Synthesis and Antitumor Activity of Lipid A Analogs Having a Phosphonooxyethyl Group with α- or β-Configuration at Position 1

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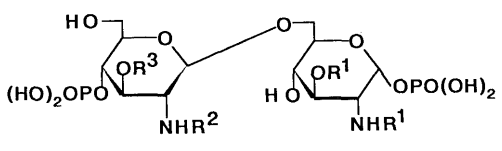
Three novel lipid A analogs, which have an α- or β-glycosidically bound phosphonooxyethyl group instead of the α-glycosyl phosphate group of natural lipid A, were synthesized. The first analog (2) had an α-phosphonooxyethyl group on the identical acylated disaccharide 4′-phosphate structure found in natural lipid A (from *Escherichia coli*) and hence differed from the latter only in the nature of the acidic group at position 1. The second one (3) had tetradecanoyl groups in place of the two (*R*)-3-hydroxytetradecanoyl groups bound to the 2- and 3-hydroxyl function of 2, retaining the α-phosphonooxyethyl group. The structure of the third analog (4) was the same as that of 3 except that the phosphonooxyethyl group of the former was β-oriented.

Compounds 2 and 3 exhibited potent activity against Meth A at the same level as natural lipid A, whereas 4 showed less activity. This fact revealed that the glycosidic phosphate is not a prerequisite for the antitumor activity of lipopolysaccharide. It can be replaced with a phosphonooxyethyl group without any loss of activity provided that the α-anomeric configuration at C-1 is retained. The replacement of the hydroxytetradecanoyl groups with tetradecanoyl groups does not change the activity either.

Keywords lipid A analogs; phosphonooxyethyl group; phosphate group; antitumor activity; Meth A fibrosarcoma; configuration; (*R*)-3-hydroxytetradecanoic acid

It is well known that lipopolysaccharide (LPS) has various potent biological activities including antitumor activity, induction of interferon, mitogenic activity for B-lymphocytes, and lethal toxicity.[1] Most of these so-called endotoxic activities have been attributed to the lipophilic moiety of the LPS molecule called lipid A.[2] The fundamental chemical structure of lipid A isolated from many *Enterobacteriae* was elucidated to have a backbone of the 1,4′-bisphosphorylated β (1→6) disaccharide of D-glucosamine.[1] The complete chemical structure of *Escherichia coli* (*E. coli*) lipid A was deduced as **1** shown in the figure below[3] and this structure was unequivocally confirmed by total synthesis.[4] The synthetic *E. coli* lipid A showed biological activity identical with its natural counterpart.[5]

Thereafter, many structural analogs of natural lipid A have been chemically synthesized[6] and tested for biological activities.[7] The results have given important information concerning the structure–activity relationship. For example, the phosphate groups seemed to play a significant role in the expression of the activity because the synthetic compound which lacks either of the two phosphates was found to have lower activity than the original bisphosphorylated compound.



1

R^1: $CH_3(CH_2)_{10}CH(OH)CH_2CO$

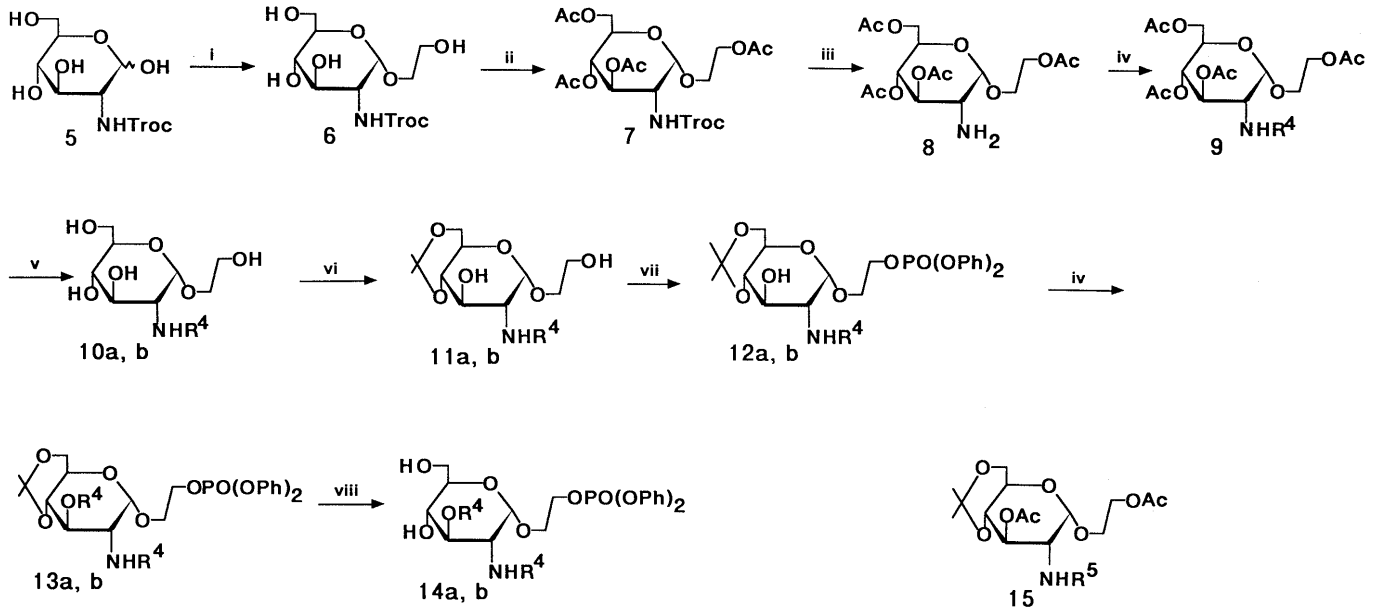
R^2: $CH_3(CH_2)_{10}OCO$, $CH_3(CH_2)_{10}CH(OH)CH_2CO$

R^3: $CH_3(CH_2)_{12}OCO$, $CH_3(CH_2)_{10}CH(OH)CH_2CO$

In the course of our synthetic efforts toward new antitumor compounds, we became interested in replacing the glycosyl phosphate moiety of lipid A. Though removal of even one of the phosphates of lipid A lowered its antitumor potency as described above, we anticipated that the substitution of them with other polar groups could not necessarily lead to similar loss of the activity. Of the two phosphate groups in the lipid A molecule, one at the glycosyl hydroxyl group is difficult to introduce synthetically and is chemically unstable. Hence, the presence of this phosphoric ester makes the synthesis itself and the purification of the product considerably difficult. We expected that substitution of this particular phosphate with a stable polar (acidic) group not only makes the synthetic strategy very flexible but also opens wide possibilities to create novel antitumor active compounds, which could be less toxic and have potential clinical application, based on the endotoxic lipid A.

According to this consideration we synthesized lipid A analogs with a glycosidically bound phosphonooxyethyl group. We expected that the acidity and the solubility of a phosphonooxyethyl glycoside resemble those of the corresponding glycosyl phosphate so that this type of substitution would not cause a serious change of the physical character of the molecules. In addition, the phosphoric ester in a phosphonooxyethyl glycoside was assumed to be more stable than a glycosyl phosphate since the phosphoric acid in the former structure is bound not directly at a glycosyl but at an alcoholic hydroxyl group of ethylene glycol.

