Synthesis and Biological Activities of Analogs of a Lipid A Biosynthetic Precursor: 1-*O*-Phosphonooxyethyl-4′-*O*-phosphono-disaccharides with (*R*)-3-Hydroxytetradecanoyl or Tetradecanoyl Groups at Positions 2, 3, 2′ and 3′

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Two novel analogs of a biosynthetic precursor of lipid A (2) were synthesized. The one analog (3) has acyl groups identical to those of 2, and the other (4) has tetradecanoyl groups in place of the (*R*)-3-hydroxytetradecanoyl groups of 2. Both 3 and 4 possess an α-glycosidically-bound phosphonooxyethyl group in place of the α-glycosyl phosphate group of 2.

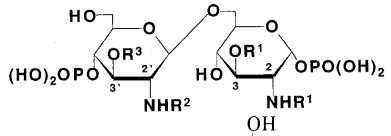
Compounds 3 and 4 exhibited definite antitumor activity against Meth A fibrosarcoma and low toxicity in rabbits, as the original compound 2 does. The replacement of the hydroxytetradecanoyl groups with tetradecanoyl groups barely affected the antitumor activity, but slightly enhanced the toxicity in rabbits.

Keywords lipid A analog; phosphate group; phosphonooxyethyl group; antitumor activity; toxicity; Meth A fibrosarcoma; tetradecanoic acid; (*R*)-3-hydroxytetradecanoic acid

Lipid A, which is the lipophilic component of bacterial lipopolysaccharide (LPS), is now known to be responsible for the various biological activities of LPS, both beneficial and toxic. Shiba and his colleagues deduced the complete chemical structure for *Escherichia coli* (*E. coli*) lipid A (**1**)[1] and unequivocally confirmed it by total synthesis.[2] They also synthesized a biosynthetic precursor (**2**)[3] which is characterized by the presence of four fatty acyl groups each with a free hydroxyl, in contrast to lipid A (**1**), which contains only two corresponding groups at positions 2 and 3 and two acyloxyacyl groups at positions 2′ and 3′ of the 1,4′-bisphosphorylated disaccharide backbone.

Biological study with pure synthetic compounds clearly demonstrated that precursor **2** is less endotoxic than the complete lipid A (**1**). Compound **2**, in spite of its low toxicity, exhibits a definite ability to induce activity of interferon-α, β and tumor necrosis activity in adequately primed mice, though in each case the potency is slightly weaker than **1**.[4] This finding suggested that compound **2**, possessing no 3-acyloxyacyl group, could serve as a "key compound" which may lead to the development of new compounds with low toxicity but which retain the beneficial bioactivities.

On the other hand, we recently reported that, with respect to antitumor activity, the 1-*O*-phosphono group of lipid A is not essential, but replaceable by other acidic groups such as a phosphonooxyethyl group.[5] 1-α-*O*-Phosphonooxyethyl derivatives could be more readily synthesized than the corresponding α-phosphate because of the chemical stability of the former. However, the phosphonooxyethyl derivatives of **1** were unfortunately toxic, though they showed high antitumor activity comparable to that of **1**. Therefore, it seemed interesting to test the α-phosphonooxyethyl derivatives which have all four linear fatty acyl groups without the branched 3-acyloxyacyl moieties on the disaccharide backbone.



1 : $R^1 = CH_3(CH_2)_{10}CHCH_2CO$ (OH; O-CO-$(CH_2)_{10}CH_3$)
$R^2 = CH_3(CH_2)_{10}CHCH_2CO$ (OH; O-CO-$(CH_2)_{12}CH_3$)
$R^3 = CH_3(CH_2)_{10}CHCH_2CO$ (O-CO-$(CH_2)_{10}CH_3$)

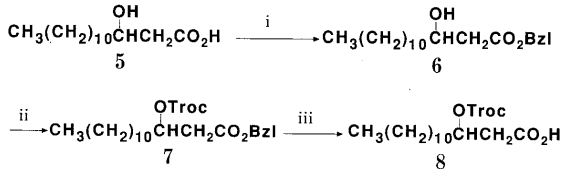
2 : $R^1 = R^2 = R^3 = CH_3(CH_2)_{10}CHCH_2CO$ (OH)

Fig. 1

In this paper we describe the synthesis as well as the toxic and antitumor activity of two analogs of **2** having either four 3-hydroxytetradecanoyl (**3**) or four tetradecanoyl groups (**4**), respectively, at positions 2, 3, 2′ and 3′ on the β(1→6)glucosamine disaccharide with 1-α-*O*-phosphonooxyethyl and 4′-*O*-phosphono groups.

Chemistry The basic strategy for the synthesis of **3** and **4** was similar to that employed in our previous synthesis of the 1-*O*-phosphonooxyethylated derivatives of lipid A.[5] In this strategy, glycosyl bromides, as an important key intermediate for the formation of disaccharides, were prepared by treatment of the corresponding glycosyl acetate with HBr–AcOH. The glycosyl bromide required for the synthesis of compound **3** contains a 3-hydroxytetradecanoyl group. By protection of this hydroxyacyl function with an acid-stable 2,2,2-trichloroethoxycarbonyl (Troc) group, the bromide was successfully obtained and used in the following glycosidation reaction. In an earlier work, where the benzyl group was employed for the protection of the hydroxyacyl function, the corresponding bromide was prepared using a Vilsmeier reagent.[6] This latter method was so moisture sensitive that the result was sometimes not satisfactory. The hydroxytetradecanoyl functions bound to the reducing glucosamine were protected with a benzyl group as in the previous works.[2,5]

