

Headline Articles

Synthesis and Biological Activities of Lipid A Analogs Possessing β-Glycosidic Linkage at 1-Position

Koichi Fukase,* Atsushi Ueno, Yoshiyuki Fukase, Masato Oikawa, Yasuo Suda, and Shoichi Kusumoto*

Department of Chemistry, Graduate School of Science, Osaka University,
1-1 Machikaneyama, Toyonaka, Osaka 560-0043

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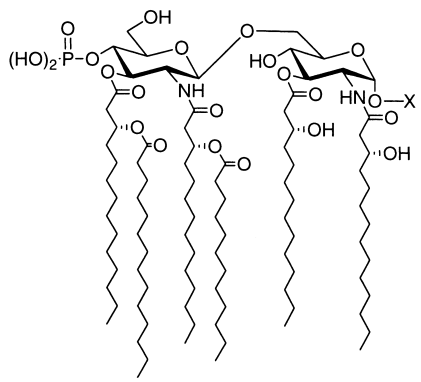
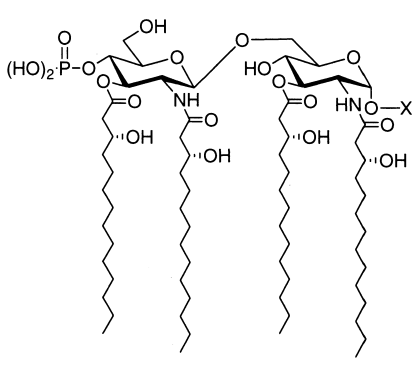
New lipid A analogs having acidic groups β-glycosidically linked at the 1-position were synthesized in order to investigate the structural requirement for immunostimulating and endotoxic activity of lipid A. The β-(phosphonoxy)ethyl (PE) and carboxymethyl (CM) analogs of *Escherichia coli* type having six acyl groups and those of the biosynthetic precursor type having four acyl groups were synthesized via a divergent synthetic route. The *E. coli* type β-(phosphonoxy)-ethyl analog, which was previously reported to be not endotoxic, showed strong immunostimulating activity comparable to the natural-type α-analog. The acidic functional groups are concluded to be essential but their strict spatial arrangement is not required for expression of the biological activity.

An innate immune system is a phylogenetically ancient defense mechanism against the invasion of microorganisms including bacteria.[1,2] This system recognizes common bacterial components such as bacterial cell wall peptidoglycan (PGN), lipopolysaccharide (LPS) of gram-negative bacteria, lipoproteins, and bacterial DNA to induce many kinds of mediators such as cytokines, prostagrandins, platelet activating factor, and NO. These mediators stimulate the immune system and also cause clinical manifestations of bacterial infections such as fever, inflammation, hypotension, and, in severe cases, lethal shock. LPS, also termed endotoxin, is a cell surface amphiphile characteristic of Gram-negative bacteria and has been known as the most potent immunostimulant originating from microorganisms.[3–8] LPS consists of a glycolipid component termed lipid A covalently bound to a polysaccharide chain. Previously, we clearly demonstrated that lipid A is the chemical entity of endotoxic activity of LPS by the total synthesis of *Escherichia coli* lipid A **1** (Fig. 1, the synthetic specimen is termed 506).[9,10] Lipid A of various bacterial origins were shown to be structurally closely related and to consist of: 1) β(1→6) disaccharide of D-glucosamines, 2) phosphono groups at the reducing end and the 4-position of the non-reducing sugar, and 3) long-chain acyl groups bound at 2,2′,3, and 3′ positions.

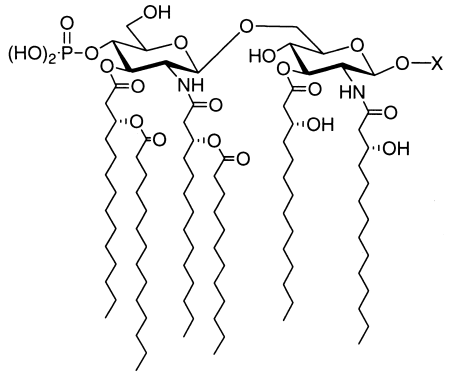


1 (**X** = **-P(O)(OH)$_2$**): *Escherichia coli* Lipid A (506)
3 (**X** = **-CH$_2$CH$_2$OP(O)(OH)$_2$**): PE analog (PE-506)
5 (**X** = **-CH$_2$COOH**): CM analog (CM-506)

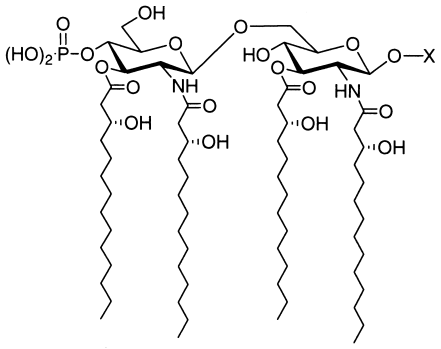


2 (**X** = **-P(O)(OH)$_2$**): Biosynthetic precursor (406)
4 (**X** = **-CH$_2$CH$_2$OP(O)(OH)$_2$**): PE analog (PE-406)
6 (**X** = **-CH$_2$COOH**): CM analog (CM-406)

Fig. 1. Structures of lipid A and analogs.



7 (**X** = **-$CH_2CH_2OP(O)(OH)_2$**): β-PE analog (β-PE-506)
9 (**X** = **-CH_2COOH**): β-CM analog (β-CM-506)



8 (**X** = **-$CH_2CH_2OP(O)(OH)_2$**): β-PE analog (β-PE-406)
10 (**X** = **-CH_2COOH**): β-CM analog (β-CM-406)

Fig. 2. Structures of lipid A analogs possessing β-glycosidic linkage at 1-position.

It has been shown that both phosphono groups at the 1- and 4′-positions and acyl groups are crucial for the biological activities of lipid A. The tetraacyl lipid A **2** (the synthetic specimen is called 406) which lacks the dodecanoyl and tetradecanoyl moieties of **1** has been identified as a biosynthetic precursor of LPS. The biosynthetic precursor **2** shows weaker but definite endotoxic activity against mice. Quite interestingly, however, **2** acts as an antagonist to LPS or lipid A **1** in human systems.[3,4,10–13] Novel precursor-type lipid A analogs which possess four (*R*)-3-hydroxyacyl moieties of shorter chain length (C10) were synthesized. Their biological tests clearly showed the critical importance of the chain length of the acyl moieties in lipid A to the antagonistic activity.[14,15]

As for the role of phosphate groups, lipid A analogs lacking 1- or 4′-phosphate showed considerably weaker activity than lipid A **1** whereas a lipid A analog lacking both phosphates did not show any activity.[11–13,16–19] Since 1-*O*-glycosyl phosphate in lipid A is chemically unstable under acidic conditions, chemically stable and biologically active analogs of lipid A had been required for the investigation of the action mechanism as well as for clinical use. In 1990, the 2-(phosphonooxy)ethyl (PE) analogs **3** and **4** were reported by Kusama et al. as chemically stable lipid A analogs. Both the *E. coli*-type (**3**: PE-506) and the biosynthetic precursor-type (**4**: PE-406) analogs showed indistinguishable activity from the activities of the corresponding natural-type compounds.[20] Kusama et al. also reported another analog in which the phosphoryl group at the 1-position is replaced with a carboxymethyl (CM) group,[21] but the CM-analog **5** with the same acylation pattern as lipid A **1** was not synthesized. We hence synthesized both the *E. coli*-type and the precursor-type analogs **5** (CM-506) and **6** (CM-406), which also showed indistinguishable activity from those of the corresponding natural-type compounds.[15,22] Kusama also prepared PE-analogs with β-configuration at the 1-position, whereas all the above analogs have the same α-configuration at 1-position as the natural lipid A.[20a] Quite interestingly, they reported that β-PE analogs showed considerably weaker activity than natural lipid A.[13,20a] We anticipated that the molecular conformation of β-PE analogs might be different from those of natural lipid A and the α-PE analogs and the difference might be reflected in the difference in the biological activity. We therefore planned to synthesize four lipid A analogs possessing β-glycosidically linked acidic groups at the 1-position, i.e., PE-analogs (β-PE-506: **7** and β-PE-406: **8**) and CM-analogs (β-CM-506: **9** and β-CM-406: **10**) having *E. coli*-type and biosynthetic precursor-type acylation pattern, respectively (Fig. 2).

Results

Synthetic Plan. In our previous studies, we elaborated divergent routes for the efficient synthesis of lipid A analogs.[14,15,22] Precursor-type analogs having shorter acyl chains were synthesized via a disaccharide 4′-phosphate as a common synthetic intermediate and each acyl moiety was introduced step by step to the respective position.[14,15] We also described another route by which *E. coli* lipid A, PE-506, and CM-506 were prepared from the same allyl glycoside via deprotective removal or oxidative cleavage of the double bond.[22,23] In the present study, we employed a new divergent synthetic route as shown in Fig. 3. The β-allyl glycoside of a disaccharide 4′-phosphate **13** was constructed as a common synthetic intermediate and then transformed to *E. coli*-type and precursor-type intermediates **14**, **15**. The β-PE- and β-CM-analogs for both types were prepared from the corresponding intermediates.

Synthesis of the Glycosyl Donor 11. The common glycosyl donor **11** was synthesized according to the method we recently reported (Scheme 1).[14,24] The allyl group of compound **16** was isomerized to the 1-propenyl group using an iridium complex ($[Ir(cod)(MePh_2P)_2]PF_6$, H_2)[25] and then an allyloxycarbonyl (Alloc) group was introduced to the 3-position. The product **17** was subjected to the reductive benzylidene-ring opening by using $Me_2NH{\cdot}BH_3$ and $Et_2O{\cdot}BF_3$ in CH_3CN to give the 6-*O*-benzylated **18** in good yield with high regioselectivity.[14,22,26] The free 4-hydroxy group was treated with Watanabe's reagent and 1-*H*-tetrazole, and then with *m*-chloroperbenzoic acid (mCPBA) to furnish the phosphate **19** in 99% yield.[27] The 1-propenyl group was smoothly deprotected with iodine and water to give **20** in 94% yield. Compound **20** was then converted in the presence of Cs_2CO_3 to the trichloroacetimidate **11** in good yield.[28]

Synthesis of the Glycosyl Acceptor 12. The acceptor **12** was prepared from *N*-acetyl glucosamine as shown in Scheme 2. The fully acetylated glucosamine **21** was treated with trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give the

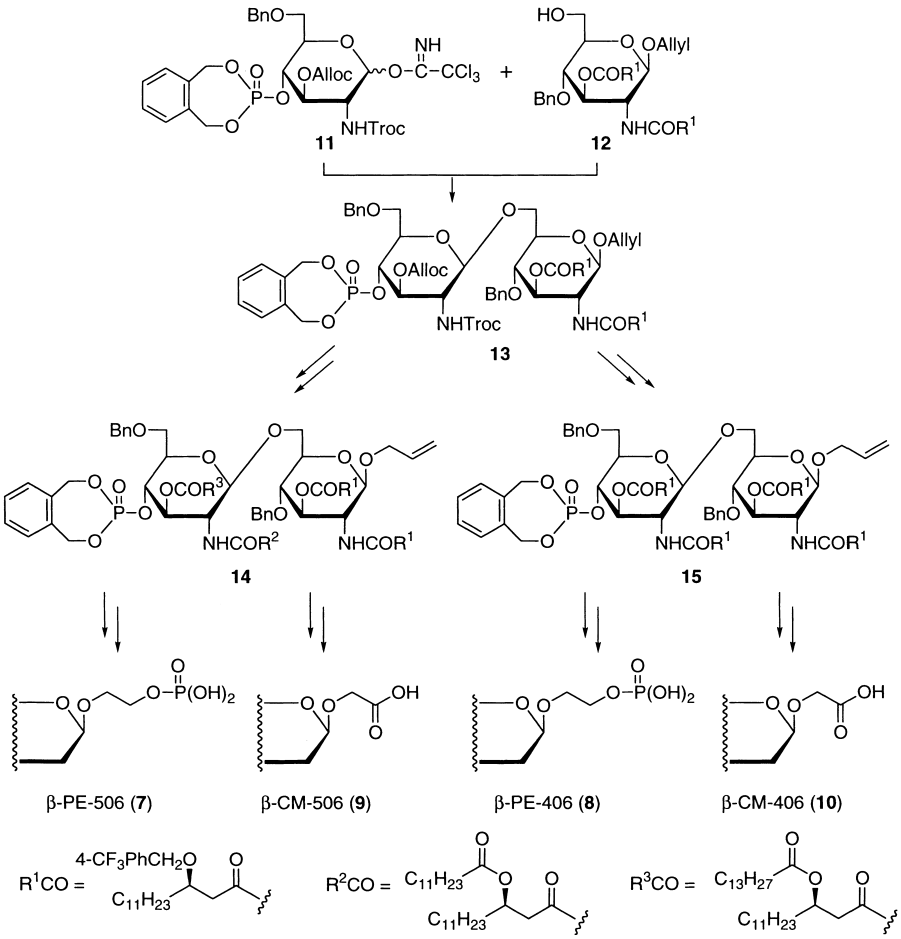
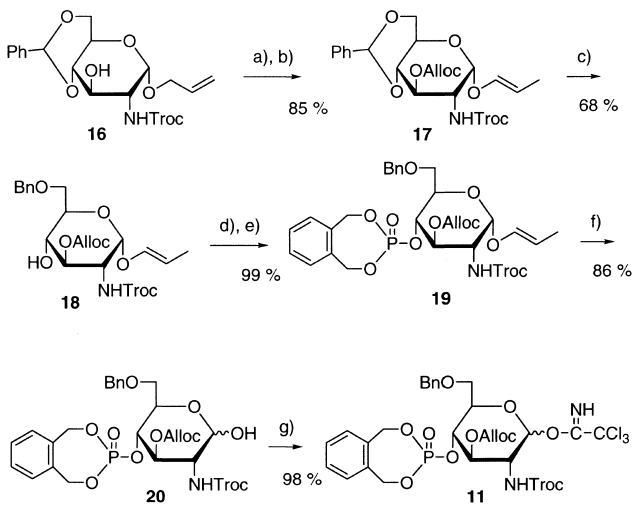
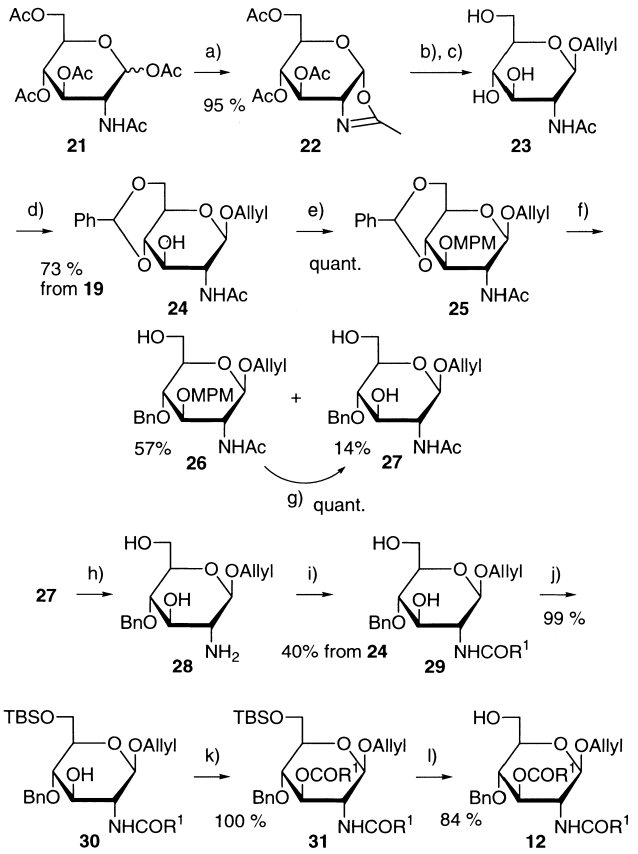


Fig. 3. Synthetic strategy for β-analogs via divergent route.



