New Potent Sialyltransferase Inhibitors—Synthesis of Donor and of Transition-State Analogues of Sialyl Donor CMP-Neu5Ac

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Abstract: Enzymatic sialyl transfer with CMP-Neu5Ac as donor can be inhibited by CDP. Therefore phosphonates **1a**,**b**, **2** and **3** were synthesized as substrate analogues. With $\alpha(2-6)$-sialyltransferase from rat liver (EC2.4.99.1) only moderate inhibition was found for these compounds. In order to obtain transition-state analogues of CMP-Neu5Ac different linkages between 2,3-dehydro-*N*-acetylneuraminol and CMP were generated, yielding **4**, (*R*)-**5** and (*R*)-**6**. Compound (*R*)-**6**, in which the CMP residue is attached to C-1 of 2,3-dehydro-*N*-acetylneuramin-1-yl phosphonate, exhibited excellent $\alpha(2-6)$-sialyltransferase inhibition in the nanomolar range ($K_i = 350$ nM), resulting in a 130-fold higher affinity for the enzyme than CMP-Neu5Ac ($K_M = 46$ μM).

Keywords: enzyme inhibitors · neuraminic acids · substrate analogues · transferases · transition states

Introduction

Sialic acids play an important role in quite a few biological processes, such as cell adhesion and inflammation.[1] Additionally, several reports indicate that there is a correlation between cell-surface sialic acid or sialyltransferase activity and the growth[2] or metastatic potential of tumour cells.[3, 4] In order to elucidate the influence of sialyl residues in biological systems it is therefore desirable to develop specific inhibitors for sialyltransferases. Various sialyltransferases, independent of their source and their acceptor specificity, employ cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac, Scheme 1) as the donor substrate;[5] donor analogues or transition-state analogues (Scheme 1, CMP-Neu5Ac‡) with high enzyme affinity could therefore become particularly versatile inhibitors. Only a few donor or acceptor analogues serving as sialyltransferase inhibitors have been reported.[6–11] We present here new CMP-Neu5Ac analogues (**1**–**3**) which are derived from cytidine diphosphate (CDP) **A**, which is a natural sialyltransferase inhibitor.[9, 12] From the potential transition state (CMP-Neu5Ac‡) in sialyltransferase catalyzed reactions (Scheme 1),[9, 13] new types of transition-state analogues are derived (**4**–**6**).[14] The synthesis of compounds **1**–**6** and their inhibition properties of $\alpha(2-6)$-sialyltransferase, obtained from rat liver (EC2.4.99.1), will be reported.

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Results and Discussion

Synthesis of sialyltransferase inhibitors based on CDP: CDP is known to be a potent sialyltransferase inhibitor which shows competitive inhibition for $\alpha(2-6)$-sialyltransferase of rat liver with a K_i value of approximately 10 μM[9, 12] (Table 1). Therefore, the corresponding methylene phosphonate analogue **B** (Scheme 1) could result in similar inhibition properties (which have not yet been determined).[15] In order to investigate the importance of the sialyl moiety for binding affinity, we recently studied various CMP-quinic acid derivatives as donor analogues.[9] The most potent inhibitor was CMP-quinic acid itself (Scheme 1, **C**) with a K_i value of 44 μM, which is practically identical to the K_M value of CMP-Neu5Ac (45 μM) in this reaction.[9] Therefore, we reasoned that an additional negative charge at the CMP residue could be sufficient for high enzyme affinity, yet the complete Neu5Ac residue may not be required. Consequently, structurally much simpler compounds were synthesized, for instance **1a**, where the distance between the two negative charges (four bonds) is similar to that in CDP. Additionally, compounds **1b**, **2** and **3** were prepared, which have a distance of five bonds between the negative charges, as in CMP-Neu5Ac. For compounds **1**–**3** K_i values at least in the range of CDP were expected.

For the synthesis of **1a**, commercially available triethyl phosphonoacetate **7a**[16] was treated with trimethylsilyl iodide in acetonitrile, resulting in phosphonate ester cleavage; subsequent addition of aniline furnished anilinium salt **8a**[17] (Scheme 2). Condensation of **8a** with 5′-*O*-unprotected cytidine derivative **9**[9, 10, 18] and DCC in pyridine afforded cytidinyl phosphonate **10a**. Deacylation with NaOMe/MeOH

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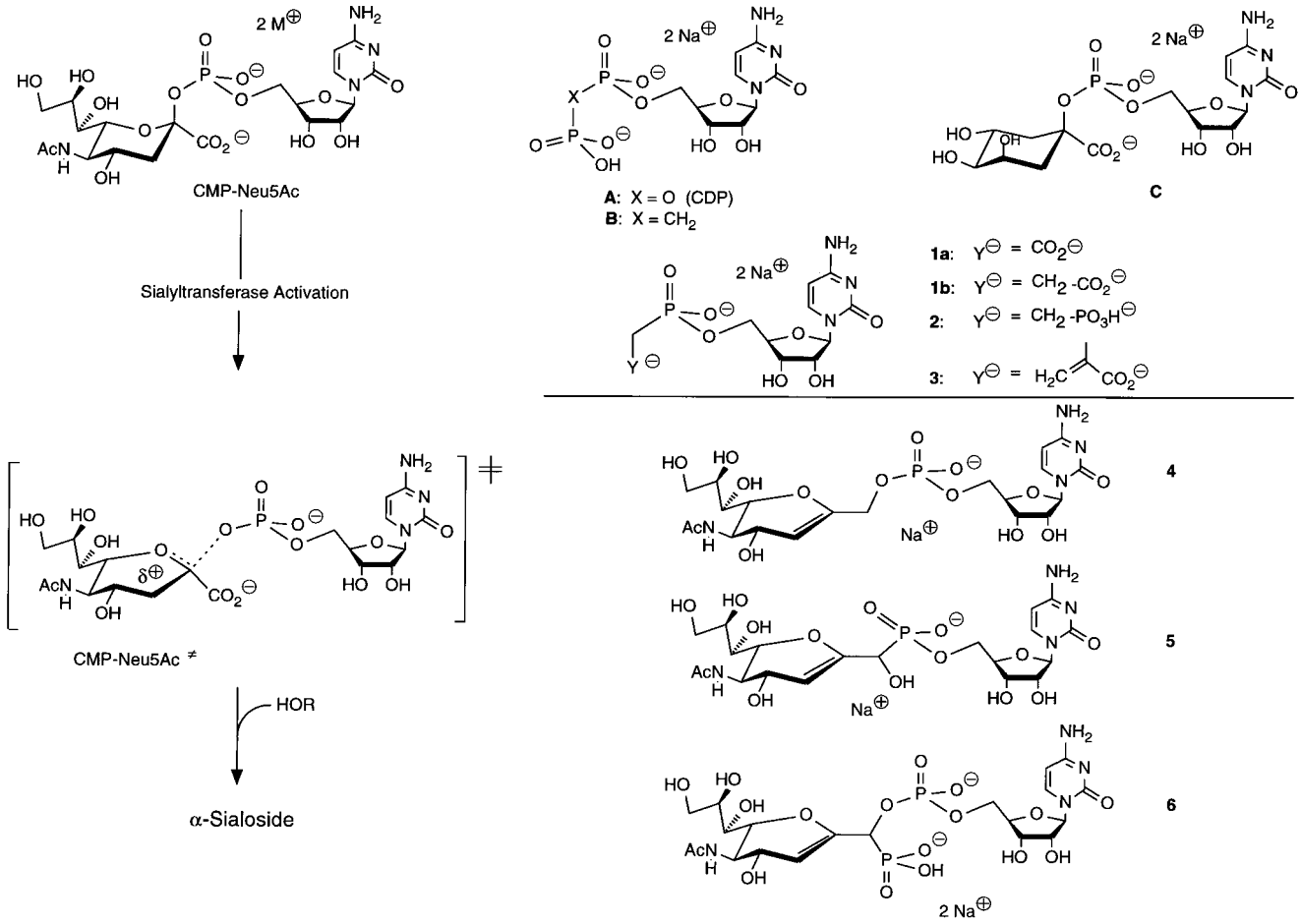


Scheme 1. Cytidine monophosphate *N*-acetylneuraminic acid (CMP-Neu5Ac) and its activated form, and analogues of the two.

Table 1. Inhibition constants (K_i) of substrate analogues **A**, **C**, and **1–3**.[a]

	K_i [μM]	Ref.
A (CDP)	10 ± 2	[9,12]
C	44 ± 7	[9]
1a	2000 ± 200	–
1b	270 ± 20	–
2	750 ± 70	–
3	250 ± 20	–

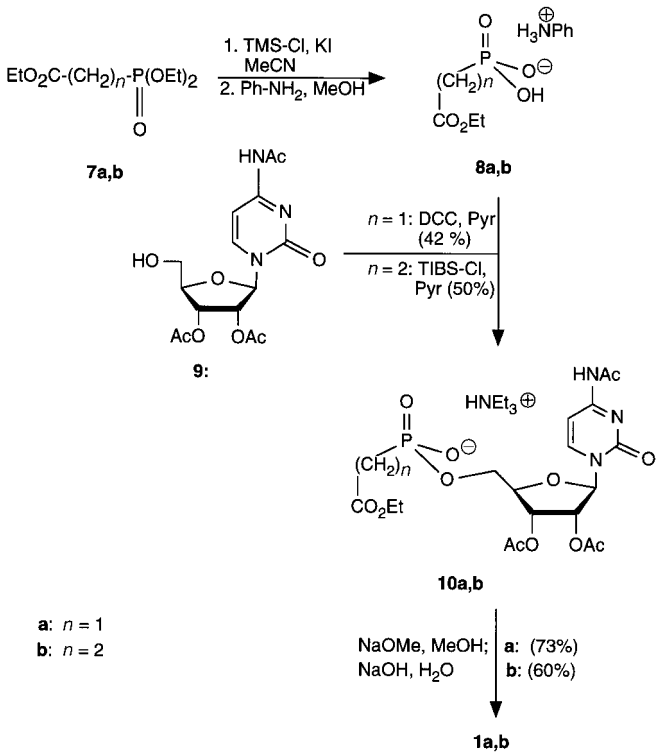
[a] For details, see Experimental Section.

Abstract in German: *Die enzymatische Übertragung von Sialylgruppen mit Hilfe von CMP-Neu5Ac als Donor kann mit CDP inhibiert werden. Deshalb wurden als Substratanaloga die Phosphonate **1a,b**, **2** und **3** synthetisiert. Sie wirkten jedoch bei der α(2–6)-Sialyltransferase aus Rattenleber (EC2.4.99.1) nur mäßig inhibierend. Um Übergangszustandsanaloga von CMP-Neu5Ac zu erhalten, wurden verschiedene Verknüpfungen zwischen 2,3-Dehydro-N-acetylneuraminol und CMP erzeugt und so die Verbindungen **4**, (R)-**5** und (R)-**6** erhalten. Verbindung (R)-**6**, bei welcher der CMP-Rest an C-1 von 2,3-Dehydro-N-acetylneuramin-1-ylphosphonat geknüpft ist, zeigte Inhibition der α(2–6)-Sialyltransferase im nanomolaren Bereich (K_i = 350 nM); (R)-**6** weist somit eine 130mal höhere Affinität zum Enzym auf als das natürliche Substrat CMP-Neu5Ac (K_M = 46 μM).*

followed by ester cleavage with aqueous NaOH yielded the desired cytidinyl phosphonate **1a**. Inhibition studies with α(2–6)-sialyltransferase[19] showed competitive inhibition with K_i = 2 mM, which is surprisingly two orders of magnitude less efficient than CDP (Table 1).

