Tetrahedron Letters,Vol.26,No.20,pp 2439-2440,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

SYNTHESIS WITH AN IMMOBILIZED ENZYME OF N-ACETYL-9-O-ACETYL-NEURAMINIC ACID, A SUGAR REPORTED AS A COMPONENT OF EMBRYONIC AND TUMOR ANTIGENS.

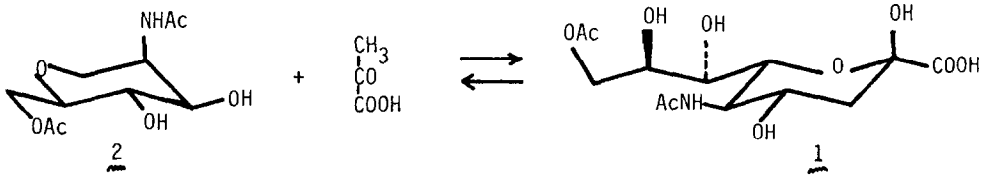
Claudine Augé, Serge David, Christine Gautheron et Alain Veyrières

Laboratoire de Chimie Organique Multifonctionnelle, Unité Associée au C.N.R.S. n° 462, Université Paris-Sud, Bt 420, 91405 Orsay Cédex, France.

Summary. Condensation of 2-acetamido-6-O-acetyl-2-deoxy-D-mannose with pyruvic acid in the presence of immobilized acylneuraminate pyruvate lyase (2.8 units) gave 5-acetamido-9-O-acetyl-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranos-1-onic acid (N-acetyl-9-O-acetylneuraminic acid) on the 0.3 mmole scale.

The sialic acids are a family of N- and O-acyl derivatives of 5-amino-3,5-dideoxy-D-glycero-D-galacto-2-nonulopyranos-1-onic acid (neuraminic acid), which occur in animal, or sometimes bacterial cells. Sialic acids are involved in the regulation of a great variety of biological phenomenons, apparently because of their peripheral position in glycoconjugates and corresponding external location in cell membranes.[1] Thus, according to some recent reports, a N-acetyl-9-O-acetyl-neuraminic acid residue is present in the antigenic epitope of a ganglioside found in the developing rat embryonic neuroectoderm and in human melanoma cells, which is recognized by a monoclonal antibody, Mab Dl-1 prepared against the rat B49 cell lines.[2] The corresponding methyl glycoside methyl ester has been synthesized some time ago.[3]

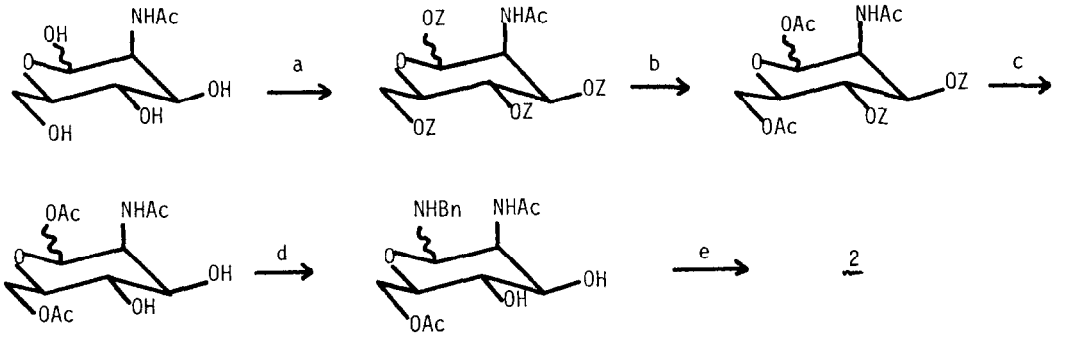
We now report a synthesis of the free sialic acid 1 by aldol condensation of 2-acetamido-6-O-acetyl-2-deoxy-D-mannose 2 with pyruvic acid in the presence of immobilized acylneuraminate pyruvate-lyase (E.C.4.1.3.3) :



The mannosamine derivative 2 was synthesized according to the Scheme given on the next page.[4,5]

The aldolase was immobilized as described previously.[6] A mixture of the immobilized enzyme (2 g; 2.8 U), compound 2 (0.5 mmol), sodium pyruvate (5 mmol), dithiothreitol (5 mol) and sodium azide (0.5 mg) was stirred in 0.05 M potassium phosphate buffer pH 7.2 at 37°C under nitrogen. The reaction was monitored by t.l.c. (propanol-water, 7:3).[1] After 8 days, the gel was removed by filtration. Chromatography of the filtrated solution, first on Dowex-1 formate (elution with a 0-2 M formic acid gradient) and then on cellulose (butanol-propanol-water, 1:2:1)[7] gave, as a first fraction N-acetyl-9-O-acetyl-neuraminic acid (1), obtained after freeze-drying as a powder (70 mg). The ^{1}H NMR spectrum was identical with the published one.[6]

A small quantity of N-acetyl-neuraminic acid present in the next fraction (23 mg) most probably came from the aqueous hydrolysis of **1**. The lability of these ester functions is a permanent problem in sialic acids biochemistry.



Z = $SiMe_3$ Bn = CH_2Ph

Scheme : a) Me_3SiCl, HMDS, pyridine, 60°C, 24 h; b) Ac_2O/AcOH, pyridine, RT, 48 h, cf[9]; c) methanol-30% aqueous acetic acid, 1:1, v/v, RT, 4 h; d) $PhCH_2NH_2$ (2 éq.), 0.2 M in THF, RT, overnight; e) IRC-50 (H^+), RT, 30 min.

Although acylneuraminate pyruvate-lyase appears to act in cells only as a catabolic enzyme, we have already used it in the in vitro synthesis of N-acetyl-neuraminic acid.[6] We see here that the same method can be readily extended to the preparation of an acetylated derivative. This synthetic pathway is completely unphysiological, since living cells make such derivatives by acetylation of completely elaborated sialic acids molecules.

References and Notes

1. R. Schauer, Adv.Carbohydr.Chem. and Biochem., 40, 131 (1982).
2. D.A. Cherech, R.A. Reisfeld and P.A. Varki, Science, 225, 844 (1984).
3. J. Haverkamp, R. Schauer, M. Wember, J.P. Kamerling and J.F.G. Vliegenthart, Z. Physiol.Chem., 356, 1575 (1975).
4. All the derivatives in the Scheme were amorphous, anomeric mixtures. N-Benzyl-2-acetamido-6-O-acetyl-2-deoxy-α,β-D-mannosylamine, the last intermediate, was characterized by its composition. The ^1H NMR spectrum of **2** was compatible with a 6-O-acetyl structure.
5. The efficiency of this route was first tested with the less expensive N-acetylglucosamine. Details of the synthesis of 6-O-acetyl-N-acetyl-glucosamine m.p. 193°C dec.; $|\alpha|_D^{25}$ +66° (3 min) → +35° (24 h), (c 1 in water) will be reported elsewhere.
6. C. Augé, S. David and C. Gautheron, Tetrahedron Letters, 25, 4663 (1984).
7. H.P. Buscher, J. Casals-Stenzel and R. Schauer, Eur.J.Biochem. 50, 71 (1974).
8. J. Haverkamp, H. Van Halbeek, L. Dorland, J.F.G. Vliegenthart, R. Pfeil and R. Schauer, Eur.J.Biochem., 122, 305 (1982).
9. E.F. Fuchs and J. Lehmann, Chem.Ber., 107, 721 (1974).

(Received in France 23 March 1985)