JOC*Article*

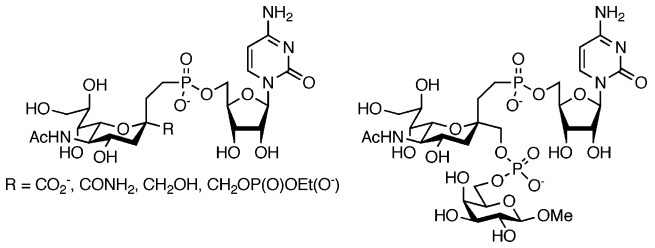
Synthesis of Bisubstrate and Donor Analogues of Sialyltransferase and Their Inhibitory Activities

Masayuki Izumi,* Katsuhiro Wada, Hideya Yuasa, and Hironobu Hashimoto

Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

masayuki.izumi@aist.go.jp

Received June 20, 2005



Sialyltransferases (STs) are involved in the biosynthesis of glycoconjugates with important biological activities. Most STs utilize cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP−Neu5Ac) as a common donor substrate. A bisubstrate analogue containing the donor substrate (CMP−Neu5Ac mimic) and the acceptor substrate (galactose) was synthesized. Four donor analogues having the partial structure of the bisubstrate analogue were also synthesized to support study of the structure−activity relationship. Each analogue contains an ethylene group in place of the exocyclic anomeric oxygen of CMP−Neu5Ac. The bisubstrate analogue exhibited only weak inhibitory activity to rat recombinant α-2,3- and α-2,6-ST (IC_{50} = 1.3, 2.4 mM). Conversion of the C-1 carboxylate of the Neu5Ac moiety to carboxyamide, hydroxymethyl, or methylene phosphate each resulted in a reduction in inhibitory activity. Among the synthesized analogues, cytidin-5′-yl sialylethylphosphonate (**4**) was the most potent inhibitor against rat recombinant α-2,3- and α-2,6-ST (IC_{50} = 0.047, 0.34 mM).

Introduction

Sialic acids, such as *N*-acetylneuraminic acid (Neu5Ac), are often located at the nonreducing end of oligosaccharides in glycoproteins and glycolipids.[1] Sialic acids are also involved in various biological events, such as bacterial or viral infection, inflammation, development, and tumor progression.[2] Sialyltransferases (STs) are the enzymes responsible for biosyntheses of the sialic acid-containing oligosaccharides.[3] Sialyltransferase inhibitors[4] are thus useful biological probes for studying sialic acid-mediated biological processes. These inhibitors are also valuable for exploring the active sites of STs and for elucidating the mechanisms of action of these enzymes.

Most STs utilize cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP−Neu5Ac **1**, Figure 1) as a glycosyl donor. We have been studying the synthesis of stable CMP−Neu5Ac analogues as potential sialyltransferase inhibitors and have reported the synthesis of **2**[5] and **3**,[6] in which the glycosidic oxygen has been removed and substituted with a methylene group, respectively. The Schmidt group also synthesized **2**, and the K_i value against rat recombinant α-2,6-ST was reported to be 0.25 mM.[7] Several stable donor analogues of glycosyltransferases having glycosyl phosphonate, glycosyl *C*-alkylphosphonate, and glycosyl *C*-alkyl phosphate have also been synthesized.[8,9] Uridine-5′-diphosphogalactose (UDP−Gal) analogues were tested as *β*-1,4-galactosyltransferase inhibitors and demonstrated K_i values similar to the K_M value of UDP−Gal.[9] This suggests that glycosyltransferases can tolerate this type of structural modification. The Schmidt group has extensively studied sialyltransferase inhibitors[10] based on CMP−Neu5Ac and its oxo-

* Current address of corresponding author: Research Center of Advanced Bionics, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan. Phone: +81-29-861-4681. Fax: +81-29-861-4680.

(1) Varki, A. In *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1999; p 195−209.
(2) Varki, A. *Glycobiology* **1993**, *3*, 97−130.
(3) Lau, J. T. Y.; Wuensch, S. A. In *Carbohydrates in Chemistry and Biology*; Ernst, B., Hart, G. W., Sinäy, P., Eds.; Wiley-VCH: Weinheim, 2000; Vol. 3, p 213−226.
(4) Wang, X.; Zhang, L.-H.; Ye, X.-S. *Med. Res. Rev.* **2003**, *23*, 32−47.
(5) Imamura, M.; Hashimoto, H. *Tetrahedron Lett.* **1996**, *37*, 1451−1454.
(6) Imamura, M.; Hashimoto, H. *Chem. Lett.* **1996**, 1087−1088.
(7) Muller, B.; Martin, T. J.; Schaub, C.; Schmidt, R. R. *Tetrahedron Lett.* **1998**, *39*, 509−512.

10.1021/jo0512608 CCC: $30.25
Published on Web 09/30/2005

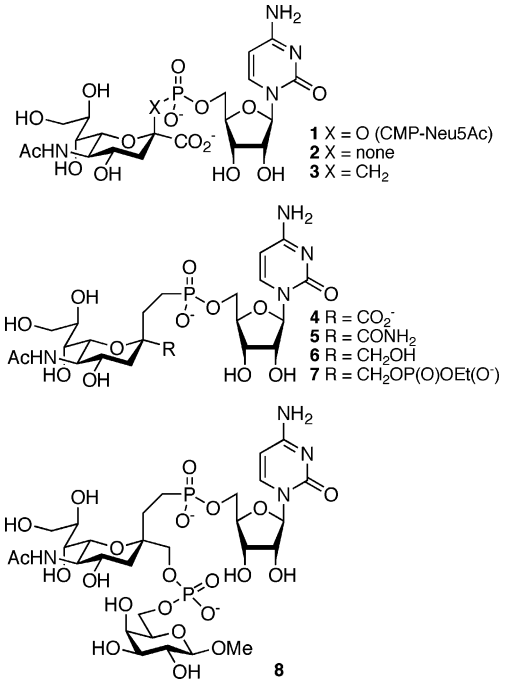


FIGURE 1. CMP−Neu5Ac and designed analogues.

carbenium transition state[11] in ST-catalyzed reactions. From these studies, they revealed that α-hydroxyphosphonate esters of CMP, having a flattened ring system resembling the transition state of glycosyl transfer, are very strong sialyltransferase inhibitors, having K_i values in the nanomolar range.[12] The most potent inhibitor reported by the group has a K_i value of 29 nM, which is roughly 4 orders of magnitude smaller than the K_M value of CMP−Neu5Ac (47 μM).[13]

There are three typical types of glycosidic linkages found in sialic acids: α-2,3-galactose (Gal); α-2,6-Gal, *N*-acetylgalactosamine, or *N*-acetylglucosamine (GlcNAc); and α-2,8-Neu5Ac. Because most STs catalyze the transfer of Neu5Ac from CMP−Neu5Ac, the specificity of STs most likely results from recognition of the glycosyl acceptor. Therefore, bisubstrate analogues in which a donor and an acceptor analogue are covalently connected, enabling interaction with both substrate-binding sites, are candidates for selective ST inhibitors. We previously reported that a bisubstrate analogue composed of UDP−Gal (donor) and GlcNAc (acceptor), connected via a methylene group, was a potent β-1,4-galactosyltransferase (GalT) inhibitor[14] but was not an inhibitor of α-1,3-GalT. Bisubstrate analogue inhibitors have also been reported for fucosyltransferases.[15] For sialyltransferases, the Schmidt group synthesized bisubstrate analogue inhibitors by adding acceptor analogues to their transition state inhibitors but reported that neither the resulting inhibitory potency nor the selectivity between rat recombinant α-2,3- and α-2,6-STs was increased.[10] Bisubstrate analogue inhibitors reported by Hinou et al. were also good inhibitors of both rat recombinant α-2,3- and α-2,6-STs, with K_i values similar to the K_M value of CMP−Neu5Ac.[16] This bisubstrate analogue strategy for the development of potent and selective glycosyltransferase inhibitors may be effective only for inverting enzymes such as sialyltransferases, β-galactosyltransferases, and fucosyltransferases, as retaining enzymes are assumed to transfer a glycosyl moiety via a glycosyl−enzyme intermediate.[17]

In our continuing synthetic studies of stable CMP-Neu5Ac analogues, we planned the synthesis of cytidin-5′-yl sialylethylphosphonate (**4**, Figure 1), which possesses an ethylene group rather than a glycosidic oxygen and resembles the transition state of glycosyl transfer, by moving CMP one bond farther from the Neu5Ac residue. We expanded our studies to include bisubstrate analogue **8**, which contains the partial structure of donor analogue **4**, and methyl β-galactoside as an acceptor analogue. Taking into consideration that α-glycoside is formed by STs, we placed an acceptor moiety at the α-face of the Neu5Ac residue via the C-1 hydroxymethyl group, which was formed via reduction of carboxylate. The donor and the acceptor analogues were linked by a phosphate group, which also mimics the negative charge of C-1 carboxylate. In this study, methyl β-galactoside was used as an acceptor analogue rather than the high-affinity acceptor LacNAc,[18] in order to simplify the synthesis. Donor analogues (**6**, **7**) possessing the partial structure of **8** were also prepared to evaluate our inhibitor design. Here, we report the synthesis of donor analogues **4**−**7**, as well as the bisubstrate analogue **8**, and their inhibitory activities toward rat recombinant α-2,3- and α-2,6-sialyltransferases.

