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CHEMICAL SYNTHESIS OF PHOSPHORYLATED FUNDAMENTAL STRUCTURE OF LIPID A

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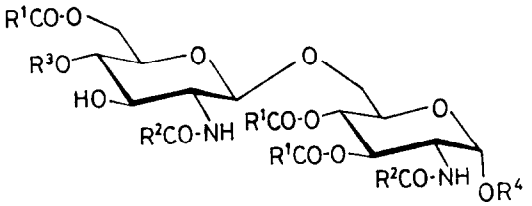
Summary: 1-α-Monophosphate (2), 4'-monophosphate (3), and 1-α,4'-diphosphate (4) of 6-O-(2-deoxy-2-tetradecanoylamino-6-O-tetradecanoyl-β-D-glucopyranosyl)-2-deoxy-2-tetradecanoylamino-3,4-di-O-tetradecanoyl-D-glucopyranose were prepared in order to elucidate the role of phosphate moieties in endotoxic activity of lipid A.

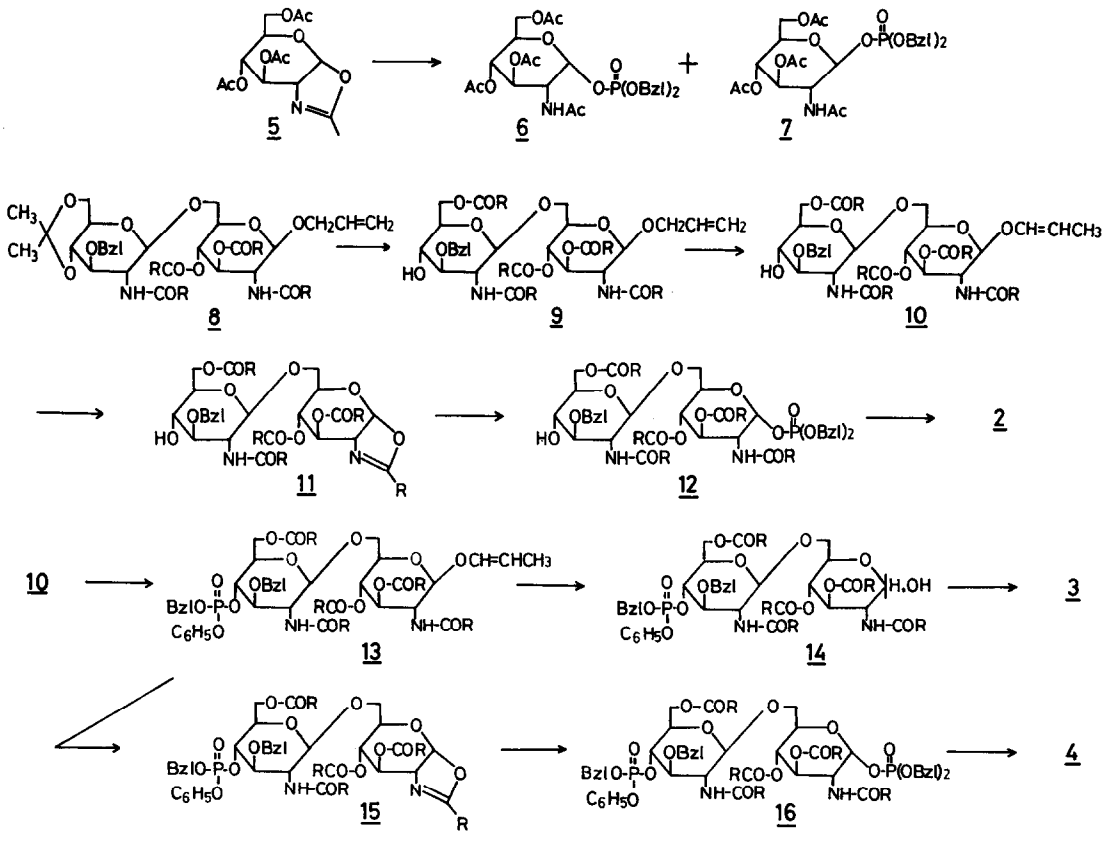
Elucidation of the chemical structure of lipid A,[1] which is the endotoxic principle of bacterial lipopolysaccharide, has enabled new investigations on the biological activity of this unique liposaccharide by synthetic approach. In our attempt of chemical synthesis of the structure 1 proposed for *Salmonella*-type lipid A, we already reported preparation of several compounds lacking the phosphate groups in 1.[2,3] However, preliminary investigations at the present time showed that these compounds represent little of the endotoxic activities, suggesting the importance of the phosphate moieties. In this investigation, phosphorylation could be accomplished on 1-α or 4'- and even both hydroxyl groups in the acyl disaccharide to form 1-α-monophosphate (2), 4'-monophosphate (3) and 1-α, 4'-diphosphate (4) respectively. This is the first chemical construction of the structure proposed for lipid A except that 3-hydroxytetradecanoyl groups attached to the amino functions in 1 were replaced with simple tetradecanoyl groups.



