J|A|C|S
ARTICLES
Published on Web 02/01/2002

Efficient Sialyltransferase Inhibitors Based on Glycosides of *N*-Acetylglucosamine

Ralf Schwörer and Richard R. Schmidt*

Contribution from the Fachbereich Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany

Received October 24, 2001

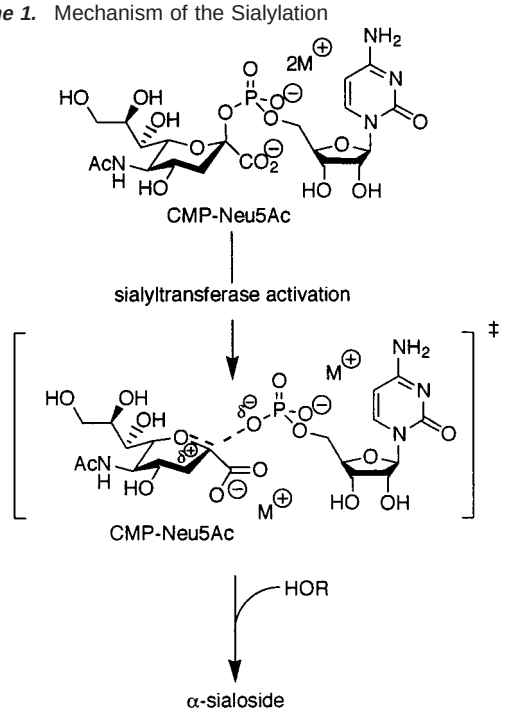
Abstract: D-Glucosamine was transformed into phenyl and 2-benzoyloxyethyl *N*-acetylglucosamine β-glycosides **6a** and **6b**, respectively. Transformation of **6a**,**b** into 6-*O*-unprotected *N*-acetylglucosamine derivatives **9a**,**b** permitted the generation of an aldehyde group in the 6-position. Treatment of these intermediates with base afforded unsaturated aldehyde derivatives **10a**,**b**, which are structural mimics of 2,3-dehydroneuraminic acid. H−Phosphonate addition to the aldehyde group and attachment of the cytidine monophosphate residue to the generated hydroxy group gave fully protected transition state analogues of cytidine monophosphate−*N*-acetylneuraminic acid **14a**,**b**. Liberation of the unprotected compounds **1a*h*,*l*** and **1b*h*,*l*** led to excellent inhibitors of α(2−6)-sialyltransferase from rat liver. Variation of the protective group cleavage procedure for **14a**,**b** led to formal loss of phosphate, thus resulting in diene derivatives (*E*)-/(*Z*)-**2a**,**b**, which also exhibited inhibitory properties.

Introduction

Sialic acid-containing glycoconjugate epitopes are involved in various biological interactions, such as the many forms of cell adhesion, thus influencing a variety of physiologically and pathologically important processes.[1] Recently, an interesting correlation between α(2−6)-sialylation of *N*-acetyllactosamine and B lymphocyte activation and immune function was reported, which could have medicinal application.[2] Therefore, to study the influence of sialyl residues in biological systems, it is highly desirable to develop efficient inhibitors for sialyltransferases.

The various sialyltransferases use, independent of their source and their acceptor specificity, cytidine monophosphate *N*-acetylneuraminic acid (CMP−Neu5Ac, Scheme 1) as the donor substrate. We recently showed[3−6] that structural analogues of the donor and particularly of the transition state CMP−Neu5Ac of the donor (Scheme 1) exhibit high affinity to sialyltransferases; therefore, they are valuable inhibitors.[3−8]

Scheme 1. Mechanism of the Sialylation



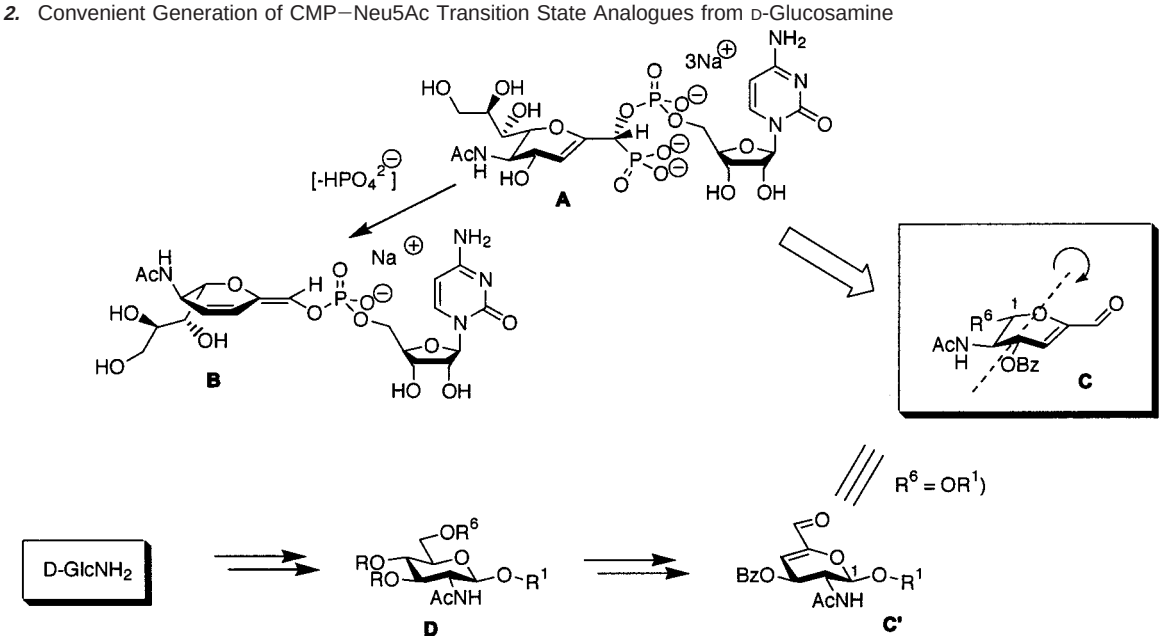
In the previous studies, transition state analogues **A** and **B** (**B** is derived from **A** through formal loss of phosphate, Scheme 2) showed up to 2 orders of magnitude higher affinity to α(2−6)-sialyltransferase of rat liver than the natural substrate CMP−Neu5Ac. Retrosynthesis of **A** exhibits that aldehyde derivative **C** is a decisive intermediate in the generation of this type of inhibitor. From these investigations it also became obvious that

* Corresponding author. E-mail: Richard.Schmidt@uni-konstanz.de.

(1) (a) Morgenthaler, J.; Kemner, J.; Brossmer, R. *Biochem. Biophys. Res. Commun.* **1990**, *171*, 860−866. (b) Rosenberg, A. *Biology of Sialic Acids*; Plenum: New York, 1995. (c) Reutter, W.; Stäsche, R.; Stehling, P.; Baum, O. In *Glycosciences;* Gabius, H. J., Gabius, S., Eds.; Chapman and Hall: Weinheim, 1997; pp 245−259. (d) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem.* **1998**, *110*, 2908−2958; *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2754−2794. (e) von Itzstein, M.; Colman, P. *Curr. Opin. Struct. Biol.* **1996**, *6*, 703−709.
(2) Hennet, T.; Chui, D.; Paulson, J. C.; Marth, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4504−4509.
(3) Schaub, C.; Müller, B.; Schmidt, R. R. *Glycoconjugate J.* **1998**, *15*, 345−354 and references therein.
(4) Müller, B.; Martin, T. J.; Schaub, C.; Schmidt, R. R. *Tetrahedron Lett.* **1998**, *39*, 509−512.
(5) Amann, F.; Schaub, C.; Müller, B.; Schmidt, R. R. *Chem. Eur. J.* **1998**, *4*, 1106−1115.
(6) Müller, B.; Schaub, C.; Schmidt, R. R. *Angew. Chem.* **1998**, *110*, 3021−3024; *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2893−2897.
(7) Schröder, P. N.; Giannis, A. *Angew. Chem.* **1999**, *111*, 1471−1472; *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1379−1380.
(8) Schaub, C.; Müller, B.; Schmidt, R. R. *Eur. J. Org. Chem.* **2000**, 1745−1758.

