Chemistry of Bacterial Endotoxins. Part 4.[1] Synthesis of Anomeric 2-Deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucopyranosyl Phosphate and Pyrophosphate Derivatives Related to 'Lipid A'

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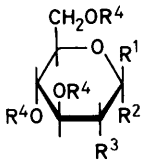
Syntheses of both anomers of 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucopyranosyl-phosphate and of 2-amino-2-deoxy-D-glucopyranosyl 2-aminoethyl phosphate are reported, as well as those of two pyrophosphate derivatives of glucosamine, namely 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl pyrophosphate and P^1-(2-aminoethyl) P^2-{2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl}pyrophosphate, structures that have been identified as being present in the hydrophobic region (Lipid A) of endotoxins.

Ethanolamine† is a common moiety of bacterial endotoxins. It has been reported to be bound, through a phosphate[2] or a pyrophosphate[3] group, to alcoholic hydroxy functions of the hydrophilic region, or to the glycosidic position of glucosamine units present in the hydrophobic region of these lipopolysaccharides where *N*-[(3*R*)-hydroxytetradecanoyl]glucosamine units carrying unsubstituted phosphate[4] or pyrophosphate[5] residues bound to the glycosidic position have also been found.

In the course of structural studies on the *Bordetella pertussis* endotoxin, we have shown that treatment of this endotoxin with dilute acetic acid at 100 °C led to the release of inorganic phosphate and of *O*-phosphoryl- and *O*-pyrophosphoryl-ethanolamine.[6] It was therefore considered necessary to examine the stability of a phosphate group, either free or esterified with ethanolamine, glycosidically bound to a glucosamine unit carrying an amide-bound (3*R*)-3-hydroxytetradecanoic acid residue, under conditions of acidity and alkalinity to which endotoxins are usually exposed during studies designed to elucidate their structure. To this end, the α-and β-1-phosphates and the α-1-pyrophosphate of 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucopyranose, compounds (**2**), (**3**), and (**26**), as well as 1-(2-aminoethoxy phosphoryl and pyrophosphoryl)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-α-D-glucopyranose (**20**) and (**28**) have been synthesized.

For the synthesis of the glucosaminyl phosphate with the β configuration (**3**), the benzyl groups of dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate[7] (**6**) were split off by hydrogenolysis, and the phthalimido and acetyl groups were then removed by cautious treatment with hydrazine. The free amino group of the resulting 2-amino-2-deoxy-β-D-glucopyranosyl dihydrogen phosphate thus formed—which was not isolated—was then *N*-acylated using (3*R*)-3-acetoxytetradecanoic anhydride[8] and, following removal of the acetyl group attached to the fatty acid, the β-D-glucopyranose ammonium hydrogen phosphate (**3**) was isolated after purification by column chromatography on silica gel in an overall yield of 41%.

The starting material for the synthesis of the corresponding α-anomer (**2**) was 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-1-diphenoxyphosphoryl-α-D-glucopyranose hydrochloride,[9] which was submitted to platinum-catalysed hydrogenolysis to remove both phenyl groups. After addition of 3 mol equiv. of triethylamine to neutralize the acidic functions thus created, and to set free the primary amino group of the sugar, the phosphomonoester was *N*-acylated with (3*R*)-3-acetoxytetradecanoic anhydride. The acetyl groups were removed and the phosphorylated sugar (**2**) was isolated in the same way as the β-anomer (**3**) in a yield of 60% from the triester.



(1) R^1 = H, R^2 = Br, R^3 = NH_2, HBr, R^4 = Ac
(2) R^1 = R^4 = H, R^2 = OPO_3HNH_4, R^3 = $NHCOCH_2CH(OH)[CH_2]_{10}Me$
(3) R^1 = OPO_3HNH_4, R^2 = R^4 = H, R^3 = $NHCOCH_2CH(OH)[CH_2]_{10}Me$
(4) R^1 = H, R^2 = $OPO(OBn)_2$, R^3 = NHZ, R^4 = Ac
(5) R^1 = $OPO(OBn)_2$, R^2 = H, R^3 = NHZ, R^4 = Ac
(6) R^1 = $OPO(OBn)_2$, R^2 = H, R^3 = phthalimido, R^4 = Ac
(7) R^1 = OH, R^2 = H, R^3 = phthalimido, R^4 = Ac

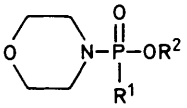
Bn = benzyl, Z = benzyloxycarbonyl

However, as it was found difficult to obtain the required pure, crystalline 1-diphenoxyphosphoryl-3,4,6-tri-*O*-acetyl-α-D-glucosamine chlorohydrate,[9] described by Maley and Lardy, in a reasonable and reproducible yield (usually < 20%), the use of the dibenzyl phosphate instead of the diphenyl phosphate group was investigated. Condensation of dibenzyl triethylammonium phosphate with 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide[9] (**1**), followed by treatment with benzyl chloroformate, gave a 50% yield of a mixture of the *N*-benzyloxycarbonyl-tri-*O*-acetyl-D-glucosamine dibenzyl phosphates (**4**) and (**5**), which were separated by column chromatography. The more abundant α-anomer (**4**) gave, after deprotection of both phosphate and amino groups (H_2, Pd/C), *N*-acylation (3-acetoxytetradecanoic anhydride), and de-*O*-acetylation, the α-phosphate (**2**) identical with that obtained by the route described above. By the same sequence of reactions, the minor compound (**5**) afforded the β-phosphate (**3**) also identical with that obtained starting with the phthalimido derivative (**6**).

It is noteworthy that all attempts to *N*-acylate the diphenyl phosphotriester were unsuccessful. However, *N*-acylation of the phosphomonoester could be performed using 3-acetoxytetradecanoic anhydride[8] [*N*-acylation using dicyclohexylcarbodiimide (DCC)–3-acetoxytetradecanoic acid[10] was excluded because of the presence of the phosphate group].

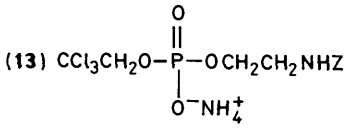
The phosphorylating agent used in the synthesis of the phosphodiester 2-aminoethyl 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl hydrogen phosphate (**20**)

† Aminoethanol.

