Supporting Information

Stereoselective synthesis of the head group of archaeal phospholipid PGP-Me to investigate bacteriorhodopsin-lipid interactions

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**General Information** Unless otherwise noted, all chemicals and solvents were purchased from Nacalai Tesque, Sigma-Aldrich, TCI, and KANTO Chemicals Inc., and were used without further purification. Thin layer chromatography (TLC) was performed on Merck pre-coated silica gel 60 F-254 plates and was visualized by UV irradiation (254 nm) or stained with phosphomolybdic acid in ethanol. Optical rotation was recorded on a JASCO P-1010 polarimeter. NMR spectra were performed on a JEOL ECA 400 (400 MHz) or JEOL ECA 500 (500 MHz) spectrometer, using the deuterated solvent as the lock. $^{31}$P NMR chemical shifts are reported using triphenylphosphate (0.0485 M in CDCl$_3$) as external standard, referenced at -16.58 ppm. Chemical shifts are given in ppm (δ) and coupling constants (J) are in Hz. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintuplet, m = multiplet, br = broad. High resolution mass spectra (HRMS) were recorded on an LTQ-Orbitrap XL instrument. Abbreviations: TBAF = tetra-butylammonium fluoride; DMAP = N,N'-dimethyl-4-aminopyridine; DCC = N,N'-dicyclohexylcarbodiimide; DPPC = dipalmitoylphosphatidylcholine; bR = bacteriorhodopsin; dbR = delipidated bR; PGP-Me = phosphatidylglycerophosphate methyl ester; S-TGA-1 = sulphated triglycosyl diphytanylglycerol; GlyC = glyco cardiolipin; PGS = phosphatidylglycerosulfate; PG = phosphatidylglycerol; DSC = differential scanning calorimetry.

**Synthesis of 2 and 3**

**Scheme S1** Synthesis of 5.

\[
\begin{align*}
\text{(R)-solketal} & \xrightarrow{\text{BnBr, NaH, DMF, 0°C to rt}} \text{BnO-C_{16}H_{33}} \\
& \xrightarrow{(i) 70\% \text{AcOH, rt}} \text{BnO-C_{16}H_{33}} \\
& \xrightarrow{(ii) C_4H_8J, NaH, DMF, 0°C to rt} \text{BnO-C_{16}H_{33}} \\
& \xrightarrow{\text{BCl}_3, \text{CH}_2\text{Cl}_2, -78°C to rt} \text{5}
\end{align*}
\]

Compound 5 was prepared from (R)-solketal using a partially modified procedures from those in previous reports as shown above.1,2)

**Scheme S2** Synthesis of 10.

\[
\begin{align*}
\text{(S)-solketal} & \xrightarrow{(i) PMBCl, NaH, DMF, 0°C to rt} \text{PMBCO-OC_{16}H_{33}} \\
& \xrightarrow{(ii) p-\text{TsOH-H}_{2}O, \text{MeOH, reflux}} \text{PMBCO-OC_{16}H_{33}} \\
& \xrightarrow{\text{Ph_3CCl, pyridine, DMAP, CH_2Cl_2, rt}} \text{PMBCO-OC_{16}H_{33}} \\
& \xrightarrow{(i) \text{NaH, BnBr, TBAI, DMF, rt}} \text{HO-OPMB}
\end{align*}
\]

Compound 10 was prepared from (S)-solketal using a partially modified procedures from those in previous reports as shown above.3,4) Compound 12 was prepared from (R)-solketal by employing the same synthetic method as for 10.
Scheme S3  Synthesis of S10.

To a solution of 10 (730 mg, 2.4 mmol) and 8 (650 mg, 2.4 mmol) in CH₂Cl₂ (8 mL) was added 1H-tetrazole (169 mg, 2.4 mmol) at 0 °C. After stirring at room temperature for 1h, t-BuOOH (436 µL, 2.4 mmol, 5.5 M in nonane) was added at 0 °C. The reaction mixture was stirred at the same temperature for 1h. 1 mL of Na₂SO₃ (1M) was added in the mixture. After vigorous stirring for 10 min, the mixture was diluted with CH₂Cl₂ and washed with sat. Na₂CO₃. The organic layer was washed with water, dried over MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 2:5) to afford 11 (1.02 g, 87%) as a colorless oil. [α]₂³D +3.8 (c 1.02, CHCl₃/CH₃OH = 1:1); ¹H NMR (400 MHz, CDCl₃): δ 3.53 (dd, J = 2.8, 5.2 Hz, 2H); 3.68 (d, J = 11.2 Hz, 3H); 3.72-3.79 (m, 1H); 3.78 (s, 3H); 4.06-4.14 (m, 1H); 4.16-4.23 (m, 1H); 4.44 (d, J = 2.8 Hz, 2H); 4.58-4.67 (m, 2H); 5.03 (d, J = 8.0 Hz, 1H); 5.04 (d, J = 8.4 Hz, 0.7H); 6.86 (dd, J = 0.8, 8.4 Hz, 2H); 7.19-7.73 (m, 12H); ¹³C NMR (100 MHz, CDCl₃): δ 54.37, 54.40, 54.43, 54.45, 55.36, 67.07, 67.13, 68.77, 69.37, 72.31, 73.21, 76.83, 77.15, 77.46, 113.86, 127.79, 127.88, 128.00, 128.45, 128.63, 128.68, 129.41, 135.91, 135.93, 135.97, 135.99, 138.20, 159.32; ³¹P NMR (100 MHz, CDCl₃): δ 1.08; HRMS Calcd. for C₂₆H₃₁O₇PNa [M+Na]+ 509.1700, found 509.1703.

