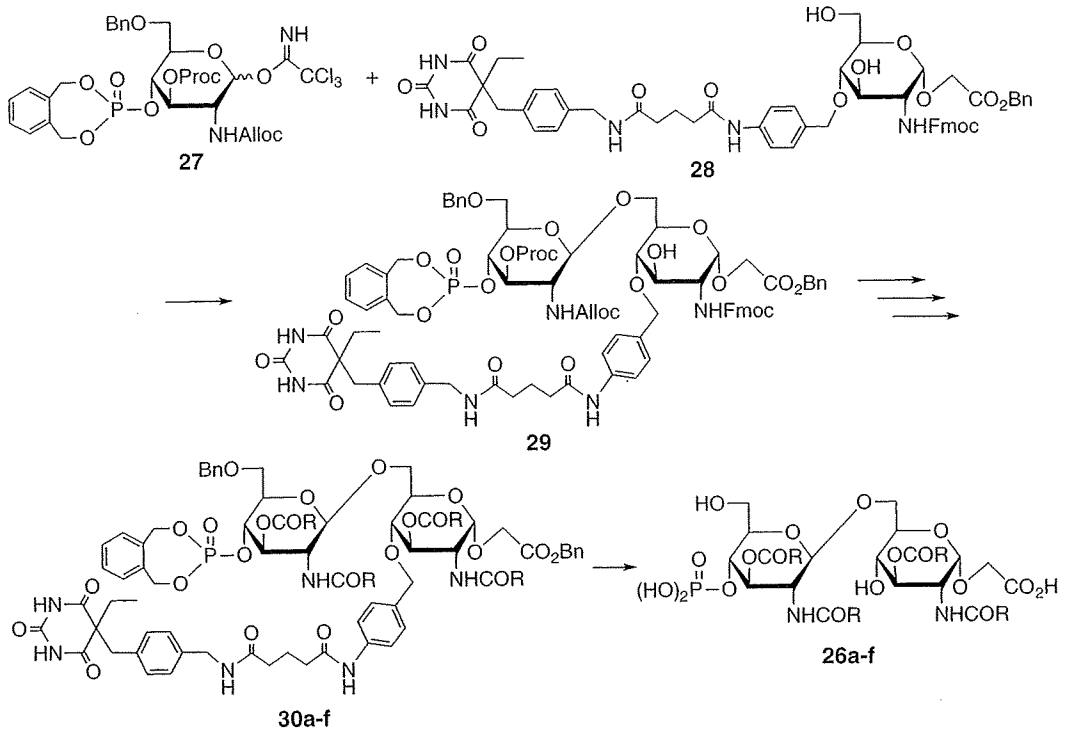
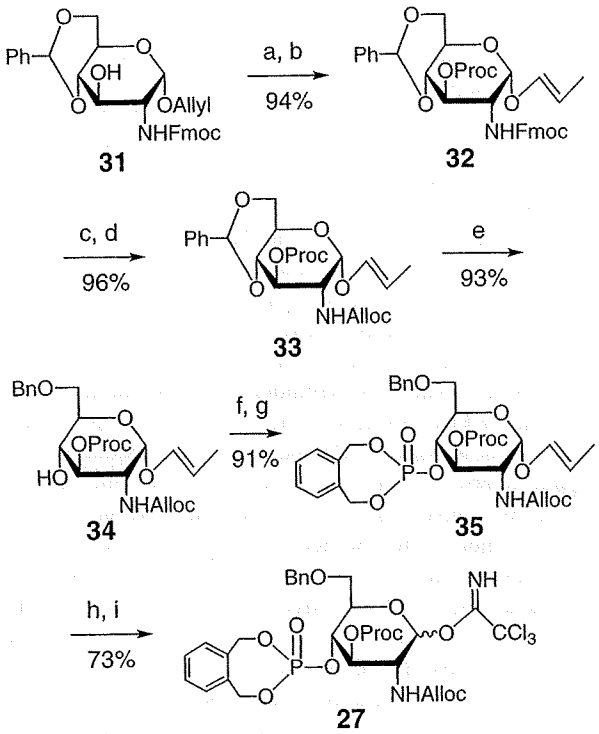


Figure 6. The basic synthesis route for the construction of the lipid A analogue library.

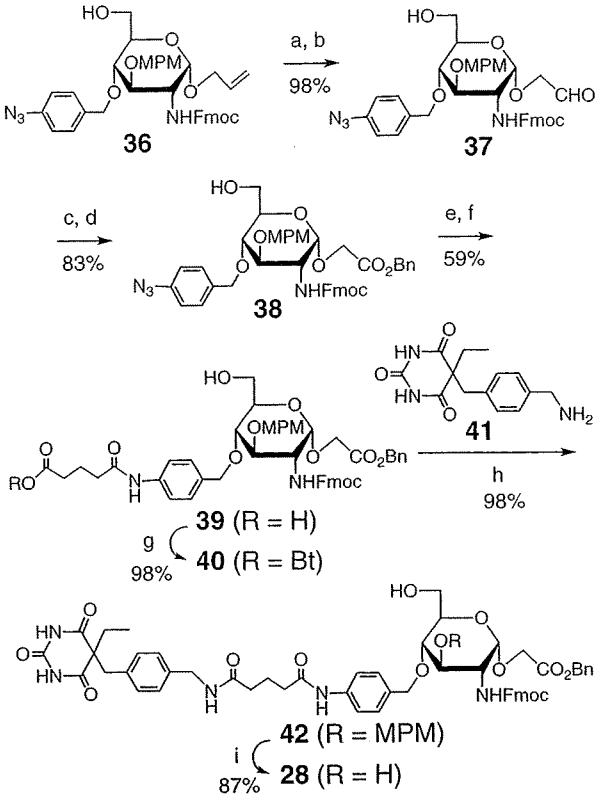


Scheme 3. Reagents and conditions: (a) [Ir(cod)-$(MePh_2P)_2$]PF_6, H_2, THF; (b) ProcCl, pyridine, DMAP, CH_2Cl_2; (c) PTBD, CH_2Cl_2; (d) AllocCl, pyridine, CH_2Cl_2; (e) Et_3SiH, $BF_3 \cdot Et_2O$, CH_2Cl_2, 0 °C; (f) *N,N*-diethyl-1,5-dihydro-3*H*-2,4,3-benzodioxaphosphepin-3-amine, 1*H*-tetrazole,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

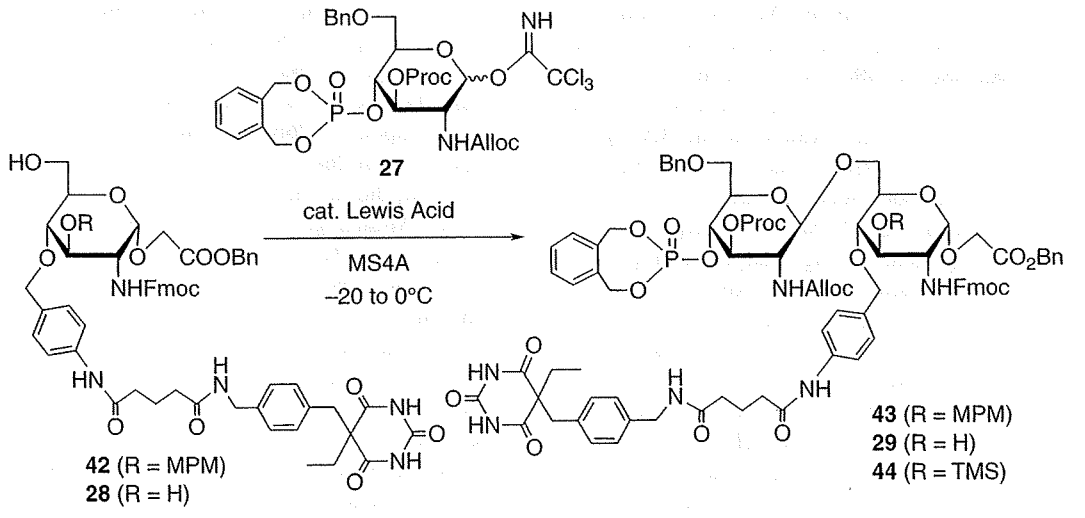
double procedure, the total yield of **43** remained as low as 60%. Glycosylation of a more reactive acceptor **28** with an additional free hydroxy group at the 3-position with the same donor **27** using TMSOTf in CH_2Cl_2 gave 3-*O*-TMS disaccharide **44** in addition to the desired **29** (80% as 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.



