Supporting Information

Design, synthesis and structure-activity relationship study of pyrilamine derivatives as histone deacetylase inhibitors

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1. Experimental procedures

HDAC-inhibitory activity assay of compounds **1**–**7**

Enzymatic activities of HDACs including HDAC1 and HDAC6 were performed by a fluorogenic assay as described previously[1]. Briefly, HDAC proteins purified from 293T cells or HeLa cells were incubated with a fluorescent peptide (2 mM Ac-KGLGK(Ac)-MCA) in 20 µL of HDAC assay buffer (20 mM Tris–HCl (pH 8.0), 150 mM NaCl, and 10% glycerol) at 37 °C for 30 min. The reaction was stopped by the addition of 20 µL of trypsin (20 mg/mL) and incubated at 37 °C for 15 min. The released aminomethylcoumarin (AMC) was measured using a fluorescence plate reader (Molecular Devices, San Jose, CA).

Statistical analysis

The parameters were represented as the mean ± S.E. Significance of differences among means of two unpaired groups and more than two groups was determined by Student's t-test and Oneway ANOVA followed by Dunnett's test, respectively.

Automated Patch clamp electrophysiological recordings

For K current measurements HEK-293 cells stably expressing human ERG potassium channel, accession number: U04270, were used. The cell line, PrecisION hERG-HEK recombinant cell line (CYL3039) was purchased from Eurofins (MO. USA) and cultured following the manufacturer instructions.

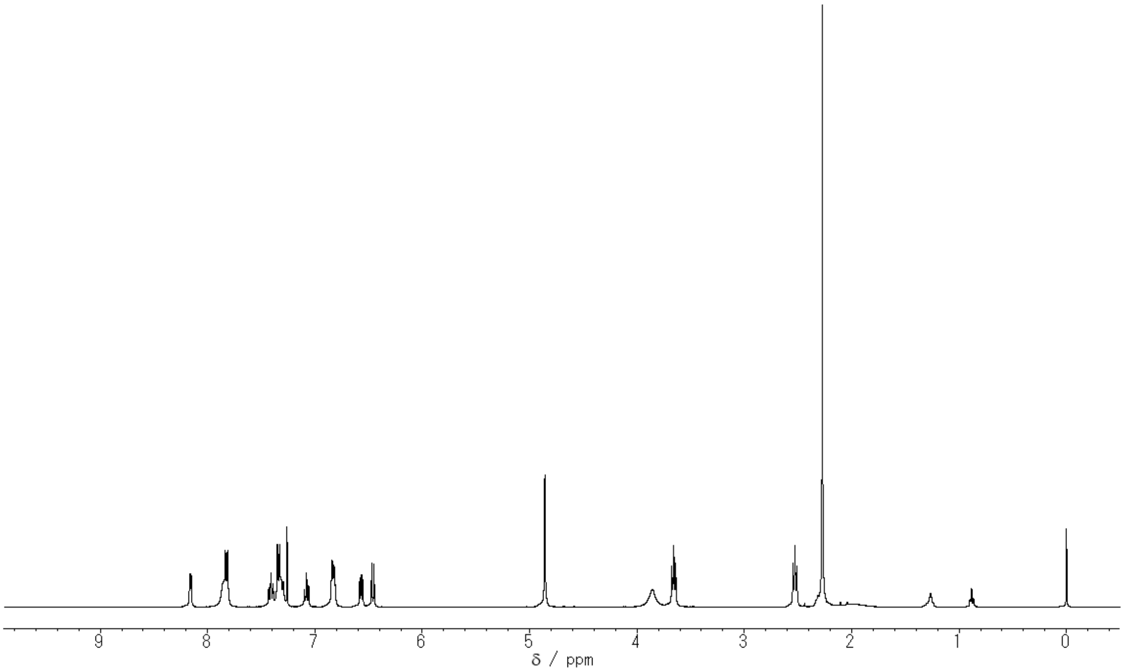
All the measurements were recorded in a whole-cell patch clamp instrument, an IonFlux 16 system (Fluxion, CA. USA) and the corresponding IonFlux 16 software for cell capture, seal formation, data acquisition, and analysis. Harvested cells were re-suspended in the extracellular solution containing: 137 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 10 mM HEPES, and 10 mM glucose, pH 7.35. The intercellular solution for recording containing: 70 mM KF, 60 mM KCl, 10 mM NaCl, 10 mM HEPES, 11 mM EGTA, and 4 mM MgATP, pH 7.30.

Cells were held at -80 mV for 100 msec before stepping to -100 mV for 90 msec and then to -50 mV for 100 msec to measure leak current, then stepped to 30 mV for 800 msec to activate and inactivate hERG current and then stepped to -50 mV for 1200 msec to elicit hERG tail current and then finally to -80 mV for 200 msec. Sweep interval was 5 sec. Test compounds were dissolved in DMSO to concentrations that were 300x the final top assay concentration. Each concentration of test compound in the extracellular solution, containing 0.33% DMSO, was applied for 2 min. Control current was determined with the extracellular solution containing 0.33% DMSO, and accuracy of the measurements was confirmed by the tail current inhibition caused by the addition of 10 μM quinidine. The hERG measurements were performed at room temperature (22 ˚C).

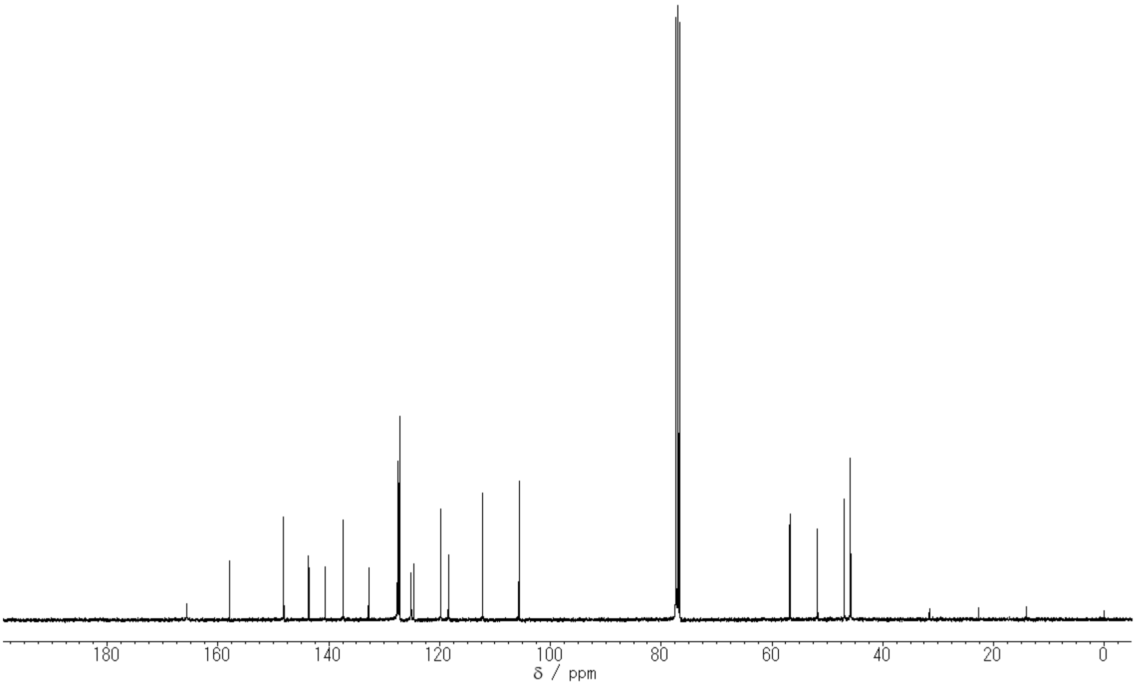
IC_{50} values were determined by the curve fitting using a variable slope model (four-parameter dose-response curve) in GraphPad Prism 6 (GraphPad, CA. USA).

2. NMR spectra of synthesized compounds

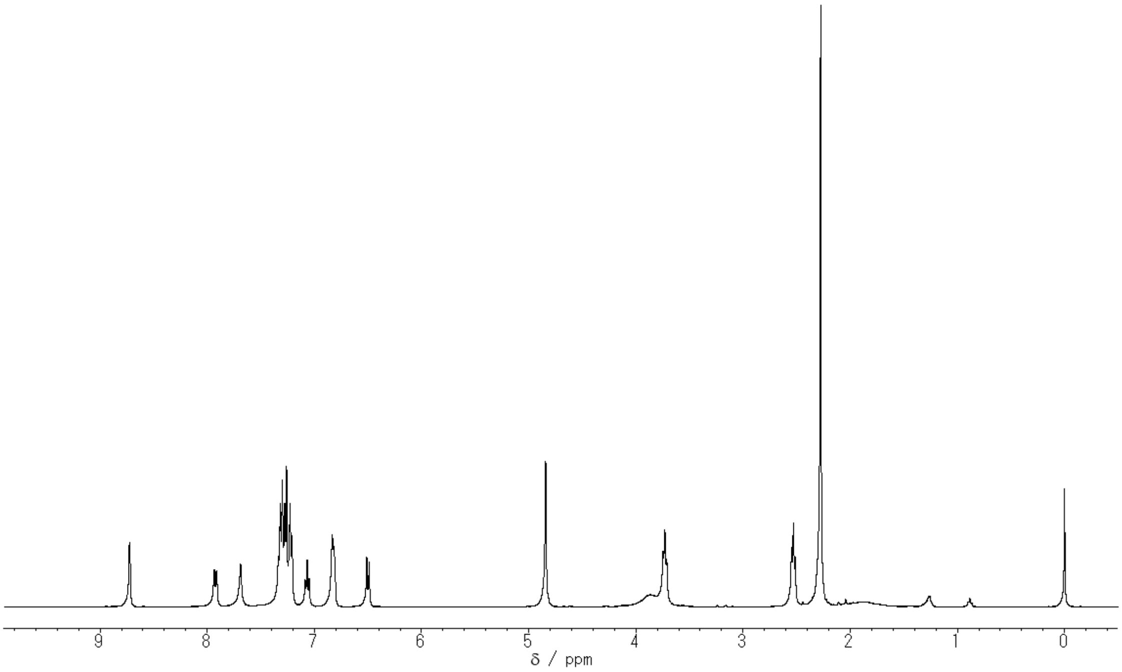
^{1}H-NMR spectrum of Compound **2** ($CDCl_3$, 400 MHz)



