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Synthesis of sialic acid-containing nucleotide sugars: CMP-sialic acid analogs

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ABSTRACT

Syntheses of some sialic acid-containing nucleotide sugars are reported. The reaction of methyl [(2-hydroxy)ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid]onate (**4**) with various fully protected hydrogen phosphonates of nucleosides (**5a–c**) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), gave, after oxidation and deprotection, the corresponding sialic acid-containing nucleotide sugar analogs (**8a–c**).

INTRODUCTION

N-Acetyl-D-neuraminic acid (Neu5Ac) and various analogs, the sialic acids present as terminal units of many oligosaccharide sequences in glycoproteins and glycolipids, play essential roles in a variety of biochemical and biological processes[1]. For example, cell-surface sialic acid residues may serve as antigens, receptors of viruses, toxins and hormones, and as mediators of cell-growth control[2]. Synthetic sialyl-glycoconjugates are therefore of interest as substrates and inhibitors for sialidases or sialyl transferases, and potential modifiers of cell-surface sialic acid. A few analogs of nucleoside–sialic acid conjugates have been synthesized[3]. As part of synthetic studies[4] on modified bacterial cell-wall components, we report herein syntheses of some sialic acid-containing nucleotide sugars: synthetic analogs of the naturally occurring cytidine 5′ (neuraminyl monophosphate) acid (CMP-Neu5Ac[5]). CMP-Neu5Ac is a substrate of CMP-sialate synthase and a key intermediate in the biosynthesis of glycoconjugates.

RESULTS AND DISCUSSION

The sialic acid unit was first prepared (Chart I). Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,5-dideoxy-3-*S*-phenyl-3-thio-D-*erythro*-β-L-*gluco*-2-nonulopyranosonate[6] (**1a**), and the corresponding glycosyl bromide (**1b**), prepared from **1a** in high yield), are well suited for stereoselective synthesis of α-glycosides of Neu5Ac. To avoid the instability of a phosphate ester at the anomeric position of CMP-Neu5Ac, we added

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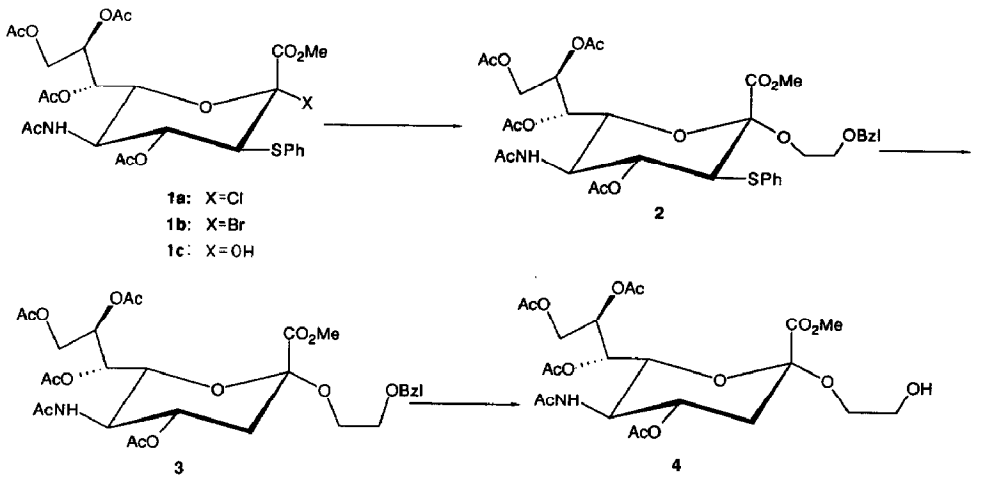
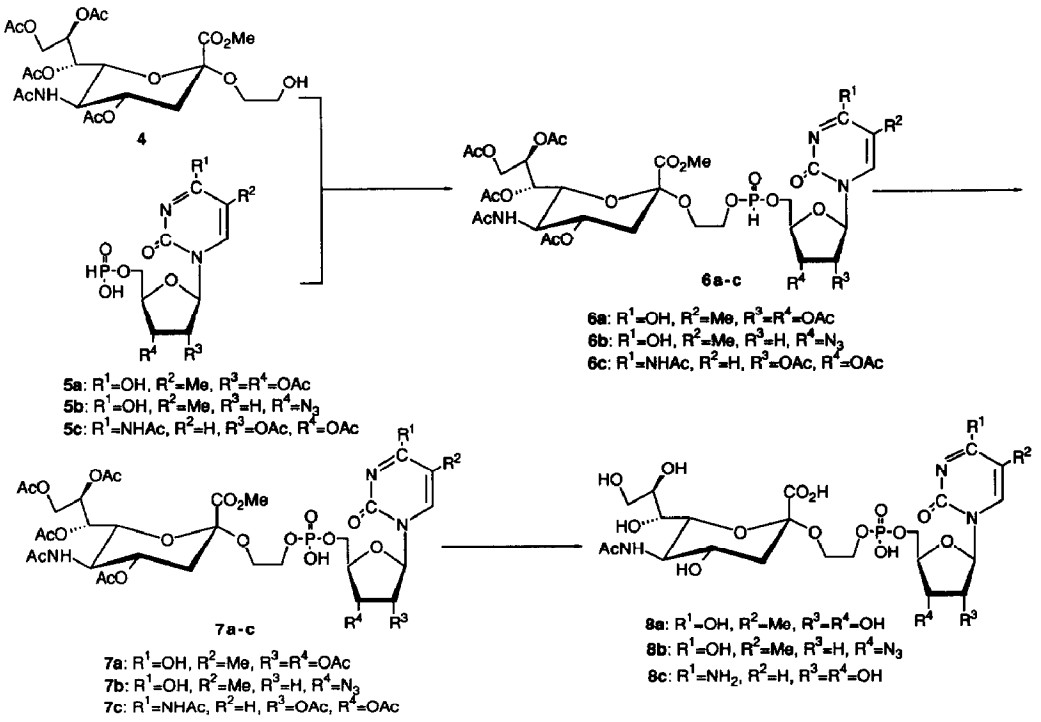


