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# Synthetic studies on glycosphingolipids from Protostomia phyla: syntheses and biological activities of amphoteric glycolipids containing a phosphocholine residue from the earthworm *Pheretima hilgendorfi*

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ABSTRACT

Two types of amphoteric glycosphingolipid found in the earthworm *Pheretima hilgendorfi*, PC(→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)Cer (**1**) and PC(→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→1)Cer (**2**), and their derivatives (**4, 5**) were synthesized. These were examined for their ability to enhance production of interleukin-8 (IL-8), a potent inflammatory cytokine involved in neutrophil chemotaxis, in a TNFα-stimulated granulocytic HL-60 cells. Compounds **1** and **2** were found to be potent enhancers of IL-8 production.



## 1. Introduction

Sialic acid-containing oligosaccharides are important constituents of gangliosides. Many carbohydrate scientists have taken an interest in the structure and functional role of mammalian glycosphingolipids, and they have been synthesized by various groups.[1] As part of our investigation of the relationship between the structure and biological function of glycolipids from invertebrate animal species that do not have gangliosides, we have synthesized glycolipids found in various protostomia phyla.[2] These compounds may function as alternative compounds to gangliosides. Sugita et al. reported[3] the neogala series of glycosphingolipids, whose structures contain a β-D-Gal*p*-(1→6)-β-D-Gal*p*- core and a mannose, glucose and a phosphocholine residue that are found in the earthworm *Pheretima* (*P.*) *hilgendorfi*, and completed a systematic diagram of the family of compounds. In our previous paper,[2f] we reported the synthesis of two phosphocholine (PC) glycolipid analogues containing octyl residues in place of ceramide, PC(→6)-β-D-Gal*p*-1→Oct and PC(→6)-β-D-Gal*p*-(1→6)-β-D-Gal*p*-1→Oct, in order to investigate the biological function of zwitterionic oligosaccharides. A number of researchers have reported the structural elucidation and immunomodulatory properties of zwitterionic glycosphingolipids from parasitic nematodes.[4] Subsequently, Harnett and co-workers examined the induced production of IL-12 and TNFα by macrophages using our synthetic PC-containing oligosaccharide compounds.[5]

In this study, we attempted total syntheses of glycosphingolipids **1** and **2** (a disaccharide- and a trisaccharide-containing PC, Fig. 1), in order to elucidate their biological functions as immunomodulatory substances in detail. In the course of these studies, **4** and **5** which were synthesized as a precursor of **2** and **1**, respectively, and **3**,[2f] as well as a commercial ceramide **6**, were used to establish the structure–activity relationships. The key reaction was coupling of the hydroxyl group at the C-6 position of the galactose moiety with phosphocholine. In our previous study,[2f] we used 2-chloro-2-oxo-1,3,2-dioxaphospholane and trimethylamine; however, this time we used a method employing a phosphorodiamidite compound.[6] We also attempted to carry out the phosphocholine coupling reaction using phosphoryl chloride and choline tosylate in order to develop a more economical method for this reaction.[7]

IL-8 is a cytokine that exerts chemotactic effects on neutrophils, T-cells, and basophils.[8] These cells play important roles in the host defense against microbial infections. IL-8 belongs to the C–X–C chemokine family and is produced by a variety of cells in response to stimulation with pro-inflammatory cytokines, such as IL-1 and TNFα.[9] In this study, we attempted to examine whether the newly synthesized glycosphingolipids and analogues have IL-8-inducing capacity using TNFα-stimulated granulocytic HL-60 cells.

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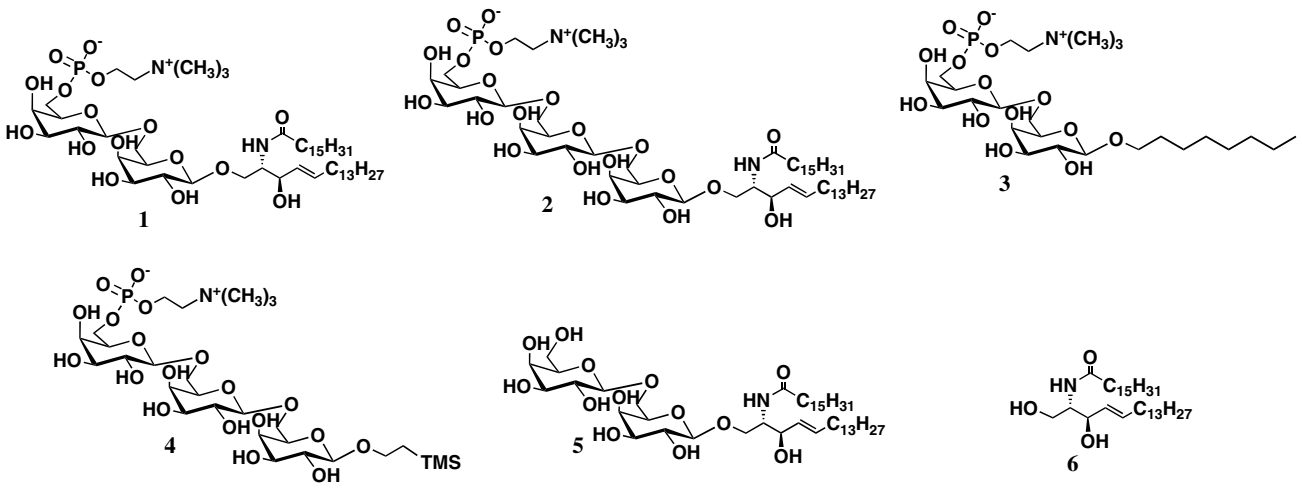
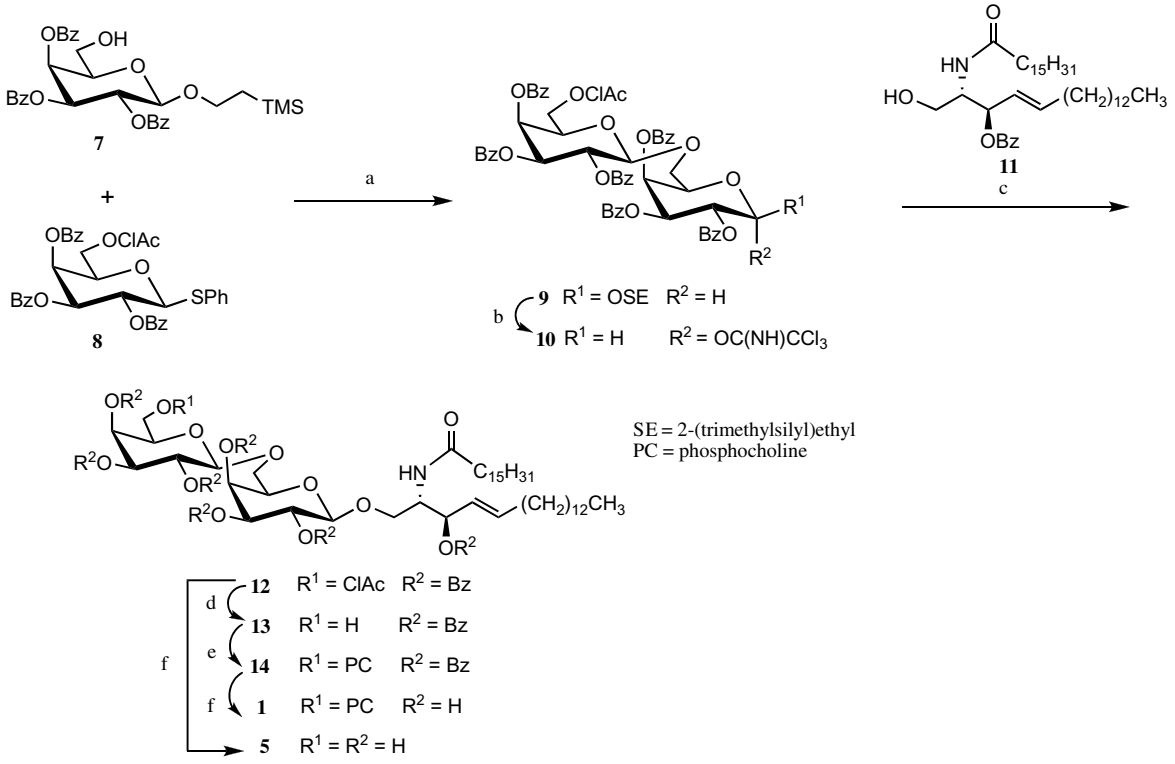


