Supporting Information

Synthesis and Characterization of New Piperazine-Type Inhibitors for Mitochondrial NADH-Ubiquinone Oxidoreductase (Complex I)

Naoya Ichimaru,[‡] Masatoshi Murai,[‡] Nobuyuki Kakutani,[‡] Junko Kako,[‡] Atsushi Ishihara,[‡] Yoshiaki Nakagawa,[‡] Takaaki Nishioka,[‡] Takao Yagi,[§] and Hideto Miyoshi[‡]

[‡]Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan and [§]Division of Biochemistry, Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037.

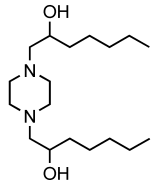
Contents

Abbreviations

DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIAD, diisopropyl azodicarboxylate; DMAP, *N,N*-4-dimethylaminopyridine; Ms, methanesulfonyl; Ph, phenyl; TBAF, tetra-*n*-butylammonium fluoride; TBS, *t*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

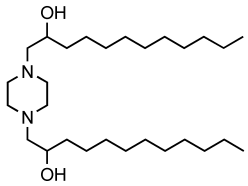
General Procedures

^{1}H NMR spectra were recorded at 400 MHz with a Bruker AVANCE 400 spectrometer using tetramethylsilane as the internal standard. ^{13}C NMR spectra were recorded at 100 MHz. The ESI-MS spectra were recorded with an Applied Biosystems API 3000 using the mixture of MeOH/H_2O/TFA (80:19.98:0.02) as a solvent. Optical rotations were measured with a JASCO P-1010 polarimeter. Column chromatography was performed on Wako silica gel (C-200, 75-150 μm) or YMC silica gel (SIL-60-S75, 42-105 μm). TLC analysis was conducted on silica gel Merck 60F_{254}, 0.25 mm precoated glass plates. Dry solvents were either used as purchased or freshly distilled using common practices where appropriate.



Compound 4

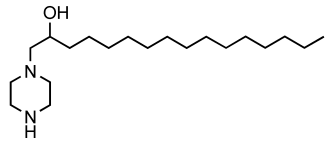
A mixture of 1,2-epoxyheptane (0.27 g, 2.36 mmol) and piperazine (50 mg, 0.58 mmol) in 2-propanol (8 mL) was refluxed at 100ºC for 24 h (*1*). After the reaction mixture was cooled down to room temperature, 2-propanol was evaporated and the residue was purified by Wako silica gel chromatography (2% MeOH/$CHCl_3$) to give **4** (164 mg, 0.52 mmol, 90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (m, 2H), 3.47 (br s, 2H), 2.69 (m, 4H), 2.40 (m, 4H), 2.35-2.25 (m, 4H), 1.45-1.29 (m, 16H), 0.89 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.14, 66.10, 64.13 (2C), 53.20 (br, 4C), 34.85 (2C), 32.01 (2C), 25.30 (2C), 22.62 (2C), 14.05 (2C); ESI-MS (*m/z*) 315.4 $[M+H]^+$.



Compound 5

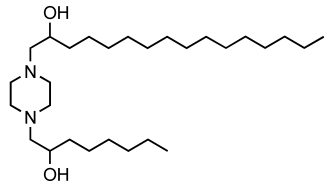
Compound **5** was synthesized by the same procedure used for the synthesis of compound **4**, except that 1,2-epoxydecane was used in place of 1,2-epoxyheptane, in a 48% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (m, 2H), 3.47 (br s, 2H), 2.69 (m, 4H), 2.39 (m, 4H), 2.35-2.22 (m, 4H), 1.46-1.12 (m, 36H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.15, 66.10, 64.14, 64.09, 53.44 (br, 4C),

34.91 (2C), 31.92 (2C), 29.81 (2C), 29.61 (4C), 29.34 (4C), 25.63 (2C), 22.70 (2C), 14.13 (2C); ESI-MS (*m/z*) 455.7 $[M+H]^+$.



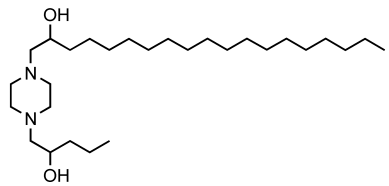
Compound 6-i

A mixture of 1,2-epoxyhexadecane (139 mg, 0.58 mmol) and piperazine (200 mg, 2.32 mmol) in 2-propanol (8 mL) was refluxed at 100ºC for 24 h and the reaction mixture was then cooled down to room temperature. To this mixture was added saturated aqueous $NaHCO_3$ and the resulting mixture was extracted with $CHCl_3$ for three times. The combined organic layer was dried over anhydrous $MgSO_4$, concentrated, and the residue was purified by Wako silica gel chromatography (20% MeOH/$CHCl_3$) to give **6-i** (165 mg, 0.50 mmol, 87%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) □ 3.66 (m, 1H), 2.94-2.84 (m, 4H), 2.90-2.10 (br s, 1H), 2.63 (m, 2H), 2.58 (br s, 1H), 2.34 (m, 2H), 2.32 (dd, *J* = 12.2, 3.0 Hz, 1H), 2.21 (dd, *J* = 12.2, 10.6 Hz, 1H), 1.46-1.20 (m, 26H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 65.91, 64.86, 54.55 (br, 2C), 46.23 (2C), 34.90, 31.92, 29.80, 29.66 (4C), 29.60 (3C), 29.35, 25.63, 22.69, 14.12; ESI-MS (*m/z*) 327.4 $[M+H]^+$.



Compound 6

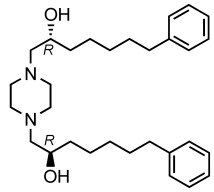
A mixture of **6-i** (50 mg, 0.153 mmol) and 1,2-epoxyoctane (78 mg, 0.61 mmol) in 2-propanol (2 mL) was refluxed at 100ºC for 12 h. After the reaction mixture was cooled down to room temperature, 2-propanol was evaporated and the residue was purified by Wako silica gel chromatography (EtOAc to 2% MeOH/$CHCl_3$) to give **6** (69 mg, 0.15 mmol, 99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) □ 3.67 (m, 2H), 3.45 (br s, 2H), 2.70 (m, 4H), 2.42 (m, 4H), 2.36-2.23 (m, 4H), 1.44-1.13 (m, 36H), 0.88 (t, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 66.15, 66.10, 64.14, 64.09, 53.04 (br, 4C), 34.90, 31.93, 31.80, 29.80, 29.67 (4C), 29.60 (4C), 29.46, 29.37, 25.62, 25.58, 22.70, 22.61, 14.13, 14.09; ESI-MS (*m/z*) 455.7 $[M+H]^+$.



Compound 7

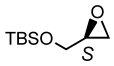
Compound **7** was synthesized by the same procedure used for the synthesis of compound **6**,

except that 1,2-epoxynonadecane and 1,2-epoxypentane were used in place of 1,2-epoxyhexadecane and 1,2-epoxyoctane, respectively, in a 66% yield (2 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67 (m, 2H), 3.48 (br s, 2H), 2.69 (m, 4H), 2.39 (m, 4H), 2.34-2.22 (m, 4H), 1.41-1.25 (m, 36H), 0.93 (t, J = 7.0 Hz, 3H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 66.10, 65.88, 64.14, 64.10, 53.34 (br, 4C), 37.04, 34.90, 31.93 (2C), 29.80, 29.70 (4C), 29.67 (2C), 29.61, 29.37 (2C), 25.63, 22.70 (2C), 18.85, 14.23, 14.13; ESI-MS (*m/z*) 455.7 $[M+H]^+$.



