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CHEMICAL SYNTHESIS OF DIPHOSPHORYLATED LIPID A DERIVATIVES

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Summary: The application of two new phosphorylation procedures enabled us to synthesize an analog of Lipid A.

Lipopolysaccharides (LPS) of many Gram-negative bacteria consist of a heteropoly-saccharide that is covalently bound to a lipid part: i.e., Lipid A. This glycophospholipid has been shown to be responsible for a variety of biological activities of LPS[1] including lethal toxicity, pyrogenicity, mitogenicity and complement activation. Furthermore, it has been established that a non-toxic fraction of Salmonella Lipid A caused tumor regression[2]. The basic structure of the Lipid A part from several Gram-negative bacteria has been elucidated[1,3,4]. Thus Lipid A (See Fig.I) contains a β(1→ 6) linked disaccharide of 2-deoxy-2-(D-3-hydroxytetradecanoyl amino)-D-glucopyranose carrying O-esterified fatty acids on the 3,4 and 6' hydroxyl functions and two phosphate groups; i.e., one at the 4' position and the other at the anomeric centre 1. The configuration of the phosphorylated anomeric centre has not yet been established.

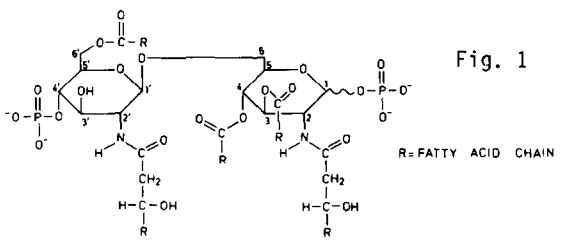
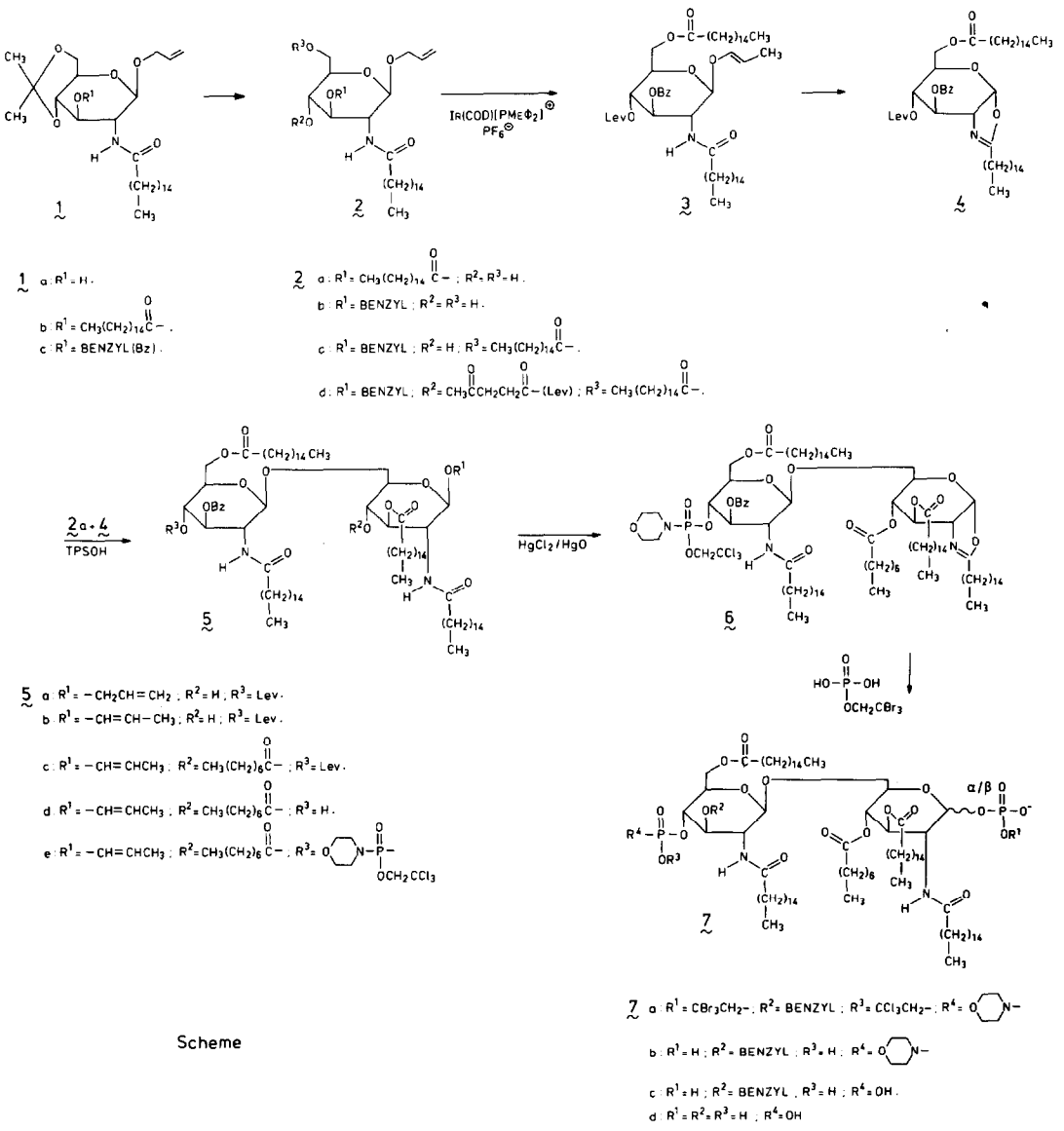


