 © 1987 The Chemical Society of Japan Bull. Chem. Soc. Jpn., 60, 2205—2214 (1987)

Total Synthesis of *Escherichia coli* Lipid A, the Endotoxically Active Principle of Cell-Surface Lipopolysaccharide

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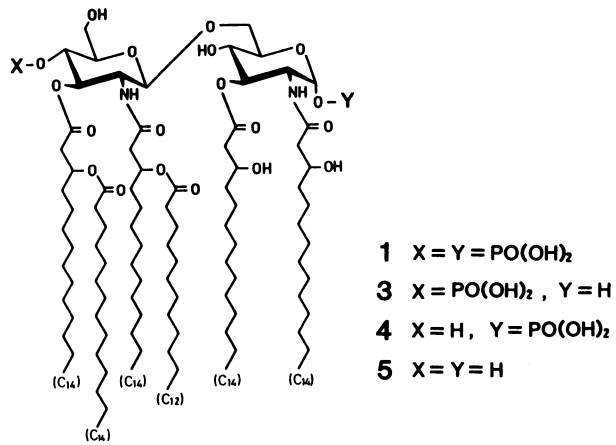
(Received December 15, 1986)

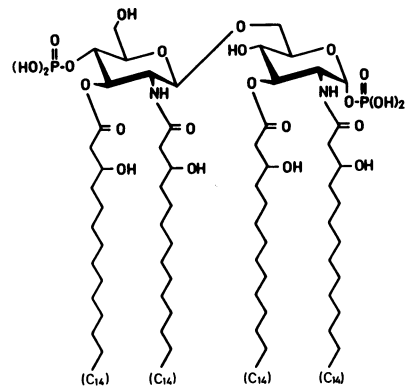
Chemical syntheses are described of polyacylated $\beta(1\rightarrow6)$ glucosamine disaccharide 1,4′-bis(phosphate), which corresponds to the proposed structure of *E. coli* lipid A, and of its dephospho derivatives. The synthetic bisphosphate proved to be identical with the corresponding natural specimen. The chemical structure of lipid A was thus established.

As described in the preceding paper,[1] lipopolysaccharide (LPS) which is located on the cell surface of gram-negative bacteria is called endotoxin and causes a diversity of toxic and beneficial biological responses in animals.[2] Its lipophilic part which could be liberated by mild acid hydrolysis of LPS was designated "lipid A" and has been believed to be responsible for most of the endotoxic activities.[2]

We recently elucidated the complete chemical structure **1** of lipid A isolated from *Escherichia coli* cells.[3] The structure contains a backbone of $1\alpha,4'$-bisphosphorylated $\beta(1\rightarrow6)$ disaccharide of D-glucosamine. The backbone is acylated at 2,2′- and 3,3′-positions with four moles of (*R*)-3-hydroxytetradecanoic acid. The 3-hydroxyl groups of amide- and ester-bound 3-hydroxytetradecanoic acid on the distal glucosamine residue are acylated with docosanoic and tetracosanoic acid respectively.



1 X = Y = PO(OH)$_2$
3 X = PO(OH)$_2$, Y = H
4 X = H, Y = PO(OH)$_2$
5 X = Y = H



2

In the meantime we synthesized a biosynthetic precursor ("Precursor Ia") (**2**) of lipid A.[1,4] This compound contains four moles of 3-hydroxytetradecanoic acid but lacks the non-hydroxylated acids found in **1**. The results of biological tests showed that the precursor Ia (**2**) exhibits not all but many of definite endotoxic activities such as lethal toxicity.[5] This fact clearly demonstrates that an acyl disaccharide phosphate like **2** is certainly responsible for the activities of LPS. Simultaneously, it could be assumed that the 3-acyloxyacyl structure which is typical of *E. coli* and other natural mature lipid A's but absent in the biosynthetic precursor **2** is important for manifestation of pyrogenicity and Shwartzman reactivity[6] which are typical endotoxic activities not observed in both synthetic and natural precursor Ia (**2**).[5] In order to confirm this assumption as well as to construct chemically a compound with full endotoxic activity and finally to confirm the proposed structure of *E. coli* lipid A, we aimed at a total synthesis of **1**. As described in our preliminary communications,[7,8] the structure **1** was first confirmed by this synthesis and synthetic **1** proved to have identical biological activities with those of lipid A isolated from *E. coli* cells.[9] It could now be concluded that **1** is indeed the active structural principle of endotoxin thirty years after the report of Westphal and Lüderitz by whom lipid A was first described.[10] In this paper we describe the details of the synthesis of **1** and its dephospho derivatives (**3**, **4**, **5**).

Most of the problems in the synthetic approach to *E. coli*-type lipid A had been already solved in our previous works.[1,11] These include, for example, preparation of optically pure (*R*)-3-hydroxytetradecanoic acid, its protection and introduction of an α-glycosyl phosphate. The distinct feature of the present synthesis lies in the following point. As seen in the structural formula, the two glucosamine residues of *E. coli* lipid A (**1**) contain different acyl groups each other. For its synthesis, therefore, two appropriately acylated glucosamine units were first prepared and then coupled together to form an acylated disaccharide. This approach, which is different from that used in our previous synthesis of **2**, seemed to be advantageous to

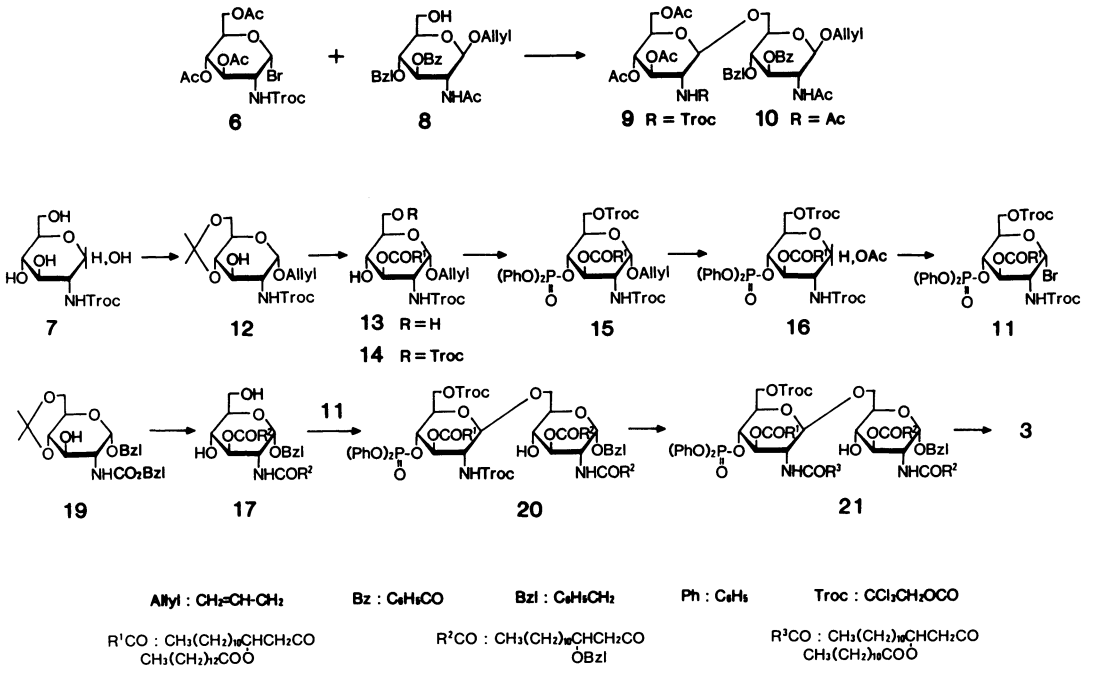
minimize the use of temporary protecting groups for the attempted synthesis of **1**.[12] The phosphate moiety on 4′-position was also introduced at the monosaccharide stage for the same reason.

