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Syntheses of glucose derivatives of E5564-related compounds and their LPS-antagonistic activities

Masao Shiozaki,[a,*] Yuji Iwano,[a] Hiromi Doi,[b] Daisuke Tanaka,[c] Takaichi Shimozato[b] and Shin-ichi Kurakata[c]

[a]*Chemistry Department, Chemtech Laboratories, Inc., Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan*
[b]*Pharmacodynamics Research Laboratories, Sankyo Co., Ltd, Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan*
[c]*Biological Research Laboratories, Sankyo Co., Ltd, Hiromachi 1-2-58, Shinagawa-ku, Tokyo 140-8710, Japan*

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Abstract—Glucose analogues **6**, **12**, **17b**, **19a**, and **19b** of E5564 were synthesized, and their LPS-antagonistic activities were measured. The antagonistic activities (IC_{50}) on LPS-induced TNFα production of these five compounds toward human whole blood were 72.8, 3.0, 0.9, 7.5, and 1.4 nM, respectively. Inhibitory doses (ID_{50}) of compounds **12**, **17b**, **19a**, and **19b** on TNFα production induced by co-injection of galactosamine and LPS in C3H/HeN mice in vivo were measured. The values of these compounds were 0.9, ND (not determined), 1.6, and 0.9 mg/kg, respectively.

Keywords: E5564; LPS-antagonist; RsDPLA; Lipid A analogue of glucose–glucose α-(1→6)-linked disaccharide

1. Introduction

Lipopolysaccharide (LPS, endotoxin) is an amphipathic glycolipid found on the outer surface of the outer membrane of Gram-negative bacteria.[1] Lipid A is the toxic component of LPS. The study of endotoxin has developed extensively since Shiba and Kusumoto's total synthesis of lipid A.[2] On the other hand, an Eisai group found a nontoxic pentaacyl diphosphoryl lipid A derived from the LPS of *Rhodobactor sphaeroides* (RsDPLA)[3] that showed strong LPS-antagonistic activity toward both human cells and murine cells. The Eisai group synthesized the reported structure of RsDPLA.[3f] Furthermore, they recognized that RsDPLA should be an effective therapeutic agent in blocking the initiation of sepsis. Namely, when LPS binds toll-like receptor 4 (TLR4), a receptor recognizing LPS, the TLR4 liberates a series of inflammatory cytokines such as IL-1 and TNFα, and this phenomenon leads to the onset of sepsis. However, RsDPLA inhibits the binding of LPS to TLR4 antagonistically and suppresses the expression of sepsis. Severe sepsis can lead to widespread organ dysfunction, and it is one of the most common causes of death in ICU patients. Unfortunately, there are no effective drugs for severe sepsis to date. In addition, the seriousness of sepsis, both in terms of cost and loss of quality of life, indeed presents a challenge. Therefore, the Eisai group started the study for developing the anti-sepsis drug. As a result, Eisai has developed a drug candidate (E5564,[4] generic name, eritoran) for treating severe sepsis and has successfully completed the Phase II trials of eritoran.

We were interested in RsDPLA-related compounds, in which the glucosamine is replaced with glucose analogues at the reducing end or at the non-reducing end or at both ends. Therefore, we synthesized some β-(1→6)-linked glucosamine–glucose disaccharides[5] and glucose–glucosamine disaccharides.[6] We also reported their activities toward both human whole blood and mice.[5,6] It was proven that these novel synthetic

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8581; e-mail: shioza@shina.sankyo.co.jp

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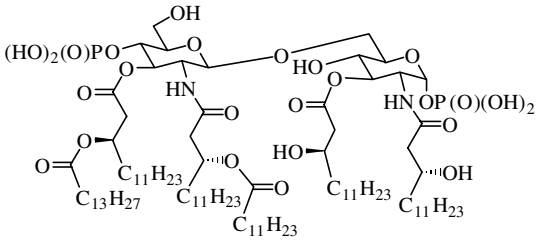
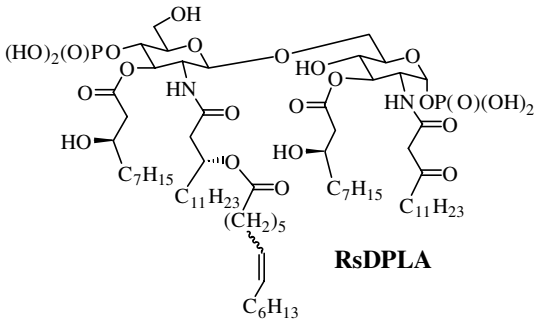
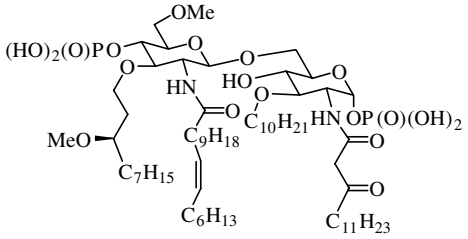


E. coli **Lipid A**



RsDPLA



E5564

Figure 1. Structures of *E. coli* Lipid A, RsDPLA, and E5564.

compounds had almost the same or stronger activities toward both human whole blood and murine macrophages than against classic glucosamine–glucosamine type disaccharides. This result aroused our interest about the biological activities of the glucose–glucose disaccharide derivatives of E5564, and we have reported the synthesis and the very strong LPS-antagonistic activity toward human whole blood concerning a glucose analogue[7] of E5564. In this paper, we describe five syntheses of other examples, **6**, **12**, **17b**, **19a**, and **19b**, and their LPS-antagonistic activities toward both human whole blood[8] by compounds **6**, **12**, **17b**, **19a**, and **19b** and galactosamine-loaded C3H/HeN mice[9] by compounds **12**, **17b**, **19a**, and **19b** (Fig. 1).

