Supporting Information

Design and Synthesis of Artificial Phospholipid for Selective Cleavage of Integral Membrane Protein

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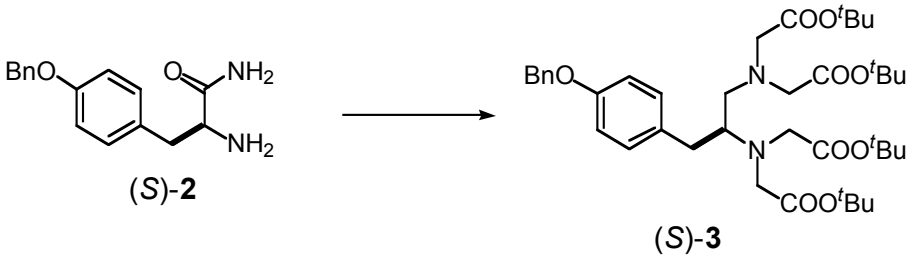
General

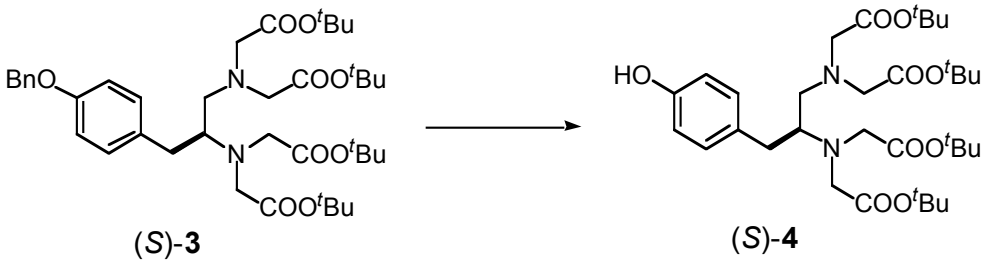
Optical rotations were measured on a JASCO P-1030 polarimeter. ^{1}H and ^{13}C NMR spectra were obtained on JEOL ECA-500 at 500 and 125 MHz, respectively, with chemical shifts being reported as ppm from tetramethylsilane as an internal standard. ^{31}P NMR spectra were taken on JEOL ECA-500 at 202 MHz and chemical shifts are referenced to H_3PO_4 as an internal standard. The mass spectra were measured on a JEOL MStation JMS-700 spectrometer. IR spectra were recorded on a JASCO WS/IR-8000. The recycling preparative HPLC was performed on JAI LC-908. THF was distilled from sodium benzophenone ketyl, CH_2Cl_2, MeOH and pyridine were from calcium hydride, magnesium and NaOH, respectively. Unless otherwise noted, all reactions were run under an argon atmosphere. All extractive organic solutions were dried over anhydrous $MgSO_4$, filtered and then concentrated under reduced pressure. Column chromatography was carried out with silica gel 60N spherical (63-210 mesh, KANTO CHEMICAL).



***t*-Bu protected EDTA derivative (*S*)-3.**

To a solution of *O*-benzyl-L-tyrosinamide (*S*)-**2** (1.89 g, 7.0 mmol) in THF (15 mL) was added 1 M BH_3-THF solution (35 mL, 35 mmol) at 0 °C and stirred under reflux for 7 h. The mixture was added MeOH at 0 °C and concentrated to give a residue. The residue was added 1 M HCl (40 mL) and Et_2O (40 mL), then stirred at room temperature for 1 h. After removal of the organic layer, the H_2O layer was basified by 2 N NaOH. The resulting H_2O layer was extracted with CH_2Cl_2 and the combined organic layer was washed with brine, dried and evaporated to give a residue (1.77 g) containing diamine derivative. The mixture of the resulted residue, Proton sponge (7.44 g, 34.7 mmol), and NaI (1.14 g, mmol) in CH_3CN (20 mL) was added *tert*-butylbromoacetate (5.12 mL, 34.6 mmol), then refluxed for 13 h. After cooling, the reaction mixture was added H_2O and EtOAc, then filtered to remove solid. The organic layer was separated from filtrate, washed with 0.2 N citric acid aq., dried and evaporated in vacuo. The residue was purified by column chromatography (SiO_2, 1% Et_3N in hexane / EtOAc = 2 / 1) to afford (S)-**3** (2.2 g, 44%) as a pale yellow oil.

