Chemistry of Bacterial Endotoxins. Part 2.[1] A Practical 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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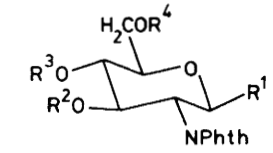
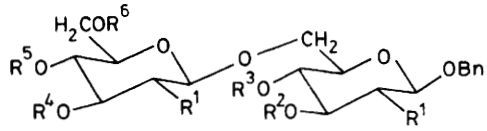
An improved method for the preparation of the phosphorylated disaccharide named in the title, *via* benzyl 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranoside, is described.

In the preceding paper of this series, a synthesis of the disaccharide β-D-glucosaminyl-(1 → 6)-D-glucosamine, phosphorylated in position 4′, and substituted by (3*R*)-3-hydroxytetradecanoic acid on both nitrogen atoms, *i.e.* compound (**14**), was described, this compound being a major constituent of the hydrophobic region of bacterial endotoxins. The synthesis was based on coupling of appropriately substituted monosaccharide units, followed by deprotection of the protected disaccharide formed initially. As the last two steps, namely coupling and deprotection, proceeded with unsatisfactory yields, an alternative route has been elaborated: in this the benzyl glycoside of the otherwise unprotected β-D-glucosaminyl-(1 → 6)-glucosamine is prepared first, and the substituents are introduced in a second series of steps. Although the number of steps in this synthesis is greater than in the previous one, the final product is obtained more easily. A further advantage of the second route is that the amide-bound fatty acids are introduced at a relatively late stage: the number of chromatographic purifications is thus greatly reduced.

Benzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**1**), prepared from the corresponding chloride,[2] was deacetylated by Zemplén's method, and the product (**2**) thus obtained was transformed into the acetylated 6-*O*-triphenylmethyl ether (**3**) by treating it, in pyridine, first with triphenylmethyl chloride, and then with acetic anhydride. This triphenylmethyl ether, when condensed with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide[3] (**4**) in the presence of silver perchlorate or silver triflate (trifluoromethanesulphonate) (the yields are the same) according to Bredereck *et al.*,[4] gave the benzyl glycoside of the fully protected disaccharide (**5**) in 65—70% yield. An alternative approach, namely de-tritylation of the glycoside (**3**) and condensation of the resulting di-*O*-acetylated derivative with the halide (**4**) failed; it was observed that the 3,4-di-*O*-acetyl derivative initially formed underwent acetyl-group migration during its isolation by chromatography on silica gel to give, presumably, the 3,6-di-*O*-acetate. The protecting acetyl and phthalimido groups of the benzyl glycoside (**5**) could be removed either sequentially, by first deacetylating it in chloroform solution with sodium methoxide, and then cleaving the phthalimido groups with hydrazine in methanol, or, simultaneously, by treatment with an excess of hydrazine in methanol. The latter method is simpler and gives a slightly better yield of the final product (**6**). Treatment, in methanol, of this unprotected benzyl glycoside with (3*R*)-3-acetoxytetradecanoic anhydride gave the amorphous diamide (**7**) in high yield; the product was characterised as its crystalline penta-acetate (**8**).



(**1**) R^1 = OBn, R^2 = R^3 = R^4 = Ac
(**2**) R^1 = OBn, R^2 = R^3 = R^4 = H
(**3**) R^1 = OBn, R^2 = R^3 = Ac, R^4 = Tr
(**4**) R^1 = Br, R^2 = R^3 = R^4 = Ac

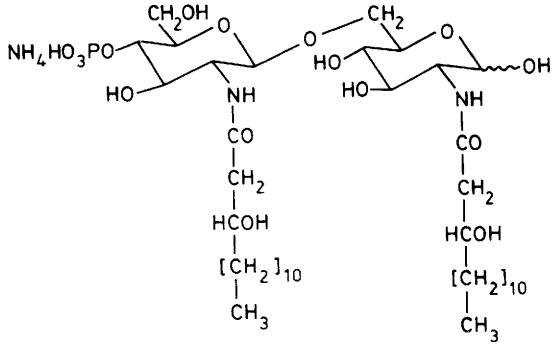


(**5**) R^1 = NPhth, R^2 = R^3 = R^4 = R^5 = R^6 = Ac
(**6**) R^1 = NH_2, R^2 = R^3 = R^4 = R^5 = R^6 = H
(**7**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = R^5 = R^6 = H
(**8**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = R^5 = R^6 = Ac
(**9**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = H, R^5R^6 = >CHPh
(**10**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = Ac, R^5R^6 = >CHPh
(**11**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = Ac, R^5 = R^6 = H
(**12**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = Ac, R^5 = H, R^6 = CH_2OCH_2Ph
(**13**) R^1 = $NHCOCH_2CH(OAc)[CH_2]_{10}CH_3$, R^2 = R^3 = R^4 = Ac, R^5 = $P(O)(OPh)_2$, R^6 = CH_2OCH_2Ph

The (3*R*)-3-acetoxytetradecanoic anhydride used to prepare the diamide (**7**) was obtained by treatment of (3*R*)-3-acetoxytetradecanoic acid with dicyclohexylcarbodi-imide. As direct acetylation of the 3-hydroxy fatty acid with acetic anhydride and various bases invariably led to tetradec-2-enoic acid, the required 3-acetoxytetradecanoic acid was synthesised by treating the potassium salt of the hydroxy acid with benzyl bromide: the benzyl ester of the hydroxy acid thus formed was then acetylated with acetic anhydride–sodium acetate, and the benzyl group was removed by hydrogenolysis.