10.1021/ja017370n CCC: $22.00

Scheme 2. Convenient Generation of CMP−Neu5Ac Transition State Analogues from D-Glucosamine



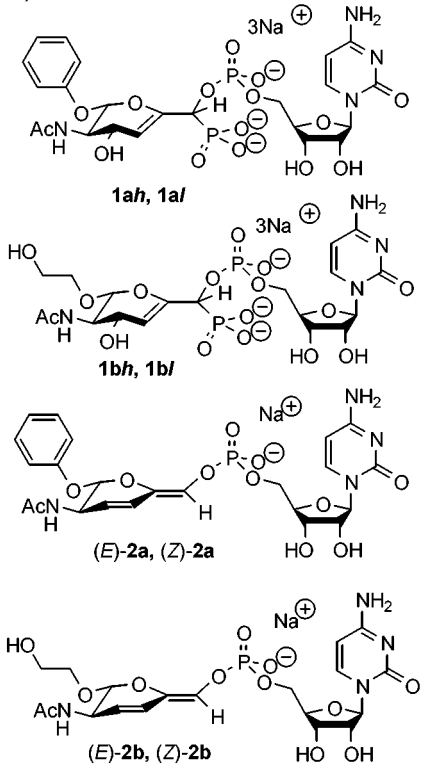
structural variations of the side chain (and also at the amino group) of the Neu5Ac residue may even increase binding to sialyltransferase, hence variations of substituent R in **C** were of interest. Thus, replacement of R by an alkoxy or an aryloxy substituent leads to **C′** which, through rotation around the C4−O axis, exhibits its relatedness to an *N*-acetyl-D-glucosamine (D-GlcNAc) derivative that should be readily available from D-GlcNAc via a glycoside intermediate **D**, as shown in Scheme 2. This synthesis design exhibits that *β*-*C*-glycosides of D-GlcNAc are also interesting precursors for Neu5Ac synthesis, as already demonstrated by Vasella and co-workers[9] starting from *N*-acetyl-1-deoxy-1-nitro-D-glucosamine as precursor.

The synthesis of compounds **C′** with R^1 being hydroxyethyl and phenyl as side chain mimics and their transformation into compounds of types **A** and **B** (i.e., **1a**,**b** and **2a**,**b**, Scheme 3) are outlined in this article; the inhibition properties of α(2−6)-sialyltransferase are also reported.

Results and Discussion

On the basis of the synthesis design of the target molecules at first, the synthesis of phenyl and 2-hydroxyethyl GlcNAc *β*-glycosides **6a** and **6b**, respectively, was performed (Scheme 4). For the synthesis of **6a** we used a four-step procedure.[10,11] For the synthesis of **6b**, glucosamine was transformed into known *N*-trichloroethoxycarbonyl (Teoc[12])-protected donor **3**.[13] Reaction with 2-benzoyloxyethanol[14] as acceptor in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as catalyst afforded *β*-glycoside **4b** in good yield (^{1}H NMR: $^3J_{1,2} = 8.4$ Hz). Exchange of the Teoc group for the acetyl group with activated zinc in the presence of acetic anhydride in tetrahydrofuran (THF)/acetic acid as solvent[13c] furnished GlcNAc derivative **5b**, which under Zemplén conditions[15] led to regioselective de-*O*-acetylation, thus affording **6b** in good yield.

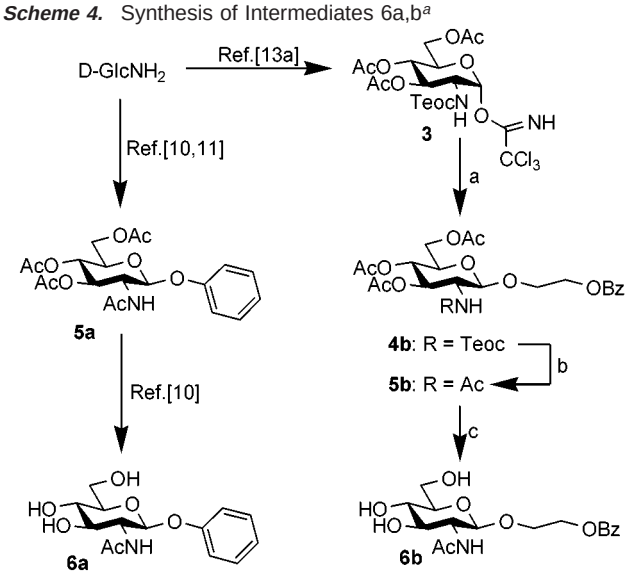
Scheme 3. Structure of the Target Molecules **1a***h*,*l*, **1b***h*,*l*, (*E*/*Z*)-**2a**, (*E*/*Z*)-**2b**



To gain regioselective access to the 6-position of **6a**,**b**, regioselective 6-*O*-silylation of **6a**,**b** with *tert*-butyldimethylsilyl chloride (TBDMS−Cl) and triethylamine and DMAP (Steglich's base) was undertaken (→ **7a**,**b**) (Scheme 5); following benzoylation with benzoyl chloride in pyridine furnished 3,4-di-*O*-benzoyl derivatives **8a**,**b**. The 6-*O*-TBDMS protective groups were then removed under acidic conditions (HCl in MeOH)[16]

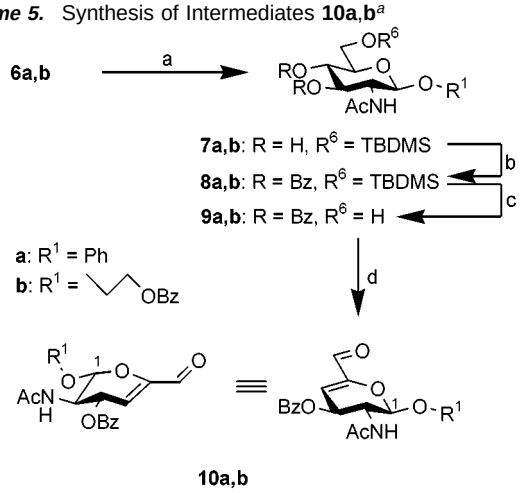
(9) Julina, R.; Müller, I.; Vasella, A.; Wyler, R. *Carbohydr. Res.* **1987**, *164*, 415−432.
(10) Roy, R.; Tropper, R. D. *Can. J. Chem.* **1991**, *69*, 817−821.
(11) Horton, D. *Methods Carbohydr. Chem.* **1972**, *6*, 282−285.
(12) Windholz, T. B.; Johnston, D. B. *Tetrahedron Lett.* **1967**, *27*, 2555−2557.
(13) (a) Paulsen, H.; Helpap, B. *Carbohydr. Res.* **1991**, *216*, 289−313. (b) Ziegler, T. *Carbohydr. Res.* **1994**, *262*, 195−212. (c) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296*, 135−137.
(14) Fischer, E. *Ber. Dtsch. Chem. Ges.* **1920**, *53*, 1634−1644.
(15) Zemplén, G. *Ber. Dtsch. Chem. Ges.* **1927**, *60*, 1554−1564.
(16) Glaudemans, C. P. J.; Nashed, E. M. *J. Org. Chem.* **1987**, *52*, 5255−5260.

