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SYNTHESIS OF *N*-ACETYLNEURAMINIC ACID 8-METHYL ETHER

A. YA. KHORLIN AND I. M. PRIVALOVA
Institute for Chemistry of Natural Products, U.S.S.R. Academy of Sciences, Moscow (U.S.S.R.)
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ABSTRACT

N-Acetylneuraminic acid 8-methyl ether (**1**) has been synthesized. Benzylation of 2-methyl-4,5-(4,6-*O*-benzylidene-2-deoxy-β-D-mannopyrano)-2-oxazoline (**2**), followed by mild, acid hydrolysis, yielded 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose (**3**). Treatment of **3** with the potassium salt of di-*tert*-butyl oxaloacetate gave 6-*O*-benzyl-7,9-*O*-benzylidene-*N*-acetylneuraminic acid γ-lactone (**4**). Methylation of **4**, followed by removal of the benzyl and benzylidene groups and opening of the γ-lactone ring, then gave **1**. The inhibition by acid **1** of the hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside by *Vibrio cholerae* neuraminidase has been investigated.

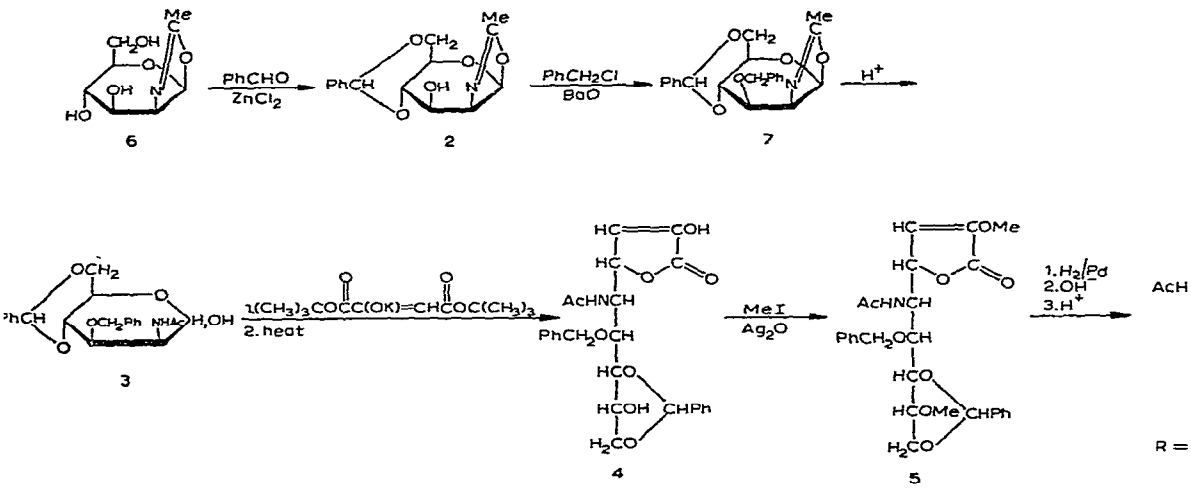
INTRODUCTION

The specific substitution of *N*-acetylneuraminic acid (5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-nonulosonic acid, NANA) is of importance in connection with studies of the mode of bonding of the sialic acid moieties in biopolymers, and with elucidation of the structural features of NANA which determine its specific receptor properties. Previous papers of this series have dealt with the synthesis of the benzhydryl ester[1] of NANA, and the acylation[2], tritylation, and glycosylation of NANA[3]. We now describe the synthesis of the 6-*O*-benzyl-7,9-*O*-benzylidene derivative (**4**) of the γ-lactone of *N*-acetylneuraminic acid which is a key intermediate for the preparation of 8-*O*-substituted derivatives of NANA. From **4**, the 8-methyl ether (**1**) of NANA (an analogue of the 8-methyl ether of *N*-glycolylneuraminic acid which was isolated from *Asterias forbesi*[4]) has been prepared.

RESULTS AND DISCUSSION

The synthesis of **1** from 2-methyl-4,5-(2-deoxy-β-D-mannopyrano)-2-oxazoline (**6**) has been accomplished according to the following scheme (on p. 374). The key compound in this reaction series was 2-acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-D-mannopyranose (**3**) obtained by conversion of the oxazoline **2** into the benzyl derivative **7** followed by mild treatment with acid to effect opening of the oxazoline ring.

The presence of the oxazoline ring in intermediates **2**, **6**, and **7** was demonstrated by the i.r. bands at 1650–1680 cm^{-1} (C=N) and by the absence of bands at 1510–



1570 cm^{-1} characteristic of secondary amides. The structure of the oxazoline **7** is also supported by the absence of OH absorption at 3200–3650 cm^{-1}. The i.r. spectrum of **3** shows bands of equal intensity at 1550 and 1665 cm^{-1} characteristic of the CONH grouping.

