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Selected Paper

Synthesis of Tetrahydropyran-2-carboxylic Acid Derivatives of Lipid A Containing an Olefin in Their Chains and Their LPS-Antagonistic Activities

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Four tetrahydropyran-2-carboxylic acid derivatives with 3-(tetradec-7-enyloxy)tetradecyl chains instead of 3-(tetradecanoyloxy)tetradecanoyl chains in lipid A were synthesized and their biological activities toward human U937 cells, human whole blood cells and mouse peritoneal resident macrophages were measured. These compounds showed LPS-antagonistic activity toward these three kinds of cells. The IC_{50} values (nM) (1 M = 1 mol dm^{-3}) of these four compounds (**21**, **21′**, **24** and **24′**) toward human monoblastic U937 cells were 2.2, 1.0, 0.017 and 0.055, respectively. However, the LPS-antagonistic activities (IC_{50} values) of these four compounds toward human whole blood cells were only 0.28, 0.21, 0.81 and 0.58 μM, respectively. The IC_{50} values (μM) toward mouse peritoneal resident macrophages were 2.49, 0.49, 0.91 and 0.69, respectively.

The study of endotoxin (lipopolysaccharide; LPS) has developed extensively[1] since Shiba and Kusumoto's[2] total synthesis of lipid A, a toxic component of LPS existing in the outer surface membrane of Gram-negative bacteria. In an earlier study, lipid A-related compounds were investigated as anticancer drugs[1] by stimulating the immune system.[3] Also, in recent years, lipid A related compounds have been studied as LPS antagonists, which may have potential as immunosuppressants in autoimmune diseases and septicemia by deactivating the LPS-induced immune system.[1] In fact, a nontoxic natural lipid A-related compound (Rs-DPLA)[4] isolated from *Rhodobacter sphaeroides* showed LPS-antagonistic activity for human macrophages, and the Eisai group has developed a related compound, E5564, as a highly potent anti-septicemia drug[5] (Fig. 1).

During our investigation of the biological activities of compounds related to lipid A, we found that lipid A type tetrahydropyran-2-carboxylic acids with ether chains, such as compound **A**, which is inactive toward mouse peritoneal resident macrophages, had LPS-antagonistic activity toward human U937 cells.[6] The Eisai group had also found that a compound (Rs-DPLA) having a unique structural feature, that is, containing a *cis*- or *trans*-alkene and a 3-oxoalkanamide in one of its long fatty acid chains, does not show LPS-agonistic activity toward mouse macrophages.[7] Furthermore, they found that many lipid A related compounds having an olefinic double bond in their molecules behave as LPS antagonists toward both human and murine macrophages.[7] Therefore, we were interested in the LPS-antagonistic activity of the lipid A type tetrahydropyran-2-carboxylic acids derivatives containing both an alkenyl and 3-oxoalkanamide in their fatty acid moieties. We thus synthesized compounds **21**, **21′**, **24** and **24′** to measure the LPS-antagonistic activity toward human U937 cells, human whole blood cells and mouse peritoneal resident macrophages. We would like to describe their syntheses and LPS-antagonistic activities in this paper.

Results and Discussion

Firstly, we tried the synthesis of a common tetrahydropyran-carboxylic acid allyl ester **6** as a glycosyl acceptor possessing a 3-oxotetradecanamide moiety in the molecule from allyl 2-azido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**).[8] The treatment of azide **1** with dodecyl methanesulfonate and sodium hydride as a base in *N*,*N*-dimethylformamide (DMF) yielded 3-*O*-dodecylglucopyranoside **2**. Deprotection of the anomeric allyl group of **2** was accomplished by double-bond isomerization to an enol ether with an iridium complex, (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate. A successive treatment of the enol ether with pyridine–iodine–water gave a pyranose derivative **3**. The treatment of **3** with trichloroacetonitrile and cesium carbonate in dichloromethane, and a successive treatment of the resulting imidate with trimethylsilyl cyanide using trimethylsilyl trifluoromethanesulfonate as a catalyst yielded an α-oriented cyano compound **4**. The azido group of **4** was treated with triphenylphosphine in tetrahydrofurane (THF) at room temperature for 1 h and then 28% ammonia water at 60 °C for 7 h to give 2-amino-2-deoxyglucopyranoside, which was successively treated with 3-oxotetradecanoic acid and 4-(dimethylamino)pyridine (DMAP) using dicyclohexylcarbodiimide (DCC) as a condensing agent to give 3-oxotetradecanamide **5**. A treatment of the nitrile group of **5** with HCl in allyl alcohol, and then water, gave

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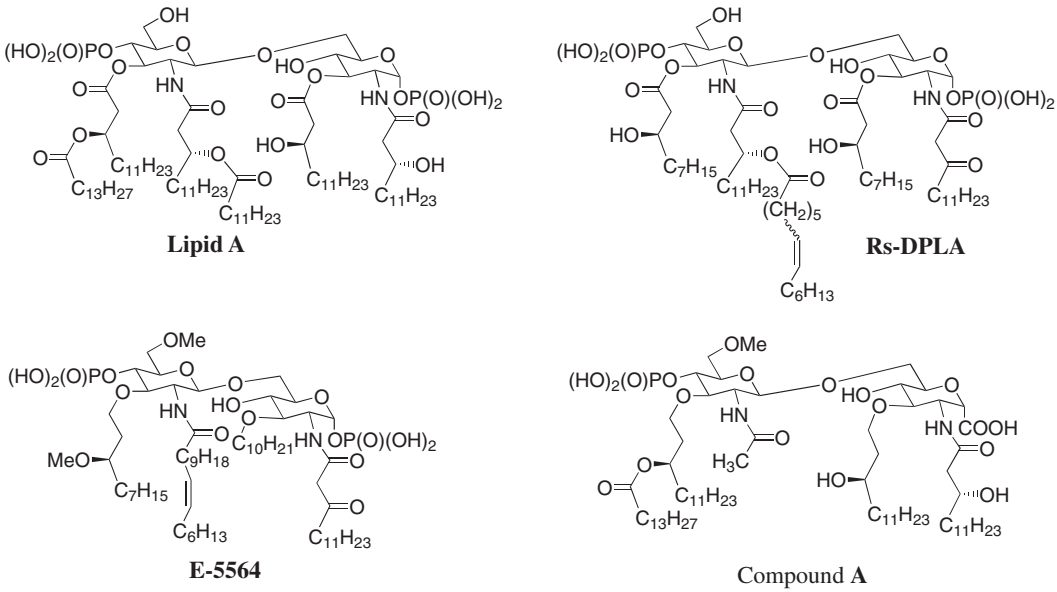
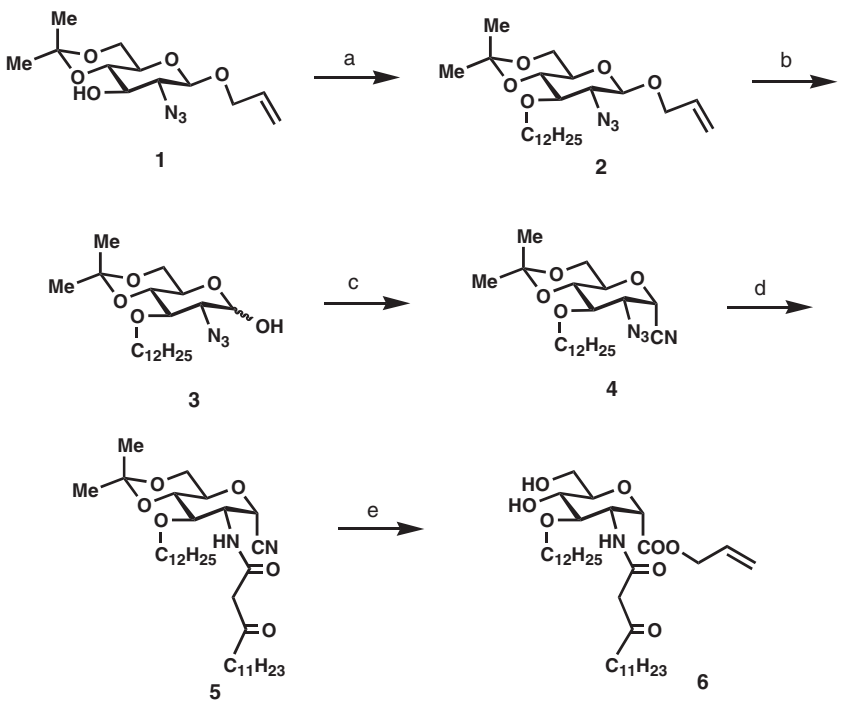


Fig. 1. Structures of lipid A, Rs-DPLA, E-5564 and compound A.



Scheme 1. Reagents and conditions: (a) dodecyl methanesulfonate, NaH, DMF, rt, 8 h, 74%; (b) $[C_8H_{12}Ir(PMePh_2)_2]PF_6$, THF, rt, 2 h, then H_2O, I_2, pyridine, rt, 30 min, 86%; (c) (i) Cl_3CCN, cat. Cs_2CO_3, CH_2Cl_2, rt, 1 h, (ii) TMSCN, cat. TMSOTf, MS4A, CH_2Cl_2, rt, 4 h, two steps 68%; (d) (i) PPh_3, THF, rt, 1 h, then 28% aq. NH_3, 60 °C, 7 h, (ii) 3-oxotetradecanoic acid, DCC, DMAP, CH_2Cl_2, rt, 4 h, two steps 66%; (e) HCl in $CH_2{=}CHCH_2OH$, rt, 2 h, then H_2O–EtOAc, rt, 1 h, 45%.

allyl ester **6** (Scheme 1).