Among the four acyl groups bound to the disaccharide, however, 3-dodecanoyloxytetradecanoyl group on the 2′-amino function had to be introduced after the formation of the disaccharide. Preliminary model experiments revealed that *N*-bound 3-acyloxyacyl group on a glucosamine molecule underwent a β-elimination to form Δ^2-acyl group when the glycosidic position was activated for glycoside bond formation.[13]

Use of 2,2,2-trichloroethoxycarbonyl (Troc) group[14] was examined for the temporary protection of the particular amino group during the glycosidation reaction with a glycosyl halogenide. The carbonyl group of 2-Troc-amino function was expected to participate the neighboring glycosyl cation in a similar manner to a usual acyl group and thus to assist the formation of a desired 1,2-trans β-glycosidic bound. Furthermore, Troc was expected to be advantageous since this urethane type group does not form an oxazoline which is one of the main side products in the glycosidation reaction with 2-acylamino glycosyl halogenides. 2-*N*-Troc-glucosamine **6** as a model donor compound was prepared from *N*-Troc-glucosamine (**7**). Thus, peracetyl derivative of **7** was converted almost quantitatively to the bromide **6** on reaction with dry hydrogen bromide in dichloromethane as monitored by TLC. Coupling of **6** with a model acceptor **8** proceeded satisfactorily in the presence of mercury(II) cyanide in boiling chloroform to give a disaccharide **9**. The glycosyl bromide of *N*-Troc-glucosamine was less reactive and hence more stable than a corresponding *N*-acetyl-type derivative. Undesirable side reactions were not obvious in the coupling reaction of the former. The $\beta(1\rightarrow6)$ structure of **9** was ascertained at the corresponding acetyl derivative which was prepared by displacing the Troc of **9** with acetyl group. The resultant bis(*N*-acetyl) disaccharide **10** was identical with a sample obtained in our previous work.[1] As expected, Troc group proved to be suitable for the amino protection: It assures formation of the desired β-glycoside and is removable selectively after formation of the disaccharide.

In order to examine the synthetic strategy according to the above considerations, we first attempted to prepare the 4′-monophosphate derivative **3** of *E. coli* lipid A. This synthesis seemed to be urgently important for the purpose of confirming the structure of lipid A because the dimethyl ester of **3** was the sole chemical entity so far derived in a pure state from a natural source.[3a,15] Direct identification of the natural and synthetic dimethyl ester of **3** was expected to provide a decisive evidence for the structure **1**.

The glycosyl donor **11** for this synthesis was prepared from *N*-Troc-D-glucosamine (**7**). Reaction of **7** with allyl alcohol in the presence of hydrogen chloride gave predominantly the α-glycoside. The α/β ratio was approximately 5/1. Isopropylidenation of the glycoside mixture followed by a simple recrystallization afforded almost pure α-allyl glycoside **12** in an acceptable yield.[16] After 3-*O*-acylation of **12** with (*R*)-3-tetradecanoyloxytetradecanoic acid which was prepared via phenacyl (*R*)-3-hydroxytetradecanoate,[1] the isopropylidene group was removed to give **13**. Selective 6-*O*-mono(trichloroethoxycarbonylation) followed by phosphorylation with diphenyl phosphorochloridate and pyridine-4-dimethylaminopyridine (DMAP).[17,18] Gave a glucosamine derivative **15** which contained all required substituents for the glycosyl donor. The allyl group in **15** was then cleaved off via



isomerization with an iridium complex to 1-propenyl group[19] followed by a reaction with iodine.[20] The product was converted via 1-*O*-acetate **16** to the glycosyl bromide **11** which was used for the coupling reaction without purification.

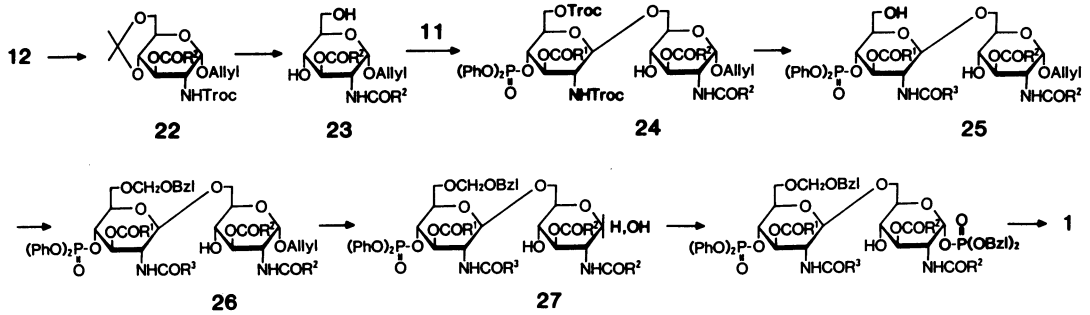
The glycosyl acceptor **17** was prepared from benzyl *N*-benzyloxycarbonyl-α-D-glucosaminide (**18**)[21] by 4,6-*O*-isopropylidenation, selective hydrogenolysis of *N*-benzyloxycarbonyl group[21] and simultaneous 3-*O*- and 2-*N*-acylation with (*R*)-3-benzyloxytetradecanoic acid[1] followed by deisopropylidenation. Because a primary 6-hydroxyl group has generally much higher reactivity than a 4-hydroxyl group in a glucopyranoside structure, preferential formation of the (1→6)-glycoside linkage was reasonably expected in the reaction of **17** without protecting the 4-position.[11] Furthermore, the 4-hydroxyl group becomes more hindered after formation of the β(1→6)disaccharide as observed in our previous work.[11] Therefore, a protection of this particular group seemed to be not necessary even during the further transformation of more reactive positions like 1- and 6′-hydroxyl groups of the disaccharide.

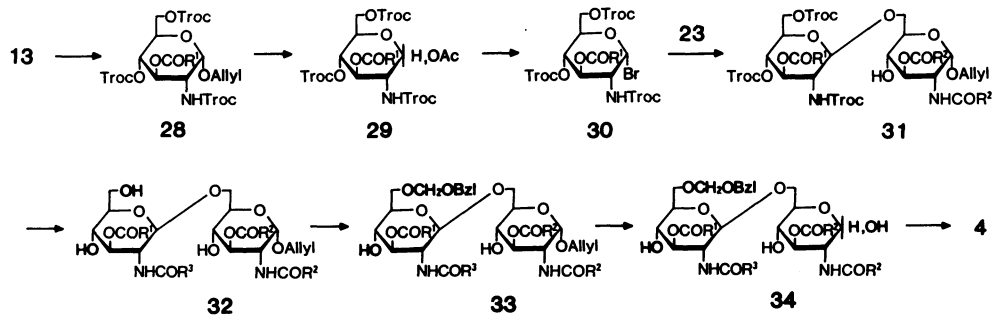
Coupling of the bromide **11** and the acceptor **17** was effected as the above model reaction with mercury(II) cyanide to give a sole disaccharide **20**, whose structure was confirmed by examination of its ^{1}H NMR spectrum. After cleavage of Troc groups of the disaccharide **20**, the last acyl group, i.e., (*R*)-3-dodecanoyloxytetradecanoyl group, was finally introduced to the 2′-amino function by use of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride. When dicyclohexylcarbodiimide was used for this step in place of the above water-soluble carbodiimide, the acylation reaction itself proceeded as well, but *N*-acyl-*N*,*N*′-dicyclohexylurea formed as a by-product made the chromatographic purification of the desired product difficult.

