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A CONVENIENT SYNTHESIS OF 2-DEOXY-2-[(*R*)-3-HYDROXYTETRADECANAMIDO]-3-*O*-[(*R*)-3-HYDROXYTETRADECANOYL]-α-D-GLUCOPYRANOSE 1-PHOSPHATE (LIPID X)

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ABSTRACT

The crystalline tris(hydroxymethyl)aminomethane ("Tris") salt of 2-deoxy-2-[(*R*)-3-hydroxytetradecanamido]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranose 1-phosphate (lipid X) has been synthesised from 2-animo-2-deoxy-D-glucose hydrochloride in five steps in ~50% overall yield. The key step was 1-*O*-(dibenzyl) phosphorylation of 4,6-*O*-benzylidene-2-[(*R*)-3-benzyloxytetradecanamido]-2-deoxy-D-glucopyranose, catalysed by butyl-lithium. The product was then 3-(*R*)-3-benzyloxytetradecanoylated, and the benzyl and benzylidene groups were removed by catalytic hydrogenation.

INTRODUCTION

Lipid X (1), discovered by Nishijima and Raetz[1], is an unusual, 2-amino-2-deoxyglucose-derived phospholipid that accumulates in *Escherichia coli* mutants defective in the pgs B gene. Takayama *et al.*[2] elucidated the structure of lipid X as **1**. Lipid X has been identified[3] as an intermediate in the biosynthesis of the lipopolysaccharide (LPS) from Gram-negative bacteria and has attracted attention as an agent that enhances non-specific resistance to experimental infections[4] and protects mice[5] and sheep[6] against a lethal challenge with LPS.

Lipid X is available commercially from the fermentation of a mutant *E. coli* strain, and two chemical syntheses have been reported. Kusumoto *et al.*[7] described a thirteen-step sequence and an overall yield of ~1.5%. Takahashi *et al.*[8] have obtained **1** by a slightly different, eleven-step route in an overall yield of 4.5%. A straightforward synthesis of the crystalline Tris salt of lipid X from 2-amino-2-deoxy-D-glucose in an overall yield of ~50% is now reported.

RESULTS AND DISCUSSION

The reaction of 2-amino-2-deoxy-D-glucose with *N*-(*R*)-3-benzyloxytetradecanoyloxysuccinimide [**6**, prepared by the reaction of *N*-hydroxysuccinimide and dicyclohexylcarbodi-imide with the known[9] (*R*)-3-benzyloxytetradecanoic acid] in

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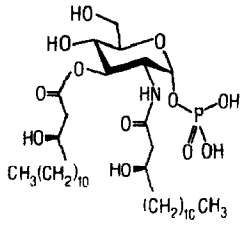
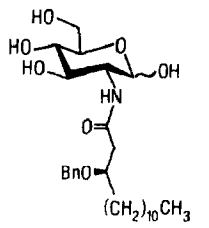
N,N-dimethylformamide afforded an almost quantitative yield of the amide derivative **2**. Compound **2** was separated from water-soluble materials by Bligh–Dyer extraction[10] and used without additional purification. 4,6-*O*-Benzylidenation of **2** to give **3** ($\alpha\beta$-ratio 4:1) was achieved by transacetalation[11] with benzaldehyde dimethyl acetal in *N,N*-dimethylformamide, catalysed by toluene-*p*-sulfonic acid. Compound **3** was converted[12] into the 1-(dibenzyl phosphate) derivative **4** which, contrary to expectations[13], was stable during chromatography over silica gel. Recovered **3** was subjected to another cycle of *O*-(dibenzyl)phosphorylation. Treatment of **4** in dichloromethane with (*R*)-3-benzyloxytetradecanoic acid[9], using dicyclohexylcarbodi-imide in the presence of 4-dimethylaminopyridine[14], gave 86% of **5**. The benzyl and benzylidene groups of **5** were removed by catalytic hydrogenation to afford the free acid form of **1** (50% overall yield). With 1.5 equiv. of tris(hydroxymethyl)aminomethane and chromatography on Sephadex LH-20, this gave the amorphous mono-Tris salt which, on addition of a further equiv. of Tris, afforded the crystalline di-Tris salt.



