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# Synthesis of 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(3*R*)-3-hydroxytetradecanyl]-α-D-glucopyranosyl dihydrogen phosphate and 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(3*R*)-3-hydroxytetradecanyl]-4-*O*-phosphono-D-glucopyranose

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ABSTRACT

Both a 3-*O*-alkyl lipid X analogue and its 4-*O*-phosphono isomer were synthesized from allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside.

## INTRODUCTION

Lipopolysaccharide (LPS), an outer membrane component of Gram-negative bacterial cells, causes fever and lethal shock in higher animals. This toxic principle is called "endotoxin". Westphal and Luderitz[1] isolated lipid A, which is the lipophilic part of LPS. Lipid A shows most of the endotoxic activities of LPS, and was first chemically synthesized by Shiba and coworkers[2]. Nishijima and Raetz[3] isolated lipid X from a mutant of *E. coli*. Lipid X, which is really the reducing-sugar part of lipid A and is a biosynthetic precursor of lipid A, demonstrates albeit weakly†, most of the activities of lipid A. Hasegawa and coworkers[4], who have synthesized various non-reducing-sugar parts of lipid X, to say nothing of lipid A analogues (reducing-sugar part), discovered that a lipid A subunit homologue, a 4-*O*-phosphono-D-glucosamine derivative (named GLA-60), elicited some distinct and beneficial biological activities of LPS. In this study, we focused on compounds which would be macrophage active and synthesized a 3-*O*-alkyl analogue of lipid X and its 4-*O*-phosphono isomer, because we reasoned that an ester bond would be easily cleaved by macrophage enzymes, while the ether bond would resist cleavage. Such an ether analogue should also increase lipophilicity, result-

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† Very recently it has been reported that lipid X is devoid of lipid A's activity. See H. Anschauer, A. Grob, J. Hildebrandt, E. Schuetze, and P. Stuetz, *J. Biol. Chem.*, 265 (1990) 9159–9164.

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ing in the compound's being stabilized and possibly showing an increase in biological activity. Herein is described the synthesis of the title compounds **8** and **14**.

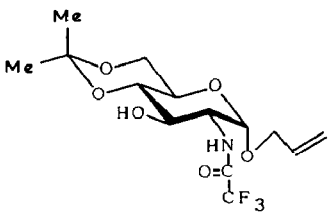
## RESULTS AND DISCUSSION

*Synthesis.* — Allyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside was successfully used as the starting material of a lipid X synthesis by Achiwa and coworkers[5]. However, allyl 2-[(3*R*)-3-(benzyloxy)tetradecanamido]-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside obtained from this amine was found not to be suitable for the synthesis of the 3-*O*-alkyl analogue of lipid X, because the strong base used in the etherification resulted in the elimination of benzyl alcohol from the 2-[3-(benzyloxy)tetradecanamido] group to give a 3-*O*-alkyl-2-α,β-unsaturated amido sugar. We therefore used the trifluoroacetyl group as a protective group for the 2-amino function. It proved to be a suitable protective group, as the deprotection was carried out under mild conditions with no other degradation.

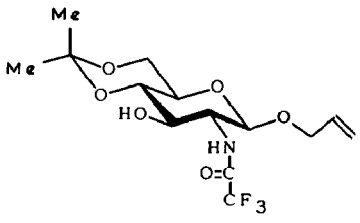
D-Glucosamine hydrochloride was converted to allyl 2-deoxy-4,6-*O*-isopropylidene-2-trifluoroacetamido-α- and -β-D-glucopyranoside (**1α** and **1β**, 29% and 22%, respectively) by the sequence as follows: (*i*) amide formation of D-glucosamine with ethyltrifluoroacetate–$Et_3N$; (*ii*) glycosidation of the 2-deoxy-2-trifluoroacetamido-D-glucopyranose with allyl alcohol containing 2% HCl, and (*iii*) 4,6-*O*-isopropylidenation of the allyl trifluoroacetamido sugar with 2,2-dimethoxypropane using pyridinium *p*-toluenesulfonate (PPTS) as a catalyst. Both anomers were thus made equally available as the starting material for further conversion to the title compounds. In this paper we describe a synthesis of lipid X analogues using the **1α** anomer (see Scheme 1).

Treatment of **1α** with (3*R*)-3-benzyloxy-1-methanesulfonyloxytetradecane in *N,N*-dimethylformamide (DMF) using NaH as a base gave **2**. Removal of the trifluoroacetyl group of **2** was successfully accomplished by M NaOH in ethanol or by $NaBH_4$ in ethanol to give **3**. The amine **3** was then easily acylated with (3*R*)-3-(benzyloxy)-tetradecanoic acid and *N,N*-dicyclohexylcarbodiimide (DCC) to produce **4a**, which was further treated with bis(methyldiphenylphosphine)cyclo-octadieneiridium(I) hexafluorophosphate[6], $[C_8H_{12}Ir(PMePh_2)_2]PF_6$, and then with $I_2$–$H_2O$–pyridine to remove the allyl group to give **5a**. Phosphorylation of the lithium salt of **5a** with dibenzyl chlorophosphate[7] was effected as described by Shiba and coworkers[8]. Debenzylation of **6a** was achieved by catalytic hydrogenolysis in THF using 10% Pd-on-carbon to give **7**. Deisopropylidenation of **7** was carried out in 80% acetic acid for 1 h at 25° to yield **8**. Alternatively, **8** was obtained from **4a** through compounds **9**, **4b**, **5b**, and **6b** as follows: Removal of the isopropylidene group from **4a** was carried out in 80% AcOH at 60°. Treatment of **9** in DMF, containing a catalytic amount of *p*-toluenesulfonic acid monohydrate, with benzaldehyde dimethylacetal gave the benzylidene compound **4b**. The same treatment of **4b** according to the prodcedure used for converting **4a** to **6a** gave **6b** through **5b**. Catalytic hydrogenolysis of **6b** using 10% Pd/C then gave **8** (Scheme 1).