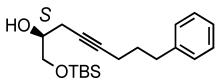
Compound 8

Compound **8** was synthesized by the same procedure used for the synthesis of compound **9**, except that 4-phenyl-1-butyne was used as a starting material in place of 5-phenyl-1-pentyne, in a 60% yield (5 steps): $[\alpha]_D^{26}$ = -11 (*c* 0.16, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.17 (m, 4H), 3.64 (m, 2H), 3.09 (br s, 2H), 2.69 (m, 4H), 2.61 (t, J = 7.7 Hz, 4H), 2.37 (m, 4H), 2.31 (dd, J = 12.2, 3.2 Hz, 2H), 2.24 (dd, J = 12.2, 10.4 Hz, 2H), 1.63 (m, 4H), 1.52-1.50 (m, 2H), 1.43-1.34 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.76 (2C), 128.38 (4C), 128.22 (4C), 125.58 (2C), 66.07 (2C), 64.09 (2C), 53.10 (br, 4C), 35.87 (2C), 34.79 (2C), 31.40 (2C), 29.40 (2C), 25.48 (2C); ESI-MS (*m/z*) 467.6 $[M+H]^+$.



Compound 9-i

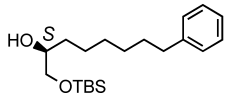
To a solution of (*R*)-(+)-glycidol (1.0 g, 13.5 mmol), DMAP (1.65 g, 13.5 mmol), and Et_3N (2.73 g, 27 mmol) in dry CH_2Cl_2 (40 mL) was added a solution of TBSCl (3.05 g, 20.3 mmol) in dry CH_2Cl_2 (60 mL) slowly over 2 h and the reaction mixture was stirred at room temperature for 18 h. The reaction mixture was then quenched and washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **9-i** (2.2 g, 11.7 mmol, 87%) as a colorless oil: $[\alpha]_D^{26}$ = -1.3 (*c* 0.79, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.85 (dd, J = 11.9, 3.2 Hz, 1H), 3.66 (dd, J = 11.9, 4.8 Hz, 1H), 3.08 (dddd, J = 4.8, 4.1, 3.2, 2.7 Hz, 1H), 2.77 (dd, J = 5.1, 4.1 Hz, 1H), 2.64 (dd, J = 5.1, 2.7 Hz, 1H), 0.90 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.75, 52.43, 44.48, 25.88 (3C), 18.37, -5.30, -5.34; ESI-MS (*m/z*) 189.3 $[M+H]^+$.



Compound 9-ii

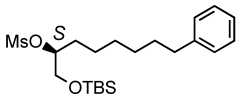
To a solution of 5-phenyl-1-pentyne (1.9 g, 13.1 mmol) in dry THF (20 mL) at -78ºC was added a solution of *n*-BuLi (1.58 M in hexane, 8.0 mL, 12.6 mmol) slowly over 5 min and the mixture was

stirred for 30 min. $BF_3{\cdot}Et_2O$ (1.46 mL, 11.6 mmol) was then added and the mixture was stirred for 15 min. To this mixture was added a solution of **9-i** (0.99 g, 5.26 mmol) in dry THF (10 mL) and the resulting mixture was stirred at -78ºC for 30 min. The mixture was quenched with saturated aqueous NH_4Cl and warmed up to room temperature. The mixture was then extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **9-ii** (1.63 g, 4.9 mmol, 93%) as a colorless oil: $[\alpha]_D^{26}$ = -10 (*c* 0.67, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.25 (m, 2H), 7.19-7.17 (m, 3H), 3.78 (dddd, *J* = 6.4, 5.8, 4.3, 4.2 Hz, 1H), 3.72, (dd, *J* = 9.8, 4.2 Hz, 1H), 3.62 (dd, *J* = 9.8, 5.8 Hz, 1H), 2.70 (t, *J* = 7.7 Hz, 2H), 2.46 (br s, 1H), 2.42 (ddt, *J* = 12.2, 6.4, 2.4 Hz, 1H), 2.37 (ddt, *J* = 12.2, 4.3, 2.4 Hz, 1H), 2.17 (ddt, *J* = 2.4, 2.4, 7.0 Hz, 2H), 1.79 (m, 2H), 0.90 (s, 9H), 0.08 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.69, 128.51 (2C), 128.33 (2C), 125.86, 82.10, 76.32, 70.51, 65.70, 34.84, 30.54, 25.89 (3C), 23.45, 18.32, 18.22, -5.36, -5.38; ESI-MS (*m/z*) 333.2 $[M+H]^+$.



Compound 9-iii

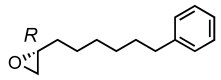
A mixture of **9-ii** (1.63 g, 4.9 mmol) and catalytic 10% Pd on carbon in EtOH (15 mL) was stirred at room temperature under a hydrogen gas atmosphere for 18 h. The mixture was then filtered to remove the catalyst and the filtrate was concentrated. The residue was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **9-iii** (1.6 g, 4.75 mmol, 97%) as a colorless oil: $[\alpha]_D^{26}$ = -0.63 (*c* 0.32, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 2H), 7.18-7.16 (m, 3H), 3.63 (m, 1H), 3.61 (dd, *J* = 10.6, 3.3 Hz, 1H), 3.37 (dd, *J* = 10.6, 8.4 Hz, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.40 (br s, 1H), 1.63-1.59 (m, 2H), 1.44-1.33 (m, 8H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.84, 128.40 (2C), 128.22 (2C), 125.57, 71.82, 67.28, 35.95, 32.77, 31.43, 29.58, 29.22, 25.89 (3C), 25.53, 18.30, -5.33, -5.39; ESI-MS (*m/z*) 337.2 $[M+H]^+$.



Compound 9-iv

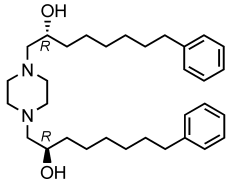
To a solution of **9-iii** (1.6 g, 4.75 mmol) and Et_3N (0.97 g, 9.6 mmol) in dry THF (45 mL) at 0ºC was added dropwise MsCl (0.56 mL, 7.2 mmol) and the mixture was stirred at 0ºC for 30 min. The reaction mixture was then quenched with H_2O, extracted with Et_2O, washed with saturated aqueous $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by YMC silica gel chromatography (10 to 15% EtOAc/hexane) to give **9-iv** (1.93 g, 4.67 mmol, 98%) as a colorless oil: $[\alpha]_D^{26}$ = +0.33 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.25 (m, 2H), 7.18-7.15 (m, 3H), 4.64 (dddd, *J* = 10.4, 6.3, 6.3, 4.1 Hz, 1H), 3.73 (dd, *J* = 11.3, 6.3 Hz, 1H), 3.69 (dd, *J* = 11.3, 4.1 Hz, 1H),

3.03 (s, 3H), 2.60 (t, *J* = 7.6 Hz, 2H), 1.67-1.61 (m, 4H), 1.44-1.39 (m, 6H), 0.89 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 142.68, 128.38 (2C), 128.23 (2C), 125.59, 84.41, 64.86, 38.56, 35.87, 31.42, 31.33, 29.24, 28.99, 25.86 (3C), 24.86, 18.34, -5.42, -5.45; ESI-MS (*m/z*) 415.3 $[M+H]^+$.



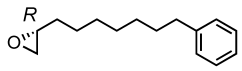
Compound 9-v

To a solution of **9-iv** (0.78 g, 1.88 mmol) in dry THF (18 mL) at 0ºC was added dropwise TBAF (1.0 M in THF, 1.88 mL, 1.88 mmol) and the mixture was stirred at 0ºC for 10 min. THF was then evaporated and the residue was purified by YMC silica gel chromatography (30 to 70% EtOAc/hexane) to give an intermediate alcohol, which was dissolved in MeOH (10 mL). To this solution was added K_2CO_3 (0.52 g, 3.76 mmol) and the resulting mixture was stirred at room temperature for 15 min. The mixture was then quenched with H_2O, extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by YMC silica gel chromatography (15% EtOAc/hexane) to give epoxide **9-v** (0.33 g, 1.6 mmol, 85%) as a colorless oil: $[□]_D^{26}$ = +7.0 (*c* 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.27-7.23 (m, 2H), 7.17-7.14 (m, 3H), 2.89-2.85 (m, 1H), 2.71 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.59 (t, *J* = 7.6 Hz, 2H), 2.43 (dd, *J* = 5.0, 2.7 Hz, 1H) 1.63-1.59 (m, 2H), 1.51-1.44 (m, 2H), 1.38-1.34 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 142.75, 128.41 (2C), 128.26 (2C), 125.63, 52.38, 47.10, 35.94, 32.47, 31.40, 29.32, 29.21, 25.93; ESI-MS (*m/z*) 205.2 $[M+H]^+$.