$[\alpha]_D^{20}$ = - 0.40 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 18H), 1.45 (s, 18H), 2.50 - 2.65 (m, 2H), 2.80 - 2.95 (m, 2H), 3.05 - 3.15 (m, 1H), 3.35 - 3.65 (m, 8H), 5.02 (s, 2H), 6.86 (d, 2H, *J* = 8.6 Hz), 7.14 (d, 2H, *J* = 8.6 Hz), 7.28 - 7.45 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 28.0, 35.8, 53.4, 54.9, 56.1, 63.2, 69.8, 80.4, 80.5, 114.5, 127.3, 127.7, 128.4, 130.0, 132.5, 137.1, 156.9, 170.7, 171.2; IR ($CHCl_3$) 1734 cm^{-1}; MS (FAB) *m/z* 713 $(M+H)^+$; HR MS Calcd for $C_{40}H_{61}N_2O_9$ $(M+H)^+$ 713.4377, Found 713.4377.

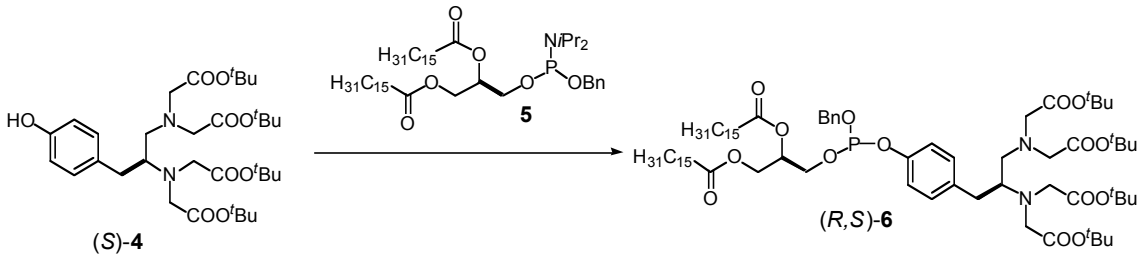


(*S*)-**3** (*S*)-**4**

Phenol (*S*)-4.

A mixture of (*S*)-**4** (1.7 g, 2.4 mmol) and 10% Pd-C (470 mg) in MeOH (10 mL) was stirred at room temperature for 2 h under hydrogen. The reaction mixture was filtered and evaporated in vacuo to give a residue. The residue was purified by recycling preparative HPLC (JAIGEL-1H and 2H, $CHCl_3$, 3.5 mL/min, t_R = 42 min) to afford (*S*)-**4** (1.2 g, 78%).

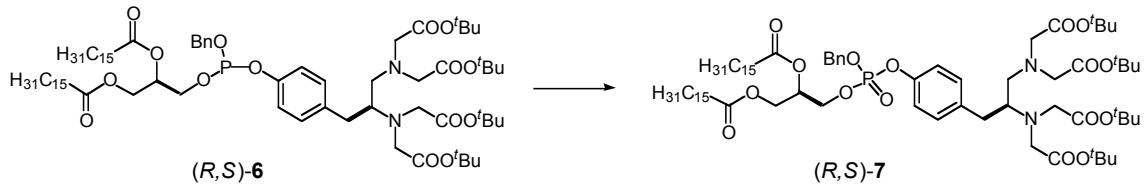
$[\alpha]_D^{20}$ = + 3.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 18H), 1.45 (s, 18H), 2.40-2.65 (m,2H), 2.77 - 2.95 (m, 2H), 3.00 - 3.15 (m, 1H), 3.48 (s, 4H), 3.50 (s, 4H), 6.75 (d, 2H, *J* = 8.0 Hz), 7.00 (d, 2H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 28.2, 35.7, 53.7, 55.2, 56.3, 63.4, 81.0, 81.2, 115.4, 130.2, 131.2, 154.7, 171.0, 171.7; IR ($CHCl_3$) 1736 cm^{-1}; MS (FAB) *m/z* 623 $(M+H)^+$; HR MS Calcd for $C_{33}H_{55}N_2O_9$ $(M+H)^+$ 623.3907, Found 623.3903.



