An easy α-glycosylation methodology for the synthesis and stereochemistry of mycoplasma α-glycolipid antigens

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Abstract

Mycoplasma fermentans possesses unique α-glycolipid antigens (GGPL-I and GGPL-III) at the cytoplasm membrane, which carry a phosphocholine group at the sugar primary (6-OH) position. This paper describes a practical synthetic pathway to a GGPL-I homologue (C16:0) and its diastereomer, in which our one-pot α-glycosylation method was effectively applied. The synthetic GGPL-I isomers were characterized with 1H NMR spectroscopy to determine the equilibrium among the three conformers (gg, gt, tg) at the acyclic glycerol moiety. The natural GGPL-I isomer was found to prefer gt (54%) and gg (39%) conformers around the lipid tail, while adopting all of the three conformers with equal probability around the sugar position. This property was very close to what we have observed with respect to the conformation of phosphatidylcholine (DPPC), suggesting that the Mycoplasma glycolipids GGPLs may constitute the cytoplasm fluid membrane together with ubiquitous phospholipids, without inducing stereochemical stress.

Introduction

Mycoplasmas constitute a family of gram-positive microbes lacking rigid cell walls. They are suspected to be associated with human immune diseases, in either direct or indirect ways, although the molecular mechanism is not fully understood [1]. In recent biochemical studies, Mycoplasma outer-membrane lipoproteins [2,3] and glycolipids [4-6] are thought to serve not only as the main antigens but also as probable pathogens. Also in our research team, Matsuda et al. [7-10] isolated a new class of α-glycolipid antigens (GGPL-I and GGPL-III, Figure 1) from M. fermentans. Another α-glycolipid (MIYL-II), which has a chemical structure very close to GGPL-III, was identified and characterized by other groups [11-14].

Keywords:
cytoplasm membrane; glycolipid antigen; glycosylation; mycoplasma; stereochemistry
Absolute chemical structures of GGPL-I [15] and GGPL-III [16] have already been established by chemical syntheses of stereoisomers; these α-glycolipids have a common chemical backbone of 3-O-(α-D-glucopyranosyl)-sn-glycerol carrying phosphocholine at the sugar primary (6-OH) position. The fatty acids at the glycerol moiety are saturated, namely palmitic acid (C_{16:0}) and stearic acid (C_{18:0}). GGPL-I has a structural feature analogous to 1,2-di-O-palmitoyl phosphatidylcholine (DPPC) as a ubiquitous cell membrane phospholipid. Apparently, GGPLs are amphiphilic compounds that can form certain self-assembled structures under physiological conditions [12,13] and may give physicochemical stress on the immune system of the host [17]. In fact, our research team has proven that these α-glycolipid antigens have certain pathogenic functions [18,19].

In order to exploit their biological functions in detail, it is necessary to obtain these α-glycolipids in sufficient amounts. Thus, both genetic [20-22] and chemical synthetic approaches [23,24] are being followed, although no practical way has yet been established. In this paper, we report a chemical access to both a natural GGPL-I homologue (C_{16:0}) and its diastereomer I-a and I-b, in which our one-pot α-glycosylation methodology [25,26] is effectively applied. The two GGPL-I isomers prepared thereby were characterized with 1H NMR spectroscopy, in terms of configuration and conformation at the asymmetric glycerol moiety.

Results and Discussion
A practical synthetic access to GGPL-I homologues

GGPL-I provides two key asymmetric centers to be controlled, literally, in the synthetic pathway. One is the configuration at the chiral glycerol moiety, and another is the sugar α-glycoside linkage. In former synthetic works on 3-O-(α-D-glucopyranosyl)-sn-glycerol [27-30], chiral 1,2-O-isopropylidene-sn-glycerol has often been employed [29,30] as the acceptor substrate for different α-glycosylation reactions. In this case, however, attention should be paid to the acid-catalyzed migration of the dimethylketal group [23,29-31]. In our synthetic pathway, chiral (S)- or (R)-glycidol is employed as an alternative source of the chiral glycerol to circumvent this problem. In an established synthetic approach, 6-O-acetyl-2,3,4-tri-O-benzyl protected sugar I [23] is used as the donor and treated with a reagent combination of CBr_4 and Ph_3P (Appel–Lee reagent) in either CH_2Cl_2 or DMF solvent, or a mixture of the two (Scheme 1).

