



Supporting Information

for

pH-Controlled isomerization kinetics of *ortho*-disubstituted benzamidines: *E/Z* isomerism and axial chirality

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Detailed experimental procedures, spectral data and HPLC charts

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1. General experimental methods

All reactions were performed under argon atmosphere. The weight of the starting materials and the products were not calibrated. Materials were purchased from commercial suppliers and used without further purification, unless otherwise noted. Solvents are distilled according to the standard protocol. All reactions requiring heating were conducted by using SynFlex, unless otherwise noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates. Normal-phase column chromatography and the filtration was performed on Merck silica gel 60 (63-200 μm) or Kanto Chemical Silica Gel 60N (spherical, neutral, 63-210 μm). Hi-flash column chromatography was performed on YAMAZEN Hi-FlashTM column silica gel (30 μm).

^1H NMR were measured in $\text{DMSO-}d_6$ solution and reported in parts per million (ppm) referenced to residual solvent peaks of $\text{DMSO-}d_6$ (2.50 ppm) using JEOL ECS 400 (400 MHz), JEOL ECX 400P (400 MHz), or JEOL ECZ 400 (400 MHz) spectrophotometers. ^{13}C NMR were measured in $\text{DMSO-}d_6$ solution and referenced to residual solvent peaks of $\text{DMSO-}d_6$ (39.52 ppm) using JEOL ECS 400 (100 MHz), JEOL ECX 400P (100 MHz) or JEOL ECZ 400 (100 MHz) spectrophotometers. Coupling constant (J) was reported in hertz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d; doublet, t: triplet, q: quartet, m: multiplet. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on ^1H - ^1H COSY NMR spectra and NOE spectra.

Mass spectra were obtained on Advion expression CMS or Thermo Scientific Exactive Plus. The mass analyzer type used for the HRMS measurements was TOF. Optical rotations were determined on Rudolph Research Analytical Autopol IV automatic polarimeter.

High performance liquid chromatography (HPLC) was performed on one of the following systems: (1) JASCO PU-2086 Plus as a pump, JASCO HV-2088-06 as a flow line selection valve, JASCO MD-2018 Plus as a detector, JASCO FC-2088-30 as a fraction collector controller, ADVANTEC CHF122SC as a fraction collector, JASCO LC-NetII/ADC as a hardware interface, and JASCO ChromNAV as a system controller; (2) JASCO PU-2086 Plus as a pump, JASCO UV-2075 Plus as a detector, JASCO FC-2088-30 as a fraction collector controller, ADVANTEC CHF122SC as a fraction collector, JASCO LC-NetII/ADC as a hardware interface, and JASCO ChromNAV as a system controller; (3) JASCO PU-4086-Binary as a pump, JASCO MD-4015 as a photo diode array detector, JASCO FCC as a fraction collector controller, ADVANTEC CHF122SC as a fraction collector, JASCO LC-NetII/ADC as a hardware interface, JASCO AS-4058 as a preparative autosampler and JASCO ChromNAV as a system controller; (4) JASCO PU-4086-Binary as a pump, JASCO UV4075 as a detector, JASCO FCC as a fraction collector controller, ADVANTEC CHF122SC as a fraction collector, JASCO LC-NetII/ADC as a hardware interface, JASCO AS-4058 as a preparative autosampler and JASCO ChromNAV as a system controller.

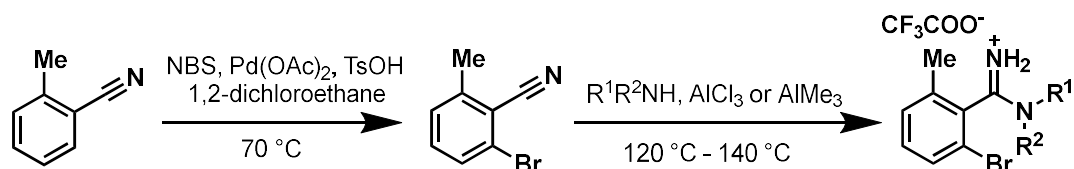
Liquid chromatography-mass spectrometry (LC-MS) was performed on either of the following systems: (1) Shimadzu LC-2050C as a HPLC system, FCV-0206 as a flow-line selection valve, LCMS-2050 as a Liquid chromatograph mass spectrometer, and LabSolutions as a system controller; (2) Shimadzu Prominence-i LC-2030C Plus as a HPLC system, FCV-20AH₂ as a flow-line selection valve, LCMS-8040 as a Liquid chromatograph mass spectrometer, and LabSolutions as a system controller.

Buffer solutions at pH 12.7 and 11.7 were prepared using aqueous solutions of Na₃PO₄ and Na₂HPO₄. A buffer solution at pH 10.3 was prepared using aqueous solutions of Na₂CO₃ and NaHCO₃. A buffer solution at pH 9.2 was prepared using aqueous solutions of NH₃ and NH₄Cl. A buffer solution at pH 6.5 was prepared using aqueous solutions of Na₂HPO₄ and NaH₂PO₄. Buffer solutions at pH 5.5, 5.0, 4.6, 4.5, and 4.0 were prepared using aqueous solutions of CH₃COONa and CH₃COOH.

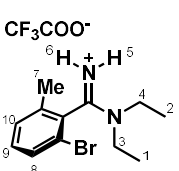
2. Preparation of compounds

All *ortho*-disubstituted benzamidines were synthesized in two steps from 2-methylbenzonitrile. 2-Bromo-6-methylbenzonitrile is a known compound reported in ref. 1.

Scheme S2.1. Synthesis of the *ortho*-disubstituted benzamidines

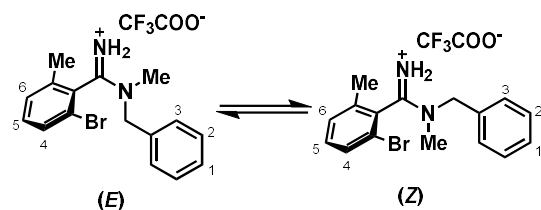


2-Bromo-*N,N*-diethyl-6-methylbenzimidamide (1) trifluoroacetate salt

 A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and diethylamine (2.19 g, 30 mmol) was treated with AlCl₃ (760 mg, 5.0 mmol) at 120 °C for 1 h under microwave irradiation. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH₂Cl₂ and 1 M *aq.* NaOH. The aqueous phase was extracted with CH₂Cl₂. The organic phase was dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The residue was purified by ODS silica gel column chromatography (φ 3.5 cm × 10 cm, 30-60-100% MeCN (+0.1% TFA)/H₂O (+0.1% TFA)) to afford **1** (332 mg, 0.87 mmol, 87%) as a trifluoroacetate salt as a yellow oil.

¹H NMR (DMSO-*d*₆, 400 MHz) δ 9.62 (s, 1H, H-5), 9.35 (s, 1H, H-6), 7.71-7.67 (m, 1H, H-8), 7.49-7.47 (m, 2H, H-9, H-10), 3.73 (dq, 1H, H-4, *J*_{gem} = 14.4, *J*_{4,2} = 7.2 Hz), 3.66 (dq, 1H, H-4, *J*_{gem} = 14.4, *J*_{4,2} = 7.2 Hz), 3.21 (dq, 1H, H-3, *J*_{gem} = 14.4, *J*_{3,1} = 7.2 Hz), 3.16 (dq, 1H, H-3, *J*_{gem} = 14.4, *J*_{3,1} = 7.2 Hz), 2.29 (s, 3H, H-7), 1.28 (dd, 3H, H-2, *J*_{2,4} = *J*_{2,4} = 7.2 Hz), 1.08 (dd, 3H, H-1, *J*_{1,3} = *J*_{1,3} = 7.2 Hz); ¹³C NMR (DMSO-*d*₆, 100 MHz) δ 161.2, 158.2, 157.9, 137.8, 132.8, 130.5, 130.4, 130.1, 120.0, 117.9, 114.9, 46.2, 42.3, 18.9, 12.6, 10.6; ESIMS-LR *m/z* 269.1 [(M+H)⁺]; ESIMS-HR calcd. for C₁₂H₁₈N₂Br 269.0648, found 269.0648.

N-Benzyl-2-bromo-*N*,6-dimethylbenzimidamide (2) trifluoroacetate salt

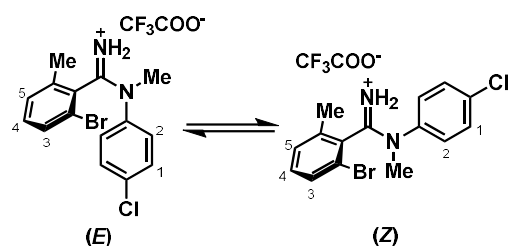


A mixture of 2-bromo-6-methylbenzonitrile (392 mg, 2.0 mmol) and *N*-methylbenzylamine (266 mg, 2.2 mmol) was treated with AlCl₃ (266 mg, 2.0 mmol) at 140 °C for 15 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH₂Cl₂ and 1 M *aq.* NaOH. The aqueous phase was extracted with CH₂Cl₂. The organic phase was dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The residue was purified by ODS silica gel column chromatography (φ 3.5 cm × 10 cm, 30-60-100% MeCN (+0.1% TFA)/H₂O (+0.1% TFA)) to afford **2** (239 mg, 0.75 mmol, 38%) as a trifluoroacetate salt as a yellow oil.

¹H NMR (DMSO-*d*₆, 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-2**: δ 9.95 (s, 1H, *N*-H), 9.84 (s, 1H, *N*-H), 7.70 (d, 1H, H-4, *J*_{4,5} = 9.0 Hz), 7.51-7.41 (m, 5H, H-1, H-2, H-5, H-6), 7.39-7.31 (m, 2H, H-3), 5.03 (d, 1H,

N-CH₂Ph, $J_{gem} = 15.5$ Hz), 4.88 (d, 1H, *N*-CH₂Ph, $J_{gem} = 15.5$ Hz), 2.80 (s, 3H, *N*-Me), 2.30 (s, 3H, *C*-Me). Selected data for **E-2**: δ 9.90 (s, 1H, *N*-H), 9.54 (s, 1H, *N*-H), 7.71 (d, 1H, H-4, $J_{4,5} = 7.2$ Hz), 7.51-7.41 (m, 3H, H-1, H-5, H-6), 7.39-3.31 (m, 2H, H-2), 7.16 (dd, 2H, H-3 $J_{3,2} = 7.4$, $J_{3,1} = 2.0$ Hz), 4.48 (d, 1H, *N*-CH₂Ph, $J_{gem} = 15.5$ Hz), 4.42 (d, 1H, *N*-CH₂Ph, $J_{gem} = 15.5$ Hz), 3.27 (s, 3H, *N*-Me), 2.20 (s, 3H, *C*-Me); ¹³C NMR (DMSO-*d*₆, 100 MHz, a mixture of *E/Z* isomers) δ 163.2, 163.0, 158.2, 157.8, 138.6, 137.7, 133.7, 133.3, 133.0, 132.9, 130.6, 130.6, 130.2, 130.1, 130.0, 129.0, 128.8, 128.5, 128.4, 128.2, 120.3, 119.6, 118.0, 115.1, 56.1, 53.2, 38.1, 36.9, 19.1, 18.9; ESIMS-LR m/z 317.1 [(M+H)⁺]; ESIMS-HR calcd. for C₁₆H₁₈N₂Br 317.0648, found 317.0641.

