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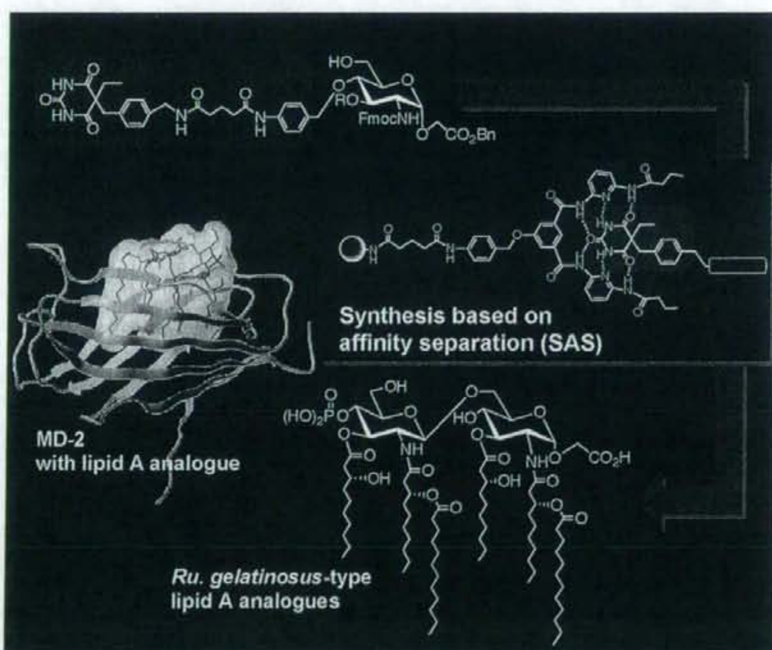

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Synthesis of *Rubrivivax gelatinosus* Lipid A and Analogues for Investigation of the Structural Basis for Immunostimulating and Inhibitory Activities

Yoshiyuki Fukase,[1] Yukari Fujimoto,*[1] Yo Adachi,[1] Yasuo Suda,[2] Shoichi Kusumoto,[1,3] and Koichi Fukase*[1]

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

[2]Department of Nanostructure and Advanced Materials, Graduate School of Science and Engineering, Kagoshima University, Kagoshima 890-0065

[3]Suntory Institute for Bioorganic Research, Shimamoto-cho, Mishima-gun, Osaka 618-8503

Received December 3, 2007; E-mail: yukarif@chem.sci.osaka-u.ac.jp

To elucidate the structural requirements for the endotoxic and antagonistic activities of lipid A derivatives, we have focused on the effects of the acyl moieties and acidic groups at the 1- and 4′-positions in the present study. We have synthesized new analogues corresponding to *Rubrivivax gelatinosus* lipid A, which has a characteristic symmetrical distribution of its acyl groups on its two glucosamine residues with shorter acyl groups (decanoyl groups (C_{10}) and lauryl groups (C_{12})) than *Escherichia coli* lipid A's. Carboxymethyl (CM) analogues in which one of the phosphates was replaced with a CM group were also synthesized with a different distribution of acyl groups. Biological tests revealed that the acyl group distribution in the lipid A analogue, strongly affected its bioactivity. The synthetic *Ru. gelatinosus* type lipid A showed potent antagonistic activity against LPS, whereas its 1-*O*-carboxymethyl analogue showed weak endotoxic activity. These results demonstrate that when lipid A has shorter (C_{10} and C_{12}) hexa-acyl groups, its bioactivity is more easily affected by small structural differences, such as differences in acidic groups or acyl group distribution, and that they can change bioactivity from endotoxic to agonistic or vice versa at this structural boundary for the bioactivity.

The innate immune system is a phylogenetically ancient defense mechanism conserved between plants and animals.[1–5] One of the important roles of innate immunity is the detection of invading pathogens (bacteria, fungi, viruses, etc.) through innate immune receptors that recognize characteristic structures that are present in microorganisms, called PAMPs (pathogen-associated molecular patterns). PAMPs are essential molecules for pathogens that are not found within the host. In vertebrates, two diverse families of receptors, i.e., the Toll-like receptor (TLR) and Nod-like receptor (NLR) families, detect PAMPs such as the bacterial cell wall peptidoglycan (PGN), lipopolysaccharide (LPS) of Gram-negative bacteria, lipoproteins, bacterial DNA, viral RNA, etc. to activate the immune system. Most PAMPs therefore show immunostimulating activity.

LPS is a cell surface glycoconjugate of Gram-negative bacteria that is also known as endotoxin,[6–12] and is sensed by a receptor complex consisting of TLR4 and its adaptor protein MD-2. Via this complex, LPS stimulates immunocompetent cells such as macrophages and monocytes to produce a variety of mediators, e.g., cytokines, prostaglandins, the platelet activating factor, oxygen free radicals, and NO. These mediators activate and modulate the immune system. If too much LPS is released during a severe Gram-negative bacterial infection, the overproduction of these mediators can lead to endotoxin-related symptoms such as high fever, serious inflammation, hypotension, and, in serious cases, lethal shock.

LPS consists of a glycolipid component termed lipid A that is covalently bound to a polysaccharide. It was unequivocally proved that lipid A is the chemical entity responsible for the biological activity of LPS by the total synthesis of *Escherichia coli* lipid A **1** (synthetic **1** is termed 506) (Figure 1) in 1984.[13,14] Lipid A specimens from various bacterial origins were shown to be closely related structurally and to consist of: 1) $\beta(1\rightarrow6)$ disaccharide of D-glucosamines, 2) phosphono groups at their reducing ends and the 4-position of their non-reducing glucosamines, and 3) long-chain acyl groups bound at 2, 2′, 3, and 3′ positions.

The recognition of LPS and lipid A by the TLR4/MD-2 receptor complex has been of major interest in the endotoxin research field.[1–12] To further our understanding of this issue, we investigated the precise structural requirements for lipid A biological activity. It has been previously shown that the acidic

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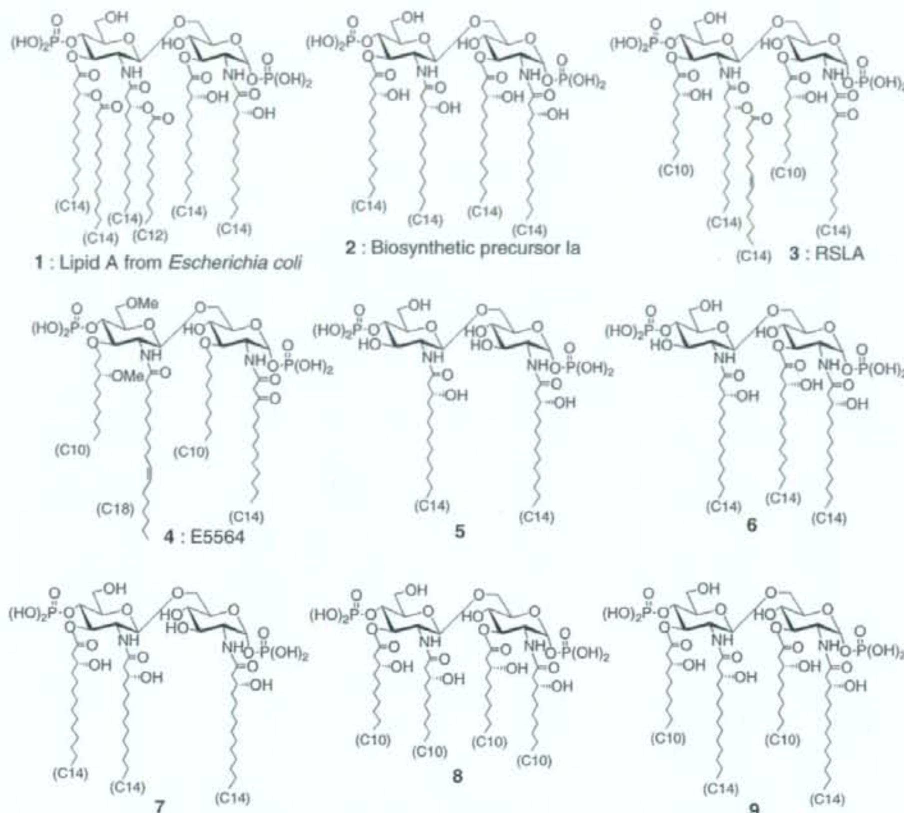


Figure 1. Structures of lipid A and its analogues with various acyl groups.

functional groups and acyl groups of lipid A are crucial for its biological activity.[15–18] Additionally, its phosphate groups can be replaced by other acidic groups (such as carboxylic acid) without decisively influencing its biological activity.[19–21] In contrast, the structure, number, and chain length of the acyl groups can dramatically influence its biological activity. The tetraacyl biosynthetic precursor of lipid A **2** (synthetic **2** is termed 406) has weaker but clear endotoxic activity in mice, but quite interestingly it also acts as an antagonist to LPS and lipid A **1** in human systems.[16,17] *Rhodobacter sphaeroides* lipid A **3** (RSLA) also shows species-related antagonistic or agonistic activities in different mammalian hosts.[22] The synthetic compounds E5531 and E5564 (Eritoran) **4** exhibit potent antagonistic activity in human systems.[23,24] RSLA, E5531, and E5564 each have acyl groups containing unsaturated and 2-keto acyl groups. E5564 is currently under development as a possible clinical therapeutic for the treatment of sepsis and septic shock. The *N,N'*-diacyl analogue **5** does not show any activity, but the triacyl-type analogues **6** and **7**, which lack acyl groups at the 3- and 3′-O-positions, show a weak but definite ability to inhibit the induction of IL-6 by LPS. Precursor-type analogues with shorter acyl chains have also been synthesized.[25] Analogue **9**, which possesses two (*R*)-3-hydroxytetradecanoic acids at the 2- and 2′-N-positions and two (*R*)-3-hydroxydecanoic acids at the 3- and 3′-O-positions, shows definite but ca. 10–100 times less potent antagonistic activity than natural-type **2**; whereas analogue **8**, which possesses four (*R*)-3-hydroxydecanoic acids, does not show this activity. On the other hand, Boons et al. revealed that an *E. coli* lipid A analogue had shorter lipids (two C14 and four C12 acids) that were ca. 100 times more active than *E. coli* lipid A.[26] They also synthesized heptaacylated *Salmonella typhimurium* lipid A, which showed much weaker activity than *E. coli* lipid A, and using its short-chain analogue they obtained similar results.

In this study, we focused on the structural requirements for the endotoxic and antagonistic activities of lipid A derivatives, and in particular, their effects on the human innate immune system. We particularly considered the effects of the acyl and acidic groups, and thus prepared and analyzed various structural analogues, including some with different numbers and distributions of acyl moieties on the lipid A backbone and some with a carboxymethyl group instead of a phosphate group.

Rubrivivax gelatinosus-type lipid A **10a** and **10b** (Figure 2) has shorter acyl groups than *E. coli* lipid A **1**, and a symmetrical $(3 + 3)$ acylation distribution. It was reported that natural lipid A isolated from *Ru. gelatinosus* showed endotoxic activity.[27] By contrast, *Chromobacterium violaceum* lipid A **11** has acyl groups that are similar to *Ru. gelatinosus* and shows antagonistic activity.[28] The only structural difference between **10** and **11** is the chain lengths of three acyl groups. Since

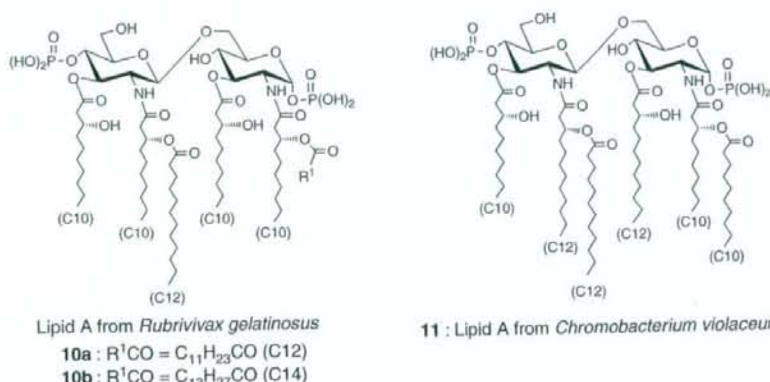
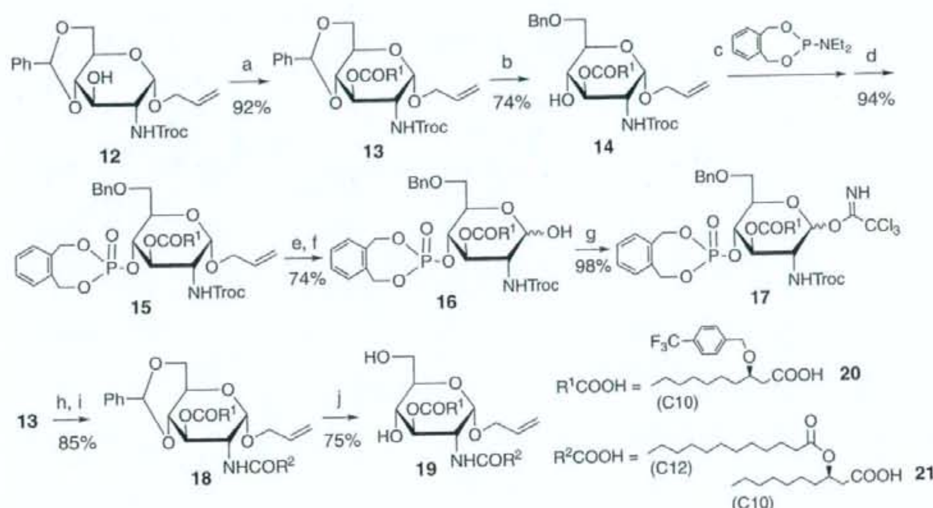


Figure 2. Structures of two natural lipid A molecules that each have six acyl groups with symmetrical distributions.



Scheme 1. Reagents and conditions: (a) R^1COOH (**20**), DCC, DMAP, CH_2Cl_2, rt, 17 h; (b) $BF_3 \cdot Et_2O$, Et_3SiH, CH_3CN, 0 °C, 1.5 h; (c) 1*H*-tetrazole, CH_2Cl_2, rt, 50 min; (d) *m*CPBA, −20 °C, 20 min; (e) $[Ir(cod)(MePh_2P)_2]PF_6$, H_2, THF, 2 h; (f) I_2, H_2O, rt, 30 min; (g) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 2 h; (h) Zn–Cu couple, AcOH, rt, 3 h; (i) R^2COOH (**21**), DCC, CH_2Cl_2, rt, 2 h; (j) TFA, H_2O, CH_2Cl_2, 0 °C, 2.5 h.

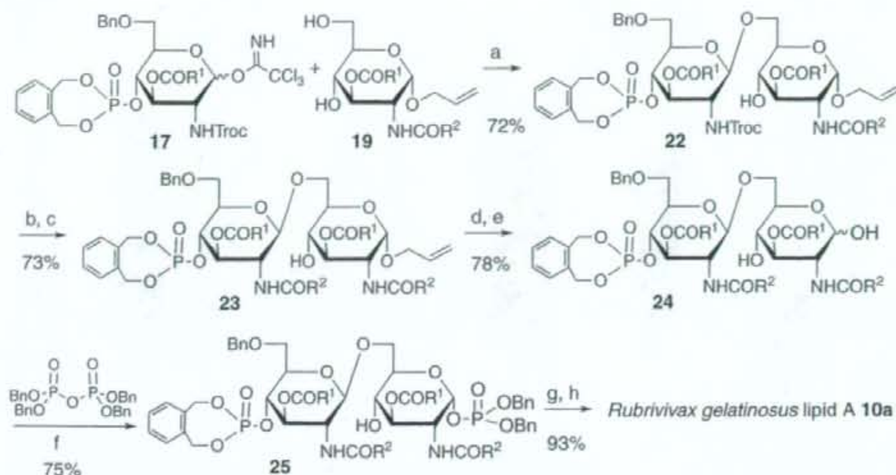
we were interested in the structural requirement for the endotoxic/antagonistic activity of lipid A with symmetrical (3 + 3) acylation distribution, *Ru. gelatinosus* lipid A **10a** was synthesized and analyzed in the present study.