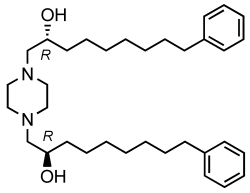
Compound 9

Compound **9** was synthesized by the same procedure used for the synthesis of compound **4**, except that **9-v** was used in place of 1,2-epoxyheptane, in a 82% yield as a white solid: $[□]_D^{26}$ = -40 (*c* 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.28-7.24 (m, 4H), 7.17-7.15 (m, 6H), 3.67-3.62 (m, 2H), 3.50 (br s, 1H), 2.68 (m, 4H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.33 (m, 4H), 2.30 (dd, *J* = 12.2, 3.2 Hz, 2H), 2.24 (dd, *J* = 12.2, 10.4 Hz, 2H), 1.63-1.59 (m, 4H), 1.50-1.25 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 142.84 (2C), 128.39 (4C), 128.22 (4C), 125.56 (2C), 66.10 (2C), 64.11 (2C), 53.17 (br, 4C), 35.95 (2C), 34.85 (2C), 31.42 (2C), 29.64 (2C), 29.24 (2C), 25.55 (2C); ESI-MS (*m/z*) 495.6 $[M+H]^+$.



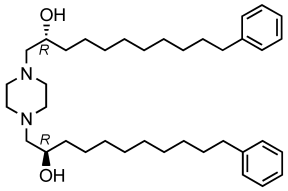
Compound 10-i

Compound **10-i** was synthesized by the same procedure used for the synthesis of compound **9-v**, except that 6-phenyl-1-hexyne was used as a starting material in place of 5-phenyl-1-pentyne, in a 58% yield (4 steps): $[\alpha]_D^{25}$ = +5.8 (*c* 0.76, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 2H), 7.17 (m, 3H), 2.90 (dddd, *J* = 5.4, 5.4, 4.0, 2.7 Hz, 1H), 2.74 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.60 (t, *J* = 7.7 Hz, 2H), 2.46 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.61 (m, 2H), 1.50 (m, 2H), 1.45 (m, 2H), 1.33 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.85, 128.39 (2C), 128.23 (2C), 125.58, 52.39, 47.13, 35.95, 32.48, 31.46, 29.41, 29.37, 29.19, 25.96; ESI-MS (*m/z*) 219.2 $[M+H]^+$.



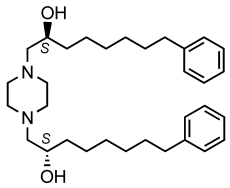
Compound 10

Compound **10** was synthesized by the same procedure used for the synthesis of compound **9**, except that **10-i** was used in place of **9-v**, in a 91% yield: $[\alpha]_D^{26}$ = -10 (*c* 0.28, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.16 (m, 6H), 3.64 (dddd, *J* = 10.4, 3.3, 3.3, 3.2 Hz, 2H), 3.39, (br s, 2H), 2.69 (m, 4H), 2.59 (t, *J* = 7.7 Hz, 4H), 2.38 (m, 4H), 2.32 (dd, *J* = 12.3, 3.2 Hz, 2H), 2.25 (dd, *J* = 12.3, 10.4 Hz, 2H), 1.61 (m, 4H), 1.46-1.24 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.88 (2C), 128.38 (4C), 128.21 (4C), 125.55 (2C), 66.10 (2C), 64.11 (2C), 53.38 (br, 4C), 35.96 (2C), 34.87 (2C), 31.48 (2C), 29.70 (2C), 29.43 (2C), 29.24 (2C), 25.60 (2C); ESI-MS (*m/z*) 523.6 $[M+H]^+$.



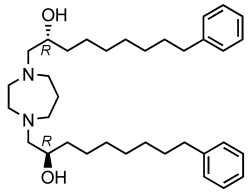
Compound 11

Compound **11** was synthesized by the same procedure used for the synthesis of compound **9**, except that 8-phenyl-1-octyne was used as a starting material in place of 5-phenyl-1-pentyne, in a 54% yield (5 steps): $[\alpha]_D^{26}$ = -9.0 (*c* 0.20, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.17 (m, 6H), 3.65 (m, 2H), 3.13 (br s, 2H), 2.70 (m, 4H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.38 (m, 4H), 2.33 (dd, *J* = 12.3, 3.2 Hz, 2H), 2.26 (dd, *J* = 12.3, 10.4 Hz, 2H), 1.58 (m, 4H), 1.51-1.38 (m, 4H), 1.35-1.24 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.95 (2C), 128.39 (4C), 128.21 (4C), 125.54 (2C), 66.13 (2C), 64.13 (2C), 52.98 (br, 4C), 35.99 (2C), 34.90 (2C), 31.52 (2C), 29.78 (2C), 29.57 (2C), 29.49 (4C), 29.33 (2C), 25.61 (2C); ESI-MS (*m/z*) 579.7 $[M+H]^+$.



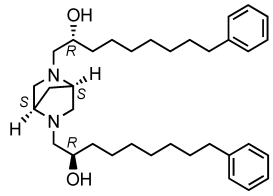
Compound 12

Compound **12** was synthesized by the same procedure used for the synthesis of compound **9**, except that (*S*)-(-)-glycidol was used as a starting material in place of (*R*)-(+)-glycidol, in a 37% yield (6 steps): $[\alpha]_D^{26}$ = +37 (*c* 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.25 (m, 4H), 7.17-7.15 (m, 6H), 3.68-3.63 (m, 2H), 3.50 (br s, 1H), 2.71 (m, 4H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.34 (m, 4H), 2.32 (dd, *J* = 12.2, 3.2 Hz, 2H), 2.26 (dd, *J* = 12.2, 10.4 Hz, 2H), 1.63-1.59 (m, 4H), 1.47-1.26 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ142.84 (2C), 128.40 (4C), 128.22 (4C), 125.57 (2C), 66.10 (2C), 64.06 (2C), 53.20 (br, 4C), 35.95 (2C), 34.86 (2C), 31.43 (2C), 29.63 (2C), 29.24 (2C), 25.54 (2C); ESI-MS (*m/z*) 495.6 $[M+H]^+$.



Compound 13

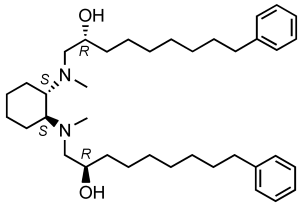
Compound **13** was synthesized by the same procedure used for the synthesis of **4**, except that homopiperazine and **10-i** were used in place of piperazine and 1,2-epoxyheptane respectively, in a 99% yield: $[\alpha]_D^{25}$ = -27 (*c* 0.43, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.16 (m, 6H), 3.90-3.20 (br s, 2H), 3.56 (m, 2H), 2.85 (m, 4H), 2.71-2.55 (m, 6H), 2.59 (t, *J* = 7.7 Hz, 4H), 2.25 (m, 2H), 1.83 (m, 2H), 1.61 (m, 4H), 1.53-1.14 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.92 (2C), 128.40 (4C), 128.22 (4C), 125.55 (2C), 66.78 (2C), 64.41 (2C), 55.98 (2C), 54.37 (2C), 35.97 (2C), 34.80 (2C), 31.50 (2C), 29.72 (2C), 29.44 (3C), 29.26 (2C), 25.70 (2C); ESI-MS (*m/z*) 537.6 $[M+H]^+$.



