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Convenient Chemical Synthesis of CMP-N-Acetylneuraminate (CMP-Neu-5-Ac)[1]

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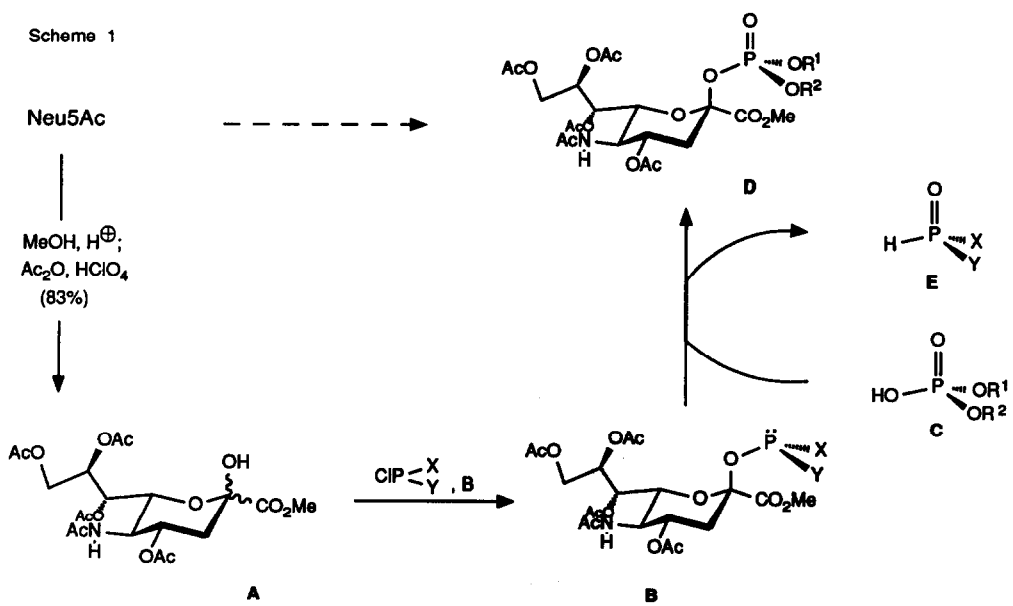
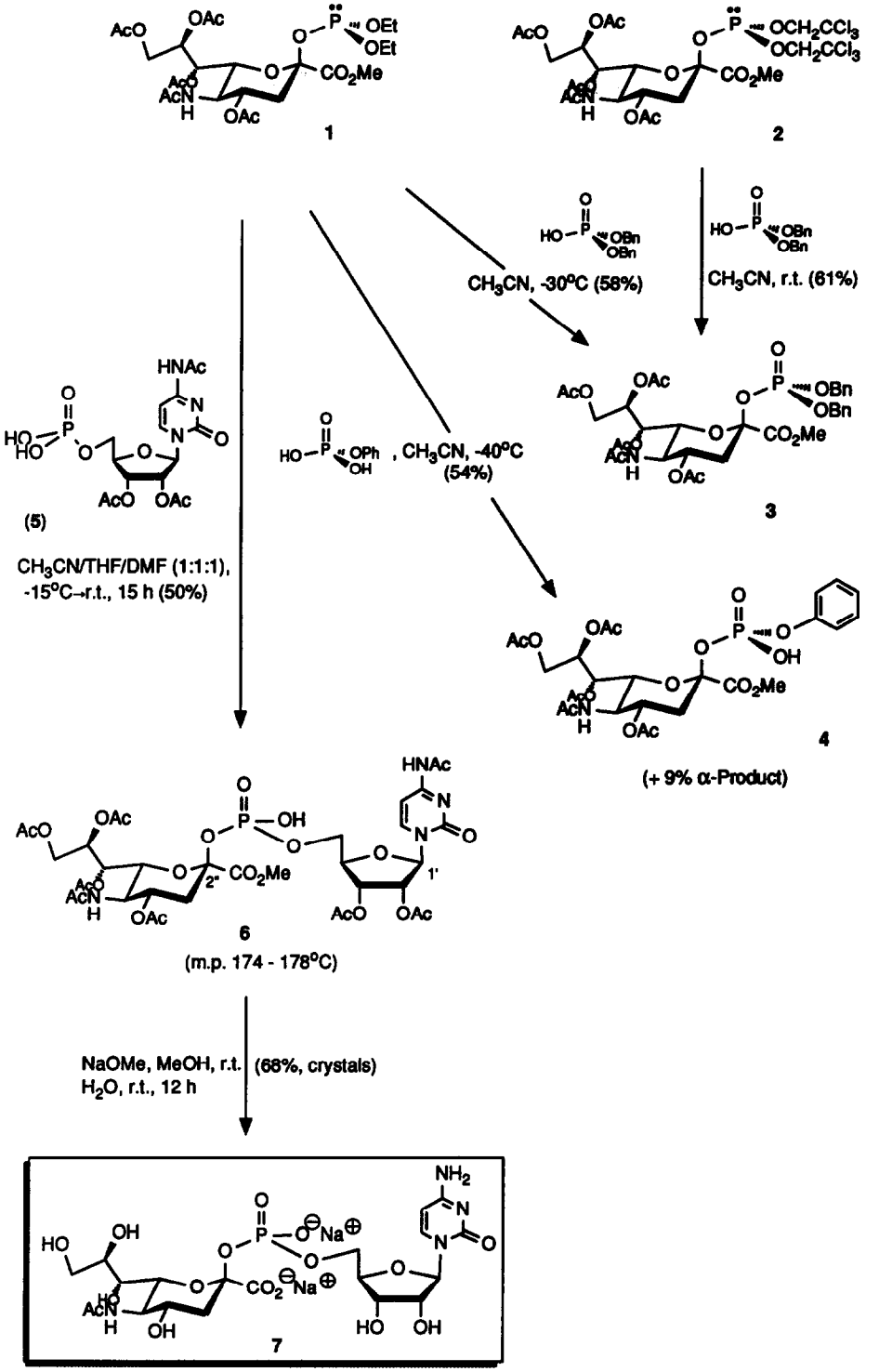
Abstract: Reaction of sialyl phosphites **1** and **2** with dibenzyl phosphate and phenyl phosphate furnished without addition of a catalyst directly the corresponding sialyl phosphates **3** and **4**, respectively, in good yields. Similarly, reaction of **1** with acetylated CMP **5** afforded exclusively β-configurated acetylated CMP-Neu-5-Ac **6**; which was readily transformed into natural sialyl donor CMP-Neu-5-Ac (**7**).