Scheme 4. Synthesis of Intermediates 6a,b[a]



[a] Reagents and conditions: (a) HO−CH_2−CH_2−OBz, TMSOTf, CH_2Cl_2 (68%). (b) Zn, Ac_2O, HOAc/THF (96%). (c) NaOMe, MeOH (68%).

Scheme 5. Synthesis of Intermediates **10a**,**b**[a]

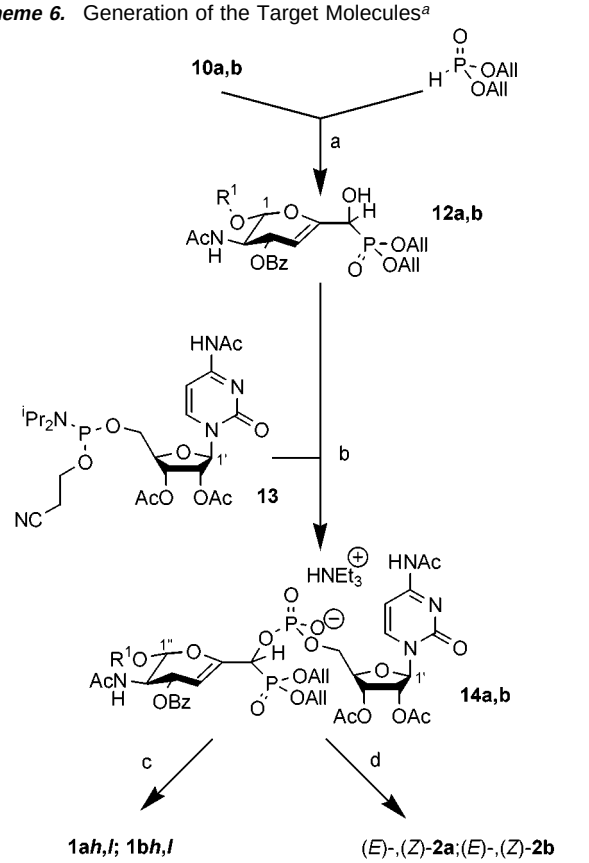


[a] Reagents and conditions: (a) TBDMS−Cl, NEt_3/DMAP (92%, 94%). (b) BzCl, pyr (93%). (c) HCl, MeOH (81%). (d) DMSO, DCC; NEt_3 (85%, 48%).

to avoid acyl group migration; thus, compounds **9a**,**b** were obtained in high yield. Oxidation of the released hydroxymethyl group was performed under Pfitzner−Moffatt conditions;[17] treatment of the crude dialdo glucosamine derivative with triethylamine as base, under β-elimination, furnished the desired unsaturated aldehyde derivatives **10a**,**b**, which correspond to the general structure **C′** in Scheme 2 [^{1}H NMR, **10a** $\delta = 9.26$ (s, 6-H); **10b** $\delta = 9.23$ (s, 6-H)].

For the transformation of **10a**,**b** into the target molecules at first addition of diallyl H-phosphonate **11**[18] in the presence of triethylamine as base was performed, furnishing α-hydroxyphosphonates **12a**,**b** as mixtures of diastereomers, which were not separated at this stage (Scheme 6). Treatment of **12a**,**b** with known cytidine-phosphite derivative **13**[19] in the presence of

Scheme 6. Generation of the Target Molecules[a]



[a] Reagents and conditions: (a) NEt_3 (quant., 87%). (b) Tetrazole; *t*-BuO_2H; NEt_3 (82%, 55%). (c) NH_3, H_2O; $Pd(PPh_3)_4$, dimedone; RP-18 HPLC; IR 120, Na^+ (**1a*h***, 36%; **1a*l***, 9%; **1b*h***, 11%; **1b*l***, 27%). (d) $Pd(PPh_3)_4$, dimedone; NH_3, H_2O; RP-18 HPLC; IR 120, Na^+ [(*E*)-**2a**, 24%; (*Z*)-**2a**, 6%; (*E*)-**2b**, 35%; (*Z*)-**2b**, 12%].

tetrazole, subsequent oxidation of the reaction product with *tert*-butyl hydroperoxide, and then addition of triethylamine to remove the cyanoethyl group led to protected target molecules **14a** and **14b**, respectively, in good yields. De-*O*-acylation with aqueous ammonia and de-*O*-allylation with catalytic amounts of $Pd(PPh_3)_4$ and dimedone as the nucleophile,[20] and then reversed-phase high-performance liquid chromatography (HPLC) with RP-18 led to clean separation of the diastereomers, thus providing from **14a** target molecules **1a*h**** (****h*** and ***l*** stand for higher and lower moving spot on TLC) and **1a*l**** and from **14b**, **1b*h**** and **1b*l**.**[1] When the sequence of steps starting from **14a**,**b** was changed, i.e., de-*O*-allylation was performed first and then de-*O*-acylation with aqueous ammonia, under formal loss of phosphate from **14a** a mixture of (*E*)- and (*Z*)-**2a** (ratio 5:1) and from **14b** a mixture of (*E*)- and (*Z*)-**2b** (ratio 3:1) was obtained that was again separated by reversed-phase HPLC on RP-18. The structural assignments of the target molecules **1a**,**b** and **2a**,**b** could be based on the NMR data. [For instance, rotating-frame Overhauser effect spectroscopy (ROESY) (600 MHz, $CDCl_3$); (*E*)-**2a**: ROE $\delta = 4.03$, 6.79 (5′-H/4″-H), no ROE signal (4″-H/6″-H); (*Z*)-**2a**: ROE $\delta = 7.23$, 3.89 (5′-H/Ph)].

Measurement of the inhibition constants K_i of compounds **1a*h*,*l***, **1b*h*,*l***, (*E*)- and (*Z*)-**2a** and (*E*)- and (*Z*)-**2b** is based on a previously reported sialyltransferase assay system.[3] As seen in Table 1, the previously investigated transition state analogue

(17) Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661−5670.
(18) (a) Felber, R.; Lohse, P. A. *Tetrahedron Lett.* **1998**, *39*, 2067−2070. (b) Gibbs, D. E.; Larsen, C. *Synthesis* **1984**, *5*, 410−413.
(19) (a) Schmidt, R. R.; Frische, K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1747−1750. (b) Amann, F. Ph.D. Dissertation, Universität Konstanz, Konstanz, Germany, 1997. (c) Kajihara, Y.; Ebata, T.; Koseki, K.; Kodama, H.; Matsushita, H.; Hashimoto, H. *J. Org. Chem.* **1995**, *60*, 5732−5735.
(20) Berkowith, D. B.; Sloss, D. G. *J. Org. Chem.* **1995**, *60*, 7047−7050.