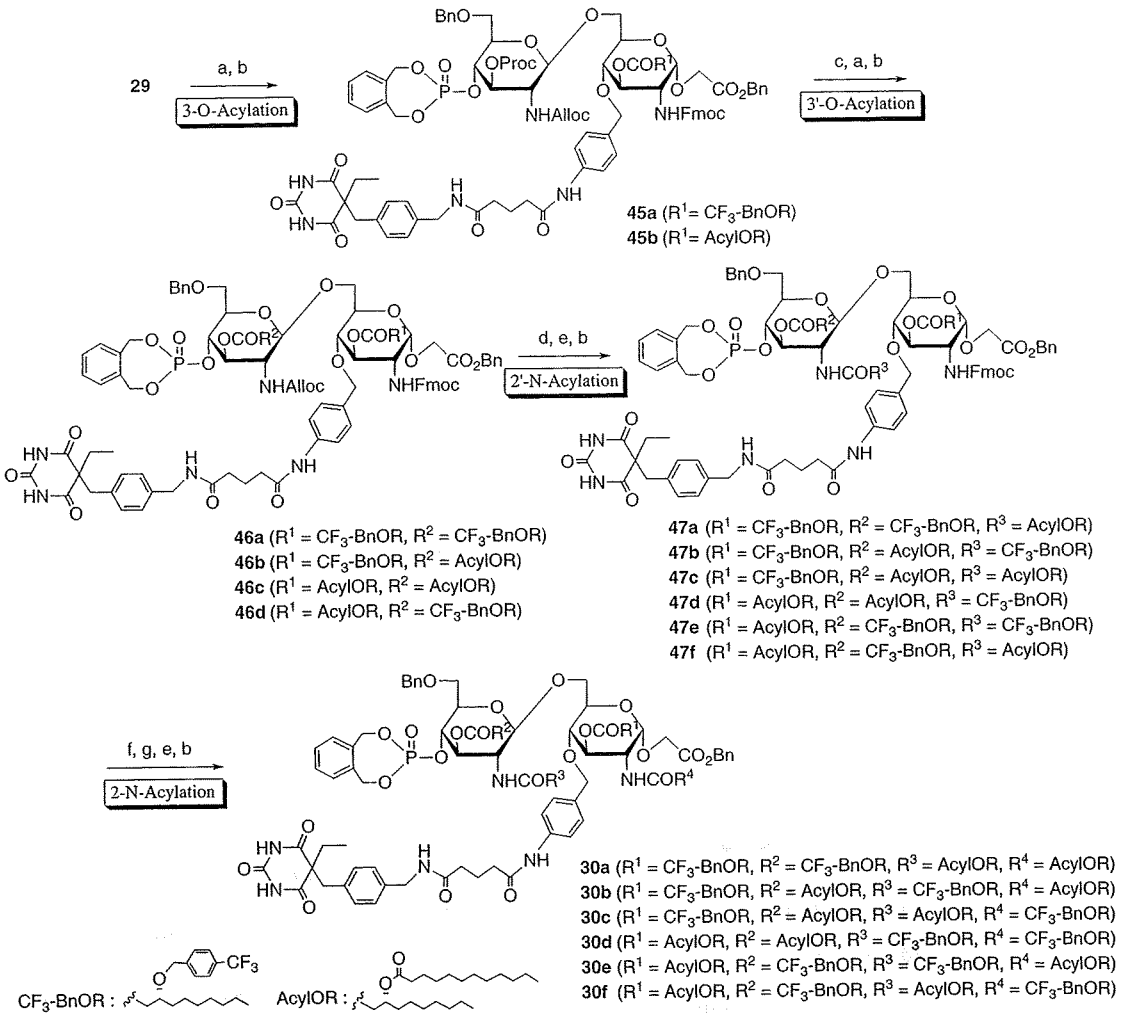
Scheme 4. Reagents and conditions: (a) OsO_4, NMO, THF/*t*-butyl alcohol/water (10:10:1); (b) $Pb(OAc)_4$, benzene/CH_2Cl_2 (2:3); (c) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, THF/*t*-butyl alcohol/water (2:4:1); (d) phenyldiazomethane, Et_2O; (e) Zn, AcOH/THF (2:1); (f) glutaric anhydride, CH_2Cl_2; (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**.

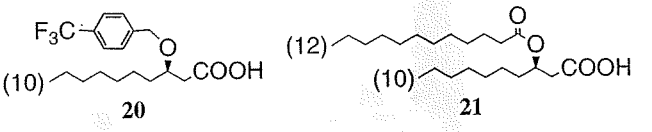
The biological activities of the six CM-analogues **26a–26f** were evaluated by measuring *Limulus* activity and cytokine (IL-6 and TNF-α) induction, in a manner similar to that mentioned above. Compounds **26e** and **26f** exhibited *Limulus* activity as strong as LPS (Table 1). The *Ru. gelatinosus*-type 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%)



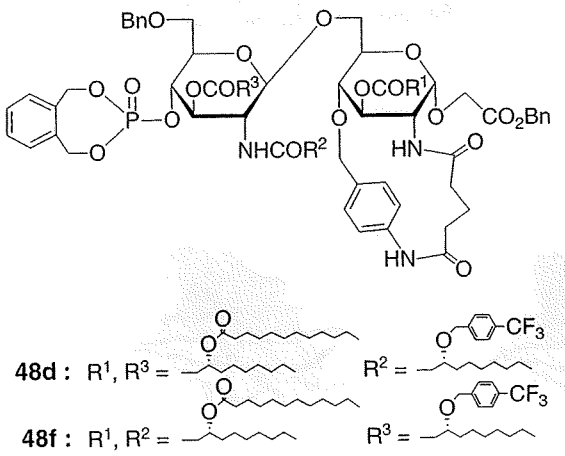
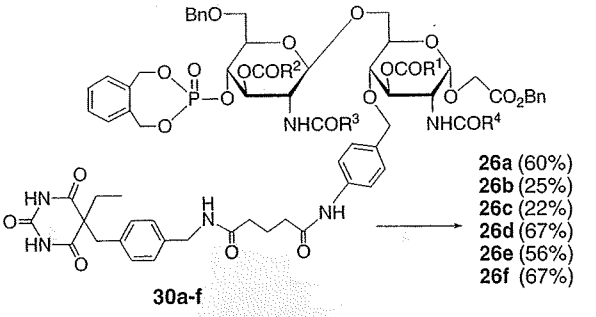


Figure 7. Structures of by-products formed during 2-N-acylation reaction.



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

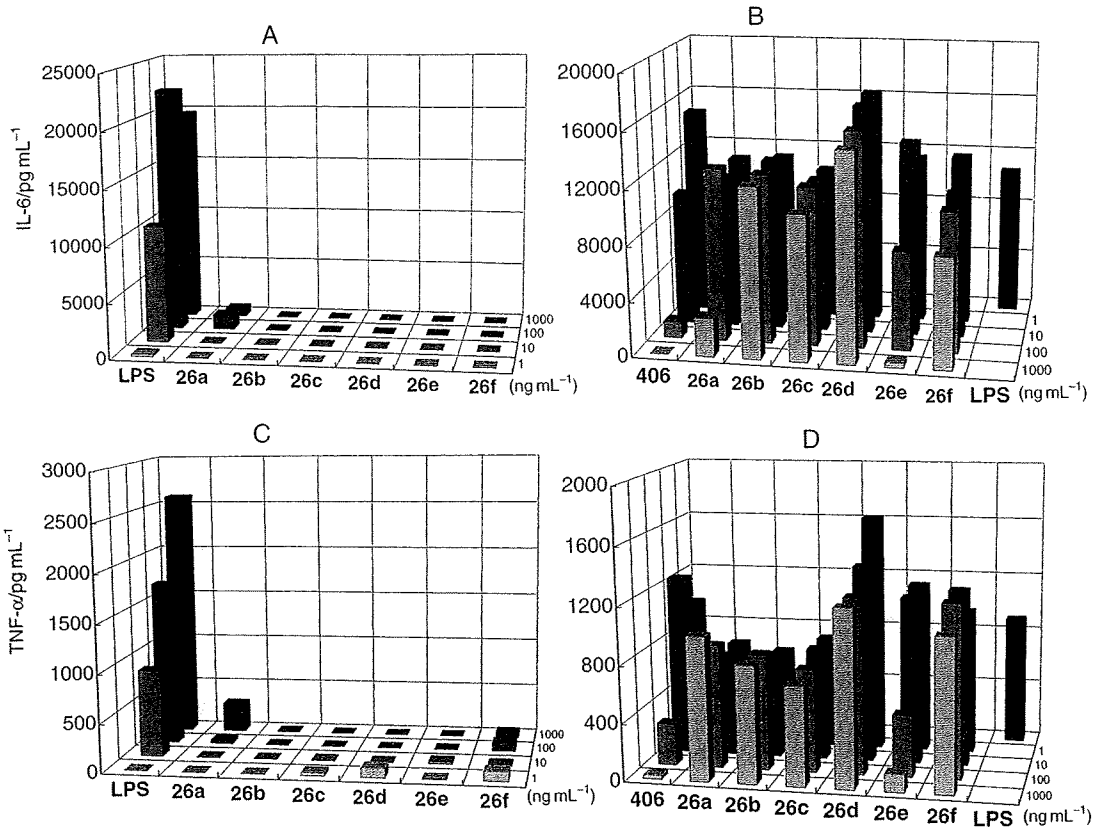


Figure 8. The cytokine inducing activity and inhibitory activities of **26a–26f**, and *E. coli* LPS 0111:B4, as measured in human peripheral whole-blood cells. A: IL-6 inducing activity, B: inhibitory activity against IL-6 induction by *E. coli* LPS 0111:B4 (10 ng mL^{-1}), C: TNF-α inducing activity,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