Results

The Synthesis and Biological Activity of *Ru. gelatinosus* Lipid A. We have established the efficient synthesis of lipid A and analogues in our previous studies.[20,21,25,29] In the present study, *Ru. gelatinosus* lipid A **10a** was synthesized using a similar strategy (Scheme 1). The hydroxy and phosphate groups were protected with benzyl-type protective groups, which were removed by catalytic hydrogenation in the last step. The $\beta(1\rightarrow6)$ disaccharide structures were constructed by glycosylation of the glycosyl acceptor **19** with the *N*-Troc trichloroacetimidate donor **17** (Troc = 2,2,2-trichloroethoxycarbonyl). A Lewis acid catalyzed activation was used for the glycosylation with the trichloroacetimidate **17**.[30]

The glycosyl donor **17** and the glycosyl acceptor **19** were synthesized as shown in Scheme 1. The hydroxy group at the 3-position of 1-*O*-allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside[20] (**12**) was acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)-decanoic acid (**20**). An unsubstituted benzyl group used for protecting the hydroxy function on the 3-hydroxyacyl residue proved to be prone to air-oxidation and gradually transformed into a corresponding benzoyl group. The *p*-trifluoromethylbenzyl group at this position was resistant to oxidation, but was readily removable by conventional hydrogenolysis.[21,30–33] Regioselective reductive opening of the benzylidene of **13** with $BF_3 \cdot OEt_2$ and Et_3SiH gave the 6-*O*-benzyl-4-OH GlcN derivative **14**.[34]

The free 4-hydroxy group of **14** was treated with Watanabe's reagent and 1-*H*-tetrazole, and then with *m*-chlo-



Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, MS4A, −20 °C, 1 h; (b) Zn, AcOH, rt, 1.5 h; (c) R^2COOH (**21**), WSCD·HCl, HOBt, CH_2Cl_2, rt, 21 h; (d) $[Ir(cod)(MePh_2P)_2]PF_6$, H_2, THF, rt, 2 h; (e) I_2, H_2O, rt, 1 h; (f) $LiN(TMS)_2$, THF, −78 °C, 1.5 h; (g) H_2 (20 kg cm^{-2}), Pd-black, THF, rt, 44 h; (h) liquid–liquid partition column chromatography using Sephadex LH-20, $CHCl_3$–MeOH–H_2O–*i*PrOH (8:8:6:1).

roperbenzoic acid (*m*CPBA) to furnish the phosphate **15** in 94% yield.[35] The 1-*O*-allyl group of **15** was removed via isomerization to a 1-propenyl group and subsequently treated with iodine.[36] The resulting 1-OH sugar **16** was then transformed into the glycosyl trichloroacetimidate **17** by treatment with CCl_3CN and Cs_2CO_3.[37]

The glycosyl acceptor **19** was synthesized as follows. The 2-*N*-Troc group of **13** was removed using Zn–Cu and acetic acid and the resulting 2-amino group was then acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to give the 2,3-diacyl derivative **18**. Deprotection of the benzylidene group of **18** under the acidic conditions gave the glycosyl acceptor **19**.

Glycosylation of the above glycosyl acceptor **19** with the glycosyl donor **17** gave the desired $\beta(1\to6)$ disaccharide **22** in 72% yield (Scheme 2). The 2′-*N*-Troc group of **22** was cleaved and the resulting amino group was acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to give the fully acylated compound **23**. The allyl group at the 1-position of **23** was cleaved via isomerization to a vinyl group with an iridium complex to give **24** in 78% yield. After selective phosphorylation at the anomeric position with tetrabenzyl pyrophosphate, all the benzyl-type protecting groups in **25** were removed by catalytic hydrogenolysis to give the desired *Ru. gelatinosus* lipid A **10a**.

The biological activities of **10a** were evaluated in comparison to the corresponding LPS (*E. coli* O111:B4) by measuring typical endotoxic activity such as *Limulus* activity and cytokine induction. Cytokine inducing activity was tested in human peripheral whole-blood cells.[38] 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 supernatants of incubated mixtures were measured using an enzyme-linked immunosorbent assay (ELISA). Antagonistic activity was examined and compared to the tetraacyl biosynthetic precursor of lipid A (compound 406)

Table 1. The *Limulus* Activity of **10a**, **26a**–**26f**, and LPS (*E. coli* 0111:B4), as Tested Using an Endospecy Test® (Seikagaku Corporation, Tokyo)

	ED50/pg mL^{-1}
Ru. gelatinosus lipid A (**10a**)	10
CM analogue **26a**	5000
26b	10000<
26c	—
26d	10000<
26e	50
26f	50
LPS (*E. coli* 0111:B4)	50

2 using an assay that measured the ability of a compound to inhibit LPS-induced cytokine production as follows. Samples, LPS (10 ng mL^{-1}) (*E. coli* O111:B4; Sigma Chemicals Co.), and heparinized human peripheral whole blood were mixed and incubated, and the levels of IL-6 and TNF-α were measured (as described above).

The *Limulus* activity, the hemolymph coagulation activity on horseshoe crab amoebocyte lysates, was evaluated by the activation of factor *C* at various concentrations using an Endospecy Test® (Seikagaku Corporation, Tokyo) with *E. coli* O111:B4 LPS as a positive standard. As clearly seen in Table 1, *Ru. gelatinosus* lipid A **10a** showed potent *Limulus* activity that was comparable to *E. coli* LPS.

Ru. gelatinosus lipid A **10a** showed no cytokine inducing activity, but a potent ability to antagonize LPS endotoxic activity that was comparable to 406 (**2**) (Figure 3). As mentioned above, natural *Ru. gelatinosus* lipid A has immunostimulatory activity. In contrast, *Chromobacterium violaceum* lipid A **11**, which has acyl groups similar to *Ru. gelatinosus*, showed antagonistic activity. Both of these lipid A molecules have shorter acyl groups than *E. coli* lipid A, and symmetric (3 + 3) acylation distribution. Therefore, our study indicated that these

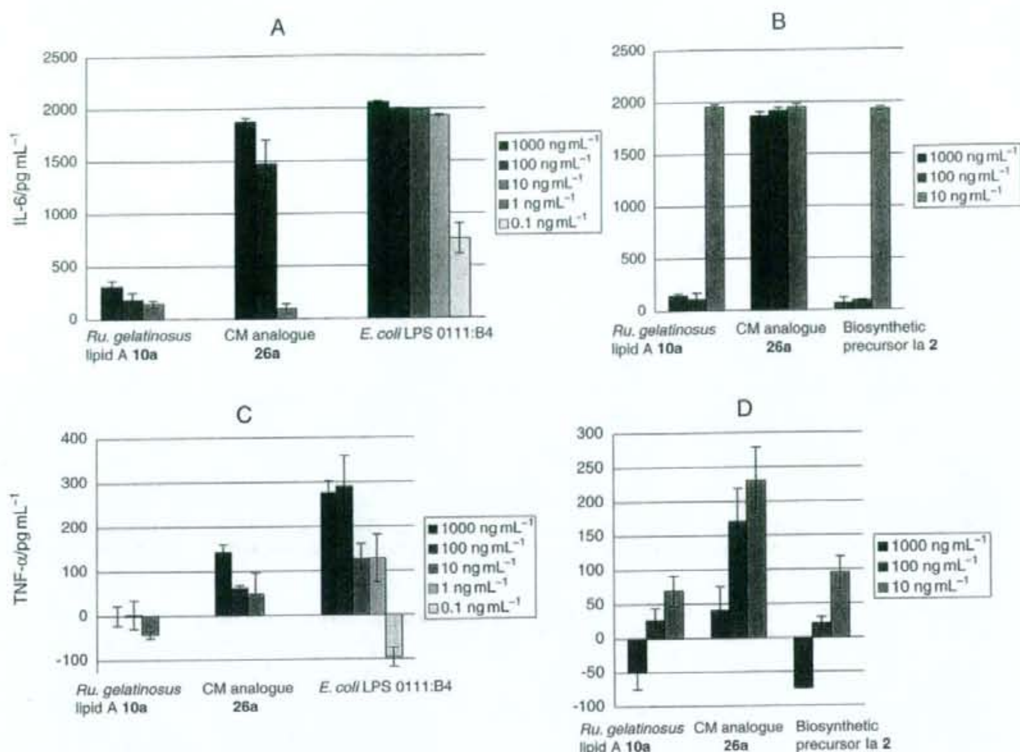


Figure 3. Cytokine inducing activity and inhibitory activity of *Ru. gelatinosus* lipid A **10a**, its CM analogue **26a**, and *E. coli* LPS 0111:B4 in human peripheral whole-blood cells. A: IL-6 inducing activity, B: inhibitory activity against IL-6 induction by *E. coli* LPS 0111:B4 (10 ng mL^{-1}), C: TNF-α inducing activity, D: Inhibitory activity against TNF-α induction by *E. coli* LPS 0111:B4 (10 ng mL^{-1}).

types of lipid A should show antagonistic activity. The reason why natural *Ru. gelatinosus* lipid A showed immunostimulatory activity will be discussed later.

Synthesis of *Ru. gelatinosus* Lipid A Analogues by Using Affinity Separation Method. We were then interested in the effect of acylation distribution on endotoxic/antagonistic activity and planned to synthesize six kinds of *Ru. gelatinosus* lipid A analogues **26a**–**26f** having hexaacyl groups (Figure 4). All of these compounds contained the same acyl groups but their distributions were different: each compound has two (*R*)-3-hydroxydecanoyl groups and two (*R*)-3-(dodecanoyloxy)decanoyl groups. Analogue **26c** had the same acylation pattern as *E. coli* lipid A **1**, so it was expected that biological tests of **26c** would give additional information on how chain length effected bioactivity.

1-*O*-Carboxymethyl (CM) analogues, which had glycosyl CM groups instead of the glycosyl phosphate moiety in natural lipid A, were chosen as targets, since they were easier to synthesize than the natural-type because of the chemical instability of the glycosyl phosphate. We previously synthesized both the *E. coli*-type and the precursor-type analogues CM-506 and CM-406 in which the phosphoryl group at the 1-position was replaced with a carboxymethyl (CM) group.[20,39] The activity of both CM-506 and CM-406 was indistinguishable from their corresponding natural-type compounds. The β-CM analogues having acidic groups β-glycosidically linked at the 1-position also showed potent activity.[21] We also synthesized two analogues that had two CM groups at 1- and 4′-positions, *E. coli*-type (Bis-CM-506) and precursor-type (Bis-CM-406), both of which showed respective activities.[40,41] The acidic functional groups are concluded to be essential,[42] but their strict type is not necessary for expression of the biological activity.

Although we had already improved the synthetic procedure for lipid A in many aspects, it still had many reaction steps and as a consequence considerable time and laborious work was required for the completion of the synthesis. In order to facilitate the synthesis, we developed a new synthetic methodology termed Synthesis based on Affinity Separation (SAS). The basic principle of SAS is as follows. A tag molecule is covalently attached to a substrate. The reactions are carried out in solutions, and the desired tagged products are rapidly isolated by solid-phase extraction using a specific affinity interaction between the tag and a ligand which is immobilized on a polymer support. So far, we have successfully used two interactions for SAS. One was an interaction of a crown ether or a podand ether tag and polymer-supported ammonium ions[43,44] and the other was the specific molecular recognition between a barbituric acid derivative and its artificial receptor which formed a tight complex with six hydrogen bonds[43b,45] (Figure 5). The versatility of SAS for glycoconjugate synthesis has already

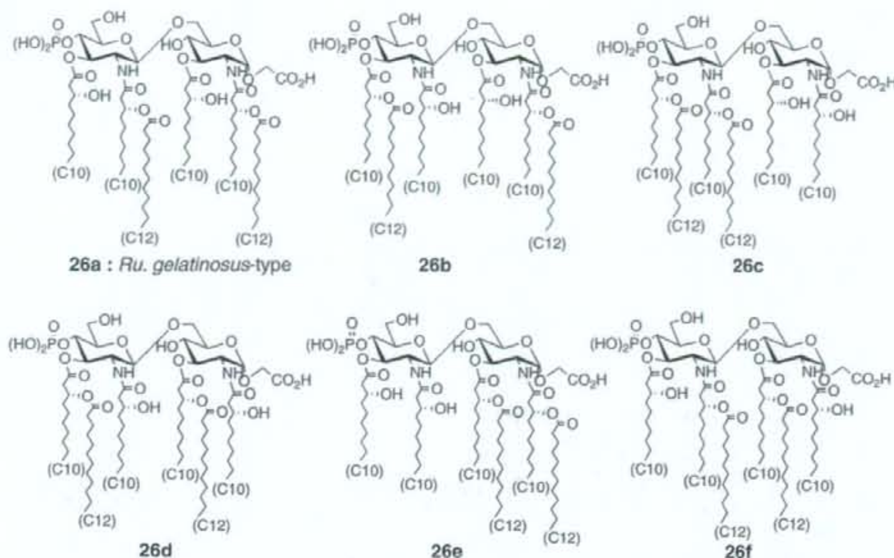


Figure 4. Lipid A library possessing six acyl groups.

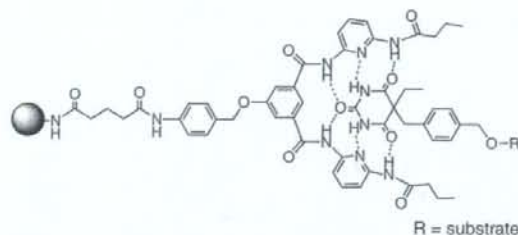


Figure 5. Host–guest interaction of a polymer-supported receptor with a barbituric acid tag.

been demonstrated by our total synthesis of *E. coli* lipid A based on the latter interaction.[31,32]

Figure 6 shows the basic synthetic route for constructing the library. A barbituric acid (BA) tag was attached to the 4-position of the glycosyl acceptor via a *p*-acylaminobenzyl linker with a glutarylamino spacer. In order to reduce the total number of reaction steps for the synthesis of the six target compounds, a $\beta(1\rightarrow6)$ disaccharide 4′-phosphate **29** was constructed as a common key synthetic intermediate by the coupling of two monosaccharides, i.e., a glycosyl trichloroacetimidate **27** as a donor and a glycosyl acceptor **28** having the BA-tag. All the acyl moieties were then introduced step by step to their respective positions. Acylation of the hydroxy group with the 3-acyloxyfatty acid in the presence of DMAP sometimes caused β-elimination of the 3-acyloxy function, especially when the hydroxy group to be acylated was sterically hindered by a neighboring long chain *N*-acyl group. Therefore, acylation of the 3- or 3′-hydroxy group with the 3-acyloxyfatty acid was carried out prior to 2- and 2′-N-acylation. After introducing the four acyl groups, simultaneous deprotection and cleavage of the linker by catalytic hydrogenolysis afforded the desired CM-analogues **26a**–**26f**. The divergent strategy was also employed by our previous synthesis and by Boons' synthesis.[25,26]

The glycosyl donor **27**, whose 2- and 3-positions are protected with the allyloxycarbonyl (Alloc) and propargyloxycarbonyl (Proc) groups respectively, was synthesized as shown in Scheme 3. The Proc group was stable to neat TFA, but could be readily cleaved by treatment with $Co_2(CO)_8$ and TFA via an alkyne–Co complex.[46] The Proc group could also be cleaved with Zn–AcOH, Pd^0–Et_3SiH, or $[Ir(cod)(MePh_2P)_2]PF_6$ that was activated with H_2 (Ir-complex).[46b] Since the 1-*O*-allyl group could not have been isomerized to a 1-propenyl group by the Ir-complex in the presence of the *N*-Alloc group, *N*-Fmoc glucosamine allyl glycoside **31** was used as a starting material. The allyl group was isomerized to a 1-propenyl group by using the Ir-complex before introduction of the Proc group, since the latter is readily cleaved with the Ir-complex. The 3-*O*-Proc derivative **32** formed in 94% yield was treated with 1,3,4,6,7,8-hexahydro-2*H*-pyrimido[1,2-α]-pyrimidine, polymer-bound, (PTBD) to remove the 2-*N*-Fmoc group.[47] The reaction was slow with PTBD and 1 day was required for the complete removal of the Fmoc group, but the solid base was removed by simple filtration and thus the work-up operation was quite simple. After the free 2-amino group was again protected with an Alloc group, reductive opening of the 4,6-*O*-benzylidene ring of **33** was effected by the use of the combination of Et_3SiH and $BF_3\cdot Et_2O$. In a small scale experiment (0.17 mmol of **33**), $BF_3\cdot Et_2O$ was added at once to a solution of the 4,6-O-benzylidenated compound **33** and Et_3SiH in CH_2Cl_2 at 0 °C to give the desired 6-O-benzylated product **34** in 93% yield. In a large scale (21.6 mmol of **33**), even when $BF_3\cdot Et_2O$ was added dropwisely, 30% of 3-*O*-Alloc derivative was formed by an undesired reduction of the Proc group. 4-O-Phosphination of **34** and a subsequent oxidation gave phosphate **35** in 91% yield. After removal of the 1-propenyl group with aqueous I_2, treatment with CCl_3CN and Cs_2CO_3 furnished glycosyl trichloroacetimidate **27**.

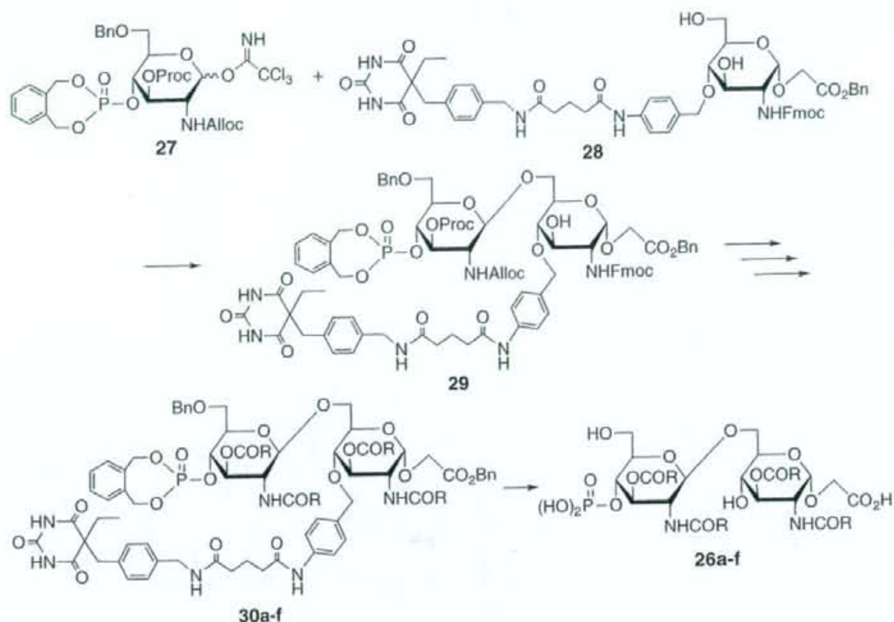
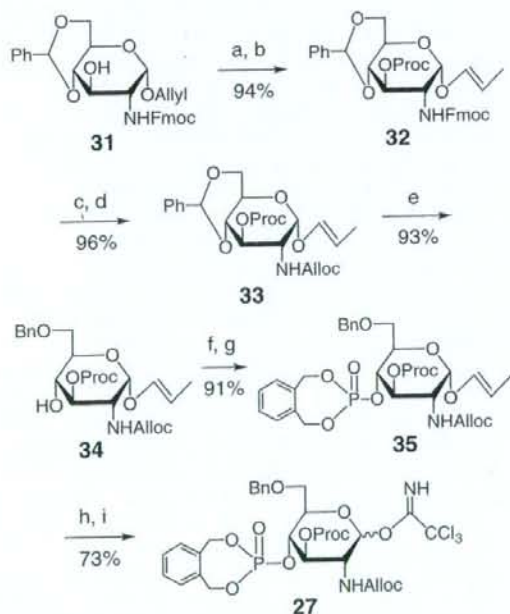


Figure 6. The basic synthesis route for the construction of the lipid A analogue library.