a short linker arm to the sialic acid moiety. 2-(Benzyloxy)ethanol reacted with **1a** and **1b** in the presence of silver triflate, disodium hydrogen phosphate, and activated 4A molecular sieves in 1,2-dichloroethane to give **2** in 67 and 52% yields, respectively. The configuration of the anomeric position of **2** was deduced to be β-L by the $J_{7,8}$ coupling constant (8.0 Hz) and the $\Delta\ \delta$ (H-9a–H-9b)[7] value (0.28 p.p.m.) in the ^{1}H-n.m.r. spectrum. The phenylthio group of **2** was readily removed by reduction with triphenyltin hydride[8] in the presence of azobis(isobutano)nitrile (AIBN) in toluene to give **3** in 97% yield. The ^{1}H-n.m.r. spectrum of **3** clearly exhibited signals for H-3*a* and H-3*e* protons at δ 1.995 and 2.638, respectively. Removal of the benzyl group from **3** with hydrogen in the presence of palladium-on-carbon in ethanol gave the glycosyl donor (**4**) in 86% yield.

Next, for synthesis of the nucleotide sugar analogs, the fully protected hydrogen phosphonate nucleoside analogs (**5a–c**) were prepared from the corresponding nucleosides [thymidine, 3′-azido-3′-deoxythymidine (AZT), and cytidine] and salicyl chlorophosphite[9]. Coupling of **4** and the hydrogen phosphonates (**5a–c**) with TPSCl[10] as the activating agent in dry pyridine for 3 h at $-20°$, and purification by flash chromatography on silica gel, gave the hydrogen phosphonate derivatives **6a–c** in 72, 78, and 65% yields, respectively (Chart II). The ^{1}H-n.m.r. spectra of **6a–c** showed signals of the equatorial H-3*e* proton at 2.608, 2.666, and 2.613 p.p.m. respectively, suggestive of the α-anomeric configuration (ranges for α-linked sialyl derivatives: H-3*e* at 2.6–2.8 p.p.m.)[11]. A P–H coupling constant of ~ 700 Hz was observed for all three hydrogen phosphonate derivatives (**6a–c**). These intermediates (**6a–c**) were then oxidized with iodine[12] in 49:1 pyridine–water, with purification by column chromatography with silanised silica-gel RP-2 (Merck) (3:7 methanol–water), to give the phosphate diesters **7a–c** in 51, 61, and 58% yields, respectively. The ^{31}P-n.m.r. spectra of **7a–c** showed signals at δ_P 0.83, 0.57, and 0.55, respectively.

Finally, ammonolysis and hydrolysis of base-labile groups of **7a–c** was performed



with 0.1M methanolic sodium methoxide and 0.1M aq. potassium hydroxide, followed by purification with Bio-Gel P-2 (H_2O) and lyophilization, to afford **8a–c** in 52, 70, and 85% yields, respectively. The fast-atom-bombardment mass spectra of **8a–c** showed molecular-ion $(M + Na)^+$ peaks at *m/z* 680, 705, and 696, respectively.

