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Synthesis of CMP-Sialic Acid Conjugates: Substrates for the Enzymatic Synthesis of Natural and Designed Sialyl Oligosaccharides#

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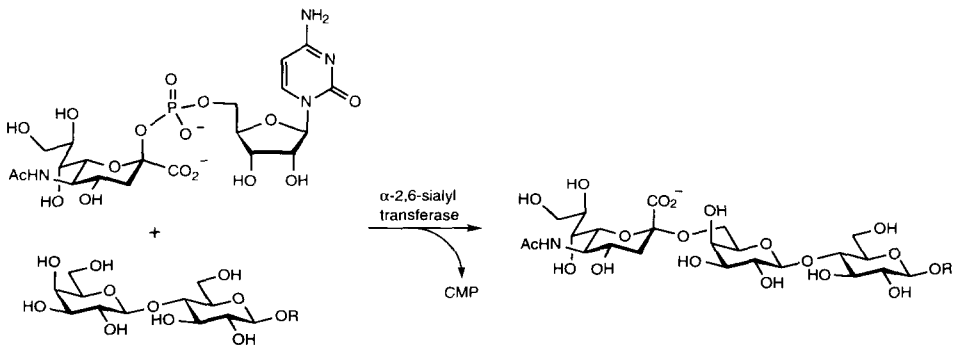
Abstract: The syntheses of several congeners of CMP-NeuAc are described. These compounds are substrates for enzymatic glycosylation. © 1997 Elsevier Science Ltd.

The use of glycosyltransferases to mediate glycosylations has emerged as one of the most powerful strategies to construct complex oligosaccharides and glycoconjugates.[1] Glycosyltransferases offer many advantages over traditional chemical methods in that the glycosylations they catalyze are regiospecific and stereospecific, and the need for cumbersome and tedious protection schemes is avoided. Another advantage is that complex biomolecules can be efficiently utilized as substrates. One current disadvantage of glycosyltransferase-based technology is the limited ability to incorporate modified sugar residues into oligosaccharides. A major reason that contributes to this drawback is the lack of availability of the requisite sugar-nucleotide glycosyl donors, but some elegant solutions to this problem have recently appeared.[2]

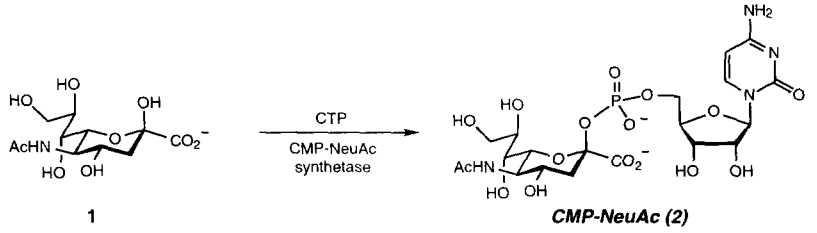
As part of our program in the *de novo* design and synthesis of bioactive glycoconjugates,[3] we required a method to enzymatically incorporate a variety of sialic acids into complex oligosaccharides using sialyltransferases (Scheme 1).[4] To accomplish this task, ready access to the corresponding CMP-NeuAc



Scheme 1

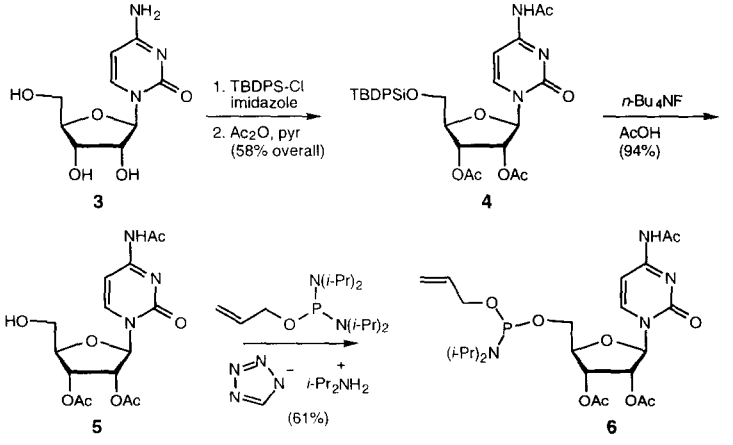
#Warmly dedicated to Professor Samuel J. Danishefsky for his inspiration, guidance, and mentorship.

(cytidine monophospho-*N*-acetylneuraminic acid) derivatives was needed. Unfortunately, the enzymatic process for producing CMP-NeuAc (**2**) from *N*-acetylneuraminic acid **1** (Scheme 2) is not generally amenable to the synthesis of derivatives with modified sialic acids.[5] Furthermore, the enzymes needed for this method, particularly CMP-NeuAc synthetase, are not readily available. To overcome these obstacles we have developed a synthetic route to CMP-NeuAc that promises to be general for the synthesis of virtually any derivative.[6]



Scheme 2

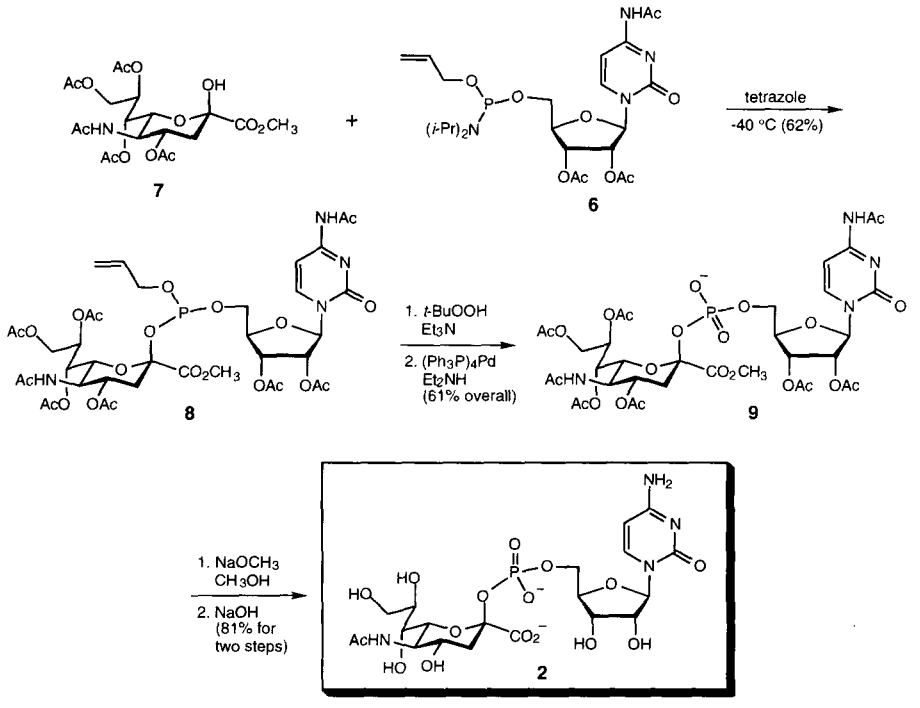
The synthesis of CMP-NeuAc hinges on a coupling of a cytidine phosphoramidite with the tertiary anomeric hydroxyl group of a selectively protected sialic acid. The cytidine component, phosphoramidite **6**, which contains an allyl protecting group for the phosphite and phosphate, was synthesized according to Scheme 3. The primary alcohol of cytidine was selectively protected as a *tert*-butyldiphenylsilyl ether and the remaining alcohols and the amine were acylated to afford compound **4**. The silyl ether was cleaved to give **5**, and the resulting primary alcohol was phosphitylated with allyl bis(diisopropylamino)phosphorimidite to give the phosphoramidite **6**.