An important direct nucleophilic substitution reaction carried out in nature is O- and N-glycoside bond formation at the anomeric carbon atom of sugars[2,3]. At this activated position as leaving groups phosphates, pyrophosphates, and their nucleoside and lipid monoester derivatives are utilized[3]. For instance, for aldoses generally nucleoside diphosphate and for 3-deoxy-2-glyculosonates (KDO, Neu-5-Ac) nucleoside monophosphate derivatives, respectively, are encountered as glycosyl donors in glycosyltransferase reactions (Leloir pathway). The importance of the synthesis of nucleoside phosphate sugars and analogs has been recently well documented[4,5].

Scheme 1



Scheme 2


1
2
CH3CN, -30°C (58%)
CH3CN, r.t. (61%)
3
(5)
CH3CN/THF/DMF (1:1:1),
-15°C→r.t., 15 h (50%)
, CH3CN, -40°C
(54%)
4
(+ 9% α-Product)
6
(m.p. 174 - 178°C)
NaOMe, MeOH, r.t. (68%, crystals)
H2O, r.t., 12 h
7

An especially interesting target is CMP-Neu-5-Ac which is required for the sialylation of glycoconjugates (biosynthesis of gangliosides and sialylated glycoproteins)[6]. Its enzymatic synthesis requires several steps with different enzymes[4], therefore other approaches might be competitive or even superior[7]. To this end we have investigated the direct chemical reaction of activated Neu-5-Ac with CMP which is based on the readily accessible sialyl phosphites **B** (via intermediate **A**, Scheme 1), which were recently introduced by us as efficient sialyl donors[8,9]. Due to their sensitivity to mild acid catalysis and the anomeric effect a direct, β-selective reaction with phosphorous acid and derivatives (**C**) is expected resulting in the desired sialyl phosphate (**D**) and phosphonate (**E**) which, because of low basicity and acidity, does not interfer in the reaction course. Similar reaction behavior has been already observed for various O-glycosyl-trichloroacetimidates[10]; however, CMP-Neu-5-Ac was not accessible via this approach.