† Preliminary accounts of this work have been presented: D. Charon and L. Szabó, *Carbohydr. Res.*, 1983, **111**, C13; Bacterial Lipopolysaccharides,' eds. L. Anderson and F. M. Unger, *Am. Chem. Soc. Symp. Ser.*, (*Washington, D.C.*), 1984, **231**, 301—316.

To obtain a derivative of the disaccharide (**7**) having only the 4′-hydroxy group free, compound (**7**) was transformed into the crystalline 4′,6′-*O*-benzylidene acetal (**9**) by treatment with α,α-dimethoxytoluene and toluene-*p*-sulphonic acid, and compound (**9**) was then acetylated to yield the corresponding penta-acetate (**10**). Subsequent removal of the benzylidene group was accomplished by hydrolysis with aqueous 80% acetic acid at 90 °C for 1 h; the resulting diol (**11**) was isolated as an amorphous powder by lyophilisation. Treatment of this diol with benzyloxymethyl bromide and tetramethylurea [5] in chloroform afforded the 6′-benzyloxymethyl ether (**12**) which was isolated and characterised. When dissolved in chloroform and treated with diphenyl phosphorochloridate and 4-dimethylaminopyridine, the phosphorylation was complete in 2 h at room temperature, whereafter the diphenyl phosphate (**13**) was isolated by column chromatography and recovered by lyophilisation. The overall yield from the diol (**11**) was 52%. The compound was fully characterised by its 400 MHz ^{1}H n.m.r. spectrum. Removal of the protecting groups was accomplished by conventional methods: benzyl and phenyl groups by hydrogenolysis catalysed by Pd–carbon and Pt, respectively, acetate groups by ammonia in methanol; the overall yield of the phosphorylated disaccharide (**14**) was 70%. It is noteworthy that the deprotection of the benzyl glycoside (**13**) was more easily accomplished, and with better yield, than that of the analogous 2-[(3*R*)-3-acetoxytetradecanamido]-6-*O*-{2-[(3*R*)-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.[1]



(**14**)

Experimental

General methods were described in ref. 1. For chromatography on silica gel, *ca.* 80 g of Kieselgel 60 (Merck), 70—230 mesh, were employed per gram of substance to be purified; the diameter-to-height ratio of the columns was 1:10, unless otherwise stated.

Benzyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranoside* (**1**).—A solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl chloride [2] (18.2 g, 40 mmol) in a mixture (1:1) of nitromethane and toluene (80 ml) was added to a stirred solution of benzyl alcohol (11 ml, 0.1 mol) in the same solvent (100 ml) containing mercury(II) cyanide (15.62 g, 62.2 mmol) and 4 Å molecular sieve (40 g). The mixture was stirred at 20 °C overnight, then diluted with ethyl acetate and filtered through a pad (diam. 12 × ht. 2 cm) of Kieselgel 60; the pooled filtrate and washings (ethyl acetate) were sequentially treated with dilute sulphuric acid, water, saturated aqueous sodium hydrogen carbonate, and water, and finally was dried before being evaporated. The residue was crystallised from diethyl ether (100 ml) by cautious addition of hexane (*ca.* 20 ml) to yield the title compound (16.6 g, 79.3%) having m.p. 109 °C (lit.,[6] 106—107 °C) (Found: C, 61.7; H, 5.1; N, 2.5. Calc. for $C_{27}H_{27}NO_{10}$: C, 61.7; H, 5.1; N, 2.7%); $[\alpha]_D^{20}$ −12° (*c* 1 in $CHCl_3$) {lit.,[6] $[\alpha]_D$ −11.3° (*c* 1.6 in $CHCl_3$)}; δ_H (90 MHz; $CDCl_3$) 1.82, 2.0, and 2.1 (total 9 H, 3 s, 3 $COCH_3$), 3.85 (1 H, m, $J_{4,5}$ 9.5, $J_{5,6}$ 4.5, $J_{5,6'}$ 2.5 Hz, 5-H), 4.1—4.4 (3 H, m, 2-, 6-, and 6′-H), 4.5 and 4.84 (each 1 H, d, J_{gem} 12.3 Hz, *H*CPh), 5.16 (1 H, t, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, 4-H), 5.36 (1 H, d, $J_{1,2}$ 8.5 Hz, 1-H), 5.8 (1 H, dd, $J_{2,3}$ 10.5, $J_{3,4}$ 9.5 Hz, 3-H), 7.07 (5 H, s, Ph), and 7.6—7.9 (4 H, m, phthalimido).