In the case of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) deprotection of p-methoxybenzyl ethers: To a solution of 11 (70 mg, 0.14 mmol) in wet CH₂Cl₂ (1 mL) was added DDQ (36 mg, 0.16 mmol) at room temperature. After stirring for 1h, the reaction mixture was washed with sat.NaHCO₃. Organic layer was concentrated. The residue was purified by flash column chromatography on silica gel (ethyl acetate) to afford 6 (45.3 mg, 86%) as a colorless oil; In the case of ceric ammonium nitrate (CAN) deprotection of p-methoxybenzyl ethers: To a solution of 11 (100 mg, 0.21 mmol) in CH₃CN/H₂O (0.9 mL/0.1 mL) was added CAN (228 mg, 0.45 mmol) at room temperature. After stirring for
3h, the reaction mixture was diluted with Et$_2$O and washed with H$_2$O. Organic layer was concentrated. The residue was purified by flash column chromatography on silica gel (ethyl acetate) to afford 6 (49.5 mg, 66%). $^1$H NMR (400 MHz, CDCl$_3$): δ 2.53 (m, 1H); 3.59-3.67 (m, 2H); 3.71 (d, $J = 11.2$ Hz, 1.5H); 3.72 (d, $J = 11.2$ Hz, 1.5H); 3.68-3.72 (m, 1H); 4.09-4.18 (m, 2H); 4.60 (ABq, 1H, $\Delta \delta_{AB} = 0.03$, $J_{AB} = 12.0$ Hz); 4.62 (ABq, 1H, $\Delta \delta_{AB} = 0.03$, $J_{AB} = 12.0$ Hz); 5.06 (d, $J = 8.4$ Hz, 1H); 5.07 (d, $J = 8.4$ Hz, 1H); 7.21-7.40 (m, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 54.50, 54.57, 61.11, 61.16, 65.82, 65.88, 69.59, 69.65, 72.25, 76.79, 77.11, 77.42, 77.61, 77.68, 127.92, 128.05, 128.09, 128.60, 128.74, 135.74, 135.80, 137.87; $^{31}$P NMR (100 MHz, CDCl$_3$): δ 1.58; HRMS Calcd. for C$_{18}$H$_{24}$O$_6$PNa $[M+Na]^{+}$ 389.1124, found 389.1129.

To a solution of 12 (186 mg, 0.62 mmol) in dry pyridine (1 mL) was added 2-chloro-4H-benzo[d][1,3,2]dioxaphosphinin-4-one (150 mg, 0.74 mmol) at 0 °C. After stirring at room temperature for 1h, Et$_3$NH$_2$CO$_3$ (2 ml, 1M) was added. The reaction mixture was stirred at the same temperature for 1h. After concentration, the residue was purified by flash column chromatography on silica gel (CH$_2$Cl$_2$/MeOH/Et$_3$N = 100:6:1) to afford 14 (244 mg, 85%) as a colorless oil. $[^\alpha]_{D}^{23}$ -0.91 (c 1.78, CHCl$_3$/CH$_3$OH = 1:1); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.21 (t, $J = 7.2$ Hz, 9H); 2.93 (q, $J = 7.2$ Hz, 6H); 3.48-3.60 (m, 2H); 3.71-3.78 (m, 1H); 6.78 (d, $J = 8.4$ Hz, 2H); 6.80 (d, $J = 646$ Hz, 1H); 7.14-7.31 (m, 7H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 8.57, 45.54, 55.32, 63.34, 63.39, 70.04, 72.07, 73.07, 76.94, 77.25, 77.58, 77.63, 77.71, 113.76, 127.46, 127.72, 128.28, 129.26, 130.48, 138.79, 159.15; $^{31}$P NMR (100 MHz, CDCl$_3$): δ 5.83; HRMS Calcd. for C$_{18}$H$_{22}$O$_6$P $[M-H]^{-}$ 365.1159, found 365.1151.

This compound was prepared by the same procedure as described for 14 using (S)-solketal. $[^\alpha]_{D}^{23}$ +0.84 (c 5.10, CHCl$_3$/CH$_3$OH = 1:1); $^1$H NMR (400 MHz, CDCl$_3$): δ 1.21 (t, $J = 7.2$ Hz, 9H); 2.93 (q, $J = 7.2$ Hz, 6H); 3.48-3.60 (m, 2H); 3.72 (s, 3H); 3.88-4.02 (m, 2H); 4.62 (ABq, 2H, $\Delta \delta_{AB} = 0.03$, $J_{AB} = 11.6$ Hz); 6.78 (d, $J = 8.4$ Hz, 2H); 6.80 (d, $J = 646$ Hz, 1H); 7.14-7.31 (m, 7H); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 8.64, 45.78, 55.35, 63.86, 63.90, 69.72, 72.19, 73.12, 76.86, 77.11, 77.37, 77.44, 113.82, 127.54, 127.80, 128.34, 129.32, 130.44, 138.66, 159.21; $^{31}$P NMR (100 MHz, CDCl$_3$): δ 5.83; HRMS Calcd. for C$_{18}$H$_{22}$O$_6$P $[M-H]^{-}$ 365.1159, found 365.1151.