For the reaction in CH_2Cl_2, N,N,N',N'-tetramethylurea (TMU) is added after in situ formation of α-glycosyl bromide 2, which
equilibrates with a more reactive β-glycosyl bromide species [32]. In the pathway using DMF, the α-glycosylation is routed via α-glycosyl cationic imidate 3, which was predicted in former studies [33] and evidenced in our preceding NMR and MS study [25,26].

The reaction between 1 and (S)-glycidol in CH$_2$Cl$_2$ (+ TMU) gave a mixture of epoxy compound 4a (60–70%) and bromide 5a (30–40%). In 5a, the oxirane ring was opened by nucleophilic Br$^-$ ions produced by Ph$_3$P and CBr$_4$. Also in the DMF-promoted reaction, a mixture of 4a (70–90%) and 5a (10–30%) was derived. In both reaction pathways, however, the glycosylation was α-selective (α:β ≥ 90:10, yields >80%) and not accompanied by isomerization at the glycerol moiety.

A mixture of 4a and 5a was used in the following chemical transformation (Scheme 2). First, a lyso-glycolipid 6a was derived after deprotection at the sugar hydroxymethyl position and S$_2$N$_2$ substitution with cesium palmitate at the glycerol sn-1 position. Then, this compound was converted to glycolipid 8a after sequential reaction of the temporary tert-butyl-dimethylsilyl (TBDMS) -protected sugar, and O-acylation at the glycerol 2-OH position to give 7a, followed by removal of the TBDMS protecting group. For introducing the phosphocholine group at the sugar 6-OH position, we employed a phosphoramidite method using 1H-tetrazole as a promoter [34]. First, 8a was treated with 2-cyanoethyl-$N,N'$-$N''$-$N'''$-tetraisopropylphosphorodiamidite in the presence of 1H-tetrazole, and then with choline tosylate to give 9a. After removal of the sugar O-benzyl group by catalytic hydrogenolysis, the GGPL-I homologue 1-a was obtained. In the same way, the GGPL-I sn-isomer 1-b was derived from a mixture of 4b and 5b available from the reaction between 1 and (R)-glycidol (Scheme 1 and Scheme 2b).

**Scheme 2:** Syntheses of GGPL-I homologue 1-a and its isomer 1-b. Conditions: (a) K$_2$CO$_3$, CH$_3$OH; (b) cesium palmitate in DMF; (c) TBDMS chloride then palmitoyl chloride in pyridine + DMAP; (d) TFA in CH$_3$OH; (e) (i) 2-cyanoethyl-$N,N'$-$N''$-$N'''$-tetraisopropylphosphorodiamidite, 1H-tetrazole and MS-4 Å in CH$_2$Cl$_2$; (ii) choline tosylate, 1H-tetrazole, (iii) mCPBA, (iv) aq. NH$_3$ in CH$_3$OH, (f) H$_2$, Pd(OH)$_2$/C in CH$_3$OH.
1H NMR characterization of I-a, I-b and the related glycerolipids

1H NMR spectroscopy provides a useful tool for discriminating between the two GGPL-I isomers as shown in Figure 2. A clear difference was observed in the chemical shifts of the glycerol methylene protons as designated by “a” and “b”. Conversely, little difference was observed between the sn-isomers at the sugar H-1 signal as well as at the glycerol H-2 (Table 1).