The acylation product **21** which contained all required functional groups on the respective positions was then subjected to two-step hydrogenolytic deprotection first with palladium and then with platinum. The deprotected compound **3** was identified by TLC with the corresponding 4′-monophosphate derived from *E. coli* lipid A.

For more strict identification by ^{1}H NMR, the product **3** was attempted to be converted to the dimethyl ester. However, the synthetic monophosphate was found to contain inorganic impurities and metal cations resulted from the hydrogenolysis catalysts. Some of cations formed salts with the phosphate group of **3**. Because the phosphate group must be converted to a free form prior to the reaction with diazomethane, removal of the inorganic contaminants was next tried. Removal of metal ions was also indispensable for preparation of triethylammonium salt which is a necessary form for biological tests.[22] Whereas the synthetic bisphosphate **2** and both its monophosphate analogs of the precursor-type could be freed from the contaminating cations by a combination of electrodialysis and acidic precipitation,[1] this method could not be applied to the present monophosphate **3** which has more lipophilic acyl groups and less solubility in water than the precursor-type derivatives. However, washing of a chloroform–methanol solution of **3** with cold hydrochloric acid under controlled conditions to avoid decomposition of the target compound was found to be effective to obtain salt-free **3**. It was then treated with diazomethane to give a compound which was identical on TLC with the corresponding dimethyl ester obtained from a natural source.[3] The synthetic and natural specimens were unequivocally identified by comparison of the 360 MHz ^{1}H NMR spectra of these dimethyl esters and their peracetates. Although the spectrum of the natural dimethyl ester still exhibited signals due to small amount of impurities, its peracetate purified and the corresponding synthetic one gave superimposable spectra.

Conclusively, the structure of *E. coli* lipid A proposed by us was confirmed by the present synthesis of 4′-monophosphate (**3**). The next step in our study was a chemical construction of the complete *E. coli* lipid A structure **1** containing both phosphate moieties. This could be performed in the same way as the above synthesis except that the glycosidic position of the disaccharide was protected with allyl group. This protecting group could be removed for the introduction of the unstable glycosyl phosphate just before the final hydrogenolysis. The corresponding glycosyl acceptor monosaccharide **23** was prepared from the same intermediate, 4,6-*O*-isopropylidenated allyl glycoside of *N*-Troc-D-glucosamine (**12**) described above. Condensation of **23** with the same glycosyl bromide (**11**) gave the disaccharide **24**. After removal of the two Troc group





and introduction of (*R*)-3-dodecanoyloxytetradecanoyl group to the 2′-amino function, the 6′-hydroxyl group was protected by benzyloxymethylation for the subsequent glycosidic phosphorylation to give **26**.[23] Cleavage of the allyl group of **26**, glycosyl phosphorylation[11] and hydrogenolysis were successively performed in the same manner as described previously.[1]

The product (**1**) was purified by silica-gel column chromatography. Owing to the improved solubility in water compared to the 4′-monophosphate (**3**) above, the product (**1**) could be freed from inorganic contaminants by electrodialysis and precipitation with cold hydrochloric acid. This synthetic compound was identified on TLC with the main component of lipid A from *E. coli* Re cells. Further identification on TLC with the natural specimen was performed after cleavage of the glycosyl phosphate and methyl esterification.

As in the case of the biosynthetic precursor described in the preceding paper,[1] both 1-monophosphate (**4**) and dephospho derivative (**5**) of *E. coli*-type lipid A were also prepared. Comparison of the biological activities of a set of pure synthetic materials which have the same acyl disaccharide skeleton, but are only different in presence of phosphate groups, could give important informations particularly concerning the effect of the phosphates on the activities.

For the preparation of **4** and **5**, a glycosyl bromide **30** devoid of 4-phosphate moiety was prepared from **13** as a glycosyl donor. The 4-hydroxyl group of **30** was also protected with Troc group during the glycosidation. After coupling of the bromide **30** with the acceptor **23** to form a disaccharide **31**, the all Troc groups were removed and the 2′-amino group was acylated. Protection of 6′-position followed by glycosyl phosphorylation as above and deprotection afforded the 1-monophosphate (**4**). Chromatographic purification and washing with hydrochloric acid as described for 4′-monophosphate (**3**) gave pure **4**. The dephospho derivative **5** was obtained by direct deprotection of a intermediate **34** without phosphorylation.

Comparative studies were carried out on the biological activities of the synthetic products (**1**, **3**—**5**) and the corresponding natural lipid A. As already described,[9] the synthetic bisphosphate (**1**) manifested complete and identical activities including pyrogenicity and Shwartzman reactivity with the natural specimen. In conclusion, this work established first a synthetic route for lipid A and consequently provided the evidence that this structure should be certainly responsible to the active principle of endotoxin. Owing to these results, it becomes now possible to prepare any desired structural analogs of lipid A in a chemically pure forms by appropriate modification of the above synthesis. In addition, precise studies will be possible on the relationships between the biological activities and chemical structures of this type of unique glycolipid.

Experimental

All melting points are uncorrected. ^{1}H NMR spectra were measured for chloroform-*d* solutions on a Varian XL-100-15 spectrometer (100 MHz) unless noted otherwise. The chemical shifts are given in δ values with TMS as the internal standard. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Conventional and medium-pressure (2—4 kg cm^{-2}) column chromatography were carried out with Kieselgel 60 (E. Merck), 0.063—0.2 and 0.040—0.063 mm, respectively. Organic solutions were dried over magnesium sulfate.

2-Deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucose (7). 2,2,2-Trichloroethoxycarbonyl chloride (50.0 g, 236 mmol) was added portionwise to an ice-cooled solution of 2-amino-2-deoxy-D-glucose hydrochloride (34.0 g, 158 mmol) and sodium hydrogencarbonate (34.0 g, 405 mmol) in water (500 ml). The mixture was stirred in an ice bath for 2 h and then at room temperature overnight. The colorless precipitate was collected by filtration, washed with water and ether, and then recrystallized from ethanol; yield 48.9 g (87%); mp 183—184 °C (decomp); $[\alpha]_D^{19}$ +50.4° (*c* 0.84, methanol). Found: C, 29.98; H, 4.19; N, 3.88; Cl, 29.20%. Calcd for $C_9H_{14}NO_7Cl_3 \cdot 0.5H_2O$: C, 29.73; H, 4.16; N, 3.85; Cl, 29.25%.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl Bromide (6). A solution of the syrupy tetraacetate (500 mg, 0.96 mmol) obtained from **7** by the usual manner was dissolved in anhydrous dichloromethane (100 ml). The solution was saturated with dry hydrogen bromide at 0 °C and left at room temperature in a sealed flask for 18 h. After evaporation of the solvent in vacuo, the residual syrup was dried in vacuo over diphosphorus pentaoxide and sodium hydroxide; yield 520 mg. It was used for the following reaction without further purification.

Allyl 2-Acetamido-6-*O*-[3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-β-D-glucopyranoside (9). A solution of the bromide **6** (520 mg, 0.96 mmol), allyl 2-acetamido-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-β-D-glucopyr-

anoside[1] (432 mg, 0.96 mmol) and mercury(II) cyanide (480 mg, 1.90 mmol) in anhydrous chloroform (10 ml) was heated under reflux for 40 min. After removal of the insoluble materials by filtration, the filtrate was washed successively with a saturated aqueous solution of sodium hydrogencarbonate and water, dried, and evaporated in vacuo. Purification by medium-pressure silica-gel column chromatography (90 g, chloroform–acetone 3:1) afforded a colorless solid; yield 390 mg (45%). ^{1}H NMR δ=1.81 (3H, s, NAc), 2.01 (6H, s, OAc), 2.04 (3H, s, OAc), and 7.1—8.2 (11H, m, aromatic H and NH).

