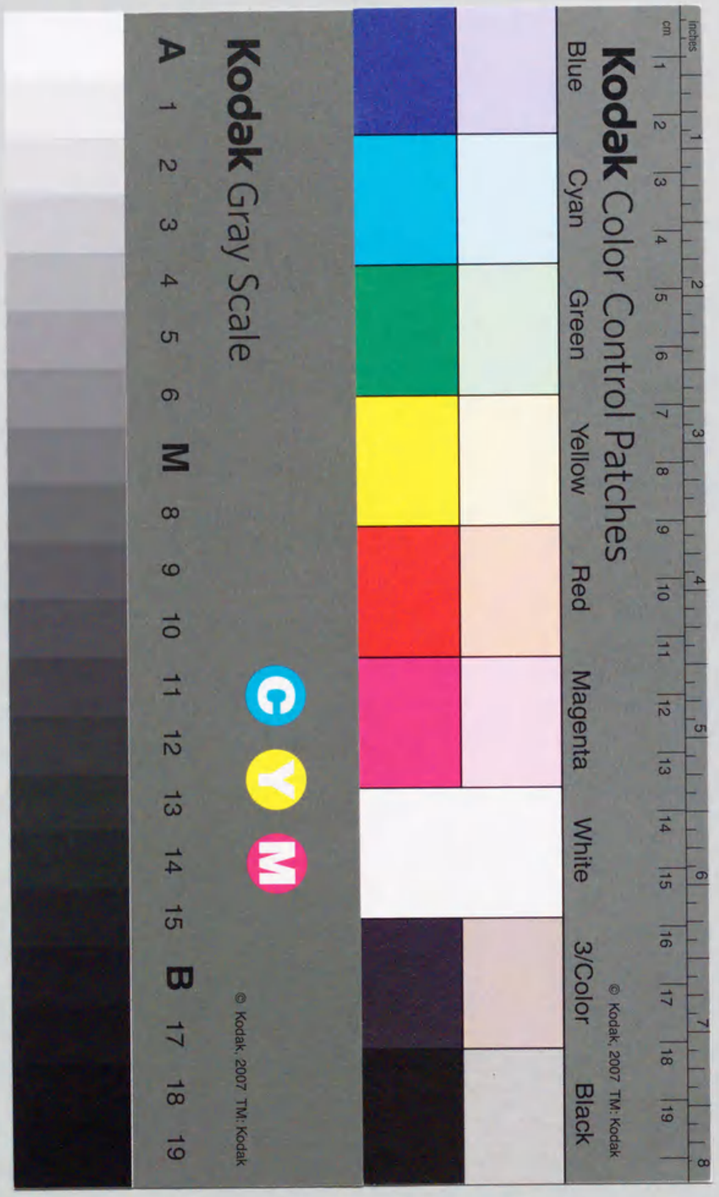

論 文 目 録

氏名　小川祐示　印

学位論文

題目　細菌内毒素成分リピドAの生物有機化学的研究と医学、生物学への応用

学位論文の基礎となる学術論文

題目　Synthesis of novel series of 4-*O*-phosphono-D-glucosamine derivatives (lipid A subunit analogs) carrying the C-branched 2-tetradecylhexadecanoyl group
著者名　Yuji Ogawa, Yushun Fujishima, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名　Carbohydrate Research　に発表
（巻・頁）　**220**, 155-164
発行年　1991

題目　Synthesis of 3-*O*-(alkyl-branched acly)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucose derivatives related to bacterial lipid A
著者名　Yuji Ogawa, Motoji Wakida, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名　Carbohydrate Research　に発表
（巻・頁）　**242**, 303-309
発行年　1993 年 4月

題目　Synthesis of novel nonreducing-sugar subunit analogs of lipid A carrying 2-acyloxytetradecanoyl and 2-hydroxyacyl groups of different carbon chain length
著者名　Yuji Ogawa, Motoji Wakida, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名　Journal of Carbohydrate Chemistry　に発表予定
（巻・頁）
発行年

既発表学術論文

題目	The chemical modification of the C-1 substituent of a 4-*O*-phosphono-D-glucosamine derivative (GLA-27) related to bacterial lipid A
著者名	Yuji Ogawa, Yushun Fujishima, Isako Konishi, Makoto Kiso, and Akira Hasegawa
学術雑誌名	Journal of Carbohydrate Chemistry　に発表
(巻・頁)	6, 399-410
発行年	1987年9月

題目	Synthesis and immunoadjuvant activity of the conjugates of 1-thio-*N*-acetyl-muramoyl dipeptide with lipid A susunit analogs
著者名	Yuji Ogawa, Masayuki Kitagawa, Yushun Fujishima, Makoto Kiso, Akira Hasegawa, Hideharu Ishida, and Ichiro Azuma
学術雑誌名	Agricultural and Biological Chemistry　に発表
(巻・頁)	53, 1025-1036
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題目	Synthesis of 3-*O*-[(3*R*)-3-acyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucose derivatives related to bacterial lipid A
著者名	Yuji Ogawa, Yushun Fujishima, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名	Carbohydrate Research　に発表
(巻・頁)	197, 281-288
発行年	1990

題目	Synthesis of 2-acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-phosphono-α-D-glucopyrsnose and the 4,6-cyclic phosphates as novel monosaccharidic lipid A analogs
著者名	Yuji Ogawa, Motoji Wakida, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名	Journal of Carbohydrate Chemistry　に発表予定
(巻・頁)	
発行年	

題目	Synthesis of 2-deoxy-2-[(3*R*)-3-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose derivatives related to bacterial lipid A
著者名	Yuji Ogawa, Yushun Fujishima, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名	Journal of Carbohydrate Chemistry　に発表
(巻・頁)	9, 643-652
発行年	1990年10月

題目	Synthesis of nonreducing-sugar subunit analogs of bacterial lipid A carrying the acyloxyacyl group of different carbon chain length
著者名	Yuji Ogawa, Motoji Wakida, Hideharu Ishida, Makoto Kiso, and Akira Hasegawa
学術雑誌名	Agricultural and Biological Chemistry　に発表
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Synthetic and biomedical studies on bacterial endotoxin lipid A

(細菌内毒素成分リピドAの生物有機化学的研究と医学・生物学への応用)

1993

The United Graduate School of Agricultural Science, Gifu University
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(2)

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CONTENTS

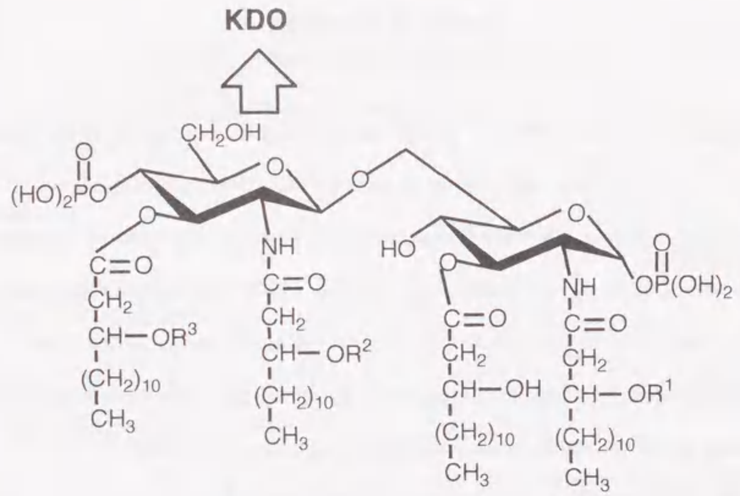
INTRODUCTION

Lipopolysaccharide (LPS),[1-5] which is an integral component of the outer membrane of gram-negative bacterial cells, expresses a variety of biological activities such as pyrogenicity, Sanarelli-Shwartzman reactivity, lethality, and mediator induction. This toxic principle is called "endotoxin". In the 1930s, immunopharmacological activities of endotoxin as non-specific protective and antitumor activities have been investigated and often submitted to clinical trial. However, these trials were unsuccessful on the basis of endotoxic activities such as pyrogenicity and lethal toxicity.

Otto Westphal and Otto Lüderitz first isolated[6] lipid A from protein-free LPS by using hot phenol-water solution. Lipid A constitutes the lipophilic part of LPS and shows most of endotoxic activities of LPS. Shiba and Kusumoto reported[7-9] that the chemical structure of lipid A of Re mutant *E. coli* consisted of two acylated glucosamines with β (1→6) glycoside linkage, containing phosphate groups at the C-1 and 4' positions, and 3-deoxy-D-manno-octulosonic acid (KDO)[10] at C-6' position. Takayama and Qureshi *et al.*[11,12], and Rietschel *et al.*[13] also reported a similar structure *Salmonella typhimurium* or *Proteus mirabilis* lipid A. Furthermore, Strain *et al.*[14] studied on LPS from mutant of *E. Coli* and Raetz *et al.*[15-17] supported the proporsal lipid A structure from his biosynthetic study of lipid A.

Nishijima and Raetz [18] isolated the called lipid X, the reducing-sugar subunit of lipid A, which is a biosynthetic lipid A precursor, and demonstrated that this showed most of the activities of lipid A. However, synthetic lipid X did not show any of lipid A's activity.[19-21]

According to the structure of natural lipid A, the synthetic[7-9,22,23] and biological[24-27] investigations were undertaken. As a result, it was proved that the



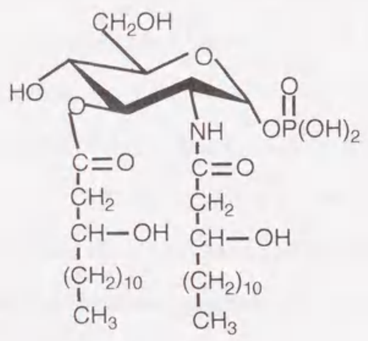
S. minuesota : $R^1 = C_{16}$, $R^2 = C_{12}$, $R^3 = C_{14}$

P. mirabilis : $R^1 = C_{16}$, $R^2 = R^3 = C_{14}$

E. coli : $R^1 = H$, $R^2 = C_{12}$, $R^3 = C_{14}$

$C_n = CH_3(CH_2)_{n-2}C(O)$-

Lipid A



Lipid X

Fig. 1 Structure of Lipid A and Lipid X

synthetic lipid A exhibited activities identical to those of natural *E. coli* lipid A in all the tests performed and in the various doses employed; it exhibited activities identical to those of natural lipid A in Shwartzman reaction, lethality, pyrogenicity, interferon (IFN)- and tumor necrosis factor (TNF)-inducing activities, as well as in polyclonal B-cell activation activity and Limulus amebocyte lysate gelation activity.

On the other hand, in the course of our synthetic approach[28-35] to clarify the molecular requirements for manifestation of biological activities of lipid A, we have found that a 4-*O*-phosphono-D-glucosamine derivative named **GLA-27**[35] (2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-[(3*R*)-3-tetradecanoyloxytetradecanamido]-D-glucopyranose) showed some distinct biological activities, such as gelating activity of Limulus amebocyte lysate, IFN- and TNF-inducing activity, and mitogenic and B-cell activation activity.[36,37] This compound, however, did not show significant pyrogenic activity, in contrast to lipid A (Table 1). This result indicates that it might be able to disassociate the desired biological activities from the toxicity. To obtain the further information on the structure-activity relationship of lipid A, a variety of monosaccharide analogs related to **GLA-27** were synthesized[38,39] (shown in Fig. 3) and investigated the biological activities.[40-45]

GLA-34 which is eliminated 3-*O*-acyl group of **GLA-27,** and **GLA-45** in which the 3-*O*-acyl group is transacylated to *O*-6 abolished any biological activities. The dephosphorylated (**GLA-25**) and 6-*O*-phosphono (**GLA-35**) derivatives showed much weaker activities than **GLA-27**. These results clearly indicate that the introduction of acyl and acyloxyacyl groups at C-2 and 3, and of phosphono group at C-4 of the D-glucosamine skeleton, are critical for expression of various biological activities.

To investigate the effect of backbone structure for biological activities, a number of carbohydrate analogs, such as 1-deoxy-glucosamine (**GLA-40**), 1-thioacetyl-

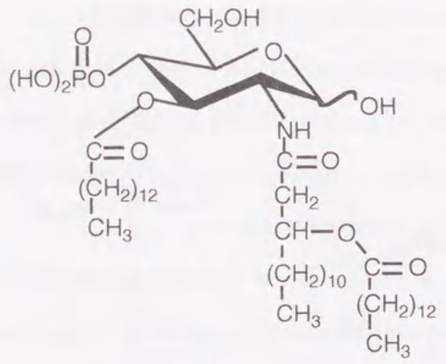
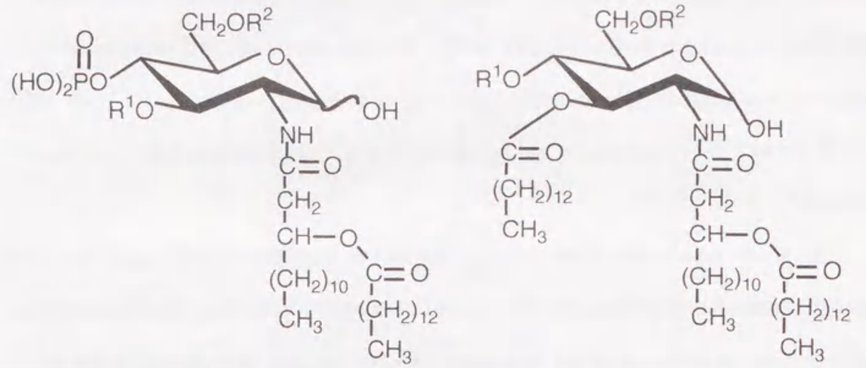


Fig. 2 GLA-27

Table 1 : Biological activities of GLA-27 and Lipid A

	GLA-27	Lipid A
Pyrogenicity		
Minimum pyrogenic dose (μg)	>10	0.001
Lethality in galactosamine-sensitized mice (LD_{50} μg)	0.54	0.0078
Shwartzman reaction		
Minimum preparatory dose (μg)	>50	1.25
TNF-inducing activity (ED_{50} μg)	3.0	0.1
IFN-inducing activity (ED_{50} μg)	0.1	0.001
Mitogenicity (SI)*	11.5	30.6
Macrophage activation		
phagocytosis (SI)*	2.6	3.5
Adjuvant activity (SI)*	3.4	3.2

*SI (Stimulation Index) : relative value to contorol.

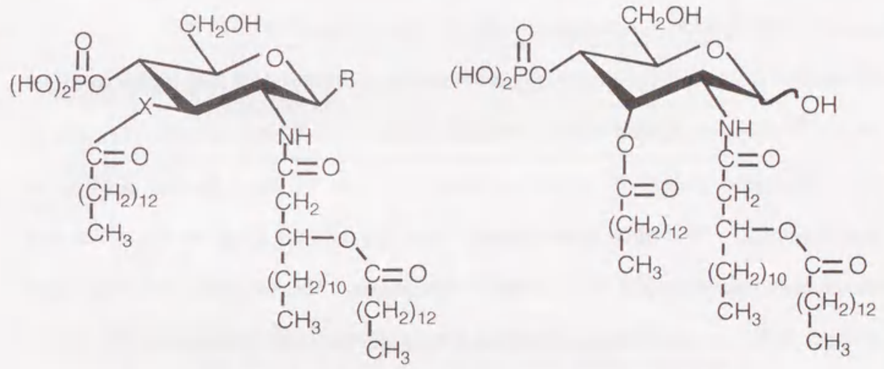


GLA-34 $R^1 = R^2 = H$

GLA-45 $R^1 = H$, $R^2 = C_{14}$

GLA-25 $R^1 = R^2 = H$,

GLA-35 $R^1 = H$, $R^2 = (HO)_2P(O)$



GLA-40 R = H, X = O

GLA-65 R = SAc, X = O

GLA-66 R = SH, X = O

GLA-48 R = OH (α,β), X = NH

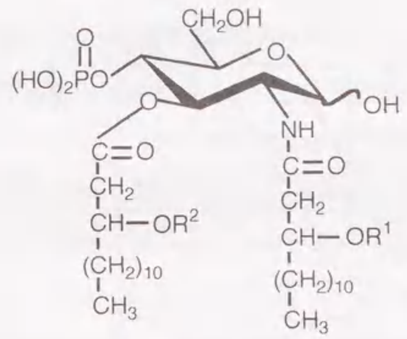
GLA-43

Fig. 3 Homologs of GLA-27

glucosamine (**GLA-65**), 1-thiol-glucosamine (**GLA-66**), 2,3-diamino-glucose (**GLA-48**), and allossamine derivative (**GLA-43**), were synthesized. All these synthetic compounds exhibited the immunopharmacological activities, especially, **GLA-40, 65,** and **66** showed B-cell activation and mitogenic activities in a similar order as those of **GLA-27**.

The above results show that the alteration of the acyl groups of **GLA-27** affects biological activities more than the alteration of the carbohydrate backbone structure. In the course of investigation of the biological effect of the fatty acyl groups in the 4-*O*-phosphono-D-glucosamine backbone, it was found[46-48] that **GLA-59** and **60** carrying 3-hydroxytetradecanoyl and 3-tetradecanoyloxytetradecanoyl groups at C-2 and 3 positions exhibited the marked immunopharmacological activities such as mitogenic, IFN-inducing, and polyclonal B cell-activation activities. Especially, **GLA-60** (2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetra-decanoyl]-D-glucopyranose) expressed 10 fold higher antiviral activity[50] than that of **GLA-27** and 1000 fold higher than *N*-acetylmuramoyl-L-alanyl-D-isoglutamine (MDP)[58] which is active principle of the adjuvant activity of peptidoglycan. We have demonstrated that the combination of **GLA-60** and recombinant interferon-γ (rIFN-γ) produced endogenous TNF more effectively than those of either rIFN-γ or **GLA-60** alone and may inhibit the lung tumor metastases.[53]

Since the lipid As of many Gram-negative bacteria, *e.g. Salmonella* species and *Escherichia coli*, have (3*R*)-3-hydroxytetradecanoic acid as a common and prominent constituent, the asymmetric carbon atom of the 3-hydroxytetradecanoyl group seems to be important for the biological activity. The stereoisomeric compounds (**GLA-27, 40, 59**, and **60**) composed of (3*R*)- and (3*S*)- configuration of 3-hydroxytetradecanoyl group were synthesized[46,59,60] and compared for their immunopharma-



GLA-59 $R^1 = C_{14}$, $R^2 = H$
GLA-60 $R^1 = H$, $R^2 = C_{14}$

Fig. 4 Structure of GLA-59 and 60

Table 2 : IFN-inducing activity of GLA-60

compound[a]	IFN titer (IU/0.1 ml)[b]	
	Exp. 1	Exp.2
MDP	<10	<10
GLA-27	640	320
GLA-60	1280	640
lipid A	2560	1280

a : Ten μg of test samples were injected intravenously into P. acnes-primed mice.

b : Serum IFN titers were assayed in L-929 cells with vesicular stomatitis virus as challenge virus. The IFN titer represents the reciprocal of the serum dilution giving a 50% reduction of the virus-induced cytopathogenicity.

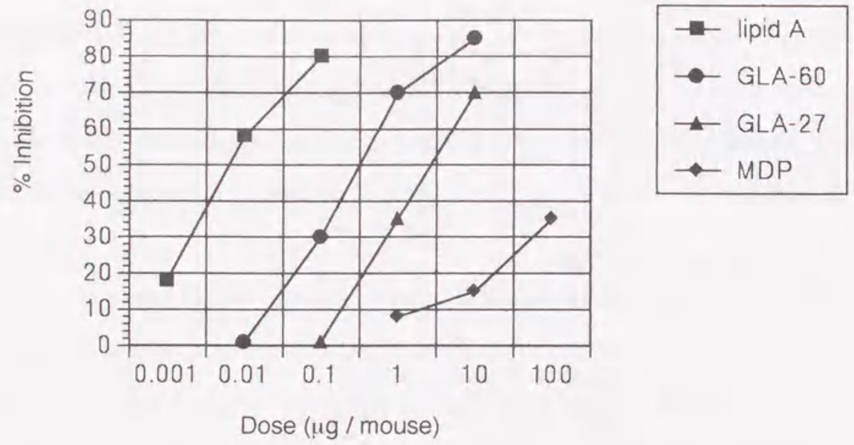


Fig. 5 Comparison of antiviral activity (against *vaccinia virus*) of GLA-27 and 60 with lipid A and MDP.

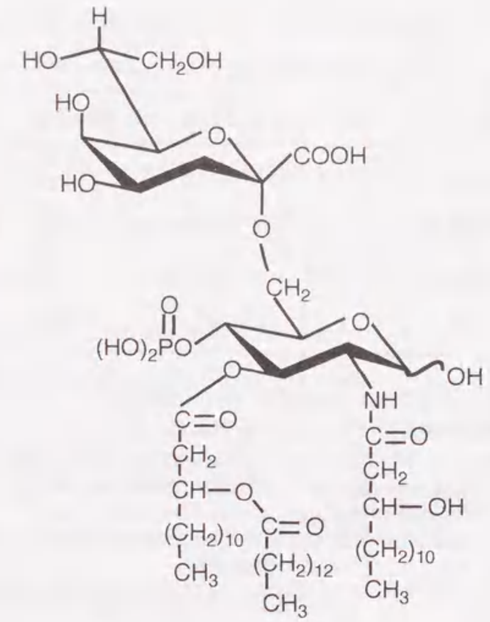


Fig. 6 KDO-(2→6)-GLA-60

cological activities.[49] Among stereoisomers of **GLA-59** and **60**, the compound with the *R* configuration elicited stronger activities *in vitro* than *S* isomers. However, *S* isomers showed biological activities as well as *R* isomers *in vivo*.

The inner-core region of bacterial LPS consists in part of an α-(2→4)-linked disaccharide KDO,[10] which is attached to *O*-6' of the *O*-(β-D-glucosaminyl)-(1→6)-D-glucosamine backbone of lipid A. The biological roles of KDO in LPS were as yet unclear, although its immunological importance has often been suggested.[61] To investigate the effect of KDO for the expression of biological activities, α-KDO-(2→6)-(4-*O*-phosphono-D-glucosamine derivatives) were synthesized[62-64] (Fig. 6) and tested for immunopharmacological activities[65] such as mitogenicity, adjuvanticity and mediator-inducing activity. It was found that KDO linked compounds did not enhance the biological activities as compared to those of the corresponding analogs without KDO. KDO-lipid A subunit analogs (named A 301-305) were also synthesized and tested a biological activities by Achiwa and Shimizu *et al*. They reported[66] that addition of KDO did not affect the biological activities. These results strongly suggest that the endotoxic activities of lipid A are affected by the constitution of the acyl and phosphono groups.