2-Bromo-*N*-(4-chlorophenyl)-*N*,6-dimethylbenzimidamide (**3**) trifluoroacetate salt

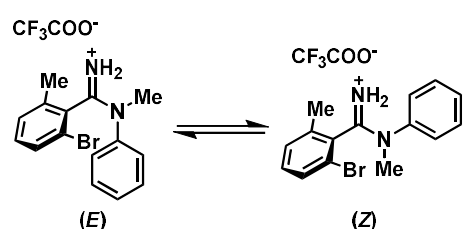


A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and 4-chloro-*N*-methylaniline (156 mg, 1.1 mmol) was treated with AlCl₃ (133 mg, 1.0 mmol) at 140 °C for 3.5 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH₂Cl₂ and 1 M *aq.* NaOH. The aqueous phase was extracted with CH₂Cl₂. The organic phase was dried (Na₂SO₄),

filtered, and concentrated *in vacuo*. The residue was purified by ODS silica gel column chromatography (ϕ 3.5 cm $\times$ 10 cm, 30-60-100% MeCN (+0.1% TFA)/H₂O (+0.1% TFA)) to afford **3** (388 mg, 0.86 mmol, 86%) as a trifluoroacetate salt as a brown oil.

¹H NMR (DMSO-*d*₆, 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-3**: δ 10.03 (s, 1H, *N*-H), 9.10 (s, 1H, *N*-H), 7.79-7.73 (m, 3H, Ar), 7.67-7.63 (m, 2H, Ar), 7.56-7.51 (m, 2H, Ar), 3.17 (s, 3H, *N*-Me), 2.46 (s, 3H, *C*-Me). Selected data for **E-3**: δ 10.37 (s, 1H, *N*-H), 10.01 (s, 1H, *N*-H), 7.50-7.45 (m, 1H, Ar), 7.41 (s, 4H, Ar), 7.30-7.25 (m, 2H, Ar), 3.64 (s, 3H, *N*-Me), 2.36 (s, 3H, *C*-Me); ¹³C NMR (DMSO-*d*₆, 100 MHz, a mixture of *E/Z* isomers) δ 163.9, 163.1, 158.1, 157.8, 140.4, 138.3, 138.1, 137.9, 134.9, 133.6, 133.2, 132.7, 131.2, 130.5, 130.4, 130.2, 130.1, 129.7, 129.6, 129.3, 128.1, 127.2, 120.3, 119.8, 118.1, 115.1, 42.0, 41.2, 19.6, 19.0; ESIMS-LR m/z 337.0 [(M+H)⁺]; ESIMS-HR calcd. for C₁₅H₁₅N₂BrCl 337.0102, found 337.0097.

2-Bromo-*N*,6-dimethyl-*N*-phenylbenzimidamide (**4**) trifluoroacetate salt



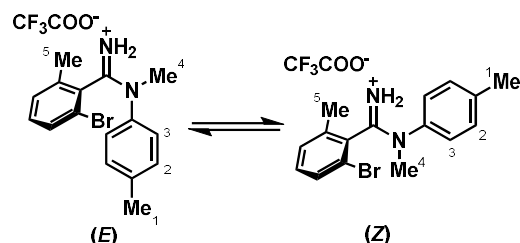
A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and *N*-methylaniline (118 mg, 1.1 mmol) was treated with AlCl₃ (133 mg, 1.0 mmol) at 140 °C for 16 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH₂Cl₂ and 1 M *aq.* NaOH. The aqueous phase was extracted with CH₂Cl₂. The organic phase was dried (Na₂SO₄), filtered, and concentrated *in vacuo*. The residue was

purified by ODS silica gel column chromatography (ϕ 3.5 cm $\times$ 10 cm, 30-60-100% MeCN (+0.1% TFA)/H₂O (+0.1% TFA)) to afford **4** (376 mg, 0.90 mmol, 90%) as a trifluoroacetate salt as a black oil.

¹H NMR (DMSO-*d*₆, 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-4**: δ 9.94 (s, 1H, *N*-H), 8.96 (s, 1H, *N*-H), 7.78-7.21 (m, 8H, Ar), 3.18 (s, 3H, *N*-Me), 2.48 (s, 3H, *C*-Me). Selected data for **E-4**: δ 10.27 (s, 1H, *N*-H),

9.90 (s, 1H, *N*-H), 7.78-7.21 (m, 8H, Ar), 3.66 (s, 3H, *N*-Me), 2.36 (s, 3H, *C*-Me); ^{13}C NMR (DMSO- d_6 , 100 MHz, a mixture of *E/Z* isomers) δ 163.5, 163.0, 158.2, 157.9, 141.6, 139.1, 138.2, 138.0, 133.1, 132.6, 131.2, 130.7, 130.5, 130.3, 130.0, 129.8, 129.6, 129.3, 129.2, 125.8, 125.1, 120.5, 119.9, 117.8, 114.9, 42.2, 41.3, 19.7, 19.0; ESIMS-LR m/z 303.1 [(*M*+*H*) $^+$]; ESIMS-HR calcd. for $\text{C}_{15}\text{H}_{16}\text{N}_2\text{Br}$ 303.0491, found 303.0488.

2-Bromo-*N*,6-dimethyl-*N*-(4-tolyl)benzimidamide (5) trifluoroacetate salt

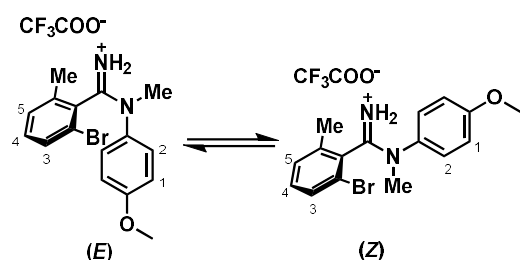


A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and *N*,4-dimethylaniline (133 mg, 1.1 mmol) was treated with AlCl_3 (133 mg, 1.0 mmol) at 140 °C for 6 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH_2Cl_2 and 1 M *aq.* NaOH. The aqueous phase was extracted with CH_2Cl_2 . The organic phase was dried (Na_2SO_4), filtered, and

concentrated *in vacuo*. The residue was purified by ODS silica gel column chromatography (ϕ 3.5 cm $\times$ 10 cm, 30-60-100% MeCN (+0.1% TFA)/ H_2O (+0.1% TFA)) to afford **5** (163 mg, 0.76 mmol, 76%) as a trifluoroacetate salt as a brown oil.

^1H NMR (DMSO- d_6 , 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-5**: δ 10.22 (s, 1H, *N*-H), 9.86 (s, 1H, *N*-H), 7.76-7.74 (m, 1H, Ar), 7.57-7.44 (m, 4H, Ar), 7.30-7.22 (m, 2H, Ar), 3.15 (s, 3H, H-4), 2.46 (s, 3H, H-1), 2.42 (s, 3H, H-5). Selected data for **E-5**: δ 9.90 (s, 1H, *N*-H), 8.90 (s, 1H, *N*-H), 7.57-7.44 (m, 2H, Ar) 7.30-7.22 (m, 3H, Ar), 7.11 (d, 2H, H-2, $J_{2,3} = 8.2$ Hz), 3.62 (s, 3H, H-4), 2.35 (s, 3H, H-5), 2.20 (s, 3H, H-1); ^{13}C NMR (DMSO- d_6 , 100 MHz, a mixture of *E/Z* isomers) δ 163.6, 163.0, 158.2, 157.9, 139.9, 139.3, 138.8, 138.1, 138.0, 136.6, 133.1, 132.5, 131.6, 130.8, 130.5, 130.2, 130.1, 129.8, 129.7, 129.6, 125.5, 124.8, 120.5, 119.9, 117.9, 114.9, 42.2, 41.4, 20.8, 20.5, 19.7, 19.0; ESIMS-LR m/z 317.1 [(*M*+*H*) $^+$]; ESIMS-HR calcd. for $\text{C}_{16}\text{H}_{18}\text{N}_2\text{Br}$ 317.0648, found 317.0644.

2-Bromo-*N*-(4-methoxyphenyl)-*N*,6-dimethylbenzimidamide (6) trifluoroacetate salt



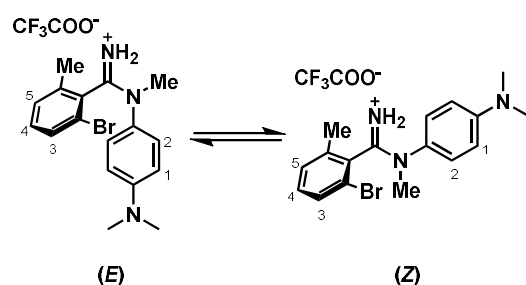
A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and 4-methoxy-*N*-methylaniline (151 mg, 1.1 mmol) was treated with AlMe_3 (1.4 M in hexane, 2 mL, 2.8 mmol) at 140 °C for 3 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH_2Cl_2 and 1 M *aq.* NaOH. The aqueous phase was extracted with CH_2Cl_2 . The organic phase was

dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified by ODS silica gel column chromatography (ϕ 3.5 cm $\times$ 10 cm, 30-60-100% MeCN (+0.1% TFA)/ H_2O (+0.1% TFA)) to afford **6** (140 mg, 0.31 mmol, 31%) as a trifluoroacetate salt as a brown oil.

^1H NMR (DMSO- d_6 , 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-6**: δ 9.87 (s, 1H, *N*-H), 8.88 (s, 1H, *N*-H), 7.75 (dd, 1H, H-3, $J_{3,5} = 1.8$, $J_{3,4} = 7.2$ Hz), 7.53 (dd, 1H, H-4, $J_{4,3} = J_{4,5} = 7.2$ Hz), 7.51 (dd, 1H, H-5, $J_{5,3} = 1.8$ Hz, $J_{5,4} = 7.2$ Hz), 7.51 (d, 2H, H-2, $J_{2,1} = 9.0$ Hz), 7.22 (d, 2H, H-1, $J_{1,2} = 9.0$ Hz), 3.85 (s, 3H, *O*-Me), 3.14 (s, 3H, *N*-Me), 2.46 (s, 3H, *C*-Me). Selected data for **E-6**: δ 10.18 (s, 1H, *N*-H), 9.81 (s, 1H, *N*-H), 7.46 (dd, 1H, H-3, $J_{3,5} =$

2.5, $J_{3,4} = 6.5$ Hz), 7.32 (d, 2H, H-2, $J_{2,1} = 9.0$ Hz). 7.27-7.24 (m, 2H, Ar), 6.84 (d, 2H, H-1, $J_{1,2} = 9.0$ Hz), 3.67 (s, 3H, *O*-Me), 3.61 (s, 3H, *N*-Me), 2.35 (s, 3H, *C*-Me); ^{13}C NMR (DMSO- d_6 , 100 MHz, a mixture of *E/Z* isomers) δ 163.8, 163.2, 160.1, 159.2, 158.3, 158.0, 138.0, 133.1, 132.5, 131.6, 130.5, 130.2, 130.1, 129.9, 129.6, 127.1, 126.3, 120.4, 119.9, 116.2, 114.3, 55.7, 55.4, 42.3, 41.6, 19.7, 19.0; ESIMS-LR m/z 333.1 [(*M*+*H*) $^+$]; ESIMS-HR calcd. for $\text{C}_{16}\text{H}_{18}\text{ON}_2\text{Br}$ 333.0597, found 333.0592.