We described in this paper a synthesis of three analogs **2**, **3**, **4** of natural lipid A. All three compounds are phosphonooxyethyl glycosides of a β(1→6)-linked glucosamine disaccharide which is phosphorylated at position 4′ and acylated at positions 2, 2′, 3, 3′. Compound **2** has the



i) $HO(CH_2)_2OH$, HCl; ii) Ac_2O, Py; iii) Zn/AcOH; iv) R^4OH–DCC or R^4Cl; v) $NaOCH_3$; vi) $(CH_3)_2C(OCH_3)_2$, H^+; vii) $(PhO)_2POCl$, DMAP; viii) 90% AcOH

Chart 1

identical composition and distribution of acyl groups as in natural *E. coli* lipid A. The phosphonooxyethyl group of **2** is in α-configuration like the α-phosphate in lipid A as well. Both **3** and **4** have again the identical acylation pattern as **2** except that the two (*R*)-3-hydroxytetradecanoyl groups on positions 2 and 3 are replaced with a tetradecanoyl group. The anomeric configurations are α in **3** but β in **4**. The two compounds are hence stereoisomers of each other with regards to the anomeric phosphonooxyethyl groups.

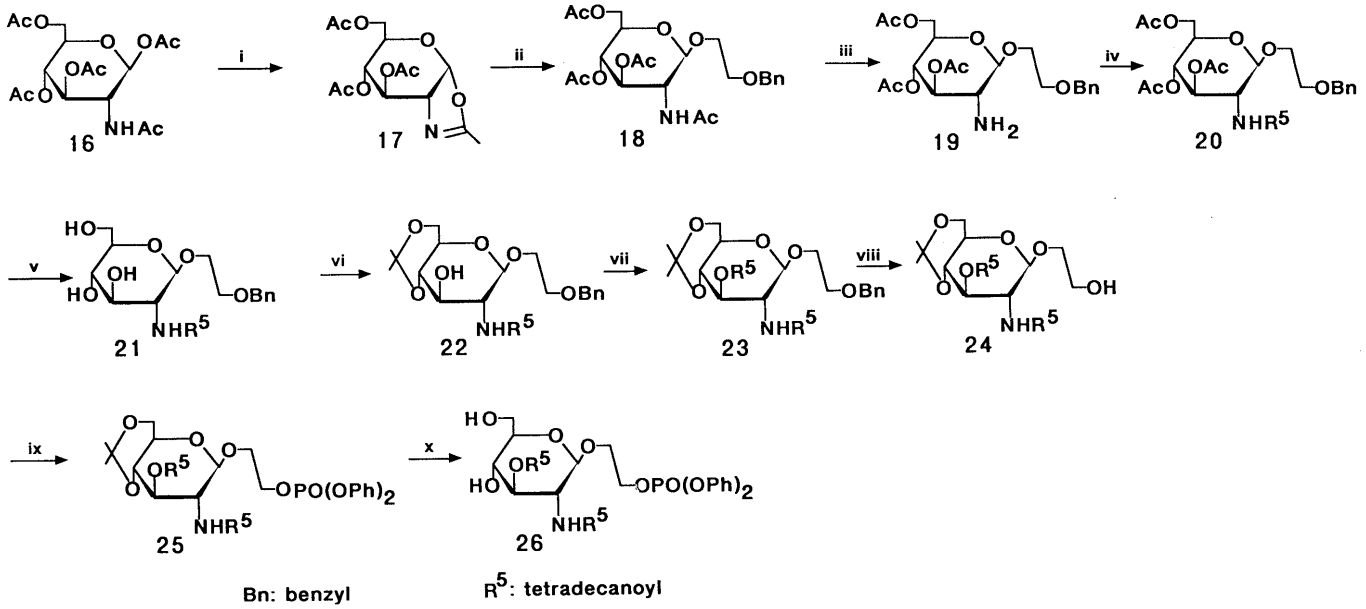
Chemistry The synthesis was performed in principle in a similar way to that reported for lipid A.[8] A distinct difference is that the phosphonooxyethyl group could be already introduced at the monosaccharide stage because of the chemical stability of this function. In case of lipid A synthesis, the glycosyl phosphate had to be introduced at the final stage just before the deprotection.

A key intermediate **14**, which served as a glycosyl acceptor to form a disaccharide **29** with α-phosphonooxyethyl group, was prepared starting from *N*-(2,2,2-trichloroethoxycarbonyl)-D-glucosamine (**5**),[4b] as shown in Chart 1.

On reaction of **5** with excess ethylene glycol in the presence of hydrogen chloride, the predominant formation of α-glycoside **6** was recognized on thin layer chromatography (TLC), but it was not isolated because of the presence of excess ethylene glycol. Thus, treatment of the reaction mixture with acetic anhydride in pyridine in order to facilitate the removal of ethylene glycol yielded the peracetylated α-glycoside **7** in a 70% yield from **5**. After cleavage of the 2,2,2-trichloroethoxycarbonyl (Troc) group of **7** with Zn dust in acetic acid, a (*R*)-3-benzyloxytetradecanoyl group was introduced to the amino group by the active-ester method to give the *N*-benzyloxytetradecanoyl derivative **9a**. Or alternatively, a tetradecanoyl group was introduced at the same position by the acid chloride method to afford **9b**. Thereafter, all acetoxy protecting groups of **9a** and **9b** were removed, and the 4- and 6-hydroxyl groups were protected by the isopropylidene formation. The position of the isopropylidene group was confirmed from the nuclear magnetic resonance (NMR) spectrum of **15**, obtained by acetylation of **11b**. The primary hydroxyl group of **11** was phosphorylated by treatment with diphenylphosphorochloridate to give **12**. The 3-hydroxyl groups of **12a** and **12b** were then acylated with (*R*)-3-benzyloxytetradecanoic acid and dicyclohexylcarbodiimide (DCC) or with tetradecanoyl chloride in the presence of 4-dimethylaminopyridine (DMAP) respectively. Hydrolysis of the isopropylidene group with 90% acetic acid afforded the glucosamine components **14a** and **14b** to be used as the glycosyl acceptors.

