HETEROCYCLES, Vol. 69, 2006, pp. 395 - 415. © The Japan Institute of Heterocyclic Chemistry
Received, 8th September, 2006, Accepted, 30th October, 2006, Published online, 31st October, 2006. COM-06-S(O)51

SYNTHESIS AND BIOLOGICAL ACTIVITIES OF BISCARBOXYMETHYL LIPID A ANALOGUES

Mikayo Kataoka, Masaya Hashimoto, Yasuo Suda, Shoichi Kusumoto, and Koichi Fukase*

Department of Chemistry, Graduate School of Science, Osaka University, 1-1 Machikaneyama, Toyonaka, Osaka 560-0043. koichi@chem.sci.osaka-u.ac.jp

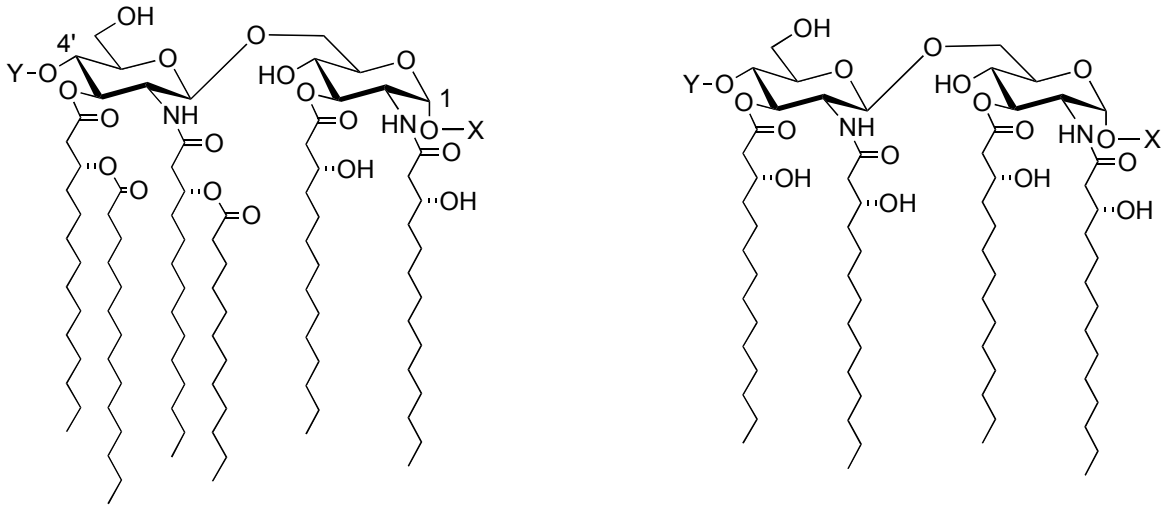
Abstract – Biscarboxymethyl analogues of *Escherichia coli* lipid A and the biosynthetic precursor-type lipid A were synthesized in order to elucidate the role of the acidic functional groups in the biological activity. Both analogues showed respective activities similar to those of their natural counterparts, i. e., the hexaacyl *E. coli* lipid A analogue showed potent immunostimulating activity, whereas the tetraacylated biosynthetic precursor-type lipid A analogue showed the antagonistic activity. The present study clearly indicates that the acidic functional groups but not phosphate groups are essential for the expression of the biological activities.

INTRODUCTION

Lipopolysaccharide (LPS), also termed endotoxin, is a cell surface component of Gram-negative bacteria and known as the strong stimulator of mammal immune system. LPS induces various inflammatory mediators such as cytokines [tumor necrosis factor α (TNF-α), interleukin (IL) 1β, 6, 8, 12, 15, 18, and etc], prostaglandins, and nitrogen oxide. These mediators stimulate immune system and also cause clinical manifestations of bacterial infections such as fever, inflammation, hypotension, and, in severe cases, lethal shock.[1] LPS consists of a glycolipid component termed lipid A and a polysaccharide part. It was unequivocally proved that lipid A is the chemical entity responsible for the biological activity of LPS by our total synthesis of *Escherichia coli* lipid A (**1**) (a synthetic **1** is termed 506) (Fig. 1).[2,3] *E. coli* lipid A consists of β(1-6) disaccharide of glucosamines, 1,4'-diphosphoryl group, long-chain acyl groups bound at 2, 2', 3, and 3' positions. A biosynthetic precursor of LPS (**2**) (a synthetic **2** is termed 406), which has four acyl groups, shows antagonistic activity in humans but displays endotoxic activity in rodents.[3-6]

LPS is sensed by a receptor complex consisting of toll like receptor 4 (TLR4)[7-11] and an adaptor protein MD-2[12-15]. Golenbock *et al*. demonstrated that the above species-specific responses to **2** are mediated by TLR4.[11] Miyake *et al.* reported that MD-2 is required for LPS signaling and is also responsible for species-specific activity of **2**.[12-14] Miyake *et al*. also demonstrated by using our synthetic lipid A and its radio-labeled analogue that TLR4-MD-2 directly associates to LPS.[15,16]

It has been shown that both phosphoryl groups at the 1-, and 4'-positions are important for the biological activities of lipid A. For example, both 1- and 4'-monophosphoryl lipid A have considerably lower activity than the natural type bisphosphorylated lipid A, and the dephosphorylated lipid A does not show any activity. To clarify the relationship between the biological activity and the role of the acidic functional groups in lipid A, we synthesized both the *E. coli*-type and the precursor-type analogues (**3**) (CM-506) and (**4**) (CM-406) in which the phosphoryl group at the 1-position is replaced with a carboxymethyl (CM) group. Both the *E. coli*-type (**3**) and the biosynthetic precursor-type (**4**) showed indistinguishable activity with the corresponding natural-type compounds.[17,18] We also synthesized lipid A analogues having acidic groups β-glycosidically linked at the 1-position.[19] The β-CM analogues also showed potent activity. The acidic functional group at 1-position are concluded to be essential but their strict spatial arrangement is not required for expression of the biological activity. In the present study, we synthesized two new analogues, *E. coli*-type (**5**) (Bis-CM-506) and precursor-type (**6**) (Bis-CM-406) having two carboxymethyl groups at 1 and 4'-positions in order to investigate the role of the acidic functional group at the 4'-position.

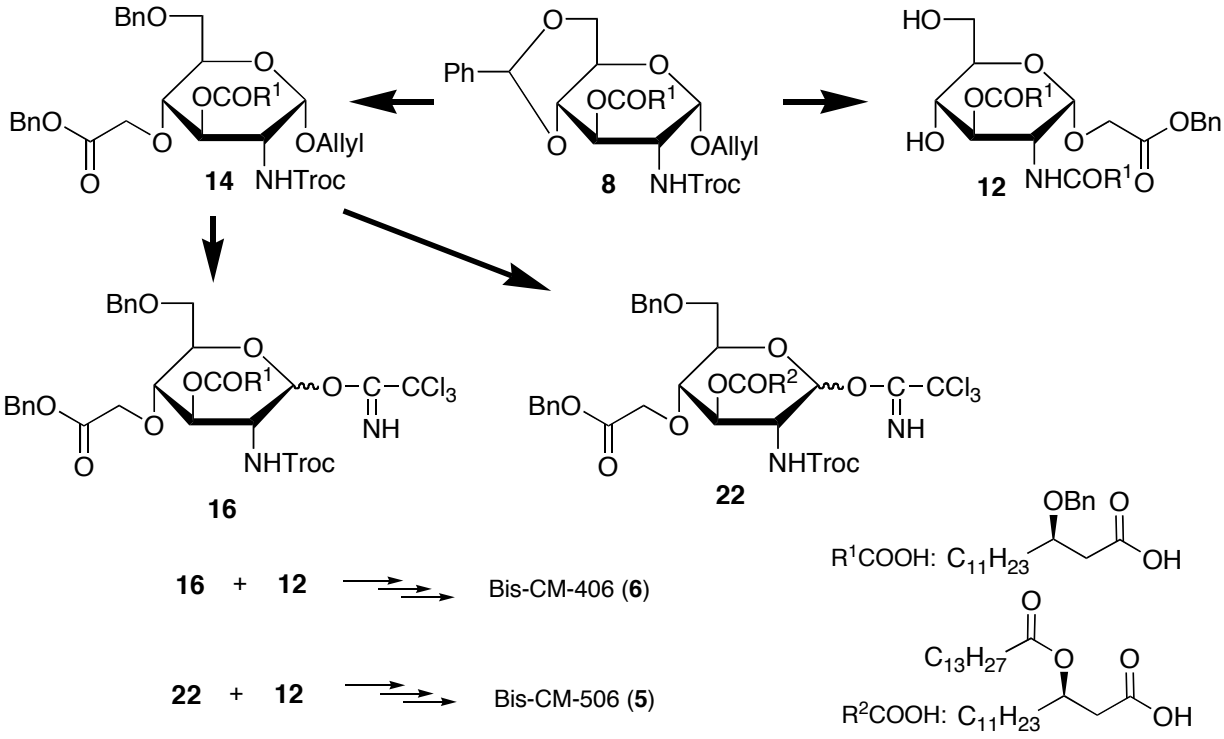


1 (X, Y = -$P(O)(OH)_2$): *Escherichia coli* Lipid A (506)
2 (X, Y = -$P(O)(OH)_2$): Biosynthetic precursor (406)
3 (X = -CH_2COOH, Y = -$P(O)(OH)_2$): CM analog (CM-506)
4 (X = -CH_2COOH, Y = -$P(O)(OH)_2$): CM analog (CM-406)
5 (X, Y = -CH_2COOH): Bis-CM analog (Bis-CM-506)
6 (X, Y = -CH_2COOH): Bis-CM analog (Bis-CM-406)

Figure 1 Structures of lipid A and analogues.

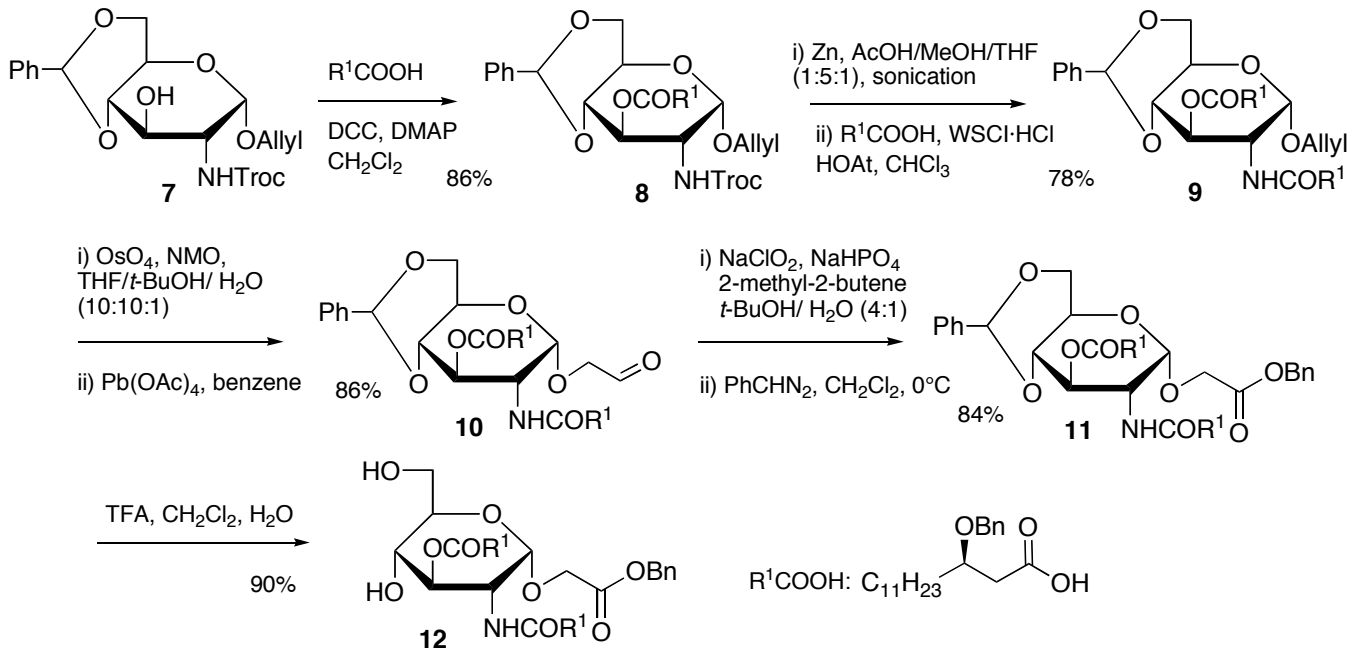
RESULTS AND DISCUSSION

We have established the efficient synthesis of lipid A and analogues in our previous studies.[17-20] In the present study, both Bis-CM-506 (**5**) and Bis-CM-406 (**6**) were synthesized via the similar strategy (Scheme 1). Hydroxy and carboxy groups were protected with benzyl groups, which were removed by catalytic hydrogenation at the last step. The β(1-6) disaccharide structures were constructed by glycosylation of the glycosyl acceptor (**12**) with the *N*-Troc trichloroacetimidate donors. Compound (**8**) was used as a common starting material for the glycosyl acceptor and the donors. The carboxymethyl group in **12** was formed by oxidative cleavage of the ally group. Both glycosyl donors (**16**) and (**22**) were derived from the key intermediate (**14**), which was synthesized from **8** via 4-*O*-carboxymethylation.