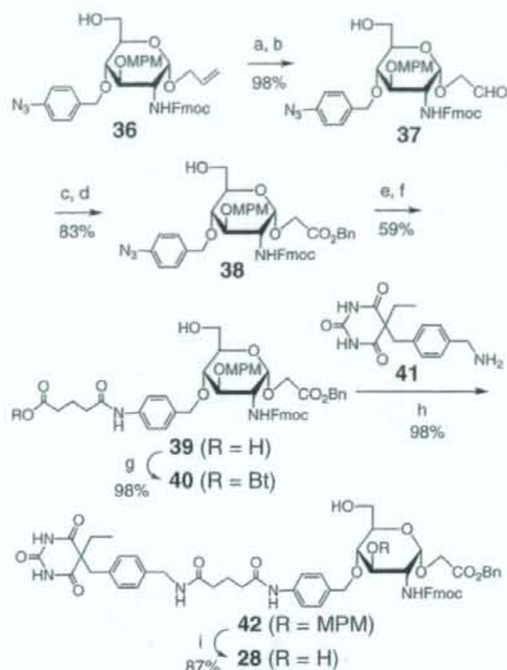


Scheme 3. Reagents and conditions: (a) [Ir(cod)-$(MePh_2P)_2]PF_6$, H_2, THF; (b) ProcCl, pyridine, DMAP, CH_2Cl_2; (c) PTBD, CH_2Cl_2; (d) AllocCl, pyridine, CH_2Cl_2; (e) Et_3SiH, $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C; (f) *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole, CH_2Cl_2; (g) *m*CPBA, −20 °C; (h) I_2, H_2O, THF; (i) CCl_3CN, Cs_2CO_3, CH_2Cl_2.

The synthesis of the glycosyl acceptor **28** is shown in Scheme 4. 4-Azidobenzylglucosamine allyl glycoside **36** was prepared as previously described.[31] The allyl group of **36** was oxidatively cleaved with OsO_4 and then with $Pb(OAc)_4$ to give aldehyde **37** in 98% yield. Further oxidation of **37** by using $NaClO_2$ gave a 1-*O*-carboxymethyl derivative.[48] Benzyl esterification by slow addition of a phenyldiazomethane solution gave the desired benzyl ester **38** in 83% yield.[49] Treatment with an excess amount of phenyldiazomethane gave an undesired N-benzylated product. The azido group of **38** was then reduced using Zn in AcOH, and the resulting amino group was acylated with glutaric anhydride to afford the carboxylic acid **39** in 59% yield. The acid **39** was converted to 1-hydroxybenzotriazole (HOBt) ester **40**, which was then coupled with the BA-tag moiety **41**. The desired BA-tagged product **42** was obtained in good yield after the affinity separation (outlined as follows). The reaction mixture in CH_2Cl_2 was applied to a short column packed with a resin immobilized artificial receptor of BA. HOBt and small amounts of **39** and **40** were efficiently removed by washing with CH_2Cl_2, while the desired **42** was retained in the column. Elution of **42** with CH_2Cl_2–MeOH (1:1) and concentration gave purified **42**. The 3-*O*-MPM group was then removed by treatment with $BF_3 \cdot Et_2O$ to afford the glycosyl acceptor **28** in 87% yield after affinity separation.

Glycosylation of the BA-tagged 3-*O*-MPM acceptor **42** with donor **27** was first attempted by using TMSOTf as a catalyst in CH_2Cl_2 at −20 °C (Scheme 5). Although glycosyl donor **27** disappeared within 1 h, glycosylation of acceptor **42** was incomplete. Hence, the reaction mixture was once subjected to the affinity separation. The recovered tagged fraction, which consisted mainly of **42** and the desired *β*-disaccharide **43** was again subjected to glycosylation with **27**. Even after this

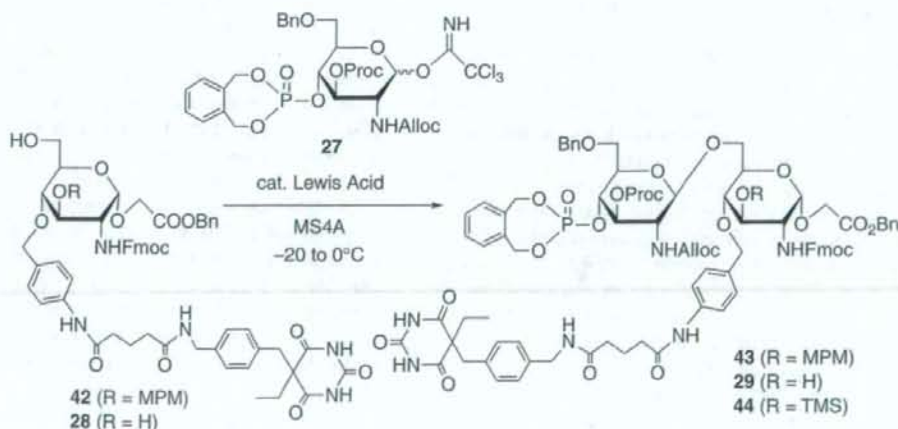
double procedure, the total yield of **43** remained as low as 60%. Glycosylation of a more reactive acceptor **28** with an additional free hydroxy group at the 3-position with the same donor **27** using TMSOTf in CH_2Cl_2 gave 3-*O*-TMS disaccharide **44** in addition to the desired **29** (80% as a mixture of **29** and **44**). Though the unexpectedly introduced TMS group can be readily cleaved to give **29**, further investigation led us to more favorable conditions for glycosylation: the desired disaccharide **29** was obtained in 96% yield by the use of $BF_3 \cdot Et_2O$ as a catalyst and THF as a solvent.



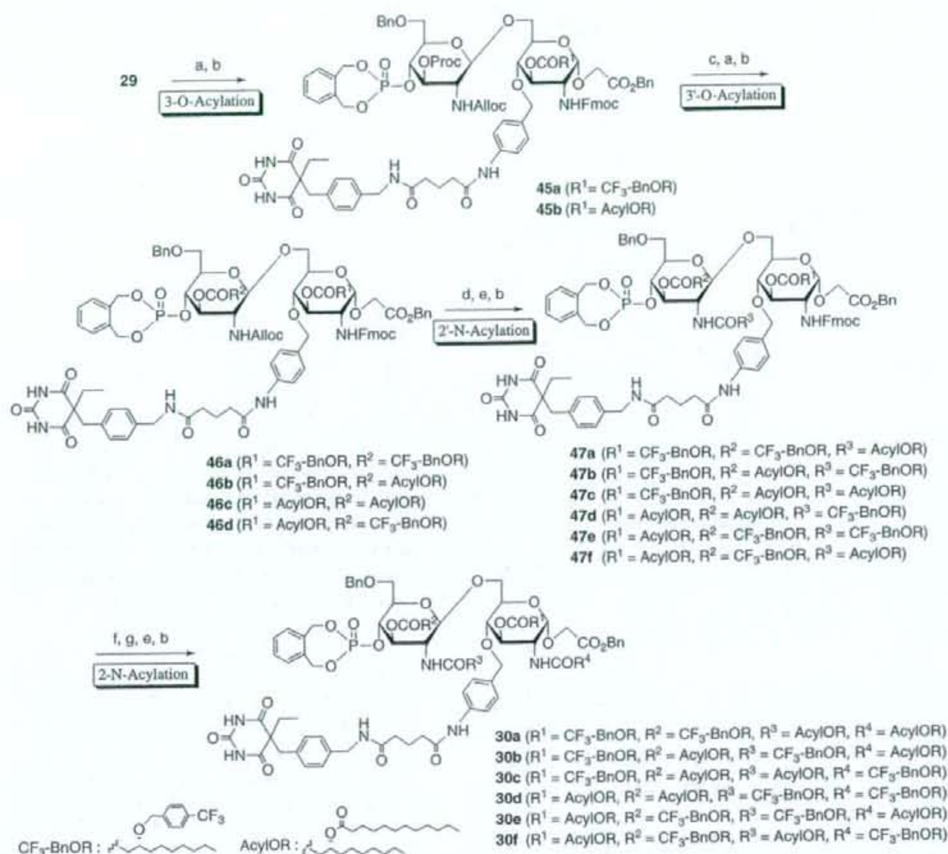
Scheme 4. Reagents and conditions: (a) OsO_4, NMO, THF/*t*-butyl alcohol/water (10:10:1); (b) $Pb(OAc)_4$, benzene/CH_2Cl_2 (2:3); (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, THF/*t*-butyl alcohol/water (2:4:1); (d) phenyldiazomethane, Et_2O; (e) Zn, AcOH/THF (2:1); (f) glutaric anhydride, CH_2Cl_2; (g) HOBt, DCC, CH_2Cl_2; (h) Et_3N, DMF, affinity separation; (i) $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C, affinity separation.

The acyl groups were then sequentially introduced to the respective positions on the disaccharide **29** (Scheme 6 and Table 2). After acylation of the 3-position of disaccharide **29**, the 3′-*O*-Proc group in **45a** or **45b** was removed by treatment with a stoichiometric amount of an Ir-complex.[46b] The 3′-*O*-Proc group was also readily cleaved by using Zn–Cu couple and AcOH. Acylation using diisopropylcarbodiimide (DIC) and DMAP gave the desired 3,3′-di-O-acylated products **46a–46d**, which were successfully separated from DMAP, DIC, and the other by-products by affinity separation. Cleavage of the 2′-*N*-Alloc group was carried out by using $Pd(PPh_3)_4$ in the presence of HCO_2H and Et_3N.[50] In contrast, complete cleavage of the 2′-*N*-Alloc group was not effected by the use of *n*-$BuNH_2$ in place of Et_3N as an additive. The third acyl group was introduced to the 2′-amino group by using DIC. Deprotection of the 2-position by treatment with DBU was followed by purification using silica-gel short column chromatography. Subsequent 2-N-acylation with DIC, affinity separation, and additional silica-gel chromatography afforded the desired fully acylated products **30a–30f**.

Table 2 summarizes the reaction time required for all the acylation steps and the yields of the two-step conversions of deprotection and acylation. The 3-O-acylation of **29** with benzyloxydecanoic acid **20** and dodecanoyloxydecanoic acid **21** gave **45a** and **45b** in good yields, respectively. The yields of the 3′-O-acylation of compound **45b**, which has a 3-*O*-acyloxyacyl group, were a little lower than those of 3-O-benzyloxyacylated compound **45a**. The 2′-N-acylation with benzyloxydecanoic acid **20** afforded **47b**, **47d**, and **47e** in lower yields than the yields of acylation with dodecanoyloxydecanoic acid **21** for the synthesis of **47a**, **47c**, **47f**. Since the reactivity of the 2-amino group was suppressed by the steric hindrance of the neighboring 3-*O*-acyl group, the yields of the 2-N-acylation reaction were generally not high. Especially, 2-N-acylation of **47d**, **47e**, and **47f** having a 3-*O*-acyloxyacyl



Scheme 5. Glycosylation of acceptors possessing BA-tag with donor **27**.



Scheme 6. Reagents and conditions: (a) (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) or (*R*)-3-(dodecanoyloxy)decanoic acid (**21**), DIC, DMAP, CH_2Cl_2; (b) affinity separation; (c) $[Ir(cod)(MePh_2P)_2]PF_6$, H_2, THF or Zn–Cu, AcOH; (d) $Pd(PPh_3)_4$, HCO_2H, Et_3N, THF; (e) (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) or (*R*)-3-(dodecanoyloxy)decanoic acid (**21**), DIC, CH_2Cl_2; (f) DBU, CH_2Cl_2; (g) silica-gel chromatography.

group gave the fully acylated products always in low yields. ESI-MS measurements suggested that the undesired by-products **48d** and **48f** were being formed in the synthesis of **30d** and **30f** (positive mode, m/z 2019.49 $[M+Na]^+$) (Figure 7). TLC analysis showed that this side reaction also occurred in the synthesis of **48a**–**48c** and **48e**. Except for the loss of material, this undesirable cyclization did not cause serious problems, since the cyclic by-products which lost the BA-tag were readily removed from the desired products by the affinity separation and therefore did not affect the purity.

Simultaneous removal of all the benzyl-type protective groups and the BA-tag moiety using catalytic hydrogenolysis was investigated. The acylaminobenzyl linker was not cleaved by the catalytic hydrogenation under neutral conditions. The acid stability of the CM-analogues allowed us a reaction under acidic conditions, so that the final deprotection and the cleavage of the BA-tag of **30a**–**30f** successfully proceeded by hydrogenolysis using $Pd(OH)_2$ in THF–AcOH (3:1) at room temperature for 1 d (Scheme 7). Subsequent purification by liquid–liquid partition column chromatography afforded the desired CM-analogues **26a**–**26f**.

The biological activities of the six CM-analogues **26a**–**26f** were evaluated by measuring *Limulus* activity and cytokine (IL-6 and TNF-α) induction, in a manner similar to that mentioned above. Compounds **26e** and **26f** exhibited *Limulus* activity as strong as LPS (Table 1). The *Ru. gelatinosus*-type analogue compound **26a** showed activity, but required concentrations 100 times higher than LPS to activate factor C. In contrast, compounds **26b** and **26d** showed very weak positive responses, but **26c**, which had the same acylation distribution as *E. coli*, did not have any activity.

The CM-analogue of *Ru. gelatinosus* lipid A **26a** had apparent IL-6 and TNF-α inducing activities, but it was much less potent than LPS (Figures 3A, 3C, 8A, and 8C). Compounds **26b**–**26f** did not induce IL-6 or TNF-α. These results clearly demonstrate that the distribution of acyl groups also plays a critical role in determining endotoxic activity under conditions where the numbers and chain lengths of the acyl groups are identical.

The inhibitory activities of analogues **26a**–**26f** were tested on the induction of IL-6 and TNF-α by LPS (Figure 8B and 8D). Compound **26e** had inhibitory activity that was compara-

Table 2. Stepwise Acylation Using **20** and **21**

3-O-Acylation			3′-O-Acylation			2′-N-Acylation			2-N-Acylation		
F.A.	Time	Yield	F.A.	Time	Yield	F.A.	Time	Yield	F.A.	Time	Yield
20	2 h	**45a** (84%)	**20**	9 h	**46a** (75%)	**21**	18 h	**47a** (70%)	**21**	14 h	**30a** (49%)
			21	10 h	**46b** (77%)	**20**	18 h	**47b** (44%)	**21**	14 h	**30b** (39%)
						21	19 h	**47c** (53%)	**20**	18 h	**30c** (35%)
21	3.5 h	**45b** (92%)	**21**	11 h	**46c** (59%)	**20**	12 h	**47d** (37%)	**20**	25 h	**30d** (14%)
			20	13 h	**46d** (59%)	**20**	15 h	**47e** (42%)	**21**	20 h	**30e** (12%)
						21	21 h	**47f** (63%)	**20**	19 h	**30f** (6%)

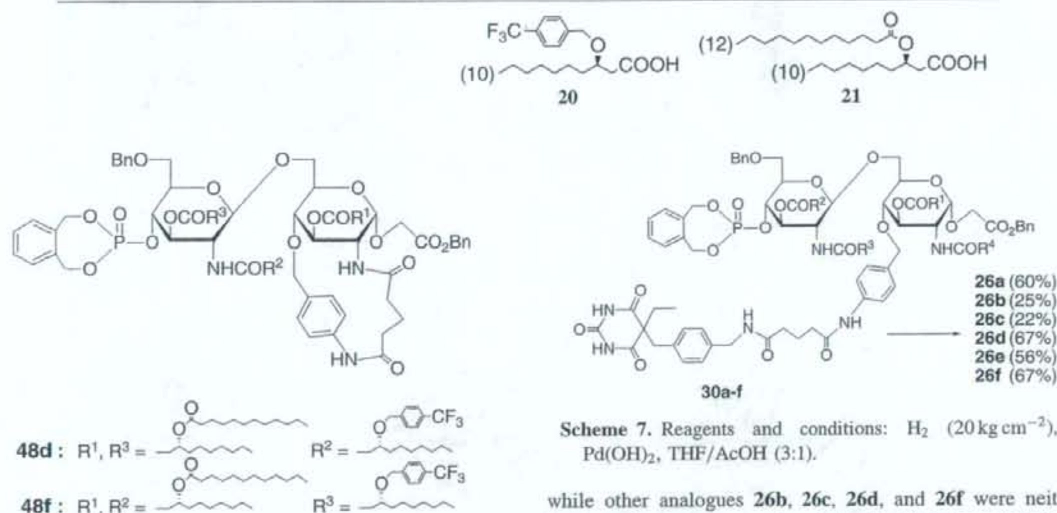
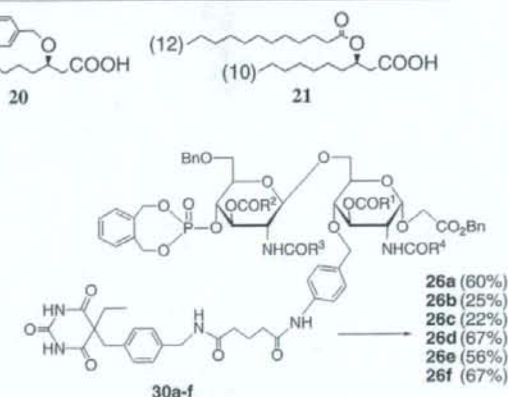


Figure 7. Structures of by-products formed during 2-N-acylation reaction.