Compound 14

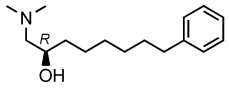
A mixture of (1*S*,2*S*)-(+)-2,5-diazabicyclo[2.2.1]heptane dihydrobromide (40 mg, 153 μmol), NaOH (6.1 mg, 153 μmol), **10-i** (100 mg, 460 μmol) in 2-propanol (1.5 mL) was refluxed at 100ºC for 22 h and then cooled to room temperature. To this mixture was added 2 M NaOH and the resulting mixture was extracted with $CHCl_3$, dried over anhydrous $MgSO_4$, and concentrated. The crude product was

purified by YMC silica gel chromatography (5% MeOH/$CHCl_3$) to give **14** (79 mg, 131 μmol, 86%) as a white solid: $[\alpha]_D^{25}$ = +3.1 (*c* 0.26, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.17 (m, 6H), 3.50 (br s, 2H), 3.48 (m, 2H), 3.32 (m, 2H), 2.90 (m, 2H), 2.64 (m, 2H), 2.59 (t, *J* = 7.7 Hz, 4H), 2.55 (dd, *J* = 11.9, 3.2 Hz, 2H), 2.47 (dd, *J* = 11.9, 9.8 Hz, 2H), 1.72 (m, 2H), 1.59 (m, 4H), 1.43 (m, 4H), 1.32 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.90 (2C), 128.40 (4C), 128.22 (4C), 125.55 (2C), 67.99 (2C), 62.88 (2C), 61.52 (2C), 58.59 (2C), 35.97 (2C), 34.88 (2C), 33.37, 31.49 (2C), 29.69 (2C), 29.43 (2C), 29.25 (2C), 25.64 (2C); ESI-MS (*m/z*) 535.6 $[M+H]^+$.



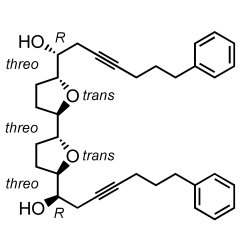
Compound 15

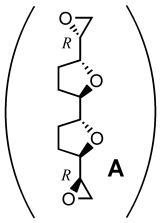
Compound **15** was synthesized by the same procedure used for the synthesis of **13**, except that (1*S*,2*S*)-(+)-*N*,*N'*-dimethylcyclohexane-1,2-diamine was used in place of homopiperazine, in a 29% yield: $[\alpha]_D^{25}$ = +17 (*c* 0.16, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.16 (m, 6H), 3.61 (m, 6H), 2.65 (dd, *J* = 12.8, 2.8 Hz, 2H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.44 (m, 2H), 2.27 (s, 6H), 2.20 (dd, *J* = 12.8, 8.8 Hz, 2H), 1.77 (m, 4H), 1.60 (m, 4H), 1.48-1.40 (m, 4H), 1.38-0.97 (m, 22H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.95 (2C), 128.40 (4C), 128.20 (4C), 125.51 (2C), 67.74 (2C), 66.07 (2C), 61.09 (br, 2C), 36.73 (br, 2C), 35.97 (2C), 35.24 (2C), 31.51 (2C), 29.76 (2C), 29.47 (2C), 29.29 (2C), 26.00 (2C), 25.50 (2C), 24.48 (2C); ESI-MS (*m/z*) 579.7 $[M+H]^+$.



Compound 16

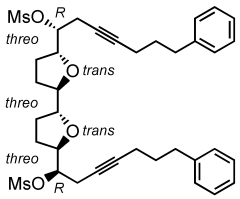
A mixture of **9-v** (0.93 g, 4.50 mmol) and dimethylamine (2.0 M in THF, 18 mL, 36 mmol) in 2-propanol (9 mL) was refluxed at 100ºC for 18 h. The reaction mixture was then cooled down to room temperature and concentrated. To this residue was added Et_2O (5 mL) and 2 M NaOH (5 mL). The resulting mixture was stirred at room temperature for 12 h. The organic layer was then separated, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by Wako silica gel chromatography (50% EtOAc/hexane to 5% MeOH/$CHCl_3$) to give **16** (0.80 g, 3.21 mmol, 71%) as a colorless oil: $[\alpha]_D^{26}$ = -4.5 (*c* 0.22, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28-7.24 (m, 2H), 7.17-7.13 (m, 3H), 3.63-3.57 (m, 1H), 3.41 (br s, 1H), 2.59 (t, *J* = 7.7 Hz, 2H), 2.25 (s, 6H), 2.24 (dd, *J* = 12.0, 10.5 Hz, 1H), 2.13 (dd, *J* = 12.0, 3.1 Hz, 1H), 1.63-1.57 (m, 2H), 1.48-1.25 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.83, 128.38 (2C), 128.20 (2C), 125.54, 66.92, 65.59, 45.47 (2C), 35.95, 34.93, 31.43, 29.66, 29.25, 25.59; ESI-MS (*m/z*) 250.3 $[M+H]^+$.





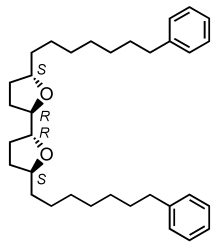
Compound 17-i

To a solution of 5-phenyl-1-pentyne (0.86 g, 6.0 mmol) in dry THF (20 mL) at –78ºC was added a solution of *n*-BuLi (1.58 M in hexane, 3.6 mL, 5.7 mmol) slowly over 5 min and the resulting mixture was stirred at –78ºC for 30 min. $BF_3{\cdot}Et_2O$ (0.50 mL, 4.3 mmol) was then added and the mixture was stirred for 15 min. To this mixture was added a solution of diepoxide **A** (0.27 g, 1.2 mmol) (*2*) in dry THF (10 mL) and the mixture was slowly warmed up to room temperature over 2 h with stirring. The mixture was quenched with saturated aqueous NH_4Cl, then extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by YMC silica gel chromatography (30 to 70% EtOAc/hexane) to give **17-i** (0.55 g, 1.07 mmol, 90%) as a colorless oil: $[\alpha]_D^{26}$ = -11 (*c* 0.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.20-7.15 (m, 6H), 4.05-4.02 (m, 2H), 3.92-3.89 (m, 2H), 3.59-3.55 (m, 2H), 2.70 (t, *J* = 7.5 Hz, 4H), 2.60 (br s, 2H), 2.42-2.40 (m, 4H), 2.20-2.16 (m, 4H), 2.00-1.98 (m, 4H), 1.81-1.67 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.75 (2C), 128.52 (4C), 128.33 (4C), 125.85 (2C), 82.05 (2C), 81.89 (2C), 81.64 (2C), 76.65 (2C), 72.48 (2C), 34.88 (2C), 30.56 (2C), 28.88 (2C), 28.35 (2C), 24.31 (2C), 18.30 (2C); ESI-MS (*m/z*) 537.3 $[M+Na]^+$.



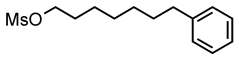
Compound 17-ii

To a solution of **17-i** (0.54 g, 1.05 mmol) and Et_3N (0.85 g, 8.4 mmol) in dry THF (11 mL) at 0ºC was added dropwise MsCl (0.49 mL, 6.3 mmol) and the resulting mixture was stirred at 0ºC for 30 min. The mixture was quenched with saturated aqueous $NaHCO_3$, then extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by YMC silica gel chromatography (20 to 50% EtOAc/hexane) to give **17-ii** (0.59 g, 0.88 mmol, 84%) as a pale yellow oil: $[\alpha]_D^{26}$ = -4.0 (*c* 0.20, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.19-7.17 (m, 6H), 4.62-4.57 (m, 2H), 4.24-4.19 (m, 2H), 3.92-3.90 (m, 2H), 3.14 (s, 6H), 2.70 (t, *J* = 7.5 Hz, 4H), 2.67-2.60 (m, 4H), 2.17-2.15 (m, 4H), 2.15-2.13 (m, 2H), 2.00-1.94 (m, 2H), 1.80-1.74 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.57 (2C), 128.51 (4C), 128.36 (4C), 125.90 (2C), 82.93 (2C), 82.83 (2C), 81.63 (2C), 79.30 (2C), 74.72 (2C), 38.94 (2C), 34.82 (2C), 30.28 (2C), 28.47 (2C), 28.26 (2C), 22.69 (2C), 18.17 (2C); ESI-MS (*m/z*) 693.4 $[M+Na]^+$.