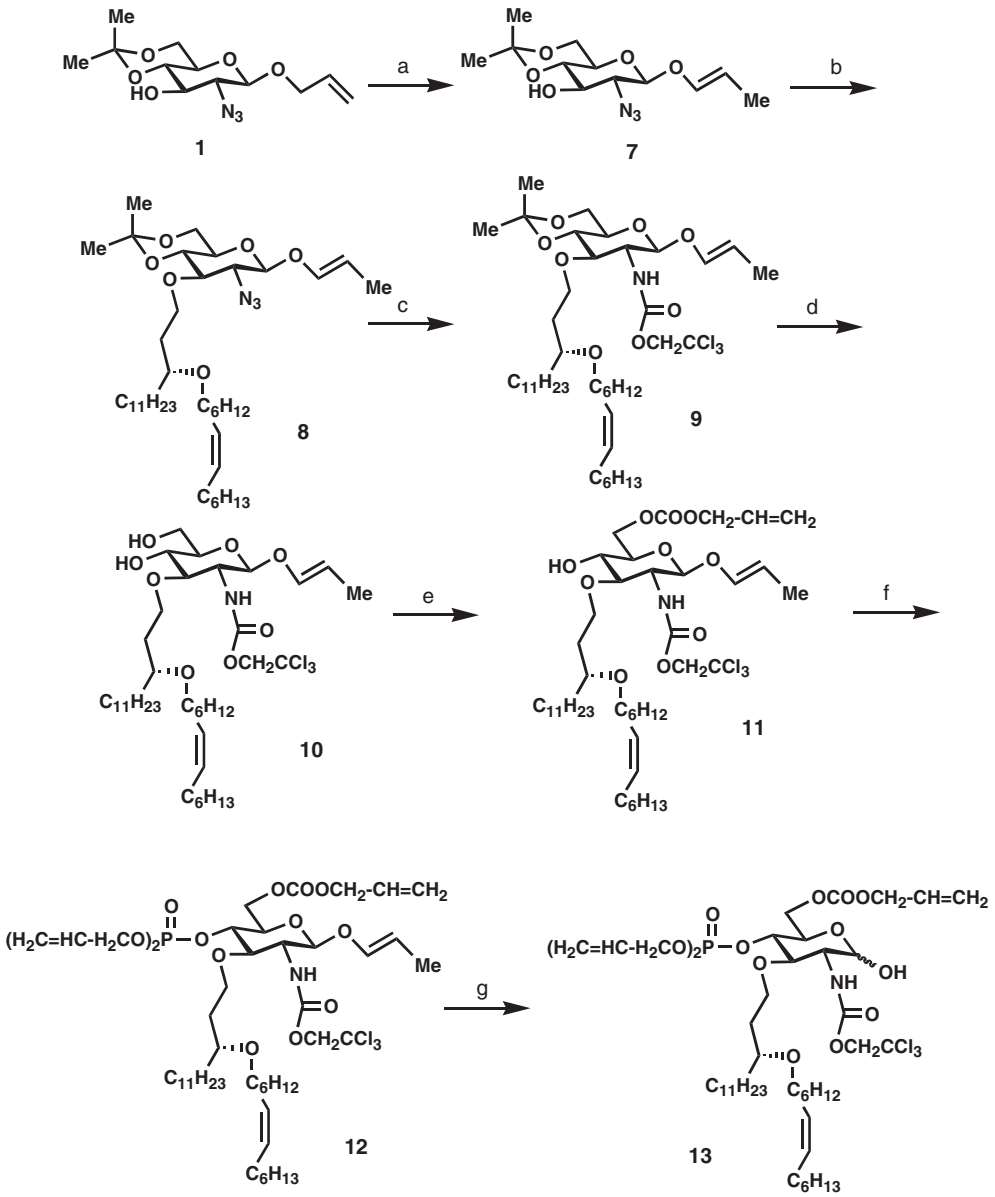
Secondly, we attempted syntheses of glycosyl doners, **13** and **18**, which were activated as their trichloroacetimidates for glycosylation with **6**. The allylic double bond of the common starting azide **1** was converted to mainly *trans*-prop-1-enyl ether **7** by the iridium complex used in the conversion from **2** to **3**. The C3-free alcohol of the pyranoside **7** was alkylated with (*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl methanesulfonate[9] and NaH in DMF to give **8**. The azido group of **8** was treated with lithium alminum hydride in THF at room temperature for 1 h to give a C2-amino compound, which was successively treated with 2,2,2-trichloroethyl chloroformate in dichloromethane using saturated aqueous sodium hydrogencarbonate as a base to afford 2,2,2-trichloroethyl carbamate **9**. The 4,6-*O*-isopropylidene group of **9** was deprotected by using *p*-toluenesulfonic acid monohydrate in MeOH to yield 4,6-diol **10** as a wax.

The route to glycosyl donors **13** and **18** was separated into

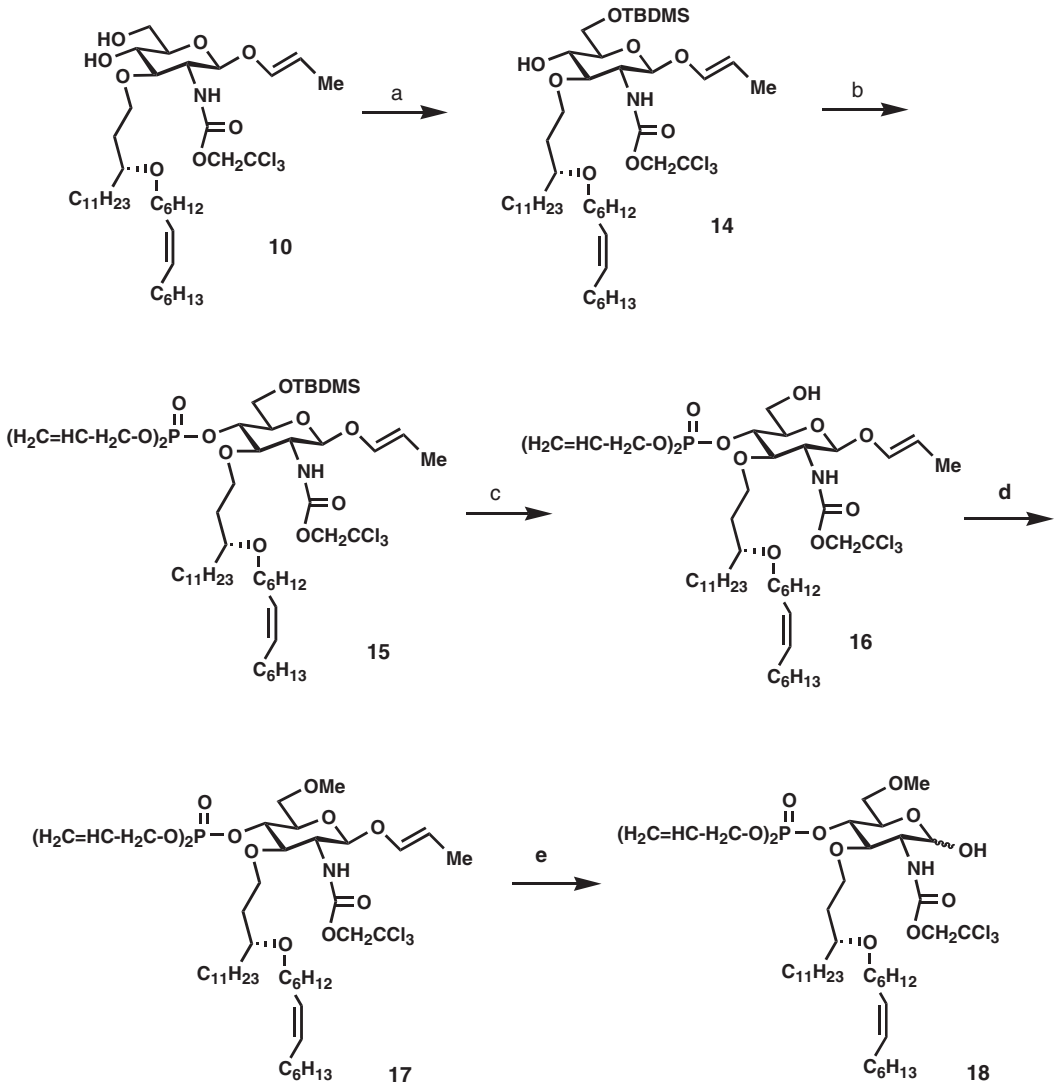
two at this stage. To prepare compound **13**, the C6-OH of **10** was protected by a treatment with allyl chloroformate in dichloromethane using pyridine as a base to give allyl carbonate **11**, which was further phosphorylated in two steps, with diallyl diisopropylaminophosphoramidite and 1*H*-tetrazole, and successive oxidation of the initially formed phosphite by hydrogen peroxide to afford phosphate **12**.[5] Compound **12** was treated with *N*-bromosuccinimide (NBS) in acetone–water to deprotect the anomeric prop-1-enyl group. The generated glucose derivative **13** was activated as trichloroacetimidate before glycosylation (Scheme 2).

To prepare compound **18**, the C6-OH of **10** was protected by a treatment with *tert*-butyldimethylsilyl chloride and DMAP to afford silyl ether **14**, which was further phosphorylated in two steps, with diallyl diisopropylaminophosphoramidite and 1*H*-tetrazole, and successive oxidation of the initially formed phosphite with hydrogen peroxide to afford phosphate **15**. Compound **15** was treated with hydrogen fluoride–pyridine in THF to deprotect C6-*O*-silyl ether without deprotecting the anomeric prop-1-enyl ether. The generated the C6-OH compound **16** was treated with trimethyloxonium tetrafluoroborate in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) to give 6-*O*-methoxy compound **17**, which was treated with NBS to give **18**, as described in the formation of **13** from **12**. The glucose derivative **18** was activated as trichloroacetimidate before glycosylation according to the same procedure as in the case of compound **13** (Scheme 3).

Thirdly, a pair of pseudo-disaccharides, **21** and **21′** (R = H



Scheme 2. Reagents and conditions: (a) $[IrC_8H_{12}(MePh_2P)_2]PF_6$, THF, N_2, rt, 5 h, 97%; (b) (*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl methanesulfonate, NaH, DMF, rt, 18 h, 78%; (c) (i) $LiAlH_4$, THF, rt, 1 h, (ii) $ClCOOCH_2CCl_3$, aq. $NaHCO_3$, CH_2Cl_2, rt, 1 h, two steps 94%; (d) *p*-TsOH, MeOH, rt, 1 h, 84%; (e) $ClCOOCH_2CH{=}CH_2$, pyridine, CH_2Cl_2, 0 °C, 2 h, 94%; (f) $(i\text{-}Pr)_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 2 h, then 30% H_2O_2, 0 °C, 1 h, 85%; (g) NBS, acetone–H_2O, 0 °C, 2 h, 88%.



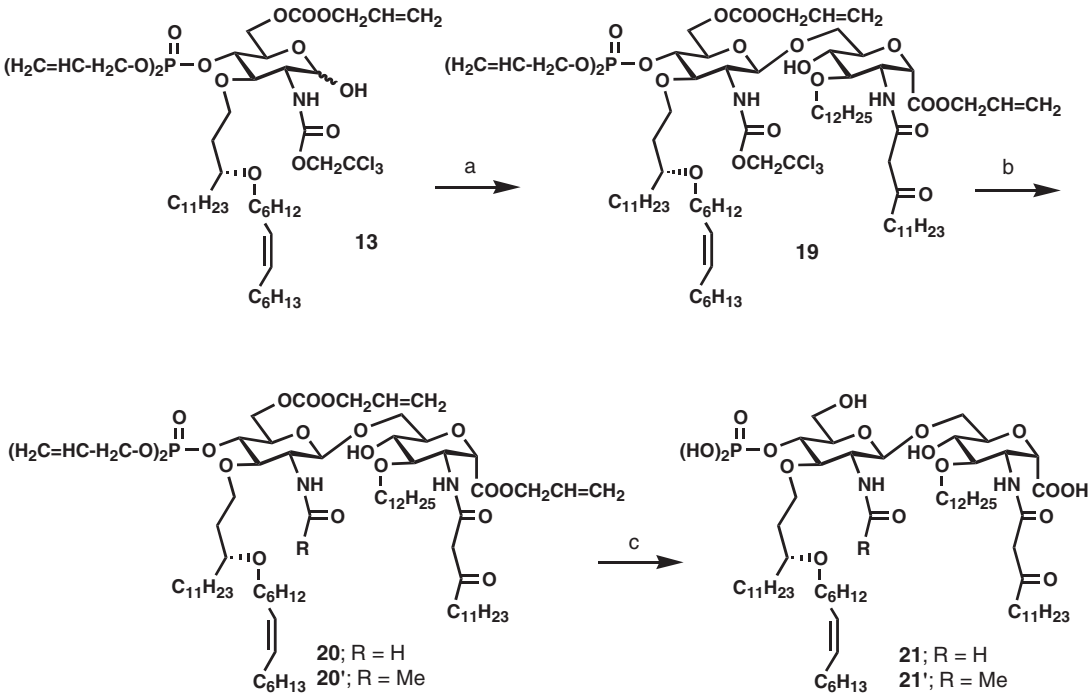
Scheme 3. Reagents and conditions: TBDMS = *tert*-$BuMe_2Si$; (a) TBDMSCl, DMAP, CH_2Cl_2, rt, 3 h, 96%; (b) $(i\text{-Pr})_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 2 h, then 30% H_2O_2, 0 °C, 1 h, 82%; (c) HF–pyridine, THF, rt, 26 h, 93%; (d) Me_3OBF_4, DTBMP, CH_2Cl_2, rt, 18 h, 90%; (e) NBS, acetone–H_2O, 0 °C, 2 h, 84%.

and Me), were synthesized from compounds **6** and **13**, as shown in Scheme 4. The reaction of **13** with trichloroacetonitrile using cesium carbonate as a base gave the trichloroacetimidate of **13**, and a successive in situ treatment of the resulting imidate with diol **6** using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst yielded β(1-6) pseudo-disaccharide **19**.[2a] A treatment of the trichloroethyl carbamate ester **19** with zinc in acetic acid gave an amine, which was formylated or acetylated with formic acid–acetic anhydride or acetic anhydride–pyridine to give formamide **20** or acetamide **20′**, respectively. Each of the four allyl groups of **20** and **20′** were deprotected in the presence of tetrakis(triphenylphosphine)palladium(0), triethylamine–formic acid and triphenylphosphine in THF to give **21** and **21′**, respectively (Scheme 4).