**Figure 1.** Target compounds.

## 2. Results and discussion

### 2.1. Syntheses of glycosphingolipids 1 and 2 and analogues 4 and 5

Syntheses of glycosphingolipid **1** and its precursor **5** were conducted as shown in Scheme 1. Glycosylation of **7**[2h] with **8**[10] in the presence of *N*-iodosuccinimide (NIS), trifluoromethanesulfonic acid (TfOH),[11] and 4 Å molecular sieves (4 Å MS) in $CH_2Cl_2$ at −60 °C to afforded the desired disaccharide **9** in 74% yield, as confirmed by $^1$H NMR spectroscopy (H-1′, $\delta$ 4.87 ppm, $J$ 7.9 Hz). Selective removal of the 2-(trimethylsilyl)ethyl group with trifluoroacetic acid in $CH_2Cl_2$, followed by reaction with trichloroacetonitrile in the presence of DBU,[12] gave the corresponding α-trichloroacetimidate **10**. Coupling of (2*S*,3*R*,4*E*)-3-*O*benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (**11**)[2c] with the glycosyl donor **10** was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) and 4 Å MS to afford the desired glycoconjugate **12** (62%). Next, selective removal of the chloroacetyl group in **12** with thiourea gave **13**. Treatment with 2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, followed by choline tosylate, gave a product that was oxidized in situ by



**Scheme 1.** Reagents: (a) NIS/TfOH, $CH_2Cl_2$, 4 Å MS, 74%; (b) (i) $CF_3CO_2H$, $CH_2Cl_2$; (ii) $CCl_3CN$, DBU, $CH_2Cl_2$, 97%; (c) TMSOTf, $CH_2Cl_2$, 4 Å MS, 62%; (d) thiourea, 5:3 EtOH–Pyr, 65%; (e) (i) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, $CH_2Cl_2$, 3 Å MS; (ii) 1*H*-tetrazole, choline tosylate; (iii) *m*-CPBA, MeOH; (iv) aq $NH_3$, 66%; (f) NaOMe, MeOH, **1**: 62%, **5**: 88%.

*m*-CPBA.[6a] After removal of the cyanoethyl group in aqueous ammonia and methanol, followed by chromatographic purification, 6-*O*-phosphocholine disaccharide **14** was obtained in 66% yield. Finally, removal of the acyl groups in **14** under Zemplén conditions, followed by column chromatography (Sephadex LH-20), furnished the target glycolipid **1**. Complete deprotection of a small amount of **12** was carried out to give PC-free glycolipid **5**. The structure and purity of **1** and **5** were demonstrated by $^1$H NMR spectroscopy and HRFABMS.

Glycosphingolipid **2** and analogue **4** were synthesized as follows: selective removal of the chloroacetyl group in **9** with thiourea gave disaccharide acceptor **15**, which was subjected to glycosylation by **8** in the presence of NIS/TfOH to afford the desired trisaccharide **16** in 60% yield, as confirmed by $^1$H NMR spectroscopy (H-1′, $\delta$ 4.82, $J$ 7.9 Hz). Compound **16** was also converted to the trichloroacetimidate derivative **19**, which was then coupled using **11** in the presence of TMSOTf to give the glycoconjugate **20** (60%). Compounds **16** and **20** were then easily converted into **2** and **4**, respectively, in a few steps as follows: (1) removal of chloroacetate with thiourea (**17** and **21**), (2) phosphorylation of the free hydroxy group (**18** and **22**) employing a phosphorodiamidite method, and (3) Zemplén O-deacylation (Scheme 2). The structures of **2** and **4** were elucidated by $^1$H NMR spectroscopy and HRFABMS.

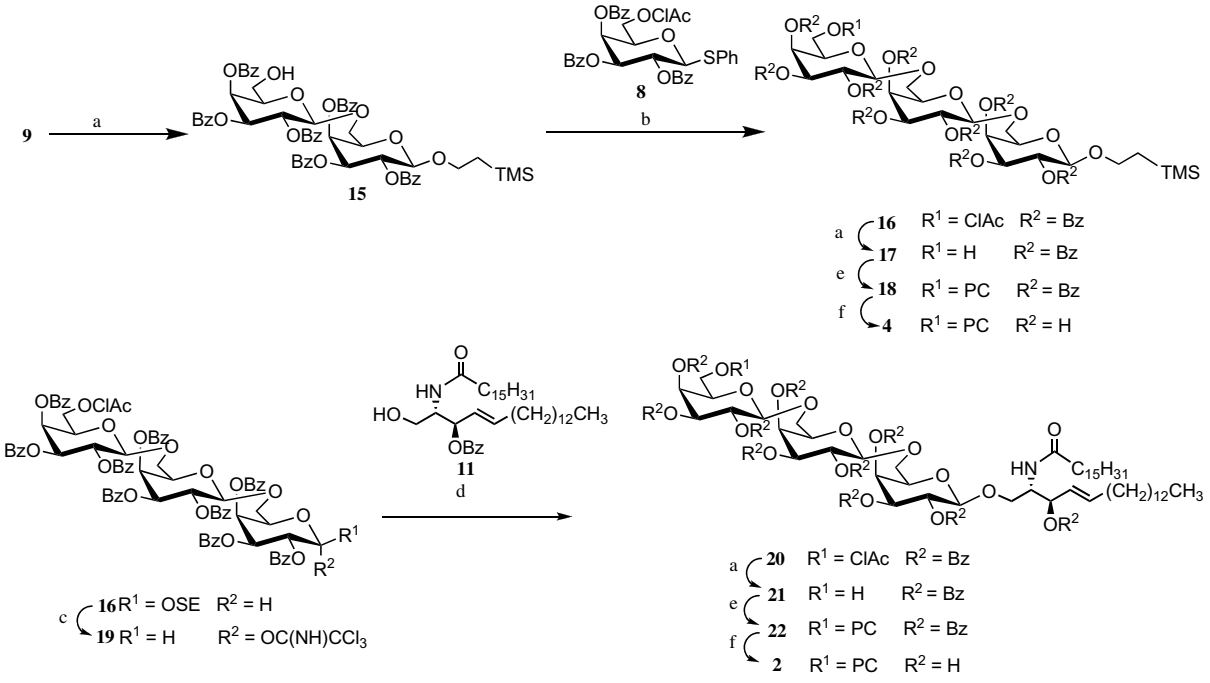
Finally, we attempted coupling with a phosphocholine group by an alternative method as follows: (1) glycosylceramide derivative **13** was esterified with phosphoryl chloride and (2) the resulting dichloroester was immediately converted to the phosphocholine derivative **14** using choline tosylate (79% yield, Scheme 3). The reagent used in this method (phosphoryl chloride) is not only inexpensive compared with the other reagent used (2-cyanoethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite), but also the former route represents a more convenient procedure for synthesis of phosphocholine glycolipids. This phosphorylation approach has not heretofore been used for a carbohydrate hydroxyl group. It is hoped that this method may be widely applied to coupling reactions of phosphocholines with carbohydrates in the future.

