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A Convenient Synthesis of Nucleoside Monophosphate-*N*-Acetylneuraminic Acids (NMP-Neu5Ac)[1]

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Abstract—Reaction of sialyl phosphites **2a,b** with acetyl-protected ribonucleoside monophosphates **3C**, **3U**, **3A** and **3G** furnished without addition of a catalyst directly the corresponding β-configurated sialyl ribonucleoside monophosphates **4C**, **4U**, **4A** and **4G**, respectively. Treatment of these compounds with sodium methanolate in methanol and sodium hydroxide afforded the disodium salts of CMP-Neu5Ac (**1C**), UMP-Neu5Ac (**1U**), AMP-Neu5Ac (**1A**), and GMP-Neu5Ac (**1G**).

Introduction

Glycoside bond formation in nature is essentially based on glycosyl donors, having phosphates, pyrophosphates, and their ribonucleoside and lipid monoester derivatives as leaving groups, and glycosyltransferases as catalysts.[2-4] For instance, for aldoses generally nucleoside diphosphate sugars and for 3-deoxy-2-glyculosonates (KDO, Neu5Ac) nucleoside monophosphate derivatives, respectively, are encountered as glycosyl donors (Leloir pathway). The different glycosyltransferases employ also different nucleoside moieties as part of the glycosyl donor leaving group. Therefore, glycosyltransferases seem to specifically recognize the sugar and the nucleoside moieties and, additionally, to generate glycosyl donor properties via activation of the phosphate moiety. These factors can be studied with the help of nucleoside phosphate sugars and especially analogs not accessible by enzymatic means. The importance of the synthesis of such compounds has been recently well documented.[4-7]

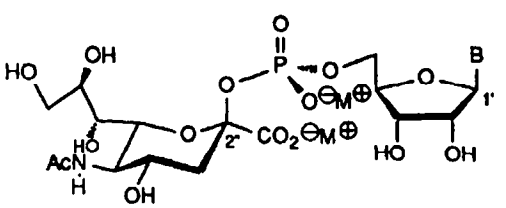
A particularly interesting target is natural CMP-Neu5Ac (**1C** in Scheme I) which is required for the enzymatic sialylation of glycoconjugates, i.e. the biosynthesis of gangliosides and sialylated glycoproteins with the help of sialyltransferases.[5,7-9] Enzymatic and chemical approaches have been reported for the synthesis of **1C**.[5,7 9] We would like to report in detail the synthesis of the nucleoside monophosphate-*N*-acetylneuraminates **1C**, **1U**, **1A** and **1G** which are derived from ribonucleoside monophosphates CMP, UMP, AMP, and GMP, respectively;[1,7] the synthesis is based on the versatile sialyl phosphites as sialyl donors,[7,10,11] thus exhibiting the ready accessibility of various types of sialyl phosphate derivatives.[12]

Results and Discussion

A particularly pleasing property of *O*-glycosyl-trichloroacetimidates as glycosyl donors **D** (Scheme II, - OX = -OC(CCl$_3$)=NH) is their direct reaction with Brønsted acids (HA) as glycosyl acceptors **A** under formation of glycosylation products **P** and trichloroacetamide [O=XH ≙ O=C(CCl$_3$)NH$_2$];[13] due to the acidity of the acceptor HA generally an additional catalyst is not required in this reaction.

Obviously, a similar reaction behaviour can be envisaged for glycosyl phosphites as glycosyl donors: protonation of the phosphite moiety with the help of the acid HA as acceptor **A** will generate an activated donor which reacts with the acceptor anion (A^-), presumably via ion pairs, under release of a phosphonate group [O=XH ≙ O=PH(OR)$_2$] to the product **P**.

As sialyl donors diethylphosphite **2a** and bis(trichloroethyl)phosphite **2b** were employed which are readily available from Neu5Ac via methyl 4,7,8,9-tetra-*O*-acetyl-*N*-acetylneuraminate[14] in three convenient steps (overall yields 80 % and 70 %, respectively) (Scheme III).[10] Direct formation of CMP-Neu5Ac (**1C**) from **2a** and CMP failed because of the insolubility of CMP in solvent systems required for the reaction to occur.[1]



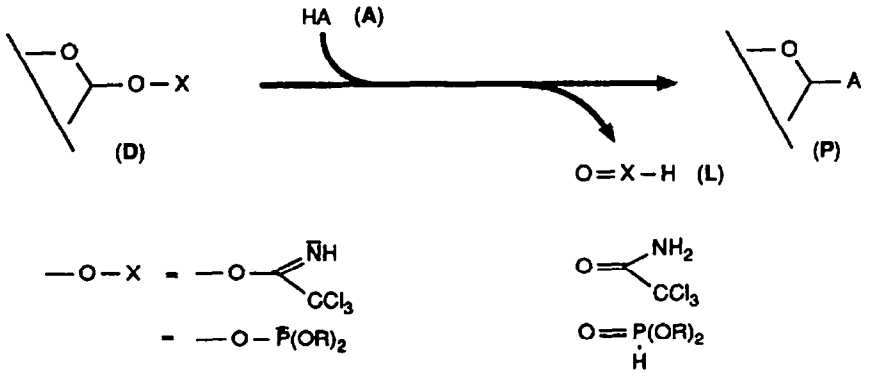
1C (CMP-Neu5Ac), B = cytosine
1U (UMP-Neu5Ac), B = uracil
1A (AMP-Neu5Ac), B = adenine
1G (GMP-Neu5Ac), B = guanine

Scheme I.