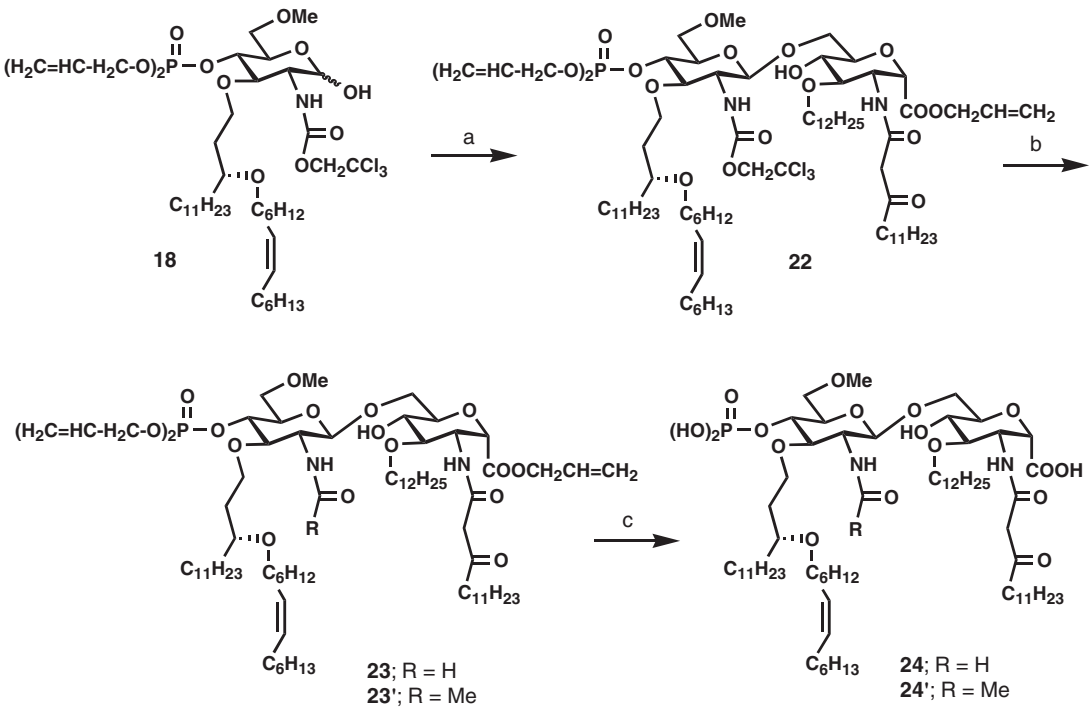
Also, a pair of pseudo-disaccharides, **24** and **24′** (R = H and Me), were synthesized from compound **6** and 6-*O*-methyl **18**, as shown in Scheme 5, through intermediates **22**, **23** and **23′** according to almost the same procedures as those for the preparation of **21** and **21′** from compounds **6** and **13**. The anomeric hydroxy group of **18** was activated with trichloroacetonitrile using cesium carbonate as a base to give the trichloroacetimidate of **18**, and a successive treatment of the resulting imidate with diol **6** using TMSOTf as a catalyst yielded β(1-6) pseudo-disaccharide **22**.[2a] A treatment of the trichloroethyl carbamate ester **22** with zinc in acetic acid gave an amine, which was formylated or acetylated with formic acid–acetic anhydride or acetic anhydride–pyridine to give formamide **23** or acetamide **23′**. Each of the four allyl groups of **23** and **23′** were deprotected in the presence of $Pd(PPh_3)_4$, Et_3N–HCOOH and PPh_3 in THF to give **24** and **24′**, respectively (Scheme 5).

Thus, we could synthesize four pseudo-disaccharide tetrahydropyran-2-carboxylic acids (**21**, **21′**, **24** and **24′**).

Biological Activity. The inhibitory activity on LPS-induced TNFα production, LPS-antagonistic activity, of the four synthesized compounds (**21**, **21′**, **24** and **24′**) was investigated in vitro using human monoblastic U937 cells, human whole blood cells and mouse peritoneal resident macrophages. The IC_{50} values (nM) of these four compounds (**21**, **21′**, **24** and



Scheme 4. Reagents and conditions: (a) (i) Cl_3CCN, cat. Cs_2CO_3, CH_2Cl_2, rt, 1 h, (ii) **6**, cat. TMSOTf, MS4A, CH_2Cl_2, −40 °C, 2 h, 76%; (b) (i) Zn, AcOH–THF (2:3), rt, 5 h; (ii) HCOOH–Ac_2O, THF, 0 °C, 2 h, or Ac_2O, pyridine, THF–H_2O, rt, 1 h, two steps 64% (**20**) or 70% (**20′**); (c) $Pd(PPh_3)_4$, PPh_3, Et_3N–HCOOH, THF, 50 °C, 4 h, 72% (**21**) or 76% (**21′**).



Scheme 5. Reagents and conditions: (a) (i) Cl_3CCN, cat. Cs_2CO_3, CH_2Cl_2, rt, 1 h; (ii) **6**, cat. TMSOTf, MS4A, CH_2Cl_2, −40 °C, 2 h, two steps 51%; (b) (i) Zn, AcOH–THF (2:3), rt, 5 h; (ii) HCOOH–Ac_2O, THF, 0 °C, 2 h, or Ac_2O, pyridine, THF–H_2O, rt, 1 h, two steps 53% (**23**) or 73% (**23′**); (c) $Pd(PPh_3)_4$, PPh_3, Et_3N–HCOOH, THF, 50 °C, 4 h, 91% (**24**) or 70% (**24′**).

24′) toward human monoblastic U937 cells were 2.2, 1.0, 0.017 and 0.055, respectively. The activities toward human monoblastic U937 cells of 6-*O*-methyl compounds **24** and **24′** were sufficiently strong. However, the LPS-antagonistic activities (IC_{50} values) of these four compounds toward human whole blood cells were only 0.28, 0.21, 0.81 and 0.58 μM, respectively. On the contrary, the activities of the methoxy compounds, **24** and **24′**, were slightly impaired toward human

whole blood cells. The influence of the C-2′-*N*-formyl and acetyl groups was minimal in both human monoblastic U-937 and whole blood cells.

Additionally, the IC_{50} values (μM) of these four compounds (**21**, **21′**, **24** and **24′**) toward mouse peritoneal resident macrophages were 2.49, 0.49, 0.91 and 0.69, respectively. The activity of these compounds toward mouse peritoneal resident macrophages was much less than that toward human monoblastic U937 cells.

Usually, lipid A analogs having six fatty acids chains show LPS-agonistic (endotoxic) activity toward both human U-937 and mouse peritoneal resident macrophages, and biosynthetic precursors of lipid A, such as lipid IVa[10] having four fatty acid chains, show LPS-antagonistic activity toward human blood cells and endotoxic activity toward mouse peritoneal resident macrophages. This fact shows, interestingly enough, that a difference exists in the molecular recognition between human and mouse LPS receptors.[11] However, the synthetic compounds this time showed LPS-antagonistic activity toward mouse peritoneal resident macrophages, as mentioned above. This tendency was the same activity as that for a nontoxic natural lipid A-related compound having a *cis*-double bond in one of the fatty acid chains isolated from *Rhodobacter sphaeroides*.[4]

Experimental

^{1}H NMR spectra were recorded with JEOL-GSX 400 and JNM-ECT 500 spectrometers using TMS as an internal standard. IR absorption spectra were measured with an IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of compounds by column chromatography was done with silica-gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (111–182 kPa) for easy elution. Commercially available anhydrous THF and dichloromethane were used for the reactions. DMF and pyridine were dried by storage over 4 Å molecular sieves.

Allyl 2-Azido-2-deoxy-3-*O*-dodecyl-4,6-*O*-isopropylidene-β-D-glucopyranoside (2). To a solution of allyl 2-azido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (**1**) (14.5 g, 50.8 mmol) in DMF (180 mL) was gradually added NaH (60% oil dispersion, 4.08 g, 102 mmol) at 0 °C with stirring. After 15 min at 0 °C, dodecyl methanesulfonate (16.1 g, 60.9 mmol) was added to the mixture. After stirring for 8 h at room temperature, the mixture was quenched with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (4:1) gave **2** (17.1 g, 74%) as an oil. IR ν_{max} ($CHCl_3$) 2927, 2855, 2114 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.6$–7.3 Hz), 1.26–1.39 (18H, m), 1.40 (3H, s), 1.49 (3H, s), 1.50–1.60 (2H, m), 3.14–3.24 (2H, m), 3.33 (1H, t, $J = 8.1$–9.5 Hz), 3.59–3.66 (2H, m), 3.75–3.80 (2H, m), 3.90 (1H, dd, $J = 5.1$, 11.0 Hz), 4.13 (1H, dd, $J = 6.6$, 12.4 Hz), 4.32 (1H, d, $J = 8.1$ Hz), 4.35 (1H, dd, $J = 5.1$, 12.4 Hz), 5.22–5.36 (2H, m), 5.93 (1H, m). FABMS (positive-ion): m/z 454 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{24}H_{44}N_3O_5$: 454.3281. Found: 454.3282. Anal. Calcd for $C_{24}H_{43}N_3O_5$ (453.6): C, 63.55; H, 9.56; N, 9.26%. Found: C, 63.55; H, 9.74; N, 9.12%.

