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STEREOSELECTIVE SYNTHESIS OF A β-D-PHOSPHATIDYLGALACTOSE

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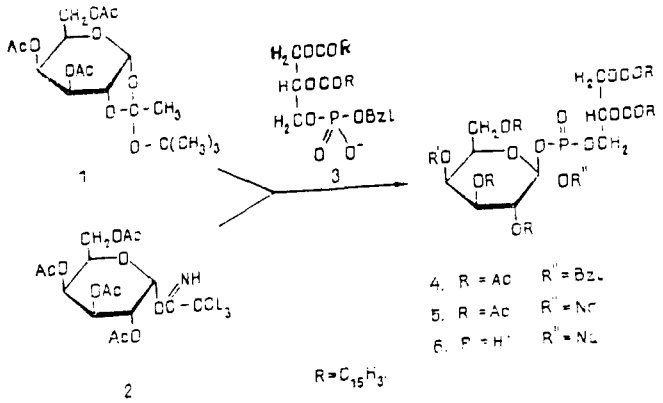
The stereoselective synthesis of a β-D-phosphatidylgalactose has been achieved by the interaction of a benzyl phosphatidate with D-galactose tert-butyl orthoacetate or trichloroacetimidate.

We have previously performed the synthesis of a β-D-phosphatidyl-glucose on the basis of the stereoselective phosphorylation of an orthoester or the trichloroacetimidate of D-glucose with a benzyl phosphatidate [1]. In a continuation of structural-functional investigations of glycophospholipids, in the present paper we describe the synthesis of a galactose-containing analogue of natural glycerophospholipids with the β-configuration of the glycosidic bond (6).

In the scheme developed, the starting compounds were D-galactose tert-butyl orthoacetate (1) and trichloroacetimidate (2) and a benzyl phosphatidate (3). The D-galactose orthoester (1) was obtained by the method of [2] from acetobromogalactose via a stage of the formation of the corresponding β-nitrate. D-Galactose trichloroacetimidate (2) was synthesized from acetobromogalactose by the saponification of the bromine [3] and treatment of the resulting 2,3,4,6-tetra-O-acetyl-D-galactopyranose with trichloroacetonitrile in the presence of sodium hydride [4]. The benzyl phosphatidate (3) was obtained by a known scheme [5].

The glycosylation of the benzyl phosphatidate (3) by the action of the D-galactose orthoester (1) and trichloroacetimidate (2) was performed at 18-20°C in anhydrous C_6H_6 and anhydrous CH_2Cl_2, respectively, with the use of a 10% excess of the phospholipid (3).



The pattern of the change in the composition of the reaction mixture was similar to that observed previously for the gluco analogue. However, the galactose derivatives (1 and 2) showed a somewhat lower reactivity on glycosylation by the benzyl phosphatidate (3) than the corresponding glucose derivatives. This was expressed in some increase in the reaction

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time and a lowering of the yield. After the elimination of the excess of benzyl phosphatidate (3) by passage through a layer of alumina, the triester (4) that had been obtained was, without isolation, converted into the sodium salt (5) with the aid of anionic debenzylation. The yields of compound (5) were 33.4 and 37.1% calculated on compounds (1) and (2), respectively.

The question of the configuration of the anomeric center was answered with the aid of NMR spectroscopy. In the ^{31}P NMR spectrum of compound (5) obtained from the orthoester (1) a single signal of the phosphorus atom was observed. The ^{13}C NMR spectrum contained two signals of a carbon atom at an anomeric center with close values of their chemical shifts (95.665 and 95.615 ppm) which may be presumably due to the presence of two isomeric forms at C-2 of the substituted glycerol or to the splitting of a signal into a doublet through the spin-spin coupling of the anomeric carbon atom with the phosphorus nucleus. These facts indicated the presence of a single isomer. Information on its configuration was obtained from the PMR spectrum, in which the signal of an anomeric proton was observed in the form of a doublet at δ 5.14 ppm. The value of the spin–spin coupling constant ($J_{1,2}$ = 6.5 Hz) showed the β-configuration of the anomeric center.

Identical signals were observed in the ^{31}P, ^{13}C, and ^{1}H NMR signals of compound (5) synthesized from the trichloroacetimidate (2).

Elimination of the acetyl groups from the molecule of the glycophospholipid (5) by the action of hydrazine hydrate in boiling methanol was achieved after 15 min. An increase in the reaction time led to the saponification of the palmitic acid residues. The yield of the phosphatidylgalactose (6) after chromatographic purification on a column was 41%.

EXPERIMENTAL

NMR spectra were taken on WM-250 pulsed NMR spectrometer (FRG) with a working frequency for ^{1}H of 250 MHz, for ^{13}C of 62.9 MHz, and for ^{31}P of 81 MHz. The internal standard for the ^{1}H and ^{13}C spectra was hexamethyldisiloxane, and for the ^{31}P NMR spectrum it was 85% phosphoric acid. Melting points were determined on a Boëtius instrument. Angles of optical rotation were measured on a Perkin–Elmer, model 241 MC, photoelectric spectropolarimeter (United Kingdom), and TLC was conducted on Silufol plates (Czechoslovakia), in the following solvent systems: 1) benzene–ethyl acetate (1:1); 2) chloroform–acetone (8:1); 3) chloroform–methanol–NH_4OH (65:18:1); 4) chloroform–methanol–NH_4OH (65:25:4). The spots were revealed by heating at 350°C or with the molybdenum blue reagent for phosphorus.