Biological activities of the nine synthetic compounds (**6–8a–c**) revealed that **7b** inhibited and the sialidase from influenza virus. The antiviral activity against HIV (Human Immunodeficiency Virus) was tested by the syncytium-formation assay method using MT-4 cells. Compounds **7b** and **8b** exhibited the most potent antiviral activity against HIV and had little or no cytotoxicity. This finding is in distinct contrast to the previous paper[3a], which indicated that *N*-acetyl-α,β-D-neuraminyl-(2→5′)-3′-azido-3′-deoxythymidines (Neu5Ac-AZT) were inactive against the influenza virus neuraminidase and HIV. Detailed biological activities will be reported elsewhere.

EXPERIMENTAL

General methods. — Melting points are uncorrected. Optical rotations were measured with a Jasco DIP-140 digital polarimeter. I.r. spectra were recorded on a Jasco IR-810 spectrometer. ^{1}H-N.m.r. spectra were recorded with either a Jeol GX 270 [^{1}H (270 MHz)] or a Jeol GX 500 [^{1}H (500 MHz)] spectrometer with Me_4Si ($\delta = 0$) in $CDCl_3$ or CD_3OD, or sodium 4,4-dimethyl-4-silapentane-1-sulfonate hydrate (DSS, $\delta = 0$ in D_2O) as internal standards at ambient temperature. ^{31}P-N.m.r. spectra were

recorded at 202.35 MHz (Jeol GX500) with external 85% H_3PO_4 in $CDCl_3$ or CD_3OD, or D_2O as reference ($\delta = 0$). Column chromatography was performed on Silica Gel Merck 60 (70–230 mesh) and Silica Gel 60 silanised (70–230 mesh, ASTM) and Bio-Gel P-2 (200–400 mesh Bio-Rad). Flash chromatography was performed on columns of Wako gel C-300 (200–300 mesh). T.l.c. was performed on aluminium sheets coated with Silica Gel $60F_{254}$ (Merck). Compounds containing sugar moieties were detected by u.v. light or by spraying t.l.c. plates with 5% H_2SO_4 in MeOH and charring at 140° for a few min.

Methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-5-deoxy-3*-S-phenyl-3-thio-D-erythro-*β*-L-gluco-*2-nonulopyranosonate* (**1c**). — The chloride **1a** (2.48 g, 4 mmol) was added to a stirred mixture of $Hg(CN)_2$ (2.02 g, 8 mmol) and $HgBr_2$ (2.16 g, 6 mmol) in $ClCH_2CH_2Cl$ (100 mL) and H_2O (1.0 mL). The mixture was stirred for 4 h at 70° and filtered. The filtrate was washed with 10% KI and brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was chromatographed on a column of silica gel that was eluted with 20:1 CH_2Cl_2–MeOH to give 2.30 g (96%) of the 2-hydroxy compound **1c** as a white powder, m.p. 88–92°, $[\alpha]_D$ +31.3° (*c* 0.40, $CHCl_3$); ν_{max} 1730 (CO) and 1640 cm^{-1} (CONH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.856, 1.880, 2.028, 2.093, 2.123 (s, each 3 H, OAc and NAc), 3.770 (d, 1 H, *J* 11.0 Hz, H-3), 3.775 (s, 3 H, CO_2Me), 3.998 (dd, 1 H, *J* 12.5 and 6.5 Hz, H-9′), 4.214 (dd, 1 H, *J* 10.5 and 2.0 Hz, H-6), 4.293 (t, 1 H, *J* 10 Hz, H-5), 4.318 (dd, 1 H, *J* 2.5 Hz, H-9), 4.60 (br s, 1 H, OH), 5.208 (ddd, 1 H, H-8), 5.276 (dd, 1 H, H-4), 5.321 (dd, 1 H, *J* 7.5 and 2.0 Hz, H-7), 5.44 (br d, 1 H, NH), and 7.24–7.39 (m, 5 H, Ph).

Anal. Calc. for $C_{26}H_{33}NO_{13}S$: C, 52.08; H, 5.55; N, 2.31. Found: C, 52.08; H, 5.41; N, 2.37.

Methyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-2-bromo-2,5-dideoxy-3*-S-*phenyl-3-thio*-D-erythro-*β*-L-gluco-*2-nonulopyranosonate* (**1b**). — To a solution of **1c** (0.6 g, 1 mmol) and CBr_4 (0.53 g, 1.6 mmol) in THF (10 mL) was added hexamethylphosphoric triamide (HMPT, 0.37 g, 1.5 mmol) at 0° under argon. After stirring for 10 h at room temperature, the mixture was evaporated and the residue was dissolved in ether. The organic layer was washed with H_2O and dried (Na_2SO_4). After removal of the solvent, the residue was purified by chromatography on silica gel in 10:1 CH_2Cl_2–acetone as the eluent, to give 0.66 g (quant.) of **1b** as a white powder, m.p. 84–88°, $[\alpha]_D$ −22.7° (*c* 2.70, $CHCl_3$); ν_{max} 1740 (CO) and 1650 cm^{-1} (CONH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.854, 1.886, 2.045, 2.106, 2.118 (s, each 3 H, OAc and NAc), 3.843 (s, 3 H, CO_2Me), 3.862 (d, 1 H, *J* 10.3 Hz, H-3), 3.995 (dd, 1 H, *J* 12.7 and 5.4 Hz, H-9′), 4.289 (dd, 1 H, *J* 2.4 Hz, H-9), 4.32–4.37 (m, 2 H, H-5 and H-6), 5.107 (ddd, 1 H, H-8), 5.35 (br d, 1 H, *J* 10 Hz, NH), 5.390 (dd, 1 H, *J* 10.3 Hz, H-4), 5.447 (dd, 1 H, *J* 8.1 and 1.9 Hz, H-7), and 7.26–7.52 (m, 5 H, Ph).

Anal. Calc. for $C_{26}H_{32}BrNO_{12}S$: C, 47.14; H, 4.87; N, 2.11. Found: C, 46.92; H, 4.76; N, 1.94.

Methyl [2-(benzyloxy)ethyl 5-acetamido-4,7,8,9-tetra-O-*acetyl-5-deoxy-3*-S-*phenyl-3-thio*-D-erythro-L-gluco-*2-nonulopyranosid]onate* (**2**). — To a stirred mixture of compound **1a** (0.92 g, 1.5 mmol), 2-(benzyloxy)ethanol (0.34 g, 2.25 mmol), Na_2HPO_4 (0.43 g, 3.0 mL) and powdered 4A molecular sieves (2.0 g) was added $AgOSO_2CF_3$

(0.77 g, 3.0 mmol) at room temperature under argon. After stirring for 15 h in the dark, the mixture was filtered through Celite. The filtrate was successively washed with aqueous saturated $NaHCO_3$ and aqueous NaCl, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by chromatography on a column of silica gel in 100:1 CH_2Cl_2–MeOH as the eluent, to give 0.73 g (67%) of the β-L-glycoside **2** as a white powder, m.p. 71–72°, $[\alpha]_D$ + 3.4° (*c* 2.38, $CHCl_3$); ν_{max} 1750 (CO), 1660, 1540 (CONH), and 690 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 1.876, 2.038, 2.096, 2.117 (s, 15 H, NHAc and OAc), 3.334 (d, 1 H, *J* 11.5 Hz, H-3*a*), 3.605 (t, 2 H, *J* 5.0 Hz, $-CH_2CH_2-$), 3.772 (t, 2 H, $-CH_2CH_2-$), 3.805 (s, 3 H, CO_2Me), 4.008 (dd, 1 H, *J* 12.5 and 6.0 Hz, H-9a), 4.285 (dd, 1 H, *J* 2.5 Hz, H-9b), 4.366 (q, 1 H, *J* 11.0 Hz, H-5), 4.446 (dd, 1 H, *J* 2.5 Hz, H-6), 5.10–5.14 (m, 1 H, H-8), 5.393 (dd, 1 H, H-4), 5.449 (dd, 1 H, *J* 8.0 Hz, H-7), 5.94 (br d, 1 H, NH), and 7.21–7.55 (m, 10 H, Ph).

Anal. Calc. for $C_{35}H_{38}ClNO_{12}S \cdot 2H_2O$: C, 54.70; H, 5.51; N, 1.82. Found: C, 54.41; H, 5.70; N, 2.02.

Glycosylation of **1b** (0.79 g, 1.2 mmol) and 2-(benzyloxy)ethanol (0.27 g, 1.8 mmol), as described for **1a**, gave **2** (0.44 g, 52%).