To a solution of 14 (53.6 mg, 0.11 mmol) in CH$_2$Cl$_2$ (1 mL) was added trifluoroacetic acid (0.2 mL) at 0 °C. After stirring at the same temperature for 1h, Et$_3$N (0.4 ml) was added slowly. After concentration, the residue was purified by flash column chromatography on silica gel (CH$_2$Cl$_2$/MeOH/Et$_3$N = 30:10:0.3) to afford 7 (31.8 mg, 80%) as a colorless oil. $[^\alpha]_{D}^{23}$ +1.49 (c 1.01, CHCl$_3$/CH$_3$OH = 1:1); $^1$H NMR (400 MHz, CDCl$_3$): δ 3.11 (t, $J = 7.6$ Hz, 9H); 3.03 (m, 6H); 3.60 (m, 1H); 3.66-3.78 (m, 2H); 4.03-4.10 (m, 2H); 4.60 (ABq, 2H, $\Delta \delta_{AB} = 0.03$, $J_{AB} = 12.0$ Hz); 6.85 (d, $J = 637$ Hz, 1H); 7.21-7.35 (m, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 8.61, 45.63, 60.30, 61.48, 61.52, 71.61, 78.28, 127.75, 127.85,
128.45, 138.43; $^{31}$P NMR (100 MHz, CDCl$_3$): $\delta$ 6.45; HRMS Calcd. for C$_{10}$H$_{14}$O$_5$P [M-H]$^-$ 245.0584, found 245.0578.

This compound was prepared by the same procedure as described for 7 using (S)-solketal. 

$[^a]^{23}D$ -1.21 (c 1.50, CHCl$_3$/CH$_3$OH = 1:1); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.26 (t, $J$ = 7.6 Hz, 9H); 3.00 (m, 6H); 3.58 (m, 1H); 3.65-3.75 (m, 2H); 4.03-4.08 (m, 2H); 4.59 (ABq, 2H, $\Delta$$\delta$$_{AB}$ = 0.03, $J_{AB}$ = 12.0 Hz); 6.85 (d, $J$ = 638 Hz, 1H); 7.20-7.35 (m, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 8.58, 45.65, 60.30, 61.70, 61.75, 71.62, 78.37, 127.72, 127.83, 128.42, 138.44; $^{31}$P NMR (100 MHz, CDCl$_3$): $\delta$ 6.45; HRMS Calcd. for C$_{10}$H$_{14}$O$_5$P [M-H]$^-$ 245.0584, found 245.0578.

![Fig. S1 Partial enlarged $^1$H NMR of (a) Chirabite-AR/7 (1/1 mol), (b) Chirabite-AR/enantiomer of 7 (1/1 mol), and (c) Chirabite-AR/7/enantiomer of 7 (1/1/1 mol) in CDCl$_3$. The CH (Hb) signal appears at 3.25 ppm in Chirabite-AR/7 (1/1 mol) (a) and 3.30 ppm in Chirabite-AR/enantiomer of 7 (1/1 mol) (b). The mixture of Chirabite-AR/7/enantiomer of 7 (1/1/1 mol) (c) shows two sets of NMR peaks. These spectra suggest that enantiomeric excess of 7 (or enantiomer of 7) is more than 95% as indicated by the absence of proton signal in the red circle of the spectra.](image)
To a solution of 7 (70 mg, 0.2 mmol) and 8 (70 mg, 0.26 mmol) in CH₂Cl₂ (1 mL) was added 1H-tetrazole (18 mg, 0.26 mmol) at 0 °C. The mixture was stirred at room temperature for 1h. After stirring at room temperature for 1h, t-BuOOH (54 µL, 0.3 mmol, 5.5 M in nonane) was added at 0 °C. The reaction mixture was stirred at the same temperature for 1h. 0.5 mL of Na₂SO₃ (1M) was added. After vigorous stirring for 10 min, the mixture was concentrated. The residue was purified by flash column chromatography on silica gel (CH₂Cl₂/MeOH/Et₃N = 100:6:1) to afford 4 (96 mg, 90%) as a colorless oil. [α]²³D +5.13 (c 0.90, CHCl₃/CH₃OH = 1:1); ¹H NMR (400 MHz, CDCl₃): δ 1.18 (t, J = 7.6 Hz, 18H; HNEt3 residue); 2.90 (m, 12H; HNEt3 residue); 3.53 (md, J = 11.2 Hz, 3H); 3.64 (m, 1H); 3.78-3.86 (m, 2H); 3.92-4.02 (m, 1H); 4.04-4.12 (m, 1H); 4.59 (ABq, 2H, ΔδAB = 0.03, JAB = 11.6 Hz); 4.85-4.91 (m, 2H); 6.71 (d, J = 6.29 Hz, 1H); 7.06-7.22 (m, 10H); 13C NMR (100 MHz, CDCl₃): δ 8.63, 45.74, 54.34, 54.39, 61.91, 61.96, 66.69, 66.74, 69.21, 69.26, 72.02, 76.61, 76.68, 76.74, 127.67, 127.71, 127.87, 128.31, 128.58, 135.72, 135.77, 137.98; ³¹P NMR (100 MHz, CDCl₃): δ 0.80, 4.89; HRMS Calcd. for C₁₈H₂₃O₈P₂ [M-H]⁻ 429.0874, found 429.0870.

