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Synthesis and absolute structures of *Mycoplasma pneumoniae* β-glyceroglycolipid antigens

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ABSTRACT

Just recently, a pair of β-glycolipids was isolated from the cell membrane of *Mycoplasma pneumoniae* as a mixture of the two compounds. They are the major immunodeterminants of this pathogenic Mycoplasma and indicate high medicinal potential. They have a β-(1→6)-linked disaccharide structure close to each other; one has β-D-galactopyranoside (β-Gal-type **1**) at the non-reducing terminal, and another has β-D-glucopyranoside (β-Glc-type **2**). In the present study, the first stereoselective synthesis was conducted for each of the two β-glycolipids **1** and **2**. ^{1}H NMR and TLC-immunostaining studies of the synthetic compounds enable us to establish the absolute structures having the β-(1→6)-linked disaccharides at the glycerol *sn*-3 position.

1. Introduction

Mycoplasma pneumoniae is a pathogenic mycoplasma that causes human respiratory diseases called *M. pneumoniae*.[1,2] This germ spreads by droplet infection or contagion and epidemics territorially. The patients may suffer from onset of fever, headache, malaise, and dry cough. Though treatment of appropriate antibiotics such as erythromycin and tetracycline can limit the term of these symptoms, a great many complications are known to occur in the nervous system (ex. transverse myelitis, peripheral neuropathies, and Guillain–Barre Syndrome) as well as in the heart (ex. Mycocarditis and pericarditis).[2–4] Many people are infected several times within a lifespan. In other words, human immunity to the *M. pneumoniae* is not strong enough for continuing through a whole lifetime. This is closely related with a lack of a rigid cell wall and LPS in an outer layer of cell membrane.

Today, diagnosis of the *M. pneumoniae* pneumonia is carried out by X-rays, culturing, antigen amplification (by the polymerase chain reaction/PCR),[5] direct antigen detection by immunofluorescence,[6] or detection of cold agglutinin.[7] However, there is no straightforward method that is valid at an early stage of infection and appearance of the pneumonia symptoms.

Just recently, one of the collaborators[8] found out the presence of a pair of β-glycolipid antigens (**1** and **2**, Fig. 1) in the cell-membrane components of *M. pneumoniae*. ^{1}H NMR and MS analyses of a mixture of **1** and **2** led us to propose that they have a β-(1→6)-linked disaccharide at the glycerol *sn*-1 or *sn*-3 position. They both have long saturated fatty acids ($C_{16:0}$ and $C_{18:0}$) on the glycerol moiety. One is β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (β-Gal-type **1**) and another is β-D-glucopyranosyl-(1→6)-β-D-galactopyranoside (β-Glc-type **2**). On the other hand, complete structure assignment has not been accomplished because they can hardly be separated from each other and purified in sufficient amounts for study. In the present study, we have performed stereoselective synthesis of each **1** and **2** ($C_{16:0}$ fatty acid homologues) and established their absolute chemical structures by ^{1}H NMR spectroscopy and TLC immunoassay.

2. Results and discussion

2.1. Total synthesis of 1 and 2 using a non-malodorous thioglycosylation method

For constructing the β-glycosyl linkages in **1** and **2**, the use of 2-*O*-acylated glycosyl donors may be a feasible approach. This is because the 2-*O*-acyl group is known to promote the β-glycosylation process via a neighboring-group participation effect.[9–11] In the present synthetic plan, however, the *O*-acyl groups seemed

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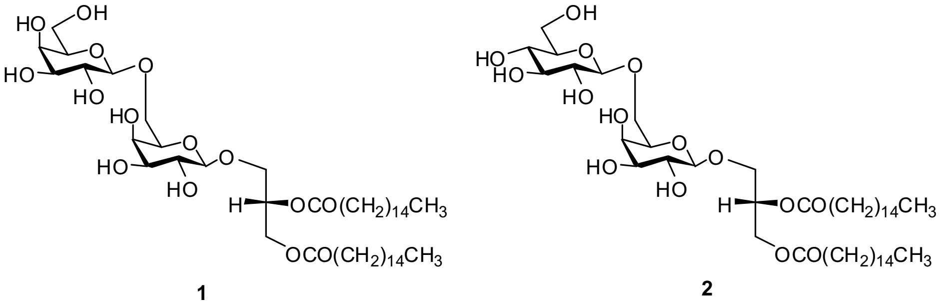


Figure 1. Proposed structures of the two β-glycolipid antigens (β-Gal-type **1** and β-Glc-type **2**) of *M. pneumoniae*.

incompatible with the glycerol di-*O*-acyl groups in **1** and **2**,[12] although chemoselective O-deacylation reactions are reported.[13] The use of a 2-*O*-benzyl group seemed more convenient in this study at the stage of introducing a β-glycoside at the sugar 6-OH position. Of course, with the 2-*O*-benzylated glycosyl donors, the neighboring-group participation effect is eliminated.

Previously, we proposed a non-malodorous thioglycosylation methodology[14–16] in which the 2-methoxycarbonylphenyl 1-thioglycosides protected with *O*-benzyl groups have been used in α-glycosylation reactions. The 2-methoxylcarbonyl group in the sulfur-containing leaving group plays a major role in suppressing the unpleasant odor. Moreover, the 2-methoxylcarbonyl group seems to play a major part in promoting β-glycosylation reactions. The carbonyl group may form a cyclic acyloxy intermediate similar to the case of the 2-*O*-acyl glycosyl donors. In the present study, we examined the reactivity of 2-methoxycarbonylohenyl 1-thioglycosides **3** and **4** (Fig. 2) by changing solvents and temperatures to establish optimal conditions for the synthesis of **1** and **2**. As the acceptor substrate, chiral *sn*-glycerol **5** having a *p*-methoxybenzyl (PMB) group was selected.

The 2-*O*-benzylated glycosyl donors **3** and **4** were prepared in the following ways (Scheme 1). 2-Methoxycarbonylphenyl 1-thio-β-D-galactopyranoside (**6**)[14] was 4,6-*O*-benzylidenated and then 2,3-di-*O*-benzylated to afford **3**. Treatment of per-*O*-acetylglucopyranosyl bromide **7** with methyl thiosalicylate and potassium bicarbonate in DMF gave thiosalicyl glycoside **8**, which was then converted to **4** by Zemplén's *O*-deacetylation and 2,3,4,6-tetra-*O*-benzylation. The acceptor **5** was prepared from 3-*O*-trityl-(*S*)-glycidol **9**.[17] The epoxy group was reacted with potassium hydroxide in dimethyl sulfoxide[18] to give **10** as the result of an S_N2 ring-opening reaction. Introduction of the PMB group and subsequent removal of trityl group afforded acceptor **5**.

With every synthetic intermediate in hand, we investigated the stereoselectivity of the glycosylation (Table 1). A combination of *N*-