Scheme 1. a) [Ir(cod)($MePh_2P$)$_2$]PF_6, H_2, THF; b) AllocCl, pyridine, CH_2Cl_2; c) $Me_2NH{\cdot}BH_3$, $Et_2O{\cdot}BF_3$, CH_3CN; d) *N*,*N*-Diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphep-in-3-amine, 1*H*-tetrazole, CH_2Cl_2; e) *m*CPBA, −20 °C; f) I_2, water, THF; g) CCl_3CN, Cs_2CO_3, CH_2Cl_2.

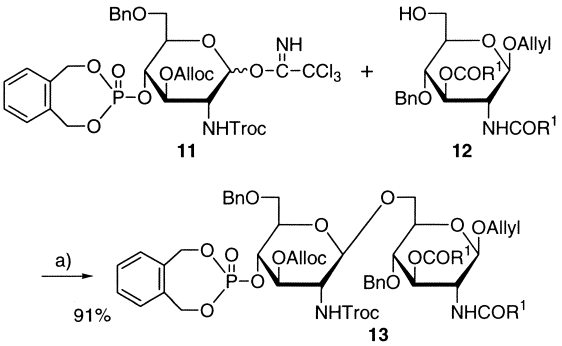
oxazoline **22**.[29] The *O*-acetyl groups of **22** was then removed by treatment with NaOAllyl in allyl alcohol. The solution was acidified with *p*-toluenesulfonic acid. Since the oxazoline derivative with free hydroxy groups is reactive, glycosylation proceeded smoothly at room temperature to give the allyl glycoside **23**, which was converted to the 4,6-*O*-benzylidene **24** in 73% yield from **22**. The 3-hydoxy group of **24** was then 4-methoxybenzylated by the trichloroacetimidate method. The product **25** was subjected to the reductive benzylidene-ring opening by using $Me_2NH{\cdot}BH_3$ and $Et_2O{\cdot}BF_3$ in CH_2Cl_2.[14,26] In this case, the 4-*O*-benzylated **26** was obtained with high regioselectivity. The 3-*O*-(4-methoxybenzyl) (MPM) group was partially cleaved during the reduction by the action of $Et_2O{\cdot}BF_3$. The remaining 3-*O*-MPM group in **26** was also cleaved with $Et_2O{\cdot}BF_3$ to give **27** quantitatively. The *N*-acetyl group of **27** was then removed by treatment with $Ba(OH)_2$ to give **28**, which was acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid by using 1-hydroxybenzotriazole (HOBt), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (WSCD·HCl) in $CHCl_3$.[30] Compound **29** was thus obtained in 40% yield from **27**. The *N*-acetyl group of **25** or **26** having 3-*O*-MPM group was found to be not cleavable at all by treatment with various bases. The 6-hydroxy group of **29** was temporarily protected with *t*-butyldimethylsilyl (TBS) group and the 3-hydroxy group of the resulting **30** was acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid and DCC in the presence of 4-(dimethylamino)pyridine (DMAP). Removal of the TBS group of the product **31** by using HF–pyridine gave the glycosyl acceptor **12**.



Scheme 2. a) TMSOTf, $(CH_2Cl)_2$, 50 °C; b) 0.1 M Allyl-ONa, Allyl-OH; c) *p*-TsOH·H_2O, Allyl-OH; d) PhCH-$(OCH_3)_2$, *p*-TsOH·H_2O, CH_3CN; e) 4-Methoxyphenylmethyl trichloroacetimidate, $Sn(OTf)_2$, THF; f) Me_2NH·BH_3, Et_2O·BF_3, CH_2Cl_2; g) Et_2O·BF_3, CH_2Cl_2; h) Ba-$(OH)_2$·$(8H_2O)$, MeOH, 70 °C; i) (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid, HOBt, WSCD·HCl, $CHCl_3$; j) *t*-butyldimethylsilyl chloride, imidazole, CH_2Cl_2; k) (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid, DCC, DMAP, CH_2Cl_2; l) HF/Pyridine.

Synthesis of the Common Allyl Glycoside Intermediate 13. In our previous studies, glycosylation of glucosamine α-allyl glycosides with 3- and 6-hydroxy groups selectively gave 6-*O*-glycosylated product in good yields.[14,22,24] By contrast, a preliminary experiment showed that the glycosylation of the corresponding β-allyl glycoside **29** gave a mixture of the desired (1→6) disaccharide and the undesired trisaccharide formed by over-glycosylation at the 3-position. Compound **12** whose 3-position was already acylated was therefore used for the present synthesis. Coupling of the trichloroacetimidate **11** with the glycosyl acceptor **12** was carried out in CH_2Cl_2 by using TMSOTf as a catalyst to give the desired disaccharide **13** in a good yield (Scheme 3).

Synthesis of β-PE-506 (7) and β-CM-506 (9) via the Common Aldehyde Intermediate 34. The common aldehyde intermediate **34** for the synthesis of hexaacyl 506-type analogs **7** and **9** were prepared from **13**, as shown in Scheme 4. The 2′-*N*-Troc group of **13** was removed with Zn–Cu in acetic acid followed by *N*-acylation with (*R*)-3-(dodecanoyloxy)tetradecanoic acid, 1-hydroxy-7-azabenzotriazole (HOAt), and



Scheme 3. a) TMSOTf (0.1 equiv), MS4A, CH_2Cl_2, −20 °C.

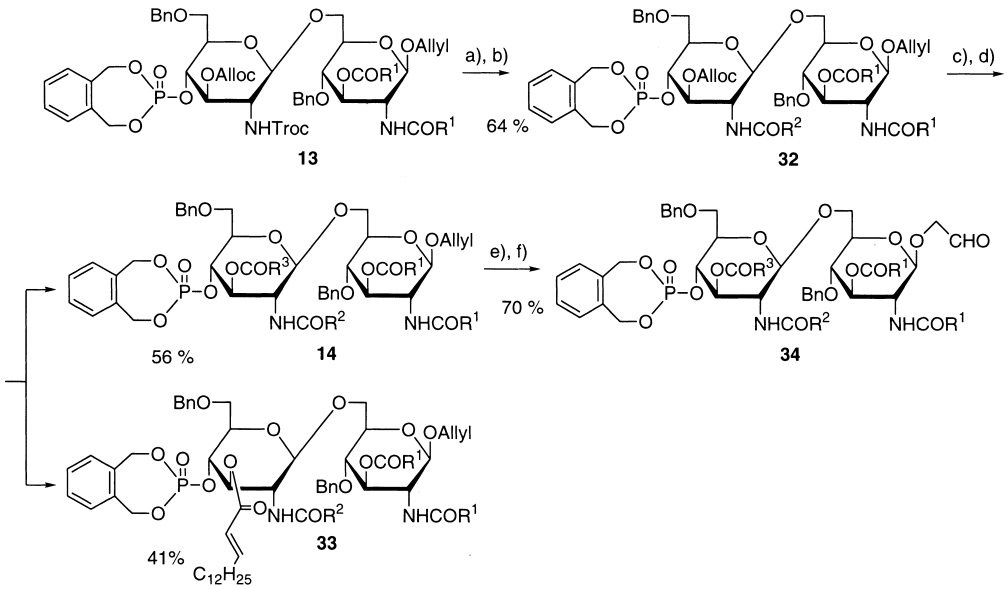
WSCD·HCl afforded **32**. The Alloc group of **32** was readily removed with $[Pd(PPh_3)_4]$, HCOOH, and butylamine. The 3′-*O*-acylation was then effected with (*R*)-3-(tetradecanoyloxy)-tetradecanoic acid by using DCC and DMAP in $CHCl_3$. The desired compound **14** was obtained in 56% yield. In this reaction, undesired **33** was formed by β-elimination of the acyloxy group in tetradecanoyloxytetradecanoic acid moiety. Dihydroxylation of the C=C double bond of **14** using catalytic amounts of osmium(VIII) oxide (OsO_4) and 4-methylmorpholine *N*-Oxide (NMO) in THF–*t*-BuOH–H_2O (10:10:1) followed by lead(IV) acetate ($Pb(OAc)_4$) oxidation afforded the aldehyde **34**.

Reduction of the aldehyde **34** with sodium tetrahydroborate ($NaBH_4$) gave the alcohol **35**. Phosphorylation of **35** gave the bisphosphate **36** in 99% yield (Scheme 5). The final deprotection was accomplished by hydrogenolysis (13 kg cm^{-2} of H_2) with Pd-black in one-step. Purification of the crude product was effected by liquid–liquid partition column chromatography using Sephadex® LH-20 to give the desired β-PE-506 (**7**) in 74% yield, whose structure was confirmed by MS and NMR spectra.

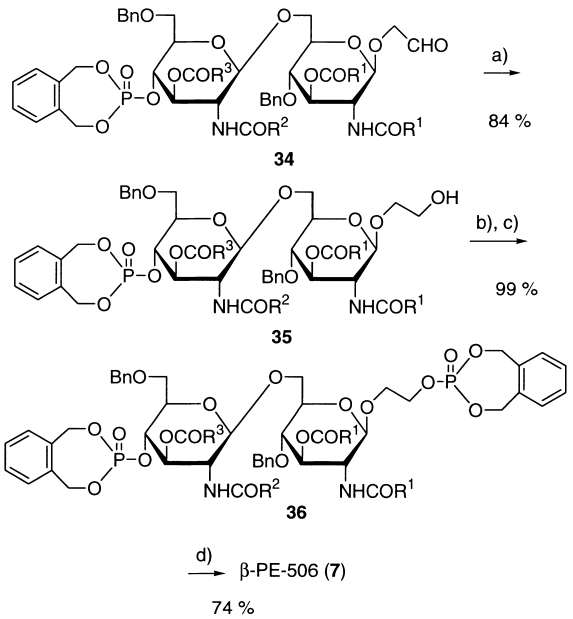
For the synthesis of β-CM-506 (**9**), the aldehyde **34** was oxidized with sodium chlorite ($NaClO_2$) (Scheme 6). Successive deprotection of **37** under hydrogenolytic conditions followed by purification by liquid–liquid partition column chromatography afforded the desired β-CM-506 (**9**) in 69% yield. The structure of **9** was confirmed by MS and NMR spectra.

Synthesis of β-PE-406 (8) and β-CM-406 (10) via the Common Aldehyde Intermediate 39. The tetraacyl 406-type analogs **8** and **10** were synthesized via the common aldehyde intermediate **39** in manners similar to the synthesis of the corresponding hexaacyl analogs. Removal of the 2′-*N*-Troc group of **13** followed by *N*-acylation with (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid gave **38**. The Alloc group of **38** was removed and the 3′-*O*-acylation with (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid gave the desired compound **15** in 86% yield. The aldehyde **39** was obtained by dihydroxylation of **15** and subsequent $Pb(OAc)_4$ oxidation in 85% yield (Scheme 7).

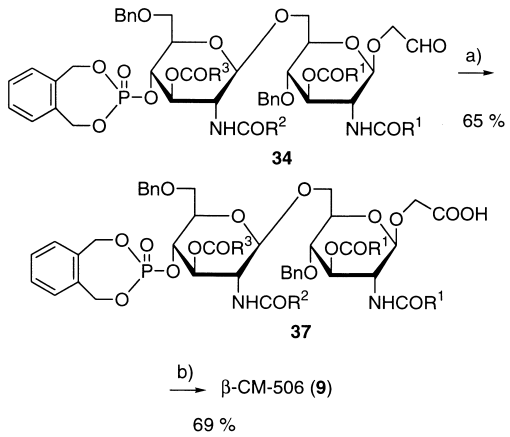
Reduction of the aldehyde **39** followed by phosphorylation gave the protected β-PE-406 (**41**). The final deprotection was carried out in THF by hydrogenolysis (13 kg cm^{-2} of H_2) with Pd-black. As the hydrogenation proceeded, parts of the products precipitated and the reaction hence was not completed. The precipitates contained the desired product accompanied



Scheme 4. a) Zn-Cu, AcOH; b) (*R*)-3-(dodecanoyloxy)tetradecanoic acid, HOBt, WSCD·HCl, $CHCl_3$; c) $[Pd(PPh_3)_4]$, $BuNH_2$, HCOOH, THF; d) (*R*)-3-(tetradecanoyloxy)tetradecanoic acid, DCC, DMAP, $CHCl_3$; e) OsO_4, NMO, THF/2-methyl-2-propanol/H_2O (10:10:1); f) $Pb(OAc)_4$, benzene.



Scheme 5. a) $NaBH_4$; b) *N*,*N*-Diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2; c) *m*CPBA, −20 °C; d) H_2 (13 kg cm^{-2}), Pd-black, THF.

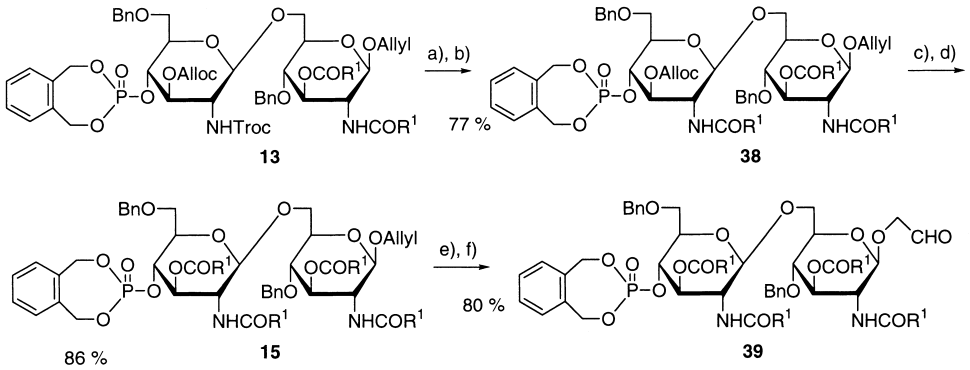


Scheme 6. a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, 2-methyl-2-propanol/water (4:1); b) H_2 (13 kg cm^{-2}), Pd-black, THF.