*Methyl [2-(benzyloxy)ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto-*2-nonulopyranosid]onate* (**3**). — To a solution of compound **2** (73 mg, 0.1 mmol) in dry toluene (0.5 mL) were added a solution of Ph_3SnH (0.35 g, 1.0 mmol) and AIBN (16 mg, 0.1 mmol) in toluene (1.0 mL) under argon. The mixture was heated for 3 h at 110°, evaporated to dryness and the residue purified by chromatography on silica gel with 100:1 CH_2Cl_2–MeOH, to give 61 mg (97%) of **3** as a white powder, m.p. 66–67°, $[\alpha]_D$ − 11.8° (*c* 0.44, $CHCl_3$); ν_{max} 1740 (CO), 1650, 1550 (CONH), and 690 cm^{-1} (Ph); ^{1}H-n.m.r. ($CDCl_3$): δ 1.878, 2.208, 2.121, 2.144 (s, 15 H, NHAc and OAc), 1.995 (t, 1 H, *J* 12.5 Hz, H-3*a*), 2.638 (dd, 1 H, *J* 4.5 Hz, H-3*e*), 3.49–3.53, 3.92–3.96 (m, 2 H, $-CH_2CH_2-$), 3.57–3.65 (m, 2 H, $-CH_2CH_2-$), 3.751 (s, 3 H, CO_2Me), 4.047 (q, 1 H, *J* 10.5 Hz, H-5), 4.083 (dd, 1 H, *J* 12.5 and 6.0 Hz, H-9a), 4.299 (dd, 1 H, *J* 2.5 Hz, H-9b), 4.545, 4.579 (d, each 1 H, *J* 12.0 Hz, $-OCH_2Ph$), 4.84–4.89 (m, 1 H, H-4), 5.13 (br d, 1 H, *J* 9.0 Hz, NH), 5.314 (dd, 1 H, *J* 8.0 and 2.5 Hz, H-7), 5.38–5.41 (m, 1 H, H-8), and 7.33–7.34 (m, 5 H, Ph).

Anal. Calc. for $C_{29}H_{34}NO_{14} \cdot H_2O$: C, 54.54; H, 5.68; N, 2.19. Found: C, 54.71; H, 6.17; N, 2.17.

*Methyl [2-(hydroxy)ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D-glycero-α-D-galacto-*2-nonulopyranosid]onate* (**4**). — A mixture of **3** (0.46 g, 0.74 mmol) and Pd–C (0.12 g) in EtOH (10 mL) was stirred for 17 h at room temperature under hydrogen, filtered through Celite, and the filtrate evaporated *in vacuo*. The residue was purified by chromatography on silica gel in 10:1 CH_2Cl_2–MeOH, to give 0.34 g (86%) of **4** as a white powder, m.p. 136–138°, $[\alpha]_D$ − 16.3° (*c* 2.6, $CHCl_3$); ν_{max} 1740 (CO), 1650, and 1550 cm^{-1} (CONH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.887, 2.032, 2.041, 2.140, 2.145 (s, each 3 H, NHAc and OAc), 1.987 (t, 1 H, *J* 12.5 Hz, H-3*a*), 2.610 (dd, 1 H, *J* 4.5 Hz, H-3*e*), 3.46–3.50 (m, 2 H, $-CH_2CH_2-$), 3.69–3.75, 3.85–3.88 (m, 2 H, $-CH_2CH_2-$), 3.813 (s, 3 H, CO_2Me), 4.070 (q, 1 H, *J* 10.5 Hz, H-5), 4.078 (dd, 1 H, *J* 12.0 and 6.0 Hz, H-9a), 4.167 (dd, 1 H, *J* 11.0 and 2.5 Hz, H-6), 4.328 (dd, 1 H, *J* 2.5 Hz, H-9b), 4.886 (ddd, 1 H, H-4), 5.10 (br d, 1 H, *J* 10.0 Hz, NH), 5.323 (dd, 1 H, *J* 8.0 Hz, H-7), and 5.400 (ddd, 1 H, H-8).

Anal. Calc. for $C_{22}H_{28}NO_{14}\cdot H_2O$: C, 48.18; H, 5.51; N, 2.55. Found: C, 48.50; H, 5.15; N, 2.58.