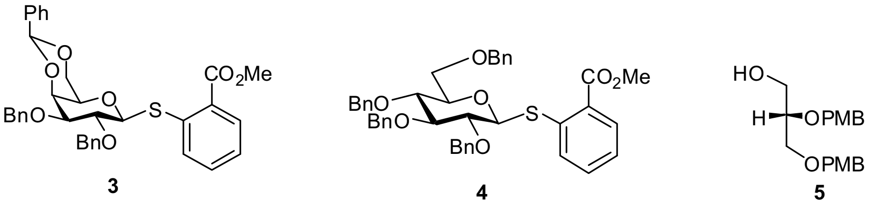
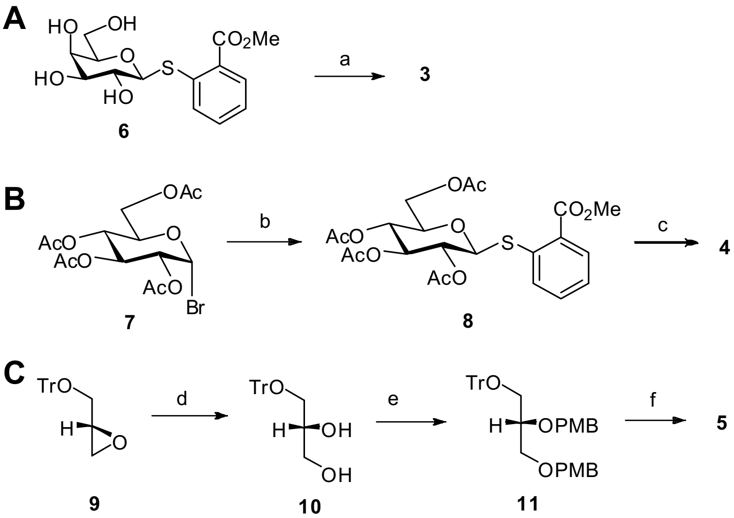
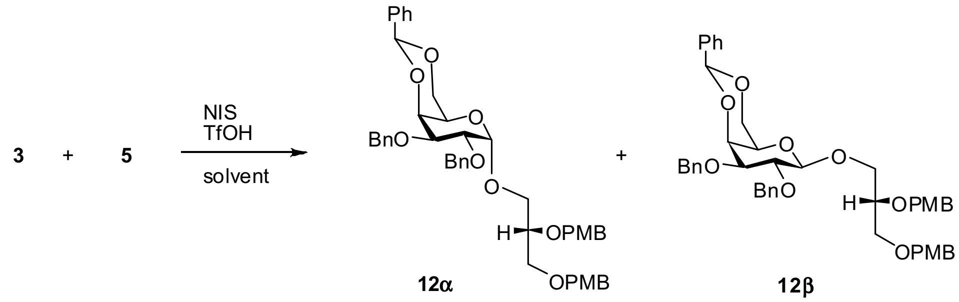


Figure 2. Building blocks **3**–**5** for total synthesis of **1** and **2**.



Scheme 1. Preparation of the glycosyl donors **3** and **4** and the acceptor **5**. Reagents and conditions: (a) (i) BDA, *p*-TsOH, DMF, 40 °C; (ii) BnBr, NaH, DMF, 59%; (b) methyl thiosalicylate, K_2CO_3, DMF, 78%; (c) (i) NaOMe, MeOH; (ii) BnBr, NaH, DMF, 64%; (d) 1 N KOH, DMSO, 70 °C, 64%; (e) NaH, PMBCl, DMF, 95%; (f) Amberlyst®, MeOH, 80%.

Table 1
Glycosylation of galactosyl donor **3** and acceptor **5**[a]



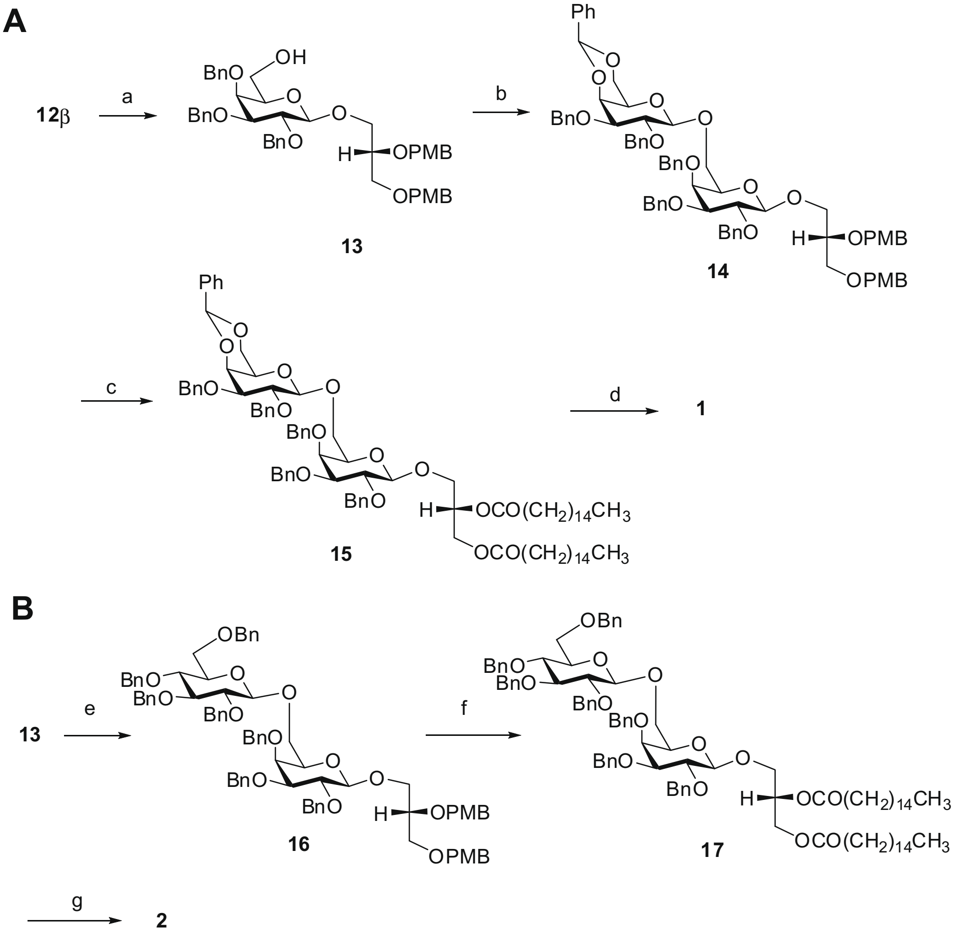
Entry	Solvent	Temp (°C)	Time	Yields[b] (%)	α/β[c]
1	CH_2Cl_2	0	<5 min	Trace	–
2	CH_2Cl_2	−40	<5 min	84	44/56
3	CH_2Cl_2	−78	8 h	87	36/64
4	4:1 CH_2Cl_2–CH_3CN	−40	<5 min	93	23/77
5	1:1 CH_2Cl_2–CH_3CN	−40	<5 min	97	10/90
6	CH_3CN	−40	30 min	50	11/89

[a] All reactions were carried out with 1.5 equiv of acceptor **5** in the presence of *N*-iodosuccinimide (1.5 equiv) and a catalytic amount of TfOH.

[b] Isolated yield.

[c] Determined by ^1H NMR spectroscopy.

iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) was employed as the promoter. Glycosylation in dichloromethane at 0 °C resulted in a poor yield due to decomposition of the PMB groups (entry 1). Consequently, the reaction temperature was lowered to −40 °C to give a mixture of glycosylated products **12**α and **12**β (44:56, isolated yield = 84%) without affecting the PMB group



Scheme 2. Syntheses of **1** and **2**. Reagents and conditions: (a) Bu_2 BOTf-Et_2O, THF, O °C, 84%; (b) **4**, NIS, TfOH, CH_2Cl_2, CH_3CN, −40 °C, 69% (α/β = 7/93); (c) (a) DDQ, CH_2Cl_2, 2-PrOH, H_2O; (b) palmitoyl chloride, CH_2Cl_2, pyridine, 80% (2 steps); (d) H_2, Pd/C, MeOH, 88%; (e) **5**, NIS, TfOH, CH_2Cl_2, CH_3CN, −40 °C, 69% (α/β = 14/86); (f) (a) DDQ, CH_2Cl_2, 2-PrOH, H_2O; (b) palmitoyl chloride, CH_2Cl_2, pyridine, 83%; (g) H_2, Pd/C, MeOH, 89%.

(entry 2). When the reaction was performed at −78 °C, the β-selectivity was slightly improved (α:β = 36:64, 87%). The cyclic acyloxy intermediate involving the 2-methoxycarbonyl group in the leaving group may be stabilized at a lower temperature to promote the β-selectivity.[19] This effect seems to work in a similar manner to the neighboring-group participation effect from 2-acylated glycosyl donors.

When a mixture of acetonitrile and dichloromethane (1:4) was used as the solvent, the β-selectivity was further improved (α:β = 23:77, entry 4). With an increase of acetonitrile in the system, higher β-selectivity resulted in up to α:β = 10:90 (entry 7). On the other hand, the yield in the glycosylation reaction decreased when the solvent system was changed to pure acetonitrile. This was due to the poor solubility of **3** without CH_2Cl_2 (entry 8). The two products, **12α** and **12β,** could be easily separated by silica gel column chromatography, and the desired **12**β was used for further reactions toward the synthesis of the desired β-glycolipids.