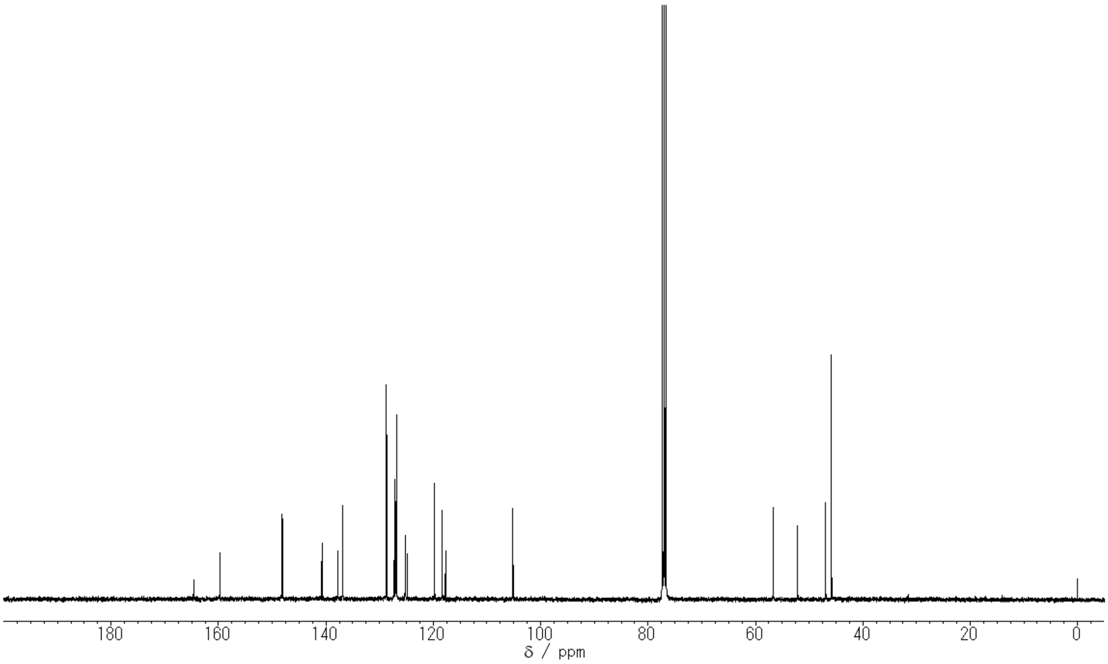
^{13}C-NMR spectrum of Compound **2** ($CDCl_3$, 100 MHz)



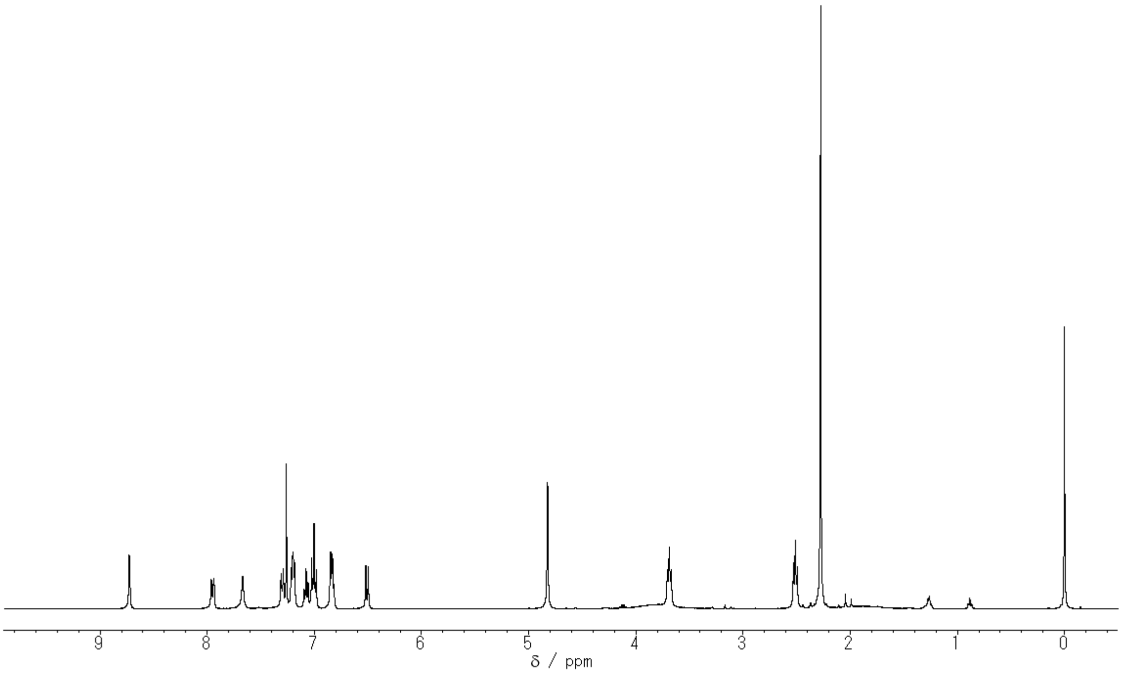
^{1}H-NMR spectrum of Compound **3** ($CDCl_3$, 400 MHz)



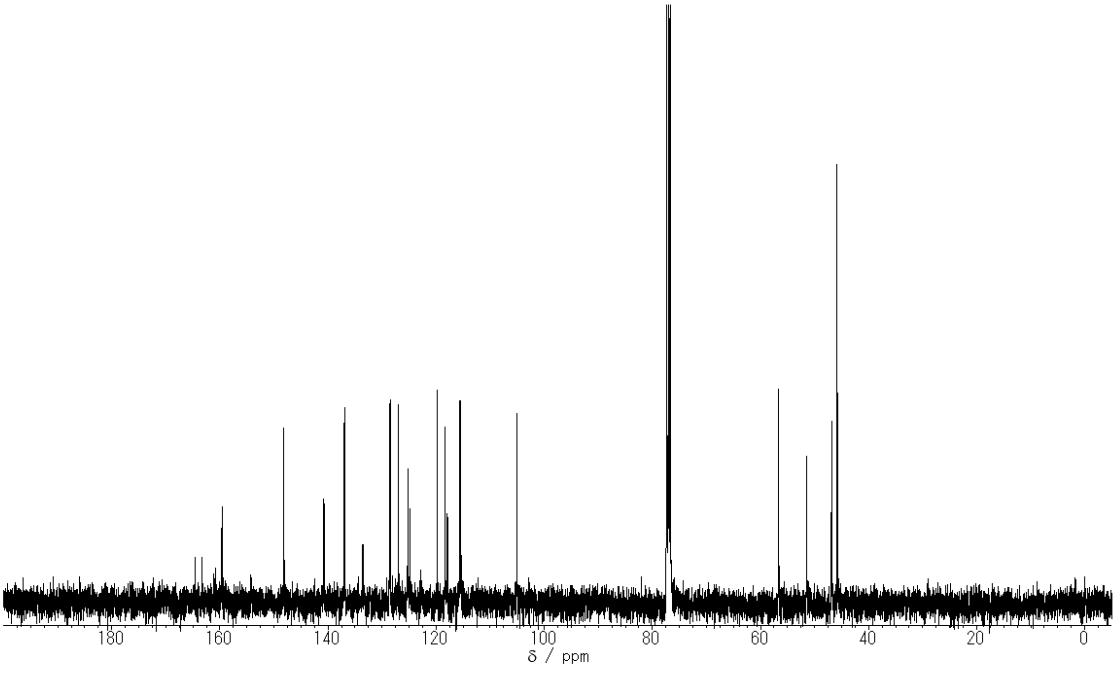
^{13}C-NMR spectrum of Compound **3** ($CDCl_3$, 100 MHz)