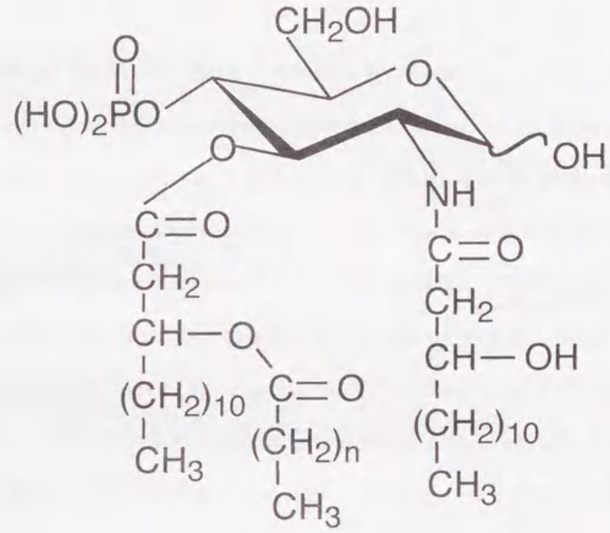
In view of these points, more study is required for the elucidation of relationship between the structure and the biological activity of lipid A analogs. In this study, a variety of non-reducing sugar subunit analogs of lipid A have been synthesized by selecting **GLA-60** as a leading compound, and their biological activities were tested. Author describes here the systematic syntheses of **GLA-60** analogs containing the different carbon chain fatty acyl groups including odd-numbered carbon-chain acids and of the derivatives carrying 2-hydroxytetradecanoyl or alkyl-branched acyl groups, together with the results of biological assays. Novel series of monosaccharidic lipid A analogs carrying three fatty acyl groups at C-1, 2, and 3, and the 4,6-cyclic phosphate analogs have also been synthesized.

CHAPTER 1

GLA-60 ANALOGS CARRYING THE FATTY ACYL GROUPS OF DIFFERENT CARBON CHAIN LENGTH

As described in this introduction, in the course of a synthetic approach to clarify the molecular requirements for manifestation of the biological activities of lipid A, it was found that GLA-60 (2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose) showed some distinct biological activities, such as *Limulus* amebocyte-lysate gelation, interferon- and tumor necrosis factor (TNF)-induction, and mitogenic and polyclonal B cell-activation activities. However, GLA-60 did not exhibit significant pyrogenic activity, in contrast to lipid A, suggesting that it might be possible to disassociate the desired biological activities from the unexpected toxicity. Recent studies[48] on the structure-activity relationship of a variety of monosaccharide analogs related to GLA-60 showed that the chain lengths of the 3-*O*-[(3*R*)-3-acyloxytetradecanoyl] group at C-3 were very important for expressing the biological activities.[52] In the series of 3-*O*-acyloxytetradecanoyl derivatives (Fig. 7), GLA-63 which has the 3-*O*-linked (3*R*)-3-dodecanoyloxytetradecanoyl group was the most beneficial compound possessing the strong immunomodulating activities. On the contrary, GLA-64 carrying (3*R*)-3-hexadecanoyloxytetradecanoyl group at *O*-3 in the sugar moiety, did not show any significant immunopharmacological activities, strongly suggesting the critical importance of the chain length of the fatty acyl groups.

In this chapter, to obtain further information on the effect of carbon chain length for immunopharmacological activity, the syntheses of a series of GLA-60 derivatives carrying the fatty acyl groups of different carbon chain length, *i.e.* 2-deoxy-2-[(3*R*)-3-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose derivatives (**14-16**)[67] and 3-*O*-acyloxyacyl-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranose derivatives (**74-91**)[68,69]



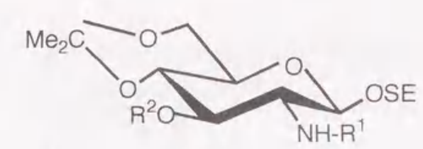
	n
GLA-63	10
GLA-60	12
GLA-64	14

Fig. 7 Structure of GLA analogs

which carry the acyl groups including odd-numbered acids, and their biological activities are described.

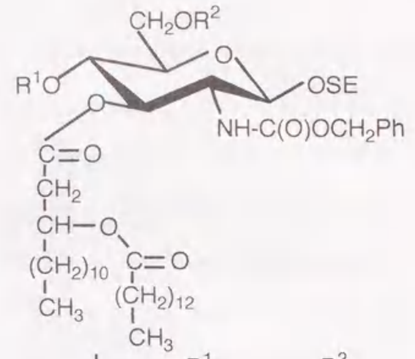
1-1. Synthesis and biological activity of 2-deoxy-2-[(3*R*)-3-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranoses

Treatment of 2-(trimethylsilyl)ethyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**)[70] with benzyloxycarbonyl chloride gave 2-(benzyloxycarbonyl)-amino derivative (**2**) in 91% yield. Compound **2** was esterified with (3*R*)-3-tetradecanoyloxytetradecanoic acid[46] and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in the presence of 4-dimethylaminopyridine (DMAP) to afford **3** in 86% yield. Hydrolytic removal of the isopropylidene group with aqueous acetic acid at 50 °C gave **4** in 91% yield. Compound **4** was treated with *tert*.-butyldimethylsilyl (TBDMS) chloride in pyridine to yield compound **5** in 98% yield. Phosphorylation at *O*-4 of **5** with diphenyl phosphorochloridate gave syrupy **6** in 93% yield. The benzyloxycarbonyl group of **6** was cleaved by hydrogenolysis with 10%-palladium on carbon to give the 2-amino derivative (**7**) in good yield. Because of its instability, compound **7** was immediately treated with (3*R*)-3-hydroxydecanoic acid, (3*R*)-3-hydroxydodecanoic acid, and (3*R*)-3-hydroxyhexadecanoic acid in the presence of WSC to give the corresponding compounds **8**, **9**, and **10** in 70-73% yields. The 2-(trimethylsilyl)ethyl and *tert* -butyldimethylsilyl groups of **8-10** were simultaneously hydrolyzed with boron trifluoride etherate to give **11-13** in 85-95% yields. These compounds could be assigned the α-D-glucopyranose form based on their ^{1}H-NMR data. Finally, hydrogenolytic deprotection of the phenyl groups from **11-13** with platinum catalyst afforded the desired compounds **14-16** in high yields. These

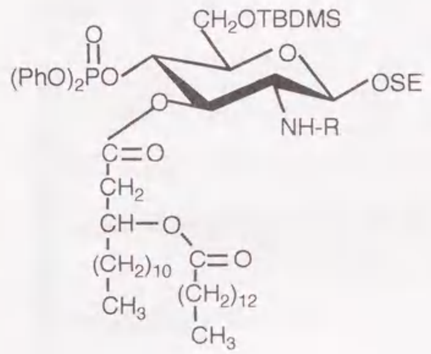


	R^1	R^2
1	H	H
2	$PhCH_2OC(O)$	H
3	$PhCH_2OC(O)$	C_{14}-O-C_{14}

SE = $Me_3SiCH_2CH_2$



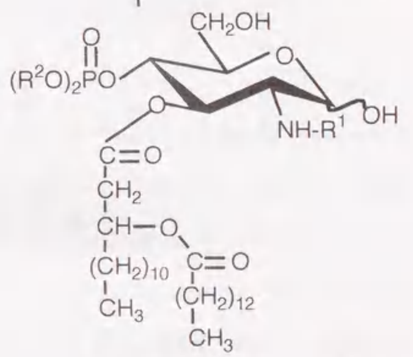
	R^1	R^2
4	H	H
5	H	TBDMS
6	$(PhO)_2P(O)$	TBDMS



	R
7	H
8	C_{10}-OH
9	C_{12}-OH
10	C_{14}-OH

TBDMS = *t*-$BuMe_2Si$

C_{14}-O-C_{14} = $CH_3(CH_2)_{10}CHCH_2C(O)$
O
$CH_3(CH_2)_{12}C(O)$



	R^1	R^2
11	C_{10}-OH	Ph
12	C_{12}-OH	Ph
13	C_{16}-OH	Ph
14	C_{10}-OH	H
15	C_{12}-OH	H
16	C_{16}-OH	H

C_n = $CH_3(CH_2)_{n-4}CHCH_2C(O)$
OH

Fig. 8

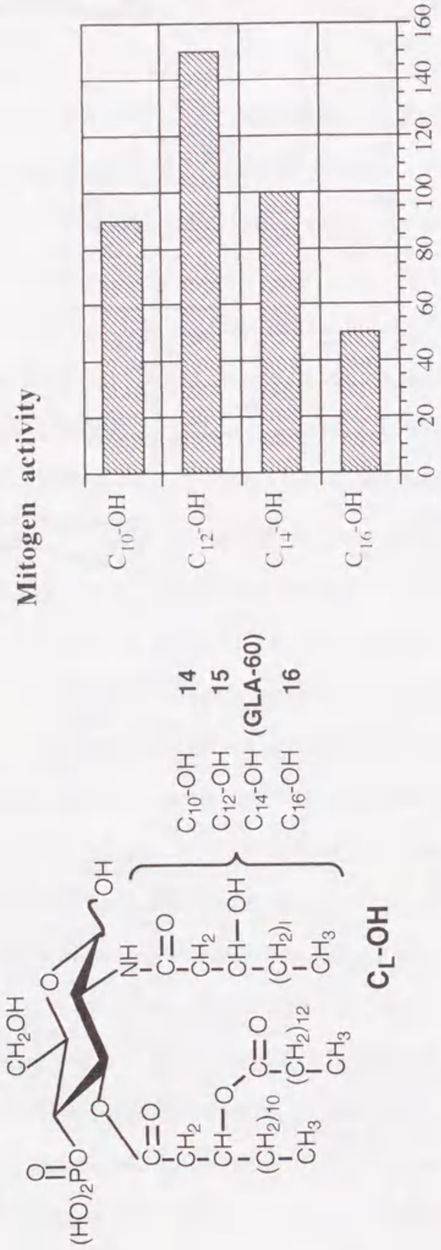


Fig. 9 Mitogenic activity of compounds 14-16

compounds gave positive test for phosphate group using the phosphomolybdate spray reagent.[71]

With regard to mitogenic activity, compound **15** carrying a (3*R*)-3-hydroxydodecanoyl group at C-2 was the most active in the series of 2-deoxy-2-[(3*R*)-3-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose derivatives (**14-16**). However, compound **16** showed weaker activity than that of GLA-60 (Fig. 9).

Experimental

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

Biological activities were assessed by the methods as described previously (see ref. 49 and 50).

2-(Trimethylsilyl)ethyl 2-(Benzyloxycarbonyl)amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (2). To a mixture of 2-(trimethylsilyl)ethyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**, 1.7 g), dichloromethane (50 mL), and M sodium hydrogen carbonate (50 mL) was added benzyloxycarbonyl chloride (2 g). The mixture was stirred vigorously for 2 h at room temperature. The organic layer was separated and washed with water, dried (sodium

sulfate) and concentrated. The residue was chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to give **2** (2.25 g, 91%) as an amorphous solid: mp 111-112 °C, $[\alpha]_D$ -30.9° (*c* 1.3, dichloromethane): IR (film) 3300 (OH, NH), 2950, 2900 (CH), 1700, 1550 (amide), 850, 830 (Me_2C, Si-C) and 750-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 9H, Me_3Si), 0.9 (m, 2H, Me_3SiCH_2), 1.43, 1.49 (2s, 6H, Me_2C), 3.25 (m, 1H, H-5), 3.3-4.0 (m, 8H, H-1, 2, 3, 4, 6, and $Me_3SiCH_2CH_2$), 5.11 (s, 2H, $PhCH_2$), and 7.33 (m, 5H, Ph).

Anal. Calcd for $C_{22}H_{35}NO_7Si$ (453.61): C, 58.25; H, 7.78; N, 3.09. Found: C, 58.43; H, 7.89; N, 3.13.

2-(Trimethylsilyl)ethyl 2-(Benzyloxycarbonyl)amino-2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (3). To a solution of **2** (2.25 g) in dichloromethane (30 mL) were added (3*R*)-3-tetradecanoyloxytetradecanoic acid (2.93 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 1.9 g) 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 500:1 dichloromethane-methanol to afford syrupy **3** (3.8 g, 86%): $[\alpha]_D$ -8.5° (*c* 0.92, dichloromethane); IR (film) 3400 (NH), 2930, 2850 (CH), 1740 (ester), 1700, 1640 (amide), 860, 840 (Si-C), and 760-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 9H, Me_3Si) 0.8-1.0 (m, 8H, Me_3SiCH_2 and Me), 1.0-1.8 (m, 42H, $-CH_2-$), 1.37, 1.47 (2s, 6H, Me_2C), 2.2-2.6 (m, 4H, $-COCH_2-$), 3.15-4.0 (m, 8H, H-1, 2, 4, 5, 6 and $Me_3SiCH_2CH_2$) 4.67 (broad s, 1H, NH), 5.0-5.3 (m, 4H, $PhCH_2$, H-3 of C_{14}-O-C_{14}, and H-3), 7.3-7.5 (m, 5H, Ph).

Anal. Calcd for $C_{50}H_{87}NO_{10}Si$ (890.33): C, 67.45; H, 9.85; N, 1.57. Found : C, 67.32; H, 9.68; N, 1.46.

2-(Trimethylsilyl)ethyl 2-(Benzyloxycarbonyl)amino-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (4). A mixture of **3** (3.8 g) and 80% aqueous acetic acid (50 mL) was stirred for 1 h at 50 °C. The mixture was concentrated and then chromatographed on a column of silica gel with 150:1 dichloromethane-methanol to obtain **4** (3.3 g, 91%), which was lyophilized from 1,4-dioxane: mp 47-49 °C, $[\alpha]_D$ -8.6° (*c* 0.89, dichloromethane); IR (film) 3450 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1700, 1540 (amide), 860, 830 (Si-C), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 9H, Me_3Si), 0.88 (m, 8H, Me_3SiCH_2 and Me), 1.1-1.7 (m, 42H, $-CH_2-$), 2.1-2.6 (m, 4H, $-COCH_2-$), 2.65 (broad s, 2H, OH), 3.4-3.6 (m, 3H, $Me_3SiCH_2CH_2$ and H-5), 3.62 (t, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.82 (dd, 1H, $J_{gem} = 12$ Hz, $J_{5,6a} = 4.4$ Hz, H-6a), 3.92 (dd, 1H, $J_{gem} = 12$ Hz, $J_{5,6b} = 3.3$ Hz, H-6b), 3.95 (m, 1H, H-2), 5.04 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 5.1 (m, 4H, $PhCH_2$, H-3 of C_{14}-O-C_{14}, and H-3), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd for $C_{47}H_{83}NO_{10}Si$ (850.26): C, 66.39; H, 9.84; N, 1.65. Found: C, 66.42; H, 10.01; N, 1.85.

2-(Trimethylsilyl)ethyl 2-(Benzyloxycarbonyl)amino-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (5). To a solution of **4** (2 g) in pyridine (40 mL) was added *tert*-butyldimethylsilyl chloride (0.53 g). The mixture was stirred overnight at room temperature. Methanol was added to decompose the excess reagent and the solvents were evaporated. The residue was extracted with dichloromethane. The extract was washed with 2M hydrochloric acid and water, dried (sodium sulfate) and concentrated. The residual syrup was chromatographed on a column of silica gel with 400:1 dichloromethane-methanol to give **5** (2.4 g, 98%) as a syrup : $[\alpha]_D$ -10.0° (*c* 1.0, dichloromethane); IR (film) 3500 (OH), 3350 (NH), 1740 (ester), 1700, 1540 (amide), 830 (Si-C), and 780-680 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 15H, MeSi), 0.8-1.0 (m,

17H, Me_3SiCH_2, *tert*-Bu, and Me), 1.1-1.7 (m, 42H, $-CH_2-$), 2.15-2.65 (m, 4H, $-COCH_2-$), 3.3-3.6 (m, 4H, $Me_3SiCH_2CH_2$, H-5, and OH), 3.63 (t, 1H, $J_{3,4} = J_{4,5} =$ 9.2 Hz, H-4), 3.85-4.0 (m, 3H, H-2 and 6), 4.98 (d, 1H, $J_{1,2} = 9.2$ Hz, H-1), 5.0-5.2 (m, 4H, $PhCH_2$, H-3 of C_{14}-O-C_{14}, and H-3), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd for $C_{53}H_{97}NO_{10}Si$ (964.53): C, 66.00; H, 10.14; N, 1.45. Found: C, 66.05; H, 10.36; N, 1.23.

2-(Trimethylsilyl)ethyl 2-(Benzyloxycarbonyl)amino-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (6). To a cooled and stirred mixture of **5** (2.2 g), DMAP (0.38 g), and pyridine (10 mL) were added diphenyl phosphorochloridate (1.7 g) and dichloromethane (10 mL); stirring was continued overnight at room temperature. Methanol was added and the solvents were evaporated. The residue was chromatographed on a column of silica gel with 8:1 hexane-ethyl acetate to give syrupy **6** (2.5 g, 93%); $[\alpha]_D$ +2.9° (*c* 0.96, dichloromethane); IR (film) 3350 (NH), 2940, 2850 (CH), 1740 (ester), 1700, 1540 (amide), 960 (P-O-Ph), 840 (Si-C), and 780-680 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 15H, MeSi), 0.8-1.0 (m, 17H, $MeSiCH_2$, *tert*-Bu, and Me), 1.1-1.7 (m, 42H, $-CH_2-$), 2.0-2.5 (m, 4H, $-COCH_2-$), 3.2-4.0 (m, 6H, $Me_3SiCH_2CH_2$, H-2, 5, and 6), 4.61 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.2$ Hz, H-4), 4.85-5.8 (m, 6H, $PhCH_2$, H-3 of C_{14}-O-C_{14}, H-1, 3, and NH), and 7.1-7.4 (m, 15H, Ph).

Anal. Calcd for $C_{65}H_{106}NO_{13}PSi_2$ (1196.70): C, 65.24; H, 8.93; N, 1.17. Found: C, 65.45; H, 8.89; N, 1.26.

2-(Trimethylsilyl)ethyl 2-Amino-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (7). To a solution of **6** (1 g) in methanol (50 mL) was added 10%-palladium on carbon (0.5 g), and the mixture was stirred for 1 h in a hydrogen atmosphere. The catalyst was filtered off, and washed with methanol. The

filtrate and washings were combined and concentrated to give **7** as a syrup (0.89 g, 98%): $[\alpha]_D$ -2.5° (*c* 0.4, dichloromethane): IR (film) 2930, 2850 (CH), 1740 (ester), 1600 (amino), 950 (P-O-Ph), 840 (Si-C), and 780-680 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 15H, Me-Si), 0.8-1.0 (m, 17H, Me_3SiCH_2, *tert*-Bu, and Me), 1.1-1.8 (m, 42H, $-CH_2-$), 2.0-2.5 (m, 4H, $-COCH_2-$), 2.9 (m, 1H, H-2), 3.1-4.0 (m, 5H, H-5, 6, and $Me_3SiCH_2CH_2$), 4.21 (d, 1H, $J_{1,2} = 8$ Hz, H-1), 4.59 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P} = 9$ Hz, H-4), 5.0-5.1 (m, 2H, H-3 of C_{14}-O-C_{14} and H-3), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{57}H_{100}NO_{11}PSi_2$ (1062.57): C, 64.43; H, 9.49; N, 1.32. Found: C, 64.34; H, 9.76; N, 1.25.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxydecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (8). A mixture of **7** (0.25 g), (3*R*)-3-hydroxydecanoic acid (0.22 g), and WSC (0.2 g) in dichloromethane (10 mL) was stirred overnight at room temperature. The reaction mixture was directly chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to afford **8** (0.2 g, 70%) as a syrup: $[\alpha]_D$ -3.6° (*c* 0.44, dichloromethane); IR (film) 3500 (OH), 3300 (NH), 1740 (ester), 1660, 1550 (amide), 960 (P-O-Ph), 840 (Si-C), and 780-680 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (s, 15H, MeSi), 0.8-1.0 (m, 20H, Me_3SiCH_2, *tert*-Bu, and Me), 1.1-1.6 (m, 54H, $-CH_2-$), 2.0-2.7 (m, 7H, OH and $-COCH_2-$), 3.4-4.0 (m, 7H, $Me_3SiCH_2CH_2$, H-3 of C_{10}-OH, H-2, 5, and 6), 4.60 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.2$ Hz, H-4), 4.99 (d, 1H, $J_{1,2} = 8.4$ Hz, H-1), 5.13 (t, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3), 6.42 (d, 1H, J = 7.3 Hz, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{67}H_{118}N0_{13}PSi_2$ (1232.82): C, 65.28; H, 9.65, N, 1.14. Found: C, 65.02; H 9.60; N, 1.02.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxydodecanamido]-3-*O*-[(3*R*)-3-tetra-

decanoyloxytetradecanoyl]-β-D-glucopyranoside (9) and 2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxyhexadecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (10). Compounds **9** and **10** were obtained by acylation of **7** with (3*R*)-3-hydroxydodecanoic acid and (3*R*)-3-hydroxyhexadecanoic acid, respectively, in 70% and 73% yields, according to the method described for **8**. IR and ^{1}H NMR data were similar to those of **8** except for the number of methylene protons at δ 1.1-1.6 ppm.

Compound **9** had $[\alpha]_D$ -5.5° (*c* 0.4, dichloromethane).

Anal. Calcd for $C_{69}H_{122}NO_{13}PSi_2$ (1260.87): C, 65.73; H, 9.75; N, 1.11. Found: C, 65.97; H, 9.64; N, 1.04.

Compound **10** had $[\alpha]_D$ -3.5° (*c* 0.46, dichloromethane).

Anal. Cacld for $C_{73}H_{130}NO_{13}PSi_2$ (1316.98): C, 66.58; H, 9.95; N, 1.06. Found: C, 66.53; H, 10.01; N, 1.12.

2-Deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxydecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (11). To a solution of **8** (0.1 g) in dichloromethane (10 mL) was added boron trifluoride etherate (0.5 mL) at 0 °C. The mixture was stirred for 1 h at the same temperature. The mixture was washed with saturated sodium hydrogen carbonate and water, dried (sodium sulfate) and concentrated. The residue was chromatographed on a column of silica gel with 50:1 dichloromethane-methanol to give **11** (0.75 g, 89%) which was lyophilized from 1,4-dioxane solution: mp 121-122 °C, $[\alpha]_D$ +5.6° (*c* 1.0, dichloromethane); IR (KBr) 3350 (OH, NH), 1740 (ester), 1650, 1550 (amide), 960 (P-O-Ph), and 780-680 cm^{-1}(Ph); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 9H, Me), 1.0-1.7 (m, 54H, -CH_2-), 2.0-2.5 (m, 6H, -$COCH_2$-), 3.0-4.2 (m, 7H, H-3 of C_{10}-OH, H-5, 6, and OH), 4.21 (m, 1H, H-2), 4.71 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.5 Hz, H-4), 5.10 (m, 1H, H-3 of C_{14}-O-C_{14}),

5.28 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 5.48 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 6.54 (d, 1H, J = 8.8 Hz, NH), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{56}H_{92}NO_{13}P$ (1018.32): C, 66.05; H, 9.11; N, 1.38. Found: C, 65.78; H, 8.96; N, 1.64.

2-Deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxydodecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (12) and 2-Deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxyhexadecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (13). Cleavage of the silyl groups of **9** and **10** as described for **11**, gave **12** (85%) and 13 (95%) respectively. IR and ^{1}H NMR spectra were consistent with the structures assigned.