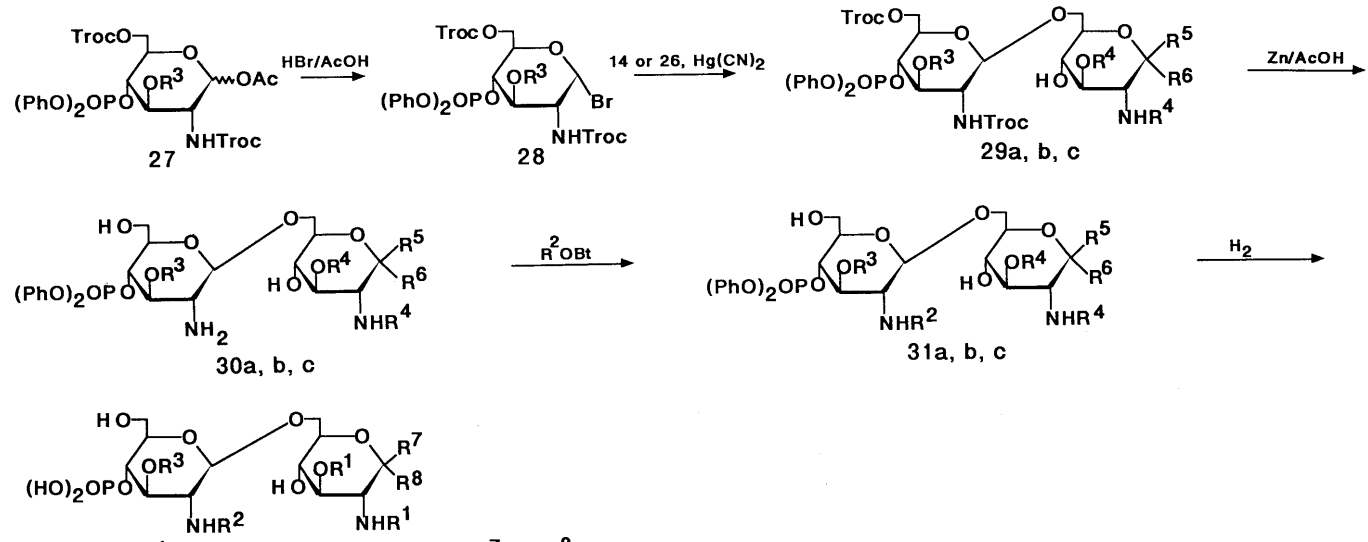
The corresponding key intermediate **26** with a β-phosphonooxyethyl group, was prepared starting from D-glucosamine β-peracetate **16**, as shown in Chart 2.

The oxazoline derivative **17**, which can be readily derived from **16**, was treated with ethylene glycol monobenzyl ether[9] in the presence of *p*-toluenesulfonic acid (*p*-TsOH) to give the β-glycoside **18** though in a rather low yield. The *N*-acetyl group of **18** was cleaved by Meerwein's reagent followed by acid hydrolysis. The free amino group of **19** was acylated with tetradecanoyl chloride to give **20**. Thereafter, all acetyl groups of **20** were removed and the 4- and 6-hydroxyl functions were protected by the isopropylidene group. The remaining 3-hydroxyl group of the 4,6-isopropylidene derivative **22** was again acylated



i) BF_3Et_2O; ii) $HO(CH_2)_2OBn$, *p*-TsOH; iii) Et_3OBF_4, HCl; iv) R^5Cl, Et_3N; v) $NaOCH_3$; vi) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH; vii) R^5Cl, Py, DMAP; viii) H_2, Pd/C; ix) $(PhO)_2POCl$, Py, DMAP; x) 90% AcOH

Chart 2



2: R^1= (*R*)-3-hydroxytetradecanoyl, R^7= H, R^8= phosphonooxyethoxy

3: R^1= tetradecanoyl, R^7= H, R^8= phosphonooxyethoxy

4: R^1= tetradecanoyl, R^7=phosphonooxyethoxy, R^8= H

R^2= (*R*)-3-dodecanoyloxytetradecanoyl, R^3= (*R*)-3-tetradecanoyloxytetradecanoyl

a: R^4= (*R*)-3-benzyloxytetradecanoyl, R^5= H, R^6= 2-(diphenylphosphonooxy)ethoxy

b: R^4= tetradecanoyl, R^5= H, R^6= 2-(diphenylphosphonooxy)ethoxy

c: R^4= tetradecanoyl, R^5= 2-(diphenylphosphonooxy)ethoxy, R^6=H

Chart 3

with tetradecanoyl chloride. The benzyl group of the acylation product **23**, was cleaved by hydrogenolysis in acetic acid in water-free conditions to give **24** in an 80% yield, leaving the isopropylidene group intact. This reaction did not proceed when ethanol or tetrahydrofuran (THF) was used as a solvent. The hydroxyl group of **24** was then phosphorylated with diphenylphosphorochloridate to give **25**, and hydrolysis with 90% acetic acid afforded the glucosamine component **26**.

The other glucosamine component **28** used as the glycosyl donor was obtained by the treatment of **27**[4b] with HBr–acetic acid. Coupling of the two glucosamine components and the subsequent conversion to **2**, **3** and **4** were performed as shown in Chart 3.

Reaction of the bromide (**28**) with **14a**, **b** or with **26** as an acceptor proceeded in the presence of mercuric cyanide

TABLE I. Antitumor Activity of Lipid A Analogs against Meth A Fibrosarcoma

Compound number	Dose (μg/mouse)	T/C (%)[a]	Mortality[b]	Complete regression[c]
2	100 × 3	14[e]	1/8	1/7
3	100 × 3	12[e]	0/8	2/8
4	100 × 3	41[d]	0/8	0/8
1	100 × 3	10[e]	0/8	1/8
Control	—	100	0/8	0/8

a) (Mean tumor weight in tested group/that in control group) × 100. *b*) Number of mice died from toxicity/ number of mice tested. *c*) Number of tumor free-mice/number of mice survived on 21th day. *d*) $p<0.01$. *e*) $p<0.001$ *vs.* control (Student's test).

to give the desired $\beta(1\rightarrow6)$disaccharide **29a**, **b** and **c**, respectively. The Troc group of **29** was cleaved by treatment with Zn dust in acetic acid, and the resultant free amino group was acylated with (*R*)-3-dodecanoyloxytetradecanoic acid by the 1-hydroxybenzotriazole (HOBt) active ester method[10] to give **31**. The benzyl and phenyl groups were removed in two steps by hydrogenolysis with palladium and platinum catalysts, respectively. The crude products **2**, **3** and **4** were purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6:4:0.5—0.7) followed by desalting with Dowex 50 (H^+ type), and the free forms of **2**, **3** and **4** were lyophilized from 0.1% aqueous triethylamine solution to give the respective triethylamine salts.

Antitumor Activity The antitumor activity of synthetic compounds was tested in BALB/c mice. Briefly, a group 8 male mice were inoculated intradermally with Meth A syngenic fibrosarcoma cells (2×10^5). The triethylamine salt of each of the compounds was dissolved in 5% (v/v) aqueous glucose containing 0.1% triethylamine solution. The resulting solution was then administered to the mice at doses of 100 μg/mouse through the tail vein on the 7th, 12th and 17th days after the implantation. The percentage antitumor effect on the growth of the Meth A was determined by dividing the average tumor weight of the test group on the 21st day by the average tumor weight of the control group and multiplying the quotient by 100. Table I shows the results.

Discussion

The three synthetic compounds, **2**, **3** and **4**, having a phosphonooxyethyl group at position 1 showed statistically significant antitumor activity in comparison with the untreated control. Among them, **2** and **3**, whose phosphonooxyethyl glycosides occupied the α-configuration, exhibited high activity of the same level as that of *E. coli*-type lipid A. By contrast, compound **4** with a β-phosphonooxyethyl group was significantly less active.