Scheme 7. Reagents and conditions: H_2 ($20\,kg\,cm^{-2}$), $Pd(OH)_2$, THF/AcOH (3:1).

ble to biosynthetic precursor **2**, but **26a–26d** and **26f** did not appear to be inhibitory.

There are two main signal transduction systems for TLR4 signals, which recruit adaptor proteins to TLR4 and induces cytokines and type I interferon (IFN) by activating the transcription factors, NF-κB and IRF-3, respectively. Seya et al. reported that compound **26a** induced IFN-β via the IRF-3 pathway, in addition to activating NF-κB, in a similar manner to *E. coli* lipid A **1**. Both 406 (**2**) and *Ru. gelatinosus* lipid A **10a** inhibited the production of IFN-β.[51]

Discussion

As described above, *Ru. gelatinosus* lipid A **10a** showed potent antagonistic activity against LPS, whereas its 1-*O*-carboxymethyl analogue **26a** showed a weak immunostimulatory activity. Compound **26e** showed potent antagonistic activity, while other analogues **26b**, **26c**, **26d**, and **26f** were neither immunostimulatory nor antagonistic. Small structural changes, i.e., acidic functional groups and acylation distribution, dramatically influenced the biological activity, as in the case of lipid A which has C10 or C12 hexa-acyl groups, which appears to be a structural boundary for the bioactivity.

The reason that changing the acylation distribution of lipid A analogues **26a–26f** effects their bioactivity can be explained as follows. Recently, Satow et al. reported the crystal structures of human MD-2 and its complex with antagonist 406 (**2**).[52] Lee et al. reported the 3D structures of the full-length ectodomain of the murine TLR4 and the MD-2 complex. They also determined the structure of the complex of human MD-2, E5564 (**4**), and TV3 (a hybrid of the partial structure of human TLR4 and variable lymphocyte receptor of hagfish).[53] In both MD-2 structures, **2** and **4** bind to the same area in MD-2. In the complex of human MD-2 with **2**, four fatty-acid chains of **2** are fully confined within a deep hydrophobic cavity that is sandwiched by two β-sheets, and phosphate and sugar moieties are located at the cavity ingress (Figure 9A). Molecular

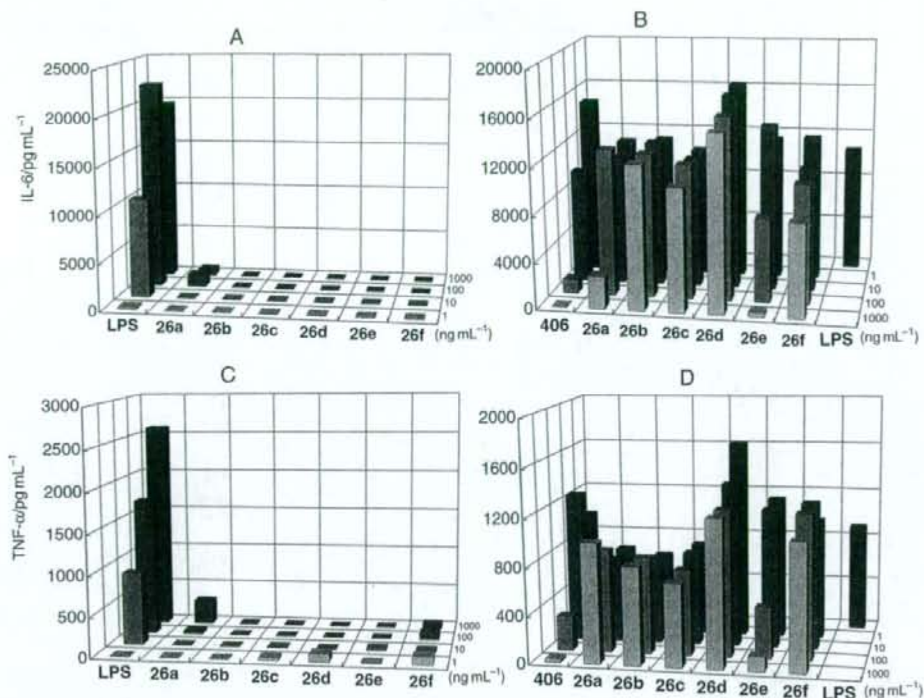


Figure 8. The cytokine inducing activity and inhibitory activities of **26a**–**26f**, and *E. coli* LPS 0111:B4, as measured in human peripheral whole-blood cells. A: IL-6 inducing activity, B: inhibitory activity against IL-6 induction by *E. coli* LPS 0111:B4 (10 ng mL^{-1}), C: TNF-α inducing activity, D: inhibitory activity against TNF-α induction by *E. coli* LPS 0111:B4 (10 ng mL^{-1}).

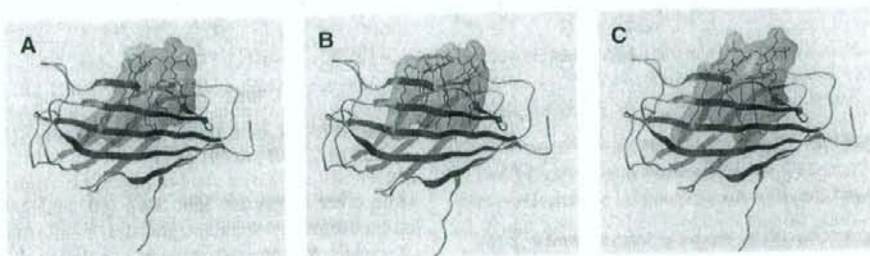


Figure 9. Stereo ribbon models of human MD-2 in complexes with 406 (**2**) (lipid IVa) (A), molecular modeling of human MD-2 in complex with *Ru. gelatinosus* lipid A **10a** (B), and with **26e** (C).

modeling using a united atom AMBER™ force field and a GB/SA continuum solvent model for water, as implemented in MacroModel (version 7.1), revealed that the *Ru. gelatinosus* lipid A **10a** and the antagonist **26e** could bind to MD-2 in a manner similar to 406 (**2**) (Figures 9B and 9C). These results indicate that the volume of the four C10 and two C12 fatty-acid chains can fit the hydrophobic pocket of MD-2.

The volume of the acyl groups in **26a**–**26f** should have been similar, but **26b**, **26c**, and **26d** are inactive in both the human peripheral blood system and *Limulus* test and **26f** is inactive in the human peripheral blood system. These results suggest that the molecular conformation is probably affected by the distribution of the acyl groups. From molecular mechanics calculations of these compounds, the biologically active compounds **26a**, **26e**, and **26f** had ordered low energy conformations, in which the acyl chains were aligned in parallel and were closely packed. On the other hand, the low energy conformations of the inactive compounds had acyl moieties with disordered structures (Figure 10). The distribution of the acylation should therefore affect the tendency of these lipids to aggregate. Seydel et al. revealed that formation of aggregates is essential for expression of the endotoxic activity; monomeric lipid A and LPS prepared by a dialysis procedure showed no activity, whereas their aggregates at the same concentrations were biologically active.[54] Monomeric lipid A and LPS molecules might be conformationally flexible due to their lack of intermolecular hydrophobic interactions and a large entropic loss should prevent their binding to the LPS receptor system, which

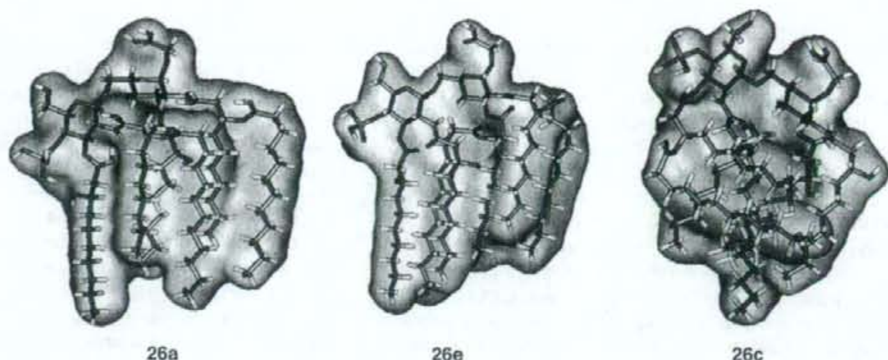


Figure 10. The lowest energy conformations of **26a**, **26e**, and **26c**, calculated with MacroModel v7.1 (Amber*, low mode, GB/SA water).

consists of LPS binding protein (LBP) in blood, the glycosyl phosphatidyl inositol (GPI) anchor protein CD14, and the TLR4/MD-2 complex. LBP binds to oligomeric LPS and should recognize the particular conformation of lipid A in the supramolecular assembly; LBP then should transfer lipid A (LPS) from the aggregates to CD14 and then lipid A (LPS) should be transferred from CD14 to TLR4/MD2. The molecular modeling study suggested that the inactive analogues **26b**, **26c**, and **26d** might not form the ordered supramolecular structure, whereas the bioactive compounds should form the supramolecular assembly.

Although the aggregate formation of lipid A and LPS is essential for their biological activity, TLR4/MD-2 should recognize them as single molecules. X-ray crystallographic analysis indicates that MD-2 binds to the antagonists 406 (**2**) and E5564 (**4**) in a 1:1 ratio, and MD-2 forms a stable complex with TLR4 (i.e., one TLR4/MD-2 binds to one antagonist). It has been reported that the binding of agonistic lipid A and LPS induces TLR4 aggregation and initiates intracellular signaling.[12,53,55–57] Immunoprecipitation assays using tritium-labeled lipid A analogues and antiTLR4/MD-2 antibodies revealed that maximal binding of the antagonistic 406 analogue to human TLR4/MD-2 was ca. 2-fold higher than that of agonistic *E. coli* lipid A **1**, suggesting that *E. coli* lipid A binds to TLR4/MD-2 in a 1:2 ratio.[55] Endotoxic lipid A should be recognized by two TLR4/MD-2 molecules and consequently induce the dimerization of TLR4/MD-2, whereas binding of antagonistic lipid A to an isolated single TLR4/MD-2 complex does not induce dimerization of the complex. Although the mode of the interaction between the TLR4-complex with the agonistic lipid As and LPS has not been clarified yet, there must be significant differences between their interactions with the antagonists and the agonists. Since the four acyl groups of 406 (**2**) and E5564 (**4**) almost occupy the hydrophobic pocket in MD-2, significant structural changes of the pocket seem to be inevitable when *E. coli* lipid A **1** binds to MD-2. This structural change in MD-2 may induce dimerization and activation of the TLR4–MD-2 complex. However, the difference between antagonistic *Ru. gelatinosus* lipid A **10a** and agonistic **26a** is only an acidic functional group at the 1 position. Similar results were obtained from our studies of lipid A analogues that contained acidic amino acid residues; immunostimulatory or antagonistic activity was observed depending on their anionic charges (carboxylic acid vs. phosphoric acid).[58,59] In addition, we recently found that synthetic tri-acyl type *Helicobacter pylori* lipid A having 1-phosphate shows antagonistic activity against the induction of inflammatory cytokines such as IL-6, whereas *H. pylori* lipid A, in which an ethanolamine group is linked to the 1-phosphate, shows weak agonistic activity.[60] The number of anionic charges in all agonists was decreased in comparison to their corresponding antagonists. It is expected that subtle difference in anionic charges decisively influences the binding manner to TLR4/MD-2 complex at around the boundary critical structure of lipid A required for endotoxic or antagonistic activity. The present work showed the volume of acyl moieties in *Ru. gelatinosus* lipid A may corresponds to the boundary structure and hence the differences in the acidic functional groups affected the bioactivity.

Experimental

General Procedures. ^{1}H NMR spectra were measured in the indicated solvents using a JEOL JNM-LA500, a JEOL JNM-GSX 400, or a Varian UNITYplus 600 spectrometer. The chemical shifts in $CDCl_3$ and DMSO-d_6 are given in δ values using tetramethylsilane (TMS) as an internal standard. Mass spectra were measured using an ESI-TOF mass spectrometer (Applied Biosystems, Mariner™). Specific rotations were measured using a Perkin-Elmer 241 polarimeter. Elemental analyses were determined using Yanaco CHN corders MT-3, MT-5, and MT-6. Recycling preparative HPLC was carried out with an LC908 (Japan Analytical Industry). Silica-gel column chromatography was carried out with Kieselgel 60 (Merck, 0.040–0.063 mm) at medium pressure (2–4 kg cm^{-2}) using the indicated solvent systems. Analytical and preparative thin layer chromatographies (TLC) were performed on precoated Kieselgel $60F_{254}$ Plates (Merck, 0.25 mm thickness) and precoated Kieselgel $60F_{254}$ Plates (Merck, 0.5 mm thickness), respectively. Anhydrous CH_2Cl_2 was distilled from CaH_2. Anhydrous $CHCl_3$, THF, Et_2O, DMF, CH_3CN, toluene, and benzene were purchased from Kanto Chemicals, Tokyo, Japan. Distilled water, purchased from Otsuka (Tokyo, Japan) or prepared by a combination of Toray Pure LV-308 (Toray) and GSL-200 (Advantec, Tokyo, Japan), was used as an eluent for the liquid–liquid partition column chromatography and as solvent for the lyophilization. Molecular sieves 4A (MS4A) was activated by heating at 250 °C in vacuo for 3 h before use. All other com-

mercially obtained materials were used as received.

Allyl 4,6-*O*-Benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (13). To a solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**12**) (3.00 g, 6.22 mmol) and (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) (2.59 g, 7.46 mmol) in anhydrous CH_2Cl_2 (200 mL) were added DCC (2.31 g, 11.2 mmol) and DMAP (75.9 mg, 0.622 mmol) at room temperature under Ar atmosphere, and the mixture was stirred for 15 h. After additional stirring for 2 h with the addition of (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) (1.14 g, 3.29 mmol), DCC (0.998 g, 4.84 mmol), and DMAP (79.2 mg, 0.648 mmol), the precipitate was filtrated off and the solution was concentrated under reduced pressure. The residue was purified by silica-gel flash chromatography (300 g, $CHCl_3$:acetone = 70:1) to give **13** (4.62 g, 92%) as a colorless solid. ESI-MS (positive) m/z 827.3 $[M+NH_4]^+$, 832.2 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.48 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.39 (dd, $J = 8.1$, 2.0 Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.30–7.24 (m, 5H, =CH–*Ph*), 5.90 (m, 1H, –OCH_2–*CH*=CH_2), 5.46 (s, 1H, =*CH*–Ph), 5.42 (t, $J = 10.0$ Hz, 1H, H-3), 5.35 (d, $J = 10.0$ Hz, 1H, NH), 5.31 (dd, $J = 17.2$, 1.4 Hz, 1H, –OCH_2–CH=CH_2), 5.25 (dd, $J = 10.5$, 1.2 Hz, 1H, –OCH_2–CH=CH_2), 4.93 (d, $J = 3.7$ Hz, 1H, H-1), 4.70 (d, $J = 12.1$ Hz, 1H, –CO–O–CH_2–CCl_3), 4.63 (d, $J = 12.1$ Hz, 1H, –CO–O–CH_2–CCl_3), 4.53 (d, $J = 12.2$ Hz, 1H, p-CF_3–C_6H_4–), 4.43 (d, $J = 12.4$ Hz, 1H, p-CF_3–C_6H_4–CH_2–), 4.29 (dd, $J = 10.3$, 4.7 Hz, 1H, H-6a), 4.22 (ddt, $J = 12.7$, 5.4, 1.2 Hz, 1H, –OCH_2CH=CH_2), 4.08 (ddd, $J = 10.1$, 10.1, 3.8 Hz, 1H, H-2), 4.03 (ddd, $J = 12.7$, 6.3, 1.2 Hz, 1H, –OCH_2CH=CH_2), 3.95 (ddd, $J = 9.8$, 9.8, 4.7 Hz, 1H, H-5), 3.82 (m, 1H, β-CH of 3-*O*-acyl), 3.78 (dd, $J = 10.3$, 10.3 Hz, 1H, H-6b), 3.71 (t, $J = 9.5$ Hz, 1H, H-4), 2.65 (dd, $J = 15.4$, 6.9 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.45 (dd, $J = 15.4$, 5.1 Hz, 1H, α-CH_2 of 3-*O*-acyl), 1.33–1.16 (m, 12H, $CH_2 \times 6$), 0.867 (t, $J = 7.3$ Hz, 3H, –CH_2–CH_3). Found: C, 55.31; H, 5.64; N, 1.92%. Calcd for $C_{37}H_{45}Cl_3F_3NO_9$: C, 54.79; H, 5.59; N, 1.73%.