Compound **12** had mp 117-119 °C, $[\alpha]_D$ +12.6° (*c* 0.87, dichloromethane).

Anal. Calcd for $C_{58}H_{96}NO_{13}P$ (1046.37): C, 66.58; H, 9.25; N, 1.34. Found: C, 66.75; H, 9.40; N, 1.56.

Compound **13** had mp 116-118 °C, $[\alpha]_D$ +5.8° (*c* 1.29, dichloromethane).

Anal. Calcd for $C_{62}H_{104}NO_{13}P$ (1102.48): C, 67.55; H, 9.51; N, 1.27. Found: C, 67.61; H, 9.26; N, 1.49.

2-Deoxy-2-[(3*R*)-3-hydroxydecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (14). To a solution of **11** (75 mg) in ethanol (60 mL) was added Adams' platinum catalyst (80 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off and washed with ethanol. The filtrate and washings were combined and concentrated to afford **14** (64 mg, quantitative) which was lyophilized from 1,4-dioxane suspension. It was positive to the specific spray-reagent for the phosphono group: mp 157-159 °C, $[\alpha]_D$ +14.3° (*c* 0.14, 3:1 chloroform-methanol); IR (KBr) 3400 (OH, NH), 2940, 2850 (CH), 1720 (ester), and 1640, 1550 cm^{-1}(amide).

Anal. Calcd for $C_{44}H_{84}NO_{13}P$ (866.12): C, 61.02; H, 9.78; N, 1.62. Found: C, 61.30; H, 9.90; N, 1.57.

2-Deoxy-2-[(3*R*)-3-hydroxydodecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (15) and 2-Deoxy-2-[(3*R*)-3-hydroxyhexadecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (16). Compounds **12** and **13** were hydrogenolyzed as described for **11**, to afford the corresponding **15** and **16**.

Compound **15** was obtained in 95% yield: mp 158-159 °C, $[\alpha]_D$ +10.0° (*c* 0.1, 3:1 chloroform-methanol); IR data was similar to that of **11**.

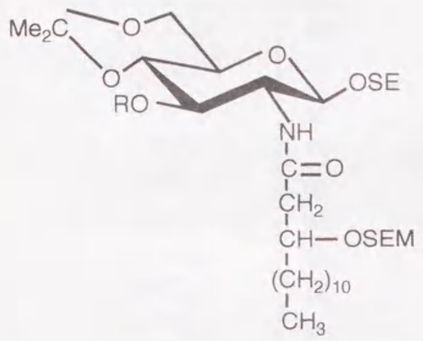
Anal. Calcd for $C_{46}H_{88}NO_{13}P$ (894.18): C, 61.79; H, 9.92; N, 1.57. Found: C, 61.71; H, 9.68; N, 1.56.

Compound **16** was obtained in 91% yield: mp 160 °C, $[\alpha]_D$ +19.6° (*c* 0.15, 3:1 chloroform-methanol); IR spectra was similar to that of **11**.

Anal. Calcd for $C_{50}H_{96}NO_{13}P$ (950.29): C, 63.20; H, 10.18; N, 1.47. Found: C, 63.36; H, 10.33; N, 1.50.

1-2. Synthesis and biological activity of 3-*O*-[(3*RS*)-3-acyloxyacyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranoses

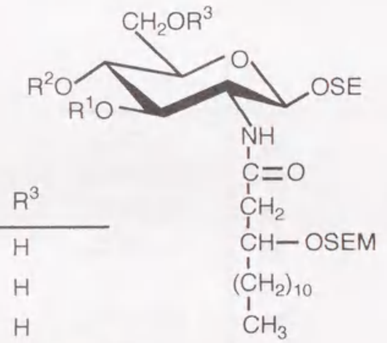
Treatment of 2-(trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (**17**)[70] with (3*RS*)-3-(benzyloxymethoxy)decanoic acid, (3*RS*)-3-(benzyloxymethoxy)dodecanoic acid, (3*R*)-3-(benzyloxymethoxy)tetradecanoic acid, or (3*RS*)-3-(benzyloxymethoxy)hexadecanoic acid, which were prepared *via* the phenacyl esters



SEM = $Me_3SiCH_2CH_2OCH_2$

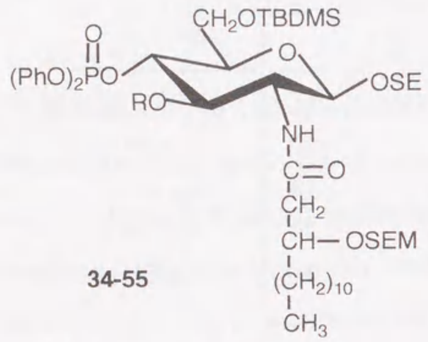
	R
17	H
18	C_{10}-OH
19	C_{12}-OH
20	C_{14}-OH
21	C_{16}-OH

C_m-OBOM = $CH_3(CH_2)_{m-4}CHCH_2C(O)$
O
$PhCH_2OCH_2$



	R^1	R^2	R^3
22	C_{10}-OBOM	H	H
23	C_{12}-OBOM	H	H
24	C_{14}-OBOM	H	H
25	C_{16}-OBOM	H	H
26	C_{10}-OBOM	H	TBDMS
27	C_{12}-OBOM	H	TBDMS
28	C_{14}-OBOM	H	TBDMS
29	C_{16}-OBOM	H	TBDMS
30	C_{10}-OBOM	$(PhO)_2P(O)$	TBDMS
31	C_{12}-OBOM	$(PhO)_2P(O)$	TBDMS
32	C_{14}-OBOM	$(PhO)_2P(O)$	TBDMS
33	C_{16}-OBOM	$(PhO)_2P(O)$	TBDMS

Fig. 10

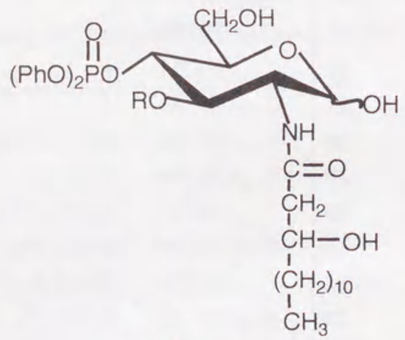


34-55

C_m-O-C_n = $CH_3(CH_2)_{m-4}CHCH_2C(O)$
O
$CH_3(CH_2)_{n-2}C(O)$

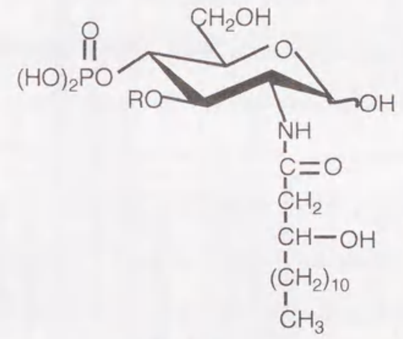
	R
34	C_{10}-OH
35	C_{12}-OH
36	C_{14}-OH
37	C_{16}-OH
38	C_{10}-O-C_{10}
39	C_{10}-O-C_{12}
40	C_{10}-O-C_{14}
41	C_{10}-O-C_{16}
42	C_{12}-O-C_{10}
43	C_{12}-O-C_{12}
44	C_{12}-O-C_{14}
45	C_{12}-O-C_{16}
46	C_{14}-O-C_2
47	C_{14}-O-C_8
48	C_{14}-O-C_{10}
49	C_{14}-O-C_{11}
50	C_{14}-O-C_{13}
51	C_{14}-O-C_{15}
52	C_{16}-O-C_{10}
53	C_{16}-O-C_{12}
54	C_{16}-O-C_{14}
55	C_{16}-O-C_{16}

	R
56	C_{10}-O-C_{10}
57	C_{10}-O-C_{12}
58	C_{10}-O-C_{14}
59	C_{10}-O-C_{16}
60	C_{12}-O-C_{10}
61	C_{12}-O-C_{12}
62	C_{12}-O-C_{14}
63	C_{12}-O-C_{16}
64	C_{14}-O-C_2
65	C_{14}-O-C_8
66	C_{14}-O-C_{10}
67	C_{14}-O-C_{11}
68	C_{14}-O-C_{13}
69	C_{14}-O-C_{15}
70	C_{16}-O-C_{10}
71	C_{16}-O-C_{12}
72	C_{16}-O-C_{14}
73	C_{16}-O-C_{16}



56-73

Fig. 11



74-91

	R
74	C_{10}-O-C_{10}
75	C_{10}-O-C_{12}
76	C_{10}-O-C_{14}
77	C_{10}-O-C_{16}
78	C_{12}-O-C_{10}
79	C_{12}-O-C_{12}
80	C_{12}-O-C_{14}
81	C_{12}-O-C_{16}
82	C_{14}-O-C_2
83	C_{14}-O-C_8
84	C_{14}-O-C_{10}
85	C_{14}-O-C_{11}
86	C_{14}-O-C_{13}
87	C_{14}-O-C_{15}
88	C_{16}-O-C_{10}
89	C_{16}-O-C_{12}
90	C_{16}-O-C_{14}
91	C_{16}-O-C_{16}

Fig. 12

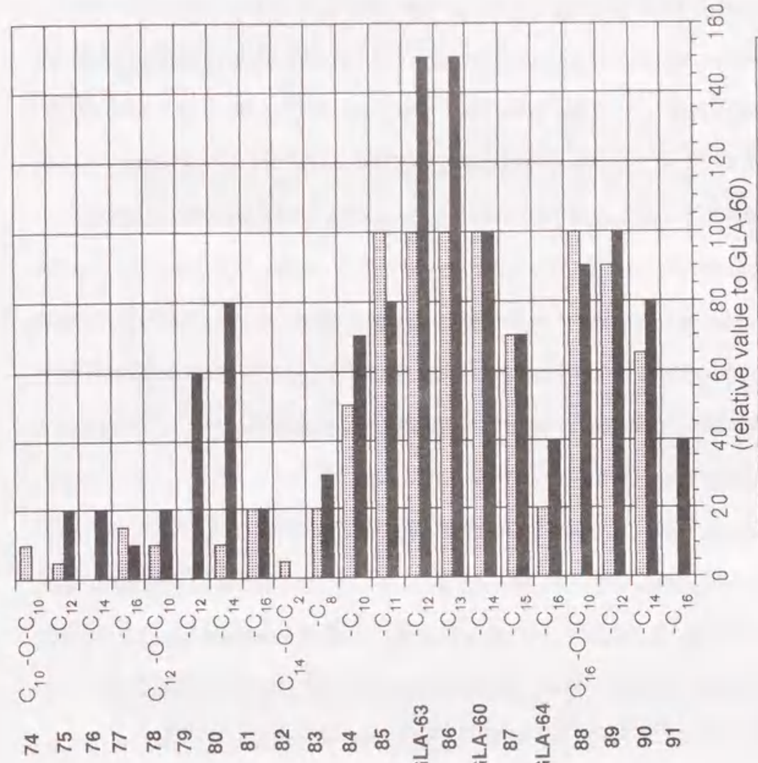
according to the procedures described previously,[46] gave the corresponding compounds **18-21**, respectively. *O*-Deisopropylidenation of compounds **18-21** with aqueous acetic acid gave **22-25**, which were subsequently treated with *tert*-butyldimethylsilyl chloride in pyridine to yield compounds **26-29**. Phosphorylation at *O*-4 of **26-29** with diphenyl phosphorochloridate gave **30-33**. Hydrogenation of **30-33** over 10% palladium on carbon gave respectively the useful intermediates **34-37**, which were esterified to afford the corresponding products **38-55**. The 2-(trimethylsilyl)ethyl, 2-(trimethylsilyl)ethoxymethyl and *tert*-butyldimethylsilyl groups of **38-55** were simultaneously removed with boron trifluoride etherate to give **56-73**. The phenyl groups were then cleaved by hydrogenation over Adams' platinum catalyst to yield the desired end products **74-91** as colorless powders, which were clearly positive to the specific spray reagent[71] for the phosphono group.

Compound **86**, obtained as just described, showed higher mitogenic activity than that of GLA-60 (Fig. 13). IL-1 inducing activity was expressed by compounds **85**, **86**, **88**, and **89**. However, no significant biological activity was expressed by compounds in which the principal chain of the acyloxyacyl group was shorter than 12 carbon atoms (**74**-**81**).

Experimental

The details of the preparation of compound **91** are given here as examples of the general procedures used.

2-(Trimethylsilyl)ethyl 3-*O*-[(3*RS*)-3-(benzyloxymethoxy)decanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (18). To a solution of 2-



IL-1 inducing activity was assessed by determining [^{3}H]thymidine uptake into thymocytes [I. Saiki *et. al*, *Vaccine* **6**, 238-244 (1988)]

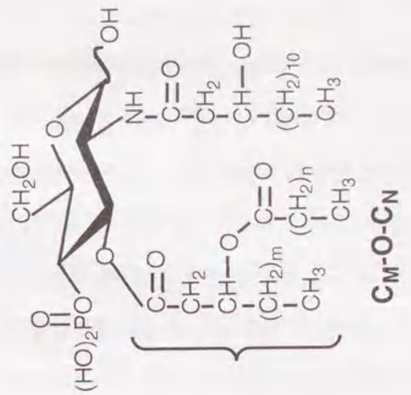


Fig. 13 Biological activities of compounds 74-91

(trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside[70] (**17**, 3 g) in dichloromethane (50 ml) were added (3*RS*)-3-(benzyloxymethoxy)decanoic acid [2.06 g; IR (film) cm^{-1}: 3600-2400 (CH, COOH), 1720 (C=O), 1050 (ether) and 770-690 (Ph)], 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 1.69 g) 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 dichloromethane-methanol (400:1) to give **18** (3.46 g, 81%) as a syrup, $[\alpha]_D$ -12° (*c* = 0.9, dichloromethane); IR (film) cm^{-1}: 3300 (NH), 2930, 2850 (CH), 1740 (ester), 1660, 1550 (amide), 860, 830 (Si-C, Me_2C) and 730-700 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 18H, Me_3Si-), 0.75-1.05 (m, 10H, Me_3Si-C*H*$_2$- and Me), 1.15-1.70 (m, 32H, -CH_2-), 1.32, 1.33, 1.43, 1.44 (4s, 6H, Me_2C), 2.30-2.70 (m, 4H, -$COCH_2$-), 3.35 (m, 1H, H-5), 3.45-4.10 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-3 of C_{10}-OBOM, H-2, 4 and 6), 4.5-4.9 (m, 7H, PhC*H*$_2$, -OCH_2O- and H-1), 5.19, 5.20 (2t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 6.2 (m, 1H, NH) and 7.2-7.4 (m, 5H, Ph).

Anal. Found: C, 64.44; H, 9.99; N, 1.40. Calcd. for $C_{52}H_{95}NO_{11}Si_2$: C, 64.62; H, 9.91; N, 1.45%.

2-(Trimethysilyl)ethyl 3-*O*-[(3*RS*)-3-(benzyloxymethoxy)decanoyl]-2-deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (22). A solution of **18** (3.46 g) in 80% aqueous acetic acid (150 ml) was stirred for 2 h at 50°C, and then concentrated to a syrup, which was chromatographed on a column of silica gel with dichloromethane-methanol (150:1) to give **22** (2.41 g, 73%) as a syrup, $[\alpha]_D$ -8.5° (*c* = 1.1, dichloromethane); IR (film) cm^{-1}: 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1650, 1540 (amide), 860, 840 (Si-C) and 680 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 18H, Me3Si-), 0.75-1.0 (m, 10H, Me_3Si-C*H*$_2$- and Me), 1.05-1.75 (m, 32H, -CH_2-), 2.15-2.65 (m, 5H, -$COCH_2$- and OH), 3.33 (m, 1H, H-5), 3.4-3.95 (m, 9H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-3 of C_{10}-OBOM, H-4 and 6), 4.07 (broad s, 1H, H-2), 4.20 (broad s, 1H, OH), 4.45-4.85 (m, 7H, PhC*H*$_2$, -OCH_2O- and H-1), 5.02 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 6.26 (m, 1H, NH) and 7.2-7.4 (m, 5H, Ph).

Anal. Found: C, 63.29; H, 10.08; N, 1.37. Calcd. for $C_{49}H_{91}NO_{11}Si_2$: C, 63.53; H, 9.90; N, 1.51%.

2-(Trimethylsilyl)ethyl 3-*O*-[(3*RS*)-3-(benzyloxymethoxy)decanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (26). To a solution of **22** (2.14 g) in pyridine (50 ml) was added *tert*-butyldimethylsilyl chloride (0.88 g). The mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residual syrup was chromatographed on a column of silica gel with dichloromethane-methanol (400:1) to obtain **26** (1.81 g, 75%) as a syrup, $[\alpha]_D$ -6.6° (*c* = 1.1, dichloromethane); IR (film) cm^{-1}: 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1640, 1540 (amide), 860, 830 (Si-C) and 780-690 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 24H, Me-Si), 0.8-1.05 (m, 19H, Me_3Si-C*H*$_2$-, *tert*-Bu and Me), 1.1-1.65 (m, 32H, -CH_2-), 2.2-2.7 (m, 4H, -$COCH_2$-), 3.3-4.2 (m, 11H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-3 of C_{10}-OBOM, H-4, 5, 6 and OH), 4.4-4.85 (m, 7H, PhC*H*$_2$, -OCH_2O- and H-1), 5.00 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 6.1 (m, 1H, NH) and 7.2-7.4 (m, 5H, Ph).

Anal. Found: C, 63.19; H, 10.22; N, 1.17. Calcd. for $C_{55}H_{105}NO_{11}Si_3$: C, 63.48; H, 10.17; N, 1.35%.

2-(Trimethylsilyl)ethyl 3-*O*-[(3*RS*)-3-(benzyloxymethoxy)decanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (30). To a cooled solution of **26** (1.76 g) and DMAP (0.41 g) in pyridine

(5 ml) was added diphenyl phosphorochloridate (0.91 g) in dichloromethane (3 ml). The mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residue was extracted with chloroform. The extract was washed with 2M-hydrochloric acid and water, dried (sodium sulfate) and concentrated. The residual syrup was chromatographed on a column of silica gel with dichloromethane-methanol (400:1) to give syrupy **30** (1.46 g, 68%), $[\alpha]_D$ +6.7° (*c* = 0.6, dichloromethane); IR (film) cm^{-1}: 3300 (NH), 2930, 2850 (CH), 1750 (ester), 1660, 1550 (amide), 960 (P-O-Ph), 860, 840 (Si-C) and 780-680 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 24H, Me-Si), 0.8-1.05 (m, 19H, Me_3Si-C*H*$_2$-, *tert*-Bu and Me), 1.1-1.7 (m, 32H, -CH_2-), 2.2-2.6 (m, 4H, -$COCH_2$-), 3.35-4.2 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-3 of C_{10}-OBOM, H-2, 5 and 6), 4.45-4.9 (m, 8H, PhC*H*$_2$, -OCH_2O-, H-1 and 4), 5.57, 5.61 (2t, 1H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3), 6.18, 6.25 (2d, 1H, J = 7.7Hz, NH) and 7.1-7.4 (m, 15H, Ph).

Anal. Found: C, 63.30; H, 9.24; N, 1.19. Calcd. for $C_{67}H_{114}NO_{14}PSi_3$: C, 63.22; H, 9.03; N, 1.10%.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(3*RS*)-3-hydroxydecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (34). A mixture of **30** (0.84 g), 10% palladium on carbon (0.3 g) and ethanol (100 ml) was stirred overnight at room temperature in a hydrogen atmosphere. The catalyst was then filtered off, and the mixture washed with ethanol. The filtrate and washings were combined and concentrated. The residue was chromatographed on a column of silica gel with dichloromethane-methanol (300:1) to give **34** (0.69 g, 91%) as an amorphous solid, $[\alpha]_D$ +8.0° (*c* = 0.9, dichloromethane); IR (film) cm^{-1}: 3500 (OH), 3300 (NH), 2930, 2850 (CH), 1740(ester), 1640, 1550 (amide), 960 (P-O-Ph), 860, 840 (Si-C) and 780-690 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 24H, Me-Si), 0.8-1.0 (m, 19H, Me_3Si-C*H*$_2$-,

tert-Bu and Me), 1.05-1.7 (m, 32H, -CH_2-), 2.2-2.4 (m, 4H, -$COCH_2$-), 3.0 (broad s, 1H, OH), 3.3-4.0 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{10}-OH, H-3 of C_{14}-OSEM, H-2, 5 and 6), 4.4-4.85 (m, 3H, H-4 and -OCH_2O-), 4.90 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.47, 5.65 (2t, 1H, $J_{2,3} = J_{3,4}$ = 9.1 Hz, H-3), 6.29, 6.40 (2d, 1H, J = 7.7 Hz, NH) and 7.1-7.4 (m, 10H, Ph).

Anal. Found: C, 61.40; H, 9.41; N, 1.36. Calcd. for $C_{59}H_{106}NO_{13}PSi_3$: C, 61.48; H, 9.27; N, 1.22%.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-butyldimethylsilyl-3-*O*-[(3*RS*)-3-decanoyloxydecanoyl]-2-deoxy-4-O-diphenylphosphono-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (38). To a solution of **34** (150 mg) in dichloromethane (5 ml) were added decanoic acid (67 mg), WSC (124 mg) and a catalytic amount of DMAP. The mixture was stirred overnight at room temperature, and concentrated. The residue was chromatographed on a column of silica gel with dichloromethane-methanol (400:1) to give **38** (140 mg, 83%) as a syrup, $[\alpha]_D$ +7.1° (*c* = 0.7, dichloromethane); IR (film) cm^{-1}: 3300 (NH), 2930, 2850 (CH), 1740 (ester), 1660, 1550 (amide), 960 (P-O-Ph), 860, 840 (Si-C) and 780-690 (Ph); NMR ($CDCl_3$) δ: 0.0 (s, 24H, Me-Si), 0.8-1.0 (m, 22H, Me_3Si-C*H*$_2$-, *tert*-Bu and Me), 1.1-1.7 (m, 48H, -CH_2-), 2.1-2.5 (m, 6H, -$COCH_2$-), 3.4-3.9 (m, 9H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-2, 5 and 6), 4.5-4.75 (m, 3H, -OCH_2O- and H-4), 4.80, 4.87 (2d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.95, 5.09 (2m, 1H, H-3 of C_{10}-O-C_{10}), 5.47, 5.52 (2t, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3),6.20, 6.30 (2d, 1H, J = 7.5 Hz, NH) and 7.1-7.4 (m, 10H, Ph).

Anal. Found: C, 63.12; H, 9.77; N, 1.31. Calcd. for $C_{69}H_{124}NO_{14}PSi_3$: C, 63.41; H, 9.56; N, 1.07%.

