Supporting Information

For

^{1}H-NMR Probe for In Situ Monitoring of Dopamine Metabolism and Its Application to Inhibitor Screening

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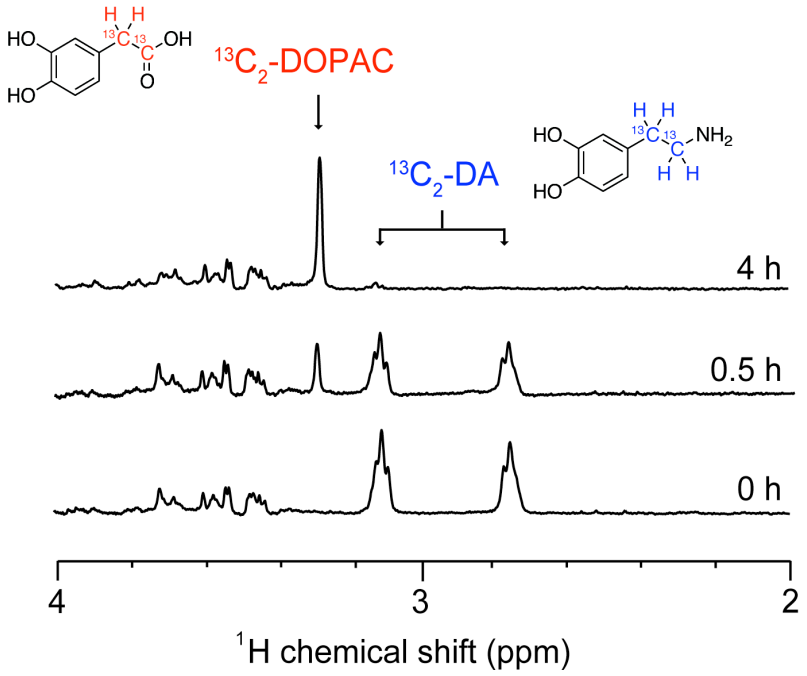


Figure S1. Time course change in the one-dimensional ^{1}H–{^{13}C} HSQC spectrum of $^{13}C_2$-DA (1 mM) incubated in the reaction buffer containing MAO-A (12 units/mL) and ALDH (5000 units/mL). The samples were measured using a Bruker Avance III spectrometer (400 MHz for ^{1}H).

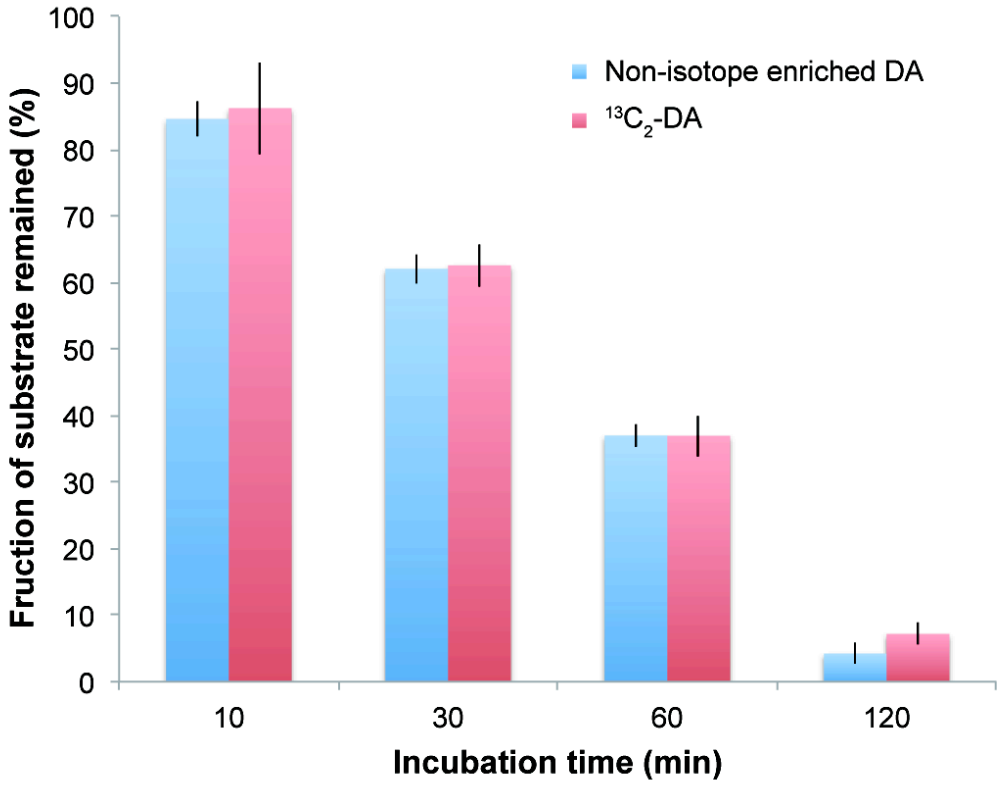
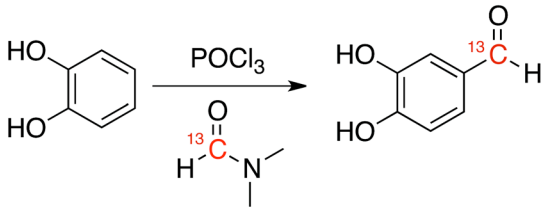