The glycerol moiety has two C–C single bonds. By free rotation, each of them is allowed to have three staggered conformers of gg (gauche-gauche), gt (gauche-trans) and tg (trans-gauche) (Figure 3). In solution and also in self-

![Figure 2: 1H NMR spectra of I-a and I-b (500 MHz, 25 °C, CDCl3/CD3OD 10:1). The assignment of sn-glycerol methylene protons (H_{proR} and H_{proS}) was performed on the basis of our preceding studies on deuterium-labeled glycerols [35-37] and α(1→6)-linked disaccharides [38-40].](image)

Table 1: 1H NMR data (500 MHz) of I-a, I-b, and their precursors (9a and 9b).

| Compound | glucose | sn-glycerol moiety |
|----------|---------|---------------------|
|          | δ [ppm] (^3J and ^2J [Hz]) |\n|          | H-1' | H-1 | H-2 | H-3 |
|          | proR | proS | proR | proS |
| I-a^a    | 4.80 (3.5) | 4.14 (6.5) | 4.40 (3.5, 12.0) | 5.24 | 3.76 (5.5) | 3.61 (5.5, 11.0) |
| 9a       | 4.70 (3.5) | 4.17 (6.5) | 4.38 (3.0, 12.0) | 5.22 | 3.72 (5.5) | 3.52 (6.0, 11.0) |
| I-b^a    | 4.80 (3.5) | 3.80 (6.0) | 3.57 (5.5, 11.0) | 5.23 | 4.34 (3.5) | 4.20 (6.5, 12.0) |
| 9b       | 4.74 (3.5) | 3.76 (5.5) | 3.57 (5.5, 11.0) | 5.23 | 4.37 (3.0) | 4.19 (6.5, 12.0) |

^aThese α-glycolipids were dissolved in a mixture of CDCl3 and CD3OD (10:1) at 11.2 mM concentration.
contacting liquid-crystalline states, these conformers are thought to equilibrate with each other. In this study, we calculated time-averaged populations of the three conformers by means of $^1$H NMR spectroscopy. As we reported in a preceding paper [41], the Karplus-type equation proposed by Haasnoot et al.[42] was adapted as follows:

$$2.8 \text{gg} + 3.1 \text{gt} + 10.7 \text{tg} = 3 \mathcal{J}_{H2,H1S} \text{(or } 3 \mathcal{J}_{H2,H3R})$$

$$0.9 \text{gg} + 10.7 \text{gt} + 5.0 \text{tg} = 3 \mathcal{J}_{H2,H1R} \text{(or } 3 \mathcal{J}_{H2,H3S})$$

$$\text{gg} + \text{gt} + \text{tg} = 1.$$

In this equation, a perfect staggering ($\Phi_1$ and $\Phi_2 = +60, -60$ or 180 degree) is assumed for every conformer. Figure 3 summarizes the results for a series of 3-substituted 1,2-di-O-palmitoyl-sn-glycerols, which involve tripalmitin (Figure 3, entry 1), DPPC (1,2-di-O-palmitoylphosphatidylcholine) (Figure 3, entry 2), and GGPL-I homologues (Figure 3, entries 3–5). In a solution state with a mixture of CDCl$_3$ and CD$_3$OD (10:1) as the solvent and at a concentration of 11 mM, tripalmitin adopts the three conformers in the ratio of $\text{gt}$ (45%), $\text{gg}$ (37%) and $\text{tg}$ (18%). In comparison with this symmetric lipid, the asymmetric phospholipid (DPPC) favors the $\text{gt}$-conformer more strongly around the tail lipid moiety along the sn-1,2 position, while disfavoring the $\text{tg}$-conformer, in the ratio of $\text{gt}$ (59%), $\text{gg}$
Figure 4: Distributions of \( gg \), \( gt \) and \( tg \)-conformers in 1-substituted \( sn \)-glycerols. In these \( sn \)-isomers, \( \Phi_1 \) and \( \Phi_2 \) represent the dihedral angles around the C–C single bond at the glycerol \( sn \)-2,3 and 1,2-position, respectively. (34%) and \( tg \) (7%). The head phosphate moiety along the \( sn \)-2,3 position adopts the three conformers in equilibrated populations (\( gg = gt = tg \)).