Scheme 3

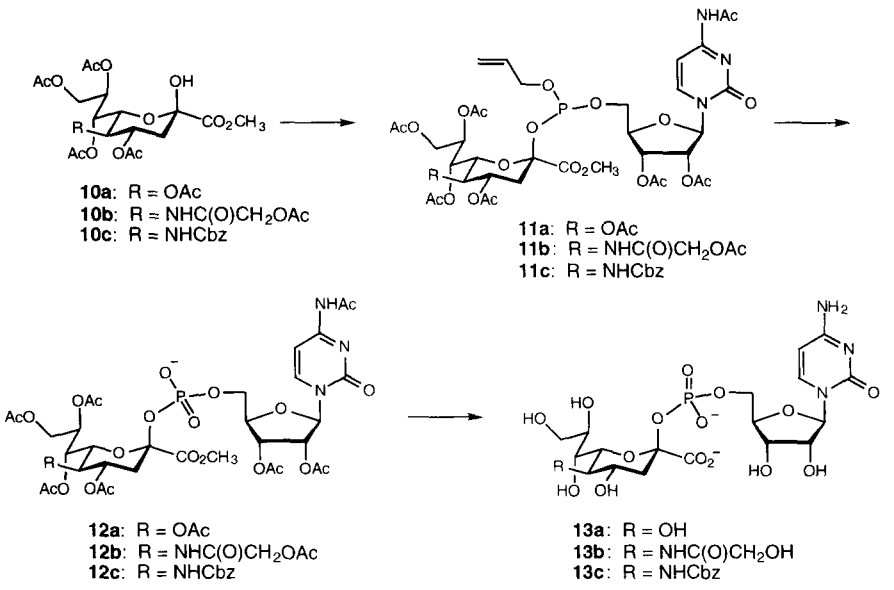
The synthesis of CMP-NeuAc is shown in Scheme 4. The NeuAc derivative **7**[7] was treated with the phosphoramidite **6**[8] and tetrazole, followed by oxidation of the resulting phosphite with *tert*-butylhydroperoxide, to provide the phosphate **8**.[9] Deallylation of **8** using catalytic palladium gave compound **9**. A central aspect of

the synthetic route was the allyl protecting group for the phosphate, which could be removed under neutral conditions prior to hydrolysis of the remaining ester blocking groups. The removal of base-labile phosphate protecting groups was problematic in our hands, and led to products derived from elimination across the C2-C3 bond of NeuAc and from cleavage of the phosphodiester linkage. Finally, cleavage of the acetates with methoxide and saponification of the methyl ester gave CMP-NeuAc **2**, which was purified by size exclusion chromatography.[10] The reaction time for the final hydrolysis step was found to be crucial, since extended exposure of **2** to NaOH led to products derived from elimination and/or phosphate hydrolysis.



Scheme 4

Following this protocol, the CMP-NeuAc derivatives **13a**, **13b**, and **13c** were synthesized from the sialic acids **10a**, **10b**, and **10c**, respectively (Scheme 5).[10] Reproducible differences in yields of both the phosphoramidite coupling (**10** → **11**) and deprotection (**12** → **13**) steps for each sialic acid derivative were observed. This is presumably related to the electron-withdrawing nature of the substitutent at C-5, however no further investigations of this observation have been carried out. Compounds **13a**[11] and **13b**[12] are themselves naturally occurring sialic acid donors. Compound **13b** is biosynthesized by hydroxylation acetyl group of CMP-NeuAc **2**, and its sialic acid moiety is found in a variety of biochemically important gangliosides and oligosaccharides.[12] Compound **13c** contains a carbobenzyloxy (Cbz) protecting group for the nitrogen moiety, which can potentially be removed under mild conditions after a glycosyltransferase reaction. This compound is significant since gangliosides that contain de-*N*-acetyl sialic acids have been shown to play important roles in cell signalling processes.[13]



Scheme 5

In summary, several derivatives of CMP-NeuAc have been synthesized for investigation as substrates for enzymatic glycosylation. The synthetic sequence is quite general, and the conditions are compatable with a number of structural groups in the sialic acid domain, including those that can be used to probe sialic acid recognition in biological systems. Current efforts in this laboratory are directed toward a detailed evaluation of these compounds as substrates for enzyme-mediated syntheses of designed sialyl glycoconjugates, and the combined chemical and enzymatic synthesis of combinatorial libraries of oligosaccharides. Furthermore, since enzymatic reactions can be performed *in vivo*, this technology, along with that developed by others,[2b,k] harnesses the power of organic synthesis to modify the antigenic properties of intact cells.

Experimental.

General Procedures: ^{1}H NMR spectra were run at 400 MHz, ^{13}C spectra at 100 MHz, and ^{31}P spectra at 162 MHz on a Bruker AM-400 spectrometer. IR was performed on Perkin Elmer 1600 spectrometer. Mass spectrometry was performed on a VG 7070 EQ spectrometer (FAB was performed in a glycerol matrix at 8 kV with Xe as the fast atom).

DMF was dried over and distilled from CaH_2 under aspirator vacuum and stored over 4Å molecular sieves. CH_2Cl_2 was dried over and distilled from CaH_2 prior to use. MeCN was dried over and distilled from P_2O_5 three times, CaH_2 one time, and stored over 3Å molecular sieves. MeOH was stored over 4Å molecular sieves prior to use. Silica gel was purchased from Scientific Adsorbents Inc. (40 μm). Size exclusion resin was purchased from Bio-rad (P-2 gel, fine, 65 ± 20 μm). Commercially available reagents were used without additional purification unless noted.