To a solution of 4 (92 mg, 0.173 mmol) and 5 (186 mg, 0.35 mmol) in dry pyridine (2 mL) at 0 °C was added pivaloyl chloride (64 µL, 0.52 mmol). The reaction mixture was stirred at room temperature for 1h. Then iodine (44 mg, 0.35 mmol) in pyridine/H₂O (1mL/50 µL) was added. The reaction mixture was stirred at the same temperature for 30 min. 1 mL of Na₂SO₃ (1M) was added. After stirring for 10 min, the mixture was diluted with water and washed with CHCl₃. The organic layer was combined, dried over MgSO₄, filtrated and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH₂Cl₂/MeOH/Et₃N = 100:6:1) to afford 15 (123 mg, 70%) as a colorless oil. [α]²³D -0.4 (c 1.27, CHCl₃/CH₃OH = 1:1); ¹H NMR (400 MHz, CDCl₃): δ 0.85 (t, J = 7.2 Hz, 6H); 1.20-1.36 (m, 52H); 1.29 (t, J = 7.2 Hz, 15H; HNEt3 residue); 1.43-1.54 (m, 4H); 3.01 (m, 10H; HNEt3 residue); 3.32-3.44 (m, 3H); 3.65 (m, J = 11.2 Hz, 15H); 3.66 (m, J = 11.2 Hz, 15H); 3.78-3.91 (m, 3H); 3.93-4.02 (m, 2H); 4.07-4.16 (m, 1H); 4.21-4.38 (m, 1H); 4.63 (ABq, 2H, ΔδAB = 0.03, JAB = 11.6 Hz); 4.95-5.05 (m, 2H); 7.15-7.40 (m, 10H); 13C NMR (100 MHz, CDCl₃): δ 8.61, 14.18, 22.75, 26.17, 26.20, 29.43, 29.63, 29.86, 29.78, 30.26, 31.99, 45.64, 54.35, 54.41, 63.59, 63.65, 65.12, 65.18, 67.24, 67.93, 69.23, 69.29, 70.63, 71.07, 71.72, 72.12, 78.07, 78.15, 127.60, 127.76, 127.95, 128.20, 128.33, 128.52, 128.61, 135.96, 136.01, 138.34; ³¹P NMR (100 MHz, CDCl₃): δ 0.74, 1.01; HRMS Calcd. for C₅₃H₉₃O₁₁P₂ [M-H]⁻ 967.6199, found 967.6205.

To a solution of 15 (100 mg, 0.09 mmol) in MeOH (2 mL) was added platinum oxide (21 mg, 0.09 mmol). The mixture was hydrogenated overnight at 1 atm of hydrogen gas. Platinum black was filtered. After concentration, the residue was purified by flash column chromatography on silica gel
(CHCl₃/MeOH/H₂O = 65:20:3) to afford corresponding deprotected product in triethylamine salt form. [As the hydrogenation reaction may be retarded due to the presence of excess amount of triethylamine salt in the reaction, a treatment of starting material with cation exchange resin in sodium form (substrate/resin (1:10 w/w)) was carried out in CHCl₃ before launching the hydrogenation.] The deprotected product was mixed with 1 mL of NaClO₄ solution (4 M). The mixture was stirred with a vortex mixer. After centrifugation the supernatant solution was removed, the precipitate was washed with distilled water, and dissolved in CHCl₃/MeOH (1:1). The organic solvent was concentrated to afford 2 (38 mg, 50% yield over two steps) as a white solid. [α]$_{23}^D$ -3.2 (c 0.20, CHCl₃/CH₃OH = 1:1); $^1$H NMR (500 MHz, CD₃OD/CDCl₃ (4/3 v/v); using CD₃OD as the lock): δ 1.20 (t, $J$ = 5.6 Hz, 6H); 1.52-1.70 (m, 52H); 1.83-1.92 (m, 4H); 3.72-3.84 (m, 3H); 3.87-3.99 (m, 4H); 3.93 (d, $J$ = 10.5 Hz, 3H); 4.20-4.34 (m, 7H); $^{13}$C NMR (125 MHz, CD₃OD/CDCl₃ (4/3 v/v)): δ 13.66, 22.57, 26.01, 26.07, 29.28, 29.47, 29.63, 29.94, 31.86, 52.40, 52.44, 64.88, 64.92, 65.35, 69.70, 70.44, 70.57, 71.67, 78.00; $^{31}$P NMR (100 MHz, CD₃OD/CDCl₃ (4/3 v/v)): δ 1.74, 3.00; HRMS Calcd. for C₃₉H₈₀O₁₁P₂Na [M-2H+Na]- 809.5079, found 809.5084.