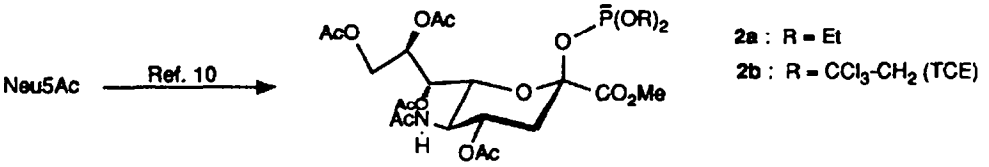
Therefore, CMP was transformed by treatment with acetic anhydride in pyridine and then ion exchange resin (Dowex 50, H^+) into triacetyl derivative **3C** (Scheme IV) which exhibited reasonable solubility in an acetonitrile:DMF mixture (1:2, by volume).

Addition of **2a** to this solution at –20 °C and slowly raising the temperature to room temperature (rt) afforded, due to thermodynamic reaction control, the β-configurated acetylated CMP-Neu5Ac derivative **4C** (Scheme V). The product was separated by flash chromatography with chloroform:methanol (4:1) and precipitated from a solution in methanol with ethyl acetate/petroleum ether and thus obtained as solid material in 50 % yield. The NMR data support the assigned structure. Reaction of the more powerful, however less basic sialyl donor **2b** which exhibits higher stability at room temperature,[10] with CMP-derivative **3C** also gave directly acylated CMP-Neu5Ac intermediate **4C**, albeit in lower yield (Scheme V). Final structural proof came from the transformation of **4C** into the desired CMP-Neu5Ac (**1C**). Treatment of **4C** with sodium methanolate/methanol and then sodium hydroxide in methanol water led to practically quantitative formation of the disodium salt of CMP-Neu5Ac (**1C**, $M^+ = Na^+$) which was neutralized (~ pH 7–8) by ion exchange resin (Amberlite IR 120, H^+). After filtration and evaporation the residue was dissolved in water. The product was isolated by precipitation with methanol and reprecipitation with methanol/diethyl ether as solid material in 68 % yield. **1C** is identical in all aspects with commercially available material.[15] The ^1H NMR spectrum recorded from a deuterium oxide solution is shown in Figure 1.

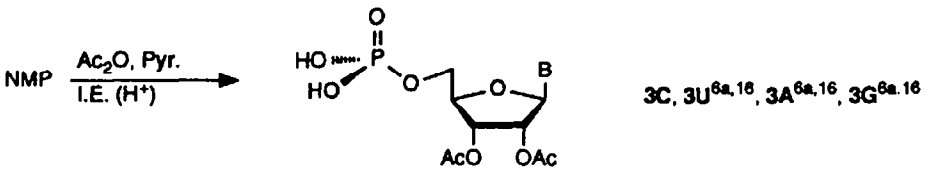
These results encouraged us to investigate the synthesis of the nucleoside monophosphate *N*-acetylneuraminates **1U**, **1A** and **1G** derived from the corresponding uridine, adenosine, and guanosine monophosphates. To this end, these compounds were converted into the known acetylated derivatives **3U**, **3A** and **3G**.[6a,16] Reaction of **3U** with **2a** gave under similar reaction conditions the acetylated intermediate **4U** in 62 % yield; again, reaction with **2b** as sialyl donor afforded **4U** in lower

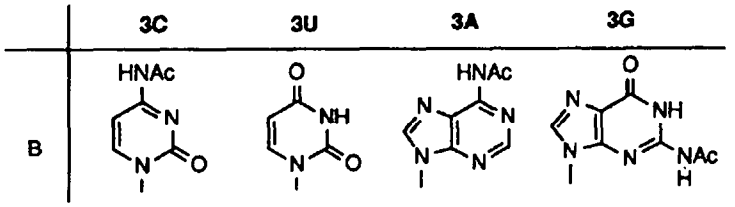


Scheme II.



Scheme III.





Scheme IV.

yield. Removal of the *O*-acetyl groups and methyl ester cleavage in the neuraminic acid moiety could be accomplished with sodium methanolate in methanol and sodium hydroxide in high yield, thus furnishing unnatural UMP-Neu5Ac as disodium salt **1U**. Similarly, triacetyl AMP (**3A**) and triacetyl GMP (**3G**) gave with **2a** directly compounds **4A** and **4G**, respectively. Their treatment with NaOMe in methanol/water furnished the disodium salts of AMP-Neu5Ac (**1A**) and GMP-Neu5Ac (**1G**) in high yields (Scheme V). The possible substrate character of **1U**, **1A** and **1G** is currently under investigation.[17]