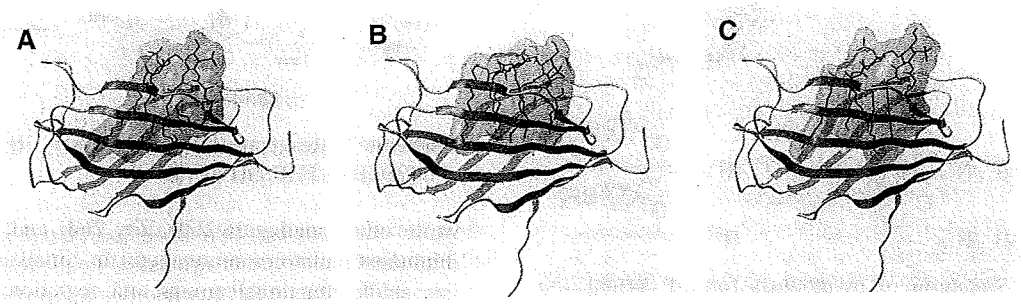


Figure 9. Stereo ribbon models of human MD-2 in complexes with 406 (**2**) (lipid IVa) (A), molecular modeling of human MD-2 in complex with *Ru. gelatinosus* lipid A **10a** (B), and with **26e** (C).

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

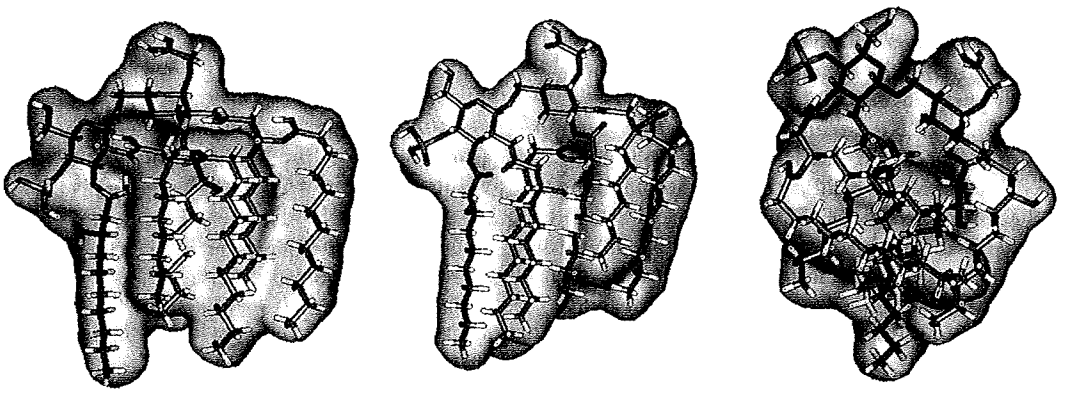


Figure 10. The lowest energy conformations of **26a**, **26e**, and **26c**, calculated with MacroModel v7.1 (Amber*, low mode, GB/SA water).

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 $[M + Na]^+$. ^{1}H 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 × 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 $[M + Na]^+$. ^{1}H 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 × 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 $[M + Na]^+$. ^{1}H 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 × 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 $[M + Na]^+$. ^{1}H 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 × 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 × 18), 0.89–0.84 (m, 9H, –CH_2–CH_3 × 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 × 18), 0.90–0.85 (m, 9H, –CH_2–CH_3 × 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)decanoyl-amino]-3-*O*-[(*R*)-3-(4-trifluoromethylbenzyloxy)decanoyl]-β-D-glucopyranosyl)-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)decanoyl-amino]-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)decanoyl-amino]-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™ ϕ11 × 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)$_2$P–), 7.28–7.26 (m, 1H, o-$C_6H_4(CH_2O)_2$P–), 7.18 (ddd, J = 7.5, 7.5, 1.2 Hz, 1H, o-$C_6H_4(CH_2O)_2$P–), 7.10 (d, J = 7.0 Hz, 1H, o-$C_6H_4(CH_2O)_2$P–), 6.74 (d, J = 7.0 Hz, 1H, o-$C_6H_4(CH_2O)_2$P–), 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-$C_6H_4(CH_2O)_2$P– and (Ph–CH_2O)$_2$P–), 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–C*H*=CH–CH_3), 5.56 (s, 1H, C_6H_5–C*H*=), 5.23 (dd, J = 12.0, 6.9 Hz, 1H, –O–CH=C*H*–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)$_2$–C*H*–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≡C*H* 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-2*H*-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–C*H*=CH–CH_3), 5.90 (dddd, J = 16.0, 10.8, 10.8, 5.6 Hz, 1H, –OCH_2–C*H*=CH_2 of Alloc), 5.52 (s, 1H, C_6H_5–C*H*=), 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=C*H*–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, $-OCH_2-C{\equiv}CH$ of Proc), 1.57 (dd, J = 6.8, 1.6 Hz, 3H, $-O-CH{=}CH-CH_3$). 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 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.27 (m, 5H, $C_6H_5-CH_2-$), 6.14 (dd, J = 12.0, 1.6 Hz, 1H, $-O-CH{=}CH-CH_3$), 5.89 (dddd, J = 17.3, 10.8, 10.8, 5.6 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.28 (dd, J = 17.3, 1.4 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.22–5.16 (m, 3H, 2-NH, $-O-CH{=}CH-CH_3$, and $-OCH_2-CH{=}CH_2$ 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, $-OCH_2-C{\equiv}CH$ of Proc), 4.69 (d, J = 2.5 Hz, 1H, $-OCH_2-C{\equiv}CH$ of Proc), 4.62–4.51 (m, 2H, $-OCH_2-CH{=}CH_2$ of Alloc), 4.59 (d, J = 12.1 Hz, 2H, $C_6H_5-CH_2-$), 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, C_4-OH), 2.51 (t, J = 2.4 Hz, 1H, $-OCH_2-C{\equiv}CH$ of Proc), 1.55 (dd, J = 6.8, 1.6 Hz, 3H, $-O-CH{=}CH-CH_3$). 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 $[M+Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.32 (m, 5H, $C_6H_4-CH_2-$ and *o*-$C_6H_4(CH_2O)_2P-$), 7.28 (d, J = 6.9 Hz, 2H, $C_6H_4-CH_2-$), 7.26–7.19 (m, 2H, *o*-$C_6H_4(CH_2O)_2P-$), 6.15 (d, J = 12.2 Hz, 1H, $-O-CH{=}CH-CH_3$), 5.90 (dddd, J = 17.2, 10.3, 5.9, 5.9 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.36 (d, J = 17.2 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.25 (d, J = 10.3 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.23–5.07 (m, 6H, H-3, *o*-$C_6H_4(CH_2O)_2P-$, and $-O-CH{=}CH-CH_3$), 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, $-OCH_2-C{\equiv}CH$ of Proc), 4.69–4.66 (m, 2H, H-4 and $-OCH_2-C{\equiv}CH$ of Proc), 4.64–4.62 (m, 2H, $-OCH_2-CH{=}CH_2$ of Alloc), 4.58 (d, J = 11.6 Hz, 1H, $C_6H_4-CH_2-$), 4.56 (d, J = 11.6 Hz, 1H, $C_6H_4-CH_2-$), 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, $-OCH_2-C{\equiv}CH$ of Proc), 1.55 (dd, J = 6.9, 1.6 Hz, 3H, $-O-CH{=}CH-CH_3$). 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 $[M+Na]^+$. ^{1}H NMR (500 MHz, $CDCl_3$) selected data for α-isomer: δ 7.35–7.27 (m, 7H, $C_6H_4-CH_2-$ and *o*-$C_6H_4(CH_2O)_2P-$), 7.21 (dd, J = 8.2, 4.6 Hz, 2H, *o*-$C_6H_4(CH_2O)_2P-$), 5.91–5.84 (m, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.28 (d, J = 17.2 Hz, 1H, $-OCH_2-CH{=}CH_2$ of Alloc), 5.23–5.06 (m, 6H, H-3, *o*-$C_6H_4(CH_2O)_2P-$, and $-OCH_2-CH{=}CH_2$ of Alloc), 4.96 (d, J = 9.6 Hz, 1H, 2-NH), 4.73 (d, J = 2.5 Hz, 1H, $-OCH_2-C{\equiv}CH$ of Proc), 4.66 (d, J = 3.8 Hz, 1H, H-1), 4.65–4.55 (m, 6H, H-4, $C_6H_4-CH_2-$, $-OCH_2-C{\equiv}CH$ of Proc, and $-OCH_2-CH{=}CH_2$ 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, C_1-OH), 2.48 (brs, 1H, $-OCH_2-C{\equiv}CH$ 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 $[M+Na]^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.65 (s, 1H,