3-\( O-(\alpha-D\)-glucopyranosyl\)-\( sn \)-glycerolipids 10 and 11 (Figure 3, entries 3 and 4) were found to have conformational properties very similar to DPPC; the lipid tail moiety prefers the gauche conformations (\( gt \) and \( gg \)), while the sugar moiety allows a random conformation. Here, it should be mentioned that the conformer distribution coincides between I-a (Figure 3, entry 5) and DPPC (Figure 3, entry 2) at the head moiety [\( gt \) (33%), \( gg \) (34%) and \( tg \) (33%)].

The above analysis was carried out also for the stereoisomer I-b and the related glycolipids (Figure 4, entries 6–8). The isomer (Figure 4, entry 8) showed an overall conformational property similar to I-a and DPPC (Figure 5), although a small difference was observed in the conformer distribution at the sugar head moiety. However, it should be recognized here that the helical direction (helicity) of the \( gt \) conformer in I-b is reversed (anti-clockwise) from the case of DPPC and GGPL-I (clockwise), as depicted in Figure 3 and Figure 4.
Conclusion
We have proposed a synthetic pathway to a GGPL-I homologue and its stereoisomer, in which our one-pot α-glycosylation methodology was effectively applied. We envisage that the simple method will allow us to prepare a variety of α-glycolipid antigens other than GGPLs and to prove their biological significance [43]. By the $^1$H NMR conformational analysis, which was based on our former studies on deuterium-labeled sn-glycerols and sugars, we have proven that GGPL-I and other 3-O-(α-D-glycopyranosyl)-sn-glycerolipids have a common conformational property at the chiral glycerol moiety: The lipid tail moiety prefers two gauche-conformations (gg and gt) in the order $gt > gg >> tg$. While the sugar head moiety adopts three conformers in an averaged population ($gg = gt = tg$). At the lipid tail position, the gt-conformer with clockwise helicity is predominant over the anticlockwise gg-conformer. The observed conformation was very close to what we have seen in DPPC (Figure 5). Although these results were based on the solution state in a solvent mixture of CHCl$_3$ and CH$_3$OH (10:1), it may be possible to assume that the mycoplasma GGPLs and the related 3-O-(α-D-glycopyranosyl)-sn-glycolipids can constitute cytoplasm membranes in good cooperation with ubiquitous phospholipids without inducing stereochemical stress at the membrane.

The GGPL-I isomer 1-b showed an overall conformational property similar to the natural isomer 1-a and DPPC. However, it should be mentioned here that the chiral helicity of gt-conformers in 1-b is reversed (anticlockwise) from the clockwise helicity of DPPC and GGPL-I. The difference in chirality seems critical in biological recognition events and also in physicochemical contact with other chiral constituents in cell membranes [44,45].

Experimental
General methods
Infrared (IR) spectra were recorded on a JASCO FT/IR-230 Fourier transform infrared spectrometer on KBr disks. All $^1$H NMR (500 MHz) spectra were recorded by using a Varian INOVA-500 or Varian Gemini 200. $^1$H chemical shifts are expressed in parts per million (δ ppm) by using an internal standard of tetramethylsilane (TMS = 0.000 ppm). Mass spectra were recorded with a JEOL JMS 700 spectrometer for fast atom bombardment (FAB) spectra. Silica gel column chromatography was performed on silica gel 60 (Merck 0.063–0.200 mm and 0.040–0.063 mm) and eluted with a mixture of toluene and ethyl acetate or a mixture of CHCl$_3$ and CH$_3$OH in gradient modes (100:0 to 80:20). For purification of phosphocholine-containing products, a chromatographic column packed with Iatrobeads (IATRON LABORATORIES INC., 6RS-8060) was applied and eluted with a mixture of CH$_3$OH and CHCl$_3$ in gradient modes. For thin-layer chromatography (TLC) analysis, Merck precoated TLC plates (silica gel 60 F$_{254}$, layer thickness 0.25 mm) and Merck TLC aluminum roles (silica gel 60 F$_{254}$, layer thickness 0.2 mm) were used. All other chemicals were purchased from Tokyo Kasei Kogyo Co., Ltd., Kishida Chemical Co., Ltd., and Sigma–Aldrich Chemical Company Co, Int., and were used without further purification.

