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Preliminary communication

Synthesis of a novel CMP-Neu5Ac analogue: CMP-[α-Neu5Ac-(2→8)-Neu5Ac]

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Sialyltransferase catalyzes the transfer of sialic acid from cytidine 5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) to an oligosaccharide [1]. The structures of these sialylated species range from the polymeric form such as colominic acid [2,3] to dimeric or monomeric forms in the various glycolipids and glycoproteins that play crucial roles in molecular recognition events and cell adhesion [4–6]. To investigate the role of sialosides, CMP-Neu5Ac analogues are potential substrates for the substitution of cell-surface oligosaccharides by sialyltransferase [7,8]. Since CMP-Neu5Ac synthetase had been obtained, enzymatic syntheses of CMP-Neu5Ac and its several analogues have been developed [7–10]. A CMP-Neu5Ac analogue having fluorescent probes at the 9-position of Neu5Ac was shown to be a good sialyl donor for sialyltransferase [7]. This fact suggests that a large substitutent attached at the 9-position of Neu5Ac may not interfere with Neu5Ac analogue transfer, and CMP-[α-Neu5Ac-(2→8)-Neu5Ac] is expected to be a novel disialyl donor for the enzymatic synthesis of sialosides containing the α-Neu5Ac-(2→8)-Neu5Ac (Neu5Ac dimer) unit. However, for synthesis of the CMP-Neu5Ac analogue, the corresponding synthetase is not generally used because the enzyme has a rather strict substrate specificity [10]. On the other hand, recently, two chemical syntheses of CMP-Neu5Ac have been reported [11,12]. However, the applicability of these methods to the synthesis of analogues is not well studied. In order to advance the enzymatic synthesis of sialosides,

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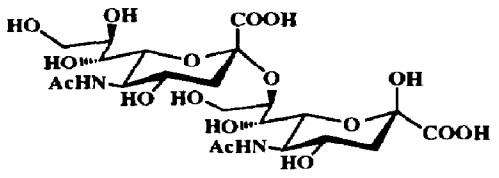
a convenient synthetic method that will be applicable to any analogue will be required. We report herein a highly efficient chemical synthesis of the CMP-Neu5Ac analogue.



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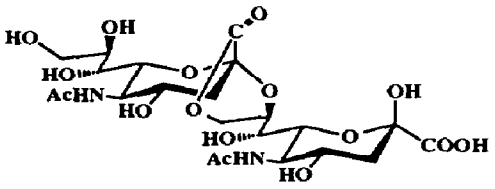