–OCH_2–C*H*O), 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, $(C_6H_4)_2$–C*H*–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 $[M+Na]^+$. ^{1}H 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, $(C_6H_4)_2$–C*H*–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 $[M-H]^-$. ^{1}H 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, $(C_6H_4)_2$–C*H*–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, $J = 6.8$,

6.8 Hz, 2H, CO-CH_2-CH_2-CH_2-CO), 2.18 (dd, J = 6.8, 6.8 Hz, 2H, CO-CH_2-CH_2-CH_2-CO), 1.98 (q, J = 7.6 Hz, 2H, -CH_2-CH_3), 1.84 (dd, J = 6.8, 6.8 Hz, 2H, CO-CH_2-CH_2-CH_2-CO), 0.78 (t, J = 7.6 Hz, 3H, -CH_2-CH_3). Anal. Calcd for $C_{85}H_{89}N_6O_{25}P$: C, 62.80; H, 5.52; N, 5.17%. Found: C, 62.78; H, 5.51; N, 5.29%.

3-O-Acylated Compounds 45a and 45b. 45a: To a solution of **29** (434 mg, 267 μmol) and (*R*)-3-(4-trifluoromethylbenzyloxy)-decanoic acid (**20**) (150 mg, 433 μmol) in anhydrous CH_2Cl_2 (10 mL) were added DMAP (3.3 mg, 27 μmol) and DIC (125 μL, 798 μmol) at room temperature under Ar atmosphere and the mixture was stirred for 2 h. To the reaction mixture was added toluene (10 mL) and the mixture was subjected to affinity separation (13 g × 3). After untagged compounds were eluted with toluene–CH_2Cl_2 (1:1) and CH_2Cl_2, **45a** was obtained as a pale yellow solid (435 mg, 84%) by elution with CH_2Cl_2–MeOH (1:1) and evaporation of the solvents. $[\alpha]_D^{23}$ = +22.9 (*c* 0.94, $CHCl_3$). ESI-MS (positive) *m/z* 1975.42 $[M + Na]^+$, 999.35 $[M + 2Na]^{2+}$. ^{1}H NMR (400 MHz, DMSO-d_6, 40 °C): δ 7.84 (d, J = 7.2 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.67 (dd, J = 8.9, 7.2 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.63 (dd, J = 7.3, 6.9 Hz, 2H, -OCH_2-$COOCH_2$-C_6H_5), 7.55 (d, J = 8.0 Hz, 2H, *p*-CF_3-C_6H_4-CH_2-), 7.52 (dd, J = 7.2, 7.2 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.41–7.31 (m, 12H, $(C_6H_4)_2$-CH-CH_2-OCO-, *p*-RCONH-C_6H_4-CH_2-, *p*-CF_3-C_6H_4-CH_2-, and C_6H_5-CH_2-), 7.30–7.25 (m, 6H, BA-CH_2-C_6H_4-CH_2NHCO-, *o*-C_6H_4-$(CH_2O)_2$P-, and -OCH_2-$COOCH_2$-C_6H_5), 7.15–7.10 (m, 4H, *o*-$C_6H_4(CH_2O)_2$P- and BA-CH_2-C_6H_4-CH_2NHCO-), 6.95 (d, J = 8.0 Hz, 2H, *p*-RCONH-C_6H_4-CH_2-), 5.95–5.83 (m, 1H, -OCH_2-CH=CH_2 of Alloc), 5.43 (d, J = 6.5 Hz, 1H, NH), 5.28 (d, J = 15.4 Hz, 1H, -OCH_2-CH=CH_2 of Alloc), 5.21 (d, J = 10.5 Hz, 1H, -OCH_2-CH=CH_2 of Alloc), 5.17–5.07 (m, 9H, NH, H-3, H-3′, *o*-$C_6H_4(CH_2O)_2$P, and -OCH_2-$COOCH_2$-C_6H_5), 4.95 (brs, 1H, H-1), 4.79 (dd, J = 10.3, 10.3 Hz, 2H, *p*-RCONH-C_6H_4-CH_2-), 4.67 (d, J = 2.8 Hz, 1H, -OCH_2-C≡CH of Proc), 4.61–4.52 (m, 8H, H-1′, H-4′, C_6H_5-CH_2-, *p*-CF_3-C_6H_4-CH_2-, -OCH_2-C≡CH of Proc, and -OCH_2-CH=CH_2 of Alloc), 4.48 (d, J = 12.5 Hz, 1H, C_6H_5-CH_2-), 4.39 (d, J = 7.2 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 4.21 (dd, J = 7.2, 7.2 Hz, 1H, $(C_6H_4)_2$-CH-CH_2-OCO-), 4.18–4.10 (m, 4H, -OCH_2-$COOCH_2$-C_6H_5 and BA-CH_2-C_6H_4-CH_2NHCO-), 3.93–3.86 (m, 2H, H-2 and H-6′a), 3.78 (brs, 1H, β-CH of 3-*O*-acyl), 3.76–3.72 (m, 3H, H-4, H-6a, and H-5′), 3.65–3.58 (m, 4H, H-5, H-6b, H-2′, and H-6′b), 3.07 (s, 2H, BA-CH_2-C_6H_4-CH_2NHCO-), 2.51–2.45 (m, 3H, -OCH_2-C≡CH of Proc and α-CH_2 of 3-*O*-acyl), 2.29 (dd, J = 6.8, 6.8 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 2.18 (dd, J = 6.8, 6.8 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 1.96 (q, J = 7.6 Hz, 2H, -CH_2-CH_3 of BA), 1.81 (dd, J = 6.8; 6.8 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 1.60–1.47 (m, 2H, γ-CH_2 of 3-*O*-acyl), 1.31–0.99 (m, 10H, CH_2 × 5), 0.79 (t, J = 7.6 Hz, 3H, -CH_2-CH_3 of 3-*O*-acyl), 0.74 (t, J = 7.2 Hz, 3H, -CH_2-CH_3 of BA).

45b: In a manner similar to the synthesis of **45a**, **29** (221 mg, 136 μmol) was acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **45b** as a pale yellow solid (247 mg, 93%). ESI-MS (positive) *m/z* 1977.87 $[M + H]^+$, 1999.81 $[M + Na]^+$, 1000.41 $[M + H + Na]^{2+}$.