3-*O*-[(3*RS*)-3-Decanoyloxydecanoyl]-2-deoxy-4-*O*-diphenylphosphono-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucopyranose (56). To a

solution of **38** (100 mg) in dichloromethane (10 ml) was added boron trifluoride etherate (0.5 ml) at 0°C. The mixture was stirred for 2 h at the same temperature. The mixture was washed with sat. sodium hydrogen carbonate and water, dried (sodium sulfate) and concentrated. The residue was chromatographed on a column of silica gel with dichloromethane-methanol (40:1) to give **56** (50 mg, 68%), which was lyophilized from a 1,4-dioxane solution, mp 97.5-99°C, $[\alpha]_D$ +2.4° (c = 0.9, dichloromethane); IR (film) cm^{-1}: 3350 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1640, 1540 (amide), 960 (P-O-Ph) and 780-690 (Ph); NMR ($CDCl_3$) δ: 0.88 (t, 9H, Me), 1.0-1.6 (m, 48H, -CH_2-), 2.1-2.5 (m, 6H, -$COCH_2$-), 3.3-4.2 (m, 6H, H-3 of C_{14}-OH, H-5, 6 and OH), 4.21 (m, 1H, H-2), 4.72 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,P}$ = 9.5 Hz, H-4), 5.01, 5.11 (2m, 1H, H-3 of C_{10}-O-C_{10}), 5.27 (broad s, 1H, H-1), 5.48, 5.51 (2t, 1H, $J_{2,3} = J_{3,4}$ = 9.5-10 Hz, H-3), 5.56 (broad s, 1H, OH), 6.45, 6.56 (2d, 1H, J = 9.1 Hz, NH) and 7.1-7.4 (m, 10H, Ph).

Anal. Found: C, 64.80; H, 9.00; N, 1.19. Calcd. for $C_{52}H_{84}NO_{13}P$: C, 64.91; H, 8.80; N, 1.46%.

3-*O*-[(3*RS*)-3-Decanoyloxydecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranose (74). To a solution of **56** (50 mg) in ethanol (30 ml) was added Adams' platinum catalyst (50 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and the mixture washed with ethanol. The filtrate and washings were combined and concentrated to afford **74** (39 mg, 94%), which was lyophilized from a 1,4-dioxane suspension, IR (KBr) cm^{-1}: 3300 (NH, OH), 2930, 2850 (CH), 1740 (ester) and 1640, 1550 (amide), and other physical data are recorded in Table 3.

Other 3-*O*-(3-acyloxyacyl)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranoses (75-91). Compounds **75-91** were

obtained by the same methods as described for **74**, and physical data are recorded in Table 3.

Table 3. Some physical properties of the compounds **74-91.**

Compd. No.	Mp (°C)[a]	$[\alpha]_D$ (°) (c)[b]	Molecular formula	Found (Calcd) % of C	H	N
74	154	+7.4 (0.8)	$C_{40}H_{76}NO_{13}P$	59.08 (59.31)	9.53 (9.46)	1.86 (1.73)
75	154	+8.7 (1.4)	$C_{42}H_{80}NO_{13}P$	60.32 (60.19)	9.77 (9.62)	1.89 (1.67)
76	151	+10.2 (1.0)	$C_{44}H_{84}NO_{13}P$	60.82 (61.02)	10.12 (9.98)	1.58 (1.62)
77	152	+9.3 (1.0)	$C_{46}H_{88}NO_{13}P$	61.77 (61.79)	9.69 (9.92)	1.74 (1.57)
78	156	+10.7 (0.9)	$C_{42}H_{80}NO_{13}P$	60.32 (60.19)	9.42 (9.62)	1.60 (1.67)
79	156	+10.6 (0.9)	$C_{44}H_{84}NO_{13}P$	61.08 (61.02)	9.78 (9.98)	1.59 (1.62)
80	155	+9.9 (0.9)	$C_{46}H_{88}NO_{13}P$	61.51 (61.79)	10.08 (9.92)	1.77 (1.57)
81	155	+9.5 (1.0)	$C_{48}H_{92}NO_{13}P$	62.60 (62.51)	10.13 (10.05)	1.70 (1.52)
82	157-159	+12.7 (0.1)	$C_{36}H_{68}NO_{13}P$	57.09 (57.35)	8.97 (9.09)	1.60 (1.86)
83	157-159	+14.6 (0.1)	$C_{42}H_{80}NO_{13}P$	60.23 (60.19)	9.49 (9.62)	1.48 (1.67)
84	157-159	+12.3 (0.15)	$C_{44}H_{84}NO_{13}P$	61.27 (61.02)	9.99 (9.98)	1.40 (1.62)
85	157-159	+10.8 (0.13)	$C_{45}H_{86}NO_{13}P$	61.15 (61.41)	10.14 (9.85)	1.83 (1.59)

Table 3 (continue). Some physical properties of the compounds **74-91.**

Compd. No.	Mp (°C)[a]	$[\alpha]_D$ (°) (*c*)[b]	Molecular formula	Found (Calcd) % of C	H	N
86	157-159	+11.4 (0.14)	$C_{47}H_{90}NO_{13}P$	61.88 (62.16)	10.08 (9.99)	1.51 (1.54)
87	157-159	+11.0 (0.22)	$C_{49}H_{94}NO_{13}P$	63.02 (62.86)	10.02 (10.12)	1.66 (1.50)
88	160	+8.6 (1.3)	$C_{46}H_{88}NO_{13}P$	61.90 (61.79)	10.10 (9.92)	1.45 (1.57)
89	159	+5.6 (0.9)	$C_{48}H_{92}NO_{13}P$	62.58 (62.51)	10.22 (10.05)	1.40 (1.52)
90	160	+10.6 (1.1)	$C_{50}H_{96}NO_{13}P$	63.03 (63.20)	10.33 (10.18)	1.58 (1.47)
91	160	+10.5 (1.2)	$C_{52}H_{100}NO_{13}P$	63.64 (63.84)	10.53 (10.30)	1.18 (1.43)

a. Decomposition b. 1:1 CH_2Cl_2-MeOH

CHAPTER 2

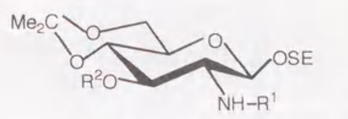
GLA-60 ANALOGS CARRYING DIFFERENT TYPES OF ACYL GROUPS

In this chapter, I describ the syntheses of a novel analogs of GLA-60 to reveal the effect of different kinds of acyl substituents. In natural lipid A, the 2-hydroxytetradecanoyl group has also been found as a minor fatty acyl component,[72,73] but the relationship with biological activities has not been investigated. In view of this point, a novel series of 3-*O*-(2-acyloxy)acylated and / or 2-*N*-(2-hydroxy)acylated GLA-60 homologues, *i. e.*, 3-*O*-[(2*RS*)-2-acyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**100a-d**), 3-*O*-[(2*RS*)-2-acyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**108a-d**), and 2-[(2*RS*)-2-hydroxyacyl]amino-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucoses (**111e-h**) were synthesized.[74]

On the other hand, it has been reported[75] that tissue toxicity is reduced after hydrolysis of 3-acyloxyacyl group of LPS by human neutrophiles. In this connection, the GLA-60 analogs containing an alkyl-branched 2-tetradecylhexadecanoyl group and 2- or 3-alkyl-branched acyl groups of type I, II. or III, in which the length of the principal alkyl chain is varied, were also synthesized. Alkyl-branched acyl groups have not only structural resemblance to the ester-branched 3-acyloxyacyl group but also resistance to the hydrolytic enzymes *in vivo*. The aim of this study was to investigate the biological influence of alkyl-branched acyl groups in GLA-60 analogs. A number of 2-(acyl)amino-2-deoxy-3-*O*-(2-tetradecylhexadecanoyl)-4-*O*-phosphono-D-glucose derivatives[76] (**120a-f**) and 3-*O*-(2- or 3-alkylacyl)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucose derivatives[77] (**126g-s**) were synthesized.

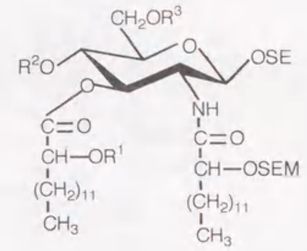
2-1. Synthesis and biological activity of the 2-acyloxytetradecanoyl and 2-hydroxyacyl derivatives

As a typical examples, a general synthetic procedures used in this study is described for the preparations of **100a** and **113e** as follows. The syntheses of other compounds all follow essentially the same pathway. Compound (**1**) was treated with (2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid in the presence of WSC in dichloromethane to give **92**, which was successively esterified at *O*-3 with (2*RS*)-2-(benzyloxymethoxy)tetradecanoic acid in the presence of WSC and a catalytic amount of DMAP in dichloromethane at room temperature, to afford **93** in 98% yield. These 2-*O*-protected 2-hydroxytetradecanoic acids were prepared *via* the phenacyl esters according to the procedures described previously[46]. Hydrolytic removal of the isopropylidene group in **93** with aqueous acetic acid and the following selective protection of the primary hydroxyl group in **94** with TBDMS chloride gave the desired compound **95** in good yield. Introduction of the diphenoxyphosphinyl group at *O*-4 was carried out by using diphenyl phosphorochloridate and DMAP in pyridine in 87% yield. Hydrogenolytic removal of the benzyloxymethyl group of **96** in the presence of 10% palladium on carbon in ethanol gave 2-(trimethylsilyl)ethyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-[(2*RS*)-2-hydroxytetradecanoyl]-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (**97**), which is a useful intermediate for syntheses of 3-*O*-[(2*RS*)-2-acyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**100a-d**). The deprotected hydroxyl group of **97** was esterified with decanoic acid, WSC, and DMAP to afford **98a**. The 2-(trimethylsilyl)ethyl, 2-(trimethylsilyl)ethoxymethyl, and *tert*-



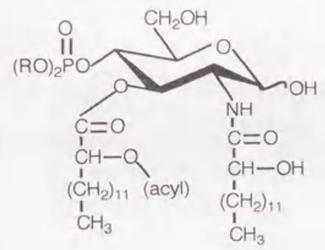
	R^1	R^2
1	H	H
92	$CH_3(CH_2)_{11}CH(OSEM)CO$	H
93	$CH_3(CH_2)_{11}CH(OSEM)CO$	$CH_3(CH_2)_{11}CH(OBOM)CO$

SE = $Me_3SiCH_2CH_2$
SEM = $Me_3SiCH_2CH_2OCH_2$
BOM = $C_6H_5CH_2OCH_2$



	R^1	R^2	R^3
94	BOM	H	H
95	BOM	H	TBDMS
96	BOM	$(PhO)_2P(O)$	TBDMS
97	H	$(PhO)_2P(O)$	TBDMS
98a-d	acyl (a-d)	$(PhO)_2P(O)$	TBDMS

TBDMS = *tert*-$BuMe_2Si$

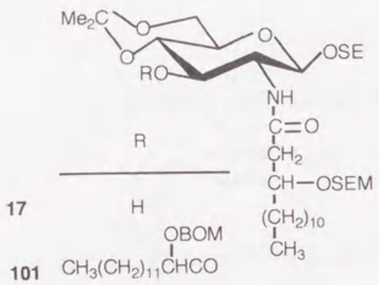


	R
99a-d	Ph
100a-d	H

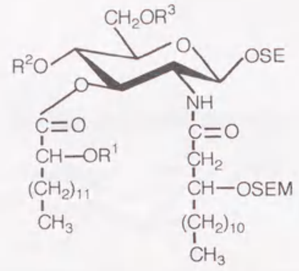
$CH_3(CH_2)_nCO$

Acyl groups	
Series	n
a	8
b	10
c	12
d	14

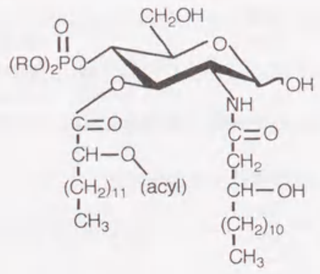
Fig. 14



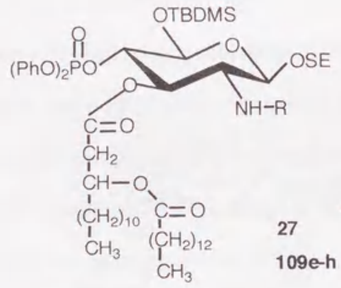
	R
17	H
101	$CH_3(CH_2)_{11}CHCO$ (OBOM)



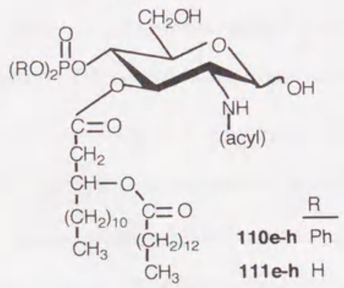
	R^1	R^2	R^3
102	BOM	H	H
103	BOM	H	TBDMS
104	BOM	$(PhO)_2P(O)$	TBDMS
105	H	$(PhO)_2P(O)$	TBDMS
106a-d	acyl (a-d)	$(PhO)_2P(O)$	TBDMS



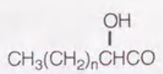
	R
107a-d	Ph
108a-d	H



	R
27	H
109e-h	acyl (e-h)



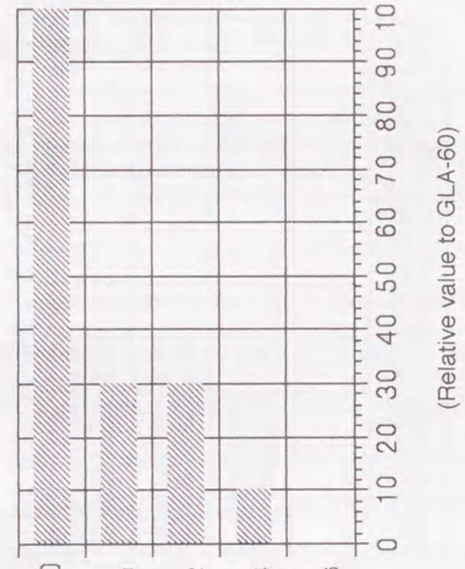
	R
110e-h	Ph
111e-h	H

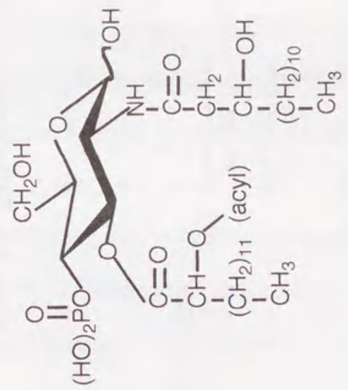


Acyl groups	
Series	n
e	7
f	9
g	11
h	13

Fig. 15

Mitogenic activity





Acyl groups

Series	n	
a	8	(C_{10})
b	10	(C_{12})
c	12	(C_{14})
d	14	(C_{16})

$CH_3(CH_2)_nCO$

Fig. 16 Mitogenic activity of compounds 108a-d

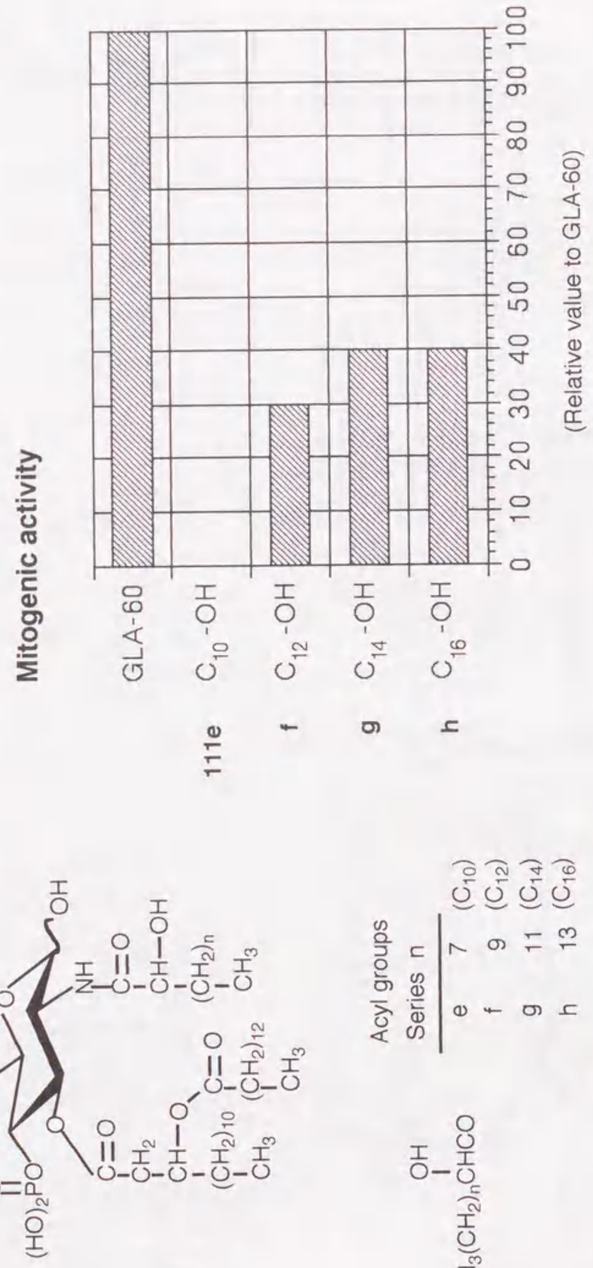


Fig. 17 Mitogenic activity of compounds 111e-h

butyldimethylsilyl groups of **98a** were simultaneously removed with boron trifluoride etherate in dichloromethane at 0 °C to give **99a** in 75% yield. In the ^{1}H NMR spectrum of **99a**, the anomeric proton appeared as a singlet at δ 5.23, showing that the α-D-pyranose configuration preponderates in chloroform-*d*. The phenoxy groups were finally cleaved by hydrogenolysis in the presence of Adams' platinum catalyst in ethanol, to afford the desired 3-*O*-[(2*RS*)-2-decanoyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranose (**100a**) in high yield. 3-*O*-[(2*RS*)-2-Acyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**108a-d**) were prepared from compound (**17**) *via* the intermediate 2-(trimethylsilyl)ethyl 6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-[(2*RS*)-2-hydroxytetradecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (**105**) by the same sequence as **100a**. 2-Deoxy-2-[(2*RS*)-2-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (**111e-h**) was obtained by using compound (**27**) as a starting material. The amino group of **27** was acylated with (2*RS*)-2-hydroxydecanoic acid and WSC to give **109e** in 79% yield. Compound **109e** was treated with boron trifluoride etherate in dichloromethane at 0 °C to give desilylated compound **110e**, which was hydrogenolyzed to afford the desired 2-deoxy-2-[(2*RS*)-2-hydroxydecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (**111e**), according to the procedures for **100a**.

These synthetic analogs (**100a-d**, **108a-d**, and **111e-h**) of GLA-60, having the 2-acyloxytetradecanoyl and the 2-hydroxyacyl groups, showed moderate mitogenicity (Fig. 16 and 17) and macrophage activation activities. However, no significant tumor necrosis factor (TNF)-inducing activity was expressed.

Experimental

2-(Trimethylsilyl)ethyl 2-Deoxy-4,6-*O*-isopropylidene-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (92). To a solution of 2-(trimethysilyl)ethyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**, 2.7 g) in CH_2Cl_2 (50 mL) were added (2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid [3.8 g, IR (film); 3600-2400 (CH, COOH), 1720 (C=O), and 860 and 830 cm^{-1}(Si-C)], and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 3.2 g). 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 **2** (4.0 g, 70 %) as a syrup: $[\alpha]_D$ -29.0° (*c* 1.2, CH_2Cl_2); IR (film); 3300 (OH, NH), 2940, 2850 (CH), 1650, 1540 (amide), and 860 and 830 (Si-C, Me_2C); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.83-1.0 (m, 7H, Me_3Si-CH_2- and Me), 1.2-1.8 (m, 22H, -CH_2-), 1.43, 1.51 (2s, 6H, Me_2C), 3.30 (m, 1H, H-5), 3.35-4.0 (m, 9H, Me_3SiCH_2-CH_2-, H-2 of C_{14}-OSEM, and ring protons H-3, 4, and 6), 4.04-4.1 (q, 1H, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 5.5 Hz, H-2), 4.25 (broad s, 1H, OH), 4.75, 4.63 (2d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.7 (m, 2H, -OCH_2O-), and 6.77, 6.80 (2d, 1H, $J_{2,NH}$ = 4.9 Hz, NH).

Anal. Calcd for $C_{34}H_{69}NO_8Si_2$: C, 60.40; H, 10.39; N, 2.09. Found: C, 60.17; H, 10.63; N, 1.98.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (93). To a solution of **92** (1.8 g) in CH_2Cl_2 (50 mL) were added (2*RS*)-2-(benzyloxymethoxy)tetradecanoic acid [1.4 g; IR (film) 3600-2400 (CH, COOH), 1720 (C=O), and 730-690 cm^{-1} (Ph)], WSC

(1.0 g) 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 **93** (2.7 g, 98 %) as a syrup: $[\alpha]_D$ -19.3° (*c* 1.0, CH_2Cl_2); IR (film) 3360 (NH), 2930, 2850 (CH), 1740 (ester) 1660, 1530 (amide), 860, 830 (Si-C, Me_2C), and 730-700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.88-1.0 (m, 10H, Me_3Si-CH_2- and Me), 1.2-1.8 (m, 44H, -CH_2-), 1.33, 1.45 (2s, 6H, Me_2C), 3.34 (m, 1H, H-5), 3.45-4.05 (m, 9H, Me_3SiCH_2-CH_2-, H-2 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-4, and 6), 4.25 (m, 1H, H-2), 4.5-4.8 (m, 7H, -OCH_2O-, $PhCH_2$-, and H-1), 5.3 (m, 1H, H-3), 6.6 (m, 1H, NH), and 7.27-7.34 (m, 5H, Ph).

Anal. Calcd for $C_{56}H_{103}NO_{11}Si_2$: C, 65.78; H, 10.15; N, 1.37. Found: C, 65.50; H, 10.40; N, 1.19.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-2-deoxy-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (94). A solution of **93** (2.6 g) in aqueous 80% acetic acid (150 mL) was stirred for 2 h at 50 °C and then concentrated to a syrup, which was chromatographed on a column of silica gel with 150:1 CH_2Cl_2-MeOH to give **94** (2.5 g, 96%) as a syrup: $[\alpha]_D$ -5.7° (*c* 1.0, CH_2Cl_2); IR (film) 3350 (OH, NH), 2930, 2850 (CH), 1740 (ester) 1650, 1530 (amide), 860, 830 (Si-C), and 760-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.88-1.0 (m, 10H, Me_3Si-CH_2- and Me), 1.2-1.8 (m, 44H, -CH_2-), 3.40 (m, 1H, H-5), 3.50-4.14 (m, 10H, Me_3SiCH_2-CH_2-, H-2 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-2, 4, and 6), 4.5-4.9 (m, 7H, -OCH_2O-, $PhCH_2$-, and H-1), 5.1 (m, 1H, H-3), 6.57 (m, 1H, NH), and 7.27-7.34 (m, 5H, Ph).