Diethyl sialyl phosphite **1** is readily available from Neu-5-Ac via methyl tetra-O-acetyl-N-acetylneuraminate (**A**)[11] in three convenient steps (overall yield 80%)[8]. Reaction of **1** with dibenzyl phosphate in dry acetonitrile at -30 °C afforded exclusively the desired β-configurated sialyl phosphate **3** (Scheme 2) which was already obtained via a different route[12,13]. Compound **3** was also accessible from dibenzyl phosphate and bis(trichloroethyl) sialyl phosphite[9] **2** at room temperature; **2** can be obtained from Neu-5-Ac as crystalline material[9]. The extension of this approach to phosphorous acid monoesters is demonstrated for the reaction of **1** with phenyl phosphate which furnished the expected phenyl sialyl phosphate **4**[13]. When the reaction was carried out at -40 °C also some α-product was found (**4β**: 54%; **4α**: 9%).

The direct formation of CMP-Neu-5-Ac from **1** and CMP failed because of insolubility of CMP in solvent systems required for the reaction. Therefore, CMP was transformed by simple acetylation into tri-acetyl derivative **5**[5,13], which exhibited reasonable solubility in an acetonitrile/THF/DMF mixture (1:1:1, by volume). Reaction of **5** with **1** at -15 °C and slowly raising the temperature to room temperature afforded the desired β-configurated acetylated CMP-Neu-5-Ac **6**[13] (separation by flash chromatography, eluent: $CHCl_3$/MeOH, 4:1) as solid material (m.p. 174-178 °C) in 50% yield. Treatment of **6** with sodium methoxide/methanol and then water led to practically quantitative formation of the disodium salt of CMP-Neu-5-Ac (**7**) which precipitated from the reaction mixture, finally by addition of ethyl acetate/petroleum ether; it was isolated as solid material by simple filtration in 68% yield. **7** is identical in all aspects with commercially available material[14].

In conclusion, an efficient and practical synthesis of CMP-Neu-5-Ac (**7**) could be developed which can be readily performed in any scale. It is based on the direct β-selective reaction of sialyl phosphite **1** with acylated CMP derivative **5** and ensuing convenient deacylation of intermediate **6**.

References and Notes

1. This work was supported by a grant from the University of Milano and by the Fonds der Chemischen Industrie. We are grateful to FIDIA Co for providing N-acetyl-neuraminic acid.

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12. Compound **3** was mentioned in ref. 7, however no data are reported.

13. NMR data:

3: ^{1}H NMR (250 MHz, $CDCl_3$) δ = 1.83 (s, 3 H, $NCOCH_3$), 1.94-2.09 (m, 13 H, H-3a, 4 $COCH_3$), 2.57 (dd, $J_{3e,4}$ = 4.9 Hz, $J_{3a,3e}$ = 13.5 Hz, 1 H, H-3e), 3.62 (s, 3 H, $COOCH_3$), 4.07-4.16 (m, 2 H, H-5, H-6), 4.22 (dd, $J_{8,9A}$ = 7.6 Hz, $J_{9A,9B}$ = 12.4 Hz, 1 H, H-9A), 4.58 (dd, $J_{8,9B}$ = 2.4 Hz, $J_{9A,9B}$ = 12.4 Hz, 1 H, H-9B), 4.96-5.05 (m, 5 H, H-4, 2 CH_2Ph), 5.23-5.32 (m, 3 H, H-7, H-8, NH), 7.27-7.39 (m, 10 H, 2 C_6H_5).

4β: ^{1}H-NMR (250 MHz, C_6D_6/CD_3OD = 2:1) δ = 1.84 (s, 3 H, $NCOCH_3$), 1.86 (s, 3 H, $COCH_3$), 1.89 (s, 3 H, $COCH_3$), 1.97-2.06 (m, 7 H, H-3a, 2 $COCH_3$), 2.94 (dd, $J_{3a,4}$ = 5.0 Hz, $J_{3e,3a}$ = 13.2 Hz, 1 H, H-3e), 3.60 (s, 3 H, $COOCH_3$), 4.31-4.40 (m, 2 H, H-5, H-9A), 4.71 (dd, $J_{5,6}$ = 10.8 Hz, $J_{6,7}$ = 2.1 Hz, 1 H, H-6), 4.83 (dd, $J_{8,9B}$ = 2.6 Hz, $J_{9A,9B}$ = 12.4 Hz, 1 H, H-9B), 5.59 (ddd, $J_{3e,4}$ = 5.0 Hz, 1 H, H-4), 5.64 (ddd, $J_{8,9B}$ = 2.6 Hz, 1 H, H-8), 5.72 (dd, $J_{6,7}$ = 2.2 Hz, 1 H, H-7), 6.95-7.01 (m, 1 H, C_6H_5), 7.26-7.29 (m, 2 H, C_6H_5), 7.44-7.47 (m, 2 H, C_6H_5). - ^{31}P-NMR (161.7 MHz, CD_3OD) δ = -11.04 (β, 100%).