Results and Discussion

For the convergent synthesis of all analogues, nonitol **15**, which contains a protected hydroxymethyl group rather than an ester, was designed as a common intermediate. The synthesis of **15** is illustrated in Scheme 1. All the hydroxyl groups of β-*C*-allyl sialoside (**9**)[19] were protected with benzyloxymethyl (BOM) groups, and reduction of the methyl ester with $LiEt_3BH$ yielded alcohol **11**. The hydroxymethyl group in **11** was protected with

(8) (a) Stolz, F.; Blume, A.; Hinderlich, S.; Reutter, W.; Schmidt, R. R. *Eur. J. Org. Chem.* **2004**, 3304−3312. (b) Schafer, A.; Thiem, J. *J. Org. Chem.* **2000**, *65*, 24−29. (c) Luengo, J. I.; Gleason, J. G. *Tetrahedron Lett.* **1992**, *33*, 6911−6914. (d) Orbe, M.; Claesson, A. *Eur. J. Med. Chem.* **1989**, *24*, 447−451. (e) Norbeck, D. W.; Kramer, J. B.; Lartey, P. A. *J. Org. Chem.* **1987**, *52*, 2174−2179.

(9) (a) Schmidt, R. R.; Frische, K. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1747−1750. (b) Vaghefi, M. M.; Bernacki, R. J.; Dalley, N. K.; Wilson, B. E.; Robins, R. K. *J. Med. Chem.* **1987**, *30*, 1383−1391.

(10) Jung, K.-H.; Schwoerer, R.; Schmidt, R. R. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 275−289.

(11) (a) Bruner, M.; Horenstein, B. A. *Biochemistry* **2000**, *39*, 2261−8. (b) Bruner, M.; Horenstein, B. A. *Biochemistry* **1998**, *37*, 289−97.

(12) Muller, B.; Schaub, C.; Schmidt, R. R. *Angew. Chem., Int. Ed.* **1998**, *37*, 2893−2897.

(13) Schwoerer, R.; Schmidt, R. R. *J. Am. Chem. Soc.* **2002**, *124*, 1632−1637.

(14) Hashimoto, H.; Endo, T.; Kajihara, Y. *J. Org. Chem.* **1997**, *62*, 1914−1915.

(15) (a) Palcic, M. M.; Heerze, L. D.; Srivastava, O. P.; Hindsgaul, O. *J. Biol. Chem.* **1989**, *264*, 17174−17181. (b) Qiao, L.; Murray, B. W.; Shimazaki, M.; Schultz, J.; Wong, C.-H. *J. Am. Chem. Soc.* **1996**, *118*, 7653−7662. (c) Jefferies, I.; Bowen, B. R. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1171−1174. (d) Pasquarello, C.; Picasso, S.; Demange, R.; Malissard, M.; Berger, E. G.; Vogel, P. *J. Org. Chem.* **2000**, *65*, 4251−4260.

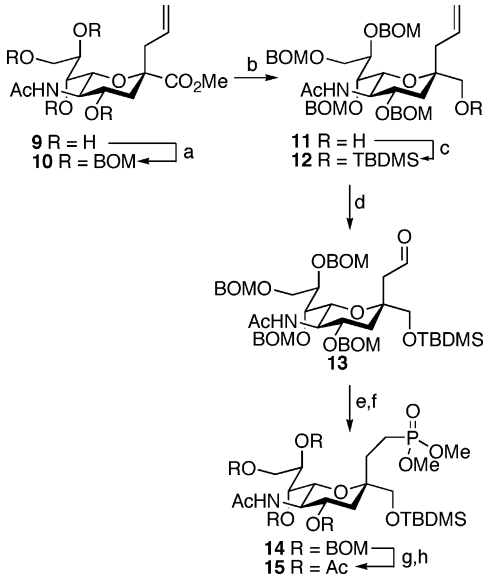
(16) Hinou, H.; Sun, X.-L.; Ito, Y. *J. Org. Chem.* **2003**, *68*, 5602−5613.

(17) Unligil, U. M.; Rini, J. M. *Curr. Opin. Struct. Biol.* **2000**, *10*, 510−517.

(18) Williams, M. A.; Kitagawa, H.; Datta, A. K.; Paulson, J. C.; Jamieson, J. C. *Glycoconjugate J.* **1995**, *12*, 755−761.

(19) Paulsen, H.; Matschulat, P. *Liebigs Ann. Chem.* **1991**, 487−495.

SCHEME 1[a]

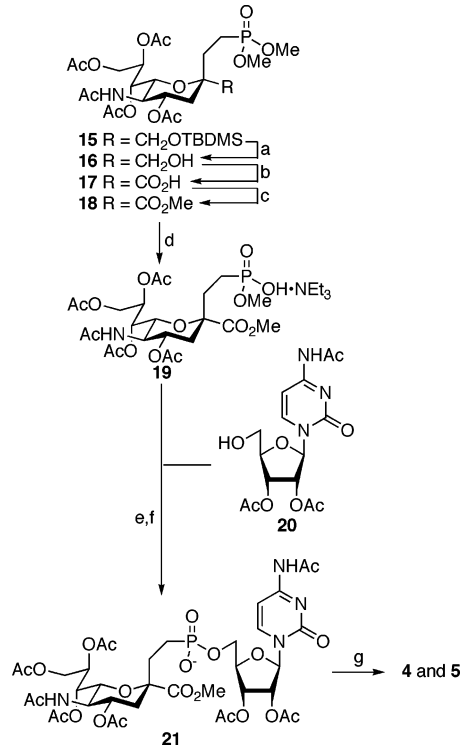


[a] Reagents and conditions: (a) BOMCl, DIEA, DMF, 60 °C, 93%; (b) $LiEt_3BH$, THF, 98%; (c) TBDMSCl, imidazole, DMF, 93%; (d) O_3, MeOH, CH_2Cl_2, −78 °C then Me_2S, 92%; (e) BuLi, $(MeO)_2POH$, THF, −78 °C then ClC(S)OPh, 92%; (f) Bu_3SnH, AIBN, toluene, 70 °C, 75%; (g) Pd−C, HCO_2NH_4, MeOH, reflux; (h) Ac_2O, pyridine, DMAP, 87% (two steps)

a *tert*-butyldimethylsilyl (TBDMS) group to yield **12**, which was then converted to aldehyde **13** by ozonolysis. Addition of dimethyl phosphite [$(MeO)_2POH$] to **13** with BuLi and subsequent phenoxythiocarbonylation of the newly formed hydroxyl group[20] yielded α-(phenoxythiocarbonyloxy)ethylphosphonate in a diastereomeric mixture. Deoxygenation with Bu_3SnH yielded nonitolylethylphosphonate **14**. BOM groups were removed by hydrogenation, and subsequent acetylation yielded common intermediate **15**.

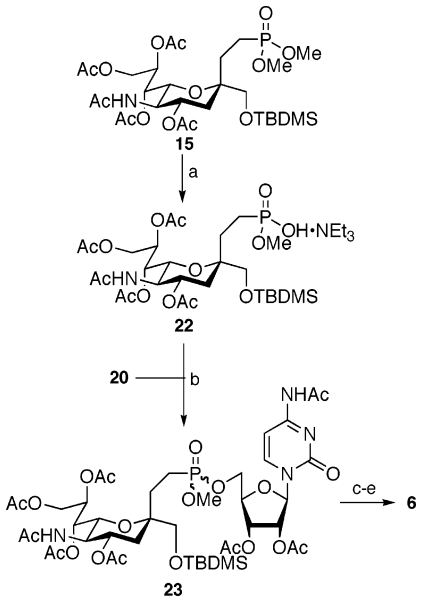
Intermediate **15** was next converted to **4** and **5** (Scheme 2). Treatment of **15** with HF−pyridine yielded alcohol **16**. Oxidation of the primary alcohol with ruthenium tetroxide[21] yielded carboxylic acid **17**, which was converted to methyl ester **18** via treatment with diazomethane. Chemoselective demethylation of ester **18**, using thiophenol (PhSH) and triethylamine (Et_3N) in dioxane[22] yielded monomethyl phosphonate **19**. Condensation of monoester **19** and triacetylcytidine (**20**)[23] using benzotriazol-1-yloxytris(dimethyamino)phosphonium hexafluorophosphate (BOP) and *N*,*N*-diisopropylethylamine (DIEA) in DMF[24] yielded the fully protected cytidin-5′-yl sialylethylphosphonate with very good yield. The methyl group of the phosphonate was removed with PhSH and Et_3N to give **21**. Simultaneous deacetylation and saponification using ammonium hydroxide (NH_4OH) and methanol (MeOH) resulted in the formation of carboxylic acid **4** and carboxyamide **5** in a 1:1 ratio. Monovalent anion **5** was eluted more rapidly from an anion-exchange column than

SCHEME 2[a]



[a] Reagents and conditions: (a) HF−pyridine, THF, 80%; (b) $RuCl_3{\cdot}nH_2O$, $NaIO_4$, CCl_4, MeCN, H_2O, 93%; (c) CH_2N_2, Et_2O, benzene, EtOH, 81%; (d) PhSH, Et_3N, dioxane, 87%; (e) BOP, DIEA, DMF; (f) PhSH, Et_3N, dioxane, 73% from **19**; (g) NH_4OH, MeOH (1:1), **4**, 32%, **5**, 30%