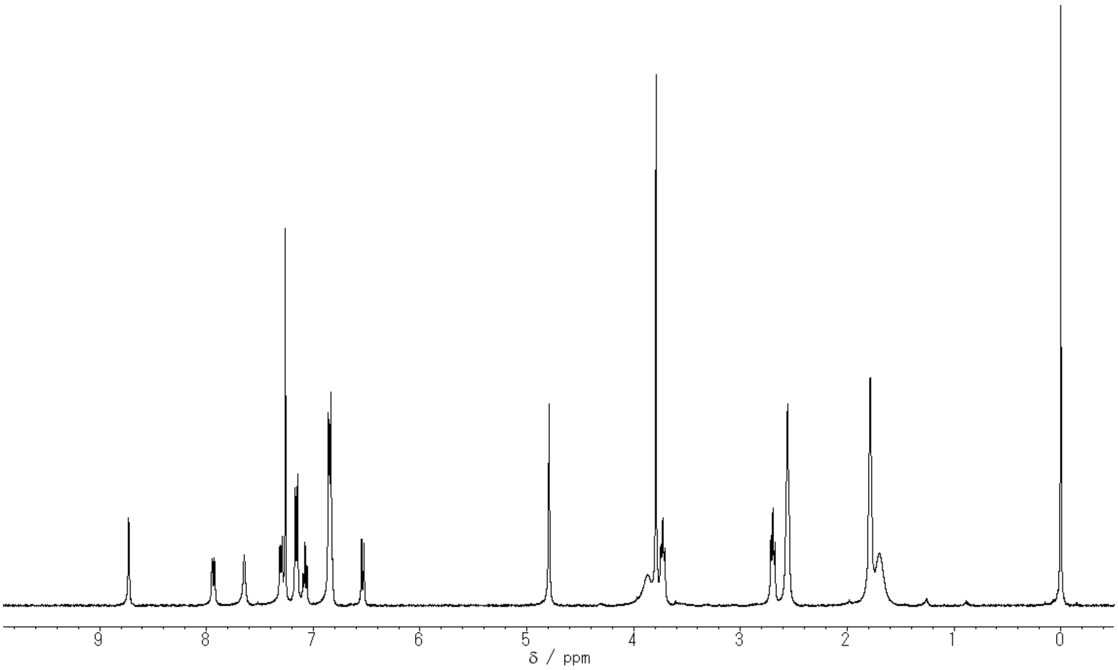
^{1}H-NMR spectrum of Compound **4** (CDCl$_3$, 400 MHz)



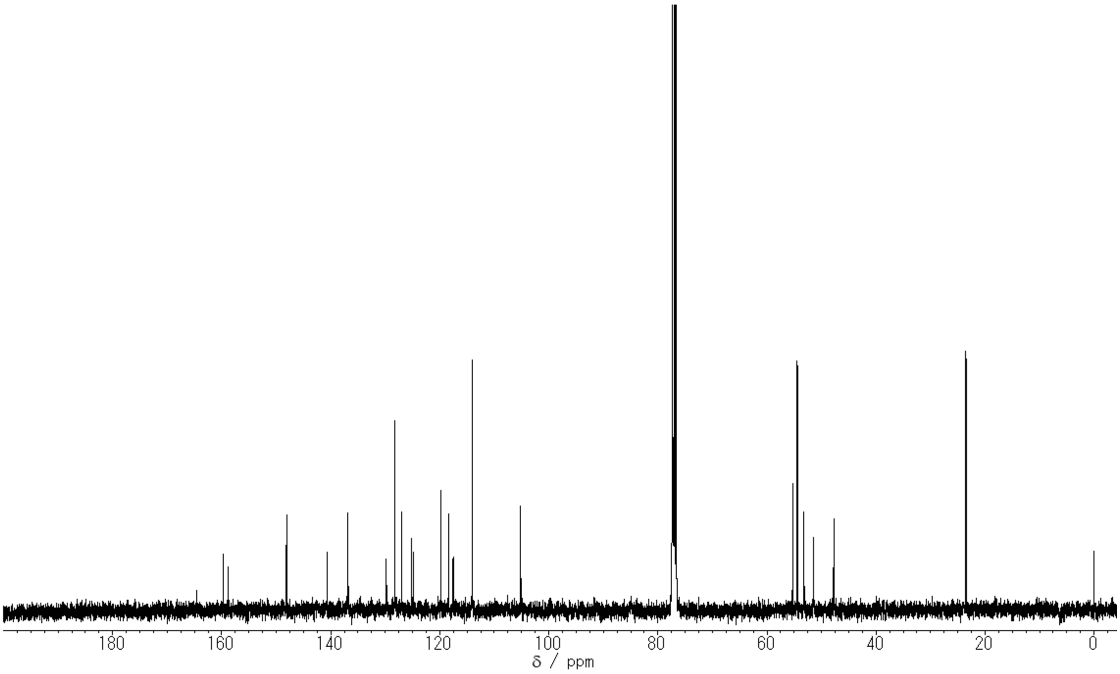
^{13}C-NMR spectrum of Compound **4** (CDCl$_3$, 100 MHz)



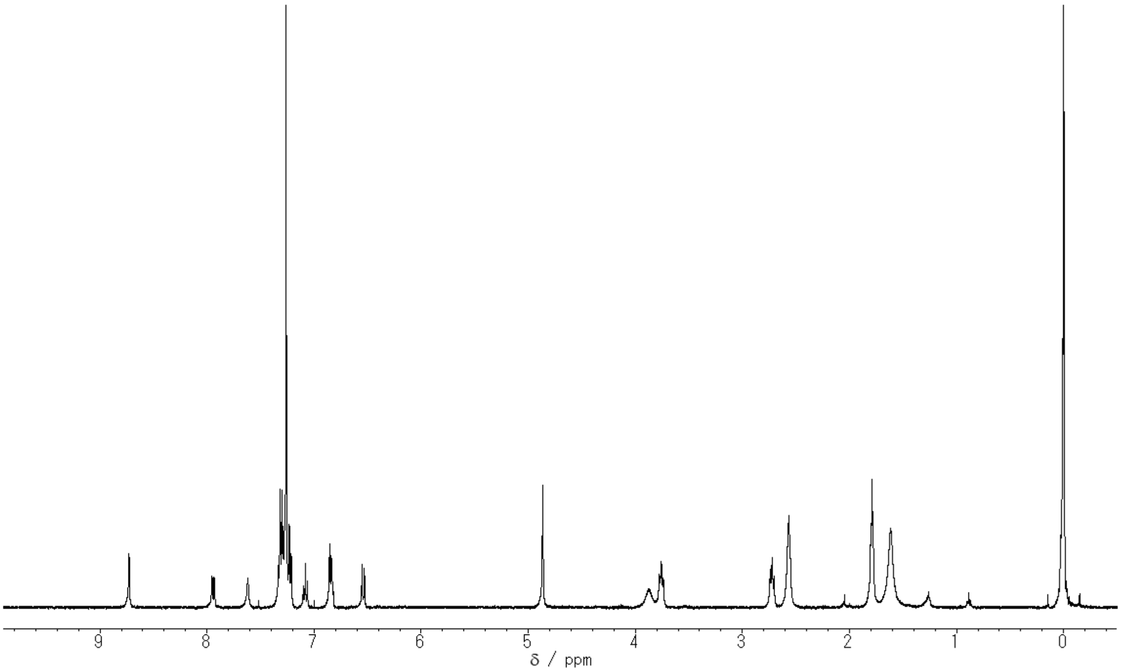
^{1}H-NMR spectrum of Compound **5** ($CDCl_3$, 400 MHz)



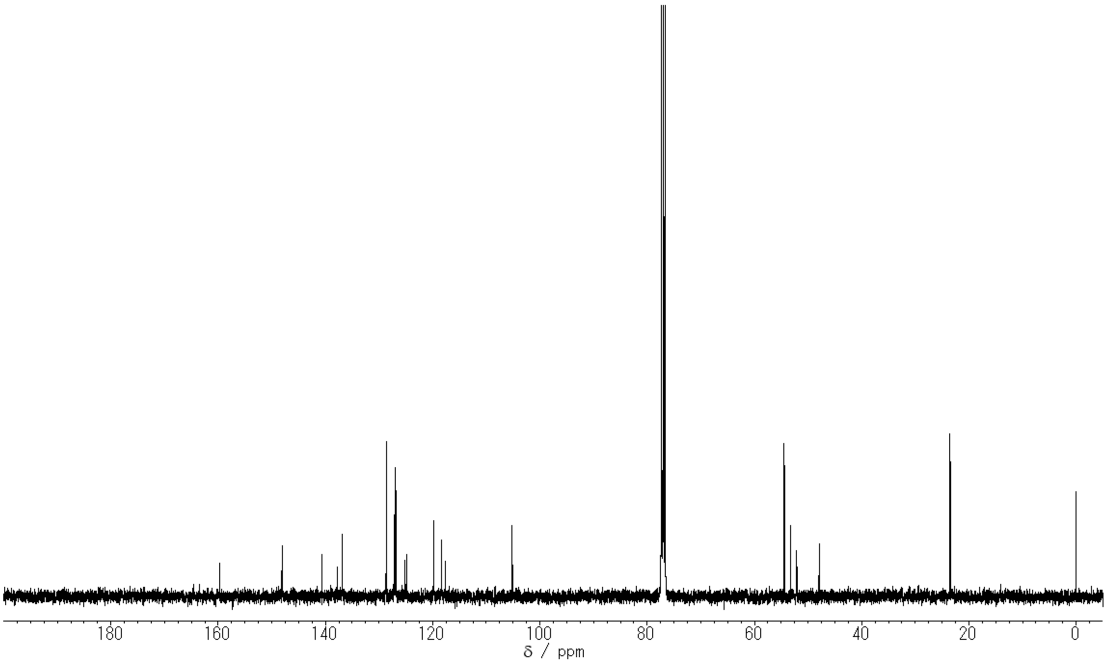
^{13}C-NMR spectrum of Compound **5** ($CDCl_3$, 100 MHz)



^{1}H-NMR spectrum of Compound **6** (CDCl$_3$, 400 MHz)



^{13}C-NMR spectrum of Compound **6** (CDCl$_3$, 100 MHz)