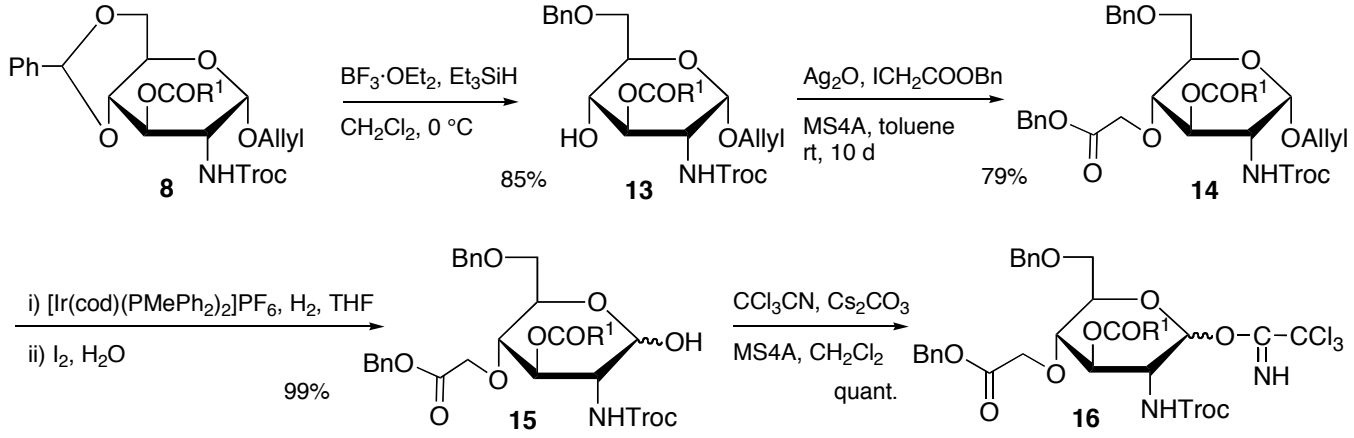
Scheme 1 Synthesis strategy of Bis-CM analogues.

The glycosyl acceptor (**12**) was synthesized as shown in Scheme 2. The hydroxy group at 3-postion of 1-*O*-allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside[17] (**7**) was acylated with (*R*)-3-benzyloxytetradecanoic acid[20]. The 2-*N*-Troc group was removed by zinc powder and acetic acid and the resulting 2-amino group was then acylated with (*R*)-3-benzyloxytetradecanoic acid to give 2,3-diacyl derivative (**9**). Allyl glycoside (**9**) was treated with OsO_4 and the resulting diol was oxidatively cleaved with $Pb(OAc)_4$ to give 1-*O*-formylmethyl glycoside (**10**), which was further oxidized with $NaClO_2$.[17] The resulting carboxyl group was protected with benzyl group by using phenyldiazomethane to give **11**. Deprotection of benzylidene group of **11** under the acidic conditions gave the glycosyl acceptor (**12**).

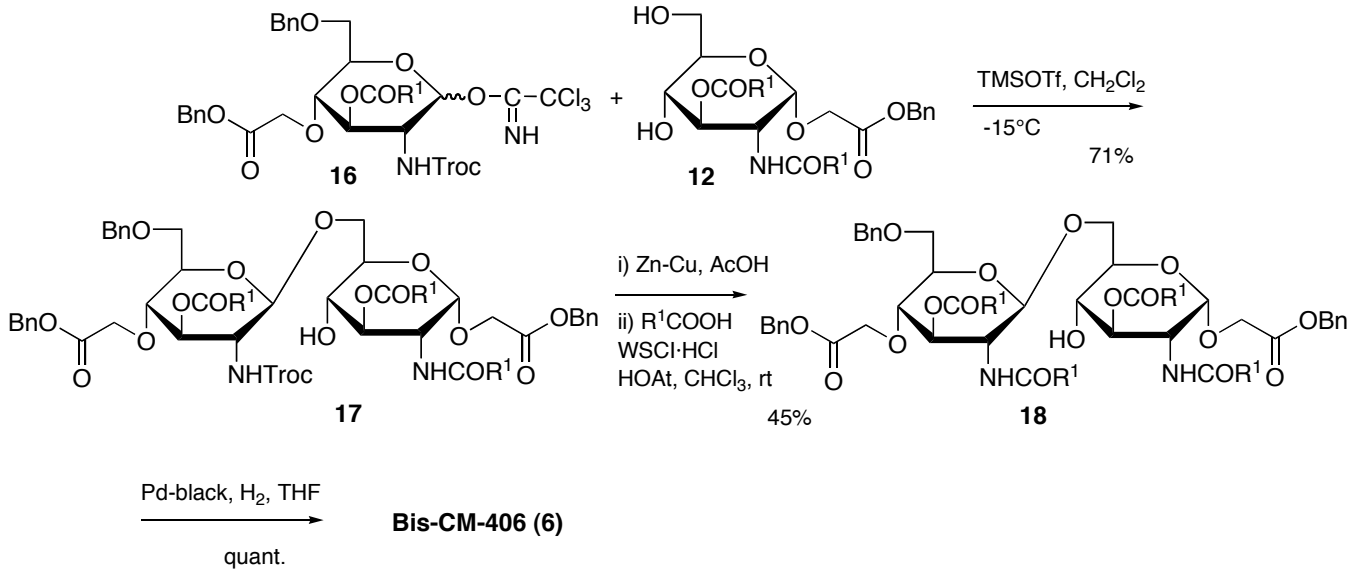


Scheme 2 Synthesis of glycosyl acceptor (**12**).

One of the key step in the synthesis of Bis-CM lipid A analogues was the introduction of the carboxymethyl group at 4'-position. Since the 4-OH group of glucosamine residue is the most sterically hindered position, we planned to introduce the carboxymethyl group before the formation of the disaccharide (Scheme 3). Regioselective reductive opening of the benzylidene (**8**) with BF_3•OEt_2 and Et_3SiH gave the 6-*O*-benzyl-4-OH GlcN derivative (**13**).[21] We then investigated the reaction conditions for the introduction of carboxymethyl group to the 4-OH group. Among the reaction conditions tested, reaction of compound (**16**) with benzyl iodoacetate and silver oxide gave 4-CM derivative (**14**) in a good yield. Other basic conditions using NaH or BuLi decomposed the starting material. The 1-*O*-allyl group of **14** was removed via isomerization to 1-propenyl group and subsequent treatment with iodine.[22] The resulting 1-OH sugar (**15**) was then transformed to the glycosyl donor (**16**).

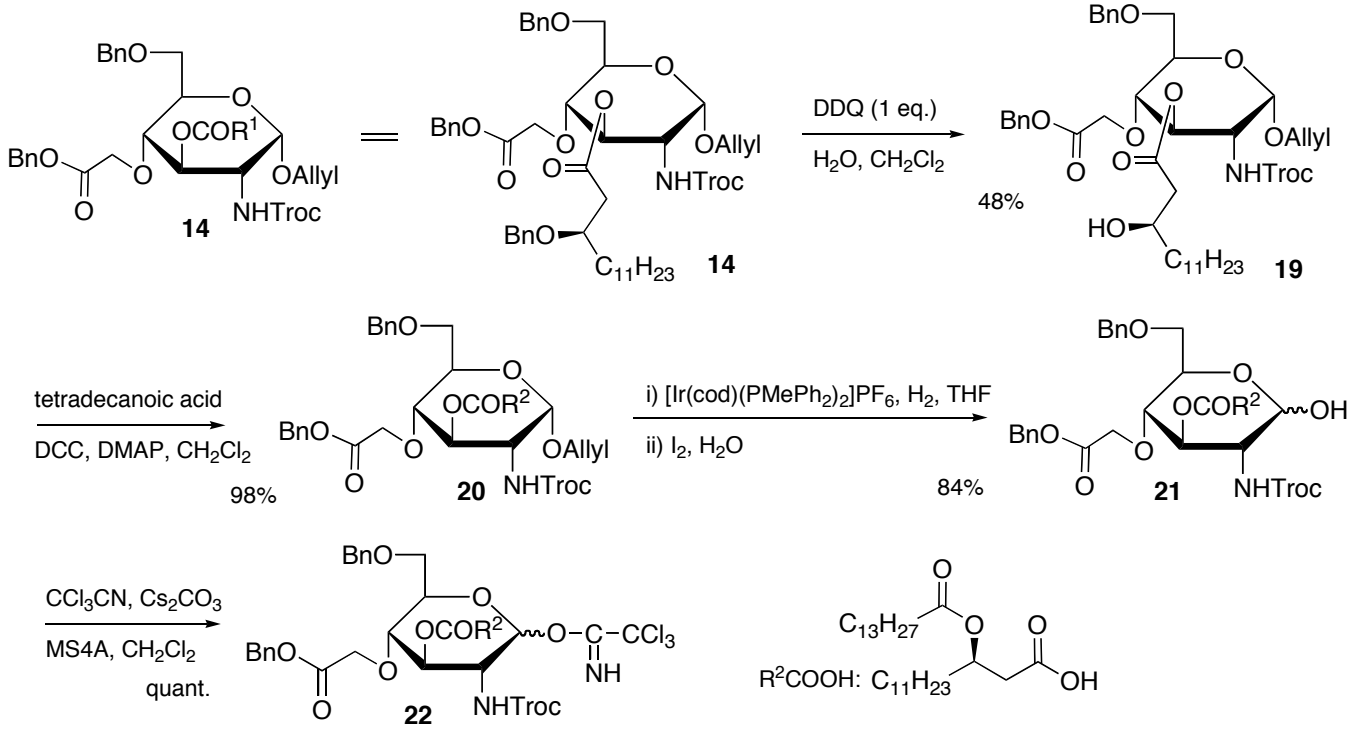


Scheme 3 Synthesis of glycosyl donor (**16**).



Scheme 4 Synthesis of Bis-CM-406 (**6**).

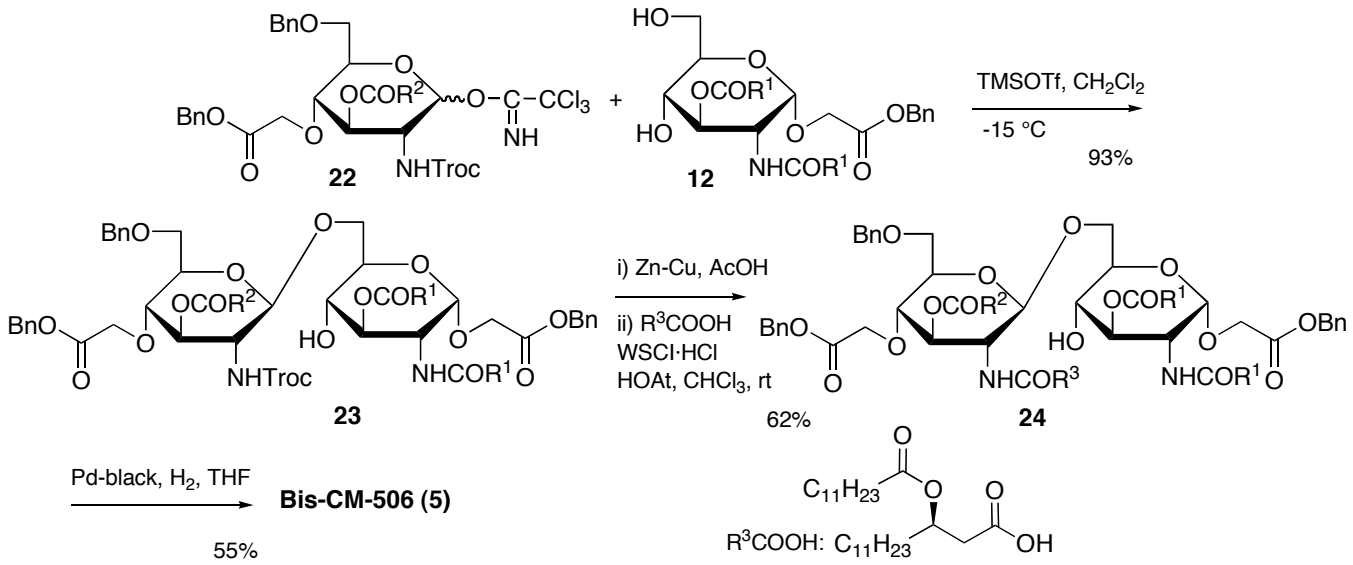
Synthesis of Bis-CM-406 was then carried out as described in Scheme 4. Glycosylation of the above glycosyl acceptor (**12**) with the glycosyl donor (**16**) gave the desired β(1-6) disaccharide (**17**) in 71% yield.[23] The 2'-*N*-Troc group of **17** was cleaved and the resulting amino group was acylated with (*R*)-3-benzyloxytetradecanoic acid to give the protected Bis-CM-406 (**18**). The final catalytic hydrogenation gave the desired Bis-CM-406 (**6**) (*m/z* = 1360.0 [(M-H)$^-$]).