Table 1. Affinity of CMP−Neu5Ac (K_M) to α(2−6)-Sialyltransferase of Rat Liver and Inhibition Constants (K_i) of A, B, **1a*h*,*l***, **1b*h*,*l***, (*E*)-,(*Z*)-**2a**, and (*E*)-,(*Z*)-**2b**

	K_M (μM)	K_i (μM)	inhibition mode	K_M/K_i	ref
CMPNeu5Ac	46				3
A (R)		3.35 ± 0.05	competitive	131.0	3
B (E)		6 ± 0.5	competitive	7.7	3
1a*h*		0.029 ± 0.006	competitive	1580.0	
1a*l*		0.69 ± 0.19	competitive	67.0	
1b*h*		0.059 ± 0.018	competitive	780.0	
1b*l*		0.038 ± 0.009	competitive	1210.0	
(*E*)-**2a**		158 ± 41α	competitive	0.3	
(*Z*)-**2a**		25 ± 7	competitive	1.8	
(*E*)-**2b**		2.4 ± 0.4	competitive	19.0	
(*Z*)-**2b**		3.5 ± 1.4	competitive	13.0	

[a] Because of low solubility this compound was dissolved in DMSO and the mixture diluted with buffer.

A[5] exhibits about 100-fold higher affinity to α(2−6)-sialyltransferase from rat liver (E.C. 2.4.99.1) than the natural substrate CMP−Neu5Ac. However, structural mimics **1a*h*** and **1b*h*,*l*** have even 1 order of magnitude higher affinity to the enzyme than **A**. Transition state analogue **1a*h*** is by now the best inhibitor of this enzyme found so far, thus exhibiting the importance of replacement of the Neu5Ac side chain for higher binding affinity. Compound **B** shows more than 10-fold lower affinity to the enzyme than **A**. The same trend is also found for compounds (*E*)-/(*Z*)-**2a** and (*E*)-/(*Z*)-**2b**. Obviously, the loss of the negatively charged phosphonate residue at C-6 leads to lower binding to the enzyme. Thus, our inhibitor design confirmed that (i) planarity at the anomeric carbon, (ii) increased distance between the anomeric carbon and the CMP leaving group, and (iii) the presence of at least two negative charges close to the glycosylation cleavage site are required for high affinity to the enzyme.[6]

Conclusion

In conclusion, β-glycosides of D-GlcNAc can be readily transformed into unsaturated aldehyde derivatives that provide, via H-phosphonate addition and attachment of the CMP residue to the generated hydroxy group, excellent inhibitors of α(2−6)-sialyltransferase. Replacement of the Neu5Ac side chain by a phenyloxy group leads to particularly high affinity to the enzyme. Obviously, the prospects for further structural variations of this type of transition state analogue are very promising.

Experimental Section

General. 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. Tetramethylsilane (TMS) or the resonance of the deuterated solvent was used as internal standard; solvents: $CDCl_3$, $\delta = 7.24$; D_2O, $\delta = 4.63$; [D_4]methanol, $\delta = 3.305$. For ^{31}P NMR phosphoric acid was used as an external standard; ^{13}C NMR spectra were broadband ^1H decoupled. Matrix-assisted laser desorption ionization mass spectra (MALDI-MS) were recorded on a Kratos Kompact Maldi 2, and 2,5-dihydroxybenzoic acid (DHB) or 6-aza-thiothymine (ATT) were used as matrixes. Fast atom bombardment mass spectra (FAB-MS) were measured on a Finnigan MAT 312/AMD 5000 (70 eV, 70 °C). Optical rotations were measured on a Büchi polar monitor in a 1 dm cell at 22 °C. Thin-layer chromatography was performed on silica gel plastic plates 60 F_{254} (Merck) or glass plates RP-18 (Merck); compounds were visualized 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 silica gel (J. T. Baker, particle size 40 μm) at a pressure of 0.3−0.4 bar. MPLC was performed at a pressure of 5−10 bar on silica gel columns LiChroprep Si 60 (Merck, 15−25 μm, 28 × 2.5 cm). Preparative HPLC was performed with a Shimadzu LC8A preparative pump and a Rainin Dynamax UV 1 detector at 260 nm; columns: (A) Eurospher 100-C18 (Knauer, 7 μm, 250 × 16 mm), (B) Eurospher 100-C18 (Knauer, 7 μm, 250 × 20 mm), (C) LiChrospher 100 RP18 (Merck, 7 μm, 250 × 25 mm). Mixtures of acetonitrile and 0.05 M triethylammonium bicarbonate (TEAB) (pH 7.2−7.5) were used as mobile phase. Analytical HPLC was performed on a Merck-Hitachi system with an L 7200 autosampler and an L 4000 UV detector; column: Eurospher 100-C18 (Knauer GmbH, 5 μm, 250 × 4 mm). Elemental analyses were measured on a Heraeus CHN−O−Rapid.

Phenyl 2-Acetamido-3-*O*-benzoyl-2,4-di-deoxy-β-L-*threo*-hex-4-ene-dialdo-1,5-pyranoside (10a). Alcohol **9a** (2.5 g, 5 mmol) was dissolved in a mixture of dimethyl sulfoxide (DMSO) (11 mL) and dry toluene (3 mL). After addition of pyridine (0.8 mL, 10 mmol), phosphoric acid (0.4 mL), and dicyclohexylcarbodiimide (DCC) (4 g, 19.4 mmol), the resulting mixture was stirred at room temperature for 72 h. The reaction mixture was filtered, washed with toluene (50 mL) and methanol (50 mL), and the filtrate and washings were combined and treated with oxalic acid monohydrate (3.8 g) for 30 min at room temperature. The mixture was diluted with dichloromethane (100 mL) and extracted with saturated sodium hydrogen carbonate solution (100 mL). The combined organic layers were dried over magnesium sulfate and concentrated. The resulting syrup was dissolved in acetone and filtered to remove dicyclohexyl urea. Solvents were evaporated, the residue was dissolved in dry dichloromethane (25 mL), and triethylamine (5 mL) was added and stirred for 1 h. Evaporation of solvents and purification by flash chromatography (toluene/acetone 9:2) yielded aldehyde **10a** (1.6 g, 84%) as a colorless syrup. $R_f = 0.51$ (toluene/ethyl acetate 2:1 v/v); $[\alpha]^{D}_{20} = +4.8$ ($c = 1$ in chloroform); ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.98$ (s, 3 H, NHAc), 4.91 (m, $^3J(1,2) = 2.5$ Hz, $^3J(2,3) = 2.3$ Hz, $^3J(2,N-H) = 8.8$ Hz, $^4J(2,4) = 1.5$ Hz, 1 H, 2-H), 5.54 (dd, $^3J(2,3) = 2.3$ Hz, $^3J(3,4) = 5.1$ Hz, 1 H, 3-H), 5.93 (d, $^3J(1,2) = 2.5$ Hz, 1 H, 1-H), 6.24 (d, $^3J(2,N-H) = 8.8$ Hz, 1 H, N−H), 6.30 (dd, $^3J(3,4) = 5.1$ Hz, $^4J(2,4) = 1.5$ Hz, 1 H, 4-H), 6.95−7.62 (m, 8 H, Ph, Bz), 8.05−8.12 (m, 2 H, Bz), 9.26 (s, 1H; 6-H); elemental analysis calcd (%) for $C_{21}H_{19}NO_6$ (381.38): C, 66.13; H, 5.02; N, 3.67; found: C, 65.82; H, 5.34; N, 3.38.