SCHEME 3[a]



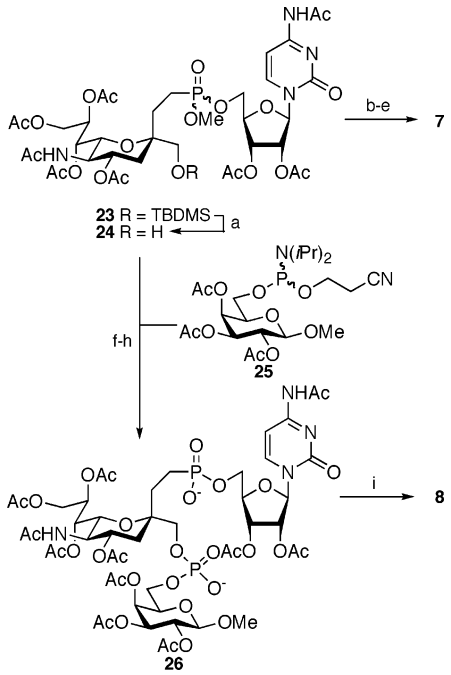
[a] Reagents and conditions: (a) PhSH, Et_3N, dioxane, 86%; (b) BOP, DIEA, DMF; (c) PhSH, Et_3N, dioxane; (d) THF, HCO_2H, H_2O (6:3:1); (e) NH_4OH, MeOH (1:1), 57% from **22**

divalent anion **4**, and the structures of **4** and **5** were elucidated by ESI-MS analysis.

Hydroxymethyl derivative **6** was next synthesized (Scheme 3). Demethylation of common intermediate **15**, using PhSH and Et_3N, yielded monoester **22**. Condensation of **22** with **20**, using BOP/DIEA, yielded protected

(20) Martin, S. F.; Dean, D. W.; Wagman, A. S. *Tetrahedron Lett.* **1992**, *33*, 1839−1842.
(21) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936−3938.
(22) Daub, G. W.; Van Tameken, E. E. *J. Am. Chem. Soc.* **1977**, *99*, 3526−3528.
(23) Baschang, G.; Kvita, V. *Angew. Chem.* **1973**, *85*, 43−44.
(24) Campagne, J.-M.; Coste, J.; Jouin, P. *J. Org. Chem.* **1995**, *60*, 5214−5223.

SCHEME 4[a]



[a] Reagents and conditions: (a) TBAF, THF, AcOH, 64%; (b) $(EtO)_2PCl$, DIEA, CH_2Cl_2; (c) tBuOOH, MeCN; (d) LiBr, MeCN, reflux; (e) NH_4OH, MeOH (1:1), 50% from **24**; (f) 1*H*-tetrazole, MeCN; (g) tBuOOH; (h) PhSH, Et_3N, dioxane, 43% from **24**; (i) NH_4OH, MeOH (1:1), 53%

cytidin-5′-yl nonitolylethylphosphonate **23**, with good yield. The methyl group was first removed by treatment with PhSH and Et_3N. The TBDMS group was then removed smoothly under acidic conditions with anchimeric assistance from the adjacent phosphonic acid.[25] Removal of all acetyl groups with NH_4OH−MeOH yielded **6**.

Finally, analogues having phosphate groups were synthesized (Scheme 4). To release the free hydroxyl group at the C-1 of the nonitol residue, the TBDMS group of **23** was removed by treatment with tetrabutylammonium fluoride (TBAF). It was necessary to use commercially available 1 M TBAF in THF without dilution to complete the deprotection, possibly due to steric hindrance at the C-1 position. Phosphorylation of alcohol **24** with diethyl chlorophosphite and DIEA, followed by oxidation with *tert*-butyl hydroperoxide, yielded the diethyl phosphate. Treatment of the diethyl phosphate with LiBr in refluxing MeCN, followed by deacetylation yielded monoethyl phosphate **7**. Alcohol **24** was also converted into bisubstrate analogue **8** in the following manner. Condensation of alcohol **24** and galactosid-6-yl phosphoramidite **25** with tetrazole and subsequent oxidation yielded a fully protected bisubstrate analogue, which was then treated with PhSH and Et_3N to give phosphodiester **26** at a 43% yield, in three steps. Deacetylation of **26** yielded **8**, which was purified by anion-exchange chromatography and gel permeation chromatography.

The inhibitory activities of CMP−Neu5Ac analogues **4**−**8** against rat recombinant α-2,3- and α-2,6-STs[18] were measured and are summarized in Table 1. The ST assay was carried out using a fluorescent acceptor, 2-[(2-pyridyl)amino]ethyl *β*-*N*-acetyllactosaminide (PA-LacNAc), in which the reaction product was quantitated via RP-HPLC.[26] To evaluate inhibitory activities, IC_{50} values were measured for all analogues under conditions for both substrates (i.e. 0.11 mM for CMP−Neu5Ac and 1.0 mM for PA-LacNAc). Only carboxylate derivative **4** exhibited significant inhibitory activities against both STs (IC_{50} = 0.047 mM for α-2,3-ST and 0.34 mM for α-2,6-ST). Since the difference between these two IC_{50} values is about 1 order of magnitude, compound **4** could be a good starting structure for the design of the selective bisubstrate analogue inhibitor. Analogues **5** and **6**, each of which has only one negative charge, were poor inhibitors, with IC_{50} values in the millimolar range. Replacing the carboxylate in **4** with a methylene phosphate group also resulted in reduced inhibitory activity, as observed for **7** and **8**. However, IC_{50} values of **7** and **8** were relatively smaller than those of **5** and **6**, suggesting the importance of the existence of the second negative charge. Schmidt et al. suggested that the distance between two negative charges should be about five bonds, to result in a strong inhibitory activity.[10] The weak inhibitory activities of **7** and **8** may result from the two negative charges being spaced too far apart. No improvement in the inhibitory activity of bisubstrate analogue **8** suggested that the galactose residue did not contribute to the additional binding. However, our linking strategy of the two substrate analogues through the 1-position of Neu5Ac may be effective, since the inhibitory potency was not interfered with by the bulky galactose residue, although connecting the donor and the acceptor through phosphate as a mimic of carboxylate was not effective. According to the structure of Schmidt's nanomolar inhibitors, the presence of two negative charges, an extended distance between the anomeric carbon and the CMP leaving group, and an anomeric carbon with sp^2 hybridization is important.[10] Recently, a crystallographic structure of the sialyltransferase CstII from *Campylobacter jejuni* in complex with CMP−3″F-Neu5Ac was reported.[27] In this structure, the pyranose ring of Neu5Ac had the $^0S_5$ skew conformation, which also suggested the importance of the flattened ring conformation for high-affinity binding. The similar affinity of **4** and CMP−Neu5Ac is probably due to the lack of an sp^2 anomeric carbon.

TABLE 1. Inhibitory Activities of Compounds 4−8 against Rat Recombinant α-2,3- and α-2,6-STs

	IC_{50}[a]	
compd	α-2,3-ST	α-2,6-ST
4	0.047	0.34
5	3.3	4.3
6	4.2	3.2
7	0.95	2.3
8	1.3	2.4

[a] All values are in mM. IC_{50} values at 0.11 mM CMP−Neu5Ac and 1.0 mM PA-LacNAc.

In conclusion, we synthesized five CMP−Neu5Ac analogues, each of which has an ethylene group rather than a glycosidic oxygen and one of which is a bisubstrate analogue. An inhibition assay for two sialyltransferases

(25) Kawahara, S.-i.; Wada, T.; Sekine, M. *J. Am. Chem. Soc.* **1996**, *118*, 9461−9468.

(26) Kajihara, Y.; Kamiyama, D.; Yamamoto, N.; Sakakibara, T.; Izumi, M.; Hashimoto, H. *Carbohydr. Res.* **2004**, *339*, 1545−1550.

(27) Chiu, C. P. C.; Watts, A. G.; Lairson, L. L.; Gilbert, M.; Lim, D.; Wakarchuk, W. W.; Withers, S. G.; Strynadka, N. C. J. *Nat. Struct. Mol. Biol.* **2004**, *11*, 163−170.

revealed that replacing the carboxylate of Neu5Ac with the methylene phosphate group resulted in a loss of affinity. Although four of the five analogues failed to exhibit substantial inhibitory activity against the STs, cytidin-5′-yl sialylethylphosphonate (**4**) was a good inhibitor against both rat recombinant α-2,3 and α-2,6-ST. The linking strategy and structure of the acceptor moiety still need to be refined for a selective bisubstrate inhibitor based on cytidin-5′-yl sialylethylphosphonate structure.

