SUPPLEMENTARY INFORMATION

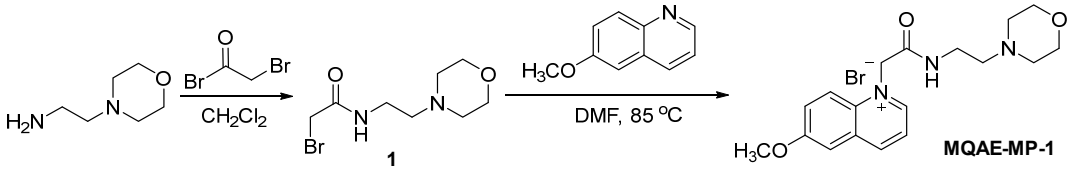
A lysosomal chloride ion-selective fluorescent probe for biological applications

Sang-Hyun Park,[+] Ji Young Hyun,[+] and Injae Shin*

Center for Biofunctional Molecules, Department of Chemistry, Yonsei University,
Seoul 03722, Republic of Korea

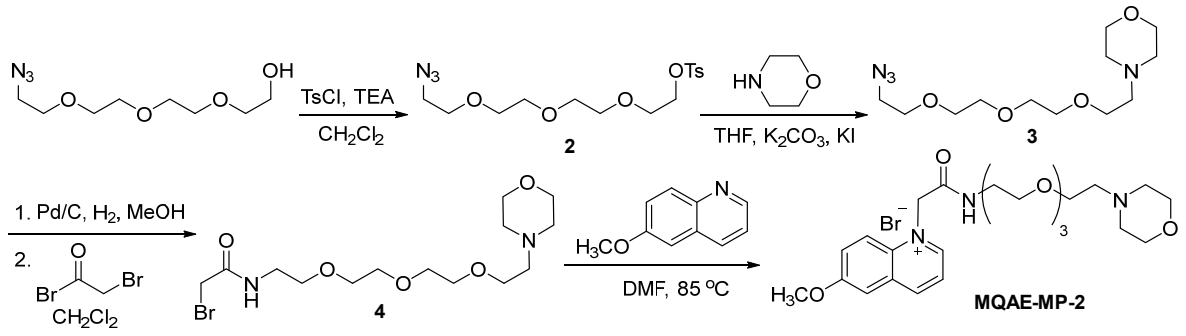
CHEMISTRY

General. Analytical thin-layer chromatography (TLC) was conducted on silica gel 60 F254 glass plates. Compound spots were visualized by UV light (254 nm) and/or by staining with 10 wt% phosphomolybdic acid in ethanol. Flash column chromatography was performed using silica gel 60 (230–400 Mesh). NMR spectra were recorded on a Bruker DRX-400 instrument. Mass spectra were obtained using a Bruker microflex MALDI-TOF mass spectrometer or Waters 3100 LC/MS System. UV and fluorescence spectra were recorded on JASCO V-650 and Perkin Elmer LS 55, respectively.



Compound 1. To a stirred solution of 2-morpholinoethylamine (0.5 g, 3.8 mmol) in CH_2Cl_2 (5 mL) was slowly added bromoacetyl bromide (0.33 mL, 3.8 mmol) at room temperature. After stirring for 1 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The organic solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 1:2) to give a product in 80% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.02–4.99 (m, 2 H), 3.14–3.12 (m, 4 H), 2.82 (t, 2 H, *J* = 6.5 Hz), 2.71–2.69 (m, 2 H), 2.52 (t, 2 H, *J* = 6.3 Hz) 2.43–2.37 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 61.2, 53.1, 52.0, 50.4, 31.2; ESI-MS calcd for $C_8H_{16}BrN_2O_2$ $[M + H]^+$ 251.0, 253.0 found 251.2, 253.3.

MQAE-MP-1. To a stirred solution of 6-methoxyquinoline (0.32 g, 2 mmol) in DMF (3 mL) was added compound **1** (0.1 g, 0.4 mmol) at room temperature. After stirring for 8 h at 85 °C, the solvent was removed under reduced pressure and the residue was dissolved into CH_2Cl_2. The organic solution was washed with water many times to remove excess 6-methoxyquinoline, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used without further purification (95% yield): ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.52 (d, 1 H, *J* = 6.1 Hz), 8.51 (d, 1 H, *J* = 8.7 Hz), 7.65 (d, 1 H, *J* = 9.6 Hz), 7.62 (dd, 1 H, *J* = 8.7, 6.5 Hz), 7.06 (dd, 1 H, *J* = 9.5, 3.6 Hz), 7.00 (d, 1 H, *J* = 3.2 Hz), 5.22 (s, 2 H), 3.22–3.21 (m, 4 H), 2.94 (t, 2 H, *J* = 6.5 Hz), 2.85–2.82 (m, 2 H), 2.60 (t, 2 H, *J* = 6.3 Hz) 2.43–2.37 (m, 2 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.2, 158.6, 145.9, 145.0, 133.3, 130.6, 127.0, 120.1, 118.8, 61.7, 54.7, 54.0, 50.4, 32.0; ESI-MS calcd for $C_{18}H_{24}N_3O_3^+$ $[M]^+$ 330.2, found 330.8.



Compound 2. To a stirred solution of 11-azido-3,6,9-trioxaundecanol (1 g, 4.5 mmol) in CH_2Cl_2 (10 mL) was added triemthylamine (0.95 mL, 6.8 mmol) and *p*-toluenesulfonyl chloride (1.2 g, 6.8 mmol) at 0 °C. After stirring for 8 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 1:1) to give a product in 85% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, 2 H, *J* = 8.6 Hz), 7.34 (d, 2 H, *J* = 8.4 Hz), 4.20–4.12 (m, 2 H), 3.75–3.55 (m, 12 H), 3.38 (t, 2 H, *J* = 5.2 Hz), 2.44 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.9, 133.1, 130.0, 128.1, 70.9, 70.8, 70.7, 70.2, 69.4, 68.8, 50.6, 21.7; ESI-MS calcd for $C_{15}H_{24}N_3O_6S$ $[M + H]^+$ 374.1, found 374.4.

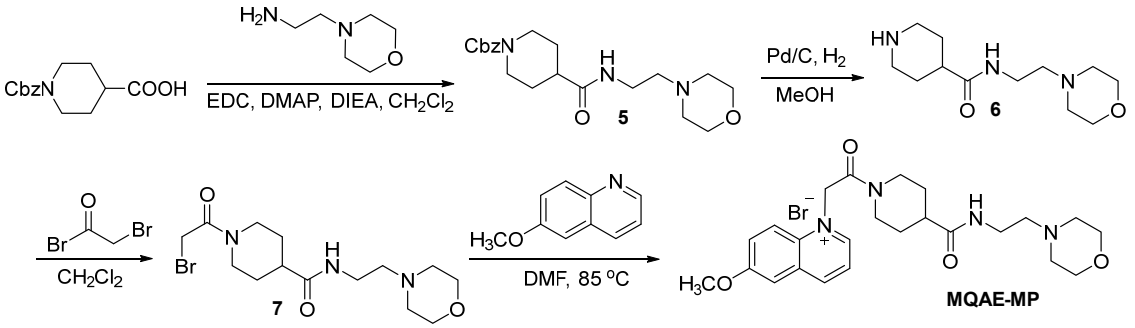
Compound 3. To a stirred solution of compound **2** (0.5 g, 1.3 mmol), K_2CO_3 (0.277 g, 2 mmol) and KI (0.215 g, 1.3 mmol) in THF (5 mL) was added morpholine (0.174 g, 2 mmol) at room temperature. After stirring for 8 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 1:4) to give a product in 68% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73–3.61 (m, 14 H), 3.40 (t, 2 H, *J* = 4.7 Hz), 2.66 (t, 4 H, *J* = 5.2 Hz), 2.60 (t, 4 H, *J* = 4.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 70.3, 70.2, 70.0, 69.8, 67.8, 66.0, 57.7, 53.6, 50.3; ESI-MS calcd for $C_{12}H_{25}N_4O_4$ $[M + H]^+$ 289.2, found 289.6.

Compound 4. Compound **3** (0.2 g, 0.7 mmol) dissolved in methanol (4 mL) was hydrogenated over 10% Pd/C (0.2 g). After 4 h, the catalyst was filtered through Celite® pad and the solvent was removed under reduced pressure to afford a reduced product with quantitative yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (t, 4 H, *J* = 3.7 Hz), 3.68–3.61 (m, 10 H), 3.53 (t, 2 H, *J* = 4.5 Hz) 2.81 (d, 2 H, *J* = 5.2 Hz), 2.62 (t, 2 H, *J* = 5.1 Hz), 2.57–2.53 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 72.0, 70.1, 69.9, 69.8, 67.9, 66.1, 57.8, 53.7, 40.7; ESI-MS calcd for $C_{12}H_{27}N_2O_4$ $[M + H]^+$ 263.2, found 263.7.

To a stirred solution of the above compound (0.1 g, 0.38 mmol) in CH_2Cl_2 (1 mL) was slowly added bromoacetyl bromide (0.033 mL, 0.38 mmol) at room temperature. After stirring for 1 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The

solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 1:2) to give a product in 80% yield: ^{1}H NMR (400 MHz, CD_3OD) δ 4.09 (dd, 2 H, *J* = 11.7, 3.2), 3.91–3.83 (m, 6 H), 3.73–3.58 (m, 12 H), 3.46–3.41 (m, 4 H), 5.32 (td, 2 H, *J* = 10.5, 4.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 168.4, 69.8, 69.0, 56.7, 52.0, 39.6, 27.4; ESI-MS calcd for $C_{14}H_{28}BrN_2O_5$ $[M + H]^+$ 383.0, 385.0 found 383.2, 385.2.