## 2.2. Biological activities

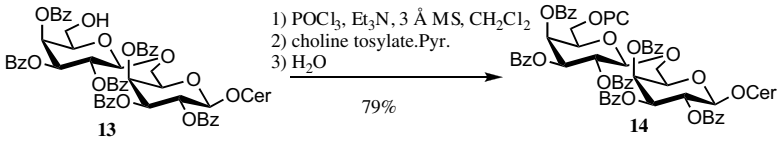
Granulocytic HL-60 cells were prepared by differentiation with all-*trans*-retinoic acid (ATRA) of HL-60 cells. These cells did not produce significant amounts of IL-8, and none of the four glycolipids containing phosphocholine (**1**–**4**) alone induced production of IL-8 in the culture supernatant (data not shown). However, when the cells were stimulated with TNFα for two days, significant IL-8 production (~29 ng/mL) was observed. Glycolipids **1**, **2**, and **3** that contained phosphocholine significantly enhanced IL-8 production in conjunction with TNFα. This enhancement was found to be dose-dependent. To examine the relationship between structure and activity, we conducted further tests of IL-8 production using glycolipid **5** and ceramide **6**, in which compound **1** showed stronger activity than **5**, indicating that the presence of phosphocholine residue increases the activity. Similarly, **1** showed stronger activity than **3**, suggesting that the ceramide is better than an octyl residue to increase activity. On the other hand, comparison of activities of **4** and **6** with **1** led to the conclusion that the ceramide and glycosyl part are necessary for IL-8 production. These results indicated that, in general, glycosyl residues and ceramide, as well as the phosphocholine residue, are the most suitable part of the structure to enhance IL-8 producing activity. In inflammatory or infected lesions, inflammatory cytokines including TNFα and IL-1 are released from activated macrophages, and then these inflammatory cytokines stimulate macrophages and granulocytes to produce IL-8. Since IL-8 is a potent neutrophil and T-lymphocyte chemotactic factor, **1** and **2** may thus lead to protection from mycobacterial[13] and fungal[14] infections by recruiting neutrophils and enhancing bactericidal activity (Fig. 2).

## 3. Conclusions

In summary, we have succeeded for the first time in carrying out total syntheses, in good yield, of phosphocholine-containing




**Scheme 2.** Reagents: (a) thiourea, 5:3 EtOH–Pyr, **15**: 88%, **17**: 85%, **21**: 99%; (b) NIS, TfOH, $CH_2Cl_2$, 4 Å MS, 60%; (c) (i) $CF_3CO_2H$, $CH_2Cl_2$; (ii) $CCl_3CN$, DBU, $CH_2Cl_2$, 99%; (d) TMSOTf, $CH_2Cl_2$, 4 Å MS, 60%; (e) (i) 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite, 1*H*-tetrazole, $CH_2Cl_2$, 3 Å MS; (ii) 1*H*-tetrazole, choline tosylate; (iii) *m*-CPBA, MeOH; (iv) aq $NH_3$, **18**: 68%, **22**: 75%; (f) NaOMe, MeOH, **4**: 69%, **2**: 81%.




**Scheme 3.**

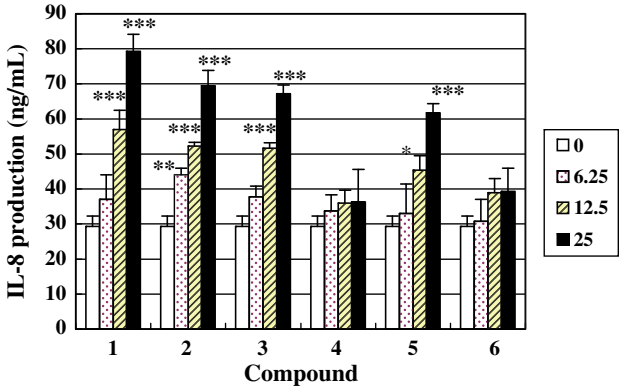



**Figure 2.** Effects of six compounds on IL-8 production in TNFα-stimulated granulocytic cells. ATRA-differentiated HL-60 cells ($5 \times 10^5$ cell/mL) were stimulated with each of the six compounds (final 6.25 μM, 12.5 μM, 25 μM) for 24 h. After that, TNFα (10 ng/mL) was added and incubated for 48 h. The IL-8 contents of culture supernatant were determined by ELISA. The values represent the mean ± SD of three independent experiments. * $P$ <0.05, ** $P$ <0.01, *** $P$ <0.005 compared with the control.

glycosphingolipids found in invertebrate species. The presence of phosphocholine and ceramide groups resulted in the enhancement of IL-8 production in a TNFα-stimulated granulocytic HL-60 cells. These target molecules lie in the class of easily accessible glycolipids in the field of carbohydrate chemistry; however, as these molecules exhibited immunomodulatory activity, they may be important and interesting for their other biological activities.

## 4. Experimental

### 4.1. General methods

Optical rotations were measured with a Jasco P-1020 digital polarimeter. $^1$H NMR and $^{13}$C NMR spectra were recorded with a JMN A500 FT NMR spectrometer with $Me_4Si$ as the internal standard for solutions in $CDCl_3$. MALDI-TOFMS was recorded on a Perseptive Voyager RP mass spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-700 under FAB conditions (HRFABMS). TLC was performed on Silica Gel 60 F254 (E. Merck) with detection by quenching of UV fluorescence and by charring with 10% $H_2SO_4$. Column chromatography was carried out on Silica Gel 60 (E. Merck). Ceramide **6** was purchased from Acros Organics Chemical Co. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**7**)[2h] and phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranoside (**8**) were prepared as reported.[9]