We can conclude from this result that the presence of the α-glycosyl phosphate is not essential for the antitumor activity of lipid A. Its substitution with α-phosphonooxyethyl glycoside proved to cause no loss of the activity. The α-orientation of the polar group at position 1 is, however, assumed to be important. Change in the anomeric configuration on this substitution resulted in a significant reduction of the activity as observed in the case of **4**, though this compound was still definitely active. It should also be noted that no significant change in activity was observed by replacement of the 3-hydroxytetradecanoyl groups with tetradecanoyl groups.

This work provided a new possibility in synthesization of a wide variety of antitumor active novel structural analogs of lipid A by replacing not only the acyl groups as already mentioned but also the phosphate function(s). Many of the stable polar groups might also serve as candidates for the substituents. Though our preliminary observation suggested that the present phosphonooxyethyl derivatives could unfortunately still be toxic, we expect that production of non-toxic lipid A analogs would be possible through synthetic approaches in this line.

Experimental

All melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined on a Varian XL-200 spectrometer (200 MHz) in chloroform-*d* solutions unless otherwise noted. The chemical shifts are given in δ values with tetramethylsilane (TMS) 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 150 A PLK5F plates (1.0 mm thickness; Whatman) were used for preparative TLC. Organic solutions were dried over sodium sulfate.

2-Acetoxyethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (7) To ethylene glycol (5.0 ml, 88 mmol) and 5 M HCl dioxane solution (0.5 ml) was added **5** (5.00 g, 14 mmol), and the mixture was stirred at 90 °C for 4 h. After cooling with ice-water, pyridine (75 ml) and acetic anhydride (30.6 g, 300 mmol) were added and the mixture was stirred under cooling for 20 min. Stirring was then continued at room temperature for an additional 16 h. The reaction mixture was poured into 350 ml of ice-water and stirred. The precipitate was collected by filtration and was washed with water. The resulting solid was dissolved in $CHCl_3$, and the solution was washed with 1 M HCl, then with saturated aqueous NaCl, and dried. After evaporation of the solvent, the residue was recrystallized from ethanol to give **7** (5.64 g, 71%) as colorless prisms. mp 138—140 °C. $[\alpha]_D$ +74.0° (c=1.2, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{26}Cl_3NO_{12}$: C, 40.26; H, 4.62; N, 2.47. Found: C, 40.48; H, 4.75; N, 2.51. IR (KBr): 3340, 1755, 1540 cm^{-1}. NMR δ: 2.02 (3H, s, OAc), 2.04 (3H, s, OAc), 2.12 (6H, s, OAc × 2), 3.7—4.3 (8H, m), 4.67 and 4.85 (each 1H, AB type d, J=12 Hz, CH_2CCl_3), 4.97 (1H, t, J=4 Hz, H-1), 5.16 (1H, t, J=10 Hz, H-3 or 4) 5.31 (1H, t, J=10 Hz, H-3 or 4). MS *m/z*: 568 [(M+2)$^+$].

2-Acetoxyethyl 3,4,6-Tri-*O*-acetyl-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (9a) To a solution of **7** (3.00 g, 5.29 mmol) in AcOH (40 ml) was added Zn dust (3.00 g), and the mixture was vigorously stirred for 1 h at room temperature. The insoluble materials were filtered off, and the filtrate was concentrated *in vacuo*. The resulting oily product was dissolved in $CHCl_3$, and the solution was washed with 5% aqueous $NaHCO_3$, and dried. The solvent was distilled off at reduced pressure to give an oil.

Separately, DCC (1.32 g, 6.40 mmol) was added to a solution of (*R*)-3-benzyloxytetradecanoic acid (1.95 g, 5.82 mmol) and HOBt (0.98 g, 6.40 mmol) in THF (30 ml) with ice-cooling, and the mixture was stirred for 3 h at room temperature. The precipitate was filtered off to give an active ester solution. The active ester solution was added to the oily amine prepared above, and *N*-methylmorpholine (0.7 ml, 64 mmol) was added to the solution with ice cooling. The mixture was stirred for 3 h at room temperature. After evaporation of the solvent, the residue was purified by silica gel column chromatography and eluted with $CHCl_3$–acetone (25:1—12:1) to give **9a** (3.70 g, 99%) as an oil. NMR δ: 0.87 (3H, t, J=6 Hz), 1.26 (18H, s, CH_2), 2.00 (3H, s, OAc), 2.04 (3H, s, OAc), 2.08 (6H, s, OAc), 2.18 (2H, $COCH_2$), 4.49 and 4.59 (each 1H, AB type d, J=12 Hz, $O\underline{CH_2}C_6H_5$), 4.76 (1H, d, J=4 Hz, H-1), 5.10 (2H, m), 6.30 (1H, d, J=10 Hz, NH), 7.36 (5H, s, arom. H).

Acetoxyethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetradecanoylamino-α-D-glucopyranoside (9b) To a solution of **7** (4.96 g, 8.75 mmol) in AcOH (60 ml) was added Zn dust (7.00 g), and the mixture was vigorously stirred for 1 h at room temperature. The insoluble materials were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in dioxane, dioxane containing dry HCl was added to the solution, and the solvent was removed by evaporation. The resulting oily product was dissolved in CH_2Cl_2 (70 ml), and *N*-methylmorpholine

(2.88 ml, 26.2 mmol) and tetradecanoyl chloride (3.24 g, 13.1 mmol) were added to the solution with ice-cooling. After stirring for 1 h, MeOH (10 ml) was added to the reaction mixture. After stirring for 10 min, the reaction mixture was diluted with $CHCl_3$, 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, 9 : 1—1 : 1) to give **9b** (4.77 g, 91%) as a colorless oil. NMR δ: 0.88 (3H, t), 1.26 (20H, s, CH_2), 2.02 (3H, s, OAc), 2.05 (3H, s, OAc), 2.13 (6H, s, OAc), 2.18 (2H, $COCH_2$), 3.7—4.5 (8H, m), 4.91 (1H, d, J=4 Hz, H-1), 5.15 (1H, t, J=10 Hz, H-3 or 4), 5.27 (1H, t, J=10 Hz, H-3 or 4), 5.78 (1H, d, J=10 Hz, NH). MS m/z: 601 (M^+), 602 [$(M+H)^+$].

2-Hydroxyethyl 2-[(*R*)-3-Benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (10a) To a solution of **9a** (3.68 g, 5.20 mmol) in dry MeOH (30 ml), a MeOH solution (3 ml) containing freshly prepared MeONa (*ca.* 2.8 mmol) was added, and the mixture was stirred for 20 min at room temperature. The solution was neutralized with Amberlist-15, and the resin was filtered off. The filtrate was concentrated at reduced pressure. The residue was washed with Et_2O to give **10a** (2.49 g, 89%) as a pale brown powder, which was recrystallized from EtOH–H_2O. mp 125—127 °C. $[\alpha]_D$ +73.3° (c=0.9, MeOH). *Anal.* Calcd for $C_{29}H_{49}NO_8$: C, 64.54; H, 9.15; N, 2.60. Found: C, 64.33; H, 8.86; N, 2.59. IR (KBr): 3310, 1645, 1555 cm^{-1}. NMR δ: 0.90 (3H, t, J=7 Hz), 1.30 (18H, s, CH_2), 2.43 (1H, dd, J=6, 14 Hz, $NCOCH_2$), 2.58 (1H, dd, J=8, 14 Hz, $COCH_2$), 4.58 (2H, $O\underline{CH_2}C_6H_5$), 4.73 (1H, d, J=4 Hz, H-1).

