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Synthesis of Novel CMP-NeuNAc Analogues Having a Glycosyl Phosphonate Structure

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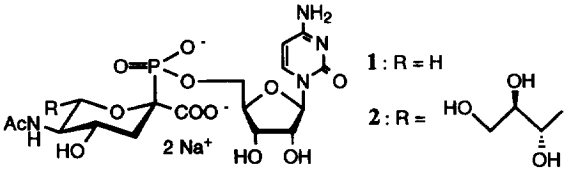
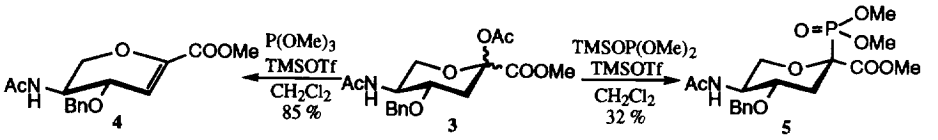
Abstract: Sialyl phosphonate was synthesized by nucleophilic substitution of sialyl phosphite with dimethyl trimethylsilyl phosphite using TMSOTf as a catalyst and converted to CMP-NeuNAc analogue **2** using Mitsunobu condensation.

Sialic acid plays important roles in biological phenomena, such as molecular recognition events and 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 analogue inhibitors of this enzyme could be potential compounds for the elucidation of the substrate recognition of sialyltransferase. While several 6'-substituted *N*-acetyllactosaminides were proved to be the acceptor-analogue inhibitors of α 2→6 sialyltransferase,[5] only protected sialic acid-nucleoside conjugates, the donor-analogues without a phosphate linkage, were reported to inhibit sialyltransferase in the cell homogenate of lymphocyte.[6] Recently, one of these analogues was elucidated to be ineffective for inhibition of sialyltransferase using the homogenate of human colonic tumor cell or the human liver.[7] In this paper, we describe a new method for the formation of a carbon-phosphorus bond at the anomeric tertiary carbon of sialic acid and the synthesis of new sugar-nucleotide analogues of a glycosyl phosphonate type (**1** and **2**).



Carbon-phosphorus bond formation at the anomeric carbon of aldopyranoses and also furanoses using trimethyl phosphite [$P(OMe)_3$] and trimethylsilyl triflate (TMSOTf) has been reported by Vasella *et al.*[8] We applied the same method to hexulosonyl acetate **3**[9] as a model compound of sialic acid. However, only the β-elimination product **4** was obtained as shown in Scheme 1 even with a reduced molar equivalent of TMSOTf. Moreover, the Arbzov type reaction between $P(OMe)_3$ and TMSOTf proceeded to give dimethyl methylphosphonate, which was confirmed by ^{31}P NMR.

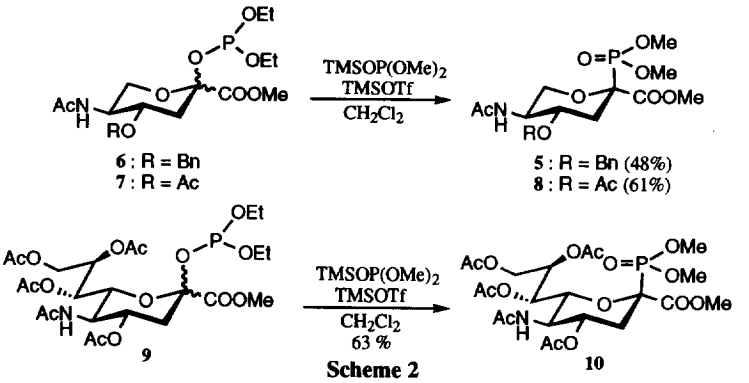
The desired ulosonyl phosphonate **5**[10] was first obtained in 32% yield (Scheme 1) using dimethyltrimethylsilyl phosphite instead of $P(OMe)_3$, thus avoiding the formation of dimethyl methylphosphonate.



Scheme 1

The ulosonyl phosphites **6**[9] and **7**[9] were found to be more active as glycosyl donors than **3** in the presence of TMSOTf and could be converted to the corresponding phosphonates **5** and **8** in better yields (48% and 61%) as shown in Scheme 2.