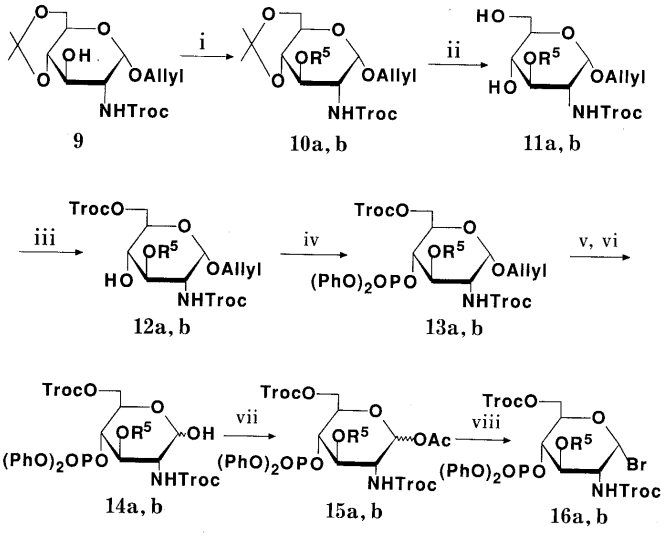
The protected fatty acid **8** with a Troc group was prepared from (*R*)-3-hydroxytetradecanoic acid (**5**), as shown in Chart 1. Reaction of (*R*)-3-hydroxytetradecanoic acid (**5**) with benzyl bromide in the presence of triethylamine (Et_3N) gave the benzyl ester **6**. Treatment of **6** with Troc–Cl in pyridine afforded a 3-*O*-Troc derivative **7**,



Bzl : $C_6H_5CH_2$ Troc : CCl_3CH_2OCO

i) BzlBr/Et_3N ii) Troc-Cl, Py iii) H_2, Pd/C

Chart 1



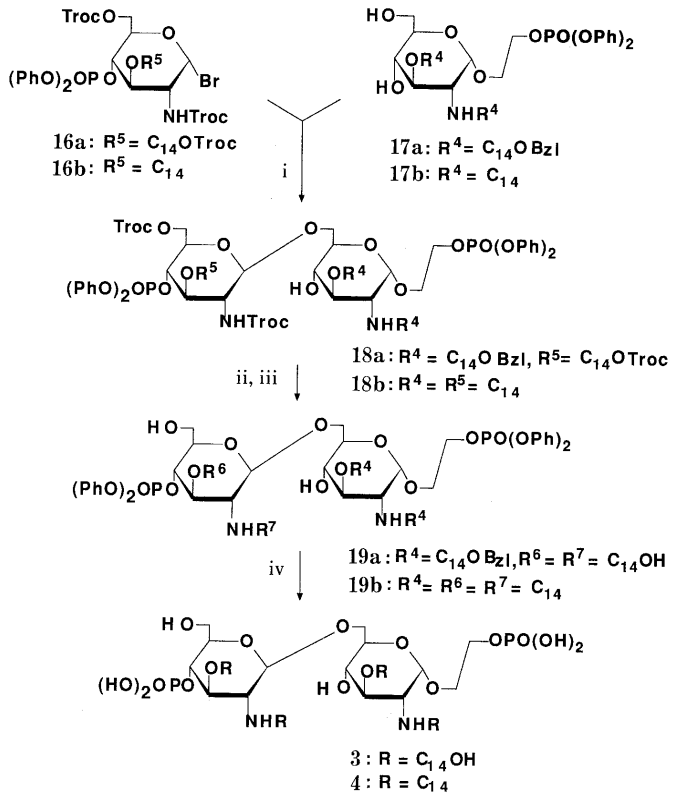
Troc : CCl_3CH_2OCO Allyl : $CH_2=CHCH_2$

a : R^5=(*R*)-3-(2, 2, 2-trichloroethoxycarbonyloxy) tetradecanoyl

b : R^5=tetradecanoyl

i) R^5OH, DMAP, DCC ii) 90% AcOH iii) Troc-Cl, Py iv) $(PhO)_2POCl$, DMAP v) (COD) Ir $[PCH_3(C_6H_5)_2]_2^+PF_6^-$ vi) I_2-H_2O vii) Ac_2O, Py viii) HBr/AcOH

Chart 2



Troc : CCl_3CH_2OCO

C_{14}OTroc : (*R*)-3-(2, 2, 2-trichloroethoxycarbonyloxy)tetradecanoyl

C_{14}OBzl : (*R*)-3-benzyloxytetradecanoyl

C_{14}OH : (*R*)-3-hydroxytetradecanoyl

C_{14} : tetradecanoyl

i) $Hg(CN)_2$ ii) Zn/AcOH iii) C_{14}OH-OBt or C_{14}-OBt iv) H_2

Chart 3

which was subjected to hydrogenolysis over palladium carbon in tetrahydrofuran (THF) to give the desired (*R*)-3-trichloroethoxycarbonyloxytetradecanoic acid (**8**). The optical purity of **8** was confirmed in the following manner. Treatment of **8** with *S*-(−)-1-phenylethylamine in the presence of dicyclohexylcarbodiimide (DCC) gave a single spot (*Rf*, 0.35) on thin layer chromatography (TLC) (benzene–EtOAc, 10 : 1), whereas, treatment of **8** with (±)-1-phenylethylamine under the same reaction conditions gave a mixture of two diastereomers (*Rf*, 0.35 and 0.39). The *Rf* value of 0.39 should correspond to (*R*)-fatty acid-(*R*)-(+)-1-phenylethylamide, which is an enantiomer of (*S*)-fatty acid-(*S*)-(−)-1-phenylethylamide, a possible contamination in the former product. Therefore, a racemization of the (*R*)-fatty acid **8** obtained above can be detected within the sensitivity of TLC.

The glycosyl donors **16a** and **16b** were synthesized from **9** by the method of Imoto *et al.*[2] as shown in Chart 2.

