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Short Communication

Synthesis of Methyl and Allyl α-Glycosides of *N*-Acetylneuraminic Acid in the Absence of Added Promoter†

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Dedicated to Professor Lennart Eberson on the occasion of his 65th birthday

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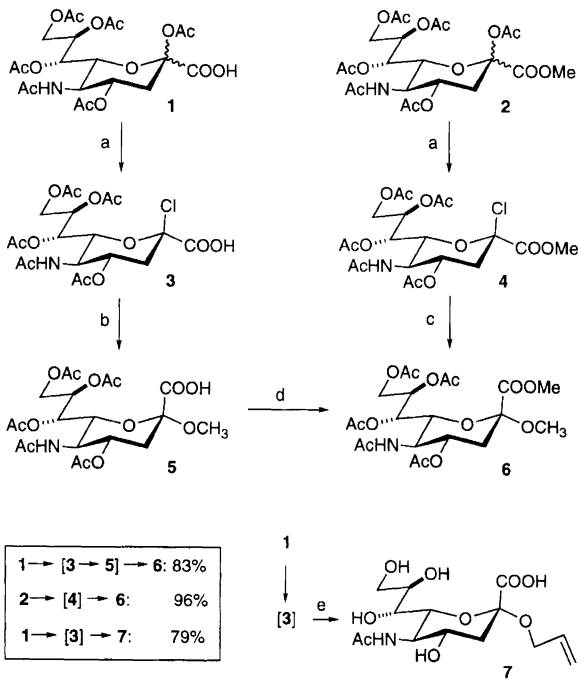
Sialic acids, especially *N*-acetylneuraminic acid, are present (as α-glycosides) on biologically important glycolipids and glycoproteins. Synthesis of sialic acid-containing oligosaccharides is therefore a prerequisite for many glycobiological studies. Such syntheses often use simple sialyl glycosides as starting materials. Their preparation usually employs heavy metal salts or other toxic and/or expensive promoters.[1] As a consequence, only few of these methods are suitable for large-scale preparations.

We wish to report a simple, α/β-selective, and high-yielding preparative procedure for the methyl sialosides **5** and **6**, and the allyl sialoside **7** (Scheme 1), that avoids the use of specially added promoter (although HCl liberated during the reaction probably promotes the reaction). A few examples of 'non-catalyzed' glycosylations with acetobromohexosides, leading to de-*O*-acetylated methyl glycosides, have been reported.[2] Furthermore, various bromo analogs of **4** were recently used for sialylation of methanol and benzyl alcohol in the absence of metal salt promoters (but in the presence of collidinium salt) to give glycosides in 70–95% yield, with an α/β ratio of 1:1–9:1.[3]

The known *O*-acetylated *N*-acetylneuraminic acid[4] (**1**) and methyl ester[5,6] (**2**) were treated separately with HCl/CH_2Cl_2 to give acetochloroneuraminic acid[4] (**3**) and methyl ester[5] (**4**) of 90–95% purity according to ^1H NMR. Compounds **3** and **4** were each dissolved in methanol and the mixtures were kept at room temperature. According to TLC analysis, compound **3** was consumed within 1 min, whereas compound **4** required a reaction time of 1 h. Three different batches of methanol were investigated, with virtually identical results.



Scheme 1. (a) HCl, CH_2Cl_2, 4 °C, 18 h. (b) MeOH, 23 °C, < 1 min. (c) MeOH, 23 °C, 1 h. (d) Me_3SiCHN_2, benzene–MeOH 4:1, 23 °C, 1 h. (e) $^{i}CH_2CHCH_2OH$, 23 °C, 4 h, ^{ii}aq. NaOH (0.2 M), 23 °C, 1 h, ^{iii}Amberlite IR-120 (NH_4-form).

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Removal of solvent from the two reaction mixtures gave the crude sialic acid **5**[7] and ester **6**[5,7,8] in practically pure form. The α/β ratio (determined by integration of the H3eq NMR signals) was in both cases ca. 30:1, which was unexpectedly high and indicates that the displacement of chloride ion by methanol proceeds via an S_N2 reaction. The sialic acid **5** was transformed into the ester **6** by treatment with trimethylsilyldiazomethane. Both batches of **6** were purified by column chromatography. Thus, pure **6** was obtained either via the route **1**→**3**→**5**→**6** or **2**→**4**→**6** in 83 or 96% overall yield, respectively; the α/β ratio was in both cases ca. 30:1. It is essential for the outcome of the sialylation reactions to keep within the reaction times given; prolonged reaction causes significant deacetylation, anomerization, and finally esterification of the carboxyl group (in **5**). The stereochemical assignments of **5**–**7** were based on the $J_{C1-H3ax}$ coupling constants.[9]

