Carbohydrate Research, 194 (1989) c15–c18
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Synthesis of the 4-acetamido-4-deoxy analogue of *N*-acetylneuraminic acid and its behaviour towards CMP-sialate synthase*†

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(Received August 23rd, 1989; accepted for publication, September 21st, 1989)

To gain more insight into the structure–activity relationship of the enzymes of sialic acid metabolism we recently prepared a series of sialic acid analogues[2–4] and determined their surface profiles[5,6]. For CMP-sialate synthase to recognize its substrate, we found that the three hydrophilic functions 2-OH β, the NH of the NHAc group, and the coaxially aligned 8-OH group as well as both hydrophobic axial hydrogens H-4 and H-6 were necessary[5,6]. On the other hand, it is known that, for the recognition of α-glycosidically bound sialic acid and its release by a sialidase, the CO-group of the 5-acetamido group and the 4-OH group are required. Exchange of the latter group by hydrogen[7] or a methoxy group[8], and the biochemical transformation into a 4-acetoxy group, prevents this cleavage[9]. Other structural relations concerning the α-face were studied systematically recently[10].

The aforementioned data prompted us to propose that the 4-acetamido-4-deoxy analogue of *N*-acetylneuraminic acid (Neu5Ac) should be transformable into a CMP-derivative. In addition it should be possible to link it to glycoproteins by means of suitable transferases in similar manner to 4-*O*-Me-Neu5Ac[8]. Such artificial sialoglycoproteins would be stable not only against sialidases but also esterases, which are known to render a 4-*O*-acetyl-sialoglycoprotein sensitive to sialidases. The results on synthesis of the title compound are shown in the scheme.

First of all the sialic acid derivative **1** was transformed via the 4-oxo-derivative **2** into the 4-epi-sialic acid derivative[1] **3**. Further reaction with triphenylphosphine–diethylazodicarboxylate (DEAD)–3M HN_3 (toluene) in THF yielded the expected compound **4** as the main product (65%) and the 3,4-didehydro sialic acid derivative **5** as a byproduct (15%)‡. Hydrogenation in the presence of Ac_2O produced com-

*Structural Variations on *N*-Acetylneuraminic Acid. Part 13. For part 12, see ref. 1.
†Presented at the 5th European Carbohydrate Symposium, Prague, August 21–25, 1989.
‡Compound **3** was treated overnight at room temp. with 1.2 equiv. Ph_3P–HN_3–DEAD. After addition of an excess of CH_2Cl_2, the solution was extracted with sat. $NaHCO_3$ and water. Chromatography over silica gel (EtOAc) yielded pure **4**: m.p. 199° (EtOAc).

0008-6215/89/$ 03.50

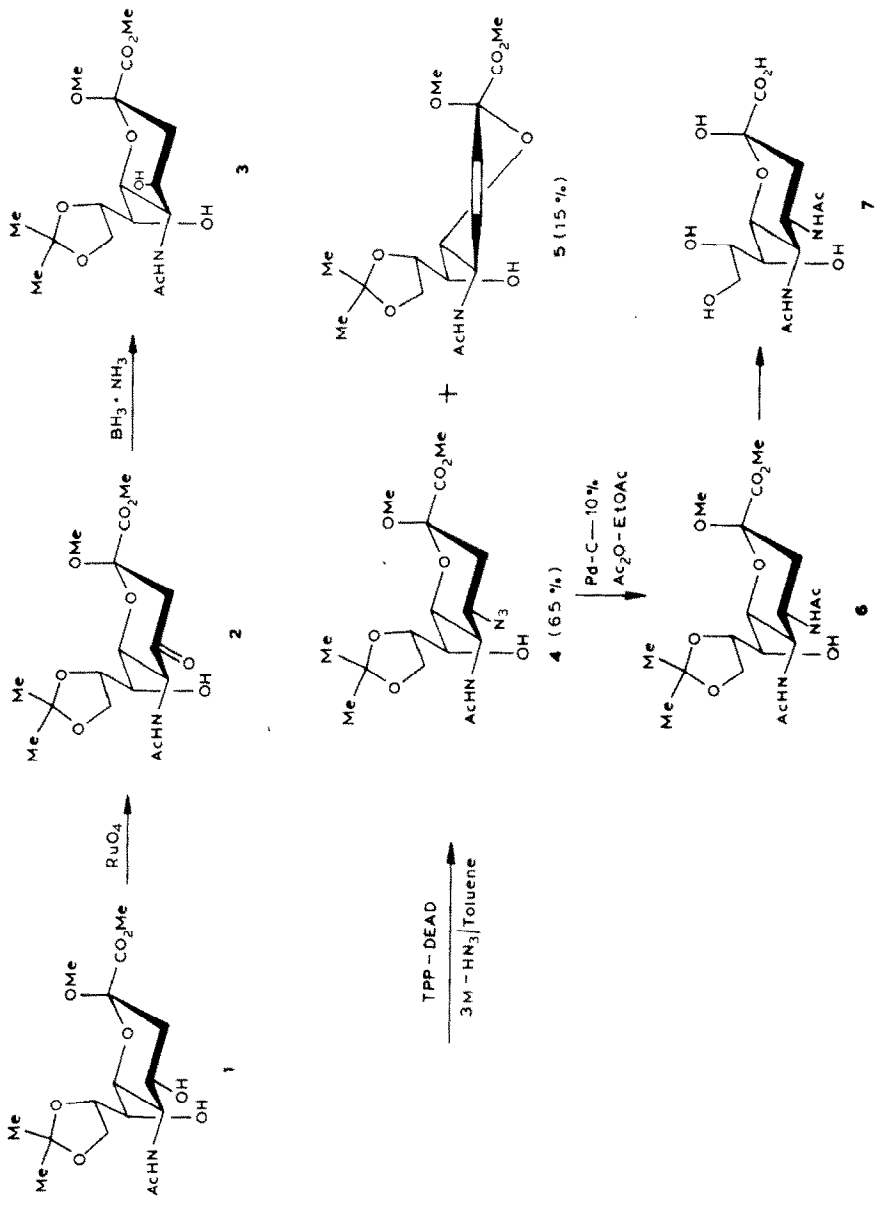
RuO4
BH3 · NH3
TPP – DEAD
3M – HN3 | Toluene
Pd-C — 10 %
Ac2O – EtOAc
5 (15 %)
4 (65 %)