Diallyl [Phenyl (6*R*,*S*)-2-acetamido-3-*O*-benzoyl-2,4-dideoxy-β-L-*threo*-hex-4-enopyranoside-6-yl]-phosphonate [(*R*,*S*)-12a]. Aldehyde **10a** (700 mg, 1.8 mmol) and diallyl phosphite **11**[18] (490 mg, 3 mmol) were dissolved in dichloromethane (2 mL) and after addition of triethylamine (0.2 mL) stirred at room temperature for 90 min. Evaporation of solvents and purification by flash chromatography (toluene/acetone 2:1 v/v) gave compound **12a** (980 mg, quant.) as a colorless glass. $R_f = 0.24$ (toluene/acetone 2:1 v/v); ^1H NMR (250 MHz, [D_4]methanol): $\delta = 1.98/1.99$ (2s, 3 H; NHAc), 4.45−4.60 (m, 6 H; 2-H, 3-H, allyl), 5.17−5.15 (m, 2 H; allyl), 5.27−5.39 (m, 2 H; allyl), 5.49 (2m, 2 H; 4-H, 6-H), 5.83 (d, $^3J(1,2) = 5.3$ Hz, 1 H; 1-H), 5.75−6.00 (m, 2 H; allyl), 7.04−7.63 (m, 8 H; Ph, Bz), 8.04−8.06 (m, 2 H; Bz); ^{31}P NMR (243 MHz, [D_4]methanol): $\delta = 23.13/23.74$ (2s); elemental analysis calcd (%) for $C_{27}H_{30}NO_9P$ (543.50): C, 59.67; H, 5.56; N, 2.58; found: C, 59.56; H, 5.57; N, 2.47.

Triethylammonium [Phenyl (6*R*,*S*)-2-acetamido-3-*O*-benzoyl-2,4-dideoxy-6-diallylphosphoryl-β-L-*threo*-hex-4-enopyranoside-6-yl](*N*-acetyl-2′,3′-di-*O*-acetylcytidine-5′-yl)phosphate (14a). Alcohol **12a** (410 mg, 0.75 mmol) and cytidine phosphoramidite **13**[19] (520 mg, 0.90 mmol) were coevaporated with dry dichloromethane and dried under high vacuum. The mixture was dissolved in dry dichloromethane (5 mL) and tetrazole (95 mg, 1.36 mmol) was added. After stirring for 3 h at room temperature, the coupling reaction was complete and *tert*-

butyl hydroperoxide (270 μL, 1.49 mmol, 5.5 M in nonane) was added. After 2 h, triethylamine (5 mL) was added, and the solution was stirred overnight. Evaporation of the solvents and purification by flash chromatography (ethyl acetate/methanol 5:1 v/v + 1% triethylamine) afforded the triethylammonium salts **(*R,S*)-14a** (600 mg, 82%, mixture of diastereomers) as a colorless lyophilisate. R_f = 0.17 (ethyl acetate/methanol 5:1 v/v + 1% triethylamine); ^{1}H NMR (250 MHz, D_2O): δ = 1.07 (2t, ^3J = 7.3 Hz, 9 H; 3 ethyl), 1.81/1.86/1.94/1.97 (8s, 12 H; NHAc, 3 acetyl), 3.00 (2q, ^3J = 7.3 Hz, 6 H; 3 ethyl), 3.75−4.07 (m, 3 H; 4′-H,5′a-H, 5′b-H), 4.25−4.51 (m, 6 H; 2″-H, 3″-H, allyl), 4.77 (dd, ^2J(6″,P) = 11.7 Hz, ^3J(6″,P) = 15.9 Hz, 1 H; 6″-H), 5.02−5.27 (m, 6 H; 2′-H, 3′-H, allyl), 5.39−5.48 (m, 2 H; 1″-H, 4″-H), 5.61 (d, ^3J(1′,2′) = 5.3 Hz, 1 H; 1′-H), 5.66−5.84 (m, 2 H; allyl), 6.75−7.51 (m, 8 H; Ph, Bz), 6,91 (d, ^3J(5,6) = 7.6 Hz, 1 H; 5-H), 7.75−7.84 (m, 2 H; Bz), 7.96 (d, ^3J(5,6) = 7.6 Hz, 1 H; 6-H); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 973 (15) [*M* − $HNEt_3$]$^-$, 933 (100) [*M* − $HNEt_3^+$ − All$^+$ + H$^+$]$^-$; MALDI-MS (positive mode, matrix: DHB): *m*/*z* (%): 997 (100) [*M* − $HNEt_3^+$ + Na$^+$ + H$^+$]$^+$, 875 (80) [*M* − $HNEt_3^+$ − PhCOOH + Na$^+$ + H$^+$]$^+$, 1075.4 for $C_{48}H_{63}N_4O_{19}P_2$.

Triethylammonium [2-Benzoyloxyethyl (6*R*,*S*)-2-acetamido-3-*O*-benzoyl-2,4-dideoxy-6-diallylphosphoryl-β-L-*threo*-hex-4-enopyranoside-6-yl](*N*-acetyl-2′,3′-di-*O*-acetylcytidine-5′-yl)phosphate (14b). Alcohol **12b** (140 mg, 0.23 mmol) and cytidine phosphoramidite **13**[19](160 mg, 0.28 mmol) were coevaporated with dry dichloromethane and dried under high vacuum. The mixture was dissolved in dry dichloromethane (2 mL) and tetrazole (32 mg, 0.44 mmol) was added. After stirring for 3 h at room temperature, the coupling reaction was complete, and *tert*-butyl hydroperoxide (70 μl, 0.38 mmol, 5.5 M in nonane) was added. After 2 h, triethylamine (4 mL) and dichloromethane (2 mL) were added and the solution stirred overnight. Evaporation of the solvents and purification by flash chromatography (ethyl acetate/methanol 5:1 v/v + 1% triethylamine) afforded the triethylammonium salts **14b** (140 mg, 55%, mixture of diastereomers) as a colorless lyophilisate. R_f = 0.78 (ethyl acetate/methanol 1:1 v/v + 1% triethylamine); ^{1}H NMR (250 MHz, D_2O): δ = 1.30 (2t, 9 H; 3 *N*-ethyl), 1.92−2.15 (8s, 12 H; NHAc, 3 acetyl), 3.00 (2q, 6 H; 3 *N*-ethyl), 3.85−4.7 (m, 11 H; 1‴a-H, 1‴b-H, 2‴a-H, 2‴b-H, 4′-H, 5′a-H, 5′b-H, allyl), 4.9−5.7 (m, 11 H; 1′-H, 2′-H, 3′-H, 2″-H, 3″-H, 4″-H, 6″-H, allyl), 5.84−6.00 (m, 2 H; allyl), 6.17 (2 d, ^3J(1″,2″) = 4.3 Hz, 1 H; 1″-H), 7.15−7.58 (m, 6 H; 5-H, Bz), 7.90−8.00 (m, 4 H; Bz), 8.40/8.44 (2d, ^3J(5,6) = 7.6 Hz, 1 H; 6-H); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 1045 (40) [*M* − $HNEt_3^+$]$^-$ 1004 (100) [*M* − $HNEt_3^+$ − All$^+$ + H$^+$]$^-$; MALDI-MS (positive mode, matrix: DHB): *m*/*z* (%): 1069 (50) [*M* − $HNEt_3^+$ + Na$^+$ + H$^+$]$^+$, 969 (100) [*M* − $HNEt_3^+$ − PhCOOH + 2Na$^+$]$^+$, 1147.4 for $C_{51}H_{67}N_4O_{21}P_2$.