MQAE-MP-2. To a stirred solution of 6-methoxyquinoline (0.1 g, 0.62 mmol) in DMF (3 mL) was added compound **4** (0.05 g, 0.12 mmol) at room temperature. After stirring for 8 h at 85 °C, the solvent was removed under reduced pressure to give a crude product. The residue was dissolved into CH_2Cl_2. The solution was washed with water many times to remove excess 6-methoxyquinoline, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used without further purification (87% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 9.27 (d, 1 H, *J* = 3.9 Hz), 9.20 (d, 1 H, *J* = 9.6 Hz), 8.30 (d, 1 H, *J* = 9.5 Hz), 8.11 (t, 1 H, *J* = 4.7 Hz), 7.89–7.87 (m, 2 H), 5.95 (s, 2 H), 4.07 (s, 3 H), 4,03–4.00 (m, 2 H), 3.89 (m, 3 H), 3.71–3.57 (m, 12 H), 3.50–3.44 (m, 4 H), 3.28 (td, 2 H, *J* = 10.9, 4.0 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 164.8, 160.1, 147.7, 146.6, 134.6, 132.1, 128.7, 121.9, 119.8, 107.6, 69.6, 68.8, 63.8, 63.5, 60.3, 59.1, 56.7, 55.9, 53.6, 52.1, 39.8 ; ESI-MS calcd for $C_{24}H_{36}N_3O_6^+$ $[M]^+$ 462.3, found 462.6.



Coumpound 5. To a stirred solution of 1-(benzyloxycarbonyl)isonipecotic acid (1 g, 3.8 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide•HCl (EDC•HCl, 0.7 g, 4.5 mmol), N,N'-dimethylaminopyridine (DMAP, 0.05 g, 0.45 mmol) and diisopropylethylamine (DIEA, 1 g, 7.6 mmol) in CH_2Cl_2 (20 mL) was added 2-morpholinoethylamine (0.5 g, 3.8 mmol) at 0 °C. After stirring for 8 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 3:1) to give a product in 84% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.28 (m, 5 H), 5.11 (s, 2 H), 4.21 (t, 2 H, *J* = 3.7 Hz), 3.70 (t, 4 H, *J* = 4.8 Hz), 3.35 (q, 2 H, *J* = 5.6 Hz), 2.81 (t, 2 H, *J* = 4.4 Hz), 2.48–2.43 (m, 6 H), 2.26 (tt, 1 H, *J* = 11.5,

3.8), 1.87–1.76 (m, 2 H), 1.69–1.58 (m, 2 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 155.1, 136.6, 128.5, 128.0, 127.8, 77.4, 77.1, 76.8, 67.1, 66.9, 56.9, 53.2, 43.4, 43.0, 35.4, 28.5; ESI-MS calcd for $C_{20}H_{30}N_3O_4$ $[M + H]^+$ 376.2, found 376.7.

Compound 6. Compound **5** (1 g, 2.6 mmol) dissolved in methanol (10 mL) was hydrogenated over 10% Pd/C (1 g). After 4 h, the catalyst was filtered through Celite® pad and the solvent was removed under reduced pressure to afford a product with quantitative yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.71 (t, 4 H, *J* = 3.8 Hz), 3.36 (t, 2 H, *J* = 6.6 Hz), 3.16–3.13 (m, 2 H), 2.71 (td, 2 H, *J* = 10.7, 2.3), 2.50–2.47 (m, 6 H), 2.36 (tt, 1 H, *J* = 11.5, 3.8), 1.81–1.63 (m, 4 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 66.3, 57.2, 53.2, 44.5, 42.2, 35.6, 28.1; ESI-MS calcd for $C_{12}H_{24}N_3O_2$ $[M + H]^+$ 242.2, found 242.6.

Compound 7. To a stirred solution of compound **6** (0.9 g, 3.7 mmol) in CH_2Cl_2 (5 mL) was slowly added bromoacetyl bromide (0.32 mL, 3.7 mmol) at room temperature. After stirring for 1 h at room temperature, the reaction was quenched by addition of CH_2Cl_2. The solution was washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (hexane : EtOAc = 1:1) to give a product in 82% yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.50–4.46 (m, 1 H), 4.17–3.89 (m, 7 H), 3.70–3.64 (m, 5 H), 3.42–3.23 (m, 9 H), 2.88 (td, 1 H, *J* = 12.7, 2.1), 2.88 (tt, 1 H, *J* = 10.4, 4.3), 2.02 (t, 2 H, *J* = 12.7 Hz), 1.83 (qd, 1 H, *J* = 11.4, 4.2), 1.61 (qd, 1 H, *J* = 11.4, 4.2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.7, 166.5, 63.6, 56.5, 53.7, 52.0, 46.2, 41.9, 41.6, 33.5, 28.5, 27.8, 25.6; ESI-MS calcd for $C_{14}H_{25}BrN_3O_3$ $[M + H]^+$ 362.1, 364.1 found 362.2, 364.2.

MQAE-MP. To a stirred solution of 6-methoxyquinoline (2.4 g, 15 mmol) in DMF (30 mL) was added compound **7** (1.1 g, 3 mmol) at room temperature. After stirring for 8 h at 85 °C, the solvent was removed under reduced pressure to give a crude product. The residue was dissolved into CH_2Cl_2. The solution was washed with water many times to remove excess 6-methoxyquinoline, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used without further purification (70% yield): ^{1}H NMR (400 MHz, CD_3OD) δ 9.20 (d, 1 H, *J* = 6.1 Hz), 9.17 (d, 1 H, *J* = 8.8 Hz), 8.26 (d, 1 H, *J* = 8.8 Hz), 8.11 (dd, 1 H, *J* = 7.6, 5.9 Hz), 7.88 (dd, 1 H, *J* = 9.5, 2.6 Hz), 7.83 (d, 1 H, *J* = 3.3 Hz), 6.33 (q, 2 H, *J* = 13.3 Hz), 4.47–4.43 (m, 1 H), 4.16–4.10 (m, 3 H), 4.08 (s, 3 H), 3.93 (t, 1 H, *J* = 12.1 Hz), 3.71–3.59 (m, 4 H), 3.49 (t, 1 H, *J* = 10.5 Hz), 3.40 (t, 2 H, *J* = 5.6 Hz), 3.28 (td, 2 H, *J* = 11.2, 4.0 Hz), 2.94 (td, 1 H, *J* = 12.0, 2.7 Hz), 3.28 (tt, 1 H, *J* = 11.4, 3.6 Hz), 2.13–1.95 (m, 3 H), 1.75 (qd, 1 H, *J* = 11.4, 4.2); ^{13}C NMR (100 MHz, CD_3OD) δ 176.7, 162.7, 159.9, 147.3, 146.5, 135.1, 132.1, 128.5, 121.8, 120.4, 107.3, 63.6, 58.8, 56.6, 55.8, 52.1, 44.7, 42.0, 41.9, 28.6, 27.7; ESI-MS calcd for $C_{24}H_{34}N_4O_4^+$ $[M]^+$ 441.2, found 441.4.

DSC4P-1 and SA-3 were synthesized according to known procedures.[1,2]

BIOLOGY

Cell culture. HeLa (human cervical cancer cells) cells were cultured in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (FBS), 50 units/mL penicillin and 50 units/mL streptomycin. HeLa cells stably expressing mRFP-EGFP-LC3 fusion protein were constructed by transfecting with the tandem mRFP-EGFP-LC3 plasmid (Addgene) using Lipofectamin 2000 (Invitrogen) and then selecting with 600 μg/mL G418 (Tocris) over 3 weeks. Cells were maintained at 37 ^{o}C under a humidified atmosphere containing 5% CO_2.

Cell viability assay. Cell viability was assessed by using an MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. HeLa cells (5 x 10^3 cells/100 μL) were plated in triplicate in 96-well plates for 24 h and were incubated with various concentrations of MQAE-MP in culture media for 24 h. 20 μL of MTT (5 mg/ml, Amresco Life Science) was added to culture media in each well and the mixture was then incubated for 2 h. After removing the culture media containing MTT, 100 μL of DMSO was added and incubated for 30 min for color development. The absorbance at 570 nm was measured using an Infinite® 200 PRO multimode microplate reader.

Effect of substances on autophagy. HeLa cells stably expressing mRFP-EGFP-LC3 fusion protein were incubated for 12 h with various concentrations of substances. After washing with DPBS three times, cells were treated with fix solution containing 4% formaldehyde and 0.1 % Triton x-100 and imaged by using confocal fluorescence microscopy (Zeiss LSM 800).