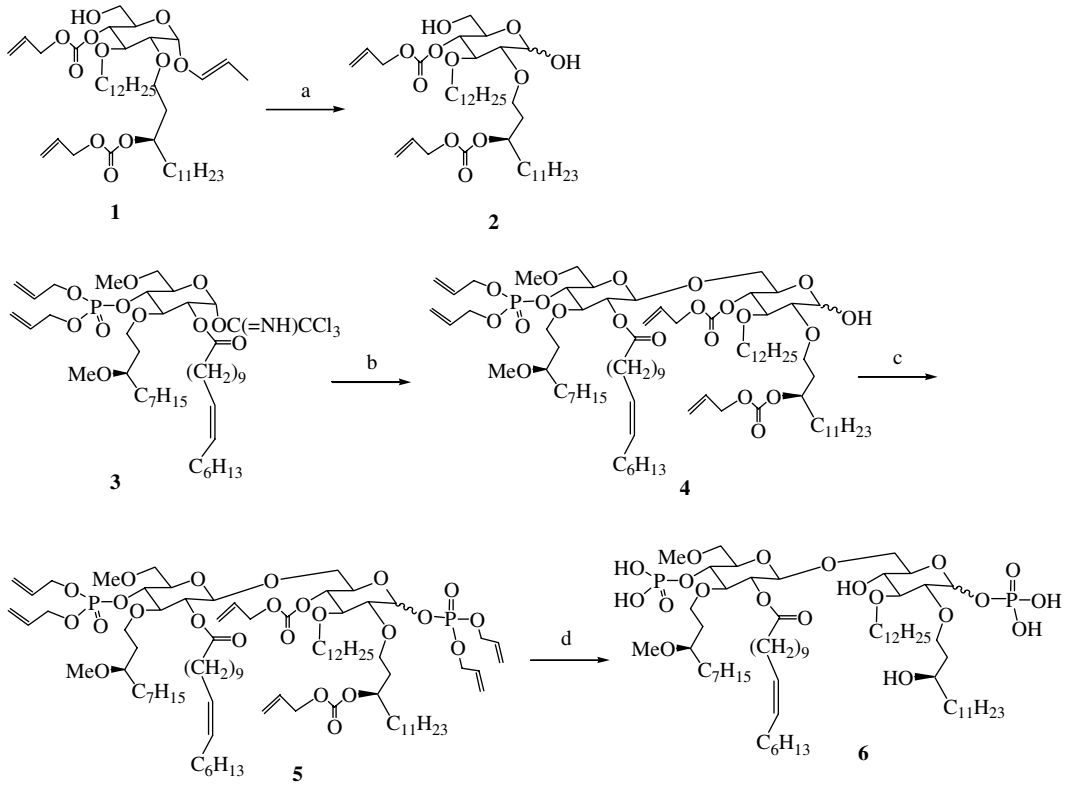
2. Results and discussion

2.1. Synthesis

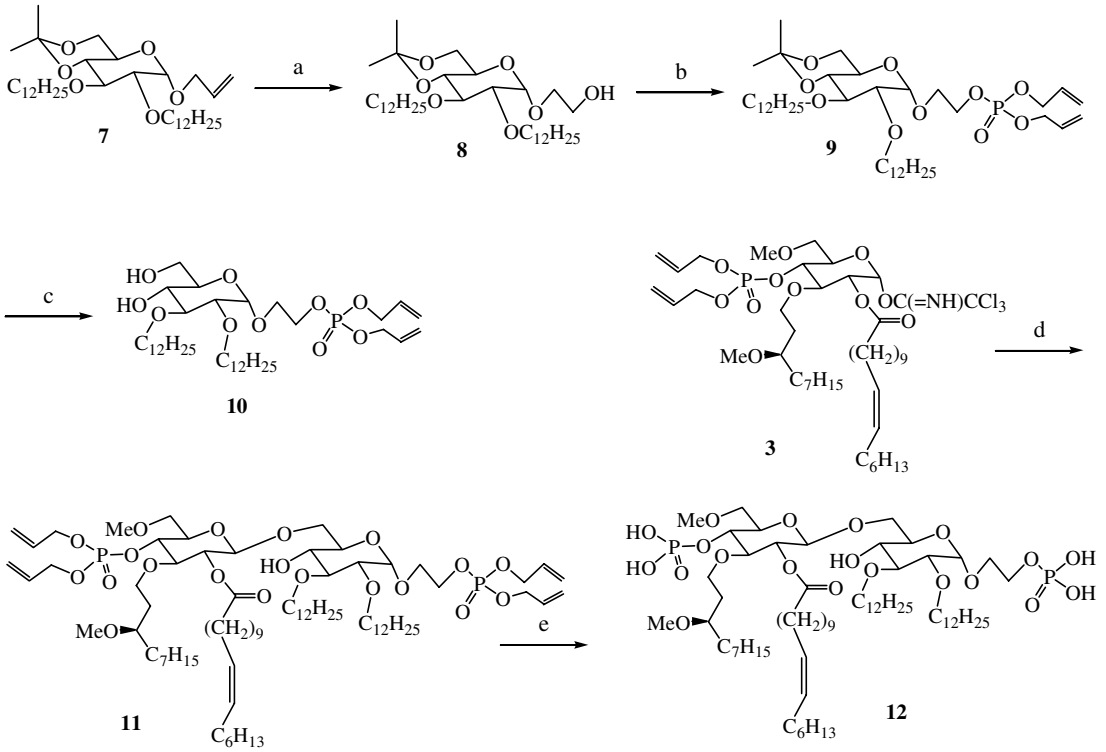
Firstly, we synthesized anomeric mixture **6** in three steps starting from the coupling of diol **2** obtained from anomeric vinyl compound **1**[5a] and imidate **3**.[6] Treatment of anomeric (*E*)-1-propenyl compound **1** with aq 48% HF in CH_3CN gave diol **2**. Reaction of **2** with trichloroacetimidate compound **3** using silver trifluoromethanesulfonate (AgOTf) as a catalyst afforded the β-(1→6) coupled compound **4**. This reaction proceeded without detection (by TLC and ^{1}H NMR) of α-(1→6)-linked disaccharide or orthoester via the anomeric oxonium ion by involvement of the C-2 acyl group. Reaction of **4** with diallyl diisopropylphosphoramidite in the presence of 1*H*-tetrazole, and successive oxidation of the resulting phosphite with hydrogen peroxide, afforded a 4:5 chromatographically inseparable mixture of α and β anomers **5**. Treatment of mixture **5** with tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd$], PPh_3, and HCO_2H–Et_3N gave a 1:1 chromatographically inseparable mixture of α and β anomers **6** (Scheme 1).

Secondly, we synthesized 2-(phosphonooxy)ethyl α-D-glucoside analogue **12** in three steps starting from the coupling of diol **10**, obtained from allyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucoside (**7**),[5a] and imidate **3** in two steps. Treatment of **7** with OsO_4 and $NaIO_4$ in THF–H_2O to cleave the allylic double bond, and successive reduction of the resulting aldehyde with $NaBH_4$ gave alcohol **8**. Phosphorylation of alcohol **8** with diallyl *N*,*N*-diisopropylphosphoramidite [*i*-$Pr_2NP(OCH_2CHCH_2)_2$] in CH_2Cl_2 using 1*H*-tetrazole as an acidic catalyst, and successive oxidation of the resulting phosphite with aq H_2O_2 yielded phosphate **9**. Deprotection of the isopropylidene group in **9** with *p*-TsOH in MeOH afforded diol **10**. Reaction of diol **10** with trichloroacetimidate compound **3** using AgOTf as a catalyst afforded the β-(1→6) coupled compound **11**. This reaction also proceeded without detection (by TLC and ^{1}H NMR) of the α-(1→6)-linked disaccharide or orthoester in the same manner mentioned in the formation of **4** from **2** and **3**. Treatment of **11** with tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd$], PPh_3, and HCO_2H–Et_3N gave **12** (Scheme 2).

Thirdly, tetraether compound **17b** was synthesized from trichloroacetimidate compound **13**[6] and alcohol **1**.[5a] Reaction of alcohol **1** with imidate **13** using AgOTf as a catalyst afforded both α-(1→6)- and β-(1→6)-linked disaccharides **14a** (37% yield) and **14b** (32% yield).