Anal. Calcd for $C_{53}H_{99}NO_{11}Si_2$: C, 64.79; H, 10.16; N, 1.43. Found: C, 65.00; H, 10.25; N, 1.40.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (95). To a solution of **94** (2.5 g) in pyridine (70 mL) was added *tert*-butyldimethylsilyl chloride (784 mg), and the mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residual syrup was chromatographed on a column of silica gel with 300:1 CH_2Cl_2-MeOH to obtain **95** (2.4 g, 83%) as a syrup: $[\alpha]_D$ -9.6° (*c* 1.0, CH_2Cl_2); IR (film) 3450 (OH), 3300 (NH), 2930, 2850 (CH), 1750 (ester), 1660, 1540 (amide), 860, 840 (Si-C), and 780-700 cm^{-1} (Ph); NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.86-1.0 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.2-1.75 (m, 44H, -CH_2-), 3.28 (d, 1H, OH), 3.35-4.2 (m, 11H, Me_3SiCH_2-C*H*$_2$-, H-2 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-2, 4, 5, and 6), 4.47-4.9 (m, 7H, -OCH_2O-, PhC*H*$_2$-, and H-1), 5.1 (m, 1H, H-3), 6.51 (m, 1H, NH), and 7.27-7.39 (m, 5H, Ph).

Anal. Calcd for $C_{59}H_{113}NO_{11}Si_3$: C, 64.61; H, 10.38; N, 1.28. Found: C, 64.44; H, 10.59; N, 1.01.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (96). To a cold solution of **95** (2.4 g) and DMAP (560 mg) in pyridine (20 mL) was added diphenyl phosphorochloridate (1.17 g) and the mixture was stirred overnight at room temperature. Methanol was added to the mixture, and it was concentrated, then the residue was extracted with $CHCl_3$. The extract was washed with 2M HCl and water, dried (Na_2SO_4) and concentrated. The residual syrup was chromatographed on a column of silica gel with 400:1 CH_2Cl_2-MeOH to give syrupy **96** (2.52 g, 87%): $[\alpha]_D$ -6.5° (*c* 1.0, CH_2Cl_2); IR (film) 3300 (NH), 2950, 2850 (CH), 1760 (ester), 1680, 1600 (amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1}(Ph); ^{1}H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.1-1.75 (m, 44H, -CH_2-), 3.48-4.02 (m, 9H, Me_3SiCH_2-C*H*$_2$-, H-2 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-5, and 6), 4.1 (m, 1H, H-2), 4.41-4.46 (q, 1H, $J_{3,4} = J_{4,5} = J_{4,p}$ = 9.5 Hz, H-4), 4.48-4.88 (m, 7H, -OCH_2O-, PhC*H*$_2$- and H-1), 5.5 (m, 1H, H-3), 6.61 (m, 1H, NH), and 7.07-7.39 (m, 15H, Ph).

Anal. Calcd for $C_{71}H_{122}NO_{14}PSi_3$: C, 64.17; H, 9.25; N, 1.05. Found: C, 64.22; H, 9.51; N, 1.20.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-[(2*RS*)-2-hydroxytetradecanoyl]-2-[(2*RS*)-2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (97). A mixture of **96** (2.91 g), 10% Pd-C (1.0 g) and EtOH (150 mL) was stirred overnight at room temperature under a hydrogen atmosphere. The catalyst was then filtered off and washed with EtOH. The filtrate and washings were combined and concentrated. The residue was chromatographed on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **97** (1.47 g, 60%), which was lyophilized from 1,4-dioxane solution: mp 87-89 °C, $[\alpha]_D$ -4.1° (*c* 0.9, CH_2Cl_2); IR (film) 3500 (OH), 3300 (NH), 2930, 2850 (CH), 1740 (ester), 1680, 1590 (amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1}(Ph); ^{1}H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.85-0.98 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.14-1.75 (m, 44H, -CH_2-), 2.96, 3.09 (2d, 1H, OH), 3.50-4.06 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-2 of C_{14}-OSEM, H-2 of C_{14}-OH, H-2, 5, and 6), 4.51-4.73 (m, 3H, -OCH_2O- and H-4), 4.85, 4.95 (2d, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 5.5 (m, 1H, H-3), 6.61, 6.65 (2d, 1H, NH), and 7.07-7.39 (m, 10H, Ph).

Anal. Calcd for $C_{63}H_{114}NO_{13}PSi_3$: C, 62.60; H, 9.51; N, 1.16. Found: C, 62.43; H, 9.79; N, 1.02.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-3-*O*-[(2*RS*)-2-decanoyloxytetradecanoyl]-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(2*RS*)-

2-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (98a). To a solution of **97** (200 mg) in CH_2Cl_2 (10 mL) were added decanoic acid (57 mg), WSC (95 mg) and a catalytic amount of DMAP. The mixture was stirred overnight at room temperature and concentrated. The residue was chromatographed on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **98a** (225 mg, 96%) as a syrup: $[\alpha]_D$ -10.5° (*c* 0.6, CH_2Cl_2); IR (film) 3350 (NH), 2930, 2850 (CH), 1740 (ester), 1680, 1590 (amide), 950 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.85-0.98 (m, 22H, *tert*-Bu, Me_3Si-CH_2- and Me), 1.12-1.75 (m, 58H, -CH_2-), 2.28-2.35 (m, 2H, -$COCH_2$-) 3.50-4.04 (m, 8H, Me_3SiCH_2-CH_2-, H-2 of C_{14}-OSEM, H-5, and 6), 4.04-4.17 (m, 1H, H-2), 4.6-4.73 (m, 3H, -OCH_2O- and H-4), 4.81-5.0 [m, 1H, H-2 of C_{14}-(O-C_{10})], 5.07, 5.17 (2d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 5.5, 5.8 (2m, 1H, H-3), 6.61, 6.82 (2m, 1H, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{73}H_{132}NO_{14}PSi_3$: C, 64.33; H, 9.76; N, 1.03. Found: C, 64.12; H, 9.90; N, 1.31.

3-*O*-[(2*RS*)-2-Decanoyloxytetradecanoyl]-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(2*RS*)-2-hydroxytetradecanamido]-D-glucopyranose (99a). To a solution of **98a** (200 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. The mixture was washed with sat $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The residue was chromatographed on a column of silica gel with 40:1 CH_2Cl_2-MeOH to give **99a** (110 mg, 75%), which was lyophilized from 1,4-dioxane solution: mp 47-49 °C, $[\alpha]_D$ -2.3° (*c* 0.3, CH_2Cl_2); IR (KBr) 3350 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1640, 1540 (amide), 950 (P-O-Ph), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.88 (m, 9H, Me), 1.12-1.75 (m, 58H, -CH_2-), 2.28-2.45 (m, 2H, -$COCH_2$-) 3.50-4.04 (m, 7H, H-2 of C_{14}-OH, OH, H-2, 5, and 6), 4.55 (broad s, 1H, OH), 4.6 (m, 1H, H-

4), 5.23 (s, 1H, H-1), 5.34 [m, 1H, H-2 of C_{14}-(O-C_{10})], 5.8 (m, 1H, H-3), 6.82 (m, 1H, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{56}H_{92}NO_{13}P$: C, 66.05; H, 9.11; N, 1.38. Found: C, 65.78; H, 9.37; N, 1.30.

3-*O*-[(2*RS*)-2-Decanoyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranose (100a). To a solution of **99a** (50 mg) in EtOH (100 mL) was added Adams' platinum catalyst (60 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off and washed with EtOH. The filtrate and washings were combined and concentrated to afford **100a** (45 mg, 95%), which was lyophilized from 1,4-dioxane suspension: IR (KBr) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), and 1680, 1590 cm^{-1}(amide). Other physical and analytical data are given in the Table 4.

Other 3-*O*-[(2*RS*)-2-Acyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranoses (100b-d). Compounds **100b-d** were prepared *via* **98b-d** and **99b-d** from **97** by the same sequence as described for **100a**, and the physical and analytical data are recorded in the Table 4.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (101). Compound **101** was obtained by treatment of 2-(trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (**17**, 2.83 g) with (2*RS*)-2-(benzyloxymethoxy)tetradecanoic acid (2 g) in nearly quantitative yield, according to the method described for **93**: $[\alpha]_D$ -10.9° (*c* 1.0, CH_2Cl_2); IR (film) 3300 (NH), 2930, 2850 (CH), 1740 (ester) 1660, 1530 (amide), 860, 830 (Si-C, Me_2C), and 730-700 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.75-1.0 (m, 10H, Me_3Si-CH_2- and Me), 1.1-1.7 (m, 42H, -CH_2-), 1.32, 1.33, 1.43, 1.44 (4s, 6H,

Me_2C), 2.2-2.35 (m, 2H, -$COCH_2$-), 3.34 (m, 1H, H-5), 3.45-4.0 (m, 9H, Me_3Si-CH_2-CH_2-, H-3 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-4, and 6), 4.23 (m, 1H, H-2), 4.5-4.9 (m, 7H, -OCH_2O-, PhCH_2-, and H-1), 5.24, 5.25 (2t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 6.18 (m, 1H, NH), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd for $C_{56}H_{103}NO_{11}Si_2$: C, 65.78; H, 10.15; N, 1.37. Found: C, 65.91; H, 10.04; N, 1.09.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-2-deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (102). Hydrolytic removal of the isopropylidene group from **101**, as described for **94**, gave **102** in 70% yield: $[\alpha]_D$ +1.6° (*c* 1.4, CH_2Cl_2); IR (film) 3300 (OH, NH), 2930, 2850 (CH), 1760 (ester) 1650, 1560 (amide), 860, 840 (Si-C), and 730-700 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3-Si-), 0.75-1.0 (m, 10H, Me_3Si-CH_2- and Me), 1.1-1.7 (m, 42H, -CH_2-), 2.15-2.4 (m, 3H, -$COCH_2$- and OH), 3.40 (m, 1H, H-5), 3.45-4.0 (m, 9H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-4, and 6), 4.1 (m, 1H, H-2), 4.5-4.85 (m, 7H, -OCH_2O-, PhCH_2-, and H-1), 5.13 (t, 1H, $J_{2,3} = J_{3,4} = 10$ Hz, H-3), 6.19, 6.28 (2d, 1H, NH), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd for $C_{53}H_{99}NO_{11}Si_2$: C, 64.79; H, 10.16; N, 1.43. Found: C, 64.99; H, 9.80; N, 1.21.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (103). Compound **103** was quantitatively obtained by treatment of **102** (2.5 g) with *tert*-butyldimethylsilyl chloride (762 mg) as described for **95**: $[\alpha]_D$ -2.9° (*c* 1.1, CH_2Cl_2); IR (film) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1640, 1540 (amide), 860, 840 (Si-C), and 780-700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 19H,

Me_3Si-CH_2-, *tert*-Bu, and Me), 1.1-1.9 (m, 42H, -CH_2-), 2.2-2.4 (m, 2H, -$COCH_2$-), 3.25-4.0 (m, 10H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2 of C_{14}-OBOM, OH, H-5, and 6), 4.1 (m, 1H, H-2), 4.5-4.9 (m, 7H, -OCH_2O-, PhCH_2-, and H-1), 5.13 (t, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 6.01, 6.11 (2d, 1H, NH), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd for $C_{59}H_{113}NO_{11}Si_3$: C, 64.61; H, 10.38; N, 1.28. Found: C, 64.50; H, 10.60; N, 1.01.

2-(Trimethylsilyl)ethyl 3-*O*-[(2*RS*)-2-(Benzyloxymethoxy)tetradecanoyl]-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (104). Compound **103** (2 g) was treated with diphenyl phosphorochloridate as described for **96**, to afford syrupy **104** (1.9 g, 80%): $[\alpha]_D$ -3.7° (*c* 1.1, CH_2Cl_2); IR (film) 3300 (NH), 2930, 2850 (CH), 1750 (ester), 1640, 1540 (amide), 950 (P-O-Ph), 860, 840 (Si-C), and 780-700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 19H, Me_3Si-CH_2-, *tert*-Bu, and Me), 1.1-1.7 (m, 42H, -CH_2-), 2.2-2.4 (m, 2H, -$COCH_2$-), 3.5-4.0 (m, 9H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2 of C_{14}-OBOM, H-5, and 6), 4.12, 4.21 (2dd, 1 H, H-2), 4.4-4.8 (m, 7H, -OCH_2O-, PhCH_2-, and H-4), 4.81 (2d, 1H, $J_{1,2} = 8$ Hz, H-1), 5.56, 5.66 (2t, 1H, $J_{2,3} = J_{3,4} =$ 10Hz, H-3), 6.21 (d, 1H, NH), and 7.2-7.4 (m, 15H, Ph).

Anal. Calcd for $C_{71}H_{122}NO_{14}PSi_3$: C, 64.17; H, 9.25; N, 1.05. Found: C, 63.89; H, 9.50; N, 0.90.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-[(2*RS*)-2-hydroxytetradecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (105). Compound **104** (1.1 g) was hydrogenolyzed in the presence of palladium catalyst as described for **97**, to give **105** (750 mg, 74%) as a syrup: $[\alpha]_D$ +8.1° (*c* 0.9, CH_2Cl_2); IR (film) 3300 (OH, NH), 2930, 2850 (CH), 1750 (ester), 1660, 1540

(amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-700 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 19H, Me_3Si-CH_2-, *tert*-Bu, and Me), 1.1-1.7 (m, 42H, -CH_2-), 2.2-2.4 (m, 2H, -$COCH_2$-), 3.05 (broad s, 1H, OH), 3.5-4.0 (m, 10H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2 of C_{14}-OH, H-2, 5, and 6), 4.6-4.8 (m, 3H, -OCH_2O- and H-4), 4.81, 4.92 (2d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 5.61, 5.64 (2t, 1H, $J_{2,3} = J_{3,4}$ = 8.8 Hz, H-3), 6.32, 6.35 (2d, 1H, NH), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{63}H_{114}NO_{13}PSi_3$: C, 62.60; H, 9.51; N, 1.16. Found: C, 62.40; H, 9.66; N, 0.98.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-3-*O*-[(2*RS*)-2-decanoyloxytetradecanoyl]-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (106a). Esterification of **105** (170 mg) was performed with decanoic acid (72 mg) in the presence of WSC (134 mg) and a catalytic amount of DMAP as described for **98a**, to give **106a** (173 mg, 90%) as a syrup: $[\alpha]_D$ +6.4° (*c* 0.8, CH_2Cl_2); IR (film) 3300 (NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 (amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 22H, Me_3Si-CH_2-, *tert*-Bu, and Me), 1.1-1.7 (m, 56H, -CH_2-), 2.2-2.4 (m, 4H, -$COCH_2$-), 3.18-4.0 (m, 9H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2, 5, and 6), 4.5-4.8 (m, 3H, -OCH_2O- and H-4), 4.87, 4.89 (2d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 5.14 [t, 1H, H-2 of C_{14}-(O-C_{10})], 5.75, 5.82 (2t, 1H, $J_{2,3} = J_{3,4}$ = 9.2 Hz, H-3), 6.30 (m, 1H, NH), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{73}H_{132}NO_{14}PSi_3$: C, 64.33; H, 9.76; N, 1.03. Found: C, 64.12; H, 9.88; N, 0.90.

3-*O*-[(2*RS*)-2-Decanoyloxytetradecanoyl]-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-hydroxytetradecanamido]-D-glucopyranose (107a). Compound **106a** (150 mg) was treated with boron trifluoride etherate (0.5 mL) as described for **99a** to give **107a** (97 mg, 86%), which was lyophilized from 1,4-dioxane solution: mp 38-39 °C, $[\alpha]_D$ -2.4° (*c* 1.0, CH_2Cl_2); IR (film) 3400 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780-690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 9H, Me), 1.1-1.7 (m, 56H, -CH_2-), 2.2-2.4 (m, 4H, -$COCH_2$-), 3.45-4.0 (m, 6H, OH, H-3 of C_{14}-OH, H-5, and 6), 4.24 (m, 1H, H-2), 4.74 (q, 1H, $J_{3,4} = J_{4,3} = J_{4,P}$ = 9.5 Hz, H-4), 4.8 (broad s, 1H, OH), 5.12 [t, 1H, H-2 of C_{14}-(O-C_{10})], 5.24 (s, 1H, H-1), 5.55, 5.59 (2t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 6.25-6.35 (m, 1H, NH), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{56}H_{92}NO_{13}P$: C, 66.05; H, 9.11; N, 1.38. Found: C, 66.00; H, 9.22; N, 1.13.

3-*O*-[(2*RS*)-2-Decanoyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranose (108a). Compound **107a** (95 mg) was hydrogenolyzed in the presence of platinum catalyst as described for **100a**, to afford the desired compound **108a** (79 mg, 97%): IR (KBr) 3400 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 cm^{-1}(amide). Other physical and analytical data are given in the Table 4.

3-*O*-[(2*RS*)-2-Acyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucopyranoses (108b-d). Compounds **108b-d** were prepared *via* **106b-d** and **107b-d** from **105** by the same sequence described for **100a**, and the physical and analytical data are recorded in Table 4.

2-(Trimethylsilyl)ethyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(2*RS*)-2-hydroxydecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (109e). A mixture of 2-(trimethylsilyl)ethyl 2-amino-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranoside (**7**, 200 mg), (2*RS*)-2-hydroxydecanoic acid (131 mg), and WSC (200 mg) in $CHCl_3$ (10 mL) was

stirred overnight at room temperature. The reaction mixture was directly chromatographed on a column of silica gel with 250:1 CH_2Cl_2-MeOH to give **109e** (182 mg,

Table 4.
Some physical properties of the compounds **100a-d, 108a-d,** and **111e-h.**

Compd. No.	Mp (°C)	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
100a	152-153	+5.5 (1.2)	$C_{44}H_{84}NO_{13}P$	60.82 (61.02)	10.02 (9.78)	1.58 (1.62)
b	151-153	+6.0 (1.4)	$C_{46}H_{88}NO_{13}P$	61.51 (61.79)	10.08 (9.92)	1.77 (1.57)
c	154-155	+5.4 (0.9)	$C_{48}H_{92}NO_{13}P$	62.60 (62.51)	10.13 (10.05)	1.70 (1.52)
d	155-156	+6.5 (1.0)	$C_{50}H_{96}NO_{13}P$	63.03 (63.20)	10.33 (10.18)	1.58 (1.47)
108a	160-161	+15.5 (0.1)	$C_{44}H_{84}NO_{13}P$	60.80 (61.02)	10.01 (9.78	1.45 (1.62)
b	158-159	+10.0 (0.1)	$C_{46}H_{88}NO_{13}P$	61.90 (61.79)	9.72 (9.92)	1.60 (1.57)
c	161	+13.3 (0.1)	$C_{48}H_{92}NO_{13}P$	62.29 (62.51)	10.25 (10.05)	1.30 (1.52)
d	159-160	+7.1 (0.1)	$C_{50}H_{96}NO_{13}P$	63.01 (63.20)	10.38 (10.18)	1.44 (1.47)
111e	162-163	+14.2 (0.2)	$C_{44}H_{84}NO_{13}P$	61.30 (61.02)	9.90 (9.78)	1.57 (1.62)
f	158	+18.5 (0.4)	$C_{46}H_{88}NO_{13}P$	61.71 (61.79)	9.68 (9.92)	1.56 (1.57)
g	161-162	+20.3 (0.1)	$C_{48}H_{92}NO_{13}P$	62.38 (62.51)	10.26 (10.05)	1.48 (1.52)
h	159-160	+16.5 (0.1)	$C_{50}H_{96}NO_{13}P$	63.36 (63.20)	10.33 (10.18)	1.50 (1.47)

a. 50:25:4:2 $CHCl_3$-MeOH-H_2O-NH_4OH

79%) as a syrup: $[\alpha]_D$ -2.2° (*c* 0.8, CH_2Cl_2); IR (film) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 (amide), 960 (P-O-Ph), 840 (Si-C), and 780-690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 15H, Me-Si-), 0.8-1.0 (m, 20H, Me_3Si-CH_2-, *tert*-Bu, and Me), 1.1-2.5 (m, 60H, -CH_2- and -$COCH_2$-), 3.2-4.2 (m, 6H, Me_3SiCH_2-CH_2-, H-2 of C_{10}-OH, H-5, and 6), 4.25 (m, 1H, H-2), 4.5-4.8 (m, 2H, H-1 and 4), 5.0-5.5 [m, 2H, OH and H-3 of C_{14}-(O-C_{14})], 6.30 (m, 1H, NH), and 7.2-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{67}H_{118}NO_{13}PSi_2$: C, 65.28; H, 9.65; N, 1.14. Found: C, 65.02; H, 9.60; N, 1.02.

2-Deoxy-4-*O*-diphenoxyphosphinyl-2-[(2*RS*)-2-hydroxydecanamido]-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (110e). Cleavage of the silyl groups of **109e** (85 mg) as described for **99a**, gave **110e** (61 mg, 85%) as amorphous: $[\alpha]_D$ +11.3° (*c* 0.6, CH_2Cl_2); IR (film) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 (amide), 960 (P-O-Ph), and 780-690 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.88 (t, 9H, Me), 1.1-2.0 (m, 56H, -CH_2-), 2.0-2.7 (m, 4H, -$COCH_2$-), 3.2-4.2 (m, 5H, OH, H-5, and 6), 4.15-4.5 (m, 2H, H-2 of C_{10}-OH, and H-2), 4.75 (m, 1H, H-4), 5.07 [m, 1H, H-3 of C_{14}-(O-C_{14})], 5.20 (m, 2H, OH and H-1), 5.46 (m, 1H, H-3), 6.55 (m, 1H, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd for $C_{56}H_{92}NO_{13}P$: C, 66.05; H, 9.11; N, 1.38. Found: C, 65.78; H, 8.96; N, 1.64.

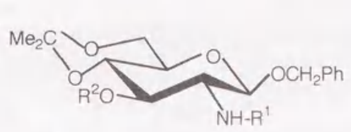
2-Deoxy-2-[(2*RS*)-2-hydroxydecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranose (111e). Cleavage of the phenyl groups of **110e** (50 mg) with platinum catalyst as described for **100a**, gave **111e** (40 mg, 93%): IR (KBr) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1660, 1540 cm^{-1} (amide). Other physical and analytical data are given in the Table 4.

2-Deoxy-2-[(2*RS*)-2-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-D-glucopyranoses (111f-h). Compounds **111f-h** were prepared *via* **109f-h** and **110f-h** from **7** by the same sequence described for **111e**. The physical and analytical data are shown in the Table 4.

2-2. Synthesis and biological activity of the alkyl-branched acyl derivatives

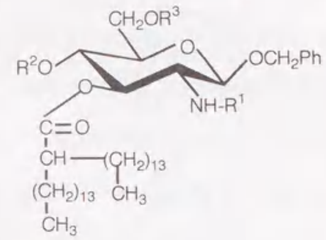
Treatment of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside[59] (**112**) with 2,2,2-trichloroethoxycarbonyl chloride gave 2-(2,2,2-trichloroethoxycarbonyl)amino derivative (**113**) in 77% yield. Compound **113** was esterified with 2-tetradecylhexadecanoic acid[78] in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and 4-dimethylaminopyridine (DMAP) to afford **114**. Hydrolytic removal of the isopropylidene group with aqueous acetic acid gave **115**, which after treatment with benzyl chloromethyl ether and 1,1,3,3-tetramethylurea in dichloromethane gave **116**. Phosphorylation of **116** at O-4 with diphenyl phosphorochloridate, and cleavage of the N-(2,2,2-trichloroethoxycarbonyl) group with zinc dust in acetic acid at 60°C gave the useful intermediate **117** in 82% yield. Compound **117** was esterified to afford **118a-f** in high yields. The benzyl and benzyloxymethyl groups of **118a-f** were simultaneously hydrogenolyzed over 10% palladium on carbon, giving **119a-f**. The phenyl groups were cleaved by hydrogenolysis in the presence of platinum catalyst, to yield the desired compounds **120a-f**.

