

## 7,9-*O*-Ethylidene and 7,9-*O*-Benzylidene Derivatives of 5-Acetamido-3,5-dideoxy-*D*-glycero-*D*-galacto-nonulosonic Acid (*N*-Acetylneuraminic Acid)

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7,9-*O*-Benzylidene and 7,9-*O*-ethylidene derivatives of *N*-acetylneuraminic acid have been prepared, using the method devised by Kuhn and Baschang for the synthesis of 5-acetamido-3,5-dideoxy-*D*-glycero-*D*-galacto-nonulosonic acid (*N*-acetylneuraminic acid). NMR studies confirm the proposed structures.

The *D*-galacto-configuration of the benzylidene derivatives was confirmed by degradation of the parent 7,9-*O*-benzylidene derivative to *N*-acetylneuraminic acid.

The 7,9-*O*-ethylidene derivatives, which were prepared in the same way as the benzylidene derivatives, are also assumed to have the *D*-galacto-configuration.

5-Acetamido-3,5-dideoxy-*D*-glycero-*D*-galacto-nonulosonic acid (*N*-acetylneuraminic acid) has been reported to act as competitive inhibitor of the neuraminidase isolated from *Vibrio cholerae*.<sup>1-2</sup> In order to investigate the effects of different derivatives of *N*-acetylneuraminic acid, the synthesis of the 7,9-*O*-ethylidene and 7,9-*O*-benzylidene derivatives of *N*-acetylneuraminic acid were undertaken, and the results are reported herein.

Kuhn and Baschang<sup>3</sup> have demonstrated that the reaction between 2-acetamido-2-deoxy-4,6-*O*-benzylidene- $\beta$ -*D*-glucose and potassium di-*t*-butyl oxalacetate in dioxane-methanol which, in principle, could give four diastereoisomeric *N*-acetylnonulosaminic acids, affords predominantly *N*-acetylneuraminic acid. In the present work, the 7,9-*O*-benzylidene derivative of 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-*D*-glycero-*D*-galacto-nonulosono-1,4-lactone, an intermediate in this reaction has been isolated.

Hydrolysis of the *t*-butoxycarbonyl group, decarboxylation and opening of the lactone ring led to the 7,9-*O*-benzylidene derivative of *N*-acetylneuraminic acid. During removal of the *t*-butoxycarbonyl group, the cyclic acetal group was hydrolysed to a considerable extent, thereby reducing the yield.

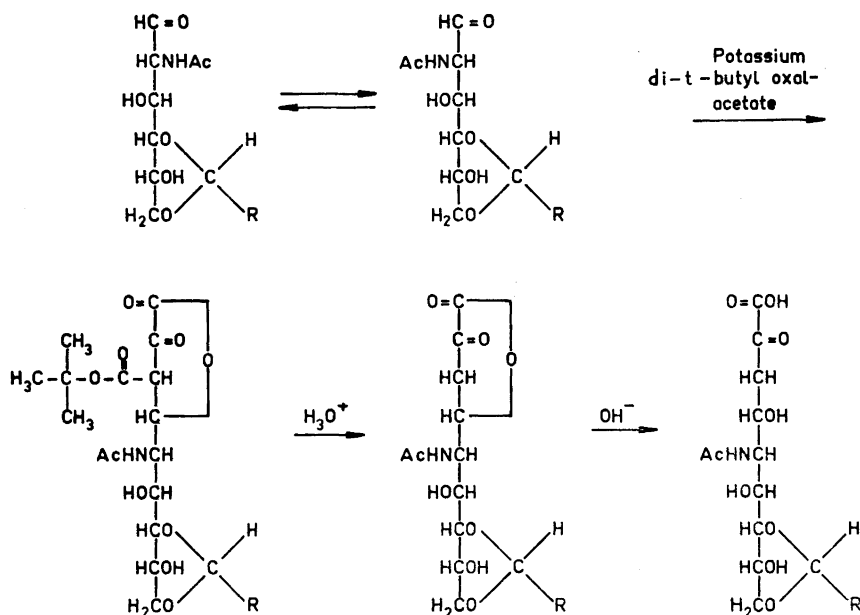


Fig. 1.

Analogously 2-acetamido-2-deoxy-4,6-*O*-ethylidene-D-glucose<sup>4</sup> afforded the 7,9-*O*-ethylidene derivative of 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-nonulosono-1,4-lactone. This lactone was isolated and treated similarly to the corresponding 7,9-*O*-benzylidene derivative.

The 7,9-*O*-benzylidene and 7,9-*O*-ethylidene derivatives of *N*-acetylneuraminic acid were isolated as stable sodium salts, the free acids being labile. Aqueous solutions of the free acids on evaporation at 40° rapidly turned red.