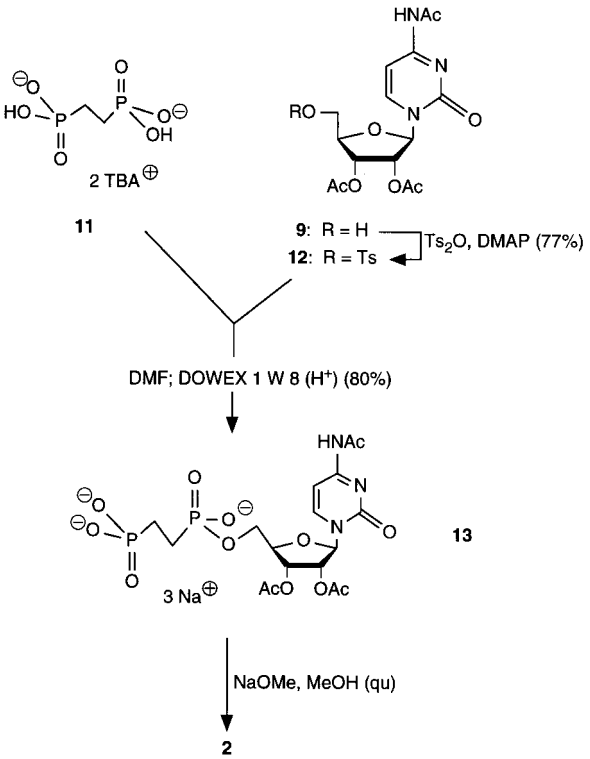
Molecular modelling calculations[20] indicated that cytidinyl phosphonopropionate **1b**, the homologue of **1a**, is structurally more closely related to CDP than **1a**. For the synthesis of **1b** practically the same methodology was applied: commercially available triethyl phosphonopropionate **7b**[21] was transformed into monoester **8b**, which was condensed with **9**[9, 18] by means of 2,4,6-triisopropylbenzenesulfonyl chloride (TIBS-Cl) in pyridine as condensing agent, to furnish **10b** in high yield; deacylation and then ester cleavage gave target molecule **1b** (Scheme 2). Substrate **1b** exhibited competitive inhibition of α(2–6)-sialyltransferase with K_i = 270 μM, thus showing markedly higher enzyme affinity than **1a**.

Replacement of the carboxylate group in **1b** by a phosphonate group led to **2**, which was readily obtained from ethane-1,2-diphosphonate **11** (Scheme 3).[22] Reaction of the bis-tetrabutylammonium salt of **11** with 5′-*O*-tosyl-cytidine derivative **12**, which was readily obtained from **9** by treatment with *p*-toluenesulfonic anhydride in the presence of DMAP, gave ethane-1,2-diphosphonate monocytidinyl ester **13** in high yield. Deacylation with NaOMe in MeOH gave target molecule **2**. Its K_i value was determined as 750 μM, suggesting

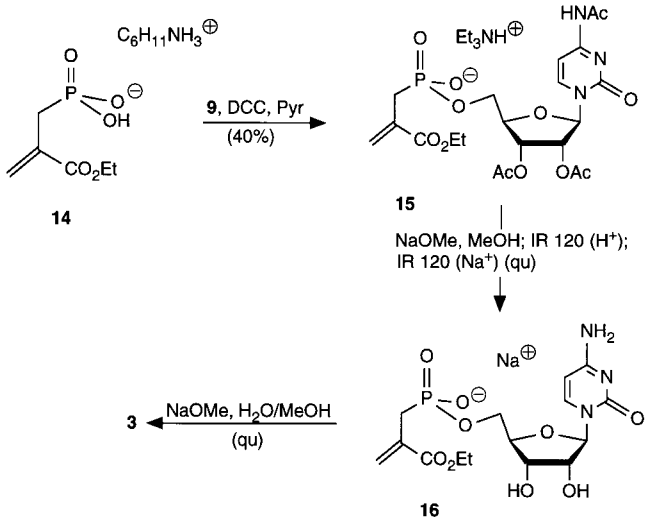


Scheme 2. Synthesis of cytidinyl phosphonate **1a** and cytidinyl phosphonopropionate **1b**.

that the phosphonate group did not contribute significantly to enzyme binding affinity. Therefore, the effect of introduction of an α-methylene group into **1b** was studied, leading to **3** as target molecule. Thus, a spatial orientation of the negative charges as in CMP-Neu5Ac should be favoured (Scheme 4). To this end, α-methylene-β-phosphonopropionate **14**[23] was condensed with **9** in the presence of DCC in pyridine as condensing agent to afford cytidinyl phosphonate **15**. Deacylation with NaOMe in MeOH, transformation into the sodium salt (→**16**) and then ethyl ester cleavage with NaOH in MeOH/water gave target molecule **3** which exhibited a K_i value of 250 μM.



Scheme 3. Synthesis of cytidin-5′-yl ethylenediphosphonate salt **2**.



Scheme 4. Synthesis of cytidin-5′-yl (2-carboxy-2-propenyl)phosphonate salt **3**.

Evidently, readily accessible but structurally simplified CMP-Neu5Ac analogues **1**–**3** are able to inhibit $\alpha(2-6)$-sialyltransferase; however, they do not reach the values of CDP or CMP-quinic acid (Scheme 1, **A**, **C**), despite being structurally similar to CDP. Therefore, a new approach was considered.

Synthesis of transition-state analogues of CMP-Neu5Ac: The proposed transition state developing from sialyltransferases with CMP-Neu5Ac in the sialyl transfer to acceptors is shown in Scheme 1 (CMP-Neu5Ac$^{\pm}$). From this model, which is based on the recently supported S_N1-type mechanism,[9, 13] phosphorus derivatives **4**–**6** were considered potentially efficient inhibitors. In compounds **4** and **6** the distance between the anomeric center (C-2 of the Neu5Ac residue) and the leaving group (CMP) is increased, compared with those expected in the transition state (CMP-Neu5Ac$^{\pm}$). In particular **6** was of interest, because it contains two negative charges separated by five bonds, as in CMP-Neu5Ac, and in addition the anomeric center is trigonal planar, the conformation assumed for an S_N1-type transition state.

Compounds **4**–**6** were prepared from 2,3-dehydroneuraminic acid. For the synthesis of **4**, Neu5Ac was transformed into 2,3-dehydro derivative **17**,[24] which, with *p*-thiocresol in the presence of carbonyl diimidazole (CDI) as condensing agent, gave the thioester derivative **18**. Reduction with $NaBH_4$ in EtOH afforded neuraminol derivative **19** (Scheme 5). Condensation with cyanoethoxy-bis(diisopropylamino)phosphane[25] in the presence of the activator system

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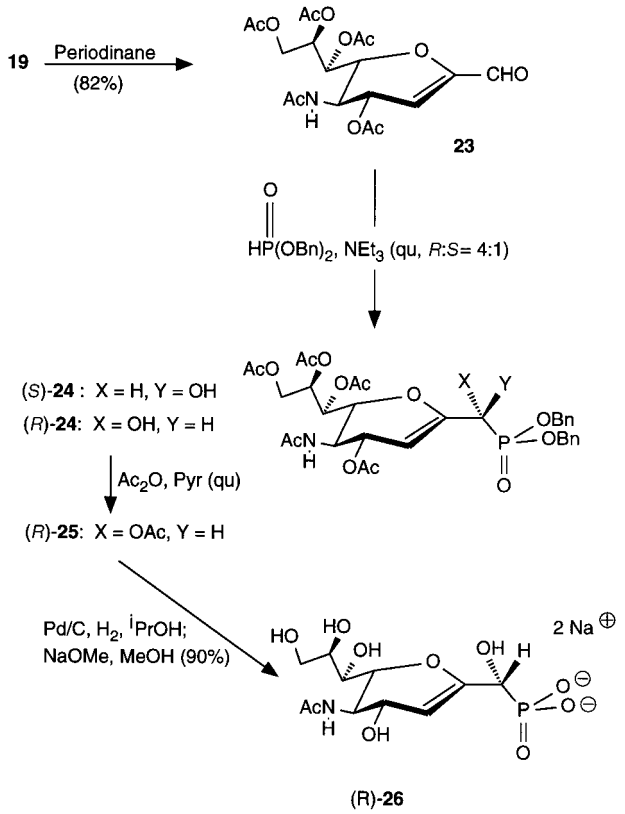
diisopropylamine/tetrazole led to phosphitamide derivative **20** in high yield. Subsequent condensation with **9** in the presence of tetrazole afforded a phosphite triester intermediate, which on oxidation with *tert*-butylhydroperoxide and then treatment with triethylamine as base led to loss of the cyanoethyl group to afford phosphorus diester **21**, but only in moderate yield. Therefore, **9** was treated with cyanoethoxy-bis(diisopropylamino)phosphane with diisopropylammonium tetrazolide to give phosphitamide derivative **22**.[26] Reaction of neuraminol derivative **19** with **22** in the presence of tetrazole, then oxidation with *tert*-butylhydroperoxide and base-catalyzed cleavage of the cyanoethyl group afforded **21** in very good overall yield. Deacylation with NaOMe in MeOH, chromatography over RP-18 with triethylammonium carbonate buffer as eluent, and then ion exchange afforded target molecule **4**. Compound **4** exhibited competitive inhibition with $K_i \approx 2$ mM, thus showing unexpectedly low affinity for the active site of $\alpha(2-6)$-sialyltransferase (Table 2).

For the synthesis of target molecules **5** and **6**, neuraminol derivative **19** was oxidized with Dess–Martin periodinane[27] leading to aldehyde **23** in high yield (Scheme 6). Reaction with dibenzyl phosphite in the presence of NEt_3 as base

Table 2. Affinity of CMP-Neu5Ac (K_M) and comparison of inhibition constants (K_i) of transition-state analogues **4**–**6** and **6′**.[a]

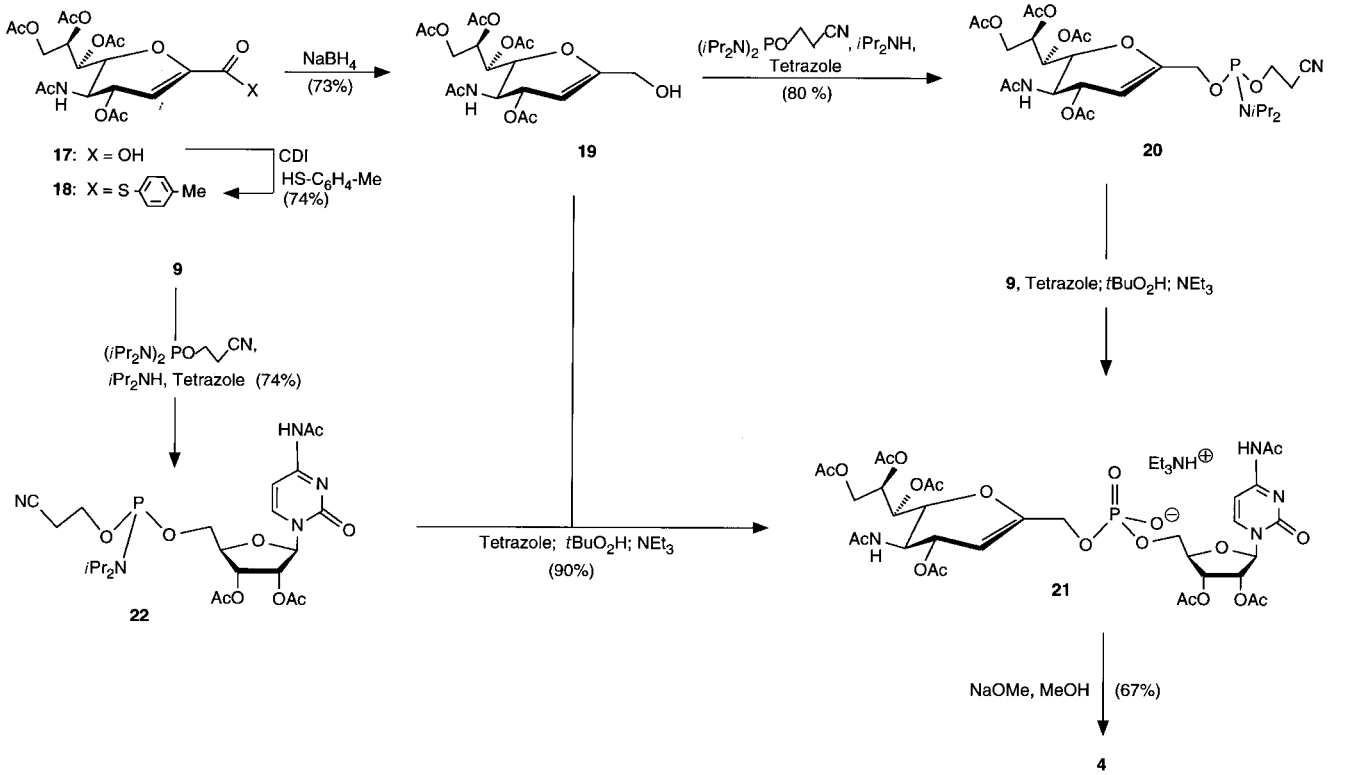
	K_M [μM]	K_i [μM]	Ref.
CMP-Neu5Ac	46 ± 7	–	[9,28]
4	–	< 2000	–
(*R*)-**5**	–	400 ± 40	–
(*E*)-**6′**	–	6 ± 0.5	–
(*R*)-**6**	–	0.35 ± 0.05	–

[a] For details, see Experimental Section.



