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Note

Synthesis of 3-*O*-(alkyl-branched acyl)-2-deoxy-2 [(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucose derivatives related to bacterial lipid A

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GLA-60 (2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucose)[1], which is an analog of the nonreducing sugar subunit of bacterial lipid A (ref 2), exhibits strong immunostimulatory activities without pyrogenicity[3]. In the course of an investigation[1,4] into structure–activity relationships in the GLA-60 series, we showed that the chain length of the 3-acyloxyacyl group at C-3 is very important for the expression of immunopharmacological activity. For example, among the compounds differing from GLA-60 in this regard, GLA-63 (ref 4b), which carries a (3*R*)-3-dodecanoyloxytetradecanoyl group, was found to be the most beneficial, possessing strong immunomodulating activities[3d]. However the derivatives[4c] which respectively carry a (3*RS*)-3-acyloxydecanoyl, (3*RS*)-3-acyloxydodecanoyl, or (3*RS*)-3-acyloxyhexadecanoyl group at O-3 in the sugar moiety were not significantly active.

We have recently found[4d] that 4-*O*-phosphono-D-glucosamine derivatives carrying an alkyl-branched 2-tetradecylhexadecanoyl group instead of a 3-acyloxyacyl group stimulate the phagocytic activity of peritoneal macrophages and their antiviral activity as strongly as GLA-60.

The aim of the present study was to investigate the biological influence of 2- or 3-alkyl-branched acyl groups of type I, II, or III (see formula chart), in which the length of the principal alkyl chain is varied. We synthesized a number of 3-*O*-(2- or 3-alkylacyl)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucose derivatives, as shown in formulas **7a–m**.

The syntheses of compounds **7a–m** all follow essentially the same pathway. 2-(Trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-2-{(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside[5] (**1**) was treated with alkyl-

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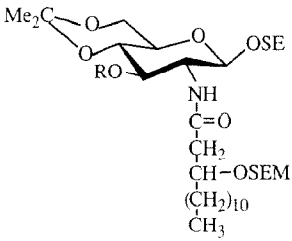


R	
1	H
2a-m	acyl (a-m)

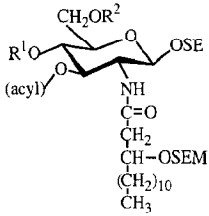
SE = $Me_3SiCH_2CH_2$

SEM = $Me_3SiCH_2CH_2OCH_2$

		Acyl groups	
		Series	n
Type I	CH_3-$(CH_2)_8$ / CH_3-$(CH_2)_n$ >CH-CH_2-CO	a	11
		b	13
		c	15
		d	17
Type II	CH_3-$(CH_2)_{10}$ / CH_3-$(CH_2)_n$ >CH-CH_2-CO	e	11
		f	13
		g	15
		h	17
Type III	CH_3-$(CH_2)_{11}$ / CH_3-$(CH_2)_n$ >CH-CO	i	9
		j	11
		k	13
		l	15
		m	17

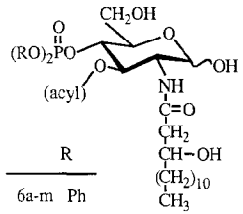
branched fatty acids (purchased from Wako Pure Chemical Industries) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and 4-dimethylaminopyridine (DMAP) to give **2a–m**. The isopropylidene group was then removed with aqueous acetic acid, giving **3a–m**. Introduction of a *tert*-butyldimethylsilyl group at O-6 and a diphenoxyphosphinyl group at O-4 were performed by the usual methods[4c] to yield **5a–m**. Hydrolytic removal of the 2-(trimethylsilyl)ethyl, 2-(trimethylsilyl)ethoxymethyl, and *tert*-butyldimethylsilyl groups with boron trifluoride etherate gave **6a–m**. Finally, the phenoxy groups of **6a–m** were hydrogenolyzed to afford the desired compounds **7a–m** as described previously[4].