3-,3′-O-Diacylated Compounds 46a, 46b, 46c, and 46d. 46a: To a degassed solution of **45a** (140 mg, 71.6 μmol) in anhydrous THF (4.0 mL) was added (1,5-cyclooctadiene)[bis(methyldiphenylphosphine)]iridium(I) hexafluorophosphate (65.0 mg, 76.9 μmol). After activation of the iridium catalyst with hydrogen three times (each 30 s), the mixture was stirred under Ar atmosphere at room temperature for 1.5 h. The mixture was concentrated in vacuo to give crude 3′-O-deprotected product. To a solution of the crude 3′-O-free product and (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) (50.0 mg, 144 μmol) in anhydrous CH_2Cl_2 (4.0 mL) were added DIC (40.0 μL, 255 μmol) and DMAP (1.0 mg, 8.2 μmol) at room temperature under Ar atmosphere. After stirring for 8 h, the reaction mixture was directly subjected to affinity separation (13 g × 1) to give **46a** as a pale yellow solid (117 mg, 75%). $[\alpha]_D^{24}$ = +18.3 (*c* 0.67, $CHCl_3$). ESI-MS (positive) *m/z* 2222.34 $[M + Na]^+$. ^{1}H NMR (400 MHz, DMSO-d_6, 40 °C): δ 9.79 (s, 1H, NH), 8.22 (t, J = 3.8 Hz, 2H, NH), 7.84 (d, J = 7.6 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.66 (dd, J = 7.3, 6.9 Hz, 2H, -OCH_2-$COOCH_2$-C_6H_5), 7.58 (dd, J = 8.1, 7.6 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.54 (d, J = 8.0 Hz, 4H, *p*-CF_3-C_6H_4-CH_2-), 7.52 (d, J = 8.1 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 7.41–7.31 (m, 14H, $(C_6H_4)_2$-CH-CH_2-OCO-, *p*-RCONH-C_6H_4-CH_2-, *p*-CF_3-C_6H_4-CH_2-, and C_6H_5-CH_2-), 7.30–7.25 (m, 6H, BA-CH_2-C_6H_4-CH_2NHCO-, *o*-C_6H_4-$(CH_2O)_2$P-, and -OCH_2-$COOCH_2$-C_6H_5), 7.14–7.10 (m, 4H, *o*-$C_6H_4(CH_2O)_2$P- and BA-CH_2-C_6H_4-CH_2NHCO-), 6.95 (d, J = 8.1 Hz, 2H, *p*-RCONH-C_6H_4-CH_2-), 5.95–5.85 (m, 1H, -OCH_2-CH=CH_2 of Alloc), 5.44 (d, J = 6.5 Hz, 1H, NH), 5.38 (dd, J = 10.1, 10.1 Hz, 1H, H-3′), 5.31 (d, J = 14.5 Hz, 1H, -OCH_2-CH=CH_2 of Alloc), 5.23 (d, J = 10.5 Hz, 1H, -OCH_2-CH=CH_2 of Alloc), 5.18–5.08 (m, 8H, NH, H-3, *o*-$C_6H_4(CH_2O)_2$P, and -OCH_2-$COOCH_2$-C_6H_5), 4.95 (brs, 1H, H-1), 4.60 (dd, J = 10.1, 10.1 Hz, 2H, *p*-RCONH-C_6H_4-CH_2-), 4.53–4.47 (m, 9H, H-1′, H-4′, C_6H_5-CH_2-, *p*-CF_3-C_6H_4-CH_2-, and -OCH_2-CH=CH_2 of Alloc), 4.39 (d, J = 7.2 Hz, 2H, $(C_6H_4)_2$-CH-CH_2-OCO-), 4.21 (dd, J = 7.2, 7.2 Hz, 1H, $(C_6H_4)_2$-CH-CH_2-OCO-), 4.15–4.07 (m, 4H, -OCH_2-$COOCH_2$-C_6H_5 and BA-CH_2-C_6H_4-CH_2NHCO-), 3.95–3.87 (m, 2H, H-2 and H-6′a), 3.81–3.74 (m, 4H, H-4, H-6a, H-5′, and β-CH of 3-*O*-acyl), 3.68–3.58 (m, 5H, H-5, H-6b, H-2′, H-6′b, and β-CH of 3′-*O*-acyl), 3.06 (d, J = 3.7 Hz, 2H, BA-CH_2-C_6H_4-CH_2NHCO-), 2.65 (dd, J = 15.0, 5.3 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.53–2.45 (m, 2H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.41 (dd, J = 15.1, 5.6 Hz, 1H, α-CH_2 of 3′-*O*-acyl), 2.28 (dd, J = 7.1, 7.1 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 2.17 (dd, J = 7.1, 7.1 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 1.96 (q, J = 7.3 Hz, 2H, -CH_2-CH_3 of BA), 1.81 (dd, J = 7.1, 7.1 Hz, 2H, -CO-CH_2-CH_2-CH_2-CO-), 1.55–1.48 (m, 4H, γ-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 1.31–0.99 (m, 20H, CH_2 × 10), 0.86–0.79 (m, 6H, -CH_2-CH_3 of 3-*O*-acyl and 3′-*O*-acyl), 0.75 (t, J = 7.1 Hz, 3H, -CH_2-CH_3 of BA).

46b: In a manner similar to the synthesis of **46a**, **45a** (288 mg, 147 μmol) was deprotected and acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **46b** as a pale yellow solid (252 mg, 77%). ESI-MS (positive) *m/z* 2249.29 $[M + Na]^+$.

46c: To a solution of **45b** (235 mg, 119 μmol) in AcOH (5.0 mL) was added Zn–Cu couple (200 mg) at room temperature, and the mixture was stirred for 2 h. After insoluble materials were filtered off, the filtrate was concentrated in vacuo, and the residual AcOH was removed by co-evaporation with toluene three times. The residue was dissolved in EtOAc, washed successively with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. To a solution of the residue and (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) (120 mg, 346 μmol) in anhydrous CH_2Cl_2 (5.0 mL) were added DMAP (1.5 mg, 12 μmol) and DIC (110 μL, 703 μmol) at room temperature under Ar atmosphere and the mixture was stirred for 11 h. To the reaction mixture

was added toluene (5.0 mL) and the mixture was subjected to affinity separation (13.0 g × 3) to give **46c** as a pale yellow solid (155 mg, 59%). ESI-MS (positive) *m/z* 2224.80 $[M + H]^+$, 2246.33 $[M + Na]^+$, 1123.62 $[M + H + Na]^{2+}$, 1134.97 $[M + 2Na]^{2+}$.

46d: In a manner similar to the synthesis of **46c**, **45b** (98.2 mg, 49.6 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **46d** as a pale yellow solid (59.6 mg, 59%). ESI-MS (positive) *m/z* 2270.29 $[M + Na]^+$, 1136.00 $[M + H + Na]^{2+}$.

2′-N-,3-,3′-O-Triacylated Compounds 47a, 47b, 47c, 47d, 47e, and 47f. 47a: To a solution of **46a** (50.0 mg, 22.7 μmol) in anhydrous THF (2.0 mL) was added Et_3N (31.7 μL, 226 μmol), HCO_2H (8.6 μL, 220 μmol), and tetrakis(triphenylphosphine)-palladium(0) (5.2 mg, 4.5 μmol) at room temperature under Ar atmosphere. After the mixture was stirred for 1 h, EtOAc was added. The organic layer was washed with 1 M HCl, saturated aqueous $NaHCO_3$, and brine. The EtOAc layer was dried over $MgSO_4$ and concentrated in vacuo to give 2′-N-deprotected product. To a solution of the crude 2-N-free product and (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) (42.1 mg, 114 μmol) in anhydrous CH_2Cl_2 (4.0 mL) were added DIC (36.0 μL, 230 μmol) at room temperature under Ar atmosphere. After stirring for 18 h, toluene (4.0 mL) was added and the reaction mixture was subjected to affinity separation (13 g × 1) to give **47a** as a pale yellow solid (38.1 mg, 70%). ESI-MS (positive) *m/z* 2268.61 $[M + H]^+$, 2490.30 $[M + Na]^+$, 1245.33 $[M + 2Na]^{2+}$. ^{1}H NMR (400 MHz, DMSO-d_6, 40 °C): δ 8.22 (s, 1H, NH), 7.83 (d, $J = 7.3$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.65 (dd, $J = 8.3$, 7.3 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.56 (dd, $J = 8.3$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.50 (d, $J = 7.3$ Hz, 4H, *p*-CF_3–C_6H_4–CH_2–), 7.39 (dd, $J = 7.3$, 7.3 Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 7.35–7.30 (m, 14H, $(C_6H_4)_2$–CH–CH_2–OCO–, *p*-RCONH–C_6H_4–CH_2–, *p*-CF_3–C_6H_4–CH_2–, and C_6H_5–CH_2–), 7.28–7.25 (m, 6H, BA–CH_2–C_6H_4–CH_2NHCO–, *o*-C_6H_4-$(CH_2O)_2$P–, and –OCH_2–$COOCH_2$–C_6H_5), 7.21 (dd, $J = 8.2$, 4.8 Hz, 2H, *o*-$C_6H_4(CH_2O)_2$P), 7.14 (d, $J = 7.8$ Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 7.08 (d, $J = 7.8$ Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 6.57 (d, $J = 8.8$ Hz, 1H, 2′-NH), 6.11 (d, $J = 8.5$ Hz, 1H, 2-NH), 5.45 (d, $J = 6.5$ Hz, 1H, NH), 5.45 (dd, $J = 10.2$, 10.2 Hz, 1H, H-3′), 5.33 (dd, $J = 10.5$, 10.5 Hz, 1H, H-3), 5.19–5.01 (m, 6H, *o*-$C_6H_4(CH_2O)_2$P and –OCH_2–$COOCH_2$–C_6H_5), 4.89 (d, $J = 3.3$ Hz, 1H, H-1), 4.81 (d, $J = 11.2$ Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.61–4.54 (m, 11H, H-1′, H-4′, C_6H_5–CH_2–, *p*-CF_3–C_6H_4–CH_2–, *p*-RCONH–C_6H_4–CH_2–, and *o*-$C_6H_4(CH_2O)_2$P), 4.38 (d, $J = 7.3$ Hz, 2H, $(C_6H_4)_2$–CH–CH_2–OCO–), 4.30–4.20 (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, $J = 13.2$, 2.8 Hz, 1H, H-6′a), 3.84–3.80 (m, 4H, H-2, β-CH of 3-*O*-acyl and 3′-*O*-acyl, and β-CH of 2′-*N*-acyl), 3.75–3.72 (m, 3H, H-4, H-6a, and H-5′), 3.68–3.65 (m, 2H, H-2′ and H-6′b), 3.45–3.41 (m, 2H, H-5 and H-6b), 3.08 (s, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 2.65 (dd, $J = 15.0$, 5.5 Hz, 1H, α-CH_2 of 3-*O*-acyl), 2.53–2.44 (m, 2H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.40 (dd, $J = 15.0$, 5.6 Hz, 1H, α-CH_2 of 3′-*O*-acyl), 2.30 (dd, $J = 6.3$, 6.3 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 2.28 (dd, $J = 14.5$, 7.0 Hz, 2H, α-CH_2 of 2′-*N*-acyl's side chain), 2.21 (dd, $J = 16.5$, 8.5 Hz, 2H, α-CH_2 of 2′-*N*-acyl's main chain), 2.17 (dd, $J = 6.3$, 6.3 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 1.97 (q, $J = 7.3$ Hz, 2H, –CH_2–CH_3 of BA), 1.92 (dd, $J = 6.3$, 6.3 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 1.55–1.01 (m, 54H, CH_2 × 27), 0.88–0.72 (m, 15H, –CH_2–CH_3 × 5).