To a solution of LiAlD₄ (227 mg, 5.4 mmol) in THF (10 mL) at 0 °C was added S1 (1.0 g, 2.7 mmol) in THF (1 mL) dropwise. After stirring at the same temperature for 2 h, the reaction mixture was quenched by ice water. The solvent was removed, and the residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:3) to afford S2 (670 mg, 70%) as a colorless oil. $^1$H NMR (400 MHz, CDCl₃): δ 1.04 (s, 9H); 1.28-1.42 (m, 4H); 1.49-1.61 (m, 4H); 3.65 (t, $J$ = 6.4 Hz, 2H); 7.34-7.44 (m, 6H); 7.65-7.69 (m, 4H); $^{13}$C NMR (100 MHz, CDCl₃): δ 19.31, 25.50, 25.68, 26.96, 32.58, 32.64, 63.93, 127.66, 129.59, 134.22, 135.66; HRMS Calcd. for C₂₂H₃₀D₂O₂SiNa [M+Na]+ 381.2189, found 381.2195.

To a solution of S2 (340 mg, 0.95 mmol) in CH₂Cl₂ (2 mL) were added $p$-toluenesulfonyl chloride (272 mg, 1.42 mmol), triethylamine (318 µL, 2.28 mmol) and N,N-dimethyl-4-aminopyridine (580 mg, 4.75 mmol) at room temperature. After stirring at the same temperature for 1 h, the solvent was removed. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:10) to afford S3 (462 mg, 95%) as a colorless semi-solid. $^1$H NMR (400 MHz, CDCl₃): δ 1.03 (s, 9H); 1.21-1.32 (m, 4H); 1.45-1.52 (m, 2H); 1.56-1.63 (m, 2H); 2.42 (s, 3H); 3.60 (t, $J$ = 6.4 Hz, 2H); 7.29-7.44 (m, 8H); 7.61-7.68 (m, 4H); $^{13}$C NMR (100 MHz, CDCl₃): δ 19.29, 21.70, 25.13, 25.27, 26.95, 28.68, 32.34, 63.73, 127.68, 127.96, 129.62, 129.87, 133.37, 134.12, 135.63, 144.68; HRMS Calcd. for C₂₉H₃₆D₂O₄SSiNa [M+Na]+ 535.2278, found 535.2285.

To a solution of S3 (300 mg, 0.59 mmol) in THF (10 mL) were added CuCl₂ (3.9 mg, 0.03 mmol) and 1-phenyl-1-propyne (14 µL, 0.11 mmol) at 0 °C under argon. Afterward the Grignard reagent (2.34 mL, 2.34 mmol; 1.0 M in diethyl ether) was added in one portion.
The ice bath was removed and the reaction mixture was stirred for 1 h. The solvent was evaporated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:20) to afford S4 (257 mg, 91%) as colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.89 (t, $J$ = 7.2 Hz, 3H); 1.05 (s, 9H); 1.21-1.39 (m, 24H); 1.51-1.60 (m, 2H); 3.66 (t, $J$ = 6.4 Hz, 2H); 7.34-7.45 (m, 6H); 7.65-7.70 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.22, 19.32, 22.79, 25.88, 26.98, 29.44, 29.46, 29.53, 29.58, 29.76, 29.81, 32.03, 32.70, 64.12, 127.64, 129.55, 134.32, 135.68; HRMS Calcd. for C$_{32}$H$_{51}$D$_2$OSi [M+H]$^+$ 483.3986, found 483.3994.

To a solution of S4 (300 mg, 0.62 mmol) in THF (10 mL) was added tetrabutylammonium fluoride (1 mL, 1M in THF). After stirring at room temperature for 4 h, the solvent was removed. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:5) to afford S5 (148 mg, 98%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.87 (t, $J$ = 6.8 Hz, 3H); 1.19-1.42 (m, 24H); 1.50-1.60 (m, 2H); 3.63 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.20, 22.78, 25.82, 29.44, 29.52, 29.69, 29.78, 32.01, 32.91, 63.20; HRMS Calcd. for C$_{16}$H$_{32}$D$_2$ONa [M+Na]$^+$ 267.2627, found 267.2625.

To a solution of S5 (140 mg, 0.57 mmol) in pyridine/CH$_2$Cl$_2$ (3 mL/3 mL) was added methanesulfonyl chloride (45 µL, 0.57 mmol) at 0 °C. After stirring at the same temperature for 2 h, white solid was filtered. The solvent was removed. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:10) to afford S6 (175 mg, 95%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.87 (t, $J$ = 6.8 Hz, 3H); 1.18-1.44 (m, 24H); 1.70-1.82 (m, 2H); 2.99 (s, 3H); 4.21 (t, $J$ = 6.4 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.20, 22.77, 25.51, 29.12, 29.22, 29.44, 29.50, 29.60, 29.77, 32.01, 37.45, 70.28; HRMS Calcd. for C$_{17}$H$_{34}$D$_2$O$_3$SNa [M+Na]$^+$ 345.2403, found 345.2405.

To a solution of 9 (80 mg, 0.61 mmol) in DMF (2 mL) was added NaH (36 mg, 0.9 mmol, 60% in oil) at 0 °C. After stirring for 30 min at 0 °C, S6 (197 mg, 0.61 mmol) was added in the mixture. After stirring for 20 h at room temperature, the mixture was diluted with water and extracted with EtOAc. The organic layer was combined and concentrated under reduced pressure. The residue was dissolved in CH$_2$Cl$_2$/MeOH (1mL/1mL) and $p$-toluenesulfonic acid monohydrate (11.6 mg, 0.06 mmol) was added. After stirring for 5 h at room temperature, the mixture concentrated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:5) to afford S7 (146 mg, 75% over two steps) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.86 (t, $J$ = 7.2 Hz, 3H); 1.17-1.35 (m, 24H); 1.50-1.60 (m, 2H); 2.16 (s, 2H); 3.42-3.54 (m, 4H); 3.59-3.73 (m, 2H); 3.83 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 14.20, 22.77, 26.17, 29.46, 29.54, 29.66, 29.71, 29.74, 29.78, 32.01, 64.36, 70.55, 71.94, 72.57; HRMS Calcd. for C$_{19}$H$_{38}$D$_2$O$_3$Na [M+Na]$^+$ 341.2995, found 341.2997.