With regard to biological activities such as mitogenicity, macrophage activation, and cytokine-induction, compound **7f** carrying a 3-undecylheptadecanoyl group at C-3 was the most active in the series of compounds having 3-alkyl-branched acyl groups (types I and II). The activities of **7f** were comparable to those of GLA-60 (ref 6). Among the compounds with 2-alkyl-branched acyl groups (type III), **7i–k** exhibited[7] more potent mitogenic activity than GLA-60, together with tumor necrosis factor (TNF)-inducing activity. However, no significant immunostimulatory activity was expressed by compounds in which the principal chain of the alkyl-branched acyl group was longer than 16 carbon atoms (**7c**, **7d**, **7g**, **7h**, **7l**, and **7m**). Overall the results indicate that compounds carrying a 2- or 3-alkyl-branched



	R^1	R^2
3a-m	H	H
4a-m	H	TBDMS
5a-m	$(PhO)_2P(O)$	TBDMS

TBDMS = *tert*-$BuMe_2Si$



R	
6a-m	Ph
7a-m	H

acyl group show biological activities similar to those of derivatives having an ester-branched 3-acyloxyacyl group.

EXPERIMENTAL

General methods. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. All evaporations were conducted in vacuo. Preparative chromatography was performed on silica gel (Wako Pure Chemical Industries, 200 mesh) with the solvent systems specified. Specific rotations were determined with a Union PM-201 polarimeter, and IR spectra were recorded with a Jasco A-100 spectrophotometer. ^1H NMR spectra were recorded with a Jeol JNM-GX 270 spectrometer.

The details of the preparation of compounds **2a–7a** are given here as examples of the general procedures used.

2-(Trimethylsilyl)ethyl 2-deoxy-4,6-O-isopropylidene-3-O-[(3RS)-3-nonylpentadecanoyl]-2-{(3R)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside (**2a**). — To a solution of 2-(trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-2-{(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside (**1**, 400 mg) in CH_2Cl_2 (20 mL) were added (3*RS*)-3-nonylpentadecanoic acid (469 mg), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 339 mg), and a catalytic amount of 4-dimethylaminopyridine (DMAP). The mixture was stirred overnight at room temperature and concentrated. The residue was chromatographed on a column of silica gel with 300:1 CH_2Cl_2–MeOH to give **2a** (620 mg, quantitative) as a syrup; $[\alpha]_D$ −12.8° (*c* 1.0, CH_2Cl_2); IR: ν_{max} 3300 (NH), 2930 and 2860 (CH), 1740 (ester), 1650 and 1550 (amide), 860 and 830 cm^{-1} (Me_2C, MeSi); ^1H NMR ($CDCl_3$): δ 0.0 (m, 18 H, 2 Me_3Si), 0.8–1.0 (m, 13 H,

Me_3SiCH_2 and terminal CH_3 of fatty acyl), 1.1–1.65 (m, 59 H, CH_2 and CH), 1.32, 1.42 (2 s, 6 H, Me_2C), 2.1–2.4 (m, 4 H, 2 CH_2CO), 3.34 (m, 1 H, H-5), 3.4–4.0 (m, 9 H, H-2,4,6, $Me_3SiCH_2CH_2$, and H-3 of C_{14}-OSEM), 4.51 (d, 1 H, $J_{1,2}$ 8.1 Hz, H-1), 4.62, 4.69 (2 d, 2 H, J_{gem} 7.0 Hz, OCH_2O), 5.11 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), and 6.20 (d, 1 H, $J_{2,NH}$ 9.5 Hz, NH). *Anal.* Calcd for $C_{58}H_{115}NO_9Si_2$ (1026.72): C, 67.85; H, 11.29; N, 1.36. Found: C, 67.58; H, 11.59; N, 1.20.