Phosphite (*R*,*S*)-6.

To a solution of amidite **5** (118 mg, 0.15 mmol) and 1*H*-tetrazole (21 mg, 0.30 mmol) in CH_2Cl_2 (3.0 mL) was added (*S*)-**4** (35 mL, 35 mmol) in CH_2Cl_2 (2.0 mL) and stirred at room temperature for 2 h. The mixture was concentrated to give a residue. The residue was purified by recycling preparative HPLC (JAIGEL-1H and 2H, $CHCl_3$, 3.5 mL/min, t_R = 37 min) to give (*R,S*)-**6** (125 mg, 64%) as a colorless oil.

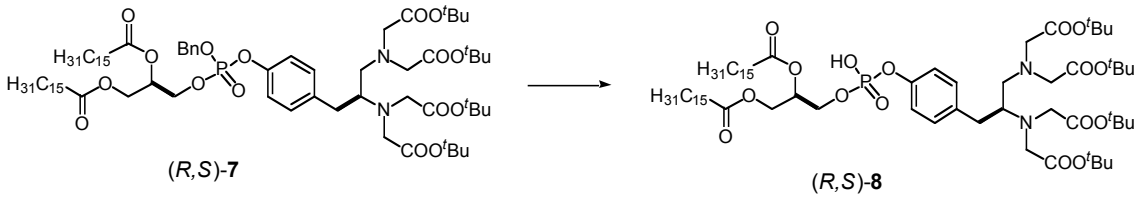
$[\alpha]_D^{20}$ = + 2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.9 Hz), 1.20 - 1.70 (m, 88H), 2.20 - 2.35 (m, 4H), 2.50 - 2.60 (m, 1H), 2.60 - 2.70 (m, 1H), 2.80 - 2.88 (m, 1H), 2.88 - 3.00 (m, 1H), 3.05 - 3.15 (m, 1H), 3.35 - 3.55 (m, 8H), 4.00 - 4.12 (m, 2H), 4.12 - 4.25 (m, 1H), 4.30 - 4.40 (m, 1H), 4.95 - 5.05 (m, 2H), 5.15-5.25 (m, 1H), 6.91 (d, 2H, *J* = 8.0 Hz), 7.15 (d, 2H, *J* = 8.0 Hz), 7.25 - 7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.8, 28.08, 28.11, 29.06, 29.10, 29.26, 29.33, 29.5, 29.6, 29.7, 31.9, 34.0, 34.2, 36.1, 53.5, 55.2, 56.3, 60.60, 60.66, 60.74, 62.1, 63.2, 64.3, 64.4, 70.08, 70.11, 70.18, 80.6, 80.7, 119.66, 119.72, 127.6, 127.9, 128.5, 130.4, 135.7, 137.57, 137.61, 150.23, 150.28, 170.9, 171.3, 172.9, 173.3; ^{31}P NMR ($CDCl_3$) δ 134.0 (s); IR ($CHCl_3$) 1734, 1716, 1699 cm^{-1}; MS (FAB) *m/z* 1328 $(M+H)^+$; HR MS Calcd for $C_{75}H_{128}N_2O_{15}P$ $(M+H)^+$ 1327.9053, Found 1327.9075.



Benzyl protected phosphodiester (*R,S*)-7.