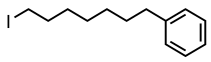
Compound 17

To a solution of **17-ii** (0.56 g, 0.84 mmol) in dry THF (4 mL) at 0ºC was added DBU (0.51 g, 3.36 mmol). The mixture was then heated to 60ºC and stirred for 24 h. The mixture was cooled down to room temperature and directly subjected to YMC silica gel chromatography (15 to 50% EtOAc/hexane) to give intermediate enyne, which was dissolved in EtOH (5 mL). To this solution was added catalytic 10% Pd on carbon and the resulting mixture was stirred at room temperature under a hydrogen gas atmosphere for 36 h. The reaction mixture was then filtered to remove the catalyst and the filtrate was concentrated. The residue was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **17** (0.26 g, 0.53 mmol, 64%) as a colorless oil: $[\alpha]_D^{26}$ = -1.1 (*c* 0.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.18-7.14 (m, 6H), 3.94-3.84 (m, 4H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.00-1.93 (m, 4H), 1.64-1.58 (m, 8H), 1.46-1.29 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ142.93 (2C), 128.40 (4C), 128.21 (4C), 125.53 (2C), 81.38 (2C), 79.75 (2C), 35.97 (2C), 35.78 (2C), 32.09 (2C), 31.51 (2C), 29.71 (2C), 29.46 (2C), 29.27 (2C), 28.54 (2C), 26.15 (2C); ESI-MS (*m/z*) 491.5 $[M+H]^+$.



Compound 18-i

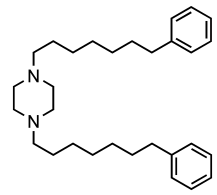
Compound **18-i** was synthesized by the same procedure used for the synthesis of compound **9-iv**, except that 7-phenyl-1-heptanol was used in place of **9-iii**, in a 99% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 2H), 7.19-7.16 (m, 3H), 4.40 (t, *J* = 6.6 Hz, 2H), 3.00 (s, 3H), 2.60 (t, *J* = 7.7 Hz, 2H), 1.75-1.70 (m, 2H), 1.63-1.58 (m, 2H), 1.41-1.33 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.65, 128.39 (2C), 128.26 (2C), 125.64, 70.11, 37.38, 35.88, 31.32, 29.10, 29.03, 28.89, 25.36; ESI-MS (*m/z*) 271.2 $[M+H]^+$.



Compound 18-ii

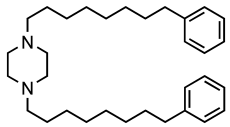
A mixture of **18-i** (0.76 g, 2.8 mmol) and NaI (2.1 g, 14 mmol) in dry acetone (14 mL) was stirred at room temperature for 16 h. The reaction mixture was diluted with Et_2O, washed with H_2O and brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was purified by Wako silica gel chromatography (hexane) to give **18-ii** (0.82 g, 2.7 mmol, 96%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 2H), 7.19-7.16 (m, 3H), 3.18, (t, *J* = 7.0 Hz, 2H), 2.60 (t, *J* = 7.7 Hz, 2H), 1.84-1.77 (m, 2H), 1.63-1.57 (m, 2H), 1.40-1.33 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.73, 128.40 (2C),

128.25 (2C), 125.62, 35.91, 33.51, 31.37, 30.42, 29.06, 28.40, 7.28; ESI-MS (*m/z*) 303.3 $[M+H]^+$.



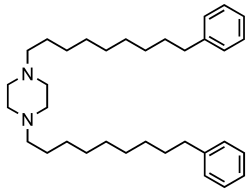
Compound 18

A mixture of piperazine (25 mg, 0.29 mmol) and **18-ii** (0.35 g, 1.16 mmol) in dry acetonitrile (4 mL) was refluxed at 100ºC for 24 h. The reaction mixture was cooled down to room temperature and quenched with 2 M NaOH, then extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$ and concentrated. The residue was purified by YMC silica gel chromatography (2 to 10% MeOH/$CHCl_3$) to give **18** (91 mg, 0.21 mmol, 72%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27-7.25 (m, 4H), 7.18-7.15 (m, 6H), 2.99-2.22 (m, 8H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.38 (t, *J* = 7.8 Hz, 4H), 1.63-1.56 (m, 4H), 1.55-1.47 (m, 4H), 1.34-1.25 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.82 (2C), 128.40 (4C), 128.23 (4C), 125.58 (2C), 58.57 (2C), 52.72 (4C), 35.94 (2C), 31.41 (2C), 29.35 (2C), 29.18 (2C), 27.42 (2C), 26.50 (2C); ESI-MS (*m/z*) 435.5 $[M+H]^+$.



Compound 19

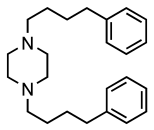
Compound **19** was synthesized by the same procedure used for the synthesis of compound **18**, except that 8-phenyl-1-octanol was used as a starting material in place of 7-phenyl-1-heptanol, in a 76% yield (3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.18-7.15 (m, 6H), 2.90-2.20 (m, 8H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.30 (t, *J* = 7.8 Hz, 4H), 1.63-1.56 (m, 4H), 1.50-1.45 (m, 4H), 1.31-1.25 (m, 16H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.90 (2C), 128.39 (4C), 128.21 (4C), 125.55 (2C), 58.90 (2C), 53.32 (4C), 35.97 (2C), 31.50 (2C), 29.51 (2C), 29.43 (2C), 29.26 (2C), 27.64 (2C), 26.93 (2C); ESI-MS (*m/z*) 463.7 $[M+H]^+$.



Compound 20

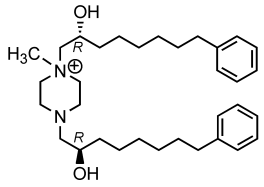
Compound **20** was synthesized by the same procedure used for the synthesis of compound **18**, except that 9-phenyl-1-nonanol was used as a starting material in place of 7-phenyl-1-heptanol, in a 75% yield (3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.25 (m, 4H), 7.18-7.16 (m, 6H), 2.90-2.25 (m, 8H), 2.59 (t, *J* = 7.8 Hz, 4H), 2.33 (t, *J* = 7.8 Hz, 4H), 1.61-1.56 (m, 4H), 1.48-1.46 (m, 4H), 1.30-1.27 (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.93 (2C), 128.39 (4C), 128.21 (4C), 125.55 (2C), 58.83 (2C),

53.15 (4C), 35.98 (2C), 31.51 (2C), 29.55 (2C), 29.49 (2C), 29.44 (2C), 29.30 (2C), 27.61 (2C), 26.82 (2C); ESI-MS (*m/z*) 491.6 $[M+H]^+$.



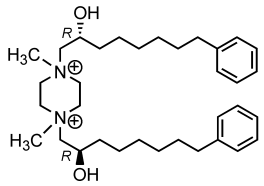
Compound 21

Compound **21** was synthesized by the same procedure used for the synthesis of compound **18**, except that 4-phenyl-1-butanol was used as a starting material in place of 7-phenyl-1-heptanol, in a 57% yield (3 steps): ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.28-7.24 (m, 4H), 7.18-7.14 (m, 6H), 2.64-2.31 (m, 8H), 2.62 (t, *J* = 7.5 Hz, 4H), 2.34 (t, *J* = 7.6 Hz, 4H), 1.66-1.58 (m, 4H), 1.54-1.48 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 142.46 (2C), 128.39 (4C), 128.25 (4C), 125.66 (2C), 58.61 (2C), 53.27 (4C), 35.85 (2C), 29.44 (2C), 26.55 (2C); ESI-MS (*m/z*) 351.4 $[M+H]^+$.



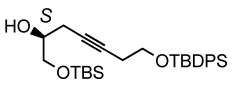
Compound 22

A mixture of **9** (20 mg, 0.04 mmol) and CH_3I (25 μL, 0.4 mmol) was stirred at room temperature for 1 h. The mixture was then directly subjected to Wako silica gel chromatography (2 to 10% $MeOH/CHCl_3$) to give **22** (15 mg, 0.023 mmol, 59%) as a white solid: ESI-MS (*m/z*) 509.6 $[M]^+$; *Anal.* Calcd for $C_{33}H_{53}N_2O_2/I$; C, 62.25; H, 8.39; N, 4.40; Found: C, 61.83; H, 8.42; N, 4.25.