After 3-*O*-acylation of **9** with (*R*)-3-trichloroethoxycarbonyloxytetradecanoic acid (**8**) or tetradecanoic acid by the 1-hydroxybenzotriazole (HOBt) active ester method, removal of the isopropylidene group gave **11a** and **11b**. Selective *O*-trichloroethoxycarbonylation at the 6-position followed by phosphorylation with diphenyl phosphorochloridate and dimethylaminopyridine (DMAP) at position 4 gave **13a** and **13b**. The allyl group in **13a** and **13b** was cleaved off with an iridium complex, followed by reaction with iodine–H_2O. After acetylation of the C-1 hydroxyl group with acetic anhydride, the resulting glycosyl acetates **15a** and **15b** were brominated with 25% HBr–AcOH.

Coupling reactions of bromides **16a** and **16b** with acceptors **17a** and **17b**, respectively, were carried out in the presence of mercuric cyanide to give the corresponding disaccharides **18a** and **18b**, as shown in Chart 3. Cleavage of two Troc groups of **18a** and **18b** was effected with zinc dust in AcOH. The resultant free amino group was acylated with an active ester of (*R*)-3-hydroxy fatty acid without a protecting group or tetradecanoic acid, respectively, to give the fully acylated compounds **19a** and **19b**. Compound **19a** was hydrogenolyzed, first over a palladium catalyst to remove benzyl protection and then over a platinum catalyst to cleave phenyl protection, to give the title compound **3**. Compound **19b** was hydrogenolyzed in the presence of platinum dioxide to give compound **4**.

Antitumor Activity Antitumor activity of the synthetic compounds was tested in BLAB/c mice as described earlier.[5] A group of 8 mice were inoculated intradermally with Meth A syngenic fibrosarcoma cells (2×10^5). The Et_3N salt of each compound was dissolved in a 5% aqueous glucose solution containing 0.1% Et_3N. The resulting solution was then administered to the mice at doses of 100 μg/mouse through the vein on the 7th, 12th and 17th days after implantation. The percentage antitumor effect on

the growth of Meth A was determined on the 21st day by dividing the average tumor weight of the tested group by the average tumor weight of the control group and multiplying the quotient by 100. Table I shows the results.

Toxicity The Et_3N salt of each compound was dissolved in 5% glucose containing 0.1% Et_3N to prepare a 100 μg/ml solution. The solution was administered to 3 NZW rabbits per group at a dose of 50 μg/kg-body weight (b.w.) through the ear vein for 3 consecutive days. Toxicity was evaluated by the number of dead test animals 24 h after the final administration. For comparison, synthetic *E. coli*-type lipid A (**1**) was administered at a level of 5 μg/kg-b.w. The results obtained are shown in Table II.

Discussion

Compounds **3** and **4** with the 1-α-phosphonooxyethyl group in place of a phosphono group exhibited definite high antitumor activity. The activities of these compounds were comparable to the activity of the corresponding original precursor type lipid A (**2**), but slightly less than that of synthetic *E. coli*-type lipid A (**1**). Compound **4**, with four tetradecanoyl groups, showed slightly higher activity than compound **3** with four (*R*)-3-hydroxytetradecanoyl groups.

Concerning toxicity in rabbits, compounds **3** and **4** were weakly toxic as was precursor-type lipid A (**2**), no rabbit being dead with a 50 μg/kg dose. Particularly, it has to be emphasized that compound **3** exhibited no detectable toxicity with that dose in spite of its significant antitumor activity. In the case of *E. coli*-type lipid A (**1**), all four rabbits died with only a single administration of a 5 μg/kg dose within 24 h, manifesting symptoms of endotoxin shock.

In view of the biological activities of compound **3** possessing the same acyl groups as that of compound **2**, the substitution of a glycosidic phosphate group with α-phosphonooxyethyl group appeared likely to yield slightly higher antitumor activity, but similar or slightly less toxicity than that of the original precursor compound **2**. On the other hand, the presence or absence of a hydroxyl group of hydroxytetradecanoic acid residue on the disaccharide back bone (**3** or **4**) exhibits little effect on the antitumor activity, although it influenced the toxicity in rabbits slightly more than the antitumor activity. It is possible that the influence on activity was caused by the capacity for hydrogen bond formation and enlargement of polarity, both by the hydroxyl in the fatty acid residue. In accordance with a previous work,[7] these results indicate that the toxicity of lipid A analogs in rabbits is affected by a slight variation of the component fatty acyl groups. In conclusion, the present study demonstrated that synthesis of lipid A analogs which contain four linear fatty acyl residues on the disaccharide back bone and have a polar moiety such as an α-*O*-phosphonooxyethyl group at the glycosidic position in place of the phosphate can be expected to give promising results in the search for low-toxicity compounds retaining significant antitumor activity.

TABLE I. Antitumor Activity of Lipid A, Its Precursor and Their Analogs Against Meth A Fibrosarcoma[a]

Compound number	Dose (μg/mouse)	T/C (%)[b]	Cured mice[c]/ treated mice
3	100 × 3	32[d]	1/8
4	100 × 3	24[d]	3/8
1	100 × 3	10[e]	1/8
Control		100	0/8
2	100 × 3	41[e]	0/8
1	100 × 3	18[e]	1/8
Control		100	0/8

a) Antitumor tests were conducted twice using compound **1** as a positive control. *b*) (Mean tumor weight in tested group/that in control group) × 100. Results given are at 21 d after tumor inoculation. *c*) Number of tumor-free mice/number of mice tested. *d*) $p<0.01$, *e*) $p<0.001$ *vs.* control (Student's *t*-test).

Experimental

All melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured on a Varian XL-200 spectrometer (200 MHz) in chloroform-*d* solutions unless otherwise noted. Tetramethylsilane (TMS) was used as the internal standard. Optical rotations were measured with a Horiba SEDA 200 polarimeter at 25 °C. Mass spectra (MS) were obtained on a JMS-HX 110 or JMX-300 instrument. Precoated Silica gel 150A PLK5F plates (1.0 mm thickness; Whatman) were used for preparative TLC. Organic solutions were dried over sodium sulfate.