To a solution of (*R,S*)-**6** (118 mg, 0.15 mmol) and $NaHCO_3$ (31 mg, 0.45 mmol) in CH_2Cl_2 (3.0 mL) was added 67 mM MCPBA in CH_2Cl_2 (1.5 mL, 0.10 mmol) dropwise at 0 °C untill starting material (*R,S*)-**6** was disappeared. The mixture was added sat. $NaHCO_3$ aq. and extracted with CH_2Cl_2.The organic layer was washed with brine, dried and evaporated in vacuo to give a residue. The residue was purified by recycling preparative HPLC (JAIGEL-1H and 2H, $CHCl_3$, 3.5 mL/min, t_R = 37 min) to give (*R,S*)-**7** (115 mg, 84%) as a colorless oil.

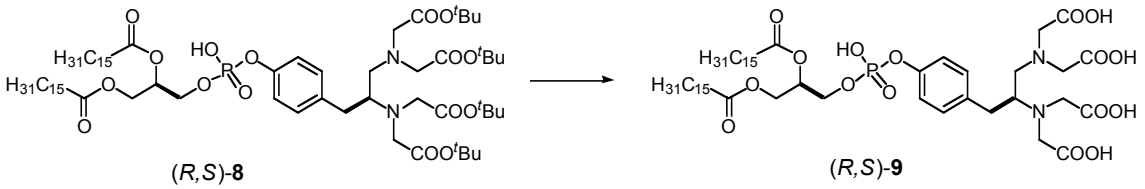
$[\alpha]_D^{20}$ = - 0.86 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.9 Hz), 1.10 – 1.80 (m, 88H), 2.20 – 2.35 (m, 4H), 2.45 – 2.60 (m, 1H), 2.65 – 2.75 (m, 1H), 2.80 – 2.90 (m, 1H), 2.90 – 3.00 (m, 1H), 3.02 – 3.15 (m, 1H), 3.35 – 3.55 (m, 8H), 4.05 – 4.35 (m, 4H), 5.05 - 5.25 (m, 3H), 7.04 (d, 2H, *J* = 8.0 Hz), 7.20 (d, 2H, *J* = 8.0 Hz), 7.30 - 7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.78, 24.82, 28.09, 28.13, 29.06, 29.11, 29.28, 29.35, 29.5, 29.65, 29.69, 31.9, 34.0, 34.1, 36.1, 53.5, 55.3, 56.3, 61.5, 63.1, 63.2, 65.86, 65.91, 69.18, 69.23, 70.08, 70.11, 80.7, 119.53, 119.57, 128.0, 128.6, 128.7, 130.5, 135.3, 148.53, 148.55, 170.8, 171.2, 172.8, 173.2; ^{31}P NMR ($CDCl_3$) δ - 5.65 (s); IR ($CHCl_3$) 1736 cm^{-1}; MS (FAB) *m/z* 1344 $(M+H)^+$; HR MS Calcd for $C_{75}H_{128}N_2O_{16}P$ $(M+H)^+$ 1343.9002, Found 1343.9015.



Phosphodiester (*R,S*)-8.

A mixture of (*R,S*)-**7** (522 mg, 0.39 mmol) and 10% Pd-C (200 mg) in MeOH (5.0 mL) was stirred at room temperature for 4.5 h under hydrogen. The reaction mixture was filtered and evaporated in vacuo to give a residue. The residue was purified by recycling preparative HPLC (JAIGEL 1H and 2H, 3.5 mL / min) to afford (*R,S*)-**8** (405 mg, 83%).