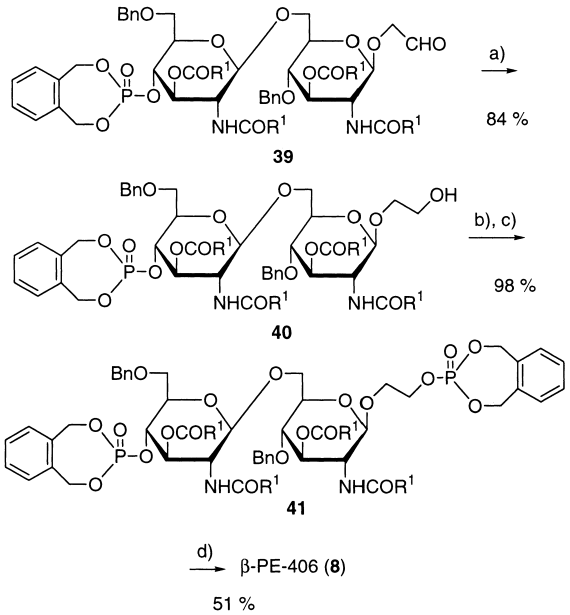
with substantial amounts of incompletely deprotected products. The latter still retained 4-trifluoromethylbenzyl groups, whose complete removal was not successful. Purification of the crude product was effected by liquid-liquid partition column chromatography using Sephadex® LH-20 to give the desired β-PE-406 (**8**) in 51% yield (Scheme 8).[31] The solubility of purified **8** was considerably lower (insoluble in $CHCl_3$–MeOH (1:1) and water) than the solubilities of the crude product and PE-406 (**4**). We recently found that the biosynthetic precursor 406 (**2**), PE-406 (**4**), and CM-406 (**6**) gave well-resolved ^1H NMR spectra in DMSO-d_6 and in SDS-d_{25}/D_2O.[15,32] However, β-PE-406 (**8**) gave analyzable spectra in neither DMSO-d_6 nor SDS-d_{25}/D_2O owing to signal broadening. These results indicate that compound **8** tends to aggregate strongly in both organic and aqueous solvents. After several solvent systems were examined, we found that an NMR spectrum of **8** in SDS-d_{25}/DMSO-d_6 afforded clear signals. The structure of **8** was thus confirmed by NMR and MS spectra.

For the synthesis of β-CM-406 (**10**), the aldehyde **39** was oxidized by the treatment with sodium chlorite ($NaClO_2$) (Scheme 9). Successive deprotection under hydrogenolytic conditions, followed by purification by liquid–liquid partition column chromatography, afforded the desired β-CM-406 (**10**) in 54% yield. The structure of **10** was confirmed by MS and NMR spectra. ^1H NMR of β-CM-406 (**10**) gave well-resolved spectra in $CHCl_3$–MeOH (1:1).

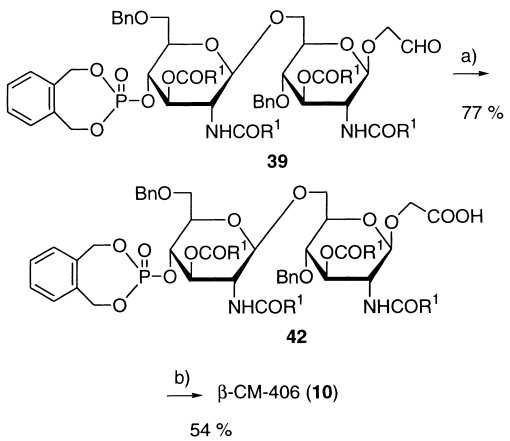
Biological Activity. Biological activities of β-type analogs (**7**, **8**, **9**, **10**) were evaluated in comparison to the corre-



Scheme 7. a) Zn-Cu, AcOH; b) (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid, HOAt, WSCD·HCl, $CHCl_3$; c) [$Pd(PPh_3)_4$], $BuNH_2$, HCOOH, THF; d) (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid, DCC, DMAP, $CHCl_3$; e) OsO_4, NMO, THF/2-methyl-2-propanol/H_2O (10:10:1); f) $Pb(OAc)_4$, benzene.



Scheme 8. a) $NaBH_4$; b) *N*,*N*-Diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2; c) *m*CPBA, −20 °C; d) H_2 (13 kg cm^{-2}), Pd-black, THF.



Scheme 9. a) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, 2-methyl-2-propanol/water (4:1); b) H_2 (13 kg cm^{-2}), Pd-black, THF.

sponding α-type analogs (**3**, **4**, **5**, **6**) and LPS (*E. coli* 0111:B4) by measuring typical endotoxic activity such as *Limulus* activity and cytokine induction. Cytokine inducing activity of the hexaacyl 506-type analogs and LPS was tested in human peripheral whole-blood cells.[33] A mixture of a test sample and heparinized human peripheral whole-blood collected from an adult volunteer in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) was incubated at 37 °C in 5% CO_2 for 24 h. The levels of cytokines, i.e., interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), in supernatant of incubation mixtures were measured by means of enzyme-linked immunosorbent assay (ELISA). Antagonistic activity of the tetraacyl 406-type analogs was examined by inhibition to cytokine induction by LPS as follows. A mixture of a test sample, LPS (2.5 ng mL^{-1}) (*E. coli* 0111:B4; Sigma Chemicals Co.), and heparinized human peripheral whole-blood was incubated and the levels of cytokines were measured in the same manner as described above. Since β-PE-406 (**8**) and β-CM-406 (**10**) were hardly soluble in water, all samples were once dissolved in DMSO and then sequentially diluted with water or saline. Several 96-well plates were required for the measurement of the dose dependency, so that 2.5 ng mL^{-1} of LPS was used as a standard for each plate.

As can be clearly seen in Figs. 4 and 5, both β-PE and β-CM 506-type analogs showed cytokine inducing activity similar to the corresponding α-analogs. On the other hand, β-CM-406 (**10**) showed definite but weaker antagonistic activity than the α-counterparts. β-PE-406 (**8**) also showed antagonistic activity, but the potency was much weaker than α-PE-406 and α-CM-406.

Limulus activity of the β-type analogs (**7**, **8**, **9**, **10**) and LPS was measured by the activation of factor *C* at various concentrations by means of Endospecy Test (Seikagaku Corporation, Tokyo).[33] The results are summarized in Table 1. All the samples were found to exhibit strong positive activity.

Discussions

We synthesized four lipid A analogs possessing β-glycosidic linkage at the 1-position, i.e., PE-analogs, β-PE-506 (**7**) and β-PE-406 (**8**), and CM-analogs, β-CM-506 (**9**) and β-CM-406 (**10**). The cytokine-inducing assay clearly demonstrated that the hexaacyl β-analogs **7** and **9** of *E. coli* lipid A-type have

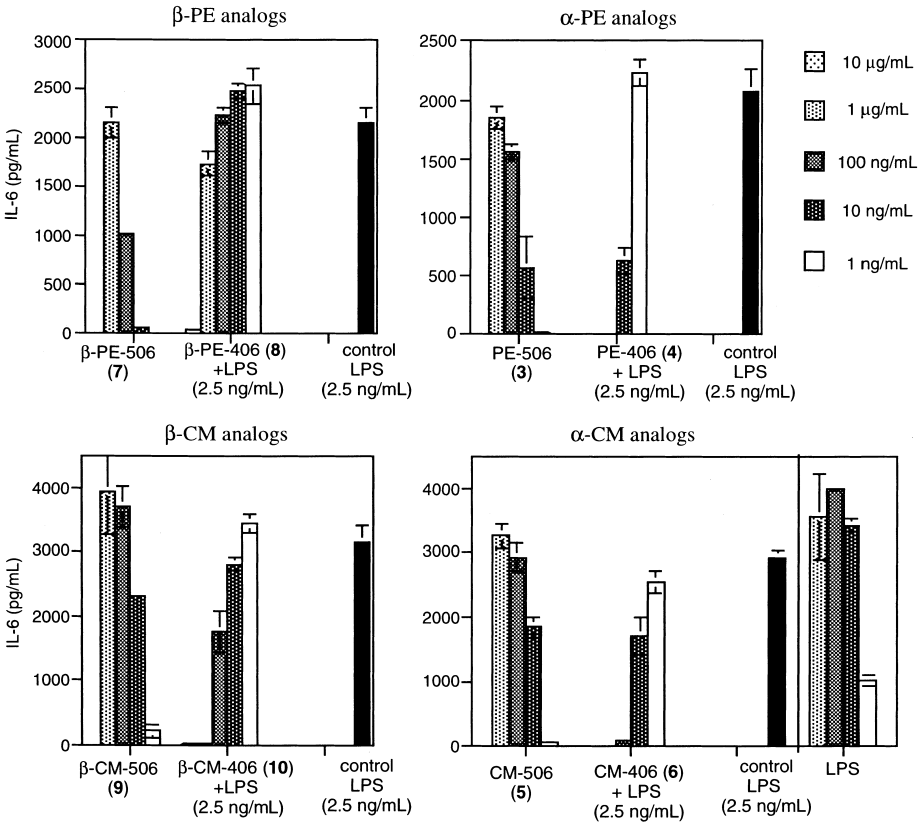


Fig. 4. IL-6 induction by **3**, **5**, **7**, **9**, and LPS and inhibitory effect of **4**, **6**, **8**, **10** to IL-6 induction by LPS. The blood donor was Y. S.

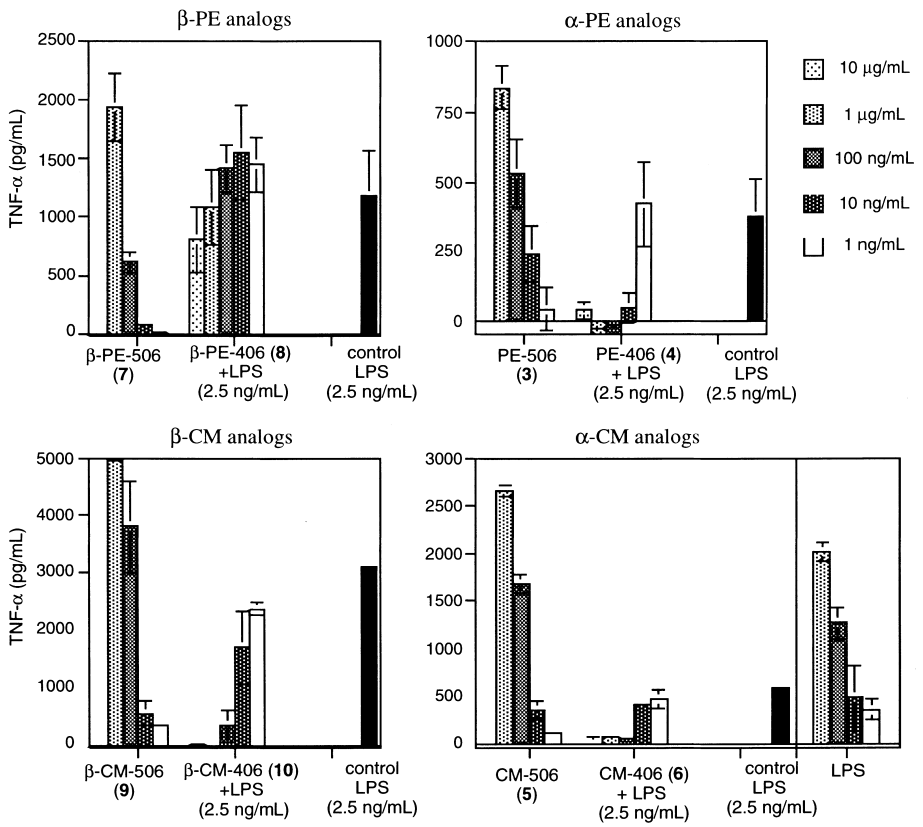


Fig. 5. TNFα induction by **3**, **5**, **7**, **9**, and LPS and inhibitory effect of **4**, **6**, **8**, **10** to TNFα induction by LPS. The blood donor was Y. S.

Table 1. *Limulus* Activity of **1**, **3**, **5**, **7**, **8**, **9**, **10**, and LPS (*E. coli* 0111:B4) as Tested by Endospecy Test® (Seikagaku Corporation, Tokyo).

	ED50/pg mL^{-1}
E. coli lipid A (**1**)	6*
PE-506 (**3**)	2*
CM-506 (**5**)	1*
β-PE-506 (**7**)	30
β-PE-406 (**8**)	30
β-CM-506 (**9**)	20
β-CM-406 (**10**)	10
LPS (*E. coli* 0111:B4)	3

*Data from previous study.[22]

strong immunostimulating activity comparable to the corresponding α-type analogs and *E. coli* lipid A itself. Kusama et al. reported that β-PE-506 (**7**) showed considerably weaker activity than natural lipid A.[13,20] The discrepancy might be owing to the solubility and aggregation problem rather than the difference in the assay systems.

LPS, lipid A, and lipid A analogs form aggregates in aqueous solution. Lipopolysaccharide binding protein (LBP) in serum is assumed to work as a shuttle that transfers LPS monomer from LPS aggregates to CD14 on the cell surface membrane of immunocompetent cells.[34,35]

Takayama et al. claimed that a monomeric LPS artificially prepared by sonication showed stronger activity than LPS aggregates,[36] which may suggest that monomeric LPS is more readily transferred to immunocompetent cells than aggregates.

LPS is then recognized by the receptor complex consisting in Toll like receptor 4 (TLR4), MD-2, CD14, and others.[3–5] CD14 and TLR4 are now considered as a capture receptor and a signaling receptor of LPS, respectively. MD-2, which is essential for LPS signaling, is an association protein to TLR4. Several other proteins involved in the signaling of LPS were also reported. Transfer of lipid A (or LPS) monomer from the aggregates to immunocompetent cells seems to be one of the critical steps for expression of endotoxic activity. It is, however, still unclear whether the receptor complex recognizes the monomeric lipid A (or LPS) or the aggregated supramolecular structure reconstructed on the cell membrane.

We thought β-analogs might form more rigid aggregates and hence be more difficult to be transferred than α-analogs. Kusama et al. used usual aqueous solution for in vivo assays and hence β-PE-506 (**7**) gave weaker response than α-PE-506 (**3**). In the present study, all the samples were once dissolved in DMSO and then diluted with water or saline for the biological assays. Under such conditions, aggregation property between α and β analogs might be similar. The biological activities of α and β analogs were hence not different in our in vitro systems.

The biological tests of biosynthetic precursor-type analogs support this assumption. As described, the solubility of β-PE-406 (**8**) was very low in both aqueous and organic solvents. It forms aggregates even in DMSO as indicated by NMR, whereas other lipid A analogs do not aggregate in DMSO. Because of its strong tendency to aggregation, β-PE-406 (**8**) is probably difficult to be transferred to the receptor complex and hence shows much weaker inhibitory activity.

The *Limulus* activity of the β-analogs was somehow weaker than that of the corresponding α-analogs. However, no significant difference was observed among the *Limulus* activities of the hexa- and tetraacylated β-analogs. This fact seems to suggest that activation of the *Limulus* enzyme, factor C, is not influenced by the stage of aggregation of lipid A.