Allyl 6-*O*-Benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (14). To a solution of **13** (1.00 g, 1.23 mmol) and triethylsilane (0.982 mL, 6.16 mmol) in dry CH_3CN (12 mL) was added diethyl ether–boron trifluoride (1/1) (0.463 mL, 3.69 mmol) dropwise and the mixture was stirred at 0 °C for 1.5 h. The reaction was then quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (50 g, $CHCl_3$: acetone = 20:1) to give **14** as a colorless syrup (0.742 g, 74%). $[\alpha]_D^{22} = +38.5$ (c 0.757, $CHCl_3$). ESI-MS (positive) m/z 829.3 $[M+NH_4]^+$, 834.3 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.41 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.36–7.25 (m, 5H, *Ph*–CH_2–), 5.88 (m, 1H, –OCH_2–*CH*=CH_2), 5.31–5.11 (m, 4H, 2-NH, H-3, and –OCH_2–CH=CH_2), 4.91 (d, $J = 3.9$ Hz, 1H, H-1), 4.66 (s, 2H, Ph–CH_2–), 4.62–4.54 (m, 4H, –CO–O–CH_2–CCl_3 and p-CF_3–C_6H_4–CH_2–), 4.53 (d, $J = 12.2$ Hz, 1H, p-CF_3–C_6H_4–CH_2–), 4.43 (d, $J = 12.4$ Hz, 1H, p-CF_3–C_6H_4–CH_2–), 4.19 (dd, $J = 13.0$, 5.2 Hz, 1H, –OCH_2CH=CH_2), 4.03–3.95 (m, 2H, –OCH_2CH=CH_2 and H-2), 3.90 (m, 1H, β-CH of 3-*O*-acyl), 3.84–3.80 (m, 1H, H-6a), 3.77–3.67 (m, 3H, H-4, H-5, and H-6b), 2.78 (d, $J = 2.9$ Hz, 1H, 4-OH), 2.65 (dd, $J = 15.0$, 7.8 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.50 (dd, $J = 15.1$, 4.4 Hz, 1H, α-CH_2 of 3-*O*-acyl), 1.72–1.49 (m, 2H, α-CH_2 of 3-*O*-acyl), 1.31–1.26 (m, 10H, $CH_2 \times 5$), 0.87 (t, $J = 6.9$ Hz, 3H, –CH_2–CH_3).

Allyl 6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (15). To a solution of **14** (1.80 g, 2.21 mmol) in anhydrous CH_2Cl_2 (30 mL) were added *N*,*N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (0.801 g, 3.34 mmol) and 1*H*-tetrazole (0.465 g, 6.64 mmol) at room temperature under Ar atmosphere. After the mixture was stirred for 50 min and then at −20 °C for 15 min, *m*CPBA (0.381 g, 2.21 mmol) was added and stirring was continued for another 20 min. The solution was quenched by addition of saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (100 g, $CHCl_3$: acetone = 30:1) to give 4-*O*-phosphate **15** (2.06 g, 94%) as a colorless syrup. $[\alpha]_D^{22} = +34.6$ (c 1.00, $CHCl_3$). ESI-MS (positive) m/z 994.2 $[M+H]^+$, 1016.2 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.43 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.39–7.24 (m, 6H, o-$C_6H_4(CH_2O)_2P$– and *Ph*–CH_2–), 7.17 (ddd, $J = 7.5$, 7.4, 1.0 Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 7.12 (d, $J = 7.8$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 6.70 (d, $J = 7.3$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 5.89 (m, 1H, –OCH_2–*CH*=CH_2), 5.40 (t, $J = 9.8$ Hz, 1H, H-3), 5.32–5.27 (m, 2H, NH and –OCH_2–CH=CH_2), 5.23 (dd, $J = 9.2$, 1.0 Hz, 1H, –OCH_2–CH=CH_2), 5.12–4.94 (m, 5H, o-$C_6H_4(CH_2O)_2P$– and H-1), 4.76 (dd, $J = 18.5$, 9.3 Hz, 1H, H-4), 4.66–4.55 (m, 6H, –CO–O–CH_2–CCl_3, p-CF_3–C_6H_4–CH_2–, and Ph–CH_2–), 4.22 (dd, $J = 12.7$, 5.3 Hz, 1H, –OCH_2CH=CH_2), 4.06–3.99 (m, 3H, –OCH_2CH=CH_2, H-2, and H-5), 3.90 (m, 1H, β-CH of 3-*O*-acyl), 3.80 (dd, $J = 11.2$, 2.0 Hz, 1H, H-6a), 3.74 (dd, $J = 10.7$, 4.9 Hz, 1H, H-6b), 2.75 (dd, $J = 17.1$, 7.8 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.56 (dd, $J = 17.1$, 3.9 Hz, 1H, α-CH_2 of 3-*O*-acyl), 1.42–1.21 (m, 12H, $CH_2 \times 6$), 0.88 (t, $J = 6.8$ Hz, 3H, –CH_2–CH_3).

6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranose (16). To a degassed solution of **15** (980.1 mg, 0.985 mmol) in dry THF (14 mL) was added $[Ir(cod)(MePh_2P)_2]PF_6$ (83.3 mg, 0.0985 mmol) activated with H_2 in THF (10 mL). After being stirred under Ar at room temperature for 2 h, iodine (300.3 mg, 1.183 mmol) and water (20 mL) were added and the reaction mixture was stirred for additional 30 min. The reaction mixture was quenched with aqueous 10% $Na_2S_2O_3$ (10%, 10 mL). The mixture was then extracted with EtOAc. The organic layer was washed with aqueous sat $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (40 g, $CHCl_3$:acetone = 10:1) to give compound **16** as a pale yellow solid (698.7 mg, 74%). $[\alpha]_D^{22} = +12.3$ (c 0.998, $CHCl_3$). ESI-MS (positive) m/z 976.3 $[M+Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, $J = 8.3$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.42–7.25 (m, 6H, o-$C_6H_4(CH_2O)_2P$– and *Ph*–CH_2–), 7.41 (d, $J = 8.0$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.17 (ddd, $J = 7.6$, 7.6, 1.2 Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 7.12 (d, $J = 7.6$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 6.70 (d, $J = 7.3$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 5.44 (t, $J = 10.0$ Hz, 1H, H-3), 5.36 (d, $J = 9.5$ Hz, 1H, NH), 5.30 (t, $J = 3.4$ Hz, 1H, H-1), 5.09–4.92 (m, 4H, o-$C_6H_4(CH_2O)_2P$–), 4.71–4.55 (m, 7H, –CO–O–CH_2–CCl_3, p-CF_3–C_6H_4–CH_2–, Ph–CH_2–, and H-4), 4.25 (m, 1H, H-

5), 3.98 (ddd, $J = 10.0, 10.0, 2.9$ Hz, 1H, H-2), 3.90 (m, 1H, β-CH of 3-*O*-acyl), 3.79 (dd, $J = 10.7, 1.8$ Hz, 1H, H-6a), 3.71 (dd, $J = 9.8, 6.0$ Hz, 1H, H-6b), 3.46 (brs, 1H, C_1–OH), 2.75 (dd, $J = 17.0, 7.9$ Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.56 (dd, $J = 17.0, 4.0$ Hz, 1H, α-CH_2 of 3-*O*-acyl), 1.36–1.27 (m, 12H, $CH_2 \times 6$), 0.88 (t, $J = 7.0$ Hz, 3H, $-CH_2-CH_3$). Found: C, 52.59; H, 5.07; N, 1.51%. Calcd for $C_{42}H_{50}Cl_3F_3NO_{12}P$: C, 52.81; H, 5.28; N, 1.47%.

6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranosyl Trichloroacetimidate (17). To a solution of 1-liberated **16** (123.0 mg, 128.8 µmol) and CCl_3CN (64.7 µL, 645 µmol) in dry CH_2Cl_2 (7 mL) were added Cs_2CO_3 (24.4 mg, 74.9 µmol) at rt. After being stirred for 1 h, to the reaction mixture were added CCl_3CN (64.7 µL, 645 µmol), Cs_2CO_3 (32.4 mg, 99.4 µmol), and the reaction mixture was stirred for an additional 45 min. The reaction mixture was quenched with aqueous 10% $Na_2S_2O_3$. The mixture was then extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and then concentrated in vacuo to give **17** (139.6 mg, 98%) as a pale yellow solid), which was used for subsequent glycosylation without purification.

Allyl 4,6-*O*-Benzylidene-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)-decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (18). To a solution of **13** (3.36 g, 4.14 mmol) in AcOH (50 mL) was added Zn–Cu (prepared from 3.5 g of Zn), and the mixture was stirred at rt for 3 h. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residual solvent was removed by coevaporation with toluene (5 mL × 3). The residue was dissolved in EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue and (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) (2.06 g, 5.55 mmol) in anhydrous CH_2Cl_2 were added DCC (1.43 g, 6.93 mmol) at room temperature under Ar atmosphere and the mixture was stirred for 2 h. The insoluble materials were filtered off, and EtOAc was added to the filtrate. The solution was washed with aqueous sat $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (270 g, toluene:AcOEt = 5:1) to give compound **18** (3.49 g, 85%) as a colorless solid. $[\alpha]_D^{22} = +26.0$ (*c* 0.998, $CHCl_3$). ESI-MS (positive) *m/z* 988.6 $[M+H]^+$, 1010.6 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48 (d, $J = 8.1$ Hz, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.39 (m, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.31–7.23 (m, 4H, =CH–C_6H_5), 7.17 (m, 1H, =CH–C_6H_5), 5.99 (d, $J = 9.5$ Hz, 1H, NH), 5.90 (m, 1H, $-OCH_2-CH=CH_2$), 5.47 (s, 1H, =CH–C_6H_5), 5.37 (t, $J = 10.0$ Hz, 1H, H-3), 5.31 (dd, $J = 17.1, 1.5$ Hz, 1H, $-OCH_2-CH=CH_2$), 5.24 (dd, $J = 10.4, 1.2$ Hz, 1H, $-OCH_2-CH=CH_2$), 5.09 (m, 1H, β-CH of 2-*N*-acyl), 4.87 (d, $J = 3.7$ Hz, 1H, H-1), 4.53 (d, $J = 12.2$ Hz, 1H, $-CH_2-C_6H_4-CF_3$), 4.42 (d, $J = 12.2$ Hz, 1H, $-CH_2-C_6H_4-CF_3$), 4.36 (ddd, $J = 6.8, 6.8, 3.8$ Hz, 1H, H-2), 4.29 (dd, $J = 10.2, 4.8$ Hz, 1H, H-6a), 4.20 (ddt, $J = 12.7, 5.2, 1.5$ Hz, 1H, $-OCH_2CH=CH_2$), 4.00 (dd, $J = 16.6, 6.4$, Hz, 1H, $-OCH_2CH=CH_2$), 3.93 (ddd, $J = 10.2, 9.8, 5.1$ Hz, 1H, H-5), 3.81 (m, 1H, β-CH of 3-*O*-acyl), 3.79–3.69 (m, 2H, H-4, H-6b), 2.67 (dd, $J = 15.3, 6.8$ Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.49–2.33 (m, 3H, α-CH_2 of 3-*O*-acyl and 2-*N*-acyl's main chain), 2.28 (t, $J = 7.4$ Hz, 2H, α-CH_2 of 2-*N*-acyl's side chain), 1.64–1.46 (m, 6H, γ-CH_2 of 3-*O*-acyl, 2-*N*-acyl's main chain, and β-CH_2 of 2-*N*-acyl's side chain), 1.37–1.15 (m, 36H, $CH_2 \times 18$), 0.89–0.84 (m, 9H, $-CH_2-CH_3 \times 3$). Found: C, 68.75; H, 9.09; N, 2.33%. Calcd for $C_{56}H_{84}F_3NO_{10}$: C, 68.06; H, 8.57; N, 1.42%.

Allyl 2-Deoxy-2-[(*R*)-3-(dodecanoyloxy)decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (19). To a solution of **18** (3.33 g, 3.37 mmol) in dry CH_2Cl_2 (72 mL) was added 90% TFA aqueous solution (3 mL) at 0 °C. The mixture was stirred under Ar for 2.5 h while warming gradually up to room temperature. The reaction mixture was quenched with aqueous sat $NaHCO_3$. The mixture was then extracted with $CHCl_3$. The organic layer was washed with aqueous sat $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (160 g, $CHCl_3$:acetone = 5:1 to 3:1) to give compound **19** as a colorless oil (2.28 g, 75%). ESI-MS (positive) *m/z* 900.6 $[M+H]^+$, 922.6 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, $J = 8.1$ Hz, 2H, $-CH_2-C_6H_4-CF_3$), 7.42 (d, $J = 8.1$ Hz, 2H, $-C_6H_4-CF_3$), 5.94 (d, $J = 9.0$ Hz, 1H, NH), 5.89 (m, 1H, $-OCH_2-CH=CH_2$), 5.30 (dd, $J = 17.3, 1.5$ Hz, 1H, $-OCH_2-CH=CH_2$), 5.23 (dd, $J = 10.5, 1.2$ Hz, 1H, $-OCH_2-CH=CH_2$), 5.15–5.05 (m, 2H, H-3 and β-CH of 2-*N*-acyl), 4.85 (d, $J = 3.7$ Hz, 1H, H-1), 4.57 (s, 2H, $-CH_2-C_6H_4-CF_3$), 4.22 (m, 1H, H-2), 4.18 (ddt, $J = 14.7, 5.1, 1.5$ Hz, 1H, $-OCH_2CH=CH_2$), 3.98 (ddt, $J = 12.8, 6.3, 1.2$ Hz, 1H, $-OCH_2CH=CH_2$), 3.90–3.70 (m, 5H, H-4, H-5, H-6ab, and β-CH of 3-*O*-acyl), 2.65 (dd, $J = 14.9, 7.8$ Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.53 (dd, $J = 14.9, 4.9$ Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.40 (dd, $J = 14.8, 7.0$ Hz, 1H, α-CH_2 of 2-*N*-acyl's main chain), 2.35–2.25 (m, 3H, α-CH_2 of 2-*N*-acyl's main chain and 2-*N*-acyl's side chain), 1.71–1.50 (m, 6H, γ-CH_2 of 3-*O*-acyl, 2-*N*-acyl's main chain, and β-CH_2 of 2-*N*-acyl's side chain), 1.37–1.16 (m, 36H, $CH_2 \times 18$), 0.90–0.85 (m, 9H, $-CH_2-CH_3 \times 3$).

Allyl 6-*O*-(6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-(2,2,2-trichloroethoxycarbonylamino)-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-β-D-glucopyranosyl)-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)-decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (22). To a mixture of the imidate **17** (139 mg, 126 µmol), the glycosyl acceptor **19** (94.3 mg, 105 µmol), and MS4A (1 g) in dry CH_2Cl_2 (7 mL) at −20 °C was added TMSOTf (2.64 µL, 14.6 µmol). After being stirred at the same temperature for 30 min, TMSOTf (2.50 µL, 13.8 µmol) was added to the solution, and the mixture was stirred further 30 min.

The reaction was quenched with aqueous $NaHCO_3$ (100 mL), and MS4A was filtered off. The mixture was extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash chromatography (20 g, $CHCl_3$:acetone = 20:1) to give **22** as a colorless solid (129 g, 72%). $[\alpha]_D^{22} = +13.5$ (*c* 0.643, $CHCl_3$). ESI-MS (positive) *m/z* 1836.0 $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56 (d, $J = 8.1$ Hz, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.52 (d, $J = 8.1$ Hz, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.44 (d, $J = 7.9$ Hz, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.41 (d, $J = 7.9$ Hz, 2H, *p*-CF_3–C_6H_4–CH_2–), 7.40–7.26 (m, 6H, *o*-$C_6H_4(CH_2O)_2P$– and *Ph*–CH_2–), 7.19 (dd, $J = 7.4, 7.4$ Hz, 1H, *o*-$C_6H_4(CH_2O)_2P$–), 7.12 (d, $J = 7.3$ Hz, 1H, *o*-$C_6H_4(CH_2O)_2P$–), 6.75 (d, $J = 7.5$ Hz, 1H, *o*-$C_6H_4(CH_2O)_2P$–), 5.91 (d, $J = 9.5$ Hz, 1H, 2-NH), 5.87 (m, 1H, $-OCH_2-CH=CH_2$), 5.42 (t, $J = 9.9$ Hz, 1H, H-3′), 5.29 (dd, $J = 17.2, 1.5$ Hz, 1H, $-OCH_2-CH=CH_2$), 5.21 (m, 2H, $-OCH_2-CH=CH_2$ and 2′-NH), 5.12 (dd, $J = 10.5, 9.3$ Hz, 1H, H-3), 5.07 (m, 1H, β-CH of 2-*N*-acyl), 5.06–4.90 (m, 4H, *o*-$C_6H_4(CH_2O)_2P$–), 4.83–4.79 (m,

2H, H-1 and H-1′), 4.67 (m, 1H, H-4′), 4.66–4.53 (m, 8H, $-CO-O-CH_2-CCl_3$, p-$CF_3-C_6H_4-CH_2-$ × 2, and $Ph-CH_2-$), 4.21 (ddd, J = 10.8, 9.4, 3.7 Hz, 1H, H-2), 4.16 (ddt, J = 12.9, 5.3, 1.4 Hz, 1H, $-OCH_2CH{=}CH_2$), 4.07 (d, J = 9.3 Hz, 1H, H-6a), 3.94 (ddt, J = 12.8, 6.3, 1.1 Hz, 1H, $-OCH_2CH{=}CH_2$), 3.90–3.86 (m, 2H, β-CH of 3-*O*-acyl and 3′-*O*-acyl), 3.84–3.76 (m, 3H, H-5, H-6′a, and H-6′b), 3.74–3.70 (m, 2H, H-6b and H-5′), 3.64 (ddd, J = 9.2, 9.2, 4.3 Hz, 1H, H-4), 3.50 (dd, J = 18.4, 8.3 Hz, 1H, H-2′), 2.84 (brs, 1H, C_4-OH), 2.74 (dd, J = 16.8, 7.5 Hz, 1H, α-CH_2 of 3′-*O*-acyl), 2.66–2.61 (m, 2H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.50 (dd, J = 15.3, 4.6 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.38 (dd, J = 14.7, 6.8 Hz, 1H, α-CH_2 of 2-*N*-acyl's main chain), 2.29 (dd, J = 14.8, 5.3 Hz, 1H, α-CH_2 of 2-*N*-acyl's main chain), 2.28–2.25 (m, 2H, α-CH_2 of 2-*N*-acyl's side chain), 1.60–1.50 (m, 6H, γ-CH_2 of 2-*N*-acyl's main chain, 3-*O*-acyl, and 3′-*O*-acyl), 1.38–1.25 (m, 48H, CH_2 × 24), 0.89–0.86 (m, 12H, $-CH_2-CH_3$ × 4).