2-(Trimethylsilyl)ethyl 2-deoxy-3-O-[(3RS)-3-nonylpentadecanoyl]-2-{(3R)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside (**3a**). — A solution of **2a** (570 mg) in aq 80% acetic acid (100 mL) was stirred for 2 h at 50°C, and then concentrated to a syrup. This was chromatographed on a column of silica gel with 200:1 CH_2Cl_2–MeOH to give **3a** (430 mg, 78%) as a syrup; $[\alpha]_D$ −1.6° (*c* 0.8, CH_2Cl_2); IR: ν_{max} 3300 (OH, NH), 2930 and 2860 (CH), 1760 (ester), 1650 and 1550 (amide), 860 and 840 cm^{-1} (MeSi); ^1H NMR ($CDCl_3$): δ 0.0 (m, 18 H, 2 Me_3Si), 0.8–1.0 (m, 13 H, Me_3SiCH_2 and terminal CH_3 of fatty acyl), 1.1–1.7 (m, 59 H, CH_2 and CH), 2.29 (m, 4 H, 2 CH_2CO), 3.10 (br s, 2 H, 2 OH), 3.40–4.0 (m, 10 H, H-2,4,5,6, $Me_3SiCH_2CH_2$, and H-3 of C_{14}-OSEM), 4.6–4.75 (m, 3 H, OCH_2O and H-1), 5.13 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), and 6.32 (d, 1 H, $J_{2,NH}$ 8.1 Hz, NH). *Anal.* Calcd for $C_{55}H_{111}NO_9Si_2$ (986.65): C, 66.95; H, 11.34; N, 1.42. Found: C, 66.82; H, 11.42; N, 1.29.

2-(Trimethylsilyl)ethyl 6-O-tert-butyldimethylsilyl-2-deoxy-3-O-[(3RS)-3-nonylpentadecanoyl]-2-{(3R)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside (**4a**). — To a solution of **3a** (390 mg) in pyridine (10 mL) was added *tert*-butylchlorodimethylsilane (116 mg). The mixture was stirred overnight at room temperature. Methanol (5 mL) was added, and the solution was then concentrated. The residual syrup was chromatographed on a column of silica gel with 300:1 CH_2Cl_2–MeOH to afford syrupy **4a** (420 mg, 96%); $[\alpha]_D$ −7.3° (*c* 1.2, CH_2Cl_2); IR: ν_{max} 3500 (OH), 3300 (NH), 2930 and 2850 (CH), 1730 (ester), 1640 and 1550 (amide), 860 and 830 cm^{-1} (MeSi); ^1H NMR ($CDCl_3$): δ 0.0 (m, 24 H, MeSi), 0.8–1.0 (m, 22 H, Me_3SiCH_2, *tert*-BuSi, and terminal CH_3 of fatty acyl), 1.1–1.65 (m, 59 H, CH_2 and CH), 2.2–2.45 (m, 4 H, 2 CH_2CO), 3.20 (d, 1 H, $J_{2,6}$ Hz, OH), 3.35–4.0 (m, 10 H, H-2,4,5,6, $Me_3SiCH_2CH_2$, and H-3 of C_{14}-OSEM), 4.53 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 4.66 (2 d, 2 H, J_{gem} 6.8 Hz, OCH_2O), 5.10 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), and 6.13 (d, 1 H, $J_{2,NH}$ 8.8 Hz, NH). *Anal.* Calcd for $C_{61}H_{125}NO_9Si_3$ (1100.92): C, 66.55; H, 11.44; N, 1.27. Found: C, 66.30; H, 11.70; N, 1.10.

2-(Trimethylsilyl)ethyl 6-O-tert-butyldimethylsilyl-2-deoxy-4-O-diphenoxyphosphinyl-3-O-[(3RS)-3-nonylpentadecanoyl]-2-{(3R)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido}-β-D-glucopyranoside (**5a**). — To a cooled solution of **4a** (360 mg) and DMAP (78 mg) in pyridine (5 mL) was added diphenyl phosphorochloridate (171 mg) in CH_2Cl_2 (2 mL). The mixture was stirred overnight at room temperature. Methanol was added to the solution, which was then concentrated. The residue was extracted with $CHCl_3$, and the extract was washed with 2