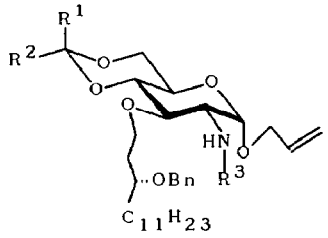
The 4-*O*-phosphono compound **14** was obtained from **9** as shown in Scheme 2. The selective benzyloxymethylation of the 6-OH group of **9** was achieved using benzyl



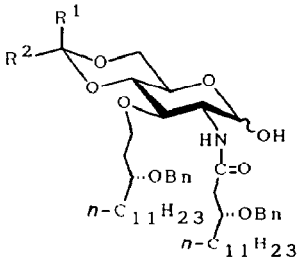
**1α**



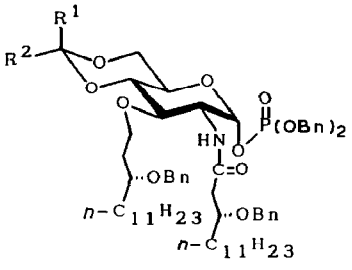
**1β**



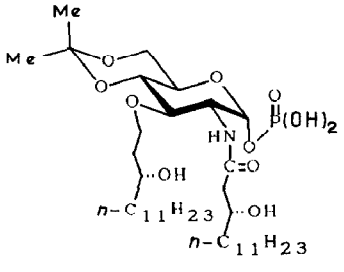
**2** : $R^1=R^2=Me$, $R^3=COCF_3$
**3** : $R^1=R^2=Me$, $R^3=H$
**4a** : $R^1=R^2=Me$, $R^3=$ (3R) , $COCH_2CH(OBn)C_{11}H_{23}$
**4b** : $R^1=H$, $R^2=Ph$, $R^3=$ (3R) , $COCH_2CH(OBn)C_{11}H_{23}$



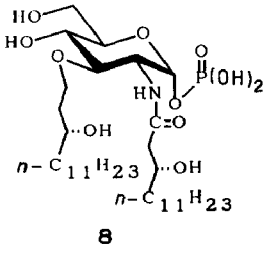
**5a** : $R^1=R^2=Me$
**5b** : $R^1=H$, $R^2=Ph$



**6a** : $R^1=R^2=Me$
**6b** : $R^1=H$, $R^2=Ph$

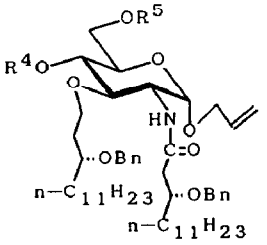


**7**

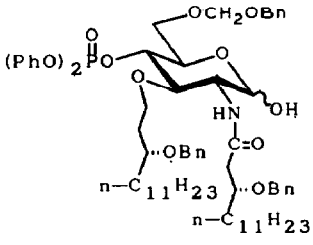


**8**

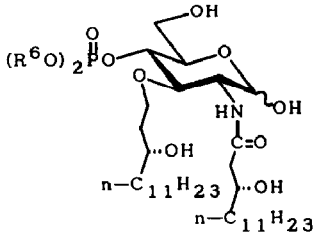
Scheme 1



**9**: $R^4=R^5=H$
**10**: $R^4=H$, $R^5=CH_2OBn$
**11**: $R^4=P(O)(OPh)_2$, $R^5=CH_2OBn$



**12**



**13**: $R^6=Ph$
**14**: $R^6=H$

Bn=benzyl
Ph=phenyl

Scheme 2

chloromethyl ether, with *N,N*-tetramethylurea ($Me_2NCONMe_2$) as a weak base to give **10**, which was further converted to the 4-*O*-(diphenylphosphono) compound **11** by treatment with diphenyl chlorophosphate and 4-dimethylaminopyridine (DMAP) in dichloromethane. Removal of the 1-allyl group of **11**, as described in the formation of **5a** from **4a**, gave **12**. Hydrogenolysis of **12** gave **13**, which was purified on a silica gel column. Hydrogenolysis of **13** using platinum as a catalyst then yielded **14**.

*Biological activity.* — The biological activities of lipopolysaccharide (LPS), lipid A, and many related compounds, which induce morphological changes (spreading), prostaglandin synthesis, and killing of tumor cells by mouse peritoneal macrophages *in vitro, etc.*, have been investigated[9]. Both lipid X and the 4-*O*-phosphono analogue of lipid X also induce, although weakly, these properties of LPS and lipid A, and have similar effects on the macrophage-like mouse cell line J744.1. However, in changing the C-3 ester bond to an ether bond on lipid X or its 4-*O*-phosphono analogue, namely, compounds **8** and **14**, resulted in the complete loss of these activities.

## EXPERIMENTAL

*General methods.* — $^1$H-N.m.r. spectra were recorded at 270 MHz on a JEOL JNN-270 insturment using tetramethylsilane as an internal standard. Preparative t.l.c. was performed on silica gel plates (E. Merck, Silica Gel 60 $F_{254}$), and column chromatography was carried out on columns packed with E. Merck Silica Gel 60 (230–400 mesh ASTM) using slightly increased pressure (1.2 atm.) for elution. Elemental analyses were performed by the Analytical Center of Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd.

*Preparation of (R)-3-benzyloxytetradecanoic acid.* — This compound was prepared essentially by the procedure of refs. 10 and 11. See also refs. 12 and 13.