2',3'-*O*,N^4-Triacetyl-5'-*tert*-Butyldiphenylsilyl Cytidine (4): A solution containing 4.95 g

(20.3 mmol) of **3** and 3.05 g (44.8 mmol) of imidazole in DMF (83 mL) was treated with 6.4 mL (24.4 mmol) of tert-butyldiphenylsilyl chloride. The solution was stirred for 20 h at room temperature and then Ac_2O (10 mL) and pyridine (20 mL) were added. The mixture was stirred for an additional 12 h, poured into H_2O (100 mL), and extracted with EtOAc (250 mL). The combined extracts were washed with H_2O, saturated $NaHCO_3$, and brine, successively. The solution was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography (2:1 EtOAc:Hexanes to 9:1 EtOAc:Hexanes) to give **4** (7.10 g) in 58% as a white solid: $[\alpha]^{25}_D$ +59.9° (c 0.7, $CHCl_3$); IR (thin film, $CHCl_3$) 3230, 3072, 2932, 2858, 1754, 1721, 1672, 1629, 1562, 1536, 1512, 1493, 1440, 1428, 1372, 1314, 1233, 1113, 1085 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 10.58 (s, 1H), 8.18 (d, J = 7.8 Hz, 1H), 7.62 (t, 4H), 7.45-7.35 (m, 6H), 7.24 (d, J = 7.8 Hz, 1H), 6.29 (d, J = 5.1 Hz, 1H), 5.47 (t, J = 5.1 Hz, 1H), 5.42 (t, J = 5.1 Hz, 1H), 4.20 (m, 1H), 4.05 (dd, J = 2.1, 13.7 Hz, 1H), 3.78 (dd, J = 2.1, 13.7 Hz, 1H), 2.26 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 1.09 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 169.5, 169.3, 163.3, 154.8, 144.0, 135.6, 135.3, 132.3, 131.7, 130.1, 128.0, 127.9, 97.4, 87.0, 82.6, 74.2, 69.9, 62.6, 26.8, 24.7, 20.5, 20.4, 19.1; HRMS (EI) calcd for $C_{31}H_{37}N_3O_8Si$ (M + H^+) 608.2428, found 608.2426.

2',3'-*O*,N^4-Triacetyl-Cytidine (5): A solution containing 2.73 g (44.9 mmol) of **4** was treated with 0.39 mL (67.4 mmol) of AcOH and 5.2 mL (179.6 mmol) tetra-N-butylammonium fluoride (1.0 M solution in THF). The solution was stirred for 18 h at room temperature and then 1.5 mL AcOH was added. The mixture was concentrated *in vacuo* and purified by silica gel chromatography (15:1 EtOAc:MeOH) to provide **5** (1.56 g) in 94% as a white solid. Spectral data were consistent with that reported.[13]

2-Allyl-2'-(2',3',N^4-Triacetyl-Cytidin-5')-yl-*N*,*N'*-diisopropylphosphoramidite (6): A solution containing 1.48 g (4.52 mmol) of **5** and 0.40 g (2.32 mmol) of diisopropylammoniumtetrazole in CH_2Cl_2 (20 mL) was treated with 2.95 mL (9.24 mmol) of allyl *N*,*N*,*N'*,*N'*-tetraisopropylphosphorodiamidite. The mixture was stirred for 2 h and washed with saturated $NaHCO_3$ (2 times) and brine, successively. The solution was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel chromatography (9:1:0.3 EtOAc:Hexanes:Et_3N) to give **6** (2.19 g) in 61% (1:1 diasteriomeric mixture) as a white foam: IR (thin film, $CHCl_3$) 3228, 3082, 2967, 2870, 1754, 1722, 1673, 1652, 1644, 1627, 1563, 1557, 1494, 1462, 1441, 1372, 1315, 1231, 1183, 1156, 1100,1076, 1026, 1000, 977 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.52 (d, J = 16.8 Hz, 2H), 8.35 (d, J = 7.8 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 7.41 (d, 7.8 Hz, 2H), 6.37 (d, J = 5.9 Hz, 2H), 5.99-5.88 (m, 2H), 5.44-5.11 (m, 9H), 4.31 (m, 3H), 4.25-4.08 (m, 4H), 4.04-3.96 (m, 2H), 3.86-3.79 (m, 3H), 3.66-4.45 (m, 6H), 2.23 (s, 3H), 2.31 (s, 3H), 2.08 (s, 3H), 2.08 (s, 3H), 2.03 (s, 6H), 1.26-1.15 (m, 24H); ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ 149.4, 149.2; MS (FAB+) calcd for $C_{24}H_{35}N_4O_9P$ (M + H^+) 557, found 557.

Methyl (5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy- β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (7): A solution containing 105 mg (0.20 mmol) Methyl-(5-Acetamido-2,4,7,8,9-penta-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate in 3:1 CH_2Cl_2:AcCl (4.0 mL) was treated with 2 drops conc. HCl. The solution was allowed to stir for 3 d with addition of 2 drops conc. HCl each day. The mixture was coevaporated with toluene and purified by silica gel chromatography (1:1 EtOAc:Hexanes) to give the anomeric chloride (90 mg) in 90% as a white foam.

A solution of the chloride containing 123 mg (0.24 mmol) in 4:1 acetone:water (5.0 mL) was treated with

Ag_2CO_3 (202 mg, 0.73 mmol). The reaction vessel was wrapped in foil and allowed to stir for 3 days. The mixture was filtered through celite, reduced *in vacuo*, and purified by silica gel chromatography (9:1 EtOAc:Hexanes) to give **7** (103 mg) in 87% as a white foam. Spectra were in accordance with the reported values.[8]

Methyl (4,5,7,8,9-penta-*O*-acetyl-3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)-onate (10a): A solution containing 207 mg (0.39 mmol) Methyl-(2,4,5,7,8,9-hexa-*O*-acetyl-3-deoxy-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate in 3:1 CH_2Cl_2:AcCl (4.0 mL) was treated with 2 drops conc. HCl. The solution was allowed to stir for 3 d with addition of 2 drops conc. HCl each day. The mixture was reduced *in vacuo* in the presence of toluene and purified by silica gel chromatography (1:1 EtOAc:Hexanes) to provide the anomeric chloride (167 mg) in 85% as a white foam.