The 4,6-*O*-benzylidene group in **12**β was subjected to reductive cleavage by a reagent combination of borane·tetrahydrofuran complex (BH_3·THF) and dibutylboryl trifluoromethanesulfonate solution ($Bu_2BOTf–Et_2O$)[20,21] to afford 6-OH β-D-galactopyranoside **13**. When other reagent combinations such as $LiAlH_4–AlCl_3$[22] or $Et_3SiH–PhBCl_2$[23] were employed, the PMB group in the glycerol moiety was cleaved to afford a mixture of products.

For the synthesis of the β-Gal-type GGL **1**, the thiogalactosyl donor **3** was coupled again with the acceptor sugar **13** under the same reaction condition as those in entry 5 (Table 1). The acceptor **13** was consumed immediately to afford the desired disaccharide **14** (α:β = 7:93). Selective deprotection of the PMB group in **14** was conducted with DDQ in a mixture of CH_2Cl_2, 2-PrOH, and water at 0 °C. Introduction of the palmitoyl groups at the derived hydroxyl groups using palmitoyl chloride then furnished **15**. The sugar *O*-benzyl and 4,6-*O*-benzylidene acetal groups of **15** were removed by catalytic hydrogenation using palladium-on-carbon in methanol to afford the β-Gal-type glycolipid **1** having a homogeneous fatty acid ($C_{16:0}$). For the synthesis of **2**, the glycosyl donor **4** was used for the glycosylation with **13** under the same reaction conditions (α:β = 14:86, Scheme 2), and thus, we have synthesized both types of β-glycolipids **1** and **2** carrying homogenous fatty acids ($C_{16:0}$).

2.2. Assignments of absolute structure of natural β-glycolipids by means of ^{1}H NMR spectroscopy and TLC-Immunostaining assay

^{1}H NMR data of the synthetic products (**1** and **2**) were completely in accord with the data for the natural products (Table 2). This means that the disaccharides in both **1** and **2** are linked to glycerol at the *sn*-3 position. Moreover, a TLC-immunostaining assay[24,25] has shown that the antigenic response of the synthetic products is also in good accord with the natural products (Fig. 3). Figure 3A gives the spots after an orcinol staining for both natural and synthetic glycolipids. Figure 3B gives the spots after immunostaining with a goat serum immunized with *M. pneumoniae* and colored with a secondary antibody conjugated with a horseradish peroxidase (HPR). By this biological assay, the β-glycolipid, which showed stronger antigenic properties to the goat-serum antibody, could be assigned to the β-Glc-type **2**, and another to the β-Gal-type **1**. Thus, we have made unambiguous the absolute structures of the natural β-glycolipids **1** and **2**.

Table 2
Selected ^{1}H NMR data of **1** and **2** (500 MHz, 98:2 DMSO-d_6–D_2O, 40 °C)

	Chemical shift (ppm)[a]			
	β-Gal-type		β-Glc-type	
	Synthetic	Natural[b]	Synthetic	Natural[b]
sn-Glycerol				
H-1_{proR}	4.14	4.14	4.14	4.14
H-1_{proS}	4.32	4.32	4.32	4.32
H-2	5.11	5.11	5.11	5.11
H-3_{proR}	3.84	3.84	3.83	3.82
H-3_{proS}	3.63	3.63	3.64	3.63
Sugars				
H-1′	4.12 (7.9)	4.11 (8.0)	4.12 (7.9)	4.11 (8.0)
H-1″	4.16 (7.0)	4.15 (7.0)	4.20 (7.0)	4.20 (7.0)

[a] Relative to DMSO (δ 2.49).
[b] ^{1}H NMR data of the natural products **1** and **2** were obtained from a mixture of **1** and **2** (ca. 1:1).

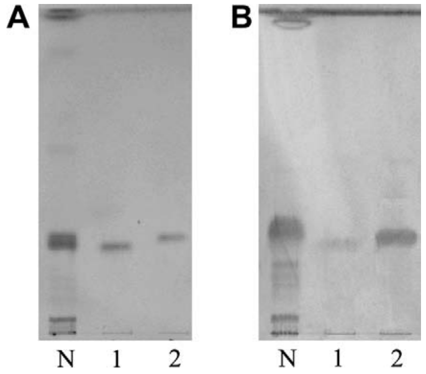


Figure 3. TLC analyses of both natural and synthetic β-glycolipids on HPTLC. [Colored chemically with orcinol (A) and stained immunologically with goat polyclonal antibodies (B). In the latter, the spots were immobilized on the HPTLC plate with poly-isobutyl-methacrylate and then visualized by the polyclonal antibodies from a goat serum infected with *M. pneumoniae*]. N: Natural products isolated from *M. pneumoniae*.

3. Conclusions

We have accomplished stereoselective synthesis for both types of the *M. pneumoniae* β-glycolipids using our non-malodorous thioglycosylation methodology. The synthetic products have enabled us to confirm the absolute structures of the natural products. That is, both of the natural products have a core structure of 3-*O*-β-D-galactopyranosyl-1,2-di-*O*-acyl-*sn*-glycerol. This structure is widely observed in plants.[26,27] Thus, the β-glycolipids of *M. pneumoniae* may be characterized by an extended β-(1→6)-linked disaccharide structure and the existence of two types, namely, β-Gal- and β-Glc-types.

4. Experimental

4.1. General methods

Reagents of the highest commercial quality were purchased and used without further purification. Anhydrous solvents were purchased from Kanto Chemical Co., Inc. All reactions were carried out under a dry argon atmosphere and monitored by thin-layer chromatography (TLC) on E. Merck aluminum-backed Silica Gel 60-F_{254} with visualization using UV light and *p*-anisaldehyde solution. E. Merck Silica Gel 60 (particle size 0.063–0.200 mm) was employed for column chromatography using hexane–EtOAc, toluene–EtOAc, and $CHCl_3$–MeOH as eluents.

^{1}H and ^{13}C NMR spectra were recorded on JEOL-JNM-LA-400, JNM-LA-400s, and JNM-LA-500 spectrometers. Unless otherwise stated, ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C in $CDCl_3$ using an internal Me_4Si standard at 0 ppm. Mass spectra were recorded on a JEOL-HX-110 spectrometer. Optical rotations were determined with JASCO DIP-1000 digital polarimeter using a water-jacketed 100-mm cell at 25 °C. IR spectra were recorded on a JASCO FT/IR-230 Fourier transform infrared spectrometer in the form of a KBr disc.

4.2. *o*-Methoxycarbonylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (3)

To a solution of *o*-methoxycarbonylphenyl 1-thio-β-D-galactopyranoside **6** (11.3 g, 34.1 mmol) and benzaldehyde dimethyl acetal (7.78 mL, 51.2 mmol) in DMF (250 mL) was added *p*-toluenesulfonic acid (648 mg, 3.41 mmol), and the reaction mixture was stirred at 40 °C under 50 mm Hg for 5 h. The reaction was quenched by addition of Et_3N and extracted with EtOAc. The organic layer was washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under diminished pressure. To a solution of the residue in DMF (200 mL) was added NaH (3.27 g, 81.8 mmol) and BnBr (9.79 mL, 81.8 mmol) slowly at 0 °C, and the reaction mixture was stirred at rt for 18 h. The reaction was quenched by addition of MeOH, and to the mixture was added EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 9:1 toluene–EtOAc) to give **3** (12.1 g, 59% 2 steps) as a colorless amorphous solid: $[\alpha]_D^{27}$ +21.2 (*c* 1.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.14–8.00 (m, 19H, Ar), 5.54 (s, 1H, Ar*CH*), 4.61–4.78 (m, 4H, ArCH_2), 4.73 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1), 4.36 (dd, 1H, $J_{5,6}$ 1.5 Hz, $J_{6,6}$ 12.5 Hz, H-6), 4.20 (dd, 1H, $J_{3,4}$ 3.5 Hz, $J_{4,5}$ 0.5 Hz, H-4), 4.05 (t, 1H, $J_{1,2}$ and $J_{2,3}$ 9.0 Hz, H-2), 4.01 (dd, 1H, $J_{5,6}$ 1.5 Hz, $J_{6,6}$ 12.5 Hz, H-6), 3.84 (s, 3H, Me), 3.65 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 3.5 Hz, H-3), 3.46 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.93, 138.14, 138.09, 137.89, 137.84, 132.13, 130.59, 129.99, 129.94, 129.00, 128.36, 128.29, 128.18, 128.15, 127.76, 127.73, 127.63, 126.44, 125.32, 101.10, 85.57, 81.15, 75.61, 75.33, 73.68, 71.84, 69.73, 69.22, 52.10; HRFABMS: *m/z* calcd for $C_{35}H_{34}KO_7S$ $[(M+K)^+]$ 637.1662; found: 637.1657.