A typical procedure for the one-pot α-glycosylation: CB$_3$Cr$_4$ (1.6 g, 6.09 mmol) and Pb$_3$P (2.02 g, 6.09 mmol) were added to a solution of 6-O-acetyl-2,3,4-tri-O-benzyl-D-glucose (1) (1.0 g, 2.03 mmol) in 10 mL of DMF and stirred for 3 h at rt. Then, (S)-glycidol (301 mg, 4.06 mmol) was added to the reaction mixture and stirred for 14 h at rt. Products were diluted with a mixture of toluene and ethyl acetate (10:1), and the solution was washed with saturated aq. NaHCO$_3$ and aq. NaCl solution, dried, and concentrated. The residue was purified by silica gel column chromatography in toluene and ethyl acetate to give a mixture of 4a and 5a (the ratio changed with reaction time) as colorless syrup. The total yield of 4a and 5a was between 80% and 90%.

3-O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-1,2-di-O-palmitoyl-sn-glycerols 8a and 8b: K$_2$CO$_3$ (379 mg, 2.74 mmol) was added to the mixture of 4a and 5a (1 g, 1.83 mmol based on 4a) in CH$_3$OH (20 mL) and stirred for 1 h at rt. The solution was heated at 100–110 °C, to which the DMF solution of the above residue was added slowly. The reaction mixture was stirred for 2 h at 110 °C, cooled to rt, and then filtered through a pad of Celite powder with ethyl acetate. The filtrate was washed with saturated aq. NaCl solution, dried, and concentrated. The residue was purified by silica gel column chromatography to give 6a as a colorless syrup (830 mg, 60% yield). To a solution of 6a (300 mg, 0.39 mmol) in pyridine (20 mL), TBDMS chloride (107 mg, 0.71 mmol) and 4-N,N-dimethylaminopyridine (cat.) were added. The reaction mixture was stirred for 12 h at rt, treated with methanol (2 mL) for 3 h and concentrated. The residue was purified by silica gel column chromatography in a mixture of toluene and ethyl acetate. The main product was dissolved in pyridine (20 mL) and then reacted with palmitoyl chloride (162 mg, 0.59 mmol) for 3 h at rt. The reaction mixture was treated with methanol (2 mL) and then concentrated with toluene. The residue was dissolved in a mixture of CH$_3$OH and CH$_2$Cl$_2$ (1:1, 20 mL) and treated with trifluoroacetic acid (1 mL) for 2 h at rt. After concentration, the residue was purified by silica gel column chromatography in a mixture of toluene and ethyl acetate to give 8a as a white waxy solid.
In the same way as derived for the synthesis of 8a, (R)-glycitol and 6b (0.30 g, 0.39 mmol) was used for the synthesis of 8b (0.30 g, 77% yield). Its [α]D20 +18.5 (c 1.0, CHCl3); IR (KBr, film): 3452, 2924, 2854, 1739, 1586, 1455, 1296, 1159, 1095, 710 cm⁻¹; 1H NMR (500 MHz, CDCl3): δH 7.40–7.23 (m, 5H × 3, -CH₂CH₂OH), 5.23 (d, 1H, 1H-2), 4.96–4.63 (2H × 2, -CH₂OCH₂CH₂-), 4.75 (d, 1H, J = 3.5 Hz, H-1), 4.38 (dd, 1H, J = 3.5 and 10.0 Hz, H-4), 3.49 (dd, 1H, J = 3.5 and 9.5 Hz, H-2), 2.29 (m, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CO₂H), 1.58 (b, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CO₂H), 1.25 (b, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CO₂H), 0.88 (t, 3H × 2, J = 7.0 Hz, -OCOC₂H₅CH₂(CH₂)₂CO₂H); FABMS m/z: [M + Na]+ 1187.8.