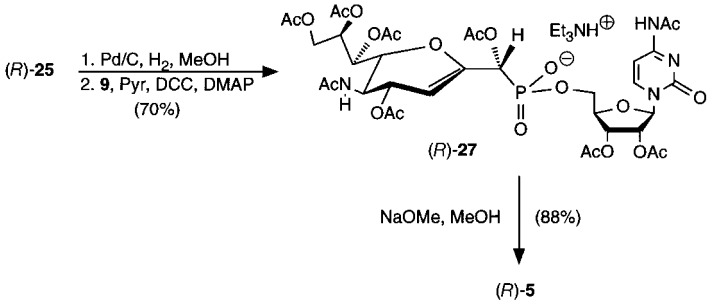
Scheme 6. Synthesis of **24** and **25**, precursors of **5** and **6**, and of α-hydroxyphosphonate (*R*)-**26** from neuraminol derivative **19**.

furnished epimeric α-hydroxyphosphonates (*R*)-**24** and (*S*)-**24** in a 4:1 ratio, which could be separated by chromatography. Only (*R*)-**24** was used in ensuing reactions because the minor



Scheme 5. Synthesis of cytidin-5′-yl phosphate **4** from 2,3-dehydroneuraminic acid.

epimer (*S*)-**24** was not available in sufficient quantity. The structural assignment was based on the Mosher method as discussed below. Full *O*-acetylation was required in order to carry out hydrogenolytic debenzylation of **24** successfully; thus, (*R*)-**24** treated with acetic anhydride in pyridine gave (*R*)-**25**; subsequent hydrogenolytic debenzylation with palladium on carbon as catalyst and then *O*-deacylation with NaOMe in MeOH afforded α-hydroxyphosphonate (*R*)-**26** in high yield; in the hydrogenation step reduction of the enol ether moiety was not observed. Hydrogenolytic debenzylation of (*R*)-**25** and immediate coupling with cytidine derivative **9** in the presence of DCC in pyridine containing DMAP gave condensation product (*R*)-**27** (Scheme 7). Deacylation of (*R*)-**27** with NaOMe in MeOH afforded target molecule (*R*)-**5**, which also exhibited competitive inhibition with a K_i value of 400 μM.

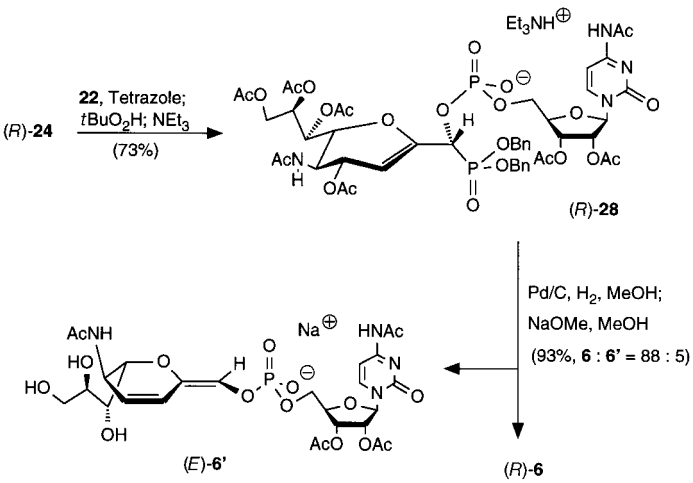


Scheme 7. Synthesis of phosphonate **5**.

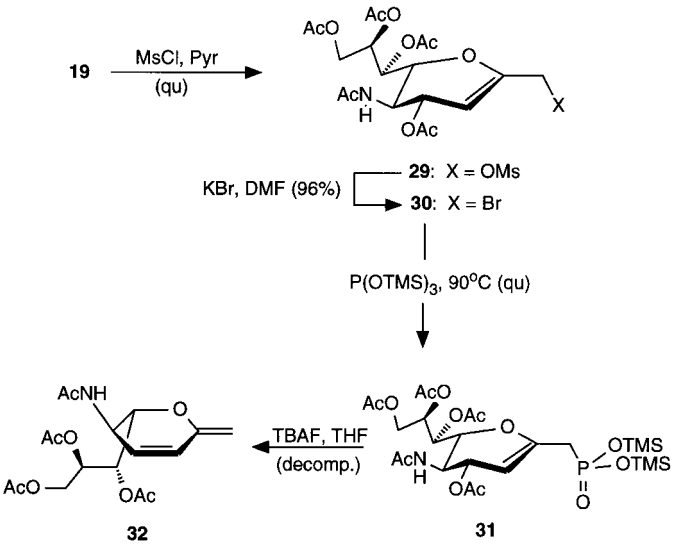
We reasoned that introduction of a second negative charge at the glycosylation site should greatly increase binding affinity to sialyltransferases. In order to prove this hypothesis, (*R*)-**24** was condensed with cytidinyl phosphitamide **22** in the presence of tetrazole as catalyst; then oxidation of the intermediate phosphite triester with *tert*-butylhydroperoxide gave the corresponding phosphate, which on treatment with NEt_3 as base led to phosphate diester (*R*)-**28** in good yield (Scheme 8). Hydrogenolytic debenzylation with Pd/C as catalyst followed by deacylation with NaOMe in MeOH furnished target molecule (*R*)-**6** in very good yield. As a minor by-product diene derivative (*E*)-**6′** was isolated, which obviously originates from a base-promoted deacetoxyphosphonylation. The configurational assignment was based on NMR data (two-dimensional ROESY spectrum); the assignment is also in accordance with the generally observed influence of stereoelectronic effects.[28, 29]

The observed deacetoxyphosphonylation is quite a facile reaction when there is no electron-withdrawing moiety in this system. Thus, transformation of neuraminol derivative **19** into bromide **30** via mesylate **29** and then Michaelis–Arbuzov reaction with tris(trimethylsilyl)phosphite gave phosphonate **31** in quantitative yield (Scheme 9). Treatment of **31** with TBAF in THF gave, on formal acetoxybis(trimethylsilyl)-phosphonate elimination, diene **32**, which turned out to be a quite labile compound.

For both compound (*R*)-**6** and by-product (*E*)-**6′** the inhibition of $\alpha(2-6)$-sialyltransferase was investigated. Competitive inhibition was found for both compounds with a K_i



Scheme 8. Synthesis of phosphate **6**.



Scheme 9. Synthesis of diene **32** from neuraminol derivative **19**.

value of 6 μM for (*E*)-**6′** and 0.35 μM for (*R*)-**6.** Thus, the transition-state analogue (*R*)-**6** exhibits excellent inhibition results; it shows a 130-fold increased affinity to the active site of $\alpha(2-6)$-sialyltransferase compared with the natural substrate CMP-Neu5Ac ($K_M = 46$ μM).[9, 30] Obviously, a planar C-2 of the Neu5Ac residue in combination with two negative charges in close proximity, which are separated by five bonds and presumably in a *syn*-arrangement, leads to high affinity for the active site of $\alpha(2-6)$-sialyltransferase. Additionally, the cytidine residue is a basic requirement for active-site recognition, as shown by Kleineidam et al.[8] Further studies with this powerful substance are in progress.

Configurational assignment of (*R*)- and (*S*)-24: The configuration of the new stereocenter in **24** was based on chemical correlation and Mosher's method.[31, 32] To this end, (*R*)-**26** was treated with ozone; the resulting ester was saponified without isolation to give (−)-α-hydroxyphosphonoacetic acid, which by comparison was found to have (*R*) configuration.[18, 32] (−)-α-Hydroxyphosphonoacetic acid was also obtained from a new galactal derivative whose configuration could be assigned by ^{31}P NMR of a corresponding Mosher ester.[18]

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Experimental Section

Solvents were purified according to the standard procedures. Melting points are reported in degrees Celsius (uncorrected). NMR measurements were recorded at 22 °C on a Bruker AC 250 Cryospec, Bruker DRX 600 or a Jeol JNM-GX 400. TMS or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, $\delta = 7.24$; CD_3OD, $\delta = 3.315$; D_2O, $\delta = 4.63$. For ^{31}P NMR phosphoric acid was used as an external standard; ^{31}P NMR spectra were broadband ^1H-decoupled. MALDI-mass spectra were recorded on a Kratos Kompact Maldi 1 and 2,5-dihydroxybenzoic acid (DHB) or 6-aza-2-thiothymine (ATT) were used as matrices. FAB mass spectra were measured on a Finnigan MAT 312/AMD 5000 (70 eV, 70 °C). Optical rotations were measured on a Perkin–Elmer polarimeter 241/MS in a 1 dm cell at 22 °C. Thin-layer chromatography was performed on Merck silica gel plastic plates $60F_{254}$ or Merck glass plates RP-18; compounds were visualised by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL). Flash chromatography was performed on J.T. Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.3 bar. Preparative HPLC separations were performed on an Autochrom System with a Shimadzu LC8A preparative pump and a Rainin Dynamax UV 1 detector at 254 nm. The column used was a Lichrosorb RP-18, 7 μm, 250 × 16 mm (Knauer, Germany). Mixtures of acetonitrile and 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.2–7.5) were used as the mobile phase.

Anilinium (ethoxycarbonylmethyl)phosphonate (**8a**) was synthesized from **7a** as previously described.[17]

Anilinium (2-ethoxycarbonylethyl)phosphonate (**8b**): The triethyl ester **7b** (3 g, 12.59 mmol) and potassium iodide (4.18 g, 25.19 mmol) were suspended in dry acetonitrile (15 mL) and TMSCl (2.74 g, 25.19 mmol) was added dropwise. After stirring for 30 min at 35 °C the suspension was filtered and the residue was washed twice with dry diethyl ether. The filtrate was concentrated under reduced pressure and the residue was dissolved in dry methanol (15 mL) containing aniline (2.29 mL, 25.19 mmol). The solvents were removed to afford a yellow solid which was purified by recrystallization from acetone to yield white needles of **8b** (2.59 g, 75%). M.p. 162–164 °C; ^1H NMR (CD_3OD): $\delta = 1.24$ (t, $J = 7.2$ Hz, 3H; OCH_2CH_3), 1.8–2.0 (m, 2H; 1a,b-H), 2.50–2.65 (m, 2H; 2a,b-H), 4.12 (q, 2H; OCH_2CH_3), 7.0–7.1 (m, 3H; 3-, 4-, 5-anilinium-H), 7.25–7.35 (m, 2H; 2-,6-anilinium-H); $C_{11}H_{18}NO_5P$ (275.24): calcd C 48.01, H 6.59, N 5.09; found C 47.89, H 6.68, N 5.06.