### 4.2. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (9)

A solution of compounds **7** (500 mg, 0.84 mmol) and **8** (669 mg, 1.01 mmol) containing activated 4 Å MS (1 g) in dry $CH_2Cl_2$ (2 mL) was stirred under an atmosphere of argon for 2 h at room temperature. After cooling to 0 °C, successively NIS (285 mg, 1.27 mmol) and TfOH (22.3 μL, 0.25 mmol) were added, stirring was continued at −60 °C for 2 h, and then the mixture was neutralized with $Et_3N$. The reaction mixture was filtered, and the filtrate was washed with aq $Na_2S_2O_3$, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 5:1 hexane–EtOAc as the eluent to give **9** (715 mg, 74%): $[\alpha]_D^{24}$ +123.6 (*c* 1.6, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$): δ 8.08–7.14 (m, 30H, 6 × Ph), 5.89 (d, 1H, $J_{3,4}$ 3.7 Hz, H-4), 5.79 (d, 1H, $J_{3',4'}$ 3.7 Hz, H-4′), 5.78–5.71 (m, 2H, H-2, 2′), 5.55–5.51 (m, 2H, H-3, 3′), 4.87 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.72 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.23–4.11 (m, 5H, H-6a, 6b, 6′a, 6′b, $-OCH_2CH_2$), 3.96–3.89 (m, 4H, H-5, 5′, $-OCH_2Cl$), 3.51–3.45 (m, 1H, $-OCH_2CH_2$), 0.87–0.70 (m, 2H, $-OCH_2CH_2$), −0.11 (s, 9H, $Si(CH_3)_3$). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.7, 165.5, 165.13 165.05, 133.6, 133.4, 133.3, 133.0, 130.0, 129.9, 129.6, 129.2, 129.1, 129.0, 128.7, 128.6, 128.5, 128.3, 128.21, 128.15, 125.2, 101.0, 100.8, 72.9, 71.9, 71.5, 71.0, 69.8, 69.6, 68.5, 67.8, 67.7, 67.5, 63.1, 40.4, 17.7, −1.5. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{61}H_{59}ClO_{18}SiNa$, 1165.3; found, 1164.4.

### 4.3. 2,3,4-Tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (10)

To a solution of **9** (100 mg, 87 μmol) in $CH_2Cl_2$ (2.0 mL), cooled to 0 °C, was added $CF_3CO_2H$ (2 mL), and the mixture was stirred for 30 min at room temperature and concentrated. EtOAc and toluene (1:2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in $CH_2Cl_2$ (2 mL) cooled at 0 °C were added trichloroacetonitrile (130 μL, 1.30 mmol) and DBU (12.5 μL, 84 μmol). The reaction mixture was stirred for 1 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (20:1 toluene–EtOAc) gave **10** (100 mg, 97%): $[\alpha]_D^{25}$ +127.0 (*c* 0.5, $CHCl_3$); $^1$H NMR ($CDCl_3$): δ 8.33 (s, 1H, NH), 8.08–7.14 (m, 30H, 6 × Ph), 6.77 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 6.08 (d, 1H, $J_{3,4}$ 3.1 Hz, H-4), 5.87 (dd, 1H, $J_{2,3}$ 10.9 Hz, H-3), 5.81 (dd, 1H, H-2), 5.81 (d, 1H, $J_{3',4'}$ 3.7 Hz, H-4′), 5.72 (dd, 1H, $J_{1',2'}$ 7.9 Hz, $J_{2',3'}$ 10.9 Hz, H-2′), 5.48 (dd, 1H, H-3′), 4.87 (d, 1H, H-1′), 4.71 (t, 1H, H-6a), 4.16–4.08 (m, 4H, H-6b, 6′a, 6′b, H-5), 3.96–3.87 (m, 4H, H-5′, $-OCH_2Cl$). $^{13}$C NMR (125 MHz, $CDCl_3$): δ 166.7, 165.6, 165.5 165.4, 165.2, 165.1, 133.6, 133.5, 133.3, 133.2, 130.1, 129.9, 129.8, 129.7, 129.3, 129.1, 129.0, 128.74, 128.69, 128.6, 128.34, 128.25, 128.2, 125.2, 101.0, 93.7, 90.7, 71.6, 71.2, 71.0, 69.4, 68.3, 68.0, 67.8, 67.5, 63.0, 40.5. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{58}H_{48}Cl_4NO_{18}Na$, 1209.2; found, 1208.3.

### 4.4. 2,3,4-Tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (12)

To a solution of **10** (65 mg, 55 μmol) and (2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol **11** (38 mg, 59 μmol) in dry $CH_2Cl_2$ (0.8 mL) was added 4 Å MS (400 mg), and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. TMSOTf (8 μL, 44 μmol) was added, and the mixture was stirred for 2 h at 0 °C, then neutralized with $Et_3N$. The solids were filtrated off and washed with $CHCl_3$. The combined filtrate and

washings were successively washed with water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 10:1 toluene–EtOAc as eluent to give **12** (57 mg, 62%): $[\alpha]_D^{25}$ +71.0 (*c* 1.2, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.12–7.21 (m, 35H, 7 × Ph), 5.87–5.82 (m, 3H, H-4′, >C=CH, –NH), 5.75 (d, 1H, H-4), 5.66–5.62 (m, 2H, H-2, 2′), 5.57–5.44 (m, 4H, H-3, 3′, >C=CH, Bz-CH), 4.60 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.48 (1H, d, $J_{1,2}$ 7.3 Hz, H-1), 4.30 (1H, b, N–C*H*), 4.12–3.89 (m, 6H, H-6, 6′, 5, 5′, $COCH_2Cl$), 3.44 (dd, 1H, O–C$H_2$), 3.30 (dd, 1H, O–C$H_2$), $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 172.5, 166.7, 165.5, 137.3, 133.6, 130.6, 130.1, 129.7, 129.6, 129.2, 128.6, 128.5, 128.4, 128.3, 125.0, 101.0 (C-1′), 100.7 (C-1), 74.0, 72.8, 71.3, 70.8, 70.5, 69.6, 68.2, 67.8, 67.4, 63.0, 50.4, 40.4, 36.5, 32.3, 31.9, 29.7, 29.5, 29.3, 29.2, 29.0, 25.5, 22.7, 14.1. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{97}H_{116}ClNO_{21}Na$, 1688.8; found, 1688.9.

### 4.5. 2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (13)

To a solution of **12** (133 mg, 80 μmol) in 3:5 pyridine–EtOH (1.6 mL) was added thiourea (31 mg, 0.40 mmol), and the mixture was stirred for 2 h at 80 °C. The mixture was diluted with $CHCl_3$, washed with $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 5:1 toluene–EtOAc as eluent to give **13** (82 mg, 65%): $[\alpha]_D^{24}$ +87.7 (*c* 2.0, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.10–7.21 (m, 35H, 7 × Ph), 5.91 (d,1H, –NH), 5.87–5.82 (m, 1H, H-4′), 5.71–5.67 (m, 2H, H-4, 2′), 5.64–5.56 (m, 2H, H-2, >C=CH), 5.54–5.44 (m, 4H, H-3, 3′, >C=CH, Bz-CH), 4.60 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.47 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4.34–4.30 (br t, 1H, N–CH), 4.01–4.00 (m, 2H, H-6′), 3.84–3.78 (m, 2H, H-6), 3.53–3.49 (q, 1H, O–$CH_2$), 3.41–3.34 (m, 3H, H-5, 5′, O–$CH_2$). $^{13}$C NMR(125 MHz, $CDCl_3$): $\delta$ 172.5, 166.6, 165.5, 165.1, 143.2, 137.3, 133.7, 133.6, 133.4, 133.3, 130.2, 130.1, 129.7, 129.6, 129.2, 129.0, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 125.0, 101.1 (C-1′), 100.7(C-1), 74.1, 73.9, 72.6, 71.6, 71.3, 70.5, 70.0, 68.8, 68.2, 67.4, 67.0, 60.7, 50.4, 36.5, 32.3, 31.9, 29.7, 29.5, 29.3, 29.2, 29.0, 25.5, 22.7, 14.1. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{95}H_{115}NO_{20}Na$, 1612.8; found, 1612.6.