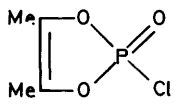


(**8**) R^1 = Cl, R^2 = CH_2CCl_3
(**9**) R^1 = OCH_2CH_2NHZ, R^2 = CH_2CCl_3
(**10**) R^1 = OBn, R^2 = CH_2CCl_3
(**11**) R^1 = O^- morpholinium, R^2 = CH_2CH_2NHZ
(**12**) $NHZCH_2CH_2OPO_3^{2-}$ Li_2^+

was obtained by condensation of 2,2,2-trichloroethyl phosphorodichloridate[11] with morpholine, to yield the crystalline phosphoramidochloridate (**8**). Reaction of this reagent with benzyloxycarbonylethanolamine in pyridine containing 4-dimethylaminopyridine (DMAP) gave the phosphotriester (**9**) from which the P–N-bound morpholine could readily be removed by treatment with a cationic-exchange resin in aqueous methanol at 60 °C; the phosphodiester (**13**) formed was isolated as its crystalline ammonium salt. It is noteworthy that while the phosphodiester (**11**) when treated with IR 77 (H^+) resin at room temperature released the amide-bound substituent immediately, removal of the same substituent from the phosphotriester (**9**) required treatment at 60 °C for 10 h with the same resin.

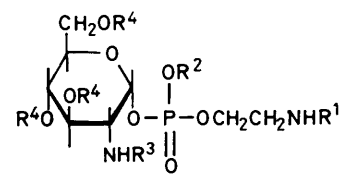


(**13**) $CCl_3CH_2O-P(=O)(O^-NH_4^+)-OCH_2CH_2NHZ$



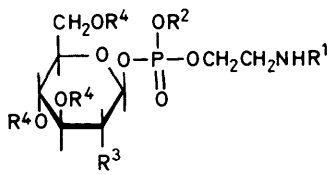
(**14**)

The anhydrous, oily triethylammonium salt of the diester (**13**) was condensed with 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide in the presence of 2 mol equiv. of triethylamine to yield a phosphotriester. Attempts to remove the trichloroethyl group from this triester with zinc powder and acetic acid[12] in pyridine failed: almost quantitative cleavage between the glucosamine unit and the phosphate radical was observed. As it was suspected that the free amino function was implicated in this reaction, and was thus responsible for the relative instability of the phosphotriester, the amino function was substituted *in situ* with a benzyloxycarbonyl group as soon as the glycosyl phosphate was formed: the phosphotriester (**15**) could then be isolated (36% yield after chromatography) as an oil but, being unstable, was not further characterized. Removal of the trichloroethyl protecting group from this material with zinc and acetic acid was readily accomplished. The acetyl groups of the resulting phosphodiester (which was not isolated) were saponified, and the ammonium salt of the diester, (**16**), an amorphous solid, was recovered by chromatography in 78% yield based on the phosphotriester (**15**). Finally, the deprotected phosphodiester (**14**) was obtained by hydrogenolysis of both benzyloxycarbonyl groups. The anomeric configuration was ascertained by ^1H n.m.r. spectroscopy; the coupling constant of 1-H (δ_H 5.40) with 2-H was small (3 Hz) as would be expected.



(**15**) R^1 = R^3 = Z, R^2 = CH_2CCl_3, R^4 = Ac
(**16**) R^1 = R^3 = Z, R^2 = NH_4, R^4 = H
(**17**) R^1 = R^2 = R^3 = R^4 = H
(**18**) R^1 = Z, R^2 = CH_2Cl_3, R^3 = $COCH_2CH(OAc)[CH_2]_{10}Me$, R^4 = Ac
(**19**) R^1 = Z, R^2 = NH_4, R^3 = $COCH_2CH(OH)[CH_2]_{10}Me$, R^4 = H
(**20**) R^1 = R^2 = R^4 = H, R^3 = $COCH_2CH(OH)[CH_2]_{10}Me$

Since substitution of the anomeric carbon of 2-deoxy-2-phthalimido-D-glucopyranose derivatives usually leads to β-anomers, 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose[13] (**7**) was chosen as starting material for the synthesis of the substituted phosphodiesters derived from 2-amino-2-deoxy-β-D-glucopyranosyl phosphate. The phosphorylating agent selected was 'acetoin enediol cyclophosphorochloridate,' 'CEP-Cl' (**14**) described by Ramirez[14] and his colleagues. With this reagent the two alcohols esterifying the phosphate group in the target phosphodiester are introduced in an uninterrupted sequence. Accordingly the acetylated phthalimido sugar derivative[13] (**7**) was treated with CEP-Cl and triethylamine, and when the reaction appeared to be complete as judged by t.l.c., 2-(benzyloxycarbonylamino)ethanol[15] was added. The phosphotriester (**21**) formed was isolated as an oil. Attempted removal of the acetoin group from the phosphotriester under the conditions employed by Ramirez, *i.e.* treatment of the triester in acetonitrile or aqueous pyridine with 2 mol equiv. of triethylamine, failed, quantitative release of the glucosamine unit being observed. It succeeded, however, when the phosphotriester (**21**) was treated with cyclohexylammonium thiocyanate[16] in anhydrous butan-2-one at 90 °C for 19 h. The phosphodiester (**22**) was isolated by column chromatography as the lithium salt in 55% yield based on starting material (**7**). Deprotection was performed by hydrogenation in the presence of palladium–charcoal catalyst which removed the benzyloxycarbonyl protecting group, followed by cautious hydrazinolysis of the internal salt (**23**) thus obtained. Despite partial dephosphorylation 2-amino-2-deoxy-β-D-glucopyranosyl 2-aminoethyl hydrogen phosphate (**24**) could be obtained in 32% yield from the phosphodiester (**23**). Selective *N*-acylation of the glucosamine moiety of this phosphodiester could not be achieved: in the major compound obtained by treatment of the diamino derivative (**24**) with acetic anhydride, the acetyl group was attached to the aminoethyl group and not to the glucosamine residue; accordingly, selective *N*-acylation with 3-hydroxytetradecanoic acid derivatives was not attempted.



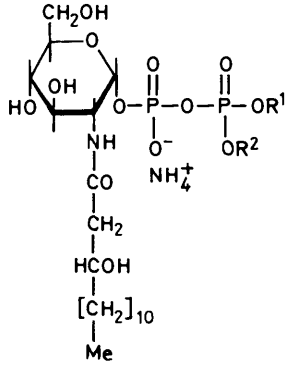
(**21**) R^1 = Z, R^2 = CH(Me)COMe, R^3 = phthalimido, R^4 = Ac
(**22**) R^1 = Z, R^2 = Li, R^3 = phthalimido, R^4 = Ac
(**23**) R^1 = R^2 = H, R^3 = phthalimido, R^4 = Ac
(**24**) R^1 = R^2 = R^4 = H, R^3 = NH_2

The α-*N*-acylated phosphodiester (**20**) was finally obtained by the following route: the glycosyl bromide (**1**) was allowed to react with the phosphodiester (**13**) and the product formed was heated, *in situ*, with 3-acetoxytetradecanoic anhydride to yield the phosphotriester (**18**). Deprotection of this by the sequence of reactions used for the obtention of the β-glycosyl phosphate (**17**) led to compound (**20**).