4.3. *o*-Methoxycarbonylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (8)

To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**7**) (20.7 g, 50.3 mmol) in DMF (200 mL) were added potassium carbonate (21.1 g, 153 mmol) and methyl thiosalicylate (8.80 g, 52.3 mmol), and the reaction mixture was stirred at rt for 3 h. The reaction mixture was filtered, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 5:1 toluene–EtOAc) to give glycosylated product **8** (19.5 g, 78%) as a colorless solid: $[\alpha]_D^{28}$ −48.1 (*c* 1.56, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.28–7.90 (m, 4H, Ar), 5.28 (t, 1H, $J_{2,3}$ and $J_{3,4}$ 9.5 Hz, H-3), 5.12 (t, 1H, $J_{3,4}$ and $J_{4,5}$ 9.5 Hz, H-4), 5.10 (t, 1H, $J_{1,2}$ and $J_{2,3}$ 9.5 Hz, H-2), 4.90 (d, 1H, $J_{1,2}$ 9.5 Hz, H-1), 4,24 (dd, 1H, $J_{5,6}$ 5.5 Hz, $J_{6,6}$ 12.0 Hz, H-6), 4.17 (dd, 1H, $J_{5,6}$ 5.5 Hz, $J_{6,6}$ 12.0 Hz, H-6), 3.90 (s, 3H, Me), 3.82 (m, 1H, H-5), 2.04 (s × 4, 3H × 4, Ac); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.47, 170.13, 169.34, 169.10, 166.72, 136.50, 132.17, 130.71, 130.65, 129.27, 126.29, 84.21, 75.65, 73.91, 69.69, 68.19, 62.26, 52.16, 20.66, 20.60, 20.53; HRFABMS: *m/z* calcd for $C_{22}H_{26}KO_{11}S$ $[(M+K)^+]$ 537.0833; found: 537.0825.

4.4. *o*-Methoxycarbonylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (4)

To a suspension of **8** (10.4 g, 20.8 mmol) in MeOH (80 mL) were added NaOMe (113 mg, 2.08 mmol), and the reaction mixture was stirred at rt for 5 h. The reaction mixture was neutralized and concentrated under diminished pressure. To a solution of the residue in DMF (100 mL) were added NaH (3.99 g, 99.8 mmol) and BnBr (11.8 mL, 99.8 mmol) slowly at 0 °C, and the reaction mixture was stirred at rt for 8 h. The reaction was quenched by addition of MeOH, and to the mixture was added EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 10:1 toluene–EtOAc) to give **4** (12.1 g, 64% 2 steps) as a colorless solid: $[\alpha]_D^{28}$ −56.0 (*c* 1.53, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.17–7.90 (m, 24H, Ar), 4.50–4.94 (m, 8H, ArCH_2), 4.80 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1), 3.87 (s, 3H, Me), 3.80 (dd, 1H, $J_{5,6}$ 1.5 Hz, $J_{6,6}$ 10.5 Hz, H-6), 3.75 (dd, 1H, $J_{2,3}$ and $J_{3,4}$ 9.0 Hz, H-3), 3.68 (dd, 1H, $J_{5,6}$ 5.0 Hz, $J_{6,6}$ 10.5 Hz, H-6), 3.65 (t, 1H, $J_{3,4}$ and $J_{4,5}$ 9.0 Hz, H-4), 3.63 (t, 1H, $J_{1,2}$ and $J_{2,3}$ 9.0 Hz, H-2), 3.61 (m, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.83, 139.33, 138.31, 138.14, 137.90, 137.79, 132.55, 130.63, 129.03, 128.42, 128.30, 127.89, 127.80, 127.76, 127.69, 127.63, 127.50, 125.13, 86.75, 85.79, 81.09, 79.05, 77.82, 75.84, 75.72, 75.04, 73.39, 69.09, 52.11; HRFABMS: *m/z* calcd for $C_{42}H_{42}KO_7S$ $[(M+K)^+]$ 729.2288; found: 729.2293.

4.5. 3-*O*-Trityl-*sn*-glycerol (10)

To a solution of 1-trityl-(*S*)-glycidol (**9**) (7.47 g, 23.6 mmol) in DMSO (100 mL) was added 1 N KOH (40 mL) at 90 °C for 20 h. The reaction mixture was extracted with EtOAc, and the organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 4:1 hexane–EtOAc) to give diol **10** (5.05 g, 64%) as a colorless solid: $[\alpha]_D^{28}$ +5.20 (*c* 0.91, 4:1$CHCl_3$–MeOH); ^1H NMR (500 MHz, $CDCl_3$): δ 7.23–7.44 (m, 15H, Ar), 3.87 (m, 1H, H-2), 3.69 (m, 1H, H-3), 3.60 (m, 1H, H-3), 3.28 (dd, 1H, $J_{1,1}$ 9.5 Hz, $J_{1,2}$ 4.5 Hz, H-1), 3.22 (dd, 1H, $J_{1,1}$ 9.5 Hz, $J_{1,2}$ 6.0 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 143.56, 128.54, 127.86, 127.12, 86.84, 71.10, 64.90, 64.20; HRFABMS: *m/z* calcd for $C_{22}H_{22}KO_3$ $[(M+K)^+]$ 373.1206; found: 373.1217.

4.6. 1,2-Di-*O*-(4-methoxybenzyl)-3-*O*-trityl-*sn*-glycerol (11)

To a solution of **10** (5.29 g, 15.8 mmol) in DMF (150 mL) were added NaH (1.90 g, 47.4 mmol) and 4-methoxybenzyl chloride (6.7 mL, 47.4 mmol) slowly at 0 °C, and the reaction mixture was stirred at rt for 4 h. The reaction was quenched by addition of MeOH, and to the mixture was added EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 10:1 hexane–EtOAc) to give **11** (8.65 g, 95%) as a colorless syrup: $[\alpha]_D^{29}$ +6.39 (*c* 1.15, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.76–7.44 (m, 23H, Ar), 4.42–4.60 (d × 4, 4H, ArCH_2), 3.79 (s × 2, 6H, Me), 3.73 (m, 1H, H-2), 3.58 (dd, 1H, $J_{1,1}$ 10.2 Hz, $J_{1,2}$ 4.6 Hz, H-1), 3.55 (dd, 1H, $J_{1,1}$ 10.2 Hz, $J_{1,2}$ 5.9 Hz, H-1), 3.23 (dd, 1H, $J_{2,3}$ 5.3 Hz, $J_{3,3}$ 9.9 Hz, H-3), 3.21 (dd, 1H, $J_{2,3}$ 5.1 Hz, $J_{3,3}$ 9.9 Hz, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.00, 144.02, 130.76, 130.41, 129.34, 129.12, 128.69, 127.69, 126.86, 113.64, 113.62, 86.52, 77.17, 72.86, 71.81, 70.22, 63.51, 55.21; HRFABMS: *m/z* calcd for $C_{38}H_{38}KO_5$ $[(M+K)^+]$ 613.2356; found: 613.2368.