Allyl 2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzoyl-4-*O*-benzyl-2-deoxy-β-D-glucopyranoside (10). Zinc powder (780 mg) was added to a solution of **9** (390 mg, 0.43 mmol) in acetic acid (10 ml). The mixture was heated at 50 °C with stirring for 30 min and then cooled to room temperature. After removal of the insoluble materials by filtration, the solvent was evaporated in vacuo. The residue was dissolved in chloroform, washed with water, and dried. The syrupy residue obtained after evaporation of the solvent in vacuo was again dissolved in chloroform and treated with triethylamine (67 μl, 0.48 mmol) and acetic anhydride (60 μl, 0.48 mmol). After usual work-up, the product was recrystallized from methanol–ether; yield 200 mg (59%); mp 220—223 °C (decomp). This product was identified with an authentic sample of **10** obtained in our previous work[1] by comparison of mps, TLC and ^{1}H NMR.

Allyl 2-Deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (12). Compound **7** (35.0 g, 98.7 mmol) was heated with stirring at 100 °C in allyl alcohol (230 ml) containing 2(w/v)% of dry hydrogen chloride for 20 min. The mixture was cooled and the solvent evaporated in vacuo. The residue dried by coevaporation with toluene three times was again dissolved in anhydrous acetone (400 ml). The solution was stirred with anhydrous calcium sulfate before 2,2-dimethoxypropane (37.8 ml, 296 mmol) and *p*-toluenesulfonic acid monohydrate (4.0 g, 19.7 mmol) were added to the solution. The mixture was stirred at room temperature for 3.5 h, neutralized by addition of aqueous sodium hydrogencarbonate and worked up as usual. The product was recrystallized from ethanol–ethyl acetate; yield 17.5 g (41%); mp 185—187 °C; $[\alpha]_D^{19}$ +59.8° (*c* 1.04, chloroform). Found: C, 41.57; H,4.93; N,3.20; Cl, 24.07%. Calcd for $C_{15}H_{22}NO_7Cl_3$: C, 41.45; H, 5.10; N, 3.22; Cl, 24.47%.

(*R*)-3-Tetradecanoyloxytetradecanoic Acid. Tetradecanoyl chloride (16.7 g, 67.6 mmol) was added dropwise to a solution of phenacyl (*R*)-3-hydroxytetradecanoate[1] (16.4 g, 45.3 mmol) in pyridine (100 ml) at room temperature. The mixture was stirred at room temperature for 1 h. Methanol (50 ml) was added and the mixture was stirred for additional 30 min. After evaporation of the solvent in vacuo, the residue was dissolved in benzene, and the solution was washed successively with 1 M† hydrochloric acid, saturated aqueous solution of sodium hydrogencarbonate, and water. The organic layer was treated with diazomethane and then dried. The syrupy residue obtained by evaporation of the solvent in vacuo was dissolved in acetic acid (300 ml). Zinc powder (45 g) was added and the mixture was stirred at 50 °C for 3 h. The mixture was filtered and the filtrate evaporated in vacuo. After coevaporation with toluene several times, the residue was subjected to column chromatography on silica gel (280 g, benzene) to give a colorless syrup; yield 17.6 g (86%); $[\alpha]_D^{17}$ −1.5° (*c* 4.99, chloroform). EI-MS: *m/z* 454 (M^+). Found: C, 73.99; H, 11.91%. Calcd for $C_{28}H_{54}O_4$: C, 73.99; H, 11.91%.

Allyl 2-Deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl] 2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (13). To a solution of **12** (10.0 g, 23.0 mmol) and (*R*)-3-tetradecanoyloxytetradecanoic acid (11.5 g, 25.3 mmol) in anhydrous dichloromethane (350 ml) were added 4-dimethylaminopyridine (DMAP) (1.41 g, 11.5 mmol) and dicyclohexylcarbodiimide (DCC) (5.22 g, 25.3 mmol). The mixture was stirred at room temperature for 2 h. The insoluble materials were removed by filtration and the filtrate was worked up as usual. After purification by silica-gel column chromatography (500 g, chloroform–acetone 30:1), the acylation product was heated in 90% acetic acid (200 ml) at 95 °C for 10 min. The solvent was evaporated in vacuo and the product recrystallized from hexane; yield 12.7 g (66%); mp 68—70 °C; $[\alpha]_D^{19}$ +43.7° (*c* 0.92, chloroform). Found: C, 57.90; H, 8.51; N, 1.75; Cl, 12.71%. Calcd for $C_{40}H_{70}NO_{10}Cl_3$: C, 57.79; H, 8.49; N, 1.68; Cl, 12.79%. ^{1}H NMR δ=0.87 (6H, t, *J*=6 Hz, CH_3), 1.1—1.45 (42H, br s, CH_2), 2.31 (2H, t, *J*=7 Hz, -COC$\underline{H}_2$CH_2-), 2.46—2.54 (2H, m, -COC$\underline{H}_2$CH(OCOR)-), 4.73 (2H, s, CH_2 of Troc), and 4.94 (1H, d, *J*=4 Hz, H-1).

Allyl 2-Deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (14). 2,2,2-Trichloroethoxycarbonyl chloride (0.89 ml, 6.5 mmol) was added to an ice-cooled solution of **13** (2.70 g, 3.25 mmol) in pyridine (60 ml). The mixture was stirred in an ice bath for 20 min. Then water was added and the mixture was stirred at room temperature. Usual work up and chromatographic purification on a silica-gel column (100 g, chloroform–acetone 30:1) gave a syrup; yield 2.00 g (61%). ^{1}H NMR δ=0.87 (3H, t, *J*=6 Hz, CH_3), 1.1—1.45 (42H, br s, CH_2), 2.27 (2H, t, *J*=7 Hz, -COC$\underline{H}_2$CH_2-), 2.52 (2H, d, *J*=6 Hz, -COC$\underline{H}_2$CH(OCOR)-), 4.80 (2H, AB-type, CH_2 of Troc), and 4.88 (2H, s, CH_2 of Troc).

Allyl 2-Deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside 4-(Diphenyl Phosphate) (15). To a solution of **14** (2.00 g, 1.99 mmol) in anhydrous dichloromethane (60 ml) were added pyridine (0.24 ml, 3.0 mmol), DMAP (0.36 g, 3.0 mmol) and diphenyl phosphorochloridate (0.62 ml, 3.0 mmol). The mixture was stirred at room temperature for 2 h. The mixture was washed successively with 1 M hydrochloric acid, water, saturated aqueous sodium hydrogencarbonate, and water, and dried. After removal of the solvent, the residue was purified by medium-pressure chromatography on silica gel (90 g, chloroform–acetone 90:1) to give a syrup; yield 2.2 g (89%). ^{1}H NMR δ=0.87 (6H, t, *J*=6 Hz, CH_3), 1.1—1.6 (42H, br s, CH_2), 2.16 (2H, t, *J*=7 Hz, -COC$\underline{H}_2$CH_2-), 2.44 (2H, d, *J*=7 Hz, -COC$\underline{H}_2$-CH(OCOR)-), 4.66 and 4.70 (each 2H, s, CH_2 of Troc), 4.98 (1H, d, *J*=4 Hz, H-1), and 7.1—7.5 (10H, m, aromatic H).

