Electronic supplementary information to the article

Membrane plasticity induced by *myo*-inositol derived Archaeal lipids: chemical synthesis and biophysical characterization

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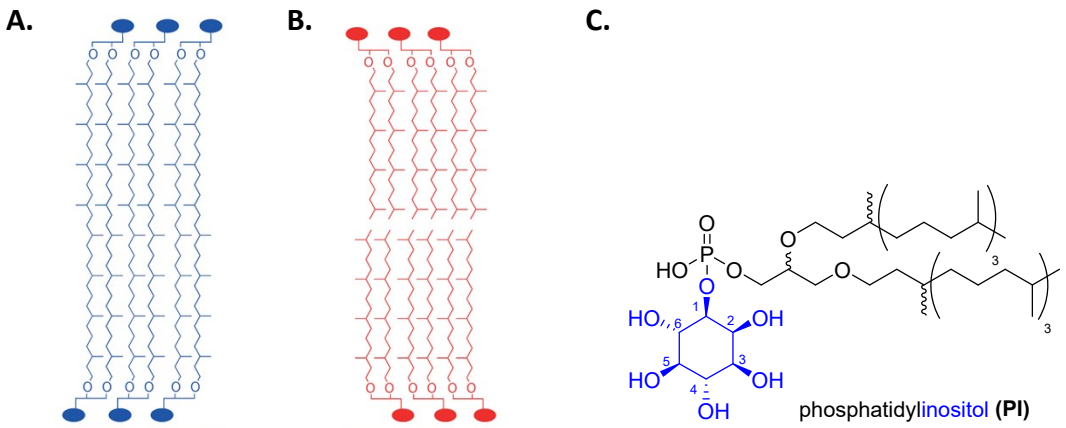


Figure S1. Schematic representation of A. bipolar tetraether lipids, B. monopolar diether lipids. C. Molecular target (1,2- di-*O*-phytanyl-*sn*-glycero-3-phosphatidylinositol).

Sample preparation for Synthesis: Reagents and solvents were supplied by Aldrich, TCI, or Alfa Aesar and purchased at the highest commercial quality to be used without further purification. NMR spectra were recorded on a Bruker 300 (^{1}H: 300 MHz; ^{13}C: 75 MHz), Bruker 400 (^{1}H: 400 MHz; ^{13}C: 100 MHz) or Bruker 500 (^{1}H: 500 MHz; ^{13}C: 125 MHz) spectrometers at 298 K, using $CDCl_3$ or DMSO-d_6 as solvent. The chemical shifts (δ, ppm) are referenced to the residual solvent peak and coupling constants (J) are reported in the standard fashion. For ^{1}H NMR chemical shift calibration the residual peak of $CHCl_3$ was set at 7.26 ppm and 2.54 ppm for DMSO. For ^{13}C NMR, chemical shift calibration the central peak of $CDCl_3$ was set at 77.16 ppm and 40.45 ppm for DMSO. The following abbreviations are used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q =quartet, m = multiplet, br = broad. High-resolution mass spectra (HRMS) were recorded on a Finnigan Mat 95xL mass spectrometer using ESI as an ionization source. Analytical thin-layer chromatography was carried out on silica gel Merck 60 D254 (0.25 mm). Flash chromatography was performed on Merck Si 60 silica gel (40−63μm).

Protocols:

Myo-inositol 1,3,5-orthobenzoate (1)[1]

A 50 mL two-necked round-bottom flask equipped with a Teflon-coated, egg-shaped magnetic stir bar was charged with *myo*-inositol (3.60 g, 20 mmol, 1 equiv.), DMSO (10 mL), and trimethyl orthobenzoate (4.01 g, 22 mmol, 1.1 equiv.). Then, CSA (92 mg, 0.4 mmol, 2 mol%) was added under nitrogen internal positive pressure and the reaction mixture was refluxed at 80 °C for 5 h. The solution was then cooled to room temperature, and saturated aqueous $NaHCO_3$ solution (10 mL) was carefully added. The solution was stirred for 10-20 min and then filtered. The filtrate was evaporated *via* a high-vacuum rotary evaporator and the crude mixture was recrystallized over MeOH/EtOAc. Compound **1** (3.68 g, 13.8 mmol, 69%) was isolated as white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.58 – 7.51 (m, 2H), 7.39 – 7.30 (m, 3H), 5.51 (d, J = 5.1 Hz, 2H), 5.33 (d, J = 6.3 Hz, 1H), 4.39 (t, J = 4.3 Hz, 2H), 4.23 – 4.18 (m, 1H), 4.18 – 4.12 (m, 2H), 4.08 (dt, J = 6.3, 1.7 Hz, 1H).

2,4,6 Tri-*O*-benzyl-*myo*-inositol 1,3,5-orthobenzoate (2)

Orthoester **1** (1.33 g, 5 mmol, 1 equiv.) in dry DMF (10 mL) was introduced in a 50 mL two-necked round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar. The solution was cooled to 0°C and then NaH (1.20 g as 60% solution in mineral oil, 30 mmol, 6 equiv.) was introduced in the flask in two portions at intervals of 5 min under nitrogen stream. The reaction was stirred at 0°C during 15 min under nitrogen stream until bubbling ceased. Then, benzyl bromide (5.12 g, 30 mmol, 6 equiv.) was added dropwise with a syringe. The nitrogen stream was replaced by a nitrogen balloon; then, the water / ice bath was removed, and the solution stirred at room temperature during 3 h. The solution was treated by careful addition

[1] a) Godage, H. Y.; Riley, A. M.; Woodman, T. J.; Potter, B. V. L. *Chem. Commun.*, **2006**, *2989*; b) Sureshan, K. N.; Riley, A. M.; Potter, B. V. L. *Tetrahedron Lett.* **2007**, *48*, 1923; c) Godage, H. Y.; Riley, A. M.; Woodman, T. J.; Thomas, M. P.; Mahon, M. F.; Potter, B. V. L. *J. Org. Chem.* **2013**, *78*, 2275.

of a minimal amount of a saturated NH_4Cl solution and vigorously stirred for ca. 15 min until bubbling ceased. The heterogeneous solution was diluted with CH_2Cl_2 (10 mL) and then filtered through Celite®. The Celite cake was rinsed with CH_2Cl_2 (3 x 10 mL) and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 95:5 to 80:20 v/v, giving compound **2** (2.21 g, 4.1 mmol, 82%) as white solid. Mp: 81 – 84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 – 7.62 (m, 2H), 7.43 – 7.18 (m, 18H), 4.65 (d, J = 15.1 Hz, 4H), 4.56 (hept, J = 2.9, 1.7 Hz, 1H), 4.54 – 4.44 (m, 6H), 4.11 (t, J = 1.6 Hz, 1H). These signals were consistent with the data provided by Murali *et al.* [2]

(1*R*,3*R*,5*S*,6*R*,7*S*,8*S*,9*S*)-6,8,9-Tris(benzyloxy)-3-phenyl-2,4-dioxabicyclo[3.3.1]nonan-7-ol (3)

