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A NEW APPROACH TO THE SYNTHESIS OF HIGHER 3-DEOXYGLYCULOSONIC ACIDS

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INTRODUCTION

3-Deoxyglyculosonic acids are currently of special interest. Higher 3-deoxyglyculosonic acids, containing 7–9 carbon atoms, play an important role in some biosynthetic processes[1] and in the formation of specific biopolymers[2–4]. The biological significance of sialic acids, which also belong to this group, is well known[5,6].

The only general method of synthesis of higher 3-deoxyglyculosonic acids involves condensation of monosaccharides with oxalacetic acid[7] or di-*tert*-butyl oxalacetate[8]. However, this method gives mixtures of several isomeric acids that are difficult to separate, and, in consequence, synthetic 3-deoxyglyculosonic acids are rather inaccessible.

We now wish to describe a new approach to the synthesis of higher 3-deoxyglyculosonic acids, including the conversion of monosaccharides (*e.g.* D-galactose) by the Wittig reaction into 3,4-dideoxyglyculos-3-enonic acids.

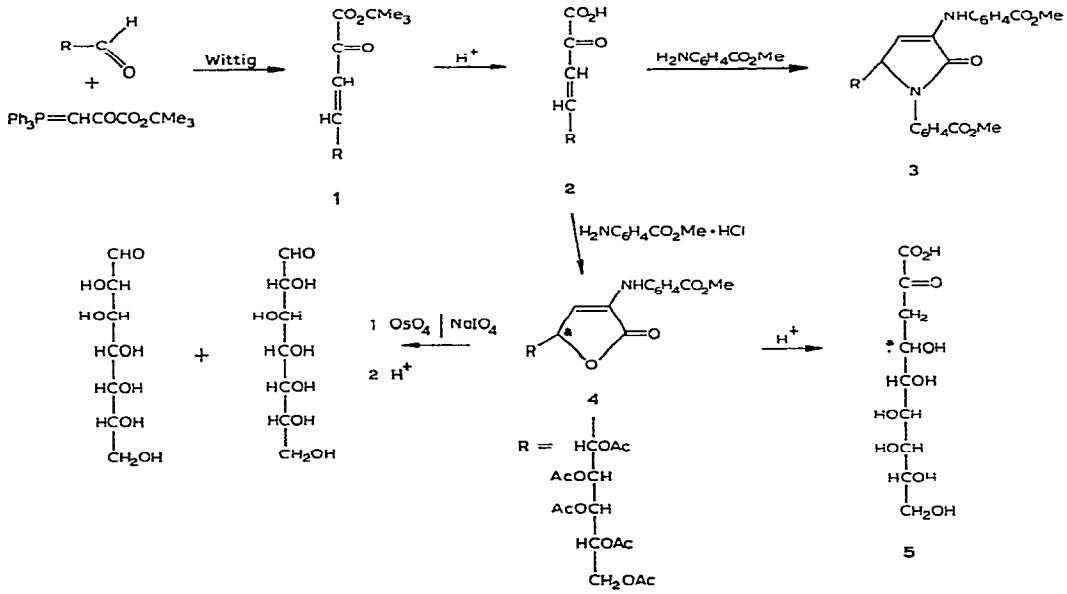
RESULTS AND DISCUSSION

The unsaturated glyculosonic acids, which are the key intermediates in the new syntheses, were obtained by the condensation of *aldehydo*-forms of monosaccharides with (alkoxyoxalyl)methylenetriphenylphosphorane, the latter being synthesized from an alkyl bromopyruvate[9]. This reaction results in elongation of the original carbon chain by three carbon atoms, with simultaneous introduction of the required functional groupings.

The reaction of penta-*O*-acetyl-*aldehydo*-D-galactose with (*tert*-butoxyoxalyl)-methylenetriphenylphosphorane proceeds smoothly in boiling benzene to give a high yield of *tert*-butyl 5,6,7,8,9-penta-*O*-acetyl-3,4-dideoxy-D-*galacto*-nonulos-*trans*-3-enonate (**1**). The use of (*tert*-butoxyoxalyl)methylenetriphenylphosphorane is of especial convenience, since the transformation of the *tert*-butyl ester **1** into the corresponding unsaturated acid **2** could be accomplished in quantitative yield with trifluoroacetic acid under mild conditions.