1-*O*-Acetyl-2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranose 4-(Diphenyl Phosphate) (16). Compound **15** (17.9 g, 14.4 mmol) was dissolved in tetrahydrofuran (THF) (150 ml) and treated

† 1 M=1 mol dm^{-3}.

with 1,5-cyclooctadienebis(methyldiphenylphosphine)-iridium hexafuluorophosphate (200 mg) under nitrogen atmosphere at 50 °C for 40 min after activation of the iridium catalyst with hydrogen.[19] After cooling, water (8 ml) and iodine (7.3 g, 28 mmol) were added and the mixture was stirred for 10 min at room temperature. Purification by medium-pressure column chromatography on silica gel (500 g, chloroform–acetone 20 : 1) afforded a syrup. It was dissolved in anhydrous chloroform (120 ml) and treated with pyridine (3.3 ml, 42 mmol) and acetic anhydride (3.9 ml, 42 mmol). After usual work-up, the product was recrystallized from hexane; yield 8.00 g (45%); mp 77—80 °C $[\alpha]_D^{29}$ +35.6° (*c* 0.55, chloroform). Found: C, 52.32; H, 6.31; N, 1.13; Cl, 17.34%. Calcd for $C_{54}H_{78}NO_{16}Cl_6$: C, 52.27; H, 6.34; N, 1.13; Cl, 17.14%. ^{1}H NMR δ=0.88 (6H, t, *J*=6 Hz, CH_3), 1.1—1.5 (42H, br s, CH_2), 2.20 (3H, s, OAc), 2.20 (2H, t, *J*=7 Hz, -$COCH_2CH_2$-), 2.46 (2H, d, *J*=7 Hz, -$COCH_2CH$(OCOR)-), 4.45 (2H, AB-type, CH_2 of Troc), 4.72 (2H, s, CH_2 of Troc), 6.33 (1H, d, *J*=3.5 Hz, H-1), and 7.0—7.5 (10H, m, aromatic H).

Benzyl 2-Benzyloxycarbonylamino-2-deoxy-4,6-*O*-isopropylidene-α-D-glucopyranoside (19). Benzyl 2-benzyloxycarbonylamino-2-deoxy-α-D-glucopyranoside (**18**)[21] (20.0 g, 49.6 mmol) was dissolved in anhydrous acetone (700 ml) and treated with 2,2-dimethoxypropane (12.1 ml, 99.2 mmol) and *p*-toluenesulfonic acid monohydrate (1.89 g, 9.94 mmol) at room temperature for 4 h. Usual work-up followed by chromatographic purification on silica gel (500 g, chloroform–acetone 5 : 1) afforded a solid; yield 16.5 g (75%).

Benzyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (17). Compound **19** (7.3 g, 16.5 mmol) was dissolved in methanol (80 ml) and hydrogenolyzed in the presence of palladium-black catalyst at room temperature under atmospheric pressure for 6 h. After usual work-up, the product was dissolved in dichloromethane (200 ml). To this solution were added (*R*)-3-benzyloxytetradecanoic acid (15.1 g, 45.1 mmol), DMAP (1.0 g, 8.3 mmol) and DCC (9.3 g, 45 mmol). The mixture was stirred at room temperature for 30 min. Usual work-up gave a syrup which was heated in 90% acetic acid at 90 °C for 20 min. After evaporation of the solvent in vacuo, the residue was recrystallized from methanol–ether–hexane; yield 7.00 g (47%); mp 88—89 °C; $[\alpha]_D^{27}$ +48.9° (*c* 0.50, chloroform). Found: C, 73.03; H, 9.13; N, 1.52%. Calcd for $C_{55}H_{83}NO_9$: C, 73.22; H, 9.27; N, 1.55%. ^{1}H NMR δ=0.89 (6H, t, *J*=6 Hz, CH_3), 1.1—1.5 (40H, br s, CH_2), 2.25—2.65 (4H, m, α-CH_2 of fatty acids), 4.88 (1H, d, *J*=4 Hz, H-1), 7.3 (15H, br s, aromatic H).

Benzyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside 4′-(Diphenyl Phosphate) (20). Bromide **11** was prepared from **16** (4.00 g, 3.22 mmol) in anhydrous dichloromethane (800 ml) as described for the preparation of **6**.

To a solution of the bromide **11** in anhydrous chloroform (60 ml) were added anhydrous calcium sulfate (4.0 g), **17** (1.45 g, 1.61 mmol) and mercury(II) cyanide (1.63 g, 6.4 mmol). The mixture was heated under reflux for 22 h. After removal of the insoluble materials by filtration, the filtrate was washed successively with aqueous solution of potassium iodide and water, dried, and the solvent evaporated in vacuo. The residue was subjected to medium-pressure column chromatography on silica gel (90 g, chloroform–acetone 15 : 1) to give a syrup; yield 2.70 g (80% from **17**). Found: C, 60.81; H, 7.66; N, 1.28; Cl, 10.39%. Calcd for $C_{107}H_{157}N_2O_{23}PCl_6 \cdot 1.5H_2O$: C, 60.91; H, 7.64; N, 1.33; Cl, 10.08%. ^{1}H NMR δ=0.88 (12H, t, *J*=6 Hz, CH_3), 1.1—1.7 (82H, broad, CH_2), 2.1—2.7 (8H, m, α-CH_2 of fatty acids), 6.11 (1H, d, *J*=9 Hz, NH-2), and 7.0—7.4 (25H, m, aromatic H).

(*R*)-3-Dodecanoyloxytetradecanoic Acid. This compound was obtained as a syrup from phenacyl (*R*)-3-hydroxytetradecanoate and dodecanoyl chloride as described for the corresponding 3-tetradecanoyloxy derivative above; yield 6.1 g (95%); $[\alpha]_D^{11}$ −1.2° (*c* 5.54, chloroform). EI-MS: *m/z* 426 (M^+). Found: C, 73.23; H, 11.77%. Calcd for $C_{26}H_{50}O_4$: C, 73.19; H, 11.81%.

Benzyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-α-D-glucopyranoside 4′-(Diphenyl Phosphate) (21). Zinc powder (5,4 g, 83 mmol) was added to a solution of **20** (2.70 g, 1.30 mmol) in acetic acid (75 ml). The mixture was stirred at room temperature for 30 min. After usual work-up, the crude product was dissolved in chloroform, and washed with 1 M hydrochloric acid and water, and dried. The solid obtained by evaporation of the solvent in vacuo was dissolved in dioxane. The solution was adjusted to pH 3 by addition of 1 M hydrochloric acid and then lyophilized to give colorless solid of hydrochloride of the 2′-amino derivative (2.18 g, 95%).

To a solution of a part of the solid (1.98 g, 1.12 mmol) in anhydrous dichloromethane (30 ml) was added triethylamine (0.16 ml, 1.15 mmol) and the solvent was evaporated. The residue was dissolved in anhydrous dichloromethane (30 ml) and dodecanoyloxytetradecanoic acid (716 mg, 1.68 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (322 mg, 1.68 mmol) were added under ice-cooling. The mixture was stirred at room temperature for 22 h and worked up as usual. The crude product was dissolved in chloroform and treated with diazomethane and then subjected to medium-pressure chromatography on silica gel (90 g, chloroform–acetone 9 : 1) to give a syrup; yield 1.29 g (49%); $[\alpha]_D^{18}$ +19.5° (*c* 0.81, chloroform). Found: C, 70.96; H, 9.59; N, 1.36%. Calcd for $C_{127}H_{203}N_2O_{22}P$: C, 71.25; H, 9.56; N, 1.31%. ^{1}H NMR δ=0.88 (18H, t, *J*=6 Hz, CH_3), 1.1—1.8 (120H, broad, CH_2), 2.1—2.6 (12H, m, α-CH_2 of fatty acids), 6.12 (1H, d, *J*=9 Hz, NH), 6.25 (1H, d, *J*=8 Hz, NH), 7.0—7.4 (25H, m, aromatic H).