The syntheses of compounds **126g-s** follows essentially the same pathway. Compounds **126g-s** were obtained by using compound **17** as a starting material. Compound **17** was treated with alkyl-branched fatty acids (purchased from Wako Pure



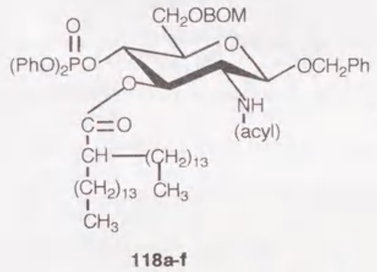
	R^1	R^2
112	H	H
113	TCEC	H
114	TCEC	TH

TCEC = $CCl_3CH_2OC(O)$
TH = $CH_3(CH_2)_{13}CHC(O)$
$CH_3(CH_2)_{13}$



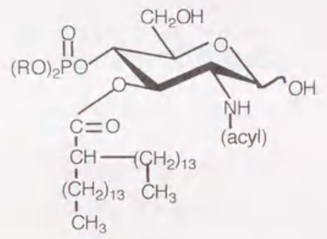
	R^1	R^2	R^3
115	TCEC	H	H
116	TCEC	H	BOM
117	H	$(PhO)_2P(O)$	BOM

BOM = $PhCH_2OCH_2$



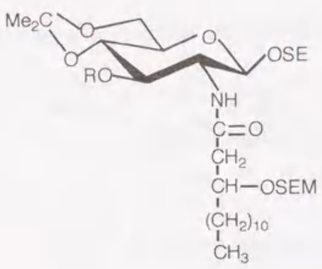
118a-f

Acyl groups	Series	n
$CH_3(CH_2)_nC(O)$	**a**	10
	b	12
	c	14
$CH_3(CH_2)_nCHCH_2CO$ (OH)	**d**	8
	e	10
	f	12



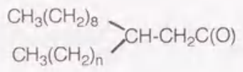
	R
119a-f	Ph
120a-f	H

Fig. 18

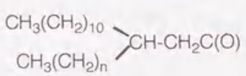


	R
17	H
121g-s	acyl (g-s)

Type I



Type II



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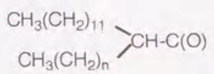
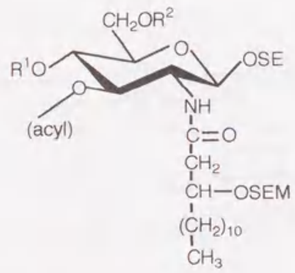
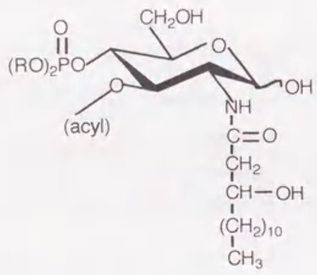


Acyl groups Series	n
g	11
h	13
i	15
j	17
k	11
l	13
m	15
n	17
o	9
p	11
q	13
r	15
s	17



	R^1	R^2
122g-s	H	H
123g-s	H	TBDMS
124g-s	$(PhO)_2P(O)$	TBDMS

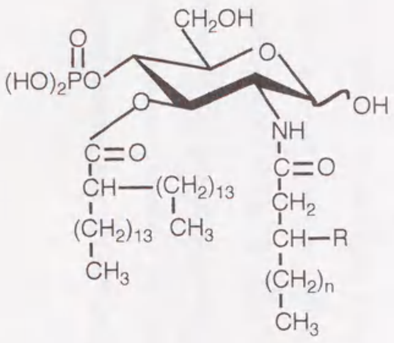


	R
125g-s	Ph
126g-s	H

Fig. 19

Chemical Industries) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) and 4-dimethylaminopyridine (DMAP), to give **121g-s**. The isopropylidene group was then removed from these compounds with aqueous acetic acid, giving **122g-s**. Introduction of a *tert*-butyldimethylsilyl group at *O*-6 and a diphenoxyphosphinyl group at *O*-4 were performed by usual methods to yield **124g-s**. Hydrolytic removal of the 2-(trimethylsilyl)ethyl, (2-trimethylsilylethoxy)methyl, and *tert*-butyldimethylsilyl groups with boron trifluoride etherate gave **125g-s**. Finally, the phenoxy groups were hydrogenolyzed to afford the desired compounds **126g-s** as described previously.

In the biological activities such as mitogenicity, IL-1 inducing, and antiviral activities, the compounds **120a**, **b**, and **e** thus obtained, showed the strong mitogenic and IL-1 inducing activities as well as those of GLA-60 (Fig. 20). The antiviral activity against vaccinia virus was expressed by the compounds **120a**, **d**, and **e**, especially the compound **120d** showed higher activity than that of GLA-60 (Fig. 21). The compound **126g** carrying a 3-undecylheptadecanoyl group at C-3 exhibited the highest activities among this series of compounds which have 3-alkyl-branched acyl groups (type I and II). The activities of **126g** were comparable to those of GLA-60 (Fig. 22). Among the compounds with 2-alkyl-branched acyl group (type III), compounds **126o-q** exhibited more potent mitogenic and IL-1 inducing activity than GLA-60 (Fig. 23). However, no significant immunostimulatory activity was expressed by compounds having a longer chain-length of alkyl-branched acyl groups. These results suggest that the naturally occurring, ester-branched 3-acyloxyacyl group can be replaced by the alkyl-branched acyl groups with retaining the biological activities of lipid A, and that these compounds with 2- or 3-alkyl-branched acyl groups showed biological activities in similar manner to those compounds with ester-branched 3-acyloxyacyl group.



	R	n	
a	H	8	C_{12}
b	H	10	C_{14}
c	H	12	C_{16}
d	OH	8	C_{12}
e	OH	10	C_{14}
f	OH	12	C_{16}

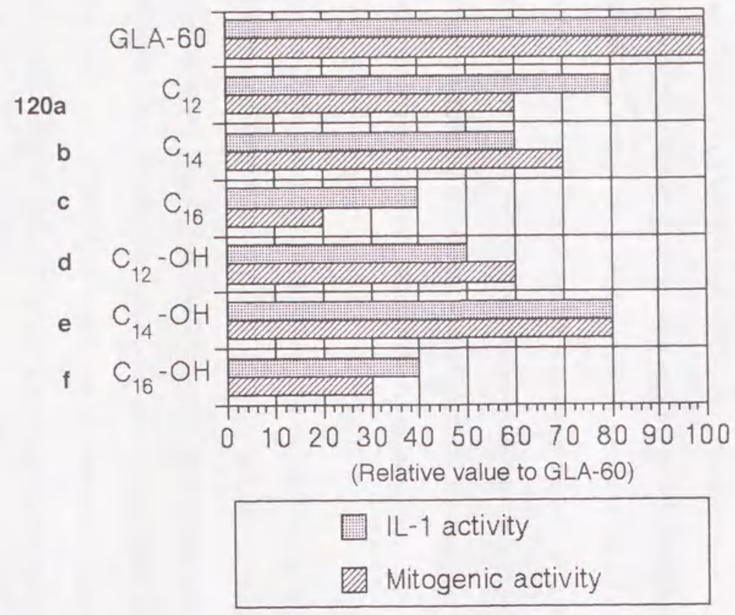
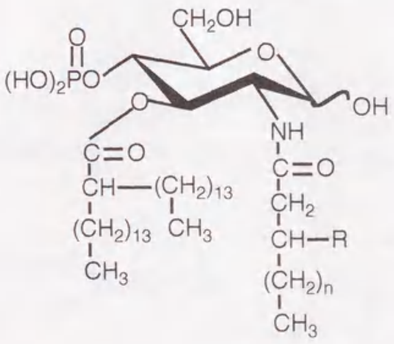
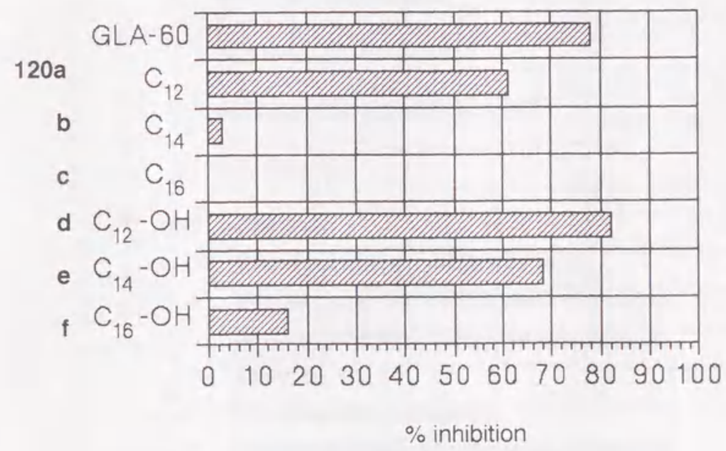


Fig. 20 Biological activities of compounds 120a-f

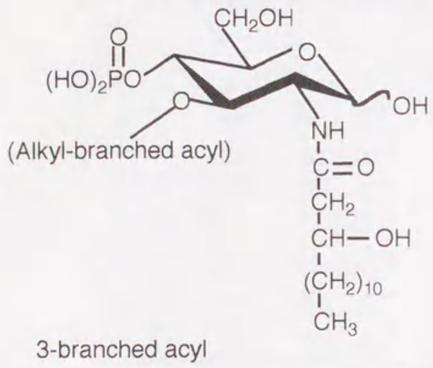


	R	n	
a	H	8	C_{12}
b	H	10	C_{14}
c	H	12	C_{16}
d	OH	8	C_{12}
e	OH	10	C_{14}
f	OH	12	C_{16}



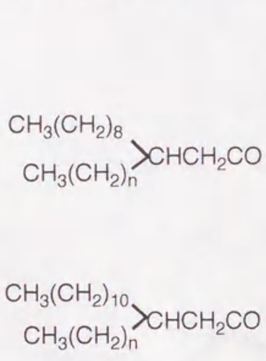
Antiviral activity was assessed by measuring the reduction of lesion numbers formed on the tail of mice infected with Vaccinia virus (see ref. 50).

Fig. 21 Antiviral activity of compounds 120a-f



3-branched acyl

	Acyl groups n
g	11
h	13
i	15
j	17
k	11
l	13
m	15
n	17



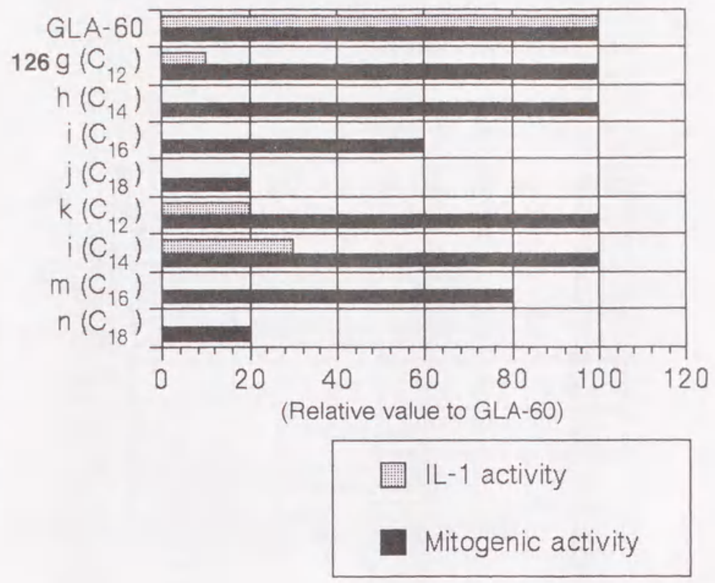
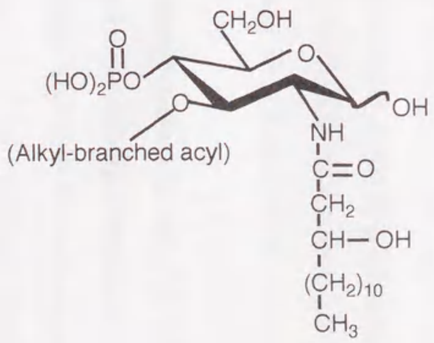
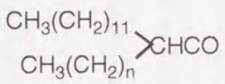


Fig. 22 Biological activities of compounds 126g-n



2-branched acyl

	Acyl groups n
o	9
p	11
q	13
r	15
s	17



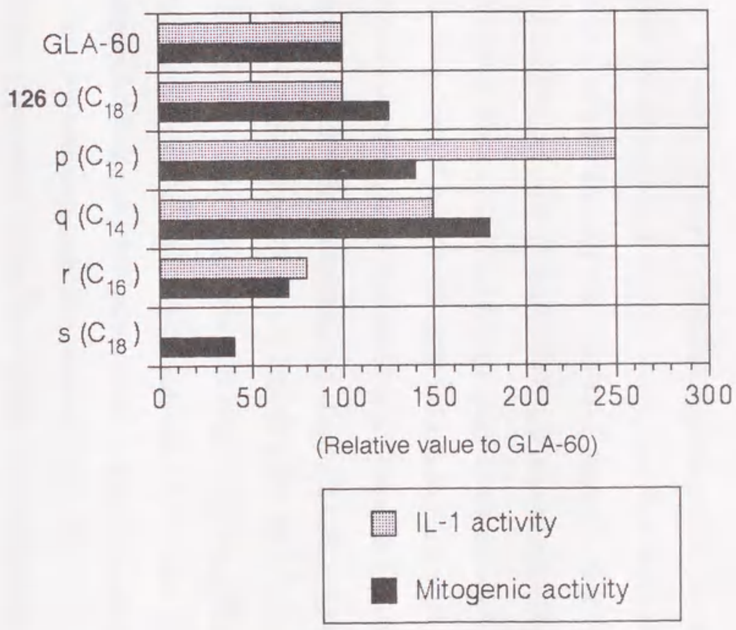


Fig. 23 Biological activities of compounds 126o-s

Experimental

The details of the preparation of compounds **120a** and **126g** are given here as examples of the general procedures used.

Benzyl 2-deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (113). To a solution of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**112**, 2 g) in dichloromethane (50 mL) were added sat. sodium hydrogen carbonate (50 mL) and 2,2,2-trichloroethoxycarbonyl chloride (2.73 g). The mixture was vigorously stirred for 2 h at room temperature. The organic layer was washed with water, dried (sodium sulfate) and concentrated. The residual syrup was chromatographed on a column of silica gel with 150:1 dichloromethane-methanol to give **113** (2.4 g, 77%), which was crystallized from ether-hexane; m.p. 157-159°C, $[\alpha]_D$ -54.9° (*c* 1.0, dichloromethane); IR 3450 (OH), 3350 (NH), 2930 and 2850 (CH), 1720 and 1550 (amide), 860 (Me_2C), and 740-700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.43, 1.52 (2 s, 6 H, Me_2C), 2.88 (broad s, 1 H, OH), 3.28 (m, 1 H, H-5), 3.45 (m, 1 H, H-2), 3.60 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.83 (t, 1 H, $J_{gem} = J_{5,6a} = 10.5$ Hz, H-6a), 3.91 (m, 1 H, H-4), 3.96 (dd, 1 H, J_{gem} 10.5, $J_{5,6b}$ 5.5 Hz, H-6b), 4.58, 4.89 (2 d, 2 H, J_{gem} 12 Hz, PhCH_2), 4.6-4.75 (m, 3 H, CCl_3CH_2 and H-1), 5.15 (m, 1 H, NH), and 7.32 (m, 5 H, Ph-*H*).

Anal. Calc. for $C_{19}H_{24}Cl_3NO_7$ (484.77): C, 47.08; H, 4.99; N, 2.89. Found: C, 47.21; H, 5.10; N, 2.66.

Benzyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (114). To a solution of **113** (2 g) in 1,2-dichloroethane (40 mL) were added 2-tetradecylhexadecanoic acid (2.4 g), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC; 1.57 g), 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 dichloromethane to give **114** (3.8 g, 97%) as a syrup; $[\alpha]_D$ -23.9° (*c* 1.0, dichloromethane); IR 3350 (NH), 2950 and 2850 (CH), 1730 (carbonyl), 1550 (NH), 860 (Me2C), and 760-700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.05-1.75 (m, 52 H, CH_2), 1.33, 1.46 (2 s, 6 H, Me_2C), 2.34 (m, 1 H, CHCO), 3.48 (m, 1 H, H-5), 3.65-3.95 (m, 3 H, H-2, 4 and 6a), 3.99 (dd, 1 H, J_{gem} 10.6, $J_{5,6b}$ 5.5 Hz, H-6b), 4.41, 4.54, 4.87, 4.93 (4 d, 4 H, J_{gem} 12 Hz, CCl_3CH_2 and PhCH_2), 4.55 (d, 1 H, $J_{1,2}$ 9.5 Hz, H-1), 5.24 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.64 (m, 1 H, NH), and 7.28 (m, 5 H, Ph-*H*).

Anal. Calc. for $C_{49}H_{82}Cl_3NO_8$ (919.56): C, 64.00; H, 8.99; N, 1.52. Found: C, 63.82; H, 9.13; N, 1.31.

Benzyl 2-deoxy-3-*O*-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (115). A solution of **114** (3.7 g) in 80% aqueous acetic acid (120 mL) was stirred for 3 h at 50°C, and then concentrated to a syrup, which was chromatographed on a column of silica gel with 100:1 dichloromethane-methanol to give **115** (3.5 g, quant.). The compound was crystallized from hexane; m.p. 73-74°C, $[\alpha]_D$ -17.2° (*c* 1.0, dichloromethane); IR 3350 (OH, NH), 2950 and 2860 (CH), 1720 (carbonyl), 1540 (NH), and 740-700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.0-1.65 (m, 52 H, CH_2), 2.38 (m, 1 H, CHCO), 2.60 (broad s, 2 H, OH), 3.42 (m, 1 H, H-5), 3.6-3.8 (m, 2 H, H-2 and 4), 3.84 (dd, 1 H, J_{gem} 12, $J_{5,6a}$ 4.5 Hz, H-6a), 3.94 (dd, 1 H, J_{gem} 12, $J_{5,6b}$ 3.3 Hz, H-6b), 4.51, 4.63, 4.83, 4.88 (4 d, 4 H, J_{gem} 12 Hz, CCl_3CH_2 and PhCH_2), 4.60 (d, 1 H, $J_{1,2}$ 8.4 Hz, H-1), 5.04 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.3 (m, 1 H, NH), and 7.31 (m, 5 H, Ph-*H*).

Anal. Calc. for $C_{46}H_{78}Cl_3NO_8$ (879.50): C, 62.82; H, 8.94; N, 1.59. Found: C, 63.01; H, 9.09; N, 1.62.

Benzyl 6-*O*-benzyloxymethyl-2-deoxy-3-*O*-(2-tetradecylhexadecanoyl)-2-(2,2,2-trichloroethoxycarbonyl)amino-β-D-glucopyranoside (116). To a cooled mixture of **115** (3.5 g), 1,1,3,3,-tetramethylurea (0.9 g), and dichloromethane (100 mL) was added benzyl chloromethyl ether (0.9 g); stirring was continued overnight at room temperature. Methanol (10 mL) was added and the solvents were evaporated. The residue was extracted with dichloromethane, washed with 2 M-hydrochloric acid and water, dried (sodium sulfate) and concentrated. The residual syrup was chromatographed on a column of silica gel with dichloromethane to afford a syrupy **116** (2.2 g, 52%); $[\alpha]_D$ -14.0°C (*c* 1.0, dichloromethane); IR 3500 (OH), 3350 (NH), 2950 and 2860 (CH), 1730 (carbonyl), 1550 (NH), and 740-700 cm^{-1} (CCl, Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.05-1.70 (m, 52 H, CH_2), 2.37 (m, 1 H, CHCO), 2.66 (broad s, 1 H, OH), 3.47 (m, 1 H, H-5), 3.75 (m, 2 H, H-2 and 4), 3.93 (d, 2 H, $J_{5,6}$ 3.7 Hz, H-6), 4.45-4.9 (m, 9 H, H-1, OCH_2O, PhCH_2, and CCl_3CH_2),5.0 (m, 1 H, NH), 5.04 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10.8 Hz, H-3), and 7.2-7.4 (m, 10 H, Ph-*H*).

Anal. Calc. for $C_{54}H_{86}Cl_3NO_9$ (999.65): C, 64.88; H, 8.67; N, 1.40. Found: C, 64.62; H, 8.79; N, 1.69.

Benzyl 2-amino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranoside (117). To a cooled solution of **116** (2.1 g) and DMAP (256 mg) in pyridine (10 mL) were added diphenyl phosphorochloridate (1.1 g) and dichloromethane (5 mL). The mixture was stirred overnight at room temperature. Methanol (5 mL) was added to the mixture, which was then concentrated. The residue was dissolved in acetic acid (100 mL), and zinc dust (16 g) was added. The mixture was vigorously stirred for 3 h at 60°C, and then filtered off and washed with dichloromethane. The filtrate and washings were combined and concentrated to a syrup, which was chromatographed on a column of silica gel with

300:1 dichloromethane-methanol to give **117** (1.8 g, 82%). The compound was crystallized from methanol; m.p. 41-43°C $[\alpha]_D$ -12.5° (*c* 1.2, dichloromethane); IR 2950 and 2860 (CH), 1750 (ester), 1600 (amine), 960 (P-O-Ph), and 780-700 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 6 H, Me), 1.0-1.65 (m, 52 H, CH_2), 2.35 (m, 3 H, CHCO and NH_2), 2.95 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 10 Hz, H-2), 3.6-3.9 (m, 3 H, H-5 and 6), 4.36 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 4.5-4.95 (m, 7 H, OCH_2O, PhCH_2, and H-4), 5.20 (t, 1 H, $J_{2,3} = J_{3,4}$ = 10 Hz, H-3), and 7.1-7.4 (m, 20 H, Ph-*H*).

Anal. Calc. for $C_{63}H_{94}NO_{10}P$ (1056.41): C, 71.63; H, 8.97; N, 1.33. Found: C, 71.84; H, 9.11; N, 1.51.

Benzyl 6-*O*-benzyloxymethyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-dodecanamido-3-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranoside (118a). To a solution of **117** (200 mg) in dichloromethane (5 mL) were added dodecanoic acid (50 mg) and WSC (60 mg). The mixture was stirred overnight at room temperature and directly chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to give **118a** (200 mg, 85%) as a syrup; $[\alpha]_D$ -13.4° (*c* 1.0, dichloromethane); IR 3300 (NH), 2950 and 2860 (CH), 1740 (ester), 1650 and 1550 (amide), 960 (P-O-Ph), and 760-700 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 9 H, Me), 1.0-1.65 (m, 70 H, CH_2), 2.07, 2.30 (2 m, 3 H, CHCO and CH_2CO), 3.72, 3.88 (2 m, 3 H, H-5 and 6), 4.98 (q, 1 H, $J_{1,2} = J_{2,3} = J_{2,NH}$ = 8.8 Hz, H-2), 4.45-4.95 (m, 8 H, H-1, 4, OCH_2O, and PhCH_2), 5.45 (t, 1 H, $J_{2,3} = J_{3,4}$ = 8.8 Hz, H-3), 5.55 (d, 1 H, $J_{2,NH}$ 8.8 Hz, NH), and 7.05-7.4 (m, 20 H, Ph-*H*).