Experimental Section

Methyl 5-Acetamido-2,6-anhydro-4,7,8,9-tetra-*O*-benzyloxymethyl-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*gluco*-nononate (10). To a solution of methyl 5-acetamido-2,6-anhydro-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*gluco*-nononate (**9**) (0.204 g, 0.59 mmol) in DMF (6.0 mL) were added DIEA (0.83 mL, 4.7 mmol) and BOMCl (0.73 mL, 4.7 mmol). After being stirred for 3 h at 60 °C, the solution was cooled and diluted with EtOAc (100 mL). The organic layer was washed with brine (50 mL × 3), dried ($MgSO_4$), and concentrated. The residue was purified on a column of silica gel (toluene−acetone 9:1) to yield **10** (452 mg, 93%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.41−7.27 (m, 20H), 5.79 (d, 1H, *J* = 7.3 Hz), 5.67−5.56 (m, 1H), 5.16−4.51 (m, 20H), 4.22 (d, 1H, *J* = 9.9 Hz), 4.06 (m, 2H), 3.84 (dd, 1H, *J* = 4.1, 9.9 Hz), 3.67 (s, 3H), 3.27 (dt, 1H, *J* = 6.9, 10.2 Hz), 2.75 (m, 2H), 2.48 (dd, 1H, *J* = 5.0, 13.2 Hz), 1.77 (dd, 1H, *J* = 10.9, 13.2 Hz), 1.61 (s, 3H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 172.0, 170.3, 137.8, 137.7, 137.6, 131.5, 128.5, 128.3, 128.0, 127.9, 127.6, 127.5, 127.4, 118.9, 96.7, 95.7, 95.1, 93.5, 79.3, 78.7, 77.4, 70.6, 69.9, 69.6, 69.4, 69.1, 68.5, 54.4, 52.0, 37.0, 36.7, 23.3; HR-ESI-MS calcd for $C_{47}H_{58}NO_{12}$ $[M + H]^+$ 828.3959, found 828.3919.

5-Acetamido-2,6-anhydro-4,7,8,9-tetra-*O*-benzyloxymethyl-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*gluco*-nonitol (11). To a solution of **10** (401 mg, 0.48 mmol) in THF (2.8 mL) was added $LiEt_3BH$ (1.0 M THF solution, 1.9 mL, 1.9 mmol) dropwise under Ar atmosphere. After being stirred for 30 min at room temperature, the solution was diluted with EtOAc (40 mL), and washed with water (10 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (toluene−acetone 5:1) to yield **11** (379 mg, 98%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.41−7.24 (m, 20H), 5.73−5.64 (m, 2H), 5.16−4.50 (m, 19H), 4.40 (d, 1H, *J* = 10.6 Hz), 4.06 (d, 1H, *J* = 10.2 Hz), 3.98 (m, 2H), 3.78 (dd, 1H, *J* = 3.2, 10.6 Hz), 3.50−3.28 (m, 2H), 3.35 (dt, 1H, *J* = 7.6, 10.2 Hz), 2.61 (dd, 1H, *J* = 7.9, 13.9 Hz), 2.50 (br, 1H), 2.33 (dd, 1H, *J* = 6.9, 13.9 Hz), 2.03 (dd, 1H, *J* = 4.9, 12.9 Hz), 1.67 (s, 3H), 1.56 (t, 1H, *J* = 12.6 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.2, 137.6, 137.50, 137.47, 137.0, 132.5, 129.9, 128.5, 128.33, 128.30, 128.28, 128.0, 127.61, 127.59, 127.58, 127.55, 127.5, 118.8, 96.8, 95.2, 94.7, 93.7, 77.6, 77.3, 70.9, 70.8, 69.7, 69.6, 69.4, 69.2, 68.5, 68.0, 54.5, 35.6, 34.7, 29.8, 23.7; HR-ESI-MS calcd for $C_{46}H_{58}NO_{11}$ $[M + H]^+$ 800.4010, found 800.3993.

5-Acetamido-2,6-anhydro-4,7,8,9-tetra-*O*-benzyloxymethyl-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-2-*C*-(2-propenyl)-D-*erythro*-L-*gluco*-nonitol (12). To a solution of **11** (1.265 g, 1.58 mmol) in DMF (5.2 mL) was added imidazole (0.742 g, 10.6 mmol). A solution of TBDMSCl (0.738 g, 4.92 mmol) in DMF (2.9 mL) was added to the solution at 0 °C. After being stirred overnight at room temperature, the solution was concentrated, and the residue was purified on a column of silica gel (toluene−acetone 7:1) to yield **12** (1.345 g, 93%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.40−7.26 (m, 20H), 5.76−5.69 (m, 2H), 5.17−4.50 (m, 19H), 4.37 (d, 1H, *J* = 10.2 Hz), 4.06 (d, 1H, *J* = 9.9 Hz), 3.98 (m, 2H), 3.79 (dd, 1H, *J* = 3.6, 9.9 Hz), 3.56 (d, 1H, *J* = 9.9 Hz), 3.37 (d, 1H, *J* = 9.9 Hz), 3.30 (m, 1H), 2.54−2.51 (m, 2H), 2.20 (dd, 1H, *J* = 5.0, 12.9 Hz), 1.64 (s, 3H), 1.37 (t, 1H, *J* = 12.2 Hz), 0.87 (s, 9H), 0.03, 0.02 (each s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.2, 137.8, 137.7, 137.2, 133.1, 128.5, 128.3, 127.9, 127.4, 118.0, 97.0, 95.7, 95.0, 93.6, 78.3, 77.7, 77.1, 71.2, 70.6, 69.5, 69.3, 69.1, 68.4, 54.7, 37.3, 34.4, 25.7, 23.4, 18.0, −5.55, −5.60; HR-ESI-MS calcd for $C_{52}H_{72}NO_{11}Si$ $[M + H]^+$ 914.4875, found 914.4877.

6-Acetamido-3,7-anhydro-5,8,9,10-tetra-*O*-benzyloxymethyl-3-*C*-*tert*-butyldimethylsilyloxymethyl-2,4,6-trideoxy-D-*erythro*-L-*manno*-decose (13). Into a solution of **12** (1.345 g, 1.47 mmol) in MeOH (11.6 mL) and CH_2Cl_2 (2.9 mL) was bubbled O_3 for 20 min at −78 °C. Then, O_2 gas was bubble into the solution for 5 min at the same temperature. Me_2S (2.3 mL) was added to the solution, and the mixture stirred for 1.5 h at the same temperature and then stirred overnight at room temperature. The solution was concentrated and the residue was purified on a column of silica gel (toluene−acetone 7:1) to yield **13** (1.246 g, 92%) as a faint yellow syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.68 (br, 1H), 7.41−7.24 (m, 20H), 5.76 (d, 1H, *J* = 7.3 Hz), 4.98−4.50 (m, 17H), 4.41 (d, 1H, *J* = 9.9 Hz), 4.01 (dd, 1H, *J* = 2.0, 10.9 Hz), 3.98−3.89 (m, 2H), 3.79 (dd, 1H, *J* = 3.6, 10.9 Hz), 3.68 (d, 1H, *J* = 9.6 Hz), 3.55 (d, 1H, *J* = 9.6 Hz), 3.33 (dt, 1H, *J* = 7.3, 9.9 Hz), 2.89 (dd, 1H, *J* = 2.0, 15.2 Hz), 2.70 (dd, 1H, *J* = 3.0, 15.2 Hz), 2.41 (dd, 1H, *J* = 4.9, 11.9 Hz), 1.65 (s, 3H), 1.42 (t, 1H, *J* = 11.9 Hz), 0.89−0.81 (s, 9H), 0.01 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 199.6, 170.2, 137.60, 137.58, 137.0, 128.5, 128.3, 128.04, 127.96, 127.53, 127.51, 127.49, 97.2, 95.9, 95.2, 93.9, 77.7, 76.9, 70.93, 70.90, 70.1, 69.8, 69.6, 69.33, 69.31, 68.0, 54.8, 44.9, 38.3, 25.8, 23.7, 18.2, −5.4, −5.5; HR-ESI-MS calcd for $C_{51}H_{70}NO_{12}Si$ $[M + H]^+$ 916.4667, found 916.4672.

Dimethyl 2-(5-Acetamido-2,6-anhydro-4,7,8,9-tetra-*O*-benzyloxymethyl-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-*C*-yl)ethylphosphonate (14). To a solution of dimethyl phosphite (76 μL, 0.83 mmol) in THF (3.9 mL) was added dropwise a solution of n-BuLi (1.6 M hexane solution, 0.52 mL, 0.83 mmol) at −78 °C under Ar atmosphere. After the mixture stirred for 7 min, a solution of **13** (0.475 g, 0.518 mmol) in THF (1.35 mL) was added dropwise to it. After the mixture stirred for 5 min at −78 °C, phenyl thionochloroformate (0.28 mL, 2.1 mmol) was added. After stirring for 30 min at the same temperature, saturated aqueous NH_4Cl was added. The aqueous layer was extracted three times with $CHCl_3$, and the combined organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (toluene−acetone 5:1→4:1) to yield dimethyl 2-(5-acetamido-2,6-anhydro-4,7,8,9-tetra-*O*-benzyloxymethyl-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-*C*-yl)-1-phenoxylthiocarbonyloxyethylphosphonate (0.519 g, 86%) as a faint yellow syrup: ^{31}P NMR ($CDCl_3$) δ 22.16, 21.71.

