Chemistry of Bacterial Endotoxins. Part 1. Block Synthesis of 6-*O*-{4-*O*-Ammonio(hydrogen)phosphono-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-β-D-glucopyranosyl}-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucose

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The synthesis of the disaccharide present in the hydrophobic region of many endotoxins, a 2-amino-2-deoxy-β-D-glucopyranosyl-(1 → 6)-2-amino-2-deoxy-D-glucose in which the amino groups are acylated by (3*R*)-3-hydroxytetradecanoic acid residues and the 4''-hydroxy group is esterified by phosphate, is described. Two synthons carrying the specific substituents were prepared and condensed; stepwise removal of the protecting groups from the disaccharide thus formed afforded the title compound.

Endotoxins isolated from cell walls of various Gram-negative bacteria possess a wide range of biological activities and are used extensively for, *inter alia*, immunological studies, *e.g.* as B-cell mitogens, adjuvants, polyclonal activators *etc.* These activities have been attributed to the hydrophobic or 'Lipid A' moiety of the lipopolysaccharides.[1]

For the 'Lipid A' of *Salmonellae*[2-4] and for that of *Escherichia coli*[1,5] it has been concluded that the fundamental structure is 6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-amino-2-deoxy-D-glucose in which the amine groups are substituted by (3*R*)-3-hydroxytetradecanoic acid residues and the disaccharide is further substituted by two phosphate groups. One of these is glycosidically bound, but the chirality of the anomeric centre bearing this group has not been established for any endotoxin preparation. According to Gmeiner *et al.*,[6,7] in the 'Lipid A' fragment of a *Salmonella minnesota* Re mutant, the second phosphate group is located at C4''. A similar assignment was made by Rosner *et al.*[5] for the 'Lipid A' fragment of the D31m4 strain of *Escherichia coli* K 12.

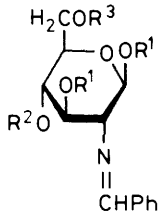
In the course of work aimed at establishing the structure of the endotoxin of *Bordetella pertussis*,[8] Caroff and Szabó have shown that the 'Lipid A' of this micro-organism also contains the glucosaminyl 1 → 6 glucosamine unit, and that this disaccharide carries a 3-hydroxytetradecanoic acid residue on the nitrogen of the reducing sugar and a phosphate group on the non-reducing sugar.[9]

We therefore adopted the working hypothesis that a phosphorylated disaccharide, similar to that identified by Rosner *et al.*,[5] was also present in the pertussis (whooping cough) endotoxin. Accordingly, the synthesis of the title compound was attempted. This synthesis could be approached in two ways. The first is to synthesize monosaccharides carrying the required substituents and then to condense them, the other is to prepare the disaccharide and then to introduce the substituents. Both approaches have been used in this laboratory;[10,11] the first is described in this paper. Kiso *et al.*[12] and Inage *et al.*[13] have recently reported syntheses of analogous structures. Furthermore, in a preliminary communication, Inage *et al.*[14] described the synthesis of the diphosphorylated disaccharide postulated to be that of the 'Lipid A' of *Salmonella* sp.[2-4] and of the *E. coli* K 12, D 31m4 strain endotoxins.[5]

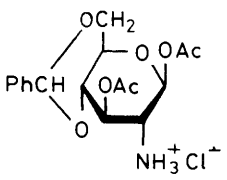
2-Amino-2-deoxy-D-glucose hydrochloride was transformed into its benzylideneamino derivative[15] (1) which was converted, by treatment with benzaldehyde and zinc chloride,[16] into the acetal (2). In preliminary experiments, the easily prepared *p*-methoxybenzylideneamino Schiff's base[17] was used in place of (1). However, during the benzylidenation reaction, the *p*-methoxybenzylidene group exchanged to a variable degree with benzaldehyde, thus giving a product which, although it could be used for the following suite of reactions, was not homogeneous. We therefore preferred to use the Schiff's base (1) which, although also easy to obtain, requires more care in its preparation due to its far greater solubility in ice–water as compared with that of the *p*-methoxybenzylidene derivative. Acetylation of the acetal (2) afforded, as sole product, the β-diacetate (3) from which the hydrochloride (4) was prepared by removal of the benzylideneamino group with one molar equivalent of acid. Starting with 1—2 mol of the free sugar, this suite of reactions gave an overall yield of 50—60% of (4) using the crude products throughout; each of these had ^{1}H n.m.r. spectra in agreement with their proposed structures. All reactions described hereafter were carried out using first a racemic mixture of 3-hydroxytetradecanoic acid (prepared by a Reformatsky reaction according to Schriner[18] and Ikawa *et al.*[19]) and, when reaction conditions were established, the pure 3*R* acid.[20]