In the present study, we clearly demonstrated that the β-configurated CM-and PE-analogs having *E. coli* acylation pattern show comparable biological activity to the corresponding α-analogs. This result indicates that the spatial arrangement of acidic functional groups is not strictly required for the activity.

Experimental

All melting points are uncorrected. ^{1}H NMR was measured with JEOL JNM-LA 600 or JEOL JNM-LA 500 spectrometers for $CDCl_3$ solutions unless otherwise noted. The chemical shifts are given in δ values from tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a JEOL JMS-SX 102 mass spectrometer and a Mariner™ Biospectrometry Workstation (Applied Biosystems) mass spectrometer. Specific rotations were measured on a Perkin-Elmer 241 polarimeter. Silica-gel column chromatography was carried out using Kieselgel 60 (E. Merck, 0.040–0.063 mm) at medium-pressure (2–4 kg cm^{-2}). Organic solutions were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated in vacuo unless otherwise noted. An LPS specimen from *E. coli* 0111:B4 (Sigma Chemicals Co) was used as a positive control for cytokine inducing assay and *Limulus* assay.

1-Propenyl 3-*O*-Allyloxycarbonyl-4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (17). To a degassed solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**16**) (38.0 g, 78.7 mmol) in anhydrous THF (400 mL) was added (1,5-cyclooctadiene)[bis(methyldiphenylphosphine)]iridium(I) hexafluorophosphate (1 g, 1.18 mmol). After activation of the iridium catalyst with hydrogen three times (each 30 s), the mixture was stirred under nitrogen atmosphere at room temperature for 1 h and then the solution was concentrated in vacuo. To the solution of the residue in CH_2Cl_2 (100 mL) and pyridine (300 mL) was added allyl chloroformate (41.8 mL, 394 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 5 h and concentrated in vacuo. EtOAc and water were added to the residue. The aqueous layer was extracted with EtOAc. The organic layer was worked up as usual. The crude product was purified by silica-gel column chromatography (500 g, toluene/EtOAc = 30:1) to give **17** as a white solid (37.1 g, 85%). Mp 96.0–98.0 °C. $[\alpha]_D^{24}$ +65.9 (*c* 1.00, $CHCl_3$). Found: C, 48.79; H, 4.53; N, 2.49%. Calcd for $C_{23}H_{26}Cl_3NO_9$: C, 48.74; H, 4.62; N, 2.47%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45–7.35 (m, 5 H, *Ph*–CH), 6.15–6.07 (m, 1 H, OC*H*=CHCH$_3$), 5.92–5.84 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.54 (d, J = 6.9 Hz, 1 H, Ph–C*H*), 5.40 (brd, 1 H, NH), 5.32 (dd, J = 17.3, 6.3 Hz, 2 H, OCH$_2$CH=C*H*$_2$), 5.27–5.19 (m, 2 H, H-3 and OCH=C*H*CH$_3$), 5.11 (d, J = 3.4 Hz, 1 H, H-1), 4.82 (d, J = 12.2 Hz, 1 H, CH$_2$ of Troc), 4.71–4.60 (m, 3 H, CH$_2$ of Troc and OC*H*$_2$CH=CH$_2$), 4.29 (dd, J = 10.3, 4.4 Hz, 1 H, H-6a), 4.17–4.13 (m, 1 H, H-2), 3.92 (ddd, J = 9.7, 9.7, 4.7 Hz, 1 H, H-6b), 3.80–3.75 (m, 2 H, H-4 and H-5), 1.68–1.56 (m, 3 H, OCH=CHC*H*$_3$).

1-Propenyl 3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside

(18). To a solution of **17** (10.0 g, 17.6 mmol) in anhydrous CH_3CN (90 mL) were added $Me_2NH{\cdot}BH_3$ (5.30 g, 88.2 mmol) and $Et_2O{\cdot}BF_3$ (11.5 mL, 88.2 mmol) at 0 °C, successively. After being stirred at 0 °C for 2 h, the solution was quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and worked up as usual. The residue was purified by silica-gel column chromatography (500 g, toluene/AcOEt/1,1,1,3,3,3-hexafluoro–2-propanol = 30:1:0.6) to give **18** as colorless oil (6.80 g, 68%). $[\alpha]_D^{24}$ = +58.7 (*c* 1.01, $CHCl_3$). Found: C, 48.28; H, 4.83; N, 2.47%. Calcd for $C_{23}H_{28}Cl_3NO_9$: C, 48.56; H, 4.96; N, 2.46%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45–7.35 (m, 5 H, *Ph*-CH_2), 6.16–6.05 (m, 1 H, OC*H*=CHCH$_3$), 5.94–5.88 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.37–5.26 (m, 3 H, NH and OCH$_2$CH=C*H*$_2$), 5.21-5.17 (m, 1 H, OCH=C*H*CH$_3$), 5.09 (d, *J* = 10.0 Hz, 1 H, H-3), 4.94 (d, *J* = 3.4 Hz, 1H, H-1), 4.84 (d, *J* = 12.0 Hz, 1 H, CH_2 of Troc), 4.64–4.53 (m, 5 H, CH_2 of Troc, Ph–C*H*$_2$, and OC*H*$_2$CH=CH$_2$), 4.07–4.06 (m, 1 H, H-2), 3.91–3.79 (m, 3 H, H-4, H-5 and H-6a), 3.70–3.68 (m, 1 H, H-6b), 1.68–1.56 (m, 3 H, OCH=CHC*H*$_3$).

1-Propenyl 3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (19). To a solution of **18** (12.0 g, 21.1 mmol) in anhydrous 1,2-dichloroethane (40 mL) were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (5.05 g, 21.1 mmol) and 1*H*-tetrazole (3.80 g, 52.7 mmol). After the mixture was stirred at room temperature for 30 min and then at −20 °C for 10 min, mCPBA (3.77 g, 21.8 mmol) was added and stirring was continued for another 30 min. The solution was quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and worked up as usual. The residue was purified by silica-gel column chromatography (500 g, $CHCl_3$/acetone = 30:1) to give **19** as colorless crystals (15.4 g, 99%). Mp 70.0–78.0 °C. $[\alpha]_D^{24}$ +56.7 (*c* 1.03, $CHCl_3$). Found: C, 48.24; H, 4.58; N, 1.99%. Calcd for $C_{31}H_{35}Cl_3NO_{11}P{\cdot}1.1H_2O$: C, 48.31; H, 4.86; N, 1.82%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.37–7.19 (m, 9 H, *Ph*-CH_2 and *o*-$C_6H_4(CH_2O)_2P$), 6.18–6.10 (m, 1 H, OC*H*=CHCH$_3$), 5.90–5.87 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.37-5.05 (m, 8 H, H-1, H-3, NH, OCH=C*H*CH$_3$ and *o*-$C_6H_4(CH_2O)_2P$), 4.85–4.76 (m, 3 H, CH_2 of Troc, OCH$_2$CH=C*H*$_2$ and H-4), 4.69–4.56 (m, 2 H, CH_2 of Troc, and OC*H*$_2$CH=CH$_2$), 4.13–4.11 (m, 1 H, H-2), 3.98 (m, 1 H, H-5), 3.79 (m, 2 H, H-6ab), 1.91–1.55 (m, 3 H, OCH=CHC*H*$_3$).

3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranose (20). To a solution of **19** (4.40 g, 5.99 mmol) in THF (15 mL) and water (15 mL) was added iodine (3.04 g, 12.0 mmol) and the reaction mixture was stirred at room temperature for 1 h. To the mixture was added 5% aqueous $Na_2S_2O_3$ and the mixture was extracted with EtOAc. The extract was washed with 5% aqueous $Na_2S_2O_3$ and brine and worked up as usual. The crude product was purified by silica-gel column chromatography (200 g, $CHCl_3$/acetone = 10:1) to give **20** as colorless crystals (3.61 g, 86%). Mp 68.5–70.0 °C. $[\alpha]_D^{24}$ +19.7 (*c* 1.14, $CHCl_3$). Found: C, 46.47; H, 4.30; N, 1.96%. Calcd for $C_{28}H_{31}Cl_3NO_{12}P{\cdot}0.7H_2O$: C, 46.48; H, 4.51; N, 1.94%. FAB-MS (positive) *m*/*z* 711.3 [(M+H)$^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38–7.26 (m, 5 H, *Ph*–CH_2), 7.21–7.17 (m, 4 H, *o*-$C_6H_4(CH_2O)_2P$), 5.92–5.84 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.37 (d, *J* = 10.6 Hz, 1 H, OCH$_2$CH=C*H*$_2$), 5.33–5.30 (m, 2 H, H-1 and NH), 5.24 (d, *J* = 10.6 Hz, 1 H, OCH$_2$CH=C*H*$_2$), 5.23 (dd, *J* = 8.9, 8.9 Hz, 1 H, H-3), 5.18–5.05 (m, 4 H, *o*-$C_6H_4(CH_2O)_2P$), 4.82 (d, *J* = 12.1 Hz, 1 H, CH_2 of Troc), 4.69–4.56 (m, 6 H, H-4, Ph–C*H*$_2$, CH_2 of Troc and OC*H*$_2$CH=CH$_2$), 3.83 (dd, *J* = 8.9, 1.9 Hz, 1 H, H-6a), 3.75 (dd, *J* = 10.8, 5.7 Hz, 1 H, H-6b), 3.40 (s, 1 H, C_1–OH).

3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl Trichloroacetimidate (11). To a solution of **20** (5.06 g, 7.12 mmol) in anhydrous CH_2Cl_2 (10 mL) at room temperature were added molecular sieves 4A, trichloroacetonitrile (7.1 mL, 71 mmol) and Cs_2CO_3 (1.16 g, 7.12 mmol). After being stirred for 2 h, saturated aqueous $NaHCO_3$ was added to the reaction mixture. The mixture was extracted with EtOAc and the extract was worked up as usual to give **11** as a pale yellow solid, which was used for the subsequent glycosylation without further purification.

4′,5′-Dihydro-2′-methyloxazolo[5′,4′:1,2]-3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranose (22). To a solution of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-acetylamino-D-glucopyranose (**21**) in $ClCH_2CH_2Cl$ (20 mL) was added TMSOTf (1.39 mL, 7.71 mmol) and the solution was stirred at 50 °C for 20 h. After the solution was cooled to room temperature, Et_3N, EtOAc, and saturated aqueous $NaHCO_3$ were added. The aqueous layer was extracted by EtOAc and the organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (100 g, toluene/EtOAc/Et_3N = 100:200:1) to give **22** as pale yellow oil (1.60 g, 95%).

Allyl 2-Acetylamino-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (24). A solution of **22** (0.18 g, 0.547 mmol) in 0.1 M Allyl-ONa in Allyl-OH (1.8 mL) was stirred at room temperature for 1 h. After addition of *p*-TsOH$\cdot H_2O$ (400 mg, 2.3 mmol), the solution was stirred at room temperature for 15 min. The crude allyl glycoside **23** was obtained by concentration. To a solution of **23** in CH_3CN (2 mL) was added benzaldehyde dimethyl acetal (0.164 mL, 1.09 mmol) and the solution was stirred at room temperature for 30 min. EtOAc and saturated aqueous $NaHCO_3$ were added. The aqueous layer was extracted with EtOAc and the organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (10 g, $CHCl_3$/MeOH = 20:1) to give **24** as a white solid (0.140 g, 73%). $[\alpha]_D^{24}$ −76.9 (*c* 1.00, MeOH). ESI-MS (positive) *m*/*z* 350.2 [(M + H)$^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 7.7, 2.2 Hz, 2 H, *Ph*CH), 7.40–7.33 (m, 3 H, *Ph*CH), 5.89 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.77 (d, *J* = 7.4 Hz, 1 H, NH), 5.58 (s, 1 H, PhC*H*), 5.32 (dd, *J* = 17.2, 1.4 Hz, 1 H, OCH$_2$CH=C*H*$_2$), 5.25 (d, *J* = 10.3 Hz, 1 H, OCH$_2$CH=C*H*$_2$), 4.77 (d, *J* = 8.4 Hz, 1 H, H-1), 4.38–4.33 (m, 2H OC*H*$_2$CH=CH$_2$ and H-6a), 4.17 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-3), 4.10 (dd, *J* = 12.8, 6.2 Hz, 1 H, OC*H*$_2$CH=CH$_2$), 3.80 (dd, *J* = 10.3, 10.3 Hz, 1 H, H-6b), 3.58 (dd, *J* = 9.1, 9.1 Hz, 1 H, H-4), 3.53–3.47 (m, 2H, H-2 and H-5), 2.05 (s, 3 H, CH_3).

Allyl 2-Acetylamino-4,6-*O*-benzylidene-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranoside (25). To a solution of **24** (6.98 g, 20.0 mmol) and 4-methoxyphenylmethyl trichloroacetimidate (8.01 mL, 40.0 mmol) in anhydrous THF (200 mL) was added $Sn(OTf)_2$ (833 mg, 2.00 mmol) at 0 °C. The solution was stirred at 0 °C for 4 h and the reaction was quenched by addition of saturated aqueous $NaHCO_3$. The mixture was extracted with $CHCl_3$ and the organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (300 g, $CHCl_3$/acetone = 10:1) to give **25** as a white solid (9.30 g, 99%). Mp 146–153 °C. $[\alpha]_D^{22}$ +1.3 (*c* 0.67, $CHCl_3$). Found: C, 65.79; H, 6.66; N, 2.96%. Calcd for $C_{26}H_{31}NO_7{\cdot}0.3\ H_2O$: C, 65.75; H, 6.71; N, 2.95%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (dd, *J* = 8.2, 1.7 Hz, 2 H, *Ph*CH), 7.40–7.35 (m, 3 H, *Ph*CH), 7.23

(d, J = 8.4 Hz, 2 H, *p*-CH_3O–*Ph*–CH_2), 6.85 (d, J = 8.4 Hz, 2 H, *p*-CH_3O–*Ph*–CH_2), 5.86 (m, 1H, OCH_2CH=CH_2), 5.59–5.58 (m, 2 H, NH and PhC*H*), 5.27 (d, J = 17.1 Hz, 1 H, OCH_2CH=CH_2), 5.19 (d, J = 10.3 Hz, 1 H, OCH_2CH=CH_2), 4.99 (d, J = 8.3 Hz, 1 H, H-1), 4.82 (d, J = 11.5 Hz, 1 H, *p*-CH_3O–Ph–CH_2), 4.59 (d, J = 11.4 Hz, 1 H, *p*-CH_3O–Ph–CH_2), 4.35–4.30 (m, 2 H, OCH_2-CH=CH_2 and H-6a), 4.26 (dd, J = 9.4, 9.4 Hz, 1 H, H-3), 4.08 (dd, J = 12.8, 6.1 Hz, 1 H, OCH_2CH=CH_2), 3.81–3.76 (m, 4 H, *p*-CH_3O–Ph–CH_2 and H-6b), 3.67 (dd, J = 9.2, 9.2 Hz, 1 H, H-4), 3.52 (m, 1 H, H-5), 3.34 (dd, J = 17.8, 8.0 Hz, 1 H, H-2), 1.82 (s, 3 H, CH_3).