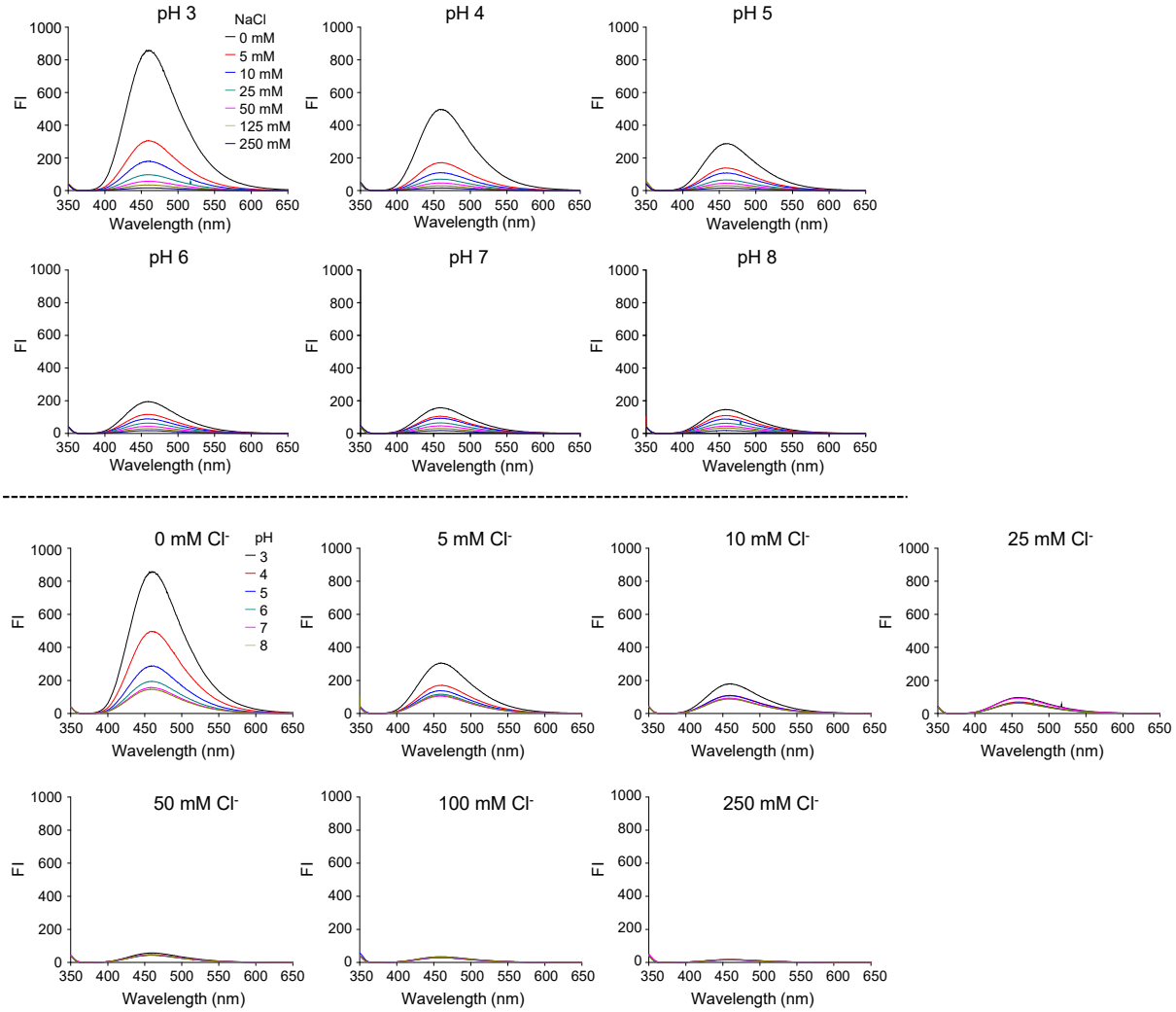


Figure S1. Effect of pH on fluorescence emission of MQAE-MP-1 at various Cl^- concentrations. Aliquots of 5 M NaCl solutions were added to solutions of MQAE-MP-1 (100 μM) in 50 mM sodium phosphate at pH 3.0-8.0. Fluorescence spectra of MQAE-MP-1 (100 μM) in 50 mM sodium phosphate buffer containing various concentrations of chloride ions (0-250 mM) at pH 3.0-8.0 were recorded on a fluorimeter upon excitation at 350 nm wavelength (FI = fluorescence intensity).

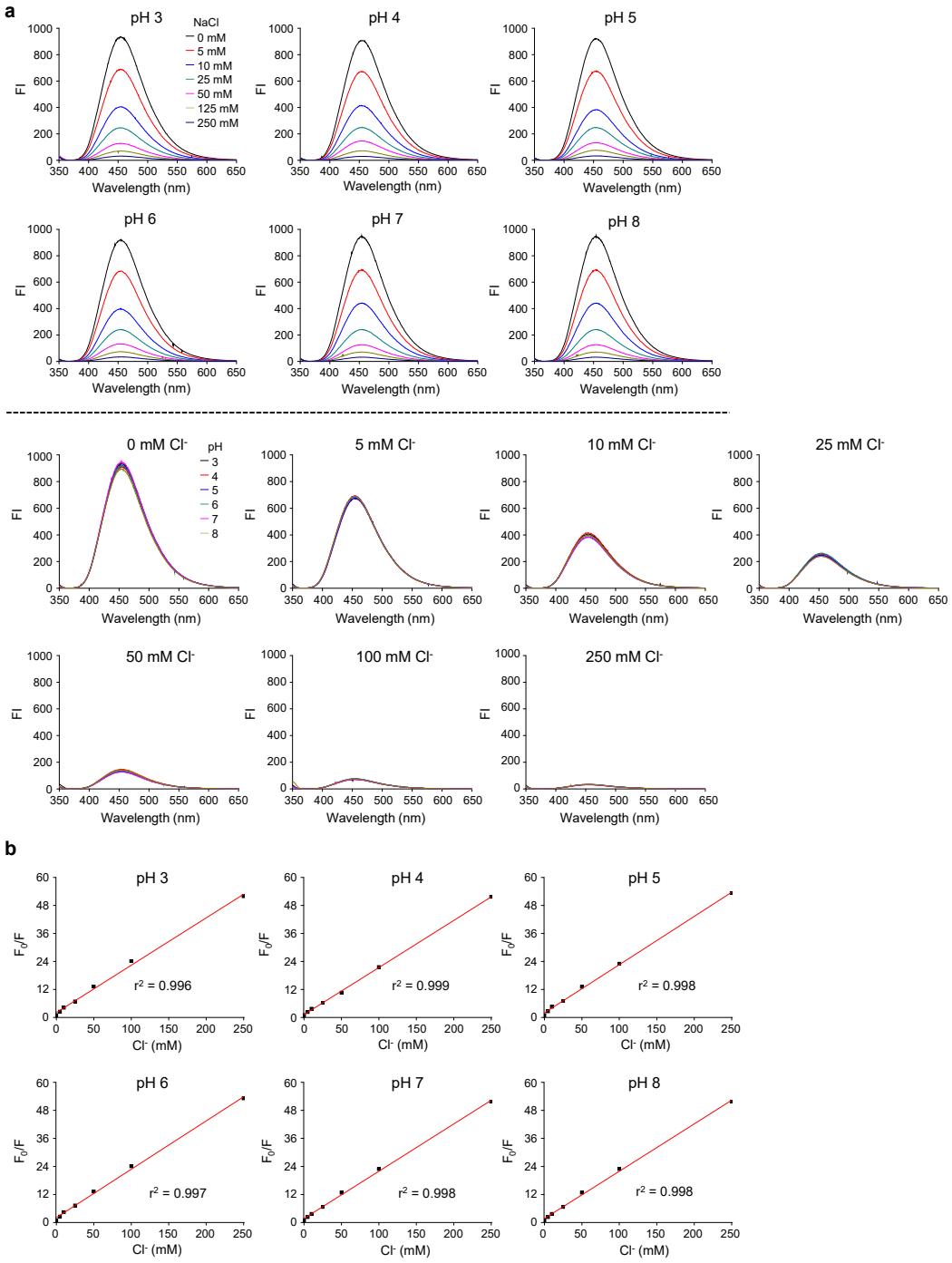


Figure S2. Effect of pH on fluorescence emission of MQAE at various Cl^- concentrations. (a) Aliquots of 5 M NaCl solutions were added to solutions of MQAE (100 μM) in 50 mM sodium phosphate at pH 3.0-8.0. Fluorescence spectra of MQAE (100 μM) in 50 mM sodium phosphate buffer containing various concentrations of chloride ions (0-250 mM) at pH 3.0-8.0 were recorded on a fluorimeter upon excitation at 350 nm wavelength. (b) Stern-Volmer plots for quenching of MQAE by Cl^- (F_0: fluorescence of buffer only, F: fluorescence of buffer containing Cl^-). Data were fitted by a linear regression.