A solution of n-Bu_3SnH (1.37 g, 4.69 mmol) and AIBN (50 mg) in dry toluene (33.6 mL) was heated to 70 °C. A solution of the yellow syrup (1.17 g, 1.01 mmol) in dry toluene (5.6 mL) was added dropwise to the solution. After being stirred for 2 h at the same temperature, the solution was concentrated, and the residue was purified on a column of silica gel (toluene−acetone 4:1→3:1) to yield **14** (0.764 g, 75%) as a colorless syrup: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (m, 20H), 5.77 (d, 1H, *J* = 7.9 Hz), 4.90−4.52 (m, 16H), 4.41 (dt, 1H, *J* = 4.7, 12.5 Hz), 4.14 (d, 1H, *J* = 10.5 Hz), 4.07 (dd, 1H, *J* = 1.8, 9.8 Hz), 4.01−3.96 (m, 2H), 3.82 (dd, 1H, *J* = 4.3, 10.5 Hz), 3.70, 3.69 (each d, 3H×2, *J* = 10.0 Hz), 3.53−3.46 (m, 2H), 3.35 (d, 1H, *J* = 9.6 Hz), 2.24 (dd, 1H, *J* = 4.7, 13.1 Hz), 2.17−1.56 (m, 7H), 1.45 (t, 1H, *J* = 12.5 Hz), 0.88 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 169.9, 137.6, 137.51, 137.47, 137.2, 129.3, 128.2, 128.1, 127.7, 127.6, 127.4, 97.0, 95.0, 94.9, 93.5, 78.2, 77.1, 76.3, 72.3, 70.6, 69.9, 69.6, 69.3, 69.1, 67.9, 67.7, 53.7, 53.3, 53.2, 52.4, 52.3, 52.2, 52.1, 38.5, 25.7, 23.5, 18.00, −5.53; ^{31}P NMR ($CDCl_3$) δ 35.70; HR-ESI-MS calcd for $C_{53}H_{77}NO_{14}PSi$ $[M + H]^+$ 1010.4851, found 1010.4847.

Dimethyl 2-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-*erythro*-

L-***gluco*-nonitol-2-yl)ethylphosphonate (15).** To a solution of **14** (0.256 g, 0.253 mmol) in MeOH (15 mL) were added Pd/C (10%, 0.5 g) and HCO_2NH_4 (0.3 g, 7.9 mmol). After being stirred for 2 h under reflux, another portion of HCO_2NH_4 (0.3 g, 7.9 mmol) was added. The suspension was stirred under reflux for 3 h and filtered through a Celite pad, and the filtrate was concentrated. The residue was acetylated with Ac_2O (2 mL), pyridine (4 mL), and DMAP (cat.) and purified on a column of silica gel (toluene−acetone 1:1) to yield **15** (0.121 g, 87%) as a colorless syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.50 (d, 1H, J = 9.9 Hz), 5.31 (dd, 1H, J = 2.0, 5.9 Hz), 5.21 (dt, 1H, J = 2.3, 6.3 Hz), 5.19−5.11 (m, 1H), 4.51 (dd, 1H, J = 2.3, 12.5 Hz), 4.02 (dd, 1H, J = 6.9, 12.5 Hz), 3.93 (q, 1H, J = 10.2 Hz), 3.81 (dd, 1H, J = 2.3, 9.2 Hz), 3.76, 3.75 (each d, 3H×2, J = 10.9 Hz), 3.53 (d, 1H, J = 10.1 Hz), 3.38 (d, 1H, J = 10.1 Hz), 2.17−1.61 (m, 6H), 2.12, 2.10, 2.02, 1.89 (each s, 3H, 3H, 6H, 3H), 0.89 (s, 9H), 0.07, 0.06 (each s, 3H×2); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.8, 170.4, 170.3, 170.1, 169.9, 77.3, 70.9, 70.8, 69.9, 68.4, 67.6, 62.4, 52.6, 52.51, 52.48, 52.38, 50.2, 37.0, 25.8, 23.2, 22.55, 22.51, 21.07, 21.05, 20.8, 18.9, 18.1, 16.8, −5.45, −5.50; ^{31}P NMR ($CDCl_3$) δ 35.56; HR-ESI-MS calcd for $C_{29}H_{52}NO_{14}PSiNa$ $[M + Na]^+$ 720.2787, found 720.2791.

Dimethyl 2-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (16). To a solution of **15** (0.121 g, 0.174 mmol) in THF (8.2 mL) was added HF−pyridine (70% HF, 0.5 mL) at 0 °C. The solution was stirred overnight at room temperature, and the reaction was quenched by adding saturated aqueous $NaHCO_3$. The aqueous layer was extracted twice with $CHCl_3$, and the combined organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (EtOAc−MeOH 10:1→9:1) to yield **16** (81.6 mg, 80%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.71 (d, 1H, J = 9.2 Hz), 5.32 (dd, 1H, J = 2.1, 5.8 Hz), 5.25−5.10 (m, 2H), 4.59 (dd, 1H, J = 2.3, 12.5 Hz), 4.13−3.86 (m, 3H), 3.77, 3.76 (each d, 3H×2, J = 10.9 Hz), 3.54−3.42 (m, 2H), 2.87 (br, 1H), 2.19−1.62 (m, 6H), 2.14, 2.11, 2.04, 2.01, 1.89 (each s, 3H×5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.7, 170.5, 170.3, 170.1, 169.8, 77.7, 71.0, 69.8, 68.6, 67.3, 62.6, 52.5, 52.4, 49.6, 34.6, 23.0, 22.2, 20.9, 20.7, 19.1, 17.0; ^{31}P NMR ($CDCl_3$) δ 34.96; HR-ESI-MS calcd for $C_{23}H_{38}NO_{14}PNa$ $[M + Na]^+$ 606.1928, found 606.1923.

Dimethyl 2-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoic acid-2-yl)-ethylphosphonate (17). To a solution of **16** (0.121 g, 0.208 mmol) in CCl_4−MeCN−H_2O (2:2:3 v/v/v, 1.5 mL) was added $NaIO_4$ (0.182 g, 0.851 mmol) and the mixture stirred until all solids dissolved. $RuCl_3{\cdot}nH_2O$ (1.2 mg, 6 μmol) was added to the solution and the mixture stirred for 3 h at room temperature. Precipitate was formed during the reaction. The suspension was diluted with $CHCl_3$ and washed with saturated aqueous $NaHCO_3$. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel ($CHCl_3$−MeOH 4:1→3:1) to yield **17** (0.116 g, 93%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$−CD_3OD 2:1) δ 5.37 (dd, 1H, J = 1.3, 4.6 Hz), 5.32−5.30 (m, 1H), 5.22 (dt, 1H, J = 4.9, 10.4 Hz), 4.78 (dd, 1H, J = 1.6, 12.2 Hz), 4.14 (dd, 1H, J = 6.9, 12.5 Hz), 3.97−3.83 (m, 2H), 3.79, 3.77 (each d, 3H×2, J = 10.9 Hz), 2.31 (dd, 1H, J = 4.9, 12.5 Hz), 2.14−1.72 (m, 5H), 2.14, 2.09, 2.03, 2.02, 1.89 (each s, 3H×5); ^{13}C NMR (67.8 MHz, $CDCl_3$−CD_3OD 2:1) δ 171.2, 170.7, 170.4, 169.8, 78.6, 71.0, 70.6, 69.4, 68.2, 62.2, 52.3, 52.2, 52.1, 49.15, 49.05, 36.3, 23.7, 22.0, 20.3, 20.2, 20.1, 20.0, 18.9, 17.3, 16.8; ^{31}P NMR ($CDCl_3$−CD_3OD 2:1) δ 39.83; HR-ESI-MS calcd for $C_{23}H_{37}NO_{15}P$ $[M + H]^+$ 598.1901, found 598.1921.

Dimethyl 2-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoate-2-yl)ethylphosphonate (18). To a solution of **17** (0.104 g, 0.173 mmol) in benzene−EtOH (4:1 v/v, 8.0 mL) was added CH_2N_2 in Et_2O by distillation, which was generated by adding 50% KOH (0.123 mL) to a solution of TsN(NO)Me (0.161 g, 0.752 mmol) in Et_2O−EtOH (2:1 v/v, 8.1 mL) and heating at 70 °C. The solution stood for 3 h. Another batch of CH_2N_2 solution was added to the solution since TLC showed remaining **17**. After standing for 1 h, the solution was still yellow and TLC showed the disappearance of **17**. AcOH was added to the solution until the yellow color disappeared. The solution was concentrated, and remaining AcOH was coevaporated with toluene. The residue was purified on a column of silica gel (EtOAc−MeOH 10:1) to yield **18** (85.5 mg, 81%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.79 (d, 1H, J = 8.2 Hz), 5.38 (d, 1H, J = 3.3 Hz), 5.24−5.20 (m, 2H), 4.81 (dd, 1H, J = 2.3, 12.5 Hz), 4.11 (dd, 1H, J = 7.9, 12.5 Hz), 3.99−3.83 (m, 2H), 3.77 (s, 3H), 3.77 (d, 3H, J = 10.6 Hz), 3.76 (d, 3H, J = 10.9 Hz), 2.30 (dd, 1H, J = 5.1, 13.6 Hz), 2.38−1.56 (m, 5H), 2.15, 2.08, 2.03, 2.02, 1.89 (each s, 3H×5); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.0, 170.55, 170.48, 170.4, 170.1, 170.0, 79.1, 78.8, 72.0, 71.6, 69.1, 68.7, 68.8, 62.6, 52.8, 52.7, 52.6, 52.5, 49.4, 36.4, 24.8, 23.2, 21.0, 20.9, 20.84, 20.80, 19.4, 17.3; ^{31}P NMR ($CDCl_3$) δ 34.19; HR-ESI-MS calcd for $C_{24}H_{39}NO_{15}P$ $[M + H]^+$ 612.2057, found 612.2096.