***N*-Acetyl-2′,3′-di-*O*-acetylcytidine** (**9**) was synthesized as previously described.[9, 18]

Triethylammonium (*N*-acetyl-2′,3′-di-O-acetylcytidin-5′-yl) (ethoxycarbonylmethyl)phosphonate (**10a**): A solution of the anilinium salt **8a** (417 mg, 1.6 mmol) in dry methanol (10 mL) was converted into the free acid by ion-exchange chromatography (Amberlite IR 120, H^+). Dry pyridine (0.5 mL) was added and the solution was evaporated. The residue was coevaporated with toluene and dissolved in dry pyridine (3 mL). DCC (495 mg, 2.4 mmol) was added to the solution and after 10 min of stirring cytidine **9** (298 mg, 0.8 mmol) was added to the red suspension. The mixture was stirred for 9 days at room temperature; water (15 mL) was then added and after 30 min the urea precipitate was filtered off. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (ethyl acetate/methanol 3:1 + 1% triethylamine) to yield the phosphonate ester **10a** (207 mg, 42%) as colourless triethylammonium salt. $R_f = 0.12$ (ethyl acetate/methanol 3:1 + 1% triethylamine); $[\alpha]_D = 28$ ($c = 0.5$ in methanol); ^1H NMR (250 MHz, CD_3OD): $\delta = 1.21–1.33$ (m, 12H; $3NCH_2CH_3$, OCH_2CH_3), 2.06, 2.10, 2.18 (3s, 9H; $COCH_3$), 2.83 (d, $^2J(1''P) = 20.5$ Hz, 2H; 1a,b″-H), 3.19 (q, $J = 7.3$ Hz, 6H; $3NCH_2CH_3$), 4.13 (q, $J = 7.2$ Hz, 2H; OCH_2CH_3), 4.15–4.30 (m, 2H; 5a′-, 5b′-H), 4.40–4.45 (m, 1H; 4′-H), 5.40–5.55 (m, 2H; 2′-, 3′-H), 6.21 (d, $J(1',2') = 4.6$ Hz, 1H; 1′-H), 7.52 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 8.48 (d, 1H; 6-H); ^{31}P NMR (161.7 MHz, CD_3OD): $\delta = 13.43$ (s, phosphonate); MS (MALDI, negative mode, matrix: ATT): $m/z = 519$ $[(M - NHEt_3^+)^-]$, 639 $[(M+H_2O)^-]$, 620.6 for $C_{25}H_{41}N_4O_{12}P$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl) (2-ethoxycarbonylethyl)phosphonate (**10b**): The anilinium salt **8b** (823 mg, 3 mmol) was converted to the free acid by ion-exchange chromatography (Amberlite IR, 120 H^+), coevaporated twice with dry pyridine and finally dissolved in dry pyridine (10 mL). TIBS-Cl (1.36 g, 4.5 mmol) was added to form a dark red solution, to which cytidine **9** (554 mg, 1.5 mmol) was added in portions. After 15 h at room temperature the reaction was quenched by addition of water (5 mL) and filtered over Celite, and the solvents were removed under reduced pressure. The residue was purified by flash chromatography (ethyl acetate/methanol 5:1 + 1% to 3:1 + 1% triethylamine) and RP-18 chromatography (water/methanol 2:1) to afford the triethylammonium salt **10b** (475 mg, 50%) contaminated with minor quantities of phosphonic acid **8b**, which could be removed by preparative HPLC (CH_3CN/water 5:95 to 30:70). $R_f = 0.11$ (ethyl acetate/methanol 3:1 + 1% triethylamine); $[\alpha]_D = +21.7$ ($c = 1$ in methanol); ^1H NMR (250 MHz, CD_3OD): $\delta = 1.22$ (t, $J = 7.2$ Hz, 3H; OCH_2CH_3), 1.32 (t, $J = 7.1$ Hz, 9H; $3NCH_2CH_3$), 1.8–2.0 (m, 2H; 1a,b″-H), 2.07, 2.10, 2.18 (3s, 9H; $3COCH_3$), 2.50–2.63 (m, 2H; 2a,b″-H), 3.19 (q, 6H; NCH_2CH_3), 4.10 (q, 2H; OCH_2CH_3), 4.06–4.25 (m, 2H; 5a,b′-H), 4.38–4.42 (m, 1H; 4′-H), 5.40–5.49 (m, 2H; 2′-, 3′-H), 6.16 (d, $J(1',2') = 4.2$ Hz, 1H; 1′-H), 7.52 (d, $J(6,5) = 7.6$ Hz, 1H; 6-H), 8.41 (d, 1H; 5-H); ^{31}P NMR (161.7 MHz, CD_3OD): $\delta = 24.46$ (s, phosphonate); MS (FAB, negative mode, matrix: 3-nitrobenzyl alcohol): $m/z = 532$ $[(M - NHEt_3^+)^-]$, 554 $[(M - NHEt_3^+ - H^+ + Na^+)^-]$, 634.3 for $C_{26}H_{43}N_4O_{12}P$.

Disodium cytidin-5′-yl carboxymethylphosphonate (**1a**): A solution of sodium methoxide (0.5 M) in dry methanol (0.2 mL) was added to a solution of ethyl ester **10a** (50 mg, 0.08 mmol) in dry methanol (5 mL). After 2 h of stirring at room temperature an additional 0.5 M solution of sodium methoxide in dry methanol (0.5 mL) was added. After 8 h of stirring the solution was neutralized with Amberlite IRC 176 (H^+), filtered and evaporated. The residue was dissolved in water/methanol (1:1, 3 mL) and NaOH (1M, 0.5 mL) was added. After stirring overnight, the solution was neutralized with Amberlite IRC 176 (H^+) and filtered and the pH adjusted to 8 with NaOH (1M). Lyophilization and subsequent purification by preparative HPLC (0.05 M TEAB + 0.5% CH_3CN, 8 mL min^{-1}, $t_R = 9$ min) yielded **1a** (24 mg, 73%) as a colourless foam. $R_f = 0.33$ (ethyl acetate/methanol/1M NH_4OAc 1:1:1); ^1H NMR (250 MHz, D_2O): $\delta = 2.61$ (d, $^2J(H,P) = 21.0$ Hz, 2H; 1a,b″-H), 3.9–4.2 (m, 5H; 2′-, 3′-, 4′-, 5a,b′-H), 5.79 (d, $^2J(1',2') = 3.5$ Hz, 1H; 1′-H), 6.0 (d, $J(6,5) = 7.6$ Hz, 1H; 6-H), 7.87 (d, 1H, 5-H); MS (MALDI, negative mode, matrix: ATT): 365 $[(M - 2Na^+ + H^+)^-]$, 409.2 for $C_{11}H_{14}N_3O_9PNa_2$.

Disodium cytidin-5′-yl (2-carboxyethyl)phosphonate (**1b**): To a solution of ethyl ester **10b** (42 mg, 0.067 mmol) in dry methanol (5 mL) a solution of sodium methoxide (0.5 M) in dry methanol (0.5 mL) was added. After stirring for 1 h, the solution was neutralized with Amberlite IRC 176 (H^+), and filtered, and the solvents were removed under reduced pressure. The residue was dissolved in water/methanol (1:1, 3 mL), and NaOH (1M, 0.5 mL) was added. After 3 days of stirring at room temperature the solution was neutralized with Amberlite IRC 176 (H^+) and filtered and the pH adjusted to a value of 8 with NaOH (1M). After lyophilization the residue was purified by preparative HPLC (0.05 M TEAB + 0.5% CH_3CN, 8 mL min^{-1}, $t_R = 9.2$ min). Ion-exchange chromatography (Amberlite IR 120, Na^+) afforded colourless disodium salt **1b** (17 mg, 60%). $R_f = 0.67$ (ethyl acetate/methanol/1M NH_4OAc 1:1:1); ^1H NMR (250 MHz, D_2O): $\delta = 1.6–1.8$ (m, 2H; 1a,b″-H), 2.1–2.3 (m, 2H; 2a,b″-H), 3.80–4.05 (m, 2H; 5a,b′-H), 4.05–4.15 (m, 3H; 2′-, 3′-, 4′-H), 5.78 (d, $^3J(1',2') = 3.3$ Hz, 1H; 1′-H), 5.92 (d, $J(6,5) = 7.4$ Hz, 1H; 6-H), 7.78 (d, 1H; 5-H); ^{31}P NMR (161.7 MHz, D_2O): $\delta = 28.19$ (s, phosphonate); MS (FAB, negative mode, matrix: glycerol): $m/z = 378$ $[(M - 2Na^+ + H^+)^-]$, 400 $[(M - Na^+)^-]$, 422 $[(M - H^+)^-]$ 423.2 for $C_{12}H_{16}N_3O_9PNa_2$.

***N*-Acetyl-2′,3′-di-*O*-acetyl-5′-*O*-tosylcytidine** (**12**): Cytidine derivative **9** (105 mg, 284 μmol) was stirred with 4-toluenesulfonic acid anhydride (139 mg, 427 μmol) and dimethylaminopyridine (35 mg, 427 μmol) in dichloromethane (4 mL) for 7 days under exclusion of moisture. Triethylamine (200 mL) was added before the solution was concentrated. The residue was purified over silica gel (toluene/acetone 3:1) to obtain **12** (114 mg, 77%) as a colourless foam. $R_f = 0.44$ (toluene/acetone 1:1), $[\alpha]_D = +33$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (250.13 MHz, $CDCl_3$): $\delta = 2.03$ (s, 3H; OAc), 2.04 (s, 3H; OAc), 2.23 (s, 3H; NHAc), 2.43 (s, 3H; $SO_2C_6H_4CH_3$), 4.26 (dd, $J(4',5a') = 3.3$ Hz, $J(5a',5b') = 10.9$ Hz, 1H; 5a′-H), 4.34 (ddd, $J(3',4') = 4.3$ Hz, $J(4',5b') = 2.3$ Hz, 1H; 4′-H), 4.41 (dd, 1H; 5b′-H), 5.24 (dd, $J(1',2') = J(2',3') = 5.0$ Hz, 1H; 2′-H), 5.28 (dd, 1H; 3′-H), 6.07 (d, 1H; 1′-H), 7.32 (d, $J(5,6) = 7.6$ Hz, 1H; 5-H), 7.35 (d, $J = 8.5$ Hz, 2H; $SO_2C_6H_4CH_3$, *meta*), 7.68 (d, 1H; 6-H), 7.79 (d, 2H; $SO_2C_6H_4CH_3$, *ortho*), 9.96 (br s, 1H; NH); $C_{22}H_{25}N_3O_{10}S$ (523.52): calcd C 50.47, H 4.81, N 8.03; found C 49.63, H 4.66, N 7.97.

Trisodium *N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl ethylene-1,2-diphosphonate (13): Ethylene-1,2-diphosphonic acid[22] (41 mg, 215 μmol) was mixed with tetrabutylammonium hydroxide (0.8 M) in methanol (537 mL). The solvent was removed and the residue was coevaporated several times with dry acetone and finally with toluene. The resulting salt **11** was stirred together with tosylate **12** (45 mg, 86 μmol) in DMF (2 mL) overnight. The reaction mixture was concentrated under reduced pressure, taken up with water and stirred with Dowex W50X2 (ammonium form). The ion-exchange resin was filtered off and the filtrate concentrated. The raw product was purified by chromatography on cellulose (acetone/0.05 M ammonium bicarbonate 3:1). The fractions containing the product were concentrated, lyophilized from water, taken up in water, stirred with IR120 (Na^+), filtered and lyophilized again to give **13** (21 mg, 42%). $R_f = 0.35$ (cellulose, acetone/0.05 M ammonium bicarbonate 2:1); ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.52-1.64$ (m, 4H; 2PCH_2), 1.95 (s, 3H; OAc), 1.97 (s, 3H; OAc), 2.04 (s, 3H; NHAc), 3.90–4.07 (m, 2H; 5a′-H, 5b′-H), 4.40 (br s, 1H; 4′-H), 5.25 (dd, $J(2',3') = J(3',4') = 5.2$ Hz, 1H; 3′-H), 5.32 (dd, $J(1',2') = 4.2$ Hz, 1H; 2′-H), 5.99 (d, 1H; 1′-H), 7.18 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 8.16 (d, 1H; 6-H); MS (FAB, negative mode, matrix: DMF/nitrobenzylalcohol): $m/z = 540$ $[(M - 3Na^+ + 2H^+)^-]$ 607.3 for $C_{17}H_{22}N_3Na_3P_2O_{13}$.