A phosphoramidite method for the synthesis of 1-a

(a) The reaction vessel was kept under anhydrous conditions with Ar gas in the presence of molecular sieves (50% w/w), and a solution of 8a (0.20 g, 0.20 mmol) and 2-cyanoethyl-N,N,N′,N′-tetraisopropylphosphoramidite (90.4 mg, 0.30 mmol) in 10 mL of CH₂Cl₂ was injected. 1H-tetrazole (28.4 mg, 0.40 mmol) was added and stirred for 2 h at rt. Then 1H-tetrazole (42.6 mg, 0.60 mmol, 3.0 equiv) and choline tosylate (220.3 mg, 0.8 mmol; thoroughly dried over night under vacuum) were added to the reaction mixture and stirred for 1.5 h at rt. The reaction was quenched by the addition of water (1 mL), and then m-chloroperoxybenzoic acid (51.8 mg, 0.5 mmol) was added at 0 °C and stirred for 10 min at rt. The reaction mixture was washed with 10%aq Na₂SO₄ solution, water and saturated aq. NaCl solution, dried and concentrated. The residue was dissolved in a mixture of CH₃OH (10 mL) and 30%aq NH₃ (1 mL) and stirred for 15 min at rt. The reaction mixture was concentrated, and the residue was purified by column chromatography (IATROBEADS in a mixture of CHCl₃ and CH₂OH) to give 9a (186 mg, 80% yield). Its [α]D20 +13.0 (c 0.45, CHCl3); IR (KBr, film): 3301, 2929, 2856, 2537, 1731, 1577, 1419, 1216, 1093, 925, 778, 746; 1H NMR (500 MHz, CDCl3): δH 7.35–7.25 (m, 5H × 3, -CH₂CH₂H₂), 5.22 (m, 1H, CHCl-2), 4.93–4.61 (d, 2H × 2, -CH₂CH₂H₂), 4.70 (d, 1H, J = 3.5 Hz, H-1), 4.38 (dd, 1H, J = 3.0 and 12.0 Hz, glycerol H-1proS), 4.19 (b, 2H, choline -CH₂CH₂N(CH₃)₂), 4.17 (dd, 1H, J = 6.5 and 12.0 Hz, glycerol H-1proR), 4.15 and 4.02 (m, 2H × 2, H-6proR and H-6proS), 3.93 (dd, 1H, J = 9.0 and 9.5 Hz, H-3), 3.72 (dd, 1H, J = 5.5 and 11.0 Hz, glycerol H-3proS), 3.71 (b, 1H, H-3S), 3.62 (t, 2H, H-4), 3.58 (b, 2H, choline -CH₂CH₂N(CH₃)₂), 3.52 (dd, 1H, J = 6.0 and 11.0 Hz, glycerol H-3proR), 3.46 (dd, 1H, J = 3.5 and 9.5 Hz, H-2), 3.15 (s, 9H, -PO(CH₂)₂N(CH₃)₂), 2.28 (m, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CH₂), 1.58 (b, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CH₂), 1.25 (b, 2H × 2, -OCOC₂H₅CH₂(CH₂)₂CH₂), 0.88 (t, 3H × 2, J = 7.0 Hz, -OCOC₂H₅CH₂(CH₂)₂CH₂); FABMS m/z: [M + Na]+ 1188.7.