Formation of pyrophosphates by condensation of a phosphoramidate with a phosphomonoester is a well known

method. To obtain 3-hydroxytetradecanoyl-α-D-glucosaminyl pyrophosphate, we chose to condense the glucosaminyl phosphate unit (**2**) with a phosphoramidate rather than an appropriate glucosaminyl phosphoramidate unit with a monophosphate, since we had already prepared the glucosaminyl phosphate (**2**) and furthermore the phosphomorpholidates (**10**) and (**11**) are readily accessible.

2,2,2-Trichloroethyl phosphoromorpholidochloridate (**8**) reacted smoothly with benzyl alcohol at 20 °C, under conditions similar to those used for the synthesis of the tribromoethyl phosphoramidate analogue described by van Boom.[17] The triester (**10**) produced was transformed directly into the acidic morpholidate (**11**) by treatment with zinc dust and this was, without isolation, condensed with the phosphomonoester (**2**) to give a 50% yield of diammonium P^1-benzyl P^2-{2-deoxy-2-[(3*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranosyl} pyrophosphate (**25**). In a similar manner, the phosphoromorpholidate of *N*-benzyloxycarbonylethanolamine, (**11**)—obtained from benzyloxycarbonylethanolamine phosphate[18] and morpholine by DCC-promoted condensation[19]—reacted with compound (**2**) to give the hygroscopic pyrophosphate diester (**27**) in a total yield of 60%. Finally the deprotected pyrophosphate (**28**) and the P^1-(2-aminoethyl) P^2-glucosaminyl pyrophosphate (**26**) were obtained by catalytic removal of the benzyl and benzyloxycarbonyl groups, respectively.



(**25**) R^1 = Bn, R^2 = NH_4
(**26**) R^1 = H, R^2 = NH_4
(**27**) R^1 = CH_2CH_2NHZ, R^2 = NH_4
(**28**) R^1 = $CH_2CH_2NH_2$, R^2 = H

Experimental

Evaporations were carried out under reduced pressure at 40 °C. Products were dried *in vacuo* (P_2O_5) and phosphates were then equilibrated in air. M.p.s were determined on a Kofler hot plate and are uncorrected. Optical rotations were determined with a Perkin-Elmer model 141 polarimeter. ^{1}H N.m.r. spectra were recorded at 90 MHz on a Varian E.M. 390 spectrometer, and at 400 MHz on a prototype I.E.F. 400 instrument,[20] using Me_4Si 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.[21] Column chromatography was performed on silica gel Merck 60 (70—230 mesh).

Ammonium 2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-α-D-glucopyranosyl Hydrogen Phosphate (**2**).—A methanolic solution (30 ml) of 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-1-diphenylphosphono-D-glucopyranose hydrobromide[9] (1.15 g, 2 mmol) was hydrogenated over Adams platinum catalyst (100 mg) until hydrogen uptake ceased (*ca.* 16 h). The catalyst was filtered off and washed with methanol. The acid solution (50 ml) was neutralized by addition of triethylamine (600 mg, 6 mmol). (3*R*)-3-Acetoxytetradecanoic anhydride (1.5 g, 3 mmol) was added; the stirred solution was kept at room temperature for 2 h, cooled (0 °C), and saturated with anhydrous ammonia gas. The container was closed and allowed to come to room temperature during 2 h; it was then kept at 4 °C overnight. After removal of the solvent, the residue was dissolved in (7.5:1.5:1) propan-2-ol–water–conc. ammonia (5–10 ml) and chromatographed on a column (20 × 4 cm) of silica gel with the same solvent. After elution of non-phosphorylated products (mainly fatty acids), the solvent mixture was changed to (7:2:1) propan-2-ol–water–conc. ammonia. Fractions giving a positive test for phosphorus[21] were collected, pooled, and brought to dryness. The *title compound* (**2**) (620 mg, 60%), triturated with acetone and collected by centrifugation, had m.p. 150—160 °C (decomp.); $[\alpha]_D^{22}$ +56° (*c* 1.4 in water) (Found: C, 46.1; H, 8.4; N, 5.6. $C_{20}H_{43}N_2O_{10}P{\cdot}H_2O$ requires C, 46.15; H, 8.65; N, 5.4%).

Ammonium 2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-β-D-glucopyranosyl Hydrogen Phosphate (**3**).—A stirred suspension of dibenzyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl phosphate (**6**)[7] (700 mg, 1 mmol) and 10% palladium–charcoal catalyst (100 mg) in methanol (20 ml) was treated with hydrogen. When removal of the benzyl groups was complete [10—15 min; t.l.c. (8:4:2) ethyl acetate–methanol–water], the catalyst was filtered off and washed with methanol (2 × 10 ml). Hydrazine hydrate (95%; 0.25 ml) was added to the pooled filtrate and washings, and the stirred mixture was kept at 80 °C for 2 h. The precipitate formed (350 mg) was collected by filtration and dried. It was dissolved in water (5 ml) and the solution was percolated through a short column (5 × 5 cm) of Amberlite IR 120 (triethylammonium) resin in (1:1) aqueous ethanol; the column was eluted with the same solvent until the effluent gave a negative test with ninhydrin (*ca.* 300 ml). The effluent was brought to dryness and the residue was dissolved in water (3 ml). Ethanol (25 ml) and triethylamine (100 mg, 1 mol equiv.) were added, followed by (3*R*)-3-acetoxytetradecanoic anhydride[8] (1 g, 2 mmol). After 2 h the solvents were removed, and methanol was added and evaporated from the residue several times. Methanol saturated with dry ammonia gas at 0 °C (25 ml) was added to the dry residue; the mixture was kept for 2 h at room temperature in a sealed bottle and then at 4 °C overnight. The *title compound* (**3**) (220 mg, 41%), isolated as described for the α-anomer, had m.p. 130—150 °C (decomp.); $[\alpha]_D^{22}$ −0.6° (*c* 1.2 in water) (Found: C, 45.2; H, 8.4; N, 5.6. $C_{20}H_{43}N_2O_{10}P{\cdot}1.5H_2O$ requires C, 45.4; H, 8.7; N, 5.3%).