4.7. 2,3-Di-*O*-(4-methoxybenzyl)-*sn*-glycerol (5)

To a solution of **11** (8.65 g, 15.1 mmol) in CH_2Cl_2 (80 mL) and MeOH (160 mL) was added Amberlyst 15E ion-exchange resin (3.0 g), and the reaction mixture was stirred at rt for 20 h. The reaction mixture was filtered, washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 4:1 hexane–EtOAc) to give **5** (4.03 g, 80%) as a colorless syrup: $[\alpha]_D^{30}$

−16.5 (*c* 1.62, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.85–7.27 (m, 8H, Ar), 4.44–4.64 (d × 4, 4H, ArCH_2), 3.80 (s × 2, 6H, Me), 3.73 (m, 1H, H-1), 3.67 (m, 1H, H-2), 3.65 (m, 1H, H-1), 3.58 (dd, 1H, $J_{2,3}$ 5.0 Hz, $J_{3,3}$ 10.0 Hz, H-3), 3.55 (dd, 1H, $J_{2,3}$ 6.0 Hz, $J_{3,3}$ 10.0 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.13, 159.10, 130.24, 129.94, 129.33, 129.18, 113.70, 113.67, 77.60, 73.01, 71.64, 69.73, 62.70, 55.11; HRFABMS: *m/z* calcd for $C_{19}H_{24}KO_5$ [$(M+K)^+$] 371.1261; found: 371.1288.

4.8. General procedure for glycosylation of galactosyl donor 3 with glycerol acceptor 5

A suspension of flame-dried 3 Å MS, thiogalactoside donor **3** and 1.2 equiv of alcohol **5** in dry solvent was stirred at rt for 3 h. After cooling to the appropriate temperature, to the mixture was added 1.5 equiv of NIS and then TfOH (catalytic amount). The reaction was stirred when donor **3** was completely consumed. The reaction was quenched by addition of Et_3N and filtered through a pad of Celite. The filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 9:1 toluene–EtOAc) to give glycosylated products **12**α and **12**β.

4.9. 3-*O*-(2′,3′-Di-*O*-benzyl-4′,6′-*O*-benzylidene-β-D-galactopyranosyl)-1,2-di-*O*-(4-methoxybenzyl)-*sn*-glycerol (12)

A suspension of flame-dried 3 Å MS (600 mg), thiogalactoside donor **3** (1.0 g, 1.67 mmol), and alcohol **5** (666 mg, 2.00 mmol) in dry CH_2Cl_2 (15 mL) and dry acetonitrile (15 mL) was stirred at rt for 3 h. To the reaction mixture was added NIS (564 mg, 2.51 mmol) at rt, followed by cooling to −40 °C. As soon as the reaction mixture was cooled, TfOH (10 μL) was added to the mixture at −40 °C, and the reaction mixture was stirred at that temperature for 30 min. The reaction was quenched by addition of Et_3N and filtered through a pad of Celite. The filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 9:1 toluene–EtOAc) to give glycosylated products **12α** (70 mg, 5.5%) and **12β** (760 mg, 60%) as a colorless syrup: **12α** $[\alpha]_D^{27}$ +62.5 (*c* 1.06, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.79–7.51 (m, 23H, Ar), 5.43 (s, 1H, ArCH), 4.94 (d, 1H, $J_{1',2'}$ 3.4 Hz, H-1′), 4.38-4.85 (d × 6, 6H, ArCH_2), 4.58 (s, 2H, CH_2 of PMB), 4.11, (m, 1H, H-4′), 4.06 (dd, 1H, $J_{1',2'}$ 3.4 Hz, $J_{2',3'}$ 10.1 Hz, H-2′), 3.94 (dd, 1H, $J_{2',3'}$ 10.1 Hz, $J_{3',4'}$ 3.4 Hz, H-3′), 3.75–4.11 (m, 4H, H-3, H-3, H-6, H-6), 3.79 (m, 1H, H-2), 3.78 (s × 2, 6H, Me of PMB), 3.56 (m, 2H, H-1), 3.53 (m, 1H, H-5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.43, 159.38, 139.09, 139.07, 138.14, 130.92, 130.62, 129.56, 129.13, 128.58, 128.55, 128.38, 128.03, 127.94, 127.81, 126.63, 114.00, 113.97, 101.31, 98.78, 76.67, 75.95, 74.98, 73.62, 73.31, 72.22, 71.98, 69.90, 69.66, 68.23, 62.85, 55.55; HRFABMS: *m/z* calcd for $C_{46}H_{50}O_{10}K$ [$(M+K)^+$] 801.3041; found: 801.3019. **12β** $[\alpha]_D^{32}$ 17.7 (*c* 0.86, $CHCl_3$); IR (disc); *v* 3453 and 1247, 1095, 1032 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ *6.80–7.56* (m, 23H, Ar), 5.50 (s, 1H, ArCH), 4.73–4.87 (d × 4, 4H, ArCH_2), 4.61 (s, 2H, CH_2 of PMB), 4.42 (s, 2H, CH_2 of PMB), 4.39 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.27 (dd, 1H, $J_{5',6'}$ 1.5 Hz, $J_{6',6'}$ 12.5 Hz, H-6′), 4.10 (dd, 1H, $J_{3',4'}$ 3.5 Hz, $J_{4',5'}$ 0.5 Hz, H-4′), 4.05 (dd, 2H, $J_{2,3}$ 5.0 Hz, $J_{3,3}$ 10.5 Hz, H-3), 4.00 (dd, 1H, $J_{5',6'}$ 2.0 Hz, $J_{6',6'}$ 12.5 Hz, H-6′), 3.80 (m, 1H, H-2), 3.76 (s × 2, 6H, Me of PMB), 3.68 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,3}$ 10.5 Hz, H-3), 3.65 (dd, 1H, $J_{1,1}$ 10.0 Hz, $J_{1,2}$ 4.5 Hz, H-1), 3.58 (dd, 1H, $J_{1,1}$ = 10.0 Hz, $J_{1,2}$ 6.0 Hz, H-1), 3.53 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 3.27 (ddd, 1 H, $J_{4',5'}$ 0.5 Hz, $J_{5',6'}$ 1.5 Hz and 2.0 Hz, H-5′); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.01, 138.83, 138.39, 137.85, 130.74, 130.44, 129.24, 129.16, 128.84, 128.27, 128.16, 128.07, 127.89, 127.67, 127.59, 127.37, 126.43, 113.64, 113.63, 103.78, 101.19, 79.10, 78.34, 75.08, 73.88, 72.88, 71.91, 71.64, 70.05, 69.15, 68.95, 66.34, 55.17, 54.33; HRFABMS: *m/z* calcd for $C_{46}H_{50}O_{10}K$ [$(M+K)^+$] 801.3041; found: 801.3116.

4.10. 3-*O*-(2′,3′,4′-Tri-*O*-benzyl-β-D-galactopyranosyl)-1,2-di-*O*-(4-methoxybenzyl)-*sn*-glycerol (13)