### 4.6. 2,3,4-Tri-*O*-benzoyl-6-*O*-phosphocholine-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (14)

#### 4.6.1. Method A

To a solution of **13** (82.1 mg, 52 μmol) and 3 Å MS (300 mg) in dry $CH_2Cl_2$ (2.0 mL) were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (24.6 μL, 77 μmol) and 1*H*-tetrazole (4.0 mg, 57 μmol) at room temperature under Ar. The solution was stirred for 0.5 h at the room temperature. To this was added 1*H*-tetrazole (10.9 mg, 0.16 mmol), followed by choline tosylate (56.9 mg, 0.21 mmol) at room temperature. This solution was stirred for 4 h at room temperature, and MeOH (1 mL) and *m*-CPBA (14.6 mg, 59 μmol) were then added, with stirring for 1 h at the room temperature. After that time, 30% aq $NH_3$ was added to mixture, and it was stirred for 1 h at room temperature. The solution was filtered and concentrated. The product was purified by Iatrobeads column chromatography using 4:5:1 $CHCl_3$–MeOH–$H_2O$ as eluent to give **14** (59.8 mg, 66%).

#### 4.6.2. Method B

To a solution of **13** (36.7 mg, 23 μmol) and 3 Å MS (130 mg) in dry $CH_2Cl_2$ (1.0 mL) were added phosphoryl chloride (2.2 μL, 23 μmol) and $Et_3N$ (3.5 μL, 25 μmol) at −10 °C under Ar. The solution was stirred for 1.5 h at the room temperature, and pyridine (1 mL) and then choline tosylate (12.7 mg, 46 μmol) were added at room temperature. This solution was stirred for 10 h at room temperature, and $H_2O$ (1 mL) was added with stirring for 1 h at the same temperature. After that time, the solution was filtered and concentrated. The product was purified by Iatrobeads column chromatography using 4:5:1 $CHCl_3$–MeOH–$H_2O$ as eluent to give **14** (32.1 mg, 79%): $[\alpha]_D^{25}$ +40.3 (*c* 0.7, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.00–6.70 (m, 35H, 7 × Ph), 5.82 (d, 1H, –NH), 5.75–5.72 (m, 2H, H-4′, >C=CH), 5.54–5.33 (m, 7H, H-2, 2′, 3, 3′, 4, >C=CH, Bz-CH), 4.65 (d, 1H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.52–4.50 (br, 1H, H-1), 4.16–4.03 (m, 4H, H-6a, N–CH, N–$CH_2$), 3.85–3.83 (m, 2H, H-6), 3.77–3.76 (q, 1H, H-6), 3.47–3.45 (br d, 2H, PO–$CH_2$) 3.33–3.24 (m, 2H, H-5, 5′), 3.07 (br s, 10H, N–$(CH_3)_3$, O–$CH_2$). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 173.5, 100.0 (C-1′), 99.7 (C-1), 74.1, 73.0, 72.2, 71.7, 70.1, 69.5, 69.4, 69.0, 67.8, 67.3, 66.7, 65.8, 65.5. MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{100}H_{128}N_2O_{23}P$, 1755.9; found, 1756.0.

### 4.7. 6-*O*-Phosphocholine-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-hexadecanamido-4-octadecene-1,3-diol (1)

To a solution of **14** (59.8 mg, 34.0 μmol) in MeOH (2.0 mL) was added NaOMe (10 mg) at 45 °C. The mixture was stirred for 1 h and then neutralized with Amberlite IR 120 [$H^+$]. The mixture was filtered and concentrated. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **1** (21.6 mg, 62%): $[\alpha]_D^{24}$ +12.0 (*c* 0.3, MeOH); $^1$H NMR (19:1 DMSO-$d_6$–$D_2O$): $\delta$ 4.17 (d, 1H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.07 (m, 3H, H-1, PO–$CH_2$). MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{51}H_{100}N_2O_{16}P$, 1027.7; found, 1028.2. HRFABMS *m*/*z*: $[M+Na]^+$ calcd for $C_{51}H_{99}N_2O_{16}PNa$, 1049.6630; found, 1049.6583.

### 4.8. β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-hexadecanamido-4-octadecene-1,3-diol (5)

To a solution of **12** (8.4 mg, 5.0 μmol) in dioxane–MeOH (2.0 mL) was added NaOMe (10 mg) at 45 °C. The mixture was stirred for 1 h and then neutralized with Amberlite IR 120 [$H^+$]. The mixture was filtered and concentrated. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **5** (3.8 mg, 88%): $[\alpha]_D^{24}$ −0.42 (*c* 0.1, 1:1 $CHCl_3$–MeOH); $^1$H NMR (19:1 DMSO-$d_6$–$D_2O$): $\delta$ 4.15 (d, 1H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.06 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1). MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{46}H_{88}NO_{13}$, 861.6; found, 861.9. HRFABMS *m*/*z*: $[M+Na]^+$ calcd for $C_{46}H_{87}NO_{13}Na$, 884.6075; found, 884.6058.

### 4.9. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (15)

To a solution of **9** (1.33 g, 1.2 mmol) in 3:5 pyridine–EtOH (6.0 mL) was added thiourea (446 mg, 5.9 mmol), and the mixture was stirred for 2.5 h at 80 °C. The mixture was diluted with $CHCl_3$, washed with aq $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 5:1 toluene–EtOAc as eluent to give **15** (1.09 g, 88%): $[\alpha]_D^{24}$ +151.0 (*c* 1.4, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.23–7.31 (30H, m, Ar-H), 6.08 (1H, d, H-4′), 5.92–5.87 (2H, m, H-4, H-2′), 5.81 (1H, dd, H-2), 5.65–5.61 (2H, m, H-3, 3′), 4.94 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.81(1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4.26–4.19 (2H, m, H-5′, H-6′a), 4.07–4.01 (3H, m, H-6′b, H-5, H-6a), 3.75–3.62 (1H, m, O–$CH_2$), 3.61–3.53 (2H, m, H-6b, O–$CH_2$). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 166.6, 165.6, 165.5, 165.21, 165.16, 133.7, 133.5, 133.2, 133.1, 130.1, 130.0, 129.7, 129.6, 129.4, 129.2, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.23, 128.16, 101.3, 100.9, 74.2, 72.9, 72.0,

71.8, 70.0, 69.9, 68.8, 68.5, 67.8, 67.5, 60.7, 17.8, −1.5. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{59}H_{58}ClO_{17}SiNa$, 1089.3; found, 1089.8.