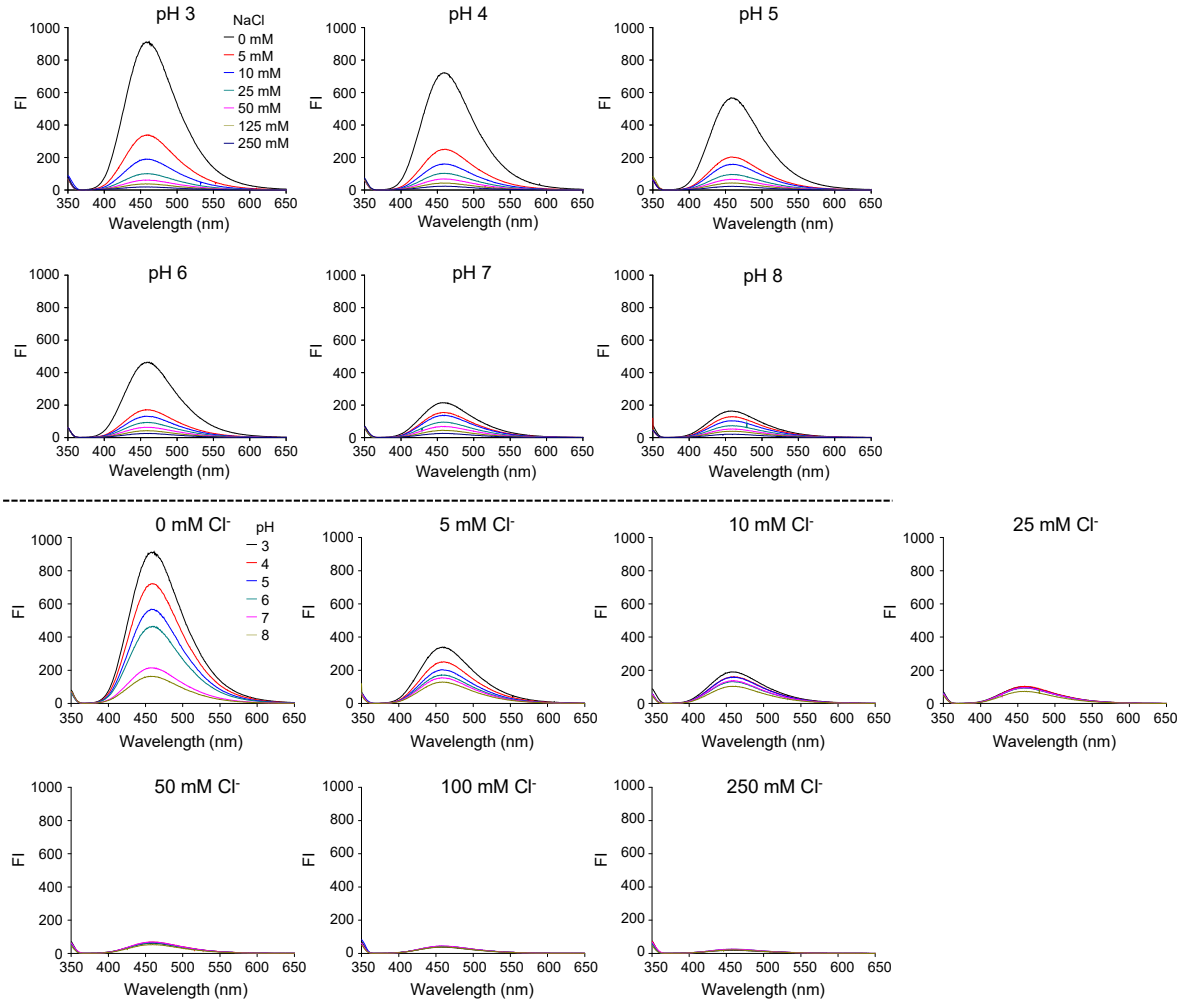


Figure S3. Effect of pH on fluorescence emission of MQAE-MP-2 at various Cl^- concentrations. (a) Aliquots of 5 M NaCl solutions were added to solutions of MQAE-MP-2 (100 μM) in 50 mM sodium phosphate at pH 3.0-8.0. Fluorescence spectra of MQAE-MP-2 (100 μM) in 50 mM sodium phosphate buffer containing various concentrations of chloride ions (0-250 mM) at pH 3.0-8.0 were recorded on a fluorimeter upon excitation at 350 nm wavelength.

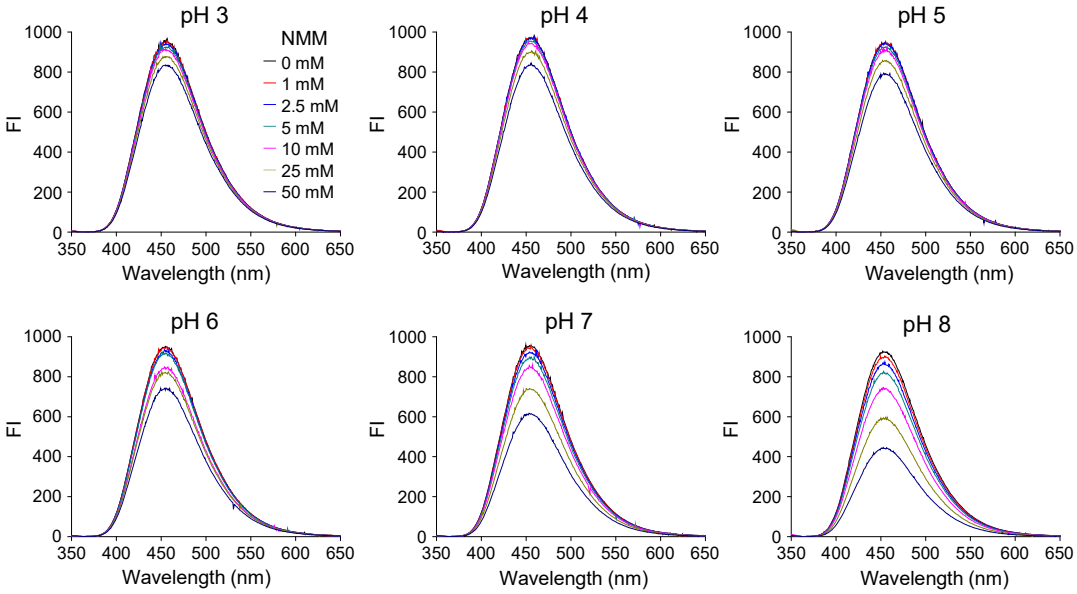


Figure S4. Effect of N-methylmorpholine (NMM) on fluorescence emission of MQAE. Fluorescence spectra of MQAE (100 μM) in 100 mM sodium phosphate buffer containing various concentrations of NMM at pH 3.0-8.0 were recorded on a fluorimeter upon excitation at 350 nm wavelength.

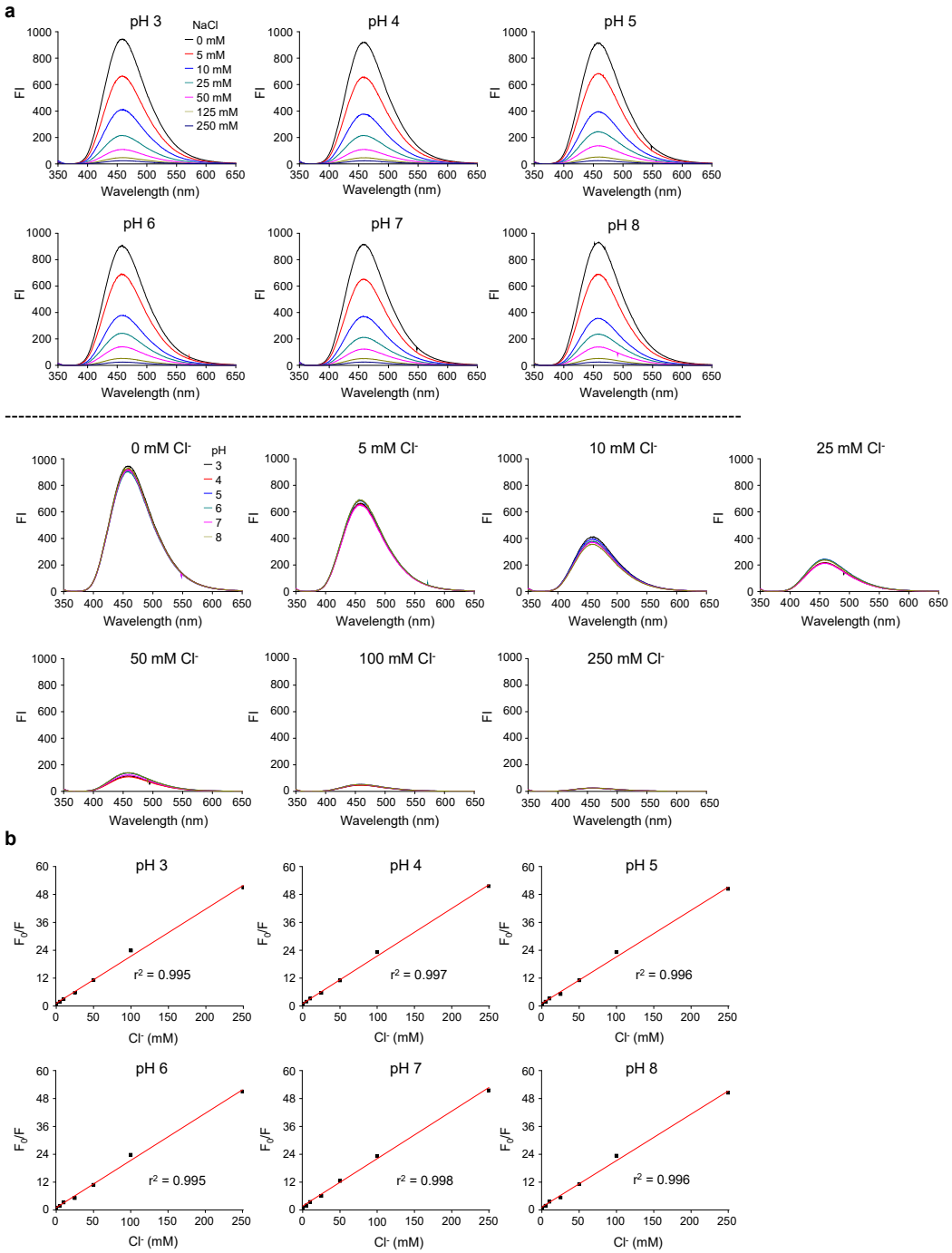


Figure S5. Effect of pH on fluorescence emission of MQAE-MP at various Cl^- concentrations. (a) Aliquots of 5 M NaCl solutions were added to solutions of MQAE-MP (100 μM) in 50 mM sodium phosphate at pH 3.0-8.0. Fluorescence spectra of MQAE-MP (100 μM) in 50 mM sodium phosphate buffer containing various concentrations of chloride ions (0-250 mM) at pH 3.0-8.0 were recorded on a fluorimeter upon excitation at 350 nm wavelength. (b) Stern-Volmer plots for quenching of MQAE-MP by Cl^- (F_0: fluorescence of buffer only, F: fluorescence of buffer containing Cl^-). Data were fitted by a linear regression.

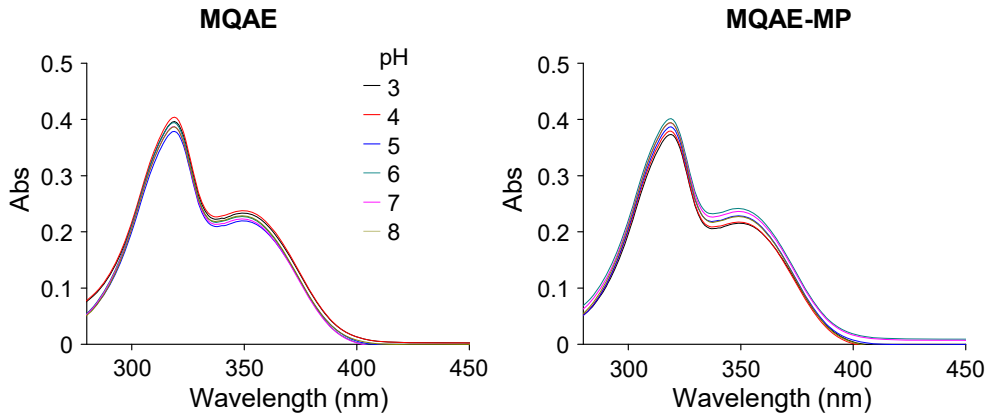


Figure S6. Absorption spectra of MQAE (100 μM) and MQAE-MP (100 μM) in 50 mM sodium phosphate at pH 3.0-8.0.