2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-D-glucose 4′-Phosphate (3). Compound **21** (860 mg, 0.402 mmol) was dissolved in a mixture of THF (22 ml) and acetic acid (1.4 ml) and hydrogenolyzed first in the presence of palladium black at room temperature under atmosphere of hydrogen (6.5 kg cm^{-2}) for 4 d. After removal of palladium and addition of platinum oxide, hydrogenolysis was continued under the same conditions for 6 h. Evaporation of the solvent followed by lyophilization from dioxane afforded colorless solid; yield 651 mg (94%).

The crude product obtained above (42.5 mg) was dissolved in chloroform–methanol (9 : 1, 6 ml). Cold 1 M hydrochloric acid (660 μl) was added and the homogeneous solution was

sonicated under ice-cooling for 5 min. The solvent was evaporated with stream of nitrogen, the residue dissolved in chloroform-methanol (9:1, 6 ml) and evaporated. The residue was again dissolved in the same solvent mixture and washed twice with water. Lyophilization from dioxane afforded a pure product; yield 39.2 mg; $[\alpha]_D^{18}$ −2.1° (*c* 0.58, chloroform). Found: C, 64.45; H, 10.31; N, 1.64%. Calcd for $C_{94}H_{177}N_2O_{22}P \cdot 2H_2O$: C, 64.35; H, 10.40; N, 1.60%.

Dimethyl Ester of 3 and Its Pentaacetate. Crude **3** (229 mg) was treated with 1 M hydrochloric acid as above and dissolved in chloroform-methanol (9:1, 9 ml). To the solution was added an ethereal solution of diazomethane. The product was purified by medium-pressure chromatography on silica gel (25 g, chloroform-methanol 15:1) and lyophilized from dioxane to give colorless solid; yield 68.2 mg (29%); $[\alpha]_D^{18}$ −2.5° (*c* 0.40, chloroform-methanol 9:1). Found: C, 65.15; H, 10.31; N, 1.55%. Calcd for $C_{96}H_{181}N_2O_{22}P \cdot 1.5H_2O$: C, 65.02; H, 10.46; N, 1.58%.

The dimethyl ester (30.0 mg, 0.017 mmol) was acetylated with acetic anhydride and pyridine in dichloromethane and purified by preparative TLC on silica gel (chloroform-acetone 5:1). The product was lyophilized from dioxane to give a colorless powder; yield 19.1 m (57%); $[\alpha]_D^{30}$ +17.9° (*c* 0.39, chloroform). Found: C, 65.17; H, 9.86; N, 1.46%. Calcd for $C_{106}H_{191}N_2O_{27}P$: C, 65.07; H, 9.84; N, 1.43%. The corresponding natural specimen: $[\alpha]_D^{30}$ +19.0.[3]

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-deoxy-4,6-*O*-isopropylidene-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (22). To a solution of **12** (13.0 g, 29.9 mmol) in anhydrous chloroform (250 ml) were added (*R*)-3-benzyloxytetradecanoic acid[1] (10.0 g. 29.9 mmol), DCC (6.17 g, 29.9 mmol), and DMAP (731 mg, 5.98 mmol). The mixture was stirred at room temperature for 1 h and worked up as usual. Purification by silica-gel column chromatography (500 g, chloroform-acetone 30:1) afforded a colorless syrup; yield 14.5 g (64%). ^{1}H NMR δ=1.42 and 1.50 (each 3H, s, CH_3), 2.67 (1H, d, *J*=2 Hz, -OH), 4.70 (2H, AB-type, CH_2 of Troc), 4.86 (1H, d, *J*=3 Hz, H-1), 5.06—5.40 (3H, m, $-OCH_2-CH=C\underline{H}_2$ and NH), and 5.67—6.07 (1H, m, $-OCH_2-C\underline{H}=CH_2$).

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (23). Compound **22** (14.5 g, 19.3 mmol) was treated with zinc powder (29 g) in acetic acid (290 ml) as described for the preparation of **21**. After washing with hydrochloric acid and water, triethylamine (2.69 ml, 19.3 mmol) was added to a solution of the product in chloroform. The solvent was once evaporated in vacuo and the residue was again dissolved in anhydrous chloroform (250 ml). (*R*)-3-Benzyloxytetradecanoic acid (7.75 g, 23.2 mmol) and DCC (4.78 g, 23.2 mmol) were added to the solution. The mixture was stirred at room temperature for 30 min. After usual work-up, the crude product was heated in 90% acetic acid (250 ml) in a boiling water bath for 30 min. The product was purified by a silica-gel column (500 g, chloroform-acetone 5:1) and recrystallized from hexane; yield 9.53 g (58%); mp 80—82 °C; $[\alpha]_D^{19}$ +37.2° (*c* 1.02, chloroform). Found: C, 71.85; H, 9.67; N, 1.62%. Calcd for $C_{51}H_{81}NO_9$: C, 71.88; H, 9.58; N, 1.64%. ^{1}H NMR δ=0.88 (6H, t, *J*=6 Hz, CH_3), 1.1—1.5 (40H, br s, CH_2), 2.25—2.63 (4H, m, α-CH_2 of fatty acids), 4.47 (4H, s, $-C\underline{H}_2C_6H_5$), 4.75 (1H, d, *J*=3 Hz, H-1), 4.98—5.26 (2H, m, $-OCH_2-CH=C\underline{H}_2$), 5.50—5.92 (1H, m, $-OCH_2-C\underline{H}=CH_2$), 6.23 (1H, d, *J*=9 Hz, NH), and 7.22 (10H, s, aromatic H).

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-6-*O*-(2,2,2-trichloroethoxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside 4′-(Diphenyl Phosphate) (24). The glycosyl bromide (**11**) prepared from the acetate **16** (3.94 g, 3.18 mmol) as described above was dissolved in anhydrous chloroform (120 ml). Compound **23** (1.35 g, 1.58 mmol), mercury(II) cyanide (1.62 g, 6.33 mmol), and anhydrous calcium sulfate (4.0 g) were added to the solution and the mixture was heated under reflux. After 20 h, **23** (1.35 g, 1.58 mmol) was added and the mixture was heated for additional 13 h. Usual work-up followed by chromatography on a silica-gel column (110 g, chloroform-acetone 15:1) afforded a solid; yield 4.54 g (70%); $[\alpha]_D^{19}$ +18.4° (*c* 1.04, chloroform). Found: C, 60.74; H, 7.69; N, 1.29; Cl, 10.70%. Calcd for $C_{103}H_{155}N_2O_{23}Cl_6P$: C, 60.85; H, 7,68; N, 1.38; Cl, 10.46%. ^{1}H NMR δ=0.87 (12H, t, *J*=6 Hz, CH_3), 1.1—1.7 (82H, br s, CH_2), 2.12—2.84 (8H, m, α-CH_2 of fatty acids), 4.47 (4H, s, $-C\underline{H}_2C_6H_5$), 6.14 (1H, d, *J*=9 Hz, NH), and 7.0—7.4 (20H, m, aromatic H).