Figure S2. Reactivity of non-isotope enriched DA (1 mM, blue bar) and $^{13}C_2$-DA (1 mM, red bar) with MAO-A (12 units/mL) and ALDH (5000 units/mL) in reaction buffer (100 mM potassium phosphate buffer, pH 7.4) containing NAD^+ (1 mM), ascorbic acid (1 mM), EDTA (0.7 mM) at 37 °C. The fraction of substrate remained was determined by HPLC-UV analysis of each enzymatic reaction solution at an indicated time point. Error bars indicate the standard deviation of three independent experiments.

2. Synthesis

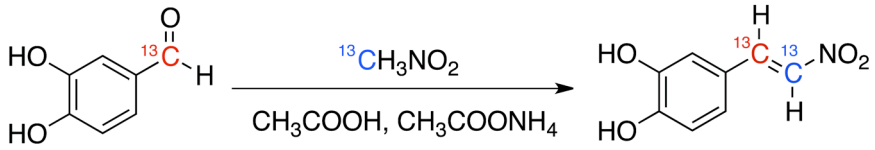
General. Reagents and solvents were purchased from standard suppliers and used without further purification. Gel permeation chromatography purification was performed on a JAIGEL-GS310 column (LC-9201, Japan Analytical Industry) using a recycle HPLC system (LC-9201, Japan Analytical Industry). NMR spectra were measured using a Bruker Avance III spectrometer (400 MHz for ^{1}H) and analyzed with solvent peaks as references. Mass spectra (MS) were measured using a JEOL JMS-HX110A (FAB).

Synthesis of 3,4-dihydroxybenzaldehyde-^{13}C.



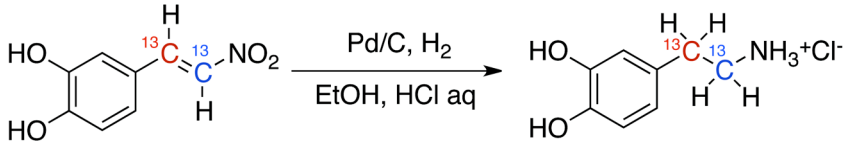
Phosphoryl chloride (2.10 g, 13.6 mmol) was added dropwise to dimethylformamide-carbonyl-^{13}C (1.00 g, 13.5 mmol) on ice bath and the mixture was stirred at room temperature. After 30 min, catechol (1.00 g, 9.1 mmol) was added to the reaction solution, and the solution was stirred at 120 °C for 4 h. After cooling to the room temperature, water (20 mL) was added and the mixture was further stirred for 1 h. This was evaporated under vacuum, resulting in a dark oily residue. The residue was purified using silica gel column chromatography (eluent: chloroform:methanol = 20:1) to give 3,4-dihydroxybenzaldehyde-^{13}C (614 mg, yield = 49%): ^{1}H NMR (CD_3OD, 400 Hz) δ = 6.90 (d, J = 8.0 Hz, 1H, aromatic), 7.26-7.30 (m, 2H, aromatics), 9.66 (d, J = 172.0 Hz, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 193.1; HRMS(FAB): m/z calc. for $C_6{}^{13}CH_7O_3^+$ $[M+H]^+$ = 140.0423, found = 140.0411.