This experimental protocol was adapted from the procedure described by Murali *et al.*[2]

Compound **2** (3.76 g, 7 mmol, 1 equiv.) dissolved in dry CH_2Cl_2 (28 mL) was introduced under nitrogen stream in a 100 mL round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar. The solution was cooled to 0°C and DIBAL-H (11.5 mL of 1.2 M solution in toluene, 13.8 mmol, 2 equiv.) was introduced dropwise under nitrogen overpressure. The solution was stirred at 0°C for 2 h and monitored by TLC. Then, the reaction was treated with saturated aqueous potassium sodium tartrate (30 mL), then saturated aqueous NH_4Cl solution (30 mL), and stirred during 2 h. Next, the solution was poured in a separating funnel, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 90:10 to 80:20 v/v, giving compound **3** as a white solid (2.58 g, 4.78 mmol, 68%). Mp: 99 – 102 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.51 (m, 2H), 7.47 – 7.27 (m, 18H), 5.72 (s, 1H),

[2] Murali, S; Shashidhar, M. S.; Gopinath, C. S. *Tetrahedron* **2007**, *63,* 4149.

4.77 (d, *J* = 11.7 Hz, 2H), 4.73 (s, 2H), 4.63 (d, *J* = 11.7 Hz, 2H), 4.42 (d, *J* = 2.4 Hz, 2H), 4.00 (d, *J* = 8.4 Hz, 2H), 3.81 (td, *J* = 8.5, 2.5 Hz, 1H), 3.62 (t, *J* = 2.5 Hz, 1H), 2.50 (d, *J* = 2.8 Hz, 1H). These signals were consistent with the data provided by Murali *et al.*[2] HRMS (ESI+) Exact mass calculated for $[M+H]^+$: 539.2428, found: 539.2420; for $[M+Na]^+$: 561.2248, found: 561.2238.

(1*R*,3*R*,5*S*,6*R*,7*R*,8*S*,9*R*)-6,7,8,9-Tetrakis(benzyloxy)-3-phenyl-2,4-dioxabicyclo[3.3.1] nonane (4)

Alcohol **3** (2.57 g, 4.77 mmol, 1 equiv.) in dry DMF (24 mL) was introduced under nitrogen stream in a 100 mL round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar. The flask was cooled at 0°C with a water/ice bath and then NaH (477 mg as 60% solution in mineral oil, 11.9 mmol, 2.5 equiv.) was introduced in one portion. The solution was stirred at 0°C under a nitrogen atmosphere for 10 min. Then, the water/ice bath was removed and benzyl bromide (1.63 g, 9.53 mmol, 2 equiv.) was introduced dropwise with a syringe. The solution was stirred under a nitrogen atmosphere at room temperature. After 1h, TLC monitoring showed an incomplete conversion. The solution was then treated by a saturated aqueous NH_4Cl solution (20 mL) and stirred for 30 min. The solution was poured in a separating funnel, the phases were separated, and the aqueous phase was extracted with EtOAc (4 x 30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 98:2 to 88:12 v/v), giving compound **4** as a thick and viscous wax (2.95 g, 4.69 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 – 7.45 (m, 2H), 7.42 – 7.36 (m, 2H), 7.35 – 7.17 (m, 21H), 5.76 (s, 1H), 4.74 – 4.61 (m, 6H), 4.54 (d, *J* = 11.6 Hz, 2H), 4.35 (d, *J* = 2.2 Hz, 2H), 4.09 (d, *J* = 7.2 Hz, 2H), 3.66 (t, *J* = 7.2 Hz, 1H), 3.59 (t, *J* = 2.3 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.32, 138.24, 138.08, 137.56, 129.42, 128.56, 128.55, 128.49,

128.46, 128.11, 128.04, 127.86, 126.66, 93.08, 82.51, 81.85, 74.57, 73.53, 71.73, 70.91, 68.46. HRMS (ESI+) Exact mass calculated for $[M+Na]^+$: 651.2717, found: 651.2710.

2,3,4,5,6-Penta-*O*-benzyl-*myo*-inositol (5)

A 100 mL round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar was charged with **4** (2.94 g, 4.67 mmol, 1 equiv.) in dry CH_2Cl_2 (24 mL) under nitrogen stream. The flask was cooled at 0°C and then DIBAL-H (8 mL of 1.2 M solution in toluene, 9.6 mmol, 2.06 equiv.) was introduced dropwise with a syringe. The solution was stirred under nitrogen atmosphere and slowly allowed to warm up to room temperature. After 17 h, TLC monitoring showed an incomplete conversion; thus, additional DIBAL-H (1 equiv.) was added dropwise to the solution at room temperature under nitrogen stream. After 1 h, the conversion was complete. The solution was treated by a saturated aqueous potassium sodium tartrate solution (30 mL), and stirred during 20 – 30 min. The solution was poured in a separating funnel, the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (4 x 30 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 94:6 to 80:20 v/v), giving compound **5** (2.62 g, 4.16 mmol, 89%) as a white/yellowish solid. Mp : 90 – 92 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 25H), 5.03 – 4.68 (m, 10H), 4.08 (t, J = 9.4 Hz, 1H), 4.04 (d, J = 2.1 Hz, 1H), 3.82 (t, J = 9.4 Hz, 1H), 3.54 – 3.43 (m, 3H). These signals were consistent with the data provided by Koto *et al.*[3] HRMS (ESI+) Exact mass calculated for $[M+H]^+$: 631.3054, found: 631.3050; for $[M+Na]^+$: 653.2874, found: 653.2869.

3-(Benzyloxy)propane-1,2-diol ((±)-6)

[3] Koto, S., Hirooka, M., Yoshida, T., Takenaka, K., Asai, C., Nagamitsu, T., Sakuma, H., Sakurai, M., Masuzawa, S., Komiya, M., Sato, T., Zen, S., Yago, K. Tomonaga, F. Bull. Chem. Soc. Jpn. 2000, 73, 2521-2529.

A 100 mL two-necked round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar was charged with (±)-solketal (3.30 g, 25 mmol, 1 equiv.) in dry DMF (50 mL). The solution was cooled to 0°C and NaH (60% in mineral oil) (1.2 g, 30 mmol, 1.2 equiv.) was introduced in the flask in one portion under internal positive pressure of nitrogen. After an additional stirring period of 40 min at 0°C, benzyl bromide (5.55 g, 32.5 mmol, 1.3 equiv.) was added dropwise to the solution. The solution was stirred at 0°C for 15 min and the water / ice bath was removed. The solution was stirred at room temperature for 22 h. The reaction was then carefully treated with a saturated NH_4Cl solution (30 ml), diluted with pentane (30 ml), and poured in a separatory funnel. The phases were separated, and the water/DMF phase was extracted with pentane (3 x 30 ml). The combined organic phases were concentrated under reduced pressure and used in the next step without further purification. The crude acetal ether intermediate was diluted with HCl 1M (100 mL) and THF (200 mL) and stirred at room temperature for 22 h. The solution was then cooled to 0°C and treated by careful addition of NaOH (solid) until neutrality. The solution was then poured in a separatory funnel, the phases were separated, and the aqueous phase was extracted with ethyl acetate (3 x 50 ml). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (pentane / EtOAc gradient, 95:5 to 50:50 v/v, and then pure EtOAc), giving diol **(±)-6** as colorless oil (4.04 g, 22.2 mmol, 89%). NMR data were identical with reported ones.[4]

(*R*)-3-(Benzyloxy)propane-1,2-diol ((*R*)-6)

[4] Gan, C. H.; Wijaya, H.; Li, L-H.; Wei, C-F.; Peng, Y-J.; Wu, S-H.; Kuo-Feng Hua, K-F.; Lam, Y.*Org. Lett.* **2020**, 22, 7, 2569–2573

The previously described procedure was applied to enantiopure (*S*)-solketal (1.32 g, 10 mmol, 1 equiv.) leading to **(*R*)-6** as colorless oil (1.72 g, 9.44 mmol, 94%).