Condensation[5] of **3** with the potassium salt of di-*tert*-butyl oxaloacetate yielded a lactone (48%) which was not purified but was treated with methyl iodide–silver oxide in methanol to give the methylated lactone **5**. The benzyl and benzylidene groups were removed in a single step by hydrogenolysis over palladium–charcoal. Subsequent opening of the lactone ring under alkaline conditions, and treatment with cation-exchange resin gave the desired 8-*O*-methyl-NANA (**1**).

Besides elemental and functional-group analysis, the structure of **1** was confirmed as follows. The acid **1** gave a colour reaction typical for sialic acid with the Svennerholm[6] and Ehrlich[7] reagents. Esterification of **1** with methanol in the presence of a cation-exchange resin, followed by acetylation with acetic anhydride in pyridine, gave 2,4,7,9-tetra-*O*-acetyl-8-*O*-methyl-*N*-acetylneuraminic acid methyl ester (**8**). The n.m.r. spectrum of **8** showed the presence of one ether and one ester methyl group (singlets at 3.42 and 3.82 p.p.m.). Comparison of the n.m.r. spectra of **8** and of 2,4,7,8,9-penta-*O*-acetyl-*N*-acetylneuraminic acid methyl ester revealed similar patterns in the region of 2.20–3.40 and 3.80–6.00 p.p.m. (*cf.* Ref. 8), confirming the D-*glycero*-D-*galacto* configuration for **8** and for **1**.

Further evidence for the stereochemistry of **1** was obtained on investigation of its inhibitory activity during enzymic hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside[9] with neuraminidase from *Vibrio cholerae*. Both **1** and NANA are competitive inhibitors and exhibit similar affinity for the active site of the neuraminidase (K_i-values 3.6×10^{-3}M and 4.9×10^{-3}M, respectively). The fact that blocking of the OH group at C-8 in NANA does not affect the affinity of the monosaccharide for the active site of the enzyme suggests that this group does not participate in formation of the enzyme–inhibitor complex.

EXPERIMENTAL

General. — Melting points were determined on a Kofler apparatus and are corrected. Optical rotations were measured with a Hilger M-142 polarimeter. Evaporation was performed at 35–40° in *vacuo*. T.l.c. was carried out on KSK silica gel with 5% gypsum by using ether (*A*), chloroform–methanol [95:5 (*B*); and 8:2 (*C*)] or, propyl alcohol–water [7:3 (*D*)]. Detection was effected with conc. sulphuric acid and Svennerholm's reagent[6]. Paper chromatography was carried out on "Goznak" paper with propyl alcohol–butyl alcohol–0.1N hydrochloric acid [2:1:1 (*E*)] and butyl alcohol–pyridine–water [6:4:3 (*F*)]. Detection was effected with aniline hydrogen phthalate and Ehrlich's reagent. N.m.r. spectra were obtained with a JNM-4H-100 spectrometer with tetramethylsilane as internal reference and chloroform-*d* as solvent. I.r. spectra were recorded with a UR-10 spectrophotometer. Absorbance at 400 nm was measured with a SF-4A spectrophotometer.

*2-Methyl-4,5-(2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**6**). — This compound, obtained as described elsewhere[10] in 98,5% yield, had m.p. 169–170°, $[\alpha]_D^{20}$ +9 ±2° (*c* 1.0, pyridine); R_F 0.12, R_{GlcNAc} 1.79 (*B*); ν_{max} 1674 cm^{-1} (C=N).

*2-Methyl-4,5-(4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**2**). — A suspension of **6** (1.05 g) and zinc chloride (0.4 g) in benzaldehyde (50 ml) was stirred magnetically overnight at room temperature in an evacuated device kept dry with phosphoric anhydride. The mixture was poured into a mixture of saturated, aqueous potassium hydrogen carbonate and ice, stirred for 30 min, and, after filtration, extracted with chloroform (5 × 50 ml). The extract was washed with water, dried ($MgSO_4$), and concentrated, and benzaldehyde was removed from the residue at 70°/1 mm. A solution of the resulting syrup in chloroform was washed with aqueous potassium hydrogen carbonate, dried, and evaporated. The residue was treated with light petroleum, and the resulting solid was recrystallised from methanol–ether–light petroleum to give the title compound (0.98 g, 68.5%), m.p. 174–175°, $[\alpha]_D^{20}$ −22,6° (*c* 0.71, chloroform), R_F 0.31 (*B*), ν_{max} 1680 cm^{-1} (C=N) (Found: C, 61.73; H, 5.95; N, 4.77. $C_{15}H_{17}NO_5$ calc.: C, 61.85; H, 5.88; N, 4.88%).