*(R)-3-Benzyloxy-1-methanesulfonyloxytetradecane.* — (*i*) To a solution of (*R*)-3-hydroxytetradecananoic acid (25 g, 0.102 mol) in THF (750 mL) was added $LiAlH_4$ (11.6 g, 0.305 mol) at 5–10° with stirring. After stirring for 2.5 h at room temperature, the reaction mixture was quenched with excess dil. HCl. The mixture was concentrated *in vacuo* and extracted with EtOAc. The organic layer was washed with aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give a crude solid which was chromatographed on a silica gel column. Elution with 7:3 cyclohexane–EtOAc gave 18.1 g (76%) of (*R*)-1,3-dihydroxytetradecane ($R_F$ = 0.15) as a solid; (*ii*) the primary alcohol of the diol (18 g, 78.1 mmol) was tritylated with trityl chloride (26 g, 93.3 mmol, 1.2 equiv.) in pyridine (250 mL) for 2 h at 60°. The reaction mixture was concentrated and extracted with EtOAc. The organic layer was washed with aq. $NH_4Cl$, dried over $MgSO_4$, concentrated, and chromatographed on a silica gel column. Elution with 19:1 cyclohexane–EtOAc gave 36.9 g of (*R*)-3-hydroxy-1-trityloxytetradecane) ($R_F$ = 0.27); (*iii*) the 3-hydroxy part of the tritylated product (36.9 g, 78.1 mmol) was benzylated with benzyl bromide (40 g, 234 mmol) and NaH (10.2 g, 55% oil dispersion, 234 mmol) in THF (650 mL) for 16 h at reflux temperature. The reaction mixture was quenched with AcOH (15 mL), concentrated *in vacuo*, diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, and dried over $MgSO_4$. The solution was concentrated to give a crude (*R*)-3-benzyloxy-1-trityloxytetradecane, which was employed for the next reaction without purification; (*iv*) the trityl group of the product obtained above was removed by treatment with *p*-TsOH·$H_2O$ (50 g, 263 mmol) in MeOH (800 mL) for 45 min at room temperature. To the reaction mixture was added $Et_3N$ (27 g, 267 mmol). This mixture was concentrated, diluted with EtOAc, washed with water, aq. $NaHCO_3$ and brine, dried over $MgSO_4$. The solution was concentrated and chromatographed on a silica gel column. Elution with 17:3 cyclohexane–EtOAc gave 14.7 g (three steps, 62%) of (*R*)-3-benzyloxy-1-hydroxytetradecane ($R_F$ = 0.26); and then (*v*) the primary alcohol (14.6 g, 45.6 mmol) obtained above was mesylated using methanesulfonyl chloride (7.3 g, 63.7 mmol) and $Et_3N$ (7.3 g, 72.1 mmol) as a base in $CH_2Cl_2$ (280 mL) for 1 h at 15–20°. The reaction mixture was concentrated, diluted with EtOAc, washed with water, dil. HCl, satd. $NaHCO_3$ and brine, concentrated, and chromatographed on a silica gel column. Elution with 17:3 cyclohexane–EtOAc gave 17.4 g (96%) of (*R*)-3-benzyloxy-1-methanesulfonyloxytetradecane ($R_F$ = 0.26) in 45% overall yield. $[\alpha]_D^{24}$ −23.1° (*c* 2.0, EtOH); $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.8–1.0 (m, 3 H), 1.05–1.66 (m, 20 H), 1.99 (t, 2 H, *J* 6.0 Hz), 2.92 (s, 3 H), 3.60 (m, 1 H), 4.35 (t, 2 H), 4.40, 4.64 (AB-q, 2 H, *J* 11.0 Hz), 7.35 (s, 5 H); i.r. $\nu_{max}^{film}$ 2930, 2865, 1735 (w) $cm^{-1}$; m.s. *m/z* 398 ($M^+$), 302.

*Anal.* Calc. for $C_{22}H_{38}O_4S$ (398.6): C, 66.29; H, 9.61; S, 8.04. Found: C, 65.84; H, 9.24; S, 8.09.

*Allyl 2-deoxy-4,6-O-isopropylidene-2-trifluoroacetamido-α- and -β-D-glucopyranosides* (**1α** *and* **1β**). — A suspension of D-glucosamine hydrochloride (21.6 g, 100 mmol), $Et_3N$ (25 g) and $CF_3COOEt$ (15.6 g, 110 mmol) in MeOH (300 mL) was stirred overnight at room temperature. The mixture was concentrated *in vacuo* and dried under pump vacuum to obtain a crude mixture. This mixture was heated under reflux for 30 min in allyl alcohol (250 mL) containing 2% HCl. The mixture was filtered through Celite, and

the filtrate was concentrated *in vacuo* and dried at oil pump vacuum to give a crude anomeric mixture. This mixture was dissolved in a mixture of DMF (100 mL) and 2,2-dimethoxypropane (50 mL) containing pyridinium *p*-toluenesulfonate (1 g). After 3 h stirring at room temperature, the mixture was concentrated *in vacuo* with a pump to give an anomeric mixture of products that was charged onto a silica gel column (300 g) with hot $CH_2Cl_2$ (100 mL). Elution with 3:2 cyclohexane–EtOAc gave 10.2 g (28.7%) of the α anomer **1α** ($R_F = 0.47$) as an oil and 7.7 g (21.7%) of the β anomer **1β** ($R_F = 0.29$) as a semi-solid.