(*S*)-3-(Benzyloxy)propane-1,2-diol ((*S*)-6)

The previously described procedure was applied to enantiopure (*R*)-solketal (1.33 g, 10 mmol, 1 equiv.) leading to **(*S*)-6** as colorless oil (1.76 g, 9.67 mmol, 97%).

((2,3-Bis((3,7,11,15-tetramethylhexadec-2-en-1-yl)oxy)propoxy)methyl)benzene (7)

This experimental procedure was adapted from the one described by van Boom et al.[5]

A 500 mL three-necked round-bottomed flask equipped with a Teflon-coated, egg-shaped magnetic stir bar was charged with **(±)-6** (3.47 g, 19.1 mmol, 1 equiv.) in dry DMF (70 mL). The solution was cooled to 0°C and NaH (60% in mineral oil) (1.72 g, 42.9 mmol, 2.24 equiv.) was introduced in one portion under internal positive pressure of nitrogen. The solution was stirred at 0°C for 2.5 h and phytyl bromide (17.12 g, 47.6 mmol, 2.49 equiv., 2:1 *E*/*Z* ratio) was added dropwise at 0°C. The flask containing phytyl bromide was rinsed with anhydrous THF (10 mL) and introduced in the reaction medium. The solution was stirred at 0°C and allowed to warm at room temperature. After 15.5 h, the reaction was treated by careful addition of a saturated NH_4Cl solution (100 mL) by using a pressure-equalizing dropping funnel and vigorously stirred for 15 min. The solution was then poured in a separatory funnel, diluted with petroleum ether (200 mL) and the minimum amount of distilled water to solubilize NaCl. The phases were separated, and the water/DMF phase was extracted with petroleum ether (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 99:1 to 80:20 v/v, giving ether **7** as a pale-yellow oil (12.02 g, 16.3 mmol, 85%, 2/1 mixture of isomers). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 – 7.27 (m, 5H), 5.34

[5] van Boeckel, C. A. A.; Westerduin, P.; van Boom, J. H. *Tetrahedron Lett.* **1981**, *22*, 2819.

(dt, *J* = 11.7, 6.9 Hz, 2H), 4.55 (s, 2H), 4.15 (t, *J* = 5.8 Hz, 2H), 4.00 (t, *J* = 6.6 Hz, 2H), 3.73 – 3.48 (m, 5H), 1.98 (dd, *J* = 12.9, 7.3 Hz, 4H), 1.72 (dd, *J* = 2.3, 1.3 Hz, 6H), 1.63 (t, *J* = 1.5 Hz, 6H), 1.53 – 0.98 (m, 38H), 0.91 – 0.78 (m, 24H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.82, 140.73, 140.57, 140.54, 140.34, 140.32, 138.60, 128.44, 127.73, 127.62, 122.11, 121.80, 121.19, 120.85, 77.04, 77.00, 73.50, 70.71, 70.63, 70.19, 70.13, 68.01, 67.73, 66.98, 66.68, 40.11, 39.52, 37.60, 37.55, 37.53, 37.48, 37.45, 37.18, 37.03, 37.00, 36.93, 36.90, 32.95, 32.93, 32.85, 32.59, 32.55, 28.13, 25.83, 25.78, 25.33, 25.31, 24.96, 24.63, 23.65, 23.63, 22.88, 22.78, 19.90, 19.87, 19.83, 19.81, 19.76, 16.59, 16.56.

(((2*R*)-2,3-Bis((3,7,11,15-tetramethylhexadec-2-en-1-yl)oxy)propoxy)methyl)benzene ((*R*)-7)

The previously described procedure was applied to enantiopure **(*R*)-6** (0.913 g, 5.01 mmol, 1 equiv.) leading to **(*R*)-7** as a pale-yellow oil (3.22 g, 4.35 mmol, 87%).

(((2*S*)-2,3-Bis((3,7,11,15-tetramethylhexadec-2-en-1-yl)oxy)propoxy)methyl)benzene ((*S*)-7)

The previously described procedure was applied to enantiopure **(*S*)-6** (0.911 g, 5 mmol, 1 equiv.) leading to **(*S*)-7** as a pale-yellow oil (3.20 g, 4.32 mmol, 86%).

2,3-Bis((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol (Archaeol) (8)

A 100 mL two-necked, round-bottom flask equipped with a Teflon-coated, egg-shaped magnetic stir bar was charged with EtOAc (7 mL) and Pd/C (15 mg, 5% w/w on charcoal, 0.007 mmol, 2 mol%). The solution was degassed by the freeze-pump thaw procedure, applying three vacuum/nitrogen cycles. A solution of **7** (268 mg, 0.366 mmol, 1 equiv.) in EtOAc (7 mL) was

degassed by the same procedure. In both solutions, dissolved nitrogen was removed by hydrogen bubbling during 5-10 additional minutes. Then, the solution of **7** was added with a syringe to the suspension of Pd/C. The solution was stirred at room temperature under 1 atm hydrogen pressure during 15 h. After 12 hours, a reload of Pd/C catalyst (2 mol%) was done for an additional stirring of 4 hours under H_2 pressure of 1 atm. Monitoring by TLC showed complete conversion of the substrate and nearly complete conversion of intermediate **8'**. After filtration over Celite® and washing with EtOAc (3 x 10 mL), the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 99:1 to 90:10 v/v), giving archaeol **8** (201 mg, 0.308 mmol, 85%) as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.77 – 3.41 (m, 9H), 2.17 (t, *J* = 6.2 Hz, 1H), 1.68 – 0.98 (m, 48H), 0.91 – 0.81 (m, 30H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 78.47, 71.15, 71.12, 70.31, 68.80, 63.25, 39.53, 37.65, 37.61, 37.55, 37.50, 37.45, 37.31, 37.23, 36.83, 36.75, 32.95, 32.93, 30.04, 29.99, 28.12, 24.96, 24.64, 24.62, 24.52, 22.87, 22.77, 19.90, 19.84, 19.79, 19.77. HRMS (ESI+) Exact mass calculated for $[M+H]^+$ 653.6806, found: 653.6806.