Allyl 2-Acetylamino-4-*O*-benzyl-2-deoxy-3-*O*-(4-methoxyphenylmethyl)-β-D-glucopyranoside (26). To a solution of **25** (16.6 g, 35.4 mmol) in anhydrous CH_2Cl_2 (180 mL) were added Me_2NH·BH_3 (10.7 g, 177 mmol) and Et_2O·BF_3 (11.5 mL, 88.4 mmol) successively at 0 °C. After the mixture was stirred for 2 h at 0 °C, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$ and the organic layer was worked up as usual. The residue was purified by silica-gel column chromatography ($CHCl_3$/acetone = 3:1) to give **26** as white powder (9.48 g, 57%) and **27** (2.32 g, 14%). $[\alpha]_D^{28}$ +5.9 (c 1.04, $CHCl_3$). Found: C, 65.17; H, 7.13; N, 2.94%. Calcd for $C_{26}H_{33}NO_7$·0.4 H_2O: C, 65.23; H, 7.12; N, 2.93%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35–7.29 (m, 5 H, *Ph*CH_2), 7.22 (d, J = 8.2 Hz, 2 H, *p*-CH_3O–*Ph*–CH_2), 6.85 (d, J = 8.0 Hz, 2 H, *p*-CH_3O–*Ph*–CH_2), 5.86 (m, 1 H, OCH_2CH=CH_2), 5.67 (d, J = 7.9 Hz, 1 H, NH), 5.25 (d, J = 17.2 Hz, 1 H, OCH_2CH=CH_2), 5.16 (d, J = 10.4 Hz, 1 H, OCH_2CH=CH_2), 4.85–4.83 (m, 2 H, PhCH_2 and H-1), 4.76 (d, J = 11.2 Hz, 1 H, Ph–CH_2), 4.67 (d, J = 11.2 Hz, 1 H, PhCH_2), 4.60 (d, J = 11.2 Hz, 1 H, Ph–CH_2), 4.29 (dd, J = 13.0, 5.3 Hz, 2 H, OCH_2CH=CH_2), 4.08–4.05 (m, 2 H, OCH_2CH=CH_2 and H-3), 3.86 (dd, J = 11.9, 2.8 Hz, 1 H, H-6a), 3.78 (s, 3 H, *p*-CH_3O–Ph–CH_2) 3.72 (dd, J = 11.9, and 3.8 Hz, 1 H, H-6b), 3.58 (dd, J = 9.0, 9.0 Hz, 1 H, H-4), 3.44–3.40 (m, 2 H, H-2 and H-5), 1.88 (s, 3 H, CH_3).

Allyl 2-Acetylamino-4-*O*-benzyl-2-deoxy-β-D-glucopyranoside (27). To a solution of **26** (6.10 g, 13.0 mmol) in anhydrous CH_2Cl_2 (60 mL) was added Et_2O·BF_3 (1.97 mL, 15.6 mmol) at 0 °C and the solution was stirred at the same temperature for 1 h. Saturated aqueous $NaHCO_3$ and $CHCl_3$ were added and the organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (300 g, $CHCl_3$/MeOH = 30:1) to give **27** as white powder (4.55 g, quant). $[\alpha]_D^{22}$ −30.7 (c 0.60, MeOH). Found: C, 60.34; H, 7.11; N, 3.87%. Calcd for $C_{18}H_{25}NO_6$·0.5 H_2O: C, 59.99; H, 7.27; N, 3.89%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45–7.26 (m, 5 H, *Ph*CH_2), 5.90 (m, 1 H, OCH_2-CH=CH_2), 5.72 (d, J = 7.9 Hz, 1 H, NH), 5.31 (dd, J = 17.2, 1.3 Hz, 1 H, OCH_2CH=CH_2), 5.24 (dd, J = 10.4, 1.3 Hz, 1 H, OCH_2CH=CH_2), 4.96 (d, J = 11.2 Hz, 1 H, PhCH_2), 4.73 (d, J = 11.3 Hz, 1 H, PhCH_2), 4.54 (d, J = 8.2 Hz, 1 H, H-1), 4.35 (dd, J = 12.8, 5.3 Hz, 1 H, OCH_2CH=CH_2), 4.10 (dd, J = 12.8 , 6.4 Hz, 1 H, OCH_2CH=CH_2), 3.92 (dd, J = 9.7, 8.4 Hz, 1 H, H-3), 3.88 (dd, J = 11.9, 2.8 Hz, 1 H, H-6a), 3.73 (dd, J = 12.0, 4.6 Hz, 1 H, H-6b), 3.56−3.48 (m, 2 H, H-2 and H-4), 3.39 (m, 1 H, H-5), 2.02 (s, 3 H, CH_3).

Allyl 4-*O*-Benzyl-2-deoxy-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (29). To a solution of **27** (2.70 g, 7.71 mmol) in MeOH (180 mL) was added $Ba(OH)_2$·$8H_2O$ (34.0 g, 108 mmol) and the reaction mixture was stirred at 70 °C for 20 h. After the solvent was removed under reduced pressure, water and EtOAc were added. The organic layer was worked up as usual to give amine **28**. To the solution of **28** and (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid (3.40 g, 8.48 mmol) in anhydrous $CHCl_3$ (30 mL) were added HOBt (1.56 g, 11.6 mmol) and 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride (WSCD·HCl) (2.95 g, 15.4 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 6 h. $CHCl_3$ and saturated aqueous $NaHCO_3$ were added and the aqueous layer was extracted with $CHCl_3$. The organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (150 g, $CHCl_3$/acetone = 10:1) to give **29** as a white solid (2.12 g, 40%). Mp 113–115 °C. $[\alpha]_D^{25}$ −11.6 (c 1.00, $CHCl_3$). Found: C, 64.88; H, 7.78; N, 2.07%. Calcd for $C_{38}H_{54}F_3NO_7$·0.5H_2O: C, 64.94; H, 7.89; N, 1.99%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.36–7.26 (m, 5 H, *Ph*CH_2), 6.45 (d, J = 5.1 Hz, 1 H, NH), 5.75 (m, 1 H, OCH_2CH=CH_2), 5.21 (dd, J = 17.2, 1.4 Hz, 1 H, OCH_2CH=CH_2), 5.13 (dd, J = 10.3, 1.4 Hz, 1 H, OCH_2CH=CH_2), 4.96 (d, J = 11.2 Hz, 1 H, PhCH_2–), 4.72 (d, J = 11.2 Hz, 1 H, PhCH_2–), 4.62–4.58 (m, 2 H, PhCH_2), 4.40 (d, J = 8.1 Hz, 1 H, H-1), 4.22 (dd, J = 12.8, 5.3 Hz, 1 H, OCH_2CH=CH_2), 3.95–3.91 (m, 2 H, OCH_2CH=CH_2 and H-3), 3.88–3.83 (m, 2 H, H-6a and β-CH of C2-*N*-acyl), 3.72 (dd, J = 12.1, 4.6 Hz, 1 H, H-6b), 3.51–3.47 (m, 2 H, H-2 and H-4), 3.37 (m, 1 H, H-5), 2.55 (dd, J = 15.0, 3.8 Hz, 1 H, α-CH of C2-*N*-acyl's main chain), 2.44 (dd, J = 15.0, 7.3 Hz, 1 H, α-CH of C2-*N*-acyl's main chain), 1.69–1.25 (m, 20 H, CH_2 × 10), 0.88 (t, J = 7.0 Hz, 3 H, CH_3).

Allyl 4-*O*-Benzyl-6-*O*-(*t*-butyldimethylsilyl)-2-deoxy-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (30). To a solution of **29** in anhydrous CH_2Cl_2 (30 mL) were added *t*-butyldimethylsilyl chloride (1.30 g, 8.65 mmol) and imidazole (589 mg, 8.65 mmol). After the solution was stirred at room temperature for 30 min, EtOAc and water were added. The organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (100 g, $CHCl_3$/acetone = 10:1) to give **30** as colorless oil (2.30 g, 99%). $[\alpha]_D^{25}$ −12.1 (c 1.00, $CHCl_3$). Found: C, 65.10; H, 8.54; N, 1.69%. Calcd for $C_{44}H_{68}F_3NO_7Si$: C, 65.40; H, 8.48; N, 1.73%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.35–7.27 (m, 5 H, *Ph*CH_2), 6.47 (d, J = 5.1 Hz, 1 H, NH), 5.70 (m, 1 H, OCH_2CH=CH_2), 5.20 (dd, J = 17.2, 1.3 Hz, 1 H, OCH_2CH=CH_2), 5.11 (dd, J = 10.3, 1.1 Hz, 1 H, OCH_2CH=CH_2), 4.93 (d, J = 11.4 Hz, 1 H, PhCH_2–), 4.69 (d, J = 11.4 Hz, 1 H, PhCH_2–), 4.62–4.58 (m, 2 H, PhCH_2), 4.40 (d, J = 8.1 Hz, 1 H, H-1), 4.21 (dd, J = 12.7, 5.1 Hz, 1 H, OCH_2CH=CH_2), 3.92 (dd, J = 12.7, 6.4 Hz, 1 H, OCH_2CH=CH_2), 3.88-3.78 (m, 4 H, H-3, H-4, H-6a and β CH of C2 *N* acyl), 3.54–3.50 (m, 2 H, H-2 and H-6b), 3.31 (m, 1 H, H-5), 2.53 (dd, J = 15.0, 3.8 Hz, 1 H, α-CH of C2-*N*-acyl's main chain), 2.44 (dd, J = 15.0, 7.3 Hz, 1 H, α-CH of C2-*N*-acyl's main chain), 1.34–1.25 (m, 20 H, CH_2 × 10), 0.91–0.87 (m, 12 H, $(CH_3)_3$-$CSi(CH_3)_2$ and CH_3), 0.06–0.05 (m, 6 H, $(CH_3)_3CSi(CH_3)_2$).

Allyl 4-*O*-Benzyl-6-*O*-(*t*-butyldimethylsilyl)-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (31). To a solution of **30** (2.90 g, 3.59 mmol) and (*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoic acid (2.17 g, 5.38 mmol) in anhydrous CH_2Cl_2 (20 mL) were added DCC (1.48 g, 7.18 mmol) and DMAP (43.8 mg, 0.359 mmol) and the reaction mixture was stirred at room temperature for 2 h. After insoluble materials were removed by filtration, EtOAc was added to the fil-

trate. The organic solution was worked up as usual. The residue was purified by silica-gel column chromatography (150 g, toluene/EtOAc = 20:1) to give **31** as a white solid (4.30 g, 100%). $[\alpha]_D^{25}$ −5.7 (*c* 1.06, $CHCl_3$). Found: C, 66.57; H, 8.60; N, 1.60%. Calcd for $C_{66}H_{99}F_6NO_9$: C, 66.47; H, 8.37; N, 1.17%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, J = 7.6 Hz, 2 H, CF_3PhCH_2–), 7.51 (d, J = 7.9 Hz, 2 H, CF_3PhCH_2–), 7.45 (d, J = 7.9 Hz, 2 H, CF_3PhCH_2–), 7.36 (d, J = 7.6 Hz, 2 H, CF_3PhCH_2–), 7.22–7.15 (m, 5 H, $PhCH_2$), 5.98 (d, J = 9.3 Hz, 1 H, NH), 5.73 (m, 1 H, $OCH_2CH{=}CH_2$), 5.18 (d, J = 17.2 Hz, 1 H, $OCH_2CH{=}CH_2$), 5.12–5.01 (m, 2 H, $OCH_2CH{=}CH_2$, H-3), 4.66–4.45 (m, 6 H, $PhCH_2$), 4.30 (d, J = 8.1 Hz, 1 H, H-1), 4.21 (dd, J = 12.8, 4.5 Hz, 1 H, $OCH_2CH{=}CH_2$), 4.02 (ddd, J = 9.3, 9.3, 9.3 Hz, 1 H, H-2), 3.93 (dd, 1 H, J = 12.8, 5.9 Hz, $OCH_2CH{=}CH_2$), 3.85–3.79 (m, 4 H, H-6a,b and β-CH of C3-*O*-acyl and C2-*N*-acyl), 3.72 (dd, J = 9.1, 9.1 Hz, 1 H, H-4), 3.29 (d, 1 H, J = 9.1 Hz, 1 H, H-5), 2.54 (dd, J = 15.8, 7.5 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.44–2.29 (m, 3 H, α-CH_2 of C3-*O*-acyl's and C2-*N*-acyl's main chain), 1.86–1.24 (m, 40 H, $CH_2 \times 20$), 0.88 (t, J = 6.7 Hz, 6 H, $CH_3 \times 2$), 0.08–0.04 (m, 9 H, TBDMS).

Allyl 4-*O*-Benzyl-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (12). To a solution of **31** (1.70 g, 1.43 mmol) in pyridine (15 mL) was added pyridine·HF (0.15 mL) and the solution was stirred at room temperature for 12 h. EtOAc and saturated aqueous $NaHCO_3$ were added to the solution. The aqueous layer was extracted with EtOAc and the organic layer was washed with 1 M HCl solution and worked up as usual. The residue was purified by silica-gel column chromatography (100 g, $CHCl_3$/acetone = 20:1) to give **12** as a white solid (1.30 g, 84%). Mp 98–106 °C. $[\alpha]_D^{25}$ −8.4 (*c* 1.00, $CHCl_3$). Found: C, 66.89; H, 7.95; N, 1.42%. Calcd for $C_{60}H_{85}F_6NO_9$: C, 66.83; H, 7.95; N, 1.30%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (d, J = 7.8 Hz, 2 H, CF_3PhCH_2–), 7.41 (d, J = 7.8 Hz, 2 H, CF_3PhCH_2–), 7.38 (d, J = 7.9 Hz, 2 H, CF_3PhCH_2–), 7.29 (d, J = 7.8 Hz, 2 H, CF_3PhCH_2–), 7.16–7.12 (m, 5 H, $PhCH_2$), 5.91 (d, J = 9.1 Hz, 1 H, NH), 5.66 (m, 1 H, $OCH_2CH{=}CH_2$), 5.14–5.08 (m, 2 H, $OCH_2CH{=}CH_2$, H-3), 5.02 (d, J = 10.4 Hz, 1 H, $OCH_2CH{=}CH_2$), 4.55–4.42 (m, 6 H, $PhCH_2$), 4.30 (d, J = 8.2 Hz, 1 H, H-1), 4.15 (dd, J = 13.0, 4.7 Hz, 1 H, $OCH_2CH{=}CH_2$), 3.94 (ddd, J = 9.1, 9.3, 9.3 Hz, 1 H, H-2), 3.85 (dd, 1 H, J = 13.0, 5.9 Hz, $OCH_2CH{=}CH_2$), 3.84–3.72 (m, 3 H, H-6a and β-CH of C3-*O*-acyl and C2-*N*-acyl), 3.65–3.58 (m, 2 H, H-4 and H-6b), 3.30 (d, 1 H, J = 9.1 Hz, 1 H, H-5), 2.48 (dd, J = 16.0, 7.8 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.35 (dd, J = 16.0, 4.4 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.24–2.23 (m, 2 H, α-CH_2 of C3-*O*-acyl's main chain), 1.53–1.06 (m, 40 H, $CH_2 \times 20$), 0.80 (t, J = 6.7 Hz, 6 H, $CH_3 \times 2$).