(b) Compound 9a (0.18 g, 0.15 mmol) was hydrogenated with Pd(OH)₂/C (8 mg) under atmospheric pressure in a mixture of CH₂OH (10 mL) and acetic acid (0.1 mL) for 7 h at rt. The reaction mixture was neutralized by the addition of Et₃N, filtered and concentrated. The residue was purified by column chromatography with IATROBEADS (CH₂OH and CH₃OH) to give 1-a (101 mg, 75% yield). Its [α]D20 +18.5 (c 1.0, CHCl₃/CH₃OH 1/1); IR (KBr, film): 3372, 2927, 2852, 1731, 1573, 1469, 1112, 975, 727; 1H NMR (500 MHz, CDCl₃/CD₂OD 1/1): δH 5.23 (m, 1H, glycerol-H-2), 4.80 (d, 1H, J = 3.5 Hz, H-1), 4.39 (dd, 1H, J = 3.5 and 12.0 Hz, glycerol H-1proS), 4.30 (b, 2H, -CH₂CH₂N(CH₃)₂), 4.24 and 3.95 (b, 2H, H-6proR and H-6proS), 4.14 (dd, 1H, J = 6.5 and 12.0 Hz, glycerol H-1proR), 3.75 (dd, 1H, J = 5.5 and 11.0 Hz, glycerol H-3proR), 3.67 (b, 2H, choline-CH₂CH₂N(CH₃)₂), 3.65 (b, 1H × 2, H-3 and H-5), 3.61 (dd, 1H, J = 5.5 and 11.0 Hz, glycerol H-3proS), 3.56 (b, 1H, H-4), 3.46 (b, 1H, H-2), 3.22 (s, 9H, -PO(CH₂)₂N(CH₃)₂), 2.31 (m, 2H × 2, -OCOC₂H₅CH₂CH₂(CH₃)₂), 1.60 (b, 2H × 2, -OCOC₂H₅CH₂CH₂(CH₃)₂), 1.25 (b, 2H × 2, -OCOC₂H₅CH₂CH₂(CH₃)₂), 0.88 (t, 3H × 2, J = 7.0 Hz, -OCOC₂H₅CH₂CH₂(CH₃)₂); HRMS-FAB (m/z): [M + Na]+ calcd for C₄₆H₆₀NO₁₅PNa, 918.6048; found, 918.6028.

In the same way as described above, 9b (180 mg) was derived from 8b (200 mg, 0.20 mmol) in 76% yield and converted to the GGPL1-isomer 1-b [70 mg, 81% yield from 9b (120 mg)]. Its [α]D20 +8.1 (c 0.62, CHCl₃); IR (KBr, film): 3413, 2923, 2857,
1735, 1461, 1241, 1097, 744, 495, 445; 1H NMR (500 MHz, CDCl₃): δ 7.35–7.23 (m, 5H × 3, -CH₂CH₂), 5.23 (b, 1H, glycerol H-2), 4.94–4.64 (d, 2H × 3, -CH₂CH₂), 4.74 (d, 1H, J = 3.5 Hz, H-1), 4.37 (dd, 1H, J = 3.0 and 12.0 Hz, glycerol H-3, proR), 4.23 (b, 2H, choline-CH₂N⁺(CH₃)₃), 4.19 (dd, 1H, J = 6.5 and 12.0 Hz, glycerol H-3, proS), 4.16 and 4.09 (b, 2H, H-6proR and H-6proS), 3.94 (dd, 1H, J = 9.5 and 9.5 Hz, H-3), 3.76 (dd, 1H, J = 5.5 and 11.0 Hz, glycerol H-1, proR) 3.72 (m, 1H, H-5), 3.61 (dd, 1H, J = 9.0 and 9.5 Hz, H-4), 3.60 (b, 2H, choline-CH₂N⁺(CH₃)₃), 3.57 (dd, 1H, J = 5.5 and 11.0 Hz, glycerol H-1, proR), 3.49 (dd, 1H, J = 3.5 and 9.5 Hz, H-2), 3.20 (s, 9H, choline-CH₂N⁺(CH₃)₃), 2.28 (2H × 2, J = 7.5 and 15 Hz, -OCOCH₂CH₂(CH₂)₂CH₃), 1.58 (b, 2H × 2, -OCOCH₂CH₂(CH₂)₂CH₃), 1.25 (b, 2H × 2, -OCOCH₂CH₂(CH₂)₂CH₃), 0.88 (s, 6H × 2, H-6proR and H-6proS), 4.20 (dd, 1H, J = 7.0 and 12.0 Hz, glycerol H-3, proS), 3.80 (dd, 1H, J = 6.0 and 11.0 Hz, glycerol H-1, proS), 3.63 (b, 1H × 2, H-3 and H-5), 3.63 (b, 2H, choline-CH₂N⁺(CH₃)₃), 3.58 (dd, 1H, J = 5.5 and 12.0 Hz, glycerol H-1, proS), 3.55 (b, 1H, H-4), 3.46 (dd, 1H, H-2), 3.22 (s, 9H, choline-CH₂N⁺(CH₃)₃), 2.31 (dd, 2H × 2, J = 7.5 and 15 Hz, -OCOCH₂CH₂(CH₂)₂CH₃), 1.60 (b, 2H × 2, -OCOCH₂CH₂(CH₂)₂CH₃), 1.26 (b, 2H × 2, -OCOCH₂CH₂(CH₂)₂CH₃), 0.88 (s, 6H × 2, J = 7.0 Hz, -OCOCH₂CH₂(CH₂)₂CH₃); HRMS–FAB (m/z): [M + Na]+ calced for C₄₆H₉₀O₁₃Na, 918.6048; found, 918.6078.