TABLE I

N.M.R. DATA (250 MHz, $CDCl_3$)

Compound	*Chemical shifts (p.p.m.)*										
	3-Ha/e	*4-H*	*5-H*	*6-H*	*7-H*	*8-H*	*9,9'-H*	*NH*	*$NCOCH_3$*	*CO_2CH_3*	*OCH_3*
4	1.84/2.50	[a]	[a]	3.64	3.47	[a]	4.00/4.13	5.67	2.08	3.79	3.29
5	[a]	[a]	4.78	3.81	3.46	4.35	4.00/4.16	5.68	2.03	3.79	3.33
6	1.83/2.27	4.48	[a]	3.65	3.43	4.25	3.97/4.10	6.52/6.73	1.97	3.77	3.27
7[b]	[a]/2.24	4.40	4.04	4.25	3.63	3.81	3.67/3.90	—	2.03/2.06	—	—
	Coupling constants (Hz)										
	$J_{3a,3e}$	$J_{3a,4}$	$J_{3e,4}$	$J_{4,NH}$	$J_{4,5}$	$J_{5,NH}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
4	−12.5	10.3	4.4	—	[a]	6.7	9.5	1.2	8.1	5.2	6.4	−8.8
5	—	—	—	—	2.4	9.2	9.5	1.6	7.9	5.6	6.6	−8.8
6	−13.0	12.0	4.5	8.8	10.5	7.3	10.2	0.5	7.9	5.4	6.2	−8.7
7[b]	−13.1	11.8	4.6	—	10.8	—	10.8	0.8	8.8	6.3	2.2	−11.7

[a]Not determined. [b]In D_2O.

pound **6** (90%), which was saponified[11] to 4,5-diacetamido-3,4,5-trideoxy-D-*glycero*-β-D-*galacto*-pyranosonic acid (**7** 60%). Structural assignments for **4**–**7** are in accordance with the 250-MHz ^{1}H-n.m.r. spectra (Table I).

The activation of **7** by CMP-sialate synthase was determined by using the enzyme kinetics as previously described[2]. As **7** does not react in the thiobarbituric acid assay[12], the determination was performed in parallel with Neu5Ac by varying both substrate concentrations. For this reason neither an inhibition constant (K_I) nor the Michaelis constant (K_m) or v_{max}-values could be determined graphically. Nevertheless these data were evaluated by simulating the distribution of the products (stochastic method[13]) and by comparing the simulated with the observed concentrations of Neu5Ac-CMP (least-square procedure). The calculated values for K_m are 0.8mM for Neu5Ac and 1.3mM for Neu5Ac4NAc. The corresponding v_{max}-values were 1.4 and 1.5 $\mu mol \cdot min^{-1}$ respectively. This good activation of the new sialic acid analogue **7** agrees with the structure–activity relationships demonstrated for a series of other sialic acid analogues[2,5,6].

ACKNOWLEDGMENTS

Supported by the Fonds zur Förderung der wissenschaftlichen Forschung in Österreich-projects P6805 and 4009. We thank Ms. S. Kotzinger and Mr. W. Schneider for competent technical assistence.

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