Synthesis of 3,4-dihydroxy-β-nitrostyrene-$^{13}C_2$.



The 3,4-dihydroxybenzaldehyde-^{13}C (373 mg, 2.68 mmol) was added to a solution of ammonium acetate (842 mg, 10.9 mmol) and nitromethane-^{13}C (1.00 g, 16.1 mmol) in glacial acetic acid (3.8 mL) and refluxed for 25 min. The solvent was evaporated under vacuum, resulting in a dark red oily residue. This was purified using silica gel column chromatography (eluent: chloroform:methanol = 10:1) to give 3,4-dihydroxy-β-nitrostyrene-^{13}C (129 mg, yield = 26%). This was further purified by gel permeation chromatography (eluent: methanol): ^{1}H NMR (CD_3OD, 400 MHz) δ = 6.82 (d, J = 8.0 Hz, 1H, aromatic), 7.05-7.10 (m, 2H, aromatics), 7.42-7.94 (m, 1H, alkene), 7.71-8.15 (m, 1H, alkene); ^{13}C NMR (CD_3OD, 100 MHz) δ = 135.5 (d, J = 77.3 Hz), 141.0 (d, J = 77.3 Hz); HRMS(FAB): m/z calc. for $C_6{}^{13}C_2H_8O_4N^+$ $[M+H]^+$ = 184.0515, found = 184.0528.

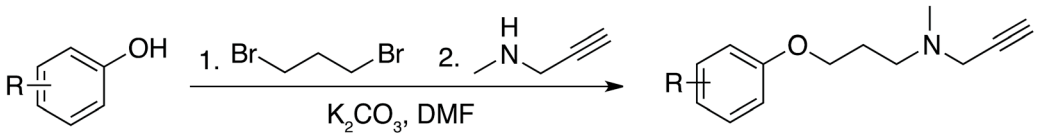
Synthesis of dopamine hydrochloride-$^{13}C_2$ ($^{13}C_2$-dopamine).



The 10% palladium on activated carbon (25 mg) was added to a solution of 3,4-dihydroxy-β-nitrostyrene-^{13}C (38 mg, 0.21 mmol) in ethanol (3.8 mL) and 6 N hydrochloric acid (0.2 mL). This was stirred for 3 h on ice bath under H_2. The insoluble materials were removed by filtration and the filtrate was evaporated under vacuum, resulting in a yellow powder. Water (15 mL) was added to the powder and washed with dichloromethane and diethyl ether (each 30 mL × 3). The aqueous phase was lyophilized to give $^{13}C_2$-dopamine hydrochloride salt (20 mg, 50%). The purity was confirmed to be more than 90%: ^{1}H NMR (D_2O, 400 MHz) δ = 2.69-3.43 (m, 4H), 6.74-6.77 (m, 1H,

aromatic), 6.84-6.98 (m, 2H, aromatics); ^{13}C NMR (CD_3OD, 100 MHz) δ = 34.1 (d, J = 34.3 Hz), 42.3 (d, J = 34.3 Hz); HRMS(FAB): m/z calc. for $C_6{}^{13}C_2H_{12}O_2N^+$ $[M+H]^+$ = 156.0930, found = 156.0937.