2-Hydroxyethyl 2-Deoxy-2-tetradecanoylamino-α-D-glucopyranoside (10b) Compound **9b** (4.77 g, 7.93 mmol) was treated with MeONa (*ca.* 9 mmol) in the same manner as described for **10a** to give **10b** (3.02 g, 88%) as a white solid. Recrystallization from EtOH–H_2O gave a purified product. mp 158—160 °C. $[\alpha]_D$ +82.1° (c=0.8, THF–H_2O, 4 : 1). *Anal.* Calcd for $C_{22}H_{43}NO_7$: C, 60.94; H, 10.00; N, 3.23. Found: C, 61.09; H, 9.95; N, 3.25. IR (KBr): 3300 (br), 1645, 1555 cm^{-1}. NMR δ: 0.90 (3H, t), 1.28 (20H, s, CH_2), 2.25 (2H, t, J=7 Hz, $COCH_2$), 4.79 (1H, d, J=4 Hz, H-1). MS m/z: 434 [$(M+H)^+$].

2-Hydroxyethyl 2-[(*R*)-3-Benzyloxytetradecanoylamino]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (11a) To a solution of **10a** (1.20 g, 2.22 mmol) in dimethylformamide (DMF) (5 ml) were added *p*-TsOH·H_2O (38 mg) and 2,2-dimethoxypropane (0.69 g, 6.66 mmol), and the mixture was stirred for 5 h at room temperature. After neutralization with 5% aqueous $NaHCO_3$, the solvent was removed by distillation at reduced pressure. The residue was dissolved in EtOAc, washed with H_2O, then with saturated aqueous NaCl, and dried. After the evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$– MeOH, 19 : 1) to give **11a** (0.98 g, 76%) as a colorless viscous oil. $[\alpha]_D$ +31.4° (c=0.9, $CHCl_3$). NMR δ: 0.89 (3H, t, J=6 Hz), 1.28 (18H, s, CH_2), 1.45 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.50 (2H, dd, $COCH_2$), 4.20 (1H, m, H-2), 4.51 and 4.63 (each 1H, AB type d, J=12 Hz, $\underline{CH_2}C_6H_5$), 4.70 (1H, d, J=4 Hz, H-1), 6.94 (1H, d, J=8 Hz, NH), 7.40 (5H, s, arom. H). MS m/z: 580 (M^+), 546 [$(M-16)^+$].

2-Hydroxyethyl 2-Deoxy-4,6-*O*-isopropylidene-2-tetradecanoylamino-α-D-glucopyranoside (11b) As described for **11a**, compound **10b** (0.87 g, 2.0 mmol) was reacted with dimethoxypropane to give **11b** (0.78 g, 82%) as a colorless viscous oil. NMR δ: 0.88 (3H, t), 1.26 (20H, s, CH_2), 1.46 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.16 (2H, t, J=7 Hz), 3.6—3.9 (9H, m), 4.10 (1H, m), 4.89 (1H, d, J=4 Hz, H-1). MS m/z: 458 [$(M-15)^+$].

2-Acetoxyethyl 3-*O*-Acetyl-2-deoxy-4,6-*O*-isopropylidene-2-tetradecanoylamino-α-D-glucopyranoside (15) To a solution of **11b** (0.12 g, 0.25 mmol) in CH_2Cl_2 (7 ml) were added pyridine (1.5 ml) and acetic anhydride (0.21 ml, 2.10 mmol), and the mixture was stirred for 21 h at room temperature. The reaction mixture was diluted with $CHCl_3$, and 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 ($CHCl_3$–acetone, 19 : 1) to give **15** (0.14 g, quant.) into a waxy solid. Recrystallization from MeOH–H_2O gave the purified product. mp 58—60 °C. *Anal.* Calcd for $C_{29}H_{51}NO_9$: C, 62.45; H, 9.22; N, 2.51. Found: C, 62.06; H, 8.91; N, 2.50. IR (KBr): 3300, 1745, 1650, 1550 cm^{-1}. NMR δ: 0.88 (3H, t, J=6 Hz), 1.26 (20H, s, CH_2), 1.38 (3H, s, CH_3), 1.48 (3H, s, CH_3), 2.06 (3H, s, OAc), 2.11 (3H, s, OAc), 2.16 (2H, t, J=7 Hz, $COCH_2$), 3.6—3.9 (6H, m, H-4, 5, 6 and $O\underline{CH_2}CH_2$), 4.30 (3H, m, H-2 and $\underline{CH_2}OAc$), 4.83 (1H, d, J=4 Hz, H-1), 5.18 (1H, m, H-3), 5.85 (1H, d, J=10 Hz, NH).

2-(Diphenylphosphonooxy)ethyl 2-[(*R*)-3-Benzyloxytetradecanoylamino]-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (12a) To a solution of **11a** (0.83 g, 1.43 mmol), pyridine (0.17 ml, 2.15 mmol) and DMAP (0.26 g, 2.15 mmol) in CH_2Cl_2 (8 ml) was added diphenylphosphorochloridate (0.42 g, 1.57 mmol) with ice cooling. After the mixture was stirred for 4 h at room temperature, MeOH (3 ml) was added. After stirring for a while, the solvent was removed by evaporation. The residue was purified by silica gel column chromatography ($CHCl_3$–acetone, 19 : 1) to give **12a** (0.98 g, 84%) as a colorless oil. NMR δ: 0.88 (3H, t, J=7 Hz), 1.25 (18H, s, CH_2), 1.46 (3H, s, CH_3), 1.53 (3H, s, CH_3), 2.47 (2H, d, J=6 Hz, $COCH_2$), 4.2 (3H, m, H-2 and CH_2OP), 4.49 and 4.57 (each 1H, AB type d, J=12 Hz, $CH_2C_6H_5$), 4.64 (1H, d, J=4 Hz, H-1), 7.2—7.4 (15H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-4,6-*O*-isopropylidene-2-tetradecanoylamino-α-D-glucopyranoside (12b) In the same manner as described for **12a**, compound **11b** (0.77 g, 1.63 mmol) was reacted with diphenylphosphorochloridate to give **12b** (0.81 g, 71%) as a colorless viscous oil. NMR δ: 0.88 (3H, t), 1.24 (20H, s, CH_2), 1.46 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.17 (2H, t, J=8 Hz), 3.6—4.0 (m), 4.2 (1H, m, H-2), 4.45 (2H, m, $\underline{CH_2}OP$), 4.79 (1H, d, J=4 Hz), 7.2—7.4 (10H, m, arom. H). MS m/z: 706 (M^+).

