Synthesis of the Isosteric Analogs of CMP-NeuNAc : Cytidine-5'-yl Sialylmethylphosphonates

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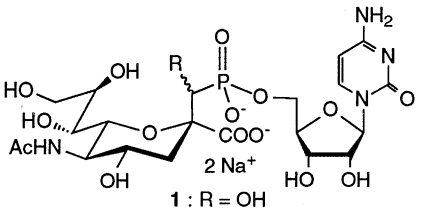
Isosteric CMP-NeuNAc phosphonate analogs (**1** and **2**) were synthesized from *C*-allyl-sialoside. These analogs have a methyl phosphonate structure instead of the phosphate linkage of CMP-NeuNAc.

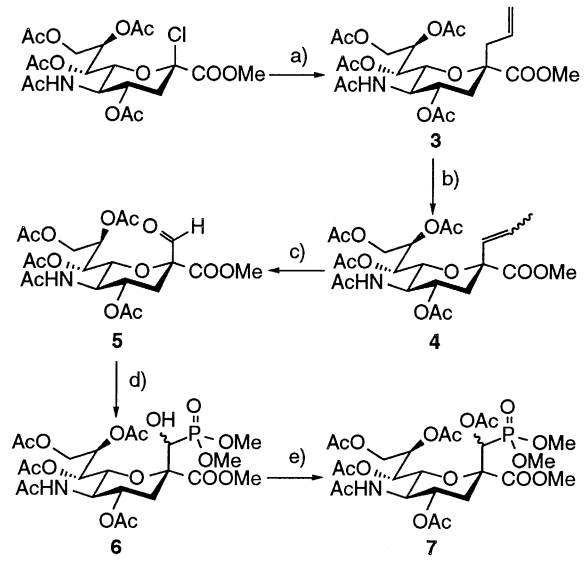
N-Acetylneuraminic acid (NeuNAc) is often situated at the non-reducing end of the glycoconjugates and plays important roles in biological phenomena, such as infection of viruses, recognition events of lectins or enzymes, and cell-cell adhesion.[1-3] Sialyltransferase catalyzes the transfer of sialic acid from cytidine 5'-monophospho-*N*-acetylneuraminic acid (CMP-NeuNAc) to an oligosaccharide.[4] Substrate-analog inhibitors of this enzyme could be potential candidates as a regulator of the biosynthesis of glycoconjugates and also as chemical tools for studies on the substrate recognition of sialyltransferase. As the acceptor-analog inhibitor of α 2→6 sialyltransferase, 6'-substituted *N*-acetyllactosaminides have been reported.[5] The sialic acid-nucleoside conjugates with protecting groups, which lack the phosphate linkage and have unnatural α-linkage, and which inhibit the formation of human hepatic metastases, were thought to be donor-analog inhibitors of a sialyltransferase.[6] The action mechanism of one of the conjugates, however, has recently been revealed to inhibit CMP-NeuNAc transport.[7] Recently, we reported the synthesis of CMP-NeuNAc analogs of sialylphosphonate type,[8] in which the phosphorus atom is directly attached to C2 of NeuNAc. In this paper, we describe the synthesis of novel isosteric phosphonate analogs (**1** and **2**) of CMP-NeuNAc (Figure 1.), where the C2"-O-P bond of CMP-NeuNAc is replaced by the C2"-C-P bond.



1 : R = OH
2 : R = H

Figure 1.

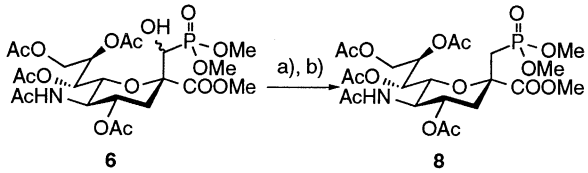
β-*C*-Allyl sialoside (**3**) was synthesized by Paulsen's method[9] from sialyl chloride and rearranged to the *C*-(1-propenyl) sialoside **4** by using bis(benzonitrile)palladium(II) chloride [$(C_6H_5CN)_2PdCl_2$] as a catalyst,[10] which was then converted to the corresponding aldehyde **5** by ozonolysis. Nucleophilic addition of dimethyl phosphite [$HP(O)(OMe)_2$] to this aldehyde with n-BuLi gave the α-hydroxylated sialylmethyl phosphonate **6** as 8 : 1 diastereomeric mixture. Acetylation of the hydroxyl group of the phosphonate **6** gave the completely protected α-hydroxy sialylmethyl phosphonate **7** as 8 : 1 diastereomeric mixture[11] (Scheme 1).



a) : (i) n-Bu_3SnAll, AIBN , THF, 60 °C, (ii) NaOMe , MeOH, (iii) Ac_2O, pyridine 52% (3 steps), b) : $(C_6H_5CN)_2PdCl_2$, benzene, reflux 95%, c) : O_3, MeOH then Me_2S 82%, d) : n-BuLi, $HP(O)(OMe)_2$, THF, -78 °C 67%, e) : Ac_2O, pyridine 88% .