Dibenzyl 3,4,6-Tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-α-and -β-D-glucopyranosyl Phosphate (**4**) *and* (**5**).—A mixture of triethylamine (4 g, 40 mmol), 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide[9] (**1**) (9 g, 20 mmol) and dibenzyl hydrogen phosphate (5.6 g, 20 mmol) in chloroform (50 ml; freshly distilled from P_2O_5) was stirred at room temperature for 1 h. Anhydrous pyridine (20 ml) and benzyl chloroformate (50% w/v solution in toluene) (10 ml) were added in portions alternately; the mixture was stirred for 1 h and was then applied to a column of silica gel (25 × 10 cm) which was eluted with (1:1) hexane–ethyl acetate. Fractions containing the *phosphotriester* (**4**) (R_F 0.45) were pooled and the solvents were removed. The residue (4 g, 29%) was crystallized from diethyl ether and had m.p. 104—106 °C; $[\alpha]_D^{22}$ +71° (*c* 2 in $CHCl_3$) (Found: C, 58.2; H, 5.5; N, 1.9. $C_{34}H_{39}NO_{13}P$ requires C, 58.3; H, 5.6; N, 2.0%); δ_H(400 MHz; $CDCl_3$) 5.75 (1 H, dd, $J_{1,2}$ 3, $J_{1,P}$ 6 Hz, 1-H).

Fractions containing the phosphotriester (**5**) (R_F 0.40) were

pooled and the *product* (800 mg, 6%) was isolated and crystallized as described above. It had m.p. 115—118 °C; $[\alpha]_D^{22}$ +1.8° (*c* 1.6 in $CHCl_3$) (Found: C, 58.0; H, 5.4; N, 2.2%); δ_H(400 MHz; $CDCl_3$) 5.50 (1 H, t, $J_{1,2}$ 8, $J_{1,P}$ 8 Hz, 1-H).

A mixture of products (**4**) and (**5**) (2 g, 14%) was isolated from the intermediate fractions.

Alternative Preparation of Ammonium 2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl Hydrogen Phosphate* (**2**) *from the Triester* (**4**).—A solution of the phosphotriester (**4**) (0.7 g, 1 mmol) in methanol (20 ml) was hydrogenated at atmospheric pressure and room temperature in the presence of 10% palladium–charcoal catalyst (0.1 g). About 1 h is required for hydrogenolysis of the two benzyl and the benzyloxy groups [t.l.c. (8:4:2) ethyl acetate–methanol–water, R_F 0.10]. The catalyst was filtered off and washed with methanol (50 ml). Triethylamine (0.2 g, 2 mmol) and (3*R*)-3-acetoxytetradecanoic anhydride (0.75 g, 1.5 mmol) were added to the combined filtrate and washings, and the stirred solution was kept for 2 h at room temperature. 1M Sodium methoxide in methanol (2 ml) was added and deacetylation was monitored by t.l.c. in (2:2:1) dichloromethane–methanol–conc. ammonia (R_F 0.66). Solvents were removed, and the residue was adsorbed onto silica gel (25 ml) and put on a column (10 × 3.3 cm) of silica gel. The product (**2**) (0.34 g, 62%), eluted with the same solvent, was isolated as already described. It had m.p. 150—160 °C (decomp.); $[\alpha]_D^{22}$ +63° [*c* 0.6 in (1:1.5:1) water–methanol–pyridine] (Found: C, 43.95; H, 8.6; N, 5.2. $C_{20}H_{43}N_2O_{10}P$·2.5 H_2O requires C, 43.9; H, 8.8; N, 5.1%).

Alternative Preparation of Ammonium 2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-β-D-*glucopyranosyl Phosphate* (**3**) *from the Triester* (**5**).—This compound (0.25 g, 47%), prepared from compound (**5**) (0.7 g, 1 mmol) as described for the α-anomer (**2**), had m.p. 130—150 °C; $[\alpha]_D^{22}$ −1.8° (*c* 1.3 in water) (Found: C, 44.75; H, 8.6; N, 7.75. $C_{20}H_{46}N_3O_{10}P$·H_2O requires C, 44.7; H, 8.9; N, 7.8%).

2,2,2-*Trichloroethyl Phosphoromorpholidochloridate* (**8**).—A solution of morpholine (17.4 g, 0.2 mol) in anhydrous diethyl ether (100 ml) was added dropwise to a stirred solution of 2,2,2-trichloroethyl phosphorodichloridate[11] (26.7 g, 0.1 mol) in anhydrous ether (400 ml) cooled to 0 °C. The precipitate (morpholine hydrochloride) was filtered off, the solvent was removed, and the residual syrup was crystallized from ether–hexane. The *product* (**8**) (19 g, 60%) had m.p. 78—79 °C (Found: C, 22.6; H, 3.2; N, 4.5. $C_6H_{10}Cl_4NO_3P$ requires C, 22.7; H, 3.15; N, 4.4%); δ_H(90 MHz; $CDCl_3$) 3.2 (4 H, m, CH_2NCH_2), 3.6 (4 H, m, CH_2OCH_2), and 4.6 (2 H, m, CH_2OP).

2-(*Benzyloxycarbonylamino*)*ethyl* 2,2,2-*Trichloroethyl Morpholinophosphate* (**9**).—A solution of the preceding compound (23.8 g, 75 mmol) in pyridine (50 ml), followed by DMAP (2 g), were added to a stirred solution of 2-(benzyloxycarbonylamino)ethanol[15] (9.75 g, 50 mmol) in anhydrous pyridine (50 ml). The mixture was stirred for 16 h, when pyridinium chloride was filtered off and washed with pyridine. The filtrate and washings were pooled, the solvent was removed, and the residue was adsorbed onto silica gel (50 ml) and applied to a column (40 × 7 cm) of silica gel in (100:3) chloroform–methanol. Fractions containing the triester (R_F 0.30) were pooled. Evaporation of the solvents left the title product (**9**) as an oil (15 g, 63%); δ_H(90 MHz; $CDCl_3$) 3.2 (4 H, m, CH_2NCH_2), 3.6 (6 H, m, CH_2OCH_2 and CH_2N), 4.0 (2 H, m, CH_2OP), 4.4 (2 H, d, *J* 6 Hz, CH_2CCl_3), 5.0 (2 H, s, CH_2Ph), and 7.2 (5 H, s, Ph).