2-(Diphenylphosphonooxy)ethyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (14a) To a solution of **12a** (0.96 g, 1.18 mmol) and (*R*)-3-benzyloxytetradecanoic acid (0.59 g, 1.78 mmol) in CH_2Cl_2 (10 ml) were added DMAP (30 mg, 0.24 mmol) and DCC (0.39 g, 1.90 mmol) with ice cooling. After the mixture was stirred for 18 h at room temperature, the insoluble materials were removed by filtration. The filtrate was washed with 0.2 M HCl, then with saturated aqueous NaCl, and dried. After the evaporation of the solvent, 90% AcOH (30 ml) was added to the residue, followed by stirring for 30 min at 90 °C. The solvent was distilled off, and the resulting residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 19 : 1) to give **14a** (1.23 g, 96%). $[\alpha]_D$ +31.4° (c=1.2, $CHCl_3$). NMR δ: 0.88 (6H, t, J=7 Hz), 1.28 (s, CH_2), 2.34 (2H, d, J=6 Hz, $NCOCH_2$), 2.6 (2H, dd, $COCH_2$), 4.2—4.3 (3H, m, H-2 and CH_2OP), 4.47 and 4.55 (each 1H, AB type d, J=12 Hz, $\underline{CH_2}C_6H_5$), 4.56 (2H, s, $\underline{CH_2}C_6H_5$), 4.71 (1H, d, J=4 Hz, H-1), 5.13 (1H, m, H-3), 6.83 (1H, d, J=10 Hz, NH), 7.2—7.4 (20H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (14b) To a solution of **12b** (0.81 g, 1.15 mmol) in CH_2Cl_2 (10 ml) were added pyridine (0.45 ml, 5.75 mmol), DMAP (50 mg) and tetradecanoyl chloride (0.45 g, 1.84 mmol) with ice cooling. The mixture was stirred for 30 min at 0 °C and then for 30 min at room temperature. After the addition of MeOH (2 ml) and stirring for a few minutes, the mixture was diluted with $CHCl_3$. The solution was washed with 1 M HCl, then with saturated aqueous NaCl, and dried. After evaporation of the solvent, 90% AcOH (30 ml) was added to the residue. The mixture was stirred for 40 min at 90 °C, and then the solvent was distilled off. The resulting residue was purified by silica gel column chromatography ($CHCl_3$–MeOH, 19 : 1) to give **14b** (0.92 g, 92%) as a semi-solid. $[\alpha]_D$ +40.8° (c=1.1, $CHCl_3$). NMR δ: 0.90 (6H, t), 1.24 (s, CH_2), 2.07 (2H, t, J=8 Hz, $COCH_2$), 2.34 (2H, t, J=8 Hz, $COCH_2$), 3.7—4.0 (6H, m), 4.4 (3H, m, H-2 and CH_2OP), 4.84 (1H, d, J=4 Hz, H-1), 5.09 (1H, m, H-3), 6.24 (1H, d, J=9 Hz, NH), 7.2—7.4 (10H, m, arom. H). MS m/z: 876 (M^+).

2-Benzyloxyethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (18) To a suspension of $CaSO_4$ (8.2 g) in CH_2Cl_2 (90 ml) were added $BF_3 \cdot Et_2O$ (3.79 ml, 30 mmol) and **16** (7.78 g, 20 mmol) at room temperature. After the reaction mixture was stirred for 2.5 h, an additional $BF_3 \cdot Et_2O$ (1.26 ml, 10 mmol) was added and the mixture was stirred for 16.5 h at room temperature. The mixture was poured into 250 ml of 5.2% aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with saturated aqueous NaCl and dried. The solvent was evaporated *in vacuo* to give a viscous oil. To a solution of the resultant oil and ethylene glycol monobenzylether (6.09 g, 40 mmol) in dry $CHCl_3$ (90 ml) was added *p*-TsOH·H_2O (190 mg, 1.00 mmol). This mixture was refluxed for 6.5 h and washed with 5% aqueous $NaHCO_3$, then with saturated aqueous NaCl, and dried. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CHCl_3$–acetone, 19 : 1—7 : 1). The resulting residue was washed with Et_2O to give **18** (3.60 g, 37%). Recrystallization from MeOH–H_2O gave a purified product of **18**. mp 106—107 °C. $[\alpha]_D$ −17.0° (c=1.2, $CHCl_3$). *Anal.* Calcd for $C_{23}H_{31}NO_{10}$: C, 57.37; H, 6.49; N, 2.91. Found: C, 57.32; H, 6.36; N, 2.95. IR (KBr): 3250, 1750, 1650, 1575 cm^{-1}. NMR δ: 1.86 (3H, s, OAc), 2.04 (6H, s, OAc × 2), 2.09 (3H, s, OAc), 3.6—4.3 (8H, m), 4.58 (2H, s, $\underline{CH_2}C_6H_5$), 4.78 (1H, d, J=8 Hz, H-1), 5.11 (1H, t, J=10 Hz, H-3), 5.28 (1H, t, J=10 Hz, H-4), 5.56 (1H, d, J=10 Hz, NH), 7.38 (5H, s, arom. H). MS m/z: 482 (M^+).

2-Benzyloxyethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-tetradecanoylamino-β-

D-glucopyranoside (20) To a solution of **18** (1.50 g, 3.1 mmol) in CH_2Cl_2 (30 ml) were added K_2CO_3 (1.55 g, 11.2 mmol) and freshly prepared $Et_3O \cdot BF_4$ at room temperature. After stirring for 17.5 h, the mixture was washed with H_2O, then with saturated aqueous NaCl, and dried. Evaporation of the solvent gave an oil. This oil was dissolved in THF (30 ml), and 1 M HCl (3.2 ml) was added to the solution with ice cooling. The solution was stirred for 40 min at room temperature and concentrated *in vacuo* to give **19** (1.56 g) as white powder. As described for **9a**, compound **19** was reacted with tetradecanoyl chloride (0.92 g, 3.7 mmol) to give **20** (1.11 g, 55%) as a white powder. Recrystallization from benzene–hexane gave a purified product of **20**. mp 101—102 °C. $[\alpha]_D$ −5.4° (c=0.9, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{55}NO_{10}$: C, 64.69; H, 8.53; N, 2.16. Found: C, 64.69; H, 8.33; N, 2.27. NMR δ: 0.88 (3H, t, J=6 Hz, CH_3), 1.26 (20H, s, CH_2), 2.02, 2.04 and 2.09 (11H, 3s, OAc×3 and $COCH_2$), 3.6—4.1 (6H, m), 4.15 (1H, dd, J=12, 2 Hz, H-6), 4.82 (1H, dd, J=12, 5 Hz, H-6), 4.58 (2H, s, $\underline{CH_2}C_6H_5$), 4.82 (1H, d, J=8 Hz, H-1), 5.12 (1H, t, J=10 Hz, H-3), 5.31 (1H, t, J=10 Hz, H-4), 5.45 (1H, d, J=8 Hz, NH), 7.38 (5H, s, arom. H). MS m/z: 649 (M^+).