M HCl and water, dried (Na_2SO_4), and concentrated. The residual syrup was chromatographed on a column of silica gel with 200:1 CH_2Cl_2–MeOH to obtain **5a** (260 mg, 60%) as a syrup; $[\alpha]_D$ +1.63° (*c* 0.8, CH_2Cl_2); IR: ν_{max} 3300 (NH), 2930 and 2850 (CH), 1740 (ester), 1660 and 1540 (amide), 950 (P–O–Ph), 860 and 830 (MeSi), and 770–680 cm^{-1} (Ph); ^1H NMR ($CDCl_3$): δ 0.0 (m, 24 H, MeSi), 0.8–1.0 (m, 22 H, Me_3SiCH_2, *tert*-BuSi, and terminal CH_3 of fatty acyl), 1.0–1.6 (m, 59 H, CH_2 and CH), 2.1–2.4 (m, 4 H, 2 CH_2CO), 3.45–3.95 (m, 9 H, H-2,4,5,6, $Me_3SiCH_2CH_2$, and H-3 of C_{14}-OSEM), 4.55–4.8 (m, 4 H, H-1,4, and OCH_2O), 5.46 (t, 1 H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), 6.10 (d, 1 H, $J_{2,NH}$ 8.8 Hz, NH), and 7.1–7.4 (m, 10 H, Ph-*H*). *Anal.* Calcd for $C_{73}H_{134}NO_{12}PSi_3$ (1333.09): C, 65.77; H, 10.13; N, 1.05. Found: C, 65.80; H, 10.00; N, 1.20.

2-Deoxy-4-O-diphenoxyphosphinyl-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3RS)-3-nonylpentadecanoyl]-D-glucopyranose (**6a**). — To a solution of **5a** (190 mg) in CH_2Cl_2 (10 mL) was added boron trifluoride etherate (0.5 mL) at 0°C. The mixture was stirred for 2 h at the same temperature, then washed with satd $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The residue was chromatographed on a column of silica gel with 45:1 CH_2Cl_2–MeOH to give **6a** (106 mg, 75%), which was lyophilized from 1,4-dioxane solution; mp 95–97°C; $[\alpha]_D$ +1.0° (*c* 1.0, CH_2Cl_2); IR: ν_{max} 3350 (OH, NH), 2930 and 2860 (CH), 1740 (ester), 1650 and 1540 (amide), 960 (P–O–Ph), and 780–680 cm^{-1} (Ph); ^1H NMR ($CDCl_3$): δ 0.88 (t, 9 H, terminal CH_3 of fatty acyl), 1.0–1.8 (m, 59 H, CH_2 and CH), 2.05–2.35 (m, 4 H, 2 CH_2CO), 3.5–4.1 (m, 7 H, H-5,6, H-3 of C_{14}-OH, and OH), 4.22 (m, 1 H, H-2), 4.74 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.5$ Hz, H-4), 5.25 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.50 (t, 1 H, $J_{2,3} = J_{3,4} = 10.3$ Hz, H-3), 6.42 (d, 1 H, $J_{2,NH}$ 9.3 Hz, NH), and 7.1–7.4 (m, 10 H, Ph-*H*). *Anal.* Calcd for $C_{56}H_{94}NO_{11}P$ (988.33): C, 68.06; H, 9.59; N, 1.42. Found: C, 67.86; H, 9.83; N, 1.31.

2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3RS)-3-nonylpentadecanoyl]-4-O-phosphono-D-glucopyranose (7a). — To a solution of **6a** (90 mg) in EtOH (50 mL) was added Adams platinum catalyst (100 mg), and the mixture was stirred overnight under H_2. The catalyst was filtered off and washed with EtOH. The filtrate and washings were combined and concentrated to afford **7a** (58 mg, 74%), which was lyophilized from a suspension in 1,4-dioxane. It gave a positive test for the phosphono group with the molybdenum spray reagent of Dittmer and Lester[8]; IR: ν_{max} 3400 (OH, NH), 2930 and 2850 (CH), 1740 (ester), and 1640 and 1550 cm^{-1} (amide). Other physical and analytical data are given in Table I.