Ammonium 2-(*Benzyloxycarbonylamino*)*ethyl* 2,2,2-*Trichloroethyl Phosphate* (**13**).—Dry Amberlite IR 77 (H^+) resin (20 ml) was added to a solution of the preceding compound (4.8 g, 10 mmol) in (1:1) methanol–water (100 ml). The stirred solution was kept at 60 °C for 10 h. The cooled mixture was filtered, and the pH of the filtrate was brought to 7 with 1M aqueous ammonia. Solvents were removed and the residual material was dissolved in the minimal amount of methanol. The *title product* (**13**) (2.5 g, 59%), which crystallized upon addition of ether, was collected and dried. It had m.p. 160—165 °C (decomp.) (Found: C, 33.9; H, 4.3; N, 6.5. $C_{12}H_{18}Cl_3N_2O_6P$ requires C, 34.0; H, 4.25; N, 6.6%); δ_H(90 MHz; CD_3OD) 3.2 (2 H, m, NCH_2), 3.8 (2 H, m, CH_2CH_2OP), 4.2 (2 H, d, *J* 6 Hz, CH_2CCl_3), 4.9 (2 H, s, CH_2Ph), and 7.2 (5 H, s, Ph).

2-(*Benzyloxycarbonylamino*)*ethyl* 3,4,6-*Tri*-O-*acetyl*-2-*benzyloxycarbonylamino*-2-*deoxy*-α-D-*glucopyranosyl* 2,2,2-*Trichloroethyl Phosphate* (**15**).—Triethylamine (1 g, 10 mmol) was added to a solution of the phosphodiester (**13**) (4.23 g, 10 mmol) in (1:1) methanol–water (100 ml). The solvents were removed, and anhydrous ethanol (50 ml) and anhydrous benzene (2 × 50 ml) were added to, and evaporated from, the residue which was then dissolved in chloroform (50 ml; freshly distilled from P_2O_5). 3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (4.5 g, 10 mmol) (**1**) followed by triethylamine (1 g, 10 mmol) were added to the stirred solution and the mixture was stirred for 1 h. Pyridine (2.5 ml) and a solution of benzyloxycarbonyl chloride (50% w/v in toluene) (5 ml) were added and both additions were repeated 10 min later. After 15 min, the solution was passed through a column (40 × 5 cm) of silica gel in (2:1) hexane–ethyl acetate. The column was washed with the same solvent mixture (1 l) and then with (1:1) hexane–ethyl acetate (1 l), followed by (1:2) hexane–ethyl acetate (1 l). Fractions eluted with the latter solvent and containing the phosphotriester (**15**) (R_F 0.15) were pooled and the solvents were removed, leaving the title product (3 g, 36%) as an oil. The material was used without undue delay.

Ammonium 2-*Benzyloxycarbonylamino*-2-*deoxy*-α-D-*glucopyranosyl* 2-(*Benzyloxycarbonylamino*)*ethyl Phosphate* (**16**).—Zinc powder (0.5 g) and acetic acid (2 ml) were added to a stirred solution of the phosphotriester (**15**) (3 g, 3.6 mmol) in anhydrous pyridine (20 ml) at room temperature, the additions being repeated 15 min later. After a further 15 min, the supernatant was removed by decantation. It was diluted with dichloromethane (200 ml), and the solution was washed with saturated aqueous ammonium hydrogen carbonate (3 × 200 ml) and dried (Na_2SO_4). The solvent was removed, the yellow residue was dissolved in 0.05M methanolic sodium methoxide (20 ml), and the mixture was kept at 4 °C overnight. Water (20 ml) was added to the mixture, which was then passed through a column (*ca.* 100 ml) of Amberlite IR 77 (H^+) resin. The resin was washed with water and the effluent was neutralized with aqueous ammonia. The residue recovered after removal of the solvents was dissolved in (8:1:1) propan-2-ol–conc. ammonia–water and chromatographed on a column (20 × 3 cm) of silica gel in the same solvent. Fractions containing the phosphodiester (**16**) (R_F 0.6) were pooled, the solvents were removed, and the residue was dissolved in methanol (5–10 ml) and applied to a column (45 × 3.6 cm) of Sephadex LH 20 (Pharmacia Fine Chemicals) prepared in (1:1) chloroform–methanol, elution being carried out with this solvent. Solvents were removed from the pooled fractions containing the phosphodiester (**16**). The residue was dissolved in acetone (10 ml) and the *title product* (**16**) (1.7 g, 78%) was precipitated by addition of ethyl acetate; it was collected by centrifugation and dried; m.p. 115—125 °C (decomp.); $[\alpha]_D^{22}$ +51° (*c* 1.6 in MeOH) (Found: C, 47.45; H, 5.9; N, 6.7. $C_{24}H_{34}N_3O_{12}P$·H_2O requires C, 47.6; H, 5.95; N, 6.9%); δ_H(90 MHz; CD_3OD) 4.5 (1 H, dd, $J_{1,2}$ 2.5, $J_{1,P}$ 7.5 Hz, 1-H).

2-Amino-2-deoxy-α-D-glucopyranosyl 2-Aminoethyl Hydrogen Phosphate (**17**).—A solution of the protected phosphodiester (**16**) (500 mg, 0.85 mmol) in methanol (100 ml) was hydrogenated in the presence of 10% palladium–charcoal catalyst (200 mg). T.l.c. [(8:1:1) propan-2-ol–conc. ammonia–water] showed removal of the benzyloxy groups to be complete within 1 h. The catalyst was filtered off and washed with methanol, the filtrate was brought to dryness, and the residue was triturated with diethyl ether. The *title product* (**17**) (250 mg, 87%), recovered by centrifugation and dried, had m.p. 145—160 °C (decomp.); $[\alpha]_D^{22}$ +85° (*c* 1.6 in water) (Found: C, 28.3; H, 6.75; N, 8.3. $C_8H_{19}N_2O_8P{\cdot}2H_2O$ requires C, 28.4; H, 6.8; N, 8.3%); δ_H(400 MHz; D_2O) 2.75 (1 H, dt, $J_{1,2}$ 3, $J_{2,3}$ 10, $J_{2,P}$ 3 Hz, 2-H), 3.15 (2 H, t, *J* 5 Hz, CH_2N), 3.30 (1 H, t, $J_{3,4}$ 10 Hz, 3- or 4-H), 3.50 (1 H, t, $J_{3,4}$ 10 Hz, 4- or 3-H), 3.65 (1 H, dd, J_{gem} 12, J_{vic} 5 Hz, 6-H), 3.72 (2 H, m, 6-H′ and 5-H), 4.05 (2 H, m, CH_2OP), and 5.40 (1 H, dd, $J_{1,2}$ 3, $J_{1,P}$ 7 Hz, 1-H).

