Tetrahedron Letters, Vol. 33, No. 3, pp. 325-328, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press plc

SYNTHESIS OF SIALIC ACID THROUGH ALDOL CONDENSATION OF GLUCOSE WITH OXALACETIC ACID

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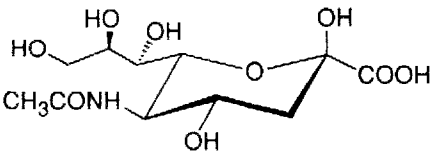
Key Words: sialic acid; N-acetylneuraminic acid; aldol condensation; glucose; phenylcarbamoyl protection.

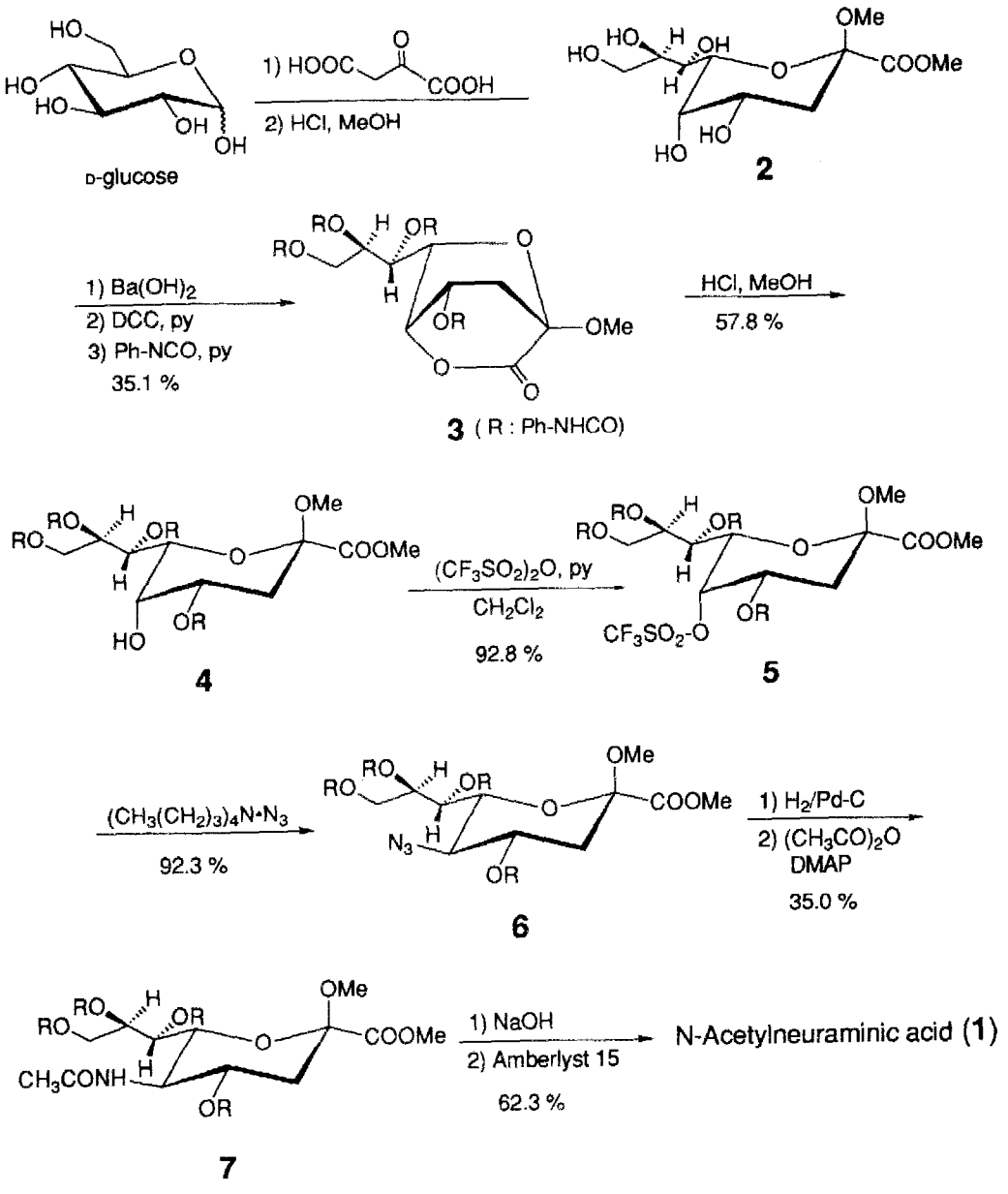
SUMMARY: N-Acetylneuraminic acid (sialic acid) **1** was synthesized through relatively short steps from D-glucose *via* a key intermediate 1,5-lactone derivative obtained from the pyranose isomer of the aldol condensation products of D-glucose with oxalacetic acid.