3'-O-Acetylthymidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-α-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate)ethyl phosphonate] (**6a**). — From a pyridine suspension of **4** (80 mg, 0.15 mmol) and **5a** (71 mg, 0.23 mmol) was evaporated anhydrous pyridine (1.0 mL) several times, and then to the suspension in pyridine (2.0 mL) was added 0.18 g (0.6 mmol) of TPSCl at −10° under argon. The mixture was stirred for 5 h at the same temperature and hydrolyzed with H_2O. The mixture was evaporated, the residue chromatographed on a column of silica gel that was eluted with 50:1 CH_2Cl_2–MeOH, to give 90 mg (72%) of **6a** as a syrup, $[\alpha]_D$ −2.3° (*c* 1.8, $CHCl_3$); ν_{max} 1740 (CO), 1680, 1650, and 1550 cm^{-1} (CONH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.883, 1.902, 2.030, 2.038, 2.040, 2.136, 2.140 (s, each 3 H, NHAc, OAc and 5-Me), 2.37–2.50 (m, 2 H, H-2'), 2.608 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*''), 3.805 (s, 3 H, CO_2Me), 4.184 (dd, 1 H, *J* 10.5 and 2.5 Hz, H-6''), 4.39–4.42 (m, 1 H, H-3'), 4.084 (dd, 1 H, *J* 12.5 and 6.5 Hz, H-9a''), 4.353 (dd, 1 H, *J* 2.5 Hz, H-9b''), 4.86–4.91 (m, 1 H, H-4''), 5.326 (dd, 1 H, *J* 8.5 Hz, H-7''), 5.36–5.40 (m, 1 H, H-8''), 5.84–5.87 (m, 1 H, H-1'), 7.58 (br s, 1 H, H-6), and 8.62 (br s, 1 H, 3-NH); ^{31}P-n.m.r. ($CDCl_3$): δ 10.4 (J_{PH} 723 Hz) and 11.1 (J_{PH} 700 Hz).

Anal. Calc. for $C_{34}H_{48}N_3O_{22}P\cdot 2H_2O$: C, 44.54; H, 5.95; N, 8.87. Found C, 44.50; H, 5.71; N, 8.58.

3'-Azido-3'-deoxythymidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate)ethyl phosphonate] (**6b**). — This compound was prepared from **4** (107 mg, 0.20 mmol) and **5b** (99 mg, 0.30 mmol) by the method described for **6a**; 132 mg (78%) of the product was obtained as a syrup; $[\alpha]_D$ −11.0° (*c* 2.1, $CHCl_3$); ν_{max} 2075 (N_3), 1740 (CO), 1680, 1640 and 1560 cm^{-1} (CONH); ^{1}H-n.m.r. ($CDCl_3$): δ 1.883, 2.036, 2.092, 2.095, 2.123, 2.129 (s, each 3 H, NHAc, OAc and 5-Me), 2.40–2.51 (m, 2 H, H-2'), 2.666 (dd, 1 H, *J* 11.5 and 5.0 Hz, H-3*e*''), 3.852 (s, 3 H, CO_2Me), 4.90–4.99 (m, 1 H, H-4''), 5.34–5.38 (m, 1 H, H-7''), 5.40–5.43 (m, 1 H, H-8''), 6.01–6.07 (m, 1 H, H-1'), 7.54 (br s, 1 H, H-6), and 8.68 (br s, 1 H, 3-NH); ^{31}P-n.m.r. ($CDCl_3$): δ 8.94 (J_{PH} 719 Hz) and 10.3 (J_{PH} 710 Hz).

Anal. Calc. for $C_{32}H_{45}N_6O_{21}P$: C, 43.64; H, 5.15; N, 9.54. Found: C, 43.61; H, 5.23; N, 9.40.

N-*Acetyl-2',3'-di-O-acetylcytidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosyloxyonate)ethyl phosphonate]* (**6c**). — This compound was prepared from **4** (91 mg, 0.17 mmol) and **5c** (108 mg, 0.25 mmol) by the method described for **6a**; 105 mg (65%) of the product was obtained as a syrup; $[\alpha]_D$ −9.4° (*c* 1.28, $CHCl_3$); ν_{max} 1740 (CO), 1680, 1650, 1550 (CONH), and 1230 cm^{-1} (P=O); ^{1}H-n.m.r. ($CDCl_3$): δ 1.881, 2.021, 2.030, 2.037, 2.071, 2.127, 2.132, 2.139 (s, each 3 H, NHAc and OAc), 2.38–2.41, 2.83–2.88 (m, 2 H, H-2' and H-3'), 2.613 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*''), 3.805 (s, 3 H, CO_2Me), 4.073 (dd, 1 H, *J* 12.5 and 6.5 Hz, H-9a''), 4.173 (dd, 1 H, *J* 10.5 and 2.5 Hz, H-6''), 4.348 (dd, 1 H, *J* 2.5 Hz, H-9b''), 4.88–4.92 (m, 1 H, H-4''), 5.32–5.34 (m, 1 H, H-7''), 5.38–5.39 (m, 1 H, H-8''), 5.771 (d, 1 H, *J* 8.0 Hz, H-1'), and 7.875 (d, 1 H, *J* 8.1 Hz, H-6); ^{31}P-n.m.r. ($CDCl_3$): δ 10.3 (J_{PH} 711 Hz) and 11.1 (J_{PH} 711 Hz).