2-Bromo-*N*-(4-(dimethylamino)phenyl)-*N*,6-dimethylbenzimidamide (**7**) trifluoroacetate salt



A mixture of 2-bromo-6-methylbenzonitrile (196 mg, 1.0 mmol) and *N*¹,*N*¹,*N*⁴-trimethylbenzene-1,4-diamine (165 mg, 1.1 mmol) was treated with AlCl_3 (133 mg, 1.0 mmol) at 140 °C for 14 h. The reaction was quenched with 1 M *aq.* NaOH (5 mL), and the mixture was partitioned between CH_2Cl_2 and 1 M *aq.* NaOH. The aqueous phase was extracted with CH_2Cl_2 . The organic phase was dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was

purified by ODS silica gel column chromatography (ϕ 3.5 cm $\times$ 10 cm, 30-60-100% MeCN (+0.1% TFA)/ H_2O (+0.1% TFA)) to afford **7** (269 mg, 0.58 mmol, 58%) as a trifluoroacetate salt as a purple oil.

^1H NMR (DMSO- d_6 , 400 MHz, a mixture of *E/Z* isomers). Selected data for **Z-7**: δ 9.77 (s, 1H, *N*-H), 8.76 (s, 1H, *N*-H), 7.74 (dd, 1H, H-3, $J_{\text{vic}} = 7.2$, $J_{\text{vic}} = 1.8$ Hz), 7.54-7.51 (m, 2H, H-4, H-5), 7.34 (d, 2H, H-2, $J_{2,1} = 9.0$ Hz), 6.92 (d, 2H, H-1, $J_{1,2} = 9.0$ Hz), 3.11 (s, 3H, *N*-Me), 3.00 (s, 6H, *N*-Me₂), 2.45 (s, 3H, *C*-Me). Selected data for **E-7**: δ 10.04 (s, 1H, *N*-H), 9.68 (s, 1H, *N*-H), 7.49-7.46 (m, 1H, H-3), 7.25-7.22 (m, 2H, H-4, H-5), 7.17 (d, 2H, H-2, $J_{2,1} = 9.2$ Hz), 6.53 (d, 2H, H-1, $J_{1,2} = 9.2$ Hz), 3.57 (s, 3H, *N*-Me), 2.82 (s, 6H, *N*-Me₂), 2.34 (s, 3H, *C*-Me); ^{13}C NMR (DMSO- d_6 , 100 MHz, a mixture of *E/Z* isomers) δ 163.7, 163.1, 158.4, 158.0, 150.9, 149.9, 137.9, 133.0, 132.3, 131.2, 130.5, 130.2, 130.2, 130.0, 129.5, 127.0, 126.0, 125.3, 120.5, 119.9, 117.3, 114.4, 113.5, 111.5, 42.4, 41.7, 19.7, 19.0; ESIMS-LR m/z 346.1 [(*M*+*H*) $^+$]; ESIMS-HR calcd. for $\text{C}_{17}\text{H}_{21}\text{N}_3\text{Br}$ 346.0913, found 346.0907.

3. Kinetic study using vt-NMR method

Variable-temperature (vt) ^1H NMR experiments were carried out to prove that protonated amidine has a higher activation energy of *E/Z* isomerization than neutral form of amidine. DMSO- d_6 was used as the solvent. In the case of neutral form, spectrum varied drastically with temperature. Then, focusing on the peaks of the two terminal methyl groups, the activation energy of *E/Z* isomerization was determined using the following equation (eq. 1). The coalescence temperature was 378 K and the difference in chemical shifts at 298 K was 88.4 Hz. From these values, the activation energy in DMSO- d_6 was determined to be 76.6 kJ·mol $^{-1}$. On the other hand, spectrum of protonated amidine showed little change with temperature.

$$\Delta G^\ddagger = RT_c \left[22.96 + \ln \left(\frac{T_c}{\Delta \nu} \right) \right] \quad (\text{eq. 1})$$

T_c = coalescence temperature (K), $\Delta \nu$ = difference in chemical shifts in hertz ($\delta_A - \delta_B$)

$R = 8.314 \text{ J} \cdot \text{K}^{-1} \text{ mol}^{-1}$, $\Delta G^\ddagger$ = activation energy

