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Preliminary communication

A convergent and flexible approach to the synthesis of enterobacterial lipid A. Fully substituted disaccharides having palmitoyl as the fatty acyl moiety

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The lipid A (1) of *Salmonella* species and *E. coli* is of considerable biochemical and pharmacological interest, because it is responsible for the endotoxic and other potent biological activities of the lipopolysaccharides from which it is derived[1—3]. Thus, compounds of the lipid A group are attractive goals for synthetic efforts. "Building blocks" for the lipid A molecule have been described[4—6], and more recently Inage *et al.*[7,8] and Kiso *et al.*[9] have synthesized derivatives of the disaccharide β-D-Glc*p*N(1 → 6)-D-Glc*p*N having fatty acyl groups at all of the same positions as the natural lipid[7,8], or on the nitrogen atoms[9]. These derivatives were made from glycosides of the disaccharide by series of deblocking, blocking, and acylation steps.

Our strategy for the synthesis of lipid A and its analogs has been first to prepare precursors of the reducing and non-reducing moieties, respectively, having their fatty acyl groups in place, and then couple these precursors. The resulting disaccharide derivatives should require only phosphorylation and deblocking for conversion into final products. This approach has the usual advantages of convergent schemes, and it exploits structural elements (fatty acyl groups) of the target compounds as protecting groups, thus minimizing the use of temporary protecting groups. In the present communication, we report the successful application of our approach to the synthesis of the disaccharide glycosides **15a** and **15b**, precursors of the palmitoyl analogs of lipid A and monodephospho lipid A, respectively. Although palmitic acid was the sole fatty acid used, the synthetic scheme provides for the selective introduction of any desired saturated fatty acid at each of the five acylated positions in the final products.

In the initial stage of the synthesis (see Scheme), allyl and benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-palmitamido-β-D-glucopyranoside (**2a**, **b**) were prepared from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride[10], as described by Kiso *et al.*[6]. The *O*-deacetylation (sodium methoxide, methanol) of **2a** and **2b** gave, respec-

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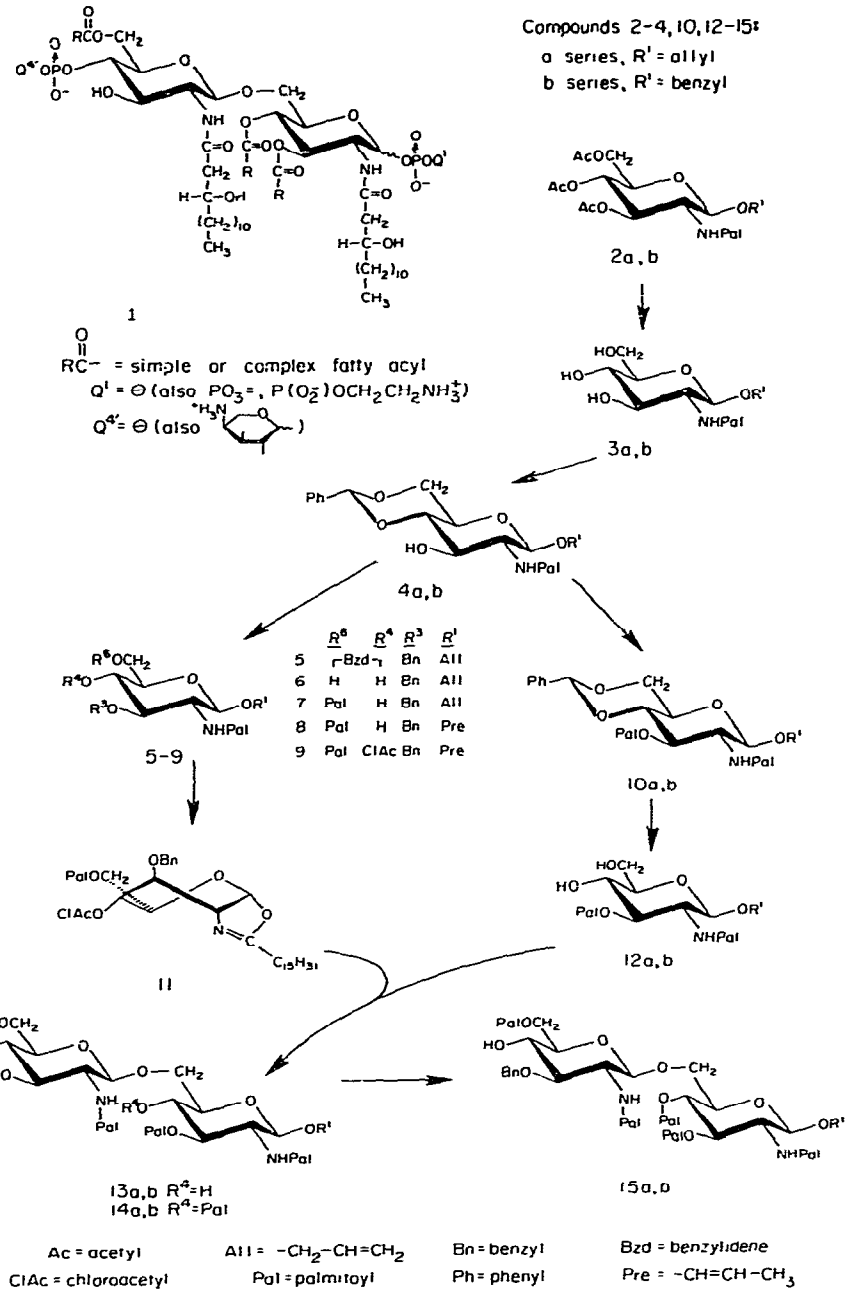
tively, allyl 2-deoxy-2-palmitamido-β-D-glucopyranoside (**3a**) and benzyl 2-deoxy-2-palmitamido-β-D-glucopyranoside (**3b**). Treatment of these glycosides with benzaldehyde and zinc chloride[11] then furnished the key, crystalline intermediates allyl 4,6-*O*-benzylidene-2-deoxy-2-palmitamido-β-D-glucopyranoside (**4a**) and benzyl 4,6-*O*-benzylidene-2-deoxy-2-palmitamido-β-D-glucopyranoside (**4b**).