2-Azido-2-deoxy-3-*O*-dodecyl-4,6-*O*-isopropylidene-D-glucopyranose (3). To a solution of **2** (17.0 g, 37.5 mmol) in THF (150 mL) was added cyclooctadienebis(methyldiphenylphosphine)-iridium(I) hexafluorophosphate, $[IrC_8H_{12}(MePh_2P)_2]PF_6$ (1.58 g). The air in the reaction flask was completely replaced with nitrogen and then further replaced with hydrogen to activate the iridium complex. Immediately after 1 or 2 min, when the red color solution of the iridium complex had become almost colorless, the hydrogen was completely replaced with nitrogen. This solution was stirred for 2 h at room temperature. After confirming a double bond shift to an enol ether (as indicated by a slightly higher R_f value) from the 1-allyloxy group by TLC, H_2O (80 mL), pyridine (4.5 g), and I_2 (19.3 g, 76.0 mmol) were added to this solution. After 30 min of stirring at room temperature, the mixture was concentrated in vacuo, diluted with EtOAc, washed with aq. 10% $Na_2S_2O_3$, satd. $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated to give a mixture that was separated on a silica-gel column. Elution with hexane–EtOAc (7:3) gave **3** (13.4 g, 86%) as a gum. IR ν_{max} ($CHCl_3$) 3599, 2927, 2855, 2114 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.9$ Hz), 1.26–1.36 (18H, m), 1.40 (1.5H, s), 1.42 (1.5H, s), 1.50 (3H, s), 1.55–1.62 (2H, m), 3.22–3.30 (1.5H, m, containing 0.5H, OH), 3.37 (0.5H, dd, $J = 3.6$, 9.8 Hz), 3.60–3.93 (7H, m, containing 0.5H, OH), 4.58 (0.5H, bs), 5.23 (0.5H, t, $J = 3.3$ Hz). FABMS (positive-ion): m/z 414 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{21}H_{40}N_3O_5$: 414.2968. Found: 414.2975. Anal. Calcd for $C_{21}H_{39}N_3O_5$ (413.6): C, 60.99; H, 9.51; N, 10.16%. Found: C, 60.39; H, 9.47; N, 10.03%.

2,6-Anhydro-3-azido-3-deoxy-4-*O*-dodecyl-5,7-*O*-isopropylidene-D-*glycero*-D-*ido*-heptononitrile (4). To a solution of **3** (13.0 g, 31.4 mmol) and trichloroacetonitrile (16 mL, 160 mmol) in CH_2Cl_2 (100 mL) was added cat. Cs_2CO_3 (3.05 g, 9.36 mmol). After stirring for 1 h at room temperature, satd. $NaHCO_3$ was added to the reaction mixture, and it was extracted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a crude imidate. To a solution of this imidate and trimethylsilyl cyanide (8.5 mL, 63.7 mmol) in CH_2Cl_2 (150 mL) was added molecular sieves 4A (10.3 g). After stirring for 1 h at room temperature, trimethylsilyl trifluoromethanesulfonate (0.60 mL) was added to the suspension. After stirring for 4 h at room temperature, satd. $NaHCO_3$ was added. The reaction mixture was filtered to remove molecular sieves 4A, diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (4:1) gave **4** (8.96 g, 68%) as a gum. IR ν_{max} ($CHCl_3$) 2927, 2855, 2119 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.6$ Hz), 1.26–1.37 (18H, m), 1.42 (3H, s), 1.49 (3H, s), 1.56–1.63 (2H, m), 3.61–3.74 (6H, m), 3.84–3.93 (2H, m), 4.78 (1H, d, $J = 5.9$ Hz). FABMS (positive-ion): m/z 423 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{22}H_{39}N_4O_4$: 423.2971. Found: 423.2977. Anal. Calcd for $C_{22}H_{38}N_4O_4$ (422.6): C, 62.53; H, 9.06; N, 13.26%. Found: C, 61.60; H, 8.87; N, 13.02%.

2,6-Anhydro-3-deoxy-4-*O*-dodecyl-5,7-*O*-isopropylidene-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptononitrile (5). To a solution of **4** (8.20 g, 19.4 mmol) in THF (50 mL) was added PPh_3 (6.11 g, 23.3 mmol). After stirring for 1 h, at room temperature, 28% aq. NH_3 (25 mL) was added to this solution. The mixture was stirred for 7 h at 60 °C, concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a residue. The residual mixture was dissolved in CH_2Cl_2 (100 mL). To this solution, 3-oxotetradecanoic acid (7.22 g, 29.8 mmol), DCC (6.05 g, 29.3 mmol), and DMAP (3.80 g, 31.1 mmol) were added with stirring. After stirring

for 4 h at room temperature, the reaction mixture was filtered, and the filtrate was concentrated in vacuo to give a mixture. The mixture was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:1) gave **5** (7.95 g, 66%) as a gum. IR ν_{max} ($CHCl_3$) 2927, 2855, 1713, 1676 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.6$–7.3 Hz), 1.26–1.32 (36H, m), 1.42 (3H, s), 1.50 (3H, s), 1.52–1.60 (2H, m), 2.51 (2H, t, $J =$ 7.3 Hz), 3.44 (2H, s), 3.54–3.76 (5H, m), 3.82–3.98 (2H, m), 4.04 (1H, m), 5.27 (1H, d, $J = 5.9$ Hz), 7.80 (1H, d, $J = 5.9$ Hz). FABMS (positive-ion): m/z 643 $(M + Na)^+$, 621 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{36}H_{65}N_2O_6$: 621.4863. Found: 621.4843. Anal. Calcd for $C_{36}H_{64}N_2O_6$ (620.9): C, 69.64; H, 10.39; N, 4.51%. Found: C, 69.49; H, 10.39; N, 4.61%.

Allyl 2,6-Anhydro-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (6). To a solution of **5** (5.80 g, 9.34 mmol) in allyl alcohol (10 mL) was added allyl alcohol (20 mL) saturated with gaseous HCl at room temperature. After stirring for 2 h at room temperature, the volatiles were concentrated in vacuo to give a residue, which was dissolved in EtOAc. Water was then added to this solution. After stirring for 1 h, this solution was neutralized with aqueous $NaHCO_3$, and extracted with EtOAc. The organic extracts were washed with brine, dried over $MgSO_4$, filtered, concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:7) gave **6** (2.67 g, 45%) as a white solid. IR ν_{max} (KBr) 3402, 3296 (broad), 3079, 2923, 2852, 1741, 1717, 1644 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$–7.3 Hz), 1.26–1.32 (36H, m), 1.52–1.59 (2H, m), 2.46 (1H, broad, OH) 2.51 (2H, t, $J = 7.3$ Hz), 3.02 (1H, s, OH), 3.37, 3.41 (2H, ABq, $J = 16.8$ Hz), 3.56–3.72 (5H, m), 3.80–3.87 (2H, m), 4.43 (1H, dt, $J = 5.1$, 9.5 Hz), 4.59 (1H, d, $J = 5.1$ Hz), 4.65 (1H, dd, $J = 5.9$, 12.5 Hz), 4.72 (1H, dd, $J = 5.9$, 13.2 Hz), 5.27–5.40 (2H, m), 5.93 (1H, m), 7.71 (1H, d, $J = 9.5$ Hz, NH). FABMS (positive-ion): m/z 662 $(M + Na)^+$, 640 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{36}H_{66}NO_8$: 640.4788. Found: 640.4791. Anal. Calcd for $C_{36}H_{65}NO_8$ (639.9): C, 67.57; H, 10.24; N, 2.19%. Found: C, 68.09; H, 10.63; N, 2.22%.

Prop-1-enyl 2-Azido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (7). A solution of $[IrC_8H_{12}(MePh_2P)_2]PF_6$ (634 mg, 0.750 mmol) in THF (200 mL) was completely replaced with nitrogen and then further replaced with hydrogen to activate the iridium complex. After 2 min, when the red color solution of iridium complex had become almost colorless, the hydrogen was completely replaced with nitrogen. To this solution was added a solution of allyl 2-azido-2-deoxy-4,6-*O*-isopropylidene-β-D-glucopyranoside (21.4 g, 75.0 mmol) in THF (50 mL) under nitrogen. The mixture was stirred for 5 h at room temperature. After confirming a double-bond shift to an enol ether (indicated by slightly higher R_f value) from the 1-allyloxy group by TLC, the reaction mixture was concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **7** (20.8 g, 97%) as a solid. IR ν_{max} ($CHCl_3$) 3594, 2890, 2116 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, s), 1.52 (3H, s), 1.58 (2.4H, dd, $J = 2.2$, 6.6 Hz), 1.64 (0.6H, dd, $J = 2.2$, 6.6 Hz), 2.86 (1H, bs, OH), 3.27 (1H, m), 3.42–3.64 (3H, m), 3.81 (1H, t, $J = 10.3$, 11.0 Hz), 3.95 (1H, dd, $J = 5.1$, 11.0 Hz), 4.55 (1H, d, $J = 8.1$ Hz), 4.66 (0.2H, m), 5.22 (0.8H, m), 6.18 (0.2H, dd, $J = 2.2$, 6.6 Hz), 6.23 (0.8H, dd, $J = 2.2$, 12.5 Hz). FABMS (positive-ion): m/z 308 $(M + Na)^+$, 286 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{12}H_{20}N_3O_5$: 286.1403. Found: 286.1410.

Prop-1-enyl 2-Azido-2-deoxy-4,6-*O*-isopropylidene-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranoside (8). To a solution of **7** (12.9 g, 45.2 mmol) in DMF (150 mL) was gradually added an excess amount of NaH (60% oil dispersion, 3.62 g, 90.5 mmol) at 0 °C with stirring. After 15 min, (*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl methanesulfonate (20.6 g, 41.0 mmol) was added to this solution, which was stirred for 18 h at room temperature. The reaction mixture was quenched with water, and extracted with EtOAc. The extract was washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (9:1) gave **8** (22.2 g, 78%). IR ν_{max} ($CHCl_3$) 2928, 2856, 2115 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.6$ Hz), 1.26–1.37 (36H, m), 1.40 (3H, s), 1.49 (3H, s), 1.57 (2.4H, dd, $J = 1.5$, 6.6 Hz), 1.64 (0.6H, dd, $J = 2.2$, 6.6 Hz), 1.69–1.75 (2H, m), 1.97–2.02 (4H, m), 3.19–3.27 (2H, m), 3.36–3.46 (4H, m), 3.62 (1H, t, $J = 9.5$ Hz), 3.72 (1H, m), 3.78 (1H, t, $J = 10.3$, 11.0 Hz), 3.86–3.94 (2H, m), 4.45 (1H, d, $J = 8.1$ Hz), 4.64 (0.2H, m), 5.19 (0.8H, m), 5.34–5.39 (2H, m), 6.16 (0.2H, dd, $J = 2.2$, 6.6 Hz), 6.21 (0.8H, dd, $J = 2.2$, 12.5 Hz). FABMS (positive-ion): m/z 692 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{40}H_{74}N_3O_6$: 692.5578. Found: 692.5553. Anal. Calcd for $C_{40}H_{73}N_3O_6$ (692.0): C, 69.42; H, 10.63; N, 6.07%. Found: C, 68.64; H, 10.60; N, 6.05%.