Compound 23

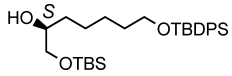
A mixture of **22** (30 mg, 47 μmol) and CH_3I (294 μL, 4.7 mmol) in acetone (1 mL) was stirred at room temperature for 3 h. The mixture was then filtered and the residue was washed with $CHCl_3$ to give **23** (15 mg, 19 □mol, 41%) as a white solid: ESI-MS (*m/z*) 637.8 $[M+CF_3COO^-]^+$; *Anal.* Calcd for $C_{34}H_{56}N_2O_2/I_2$; C, 52.45; H, 7.25; N, 3.60; Found: C, 52.23; H, 7.23; N, 3.58.



AFP-i

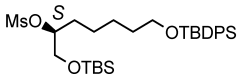
AFP-i was synthesized by the same procedure used for the synthesis of compound **9-ii**, except that 1-(*t*-butyldiphenylsilyloxy)-3-butyne was used in place of 5-phenyl-1-pentyne, in a 76% yield: $[\alpha]_D^{27}$ = +5.7 (*c* 0.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.68-7.66 (m, 4H), 7.44-7.36 (m, 6H), 3.73 (t, *J* =

7.2 Hz, 2H), 3.72 (m, 1H), 3.66 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.57 (dd, *J* = 9.9, 5.8 Hz, 1H), 2.44 (br s, 1H), 2.43 (m, 2H), 2.34 (m, 2H), 1.05 (s, 9H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 135.55 (4C), 133.64 (2C), 129.65 (2C), 127.66 (4C), 79.32, 77.07, 70.37, 65.61, 62.77, 26.78 (3C), 25.86 (3C), 23.43, 22.90, 19.19, 18.28, -5.39, -5.42; ESI-MS (*m/z*) 497.5 $[M+H]^+$.



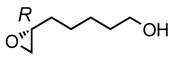
AFP-ii

AFP-ii was synthesized by the same procedure used for the synthesis of compound **9-iii** in a 90% yield: $[]_D^{27}$ = +0.75 (*c* 0.21, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.68-7.65 (m, 4H), 7.42-7.35 (m, 6H), 3.65 (t, *J* = 6.5 Hz, 2H), 3.63-3.59 (m, 1H), 3.61 (dd, *J* = 10.6, 3.2 Hz, 1H), 3.37 (dd, *J* = 10.6, 8.0 Hz, 1H), 2.40 (br s, 1H), 1.58-1.55 (m, 2H), 1.42-1.36 (m, 6H), 1.26 (s, 9H), 0.90 (s, 9H), 0.07 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 135.58 (4C), 134.15 (2C), 129.49 (2C), 127.57 (4C), 71.78, 67.28, 63.91, 32.79, 32.52, 26.87 (3C), 25.94, 25.89 (3C), 25.36, 19.22, 18.30, -5.32, -5.38; ESI-MS (*m/z*) 501.6 $[M+H]^+$.



AFP-iii

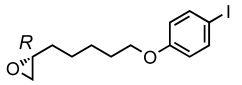
AFP-iii was synthesized by the same procedure used for the synthesis of compound **9-iv** in a 92% yield: $[]_D^{26}$ = +1.8 (*c* 0.11, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.67-7.65 (m, 4H), 7.42-7.36 (m, 6H), 4.63 (dddd, *J* = 10.4, 6.3, 6.3, 4.3 Hz, 1H), 3.74 (dd, *J* = 11.4, 6.3 Hz, 1H), 3.69 (dd, *J* = 11.4, 4.2 Hz, 1H), 3.65 (t, *J* = 6.4 Hz, 2H), 3.03 (s, 3H), 1.67-1.62 (m, 2H), 1.60-1.53 (m, 2H), 1.44-1.31 (m, 4H), 1.04 (s, 9H), 0.89 (s, 9H), 0.08 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 135.56 (4C), 134.06 (2C), 129.52 (2C), 127.59 (4C), 84.41, 64.86, 63.75, 38.55, 32.32, 31.48, 26.87 (3C), 25.87 (3C), 25.68, 24.77, 19.21, 18.34, -5.41, -5.45; ESI-MS (*m/z*) 579.4 $[M+H]^+$.



AFP-iv

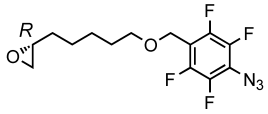
To a solution of **AFP-iii** (4.37 g, 7.6 mmol) in dry THF (20 mL) at 0ºC was added dropwise TBAF (1.0 M in THF, 19 mL, 19 mmol) and the mixture was stirred at room temperature for 2 h. To this mixture was added a solution of K_2CO_3 (2.1 g, 15.2 mmol) in MeOH (10 mL) and the resulting mixture was stirred at 35ºC for 1 h. The mixture was then diluted with H_2O, extracted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The crude product was purified by YMC silica gel chromatography (30 to 50% EtOAc/hexane) to give **AFP-iv** (0.73 g, 5.6 mmol, 74%) as a colorless oil: $[]_D^{27}$ = +11 (*c* 0.79, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 3.65 (t, *J* = 6.5 Hz, 2H), 2.94-2.89 (m, 1H),

2.75 (dd, *J* = 5.0, 4.1 Hz, 1H), 2.47 (dd, *J* = 5.0, 2.8 Hz, 1H), 1.71 (br s, 1H), 1.61-1.42 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) 62.81, 52.32, 47.12, 32.63, 32.42, 25.82, 25.58; ESI-MS (*m/z*) 131.3 $[M+H]^+$.



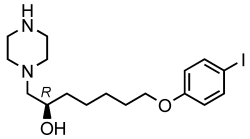
AFP-v

To a mixture of **AFP-iv** (0.30 g, 2.3 mmol), 4-iodophenol (0.76 g, 3.5 mmol), and PPh_3 (1.2 g, 4.6 mmol) in dry THF (5 mL) at 0ºC was added dropwise DIAD (0.93 g, 4.6 mmol) slowly over 10 min and the mixture was stirred at room temperature for 30 min. THF was then evaporated and the residue was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **AFP-v** (0.75 g, 2.2 mmol, 98%) as a colorless oil: $[]_D^{27}$ = +5.1 (*c* 0.35, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.54 (d, *J* = 6.8 Hz, 2H), 6.67 (d, *J* = 6.8 Hz, 2H), 3.92 (t, *J* = 6.4 Hz, 2H), 2.92 (m, 1H), 2.76 (dd, *J* = 4.9, 4.0 Hz, 1H), 2.48 (dd, *J* = 4.9, 2.7 Hz, 1H), 1.80-1.77 (m, 2H), 1.60-1.51 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) 158.91, 138.17 (2C), 116.90 (2C), 82.50, 67.85, 52.35, 47.15, 32.37, 29.06, 25.87, 25.76; ESI-MS (*m/z*) 333.1 $[M+H]^+$.