1	R^1CO = acyl,	R^2CO = (*R*)-3-hydroxyacyl,	$R^3 = R^4 = PO(OH)_2$*
2	$R^1CO = R^2CO$ = tetradecanoyl,	R^3 = H,	$R^4 = PO(OH)_2$
3	" = " = " ,	$R^3 = PO(OH)_2$,	R^4 = H*
4	" = " = " ,	$R^3 = R^4 = PO(OH)_2$	

* The configuration of C-1 is not known.

For preparation of 1-phosphate of glucosamine moiety, the reaction of the corresponding oxazoline derivative with dibenzyl phosphate[4] seemed to be most promising.[5] Moreover, the oxazoline required for the present synthesis can be readily derived from the allyl glycoside[6,7] prepared in our previous work.[2] A preliminary reaction of the phosphorylation was first examined with a model oxazoline derivative of glucosamine (5). When 5 was treated with dibenzyl phosphate (in CH_2ClCH_2Cl at room temperature), the presence of two products containing phosphate was observed on TLC in the early stage of the reaction.[8] However, one of them disappeared in the reaction course and only the other remained after 24 hr. The latter proved to be the α-phosphate (6) by means of NMR after hydrolysis and neutralization with triethylamine (H-1 δ 5.45ppm dd, $J_{1,2}$=3.0, $J_{1,p}$=7.5Hz). These observations might be explained by assuming that dibenzyl β-phosphate (7) formed kinetically, anomerized to the more stable α-anomer (6) thermodynamically.[9] Therefore, in the following synthesis, enough long reaction time was employed in the phosphorylation step to obtain a single



product of the phosphate with α-configuration. This seemed to be advantageous to avoid the tedious separation of anomers, though the intrinsic configuration of C-1 in natural lipid A has not been known yet.

For the phosphorylation of the fundamental structure of lipid A, a synthetic intermediate 8 in the previous work was used as the starting material. Removal of the isopropylidene group in 8 (90% AcOH at 90°C for 30 min) followed by selective 6-O-acylation (tetradecanoyl chloride in pyridine-THF at 10°C) afforded 9 (93% from 8, mp 186-188°C, $[\alpha]_D^{14}$-4.38°).[10] It was converted into vinyl glycoside (10) (isomerization with $RhCl(PPh_3)_3$)[6] and then treated with $HgCl_2$-HgO (in CH_3CN-$CHCl_3$ 2:1 at 70°C for 1 hr) to give enough pure oxazoline (11), which was directly used for the successive coupling with dibenzyl phosphate (1.1 equivalents in CH_2ClCH_2Cl at room temperature). The progress of the reaction was monitored with TLC (silica gel, $CHCl_3$-MeOH 50:1). When only a single glycosyl phosphate (12) was recognized in the mixture after 24 hr, the reaction was stopped by removing dibenzyl phosphate with a silica gel column (elution with $CHCl_3$-MeOH 50:1). The mixture was immediately subjected to hydrogenolysis (Pd-black in THF) and neutralized with triethylamine(TEA). The desired 1-α-phosphate (2) was isolated as its triethylamine salt after silica gel column chromatography ($CHCl_3$-MeOH-TEA 5:1:0.02) and lyophilization from dioxane (35% from 9, mp 145-150°C $[\alpha]_D^{18}$+12.9°).[10,11] The structure of 2 was further confirmed by conversion into dimethyl ester (mp 118-123°C)[12] with diazomethane.