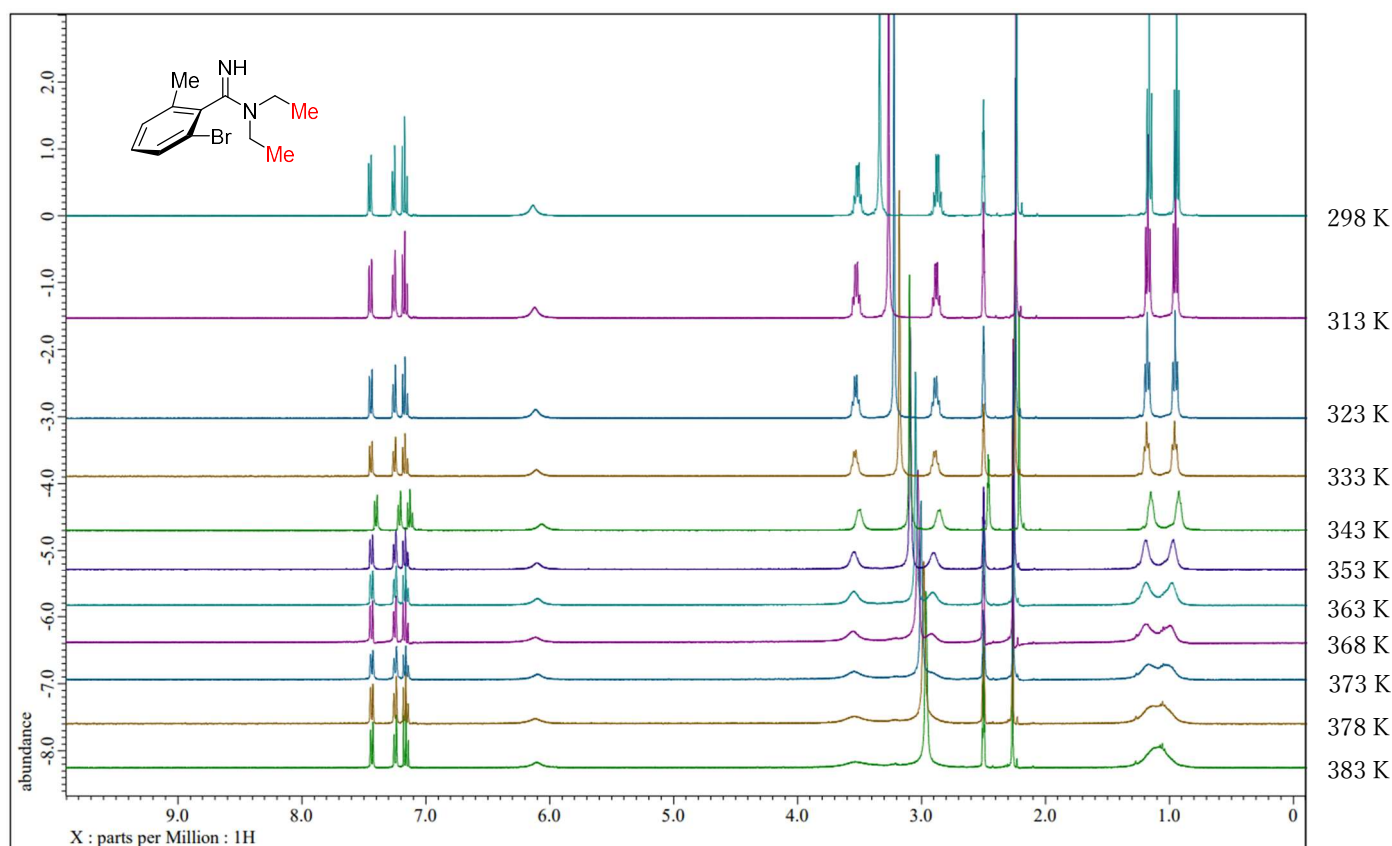


Figure S3.1. Overall view of vt- ^1H NMR spectrum of the neutral form of **1**

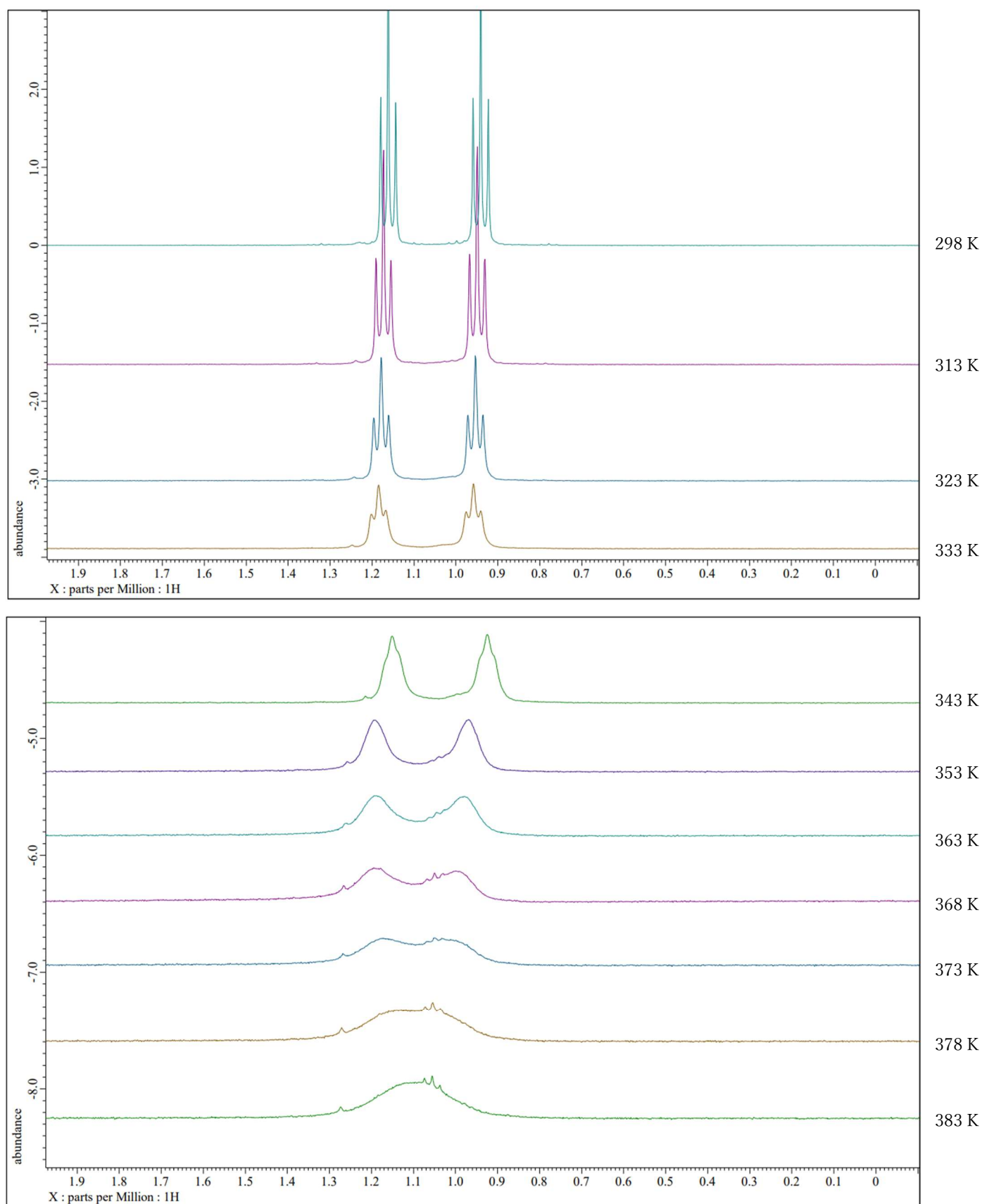


Figure S3.2. Enlarged view of vt- ^1H NMR spectrum of neutral form of **1**

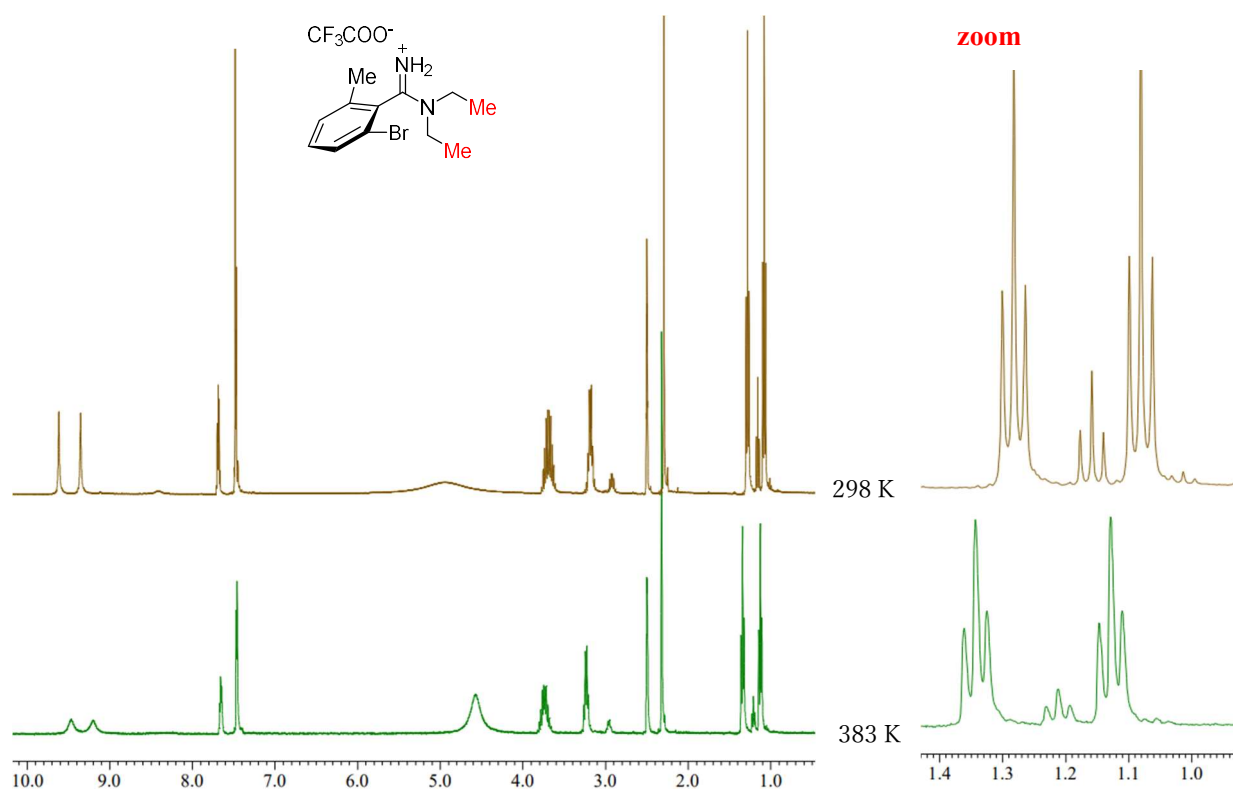


Figure S3.3. Overall and enlarged view of vt-¹H NMR spectrum of trifluoroacetate salt of **1**

4. Separation of the *E/Z* isomers

A *E/Z* mixture of synthesized amidines was purified by RP-HPLC. The purity of the isomers was determined by LC-MS. The Purification by RP-HPLC was performed under the following conditions. Since the eluent contained trifluoroacetic acid, each amidine was observed as its trifluoroacetate salt in the HPLC chart shown in Figure S4.1-4.6.

Conditions for compound 2

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm).

Conditions for compound 3

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 32% B in A isocratic elution, detection: UV (254 nm).

Conditions for compound 4

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm).

Conditions for compound 5

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm).

Conditions for compound 6

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm).

Conditions for compound 7

column: COSMOSIL Packed Column 5C₁₈-AR-II 20 mmID × 250 mm, flow rate: 8.0 mL/min, eluent: A; 0.1% trifluoro acetic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm).

