaction of stannylene-activated galactosides with alkyl halides normally gives 3-*O*-alkyl derivatives in excellent yield[8–10]. Our observation of substitution at O-6 thus represents an altered regioselectivity, for which we have no explanation.

Compound **2** was prepared as fine crystals from 2-(trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside[11] by Zemplén deacetylation. Treatment[12] of **2** with dibutyltin oxide in methanol gave the corresponding dibutylstannylene derivative, which was coupled with methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate[13] (**1**) at 25–30° in the presence of tetrabutylammonium bromide and molecular sieves 4A in benzene. The resulting disaccharides were isolated by chromatography to give the α-glycoside **3**, β-glycoside **5**, and their mixture in 36%, 23%, and 4% yields, respectively, based on the acceptor **2**. The 2,3-dehydro derivative of acetylated sialic acid methyl ester (**7**), derived from glycosyl donor **1**, was obtained as a major by-product. The details are described in the Experimental section.

In the ^{1}H-n.m.r. spectra, signals for the C-3 equatorial proton and the C-4 axial proton of the Neu5Ac moiety appeared at δ 2.55 and 4.86, respectively, for **3**,

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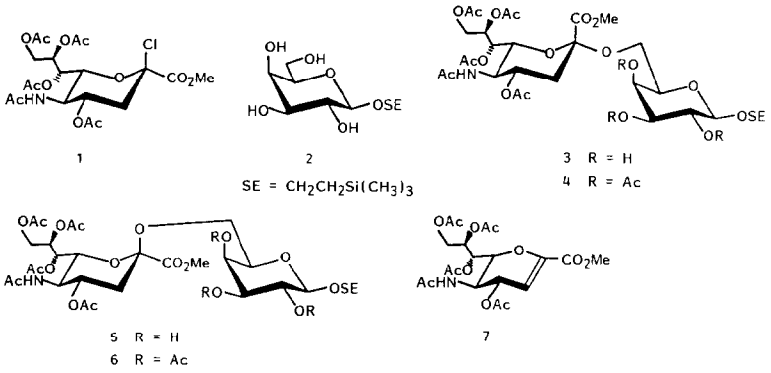
and at 2.44 and 5.16 for **5**, showing the patterns in chemical shifts characteristic for α- and β-ketosidic derivatives of Neu5Ac[2]. The C-2, C-3, and C-4 protons of the galactose moiety were confirmed by acetylation of the hydroxyl groups in **3** and **5**. The distinctive n.m.r. patterns of the sialic acid residue were maintained in the fully acetylated derivatives **4** and **6**.



In conclusion, the primary hydroxyl group of unprotected 2-(trimethylsilyl)ethyl β-D-galactopyranoside was readily sialylated by using the stannylene-activation method. This procedure may be useful for incorporation into short synthesis of Neu5Ac-(2→6)-Gal derivatives.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. T.l.c. was performed on Silica Gel 60 (Merck, aluminum sheets), and column chromatography on silica gel (Wako Co., 200 or 300 mesh) was accomplished with the solvent systems (v/v) specified. Evaporations were conducted *in vacuo*. Specific rotations were determined with a Union PM201 polarimeter, and i.r. spectra were recorded with a Jasco IRA-1 spectrophotometer. ^{1}H-N.m.r. spectra were recorded at 270 MHz with a JEOL JNM-270 spectrometer.

2-(Trimethylsilyl)ethyl β-D-galactopyranoside (**2**). — Tetra-*O*-acetyl-α-D-galactopyranosyl bromide was treated with 2-(trimethylsilyl)ethanol in the same manner as described[14] previously for the preparation of 2-(trimethylsilyl)ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-β-D-lactoside, to give 2-(trimethylsilyl)ethyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside in 79% yield, $[\alpha]_D$ −4.3° (*c* 0.5, dichloromethane); ^{1}H-n.m.r. data ($CDCl_3$): δ 4.46 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1). Zemplén deacetylation of this tetraacetate gave **2** (quantitative) as fine crystals from methanol–ether, m.p. 113–114°, $[\alpha]_D$ −16° (*c* 0.57, methanol); ^{1}H-n.m.r. data (CD_3OD): δ 0.98 (m, 2 H, CH_2SiMe_3), 3.28 (m, 1 H, H-5), 3.60, 3.97 (2 m, 2 H, $OCH_2CH_2SiMe_3$), 3.80 (m, 1 H, H-4), and 4.19 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1).