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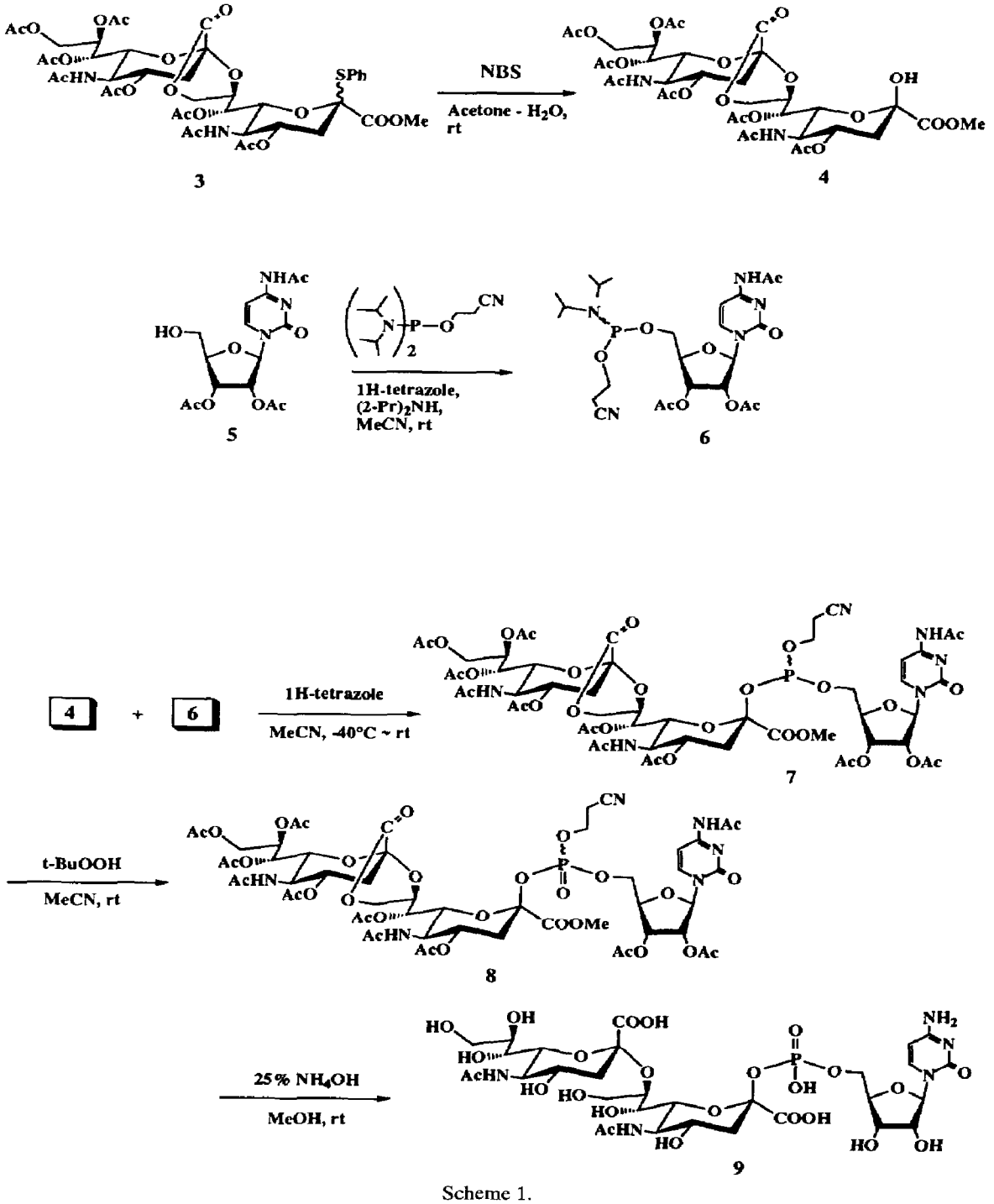
The synthesis of the CMP-(Neu5Ac dimer) was examined by enzymatic [13] as well as by two chemical methods. Because of the negative charge of the carboxyl group at the nonreducing end of the Neu5Ac dimer, the compound was thought to disturb binding of the Neu5Ac dimer to CMP-Neu5Ac synthetase. Therefore, the 2′,8-lactone **2** of the Neu5Ac dimer **1** was used as substrate in addition to commercially available Neu5Ac dimer **1** (Nacalai Tesque, Inc.). The esterified Neu5Ac dimer **2** was prepared by treatment of **1** with Dowex-50W×8 in Me_2NCHO at room temperature. However, these substrates have no activities toward CMP-Neu5Ac synthetase [(EC 2.7.7.43), Genzyme, assay conditions: Neu5Ac dimer **1** or **2** (10 mM), CTP (100 mM), synthetase (100 mU), Tris-HCl buffer, $MgCl_2$, BSA, 37°C, 48 h] in the pH range 7.0 to 8.0. In the first chemical method using the 2-diethylphosphite derivative of Neu5Ac [11], the corresponding diethylphosphite of **4** could not be coupled with tri-*O*-acetyl-CMP. Thus, the second chemical method which uses the allyloxycarbonyl protecting group [12] was examined. However, allyloxycarbonylation did not proceeded to completion in the case of the Neu5Ac dimer. In order to develop a suitable method for the chemical synthesis of the CMP-Neu5Ac dimer, we have investigated both the stability of CMP-Neu5Ac under several different conditions and the coupling of Neu5Ac dimer with cytidine-5′-*O*-phospho derivatives by several different methods. It is noteworthy that CMP-Neu5Ac is very stable under strong basic conditions such as NH_4OH (25%) or Na_2CO_3 solution (pH > 11). Therefore, we planned our synthetic route using the acetyl group for a protecting group, as shown in Scheme 1.

