Electronic Supplementary Information (ESI):

Highly efficient synthesis of phosphatidylserine in the eco-friendly solvent γ-valerolactone

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Experimental section

Biological and chemical materials

PC (≥99%, from Soybean), PS (≥97%, from Soybean), PA (98%, from Soybean), γ-valerolactone (99%), PLD-3 (from Arachis hypogaea, 32.8 U mg^{-1}), PLD-4 (from Cabbage, 335 U mg^{-1}) and PLD-5 (from *Streptomyces* sp., 156 U mg^{-1}) were purchased from Sigma-Aldrich (USA). PLD-1 (from *Streptomyces chromofuscus*, 60 U mg^{-1}) and PLD-2 (from *Streptomyces* sp., 100 U mg^{-1}) were purchased from Asahi-kasei pharma corporation (Japan). All other chemicals and reagents used were obtained from commercial sources and were of analytical grade.

All the enzymes are lyophilized powder. The enzyme activity determination approach was as follows: 3.0 mL γ-valerolactone containing 0.05 mmol PC, 0.15 mmol L-serine, 1 mg enzyme and 0.5% water (based on the total weight of the reaction system) were incubated in an air-bath rotary shaker at 180 rpm at 40 ^{o}C. One unit was defined as the amount of enzyme which catalyzed 1 μmole PC to form PS per hour at 40 ^{o}C. The enzymatic activity profiles of the PLDs used were depicted in Fig. 1s. All the data were averages of experiments performed at least in duplicate.

The thermal stability of PLD-1 was also investigated (as presented in Fig. 2s). PLD-1 was incubated in an air-bath rotary shaker at 20 ^{o}C, 30 ^{o}C, 40 ^{o}C, 50 ^{o}C, 60 ^{o}C and 70 ^{o}C, respectively, for 1 h. The enzyme residual activity was calculated by the enzyme activity determination method as described above. All the data were averages of experiments performed at least in duplicate.

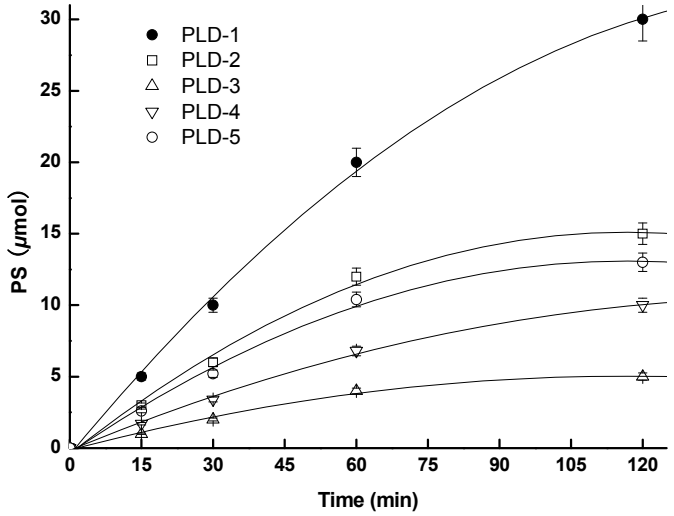

Fig. 1s The enzymatic activity of PLDs.

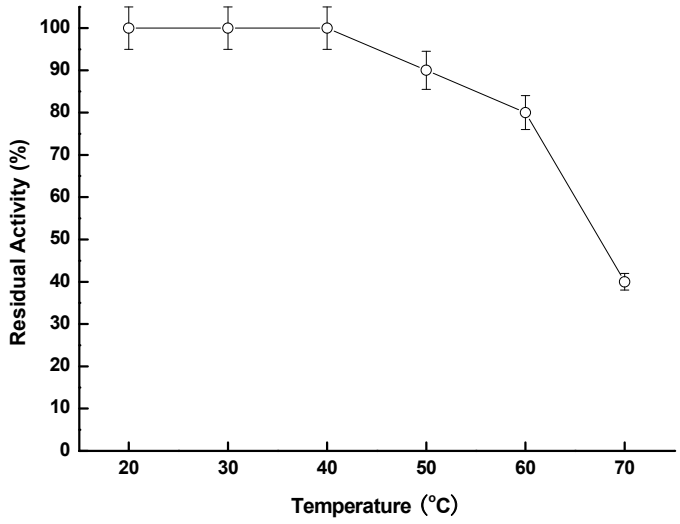

Fig. 2s The thermal stability of PLD-1.

General procedure for enzymatic synthesis of PS

In a typical experiment, the enzymatic transphosphatidylation was performed in a 10 mL flask on an air-bath rotary shaker at 180 rpm. The compositions of the reaction mixtures were as follows: 3.0 mL of γ-valerolactone, 0.05 mmol PC, a certain amount of L-serine, enzyme and 0.5% water (based on the total weight of the reaction system). 50 μL samples were taken from the reaction mixture at specified

time intervals, centrifuged to obtain the upper layer and analyzed by HPLC.