Benzyl 2-*Deoxy*-2-*phthalimido*-β-D-*glucopyranoside* (**2**).—Methanolic sodium methoxide (1M; 8 ml) was added to a cold solution (0.5 °C) of the glycoside (**1**) (52.5 g, 0.1 mol) in methanol (800 ml). After 4 h, deacetylation appeared to be complete [t.l.c.; (8:2:4) ethyl acetate–ethanol–hexane] and Dowex 50 (H^+) resin (15 ml) was added, the mixture was filtered, and the solvent was evaporated off. The crystalline residue was recrystallized from ethyl acetate to yield, in two crops (the second crop being obtained by addition of hexane to the mother liquors), the product (**2**) (27.1 and 8.9 g, total yield 90.2%), m.p. 171—172 °C (lit.,[6] 169—170 °C) (Found: C, 63.4; H, 5.3; N, 3.3. Calc. for $C_{21}H_{21}NO_7$: 63.2; H, 5.3; N, 3.5%); $[\alpha]_D^{20}$ −58° (*c* 2.2 in MeOH) {lit.,[6] $[\alpha]_D^{20}$ −50.7° (*c* 0.38 in $CHCl_3$)}; δ_H [90 MHz; $(CD_3)_2SO$] (D_2O-exchanged sample with internal acetone used as reference) 4.45 and 4.75 (each 1 H, d, J_{gem} 12 Hz, *H*CPh), 5.15 (1 H, d, $J_{1,2}$ 8.5 Hz, 1-H), 7.0 (5 H, s, Ph), and 7.8 (4 H, m, phthalimido); the remainder of the spectrum was not interpreted.

Benzyl 3,4-*Di*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-6-O-*triphenylmethyl*-β-D-*glucopyranoside* (**3**).—Triphenylmethyl chloride (29.3 g, 105 mmol) was added to a solution of the benzyl glycoside (**2**) (30 g, 75 mmol) in pyridine (150 ml), and the mixture was left at room temperature overnight; t.l.c. [(7:3) ethyl acetate–hexane] then showed the reaction to be complete. Acetic anhydride (150 ml) was added to the reaction mixture and, after 24 h, ice–water (2 000 ml) was added slowly to the constantly stirred mixture. The resulting precipitate was filtered off, washed with water, dried *in vacuo*, and then crystallized from a hot mixture of carbon tetrachloride (250 ml) and methanol (250 ml). The *acetylated trityl ether* (**3**) [50 g in two crops (43 + 7g), 92%] had m.p. 198—200 °C (Found: C, 72.6; H, 5.35; N, 2.05. $C_{44}H_{39}NO_9$ requires C, 72.8; H, 5.4; N, 1.9%); $[\alpha]_D^{20}$ +20° (*c* 2.3 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 1.7 and 1.8 (total 6 H, 2 s, 2 $COCH_3$), 3.25 (2 H, m, 6- and 6′-H), 3.75, (1 H, m, 5-H), 4.4 (1 H, dd, *J* 8.5 and 10.5 Hz, 2-H), 4.6 and 4.9 (each 1 H, d, J_{gem} 12 Hz, *H*CPh) 5.25 (1 H, t, *J* 9 Hz, 4-H), 5.4 (1 H, d, $J_{1,2}$ 8.5 Hz, 1-H), 5.72 (1 H, dd, *J* 9 and 10.5 Hz, 3-H), and 7.0—7.9 (24 H, m, ArH).

Benzyl 3,4-*Di*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-6-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-β-D-*glucopyranoside* (**5**).—To a suspension of 4Å molecular sieves (100 g) in nitromethane (140 ml) was added freshly crystallized and dried silver perchlorate (12.4 g, 60 mmol) and the mixture was stirred for 30 min. Acetylated trityl ether (**3**) (29 g, 40 mmol) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide [3] (28 g, 56 mmol) were added and the mixture was stirred for 1 h, after which time t.l.c. [(6:4) ethyl acetate–hexane] indicated that some bromide remained, so more acetylated trityl ether (3 g, 4 mmol) was added, and the mixture was stirred overnight at room temperature. Insoluble solids were filtered off and washed with nitromethane, and the clear filtrate and washings were diluted with chloroform (500 ml). The mixture was washed successively with saturated aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and

evaporated. The dry residue, when crystallized from methanol, gave the title disaccharide (23 g). From the mother liquors, containing mainly the disaccharide and triphenylmethanol, further disaccharide (3.6 g) could be isolated by column chromatography: triphenylmethanol was eluted first with benzene, and then the disaccharide with ethyl acetate–hexane (6:4). The *disaccharide* (**5**) [26.6 g, 67.2% with respect to (**3**)] had m.p. 132—135 °C (Found: C, 60.0; H, 5.0; N, 3.4. $C_{45}H_{44}N_2O_{18}$ requires C, 60.0; H, 4.9; N, 3.1%); $[\alpha]_D^{20}$ +6.6 ° (*c* 1.3 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$)* 1.79, 1.86, 1.96, 2.04, and 2.14 (total 15 H, 5 s, 5 $COCH_3$), 3.70 (1 H, dd, J_{gem} 11, J_{vic} 7 Hz, 6-H_a), 3.75 (1 H, m, 5-H), 3.82 (1 H, ddd, *J* 2, 5, and 10 Hz, 5′-H), 3.88 (1 H, dd, J_{gem} 11, J_{vic} 2 Hz, 6-H_b), 4.20 (3 H, m, 2- or 2′-H, 6′-H_A, and *H*CPh), 4.36 (2 H, m, 2′- or 2-H and 6′-H_B), 4.48 (1 H, d, J_{gem} 12 Hz, *H*CPh), 4.88 (1 H, dd, *J* 9 and 10 Hz, 4- or 4′-H), 5.18 (1 H, t, *J* 9 Hz, 4′-H or 4-H), 5.20 (1 H, d, $J_{1',2'}$ 8 Hz, 1′-H), 5.5 (1 H, d, $J_{1,2}$ 8.5 Hz, 1-H), 5.66 and 5.73 (2 × 1 H, both dd, J_{vic} 9 and 11 Hz, respectively, 3- and 3′-H), and 6.94—7.82 (13 H, m, ArH).