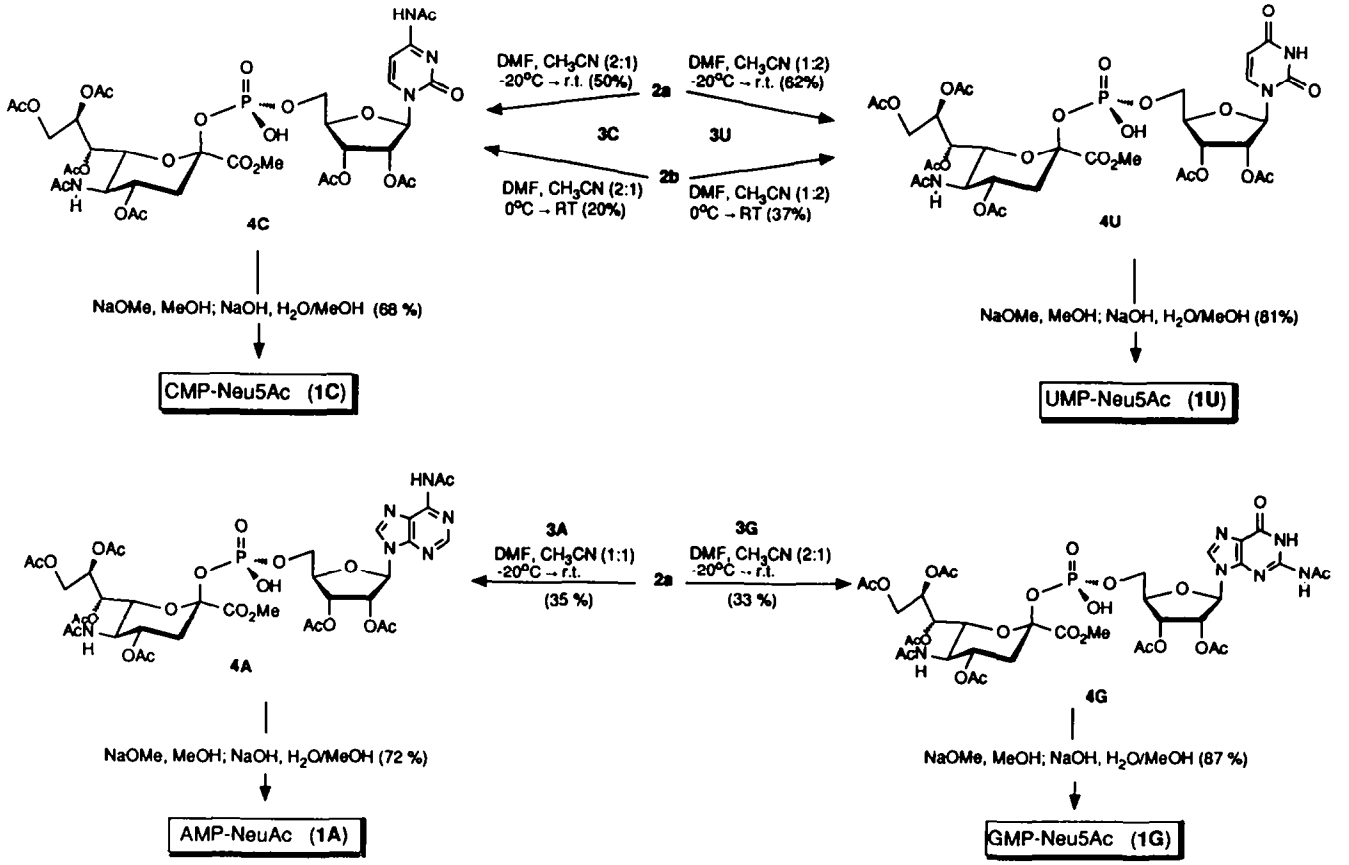
In conclusion, a convenient and practical synthesis of ribonucleoside monophosphate *N*-acetylneuraminates **1** could be accomplished which was performed for CMP-Neu5Ac (**1C**) already in 0.5 g scale of final product.[1] The procedure is based on the direct β-selective reaction of sialyl β-phosphites **2a,b** with acetylated nucleoside monophosphates **3** and ensuing convenient deacetylation and methyl ester cleavage in intermediate **4**. Obviously, this method can be extended to various phosphorous acid derivatives[7] and also to other Brønsted acids.

Experimental Section

Solvents were purified and dried in the usual way; the boiling range of the petroleum ether used was 35–65 °C. ^{1}H NMR spectra: Bruker WM 250 cryospec, internal standard tetramethylsilane (TMS). Flash chromatography: J. T. Baker silica gel (30–60 μm) at a pressure of 0.3 bar. Thin-layer chromatography (TLC): Merck glass plates NH_2 F_{254}, layer thickness 0.2 mm. Merck plastic plates Cellulose F, layer thickness 0.1 mm; Merck plastic plates silica gel 60 F_{254}, layer thickness 0.2 mm, detection by treatment with a solution of 15 % H_2SO_4, followed by heating at 120 °C. Optical rotations: Perkin–Elmer polarimeter 241/MS, 1 dm cell.

Triacetyl-CMP 3C

To a solution of cytidine 5'-monophosphate (CMP; lg, 3.09 mmol) in pyridine:water (50 mL, 4:1) was added tributylamine (1.47 mL, 6.18 mmol). The mixture was evaporated and coevaporated with dry pyridine (20 mL, three times). The residue was treated with acetic anhydride:pyridine (75 mL, 1:2) and stirred for 18 h at room temperature. After addition of water (75 mL) at 0 °C and stirring for 2 h, the solution was evaporated. Again, the residue was dissolved in water (5 mL); the salt was converted to the free acid by an ion exchange resin column (Dowex 50, H^+). Water (60 mL) was removed by freeze drying for 2 days, to give the dry crude product (1.3 g, 96 %) with 5 to 10 % impurity. A further purification was not necessary. R_f [cellulose, *n*-butanol:acetone:acetic acid:5 % NH_3:water (7:5:3:3:2)] = 0.78. ^{1}H NMR (250 MHz, D_2O), δ 1.97–1.98 (2 s, 6H, 2 $COCH_3$), 2.10 (s, 3H, $COCH_3$), 3.91–3.99 (m, 1H, 5'$_A$-H), 4.01–4.13 (m, 1H, 5'$_B$-H), 4.40–4.44 (m, 1H, 4'-H),



Scheme V.