(*2R*)-2,3-Bis((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol (Archaeol) ((*R*)-8)

The previously described procedure was applied to enantiopure **(*S*)-7** (0.707 g, 0.957 mmol, 1 equiv.) leading to **(*R*)-8** as a colorless oil (0.495 g, 0.757 mmol, 79%).

(*2S*)-2,3-Bis((3,7,11,15-tetramethylhexadecyl)oxy)propan-1-ol (Archaeol) ((*S*)-8)

The previously described procedure was applied to enantiopure **(*R*)-7** (1.51 g, 2.04 mmol, 1 equiv.) leading to **(*S*)-8** as a colorless oil (0.96 g, 1.47 mmol, 74%).

Benzyl (2,3-bis((3,7,11,15-tetramethylhexadecyl)oxy)propyl) (2,3,4,5,6-benzyl-1-yl-myo-inositol) phosphate (10)

Archaeol **8** (326 mg, 0.500 mmol, 1 equiv.), and phosphoradiamidite **4** (194 mg, 0.575 mmol, 1.15 equiv.) were introduced in a 50 mL round-bottom flask and dissolved in dry toluene (3 mL). The reagents were dried by azeotropic evaporation of toluene under reduced pressure. The operation was repeated three additional times. A Teflon-coated, egg-shaped magnetic stir bar was then introduced and the flask was flushed with nitrogen by three vacuum/nitrogen cycles. The flask was sealed with a septum and dry CH_2Cl_2 (10 mL) was introduced with a syringe under nitrogen stream. The solution was stirred at room temperature and then tetrazole (1.33 mL of 0.45 M solution in acetonitrile, 0.600 mmol, 1.2 equiv.) was introduced dropwise. Monitoring by TLC shows nearly complete conversion after 45 min. Then, compound **5** (410 mg, 0.65 mmol, 1.3 equiv.) was introduced in one portion followed by dropwise addition of tetrazole (1.33 mL of 0.45 M solution in acetonitrile, 0.6 mmol, 1.2 equiv.) under a nitrogen stream. Monitoring by TLC showed a partial conversion of the phosphoramidite intermediate after 1.5 h. Therefore, another 1.2 equiv. of tetrazole solution was introduced and the reaction stirred during 16 h. Then, TBHP (0.200 mL of a 5M solution in decane, 1.00 mmol, 2 equiv.) was introduced with a syringe and the solution was stirred for 2 additional hours. Monitoring by TLC shows complete conversion of the phosphite intermediate. The solution was then treated by 10% $NaHSO_3$ (5 mL), 10% $NaHCO_3$ (5 mL), poured in a separatory funnel and diluted with distilled water (10 mL). The phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was diluted in pentane/Et_2O solution (40 mL, 1:3 v/v), washed with DMSO (4 x 4 mL) to remove the excess of **5**, and then washed with H_2O (4 x 4 mL). The organic phase was dried with Na_2SO_4, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (pentane / EtOAc gradient, 95:5 to 80:20 v/v), giving compound **10** (354 mg, 0.246 mmol, 49%) as colorless oil. ^{1}H NMR (300 MHz, $CHCl_3$) ẟ 7.42 – 7.18 (m,

30H), 5.09 – 4.56 (m, 12H), 4.41 – 4.20 (m, 2H), 4.15 – 3.90 (m, 4H), 3.56 – 3.26 (m, 9H), 1.55 – 0.98 (m, 48H), 0.90 – 0.78 (m, 30H). ^{13}C NMR (75 MHz, $CDCl_3$) ẟ 138.95, 138.91, 138.78, 138.68, 138.66, 138.60, 138.27, 136.04, 136.03, 136.01, 135.99, 135.93, 135.92, 135.89, 128.67, 128.60, 128.48, 128.46, 128.43, 128.36, 128.30, 128.27, 128.17, 128.16, 127.99, 127.97, 127.94, 127.89, 127.83, 127.79, 127.71, 127.66, 127.59, 127.53, 127.47, 83.27, 81.42, 80.73, 80.70, 80.67, 80.59, 80.26, 80.22, 80.20, 80.16, 78.88, 78.79, 78.75, 78.72, 77.36, 76.63, 76.53, 76.14, 76.00, 75.69, 75.63, 75.57, 75.29, 75.19, 72.88, 72.85, 72.82, 70.28, 70.23, 69.99, 69.53, 69.49, 69.46, 69.42, 69.36, 69.29, 69.19, 69.09, 67.55, 67.54, 67.52, 67.47, 67.44, 67.35, 67.28, 67.26, 39.51, 37.79, 37.72, 37.67, 37.63, 37.61, 37.56, 37.54, 37.43, 37.27, 37.20, 36.84, 36.75, 32.94, 30.08, 29.99, 29.92, 29.84, 28.11, 24.95, 24.64, 24.52, 22.87, 22.78, 19.90, 19.82, 19.77, 19.71, 19.66, 19.59. HRMS (ESI+) Exact mass calculated for $[M+H]^+$: 1435.9815, found: 1435.9790; for $[M+Na]^+$: 1457.9634, found: 1457.9606.

(2,3-Bis((3,7,11,15-tetramethylhexadecyl)oxy)propyl) ((1*R*,2*S*,3*R*,4*R*,5*S*,6*S*)-2,3,4,5,6-pentahydroxycyclohexyl) hydrogen phosphate (11)

Compound **10** (246 mg, 0.171 mmol, 1 equiv.) and Pd/C (182 mg, 10% w/w on charcoal, 0.171 mmol, 1 equiv.) in THF/H_2O (17 mL, 3:1 v/v) were introduced in a 50 mL round-bottom flask equipped with a Teflon-coated, egg-shaped magnetic stir bar and the flask was sealed with a septum. The solution was degassed by hydrogen bubbling during 15 min, and then stirred at room temperature under 1 atm hydrogen pressure during 24 h. The solution was then filtered over a Celite cake, the latter was washed with EtOAc (3 x 50 mL), and the filtrate was concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel ($CHCl_3$ / MeOH gradient, 90:10 then 80:20 v/v, and finally $CHCl_3$ / MeOH / H_2O 65:35:4 v/v/v). After purification, the product was dissolved in $CHCl_3$/MeOH 90:10 v/v and filtered to remove residual silica. The filtrate was then concentrated under reduced pressure, giving **11** (121 mg, 0.135 mmol, 79%) as white foam. ^{1}H NMR (300 MHz,

$CDCl_3$) δ 3.65 – 3.19 (m, 9H), 1.21 (q, *J* = 52.6, 48.8 Hz, 48H), 0.76 (s, 30H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 39.31, 37.54, 37.42, 37.24, 32.81, 32.74, 29.98, 27.89, 24.74, 24.48, 24.37, 22.57, 22.48, 19.61, 19.54, 19.45. HRMS (ESI+) Exact mass calculated for $[M+H]^+$: 893.6852, found: 893.6841.

