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Chemical Synthesis of Cytidine-5'-monophosphono-*N*-acetylneuraminic Acid (CMP-Neu5Ac)

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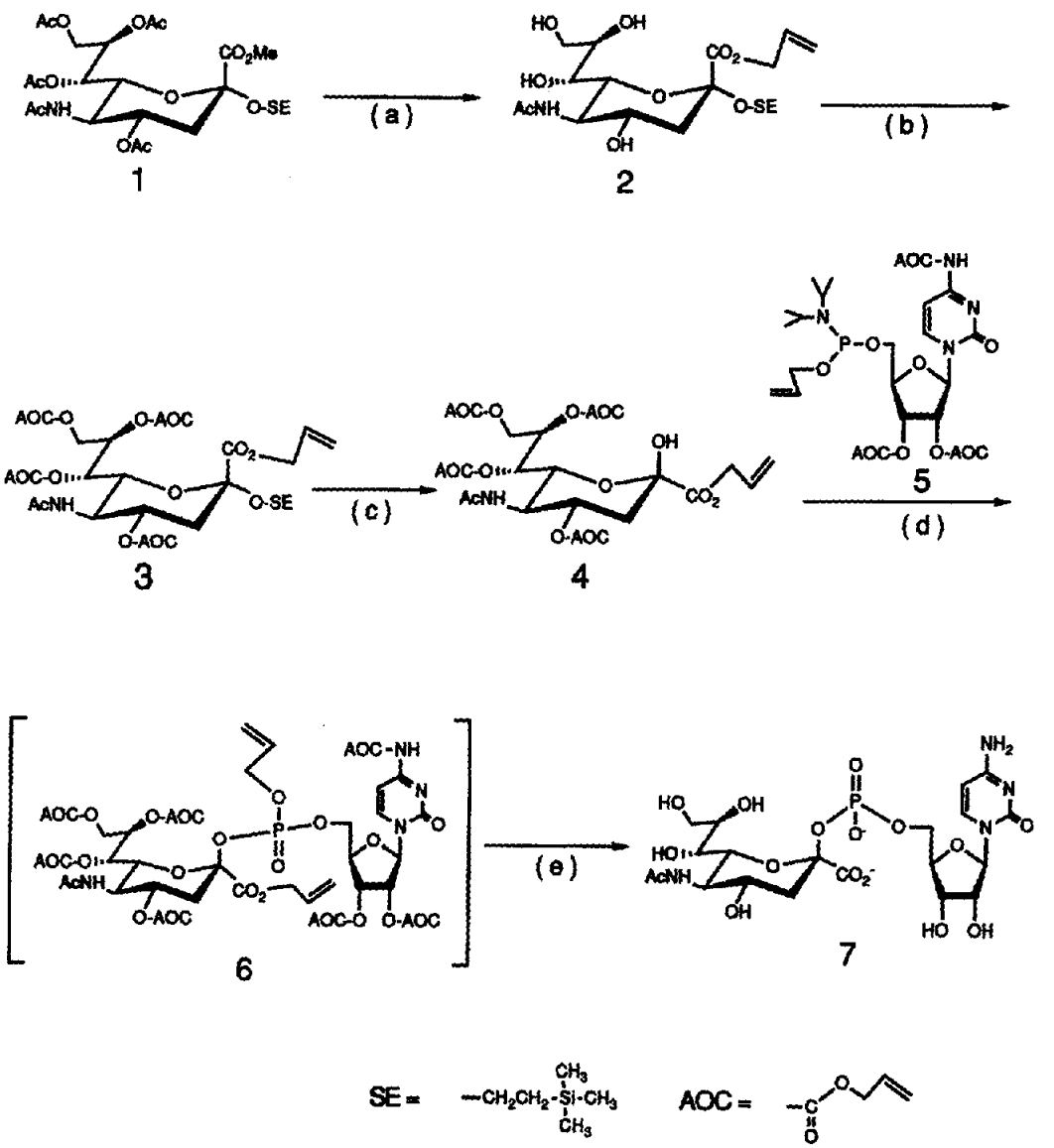
Abstract: CMP-Neu5Ac was synthesized for the first time from the cytidine 5'-phosphoramidite and the Neu5Ac derivative bearing allyl and allyloxycarbonyl groups as the protecting groups.