Treatment of lactonated Neu5Ac dimer thioglycoside **3** [14] with *N*-bromosuccinimide afforded **4** (only β configuration) in 65% yield. Introduction of the amidite group to the 5′-position of cytidine derivative **5** [15] with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropyldiamidite in the presence of diisopropylamine and 1*H*-tetrazole afforded the 5′-amidite **6** as a diastereomeric mixture (1:1) in 65% yield. ^{1}H NMR ($CDCl_3$): δ 9.40 (br s, 1 H, NH-4), 8.26, 8.24 (each d, each 0.5 H, *J* 7.6 Hz, H-6), 7.44 (d, 1 H, H-5), 6.37, 6.30 (each d, each 0.5 H, *J* 4.9 Hz, H-1′); ^{31}P NMR ($CDCl_3$, H_3PO_4 = 0.00): δ 150.51, 149.67. Coupling of **4** with **6** in the presence of 1*H*-tetrazole led quantitatively to phosphite derivative **7**. Purification of the crude phosphite on a column of silica gel afforded pure **7** in 47% yield (1:1 diastereomeric mixture). Although the phosphite derivative **7** was stable even in the crude state in several organic solvents at least for one week, it readily decomposed on silica gel. Purification by chromatography on Sephadex LH-20 (MeOH) gave pure phosphite **7** in 85% yield. ^{1}H NMR ($CDCl_3$): δ 9.38, 9.14 (each br s, each 0.5 H, each NH-4), 7.84 (d, 0.5 H, *J* 7.5 Hz, H-6), 7.57 (d, 0.5 H, *J* 7.4 Hz, H-6), 7.46, 7.42 (each d, each 0.5 H, each H-5), 3.89 (s, 3 H, Me), 2.60–2.25 (m, 2 H, H-3″*eq*,3‴*eq*), 1.83 (t, 0.5 H, *J* 12.0 Hz, H-3″*ax*), 1.51 (t, 0.5 H, *J* 11.5 Hz, H-3″*ax*); ^{31}P NMR: δ 135.25, 133.47. Oxidation of the



Scheme 1.

phosphite **7** to the phosphate derivative **8** (1.6:1 diastereomeric mixture) was performed with *tert*-butylhydroperoxide in 73% yield [Sephadex LH-20 (CH_2Cl_2)]. ^1H NMR ($CDCl_3$): δ 9.40 (br s, 0.6 H, NH-4), 8.99 (br s, 0.4 H, NH-4), 7.56 (d, 0.4 H, J 7.6 Hz,

H-6), 7.49–7.43 (m, 1.6 H, H-6, -5×2), 2.92 (dd, 0.4 H, *J* 4.8, 13.8 Hz, H-3‴*eq*), 2.60 (dd, 0.6 H, *J* 5.1, 13.1 Hz, H-3‴*eq*), 2.39 (dd, 0.4 H, *J* 5.8, 13.7 Hz, H-3″*eq*), 1.66 (t, 0.4 H, *J* 10.3 Hz, H-3″*ax*), 1.41 (t, 0.6 H, *J* 9.5 Hz, H-3″*ax*); ^{31}P NMR: δ −6.68, −8.31. O- and N-deacetylation with 20:1 NH_4OH (25%)–MeOH, and subsequent purification using an anion-exchange column (formate form) and a gel-permeation column (Sephadex G-15, water, 4°C) afforded the desired CMP-(Neu5Ac dimer) **9** in 67% yield (>90% purity). ^{1}H NMR (D_2O, HOD = 4.81): δ 7.99 (d, 1 H, *J* 7.6 Hz, H-6), 6.13 (d, 1 H, H-5), 6.02 (bs, 1 H, H-1′), 2.87 (dd, 1 H, *J* 4.5, 13.2 Hz, H-3‴*eq*), 2.58 (dd, 1 H, *J* 4.5, 13.1 Hz, H-3″*eq*), 2.11, 2.07 (each s, each 3 H, Ac), 1.87 (t, 1 H, *J* 13.2 Hz, H-3‴*ax*), 1.77 (ddd, 1 H, *J* 6.6, 13.1 Hz, H-3″*ax*); ^{31}P NMR (D_2O, H_3PO_4 = 0.00): δ −5.65. ^{1}H NMR spectral data, especially the $J_{3''ax,P}$ value of 6.6 Hz and chemical shifts of both H-3″*ax* and H-3″*eq* suggested that the reducing-end residue of the Neu5Ac dimer had the β configuration [16].

In summary, we have developed a highly efficient chemical synthesis of the CMP-(Neu5Ac dimer). This methodology also proved to be applicable to the synthesis of CMP-Neu5Ac itself. The synthesis of CMP-Neu5Ac was performed in 56% overall yield from methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-β-D-*glycero*-D-*galacto*-2-nonulopyranosonate. The other advantage of this methodology is the easy purification of the synthetic intermediates. The demonstrated convenient isolation of the intermediates augur well for a successful scale-up to any desired large reaction size. The synthesis of other CMP-Neu5Ac analogues and the transfer assay of Neu5Ac dimer with sialyltransferase are in progress.

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