To a solution of S7 (100 mg, 0.61 mmol) in toluene (2 mL) was added $p$-methoxybenzyltrichloroacetimidate (0.13 mL, 0.61 mmol) and lanthanum triflate.
(35.2 mg, 0.06 mmol) at room temperature. After stirring at the same temperature for 30 min, the reaction mixture was washed with water. The combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:10) to afford S8 (89 mg, 65%) as a colorless solid. 

\[\text{\textsuperscript{1}H NMR (400 MHz, CDCl₃): } \delta 0.87 (t, J = 7.2 Hz, 3H); 1.17-1.35 (m, 24H); 1.50-1.60 (m, 2H); 3.39-3.54 (m, 6H); 3.80 (s, 3H); 3.95 (m, 1H); 4.48 (s, 2H); 6.87 (d, J = 8.4 Hz, 2H); 7.24 (d, J = 8.4 Hz, 2H); \text{\textsuperscript{13}C NMR (100 MHz, CDCl₃): } \delta 14.21, 22.78, 26.19, 29.44, 29.51, 29.55, 29.74, 29.79, 32.01, 55.36, 69.63, 71.19, 71.78, 71.88, 73.19, 113.90, 129.45, 130.21, 159.37; \text{HRMS Calcd. for C}_{27}H_{46}D_{2}O_{4}Na [M+Na]^+ 461.3570, found 461.3573.\]

\[\text{To a solution of S8 (60 mg, 0.14 mmol) in DMF (1.5 mL) was added NaH (8.4 mg, 0.21 mmol, 60% in oil) at 0 °C. After stirring for 30 min at 0 °C, MsOC}_{16}H_{33} (90 mg, 0.28 mmol) was added in the mixture. After stirring for 24 h at room temperature, the mixture was diluted with water and extracted with EtOAc. The organic layer was combined and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:10) to afford S9 (66.8 mg, 72%) as a colorless solid. \]

\[\text{\textsuperscript{1}H NMR (400 MHz, CDCl₃): } \delta 0.87 (t, J = 7.2 Hz, 6H); 1.18-1.35 (m, 50H); 1.50-1.61 (m, 4H); 3.40-3.60 (m, 9H); 3.79 (s, 3H); 4.47 (s, 2H); 6.85 (d, J = 8.4 Hz, 2H); 7.25 (d, J = 8.4 Hz, 2H); \text{\textsuperscript{13}C NMR (100 MHz, CDCl₃): } \delta 14.20, 22.78, 26.19, 26.23, 29.45, 29.55, 29.58, 29.61, 29.75, 29.80, 30.20, 32.02, 55.33, 70.06, 70.67, 70.89, 71.75, 73.09, 78.01, 113.80, 129.29, 130.64, 159.21; \text{HRMS Calcd. for C}_{43}H_{78}D_{2}O_{4}Na [M+Na]^+ 685.6074, found 685.6085.\]

\[\text{To a solution of S9 (57 mg, 0.08 mmol) in wet CH₂Cl₂ at room temperature was added DDQ (22.7 mg, 0.1 mmol). After stirring for 1 h at room temperature, the mixture was diluted with CH₂Cl₂ and washed with sat.NaHCO₃. Organic layer was concentrated. The residue was purified by flash column chromatography on silica gel (ethyl acetate/hexane = 1:6) to afford S10 (42 mg, 90%) as a white solid. \]

\[\text{\textsuperscript{1}H NMR (400 MHz, CDCl₃): } \delta 0.89 (t, J = 7.2 Hz, 6H); 1.20-1.36 (m, 50H); 1.29 (t, J = 7.2 Hz, 15H; HNEt₃ residue); 1.43-1.54 (m, 4H); 3.01 (m, 10H; HNEt₃ residue); 3.32-3.44 (m, 3H); 4.63 (ABq, 2H, \Delta \delta_{AB} = 0.03, J_{AB} = 11.6 Hz); 4.95-5.05 (m, 2H); \text{HRMS Calcd. for C}_{35}H_{70}D_{2}O_{3}Na [M+Na]^+ 565.5499, found 565.5498.\]

This compound was prepared in 68% yield over 2 steps as a colorless oil by following the same procedure as described for 15. 

\[\text{\textsuperscript{1}H NMR (400 MHz, CDCl₃): } \delta 0.85 (t, J = 7.2 Hz, 6H); 1.20-1.36 (m, 50H); 1.29 (t, J = 7.2 Hz, 15H; HNEt₃ residue); 1.43-1.54 (m, 4H); 3.01 (m, 10H; HNEt₃ residue); 3.32-3.44 (m, 3H); 3.46-3.60 (m, 4H); 3.65 (d, J = 11.2 Hz, 1.5H); 3.66 (d, J = 11.2 Hz, 1.5H); 3.78-3.91 (m, 3H); 3.92-4.02 (m, 2H); 4.07-4.16 (m, 1H); 4.21-4.28 (m, 1H); 4.63 (ABq, 2H, \Delta \delta_{AB} = 0.03, J_{AB} = 11.6 Hz); 4.95-5.05 (m, 2H); 7.15-7.39 (m, 10H); \text{HRMS Calcd. for C}_{35}H_{70}D_{2}O_{3}Na [M+Na]^+ 565.5499, found 565.5498.\]
Calcd. for C_{53}H_{91}D_2O_{11}P_2 [M-H]- 969.6324, found 969.6330.