Benzyl (*R*)-3-Hydroxytetradecanoate (6) Benzyl bromide (4.20 g, 24.5 mmol) and Et_3N (3.44 ml, 24.5 mmol) were added to a suspension of

TABLE II. Toxicity of Precursor Lipid A Analogs in Rabbits

Observations	Compound				
	3 (Dose 50	**4** 50	**1** 1	5	**2** 50 μg/kg)
Mortality[a]	0/3	0/3	0/5	4/4	0/4
Clinical signs[b]	NO	PT, AD, LY, HE	AD, HE	AD, HE, LY	NO
Decrease in body weight (g)[c]	NO	300—400	0—180	NE	0—160
Decrease in platelets (%)[d]	NO	52	56	NE	33
Hematological examination[e]	NO	GOT↑, GPT↑, UN↑, CRE↑	GOT↑, GPT↑, UN↑, CRE↑	NE	(NE)
Pathology[a]					
Thrombus	NO	3/3 (3+)	4/5 (2+—3+)	4/4 (+—2+)	NO
Liver change[f]	NO	3/3 (2+—3+)	4/5 (+)	4/4 (+)	NO
Kidney change[g]	NO	NO	4/5 (+—3+)	NO	NO
Heart change[h]	NO	3/3 (+—3+)	4/5 (+—3+)	NO	NO

a) Mortality and pathology: Number of rabbits changed/number of rabbits tested. *b*) PT: ptosis. AD: decrease in locomotor activity. HE: hyperemia of eye. LY: lying on side. *c*) 24 h after final injection. *d*) 24 h after first injection. *e*) GOT: glutamate oxaloacetate transaminase. GPT: glutamate pyruvate transaminase. UN: urea nitrogen. CRE: creatinine. *f*) Liver change: degeneration and necrosis of liver cells. *g*) Kidney change: degeneration and necrosis of uriniferous tubular epithelia. *h*) Heart change: degeneration and necrosis of muscle fiber. NO: no change, NE: not examined for early death, (NE): not examined. +: slight. 2+: moderate. 3+: severe.

(*R*)-3-hydroxytetradecanoic acid (2.00 g, 8.18 mmol) in ethyl acetate (EtOAc) (20 ml). The mixture was stirred for 3 d at room temperature. Any insoluble matter was filtered off and the filtrate was concentrated. The residue was purified by silica gel column chromatography (benzene–EtOAc, 19 : 1) to give an oil. Hexane was added to the oil and the resulting precipitate was collected to give **6** as a white powder (1.68 g, 61%). mp 43—44 °C. $[\alpha]_D$ −13.6° (c=1.3, $CHCl_3$). IR (KBr): 3560, 1715, 1470, 1410 cm^{-1}. ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.25 (s) and 1.7 (m) (total 20H), 2.50 (2H, m, CH_2CO_2), 4.00 (1H, m, CH–OH), 5.15 (2H, s, $CH_2C_6H_5$), 7.35 (5H, s, arom. H).

(*R*)-3-(2,2,2-Trichloroethoxycarbonyloxy)tetradecanoic Acid (8) A solution of Troc–Cl (1.32 ml, 9.56 mmol) in THF (2 ml) was added to a solution of **6** (1.60 g, 4.78 mmol) in 20 ml of pyridine with ice-cooling. The mixture was stirred for 1 h at room temperature, then methanol (4 ml) was added to the reaction mixture and stirred for 1 h. After evaporation of the solvent, the residue was diluted with benzene. The solution was washed with 1 M HCl and then with saturated aqueous NaCl, and dried. Evaporation of the solvent gave a pale yellow oil of crude **7**. Palladium carbon (5%, 0.2 g) was added to a solution of the above oily substance in THF (30 ml), and the mixture was stirred under a hydrogen atmosphere for 2 h. The catalyst was filtered off and the filtrate was concentrated. The resulting oily substance was purified by silica gel column chromatography (benzene–EtOAc, 9 : 1—1 : 1) to give **8** (1.43 g, 71%) as a pale yellow oil. $[\alpha]_D$ 2.7° (c=1.2, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.25 (s) and 1.7 (m) (total 20H), 2.72 (2H, m, CH_2CO_2), 4.80 (2H, s, CH_2CCl_3), 5.18 (1H, m, CHOTroc). MS m/z: 418 (M^+).

Allyl 2-Deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-α-D-glucopyranoside (10a) DMAP (67 mg, 0.55 mmol) and DCC (0.68 g, 3.31 mmol) were added to a solution of **8** (1.27 g, 3.04 mmol) and **9** (1.20 g, 2.76 mmol) in CH_2Cl_2 (20 ml) with ice-cooling, and the mixture was stirred for 1 h at room temperature. The precipitate was filtered off, and the filtrate was concentrated by evaporation. The residue was purified by silica gel column chromatography (benzene–EtOAc, 19 : 1) to give **10a** (1.74 g, 75%) as a colorless oil. $[\alpha]_D$ +31.2° (c=1.1, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.26 (18H, s, CH_2), 1.39 and 1.49 (each 3H, s, CCH_3), 1.7 (2H, br, CH_2), 2.59 (1H, dd, J=16, 6 Hz, CH_2CO_2), 2.81 (1H, dd, J=16, 8 Hz, CH_2CO_2), 4.22 (1H, m), 4.76 (2H, s, CH_2CCl_3), 4.73 and 4.93 (each 1H, AB type d, J=12 Hz, CH_2CCl_3), 4.92 (1H, d, J=4 Hz, H-1), 5.9 (1H, m, $\underline{CH}=CH_2$). MS m/z: 833 (M^+).