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-α-D-glucopyranoside 4′-(Diphenyl Phosphate) (25). Compound **24** (4.48 g, 2.20 mmol) was treated with zinc powder (9.0 g) in acetic acid (140 ml) and then with triethylamine (0.31 ml, 2.2 mmol) as described above for the preparation of **23**. To a solution of the product in anhydrous chloroform (80 ml) were added (*R*)-3-dodecanoyloxytetradecanoic acid (1.13g, 2.65 mmol) and DCC (546 mg, 2.65 mmol). The mixture was stirred at room temperature for 17 h. Usual work-up and chromatography on a silica-gel column (90 g, chloroform-acetone 9:1) afforded a colorless syrup; yield 3.25 g (71%). A part of the syrup was lyophilized from dioxane to give a colorless powder which was used for elemental analysis. Found: C, 69.97; H, 9.62; N, 1.43%. Calcd for $C_{123}H_{201}N_2O_{22}P \cdot H_2O$: C, 70.05; H, 9.70; N, 1.47%. ^{1}H NMR δ=0.87 (18H, t, *J*=6 Hz, CH_3), 1.0—1.7 (120H, br s, CH_2), 2.08—2.66 (12H, m, α-CH_2 of fatty acids) 4.47 (4H, s, $-C\underline{H}_2C_6H_5$), 6.18 (1H, d, *J*=9 Hz, NH), 6.36 (1H, d, *J*=8 Hz, NH), and 7.0—7.4 (20H, m, aromatic H).

Allyl 6-*O*-[6-*O*-Benzyloxymethyl-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside 4′-(Diphenyl Phosphate) (26). To a solution of **25** (3.14 g, 1.50 mmol) in anhydrous dichloromethane (100 ml) were added benzyloxymethyl chloride (352 mg, 2.25 mmol) and *N*,*N*-diisopropylethylamine (0.39 ml, 2.2 mmol). The mixture was stirred at room temperature for 48 h with portionwise addition of total 2.5 equivalent of each reagent. After usual work-up, the product was purified by silica-gel column chromatography (180 g, chloroform-acetone 15:1) to give a syrup; yield 2.17 g (65%). A part of the syrup was lyophilized from dioxane to give a colorless powder which was used for elemental analysis. Found: C, 70.80; H, 9.56; N, 1.30%. Calcd for $C_{131}H_{209}N_2O_{23}P \cdot 0.5H_2O$: C, 70.87; H, 9.53; N, 1.26%. $[\alpha]_D^{19}$ +18.3° (*c* 1.01, chloroform). ^{1}H NMR δ=0.88 (18H, t, *J*=6 Hz, CH_3), 1.1—1.7 (120H, br s, CH_2), 2.12—2.64 (12H, α-CH_2 of fatty acids), 6.11 (1H, d, *J*=9 Hz, NH), 6.24 (1H, d, *J*=8 Hz, NH), and 7.0—7.4 (25H, m,

aromatic H).

6-*O*-[6-*O*-Benzyloxymethyl-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-D-glucose 4′-(Diphenyl Phosphate) (27). Compound **26** (2.11 g, 0.954 mmol) was treated with the iridium complex[19] (105 mg) in THF and then with water and iodine (500 mg) as described above for the preparation of **16**. Purification by medium-pressure column chromatography on silica gel (80 g, chloroform–acetone 10 : 1) followed by lyophilization from dioxane afforded a pale yellow powder; 1.31 g (63%). Found: C, 70.40; H, 9.44; N, 1.33%. Calcd for $C_{128}H_{205}N_2O_{23}P \cdot 0.5H_2O$: C, 70.52; H, 9,52; N, 1.29%. ^{1}H NMR δ=0.87 (18H, t, *J*=6 Hz, CH_3), 1.1—1.8 (120H, br s, CH_2), 2.10—2.62 (12H, m, α-CH_2 of fatty acids), 7.0—7.4 (25H, m, aromatic H).

2-Deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucopyranose 1,4′-Bis(phosphate) (1). A solution of **27** (600 mg, 0.276 mmol) in anhydrous THF (12 ml) was cooled to −70 °C under argon atmosphere. To this solution were added 10(w/v)% hexane solution of butyllithium (210 μl, 0.33 mmol) and dibenzyl phosphorochloridate (85 μl) as described in our preceding paper.[1] After 20 min, palladium black was added to the cold mixture and the mixture was subjected to hydrogenolysis under 8 kg cm^{-2} of hydrogen at room temperature for 2 h. The catalyst was substituted with platinum oxide and then hydrogenolysis was continued under the same conditions. After purification by medium-pressure chromatography on silica gel (80 g, chloroform–methanol–water–triethylamine 200 : 100 : 20 : 1), the product was subjected to electrodialysis and acid precipitation as described previously[1] to afford colorless powder; yield 137 mg (27%). Found: C, 61.04; H, 10.05; N, 1.46%. Calcd for $C_{94}H_{178}N_2O_{25}P_2 \cdot 3H_2O$: C, 60.95; H, 10.01; N, 1.51%. $[\alpha]_D^{15}$ +11.3° (*c* 0.56, chloroform–methanol 9 : 1).

Allyl 2-Deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4,6-bis[*O*-(2,2,2-trichloroethoxycarbonyl)]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (28). A solution of 2,2,2-trichloroethoxycarbonyl chloride (3.7 ml, 27 mmol) in THF (20 ml) was added to a solution of **13** (9.00 g, 10.8 mmol) in anhydrous pyridine (150 ml) cooled in an ice bath. The mixture was then stirred at room temperature for 50 min and worked up as usual. Chromatographic purification on a silica-gel column (500g, chloroform) afforded a colorless syrup; yield 12.8 g (quantitative). ^{1}H NMR δ=0.87 (6H, t, *J*=6 Hz, CH_3), 1.1—1.6 (42H, broad, CH_2), 2.24 (2H, t, *J*=7 Hz, -COC$\underline{H_2}$CH_2-), 2.50 (2H, d, *J*=6 Hz, -COC$\underline{H_2}$CH(OCOR)-), 4.70 (2H, br s, CH_2 of Troc), and 4.72 and 4.73 (each 2H, s, CH_2 of Troc).

1-*O*-Acetyl-2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4,6-bis[*O*-(2,2,2-trichloroethoxycarbonyl)]-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranose (29). Compound **28** obtained above (12.8 g) was treated with the iridium complex[19] (200 mg) in THF and then with iodine (5.5 g) and water as described for the preparation of **16**. Purification by column chromatography on silica gel (500 g, chloroform–acetone 50 : 1) afforded a pale yellow syrup (13.1 g), which was dissolved in anhydrous dichloromethane (200 ml) and treated with pyridine (3.7 ml) and acetic anhydride (2.2 ml, 23 mmol). Usual work-up and chromatographic purification on a silica-gel column (500 g, chloroform–acetone 100 : 1) gave colorless syrup; 12.6 g (93%). Found: C, 45.95; H, 6.06; N, 1.20; Cl, 27.16%. Calcd for $C_{45}H_{70}NO_{15}Cl_9$: C, 45.65; H, 5.96; N, 1.18; Cl, 26.95%. ^{1}H NMR δ=0.87 (6H, t, *J*=6 Hz, CH_3), 1.1—1.7 (42H, broad, CH_2), 2.15 (3H, s, OAc), 2.26 (2H, t, *J*=7 Hz, -COC$\underline{H_2}$CH_2-), 2.50 (2H, d, *J*=6 Hz, -COC$\underline{H_2}$CH(OCOR)-), 4.66 (2H, AB-type, CH_2 of Troc), 4.71 and 4.74 (each 2H, s, CH_2 of Troc), and 6.25 (1H, d, *J*=3 Hz, H-1).