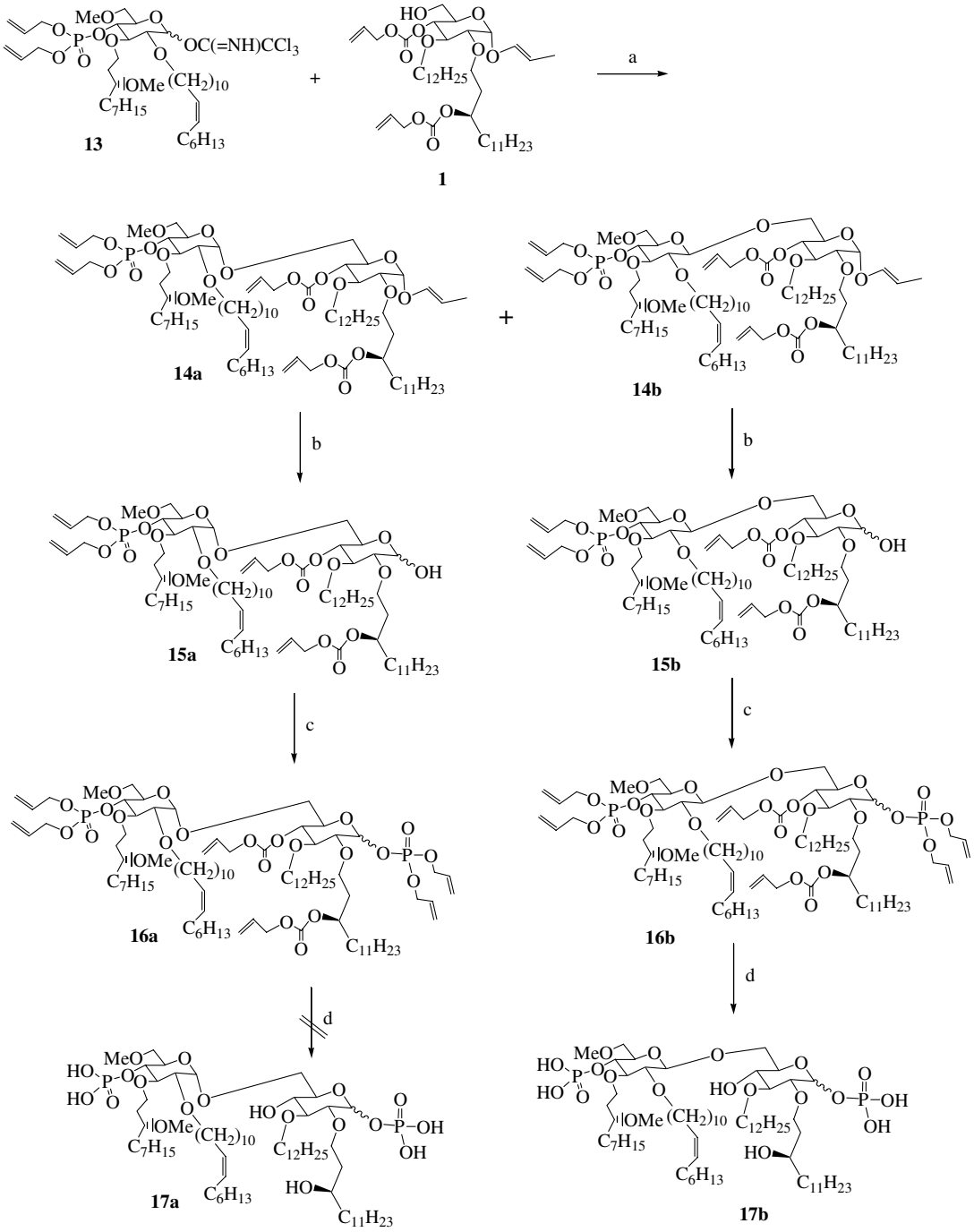


Scheme 1. Reagents and conditions: (a) aq 48% HF, MeCN, rt, 12 h, 72%; (b) **2**, AgOTf, 4 Å MS, CH_2Cl_2, rt, 20 min, N_2, 52%; (c) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, Na_2SO_4, CH_2Cl_2, rt, 30 min, then, aq 30% H_2O_2, THF, rt, 45 min, a 4:5 (α:β) inseparable anomeric mixture, two steps, 90%; (d) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 36 °C, 16 h, a 1:1 (α:β) inseparable anomeric mixture, 75%.



Scheme 2. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF–H_2O (3:1), *n*-BuOH, rt, 1.5 h, then $NaBH_4$, EtOH, rt, 10 min, 69%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 20 min, then H_2O_2, CH_2Cl_2–THF, rt, 20 min, 87%; (c) *p*-TsOH, MeOH, rt, 30 min, 96%; (d) **10**, AgOTf, TMSOTf, 4 Å MS, CH_2Cl_2, rt, 16 h, 52%; (e) $Pd(PPh_3)_4$, Ph_3P, Et_3N, HCOOH, N_2, THF, 55 °C, 16 h, 65%.

It was anticipated that the ether C-2 substituent in **13** does not affect the coupling stereochemistry. In fact, this coupling reaction showed no selectivity. Treatment of **14a** and **14b** with iodine in THF–H_2O yielded glucose analogues, **15a** and **15b**, respectively. Treatment of **15a** and **15b** according to the same procedure for the synthesis of **5** from **4** gave a 4:5 mixture of α and β anomers **16a** and a 1:2 mixture of α and β anomers **16b**, respectively. Neither anomeric mixture could be separated chromatographically. Deprotection of the allyl groups of **16b** with tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd$], PPh_3, and HCO_2H–Et_3N gave a 1:2 mixture of α and β anomers **17b**. However, the same treatment of **16a** gave many unknown degradation



Scheme 3. Reagents and conditions: (a) AgOTf, 4 Å MS, CH_2Cl_2, rt, 1 h, 37% (**14a**) and 32% (**14b**); (b) I_2, H_2O, THF, 54% (**15a**) or 69% (**15b**); (c) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, Na_2SO_4, CH_2Cl_2, rt, 30 min, then aq 30% H_2O_2, THF, rt, 45 min, a 4:5 (α:β) inseparable anomeric mixture, 84% (**16a**) or a 1:2 (α:β) inseparable anomeric mixture, 86% (**16b**); (d) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 36 °C, 16 h, decomposed (**17a**) or a 1:2 (α:β) inseparable anomeric mixture, 73% (**17b**).

products without yielding **17a**. This unexplainable instability of **17a** led to the same result as in allyl deprotection reaction of the α-(1→6)-linked disaccharides reported previously[6] (Scheme 3).

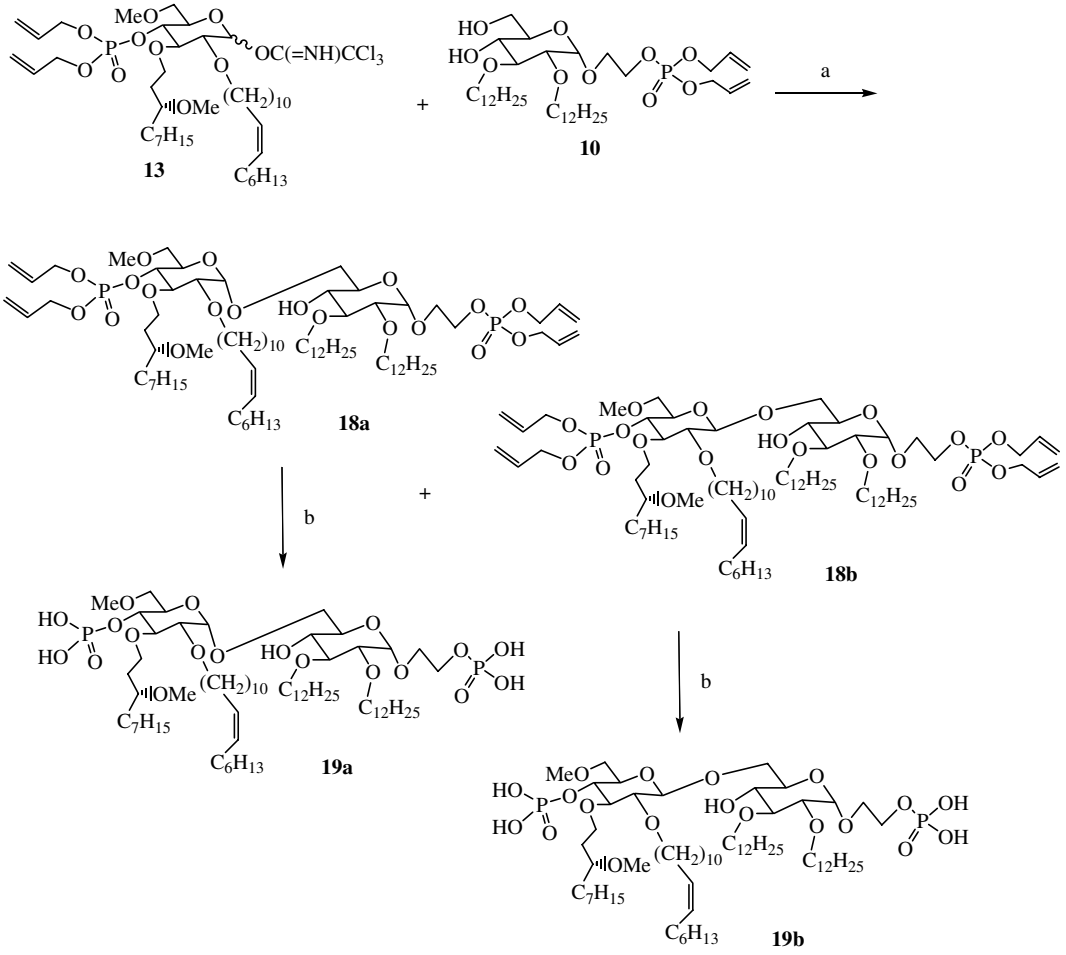
Finally, both compounds **19a** and **19b** were synthesized from diol **10** and imidate **13**. Coupling reaction of **10** with **13** using AgOTf as a catalyst afforded both α-(1→6)- and β-(1→6)-linked disaccharides **18a** (14% yield) and **18b** (22% yield). Deprotection of the allyl groups of **18a** and **18b** with tetrakis(triphenylphosphine)palladium(0) [$(PPh_3)_4Pd$], PPh_3, and HCO_2H–Et_3N gave **19a** and **19b**, respectively. In this case, α-(1→6)-linked disaccharide **19a** was obtained in 84% yield (Scheme 4).

