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 tetramethyldisilane as an internal standard. $^{31}$P NMR spectra were taken on JEOL ECA-500 at 202 MHz and chemical shifts are referenced to H$_3$PO$_4$ 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$_2$Cl$_2$, 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$_2$O (40 mL), then stirred at room temperature for 1 h. After removal of the organic layer, the H$_2$O layer was basified by 2 N NaOH. The resulting H$_2$O layer was extracted with CH$_2$Cl$_2$ 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$_3$CN (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$_2$O 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$_3$N 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$_3$) $\delta$ 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$_3$) $\delta$ 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$_2$O$_9$ (M+H)$^+$ 713.4377, Found 713.4377.
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, CHCl₃, 3.5 mL/min, tᵣ = 42 min) to afford (S)-4 (1.2 g, 78%).

\[ \alpha \] D²⁰ = +3.8 (c 1.0, CHCl₃); ¹H NMR (CDCl₃) 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); ¹³C NMR (CDCl₃) 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 (CHCl₃) 1736 cm⁻¹; MS (FAB) m/z 623 (M+H)⁺; HR MS Calcd for C₃₃H₅₅N₂O₉ (M+H)⁺ 623.3907, Found 623.3903.
Phosphite \((R,S)-6\).

To a solution of amidite \(5\) (118 mg, 0.15 mmol) and \(1H\)-tetrazole (21 mg, 0.30 mmol) in \(\text{CH}_2\text{Cl}_2\) (3.0 mL) was added \((S)-4\) (35 mL, 35 mmol) in \(\text{CH}_2\text{Cl}_2\) (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, \(\text{CHCl}_3\), 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, \(\text{CHCl}_3\)); \(^1H\) NMR (CDCl\(_3\)) \(\delta\) 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\(_3\)) \(\delta\) 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\(_3\)) \(\delta\) 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\(_2\)O\(_{15}\)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\(_2\)Cl\(_2\) (3.0 mL) was added 67 mM MCPBA in CH\(_2\)Cl\(_2\) (1.5 mL, 0.10 mmol) dropwise at 0 °C until starting material \((R,S)-6\) was disappeared. The mixture was added sat. NaHCO\(_3\) aq. and extracted with CH\(_2\)Cl\(_2\). 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.

\([\alpha]^{20}_D = -0.86\) (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\(_2\)O\(_{16}\)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$_2$O$_{16}$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.

\[ \left[ \alpha \right]_D^{20} = +3.5 \ (c = 1.2, \text{CHCl}_3 : \text{MeOH} = 6 : 1) \]; \(^1\)H NMR (CDCl\textsubscript{3}) \( \delta \) 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); \(^3\)P NMR (CDCl\textsubscript{3} : CD\textsubscript{3}OD = 6 : 1) \( \delta \) - 5.60 (s); MS (FAB) \( m/z \) 1030 (M+H)\(^+\), 1052 (M+Na)\(^+\); HR MS \( m/z \) Calcd for C\textsubscript{52}H\textsubscript{90}N\textsubscript{2}O\textsubscript{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\textsubscript{3} (40 µL) / MeOH (20 µL) was added 134 mM FeCl\textsubscript{3} in MeOH (18 µL, 2.36 µmol) in the presence of \(^3\)Pr\textsubscript{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$_2$O$_2$ (pH 7.0, 11.5 µL) were successively added to initiate cleavage reaction (final conc. of sodium ascorbate and H$_2$O$_2$: 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$_2$O$_2$.

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 H₂O₂ (pH 7.0, 11.5 µL) were successively added to initiate cleavage reaction (final conc. of sodium ascorbate and H₂O₂: 4.4 mM). After incubation for 1 and 2 h 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 5 min 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 H₂O₂ (pH 7.0, 23 µL) (final conc. of sodium ascorbate and H₂O₂: 4.4 mM). After incubation for 30 s, 10, 20, 30, 40, 50 and 60 min 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] (16.9 µL) to quench the cleavage reaction. The quenched reaction mixture was heated at 100 °C for 5 min 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.