Scheme 5 Synthesis of glycosyl donor (**22**).

Synthesis of Bis-CM-506 (**5**) is shown in Scheme 5 and 6. In our previous study, we found that the benzyl group on 3-benzyloxyalkanoyl moiety was more prone to oxidation than usual *O*-benzyl group.[19] We hence attempted to covert bezyloxytetradecanoate (**14**) to tetradecanoyloxytetradecanoate (**20**) via selective cleavage of the benzyl group on benzyloxyalkanoyl moiety by using 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). Benzyl group on 3-benzyloxytetradecanoyl moiety in **14** was thus cleaved by DDQ to give **19** in 48% yield with 17% recovery of **14** and other undesired 6-hydroxyl products. The hydroxy group of resulting **19** was then acylated with tetradecanoic acid to give **20**. Deprotection of 1-*O*-allyl group of **20** and subsequent formation to 1-*O*-trichloroacetoimidate afforded glycosyl donor (**22**).

Glycosylation of the acceptor (**12**) with **22** gave the disaccharide (**23**) in 93% yield. The 2'-*N*-Troc group of **23** was cleaved by Zn-Cu and acetic acid and 3-(decanoyloxy)tetradecanoic acid was then introduced to the 2'-amino group. The fully protected Bis-CM-506 (**24**) was thus obtained in 62% yield. Finally, catalytic hydrogenation of **24** removed all the benzyl groups to give the desired Bis-CM-506 (**5**) (*m/z* = 1753.1 [$(M-H)^-$]).



Scheme 6 Synthesis of Bis-CM-506 (**5**).

Biological activities of the hexaacyl Bis-CM-506 (**5**), CM-506 (**3**), and *E. coli* lipid A 506 (**1**) were evaluated by measurement of cytokine inducing activity (IL-6, TNF-α) in human peripheral whole-blood cells (Figure 2).[24] The levels of induced cytokines were measured by means of the enzyme-linked immunosorbent assay (ELISA). Bis-CM-506 (**5**) showed potent but slightly weaker activity than both natural type lipid A (**1**) and CM-506 (**3**).

Antagonistic activity of the tetraacyl Bis-CM-406 (**6**) and the biosynthetic precursor 406 (**2**) was examined by inhibition to cytokine induction by LPS. A mixture of a test sample, LPS (10 ng ml^{-1}) (*E. coli*

0111:B4; Sigma Chemicals Co.), and human peripheral whole-blood was incubated and the levels of cytokines were measured by ELISA. Bis-CM-406 (**6**) showed definite antagonistic activity but was 10-fold less active than 406 (**2**) (Figure 3).

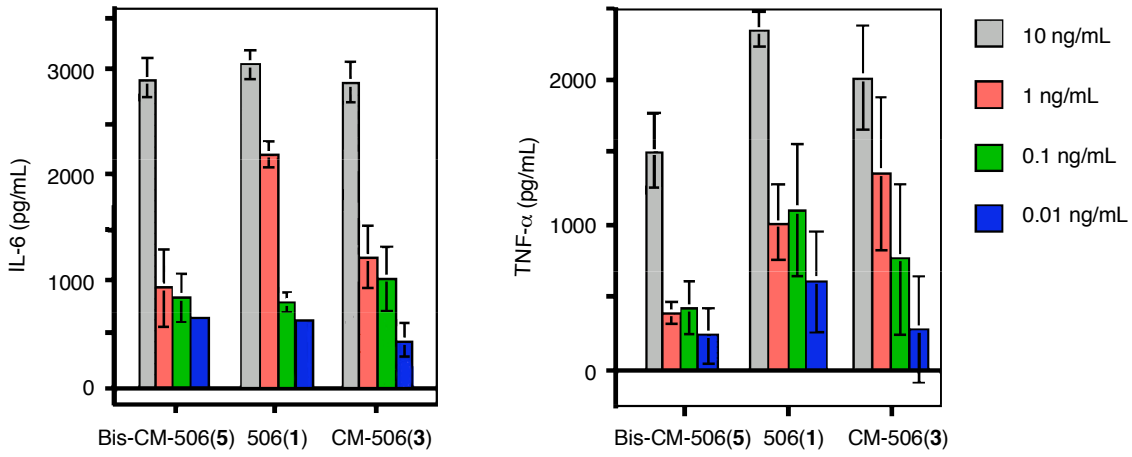


Figure 2 IL-6 and TNF-α inducing activity.

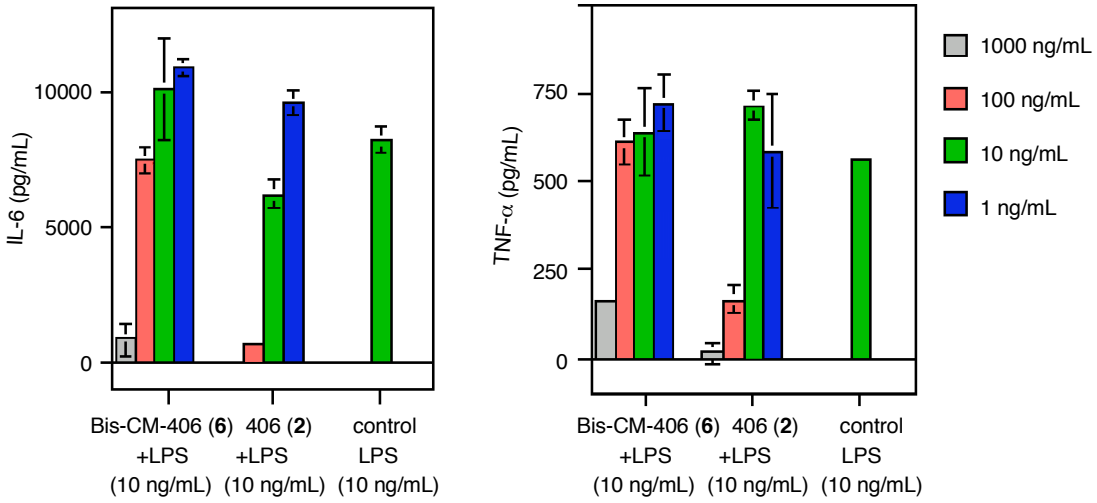


Figure 3 Inhibitory activity of Bis-CM-406 and 406.

As described, we synthesized two new lipid A analogues *E. coli*-type (**5**) (Bis-CM-506) and precursor-type (**6**) (Bis-CM-406) having two carboxy groups in place of two phosphates in natural compounds. Both analogues showed potent but slightly weaker activities than natural types. These results clearly indicated that the acidic functional groups but phosphate groups are essential for expression of the biological activities. The receptor complex should recognize negative charges on two acidic functional groups at 1 and 4'-positions. The precise biological activities and biophysical properties of two Bis-CM analogues have been reported separately.[25]

EXPERIMENTAL

General methods

NMR spectra were measured with a JEOL JNM-GX400 and JEOL JMM-GX270 at 30 °C unless otherwise specified, and analyzed using Alice® program (version 2.0). The proton chemical shifts in $CDCl_3$ are given in δ values from tetramethylsilane as an internal standard, and the chemical shifts in other solvents or conditions are given in δ values from the residual proton signal of the solvent. Mass spectra were obtained with ESI-TOF mass spectrometer (Applied Biosystems, Mariner™). Specific optical rotations were measured on a Perkin-Elmer 241 polarimeter. Silica-gel chromatography was performed using Kieselgel 60 (Merck, 0.040-0.063 mm) under medium pressure (2-4 kg/cm^2) using the indicated solvent systems. Analytical and preparative thin-layer chromatographies (TLC) were performed on Kieselgel $60F_{254}$ Plates (Merck, 0.25 mm thickness) and precoated Kieselgel $60F_{254}$ Plates (Merck, 0.5 mm thickness), respectively. Recycling preparative HPLC was carried out with Japan Analytical Industry LC908 by using $CHCl_3$ as an eluent. Nonaqueous reactions were carried out under argon atmosphere unless otherwise noted. Anhydrous dichloromethane (CH_2Cl_2) was prepared by distillation from calcium hydride and phosphorus pentoxide. Anhydrous acetonitrile, tetrahydrofuran (THF), and toluene were purchased from Kanto Chemicals Co. Distilled water was purchased from Otsuka (Tokyo, Japan) or prepared by a combination of Toray Pure LV-308 (Toray) and GSL-200 (Advantec, Tokyo, Japan). Molecular sieves 4A was activated *in vacuo* at 250 °C for 3 h before use. All other commercially obtained materials were used as received.

Allyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (**8**). To a solution of allyl 4,6-*O*-benzylidene-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranoside (**7**) (6.47 g, 13.4 mmol) and (*R*)-3-benzyloxytetradecanoic acid (5.38 g, 16.1 mmol) in dry CH_2Cl_2(100 mL) were added DMAP (318 mg, 2.60 mmol) and DCC (4.02 g, 19.5 mmol) at room temperature under N_2 atmosphere. After being stirred for 2 h, the insoluble materials were removed by filtration and the filtrate was diluted with $CHCl_3$. The organic solution was washed with aqueous 10% citric acid, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The filtrate was concentrated *in vacuo* and the residue was purified by silica-gel column chromatography (500 g, toluene/AcOEt = 50/1) to give **8** as a white solid (9.20 g, 86%). MALDI-TOF (positive) *m/z* 822.3 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.21 (m, 10H, $OCH_2\underline{Ph}$, $\underline{Ph}CH$), 5.95-5.85 (m, J_{trans} = 17.1 Hz, J_{cis} = 10.4 Hz, J_{vic} = 5.3 Hz, 1H, $OCH_2C\underline{H}=CH_2$), 5.47 (s, 1H, $PhC\underline{H}$), 5.47-5.39 (dd, $J_{3,2}$ = 10.1 Hz, $J_{3,4}$ = 9.9 Hz, 1H, H-3), 5.37-5.34 (d, $J_{N,2}$ = 10.1 Hz, 1H, $N\underline{H}$), 5.34-5.29 (dd, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 5.26-5.23 (dd, J_{cis} = 10.4 Hz, J_{gem} = 1.5 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 4.94-4.93 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.74-4.71 (d, J_{jem} = 12.1 Hz, 1H, $OC\underline{H}_2Ph$), 4.60-4.57 (d, J_{jem} = 12.1 Hz, 1H, $OC\underline{H}_2Ph$), 4.52-4.49 (d, J_{jem} = 12.1 Hz, 1H, $C\underline{H}_2$ of Troc),

4.41-4.38 (d, J_{jem} = 12.1 Hz, 1H, C$\underline{H}_2$ of Troc), 4.30-4.27 (m, $J_{6a,5}$ = 4.7 Hz, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6a), 4.24-4.19 (dd, J_{jem} = 12.7 Hz, J_{vic} = 5.3 Hz, 1H, OC$\underline{H}_2$CH=CH_2), 4.10-4.00 (m, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.1, $J_{2,N}$ = 10.1 Hz, J_{jem} = 12.8 Hz, J_{vic} = 6.4 Hz, 2H, OC$\underline{H}_2$CH=CH_2, H-2), 3.98-3.92 (ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6a}$ = 4.7 Hz, $J_{5,6b}$ = 9.9 Hz, 1H, H-5), 3.83-3.68 (m, 3H, $C^3\underline{H}$ of acyl, H-6b, H-4), 2.70-2.64 (dd, J_{jem} = 15.3 Hz, J_{vic} = 6.4 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.45-2.40 (dd, J_{jem} = 15.3 Hz, J_{vic} = 6.0 Hz, 1H, $C^2\underline{H}_2$ of acyl), 1.53-1.41 (m, 2H, $C^4\underline{H}_2$ of acyl), 1.32-1.18 (m, 18H, $C^{5-13}\underline{H}_2$ of acyl), 0.90-0.86 (t, J_{vic} = 7.2 Hz, 3H, $C^{14}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 60.11; H, 6.81; N, 1.75%. Found: C, 60.61; H, 6.70; N, 1.74. $[\alpha]_D^{25}$ = +35.8 (c 1.00, $CHCl_3$).

Allyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (9). To a solution of **8** (2.00 g, 2.51 mmol) in AcOH/MeOH/THF = 1/5/1 (21 mL) was added zinc powder (2.07 g). The suspension was sonicated for 30 sec. and stirred at room temperature for 20 min. After additional sonication for 30 min and being stirred for 2h, the mixture was filtered through cerite pad. To the filtrate was added saturated aqueous $NaHCO_3$ (20 mL) and then the mixture was extracted with AcOEt. Combined extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give crude amine. To the solution of the amine, (*R*)-3-benzyloxytetradecanoic acid (78.8 mg, 2.36 mmol) and HOAt (440 mg, 3.23 mmol) in dry $CHCl_3$ (22 mL) was added WSCI•HCl (620 mg, 3.23 mmol) at room temperature under N_2 atmosphere. After being stirred over night, the mixture was quenched with water (30 mL) and extracted with $CHCl_3$. Combined organic layer was washed with water, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (80 g, toluene/AcOEt = 50/1) to give **9** as a white solid (1.84 g, 78%). MALDI-TOF (positive), *m/z* 962.7 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.20 (m, 15H, $OCH_2\underline{Ph}$ x 2, $\underline{Ph}CH$), 5.37-5.34 (d, $J_{N,2}$ = 10.1 Hz, 1H, N$\underline{H}$), 5.78-5.68 (m, 1H, $OCH_2C\underline{H}$=CH_2), 5.46 (s, 1H, PhC$\underline{H}$), 5.41-5.36 (dd, $J_{3,2}$ = 10.1 Hz, $J_{3,4}$ = 9.9 Hz, 1H, H-3), 5.22-5.17 (dd, J_{trans} = 17.2 Hz, J_{gem} = 1.5 Hz, 1H, OCH_2CH=C$\underline{H}_2$), 5.14-5.10 (dd, J_{cis} = 10.4 Hz, J_{gem} = 1.4 Hz, 1H, OCH_2CH=C$\underline{H}_2$), 4.80-4.79 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.56-4.48 (m, J_{jem} = 11.6 Hz, 3H, OC$\underline{H}_2$Ph, OC$\underline{H}_2$Ph), 4.43-4.36 (m, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.5, $J_{2,N}$ = 9.6 Hz, J_{jem} = 11.6 Hz, 2H, H-2, OC$\underline{H}_2$Ph), 4.06-4.00 (dd, J_{jem} = 12.7 Hz, J_{vic} = 5.5 Hz, 1H, OC$\underline{H}_2$CH=CH_2), 3.94-3.88 (ddd, $J_{5,4}$ = 9.8 Hz, $J_{5,6a}$ = 4.6 Hz, $J_{5,6b}$ = 9.8 Hz, 1H, H-5), 3.84-3.68 (m, 5H, OC$\underline{H}_2$CH=CH_2, $C^3\underline{H}$ of acyl x 2, H-6b, H-4), 2.70-2.64 (m, J_{jem} = 15.3 Hz, J_{vic} = 6.3 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.44-2.39 (dd, J_{jem} = 15.3 Hz, J_{vic} = 6.1 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.37-3.27 (m, 2H, $C^2\underline{H}_2$ of acyl), 1.61-1.20 (m, 40H, $C^{4-13}\underline{H}_2$ of acyl x 2), 0.90-0.86 (m, 3H, $C^{14}\underline{H}_3$ of acyl x 2). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 74.09; H, 9.11; N, 1.49%. Found: C, 73.81; H, 9.06; N, 1.50. $[\alpha]_D^{25}$ = +36.3 (c 1.00, $CHCl_3$).

Formylmethyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (10). To a solution of **9** (126 mg, 0.134 mmol) in

THF/*t*-BuOH/H_2O = 10/10/1 (20.1 mL) were added 4-methylmorpholine *N*-oxide (64.7 mg, 0.536 mmol) and OsO_4 (1 M, 26.9 mL, 26.9 mmol) at room temperature. After being stirred for 8 h, the reaction was quenched with 10% aqueous $Na_2S_2O_3$. The mixture was extracted with AcOEt, washed with 10% aqueous $Na_2S_2O_3$, and concentrated *in vacuo* to give the crude diol. To the solution of the diol in anhydrous benzene (1.2 mL) was added $Pb(OAc)_4$ (93%, 76.8 mg, 0.161 mmol) at room temperature under N_2 atmosphere. After being stirred for 1 h, the mixture was filtrated through cerite pad and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10 g, $CHCl_3$/acetone = 20/1) to give **10** as a white solid (109 mg, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.41 (s, 1H, C<u>H</u>O), 7.37-7.23 (m, 15H, OCH$_2$<u>Ph</u> x 2, CH<u>Ph</u>), 6.58-6.55 (d, $J_{N,2}$ = 9.5 Hz, N<u>H</u>), 5.45-5.35 (m, 2H, C<u>H</u>Ph, H-3), 4.73-4.72 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.58-4.37 (m, 5H, OC<u>H</u>$_2$Ph x 2, H-2), 4.24-4.20 (dd, $J_{6a,5}$ = 4.9 Hz, $J_{6a,6b}$ = 10.2 Hz, 1H, H-6a), 3.94-3.67 (m, 7H, H-5, C<u>H</u>$_2$ of formylmethyl, C^3<u>H</u> of acyl x 2, H-6b, H-4), 2.70-2.65 (dd, J_{jem} = 14.8 Hz, J_{vic} = 6.0 Hz, 1H, C^2<u>H</u>$_2$ of acyl), 2.46-2.28 (m, 3H, C^2<u>H</u>$_2$of acyl x 3), 1.47-1.18 (m, 40H, C$^{4\text{-}13}$<u>H</u>$_2$ of acyl x 2), 0.90-0.86 (t, 6H, C^{14}<u>H</u>$_3$ of acyl x 2).

Benzyloxycarbonylmethyl 4,6-*O*-benzylidene-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (11). To a suspension of **10** (942 mg, 1.00 mmol), NaH_2PO_4 (120 mg, 1.0 mmol), and 2-methyl-2-butene (530 mL, 5.00 mol) in *t*-BuOH/H_2O = 4/1 (10 mL) was added $NaClO_2$ (80% purity, 113 mg, 1.00 mmol) at room temperature. After being stirred over night, the mixture was neutralized with aqueous 1 M HCl and extracted with AcOEt three times. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The residue was freezed-dried from dioxane to give the crude carboxylic acid. To the suspension of the carboxylic acid in CH_2Cl_2 (10 mL) was added the Et_2O solution of phenyl diazomethane (0.46 M) at room temperature until the suspension turned a pale red. The excess reagent was quenched with AcOH and AcOEt was added. The organic solution was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (40 g, toluene/AcOEt = 10/1) to give **11** as a white solid (886 mg, 84%). MALDI-TOF (positive) *m/z* 1070.7 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.21 (m, 20H, OCH$_2$<u>Ph</u> x 3, <u>Ph</u>CH), 6.71-6.69 (d, $J_{N,2}$ = 9.5 Hz, 1H, N<u>H</u>), 5.44 (s, 1H, PhC<u>H</u>), 5.43-5.38 (dd, $J_{3,2}$ = 9.9 Hz, $J_{3,4}$ = 10.1 Hz, 1H, H-3), 5.12 (s, 2H, COOCH$_2$Ph), 4.80-4.79 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.59-4.47 (m, 3H, OC<u>H</u>$_2$Ph, OC<u>H</u>$_2$Ph), 4.45-4.39 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 9.9 Hz, $J_{2,N}$ = 9.5 Hz, 1H, H-2), 4.38-4.35 (d, J_{jem} = 11.6 Hz, 1H, OC<u>H</u>$_2$Ph), 4.22-4.18 (dd, $J_{6a,5}$ = 4.9 Hz, $J_{6a,6b}$ = 10.4 Hz, 1H, H-6a), 4.01-3.92 (m, 3H, C<u>H</u>$_2$ of CM, H-6b), 3.85-3.78 (m, 2H, H-5, H-4), 2.70-2.64 (m, J_{jem} = 15.1 Hz, J_{vic} = 6.6 Hz, 1H, C^2<u>H</u>$_2$ of acyl), 2.43-2.37 (m, J_{jem} = 15.1 Hz, J_{vic} = 5.8 Hz, 3H, C^2<u>H</u>$_2$ of acyl x 3), 1.48-1.43 (m, 40H, C$^{4\text{-}13}$<u>H</u>$_2$ of acyl x 2), 0.90-0.86 (m, 6H, C^{14}<u>H</u>$_3$ of acyl x 2). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 73.32; H, 8.56; N, 1.34%. Found: C, 72.94; H, 5.89; N, 1.36. $[\alpha]_D^{25}$ = +34.8 (c 1.00, $CHCl_3$).

Benzyloxycarbonylmethyl 3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoyl-amino)-2-deoxy-α-D-glucopyranoside (12). To the suspension of **11** (56.0 mg, 53.4 mmol) in CH_2Cl_2/H_2O = 50/1 (0.25 mL) was added the CH_2Cl_2 solution of trifluoroacetic acid (10%, 250 mL) at room temperature under N_2 atmosphere. After being stirred for 40 min, the reaction was quenched with aqueous saturated $NaHCO_3$ (1 mL) and extracted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (5 g, $CHCl_3$/acetone = 10/1) to give **12** as a white solid (46.2 mg, 90%). MALDI-TOF (positive) *m/z* 982.7 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44-7.20 (m, 15H, $OCH_2\underline{Ph}$ x 3), 6.68-6.66 (d, $J_{N,2}$ = 9.5 Hz, 1H, N$\underline{H}$), 5.16-5.11 (dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 9.0 Hz, 1H, H-3), 5.11 (s, 2H, COOC$\underline{H}_2$Ph), 4.79-4.78 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.55-4.50 (m, 4H, OC$\underline{H}_2$Ph x 2), 4.32-4.26 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.7 Hz, $J_{2,N}$ = 9.5 Hz, 1H, H-2), 4.03-3.98 (d, J_{jem} = 16.6 Hz, 1H, C$\underline{H}_2$ of CM) , 3.97-3.93 (d, J_{jem} = 16.6 Hz, 1H, C$\underline{H}_2$ of CM), 3.90-3.84 (m, 1H, $C^3\underline{H}$ of acyl), 3.83-3.79 (m, 1H, $C^3\underline{H}$ of acyl), 3.75-3.68 (m, 3H, H-5, H-6a, H-6b), 3.65-3.60 (dd, $J_{4,3}$ = 9.3 Hz, $J_{4,5}$ = 9.3 Hz, 1H, H-4), 2.64-2.59 (dd, J_{jem} = 14.7 Hz, J_{vic} = 8.1 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.48-2.44 (dd, J_{jem} = 14.7 Hz, J_{vic} = 4.6 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.38-2.37 (d, J_{vic} = 5.9 Hz, 2H, $C^2\underline{H}_2$ of acyl), 1.65-1.42 (m, 2H, $C^4\underline{H}_2$ of acyl x 2), 1.30-0.90 (m, 36H, $C^{5\text{-}13}\underline{H}_2$ of acyl x 2), 0.88-0.86 (t, J_{vic} = 6.1 Hz, 6H, $C^{14}\underline{H}_3$ of acyl x 2). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 71.29; H, 8.92; N, 1.46%. Found: C, 70.92 ; H, 8.94; N, 1.51. $[\alpha]_D^{25}$ = +34.2 (c 1.00, $CHCl_3$).