Triethylammonium Methyl 2-(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoate-2-yl)ethylphosphonate (19). To a solution of **18** (0.114 g, 0.187 mmol) in 1,4-dioxane (0.95 mL) were added PhSH (0.74 mL) and Et_3N (1.5 mL). After the reaction stirred for 4 h at room temperature, MeOH was added to the solution. The solution was concentrated, and the residue was purified on a column of silica gel (EtOAc−MeOH−Et_3N 4:1:0.1→3:1:0.1) to yield **19** (0.116 g, 87%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 6.70 (br, 1H), 5.40 (t, 1H, J = 2.3 Hz), 5.24−5.17 (m, 2H), 4.90 (dd, 1H, J = 2.0, 12.5 Hz), 4.21 (dd, 1H, J = 8.2, 12.5 Hz), 4.16 (overlap, 1H), 4.03 (q, 1H, J = 10.2 Hz), 3.73 (s, 3H), 3.57 (d, 3H, J = 10.6 Hz), 3.04 (q, 5H, J = 7.3 Hz), 2.36 (dd, 1H, J = 4.6, 12.9 Hz), 2.51−2.33, 2.08−1.81, 1.58−1.37 (each m, 1H, 3H, 1H), 2.14, 2.06, 2.02, 1.99, 1.88 (each s, 3H×5), 1.31 (t, 9H, J = 7.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.6, 170.24, 170.19, 170.10, 170.08, 169.9, 79.3, 79.1, 72.3, 71.1, 69.7, 68.9, 62.7, 52.2, 51.4, 51.3, 48.7, 45.1, 36.2, 26.1, 23.0, 20.9, 20.8, 20.7, 18.7, 8.4; ^{31}P NMR ($CDCl_3$) δ 24.96; HR-ESI-MS calcd for $C_{23}H_{37}NO_{15}P$ $[M - Et_3N + H]^+$ 598.1901, found 598.1904.

Sodium Cytidin-5′-yl 2-(Sodium 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoate-2-yl)ethylphosphonate (4) and Sodium Cytidin-5′-yl 2-(5-Acetamido-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanamide-2-yl)ethylphosphonate (5). Compound **19** and 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidine (**20**) were dried by concentrating three times from dry DMF in vacuo prior to use. To a solution of **19** (0.112 g, 0.158 mmol) and **20** (0.175 g, 0.474 mmol) in DMF (0.87 mL) were added BOP (0.210 g, 0.475 mmol) and DIEA (87 μL, 0.63 mmol). After being stirred for 3 h at room temperature, the solution was diluted with EtOAc, and washed twice with brine. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified on a column of silica gel (EtOAc−MeOH 10:1→7:1), first to yield unreacted **20**, and next 4-*N*-acetyl-2′,3′-di-*O*-acetyl-cytidin-5′-yl methyl 2-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoate-2-yl)ethylphosphonate (0.169 g, HMPA contamination, <90%) as a syrup in a diastereomer mixture on P: ^{31}P NMR ($CDCl_3$) δ 33.83, 33.27; HR-ESI-MS calcd for $C_{38}H_{54}N_4O_{22}P$ $[M + H]^+$ 949.2967, found 949.2959.

To a solution of the syrup (0.169 g, HMPA, contamination, <0.142 mmol) in 1,4-dioxane (0.72 mL) were added PhSH (0.56 mL) and Et_3N (1.1 mL). After being stirred overnight at room temperature, MeOH was added to the solution. The solution was concentrated, and the residue was purified on a column of silica gel (EtOAc−MeOH 2% Et_3N 4:1→3:1) to yield triethylammonium 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl 2-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonanoate-2-yl)ethylphosphonate (**21**, 0.130 g, <81%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.45 (d, 1H, J = 7.6 Hz), 7.82, 7.63 (each d, 1 H, J = 6.6 Hz), 7.39 (d, 1H,

J = 7.3 Hz), 6.94 (br, 1H), 6.24 (d, 1H, J = 5.3 Hz), 5.61−5.48 (m, 3H), 5.17−5.10 (m, 2H), 4.87 (d, 1H, J = 10.6 Hz), 4.38−4.07 (m, 6H), 3.73 (s, 3H), 2.99 (q, 6H, J = 7.3 Hz), 2.37−1.86 (m, 6H), 2.23, 2.14, 2.11, 2.07, 2.02, 1.982, 1.976, 1.86 (each s, 3H×8), 1.25 (9H, t, J = 7.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.5, 171.2, 170.5, 170.3, 170.2, 169.9, 169.8, 162.9, 155.5, 145.6, 97.4, 87.6, 82.2, 82.0, 79.7, 79.5, 74.4, 72.6, 71.7, 71.4, 70.0, 69.3, 63.0, 62.9, 52.4, 48.8, 45.4, 35.7, 26.89, 26.85, 24.9, 23.1, 22.50, 22.45, 21.0, 20.9, 20.7, 20.6, 8.8; ^{31}P NMR ($CDCl_3$) δ 22.94; HR-ESI-MS calcd for $C_{37}H_{52}N_4O_{22}P$ $[M + H]^+$ 935.2811, found 935.2821.

To a solution of the syrup (0.129 g, 0.114 mmol) in MeOH (5 mL) was added 28% NH_4OH (5 mL) and the mixture stirred for 6 h at room temperature. The solution was concentrated in vacuo, and the residue was applied to a DEAE-Sephadex A-25 column. The column was first washed with water and then eluted with a 0−1 M linear gradient of HCO_2Na solution. Two peaks were detected by UV absorbance at 275 nm around 0.1 and 0.3 M. Fractions containing each peak were collected, separately, and lyophilized. Each residue was purified by Sephadex G-15 chromatography (water) and lyophilized to yield **5** (21.9 mg, 30%), which was the first peak, and **4** (24.5 mg, 32%), which was the second peak, as white solids. Compound **4**: ^{1}H NMR (400 MHz, D_2O, COSY) δ 8.09 (d, 1H, J = 7.6 Hz, H6), 6.24 (d, 1H, J = 7.6 Hz, H5), 6.01 (d, 1H, J = 3.4 Hz, H1′), 4.37−4.33 (m, 2H, H2′,3′), 4.30 (br, 1H, H4′), 4.17 (m, 1H, H5′a), 4.07 (m, 1H, H5′b), 3.97−3.84 (m, 3H, H4″,8″,9″a), 3.81 (t, 1H, J = 10.1 Hz, H5″), 3.66 (d, 1H, J = 9.0 Hz, H6″), 3.66 (dd, 1H, J = 5.5, 11.9 Hz, H9″b), 3.48 (d, 1H, J = 9.3 Hz, H7″), 2.27 (dd, 1H, J = 4.6, 13.1 Hz, H3″eq), 2.06 (s, 3H, Ac), 2.22−1.91 (m, 2H, CH_2P), 1.68 (t, 1H, J = 12.3 Hz, H3″ax), 1.84−1.73, 1.44−1.33 (each m, 1H×2, CH_2C); ^{13}C NMR (100 MHz, D_2O) δ 180.7, 175.6, 166.3, 157.7, 142.7, 97.3, 90.2, 84.06, 83.98, 81.41, 81.21, 75.0, 71.0, 70.9, 70.3, 69.7, 68.8, 64.5, 63.52, 63.47, 53.6, 40.9, 26.5, 23.1, 21.8, 20.5; ^{31}P NMR (D_2O) δ 28.47; HR-ESI-MS calcd for $C_{22}H_{36}N_4O_{15}P$ $[M + H]^+$ 627.1909, found 627.1924. Compound **5**: ^{1}H NMR (400 MHz, D_2O, COSY) δ 7.98 (d, 1H, J = 7.5 Hz, H6), 6.17 (brd, 1H, J = 6.4 Hz, H5), 6.03 (d, 1H, J = 3.8 Hz, H1′), 4.37−4.30 (m, 2H, H2′,3′), 4.27 (br, 1H, H4′), 4.13 (m, 1H, H5′a), 4.05 (m, 1H, H5′b), 3.94−3.82 (m, 4H, H4″,5″,8″,9″a), 3.74 (d, 1H, J = 9.8 Hz, H6″), 3.67 (dd, 1H, J = 5.3, 11.9 Hz, H9″b), 3.58 (d, 1H, J = 9.6 Hz, H7″), 2.25 (dd, 1H, J = 4.0, 13.2 Hz, H3″eq), 2.06 (s, 3H, Ac), 2.20−1.92 (m, 2H, CH_2P), 1.70 (t, 1H, J = 11.7 Hz, H3″ax), 1.89−1.77, 1.43−1.31 (each m, 1H×2, CH_2C); ^{13}C NMR (100 MHz, D_2O) δ 178.9, 175.9, 166.6, 158.1, 142.7, 97.5, 90.3, 83.98, 83.90, 80.64, 80.47, 75.0, 71.5, 70.6, 70.5, 69.1, 68.3, 64.4, 63.90. 63.85, 53.4, 40.3, 25.9, 23.1, 21.0, 19.6; ^{31}P NMR (D_2O) δ 27.56; HR-ESI-MS calcd for $C_{22}H_{37}N_5O_{15}P$ $[M + H]^+$ 626.2069, found 626.2077.