Preliminary trials to acylate the amino group with 3-hydroxytetradecanoic acid were carried out using 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranose hydrochloride.[17] The method of Gorbach *et al.*,[21] in which the sugar hydrochloride is condensed with the hydroxy acid in aqueous pyridine in the presence of one molar equivalent of triethylamine and of dicyclohexylcarbodi-imide, afforded yields of the desired acylamido sugar not exceeding 20%. When dichloromethane or tetrahydrofuran were employed as solvents, yields of about 30% were obtained. Similarly, reaction of the *N*-hydroxysuccinimide ester of the fatty acid[20] with the hydrochloride gave a relatively low yield (*ca.* 30%) of the desired acylamide. Using the azide method[22] with equimolar amounts of both the azide and the hydrochloride (4), yields of the acetylated acylamido sugar (5) were *ca.* 40%. Although yields of 70—80% could be isolated if the molar ratio of azide to amine hydrochloride were increased to 3 : 1, the method was abandoned because of the large amounts of the expensive reagent required. The best yields for the condensation of the hydroxy acid with the hydrochloride (4) were obtained by performing the reaction in anhydrous pyridine in the presence of triethylamine and dicyclohexylcarbodi-imide using equimolar proportions of all reagents. Acetylation of the crude amide afforded the pure triacetate (5) in 70% yield after silica gel chromatography.

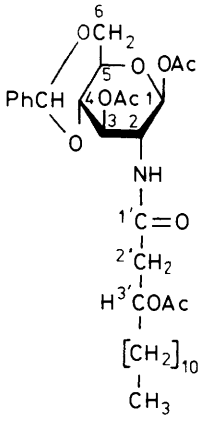
Removal of the acetal group from (5) with acid failed, the labile β-anomeric acetyl group being lost concomitantly; it could, however, be achieved by hydrogenolysis in the presence of palladium at 50 °C. The 4,6-diol (6) was selectively *O*-



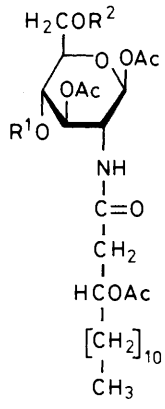
(1) $R^1 = R^2 = R^3 = H$
(2) $R^1 = H, R^2R^3 = PhCH$
(3) $R^1 = Ac, R^2R^3 = PhCH$



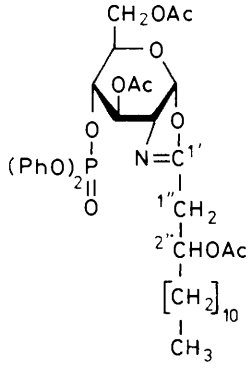
(4)



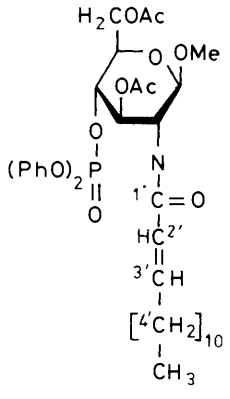
(5)



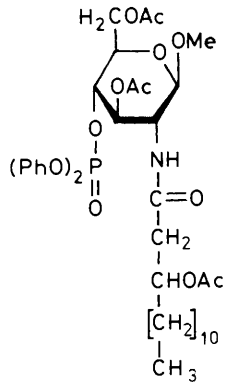
(6) $R^1 = R^2 = H$
(7) $R^1 = H, R^2 = Ac$
(8) $R^1 = (PhO)_2PO, R^2 = Ac$



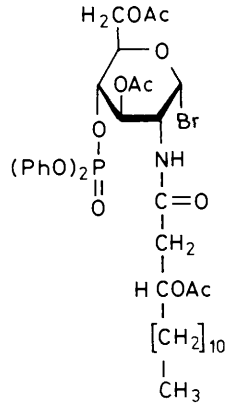
(9)



(10)



(11)



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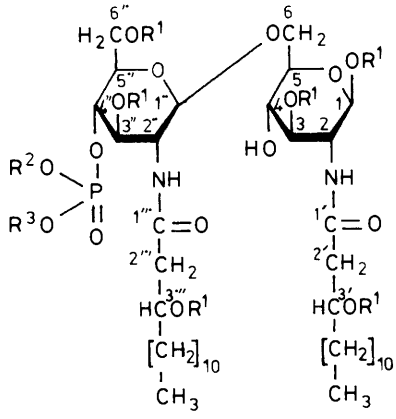
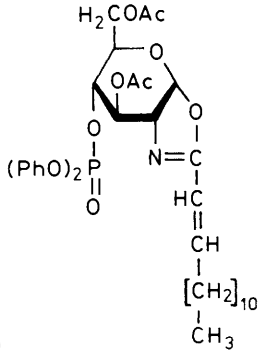


(13) $R^1 = Ac, R^2 = R^3 = Ph$
(14) $R^1 = R^2 = H, R^3 = NH_4$



(15)

acetylated at position 6 using acetyl chloride and pyridine at low temperatures. This procedure was far more rapid and gave higher yields than selective acetylation using the imidazole method.[23] Phosphorylation of the resulting tetra-acetate (7) in benzene with a slight excess of diphenyl phosphonochloridate in the presence of two equivalents of 4-dimethylaminopyridine proceeded rapidly to give a high yield of the 4-diphenyl phosphate (8). In the absence of the latter reagent phosphorylation was slow, did not go to completion, and gave highly coloured reaction mixtures. Inage *et al.*[13] noted that phosphorylation at position 4″ of an otherwise completely protected 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranoside derivative required 'forced reaction conditions.'