Allyl 6-*O*-[3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (13). The imidate **11** (1.44 g, 1.69 mmol), the acceptor **12** (1.30 g, 1.21 mmol), and the molecular sieves 4A in anhydrous CH_2Cl_2 (10 mL) were stirred at room temperature for 1 h. To this mixture was added TMSOTf (21 µL, 0.12 mmol) and the mixture was stirred at −20 °C for 1 h. The reaction mixture was neutralized with saturated aqueous $NaHCO_3$, extracted with EtOAc, and worked up as usual. The residue was purified by silica-gel column chromatography (100 g, $CHCl_3$/acetone = 25:1) to give **13** as a white solid (1.95 g, 91%). Mp 123–127 °C. $[\alpha]_D^{26}$ −5.0 (*c* 1.00, $CHCl_3$). Found: C, 56.51; H, 6.39; N, 1.80%. Calcd for $C_{88}H_{114}Cl_3F_6N_2O_{20}P{\cdot}5H_2O$: C, 56.79; H, 6.72; N, 1.51%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.59 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.48 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.36–7.26 (m, 10 H, $PhCH_2$, CF_3PhCH_2– and o-$C_6H_4(CH_2O)_2P$), 7.22–7.15 (m, 6 H, $PhCH_2$), 5.92–5.88 (m, 2 H, NH, $CH_2CH{=}CH_2$ of Alloc), 5.75 (m, 1 H, $OCH_2CH{=}CH_2$), 5.36 (dd, J = 17.2, 1.3 Hz, 1 H, $CH_2CH{=}CH_2$ of Alloc), 5.29–5.07 (m, 10 H, $CH_2CH{=}CH_2$ of Alloc, $OCH_2CH{=}CH_2$, H-3, H-3′, N-H′ and o-$C_6H_4(CH_2O)_2P$), 4.73 (d, J = 8.2 Hz, 1 H, H-1′), 4.64–4.47 (m, 13 H, $PhCH_2 \times 4$, $CH_2CH{=}CH_2$ of Alloc, H-4′ and CH_2 of Troc), 4.38 (d, J = 8.2 Hz, 1 H, H-1), 4.23 (dd, J = 13.2, 4.6 Hz, 1 H, $OCH_2CH{=}CH_2$), 4.08 (d, J = 9.9 Hz, 1 H, H-6a), 4.00–3.95 (m, 2 H, H-2 and $OCH_2CH{=}CH_2$), 3.86–3.78 (m, 3 H, H-6a′,b′ and β-CH), 3.74–3.68 (m, 3 H, H-5′, H-6b, β-CH of C2′-*N*-acyl and β-CH of C3-*O*-acyl), 3.53 (dd, 1 H, J = 9.2, 9.2 Hz, 1 H, H-4), 3.53–3.50 (m, 2 H, H-2′, and H-5), 2.54 (dd, J = 15.9, 7.8 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.41 (dd, J = 15.9, 4.4 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.29–2.27 (m, 2 H, α-CH_2 of C3-*O*-acyl's main chain), 1.64–1.23 (m, 40 H, $CH_2 \times 20$), 0.88 (t, J = 6.8 Hz, 6 H, $CH_3 \times 2$).

Allyl 6-*O*-{3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)-tetradecanoylamino]-α-D-glucopyranoside (32). To a solution of **13** (1.00 g, 565 µmol) in AcOH (20 mL) was added zinc-copper couple (5 g) and the mixture was stirred at room temperature for 1 h. After the insoluble material was removed by filtration, the filtrate was concentrated in vacuo, and the residual solvent was coevaporated with toluene three times. The crude product was dissolved in EtOAc and worked up as usual to give the *N*-deprotected product. The crude amine thus obtained was dissolved in anhydrous $CHCl_3$ (15 mL). To this solution were added (*R*)-3-(dodecanoyloxy)tetradecanoic acid (465 mg, 1.13 mmol), HOBt (115 mg, 848 µmol), and WSCD·HCl (270 mg, 1.41 mmol). After the mixture was stirred at room temperature for 19 h, saturated aqueous $NaHCO_3$ and EtOAc were added to the reaction mixture. The organic layer was worked up as usual and the residue was purified by silica-gel column chromatography (300 g, $CHCl_3$/acetone/1,1,1,3,3,3-hexafluoro-2-propanol = 10:1:0.6) to give **32** as a white solid (724 mg, 64%). $[\alpha]_D^{26}$ −4.1 (*c* 1.00, $CHCl_3$). Found: C, 65.80; H, 8.27; N, 1.68%. Calcd for $C_{111}H_{161}F_6N_2O_{21}P{\cdot}H_2O$: C, 65.92; H, 8.12; N, 1.39%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.49 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.43 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.36–7.16 (m, 16 H, $PhCH_2$, CF_3PhCH_2– and o-$C_6H_4(CH_2O)_2P$), 5.98 (d, J = 7.7 Hz, 1 H, NH′), 5.92–5.88 (m, 2 H, NH and $CH_2CH{=}CH_2$ of Alloc), 5.73 (m, 1 H, $OCH_2CH{=}CH_2$), 5.43 (dd, J = 10.3, 8.9 Hz, 1 H, H-3′), 5.36 (dd, J = 17.3, 1.5 Hz, 1 H, $CH_2CH{=}CH_2$ of Alloc), 5.25–5.19 (m, 2 H, $CH_2CH{=}CH_2$ of Alloc and $OCH_2CH{=}CH_2$), 5.15–5.00 (m, 8 H, H-3, H-1′, $OCH_2CH{=}CH_2$ β-CH of C2′-*N*-acyl and o-$C_6H_4(CH_2O)_2P$), 4.61–4.46 (m, 11 H, $PhCH_2 \times 4$, $CH_2CH{=}CH_2$ of Alloc and H-4′), 4.37 (d, J = 8.1 Hz, 1 H, H-1), 4.22 (dd, J = 13.1, 4.8 Hz, $OCH_2CH{=}CH_2$), 4.05–4.01 (m, 2 H, H-2 and H-6a), 3.94 (dd, J = 13.1, 6.1 Hz, 1 H, $OCH_2CH{=}CH_2$), 3.85–3.80 (m, 3 H, H-6a′,b′ and β-CH of C3-*O*-acyl), 3.75–3.71 (m, 3 H, H-5′, H-6b and β-CH of C2-*N*-acyl),

3.60–3.51 (m, 3 H, H-2′, H-4 and H-5), 2.52 (dd, J = 16.0, 7.7 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.44–2.38 (m, 2 H, α-CH_2 of C2-*N*-acyl's main chain and α-CH_2 of C2′-*N*-acyl's main chain), 2.31–2.24 (m, 5 H, α-CH_2 of C2′-*N*-acyl's main chain, α-CH_2 of C3-*O*-acyl's main chain and α-CH_2 of C2′-*N*-acyl's side chain), 1.60–1.48 (m, 8 H, $CH_2 \times 4$), 1.40–1.23 (m, 80 H, CH_2), 0.88 (m, 12 H, $CH_3 \times 4$).

Allyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (14). To a solution of **32** (720 mg, 359 μmol) in anhydrous THF (5 mL) were added *n*-BuNH_2 (71 μL, 718 μmol), HCOOH (27 μL, 718 μmol), and tetrakis(triphenylphosphine)palladium(0) (41.5 mg, 35.9 μmol). After the mixture was stirred at room temperature for 1 h, EtOAc was added. The organic layer was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine and worked up as usual. The residue was purified by silica-gel column chromatography (35 g, $CHCl_3$/acetone = 10:1) to give 3′-hydroxy compound. To a solution of the residue in anhydrous $CHCl_3$ (3 mL) were added (*R*)-3-(tetradecanoyloxy)tetradecanoic acid (105 mg, 230 μmol), DCC (59.4 mg, 288 μmol), and DMAP (1.40 mg, 11.5 μmol). The mixture was stirred at room temperature for 3 h and the insoluble materials were removed by filtration. The filtrate was worked up as usual. The residue was purified by silica-gel column chromatography (250 g, $CHCl_3$/acetone/1,1,1,3,3,3-hexafluoro-2-propanol = 10:1:0.6) to give **14** as a white solid (151 mg, 56%). $[\alpha]_D^{26}$ −3.5 (*c* 1.00, $CHCl_3$). Found: C, 69.19; H, 9.29%. Calcd for $C_{135}H_{209}F_6N_2O_{22}P$: C, 68.79; H, 8.94%. ESI-MS (positive) *m/z* 2379.3 $[(M + Na)^+]$. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.49 (d, J = 7.1 Hz, 2 H, CF_3PhCH_2–), 7.43 (d, J = 7.4 Hz, 2 H, CF_3PhCH_2–), 7.37–7.18 (m, 16 H, $PhCH_2 \times 2$, CF_3PhCH_2– and *o*-$C_6H_4(CH_2O)_2P$), 6.14 (d, J = 7.1 Hz, 1 H, NH′), 5.90 (d, J = 9.2 Hz, 1 H, NH), 5.76 (m, 1 H, $OCH_2CH{=}CH_2$), 5.50 (dd, J = 8.3, 8.3 Hz, 1 H, H-3′), 5.26–4.98 (m, 10 H, H-3, H-1′, $OCH_2CH{=}CH_2$, β-CH of C3′-*O*-acyl, β-CH of C2′-*N*-acyl and *o*-$C_6H_4(CH_2O)_2P$), 4.61–4.46 (m, 9 H, $PhCH_2 \times 4$ and H-4′), 4.38 (d, J = 8.1 Hz, 1 H, H-1), 4.26 (d, J = 13.3 Hz, 1 H, $OCH_2CH{=}CH_2$), 4.08–3.95 (m, 3 H, H-2, H-6a and $OCH_2CH{=}CH_2$), 3.82–3.68 (m, 5 H, H-6b, H-6a′,b′, H-5′ and β-CH of C3-*O*-acyl), 3.60–3.54 (m, 3 H, H-4, H-5 and β-CH of C2-*N*-acyl), 3.48 (dd, J = 16.3, 8.4 Hz, 1 H, H-2′), 2.65–2.61 (m, 2 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.53 (dd, J = 16.2, 7.7 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.40 (d, J = 16.2 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.33–2.19 (m, 8 H, α-CH_2 of acyl), 1.61–1.45 (m, 12 H, $CH_2 \times 6$), 1.34–1.09 (m, 108 H, $CH_2 \times 54$), 0.88 (t, J = 6.7 Hz, 18 H, $CH_3 \times 6$).

Formylmethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (34). To a solution of the residue in THF/2-methyl-2-propanol/water (10:10:1, 2.1 mL) were added 4-methylmorpholine *N*-oxide (NMO) (24.6 mg, 204 μmol) and OsO_4 in water (2.5%, 0.10 mL, 10 μmol) at room temperature. After the mixture was stirred for 6 h, 10% aqueous $Na_2S_2O_3$ was added, and the mixture was extracted with EtOAc. The organic layer was worked up as usual. The crude diol thus obtained was dissolved in anhydrous benzene (1.5 mL). To this solution was added lead(IV) acetate ($Pb(OAc)_4$) (30.1 mg, 60.1 μmol) and the mixture was stirred at room temperature for 30 min. The mixture was filtered through a silica-gel column (2 g) using EtOAc as an eluent. After removal of the solvent in vacuo, the residue was purified by silica-gel column chromatography (10 g, $CHCl_3$/acetone = 10:1) to give **34** as a white solid (84 mg, 70%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.50 (s, 1 H, CHO), 7.58 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.48 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 8.0 Hz, 2 H, CF_3PhCH_2–), 7.42–7.15 (m, 16 H, $PhCH_2 \times 2$, CF_3PhCH_2– and *o*-$C_6H_4(CH_2O)_2P$), 6.35 (d, J = 7.8 Hz, 1 H, NH′), 6.19 (d, J = 8.9 Hz, 1 H, NH), 5.54 (dd, J = 9.3, 9.3 Hz, 1 H, H-3′), 5.28–4.99 (m, 8 H, H-3, H-1′, β-CH of C3′-*O*-acyl, β-CH of C2′-*N*-acyl and *o*-$C_6H_4(CH_2O)_2P$), 4.63–4.46 (m, 9 H, $PhCH_2 \times 4$ and H-4′), 4.42 (d, J = 8.3 Hz, 1 H, H-1), 4.15–3.97 (m, 2 H, H-2 and H-6a), 3.84–3.76 (m, 4 H, H-6a′,b′ and OCH_2CHO), 3.72–3.45 (m, 7 H, H-4, H-5, H-6b, H-2′, H-5′, β-CH of C3-*O*-acyl and β-CH of C2-*N*-acyl), 2.68–2.60 (m, 2 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.55 (dd, J = 15.4, 7.0 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.43 (dd, J = 16.2, 4.6 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.40-2.21 (m, 8 H, α-CH_2 of acyl), 1.58–1.43 (m, 12 H, $CH_2 \times 6$), 1.28–1.24 (m, 108 H, $CH_2 \times 54$), 0.88 (t, J = 6.8 Hz, 18 H, $CH_3 \times 6$).

2-Hydroxyethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (35). To a solution of **34** (25.0 mg, 10.6 μmol) in 2-propanol/MeOH/CH_2Cl_2 (5:1:1, 1.4 mL) was added $NaBH_4$ (0.4 mg, 11 μmol) at 0 °C. After the mixture was stirred for 1h, the reaction was quenched by the addition of saturated aqueous NH_4Cl, and the mixture was extracted with EtOAc. The organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (10 g, $CHCl_3$/acetone = 5:1) to give **35** as a white solid (21.0 mg, 84%). $[\alpha]_D^{27}$ −1.7 (*c* 0.59, $CHCl_3$). Found: C, 67.87; H, 8.96; N, 1.22%. Calcd for $C_{134}H_{209}F_6N_2O_{23}P$: C, 68.17; H, 8.92; N, 1.19%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.49 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.37–7.13 (m, 16 H, $PhCH_2 \times 2$, CF_3PhCH_2– and *o*-$C_6H_4(CH_2O)_2P$), 6.66 (d, J = 7.6 Hz, 1 H, NH′), 6.02 (d, J = 9.2 Hz, 1 H, NH), 5.59 (dd, J = 9.3, 9.3 Hz, 1 H, H-3′), 5.17–5.00 (m, 8 H, H-3, H-1′, β-CH of C3′-*O*-acyl, β-CH of C2′-*N*-acyl and *o*-$C_6H_4(CH_2O)_2P$), 4.61–4.41 (m, 10 H, $PhCH_2 \times 4$, H-1 and H-4′), 4.12–4.03 (m, 2 H, H-2 and H-6a), 3.84–3.67 (m, 5 H, H-6b, H-5′, H-6a′, β-CH of C3-*O*-acyl and β-CH of C2-*N*-acyl), 3.65–3.42 (m, 8 H, H-4, H-5, H-2′, H-6b′ and $-O(CH_2)_2OH$), 2.64–2.61 (m, 2 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.53 (dd, J = 16.1, 7.7 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.45–2.24 (m, 9 H, α-CH_2 of acyl), 1.57–1.41 (m, 12 H, $CH_2 \times 6$), 1.35–1.20 (m, 108 H, $CH_2 \times 54$), 0.88 (m, 18 H, $CH_3 \times 6$).