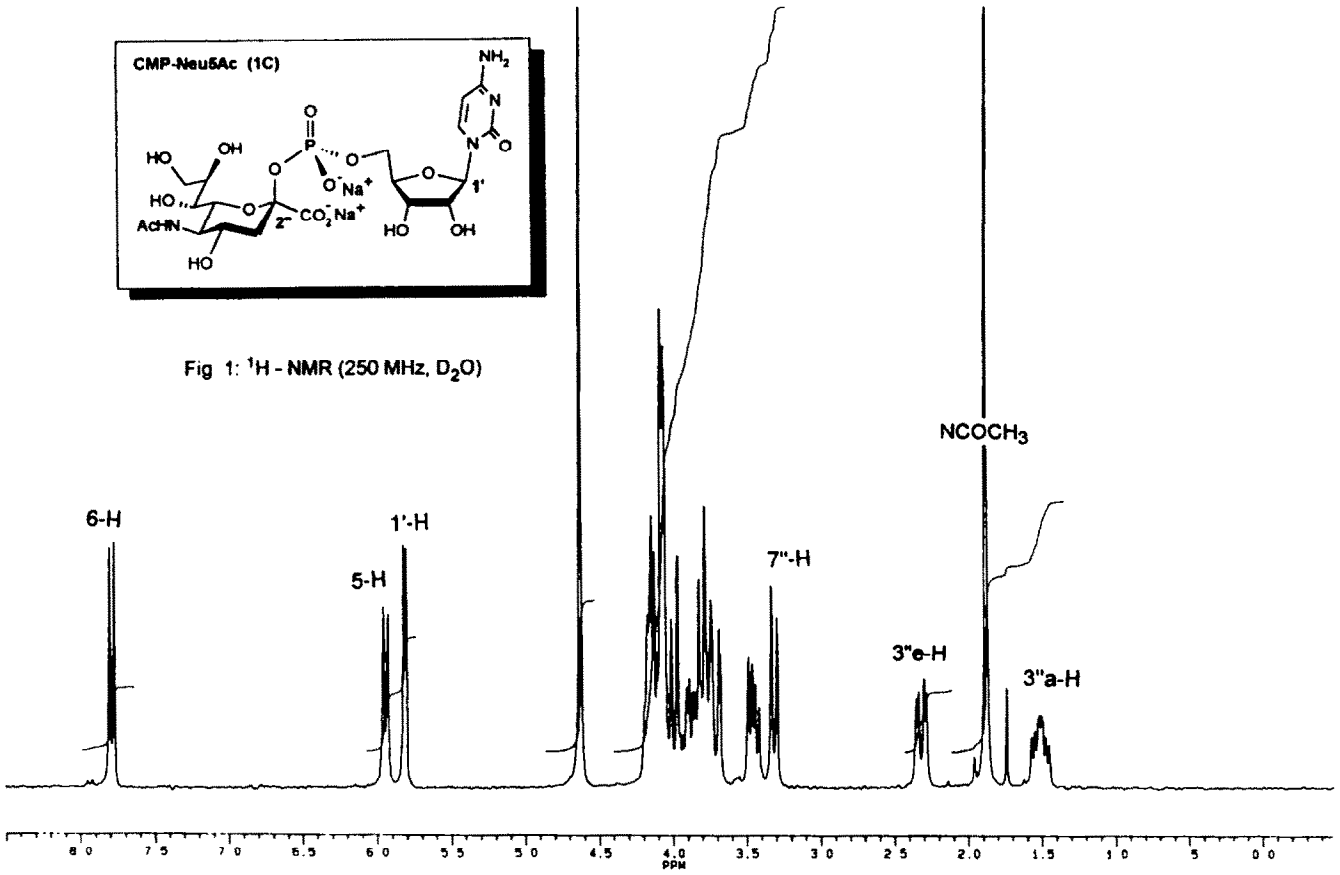


Figure 1. ^{1}H NMR (250 MHz, D_2O).

5.25–5.31 (m, $J_{3',4'}$ = 2.6 Hz, $J_{2',3'}$ = 5.3 Hz, 1H, 3'-H), 5.36 (dd, $J_{2',3'}$ = 5.3 Hz, 1H, 2'-H), 6.04 (d, $J_{1,2}$ = 4.5 Hz, 1H, 1'-H), 7.00 (d, $J_{5,6}$ = 7.6 Hz, 1H, 5-H), 8.31 (d, $J_{5,6}$ = 7.6 Hz, 1H, 6-H).

General procedure for the synthesis of compounds 4C, 4U, 4A and 4G

From 2a. To a solution of **3C**, **3U**, **3A** or **3G** (0.5 mmol) in a mixture of dry DMF:CH_3CN (5 mL, ratio: see Scheme V) was injected under stirring a solution of **2a** (0.8 mmol) in dry acetonitrile (15 mL) at –20 °C. Within 12 h the mixture was slowly warmed up to room temperature. The solvents were evaporated *in vacuo*, finally at 10^{-2} torr. The residue was purified by flash chromatography with chloroform:methanol as eluents (ratio: see Table 1). The product was dissolved in methanol and obtained pure by precipitation with ethyl acetate/petroleum ether. For yields, see Scheme V. Physical data are recorded in Table 1.