Trisodium [Phenyl (6*R*,*S*)-2-acetamido-3-*O*-benzoyl-2,4-dideoxy-6-phosphoryl-β-L-*threo*-hex-4-enopyranoside-6-yl](cytidine-5′-yl)-phosphate (1a*h*,*l*). A solution of **14a** (50 mg, 0.046 mmol) in aqueous ammonia (25%, 3 mL) was stirred for 4 h at room temperature. The mixture was concentrated and lyophilized from water. To a solution of the resulting lyophilisate and dimedone (30 mg, 0.22 mmol) in THF (2 mL) was added $Pd(PPh_3)_4$ (15 mg, 12 μmol) in the dark. After 5 h the solvents were evaporated and dimedone was removed by RP-18 chromatography (ethanol/water 1:3 v/v). After lyophilization from water, purification and separation of diastereomers by RP-18 HPLC (0.05 M TEAB) the products were converted into their sodium salts by IR 120 (Na$^+$) and lyophilized to yield **1a*h*** (12 mg, 36%) and **1a*l*** (3 mg, 9%).

1a*h*. HPLC: prep. RP-18, column A (flow 15 mL min^{-1}, 0.05 M TEAB buffer, 6% acetonitrile): t_R = 10 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.95 (s, 3H; NHAc), 3.93 (m, ^2J(5′a,5′b) = 14 Hz, 1 H; 5′a-H), 4.09 (dd, ^3J(1′,2′) = 3 Hz, ^3J(2′,3′) = 4.8 Hz, 1 H; 2′-H), 4.12 (m, ^3J(3′,4′) = 4.8 Hz, 1 H; 4′-H), 4.14 (m, ^2J(5′a,5′b) = 14 Hz, 1 H; 5′b-H), 4.19 (dd, ^3J(2′,3′) = 4.8 Hz, ^3J(3′,4′) = 4.8 Hz, 1 H; 3′-H), 4.22 (m, ^3J(2″,3″) = 6.1 Hz, 1 H; 3″-H), 4.36 (dd, ^3J(2″,3″) = 6.1 Hz, ^3J(1″,2″) = 6.4 Hz, 1 H; 2″-H), 4.61 (m, ^2J(6″,P) = 12.1 Hz, 1 H; 6″-H), 5.19 (m, 1 H; 4″-H), 5.54 (d, ^3J(1″,2″) = 6.4 Hz, 1 H, 1″-H), 5.80 (d, ^3J(1′,2′) = 3 Hz, 1 H, 1′-H), 6.03 ((d, ^3J(5,6) = 7.8 Hz, 1 H, 5-H), 7.01−7.28 (m, 5 H, Ph), 8.09 (d, ^3J(5,6) = 7.8 Hz, 1 H, 6-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 52.2 (2″-C), 63.5 (^2J(C,P) = 4.6 Hz; 5′-C), 65.3 (3″-C), 68.4 (3′-C), 74.6 (2′-C), 73.4 (^1J(C,P) = 160 Hz, 6″-C), 82.6 (4′-C), 89.7 (1′-C), 95.1 (6-C), 98.4 (1″-C), 103.7 (4″-C), 116.8/123.2/129.8 (Ph), 143.9 (5-C), 148.2 (5″-C), 156.7 (4-C), 174.5 (2-C); ^{31}P NMR (243 MHz, D_2O): δ = 1.52 (bd, ^3J(P,P) = 25 Hz, PO_4), 13.44 (bd, ^3J(P,P) = 25 Hz, PO_3); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 663 (100) [*M* − 3Na$^+$ + 2H$^+$]$^-$, 730.4 for $C_{23}H_{27}N_4Na_3O_{15}P_2$.

1a*l*. HPLC: prep. RP-18, column A (flow 15 mL min^{-1}, 0.05 M TEAB buffer, 6% acetonitrile): t_R = 11.5 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.94 (s, 3H; NHAc), 4.01 (m, ^2J(5′a,5′b) = 11.4 Hz, 1 H; 5′a-H), 4.16 (m, 1 H; 2′-H), 4.19 (m, 1 H; 4′-H), 4.22 (m, 1 H; 3′-H), 4.28 (m, ^2J(5′a,5′b) = 11.4 Hz, 1 H; 5′b-H), 4.33 (m, 1 H; 3″-H), 4.37 (m, ^3J(1″,2″) = 7.3 Hz, 1 H; 2″-H), 4.41 (m, ^2J(6″,P) = 12.9 Hz, 1 H; 6″-H), 5.20 (dd, ^3J(3″,4″) = ^4J(4″,6″) = 3.2 Hz, 1 H; 4″-H), 5.43 (d, ^3J(1″,2″) = 7.3 Hz, 1 H, 1″-H), 5.88 (d, ^3J(1,2) = 2.5 Hz, 1 H, 1′-H), 6.00 (d, ^3J(5,6) = 7.9 Hz, 1 H, 5-H), 6.99−7.26 (m, 5 H, Ph), 8.12 (d, ^3J(5,6) = 7.9 Hz, 1 H, 6-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 52.5 (2″-C), 63.4 (5′-C), 66.26 (3″-C), 67.9 (3′-C), 71.6 (^1J(C,P) = 160 Hz, 6″-C), 74.6 (2′-C), 82.1 (4′-C), 89.9 (1′-C), 94.7 (6-C), 98.4 (1″-C), 101.5 (4″-C), 116.5/123.2/129.8 (Ph), 144.2 (5-C), 149.4 (5″-C), 158.4 (4-C), 174.6 (2-C); ^{31}P NMR (243 MHz, D_2O): δ = 1.45 (bd, ^3J(P,P) = 25 Hz, PO_4), 13.34 (bd, ^3J(P,P) = 25 Hz, PO_3); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 663 (100) [*M* − 3Na + 2H]$^-$; FAB-MS (positive mode, matrix: glycerol/acetonitrile/0.1% trifluoroacetic acid 1:1:1): 687 (20) [*M* − 2Na$^+$ + 3H$^+$]$^+$, 709 (40) [*M* − Na$^+$ + 2H$^+$]$^+$, 725 (35) [*M* − 2Na$^+$ + K$^+$ + 2H$^+$]$^+$, 731 (35) [*M* + H$^+$]$^+$, 730.4 for $C_{23}H_{27}N_4Na_3O_{15}P_2$.

Trisodium [2′-Hydroxyethyl (6*R*,*S*)-2-acetamido-3-*O*-benzoyl-2,4-dideoxy-6-phosphoryl-β-L-*threo*-hex-4-enopyranoside-6-yl]-(cytidine-5′-yl)phosphate (1b*h*,*l*). A solution of **14b** (30 mg, 0.026 mmol) in aqueous ammonia (25%, 4 mL) was stirred for 3 days at room temperature. The mixture was concentrated and lyophilized from water. To a solution of the resulting lyophilisate and dimedone (50 mg, 0.37 mmol) in THF (2.5 mL), was added $Pd(PPh_3)_4$ (15 mg, 12 μmol) in the dark. After 5 h the solvents were evaporated and dimedone was removed by RP-18 chromatography (ethanol/water 1:3 v/v). After lyophilization from water, separation of diastereomers by RP-18 HPLC, conversion into the sodium salts by IR 120 (Na$^+$) and lyophilization from water **1b*h*** (2 mg, 11%) and **1b*l*** (5 mg, 27%) were collected.