Synthesis of chlorgyline derivatives a–g.[1]



Chlorgyline derivatives **a–g** were synthesized from the corresponding phenol derivatives in a one-step procedure. Typically, phenol derivative (1.9 mmol), 1,3-dibromopropane (3.8 mmol), and potassium carbonate (1.9 mmol) were dissolved in DMF (3 mL) and the mixture was stirred for 3 h at 80 °C under N_2. After cooling to room temperature, *N*-methylpropagylamine (5.7 mmol) and potassium carbonate (5.7 mmol) were added to the reaction solution, and the solution was stirred at 40 °C. After 24 h, water (15 mL) was added to the reaction solution and extracted with ethylacetate (15 mL × 2). The organic phase was washed with water (30 mL × 2) and dried with magnesium sulfate, and the solvent was removed *in vacuo*. The resulting residue was purified using silica gel column chromatography (eluent: chloroform:methanol = 100:1 to 20:1) to give free bases **a–g** (yield = 31-56%). Each free base was converted to hydrochloride salt before use.

N-[3-(2-Fluorophenoxy)propyl]-*N*-methyl-2-propagylamine (**a**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.27 (dt, J = 5.6 Hz, 7.6 Hz, 2H), 3.02, (s, 3H), 3.40 (t, J = 2.8 Hz, 1H), 3.49 (t, J = 7.6 Hz, 2H), 4.19 (t, J = 5.6 Hz, 2H), 4.19 (d, J = 2.8 Hz, 2H), 6.93-6.99 (m, 1H), 7.08-7.15 (m, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 25.7, 40.8, 46.3, 54.5, 67.5, 72.8, 81.4, 116.5, 117.1 (d, J = 18.2 Hz), 123.0

(d, J = 6.6 Hz), 125.7 (d, J = 3.6 Hz), 147.8 (d, J = 10.9 Hz), 154.1 (d, J = 242.8 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{17}F_1NO^+$ $[M+H]^+$ = 222.1289, found = 222.1278.

N-[3-(4-Fluorophenoxy)propyl]-*N*-methyl-2-propagylamine (**b**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.22 (dt, J = 5.6 Hz, 8.0 Hz, 2H), 3.00, (s, 3H), 3.41 (t, J = 2.4 Hz, 1H), 3.45 (t, J = 8.0 Hz, 2H), 4.09 (t, J = 5.6 Hz, 2H), 4.18 (d, J = 2.4 Hz, 2H), 6.91-6.96 (m, 2H), 6.99-7.05 (m, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 25.7, 40.8, 46.3, 54.5, 66.5, 73.0, 81.3, 116.7 (d, J = 13.1 Hz), 116.9 (d, J = 2.2 Hz), 156.1, 159.0 (d, J = 236.2 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{17}F_1NO^+$ $[M+H]^+$ = 222.1289, found = 222.1295.

N-[3-(2,6-Difluorophenoxy)propyl]-*N*-methyl-2-propagylamine (**c**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.21 (dt, J = 5.6 Hz, 8.0 Hz, 2H), 2.99 (s, 3H), 3.39 (t, J = 2.4 Hz, 1H), 3.50 (t, J = 8.0 Hz, 2H), 4.16 (d, J = 2.4 Hz, 2H), 4.24 (t, J = 5.6 Hz, 2H), 6.98-7.14 (m, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 26.3, 40.7, 46.4, 54.2, 72.6, 72.8, 81.4, 113.4 (dd, J = 6.6 Hz, 17 Hz), 125.1 (t, J = 9.1 Hz), 136.3 (t, J = 14.6 Hz), 157.5 (dd, J = 5.1 Hz, 245.7 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{16}F_2NO^+$ $[M+H]^+$ = 204.1194, found = 204.1200.

N-[3-(3,5-Difluorophenoxy)propyl]-*N*-methyl-2-propagylamine (**d**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.23 (dt, J = 5.6 Hz, 8.0 Hz, 2H), 3.00 (s, 3H), 3.40 (t, J = 2.4 Hz, 1H), 3.43 (t, J = 8.0 Hz, 2H), 4.12 (t, J = 5.6 Hz, 2H), 4.17 (d, J = 2.4 Hz, 2H), 6.52-6.63 (m, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 25.4, 40.8, 46.3, 54.1, 66.7, 72.9, 81.4, 97.4 (t, J = 26 Hz), 99.5 (dd, J = 8.0 Hz, 20 Hz), 162.0 (t, J = 14 Hz), 165.2 (dd, J = 15.3 Hz, 243.5 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{16}F_2NO^+$ $[M+H]^+$ = 204.1194, found = 204.1201.

