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Note

A new approach to the synthesis of 5-*N*-acetyl-D-neuraminic acid α-ketosides

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Synthetic α-ketosides of 5-*N*-acetyl-D-neuraminic acid (NeuNAc), which are useful tools for studies of neuraminidases, are normally prepared by the Koenigs–Knorr procedure, often very successfully[1]. However, difficulties may be encountered with complex aglycons, and as the ratio of aglycon to glycosyl halide decreases. We now report a new approach, some details of which have been published[2].

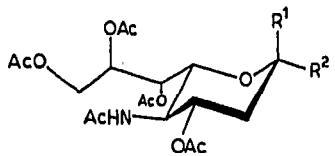
The method is based on the results of Wulff *et al.*[3], who found that glycosyl halides react smoothly with alcohols in the presence of an insoluble silver salt (*e.g.*, of a 1,4-dicarboxylic acid) to yield the respective glycosides. The good yields were explained by participation of a hydroxyl or carboxyl group with the carboxyl anion[4]. Such participation should also occur on the surface of a solid polymer having a steric orientation of functional groups similar to maleic acid or 4-hydroxyvaleric acid. Moreover, such a resin-type reagent should expedite and simplify the experimental method. In seeking suitable, stable and insoluble reagents, several polymeric acids were investigated, including carboxycellulose, sulphonated polystyrene, polymethacrylate, and polymaleate. Most of these polymers lacked the required steric orientation of functional groups and also shrank extensively when converted into the (Ag^+)-form. Thus, formation of the glycosidic linkage and steric control were difficult to achieve, even in the presence of swelling solvents. The best polymer was a cross-linked polymaleic acid prepared from polymaleic acid anhydride[2]. Preliminary experiments confirmed that, under the reaction conditions selected, the α linkage is largely unaffected by the weakly acid ion-exchanger.

In a series of comparative experiments, freshly prepared acetochloroneuraminic acid, or the respective methyl ester, was treated with various aliphatic alcohols at room temperature in the presence of silver polymaleate and silver polymethacrylate. The reactions were monitored by t.l.c. and shown to be complete in 5–12 h. The best results were obtained with an excess of polymeric silver salt, and the yields of products, 50–70%, were higher than those obtained with silver carbonate.

With an excess of alcohol, both polymers yielded similar results, but the polymethacrylate (70–150-mesh beads) was easier to handle than polymaleate. On the

TABLE I

ALCOHOLYSIS OF (5-ACETAMIDO-4,7,8,9-TETRA-*O*-ACETYL-3,5-DIDEOXY-β-D-*glycero*-D-*galacto*-NONULOPYRANOSYL CHLORIDE)ONIC ACID[a] AND ITS METHYL ESTER IN THE PRESENCE OF POLYMERIC ACID-BINDING AGENTS AT 20°



Compound		*Solvent*	*M.p. (degrees)*	$[\alpha]_D^{25}$ *(methanol) (degrees)*	*Yield (%)*	*Ref.*	*Formula*	*Found (%)*			*Calc. (%)*		
R[1]	*R*[2]							*C*	*H*	*N*	*C*	*H*	*N*
CO_2H	OMe	Methanol	185–188 (dec.)	−16.5	63	1	$C_{20}H_{29}NO_{13}$	48.68	6.20	2.60	48.87	5.94	2.85
CO_2Me	OMe	Methanol	135–137	−19	55		$C_{21}H_{31}NO_{13}$	49.17	6.54	2.83	49.89	6.18	2.77
CO_2H	O-$CH_2CH=CH_2$	2-Propenol	167–169 (dec.)	−14	57		$C_{22}H_{31}NO_{13}$	50.90	6.34	2.65	51.05	6.03	2.70
CO_2Me	O-$CH_2CH=CH_2$	2-Propenol	154–156	−13	51		$C_{23}H_{33}NO_{13}$	51.67	6.60	2.76	51.97	6.25	2.63
CO_2H	OBzl	Benzyl alcohol	194–195 (dec.)	− 7.5	64	1	$C_{26}H_{33}NO_{13}$	54.99	6.14	2.21	55.03	5.86	2.47
CO_2Me	OBzl	Benzyl alcohol	85–89	− 3.5	53	5	$C_{27}H_{35}NO_{13}$	55.38	5.80	2.77	55.75	6.07	2.41

[a]Acetochloroneuraminic acid.

other hand, polymethacrylate shrinks substantially on conversion into its (Ag^+)-form. However, although diffusion and steric hindrance are important factors, they do not prevent the desired reactions from proceeding to completion. The conditions permitted all of the reactions to occur at useful rates.

Comparison of the results obtained with the esterified and unesterified 5-*N*-acetyl-D-neuraminic acid derivatives reveals remarkable differences. In t.l.c., the esterified derivatives show less by-products and higher yields (up to 70%), but isolation of pure, crystalline α-ketoside is difficult. The main impurities in both preparations are those leading to 5-*N*-acetyl-2,3-dehydro-2-deoxy-D-neuraminic acid. These findings accord with the results obtained by the Koenigs–Knorr procedure. The ratio of α-ketoside to dehydro compound is influenced by temperature, the solvent, and the amount and basicity of the acid-binding agent. With the aglycon as solvent and a slight excess of polymeric silver salt, the undesired elimination was almost completely prevented.