Triethylammonium Methyl 2-(5-Acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (22). A solution of **15** (0.214 g, 0.306 mmol) in 1,4-dioxane (1.6 mL) was treated with PhSH (1.2 mL) and Et_3N (2.5 mL), as mentioned for **18**. Purification by silica gel column chromatography (EtOAc−MeOH−Et_3N 4:1:0.1→3:1:0.1) yielded **22** (0.208 g, 86%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.15 (d, 1H, J = 9.9 Hz), 5.34 (dd, 1H, J = 2.0, 5.0 Hz), 5.27−5.13 (m, 2H), 4.55 (dd, 1H, J = 2.3, 12.2 Hz), 4.13−3.92 (m, 3H), 3.62 (d, 3H, J = 10.6 Hz), 3.52 (d, 1H, J = 9.9 Hz), 3.39 (d, 1H, J = 9.9 Hz), 3.09 (q, 6H, J = 7.3 Hz), 2.12−1.87 (m, 5H), 2.12, 2.10, 2.01, 1.99, 1.87 (each s, 3H×5), 1.64 (t, 1H, J = 12.2 Hz), 1.33 (t, 9H, J = 7.3 Hz), 0.88 (s, 9H), 0.06, 0.05 (each s, 3H×2); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.4, 170.3, 170.2, 169.9, 77.2, 70.9, 70.3, 68.6, 67.6, 62.5, 51.64, 51.56, 49.3, 45.4, 36.3, 25.8, 23.4, 23.0, 21.1, 20.8, 20.7, 20.0, 18.1, 18.0, 8.5, −5.4, −5.5; ^{31}P NMR ($CDCl_3$) δ 28.42; HR-ESI-MS calcd for $C_{28}H_{51}NO_{14}PSi$ $[M - NEt_3 + H]^+$ 684.2816, found 684.2846.

Sodium Cytidin-5′-yl 2-(5-Acetamido-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (6). A solution of **22** (85.9 mg, 0.109 mmol) and **20** (0.121 g, 0.328 mmol) in DMF (0.6 mL) was treated with DIEA (60 μL, 0.433 mmol) and BOP (0.147 g, 0.332 mmol) as mentioned for the synthesis of **21**. Purification by silica gel column chromatography (EtOAc−MeOH 20:1→15:1→10:1→9:1→8:1→7:1) yielded 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl methyl 2-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-*O*-*tert*-butyldimethylsilyl-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (**23**, 0.115 g, HMPA contamination, <89%) as a syrup, which was a diastereomer mixture on P: ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.24 (brs, 1H), 7.70−7.11 (m, 3H), 5.87−5.70 (m, 3H), 5.45 (d, 1H, J = 2.6 Hz), 5.11−5.22 (m, 2H), 4.58−3.92 (m, 7H), 3.75−3.68 (m, 3H), 3.54−3.33 (m, 2H), 2.17−1.60 (m, 30H), 0.886, 0.880 (each s, 9H), 0.08, 0.06 (each s, 6H); ^{31}P NMR ($CDCl_3$) δ 35.45, 35.33; HR-ESI-MS calcd for $C_{43}H_{68}N_4O_{21}PSi$ $[M + H]^+$ 1035.3883, found 1035.3898.

A solution of **23** (23.9 mg, HMPA contamination, <20 μmol) in 1,4-dioxane (0.1 mL) was treated with PhSH (78 μL) and Et_3N (0.16 mL) as mentioned for **18**. Purification by silica gel column chromatography (EtOAc−MeOH 6:1→EtOAc−MeOH−Et_3N 4:1:0.2→3:1:0.2→5:2:0.4) yielded triethylammonium 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl 2-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-*O*-*tert*-butylsimethylsilyl-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (23.8 mg, HMPA contamination, <20 μmol, <99%) as a syrup: ^{31}P NMR ($CDCl_3$) δ 26.28; HR-ESI-MS calcd for $C_{42}H_{66}N_4O_{21}PSi$ $[M + H]^+$ 1021.3727, found 1021.3812.

A solution of the syrup (23.8 mg, HMPA contamination, <20 μmol) in THF−HCO_2H−H_2O (6:3:1 v/v/v, 2.0 mL) was stirred for 5 h at room temperature. MeOH was added to the solution, and the solution was concentrated in vacuo. The residue was purified on a column of spherical silica gel (EtOAc−MeOH−Et_3N 4:1:0.2→3:1:0.1→2:1:0.1) to yield triethylammonium 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl 2-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (21 mg, small HMPA contamination, 99%) as a syrup: ^{31}P NMR ($CDCl_3$) δ 23.85; HR-ESI-MS calcd for $C_{36}H_{52}N_4O_{21}P$ $[M + H]^+$ 907.2862, found 907.2867.

A solution of the syrup (21 mg, <20 μmol) in MeOH (2 mL) and NH_4OH (28%, 2 mL) was treated as mentioned for the synthesis of **4**. Purification by a DEAE-Sephadex A-25 column (0−1 M HCO_2Na linear gradient) and Sephadex G-15 chromatography (water) as mentioned for **4** yielded **6** (8.3 mg, 63%) as a white solid after lyophilization: ^{1}H NMR (400 MHz, D_2O, COSY) δ 7.96 (d, 1H, J = 7.6 Hz, H6), 6.13 (d, 1H, J = 7.6 Hz, H5), 6.03 (d, 1H, J = 3.8 Hz, H1′), 4.37−4.33 (m, 2H, H2′,3′), 4.29 (br, 1H, H4′), 4.14 (m, 1H, H5′a), 4.08 (m, 1H, H5′b), 3.89 (m, 1H, H4″), 3.84−3.79 (m, 2H, H8″,9″a), 3.76 (t, 1H, J = 10.2 Hz, H5″), 3.66 (d, 1H, J = 10.2 Hz, H6″), 3.66 (dd, 1H, J = 6.6, 12.6 Hz, H9″b), 3.60 (d, 1H, J = 12.0 Hz, H1″a), 3.50 (d, 1H, J = 9.0 Hz, H7″), 3.45 (d, 1H, J = 12.0 Hz, H1″b), 2.06 (s, 3H, Ac), 2.05−1.96 (m, 1H, CH_2CH_2), 1.92 (dd, 1H, J = 4.6, 12.5 Hz, H3″eq), 1.72 (t, 1H, J = 12.5 Hz, H3″ax), 1.81−1.53 (m, 3H, CH_2CH_2); ^{13}C NMR (100 MHz, D_2O) δ 175.9, 167.2, 158.8, 142.5, 97.4, 90.4, 83.91, 83.82, 77.63, 77.47, 75.0, 70.9, 70.8, 70.5, 69.6, 68.6, 67.0, 64.5, 63.92, 63.87, 53.7, 37.9, 25.2, 23.1, 20.6, 19.3; ^{31}P NMR (D_2O) δ 28.84; HR-ESI-MS calcd for $C_{22}H_{38}N_4O_{14}P$ $[M + H]^+$ 613.2122, found 613.2121.

Sodium Cytidin-5′-yl 2-{5-Acetamido-2,6-anhydro-1-(sodium ethyl phosphate)-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl}ethylphosphonate (7). A solution of **23** (0.228 g, HMPA contamination, <0.145 mmol) in TBAF in THF (1 M, 1.0 mL, pH adjusted to 4−5 with AcOH) was stirred at room temperature for 22 h. The solution was concentrated, and the residue was purified on a column of silica gel (EtOAc−MeOH 15:1→10:1→9:1) to yield 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl methyl 2-(5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl)ethylphosphonate (**24**, 0.129 g, HMPA contamination, <64%) as a syrup, which was a diastereomer mixture on P: ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.25 (brs, 1H), 7.73−7.20 (m, 3H), 5.86−5.69 (m, 3H), 5.47, 5.36 (each d, 1H, J = 2.6 Hz), 5.21−5.11 (m, 2H), 4.70−4.03 (m, 6H), 3.90−3.68 (m, 6H), 2.31−1.57 (m, 30H); ^{31}P NMR

($CDCl_3$) δ 34.47, 34.08; HR-ESI-MS calcd for $C_{37}H_{54}N_4O_{21}P$ [M + H]$^+$ 921.3018, found 921.3007.

To a solution of **24** (44.2 mg, HMPA contamination, <31.9 μmol) in CH_2Cl_2 (0.5 mL) were added DIEA (18 μL) and diethyl phosphorchloridite (14 μL) under Ar atmosphere. After being stirred for 2 h at room temperature, the solution was concentrated. The residue was dissolved in MeCN (0.5 mL), and to the solution was added tBuOOH (2.5 M decane solution, 80 μL). After being stirred for 30 min at room temperature, the solution was concentrated, and the residue was purified on a column of silica gel (EtOAc−MeOH 10:1) to yield 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl methyl 2-{5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-(diethyl phosphate)-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl}ethylphosphonate (39.5 mg, HMPA contamination) as a syrup: HR-ESI-MS calcd for $C_{41}H_{63}N_4O_{24}P_2$ [M + H]$^+$ 1057.3308, found 1057.3291.

To a solution of the syrup (39.5 mg, HMPA contamination) in MeCN (2 mL) was added LiBr (11 mg). After being stirred under reflux overnight, the solution was concentrated to yield crude lithium 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl 2-{5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-1-(lithium ethyl phosphate)-D-*erythro*-L-*gluco*-nonitol-2-yl}ethylphosphonate as a syrup: *m/z* (ESI) 1015.0 (M − 2Li + 3H$^+$).