Prop-1-enyl 2-Deoxy-4,6-*O*-isopropylidene-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (9). To a suspension of $LiAlH_4$ (855 mg, 22.5 mmol) in THF (75 mL) was added **8** (10.4 g, 15.0 mmol) at 0 °C with stirring. After stirring for 1 h at room temperature, the solution was diluted with Et_2O (75 mL), quenched with H_2O (1 mL), 15% NaOH aq (1 mL), and H_2O (3 mL). After stirring for 30 min, this mixture was filtered, extracted with ether, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue, which was dissolved in CH_2Cl_2 (50 mL). To this solution were added satd. $NaHCO_3$ (50 mL) and 2,2,2-trichloroethyl chloroformate (4.80 g, 22.7 mmol). After stirring for 1 h at room temperature, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica-gel column. Elution with hexane–EtOAc (4:1) gave **9** (11.9 g, 94%) as a gum. IR ν_{max} ($CHCl_3$) 3454, 2928, 2856, 1739, 1679 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, $J = 6.6$ Hz), 1.26–1.32 (36H, m), 1.40 (3H, s), 1.49 (3H, s), 1.53 (2.4H, dd, $J = 1.5$, 6.6 Hz), 1.57 (0.6H, dd, $J = 1.5$, 6.6 Hz), 1.66–1.72 (2H, m), 1.97–2.02 (4H, m), 3.29–3.42 (5H, m), 3.59–3.66 (2H, m), 3.76–3.86 (3H, m), 3.94 (1H, dd, $J = 5.1$, 11.0 Hz), 4.56 (0.2H, m), 4.73 (2H, s), 4.92 (0.2H, d, $J = 7.3$ Hz), 4.96 (0.8H, d, $J = 7.3$ Hz), 5.12 (0.8H, m), 5.31–5.39 (3H, m, containing NH), 6.13 (0.2H, dd, $J = 1.5$, 6.6 Hz), 6.17 (0.8H, dd, $J = 1.5$, 11.7 Hz). FABMS (positive-ion): m/z 878 $(M + K)^+$ (on addition of KI), 840 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{43}H_{76}NO_8Cl_3K$: 878.4274. Found: 878.4260. Anal. Calcd for $C_{43}H_{76}NO_8Cl_3$ (841.4): C, 61.38; H, 9.10; N, 1.66; Cl, 12.64%. Found: C, 61.87; H, 9.49; N, 1.75; Cl, 13.11%.

Prop-1-enyl 2-Deoxy-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]-tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (10). To a solution of **9** (11.5 g, 13.7 mmol) in MeOH (70 mL) was added *p*-TsOH·H_2O (520 mg, 2.74 mmol) at room temperature. After stirring for 1 h, the solution was diluted with EtOAc, washed with satd. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated in vacuo to give a mixture, which

was chromatographed on a silica-gel column. Elution with hexane–EtOAc (1:1) gave **10** (9.25 g, 84%) as a wax. IR ν_{max} ($CHCl_3$) 3601, 3450, 3350, 2928, 2856, 1741, 1679 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$–7.3 Hz), 1.26–1.46 (36H, m), 1.53 (2.4H, dd, $J = 2.2$, 6.6 Hz), 1.57 (0.6H, dd, $J = 2.2$, 6.6 Hz), 1.69–1.78 (2H, m), 1.97–2.02 (4H, m), 2.33 (1H, bs, OH), 3.32–3.47 (5H, m), 3.55–3.73 (3H, m), 3.78–3.95 (4H, m, containing OH), 4.57 (0.2H, m), 4.75 (2H, s), 4.86 (0.2H, d, $J =$ 8.1 Hz), 4.91 (0.8H, d, $J = 7.3$ Hz), 5.10 (0.8H, m), 5.31–5.39 (3H, m, containing NH), 6.14 (0.2H, dd, $J = 2.2$, 6.6 Hz), 6.19 (0.8H, dd, $J = 2.2$, 12.5 Hz). FABMS (positive-ion): m/z 822 $(M + Na)^+$, 800 $(M + H)^+$; HRFABMS (positive-ion); Calcd for $C_{40}H_{72}NO_8Cl_3Na$: 822.4221. Found: 822.4219. Anal. Calcd for $C_{40}H_{72}NO_8Cl_3$ (801.4): C, 59.95; H, 9.06; N, 1.75; Cl, 13.27%. Found: C, 59.74; H, 9.24; N, 1.82; Cl, 14.19%.

Prop-1-enyl 6-*O*-Allyloxycarbonyl-2-deoxy-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (11). To a solution of **10** (3.21 g, 4.01 mmol) in CH_2Cl_2 (15 mL) were added pyridine (0.50 mL, 6.18 mmol) and allyl chloroformate (0.51 mL, 4.81 mmol) at 0 °C. After stirring for 2 h at 0 °C, the solution was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:1) gave **11** (3.35 g, 94%) as an amorphous. IR ν_{max} ($CHCl_3$) 3451, 3350, 2928, 2856, 1746, 1679 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.9$ Hz), 1.26–1.45 (36H, m), 1.53 (2.4H, dd, $J = 1.2$, 6.8 Hz), 1.56 (0.6H, dd, $J = 1.2$, 6.8 Hz), 1.70–1.78 (2H, m), 1.96–2.03 (4H, m), 3.31–3.43 (4H, m), 3.51 (1H, m), 3.58 (1H, m), 3.65–3.74 (2H, m), 3.79 (1H, bs, OH), 3.87 (1H, m), 4.38 (1H, dd, $J = 5.7$, 11.7 Hz), 4.49 (1H, dd, $J = 1.8$, 11.7 Hz), 4.56 (0.2H, m), 4.63 (2H, d, $J = 5.8$ Hz), 4.74 (2H, s), 4.83–4.89 (1H, m), 5.10 (0.8H, m), 5.26–5.40 (5H, m, containing NH), 5.92 (1H, m), 6.16 (0.2H, dd, $J = 1.7$, 6.3 Hz), 6.20 (0.8H, dd, $J = 1.5$, 12.3 Hz). FABMS (positive-ion): m/z 906 $(M + Na)^+$, 884 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{44}H_{76}NO_{10}Cl_3Na$: 906.4433. Found: 906.4433. Anal. Calcd for $C_{44}H_{76}NO_{10}Cl_3$ (885.4): C, 59.69; H, 8.65; N, 1.58; Cl, 12.01%. Found: C, 59.72; H, 8.81; N, 1.61; Cl, 12.18%.

Prop-1-enyl 6-*O*-Allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (12). To a solution of **11** (3.00 g, 3.39 mmol) and 1*H*-tetrazole (356 mg, 5.08 mmol) in THF (15 mL) was added bis(allyloxy)(diisopropylamino)phosphine (1.00 g, 4.08 mmol). After stirring for 2 h at room temperature, 30% H_2O_2 (2 mL) was added to the reaction mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq. 10% $Na_2S_2O_3$, diluted with EtOAc, washed with water, satd. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (7:3) gave **12** (3.02 g, 85%) as a gum. IR ν_{max} ($CHCl_3$) 3450, 2928, 2856, 1746, 1679 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$ Hz), 1.26–1.45 (36H, m), 1.52 (2.4H, dd, $J = 1.5$, 7.3 Hz), 1.56 (0.6H, dd, $J = 1.5$, 7.3 Hz), 1.71–1.74 (2H, m), 1.96–2.02 (4H, m), 3.28–3.38 (4H, m), 3.71–3.81 (3H, m), 3.93 (1H, m), 4.28–4.39 (2H, m), 4.51–4.64 (7.2H, m), 4.70, 4.75 (2H, ABq, $J = 11.7$ Hz), 5.01–5.05 (1H, m), 5.09 (0.8H, m), 5.24–5.39 (8H, m), 5.53 (1H, br, NH), 5.88–5.99 (3H, m), 6.13 (0.2H, d, $J = 1.5$, 5.9 Hz), 6.17 (0.8H, dd, $J = 1.5$, 12.5 Hz). FABMS (positive-ion): m/z 1066 $(M + Na)^+$, 1044 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{50}H_{86}NO_{13}Cl_3P$: 1044.4902. Found: 1044.4929.

6-*O*-Allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (13). To a solution of **12** (2.55 g, 2.44 mmol) in acetone (8 mL) and H_2O (2 mL) was added NBS (521 mg, 2.93 mmol) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was diluted with EtOAc, washed with 10% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to give a residue, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **13** (2.16 g, 88%) as a gum. IR ν_{max} ($CHCl_3$) 3598, 3435, 2928, 2856, 1747, 1651 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J =$ 6.6 Hz), 1.21–1.50 (36H, m), 1.70–1.73 (2H, m), 1.99–2.02 (4H, m), 3.27–3.36 (3H, m), 3.63–3.69 (2H, m), 3.83–3.91 (2H, m), 4.17 (1H, m), 4.30–4.37 (2H, m), 4.49–4.63 (8H, m, containing OH), 4.73 (2H, s), 5.23–5.39 (9H, m), 5.54 (1H, d, $J = 8.8$ Hz, NH), 5.87–5.98 (3H, m). FABMS (positive-ion): m/z 1026 $(M + Na)^+$, 1004 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{47}H_{82}NO_{13}Cl_3P$: 1004.4589. Found: 1004.4587. Anal. Calcd for $C_{47}H_{81}NO_{13}Cl_3P$ (1005.5): C, 56.14; H, 8.12; N, 1.39; Cl, 10.58; P, 3.08%. Found: C, 57.09; H, 8.27; N, 1.56; Cl, 10.31; P, 3.01%.

Prop-1-enyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (14). To a solution of **10** (5.21 g, 6.50 mmol) in CH_2Cl_2 (20 mL) were added *tert*-butyldimethylsilyl chloride (1.18 g, 7.83 mmol) and DMAP (1.03 g, 8.43 mmol). After stirring for 3 h at room temperature, the solution was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (4:1) gave **14** (5.72 g, 96%) as a gum. IR ν_{max} ($CHCl_3$) 3453, 2954, 2929, 2857, 1741, 1680 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.02 (6H, s), 0.69–0.94 (15H, m), 1.17–1.34 (36H, m), 1.44 (2.4H, dd, $J = 2.0$, 6.8 Hz), 1.47 (0.6H, dd, $J = 2.0$, 6.8 Hz), 1.65–1.67 (2H, m), 1.88–1.93 (4H, m), 3.22–3.34 (5H, m), 3.47–3.56 (2H, m), 3.61 (1H, bs, OH), 3.70–3.72 (2H, m), 3.77–3.82 (2H, m), 4.46 (0.2H, m), 4.65 (2H, s), 4.75 (1H, m), 5.01 (0.8H, m), 5.18 (1H, bs, NH), 5.23–5.30 (2H, m), 6.05 (0.2H, dd, $J = 2.0$, 5.9 Hz), 6.10 (0.8H, dd, $J = 2.0$, 10.7 Hz). FABMS (positive-ion): m/z 936 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{46}H_{86}NO_8Cl_3SiNa$: 936.5086; Found: 936.5078. Anal. Calcd for $C_{46}H_{86}NO_8Cl_3Si$ (915.6): C, 60.34; H, 9.47; N, 1.53; Cl, 11.62%. Found: C, 59.87; H, 9.59; N, 1.66; Cl, 12.11%.

Prop-1-enyl 6-*O*-*tert*-Butyldimethylsilyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (15). Compound **14** (5.52 g, 6.03 mmol) was treated as described in the formation of **12** from **11** to give **15** (5.33 g, 82%) as a gum. IR ν_{max} ($CHCl_3$) 3450, 2954, 2929, 2856, 1739, 1680 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.05 (6H, s), 0.86–0.89 (15H, m), 1.26–1.31 (36H, m), 1.52 (2.4H, dd, $J = 1.5$, 6.6 Hz), 1.56 (0.6H, dd, $J = 1.5$, 7.3 Hz), 1.72–1.78 (2H, m), 1.96–2.02 (4H, m), 3.28–3.38 (4H, m), 3.57 (1H, m), 3.70–3.79 (3H, m), 3.87 (1H, m), 4.02 (1H, m), 4.28 (1H, q, $J = 8.8$ Hz), 4.54–4.58 (4.2H, m), 4.73 (2H, s), 4.96 (1H, m), 5.08 (0.8H, m), 5.23–5.39 (6H, m), 5.46 (1H, bs, NH), 6.11 (0.2H, dd, $J = 1.5$, 6.6 Hz), 6.17 (0.8H, dd, $J = 1.5$, 12.5 Hz). FABMS (positive-ion): m/z 1096 $(M + Na)^+$, 1074 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{52}H_{96}NO_{11}Cl_3PSi$: 1074.5556. Found: 1074.5549.