AFP-vi

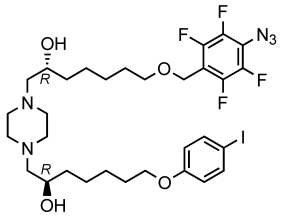
To a solution of **AFP-iv** (116 mg, 0.89 mmol) in dry THF (5 mL) at 0ºC was added *t*-BuOK (100 mg, 0.89 mmol) and the mixture was stirred at 0ºC for 30 min. To this mixture was added a solution of 1-azido-4-(bromomethyl)-2,3,5,6-tetrafluorobenzene (369 mg, 1.3 mmol) (*3*, *4*) in dry THF (3 mL) at 0ºC and the resulting mixture was stirred at room temperature for 1 h in the dark. The mixture was then diluted with Et_2O, washed with brine, dried over anhydrous $MgSO_4$ and concentrated. The crude product was purified by YMC silica gel chromatography (10% EtOAc/hexane) to give **AFP-vi** (157 mg, 0.47 mmol, 53%) as a pale yellow oil: $[]_D^{27}$ = +5.0 (*c* 0.12, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 4.56 (s, 2H), 3.48 (t, *J* = 6.5 Hz, 2H), 2.90-2.89 (m, 1H), 2.89 (dd, *J* = 5.0, 4.0 Hz, 1H), 2.46 (dd, *J* = 5.0, 2.7 Hz, 1H), 1.61-1.41 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$) 144.92 (d, *J* = 254 Hz, 2C), 140.50 (d, *J* = 248 Hz, 2C), 120.69 (t, *J* = 12 Hz, 1C), 112.32 (t, *J* = 17 Hz, 1C), 70.80, 59.61, 52.26, 47.09, 32.39, 29.45, 25.75, 25.70; ESI-MS (*m/z*) 334.2 $[M+H]^+$.



AFP-vii

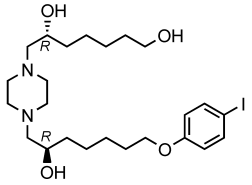
AFP-vii was synthesized by the same procedure used for the synthesis of compound **6-i**, except that **AFP-v** was used in place of 1,2-epoxyhexadecane, in a 87% yield: $[]_D^{27}$ = -21 (*c* 0.32, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.53 (d, *J* = 6.8 Hz, 2H), 6.66 (d, *J* = 6.8 Hz, 2H), 3.91 (t, *J* = 6.5 Hz, 2H),

3.69-3.65 (m, 1H), 2.94-2.80 (m, 4H), 2.65-2.15 (br, 2H), 2.64 (m, 2H), 2.35 (m, 2H) 2.33 (dd, *J* = 12.2, 3.1 Hz, 1H), 2.22 (d, *J* = 12.2, 10.6 Hz, 1H), 1.80-1.76 (m, 2H), 1.49-1.39 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 158.97, 138.15 (2C), 116.92 (2C), 82.42, 67.96, 65.79, 64.80, 54.42 (2C), 46.15 (2C), 34.77, 29.09, 26.16, 25.40; ESI-MS (*m/z*) 419.2 $[M+H]^+$.



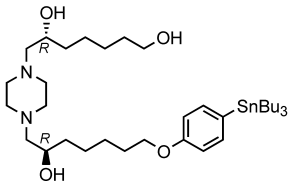
AFP

AFP was synthesized by the same procedure used for the synthesis of compound **6**, except that **AFP-vi** and **AFP-vii** were used in place of 1,2-epoxyoctane and **6-i**, respectively, in a 48% yield: $[\square]_D^{27}$ = +2.0 (*c* 0.20, EtOH); ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.54 (d, *J* = 6.8 Hz, 2H), 6.66 (d, *J* = 6.8 Hz, 2H), 4.56 (s, 2H), 3.91 (t, *J* = 6.5 Hz, 2H), 3.68-3.62 (m, 2H), 3.48 (t, *J* = 6.5 Hz, 2H), 3.45 (br s, 2H), 2.70 (m, 4H), 2.34 (m, 4H), 2.32-2.30 (m, 2H), 2.30-2.24 (m, 2H), 1.78-1.76 (m, 2H), 1.59-1.57 (m, 2H), 1.48-1.35 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 158.97, 145.54 (d, *J* = 254 Hz, 2C), 140.38 (d, *J* = 248 Hz, 2C), 138.15 (2C), 119.99, 116.92 (2C), 112.04 (t, *J* = 18 Hz, 1C), 82.43, 70.88, 67.95, 66.01, 65.99, 64.09, 64.07, 59.57, 53.17 (br, 4C), 34.76 (2C), 29.45, 29.08, 26.14 (2C), 25.39, 25.38; ESI-MS (*m/z*) 752.5 $[M+H]^+$.



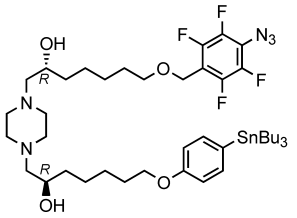
[^{125}I]AFP-i

[^{125}I]AFP-i was synthesized by the same procedure used for the synthesis of compound **6**, except that **AFP-iv** and **AFP-vii** were used in place of 1,2-epoxyoctane and **6-i**, respectively, in a 98% yield: $[\square]_D^{26}$ = -33 (*c* 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) □ 7.53 (d, *J* = 6.8 Hz, 2H), 6.66 (d, *J* = 6.8 Hz, 2H), 3.91 (t, *J* = 6.5 Hz, 2H), 3.69-3.63 (m, 2H), 3.65 (t, *J* = 6.6 Hz, 2H), 2.94-2.07 (br, 3H), 2.70 (m, 4H), 2.55-2.21 (m, 4H), 2.32 (dd, *J* = 12.2, 3.0 Hz, 2H), 2.26 (*J* = 12.2, 10.2 Hz, 2H), 1.78-1.76 (m, 2H), 1.62-1.57 (m, 2H), 1.48-1.37 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) □ 158.96, 138.15 (2C), 116.92 (2C), 82.43, 67.95, 66.04, 66.00, 64.10 (2C), 62.88, 53.23 (br, 4C), 34.76 (2C), 32.65, 29.08, 26.15, 25.84, 25.40, 25.39; ESI-MS (*m/z*) 549.4 $[M+H]^+$.



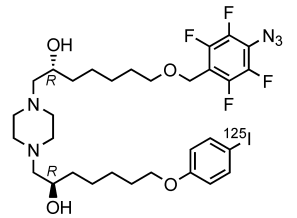
[^{125}I]AFP-ii

A mixture of **[^{125}I]AFP-i** (180 mg, 0.32 mmol) and $Pd(PPh_3)_4$ (75 mg, 0.06 mmol) in anhydrous dioxane (3.5 mL) was made anaerobic by freezing, vacuum degassing and introducing argon atmosphere three times at 0ºC (*5*). To this mixture was added bis(tributyltin) (0.96 mL, 1.92 mmol) and the resulting mixture was stirred at 50ºC for 20 h. The mixture was then cooled to room temperature and directly subjected to Wako silica gel chromatography ($CHCl_3$ to 10% MeOH/$CHCl_3$) to give **[^{125}I]AFP-ii** (97 mg, 0.136 mmol, 43%) as a brown oil: $[\alpha]_D^{26}$ = -27 (*c* 0.95, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36-7.33 (m, 2H), 6.90-6.87 (m, 2H), 3.95 (t, *J* = 6.5 Hz, 2H), 3.72-3.63 (m, 2H), 3.68 (br s, 1H), 3.66 (t, *J* = 6.6 Hz, 2H), 3.49 (br s, 2H), 2.70 (m, 4H), 2.38 (m, 4H), 2.34 (dd, *J* = 12.2, 3.0 Hz, 2H), 2.28 (dd, *J* = 12.2, 10.2 Hz, 2H), 1.79 (m, 2H), 1.58-1.41 (m, 14H), 1.54 (m, 6H), 1.48 (m, 6H), 1.04-1.00 (m, 6H), 0.88 (t, *J* = 7.3 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.20, 137.46 (2C), 131.76, 114.47 (2C), 67.48, 66.04 (2C), 64.11 (2C), 62.87, 53.15 (br, 4C), 34.80, 34.76, 32.66, 29.26, 29.10 (3C), 29.00, 27.38 (3C), 26.26, 25.84, 25.42, 13.69 (3C), 9.57 (3C); ESI-MS (*m/z*) 713.6 $[M+H]^+$ (^{120}Sn isotope).