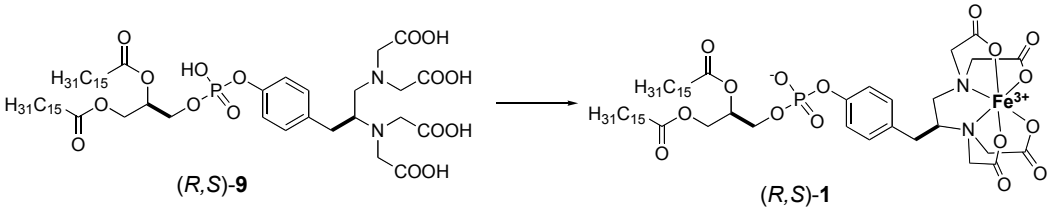
$[\alpha]_D^{20}$ = +13.8 (c = 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 6H, *J* = 6.3 Hz), 1.16 - 1.70 (m, 88H), 1.43 (s, 18H), 1.44 (s, 18H), 1.48 - 1.61 (m, 4H), 2.18 - 2.28 (m, 4H), 2.33 - 2.46 (m, 1H), 2.84 - 2.93 (m, 1H), 3.17 - 4.50 (m, 15H), 5.23 (m, 1H), 6.99 (d, 2H, *J* = 8.0 Hz), 7.12 (d, 2H, *J* = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.6, 24.8, 27.8, 27.9, 28.0, 29.10, 29.14, 29.32, 29.5, 29.62, 29.65, 29.67, 31.9, 34.1, 34.2, 52.9, 55.5, 55.6, 62.6, 63.9, 70.2, 81.8, 83.1, 120.7, 129.6, 131.9, 160.9, 170.5, 172.9, 173.3; ^{31}P NMR ($CDCl_3$) δ - 6.63 (s); IR ($CHCl_3$) 1734 cm^{-1}; MS (FAB) *m/z* 1254 $(M+H)^+$; HR MS *m/z* Calcd for $C_{68}H_{122}N_2O_{16}P$ $(M+H)^+$ 1253.8532 Found 1253.8555.



EDTA modified phospolipid (*R,S*)-9.

The solution of (*R,S*)-**8** (45 mg, 35.8 μmol) in TFA (1.0 mL) was stirred at room temperature for 10 h in the dark. The mixture was evaporated in vacuo to give (*R,S*)-**9** (37 mg, quant.) as a colorless amorphous**.**

$[\alpha]_D^{20}$ = +3.5 (c = 1.2, $CHCl_3$: MeOH = 6 : 1); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6H, *J* = 6.9 Hz), 1.20 - 1.45 (m, 48H), 1.50 – 1.70 (m, 4H), 2.20 - 2.40 (m, 4H), 2.40 - 4.50 (m, 17H), 5.26 (brs, 1H), 7.00 – 7.30 (m, 4H); ^{31}P NMR ($CDCl_3$: CD_3OD = 6 : 1) δ - 5.60 (s); MS (FAB) *m/z* 1030 $(M+H)^+$, 1052 $(M+Na)^+$; HR MS *m/z* Calcd for $C_{52}H_{90}N_2O_{16}P$ $(M+H)^+$ 1029.6028 Found 1029.6038.



Fe(III)-EDTA modified phospolipid (*R,S*)-1.

To a solution of (*R,S*)-**9** (3.0 mg, 2.4 μmol) in $CHCl_3$ (40 μL) / MeOH (20 μL) was added 134 mM $FeCl_3$ in MeOH (18 μL, 2.36 μmol) in the presence of ^{i}Pr$_2$NEt (3.3 μL, 19 μmol) and stirred at room temperature for 1h. The mixture was evaporated in vacuo to give (*R,S*)-**1** as a yellow amorphous**.**

MS (FAB) *m/z* 1083 $[(M-2)+Fe]^+$, 1105 $[(M-3)+Fe+Na]^+$, 1121 $[(M-3)+Fe+K]^+$.

Cleavage reaction of hemagglutinin with (*R,S*)-1 (Figure. 2b).