Scheme 1.

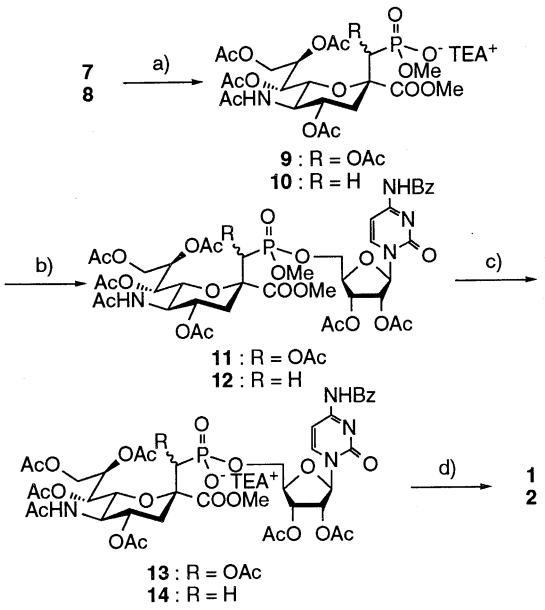
Phenyloxythiocarbonylation of the hydroxyl group of **6** followed by deoxygenation with n-Bu_3SnH gave the sialylmethyl phosphonate **8**[12] (Scheme 2).



a) : PhOC(S)Cl, pyridine, CH_2Cl_2, b) : n-Bu_3SnH, AIBN, toluene, reflux 59% (2 steps).

Scheme 2.

The phosphonates **7** and **8** were subjected to chemoselective demethylation by using thiophenol and triethylamine in dioxane[13] to give monomethyl esters **9** and **10**. The monomethyl esters **9** and **10** were coupled with 2',3'-di-*O*-acetyl-*N*-benzoylcytidine by Mitsunobu reaction[14,15] (PPh_3 and DIAD in THF) to give the protected CMP-NeuNAc analogs **11** and **12**. Further demethylation of these methyl phosphonates under the same condition as described above gave **13** and **14**, respectively. Simultaneous *O*-deacetylation, *N*-debenzoylation, and hydrolysis of the methyl carboxylate of the compounds **13** and **14** with 10:1 NH_4OH (28%) - MeOH afforded the desired CMP-NeuNAc analogs **1**[16] and **2**[17] as NH_4^+ salts (Scheme 3).



a) : PhSH, Et_3N, dioxane 94% (**9**), 95% (**10**), b) : 2',3'-di-*O*-acetyl-*N*-benzoylcytidine, PPh_3, DIAD, THF, c) : PhSH, Et_3N, dioxane 56% (**13**), 56% (**14**) (2 steps), d) : NH_4OH:MeOH = 10:1 54% (**1**), 47% (**2**).

Scheme 3.

The purification of these NH_4^+ salts was carried out on a column of cation-exchange resin (Dowex 50W x8, sodium form), and gel-permeator (Sephadex G15) to give the CMP-NeuNAc analogs **1** (10 : 1 diastereomeric mixture) and **2** . Biological evaluation of these CMP-NeuNAc analogs **1** and **2** are under current investigation.