Thus, we could synthesize five disaccharides **6**, **12**, **17b**, **19a**, and **19b**.

2.2. Biological activity

The inhibitory activity on LPS-induced TNFα production, LPS-antagonistic activity, of synthetic five compounds, **6**, **12**, **17b**, **19a**, and **19b**, was investigated in vitro using human whole blood by comparison with E5564. The IC_{50} values (nM) of the compounds (**6**, **12**, **17b**, **19a**, and **19b**) and E5564 toward human whole blood were 72.8, 3.0, 0.9, 7.5, 1.4, and 0.97–2.94 nM, respectively. In our previous study, the β anomer had almost the same activity as the α anomer.[5a] The activity of a 1:2 mixture of α and β anomers **17b** was the same or stronger than that of E5564. Therefore, in this case the β anomer in **17b** should be active enough. The difference between the ester group in the C-2′ position of **6** and the ether group in the C-2′ position of **17b** affected the activity largely despite almost the same activity between compounds **12** and **19a** or **19b**. It is interesting to note that the α-(1→6)-linked disaccharide **19a** still has activity toward human whole blood.

Inhibitory doses (ID_{50}) of compounds **12**, **17b**, **19a**, and **19b** on TNFα production induced by co-injection of galactosamine and LPS in C3H/HeN mice were measured by comparison with E5564. The values of these four compounds and E5564 were 0.9, ND, 1.6, 0.9, and 3.3 mg/kg, respectively. Compounds **12** and **19b** were more potent than E5564. Compound **17b** showing



Scheme 4. Reagents and conditions: (a) AgOTf, 4 Å MS, CH_2Cl_2, rt, 1 h, 14% (**18a**) and 22% (**18b**); (b) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 36 °C, 16 h, 84% (**19a**) or 63% (**19b**).

strong activity toward human whole blood was not very strong toward mouse macrophages.

Usually, lipid A analogues having six fatty acids chains show LPS-agonistic (endotoxic) activity toward both human U-937 and mouse peritoneal resident macrophages, and lipid IVa[3a,10] having four fatty acid chains shows LPS-antagonistic activity toward human blood cells but adverse 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 both human and mouse blood cells. This tendency was the same activity as those for artificial E5564 and the nontoxic natural RsDPLA[3] having a cis-double bond in one of the fatty acid chains.

3. Conclusions

Thus, we could synthesize five glucose derivatives of E5564, compounds **6**, **12**, **17b**, **19a**, and **19b**. As a result, it was proved that these novel synthetic glucose–glucose disaccharides except for **6** were effective toward blocking the agonistic effects of LPD in the human whole blood assay and the TNFα production in C3H/HeN mice. These compounds except for **6** were still active as the classic glucosamine–glucosamine type disaccharides.[12] The activity of **17b** for human whole blood was about the same or stronger than that of E5564.

4. Experimental

4.1. General procedures

^{1}H NMR spectra were recorded with JEOL-GSX 400 and JNM-ECT 500 spectrometers using Me_4Si as the 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 carried out with Silica Gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (111–182 kPa) for easy elution. Commercially available anhydrous THF and CH_2Cl_2 were used for the reactions. DMF and pyridine were dried by storage over molecular sieves (4 Å MS).

4.1.1. 4-*O*-Allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-α,β-D-glucopyranose (2). To a solution of (*E*)-1-propenyl 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-α-D-glucopyranoside **1** (500 mg, 0.651 mmol) in MeCN (25 mL) was added 48% aq HF (1.46 mL). This mixture was stirred for 16 h at room temperature, diluted with CH_2Cl_2, which was washed with satd aq $NaHCO_3$ 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 2:1 hexane–EtOAc gave **2** (340 mg, 72%) as an oil: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.86 (6H, t, *J* 6.6 Hz), 1.25 (36H, br s), 1.49–1.65 (4H, m), 1.80–2.00 (2H, m), 2.39 (1H, m), 3.20 (1H, dd, *J* 2.9, 9.5 Hz), 3.60–4.00 (8H, m), 4.58–4.72 (5H, m), 4.80–4.90 (1H, m), 5.25–5.40 (4H, m), 5.88–6.00 (2H, m). FABMS (positive-ion): *m*/*z* 751 $[M+Na]^+$.

4.1.2. 4-*O*-Allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-D-glucopyranose (4). To a solution of 6-*O*-[2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-(octadecenoyl)oxy]-α-D-glucopyranosyl trichloroacetimidate (**3**, 666 mg, 0.700 mmol) and **2** (520 mg, 0.700 mmol) in CH_2Cl_2 (20 mL) was added 4 Å MS (400 mg) under nitrogen. After the mixture was stirred for 20 min at room temperature, AgOTf (20 mg, 0.070 mmol) was added to this mixture, which was stirred for 20 min at room temperature under nitrogen, and diluted with CH_2Cl_2. The solution was washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column. Elution with 2:1 cyclohexane–EtOAc gave **4** (520 mg, 52%) as a gum: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.88 (12H, t, *J* 6.6 Hz), 1.25 (66H, br s), 1.40–1.90 (10H, m), 1.90–1.96 (2H, m), 1.98–2.06 (4H, m), 2.30–2.43 (2H, m), 3.20–3.90 (22H, m, containing two 3H, s, at 3.26 and 3.39 ppm), 4.10–4.70 (12H, m), 4.81 (1H, m), 4.91 (1H, m), 5.20–5.40 (10H, m), 5.90–6.03 (4H, m). FABMS (positive-ion): *m*/*z* 1521 $[M+Na]^+$. HRFABMS, calcd for $C_{82}H_{147}NaO_{21}P$: 1522.007, found: 1522.0068.

4.1.3. Diallylphosphono 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl}-α,β-D-glucopyranoside (5). To a solution of **4** (430 mg, 0.287 mmol) in CH_2Cl_2 (60 mL) were added Na_2SO_4 (900 mg), 1*H*-tetrazole (84.3 mg, 1.20 mmol) and diallyl diisopropylphosphoramidite (105 mg, 0.430 mmol) under nitrogen at room temperature. After stirring for 30 min, the reaction suspension was charged on a silica gel short column, and eluted with 2:1 hexane–EtOAc to give an α,β-anomeric mixture of phosphites (500 mg), which was dissolved in THF (25 mL), and 30% aq H_2O_2 (500 mg, 4.412 mmol) was added to this solution. After stirring for 30 min at 0 °C, the reaction mixture was diluted with EtOAc, which was washed with 10% aq $Na_2S_2O_3$ and brine, dried over $MgSO_4$,

and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column. Elution with 3:2 hexane–EtOAc, then EtOAc, gave a 4:5 mixture of α and β anomers **5** (430 mg, 90%) as an oil. This anomeric mixture could not be chromatographically separated. 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.80–1.90 (12H, m), 1.26 (66H, br s), 1.40–1.72 (10H, m), 1.83–1.88 (2H, m), 1.98–2.03 (4H, m), 2.26–2.48 (2H, m), 3.18–3.85 (23H, m, containing two 3H, s at 3.24 and 3.36 ppm), 3.94–4.00 (1H, m), 4.25–4.29 (1H, m), 4.36 (4/9H, d, *J* 7.8 Hz, H-1′), 4.43 (5/9H, d, *J* 7.8 Hz, H-1′), 4.53–4.62 (14H, m), 4.74–4.78 (1H, m), 4.85–4.91 (1H, m), 5.01 (5/9H, dd, *J* 6.4, 7.2 Hz, H-1, β anomer), 5.17–5.39 (14H, m), 5.75 (4/9H, dd, *J* 3.2, 7.0 Hz, H-1, α anomer), 5.86–5.98 (6H, m). FABMS (positive-ion): *m*/*z* 1681 $[M+Na]^+$.