CMP-Neu5Ac **6** is known to be a substrate of sialyltransferase, which transfers *N*-acetylneuraminic acid (Neu5Ac)[1] to oligosaccharides, glycolipids and glycoproteins.[2] CMP-Neu5Ac has been enzymatically synthesized using CMP-Neu5Ac synthetase.[3] But, this method can hardly be applied for CMP-Neu5Ac derivatives,[4] because CMP-Neu5Ac synthetase strictly recognizes the substrates. Therefore, the chemical synthesis is the most promising method for the preparations of CMP-Neu5Ac derivatives. In the chemical synthesis, there are two problems. One problem is the instability of CMP-Neu5Ac under both basic and acidic conditions. Another is the low reactivity of the hindered anomeric hydroxyl group of Neu5Ac for the phosphorylation. Very recently, Wong *et al.*[5] have synthesized a fully protected CMP-Neu5Ac derivative via the phosphoramidite method. However, they did not perform deprotection. In this paper, we wish to report the synthesis of CMP-Neu5Ac by the phosphoramidite method employing allyl and allyloxycarbonyl (AOC)[6] groups as the protecting groups which can be removed smoothly by treatment with palladium(0) catalyst under neutral conditions.[7]

Both the methoxy and acetyl groups could be removed from methyl [2-(trimethylsilyl)ethyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-nonulopyranosid]onate[8] **1** under alkaline conditions, and subsequently, the carboxyl group was protected as the allyl ester to give **2** in 90% yield according to the literature procedure.[9] AOC groups at 4, 7, 8 and 9 positions could be successfully introduced to **2** with 50 equiv each of AOC-tetrazolide and 2,6-lutidine in a very small amount of DMF at room temperature for 24 h to give **3** in 94% yield.[10] Removal of the trimethylsilylethyl (SE) group from **3** was carried out by treatment with trifluoroacetic acid[11] at room temperature for 30 min to give **4** in 97% yield. **4** was treated with 6 equiv of an appropriately protected cytidine 5'-phosphoramidite **7**[12] in the presence of 15 equiv of tetrazole at 0 °C for 10 min. Successive addition of triethylamine could stabilize the phosphite intermediate.[13] Oxidation of the phosphite was performed by *tert*-butyl hydroperoxide.[14]



Scheme 1. (a) (i) $NaOCH_3$ / CH_3OH; (ii) NaOH / H_2O; (iii) Dowex 50Wx8 (H^+ form); (iv) Cs_2CO_3; (v) allyl bromide (8 equiv) in DMF. (b) AOC-tetrazolide (50 equiv) / 2,6-lutidine (50 equiv) in DMF. (c) trifluoroacetic acid. (d) (i) compound **7** (6 equiv), tetrazole (15 equiv) in CH_3CN (8ml) at 0°C; (ii) Et_3N (30 equiv), 80% tBuOOH (20 equiv), at 0 °C to room temperature. (e) (i) $HCOO^-$ $nBuNH_3^+$ (60 equiv); (ii) triphenylphosphine (1.5 equiv) / tetrakis(triphenylphosphine) palladium(0) (3 equiv) in THF (3ml).

The structure of **5** was supported by ^{31}P NMR.[15] Isolation of **5** was tried by silica gel column chromatography, but could not be achieved due to the instability of **5** bearing the protecting groups. Therefore, **5** was immediately deprotected by addition of a solution of triphenylphosphine and tetrakis(triphenylphosphine)palladium(0) in tetrahydrofuran. Purification of the reaction mixture by Sephadex G-15 column chromatography[16] gave **6** in 25% yield (14mg) based on **4**. Structure of **6** was supported by its mobility on TLC, and confirmed by ^{1}H and ^{31}P NMR spectra.[17] The ^{1}H NMR spectrum (Figure 1) was identical with that of the enzymatically synthesized authentic sample.