Supplementary DLS data:

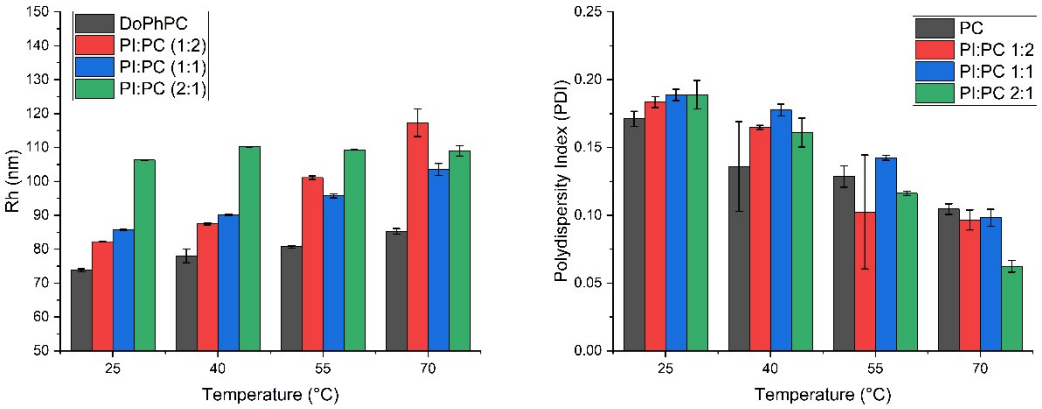


Figure S2: *Characterization of unilamellar vesicles (ULVs) containing DoPhPI by dynamic light scattering. Hydrodynamic radius (Rh) calculated from DLS data for ULVs containing DoPhPI at 25-70°C (left). Polydispersity index (PDI) calculated from DLS data for ULVs containing DoPhPI at 25-70°C (right).*

Supplementary Neutron data:

In this experiment, we also wanted to control the pressure (up to 1000 bars), which adds an important difficulty as lipids need to be in contact with a transmitting pressure medium. For this, we deposited 3 mg of lipid on two different wafers, and after they were completely hydrated, we placed the wafers face to face and we put them in the compartment of the high-pressure cell [6]. We added 5 µl of D_2O contrast and we let them rehydrate in the high-pressure (HP) cell. Once lipids were completely hydrated, we used fluorinert as transmitting medium

[6] Peters, J.; Golub, M.; Demé, B.; Gonthier, J.; Payre, C.; Maurice, J.; Sadykov, R.; Lelièvre-Berna, E. New pressure cells for membrane layers and systems in solutions up to 100°C. J. Neutron Res. 2018, 20, 3 - 12.

inside the cell. We controlled the level of hydration of lipids comparing the d-spacing obtained with the values of previous experiments. The avoid freezing of the pressure transmitting medium, we used a specific stick, which permits to heat and control the medium *in-situ* [7].

Pure DoPhPI membrane as a function of temperature and pressure

Diffraction data was also collected at pressures up to 1000 bar using a high pressure cell over the same temperature range. Diffractograms at high pressure can be found in Figure S3. Pressure and temperature had opposing effects on the membrane d-spacing. While increasing temperature led to a decrease in d-spacing, increasing pressure led to an increase in d-spacing (see Table S1). Increasing the pressure to 1000 bar also allowed for Phase 2 to be detected at higher temperatures (Table S1). A phase diagram was generated to show the transition from a mixture of the two phases at low T, high P to solely Phase 1 at high T, low P (see Figure S4).

[7] Lelièvre-Berna, E.; Demé, B.; Gonthier, J.; Gonzales, J. P.; Maurice, J.; Memphis, Y.; Payre, C.; Oger, P.; Peters, J.; Vial, S. 700 MPa sample stick for studying liquid samples or solid-gas reactions down to 1.8 K and up to 550 K. Journal of Neutron Research 2017, 19, 77 - 84.

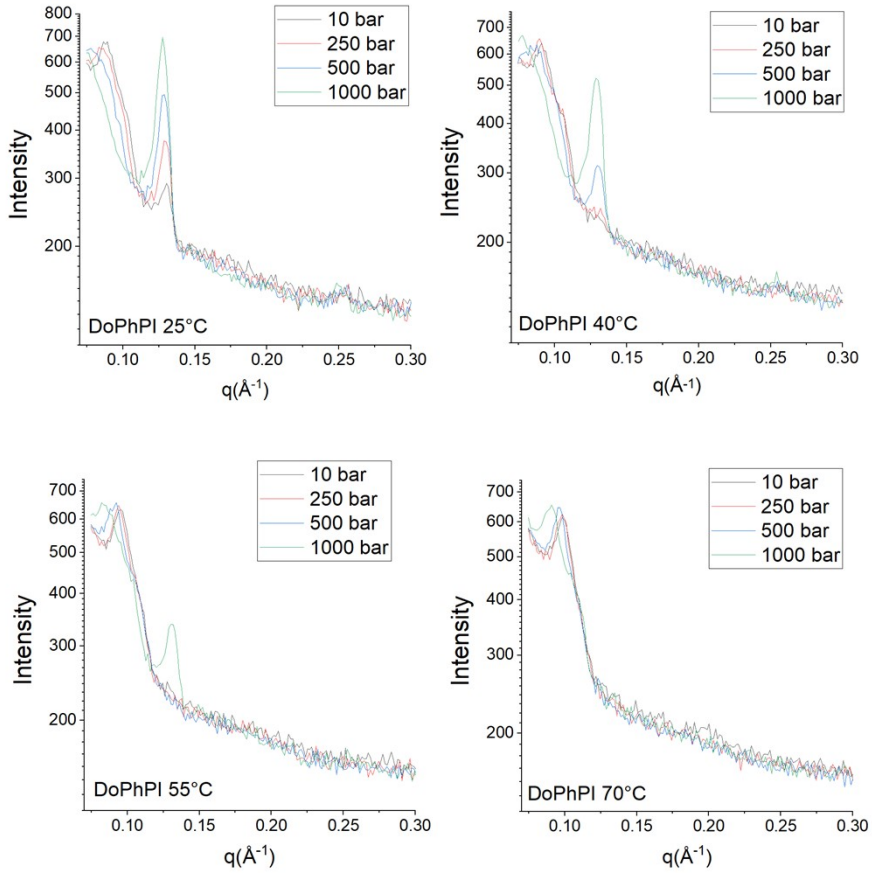


Figure S3: *1D intensity profiles of neutron diffraction from DoPhPI membrane multistacks with increasing T and P.*

Table S1: *DoPhPI membrane d-spacing estimated from the first order Bragg peak. Errors are ~ 1Å. Data was collected in HP Cell with the exception of data noted with a * which was collected in a flat aluminum cell*

Phase 1	25°C	40°C	55°C	70°C	85°C
1 bar*	69.1 Å	65.6 Å	64.0 Å	62.2 Å	59.8 Å
10 bar	70.3	68.0	65.5	62.9	60.9
250 bar	71.4	64.5	66.3	63.2	61.2
500 bar	74.0	71.4	68.5	65.0	62.2
1000 bar	78	75.4	73.5	68.9	65.4
Phase 2	25°C	40°C	55°C	70°C	85°C
1 bar*	48.7 Å	--	--	--	--
10 bar	48.4	--	--	--	--
250 bar	48.6	47.9	--	--	--
500 bar	48.9	48.3	--	--	--
1000 bar	49.3	48.8	48.0	--	--