Allyl 6-*O*-benzyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarboyl-amino)-α-D-glucopyranoside (13). To a solution of **8** (2.00 g, 2.56 mmol) in dry CH_2Cl_2 (24 mL) were added triethylsilane (4.8 mL, 30.0 mmol) and boron trifruoride diethyl etherate (614 mL, 5.0 mmol) at 0 °C under N_2 atmosphere. After being stirred for 5 h at room temperature, the reaction was quenched with saturated aqueous $NaHCO_3$ (20 mL) and extracted with AcOEt. 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 column chromatography (180 g, toluene/AcOEt = 15/1) to give **13** as colorless oil (1.70 g, 85%). A part of the product was acetylated for NMR analysis. MALDI-TOF (positive) *m/z* 822.3 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) of 4-*O*-acetylate: δ 7.35-7.22 (m, 10H, $OCH_2\underline{Ph}$ x 2), 5.95-5.85 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.34-5.29 (dd, $J_{3,2}$ = 11.0 Hz, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 5.33-5.28 (dd, J_{jem} = 1.4 Hz, J_{vic} = 17.1 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 5.27-5.25 (d, $J_{N,2}$ = 10.1 Hz, 1H, N$\underline{H}$), 5.25-5.22 (dd, J_{jem} = 1.2 Hz, J_{vic} = 10.7 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 5.17-5.12 (dd, $J_{4,3}$ = 9.7 Hz, $J_{4,5}$ = 9.9 Hz, 1H, H-4), 4.96-4.95 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.70-4.67 (d, J_{jem} = 12.1 Hz, 1H, C$\underline{H}_2$ of Troc), 4.60-4.58 (d, J_{jem} = 12.1 Hz, 1H, C$\underline{H}_2$ of Troc), 4.57-4.56 (m, 4H, OC$\underline{H}_2$Ph x 2), 4.24-4.19 (dd, J_{jem} = 11.4 Hz, J_{vic} = 5.3 Hz, 1H, $OC\underline{H}_2CH=CH_2$), 4.08-4.00 (m, J_{jem} = 11.4 Hz, J_{vic} = 6.1 Hz, 2H, $OC\underline{H}_2CH=CH_2$, H-2), 3.98-3.93 (ddd, $J_{5,4}$ = 10.1 Hz, $J_{5,6a}$ = 3.7 Hz, $J_{5,6b}$ = 4.1 Hz, 1H, H-5), 3.83-3.77 (m, 1H, $C^3\underline{H}$ of acyl), 3.56-3.49 (m, J_{jem} = 2.1 Hz, $J_{6a,5}$ = 3.7 Hz,

$J_{6b,5}$ = 4.1 Hz, 2H, H-6a, H-6b), 2.59-2.53 (dd, J_{jem} = 15.7 Hz, J_{vic} = 7.0 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.45-2.40 (dd, J_{jem} = 15.7 Hz, J_{vic} = 5.2 Hz, 1H, $C^2\underline{H}_2$ of acyl), 1.75 (s, 3H, $C\underline{H}_3$ of Ac), 1.71-1.38 (m, 2H, $C^4\underline{H}_2$ of acyl), 1.32-1.25 (m, 18H, $C^{5\text{-}13}\underline{H}_2$ of acyl), 0.90-0.86 (t, J_{vic} = 6.7 Hz, 3H, $C^{14}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 59.96; H, 7.04; N, 1.75%. Found: C, 59.97; H, 7.07; N, 1.78. $[\alpha]_D^{25}$ = +36.7 (c 1.00, $CHCl_3$).

Allyl 6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranoside (14). To the suspension of **13** (335 mg, 0.418 mmol), silver oxide (484 mg, 2.09 mmol) and MS4A in anhydrous toluene (4.2 mL) was added benzyl iodoacetate (571 mg, 2.09 mmol) at room temperature under N_2 atmosphere. After being stirred for 10 d, AcOEt was added to the reaction mixture and the insoluble materials were removed by filtration. The filtrate was washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (80 g, toluene/AcOEt = 20/1) and by recycling HPLC to give **14** as a colorless oil (282 mg, 79%). MALDI-TOF (positive) *m/z* 970.4 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.20 (m, 15H, $OCH_2\underline{Ph}$ x 3), 5.91-5.81 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.39-5.34 (dd, $J_{3,2}$ = 10.4 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.31-5.25 (m, $J_{N,2}$ = 10.1 Hz J_{jem} = 1.5 Hz, J_{vic} = 17.2 Hz, 2H, N$\underline{H}$, $OCH_2CH=C\underline{H}_2$), 5.24-5.18 (dd, J_{jem} = 1.3 Hz, J_{vic} = 10.3 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 5.06-5.03 (d, J_{jem} = 12.3 Hz, 1H, $COOC\underline{H}_2Ph$), 5.02-4.99 (d, J_{jem} = 12.3 Hz, 1H, $COOC\underline{H}_2Ph$), 4.90-4.89 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.69-4.44 (m, 6H, $OC\underline{H}_2Ph$ x 2, $C\underline{H}_2$ of Troc), 4.22-4.04 (m, $J_{jem\ of\ CM}$ = 16.1 Hz, J_{jem} = 12.6 Hz, J_{vic} = 5.3 Hz, 3H, $C\underline{H}_2$ of CM, $OC\underline{H}_2CH=CH_2$), 4.01-3.97 (dd, J_{jem} = 12.6 Hz, J_{vic} = 6.2 Hz, 1H, $OC\underline{H}_2CH=CH_2$), 3.96-3.82 (m, 4H, H-2, H-5, H-6a, $C^3\underline{H}$ of acyl), 3.74-3.67 (m, $J_{4,3}$ = 9.5 Hz, 2H, H-4, H-6b), 2.64-2.58 (dd, J_{jem} = 15.8 Hz, J_{vic} = 7.1 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.46-2.41 (dd, J_{jem} = 15.8 Hz, J_{vic} = 5.1 Hz, 1H, $C^2\underline{H}_2$of acyl), 1.56-1.45 (m, 2H, $C^4\underline{H}_2$ of acyl), 1.30-1.25 (m, 18H, $C^{5\text{-}13}\underline{H}_2$ of acyl), 0.90-0.86 (t, J_{vic} = 6.8 Hz, 3H, $C^{14}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 61.99; H, 6.79; N, 1.48%. Found: C, 61.91; H, 6.75; N, 1.47.

6-*O*-Benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranose (15). To a degassed solution of **14** (606 mg, 0.638 mmol) in anhydrous THF (4 mL) was added [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)-iridium(I) hexafluorophosphate (13.4 mg, 15.8 mmol). After activation of the iridium catalyst with hydrogen three times (each 30 sec), the mixture was stirred under nitrogen atmosphere at room temperature for 30 min. To the mixture were added successively H_2O (5 mL) and iodine (335 mg, 1.32 mmol), and the mixture was stirred for additional 30 min. After quenching with aqueous 10% $Na_2S_2O_3$ (8 mL), the mixture was extracted with AcOEt. Combined extracts were washed with aqueous 10% $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (40 g, toluene/AcOEt = 7/1) to give **15** as a white solid (575 mg, 99%). MALDI-TOF

(positive) *m/z* 930.4 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ 7.36-7.19 (m, 15H, $OCH_2\underline{Ph}$ x 3), 5.45-5.39 (m, $J_{N,2}$ = 9.5 Hz, $J_{3,2}$ = 11.0 Hz, $J_{3,4}$ = 9.5 Hz, 2H, N$\underline{H}$, H-3), 5.26-5.26 (d, $J_{1,2}$ = 3.2 Hz, 1H, H-1), 5.07-5.04 (d, J_{jem} = 12.2 Hz, 1H, COOC$\underline{H}_2$Ph), 5.03-4.99 (d, J_{jem} = 12.2 Hz, 1H, COOC$\underline{H}_2$Ph), 4.66-4.45 (m, 6H, C$\underline{H}_2$ of Troc, OC$\underline{H}_2$Ph x 2), 4.21-4.17 (m, $J_{jem\ of\ CM}$ = 16.4 Hz, 1H, C$\underline{H}_2$ of CM), 4.14-4.11 (ddd, $J_{5,4}$ = 10.0 Hz, $J_{5,6a}$ = 4.2 Hz, $J_{5,6b}$ = 1.7 Hz, 1H, H-5), 4.10-4.06 (m, $J_{jem\ of\ CM}$ = 16.4 Hz, 1H, C$\underline{H}_2$ of CM), 3.92-3.85 (m, 2H, H-2, C$^3\underline{H}$ of acyl), 3.84-3.80 (dd, $J_{6a,5}$ = 4.2 Hz, $J_{6a,6b}$ = 11.0 Hz, 1H, H-6a), 3.77-3.74 (dd, $J_{6b,5}$ = 1.7 Hz, $J_{6b,6a}$ = 11.0 Hz, 1H, H-6b), 3.66-3.61 (m, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 10.0 Hz, 2H, H-4,), 3.16 (s, 1H, O$\underline{H}$-1), 2.64-2.58 (dd, J_{jem} = 15.9 Hz, J_{vic} = 7.6 Hz, 1H, C$^2\underline{H}_2$ of acyl), 2.46-2.41 (dd, J_{jem} = 15.9 Hz, J_{vic} = 5.1 Hz, 1H, C$^2\underline{H}_2$ of acyl), 1.53-1.45 (m, 2H, C$^4\underline{H}_2$ of acyl), 1.32-1.25 (m, 18H, C$^{5\text{-}13}\underline{H}_2$ of acyl), 0.90-0.86 (t, J_{vic} = 7.1 Hz, 3H, C$^{14}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 60.76; H, 6.65; N, 1.54%. Found: C, 60.31; H, 6.58; N, 1.55.

Benzyloxycarbonylmethyl 6-*O*-[6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranoside (17). To a suspension of **15** (341 mg, 0.375 mmol) and MS4A in dry CH_2Cl_2 (3.7 mL) were added trichloroacetonitrile (376 mL, 3.75 mmol) and cesium carbonate (61.3 mg, 0.188 mmol) at room temperature under N_2 atmosphere. After being stirred for 1 h, the reaction was quenched with aqueous saturated $NaHCO_3$ (10 mL) and extracted with AcOEt. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude imidate (**16**) (396 mg, quant.). To the suspension of **16** and the glycosyl acceptor (**12**) (293 mg, 0.306 mmol) and MS4A in dry CH_2Cl_2 (4 mL) was added trimethylsilyl triflate (11.9 mL, 61.2 mmol) at -20 ˚C under N_2 atmosphere. After being stirred for 10 min, the mixture was quenched by addition of aqueous saturated $NaHCO_3$ (10 mL) and extracted with AcOEt. The organic layer was washed with aqueous saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (30 g, toluene/AcOEt = 7/1) to give **17** as a white solid (427 mg, 71%). MALDI-TOF (positive) *m/z* 1873.4 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.20 (m, 15H, $OCH_2\underline{Ph}$ x 6), 6.61-6.59 (d, $J_{N,2}$ = 9.3 Hz, 1H, N$\underline{H}$), 5.22-5.18 (m, 2H, N'$\underline{H}$, H-3'), 5.15-5.07 (m, $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 9.3 Hz, J_{jem} = 11.9 Hz, 3H, H-3, COOC$\underline{H}_2$Ph), 5.03-5.00 (d, J_{jem} = 12.5 Hz, 1H, COOC$\underline{H}_2$Ph'), 4.76-4.75 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.68-4.43 (m, $J_{1',2'}$ = 8.5 Hz, 11H, C$\underline{H}_2$ of Troc, H-1', OC$\underline{H}_2$Ph x 4), 4.30-4.24 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.8 Hz, $J_{2,N}$ = 9.3 Hz, 1H, H-2), 4.18-4.14 (m, J_{jem} = 16.2 Hz, 1H, C$\underline{H}_2$ of 4-*O*-carboxymethyl(CM')), 4.09-4.05 (m, J_{jem} = 16.2 Hz, 1H, C$\underline{H}_2$ of CM'), 4.02-3.98 (m, J_{jem} = 16.8 Hz, 2H, H-6a, C$\underline{H}_2$ of CM), 3.96-3.92 (m, $J_{jem\ of\ CM}$ = 16.8 Hz, 1H, C$\underline{H}_2$ of CM), 3.83-3.80 (m, 6H, H-5, H-6a, H-6a', C$^3\underline{H}$ of acyl x 3), 3.73-3.69 (dd, $J_{6b,5}$ = 5.0 Hz, $J_{6b,6a}$ = 10.8 Hz, 1H, H-6b), 3.64-3.52 (m, 4H, H-4, H-4', H-2', H-5'), 2.65-2.56 (m, J_{jem} = 15.3 Hz, J_{jem} = 15.4 Hz , J_{vic} = 7.8 Hz, J_{vic} = 7.0 Hz, 2H, C$^2\underline{H}_2$ of acyl x 2), 2.50-2.42

(m, J_{jem}= 15.4 Hz, J_{jem}= 15.3 Hz, $J_{vi\,c}$= 5.0 Hz, J_{vic}= 4.9 Hz, 2H, $C^2\underline{H}_2$ of acyl x 2), 2.35-2.31 (m, 2H, $C^2\underline{H}_2$ of acyl), 1.59-1.48 (m, 6H, $C^4\underline{H}_2$ of acyl x 3), 1.30-1.25 (m, 54H, $C^{5\text{-}13}\underline{H}_2$ of acyl x 3), 0.90-0.86 (t, J_{vic}= 7.1 Hz, 9H, $C^{14}\underline{H}_3$ of acyl x 3). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 66.81; H, 7.78; N, 1.51%. Found: C, 66.63; H, 7.76; N, 1.58. $[\alpha]_D^{25}$= +20.9 (c 1.00, $CHCl_3$).