The conversion of the unsaturated acid **2** into a 3-deoxyglyculosonic acid might be achieved either by hydration of the double bond or by cyclisation, the latter process giving the corresponding lactone. The hydration and/or lactonisation of β,γ-enoic

acids proceeds in the presence of concentrated mineral acids under drastic conditions that cannot be applied to the carbohydrate derivatives. Arylidenepyruvic acids have been converted into *N*-aryl-enaminolactones by the action of aromatic amines[10]. However, under these conditions, the unsaturated acid **2** gave mainly the enaminolactam instead of the expected enaminolactone. Treatment of acid **2** with methyl



p-aminobenzoate resulted in formation of the *N,N'*-di[(*p*-methoxycarbonyl)phenyl]-enaminolactam **3** as the major product, the *N*-[(*p*-methoxycarbonyl)phenyl]enaminolactone **4** being isolated in much lower yield. Structure **3** follows from the n.m.r. spectrum (Fig. 1), which showed peaks at δ *ca.* 2 (five *O*-acetyl groups), 3.88 and 3.91 (two methoxy-groups), 4.97–5.40 (complex multiplet, C-4–C-8 protons), 6.15 (singlet, olefinic proton), and 6.98 (NH proton). Two doublets centered at δ 7.00 and 7.93 (J 8 Hz) correspond to protons of the aromatic nucleus linked with the nitrogen atom of the enamino group, and the protons of the aromatic nucleus linked to the nitrogen atom of the lactam ring gave two doublets centered at δ 7.60 and 8.67 (J 9 Hz).

Formation of the enaminolactam **3** results from nucleophilic addition of the amine to the conjugated double bond, followed by cyclisation of the γ-amino acid. In the case of arylidenepyruvic acids, no addition to the double bond occurs, and the reaction appears to proceed *via* anil formation. In order to obtain the enaminolactone **4** from the unsaturated acid **2**, nucleophilic attack must be prevented, *e.g.*, by use of the amine salt instead of the free base. Indeed, the condensation of the unsaturated acid **2** with methyl *p*-aminobenzoate hydrochloride in glacial acetic acid at 70° resulted in formation of the enaminolactone **4**, isolated in *ca.* 45% yield after chromatography. The n.m.r. spectrum of **4** (Fig. 2) supports the suggested structure and contains two doublets centered at δ 6.98 and 7.97 (J 8.0 Hz, 4 aromatic protons), peaks corresponding to five *O*-acetyl groups and one methoxyl group (δ 3.84), a

complex multiplet (δ 5.07–5.66, H-4–H-8), and an AB octet of an ABX system (δ 3.67–4.47, C-9 methylene protons). The singlet at δ 6.18 is assigned to the olefinic

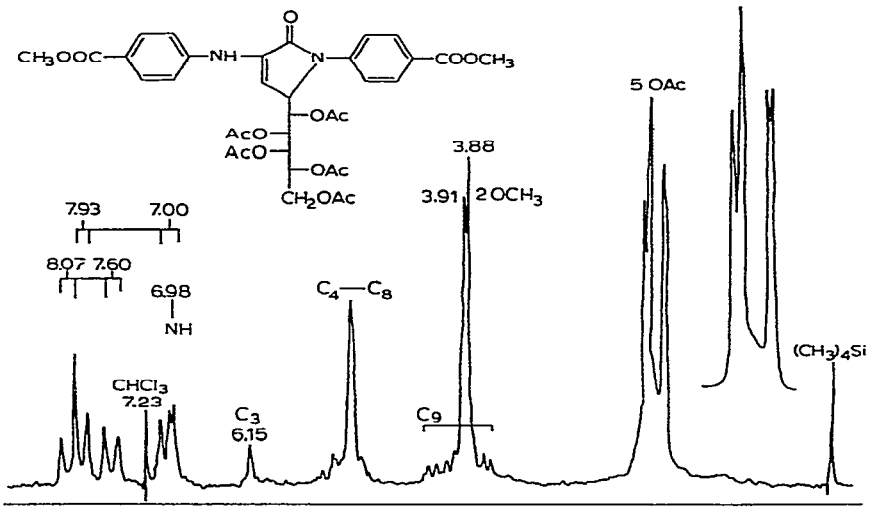


Fig. 1. N.m.r.-spectrum of *N,N'*-di[(*p*-methoxycarbonyl)phenyl]-2-enamino-4-(D-*galacto*-pentaacetoxypentyl)-4-butanelactam (**3**). (The right-hand, upper portion shows the signals for the *O*-acetyl groups recorded at smaller sweep-width).

proton at C-3, whilst that at 6.91 corresponds to the NH-group at C-2, since the latter signal showed an upfield shift on dilution.