From 2b. To a solution of **3C** or **3U** (0.5 mmol) in a mixture of dry DMF:CH_3CN (5 mL, ratio: see Scheme V) was injected under stirring a solution of **2b** (0.8 mmol) in dry acetonitrile (1.5 mL) at 0 °C. After 12 h, the mixture was allowed to warm up to room temperature and kept there for 2 days. Then the solvents were evaporated *in vacuo* and work up was performed as described above.

General procedure for the synthesis of compounds 1C, 1U, 1A and 1G

To a solution of **4C**, **4U**, **4A** or **4G** (0.1 mmol) in dry methanol (3 mL) was added under stirring sodium methanolate (0.5 mL of a 0.1 M solution in methanol) at room temperature. After 3 to 4 h (TLC monitoring) the reaction mixture was neutralized (pH 7–8) by adding a cation exchange resin (Amberlite IR 120, H^+) and then concentrated *in vacuo*. Addition of ethanol and diethyl ether gave solid materials of the methyl ester, which were slurried in methanol (3 mL) and combined with a solution of sodium hydroxide (2 mL, 1 M) at room temperature. After 3 to 4 days (TLC-monitoring), the mixture was neutralized (pH ~ 7–8) by adding a cation exchange resin (Amberlite IR, H^+). After filtration, the solvents were evaporated *in vacuo*. The residue was dissolved in less water, the products were precipitated by slow addition of methanol. The precipitate was again dissolved in less water and the product reprecipitated by the addition of methanol and finally diethyl ether. Filtration led to pure product.

Physical Data of Compounds 4C, 4U, 4A, 4G, 1C, 1U, 1A, 1G

4C: $[\alpha]_D^{20}$ –19.9° (c = 1, $CHCl_3$). Mp 176 °C (decomp.) R_f [silica gel plates, $CHCl_3$:MeOH (4:1)] 0.25. ^{1}H NMR

(250 MHz, MeOD): δ 1.90 (s, 3H, $NCOCH_3$), 2.01–2.15 (6 s, 19H, H-3"a, 6 $COCH_3$), 2.24 (s, 3H, $COCH_3$), 2 74 (dd, $J_{3"e,4"}$ = 4.8 Hz, $J_{3"e,3"a}$ = 13.2 Hz, 1H, 3"e-H), 3.84 (s, 3H, $COOCH_3$), 4.05 (dd, $J_{5",6"}$ = 10.6 Hz, 1H, 5"-H), 4.23 (dd, $J_{8",9"B}$ = 7.1 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 2H, H-5'B, 9"B-H), 4.34–4.39 (m, 1H, 5'A-H), 4.43 (m, 1H, 4'-H), 4.49 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{5",6"}$ = 10.6 Hz, 1H, 6"-H), 4.66 (dd, $J_{8"9A"}$= 2.4 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1H, 9"A-H), 5.28–5.38 (m, $J_{3"e,4"}$ = 4.8 Hz, $J_{7",8"}$ = 4.7 Hz, 2H, 4"-H, 8"-H), 5.48 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{7",8"}$ = 4.7 Hz, lH, 7"-H), 5.55–5.61 (m, 2H, 2'-H, 3'-H), 6.17 (d, $J_{1',2'}$ = 3.7 Hz, lH, l'-H), 7.44 (d, J = 7.5 Hz, 1H, 5-H), 8.43 (d, J = 7.5 Hz, 1H, 6-H). ^{31}P NMR (161.7 MHz, MeOD): δ –3.59 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 921.

4U: R_f [silica gel plates, $CHCl_3$:MeOH (3:1)] 0.35. ^{1}H NMR (250 MHz, MeOD): δ = 1.90 (s, 3H, $NCOCH_3$), 2.00 (s, 3H, $COCH_3$), 2.06–2.17 (5 s, 16H, 3"a-H, 5 $COCH_3$), 2.76 (dd, $J_{3"e,4"}$ = 4.8 Hz, $J_{3"e,3"a}$ = 13.0 Hz, 1H, H-3"e), 3.84 (s, 3H, $COOCH_3$), 4.05 (dd, $J_{5",6"}$ = 10.6 Hz, 1H, 5"-H), 4.24 (m, 2H, 5'A-H, 5'B-H), 4.28 (dd, $J_{8",9"B}$ = 7.3 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1H, 9"B-H), 4.51 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{5",6"}$ = 10.6 Hz, 1H, 6"-H), 4.66 (dd, $J_{8",9"A}$ = 2.5 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1H, 9"A-H), 5.28–5.36 (m, $J_{3"e,4"}$ = 4.8 Hz, 2H, 4"-H, 8"-H), 5.47–5.51 (m, 2H, H-2', H-3'), 5.62 (dd, $J_{6",7"}$ = 2.2 Hz, lH, 7"-H), 5.90 (d, J = 8.1 Hz, lH, 5-H), 6.21 (d, $J_{1',2'}$ = 6.6 Hz, lH, l'-H), 8.08 (d, J = 8.1 Hz, lH, 6-H). ^{31}P NMR (161.7 MHz, MeOD): δ – 4.87 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 880.