The proposed structures of the lactones and acids prepared herein were confirmed by NMR studies. Chemical shifts were consistent with expected values. The lactones, like the di-*t*-butyl oxalacetate, were weakly acidic and react as enols, giving a red colour with ferric ion. NMR spectra of the lactones in hexadeuteriomethylsulfoxide showed minor peaks which probably originated from enolic structures. The intensities of these peaks were diminished when deuteriumoxide was used as solvent.

The *D*-galacto configuration of the benzylidene derivatives was determined after hydrogenolysis of the benzylidene group. The pure 7,9-*O*-benzylidene derivative of 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-nonulosono-1,4-lactone was hydrogenated at room temperature and atmospheric pressure, using a palladium on charcoal catalyst. On chromatography of the hydrogenated product, only one spot could be detected with the different reagents for *N*-acetylnonulosonolactones. The observed spot and authentic 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-*D*-glycero-*D*-galacto-nonulosono-1,4-lactone had the same  $R_F$ -value, which is much lower than that of the correspond-

ing *D-gulo* derivative.<sup>3</sup> Hydrolysis and decarboxylation of the hydrogenated product gave 5-acetamido-3,5-dideoxy-*D-glycero-D-galacto*-nonulosonic acid, indistinguishable from an authentic sample. This confirms the proposed *D-galacto* configuration of the benzylidene derivative, which may possibly be contaminated with the corresponding *D-talo* derivative.

As the 7,9-*O*-ethylidene derivatives were prepared under the same conditions as the benzylidene derivatives, it is reasonable to assume that they also have the *D-galacto* configuration.

## EXPERIMENTAL

*General methods.* Concentrations were carried out under reduced pressure at a bath temperature not exceeding 40°. Melting points (dec.) were determined as previously described<sup>4</sup> and are corrected.

NMR-Analyses were performed with a Varian A-60 A instrument, and elemental analyses with a Hewlett Packard Model 185 CNH Analyser. Optical rotations were determined with a Perkin-Elmer 141 polarimeter, using a 10 cm micro cell.

Reactions and purifications were followed by TLC, using silica gel (Merck HR and Merck HF<sub>254</sub>) activated at 120° for 30 min, and the solvents: (a) ethyl acetate-acetic acid-water, 9:2:2; (b) tetrahydrofuran. Paper chromatography was performed on Whatman No. 1 paper, using ethyl acetate-acetic acid-water, 9:2:2, as solvent. Spray reagents: (a) 2,4-dinitrophenylhydrazine-orthophosphoric acid reagent<sup>5</sup> and heating for 10–20 min at 125°; (b) *p*-dimethylaminobenzaldehyde-trichloroacetic acid reagent<sup>6</sup> and heating as under (a); and (c) ferric chloride in water.

Column chromatography was carried out on silica gel (Merck 0.05–0.20 mm) washed with conc. hydrochloric acid<sup>7</sup> to remove traces of iron. Tetrahydrofuran was used as solvent with a flow rate of 0.1–0.2 ml cm<sup>-2</sup> min<sup>-1</sup>. The ratio of sample to silica gel was 1:30 (w/w).

## Synthesis

*7,9-O-Ethylidene and 7,9-O-benzylidene derivatives of 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-*D*-glycero-*D*-galacto-nonulosono-1,4-lactone.* A mixture of the 4,6-*O*-benzylidene or 4,6-*O*-ethylidene derivative of 2-acetamido-2-deoxy-*D*-glucose (10.0 g) and potassium di-*t*-butyl oxalacetate (10.0 and 13.0 g, respectively) in dioxane-anhydrous methanol (1:1, 300 ml) was shaken for 14 days at 22°. The homogeneous solution was then passed through a column (3 × 10 cm) of Dowex 50W-X8 (H<sup>+</sup>), 100–200 mesh, previously equilibrated with methanol. The resin was washed with methanol until the eluate gave a negative reaction with the *p*-dimethylaminobenzaldehyde reagent.

After evaporation of the solvent, the residue was extracted with ethyl acetate (15 ml per g product). The solution was filtered, and the lactone was precipitated with five volumes of ligroin (80–110°). After 24 h at 5°, the precipitate was filtered off, washed with ligroin and dried, giving 10.3 g (67 %) of the benzylidene derivative and 11.1 g (65 %) of the ethylidene derivative.

The crystalline product was further purified on a silica gel column, using tetrahydrofuran as solvent. The recovery of chromatographically homogeneous compound from the column was approximately 75 %. The compound was dissolved in a small amount of ethyl acetate and precipitated as above. Physical constants and analyses are given in Table 1.