A solution of the chloride (164 mg, 0.32 mmol) in 4:1 acetone:water (5.0 mL) was treated with Ag_2CO_3 (266 mg, 0.96 mmol). The reaction vessel was wrapped in foil and allowed to stir for 3 days. The mixture was filtered through celite, concentrated *in vacuo*, and purified by silica gel chromatography (1:1 EtOAc:Hexanes) to give **10a** (134 mg) in 85% as a white foam: $[\alpha]^{25}_D$ -2.1° (c 3.2, $CHCl_3$); IR (thin film, $CHCl_3$) 3448, 2958,1747, 1508, 1437, 1371, 1224, 1150, 1052 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.28-5.22 (m, 3H), 4.86 (t, J = 9.8 Hz, 1H), 4.28-4.18 (m, 3H), 4.03 (dd, J = 5.5, 12.5 Hz, 1H), 3.80 (s, 3H), 2.22-2.14 (m, 2H), 2.05 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 170.1, 170.0, 169.9, 169.8, 168.8, 94.6, 69.5, 69.2, 68.9, 67.9, 66.7, 62.3, 53.5, 35.6, 20.8, 20.8, 20.6, 20.6, 20.5; HRMS (CI) calcd for $C_{20}H_{28}O_{14}$ ($M + H^+$) 493.1557, found 493.1450.

Methyl (5-(2-(acetoxy)acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (10b): A solution containing 112 mg (0.19 mmol) Methyl (5-(2-hydroxyacetyl)-2,4,7,8,9-penta-O-acetyl-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosid)onate in 3:1 CH_2Cl_2:AcCl (4.0 mL) was treated with 2 drops conc. HCl. The solution was allowed to stir for 3 d with addition of 2 drops conc. HCl each day. The mixture was coevaporated with toluene and purified by silica gel chromatography (4:1 EtOAc:Hexanes) to obtain the anomeric chloride (92 mg) in 86% as a white foam.

A solution of the chloride (90 mg, 0.16 mmol) in 4:1 acetone:water (5.0 mL) was treated with Ag_2CO_3 (136 mg, 0.49 mmol). The reaction vessel was wrapped in foil and allowed to stir for 3 days. The mixture was filtered through celite, concentrated *in vacuo*, and purified by silica gel chromatography (4:1 EtOAc:Hexanes) to give **10b** (77 mg) in 88% as a white foam: $[\alpha]^{25}_D$ -9.9° (c 1.0, $CHCl_3$); IR (thin film, $CHCl_3$) 3363, 2958, 1746, 1535, 1438, 1372, 1231, 1156, 1122, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.20 (d, J = 9.8 Hz, 1H), 5.30-5.19 (m, 3H), 4.56 (d, J = 14.9 Hz, 1H), 4.45 (dd, J = 2.3, 6.6 Hz, 1H), 4.29 (d, J = 15.2 Hz, 1H), 4.22-4.13 (m, 2H), 3.99 (dd, J = 7.4, 12.5 Hz, 1H), 33.84 (s, 3H), 2.23 (dd, J = 11.3, 12.5 Hz, 1H), 2.18-2.13 (m, 4H), 2.10 (s, 3H), 2.08 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.8, 170.7, 170.3, 169.8, 169.0, 167.6 94.8, 71.0, 70.8, 68.5, 67.8, 62.7, 62.6, 53.5, 49.4, 36.1, 21.0, 20.8, 20.8, 20.8, 20.6; HRMS (FAB+) calcd for $C_{22}H_{31}NO_{15}$ ($M + H^+$) 550.1772 found 550.1806.

Methyl (4,7,8,9-tetra-*O*-acetyl-5-amino-5-*N*-benzyloxycarbonyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate (10c): A solution containing 132 mg (0.21 mmol) Methyl (*N*-benzyloxycarbonyl-2,4,7,8,9-penta-*O*-acetyl-5-amino-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid)onate in 3:1 CH_2Cl_2:AcCl (4.0 mL) was treated with 2 drops conc. HCl. The solution was

allowed to stir for 2 d with addition of 2 drops conc. HCl after 17 hours. The mixture was coevaporated with toluene and used in the next step without purification.

A solution of the crude chloride in 4:1 acetone:water (5.0 mL) was treated with Ag_2CO_3 (196 mg, 0.71 mmol). The reaction vessel was wrapped in foil and allowed to stir for 2 days. The mixture was filtered through celite, concentrated *in vacuo*, and purified by silica gel chromatography (1:1 EtOAc:Hexanes) to give **10c** (104 mg) in 75% as a white foam: $[\alpha]^{25}_D$ +0.9° (c 1.2, $CHCl_3$); IR (thin film, $CHCl_3$) 3382, 2958, 1745, 1648, 1534, 1499, 1438, 1371, 1313, 1227, 1155, 1074, 1038 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.31-7.22 (m, 5H), 5.41 (dd, J = 2.0, 4.7 Hz, 1H), 5.38 (d, J = 10.2 Hz, 1H), 5.25-5.21 (m, 2H), 5.16 (m, 3H), 4.87 (d, J = 12.5 Hz, 1H), 4.47 (dd, J = 2.3, 12.5 Hz, 1H), 4.00 (dd, J = 7.8, 12.5 Hz, 1H), 3.83-3.73 (m, 4H), 2.17 (dd, J = 5.5, 12.9 Hz, 1H), 2.13-2.07 (m, 4H), 2.02 (s, 3H), 1.97 (s, 3H), 1.76 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 171.0, 170.5, 170.0, 169.0, 156.0, 136.5, 128.3, 127.9, 127.8, 94.8, 71.2, 71.1, 69.2, 68.3, 66.6, 62.5, 53.2, 51.2, 36.1, 20.8, 20.7, 20.6, 20.5; HRMS (EI) calcd for $C_{26}H_{33}NO_{14}$ (M + H^+) 584.1979, found 584.1992.

General Procedure for Cytidine Phosphoramidite-Sialic Acid Coupling: A solution of **7**, **10a**, **10b**, or **10c** was prepared in 1.0 mL MeCN and stirred with one weight equivalent of activated, crushed 3Å molecular sieves for 1 hour. Compound **6** was dissolved in 1.0 mL MeCN and both solutions were cooled to -43°C (dry ice/MeCN) for 30 minutes. Compound **6** was added via syringe to the sialic acid solution at -43°C and was warmed to RT and stirred for 1 hour. Triethylamine (10 eq) was added, the solution was filtered through celite, and then concentrated *in vacuo*. The syrup was redissolved in 10 mL EtOAc and washed with H_2O (2x), and brine, successively. The solution was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography to give **8**, **11a**, **11b**, or **11c**, respectively..