### 4.10. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (16)

A solution of compounds **15** (300 mg, 0.28 mmol) and **8** (223 mg, 0.34 mmol) containing activated 4 Å MS (400 mg) in dry $CH_2Cl_2$ (1.5 mL) was stirred under an atmosphere of Ar for 1.5 h at room temperature. After cooling to 0 °C, successively NIS (127 mg, 0.56 mmol) and TfOH (14.9 μL, 0.17 mmol) were added, stirring was continued at 0 °C for 2 h, and then the mixture was neutralized with $Et_3N$. The reaction mixture was filtered, and the filtrate was washed with $Na_2S_2O_3$ and water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 5:1 toluene–EtOAc as the eluent to give **16** (273 mg, 60%): $[\alpha]_D^{24}$ +67.0 (*c* 6.6, $CHCl_3$). $^1H$ NMR (500 MHz, $CDCl_3$): δ 8.16–7.30 (45H, m, 9 × Ph), 6.01–6.00 (2H, d, H-4, 4′), 5.88–5.87 (1H, d, H-4″), 5.82–5.72 (3H, m, H-2, 2′, 2″), 5.63–5.56 (3H, m, H-3, 3′, 3″), 4.87 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4.82 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.71 (1H, d, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.24–4.15 (4H, m, H-6, 6′), 4.09–3.90 (7H, m, H-6″, 5, 5′, O–$CH_2$, C–$CH_2$–Cl), 3.67 (1H, q, O–$CH_2$) 3.58–3.55 (1H, m, H-5″), $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 166.6, 165.5, 165.4, 165.2, 165.1, 165.0, 164.9, 133.5, 133.3, 133.2, 133.1, 133.0, 130.04, 129.96, 129.7, 129.64, 129.55, 129.38, 129.35, 129.2, 129.0, 128.7, 128.64, 128.56, 128.5, 128.39, 128.36, 128.3, 128.2, 128.1, 100.8 (C-1,1′), 100.7 (C-1″), 72.7, 72.5, 71.9, 71.6, 71.5, 70.9, 69.9, 69.6, 68.4, 67.9, 67.7, 67.4, 67.2, 66.5, 62.9, 40.4, 17.7, −1.5. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{88}H_{81}ClO_{26}SiNa$, 1639.5; found, 1639.5.

### 4.11. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (17)

To a solution of **16** (273 mg, 0.17 mmol) in 3:5 pyridine–EtOH (3.2 mL) was added thiourea (64.2 mg, 0.84 mmol), and the mixture was stirred for 2.5 h at 80 °C. The mixture was diluted with $CHCl_3$, washed with $NaHCO_3$ and water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 10:1 toluene–acetone as eluent to give **17** (222 mg, 85%): $[\alpha]_D^{24}$ +123.7 (*c* 3.9, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 8.10–7.13 (45H, m, Ar-H), 5.95 (1H, d, H-4″), 5.91 (1H, d, H-4′), 5.73–5.65 (4H, m, H-4, H-2″, 2′, 2), 5.53–5.47 (3H, m, H-3″, 3′, 3), 4.76 (1H, d, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.71 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.58 (1H, d, $J_{1,2}$ 7.9 Hz, H-1), 4.13–4.02 (3H, m, H-5″, 5′ H-6″a), 3.95–3.90 (1H, m, O–$CH_2$), 3.86–3.81 (3H, m, H-6″b, H-5, H-6′a), 3.55–3.45 (3H, m, H-6′b H-6a, O–$CH_2$), 3.35–3.31 (1H, m, H-6b). $^{13}C$ NMR (125 MHz, $CDCl_3$): δ 166.5, 165.4, 165.3, 165.18, 165.15, 165.1, 133.5, 133.4, 133.2, 133.0, 130.1, 130.0, 129.71, 129.66, 129.6, 129.4, 129.2, 129.0, 128.9, 128.7, 128.5, 128.41, 128.36, 128.3, 128.2, 128.1, 101.0, 100.8, 74.1, 72.7, 72.3, 71.9, 71.74, 71.69, 69.9, 68.7, 68.4, 67.9, 67.4, 67.0, 66.7, 60.7, 17.7, −1.5. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{86}H_{80}O_{25}SiNa$, 1563.5; found, 1564.0.

### 4.12. 2-(Trimethylsilyl)ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-phosphocholine-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (18)

To a solution of **13** (100 mg, 65 μmol) and 3 Å MS (300 mg) in dry $CH_2Cl_2$ (2.0 mL) were added 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (30.9 μL, 97 μmol) and 1*H*-tetrazole (4.5 mg, 64 μmol) at room temperature under Ar. The solution was stirred for 0.5 h at room temperature, and 1*H*-tetrazole (13.6 mg, 0.19 mmol) and choline tosylate (71.5 mg, 0.26 mmol) were added at room temperature. This solution was stirred for an additional 4 h at room temperature, and MeOH (1 mL) and *m*-CPBA (19.2 mg, 78 μmol) were added with stirring for 1 h at the same temperature. After that, 30% aq $NH_3$ was added and the mixture was stirred for 1 h at room temperature. The solution was filtered through a pad of Celite and concentrated. The product was purified by Iatrobeads column chromatography using 4:5:1 $CHCl_3$–MeOH–$H_2O$ as eluent to give **8** (75.5 mg, 68%): $[\alpha]_D^{25}$ +40.3 (*c* 0.7, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 8.02–6.95 (45H, m, 9 × Ph), 5.87 (1H, d, H-4), 5.80 (1H, d, H-4′) 5.76 (1H, d, H-4″) 5.69–5.45 (6H, m, H-2, 2′, 2″, 3, 3′, 3″), 5.00 (2H, d, $J_{1,2\,1',2'}$ 8.6 Hz, H-1, 1′), 4.84 (1H, d, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.40–4.39 (1H, t, H-6a), 4.30–4.20 (4H, m, H-6b, 6′a, PO–$CH_2$), 4.11–4.06 (1H, m, H-6′b), 4.00–3.93 (5H, m, H-6″, 5, 5″, O–$CH_2$), 3.60–3.50 (4H, m H-5″, O–$CH_2$, N–$CH_2$), 3.08 (9H, s, N($CH_3$)). $^{13}C$ NMR (125 MHz, $CDCl_3$): 100.7 (C-1), 100.3 (C-1′), 100.3 (C-1″), 73.3, 72.2, 72.0, 71.8, 71.6, 71.5, 71.1, 70.0, 69.7, 68.3, 68.1, 67.9, 67.8, 67.2, 65.9, 62.5, 58.8, 53.3. MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{91}H_{93}NO_{28}PSi$, 1706.5; found, 1706.8.

### 4.13. 2-(Trimethylsilyl)ethyl 6-*O*-phosphocholine-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (4)

To a solution of **18** (75.5 mg, 44 μmol) in MeOH (3.0 mL) was added NaOMe (15 mg) at 45 °C, and the mixture was stirred for 1 h, then neutralized with Amberlite IR 120 [$H^+$]. The mixture was filtered and concentrated. The product was purified by Sephadex LH-20 column chromatography in MeOH to give **4** (23.6 mg, 69%): $[\alpha]_D^{24}$ −17.4 (*c* 0.6, MeOH); $^1H$ NMR ($CD_3OD$): δ 4.41–4.27 (2H, m, H-1, 1″), 4.20–4.18 (1H, t, H-1″). $^{13}C$ NMR (125 MHz, $CD_3OD$): δ 105.4 (C-1), 105.2 (C-1′), 104.4 (C-1″), 75.34, 75.27, 75.0, 74.83, 74.76, 74.7, 72.5, 72.4, 70.1, 70.01, 69.98, 69.8, 69.7, 68.2, 67.5, 66.0, 60.1, 54.8, 19.2, −1.3. MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{28}H_{57}NO_{19}PSi$, 770.3; found, 770.0. HRFABMS *m*/*z*: $[M+Na]^+$ calcd for $C_{28}H_{56}NO_{19}PSiNa$, 792.2851; found, 792.2871.