Anal. Calc. for $C_{11}H_{24}O_6Si$ (280.39): C, 47.12; H, 8.63. Found: C, 47.35; H, 8.51.

2-(Trimethylsilyl)ethyl O-[*methyl (5-acetamido-4,7,8,9-tetra*-O*-acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)*-β-D-*galactopyranoside* (**3**) *and 2-(trimethylsilyl)ethyl* O-[*methyl (5-acetamido-4,7,8,9-tetra*-O*-acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)*-β-D-*galactopyranoside* (**5**). — To a solution of **2** (0.56 g, 2.0 mmol) in dry methanol (25 mL) was added dibutyltin oxide (0.55 g, 2.2 mmol), and the mixture was heated for 2 h at reflux temperature. The solvent was removed by evaporation, and the residue was lyophilized from 1,4-dioxane solution to give the corresponding powdered stannylene derivative. To a solution of the stannylene derivative in benzene (15 mL) were added methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl 3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosyl chloride)onate (**1**; 1.5 g, 2.9 mmol), tetrabutylammonium bromide (0.65 g, 2.0 mmol), and molecular sieves 4A (1 g), and the mixture was stirred for 2 days at 25–30°, the course of the reaction being monitored by t.l.c. (5:1 dichloromethane-methanol or 5:1 toluene-ethanol). Compound **1** (0.54 g, 1.1 mmol) was further added, the mixture was stirred for another 2 days at 25–30°, and then filtered. The solid was washed with acetone, and the filtrate and washings were combined and evaporated. The residue was chromatographed on a column of silica gel with dichloromethane-methanol (100:1→30:1) to give **3** (0.55 g, 36%), **5** (0.34 g, 23%), and a mixture of **3** and **5** (60 mg, 4%); yields based on **2**. Also obtained was the 2,3-dehydro derivative **7** (1.2 g, 2.53 mmol).

Compound **3** had $[\alpha]_D$ −29° (*c* 0.7, chloroform); ν_{max} 3700–3100 (OH, NH), 1750, 1220 (ester), and 860 and 840 cm^{-1} (TMS ethyl); ^{1}H-n.m.r. data ($CDCl_3$): δ (aglycon) 0.97 (m, 2 H, CH_2SiMe); (Neu5Ac moiety) 1.86, 2.01, 2.02, 2.11, 2.13 (5 s, 15 H, $COCH_3$), 2.55 (dd, 1 H, J_{gem} 12.7, $J_{3eq,4}$ 4.7 Hz, H-3*eq*), 3.8 (s, 3 H, CO_2CH_3), 4.37 (dd, 1 H, J_{gem} 12.3, $J_{8,9}$ 2.2 Hz, H-9), 4.86 (m, 1 H, H-4), 5.31 (m, 2 H, H-7,8), and 5.44 (d, 1 H, $J_{5,NH}$ 9.2 Hz, N*H*).

Anal. Calc. for $C_{31}H_{51}NO_{18}Si$ (753.83): C, 49.39; H, 6.82; N, 1.86. Found: C, 49.58; H, 6.69; N, 1.90.

Compound **5** had $[\alpha]_D$ −27° (*c* 0.7, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ (aglycon) 0.96 (m, 2 H, CH_2SiMe_3); (Neu5Ac moiety) 1.88, 2.00, 2.04, 2.09, 2.12 (5 s, 15 H, $COCH_3$), 2.44 (dd, 1 H, J_{gem} 12.8, $J_{3eq,4}$ 4.8 Hz, H-3*eq*), 3.80 (s, 3 H, CO_2CH_3), 5.16 (m, 1 H, $J_{3ax,4} = J_{4,5}$ 10.8, $J_{3eq,4}$ 4.8 Hz, H-4), 5.25 (m, 1 H, H-8), and 5.53 (m, 1 H, H-7).