Disodium cytidin-5′-yl ethylenediphosphonate (2): Acetylated starting material **13** (20 mg, 33 μmol) was taken up in methanol (5 mL) and treated with sodium methoxide (1M) in methanol (0.5 mL). After ten minutes methanol-washed IRC176 (H^+) was added. The mixture was filtered and concentrated and the residue was lyophilized from water to give **2** (15 mg, quant.) of a powder. $R_f = 0.06$ (cellulose, acetone/0.05 M ammonium bicarbonate 2:1); ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.41-1.71$ (m, 4H; 2PCH_2), 3.88 (ddd, $J(4',5a') = 2.4$ Hz, $J(5a',5b') = 11.8$ Hz, $J(5a',P) = 5.2$ Hz, 1H; 5a′-H), 3.98 (ddd, $J(4',5b') = 1.9$ Hz, $J(5b',P) = 3.9$ Hz, 1H; 5b′-H), 4.07–4.14 (m, 3H; 2′-H, 3′-H, 4′-H), 5.77 (d, $J(1',2') = 3.6$ Hz, 1H; 1′-H), 5.92 (d, $J(5,6) = 7.6$ Hz, 1H; 5-H), 7.75 (d, 1H; 6-H); ^{31}P NMR (242.55 MHz, D_2O): $\delta = 27.45$ (d, $^2J = 73.1$ Hz, phosphonate), 31.65 (d, phosphonate); MS (FAB, negative mode, matrix: water/DMSO/acetic acid/glycerol 1:1:1:1): $m/z = 414$ $[M - 2Na^+ + H^+]$, 459.2 for $C_{11}H_{17}N_3Na_2P_2O_{10}$.

Monocyclohexylammonium (2-ethoxycarbonyl-2-propenyl)phosphonate (14) was synthesized as previously described.[23]

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′yl) (2-ethoxycarbonyl-2-propenyl)phosphonate (15): A solution of the monocyclohexylammonium salt **14** (500 mg, 1.8 mmol) in dry methanol (5 mL) was converted into the free acid by ion-exchange chromatography (Amberlite IR120, H^+). Dry pyridine (0.5 mL) was added and the solution was evaporated. The residue was coevaporated with toluene and dissolved in dry pyridine (3 mL). DCC (1.2 g, 5.8 mmol) was added to the solution. After 10 min of stirring, cytidine **9**, (1.5 g, 4 mmol) was added to the white suspension. The mixture was stirred for 7 days at room temperature, then water (15 mL) was added and after 30 min the urea precipitate was filtered off. The solvents were removed under reduced pressure and the residue was purified by flash chromatography (ethyl acetate/methanol 5:1 + 1% triethylamine) to yield phosphonate ester **15** (500 mg, 43%) as a colourless salt. Triethylammonium salt **15** was contaminated with minor quantities of phosphonic acid **14**. $R_f = 0.11$ (ethyl acetate/methanol 3:1 + 1% triethylamine); ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 1.25$ (m, 12H; 3 NCH_2CH_3, OCH_2CH_3), 1.99, 2.06, 2.18, (3s, 9H; 3OAc), 2.88 (d, $^2J(CH_2,P) = 21.0$ Hz, 2H; CH_2-P), 3.15 (q, $J = 7.3$ Hz, 6H; 3NCH_2CH_3), 4.2 (m, 5H; 4′-H, 5a′,b′-H, OCH_2CH_3), 5.40 (m, 2H; 2′-,3′-H), 5.78 (d, $^2J(3b'',3a'') = 5.1$ Hz, 1H; 3b″-H), 6.15 (d, $J(1',2') = 3.9$ Hz, 1′-H), 6.21(d, $^2J(3a'',3b'') = 5.1$ Hz, 1H; 3a″-H) , 7.41 (d, $^3J(5,6) = 7.5$ Hz, 1H; 5-H), 8.14 (d, 1H; 6-H); MS (MALDI, negative mode, matrix: ATT): $m/z = 566$ $[(M - H^+)^-]$, 567 for $C_{21}H_{27}N_3NaO_{12}P$.

Sodium cytidin-5′-yl (2-ethoxycarbonyl-2-propenyl)phosphonate (16): To a solution of ethyl ester **15** (100 mg, 0.16 mmol) in dry methanol (4 mL) a solution of sodium methoxide (1M) in dry methanol (0.2 mL) was added. After 30 min of stirring at room temperature the solution was neutralized with Amberlite IR120 (H^+), and evaporated. The residue was dissolved in methanol (3 mL) and converted with Amberlite IR120 (Na^+) into the sodium salt. It was purified by crystallization from chloroform to yield **16** as colourless crystals (50 mg, 73%). M.p. 163–166 °C; $R_f = 0.68$ (RP-18, water/ethanol 3:1); ^{1}H NMR (250 MHz, CD_3OD): $\delta = 1.25$ (t, $J = 7.1$ Hz, 3H; OCH_2CH_3), 2.8 (d, $^2J(CH_2,P) = 21.0$ Hz, 2H; CH_2-P), 4.02–4.21 (m, 7H; 2′-,3′-,4′-,5a′,b′-H, OCH_2CH_3), 5.81 (d, $^2J(3a'',3b'') = 5.1$ Hz, 1H; 3a″-H), 5.87 (d, $J(1',2') = 3.1$ Hz, 1H; 1′-H), 6.09 (d, $J(5,6) = 7.8$ Hz, 1H; 5-H), 6.19 (d, 1H; 3b″-H), 8.28 (d, 1H; 6-H).

Disodium cytidin-5′-yl (2-carboxy-2-propenyl)phosphonate (3): NaOH (1M, 0.5 mL) was added to a solution of ethyl ester **16** (50 mg, 0.11 mmol) in water/methanol (2:1, 3 mL). After stirring overnight, the solution was neutralized with Amberlite IR120 (H^+), filtered and converted into the sodium salt with Amberlite IR120 (Na^+). The filtrate was concentrated under reduced pressure and the residue was dissolved in 1 mL of water. Addition of 10 mL of ethanol caused **3** (30 mg, 63%) to precipitate as a white powder. $R_f = 0.81$ (RP-18 water/ethanol 3:1); $[\alpha]_D = -12.8$ ($c = 0.05$, water); ^{1}H NMR (250 MHz, D_2O): $\delta = 2.62$ (d, $^2J(CH_2,P) = 21.0$ Hz, 2H; CH_2-P), 3.86–4.08 (m, 5H; 2′-,3′-,4′-,5a′,b′-H), 5.57 (d, $^2J(3a'',3b'') = 5.1$ Hz, 1H; 3a″-H), 5.74 (d, $J(1',2') = 3.1$ Hz, 1H; 1′-H), 5.98 (d, 1H, 3b″-H), 6.01 (d, $J(5,6) = 7.8$ Hz, 1H, 5-H), 7.91 (d, 1H, 6-H). ^{31}P NMR (161.70 MHz, D_2O): $\delta = 25.86$ (s, phosphonate); MS (FAB, negative mode, matrix: glycerol/3-nitrobenzyl alcohol): $m/z = 390$ $[(M - H^+)^-]$, 482 $[(M + \text{glycerol} - H^+)^-]$, 391.25 for $C_{13}H_{18}N_3O_9P$.

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enopyranosonic acid (17): The compound was synthesized from 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid as previously described.[24] $R_f = 0.39$ (ethyl acetate/methanol 7:1 + 3% acetic acid); $[\alpha]_D = +65$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.91$ (s, 3H; NHAc), 2.01 (s, 3H; OAc), 2.04 (s, 3H; OAc), 2.05 (s, 3H; OAc), 2.09 (s, 3H; OAc), 4.09 (dd, $J(8,9a) = 5.8$ Hz, $J(9a,9b) = 11.0$ Hz, 1H; 9a-H), 4.31–4.41 (m, 2H; 5-H, 6-H), 4.55 (dd, $J(8,9b) = 3.0$ Hz, 1H; 9b-H), 5.34 (ddd, $J(7,8) = 4.8$ Hz, 1H; 8-H), 5.45–5.52 (m, 2H; 4-H, 7-H), 6.03 (d, $J(3,4) = 3.1$ Hz, 1H; 3-H), 6.12 (d, $J(5,NH) = 9.1$ Hz, 1H; NH), 10.71 (br s, 1H; COOH); $C_{19}H_{25}NO_{12} \cdot H_2O$ (459.41): calcd C 47.80, H 5.70, N 2.93; found C 48.05, H 5.65, N 3.21.

4-Methylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enpyranosonothioate (18): Acid **17** (95 mg, 20 μmol) was dissolved in DMF (20 mL) and cooled to 0 °C. Carbonyldiimidazole (35 mg, 219 μmol) was then added. The cooling bath was removed and the solution stirred for 2 h under exclusion of moisture. 4-Thiocresol was added to the solution, which was diluted with ethyl acetate (100 mL) after another hour. The solution was washed with brine and several times with water. The aqueous layers were combined and extracted again with ethyl acetate. The combined organic layers were finally washed with water, dried over magnesium sulfate and concentrated. The remaining oil was purified by chromatography over silica gel (toluene/ethyl acetate/methanol 20:4:1) and yielded 1.12 g (74%) crystals. M.p. 139 °C; $R_f = 0.49$ (toluene/acetone 1:1), $[\alpha]_D = +62$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.93$ (s, 3H; NHAc), 2.03 (s, 3H; OAc), 2.06 (s, 3H; OAc), 2.09 (s, 3H; OAc), 2.15 (s, 3H; OAc), 2.36 (s, 3H; $SC_6H_4CH_3$), 4.23 (dd, $J(8,9a) = 6.0$ Hz, $J(9a,9b) = 12.4$ Hz, 1H; 9a-H), 4.38 (ddd, $J(5,6) = J(5,NH) = 9.0$ Hz, $J(4,5) = 7.2$ Hz, 1H; 5-H), 4.51 (dd, $J(6,7) = 3.5$ Hz, 1H; 6-H), 4.52 (dd, $J(8,9b) = 3.4$ Hz, 1H; 9b-H), 5.50–5.56 (m, 3H; 4-H, 7-H, NH), 5.51 (ddd, $J(7,8) = 6.0$ Hz, 1H; 8-H), 5.87 (d, $J(3,4) = 3.3$ Hz, 1H; 3-H), 7.19–7.29 (m, 4H; $SC_6H_4CH_3$); $C_{26}H_{31}NO_{11}S$ (565.60): calcd C 55.21, H 5.52, N 2.48; found C 55.31, H 5.59, N 2.66.

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enitol (19): Compound **18** (85 mg, 150 μmol) was dissolved in ethanol (5 mL) and cooled to 0 °C under exclusion of moisture. Sodium borohydride (144 mg, 3.81 mmol) was added under rapid stirring. After three hours, concentrated ammonium chloride solution was poured into the reaction mixture. When the evolution of gas ceased, water (20 mL) was added. The solution was extracted with ethyl acetate several times. The organic layers were collected, dried over magnesium sulfate and concentrated. Chromatography over silica gel (toluene/acetone 1:1) gave **19** (49 mg, 73%), a colourless foam. $R_f = 0.38$ (toluene/ethyl acetate/methanol 5:2:1), $[\alpha]_D = +41$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.89$ (s, 3H; NHAc), 2.02 (s, 3H; OAc), 2.03 (s, 3H; OAc), 2.06 (s, 3H; OAc), 2.09 (s, 3H; OAc), 2.98 (br s, 1H; OH), 3.88 (br d, $J(1a,1b) = 13.6$ Hz, 1H; 1a-H), 3.97 (dd, $J(8,9a) = 7.2$ Hz, $J(9a,9b) = 12.4$ Hz, 1H; 9a-H), 4.09 (br d, 1H; 1b-H), 4.19 (dd, $J(5,6) = 9.6$ Hz, $J(6,7) = 2.9$ Hz, 1H; 6-H), 4.36 (ddd, $J(5,NH) = 9.7$ Hz, $J(4,5) = 7.9$ Hz, 1H; 5-H), 4.60 (dd, $J(8,9b) = 2.5$ Hz, 1H; 9b-H), 4.87 (d, $J(3,4) = 2.7$ Hz, 1H; 3-H), 5.35 (ddd, $J(7,8) = 7.2$ Hz, 1H; 8-H), 5.37–5.42 (m, 2H, 4-H; 7-H), 5.66 (d, 1H; NH); $C_{19}H_{27}NO_{11}$ (445.43): calcd C 51.23, H 6.11, N 3.14; found C 50.91, H 6.16, N 3.17.