Benzyl 2-*Deoxy*-6-O-((2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl*)-2-*phthalimido*-β-D-*glucopyranoside*.—A solution of sodium methoxide in methanol (1M; 1.5 ml) was added to a solution of the acetylated disaccharide (**5**) (9 g, 10 mmol) in a mixture of chloroform and methanol (1:1) (100 ml) at 0—4 °C, and the mixture was kept at that temperature for *ca.* 4 h after which time deacetylation appeared to be complete [t.l.c.; (8:2:4) ethyl acetate–ethanol–hexane]. Dowex 50 (H^+) resin (3 ml), previously washed with methanol, was added, and the mixture was shaken until the pH reached 7 (indicator paper). The resin was filtered off and washed, and the combined filtrate and washings concentrated to give a white solid, which was suspended in diethyl ether, collected by filtration, washed with the solvent, and dried to yield the *title compound*, (6.4 g, 92.7%), m.p. 134—150 °C (decomp.) (Found: C, 60.8; H, 5.05; N, 4.0 $C_{35}H_{34}N_2O_{13}$ requires C, 60.9; H, 4.9; N, 4.1%); $[\alpha]_D^{20}$ −46 ° (*c* 1 in MeOH).

Benzyl 2-*Amino*-6-O-(2-*amino*-2-*deoxy*-β-D-*glucopyranosyl*)-2-*deoxy*-β-D-*glucopyranoside* (**6**).—A molar solution of hydrazine in methanol (10 ml) was added to a solution of the preceding phthalimido disaccharide (3.45 g) in methanol, and the mixture was heated under reflux for 4 h, after which time t.l.c. [(8:8:8:4:1) ethyl acetate–propan-1-ol–ethanol–water–aqueous ammonium hydroxide (*d* 0.91)] showed the reaction to be complete; the mixture was then kept overnight at room temperature. The solid material was filtered off and washed with methanol (2 × 20 ml), the pooled filtrate and washings were evaporated to dryness, and the residue was crystallized from methanol to yield the *product* (**6**) (1.99 g, 88%) slightly contaminated with a u.v-absorbing non-charring material. An analytical sample (from MeOH–EtOH) had m.p. 227—230 °C (Found: C, 53.0; H, 7.0; N, 6.5. $C_{19}H_{30}N_2O_9$ requires C, 53.1; H, 6.8; N, 6.5%); $[\alpha]_D^{20}$ −65.4 ° (*c* 0.97 in water); δ_H (400 MHz; D_2O; internal reference: acetone) 2.49 (2 H, m, 2-H and 2′-H), 3.23 (5 H, m, 5-H or 5′-H, 4- and 4′-H, and 3- and 3′-H), 3.43 (1 H, m, *J* 2, 5.5, and 10 Hz, 5′-H or 5-H), 3.6 (1-H, dd, J_{gem} 12.5, J_{vic} 5.5 Hz, 6′-H_b), 3.7 (1 H, dd, J_{gem} 11.5, J_{vic} 6 Hz, 6-H_b), 3.76 (1 H, dd, J_{gem} 12.5, J_{vic} 2 Hz, 6′-H_a), 4.07 (1 H, dd, J_{gem} 11.5, J_{vic} 2 Hz, 6-H_a), 4.24 (1 H, d, $J_{1',2'}$ 8 Hz, 1′-H), 4.3 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 4.5 (1 H, d, J_{vic} 12 Hz, *H*CPh), 4.75 (1 H, d, J_{vic} 12 Hz, *H*CPh), and 7.29 (5 H, m, ArH).

Alternatively, compound (**6**) was obtained directly from the protected disaccharide (**5**) (3.6 g, 4 mmol) by treatment with hydrazine (1.5 g) in methanol under reflux for 4 h. After removal of the solvents, the residue was taken up in water, the pH was brought to *ca* 4 by addition of dilute HCl, insoluble material was removed by filtration, and the filtrate was treated with Dowex 1 X 8 (OH^-) resin (30 ml) to eliminate anions. After removal of the resin, solvents were distilled off, and the residue was dried under oil-pump vacuum at 80 °C and then crystallized in a mixture of methanol–ethanol (1:1; 50 ml) to yield the product (1.4 g, 84%).