Anal. Found: C, 49.60; H, 6.80; N, 1.82.

2-(Trimethylsilyl)ethyl O-[*methyl (5-acetamido-4,7,8,9-tetra*-O*-acetyl-3,5-dideoxy*-D-glycero-α-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-2,3,4-tri*-O*-acetyl*-β-D-*galactopyranoside* (**4**) *and 2-(trimethylsilyl)ethyl* O-[*methyl (5-acetamido-4,7,8,9-tetra*-O*-acetyl-3,5-dideoxy*-D-glycero-β-D-galacto-*2-nonulopyranosyl)onate*]-*(2→6)-2,3,4-tri*-O*-acetyl*-β-D-*galactopyranoside* (**6**). — Compounds **3** and **5** were acetylated with acetic anhydride in pyridine. After extractive processing, the products were purified by chromatography on a column of silica gel (elution with 80:1 di-

chloromethane–methanol) to afford **4** and **6**, respectively in quantitative yields.

Compound **4** had $[\alpha]_D$ −30° (*c* 0.8, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ (aglycon) 0.94 (m, 2 H, CH_2SiMe_3), 3.55 (m, 1 H, $OCH_2CH_2SiMe_3$); (Gal moiety) 4.53 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 5.03 (dd, 1 H, $J_{2,3}$ 10.4, $J_{3,4}$ 3.5 Hz, H-3), 5.17 (dd, 1 H, H-2), and 5.43 (d, 1 H, H-4); (Neu5Ac moiety) 2.52 (dd, 1 H, J_{gem} 12.9, $J_{3eq,4}$ 4.6 Hz, H-3*eq*), 3.78 (s, 3 H, CO_2CH_3), 4.31 (dd, 1 H, J_{gem} 12.5, $J_{8,9}$ 8.9 Hz, H-9), 4.83 (m, 1 H, H-4), 5.27 (dd, 1 H, $J_{6,7}$ 1.9, $J_{7,8}$ 8.9 Hz, H-7), and 5.34 (m, 1 H, H-8); 1.87, 1.96, 2.01, 2.02, 2.04, 2.11, 2.13, and 2.17 (8 s, 24 H, $COCH_3$).

Anal. Calc. for $C_{37}H_{57}NO_2Si$ (879.94): C, 50.50; H, 6.53; N, 1.59. Found: C, 50.34; H, 6.44; N, 1.53.

Compound **6** had $[\alpha]_D$ −24° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ (aglycon) 0.91 (m, 2 H, CH_2SiMe_3), 3.96 (m, 1 H, $OCH_2CH_2SiMe_3$); (Gal moiety) 4.49 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 5.07 (dd, 1 H, $J_{2,3}$ 10.3, $J_{3,4}$ 3.1 Hz, H-3), 5.18 (dd, 1 H, H-2), and 5.58 (d, 1 H, *J* 3 Hz, H-4); (Neu5Ac moiety) 2.43 (dd, 1 H, J_{gem} 12.7, $J_{3eq,4}$ 5.0 Hz, H-3*eq*), 3.81 (s, 3 H, CO_2CH_3), 4.02 (dd, 1 H, J_{gem} 12.3, $J_{8,9a}$ 7.3 Hz, H-9a), 4.14 (q, 1 H, $J_{4,5} = J_{5,6} = J_{5,NH}$ 10.3 Hz, H-5), 4.69 (dd, 1 H, J_{gem} 12.3, $J_{8,9b}$ 2.4 Hz, H-9b), 5.07 (m, 1 H, H-8), 5.19 (m, 1 H, H-4), 5.33 (dd, 1 H, $J_{6,7}$ 2.4, $J_{7,8}$ 4.6 Hz, H-7), and 5.71 (d, 1 H, N*H*); 1.88 (s, 3 H, $NCOCH_3$), 1.99 (s, 6 H, $OCOCH_3$), 2.02, 2.04, 2.07, 2.14, and 2.30 (5 s, 15 H, $OCOCH_3$).

Anal. Found: C, 50.28; H, 6.48; N, 1.65.

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