^{1}H NMR (250 MHz, MeOD:$CDCl_3$ = 1:1): δ 1.86–2.11 (m, 11H, 7 $COCH_3$, 3"e-H), 2.70 (dd, $J_{3e,4}$ = 13.1 Hz, 1H, 3e-H), 3.79 (s, 3H, $COOCH_3$), 4.04 (dd, $J_{4",5"}$ = $J_{5",6"}$ = 10.5 Hz, 1H, 5"-H), 4.10–4.22 (m, 3H, 5'A-H, 5'B-H, 9"B-H), 4.27–4.28 (m, 1H, 4'-H), 4.37 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{5",6"}$ = 10.5 Hz, 1H, 6"-H), 4.59 (d, $J_{8",9"A}$ = 2.5 Hz, 1H, 9"A-H), 5.20 (ddd, $J_{3"e,4"}$ = 4.9 Hz, $J_{3"a,4"}$ = 11.1 Hz, 1H, 4"-H), 5.30–5.41 (m, 3H, 2'-H, 3'-H, 8"-H), 5.57 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{7",8"}$ = 5.5 Hz, lH, 7"-H), 5.82 (d, J = 8.1 Hz, 1H, 5-H), 6.22 (d, $J_{1',2'}$ = 7.1 Hz, 1H, l'-H), 7.96 (d, J = 8.1 Hz, 1H, 6-H).

4A: R_f [silica gel plates, $CHCl_3$:MeOH (6:1)] 0.25. ^{1}H NMR (250 MHz, MeOD): δ = 1.84–2.30 (8 s, 25H, 3"aH, 8 $COCH_3$), 2.74 (dd, $J_{3"e,4"}$ = 4.9 Hz, $J_{3"a,3"e}$ = 13.2 Hz, 1H, 3"e-H), 3.77 (s, 3H, $COOCH_3$), 4.00 (dd, $J_{4",5"}$ = $J_{5",6"}$ = 10.6 Hz, 1H, 5"-H), 4.18–4.28 (m, $J_{8",9"B}$ = 7.1 Hz, 3H, 5'A-H, 5'B-H, 9"B-H), 4.46 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{5",6"}$ = 10.6 Hz, 1H, 6"-H), 4.46–4.51 (m, 1H, 4'-H), 4.60 (dd, $J_{8",9"A}$ = 2.7 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1H, 9" A-H), 5.25–5.36 (m, $J_{3"e,4"}$ = 4.9 Hz, $J_{3"a,4"}$ = 11.5 Hz, $J_{8",9"A}$ = 2.7 Hz, $J_{7",8"}$ = 5.0 Hz, 2H 4"-H 8"-H) 5.44 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{7",8"}$ = 5.0 Hz, 1H, 7"-H), 5.78 (dd, $J_{2',3'}$ = 5.6 Hz, $J_{3',4'}$ = 3.1 Hz, 1 H, 3'-H), 5.98 (dd, $J_{1',2'}$ = 6.1 Hz, $J_{2',3'}$ = 5.6 Hz, 1 H, 2'-H), 6.48 (d, $J_{1',2'}$ = 6.1 Hz, 1H, l'-H), 8.99 (s, 1H, 2-H), 9.06 (s, 1H, 8-H). ^{31}P NMR (161.7 MHz, MeOD): δ = –5.24 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 945, $(MNa - H)^-$ 967.

4G: R_f [silica gel plates, $CHCl_3$/MeOH (3:1)] 0.30. ^{1}H NMR (250 MHz, MeOD:$CDCl_3$ = 3:1): δ = 1.85–2.25 (8 s, 25H, 3"a-H, 8 $COCH_3$), 2.73 (dd, $J_{3"e,4"}$ = 5.0 Hz, $J_{3"a,3"e}$ = 13.1 Hz, 1H, 3"e-H), 3.78 (s, 3H, $COOCH_3$), 4.01 (dd $J_{4",5"}$ = $J_{5",6"}$ = 10.5 Hz, lH, 5"-H), 4.19–4.27 (m, $J_{8",9"B}$ = 7.4 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 3H, 5'A-H,5'B-H, 9"B-H), 4.37 (m, 1H, 4'-H), 4.47 (dd, $J_{5",6"}$ = 10.5 Hz, $J_{6",7"}$ = 2.1 Hz, 1H, 6"-H), 4.64 (dd, $J_{8",9"A}$ = 2.6 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1H, 9"A-H), 5.27 (ddd, $J_{3"e,4"}$ = 5.0 Hz, $J_{4",5"}$ = 10.5 Hz, 1H, 4"-H), 5.34 (ddd, $J_{8",9"A}$ = 2.6 Hz, $J_{7",8"}$ = 4.5 Hz, $J_{8",9"B}$ = 7.4 Hz, 1H, 8"-H), 5.43 (dd, $J_{6",7"}$ = 2.1 Hz, $J_{7",8"}$ = 4.5, 1H, 7"-H), 5.72 (dd, $J_{3',4'}$ = 2.1 Hz, $J_{2',3'}$ = 5.3 Hz, lH, 3'-H), 6.09 (d, $J_{1',2'}$ =7.1 Hz, lH, l'-H), 6.20 (dd, $J_{1',2'}$ = 7.1 Hz, $J_{2',3'}$ = 5.3Hz, 1H, 2'-H), 8.17 (s, lH, 8-H). ^{31}P NMR (161.7 MHz, MeOD): δ –5.36 (δ, 100 %). MS (FAB, negative mode): $(M - H)^-$ 961, M^- 962.