Benzyl (3R)-3-*Hydroxytetradecanoate*.—Potassium carbonate (14 g) and benzyl chloride (23 ml) were added to a stirred solution of (3*R*)-3-hydroxytetradecanoic acid (24 g, 0.1 mol) in a mixture (1:1) of tetrahydrofuran (THF) and dimethyl sulphoxide (200 ml). The stirred mixture was kept at 50 °C overnight, cooled, diluted with methylene dichloride (250 ml), and thoroughly washed with water. The organic layer was dried (Na_2SO_4), and the solvent was evaporated off (0.1 Torr) to leave a white residue which was triturated with water, collected by filtration, and dried (32 g, 97%). The *product* was homogeneous by t.l.c. [(50:75:5) diethyl ether–hexane–formic acid]. A sample, recrystallized from hexane, had m.p. 45—47 °C (Found: C, 75.5; H, 9.9. $C_{21}H_{34}O_3$ requires C, 75.45; H, 10.2%); $[\alpha]_D^{20}$ −12.8 ° (*c* 4.7 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 0.9 (3 H, t, terminal CH_3), 1.1—1.7 (20 H, br s, $[CH_2]_{10}$), 2.5 (2 H, m, H_2CCO_2), 3.25 [1 H (exchangeable), br s, OH], 4.05 (1 H, m, *H*–COH), 5.15 (2 H, s, H_2CPh), and 7.3 (5 H, s, 5 ArH).

Benzyl (3R)-3-*Acetoxytetradecanoate*.—Benzyl (3*R*)-3-hydroxytetradecanoate (32 g) was treated with acetic anhydride (200 ml) and sodium acetate (4 g) at 100 °C for 2 h. Acetic anhydride was removed by co-distillation with toluene under reduced pressure, and the residue was extracted with diethyl ether. The extract was treated with charcoal, filtered, and the solvent was evaporated off: the remaining oil (35 g) was homogeneous by t.l.c. [(1:2) diethyl ether–hexane]. An *analytical sample* was obtained by column chromatography on silica gel (same solvent), and drying first at 60 °C *in vacuo*, and then at room temperature over paraffin shavings (Found: C, 73.2; H, 9.3. $C_{23}H_{36}O_4$ requires C, 73.4; H, 9.6%); $[\alpha]_D^{20}$ 0° (*c* 6.2 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 0.9 (3 H, t, terminal CH_3), 1.2—1.7 (20 H, br s, $[CH_2]_{10}$), 1.9 (3 H, s, $COCH_3$), 2.6 (2 H, d, *J* 6.5 Hz, CH_2CO_2), 5.1 (2 H, s, CH_2Ph), 5.2 (1 H, m, *H*COAc), and 7.3 (5 H, s, ArH).

(3R)-3-*Acetoxytetradecanoic Acid*.—A solution of benzyl (3*R*)-3-acetoxytetradecanoate (35 g) in ethyl acetate (250 ml) was hydrogenated in the presence of Pd—C catalyst (5% Pd; 3 g) under a pressure of 50 bars at 50 °C. Usually the reaction was complete within 2 h as observed by t.l.c. [(50:75:5) diethyl ether–hexane–formic acid]. In some cases an unidentified catalyst poison was present in the starting material: it could be removed by either filtration of the ethereal solution of the benzyl ether through a pad of silica gel, or by shaking the solution with Pd—C for some time; the filtered solution could then be hydrogenated with fresh catalyst. After removal of catalyst and solvent, the *acid* (24.5 g, 95%) was obtained as a white solid, very soluble in hexane, diethyl ether, benzene, and methanol. An analytical sample was obtained by crystallization from pentane at −20 °C; it had m.p. 31—32 °C (Found: C, 66.8; H, 10.4. $C_{16}H_{30}O_4$ requires C, 67.1; H, 10.5%); $[\alpha]_D^{20}$ −2.5 ° (*c* 5.6 in $CHCl_3$); δ_H (90 MHz; $CDCl_3$) 0.85 (3 H, t, terminal CH_3), 1.1—1.8 (20 H, br s, $[CH_2]_{10}$), 2.0 (3 H, s, $COCH_3$), 2.6 (2 H, d, *J* 6 Hz, CH_2CO_2), 5.2 (1 H, m, *H*COAc), and 10.8 [1 H (exchangeable), br s, HO_2C].

(3R)-3-*Acetoxytetradecanoic Anhydride*.—The anhydride was prepared immediately before use by the addition of dicyclohexylcarbodi-imide (0.5 mmol) to a solution of the acid

* For the *disaccharides*, unprimed numbers refer to atoms of one ring, and primed numbers to atoms of the other one.

(1 mmol) in anhydrous diethyl ether (10 ml). After 1 h the precipitate (dicyclohexylurea) formed was filtered off, and the solvent was removed under reduced pressure. The absence of an acidic proton was ascertained by n.m.r. spectroscopy. The anhydride forms a white solid at +4 °C; it is a liquid at room temperature.