The role of precursor of the non-reducing half of the target disaccharides demanded an activated derivative of 2-deoxy-2-palmitamido-D-glucose carrying an *O*-palmitoyl group at position 6, and differentially removable protecting groups at positions 3 (unsubstituted in lipid A) and 4 (phosphorylated in lipid A). Oxazoline **11** was designed to meet these specifications and obtained from **4a** *via* intermediates **5**–**9**. The benzylation of **4a** (benzyl bromide, barium oxide, barium hydroxide, *N,N*-dimethylformamide)[12] furnished allyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-palmitamido-β-D-glucopyranoside (**5**). On boiling with 80% aqueous acetic acid, compound **5** was quickly hydrolyzed to allyl 3-*O*-benzyl-2-deoxy-2-palmitamido-β-D-glucopyranoside (**6**). Acylation of the primary hydroxyl was accomplished by treating **6** with 1.2 molar portions of palmitoyl chloride [pyridine, ~2 h, −15° → room temperature (r.t.)], which gave allyl 3-*O*-benzyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranoside (**7**) in high yield. Next, rearrangement of the glycosidic allyl group in **7**, catalyzed by tris(triphenylphosphine)-rhodium(I) chloride[13], provided 1-propenyl 3-*O*-benzyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranoside (**8**). The fully substituted glycoside, 1-propenyl 3-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranoside (**9**), was then generated by the reaction of **8** with chloroacetyl chloride (dichloromethane, pyridine, 10 min, 0°). Cyclization, by the procedure developed in this laboratory[14] (mercuric chloride, mercuric oxide, acetonitrile), converted **9** into 2-pentadecyl-(3-*O*-benzyl-4-*O*-chloroacetyl-1,2-dideoxy-6-*O*-palmitoyl-α-D-glucopyrano)-[2,1-*d*]-2-oxazoline (**11**).

Our choice of glycosyl acceptors (reducing-end units) was influenced by earlier, unsuccessful attempts (unpublished) to couple an oxazoline[5] related to **11** to derivatives of 2-deoxy-2-palmitamido-D-glucose bearing *O*-palmitoyl groups at positions 3 and 4. These attempts were thwarted by migration of the 4-*O*-palmitoyl group to O-6. Hence, we decided to use acceptors having only one *O*-palmitoyl group, at position 3. The synthesis of the acceptors involved first the acylation of **4a** and **4b** with palmitoyl chloride (pyridine, r.t.), which gave allyl 4,6-*O*-benzylidene-2-deoxy-2-palmitamido-3-*O*-palmitoyl-β-D-glucopyranoside (**10a**) and benzyl 4,6-*O*-benzylidene-2-deoxy-2-palmitamido-3-*O*-palmitoyl-β-D-glucopyranoside (**10b**). Brief heating of **10a** and **10b** in 80% aqueous acetic acid gave the desired allyl 2-deoxy-2-palmitamido-3-*O*-palmitoyl-β-D-glucopyranoside (**12a**) and benzyl 2-deoxy-2-palmitamido-3-*O*-palmitoyl-β-D-glucopyranoside (**12b**).

The reaction of oxazoline **11** with **12a** and **12b**, under conditions devised in this laboratory[15] (*p*-toluenesulfonic acid, 1,2-dichloroethane, 6–8 h, 70°), furnished disaccharide products in 50–70% yield. It was expected that glycosylation would occur preferentially at position 6 of the acceptors, and evidence for this was secured when the remaining free hydroxyl group in the products was acylated with palmitoyl chloride



	R6	R4	R3	R'
5	Bzd		Bn	All
6	H	H	Bn	All
7	Pal	H	Bn	All
8	Pal	H	Bn	Pre
9	Pal	ClAc	Bn	Pre