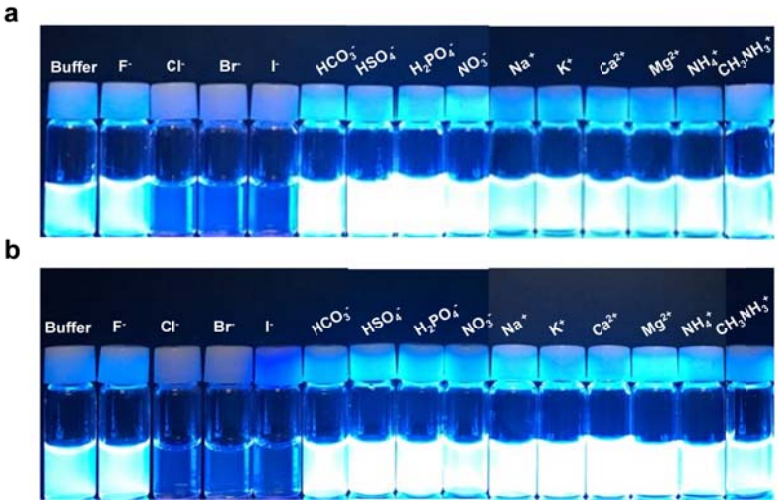


Figure S7. Visual emission of (a) 100 μM MQAE and (b) 100 μM MQAE-MP in the presence of indicated ions (50 mM) under irradiation at 365 nm using a handheld UV lamp.

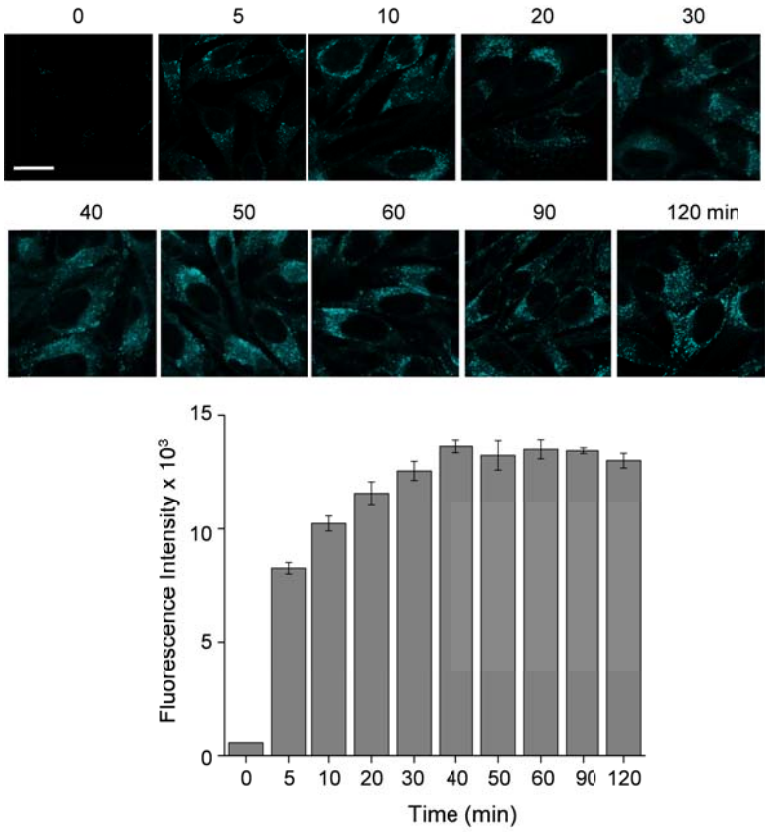


Figure S8. Time-dependent detection of lysosomal Cl^- ions using MQAE-MP. HeLa cells cultured in Cl^--deficient buffer for 6 h were treated with 5 mM MQAE-MP for indicated times. Cell images were obtained by using confocal fluorescence microscopy (scale bar: 10 μm). Graph shows fluorescence intensity of MQAE-MP in cells which were analyzed by using the ZEN software (mean ± s.d., n = 3).

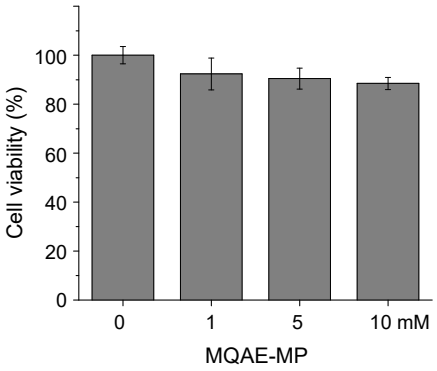


Figure S9. Effect of MQAE-MP on cytotoxicity. HeLa cells were incubated with various concentrations of MQAE-MP for 24 h. Cell viabilities were measured by using an MTT assay (mean ± s.d., n = 3)

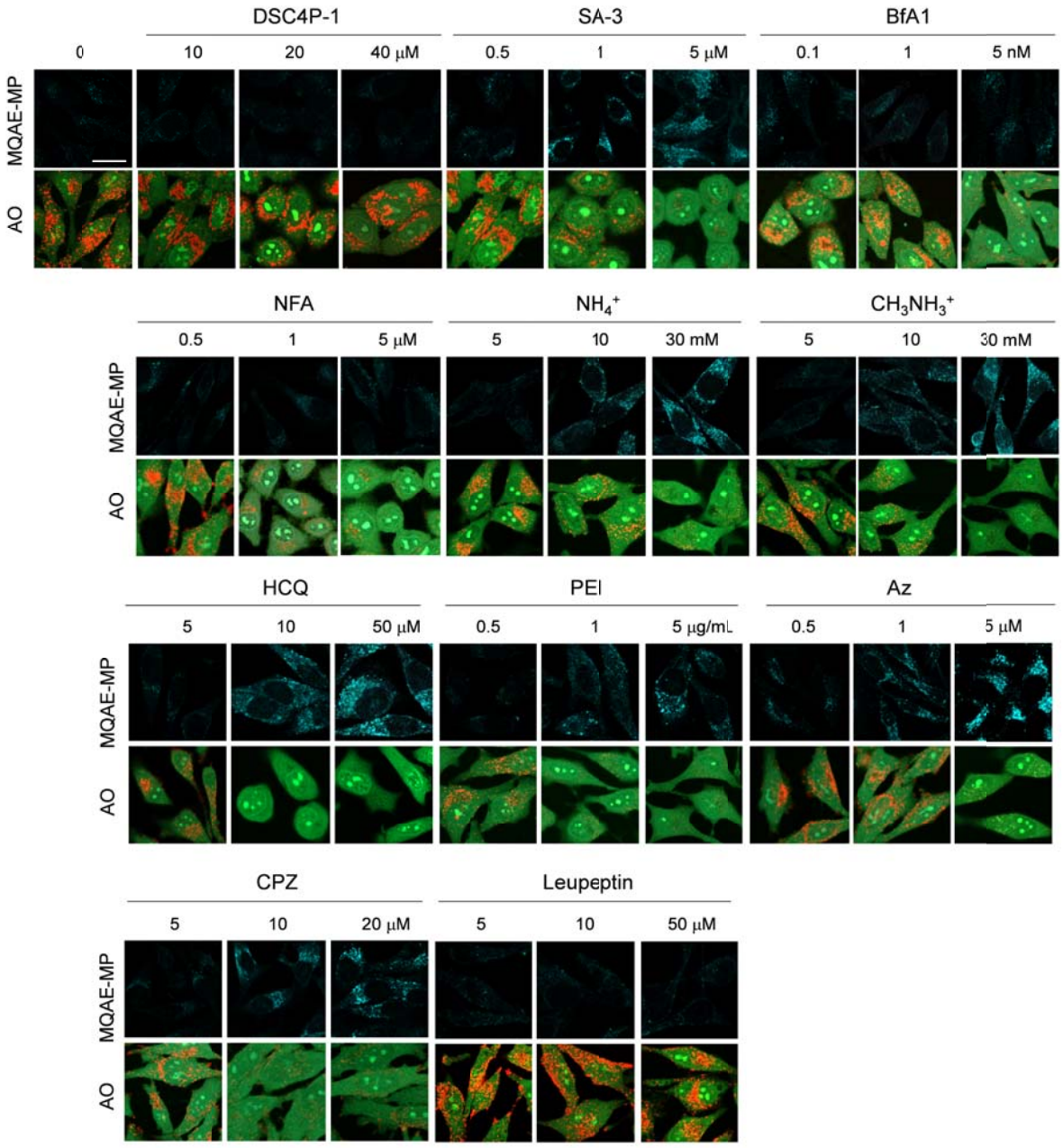


Figure S10. Detection of lysosomal chloride ions in cells treated with various substances using MQAE-MP. HeLa cells were incubated with indicated concentrations of each substance for 6 h and then treated for 30 min with 5 mM MQAE-MP or 100 nM acridine orange (AO). Cell images were obtained by using confocal fluorescence microscopy (scale bar: 10 μm).