1 (Lipid X)



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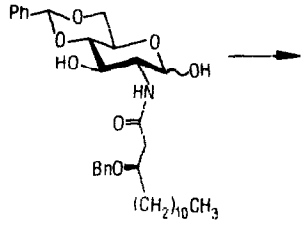

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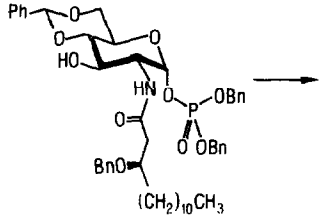

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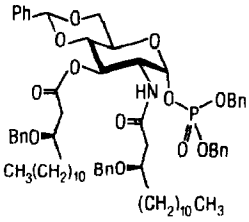

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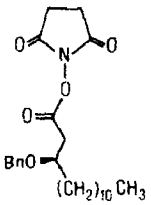

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EXPERIMENTAL

General. — Melting points were determined with a Kofler hot-stage and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarime-

ter at 20°. N.m.r. spectra (internal Me_4Si) were recorded with a Bruker WH-250 spectrometer (250 MHz for ^1H, and 62.89 MHz for ^{13}C). T.l.c. was performed on Silica Gel 60 F_{254} (Merck) with detection by u.v. light or with vanillin–sulfuric acid. Column chromatography was performed on Merck-Lichroprep columns (silica gel, 40–63 μm), under pressure (~0.2 MPa). Elemental analyses were performed by Dr. J. Zak (Mikroanalytisches Laboratorium am Institut für Physikalische Chemie, Universität Wien).

*2-[(R)-3-Benzyloxytetradecanamido]-2-deoxy-*D*-glucopyranose* (**2**). — A mixture of 2-amino-2-deoxy-D-glucose hydrochloride (5.4 g), **6** (10.8 g), and ethyldiisopropylamine (5 mL) in dry *N,N*-dimethylformamide (25 mL) was stirred for 24 h at room temperature, and then concentrated, and the residue was dissolved in a mixture of chloroform (150 mL), methanol (300 mL), and water (120 mL). Following addition of a further 150 mL each of chloroform and water, the lower phase was separated, washed twice with the upper phase of the system chloroform (100 mL), methanol (100 mL), and water (90 mL), and then concentrated, and the residue was dried under high vacuum. The product (12.4 g, ~100%) was sufficiently pure for use in the next step. Column chromatography (9:1 toluene–ethanol) of a sample gave material with m.p. 150–158°, $[\alpha]_D$ +53° (*c* 1, *N,N*-dimethylformamide); R_F 0.3 (9:1 chloroform–methanol). ^{13}C-N.m.r. data [$(CD_3)_2SO$, 50°]: αβ-**2** (αβ-ratio 4:1): δ 13.78 (CH_3), 21.98 (ω−1 CH_2), 24.71 (δ CH_2), 28.59, 28.95, 29.00 (CH_2), 31.21 (ω−2 CH_2), 34.03 (γ CH_2), 41.01, 41.50 (α CH_2), 54.31 (C-2α), 57.56 (C-2β), 61.35 (C-6), 70.29 (Ph*C*H_2O), 70.78 (C-4α), 71.14 (C-4β), 71.41 (C-3α), 71.99 (C-5α), 74.49 (C-3β), 76.11 (β CHO), 76.70 (C-5β), 90.80 (C-1α), 95.76 (C-1β), 127.03, 127.45, 127.95, 139.13 (Ph), 170.49 (CON).

Anal. Calc. for $C_{27}H_{45}NO_7$: C, 65.42; H, 9.15; N, 2.83. Found: C, 65.48; H, 9.04; N, 2.78.