(pyridine, r.t.). An additional, downshifted triplet signal for sugar C*H* was clearly visible in the n.m.r. spectrum of each of the acylated products (**14a** and **b**, see Table I). This observation established the site of acylation as OH-4. Hence the coupling products are formulated as allyl *O*-(3-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-2-palmitamido-3-*O*-palmitoyl-β-D-glucopyranoside (**13a**) and the corresponding benzyl glycoside (**13b**). The fully acylated compounds are allyl *O*-(3-*O*-benzyl-4-*O*-chloroacetyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranosyl)-

TABLE I

CHARACTERIZING DATA

Compound[a]	*Yield (%)*	*M.p. (°C)*	$[\alpha]_D^{25}$ *(degrees)*[b]	*^{1}H-N.m.r. at 270 MHz*[c]
3a	95	180–181	−20.2 (MeOH)	(Me_2SO-d_6) No CH_3CO
3b	98	amorph.	−28.2 (MeOH)	(Me_2SO-d_6) No CH_3CO
4a*	85	220–221	−45.8	δ 7.61–7.32 (5 Ph-H), 5.58 (s, PhC*H*)
4b*	90	225–226	−62.6	δ 7.61–7.32 (10 Ph-H), 5.59 (s, PhC*H*)
5*	94	amorph.	−1.8	δ 7.52–7.08 (10 Ph-H), 4.76 (AB, PhCH_2)
6*	80	amorph.	−8.1	δ 7.42–7.33 (5 Ph-H), no PhC*H*
7*	88	amorph.	−10.5	δ 3.94–3.70 (m, H-6, H-6'), 2.10 (t, *J* 6.5 Hz, CH_2CO)
8	>90	amorph.		δ 6.28–6.11 (m, OC*H*=), no OCH$_2$C*H*=CH_2
9*	84	95–96	+5.9	δ 5.34 (m, H-4)
10a*	95	amorph.	−40.0	δ 5.28 (t, *J* 9.0 Hz, H-3), 2.32 (t, *J* 6.5 Hz, CH_2CO)
10b*	85	171–172	−56.0	δ 5.21 (t, *J* 9.3 Hz, H-3), 2.15 (t, *J* 7.0 Hz, CH_2CO)
11	>90	amorph.		δ 6.01 (d, *J* 7.5 Hz, H-1)
12a*	80	amorph.	−18.7	No Ph-H or PhC*H*
12b*	82	amorph.	−30.9	5 Ph-H, no PhC*H*
13a	40–60	amorph.	−1.6	δ 7.44–7.32 (Ph-H), 5.80 (m, −C*H*=), 5.95 and 5.40 (2 d, N*H*); after exchange of N*H* and O*H*, δ 5.08 and 5.40 (2 t, *J* 8–10 Hz, H-3 and H-4')
13b	40–60	amorph.	−6.5	δ 5.35–5.24 (2 t, *J* 8–10 Hz, H-3 and H-4'), 5.77 (d, *J* 6.4 Hz, N*H*), 5.48 (d, *J* 6.8 Hz, N*H*)
14a	90	amorph.		δ 5.09–4.85 (3 t, *J* 8–10 Hz, H-3, H-4, and H-4')
14b	85	amorph.		δ 5.35–5.14 (3 t, *J* 8–10 Hz, H-3, H-4, and H-4')
15a		amorph.	−7.6	δ 5.30, 5.03 (2 t, *J* 9 Hz, H-3 and H-4)
15b		amorph.	−8.4	δ 5.15, 4.98 (2 t, *J* 9.0 Hz, H-3 and H-4)

[a] An asterisk (*) indicates that analyses for C, H, and N were performed. [b] In chloroform, unless otherwise indicated. [c] Determined in $CDCl_3$, unless otherwise indicated. Referenced to internal tetramethylsilane. The listing gives the most significant differences between the spectrum of each compound and the spectrum of its precursor, except for 13a and 13b. For these compounds, signals characteristic of each of the sugar units are listed.

(1→6)-2-deoxy-2-palmitamido-3,4-di-*O*-palmitoyl-β-D-glucopyranoside (**14a**) and the corresponding benzyl glycoside (**14b**).

Removal of the 4′-*O*-chloroacetyl group from **14a** and **14b** was effected with thiourea[16] (chloroform–methanol, overnight, r.t.), giving the selectively deprotected products allyl *O*-(3-*O*-benzyl-2-deoxy-2-palmitamido-6-*O*-palmitoyl-β-D-glucopyranosyl)-(1→6)-2-deoxy-2-palmitamido-3,4-di-*O*-palmitoyl-β-D-glucopyranoside (**15a**) and the corresponding benzyl glycoside (**15b**). The presence of two doublet signals for anomeric protons, J = 7.7–8.6 Hz, in the spectra of **13a**–**15a** and **13b**–**15b** established the configuration of the intersugar linkage in these compounds as β.

The compounds described here were purified to homogeneity (t.l.c.) by recrystallization, or by chromatography on columns of silica gel when necessary. All were well characterized by ^{1}H-n.m.r. spectroscopy, and when elemental analyses were obtained (see Table I) the values were within ±0.4% of the theoretical. Yields, specific rotations, melting points (for crystalline products), and selected n.m.r. data are presented in Table I.

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