Benzyloxycarbonylmethyl 6-*O*-[6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (18). To the solution of **17** (281 mg, 0.152 mmol) in AcOH (3 mL) was added zinc-copper couple [Zn (200 mg) was activated with aqueous 10% $CuSO_4$ solution]. The mixture was stirred for 30 min and AcOEt was added. After the in soluble materials were removed by filtration, the filtrate was washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. To the solution of the residue, (*R*)-3-benzyloxytetradecanoic acid (62.8 mg, 0.182 mmol), and HOAt (31.0 mg, 28.0 mmol) in dry $CHCl_3$ (2 mL) was added WSCI•HCl (43.7 mg, 0.228 mmol) at room temperature under N_2 atmosphere. After being stirred for 2 d, the mixture was purified by silica-gel column chromatography (40 g, $CHCl_3$/ acetone = 20/1) and recycling HPLC to give **18** as a white solid (137 mg, 45%). MALDI-TOF (positive) *m/z* 2013.98 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.20 (m, 15H, $OCH_2\underline{Ph}$ x 7), 6.62-6.60 (d, $J_{N,2}$= 9.3 Hz, 1H, N$\underline{H}$), 6.29-6.27 (d, $J_{N',2'}$= 9.3 Hz, 1H, N'$\underline{H}$), 5.17-5.12 (dd, $J_{3,2}$= 10.5 Hz, $J_{3,4}$= 9.3 Hz, 1H, H-3), 5.08 (s, 1H, COOC$\underline{H}_2$Ph), 5.06-4.97 (m, 3H, COOC$\underline{H}_2$Ph', H-3'), 4.74-4.73 (d, $J_{1,2}$= 3.7 Hz, 1H, H-1), 4.54-4.39 (m, 10H, OC$\underline{H}_2$Ph x 5), 4.29-4.23 (m, $J_{2,1}$= 3.7 Hz, $J_{2,3}$= 10.7 Hz, $J_{1',2'}$= 8.3 Hz, 2H, H-2 , H-1'), 4.15-4.11 (m, J_{jem}= 16.4 Hz, 1H, C$\underline{H}_2$ of CM'), 4.04-4.00 (m, J_{jem}= 16.4 Hz, 1H, C$\underline{H}_2$ of CM'), 3.97-3.62 (m, 12H, C$\underline{H}_2$ of CM, H-2', $C^3\underline{H}$ of acyl x 3, H-6a, H-5, H-6a', H-6b', H-6b, H-4'), 3.60-3.55 (dd, $J_{4,3}$= 9.0 Hz, $J_{4,5}$= 9.0 Hz, 1H, H-4), 3.48-3.44 (m, 1H, H-5'), 2.64-2.54 (m, J_{jem}= 15.6 Hz, J_{jem}= 15.6 Hz , J_{vic}= 7.3 Hz, J_{vic}= 7.6 Hz, 2H, $C^2\underline{H}_2$ of acyl x 2), 2.46-2.40 (m, 2H, $C^2\underline{H}_2$ of acyl x 2), 2.39-2.17 (m, 4H, $C^2\underline{H}_2$ of acyl x 2), 1.59-1.24 (m, 80H, $C^{5\text{-}13}\underline{H}_2$ of acyl x 4), 0.90-0.86 (t, J_{vic}= 6.8 Hz, 12H, $C^{14}\underline{H}_3$ of acyl x 4). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 72.93; H, 8.80; N, 1.41%. Found: C, 72.32; H, 8.78; N, 1.43. $[\alpha]_D^{25}$= +16.4 (c 1.00, $CHCl_3$).

Carboxymethyl 6-*O*-[4-*O*-carboxymethyl-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-2-deoxy-β-D-glucopyranosyl]-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (Bis-CM-406, 6). To a solution of **18** (61.5 mg, 30.9 mmol) in distilled THF (0.7 mL) was added Pd-black (90.3 mg). The mixture was stirred under 7 kg/cm^2 of hydrogen at room temperature overnight. After removal of the Pd catalyst by filtration, the solution was concentrated *in vacuo*. To the residue was added distilled water and *t*-BuOH and the suspension was lyophilized to give Bis-CM-406 (**6**) as a white powder (41.5 mg, quant.). ESI-MS (negative) *m/z* 1360.04[$(M-H)^-$], 679.45 [$(M-2H)^{2-}$]. ^{1}H NMR (500 MHz, $CDCl_3$/CD_3OD = 1/1) δ 5.16-5.10 (m, $J_{3',2'}$

= 10.5 Hz, $J_{3',4'}$ = 9.1 Hz $J_{3,2}$ = 10.5 Hz, $J_{3,4}$ = 9.3 Hz, 2H, H-3', H-3), 4.70-4.69 (d, $J_{1,2}$ = 3.5 Hz, 1H, H-1), 4.12-4.07 (m, $J_{jem\ of\ CM}$ = 16.3 Hz, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.6 Hz, 2H, C$\underline{H}_2$ of CM', H-2), 4.05-4.01 (m, $J_{jem\ of\ CM}$ = 15.8 Hz, $J_{jem\ of\ CM'}$ = 16.3 Hz, 2H, C$\underline{H}_2$ of CM', C$\underline{H}_2$ of CM), 4.00-3.98 (m, $J_{6a,6b}$ = 10.8 Hz, 1H, H-6a), 3.94-3.78 (m, $J_{jem\ of\ CM'}$ = 16.3 Hz, $J_{2',1'}$ = 8.5 Hz, $J_{2',3'}$ = 10.6 Hz, 7H, $C^3\underline{H}$ of acyl x 4, C$\underline{H}_2$ of CM, H-6a', H-2'), 3.76-3.71 (m, 2H, H-6b', H-5), 3.70-3.67 (dd, $J_{6b,5}$ = 4.5 Hz , $J_{6b,6a}$ = 10.8 Hz, 1H, H-6b), 3.60-3.56 (dd, $J_{4',3'}$ = 9.3 Hz, $J_{4',5'}$ = 9.3 Hz, 1H, H-4'), 3.55-3.51 (dd, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.6 Hz, 1H, H-4), 3.35-3.32 (m, $J_{5',4'}$ = 9.4 Hz, 1H, H-5'), 2.45-2.10 (m, 8H, $C^2\underline{H}_2$ of acyl x 4), 1.37-1.11 (m, 80H, $C^{4\text{-}13}\underline{H}_2$ of acyl x 4), 0.81-0.78 (t, 12H, $C^{14}\underline{H}_3$ of acyl x 4).

Allyl 6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranoside (19). To a suspension of **14** (1.38 g, 1.45 mmol) in CH_2Cl_2/H_2O = 20/1 (14.5 mL) was added DDQ (330 mg, 1.45 mmol) at room temperature. After being stirred for 1 d, the reaction was quenched with aqueous 10% $Na_2S_2O_3$ (10 mL) and extracted with CH_2Cl_2. Combined extracts were washed with aqueous saturated $NaHSO_4$, water, and brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (40 g, toluene/AcOEt = 20/1) to give **19** as colorless oil (601 mg, 48%). MALDI-TOF (positive) *m/z* 880.32 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.23 (m, 10H, $OCH_2\underline{Ph}$ x 2), 5.92-5.82 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.40-5.35 (dd, $J_{3,2}$ = 10.3 Hz, $J_{3,4}$ = 9.9 Hz, 1H, H-3), 5.34-5.31 (d, $J_{N,2}$ = 10.1 Hz, 2H, N$\underline{H}$), 5.30-5.26 (dd, J_{gem} = 1.5 Hz, J_{trans} = 17.3 Hz, $OCH_2CH=C\underline{H}_2$), 5.23-5.20 (dd, J_{gem} = 1.5 Hz, J_{cis} = 10.3 Hz, 1H, $OCH_2CH=C\underline{H}_2$), 5.16-5.12 (d, J_{jem} = 12.2 Hz, 1H, $COOC\underline{H}_2Ph$), 5.12-5.09 (d, J_{jem} = 12.2 Hz, 1H, $COOC\underline{H}_2Ph$), 4.90-4.90 (d, $J_{1,2}$ = 3.4 Hz, 1H, H-1), 4.73-4.70 (d, J_{jem} = 12.0 Hz, 1H, $OC\underline{H}_2Ph$), 4.68-4.65 (d, J_{jem} = 12.0 Hz, 1H, $OC\underline{H}_2Ph$), 4.63-4.60 (d, J_{jem} = 12.0 Hz, 1H, C$\underline{H}_2$ of Troc), 4.50-4.46 (d, J_{jem} = 12.0 Hz, 1H, C$\underline{H}_2$ of Troc), 4.23- 4.15 (m, $J_{jem\ of\ CM}$ = 16.1 Hz, J_{jem} = 12.7 Hz, J_{vic} = 5.4 Hz, 2H, C$\underline{H}_2$ of CM, $OC\underline{H}_2CH=CH_2$), 4.13-4.09 (d, J_{jem} = 16.1 Hz, 1H, C$\underline{H}_2$ of CM), 4.04-3.94 (m, 3H, $OC\underline{H}_2CH=CH_2$, H-2, $C^3\underline{H}$ of acyl), 3.89-3.81 (m, $J_{5,4}$ = 10.5 Hz, $J_{5,6a}$ = 3.2 Hz, $J_{5,6b}$ = 1.5 Hz, $J_{6a,5}$ = 3.2 Hz, 2H, H-5, H-6a), 3.74-3.70 (m, 2H, H-4, H-6b), 2.81-2.80 (d, $J_{OH,C3H}$ = 3.9 Hz, 1H, $C^3O\underline{H}$ of acyl), 2.48-2.44 (dd, J_{jem} = 16.1 Hz, J_{vic} = 2.7 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.40-2.34 (dd, J_{jem} = 16.3 Hz, J_{vic} = 9.4 Hz, 1H, $C^2\underline{H}_2$ of acyl), 1.49-1.26 (m, 20H, $C^{4\text{-}13}\underline{H}_2$ of acyl), 0.90-0.86 (t, J_{vic} = 7.1 Hz, 3H, $C^{14}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 58.71; H, 6.80; N, 1.63%. Found: C, 58.56; H, 6.77; N, 1.62. $[\alpha]_D^{25}$ = +53.9 (c 1.00, $CHCl_3$).