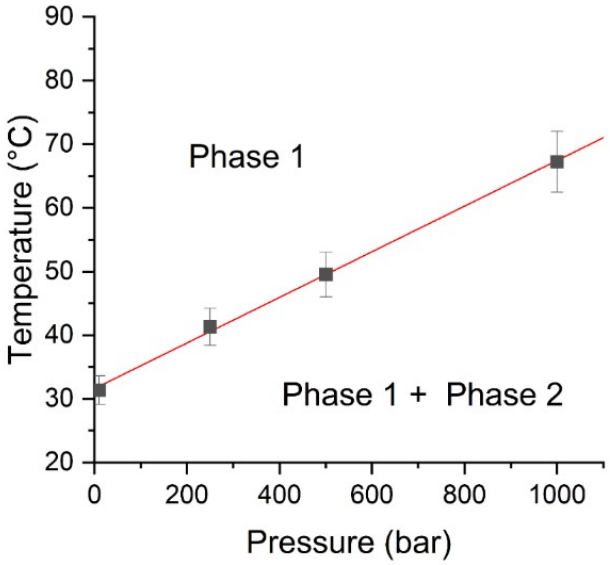


Figure S4: *Diagram indicating the P/T at which Phase 2 is no longer detected via diffraction in the high pressure cell.*

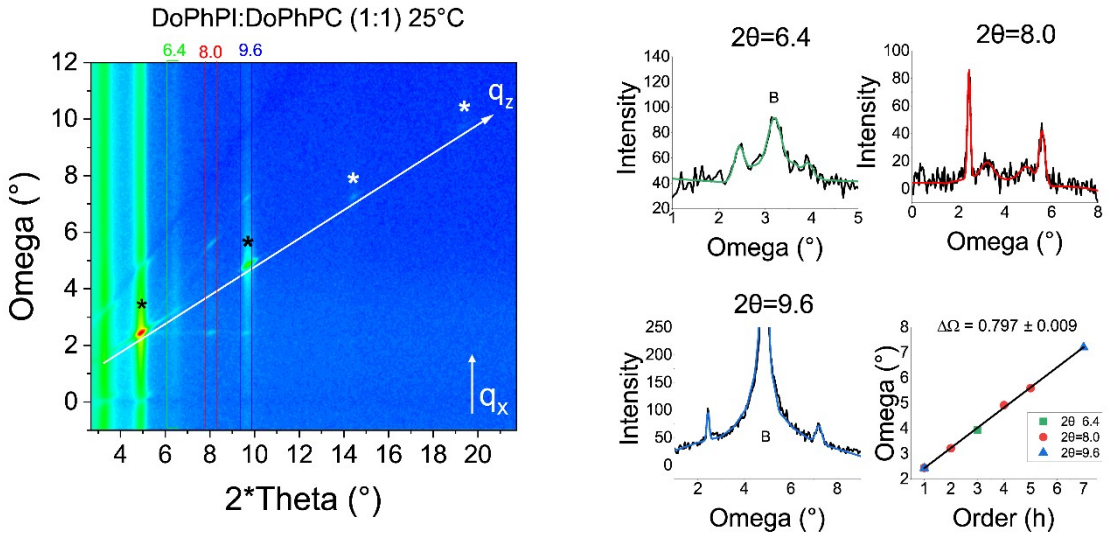


Figure S5*: Lateral Organization in DoPhPI:DoPhPC (1:1) membrane at 25°C.*

Supplementary NMR Data:

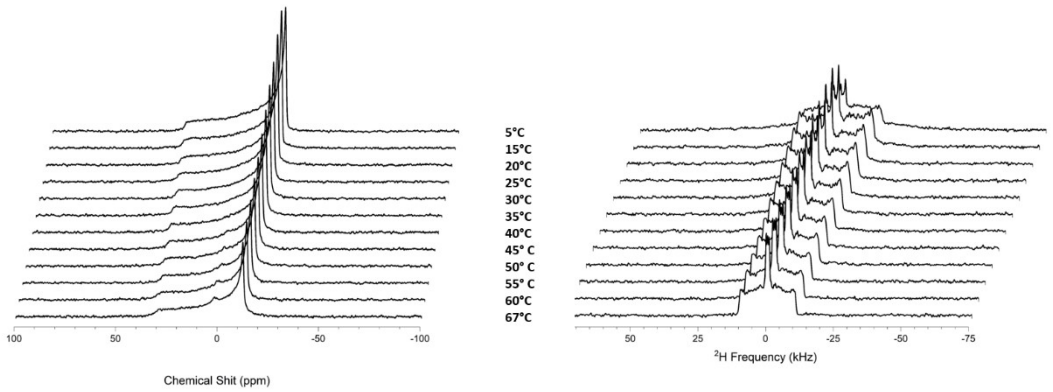


***Figure S6**. Left, ^{31}P-ssNMR spectra and right, ^{2}H-ssNMR spectra of a temperature run of water dispersions of DoPhPC + 20 mol% $^{2}H_{31}$-DPPC. 20 minutes were waited after temperature equilibrium and before acquisition. Acquisition time at a given temperature is 42 min for ^{31}P-NMR and 34 min for ^{2}H-NMR representing a total time of 27hr for a full range of temperatures. Acquisition parameters were kept constant for all experiments and are given in materials and methods.*

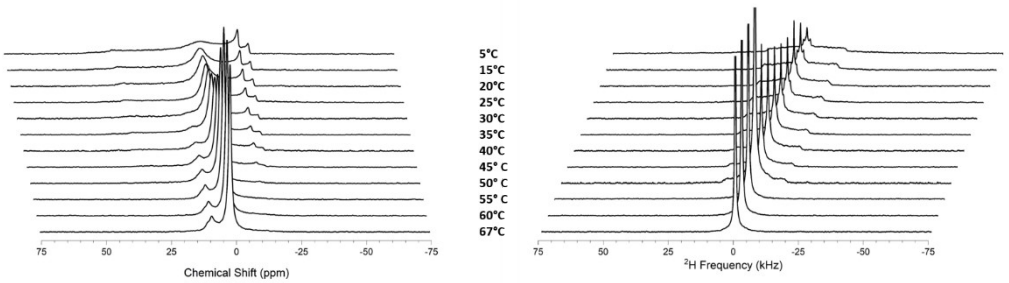


***Figure S7**. Left, ^{31}P-ssNMR spectra and right, ^{2}H-ssNMR spectra of a temperature run of water dispersions of DoPhPC:DoPhPI (1:1) + 10 mol% $^{2}H_{31}$-DPPC. Experimental parameters as in Fig. S4.*

Figure S8 a)