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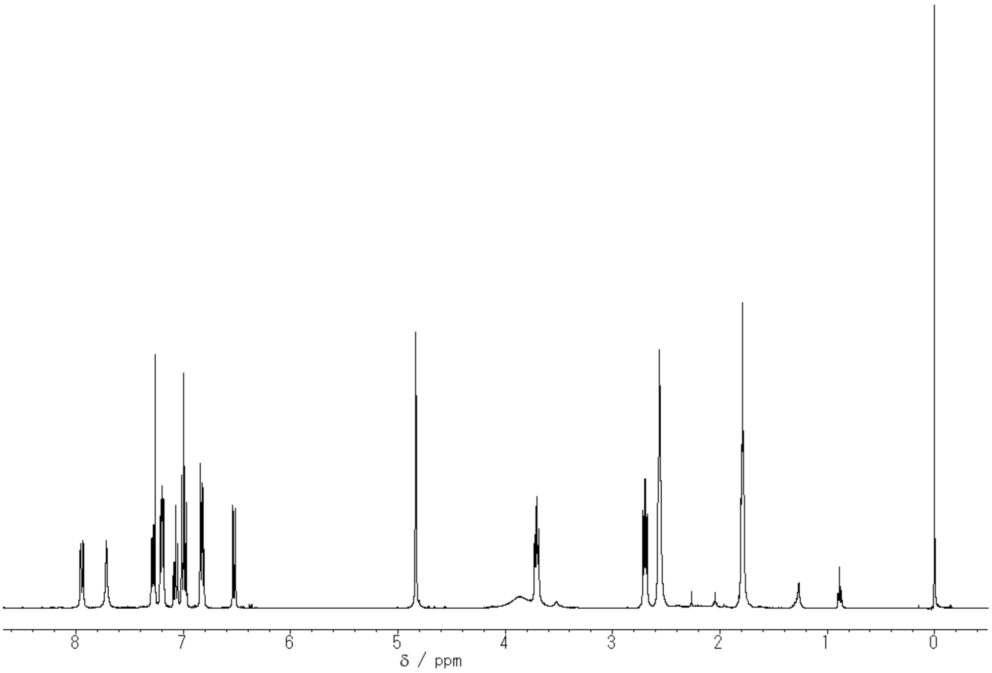
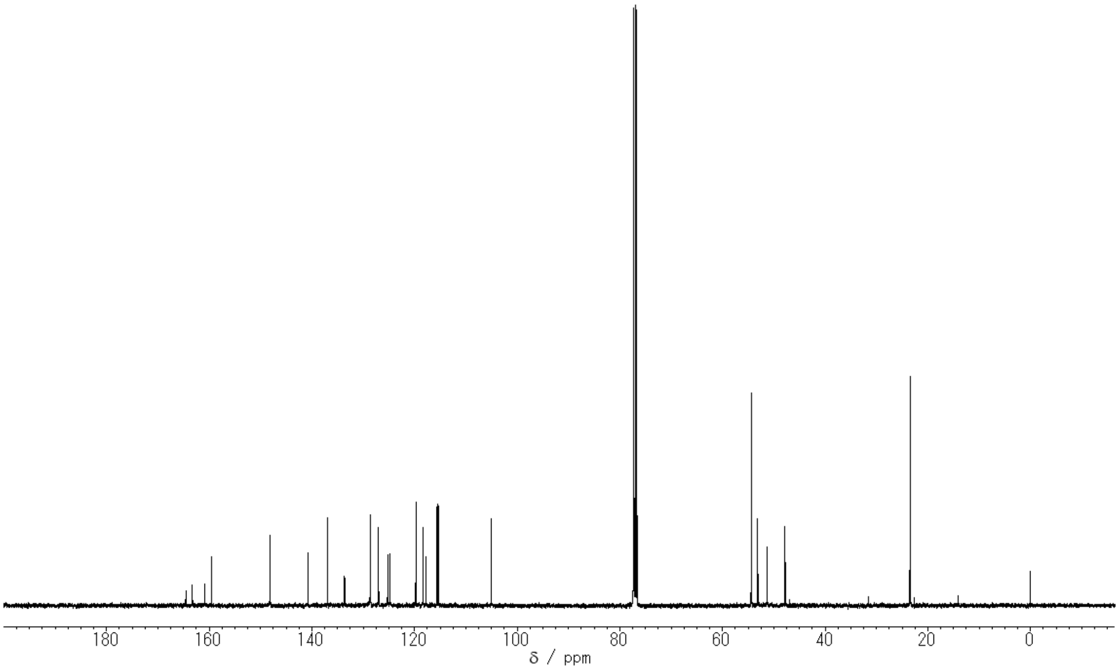
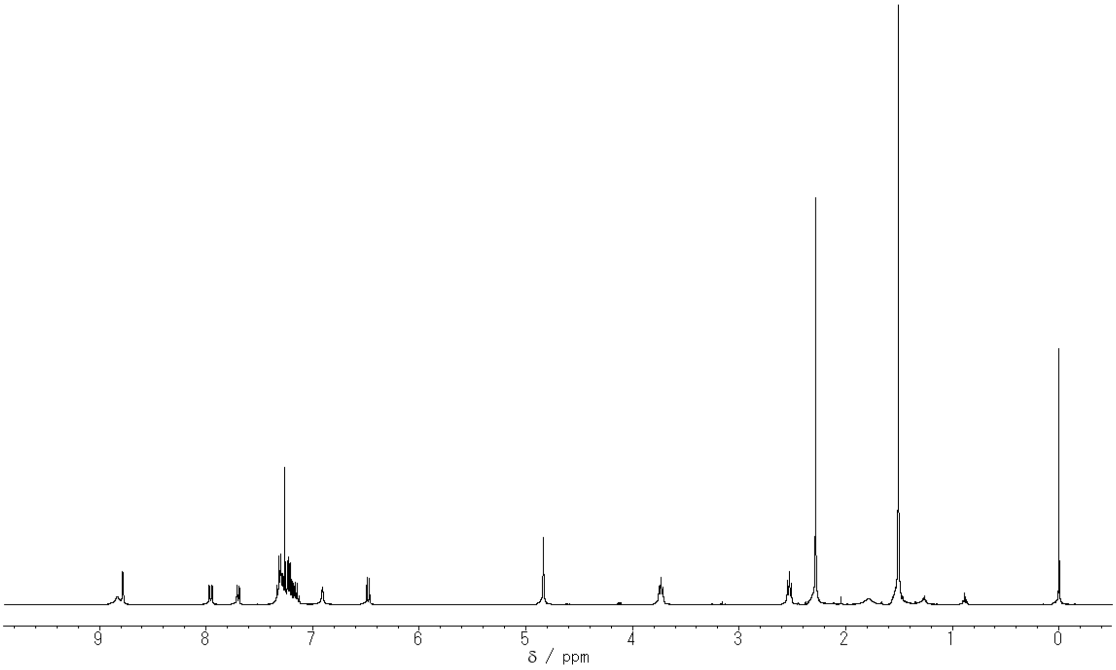


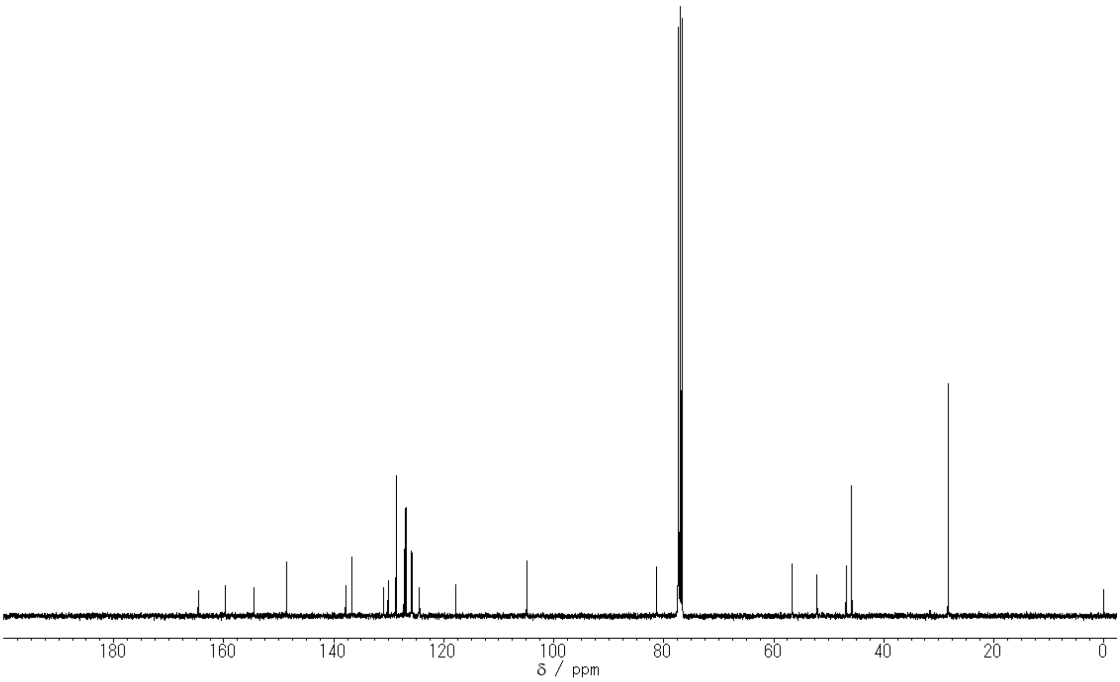
^{13}C-NMR spectrum of Compound **7** ($CDCl_3$, 100 MHz)