Allyl 6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranoside (20). To the solution of **19** (520 mg, 0.605 mmol) and tetradecanoic acid (276 mg, 1.21 mmol) in dry CH_2Cl_2 (6 mL) were added DCC (312 mg, 1.51 mmol) and DMAP (14.8 mg, 0.121 mmol) at room temperature under N_2 atmosphere. After being stirred for 4 h, the mixture was filtered through silica gel pad and concentrated *in vacuo*. The

residue was purified by silica-gel column chromatography (10 g, toluene/AcOEt = 20/1) and recycling HPLC to give **20** as a white solid (636 mg, 98%). MALDI-TOF (positive) *m/z* 1090.70 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34-7.23 (m, 10H, $OCH_2\underline{Ph}$ x 2), 5.89-5.79 (m, 1H, $OCH_2C\underline{H}=CH_2$), 5.35-5.29 (m, $J_{N,2}$ = 9.7 Hz, 2H, N$\underline{H}$, H-3), 5.28-5.23 (dd, J_{gem} = 1.4 Hz, J_{trans} = 17.3 Hz, $OCH_2CH=C\underline{H}_2$), 5.20-5.14 (dd, J_{gem} = 1.4 Hz, J_{cis} = 10.2 Hz, 2H, $OCH_2CH=C\underline{H}_2$, C$^3\underline{H}$ of acyl), 5.12 (s, 2H, $COOC\underline{H}_2Ph$), 4.89-4.89 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.73-4.70 (d, J_{jem} = 12.0 Hz, 1H, $OC\underline{H}_2Ph$), 4.66-4.63 (d, J_{jem} = 12.0 Hz, 1H, $OC\underline{H}_2Ph$), 4.61-4.58 (d, J_{jem} = 12.0 Hz, 1H, $C\underline{H}_2$ of Troc), 4.48-4.45 (d, J_{jem} = 12.0 Hz, 1H, $C\underline{H}_2$ of Troc), 4.26-4.22 (d, J_{jem} = 16.0 Hz, 1H, $C\underline{H}_2$ of CM), 4.17-4.09 (m, J_{jem} = 12.8 Hz, J_{vic} = 5.3 Hz, $J_{jem\,of\,CM}$ = 16.0 Hz, 2H, $OC\underline{H}_2CH=CH_2$, $C\underline{H}_2$of CM), 3.99-3.94 (dd, J_{jem} = 12.7 Hz, J_{vic} = 6.3 Hz, 1H, $OC\underline{H}_2CH=CH_2$), 3.93-3.82 (m, 3H, H-2, H-6a, H-6b), 3.72-3.66 (m, 2H, H-4, H-5), 2.62-2.56 (dd, J_{jem} = 16.2 Hz, J_{vic} = 7.2 Hz, 1H, C$^2\underline{H}_2$ of acyl), 2.53-2.57 (dd, J_{jem} = 16.2 Hz, J_{vic} = 5.4 Hz, 1H, C$^2\underline{H}_2$ of acyl), 2.24-2.21 (t, J_{vic} = 6.6 Hz, 2H, C$^{2'}\underline{H}_2$ of acyl), 1.55-1.25 (m, 42H, C$^{4\text{-}13}\underline{H}_2$ of acyl, C$^{3'\text{-}13'}\underline{H}_2$ of acyl), 0.89-0.86 (t, J_{vic} = 6.6 Hz, 6H, C$^{14}\underline{H}_3$ of acyl, C$^{14'}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 62.88; H, 7.92; N, 1.31%. Found: C, 62.82; H, 7.92; N, 1.27. $[\alpha]_D^{25}$ = +55.0 (c 1.00, $CHCl_3$).

6-*O*-Benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-α-D-glucopyranose (21). To a degassed solution of **20** (518 mg, 0.484 mmol) in anhydrous THF (3 mL) was added [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)-iridium(I) hexafluorophosphate (13.4 mg, 15.8 mmol). After activation of the iridium catalyst with hydrogen three times (each 30 sec), the mixture was stirred under nitrogen atmosphere at room temperature for 30 min. To the mixture were added successively H_2O (4 mL) and iodine (305 mg, 1.20 mmol), and stirred for additional 25 min. After the reaction was quenched with aqueous 10% $Na_2S_2O_3$ (10 mL), the mixture was extracted with AcOEt. Combined extracts were washed with aqueous 10% $Na_2S_2O_3$, aqueous saturated $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (40 g, toluene/AcOEt = 10/1) to give **21** as a white solid (417 mg, 84%). MALDI-TOF (positive) *m/z* 1050.05 [(M+Na)$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) for α-anomer: δ 7.38-7.22 (m, 10H, $OCH_2\underline{Ph}$ x 2), 5.53-5.50 (d, $J_{N,2}$ = 9.8 Hz, 1H, N$\underline{H}$), 5.39-5.34 (dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 5.28-5.26 (dd, $J_{1,2}$ = 3.7 Hz, $J_{1,OH}$ = 3.4 Hz, 1H, H-1), 5.17-5.12 (m, 3H, C$^3\underline{H}$ of acyl, $COOC\underline{H}_2Ph$), 4.69 (s, 2H, $OC\underline{H}_2Ph$), 4.59-4.56 (d, J_{jem} = 11.9 Hz, 1H, $C\underline{H}_2$ of Troc), 4.49-4.46 (d, J_{jem} = 11.9 Hz, 1H, $C\underline{H}_2$ of Troc), 4.26-4.08 (m, J_{jem} = 16.2 Hz, $J_{5,4}$ = 9.9 Hz, $J_{5,6a}$ = 4.3 Hz, $J_{5,6b}$ = 1.8 Hz, 3H, $C\underline{H}_2$ of CM, H-5), 3.90-3.85 (m, 1H, H-2), 3.82-3.78 (dd, $J_{6a,5}$ = 4.3 Hz, $J_{6a,6b}$ = 11.0 Hz, 1H, H-6a), 3.75-3.73 (m, 1H, H-6b), 3.66-3.61 (dd, $J_{4,3}$ = 9.5 Hz, $J_{4,5}$ = 9.8 Hz, 1H, H-4), 3.29-3.29 (d, $J_{OH,1}$ = 3.4 Hz, 1H, O$\underline{H}$-1), 2.62-2.56 (dd, J_{jem} = 16.2 Hz, J_{vic} = 7.3 Hz, 1H, C$^2\underline{H}_2$ of acyl), 2.55-2.47 (dd, J_{jem} = 16.2 Hz, J_{vic} = 5.2 Hz, 1H, C$^2\underline{H}_2$ of acyl), 2.25-2.21 (t, J_{vic} = 7.9 Hz, 2H, C$^{2'}\underline{H}_2$ of acyl), 1.56-1.55 (m, 4H, C$^4\underline{H}_2$ of acyl, C$^{3'}\underline{H}_2$ of acyl), 1.25-1.21 (m, 38H, C$^{5\text{-}13}\underline{H}_2$ of acyl, C$^{4'\text{-}13'}\underline{H}_2$ of acyl), 0.90-0.86 (t, 6H, C$^{14}\underline{H}_3$ of acyl,

$C^{14'}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 61.83; H, 7.83; N, 1.36%. Found: C, 61.82; H, 7.80; N, 1.36.

Benzyloxycarbonylmethyl 6-*O*-[6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-2-deoxy-2-(2,2,2-trichloroethoxycarboylamino)-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (23). To a suspension of **21** (37.9 mg, 36.8 μmol) in anhydrous CH_2Cl_2 (0.4 mL) and MS4A were added trichloroacetonitrile (36.9 μL, 0.368 mmol) and cesium carbonate (6.0 mg, 18.4 μmol) at room temperature under argon atmosphere. After being stirred for 1 h, the mixture was filtered with silica gel and concentrated *in vacuo* to give the imidate (**22**) (44 mg, quant.), which was subjected to the following glycosylation without further purification. To the suspension of imidate (**22**), the glycosyl acceptor (**12**) (23.7 mg, 24.7 μmol) and MS4A in anhydrous CH_2Cl_2 (0.3 mL) was added trimethylsilyl triflate (1.0 μL, 4.9 μmol) at -15 ˚C under argon atmosphere. After stirring for 15 min, the mixture was quenched with aqueous saturated $NaHCO_3$ (1.5 mL) and extracted with AcOEt. Combined extracts were washed with aqueous saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (30 g, $CHCl_3$/acetone = 20/1) to give **23** as a white solid (45.2 mg, 93%). MALDI-TOF (positive) *m/z* 1991.89 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38- 7.19 (m, 25H, $OCH_2\underline{Ph}$ x 5), 6.65- 6.63 (d, $J_{N,2}$ = 9.3 Hz, 1H, N$\underline{H}$), 5.45- 5.43 (d, $J_{N',2'}$ = 8.5 Hz, 1H, N'$\underline{H}$), 5.20- 5.06 (m, 7H, $C^3\underline{H}$, H-3, H-3', $COOC\underline{H}_2Ph$ x 2), 4.76- 4.74 (m, $J_{1,2}$ = 3.7 Hz, 2H, H-1, $OC\underline{H}_2Ph$), 4.68- 4.44 (m, $J_{1',2'}$ = 9.2 Hz $J_{jem\ of\ Troc}$ = 12.1 Hz, 8H, H-1', $OC\underline{H}_2Ph$, $OC\underline{H}_2Ph$ x 2, $C\underline{H}_2$ of Troc), 4.29- 4.33 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.7 Hz, $J_{2,N}$ = 9.5 Hz, 1H, H-2), 4.23-4.19 (d, J_{jem} = 16.2 Hz, 1H, $C\underline{H}_2$ of CM), 4.14-4.10 (d, J_{jem} = 16.2 Hz, 1H, $C\underline{H}_2$ of CM), 4.02-3.98 (m, $J_{jem\ of\ CM}$ = 16.9 Hz, $J_{6a,5}$ = 2.7 Hz, 2H, $C\underline{H}_2$ of CM, H-6a), 3.97-3.93 (d, J_{jem} = 16.9 Hz, 1H, $C\underline{H}_2$ of CM), 3.88- 3.78 (m, 5H, $C^3\underline{H}$ of acyl × 2, H-5, H-6a', H-6b'), 3.74-3.70 (dd, $J_{6a,5}$ = 4.9 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H, H-6b), 3.65-3.58 (m, 3H, H-4', H-2', H-4), 3.54-3.50 (ddd, $J_{5',4'}$ = 9.5 Hz, $J_{5',6a'}$ = 2.7 Hz, $J_{5,6b'}$ = 3.2 Hz, 1H, H-5'), 3.03 (s, 1H, O$\underline{H}$-4), 2.65- 2.59 (dd, J_{jem} = 15.1 Hz, J_{vic} = 7.9 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.60-2.54 (dd, J_{jem} = 15.9 Hz, J_{vic} = 7.2 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.54-2.48 (dd, J_{jem} = 16.0 Hz, J_{vic} = 5.0 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.47-2.42 (dd, J_{jem} = 15.1 Hz, J_{vic} = 4.7 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.35-2.33 (m, 2H, $C^2\underline{H}_2$ of acyl), 2.31-2.22 (m, 2H, $C^{2'}\underline{H}_2$ of acyl), 1.61-1.42 (m, 8H, $C^4\underline{H}_2$ of acyl × 3, $C^{3'}\underline{H}_2$ of acyl), 1.30-1.25 (m, 76H, $C^{5\text{-}13}\underline{H}_2$ of acyl × 3, $C^{4'\text{-}13'}\underline{H}_2$ of acyl), 0.92-0.86 (t, J_{vic} = 7.0 Hz, 12H, $C^{14}\underline{H}_3$ of acyl × 3, $C^{14'}\underline{H}_3$ of acyl). Anal. Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 67.00; H, 8.33; N, 1.42%. Found: C, 66.83; H, 8.35; N, 1.42. $[\alpha]_D^{25}$ = +18.2 (c 1.00, $CHCl_3$).