During the experiments of comparing the reaction systems, the γ-valerolactone system containing 3.0 mL of γ-valerolactone, 0.05 mmol PC, 0.15 mmol L-serine, 60 U PLD-1 and 0.5% water (based on the total weight of the reaction system) was incubated in an air-bath rotary shaker at 180 rpm at 40 ^{o}C. By contrast, in the biphasic system, the reaction mixtures were replaced by 1.5 mL ethyl acetate (containing 0.05 mmol PC) and 1.5 mL 0.2 M acetate buffer (pH=5.5, containing 0.15 mmol L-serine and 60 U PLD-1), and the others were the same; in the purely aqueous system, the reaction mixtures were replaced by 3.0 mL 0.2 M acetate buffer (pH=5.5, containing 0.05 mmol PC, 0.15 mmol L-serine and 60 U PLD-1), and the others were the same.

In the experiments of investigating the temperature effect on the reaction, the conditions were as follows: 3.0 mL of γ-valerolactone, 0.05 mmol PC, 0.15 mmol L-serine, 60 U PLD-1, 0.5% water (based on the total weight of the reaction system).

In the experiments of investigating the substrate molar ratio effect on PS yield, the conditions were as follows: 3.0 mL of γ-valerolactone, 0.05 mmol PC, 60 U PLD-1, 0.5% water (based on the total weight of the reaction system), 40 ^{o}C, 12 h.

In the experiments of PS synthesis catalyzed by various PLDs, the conditions were as follows: 3.0 mL of γ-valerolactone, 0.05 mmol PC, 0.15 mmol L-serine, 60 U enzyme, 0.5% water (based on the total weight of the reaction system), 40 ^{o}C, 12 h. The control reaction was performed by following the above procedure except that no enzyme was added.

HPLC analysis

The samples were detected using the method described by Agilent Technologies.[1] The sample was analyzed by a Waters e2695 HPLC system (Waters, Milford, USA) with an evaporative light scattering detector (ELSD). External standards of PC, PS and PA were used to prepare calibration solutions at five different concentrations. 2 μL sample and 1 mL hexane-isopropane (1/1, v/v) were precisely measured and mixed thoroughly. 10 μL of the aforementioned mixture was injected. The stationary and mobile phases were a Lichrospher 100 Diol column (5 μm, 250 mm × 4.6 mm) (Merck

Chemicals, Germany) and a gradient elution program (Table 1s) at 1.5 mL min^{-1}, respectively. The column temperature and drift pipe temperature were controlled at 25 and 65 ^{o}C, respectively, and the nitrogen pressure was controlled at 40 psi. The retention times were 2.520, 2.948, and 23.826 min for PA, PS and PC, respectively (Fig. 3s). PS yield was calculated from the HPLC data. The average error for this assay is less than 0.5%. All the reported data are averages of experiments performed at least in duplicate.

Table 1s Gradient elution program*

Time (min)	Flow rate (mL min^{-1})	Hexane-isopropanol-methanol (95/2.5/2.5) (v/v, %)	Isopropanol-methanol (40/60) (v/v, %)
0	1.50	100	0
20.0	1.50	81.3	18.7
20.2	1.50	0	100
25.0	1.50	0	100
25.1	1.50	100	0
30.0	1.50	100	0

* Each mobile phase contained 1 mM ammonium acetate.

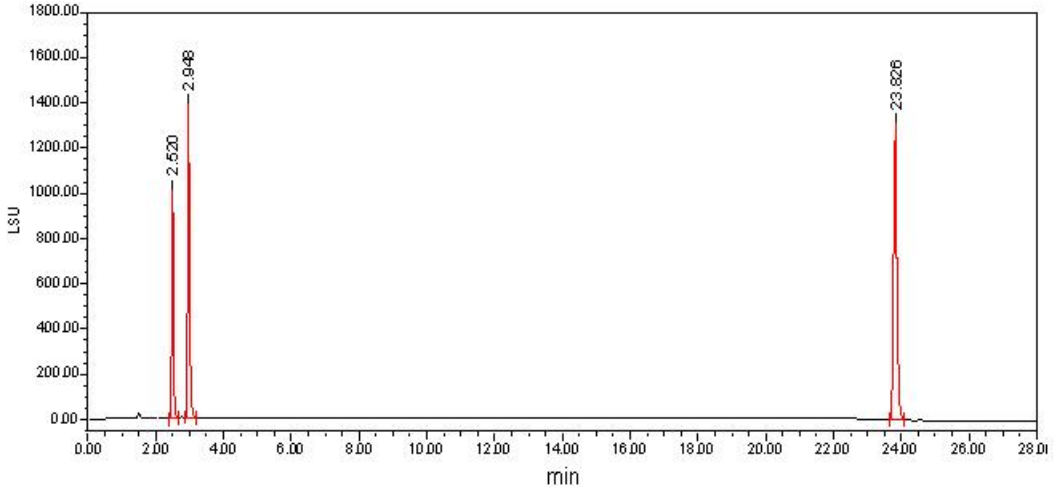

Fig. 3s The sample chromatogram with a mixture of PA, PS and PC.

1 C. Woodward and R. Majors. The HPLC preparative scale-up of soybean phospholipids. Agilent Technologies Publications 5989-2848EN, www.agilent.com/chem/cn.