Disodium (5-acetamido-3,5-dideoxy-δ-D-glycero-D-galacto-2-nonulopyranosyl)onate cytidine phosphate (CMP-Neu5Ac **1C**)

R_f [aminophase, EtOH:H_2O (3:7, 0.2 M NaCl-solution)] 0.72. ^{1}H NMR (250 MHz, D_2O): δ 1.49 (ddd, $J_{3"a,4"}$ = 11.1 Hz, $J_{3"e,3a"}$ = 13.3 Hz, $J_{3"a,P}$ = 5.8 Hz, 1H, 3"a-H), 1.86 (s, 3H, $NCOCH_3$) 2.30 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{3"e,3"a}$ = 13.3 Hz, 1H, 3"e-H), 3.33 (d, J = 9.3 Hz, 1H, 7"-H), 3.43 (dd, $J_{8",9"B}$ = 6.7 Hz, $J_{9"A,9"B}$ = 11.9 Hz, 1H, 9"B-H), 3.68 (dd, $J_{8",9"A}$ = 2.4 Hz, $J_{9"A,9"B}$ = 11.9 Hz, 1H, 9"A-H), 3.79 (d, $J_{5",6"}$ = 10.0 Hz, 1H, 5"-H), 3.90 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{4",5"}$ = 10.2 Hz, 1H, 4"-H), 4.00 (d, $J_{5",6"}$ = 10.0 Hz,1H, 6"-H), 4.05–4.17 (m, 5H, 2'-H, 3'-H, 4'-H, 5'A-H, 5'B-H), 5.79 (d, $J_{1',2'}$ = 4.1 Hz, 1H, l'-H), 5.92 (d, J = 7.6 Hz, 1H, 5-H), 7.77 (d, J = 7.6 Hz, 1H, 6-H). ^{31}P NMR (161.7 MHz, D_2O): δ –4.06 (β, 100 %).

^{1}H NMR data, ^{31}P NMR data and R_f value of compound **1C** are identical with commercially available CMP-Neu5Ac.[15]

Disodium (5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)onate uridine phosphate (UMP-Neu5Ac 1U)

R_f [aminophase, EtOH:H_2O (3:7, 0.2 M NaCl-solution)] 0.68. ^{1}H NMR (250 MHz, D_2O): δ 1.48 (ddd, $J_{3"a,4"}$ = 11.0 Hz, $J_{3"e,3"a}$ = 13.3 Hz, $J_{3"a,P}$ = 5.8 Hz, 1H, 3"a-H), 1.88 (s, 3H, $NCOCH_3$), 2.32 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{3"a,3"e}$ = 13.3 Hz, 1H, 3"e-H), 3.28 (d, J = 9.7 Hz, 1H, 7"-H), 3.45 (dd, $J_{8",9"B}$ = 6.8 Hz, $J_{9"A,9"B}$ = 12.1 Hz, 1H, 9"B-H), 3.72 (dd, $J_{9"A,8}$ = 2.4 Hz, $J_{9"A,9"B}$ = 12.1 Hz, 1H, 9"A-H), 3.80 (d, $J_{5",6"}$ = 10.2 Hz, 1H, 5"-H), 3.76–3.82 (m, 1H, 8"-H), 3.88 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{3"a,4"}$ = 11.0 Hz, 1H, 4"-H), 3.97 (d, $J_{5",6"}$ = 10.2 Hz, 1H, 6"-H), 4.01–4.11 (m, 3H, 4'-H, 5'A, 5'B), 4.15–4.22 (m, 2H, 2'-H, 3'-H), 5.74 (d, J = 7.8 Hz, 1H, 5-H), 5.84 (d, $J_{1',2'}$ = 4.6 Hz, 1H, l'-H), 7.71 (d, J = 7.8 Hz, 1H, 6-H). ^{31}P NMR (161.7 MHz, D_2O): δ –4.02 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 614.

Disodium (5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)onate adenosine phosphate (AMP-Neu5Ac lA)

R_f [aminophase, EtOH:H_2O M NaCl-solution)] 0.73. ^1H NMR (250 MHz, D_2O): δ 1.48 (ddd, $J_{3"a,4"}$ = 11.2 Hz, $J_{3"e,3"a}$ = 13.2 Hz, $J_{3"a,P}$ = 5.9 Hz, 1H, 3"a-H), 1.86 (s, 3H, $NCOCH_3$), 2.32 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{3"a,3"e}$ = 13.2 Hz, 1H, 3"e-H), 3.27 (d, J = 9.6 Hz, 1H, 7"-H), 3.43 (dd, $J_{8",9"B}$ = 6.5 Hz, $J_{9"A,9"B}$ = 11.7 Hz, 1H, 9"B-H), 3.69 (dd, $J_{9"A,8}$ = 2.3 Hz, $J_{9"A,9"B}$ = 11.7 Hz, 1H, 9"A-H), 3.73–3.79 (m, 1H 8"-H) 3.79 (d, $J_{5",6"}$ = 10.2 Hz, 1H, 5"-H), 3.87 (dd, $J_{3"e,4"}$ = 4.5 Hz, $J_{4",5"}$ = 10.7 Hz, 1H, 4"-H), 3.97 (d, $J_{5",6"}$ = 10.2 Hz, 1H, 6"-H), 4.01–4.12 (m, 2H, 5'A-H, 5'B-H), 4.18 (m, 1H, 4'-H), 4.35 (dd, J = 3.6 Hz, J = 5.2 Hz, 1H, 3'-H), 4.55 (dd, $J_{2',3'}$ = 5.2 Hz, $J_{2',2'}$ = 5.8 Hz, 1H, 2'-H), 5.93 (d, $J_{1',2'}$ = 5.8 Hz, 1H, 1'-H), 8.01 (s, 1H, 2-H), 8.34 (s, 1 H, 8-H). ^{31}P NMR (161.7 MHz, D_2O) δ −3.85 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 637.