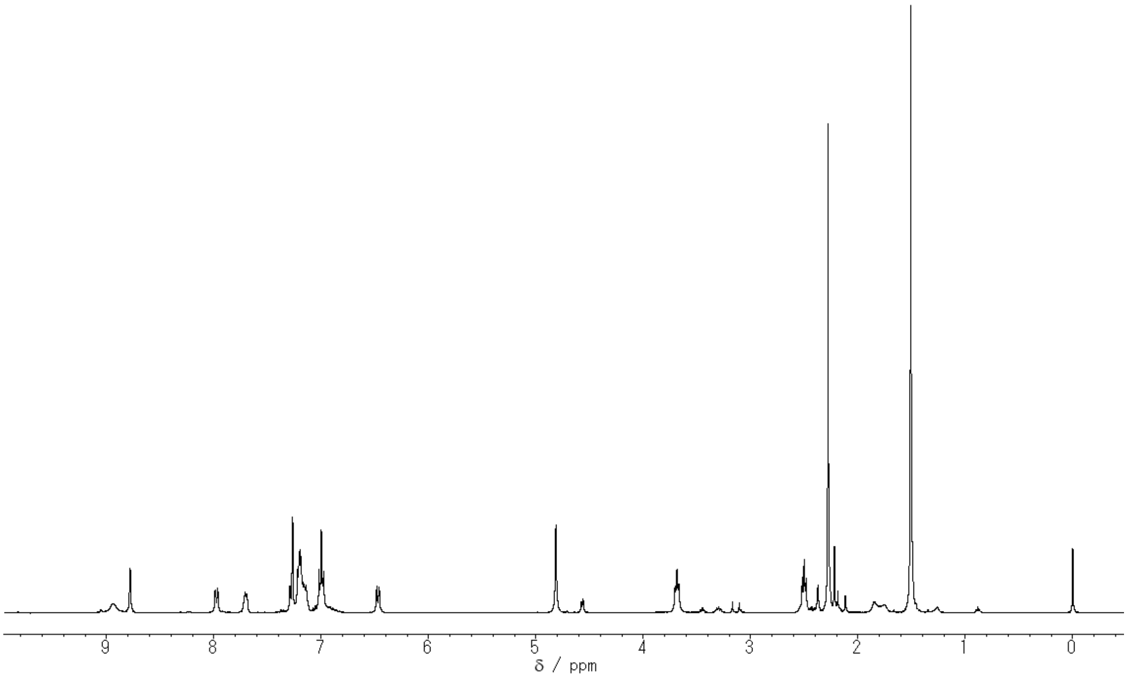
^{1}H-NMR spectrum of Compound **10a** ($CDCl_3$, 400 MHz)



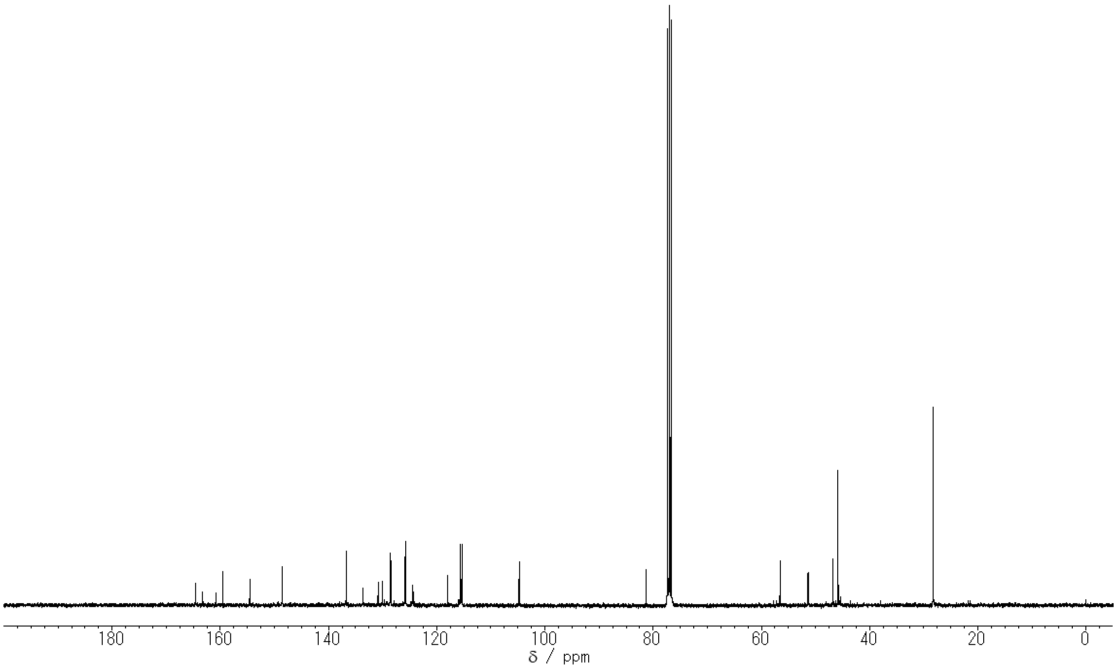
^{13}C-NMR spectrum of Compound **10a** ($CDCl_3$, 100 MHz)



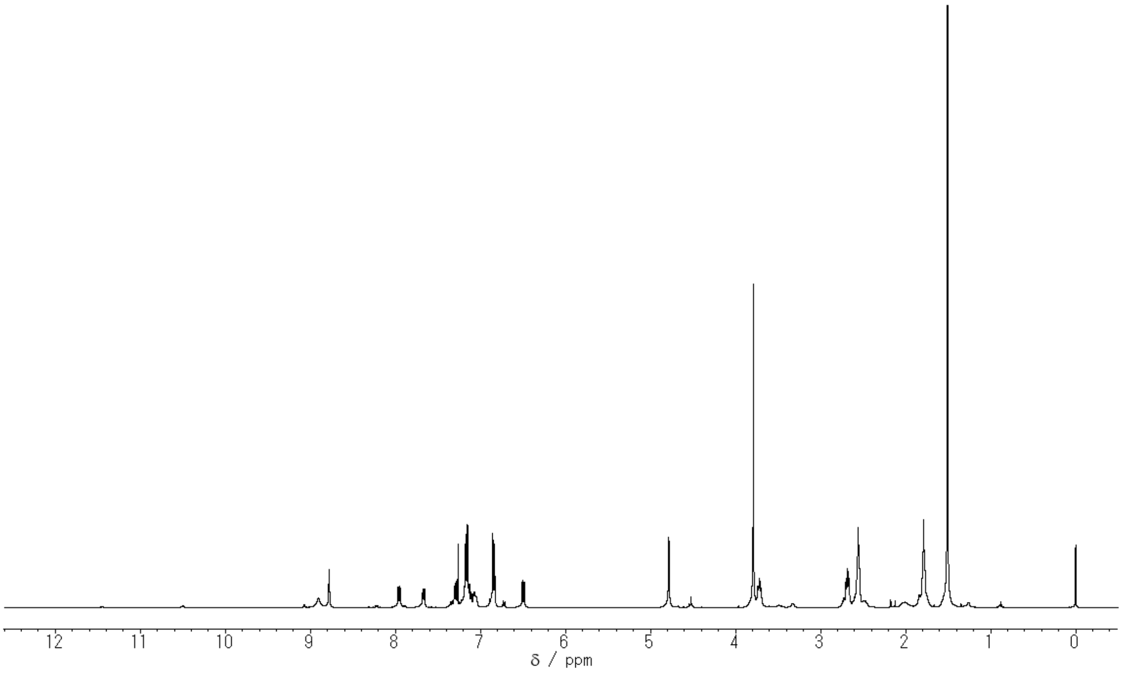
^{1}H-NMR spectrum of Compound **10b** (CDCl$_3$, 400 MHz)



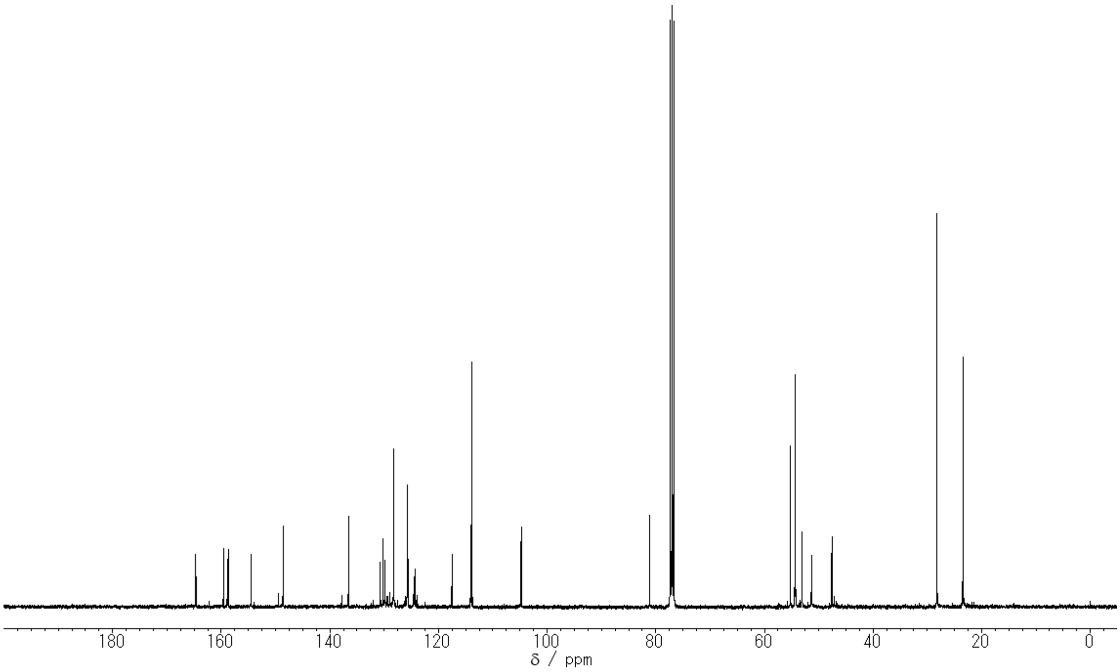
^{13}C-NMR spectrum of Compound **10b** (CDCl$_3$, 100 MHz)