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-2-*deoxy*-β-D-*glucopyranosyl*}-2-*deoxy*-β-D-*glucopyranoside* (**7**).—A suspension of the diaminodisaccharide (**6**) (1.29 g, 3 mmol) in methanol (250 ml) was stirred until the major part of the substance had dissolved. A solution of (3*R*)-3-acetoxytetradecanoic anhydride (5.7 g, 6.6 mmol) in THF (10 ml) was then added dropwise: soon all the diaminodisaccharide had dissolved and then gelatinous granules appeared. The mixture was left at room temperature overnight: t.l.c. [(65:25:4) chloroform–methanol–aqueous ammonium hydroxide (*d* 0.91)] then indicated that all the diaminodisaccharide had reacted. Solids were collected by centrifugation; the supernatant was concentrated, triturated with methanol, and the solid *product* (**7**) collected by filtration on a sintered glass filter (combined yield 2.458 g, 85%), m.p. 218—220 °C (Found: C, 63.2; H, 8.9; N, 2.9. $C_{51}H_{86}N_2O_{15}$ requires C, 63.35; H, 8.9; N, 2.9%); $[\alpha]_D^{20}$ −23 ° [*c* 0.45 in (3:2) THF–propan-1-ol].

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*}-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**8**).—The disaccharide (**7**) (100 mg) was treated with a mixture (1 ml) of acetic anhydride and pyridine (1:1) at room temperature for 48 h. The solvents were evaporated off, and the residue was purified by column chromatography [(1:1) ethyl acetate–methylene dichloride] to yield the crystalline *product* (**8**) (100 mg, 82%), m.p.194—196 °C (Found: C, 62.6; H, 8.4; N, 2.4. $C_{61}H_{96}N_2O_{20}$ requires C, 62.2; H, 8.2; N, 2.4%); $[\alpha]_D^{20}$ −13 ° (*c* 1 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 0.89 (6 H, t, *J* 7 Hz, 2 $[CH_2]_{10}CH_3$), 1.26 (36 H, br, 2 $[CH_2]_9CH_3$), 1.57 [4 H, br, 2 $CH_2CH_2CH(OAc)$], 2.00, 2.02, 2.037, 2.047, 2.06, and 2.10 (total 21 H, 6 s, 7 $COCH_3$), 2.29—2.53 (4 H, m, 2 $COCH_2CH(OAc)$], 3.57 (1 H, dd, J_{gem} 12, J_{vic} 5Hz, 6-H_a), 3.68 (2 H, m, 2 5-H), 3.9 (2 H, m, 2 2-H), 4.04 (1 H, br d, J_{gem} 12 Hz, 6-H_b), 4.15 (1 H, dd, J_{gem} 12, J_{vic} 2 Hz, 6′-H_a), 4.29 (1 H, dd, J_{gem} 12, J_{vic} 5 Hz, 6′-H_b), 4.60 (1 H, d, *J* 8 Hz, 1-H), 4.63 (1 H, d, *J* 12 Hz, C*H*Ph), 4.78 (1 H, d, *J* 8 Hz, 1′-H), 4.91 (1 H, d, *J* 12 Hz, C*H*Ph), 5.07 {4 H, m (2 triplets centred at 5.04 and 5.08), *J* 10 Hz, 4-H and 4′-H, and 2 (*H*COAc) $[CH_2]_{10}$}, 5.235 and 5.295 (each 1 H, t, *J* 10 Hz, together 3- and 3′-H), 5.92 (1 H, d, *J* 9 Hz, NH), 6.09 (1 H, d, *J* 8 Hz, NH), and 7.36 (5 H, ArH).

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-4,6-O-*benzylidene*-2-*deoxy*-β-D-*glucopyranosyl*}-2-*deoxy*-β-D-*glucopyranoside* (**9**).—α,α-Dimethoxytoluene (0.7 ml) was added to a solution of the disaccharide (**7**) (1.93 g, 2 mmol) in dimethylformamide (DMF) (10 ml) containing toluene-*p*-sulphonic acid (50 mg) maintained at 50 °C. The addition was repeated 1 h later. After a further 1 h, the mixture was cooled and then kept at +4 °C overnight. The colourless, *crystalline product* (1.7 g) was collected by filtration, washed with water, and dried *in vacuo* over phosphoric anhydride; it had m.p. 218—220 °C. Addition of water to the mother liquors gave an additional crop (0.3 g) (m.p. 190—198 °C; raised to 221 °C upon recrystallization from DMF) (total yield 2.0 g, 95%), which was collected by centrifugation. The compound appeared to be homogeneous by t.l.c. [(4:3:1) methylene dichloride–ethyl acetate–toluene]. An analytical sample had m.p. 220—221 °C (Found: C, 66.2; H, 8.4; N, 2.8. $C_{58}H_{90}N_2O_{15}$ requires C, 66.0; H, 8.50; N, 2.7%); $[\alpha]_D^{20}$ −31.6 ° (*c* 0.56 in THF).