Prop-1-enyl 2-Deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-

tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (16). To a solution of **15** (4.02 g, 3.74 mmol) in THF (15 mL) was added HF·Py (20 drops) at room temperature. After stirring for 26 h at room temperature, the reaction mixture was quenched with satd. $NaHCO_3$, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **16** (3.35 g, 93%) as a gum. IR ν_{max} ($CHCl_3$) 3451, 2928, 2856, 1739, 1679 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$ Hz), 1.26–1.39 (36H, m), 1.52 (2.4H, dd, $J = 1.5$, 7.3 Hz), 1.56 (0.6H, dd, $J = 1.5$, 6.6 Hz), 1.68–1.75 (2H, m), 1.97–2.03 (4H, m), 3.24–3.45 (5H, m), 3.67–3.74 (2H, m, containing OH), 3.80–3.95 (4H, m), 4.36 (1H, q, $J = 9.5$ Hz), 4.55–4.64 (4.2H, m), 4.70, 4.75 (2H, ABq, $J = 12.1$ Hz), 5.02 (1H, m), 5.05–5.12 (0.8H, m), 5.25–5.42 (6H, m), 5.48 (1H, bs, NH), 5.89–5.98 (2H, m), 6.13 (0.2H, dd, $J = 1.5$, 6.6 Hz), 6.18 (0.8H, dd, $J = 1.5$, 12.5 Hz). FABMS (positive-ion): m/z 982 $(M + Na)^+$; 960 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{46}H_{81}NO_{11}Cl_3PNa$: 982.4511. Found: 982.4482. Anal. Calcd for $C_{46}H_{81}NO_{11}Cl_3P$ (961.5): C, 57.46; H, 8.49; N, 1.46; Cl, 11.06; P, 3.22%. Found: C, 55.46; H, 8.29; N, 1.43; Cl, 11.11; P, 3.08%.

Prop-1-enyl 2-Deoxy-4-*O*-diallylphosphono-6-*O*-methyl-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (17). To a solution of **16** (3.15 g, 3.28 mmol) in CH_2Cl_2 (10 mL) were added 2,6-di-*tert*-butyl-4-methylpyridine (1.02 g, 4.97 mmol) and trimethyloxonium tetrafluoroborate (728 mg, 4.92 mmol) at room temperature. After 18 h at room temperature, the reaction mixture was diluted with EtOAc, washed with satd. $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated, and chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **17** (2.88 g, 90%) as a gum. IR ν_{max} ($CHCl_3$) 3450, 2928, 2856, 1741, 1679 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$ Hz), 1.26–1.45 (36H, m), 1.52 (2.4H, dd, $J = 1.5$, 6.6 Hz), 1.56 (0.6H, dd, $J = 1.5$, 6.6 Hz), 1.72–1.74 (2H, m), 1.96–2.02 (4H, m), 3.27–3.40 (7H, m, containing 3H, s, at 3.38 ppm), 3.61–3.66 (2H, m), 3.68–3.81 (3H, m), 3.91 (1H, m), 4.35 (1H, q, $J = 9.5$ Hz), 4.53–4.59 (4.2H, m), 4.68–4.77 (2H, m), 4.99–5.05 (1H, m), 5.08 (0.8H, m), 5.24–5.40 (6H, m), 5.48 (1H, m, NH), 6.15 (0.2H, dd, $J = 1.5$, 6.6 Hz), 6.19 (0.8H, dd, $J = 1.5$, 12.5 Hz). FABMS (positive-ion): m/z 996 $(M + Na)^+$; 974 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{47}H_{84}NO_{11}Cl_3P$: 974.4848. Found: 974.4848. Anal. Calcd for $C_{47}H_{83}NO_{11}Cl_3P$ (975.5): C, 57.87; H, 8.58; N, 1.44; Cl, 10.90; P, 3.18%. Found: C, 57.97; H, 8.55; N, 1.58; Cl, 11.11; P, 3.16%.

2-Deoxy-4-*O*-diallylphosphono-6-*O*-methyl-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (18). Compound **17** (2.55 g, 2.61 mmol) was treated as described in the formation of **13** from **12** to give **18** (2.04 g, 84%) as a gum. IR ν_{max} ($CHCl_3$) 3600, 3435, 2929, 2856, 1742 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (6H, t, $J = 6.6$–7.3 Hz), 1.26–1.52 (36H, m), 1.70–1.75 (2H, m), 1.96–2.02 (4H, m), 3.26–3.37 (3H, m), 3.40 (3H, s), 3.61–3.73 (4H, m), 3.83–3.92 (2H, m), 4.11 (1H, m), 4.29 (1H, q, $J = 9.5$ Hz), 4.56–4.62 (4H, m), 4.73 (2H, s), 5.24–5.40 (8H, m, containing 1H, NH), 5.90–6.00 (2H, m). FABMS (positive-ion): m/z 956 $(M + Na)^+$, 924 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{44}H_{79}NO_{11}Cl_3PNa$: 956.4354. Found: 956.4341.

Allyl 2,6-Anhydro-7-*O*-[6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (19). To a solution of **13** (941 mg, 0.936 mmol) in CH_2Cl_2 (5 mL) were added Cl_3CCN (0.50 mL, 4.99 mmol) and cat. Cs_2CO_3 (62 mg, 0.190 mmol) at room temperatrue. After stirring for 1 h at room temperature, the reaction mixture was quenched with satd. $NaHCO_3$, extracted with EtOAc, washed with brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude imidate, which was immediately used for next reaction without further purification. In a nitrogen atmosphere, a solution of thus-obtained imidate (1.05 g), diol **6** (500 mg, 0.782 mmol), and molecular sieves 4A (820 mg) in CH_2Cl_2 (10 mL) was stirred at room temperature. After stirring for 1 h, TMSOTf (10 μL, 0.055 mmol) was added to the mixture at −40 °C. After stirring 2 h at −40 °C, the mixture was quenched with satd. $NaHCO_3$, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (3:2) gave **19** (972 mg, 76%) as an amorphous. IR ν_{max} ($CHCl_3$) 3446, 2928, 2856, 1743, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (12H, t, $J = 6.6$ Hz), 1.26–1.77 (76H, m), 1.97–2.02 (4H, m), 2.50 (2H, t, $J = 7.3$ Hz), 2.80 (1H, d, $J = 3.7$ Hz, OH), 3.26–3.42 (5H, m), 3.51–3.84 (11H, m), 4.02 (1H, m), 4.26 (1H, q, $J = 9.5$ Hz), 4.31–4.50 (2H, m), 4.53 (1H, d, $J = 5.1$ Hz), 4.56–4.79 (11H, m), 4.91 (1H, m), 5.23–5.39 (10H, m), 5.65 (1H, d, $J = 7.3$ Hz, NH), 5.89–5.99 (4H, m), 7.61 (1H, d, $J = 8.8$ Hz, NH). FABMS (positive-ion): m/z 1647 $(M + Na)^+$, 1625 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{83}H_{144}N_2O_{20}Cl_3PNa$: 1647.9013. Found: 1647.8931. Anal. Calcd for $C_{83}H_{144}N_2O_{20}Cl_3P$ (1627.4): C, 61.26; H, 8.92; N, 1.72; Cl, 6.54; P, 1.90%. Found: C, 60.99; H, 8.88; N, 1.78; Cl, 6.49; P, 1.88%.

Allyl 2,6-Anhydro-7-*O*-[6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-2-formamido-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (20). To a solution of **19** (387 mg, 0.238 mmol) in THF (3 mL) and acetic acid (2 mL) was added zinc dust (312 mg, 4.78 mmol). After vigorously stirring for 5 h at room temperature, the solution was filtered to remove the zinc dust, concentrated in vacuo, diluted with EtOAc, washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product (344 mg). Formic acid (55 μL, 1.46 mmol) was added dropwise to acetic anhydride (90 μL, 0.954 mmol) at 0 °C and then the mixture was heated at 60 °C for 30 min. The mixture was cooled to 0 °C and THF (2 mL) was added. To this solution was added the above obtained crude amine (344 mg) in THF (3 mL). After stirring for 3 h at room temperature, the mixture was concentrated in vacuo, diluted with EtOAc, washed with satd. $NaHCO_3$ and brine, and chromatographed on a silica-gel column. Elution with hexane–EtOAc (1:1) gave **20** (226 mg, 64%, 2 steps). IR ν_{max} ($CHCl_3$) 3606, 3439, 2928, 2856, 1745, 1688 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (12H, t, $J = 6.6$–7.3 Hz), 1.25–1.79 (76H, m), 1.98–2.02 (4H, m), 2.50 (2H, t, $J = 7.3$ Hz), 3.01 (1H, s, OH), 3.23–3.43 (6H, m), 3.47–3.89 (10H, m), 4.04 (1H, dd, $J = 2.2$, 11.7 Hz), 4.23 (1H, q, $J = 9.5$ Hz), 4.32 (1H, dd, $J = 5.9$, 11.7 Hz), 4.41 (1H, dt, $J = 5.1$, 9.5 Hz), 4.53–4.72 (10H, m), 5.13 (1H, d, $J = 8.1$ Hz), 5.23–5.39 (10H, m), 5.88–5.99 (4H, m), 6.56 (0.7H, d, $J = 5.9$ Hz, NH), 7.62 (1H, d, $J = 8.8$ Hz, NH), 7.72 (0.3H, m, NH), 8.02 (0.3H, d, $J = 11.0$ Hz, CHO), 8.12 (0.7H, s, CHO). FABMS (positive-ion): m/z 1501 $(M + Na)^+$; 1479 $(M + H)^+$. HRFABMS (positive-ion); Calcd for

$C_{81}H_{143}N_2O_{19}PNa$: 1501.9920. Found: 1501.9955.

Allyl 2,6-Anhydro-7-*O*-[2-acetamido-6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (20′). To a solution of **19** (408 mg, 0.251 mmol) in THF (3 mL) and acetic acid (2 mL) was added zinc dust (329 mg, 5.03 mmol). After vigorously stirring for 4 h at room temperature, the solution was filtered to remove the zinc dust. The filtrate was concentrated in vacuo to give a residue, which was diluted with EtOAc. The solution was washed with satd. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a crude product (364 mg). Then, the crude product was dissolved in THF (3 mL), and H_2O (2 mL), pyridine (0.12 mL, 1.48 mmol) and Ac_2O (0.12 mL, 1.27 mmol) were added to this solution. After stirring for 1 h at room temperature, the mixture was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica-gel column. Elution with hexane–EtOAc (2:3) gave **20′** (264 mg, 2 steps 70%) as a gum. IR ν_{max} ($CHCl_3$) 3452, 3345, 2928, 2856, 1746, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (12H, t, J = 6.6 Hz), 1.26–1.81 (76H, m), 1.98–2.12 (7H, m, containing 3H, s, at 2.01 ppm), 2.50 (2H, t, J = 7.3 Hz), 3.14 (1H, m), 3.32–3.41 (5H, m, containing OH), 3.52–3.76 (8H, m), 3.79–3.83 (2H, m), 3.91 (1H, t, J = 8.8–9.5 Hz), 4.05 (1H, dd, J = 2.2, 11.7 Hz), 4.22 (1H, q, J = 8.8–9.5 Hz), 4.32 (1H, dd, J = 5.9, 11.7 Hz), 4.37 (1H, dt, J = 3.7, 9.5 Hz), 4.52–4.73 (10H, m), 5.20 (1H, d, J = 8.1 Hz), 5.23–5.39 (10H, m), 5.88–5.98 (4H, m), 6.25 (1H, d, J = 6.6 Hz, NH), 7.57 (1H, d, J = 8.8 Hz, NH). FABMS (positive-ion): m/z 1515 $(M + Na)^+$; 1493 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{82}H_{145}N_2O_{19}PNa$: 1516.0077. Found: 1516.0052. Anal. Calcd for $C_{82}H_{145}N_2O_{19}P$ (1494.0): C, 65.92; H, 9.78; N, 1.88; P, 2.07%. Found: C, 65.54; H, 9.90; N, 1.86; P, 2.09%.