Sialic acid is an essential component of ganglioside (sialoglycosphingolipid) which is involved in cellular interactions, differentiation, and growth.[1] Further, sialic acid as a sole molecule exerts an important effect in variable biological phenomena.[2,3] Therefore, many researchers pursued anxiously an efficient way which can supply enough amount of sialic acid by either chemical or enzymatic synthesis during the last three decades.[4-9] However, the preparative methods so far reported are not satisfactory from a view point of practical supply of a large amount of sialic acid economically. Among them, How's method[5] by coupling of N-acetyl-D-mannosamine and oxalacetic acid seems to be the most attractive way to obtain sialic acid but with a disadvantage that the starting material, N-acetyl-D-mannosamine is considerably expensive. We thus adopted D-glucose, as a starting material for the aldol condensation, whose price is negligibly low compared with N-acetyl-D-mannosamine.



N-acetylneuraminic acid (**1**)

D-Glucose was coupled with oxalacetic acid in alkaline solution.[10] The product was then decarboxylated under acidic condition with Dowex 50 (H^+) resin to form 3-deoxynonulosonic acid. After removal of excess glucose by use of Dowex 1 ($HCOO^-$) resin, a mixture of the stereoisomers was treated with HCl in methanol. Five isomers including the desired pyranose compound **2** were obtained in 20% yield and separated each other by using HPLC.[11] The structures of those compounds were determined mainly by analysis of ^{1}H-NMR spectra of their acetylated derivatives respectively.[12] A ratio of the configurational isomers at C4 (4*S* to 4*R*) throughout the product was 7 to 3.


D-glucose
1) HOOC COOH
2) HCl, MeOH
2
1) Ba(OH)2
2) DCC, py
3) Ph-NCO, py
35.1 %
3 (R : Ph-NHCO)
HCl, MeOH
57.8 %
4
(CF3SO2)2O, py
CH2Cl2
92.8 %
5
(CH3(CH2)3)4N•N3
92.3 %
6
1) H2/Pd-C
2) (CH3CO)2O
DMAP
35.0 %
7
1) NaOH
2) Amberlyst 15
62.3 %
N-Acetylneuraminic acid (1)

For the selective protection of C5-hydroxyl group in the compound **2**, we employed a lactone formation between a carboxyl and an axial C5-hydroxyl groups. Thus, the pure ester **2** was saponified with barium hydroxide, and then lactonized with dicyclohexylcarbodiimide (DCC) in pyridine. Thereafter, all other hydroxyl groups at C4, C7, C8, and C9 were protected by phenyl isocyanate simultaneously.[13] The one-pot reaction mentioned above gave a key intermediate lactone **3**. An advantage for use of phenylcarbamoyl group is not only in its high stability under acidic condition required for opening of the lactone ring, but in an applicability of a single solvent of pyridine through the one-pot reaction. Lactone **3** was treated with HCl in methanol to convert to 5-hydroxy methyl ester **4** without cleavage of phenylcarbamoyl groups.

The free 5-hydroxyl group in the compound **4** was sulfonylated with trifluoromethanesulfonic anhydride in the presence of pyridine to give the compound **5**.[14] By use of tetrabutylammonium azide[7] in benzene, the triflate **5** was converted to azide **6**, being accompanied by inversion of the configuration at C5.[15] Catalytic hydrogenation of the azide **6** gave the primary amine, which was then acetylated with acetic anhydride and 4-dimethylaminopyridine (DMAP) in dichloromethane to form the protected N-acetylneuraminic acid **7**. Finally, deprotection of all of the protecting groups was performed by two steps. Phenylcarbamoyl group and methyl ester were cleaved with sodium hydroxide. The methyl glycoside obtained was hydrolyzed in the presence of Amberlyst 15 (H^+) resin to give N-acetylneuraminic acid (**1**). Synthetic N-acetylneuraminic acid (**1**) thus obtained, was completely identical with natural one in all respects (TLC, HPLC, and ^{1}H-NMR spectra).[16]

After optimization of the reaction conditions in aldol condensation as well as decarboxylation, this process may provide us the most convenient and valuable synthetic way for preparation of sialic acid with different kinds of N-acyl group from D-glucose. Moreover, syntheses of analogs of N-acetylneuraminic acid (**1**) are made possible from other stereoisomers of the compound **2**, which were obtained by the aldol condensation.[17]