A solution of 1.1 M BH_3 in THF (5.1 mL) was added to a flask containing compound **12β** (427 mg, 0.560 mmol) at 0 °C and the solution was stirred for 5 min. A solution of 1.0 M Bu_2BOTf in Et_2O (560 μL) was then slowly added to the solution. After 1 h at 0 °C, Et_3N was added to the reaction mixture, followed by addition of MeOH until the evolution of H_2 had ceased. The reaction mixture was then coevaporated with MeOH, and the residue was purified by chromatography (silica gel, 4:1 toluene–EtOAc) to give alcohol **13** (360 mg, 84%) as a colorless syrup: $[\alpha]_D^{29}$ −13.5 (*c* 1.40, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 6.80–7.37 (m, 24H, Ar), 4.64–4.96 (d × 6, 6H, ArCH_2), 4.58 and 4.41 (s × 2, 4H, ArCH_2), 4.34 (d, 1H, $J_{1',2'}$ 7.7 Hz, H-1′), 3.96 (dd, 1H, $J_{2,3}$ 4.7 Hz, $J_{3,3}$ 10.3 Hz, H-3), 3.81 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.8 Hz, H-2′), 3.72–3.78 (m, 3H, H-2, H-4′, H-6′), 3.77 (s, 6H, Me), 3.66 (dd, 1H, $J_{2,3}$ 3.1 Hz, $J_{3,3}$ 10.3 Hz, H-3), 3.65 (dd, 1H, $J_{1,1}$ 10.4 Hz, $J_{1,2}$ 5.2 Hz, H-1), 3.55 (dd, 1H, $J_{1,1}$ 10.4 Hz, $J_{1,2}$ 5.8 Hz, H-1), 3.50 (dd, 1H, $J_{2',3'}$ 2.9 Hz, $J_{3',4'}$ 9.8 Hz, H-3′), 3.33 (m, 1H, H-5′); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.05, 138.64, 138.35, 138.17, 130.65, 130.32, 129.28, 129.28, 129.20, 128.63, 128.42, 128.40, 128.21, 128.01, 127.95, 127.65, 127.59, 127.46, 113.68, 113.65, 104.26, 82.15, 79.51, 75.06, 74.62, 74.11, 73.39, 72.91, 72.81, 71.71, 70.57, 70.14, 69.16, 61.93, 55.21, 55.19; HRFABMS: *m/z* calcd for $C_{46}H_{52}$ KO_{10} $[M+K]^+$ 803.3198; found: 803.3239.

4.11. 3-*O*-[(2″,3″-Di-*O*-benzyl-4″,6″-*O*-benzylidene-β-D-galactopyranosyl)-(1→6)-(2′,3′,4′-tri-*O*-benzyl-β-D-galactopyranosyl)]-1,2-di-*O*-(4-methoxybenzyl)-*sn*-glycerol (14)

A suspension of flame-dried 3 Å MS (50 mg), thiogalactoside donor **3** (53.6 mg, 89.6 μmol), and alcohol **13** (54.7 mg, 71.5 μmol) in dry CH_2Cl_2 (1 mL) and dry acetonitrile (1 mL) was stirred at rt for 3 h. To the reaction mixture was added NIS (25.2 mg, 112 μmol) at rt, followed by cooling to −40 °C. As soon as the reaction mixture was cooled, TfOH (2 μL) was added to the mixture, and the reaction mixture was stirred at that temperature for 30 min. The reaction was quenched by addition of Et_3N, and filtered through a pad of Celite, and the filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 8:1 toluene–EtOAc) to give glycosylated product **14** (59.1 mg, 69%) as a colorless syrup: $[\alpha]_D^{30}$ +16.6 (*c* 1.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.76–7.53 (m, 38H, Ar), 5.46 (s, 1H, ArCH), 4.33-4.94 (m, 14H, ArCH_2), 4.45 (d, 1H, $J_{1'',2''}$ 7.8 Hz, H-1′), 4.28 (d, 1H, $J_{1',2'}$ 7.7 Hz, H-1″), 4.18 (dd, 1H, $J_{5'',6''}$ 1.4 Hz, $J_{6'',6''}$ 12.2 Hz, H-6″), 4.03 (m, 1H, H-4″), 3.96 (dd, 1H, $J_{2,3}$ 4.9 Hz, $J_{3,3}$ 10.4 Hz, H-3), 3.92 (dd, 1H, $J_{5'',6''}$ 2.0 Hz, $J_{6'',6''}$ 12.0 Hz, H-6″), 3.91 (dd, 1H, $J_{5',6'}$ 5.6 Hz, $J_{6',6'}$ 10.5 Hz, H-6′), 3.70–3.80 (m, 5H, H-2, H-2′, H-4′, H-5′, H-6′, H-2″), 3.74 and 3.73 (s × , 6H, Me), 3.49-3.54 (m, 4H, H-1 × 2, H-3, H-3″), 3.44 (dd, 1H, $J_{2',3'}$ 9.8 Hz, $J_{3',4'}$ 3.0 Hz, H-3′), 3.14 (m, 1H, H-5″); ^{13}C NMR (125 MHz, $CDCl_3$); δ 159.02, 158.97, 138.97, 138.757, 138.52, 138.46, 138.42, 137.86, 130.84, 130.48, 129.25, 129.12, 128.86, 128.38, 128.29, 128.28, 128.22, 128.15, 128.06, 128.04, 127.75, 127.64, 127.59, 127.54, 127.50, 127.45, 127.44, 127.37, 126.41, 113.66, 113.58, 104.11, 103.32, 101.19, 81.97, 79.32, 78.47, 76.80, 75.01, 74.95, 74.37, 74.00, 73.85, 73.60, 73.03, 72.84, 71.96, 71.62, 70.35, 69.21, 69.07, 67.95, 66.25, 55.18; HRFABMS: *m/z* calcd for $C_{60}H_{66}KO_{13}$ $[M+K]^+$ 1233.4978; found: 1233.4990.

4.12. 3-*O*-[(2″,3″-Di-*O*-benzyl-4″,6″-*O*-benzylidene-β-D-galactopyranosyl)-(1→6)-(2′,3′,4′-tri-*O*- benzyl-β-D-galactopyranosyl)]-1,2-di-*O*-palmitoyl-*sn*-glycerol (15)

To a solution of compound **14** (30 mg, 25.1 μmol) in a mixture of CH_2Cl_2 (1.0 mL), 2-PrOH (500 μL), and H_2O (500 μL) was added DDQ (15.2 mg, 60.2 μmol) at 0 °C, and the mixture was stirred at rt for 5 h. To the reaction mixture was added satd $NaHCO_3$, followed by washing with brine. The organic extract was dried over $MgSO_4$, and concentrated under diminished pressure. The residue was dissolved in pyridine (500 μL), and to the solution was added a solution of palmitoyl chloride (22.5 μL, 75.3 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C. After stirring at rt for 15 h, the reaction was quenched by addition of MeOH at 0 °C, and the mixture was extracted with toluene. The organic layer was washed with satd $NaHCO_3$ and satd brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 15:1 toluene–EtOAc) to give diacylglycerogalactoside **15** (28.7 mg, 80%) as a colorless amorphous solid: $[\alpha]_D^{30}$ +11.5 (*c* 0.954, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.20–7.54 (m, 30H, Ar), 5.49 (s, 1H, Ar*CH*), 5.12 (m, 1H, H-2), 4.60-4.93 (d × 10, 10H, ArCH_2), 4.45 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.25 (dd, 1H, $J_{1,1}$ 12.0 Hz, $J_{1,2}$ 3.5 Hz, H-1), 4.23 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.23 (dd, 1H, $J_{5'',6''}$ 1.5 Hz, $J_{6'',6''}$ 11.5 Hz, H-6″), 4.13 (dd, 1H, $J_{1,1}$ 12.0 Hz, $J_{1,2}$ 7.0 Hz, H-1), 4.12 (bd, 1H, $J_{3'',4''}$ 3.0 Hz, H-4″), 4.00 (dd, 1H, $J_{5'',6''}$ 2.0 Hz, $J_{6'',6''}$ 11.5 Hz, H-6″), 3.91 (dd, 1H, $J_{2,3}$ 5.0 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.90 (dd, 1H, $J_{5',6'}$ 5.0 Hz, $J_{6',6'}$ 11.0 Hz, H-6′), 3.74-3.79 (m, 4H, H-2″, H-2′, H-4′, H-6′), 3.55 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, $J_{3'',4''}$ 3.5 Hz, H-3″), 3.50 (m, 1H, H-5′), 3.47 (dd, 1H, $J_{2,3}$ 5.0 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.44 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$ 3.0 Hz, H-3′), 3.31 (ddd, 1H, $J_{4'',5''}$ 0.5 Hz, $J_{5'',6''}$ 1.5 Hz,2.0 Hz, H-5″), 2.34 and 2.23 (t × 2, 4 H, $-OCOCH_2-$), 1.63 (m, 4H, $-OCOCH_2CH_2-$), 1.25 (b, 48H, $-COCH_2CH_2(CH_2)_{12}-$), 0.88 (t × 2, 6H, $-CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.35, 173.02, 139.01, 138.68, 138.43, 137.90, 128.92, 128.53, 128.39, 128.36, 128.31, 128.28, 128.24, 128.14, 128.10, 127.78, 127.74, 127.69, 127.62, 127.58, 127.55, 127.52, 126.48, 104.18, 103.51, 101.29, 81.94, 79.30, 79.09, 78.49, 77.26, 75.05, 74.45, 74.17, 73.92, 73.46, 73.17, 72.00, 69.97, 69.18, 68.13, 67.92, 66.40, 62.86, 34.29, 34.13, 31.97, 29.75, 29.73, 29.70, 29.64, 29.56, 29.41, 29.37, 29.35, 29.20, 29.15, 24.93, 24.90, 22.74, 14.17; MALDI-TOFMS *m/z* calcd for $C_{89}H_{122}O_{15}Na$ $[M+Na]^+$ 1454; found: 1454.