Allyl 2',3'-*O*,N^4-Triacetylcytidin-5'-yl Methyl 5-Acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphite (8): This compound was synthesized according to the general coupling procedure. The crude solid was purified by silica gel chromatography (15:1:0.1 EtOAc:MeOH:Et_3N) to give **8** (91mg) in 62% yield (2.4:1 diastereomeric mixture) as a white foam. The major diastereomer was characterized: IR (thin film, $CHCl_3$) 3316, 3019, 1746, 1671, 1630, 1556, 1483, 1439, 1373, 1323, 1224, 1218, 1207, 1114, 1046 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.55 (s, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.41 (d, J = 7.4 Hz, 1H), 7.32 (d, J = 9.8 Hz, 1H), 5.96 (,d, J = 4.7 Hz, 1H), 5.94-5.83 (m, 1H), 5.60-5.54 (m, 3H), 5.27 (ddd, J = 1.6, 3.1, 17.2 Hz, 1H), 5.14 (dd, J = 1.2, 10.2 Hz, 1H), 5.11-5.05 (m, 2H), 4.54 (dd, J = 2.7, 12.1 Hz, 1H), 4.41-4.28 (m, 6H), 4.25-4.16 (m, 2H), 3.99 (dd, J = 8.2, 12.1 Hz, 1H), 3.77 (s, 3H), 2.45 (dd, J = 5.1, 13.3 Hz, 1H), 2.21 (s, 3H), 2.11 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 1.96 (s, 6H), 1.85 (s, 3H), 1.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.7, 170.5, 170.4, 170.3, 170.3, 170.2, 170.0, 167.5, 162.9, 155.4, 146.2, 134.1 (d, J_{C-P} = 5.8 Hz), 117.5, 98.2 (d, J_{C-P} = 5.8 Hz), 97.2, 82.4 (d, J_{C-P} = 4.4 Hz), 73.9, 72.6, 72.0 (d, 2.9 Hz), 71.0, 69.4, 69.0, 64.9 (d, J_{C-P} = 16.0 Hz), 62.8, 60.0, 53.2, 48.3, 37.8, 24.7, 23.1, 20.9, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6; ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ 134.9; MS (FAB+) calcd for $C_{38}H_{51}N_4O_{22}P$ (M + H^+) 947, found 947.

Allyl 2',3'-*O*,N^4-Triacetylcytidin-5'-yl Methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphite (11a): This compound was synthesized

according to the general coupling procedure. The crude solid was purified by silica gel chromatography (9:1:0.1 EtOAc:Hexanes:Et_3N to 9:1:0.1 EtOAc:MeOH:Et_3N) to provide **11a** (243 mg) in 82% yield (1.1:1 inseparable mixture of diastereomers): IR (thin film, $CHCl_3$) 3144, 2958, 1749, 1674, 1628, 1559, 1491, 1437, 1371, 1314, 1232, 1115, 1057 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (s, 2.1 H), 8.17 (d, J = 7.4 Hz, 1.1 H), 8.06 (d, J = 7.4 Hz, 1H), 7.47 (m, 2.1H), 6.32 (d, J = 5.9 Hz, 1.1H), 6.26 (d, J = 5.1 Hz, 1.0H), 6.02-5.86 (m, 2.1H), 5.42-5.13 (m, 16.1H), 4.93 (dd, J = 9.8, 10.2 Hz, 2.1H), 4.51-4.26 (m, 18.3H), 3.83 (s, 3.3H), 3.79 (s, 3.0H), 2.58 (dd, J = 4.7, 13.3 Hz, 1.0H), 2.47 (dd, J = 5.1, 12.9 Hz, 1.1H), 2.24 (s, 6.3H), 2.12-1.99 (m, 44.1H), 1.96 (s, 6.3H); ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ 137.2, 136.6; MS (FAB-) calcd for $C_{38}H_{49}N_3O_{22}P$ (M - H^+) 946, found (M - H^+) 946.

Allyl 2',3'-*O*,N^4-Triacetylcytidin-5'-yl Methyl 5-(2-(acetoxy) acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphite (11b): This compound was synthesized according to the general coupling procedure. The crude solid was purified by silica gel chromatography (20:1:0.1 EtOAc:MeOH:Et_3N) to give **11b** (69 mg) in 49% yield (6.2:1 diastereomeric ratio) as a white foam: $[\alpha]^{25}_D$ -21.5° (c 1.6, $CHCl_3$); IR (thin film, $CHCl_3$) 3318, 2957, 1747, 1665, 1626, 1560, 1488, 1437, 1372, 1323, 1229, 1115, 1040 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.63 (d, J = 7.4 Hz, 1H), 7.44 (d, J = 7.4 Hz, 1H), 5.93-5.79 (m, 2H), 5.55-5.52 (m, 3H), 5.28 (dd, J = 1.6, 11.3 Hz, 1H), 5.23-5.13 (m, 2H) 5.01 (dt, J = 3.1, 8.6, 1H), 4.86 (d, J = 14.9 Hz, 1H), 4.50 (dd, J = 2.3, 12.1 Hz, 1H), 4.37-4.27 (m, 9H), 3.95 (dd, J = 8.6, 12.1 Hz, 1H), 3.78 (s, 3H), 2.46 (dd, J = 4.7, 12.9 Hz, 1H), 2.23 (s, 3H), 2.10 (s, 6H), 2.07 (s, 3H), 2.06 (s, 3H), 1.99 (s, 3H), 1.92 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 171.0, 170.7, 170.5, 170.4, 170.4, 170.0, 170.0, 170.0, 167.9, 167.6, 162.9, 147.8, 134.3 (d, J_{C-P} = 5.8 Hz), 116.8, 98.1 (d, J_{C-P} = 7.4 Hz), 97.2, 82.2 (d, J_{C-P} = 4.4 Hz), 73.4, 72.2, 71.9 (d, J_{C-P} = 2.2 Hz), 71.0, 69.6, 68.9, 64.4 (d, J_{C-P} = 13.8 Hz), 63.0, 62.8, 60.0, 53.2, 48.1, 38.1, 24.7, 20.9, 20.9, 20.7, 20.6, 20.6, 20.5, 20.5, 20.5; ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ 134.2; MS (FAB+) calcd for $C_{40}H_{53}N_4O_{24}P$ (M + H) 1005, found (M + H) 1005.