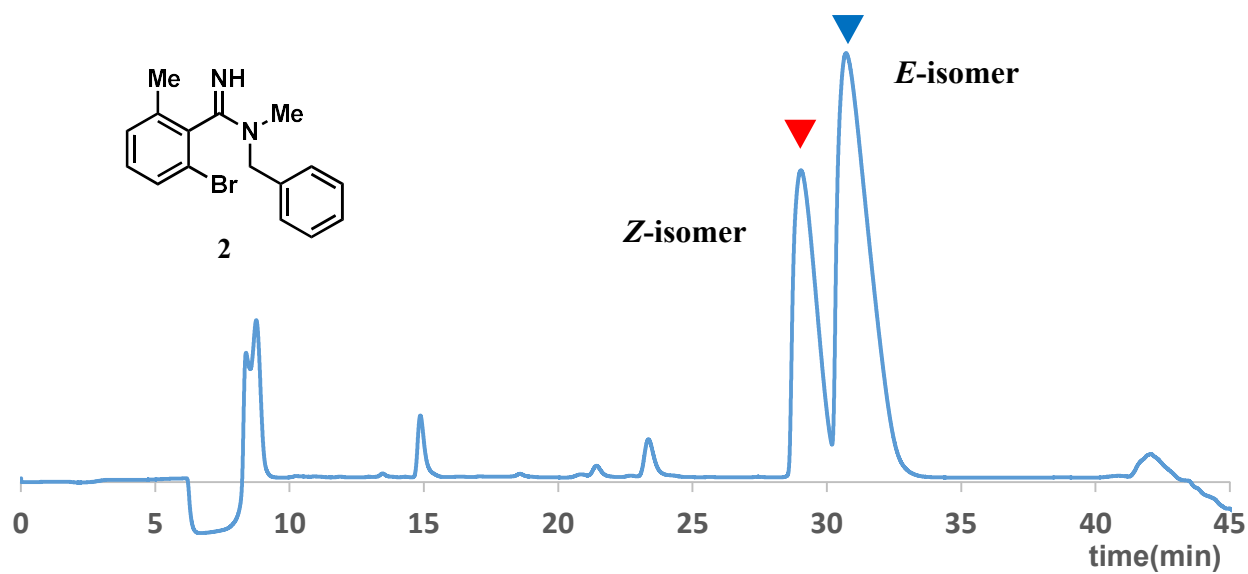


Figure S4.1. HPLC chart of *E*- and *Z*- isomer of amide 2

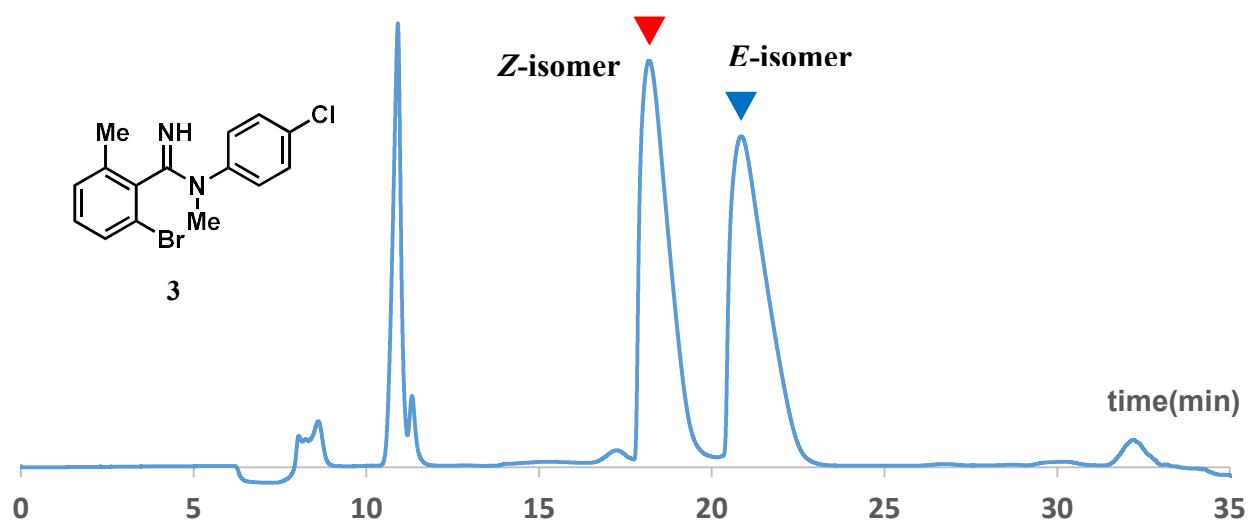


Figure S4.2. HPLC chart of *E*- and *Z*- isomer of amide 3

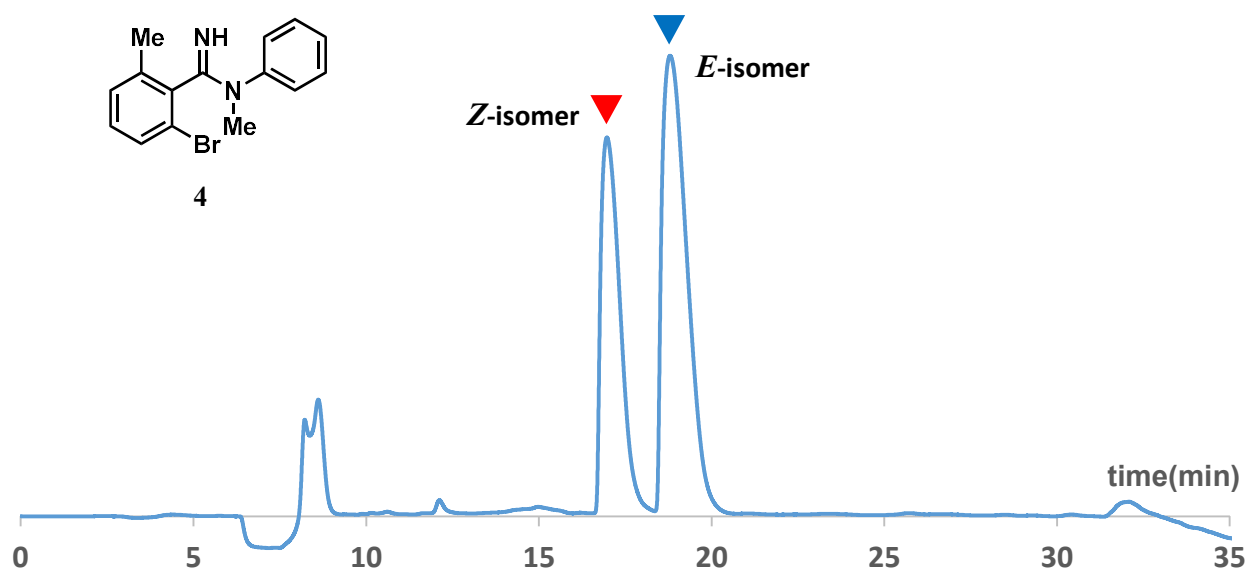


Figure S4.3. HPLC chart of *E*- and *Z*- isomer of amide 4

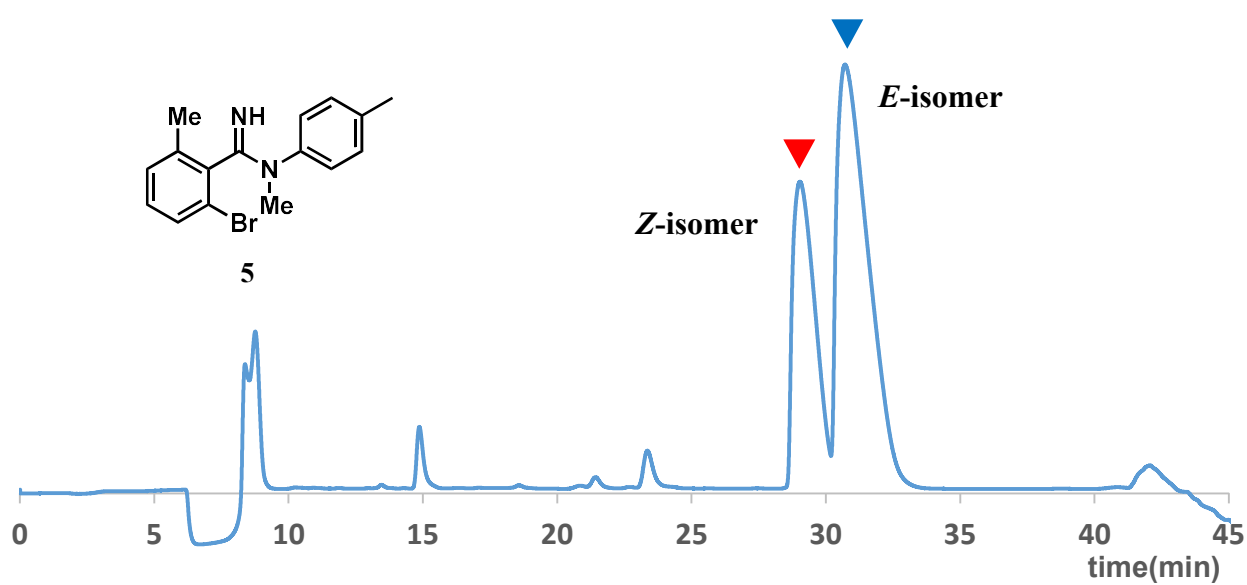


Figure S4.4. HPLC chart of *E*- and *Z*- isomer of amide 5

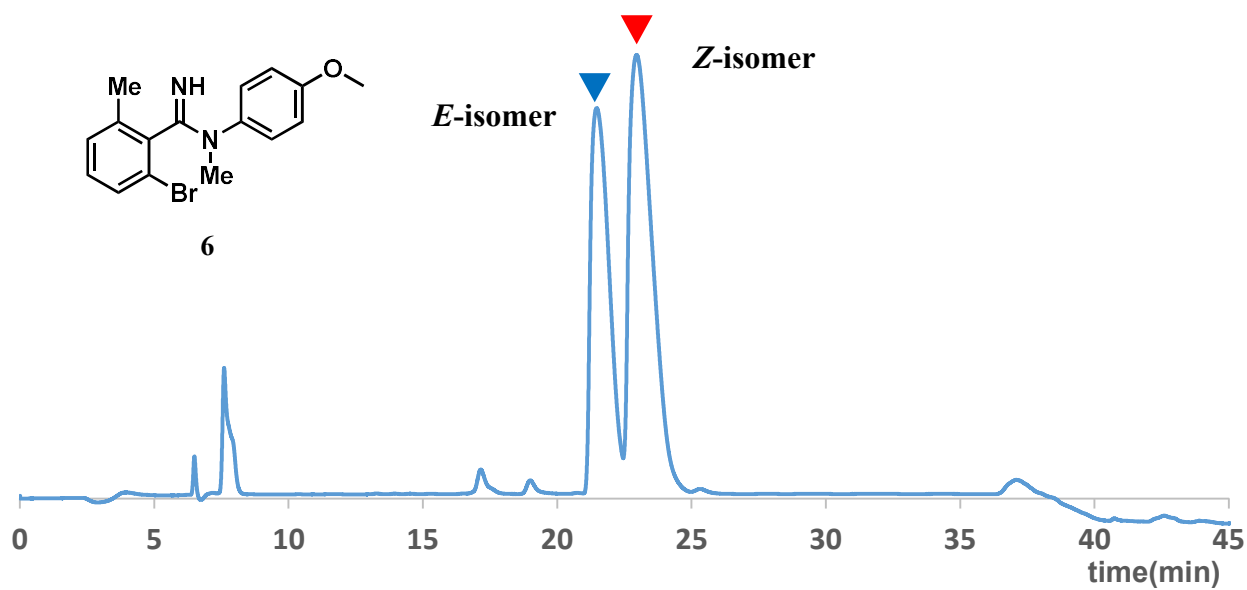


Figure S4.5. HPLC chart of *E*- and *Z*- isomer of amide 6

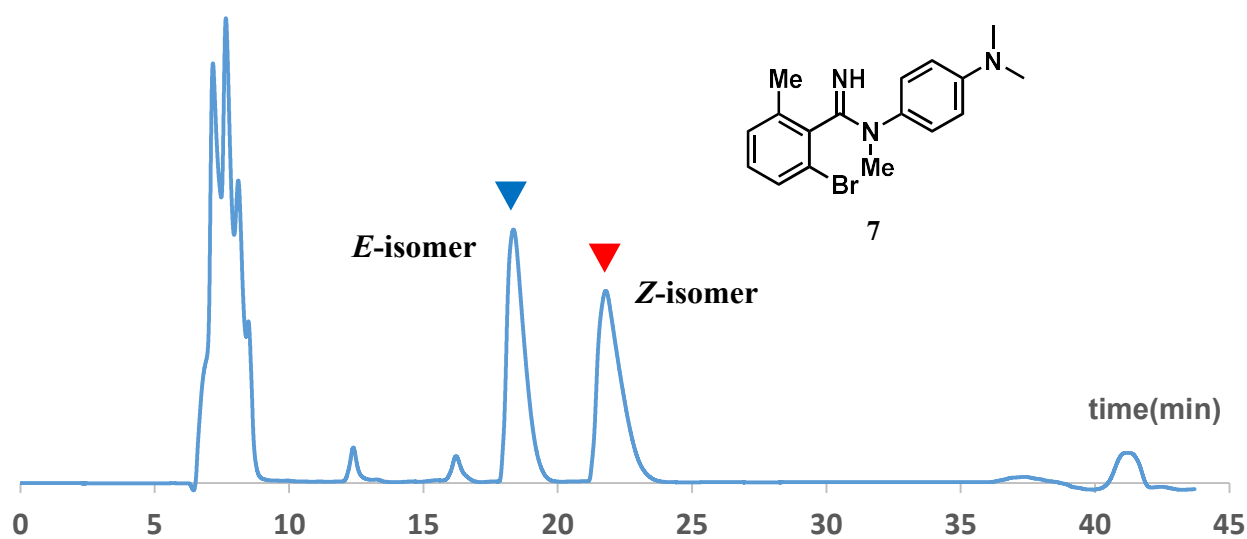


Figure S4.6. HPLC chart of *E*- and *Z*- isomer of amide 7

5. Monitoring of the *E/Z*-isomerization

Procedure A (for compound 2)

After the separation of the *E/Z* isomers, a solution of the *Z* isomer was concentrated *in vacuo* and diluted with a buffer, and the resulting solution was maintained at 20 °C. The progress of the isomerization was monitored by LC-MS analysis.

Procedure B (for compounds 3-7)

After the separation of the *E/Z* isomers, a solution of the *Z* isomer was diluted with a buffer and MeCN (final ratio: buffer/MeCN = 95/5), and the resulting solution was maintained at 30 °C. The progress of the isomerization was monitored by LC-MS analysis.