Allyl 6-*O*-(6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)-decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-β-D-glucopyranosyl)-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)-decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranoside (23). To a solution of **22** (104.9 mg, 57.1 µmol) in AcOH (3 mL) was added Zn powder (400 mg), and the mixture was stirred at rt for 1.5 h. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo. The residual solvent was removed by coevaporation with toluene (5 mL × 3). The residue was dissolved in EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated in vacuo. To a solution of the residue and (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) (29.5 mg, 79.6 µmol) in anhydrous CH_2Cl_2 were added HOBt (6.54 mg, 48.4 µmol) and WSCD·HCl (21.0 mg, 110 µmol) at room temperature under Ar atmosphere, and the mixture was stirred for 21 h. Aqueous sat $NaHCO_3$ was added to the mixture, and the mixture was extracted with EtOAc. The organic layer was washed with aqueous sat $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (9 g, $CHCl_3$:acetone = 10:1) to give compound **23** (78.7 g, 73%) as a colorless oil. $[\alpha]_D^{22} = +16.4$ (*c* 0.700, $CHCl_3$). ESI-MS (positive) *m/z* 2014.4 $[M+H]^+$, 2035.4 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55 (d, J = 8.3 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.51 (d, J = 8.0 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.43 (d, J = 8.3 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.40 (d, J = 8.0 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.37–7.24 (m, 6H, o-$C_6H_4(CH_2O)_2P-$ and $Ph-CH_2-$), 7.18 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 7.11 (d, J = 7.3 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 6.73 (d, J = 7.5 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 6.01 (d, J = 7.8 Hz, 1H, 2′-NH), 5.92 (d, J = 9.3 Hz, 1H, 2-NH), 5.86 (m, 1H, $-OCH_2-CH{=}CH_2$), 5.41 (dd, J = 10.4, 9.2 Hz, 1H, H-3′), 5.27 (ddd, J = 17.3, 2.9, 1.5 Hz, 1H, $-OCH_2-CH{=}CH_2$), 5.19 (dd, J = 10.8, 1.5 Hz, 1H, $-OCH_2-CH{=}CH_2$), 5.15 (dd, J = 10.3, 8.8 Hz, 1H, H-3), 5.10–5.03 (m, 2H, β-CH of 2-*N*-acyl and 2′-*N*-acyl), 4.99–4.91 (m, 5H, o-$C_6H_4(CH_2O)_2P-$ and H-1′), 4.81 (d, J = 3.7 Hz, 1H, H-1), 4.67–4.50 (m, 7H, p-$CF_3-C_6H_4-CH_2-$ × 2, $Ph-CH_2-$, and H-4′), 4.21 (ddd, J = 9.3, 9.3, 3.7 Hz, 1H, H-2), 4.15 (dd, J = 12.9, 5.3 Hz, 1H, $-OCH_2CH{=}CH_2$), 4.02 (dd, J = 10.7, 1.9 Hz, 1H, H-6a), 3.94 (dd, J = 12.9, 5.3 Hz, 1H, $-OCH_2CH{=}CH_2$), 3.91–3.84 (m, 2H, β-CH of 3-*O*-acyl and 3′-*O*-acyl), 3.83 (m, 1H, H-6′a), 3.74–3.61 (m, 7H, H-4, H-5, H-6b, H-2′, H-5′, H-6′b, and C_4-OH), 2.75–2.60 (m, 3H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.47 (dd, J = 15.7, 4.8 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.38–2.33 (m, 2H, α-CH_2 of 2-*N*-acyl's main chain and 2′-*N*-acyl's main chain), 2.30–2.21 (m, 6H, α-CH_2 of 2-*N*-acyl's main chain, 2-*N*-acyl's side chain, 2′-*N*-acyl's main chain, and 2-*N*-acyl's side chain), 1.60–1.51 (m, 8H, γ-CH_2 of 2-*N*-acyl's main chain, 2-*N*′-acyl's main chain, 3-*O*-acyl, and 3′-*O*-acyl), 1.38–1.25 (m, 76H, CH_2 × 38), 0.89–0.86 (m, 18H, $-CH_2-CH_3$ × 6).

6-*O*-(6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-β-D-glucopyranosyl)-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranose (24). To a solution of **23** (70.4 mg, 34.9 µmol) in dry THF (4 mL) was added $[Ir(cod)(MePh_2P)_2]PF_6$ (9.3 mg, 11 µmol) activated with H_2 in THF (4 mL). After being stirred under Ar at room temperature for 2 h, iodine (9.5 mg, 37 µmol) and water (4 mL) were added and the reaction mixture was stirred for an additional 1 h. The reaction mixture was quenched with aqueous 10% $Na_2S_2O_3$. The mixture was then extracted with EtOAc. The organic layer was washed with aqueous sat $NaHCO_3$ and brine, dried over $MgSO_4$, and then concentrated in vacuo. The residue was purified by silica-gel flash chromatography (5 g, $CHCl_3$:acetone = 5:1) to give compound **24** as a pale yellow solid (53.8 mg, 78%). ESI-MS (positive) *m/z* 988.0 $[M+2H]^{2+}$, 1975.2 $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (d, J = 8.1 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.52 (d, J = 8.1 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.44 (d, J = 8.4 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.41 (d, J = 8.1 Hz, 2H, p-$CF_3-C_6H_4-CH_2-$), 7.38–7.31 (m, 4H, $Ph-CH_2-$), 7.29–7.25 (m, 3H, o-$C_6H_4(CH_2O)_2P-$ and $Ph-CH_2-$), 7.19 (dd, J = 7.7, 7.7 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 7.12 (d, J = 7.5 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 6.76 (d, J = 7.8 Hz, 1H, o-$C_6H_4(CH_2O)_2P-$), 6.03 (d, J = 7.9 Hz, 1H, 2′-NH), 5.92 (d, J = 9.1 Hz, 1H, 2-NH), 5.45 (dd, J = 10.4, 9.2 Hz, 1H, H-3′), 5.19 (d, J = 7.9 Hz, 1H, H-1′), 5.15 (brs, 1H, H-1), 5.13–5.06 (m, 2H, H-3 and β-CH of 2′-*N*-acyl), 5.03–4.88 (m, 5H, β-CH of 2-*N*-acyl and o-$C_6H_4(CH_2O)_2P-$), 4.67–4.52 (m, 8H, p-$CF_3-C_6H_4-CH_2-$ × 2, $Ph-CH_2-$, H-4′, and C_1-OH), 4.14 (dd, J = 9.4, 9.4 Hz, 1H, H-2), 4.04–3.98 (m, 2H, H-5 and H-6′a), 3.91–3.84 (m, 2H, β-CH of 3-*O*-acyl and 3′-*O*-acyl), 3.83 (m, 1H, H-6′b), 3.73–3.70 (m, 3H, H-6a, H-6b, and H-5′), 3.50 (ddd, J = 8.6, 7.9, 7.9 Hz, 1H, H-2′), 3.42 (ddd, J = 9.4, 9.4, 4.1 Hz, 1H, H-4), 2.91 (d, J = 4.4 Hz, 1H, C_4-OH), 2.74–2.62 (m, 3H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.50 (dd, J = 15.1, 4.9 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.40–2.34 (m, 2H, α-CH_2 of 2-*N*-acyl's main chain and 2′-*N*-acyl's main chain), 2.32–2.21 (m, 6H, α-CH_2 of 2-*N*-acyl's main chain, 2-*N*-acyl's side chain, 2′-*N*-acyl's main chain, and 2-*N*-acyl's side chain), 1.58–1.53 (m, 8H, γ-CH_2 of 2-*N*-acyl's main chain, 2-*N*′-acyl's main chain, 3-*O*-acyl, and 3′-*O*-acyl), 1.38–1.25 (m, 76H, CH_2 × 38), 0.89–0.86 (m, 18H, $-CH_2-CH_3$ × 6).

6-*O*-[6-*O*-Benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-2-[(*R*)-3-(dodecanoyloxy)decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-β-D-glucopyranosyl]-1-*O*-bis(benzyloxy)phosphoryl-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)decanoylamino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-α-D-glucopyranose (25). To a mixture of **24** (33.1 mg, 16.8 µmol) and tetrabenzyl diphosphate (13.5 mg, 25.1 µmol) in dry THF (4 mL) was added 1.08 M (1 M = 1 mol dm^{-3}) $LiN(TMS)_2$ (22.0 µL, 23.8 µmol) at −78 °C and the mixture was stirred at −78 °C for 40 min. After addition of tetrabenzyl diphosphate (12.1 mg, 22.5 µmol) in dry THF (4 mL) was

added 1.08 M LiN(TMS)$_2$ (10.0 µL, 10.8 µmol), the reaction mixture was further stirred for 50 min. After addition of saturated aqueous $NaHCO_3$, the mixture was extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by ULTRA PACK™ $\phi 11 \times 150$ mm (YAMAZEN Co., Tokyo, $CHCl_3$:acetone:Et_3N = 10:1:0.002) to give **25** as a yellowish oil (28.2 mg, 75%). ESI-MS (positive) m/z 2234.4 $[M+H]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.57 (d, $J = 8.1$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.49 (d, $J = 8.1$ Hz, 2H, p-CF_3–C_6H_4–CH_2–), 7.44–7.41 (m, 4H, p-CF_3–C_6H_4–CH_2–), 7.41–7.31 (m, 15H, Ph–CH_2– and $(Ph$–$CH_2O)_2P$–), 7.28–7.26 (m, 1H, o-$C_6H_4(CH_2O)_2P$–), 7.18 (ddd, $J = 7.5$, 7.5, 1.2 Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 7.10 (d, $J = 7.0$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 6.74 (d, $J = 7.0$ Hz, 1H, o-$C_6H_4(CH_2O)_2P$–), 6.52 (d, $J = 8.1$ Hz, 2′-NH), 5.93 (d, $J = 8.7$ Hz, 1H, 2-NH), 5.66 (dd, $J = 5.0$, 3.4 Hz, 1H, H-1), 5.33 (dd, $J = 10.7$, 10.5 Hz, 1H, H-3′), 5.11 (dd, $J = 9.5$, 9.5 Hz, 1H, H-3), 5.09 (m, 1H, β-CH of 2′-N-acyl), 5.05–4.99 (m, 8H, o-$C_6H_4(CH_2O)_2P$– and (Ph–$CH_2O)_2P$–), 4.95–4.88 (m, 2H, β-CH of 2-N-acyl and H-1′), 4.66–4.50 (m, 7H, p-CF_3–C_6H_4–CH_2– × 2, Ph–CH_2–, and H-4′), 4.22 (m, 1H, H-2), 3.98–3.96 (m, 2H, H-5 and H-6′a), 3.93–3.85 (m, 2H, β-CH of 3-O-acyl and 3′-O-acyl), 3.82–3.79 (m, 2H, H-6a and H-6′b), 3.72–3.68 (m, 2H, H-6b and H-2′), 3.67–3.61 (m, 2H, H-4 and H-5′), 2.72 (dd, $J = 16.8$, 7.5 Hz, 1H, α-CH_2 of 3′-O-acyl), 2.66–2.61 (m, 2H, α-CH_2 of 3-O-acyl and 3′-O-acyl), 2.55 (dd, $J = 15.6$, 4.7 Hz, 1H, α-CH_2 of 3-O-acyl), 2.38 (dd, $J = 15.6$, 6.1 Hz, 1H, α-CH_2 of 2′-N-acyl's main chain), 2.30–2.21 (m, 6H, α-CH_2 of 2-N-acyl's main chain and side chain, 2′-N-acyl's main chain and side chain), 2.11 (m, 1H, α-CH_2 of 2-N-acyl's main chain), 1.58–1.50 (m, 8H, γ-CH_2 of 2-N-acyl's main chain, 2-N'-acyl's main chain, 3-O-acyl, and 3′-O-acyl), 1.36–1.22 (m, 76H, CH_2 × 38), 0.89–0.86 (m, 18H, –CH_2–CH_3 × 6).

6-O-{2-Deoxy-2-[(R)-3-(dodecanoyloxy)decanoylamino]-3-O-[(R)-3-hydrodecanoyl]-β-D-glucopyranosyl}-2-deoxy-2-[(R)-3-(dodecanoyloxy)decanoylamino]-3-O-[(R)-3-hydrodecanoyl]-α-D-glucopyranose 1,4′-Bisphosphate (10a). To a solution of **25** (37.0 mg, 16.6 µmol) in dry THF (4 mL) was added Pd-black (42 mg) at rt and the mixture was stirred at rt under H_2 (20 atm) for 44 h. After addition of 10% Et_3N–THF solution (46.3 µL), Pd-black was filtered off with a membrane filter. The organic layer was concentrated under reduced pressure. The residue was lyophilized with water to give crude **10a**. The compound **10a** was purified by liquid–liquid partition column chromatography (5 g of Sephadex® LH-20, $CHCl_3$:MeOH:^{i}PrOH:H_2O = 8:8:1:6), wherein organic and aqueous layers were used for stationary and mobile phases, respectively, to give **10a** (23.9 mg, 93%) as a white powder. ESI-MS (negative) m/z 771.5 $[M-2H]^{2-}$, 1543.9 $[M-H]^-$. ^{1}H NMR (500 MHz, $CDCl_3$:MeOH-d_4 = 1:1): δ 5.40–5.00 (m, 4H), 4.80–4.4 (m, 2H), 4.4–4.18 (m, 3H), 4.18–3.78 (m, 4H), 3.6–3.4 (m, 1H), 3.4–3.06 (m, 7H), 2.59–2.26 (m, 12H), 1.62–1.38 (m, 8H, γ-CH_2 of 3-O-acyl, 3′-O-acyl, 2-N-acyl's main chain, and 2′-N-acyl's main chain), 1.38–1.0 (m, 66H, –CH_2– × 33), 0.89 (t, $J = 6.3$ Hz, 18H, –CH_3 × 6).

1-Propenyl 4,6-O-Benzylidene-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-O-(2-propynyloxycarbonyl)-α-D-glucopyranoside (32). To a degassed solution of **31** (13.1 g, 24.7 mmol) in anhydrous THF (300 mL) was added (1,5-cyclooctadiene)[bis(methyldiphenylphosphine)]iridium(I) hexafluorophosphate (500 mg, 591 µmol). After activation of the iridium catalyst with H_2 three times (each 30 s), the mixture was stirred under Ar atmosphere at room temperature for 1.5 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in anhydrous CH_2Cl_2 (380 mL), and then DMAP (50.0 mg, 409 µmol), pyridine (20.0 mL, 247 mmol), and 2-propynyl chloroformate (7.20 mL, 74.2 mmol) were added to the solution at 0 °C under Ar atmosphere. After stirring at room temperature for 1 h, the reaction was quenched by addition of water. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with 0.5 M HCl and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (400 g, $CHCl_3$:acetone = 40:1) to give **32** as colorless powder (14.1 g, 94%). $[\alpha]_D^{22} = +52.0$ (c 1.00, $CHCl_3$). ESI-MS (positive) m/z 634.22 $[M+Na]^+$, 1246.23 $[2M+Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.77 (d, $J = 7.6$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.58 (dd, $J = 8.8$, 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.49 (dd, $J = 5.2$, 2.0 Hz, 2H, C_6H_5–CH=), 7.41 (dd, $J = 7.3$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.37 (dd, $J = 5.2$, 2.0 Hz, 3H, C_6H_5–CH=), 7.32 (d, $J = 8.8$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 6.12 (dd, $J = 12.0$, 1.6 Hz, 1H, –O–CH=CH–CH_3), 5.56 (s, 1H, C_6H_5–CH=), 5.23 (dd, $J = 12.0$, 6.9 Hz, 1H, –O–CH=CH–CH_3), 5.20 (d, $J = 10.8$ Hz, 1H, 2-NH), 5.17 (dd, $J = 10.1$, 10.1 Hz, 1H, H-3), 4.91 (d, $J = 3.5$ Hz, 1H, H-1), 4.76 (d, $J = 2.3$ Hz, 1H, –OCH_2–C≡CH of Proc), 4.64 (d, $J = 2.3$ Hz, 1H, –OCH_2–C≡CH of Proc), 4.38 (dd, $J = 10.5$, 10.5 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 4.29 (dd, $J = 10.3$, 4.8 Hz, 1H, H-6a), 4.22 (dd, $J = 10.5$, 10.5 Hz, 1H, $(C_6H_4)_2$–CH–CH_2–OCO), 4.14 (ddd, $J = 10.1$, 10.1, 3.5 Hz, 1H, H-2), 3.93 (ddd, $J = 9.9$, 9.9, 4.8 Hz, 1H, H-5), 3.80–3.73 (m, 2H, H-4 and H-6b), 2.33 (s, 1H, –OCH_2–C≡CH of Proc), 1.57 (dd, $J = 6.9$, 1.6 Hz, 3H, –O–CH=CH–CH_3). Anal. Calcd for $C_{35}H_{33}NO_9$: C, 68.73; H, 5.44; N, 2.29%. Found: C, 68.65; H, 5.58; N, 2.29%.