Originally, it was planned to synthesize the fully protected disaccharide (13) by condensation of the oxazoline (dihydrooxazole) (9), readily prepared in high yield by reaction of the β-acetate (8) with iron(III) chloride, and the diol (6) in the presence of toluene-*p*-sulphonic acid. In a preliminary experiment, reaction of (9) with a large excess of methanol in (1 : 1) nitromethane–toluene in the presence of a catalytic amount of undried toluene-*p*-sulphonic acid led to a 67% yield of a methyl β-D-glycoside (10) in which the acetoxy group of the amide substituent had been eliminated to give the *trans*-olefinic derivative. Reactions of this type have been examined in more detail in this laboratory.[24] When the condensation was repeated in 1,2-dichloroethane at 50 °C using anhydrous toluene-*p*-sulphonic acid under conditions described by Kiso *et al.*,[12] with the exception that the oxazoline and acceptor were used in a ratio of 1 : 1 instead of 3 : 1, after a reaction time of 1 h (overnight reaction as described by Kiso led to a reaction mixture containing a large number of products), a 40% yield of the methyl β-D-glycoside (11) was isolated and there was no evidence for the formation of the olefinic glycoside (10). Under the same conditions, using the diol (6) as aglycone, after a reaction time of 1 h, no acceptor diol (6) and practically no oxazoline (9) remained, nor could any olefinic oxazoline (15) be detected; however, the desired disaccharide (13) was isolated in only 4% yield. Since, under the above conditions, it was observed that the acidity of the reaction mixture was sufficient to cause loss of the β-acetate group of the diol (6), this condensation was repeated using 2,6-lutidine toluene-*p*-sulphonate as catalyst.[25] The reaction was slow; after 8 h, un-

changed oxazoline (9) remained, and there was no apparent degradation of the diol (6). After 24 h, the oxazoline (9) was depleted and the olefinic oxazoline (15) could be identified in the reaction mixture. The yield of isolated disaccharide (13) was 7% if calculated from the amount of starting oxazoline or, as unchanged diol (6) could be recovered, 10% on the basis of consumed (6). [All of these experiments involved synthons (6) and (9) containing racemic 3-acetoxytetradecanoic acid residues.]

An alternative approach to the synthesis of the title disaccharide using the synthons (6) and (8) was adopted. The β-acetate (8) was transformed quantitatively into the α-bromide (12), which was too unstable to be manipulated, but which was fully characterised by ^{1}H n.m.r. spectroscopy. It was immediately treated with the diol (6) in (1 : 1) nitromethane–toluene in the presence of mercury(II) cyanide as catalyst. When compounds (6) and (12) carried (*RS*)-3-acetoxytetradecanamido groups, the yield of disaccharide (13) was 20%, but only 7% when the condensation was carried out with derivatives of the (*R*)-acid. It could be that the lower solubility of the synthon (6) derived from the (*R*)-acid in the solvent system used may account for this difference in yield.

A secondary reaction of the bromide (12) is probably responsible for the low yields of disaccharide (13) in both cases; indeed, after work-up and column chromatography, in addition to the disaccharide (13), two major products were isolated and identified by their ^{1}H n.m.r. spectra as being the oxazoline (9) and a second oxazoline (15).

Attempts to improve the yields of the disaccharide using the same catalyst but varying the solvent (acetonitrile, dichloromethane) or the temperature (35—60 °C) of the reaction failed, and the same two major products derived from the bromide (12) were isolated in every case. When silver trifluoromethanesulphonate, with *s*-collidine, was used for the condensation in (1 : 1) nitromethane–toluene at −30 °C, a very low yield of a disaccharide, different (^{1}H n.m.r.) from (13), was obtained. This reaction was not further investigated.

The title disaccharide (14) was obtained by hydrogenolysis and deacetylation of (13), followed by column chromatographic purification. Because of its amphipathic nature, considerable losses in yield were incurred during the isolation procedure. Similarly, problems of solubility rendered characterisation of the deprotected compound by ^{1}H n.m.r. spectroscopy impracticable. The final product had chromatographic properties (t.l.c. on DEAE cellulose) similar to those of ' Compound II ' isolated by Rosner *et al.*[5] to which the structure of the disaccharide (14) was assigned. In addition, it was identical with the product obtained in this laboratory [11] by the ' disaccharide route.'

Experimental

Evaporations were carried out under reduced pressure at 40 °C. M.p.s were determined on a Kofler hot-plate and are uncorrected. Optical rotations were determined with a Perkin-Elmer model 141 polarimeter. Except where mentioned, all ^{1}H n.m.r. spectra were recorded at 90 MHz on a Varian E.M. 390 spectrometer. A prototype I.E.F. 400 instrument [26] was used for recording the 400 MHz ^{1}H spectra. ^{13}C N.m.r. spectra were recorded on a Varian CFT 20 spectrometer operating at 20 MHz in the Fourier-transform mode. For all n.m.r. studies, tetramethylsilane was used as internal standard. T.l.c. was performed on silica gel (60 F_{254} on aluminium foil, Merck); all compounds were located by spraying with sulphuric acid (10%) in ethanol and heating on a hot plate. Phosphorus-containing compounds were revealed by spraying with the reagent of Dittmer and Lester.[27] Column chromatography was performed on silica gel (Merck 60, 70—230 mesh).