2-[(3R)-3-*Acetoxytetradecanamido*]-3,4-6-*tri*-O-*acetyl*-2-*deoxy*-α-D-*glucopyranosyl* 2-(*Benzyloxycarbonylamino*)*ethyl* 2,2,2-*Trichloroethyl Phosphate* (**18**).—Triethylamine (0.5 g, 5 mmol) was added to a solution of the phosphodiester (**13**) (2.1 g, 5 mmol) in (1:1) methanol–water (100 ml). The solvents were removed, and anhydrous ethanol (50 ml) and anhydrous benzene (2 × 50 ml) were added to, and removed from, the residue which was then dissolved in chloroform (25 ml; freshly distilled from P_2O_5). 3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide (**1**) (2.25 g, 5 mmol) followed by triethylamine (0.5 g, 5 mmol) were added to the stirred solution and the mixture was stirred for 1 h. A solution of (3*R*)-3-acetoxytetradecanoic anhydride (3.5 g, 7 mmol) in ethyl acetate (25 ml) was added. After 1 h, the solution was passed through a column (40 × 5 cm) of silica gel in (1:1) hexane–ethyl acetate and the column was eluted with the same solvent. Fractions containing the phosphotriester (**18**) (t.l.c. in the same solvent, R_F 0.20) were pooled. The solvents were removed, leaving the title product (**18**) (1.5 g, 31%) as an oil, δ_H(90 MHz; $CDCl_3$ containing 5% CD_3OD) 5.7 (1 H, dd, $J_{1,2}$ 3, $J_{1,P}$ 6 Hz, 1-H).

Ammonium 2-(*Benzyloxycarbonylamino*)*ethyl* 2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl Phosphate* (**19**).—Zinc powder (0.5 g) and acetic acid (2 ml) were added to a stirred solution of the phosphotriester (**18**) (1.5 g, 1.6 mmol) in anhydrous pyridine (20 ml) at room temperature, the additions being repeated 15 min later. After a further 15 min the solid was allowed to settle, and the solution was decanted; it was diluted with dichloromethane (200 ml), washed with saturated aqueous sodium hydrogen carbonate (3 × 100 ml), then dried (Na_2SO_4) and the solvents were removed. The yellow residue was dissolved in 0.05M methanolic sodium methoxide (20 ml) and the mixture was kept at 4 °C overnight. Solvents were removed (30 °C) and the residue was dissolved in (65:25:4) chloroform–methanol–conc. ammonia (5 ml) and applied to a column of silica gel (45 × 2 cm) prepared and eluted with the same solvent. Fractions containing the phosphodiester (**19**) were pooled, the solvents were removed, and the oily residue was dissolved in the minimum amount of methanol (4—5 ml) and precipitated by addition of acetone. The *title compound* (**19**) (0.7 g, 63%), collected by centrifugation and dried, had m.p. 140—150 °C (decomp.); $[\alpha]_D^{22}$ +47° [*c* 1 in (2:1) methanol–pyridine] (Found: C, 50.5; H, 8.1; N, 5.7. $C_{30}H_{54}N_3O_{12}P{\cdot}2H_2O$ requires C, 50.35; H, 8.1; N, 5.9%).

2-Aminoethyl 2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl Hydrogen Phosphate* (**20**).—A solution of the preceding compound (0.3 g, 0.42 mmol) in methanol (20 ml) was hydrogenated in the presence of 10% palladium–charcoal catalyst (0.1 g). After removal of the benzyloxycarbonyl group [t.l.c. (65:25:4) chloroform–methanol–conc. ammonia, R_F 0.04], the catalyst was filtered off and washed with methanol (20 ml). The solvents were removed and the *title product* (**20**) (0.19 g, 82%) was precipitated by addition of acetone, recovered by centrifugation, and dried; m.p. 160—170 °C (decomp.); $[\alpha]_D^{22}$ +60° (*c* 1.1 in water) (Found: C, 47.9; H, 8.6; N, 5.25. $C_{22}H_{45}N_2O_{10}P{\cdot}1.5H_2O$ requires C, 47.6; H, 8.65; N, 5.0%); δ_H[400 MHz; (1:1) D_2O–CD_3OD] 0.89 (3 H, t, *Me*CH_2), 1.29 (18 H, br peak, $[CH_2]_9$), 1.46 (2 H, br peak, $CH_2[CH_2]_9$), 2.35 (2 H, d, CH_2CO), 3.12 (2 H, m, CH_2ND_2), 3.20 (1 H, t, $J_{3,4}$ 10 Hz, 3- or 4-H), 3.60 (1 H, dd, J_{gem} 12, J_{vic} 8 Hz, 6-H′), 3.72 (1 H, dd, $J_{3,4}$ 10 Hz, 4- or 3-H), 3.87 (1 H, m, *H*COD$[CH_2]_{10}$), 3.92 (1 H, m, $J_{1,2}$ 3, $J_{2,3}$ 10 Hz, 2-H), 4.0 (2 H, m, 6- and 5-H), 4.05 (2 H, m, CH_2OP), and 5.55 (1 H, dd, $J_{1,2}$ 3, $J_{1,P}$ 8 Hz, 1-H).

2-(*Benzlyoxycarbonylamino*)*ethyl* 1-*Methyl*-2-*oxopropyl* 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalamido*-β-D-*glucopyranosyl Phosphate* (**21**).—A solution of freshly distilled triethylamine (3 g, 30 mmol) in dichloromethane (10 ml) was added to a stirred suspension of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalamido-β-D-glucopyranose (**7**) (13.1 g, 30 mmol) in anhydrous dichloromethane (50 ml) followed by a solution of CEP-Cl [14] (**14**) (5.1 g, 30 mmol) in dichloromethane (50 ml). The mixture was stirred for 2 h, then a solution of 2-(benzyloxycarbonylamino)ethanol [15] (5.9 g, 30 mmol) in dichloromethane (25 ml) was added and the reaction (whose progress can be monitored by t.l.c. in ethyl acetate) was allowed to continue overnight. Diethyl ether (300 ml) was added to the mixture, the precipitate formed (triethylamine hydrochloride) was filtered off, washed with diethyl ether (2 × 200 ml), and the filtrate was dried (Na_2SO_4). The yellow oil remaining after removal of the solvent was the crude phosphotriester (**21**) (21.2 g) [*R* (triacetyl phthalimido glucose) 0.8] contaminated with an estimated 5—10% of triacetyl(phthalimido)glucose. It was used without delay.