Allyl 2-Deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (10b) As described for **10a**, compound **9** (15.0 g, 34.5 mmol) was treated with tetradecanoic acid (9.46 g, 41.4 mmol) in the presence of DCC (8.54 g, 41.4 mmol) and DMAP (0.84 g, 6.9 mmol) to give **10b** (19.8 g, 89%) as a colorless oil. $[\alpha]_D$ +46.4° (c=1.2, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{48}Cl_3NO_8$: C, 54.00; H, 7.50; N, 2.17. Found: C, 54.07; H, 7.54; N, 2.21. ^{1}H-NMR δ: 0.87 (3H, t, J=6 Hz, CH_3), 1.26 (18H, s, CH_2), 1.38 and 1.49 (each 3H, s, CCH_3), 1.60 (2H, br, CH_2), 2.30 (2H, m, CH_2CO), 4.72 (2H, s, CH_2CCl_3), 4.92 (1H, d, J=4 Hz, H-1), 5.90 (1H, m, $\underline{CH}=CH_2$). MS m/z: 643 (M^+).

Allyl 2-Deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-α-D-glucopyranoside (11a) A solution of **10a** (1.72 g, 2.06 mmol) in 90% AcOH (40 ml) was heated at 90 °C for 30 min. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 19 : 1) to give **11a** as a colorless, viscous oil (1.49 g, 91%). $[\alpha]_D$ +53.4° (c=2.0, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz), 1.26 (18H, s, CH_2), 1.70 (2H, br, CH_2), 1.94 (1H, t, J=6 Hz, OH), 2.60 (1H, dd, J=16, 4 Hz, CH_2CO_2), 2.8 (2H, m, CH_2CO_2 and OH), 4.24 (1H, m), 4.70 and 4.80 (each 1H, AB type d, J=12 Hz, CH_2CCl_3), 4.73 and 4.88 (each 1H, AB type d, J=12 Hz, CH_2CCl_3), 4.95 (1H, J=4 Hz, H-1), 5.1—5.4 (5H, m, H-3, CHOTroc, NH and $CH=\underline{CH_2}$), 5.9 (1H, m, $\underline{CH}=CH_2$). MS m/z: 793 (M^+).

Allyl 2-Deoxy-3-*O*-tetradecanoyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (11b) As described for **11a**, compound **10b** (4.43 g, 6.78 mmol) was treated with 90% AcOH to give **11b** (3.20 g, 77%) as a colorless oil. $[\alpha]_D$ +54.4° (c=1.0, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{44}Cl_3NO_8$: C, 51.62; H, 7.33; N, 2.32. Found: C, 51.87; H, 7.30; N, 2.26. ^{1}H-NMR δ: 0.90 (3H, t, J=6 Hz), 1.26 (18H, s, CH_2), 1.60 (2H, br, CH_2), 2.35 (2H, m, CH_2CO), 4.75 (2H, m, CH_2CCl_3), 4.97 (1H, d, J=4 Hz, H-1), 5.92 (1H, m, $\underline{CH}=CH_2$). MS m/z: 603 (M^+).

Allyl 2-Deoxy-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)-tetradecanoyl]-α-D-glucopyranoside (12a) Troc–Cl (0.57 ml, 4.19 mmol) was gradually added to a solution of **11a** (1.44 g, 1.81 mmol) in pyridine (20 ml) with ice cooling. After the mixture was stirred for 6 h at room temperature, the mixture was diluted with EtOAc. The solution was washed with 1 M HCl, then with saturated aqueous NaCl and dried. After evaporation of the solvent, the residue was purified by silica gel column chromatography (benzene–EtOAc, 19 : 1—9 : 1) to give **12a** (1.50 g, 85%) as a pale yellow oil. $[\alpha]_D$ +46.2° (c=1.8, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz), 1.28 (18H, s, CH_2), 1.70 (2H, br, CH_2), 2.60 (1H, dd, J=16, 4 Hz, CH_2CO_2), 2.78 (1H, dd, J=16, 8 Hz, CH_2CO_2), 2.86 (1H, m, OH), 4.57 (2H, d, J=4 Hz, H-6), 4.8 (6H, m, $CH_2CCl_3\times3$), 4.96 (1H, d, J=4 Hz, H-1), 5.9 (1H, m, $\underline{CH}=CH_2$). MS m/z: 967 (M^+).

Allyl 2-Deoxy-3-*O*-tetradecanoyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (12b) As described for **12a**, compound **11b** (3.20 g, 5.29 mmol) was treated with Troc–Cl (1.20 ml, 8.95 mmol) to give **12b** (3.00 g, 73%) as an oil. $[\alpha]_D$ +45.4° (c=1.1, $CHCl_3$). *Anal.* Calcd for $C_{29}H_{45}Cl_6NO_{10}$: C, 44.63; H, 5.81; N, 1.79. Found: C, 44.76; H, 5.67; N, 1.75. ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.28 (18H, s, CH_2), 1.60 (2H, br, CH_2), 2.36 (2H, t, J=7 Hz, CH_2CO), 4.58 (2H, d, J=4 Hz, H-6), 4.8 (4H, m, CH_2CCl_3), 4.96 (1H, d, J=4 Hz, H-1), 5.9 (1H, m, $\underline{CH}=CH_2$). MS m/z: 777 (M^+).

Allyl 2-Deoxy-4-*O*-diphenylphosphono-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-α-D-glucopyranoside (13a) Diphenyl phosphorochloridate (0.73 g, 2.75 mmol), DMAP (0.33 g, 2.75 mmol) and pyridine (0.18 ml, 2.25 mmol) were added to a solution of **12a** (1.46 g, 1.50 mmol) in CH_2Cl_2. The mixture was stirred for 22 h at room temperature, then $CHCl_3$ was added to the mixture. The solution was washed successively with 1 M HCl, H_2O, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried. After evaporation of the solvent, the resultant residue was purified by silica gel column chromatography (benzene–EtOAc, 19 : 1) to give **13a** (1.72 g, 95%) as a colorless oil. $[\alpha]_D$ +36.8° (c=1.4, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz), 1.26 (18H, s, CH_2), 1.52 (2H, br, CH_2), 2.55 (2H, m, CH_2CO_2), 4.0—4.3 (4H, m, H-2, H-5, and $O\underline{CH_2}CH=CH_2$), 4.42 (2H, m, H-6), 4.60—4.95 (7H, m, H-4, $CH_2CCl_3\times3$), 5.00 (1H, d, J=4 Hz, H-1), 5.10 (1H, m, CHOTroc), 5.3—5.6 (4H, m, H-3, NH and $CH=\underline{CH_2}$), 5.9 (1H, m, $\underline{CH}=CH_2$), 7.2—7.4 (m). MS m/z: 1199 (M^+).