2-Benzyloxyethyl 2-Deoxy-2-tetradecanoylamino-β-D-glucopyranoside (21) In a manner similar to that for **10a**, compound **20** (1.05 g, 1.6 mmol) was treated with MeONa (*ca.* 2.2 mmol) in MeOH (20 ml) to give **21** (0.74 g, 87%) as a white powder. Recrystallization from EtOH–H_2O gave a purified product of **21**. mp 155—156 °C. $[\alpha]_D$ −18.1° (c=0.5, MeOH). *Anal.* Calcd for $C_{29}H_{49}NO_7$: C, 66.51; H, 9.43; N, 2.67. Found: C, 66.63; H, 9.43; N, 2.75. IR (KBr): 3451, 3390, 1665, 1535 cm^{-1}. NMR δ: 0.88 (3H, t, J=6 Hz), 1.28 (20H, s, CH_2), 2.04 (2H, t, J=7 Hz), 3.5—4.1 (m), 4.55 (1H, d, J=8 Hz, H-1), 4.62 (2H, s, $\underline{CH_2}C_6H_5$), 7.40 (5H, s, arom. H). MS m/z: 525 [$(M+H)^+$].

2-Benzyloxyethyl 2-Deoxy-4,6-*O*-isopropylidene-2-tetradecanoylamino-β-D-glucopyranoside (22) As described for **11**, compound **21** (710 mg, 1.36 mmol) was reacted with dimethoxypropane (0.42 g, 4.1 mmol) in CH_2Cl_2 (30 ml) in the presence of p-TsOH·H_2O to give **22** (710 mg, 93%) as a waxy solid. NMR δ: 0.88 (3H, t, J=6 Hz), 1.26 (20H, s, CH_2), 1.46 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.02 (2H, t, J=8 Hz), 3.3 (1H, m), 3.5—4.1 (9H, m), 4.62 (1H, d, J=8 Hz, H-1), 4.62 (2H, s, $\underline{CH_2}C_6H_5$), 7.38 (5H, m, arom. H). MS m/z: 565 [$(M+H)^+$].

2-Benzyloxyethyl 2-Deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranoside (23) As described for **13b**, compound **22** (0.70 g, 1.24 mmol) was treated with tetradecanoyl chloride (0.46 g, 1.86 mmol) to give **23** (0.79 g, 82%). Recrystallization from hexane gave a purified product of **23**. mp 88—89 °C. $[\alpha]_D$ −29.0° (c=0.8, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{79}NO_8$: C, 71.37; H, 10.29; N, 1.81. Found: C, 71.32; H, 10.19; N, 1.82. IR (KBr): 1740, 1665, 1530 cm^{-1}. NMR δ: 0.89 (6H, t, J=7 Hz), 1.26 (40H, s, CH_2), 1.38 (3H, s, CH_3), 1.47 (3H, s, CH_3), 2.05 (2H, t, J=8 Hz), 2.32 (2H, m), 3.38 (1H, m), 3.6—4.2 (8H, m), 4.57 (2H, s, $\underline{CH_2}C_6H_5$), 4.62 (1H, d, J=8 Hz, H-1), 5.08 (1H, t, J=10 Hz, H-3), 5.54 (1H, d, J=10 Hz, NH), 7.38 (5H, m, arom. H). MS m/z: 774 (M^+).

2-Hydroxyethyl 2-Deoxy-4,6-*O*-isopropylidene-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranoside (24) A suspension of **23** (745 mg, 0.96 mmol) in AcOH (50 ml) was hydrogenolyzed over 5% palladium carbon as a catalyst at room temperature for 15 h (atmospheric pressure). The catalyst was removed by filtration, and the filtrate was freeze-dried to give a powder. The catalyst on the filter was washed with $CHCl_3$. The freezed-dried powder was dissolved in the $CHCl_3$ washing and the resulting solution was washed with 5% aqueous $NaHCO_3$, then with H_2O, and dried. After evaporation of the solvent, the residue was purified by short silica gel column chromatography ($CHCl_3$–acetone, 9 : 1) to give **24** (529 mg, 80%) as a white powder, which gradually softened on heating without showing a definite melting point. IR (KBr): 3320, 1740, 1660, 1535 cm^{-1}. NMR δ: 0.89 (6H, t, J=7 Hz), 1.26 (40H, s, CH_2), 1.38 (3H, s, CH_3), 1.48 (3H, s, CH_3), 2.15 (2H, t, J=7 Hz), 2.33 (2H, m), 3.40 (1H, m), 3.7—4.2 (9H, m), 4.50 (1H, d, J=8 Hz, H-1), 5.08 (1H, t, J=10 Hz, H-3), 5.28 (1H, d, J=8 Hz, NH). MS m/z: 684 (M^+).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranoside (26) As described for **12a** and **14a**, compound **24** (515 mg, 0.75 mmol) was reacted with diphenylphosphorochloridate (304 mg, 1.13 mmol), and the resulting oily **25** was treated with 90% AcOH to give **26** (491 mg, 74%) as an oil. $[\alpha]_D$ −19.5° (c=0.9, $CHCl_3$). NMR δ: 0.88 (6H, t, J=7 Hz), 1.24 (40H, s, CH_2), 2.08 (2H, t, J=8 Hz), 2.36 (2H, t, J=8 Hz), 3.40 (1H, m), 3.6—4.0 (8H, m), 4.20 (2H, m, CH_2OP), 4.62 (1H, d, J=8 Hz, H-1), 5.02 (3H, t, J=10 Hz, H-3), 5.96 (1H, d, J=8 Hz, NH), 7.2—7.4 (10H, m, arom. H). MS m/z: 875 (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-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside (29a) To a solution of **27** (450 mg, 0.36 mmol) in CH_2Cl_2 (2 ml) was added 25% HBr–AcOH (6 ml), and the mixture was stirred for 1.5 h at room temperature. After the reaction mixture was diluted with $CHCl_3$, the solution was washed successively with ice-water, 5% aqueous $NaHCO_3$ and saturated aqueous NaCl, and dried. Evaporation of the solvent gave **28** as an oil. The oil and **14a** (392 mg, 0.36 mmol) were dissolved in CH_2Cl_2 (5 ml). To the soltuion were added $CaSO_4$ (0.5 g) and mercuric cyanide (182 mg, 0.72 mmol), and the mixture was refluxed for 14 h. The insoluble materials were removed by filtration through Celite 545, and the filtrate was washed with 5% aqueous potassium iodide, then with 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 **29a** (554 mg, 67%) as a colorless oil. NMR δ: 0.89 (12H, t, J=7 Hz), 1.28 (s, CH_2), 2.2—2.7 (8H, m, $COCH_2 \times 4$), 5.66 (1H, m, H′-3), 7.2—7.4 (30H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (29b) As was described for **29a**, compound **28**, obtained from **27** (400 mg, 0.32 mmol), was reacted with **14b** (276 mg, 0.32 mmol) to give **29b** (537 mg, 81%) as an oil. NMR δ: 0.88 (12H, t), 1.24 (78H, s, CH_2), 2.07 (2H, t, J=8 Hz), 2.3 (6H, m), 4.63 and 4.76 (AB type d, J=12 Hz), 5.63 (1H, m), 7.2—7.4 (20H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphospho-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranoside (29c) As described for **29a**, compound **28** (*ca.* 0.32 mmol) was treated with **26** (280 mg, 0.32 mmol) to give **29c** (522 mg, 79%) as a colorless viscous oil. NMR δ: 0.89 (12H, t, J=7 Hz), 1.24 (78H, s, CH_2), 2.07 (2H, t), 2.22 (2H, t), 2.38 (4H, m), 5.23 (1H, m), 5.58 (1H, t, J=10 Hz), 7.2—7.4 (20H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-α-D-glucopyranoside (31a) As described for **9a**, compound **29a** (520 mg, 0.32 mmol) was treated with Zn dust, and the resulting oil was condensed with the HOBt active ester of (*R*)-3-dodecanoyloxytetradecanoic acid (149 mg, 0.35 mmol) to give **31a** (327 mg, 61%) as a colorless oil. NMR δ: 0.89 (18H, t, J=7 Hz), 1.28 (108H, s, CH_2), 2.2—2.7 (12H, m, $COCH_2 \times 6$), 5.58 (1H, m, H-3), 7.2—7.4 (30H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (31b) In the manner described for **9a**, compound **29b** (527 mg, 0.26 mmol) was treated with Zn powder, and the resulting oil was condensed with the HOBt active ester of (*R*)-3-dodecanoyloxytetradecanoic acid (166 mg, 0.39 mmol) to give **31b** (286 mg, 53%) as an oil. $[\alpha]_D$ +13.3° (c=0.5, $CHCl_3$). NMR δ: 0.88 (18H, t), 1.24 (112H, s, CH_2), 2.03 (2H, m), 2.3 (10H, m), 5.57 (1H, m), 7.2—7.4 (20H, m, arom. H).