Lithium 2-(*Benzyloxycarbonylamino*)*ethyl* 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl Phosphate* (**22**).—Cyclohexylammonium thiocyanate (4.68 g, 30 mmol) (*vide infra*) was added to a solution of the crude phosphotriester (**21**) (21.2 g) in butan-2-one (170 ml; freshly distilled from P_2O_5) and the mixture was kept at 90 °C for 19 h. The yellow solution was cooled and the solvents were removed. A solution of the residue in ethyl acetate (50 ml) was applied to a column (75 × 5 cm) of silica gel (PF 254, Merck) under a pressure of 4 bars. The column was eluted under the same pressure first with ethyl acetate (2 l), then with (8:2) ethyl acetate–methanol (1 l), and finally with (6:4) ethyl acetate–methanol (1.5 l), the phosphodiester cyclohexylammonium salt being eluted with the latter solvent. The fractions containing this product were pooled (*ca.* 500 ml) and the solvents were removed. The residue was taken up in methanol (50 ml) and percolated through a column (50 × 4 cm) of Amberlite IR 120 (Li^+) resin in methanol. The column was eluted with methanol (*ca.* 300 ml) and the solvent was removed from the eluate. Benzene (3 × 100 ml) was evaporated from the residue to remove any water present and the oily remanent substance was triturated with anhydrous ether until it solidified. The *title product* (**22**) (11.6 g, 55%), collected by filtration and dried, had m.p. 137 °C (decomp.); $[\alpha]_D^{22}$ +34.5° (*c* 1 in MeOH) (Found: C, 51.8; H, 4.8; N, 4.2. $C_{30}H_{32}LiN_2O_{15}P$ requires C, 51.6; H, 4.6; N, 4.0%).

2-Aminoethyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*phthalimido*-β-D-*glucopyranosyl Hydrogen Phosphate* (**23**).—A solution of the lithium salt (**22**) (2 g, 2.86 mmol) in methanol (20 ml) was percolated through a column of Amberlite IR 120 (H^+) resin (25 ml) in methanol. The column was washed with methanol and the acidic effluent (*ca.* 100 ml) was hydrogenated in the

presence of 10% palladium–charcoal catalyst (100 mg), the progress of the hydrogenation, which lasted *ca.* 3 h, being monitored by t.l.c. [(8:4:2) ethyl acetate–ethanol–water]; R_F (**23**) 0.72, R_F (**22**) 0.40. The catalyst was filtered off, and solvents were removed from the neutral solution. The white solid (1.2 g, 72%) formed upon trituration of the residue with 2-isopropoxypropane was collected by filtration and dried. The *title product* (**23**) had m.p. 190 °C (decomp.); $[\alpha]_D^{22}$ +35° (*c* 1.2 in methanol) (Found: C, 45.8; H, 5.05; N, 4.65. $C_{22}H_{27}N_2O_{13}P{\cdot}H_2O$ requires C, 45.8; H, 5.0; N, 4.9%).

2-*Amino*-2-*deoxy*-β-D-*glucopyranosyl* 2-*Aminoethyl Hydrogen Phosphate* (**24**).—Compound (**23**) (0.5 g, 0.87 mmol) in 2-methoxyethanol (3.5 ml) was heated for 9 min at 100 °C with anhydrous hydrazine (0.25 ml). After removal of the solvents, the residue was taken up in water (12 ml) and the pH of the solution was adjusted to 6 with acetic acid. The resulting precipitate was removed and washed with water (2 × 10 ml). The filtrate was concentrated to 2—3 ml and precipitation was induced by addition of ethanol (20 ml). The *hygroscopic compound* (**24**) (100 mg, 35%) was recovered by centrifugation, washed with acetone, and dried; $[\alpha]_D^{22}$ +3° (*c* 1 in water) (Found: C, 30.3; H, 6.6; N, 8.6. $C_8H_{19}N_2O_8P{\cdot}H_2O$ requires C, 30.0; H, 30.0; H, 6.6; N, 8.75%); δ_H(400 MHz; D_2O) 2.6 (1 H, t, $J_{1,2}$ 8, $J_{2,3}$ 8 Hz, 2-H), 3.1 (2 H, t, *J* 5 Hz, CH_2N), 3.30 (2 H, m, 3- or 4-H), 3.40 (1 H, m, 5-H), 3.60 (1 H, dd, J_{gem} 12, J_{vic} 6 Hz, 6-H), 3.75 (1 H, dd, $J_{H,H}$ 5, $J_{H,P}$ 11 Hz, CH_2OP), and 4.75 (1 H, t, $J_{1,2}$ 8, $J_{1,P}$ 8 Hz, 1-H).

Benzyl 2,2,2-*Trichloroethyl Morpholinophosphate* (**10**).—2,2,2-Trichloroethyl phosphomorpholidochloridate (**8**) (3.1 g, 10 mmol) and DMAP (0.2 g) were added to anhydrous benzyl alcohol (2.2 g, 20 mmol) containing 4 Å molecular sieves (5 g). After 2 h, t.l.c. [(1:2) ethyl acetate–hexane] showed the reaction to be complete; pyridine was removed by evaporation from the stirred mixture and the residue was purified by chromatography, on a column (30 × 5 cm) of silica gel, using the same solvent. The fractions containing the phosphotriester (**10**) were pooled and solvents were removed to give the title compound (**10**) (3 g, 77%) as an oil, δ_H(90 MHz; $CDCl_3$) 3.1 (4 H, m, CH_2NCH_2), 3.65 (4 H, m, CH_2OCH_2), 4.75 (2 H, d, *J* 6 Hz, CH_2CCl_3), 5.1 (2 H, d, *J* 8 Hz, CH_2Ph), and 7.4 (5 H, s, Ph).

Diammonium P^1-*Benzyl* P^2-{2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl*} *Pyrophosphate* (**25**).—Activated zinc dust (100 mg) was added to a solution of the phosphoromorpholidate (**10**) (390 mg, 1 mmol) in pyridine (5 ml) containing acetic acid (0.5 ml). After 0.5 h, when t.l.c. [(1:1) ethyl acetate–hexane] showed removal of the trichloroethyl group to be complete, the excess of zinc was filtered off and morpholine (1 ml) was added to the filtrate which was then concentrated to dryness. A solution of the residue in anhydrous pyridine (5 ml) was added to a mixture of tributylamine (83 mg, 1 mmol) and the 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-α-D-glucopyranosyl phosphate (**2**) (260 mg, 0.5 mmol), previously made anhydrous by evaporation with anhydrous pyridine (2 × 10 ml), in pyridine (10 ml). After 72 h the solvent was removed and the residue was chromatographed on a column (35 × 3 cm) of silica gel, using (6.5:3.5:1) chloroform–methanol–conc. ammonia. Fractions containing the pyrophosphate (**25**) (R_F 0.43) were pooled and the solvents were evaporated off. The *title pyrophosphate* (**25**) (180 mg, 60%) was dissolved in methanol, precipitated by addition of acetone, recovered by centrifugation, and dried, $[\alpha]_D^{22}$ +44° (*c* 1.3 in methanol) (Found: C, 44.7; H, 7.8; N, 6.1. $C_{27}H_{53}N_3O_{13}P_2{\cdot}2H_2O$ requires C, 44.7; H, 7.9; N, 5.8%).