Anal. Calc. for $C_{37}H_{51}N_4O_{25}P \cdot H_2O$: C, 44.40; H, 5.34; N, 5.60. Found: C, 44.43; H, 5.65; N, 5.36.

*3'-O-Acetylthymidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyloxyonate)ethyl phosphate]* (**7a**). — To a solution of **6a** (77 mg, 0.09 mmol) in pyridine (0.5 mL) was added a 0.5M solution of iodine in pyridine–water (98:2; v/v) (0.22 mL, 0.11 mmol) at 0°. After stirring for 1 h, the mixture was evaporated and the residue purified by column chromatography using silanised silica gel as the stationary phase (3:7 MeOH:H_2O) to give 40 mg (51%) of **7a** as a white powder, m.p. 31–32°, $[\alpha]_D$ −20.3° (*c* 0.66, MeOH); ν_{max} 1740 (CO), 1680, 1650, 1550 (CONH), 1220 cm^{-1} (P=O); ^{1}H-n.m.r. (CD_3OD): δ 1.839, 1.964, 1.992, 2.010, 2.098, 2.115, 2.125 (s, 24 H, NHAc, OAc, and 5-Me), 2.32–2.44 (m, 2 H, H-2'), 2.629 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*''), 3.824 (s, 3 H, CO_2Me), 4.077 (dd, 1 H, *J* 12.5 and 5.0 Hz, H-9a''), 4.280 (dd, 1 H, *J* 2.5 Hz, H-9b''), 5.318 (dd, 1 H, *J* 9.0 and 2.5 Hz, H-7''), 5.36, 5.41 (m, 1 H, H-8''), 6.34–6.37 (m, 1 H, H-1'), and 7.86 (br s, 1 H, H-6); ^{31}P-n.m.r. (CD_3OD): δ 0.83.

Anal. Calc. for $C_{34}H_{48}N_3O_{23}P \cdot 3H_2O$: C, 42.02; H, 5.60; N, 4.32. Found: C, 42.35; H, 6.00; N, 4.05.

*3'-Azido-3'-deoxythymidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyloxyonate)ethyl phosphate]* (**7b**). — This compound was prepared from **6b** (42 mg, 0.05 mmol) by the method described for **7a**; 57 mg (61%) of the product was obtained as white powder, m.p. 101–103°, $[\alpha]_D$ −11.3° (*c* 1.1, MeOH); ν_{max} 2100 (N_3), 1730 (CO), 1690, 1650, 1550 (CONH), and 1220 cm^{-1} (P=O); ^{1}H-n.m.r. (CD_3OD): δ 1.854, 1.938, 2.003, 2.024, 2.126, 2.145 (s, each 3 H, NHAc, OAc, and 5-Me), 2.37–2.48 (m, 2 H, H-2'), 2.648 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*''), 3.832 (s, 3 H, CO_2Me), 3.973 (t, 1 H, *J* 10.0 Hz, H-5''), 4.076 (dd, 1 H, *J* 12.5 and 5.5 Hz, H-9a''), 4.127 (dd, 1 H, *J* 10.5 Hz, H-6''), 4.284 (dd, 1 H, *J* 2.5 Hz, H-9b''), 4.48–4.51 (m, 1 H, H-4''), 5.331 (dd, 1 H, *J* 9.0 and 2.0 Hz, H-7''), 5.38–5.41 (m, 1 H, H-8''), 6.22–6.27 (m, 1 H, H-1'), 7.72 (br s, 1 H, H-6), and 8.86 (br s, 1 H, 3-NH); ^{31}P-n.m.r. (CD_3OD): δ 0.57.

Anal. Calc. for $C_{32}H_{45}N_6O_{20}P \cdot 2H_2O$: C, 42.67; H, 5.48; N, 9.33. Found: C, 42.21; H, 5.01; N, 9.12.

*N-Acetyl-2',3'-di-O-acetylcytidine 5'-[2-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonulopyranosyloxyonate)ethyl phosphate]* (**7c**). — This compound was prepared from **6c** (106 mg, 0.11 mmol) by the method described for **7a**; 63 mg (58%) of the product was obtained as a white powder, m.p. 105–108°, $[\alpha]_D$ −2.1° (*c* 0.78, MeOH); ν_{max} 1740 (CO), 1680, 1660, 1540 (CONH), and 1230 cm^{-1} (P=O); ^{1}H-n.m.r. (CD_3OD): δ 1.841, 1.992, 2.011, 2.058, 2.112, 2.129 (s, 24 H, NHAc and OAc), 2.645 (s, 3 H, *J* 12.5 and 5.0 Hz, H-3*e*''), 3.829 (s, 3 H, CO_2Me), 5.324 (dd, 1 H, *J* 9.0 and 2.0 Hz, H-7''), 6.140 (d, 1 H, *J* 6.5 Hz, H-1'), 7.93 (br s, 1 H, H-6), and 8.79 (br s, 1 H, 3-NH); ^{31}P-n.m.r. (CD_3OD): δ 0.55.