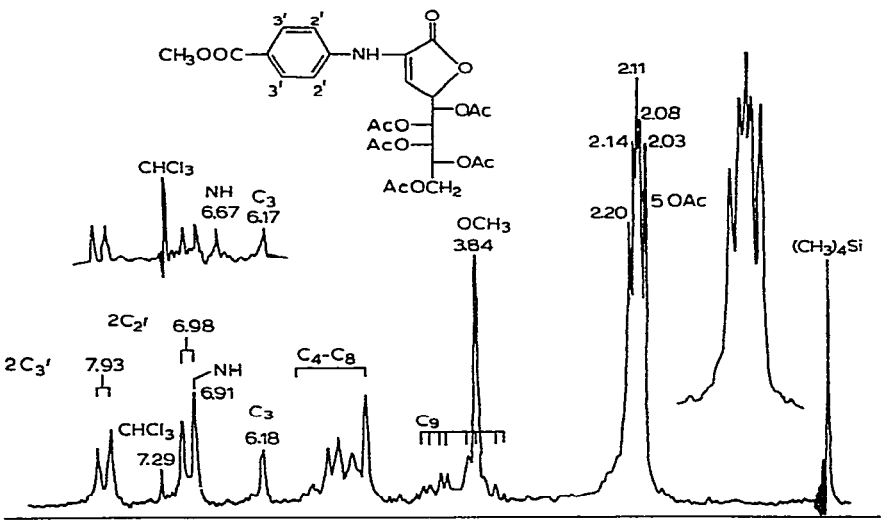


Fig. 2. N.m.r.-spectrum of 2-[*N*-(*p*-methoxycarbonyl)phenyl]enamino-4-(D-*galacto*-pentaacetoxypentyl)-4-butanolide (**4**) (the left-hand upper portion corresponds to the diluted solution, the right-hand one to the *O*-acetyl signals recorded at smaller sweep-width).

The presence of the enamino group in the lactone **4** was proved by identification of methyl *p*-aminobenzoate formed on mild acid hydrolysis. The condensation of the acid **2** with methyl *p*-aminobenzoate hydrochloride may give rise to two enaminolactones isomeric at C-4. Indeed, the product obtained, in spite of its apparent homogeneity on t.l.c., was shown to be a mixture of epimers by the formation of two heptoses on scission of the double bond in lactone **4** by treatment with sodium metaperiodate–osmium tetroxide in aqueous *N,N*-dimethylformamide. Methanolysis and hydrolysis of the product gave a mixture (identified by paper chromatography[11]) of D-*glycero*-L-*manno*-heptose, D-*glycero*-L-*gluco*-heptose, and D-galactose. The above heptoses were synthesized from D-galactose by Sowden's method[12]. The appearance of D-galactose in the degradation mixture is apparently due to hydrolytic elimination of *p*-(methoxycarbonyl)oxanilic acid with subsequent periodate oxidation of the sugar moiety.

The final step in the synthesis, involving conversion of enaminolactone **4** into free acid **5**, was accomplished by the action of 0.2N methanolic hydrogen chloride at 75°, followed by hydrolysis. The acids **5**, isolated as the barium salts, could be separated by paper chromatography (see Experimental) and gave positive periodate–thiobarbituric acid[13] (λ_{max} 550 nm) and *o*-phenylenediamine tests[14] (λ_{max} 335 nm), thus proving them to be 3-deoxyglyculosonic acids. The isolation of individual acids is now in progress.

EXPERIMENTAL

General. — Paper chromatograms were run on the Leningrad factory No. 2 paper "M" by the ascending technique with the following systems (v/v): (*A*) butyl alcohol–acetic acid–water (4:1:1), and (*B*) butyl alcohol–pyridine–water (5:3:2), and by the descending technique in (*C*) butyl alcohol–pyridine–water (6:4:3), (*D*) propyl alcohol–benzyl alcohol–85% formic acid–water (5:7:2:2), and (*E*) butyl alcohol–pyridine–0.1N hydrochloric acid (5:3:2). Paper electrophoresis was performed in 0.01M pyridine-acetate buffer, pH 4.5, at 20 volts/cm for 0.5–1 h. Substances were detected on paper by the following spray reagents: periodate–benzidine, alkaline silver nitrate, sodium periodate–thiobarbituric acid (Warren's reagent[15]), *p*-anisidine phosphate, and a specific reagent[16] for heptoses. Thin-layer chromatography (t.l.c.) was performed on silica gel KSK in chloroform–methanol (19:1), and detection was effected with conc. sulfuric acid, aqueous potassium permanganate, or iodine vapour. Evaporations were carried out under diminished pressure at temperatures not exceeding 40°. Melting points were determined on a Kofler micro-heating stage. N.m.r. spectra were measured on an RS-60 NMR spectrometer on solutions in deuteriochloroform with tetramethylsilane as the internal standard.

