Tetrahedron Letters, Vol. 33, No. 52, pp. 8031-8034, 1992
Printed in Great Britain

0040-4039/92 $5.00 + .00
Pergamon Press Ltd

Synthesis of the C-5 Homologue of N-Acetylneuraminic Acid by Enzymatic Chain Elongation of 2-C-Acetamidomethyl-2-deoxy-D-mannose

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Key words: neuraminic acid homologue; branched chain mannosamine; nitromethane condensation; aldolase; enzymatic chain elongation

Abstract: *Condensation of hexopyranosid-2-ulose **1** with nitromethane afforded after acetylation **2**. Treatment with borohydride yielded stereoselectively **4**. Reduction, acetylation, deblocking, and acid hydrolysis gave then C-2 branched N-acetylmannosamine **7** that was enzymatically converted to the first known C-5 branched homologue of **8**.*

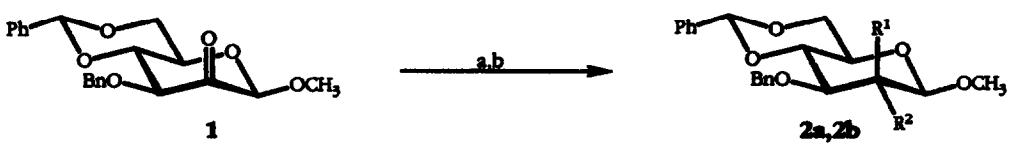
As part of our studies on the chemistry and biological activity of sialic acids and its activated form[1] we were also interested in obtaining sialic acid analogues modified at C-5. There are indications that this region of the molecule plays an important role in different ligand-receptor based biological systems.

The synthesis of **8** first required the preparation of the corresponding C-2 branched N-acetylmannosamine homologue **7** since in this instance preformed N-acetylneuraminic acid cannot serve as starting material. **7** itself is an interesting compound with biological potential. It represents the first example of an unsubstituted C-2 branched hexosamine.

In the second part the C-2 branched amino sugar was chain elongated employing N-acetylneuraminate pyruvate lyase (EC 4.1.3.3).

A general method to achieve C-C branching is the condensation of nitromethane with a ketone. We applied the *Henry* reaction to a suitably protected hexopyranosid-2-ulose. Methyl 3-O-benzyl-β-D-glucopyranoside[2] was converted to the 4,6-O-benzylidene derivative, and subsequently oxidised to give **1**. Compared to the literature[3] the yield of both steps could be improved substantially. As expected, condensation of **1** with nitromethane produced a mixture of the tertiary nitroalcohols which were acetylated to yield the epimeric nitromethyl acetates **2a,2b**.