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(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enitol-1-yl) 2-cyanoethyl phosphite diisopropylamide (20): Alcohol **19** (117 mg, 263 μmol) was dried under reduced pressure together with diisopropylammonium tetrazolide (23 mg, 131 μmol). This mixture was taken up in dichloromethane (4 mL) under exclusion of moisture. 2-Cyanoethyl phosphite bisdiisopropylamide[25] (167 mL, 525 mmol) was added. After the mixture had been stirred for one hour, concentrated sodium bicarbonate solution (5 mL), water and dichloromethane were added. The organic layer was dried over magnesium sulfate and concentrated. The raw product was quickly filtered over silica gel (toluene/acetone 1:1) to give a mixture of the two diastereomers of **20** and 2-cyanoethyl phosphite diisopropylamide, which does not interfere in the next step (in total 228 mg of a colourless oil). $R_f = 0.6$ (toluene/acetone 1:1).

Triethylammonium (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enitol-1-yl) (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl) phosphate (21):

a) From phosphite **20**: The raw product **20** (225 mg, approximately 260 mmol) and compound **9** (96 mg, 260 mmol) were coevaporated several times with toluene and finally with dichloromethane and dried under reduced pressure. This mixture was taken up with dichloromethane (10 mL) under a nitrogen atmosphere. 1H-Tetrazole (27 mg, 390 mmol) was added and dissolved slowly. The solution was cooled to $-10\,^{\circ}C$ after stirring overnight. *Tert*-butylhydroperoxide solution (3 M) in toluene (173 mL) was added. 3 h later, triethylamine (1 mL) diluted with dichloromethane (5 mL) was dropped into the reaction mixture, which was concentrated after 2 h. The residue was purified over silica gel (ethyl acetate/methanol 3:1 + 2% triethylamine) to give 110 mg of the product which could not be separated from an impurity (probably 1-phosphite of compound **19**).

b) From phosphite **22**: Phosphite **22** (63 mg, 141 μmol) and alcohol **19** (73 mg, 128 μmol) were taken up in dichloromethane, foamed and dried in vacuum. The foam was dissolved in dry dichloromethane (5 mL) under nitrogen. 1H-Tetrazole (13 mg, 192 μmol) and, after this had been stirred for three hours, *tert*-butylhydroperoxide solution in toluene (3 M, 85 mL, 256 mmol) were added. After another hour triethylamine (1 mL) was added. After stirring overnight, the reaction mixture was carefully concentrated and chromatographed over silica gel (ethyl acetate/methanol 5:1 + 1% triethylamine) to yield **21** (122 mg, ca. 90%) of a pale yellow syrup. $R_f = 0.11$ (ethyl acetate/methanol 3:1); ^{1}H NMR (250.13 MHz, CD_3OD): $\delta = 1.36$ (t, $J = 7.3$ Hz, 9H; $3NCH_2CH_3$), 1.90 (s, 3H; NHAc), 2.03 (s, 3H; OAc), 2.04 (s, 3H; OAc), 2.07 (s, 3H; OAc), 2.12 (s, 3H; OAc), 2.13 (s, 3H; OAc), 2.15 (s, 3H; OAc), 2.23 (s, 3H; NHAc), 3.25 (q, 6H; $3NCH_2CH_3$), 4.08–4.46 (m, 9H; 4′-H, 5a′-H, 5b′-H, 1a″ 1b″-H, 5″-H, 6″-H, 9a″-H, 9b″-H), 5.04 (d, $J(3'',4'') = 2.4$ Hz, 1H; 3″-H), 5.23 (ddd, $J(7'',8'') = 7.2$ Hz, $J(8'',9a'') = 10.0$ Hz, $J(8'',9b'') = 2.8$ Hz, 1H; 8″-H), 5.42–5.52 (m, 4H, 2′-H, 3′-H, 4″-H, 7″-H), 6.24 (d, $J(1',2') = 3.8$ Hz, 1H; 1′-H), 7.57 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 8.47 (d, 1H; 6-H); MS (MALDI, negative mode, matric:ATT): $m/z = 875$ $[(M - HNEt_3^+)^-]$, 976.9 for $C_{40}H_{59}N_5O_{21}P$.

(*N*-Acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl) 2-cyanoethyl phosphite diisopropylamide (22): Alcohol **9** (95 mg, 257 μmol) was dried under reduced pressure together with diisopropylammonium tetrazolide. The mixture was taken up with dichloromethane (3 mL) under exclusion of moisture. 2-Cyanoethyl phosphite bisdiisopropylamide (163 μL, 514 μmol) was added. After stirring overnight, the reaction mixture was concentrated and purified over silica gel (toluene/acetone 3:1) to give **22** (108 mg, 74%), a colourless foam containing both diastereomers. $R_f = 0.46$ (toluene/acetone 1:1); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.14$–1.26 (m, 12H; $4NCHCH_3$), 2.02–2.09 (m, 6H; 2OAc), 2.24 (s, 3H; NHAc), 2.65 (m, 2H; OCH_2CH_2CN), 3.54–3.66 (m, 2H; $2NCHCH_3$), 3.72–4.04 (m, 4H; 5a′-H, 5b′-H, OCH_2CH_2CN), 4.32 (br s, 1H; 4′-H), 5.31–5.46 (m, 2H; 2′-H, 3′-H), 6.28–6.35 (d, $J(1',2') = 5.0$ Hz, 1H; 1′-H), 7.42 (d, $J(5,6) = 7.6$ Hz, 1H; 5-H), 8.22–8.23 (d, 1H; 6-H), 9.92 (br s, 1H; NH); $C_{24}H_{36}N_5O_9P$ (569.56): calcd C 50.61, H 6.37, N 12.30; found C 50.55, H 6.44, N 12.15.

Triethylammonium (5-acetamido-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enitol-1-yl) (cytidin-5′-yl) phosphate (4): Compound **21** (120 mg, 122 μmol) was dissolved in methanol (3 mL) and treated with sodium methoxide (1 M) in methanol (0.5 mL). After stirring overnight, ammonium chloride (100 mg) was added. The solvent was removed and the residue was purified by HPLC over RP-18 silica gel. Lyophilization gave **4** (56 mg, 67%). HPLC: prep. RP-18 column (flow: 7 mL min^{-1}, 0.05 M triethylammonium bicarbonate buffer + 7% acetonitrile): $t_R = 13.5$ min; ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.12$ (t, $J = 7.4$ Hz, 9H; $3NCH_2CH_3$), 1.90 (s, 3H; NHAc), 3.04 (q, 6H; $3NCH_2CH_3$), 3.44 (br d, $J(7'',8'') = 9.5$ Hz, 1H; 7-H), 3.47 (dd, $J(8'',9a'') = 6.6$ Hz, $J(9a'',9b'') = 12.5$ Hz, 1H; 9a″-H), 3.66–3.73 (m, 2H; 8″-H, 9b″-H), 3.87 (dd, $J(4'',5'') = 8.6$ Hz, $J(5'',6'') = 10.8$ Hz, 1H; 5″-H), 3.86–4.76 (m, 7H; 2′-H, 3′-H, 4′-H, 5a′-H, 5b′-H, 1a″-H, 1b″-H), 4.03 (br d, 1H; 6″-H), 4.16 (d, $J(3'',4'') = 1.7$ Hz, 1H; 4″-H), 4.83 (d, 1H; 3″-H), 5.82 (d, $J(1',2') = 3.5$ Hz, 1H; 1′-H), 5.94 (d, $J(5,6) = 8.0$ Hz, 1H; 5-H), 7.76 (d, 1H; 6-H); ^{31}P NMR (242.55 MHz, D_2O): $\delta = -3.71$ (s, phosphate); MS (FAB, negative mode, matrix: glycerol/water 1:1): $m/z = 581$ $[(M - HNEt_3^+)^-]$ 682.7 for $C_{26}H_{45}N_5O_{14}P$.

5-Acetamido-4,7,8,9-tetra-*O*-acetylaldehydo-2,6-anhydro-3,5-dideoxy-D-glycero-D-galacto-non-2-enose (23): Alcohol **19** (235 mg, 528 mmol) was dissolved in dichloromethane (10 mL). Periodinane (257 mg, 607 μmol) was added and the solution stirred for 20 min. Subsequently the mixture was diluted with dichloromethane (100 mL) and stirred for another 20 min with a mixture of sodium thiosulfate (2.2 g), water (5 mL), and concentrated sodium bicarbonate solution (5 mL). The organic layer was separated, washed with water and dried over magnesium sulfate. The crude product was purified over silica gel (toluene/acetone 1:1) to give **23** (193 mg, 82%) as a colourless foam. $R_f = 0.39$ (toluene/acetone 1:1), $[\alpha]_D = +102$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.93$ (s, 3H; NHAc), 2.03 (s, 3H; OAc), 2.04 (s, 3H; OAc), 2.08 (s, 3H; OAc), 2.10 (s, 3H; OAc), 4.19 (dd, $J(8,9a) = 6.5$ Hz, $J(9a,9b) = 12.3$ Hz, 1H; 9a-H), 4.38 (ddd, 1H; $J(4,5) = 6.6$ Hz, $J(5,6) = 8.3$ Hz, $J(5,NH) = 8.3$ Hz, 5-H), 4.41 (dd, $J(6,7) = 3.9$ Hz, 1H; 6-H), 4.48 (dd, $J(8,9b) = 3.3$ Hz, 1H; 9b-H), 5.38 (ddd, $J(7,8) = 5.9$ Hz, 1H; 8-H), 5.46 (dd, 1H; 7-H), 5.56 (dd, $J(3,4) = 3.2$ Hz, 1H; 4-H), 5.60 (d, 1H; NH), 5.82 (d, 1H; 3-H), 9.20 (s, 1H; CHO); $C_{19}H_{25}NO_{11}$ (443.41): calcd C 51.47, H 5.68, N 3.16; found C 51.09, H 5.76, N 3.23.

Dibenzyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl)phosphonate [(*R*)-**24**] and **dibenzyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-erythro-L-manno-non-2-enitol-1-yl)phosphonate** [(*S*)-**24**]: Aldehyde **23** (53 mg, 119 μmol) was dissolved in dichloromethane (3 mL). Dibenzyl phosphite (40 μL, 179 μmol) and triethylamine (5 μL) were added. The solution was stirred for one hour, concentrated and chromatographed over silica gel (toluene/acetone 1:1) to give 83 mg (99%) of the product as a colourless foam. The two diastereomers were formed in a ratio of $R{:}S = 4{:}1$; they could be separated by MPLC over silica gel (toluene/acetone 2:3).

(*R*)-**24**: $R_f = 0.44$ (toluene/acetone 2:3), $[\alpha]_D = +56$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.85$ (s, 3H; NHAc), 1.98 (s, 6H; 2OAc), 2.00 (s, 3H; OAc), 2.04 (s, 3H; OAc), 3.86 (dd, $J(8,9a) = 7.4$ Hz, $J(9a,9b) = 12.4$ Hz, 1H; 9a-H), 4.13 (dd, $J(1,OH) = 9.4$ Hz, $J(OH,P) = 6.5$ Hz, 1H; OH), 4.19 (dd, $J(5,6) = 9.1$ Hz, $J(6,7) = 3.5$ Hz, 1H; 6-H), 4.31 (dd, $J(1,P) = 14.6$ Hz, 1H; 1-H), 4.42 (ddd, $J(4,5) = 7.5$ Hz, $J(5,NH) = 9.1$ Hz, 1H; 5-H), 4.61 (dd, $J(8,9b) = 2.6$ Hz, 1H; 9b-H), 5.02 (dd, $J(3,4) = J(3,P) = 3.1$ Hz, 1H; 3-H), 5.06–5.11 (m, 4H; $2CH_2C_6H_5$), 5.29 (ddd, $J(7,8) = 4.9$ Hz, 1H; 8-H), 5.36 (dd, 1H, 4-H), 5.40 (dd, 1H; 7-H), 5.83 (d, 1H; NH), 7.30–7.34 (m, 10H; $2CH_2C_6H_5$); $C_{33}H_{40}NO_{14}P \cdot {}^3/_2H_2O$ (705.66): calcd C 54.10, H 5.91, N 1.91; found C 54.12, H 5.72, N 2.11.