1-Propenyl 2-Allyloxycarbonylamino-4,6-O-benzylidene-2-deoxy-3-O-(2-propynyloxycarbonyl)-α-D-glucopyranoside (33). To a solution of **32** (116 mg, 190 µmol) in CH_2Cl_2 (1.5 mL) was added 1,3,4,6,7,8-hexahydro-2H-pyrimido[1,2-α]pyrimidine polymer-bound (PTBD) (30.0 mg, 240 µmol) at room temperature and the mixture was shaken for 1 d. PTBD was removed by filtration and the filtrate was concentrated in vacuo to give 2-N-deprotected product as a pale yellow solid: Yield 75.2 mg (quant.). To a solution of the 2-N-free product (74.0 mg, 190 µmol) in anhydrous CH_2Cl_2 (1.5 mL) were added allyl chloroformate (30.0 µL, 283 µmol) and pyridine (25.0 µL, 309 µmol) at 0 °C under Ar atmosphere. After stirring for 1 h, the reaction was quenched by addition of water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (12 g, $CHCl_3$:acetone = 40:1) to give **33** as a colorless solid (86.3 mg, 96%). $[\alpha]_D^{21} = +79.2$ (c 0.97, $CHCl_3$). ESI-MS (positive) m/z 474.20 $[M+H]^+$, 496.17 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (dd, $J = 4.0$, 2.4 Hz, 2H, C_6H_5–CH=), 7.35 (dd, $J = 4.0$, 2.4 Hz, 3H, C_6H_5–CH=), 6.12 (dd, $J = 12.0$, 1.6 Hz, 1H, –O–CH=CH–CH_3), 5.90 (dddd, $J = 16.0$, 10.8, 10.8, 5.6 Hz, 1H, –OCH_2–CH=CH_2 of Alloc), 5.52 (s, 1H, C_6H_5–CH=), 5.30 (dd, $J = 16.0$, 1.4 Hz, 1H, –OCH_2–CH=CH_2 of Alloc), 5.23–5.11 (m, 4H, 2-NH, H-3, –O–CH=CH–CH_3, and –OCH_2–CH=CH_2 of Alloc), 5.08 (d, $J = 3.6$ Hz, 1H, H-1), 4.72 (d, $J = 2.5$ Hz, 1H, –OCH_2–C≡CH of Proc), 4.68 (d, $J = 2.5$ Hz, 1H, –OCH_2–C≡CH of Proc), 4.61–4.54 (m, 2H, –OCH_2–CH=CH_2 of Alloc), 4.28 (dd, $J = 10.0$, 4.4 Hz, 1H, H-6a), 4.13 (ddd, $J = 10.4$, 10.4, 3.6 Hz, 1H, H-2), 3.91 (ddd,

$J = 9.6$, 9.6, 4.4 Hz, 1H, H-5), 3.77 (dd, $J = 10.0$, 4.4 Hz, 1H, H-6b), 3.75 (dd, $J = 9.6$, 9.6 Hz, 1H, H-4), 2.46 (t, $J = 2.4$ Hz, 1H, –OCH₂–C≡C*H* of Proc), 1.57 (dd, $J = 6.8$, 1.6 Hz, 3H, –O–CH=CH–C*H*₃). Anal. Calcd for $C_{24}H_{27}NO_9$: C, 60.88; H, 5.75; N, 2.96%. Found: C, 60.76; H, 5.89; N, 3.02%.

1-Propenyl 2-Allyloxycarbonylamino-6-*O*-benzyl-2-deoxy-3-*O*-(2-propynyloxycarbonyl)-α-D-glucopyranoside (34). To a solution of **33** (82.3 mg, 174 µmol) in anhydrous CH_2Cl_2 (1.5 mL) were added triethylsilane (260 µL, 1.63 mmol) and boron trifluoride diethyl etherate (40.0 µL, 316 µmol) at 0 °C under Ar atmosphere. After stirring for 2 h, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (11 g, $CHCl_3$:acetone = 40:1) to give **34** as a colorless solid (76.8 mg, 93%). $[\alpha]_D^{21} = +40.0$ (*c* 0.64, $CHCl_3$). ESI-MS (positive) *m/z* 498.21 $[M + Na]^+$. ¹H NMR (400 MHz, $CDCl_3$): δ 7.37–7.27 (m, 5H, C_6H_5–CH₂–), 6.14 (dd, $J = 12.0$, 1.6 Hz, 1H, –O–C*H*=CH–CH₃), 5.89 (dddd, $J = 17.3$, 10.8, 10.8, 5.6 Hz, 1H, –OCH₂–C*H*=CH₂ of Alloc), 5.28 (dd, $J = 17.3$, 1.4 Hz, 1H, –OCH₂–CH=C*H*₂ of Alloc), 5.22–5.16 (m, 3H, 2-NH, –O–CH=C*H*–CH₃, and –OCH₂–CH=C*H*₂ of Alloc), 5.06 (d, $J = 3.2$ Hz, 1H, H-1), 4.96 (dd, $J = 10.8$, 10.8 Hz, 1H, H-3), 4.73 (d, $J = 2.5$ Hz, 1H, –OC*H*₂–C≡CH of Proc), 4.69 (d, $J = 2.5$ Hz, 1H, –OC*H*₂–C≡CH of Proc), 4.62–4.51 (m, 2H, –OC*H*₂–CH=CH₂ of Alloc), 4.59 (d, $J = 12.1$ Hz, 2H, C₆H₅–C*H*₂–), 4.02 (ddd, $J = 10.8$, 10.8, 3.2 Hz, 1H, H-2), 3.88 (ddd, $J = 10.8$, 9.5, 3.2 Hz, 1H, H-4), 3.82–3.78 (m, 2H, H-5 and H-6a), 3.67 (dd, $J = 10.1$, 3.2 Hz, 1H, H-6b), 2.72 (d, $J = 3.2$ Hz, 1H, C₄-OH), 2.51 (t, $J = 2.4$ Hz, 1H, –OCH₂–C≡C*H* of Proc), 1.55 (dd, $J = 6.8$, 1.6 Hz, 3H, –O–CH=CH–C*H*₃). Anal. Calcd for $C_{24}H_{29}NO_9$: C, 60.62; H, 6.15; N, 2.95%. Found: C, 60.71; H, 6.19; N, 2.99%.

1-Propenyl 2-Allyloxycarbonylamino-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-3-*O*-(2-propynyloxycarbonyl)-α-D-glucopyranoside (35). To a solution of **34** (4.03 g, 8.48 mmol) in anhydrous CH_2Cl_2 (100 mL) were added *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine (2.10 g, 8.78 mmol) and 1*H*-tetrazole (2.97 g, 42.4 mmol) at room temperature under Ar atmosphere. After the mixture was stirred for 30 min and then at −20 °C for 10 min, *m*CPBA (2.10 g, 8.52 mmol) was added and stirring was continued for another 20 min. The solution was quenched by addition of saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (300 g, $CHCl_3$:acetone = 20:1) to give **35** as a colorless foamy solid (5.01 g, 91%). $[\alpha]_D^{21} = +37.0$ (*c* 1.00, $CHCl_3$). ESI-MS (positive) *m/z* 658.22 $[M + H]^+$, 680.20 $[M + Na]^+$. ¹H NMR (500 MHz, $CDCl_3$): δ 7.37–7.32 (m, 5H, C_6H_4–CH₂– and *o*-C_6H_4(CH₂O)₂P–), 7.28 (d, $J = 6.9$ Hz, 2H, C_6H_4–CH₂–), 7.26–7.19 (m, 2H, *o*-C_6H_4(CH₂O)₂P–), 6.15 (d, $J = 12.2$ Hz, 1H, –O–C*H*=CH–CH₃), 5.90 (dddd, $J = 17.2$, 10.3, 5.9, 5.9 Hz, 1H, –OCH₂–C*H*=CH₂ of Alloc), 5.36 (d, $J = 17.2$ Hz, 1H, –OCH₂–CH=C*H*₂ of Alloc), 5.25 (d, $J = 10.3$ Hz, 1H, –OCH₂–CH=C*H*₂ of Alloc), 5.23–5.07 (m, 6H, H-3, *o*-C₆H₄(C*H*₂O)₂P–, and –O–CH=C*H*–CH₃), 5.03 (d, $J = 9.6$ Hz, 1H, 2-NH), 4.93 (d, $J = 3.5$ Hz, 1H, H-1), 4.73 (d, $J = 2.5$ Hz, 1H, –OC*H*₂–C≡CH of Proc), 4.69–4.66 (m, 2H, H-4 and –OC*H*₂–C≡CH of Proc), 4.64–4.62 (m, 2H, –OC*H*₂–CH=CH₂ of Alloc), 4.58 (d, $J = 11.6$ Hz, 1H, C₆H₄–C*H*₂–), 4.56 (d, $J = 11.6$ Hz, 1H, C₆H₄–C*H*₂–), 4.09 (ddd, $J = 10.3$, 9.6, 3.5 Hz, 1H, H-2), 3.99 (ddd, $J = 9.9$, 9.9, 4.8 Hz, 1H, H-5), 3.83 (d, $J = 10.3$ Hz, 1H, H-6a), 3.77 (dd, $J = 10.3$, 4.8 Hz, 1H, H-6b), 2.43 (t, $J = 2.5$ Hz, 1H, –OCH₂–C≡C*H* of Proc), 1.55 (dd, $J = 6.9$, 1.6 Hz, 3H, –O–CH=CH–C*H*₃). Anal. Calcd for $C_{32}H_{36}NO_{12}P$: C, 58.45; H, 5.52; N, 2.13%. Found: C, 58.45; H, 5.64; N, 2.11%.

2-Allyloxycarbonylamino-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-3-*O*-(2-propynyloxycarbonyl)-D-glucopyranosyl Trichloroacetimidate (27). To a solution of **35** (4.69 g, 7.13 mmol) in THF (150 mL) were added water (100 mL) and iodine (1.82 g, 7.17 mmol) at room temperature. After the mixture was stirred for 30 min, aqueous 10% $Na_2S_2O_3$ was added to quench the reaction. The mixture was extracted with EtOAc and the organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (250 g, $CHCl_3$:acetone = 5:1 to 3:1) to give 1-OH product as a colorless foamy solid (3.16 g, 73%). ESI-MS (positive) *m/z* 618.31 $[M + H]^+$, 640.29 $[M + Na]^+$. ¹H NMR (500 MHz, $CDCl_3$) selected data for α-isomer: δ 7.35–7.27 (m, 7H, C_6H_4–CH₂– and *o*-C_6H_4(CH₂O)₂P–), 7.21 (dd, $J = 8.2$, 4.6 Hz, 2H, *o*-C_6H_4(CH₂O)₂P–), 5.91–5.84 (m, 1H, –OCH₂–C*H*=CH₂ of Alloc), 5.28 (d, $J = 17.2$ Hz, 1H, –OCH₂–CH=C*H*₂ of Alloc), 5.23–5.06 (m, 6H, H-3, *o*-C₆H₄(C*H*₂O)₂P–, and –OCH₂–CH=C*H*₂ of Alloc), 4.96 (d, $J = 9.6$ Hz, 1H, 2-NH), 4.73 (d, $J = 2.5$ Hz, 1H, –OC*H*₂–C≡CH of Proc), 4.66 (d, $J = 3.8$ Hz, 1H, H-1), 4.65–4.55 (m, 6H, H-4, C₆H₄–C*H*₂–, –OC*H*₂–C≡CH of Proc, and –OC*H*₂–CH=CH₂ of Alloc), 4.22 (ddd, $J = 9.9$, 9.9, 4.8 Hz, 1H, H-5), 4.09 (ddd, $J = 9.6$, 9.6, 3.8 Hz, 1H, H-2), 3.83 (dd, $J = 10.8$, 4.8 Hz, 1H, H-6a), 3.74 (dd, $J = 10.8$, 9.9 Hz, 1H, H-6b), 3.40 (brs, 1H, C₁-OH), 2.48 (brs, 1H, –OCH₂–C≡C*H* of Proc). Anal. Calcd for $C_{29}H_{32}NO_{12}P$: C, 56.40; H, 5.22; N, 2.27%. Found: C, 56.46; H, 5.23; N, 2.21%.

To a solution of the 1-OH product (2.66 g, 4.31 mmol) in anhydrous CH_2Cl_2 (50 mL) were added trichloroacetonitrile (9.32 mL, 43.1 mmol) and Cs_2CO_3 (700 mg, 2.15 mmol). After stirring for 1 h, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give **27** (3.25 g, 99%) as a pale yellow solid, which was used for the subsequent glycosylation without further purification.

Formylmethyl 4-*O*-(4-Azidophenylmethyl)-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-*O*-(4-methoxyphenylmethyl)-α-D-glucopyranoside (37). To a solution of **36** (4.58 g, 6.61 mmol) in THF–*t*-BuOH–water (10:10:1) (84 mL) were added NMO (3.00 g, 25.6 mmol) and OsO_4 in water (25 g L⁻¹, 10.0 mL, 984 µmol) at room temperature. After stirring for 4 h, the mixture was added to 10% aqueous $Na_2S_2O_3$ and extracted with EtOAc. The organic layer was washed successively with 10% aqueous $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo to give the crude diol (4.88 g), which was subjected to the following oxidation without further purification. To a suspension of crude diol thus obtained in anhydrous benzene–CH_2Cl_2 (2:3) (100 mL) was added $Pb(OAc)_4$ (90% purity, 4.40 g, 9.92 mmol) at room temperature under Ar atmosphere. After stirring for 4 h, the mixture was filtered through a short silica-gel column (30 g) using EtOAc as an eluent. The filtrate was concentrated in vacuo and then the residue was purified by silica-gel flash column chromatography (200 g, $CHCl_3$:acetone = 5:1) to give **37** (4.49 g, 98%) as a pale brown foamy solid. ESI-MS (positive) *m/z* 717.31 $[M + Na]^+$. ¹H NMR (400 MHz, $CDCl_3$): δ 9.65 (s, 1H,

–OCH_2–CHO), 7.76 (d, $J = 7.3$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.59 (dd, $J = 7.6$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.41 (dd, $J = 7.3$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.35 (d, $J = 8.3$ Hz, 2H, p-N_3–C_6H_4–CH_2–), 7.32 (d, $J = 7.6$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.16 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 7.01 (d, $J = 8.3$ Hz, 2H, p-N_3–C_6H_4–CH_2–), 6.75 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 4.85 (d, $J = 10.3$ Hz, 1H, 2-NH), 4.83 (d, $J = 3.3$ Hz, 1H, H-1), 4.80 (d, $J = 11.2$ Hz, 1H, p-N_3–C_6H_4–CH_2–), 4.70 (d, $J = 11.5$ Hz, 1H, p-N_3–C_6H_4–CH_2–), 4.66 (d, $J = 11.3$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.62 (d, $J = 11.3$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.44 (dd, $J = 10.7$, 6.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 4.21 (dd, $J = 6.3$, 6.3 Hz, 1H, $(C_6H_4)_2$–CH–CH_2–OCO), 3.94 (ddd, $J = 10.3$, 10.3, 3.3 Hz, 1H, H-2), 3.85–3.78 (m, 2H, H-3, H-6a, and –OCH_2–CHO), 3.75–3.65 (m, 5H, H-5, H-6b, and p-CH_3O–C_6H_4–CH_2–), 3.60 (dd, $J = 8.7$, 8.7 Hz, 1H, H-4).