4,6-O-*Benzylidine*-2-*benzylideneamino*-2-*deoxy*-β-D-*glucopyranose* (2).—2-Benzylideneamino-2-deoxy-β-D-glucopyranose [15] (1) (54 g) was added rapidly to a vigorously stirred mixture of benzaldehyde (180 ml) and zinc chloride (54 g), the mixture was stirred for a further 5 h, and then left at room temperature for 48 h. It was then poured into stirred hexane (500 ml). The hexane was decanted and the residue was stirred with fresh hexane (500 ml) which was then decanted. The residue was dissolved in acetone (400 ml) and the solution was added dropwise to vigorously stirred ice–water (4 l). When the oily precipitate had solidified it was filtered off, washed with water, and suspended in hexane. The *precipitate* (52.3 g, 73%) was filtered off, washed with hexane, and dried. It was not possible to recrystallise the compound owing to its insolubility in all of the usual solvents: δ_H [$(CD_3)_2SO$] 2.9 (1 H, t, $J_{1,2} = J_{2,3} = 9$ Hz, 2-H), 4.9 (1 H, t, $J_{1,2} = J_{1,1\text{-OH}} = 9$ Hz, 1-H), 5.2 (1 H, d, $J_{3,3\text{-OH}}$ 5—6 Hz, 3-OH), 5.6 [1 H, s, Ph*H*C(−O)O], 6.7 (1 H, d, $J_{1,2\text{-OH}}$ 9 Hz, 1-OH), 7.4 [8 H, m, *Ph*HC(−O)O and 3 H, of *Ph*−CH=N], 7.7 (2 H, m, 2 *o*-H of *Ph*CH=N), and 8.2 (1 H, s, PhC*H*=N).

1,3-*Di*-O-*acetyl*-4,6-O-*benzylidene*-2-*benzylideneamino*-2-*deoxy*-β-D-*glucopyranose* (3).—Acetic anhydride (127 ml), cooled to 0 °C, was added to stirred pyridine (470 ml) cooled in an ice–salt bath, followed by compound (2) (94.7 g). The mixture was stirred overnight, the reaction mixture being allowed to come to room temperature. The resulting solution was added dropwise to vigorously stirred ice–water (8 l). When the precipitate had solidified, it was filtered off and dissolved in dichloromethane. The solution was washed with ice–water and dried (Na_2SO_4). All attempts to crystallise the residue (105 g, 90%) remaining after removal of the solvents resulted in decomposition of the compound: δ_H ($CDCl_3$) 1.9 and 2.0 (6 H, 2 s, 2 Ac), 5.5 [1 H, s, Ph*H*C(−O)O], 6.0 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 7.4 [8 H, m, *Ph*HC(−O)O and 3 H of *Ph*CH=N], 7.7 (2 H, m, 2 *o*-H of *Ph*CH=N), and 8.2 (1 H, s, PhC*H*=N).

1,3-*Di*-O-*acetyl*-2-*amino*-4,6-O-*benzylidene*-2-*deoxy*-β-D-*glucopyranose Hydrochloride* (4).—Acetone (340 ml) containing 5M HCl (22.8 ml) was added rapidly to a solution of compound (3) (50 g) in acetone (797 ml) cooled in an ice-bath. The mixture was kept in the ice-bath for 15 min and occasionally swirled, then diethyl ether (1.3 l) was added and the mixture was left for a further 30 min in the ice-bath. The precipitate (35.6 g, 80%) was filtered off, washed with diethyl ether, and dried. Attempted crystallisation resulted in decomposition of the compound: δ_H [$(CD_3)_2SO$] 2.1 and 2.2 (6 H, 2 s, 2 Ac), 5.8 [1 H, s, Ph*H*C(−O)O], 6.0 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 7.3 (5 H, s, Ph), and 8.9 (3 H, m, NH_3^+).

2-[(3R)-3-*Acetoxytetradecanamido*]-1,3-*di*-O-*acetyl*-4,6-O-*benzylidene*-2-*deoxy*-β-D-*glucopyranose* (5).—Triethylamine (5 g, 49.5 mmol) was added dropwise to a suspension of compound (4) (20 g, 51.6 mmol) in anhydrous pyridine (400 ml). When dissolution was almost complete, (3*R*)-3-hydroxytetradecanoic acid [20] (12.5 g, 51.2 mmol) followed by *NN*-dicyclohexylcarbodi-imide (13 g, 63 mmol) were added and the reaction mixture was stirred overnight. The precipitate was filtered off and washed with ethyl acetate. The combined filtrate and washings were concentrated to dryness. Anhydrous sodium acetate (2 g) was added to a solution of the residue in acetic anhydride (80 ml) and the reaction mixture was heated at 100 °C for 1 h. The solvents were removed, the last traces by repeated addition and removal of toluene.