Fig. 1

As part of our programme to synthesize cell-wall substances[5], we now wish to report the synthesis of a Lipid A analog which does not contain the β-hydroxy fatty acids (i.e. compound 7d). The strategy we followed for the synthesis of Lipid A analog 7d is outlined in the Scheme. The key intermediate 5d was prepared starting from allyl 3,4,6-tri-O-acetyl-2-deoxy-2-palmitamido-β-D-glucopyranoside[6]. The latter compound was O-deacetylated and treated with 2,2-dimethoxypropane in dry DMF in the presence of a catalytic amount of p-toluenesulfonic acid[7], to afford compound 1a[8] in 94% yield. Compound 1a thus obtained was used for the synthesis of two precursors: i.e., the non-reducing unit 4 and the reducing unit 2a. The latter derivatives were applied for the construction of disaccharide 5a. In our strategy, we chose the benzyl group as a persistent protective group for the 3-hydroxyl function of the non-reducing part. Thus benzylation of 1a followed by acidolysis[8] of the isopropylidene function and subsequent selective acylation of the primary hydroxyl group afforded 2c. The 4' hydroxyl group of 2c, which in a later stage of the synthesis has to be phosphorylated, was, for the reasons given below, protected with the temporary levulinoyl group[9]. Thus the levulinoyl group proved to be stable under the conditions necessary for the formation of oxazoline 4 and disaccharide 5a and could also be removed fast and selectively in the presence of other ester functions. The 3 hydroxyl function of the reducing unit 1a had to be esterified



Scheme

by a fatty acid residue, to afford, after removal of the isopropylidene group, compound 2a.

Thus benzylation of compound 1a with benzylbromide in DMF in the presence of $BaO/Ba(OH)_2$ [12] gave 1c in 96% yield (m.p. 83°C), while acylation of 1a with palmitoyl chloride (1.1eq.) in pyridine afforded 1b in 93% yield. The isopropylidene group was now removed from 1b and 1c by acetic acid (90%) treatment for 20 min. at 90°C, to afford 2a [10,11] and 2b [10,11], respectively, in a quantitative yield. Compound 2b was selectively acylated [11] with palmitoyl chloride in pyridine at -15°C to afford 2c in 85% yield. Compound 2c was treated with levulinic acid anhydride in the presence of 4-dimethylaminopyridine (DAP) to afford, after column chromatography, compound 2d [10] in 88% yield. The allyl derivative was quantitatively isomerized into the trans-1-propenyl derivative 3 [10] (m.p. 84°C) by treatment of a solution of 2d in

peroxide free THF with a catalytic amount of hydrogen activated 1,5-cyclooctadiene- bis {methyldiphenyl phosphine} Iridium hexafluorophosphate[13] for 4 hr at 35°C. The 1-propenyl derivative 3 was converted into oxazoline 4 by the action of $HgCl_2/HgO$ in acetonitrile at 80°C[14] for 40 min. to give, after short column chromatography, compound 4[10] in 85% yield as an oil. The oxazoline 4 was now selectively condensed with the primary hydroxyl function of 2a. Thus 2a (1.2 mmole) in 1,2-dichloroethane (10 ml) containing 2,4,6-triisopropylbenzenesulfonic acid[15] (TPSOH) and oxazoline 4 (0.96 mmole) were left for 20 h. at 65°C to afford, after purification by column-chromatography, disaccharide 5a[10] as an amorphous solid {0.62 mmole ^{1}H-NMR:H1' δ= 4.41 (d), H3 δ= 4.90(t), H4' δ= 5.00(t)}. The allyl ether of derivative 5a was now quantitatively isomerized under the conditions as described earlier[13] (see isomerization of 2d into 3) to give 5b[10] {^{1}H-NMR: $OCH=CHCH_3$ = 6.22(dq)}.