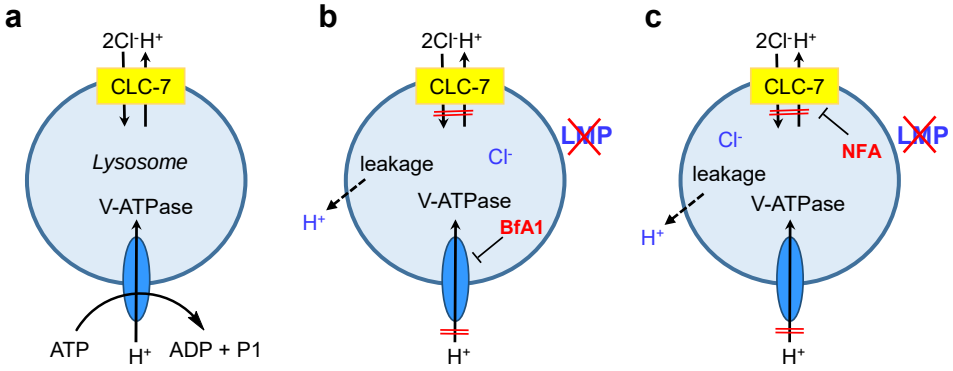


Figure S11. Schematic representation of changes in lysosomal Cl^- concentrations and pHs induced by substances. (a) Lysosomal pH is regulated by action of both proton pump V-ATPase and chloride transporter CLC-7 that maintains electroneutrality during proton pumping into lysosomes. (b) BfA1 and (c) niflumic acid do not change lysosomal Cl^- concentrations, but increase lysosomal pHs via leakage of protons from lysosomes without compensation for proton influx into lysosomes. In addition, BfA1 and niflumic acid do not induce LMP.

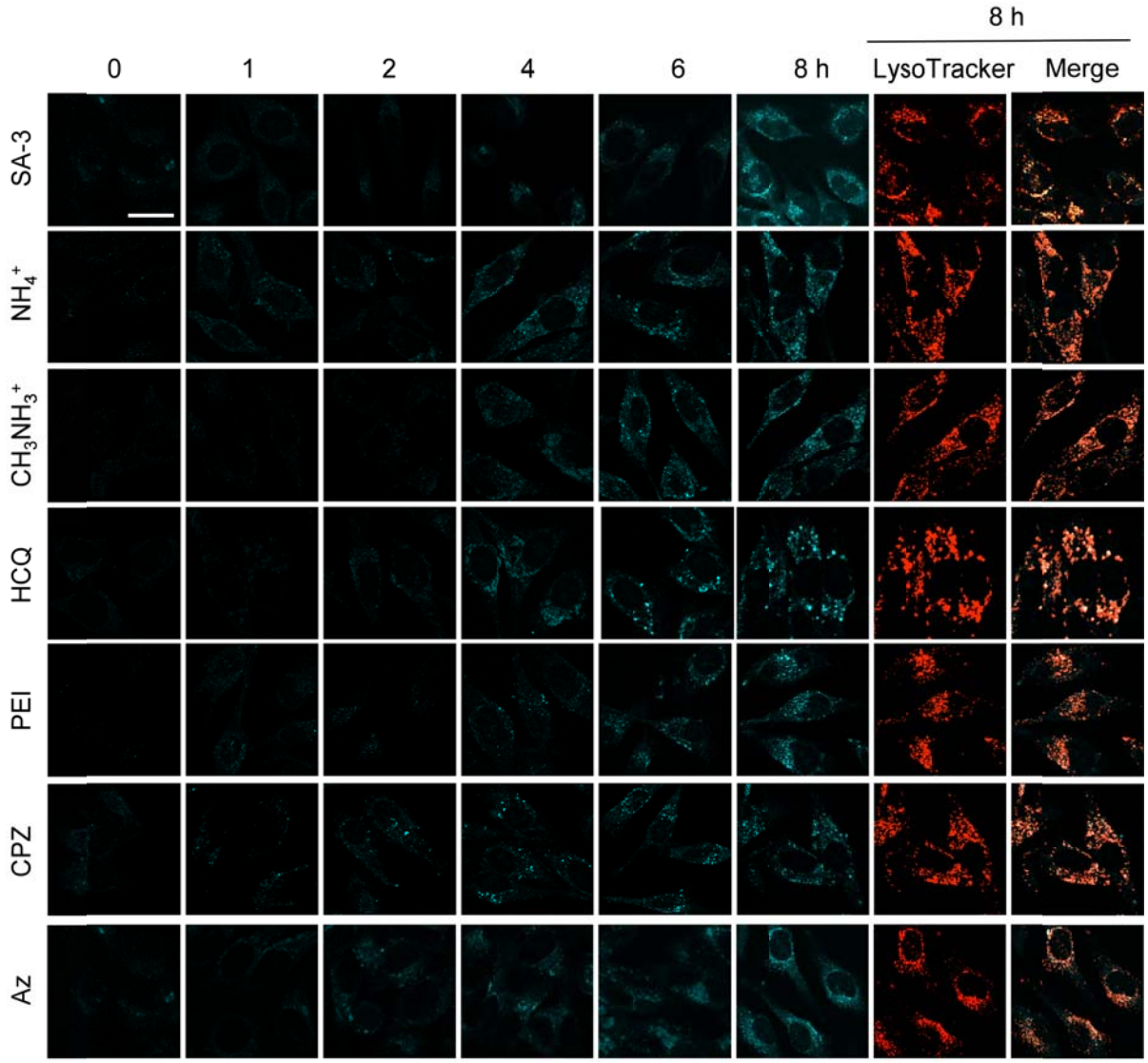


Figure S12. Time-dependent detection of lysosomal chloride ions in cells treated with various substances using MQAE-MP. HeLa cells were incubated with each compound (concentration: 5 μM SA-3, 30 mM ammonium nitrate, 30 mM methylammonium phosphate, 50 μM HCQ, 5 μg/mL PEI, 50 μM LP, 20 μM CPZ and 5 μM Az) for indicated times followed by co-treatment with 5 mM MQAE-MP and 100 nM LysoTracker red for 30 min. Cell images were obtained by using confocal fluorescence microscopy (scale bar: 10 μm). (Right) Merged cell images of fluorescence of MQAE-MP and LysoTracker red after treatment of cells with substances for 8 h.

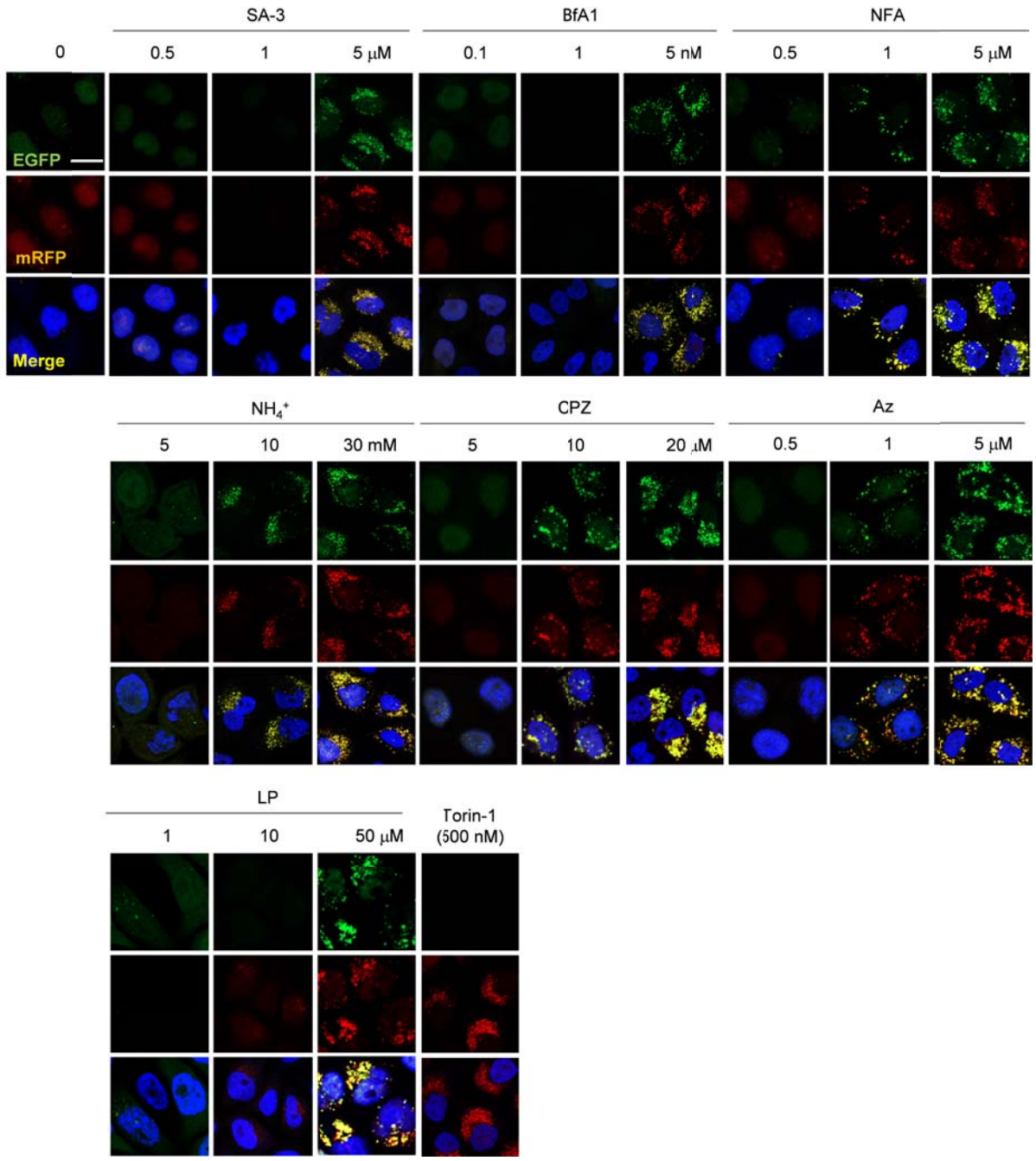


Figure S13. Effect of substances on autophagy. HeLa cells stably expressing EGFP-mRFP-LC3 were incubated with indicated concentrations of each substance for 12 h. Cell images were obtained by using confocal fluorescence microscopy (scale bar: 10 μm). Merged cell images of EGFP and mRFP fluorescence are shown in the bottom. Torin-1 was used as a control of an autophagy induction.