2,6-Anhydro-3-deoxy-7-*O*-[2-deoxy-2-formamido-4-*O*-phosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonic Acid (21). To a solution of **20** (165 mg, 0.111 mmol) in THF (3 mL) were added PPh_3 (14.8 mg, 0.056 mmol), Et_3N (77 μL, 0.552 mmol), HCOOH (42 μL, 1.11 mmol), and $Pd(PPh_3)_4$ (6.4 mg). The reaction mixture was stirred for 4 h at 50 °C, and concentrated in vacuo to give a residue. The residue was dissolved in $CHCl_3$–MeOH (2:1) and applied to a DEAE (diethylaminoethyl)-cellulose column (acetate form). The column was eluted with $CHCl_3$/MeOH (2:1), then 0.05 M ammonium acetate in $CHCl_3$/MeOH/H_2O (2:3:1). The fractions were analyzed by silica-gel TLC and a solvent system of $CHCl_3$/EtOH/AcOH/H_2O (8:5:1:1). The product-containing fractions were concentrated in vacuo to give a residue, which was dissolved in $CHCl_3$ (4 mL), MeOH (8 mL) and aqueous 0.1 M HCl (3.2 mL). To this solution was added another volume of $CHCl_3$ (4 mL) and aqueous 0.1 M HCl (4 mL) to separate the solution into two phases. The lower $CHCl_3$ phase was collected and concentrated to give **21** (102 mg, 72%) as a white powder, mp 173.5–175.0 °C. $[\alpha]_D^{25}$ +2.3 (c 0.40, $CHCl_3$). IR ν_{max} (KBr) 3308 (broad), 3005, 2924, 2853, 1717, 1658 cm^{-1}. ^1H NMR (500 MHz, CD_3OD:$CDCl_3$ = 1:5) δ 0.89 (12H, t, J = 6.6 Hz), 1.17–1.75 (76H, m), 2.00–2.03 (4H, m), 2.55 (2H, t, J = 7.3 Hz), 3.20 (1H, m), 3.33–3.48 (6H, m), 3.54–3.96 (11H, m), 4.02–4.16 (2H, m), 4.25 (1H, m), 4.48 (1H, d, J = 5.1 Hz), 4.52 (0.25H, d, J = 8.8 Hz), 4.64 (0.75H, d, J = 8.8 Hz), 5.30–5.38 (2H, m), 7.96 (0.25H, s, CHO), 8.14 (0.75H, s, CHO). FABMS (positive-ion): m/z 1297 $(M + Na)^+$; 1275 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{68}H_{127}N_2O_{17}PNa$: 1297.8770. Found: 1297.8770. Anal. Calcd for $C_{68}H_{127}N_2O_{17}P$ (1275.7): C, 64.02; H, 10.03; N, 2.20; P, 2.43%. Found: C, 63.08; H, 10.37; N, 2.19; P, 2.35%.

2,6-Anhydro-7-*O*-[2-acetamido-2-deoxy-4-*O*-phosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonic Acid (21′). Compound **20′** (235 mg, 0.157 mmol) was treated as described in the formation of **21** from **20**, to give **21′** (153 mg, 76%) as a white powder, mp 158.0–159.5 °C. $[\alpha]_D^{25}$ +3.0 (c 0.20, $CHCl_3$). IR ν_{max} (KBr) 3297 (broad), 3070, 2924, 2853, 1719, 1649 cm^{-1}. ^1H NMR (500 MHz, CD_3OD:$CDCl_3$ = 1:5) δ 0.90 (12H, t, J = 7.3 Hz), 1.29–1.57 (74H, m), 1.68–1.78 (2H, m), 1.99–2.04 (7H, m, containing 3H, s, at 2.00 ppm), 2.55 (2H, t, J = 7.3 Hz), 3.40–3.48 (6H, m), 3.52–3.69 (6H, m), 3.73–3.88 (6H, m), 4.02 (1H, dd, J = 2.0, 11.7 Hz), 4.10 (1H, q, J = 9.3 Hz), 4.23 (1H, dd, J = 4.9, 8.8 Hz), 4.48 (1H, d, J = 4.9 Hz), 4.59 (1H, d, J = 8.8 Hz), 5.31–5.39 (2H, m). FABMS (positive-ion): m/z 1311 $(M + Na)^+$; 1289 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{69}H_{129}N_2O_{17}PNa$: 1311.8927. Found: 1311.8889. Anal. Calcd for $C_{69}H_{129}N_2O_{17}P$ (1289.7): C, 64.26; H, 10.08; N, 2.17; P, 2.40%. Found: C, 63.49; H, 10.43; N, 2.18; P, 2.34%.

Allyl 2,6-Anhydro-3-deoxy-7-*O*-[2-deoxy-4-*O*-diallylphosphono-6-*O*-methyl-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (22). Compound **18** (1.04 g, 1.11 mmol) was treated as described in the formation of **19** from **13** to give **22** (749 mg, 51%) as an amorphous. IR ν_{max} ($CHCl_3$) 3445, 2928, 2856, 1735, 1675 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 0.88 (12H, t, J = 6.6–7.3 Hz), 1.26–1.60 (74H, m), 1.72–1.77 (2H, q, J = 6.6 Hz), 1.99–2.02 (4H, m), 2.50 (2H, t, J = 7.3 Hz), 2.93 (1H, s, OH), 3.26–3.38 (5H, m), 3.39 (3H, s), 3.50–3.87 (12H, m), 4.03 (1H, m), 4.30 (1H, q, J = 9.5 Hz), 4.38 (1H, dt, J = 5.1, 9.5 Hz), 4.53 (1H, d, J = 5.1 Hz), 4.54–4.78 (9H, m), 4.86 (1H, m), 5.24–5.39 (8H, m), 5.54 (1H, d, J = 8.1 Hz, NH), 5.87–5.99 (3H, m), 7.58 (1H, d, J = 9.5 Hz, NH). FABMS (positive-ion): m/z 1577 $(M + Na)^+$; 1555 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{80}H_{142}N_2O_{18}Cl_3PNa$: 1577.8959. Found: 1577.8917. Anal. Calcd for $C_{80}H_{142}N_2O_{18}Cl_3P$ (1557.3): C, 61.70; H, 9.19; N, 1.80; Cl, 6.83; P, 1.99%. Found: C, 61.35; H, 9.21; N, 1.85; Cl, 6.82; P, 1.71%.

Allyl 2,6-Anhydro-3-deoxy-7-*O*-[2-deoxy-4-*O*-diallylphosphono-2-formamido-6-*O*-methyl-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (23). Compound **22** (291 mg, 0.187 mmol) was treated as described in the formation of **20** from **19**, to give **23** (139 mg, 2 steps 53%). IR ν_{max} ($CHCl_3$) 3430, 3345, 3289, 2928, 2856, 1731, 1688 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (12H, t, J = 6.9 Hz), 1.25–1.77 (76H, m), 1.97–2.02 (4H, m), 2.50 (2H, t, J = 7.4 Hz), 2.86 (1H, br, OH), 3.21–3.43 (9H, m, containing 3H, s, at 3.39 ppm), 3.47–3.87 (12H, m), 4.05 (1H, m), 4.27 (1H, q, J = 9.2 Hz), 4.40 (1H, m), 4.53 (1H, d, J = 4.8 Hz), 4.55–4.59 (4H, m), 4.60–4.74 (2H, m), 5.06 (1H, d, J = 8.2 Hz), 5.24–5.39 (8H, m), 5.90–5.98 (3H, m), 6.39 (0.6H, m, NH), 7.57 (1H, d, J = 9.3 Hz, NH), 7.70 (0.4H, m, NH), 8.03 (0.4H, d, J = 11.4 Hz, CHO), 8.13 (0.6H, s, CHO). FABMS (positive-ion): m/z 1431 $(M + Na)^+$. HRFABMS (positive-ion); Calcd for $C_{78}H_{141}N_2O_{17}PNa$: 1431.9866. Found: 1431.9857.

Allyl 2,6-Anhydro-7-*O*-[2-acetamido-2-deoxy-4-*O*-diallyl-

phosphono-6-*O*-methyl-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonate (23′). Compound **22** (286 mg, 0.184 mmol) was treated as described in the formation of **20′** from **19**, to give **23′** (191 mg, 73%, 2 steps). IR ν_{max} ($CHCl_3$) 3452, 3351, 2928, 2856, 1729, 1715, 1675 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 0.88 (12H, t, $J = 6.9$ Hz), 1.26–1.79 (76H, m), 1.96–2.05 (7H, m, containing 3H, s, at 2.01 ppm), 2.50 (2H, t, $J = 7.4$ Hz), 3.25 (1H, m), 3.31–3.40 (7H, m, containing 3H, s, at 3.38 ppm), 3.48 (1H, bs, OH), 3.52–3.88 (13H, m), 4.05 (1H, dd, $J = 2.6$, 11.8 Hz), 4.26 (1H, q, $J = 9.1$ Hz), 4.37 (1H, dt, $J = 5.2$, 9.2 Hz), 4.53 (1H, d, $J = 5.2$ Hz), 4.54–4.59 (4H, m), 4.64 (1H, dd, $J = 5.9$, 13.0 Hz), 4.70 (1H, dd, $J = 5.9$, 13.0 Hz), 5.11 (1H, d, $J = 8.1$ Hz), 5.24–5.39 (8H, m), 5.89–5.97 (3H, m), 6.19 (1H, d, $J = 7.0$ Hz, NH), 7.55 (1H, d, $J = 9.2$ Hz, NH). FABMS (positive-ion): m/z 1445 $(M + Na)^+$; 1423 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{79}H_{143}N_2O_{17}PNa$: 1446.0022. Found: 1446.0045. Anal. Calcd for $C_{79}H_{143}N_2O_{17}P$ (1424.0): C, 66.63; H, 10.12; N, 1.97; P, 2.18%. Found: C, 66.05; H, 10.08; N, 1.98; P, 2.01%.