2-(1,5-Dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yloxy)ethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyrano-

side (36). To a solution of **35** (21.0 mg, 8.89 μmol) in anhydrous CH_2Cl_2 (1 mL) were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (6.38 mg, 26.7 μmol) and 1*H*-tetrazole (1.87 mg, 26.7 μmol). After the mixture was stirred at room temperature for 1 h, mCPBA (3.77 g, 21.8 mmol) was added at −20 °C and stirring was continued for another 30 min. The reaction was quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and worked up as usual. The residue was purified by silica-gel column chromatography (15 g, $CHCl_3$/acetone = 10:1) to give **36** as colorless oil (22.4 mg, 99%). ESI-MS (positive) *m/z* 2564.3 [(M + Na)$^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.55 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.48 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2), 7.43 (d, J = 8.2 Hz, 2 H, CF_3PhCH_2–), 7.37 (d, J = 7.1 Hz, 2 H, CF_3PhCH_2–), 7.33–7.15 (m, 18 H, $PhCH_2$ × 2, CF_3PhCH_2– and *o*-$C_6H_4(CH_2O)_2P$), 6.78 (d, J = 8.0 Hz, 1 H, NH′), 6.41 (d, J = 9.1 Hz, 1 H, NH), 5.52 (dd, J = 9.1, 9.1 Hz, 1 H, H-3′), 5.24–4.95 (m, 12 H, H-3, H-1′, β-CH of C3′-*O*-acyl, β-CH of C2′-*N*-acyl and *o*-$C_6H_4(CH_2O)_2P$ × 2), 4.61–4.44 (m, 10 H, PhCH_2 × 4, H-1 and H-4′), 4.24–4.20 (m, 2 H, OCH_2CH_2OP), 4.10 (ddd, J = 8.9, 8.9, 8.0 Hz, 1 H, H-2), 4.03 (d, J = 11.0 Hz, 1 H, H-6a), 3.87–3.67 (m, 7 H, H-6b, H-5′, H-6a′,b′, OCH_2CH_2OP, β-CH of C3-*O*-acyl and β-CH of C2-*N*-acyl), 3.58–3.52 (m, 2 H, H-5 and H-2′), 3.05 (m, 1 H, OCH_2CH_2OP), 2.65–2.61 (m, 2 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.52 (dd, J = 16.3, 7.8 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.41–2.23 (m, 9 H, α-CH_2 of acyl), 1.68–1.40 (m, 12 H, CH_2 × 6), 1.35–1.20 (m, 108 H, CH_2 × 54), 0.88 (t, J = 6.9 Hz, 18 H, CH_3 × 6).

2-(Phosphonooxy)ethyl 2-Deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranoside (β-PE-506, 7). To a solution of **36** (20.0 mg, 7.86 μmol) in anhydrous THF (2 mL) was added Pd-black (150 mg). The mixture was stirred under 13 kg cm^{-2} of hydrogen at room temperature for 2 d. After removal of the Pd catalyst by filtration, the solvent was evaporated in vacuo. The crude product was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/MeOH/water/2-propanol = 15:15:13:2). The organic layer was the stationary phase, and the aqueous layer was the mobile phase in this chromatography. Removal of the solvent in vacuo followed by lyophilization gave **7** as a colorless solid (10.7 mg, 74%). ESI-MS (negative) *m/z* 1841.4 [(M − H)$^-$]. ^{1}H NMR (500 MHz, $CDCl_3$: MeOH = 3:2) δ 5.22 (m, 1 H, β-CH of C3′-*O*-acyl), 5.16 (m, 1 H, β-CH of C2′-*N*-acyl), 5.10 (t, 1 H, H-3′), 5.05 (t, 1 H, H-3), 4.67 (m, 1 H, H-1), 4.53 (m, 1 H, H-1′), 4.27 (m, 1 H, H-4′), 4.18–3.89 (m, 9 H, OCH_2CH_2OP, OCH_2CH_2OP, H-6a, H-6a′, H-2, H-2′, β-CH of C3-*O*-acyl and β-CH of C2-*N*-acyl), 3.88 (m, 1 H, H-6b), 3.78 (m, 1 H, H-6b′), 3.67 (m, 1 H, H-4), 3.60 (m, 1 H, OCH_2CH_2OP), 3.50 (m, 1 H, H-5), 3.38 (m, 1 H, H-5′), 2.72 (dd, 1 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.65 (dd, 1 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.53–2.47 (m, 2 H, α-CH_2 of C2-*N*-acyl's main chain and α-CH_2 of C2′-*N*-acyl's main chain), 2.44–2.37 (m, 2 H, α-CH_2 of C2-*N*-acyl's main chain and α-CH_2 of C2′-*N*-acyl's main chain), 2.36–2.24 (m, 6 H, α-CH_2 of acyl), 1.68–1.40 (m, 12 H, CH_2 × 6), 1.39–1.12 (m, 108 H, CH_2 × 54), 0.88 (t, 18 H, CH_3 × 6).

4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyloxyacetic Acid (37). To a solution of the aldehyde **34** (63.0 mg, 26.7 μmol), NaH_2PO_4 (3.20 mg, 26.7 μmol), and 2-methyl-2-butene (14.2 μL, 134 μmol) in water/2-methyl-2-propanol (1:4, 4.5 mL) was added $NaClO_2$ (80%, 9.0 mg, 80 μmol) at room temperature. After being stirred for 12 h, the reaction mixture was acidified with hydrochloric acid (1 mol dm^{-3}) and extracted with EtOAc. The organic layer was worked up as usual. The residue was purified by silica-gel column chromatography (20 g, $CHCl_3$/acetone = 10:1) to give **37** as colorless oil (41.2 mg, 65%). ESI-MS (positive) *m/z* 2396.3 [(M + Na)$^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.50 (d, J = 7.4 Hz, 2 H, CF_3PhCH_2–), 7.49 (d, J = 7.9 Hz, 2 H, CF_3PhCH_2–), 7.44 (d, J = 7.1 Hz, 2 H, CF_3PhCH_2–), 7.34–7.09 (m, 16 H, $PhCH_2$ × 2, CF_3PhCH_2– and *o*-$C_6H_4(CH_2O)_2P$), 6.81 (brs, 1 H, NH′), 6.35 (brs, 1 H, NH), 5.55 (brs, 1 H, H-3′), 5.25–4.89 (m, 8 H, H-3, H-1′, β-CH of C3′-*O*-acyl, β-CH of C2′-*N*-acyl and *o*-$C_6H_4(CH_2O)_2P$), 4.60–4.47 (m, 10 H, PhCH_2 × 4, H-1 and H-4′), 4.28–4.00 (m, 4 H, H-2, H-6a, H-6a′ and OCH_2COOH), 3.81–3.65 (m, 7 H, H-6b, H-5′, H-6b′, H-2′ OCH_2COOH, β-CH of C3-*O*-acyl and β-CH of C2-*N*-acyl), 3.58–3.50 (m, 2 H, H-4 and H-5), 2.64-2.18 (m, 10 H, α-CH_2 of acyl), 1.57–1.54 (m, 12 H, CH_2 × 6), 1.45–1.29 (m, 108 H, CH_2 × 54), 0.88 (td, J = 7.1, 2.0 Hz, 18 H, CH_3 × 6).

2-Deoxy-6-*O*-{2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-4-*O*-phosphono-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl}-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyloxyacetic Acid (β-CM-506, 9). In a manner similar to the synthesis of **7**, **37** (20.0 mg, 8.42 μmol) was deprotected. The crude product was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/MeOH/water/2-propanol = 7:7:6:1) to yield **9** as white powder (10.3 mg, 69%). ESI-MS (negative) *m/z* 887.1 [(M − 2H)$^{2-}$]. ^{1}H NMR (500 MHz, $CDCl_3$:MeOH = 1:1) δ 5.25–5.09 (m, 3 H, H-3′, β-CH of C3′-*O*-acyl and β-CH of C2′-*N*-acyl), 5.01 (dd, J = 9.8, 9.8 Hz, 1 H, H-3), 4.59–4.52 (m, 2 H, H-1 and H-1′), 4.28 (m, 1 H, H-4′), 4.22 (d, J = 18.0 Hz, 1 H, OCH_2COOH), 4.11 (d, J = 18.0 Hz, 1 H, OCH_2COOH), 4.05–3.96 (m, 3 H, H-2, H-6a and β-CH of C2-*N*-acyl), 3.95–3.84 (m, 3 H, H-2′, H-6b and β-CH of C3-*O*-acyl), 3.77 (m, 1 H, H-6a′), 3.63 (m, 1 H, H-4), 3.51–3.46 (m, 2 H, H-5 and H-6b′), 3.38 (m, 1 H, H-5′), 2.72 (dd, J = 18.6, 7.3 Hz, 1 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.64 (dd, J = 18.6, 5.4 Hz, 1 H, α-CH_2 of C3′-*O*-acyl's main chain), 2.54–2.37 (m, 4 H, α-CH_2 of C2′-*N*-acyl's main chain and α-CH_2 of C2-*N*-acyl's main chain), 2.36–2.28 (m, 5 H, α-CH_2 of acyl), 2.23 (dd, J = 14.7, 9.0 Hz, 1 H, α-CH_2 of C3-*O*-acyl's side chain), 1.68–1.40 (m, 12 H, CH_2 × 6), 1.39–1.20 (m, 108 H, CH_2 × 54), 0.89 (t, J = 7.0 Hz, 18 H, CH_3 × 6).

Allyl 6-*O*-{3-*O*-Allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-4-*O*-benzyl-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (38). In a manner similar to the synthesis of **32**, removal of Troc group of the common synthetic intermediate **13** (80.3 mg, 45.2 μmol) and subsequent acylation gave **38** as a white solid (68.8 mg, 77%). $[\alpha]_D^{25}$ −3.5 (*c* 0.37, $CHCl_3$). Found: C, 64.27; H, 7.12; N, 1.14%. Calcd for $C_{107}H_{144}F_9N_2O_{20}P\cdot 1H_2O$: C, 64.31; H, 7.36; N, 1.40%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.58 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.48 (d, J =

8.1 Hz, 2 H, CF_3PhCH_2–), 7.45 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.42 (d, J = 8.1 Hz, 2 H, CF_3PhCH_2–), 7.34–7.14 (m, 16 H, $PhCH_2$, CF_3PhCH_2– and o-$C_6H_4(CH_2O)_2P$), 6.34 (d, J = 8.1 Hz, 1 H, NH′), 5.88–5.82 (m, 2 H, NH and CH_2CH=CH_2 of Alloc), 5.70 (m, 1 H, OCH_2CH=CH_2), 5.36–5.22 (m, 2 H, H-3′ and CH_2-CH=CH_2 of Alloc), 5.20–5.17 (m, 2 H, CH_2CH=CH_2 of Alloc, OCH_2CH=CH_2), 5.11–5.04 (m, 6 H, H-3, N-H′ and o-C_6H_4-$(CH_2O)_2P$), 4.82 (d, J = 8.2 Hz, 1 H, H-1′), 4.62–4.48 (m, 13 H, $PhCH_2$ × 5, CH_2CH=CH_2 of Alloc and H-4′), 4.30–4.19 (m, 2 H, H-1 and OCH_2CH=CH_2), 4.04–3.96 (m, 2 H, H-2 and H-6a), 3.90 (dd, J = 13.2, 6.1 Hz, 1 H, OCH_2CH=CH_2), 3.83–3.76 (m, 3 H, H-6a′,b′ and β-CH of C2-*N*-acyl), 3.75–3.62 (m, 5 H, H-2′, H-5′, H-6b and β-CH × 2), 3.53 (dd, 1 H, J = 8.9, 8.9 Hz, 1 H, H-4), 3.46 (m, 1 H, H-5), 2.51 (dd, J = 16.0, 7.7 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.39 (dd, J = 16.0, 4.6 Hz, 1 H, α-CH_2 of C2-*N*-acyl's main chain), 2.36–2.33 (m, 2 H, α-CH_2 of C2′-*N*-acyl's main chain), 2.27–2.25 (m, 2 H, α-CH_2 of C3-*O*-acyl's main chain), 1.55–1.22 (m, 60 H, CH_2 × 30), 0.88 (t, J = 6.9 Hz, 9 H, CH_3 × 3).

Allyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (15). In a manner similar to the synthesis of **14**, 3′-*O*-alloc group in **38** (220 mg, 111 μmol) was removed to give the 3′-hydroxy compound (210 mg, 100%), which was then acylated to give **15** as a white solid (197 mg, 86%). Mp 110–113 °C. $[\alpha]_D^{26}$ −6.5 (c 1.00, $CHCl_3$). Found: C, 65.97; H, 7.67; N, 1.55%. Calcd for $C_{125}H_{171}F_{12}N_2O_{20}P$: C, 65.83; H, 7.56; N, 1.23%. ESI-MS (positive) m/z 2303.0 [$(M + Na)^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.60–7.57 (m, 4 H, CF_3PhCH_2–), 7.51–7.42 (m, 10 H, CF_3PhCH_2–), 7.34–7.11 (m, 15 H, $PhCH_2$ × 2, CF_3PhCH_2– and o-$C_6H_4(CH_2O)_2P$), 6.75 (d, J = 7.3 Hz, 1 H, o-$C_6H_4(CH_2O)_2P$), 6.09 (d, J = 8.8 Hz, 1 H, NH′), 5.83 (d, J = 9.4 Hz, 1 H, NH), 5.70 (m, 1 H, OCH_2CH=CH_2), 5.32 (dd, J = 9.3, 9.3 Hz, 1 H, H-3′), 5.20 (dd, J = 17.4, 1.6 Hz, 1 H, OCH_2-CH=CH_2), 5.07–4.65 (m, 6 H, H-3, OCH_2CH=CH_2 and o-C_6H_4-$(CH_2O)_2P$), 4.63–4.45 (m, 14 H, $PhCH_2$ × 6, H-4′ and H-1′), 4.27 (d, J = 8.2 Hz, 1 H, H-1), 4.23 (dd, J = 16.9, 3.7 Hz, 1 H, OCH_2CH=CH_2), 4.01 (dd, J = 9.3, 9.3 Hz, 1 H, H-2), 3.95–3.85 (m, 7 H, OCH_2CH=CH_2, H-6a, H-6a′,b′, H-2′ and β-CH × 2), 3.72–3.61 (m, 3 H, H-6b, H-5′ and β-CH), 3.53–3.44 (m, 3 H, H-4, H-5 and β-CH), 2.72 (dd, J = 16.8, 7.7 Hz, 1 H, α-CH_2 of acyl), 2.57-2.48 (m, 2 H, α-CH_2 of acyl), 2.44 (dd, J = 15.1, 7.1 Hz, 1 H, α-CH_2 of acyl), 2.31–2.17 (m, 3 H, α-CH_2 of acyl), 1.94 (d, J = 9.4 Hz, 1 H, α-CH_2 of acyl), 1.68–1.04 (m, 80 H, CH_2 × 40), 0.87 (m, 12 H, CH_3 × 4).

Formylmethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (39). In a manner similar to the preparation of **34**, the allyl glycoside of **15** (33.0 mg, 14.5 μmol) was oxidatively cleaved to give colorless oil **39** (26.4 mg, 80%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.52 (s, 1 H, OCH_2CHO), 7.60–7.11 (m, 29 H, $PhCH_2$ × 2, CF_3PhCH_2 × 4 and o-$C_6H_4(CH_2O)_2P$), 6.74 (d, J = 7.4 Hz, 1 H, o-$C_6H_4(CH_2O)_2P$), 6.40 (d, J = 8.7 Hz, 1 H, NH′), 5.92 (d, J = 9.0 Hz, 1 H, NH), 5.45 (dd, J = 9.4, 9.4 Hz, 1 H, H-3′), 5.06-4.89 (m, 5 H, H-3 and o-$C_6H_4(CH_2O)_2P$), 4.66–4.41 (m, 14 H, $PhCH_2$ × 6, H-4′ and H-1′), 4.20 (d, J = 8.6 Hz, 1 H, H-1), 4.07 (q, J = 9.1 Hz, 1 H, H-2), 4.00–3.80 (m, 6 H, H-6a, H-6a′,b′, H-2′ and β-CH × 2), 3.70-3.65 (m, 5 H, H-6b, H-5′ OCH_2CHO and β-CH), 3.59–3.32 (m, 3 H, H-4, H-5 and β-CH), 2.73 (dd, J = 17.0, 7.7 Hz, 1 H, α-CH_2 of acyl), 2.58 (dd, J = 17.0, 4.1 Hz, 1 H, α-CH_2 of acyl), 2.51 (dd, J = 16.1, 7.7 Hz, 1 H, α-CH_2 of acyl), 2.40 (dd, J = 16.1, 4.6 Hz, 1 H, α-CH_2 of acyl), 2.35–2.22 (m, 3 H, α-CH_2 of acyl × 3), 1.92 (d, J = 12.6 Hz, 1 H, α-CH_2 of acyl), 1.62–1.15 (m, 80 H, CH_2 × 40), 0.88 (m, 12 H, CH_3 × 4).

2-Hydroxyethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (40). In a manner similar to the synthesis of **35**, the aldehyde **39** (89.0 mg, 39.0 μmol) was reduced with $NaBH_4$ to give **40** as a white solid (74.8 mg, 84%). $[\alpha]_D^{26}$ −2.2 (c 0.81, $CHCl_3$). Found: C, 64.56; H, 7.49; N, 1.24%. Calcd for $C_{124}H_{171}F_{12}N_2O_{21}P\cdot 1H_2O$: C, 64.68; H, 7.57; N, 1.22%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58 (d, J = 8.1 Hz, 4 H, CF_3PhCH_2–), 7.50 (d, J = 13.1 Hz, 4 H, CF_3PhCH_2–), 7.37–7.10 (m, 21 H, $PhCH_2$ × 2, CF_3PhCH_2 × 2 and o-$C_6H_4(CH_2O)_2P$), 6.78 (d, J = 7.4 Hz, 1 H, NH′), 6.74 (d, J = 7.4 Hz, 1 H, o-$C_6H_4(CH_2O)_2P$), 5.77 (d, J = 9.2 Hz, 1 H, NH), 5.50 (dd, J = 9.2, 9.2 Hz, 1 H, H-3′), 5.05–4.90 (m, 5 H, H-3 and o-$C_6H_4(CH_2O)_2P$), 4.70 (d, J = 8.3 Hz, 1 H, H-1′), 4.66–4.39 (m, 13 H, $PhCH_2$ × 6 and H-4′), 4.19 (d, J = 8.5 Hz, 1 H, H-1), 4.04–3.98 (m, 2 H, H-2 and H-6a), 3.84–3.70 (m, 8 H, H-6a′, $O(CH_2)_2OH$, H-2′, H-5′ and β-CH), 3.58–3.42 (m, 6 H, H-6b, H-6b′, H-5′ and β-CH × 3), 3.30 (dd, J = 9.0, 9.0 Hz, 1 H, H-4), 2.71 (dd, J = 17.0, 7.8 Hz, 1 H, α-CH_2 of acyl), 2.53 (dd, J = 17.0, 4.3 Hz, 1 H, α-CH_2 of acyl), 2.51 (dd, J = 16.5, 7.6 Hz, 1 H, α-CH_2 of acyl), 2.39 (dd, J = 16.0, 4.6 Hz, 1 H, α-CH_2 of acyl), 2.31–2.27 (m, 3 H, α-CH_2 of acyl × 3), 2.21 (dd, J = 14.7, 4.1 Hz, 1 H, α-CH_2 of acyl), 1.70–1.24 (m, 80 H, CH_2 × 40), 0.87 (t, J = 6.9 Hz, 12 H, CH_3 × 4).

2-(1,5-Dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yloxy)ethyl 4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranoside (41). In a manner similar to the synthesis of **36**, the alcohol **40** (72.0 mg, 31.5 μmol) was phosphorylated to give **41** as colorless oil (76.2 mg, 98%). ESI-MS (positive) m/z 2488.2 [$(M + Na)^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58–7.54 (m, 4 H, CF_3PhCH_2–), 7.49–7.10 (m, 30 H, $PhCH_2$ × 2, CF_3PhCH_2 × 3, o-$C_6H_4(CH_2O)_2P$ × 2 and NH′), 6.70 (d, J = 7.4 Hz, 1 H, o-$C_6H_4(CH_2O)_2P$), 6.06 (d, J = 9.2 Hz, 1 H, NH), 5.46 (dd, J = 9.3, 9.3 Hz, 1 H, H-3′), 5.23–4.84 (m, 9 H, H-3 and o-$C_6H_4(CH_2O)_2P$ × 2), 4.83 (d, J = 8.3 Hz, 1 H, H-1′), 4.64–4.44 (m, 13 H, $PhCH_2$ × 6 and H-4′), 4.23 (m, 1 H, OCH_2CH_2OP), 4.18 (d, J = 8.2 Hz, 1 H, H-1), 4.05–4.00 (m, 2 H, H-2 and H-6a), 3.87–3.78 (m, 8 H, H-6a′,b′, H-6b, H-2′ and β-CH × 4), 3.74–3.68 (m, H-5′ and OCH_2CH_2OP), 3.65 (m, 1 H, OCH_2CH_2OP), 3.60 (dd, J = 11.2, 6.5 Hz, 1 H, H-5), 3.49 (m, 1 H, H-4), 3.06 (m, 1 H, OCH_2CH_2OP), 2.70 (dd, J = 17.0, 7.9 Hz, 1 H, α-CH_2 of

acyl), 2.53 (dd, J = 17.0, 4.1 Hz, 1 H, α-CH_2 of acyl), 2.49 (dd, J = 16.1, 7.7 Hz, 1 H, α-CH_2 of acyl), 2.38–2.30 (m, 3 H, α-CH_2 of acyl), 2.30–2.22 (m, 2 H, α-CH_2 of acyl), 1.82–1.23 (m, 80 H, $CH_2 \times 40$), 0.87 (t, J = 6.9 Hz, 12 H, $CH_3 \times 4$).

2-(Phosphonooxy)ethyl 2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranoside (β-PE-406, 8). In a manner similar to the synthesis of **7**, all benzyl-type protective groups of **41** (20.0 mg, 8.12 μmol) were removed by catalytic hydrogenation. The crude product was purified by liquid-liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/MeOH/water/2-propanol = 10:8:8:1) to yield **8** as white powder (5.99 mg, 51%). ESI-MS (negative) m/z 1447.98 [$(M - H)^-$]. ^{1}H NMR (600 MHz, **8** (1 mg) and SDS-d_{25} (19.3 mg) were dissolved in DMSO-d_6 (0.7 mL)) δ 7.87 (m, 1 H, NH), 7.84 (m, 1 H, NH′), 5.10 (t, 1 H, H-3′), 4.87 (t, 1 H, H-3), 4.58 (m, 1 H, H-1′), 4.53 (m, 1 H, H-1), 4.13 (m, 1 H, OCH_2CH_2OP), 4.09 (m, 1 H, H-4′), 4.04 (m, 1 H, H-6a), 3.85 (m, 1 H, OCH_2CH_2OP), 3.83–3.80 (m, 2 H, H-2′ and H-6a′), 3.76 (m, 1 H, OCH_2CH_2OP), 3.75–3.74 (m, 2 H, β-CH of acyl), 3.74 (m, 1 H, H-6b′), 3.70 (m, 1 H, H-2), 3.69 (m, 1 H, H-6b), 3.65 (m, 2 H, β-CH of acyl), 3.48 (m, 1 H, H-4), 3.46 (m, 2 H, OCH_2CH_2P and H-5′), 3.44 (m, 1 H, H-5), 2.40–1.96 (m, 8 H, α-CH_2 of acyl), 1.37–0.98 (m, 80 H, $CH_2 \times 40$), 0.78–0.64 (m, 12 H, $CH_3 \times 4$).

4-*O*-Benzyl-6-*O*-{6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3λ^5-3*H*-2,4,3-benzodioxaphosphepin-3-yl)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyl}-2-deoxy-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoyl]-2-[(*R*)-3-(4-trifluoromethylbenzyloxy)tetradecanoylamino]-β-D-glucopyranosyloxyacetic Acid (42). In a manner similar to the synthesis of **37**, the aldehyde **39** (47.0 mg, 20.6 μmol) was oxidized with $NaClO_2$ to give **42** (36.2 mg, 77%). ESI-MS (positive) m/z 2320.1 [$(M + Na)^+$]. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.58–7.19 (m, 29 H, $PhCH_2 \times 2$, $CF_3PhCH_2 \times 4$ and o-C_6H_4-$(CH_2O)_2P$), 6.94 (brs, 1 H, o-$C_6H_4(CH_2O)_2P$), 6.82 (brs, 1 H, NH′), 6.14 (brs, 1 H, NH), 5.58 (brs, 1 H, H-3′), 4.93–4.86 (m, 5 H, H-3 and o-$C_6H_4(CH_2O)_2P$), 4.63–4.44 (m, 14 H, H-1′, $PhCH_2 \times 6$ and H-4′), 4.28 (brs, 1 H, H-1), 4.14 (brs, 1 H, OCH_2COOH), 4.06–3.74 (m, 10 H, H-2, H-6a, H-6a′,b′, OCH_2COOH, H-2′, H-5′ and β-CH × 3), 3.65–3.56 (m, 2 H, H-5 and β-CH), 3.41 (brs, 1 H, H-4), 3.35 (brs, 1 H, H-6b), 2.69 (dd, J = 16.7, 7.6 Hz, 1 H, α-CH_2 of acyl), 2.55–2.24 (m, 7 H, α-CH_2 of acyl), 1.52–1.18 (m, 80 H, $CH_2 \times 40$), 0.88 (t, J = 6.9 Hz, 12 H, $CH_3 \times 4$).

2-Deoxy-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxytetradecanoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-(*R*)-3-hydroxytetradecanoylamino]-β-D-glucopyranosyloxyacetic Acid (β-CM-406, 10). In a manner similar to the synthesis of **7**, all benzyl-type protective groups of **42** (25.0 mg, 10.9 μmol) were removed by catalytic hydrogenation. The crude product was purified by liquid–liquid partition column chromatography (20 g of Sephadex® LH-20, $CHCl_3$/MeOH/water/2-propanol = 10:9:8:1) to yield **10** as white powder (8.1 mg, 54%). ESI-MS (negative) m/z 690.5 [$(M - 2H)^{2-}$]. ^{1}H NMR (600 MHz, **10** (1 mg) and SDS-d_{25} (19.3 mg) were dissolved in DMSO-d_6 (0.7 mL)) δ 7.80 (NH′ and NH), 5.05 (t, 1 H, H-3′), 4.85 (t, 1 H, H-3), 4.52 (m, 1 H, H-1), 4.50 (m, 1 H, H-1′), 4.15 (m, 1 H, H-6a), 4.03 (m, 2 H, CH_2COOH and H-6b), 4.02 (m, 1 H, H-4′), 3.97 (m, 1 H, H-6a′), 3.80 (m, 2 H, β-CH of acyl), 3.76 (m, 2 H, H-2′ and CH_2COOH), 3.74 (m, 2 H, β-CH of acyl), 3.68 (m, 2 H, H-2), 3.61 (m, 1 H, H-6b′), 3.35 (m, 1 H, H-4), 3.26 (m, 1 H, H-5), 3.25 (m, 1 H, H-5′), 2.66–2.01 (m, 8 H, α-CH_2 of acyl), 1.51–1.00 (m, 80 H, $CH_2 \times 40$), 0.88–0.77 (m, 12 H, $CH_3 \times 4$).

Cytokine Induction in Human Peripheral Whole-Blood Cell Cultures. An aqueous 5% DMSO solution (50 μg/mL) of each sample was prepared by dilution of DMSO solution (1 mg/mL) with saline (Otsuka Pharmaceuticals Co., Ltd.). Each sample was further diluted stepwise with saline on a 96-well plastic plate (#2870-096, Iwaki Glass Co., Ltd). The mixture consisting of test sample (25 μL), RPMI 1640 medium (75 μL; Flow Laboratories, Irvine, Scotland), and heparinized human peripheral whole-blood (25 μL) collected from an adult volunteer was incubated in triplicate at 37 °C in 5% CO_2 for 24 h. The plate was centrifuged at 300 × g for 2 min. The levels of TNFα and IL-6 in culture supernatant were measured by means of enzyme-linked immunosorbent assay (ELISA) as described in the previous paper.

Inhibition Assay in Cytokine Induction. The mixture consisting of test sample (25 μL) prepared in the same manner as above, LPS (25 μL, *E. coli* 0111:B4; Sigma Chemicals Co.), RPMI 1640 medium (75 μL; Flow Laboratories, Irvine, Scotland), and heparinized human peripheral whole-blood (25 μL) collected from an adult volunteer was incubated in triplicate at 37 °C in 5% CO_2 for 24 h. The plate was centrifuged at 300 × g for 2 min. The levels of TNFα and IL-6 in culture supernatant were measured by means of enzyme-linked immunosorbent assay (ELISA) as described in the previous paper.

***Limulus* Assay.** DMSO solution of each sample (1 mg/mL) was diluted stepwise with distilled water (Otsuka Pharmaceuticals Co., Ltd.) on a 96-well plastic plate (#2870-096, Iwaki Glass Co., Ltd). *Limulus* activity was measure by means of Endospecy Test® (Seikagaku Kogyo).

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