Explanation: It is known that pH-sensitive fluorescence of EGFP is efficiently quenched in acidic autolysosomes but the pH-insensitive fluorescence of mRFP is not.[3] On this basis, HeLa cells stably expressing mRFP-EGFP-LC3 were separately treated for 12 h with seven substances (SA-3, BfA1,

niflumic acid, NH_4^+, chlorpromazine, apoptozole and leupeptin) along with an autophagy inducer, torin-1,[1] as a control. Cell images were then obtained by using confocal fluorescence microscopy. As expected, red fluorescence puncta were seen in cells treated with torin-1. In marked contrast, treatment with each of seven substances led to a conspicuous increase in yellow fluorescence puncta in cells, which resulted from colocalization of EGFP and mRFP fluorescence. The findings indicate that the tested substances act as autophagy inhibitors.

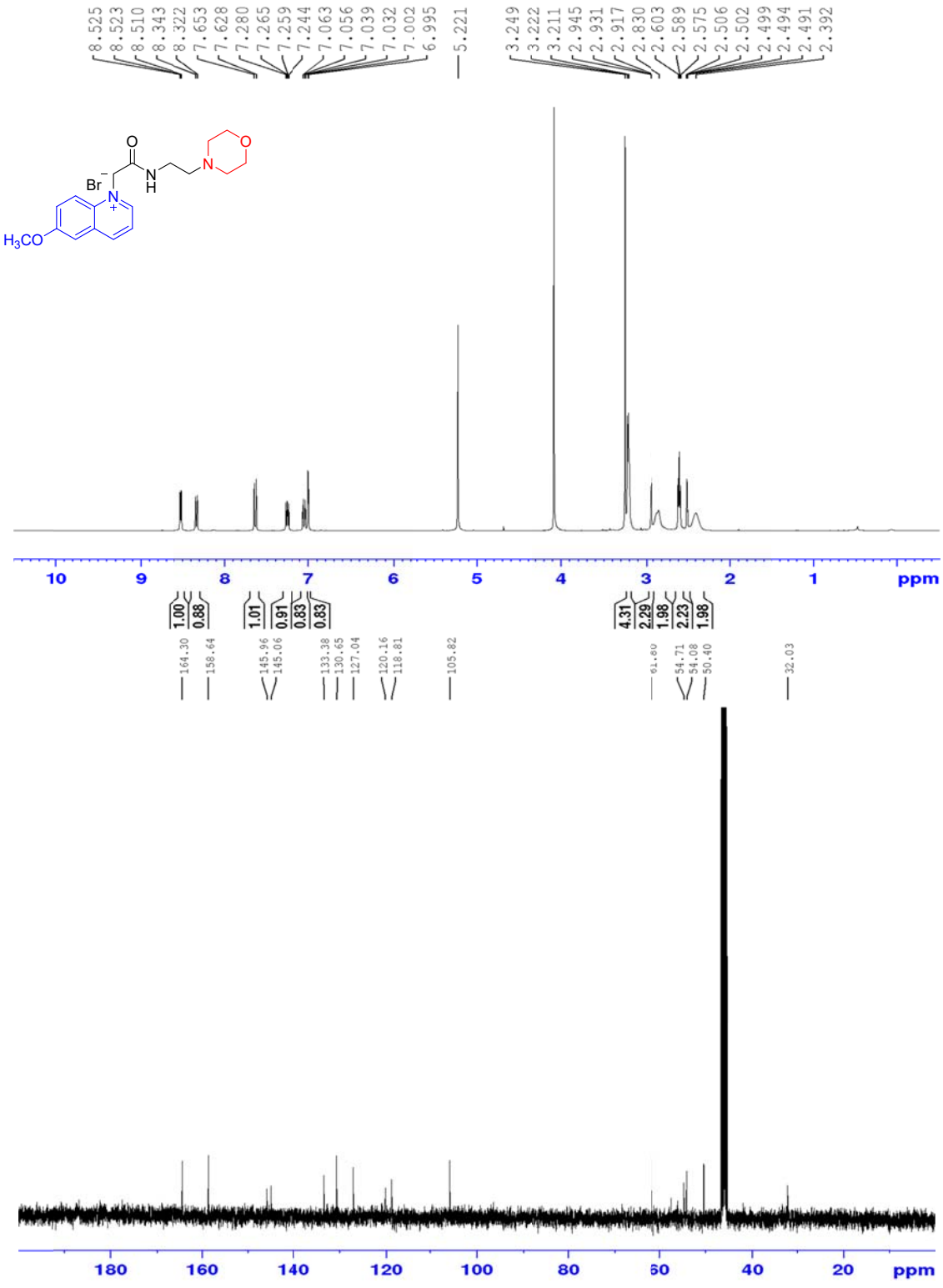
In short, SA-3,[1] which increased lysosomal pH and decreased lysosomal Cl^- concentration, inhibited the autophagy process through disruption of lysosome function. In addition, BfA1[4] and niflumic acid, which increased lysosomal pH without affecting lysosomal Cl^- concentrations, disrupted autophagy by perturbing lysosomal homeostasis. Although SA-3, BfA1 and niflumic acid did not induce LMP, they efficiently blocked the autophagy process. Ammonium ions,[5] chlorpromazine[6] and apoptozole,[7] which caused increases in lysosomal pH and decreases in lysosomal Cl^- concentrations by inducing LMP, suppressed autophagy. Leupeptin,[8] which did not affect lysosomal pH and Cl^- concentration nor induced LMP, inhibited the autophagy process. It is known that leupeptin inhibits degradation catalyzed by lysosomal proteases and causes accumulation of non-degraded proteins in lysosomes, thereby leading to disruption of the autophagy process.[9]

Supplementary References

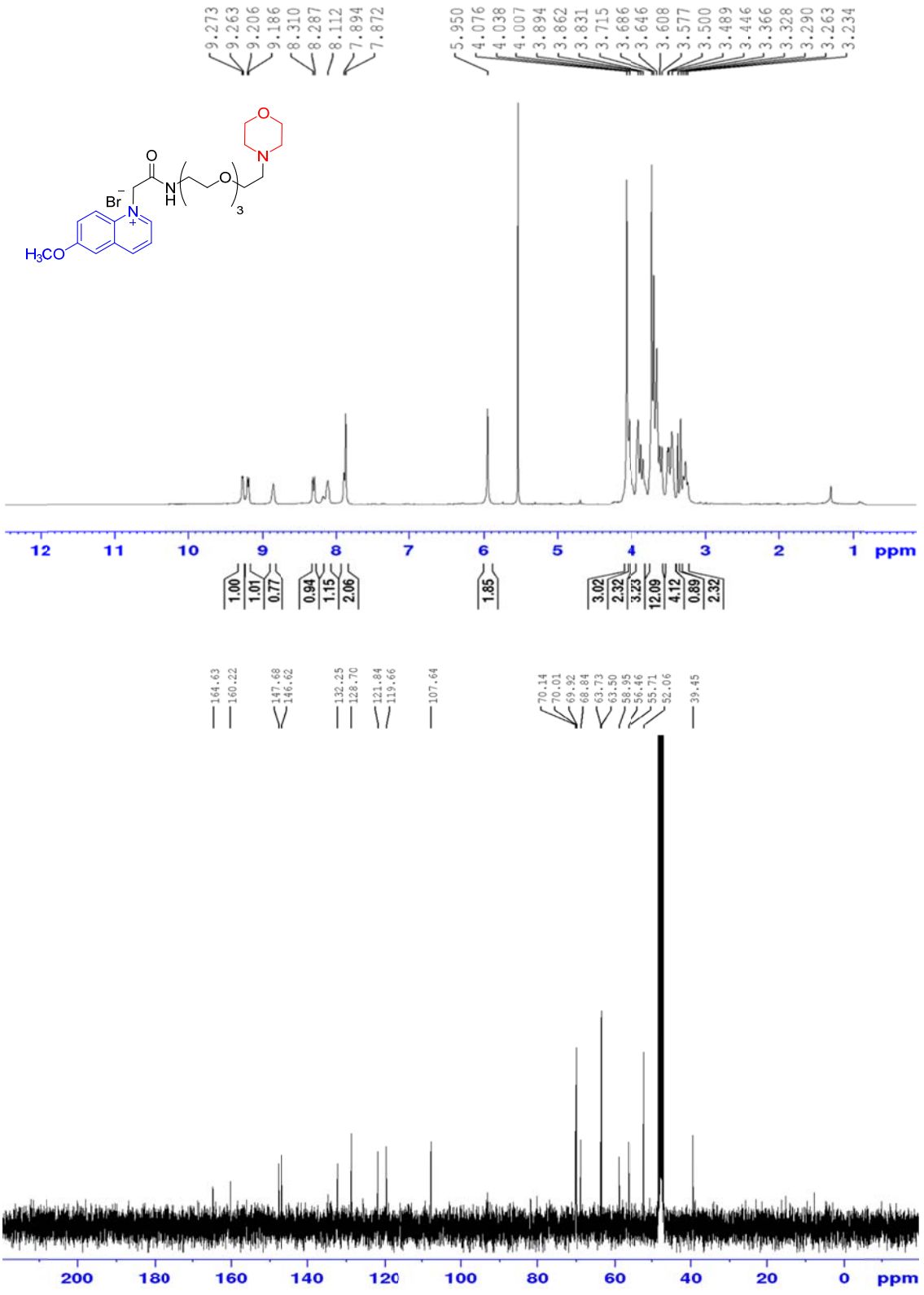
(1) N. Busschaert, S. H. Park, K. H. Baek, Y. P. Choi, J. Park, E. N. W. Howe, J. R. Hiscock, L. E. Karagiannidis, I. Marques, V. Felix, W. Namkung, J. L. Sessler, P. A. Gale and I. Shin, *Nat. Chem.*, 2017, **9**, 667.
(2) S. K. Ko, S. K. Kim, A. Share, V. M. Lynch, J. Park, W. Namkung, W. Van Rossom, N. Busschaert, P. A. Gale, J. L. Sessler and I. Shin, *Nat. Chem.*, 2014, **6**, 885.
(3) K. H. Baek, J. Park and I. Shin, *Chem. Soc. Rev.*, 2012, **41**, 3245.
(4) A. Yamamoto, Y. Tagawa, T. Yoshimori, Y. Moriyama, R. Masaki and Y. Tashiro, *Cell Struct. Funct.*, 1998, **23**, 33.
(5) P. Boya and G. Kroemer, *Oncogene*, 2008, **27**, 6434.
(6) X. Li, M. Xu, A. L. Pitzer, M. Xia, K. M. Boini, P. L. Li and Y. Zhang, *J. Mol. Med.*, 2014, **92**, 473.
(7) S. H. Park, K. H. Baek, I Shin and I. Shin, *Cell Chem. Biol.*, 2018, DOI: 10.1016/j.chembiol.2018.06.010.
(8) J. Haspel, R. S. Shaik, E. Ifedigbo, K. Nakahira, T. Dolinay, J. A. Englert and A. M. Choi, *Autophagy*, 2011, **7**, 629.
(9) J. Kopitz, G. O. Kisen, P. B. Gordon, P. Bohley and P. O. Seglen, *J. Cell Biol.*, 1990, **111**, 941.