Allyl 2-Deoxy-4-*O*-diphenylphosphono-3-*O*-tetradecanoyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (13b) As described for **13a**, compound **12b** (2.95 g, 3.78 mmol) was reacted with diphenylphosphorochloridate (2.40 g, 11.4 mmol) to give **13b** (3.58 g, 94%) as a colorless oil. $[\alpha]_D$ +42.4° (c=0.2, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz), 1.1—1.3 (20H, br, CH_2), 2.14 (2H, m, CH_2CO_2), 4.63—4.86 (4H, m, $CH_2CCl_3\times2$), 4.98 (1H, d, J=4 Hz, H-1), 5.9 (1H, m, $CH=\underline{CH_2}$), 7.14—7.46 (10H, m, arom. H). MS m/z: 1009 (M^+).

2-Deoxy-4-*O*-diphenylphosphono-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-D-glucose (14a) 1,5-Cyclooctadienebis(methyldiphenylphosphine)iridium hexafluorophosphate (20 mg) was added to a solution of **13a** (1.68 g, 1.40 mmol) in THF (20 ml) under nitrogen. The mixture was heated at 50 °C under a nitrogen atmosphere for 2 h after activation of the iridium catalyst with hydrogen for 1 min. After cooling, iodine (0.71 g) and H_2O (2.5 ml) were added to the solution and the mixture was stirred for 20 min at room temperature. The solution was neutralized with 5% aqueous Na_2SO_3 and concentrated by evaporation. The residue was dissolved in $CHCl_3$ and the solution was washed with saturated aqueous NaCl, and dried. After evaporation of the solvent, the residue was purified by silica gel column chromatography (benzene–EtOAc, 9 : 1—4 : 1) to give **14a** (1.13 g, 70%). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz), 1.26 (18H, s, CH_2), 1.52 (2H, br, CH_2), 2.56 (2H, m, CH_2CO_2), 3.44 (1H, br, OH), 4.06 (1H, m, H-2 or H-5), 4.4 (3H, m), 4.6—5.0 (7H, m, H-4 and $CH_2CCl_3\times3$), 5.11 (1H, m, CHOTroc), 5.40 (1H, m, H-1), 5.55 (2H, m, H-3 and NH), 7.2—7.4 (10H, m, arom. H). MS m/z: 1159 (M^+).

2-Deoxy-4-*O*-diphenylphosphono-3-*O*-tetradecanoyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucose (14b) As described for **14a**, compound **13b** (2.70 g, 2.98 mmol) was treated with 1,5-cyclooctadienebis(methyldiphenylphosphine)iridium hexafluorophosphate (59 mg) to give **14b** (2.32 g, 89%) as a caramel. *Anal.* Calcd for $C_{38}H_{50}Cl_6NO_{13}P$: C, 46.93; H, 5.18; Cl, 21.87; N, 1.44. Found: C, 47.16; H, 5.18; Cl, 22.00; N, 1.42. $[\alpha]_D$ +26.4° (c=0.3, $CHCl_3$). ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.1—1.5 (20H, br, CH_2), 2.08—2.30 (2H, m, CH_2CO_2), 4.60—4.90 (4H, m, $CH_2CCl_3\times2$), 7.14—7.46 (10H, m, arom.

H). MS *m/z*: 969 (M^+).

1-*O*-Acetyl-2-deoxy-4-*O*-diphenylphosphono-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-D-glucopyranose (15a) Acetic anhydride (0.39 g, 3.82 mmol) and pyridine (0.30 ml, 3.82 mmol) were added to a solution of **14a** (0.89 g, 0.76 mmol) in CH_2Cl_2. The mixture was stirred for 15 h at room temperature and diluted with $CHCl_3$. The solution was washed with 1 M HCl, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl. The solvent was evaporated *in vacuo* to give **15a** (0.92 g, quant.) as an oil. $[\alpha]_D$ +43.2° (c=1.2, $CHCl_3$). *Anal.* Calcd for $C_{43}H_{53}Cl_9NO_{17}P$: C, 42.83; H, 4.43; N, 1.16. Found: C, 42.66; H, 4.09; N, 1.16. ^{1}H-NMR δ: 0.89 (3H, t, J=6 Hz), 1.2—1.3 (20H, s, CH_2), 1.52 (2H, br, CH_2), 2.22 (3H, s, OAc), 2.56 (2H, m, CH_2CO_2), 4.1—4.3 (2H, m, H-2 and H-5), 4.40 (2H, m, H-6), 4.6—5.0 (7H, m, H-4 and $CH_2CCl_3\times3$), 5.12 (1H, m, CHOTroc), 5.32 (1H, d, J=10 Hz, NH), 5.50 (1H, t, J=10 Hz, H-3), 6.32 (1H, d, J=4 Hz, H-1), 7.2—7.4 (10H, m, arom. H). MS *m/z*: 1201 (M^+).

1-*O*-Acetyl-2-deoxy-4-*O*-diphenylphosphono-3-*O*-tetradecanoyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranose (15b) In the same manner as described for **15a**, compound **14b** (2.28 g, 3.34 mmol) was reacted with acetic anhydride to give **15b** (2.18 g, 91.6%) as an oil. $[\alpha]_D$ +37.2° (c=0.8, $CHCl_3$). *Anal.* Calcd for $C_{40}H_{52}Cl_6NO_{14}P$: C, 47.36; H, 5.17; N, 1.38. Found: C, 47.78; H, 4.95; N, 1.33. ^{1}H-NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.1—1.5 (20H, br, CH_2), 2.16 (2H, CH_2CO_2), 2.24 (3H, s, OAc), 4.60—5.00 (4H, m, $CH_2CCl_3\times2$), 5.50 (1H, t, J=10 Hz, H-3), 6.29 (1H, d, J=4 Hz, H-1), 7.04—7.46 (10H, m, arom. H). MS *m/z*: 1011 (M^+).