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10) The best condensation condition so far obtained is as follows: One equivalent of oxalacetic acid and three equivalents of glucose were condensed in 0.6M sodium borate buffer (pH 11.0) at r.t. for 72 hours.
11) There should be eight isomers of 3-deoxynonulosonic acid theoretically (configurational isomers at C2, C4 as well as structural isomers of pyranose and furanose). HPLC conditions [Column: Cosmosil 5C_{18}-AR (10 x 250 mm), Solvent: 1% CH_3CN-H_2O (isocratic), Flow rate: 3.0 ml/min, Detection: UV(210 nm), Retention time (min): 19.3, 20.4, 21.0, 23.2, 26.4 (compound **2**)]
12) Peracetylations of all of the isomers for their identifications were carried out with acetic anhydride and pyridine. According to 2D ^{1}H-NMR data, those structures of five isomers were assigned. Compound **2** which accounts for 10% of all of the isomers, is unambiguously identified as methyl (methyl 3-deoxy-D-*glycero*-D-*gulo*-2-nonulopyranosid)onate[4*S*-isomer]. ^{1}H-NMR data of the peracetate of the compound **2** (in $CDCl_3$, 270 MHz): δ2.00-2.15 (2H, m, H3), δ5.28 (1H, ddd, J= 3.0, 6.4, 10.0 Hz, H4), δ5.49 (1H, dd, J= 1.0, 3.0 Hz, H5), δ3.93 (1H, dd, J= 1.0, 8.4 Hz, H6), δ5.54 (1H, dd, J= 3.0, 8.4 Hz, H7), δ5.00 (1H, ddd, J= 3.0, 5.4, 5.9 Hz, H8), δ4.21 (1H, dd, J= 5.9, 11.9 Hz, H9), δ4.29 (1H, dd, J= 5.4, 11.9 Hz, H9), δ3.24 (3H, s, C2-OMe), δ3.80 (3H, s, COOMe), δ1.98 (3H, s, OAc), δ2.01 (3H, s, OAc), δ2.10 (3H, s, OAc), δ2.13 (3H, s, OAc), δ2.22 (3H, s, OAc). The assigned structures of four other isomers will be reported elsewhere.
13) K. L. Agarwal and H. G. Khorana, *J. Am. Chem. Soc.*, **94**, 3578-3585 (1972).
14) ^{1}H-NMR data of the compound **5** (in $CDCl_3$, 270 MHz): δ2.23 (1H, dd, J= 12.5, 12.9 Hz, H3a), δ2.35 (1H, dd, J= 5.2, 12.9 Hz, H3e), δ5.28 (1H, dd, J= 1.7, 5.2, 12.5 Hz, H4), δ5.78 (1H, br.s, H5), δ4.18 (1H, d, J= 9.0 Hz, H6), δ5.65 (1H, dd, J= 5.2, 9.0 Hz, H7), δ5.32 (1H, m, H8), δ4.33 (1H, dd, J= 3.3, 11.4 Hz, H9), δ4.65 (1H, dd, J= 6.5, 11.4 Hz, H9), δ3.27 (3H, s, C2-OMe), δ3.69 (3H, s, COOMe), δ6.77-7.64 (24H, m, Ph and NH).
15) ^{1}H-NMR data of the compound **6** (in $CDCl_3$, 270 MHz): δ1.79 (1H, dd, J= 11.5, 12.9 Hz, H3a), δ 2.70 (1H, dd, J= 5.1, 12.9 Hz, H3e), δ5.32 (1H, ddd, J= 5.1, 9.8, 11.5 Hz, H4), δ3.47 (1H, dd, J= 9.8, 10.0 Hz, H5), δ3.83 (1H, dd, J= 1.2, 10.0 Hz, H6), δ5.65 (1H, dd, J= 1.2, 7.8 Hz, H7), δ5.45 (1H, ddd, J= 2.7, 4.6, 7.8 Hz, H8), δ4.24 (1H, dd, J= 4.6, 12.5 Hz, H9), δ4.65 (1H, dd, J= 2.7, 12.5 Hz, H9), δ3.23 (3H, s, C2-OMe), δ3.81 (3H, s, COOMe), δ6.80-7.45 (24H, m, Ph and NH).
16) ^{1}H-NMR data of the compound **1** (in 1% CF_3COOD, 270 MHz): δ1.80 (1H, dd, J= 11.5, 13.2 Hz, H3a), δ2.24 (1H, dd, J= 4.7, 13.2 Hz, H3e), δ3.99 (1H, ddd, J= 4.7, 10.0, 11.5 Hz, H4), δ3.84 (1H, dd, J= 10.0, 10.3 Hz, H5), δ3.98 (1H, dd, J= 1.2, 10.3 Hz, H6), δ3.48 (1H, dd, J= 1.2, 9.0 Hz, H7), δ3.67 (1H, ddd, J= 2.7, 6.1, 9.0 Hz, H8), δ3.54 (1H, dd, J= 6.1, 11.5 Hz, H9), δ3.75 (1H, dd, J= 2.7, 11.5 Hz, H9), δ1.97 (3H, s, NAc).
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(Received in Japan 9 September 1991)