Diammonium P^1-{2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl* P^2-*Hydrogen Pyrophosphate* (**26**).—A solution of the preceding compound (150 mg, 0.21 mmol) in methanol (20 ml) was stirred with 10% palladium–charcoal catalyst (50 mg) under a slight pressure of hydrogen. After 1 h, t.l.c. [(5:5:2) chloroform–methanol–conc. ammonia] showed the conversion of (**25**) the starting pyrophosphate (R_F 0.82) into (**26**) (R_F 0.21) to be complete. The catalyst was removed by filtration, and the filtrate was concentrated below 30 °C; the dry residue, upon trituration with acetone, gave the *pyrophosphate* (**26**) (100 mg, 75%) as a hygroscopic powder, $[\alpha]_D^{22}$ +46° (*c* 1 in water) (Found: C, 37.5; H, 7.8; N, 6.6. $C_{20}H_{47}N_3O_{13}P_2{\cdot}2H_2O$ requires C, 37.8; H, 8.0; N, 6.6%).

Dilithium 2-(*Benzyloxycarbonylamino*)*ethyl Phosphate* (**12**).—A solution of benzyl chloroformate in benzene (50% w/v; 80 mmol) was added to a vigorously stirred solution of *O*-phosphorylethanolamine (2-aminoethyl dihydrogen phosphate) (14.1 g, 100 mmol) in water (500 ml) the pH of which had been adjusted to 12 with saturated aqueous lithium hydroxide. The reaction mixture was stirred for 2 h, a pH of 12 being maintained by further addition of aqueous lithium hydroxide. The benzene layer was discarded and the aqueous phase was washed with toluene (200 ml) and heated to 100 °C on a steam-bath until crystallization occurred. The *title compound* (**12**) (14 g, 47%), recovered as white crystals by filtration, was washed with hot water and dried. T.l.c. [(7:1:2) propan-2-ol–conc. ammonia–water] *R* (picric acid) 0.58 (Found: C, 40.5; H, 4.0; N, 5.0. $C_{10}H_{12}Li_2NO_6P{\cdot}0.5H_2O$ requires C, 40.5; H, 4.4; N, 4.7%). A further crop (6 g, total yield 67%) of the product, slightly contaminated with ethanolamine phosphate, could be recovered from the mother liquors.

Morpholinium 2-(*Benzyloxycarbonylamino*)*ethyl Morpholinophosphate* (**11**).—Amberlite IR 120 (H^+) resin (20 ml) was added to a stirred suspension of the preceding compound (2.96 g, 10 mmol) in water (20 ml). When all of the phosphate had dissolved (*ca.* 15 min), the resin was filtered off and washed with water until the washings were neutral (pH paper). Morpholine (3.9 g, 45.3 mmol) was added to the filtrate (100 ml) previously diluted with tertiary butyl alcohol (100 ml). A solution of DCC (8.24 g, 40 mmol) in t-butyl alcohol (150 ml) was added dropwise to the reaction mixture which was then refluxed for 1 h and allowed to come to room temperature. Dicyclohexylurea was filtered off and the filtrate was concentrated until removal of t-butyl alcohol was complete. The remaining aqueous solution was diluted with water (200 ml), washed with ether (3 × 200 ml), and concentrated to dryness. A solution of the residue in (1:1) methanol–water (50 ml) was passed through a column of Amberlite IR 120 (morpholinium form) resin (50 ml) and the eluate was concentrated to dryness. The *title compound* (**11**) (3 g, 70%) was crystallized by dissolution of the residue in the minimum amount of ethanol, addition of ether to persistent turbidity, and storage at 4 °C overnight. It had m.p. 104—105 °C; t.l.c. [(7:1:2) propan-2-ol–conc. ammonia–water] *R* (picric acid) 0.9 (Found: C, 50.2; H, 6.9; N, 9.6. $C_{18}H_{29}N_3O_7P$ requires C, 50.2; H, 6.7; N, 9.8%).

P^1-[2-(*Benzyloxycarbonylamino*)*ethyl*]P^2-{2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl*} *Pyrophosphate* (**27**).—Tributylamine (165 mg, 1 mmol) was added to a suspension of compound (**2**) (520 mg, 1 mmol) in pyridine (10 ml) and the solvent was removed. Anhydrous pyridine (2 × 10 ml) was added to and removed from the residue, then a solution of compound (**11**) (860 mg, 2 mmol) in pyridine (10 ml) was added. After 72 h, solvents were removed and the residue was purified on a column of silica gel (35 × 3 cm) in (6.5:3.5:1) chloroform–methanol–conc. ammonia, elution being carried

out in the same solvent. Fractions containing the desired product (R_F 0.37) were pooled and concentrated to dryness. The residue was dissolved in the minimum amount of methanol and acetone was added to precipitate the *title compound* (**27**) (350 mg, 44%), which was recovered as a hygroscopic white powder by centrifugation, and dried; $[\alpha]_D^{22}$ +40° (*c* 1.3 in methanol) (Found: C, 45.1; H, 7.4; N, 6.7. $C_{30}H_{58}N_4O_{15}P_2 \cdot H_2O$ requires C, 45.3; H, 7.6; N, 7.05%).

P^1-*Ammonium* P^2-(2-*Aminoethyl*) P^1-{2-*Deoxy*-2-[(3R)-3-*hydroxytetradecanamido*]-α-D-*glucopyranosyl*} P^2-*Hydrogen Pyrophosphate* (**28**).—A solution of the preceding compound (300 mg, 0.38 mmol) in methanol (20 ml) was stirred with 10% palladium–charcoal catalyst (50 mg) under a slight pressure of hydrogen. After 1 h, t.l.c. [(7.5:1.5:1) propan-2-ol–water–conc. ammonia] showed the removal of the benzyloxycarbonyl group to be complete. The catalyst was removed by filtration, and the filtrate was concentrated (30 °C); the dry residue, upon trituration with acetone, gave the *title pyrophosphate* (**28**) (200 mg, 79%) as a hygroscopic powder, $[\alpha]_D^{22}$ +49° (*c* 1 in water) (Found: C, 39.4; H, 8.0; N, 6.0. $C_{22}H_{49}N_3O_{13}P_2 \cdot 2.5H_2O$ requires C, 39.4; H, 8.1; N, 6.3%).

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