4.13. 3-*O*-[β-D-Galactopyranosyl-(1→6)-β-D-galactopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (1)

To a solution of benzyl ether **15** (55 mg, 38 μmol) in a mixture of THF (1.5 mL) and MeOH (10 mL) was added 10% Pd/C (catalytic amount). (Caution! Extreme fire hazard!) The reaction mixture was stirred vigorously under an H_2 atmosphere at rt for 8 h. The reaction mixture was filtered through a glass filter, and the filtrate was concentrated under diminished pressure, and the residue was purified by chromatography (Iatrobeads, 10:1 $CHCl_3$–MeOH) to give **1** (27 mg, 88%) as a white crystal: $[\alpha]_D^{24}$ −0.82 (*c* 0.1, 2:1 $CHCl_3$–MeOH); IR (film): ν 3415, 1733, 1070 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$–CD_3OD = 4:1): δ 5.25 (m, 1H, H-2), 4.37 (dd, 1H, $J_{1,1}$ 12.0 Hz, $J_{1,2}$ 3.5 Hz, H-1), 4.30 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.23 (dd, 1H, $J_{1,1}$ 11.5 Hz, $J_{1,2}$ 6.5 Hz, H-1), 4.22 (d, 1H, $J_{1',2'}$ 7.0 Hz, H-1′), 4.03 (dd, 1H, $J_{5',6'}$ 6.5 Hz, $J_{6',6'}$ 10.5 Hz, H-6′), 3.97 (br d, 1H, $J_{3',4'}$ 3.0 Hz, H-4′), 3.91 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.88 (br d, 1H, $J_{3'',4''}$ 4.0 Hz, H-4″), 3.86 (dd, 1H, $J_{5',6'}$ 6.0 Hz, $J_{6',6'}$ 11.0 Hz, H-6′), 3.84 (dd, 1H, $J_{5'',6''}$ 7.0 Hz, $J_{6'',6''}$ 11.5 Hz, H-6″), 3.76 (dd, 1H, $J_{5'',6''}$ 5.0 Hz, $J_{6'',6''}$ 12.0 Hz, H-6″), 3.73 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.67 (m, 1H, H-5′), 3.56 (dd, 1H, $J_{1'',2''}$ 7.5 Hz, $J_{2'',3''}$ 9.5 Hz, H-2″), 3.54 (dd, 1H, $J_{1',2'}$ 7.5 Hz, $J_{2',3'}$ 9.5 Hz, H-2′), 3.53 (m, 1H, H-5″), 3.50 (dd, 1H, $J_{2',3'}$ 9.5 Hz, $J_{3',4'}$ 3.5 Hz, H-3′), 3.49 (dd, 1H, $J_{2'',3''}$ 9.5 Hz, $J_{3'',4''}$ 4.0 Hz, H-3″), 2.32 and 2.30 (t × 2, 4H, $-OCOCH_2-$), 1.61 (m, 4H, $-OCOCH_2CH_2-$), 1.27 (b, 48H, $-OCOCH_2CH_2(CH_2)_{12}-$), 0.88 (t × 2, 6H, $-CH_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD = 10:1): δ 174.12, 173.88, 103.98, 103.61, 74.99, 73.55, 73.26, 73.11, 71.28, 70.33, 69.16, 68.07, 67.77, 67.41, 62.83, 61.77, 34.40, 34.24, 32.03, 29.81, 29.77, 29.63, 29.47, 29.42, 29.25, 29.22, 25.00, 24.98, 22.79, 14.16; HRFABMS: *m/z* calcd for $C_{47}H_{88}O_{15}Na$ $[M+Na]^+$ 915.6021; found: 915.5977.

4.14. 3-*O*-[(2″,3″,4″,6″-Tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-(2′,3′,4′-tri-*O*-benzyl-β-D- galactopyranosyl)]-1,2-di-*O*-(4-methoxybenzyl)-*sn*-glycerol (16)

A suspension of flame-dried 3 Å MS (50 mg), thioglucoside donor **4** (59.3 mg, 85.8 μmol), and acceptor alcohol **13** (52.4 mg, 68.5 μmol) in dry CH_2Cl_2 (1.0 mL) and dry acetonitrile (1.0 mL) was stirred at rt for 3 h. To the reaction mixture was added NIS (24.1 mg, 107 μmol) at rt, followed by cooling to −40 °C. As soon as the reaction mixture was cooled, to the mixture was added TfOH (2.0 μL), and the reaction mixture was stirred at that temperature for 15 min. The reaction was quenched by addition of Et_3N, and the mixture was filtered through a pad of Celite. The filtrate was washed with 5% aq $Na_2S_2O_3$, satd $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 10:1 toluene–EtOAc) to give glycosylated product **16** (60.8 mg, 69%) as a colorless syrup: $[\alpha]_D^{30}$ +5.16 (*c* 1.440, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 6.75–7.36 (m, 39H, Ar), 4.39–4.93 (d × 18, 18H, ArCH_2), 4.43 (d, 1H, $J_{1'',2''}$ 7.7 Hz, H-1″), 4.31 (d, 1H, $J_{1',2'}$ 7.4 Hz, H-1′), 3.96 (dd, 1H, $J_{5',6'}$ 4.5 Hz, $J_{6',6'}$ 10.4 Hz, H-6′), 3.88 (dd, 1H, $J_{5',6'}$ 5.4 Hz, $J_{6',6'}$ 10.4 Hz, H-6′), 3.74 and 3.72 (s × 2, 6H, Me), 3.52-3.81 (m, 11H, H-1 × 2, H-2, H-3 × 2, H-4′, H-2″, H-3″, H-4″, H-6″ × 2), 3.49 (m, 1H, H-5′), 3.44 (dd, 1H, $J_{2',3'}$ 9.9 Hz, $J_{3',4'}$ 2.9 Hz, H-3′), 3.36-3.41 (m, 2H, H-2′, H-5″); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.01, 158.96, 138.72, 138.55, 138.51, 138.46, 138.15, 138.05, 130.77, 130.47, 129.19, 129.12, 128.40, 128.33, 128.30, 128.17, 128.04, 127.86, 127.81, 127.75, 127.67, 127.58, 127.56, 127.52, 127.48, 127.39, 113.65, 113.60, 104.16, 103.61, 84.67, 82.15, 81.97, 79.33, 77.65, 75.63, 74.97, 74.92, 74.67, 74.55, 74.38, 73.82, 73.58, 73.43, 73.08, 72.83, 71.54, 70.30, 69.07, 68.71, 68.40, 55.16; HRFABMS: *m/z* calcd for $C_{80}H_{86}O_{15}K$ $[M+K]^+$ 1325.5604; found: 1325.5701.