Compound **1α**: $^1$H-N.m.r. data ($CDCl_3$): δ 1.44 (s, 3 H), 1.52 (s, 3 H), 2.50 (br s, 1 H, OH), 3.57–3.90 (m, 5 H), 3.99 (m, 1 H), 4.15–4.24 (m, 2 H), 4.91 (d, 1 H, *J* 4.0 Hz, H-1), 5.23–5.33 (m, 2 H), 5.79–5.93 (m, 1 H), 6.55 (d, 1 H, *J* 8.8 Hz, NH); i.r. $\nu_{max}^{KBr}$ 3460–3300, 1733 (shoulder), 1716, 1560 $cm^{-1}$; m.s. *m/z* 356 ($M^+ + 1$), 340.

*Anal.* Calc. for $C_{14}H_{20}F_3NO_6$ (355.3): C, 47.33; H, 5.67; F, 16.04; N, 3.94. Found: C, 47.05; H, 5.76; F, 16.02; N, 3.86.

Compound **1β**: $^1$H-N.m.r. data ($CDCl_3$): δ 1.32 (s, 3 H), 1.50 (s, 3 H), 3.23 (dt, 1 H, *J* 6.2, 9.5 Hz), 3.60 (t, 1 H, *J* 9.2 Hz), 3.74–3.87 (m, 4 H), 4.04 (m, 1 H), 4.27 (m, 1 H), 4.65 (d, 1 H, *J* 4.8 Hz, OH), 4.69 (d, 1 H, *J* 8.1 Hz, H-1), 5.11 (m, 1 H), 5.25 (m, 1 H), 5.78–5.92 (m, 1 H), 8.48 (d, 1 H, *J* 7.0 Hz, NH); i.r. $\nu_{max}^{KBr}$ 3490, 3330, 1707, 1560 $cm^{-1}$; m.s. *m/z* 356 ($M^+ + 1$), 340.

*Anal.* Calc. for $C_{14}H_{20}F_3NO_6$ (355.3): C, 47.33; H, 5.67; F, 16.04; N, 3.94. Found: C, 47.42; H, 5.96; F, 16.29; N, 3.91.

*Allyl 3-O-[(3R)-3-benzyloxytetradecanyl]-2-deoxy-4,6-O-isopropylidene-2-trifluoroacetamido-α-D-glucopyranoside* (**2**). — To a solution of **1α** (3.55 g, 10 mmol) and (*R*)-3-benzyloxy-1-methanesulfonyloxytetradecane (3.78 g, 9.50 mmol) in DMF (20 mL) was gradually added NaH (55% oil dispersion, 1.8 g, 41.3 mmol), at 0–5° with stirring. After 3 h at room temperature, the mixture was diluted with EtOAc, quenched with AcOH (4 mL), washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), filtered, and concentrated, to give a residue that was purified on a silica gel column. Elution with 3:1 cyclohexane–EtOAc gave 3.32 g (53%) of **2** as a viscous oil; $^1$H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 3 H, *J* 6.6 Hz), 1.2–1.6 [m, 26 H (containing br s, 18 H, δ 1.26; s, 3 H, δ 1.40; and s, 3 H, δ 1.48)]. 1.6–1.8 (m, 2 H), 3.40–4.02 (m, 9 H), 4.11–4.22 (m, 2 H), 4.42, 4.50 (AB-q, 2 H, *J* 11.7 Hz), 4.86 (d, 1 H, *J* 3.7 Hz, H-1), 5.24–5.33 (m, 2 H, olefinic), 5.80–5.95 (m, 1 H, olefinic), 6.37 (d, 1 H, *J* 9.2 Hz, NH), 7.24–7.34 (m, 5 H); i.r. $\nu_{max}^{film}$ 3500–3300, 2930, 2860, 1710 $cm^{-1}$.

*Anal.* Calc. for $C_{35}H_{54}F_3NO_7$ (657.8): C, 63.91; H, 8.27; F, 8.66; N, 2.13. Found: C, 63.90; H, 8.28; F, 8.43; N, 2.03.

*Allyl 2-amino-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside* (**3**). — A solution of **2** (3.3 g, 5.0 mmol) in EtOH (80 mL) and M NaOH (40 mL) was heated under reflux for 1.5 h, concentrated *in vacuo*, extracted with ether, washed with water and brine, dried ($Na_2SO_4$), and concentrated, to give a residue that was chromatographed on a silica gel column. Elution with 2:1 cyclohexane–EtOAc gave 2.6 g (92%) of **3** as a gum; $^1$H-n.m.r. data ($CDCl_3$): δ 0.86 (t, 3 H, *J* 6.6 Hz), 1.26 (br s, 18 H), 1.39 (s, 3 H), 1.47 (s, 3 H), 1.54 (br s, 4 H), 1.75–1.83 (m, 2 H), 2.71 (dd, 1

H, *J* 3.8, 9.7 Hz, H-2), 3.25 (t, 1 H, *J* 9.2 Hz), 3.50–3.86 (m, 6 H), 3.92–4.02 (m, 2 H), 4.14–4.21 (m, 1 H), 4.47, 4.54 (AB-q, 2 H, *J* 11.7 Hz), 4.85 (d, 1 H, *J* 3.7 Hz, H-1), 5.21 (m, 1 H, olefinic), 5.30 (m, 1 H), 5.70–5.85 (m, 1 H), 7.28–7.35 (m, 5 H); m.s. *m/z* 562 ($M^+$ + 1), 546, 504, 470, 455, 415.

*Anal.* Calc. for $C_{33}H_{55}NO_6$ (561.7): C, 70.55; H, 9.87; N, 2.49. Found: C, 70.56; H, 9.87; N, 2.37.