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-4,6-bis[*O*-(2,2,2-trichloroethoxycarbonyl)]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-α-D-glucopyranoside (31). Syrupy **29** (12.6 g, 10.6 mmol) was converted to glycosyl bromide **30** and treated with **23** (5.50 g, 6.45 mmol) in the presence of mercury(II) cyanide (5.38 g) and anhydrous calcium sulfate in anhydrous chloroform (200 ml) under the same conditions as for the synthesis of **20**. Chromatography on a silica-gel column (500 g, chloroform–acetone 15 : 1) afforded a colorless syrup; yield 6.97 g (54% from **23**). A portion of the syrup was lyophilized to give colorless powder; $[\alpha]_D^{33}$ +19.3° (*c* 0.83, chloroform). Found: C, 56.82; H, 7.48; N, 1.44; Cl, 16.30%. Calcd for $C_{94}H_{147}N_2O_{22}Cl_9$: C, 57.13; H, 7.50; N, 1.42; Cl, 16.15%. ^{1}H NMR δ=0.88 (12H, t, *J*=6 Hz, CH_3), 1.1—1.7 (82H, broad, CH_2), 2.18—2.6 (8H, m, α-CH_2 of fatty acids), 4.47 (4H, s, $C_6H_5C\underline{H_2}$), 6.16 (1H, d, *J*=9 Hz, NH-2), and 7.22 (10H, br s, aromatic H).

Allyl 3-*O*-[(*R*)-3-Benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-6-*O*-[2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-α-D-glucopyranoside (32). This compound was prepared from **31** (6.97 g, 3.53 mmol) as described for the preparation of **21**, i.e., removal of Troc group with zinc (14 g) followed by acylation with (*R*)-3-dodecanoyloxytetradecanoic acid (2.26 g, 5.30 mmol) and DCC (1.09 g, 5.28 mmol) in dichloromethane. Purification by medium-pressure chromatography on silica gel (190 g, chloroform–acetone 6 : 1) followed by precipitation from methanol gave colorless solid; yield 3.00 g (46%); mp 99—102 °C (with sintering at 85 °C; $[\alpha]_D^{27}$ +15.1° (*c* 1.04, chloroform). Found: C, 71.27; H, 10.35; N, 1.51%. Calcd for $C_{111}H_{192}N_2O_{19} \cdot 0.5H_2O$: C, 71.38; H, 10.36; N, 1.50%. ^{1}H NMR δ=0.88 (18H, t, *J*=6 Hz, CH_3), 1.1—1.8 (122H, broad, CH_2), 2.2—2.65 (12H, m, α-CH_2 of fatty acids), 4.48 (4H, s, $C_6H_5C\underline{H_2}$), 5.96 (1H, d, *J*=9 Hz, NH), 6.14 (1H, d, *J*=8 Hz, NH), and 7.22 (10H, br s, aromatic H).

Allyl 6-*O*-[6-*O*-Benzyloxymethyl-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-α-D-glucopyranoside (33). To a solution of **32** (3.00 g, 1.61 mmol) in anhydrous dichloromethane (100 ml) were added *N,N*-diisopropylethylamine (1,26 ml, 7.2 mmol) and benzyloxymethyl chloride (1.13 ml, 7.2 mmol). After 30 h at room temperature, the mixture was worked up as usual. The product was purified by medium-pressure chromatography on silica gel (90 g, chloroform–acetone 10 : 1) and recrystallized from methanol; yield 2.37 g (74%); mp 92—95 °C; $[\alpha]_D^{27}$ +14.5° (*c* 1.07, chloroform). Found: C, 71.51; H, 10.04; N, 1.42%. Calcd for $C_{119}H_{200}N_2O_{20}$: C, 71.58; H, 10.09; N, 1.40%. ^{1}H NMR δ=0.88 (18H, t, *J*=6 Hz, CH_3), 1.1—1.8 (122H, broad, CH_2), 2.2—2.5 (12H, m, α-CH_2 of fatty acids), 4.48

(4H, s, $C_6H_5C\underline{H}_2$), 4.58 (2H, s, $C_6H_5C\underline{H}_2OCH_2O$-), 4.76 (2H, s, $C_6H_5CH_2OC\underline{H}_2O$-), 7.1—7.4 (15H, aromatic H).

6-*O*-[6-*O*-Benzyloxymethyl-2-deoxy-2-[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-benzyloxytetradecanoyl]-2-[(*R*)-3-benzyloxytetradecanoylamino]-2-deoxy-D-glucose (34). This compound was prepared from **33** (3.30 g, 1.67 mmol) as described for **16** and purified by silica-gel column chromatography (90 g, chloroform–acetone 6 : 1) followed by recrystallization from methanol; yield 1.77 g (55%); mp 115—117 °C (with sintering at 81 °C); $[\alpha]_D^{19}$ +2.6° (*c* 0.66, chloroform). Found: C, 71.48; H, 10.19; N, 1.42%. Calcd for $C_{116}H_{196}N_2O_{20}\cdot 0.5H_2O$: C, 71.53; H, 10.14; N, 1.44%. ^{1}H NMR δ=0.88 (18H, t, *J*=6 Hz, CH_3), 1.1—1.8 (122H, broad, CH_2), 2.1—2.6 (12H, m, α-CH_2 of fatty acids), 4.45 and 4.47 (each 2H, s, $C_6H_5C\underline{H}_2$), 4.57 (2H, s, $C_6H_5C\underline{H}_2OCH_2O$-), 4.76 (2H, s, $C_6H_5CH_2OC\underline{H}_2O$-), 7.1—7.4 (15H, aromatic H).

2-Deoxy-6-*O*-[2-deoxy-2[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-α-D-glucose 1-Phosphate (4). A solution of **34** (200 mg, 0.103 mmol) in anhydrous THF (10 ml) was treated under the same conditions as described for **1** with a solution of butyllithium in hexane (66 μl, 0.10 mmol) and dibenzyl phosphorochloridate (26 μl, 0.10 mmol). Hydrogenolysis with palladium black at room temperature at 6 kg cm^{-2} followed by medium-pressure chromatography on silica gel (15 g, chloroform–methanol–water–triethylamine 500 : 100 : 10 : 1) gave a crude product as colorless solid; 63.7 mg. A portion (58 mg) of the crude product was dissolved in chloroform–methanol (9 : 1, 5.8 ml) and treated with cold 1 M hydrochloric acid as described for the purification of **3** and lyophilized from dioxane; yield 43 mg. $[\alpha]_D^{18}$ +6.0° (*c* 0.42, chloroform–methanol 9 : 1). Found: C, 63.58; H, 10.27; N, 1.50%. Calcd for $C_{94}H_{177}N_2O_{22}P\cdot 3H_2O$: C, 63.70; H, 10.41; N, 1.58%.

2-Deoxy-6-*O*-[2-deoxy-2[(*R*)-3-dodecanoyloxytetradecanoylamino]-3-*O*-[(*R*)-3-tetradecanoyloxytetradecanoyl]-β-D-glucopyranosyl]-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-D-glucose (5). Hydrogenolysis of **34** (217 mg) in THF (5 ml) and acetic acid (0.2 ml) under conditions described for **4** followed by recrystallization from methanol afforded the product; yield 155 mg (85%); mp 169—173 °C (decomp), $[\alpha]_D^{19}$ −5.5° (*c* 0.56, chloroform). Found: C, 68.29; H, 10.76; N, 1.70%. Calcd for $C_{94}H_{176}N_2O_{19}\cdot H_2O$: C, 68.16; H, 10.71; N, 1.69%.

A part of this work was supported by Grant-in-Aid for Scientific Research from Ministry of Education, Science and Culture (Nos. 58122002, 59116004, and 60108004). The work was also partly supported by a Joint Research Project from Japan Society for Promotion of Science (JSPS). One of the authors (M. I.) thanks for the financial support by Fellowship for Japanese Junior Scientist from JSPS. The authors are grateful to Mr. Hideo Naoki, Suntory Institute for Bioorganic Research, for his kind measurement of 360 MHz ^{1}H NMR spectra.

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