Compound (5) (22.5 g, 70%), after purification on a column (75 × 6.5 cm) of silica gel [(5 : 1) dichloromethane–ethyl acetate], had m.p. 190—195 °C (Found: C, 64.2; H, 8.1; N, 2.3. $C_{33}H_{49}NO_{10}$ requires C, 63.95; H, 8.0; N, 2.3%); $[\alpha]_D^{25}$ −23.6° (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 0.9 (3 H, t, terminal CH_3), 1.25 (18 H, s, $[CH_2]_9$), 1.53 (2 H, m, 4′-H_2), 2.0 (3 H, s, Ac), 2.05 (6 H, s, 2 Ac), 2.35 (2 H, s, 2′-H_2), 5.45 [1 H, s, PhC(=O)O], 5.65 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 5.95 (1 H, d, $J_{2,NH}$ 9 Hz), and 7.3 (5 H, m, Ph).

2-[(3R)-3-*Acetoxytetradecanamido*]-1,3-*di*-O-*acetyl*-β-D-*glucopyranose* (6).—A solution of compound (5) (22.5 g) in ethanol (700 ml) was hydrogenated at 50 °C and atmospheric pressure in the presence of 10% palladium–charcoal catalyst (1.5 g), the reaction being monitored by t.l.c. [(99 : 1) ethyl acetate–ethanol]. The catalyst was filtered off and the filtrate was concentrated. The *triacetate* (6) (8 g, 41%), after purification on a column (73 × 6.5 cm) of silica gel, had m.p. 125—128 °C (Found: C, 58.8; H, 8.6; N, 2.6. $C_{26}H_{45}NO_{10}$ requires C, 58.7; H, 8.5; N, 2.6%); $[\alpha]_D^{25}$ −6.8° (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 0.86 (3 H, t, terminal CH_3), 1.23 (18 H, s, $[CH_2]_9$), 1.53 (2 H, m, 4′-H_2), 2.01, 2.04, and 2.05 (each 3 H, s, Ac), 2.38 (2 H, d, 2′-H_2), 5.7 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), and 6.4 (1 H, d, $J_{2,NH}$ 9 Hz, NH).

2-[(3R)-3-*Acetoxytetradecanamido*]-1,3,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranose* (7).—A solution [10 ml, of acetyl chloride (1.25 ml) in dichloromethane (20 ml) freshly distilled from P_2O_5] was added to a solution of compound (6) (4.5 g) in dichloromethane (20 ml); the mixture was then cooled to −30 °C and a solution (10 ml) of pyridine (1.41 ml) in dichloromethane (20 ml) was injected slowly. The reaction mixture was left overnight and allowed to come to room temperature. Solvents were removed under reduced pressure and the residue was purified on a column (55 × 4.5 cm) of silica gel (ethyl acetate). The *tetra-acetate* (7) (2.4 g, 50%) had m.p. 124—125 °C (Found: C, 58.6; H, 8.2; N, 2.4. $C_{28}H_{47}NO_{11}$ requires C, 58.6; H, 8.3; N, 2.4%); $[\alpha]_D^{25}$ −20.8° (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 0.87 (3 H, t, terminal CH_3), 1.27 (18 H, s, $[CH_2]_9$), 1.53 (2 H, m, 4′-H_2), 2.05, 2.12, and 2.15 (each 3 H, s, Ac), 2.34 (2 H, d, 2′-H_2), 5.6 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), and 6.1 (1 H, d, $J_{2,NH}$ 9 Hz, NH).

2-[(3R)-3-*Acetoxytetradecanamido*]-1,3,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranose* 4-*Diphenylphosphate* (8).—Anhydrous pyridine (1.42 ml, 17.6 mmol) and dimethylaminopyridine (2.15 g, 17.6 mmol) were added to a stirred solution of compound (7) (8.4 g, 14.66 mmol) in dry benzene (75 ml) containing diphenyl phosphonochloridate (2.87 g, 29.32 mmol), the reaction being monitored by t.l.c. [(3 : 1) dichloromethane–ethyl acetate]. After complete disappearance of the starting material, water (1 ml) was added to the cooled solution. After 2 h the mixture was diluted with chloroform (500 ml). After the usual work-up,[28] the residue was purified on a column (60 × 6.5 cm) of silica gel (eluant as for t.l.c.). The *diphenyl phosphate* (8) (10.07 g, 85%) had m.p. 79—81 °C (Found: C, 59.7; H, 7.1; N, 1.85. $C_{40}H_{56}NO_{14}P$ requires C, 59.6; H, 7.0; N, 1.7%); $[\alpha]_D^{25}$ −1.1° (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 0.87 (3 H, t, terminal CH_3), 1.27 (18 H, s, $[CH_2]_9$), 1.53 (2 H, m, 4′-H_2), 1.9, 2.0, 2.03, and 2.08 (each 3 H, s, Ac), 2.33 (2 H, d, 2′-H_2), 5.73 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 6.21 (1 H, d, $J_{2,NH}$ 9 Hz, NH), and 7.25 (10 H, m, 2 Ph).