*Allyl 2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranoside* (**4a**). — To a solution of **3** (2.60 g) in $CH_2Cl_2$ (30 mL) was added (*R*)-3-(benzyloxy)tetradecanoic acid (1.62 g, 1.05 equiv.) and *N,N*-dicyclohexylcarbodiimide (1.05 g, 1.10 equiv.). After 30 min stirring at 20°, the mixture was filtered to remove *N,N*-dicyclohexylurea. The filtrate was concentrated and chromatographed on a silica gel column. Elution with 3:1 cyclohexane–EtOAc gave 3.77 g (93%) of **4a** as a wax; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, *J* 6.6 Hz), 1.25 (br s, 38 H), 1.38 (s, 3 H), 1.46 (s, 3 H), 1.46–1.78 (m, 4 H), 2.31 (dd, 1 H, *J* 7.3, 15.0 Hz), 2.42 (dd, 1 H, *J* 3.7, 15.0 Hz), 3.36–3.85 (m, 10 H), 4.00–4.07 (m, 1 H), 4.16–4.24 (m, 1 H), 4.46 (s, 2 H), 4.52 (s, 2 H), 4.78 (d, 1 H, *J* 3.7 Hz), 5.10–5.24 (m, 2 H), 5.72–5.86 (m, 1 H), 6.38 (d, 1 H, *J* 9.2 Hz, NH), 7.23–7.38 (m, 10 H); i.r. $\nu_{max}^{neat}$ 3310, 1642 $cm^{-1}$.

*Anal.* Calc. for $C_{54}H_{87}NO_8$ (878.2): C, 73.85; H, 9.98; N, 1.59. Found: C, 74.04; H, 10.13; N, 1.45.

*Allyl 4,6-O-[(3R)-benzylidene]-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-α-D-glucopyranoside* (**4b**). — A solution of **9** (838 mg, 1 mmol), benzaldehyde dimethylacetal (304 mg, 2 mmol), and *p*-TsOH (50 mg) in DMF (20 mL) was allowed to stand overnight at 20–25°. The mixture was concentrated under pump vacuum and diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated, to give a residue that was purified by chromatography on a silica gel column. Elution with 3:1 cyclohexane–EtOAc gave 920 mg (99%) of **4b** as a gum; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, *J* 6.6 Hz), 1.2–1.8 (m, 42 H), 2.27–2.48 (m, 2 H, $NCOCH_2$), 3.4–3.9 (m, 9 H), 4.02–4.09 (m, 1 H), 4.20–4.27 (m, 2 H), 4.42 (s, 2 H), 4.48–4.58 (AB-q, 2 H, *J* 11.0 Hz), 4.82 (d, 1 H, *J* 3.7 Hz, H-1), 5.11–5.86 (m, 1 H), 6.34 (d, 1 H, *J* 9.2 Hz, NH), 7.23–7.34 (m, 13 H), 7.44–7.48 (m, 2 H); i.r. $\nu_{max}^{CHCl_3}$ 3350, 2930, 2860, 1665, 1592 $cm^{-1}$.

*Anal.* Calc. for $C_{58}H_{87}NO_8$ (926.3): C, 75.20; H, 9.47; N, 1.51. Found: C, 75.13; H, 9.37; N, 1.42.

*2-[(3R)-3-(Benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4,6-O-isopropylidene-D-glucopyranose* (**5a**). — To a solution of **4a** (1.76 g, 2 mmol) in THF 80 mL, freshly distilled from $LiAlH_4$) was added bis(methyldiphenylphosphine)cyclo-octadieneiridium(I) hexafluorophosphate[6], $[C_8H_{12}Ir(PMePh_2)_2]PF_6$ (100 mg). The air in the reaction flask was completely replaced with nitrogen and then further replaced with hydrogen to activate the iridium complex. Immediately after 1 or 2 min, when the red color solution of the iridium complex had become almost colorless, the hydrogen was completely replaced with nitrogen. This solution was stirred for 2 h at 20°. After confirming a double bond shift to an enol ether (as indicated by a slightly

higher $R_F$ value) from the 1-allyloxy group by t.l.c., $H_2O$ (8 mL), pyridine (0.56 g), and $I_2$ (1.0 g) were added to this solution. After 20 min stirring at 20°, the mixture was concentrated *in vacuo*, diluted with EtOAc, washed with aq. 5% $Na_2SO_3$, satd. $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to give a mixture that was separated on a silica gel column. Elution with 1:3 cyclohexane–EtOAc gave 1.5 g (89%) of **5a** as gum, which gradually decomposed on standing at room temperature for several weeks; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.2–1.8 [m, 48 H (containing s, 3 H, $\delta$ 1.37 and s, 3 H, $\delta$ 1.45)], 2.24–2.44 (m, 2 H), 3.42–4.04 (m, 11 H), 4.42–4.59 (m, 4 H), 5.06 (t, 1 H, $J$ 1.0 Hz), 6.39 (d, 1 H, $J$ 8.8 Hz), 7.31 (s, 5 H), 7.33 (s, 5 H); i.r. $\nu_{max}^{film}$ 3300, 2920, 2850, 1640 cm$^{-1}$.

*Anal.* Calc. for $C_{51}H_{83}NO_8$ (838.2): C, 73.08; H, 9.98; N, 1.67. Found: C, 72.72; H, 10.08; N, 1.57.

*4,6-O-[(R)-Benzylidene]-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-D-glucopyranose* (**5b**). — Compound **4b** (1.05 g, 1.13 mmol) was treated as described in the formation of **5a** from **4a**, to give 629 mg (63%) of **5b** as a gum; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.2–1.8 (m, 42 H), 2.28 (dd, 1 H, $J$ 8.1, 14.7 Hz), 2.40 (dd, 1 H, $J$ 3.7, 14.7 Hz), 2.75 (br s, 1 H, OH), 3.44–4.07 (m, 8 H), 4.23 (dd, 1 H, $J$ 4.8, 9.9 Hz), 4.37–4.61 [m, 5 H (containing s, 2 H, $\delta$ 4.42)], 5.09 (d, 1 H, $J$ 3.7 Hz, H-1), 5.52 (s, 1 H, C*H*Ph), 6.38 (d, 1 H, $J$ 8.8 Hz, NH), 7.24–7.35 (m, 13 H), 7.42–7.47 (m, 2 H); i.r. $\nu_{max}^{CHCl_3}$ 3350, 2930, 2860, 1660 cm$^{-1}$.