We now investigated the esterification of the sterically hindered 4-hydroxyl function of 5b. Initially, we applied a known acylation procedure[11,16] using palmitoyl chloride (2 eq.) in pyridine for 5 h. at 20°C. However, TLC analysis as well as ^{1}H-NMR spectroscopy of the reaction mixture revealed the presence of a considerable amount of side products. The formation of these side products is due to the acylation of the amides, which are converted into imide functions. Fortunately, acylation of 5b (0.49 mmole), for 1 h. at 40°C, using octanoic acid anhydride in THF/pyridine and in the presence of a catalytic amount of DAP, proceeded without the formation of side products. In this way, pure compound 5c[10] was obtained in good yield {0.44 mmole; ^{1}H-NMR: H4 δ= 5.25(t), H4' δ= 5.00(t), H3 δ= 4.94(t)}. Removal of the levulinoyl group was achieved by hydrazinolysis of 5c (0.389 mmole) in chloroform/pyridine/acetic acid (4:7:1, v/v; 0.25M hydrazine hydrate) for 7 min. at 40°C to give, after column chromatography, Lipid A precursor 5d[10] {0.34 mmole; ^{1}H-NMR: H4 δ= 5.21(t), H3 δ= 4.98(t)}.

The non reactive[17] 4'-hydroxyl function of 5d was easily phosphorylated by a recently developed phosphorylating technique[18]. Thus, the dihydroxybenzotriazole[18] derivative of 2,2,2-trichloroethyl phosphodichloridate (0.64 mmole) in THF/pyridine was added to 5d (0.318 mmole). After 30 min. at 35°C, morpholine was added to the mixture, to give after work-up and short column chromatography compound 5e[10] (0.286 mmole) as a waxy solid. {^{31}P-NMR: δ= 4.97, 5.72: two diastereomers}. Compound 5e (0.134 mmole) was converted into the oxazoline 6 by $HgCl_2/HgO$ treatment[14]. Purification by short column chromatography afforded pure 6 (0.094 mmole) as an oil {^{1}H-NMR: H1 δ= 5.96(d)}.

At this stage, we turned our attention to the phosphorylations on the anomeric centre of glucoseamines[19]. Up to now, the most promising results were obtained with oxazoline derivatives, which were reacted together with dibenzylphosphate to afford α-phosphotriesters on the anomeric centre[20,21]. Unfortunately, the latter derivatives are quite labile and had to be hydrogenolyzed immediately[20]. On the other hand, phosphodiesters on the anomeric centre are more stable and the phosphorylation of this centre can easily be accomplished by treating the oxazoline with a phosphoric acid monoester[19]. Thus compound 6 (0.094 mmole) in DMF/toluene (2:1, v/v) was reacted together with 2,2,2-tribromoethylphosphoric acid (0.37 mmole) to afford after 3 days at 35°C, compound 7a (0.063 mmole) as a mixture of anomers[22] (α:β= 7:2). The latter anomers could easily be separated by short column chromatography: 7a (α- anomer ^{1}H-NMR: H1 δ= 5.57(dd), H3 δ= 5.30(t), H4 δ= 5.09(t); 7a (β-anomer) ^{1}H-NMR: H1 δ= 5.04(t).

The α-anomer of 7a (0.045 mmole) was now deblocked in three distinct steps. Firstly, the

2,2,2-tribromoethyl and 2,2,2-trichloroethyl groups were removed simultaneously with activated zinc dust in the presence of 2,4-pentanedione[23] to afford 7b. After work-up compound 7b was immediately dissolved in THF/90% acetic acid (1:1, v/v) to remove the acid labile morpholine group. After three hours at room temperature followed by work-up, compound 7c (0.042 mmole; α-anomer) was isolated as its triethylammonium salt {^{13}C-NMR:C1 δ= 93.9 ppm; C1' δ= 99.5 ppm}. Finally, the benzyl group was removed from compound 7c (α-anomer) by hydrogenolysis on Palladium Charcoal (10%) to afford Lipid A analog 7d as a waxy solid.

In conclusion, our synthesis of Lipid A precursor 5d as well as analog 7d differs from recently reported syntheses - bisdephospho Lipid A derivatives[11,16,24,25] and a phosphorylated Lipid A analog[17] - on several points: (a) the introduction of the levulinoyl group[9] for the temporary protection of the 4'hydroxyl group; (b) an improved isomerization of the allyl ethers with an Iridium catalyst[13]; (c) introduction of TPSOH as an improved acid catalyst for the oxazoline condensation procedure (preparation of 5a); (d) acylation of the 4-hydroxyl function with carboxylic acid anhydride avoids acylation of the amides (conversion of 5b into 5c). (e) effective phosphorylation of the 4'hydroxyl function with the recently developed dihydroxybenzotriazole method[18,26] (phosphorylation of 5d to give 5e); (f) new phosphorylation procedure to introduce phosphate at the anomeric centre (conversion of 6 into 7a) and isolation, in good yield, of α and β phosphorylated derivatives of 7a.

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