2-(Diphenylphosphonooxy)ethyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(2,2,2-trichloroethoxycarbonyloxy)tetradecanoyl]-β-D-glucopyranosyl]-α-D-glucopyranoside (18a) HBr–AcOH (25%, 6 ml) was added to a solution of **15a** (409 mg, 0.34 mmol) in CH_2Cl_2 (2 ml), and the mixture was stirred for 1.5 h at room temperature. The reaction mixture was diluted with $CHCl_3$ and the solution was washed with a mixture of ice-H_2O, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried. Evaporation of the solvent gave **16a** as an oil. This oil and **17a** (370 mg, 0.34 mmol) were dissolved in CH_2Cl_2 (5 ml), then $CaSO_4$ (0.5 g) and mercuric cyanide (172 mg, 0.68 mmol) were added to the mixture. After being refluxed for 18 h, the reaction mixture was filtered through Celite 545, and the filtrate was washed with 5% aqueous potassium iodide and saturated aqueous NaCl, and dried. After evaporation of the solvent, the residue was purified by silica gel column chromatography (benzene–EtOAc, 9:1—2:1) to give **18a** (577 mg, 76%) as a pale yellow viscous oil. $[\alpha]_D$ +20.2° (c=0.2, $CHCl_3$). ^{1}H-NMR δ: 0.88 (9H, t, J=7 Hz), 1.26 (54H, s, CH_2), 1.56 (6H, br, CH_2), 2.3—2.7 (6H, m), 5.70 (1H, m, H-3′), 7.2—7.4 (30H, m, arom. H). MS *m/z*: 2228 (M^+).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-3-*O*-tetradecanoyl-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (18b) As described for **18a**, compound **16b**, obtained from **15b** (706 mg, 0.70 mmol), was reacted with **17b** (543 mg, 0.62 mmol) to give **18b** (885 mg, 78%) as an oil. $[\alpha]_D$ +15.1° (c=0.8, $CHCl_3$). *Anal.* Calcd for $C_{86}H_{126}Cl_6N_2O_{23}P_2\cdot H_2O$: C, 55.88; H, 6.98; N, 1.52. Found: C, 55.64; H, 6.84; N, 1.51. ^{1}H-NMR δ: 0.89 (9H, t, J=6 Hz), 1.26 (54H, s, CH_2), 1.6 (6H, br, CH_2), 2.01—2.46 (6H, m, $CH_2CO_2\times3$), 4.68 (2H, m, CH_2CCl_3), 4.76 (2H, m, CH_2CCl_3), 5.06 (1H, t, J=10 Hz, H-3), 5.60 (1H, t, J=10 Hz, H-3′), 5.75 (1H, d, NH), 6.24 (1H, d, NH), 7.15—7.52 (20H, m, arom. H). MS *m/z*: 1826 (M^+).

2-(Diphenylphosphonooxy)ethyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyl]-α-D-glucopyranoside (19a) Zinc powder (0.6 g) was added to a solution of **18a** (555 mg, 0.25 mmol) in AcOH (12 ml), and the mixture was vigorously stirred for 2 h at room temperature. The reaction mixture was filtered off and the filtrate was concentrated *in vacuo* to give an oil. After dissolution in $CHCl_3$, the solution was washed with 1 M HCl, H_2O, and 5% aqueous $NaHCO_3$ and dried. Separately, (*R*)-3-hydroxytetradecanoic acid (93 mg, 0.38 mmol) was dissolved in THF (2 ml), then 1-hydroxybenzotriazole (61 mg, 0.4 mmol) and DCC (82 mg, 0.4 mmol) were added to the solution with ice cooling. The mixture was stirred for 3 h at room temperature and the precipitate was filtered off to give an active ester solution. The active ester solution was added to the solution of the oily substance mentioned above in CH_2Cl_2 (5 ml), then *N*-methylmorpholine (44 μl, 0.4 mmol) was added to the mixture with ice cooling, and the final mixture was stirred for 13 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 50:1—20:1) to give **19a** (312 mg, 65%) as a colorless oil. $[\alpha]_D$ +6.3° (c=0.7, $CHCl_3$). ^{1}H-NMR δ: 0.90 (12H, t, J=7 Hz), 1.26 (72H, s, CH_2), 1.56 (8H, br, CH_2), 1.9—2.7 (8H, m), 4.7 (2H, m, H-1 and H-4′), 4.84 (1H, d, J=8 Hz, H-1′), 5.16 (1H, m, H-3), 5.59 (1H, m, H-3′), 7.2—7.4 (30H, m, arom. H). MS *m/z*: 1932 (M^+).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-(2-deoxy-4-*O*-diphenylphosphono-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranosyl)-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (19b) As described for **19a**, compound **18b** (402 mg, 0.22 mmol) was treated with Zn dust, and the resulting oil was condensed with the HOBt active ester of tetradecanoic acid (75 mg, 0.33 mmol) to give **19b** (308 mg, 83%) as a white powder. mp 47.0—50.0 °C. *Anal.* Calcd for $C_{94}H_{150}N_2O_{20}P_2\cdot H_2O$: C, 66.09; H, 8.97; N, 1.64. Found: C, 66.30; H, 8.82; N, 1.69. $[\alpha]_D$ +5.9° (c=0.7, $CHCl_3$). ^{1}H-NMR δ: 0.88 (12H, t, J=6 Hz, $CH_3\times4$), 1.26 (72H, s, CH_2), 1.5 (8H, br, CH_2), 1.96—2.46 (8H, m, CH_2CO_2), 4.64—4.94 (3H, H-4′, H-1 and H-1′), 5.10 (1H, t, J=10 Hz, H-3), 5.50 (1H, t, J=10 Hz, H-3′), 5.90 (1H, d, NH), 6.29 (1H, d, NH), 7.10—7.50 (20H, m, arom. H). IR (KBr): 3440, 2930, 1745, 1670, 1630, 1495, 1290, 1195 cm^{-1}. MS *m/z*: 1688 (M^+).