### 4.14. 2,3,4-Tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (19)

To a solution of **16** (100 mg, 62 μmol) in $CH_2Cl_2$ (2.0 mL), cooled to 0 °C, was added $CF_3CO_2H$ (2 mL), and the mixture was stirred for 30 min at room temperature and concentrated. EtOAc and toluene (1:2) were added and evaporated to give the 1-hydroxy compound. To a solution of the residue in $CH_2Cl_2$ (2 mL) cooled at 0 °C were added trichloroacetonitrile (62.4 μL, 0.62 mmol) and DBU (18.5 μL, 124 μmol). The reaction mixture was stirred for 1 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography of the residue on silica gel (20:1 toluene–EtOAc) gave **19** (102.3 mg, 99%): $[\alpha]_D^{25}$ +100.9 (*c* 3.3, $CHCl_3$); $^1H$ NMR ($CDCl_3$): δ 8.36 (s, 1H, NH), 6.76 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.74 (d, 1H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.57 (d, 1H, $J_{1'',2''}$ 7.9 Hz, H-1″). The product was used directly in the next step (Section 4.15).

### 4.15. 2,3,4-Tri-*O*-benzoyl-6-*O*-chloroacetyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (20)

To a solution of **19** (82.0 mg, 49 μmol) and (2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (**11**, 47.5 mg,

74 μmol) in dry $CH_2Cl_2$ (1 mL) was added 4 Å MS (400 mg), and the mixture was stirred for 2 h at room temperature, then cooled to 0 °C. TMSOTf (10.8 mg, 59 μmol) was added, and the mixture was stirred for 2.5 h at 0 °C, then neutralized with $Et_3N$. The solids were filtrated off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried ($MgSO_4$), and concentrated. The product was purified by silica gel column chromatography using 6:1 toluene–EtOAc as eluent to give **20** (63.0 mg, 60%): $[\alpha]_D^{25}$ +73.8 (*c* 2.9, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.08–7.21 (50H, m, 10 × Ph), 5.88–5.76 (4H, m, H-4, 4′, 4″, –NH), 5.65–5.53 (5H, m, H-2, 2′, 2″, C=CH), 5.48–5.42 (4H, m, H-3, 3′, 3″, Bz-CH), 4.60 (1H, dd, $J_{1,2}$ 7.3 Hz, H-1), 4.58 (2H, dd, $J_{1'2'}$ 7.9 Hz, H-1′), 4.37 (1H, d, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.26 (1H, br s, N–CH), 4.04-3.76 (10H, m, H-6, 6′, 6″, 5, 5′, O–$CH_2$), 3.48-3.45 (1H, q, O–$CH_2$), 3.35–3.31 (1H, q, H-5′), 3.28–3.26 (1H, q, H-5″). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 172.4, 166.7, 165.4, 165.2, 165.0, 137.2, 133.3, 130.6, 130.1, 130.0, 129.8, 129.7, 129.3, 129.0, 128.6, 128.54, 128.46, 128.4, 128.3, 125.0, 100.9 (C-1), 100.8 (C-1′), 100.7 (C-1″), 73.8, 72.6, 72.3, 71.6, 71.4, 70.9, 70.5, 69.9, 69.8, 68.2, 67.7, 67.2, 66.2, 62.9, 50.6, 50.4, 40.4, 36.5, 32.3, 31.9, 29.70, 29.65, 29.5, 29.3, 29.2, 29.0, 25.5, 22.7, 14.1. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{124}H_{138}ClNO_{29}Na$, 2162.9; found, 2162.7.

### 4.16. 2,3,4-Tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (21)

Compound **21** was prepared from **20** (132.1 mg) as described for preparation of **13**, yielding 100.5 mg (79%): $[\alpha]_D^{24}$ +79.5 (*c* 2.5, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.10–7.20 (75H, m, Ar-H), 5.91–5.82 (4H, m, H-4, 4′, 4″, –NH), 5.71–5.43 (9H, m, H-2, 2′, 2″, 3, 3′ 3″, >C=CH, Bz-CH), 4.59 (1H, d, $J_{1,2}$ 7.3 Hz, H-1), 4.55 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.36 (1H, d, $J_{1'',2''}$ 7.3 Hz, H-1″), 4.31 (1H, br s, N–CH), 4.00–3.94 (2H, m, H-6), 3.87–3.78 (4H, m, H-6′,6″), 3.69 (1H, t, O–$CH_2$) 3.34–3.28 (4H, m, H-5, 5′, 5″, O–$CH_2$). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 172.4, 166.5, 165.5, 165.4, 165.3, 165.1, 165.0, 164.9, 137.2, 133.4, 133.2, 133.1, 132.8, 130.6, 130.2, 130.0, 129.74, 129.69, 129.6, 129.5, 129.3, 129.2, 129.00, 128.95, 128.80, 128.76, 128.6, 128.5, 128.4, 128.3, 128.20, 128.15, 125.0, 101.0 (C-1), 100.8 (C-1′), 100.7 (C-1″), 74.2, 73.8, 72.5, 72.0, 71.8, 71.5, 71.3, 70.5, 69.9, 68.7, 68.0, 67.8, 67.0, 66.9, 66.4, 60.7, 50.4, 36.5, 32.3, 31.9, 29.7, 29.5, 29.32, 292.26, 29.2, 29.0, 25.5, 22.7, 14.1. MALDI-TOFMS *m*/*z*: $[M+Na]^+$ calcd for $C_{122}H_{137}NO_{28}Na$, 2086.9; found, 2087.5.

### 4.17. 2,3,4-Tri-*O*-benzoyl-6-*O*-phosphocholine-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-3-*O*-benzoyl-2-hexadecanamido-4-octadecene-1,3-diol (22)

Compound **22** was prepared from **21** (100.5 mg) as described for preparation of **14**, yielding 81 mg (75%): $[\alpha]_D^{25}$ +53.8 (*c* 2.0, $CHCl_3$); $^1$H NMR ($CDCl_3$): $\delta$ 8.84–8.02 (50H, m, 10;× Ph), 5.86–5.64 (5H, m, H-2, 4, 4′, 4″, –NH), 5.60–5.47 (7H, m, H-2′, 2″, 3, 3′ 3″, –CH=CH), 5.38 (1H, q, Bz-CH), 4.89 (1H, d, $J_{1,2}$ 7.3 Hz, H-1), 4.76 (1H, d, $J_{1',2'}$ 7.9 Hz, H-1′), 4.66 (1H, d, $J_{1'',2''}$ 6.7 Hz, H-1″), 4.28–4.00 (7H, m, H-6, 6′, PO–$CH_2$, N–CH), 3.77–3.11 (8H, m, H-6″, 5, 5′, 5″, N–$CH_2$, O–$CH_2$), 3.15 (1H, t, O–$CH_2$). $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 173.8, 165.7, 165.6, 165.1, 164.9, 143.0, 141.2, 139.9, 136.5, 133.0, 132.9, 132.4, 129.7, 129.4, 129.3, 129.1, 129.0, 128.9, 128.7, 128.6, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 125.2, 124.1, 100.8 (C-1), 100.2 (C-1′), 99.9 (C-1″), 73.3, 71.8, 71.6, 71.3, 71.1, 70.1, 69.6, 69.5, 67.9, 67.7, 67.6, 66.8, 65.81, 65.76, 65.1, 58.6, 53.3, 42.0 35.7, 31.7, 31.3, 29.0, 28.9, 28.7, 28.5, 28.3, 25.2, 22.0, 20.2, 13.2. MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{127}H_{150}N_2O_{31}P$, 2229.9; found, 2230.4.