This compound was prepared in 46% yield over 2 steps as a white solid by following the same procedure as described for 2. ¹H NMR (400 MHz, CD₃OD/CDCl₃ (4/3 v/v); using CD₃OD as the lock): δ 1.20 (t, J = 5.6 Hz, 6H); 1.52-1.71 (m, 50H); 1.82-1.92 (m, 4H); 3.72-3.84 (m, 3H); 3.87-3.99 (m, 4H); 3.93 (d, J = 10.5 Hz, 3H); 4.20-4.35 (m, 7H); HRMS Calcd. for C_{39}H_{78}D_2O_{11}P_2Na [M-2H+Na]- 811.5205, found 811.5212.

**Preparation of PM** PM was prepared from cultured *Halobacterium salinarum* (strain R₁M₁) according to a standard method.⁶,⁷) After resuspension of PM in 100 mM sodium phosphate buffer (pH 7.0), the concentration of bR were determined calorimetrically using the absorption coefficient ε = 63,000 M⁻¹·cm⁻¹ at 570 nm of PM⁸) and ε = 57,000 M⁻¹·cm⁻¹ at 560 nm of dbR with Shimadzu UV-3150 (Kyoto, Japan).

**Preparation of dbR** Delipidation of bR was performed with 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPS) and dodecyl-β-D-maltoside (DM) as described previously⁹) with slight modifications as follows; PM (bR, 1 mg/mL) was incubated with 5% (w/v) CHAPS in 10 mM sodium phosphate buffer (pH 7.0) at room temperature for 48 h. The CHAPS-treated bR was then washed and incubated with 5% (w/v) DM in 10 mM sodium phosphate buffer (2 M NaCl pH 7.0) at room temperature for 48 h. The DM-treated bR was then washed, and dialysed against pure water, while being stirred with Bio-Beads at 4 °C for 1 day. The dbR was washed and resuspended in 10 mM sodium phosphate buffer (pH 7.0). The recovery of dbR was found to be ~ 95% according to the colorimetric method.

**Purification of PM lipids** To 0.2 mL of dbR solution (0.05 ~ 0.1 mg bR) was added 0.75 mL of CHCl₃/MeOH (1:1). The mixture was vortex-mixed and centrifuged. Chromatographic separation was performed using CAPCELL PAK C8 UG120 (150 mm × 2.0 mm) at 40 °C. PM lipids were separated in gradient solvent system using methanol and water with 10mM ammonium acetate at a flow rate of 0.2 mL/min. The collected fractions contain PGP-Me (m/z 899.7 [M-H]⁻), S-TGA-1 (m/z 1217.8 [M-H]⁻), GlyC (966.2), PGS (m/z 885.6 [M-H]⁻), and PG (m/z 805.6 [M-H]⁻) as determined by LC-MS (LC-20A and Model 2020, Shimadzu, Kyoto).

**Sample Preparation for CD Measurements and Flash Laser Photolysis** 5.0 mg of 2 or DPPC was dissolved in MeOH/CHCl₃ (1:1 v/v). After removing the solvent in vacuo for 20 h, the dried membrane film was hydrated with 0.5 mL of distilled water and vigorously vortexed at 65 °C to make multilamellar vesicles. The sample was freeze–thawed six times. The solubilized dbR was added in the 2 or DPPC suspension at the molar ratio of 1:100. The mixture was vigorously vortexed, freeze–thawed, lyophilized, and resuspended in phosphate buffer (3.0 mL,
100 mM, pH 7.0) for CD measurement. The sample of 2/bR used for CD measurement was diluted over 5-fold for flash laser photolysis. In the case of dbR and PM samples, the concentration of bR is the same as that of reconstituted proteoliposomes for measurements of CD spectra and flash laser photolysis.

**CD Measurement** A J-720W spectropolarimeter equipped with a JWJTC-484 Peltier temperature controller (JASCO Co., Tokyo, Japan) was employed for obtaining visible CD spectra. The visible CD spectra in the region of 400-700 nm were measured with the 10-mm cuvette. Eight scans were averaged for each spectrum.

**Flash Laser Photolysis Data** Flash-induced absorption changes of the intermediates of bR were analyzed using a flash photolysis system (Unisoku Science Institute, Osaka, Japan) using laser excitation at 532 nm with pulses of a Nd-YAG laser (10 ns, 2 mJ). Sample concentration is approximately 0.15 mg bR/mL. All measurements were performed at 25 °C with a quartz cuvette (path length = 10 × 10 mm, 3 mL) without stirring. The M intermediate, O intermediate, and ground state of the bR photochemical reaction were measured by probing absorption at 410 nm, 640 nm, and 560 nm, respectively, with 128 laser shots averaging from 1 to 50 ms.