*Anal.* Calc. for $C_{55}H_{83}NO_8$ (886.3): C, 74.54; H, 9.44; N, 1.58. Found: C, 74.30; H, 9.38; N, 1.86.

*2-[(3R)-3-(Benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4,6-O-isopropylidene-α-D-glucopyranosyl dibenzyl phosphate* (**6a**). — To a solution of **5a** (1.01 g, 1.2 mmol) in THF (15 mL) was added a solution of BuLi (0.9 mL, 1.6M solution in hexane, 1.2 equiv.), and then a solution of $ClP(O)(OCH_2Ph)_2$ (463 mg, 1.58 mmol, 1.3 equiv.) at −78° under nitrogen with stirring. After 10 min, the mixture was quenched with a solution of AcOH (0.5 mL) in THF (5 mL), diluted with EtOAc, washed with $H_2O$, aq. $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated to give a residue that was chromatographed on a silica gel column to give 964 mg (73%) of **6a** as a gum; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6–6.9 Hz), 1.2–1.8 [m, 48 H (containing s, 3 H, $\delta$ 1.37 and s, 3 H, $\delta$ 1.43)], 2.24–2.27 (m, 2 H), 3.23 (dd, 1 H, $J$ 8.4, 10.3 Hz), 3.3–3.5 (m, 2 H), 3.6–3.75 (m, 6 H), 4.1–4.22 (m, 1 H), 4.35–4.47 (m, 4 H, 2 × OC$H_2$Ph), 4.99 (d, 2 H, $J$ 4.8 Hz, POC$H_2$Ph), 5.03 (d, 2 H, $J$ 5.1 Hz), 5.73 (dd, 1 H, $J$ 3.4, 5.7 Hz, H-1), 6.48 (d, 1 H, $J$ 8.1 Hz, NH), 7.30–7.33 (m, 20 H); i.r. $\nu_{max}^{film}$ 3310, 2930, 2860, 1660 cm$^{-1}$.

*Anal.* Calc. for $C_{65}H_{96}NO_{11}P$ (1098.4): C, 71.07; H, 8.81; N, 1.28; P, 2.82. Found: C, 70.72; H, 8.68; N, 1.18; P, 2.63.

*4,6-O-[(R)-Benzylidene]-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-α-D-glucopyranosyl dibenzyl phosphate* (**6b**). — Compound **5b** (579 mg, 0.653 mmol) was treated as described in the formation of **6a** from **5a** to give 484 mg (63%) of **6b** as powder and recovered **5b** (123 mg, 21%), after chromatography on a silica gel column eluted by 2:1 cyclohexane–EtOAc.

Compound **6b**: $^1$H-N.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.2–1.8 (m, 42

H), 2.26–2.28 (m, 2 H), 3.34–3.44 (m, 3 H), 3.57–3.75 (m, 4 H), 3.85–3.90 (m, 1 H), 4.08 (dd, 1 H, *J* 4.6, 10.1 Hz), 4.19–4.26 (m, 1 H), 4.38 (s, 2 H, $OCH_2Ph$), 4.39, 4.45 (AB-q, 2 H, *J* 11.2 Hz, $OCH_2Ph$), 5.00 (d, 2 H, *J* 6.2 Hz, $POCH_2Ph$), 5.03 (d, 2 H, *J* 5.9 Hz, $POCH_2Ph$), 5.48 (s, 1 H, C*H*Ph), 5.77 (dd, 1 H, *J* 3.3, 5.9 Hz, H-1), 6.52 (d, 1 H, *J* 8.1 Hz, NH), 7.23–7.33 (m, 23 H), 7.42–7.45 (m, 2 H); i.r. $\nu_{max}^{Nujol}$ 3310, 1643 $cm^{-1}$.

*Anal.* Calc. for $C_{69}H_{96}NO_{11}P$ (1146.5): C, 72.29; H, 8.44; N, 1.22; P, 2.70. Found: C, 72.49; H, 8.35; N, 1.36; P, 2.40.

*2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-*O*-[(3R)-3-hydroxytetradecanyl]-4,6-*O*-isopropylidene-α-*D*-glucopyranosyl dihydrogen phosphate* (**7**). — A solution of **6a** (380 mg, 0.346 mmol) in THF (15 mL) was hydrogenolyzed using 10% Pd/C (1.0 g, Type A, Kawaken Fine Chemical Co.) as a catalyst for 3 h at 20–25° under a hydrogen atmosphere. The mixture was filtered and concentrated *in vacuo* to give 235 mg (92%) of **7** as a powder, which gradually changed to **8** on standing.

Compound **7**: $^1$H-N.m.r. data (1:1 $CDCl_3$–$CD_3OD$): δ 0.87–0.91 (m, 6 H), 1.27–1.80 [m, 48 H (containing s, 3 H, δ 1.41 and s, 3 H, δ 1.53)], 2.30–2.45 (m, 2 H), 3.45–4.20 (m, 10 H), 5.54 (m, 1 H, H-1); i.r. $\nu_{max}^{CHCl_3}$ 3300, 2930, 2860, 1650, 1590 $cm^{-1}$; f.a.b.-m.s. (negative): *m/z* 736 $(M - H)^-$, 696, 297, 148.