(*S*)-**24** $R_f = 0.44$ (toluene/acetone 2:3), $[\alpha]_D = +69$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.85$ (s, 3H; NHAc), 1.99 (s, 3H; OAc), 2.00 (s, 3H; OAc), 2.02 (s, 6H; 2 OAc), 3.55 (br s, 1H; OH), 4.08 (dd, $J(8,9a) = 6.8$ Hz, $J(9a,9b) = 12.0$ Hz, 1H; 9a-H), 4.24 (dd, $J(5,6) = J(6,7) = 4.7$ Hz, 1H; 6-H), 4.40 (dd, $J(8,9b) = 4.0$ Hz, 1H; 9b-H), 4.43 (d, $J(1,P) = 13.2$ Hz, 1H; 1-H), 4.52 (ddd, $J(4,5) = 4.7$ Hz, $J(5,NH) = 9.3$ Hz, 1H; 5-H), 5.00–5.17 (m, 6H; 3-H, 4-H, $2CH_2C_6H_5$), 5.40 (ddd, $J(7,8) = 4.1$ Hz, 1H; 8-H), 5.50 (dd, 1H; 7-H), 6.37 (d, 1H; NH), 7.25–7.34 (m, 10H; $2CH_2C_6H_5$); $C_{33}H_{40}NO_{14}P \cdot {}^3/_4H_2O$ (705.66): calcd C 55.11, H 5.82, N 1.95; found C 55.08, H 5.94 N 2.09.

Dibenzyl (5-acetamido-1,4,7,8,9-penta-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl)phosphonate [(*R*)-**25**]: Compound (*R*)-**24** (153 mg, 217 μmol) was mixed with pyridine and acetic anhydride (2 mL each). After stirring for 2 h the solution was concentrated under vacuum and filtered over silica gel (toluene/acetone 2:1) to give (*R*)-**25** (150 mg, 92%) as a colourless foam. $R_f = 0.45$ (toluene/acetone 1:1), $[\alpha]_D = +62$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.89$ (s, 3H; NHAc), 1.90 (s, 3H; OAc), 2.00 (s, 6H; 2OAc), 2.01 (s, 6H; 2OAc), 4.09 (dd, $J(8,9a) = 6.2$ Hz, $J(9a,9b) = 12.2$ Hz, 1H; 9a-H), 4.17 (dd, $J(5,6) = J(6,7) = 6.0$ Hz, 1H; 6-H), 4.33 (dd, $J(8,9b) = 3.9$ Hz, 1H; 9b-H), 4.42 (ddd, $J(4,5) = 6.0$ Hz, $J(5,NH) = 9.5$ Hz, 1H; 5-H), 5.00–5.15 (m, 5H; 3-H,

2CH_2C$_6$H$_5$), 5.21 (ddd, $J(3,4) = J(4,P) = 6.0$ Hz, 1H; 4-H), 5.36 (dd, $J(7,8) = 5.2$ Hz, 1H; 8-H), 5.47 (dd, 1H; 7-H), 5.53 (d, $J(1,P) = 14.0$ Hz, 1H; 1-H), 5.93 (d, 1H; NH), 7.27–7.39 (m, 10H; 2CH$_2$C$_6$H$_5$); $C_{35}H_{42}NO_{15}P$ (747.70): calcd C 56.22, H 5.66, N 1.87; found C 56.01, H 5.74, N 1.91.

Disodium (5-acetamido-2,6-anhydro-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl)phosphonate [(*R*)-**26**]: Compound (*R*)-**25** (48 mg, 64 μmol) was dissolved in isopropanol (10 mL). Palladium on charcoal (5 mg, 10% Pd) was added and the mixture was stirred vigorously under hydrogen at normal pressure. After 40 min the catalyst was filtered off and washed with methanol. Filtrate and washings were combined and sodium methoxide in methanol (1M, 0.2 mL) was added. After 10 min the solution was concentrated to a volume of about 3 mL and quickly diluted with acetone (15 mL). The precipitate was centrifuged, washed with acetone and dried under reduced pressure to give (*R*)-**26** (23 mg, 90%) of an amorphous powder. $R_f = 0.70$ (cellulose, acetone/0.05M ammonium bicarbonate 1:1); ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.90$ (s, 3H, NHAc), 3.40 (dd, $J(6,7) = 1.7$ Hz, $J(7,8) = 9.8$ Hz, 1H; 7-H), 3.44 (dd, $J(8,9a) = 6.9$ Hz, $J(9a,9b) = 12.0$ Hz, 1H; 9a-H), 3.69 (dd, $J(8,9b) = 2.6$ Hz, 1H; 9b-H), 3.74 (ddd, 1H; 8-H), 3.87–4.01 (m, 3H; 1-H, 5-H, 6-H), 4.22 (ddd, $J(3,4) = J(4,6) = 2.7$ Hz, $J(4,5) = 7.0$ Hz, 1H; 4-H), 4.77 (dd, $J(3,P) = 2.7$ Hz, 1H; 3-H); MS (FAB, negative mode, matrix: glycerol/acetic acid/DMSO/water 1:1:1:1): $m/z = 378$ [$(M - Na^+)^-$] 401.2 for $C_{11}H_{18}NNa_2O_{10}P$.

Triethylammonium (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl) (5-acetamido-1,4,7,8,9-penta-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl)phosphonate [(*R*)-**27**]: The peracetylated phosphonate (*R*)-**25** (125 mg, 167 mmol) was taken up in methanol (15 mL) and stirred for 30 min with palladium on charcoal (10 mg, 10% Pd) under a hydrogen atmosphere at normal pressure. The mixture was filtered and washed with methanol. Pyridine (200 mL) was added before the solvent was removed. The residue was coevaporated with pyridine several times, dried under reduced pressure and taken up with pyridine again. Dicyclohexylcarbodiimide (103 mg, 501 μmol), dimethylaminopyridine (2 mg, 17 μmol) and compound **9** (93 mg, 251 mmol) were added under exclusion of moisture. After stirring overnight, water (2 mL) was added and the reaction mixture was filtered, washed with pyridine and concentrated. Chromatography over silica gel (ethyl acetate/methanol 5:1 to 3:1 + 1% triethylamine) afforded (*R*)-**27** (111 mg, 65%) of a colourless glass. $R_f = 0.22$ (ethyl acetate/methanol 2:1 + 1% triethylamine); ^{1}H NMR (250.13 MHz, CD_3OD): $\delta =$ 1.35 (t, $J = 7.3$ Hz, 9H; 3NCH$_2$CH_3), 1.90 (s, 3H; NHAc), 2.02 (s, 3H; OAc), 2.03 (s, 3H; OAc), 2.07 (s, 3H; OAc), 2.11 (s, 3H; OAc), 2.12 (s, 3H; OAc), 2.15 (s, 3H; OAc), 2.17 (s, 3H; OAc), 2.23 (s, 3H; NHAc), 3.24 (q, 6H, 3NCH_2CH$_3$), 4.23 (dd, $J(8'',9a'') = 6.6$ Hz, $J(9a'',9b'') = 12.4$ Hz, 1H; 9a″-H), 4.25–4.46 (m, 5H; 4′-H, 5a′-H, 5b′-H, 5″-H, 6″-H), 4.52 (dd, $J(8'',9b'') = 2.8$ Hz, 1H; 9b″-H), 5.14 (dd, $J(3'',4'') = J(3'',P) = 2.8$ Hz, 1H; 3″-H), 5.37 (d, $J(1'',P) = 14.3$ Hz, 1H; 1″-H), 5.38–5.55 (m, 5H; 2′-H, 3′-H, 4″-H, 7″-H, 8″-H), 6.25 (d, $J(1',2') = 4.5$ Hz, 1H; 1′-H), 7.58 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 8.47 (d, 1H; 6-H); MS (MALDI, negative mode, matrix: ATT): $m/z = 917$ [$(M - HNEt_3^+)^-$], 1019.9 for $C_{42}H_{62}N_5O_{22}P$.

Sodium cytidin-5′-yl (5-acetamido-2,6-anhydro-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl)phosphonate [(*R*)-**5**]: Compound **27** (85 mg, 83 mmol) was dissolved in methanol (6 mL) and treated with sodium methoxide (1M) in methanol (0.2 mL). After reaction overnight, the solution was diluted with acetone (14 mL). Centrifugation, washing with acetone and drying under reduced pressure afforded **5** (44 mg, 88%), an amorphous powder. $R_f = 0.71$ (cellulose, acetone/0.05M ammonium bicarbonate 1:1); ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.90$ (s, 3H; NHAc), 3.43 (br d, $J(7'',8'') = 8.6$ Hz, 1H; 7″-H), 3.46 (dd, $J(8'',9a'') = 6.7$ Hz, $J(9a'',9b'') = 13.7$ Hz, 1H; 9a″-H), 3.68 (br d, 1H; 9b″-H), 3.71 (ddd, 1H; 8″-H), 3.91 (dd, $J(4'',5'') = 8.1$ Hz, $J(5'',6'') = 10.8$ Hz, 1H; 5″-H), 3.98–4.20 (m, 7H; 2′-H, 3′-H, 4′-H, 5a′-H, 5b′-H, 1″-H, 6″-H), 4.25 (ddd, $J(3'',4'') = 1.0$ Hz, $J(4'',6'') = 5.5$ Hz, 1H; 4″-H), 4.83 (dd, $J(3'',P) = 1.0$ Hz, 1H; 3″-H), 5.82 (d, $J(1',2') = 3.4$ Hz, 1H; 1′-H), 5.92 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 7.83 (d, 1H; 6-H); ^{31}P NMR (161.70 MHz, D_2O): $\delta = 16.69$ (s, phosphonate); MS (FAB, negative mode, matrix: glycerol/acetic acid/DMSO 1:1:1): $m/z = 581$ [$(M - Na^+)^-$] 604.4 for $C_{20}H_{30}N_4NaO_{14}P$.

Triethylammonium (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-dibenzylphosphoryl-3,5-dideoxy-D-erythro-L-gluco-non-2-enitol-1-yl) (*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl) phosphate [(*R*)-**28**]: Compound (*R*)-**24** (111 mg, 157 μmol) was treated as described for compound **21** (route b). A colourless syrup of (*R*)-**28** (143 mg, 73%) was obtained. $R_f = 0.30$ (ethyl acetate/methanol 2:1); ^{1}H NMR (250.13 MHz, CD_3OD): $\delta = 1.33$ (t, $J =$ 7.3 Hz, 9H; 3NCH$_2$CH_3), 1.88 (s, 3H; NHAc), 1.98 (s, 3H; OAc), 2.00 (s, 6H; 2OAc), 2.07 (s, 3H; OAc), 2.11 (s, 3H; OAc), 2.12 (s, 3H; OAc), 2.20 (s, 3H; NHAc), 3.21 (q, 6H; 3NCH_2CH$_3$), 4.11 (dd, $J(8'',9a'') = 6.0$ Hz, $J(9a'',9b'') = 11.1$ Hz, 1H; 9a″-H), 4.17 (ddd, $J(4',5a') = 4.5$ Hz, $J(5a',5b') = 12.7$ Hz, $J(5a',P) = 1.8$ Hz, 1H; 5a′-H), 4.26–4.50 (m, 4H; 4′-H, 5b′-H, 5″-H, 6″-H), 4.46 (dd, $J(8'',9b'') = 2.2$ Hz, 1H; 9b″-H), 5.15 (dd, $J(1'',P) = 11.1$ Hz, $J(1'',P) = 15.6$ Hz, 1H; 1″-H), 5.19–5.31 (m, 6H; 3″-H, 8″-H, 2CH_2C$_6$H$_5$), 5.45–5.52 (m, 4H; 2′-H, 3′-H, 4″-H, 7″-H), 6.24 (d, $J(1',2') = 4.0$ Hz, 1H; 1′-H), 7.34–7.44 (m, 10H; 2CH$_2$C$_6$H$_5$), 7.54 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 8.18 (d, $J(5'',NH) = 9.4$ Hz, 1H; NH), 8.48 (d, 1H; 6-H); MS (MALDI, positive mode, matrix: ATT): $m/z = 1161$ [$(M -$ NEt$_3$+Na$^+$)$^+$], 1238.1 for $C_{54}H_{73}N_5O_{24}P_2$.