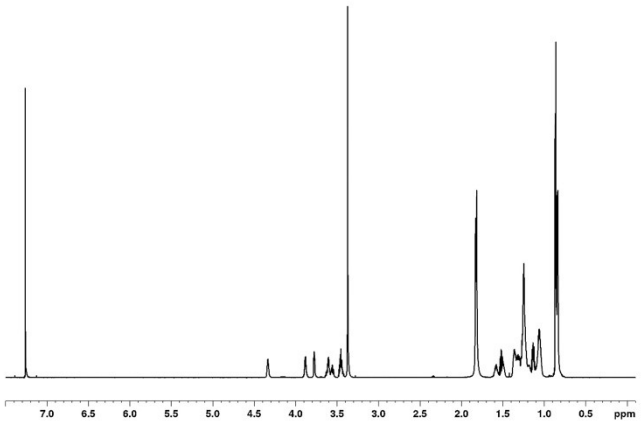


Figure S8 b

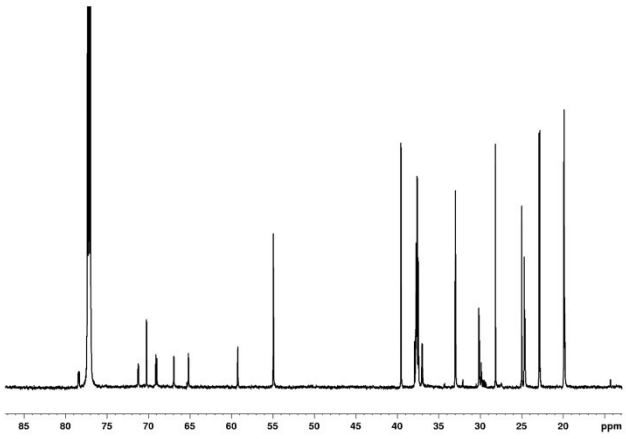


Figure S8 c)

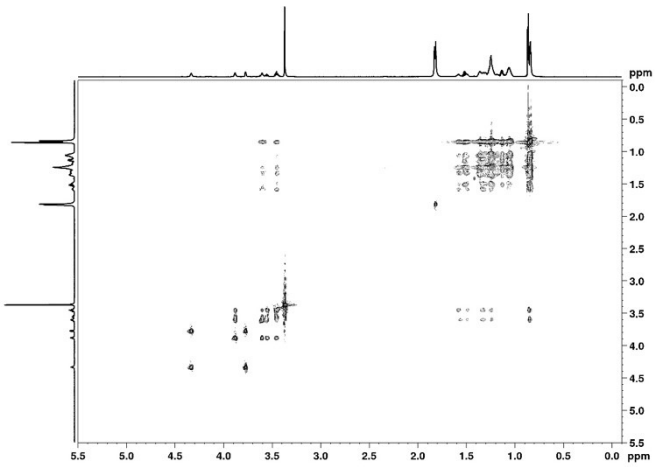


Figure S8

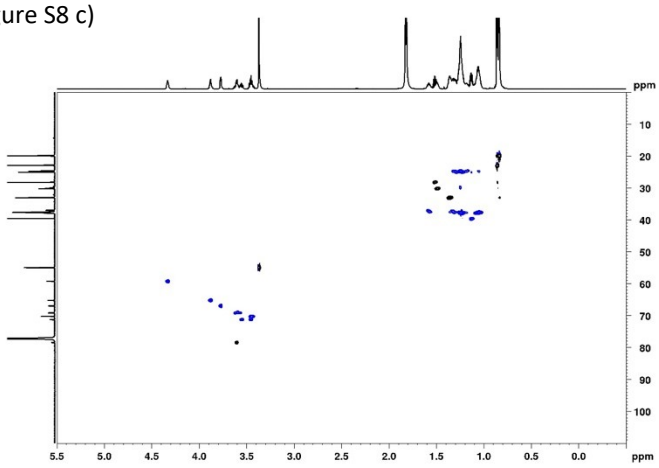


Figure S8 e)

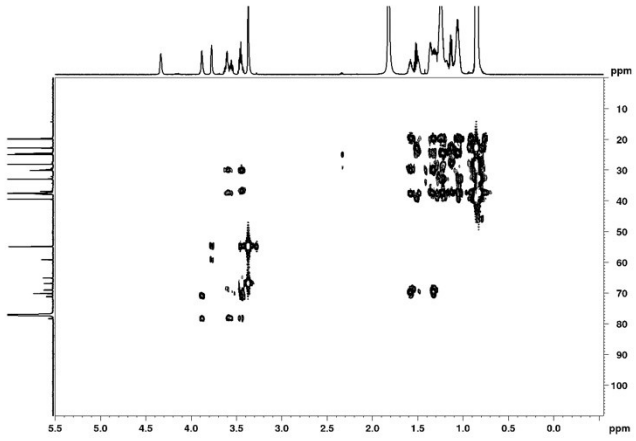


Figure S8. *Liquid state NMR spectra of DoPhPC (1-2mg in $CDCL_3$, 25°C) performed at 800MHz (proton frequency) on a cryogenic probe CP TXI 2H/1H/13C/15N. a) ^{1}H-NMR spectrum, spectral width of 15ppm, 8 scans, recycling delay of 2s, b) ^{13}C-NMR spectrum, spectral width of 240ppm, 8k scans, recycling delay of 5s, c) 2D ^{1}H-^{1}H Total Correlation SpectroscopY (TOCSY) NMR map, spectral width of 12ppm, 16 scans, Time domain in F1 256points, TOCSY spinlock of 300ms, recycling delay of 1.5s, d) 2D ^{1}H-^{13}C Heteronuclear Single Quantum Coherence (HSQC) NMR map, spectral width of 12ppm in F2, 170ppm in F1, 32 scans, Time domain in F1 256points, recycling delay of 1.5s, e) 2D ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation (HMBC) NMR map, spectral width of 13ppm in F2, 220ppm in F1, 48 scans, Time domain in F1 128points, recycling delay of 1.5s*

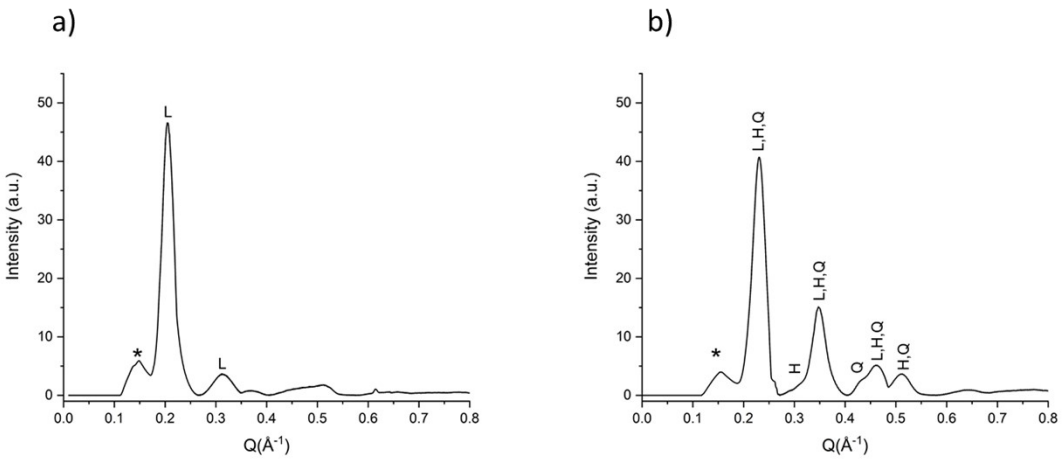


***Figure S9**. SAXS data on a) DoPhPC b) DoPhPI:DoPhPC at 25°C. Indexation is made according to Bragg peak occurrence in Lamellar (L, lattice parameter aLam= 54.5 Å), Hexagonal of type II (H, aHex = 47.5 Å), and Cubic Pn3m (Q, aQ_{Pn3m} = 54.5 Å). *: beam stop.*