Benzyloxycarbonylmethyl 4-*O*-(4-Azidophenylmethyl)-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-*O*-(4-methoxyphenylmethyl)-α-D-glucopyranoside (38). To a solution of **37** (8.15 g, 11.7 mmol), NaH_2PO_4 (2.20 g, 18.3 mmol), and 2-methyl-2-butene (6.22 mL, 58.7 mmol) in THF–t-BuOH–water (2:4:1) (280 mL) was added $NaClO_2$ (80% purity, 4.0 g, 35.4 mmol) at room temperature and the mixture was stirred for 9 h. The reaction mixture was acidified by addition of 1 M HCl and extracted with $CHCl_3$. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give crude carboxylic acid product. To a suspension of the crude carboxylic product in Et_2O (100 mL) was added solution of phenyldiazomethane in Et_2O (0.24 M, 60 mL, 14.4 mmol) at room temperature and the mixture was stirred for 1 h. After another solution of phenyldiazomethane (=(diazo)phenylmethane) in Et_2O (0.24 M, 60 mL, 14.4 mmol) was added, the mixture was stirred for an additional 1 h and then concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (450 g, $CHCl_3$:acetone = 40:1 to 3:1) to give **38** (7.79 g, 83%) as a pale yellow solid. $[\alpha]_D^{21} = +21.6$ (c 0.99, $CHCl_3$). ESI-MS (positive) m/z 801.27 $[M + H]^+$, 823.32 $[M + Na]^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, $J = 7.2$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.63 (dd, $J = 7.2$, 7.0 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.59 (dd, $J = 7.8$, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.37 (d, $J = 8.3$ Hz, 2H, p-N_3–C_6H_4–CH_2–), 7.32 (dd, $J = 7.2$, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.30–7.25 (m, 3H, –OCH_2–$COOCH_2$–C_6H_5), 7.16 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 6.98 (d, $J = 8.3$ Hz, 2H, p-N_3–C_6H_4–CH_2–), 6.74 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 5.45 (d, $J = 9.2$ Hz, 1H, 2-NH), 5.18 (d, $J = 2.4$ Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 4.86 (d, $J = 3.6$ Hz, 1H, H-1), 4.81 (d, $J = 11.3$ Hz, 1H, p-N_3–C_6H_4–CH_2–), 4.70 (d, $J = 11.5$ Hz, 1H, p-N_3–C_6H_4–CH_2–), 4.66 (d, $J = 13.8$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.62 (d, $J = 13.8$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.40 (dd, $J = 12.8$, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 4.23–4.20 (m, 3H, $(C_6H_4)_2$–CH–CH_2–OCO and –OCH_2–$COOCH_2$–C_6H_5), 3.98 (ddd, $J = 10.5$, 9.2, 3.6 Hz, 1H, H-2), 3.81–3.75 (m, 4H, H-3, H-5, and H-6a,b), 3.70 (s, 3H, p-CH_3O–C_6H_4–CH_2–), 3.60 (dd, $J = 8.8$, 8.8 Hz, 1H, H-4), 1.79 (brs, 1H, C_6-OH). Anal. Calcd for $C_{45}H_{44}N_4O_{10}$: C, 67.49; H, 5.54; N, 7.00%. Found: C, 67.42; H, 5.53; N, 6.87%.

Benzyloxycarbonylmethyl 4-*O*-[4-(4-Carboxylbutyrylamino)phenylmethyl]-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-*O*-(4-methoxyphenylmethyl)-α-D-glucopyranoside (39). To a suspension of **38** (3.05 g, 3.81 mmol) in AcOH–THF (2:1) (60 mL) was added zinc powder (2.50 g), and the mixture was stirred at room temperature for 2.5 h. After the insoluble materials were removed by filtration, the filtrate was concentrated in vacuo. The residual AcOH was removed by co-evaporation with toluene three times. The residue was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. To a solution of the residue in CH_2Cl_2 (30 mL) was added glutaric anhydride (520 mg, 4.56 mmol) at room temperature, and the mixture was stirred for 1 d. The reaction mixture was concentrated in vacuo. The residue was purified by silica-gel flash column chromatography (150 g, $CHCl_3$:acetone = 5:1 to $CHCl_3$:MeOH = 5:1) to give **39** (1.95 g, 59%) as a colorless solid. ESI-MS (negative) m/z 887.353 $[M - H]^-$. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, $J = 7.3$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.63 (dd, $J = 7.3$, 7.0 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.58 (dd, $J = 7.8$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.32 (dd, $J = 7.3$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 7.30–7.25 (m, 3H, –OCH_2–$COOCH_2$–C_6H_5), 7.29 (d, $J = 8.3$ Hz, 2H, p-RCONH–C_6H_4–CH_2–), 7.23 (d, $J = 8.3$ Hz, 2H, p-RCONH–C_6H_4–CH_2–), 7.16 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 6.74 (d, $J = 8.8$ Hz, 2H, p-CH_3O–C_6H_4–CH_2–), 5.22 (d, $J = 9.2$ Hz, 1H, 2-NH), 5.18 (d, $J = 2.5$ Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 4.86 (d, $J = 3.4$ Hz, 1H, H-1), 4.79 (d, $J = 11.5$ Hz, 1H, p-RCONH–C_6H_4–CH_2–), 4.73 (d, $J = 11.5$ Hz, 1H, p-RCONH–C_6H_4–CH_2–), 4.64 (d, $J = 13.8$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.59 (d, $J = 13.8$ Hz, 1H, p-CH_3O–C_6H_4–CH_2–), 4.40 (dd, $J = 12.8$, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO), 4.23–4.20 (m, 3H, $(C_6H_4)_2$–CH–CH_2–OCO and –OCH_2–$COOCH_2$–C_6H_5), 3.98 (ddd, $J = 10.5$, 9.2, 3.4 Hz, 1H, H-2), 3.80–3.75 (m, 2H, H-3 and H-6a), 3.73–3.63 (m, 5H, H-5, H-6b, and p-CH_3O–C_6H_4–CH_2–), 3.60 (dd, $J = 8.8$, 8.8 Hz, 1H, H-4), 2.45 (dd, $J = 5.9$, 5.9 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.42 (dd, $J = 5.9$, 5.9 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.00 (dd, $J = 5.9$, 5.9 Hz, 2H, CO–CH_2–CH_2–CH_2–CO). Anal. Calcd for $C_{50}H_{52}N_2O_{13}$: C, 67.56; H, 5.90; N, 3.15%. Found: C, 67.59; H, 5.86; N, 3.27%.

Benzyloxycarbonylmethyl 4-*O*-{4-[4-(Benzotriazolyloxycarboxyl)butyrylamino]phenylmethyl}-2-deoxy-2-(9-fluorenylmethoxycarbonylamino)-3-*O*-(4-methoxyphenylmethyl)-α-D-glucopyranoside (40). To a mixture of **39** (1.00 g, 1.12 mmol) and HOBt (182 mg, 1.35 mmol) in anhydrous CH_2Cl_2 (20 mL) was added DCC (340 mg, 1.65 mmol), and the mixture was stirred at room temperature for 5 h. After the insoluble materials were removed by filtration, the filtrate was concentrated in vacuo to give **40** (1.10 g, 98%) as a pale yellow solid, which was used for the subsequent coupling reaction without further purification.

General Procedure for Affinity Separation. After completion of the reaction, the reaction mixture was directly applied to the resin column (7.0 g: 1.5 cm × 7 cm; 13 g: 2.5 cm × 10 cm, CH_2Cl_2) unless otherwise noted. After untagged compounds were washed off with toluene–CH_2Cl_2 (1:1) then CH_2Cl_2, the tagged compound was eluted with CH_2Cl_2–MeOH (1:1). Evaporation of the solvents afforded the desired product having the BA-tag.

Benzyloxycarbonylmethyl 2-Deoxy-4-*O*-(4-{4-[4-(1-ethyl-2,4,6-trioxo-3,5-diazacyclohexylmethyl)phenylmethylaminocarbonyl]butyrylamino}phenylmethyl)-2-(9-fluorenylmethoxycarbonylamino)-3-*O*-(4-methoxyphenylmethyl)-α-D-glucopyranoside (42). To a solution of **41** (637 mg, 2.32 mmol) and activated ester **40** (2.80 g, 2.78 mmol) in anhydrous DMF (40 mL) was added Et_3N (420 µL, 3.01 mmol) at room temperature under Ar atmosphere and the mixture was stirred for 1.5 h. EtOAc was added to the mixture and the organic layer was washed with 10% aqueous citric acid and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then subjected to

affinity separation (13 g × 4) to give a mixture of **42** and **41**. The mixture thus obtained was dissolved in anhydrous DMF (40 mL) and activated ester **40** (2.80 g, 2.78 mmol) and Et_3N (420 mL, 3.01 mmol) were added at room temperature under Ar atmosphere. After stirring for 1.5 h, EtOAc was added to the mixture and the mixture was washed with 10% aqueous citric acid and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then subjected to affinity separation (13 g × 4) to give **42** (2.61 g, 98%) as a colorless foamy solid. $[\alpha]_D^{21} = +26.7$ (*c* 1.03, $CHCl_3$). ESI-MS (positive) *m/z* 1168.41 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (brs, 2H, CONHCO × 2), 7.74 (d, *J* = 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.61 (dd, *J* = 8.0, 6.6 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.48 (dd, *J* = 8.3, 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.37 (dd, *J* = 7.6, 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.32 (d, *J* = 7.3 Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 7.30–7.25 (m, 3H, –OCH_2–$COOCH_2$–C_6H_5), 7.23 (d, *J* = 7.3 Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 7.19 (d, *J* = 7.8 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 7.16 (d, *J* = 8.6 Hz, 2H, *p*-CH_3O–C_6H_4–CH_2–), 7.04 (d, *J* = 7.8 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 6.74 (d, *J* = 8.6 Hz, 2H, *p*-CH_3O–C_6H_4–CH_2–), 6.53 (brs, 1H, –CH_2–NHCO), 5.38 (d, *J* = 9.3 Hz, 1H, 2-NH), 5.17 (d, *J* = 2.1 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 4.86 (d, *J* = 3.8 Hz, 1H, H-1), 4.79 (d, *J* = 10.8 Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.71 (d, *J* = 10.8 Hz, 1H, *p*-CH_3O–C_6H_4–CH_2–), 4.64 (d, *J* = 10.8 Hz, 1H, *p*-CH_3O–C_6H_4–CH_2–), 4.59 (d, *J* = 11.3 Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.39 (dd, *J* = 12.8, 7.8 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 4.26 (d, *J* = 5.6 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 4.20–4.19 (m, 3H, $(C_6H_4)_2$–CH–CH_2–OCO– and –OCH_2–$COOCH_2$–C_6H_5), 3.96 (ddd, *J* = 11.3, 9.3, 3.8 Hz, 1H, H-2), 3.76 (dd, *J* = 11.3, 9.6 Hz, 1H, H-3), 3.71–3.66 (m, 3H, H-5 and H-6a,b), 3.65 (s, 3H, *p*-CH_3O–C_6H_4–CH_2–), 3.57 (dd, *J* = 9.6, 9.6 Hz, 1H, H-4), 3.20 (s, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 2.34 (dd, *J* = 7.2, 7.2 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.24 (dd, *J* = 7.2, 7.2 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.15 (q, *J* = 7.2 Hz, 2H, –CH_2–CH_3), 1.94 (dd, *J* = 7.2, 7.2 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 0.88 (t, *J* = 7.2 Hz, 3H, –CH_2–CH_3). Anal. Calcd for $C_{64}H_{67}N_5O_{15}$: C, 67.06; H, 5.89; N, 6.11%. Found: C, 67.09; H, 5.87; N, 6.01%.

Benzyloxycarbonylmethyl 2-Deoxy-4-*O*-(4-{4-[4-(1-ethyl-2,4,6-trioxo-3,5-diazacyclohexylmethyl)phenylmethylaminocarbonyl]butyrylamino}phenylmethyl)-2-(9-fluorenylmethoxycarbonylamino)-α-D-glucopyranoside (28). To a solution of **42** (130 mg, 113 µmol) in anhydrous CH_2Cl_2 (5.0 mL) was added diethyl ether–boron trifluoride (1/1) (15.0 µL, 118 µmol) at 0 °C under Ar atmosphere. After stirring at 0 °C for 3 h, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then subjected to affinity separation (13 g) to give **28** (101 mg, 87%) as a pale yellow foamy solid. $[\alpha]_D^{21} = +28.1$ (*c* 0.96, $CHCl_3$). ESI-MS (positive) *m/z* 1048.47 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (brs, 2H, CONHCO × 2), 7.74 (d, *J* = 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.62 (dd, *J* = 8.0, 6.6 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.48 (dd, *J* = 8.7, 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.37 (dd, *J* = 7.6, 7.6 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.32 (d, *J* = 8.3 Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 7.30–7.25 (m, 3H, –OCH_2–$COOCH_2$–C_6H_5), 7.23 (d, *J* = 8.3 Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 7.19 (d, *J* = 7.8 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 7.00 (d, *J* = 7.8 Hz, 2H, BA–CH_2–C_6H_4–CH_2–NHCO–), 6.72 (brs, 1H, –CH_2–NHCO), 5.95 (brs, 1H, 2-NH), 5.16 (d, *J* = 2.5 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 4.87 (d, *J* = 3.3 Hz, 1H, H-1), 4.79 (d, *J* = 10.8 Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.65 (d, *J* = 10.8 Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.36 (dd, *J* = 12.8, 7.8 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 4.26 (d, *J* = 5.8 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 4.21–4.19 (m, 3H, $(C_6H_4)_2$–CH–CH_2–OCO– and –OCH_2–$COOCH_2$–C_6H_5), 3.92 (ddd, *J* = 10.5, 10.5, 3.3 Hz, 1H, H-2), 3.84 (ddd, *J* = 11.3, 9.6, 3.8 Hz, 1H, H-5), 3.76–3.70 (m, 3H, H-3 and H-6a,b), 3.50 (dd, *J* = 9.6, 9.6 Hz, 1H, H-4), 3.17 (s, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 2.29 (dd, *J* = 7.3, 7.3 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.24 (dd, *J* = 7.3, 7.3 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 2.14 (q, *J* = 7.3 Hz, 2H, –CH_2–CH_3), 1.89 (dd, *J* = 7.3, 7.3 Hz, 2H, CO–CH_2–CH_2–CH_2–CO), 0.86 (t, *J* = 7.3 Hz, 3H, –CH_2–CH_3). Anal. Calcd for $C_{56}H_{59}N_5O_{14}$: C, 65.55; H, 5.80; N, 6.83%. Found: C, 65.43; H, 5.96; N, 6.78%.

Benzyloxycarbonylmethyl 6-*O*-[2-Allyloxycarbonylamino-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3*H*-2,4,3λ^5-benzodioxaphosphepin-3-yl)-3-*O*-(2-propynyloxycarbonyl)-β-D-glucopyranosyl]-2-deoxy-4-*O*-(4-{4-[4-(1-ethyl-2,4,6-trioxo-3,5-diazacyclohexylmethyl)phenylmethylaminocarbonyl]butyrylamino}phenylmethyl)-2-(9-fluorenylmethoxycarbonylamino)-α-D-glucopyranoside (29). To a mixture of donor **27** (505 mg, 663 µmol), acceptor **28** (454 mg, 442 µmol) and MS4A (1.0 g) in anhydrous THF (15.0 mL) was added diethyl ether–boron trifluoride (1/1) (30.0 µL, 237 µmol) at 0 °C under Ar atmosphere. After stirring for 1.5 h, another donor **27** (170 mg, 223 µmol) was added, and the mixture was stirred for additional 1 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$. After removal of insoluble materials by filtration, the filtrate was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 and then subjected to affinity separation (13 g × 1) to give **29** (689 mg, 96%) as a colorless solid. ESI-MS (positive) *m/z* 1657.54 $[M + Na]^+$, 835.29 $[M + 2Na]^{2+}$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.27 (d, *J* = 5.6 Hz, 2H, CONHCO × 2), 7.87 (d, *J* = 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.75 (d, *J* = 6.4 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.53 (dd, *J* = 8.3, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.40 (dd, *J* = 7.2, 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.36–7.33 (m, 9H, $(C_6H_4)_2$–CH–CH_2–OCO–, *p*-RCONH–C_6H_4–CH_2–, and C_6H_4–CH_2–), 7.32–7.27 (m, 7H, BA–CH_2–C_6H_4–CH_2NHCO–, *o*-$C_6H_4(CH_2O)_2P$–, and –OCH_2–$COOCH_2$–C_6H_5), 7.26 (d, *J* = 7.2 Hz, 2H, *o*-$C_6H_4(CH_2O)_2P$–), 7.12 (d, *J* = 8.0 Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 6.96 (d, *J* = 7.6 Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 5.80 (dddd, *J* = 15.8, 10.5, 10.5, 5.8 Hz, 1H, –OCH_2–CH=CH_2 of Alloc), 5.29 (d, *J* = 15.8 Hz, 1H, –OCH_2–CH=CH_2 of Alloc), 5.20 (d, *J* = 10.5 Hz, 1H, –OCH_2–CH=CH_2 of Alloc), 5.18–5.11 (m, 9H, 2-NH, 2′-NH, H-3′, *o*-$C_6H_4(CH_2O)_2P$, and –OCH_2–$COOCH_2$–C_6H_5), 4.86 (d, *J* = 2.8 Hz, 1H, H-1), 4.80 (d, *J* = 11.2 Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.68 (d, *J* = 2.5 Hz, 1H, –OCH_2–C≡CH of Proc), 4.61–4.59 (m, 7H, H-1′, H-4′, C_6H_4–CH_2–, *p*-RCONH–C_6H_4–CH_2–, –OCH_2–C≡CH of Proc, and –OCH_2–CH=CH_2 of Alloc), 4.56 (d, *J* = 12.5 Hz, 1H, C_6H_4–CH_2–), 4.38 (d, *J* = 7.2 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 4.26–4.22 (m, 5H, $(C_6H_4)_2$–CH–CH_2–OCO, –OCH_2–$COOCH_2$–C_6H_5, and BA–CH_2–C_6H_4–CH_2NHCO–), 4.01 (dd, *J* = 8.9, 2.5 Hz, 1H, H-6′a), 3.85 (brd, *J* = 8.8 Hz, 1H, H-3), 3.80 (brd, *J* = 9.5 Hz, 1H, H-2), 3.78–3.70 (m, 3H, H-4, H-6a, and H-5′), 3.68–3.65 (m, 2H, H-2′ and H-6′b), 3.45–3.40 (m, 2H, H-5 and H-6b), 3.08 (s, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 2.46 (t, *J* = 2.5 Hz, 1H, –OCH_2–C≡CH of Proc), 2.36 (dd, *J* = 6.8,