Treatment of **3** with allyl alcohol gave a much slower sialylation than with methanol. After 4 h, the solvent was removed to leave a crude material, mainly consisting of the allyl counterpart of methyl glycoside **5**. An S_N2-type reaction at the crowded anomeric position of **3** is consistent with the difference in reaction time between methanol and the more sterically demanding allyl alcohol. The crude material was *O*-deacetylated and purified by ion-exchange chromatography to give known[10] **7** (79%) as the pure ammonium salt. The α/β ratio was also in this case ca. 30:1. Allyl glycosides, including allyl sialosides, have been used in the synthesis of various neoglycoconjugates.[10–14]

In summary, the sialylation procedure described here is simple and efficient, both in terms of yield and stereoselectivity, and suitable for large-scale synthesis. It seems to be limited to simple alcohols that can be used as solvent. Attempted sialylations using a limited amount of alcohol in an inert solvent resulted in longer reaction times, lower yields, and poor stereoselectivity.

Experimental

NMR spectra were recorded at 23 °C with a Varian XL-300 spectrometer. Signal assignments were confirmed by 2D ^1H–^1H and ^1H–^{13}C correlation experiments. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. TLC analysis was performed with Merck F_{254} silica gel precoated aluminium sheets, and spots were visualized with UV light and by charring with 10% H_3PO_4 in 95% ethanol. Visualization of compound **7** was also performed with 5% $KMnO_4$ in 2% aqueous $NaHCO_3$. Column chromatography was performed on Matrex 60 (35–70 μm) silica gel (Grace). Methanol was p.a. or HPLC grade (J. T. Baker B. V., Deventer, Holland; Catalog no. 8045 and 8402, respectively). Allyl alcohol was distilled from CaO and stored over 4 Å molecular sieves. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. Compounds **1** and **2** were prepared as described in Refs. 4–6.

5-Acetamido-4,7,8,9-tetra-O-*acetyl-2-chloro-2,3,5-trideoxy*-D-*glycero*-β-D-*galacto*-2-*nonulopyranosonic acid* (**3**). HCl (g) was bubbled for 1 h into a cooled (4 °C) solution of compound **1** (58.0 mg, 0.113 mmol) in CH_2Cl_2 (10 mL). The mixture was kept at 4 °C for 18 h, the solvent was removed at room temperature and the residue was co-concentrated three times with CCl_4 to give crude **3** (59.8 mg). The relative mobility of **3** and **1** on TLC (*n*-BuOH/*n*-PrOH/0.1 M HCl, 1:2:1) was 1.19 (lit:[4] 1.17). ^1H NMR (acetone-d_6): δ 5.50 (dd, 1 H, *J* 7.9, 2.4 Hz, H-7), 5.37 (ddd, 1 H, *J* 14.0, 10.7, 4.8 Hz, H-4), 5.21 (ddd, 1 H, *J* 7.9, 5.9, 2.9 Hz, H-8), 4.54 (dd, 1 H, *J* 10.7, 2.4 Hz, H-6), 4.32 (dd, 1 H, *J* 12.4, 2.9 Hz, H-9′), 4.21 (t, 1 H, *J* 10.7 Hz, H-5), 4.07 (dd, 1 H, *J* 12.4, 5.9 Hz, H-9), 2.81 (dd, 1 H, *J* 14.0, 4.8 Hz, H-3_{eq}), 2.26 (t, 1 H, *J* 14.0 Hz, H-3_{ax}), 2.06, 2.03, 1.99, 1.97 (4 s, 3 H each, AcO), 1.84 (s, 3 H, AcN). ^{13}C NMR (acetone-d_6): δ 171.3, 170.6, 170.5, 170.2, 169.8, 166.3 (C-1), 98.4 (C-2), 73.4 (C-6), 69.8 (C-8), 69.6 (C-4), 67.7 (C-7), 62.6 (C-9), 49.0 (C-5), 41.4 (C-3), 22.7 (AcNH), 20.9, 20.8, 20.7, 20.6.

Methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-2-chloro-2,3,5-trideoxy*-D-*glycero*-β-D-*galacto*-2-*nonulopyranosonate* (**4**). Compound **2** (16.14 g, 30.25 mmol) was dissolved in CH_2Cl_2 (300 mL), and the mixture was treated as in the preparation of **3** to give **4** (15.9 g). The relative mobility of **4** and **2** on TLC (EtOAc) was 1.80. ^1H NMR (acetone-d_6): δ 7.15 (d, 1 H, *J* 9.6 Hz, NH), 5.50 (dd, 1 H, *J* 7.6, 2.4 Hz, H-7), 5.35 (ddd, 1 H, *J* 11.1, 10.4, 4.9 Hz, H-4), 5.15 (ddd, 1 H, *J* 7.6, 5.9, 3.0 Hz, H-8), 4.52 (dd, 1 H, *J* 10.8, 2.4 Hz, H-6), 4.35 (dd, 1 H, *J* 12.4, 3.0 Hz, H-9′), 4.21 (q, 1 H, *J* 10.4 Hz, H-5), 4.04 (dd, 1 H, *J* 12.4, 5.9 Hz, H-9), 3.85 (s, 3 H, OMe), 2.81 (dd, 1 H, *J* 13.9, 4.9 Hz, H-3_{eq}), 2.27 (dd, 1 H, *J* 13.9, 11.1 Hz, H-3_{ax}), 2.06, 2.01, 2.00 (3 s, 12 H, AcO), 1.82 (s, 3 H, AcN). ^{13}C NMR (acetone-d_6): δ 170.6, 170.5, 170.4, 170.1, 169.5, 166.2 (C-1), 98.3 (C-2), 74.6 (C-6), 70.0 (C-8), 69.5 (C-4), 67.7 (C-7), 62.6 (C-9), 53.9 (OMe), 48.8 (C-5), 41.4 (C-3), 22.9 (AcNH), 20.8, 20.7, 20.5. For NMR data in $CDCl_3$, see Ref. 15.

Methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-*glycero*-α-D-*galacto*-2-*nonulopyranosonic acid* (**5**). Compound **3** (59.8 mg, 0.113 mmol) was dissolved in MeOH (5 mL) at room temperature, which gave **5** in <1 min, according to TLC (SiO_2, BuOH/PrOH/0.1 M HCl, 1:2:1). The solvent was removed and the residue was co-concentrated with CCl_4 (3 × 5 mL) to give crude **5** (57.6 mg, α/β ~30:1). $[\alpha]_D$ −16.5° (*c* 1.0, MeOH) {lit.:[7] $[\alpha]_D$ −16.5° (MeOH)}. The relative mobility of **5** and **1** on TLC (BuOH/PrOH/0.1 M HCl, 1:2:1) was 1.30 (lit:[4] 1.29). ^1H NMR (CD_3OD): δ 5.40 (ddd, 1 H, *J* 9.1, 5.1, 2.6 Hz, H-8), 5.33 (dd, 1 H, *J* 9.1, 2.1 Hz, H-7), 4.89 (m, 1 H, H-4), 4.30 (dd, 1 H, *J* 12.4, 2.6 Hz, H-9′), 4.23 (dd, 1 H, *J* 10.8, 2.1 Hz, H-6), 4.07 (dd, 1 H, *J* 12.4, 5.1 Hz, H-9), 3.94 (dd, 1 H, *J* 10.8, 10.5 Hz, H-5), 3.33 (s, 3 H, OMe), 2.61 (dd, 1 H, *J* 12.5, 4.7 Hz, H-3_{eq}),

2.10, 2.00, 1.98 (3 s, 12 H, AcO), 1.83 (s, 3 H, AcN), 1.75 (t, 1 H, J 12.5 Hz, H-3_{ax}). ^{13}C NMR (CD_3OD): δ 173.5, 172.4, 170.8, 171.7, 171.6, 170.2 (C-1), 100.1 (C-2), 73.0 (C-6), 71.0 (C-4), 69.5 (C-8), 68.7 (C-7), 63.5 (C-9), 52.6 (OMe), 50.2 (C-5), 39.1 (C-3), 22.7 (AcNH), 21.2, 20.8, 20.7, 20.6. ^{13}C NMR ($CDCl_3$): δ 171.4, 171.3, 170.9, 170.7, 169.4 (C-1, $J_{C1,H3ax}$ 6.5 Hz)[9], 98.9 (C-2), 72.6 (C-6), 69.8 (C-4), 69.7 (C-8), 67.9 (C-7), 62.6 (C-9), 52.3 (OMe), 49.1 (C-5), 37.3 (C-3), 23.0 (AcNH), 21.1, 21.0, 20.8.

Methyl (methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-3,5-dideoxy*-D-*glycero*-α-D-*galacto-2-nonulopyranosid)onate* (**6**). (a) Compound **4** (61.1 mg, 0.120 mmol) was dissolved in MeOH (5 mL) at room temperature, which gave **6** in 1 h, according to TLC (SiO_2, EtOAc). The solvent was removed and the residue was co-concentrated with CCl_4 (3 × 5 mL) to give crude **6** (64.9 mg, α/β ~30:1), which was then treated with acetic anhydride–pyridine (2 mL, 1:1) for 18 h. The solvent was removed and the residue was co-concentrated with toluene and chromatographed (SiO_2, EtOAc–heptane 95:5) to give pure **6** (58.5 mg, 96%, α/β ~30:1).