The ratio of the absorbance coefficients at 254 nm for each geometric isomer was determined by comparing the integral ratio of ¹H NMR spectra and the area ratio of UV chromatogram of the LC-MS analysis (detailed conditions were described below). The ratio of the absorbance coefficients was used to calculate the molar ratios at each time point from the UV chromatogram of the LC-MS analysis. The ratio of each isomer was plotted as a function of time, and curve-fitting analysis of the experimental results using differential equations describing the isomerization (eq. 2, 3), to calculate kinetic constant *k* (Figure S5.1-5.23).

$$\frac{d[E]}{dt} = k_{Z \rightarrow E}[Z] - k_{E \rightarrow Z}[E] \quad (\text{eq. 2})$$

$$\frac{d([E]+[Z])}{dt} = 0 \quad (\text{eq. 3})$$

LC-MS conditions:

For compound **2**

column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 25% B in A isocratic elution, detection: UV (254 nm)

For compound **3**

column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm)

For compound **4**

column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 24% B in A isocratic elution, detection: UV (254 nm)

For compound **5**

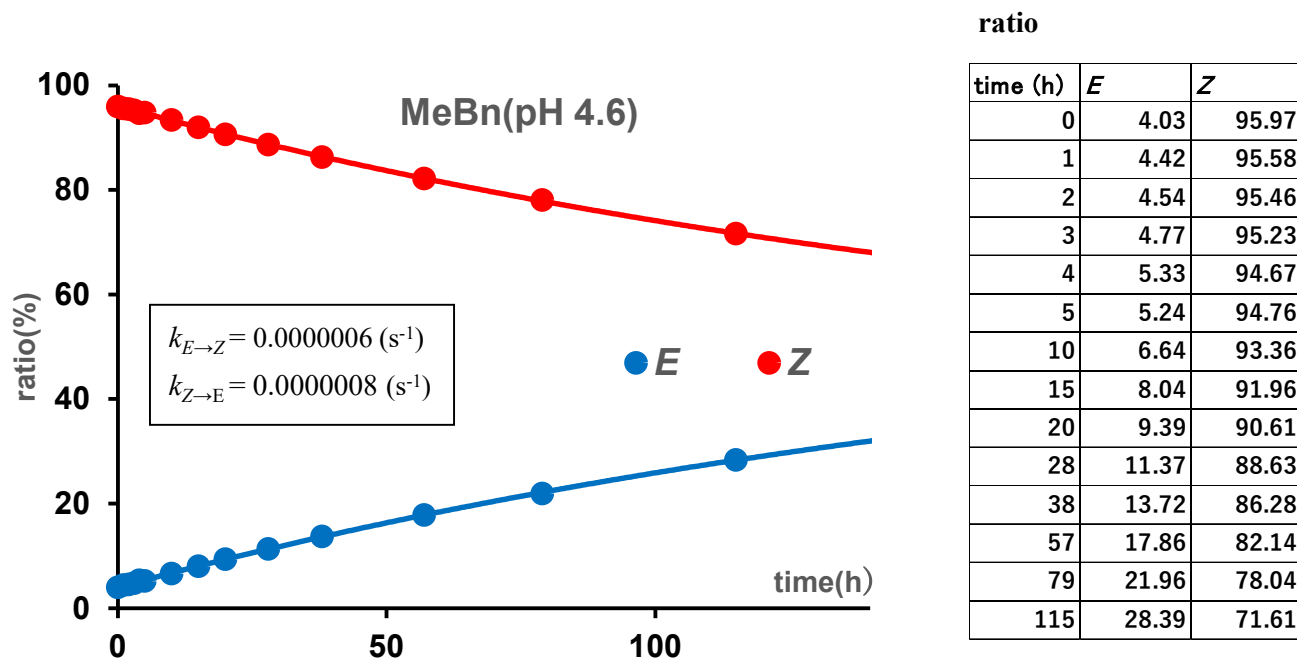
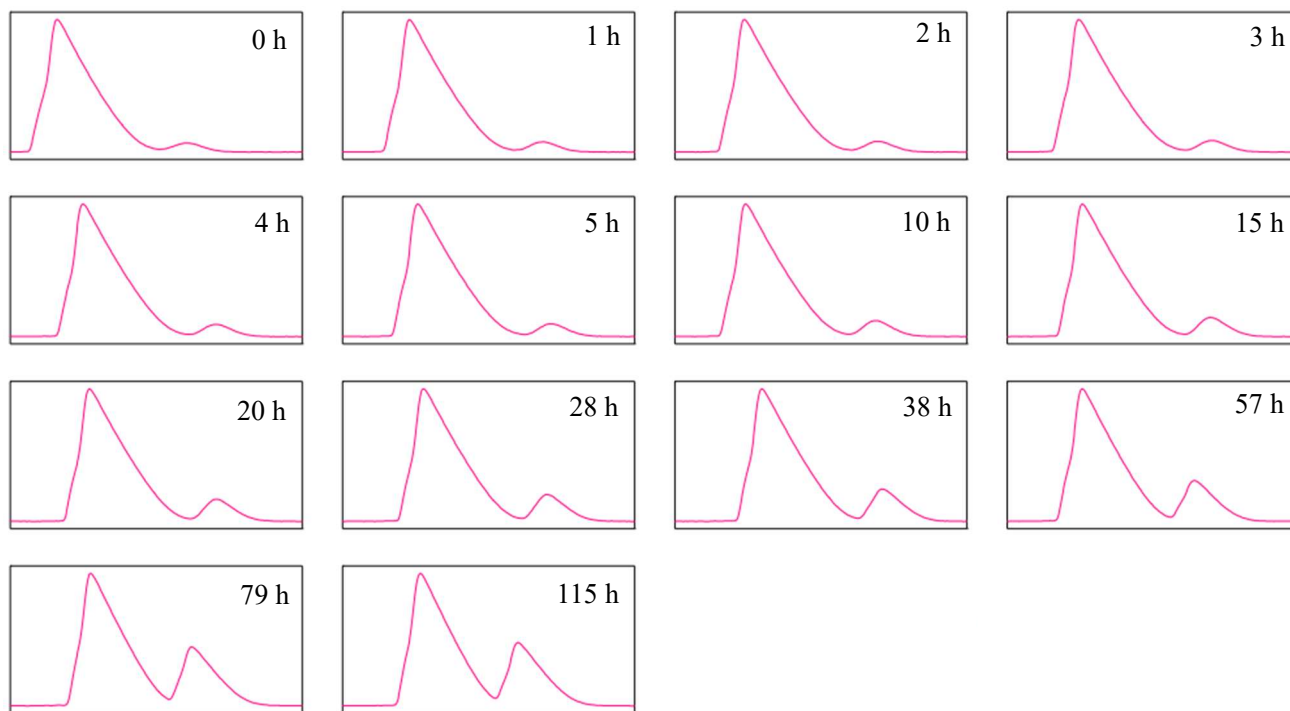
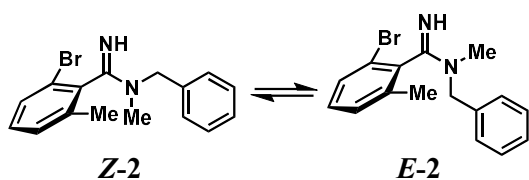
column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 24% B in A isocratic elution, detection: UV (254 nm)

For compound **6**

column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 24% B in A isocratic elution, detection: UV (254 nm)

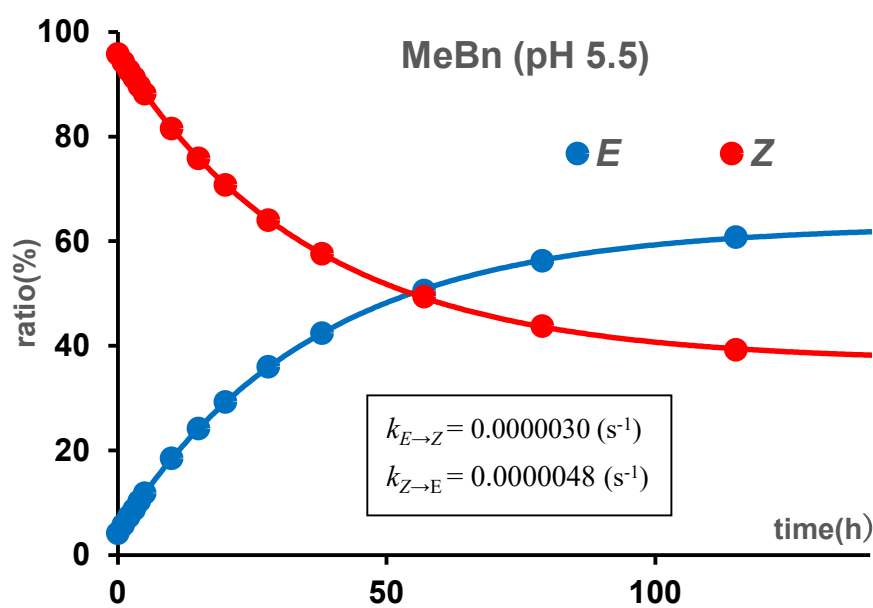
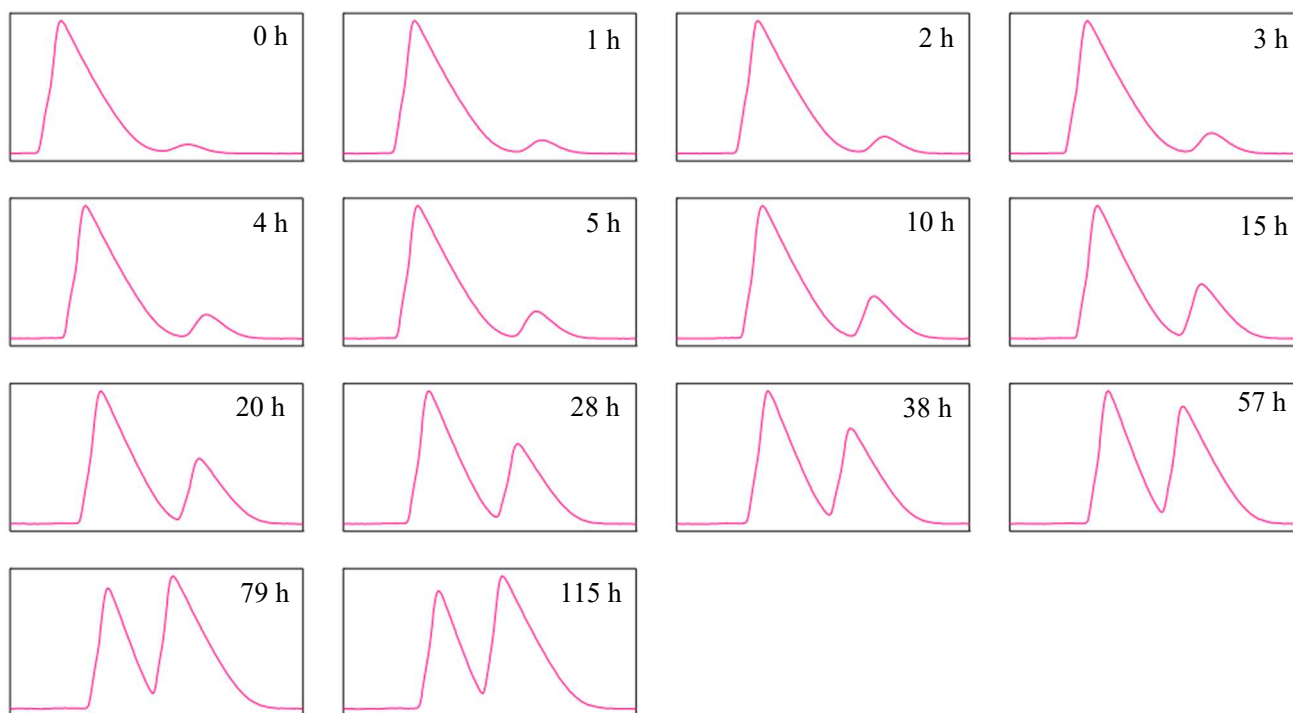
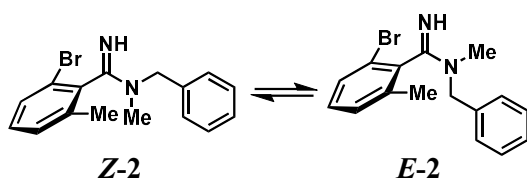
For compound **7**

column: J'sphere ODS-M80, 150 × 4.6 mmI.D., S-4 μm, 8 nm, flow rate: 0.4 mL/min, eluent: A; 0.1% aqueous formic acid in H₂O, B; MeCN, 28% B in A isocratic elution, detection: UV (254 nm)



