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Preliminary communication

A new synthetic route to 2-(acylamino)-2-deoxy-α-D-glucopyranosyl phosphates, and their endotoxic activity related to the *Salmonella*-type lipid A

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Bacterial lipopolysaccharides (LPS) possess a variety of interesting biological activities, e.g., endotoxicity, tumor necrotic activity, adjuvanticity, and B lymphocyte mitogenicity, and it has been suggested[1] that most of these activities are localized in the unique, hydrophobic component called lipid A. Although the chemical structure of the *Salmonella*-type lipid A has been established[1–3], the moiety of the structure that is required for manifestation of the activity still remains obscure.

In view of this situation, we have synthesized[4] the fundamental structures of the lipid A (recently, the bisdephosphoryl lipid A and its analogs were synthesized by other groups[5]), and have demonstrated[6] that 2-deoxy-2-(D-3-hydroxytetradecanoylamino)-D-glucose[4b] (**10**, see Table I) and one of its β-(1→6)-linked disaccharide derivatives exhibit antitumor, as well as *Limulus*-lysate gelation, activities. In this connection, we recently prepared[7] some phosphate derivatives of *N*-fatty acylated 2-amino-2-deoxy-D-glucose from the corresponding oxazolines by the method described by Khorlin *et al.*[8], and then by Jeanloz *et al.*[9]. However, the synthesis of the 3-hydroxytetradecanoyl derivative by this method was unsuccessful. We now report a new, and apparently general, procedure for the synthesis of 2-(acylamino)-2-deoxy-α-D-glucopyranosyl phosphates, and their endotoxic activities.

3,4,6-Tri-*O*-acetyl-2-deoxy-2-(*p*-methoxybenzylideneamino)-α-D-glucopyranosyl bromide[10] (**1**) (1 mol. equiv.) was treated with dibenzyl tributylstannyl phosphate* (1.1 mol. equiv.) in 1,2-dichloroethane containing a catalytic amount of tetraethylammonium bromide for 3 h at 40° (a similar procedure was employed for the synthesis of glycosyl esters by Ogawa *et al.*[13]), to give two major glycosyl phosphates (2 and 3) which showed, in t.l.c., the characteristic blue color with the phosphate-specific spray-reagent described by Dittmer and Lester[14].

*Prepared by treatment of dibenzyl phosphate with tributyltin methoxide[11] according to the method of Yamaguchi *et al.*[12], and the crude syrup obtained was used without purification.

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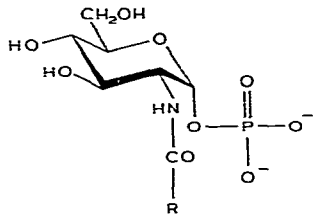
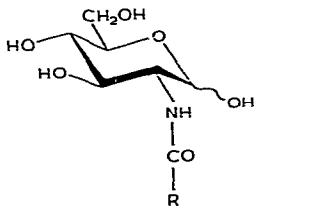
TABLE I

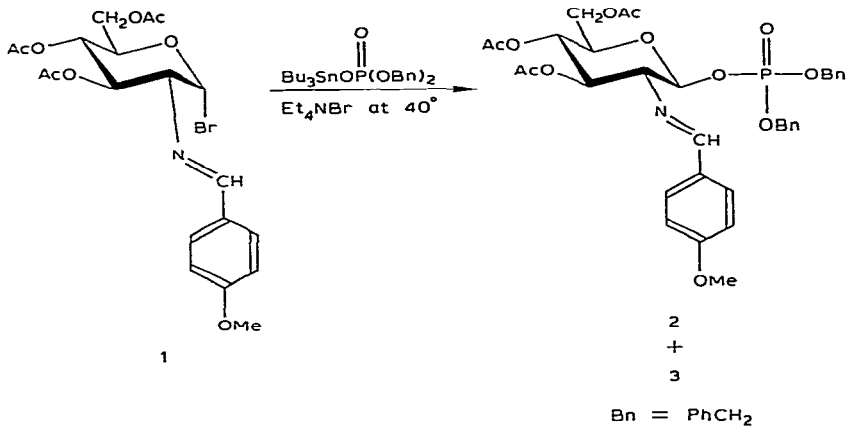
GELATION SENSITIVITY BY THE LIMULUS TEST[a]

Compound			*Concentration (μg/mL)*		
Formula	*No.*	*R*	10^{-1}	10^{-3}	10^{-5}
	7	$CH_3(CH_2)_{10}CHCH_2$ (D), OH	+	+	+
	8	$CH_3(CH_2)_{12}$	+	±	–
	9	$CH_3(CH_2)_{14}$	+	–	–
	10[b]	$CH_3(CH_2)_{10}CHCH_2$ (D), OH	+	+	+
	11[b]	$CH_3(CH_2)_{12}$	+	±	–
	12[b]	$CH_3(CH_2)_{14}$	±	–	–
LPS (*E. coli* 055:B5)			+	+	+

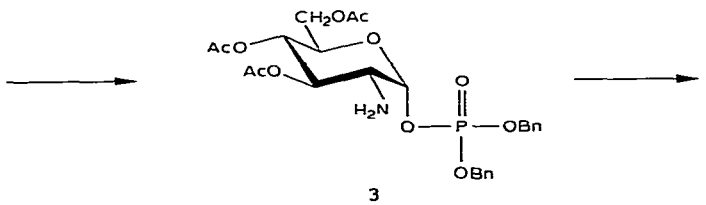
[a] Gelation sensitivity was examined with the *Limulus* amebocyte lysate (Associates of Cape Cod, Inc.). [b] Prepared by the stepwise deprotection of recrystallized benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(tetra- and hexa-decanoylamino)-β-D-glucopyranoside[4a], respectively.