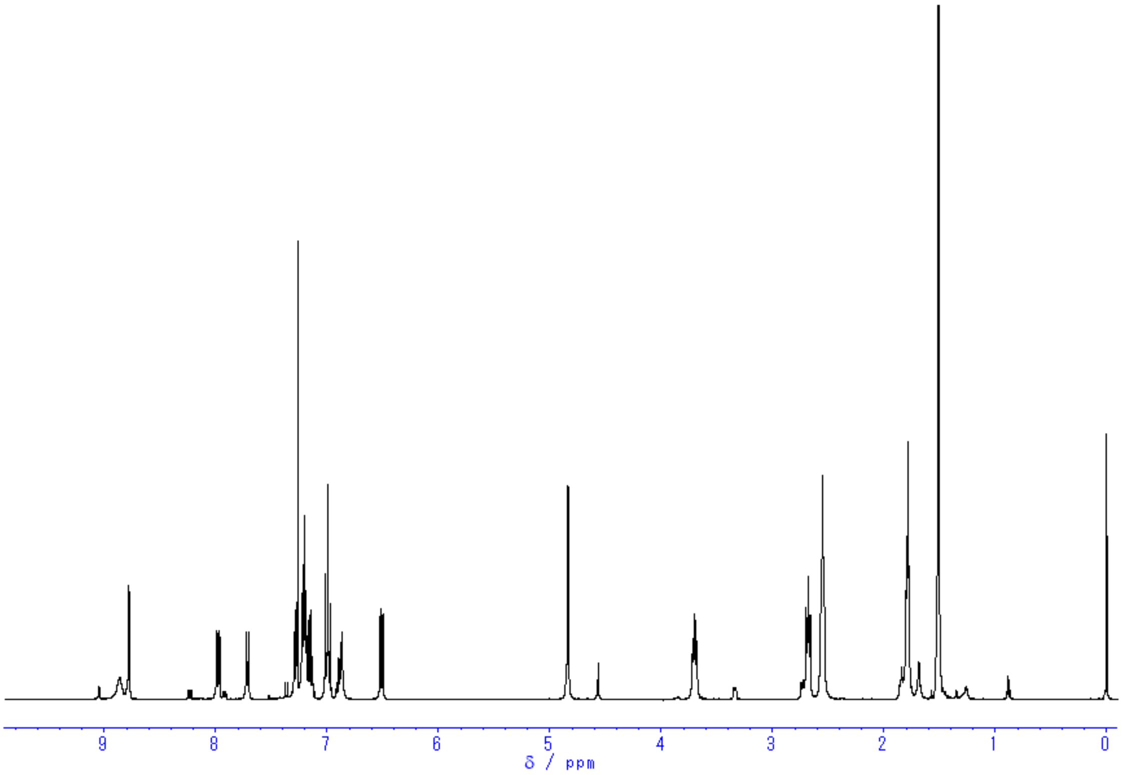
^{1}H-NMR spectrum of Compound **10c** (CDCl$_3$, 400 MHz)



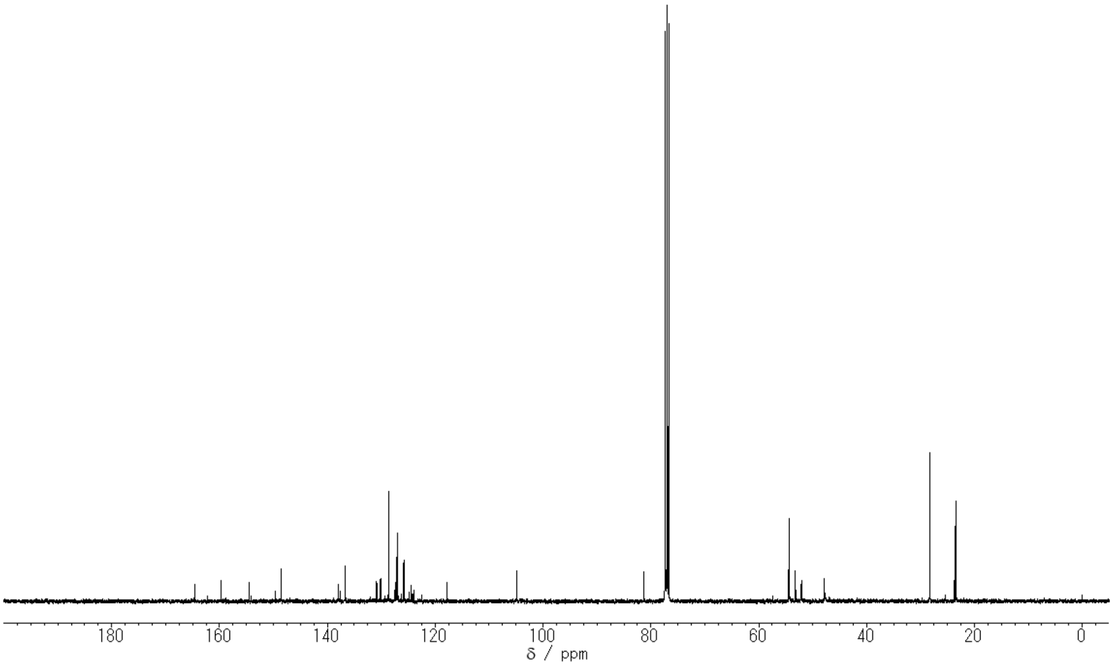
^{13}C-NMR spectrum of Compound **10c** (CDCl$_3$, 100 MHz)



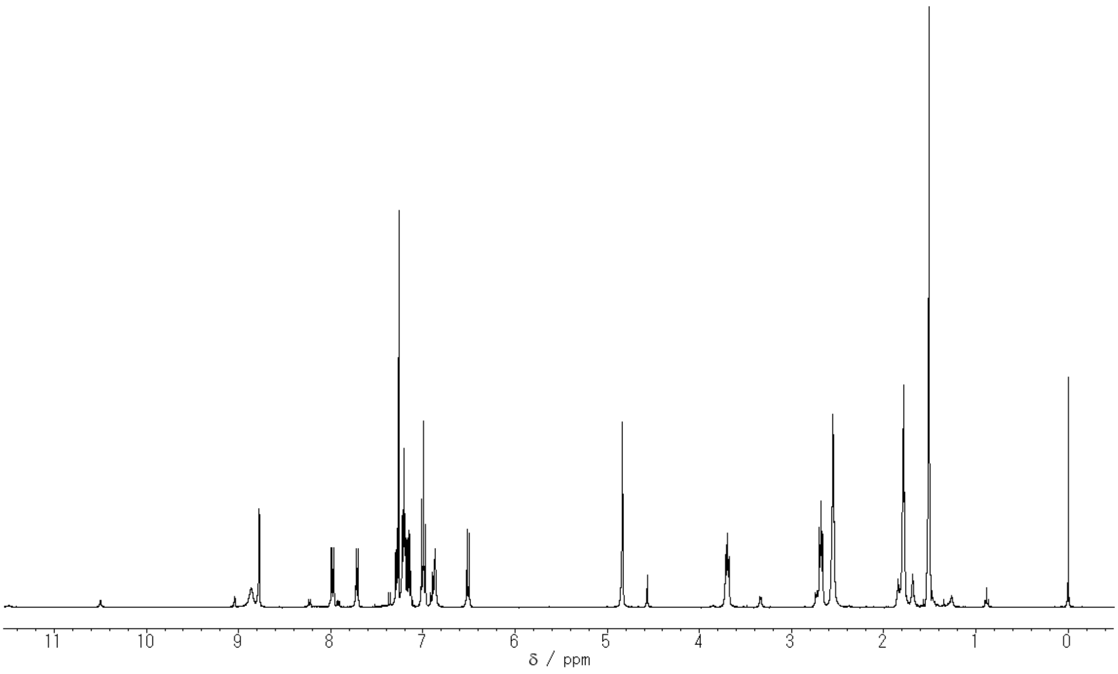
^{1}H-NMR spectrum of Compound **10d** (CDCl$_3$, 400 MHz)



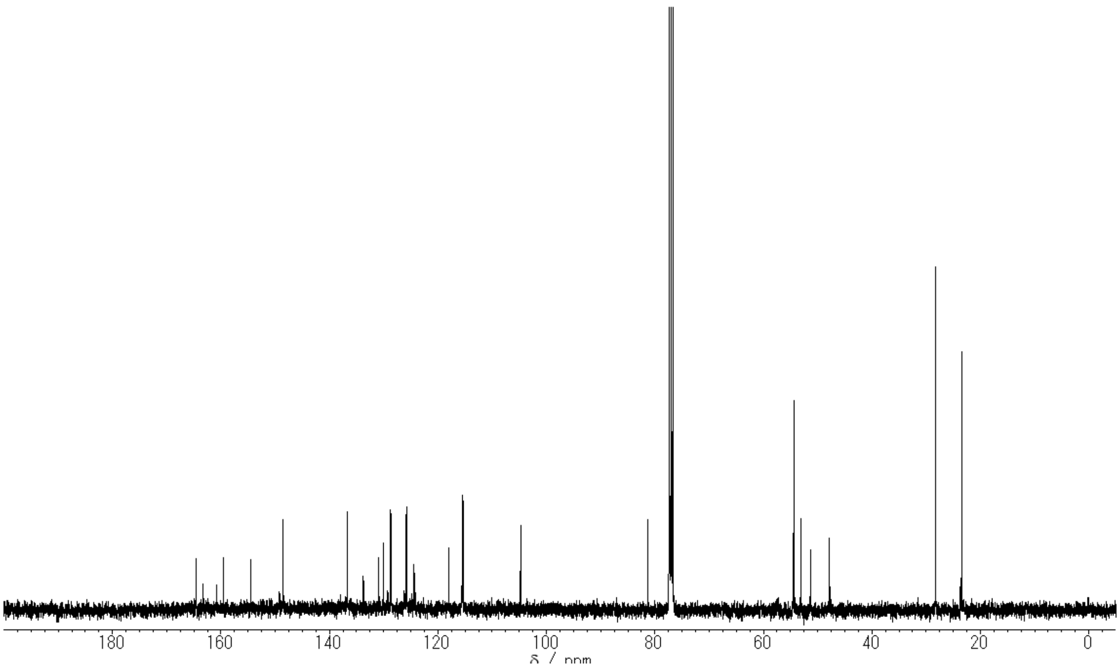
^{13}C-NMR spectrum of Compound **10d** (CDCl$_3$, 100 MHz)