2′-[(2RS)-2-*Acetoxytridecyl*]-(3,6-*di*-O-*acetyl*-1,2-*dideoxy*-4-O-*diphenylphosphono*-α-D-*glucopyranoso*[2,1-d]-Δ^2-*oxazoline* (9).—Anhydrous iron(III) chloride (2.5 g) was added to a solution of (8) [*N*-substituted with (*RS*)-3-acetoxytetradecanoic acid] (395 mg) in dry dichloromethane (10 ml) and the mixture was stirred at room temperature for 2 h when t.l.c. [(7 : 7 : 1) benzene–diethyl ether–methanol] showed the reaction to be complete. Solids were filtered off and the filtrate was washed three times with ice–water, dried (Na_2SO_4), and concentrated to dryness. The residue was purified on a column of silica gel (37 × 2.2 cm) [(3 : 1) dichloromethane–ethyl acetate] to give an oil (255 mg, 70%), $\nu_{max.}$ ($CHCl_3$) 1 735 (C=O) and 1 665 (C=N); δ_H ($CDCl_3$) 0.88 (3 H, t, termina, CH_3), 1.23 (18 H, s, $[CH_2]_9$], 1.63 (2 H, m, 3″-H_2), 2.03 (9 H, s, 3 Ac), 2.6 (2 H, t, 1″-H_2), 3.75 (1 H, m, 5-H), 4.13 (3 H, m, 2-H and 6-H_2), 4.68 (1 H, m, 4-H), 5.13 (1 H, m, 2″-H), 5.43 (1 H, m, 3-H), 5.88 (1 H, d, $J_{1,2}$ 7.5 Hz, 1-H), and 7.2 (10 H, m, 2 Ph); δ_C ($CDCl_3$; reference—centre peak of $CDCl_3$) 170.43 and 170.25 (C=N), 168.95, 166.26, and 166.07 (CH_3C=O), 150.34 and 149.98 (2 *ipso*-aromatics), 129.73 (4 *m*-aromatics), 125.45 (2 *p*-aromatics), 120.08, 119.94, 119.85, and 119.71 (4 *o*-aromatics), 99.69 and 99.42 (C-1), 73.61, 73.46, 73.33, and 73.18 (C-4, J_{CP} 3 Hz), 71.00, 70.66, 70.52, 70.37, 68.41, and 68.03 (C-3, -5, and -2″), 65.14 (C-2), 62.61 (C-6), 34.05 and 33.96 (C-1″), 29.45 (CH_2 of chain), 20.92, 20.70, and 20.55 (3 CH_3CO), and 13.99 p.p.m. (terminal CH_3).

Methyl 3,6-*Di*-O-*acetyl*-2-*deoxy*-4-O-*diphenylphosphono*-2-[(E)-*tetradec*-2-*enamido*]-β-D-*glucopyranoside* (10).—A solution of compound (9) (0.45 g) in anhydrous toluene (2.5 ml) and anhydrous nitromethane (2.5 ml) was stirred at 35 °C with toluene-*p*-sulphonic acid (10 mg) and anhydrous methanol (1 ml). T.l.c. [(7 : 7 : 1) benzene–diethyl ether–methanol] showed the reaction to be complete after 1 h. The reaction mixture was diluted with chloroform (100 ml) and washed in turn with ice-cold, saturated aqueous sodium hydrogen carbonate and ice–water, and dried (Na_2SO_4). Solvents were removed under reduced pressure and the residue was purified on silica gel (30 × 2 cm) (same solvent as for t.l.c.) to give the *methyl glycoside* (10) (292 mg, 67%) (Found: C, 62.0; H, 7.1; N, 2.05. $C_{37}H_{52}NO_{11}P$ requires C, 61.9; H, 7.3; N, 1.95%); $[\alpha]_D^{20}$ −1.5° (*c* 1 in $CHCl_3$); δ_H ($CDCl_3$) 0.88 (3 H, t, terminal CH_3), 1.3 (18 H, s, $[CH_2]_9$), 1.83—2.13 (total 8 H, m + 2 s, 4′-CH_2 and 2 Ac), 3.43 (3 H, s, OCH_3), 3.8—4.4 (4 H, m, 2-H, 5-H, and 6-H_2), 4.63 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 4.80 (1 H, q, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9 Hz, 4-H), 5.48 (1 H, t, $J_{2,3} = J_{3,4}$ = 9 Hz, 3-H), 5.7 (1 H, d, $J_{2',3'}$ 15 Hz, 2′-H), 6.03 (1 H, d, $J_{1,NH}$ 9 Hz, NH), 6.87 (1 H, q, $J_{2',3'}$ 15, $J_{3',4'a} = J_{3',4'b}$ = 7 Hz, 3′-H), and 7.2 (10 H, m, 2 Ph); δ_C ($CDCl_3$) 170.99 and 170.56 (CH_3C=O), 166.44 (NC=O), 150.62—150.23 (2 *ipso*-aromatics), 145.70 (C=), 129.94 (4 *m*-aromatics), 125.70 (2 *p*-aromatics), 123.25 (C=), 120.13 and 119.90 (4 *o*-aromatics), 101.78 (C-1), 75.03, and 72.92 (C-3 and C-5), 72.15 and 71.82 (C-4), 62.27 (C-6), 56.69 (OCH_3), 54.34 (C-2), 29.62 (CH_2), 20.65 ($COCH_3$), and 14.10 p.p.m. (terminal CH_3).