For complex aglycons, the choice of reaction solvent is important. Its effectiveness depends partly on its ability to swell the resin and partly on other factors such as dielectric constant and solvent power. A standard method for glycosylation was developed, involving acetone, an excess of silver polymaleate, and a ratio of glycosyl halide to aglycon of 1 : 10. In this way, it was possible, for example, to improve the yield of the 2-benzyloxycarbonylaminoethyl α-ketoside of 5-*N*-acetyl-D-neuraminic acid[6] from 30% to 60%. The formation of 5-*N*-acetyl-2,3-dehydro-2-deoxy-D-neuraminic acid was suppressed in favour of α-ketoside synthesis even at a minor ratio of the starting materials. The smooth reaction conditions and simplicity of manipulation allow the synthesis even of sensitive NeuNAc α-ketosides, and the method has been applied[7] in the synthesis of several disaccharides containing 5-*N*-acetylneuraminic acid.

EXPERIMENTAL

M.p.s. were determined with a Tottoli–Büchi apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 141 polarimeter, using a 10-cm micro-cell. I.r. spectra (KBr discs) were recorded with a Beckman IR-8 spectrometer. T.l.c. was performed on silica gel (Merck PF_{254}) with *A*, ethyl acetate; *B*, ether; *C*, 1-butanol–1-propanol–0.1M HCl (1:2:2); and detection with 2,4-dinitrophenylhydrazine–orthophosphoric acid at 150° for 10–20 min. Polymethacrylic acid (No. 5236) and polymaleic acid anhydride (No. 10272) were purchased from Merck, Darmstadt, Germany. 5-*N*-Acetyl-D-neuraminic acid (m.p. 184–185°) was prepared from meconium[8]. *Vibrio cholerae* neuraminidase was obtained from Behring-Werke, Marburg; 1 ml contained 500 units (manufacturer's specification).

Treatment with neuraminidase was carried out as follows. A solution of the saponified, deacetylated α-ketoside (200–600 μg) in 0.05M Tris-maleate buffer (pH 6.4, 0.5 ml) containing 5mM calcium chloride and 100 μl of neuraminidase was

incubated at 37°. Samples (100 µl) taken at intervals were analysed with the thiobarbiturate assay[9].

Polymaleic acid. — (*a*) *Sodium salt.* A suspension of cross-linked polymaleic acid anhydride (10 g) in water (200 ml) was separated from fines and then shaken with M sodium hydroxide (50 ml) for several hours at room temperature. The solids were collected, and thoroughly washed with water, acetone, and ether, to give the sodium salt as a white, amorphous substance.

(*b*) *Silver salt.* A mixture of sodium polymaleate (4 g) in water (100 ml) was adjusted to pH 7 followed by the dropwise addition of a solution of silver nitrate (12 g) in water (50 ml). After being shaken for several hours in the dark, the resin was collected, washed twice with the filtrate, then with water, acetone, and ether. The light-sensitive silver salt was obtained as an amorphous, white product, which was stored in the dark over P_4O_{10}.

(*c*) *Silver polymethacrylate.* The ammonium salt of the resin, prepared from the (H^+)-form (25 ml) by treatment with M ammonium hydroxide, was added in portions to a solution of silver nitrate (10 g) in water (200 ml). After stirring in the dark for 1 h, the macroporous gel was collected, washed with the filtrate, then with water, acetone, and ether. The light-sensitive, yellowish beads were dried over P_4O_{10}. Activity is lost on prolonged storage.

5-N-Acetyl-4,7,8,9-tetra-O-acetyl-α-D-neuraminic acid alkyl glycosides. — Acetochloroneuraminic acid, freshly prepared from peracetylated neuraminic acid (2 mmol), was dissolved in the appropriate alcohol (50–250 ml), silver polymaleate (4 ml) was added, and the suspension was stirred in the dark for 12 h. The precipitate was collected over Celite and washed with acetone. The combined filtrates and washings were concentrated, and a solution of the residue in methanol was deionised with Dowex-50W X8(H^+) resin at −10° and then concentrated to dryness. The residue was crystallised from methanol–ether (1 : 1) by the dropwise addition of light petroleum (b.p. 40°). The yields of the products are given in Table I.

Methyl 5-N-acetyl-4,7,8,9-tetra-O-acetyl-α-D-neuraminate alkyl glycosides. — Using methyl 2,4,7,8,9-penta-*O*-acetyl-D-neuraminate (obtained from the acid by esterification with diazomethane) and following the procedure described above, the title compounds (the data for which are given in Table I) were prepared.

2-Aminoethyl α-glycoside of 5-N-acetyl-D-neuraminic acid. — Methyl acetochloroneuraminate, prepared from peracetylated methyl 5-*N*-acetyl-D-neuraminate (0.5 g, 0.94 mmol), was dissolved in a solution of 2-(benzyloxycarbonylamino)ethanol (2 g, 10 mmol) in acetone (2 ml) and stirred with silver polymaleate (1 g) at room temperature for 15 h. The solids were collected, and washed with nitromethane, and the combined filtrate and washings were concentrated to dryness. The crude material was eluted from silica gel with ether. *O*-Deacetylation was effected by treatment with M NaOH (5 ml) for 15 min. The solution was deionised with Dowex-50W X8(H^+) resin, concentrated, and freeze-dried. To a solution of the residue (365 mg) in ethanol–water (1 : 1) was added palladium oxide (100 mg), and hydrogenation was carried out overnight at room temperature and atmospheric pressure. The mixture was

filtered and concentrated, and a solution of the resulting, colourless glass in water was passed through a column of Dowex-1 X4(AcO^-) resin which was washed with water until a negative reaction with the Ehrlich reagent was obtained. Evaporation of solvent and freeze-drying gave the chromatographically homogeneous, title product (192 mg, 58%), $[\alpha]_D^{25}$ −17° (*c* 0.5, water). Using silver polymethacrylate, the yield was 20.3%, and 25% was obtained with silver carbonate.

Anal. Calc. for $C_{13}H_{24}N_2O_9 \cdot 2H_2O$: C, 40.21; H, 7.26; N, 7.22. Found: C, 40.42; H, 7.22; N, 7.32.

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