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3-O-*acetyl*-4,6-O-*benzylidene*-2-*deoxy*-β-D-*glucopyranosyl*}-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**10**).—The disaccharide (**9**) (1.6 g, 1.5 mmol) was treated with acetic anhydride (20 ml) containing sodium acetate (300 mg) at 120 °C for 2 h. After removal of excess of acetic anhydride by co-distillation under reduced pressure with toluene, the residue was taken up in methylene dichloride and the *title compound* (**10**) (1.6 g, 89%) was isolated by column chromatography [(4:3:1) methylene dichloride–ethyl acetate–toluene]. Crystallization from $CHCl_3$ gave compound (**10**), m.p. 212—215 °C (Found: C, 65.1; H, 8.25; N, 2.5. $C_{64}H_{96}N_2O_{18}$ requires C, 65.1; H, 8.1; N, 2.4%); $[\alpha]_D^{20}$ −34 ° (*c* 0.77 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) (D_2O-exchanged sample) 0.89 (6 H, t, *J* 7 Hz, 2 terminal $CH_3[CH_2]_{10}$), 1.25 (36 H, br s, 2 $[CH_2]_9CH_3$), 1.54 [4 H, br s, 2 $[CH_2]_8CH_2CH(OAc)$], 1.98, 2.02, 2.03, 2.06, and 2.09 (total 15 H, 5 s, 5 $COCH_3$), 2.32—2.54 [4 H, m, 2 $O_2CCH_2CH(OAc)$], 3.55 and 3.72 (total 4 H, 2 m, 5-, 5′-, 6-H_b, and 6′-H_{ax}), 3.82 (1 H, t, *J* 10 Hz, 4′-H), 3.92 (1 H, dd, *J* 8 and 10 Hz, 2-H), 3.97 (1 H, dd, *J* 2 and 11 Hz, 6-H_a), 4.09 (1 H, dd, *J* 8 and 10 Hz, 2′-H), 4.36 (1 H, dd, $J_{6'eq,6'ax}$ 10.5 $J_{6'eq,5'}$ 5 Hz, 6′-H_{eq}), 4.57 (1 H, d, $J_{1,2}$ 8 Hz, 1-H), 4.60 (1 H, d, J_{gem} 12 Hz, OC*H*HPh), 4.74 (1 H, d, $J_{1',2'}$ 8 Hz, 1′-H), 4.89 (1 H, d, J_{gem} 12 Hz, OCH*H*Ph), 4.97 (1 H, t, *J* 10 Hz, 4-H), 5.08 and 5.13 (2 H, 2 m, 2 $CH_2CH(OAc)CH_2$], 5.24 (2 H, t, *J* 10 Hz, 3- and 3′-H), 5.55 (1 H, s, benzylidene *H*CPh), and 7.32—7.5 (10 H, m, ArH).

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*}-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**11**).—The benzylidene acetal (**10**) (1.2 g, 1 mmol) was dissolved with the aid of dioxane (2 ml) in 80% aqueous acetic acid (50 ml) and the solution was kept at 90 °C for 1 h. The solvents were removed under reduced pressure the residual gum was dissolved in dioxane and the solution was freeze-dried to yield the *title compound* (**11**) (983 mg, 90%) as a white powder. An analytical sample was obtained after purification by column chromatography [(15:2) chloroform–methanol]. The amorphous solid, obtained by freeze-drying from dioxane, liquefied at 180—185 °C (Found: C, 62.5; H, 8.4; N, 2.7. $C_{57}H_{92}N_2O_{18}$ requires C, 62.6; H, 8.4; N, 2.5%); $[\alpha]_D^{20}$ −25.4 ° (*c* 1.14 in $CHCl_3$).

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3-O-*acetyl*-6-O-*benzyloxymethyl*-2-*deoxy*-β-D-*glucopyranosyl*}-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**12**).—The carefully dried diol (**11**) (860 mg, 0.79 mmol) was dissolved in chloroform (25 ml) freshly distilled from P_2O_5, and the solution was cooled to 0 °C. 4Å Molecular sieve (*ca.* 5 g), and *NNN′N′*-tetramethylurea (0.5 ml) were added, followed by freshly distilled benzyloxymethyl bromide (0.150 ml). After 30 min at 0—5 °C monoalkylation was complete, as judged by t.l.c. [(150:15) chloroform–methanol]. The mixture was diluted with chloroform (50 ml), decanted from the solids, and directly percolated through a short (diam. 4 cm × ht. 10 cm) column of silica gel, which was first eluted with chloroform (100 ml) and then with 95:5 chloroform–methanol. Dialkylated material (108 mg) was eluted first, followed by the *title compound*. Fractions containing the latter were pooled and brought to dryness to yield the benzyloxymethyl ether (**12**) as a white solid (830 mg, 87%). A sample, crystallized from diisopropyl ether containing a few drops of methanol, had m.p. 182—183 °C (Found: C, 64.25; H, 8.3; N, 2.4. $C_{65}H_{100}N_2O_{19}$ requires C, 64.35; H, 8.25; N, 2.3%); $[\alpha]_D^{20}$ −20.6 ° (*c* 1.8 in $CHCl_3$).