4.15. 3-*O*-[(2″,3″,4″,6″-Tetra-*O*-benzyl-β-D-glucopyranosyl)-(1→6)-(2′,3′,4′-tri-*O*-benzyl-β-D- galactopyranosyl)]-1,2-di-*O*-palmitoyl-*sn*-glycerol (17)

To a solution of compound **16** (29 mg, 22.5 μmol) in a mixture of CH_2Cl_2 (1.0 mL), 2-PrOH (500 μL) and H_2O (500 μL) was added DDQ (13.6 mg, 53.7 μmol) at 0 °C, and the mixture was stirred at rt for 5 h. To the reaction mixture was added satd $NaHCO_3$, and the organic extract was then washed with brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was dissolved in pyridine (500 μL), and to the solution was added a solution of palmitoyl chloride (20.3 μL, 67.2 μmol) in CH_2Cl_2 (1.0 mL) at 0 °C. After stirring at rt for 18 h, the reaction was quenched by addition of MeOH at 0 °C, followed by extraction with toluene. The organic layer was washed with satd $NaHCO_3$ and satd brine, dried over $MgSO_4$, and concentrated under diminished pressure. The residue was purified by chromatography (silica gel, 15:1 toluene–EtOAc) to give diacylglycerogalactoside **17** (28.5 mg, 83% 2 steps) as a colorless amorphous solid: $[\alpha]_D^{27}$ +5.49 (*c* 0.760, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.13–7.36 (m, 35H, Ar), 5.13 (m, 1H, H-2), 4.45–4.92 (d × 14, 14H, ArCH_2), 4.41 (d, 1H, $J_{1'',2''}$ 7.5 Hz, H-1″), 4.26 (d, 1H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.25 (dd, 1H, $J_{1,1}$ 12.5 Hz, $J_{1,2}$ 3.0 Hz, H-1), 4.12 (dd, 1H, $J_{1,1}$ 12.5 Hz, $J_{1,2}$ 6.5 Hz, H-1), 3.93 (dd, 1H, $J_{2,3}$ 4.0 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.87 (dd, 1H, $J_{5',6'}$ 5.0 Hz, $J_{6',6'}$ 11.0 Hz, H-6′), 3.75-3.80 (m, 3H, H-2′, H-4′, H-6′), 3.68 (m, 2H, H-4″, H-6″), 3.62 (t, 1H, $J_{2'',3''}$ $J_{3'',4''}$ 7.0 Hz, H-3″), 3.61 (dd, 1H, H-6″), 3.51 (dd, 1H, $J_{2,3}$ 5.0 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.49 (b, 1H, H-5′), 3.44 (dd, 1H, $J_{2',3'}$ 10.0 Hz, $J_{3',4'}$ 3.0 Hz, H-3′), 3.37–3.42 (m, 2H, H-2″, H-5″), 2.34 (t × 2, 4H, $-OCOCH_2-$), 1.63 (m, 4H, $-OCOCH_2CH_2-$), 1.22 (b, 48H, $-OCOCH_2CH_2(CH_2)_{12}-$), 0.88 (t × 2, 6H, $-CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.25, 172.93, 138.61, 138.53, 138.50, 138.37, 138.32, 138.11, 138.02, 128.47, 128.36, 128.35, 128.33, 128.22, 128.10, 128.04, 127.90, 127.83, 127.76, 127.73, 127.70, 127.68, 127.61, 127.57, 127.53, 127.50, 104.17, 103.64, 84.66, 82.16, 81.90, 79.08, 77.64, 77.20, 75.68, 75.03, 74.97, 74.69, 74.54, 74.42, 73.95, 73.47, 73.16, 69.87, 68.71, 68.45, 67.93, 34.22, 34.08, 31.93, 29.71, 29.67, 29.60, 29.52, 29.43, 29.36, 29.32, 29.30, 29.21, 29.15, 29.10, 24.87, 24.86, 22.69, 14.13; MALDI-TOFMS: *m/z* calcd for $C_{96}H_{130}O_{15}Na$ $[M+Na]^+$ 1547; found: 1547.

4.16. 3-*O*-[β-D-Glucopyranosyl-(1→6)-β-D-galactopyranosyl]-1,2-di-*O*-palmitoyl-*sn*-glycerol (2)

To a solution of benzyl ether **17** (30 mg, 20 μmol) in a mixture of THF (1 mL) and MeOH (7 mL) was added 10% Pd/C (catalytic amount). (Caution! Extreme fire hazard!) The reaction mixture was stirred vigorously under an H_2 atmosphere at rt for 8 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated under diminished pressure, and the residue was purified by chromatography (Iatrobeads, 15:1 $CHCl_3$–MeOH) to give **2** (16 mg, 89%) as a white crystalline product: $[\alpha]_D^{25}$ −8.94 (*c* 0.1, 2:1 $CHCl_3$–MeOH); IR (film): ν 3350, 1737, 1069 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD = 10:1): δ 5.26 (m, 1H, H-2), 4.35 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1″), 4.33 (dd, 1H, $J_{1,1}$ 12.0 Hz, $J_{1,2}$ 3.5 Hz, H-1), 4.23 (dd, 1H, $J_{1,1}$ 12.0 Hz, $J_{1,2}$ 7.0 Hz, H-1), 4.21 (d, 1H, $J_{1',2'}$ 6.5 Hz, H-1′), 4.01 (dd, 1H, $J_{5',6'}$ 7.5 Hz, $J_{6',6'}$ 10.5 Hz, H-6′), 3.99 (b, 1H, H-4′), 3.94 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.88 (dd, 1H, $J_{5'',6''}$ 3.0 Hz, $J_{6'',6''}$ 12.0 Hz, H-6″), 3.86 (dd, 1H, $J_{5',6'}$ 6.0 Hz, $J_{6',6'}$ 10.0 Hz, H-6′), 3.73 (dd, 1H, $J_{5'',6''}$ 5.0 Hz, $J_{6'',6''}$ 12.0 Hz, H-6″), 3.70 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,3}$ 11.0 Hz, H-3), 3.64 (m, 1H, H-5′), 3.50–3.56 (m, 2H, H-2′, H-3′), 3.43 (m, 2H, H-3″, H-4″), 3.32 (m, 1H, H-5″), 3.28 (dd, 1H, $J_{1'',2''}$ $J_{2'',3''}$ 8.5 Hz, H-2″), 2.32 and 2.31 (t × 2, 4H, $-OCOCH_2-$), 1.60 (m, 4H, $-OCOCH_2CH_2-$), 1.25 (b, 48H, $-OCOCH_2CH_2(CH_2)_{12}-$), 0.88 (t × 2, 6H, $-CH_2CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD = 10:1): δ 174.29, 174.04, 104.08, 103.27, 76.65, 76.28, 73.59, 73.50, 73.24, 71.34, 70.43, 70.30, 68.11, 68.00, 67.75, 62.91, 61.75, 34.47, 34.30, 32.10, 29.88, 29.86, 29.84, 29.70, 29.69, 29.54, 29.50, 29.31, 29.28, 25.07, 25.05, 22.86, 14.19; HRFABMS: *m/z* calcd for $C_{47}H_{88}O_{15}Na$ $[M+Na]^+$ 915.6021; found: 915.6000.

4.17. High-performance thin-layer chromatography (HPTLC) and immunostaining of the synthetic glyceroglycolipids on an HPTLC plate

The synthetic GGLs and other glycolipids extracted from *M. pneumoniae* were chromatographed with an HPTLC-plate in 80:20 chloroform–methanol. Those glycolipids were visualized by the orcinol reagent. Immunostaining of the glycolipid on the HPTLC-plate was performed according to the method of Matsuda et al. The glycolipids from *M. pneumoniae* and the synthetic products were chromatographed with an HPTLC-plate. The plates were dipped in 0.4% poly-isobutyl-methacrylate solution (diluted 2.5% polymer solution in chloroform with hexane, by volume) for 1 min, followed by drying. The HPTLC-plates were blocked with 2% BSA (bovine serum albumin fraction V) in PBS for 3 h, and the fluid was aspirated. Then the diluted polyclonal antibody which was isolated from the blood serum of a goat infected with *M. pneumoniae* was overlaid on the HPTLC-plate and incubated at 4 °C overnight. After the plates were rinsed with PBS three times, the secondary horseradish peroxidase (HPR) conjugates were added and incubated at rt for 2 h. The HPTLC-plates were then rinsed with PBS three more times, and visualized with the Konica Immunostaining HRP Kit (Konica, Tokyo).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2008.09.028.

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