Anal. Calc. for $C_{75}H_{116}NO_{11}P$ (1238.72): C, 72.72; H, 9.44; N, 1.13. Found: C, 72.83; H, 9.23; N, 1.20.

Other benzyl 2-acylamino-6-*O*-benzyloxymethyl-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-(2-tetradecylhexadecanoyl)-β-D-glucopyranoside (118b-f). Compound **117** was respectively esterified with tetradecanoic acid,

hexadecanoic acid, (3*RS*)-3-hydroxydodecanoic acid, (3*RS*)-3-hydroxytetradecanoic acid, and (3*RS*)-3-hydroxyhexadecanoic acid to afford the corresponding **118b-f** as described for **118a**.

2-Deoxy-4-*O*-diphenoxyphosphinyl-2-dodecanamido-3-*O*-(2-tetradecylhexadecanoyl)-D-glucopyranose (119a). A mixture of **118a** (80 mg), 10% palladium on carbon (80 mg), and ethanol (30 mL) was stirred overnight at room temperature in a hydrogen atmosphere. The catalyst was filtered off, and washed with ethanol. The filtrate and washings were combined, and concentrated. The residue was chromatographed on a column of silica gel with 50:1 dichloromethane-methanol to give **119a** (40 mg, 60%), which was lyophilized from 1,4-dioxane solution; m.p. 44-45°C $[\alpha]_D$ +8.3° (*c* 0.4, dichloromethane); IR 3400 (OH, NH), 2950 and 2860 (CH), 1760 (ester), 1660 and 1550 (amide), 980 (P-O-Ph), and 780-700 cm^{-1} (Ph); ^{1}H-n.m.r. data ($CDCl_3$): δ 0.88 (t, 9 H, Me), 1.0-1.65 (m, 70 H, CH_2), 2.05-2.35 (m, 3 H, CHCO and CH_2CO), 3.63 (broad s, 2 H, H-6), 4.00 (d, 1 H, $J_{4,5}$ 10 Hz, H-5), 4.26 (m, 1 H, $J_{1,2}$ 3.3, $J_{2,3}$ 9.5, $J_{2,NH}$ 8.8 Hz, H-2), 4.76 (q, 1 H, $J_{3,4} = J_{4,5} = J_{4,P} = 9.5$ Hz, H-4), 5.28 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 5.51 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.95 (d, 1 H, $J_{2,NH}$ 8.8 Hz, NH), and 7.1-7.4 (m, 10 H, Ph-*H*).

Anal. Calc. for $C_{60}H_{102}NO_{10}P$ (1028.44): C, 70.07; H, 10.00; N, 1.36. Found: C, 69.81; H, 10.20; N, 1.19.

Other 2-acylamino-2-deoxy-4-*O*-diphenoxyphosphinyl-3-*O*-(2-tetradecylhexadecanoyl)-D-glucopyranose (119b-f). These compounds were prepared by hydrogenolysis of **118b-f**, according to the method described for **119a**.

2-Deoxy-2-dodecanamido-4-*O*-phosphono-3-*O*-(2-tetradecylhexadecanoyl)-D-glucopyranose (120a). To a solution of **119a** (40 mg) in ethanol (30 mL) was added Adams' platinum catalyst (40 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with ethanol. The

filtrate and washing were combined and concentrated to afford **120a** (30 mg, 88%) which was lyophilized from 1,4-dioxane suspension. It gave positive test for the phosphono group using the phosphomolybdate spray-reagent; IR 3350 (OH, NH), 2940 and 2860 (CH), 1730 (ester), and 1650 and 1550 cm^{-1} (amide), and other physical data are given in Table 5.

Other 2-acylamino-2-deoxy-4-*O*-phosphono-3-*O*-(2-tetradecylhexadecanoyl)-D-glucopyranose (120b-f). Compounds **119b-f** were hydrogenated to yield **120b-f**, as described for **120a**. The physical data are recorded in Table 5.

Table 5. Some physical properties of the compounds **120a-f.**

Compd. No.	Mp (°C)[a]	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
120a	140-141	+20.5 (0.1)	$C_{48}H_{94}NO_{10}P$	66.01 (65.80)	10.93 (10.81)	1.78 (1.60)
b	159-161	+21.3 (0.1)	$C_{50}H_{98}NO_{10}P$	66.43 (66.41)	10.80 (10.92)	1.49 (1.55)
c	174-175	+23.3 (0.1)	$C_{52}H_{102}NO_{10}P$	70.10 (66.99)	11.25 (11.03)	1.63 (1.50)
d	162-163	+18.0 (0.1)	$C_{48}H_{94}NO_{11}P$	64.57 (64.62)	10.49 (10.62)	1.49 (1.57)
e	164-165	+17.0 (0.1)	$C_{50}H_{98}NO_{11}P$	65.20 (65.26)	10.99 (10.73)	1.28 (1.52)
f	170-172	+12.5 (0.1)	$C_{52}H_{102}NO_{11}P$	65.90 (65.86)	11.10 (10.84)	1.20 (1.48)

a. 1:1 CH_2Cl_2-MeOH

2-(Trimethylsilyl)ethyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(3*RS*)-3-nonylpentadecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (121g). To a solution of 2-(trimethylsilyl)-

ethyl 2-deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]-tetradecanamido]-β-D-glucopyranoside (**17**, 400 mg) in dichloromethane (20 mL) were added (3*RS*)-3-nonylpentadecanoic acid (469 mg), WSC (339 mg), and a catalytic amount of DMAP. The mixture was stirred overnight at room temperature and concentrated. The residue was chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to give **121g** (620 mg, quant.) as a syrup, $[\alpha]_D$ -12.8° (*c* 1.0, CH_2Cl_2); IR: 3300 (NH), 2930 and 2860 (CH), 1740 (ester), 1650 and 1550 (amide), and 860 and 830 cm^{-1} (Me_2C, MeSi); ^{1}H-NMR ($CDCl^3$): δ 0.0 (m, 18 H, 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, 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. Calc. 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*-[(3*RS*)-3-nonylpentadecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (122g). A solution of **121g** (570 mg) in 80% aqueous 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 dichloromethane-methanol to give **122g** (430 mg, 78%) as a syrup, $[\alpha]_D$ -1.6° (*c* 0.8, CH_2Cl_2); IR: 3300 (OH, NH), 2930 and 2860 (CH), 1760 (ester), 1650 and 1550 (amide), and 860 and 840 cm^{-1} (MeSi); ^{1}H-NMR ($CDCl_3$): δ 0.0 (m, 18 H, 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, CH_2CO), 3.10 (broad s, 2 H, 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. Calc. 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*-[(3*RS*)-3-nonylpentadecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (123g). To a solution of **122g** (390 mg) in pyridine (10 mL) was added *tert*-butyldimethylsilyl chloride (116 mg). The mixture was stirred overnight at room temperature. Methanol (5 mL) was added to the mixture, which was then concentrated. The residual syrup was chromatographed on a column of silica gel with 300:1 dichloromethane-methanol to afford syrupy **123g** (420 mg, 96%), $[\alpha]_D$ -7.3°C (*c* 1.2, CH_2Cl_2); IR: 3500 (OH), 3300 (NH), 2930 and 2850 (CH), 1730 (ester), 1640 and 1550 (amide), and 860 and 830 cm^{-1} (MeSi); ^{1}H-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, 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. Calc. 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*-[(3*RS*)-3-nonylpentadecanoyl]-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (124g). To a cooled solution of **123g** (360 mg) and DMAP (78 mg) in pyridine (5 mL) was added diphenyl phosphorochloridate (171 mg) in dichloromethane (2 mL). The

mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residue was extracted with chloroform. The extract was washed with 2M-hydrochloric acid and water, dried (sodium sulfate) and concentrated. The residual syrup was chromatographed on a column of silica gel with 200:1 dichloromethane-methanol to obtain **124g** (260 mg, 60%) as a syrup, $[\alpha]_D$ +1.6° (*c* 0.8, CH_2Cl_2); IR: 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); ^{1}H-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, CH_2CO), 3.45-3.95 (m, 9 H, H-2,5,6, $Me_3SiCH_2CH_2$, 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. Calc. 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-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(3*RS*)-3-nonylpentadecanoyl]-D-glucopyranose (125g). To a solution of **124g** (190 mg) in dichloromethane (10 mL) was added boron trifluoride etherate (0.5 mL) at 0°C. The mixture was stirred for 2 h at the same temperature. The mixture was washed with sat. sodium hydrogen carbonate and water, dried (sodium sulfate) and concentrated. The residue was chromatographed on a column of silica gel with 45:1 dichloromethane-methanol to give **125g** (106 mg, 75%), which was lyophilized from 1,4-dioxane solution, m.p. 95-97°C $[\alpha]_D$ +1.0° (*c* 1.0, CH_2Cl_2); IR: 3350 (OH, NH), 2930 and 2860 (CH), 1740 (ester), 1650 and 1540 (amide), 960 (P-O-Ph), and 780-680 cm^{-1}(Ph); ^{1}H-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, 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. Calc. 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-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(3*RS*)-3-nonylpentadecanoyl]-4-*O*-phosphono-D-glucopyranose (126g). To a solution of **125g** (90 mg) in ethanol (50 mL) was added Adams' platinum catalyst (100 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and washed with ethanol. The filtrate and washings were combined and concentrated to afford **126g** (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, IR: 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 6.

Other alkyl-branched acyl derivatives (126h-s). The physical and analytical data are recorded in Table 6.

Table 6. Some physical properties of the compounds **126g-s.**

Compd. No.	Mp (°C)[a]	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
126g	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)
h	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)
i	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)
j	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)

Table 6 (continue). Some physical properties of the compounds **126g-s.**

Compd. No.	Mp (°C)[a]	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
126k	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)
l	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)
m	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)
n	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)
o	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)
p	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)
q	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)
r	168-170	+9.4 (0.1)	$C_{50}H_{98}NO_{11}P$	64.99 (65.24)	10.98 (10.73)	1.58 (1.52)
s	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)

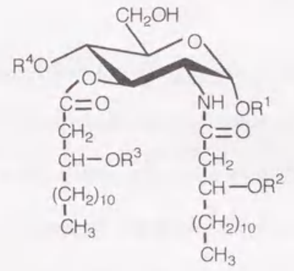
a. 50:25:4:2 $CHCl_3$-MeOH-H_2O-NH_4OH

CHAPTER 3

OTHER MONOSACCHARIDE LIPID A ANALOGS

As described for introduction, the significant biological activities of GLA-60 [2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyl-oxytetradecanoyl]-D-glucopyranose] which is an analog of the nonreducing-sugar subunit of bacterial lipid A, were discovered. In order to clarified the structure-biofunction relationship of GLA-60, a variety of 4-*O*-phosphono-D-glucosamine derivatives have been synthesized and their biological activities were investigated. We have reported that three fatty acyl chains, including one 3-acyloxyacyl or one (2 or 3)-alkyl-branched acyl groups at C-2 and 3 positions of the glucosamine skeleton, are the required features for expression of significant biological activities but no toxicity (chapter 1 and 2). On the contrary, lipid X and GLA-46, both carrying two 3-hydroxytetradecanoyl groups, did not show any activity.[21,40] In addition, GLA-47, which corresponds to the nonreducing-sugar subunit of natural lipid A and carries two 3-tetradecanoyloxytetradecanoyl groups (total of four acyl chains), had only weak immunopharmacological activities. Recently Sandoz group has showed[79,80] that the synthetic lipid A analog, 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-3,4-bis-*O*-[(3*R*)-3-hydroxytetradecanoyl]-1-*O*-phosphono-α-D-glucopyranose (SDZ MRL 935), expresses immunopharmacological activities. It has also three fatty acyl chains which are composed of three (3*R*)-3-hydroxytetradecanoyl groups.

I described[81] here the syntheses of a novel series of monosaccharidic lipid A analogs carrying three fatty acyl groups at the C-1, 2, and 3 positions (2-acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-phosphono-α-D-glucopyranoses: **135a-h**), as a part of study to elucidate the relationship between the linkage position of fatty acyl group in the



	R^1	R^2	R^3	R^4
Lipid X	$(HO)_2P(O)$	H	H	H
GLA-46	H	H	H	$(HO)_2P(O)$
GLA-47	H	C_{14}	C_{14}	$(HO)_2P(O)$
GLA-60	H	H	C_{14}	$(HO)_2P(O)$
SDZ MRL 953	$(HO)_2P(O)$	H	H	C_{14}-OH

$C_{14} = CH_3(CH_2)_{12}C(O)$

C_{14}-OH = $CH_3(CH_2)_{10}\underset{\displaystyle OH}{C}HCH_2C(O)$

Fig. 24 Structure of synthetic lipid A analogs

glucosamine skeleton and the biological activity. A facile synthesis of various 4,6-cyclic phosphate analogs (**136-139** and **140a-h**) were also described.

3-1. Synthesis and biological activity of 2-acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-phosphono-α-D-glucopyranoses

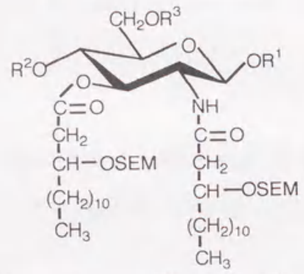
As a typical examples, general synthetic procedure used in this study is described for the preparation of **135a** as follows. Treatment of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**112**) with (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC) in dichloromethane at room temperature gave **127** in 74% yield. Compound **127** was esterified with (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid, WSC, and 4-dimethylaminopyridine (DMAP) in dichloromethane at room temperature to afford **128** in 74% yield. Hydrolytic removal of the isopropylidene group of **128** with aqueous acetic acid gave **129**, which after treatment with *tert*-butyldimethylsilyl (TBDMS) chloride in pyridine gave **130** in 92% yield. Compound **130** was phosphorilated at *O*-4 with diphenyl phosphorochloridate and DMAP in 80% yield. Hydrogenolytic removal of the benzyl group of **131** in the presence of 10% palladium on carbon in ethanol gave **132** in 61% yield. Esterification of **132** with (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid, WSC, and DMAP in dichloromethane at room temperature gave the desired **133** in 79% yield. The observed chemical shift and coupling constant of **133** in *H*-1 proton (δ 6.17, $J_{1,2}$ = 3.3 Hz) is characteristic of the α-D-pyranose configuration. Treatment of compound **133** with trifluoroacetic acid in dichloromethane for 1 h at 0°C gave 2-deoxy-4-*O*-

	R^1	R^2
112	H	H
127	C_{14}-OSEM	H
128	C_{14}-OSEM	C_{14}-OSEM

Bn = $C_6H_5CH_2$-

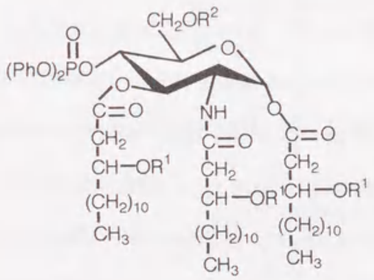
C_{14}-OSEM = $CH_3(CH_2)_{10}CH(OSEM)CH_2CO$

SEM = $Me_3SiCH_2CH_2OCH_2$-

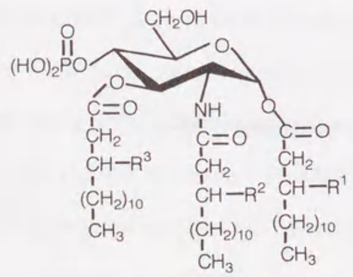


	R^1	R^2	R^3
129	Bn	H	H
130	Bn	H	TBDMS
131	Bn	$(PhO)_2P(O)$	TBDMS
132	H (α,β)	$(PhO)_2P(O)$	TBDMS

TBDMS = t-$BuMe_2Si$-



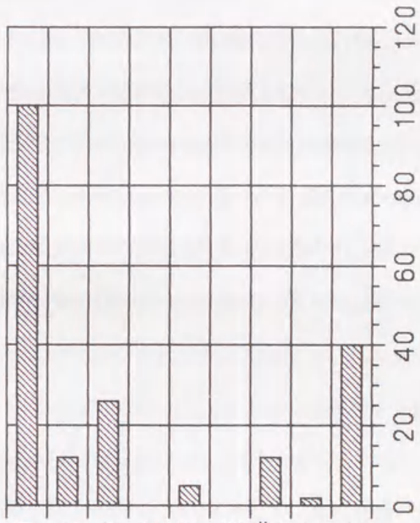
	R^1	R^2
133	SEM	TBDMS
134	H	H

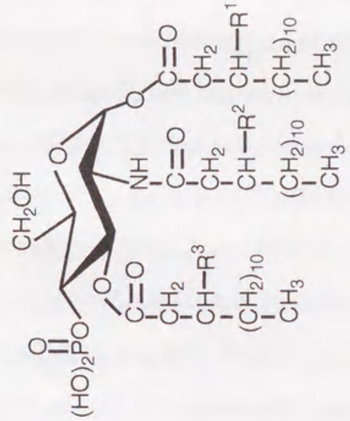


	R^1	R^2	R^3
a	OH	OH	OH
b	OH	OH	H
c	OH	H	OH
d	OH	H	H
e	H	OH	OH
f	H	OH	H
g	H	H	OH
h	H	H	H

135a-h

Fig. 25





	R^1	R^2	R^3
a	OH	OH	OH
b	OH	OH	H
c	OH	H	OH
d	OH	H	H
e	H	OH	OH
f	H	OH	H
g	H	H	OH
h	H	H	H

Fig. 26 Mitogenic activity of compounds 135a-h

diphenoxyphosphinyl-2-[(3*R*)-3-hydroxytetradecanamido]-1,3-*bis*-*O*-[(3*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranose (**134**) in 80% yield. The phenoxy groups were finally cleaved by hydrogenolysis in the presence of Adams' platinum catalyst in ethanol, to afford the desired 2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-1,3-bis-*O*-[(3*R*)-3-hydroxytetradecanoyl]-4-*O*-phosphono-α-D-glucopyranose (**135a**) in 85% yield. The syntheses of other compounds (**135b-h**) all follow essentially the same pathway.

2-acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-phosphono-α-D-glucopyranoses (**135b-h**) obtained as just described, did not show any significant immunopharmacological activities (Fig. 26).

Experimental

Benzyl 2-Deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanamido]-β-D-glucopyranoside (127). To a solution of benzyl 2-amino-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**112**, 1 g) in CH_2Cl_2 (25 mL) were added (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid (1.2 g) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSC, 1.2 g). The mixture was stirred overnight at room temperature and concentrated. The residue was chromatographed on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **127** (1.6 g, 74 %) as a syrup, $[\alpha]_D$ -46.0° (*c* 1.0, CH_2Cl_2); IR (film) 3470 (OH), 3300 (NH), 2940, 2850 (CH), 1650, 1540 (amide), 860, 840 (Si-C, Me_2C), and 730-700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 9H, Me_3Si-), 0.8-1.0 (m, 5H, Me_3Si-CH_2- and Me), 1.15-1.4 (m, 20H, -CH_2-), 1.43, 1.53 (2s, 6H, Me_2C), 2.32, 2.42 (2dd, 2H, J_{gem} = 14.7 Hz, -$COCH_2$-), 3.29 (m, 1H, H-5), 3.44-4.0 (m, 8H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, and ring protons H-2, 3, 4, and 6), 4.45-4.8 (m, 5H, PhCH_2-, -OCH_2O-, and OH), 4.88 (d, 1H, $J_{1,2}$ = 12.1 Hz, H-1), 6.44 (d, 1H, $J_{2,NH}$ = 5.9 Hz, NH), and 7.2-7.4 (m, 5H, Ph).

Anal. Calcd. for $C_{36}H_{63}NO_8Si$: C, 64.93; H, 9.53; N, 2.10. Found: C, 64.66; H, 9.70; N, 1.98.

Benzyl 2-Deoxy-4,6-*O*-isopropylidene-2-[(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanamido]-3-*O*-[(3*R*)-3-[2-(trimethylsilyl)ethoxy-methoxy]tetradecanoyl]-β-D-glucopyranoside (128). To a solution of **127** (800 mg) in CH_2Cl_2 (50 mL) were added (3*R*)-3-[2-(trimethylsilyl)ethoxy-methoxy]tetradecanoic acid (450 mg), WSC (1.0 g), 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 **128** (910 mg, 74 %) as a syrup, $[\alpha]_D$ -9.7° (*c* 1.0, CH_2Cl_2); IR (film): 3300 (NH), 2930, 2850 (CH), 1740 (ester) 1650, 1550 (amide), 860, 840 (Si-C, Me_2C), and 730-700 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.88-1.0 (m, 10H, Me_3Si-CH_2- and Me), 1.1-1.24 (m, 40H, -CH_2-), 1.34, 1.45 (2s, 6H, Me_2C), 2.25-2.65 (m, 4H, -$COCH_2$-), 3.30 (m, 1H, H-5), 3.45-4.1 (m, 10H, Me_3SiCH_2-CH_2-, H-3 of C_{14}-OSEM, H-2, 4, and 6), 4.5-4.7 (m, 6H, -OCH_2O- and PhCH_2-), 4.86 (d, 1H, $J_{1,2}$ = 12.3 Hz, H-1), 5.09 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 6.12 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH), and 7.2-7.35 (m, 5H, Ph).

Anal. Calcd. for $C_{56}H_{103}NO_{11}Si_2$: C, 65.78; H, 10.15; N, 1.37. Found: C, 65.50; H, 10.40; N, 1.19.

Benzyl 2-Deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetrade-canamido]-3-*O*-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoyl]-β-D-glucopyranoside (129). A solution of **128** (900 mg) in 80% aqueous acetic acid (100 mL) was stirred for 2 h at 50°C, and then concentrated to a syrup, which was chromatographed on a column of silica gel with 100:1 CH_2Cl_2-MeOH to give **129** (735

mg, 85%) as a syrup, $[\alpha]_D$ -22.1° (*c* 0.9, CH_2Cl_2); IR (film): 3350 (OH, NH), 2930, 2850 (CH), 1720 (ester) 1650, 1560 (amide), 860, 830 (Si-C), and 760-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 18H, Me_3Si-), 0.88-1.0 (m, 10H, Me_3Si-C*H*$_2$- and Me), 1.2-1.8 (m, 40H, -CH_2-), 2.0 (s, 1H, OH), 2.25-2.6 (m, 4H, -$COCH_2$-), 3.40 (m, 1H, H-5), 3.50-4.14 (m, 11H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-2, 4, 6 and OH), 4.5-4.9 (m, 6H, -OCH_2O- and PhC*H*$_2$-), 4.85 (d, 1H, $J_{1,2}$ = 12.3 Hz, H-1), 5.03 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.7 Hz, H-3), 6.22 (d, 1H, $J_{2,NH}$ = 9.0 Hz, NH), and 7.27-7.34 (m, 5H, Ph).