tert-*Butyl 5,6,7,8,9-penta*-O-*acetyl-3,4-dideoxy*-D-galacto-*nonulos*-trans-3-*enonate* (**1**)[9]. — A solution of penta-*O*-acetyl-*aldehydo*-D-galactose (0.39 g) and (*tert*-butoxy oxalyl)methylenetriphenylphosphorane[9] (0.5 g, 25% excess) in dry benzene (50 ml) was refluxed for 20 h. The cooled mixture was concentrated and placed on a column

of silicic acid (50 g). The column was washed with gradients of increasing concentration of ether in benzene from 0 to 50%. Fractions were monitored by t.l.c., and those containing pure substance (R_F 0.80) were combined and evaporated to dryness. Crystallization of the residue from ethyl acetate–hexane gave the ester **1** (425 mg, 80%), m.p. 102–104°, $[\alpha]_D^{20}$ +27.2° (*c* 4.18, benzene).

Anal. Calc. for $C_{23}H_{32}O_{13}$: C, 53.48; H, 6.25. Found: C, 53.89; H, 6.61.

*5,6,7,8,9-Penta-O-acetyl-3,4-dideoxy-*D-galacto-*nonulose-*trans-*3-enonic acid* (**2**). — Ester **1** (100 mg) was dissolved in trifluoroacetic acid (1 ml), the clear solution was kept for 40 min at room temperature and evaporated to dryness, and benzene (5 ml) was distilled from the residue. After threefold evaporation with benzene, the residue was dried *in vacuo* at 40°, and the acid **2** was obtained in quantitative yield as a white, amorphous powder. The analytical sample was obtained as follows: a solution of the dry residue in saturated, aqueous sodium hydrogen carbonate (5 ml) was washed with chloroform (2 × 5 ml), acidified to pH 3 with 2N hydrochloric acid, and extracted with chloroform. The extract was dried, evaporated, and crystallized from ethyl acetate–hexane to give **2** (40 mg), m.p. 98–104°, $[\alpha]_D^{20}$ +26.9° (*c* 3.22, chloroform).

Anal. Calc. for $C_{19}H_{24}O_{13}$: C, 49.56; H, 5.25. Found: C, 49.53; H, 5.63.

N,N′-*Di-*[(p-*methoxycarbonyl)phenyl*]-*2-enamino-4-*(D-galacto-*pentaacetoxypentyl*)-*4-butanelactam* (**3**). — To a solution of acid **2** (from 256 mg of ester **1**) in glacial acetic acid (2.5 ml) was added methyl *p*-aminobenzoate (75.5 mg), and the solution was kept for 5 days at room temperature. The reaction mixture was evaporated to dryness, the residue was dissolved in chloroform (10 ml), and the solution was washed successively with 2N hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried with magnesium sulfate, and evaporated. The residue was dissolved in a few ml of benzene and chromatographed on a column of silica gel (20 g), using benzene–ether mixtures with increasing proportions of ether (0 to 50%, v/v). Fractions were monitored by t.l.c., and evaporation of appropriate fractions gave 200 mg of crystalline residue, which, on recrystallization from 4 ml of methanol, gave lactam **3** (140 mg) as needles, m.p. 207–209°, $[\alpha]_D^{20}$ −3.4° (*c* 4.14, chloroform).

Anal. Calc. for $C_{35}H_{38}N_2O_{15}$: C, 57.85; H, 5.27; N, 3.86. Found: C, 57.91; H, 5.45; N, 3.71.

Evaporation of the mother liquor and recrystallization from methanol (2 ml) gave lactone **4** (40 mg) as rosettes, m.p. 180°.