47b: In a manner similar to the synthesis of **47a**, **46b** (111 mg, 49.8 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **47b** as a pale yellow solid (54.5 mg, 44%). ESI-MS (positive) *m/z* 2468.98 $[M + H]^+$, 2489.97 $[M + Na]^+$.

47c: In a manner similar to the synthesis of **47a**, **46b** (110 mg, 49.7 μmol) was deprotected and acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **47c** as a pale yellow solid (65.9 mg, 53%). ESI-MS (positive) *m/z* 2492.93 $[M + H]^+$, 2514.23 $[M + Na]^+$.

47d: In a manner similar to the synthesis of **47a**, **46c** (59.6 mg, 26.5 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **47d** as a pale yellow solid (24.5 mg, 37%). ESI-MS (positive) *m/z* 1244.15 $[M + 2H]^{2+}$.

47e: In a manner similar to the synthesis of **47a**, **46d** (78.0 mg, 35.1 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **47e** as a pale yellow solid (41.2 mg, 42%). ESI-MS (positive) *m/z* 2469.18 $[M + H]^+$, 2489.74 $[M + Na]^+$, 1245.40 $[M + H + Na]^{2+}$, 1256.35 $[M + 2Na]^{2+}$.

47f: In a manner similar to the synthesis of **47a**, **46d** (78.0 mg, 35.1 μmol) was deprotected and acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **47f** as a pale yellow solid (67.4 mg, 63%). ESI-MS (positive) *m/z* 2515.20 $[M + Na]^+$, 1256.95 $[M + H + Na]^{2+}$, 1268.40 $[M + 2Na]^{2+}$.

2-,2′-N-,3-,3′-O-Tetraacylated Compounds 30a, 30b, 30c, 30d, 30e, and 30f. 30a: To a solution of **47a** (38.0 mg, 15.4 μmol) in CH_2Cl_2 (2.0 mL) was added DBU (2.5 μL, 16.7 μmol) at room temperature and the mixture was stirred for 1.5 h. The reaction mixture was directly subjected to silica-gel column chromatography (5.0 g, $CHCl_3$:MeOH = 10:1) to give 2-N-deprotected product as a colorless solid: Yield 19.0 mg (63%). To a solution of the 2-N-free product and (*R*)-3-(dodecanoyloxy)-decanoic acid (**21**) (28.0 mg, 75.6 μmol) in anhydrous CH_2Cl_2 (2.0 mL) were added DIC (25.0 μL, 160 μmol) at room temperature under Ar atmosphere. After stirring for 14 h, to the reaction mixture was added toluene (2.0 mL) and the mixture was subjected to affinity separation (13 g × 1) to give **30a** as a pale yellow solid (18.7 mg, 49%). ESI-MS (positive) *m/z* 2620.88 $[M + Na]^+$, 1321.05 $[M + 2Na]^{2+}$. ^{1}H NMR (400 MHz, DMSO-d_6, 40 °C): δ 9.81 (s, 1H, NH), 8.25 (s, 1H, NH), 7.65 (dd, $J = 8.5$, 7.3 Hz, 2H, –OCH_2–$COOCH_2$–C_6H_5), 7.55 (d, $J = 7.3$ Hz, 4H, *p*-CF_3–C_6H_4–CH_2–), 7.35–7.29 (m, 12H, *p*-RCONH–C_6H_4–CH_2–, *p*-CF_3–C_6H_4–CH_2–, and C_6H_5–CH_2–), 7.26–7.20 (m, 7H, BA–CH_2–C_6H_4–CH_2NHCO–, *o*-$C_6H_4(CH_2O)_2$P–, and –OCH_2–$COOCH_2$–C_6H_5), 7.16 (d, $J = 7.8$ Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 7.02 (d, $J = 7.8$ Hz, 2H, *p*-RCONH–C_6H_4–CH_2–), 6.39 (d, $J = 8.3$ Hz, 1H, 2′-NH), 6.20 (d, $J = 7.3$ Hz, 1H, 2-NH), 5.60 (d, $J = 6.5$ Hz, 1H, NH), 5.48 (dd, $J = 10.5$, 9.3 Hz, 1H, H-3′), 5.36 (dd, $J = 10.5$, 10.5 Hz, 1H, H-3), 5.23–5.01 (m, 6H, *o*-$C_6H_4(CH_2O)_2$P and –OCH_2–$COOCH_2$–C_6H_5), 4.85 (d, $J = 3.5$ Hz, 1H, H-1), 4.75 (d, $J = 10.5$ Hz, 1H, *p*-RCONH–C_6H_4–CH_2–), 4.62–4.50 (m, 11H, H-1′, H-4′, C_6H_5–CH_2–, *p*-CF_3–C_6H_4–CH_2–, *p*-RCONH–C_6H_4–CH_2–, and *o*-$C_6H_4(CH_2O)_2$P), 4.33–4.20 (m, 4H, –OCH_2–$COOCH_2$–C_6H_5 and BA–CH_2–C_6H_4–CH_2NHCO–), 4.01 (dd, $J = 13.2$, 1.8 Hz, 1H, H-6′a), 3.88–3.79 (m, 4H, H-2, β-CH of 3-*O*-acyl and 3′-*O*-acyl, and β-CH of 2′-*N*-acyl), 3.75–3.72 (m, 5H, H-4, H-6a, H-5′, H-6′b, and β-CH of 2-*N*-acyl), 3.68–3.64 (m, 2H, H-2′ and H-6′b), 3.45–3.40 (m, 2H, H-5 and H-6b), 3.12 (d, $J = 3.1$ Hz, 2H, BA–CH_2–C_6H_4–CH_2NHCO–), 2.65 (dd, $J = 15.1$, 5.5 Hz, 1H, α-CH_2 of 3-*O*-

acyl), 2.53–2.43 (m, 2H, α-CH_2 of 3-*O*-acyl and 3′-*O*-acyl), 2.40 (dd, J = 15.0, 5.6 Hz, 1H, α-CH_2 of 3′-*O*-acyl), 2.31 (dd, J = 7.0, 7.0 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 2.28–2.20 (m, 8H, α-CH_2 of 2′-*N*-acyl's main and side chains, and α-CH_2 of 2′-*N*-acyl's main and side chains), 2.16 (dd, J = 7.0, 7.0 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 2.08 (q, J = 6.9 Hz, 2H, –CH_2–CH_3 of BA), 1.97 (dd, J = 7.0, 7.0 Hz, 2H, –CO–CH_2–CH_2–CH_2–CO–), 1.61–1.43 (m, 12H, γ-CH_2 of 2-*N*-acyl, 2′-*N*-acyl, 3-*O*-acyl, and 3′-*O*-acyl), 1.35–1.08 (m, 76H, –CH_2– × 38), 0.88 (t, J = 6.9 Hz, 21H, –CH_2–CH_3 × 7).

30b: In a manner similar to the synthesis of **30a**, **47b** (55.0 mg, 22.3 μmol) was deprotected and acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **16b** as a pale yellow solid: Yield 19.7 mg (39%); ESI-MS (positive) m/z 2622.53 $[M + Na]^+$.

30c: In a manner similar to the synthesis of **30a**, **47c** (66.0 mg, 26.8 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **30c** as a pale yellow solid (17.9 mg, 35%). ESI-MS (positive) m/z 2620.30 $[M + Na]^+$, 1299.57 $[M + 2H]^{2+}$.