*Anal.* Calc. for $C_{37}H_{72}NO_{11}P$ (737.9): C, 60.22; H, 9.83; N, 1.90; P, 4.20. Found: C, 58.96; H, 9.44; N, 1.72; P, 4.28.

*2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-*O*-[(3R)-3-hydroxytetradecanyl]-α-*D*-glucopyranosyl dihydrogen phosphate* (**8**). — Procedure A. A suspension of **7** (113 mg, 0.153 mmol) in 80% AcOH (30 mL) was stirred vigorously for 1 h at 25°. The suspension was concentrated *in vacuo* to give **8** quantitatively as a white powder; $^1$H-n.m.r. data (1:1 $CDCl_3$–$CD_3OD$): δ 0.89–0.93 (m, 12 H), 1.35–2.05 (m, 74 H), 2.6–2.9 (m, 6 H), 3.8–5.0 (m, 19 H), 5.65 (m, 1 H); i.r. $\nu_{max}^{Nujol}$ 3500–3200, 1640 $cm^{-1}$; f.a.b.-m.s. (negative, triethanolamine) *m/z* 696 $(M - H)^-$.

*Anal.* Calc. for $C_{34}H_{68}NO_{11}P$ (697.9): C, 58.52; H, 9.82; N, 2.01; P, 4.44. Found: C, 58.01; H, 9.69; N, 2.19; P, 4.09.

Procedure **B**. A solution of **6b** (370 mg, 0.323 mmol) in THF (40 mL) containing 10% Pd/C (750 mg, Type A; Kawaken Fine Chemical Co.) was vigorously stirred under a hydrogen atmosphere for 10 h at 25°. The mixture was filtered, and the filtrate was concentrated to give 28 mg of a mixture (non-acidic material and **8**). This mixture was discarded. Compound **8**, which was adsorbed onto the filter carbon, was washed well with 1:1 $CHCl_3$–MeOH (3 × 50 mL) and filtered. The combined filtrate was concentrated to give 130 mg (58%) of **8** as powder, which was identical in all respects with **8** obtained in procedure A.

The powder **8** (30 mg) was suspended in 0.1M HCl (8 mL) and 1:2 $CHCl_3$–MeOH (30 mL) and dissolved with aid of an ultrasonic device. Additional $CHCl_3$ (10 mL) and 0.1M HCl (10 mL) were added to this solution to ensure separation into two phases. The lower chloroform phase was collected and concentrated to give 28 mg of **8**, which was soluble in 0.1% $Et_3N$ water (v/v).

*Allyl 2-[(3R)-3-(benzyloxy)tetradecanamido]-3-*O*-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-α-*D*-glucopyranoside* (**9**). — A solution of **4a** (1.92 g, 2.19 mmol) in

80% AcOH (60 mL) was stirred for 30 min at 60°. The solution was concentrated *in vacuo* and dried with a pump to give 1.83 g (quantitative) of **9** as a powder; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.2–1.8 (m, 42 H), 2.02 (br s, 1 H, OH), 2.35 (dd, 1 H, $J$ 7.3, 15.0 Hz), 2.45 (d, 1 H, $J$ 3.8, 15.0 Hz), 3.37–3.85 (m, 11 H, containing OH), 4.06 (tdd, 1 H, $J$ 1.0, 5.5, 12.8 Hz), 4.18 (dt, 1 H, $J$ 3.6, 9.9 Hz), 4.46 (s, 2 H, $CH_2Ph$), 4.50, 4.55 (AB-q, 2 H, $J$ 11.0 Hz, $CH_2Ph$), 4.76 (d, 1 H, $J$ 3.7 Hz, H-1), 5.10–5.24 (m, 2 H), 5.71–5.83 (m, 1 H), 6.48 (d, 1 H, $J$ 9.5 Hz, NH), 7.30–7.33 (m, 10 H); i.r. $\nu_{max}^{Nujol}$ 3325, 1640 cm$^{-1}$.

*Anal.* Calc. for $C_{51}H_{83}NO_8$ (838.2): C, 73.08; H, 9.98; N, 1.67. Found: C, 72.65; H, 9.82; N, 1.91.

*Allyl 6-O-benzyloxymethyl-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-α-D-glucopyranoside* (**10**). — A solution of **9** (900 mg, 1.07 mmol), $PhCH_2OCH_2Cl$ (185 mg, 1.18 mmol), and $Me_2NCONMe_2$ (137 mg, 1.18 mmol) in $CH_2Cl_2$ (9 mL) was stirred for 15 h at 25°. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated to give a mixture that was chromatographed on a silica gel column. Elution with 2:1 cyclohexane–EtOAc gave the recovered **9** (230 mg) and 387 mg (38%) of **10** as powder; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.20–1.80 (m, 42 H), 2.34 (dd, 1 H, $J$ 7.3, 15.0 Hz), 2.42 (dd, 1 H, $J$ 4.0, 15.0 Hz), 3.18 (br s, 1 H, OH), 3.38–3.86 (m, 10 H), 4.06 (dd, 1 H, $J$ 5.6, 7.1 Hz), 4.25 (dt, 1 H, $J$ 3.3, 6.8 Hz), 4.41–4.85 (m, 9 H), 5.09–5.24 (m, 2 H), 5.73–5.80 (m, 1 H), 6.44 (d, 1 H, $J$ 9.5 Hz, NH), 7.20–7.38 (m, 15 H); i.r. $\nu_{max}^{Nujol}$ 3450, 3320, 1640 cm$^{-1}$.