Other alkyl-branched acyl derivatives. — The additional compounds of the series were as follows: The physical and analytical data are recorded in Table I.

7b. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3RS)-3-nonylheptadecanoyl]-4-O-phosphono-D-glucopyranose.*

7c. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3RS)-3-nonylnonadecanoyl]-4-O-phosphono-D-glucopyranose.*

7d. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-3-O-[(3RS)-3-nonylheneicosanoyl]-4-O-phosphono-D-glucopyranose.*

TABLE I

Some physical properties of compounds **7a–m**

Compound	Mp (°C)	$[\alpha]_D$ (°) (c in 50:25:4:2 $CHCl_3$–MeOH–H_2O–NH_4OH)	Molecular formula	Composition found (calcd) (%)		
				C	H	N
7a	163–165	+13.3 (0.1)	$C_{44}H_{86}NO_{11}P$	62.99 (63.21)	10.55 (10.37)	1.39 (1.68)
b	161–163	+4.1 (0.6)	$C_{46}H_{90}NO_{11}P$	63.88 (63.93)	10.63 (10.50)	1.39 (1.62)
c	164–166	+5.1 (0.8)	$C_{48}H_{94}NO_{11}P$	64.76 (64.62)	10.90 (10.62)	1.66 (1.57)
d	167–169	+5.0 (0.4)	$C_{50}H_{98}NO_{11}P$	65.04 (65.26)	10.96 (10.73)	1.66 (1.52)
e	157–160	+7.4 (0.4)	$C_{46}H_{90}NO_{11}P$	64.10 (63.93)	10.38 (10.50)	1.46 (1.62)
f	160–163	+9.7 (0.4)	$C_{48}H_{94}NO_{11}P$	64.47 (64.62)	10.88 (10.62)	1.49 (1.57)
g	163–165	+12.5 (0.1)	$C_{50}H_{98}NO_{11}P$	65.50 (65.26)	10.89 (10.73)	1.27 (1.52)
h	166–168	+11.9 (0.3)	$C_{52}H_{102}NO_{11}P$	65.61 (65.86)	10.98 (10.84)	1.44 (1.48)
i	167–168	+5.7 (0.5)	$C_{44}H_{86}NO_{11}P$	63.50 (63.21)	10.54 (10.37)	1.60 (1.68)
j	166–167	+12.0 (0.3)	$C_{46}H_{90}NO_{11}P$	64.11 (63.93)	10.80 (10.50)	1.37 (1.62)
k	167–168	+12.0 (0.3)	$C_{48}H_{94}NO_{11}P$	64.83 (64.62)	10.81 (10.62)	1.29 (1.57)
l	168–170	+9.4 (0.1)	$C_{50}H_{98}NO_{11}P$	64.99 (65.26)	10.98 (10.73)	1.58 (1.52)
m	169–172	+13.1 (0.2)	$C_{52}H_{102}NO_{11}P$	66.10 (65.86)	10.62 (10.84)	1.20 (1.48)

7e. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-O-[(3RS)-3-undecylpentadecanoyl]-D-glucopyranose.*

7f. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-O-[(3RS)-3-undecylheptadecanoyl]-D-glucopyranose.*

7g. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-O-[(3RS)-3-undecylnonadecanoyl]-D-glucopyranose.*

7h. *2-Deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-3-O-[(3RS)-3-undecylheneicosanoyl]-D-glucopyranose.*

7i. *3-O-[(2RS)-2-Decyltetradecanoyl]-2-deoxy-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucopyranose..*

7j. *2-Deoxy-3-O-(2-dodecyltetradecanoyl)-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucopyranose.*

7k. *2-Deoxy-3-O-[(2RS)-2-dodecylhexadecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucopyranose.*

7l. *2-Deoxy-3-O-[(2RS)-2-dodecyloctadecanoyl]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucopyranose.*

7m. *2-Deoxy-3-O-[(2RS)-2-dodecyleicosanoyl]-2-[(3R)-3-hydroxytetradecanamido]-4-O-phosphono-D-glucopyranose.*

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