To a solution of the crude syrup in MeOH (2 mL) was added 28% NH_4OH (2 mL). After being stirred for 8 h at room temperature, the solution was concentrated. Purification of the residue by DEAE-Sephadex A-25 column (0−1 M HCO_2Na linear gradient) and Sephadex G-15 chromatography (water) as mentioned for **4** yielded **7** (12.1 mg, 50% from **24**) as a white solid: ^{1}H NMR (400 MHz, D_2O, COSY) δ 8.00 (d, 1H, J = 7.6 Hz, H6), 6.16 (d, 1H, J = 7.5 Hz, H5), 6.04 (d, 1H, J = 3.8 Hz, H1′), 4.36−4.32 (m, 2H, H2′,3′), 4.30 (br, 1H, H4′), 4.16 (ddd, 1H, J = 2.4, 3.8, 11.7 Hz, H5′a), 4.10 (ddd, 1H, J = 2.4, 4.8, 11.7 Hz, H5′b), 3.94 (qu, 2H, J = 7.2 Hz, Et), 3.91 (dt, 1H, J = 5.0, 11.4 Hz, H4″), 3.84−3.76 (m, 5H, H1″a,b,5″,8″,9″a), 3.69 (d, 1H, J = 11.0 Hz, H6″), 3.63 (dd, 1H, J = 6.6, 12.6 Hz, H9″b), 3.48 (d, 1H, J = 8.9 Hz, H7″), 2.08−2.03 (m, 2H, H3″eq, CH_2-CH_2), 2.06 (s, 3H, Ac), 1.80−1.62 (m, 3H, CH_2CH_2), 1.67 (t, 1H, J = 12.1 Hz, H3″ax), 1.27 (t, 3H, J = 7.1 Hz, Me); ^{13}C NMR (100 MHz, D_2O) δ 175.8, 167.1, 158.7, 142.3, 97.5, 90.1, 83.98, 83.89, 76.89, 76.80, 76.73, 76.63, 75.1, 71.0, 70.8, 70.5, 70.26, 70.21, 69.6, 68.5, 64.4, 63.68, 63.63, 63.41, 63.36, 53.7, 38.9, 24.9, 23.1, 20.8, 19.4, 16.69, 16.62; ^{31}P NMR (D_2O) δ 28.69, 1.21; HR-ESI-MS calcd for $C_{24}H_{43}N_4O_{17}P_2$ [M − 2Na + 3H]$^+$ 721.2099, found 721.2103.

Ammonium Cytidin-5′-yl 2-[5-Acetamido-2,6-anhydro-3,5-dideoxy-1-{ammonium (methyl β-D-galactopyranosid-6-yl) phosphate}-D-*erythro*-L-*gluco*-nonitol-2-yl]ethylphosphonate (8). Compound **24** and 2-cyanoethyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid-6-yl) *N,N*-diisopropylphosphoramidite (**25**) were dried by concentrating twice from dry toluene then in vacuo prior to use. To a solution of **24** (29.1 mg, HMPA contamination, <26 μmol) and **25** (27.5 mg, 52.8 μmol) in dry MeCN (0.35 mL) was added 1*H*-tetrazole (5.5 mg, 78 μmol) under Ar atmosphere. After stirring for 50 min at room temperature, tBuOOH (2.5 M decane solution, 30 μL, 75 μmol) was added. The solution was stirred for 30 min at room temperature and then concentrated in vacuo to yield 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl methyl 2-[5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-1-{2-cyamoethyl (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid-6-yl) phosphate}-3,5-dideoxy-D-*erythro*-L-*gluco*-nonitol-2-yl]ethylphosphonate as a syrup: *m/z* (ESI) 1356.4 (M + H$^+$).

A solution of the syrup in 1,4-dioxane (0.2 mL) was treated with PhSH (0.1 mL) and Et_3N (0.32 mL) as mentioned for **18**. Purification by silica gel column chromatography (EtOAc−MeOH 2:1→1:1, containing 1% Et_3N) yielded triethylammonium 4-*N*-acetyl-2′,3′-di-*O*-acetylcytidin-5′-yl 2-[5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,6-anhydro-3,5-dideoxy-1-{triethylammonium (methyl 2,3,4-tri-*O*-acetyl-β-D-galactopyranosid-6-yl) phosphate}-D-*erythro*-L-*gluco*-nonitol-2-yl]ethylphosphonate (**26**, 16.9 mg, 43%) as a syrup: ^{1}H NMR (270 MHz, $CDCl_3$) δ 8.40 (brs, 1H), 7.30 (overlap), 6.88 (brs, 1H), 6.15 (brs, 1H), 5.57−4.95 (m, 10H), 4,52−3.50 (m, 12H), 3.50 (s, 3H), 3.05 (q, J = 7.3 Hz), 2.25−1.53 (m, 39H), 1.29 (t, J = 7.3 Hz); ^{31}P NMR ($CDCl_3$) δ 24.33, −0.44; *m/z* (ESI) 1289.3 (M − 2Et$_3$N + H$^+$).

To a solution of **26** (16.9 mg) in MeOH (2 mL) was added NH_4OH (28%, 2 mL), and the mixture stirred overnight at room temperature. The solution was concentrated in vacuo. Purification of the residue by DEAE-Sephadex A-25 column (0−1 M HCO_2NH_4 linear gradient) and Sephadex G-15 chromatography (water) as mentioned for **4** yielded **8** (5.4 mg, 53%) as a white solid: ^{1}H NMR (400 MHz, D_2O, COSY) δ 8.04 (d, 1H, J = 7.6 Hz, H6), 6.20 (brd, 1H, J = 6.4 Hz, H5), 6.03 (d, 1H, J = 3.7 Hz, H1′), 4.36−4.33 (m, 3H, H2′,3′,1‴), 4.30 (br, 1H, H4′), 4.16 (m, 1H, H5′a), 4.09 (m, 1H, H5′b), 4.03 (t, 2H, J = 6.6 Hz, H6‴a,b), 3.99 (d, 1H, J = 3.5 Hz, H4‴), 3.91 (dt, 1H, J = 4.6, 11.0 Hz, H4″), 3.86−3.76 (m, 6H, H1″a,b,5″,8″,9″a,-5‴), 3.69 (d, 1H, J = 9.3 Hz, H6″), 3.67 (dd, 1H, J = 3.4, 10.1 Hz, H3‴), 3.62 (dd, 1H, J = 5.8, 11.8 Hz, H9″b), 3.58 (s, 3H, OMe), 3.52 (dd, 1H, J = 7.9, 9.9 Hz, H2‴), 3.47 (d, 1H, J = 9.6 Hz, H7″), 2.12−2.03 (m, 2H, H3″eq, CH_2CH$_2$), 2.05 (s, 3H, Ac), 1.78−1.74 (m, 3H, CH_2CH_2), 1.66 (t, 1H, J = 12.2 Hz, H3″ax); ^{13}C NMR (100 MHz, D_2O) δ 175.8, 166.0, 157.3, 142.8, 104.8, 97.5, 90.2, 84.07, 84.00, 76.89, 76.80, 76.72, 76.63, 75.1, 74.57, 74.49, 73.6, 71.7, 71.0, 70.8, 70.4, 69.6, 69.2, 68.5, 65.08, 65.03, 64.4, 63.65, 63.61, 58.2, 53.7, 39.0, 24.9, 23.1, 20.7, 19.4; ^{31}P NMR (D_2O) δ 28.75, 1.04; HR-ESI-MS calcd for $C_{29}H_{51}N_4O_{22}P_2$ [M − 2NH$_3$ + H]$^+$ 869.2470, found 869.2461.

α-2,3-ST Assay. The reaction mixture containing 0.11 mM CMP−Neu5Ac, 1.0 mM PA-LacNAc, 1 mg/mL BSA, 0.5% Triton X-100, 350 μU α-2,3-ST, and inhibitor in 50 mM HEPES buffer (pH 7.4) was adjusted to total volume of 20 μL. The reaction solution was incubated at 25 °C for 20 min. An aliquot (10 μL) of the reaction mixture was added to 0.1 M AcOH (70 μL) to stop the reaction, and the amount of the reaction product was analyzed by RP-HPLC (Inertsil-ODS-3 ϕ4.6 mm×100 mm). The column was heated at 40 °C and eluted with 0.1 M NH_4OAc (pH 4.5) containing 0.025% 1-BuOH at the flow rate of 1.5 mL/min. The reaction product was quantitated with fluorescence detector with excitation at 320 nm and emission at 400 nm. Retention time of the reaction product was 11.09 min. In each assay, the amount of the product formed was less than 10% of the amount of added CMP−Neu5Ac.

α-2,6-ST Assay. The reaction mixture containing 0.11 mM CMP−Neu5Ac, 1.0 mM PA-LacNAc, 1 mg/mL BSA, 0.5% Triton X-100, 227 μU α-2,6-ST, and inhibitor in 25 mM sodium cacodylate buffer (pH 6.8) was adjusted to a total volume 20 μL. The reaction solution was incubated at 25 °C for 15 min. The reaction was stopped and the amount of the product was quantitated as mentioned for the α-2,3-ST assay. The retention time of the reaction product was 11.54 min. In each assay, the amount of the product formed was less than 10% of the amount of added CMP−Neu5Ac.

Acknowledgment. This research was partially supported by a Grant-in-Aid for Young Scientists (B) from the Ministry of Education, Culture, Sports, Science and Technology (14780457).

Supporting Information Available: General experimental procedures and ^{1}H and ^{13}C NMR spectra of compounds **4**−**8**, **10**, **12**−**19**, **21**−**24**, and **26**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0512608