Anal. Calcd. for $C_{53}H_{99}NO_{11}Si_2$: C, 64.79; H, 10.16; N, 1.43. Found: C, 65.00; H, 10.25; N, 1.40.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-3-*O*-[(3*R*)-3-[2-(trimethylsilyl)-ethoxymethoxy]tetradecanoyl]-β-D-glucopyranoside (130). To a solution of **129** (700 mg) in pyridine (50 mL) was added *tert*-butyldimethylsilyl chloride (310 mg). The mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residual syrup was chromatographed on a column of silica gel with 300:1 CH_2Cl_2-MeOH to obtain **130** (718 mg, 92%) as a syrup, $[\alpha]_D$ -18.4° (*c* 1.0, CH_2Cl_2); IR (film): 3500 (OH), 3300 (NH), 2930, 2850 (CH), 1750 (ester), 1660, 1540 (amide), 860, 840 (Si-C), and 780-700 cm^{-1} (Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.86-1.0 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.2-1.75 (m, 40H, -CH_2-), 2.3-2.65 (m, 4H, -$COCH_2$-), 3.36 (m, 1H, H-5), 3.45-3.7 (m, 6H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM), 3.8-4.1 (m, 5H, H-2,4,6, and OH), 4.45-4.75 (m, 6H, -OCH_2O- and $PhCH_2$-), 4.86 (d, 1H, $J_{1,2}$ = 12.1 Hz, H-1), 4.99 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.8 Hz, H-3), 6.06 (d, 1H, $J_{2,NH}$ = 9.2 Hz, NH), and 7.27-7.39 (m, 5H, Ph).

Anal. Calcd. for $C_{59}H_{113}NO_{11}Si_3$: C, 64.61; H, 10.38; N, 1.28. Found: C, 64.44; H, 10.59; N, 1.01.

Benzyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-3-*O*-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoyl]-β-D-glucopyranoside (131). To a cold solution of **130** (700 mg) and DMAP (198 mg) in pyridine (20 mL) was added diphenyl phosphorochloridate (480 mg). The mixture was stirred overnight at room temperature. Methanol was added to the mixture, which was then concentrated. The residue was extracted with $CHCl_3$. The extract was washed with 2M-hydrochloric acid and water, dried (Na_2SO_4) and concentrated. The residual syrup was chromatographed on a column of silica gel with 400:1 CH_2Cl_2-MeOH to give syrupy **131** (678 mg, 80%), $[\alpha]_D$ +2.6° (*c* 0.8, CH_2Cl_2); IR (film): 3300 (NH), 2950, 2850 (CH), 1740 (ester), 1650, 1560 (amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.8-1.0 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.1-1.75 (m, 40H, -CH_2-), 2.25-2.4 (m, 4H, -$COCH_2$-), 3.48-4.02 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-2, 5, and 6), 4.48-4.92 (m, 8H, -OCH_2O-, PhC*H*$_2$-, H-1 and 4), 5.5 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.5 Hz, H-3), 6.29 (d, 1H, $J_{2,NH}$ = 8.4 Hz, NH), and 7.07-7.39 (m, 15H, Ph).

Anal. Calcd. for $C_{71}H_{122}NO_{14}PSi_3$: C, 64.17; H, 9.25; N, 1.05. Found: C, 64.22; H, 9.51; N, 1.20.

6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-3-*O*-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoyl]-D-glucopyranose (132). A mixture of **131** (650 mg), 10% palladium on carbon (250 mg) and EtOH (30 mL) was stirred overnight at room temperature in a hydrogen atmosphere. The catalyst was then filtered off, and the mixture was washed with ethanol. The filtrate and washings were

combined and concentrated. The residue was chromatographed on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **132** (369 mg, 61%) as a syrup, $[\alpha]_D$ +34.8° (*c* 0.9, CH_2Cl_2); IR (film): 3500 (OH), 3300 (NH), 2930, 2850 (CH), 1750 (ester), 1660, 1540 (amide), 960 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 24H, Me-Si-), 0.85-0.98 (m, 19H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.14-1.75 (m, 40H, -CH_2-), 2.26-2.42 (m, 4H, -$COCH_2$-), 3.43 (m, 1H, H-5), 3.50-4.06 (m, 10H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-2, 4, and 6), 4.49-4.68 (m, 4H, -OCH_2O-), 4.75 (broad s, 1H, OH), 5.26 (broad s, 1H, H-1), 5.52 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.8 Hz, H-3), 6.29 (d, 1H, $J_{2,NH}$ = 8.6 Hz, NH), and 7.07-7.39 (m, 10H, Ph).

Anal. Calcd. for $C_{64}H_{116}NO_{14}PSi_3$: C, 62.05; H, 9.44; N, 1.13. Found: C, 62.13; H, 9.66; N, 1.02.

6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanamido]-1,3-bis-*O*-[(3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoyl]-α-D-glucopyranose (133). To a solution of **132** (100 mg) in CH_2Cl_2 (30 mL) were added (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid (34 mg), WSC (31 mg), and a catalytic amount of DMAP. The mixture was stirred overnight at room temperature, and concentrated. The residue was chromatographed on a column of silica gel with 200:1 CH_2Cl_2-MeOH to give **133** (100 mg, 79%) as a syrup, $[\alpha]_D$ +54.2° (*c* 0.8, CH_2Cl_2); IR (film): 3350 (NH), 2930, 2850 (CH), 1740 (ester), 1680, 1590 (amide), 950 (P-O-Ph), 860, 840 (Si-C), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.0 (m, 33H, Me-Si-), 0.85-0.98 (m, 24H, *tert*-Bu, Me_3Si-C*H*$_2$- and Me), 1.12-1.75 (m, 60H, -CH_2-), 2.3-2.6 (m, 6H, -$COCH_2$-) 3.34-4.10 (m, 13H, Me_3SiCH_2-C*H*$_2$-, H-3 of C_{14}-OSEM, H-2, 5, and 6), 4.36-4.93 (m, 7H, -OCH_2O- and H-4), 5.43 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz, H-3), 6.17 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 6.40 (d, 1H, $J_{2,NH}$ = 8.8 Hz, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd. for $C_{84}H_{156}NO_{17}PSi_4$: C, 63.24; H, 9.85; N, 0.88. Found: C, 63.01; H, 9.90; N, 0.69.

2-Deoxy-4-*O*-diphenoxyphosphinyl-2-[(3*R*)-3-hydroxytetradecanamido]-1,3-bis-*O*-[(3*R*)-3-hydroxytetradecanoyl]-α-D-glucopyranose (134). To a solution of **133** (80 mg) in CH_2Cl_2 (4 mL) was added trifluoroacetic acid (2 mL) at 0°C. The mixture was stirred for 1 h at the room temperature, and then concentrated. The residue was chromatographed on a column of silica gel with 40:1 CH_2Cl_2-MeOH to give **134** (43 mg, 80%)as a syrup, $[\alpha]_D$ +10.9° (*c* 0.3, CH_2Cl_2); IR (KBr) 3600-3200 (OH, NH), 2930, 2850 (CH), 1740 (ester), 1640, 1540 (amide), 950 (P-O-Ph), and 780-690 cm^{-1}(Ph); ^1H NMR ($CDCl_3$) δ 0.88 (m, 9H, Me), 1.12-1.75 (m, 60H, -CH_2-), 2.28-2.45 (m, 6H, -$COCH_2$-) 3.65-4.85 (m, 7H, H-3 of C_{14}-OH, H-2, 5, 6, and OH), 5.08 (q, 1H, $J_{3,4}$ = $J_{4,5}$ = $J_{4,P}$ = 9-10 Hz, H-4), 5.43 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 10.5 Hz, H-3), 5.60 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 6.25 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NH), and 7.1-7.4 (m, 10H, Ph).

Anal. Calcd. for $C_{60}H_{100}NO_{14}P$: C, 66.09; H, 9.24; N, 1.28. Found: C, 65.98; H, 9.37; N, 1.30.

2-Deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-1,3-bis-*O*-[(3*R*)-3-hydroxytetradecanoyl]-4-*O*-phosphono-α-D-glucopyranose (135a). To a solution of **134** (15 mg) in EtOH (20 mL) was added Adams' platinum catalyst (10 mg), and the mixture was stirred overnight in a hydrogen atmosphere. The catalyst was filtered off, and the mixture was washed with EtOH. The filtrate and washings were combined and concentrated to afford **135a** (13 mg, 85%), which was lyophilized from 1,4-dioxane suspension; IR (KBr) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), and 1680, 1590 cm^{-1}(amide). Other physical and analytical data are given in Table 7.

Other 2-Acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-phosphono-α-D-glucopyranoses (135b-h). Compounds (**135b-h**) were obtained from compound **112** and tetradecanoic acid and/or (3*R*)-3-[2-(trimethylsilyl)ethoxymethoxy]tetradecanoic acid, respectively, according to the methods described for **135a**. The physical and analytical data are shown in Table 7.

Table 7. Some physical properties of the compounds **135a-h**.

Compd. No.	Mp (°C)	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
135a	161-164	+41.7 (0.2)	$C_{48}H_{92}NO_{14}P$	61.25 (61.45)	9.98 (9.88)	1.33 (1.49)
b	146-148	+18.7 (0.2)	$C_{48}H_{92}NO_{13}P$	62.78 (62.51)	10.26 (10.05)	1.33 (1.52)
c	133-136	+21.9 (0.2)	$C_{48}H_{92}NO_{13}P$	62.38 (62.51)	10.30 (10.05)	1.44 (1.52)
d	151-153	+12.5 (0.2)	$C_{48}H_{92}NO_{13}P$	63.77 (63.62)	10.01 (10.23)	1.63 (1.55)
e	132-135	+41.4 (0.2)	$C_{48}H_{92}NO_{13}P$	62.36 (62.51)	10.20 (10.05)	1.44 (1.52)
f	174-177	+60.0 (0.2)	$C_{48}H_{92}NO_{12}P$	63.60 (63.62)	10.33 (10.23)	1.50 (1.55)
g	176-178	+33.3 (0.2)	$C_{48}H_{92}NO_{12}P$	63.38 (63.62)	10.50 (10.23)	1.44 (1.55)
h	106-108	+26.3 (0.2)	$C_{48}H_{92}NO_{11}P$	64.88 (64.76)	10.19 (10.42)	1.63 (1.57)

a. 1:1 $CHCl_3$-MeOH

3-2. Synthesis of novel monosaccharide lipid A analogs containing 4,6-cyclic phosphate

The nonprotected 4-*O*-phosphono-D-glucosamine derivatives (**108c, GLA-60, 126l, 126q,** and **135a-h**) was employed as a starting material for the synthesis of the desired 4,6-cyclic phosphate (**136-139** and **140a-h**). The preparation of **108c, 126l, 126q,** and **135a-h** were described previously. Treatment of the nonprotected 4-*O*-phosphono-D-glucosamine derivatives (**108c, GLA-60, 126l, 126q,** and **135a-h**) with 1,3-dicyclohexylcarbodiimide in a large quantity of chloroform at room temperature gave **136-139** and **140a-h** in 92-95% yields. When 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) was used as the cyclization reagent, the coupled compound with 4-*O*-phosphono-D-glucosamine derivative and WSC was just formed, not cyclic phosphate.

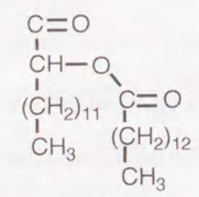
The cyclic phosphates as just described, are chemically more stable than the corresponding 4-*O*-phosphono-D-glucosamine derivatives. It might affect the immunopharmacological activities.

Experimental

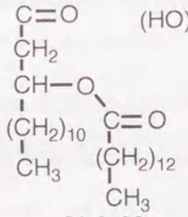
The details of the preparation of compound **136** is given here as examples of the general procedures used.

2-Deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(2*RS*)-2-tetradecanoyloxytetradecanoyl]-D-glucopyranose 4,6-Cyclic Phosphate (136). To a solution of 2-Deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-3-*O*-[(2RS)-2-tetradeca-

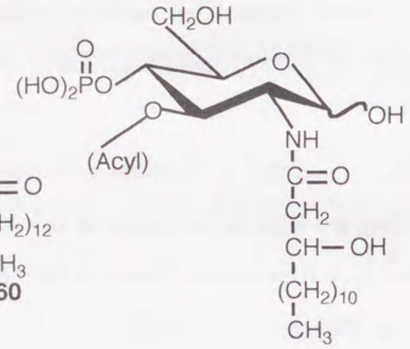
Acyl =

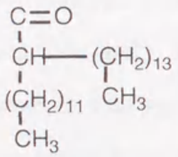


108c

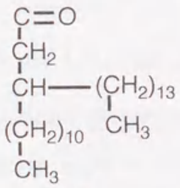


GLA-60

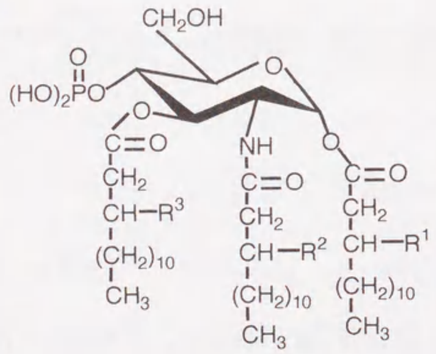




126q



126l

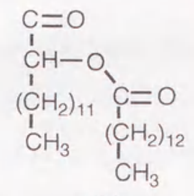


135a-h

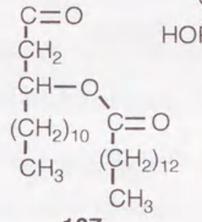
	R^1	R^2	R^3
a	OH	OH	OH
b	OH	OH	H
c	OH	H	OH
d	OH	H	H
e	H	OH	OH
f	H	OH	H
g	H	H	OH
h	H	H	H

Fig. 27

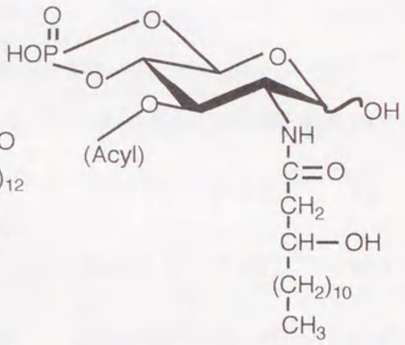
Acyl =



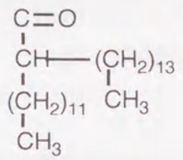
136



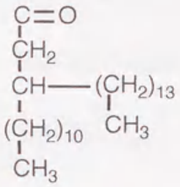
137



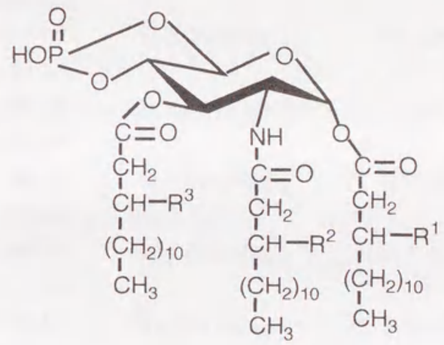
136-139



138



139



140a-h

Fig. 28

Table 8. Some physical properties of the compounds **136-139**. and **140a-h**

Compd. No.	Mp (°C)	$[\alpha]_D$ (°) (*c*)[a]	Molecular formula	Found (Calcd) % of C	H	N
136	158-160	-3.8 (0.5)	$C_{48}H_{90}NO_{12}P$	63.80 (63.76)	10.26 (10.03)	1.40 (1.55)
137	168-170	-2.0 (0.5)	$C_{48}H_{90}NO_{12}P$	63.88 (63.76)	10.28 (10.03)	1.50 (1.55)
138	175-177	+9.4 (0.6)	$C_{48}H_{92}NO_{10}P$	66.08 (65.95)	10.50 (10.61)	1.44 (1.60)
139	176-179	+10.3 (0.2)	$C_{48}H_{92}NO_{10}P$	66.20 (65.95)	10.59 (10.61)	1.63 (1.60)
140a	141-144	+25.0 (0.1)	$C_{48}H_{90}NO_{13}P$	62.45 (62.65)	9.98 (9.86)	1.33 (1.52)
b	134-136	+15.0 (0.1)	$C_{48}H_{90}NO_{12}P$	63.88 (63.76)	10.26 (10.03)	1.33 (1.55)
c	170-173	+13.6 (0.1)	$C_{48}H_{90}NO_{12}P$	63.48 (63.76)	10.30 (10.03)	1.44 (1.55)
d	176-179	+26.0 (0.2)	$C_{48}H_{90}NO_{11}P$	64.77 (64.91)	10.01 (10.21)	1.63 (1.58)
e	181-184	+25.0 (0.1)	$C_{48}H_{90}NO_{12}P$	63.90 (63.76)	10.26 (10.03)	1.49 (1.55)
f	190-193	+22.7 (0.2)	$C_{48}H_{90}NO_{11}P$	64.88 (64.91)	10.38 (10.21)	1.50 (1.58)
g	213-216	+31.7 (0.1)	$C_{48}H_{90}NO_{11}P$	64.98 (64.91)	10.50 (10.21)	1.44 (1.58)
h	193-196	+38.0 (0.2)	$C_{48}H_{90}NO_{10}P$	66.28 (66.10)	10.19 (10.40)	1.63 (1.61)

a. 1:1 $CHCl_3$-MeOH

noyloxytetradecanoyl]-D-glucopyranose (**108c**, 20 mg) in $CHCl_3$ (10 mL) was added 1,3-dicyclohexylcarbodiimide (DCC: 5 mg). The mixture was performed by gel filtration (Sephadex LH-20) with $CHCl_3$:MeOH (1:1) to give **136** (18 mg, 95 %), which was

lyophilized from 1,4-dioxane suspension; IR (KBr) 3300 (OH, NH), 2930, 2850 (CH), 1740 (ester), and 1680, 1590 cm^{-1}(amide). Other physical and analytical data are given in Table 8.

Other 4,6-Cyclic Phosphates (**137-139** and **140a-h**). Compounds (**137-139** and **140a-h**) were obtained from corresponding compounds **GLA-60, 126l, 126q** and **135a-h** in a high yields, according to the methods described for **136**. The physical and analytical data are shown in Table 8.

SUMMARY

In this study the author synthesized a number of novel non-reducing sugar subunit analogs to investigate the structure-activity relationship of bacterial lipid A, and demonstrated clearly that the importance of kinds and binding sites of acyl groups for expressing immunopharmacological activity.

With respect to the carbon chain length of fatty acids substituents, a series of 2-deoxy-2-[(3*R*)-3-hydroxyacyl]amino-4-*O*-phosphono-3-*O*-[(3*R*)-3-tetradecanoyloxy-tetradecanoyl]-D-glucoses (**14-16**) and 3-*O*-acyloxyacyl-2-deoxy-2-[(3*R*)-3-hydroxy-tetradecanamido]-4-*O*-phosphono-D-glucoses (**74-91**) were synthesized. In biological activities, compound (**15**) carrying 3-hydroxydodecanoyl group at C-2 position showed stronger mitogenic activity than that of GLA-60. Compound (**86**) which has odd-numbered tridecanoyloxytetradecanoyl group at C-3 position also exhibited significant mitogenic and IL-1 inducing activities. However, no significant biological activity was expressed by compounds in which the principal chain of acyloxyacyl group was shorter than 12 carbon atoms. This result indicates that the carbon chain length of acyl group strongly affects the expression of the biological activity. In various carbon chain length, C_{12} and C_{14} carbon chain length of 3-hydroxyacyl group at C-2 position, C_{14} carbon atoms of the principal chain of acyloxyacyl group at C-3, and C_{12} to C_{14} carbon chain length of 3-acyloxytetradecanoyl group of GLA-60 derivatives are preferred for expression of beneficial lipid A's activities. Since the immunopharmacological activities of synthetic *Salmonella*-type lipid A which is composed of C_{12}, C_{14}, and C_{16} carbon chain length of acyl group are significantly less[82-84] active than those of synthetic *E. Coli* lipid A having only C_{12} and C_{14} carbon chain length of acyl constituents, the results for compounds (**15** and **86**) can be considered reasonable.

Three types of analogs containing 2-hydroxyacyl and 2-acyloxytetradecanoyl groups [3-*O*-[(2*RS*)-2-acyloxytetradecanoyl]-2-deoxy-2-[(2*RS*)-2-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**100a-d**), 3-*O*-[(2*RS*)-2-acyloxytetradecanoyl]-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**108a-d**), and 2-[(2*RS*)-2-hydroxyacyl]amino-2-deoxy-3-*O*-[(3*R*)-3-tetradecanoyloxytetradecanoyl]-4-*O*-phosphono-D-glucoses (**111e-h**)] and analogs carrying an alkyl-branched acyl group at C-3 of GLA-60 [2-(acyl)amino-2-deoxy-3-*O*-(2-tetradecylhexadecanoyl)-4-*O*-phosphono-D-glucoses (**120a-f**) and 3-*O*-(2- or 3-alkylacyl)-2-deoxy-2-[(3*R*)-3-hydroxytetradecanamido]-4-*O*-phosphono-D-glucoses (**126g-s**)] were synthesized to elucidate the biological effect of different kinds of acyl substituents. The synthetic analogs of GLA-60 having the 2-hydroxyacyl and 2-acyloxytetradecanoyl groups showed weaker activities than GLA-60. This suggests the importance of the presence of 3-*O*-hydroxyacyl and 3-acyloxytetradecanoyl groups in GLA-60 homologs.

Concerning the alkyl-branched acyl substituents, very interesting results were obtained. Some of the synthetic compounds (**120a,b,d**, and **e**) having 2-tetradecylhexadecanoyl group showed the strong mitogenic, IL-1 inducing, and antiviral activities, especially compounds (**120d**) exhibited higher antiviral activity than GLA-60. The biological activities were also observed by the synthetic analogs linking 2- or 3-alkyl-branched acyl groups. These results suggest that the introduction of the suitable carbon chain length of alkyl-branched acyl groups in GLA-60 homologs enhance the immunostimulate potency, although the introduction of 2-acyloxytetradecanoyl groups reduce that potency.

As a part of study to elucidate the relationship between the linkage position of fatty acyl groups and the biological activities, 2-acylamino-1,3-bis-*O*-acyl-2-deoxy-4-*O*-

phosphono-α-D-glucoses (**135a-h**) were synthesized. All the synthetic derivatives did not show any lipid A's activities, indicating that the binding of acyl groups at C-2 and 3 positions of 4-*O*-phosphono-D-glucosamine skeleton are essentially required for expression of the biological activities.

To obtain the chemical stable analogs, the method of the cyclization of 4-*O*-phosphono group has been established and various 4,6-cyclic phosphate analogs (**136-139** and **140a-h**) were synthesized.

In conclusion, I have systematically synthesized a series of GLA-60 homologs and demonstrated the biological requirement for expression of beneficial lipid A activities. I believe that these resulting structural compounds should give a important information for the development of new type of biological response modifiers and might be applied to clinical trial in the near future.

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