**Fig. S2** Flash-induced absorbance changes at 410 nm (M intermediate), 640 nm (O intermediate) and 560 nm (ground state) for dbR (green), PM (blue), and 2/dbR (100/1 mol) (red) at 50 °C.

**DSC Measurement** The phase transition temperature of target molecule was measured by nanodifferential scanning calorimeter (Calorimetry Science Corp., UT). 1 mg of 2 dissolved in MeOH/CHCl₃ (1:4 v/v) were mixed in a glass vial. The solution was dried under a flow of nitrogen and then under high vacuum for at least 24 h. The resulting lipid film was dispersed into distilled and deionized water (0.5 mL) and incubated for 30 min at 65 °C with intermittent vortexing. 330 μL of the sample were used for DSC measurements with a scanning rate of 0.5 °C/min.
Sample Preparation for NMR Measurements For the sample of 3 bilayer (or 3/PM lipids mixture): 5.0 mg of 3 (or 3/PM lipids (100/4 w/w)) was dissolved in MeOH/CHCl₃ (1:1 v/v). After removing the solvent in vacuo for 20 h, the dried membrane film was hydrated with 0.5 mL of distilled water and vigorously vortexed at 65 °C to make multilamellar vesicles. The sample was freeze–thawed six times, lyophilized, and rehydrated with deuterium-depleted water to make 60% water (w/w). Then the mixture was again freeze–thawed and transferred into the open-ended NMR tube (3 cm long, 4 mm o.d.). After transferring, the tube was sealed with epoxy glue. 

For the mixture of 3/dbR: 5.0 mg of 3 was dissolved in MeOH/CHCl₃ (1:1 v/v). After removing the solvent in vacuo for 20 h, the dried membrane film was hydrated with 0.5 mL of distilled water and vigorously vortexed at 65 °C to make multilamellar vesicles. The sample was freeze–thawed six times. The solublized dbR was added in the 3 suspension at the molar ratio of 1:100. The mixture was rigorously vortexed, freeze–thawed, lyophilized, and rehydrated with deuterium-depleted water to make 60% water (w/w). Then the mixture was again freeze–thawed and transferred into the open-ended NMR tube (3 cm long, 4 mm o.d.). After transferring, the tube was sealed with epoxy glue.

²H NMR Measurements ²H NMR spectra were acquired on a Bruker Ultrashield 400 MHz spectrometer with a Bruker 5 mm ²H static probe using a quadrupolar echo sequence.¹⁰ The 90° pulse width was 5 µs, interpulse delay was 24 µs, and recycle delay was 0.5 s. The sweep width was 250 kHz, and the number of scans was 200000.

Fig. S3 DSC heating thermograms of pure 2 bilayers.

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¹⁰ [Ref: Specific Reference]
Sample Preparation for $^{31}$P NMR Measurements

a: PGP-Me analogue 2 (4 mg) was dispersed in 393 μL of distilled water and 6 μL of 150 mM HEPES buffer (pH 7.0) by sonication and vortex. The sample was freeze–thawed five times, lyophilized, and rehydrated with distilled water (6 μL) to make 60% water (w/w). Then the mixture was again freeze–thawed and transferred into the HR-MAS insert (Bruker). After transferring, the insert was sealed with epoxy glue.

b: PGP-Me analogue 2 (4 mg) was dispersed in 393 μL of distilled water and 7.9 μL of 150 mM HEPES buffer (pH 7.0) by sonication and vortex. Then 1.28 mg of dbR in water (99 μL) was added in the suspension at the molar ratio of 1:100. The mixture was sonicated, freeze–thawed, lyophilized, and rehydrated with deuterium-depleted water (7.9 μL) to make 60% water (w/w). Then the mixture was again freeze–thawed and transferred into the HR-MAS insert (Bruker). After transferring, the insert was sealed with epoxy glue.

c: PGP-Me analogue 2 (4 mg) was dispersed in 393 μL of distilled water by sonication and vortex. Then 1.28 mg of dbR in water (99 μL) was added in the suspension at the molar ratio of 1:100. The mixture was sonicated, freeze–thawed, lyophilized, and rehydrated with deuterium-depleted water (7.9 μL) to make 60% water (w/w). Then the mixture was again freeze–thawed and transferred into the HR-MAS insert (Bruker). After transferring, the insert was sealed with epoxy glue.

$^{31}$P NMR Measurements $^{31}$P NMR spectra were acquired on a Bruker Ultrashield 400 MHz spectrometer with a Bruker 5 mm $^{31}$P static probe using a single pulse experiment. The 90° pulse width was 5.5 μs, and recycle delay was 2 s. The sweep width was 64 kHz, and the number of scans was 1200.

Fig. S4 $^{31}$P NMR spectra of multilamellar dispersions of PGP-Me analogue 2 in 150 mM HEPES buffer at pH 7.0 (a), 2/dbR (100/1 mol) in 150 mM HEPES buffer at pH 7.0 (b), and 2/dbR (100/1 mol) in plain water (c). The spectra were measured with 60% hydrated membrane dispersions at 50°C.
References


$^1$H and $^{13}$C NMR spectra of synthetic intermediates and products
enantiomer of 14
enantiomer of 7