*4,6-*O*-Benzylidene-2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-*D*-glucopyranose* (**3**). — A solution of **2** (5.83 g), benzaldehyde dimethyl acetal (3 g), and toluene-*p*-sulfonic acid monohydrate (500 mg) in dry *N,N*-dimethylformamide (200 mL) was rotated for 3 h at 55–60° and 3–4 kPa. T.l.c. (1:1 toluene–ethyl acetate) then showed that all **2** had reacted. The major part of the *N,N*-dimethylformamide was evaporated, dichloromethane (500 mL) was added, and the solution was extracted twice each with dilute aqueous sodium hydrogencarbonate (200 mL) and water (200 mL), then dried (Na_2SO_4), and concentrated. Column chromatography (6:4 toluene–ethyl acetate) of the residue gave **3** (5.1 g, 74.3%), m.p. 162–165°, $[\alpha]_D$ −5.5° (*c* 1, chloroform); R_F 0.2 (1:1 toluene–ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): αβ-**3** (αβ-ratio 4:1), δ 14.02 (CH_3), 22.59 (ω−1 CH_2), 25.07 (δ CH_2), 29.25, 29.52, 29.56 (CH_2), 31.82 (ω−2 CH_2), 33.77 (γ $CH_2\alpha$), 33.86 (γ $CH_2\beta$), 41.24 (α $CH_2\beta$), 41.42 (α $CH_2\alpha$), 54.55 (C-2α), 58.80 (C-2β), 62.28 (C-6α), 66.23 (C-6β), 68.48 (C-3β), 68.82 (C-3α), 69.96 (C-5α), 71.01 (C-5β), 71.50 (Ph*C*H_2O), 76.63 (β CHOβ), 76.82 (β CHOα), 81.23 (C-4β), 82.12 (C-4α), 92.09 (C-1α), 97.58 (C-1β), 101.72 (Ph*C*HOβ), 101.79 (Ph*C*HOα), 126.23–138.18 (Ph), 173.12 (CONα), 175.12 (CONβ).

Anal. Calc. for $C_{34}H_{49}NO_7$: C, 69.95; H, 8.46; N, 2.40; Found: C, 70.00; H,

8.40; N, 2.42.

4,6-O-Benzylidene-2-[(R)-3-benzyloxytetradecanamido]-2-deoxy-α-D-glucopyranose 1-(dibenzyl phosphate) (**4**). — To a solution of dibenzyl phosphite (3.55 g, 12.8 mmol) in benzene (10 mL) was added *N*-chlorosuccinimide (1.72 g, 12.8 mmol) in portions. After 1 h, the succinimide formed was filtered off and the solution of dibenzyl chlorophosphonate[15] used directly as required. A solution of butyl-lithium in hexane (8 mL, 1.6M) was added dropwise at −70° to a solution of **3** (4.85 g) in dry tetrahydrofuran (40 mL). After 5 min, a solution of dibenzyl chlorophosphonate (3.8 g) in dry benzene (10 mL) was added dropwise at the same temperature, the mixture was stirred at −70° for 10 min, then acetic acid (0.3 mL) was added, and the solution was concentrated to quarter volume, diluted with dichloromethane (200 mL), washed with water (50 mL), dilute aqueous sodium hydrogencarbonate (50 mL), and aqueous sodium chloride (50 mL), dried (Na_2SO_4), and concentrated. Column chromatography (7:3 toluene–ethyl acetate) of the residue gave **4** (4.1 g, 58.6%), m.p. 102–105°, $[\alpha]_D$ +32° (*c* 1, chloroform); R_F 0.32 (1:1 toluene–ethyl acetate). Analogous treatment of the mixed column fractions containing both **3** and **4** afforded more **4** (1.8 g, total yield of 82.7%). N.m.r. data ($CDCl_3$): ^1H, δ 2.35 (d, 2 H, *J* 5 Hz, α CH_2), 3.55 (t, 1 H, $J_{6,6'}$ 10 Hz, H-6), 3.62 (t, 1 H, $J_{3,4}$ 9.5 Hz, H-3), 3.73 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4), 3.5–3.8 (m, 2 H, β CHO and HO-3), 3.87 (dt, 1 H, $J_{5,6}$ 10 Hz, H-5), 4.04 (dd, 1 H, $J_{5,6'}$ 5 Hz, H-6'), 4.27 (m, 1 H, H-2), 4.42, 4.48 (ABq, 2 H, J_{AB} 11.5, PhCH_2O), 4.96 (d, 2 H, $J_{H,P}$ 8 Hz, PhCH_2OP), 4.98 (d, 2 H, $J_{H,P}$ 8 Hz, PhCH_2OP), 5.46 (s, 1 H, PhC*H*O), 5.75 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 6.5 Hz, H-1), 6.78 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 7.2–7.5 (20 H, 4 Ph); ^{13}C, δ 13.95 (CH_3), 22.50 ($\omega-1$ CH_2), 25.04 (δ CH_2), 29.16, 29.42, 29.45, 29.48 (CH_2), 31.73 ($\omega-2$ CH_2), 33.58 (γ CH_2), 40.68 (α CH_2), 53.78 (d, $J_{C-2,P}$ 6.9 Hz, C-2), 64.31 (C-6), 68.11 (C-3), 68.77 (C-5), 69.56 (d, $J_{C,P}$ 5.4 Hz, PhCH_2OP), 70.81 (PhCH_2O), 75.89 (β CHO), 81.08 (C-4), 96.79 (d, $J_{C-1,P}$ 6.5 Hz, C-1), 101.63 (PhCHO), 126.18–138.18 (4 Ph), and 172.24 (CON).