1b*h*. HPLC: prep. RP-18 column (flow 15 mL min−^{1}, 0.05 m TEAB buffer, 1% acetonitrile): t_R = 11 min; ^{1}H NMR (250 MHz, D_2O): δ = 1.92 (s, 3H; NHAc), 3.45−4.30 (m, 12 H; 1‴a,b−H,2‴a,b−H, 3″-H, 2″-H, 5′a,b−H, 4′-H, 2′-H, 3′-H, 6″-H), 4.95 (d, 1 H; 1″-H), 5.20 (m, 1 H; 4″-H), 5.84 (d, ^3J(1′,2′) = 3.5 Hz, 1 H; 1′-H), 6.16 (d, ^3J(5,6) = 7.8 Hz, 1 H; 5-H), 8.02 (d, ^3J(5,6) = 7.8 Hz, 1 H; 6-H); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 631 (100) [*M* − 3Na$^+$ + 2H$^+$]$^-$, 698.4 for $C_{19}H_{27}N_4Na_3O_{16}P_2$.

1b*l*. HPLC: prep. RP-18 column (flow 15 mL min−^{1}, 0.05 M TEAB buffer, 1% acetonitrile): t_R = 12 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.94 (s, 3H; NHAc), 3.64−3.90 (m, 4 H; 1‴a,b−H,2‴a,b−H), 4.02−4.04 (m, 2 H; 2″-H, 3″-H), 4.10−4.20 (m, ^2J(5′a,5′b) = 12.7 Hz, 3 H; 5′a,b−H, 4′-H), 4.26−4.27 (m, 2 H; 2′-H, 3′-H), 4.45 (dd, ^1J(6″,P) = 12 Hz, ^4J(4″,6″) ≈ 5 Hz), 1 H; 6″-H), 5.01 (d, ^3J(1″,2″) = 5.6 Hz, 1 H; 1″-H), 5.12 (dd, ^3J(3″,4″) ≈ ^4J(4″,6″) ≈ 5 Hz, 1 H; 4″-H), 5.85 (d, ^3J(1′,2′) = 3.6 Hz, 1 H; 1′-H), 6.23 (d, ^3J(5,6) = 7.8 Hz, 1 H; 5-H), 8.11 (d, ^3J(5,6) = 7.8 Hz, 1 H; 6-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 52.4 (2″-C), 60.5 (2‴-C), 63.9 (^2J(C,P) = 4.6 Hz; 5′-C), 65.3 (3″-C), 68.7/73.4 (2′-C,3′-C), 70.7 (1‴-C), 72.6 (^1J(C,P) = 155 Hz, ^2J(C,P) = 9.2 Hz; 6″-C), 83.1 (^4J(C,P) = 7.8 Hz; 4′-C), 89.6 (1′-C), 95.4 (6-C), 99.2 (1″-C), 101.3 (^3J(C,P) = 7.5 Hz; 4″-C), 143.5 (5-C), 149.3 (^2J(C,P) = 72 Hz; 5″-C), 160.0 (4-C), 174 (2-C); ^{31}P NMR (243 MHz, D_2O): δ = 1.23 (b, PO_4), 12.9 (b, PO_3); MALDI-MS (negative mode,

matrix: ATT): *m*/*z* (%): 653 (15) [$M - 2Na^+ + H^+]^-$, 631 (100) [$M - 3Na^+ + 2H^+]^-$, 698.4 for $C_{19}H_{27}N_4Na_3O_{16}P_2$.

Sodium [Phenyl (6*R*,*S*)-2-acetamido-2,3,5-trideoxy-6-phosphoryl-β-D-*glycero*-hex-3,5-dienopyranoside-6-yl]-(cytidine-5′-yl)phosphate (*E*)-2a,(*Z*)-2a. To a solution of **14a** (60 mg, 0.056 mmol) and dimedone (54 mg, 0.39 mmol) in THF (2.5 mL) was added $Pd(PPh_3)_4$ (22 mg, 18 μmol) in the dark. After 18 h the solvents were evaporated and dimedone was removed by RP-18 chromatography (ethanol/water 1:3 v/v). The crude product was dissolved in aqueous ammonia (25%, 2 mL) and stirred for 4 h at room temperature. The mixture was concentrated and lyophilized from water. Purification and separation of diastereomers by RP-18 HPLC (0.05 M TEAB) ion exchange by IR 120 (Na^+) and lyophilization from water yielded (*Z*)-**2a** (2 mg, 6%) and (*E*)-**2a** (8 mg, 24%)

(*Z*)-2a. HPLC: prep. RP-18, column B [flow 18 mL min^{-1}, 0.05 M TEAB buffer, 7−15% acetonitrile (0−30 min, linear gradient)]: t_R = 24 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.91 (s, 3H; NHAc), 3.61 (dd, ^2J(5′a,5′b) = 11.8 Hz, ^3J(4′,5′a) = 3.3 Hz, 1 H; 5′a-H), 3.83 (dd, 1 H; 2′-H), 3.88 (dd, 1 H; 3′-H), 3.89 (dd, ^2J(5′a,5′b) = 11.8 Hz, 1 H; 5′b-H), 3.91 (m, 1 H; 4′-H), 4.51 (dd, 1 H; 2″-H), 5.55 (d, 1 H; 1″-H), 5.72 (d, 1 H, 1′-H), 5.74 (dd, ^3J(3″,4″) = 9.8 Hz 1 H; 3″-H), 5.83 (d, ^3J(5,6) = 7.5 Hz, 1 H, 5-H), 6.18 (m, ^3J(6″,P) = 6.1 Hz, ^4J(4″,6″) ≤ 1 Hz, 1 H; 6″-H), 6.24 (dd, ^3J(3″,4″) = 9.8 Hz, ^4J(4″,6″) ≤ 1 Hz, 1 H, 4″-H), 6.98−7.23 (m, 5 H, Ph), 7.52 (d, ^3J(5,6) = 7.5 Hz, 1 H, 6-H); ROESY (600 Hz, D_2O: δ = 7.29, 3.89 Ph, 5′-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 46.4 (2″-C), 64.6 (5′-C), 69.3 (3′-C), 74.5 (2′-C), 82.6 (4′-C), 89.5 (1′-C), 96.8 (6-C), 97.4 (1″-C), 118.9 (3″-C), 123.4 (4″-C), 125.6 (6″-C), 117.8/123.8/130.1 (Ph), 141.0 (5-C); ^{31}P NMR (243 MHz, D_2O): δ = −1.84 (s, PO_4); MALDI-MS (positive mode, matrix: DHB): 589 (30) [$M + H^+]^+$, 611 (100) [$M + Na^+]^+$, 627 (80) [$M + K^+]^+$, 588.1 for $C_{23}H_{26}N_4NaO_{11}P$.