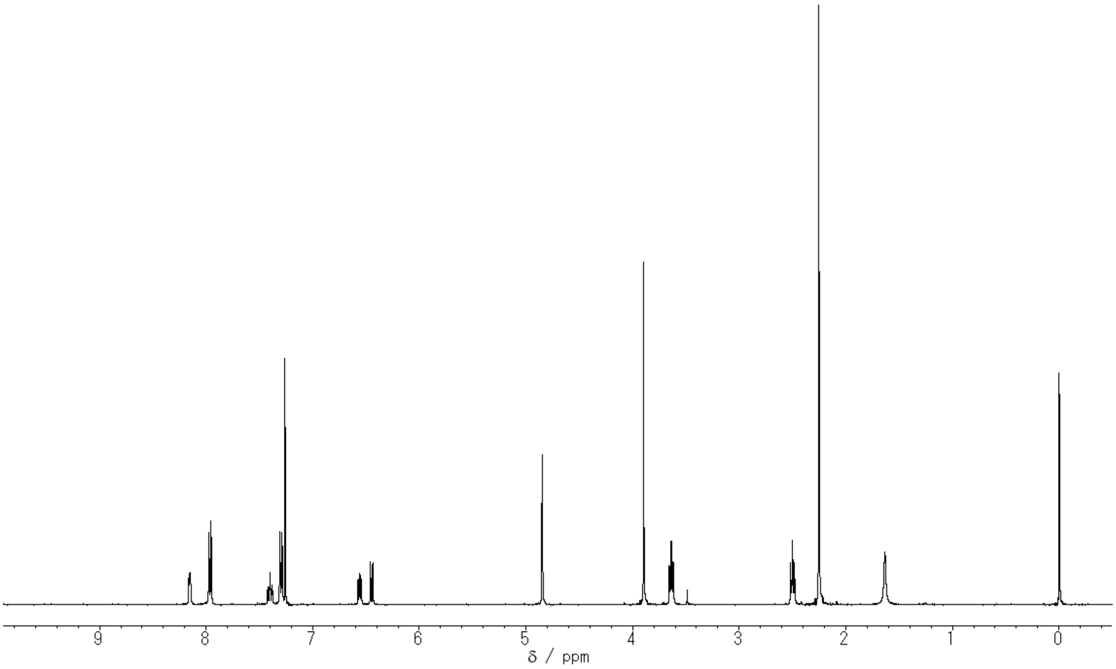
^{1}H-NMR spectrum of Compound **10e** (CDCl$_3$, 400 MHz)



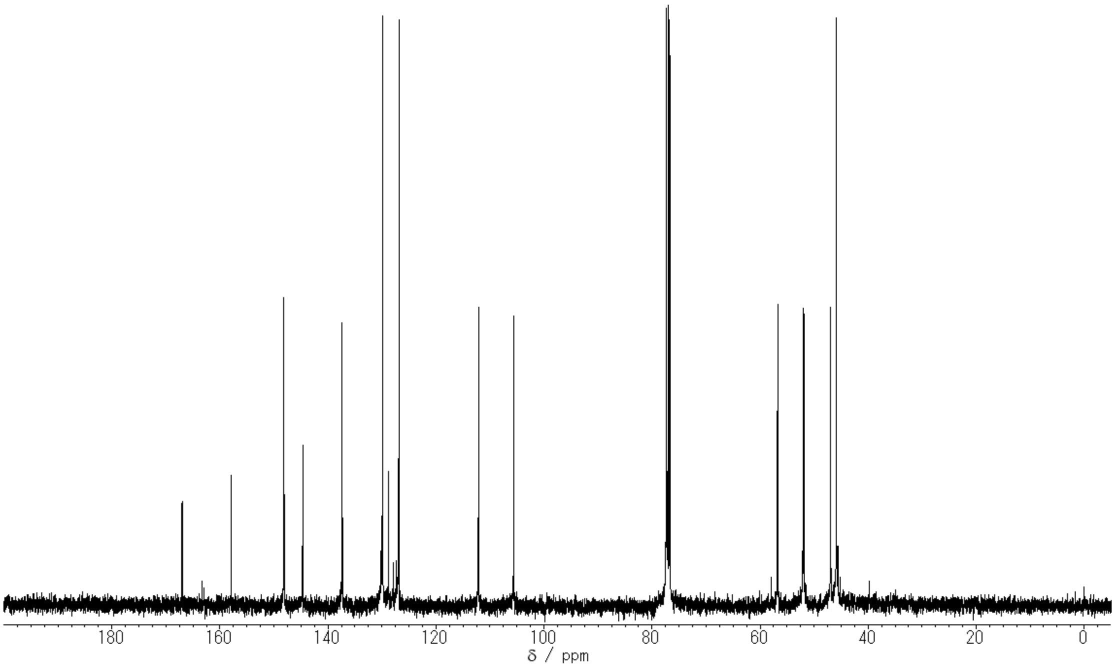
^{13}C-NMR spectrum of Compound **10e** (CDCl$_3$, 100 MHz)



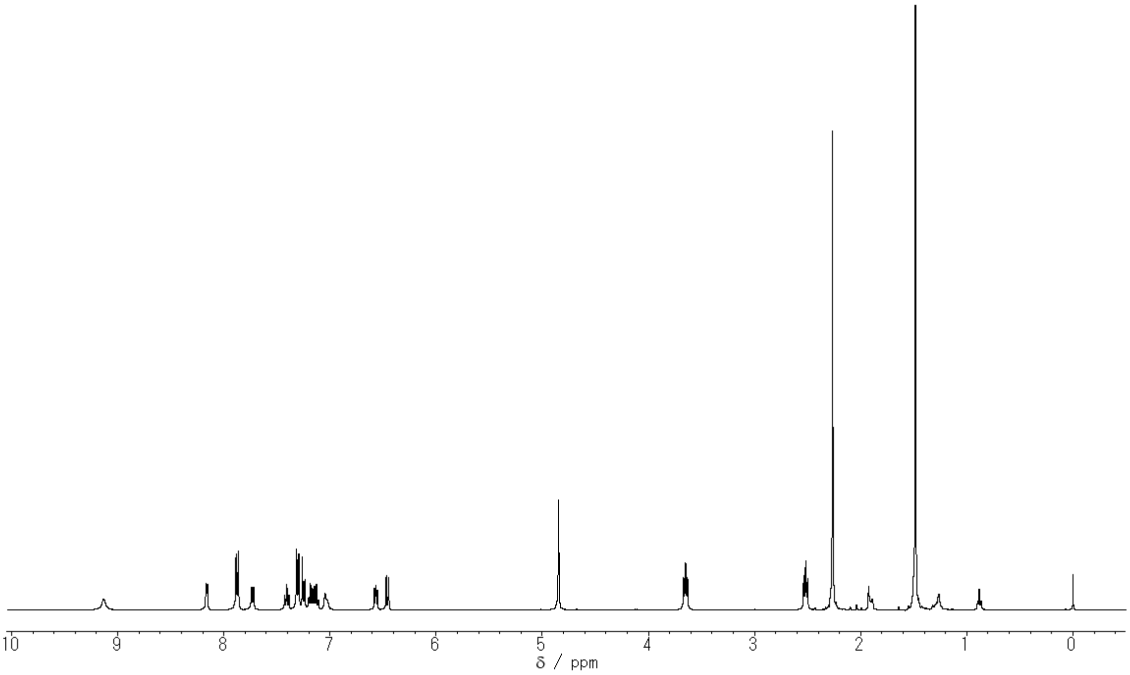
^{1}H-NMR spectrum of Compound **12** ($CDCl_3$, 400 MHz)



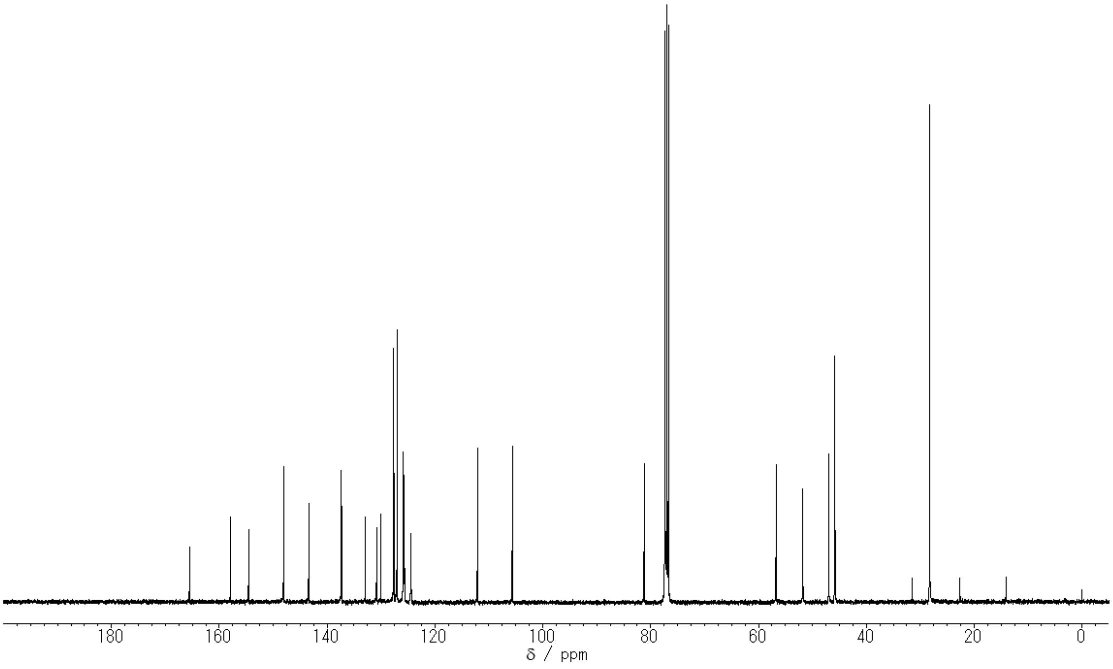
^{13}C-NMR spectrum of Compound **12** ($CDCl_3$, 100 MHz)