4α: ^{1}H-NMR (250 MHz, CD_3OD) δ = 1.87 (s, 3 H, $NCOCH_3$), 2.00 (s, 3 H, $COCH_3$), 2.02 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.11 (s, 3 H, $COCH_3$), 2.22 (dd, $J_{3a,3e}$ = 12.8 Hz, 1 H, H-3a), 2.94 (dd, $J_{3a,3e}$ = 12.8 Hz, $J_{3e,4}$ = 4.6 Hz, 1 H, H-3e), 3.82 (s, 3 H, $COOCH_3$), 3.99 (dd, $J_{5,6}$ = 10.3 Hz, 1 H, H-5), 4.11-4.17 (m, 2 H, H-6, H-9A), 4.43 (dd, $J_{8,9B}$ = 2.4 Hz, $J_{9A,9B}$ = 12.4 Hz, 1 H, H-9B), 5.04 (ddd, $J_{3e,4}$ = 4.6 Hz, 1 H, H-4), 5.30-5.36 (m, 2 H, H-7, H-8), 7.07-7.13 (m, 2 H, C_6H_5), 7.24-7.37 (m, 3 H, C_6H_5).

5: ^{1}H-NMR (250 MHz, D_2O) δ = 1.97-1.98 (2 s, 6 H, 2 $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 3.91-3.99 (m, 1H, H-5'$_A$), 4.01-4.13 (m, 1 H, H-5'$_B$), 4.40-4.44 (m, 1 H, H-4'), 5.25-5.31 (m, $J_{3',4'}$ = 2.6 Hz, $J_{2',3'}$ = 5.3 Hz, 1 H, H-3'), 5.36 (dd, $J_{2',3'}$ = 5.3 Hz, 1 H, H-2'), 6.04 (d, $J_{1',2'}$ = 4.5 Hz, 1 H, H-1'), 7.00 (d, $J_{5,6}$ = 7.6 Hz, 1 H, H-5), 8.31 (d, $J_{5,6}$ = 7.6 Hz, 1 H, H-6).

6: ^{1}H-NMR (250 MHz, CD_3OD) δ = 1.90 (s, 3 H, $NCOCH_3$), 2.01-2.15 (m, 19 H, H-3"a, 6 $COCH_3$), 2.24 (s, 3 H, $COCH_3$), 2.74 (dd, $J_{3"a,4"}$ = 4.8 Hz, $J_{3"e,3"a}$ = 13.2 Hz, 1 H, H-3"e), 3.84 (s, 3 H, $COOCH_3$), 4.05 (dd, $J_{5",6"}$ = 10.7 Hz, 1 H, H-5"), 4.23 (dd, $J_{8",9"B}$ = 12.2 Hz, 2 H, H-5'B, H-9"B), 4.34-4.39 (m, 1 H, H-5'A), 4.43 (m, 1 H, H-4'), 4.49 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{5",6"}$ = 10.7 Hz, 1 H, H-6"), 4.66 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{9"A,9"B}$ = 12.2 Hz, 1 H, H-9"A), 5.28-5.38 (m, $J_{3"e,4"}$ = 4.8 Hz, 2 H, H-4", H-8"), 5.48 (dd, $J_{6",7"}$ = 2.2 Hz, $J_{7",8"}$ = 4.8 Hz, 1 H, H-7"), 5.55-5.61 (m, 2 H, H-2', H-3'), 6.17 (d, $J_{1',2'}$ = 3.7 Hz, 1 H, H-1'), 7.44 (d, $J_{5,6}$ = 7.5 Hz, 1 H, H-5), 8.43 (d, $J_{5,6}$ = 7.5 Hz, 1 H, H-6). - ^{31}P-NMR (161.7 MHz, CD_3OD) δ = -3.59 (β, 100%).

14. Purchased from SIGMA Co.

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