30d: In a manner similar to the synthesis of **30a**, **47d** (24.7 mg, 9.91 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **30d** as a pale yellow solid (3.3 mg, 14%). ESI-MS (positive) m/z 2621.46 $[M + Na]^+$, 1311.14 $[M + H + Na]^{2+}$, 1321.56 $[M + 2Na]^{2+}$.

30e: In a manner similar to the synthesis of **30a**, **47e** (42.7 mg, 17.3 μmol) was deprotected and acylated with (*R*)-3-(dodecanoyloxy)decanoic acid (**21**) to yield **30e** as a pale yellow solid (5.2 mg, 12%). ESI-MS (positive) m/z 2620.55 $[M + Na]^+$, 1311.06 $[M + H + Na]^{2+}$, 1321.42 $[M + 2Na]^{2+}$.

30f: In a manner similar to the synthesis of **30a**, **47f** (68.4 mg, 27.4 μmol) was deprotected and acylated with (*R*)-3-(4-trifluoromethylbenzyloxy)decanoic acid (**20**) to yield **30f** as a pale yellow solid (3.0 mg, 6.0%). ESI-MS (positive) m/z 1312.33 $[M + H + Na]^{2+}$, 1321.77 $[M + 2Na]^{2+}$.

CM-Analogues 26a, 26b, 26c, 26d, 26e, and 26f. 2-Deoxy-6-*O*-[2-deoxy-2-((*R*)-3-(dodecanoyloxy)decanoylamino)-3-*O*-((*R*)-3-hydroxydecanoyl)-4-*O*-phosphono-β-D-glucopyranosyl]-2-((*R*)-3-(dodecanoyloxy)decanoylamino)-3-*O*-((*R*)-3-hydroxydecanoyl)-α-D-glucopyranosyloxyacetic Acid (26a): To a solution of **30b** (18.0 mg, 6.93 μmol) in THF–AcOH (3:1) (2.4 mL) was added $Pd(OH)_2$ (25.0 mg). The mixture was stirred under 19 kg cm^{-2} of hydrogen at room temperature for 1 d. After removal of the Pd catalyst by filtration, the solvent was evaporated in vacuo. The crude product was purified by liquid–liquid partition column chromatography (5.0 g of Sephadex® LH-20, $CHCl_3$:MeOH:water:*i*-PrOH = 10:10:10:1.3). The organic layer was the stationary phase, and the aqueous layer was the mobile phase in this chromatography. After removal of the solvent in vacuo, the residue was lyophilized from sterilized water to afford **26a** as a colorless solid (4.0 mg, 60%). ESI-MS (negative) m/z 1521.77 $[M - H]^-$, 760.35 $[M - 2H]^{2-}$. ^1H NMR (600 MHz, $CDCl_3$:MeOH-d_4 = 1:1) δ 5.20–5.04 (m, 2H, β-CH of 2-*N*-acyl and 2′-*N*-acyl), 5.19 (dd, J = 8.7, 8.7 Hz, 1H, H-3), 5.14 (dd, J = 8.9, 8.9 Hz, 1H, H-3′), 4.74 (d, J = 3.1 Hz, 1H, H-1), 4.66 (d, J = 7.4 Hz, 1H, H-1′), 4.20–4.16 (m, 2H, H-2′ and H-4′), 4.03 (d, J = 12.3 Hz, 1H, –OCH_2–COOH), 4.01–3.96 (m, 3H, H-6a, H-6′a, and β-CH of 3′-*O*-acyl), 3.83 (d, J = 12.3 Hz, 1H, –OCH_2–COOH), 3.81–3.68 (m, 5H, H-2′, H-5, H-6b, H-6′b, and β-CH of 3-*O*-acyl), 3.56 (dd, J = 8.7, 8.7 Hz, 1H, H-4), 3.46 (dd, J = 6.9, 6.9 Hz, 1H, H-5′), 2.45 (dd, J = 12.8, 6.3 Hz, 1H, α-CH_2 of 2′-*N*-acyl's main chain), 2.38 (dd, J = 12.9, 3.8 Hz, 1H, α-CH_2 of 2-*N*-acyl's main chain), 2.28–2.21 (m, 10H, α-CH_2 of 3-*O*-acyl, 3′-*O*-acyl, 2-*N*-acyl's main and side chain, and 2′-*N*-acyl's main and side chain), 1.60–1.52 (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), 1.31–1.16 (m, 66H, –CH_2– × 33), 0.85 (t, J = 6.6 Hz, 18H, –CH_3 × 6).

26b: In a manner similar to the synthesis of **26a**, **30b** (18.0 mg, 6.93 μmol) was hydrogenolytically deprotected to give **26b** as a colorless solid (2.7 mg, 25%). ESI-MS (negative) m/z 1521.24 $[M - H]^-$, 760.26 $[M - 2H]^{2-}$.

26c: In a manner similar to the synthesis of **26a**, **30c** (17.0 mg, 6.54 μmol) was hydrogenolytically deprotected to give **26c** as a colorless solid (2.1 mg, 22%). ESI-MS (negative) m/z 1521.54 $[M - H]^-$, 760.39 $[M - 2H]^{2-}$.

26d: In a manner similar to the synthesis of **26a**, **30d** (3.3 mg, 1.27 μmol) was hydrogenolytically deprotected to give **26d** as a colorless solid (1.3 mg, 67%). ESI-MS (negative) m/z 761.23 $[M - 2H]^{2-}$.

26e: In a manner similar to the synthesis of **26a**, **30e** (5.2 mg, 2.00 μmol) was hydrogenolytically deprotected to give **26e** as a colorless solid (1.7 mg, 56%). ESI-MS (negative) m/z 1522.11 $[M - H]^-$, 760.46 $[M - 2H]^{2-}$.

26f: In a manner similar to the synthesis of **26a**, **30f** (3.8 mg, 1.46 μmol) was hydrogenolytically deprotected to give **26f** as a colorless solid (1.5 mg, 67%). ESI-MS (negative) m/z 1521.93 $[M - H]^-$, 760.44 $[M - 2H]^{2-}$.

***Limulus* Assay.** *Limulus* activity of synthetic samples were measured by means of the Endospecy Test® (Seikagaku Kogyo, Tokyo, Japan) using an LPS specimen from *E. coli* O111:B1 (Sigma-Aldrich Chemical Co.) as a reference standard. A solution of a test sample in 1% DMSO in distilled water (30 μL) was mixed with the reagent in the Endospecy ES-50M set (30 μL) and incubated in duplicate in a 96-well plastic plate (Toxipet plate 96F, Seikagaku Kogyo) at 37 °C for 30 min. Sodium nitrate (75 μL, 0.04% in 0.48 mol dm^{-3} hydrochloric acid), 75 μL of 0.3% ammonium sulfate, and 75 μL of 0.07% *N*-1-naphthylethylenediamine dichloride were added successively. The absorbance at 414 nm of each well was measured using a micro plate reader.

***Cytokine* Assay.** Heparinized human whole blood diluted with RPMI 1640 (Biken, Osaka, Japan) (v/v, 1/4) was incubated at 37 °C for 24 h in humidified air containing 5% (v/v) CO_2 in a 96-well culture plate (Becton Dickson) with or without various doses of test specimens for interleukin-6 (IL-6) assay. The amounts of cytokine induced were measured from the culture supernatants using the appropriate ELISA kit systems (IL-6 and TNF-α, ELISA Development Kit human IL-6 and ELISA Development Kit human TNF-α, Genzyme TECHNE Co., Minneapolis, MN, USA). These assays were performed according to the manufacturer's instructions, and the cytokine amount was determined from a standard curve prepared for each assay. Assays were repeated three times. Similar results were obtained in repeated experiments.

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Age-related maculopathy and sunlight exposure evaluated by objective measurement

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ABSTRACT

Aim: To study the relationship between age-related maculopathy (ARM) and exposure to sunlight using an objective method.

Methods: In a case–control study of Japanese men aged ≥50 years (67 controls without ophthalmic disease and 148 with ARM), those with ARM were separated into groups of early (n = 75) and late (n = 73) ARM. Facial wrinkle length and area of hyperpigmentation, which are considered to be associated with exposure to sun, were measured using imaging with computer-based image analysis. Skin tone was also measured on the upper inner arm, which is not exposed to sun. Early and late ARM association with skin measurements was then evaluated.

Results: Significantly more facial wrinkling (p = 0.047, odds ratio 3.8; 95% CI 1.01 to 13.97) and less facial hyperpigmentation (p = 0.035, odds ratio 0.3; 95% CI 0.08 to 0.92) was present in late ARM cases. The relationship between skin tone and ARM risk was not statistically significant.

Conclusions: This objective method showed that lifetime exposure to sunlight is an important factor in the progression of late ARM. An individual's reaction to sunlight exposure may have a role in ARM progression in addition to total lifetime exposure to sunlight.