Allyl 2',3'-*O*,N^4-Triacetylcytidin-5'-yl Methyl 4,7,8,9-tetra-*O*-acetyl-5-amino-5-*N*-benzyloxycarbonyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphite (11c): This compound was synthesized according to the general coupling procedure. The crude solid was purified by silica gel chromatography (20:1 EtOAc:MeOH) to provide **11c** (92 mg) in 43% yield (2.9:1 diastereomeric ratio) as a white foam: IR (thin film, $CHCl_3$) 3330, 2957, 1744, 1670, 1628, 1560, 1534, 1490, 1438, 1371, 1314, 1229, 1113, 1039 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.64 (s, 1H), 7.94 (d, J = 7.4 Hz, 1H), 7.41 (d, J = 7.4 Hz, 1H), 7.32-7.21 (m, 5H), 6.49 (d, J = 10.2 Hz, 1H), 6.17 (d, J = 5.5 Hz, 1H), 5.86 (m, 1H), 5.57-5.52 (m, 2H), 5.44 (t, J = 5.9 Hz, 1H), 5.27 (dd, J = 1.6, 17.2 Hz, 1H), 5.17-5.06 (m, 4H), 4.89 (d, J = 12.5 Hz, 1H), 4.58 (dd, J = 2.3, 12.1 Hz, 1H), 4.44-4.26 (m, 5H), 4.12-4.05 (m, 2H), 3.84 (dd, J = 10.2, 20.7 Hz, 1H), 3.77 (s, 3H), 2.49 (dd, J = 5.1, 13.3 Hz, 1H), 2.15 (s, 3H), 2.11 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H),1.98 (s, 3H), 1.90 (s, 3H), 1.76 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 170.6, 170.1, 170.0, 170.0, 167.3, 162.8 (d, J_{C-P} = 2.1 Hz), 156.2, 145.3, 136.7, 134.0 (d, J_{C-P} = 5.8 Hz), 128.4, 128.3, 127.8, 127.7, 127.6, 117.1, 98.0 (d, J_{C-P} = 5.1 Hz), 97.2, 89.1, 82.5, 74.0, 72.5, 71.7 (d, J_{C-P} = 2.1 Hz), 70.8, 68.8, 68.7, 66.5, 65.3 (d, J_{C-P} = 19.0 Hz), 62.5, 60.1, 53.1, 50.9, 37.8, 24.7, 21.0, 20.9, 20.7,

20.7, 20.5, 20.5, 20.4; ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ 135.2; MS (ES) calcd for $C_{44}H_{55}N_4O_{23}P$ (M + H^+) 1039, found (M + H^+) 1039.

General Procedure for the Oxidation of CMP-Sialic Acid Phosphites. A solution of **8**, **11a**, **11b**, or **11c** was prepared in 1.0 mL MeCN and cooled to 0°C for 30 minutes. Et_3N (30 eq) and tert-butyl hydroperoxide (20 eq) were added and the solution was warmed to RT and stirred for 1 hour. Upon completion of the reaction as monitored by TLC, dimethylsulfide (30 eq) was added and the solution was stirred for 15 minutes. The mixture was concentrated *in vacuo* and taken to the next step without purification.

General Procedure for Palladium Catalyzed Deallylation: A solution of the crude CMP-Sialic Acid phosphate was prepared in 1.0 mL MeCN and 5 eq. of diisopropyamine were added. Tetrakistriphenylphosphinepalladium(0) (0.05 eq) was added and the solution was allowed to stir for 30 minutes. The mixture was concentrated *in vacuo* and purified by silica gel chromatography (3:1:0.1 EtOAc:MeOH:Et_3N).

2',3'-*O*,*N*^{4}-Triacetylcytidin-5'-yl Methyl 5-(2-(acetoxy)acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2"-yl Phosphate (9): This compound was synthesized according to the general oxidation and deallylation procedures to provide **9** (24.9 mg) in 61% yield as a white solid. A small portion of the allyl phosphate intermediate was purified and characterized (major diastereomer): $[\alpha]^{25}_D$ -21.4° (c 0.8, $CHCl_3$); IR (thin film, $CHCl_3$) 3316, 3028, 3003, 2957, 2854, 1744, 1671, 1630, 1556, 1483, 1438, 1410, 1372, 1324, 1232, 1224, 1216, 1210, 1203, 1112, 1073, 1037 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1H), 7.68 (d, J = 6.6 Hz, 1H), 7.48 (d, J = 10.6 Hz, 1H), 7.42 (d, J = 7.0 Hz, 1H), 5.97-5.86 (m, 1H), 5.74-5.69 (m, 2H), 6.64-5.61 (m, 2H), 5.37-5.32 (m, 1H), 5.26-5.17 (m, 3H), 4.63-4.59 (m, 3H), 4.52 (dd, J = 2.7, 10.6, 1H), 4.40-4.35 (m, 2H), 4.31-4.23 (m, 2H), 4.15 (dd, J = 8.6, 12.1 Hz, 1H), 3.82 (s, 3H), 2.63 (dd, J = 5.1, 13.3 Hz, 1H), 2.21 (s, 3H), 2.15 (s, 3H), 2.09 (s, 6H), 1.99 (s, 3H), 1.94 (m, 4H), 1.89 (s, 3H), 1.85 (s, 3H); ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ -6.9; MS (FAB) calcd for $C_{38}H_{51}N_4O_{23}P$ (M + H^+) 963, found (M + H^+) 963.

Compound 9: $[\alpha]^{25}_D$ 2.8° (c 1.1, $CHCl_3$); IR (thin film, $CHCl_3$) 3266, 2926, 1746, 1659, 1564, 1493, 1438, 1371, 1314, 1233, 1166, 1120, 1089, 1038 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (d, J =7.8 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 6.08 (d, J = 4.8 Hz, 1H), 5.41-5.36 (m, 2H), 5.32 (dd, J = 2.4, 4.8 Hz, 1H), 5.20-5.10 (m, 2H), 4.45 (dd, J = 2.9, 12.3 Hz, 1H), 4.33 (dd, J = 2.4, 11.0 Hz, 1H), 4.29-4.27 (m, 1H), 4.17-4.05 (m, 4H), 3.88 (t, J = 10.4 Hz, 1H), 3.66 (s, 3H), 2.60 (dd, J = 4.8, 13.1, 1H), 2.05 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.95 (s, 3H), 1.90 (s, 3H), 1.87 (s, 3H), 1.82 (s, 3H), 1.72 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD, 85% H_3PO_4 external standard = 0.00) δ -4.8; MS (FAB-) calcd for $C_{35}H_{46}N_4O_{23}P$ (phosphate anion = M) 921, found (M) 921.