Benzyl 2-[(3R)-3-*Acetoxytetradecanamido*]-6-O-{2-[(3R)-3-*acetoxytetradecanamido*]-3-O-*acetyl*-6-O-*benzyloxymethyl*-2-*deoxy*-4-O-*diphenylphosphono*-β-D-*glucopyranosyl*-}-3,4-*di*-

O-*acetyl*-2-*deoxy*-β-D-*glucopyranoside* (**13**).—A solution of the benzyloxymethyl ether (**12**) was prepared from the diol (**11**) (1.1 g, 1 mmol) as described above, except that, when alkylation appeared to be complete, the diluted reaction mixture, decanted from the solids, was sequentially washed with 2M sulphuric acid, water, and aqueous sodium hydrogen carbonate, and then thoroughly dried (Na_2SO_4). The volume of the solution was reduced to *ca.* 5 ml, and to it was added diphenyl phosphorochloridate (1.2 g, 4.5 mmol) in dry chloroform (50 ml) containing 4-dimethylaminopyridine (500 mg, 4.1 mmol). The mixture was kept at room temperature for 2 h, cooled to 0 °C, and the excess of the phosphorylating agent was destroyed by addition of a small amount of ice. After a short time, the mixture was diluted with methylene dichloride, the solution was washed successively with dilute sulphuric acid, water, aqueous sodium hydrogen carbonate, and water, and dried (Na_2SO_4). The syrup obtained after removal of the solvents was applied to the top of a dry column of silica gel (100 g), and the column was eluted with 8:5 ethyl acetate–hexane. Fractions containing phosphorus (detected by spotting on t.l.c. plates and spraying with Dittmer and Lester's reagent [7]) were pooled, the solvents were removed under reduced pressure, the residue was dissolved in benzene, and the solution was freeze-dried to yield the *product* (**13**) [760 mg, 52.6% from (**11**)] which liquefied at 143 °C (Found: C, 64.1; H, 7.4; N, 1.9. $C_{77}H_{109}N_2O_{22}P$ requires C, 64.0; H, 7.55; N, 1.95%); $[\alpha]_D^{20}$ − 8.5° (*c* 0.8 in $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 0.885 (6 H, t, *J* 6 Hz, 2 terminal CH_3), 1.245 (36 H, br s, 2 $[CH_2]_9CH_3$), 1.56 [4 H, br d, 2 $[CH_2]_8CH_2CH(OAc)$], 1.89, 1.99, 2.00, 2.01, and 2.06 (total 15 H, 5 s, 5 $COCH_3$), 2.28—2.55 (4 H, m, 2 $COCH_2CH_2$), 3.52 (1 H, dd, J_{gem} 11.5, J_{vic} 6 Hz, 6-H_b), 3.58—3.64 (3 H, m, 2′-, 5-, and 5′-H), 3.82 (1 H, dd, *J* 2 and 10.5 Hz, 6′-H_b), 3.88 (1 H, ddd, 2-H), 3.97—4.08 (2 H, m, 6′-H_a and 6-H_a), 4.52 (1 H, d, *J* 8.5 Hz, 1-H), 4.56—4.8 (7 H, m, 4′-H, 1′-H, (CH_2OCH_2Ph, $OCHHPh$, and OCH_2O), 4.87 (1 H, d, J_{gem} 12 Hz, $OCHHPh$), 5.02 (1 H, t, *J* 9 Hz, 4-H), 5.04 [1 H, m, $CH_2CH(OAc)CH_2$], 5.12 [1 H, m, $CH_2CH(OAc)CH_2$], 5.28 (1 H, t, *J* 9.5 Hz, 3′- or 3-H), 5.32 (1 H, t, *J* 9 Hz, 3′-H or 3-H), 5.80 (1 H, d, *J* 9 Hz, NH), 6.16 (1 H, *J* 9 Hz, NH), and 7.12—7.42 (20 H, m, ArH).

6-O-{4-O-*Ammonio*(*hydrogen*)*phosphono*-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucopyranosyl*}-2-*deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-D-*glucose* (**14**).—A solution of the diphenyl phosphate (**13**) (200 mg) in methanol (20 ml) was treated with hydrogen at room temperature under slight pressure (1 bar) in the presence of Pd–C (10% Pd; 100 mg); within 3 h, the benzyl groups were removed [t.l.c.; (8:2) chloroform–methanol]. Hydrogenolysis was continued in the presence of Adam's platinum catalyst (50 mg) overnight; according to t.l.c. [(8:2) chloroform–methanol; R_F 0.13] a single, phosphorus-containing substance, that did not absorb u.v. light, was then present. The catalyst was filtered off and thoroughly washed with methanol. The filtrate and washings were pooled, cooled to 0 °C, saturated with ammonia, and kept overnight at 0 °C and for 1 day at room temperature. The mixture was then concentrated to a small volume, and acetone was added to precipitate the *ammonium hydrogen salt* (86 mg, 70%). An analytical sample, obtained by column chromatography (10 × 1 cm) on silica gel [(7:2:1) propan-1-ol–aqueous ammonia (*d* 0.91)–water] had m.p. 160—166 °C (decomp.) (Found: C, 50.6; H, 9.1; N, 4.5. $C_{40}H_{80}N_3O_{16}P{\cdot}3H_2O$ requires C, 50.9; H, 9.1; N, 4.45%); $[\alpha]_D^{25}$ +19° [*c* 0.25 in (1:1) pyridine–methanol].

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