Benzyloxycarbonylmethyl 6-*O*-[6-*O*-benzyl-4-*O*-benzyloxycarbonylmethyl-2-deoxy-2-[(*R*)-3-(dodecanoloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-((*R*)-3-benzyloxytetradecanoyl)-2-((*R*)-3-benzyloxytetradecanoylamino)-2-deoxy-

α-D-glucopyranoside (24). To a solution of **23** (43.8 mg, 22.2 μmol) in AcOH/MeOH/THF = 15/10/3 (280 μL) was added zinc powder (50 mg). The suspension was sonicated for 30 sec and stirred at room temperature for 20 min. After additional sonication for 30 min, the mixture was stirred for 2h and then filtered through cerite pad. The filtrate was concentrated and co-evaporated with toluene three times. The residue was purified by silica-gel column chromatography (5 g, $CHCl_3$/acetone = 10/1). To the solution of the residue, (*R*)-3-(dodecanoyloxy)tetradecanoic acid (18.9 mg, 22.2 μmol) and HOAt (7.6 mg, 5.5 μmol) in dry $CHCl_3$ (0.22 mL) was added WSCD•HCl (10.6 mg, 55.5 μmol) at room temperature under N_2 atmosphere. The mixture was stirred for 2 h and then applied to silica-gel column chromatography (80 g, toluene/AcOEt = 50/1) to give **24** as a white solid (30.2 mg, 62%). MALDI-TOF (positive) *m/z* 2225.69 [$(M+Na)^+$]. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.19 (m, 25H, $OCH_2\underline{Ph}$ × 5), 6.64-6.61 (d, $J_{N,2}$ = 9.2 Hz, 1H, N$\underline{H}$), 6.11-6.09 (d, $J_{N',2'}$ = 8.2 Hz, 1H, N'$\underline{H}$), 5.20-5.05 (m, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 8.9 Hz, 8H, $C^3\underline{H}$ x 2, H-3, H-3', $COOC\underline{H}_2Ph$ × 2), 4.78-4.77 (d, $J_{1,2}$ = 3.7 Hz, 1H, H-1), 4.62-4.60 (d, $J_{1',2'}$ = 7.9 Hz, 1H, H-1'), 4.57-4.41 (m, 6H, $OC\underline{H}_2Ph$ × 3), 4.31- 4.25 (ddd, $J_{2,1}$ = 3.7 Hz, $J_{2,3}$ = 10.7 Hz, $J_{2,N}$ = 9.2 Hz, 1H, H-2), 4.21-4.17 (d, J_{jem} = 16.2 Hz, 1H, $C\underline{H}_2$ of CM), 4.12-4.08 (d, J_{jem} = 16.2 Hz, 1H, $C\underline{H}_2$ of CM), 3.98-3.91 (m, 3H, $C\underline{H}_2$ of CM, H-6a), 3.88-3.69 (m, 7H, $C^3\underline{H}$ of acyl × 2, H-2', H-5, H-6a', H-6b, H-4), 3.63-3.53 (m, 3H, H-4', H-6b', H-5'), 2.66-2.60 (dd, J_{jem}= 15.3 Hz, J_{vic}= 7.3 Hz, 1H, $C^2\underline{H}_2$ of acyl), 2.55-2.54 (m, 2H, $C^2\underline{H}_2$ of acyl), 2.45-2.21 (m, 9H, $C^2\underline{H}_2$ of acyl × 2, $C^2\underline{H}_2$ of acyl, $C^{2'}\underline{H}_2$ of acyl × 2), 1.56-1.44 (m, 12H, $C^4\underline{H}_2$ of acyl × 4, $C^{3'}\underline{H}_2$ of acyl × 2), 1.25-1.21 (m, 108H, $C^{5-13}\underline{H}_2$ of acyl × 4, $C^{4'-13'}\underline{H}_2$ of acyl, $C^{4'-11'}\underline{H}_2$ of acyl), 0.89-0.86 (t, 18H, $C^{14}\underline{H}_3$ of acyl × 4, $C^{14'}\underline{H}_3$ of acyl, $C^{12'}\underline{H}_3$ of acyl). Calcd for $C_{87}H_{119}N_2O_{17}P$: C, 72.44; H, 9.62; N, 1.27%. Found: C, 71.94; H, 9.60; N, 1.27. $[\alpha]_D^{25}$ = +17.0 (c 1.00, $CHCl_3$).

Carboxymethyl 6-*O*-[4-*O*-carbooxymethyl-2-deoxy-2-[(*R*)-3-(dodecanoyloxy)tetradecanoylamino]-3-*O*-[(*R*)-3-(tetradecanoyloxy)tetradecanoyl]-β-D-glucopyranosyl]-3-*O*-((*R*)-3-hydroxytetradecanoyl)-2-((*R*)-3-hydroxytetradecanoylamino)-2-deoxy-α-D-glucopyranoside (Bis-CM-506, 5). To a solution of **24** (55.2 mg, 25.0 μmol) in distilled THF (1 mL) was added Pd-black (53.4 mg). The mixture was stirred under 7 kg cm^{-2} of hydrogen at room temperature overnight. After the removal of the Pd catalyst by filtration, the solvent was evaporated *in vacuo*. The residue was purified by silica-gel column chromatography [silica-gel (4 g) inactivated H_2O (150 μL) overnight, 1% hexafluoroisopropanol, $CHCl_3$/MeOH = 7/1, then 1% hexafluoroisopropanol, MeOH] and concentrated. The residue was dissolved in $CHCl_3/H_2O$ = 5/4 (9mL). The organic layer was washed with aqueous 0.1 N HCl and water, and concentrated *in vacuo*. Lyophilization with water gave Bis-CM-506 (**5**) as a white powder (24.3 mg, 55%). ESI-TOF Mass (negative) *m/z* 1753.1 [$(M-H)^-$], 876.1 [$(M-2H)^{2-}$]. ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$ = 1/1) δ 5.16-5.05 (m, 4H, H-3', H-3, $C^3\underline{H}$ × 2 of acyl), 4.70 (m, 1H, H-1), 4.52 (m, 1H, H-1'), 4.20-4.00 (m, 4H, $C\underline{H}_2$ of CM', $C\underline{H}_2$ of CM, H-2), 3.93-3.91 (m, 4H, H-5, H-6a, H-6a', $C^3\underline{H}$ of

acyl), 3.81 (m, 3H, H-6b' C$\underline{H}_2$ of CM, $C^3\underline{H}$ of acyl), 3.71 (m, 3H, H-2', H-4', H-6b), 3.30-3.26 (m, 2H, H-5', H-4), 2.58-2.18 (m, 10H, $C^2\underline{H}_2$ of acyl × 4, $C^{2'}\underline{H}_2$ of acyl × 2), 1.52 (m, 4H, $C^4\underline{H}_2$ of acyl × 2), 1.37-1.20 (m, 116H, $C^{4\text{-}13}\underline{H}_2$ of acyl × 2, $C^{5\text{-}13}\underline{H}_2$ of acyl × 2, $C^{3'\text{-}13'}\underline{H}_2$ of acyl, $C^{3'\text{-}11'}\underline{H}_2$ of acyl), 0.82-0.79 (t, 18H, $C^{14}\underline{H}_3$ of acyl × 4, $C^{14'}\underline{H}_3$ of acyl, $C^{12'}\underline{H}_3$ of acyl).

Cytokine induction in human peripheral whole-blood cell cultures. Aqueous 5% DMSO solution (50 mg/mL) of each sample was prepared by dilution of DMSO solution (1 mg/mL) with saline (Otsuka Pharmaceuticals Co., Ltd.). Each sample was further diluted stepwise with saline on a 96-well plastic plate (#2870-096, Iwaki Glass Co., Ltd). The mixture consisting of test sample (25 mL), RPMI 1640 medium (75 mL; Flow Laboratories, Irvine, Scotland), and heparinized human peripheral whole-blood (25 mL) collected from an adult volunteer was incubated in triplicate at 37°C in 5% CO_2 for 24 h. The plate was centrifuged at 300 X *g* for 2 min. The levels of TNF-α and IL-6 in culture supernatant were measure by means of enzyme-linked immunosorbent assay (ELISA).

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

ACKNOWLEDGEMENTS

We are grateful to Prof. T. Tamura and Ms. K. Aoyama at Hyogo medical college for their assistance in the cytokine induction assay. We also thank Mr. S. Adachi for NMR measurements, and Ms. K. Hayashi and Ms. T. Ikeuchi for elemental analysis at Osaka University. The present work was financially supported in part by Grants-in Aid for Scientific Research (No.13NP0401, No.17035050, No.17310128) from the Japan Society for the Promotion of Science, CREST of JST, Suntory Institute for Bioorganic Research (SUNBOR) Grant, and Houansha Foundation Grant.

REFERENCES (AND NOTES)

1. 'Endotoxin in Health and Disease, ' ed. by H. Brade, S. M. Opal, S. N. Vogel, and D. C. Morrison, Marcel Dekker, Inc., NewYork, Basel, 1999.
2. M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, 1984, **25**, 2667; M. Imoto, H. Yoshimura, M. Yamamoto, T. Shimamoto, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 2197.
3. M. Imoto, H. Yoshimura, M. Yamamoto, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Tetrahedron*

Lett., 1985, **26**, 1545; M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 2205.

4. H. Loppnow, H. Brade, I. Durrbaum, C. A. Dinarello, S. Kusumoto, E. Th. Rietschel, and H. D. Flad, *J. Immunol.*, 1989, **142**, 3229.
5. E. Th. Rietschel, T. Kirikae, F. U. Schade, A. J. Ulmer, O. Holst, H. Brade, G. Schmidt, U. Mamat, H.-D. Grimmecke, S. Kusumoto, and U. Zähringer, *Immunobiol.*, 1993, **187**, 169.
6. T. Kirikae, F. U. Shade, U. Zähringer, F. Kirikae, H. Brade, S. Kusumoto, T. Kusama, and E. Th. Rietschel, *FEMS Immunol. Med. Microbiol.*, 1994, **8**, 13.
7. A. Poltorak, X. He, I. Smirnova, M. Y. Liu, C. V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton, and B. Beutler, *Science*, 1998, **282**, 2085.
8. S. T. Qureshi, L. Larivière, G. Leveque, S. Clermont, K. J. Moore, P. Gros, and D. Malo, *J. Exp. Med.*, 1999, **189**, 615.
9. K. Hoshino, O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira, *J. Immunol.*, 1999, **162**, 3749.
10. J. C. Chow, D. W. Young, D. T. Golenbock , W. J. Christ, and F. Gusovsky, *J. Bio. Chem.*, 1999, **274**, 10689.
11. E. Lien, T. K. Means, H. Heine, A. Yoshimura, S. Kusumoto, K. Fukase, M. J. Fenton, M. Oikawa, N. Qureshi, B. Monks, R. W. Finberg, R. R. Ingalls, and D. T. Golenbock, *J. Clin. Invest.*, 2000, **105**, 497.
12. R. Shimazu, S. Akashi, H. Ogata, Y. Nagai, K. Fukudome, K. Miyake, and M. Kimoto, *J. Exp. Med.*, 1999, **189**, 1777.
13. S. Akashi, R. Shimazu, H. Ogata, Y. Nagai, K. Takeda, M. Kimoto, and K. Miyake, *J. Immunol.*, 2000, **164**, 3471.
14. S. Akashi, Y. Nagai, H. Ogata, M. Oikawa, K. Fukase, S. Kusumoto, K. Kawasaki, M. Nishijima, S. Hayashi, M. Kimoto, and K. Miyake, *Int. Immunol.*, 2001, **13**, 1595.
15. S. Akashi, S. Saitoh, Y. Wakabayashi, T. Kikuchi, N. Takamura, Y. Nagai, Y. Kusumoto, K. Fukase, S. Kusumoto, Y. Adachi, A. Kosugi, and K. Miyake, *J. Exp. Med.*, 2003, **198**, 1035.
16. K. Fukase, T. Kirikae, F. Kirikae, W.-C. Liu, M. Oikawa, Y. Suda, M. Kurosawa, Y. Fukase, H. Yoshizaki, and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, 2001, **74**, 2189.
17. W.-C. Liu, M. Oikawa, K. Fukase, Y. Suda, and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, 1999, **72**, 1377.
18. K. Fukase, M. Oikawa, Y. Suda, W.-C. Liu, Y. Fukase, T. Shintaku, H. Sekljic, H. Yoshizaki, and S. Kusumoto, *J. Endotoxin Res.*, 1999, **5**, 46.

19. K. Fukase, A. Ueno, Y. Fukase, M. Oikawa, Y. Suda, and S. Kusumoto, *Bull. Chem. Soc. Jpn.*, 2003, **76**, 485.
20. K. Fukase, Y. Fukase, M. Oikawa, W.-C. Liu, Y. Suda, and S. Kusumoto, *Tetrahedron*, 1998, **54**, 4033.
21. S. D. Debenham and E. J. Toone, *Tetrahedron: Asymmetry*, 2000, **11**, 385.
22. J. J. Oltvoort, C. A. A. v. Boeckel, J. H. d. Koning, and J. H. v. Boom, *Synthesis*, 1981, 305.
23. R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21.
24. Y. Suda, H. Tochio, K. Kawano, H. Takada, H. Yoshida, S. Kotani, and S. Kusumoto, *FEMS Immun. Med. Microbiol.*, 1995, **12**, 97.
25. U. Seydel, A. B. Schromm, L. Brade, S. Gronow, J. Andrae, M. Mueller, M. H. J. Koch, K. Fukase, M. Kataoka, M. Hashimoto, S. Kusumoto, and K. Brandenburg, *FEBS Journal*, 2005, **272**, 327.