2',3'-*O*,*N*^{4}-Triacetylcytidin-5'-yl Methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2"-yl Phosphate (12a): This compound was synthesized according to the general oxidation and deallylation procedures to give **12a** (98 mg) in 59% yield as a white solid. A small portion of the allyl phosphate intermediate was purified and characterized (1:1 mixture of diastereomers): IR (thin film, $CHCl_3$) 3222, 3087, 2968, 2934, 1750, 1672, 1628, 1560, 1491, 1438, 1371, 1313, 1234, 1175, 1115, 1041cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.30 (s, 2H), 8.01-7.99 (m, 2H), 7.46-7.43 (m, 2H),

6.21-6.18 (m, 2H), 6.02-5.88 (m, 2H), 5.45-5.19 (m, 17H), 4.97-4.92 (m, 2H), 4.69-4.58 (m, 4H), 4.48-4.33 (m, 12H), 4.19-4.13 (m, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 3.34 (dd, J = 7.4, 14.5 Hz, 1H), 3.26 (dd, J = 7.0, 14.5 Hz, 1H), 2.79-2.72 (m, 2H), 2.23 (s, 6H), 2.08-2.05 (m, 29H), 2.05-1.95 (m, 19H); ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ -5.2, -5.8; MS (FAB) calcd for $C_{38}H_{49}N_3O_{24}P$ (M - H^+) 962, found (M - H^+) 962.

Compound 12a: $[\alpha]^{25}_D$ 2.6° (c 1.6, $CHCl_3$); IR (thin film, $CHCl_3$) 2956, 1749, 1669, 1636, 1624, 1559, 1493, 1456, 1437, 1372, 1312, 1238, 1166, 1124, 1078, 1056, 1010 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.45 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 6.00 (d, J = 3.5 Hz, 1H), 5.48 (dd, J = 3.7, 5.4 Hz, 1H), 5.36-5.33 (m, 2H), 5.28-5.17 (m, 2H), 4.49-4.44 (m, 2H), 4.36-4.34 (m, 1H), 4.00 (ddd, J = 2.4, 4.8, 11.7 Hz, 1H), 4.14 (dd, J = 7.0, 12.3 Hz, 1H), 4.06 (ddd, J = 2.7, 5.1, 11.8 Hz, 1H), 3.71 (s, 3H), 2.64 (dd, J = 5.4, 13.6 Hz, 1H), 2.11 (s, 3H), 2.02 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.84 (s, 3H), 1.82 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD, 85% H_3PO_4 external standard = 0.00) δ -4.8; MS (ES) calcd for $C_{35}H_{45}N_3O_{24}P$ (phosphate anion = M) 922, found (M) 922.

2',3'-*O*,*N*^{4}-Triacetylcytidin-5'-yl Methyl 5-(2-(acetoxy)acetamido)-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphate (12b): This compound was synthesized according to the general oxidation and deallylation procedures to provide **12b** (11.3 mg) in 47% yield as a white solid. A small portion of the allyl phosphate intermediate was purified and characterized: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1H), 8.09 (d, J = 9.8 Hz, 1H), 7.56 (d, J = 6.6 Hz, 1H), 7.46 (d, J = 7.4 Hz, 1H), 5.97-5.78 (m, 2H), 5.62-5.57 (m, 2H), 5.42-5.32 (m, 2H), 5.26-5.16 (m, 3H), 4.85 (d, J = 14.9 Hz, 1H), 4.62-4.59 (m, 3H), 4.53 (dd, J = 2.7, 11.0 Hz, 1H), 4.43-4.29 (m, 6H), 4.15 (dd, J = 8.6, 11.7 Hz, 1H), 3.82 (s, 3H), 2.62 (dd, J = 5.1, 13.3 Hz, 1H), 2.25 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.93 (s, 3H), 1.82 (s, 3H); ^{31}P NMR (162 MHz, $CDCl_3$, 85% H_3PO_4 external standard = 0.00) δ -6.9; MS (ES) calcd for $C_{40}H_{53}N_4O_{25}P$ (M - H^+) 1019, found (M - H^+) 1019.

Compound 12b: $[\alpha]^{25}_D$ -2.7° (c 1.0, $CHCl_3$); IR (thin film, $CHCl_3$) 3224, 2955, 1747, 1694, 1660, 1651, 1621, 1566, 1556, 1538, 1494, 1434, 1372, 1316, 1235, 1167, 1118, 1039 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (d, J = 7.4 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 6.05 (d, J = 4.3 Hz, 1H), 5.41-5.36 (m, 2H), 5.28 (dd, J = 2.3, 5.1 Hz, 1H), 5.24 (ddd, J = 5.1, 10.6, 11.3 Hz, 1H), 5.14-5.10 (m, 1H), 4.46-4.40 (m, 2H), 4.31 (d, J = 14.9 Hz, 1H), 4.28 (m, 1H), 4.22 (d, J = 14.9 Hz, 1H), 4.14 (ddd, J = 2.7, 5.1, 12.1 Hz, 1H), 4.12-4.06 (m, 2H), 3.93 (t, J = 10.6 Hz, 1H), 3.67 (s, 3H), 2.58 (dd, J = 5.1, 13.3 Hz, 1H), 2.06 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.88 (s, 3H), 1.82 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD, 85% H_3PO_4 external standard = 0.00) δ -4.8; MS (FAB) calcd for $C_{37}H_{48}N_4O_{25}P$ (phosphate anion = M) 979, found (M) 979.

2',3'-*O*,*N*^{4}-Triacetylcytidin-5'-yl Methyl 4,7,8,9-tetra-*O*-acetyl-5-amino-5-*N*-benzyloxycarbonyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2''-yl Phosphate (12c): This compound was synthesized according to the general oxidation and deallylation procedures to give **12c** (47 mg) in 59% yield as a white solid. Due to the instability of the allyl phosphite intermediate to silica gel, no phosphate sample was purified for characterization: MS (ES) calcd for $C_{44}H_{55}N_4O_{24}P$ (M - H^+) 1053, found (M - H^+) 953.

Compound 12c: $[\alpha]^{25}_D$ 10.9° (c 1.8, $CHCl_3$); IR (thin film, $CHCl_3$) 3568, 2956, 1744, 1736, 1685, 1670, 1664, 1654, 1648, 1637, 1624, 1618, 1570, 1560, 1534, 1430, 1370, 1312, 1234, 1170, 1122, 1078, 1039 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) δ 8.40 (d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.5 Hz, 1H), 7.24-7.23 (m, 5H), 6.08 (d, J = 4.3 Hz, 1H), 5.45-5.39 (m, 3H), 5.20-5.13 (m, 2H), 5.00 (d, J = 12.3 Hz, 1H), 4.84 (d, J = 12.6 Hz, 1H), 4.48 (dd, J = 2.9, 12.3 Hz, 1H), 4.34 (dd, J= 2.4, 10.7 Hz, 1H), 4.30-4.24 (m, 1H), 4.21-4.03 (m, 3H), 3.69 (s, 3H), 2.60 (dd, J = 5.1, 13.4 Hz, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.96 (s, 3H), 1.92 (s, 3H), 1.67 (s, 3H); ^{31}P NMR (162 MHz, CD_3OD, 85% H_3PO_4 external standard = 0.00) δ -4.86; MS (ESI-) calcd for $C_{41}H_{50}N_4O_{24}P$ (phosphate anion = M) 1013, found (M) 1013.