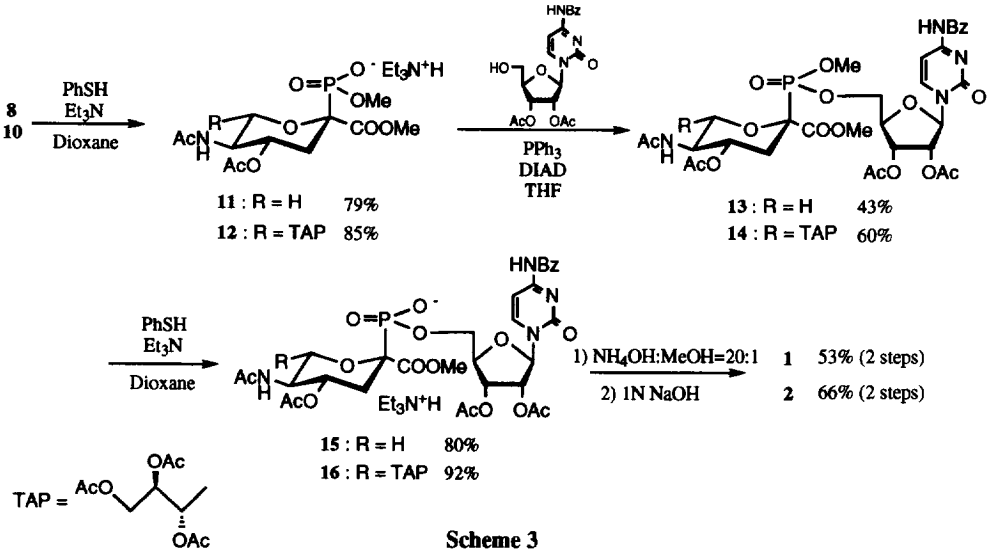
This glycosyl phosphite method was proved to be also effective for the conversion of sialyl phosphite **9**[11] to sialyl phosphonate **10**[12] (Scheme 2).



Scheme 2

The dimethyl phosphonates **8** and **10** were half-deesterified with thiophenol and triethylamine in dioxane[13] to give monomethyl esters **11** and **12**. Mitsunobu condensation[14] (PPh_3 and DIAD in THF) of **11** and **12** with 2', 3'-di-*O*-acetyl-*N*-benzoylcytidine gave the protected CMP-NeuNAc analogues **13** and **14**. Further deesterification of these methyl phosphonates with the same reagents as described as above gave **15** and **16**, respectively. Successive *O*-deacetylation and *N*-debenzoylation with 20:1 NH_4OH (28%) - MeOH, and hydrolysis of methyl carboxylate using 1M NaOH, afforded the desired CMP-NeuNAc analogues **1**[15] and **2**[16] (Scheme 3).

Subsequent purification of **1** and **2** was carried out on a column of anion-exchange resin (formate form), a gel-permeator (Biogel P-2) and cation-exchange resin (sodium form). Futher studies to assay the inhibition of **1** and **2** against sialyltransferase are now in progress.



Scheme 3

Thus, the carbon-phosphorus bond formation at the anomeric carbon of sialic acid was made possible by nucleophilic substitution of glycosyl phosphite with dimethyl trimethylsilyl phosphite using trimethylsilyl triflate as a catalyst and the obtained glycosyl phosphonates were converted to novel CMP-NeuNAc analogues.

Acknowledgment

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9. Compounds **3**, **6** and **7** were synthesized from GlcNAc (11 steps, 13 steps and 14 steps).

NMR Data of Compound **3**; ^{1}H-NMR (270 MHz, $CDCl_3$) : δ 4.49(dd, 1H, $J_{6eqβ,5β}$ 2.2, $J_{6eqβ,6axβ}$ 12.0 Hz, H-6eqβ), 4.33(dd, 1H, $J_{6eqα,5α}$ 5.0, $J_{6eqα,6axα}$ 11.2 Hz, H-6eqα), 3.80(s, 3H, COOCH_3α), 3.79(s, 3H, COOCH_3β), 3.71(dd, 1H, $J_{6axβ,5β}$ 1.0 Hz, H-6axβ), 3.53(dd, 1H, $J_{6axα,5α}$ 9.6 Hz, H-6axα), 2.64(dd, 1H, $J_{3eqα,4α}$ 4.6, $J_{3eqα,3axα}$ 13.9 Hz, H-3eqα), 2.44(dd, 1H, $J_{3eqβ,4β}$ 2.3, $J_{3eqβ,3axβ}$ 15.5 Hz, H-3eqβ), 2.18(dd, 1H, $J_{3axβ,4β}$ 4.0 Hz, H-3axβ), 1.96(dd, 1H, $J_{3axα,4α}$ 9.6 Hz, H-3axα).
NMR Data of Compound **6**; ^{31}P-NMR (109.25 MHz, $CDCl_3$, H_3PO_4 as an external standard) δ 139.87, 138.24(α and β phosphites).
NMR Data of Compound **7**; ^{31}P-NMR (109.25 MHz, $CDCl_3$) δ 139.45(α and β phosphites).