2-[N-(p-*Methoxycarbonyl)phenyl*]*enamino-4-*(D-galacto-*pentaacetoxypentyl*)-*4-butanolide* (**4**). — To a solution of acid **2** (from 520 mg of ester **1**) in glacial acetic acid (5 ml) was added methyl *p*-aminobenzoate hydrochloride (190 mg), and the solution was kept for 6 h at 70°. The darkened mixture was evaporated to dryness, and a solution of the residue in chloroform (10 ml) was washed with water, saturated aqueous sodium hydrogen carbonate (4 × 25 ml), and water, dried with magnesium sulfate, and evaporated. The residue was chromatographed on silica gel (30 g), using benzene–ether mixtures, to yield 270 mg (45%) of lactone **4**. It was twice recrystallized from methanol, and 250 mg of analytically pure lactone **4** were obtained as rosettes, m.p. 181° (sinters at 168–170°), $[\alpha]_D^{23}$ +57.0° (*c* 3.38, chloroform).

Anal. Calc. for $C_{27}H_{31}NO_{14}$: C, 54.63; H, 5.26; N, 2.36. Found: C, 54.45; H, 5.24; N, 2.52.

Conversion of enaminolactone **4** *into heptoses.* — To a stirred solution of enaminolactone **4** (10 mg) in 80% aqueous *N*,*N*-dimethylformamide (1 ml) was added one crystal of osmium tetroxide. After 15 min, small portions of sodium metaperiodate (total, 50 mg) were added to the darkened mixture, which was stirred for 2 h. The colourless solution was filtered, diluted with chloroform (5 ml), and washed with 5% aqueous sodium thiosulfate and water, dried with magnesium sulfate, and evaporated to dryness. A solution of the syrupy residue in 0.2N methanolic hydrogen chloride (1 ml) was boiled under reflux for 2 h and then evaporated to dryness. The residue was heated with 2N hydrochloric acid (1 ml) for 3 h at 100°. The cooled solution was diluted with water (10 ml) and passed through columns of Amberlite IRA-400 (AcO^-, 3 ml) and KU-2 (H^+, 2 ml), and the eluate was concentrated to 1 ml. This hydrolyzate contained D-galactose, D-*glycero*-L-*manno*-heptose, and D-*glycero*-L-*gluco*-heptose, indistinguishable from authentic sugars on paper chromatograms in systems *B*, *C*, and *D*.

3-Deoxy-D-glycero-L-manno- *and* -L-gluco-*nonulosonic acids* (**5**). — A solution of enaminolactone **4** (0.2 g) in 0.2N methanolic hydrogen chloride (100 ml) was boiled under reflux for 2 h, water (20 ml) was added, and the mixture was heated for 1 h at 80°. The colourless solution was cooled, the methanol was evaporated *in vacuo* with addition of water, and the solution was neutralized with sodium hydrogen carbonate and extracted with chloroform (3 × 25 ml). The chloroform extract contained (t.l.c. and electrophoresis) methyl *p*-aminobenzoate. The aqueous solution was treated (to pH 3) batchwise with cation-exchanger KU-2 (H^+), filtered, stirred with excess of silver carbonate, filtered, and passed through a column of cation-exchanger KU-2 (H^+). The eluate was concentrated *in vacuo* to a small volume and then freeze-dried. The resulting product contained the free acids **5** and their lactones (R_F 0.10 and 0.20 in system *A*; two spots, one moving towards the anode and the second being immobile, were detected on a paper electrophoretogram). The product was dissolved in water (5 ml), 0.1N barium hydroxide was added with stirring to pH 7.5, and the stirring was continued for 20 min. The mixture was neutralized with carbon dioxide, filtered, concentrated *in vacuo*, and filtered, and the clear solution was freeze-dried. The resulting white solid (94.3 mg) was the trihydrate, $[\alpha]_D^{20}$ −21.0° (*c* 4.27, water), of the barium salt of the title compound.

Anal. Calc. for $(C_9H_{15}O_9)_2Ba.3H_2O$: C, 29.78; H, 5.00; Ba, 18.93. Found: C, 29.77; H, 4.85; Ba, 19.59.

The product migrated as a single spot (R_F 0.07) on chromatography in system *A*, and on electrophoresis, but could be separated in system *E* (Whatman No. 2 paper, 3 days) into two spots, with mobilities of 14.7 and 19.3 cm, in the ratio of *ca.* 10:1.

SUMMARY

A novel approach to the synthesis of 3-deoxyglyculosonic acids is reported. The key step in this synthesis involves the transformation of a monosaccharide by the

Wittig reaction into a 3,4-dideoxyglyculos-3-enonic acid, and subsequent lactonisation.

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