3-O-(6-O-palmitoyl-α-D-glucopyranosyl)-1,2-di-O-palmitoyl-sn-glycerol (11, entry 4): A mixture of 8a (120 mg, 0.12 mmol) and palmitoyl chloride (165 mg, 0.6 mmol) in pyridine was stirred at rt for 3 h and then treated with CH₃OH (1 mL) for 3 h. After concentration in vacuo, the residue was purified on silica gel (toluene/ethyle acetate). The main product (138 mg) was dissolved in a mixture of cyclohexene/ethanol 1:4 and subjected to catalytic hydrogenation at atmospheric pressure in the presence of Pd(OH)₂C (50 mg). The product was purified by silica gel column chromatography (CH₂OH/CHCl₃) to afford 11 (99 mg, 85% yield from 8a). [α]D₂⁰ +20.9 (c 1.0, CHCl₃/ CHOH 10:1); IR (KBr film): 3414, 2919, 2851, 1731, 1465, 1375, 1176, 1054, 720 cm⁻¹; 1H NMR (500 MHz, CDCl₃/CD₃OD 10:1): δ 1.0, CHCl₃/CH₂OH 10:1: δ 5.25 (m, 1H, glycerol H-2), 4.82 (d, 1H, J = 4.0 Hz, H-1), 4.40 (dd, 1H, J = 3.5 and 12.0 Hz, glycerol H-1, proS), 4.34 and 4.30 (dd × 2, 2H, J = 5.0 and 12.0 and 12.0 Hz, H-6proR and H-6proS), 4.16 (dd, 1H, J = 6.5 and 12.0 Hz, glycerol H-1, proR), 3.82 (dd, 1H, J = 1.0 and 10.5 Hz, glycerol H-3, proS), 3.45 (dd, 1H, J = 4.0 and 9.5 Hz, H-2), 3.33 (dd, 1H, J = 9.0 and 10.0 Hz, H-4), 2.33 (m, 2H × 3, -OCOCH₂CH₂(CH₂)₂CH₃), 1.61 (b, 2H × 3, -OCOCH₂CH₂(CH₂)₂CH₃), 1.26 (b, 2H × 3, -OCOCH₂CH₂(CH₂)₂CH₃), 0.88 (s, 6H × 2, J = 7.0 Hz, -OCOCH₂CH₂(CH₂)₂CH₃); HRMS–FAB (m/z): [M + Na]+ calced for C₅₇H₁₀⁸O₁₁Na, 991.7789; found, 991.7832.

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References
1. Rawadi, G. Microbes Infect. 2000, 2, 955–964. doi:10.1016/S1218-4579(00)00395-6
2. Shimizu, T.; Kida, Y.; Kuwano, K. Immunology 2004, 113, 121–129. doi:10.1111/j.1365-2567.2004.01937.x
3. Romero, F.; Moreno, E.; Ruiz-Bravo, A.; Jiménez-Valera, M. Curr. Microbiol. 2004, 48, 237–239. doi:10.1007/s00284-003-4134-1
4. Rötter, S. Biochim. Biophys. Acta. Rev. Biomembr. 1980, 604, 65–90. doi:10.1016/0005-2736(80)80585-4
5. Boggs, J. M. Biochim. Biophys. Acta 1987, 906, 353–404.
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