Conditions: 20 °C in 100 mM acetate buffer

Figure S5.1. Isomerization of compound 2 at pH 4.6

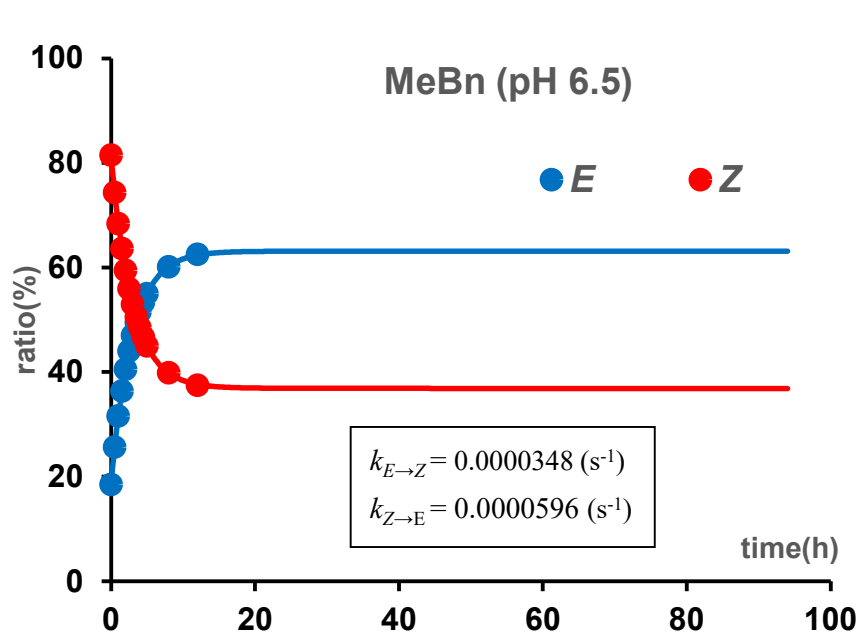
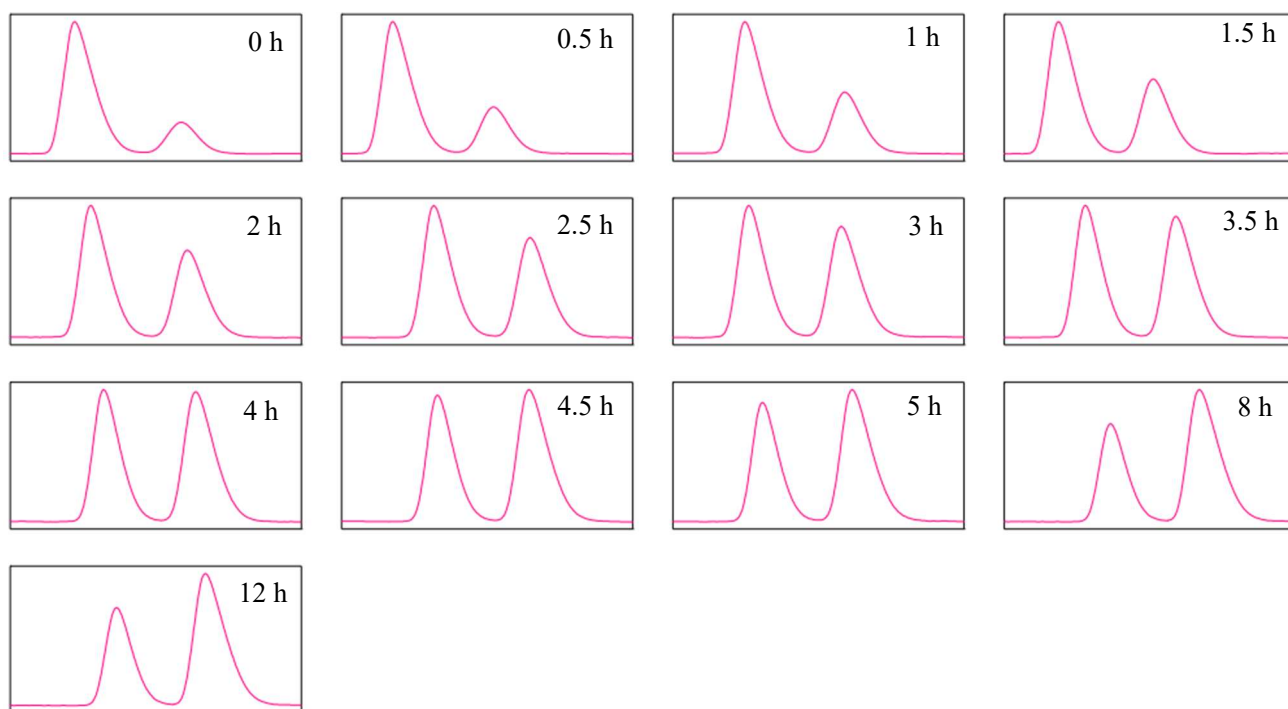
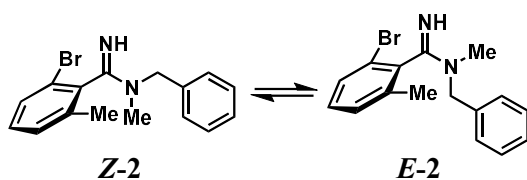


ratio

time (h)	<i>E</i>	<i>Z</i>
0	4.18	95.82
1	5.74	94.26
2	7.32	92.68
3	8.71	91.29
4	10.29	89.71
5	11.81	88.19
10	18.48	81.52
15	24.16	75.84
20	29.25	70.75
28	35.98	64.02
38	42.42	57.58
57	50.57	49.43
79	56.23	43.77
115	60.78	39.22

Conditions: 20 °C in 100 mM acetate buffer

Figure S5.2. Isomerization of compound 2 at pH 5.5

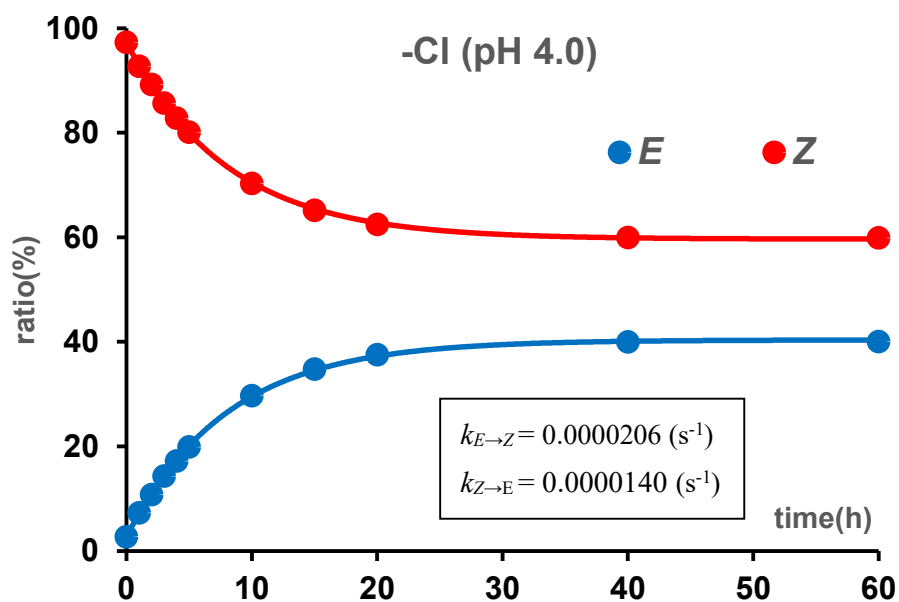
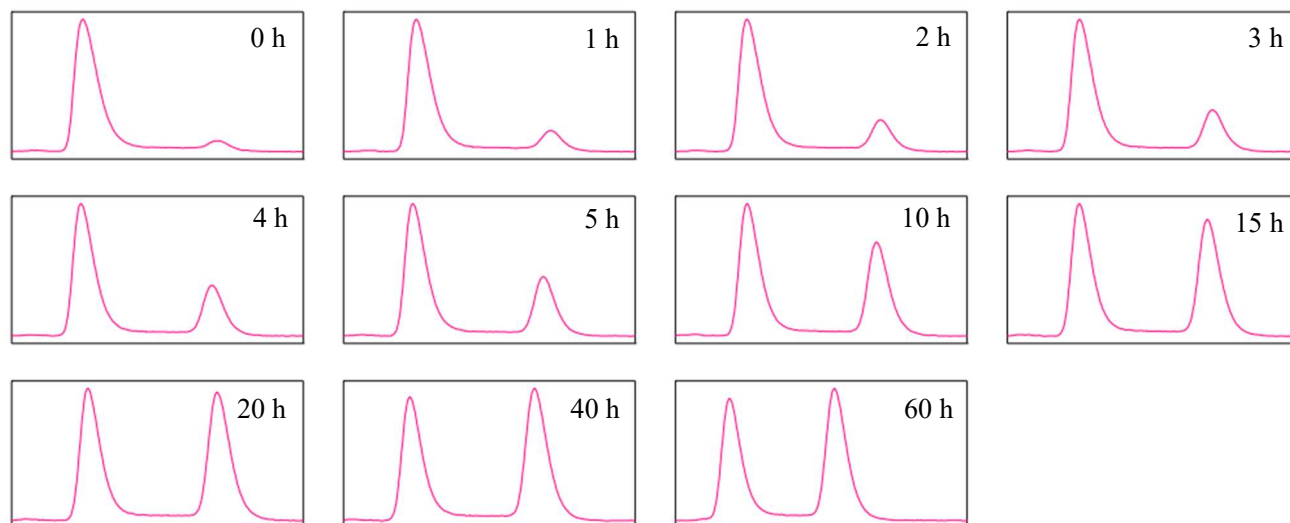
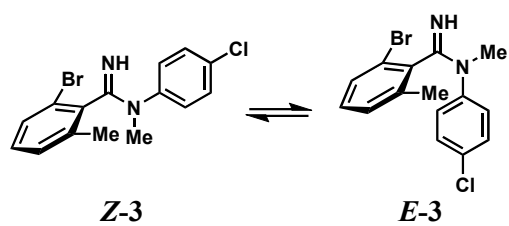


ratio

time (h)	<i>E</i>	<i>Z</i>
0	18.51	81.49
0.5	25.68	74.32
1	31.61	68.39
1.5	36.36	63.64
2	40.54	59.46
2.5	44.01	55.99
3	47.03	52.97
3.5	49.50	50.50
4	51.48	48.52
4.5	53.31	46.69
5	54.96	45.04
8	60.13	39.87
12	62.50	37.50