4.1.4. Phosphono 3-*O*-dodecyl-6-*O*-[3-*O*-[(*R*)-3-(methoxy)-decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-4-*O*-phosphono-β-D-glucopyranosyl]-2-*O*-[(*R*)-3-(hydroxy)tetradecyl]-α,β-D-glucopyranoside (6). To a solution of anomeric mixture **5** (180 mg, 0.090 mmol) in dry THF (10 mL) were added PPh_3 (30 mg, 0.114 mmol), Et_3N (111 mg, 1.10 mmol), HCO_2H (93 mg, 2.02 mmol), and $Pd(PPh_3)_4$ (30 mg, 0.026 mmol) in this sequence. The solution was stirred for 16 h at 36 °C under nitrogen and concentrated in vacuo to give a mixture that was chromatographed on a DEAE-cellulose (Whatman Ion-Exchange Cellulose, wet 8 g) column. The column was prepared by preliminary consecutive washing with 80 mL each of 0.5 M HCl, H_2O, 0.5 M NaOH, and H_2O, 32 mL each of 1 M AcOH and H_2O, 80 mL of aq 0.05 M AcO·NH_4, and 60 mL each of 2:3:1 $CHCl_3$–MeOH–H_2O and finally 2:1 $CHCl_3$–MeOH. The column was eluted with 5 mL each of 2:1 $CHCl_3$–MeOH, then 0.05 M AcO·NH_4 in 2:3:1 $CHCl_3$–MeOH–H_2O. Six fractions were collected. To this solution were added additional $CHCl_3$ (5 mL) and 0.15 M aq HCl (10 mL), and the mixture was shaken well. The lower $CHCl_3$ layer was separated and concentrated in vacuo to give a 1:1 anomeric mixture **6** (90 mg, 75%) as a wax that could not be separated: IR ν_{max} (KBr): 3506 (w), 3345 (w), 2923, 2853, 1720, 1465, 1466 cm^{-1}; 400 MHz ^{1}H NMR ($CDCl_3$–CD_3OD, 5:1): δ 0.88 (12H, t, *J* 6.7 Hz, fatty acid terminal $CH_3 \times 4$), 1.26 (66H, br s), 1.38–1.76 (12H, m), 1.99–2.04 (4H, m, C2′ side chain C10–H_2 and C13–H_2), 2.30–2.39 (2H, m, C-2′ side chain C2–H_2), 3.11–4.02 (24H, m, C2–H, C2 side chain C1–H_2, C2 side chain C3–H, C3–H, C3 side chain C1–H_2, C4–H, C5-H, C6–H_2, C3′–H, C37 side chain C1–H_2, C3′ side chain C3–H, C5′–H, C6′–H_2, containing two 3H, s at 3.30 and 3.41 ppm, C6′–OCH_3 and C3′ side chain C3–OCH_3), 4.20 (1H, q, *J* 10.2 Hz, C4′–H), 4.51 (0.5H, d, *J* 7.3 Hz, C1′ anomeric H), 4.63 (0.5H, d, *J* 7.3 Hz, C1′ anomeric H), 4.87 (0.5H, dd, *J* 7.5, 8.0 Hz, C2′–H), 4.89 (0.5H, dd, *J* 7.5, 8.0 Hz, C2′–H), 4.98 (0.5H, dd, *J* 7.5, 8.0 Hz, C1–H, β anomer), 5.33–5.36 (2H, m, C2′ side chain olefinic C11–H and C12–H), 5.74 (0.5H, dd, *J* 3.3, 6.6 Hz, C1–H, α anomer). FABMS (negative-ion): *m*/*z* 1329 $[M-H]^-$, 1322. Anal. Calcd for $C_{68}H_{132}O_{20}P_2 \cdot 3H_2O$: C, 58.99; H, 10.00; P, 4.23. Found: C, 58.94; H, 10.04; P, 4.47.

4.1.5. 2-Hydroxyethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (8). To a solution of **7** (2.39 g, 4.00 mmol) and $NaIO_4$ (4.00 g) in EtOH (30 mL)–H_2O (10 mL) was added OsO_4 (2.5% solution in *t*-BuOH, 80 mg). After stirring for 2 h at room temperature, the reaction mixture was diluted with EtOAc, which was washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give the aldehyde, which was dissolved in 99% EtOH (50 mL), and $NaBH_4$ (200 mg) was added. The mixture was stirred for 10 min at room temperature and quenched with AcOH. The reaction mixture was diluted with EtOAc, which was washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue that was chromatographed on a silica gel column, eluting with 2:1 cyclohexane–EtOAc to give **8** (1.66 g, 69% yield): IR ν_{max} (film) 3452 (br), 2926, 2855, 1466, 1380, 1370 cm^{-1}; 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.88 (6H, t, *J* 6.6 Hz), 1.26 (36H, br s), 1.40 (3H, s), 1.48 (3H, s), 1.51–1.57 (4H, m), 2.82 (1H, br s, OH), 3.30 (1H, m), 3.51–3.85 (13H, m), 4.89 (1H, d, *J* 3.7 Hz, C1 anomeric H). FABMS (positive-ion): *m*/*z* 601 $[M+H]^+$, 623 $[M+Na]^+$.

4.1.6. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (9). To a solution of **8** (1.47 g, 2.45 mmol) in CH_2Cl_2 (30 mL) were added Na_2SO_4 (1.5 g), 1*H*-tetrazole (400 mg, 5.71 mmol) and diallyl diisopropylphosphoramidite (1.20 g, 4.89 mmol). After stirring for 20 min at room temperature, the reaction mixture was diluted with THF (30 mL). To this mixture, 31% H_2O_2 (0.5 g) was added. After stirring for 20 min at room temperature, the reaction mixture was diluted with EtOAc, which was washed with aq 10% $Na_2S_2O_3$, satd aq $NaHCO_3$, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column, eluting with 2:1 cyclohexane–EtOAc to give **9** (1.62 g, 87%) as an oil: IR ν_{max} (film) 2925, 2854, 1466 cm^{-1}; 400 MHz ^{1}H NMR ($CDCl_3$): 0.88 (6H, t, *J* 6.6 Hz), 1.26 (36H, br s), 1.39 (3H, s). 1.47 (3H, s), 1.54–1.59 (4H, m), 3.29 (1H, dd, *J* 3.7, 8.8 Hz), 3.50–3.88 (11H, m), 4.23–4.27 (2H, m), 4.50–4.59 (4H, m), 4.90 (1H, d, *J* 3.7 Hz, C1 anomeric H), 5.25–5.42 (4H, m), 5.90–6.00 (2H, m). FABMS (positive-ion): *m*/*z* 761 $[M+H]^+$, 783 $[M+Na]^+$.

4.1.7. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (10). To a solution of **9** (6.60 g, 9.10 mmol) in MeOH (60 mL) was added *p*-TsOH (412 mg). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column, eluting with EtOAc and then 10% MeOH in EtOAc to give **10** (6.01 g, 96%) as a wax: IR ν_{max} (film) 3410 (br), 2926, 2855, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$): δ 0.88 (6H, t, *J* 6.6 Hz), 1.26 (36H, br s), 1.52–1.60 (4H, m), 3.27 (1H, dd, *J* 3.7, 8.8 Hz), 3.41–3.91 (11H, m), 4.24–4.28 (2H, m), 4.48–4.59 (4H, m), 4.95 (1H, d, *J* 3.7 Hz, C1 anomeric H), 5.19–5.40 (4H, m), 5.90–6.00 (2H, m). FABMS (positive-ion): *m/z* 721 $[M+H]^+$, 753 $[M+Na]^+$.