N-[3-(2-Fluoro-4-nitrophenoxy)propyl]-*N*-methyl-2-propagylamine (**e**):

^{1}H NMR (CD_3OD, 400 MHz) δ = 2.34 (dt, J = 5.8 Hz, 7.8 Hz, 2H), 3.03 (s, 3H), 3.42 (t, J = 2.4 Hz, 1H), 3.49 (t, J = 7.8 Hz, 2H), 4.21 (d, J = 2.4 Hz, 2H), 4.35 (t, J = 5.8 Hz, 2H), 7.31-7.35 (m, 1H), 8.05-8.09 (m, 1H), 8.11-8.14 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 25.4, 40.8, 46.3, 54.0, 67.9, 72.8, 81.5, 113.0 (d, J = 22.6 Hz), 114.9, 122.1 (d, J = 3.6 Hz), 142.5 (d, J = 7.3 Hz), 152.5 (d, J = 248.6 Hz), 153.5 (d, J = 10.9 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{16}F_1O_3N_2^+$ $[M+H]^+$ = 267.1139, found = 267.1165.

N-[3-(4-Fluoro-2-nitrophenoxy)propyl]-*N*-methyl-2-propagylamine (**f**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.29 (dt, J = 5.6 Hz , 7.6 Hz, 2H), 3.01 (s, 3H), 3.38 (t, J = 2.4 Hz, 1H), 3.49 (t, J = 7.6 Hz, 2H), 4.19 (d, J = 2.4 Hz, 2H), 4.30 (t, J = 5.6 Hz, 2H), 7.33-7.37 (m, 1H), 7.43-7.48 (m, 1H), 7.73-7.76 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 25.3, 40.9, 46.4, 54.4, 68.5, 72.6, 81.5, 113.6 (d, J = 27.7 Hz), 118.0 (d, J = 8.1 Hz), 122.4 (d, J = 23.3 Hz), 140.9, 149.5 (d, J = 2.9 Hz), 157.1 (d, J = 241.3 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{16}F_1O_3N_2^+$ $[M+H]^+$ = 267.1139, found = 267.1134.

N-[3-(2-Fluoro-6-nitrophenoxy)propyl]-*N*-methyl-2-propagylamine (**g**): ^{1}H NMR (CD_3OD, 400 MHz) δ = 2.28 (dt, J = 5.6 Hz, 7.8 Hz, 2H), 3.02 (s, 3H), 3.41 (t, J = 2.4 Hz, 1H), 3.53 (t, J = 7.8 Hz, 2H), 4.20 (d, J = 2.4 Hz, 2H), 4.37 (t, J = 5.6 Hz, 2H), 7.29-7.34 (m, 1H), 7.53-7.58 (m, 1H), 7.70-7.73 (m, 1H); ^{13}C NMR (CD_3OD, 100 MHz) δ = 26.2, 40.7, 46.4, 54.2, 72.7, 73.4 (d, J = 5.8 Hz), 81.5, 121.7 (d, J = 3.6 Hz), 122.6 (d, J = 19.7 Hz), 125.7 (d, J = 8.0 Hz), 141.5 (d, J = 14.5 Hz), 145.9 (d, J = 1.5 Hz), 157.4 (d, J = 247.9 Hz); HRMS(FAB): m/z calc. for $C_{13}H_{16}F_1O_3N_2^+$ $[M+H]^+$ = 267.1139, found = 267.1142.

3. HPLC analysis

HPLC analysis was performed on a TCI Dual ODS CX-10 (4.6 mm × 150 mm) column (Tokyo Chemical Industry) using an HPLC system composed of a pump (LC-20AT, Shimadzu) and a detector (SPD-M20A, Shimadzu). Samples were analyzed with a combination of isocratic elution (5 mM ammonium formate buffer (pH 3.5) for first 2 min) and linear gradient elution (5 mM ammonium formate buffer (pH 3.5):CH_3CN = 100:0 to 65:35 for next 23 min) at a flow rate of 1.0 mL/min. Monitored wavelength was λ_{max} of DA (280 nm).