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11 Selected NMR Data of major stereoisomer of compound **7** ; ^1H NMR (270 MHz, $CDCl_3$) : δ 5.76(d, 1H, $J_{AcOCH,P}$ 12.5 Hz, AcOC*H*P), 5.08-5.12(m, 1H, H-4), 4.76(dd, 1H, $J_{9a,8}$ 2.0, $J_{9a,9a}$ 12.2 Hz, H-9a), 4.36(dd, 1H, $J_{6,5}$ 9.9, $J_{6,7}$ 3.0 Hz, H-6), 4.28(dd, 1H, $J_{9b,8}$ 8.2 Hz, H-9b), 4.04-4.16(m, 1H, H-5), 3.80, 3.78(each d, each 3H, $J_{Me,P}$ 10.6 Hz, 2POMe), 3.78(s, 3H, COOMe), 2.69(dd, 1H, $J_{3eq,3ax}$ 14.2, $J_{3eq,4}$ 4.6 Hz, H-3eq). ^{31}P NMR (109.25 MHz, $CDCl_3$, H_3PO_4 as an external standard) : δ 19.16.
12 Selected NMR Data of compound **8** ; ^1H NMR (400 MHz, $CDCl_3$) : δ 5.24(dt, $J_{4,3ax}$ 11.4, $J_{4,3eq}$ 4.7, $J_{4,5}$ 10.4 Hz, H-4), 4.77(dd, 1H, $J_{9a,8}$ 2.0, $J_{9a,9b}$ 12.1 Hz, H-9a), 4.27(dd, 1H, $J_{9b,8}$ 8.1 Hz, H-9b), 4.21(dd, 1H, $J_{6,5}$ 10.5 $J_{6,7}$ 2.4 Hz, H-6), 3.92(dt, 1H, H-5), 3.81(s, 3H, COOMe), 3.73(d, 6H, $J_{Me,P}$ 11.0 Hz, 2POMe), 2.69, 2.58(each dd, each 1H, J_{gem} 15.6, $J_{H,P}$ 17.9 20.8 Hz, CH_2P), 2.31(dd, 1H, $J_{3eq,3ax}$ 13.0 Hz, H-3eq), 1.96(ddd, 1H, $J_{3ax,P}$ 5.6 Hz, H-3ax). ^{31}P NMR (109.25 MHz, $CDCl_3$) : δ 27.25.
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16 Selected NMR Data of major stereoisomer of compound **1** ; ^1H NMR (400 MHz, D_2O, 25 °C, HDO=4.81 ppm) : δ 8.13(d, 1H, $J_{6,5}$ 7.6 Hz, H-6), 6.18(d, 1H, H-5), 6.02(d, 1H, $J_{1'2'}$ 3.7 Hz, H-1'), 4.13-4.19(m, 1H, H-4"), 3.67(dd, 1H, $J_{9"b,8"}$ 7.2, $J_{9"b,9"a}$ 11.9 Hz, H-9"b), 3.52(dd, 1H, $J_{7",6"}$ 0.8, $J_{7",8"}$ 9.7 Hz, H-7"), 2.93(dd, 1H, $J_{3"eq,3"ax}$ 14.0, $J_{3"eq,4"}$ 4.9 Hz, H-3"eq), 2.11(s, 3H, NAc), 1.71(ddd, 1H, $J_{3"ax,4}$ 11.0, $J_{3"ax,P}$ 3.4 Hz, H-3"ax). ^{31}P NMR (109.25 MHz, D_2O, H_3PO_4 as an external standard) : δ 16.25.
17 Selected NMR Data of compound **2** ; ^1H NMR (400 MHz, D_2O, 25 °C) : δ 8.03(d, 1H, $J_{6,5}$ 7.6 Hz, H-6), 6.18(d, 1H, H-5), 6.02(d, 1H, $J_{1'2'}$ 3.8 Hz, H-1'), 4.29-4.30(m, 1H, H-4'), 4.21(ddd, 1H, $J_{5'a,4'}$ 2.6, $J_{5'a,5'b}$ 12.1, $J_{5'a,P}$ 5.3 Hz, H-5'a), 3.68(dd, 1H, $J_{9"b,8}$ 6.6, $J_{9"b,9"a}$ 11.6 Hz, H-9"b), 3,53(dd, 1H, $J_{7",6"}$ 0.8, $J_{7",8"}$ 10.2 Hz, H-7"), 2.60(t, 1H, J_{gem} $J_{CH2,P}$ 15.9 Hz, CH_2P), 2.48(dd, 1H, $J_{CH2,P}$ 19.2 Hz, CH_2P), 2.27(dd, 1H, $J_{3"eq,3"ax}$ 13.1, $J_{3"eq,4}$ 4.6 Hz, H-3"eq), 2.12(s, 3H, NAc), 1.73(ddd, 1H, $J_{3"ax,4}$ 11.8, $J_{3"ax,P}$ 7.0 Hz, H-3"ax), ^{31}P NMR (109.25 MHz, D_2O) : δ 19.65