4.1.8. 2-[(Diallylphosphono)oxy]ethyl 6-*O*-[4-*O*-(diallylphosphono)-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-β-D-glucopyranosyl]-2,3-di-*O*-dodecyl-α-D-glucopyranoside (11). To a solution of imidate **3** (260 mg, 0.279 mmol) and diol **10** (197 mg, 0.279 mmol) in CH_2Cl_2 (10 mL) was added 4 Å MS (500 mg) under nitrogen. After the mixture was stirred for 30 min at room temperature, AgOTf (180 mg, 0.701 mmol) and TMSOTf (20 mg, 0.090 mmol) were added to this mixture, which was stirred for 16 h at room temperature under nitrogen, and then diluted with CH_2Cl_2. The solution was washed with satd aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column. Elution with 1:2 cyclohexane–EtOAc to give **11** (215 mg, 52%) as a gum: IR ν_{max} (film) 3412 (br), 2926, 2855, 1750, 1652 (w), 1465 cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$): δ 0.88 (12H, t, *J* 6.6 Hz), 1.10–1.43 (70H, m), 1.55–1.70 (6H, m), 1.99–2.02 (4H, m), 2.29–2.36 (2H, m), 2.82 (1H, br s, OH), 3.22 (1H, m), 3.25 (3H, s), 3.39 (3H, s), 3.47–3.84 (18H, m), 3.96 (1H, dd, *J* 1.0, 8.6 Hz), 4.19–4.25 (2H, m), 4.32 (1H, q, *J* 9.4 Hz, C4′–H), 4.47 (1H, d, *J* 7.8 Hz, C1′–H), 4.54–4.59 (8H, m), 4.89 (1H, d, *J* 3.5 Hz, C1 anomeric H), 4.97 (1H, t, *J* 8.6 Hz, C2′–H), 5.24–5.40 (10H, m), 5.89–5.99 (4H, m). FABMS (positive-ion): *m/z* 1513 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{148}O_{20}P_2Na$: 1513.9937, found: 1513.9934.

4.1.9. 2-[(Phosphono)oxy]ethyl 6-*O*-[3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenoyl]-4-*O*-(phosphono)-β-D-glucopyranosyl]-2,3-di-*O*-dodecyl-α-D-glucopyranoside (12). To a solution of **11** (140 mg, 0.094 mmol) in dry THF (8 mL) were added PPh_3 (16 mg, 0.061 mmol), Et_3N (65 mg, 0.642 mmol), HCO_2H (54 mg, 0.843 mmol), and $Pd(PPh_3)_4$ (16 mg, 0.014 mmol) in this sequence. The solution was stirred for 16 h at 55 °C under nitrogen and concentrated in vacuo to give a mixture that was chromatographed on a DEAE-cellulose (Whatman Ion-Exchange Cellulose, wet 6 g) column. The column was prepared by preliminary consecutive washing with 60 mL each of 0.5 M aq HCl, H_2O, 0.5 M aq NaOH, H_2O, 24 mL of 1 M aq AcOH, 60 mL each of H_2O, 0.05 M aq $AcONH_4$, 2:3:1 $CHCl_3$–MeOH–H_2O, and finally 2:1 $CHCl_3$–MeOH. The column was eluted with 5 mL each of 2:1 $CHCl_3$–MeOH, then 0.05 M aq $AcONH_4$ in 2:3:1 $CHCl_3$–MeOH–H_2O. Six fractions containing **12** were collected. To this solution were added further $CHCl_3$ (5 mL) and 0.15 M aq HCl (10 mL), and the mixture was shaken well to adjust to pH 2–3. The lower $CHCl_3$ layer was separated and concentrated in vacuo to give **12** (81 mg, 65%) as a wax: IR ν_{max} (KBr): 3500–2500 (br), 2924, 2853, 1726, 1467 cm^{-1}; 400 MHz ^1H NMR ($CDCl_3$+CD_3OD, 5:1): δ 0.86–0.90 (12H, m, fatty acid terminal $CH_3 \times 4$), 1.26 (66H, br s), 1.40–1.44 (2H, m), 1.54–1.62 (6H, m), 1.71–1.75 (2H, m), 2.00–2.04 (4H, m, C2′ side chain C10–H_2 and C13–H_2), 2.32–2.38 (2H, m, C2′ side chain C2–H_2), 3.24–3.82 (24H, m, C1 side chain C1–H_2, C2 side chain C1–H_2, C3–H, C3 side chain C1–H_2, C4–H, C5–H, C6–H_2, C3′–H, C3′ side chain C1–H_2, C3′ side chain C3–H, C5′–H, C6′–H_2, containing two 3H, s at 3.30 and 3.41 ppm, C6′–OCH_3 and C3′ side chain C3–OCH_3), 4.02 (1H, dd, *J* 2.0, 10.2 Hz, C2–H), 4.16–4.21 (3H, m, C1 side chain C2–H_2 and C4′–H), 4.49 (1H, d, *J* 7.8 Hz, C1′–H, anomeric H), 4.87 (1H, d, *J* 2.0 Hz, C1–H, anomeric H), 4.91 (1H, t, *J* 8.4 Hz, C2′–H), 5.33–5.37 (2H, m, C2′ side chain olefinic C11–H and C12–H). FABMS (negative-ion): *m/z* 1329 $[M-H]^-$, 1351 $[M-2H+Na]^-$. Anal. Calcd for $C_{68}H_{132}O_{20}P_2 \cdot 0.4H_2O$: C, 61.00; H, 10.00; P, 4.63. Found: C, 61.08; H, 9.65; P, 4.56.

4.1.10. (*E*)-1-Propenyl 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl}-α-D-glucopyranoside (14a) and (*E*)-1-propenyl 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-α-D-glucopyranoside (14b). To a solution of 4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]}-α,β-D-glucopyranosyl trichloromethylimidate **13** (760 mg, 0.840 mmol) and alcohol **1** (645 mg, 0.840 mmol) in CH_2Cl_2 (50 mL) was added 4 Å MS (400 mg) under nitrogen. After the mixture was stirred for 20 min at room temperature, AgOTf (21 mg, 0.084 mmol) was added, and the mixture was stirred for 1 h at room temperature under nitrogen and then diluted with CH_2Cl_2. The solution was washed with satd aq $NaHCO_3$, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column, eluting with 4:1 hexane–EtOAc to give **14b** (400 mg, 32%) and **14a** (460 mg, 37%) as gums. Physical

data for **14b**: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.88 (12H, t, J 6.6 Hz), 1.25 (66H, br s), 1.40–1.80 (13H, m, containing 3H, dd, J 1.5, 6.6 Hz at 1.55 ppm), 1.82–1.89 (2H, m),1.98–2.02 (4H, m) 3.1–3.2 (2H, m), 3.22–3.79 (20H, m, containing two 3H, s, at 3.28 and 3.37 ppm), 3.80–4.01 (4H, m), 4.19–4.30 (2H, m), 4.53–4.87 (12H, m), 5.07 (1H, d, J 3.2 Hz, C1–H), 5.13–5.40 (11H, m), 5.88–5.97 (4H, m), 6.13 (1H, qd, J 1.5, 12.5 Hz, C1 side chain olefinic C1–H). FABMS (positive-ion): m/z 1547 $[M+Na]^+$. HRFABMS, calcd for $C_{85}H_{153}O_{20}PNa$: 1548.0591, found: 1548.0624. Physical data for **14a**: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.88 (12H, t, J 6.6 Hz), 1.25 (66H, br s), 1.40–1.80 (13H, m, containing 3H, dd, J 1.5, 6.6 Hz at 1.55 ppm), 1.82–2.02 (6H, m), 3.22–4.00 (22H, m, containing two 3H, s, at 3.28 and 3.37 ppm), 4.23 (1H, m), 4.53–4.80 (11H, m), 4.86 (1H, d, J 3.5 Hz, C1′–H, α anomer), 5.06 (1H, d, J 3.5 Hz, C1–H), 5.15–5.40 (11H, m), 5.89–5.98 (4H, m), 6.13 (1H, qd, J 1.5, 12.5 Hz, C1 side chain olefinic C1–H). FABMS (positive-ion): m/z 1547 $[M+Na]^+$. HRFABMS, calcd for $C_{85}H_{153}O_{20}PNa$: 1548.0591, found: 1548.0582.

4.1.11. 4-*O*-Allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-α,β-D-glucopyranose (15b). To a solution of **14b** (370 mg, 0.242 mmol) in THF (20 mL) and H_2O (0.5 mL) was added iodine (92.5 mg, 0.365 mmol). The mixture was stirred for 4 h at room temperature, diluted with EtOAc, and then washed with aq $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column, eluting with 3:1 hexane–EtOAc to give **15b** (250 mg, 69%) as a gum: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.86–0.89 (12H, m), 1.25 (66H, br s), 1.40–2.02 (17H, m, containing OH), 3.10–3.90 (20H, m, containing two 3H, s, at 3.17 and 3.37 ppm), 4.0–4.70 (14H, m), 4.81 (1H, m), 5.18–5.42 (11H, m), 5.89–6.00 (4H, m), FABMS (positive-ion): m/z 1507 $[M+Na]^+$. HRFABMS, calcd for $C_{82}H_{149}O_{20}PNa$: 1508.0277, found: 1508.0294.