(b) Crude **5** (57.6 mg, 0.12 mmol) was dissolved in a mixture of benzene and MeOH (10 mL, 4:1) and trimethylsilyldiazomethane (0.26 mmol; 0.13 mL of a 2 M solution in hexane) was added. The mixture was kept at room temperature for 1 h, the solvent was removed, and the residue was treated with acetic anhydride–pyridine (2 mL, 1:1) as above. The solvent was removed and the residue was purified as above to give pure **6** (47.2 mg, 83%, α/β ~30:1). $[\alpha]_D$ −19.0° (*c* 4.0, MeOH) {lit:[5] $[\alpha]_D$ −18° (*c* 4.0, MeOH), lit:[7] $[\alpha]_D$ −19° (MeOH), lit:[8] $[\alpha]_D$ −5.0° (*c* 4.0, MeOH)}. The relative mobility of **6** and **2** on TLC (EtOAc) was 1.43. ^{1}H NMR ($CDCl_3$): δ 5.43 (ddd, 1 H, J 8.5, 5.5, 2.7 Hz, H-8), 5.33 (dd, 1 H, J 8.5, 2.0 Hz, H-7), 5.14 (d, 1 H, J 9.3 Hz, NH), 4.85 (ddd, 1 H, J 12.9, 9.8, 4.6 Hz, H-4), 4.31 (dd, 1 H, J 12.4, 2.7 Hz, H-9′), 4.11 (m, 1 H, H-6), 4.13 (m, 1 H, H-9), 4.08 (m, 1 H, H-5), 3.81 (s, 3 H, COOMe), 3.32 (s, 3 H, OMe), 2.56 (dd, 1 H, J 12.9, 4.6 Hz, H-3_{eq}), 2.16, 2.14, 2.04, 2.03 (4 s, 3 H each, AcO), 1.94 (t, 1 H, J 12.9 Hz, H-3_{ax}), 1.88 (s, 3 H, AcN). ^{13}C NMR ($CDCl_3$): δ 171.0, 170.7, 170.3, 170.14, 170.09, 168.2 (C-1, $J_{C1,H3ax}$ 5.7 Hz),[9] 99.0 (C-2), 72.5 (C-6), 69.1 (C-4), 68.6 (C-8), 67.4 (C-7), 62.4 (C-9), 52.7 ($COOCH_3$), 52.4 (OMe), 49.4 (C-5), 37.8 (C-3), 23.1 (AcNH), 21.1, 20.83, 20.80, 20.7. For previously reported NMR data in $CDCl_3$, see Refs. 8, 16 and 17.

Allyl 5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto-2-nonulopyranosonic acid* (**7**). Crude **3** (52.4 mg, 0.103 mmol) was dissolved in dry allyl alcohol (1.5 mL) at room temperature. After 4 h, the mixture was concentrated and co-concentrated with CCl_4 (4 × 5 mL) and the residue was kept under vacuum for 1 h to give a material (56.4 mg) that consisted mainly (~85% yield) of the tetra-*O*-acetyl analog of **7**, according to NMR analysis. The crude material was dissolved in MeOH (4 mL), the mixture was cooled (ice-water bath), and cold aqueous NaOH (1 mL, 1 M) was added. The mixture was stirred at 4 °C for 1 h and then neutralized by addition of Amberlite IR-120 resin (NH_4-form). The resin was removed and washed with water. The combined aqueous solutions were freeze-dried and the residue was purified by ion-exchange chromatography (DEAE-Sepharose CL-6B, AcO^--form; column size: 150 × 16 mm; aqueous NH_4OAc gradient 0.001 → 0.015 M, then isocratic elution with 0.015 M NH_4OAc). Appropriate fractions were pooled and freeze-dried to the give pure NH_4 salt of **7** (29 mg, 79% overall yield from **1**); $[\alpha]_D$ −1.6° (*c* 0.7, H_2O) {lit:[10] for the Na salt: $[\alpha]_D$ −9.1° (H_2O). ^{1}H NMR (D_2O): δ 5.90 (dddd, 1 H, J 17.0, 10.4, 6.2, 6.0 Hz, vinyl-H), 5.29 (dd, 1 H, J 17.0, 1,4 Hz, vinyl-H), 5.19 (dd, 1 H, J 10.4, 0.7 Hz, vinyl-H), 4.20 (dd, 1 H, J 12.0, 6.2 Hz, allyl-CH_2), 3.98 (dd, 1 H, J 12.0, 6.0 Hz, Allyl-CH_2), 2.71 (dd, 1 H, J 12.5, 4.5 Hz, H-3_{eq}), 1.61 (t, 1 H, J 12.0 Hz, H-3_{ax}). ^{13}C NMR (D_2O): δ 175.8 (CONH), 174.2 (C-1), 134.5 (CH_2=CH), 119.0 (CH_2=CH), 101.3 (C-2), 73.4 (C-6), 72.4 (C-8), 68.9 (C-4,7), 66.7 (CH_2–CH=), 63.3 (C-9), 52.6 (C-5), 41.2 (C-3), 22.7 (AcNH).

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