Trisodium 5-acetamido-2,6-anhydro-3,5-dideoxy-1-phosphoryl-D-erythro-L-gluco-non-2-enitol-1-yl cytidin-5′-yl phosphate [(*R*)-**6**] and **sodium (1*E*) 5-acetamido-2,6-anhydro-3,4,5-trideoxy-D-manno-non-1,3-dienitol-1-yl cytidin-5′-yl phosphate** [(*E*)-**6′**]: Freshly prepared compound (*R*)-**28** (81 mg, 65 μmol) was taken up with methanol (5 mL), and palladium on charcoal (10 mg, 10% Pd) was added. The mixture was stirred vigorously under a hydrogen atmosphere at normal pressure. The catalyst was filtered off and washed. Filtrate and washings were combined and sodium methoxide (1M, 0.3 mL) was added. After 15 min acetone (20 mL) was quickly added. The precipitate was centrifuged, washed with acetone and dried under reduced pressure. The crude product was purified by HPLC over RP-18 silica gel. The fractions containing the product were combined and lyophilized, taken up in water and stirred with IR120 (Na^+). Filtration and lyophilization afforded 42 mg (88%) (*R*)-**6**. A second compound was eluted from the column (after the product), which may have been formed from the unstable intermediate after the hydrogenolysis. It was also transformed into the sodium form and lyophilized to give (*E*)-**6′** (2 mg, 5%).

(*R*)-**6**: HPLC: prep. RP-18 column (flow: 8 mL min^{-1}, 0.1M triethylammonium hydrogencarbonate buffer + 2% acetonitrile): $t_R = 6.5$ min; ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.94$ (s, 3H; NHAc), 3.42 (br d, $J(7,8) = 9.2$ Hz, 1H; 7-H), 3.49 (dd, $J(8'',9a'') = 6.6$ Hz, $J(9a'',9b'') = 11.8$ Hz, 1H; 9a″-H), 3.74 (dd, $J(8'',9b'') = 2.6$ Hz, 1H; 9b″-H), 3.82 (ddd, 1H; 8″-H), 3.87 (dd, $J(4'',5'') = 4.0$ Hz, $J(5'',6'') = 7.7$ Hz, 1H; 5″-H), 4.05 (dd, 1H; 6″-H), 4.10–4.33 (m, 7H; 2′-H, 3′-H, 4′-H, 5a′-H, 5b′-H, 1″-H, 4″-H), 4.86 (dd, $J(3'',4'') = J(3'',P) = 2.4$ Hz, 1H; 3″-H), 6.02 (d, $J(1',2') = 3.2$ Hz, 1H; 1′-H), 6.03 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 7.92 (d, 1H; 6-H); ^{31}P NMR (161.70 MHz, D_2O): $\delta = 1.00$ (d, phosphate), 11.28 (d, phosphonate); MS (FAB, negative mode, matrix: glycerol/acetic acid/DMSO 1:1:1): $m/z = 683$ [$(M - 2Na^+ + H^+)^-$] 728.4 for $C_{20}H_{29}N_4Na_3O_{17}P_2$.

(*E*)-**6′**: HPLC: prep. RP-18 column (flow: 8 mL min^{-1}, 0.1M triethylammonium bicarbonate buffer + 2% acetonitrile): $t_R = 21.6$ min; ^{1}H NMR (250.13 MHz, D_2O): $\delta = 1.95$ (s, 3H; NHAc), 3.49 (dd, $J(6'',7'') \approx 1.0$ Hz, $J(7'',8'') = 8.9$ Hz, 1H; 7″-H), 3.57 (dd, $J(8'',9a'') = 6.9$ Hz, $J(9a'',9b'') = 12.6$ Hz, 1H; 9a″-H), 3.74–3.79 (m, 3H; 6″-H, 8″-H, 9b″-H), 4.03–4.22 (m, 5H; 2′-H, 3′-H, 4′-H, 5a′-H, 5b′-H), ≈4.6 (m, 1H; 5″-H), 5.70 (ddd, $J(3'',4'') = 10.2$ Hz, $J(1'',3'') \approx J(3'',5'') 1.0$ Hz, 1H; 3″-H), 5.90 (d, $J(1',2') = 4.2$ Hz, 1H; 1′-H), 6.01 (d, $J(5,6) = 7.5$ Hz, 1H; 5-H), 6.43 (br d, $J(1'',P) = 4.6$ Hz, 1H; 1″-H), 6.50 (dd, $J(4'',5'') = 2.1$ Hz, 1H; 4″-H), 7.77 (d, 1H; 6-H); ^{31}P NMR (161.70 MHz, D_2O): $\delta = -2.23$ (s, phosphate); MS (FAB, negative mode, matrix: glycerol/acetic acid/DMSO/water 1:1:1:1): $m/z = 563$ [$(M - Na^+)^-$] 586.4 for $C_{20}H_{28}N_4NaO_{13}P$.

5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-1-*O*-mesyl-D-glycero-D-galacto-non-2-enitol (**29**): Alcohol **19** (354 mg, 794 μmol) was taken up in a mixture of dichloromethane (10 mL) and triethylamine (223 μL, 1.59 mmol). The solution was cooled to 0°C and mesylchloride (109 μL, 1.41 mmol) dissolved in dichloromethane (0.5 mL) was added. The reaction mixture was diluted with dichloromethane (50 mL), after 1 h, washed with water, dried over magnesium sulfate and concentrated to give **29** (422 mg, quant) as a colourless foam. $R_f = 0.40$ (toluene/ethyl acetate/methanol 5:2:1), $[\alpha]_D = +50$ ($c = 1.0$ in $CHCl_3$);^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.92$ (s, 3H; NHAc), 2.03 (s, 3H; OAc), 2.04 (s, 3H; OAc), 2.05 (s, 3H; OAc), 2.11 (s, 3H; OAc), 3.07 (s, 3H; SO_2CH_3), 4.10 (dd, $J(8,9a) = 6.2$ Hz, $J(9a,9b) = 12.2$ Hz, 1H; 9a-H), 4.29 (dd, $J(5,6) = 7.9$ Hz, $J(6,7) = 4.5$ Hz, 1H; 6-H), 4.35 (dd, $J(8,9b) = 2.6$ Hz, 1H; 9b-H), 4.41 (ddd, $J(4,5) = 6.1$ Hz, $J(5,NH) = 9.4$ Hz, 1H; 5-H), 4.48 (d, $J(1a,1b) = 12.4$ Hz, 1H; 1a-H), 4.56 (d, 1H; 1b-H), 5.14 (d, $J(3,4) = 3.4$ Hz, 1H; 3-H), 5.33 (dd, 1H; 4-H), 5.38 (ddd, $J(7,8) = 6.2$ Hz, 1H; 8-H), 5.46 (dd, 1H; 7-H), 5.63 (d, 1H; NH); $C_{20}H_{29}NO_{13}S$ (523.51): calcd C 45.89, H 5.58, N 2.68; found C 45.30, H 5.64, N 2.84.

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5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-bromo-1,3,5-trideoxy-D-glycero-D-galacto-non-2-enitol (**30**): Compound **29** (180 mg, 344 μmol) was dissolved in DMF (15 mL). Powdered potassium bromide (164 mg, 1.37 mmol) was added. After stirring overnight, the reaction mixture was concentrated under reduced pressure. Purification over silica gel (toluene/acetone 1:1) afforded **30** (168 mg, 96 %) as a colourless foam. $R_f = 0.43$ (toluene/ethyl acetate/methanol 5:2:1), $[\alpha]_D = +51$ ($c = 1.0$ in $CHCl_3$); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.92$ (s, 3H; NHAc), 2.04 (s, 6H; 2OAc), 2.06 (s, 3H; OAc), 2.11 (s, 3H; OAc), 3.74 (d, J(1a,1b) = 11.0 Hz, 1H; 1a-H), 3.81 (d, 1H; 1b-H), 4.12 (dd, J(8,9a) = 5.9 Hz, J(9a,9b) = 12.4 Hz, 1H; 9a-H), 4.31–4.40 (m, 2H; 5-H, 6-H), 4.44 (dd, J(8,9b) = 3.0 Hz, 1H; 9b-H), 5.03 (d, J(3,4) = 3.0 Hz, 1H; 3-H), 5.38 (ddd, J(7,8) = 5.9 Hz, 1H; 8-H), 5.34–5.39 (m, 2H; 4-H, NH), 5.45 (dd, J(6,7) = 3.1 Hz, 1H; 7-H); $C_{19}H_{26}BrNO_{10}$ (508.32): calcd C 44.89, H 5.16, N 2.76; found C 44.32, H 5.13, N 3.21.

Bistrimethylsilyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1,3,5-trideoxy-D-glycero-D-galacto-non-2-enitol-1-yl)phosphonate (**31**): Bromide **30** (12 mg, 24 μmol) was foamed with dichloromethane and dried under reduced pressure. Subsequently tristrimethylsilyl phosphite (100 μL) was added. This mixture was heated to 90 °C under a pressure of 25 mbar for two hours. Then excess phosphite was removed by distillation under a pressure of approximately 1 mbar. The product (approximately 20 mg) is a colourless oil sensitive to moisture. $R_f \approx 0$ (toluene/acetone 1:1); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 0.25$–0.29 (m, 18H; 6CH_3), 1.91 (s, 3H; NHAc), 2.01 (s, 3H; OAc), 2.02 (s, 3H; OAc), 2.03 (s, 3H; OAc), 2.09 (s, 3H; OAc), 2.45 (dd, J(1a,1b) = 14.9 Hz, J(1a,P) = 22.0 Hz, 1H; 1a-H), 2.63 (dd, J(1b,P) = 21.8 Hz, 1H; 1b-H), 4.17 (dd, J(8,9a) = 7.0 Hz, J(9a,9b) = 12.0 Hz, 1H; 9a-H), 4.24 (d, J(5,6) = J(6,7) = 6.2 Hz, 1H; 6-H), 4.40–4.49 (m, 2H; 5-H, 9b-H), 4.93 (dd, J(3,4) = J(3,P) = 4.3 Hz, 1H; 3-H), 5.15 (ddd, J(4,5) = 9.6 Hz, J(4,P) = 3.0 Hz, 1H; 4-H), 5.42 (ddd, J(7,8) = 4.6 Hz, J(8,9b) = 3.5 Hz, 1H; 8-H), 5.51 (dd, 1H; 7-H), 6.59 (d, J(5,NH) = 9.1 Hz, 1H; NH).

5-Acetamido-1,2,3-tri-*O*-acetyl-4,8-anhydro-5,6,7,9-tetradeoxy-D-manno-non-6,8-dienitol (**32**): Ester **31** (19 mg, 24 μmol) was dissolved in dry acetone (1 mL). The solution was cooled to 0 °C and treated with tetrabutylammonium fluoride solution (1M) in THF (100 μL). After stirring for 30 min it was concentrated and purified over silica gel (toluene/acetone 1:1). Product **32** is a colourless oil which decomposes on storage. $R_f = 0.48$ (toluene/acetone 1:1); ^{1}H NMR (250.13 MHz, $CDCl_3$): $\delta = 1.96$ (s, 3H; NHAc), 2.03 (s, 3H; OAc), 2.05 (s, 3H; OAc), 2.08 (s, 3H; OAc), 3.99 (dd, J(4,5) = 7.1 Hz, J(3,4) = 2.7 Hz, 1H; 4-H), 4.16 (dd, J(1a,1b) = 12.5 Hz, J(1a,2) = 5.6 Hz, 1H; 1a-H), 4.26 (s, 1H; 9a-H), 4.44 (dd, J(1b,2) = 2.2 Hz, 1H; 1b-H), 4.54 (s, 1H; 9b-H), 4.57–4.58 (m, 1H; 5-H), 5.31 (dd, J(2,3) = 6.8 Hz, 1H; 3-H), 5.36 (ddd, 1H; 2-H), 5.55 (d, J(5,NH) = 8.8 Hz, 1H; NH), 5.77 (br dd, J(6,7) = 3.3 Hz, J(5,7) = 1.0 Hz, 1H; 7-H), 6.10 (dd, J(5,6) = 2.0 Hz, 1H; 6-H).

Sialyltransferase assay: The inhibitors **1a**, **1b**, **2**, **3**, **4**, (*R*)-**5**, (*R*)-**6** and (*E*)-**6′** were tested according to the published procedure;[9] α-(2,6)-sialyltransferase from rat liver (EC2.4.99.1) was purchased from Sigma.

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