[^{125}I]AFP-iii

[^{125}I]AFP-iii was synthesized by the same procedure used for the synthesis of compound **AFP-vi** in a 35% yield: $[\alpha]_D^{26}$ = -28 (*c* 0.30, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40-7.28 (m, 2H), 6.94-6.87 (m, 2H), 4.63 (s, 2H), 3.95 (t, *J* = 6.4 Hz, 2H), 3.66-3.64 (m, 2H), 3.65 (br s, 1H), 3.46 (t, *J* = 6.5 Hz, 2H), 3.42 (br s, 1H), 2.71 (m, 4H), 2.42 (m, 4H), 2.32-2.27 (m, 4H), 1.79 (m, 2H), 1.70-1.22 (m, 14H), 1.59 (m, 6H), 1.50 (m, 6H), 1.36-1.29 (m, 6H), 0.88 (t, *J* = 7.3 Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.21, 144.22 (d, *J* = 254 Hz, 2C), 140.67 (d, *J* = 248 Hz, 2C), 137.47 (2C), 137.76, 120.69 (t, *J* = 12 Hz, 1C), 114.47 (2C), 112.04 (t, *J* = 17 Hz, 1C), 70.89 (2C), 67.48, 66.02 (2C), 64.11, 59.57, 53.10 (br, 4C), 34.81, 34.77, 29.46, 29.27, 29.11 (3C), 27.39 (3C), 26.27, 26.15, 25.43, 25.38, 13.69 (3C), 9.58 (3C); ESI-MS (*m/z*) 916.7 $[M+H]^+$ (^{120}Sn isotope).



[^{125}I]AFP

To a mixture of **[^{125}I]AFP-iii** (1 mM in EtOH, 10 μL) and 0.02 M HCl (20 μL) in a 1.5 mL screw-capped tube was added a solution of [^{125}I]NaI (PerkinElmer NEZ033H, 3 μL, 37 MBq, 80.5

TBq/mmol) in H_2O (17 μL) (*6*). To this mixture was added 30% H_2O_2 (5 μL) and the resulting mixture was vortexed, spin-downed and incubated at room temperature for 1.5 min. The mixture was then quenched with 0.1 M NaOH (10 μL), extracted with $CHCl_3$ (50 μL x 4), and the combined organic layer was removed under a stream of N_2. The residue was dissolved in 75% MeOH/0.01% aq. TFA (40 μL) and subjected to HPLC (Shimadzu LC-10AS) purification using C18 column (COSMOSIL $5C_{18}$-MS-II, 4.6 x 150 mm, Nacalai Tesque) with the mixture of MeOH/H_2O/TFA (75:24.9975:0.0025) as a solvent at 0.8 mL/min flow rate.

The fractions were collected every 30 s (400 μL), and radioactivity of each fraction was measured with the γ-counting system (COBRA^{TM} II, Packard). The solvent of the most radioactive fraction (No. 16, Figure S1), which corresponded to the retention time of AFP (7.5 min), was removed under a stream of N_2. Purified [^{125}I]AFP was stored at 4ºC as an ethanol solution (37 MBq/mL). The radiochemical yield of [^{125}I]AFP from the initial [^{125}I]NaI was 70-80%. The radiochemical purity and specific activity were >99% and ~80 TBq/mmol, respectively.

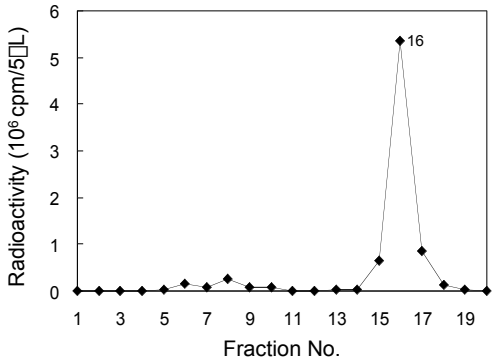


Figure S1: Radioactivity detection of [^{125}I]AFP in HPLC chromatogram.

References

1. Annoura, H., Nakanishi, K., Toba, T., Takemoto, N., Imajo, S., Miyajima, A., Tamura-Horikawa, Y., and Tamura, S. (2000) *J. Med. Chem. 43*, 3372-3376.
2. Motoyama, T., Yabunaka, H., and Miyoshi, H. (2002) *Bioorg. Med. Chem. Lett. 12*, 2089-2092.
3. Keana, J. F. W. and Cai, S.-X. (1990) *J. Org. Chem. 55*, 3640-3647.
4. Lei, H., and Atkinson, J. (2000) *J. Org. Chem. 65*, 2560-2567.
5. Latli, B., Tomizawa, M., and Casida, J. E. (1997) *Bioconjugate Chem. 8*, 7-14.
6. Hocke, C., Prante, O., Löber, S., Hübner, H., Gmeiner, P., and Kuwert, T. (2004) *Bioorg. Med. Chem. Lett. 14*, 3963-3966.

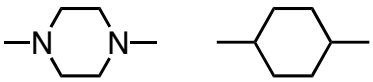


Figure S2. Structures of 1,4-dimethylpiperazine (left) and 1,4-dimethylcyclohexane (right).

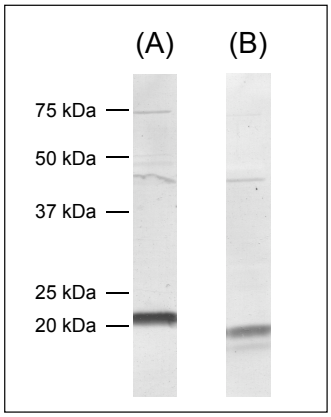


Figure S3. Western blotting analysis of complex I using anti-*Pd* NQO9 and NQO6 antibodies. Complex I isolated from BN-PAGE (equivalent to 30 μg of SMP) was loaded on Laemmli 12.5% polyacrylamide gel, followed by electrophoresis and western blotting with anti-*Pd* NQO9 (for bovine TYKY, panel A) and NQO6 (PSST, panel B) antibodies.

Table S1: Identification of tryptic proteins of complex I by MALDI-TOF MS analysis.

Protein	NCBI GI number	MALDI -TOF MS Data[a]					
		MASCOT score[b]		Mr		Residues	Peptide
			m/z (MH^+)	Expected	Calculated		
49 kDa	833783	186	2483.13	2482.12	2482.13	1-21	(-)QWQPDVEWAEQYGGAVMYPTK(E)
			2013.98	2012.97	2012.97	22-38	(K)ETAHWKPPPWNDVDPPK(D)
			1263.65	1262.64	1262.64	62-72	(R)LVMELSGEMVR(K)
			1386.72	1385.71	1385.70	93-103	(K)TYLQALPYFDR(K)
			1075.68	1074.67	1074.67	123-131	(K)LLNIRPPPR(A)
			934.54	933.53	933.53	137-144	(R)VLFGEITR(L)
			3291.60	3290.60	3290.61	145-172	(R)LLNHIMAVTTHALDIGAMTPFFWMFEER(E)
			1021.45	1020.44	1020.44	175-181	(K)MFEFYER(V)
			3159.53	3158.52	3158.53	187-214	(R)MHAAYVRPGGVHQDLPLGLMDDIYEFSK(N)
			1476.70	1475.70	1475.69	220-231	(R)IDELEEMLTNNR(I)
			2441.24	2440.24	2440.23	237-259	(R)TVDIGIVTAEDALNYGFSGVMLR(G)
			1031.54	1030.53	1030.52	260-268	(R)GSGIQWDLR(K)
			1159.63	1158.62	1158.61	260-269	(R)GSGIQWDLRK(T)
			2356.14	2355.13	2355.14	269-288	(R)KTQPYDVYDQVEFDVPIGSR(G)
			1329.64	1328.64	1328.65	338-348	(K)TSMESLIHHFK(L)
			1888.92	1887.92	1887.91	370-386	(K)GEFGVYLVSDGSSRPYR(C)
			1196.64	1195.64	1195.63	391-402	(K)APGFAHLAGLDK(M)
			2455.27	2454.26	2454.26	406-428	(K)GHMLADVVAIIGTQDIVFGEVDR(-)

[a] The data were used to search a complied protein database that was composed of the protein database NCBInr, which is publicly available (http://www.ncbi.nlm.nih.gov/).

[b] Proteins score is -10*Log(P), where P is the probability that the observed match is a random event. Protein scores greater than 58 are significant ($p < 0.05$).