*7,9-O-Ethylidene and 7,9-O-benzylidene derivatives of 5-acetamido-3,5-dideoxy-*D*-glycero-*D*-galacto-nonulosono-1,4-lactone.* The pure *t*-butoxycarbonyl lactone (1.5 g) was added to a boiling mixture of methanol-water (1:9, 80–100 ml) which was then heated on a boiling water bath for 15 min. Isobutene and carbon dioxide were liberated. A small amount of activated carbon was added, and the solution was rapidly cooled to room temperature, filtered and concentrated. The resulting solid on chromatography showed only traces of the *t*-butoxycarbonyl lactone, but in addition to the main product, an impurity with a low *R<sub>F</sub>*-value was observed. The latter did not contain a cyclic acetal group.

The residue was extracted with acetone, and insoluble material removed by centrifugation. The solvent was evaporated and the residue dried, giving 0.87 g (73 %) of the ethylidene and 0.35 g (28 %) of the benzylidene derivative (monohydrate). The product was further purified on a silica gel column with tetrahydrofuran as solvent. The chromatographically homogeneous compound was dissolved in a small amount of acetone and precipitated with 10 volumes of ethyl ether. After 24 h at 5°, the precipitate was collected and dried.

The recovery of pure product from the column was approximately 80 % for the ethylidene and 50 % for the benzylidene derivative. Physical constants and analyses are given in Table 1.

Table 1. Properties of derivatives of *N*-acetylneuraminic acid.

Compound	m.p. <sup>a</sup> (dec.)°	[α] <sub>D</sub> <sup>25</sup> (c 1.0) Methanol	Calc.			Found		
			C	H	N	C	H	N
R <sub>1</sub> -Benzylidene-R <sub>2</sub>	151–152	–42°	57.6	6.10	2.92	58.3	6.17	2.81
R <sub>1</sub> -Benzylidene-R <sub>3</sub>	159–160	–92°	54.4	5.83	3.52	54.1	5.77	3.32
R <sub>4</sub> -Benzylidene-R <sub>5</sub>	150–170	–50°	49.3	5.75	3.19	48.8	5.84	2.67
R <sub>1</sub> -Ethylidene-R <sub>2</sub>	126–127	–24°	51.8	6.52	3.35	51.9	6.82	3.18
R <sub>1</sub> -Ethylidene-R <sub>3</sub>	149–150	–66°	46.6	6.31	4.18	46.5	6.48	3.96
R <sub>4</sub> -Ethylidene-R <sub>5</sub>	150–170	–44°	41.5	6.16	3.72	41.0	5.95	3.50

R<sub>1</sub> = 5-Acetamido-3,5-dideoxy-7,9-*O*.

R<sub>2</sub> = 3-*t*-Butoxycarbonyl-D-glycero-D-galacto-nonulosono-1,4-lactone.

R<sub>3</sub> = D-glycero-D-galacto-Nonulosono-1,4-lactone monohydrate.

R<sub>4</sub> = Sodium 5-acetamido-3,5-dideoxy-7,9-*O*.

R<sub>5</sub> = D-glycero-D-galacto-Nonulosonate dihydrate.

<sup>a</sup> = By evolution of gas.

*7,9-O-Ethylidene and 7,9-O-benzylidene derivatives of sodium 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-nonulosonate.* To an aqueous solution of the lactone at room temperature an equivalent amount of 2 M sodium hydroxide was added dropwise at such rate that the pH never exceeded 9.0. One hour after the last addition, the neutral solution was concentrated and the residue dried over diphosphorus pentoxide. The solid was then dissolved in a small amount of methanol and precipitated with ethyl ether. The precipitate was collected by centrifugation and dried. Physical constants and analyses are given in Table 1.

*Isolation of 5-acetamido-3,5-dideoxy-D-glycero-D-galacto-nonulosonic acid.* The pure 7,9-*O*-benzylidene acetal (500 mg) of 5-acetamido-3,5-dideoxy-3-*t*-butoxycarbonyl-nonulosono-1,4-lactone was dissolved in a mixture of methanol-water (9:1, 2.0 ml) and hydrogenated at 22° and atmospheric pressure, using a 10 % palladium on charcoal catalyst (200 mg). After 24 h, the catalyst was removed by centrifugation and the solvent was evaporated. The residue was dissolved in water (10–20 ml), hydrolysed and decarboxylated as described above.

The resulting solution was treated as described by Kuhn and Baschang,<sup>3</sup> giving a product which was indistinguishable from an authentic sample of *N*-acetylneuraminic acid.

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