<NMR spectra>

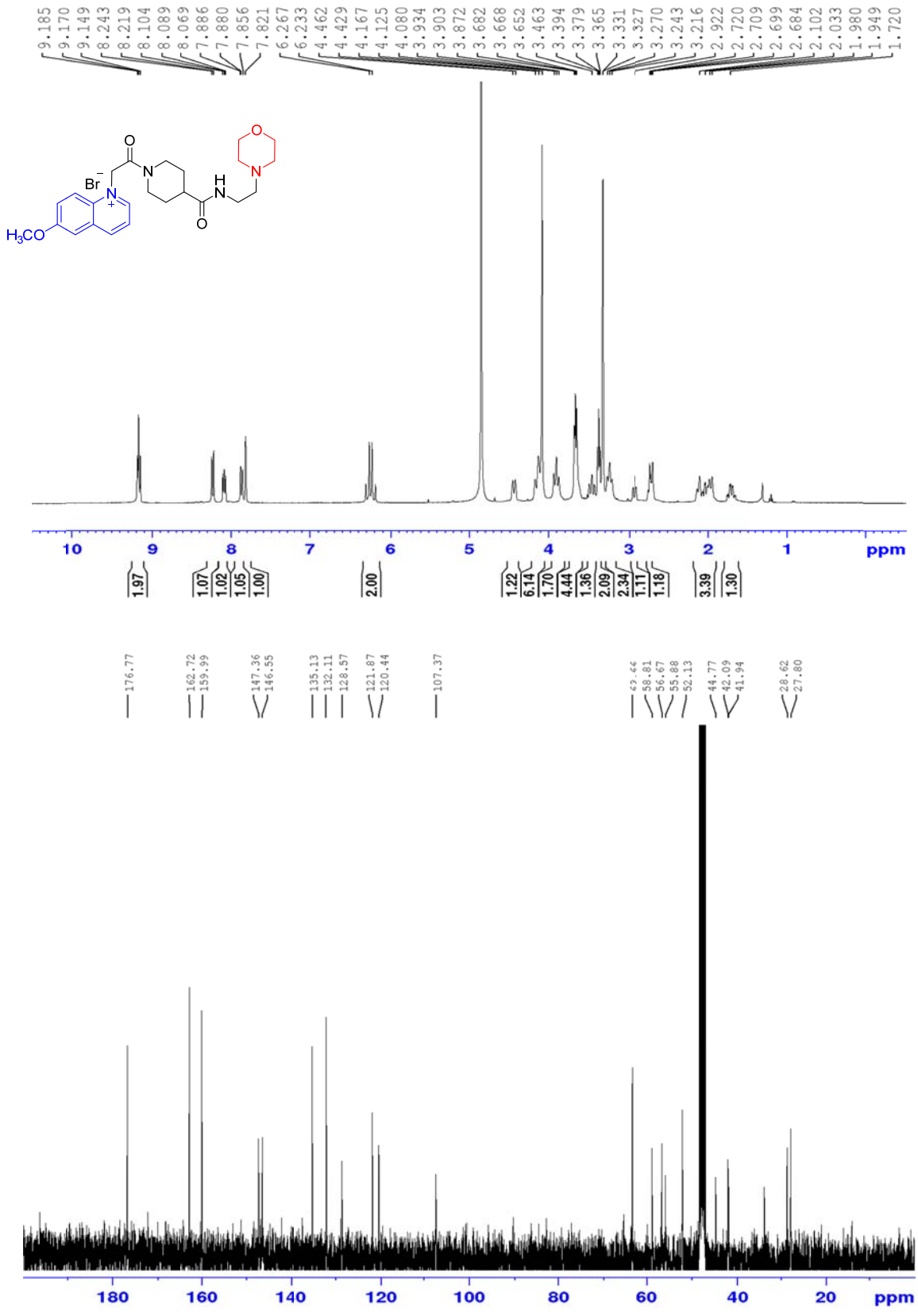
MQAE-MP-1 (DMSO-d_6 / ^{1}H: 400 MHz / ^{13}C: 100 MHz)



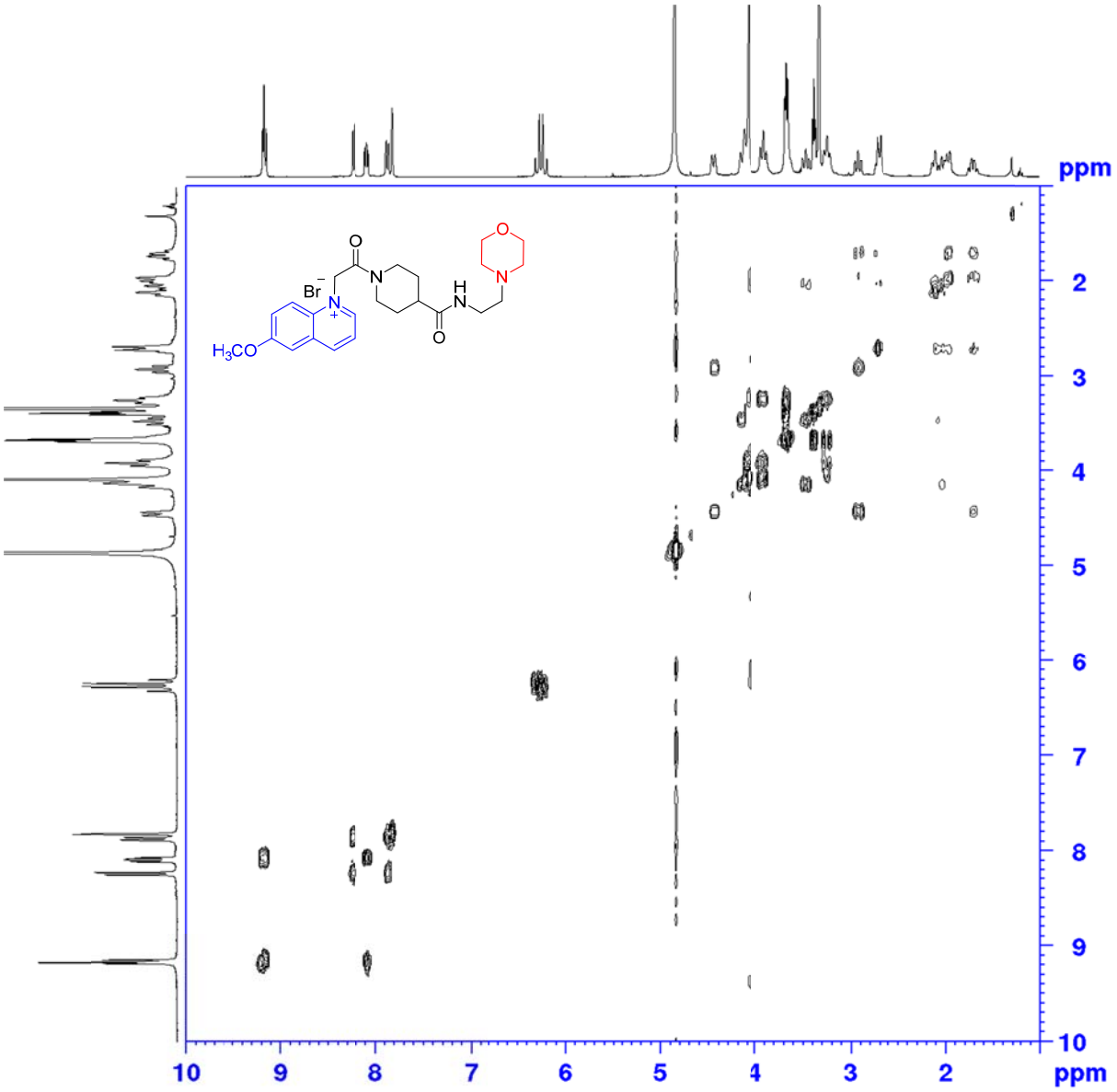
MQAE-MP-2 (CD_3OD / ^1H: 400 MHz / ^{13}C: 100 MHz)



MQAE-MP (CD_3OD / ^{1}H: 400 MHz / ^{13}C: 100 MHz)



MQAE-MP (CD_3OD / COSY: 400 MHz)



MQAE-MP (CD_3OD / HSQC: 400 MHz)