2,6-Anhydro-3-deoxy-7-*O*-[2-deoxy-2-formamido-6-*O*-methyl-4-*O*-phosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonic Acid (24). Compound **23** (105 mg, 0.075 mmol) was treated as described in the formation of **21** from **20** to give **24** (87 mg, 91%) as a white powder, mp 152.5–153.5 °C. $[\alpha]_D^{25}$ +10.9 (*c* 0.20, $CHCl_3$). IR ν_{max} (KBr) 3309 (broad), 2924, 2853, 1717, 1652 cm^{-1}. ^1H NMR (500 MHz, CD_3OD:$CDCl_3$ = 1:5) δ 0.89 (12H, t, $J = 6.6$–7.3 Hz), 1.17–1.56 (74H, m), 1.64–1.73 (2H, m), 1.98–2.03 (4H, m), 2.55 (2H, t, $J = 7.3$ Hz), 3.20 (1H, m), 3.35–3.87 (20H, m, containing 3H, s, at 3.40 ppm), 4.01–4.16 (2H, m), 4.27 (1H, dd, $J = 5.1$, 8.8 Hz), 4.50 (1H, d, $J = 4.4$ Hz), 4.54 (0.25H, d, $J = 8.8$ Hz), 4.64 (0.75H, d, $J = 8.8$ Hz), 5.30–5.38 (2H, m), 7.96 (0.25H, s, CHO), 8.14 (0.75H, s, CHO). FABMS (positive-ion): m/z 1311 $(M + Na)^+$; 1289 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{69}H_{129}N_2O_{17}PNa$: 1311.8927. Found: 1311.8918. Anal. Calcd for $C_{69}H_{129}N_2O_{17}P$ (1289.7): C, 64.26; H, 10.08; N, 2.17; P, 2.40%. Found: C, 64.08; H, 10.51; N, 2.11; P, 2.34%.

2,6-Anhydro-7-*O*-[2-acetamido-2-deoxy-6-*O*-methyl-4-*O*-phosphono-3-*O*-{(*R*)-3-[(*Z*)-tetradec-7-enyloxy]tetradecyl}-β-D-glucopyranosyl]-3-deoxy-4-*O*-dodecyl-3-(3-oxotetradecanamido)-D-*glycero*-D-*ido*-heptonic Acid (24′). Compound **23′** (177 mg, 0.124 mmol) was treated as described in the formation of **21** from **20**, to give **24′** (114 mg, 70%) as a white powder; mp 164.0–165.0 °C. $[\alpha]_D^{25}$ +1.7 (*c* 0.23, $CHCl_3$). IR ν_{max} (KBr) 3298 (broad), 3072, 2924, 2853, 1719, 1653 cm^{-1}. ^1H NMR (500 MHz, CD_3OD:$CDCl_3$ = 1:5) δ 0.90 (12H, t, $J = 6.9$ Hz), 1.29–1.60 (74H, m), 1.72–1.75 (2H, m), 1.97–2.04 (7H, m, containing 3H, s, at 2.00 ppm), 2.55 (2H, t, $J = 7.3$ Hz), 3.40 (3H, s), 3.37–3.87 (18H, m), 4.00 (1H, dd, $J = 2.3$, 11.5 Hz), 4.08 (1H, q, $J = 9.6$ Hz), 4.26 (1H, dd, $J = 5.0$, 8.5 Hz), 4.49 (1H, d, $J = 4.9$ Hz), 4.59 (1H, d, $J = 8.3$ Hz), 5.31–5.38 (2H, m). FABMS (positive-ion): m/z 1325 $(M + Na)^+$; 1303 $(M + H)^+$. HRFABMS (positive-ion); Calcd for $C_{70}H_{131}N_2O_{17}PNa$: 1325.9083. Found: 1325.9069. Anal. Calcd for $C_{70}H_{131}N_2O_{17}P$ (1303.8): C, 64.49; H, 10.13; N, 2.15; P, 2.38%. Found: C, 63.72; H, 10.14; N, 2.24; P, 2.25%.

Methods for Measurement of Biological Activity.[12] The sources of the materials used in the study are as follows: lipopolysaccharide (LPS) from *E. coli* serotype 026:B6 and 12-*O*-tetradecanoylphorbol acetate (TPA) were from Sigma, St. Louis, MO; RPMI-1640 medium, fetal bovine serum (FBS), and newborn calf serum (NBCS) were from Gibco, Grand Island, NY; and human TNFα ELISA kit and mouse TNFα ELISA kit were from Genzyme-Techne, Mineapolis, MN.

Cell Culture: Human monoblastic U937 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, 100 U/mL of penicillin and 100 μg/mL of streptomycin (growth medium).

Production of TNFα by U937 Cells: U937 cells (1×10^4/200 μL/well) were plated in 96-well plates (Corning, Cambridge, MA) and cultured in the presence of TPA (30 ng/mL) for 72 h at 37 °C. After removing the supernatant, the cells were incubated in 200 μL of fresh RPMI-1640 medium containing 10% NBCS, in the absence or the presence of 30 ng/mL of LPS with graded concentrations of the compounds in a humidified atmosphere of 5% CO_2 for 4.5 h at 37 °C. After incubation, the amount of TNFα produced in the culture supernatants was determined using the TNFα ELISA kits. As a control, the amount of TNFα produced by U937 cells, which were stimulated with 30 ng/mL of LPS in the absence of the test compounds, was used. The concentrations (nM) of the compounds required to inhibit the LPS-induced TNFα production by U937 cells by 50% (IC_{50}) was calculated from the control amount. All experiments were carried out at least twice, demonstrating that the data are reproducible.

Production of TNFα by Mouse Peritoneal Macrophage: C57BL/6 female mice (6–7 weeks old) were obtained from Charles River Japan, Inc., Yokohama, Japan. Peritoneal resident macrophages were collected by peritoneal lavage with ice-cold saline. After washing, cells were resuspended in RPMI-1640 medium supplemented with 10% NBCS, 100 U/mL of penicillin and 100 μg/mL of streptomycin, and were plated in 96-well plates (5×10^4/100 μL/well). After incubation overnight at 37 °C, nonadherent cells were removed by washing three times with RPMI-1640 medium containing 10% NBCS, and adherent cells were incubated in 100 μL of the same medium, in the absence or presence of 10 ng/mL of LPS, with graded concentrations of the compounds in the humidified atmosphere of 5% CO_2 for 4.5 h at 37 °C. After incubation, the amount of TNFα produced in the culture supernatants was determined using the mouse TNFα ELISA kits. The IC_{50} of the compounds was calculated as described above.

Production of TNFα by Human Whole Blood: Materials: Lipopolysaccharide (LPS, lot 50K4117, *E. coli* 026:B6), human tumor necrosis factor alpha (TNFα) immunoassay kit and 96-well assay plates were purchased from Sigma, BioSource International, Inc. and Corning Inc. (Cat. No. 3956), respectively.

Whole blood TNFα production: Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. The subjects did not have any apparent inflammatory conditions and had taken no drugs for at least 7 days prior to blood collection. Written informed consent was obtained from all volunteers before the experiment. In each well of the plates, 360 μL aliquots of blood were mixed with 20 μL of LPS solution (200 ng/mL) dissolved in PBS in the presence (for test sample) or absence (for positive control sample) of test compounds solution (dissolved in 10% DMSO/PBS solution). For the negative control samples, the same amount of blood was cultured without either LPS or a test compound solution. After 6 h of incubation at 37 °C, the plates were centrifuged at 490×g for 15 min, and the plasma was collected and stored at −20 °C. The concentrations of TNFα in the plasma were measured with commercially available immunoassay kits.

Statistical analysis: The percentage of inhibition of TNFα production was calculated by the following formula: {1 − (concentra-

tion of TNFα in the test sample − concentration of TNFα in the negative control sample) / (concentration of TNFα in the positive control sample − concentration of TNFα in the negative control sample)} × 100. The suppressive activities of test compounds are expressed as the fifty percent inhibitory concentration (IC_{50}) of the test compound, the concentration at which the test compound suppresses TNFα production by 50%. The IC_{50} was calculated from the percentage of inhibition using the SAS System for Windows. The results are expressed as the mean IC_{50} of triplicate experiments.

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References

1 "Endotoxin in Health and Diseases," ed by H. Brade, S. M. Opal, S. N. Vogel, and D. C. Morrison, Marcel Dekker Inc., New York, Basel (1999).

2 a) M. Imoto, H. Yoshimura, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Tetrahedron Lett.*, **26**, 1545 (1985). b) M. Imoto, H. Yoshimura, T. Shimamoto, N. Sakaguchi, S. Kusumoto, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **60**, 2205 (1987). c) Cf. T. Takahashi, S. Nakamoto, K. Ikeda, and K. Achiwa, *Tetrahedron Lett.*, **27**, 1819 (1986).

3 C. Galanos, O. Luderitz, E. T. Rietschel, and O. Westphal, *Int. Rev. Biochem.*, **14**, 293 (1977).

4 a) N. Qureshi, J. P. Honovich, H. Hara, R. J. Cotter, and K. Takayama, *J. Biol. Chem.*, **263**, 5502 (1988). b) N. Qureshi, K. Takayama, and R. Kurtz, *Infect. Immun.*, **51**, 441 (1991). c) N. Qureshi, K. Takayama, K. C. Meyer, T. N. Kirkland, C. A. Bush, L. Chen, R. Wang, and R. J. Cotter, *J. Biol. Chem.*, **266**, 6532 (1991). d) W. J. Christ, P. D. McGuinness, O. Asano, Y. Wang, M. A. Mullarkey, M. Perez, L. D. Hawkins, T. A. Blythe, G. R. Dubuc, and A. L. Robidoux, *J. Am. Chem. Soc.*, **116**, 3637 (1994). e) I. A. Kaltashov, V. Doroshenco, R. J. Cotter, K. Takayama, and N. Qureshi, *Anal. Chem.*, **69**, 2317 (1997).

5 W. J. Christ, D. P. Rossignol, S. Kobayashi, and T. Kawata, U. S. Patent 5935938 (Aug. 10, 1999).

6 Y. Watanabe, T. Mochizuki, M. Shiozaki, S. Kanai, S. Kurakata, and M. Nishijima, *Carbohydr. Res.*, **333**, 203 (2001).

7 D. P. Rossignol, L. D. Hawkins, W. J. Christ, K. Kobayashi, T. Kawata, M. Lynn, I. Yamatsu, and Y. Kishi, Chapter 47 in "Endotoxin in Health and Diseases," ed by H. Brade, S. M. Opal, S. N. Vogel, and D. C. Morrison, Marcel Dekker, Inc., New York, Basel (1999).

8 Y. Watanabe, K. Miura, M. Shiozaki, S. Kanai, S. Kurakata, and M. Nishijima, *Carbohydr. Res.*, **332**, 257 (2001).

9 T. Nakamura, Y. Watanabe, M. Shiozaki, S. Kanai, and S. Kurakata, *Bull. Chem. Soc. Jpn.*, **76**, 1011 (2003).

10 D. T. Golenbock, R. Y. Hampton, N. Qureshi, K. Takayama, and C. R. Raetz, *J. Biol. Chem.*, **266**, 19490 (1991).

11 a) 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.*, **13**, 1595 (2001). b) 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.*, **105**, 497 (2000).

12 a) K.-Y. Wang, N. Arima, S. Higuchi, S. Shimajiri, A. Tanimoto, Y. Murata, T. Hamada, and Y. Sasaguri, *FEBS Lett.*, **473**, 345 (2000). b) R. Ikegami, Y. Sugimoto, E. Segi, M. Katsuyama, H. Karahashi, F. Amano, T. Maruyama, H. Yamane, S. Tsuchiya, and A. Ichikawa, *J. Immunol.*, **166**, 4689 (2001). c) M. P. Jagger, Z. Huo, and P. G. Riches, *Clin. Exp. Immunol.*, **130**, 467 (2002).