Scheme 1

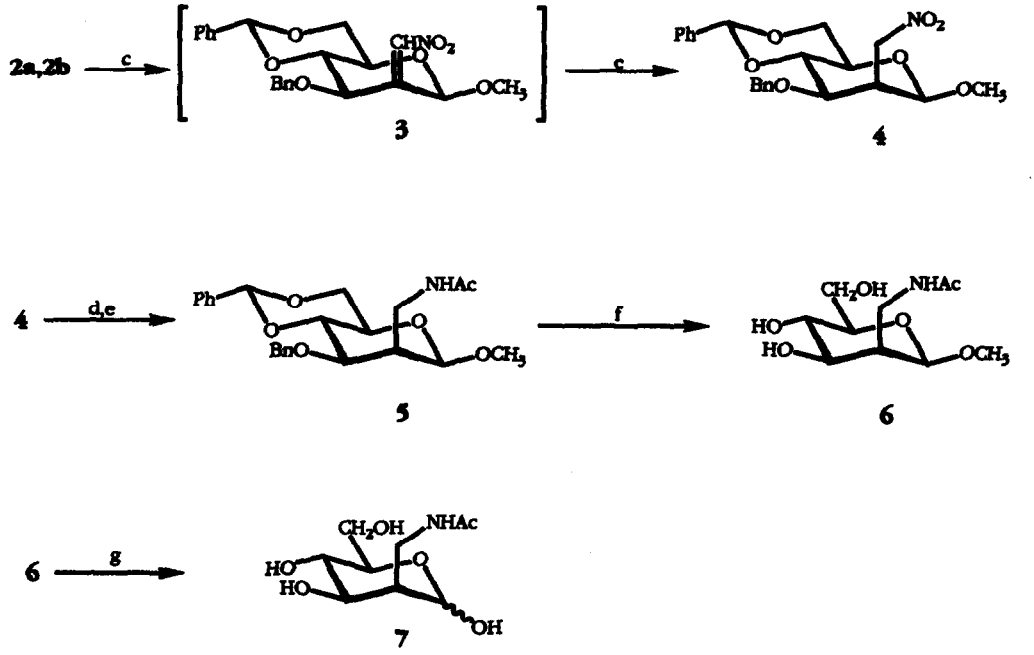


1 **2a,2b**

2a: $R^1 = OAc$, $R^2 = CH_2NO_2$
2b: $R^1 = CH_2NO_2$, $R^2 = OAc$

a) nitromethane, MeOH/NaOMe, 0°C; b) Ac_2O, TsOH

Scheme 1 (continued)



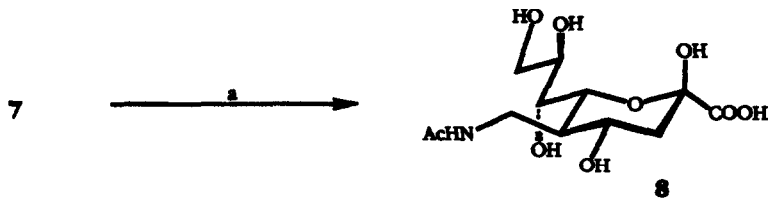
c) $NaBH_4$-EtOH 0°C; d) $LiAlH_4$-THF (reflux, 2h); e) Ac_2O-MeOH; f) H_2, Pd/C, EtOH-dioxane; g) 0.01n HCl, 60°C (30 min)

Treatment with sodium borohydride afforded in a one-pot-reaction intermediate nitroolefin **3**. Due to the anomeric configuration, **3** was stereoselectively converted to methyl 3-O-benzyl-4,6-O-benzylidene-2-deoxy-2-C-nitromethyl-β-D-mannopyranoside **4** in good yield.[4] Reduction of the nitro group with lithium aluminium hydride gave the corresponding aminomethyl compound which, without further purification, was acetylated to yield **5**. Hydrogenolytic cleavage of benzyl and benzylidene groups produced crystalline **6** (Fp. 154-154.5 °C) which was converted to the branched amino sugar **7** by mild acid hydrolysis.

Interestingly, ^1H NMR data of **7** exhibit four signals in the region of the anomeric protons which were assigned to α,β-pyranose and α,β-furanose[5]. The equilibrium mixture contained 27% of furanoses. This is in contrast to the ^1H NMR spectrum of N-acetylmannosamine which does not show any signal attributable to a furanoid ring structure.

Incubation of N-acetylneuraminate pyruvate lyase (EC 4.1.3.3) with **7** and pyruvate (10 fold excess) provided, after ion exchange chromatography, 5-C-acetamidomethyl-5-deamino-neuraminic acid **8** in 75% yield. NMR data[6] and elemental analyses were in agreement with the proposed structure.

Scheme 2



a) 1 mmol **7**, 10 mmol Na-pyruvate, K-phosphate buffer (50 mM, pH 7.5), NeuAc-aldolase (EC 4.1.3.3, 10 U, N_2, 37°C, 7 days)

Studies on the biological properties of the sialic acid analogue **8** as well as enzymatic syntheses of additional sialic acid analogues are in progress. Detailed results will be reported elsewhere.

In conclusion, we have succeeded in preparing the N-acetylmannosamine homologue[7] **7** in a straightforward synthesis (7 steps, overall yield 55% relative to **1**) that can easily be adapted to multigram scale. In addition the enzymatic synthesis of the C-5 homologue of N-acetylneuraminic acid has been accomplished in good yield.