The aetiology of age-related maculopathy (ARM), which is the most common cause of vision loss in older people in developed countries, remains unclear, but is suspected to involve both external and internal factors.[1–7] Of the external factors, smoking is the most well-established independent risk factor.[4–7] In contrast, there is controversy over the role of other potential external factors, such as exposure to sunlight or ultraviolet radiation (UV).[8–16] It has been reported that abnormal skin sensitivity to sunlight or a propensity to tan is associated with ARM.[11–13] However, there are several reports that sunlight exposure is not a risk factor related to ARM.[14–17]

The controversy is probably due to the methods used to measure lifetime exposure to sunlight. Most studies assessed total sunlight exposure by using questionnaires, and the accuracy of the data obtained depends heavily on question "quality" and respondents' memory. This is an inevitable and unsolvable problem of questionnaire methodology.[8–17]

We previously reported[18 19] that people with different lifetime exposures to sunlight have correspondingly different severities of facial skin wrinkling and hyperpigmentation. In those earlier studies, we used video imaging combined with image analysis to objectively quantify skin features, reasoning that wrinkling and hyperpigmentation were quantitative, objective biomarkers of the exposure of people of the same gender and ethnic group, and thus measured true lifetime exposure more accurately than questionnaires. We used these measurements to evaluate the relationship between facial wrinkling and hyperpigmentation and ARM.

SUBJECTS AND METHODS

This case–control study of ARM and healthy controls involved subjects seen at Kagoshima University Hospital or Kagoshima Kouseiren Hospital Health Care Center between May 2005 and February 2006 who met the inclusion criteria below, and were asked to participate after the study was carefully explained. Inclusion criteria were as follows:

1. Life-long residence in Kagoshima prefecture
2. Aged 50 years or older and male
3. Fundus photographs could be taken
4. Ocular fundi were observable
5. Absence of self-reported ocular disease, eg, glaucoma or diabetic retinopathy

Late ARM cases were those diagnosed at Kagoshima University Hospital during the study. Controls and early ARM cases had undergone health checks at Kagoshima Kouseiren Hospital Health Care Center during the same period.

An initial assessment of 259 participants excluded 44: 18 had media opacity and 26 had ocular diseases (four with diabetic retinopathy, one with branch retinal vein occlusion, three with glaucoma, five with epiretinal membrane, and 13 with polypoidal choroidal vasculopathy). The 215 subjects who met the inclusion criteria comprised 67 controls, 75 with early ARM and 73 with late ARM. All subjects with late ARM had neovascular membrane confirmed by angiography. No geographic atrophy was seen.

Our research followed the tenets of the Declaration of Helsinki, with informed consent obtained from the subjects, and was approved by all of the institutional review boards involved.

Fundus examination

Fundus colour photographs (45°) of the macula (Canon CR-DG10, Tokyo, Japan) were graded by two independent qualified judges (MH, AO), who had no contact with the subjects. ARM was defined on the basis of the International ARM Epidemiological Study Group classification[20]: early ARM by the presence of soft drusen (≤63 μm) or retinal pigment epithelium pigmentation abnormalities within the grid, and late ARM by either

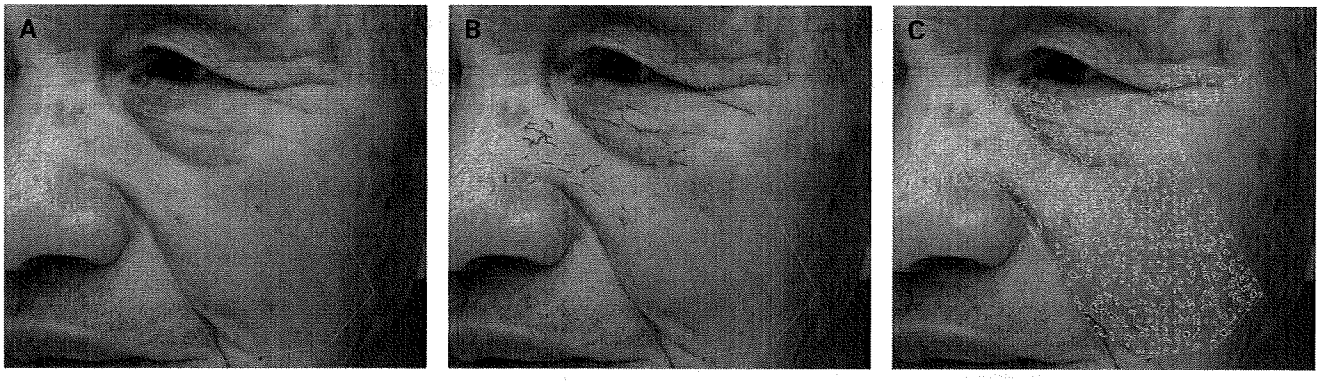

Figure 1 Representative images used to quantify facial wrinkling and hyperpigmentation. (A) The region of interest (ROI) was demarcated manually as shown by the green line. (B) The facial wrinkles detected in the ROI are shown (blue lines). (C) The hyperpigmented regions detected in the ROI are shown (yellow). Patient consent has been obtained for publication of this figure.

neovascular age-related macular degeneration or geographic atrophy involving the fovea. Minimum geographic atrophy was a circle of 175 μm or more in diameter. Those with fundus inflammatory or retinovascular disease, choroidal neovascularisation due to high myopia, or polypoidal choroidal vasculopathy confirmed by fluorescein and indocyanine green angiography were excluded. Classification was based on the subject's worst eye.

Smoking

Smoking history was obtained from questionnaires, with lifetime smoking exposure quantified in "pack-years", one "pack year" being 20 cigarettes smoked per day for one year.[21]

Hypertension

Blood pressure was measured three times with the subject in a sitting position, and the mean was used for analysis. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or current use of antihypertensive drugs.

Skin examination

Wrinkles

The total length of facial wrinkles in the region of the upper cheek and temporal areas next to the eyes was objectively measured using a two-dimensional imaging system using a commercially available high-resolution digital camera equipped with a close-up lens mounted in a standardised illumination box fitted with head-positioning aids (Beauty Imaging System; Procter & Gamble, Cincinnati, Ohio, USA). The camera was calibrated daily using a GretagMacbeth neutral 8.0 grey colour board in front of the camera. Left and right views of the face were standardised—that is, the same focal distance from the camera lens to the face, same magnification, same head position so that the camera angle was the same relative to the face surface, and exactly the same lighting.[18 19 22 23] The region of interest (ROI) was marked manually based on 12 predefined facial landmarks around the eye and cheek—for example, corners of the eye, bridge of the nose, corners of the mouth (fig 1). The lengths of facial wrinkles (fine lines) in the ROI were quantified objectively using image analysis algorithms based on an Optimus software platform, which automatically locates each facial line and quantifies the total number, length and area of facial lines longer than 5 mm and more than 0.16 mm wide, known magnification used to convert pixel data to actual length and area data. Thresholds were based on "clinically important" wrinkling—that is, excluding lines shorter than 5 mm and narrower than 0.16 mm, which fall under the heading of surface "texture".

Because the ROI varies in shape and size, total wrinkle area was normalised to total ROI size to yield a wrinkle area fraction (WAF)—that is, fractional ROI area occupied by wrinkles or fine lines. WAF varied from 0.05 (5% of ROI) to 0.2 (20% of ROI) depending on individual severity of wrinkling. Group statistical analysis used the mean WAF on the left and right sides of the face for each subject. The intraindividual coefficient of variation of imaging (within-subject reproducibility) quantifying wrinkling was found previously to be 5.2%.[23] Accuracy was confirmed using mannequins with artificial wrinkles of known length and width. Imaging accuracy was ±5% of the actual value.[23]

Pigmentation

Total facial hyperpigmentation on the left and right sides was objectively measured using the Beauty Imaging System. The region hyperpigmentation was defined as a localised region of darker skin. Hyperpigmentation is often observed after inflammation, melasma and senile lentigines, and can be exacerbated by exposure to sun.[18 19] The ROI in each image was defined manually and then automatically analysed using customised software that locates and quantifies the total area of hyperpigmented spots. The total area of spots was then normalised to the total area of the region analysed. This analysis was conducted on both the left and right sides of the face, and the mean of the two sides was used as the final measure of hyperpigmentation for each subject in the group statistical analysis.

Skin tone

Skin tone was measured on the upper inner arm using a colour reader (CR-13; Minolta, Tokyo, Japan), which was calibrated using the standard white plates supplied with the instrument,[18 19 22] to obtain three skin tone indices L*, a* and b*, ie, lightness, redness and yellowness. Triplicate measurements at each site were averaged and analysed. Skin on the inner arm represents constitutive skin colour because it is not exposed to sun.