10. NMR Data of Compound **5**; ^{1}H-NMR (270 MHz, $CDCl_3$) : δ 4.25(dd, 1H, $J_{6eq,5}$ 4.6, $J_{6eq,6ax}$ 12.9 Hz, H-6eq), 3.87, 3.86(each d, each 3H, $J_{P,Me}$ 10.2 Hz, 2P-O-Me), 3.85(s, 3H, COOMe), 3.84–3.89(m, 1H, H-5), 3.21(t, 1H, $J_{6ax,5}$ 10.6 Hz, H-6ax), 3.17-3.31(m, 1H, H-4), 2.98(ddd, $J_{3eq,3ax}$ 12.9, $J_{3eq,4}$ 4.3 Hz, $J_{3eq,P}$ 2.0 Hz, H-3eq), 2.01(dt, $J_{3ax,4}$ 12.8, $J_{3ax,P}$ 11.2 Hz, H-3ax), 1.87(s, 3H, N-Ac). ^{31}P-NMR (109.25 MHz, $CDCl_3$, H_3PO_4) δ 17.49.
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12. NMR Data of Compound **10**; ^{1}H-NMR (270 MHz, $CDCl_3$) : δ 5.38-5.32(m, 3H, H-7, H-8, NH), 4,86(m, 1H, H-4), 4.42(dd, 1H, $J_{9a,8}$ 2.3, $J_{9a,9b}$ 13.2 Hz, H-9a), 4.17-4.01(m, 3H, H-5, H-6, H-9b), 3.90, 3.84(each d, each 3H, $J_{P,Me}$ 10.7 Hz, 2P-O-Me), 3.86(s, 3H, COOMe), 2.75(ddd, $J_{3eq,3ax}$ 13.0, $J_{3eq,4}$ 3.6, $J_{3eq,P}$ 1.0 Hz, H-3eq), 2.25(dt, $J_{3ax,4}$ 11.7, $J_{3ax,P}$ 11.7 Hz, H-3ax), 2.15, 2.13, 2.04, 2.03, 1.89(each s, each 3H, 5Ac). ^{31}P-NMR (109.25 MHz, $CDCl_3$) δ 15.95.
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15. NMR Data of Compound **1**; ^{1}H-NMR (400 MHz, D_2O, 25 °C, HDO=4.81 ppm) : δ 8.06(d, 1H, $J_{6,5}$ 7.6 Hz, H-6), 6.19(d, 1H, H-5), 6.05(d, 1H, $J_{1',2'}$ 4.4 Hz, H-1'), 4.40(t, 1H, $J_{3',2'}$ 4.4, $J_{3',4'}$ 4.4 Hz, H-3'), 4.37(t, 1H, H-2'), 4.33(ddd, 1H, $J_{5'a,4'}$ 4.7, $J_{5'a,5'b}$ 11.8, $J_{5'a,P}$ 2.3 Hz, H-5'a), 4.28(dt, 1H, $J_{4',5'b}$ 2.4 Hz, H-4'), 4.23(ddd, 1H, $J_{5'b,P}$ 5.8 Hz, H-5'b), 3.85(dd, 1H, $J_{6"eq,5"}$ 5.2, $J_{6"eq,6"ax}$ 11.4 Hz, H-6"eq), 3.78(dt, 1H, $J_{5",4"}$ 10.4, $J_{5",6"ax}$ 10.4 Hz, H-5"), 3.62(dt, 1H, $J_{4",3"ax}$ 10.5, $J_{4",3"eq}$ 4.3 Hz, H-4"), 3.39(t, 1H, H-6"ax), 2.85(dd, 1H, $J_{3"eq,3"ax}$ 10.5 Hz, H-3"eq), 2.05(s, 3H, N-Ac), 1.91(dt, 1H, $J_{3ax,P}$ 10.4 Hz, H-3"ax). ^{31}P-NMR (109.25 MHz, D_2O) δ 15.95.
16. NMR Data of Compound **2**; ^{1}H-NMR (400 MHz, D_2O, 25 °C, HDO=4.81 ppm) : δ 8.07(d, 1H, $J_{6,5}$ 7.6 Hz, H-6), 6.20(d, 1H, H-5), 6.01(d, 1H, $J_{1',2'}$ 4.4Hz, H-1'), 4.38-4.19(m, 5H, H-2', H-3', H-4', H-5'a, H-5'b), 3.86-3.80(m, 2H, H-5", H-8"), 3.71-3.58(m, 5H, H-4", H-6", H-7", H-9"a, H-9"b), 2.84(dd, 1H, $J_{3"eq,3"ax}$ 12.8, $J_{3"eq,4"}$ 4.7 Hz, H-3"eq), 2.06(s, 3H, N-Ac), 1.95(dt, 1H, $J_{3"ax,4"}$ 11.8 Hz, $J_{3"ax,P}$ 11.8 Hz, H-3"ax) . ^{31}P-NMR (109.25 MHz, D_2O) δ 16.00

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