^{1}H-NMR spectrum of Compound **13** (CDCl$_3$, 400 MHz)



^{13}C-NMR spectrum of Compound **13** (CDCl$_3$, 100 MHz)



3. Synthesis of starting materials

N^2-Benzyl-*N*^{1},*N*^{1}-dimethylethane-1,2-diamine (8a)

A mixture of benzaldehyde (1.1 mL, 11 mmol), *N*,*N*-dimethylethylenediamine (0.95 mL, 8.8 mmol), $NaBH(OAc)_3$ (2.8 g, 13 mmol), AcOH (0.75 mL, 13 mmol) and CH_2Cl_2 (25 mL) was stirred for 8 h at rt. Then, H_2O (20 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 7.0. Then, the mixture was extracted with EtOAc (2 × 30 mL). After confirming depletion of dimer in the aqueous layer using TLC, K_2CO_3 was added until the aqueous layer became pH 12. And then, the adjusted aqueous layer was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Evaporation of the washed organic extract gave **7** (1.1 g, 59%) as brown oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.87 (s, 1H), 2.19 (s, 6H), 2.42 (t, *J* = 5.6 Hz, 2H), 2.69 (t, *J* = 5.6 Hz, 2H), 3.77 (s, 2H), 7.20-7.26 (s, 2H), 7.29-7.34 (d, *J* = 4.4, 4H).

HRMS (ESI) *m/z* calcd for: $C_{11}H_{19}N_2(M+H)^+$: 179.2863, found: 179.1552.

N^2-[(4-fluorophenyl)methyl]- *N*^{1},*N*^{1}-dimethylethane-1,2-diamine (8b)

A mixture of 4-fluorobenzaldehyde (1.1 mL, 11 mmol), *N*,*N*-dimethylethylenediamine (0.95 mL, 8.8 mmol), $NaBH(OAc)_3$ (2.8 g, 13 mmol), AcOH (0.75 mL, 13 mmol) and CH_2Cl_2 (25 mL) was stirred for 8 h at rt. Then, H_2O (20 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 7.0. Then, the mixture was extracted with EtOAc (2 × 30 mL). After confirming depletion of dimer in the aqueous layer using TLC, K_2CO_3 was added until the aqueous layer became pH 12. And then, the adjusted aqueous layer was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Evaporation of the washed organic extract gave **8** (0.94 g, 46%) as brown oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.87 (s, 1H), 2.22 (s, 6H), 2.42 (t, *J* = 6.4 Hz, 2H), 2.67(t, *J* = 6.4 Hz, 2H), 3.77 (s, 2H), 7.00 (t, *J* = 8.8 Hz, 2H), 7.27-7.30 (m, 2H).

HRMS (ESI) *m/z* calcd for: $C_{11}H_{18}FN_2(M+H)^+$:197.1449, found:197.1456.

N-[(4-Methoxyphenyl)methyl]-2-(pyrrolidin-1-yl)ethan-1-amine (8c)

A mixture of 4-methoxybenzaldehyde (1.3 mL, 11 mmol), 1-(2-aminoethyl)pyrrolidine (1.0 g, 8.8 mmol), $NaBH(OAc)_3$ (2.8 g, 13 mmol), AcOH (0.75 mL, 13 mmol) and CH_2Cl_2 (25 mL) was stirred for 8 h at rt. Then, H_2O (20 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 7.0. Then, the mixture was extracted with EtOAc (2 × 30 mL).

After confirming depletion of dimer in the aqueous layer using TLC, K_2CO_3 was added until the aqueous layer became pH 12. And then, the adjusted aqueous layer was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Evaporation of the washed organic extract gave **9** (1.2 g, 51%) as brown oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.73-1.78 (m, 4H), 1.88 (s, 1H), 2.45-2.47 (m, 4H), 2.60 (t, *J* = 6.3 Hz, 2H), 2.72 (t, *J* = 6.3 Hz, 2H), 3.77 (s, 2H), 3.86 (s, 3H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.23 (d, *J* = 8.6 Hz, 2H).

HRMS (ESI) *m/z* calcd for: $C_{14}H_{23}N_2O(M+H)^+$:235.1805, found:235.1803.

N-Benzyl-2-(pyrrolidin-1-yl)ethan-1-amine (8d)

A mixture of benzaldehyde (1.1 mL, 11 mmol), 1-(2-aminoethyl)pyrrolidine (1.0 g, 8.8 mmol), $NaBH(OAc)_3$ (2.8 g, 13 mmol), AcOH (0.75 mL, 13 mmol) and CH_2Cl_2 (25 mL) was stirred for 8 h at rt. Then, H_2O (20 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 7.0. Then, the mixture was extracted with EtOAc (2 × 30 mL). After confirming depletion of dimer in the aqueous layer using TLC, K_2CO_3 was added until the aqueous layer became pH 12. And then, the adjusted aqueous layer was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Evaporation of the washed organic extract gave **10** (1.4 g, 68%) as brown oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.73-1.79 (m, 4H), 1.82 (s, 1H), 2.45-247 (m, 4H), 2.61 (t, *J* = 6.6 Hz, 2H), 2.74 (t, J = 6.6 Hz, 2H), 3.81 (s, 2H), 7.11-7.25 (m, 1H), 7.31 (d, *J* = 4.4 Hz, 4H).

HRMS (ESI) *m/z* calcd for: $C_{13}H_{21}N_2(M+H)^+$:205.1699, found:205.1704.

N-[(4-Fluorophenyl)methyl]-2-(pyrrolidin-1-yl)ethan-1-amine (8e)

A mixture of 4-fluorobenzaldehyde (1.1 mL, 11 mmol), 1-(2-aminoethyl)pyrrolidine (1.0 g, 8.8 mmol), $NaBH(OAc)_3$ (2.8 g, 13 mmol), AcOH (0.75 mL, 13 mmol) and CH_2Cl_2 (25 mL) was stirred for 8 h at rt. Then, H_2O (20 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 7.0. Then, the mixture was extracted with EtOAc (2 × 30 mL). After confirming depletion of dimer in the aqueous layer using TLC, K_2CO_3 was added until the aqueous layer became pH 12. And then, the adjusted aqueous layer was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Evaporation of the washed organic extract gave **11** (1.5 g, 64%) as brown oil.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.74-1.76 (m, 4H), 1.82 (s, 1H), 2.45-2.47 (m, 4H), 2.60 (*J* = 6.6 Hz, 2H), 2.72 (t, *J* = 6.1 Hz, 2H), 3.77 (s, 2H), 7.00(t, *J* = 8.8 Hz, 2H), 7.06-7.30 (m, 2H).

HRMS (ESI) *m/z* calcd for: $C_{13}H_{20}FN_2$ $(M+H)^+$:223.1605, found:223.1604.

Methyl 4-{[(2-dimethylaminoethyl)amino]methyl}benzoate (11)

A mixture of methyl terephthalaldehydate (6.0 mL, 37 mmol), *N*,*N*-dimethylethylenediamine (4.8 mL, 44 mmol), $NaBH(OAc)_3$ (8.7 g, 55 mmol), AcOH (3.14 mL, 55 mmol) and CH_2Cl_2 (90 mL) was stirred for 8 h at rt. Then, H_2O (30 mL) was added, and the mixture was evaporated to remove CH_2Cl_2. After that, K_2CO_3 was added until the mixture became pH 12. Then, the mixture was extracted with EtOAc (2 × 30 mL). The organic extract was washed with brine (2 × 30 mL), dried over Na_2SO_4. Amino-silica gel column chromatography (hexane/EtOAc = 70/30) of the residue gave **24** (4.2 g, 55%) as a white solid.

^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.82 (s, 1H), 2.20 (s, 6H), 2.42 (t, *J* = 5.8 Hz, 2H), 2.68(t, *J* = 6.4 Hz, 2H), 3.86 (s, 2H), 3.91 (s, 3H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.99 (d, *J* = 6.6 Hz, 1H).

HRMS (ESI) *m/z* calcd for: $C_{13}H_{21}N_2O_2$ $(M+H)^+$:237.1598, found:237.1545.

4. Reference

(1) Narita, K.; Matsuhara, K.; Itoh, J.; Akiyama, Y.; Dan, S.; Yamori, T.; Ito, A.; Yoshida, M.; Katoh, T. Synthesis and biological evaluation of novel FK228 analogues as potential isoform selective HDAC inhibitors. *Eur. J. Med. Chem*. **2016**, *121*, 592–609.