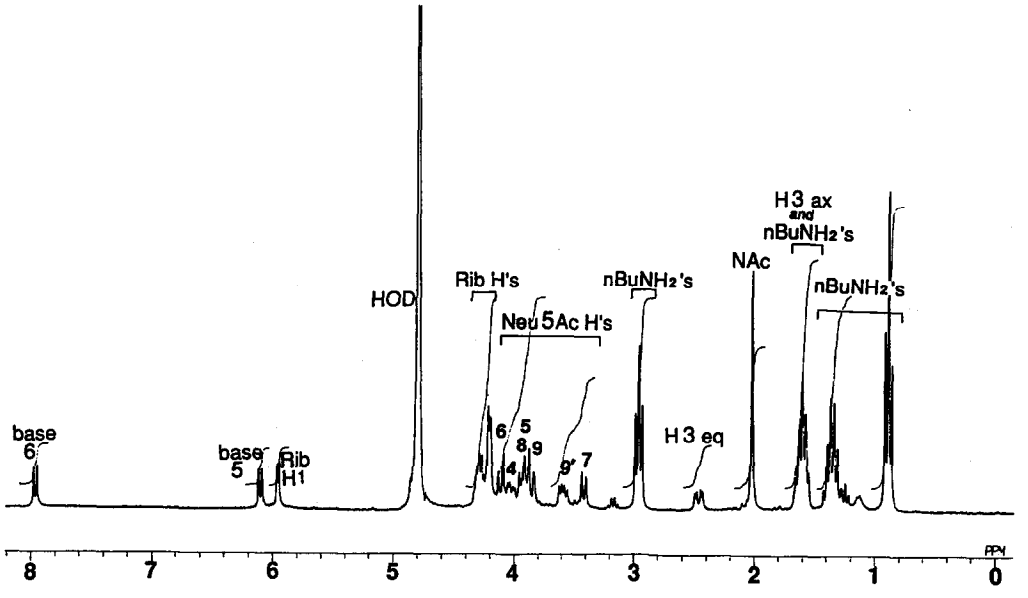


Figure 1 ^{1}H NMR spectrum of CMP-Neu5Ac ($nBuNH_3^+$ salt)

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10) ^{1}H NMR (d, ppm, $CDCl_3$) 1.92 (1H, s, NAc), 2.28-2.36 (2H, m, H-3ax and H-3eq), 4.22 (1 H, d, J=10.6 Hz, H-5), 4.28 (1H, d, J=9.2 Hz, H-9'), 4.38 (1H, d, J=10.5 Hz, H-6), 4.53-4.73 (11H, m, H-9 and allyl), 5.10-5.43 (13H, m, H-8, H-4, H-7 and allyl), 5.83-6.02 (5H, m, allyl), 6.14 (1H, d, J=9.6 Hz, NH).
^{13}C NMR (d, ppm, $CDCl_3$) 23.3 (NAc), 36.0 (C-3), 49.5 (C-5), 65.7 (C-9), 67.0, 68.7, 68.7, 69.1, 69.4 (allyl), 70.5 (C-6), 72.0 (C-7), 73.3 (C-4), 74.8 (C-8), 95.0 (C-2), 118.9, 118.9, 119.2, 119.3, 119.3 (allyl), 130.9, 131.2, 131.3, 131.5, 131.6 (allyl), 154.2, 154.4, 154.6, 154.9 (carbonate), 168.0 (NCO), 171.0 (C-1).
The chemical shifts were assigned by H-H COSY and H-C COSY.

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13) ^{31}P NMR before oxidation (d, ppm, CH_3CN-$CDCl_3$, 1:1, v/v) 136.5, 135.1.

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17) ^{31}P NMR (d, ppm, D_2O) -4.0 R_F=0.2. The sample was loaded on HPTLC-Fertigplatten Cellulose F_{254S}(Merck) and developed with ethanol-1 M ammonium acetate (7:3, v/v). Then, cytidine component was detected by UV light and N-acetylneuraminic acid component was detected by the periodate resorcinol method.[18]

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