Trichloroacetonitrile (Fluka) was used without additional purification; $Hg(CN)_2$ (Merck), $HgBr_2$ (Aldrich) and boron trifluoride ethereate (USSR) were redistilled over sodium; NaH (60% suspension in mineral oil, Aldrich) was washed with dry hexane before use; and hydrazine hydrate (USSR) was used without additional distillation.

The substances were purified by column chromatography on silica gel L 40/100 μ (Czechoslovakia).

The results of the analysis of all the compounds corresponded to the calculated values.

3,4,6-Tri-O-acetyl-α-D-galactopyranose-1,2-(tert-butyl orthoacetate) (1) was obtained by the method of (2) with a yield of 67%, mp 88-89°C, $[\alpha]_D^{20}$ +79.5° (c 0.3; $CHCl_3$), R_f 0.81 (system 2). According to the literature [2], mp 89-90°C, $[\alpha]_D^{20}$ + 80° (c 1.0; $CHCl_3$).

2,3,4,6-Tetraacetyl-α-D-galactopyranosyl trichloroacetimidate (2) was synthesized as described in [4]. Yield 1.9 g (74.6%), mp 119-121°C, R_f 0.85 (system 2). ^{1}H NMR spectrum (250 MHz, $CDCl_3$); 2.03; 2.04; 2.18 (12H, 3s, $COCH_3$), 4.04-4.23; 4.40-4.50; 5.30-5.46; 5.56-5.59 (6H, 4m, H-2, H-3, H-4, H-5, H-6, H-6'), 6.60 (1H, d, $J_{1,2}$ = 3.1 Hz, H-1), 8.68 (1H, s, NH).

Sodium benzyl 1,2-di-O-palmitoyl-rac-glyceryl phosphate was obtained through a series of stages [5], starting from 1,2-di-O-palmitoyl-3-iodo-3-deoxyglycerol and silver dibenzyl phosphate.

Benzyl 1,2-Di-O-palmitoyl-rac-glyceryl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl Phosphate (4). A. Orthoester Method. A solution of 0.88 g of the Na salt of benzyl 1,2-di-O-palmitoyl-rac-glyceryl phosphate in a mixture of 40 ml of dry acetone and 40 ml of dry methylene chloride was stirred at 18-20°C with Dowex 50WX8 ion-exchange resin (H^+). After 60-80 min the resin was filtered off and the solution was evaporated in a rotary

evaporator at a temperature not exceeding 40°C. The residue was dissolved in 12 ml of dry benzene and, with stirring, at 18-20°C, 0.44 g of the D-galactose tert-butyl orthoacetate (1) was added in portions. After 60-80 min, the reaction mixture was passed through a layer of alumina (Brockmann activity grade II), which was then washed with benzene, and evaporation of the whole was performed in a rotary evaporator. This gave 0.83 g of compound (4), R_f 0.68 (system 2), which was used in the following stage without purification.

B. Trichloroacetimidate Method. With stirring at 18-20°C, 0.4 g of the D-galactose trichloroacetimidate (2) was added to a solution in 10 ml of dry methylene chloride of the benzyl phosphatidate (3) obtained from 0.26 g of its sodium salt as described above. After 2.5 h the reaction mixture was worked up as described for case A. The yield of compound (4) was 0.4 g, R_f 0.68 (system 2). The substance was used in the following stage without additional purification.

Sodium 1,2-Di-O-palmitoyl-rac-glyceryl 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl Phosphate (5). A solution of 0.7 g of compound (4) in 7 ml of dry acetone was boiled with 1.3 g of calcined sodium iodide for 1-1.5 h. The reaction mixture was kept overnight in the refrigerator, and the precipitate was filtered off, washed with cold acetone, and dried over Na_2SO_4. Yield 0.3 g (33.4% on the orthoester (1) and 37.1% on the imidate (2)), mp 126-129°C, $[\alpha]_D^{20}$ +10.8° (c 1.0; $CHCl_3$), R_f 0.85 (system 4). Found, %: C 58.52, H 8.66, P 3.08. $C_{49}H_{86}NaO_{17}P$. Calculated, %: C 58.79, H 8.66, P 3.09. ^1H NMR spectrum (250 Mhz, $CDCl_3$): 0.64 (6H, t, CH_3), 1.05 (48H, s, CH_2), 1.74; 1.80; 1.85; 1.92 (12H, 4s, $COCH_3$), 5.15 (0.5H, d, $J_{1,2}$ = 6.5 Hz, H-1), 5.17 (0.5H, d, $J_{1,2}$ = 6.5 Hz, H-1). ^{13}C NMR spectrum (62.9 MHz, $CDCl_3$): 173.4; 173.01 (C=O, palmitoyl), 170.3; 170.1; 169.9; 169.8 (C=O, acetyl), 95.67; 95.62 (C-1).

Sodium 1,2-Di-O-palmitoyl-rac-glyceryl β-D-Galactopyranosyl Phosphate (6). A solution of 0.4 g of compound (5) in 13 ml of methanol was treated with 0.16 ml of hydrazine hydrate, and the mixture was boiled for 15 min. The cooled mixture was neutralized with 85% formic acid and was kept in the refrigerator for 10-15 h. The precipitate that had deposited was filtered off and washed with cold methanol. The substance was purified by column chromatography on silica gel L 40/100 μm, with elution by chloroform–methanol (85:15). Yield 0.14 g (41%), mp 142-153°C, $[\alpha]_D^{20}$ +5.64° (c 1.3; $CHCl_3$–CH_3OH, 2:1), R_f 0.3 (system 3), 0.79 (system 4). Found, %: C 58.87, H 10.11, P 3.79. $C_{41}H_{78}NaO_{13}P$. Calculated, %: C 59.11, H 9.44, P 3.72.

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