4.1.12. 4-*O*-Allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl}-D-glucopyranose (15a). Compound **14a** (460 mg, 0.301 mmol) was treated as described for the formation of **15b** from **14b** to give **15a** (250 mg, 54%) as a gum: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.86–0.89 (12H, m), 1.25 (66H, br s), 1.40–1.90 (13H, m, containing OH), 1.92–2.02 (4H, m), 3.10–3.90 (20H, m, containing two 3H, s, at 3.17 and 3.37 ppm), 4.10–4.70 (13H, m), 4.81 (1H, m), 4.87 (1H, d, J 3.1 Hz, C1′–H, α anomer), 5.23–5.40 (10H, m), 5.89–6.00 (4H, m). FABMS (positive-ion): m/z 1507 $[M+Na]^+$. HRFABMS, calcd for $C_{82}H_{149}O_{20}PNa$: 1508.0277, found: 1508.0259.

4.1.13. Diallylphosphono 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-{4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-β-D-glucopyranosyl}-α,β-D-glucopyranoside (16b). To a solution of **15b** (250 mg, 0.168 mmol) in CH_2Cl_2 (30 mL) were added Na_2SO_4 (540 mg), 1*H*-tetrazole (51 mg, 0.700 mmol), and diallyl diisopropylphosphoramidite (64 mg, 0.250 mmol) under nitrogen at room temperature. After stirring for 30 min, the reaction suspension was charged onto a short silica gel column, eluting with 2:1 hexane–EtOAc to give the phosphite (268 mg), which was employed for the next reaction without further purification. The above-obtained phosphite was dissolved in THF (25 mL), and 30% aq H_2O_2 (500 mg) was added to this solution. After stirring for 30 min at 0 °C, the reaction mixture was diluted with EtOAc, which was washed with 10% aq $Na_2S_2O_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column, eluting with 2:1 hexane–EtOAc, then EtOAc to give a 1:2 mixture of α and β anomers **16b** (240 mg, 86%;) as an oil that could not be separated: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.80–1.90 (12H, m), 1.26 (66H, br s), 1.40–1.70 (10H, m), 1.70–1.80 (2H, m), 1.80–1.90 (2H, m), 1.90–2.05 (4H, m), 3.10 (1H, m), 3.27 (3H, s), 3.33 (1H, m), 3.38 (3H, m), 3.40–3.90 (14H, m), 4.18 (1H, m), 4.28 (1H, d, J 7.8 Hz, C1′–H, anomeric H), 4.40–4.80 (14H, m), 5.06 (2/3H, t, J 7.4 Hz, C1–H, β anomer), 5.19–5.40 (14H, m), 5.86–5.98 (6H, m), 5.81 (1/3H, dd, J 3.1, 7.4 Hz, C1–H, α anomer). FABMS (positive-ion): m/z 1667 $[M+Na]^+$. HRFABMS, calcd for $C_{88}H_{158}NaO_{23}P_2$: 1668.0567, found: 1668.0590.

4.1.14. Diallylphosphono 4-*O*-allyloxycarbonyl-2-*O*-[(*R*)-3-(allyloxycarbonyloxy)tetradecyl]-3-*O*-dodecyl-6-*O*-[4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-α-D-glucopyranosyl]-α,β-D-glucopyranoside (16a). Compound **15a** was treated as described for the formation of **16b** from **15b** to give a 4:5 mixture of α and β anomers **16a** (84%;) as an oil that could not be separated by chromatography: 400 MHz ^{1}H NMR ($CDCl_3$): δ 0.88 (12H, t, J 6.9 Hz), 1.26 (66H, br s), 1.39–2.02 (16H, m), 3.23 (1H, m), 3.27 (3H, s), 3.38 (3H, m), 3.48–3.90 (14H, m), 4.08 (1H, m), 4.25 (1H, dd, J 9.5, 18.3 Hz), 4.40–4.61 (13H, m), 4.79 (1H, m), 4.85 (1H, d, J 3.1 Hz, C1′–H), 5.06 (5/9H, t, J 7.4 Hz, C1–H, β anomer), 5.20–5.41 (14H, m), 5.80 (4/9H, dd, J 3.1, 6.6 Hz, C1–H, α anomer), 5.86–5.98 (6H, m). FABMS (positive-ion): m/z 1667 $[M+Na]^+$.

4.1.15. Phosphono 3-*O*-dodecyl-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-phosphono-β-D-glucopyranosyl}-2-*O*-[(*R*)-3-hydroxytetradecyl]-α,β-D-glucopyranoside (17b). Compound **16b** (150 mg, 0.091 mmol) was treated as described in the formation of **6** from **5** to give a 1:2 mixture of α and β anomers **17b** (88 mg, 73%) as a wax that could not be separated by chromatography: IR ν_{max} (KBr): 3402 (w), 3130 (w), 2955, 2922, 2853, 1647 (w), 1466 cm^{-1}; 400 MHz ^1H NMR (9:1 $CDCl_3$–CD_3OD): δ 0.86–0.90 (12H, m, fatty acid terminal $CH_3 \times 4$), 1.27 (66H, br s), 1.40–1.90 (12H, m), 1.99–2.03 (4H, m, C2′ side chain C10–H_2 and C13–H_2), 3.10–4.12 (27H, m, C2–H, C2 side chain C1–H_2, C2 side chain C3–H, C3–H, C3 side chain C1–H_2, C4–H, C5–H, C6–H_2, C2′–H, C2′ side chain C1–H_2, C3′–H, C3′ side chain C1–H_2, C3′ side chain C3–H, C5′–H, C6′–H_2, containing two 3H, s at 3.30 and 3.40 ppm, C6′–OCH_3 and C3′ side chain C3–OCH_3), 4.43 (1/3H, d, *J* 7.4 Hz, C1′–H), 4.51 (2/3H, d, *J* 7.4 Hz, C1′–H), 5.00 (2/3H, *J* 8.2 Hz, C1–H, β anomer), 5.30–5.40 (2H, m, C2′ side chain olefinic C11–H and C12–H), 5.76 (1/3H, dd, *J* 3.1, 7.4 Hz, C1–H, α anomer). FABMS (negative-ion): *m*/*z* 1315 $[M-H]^-$. Anal. Calcd for $C_{68}H_{134}O_{19}P_2 \cdot 3H_2O$: C, 59.54; H, 10.29; P, 4.52. Found: C, 59.52; H, 9.93; P, 4.42.

4.1.16. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-6-*O*-{6-*O*-methyl-3-*O*-(3-methoxydecyl)-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-(diallylphosphono)-α-D-glucopyranocyl}-α-D-glucopyranoside (18a) and 2-(Diallylphosphonooxy)-ethyl 2,3-di-*O*-dodecyl-6-*O*-{6-*O*-methyl-3-*O*-(3-methoxydecyl)-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-(diallylphosphono)-β-D-glucopyranosyl}-α-D-pyranoside (18b). To a solution of trichloromethylimidate **13** (500 mg, 0.552 mmol) and **10** (380 mg, 0.552 mmol) in CH_2Cl_2 (20 mL) were added 4 Å MS (630 mg) and AgOTf (12.5 mg, 0.055 mmol). The mixture was stirred for 1 h at room temperature under nitrogen, and diluted with EtOAc, which was washed with aq $NaHCO_3$ and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo to give a mixture that was chromatographed on a silica gel column, eluting with 1:2 EtOAc–CH_2Cl_2 gave **18b** (180 mg, 22%) and **18a** (120 mg, 14%) as a gum. Physical data for **18b**: 400 MHz ^1H NMR ($CDCl_3$): δ 0.88 (12H, t, *J* 6.6 Hz), 1.26 (68H, br s), 1.40–1.79 (10H, m), 1.99–2.07 (4H, m), 2.60 (1H, br s, OH), 3.17–3.90 (26H, m, containing two 3H, s, at 3.28 and 3.39 ppm), 4.05 (1H, m), 4.20–4.30 (4H, m), 4.35 (1H, d, *J* 7.8 Hz, C1′–H), 4.53–4.60 (8H, m), 4.92 (1H, d, *J* 3.7 Hz, C1–H), 5.21–5.24 (4H, m), 5.30–5.40 (8H, m), 5.89–6.00 (4H, m). FABMS (positive-ion): *m*/*z* 1499 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{150}NaO_{19}P_2$: 1500.0144, found: 1500.0145. Physical data for **18a**: 400 MHz ^1H NMR ($CDCl_3$): δ 0.88 (12H, t, *J* 6.6 Hz), 1.26 (68H, br s), 1.40–1.79 (10H, m), 1.99–2.07 (4H, m) 3.17–3.90 (26H, m, containing two 3H, s, at 3.28 and 3.39 ppm), 3.99 (1H, m), 4.20–4.30 (4H, m), 4.53–4.58 (8H, m), 4.88 (1H, d, *J* 3.7 Hz, C1–H or C1′–H), 4.92 (1H, d, *J* 3.7 Hz, C1–H or C1′–H), 5.20–5.30 (4H, m), 5.89–6.00 (4H, m). FABMS (positive-ion): *m*/*z* 1499 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{150}NaO_{19}P_2$: 1500.0144, found: 1500.0121.

