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 Ebersson on the occasion of his 65th birthday


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 acid4 (1) and methyl ester5,6 (2) were treated separately with HCl/CH₂Cl₂ to give acetochloroneuraminic acid4 (3) and methyl ester6 (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.


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Removal of solvent from the two reaction mixtures gave the crude sialic acid 5 and ester 6 in practically pure form. The \(\alpha/\beta\) 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 SN2 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 \(\rightarrow\) 3 \(\rightarrow\) 5 \(\rightarrow\) 6 or 2 \(\rightarrow\) 4 \(\rightarrow\) 6 in 83 or 96% overall yield, respectively; the \(\alpha/\beta\) 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 decetylation, anomerization, and finally esterification of the carboxyl group in 5. The stereochemical assignments of 5–7 were based on the J\(_{\text{1,2H}}\) 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 alyl counterpart of methyl glycoside 5. An SN2-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 7 (79%) as the pure ammonium salt. The \(\alpha/\beta\) 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–13C correlation experiments. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. TLC analysis was performed with Merck F254 silica gel precoated aluminium sheets, and spots were visualized with UV light and by charring with 10% \(\text{H}_2\text{PO}_4\) in 95% ethanol. Visualization of compound 7 was also performed with 5% KMnO\(_4\) in 2% aqueous NaHCO\(_3\). Column chromatography was performed on Matrix 30 (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-\(\beta\)-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 \(\text{CH}_2\text{Cl}_2\) (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-ButOH/n-PrOH/0.1 M HCl, 1:2:1) was 1.19 (lit.\(^4\) 1.17).\(^{11}\) 1H NMR (acetone-\(d_6\)): β 5.50 (dd, 1H, J 7.9, 2.4 Hz, H-7), 5.37 (ddd, 1H, J 14.0, 10.7, 4.8 Hz, H-4), 5.21 (ddd, 1H, J 7.9, 5.9, 2.9 Hz, H-8), 4.54 (dd, 1H, J 10.7, 2.4 Hz, H-6), 4.32 (dd, 1H, J 12.4, 2.9 Hz, H-9), 4.21 (t, 1H, J 10.7 Hz, H-5), 4.07 (ddd, 1H, J 12.4, 5.9 Hz, H-9), 2.81 (dd, 1H, J 14.0, 4.8 Hz, H-3\(_{\alpha}\)), 2.26 (t, 1H, J 14.0 Hz, H-3\(_{\alpha}\)), 2.06, 2.03, 1.99, 1.97 (4s, 3H each, AcO). 15C 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-\(\beta\)-d-galacto-2-nonulopyranosonate (4). Compound 2 (16.14 g, 30.25 mmol) was dissolved in \(\text{CH}_2\text{Cl}_2\) (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.\(^{12}\) 1H NMR (acetone-\(d_6\)): δ 7.15 (d, 1H, J 9.6 Hz, NH), 5.50 (dd, 1H, J 1.7, 2.4 Hz, H-7), 5.35 (ddd, 1H, J 11.1, 10.4, 4.9 Hz, H-4), 5.15 (ddd, 1H, J 11.7, 5.9, 3.0 Hz, H-8), 4.52 (dd, 1H, J 10.8, 2.4 Hz, H-6), 4.35 (dd, 1H, J 12.4, 3.0 Hz, H-9), 4.21 (q, 1H, J 10.4 Hz, H-5), 4.04 (dd, 1H, J 12.4, 5.9 Hz, H-9), 3.85 (s, 3H, OMe), 2.81 (dd, 1H, J 13.9, 4.9 Hz, H-3\(_{\alpha}\)), 2.27 (dd, 1H, J 13.9, 11.1 Hz, H-3\(_{\alpha}\)), 2.06, 2.01, 2.00 (3s, 12H, AcO), 1.82 (s, 3H, AcN).\(^{13}\) 13C 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-\(\beta\)-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 x 5 mL) to give crude 5 (57.6 mg, \(\alpha/\beta\) ~30:1). [\(\alpha\)\(_{\text{D}}\) +16.5° (c 1.0, MeOH) [lit.\(^2\) [\(\alpha\)\(_{\text{D}}\) +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).\(^{14}\) 1H NMR (CD\(_3\)OD): δ 5.40 (ddd, 1H, J 9.1, 5.1, 2.6 Hz, H-8), 5.33 (ddd, 1H, J 9.1, 2.1 Hz, H-7), 4.89 (m, 1H, H-4), 4.30 (dd, 1H, J 12.4, 2.6 Hz, H-9), 4.23 (dd, 1H, J 10.8, 2.1 Hz, H-6), 4.07 (dd, 1H, J 12.4, 5.1 Hz, H-9), 3.94 (dd, 1H, J 10.8, 10.5 Hz, H-5), 3.33 (s, 3H, OMe), 2.61 (dd, 1H, J 12.5, 4.7 Hz, H-3\(_{\alpha}\)).
Methyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-D-D-galacto-2-nonulopyranosiduronate (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 (SiO₂, EtOAc). The solvent was removed and the residue was co-concentrated with CCl₄ (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 (SiO₂, 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). [α]D -19.0° (c 4.0, MeOH) [lit.5 [α]D -18° (c 4.0, MeOH) lit.7 [α]D -19° (MeOH)] lit.8 [α]D -5.0° (c 4.0, MeOH)). The relative mobility of 6 and 2 on TLC (EtOAc) was 1.43. 1H NMR (CDCl₃): δ 4.53 (ddd, 1 H, J 8.5, 5.5, 2.7 Hz, H-8), 5.33 (ddd, 1 H, J 8.5, 2.0 Hz, H-7), 5.14 (dd, 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-3eq), 2.16, 2.14, 2.04, 2.03 (4 s, 3 H each, AcO), 1.94 (t, 1 H, J 12.9 Hz, H-3ax), 1.88 (s, 3 H, AcN). 13C NMR (CDCl₃): δ 171.0, 170.7, 170.14. 19.07, 5.7 Hz, 9.90 (C-2), 72.5 (C-6), 69.1 (C-4), 68.6 (C-8), 67.4 (C-7), 62.4 (C-9), 52.7 (COOCCH₃), 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 CDCl₃, see Refs. 8, 16 and 17.

Allyl 5-acetamido-3,5-dideoxy-D-glycero-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 CCl₄ (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 (NH₄-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 NaH₂OAc gradient 0.001 → 0.015 M, then isotropic elution with 0.015 M NaH₂OAc). Appropriate fractions were pooled and freeze-dried to give pure NH₄ salt of 7 (29 mg, 79% overall yield from 1); [α]D -1.6° (c 0.7, H₂O) [lit.10 for the Na salt: [α]D -9.1° (H₂O)]. 1H NMR (D₂O): δ 8.90 (dd, 1 H, J 17.5, 10.4, 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-CH₂), 3.98 (dd, 1 H, J 12.0, 6.0 Hz, Allyl-CH₂), 2.71 (dd, 1 H, J 12.5, 4.5 Hz, H-3eq), 1.61 (t, 1 H, J 12.0 Hz, H-3ax). 13C NMR (D₂O): δ 175.8 (CONH), 174.2 (C-1), 134.5 (CH₂-CH), 119.0 (CH=CH), 101.3 (C-2), 73.4 (C-6), 72.4 (C-8), 68.9 (C-4), 66.7 (CH₂-CH=), 63.3 (C-9), 52.6 (C-5), 41.2 (C-3), 22.7 (AcNH).

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