Methyl 2-[(3RS)-3-*Acetoxytetradecanamido*]-3,6-*di*-O-*acetyl*-2-*deoxy*-4-O-*diphenylphosphono*-β-D-*glucopyranoside* (11).—A solution of the oxazoline (9) (0.42 g, 0.56 mmol) in 1,2-dichloroethane (9 ml) containing anhydrous methanol (39 μl, 0.56 mmol) and anhydrous toluene-*p*-sulphonic acid (31 mg, 0.185 mmol; concentration 20 mM) was stirred at 50 °C. After 1 h, t.l.c. [(7 : 7 : 1) benzene–diethyl ether–methanol] showed the reaction to be complete. Work-up and purification as for compound (10) gave the methyl glycoside (11) (188 mg, 40%); δ_H ($CDCl_3$) 0.87 (3 H, t, terminal CH_3), 1.27 (18 H, s, $[CH_2]_9$), 1.53 (2 H, m, 4′-H_2), 1.9, 2.0, and 2.05 (each 3 H, s, φAc), 2.4 (2 H, d, 2′-CH_2), 3.45 (3 H, s, OCH_3), 3.7—4.4 (4 H, m, 2-H, 5-H, and 6-H_2), 4.58 (1 H, d, $J_{1,2}$ 9 Hz, 1-H), 4.7 (1 H, q, 4-H), 5.07 (1 H, m, 3′-H), 5.42 (1 H, t, 3-H), 6.07 (1 H, d, $J_{1,NH}$ 9 Hz, NH), and 7.18 (10 H, m, 2 Ph).

2-[(3R)-3-*Acetoxytetradecanamido*]-3,6-*di*-O-*acetyl*-2-*deoxy*-4-O-*diphenylphosphono*-α-D-*glucopyranosyl Bromide* (12).—Acetic anhydride (2 ml) and a solution of hydrobromic acid in acetic acid (7.7 ml; 33% w/w) were added to a solution of compound (8) (6.2 g, 7.7 mmol) in freshly distilled (P_2O_5) chloroform (50 ml) and the mixture was kept at room temperature. After 1 h, t.l.c. [(3 : 1) dichloromethane–ethyl acetate] showed the transformation of (8) to (12) to be complete. Toluene was added to the solution, and the solvents were removed under reduced pressure. Toluene was added to the residue and evaporated several times to remove the last traces of acetic anhydride. The crude bromide, δ_H ($CDCl_3$) 0.86 (3 H, t, terminal CH_3), 1.27 (18 H, s, $[CH_2]_9$), 1.57 (2 H, m, 4′-H_2), 1.87, 2.0, and 2.05 (each 3 H, s, Ac), 2.38 (2 H, d, 2′-H_2), 6.13 (1 H, d, $J_{1.NH}$ 9 Hz, NH), 6.47 (1 H, d, $J_{1.2}$ *ca.* 3 Hz, 1-H), and 7.1 (10 H, m, 2 Ph), was used immediately for the next step.

2-[(3R)-3-*Acetoxytetradecanamido*[6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3,6-*di*-O-*acetyl*-2-*deoxy*-4-O-*diphenylphosphono*-β-D-*glucopyranosyl*}-1,3-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranose* (13).—The whole of the above crude bromide (12) in nitromethane (10 ml) was added to a solution of compound (6) (4 g, 7.53 mmol) in toluene (50 ml) and nitromethane (30 ml) previously stirred for 2 h with mercury(II) cyanide (3.53 g, 14 mmol) and molecular sieves (3 Å and 4 Å) and the mixture was then vigorously stirred for 72 h. The reaction mixture was diluted with dichloromethane, solids were filtered off and washed with dichloromethane, and the combined filtrate and washings were washed with an ice-cold solution of sodium hydrogen carbonate, then with ice–water, and dried (Na_2SO_4). Solvents were removed under reduced pressure and the residue was purified on a column (70 × 4.5 cm) of silica gel [(3 : 1) ethyl acetate–dichloromethane]. Three major products were recovered. ^{1}H N.m.r. spectroscopy of the first compound to be eluted showed it to have structure (15); δ_H ($CDCl_3$) 0.88 (3 H, t, terminal CH_3), 1.27 (18 H, s, $[CH_2]_9$), 1.82 (2 H, m, 3″-H_2), 2.03 (6 H, 2 s, 2 Ac), 3.5 (1 H, m, 5-H), 4.17 (3 H, m, 2-H and 6-H_2), 4.73, (1 H, td, 4-H), 5.48 (1 H, t, 3-H), 5.9 (2 H, dd, $J_{1.2}$ 7.5, $J_{1'',2''}$ 15 Hz, 1- and 1″-H), 6.77 (1 H, dt, $J_{1'',2''}$ 15, $J_{2'',3''}$ 7.5 Hz, 2″-H), 7.17 (10 H, m, 2 Ph).