The suspension (5.8 μL) of influenza virus in PBS buffer (1.3 mg / mL buffer) was centrifuged to give a pellet, which was washed with 10 mM MOPS buffer (80 μL x 3). After the pellet was suspended in 10 mM MOPS buffer (80 μL), 6.4 mM solution of (*R,S*)-**1** in DMSO (8 μL) was added (final conc. of (*R,S*)-**1**: 0.58 mM). The mixture was incubated at 30 °C for 1h to incorporate (*R,S*)-**1** into viral membrane, then washed with 10 mM MOPS buffer (80 μL x 3) by centrifugation and resuspension to remove excess (*R,S*)-**1**. The resulted pellet was suspended in 10 mM MOPS buffer (80 μL), then 40 mM sodium ascorbate (pH 7.0, 11.5 μL) and 40 mM H_2O_2 (pH 7.0, 11.5 μL) were successively added to initiate cleavage reaction (final conc. of sodium ascorbate and H_2O_2: 4.0 mM). After incubation for 1 and 2h at 30 °C, the aliquot (26 μL) of reaction mixture was collected and added to SDS-PAGE sample loading buffer [200 mM Tris·HCl (pH 6.8) / 48% glycerol / 16% SDS / 8% 2-mercaptoethanol / 0.04% bromophenol blue] (8.7 μL) to quench the cleavage reaction. The quenched reaction mixture was heated at 100 °C for 5min and the aliquot (15 μL) was analyzed by SDS-PAGE stained with Coomassie-blue.

For control experiment (lane 2), 10 mM MOPS buffer was added to the reaction mixture instead of 40 mM sodium ascorbate and 40 mM H_2O_2.

Cleavage reaction of hemagglutinin with Fe(III)-EDTA complex (Figure. 2b).

The suspension (5.8 μL) of influenza virus in PBS buffer (1.3 mg / mL buffer) was centrifuged to give a pellet, which was washed with 10 mM MOPS buffer (80 μL x 3). The pellet was suspended in 10 mM MOPS buffer (88 μL), then incubated at 30 °C for 1h. The incubated solution was washed with 10 mM MOPS buffer (80 μL x 3). After the resulted pellet was suspended in 10 mM

MOPS buffer (80 μL), 6.4 mM solution of Fe(III)-EDTA complex in 10 mM MOPS buffer (8 μL), 40 mM sodium ascorbate (pH 7.0, 11.5 μL) and 40 mM H_2O_2 (pH 7.0, 11.5 μL) were successively added to initiate cleavage reaction (final conc. of sodium ascorbate and H_2O_2: 4.4 mM). After incubation for 1 and 2h at 30 °C, the aliquot (26 μL) of reactiom mixture was collected and added to SDS-PAGE sample loading buffer [200 mM Tris·HCl (pH 6.8) / 48% glycerol / 16% SDS / 8% 2-mercaptoethanol / 0.04% bromophenol blue] (8.7 μL) to quench the cleavage reaction. The quenched reaction mixture was heated at 100 °C for 5min and the aliquot (15 μL) was analyzed by SDS-PAGE stained with Coomassie-blue.

Cleavage reaction of bovine fetuin with (*R,S*)-1 and Fe(III)-EDTA complex (Figure. 3).

The solution (11.6 μL) of fetuin in 10 mM MOPS buffer (14.1 mg / mL) was diluted with 10 mM MOPS buffer (144 μL). The cleavage reaction was initiated by adding 6.4 mM solution of (*R,S*)**-1** in DMSO (16 μL), 40 mM sodium ascorbate (pH 7.0, 23 μL) and 40 mM H_2O_2 (pH 7.0, 23 μL) (final conc. of sodium ascorbate and H_2O_2: 4.4 mM). After incubation for 30 s, 10, 20, 30, 40, 50 and 60 min at 30 °C, the aliquot (26 μL) of reactiom mixture was collected and added to SDS-PAGE sample loading buffer [200 mM Tris·HCl (pH 6.8) / 48% glycerol / 16% SDS / 8% 2-mercaptoethanol / 0.04% bromophenol blue] (16.9 μL) to quench the cleavage reaction. The quenched reaction mixture was heated at 100 °C for 5min and the aliquot (15 μL) was analyzed by SDS-PAGE stained with Coomassie-blue.

In the case of cleavage with Fe(III)-EDTA complex (Figure 3b), the solution of 6.4 mM Fe(III)-EDTA complex in 10 mM MOPS buffer (16 μL) was added instead of 6.4 mM solution of (*R,S*)**-1** in DMSO.