2-(Diphenylphosphonooxy)ethyl 2-Deoxy-6-*O*-[2-deoxy-4-*O*-diphenylphosphono-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-β-D-glucopyranoside (31c) As in the case of **9a**, compound **29c** (509 mg, 0.25 mmol) was condensed with Zn dust, and the resulting oil was reacted with the HOBt active ester of (*R*)-3-dodecanoyloxytetradecanoic acid (160 mg, 0.37 mmol) to give **31c** (286 mg, 55%) as a colorless viscous oil. $[\alpha]_D$ −16.5° (c=0.7, $CHCl_3$). NMR δ: 0.88 (18H, t, J=7 Hz), 1.24 (112H, s, CH_2), 2.05 (2H, m), 2.3 (10H, m), 5.56 (1H, t, J=10 Hz), 7.2—7.4 (20H, m, arom. H).

2-Phosphonooxyethyl 2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranoside (2) Compound **31a** (314 mg, 0.13 mmol) was dissolved in AcOH (12 ml) and hydrogenolyzed in the presence of 5% palladium–carbon for 6 h at room temperature (atmospheric pressure). The catalyst was removed by filtration and the filtrate was condensed by evaporation *in vacuo*. The residue was hydrogenolyzed over platinum dioxide (atmospheric pressure), and

the resulting powder was purified by preparative TLC using $CHCl_3$–MeOH–H_2O (6:4:0.7). After extraction with $CHCl_3$–MeOH–H_2O–Et_3N (6:4:1:0.02), the solvent was evaporated *in vacuo*. The residue was dissolved in $CHCl_3$–MeOH–H_2O (6:4:1), and the solution was desalted with Dowex 50 (H^+ type). The desalted solution was concentrated, and the residue was lyophilized from 0.1% aqueous Et_3N to give the Et_3N salt of **2** (141 mg) as a white powder. The physical data given below are those of the free acid form of **2**. mp 144—147 °C (dec.). $[\alpha]_D$ +9.8° (c=0.5, $CHCl_3$–MeOH, 3:1). IR (KBr): 1740, 1660 cm^{-1}. NMR ($CDCl_3$–CD_3OD, 1:1) δ: 0.90 (18H, t, J=6 Hz), 1.30 (108H, s, CH_2), 2.3—2.7 (12H, m, $COCH_2 \times 6$), 5.2 (4H, m, $CHCO \times 4$).

2-Phosphqnooxyethyl 2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-tetradecanoylamino-α-D-glucopyranoside (3) Compound **31b** (260 mg, 0.12 mmol) was dissolved in THF (15 ml) and hydrogenolyzed with the mixture of platinum dioxide (290 mg) and 5% palladium–carbon (140 mg) as a catalyst at room temperature for 38 h (atmospheric pressure). The catalyst was removed by filtration, and washed with $CHCl_3$–MeOH–H_2O (6:4:0.5). The filtrate and the washing solution were combined, and the solvent was evaporated at reduced pressure. The residue was purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6:4:0.5), and the resultant extracted solution, in the same manner as described for **2**, was desalted with Dowex 50 (H^+ type). A portion of the desalted solution was concentrated, and the residue was lyophilized from dioxane suspension to give **3** (20 mg) as a white powder. mp 163—167 °C (dec.). $[\alpha]_D$ +15.0° (c=0.6, $CHCl_3$–MeOH, 3:1). *Anal.* Calcd for $C_{96}H_{182}N_2O_{24}P_2 \cdot 2H_2O$: C, 62.45; H, 10.15; N, 1.52. Found: C, 62.13; H, 9.87; N, 1.52. IR (KBr): 1735, 1660, 1565 cm^{-1}. NMR ($CDCl_3$–CD_3OD, 1:1) δ: 0.90 (18H, t, J=6 Hz), 1.30 (112H, s, CH_2), 2.19 (2H, m), 2.3—2.4 (8H, m), 2.65 (2H, m), 5.2 (m).

The residual desalted solution was adjusted with $CHCl_3$–MeOH–H_2O–Et_3N (6:4:1:0.02) to about pH 8 with ice-cooling. After evaporation of the solvent, the residue was lyophilized from 0.1% aqueous Et_3N to give 36 mg of Et_3N salt of **3** as a white powder.

2-Phosphonooxyethyl 2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-tetradecanoyl-2-*O*-tetradecanoylamino-β-D-glucopyranoside (4) In the same manner as described for **2**, compound **31c** (260 mg, 1.23 mmol) was hydrogenolyzed in the presence of a catalyst, and the resulting powder was purified by preparative TLC ($CHCl_3$–MeOH–H_2O, 6:4:0.6) and desalted. The product was lyophilized from 0.1% aqueous Et_3N to give the Et_3N salt of **4** (115 mg) as a white powder. mp 145—150 °C (dec.). $[\alpha]_D$ −11.8° (c=0.5, $CHCl_3$–MeOH, Et_3N salt of **4**). IR (KBr): 1730, 1635, 1560 cm^{-1}. NMR ($CDCl_3$–CD_3OD, 1:1) δ: 0.89 (18H, t), 1.26 (112H, s, CH_2), 2.06 (2H, m), 2.3—2.5 (8H, m), 2.66 (2H, m).

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