*2-Methyl-4,5-(3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyrano)-2-oxazoline* (**7**). — To a stirred suspension of **2** (0.5 g) and barium oxide (0.5 g) in methyl sulphoxide (20 ml), benzyl chloride (10 ml) was added dropwise. After 3 h, more barium oxide (0.5 g) was added, and the mixture was stirred overnight. The precipitate was filtered off, and washed with chloroform, and the combined filtrates (containing benzyl chloride) were evaporated. The residue was twice recrystallised from methanol to give **7** as colorless needles (0.42 g, 64%), m.p. 169°, $[\alpha]_D^{20}$ −18 ±2° (*c* 1.5, chloroform); R_F 0.47 (*B*), 0.58 (*C*); ν_{max} 1678 cm^{-1} (C=N) (Found: C, 69,03; H, 5.94; N, 3.38. $C_{22}H_{23}NO_5$ calc.: C, 69.29; H, 6.03; N, 3.67%).

*2-Acetamido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-*D*-mannopyranose* (**3**). — To a solution of **7** (0.3 g) in 80% aqueous acetone (5 ml), 0.1N hydrochloric acid (1 ml) was added. After 3 h at 20°, the mixture was evaporated by co-distillation with ethanol, and the residue was recrystallised from ethanol to give **3** (0.30 g, 96%),

m.p. (dec.) *ca.* 120°, $[\alpha]_D^{20}$ −20° (*c* 1.0, methanol), R_F 0.43 (*C*), ν_{max} 1540 and 1645 cm^{-1} (CONH) (Found: C, 65.98; H, 5.97; N, 3.26. $C_{22}H_{25}NO_6$ calc.: C, 66.17; H, 6.26; N, 3.50%).

6-O-Benzyl-7,9-O-benzylidene-8-O-methyl-N-acetylneuraminic acid γ-lactone (**5**). — A suspension of **3** (0.2 g) and the potassium salt of di-*tert*-butyl oxaloacetate[5] (0.15 g) in 1:1 methanol–*p*-dioxane (10 ml) was stirred for 5 days at room temperature, filtered, and, after deionisation with Amberlite IR-120 (H^+), evaporated to dryness. The residue was repeatedly washed with ether, dissolved in *p*-dioxane (10 ml) and heated for 15–20 min at 100° until no more gas was evolved. The solution was decolorised with charcoal and evaporated to dryness to give lactone **4** (0.115 g, 48%), R_F 0.53 (*C*).

Lactone **4** (0.10 g) and freshly prepared silver oxide (3 g) were stirred in dry methanol (3 ml) and methyl iodide (10 ml) for 3 h at 40–45° and then overnight at 20°. After filtration, the precipitate was washed with boiling chloroform (3 × 40 ml), and the combined filtrates were evaporated to dryness. The residue was treated as described above to give the title compound (0.11 g, 92%) as a colourless solid, $[\alpha]_D^{20}$ −4.4° (*c* 1.3, methanol), R_F 0.80 (*D*) (Found: C, 66.01; H, 6.24; N, 2.81. $C_{28}H_{33}NO_8$ calc.: C, 65.74; H, 6.50; N, 2.74%).

N-*Acetylneuraminic acid 8-methyl ether* (**1**). — Lactone **5** (0.10 g) was hydrogenated over 5% palladium-on-carbon (3 g) in methanol (10 ml) for 2 days at room temperature. The catalyst was filtered off and washed with methanol (3 × 50 ml), and the combined filtrates were evaporated. A solution of the residue in 50% aqueous methanol was brought to pH 9 with N sodium hydroxide, kept overnight at 0°, deionized with Amberlite IR-120 (H^+), decolourized with charcoal, and freeze-dried to give **1** (0.05 g, 83%) as a colourless powder, $[\alpha]_D^{20}$ −28° (*c* 1.0, water), R_{NANA} 0.27 (*D*), 0.39 (*E*) (Found: C, 44.16; H, 6.57; N, 4.12; OCH_3, 9.41. $C_{12}H_{21}NO_9$ calc.: C, 44.58; H, 6.55; N, 4.33; OCH_3, 9.60%).

Determination of inhibition activity of NANA and 8-O-methyl-NANA (**1**). — The enzymic hydrolysis of *p*-nitrophenyl *N*-acetyl-α-D-neuraminoside was carried out as described elsewhere[9] in 0.1M acetate buffer (pH 5.6) containing 0.9% of sodium chloride and 0.1% of calcium chloride with 0.3 mg/ml of neuraminidase (from *Vibrio cholerae*, N. V. Philips–Duphar, Holland) at substrate concentrations 0.58, 1.17, 1.75, 2.30, and 3.50 mM at 37°. The substrate was incubated in the presence of NANA or **1** (1.10 mM). *p*-Nitrophenol was determined[11] in incubated and control mixtures by spectrophotometry at 400 nm. The initial rate of hydrolysis was determined by extrapolation to zero time[12]. A plot[13] of $1/V$ against $1/S$ gave, in all the experiments, equal V_{max}-values (53 μmoles/min) and K_i-values of 3.6×10^{-3}M and 4.9×10^{-3}M for **1** and NANA, respectively.

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