### 4.18. 6-*O*-Phosphocholine-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-(1→1)-(2*S*,3*R*,4*E*)-2-hexadecanamido-4-octadecene-1,3-diol (2)

Compound **2** was prepared from **22** (41.9 mg) as described for preparation of **1**, yielding 18.0 mg (81%). $[\alpha]_D^{24}$ +24.7 (*c* 0.1, MeOH); $^1$H NMR (19:1 DMSO-$d_6$–$D_2O$): $\delta$ 4.18 (d, 1H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.17 (d, 1H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.07 (d, 1H, $J_{1,2}$ 7.3 Hz, H-1). MALDI-TOFMS *m*/*z*: $[M+H]^+$ calcd for $C_{57}H_{110}N_2O_{21}P$, 1189.7; found, 1190.5. HRFABMS *m*/*z*: $[M+Na]^+$ calcd for $C_{57}H_{109}N_2O_{21}PNa$, 1211.7158; found, 1211.7140.

### 4.19. Biological activities

HL-60 cells were suspended in complete medium (RPMI1640 containing 5% FBS) (FBS, Nippon Bio Supply Center, Tokyo). Exponentially growing cells were incubated in fresh media at a concentration at a $5 \times 10^5$/mL in the presence of ATRA (1 μM) for 2 days. Each test compound was added at various doses. After 1 day of addition of test compounds, TNFα (10 ng/mL) was added, and the mixture was incubated for further 2 days. The supernatants were obtained, and IL-8 was quantitated using an ELISA method.[8]

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## References

1. (a) De Meo, C.; Boons, G.-J.; Demchenko, A. V. In *Comprehensive Glycoscience*; Kamerling, J. P., Ed.; Elsevier Ltd. 2007; Vol. 1, pp 583–604; (b) Ando, T.; Imamura, A.; Ishida, H.; Kiso, M. In *Comprehensive Glycoscience*; Kamerling, J. P., Ed.; Elsevier Ltd., 2007; Vol. 1, pp 797–813.
2. (a) Hada, N.; Nakashima, T.; Shrestha, S. P.; Masui, R.; Narukawa, Y.; Tani, K.; Takeda, T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5912–5915; (b) Hada, N.; Sonoda, Y.; Takeda, T. *Carbohydr. Res.* **2006**, *341*, 1341–1352; (c) Yamamura, T.; Hada, N.; Kaburaki, A.; Yamano, K.; Takeda, T. *Carbohydr. Res.* **2004**, *339*, 2749–2759; (d) Ohtsuka, I.; Hada, N.; Sugita, M.; Takeda, T. *Carbohydr. Res.* **2003**, *337*, 2037–2047; (e) Ohtsuka, I.; Hada, N.; Ohtaka, H.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2002**, *50*, 600–604; (f) Hada, N.; Sato, K.; Sakushima, J.-I.; Goda, Y.; Sugita, M.; Takeda, T. *Chem. Pharm. Bull.* **2001**, *49*, 1464–1467; (g) Hada, N.; Ohtsuka, I.; Sugita, M.; Takeda, T. *Tetrahedron Lett.* **2000**, *41*, 9065–9068; (h) Hada, N.; Hayashi, E.; Takeda, T. *Carbohydr. Res.* **1999**, *316*, 58–70.
3. (a) Sugita, M.; Fujii, H.; Dulaney, T. J.; Inagaki, F.; Suzuki, M.; Suzuki, A.; Ohta, S. *Biochim. Biophys. Acta* **1995**, *1259*, 220–226; (b) Sugita, M.; Ohta, S.; Morikawa, A.; Dulaney, T. J.; Ichkawa, S.; Kushida, S.; Inagaki, F.; Suzuki, M.; Suzuki, A. *J. Jpn. Oil Soc.* **1997**, *46*, 15–26.
4. (a) Houston, K. M.; Harnett, W. *Parasitology* **2004**, *129*, 655–661; (b) Friedl, C. H.; Lochnit, G.; Zähringer, U.; Bahr, U.; Bahr, U.; Geyer, R. *Biochem. J.* **2003**, *369*, 89–102; (c) Deehan, M. R.; Goodridge, H. S.; Blair, D.; Lochnit, G.; Dennis, R. D.; Geyer, R.; Harnett, M. M.; Harnett, W. *Parasite Immunol.* **2002**, *24*, 463–469; (d) Wuhrer, M.; Rickhoff, S.; Dennis, R. D.; Lochnit, G.; Soboslay, P. T.; Baumeister, S.; Geyer, R. *Biochem. J.* **2000**, *348*, 417–423; (e) Locnit, G.; Dennis, R. D.; Ulmer, A. J.; Geyer, R. *J. Biol. Chem.* **1998**, *273*, 466–474.
5. Kean, D. E.; Ohtsuka, I.; Sato, K.; Hada, N.; Takeda, T.; Lochnit, G.; Geyer, R.; Harnett, M. M.; Harnett, W. *Parasite Immunol.* **2006**, *28*, 69–76.
6. (a) Pekari, K.; Tailler, D.; Weingart, R.; Schmidt, R. R. *J. Org. Chem.* **2001**, *66*, 7432–7442; (b) Nishida, Y.; Takamori, Y.; Matsuda, K.; Ohrui, H.; Yamada, T.; Kobayashi, K. *J. Carbohydr. Chem.* **1999**, *18*, 985–997.
7. Mickeleit, M.; Wieder, T.; Arnold, M.; Geilen, C. C.; Mulzer, J.; Reutter, W. *Angew. Chem., Int. Ed.* **1998**, *37*, 351–353.
8. Matsushima, K.; Morishita, K.; Yoshimura, T.; Lavu, S.; Kobayashi, Y.; Lew, W.; Appella, E.; Kung, H. F.; Leonard, E. J. *J. Exp. Med.* **1988**, *167*, 1883–1893.

9. (a) Matsushima, K.; Larsen, C. G.; DuBois, G. C.; Oppenheim, J. J. *J. Exp. Med.* **1989**, *169*, 1485–1490; (b) Larsen, C. G.; Anderson, A. O.; Appella, E.; Oppenheim, J. J.; Matsushima, K. *Science* **1989**, *243*, 1464–1466; (c) Furutani, Y.; Nomura, H.; Notake, M.; Oyamada, Y.; Fukui, T.; Yamada, M.; Larsen, C. G.; Oppenheim, J. J.; Matsushima, K. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 249–255; (d) Kasahara, T.; Mukaida, N.; Yamashita, K.; Yagisawa, H.; Akahoshi, T.; Matsushima, K. *Immunology* **1991**, *74*, 60–67.
10. Hanashima, S.; Manabe, S.; Ito, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 4218–4224.
11. Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313–4316.
12. Schmidt, R. R.; Grundler, G. *Synthesis* **1985**, 885–887.
13. Nibbering, P. H.; Pos, O.; Stevenhagen, A.; Van Furth, R. *Infect. Immun.* **1993**, *61*, 3111–3116.
14. Djeu, J. Y.; Matsushima, K.; Oppenheim, J. J.; Shiotsuki, K.; Blanchard, D. K. *J. Immunol.* **1990**, *144*, 2205–2210.