Although t.l.c. revealed the favoured formation of 2 {m.p. 128–130°, $[\alpha]_D$ +4.7° (*c* 1, chloroform); ν_{max}^{KBr} 1640, 1610 (C=N), and 1300–1250 cm^{-1} (P=O); ^{1}H-n.m.r. data (in $CDCl_3$): δ 5.6 (t, 1 H, $J_{1,2} \simeq J_{1,P}$ 8.0 Hz, H-1) and 6.8–7.7 (m, 14 H, Ph)}, its isolated yield (33%), after chromatography on a column of silica gel (Waco gel C-300) with 100:1 chloroform–methanol, was less than that of 3 (36%), m.p. 87–89°, $[\alpha]_D$ +80.9° (*c* 2.4, chloroform); ν_{max}^{KBr} 3680–3240 and 1600 cm^{-1} (NH_2); ^{1}H-n.m.r. data (in $CDCl_3$): δ 5.66 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 6 Hz, H-1) and 7.3 (s, 10 H, Ph). In fact, we found that, when the reaction mixture was stirred, before chromatography, with the silica gel in 100:1 chloroform–methanol for 24–48 h at room temperature, compound 2 was converted completely into 3.

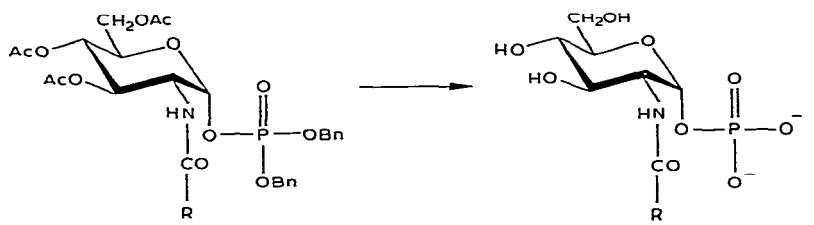
Treatment of 3 in 1:1 dichloromethane–2,6-lutidine containing a trace of *N,N*-diisopropylethylamine with freshly prepared D-3-acetoxytetradecanoyl chloride (prepared by a slight modification of the method employed for the synthesis of acetylmandelyl chloride[15]) during 3 h at 0° gave, after chromatographic separation, a syrup of 4 (40%), $[\alpha]_D$ +48.4° (*c* 1.30, chloroform); ν_{max}^{film} 3240, 1670, and 1540 cm^{-1} (amide); ^{1}H-n.m.r. data (in $CDCl_3$): δ 0.75–2.3 (25 H, CH_3 and CH_2), 1.98–2.02 (12 H, CH_3CO), 5.65 (dd, 1 H, $J_{1,2}$ 3.2, $J_{1,P}$ 6 Hz, H-1), and 7.30 and 7.32 (2 s, 10 H, Ph). Similar treat-



1



3



4 R = $CH_3(CH_2)_{10}CHCH_2$ (OAc)
5 R = $CH_3(CH_2)_{12}$
6 R = $CH_3(CH_2)_{14}$

7 R = $CH_3(CH_2)_{10}CHCH_2$ (OH)
8 R = $CH_3(CH_2)_{12}$
9 R = $CH_3(CH_2)_{14}$

ment of **3** with tetra- and hexa-decanoyl (myristoyl and palmitoyl) chloride, respectively, gave the desired dibenzyl phosphate derivatives (**5** and **6**). Their physical properties and spectral data were identical with those of the same compounds[7] prepared by the oxazoline method.

Finally, the dibenzyl phosphate derivatives (**4–6**) were hydrogenolyzed in methanol in the presence of 10% palladium–carbon catalyst, and the free phosphates formed were neutralized with a cation-exchange resin (Na^+). Mild *O*-deacetylation of the

salts with dilute, methanolic sodium methoxide at 0° afforded 7, $[\alpha]_D$ +35.3° (*c* 0.4 water); 8, $[\alpha]_D$ +26.4° (*c* 0.307, water); and 9, $[\alpha]_D$ +31.1° (*c* 0.457, water), as amorphous materials that gave clearly positive tests with the specific spray-reagent[14] for the phosphoric group. They were then lyophilized for biological tests.

The endotoxic activity of 7–9 was examined by the limulus test. As shown in Table I, compound 7 exhibited potent gelation-activity comparable to that of LPS, but its analogs 8 and 9 were less active in this assay by a factor of 10^{-2}–10^{-4}; a similar relationship was also observed for the corresponding, dephosphorylated derivatives (10–12). In view of this fact, the amide-linked D-3-hydroxytetradecanoic acid, rather than the phosphate group, seems to be essential for the endotoxic activity.

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