*Anal.* Calc. for $C_{59}H_{91}NO_9$ (958.4): C, 73.94; H, 9.57; N, 1.46. Found: C, 73.72; H, 9.56; N, 1.56.

*Allyl 6-O-benzyloxymethyl-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4-O-diphenylphosphono-α-D-glucopyranoside* (**11**). — A solution of **10** (276 mg, 0.288 mmol), DMAP (105 mg, 3 equiv.), and $(PhO)_2P(O)Cl$ (235 mg, 3 equiv.) in $CH_2Cl_2$ (3 mL) was stirred for 15 h at 25°. The mixture was diluted with EtOAc, washed with aq. $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated to give a mixture that was chromatographed on a silica gel column. Elution with 2:1 cyclohexane–EtOAc gave 340 mg (99%) of **11** as powder; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 6.6 Hz), 1.1–1.8 (m, 42 H), 2.2–2.4 (m, 2 H), 3.30–4.82 (m, 21 H), 5.13 (dd, 1 H, $J$ 1.5, 10.3 Hz), 5.20 (dd, 1 H, $J$ 1.5, 17.3 Hz), 5.71–5.86 (m, 1 H), 6.42 (d, 1 H, $J$ 8.2 Hz, NH), 7.11–7.65 (m, 25 H); i.r. $\nu_{max}^{Nujol}$ 3340, 1645, 1590 cm$^{-1}$.

*Anal.* Calc. for $C_{71}H_{100}NO_{12}P$ (1190.5): C, 71.63; H, 8.46; N, 1.17; P, 2.60. Found: C, 71.90; H, 8.32; N, 1.09; P, 2.53.

*6-O-Benzyloxymethyl-2-[(3R)-3-(benzyloxy)tetradecanamido]-3-O-[(3R)-3-(benzyloxy)tetradecanyl]-2-deoxy-4-O-diphenylphosphono-D-glucopyranose* (**12**). — Compound **11** (360 mg, 0.302 mmol) was treated as described in the formation of **5a** from **4a**, to give 231 mg (66%) of **12** as a gum after chromatography on a silica gel column eluted by 2:1 cyclohexane–EtOAc; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (t, 6 H, $J$ 7.0 Hz), 1.16–1.71 (m, 42 H), 2.17–2.36 (m, 2 H), 2.91 (br s, 1 H, OH), 3.39 (m, 1 H), 3.59–3.87 (m, 6 H), 4.08–4.22 (m, 2 H), 4.35–4.76 (m, 9 H), 5.07 [t, 1 H, $J$ 3.3 Hz (d, $J$ 3.3

Hz with $D_2O$), H-1)], 6.39 (d, 1 H, *J* 8.8 Hz, NH), 7.11–7.36 (m, 25 H); i.r. $\nu_{max}^{film}$ 3300, 2910, 2840, 1640, 1590 $cm^{-1}$.

*Anal.* Calc. for $C_{68}H_{96}NO_{12}P$ (1150.5): C, 70.99; H, 8.41; N, 1.21; P, 2.69. Found: C, 70.59; H, 8.42; N, 1.18; P, 2.62.

*2-Deoxy-4-O-diphenylphosphono-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3R)-3-hydroxytetradecanyl]-D-glucopyranose* (**13**). — A solution of **12** (210 mg, 0.183 mmol) in MeOH (20 mL) containing a drop of formic acid was hydrogenolyzed using 10% Pd/C (350 mg, Type A; Kawaken Fine Chemical Co.) for 6 h at 20–25° under a hydrogen atmosphere. The mixture was filtered and concentrated *in vacuo* to give a residue, which was chromatographed on a silica gel column. Elution with 24:1 EtOAc–MeOH gave 105 mg (68%) of **13** ($R_F$ = 0.39) as powder; $^1$H-n.m.r. data ($CDCl_3$): $\delta$ 0.88 (m, 6 H), 1.2–1.8 (m, 42 H), 2.1–2.4 (m, 2 H), 3.5–4.2 (m, 9 H), 4.69 (m, 1 H), 4.92 (br s, 1 H, OH), 5.45 (d, 1 H, *J* 3.3 Hz, H-1), 6.77 (br s, 1 H, OH), 6.69 (d, 1 H, *J* 8.8 Hz, NH), 7.1–7.7 (m, 10 H).

*Anal.* Calc. for $C_{46}H_{76}NO_{11}P$ (850.1): C, 64.99; H, 9.01; N, 1.65; P, 3.64. Found: C, 64.46; H, 8.78; N, 1.65; P, 3.32.

*2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3R)-3-hydroxytetradecanyl]-4-O-phosphono-D-glucopyranose* (**14**). — A solution of **13** (100 mg, 0.118 mmol) in MeOH (10 mL) was hydrogenolyzed using $PtO_2$ (15 mg) for 3 h at 20–25° under a hydrogen atmosphere. The reaction mixture was filtered and concentrated *in vacuo* to give 59 mg (72%) of **14** as powder; $^1$H-n.m.r. data ($CDCl_3$–$CD_3OD$): $\delta$ 0.89 (t, 6 H, *J* 6.6 Hz), 1.20–1.83 (m, 42 H), 2.32 (dd, 1 H, *J* 8.8, 15.4 Hz), 2.43 (dd, 1 H, *J* 3.3, 15.4 Hz), 3.50–4.75 (m, 10 H), 5.11 (d, 1 H, *J* 3.3 Hz, H-1); i.r. $\nu_{max}^{Nujol}$ 3300, 1650 $cm^{-1}$.

*Anal.* Calc. for $C_{34}H_{68}NO_{11}P$ (697.9): C, 58.52; H, 9.82; N, 2.01; P, 4.44. Found: C, 58.01; H, 9.54; N, 2.08; P, 3.99.

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