4.1.17. 2-(Phosphonooxy)ethyl 2,3-di-*O*-dodecyl-6-*O*-{6-*O*-methyl-3-*O*-(3-methoxydecyl)-2-*O*-[(*Z*)-11-octadecenyl]-4-*O*-phosphono-β-D-glucopyranocyl}-α-D-glucopyranoside (19b). Compound **18b** (180 mg, 0.120 mmol) was treated as described in the formation of **6** from **5** to give **19b** (100 mg, 63%) as a wax: IR ν_{max} (KBr): 2954, 2923, 2853, 1654 cm^{-1}; 400 MHz ^1H NMR (5:1 $CDCl_3$–CD_3OD): δ 0.88 (12H, m, fatty acid terminal $CH_3 \times 4$), 1.26 (68H, br s), 1.39–1.75 (10H, m), 1.75–1.90 (2H, m), 1.98–2.04 (4H, m, C2′ side chain C10–H_2 and C13–H_2), 3.13–4.25 (31H, m, C1 side chain C1–H_2, C1 side chain C2–H_2, C2–H, C2 side chain C1–H_2, C3–H, C3 side chain C1–H_2, C4–H, C5–H, C6–H_2, C2′–H, C2′ side chain C1–H_2, C3′–H, C3′ side chain C1–H_2, C3′ side chain C3–H, C4′–H, C5′–H, C6′–H_2, containing two 3H, s at 3.28 and 3.39 ppm, C6′–OCH_3 and C3′ side chain C3–OCH_3), 4.40 (1H, d, *J* 7.8 Hz, C1′–H), 4.94 (1H, d, *J* 3.7 Hz, C1–H), 5.35 (2H, t, *J* 5.7 Hz, C2′ chain olefinic C11–H and C12–H). FABMS (negative-ion): *m*/*z* 1315. Anal. Calcd for $C_{68}H_{134}O_{19}P_2 \cdot 5/2H_2O$: C, 60.01; H, 10.31; P, 4.33. Found: C. 59.93; H, 10.28; P, 4.55.

4.1.18. 2-(Phosphonooxy)ethyl 2,3-di-*O*-dodecyl-6-*O*-{(6-*O*-methyl)-3-*O*-(3-methoxydecyl)-2-*O*-[(*Z*)-octadec-11-enyl]-4-*O*-phosphono-α-D-glucopyranocyl}-α-D-glucopyranoside (19a). Compound **18a** (120 mg, 0.081 mmol) was treated as described in the formation **6** from **5** to give **19a** (90 mg, 84%) as a wax: IR ν_{max} (KBr): 3480, 2955, 2852, 1654 cm^{-1}; 400 MHz ^1H NMR (5:1 $CDCl_3$–CD_3OD): δ 0.88 (12H, t, *J* 6.6 Hz, fatty acid terminal $CH_3 \times 4$), 1.26 (68H, br s), 1.39–1.70 (10H, m), 1.75–1.90 (2H, m), 1.98–2.04 (4H, m, C2′ side chain C10–H_2 and C13–H_2), 3.25–4.11 (28H, m, C1 side chain C1–H_2, C2–H, C2 side chain C1–H_2, C3–H, C3 side chain C1–H_2, C4–H, C5–H, C6–H_2, C2′–H, C2′ side chain C1–H_2, C3′–H, C3′ side chain C1–H_2, C3′ side chain C3–H, C5′–H, C6′–H_2, containing two 3H, s at 3.28 and 3.39 ppm, C6′–OCH_3 and C3′ side chain C3–OCH_3), 4.15–4.24 (3H, m, C1 side chain C2–H_2, C4′–H), 4.90 (1H, d, *J* 3.7 Hz, C1–H or C1′–H), 4.99 (1H, d, *J* 3.7 Hz, C1–H or C1′–H) 5.35 (2H, t, *J* 5.5 Hz, C2′ side chain olefinic C11–H and C12–H). FABMAS (negative ion): *m*/*z* 1315. Anal. Calcd for $C_{68}H_{134}O_{19}P_2 \cdot H_2O$: C, 60.01; H, 10.31; P, 4.33. Found: C, 59.93; H, 10.28; P, 4.55.

4.2. Methods for measurement of biological activity

4.2.1. Production of TNFα by human whole blood[8]

4.2.1.1. Materials. Lipopolysaccharide (LPS, *Escherichia coli* 026:B6), human tumor necrosis factor alpha (TNFα) immunoassay kit and 96-well assay plates were purchased from Sigma Chemical Co., BioSource International, Inc. and Corning, Inc. (Cat. No. 3956), respectively.

4.2.1.2. 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 96-well assay 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 samples) of test compound (dissolved in 10% DMSO/PBS solution). For the negative control samples, the same amount of blood was cultured with PBS and 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.

4.2.1.3. Statistical analysis. The percentage inhibition of TNFα production was calculated by the following formula: [1 − (concentration 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 activity of each test compound was expressed as the 50% 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 inhibition using the SAS System for Windows. The results are expressed as the mean IC_{50} of triplicate experiments.

4.2.2. Production of TNFα in C3H/HeN mice co-injected with galactosamine and LPS[9]

4.2.2.1. Animals. Male C3H/HeN mice were purchased from Charles River Japan (Tokyo, Japan). All mice were used at the age of 7 weeks and housed at Sankyo Laboratories (Tokyo, Japan) with free access to standard rodent chow diet.

4.2.2.2. Reagents. Lipopolysaccharide (LPS, from *E. coli* O26:B6) and D-galactosamine (GalN) were purchased from Sigma Chemical Co. (St Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits of murine TNFα were from R&D Systems (Minneapolis, MN).

4.2.2.3. TNFα production. Naïve C3H/HeN mice (five per group) were intravenously injected with the test compound solution dissolved in 0.1% Et_3N–saline solution (0.2, 1.0, and 5.0 mg/10 mL/kg), and immediately after, mice were intravenously injected with a mixture of LPS (0.05 mg/10 mL saline/kg) and GalN (1 g/10 mL saline/kg). Mice were injected with vehicle (0.1% Et_3N–saline solution) and saline for negative control samples, and with vehicle and LPS/GalN for positive control samples. One hour after injection, venous blood was collected from the abdominal vein under ether anesthesia with heparinized syringes fitted with 23-gauge needles, and was centrifuged at 4 °C for 3 min at 13,230*g* to obtain the plasma. Plasma was stored at −30 °C before measuring TNFα levels by ELISA. The concentrations of TNFα of mouse plasma were measured using ELISA analysis according to the manufacturer's instructions.

4.2.2.4. Statistical analysis. The percentage inhibition of TNFα production was calculated by the following formula: [1 − (concentration 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 activity of each test compound is expressed as the 50% inhibitory dose (ID_{50}) of the test compound, the dose at which the test compound suppresses TNFα production by 50%. The ID_{50} was calculated from the percentage inhibition using the SAS System for Windows (v. 5).

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