***Table S2**. Amount of the different lipid phases determined from ssNMR*

	PC			PC/PI		
T(°C)	L	H	Q	L	H	Q
25	100			43	7	50
5	100			80	0	20
15	100			58	0	42
20	100			53	0	47
25	100			45	0	55
30	100			35	5	60
35	100			35	15	50
40	100			35	15	50
45	100			20	21	59
50	100			0	34	66
55	100			0	28	72
60	100			0	28	72
67	100			0	35	65
25	100			0	0	100

Percentages are estimated from spectral simulations (vide supra) or spectral subtraction of an isotropic line (with matching width) to the total spectrum. Accuracy 5%.

***Table S3.** Lattice parameters (Å) at 25°C*

	PC	PC/PI
a_{lam}	61.0	54.5
a_{hex}		47.5
a_{cub}		54.5

Lattice parameters have been calculated by fitting the Bragg orders the equation $q(n) = 2\pi n/a_i$ *where* a_i *is the lattice parameter with i = lamellar, hexagonal, cubic. For lamellar symmetry,* $n^2 = h^2$*, k = l = 0, for hexagonal,* $n^2 = h^2 + k^2 + hk$*, l = 0,* $q(n) = 2\pi n/a_{hexagonal} 2/\sqrt{3}$*, for cubic* $n^2 = h^2 + k^2 + l^2$*. In the latter case the permitted combinations depend on the cubic phase of interest, for Pm3n,* n^2 *= 2, 4, 5, 6, 8, 10, 12, 13, … Calculations were performed using a home-made program written with MATLAB 2021a and compared to the experimental SAXS curves to obtain the best fit. Accuracy is ca. 1 Å.*

Table S4. *Simulation parameters for the DoPhPC:DoPhPI (1:1) + 10 mol% $^2H_{31}$-DPPC deuterium NMR spectrum at 25°C.*

Phase	Labelled Carbon Position	Number of deuterons	Quadrupolar Splitting (kHz)	Line width (Hz)	S_{CD}	S_{CC}	weight %
Lamellar	2	2	54.0	800	-0.216	0.196	90
	3	2	54.0	800	-0.216	0.235	
	4	2	54.0	800	-0.216	0.196	
	5	2	54.0	800	-0,216	0.235	
	6	2	52.0	600	-0.208	0,196	
	7	2	52.0	600	-0.208	0.220	
	8	2	48.0	600	-0.192	0.196	
	9	2	44.0	600	-0.176	0.188	
	10	2	38.0	600	-0.152	0.164	
	11	2	36.0	600	-0.144	0.140	
	12	2	32.0	600	-0.128	0.148	
	13	2	28.0	600	-0.112	0.108	
	14	2	22.0	600	-0.088	0.116	
	15	2	14.0	600	-0.056	0.060	
	16	3	4.0	350	-0.016	0.052	
Isotropic			0.0	300			10

Procedure as in Douliez et al, ref. 25. Accuracy 1%.

Table S5. *Simulation parameters for the DoPhPC:DoPhPI (1:1) + 10 mol% + 10 mol% $^2H_{31}$-DPPC phosphorus-NMR spectrum at 25°C*

Phase	Chemical Shift Anisotropy (ppm)	Line width (Hz)	Weight (%)
Lamellar DoPhPI	-61.1	250	19
Lamellar DoPhPC+DPPC	-48.7	250	30
Isotropic	0	1300	51

Procedure as in Pott & Dufourc, ref.22. Accuracy 5%.

Table S6. *Average DPPC Chain length as calculated from order parameters.*

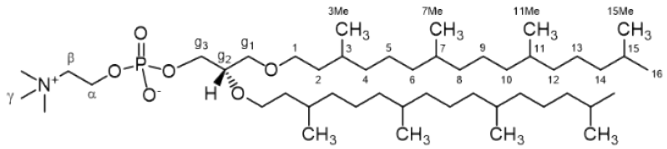
Systems/ Temperature	60°C	40°C	25°C
DoPhPC:DoPhPI(1:1) + 10% $^{2}H_{31}$DPPC		12.8[b]	13.2[b]
DoPhPC + 10% $^{2}H_{31}$DPPC	12.7[c]	13.0[c]	13.4[c]
$^{62}H_{31}$DPPC	13.2[c]	13.8[c]	

[b]*Lamellar phase in the two-phase system L_α/Q*

[c]*Lamellar phase, L_α (one phase system)*

Table S7. *DoPhPC ^{1}H and ^{13}C chemical shift assignment based on 1D and 2D experiments. ^{1}H and ^{13}C chemical shifts are referenced to $CHCl_3$ residual signal calibrated at 7.26ppm (^{1}H) and 77.10ppm in (^{13}C). Accuracy 0.01 and 0.02 ppm for ^{1}H and ^{13}C chemical shifts, respectively.*

The carbon resonances of the two phytanyl chains are indistinguishable except when indicated by (a) where *two ^{13}C chemical shifts are detected, probably due to the presence of two diastereoisomers, and by a prime (') for carbons C1 and C2 of the sn-2 chain. Phosphorus-carbon coupling constants at 2, 3, 4 bonds are given as well as multiplicity (d: doublet).*



Atom Numbering	^{1}H chemical shift (ppm)	^{13}C chemical shift (ppm)
α	4.33	59.15 (d, $^{2}J_{CP}$=4.5Hz)
β	3.77	66.88 (d, $^{3}J_{CP}$=6.2Hz)
γ	3.37	54.88
g1[(a)]	3.55/3.45	71.21 (d, $^{4}J_{CP}$=3.0Hz) 71.17 (d, $^{4}J_{CP}$=4.3Hz)
g2[(a)]	3.60	78.40 (d, $^{3}J_{CP}$=7.9Hz) 78.30 (d, $^{3}J_{CP}$=8.4 Hz)
g3[(a)]	3.88	65.10 (d, $^{2}J_{CP}$=6.1Hz) 65.07 (d, $^{2}J_{CP}$=5.4Hz)
1	3.45	70.15
1'[(a)]	3.60	69.04 68.91
2,2'	1.58	36.96 36.86
3	1.50	30.08
3Me,7Me,11Me	0.86-0.83	19.85-19.69
7,11	1.36	32.94-32.90
14	1.13	39.47
15	1.51	28.19
15Me,16	0.86	22.83 22.73
4,6,8,10,12	1.32-1.01	37.85-37.34
5,9,13	1.30-1.05	24.91-24.50