4. $^{1}H–\{^{13}CA–^{13}CO\}$ NMR analysis

$^{1}H–\{^{13}CA–^{13}CO\}$ NMR spectra were acquired at 298 K using a Bruker Avance III 600 spectrometer equipped with a 5 mm TCI CryoProbe. All one-dimensional $^{1}H–\{^{13}CA–^{13}CO\}$ spectra were obtained using a 3D HCACO pulse sequence, which is commonly used to assign backbone chemical shifts of proteins, with a slight modification for the one-dimensional proton experiments.[2]

5. Enzymatic reactions

General. Human MAO-A (recombinant, microsomes from baculovirus-infected insect cells) was purchased from Sigma Aldrich. One unit is defined as the activity needed to convert 1 nmol of kynuramine per minute. Aldehyde dehydrogenase (ALDH from yeast) was purchased from MP Biomedicals. One unit is defined as the activity needed to convert 1 nmol of acetaldehyde per minute. Enzymatic units used were calculated according to the enzymatic units determined by manufacture. Enzymatic reactions were typically performed as follows: MAO-A (12 units/mL) and ALDH (5000 units/mL) were added to the reaction buffer (100 mM potassium phosphate buffer, pH 7.4) containing NAD^+ (1 mM), ascorbic acid (1 mM), EDTA (0.7 mM). The $^{13}C_2$-DA (1 mM) was added to the solution and incubated at 37 °C. After an appropriate incubation time, the enzymatic

reaction was stopped by addition of clorgyline (200 μM). In the case of NMR monitoring, D_2O was added to the reaction solution.

5-1. Dopamine metabolism: Figure 2b.

Enzymatic reactions were performed in a volume of 600 μL under the condition described above. At the appropriate incubation time, the reaction was stopped by addition of clorgyline (200 μM) and 60 μL of D_2O was added to the solution. The 550 μL aliquot was used for NMR analysis and the resulting solution was subjected to HPLC analysis.

5-2. Preparation of mouse liver homogenate.

The liver tissue of BALB/c mice weighing approx. 1 g was harvested and homogenized in 9 mL of potassium phosphate buffer (100 mM, pH 7.4) containing EDTA (0.5 mM) using potter homogenizer. The pestle of the homogenizer was rotated at 600 to 1000 r.p.m.. The homogenate were centrifuged at 1000 g for 5 min. The supernatant fluid was collected and used as the mouse liver homogenate. Animal experiments were performed according to the Institutional Guidance of Kyushu University on Animal Experimentation and under permission by the animal experiment committee of Kyushu University.

5-3. NMR analysis of $^{13}C_2$-DOPAC in mouse liver homogenate: Figure 2c.

The 60 μL of enzymatic reaction solution containing $^{13}C_2$-DOPAC (832 μM), prepared as described above, was added to 440 μL of the mouse liver homogenate. Final concentration of $^{13}C_2$-DOPAC was 100 μM. A 50 μL of D_2O was added to the homogenate and then the resulting mixture was subjected to NMR analysis (actual concentration of $^{13}C_2$-DOPAC measured by NMR was approximately 91 μM).

5-4. Evaluation of candidate MAO inhibitors in mouse liver homogenate: Figure 3c.

Each candidate MAO inhibitor (10 μM) was incubated with 100 μL of the mouse liver homogenate in 500 μL of reaction buffer (100 mM potassium phosphate buffer, pH 7.4) containing NAD^+ (1 mM), ascorbic acid (1 mM), EDTA (0.7 mM) at 37 °C. After 15 min, $^{13}C_2$-DA (1 mM) was added to the solution and further incubated at 37 °C for 5 h. A 50 μL of D_2O was added to the reaction solution and then the resulting mixture was subjected to NMR analysis.

6. References

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(2) Yamada, H.; Mizusawa, K.; Igarashi, R.; Tochio, H.; Shirakawa, M.; Tabata, Y.; Kimura, Y.; Kondo, T.; Aoyama, Y.; Sando, S. *ACS Chem. Biol*. **2012**, *7*, 535-542.