Anal. Calc. for $C_{48}H_{62}NO_{10}P$: C, 68.31; H, 7.41; N, 1.66; P, 3.67. Found: C, 68.30; H, 7.46; N, 1.76; P, 3.72.

4,6-O-Benzylidene-2-[(R)-3-benzyloxytetradecanamido]-3-O-[(R)-3-benzyloxytetradecanoyl]-2-deoxy-α-D-glucopyranose 1-(dibenzyl phosphate) (**5**). — To a cooled (−10°) solution of **4** (3.9 g), (*R*)-3-benzyloxytetradecanoic acid (2 g), and 4-dimethylaminopyridine (50 mg) in dry dichloromethane (20 mL) was added dicyclohexylcarbodi-imide (1.25 g). The mixture was kept at 4° overnight, then filtered, and concentrated, and the residue was taken up in a small volume of 8:2 toluene–ethyl acetate, filtered, and chromatographed, using the same solvent system, to give **5** (4.62 g, 86%), m.p. 96–98°, $[\alpha]_D$ +33° (*c* 1, chloroform); R_F 0.5 (2:1 toluene–ethyl acetate). N.m.r. data ($CDCl_3$): ^1H, δ 2.16 (d, 2 H, $J_{\alpha,\beta}$ 5 Hz, α CH_2CON), 2.36 (dd, 1 H, $J_{\alpha,\alpha'}$ 15.5 Hz, $J_{\alpha,\beta}$ 5.5 Hz, α C*H*H'COO), 2.60 (dd, 1 H, $J_{\alpha',\beta}$ 6 Hz, α CH*H'*COO), 3.55–3.80 (m, 4 H, H-4,6 and 2 β CHO), 3.90 (dt, 1 H, $J_{4,5} = J_{5,6}$ = 10 Hz, H-5), 4.02 (dd, 1 H, $J_{5,6'}$ 5, $J_{6,6'}$ 10 Hz, H-6'), 4.32, 4.47 (ABq, 2 H, J_{AB} 12 Hz, PhCH_2O), 4.36, 4.43 (ABq, 2 H, J_{AB} 11.5 Hz, PhCH_2O), 4.4 (m, 1 H, H-2),

4.94 (d, 2 H, $J_{H,P}$ 8 Hz, PhCH_2OP), 4.98 (d, 2 H, $J_{H,P}$ 8 Hz, PhCH_2OP), 5.29 (t, 1 H, $J_{2,3} = J_{3,4} =$ 9.5 Hz, H-3), 5.38 (s, 1 H, PhC*H*O), 5.75 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 6 Hz, H-1), 6.26 (d, 1 H, $J_{2,NH}$ 8.5 Hz, NH), 7.15–7.40 (m, 25 H, 5 Ph); ^{13}C, δ 14.04 (CH_3), 22.60 ($\omega-1$ CH_2), 25.06, 25.22 (2 δ CH_2), 29.28, 29.57 (CH_2), 31.86 ($\omega-2$ CH_2), 33.83, 34.47 (2 γ CH_2), 39.60, 40.90 (2 α CH_2), 52.14 (d, $J_{C-2,P}$ 8.4 Hz, C-2), 64.49 (C-6), 68.26 (C-3), 68.91 (C-5), 69.70 (d, $J_{C,P}$ 4.9 Hz, PhCH_2OP), 70.88, 71.04 (2 PhCH_2O), 75.33, 75.53 (2 β CHO), 78.58 (C-4), 96.60 (d, $J_{C-1,P}$ 5.7 Hz, C-1), 101.51 (PhCHO), 126.05–138.45 (5 Ph), 171.24 and 171.48 (2 CO).

Anal. Calc. for $C_{69}H_{94}NO_{12}P$: C, 71.41; H, 8.16; N, 1.21; P, 2.67. Found: C, 71.56; H, 8.28; N, 1.18; P, 2.65.