Acknowledgements - This work was supported by the Fonds der Chemischen Industrie. We are indebted to Mr. H. Großkurth, Max-Planck-Institut für Medizinische Forschung, Heidelberg, for recording 500 MHz ^{1}H NMR spectra.

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4 ^{1}H NMR data (300 MHz, $CDCl_3$), compound **4**: δ 7.50-7.26 (m, 10 H, 2 Ph) 5.58 (s, 1 H, 7-H) 4.76 (dd, 1 H, 8-H, $J_{8,8'}$ 14.0 Hz) 4.74 (s, 2 H, PhCH_2) 4.65 (dd, 1 H, 8'-H) 4.56 (d, 1 H, 1-H,

$J_{1,2}$ 2.4 Hz) 4.33 (dd, 1 H, 6_{eq}-H, $J_{6eq,6ax}$ 10.4 Hz) 3.89 (dd, 1 H, 3-H, $J_{3,4}$ 9.7 Hz) 3.79 (dd, 1 H, 6_{ax}-H) 3.55 (dd, 1 H, 4-H, $J_{4,5}$ 9.8 Hz) 3.52 (m, 1 H, 2-H, $J_{2,3}$ 5.9 Hz) 3.46 (s, 3 H, OMe) 3.35 (ddd, 1 H, 5-H, $J_{5,6ax}$ 10.3 Hz, $J_{5,6eq}$ 4.9 Hz)

5 Selected ^{1}H NMR data (500 MHz, D_2O), compound **7**: δ 5.42 (d, 0.06 H, 1-H_{fu}, $J_{1,2}$ 5.6 Hz), 5.30 (d, 0.21 H, 1-H_{fu}, $J_{1,2}$ 6.5 Hz), 5.21 (d, 0.60 H, 1-$H_{\alpha\text{-py}}$, $J_{1,2}$ 1.3 Hz), 5.06 (d, 0.13 H, 1-$H_{\beta\text{-py}}$, $J_{1,2}$ 2.1 Hz), 2.45 (m, 0.06 H, 2-H_{fu}, 2.42 (m, 0.21 H, 2-H_{fu}, 2.32 (m, 0.13 H, 2-$H_{\beta\text{-py}}$, 2.26 (m, 0.60 H, 2-$H_{\alpha\text{-py}}$, 2.02-2.00 (4s, 3H, $NHAc_{fu,fu,\alpha\text{-py},\beta\text{-py}}$)

6 ^{1}H NMR data (500 MHz, D_2O), compound **8**: δ 4.06 (d, 1 H, 6-H) 4.02 (ddd, 1 H, 4-H, $J_{4,5}$ 10.9 Hz) 3.87 (dd, 1 H, 9'-H) 3.83 (d, 1 H, 7-H, $J_{7,8}$ 8.9 Hz) 3.80 (ddd, 1 H, 8-H, $J_{8,9}$ 6.0 Hz, $J_{8,9'}$ 2.5 Hz) 3.66 (dd, 1 H, 9-H, $J_{9,9'}$ 11.9 Hz) 3.60 (dd, 1H, 10-H, $J_{10,10'}$ 14.8 Hz), 3.37 (dd, 1 H, 10'-H), 2.24 (dd, 1 H, 3_e-H, $J_{3e,3a}$ 12,6, Hz $J_{3e,4}$ 4.7 Hz), 2.02 (s, 3 H, NHAc), 1.88 (dddd, 1 H, 5-H, $J_{5,6}$ 10.9 Hz, $J_{5,10}$ 3.3 Hz, $J_{5,10'}$ 3.9 Hz), 1.78 (dd, 1 H, 3_a-H, $J_{3a,4}$ 11.8 Hz)

7 After completion of this work the preparation of the methyl α-anomer of **6** has been reported. Sarda, P.; Olesker, A.; Lukas, G. *Carbohydr. Res.* **1992**, 229, 161-165.

(Received in Germany 30 July 1992)