Phosphorylation of the secondary 4'-hydroxyl group in 6'-O-acylated disaccharide such as 10 required rather forced reaction condition. Thus, 10 was treated with phenyl phosphate and DCC (in pyridine at room temperature for 48 hr). After addition of phenyldiazomethane, the benzyl phenyl diester of 4'-monophosphate (13) formed was isolated by silica gel column chromatography ($CHCl_3$-MeOH 150:1) (78% from 10). In contrast with the case of 1-phosphates, the diester of 4'-phosphate was quite stable and could be handled easily. Removal of vinyl glycoside ($HgCl_2$-HgO in acetone-H_2O 18:1) afforded 14 (87%, mp 129-137°C, $[\alpha]_D^{18}$+11.7°).[10] It was hydrogenolyzed with PtO_2 in THF-EtOH (5:3) to yield the desired 4'-phosphate (3), which was isolated as triethylamine salt after precipitation from acetone (92%, mp 187-191°C, $[\alpha]_D^{14}$+10.6°).[10] It was also converted into dimethyl ester (mp 110-114°C)[12] for further characterization.

On the basis of the above results, the synthetic route to 1,4'-diphosphate (4) was constructed as shown in the scheme.[13] The benzyl phenyl ester (13) was converted into the corresponding oxazoline (15), and coupled with dibenzyl phosphate as above to give the protected 1-α,4'-diphosphate (16). The crude reaction product was immediately hydrogenolyzed in THF-MeOH (10:1) first with Pd-black (at 4 atm for 2.5 hr) and then with PtO_2 (at 1 atm 4 hr). After neutralization with TEA the desired diphosphate (4) was purified with a silica gel column ($CHCl_3$-MeOH-H_2O-TEA 30:10:1:0.1) and isolated as tetrasodium salt (18% from 13, mp 208-212°C dec, $[\alpha]_D^{13}$+14.7°).[10,12]

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References and Notes

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5) Other usual phosphorylation methods with phosphorochloridate, sulfonyl chloride and so on, could not be satisfactorily applied to 1-hydroxyl group.

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8) Detected with Dittmer-Lester reagent: J. C. Dittmer and R. L. Lester, J. Lipid Res., 5, 126 (1964).

9) In fact, a mixture of α- and β-phosphate could be obtained when the reaction was interrupted in an early stage. The unstable diester of β-phosphate (7) was characterized by conversion to sodium salt of N-acetyl-β-glucosamine 1-phosphate (NMR : H-1 4.96ppm t, $J_{1,2}=J_{1,p}=7.9$Hz), through hydrogenation followed by de-O-acetylation. The previous authors[4] did not notice the occurrence of β-phosphate, probably because of their long reaction period.

10) Solvents used for $[\alpha]_D$ measurements were as follows. Compounds 2, 9, and 14 in $CHCl_3$-MeOH (5:1); 3 in $CHCl_3$-MeOH-H_2O (15:5:1); 4 in $CHCl_3$-MeOH-H_2O (50:10:1).

11) Although 1-phosphates and their benzyl esters are unstable and decomposed spontaneously on standing at room temperature even in crystalline state, their triethylamine and sodium salts can be stored unchanged.

12) Satisfactory result was obtained in elemental analysis (C,H,N, and P) for this compound.

13) The 4'-phosphorylation of 12 attempted as an alternative route failed presumably because of low stability of the 1-phosphate moiety in the molecule.

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