2-Phosphonooxyethyl 2-Deoxy-6-*O*-[2-deoxy-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-4-*O*-phosphono-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-*O*-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranoside (3) Palladium–carbon (5%, 0.3 g) was added to a solution of **19a** (294 mg, 0.15 mmol) in AcOH (10 ml), and the mixture stirred under a hydrogen atmosphere for 7 h. The catalyst was filtered off and the filtrate was concentrated under reduced pressure, then the resulting oil was dissolved in THF (20 ml). Next, platinum dioxide (180 mg) was added to the solution and the mixture was stirred under a hydrogen atmosphere for 1.5 h. After filtration of the catalyst, the filtrate was concentrated, and the resulting residue was purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6:4:0.7). The extracted solution with $CHCl_3$–MeOH–H_2O–Et_3N (6:4:1:0.02) was concentrated by evaporation. After dissolution in $CHCl_3$–MeOH–H_2O (6:4:1), the solution was desalted with Dowex 50 (H^+). A part of the desalted solution was concentrated and freeze-dried from a dioxane suspension to give **3** as a white powder. mp 155—158 °C (dec). $[\alpha]_D$ −1.8° (c=0.5, $CHCl_3$–MeOH, 3:1). IR (KBr): 3440, 1740, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD, 3:1) δ: 0.90 (12H, t, J=6 Hz, CH_3), 1.28 (72H, br, CH_2), 1.48 (8H, br, CH_2), 2.3—2.5 (8H, m), 5.2 (2H, m).

The remaining desalted solution was neutralized with MeOH containing 1% Et_3N under ice-cooling to about pH 8. After evaporation of the solvent, the residue was dissolved in 0.1% Et_3N. The solution was lyophilized to give 76 mg of Et_3N salt as a white powder.

2-Phosphonooxyethyl 2-Deoxy-6-*O*-(2-deoxy-4-*O*-phosphono-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranosyl)-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (4) In similar manner to that described for **3**, compound **19b** (215 mg, 0.13 mmol) was hydrogenolyzed in the presence of platinum dioxide (210 mg), and the resulting powder was purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6:4:0.8), and desalted and freezed-dried from a dioxan suspension to give **4** (80 mg, 45%) as a white powder. mp 153.5—155.0 °C (dec). $[\alpha]_D$ +13.3° (c=0.6, $CHCl_3$–MeOH, 9:1). IR (KBr): 3445, 2930, 1740, 1660, 1560 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD, 3:1) δ: 0.90 (12H, CH_3), 1.28 (72H, br, CH_2), 1.60 (8H, br, CH_2), 2.10—2.26 (4H, m, CH_2CO_2), 2.26—2.46 (4H, m, CH_2CO_2).

Et_3N salt was prepared in a manner similar to that described for **3**.

References

1) M. Imoto, S. Kusumoto, T. Shiba, H. Naoki, T. Iwashita, E. Th. Rietschel, H. W. Wollenweber, C. Galanos and O. Luderiz, *Tetrahedron Lett.*, **24**, 4017 (1983); M. Imoto, S. Kusumoto, T. Shiba, E. Th. Rietschel, C. Galanos and O. Luderiz, *ibid.*, **26**, 907 (1985).
2) M. Imoto, H. Yoshimura, S. Kusumoto and T. Shiba, *Proc. Jpn. Acad., Ser. B*, **60**, 285 (1984); M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto and T. Shiba, *Tetrahedron Lett.*, **26**, 1545 (1985); M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2205 (1987).
3) M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2197 (1987); *idem*, *Tetrahedron Lett.*, **25**, 2267 (1984).
4) C. Galanos, V. Lehmann, O. Luderitz, E. Th. Rietschel, O. Westphal, H. Brade, L. Brade, M. A. Freudenberg, T. Hansen-Hagge, T.

Luderitz, G. Mckenzie, U. Schade, W. Strittmatter, K. Tanamoto, U. Zahringer, M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto and T. Shiba, *Eur. J. Biochem.*, **140**, 221 (1984); S. Kotani, H. Takada, M. Tsujimoto, T. Ogawa, K. Harada, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura, M. Yamamoto and T. Shimamoto, *Infect. Immun.*, **45**, 293 (1984); H. Takada, S. Kotani, M. Tsujimoto, T. Ogawa, I. Takahashi, K. Harada, C. Katsukawa, S. Tanaka, T. Shiba, S. Kusumoto, M. Imoto, H. Yoshimura, M. Yamamoto and T. Shimamoto, *ibid.*, **48**, 219 (1985); S. Kanegasaki, Y. Kojima, M. Matsuura, J. Y. Homma, A. Yamamoto, Y. Kumazawa, K. Tanamoto, T. Yasuda, T. Tsumita, M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto and T. Shiba, *Eur. J. Biochem.*, **143**, 237 (1984).

5) T. Kusama, T. Soga, E. Shioya, H. Nakayama, Y. Osada, Y. Ono, S. Kusumoto and T. Shiba, *Chem. Pharm. Bull.*, **38**, 3366 (1990).
6) M. Imoto, N. Kusunose, S. Kusumoto and T. Shiba, *Tetrahedron Lett.*, **29**, 2227 (1988).
7) S. Kusumoto, N. Kusunose, M. Imoto, T. Shimamoto, T. Kamikawa, H. Takada, S. Kotani, E. Th. Rietschel and T. Shiba, *Pure Appl. Chem.*, **61**, 461 (1989).