Disodium (5-acetamido-3,5-dideoxy-β-D-glycero-D-galacto-2-nonulopyranosyl)onate guanosine phosphate (GMP-Neu5Ac 1G)

R_f [aminophase, EtOH:H_2O (3:7, 0.2 M NaCl-solution)] 0.50. ^1H NMR (250 MHz, D_2O): δ = 1.48 (ddd, $J_{3"a,4"}$ = 11.0 Hz, $J_{3"a,3"e}$ = 13.1 Hz, $J_{3"a,P}$ = 5.6 Hz, 1H, 3"a-H), 1.88 (s, 3H, $NCOCH_3$), 2.33 (dd, $J_{3"e,4"}$ = 4.4 Hz, $J_{3"a,3"e}$ = 13.1 Hz, 1H, 3"e-H), 3.28 (d, J = 9.4 Hz, 1H, 7"-H), 3.44 (dd, $J_{8",9"B}$ = 6.4 Hz, $J_{9"A,9"B}$ = 11.8 Hz, 1H, 9"B-H), 3.71 (dd, $J_{9"A,9"B}$ = 11.8 Hz, 1H, 9"A-H), 3.80 (dd, $J_{5",6"}$= 10.3 Hz, 1H, 5"-H), 3.78–3.82 (m, 1H, 8"-H), 3.89 (dd, $J_{3"e,4"}$ = 4.4 Hz, $J_{4",5"}$ = 10.3 Hz, 1H, 4"-H), 3.97 (d, $J_{5",6"}$ = 10.3 Hz, 1H,6"-H), 4.02–4.11 (m, 2H, 5'A-H, 5'B-H), 4.15 (m, 1H,4'-H), 4.34 (dd, J = 3.6 Hz, J = 4.2 Hz, 1H, 3'-H), 4.52 (m, 1H, 2'-H), 5.76 (d, J = 6.1 Hz, 1H, 1'-H), 7.98 (s, 1 H, 8-H). ^{31}P NMR (161.7 MHz, D_2O) δ −4.00 (β, 100 %). MS (FAB, negative mode): $(M - H)^-$ 653, $(MNa - H)^-$ 675, $(MNa_2 - H)^-$ 697.

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References and Notes

1. Martin, T. J. Ph.D. Thesis, Univ. Konstanz, 1994.

2. Westheimer, F. M. *Science* **1987**, *235*, 1173.

3. Sinnot, L. M. In: *Enzyme Mechanisms*, pp. 259–297, Page, M. I.; Williams, A., Eds; The Royal Society of Chemistry; London 1987; *Chem. Rev.* **1990**, *90*, 1171 and references cited therein.

4. Schmidt, R. R. In: *Carbohydrates—Synthetic Methods and Application in Medicinal Chemistry* pp. 68–88, Ogura, H.; Hasegawa, A.; Suami, T., Eds; Kodanasha Ltd; Tokyo 1992; Frische K.; Schmidt, R. R. *Liebigs Ann. Chem.* **1994**, 297.

5. Heidlas, J. E.; Williams, K. W.; Whitesides, G. M. *Acc. Chem. Res.* **1992**, *25*, 307 and references cited therein.

6. (a) Schmidt, R. R.; Braun, H.; Jung, K.-H. *Tetrahedron Lett.* **1992**, *33*, 1585; (b) Schmidt, R. R.; Frische K. *BioMed. Chem. Lett.* **1993**, *3*, 1747.

7. Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1993**, *34*, 1765.

8. Schauer, R. *Adv. Carbohydr. Chem. Biochem.* **1982**, *40*, 131; Cell Biology Monographs, Vol. 10, *Sialic Acids Chemistry, Metabolism and Function*, Springer Verlag; Vienna, 1982.

9. Makino, S.; Ueno, Y.; Ishikawa, M.; Hayakawa, Y.; Hata, T. *Tetrahedron Lett.* **1993**, *34*, 2775; Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159; Thiem, J. Treder, W. *Angew. Chem.* **1986**, *98*, 1100; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 1096.

10. Schmidt, R. R. XIVth Int. Carbohydr. Symp., Paris, 7 July, 1992; Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123; Martin, T. J.; Brescello, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16; Greilich, U.; Zimmermann, P.; Jung, K.-H.; Schmidt, R. R. *Liebigs Ann. Chem.* **1993**, 859; Schmidt, R. R. ACS-Symposium Series, **1994**, *560*, 276

11. Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260; Kondo, H.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1992**, *114*, 8748.

12. For a preliminary publication of this approach see Ref. 7.

13. Schmidt, R. R. *Angew. Chem.* **1986**, *98*, 213; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212; *Pure Appl. Chem.* **1989**, *61*, 1257; and references cited therein.

14. Paulsen H.; Tietz H. *Carbohydr. Res.* **1984**, *125*, 47; Kuhn, R.; Lutz, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611; Bagget, N.; Marsden, B. J. *Carbohydrate Res.* **1982**, *110*, 11.

15. Purchased from SIGMA Co.

16. Marian, M. *Microchem. J.* **1984**, *29*, 219; Braun, H. Diplomarbeit, Univ. Konstanz, 1991.

17. Van den Eijnden, D. H.; to be submitted.

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