General Procedure for Acetate and Methyl Ester Hydrolysis: A solution of **2**, **13a**, **13b**, or **13c** was prepared in 0.5 mL MeOH, treated with 1 mg NaOMe, and allowed to stir for 30 minutes. The reaction was directly purified by size exclusion chromatography (Biogel P-2). Fractions containing product were combined, reduced, and taken onto the saponification without further characterization. The ester was dissolved in 1.0 mL of H_2O to which 0.5 mL of 1.0N NaOH was added and stirred for 10 minutes. The reaction was directly subjected to size exclusion chromatography (Biogel P-2) and fractions containing product were combined and lyophilized.

Cytidin-5'-yl 5-Acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2"-yl Phosphate (2): This compound was synthesized according to the general hydrolysis procedure to give **2** (11.2 mg) in 81% yield as a white foam. The ^1H NMR spectrum and MS were in good agreement with the reported data.[6]

Cytidin-5'-yl 3-deoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2"-yl Phosphate (13a): This compound was synthesized according to the general hydrolysis procedure to provide **13a** (28.3 mg) in 63% yield as a white foam: ^1H NMR (400 MHz, D_2O) δ 7.91 (d, J = 7.4 Hz, 1H), 6.06 (d, J = 7.4 Hz, 1H), 5.93 (d, J = 4.3 Hz, 1H), 4.28 (t, J = 4.7 Hz, 1H), 4.24 (dd, J = 4.3, 5.1 Hz, 1H), 4.18 (m, 3H), 4.05 (d, J = 10.2 Hz, 1H), 4.00-3.93 (m, 1H), 3.90-3.51 (m, 2H), 3.69 (d, J = 10.2 Hz, 1H), 3.61 (dd, J = 7.0, 12.5 Hz, 1H), 3.53 (t, J = 9.8 Hz, 1H), 2.37 (dd, J = 4.7, 12.9 Hz, 1H), 1.54 (ddt, J = 1.2, 5.9, 12.5, 1H); ^{13}C NMR (HMQC, 100 MHz, D_2O) δ 142.8, 98.0, 90.5, 84.1, 75.4, 74.2, 71.0, 70.5, 69.8, 69.4, 66.0, 64.2, 54.7, 41.9; ^{31}P NMR (162 MHz, D_2O, 85% H_3PO_4 external standard = 0.00) δ -3.9; HRMS (FAB-) calcd for $C_{18}H_{26}N_3O_{16}P$ (M - H_2 + Na^+) 594.0948, found (M - H_2 + Na^+) 594.0953.

Cytidin-5'-yl 5-(2-(acetoxy)acetamido-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosid-2"-yl Phosphate (13b): This compound was synthesized according to the general hydrolysis procedure to provide **13b** (12.3 mg) in 88% as a white foam: ^1H NMR (400 MHz, D_2O) δ 7.90 (d, J = 7.4 Hz, 1H), 6.05 (d, J = 7.4 Hz, 1H), 5.92 (d, J = 4.3 Hz, 1H), 4.27 (t, J = 4.7 Hz, 1H), 4.23 (dd, J = 4.3, 4.7 Hz, 1H), 4.17 (m, 4H), 4.13-4.06 (m, 3H), 3.96 (dd, J = 9.8, 10.6 Hz, 1H), 3.89-3.85 (m, 1H), 3.81 (dd, 2.4, 12.1 Hz, 1H), 3.55 (dd, J = 6.6, 12.1 Hz, 1H), 3.36 (d, J = 9.8 Hz, 1H), 2.43 (dd, J = 4.7, 13.7 Hz, 1H), 1.59 (ddt, J = 1.2, 5.9, 12.9 Hz, 1H); ^{13}C NMR (HMQC, 100 MHz, D_2O) δ 143.1, 130.3, 98.0, 90.0, 84.3, 75.6, 73.0, 71.0, 70.5, 70.1, 66.1, 64.2, 62.3, 52.7, 42.2; ^{31}P NMR (162 MHz, D_2O, 85% H_3PO_4 external standard = 0.00) δ -4.0; HRMS (FAB-) calcd for $C_{20}H_{29}N_4O_{17}P$ (MH^-) 629.1344, found (MH^-) 629.1442.

Cytidin-5'-yl 5-*N*-Carbobenzyloxylamino-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-

nonulopyranosid-2"-yl Phosphate (13c): This compound was synthesized according to the general hydrolysis procedure to give **13c** (6.3 mg) in 85% yield as a white foam: ^{1}H NMR (400 MHz, D_2O) δ 7.88 (d, J = 7.8 Hz, 1H), 7.40-7.25 (m, 5H), 6.02 (d, J = 7.4 Hz, 1H), 5.90 (d, 4.3 Hz, 1H), 5.06 (dd, J = 12.9, 26.6 Hz, 2H), 4.27-4.24 (m, 1H), 4.20 (dd, J = 4.7, 5.1 Hz, 1H), 4.17-4.12 (m, 3H), 4.65 (d, J = 10.6 Hz, 1H), 3.96 (dt, J = 4.7, 11.0 Hz, 1H), 3.84 (m, 1H), 3.77 (dd, J = 2.0, 12.2 Hz, 1H), 3.60 (dd, J = 10.2, 10.6 Hz, 1H), 3.51-3.46 (m, 1H), 3.38 (d, J = 9.8 Hz, 1H), 2.39 (dd, J = 4.3, 12.9 Hz, 1H), 1.56 (ddt, J = 0.8, 5.1, 12.1 Hz, 1H); ^{13}C NMR (HMQC, 100 MHz, D_2O) δ 143.2, 129.6, 129.6, 128.9, 97.7, 90.0, 84.6, 75.8, 73.5, 71.2, 70.8, 70.4, 68.5, 68.5, 66.5, 64.6, 54.8, 42.2; ^{31}P NMR (162 MHz, D_2O, 85% H_3PO_4 external standard = 0.00) δ -4.0; HRMS (FAB-) calcd for $C_{26}H_{33}N_4O_{17}P$ (MH^-) 705.1657, found (MH^-) 705.1702.

References and Notes

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