(*E*)-2a. HPLC: prep. RP-18, column B (flow 18 mL min^{-1}, 0.05 m TEAB buffer, 7−15% acetonitrile (0−30 min, linear gradient): t_R = 26 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.90 (s, 3H; NHAc), 3.89 (dd, ^3J(1′,2′) = 2.6 Hz, ^3J(2′,3′) = 4.7 Hz, 1 H; 2′-H), 3.95 (dd, ^2J(5′a,5′b) = 14 Hz, 1 H; 5′a-H), 3.98 (dd, ^3J(2′,3′) = 4.7 Hz, 1 H; 3′-H), 4.02 (m, 1 H; 4′-H), 4.03 (dd, ^2J(5′a,5′b) = 14 Hz, 1 H; 5′b-H), 4.44 (dd, ^3J(2″,3″) = 5.8 Hz, 1 H; 2″-H), 5.47 (d, ^3J(1′,2′) = 2.6 Hz, 1 H; 1′-H), 5.52 (d, 1 H, 1″-H), 5.73 (d, ^3J(5,6) = 7.5 Hz, 1 H, 5-H), 5.86 (d, ^3J(3″,4″) = 10 Hz, ^3J(2″,3″) = 5.8 Hz, 1 H; 3″-H), 6.37 (m, ^3J(6″,P) = 4.7 Hz, ^4J(4″,6″) ≤ 1 Hz, 1 H; 6″-H), 6.79 (dd, ^3J(4″,3″) = 10 Hz, ^4J(4″,6″) ≤ 1 Hz, 1 H, 4″-H), 6.89−7.11 (m, 5 H, Ph), 7.52 (d, ^3J(5,6) = 7.5 Hz, 1 H, 6-H); ROESY (600 Hz, D_2O): δ = 4.03, 6.79 (5′-H,4″-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 46.5 (2″-C), 63.9 (5′-C), 68.3 (3′-C), 74.8 (2′-C), 81.8 (4′-C), 90.2 (1′-C), 96.3 (6-C), 97.3 (1″-C), 120.5 (4″-C), 120.6 (3″-C), 127.0 (6″-C), 116.6/123.6/129.8 (Ph), 140.8 (5-C); ^{31}P NMR (243 MHz, D_2O): δ = −2.18 (s, PO_4); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 565 [$M - Na^+]^-$; MALDI-MS (positive mode, matrix: DHB): 589 (30) [$M + H^+]^+$, 611 (100) [$M + Na^+]^+$, 588.1 for $C_{23}H_{26}N_4NaO_{11}P$.

Sodium [2′-Hydroxyethyl 2-acetamido-2,3,5-trideoxy-6-phosphoryl- β-D-*glycero*-hex-3,5-dienopyranoside-6-yl]-(cytidine-5′-yl)-phosphate (*E*)-2b,(*Z*)-2b. To a solution of **14b** (34 mg, 0.029 mmol) and dimedone (20 mg, 0.15 mmol) in THF (1.5 mL) was added $Pd(PPh_3)_4$ (10 mg, 8 μmol) in the dark. After 36 h the solvents were evaporated and dimedone was removed by RP-18 chromatography (ethanol/water 1:3 v/v). The crude product was dissolved in aqueous ammonia (25%, 3 mL) and stirred for 4 h at room temperature. The mixture was concentrated and lyophilized from water. Purification and separation of diastereomers by RP-18 HPLC (0.05 M TEAB) conversion into the sodium salts by IR 120 (Na^+) and lyophilization from water yielded **(*Z*)-2b** (2 mg, 12%) and **(*E*)-2b** (6 mg, 35%)

(*Z*)-2b. HPLC: prep. RP-18, column C (flow 18 mL min^{-1}, 0.05 M TEAB buffer, 1% acetonitrile): t_R = 22 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.92 (s, 3H; NHAc), 3.63 (2 ddd, ^2J(2‴a,2‴b) = 12.4 Hz, ^3J(1‴a,2‴a) = 7.0 Hz, ^3J(1‴a,2‴b) = 3.2 Hz, ^3J(1‴b,2‴a) = 3.3 Hz, ^3J(1‴b,2‴b) = 5.4 Hz, 2 H; 2‴a-H, 2‴b-H), 3.65 (ddd, ^2J(1‴a,1‴b) = 11.3 Hz, ^3J(1‴a,2‴a) = 7.0 Hz, ^3J(1‴a,2‴b) = 3.2 Hz, 1 H; 1‴a-H), 3.80 (ddd, ^2J(1‴a,1‴b) = 11.3 Hz, ^3J(1‴b,2‴a) = 3.3 Hz, ^3J(1‴b,2‴b) = 5.4 Hz, 1 H; 1‴b-H), 4.04 (dd, ^2J(5′a,5′b) = 12.5 Hz, ^3J(5a′,P) = 5 Hz, ^3J(4′,5a′) = 3 Hz, 1 H; 5′a-H), 4.18−4.24 (m, 4 H; 2′-H, 3′-H, 4′-H, 5b′-H), 4.32 (dd, ^3J(2″,3″) = 5.7 Hz, 1 H; 2″-H), 4.98 (d, 1 H; 1″-H), 5.65 (dd, ^3J(3″,4″) = 10 Hz, ^3J(2″,3″) = 5.7 Hz, 1 H; 3″-H), 5.90 (d, 1 H, 1′-H), 6.09 (d, ^3J(5,6) = 7.8 Hz, 1 H, 5-H), 6.14 (d, ^3J(3″,4″) = 10 Hz, 1 H, 4″-H), 6.17 (d, ^3J(6″,P) = 6 Hz, 1 H; 6″-H), 7.90 (d, ^3J(5,6) = 7.8 Hz, 1 H, 6-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 46.1 (2″-C), 60.8 (2‴-C), 65 (5′-C), 69.5 (2′-C), 70.2 (1‴-C), 74.1 (3′-C), 83.0 (4′-C), 89.4 (1′-C), 95.5 (5-C), 98.6 (1″-C), 118.9 (3″-C), 123.3 (4″-C), 125.9 (6″-C), 142.8 (6-C); ^{31}P NMR (243 MHz, D_2O): δ = −4.46 (bs, PO_4); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 633 [$M - Na^+]^-$, 656.1 for $C_{19}H_{26}N_4NaO_{12}P$.

(*E*)-2b. HPLC: prep. RP-18, column C (flow 18 mL min^{-1}, 0.05 M TEAB buffer, 1% acetonitrile): t_R = 26 min; ^{1}H NMR (600 MHz, D_2O): δ = 1.93 (s, 3H; NHAc), 3.61 (2 ddd, 2 H; 2‴a-H, 2‴b-H), 3.63 (ddd, 1 H; 1‴a-H), 3.73 (ddd, 1 H; 1‴b-H), 4.06−4.23 (m, ^2J(5′a,5′b) = 11.8 Hz, 5 H; 2′-H, 3′-H, 4′-H, 5′a-H, 5′b-H), 4.29 (dd, ^3J(2″,3″) = 5.5 Hz, 1 H; 2″-H), 4.94 (d, J < 2 Hz, 1 H; 1″-H), 5.75 (dd, ^3J(3″,4″) = 9.6 Hz, ^3J(2″,3″) = 5.5 Hz, 1 H; 3″-H), 5.89 (d, ^3J(1′,2′) = 2.3 Hz, 1 H, 1′-H), 6.13 ((d, ^3J(5,6) = 7.8 Hz, 1 H, 5-H), 6.46 (d, ^3J(6″,P) = 4.5 Hz, 1 H; 6″-H), 6.68 (d, ^3J(3″,4″) = 9.6 Hz, 1 H, 4″-H), 7.92 (d, ^3J(5,6) = 7.8 Hz, 1 H, 6-H); ^{13}C NMR (150.9 MHz, D_2O): δ = 46.0 (2″-C), 60.3 (2‴-C), 64.4 (5′-C), 69 (2′-C), 69.1 (1‴-C), 74 (3′-C), 82.8 (4′-C), 89.5 (1′-C), 95.9 (5-C), 98.9 (1″-C), 119.5 (4″-C), 120.5 (3″-C), 126.6 (6″-C), 142.5 (6-C); ^{31}P NMR (243 MHz, D_2O): δ = −4.19 (bs, PO_4); MALDI-MS (negative mode, matrix: ATT): *m*/*z* (%): 533 [$M - Na^+]^-$, 556.1 for $C_{19}H_{26}N_4NaO_{12}P$.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Supporting Information Available: Experimental procedures and analytical data for new compounds (**4b**−**10b**, **7a**−**9a**, **12b**) and reproductions of ^{1}H, ^{13}C, ^{31}P NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA017370N