The second compound eluted had an ^{1}H n.m.r. spectrum identical to that of the oxazoline (9).

The third compound was the *disaccharide* (13) (685 mg, 7%) (Found: C, 59.8; H, 7.8; N, 2.3. $C_{64}H_{97}N_2O_{22}P$ requires C, 60.2; H, 7.65; N, 2.2%); $[\alpha]_D^{20}$ −7.85° (*c* 1 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 0.84 (6 H, t, 2 terminal CH_3), 1.27 (36 H, s, 2 $[CH_2]_9$), 1.58 (4 H, m, 4′- and 4‴-H_2), 1.90, 2.00, 2.03, 2.07, 2.08, and 2.19 (each 3 H, s, Ac), 2.43 (4 H, m, 2′- and 2‴-H_2), 4.70 (1 H, d, $J_{1'',2''}$ 9 Hz, 1″-H), 5.46 (1 H, d, $J_{1.2}$ 9 Hz, 1-H), 6.59 and 6.94 (2 × 1 H, 2 d, $J_{1.NH} = J_{1'',N''H}$ = 9 Hz, 2 NH), and 7.17 and 7.34 (10 H, 2 m, 2 Ph). The remainder of the spectrum was not interpreted.

2-[(3RS)-3-*Acetoxytetradecanamido*]-6-O-{2-[3RS]-3-*acetoxytetradecanamido*]-3,6-*di*-O-*acetyl*-2-*deoxy*-4-*diphenylphosphono*-β-D-*glucopyranosyl*}-1,3-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranose*.—(a) The title compound (yield 20%) was first synthesized by the method described for compound (13) but using synthons *N*-substituted with racemic 3-acetoxytetradecanoic acid residues. It had a ^{1}H n.m.r. spectrum compatible with its proposed structure, similar to that of compound (13) but more complicated due to the presence of racemic fatty acid residues.

(b) A solution of the oxazoline (9) (0.4 g, 0.54 mmol) in 1,2-dichloroethane (9 ml) containing the diol (6) *N*-substituted with a racemic 3-acetoxytetradecanoic acid residue (574 mg, 1.08 mmol) and anhydrous toluene-*p*-sulphonic acid (obtained by fusing the hydrate at 110 °C *in vacuo* over P_2O_5) (30 mg, 0.18 mmol; concentration 20 mM) was stirred at 50 °C. T.l.c. [(7 : 7 : 1) benzene–diethyl ether–methanol] showed the reaction to be complete within 1 h. The reaction mixture was worked up as for compound (11). The disaccharide (30 mg, 4.4%), isolated as for compound (13), had an ^{1}H n.m.r. spectrum identical with that of the disaccharide obtained by method (a).

(c) A solution of the oxazoline (9) (0.34 g, 0.456 mmol) in 1,2-dichloroethane (7 ml) containing the same diol (6) as used in methods (a) and (b) (0.266 g, 0.5 mmol) and 2,6-lutidine toluene-*p*-sulphonate [25] (0.1 g, 0.36 mmol) was heated for 24 h at 50 °C. The solution was washed twice with ice–water and dried (Na_2SO_4). After removal of the solvents, the residue was purified on a column (43 × 2.3 cm) of silica gel as for compound (13). After elution of the disaccharide (41 mg) [^{1}H n.m.r. spectrum identical with those of the disaccharides obtained in (a) and (b)], elution was continued with (99 : 1) ethyl acetate–ethanol to afford the starting diol (6) (101 mg). Yield of disaccharide based on oxazoline, 7%; based on consumed diol (6), 10%.

6-O-{4-O-*Ammonio*(*hydrogen*)*phosphono*-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucopyranosyl*}-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucose* (14).—A solution of compound (13) (350 mg) in ethanol (12 ml) was treated with hydrogen and Adams platinum catalyst. The catalyst was filtered off and the solution was neutralised with ammonia. Solvents were removed under reduced pressure and the residue was dried under a stream of nitrogen, dissolved in absolute methanol (12 ml), cooled in an ice-bath, and the solution saturated with gaseous ammonia. The reaction mixture was left overnight at room temperature, ammonia and solvents were removed under a stream of nitrogen, and the residue was purified by dry column chromatography on silica gel [(7 : 2 : 1) propan-1-ol–conc. ammonia–water]. The pure disaccharide (14) (40 mg), after being washed with (1 : 1) methanol–acetone, had $[\alpha]_D^{20}$ +21° [*c* 0.225 in (1 : 1) pyridine–methanol] (lit.,[11] +19°) (Found: C, 50.4; H, 9.0; N, 4.4. Calc. for $C_{40}H_{80}N_3O_{16}P{\cdot}3.5H_2O$: C, 50.4; H, 9.2; N, 4.4%).

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