Anal. Calc. for $C_{37}H_{51}N_4O_{24}P \cdot 2H_2O$: C, 44.32; H, 5.53; N, 5.59. Found: C, 44.13; H, 5.86; N, 5.31.

*Thymidine 5'-[2(N-acetyl-α-*D*-neuraminyloxy)ethyl phosphate]* (**8a**). — To a

solution of compound **7a** (43 mg, 0.05 mmol) in dry MeOH (1.5 mL) was added a solution of NaOMe (9.1 mg) in dry MeOH (1.5 mL), and the solution was stirred for 2 h at 0°, and treated with Amberlite IRC-50 (0.5 g) resin to remove sodium ions. The mixture was filtered, the filtrate evaporated, and the residue dissolved in MeOH (1.5 mL). To this solution was added 0.1 M KOH (1.5 mL) and it was then stirred for 16 h at room temperature. This solution was cooled to 0°, adjusted to pH 3 with Amberlite IR-120 (H^+) resin, and evaporated. The residue was purified on a column of Bio-Gel P-2 using water as eluant. After freeze drying, **8a** (17 mg, 52%) was obtained as a white powder, $[\alpha]_D$ −7.1° (*c* 0.42, MeOH); ν_{max} 1680, 1550 (CONH), and 1270 cm^{-1} (P=O); ^{1}H-n.m.r. (D_2O): δ 1.917 (s, 3 H, 5-Me), 2.027 (s, 3 H, NAc), 2.36–2.38 (m, 2 H, H-2′), 2.679 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*″), 3.634 (dd, 1 H, *J* 12.5 and 6.5 Hz, H-9a″), 4.04–4.11 (m, 2 H, H-4″ and H-8″), and 7.73 (br s, 1 H, H-6); ^{31}P-n.m.r. (D_2O): δ 0.67. Positive f.a.b.-m.s.: $(M + H)^+$ *m/z* 658 and $(M + Na)^+$ 680.

*3′-Azido-3′-deoxythymidine 5′-[2-(*N*-acetyl-α-*D*-neuraminyloxy)ethyl phosphate]* (**8b**). — This compound was prepared from **7b** (51 mg, 0.06 mmol) by the method described for **8a**; 28 mg (70%) of the product was obtained as à syrup, $[\alpha]_D$ +13.6° (*c* 0.66, MeOH); ν_{max} 2100 (N_3), 1690, 1550 (CONH), and 1270 cm^{-1} (P=O); ^{1}H-n.m.r. (D_2O): δ 1.920 (s, 3 H, 5-Me), 2.029 (s, 3 H, NHAc), 2.48–2.51 (m, 2 H, H-2′), 2.683 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*″), 3.638 (dd, 1 H, *J* 12.0 and 6.0 Hz, H-9a″), 6.267 (t, 1 H, *J* 6.5 Hz, H-1′), and 7.72 (br s, 1 H, H-6); ^{31}P-n.m.r. (D_2O): δ 0.62. Positive f.a.b.-m.s. (NBA): $(M + H)^+$ *m/z* 683 and $(M + Na)^+$ 705.

*Cytidine 5′-[2(*N*-acetyl-α-*D*-neuraminyloxy)ethyl phosphate]* (**8c**). — This compound was prepared from **7c** (40 mg, 0.41 mmol) by the method described for **8a**: 23 mg (85%) of the product was obtained as a syrup, $[\alpha]_D$ −2.0° (*c* 0.3, MeOH); ν_{max} 1680, 1550 (CONH), and 1270 cm^{-1} (P=O); ^{1}H-n.m.r. (D_2O): δ 2.034 (s, 3 H, NAc), 2.698 (dd, 1 H, *J* 12.5 and 4.5 Hz, H-3*e*″), 3.641 (dd, 1 H, *J* 12.5 and 7.0 Hz, H-9a″), 5.94–5.97 (m, 1 H, H-1′), and 7.91 (d, 1 H, *J* 8.0 Hz, H-6); ^{31}P-n.m.r. (D_2O): δ 0.68. Positive f.a.b.-m.s. (NBA): $(M + K)^+$ *m/z* 696.

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