2-Deoxy-2-[(R)*-3-hydroxytetradecanamido*]*-3-*O-[(R)*-3-hydroxytetradecanoyl*]*-α-*D*-*glucopyranose 1-phosphate* (**1**). — A solution of **5** (4.59 g) in 85:15 tetrahydrofuran–pyrogen-free water (900 mL) was hydrogenated for 2 h at 40° and 1 MPa over 10% Pd/C (1.5 g), and then filtered. The tetrahydrofuran was evaporated, and the aqueous suspension was lyophilised to give **1** (96%); R_F 0.56 (125:75:10:20 chloroform–methanol–acetic acid–water). For final purification, lipid X (10 mg/mL) was suspended in a solution of 1.5 mol of tris(hydroxymethyl)aminomethane in pyrogen-free water. The suspension was sonicated (Bandelin Sonorex sonicator bath, 35 kHz, 350 W, room temperature) until clear, and then lyophilised. A solution of the residue in methanol (20 mg/mL) was chromatographed over Sephadex LH-20, using methanol. The fractions containing lipid X were combined and concentrated, to give a practically quantitative yield of **1** as the amorphous mono-Tris salt. N.m.r. data (CD_3OD): ^{1}H, δ 3.63 (dd, 1 H, $J_{4,5}$ 10 Hz, H-4), 3.71 (s, 6 H, 3 CH_2OH), 3.75 (dd, 1 H, $J_{6,6'}$ 12 Hz, H-6), 3.90 (dd, 1 H, $J_{5,6'}$ 2 Hz, H-6'), 4.00 (ddd, 1 H, $J_{5,6}$ 5.5 Hz, H-5), 4.0 (m, 2 H, 2 β CHOH), 4.22 (ddd, 1 H, $J_{2,3}$ 10.5, $J_{2,P}$ 2.5 Hz, H-2), 5.27 (dd, 1 H, $J_{3,4}$ 9.5 Hz, H-3), and 5.25 (dd, 1 H, $J_{1,2}$ 3.5, $J_{1,P}$ 7.5 Hz, H-1); ^{13}C, 14.40 (CH_3), 23.60 ($\omega-1$ CH_2), 26.59 (δ CH_2), 30.35, 30.66, 30.69 (CH_2), 32.94 ($\omega-2$ CH_2), 38.04, 38.40 (2 γ CH_2), 43.41, 44.67 (2 α CH_2), 53.32 (d, $J_{C-2,P}$ 7.9 Hz, C-2), 61.01 (CH_2OH), 62.36 (C-6), 62.60 (C-N), 69.11 (C-4), 69.54 (β CHOH), 74.27, 75.10 (C-3, C-5), 95.10 (d, $J_{C-1,P}$ 5.8 Hz, C-1), 173.34 and 174.54 (COO and CON).

Addition of 1 equiv. of tris(hydroxymethyl)aminomethane to a solution of lipid X mono-Tris salt in methanol at 50° (70 mg/mL) gave the di-Tris salt of **1**, m.p. 183–185° (dec.), $[\alpha]_D$ +15° (*c* 1, water).

Anal. Calc. for $C_{42}H_{88}N_3O_{18}P$: C, 52.92; H, 9.31; N, 4.41; P, 3.25. Found: C, 52.61; H, 9.11; N, 4.45; P, 3.34.

N-(R)*-3-Benzyloxytetradecanoyloxysuccinimide* (**6**). — To a solution of (*R*)-3-benzyloxymyristic acid[9] and *N*-hydroxysuccinimide (equimolar amounts) in ethyl acetate at 0° was added 1 equiv. of dicyclohexylcarbodi-imide. The mixture was allowed to attain room temperature overnight, then filtered, and concentrated, and the residue was crystallised from methanol to give **6** (75%), m.p. 41.5–42.5°. ^{1}H-N.m.r. data: δ 0.88 (t, 3 H, CH_3), 1.20–1.50 (m, 18 H, 9 CH_2), 1.65 (m, 2 H, γ CH_2), 2.76 (dd, 1 H, $J_{\alpha,\alpha'}$ 15.5, $J_{\alpha,\beta}$ 5.5 Hz, α C*H*H'), 2.83 (s, 4 H, succinimide),

2.90 (dd, 1 H, $J_{\alpha',\beta}$ 7.5 Hz, α CHH'), 3.95 (m, 1 H, β CHO), 4.53, 4.66 (ABq, 2 H, J_{AB} 11.5 Hz, PhCH_2O), and 7.2–7.4 (m, 5 H, Ph).

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