Conditions: 20 °C in 100 mM phosphate buffer

Figure S5.3. Isomerization of compound **2** at pH 6.5

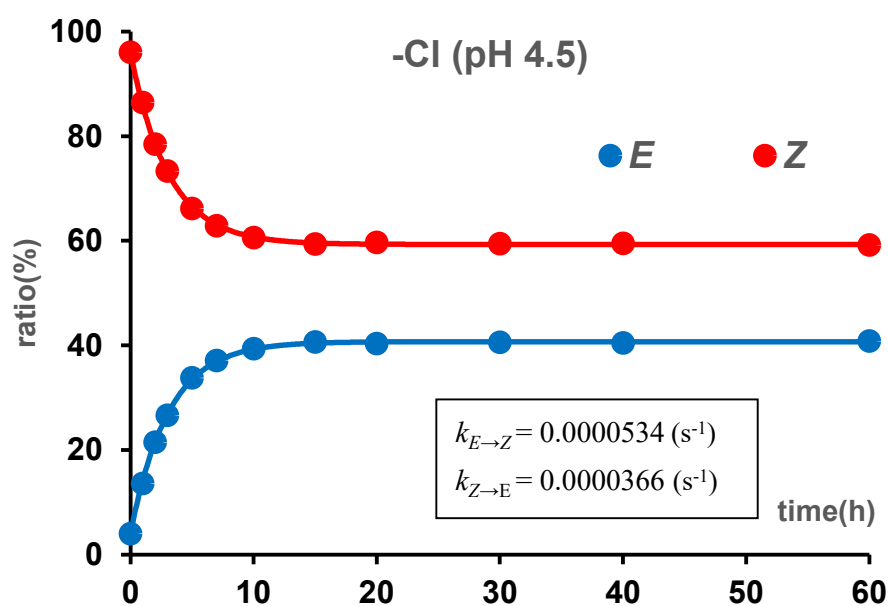
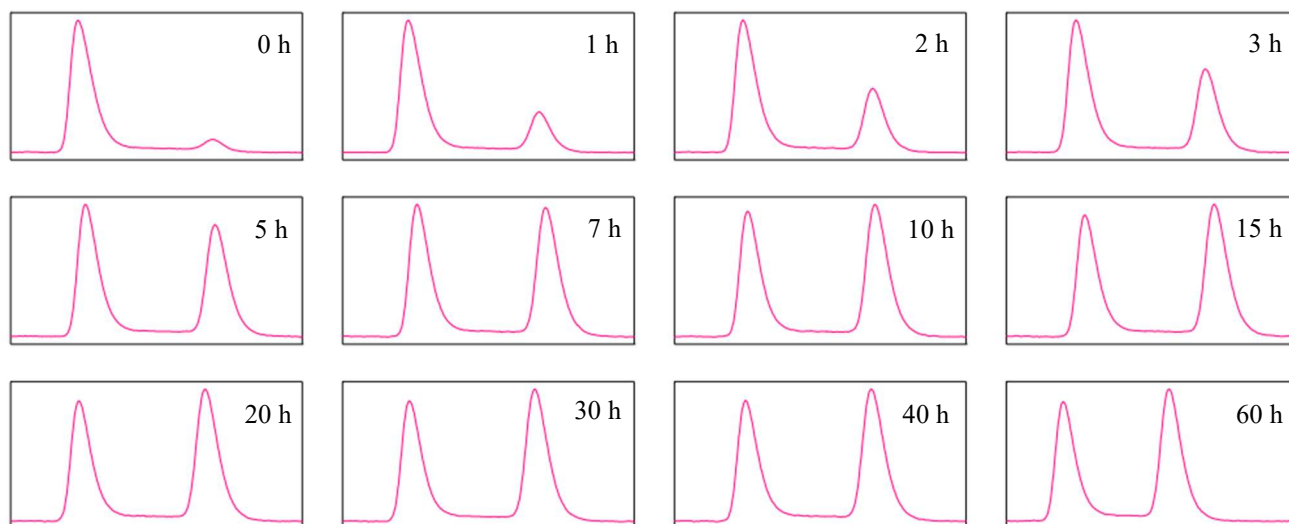
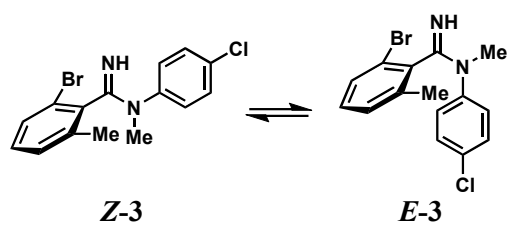


ratio

time (h)	<i>E</i>	<i>Z</i>
0	2.69	97.31
1	7.32	92.68
2	10.79	89.21
3	14.35	85.65
4	17.24	82.76
5	19.92	80.08
10	29.69	70.31
15	34.83	65.17
20	37.53	62.47
40	40.02	59.98
60	40.08	59.92

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.4. Isomerization of compound 3 at pH 4.0

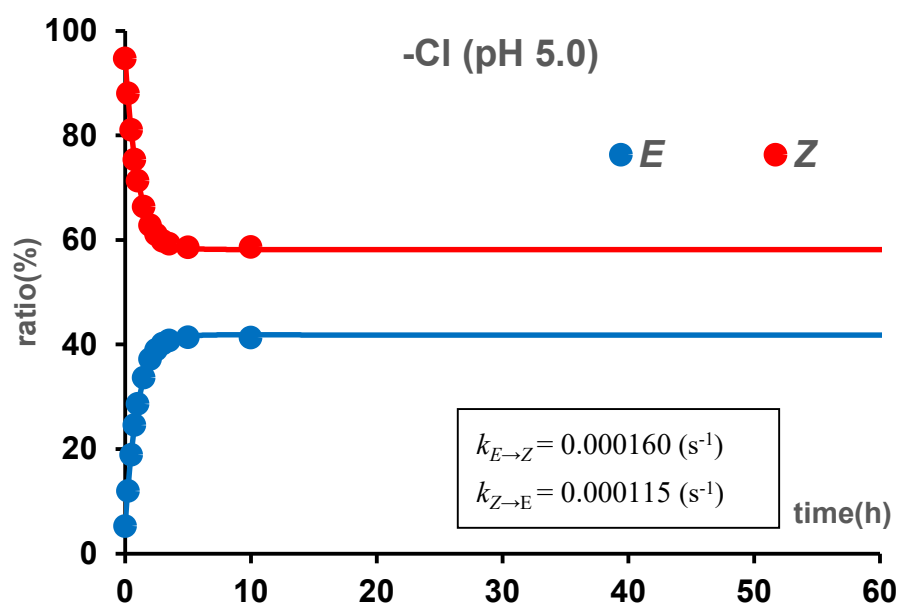
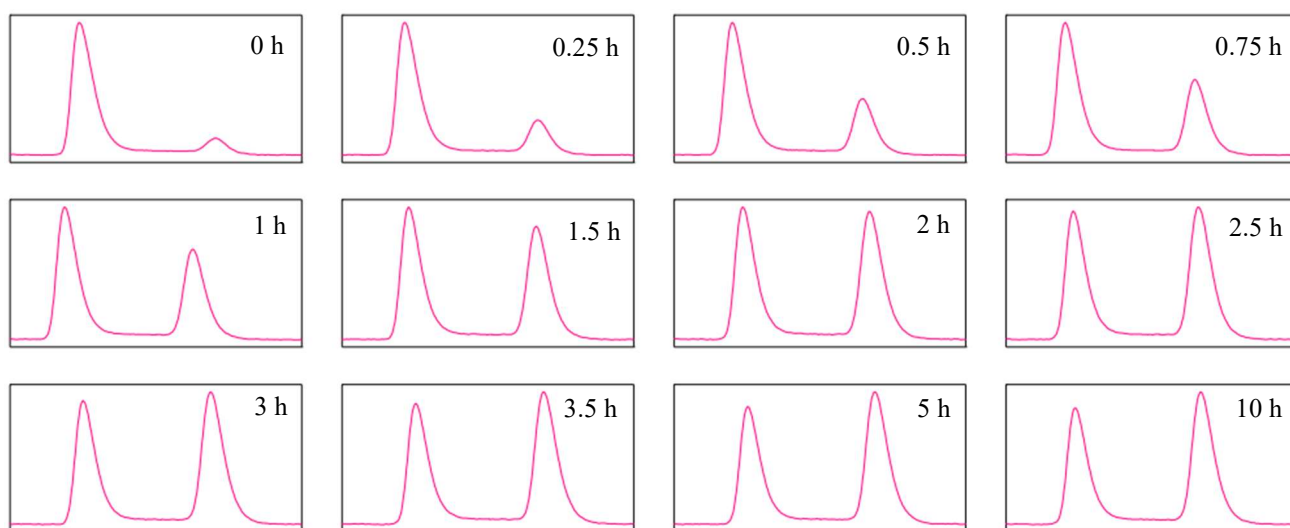
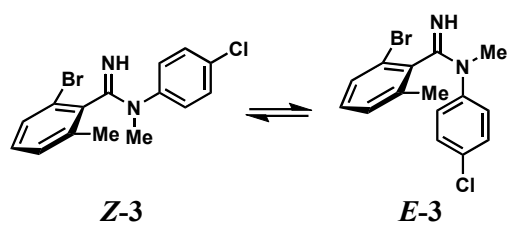


ratio

time (h)	<i>E</i>	<i>Z</i>
0	3.96	96.04
1	13.57	86.43
2	21.52	78.48
3	26.64	73.36
5	33.80	66.20
7	37.11	62.89
10	39.37	60.63
15	40.64	59.36
20	40.32	59.68
30	40.57	59.43
40	40.50	59.50
60	40.82	59.18

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

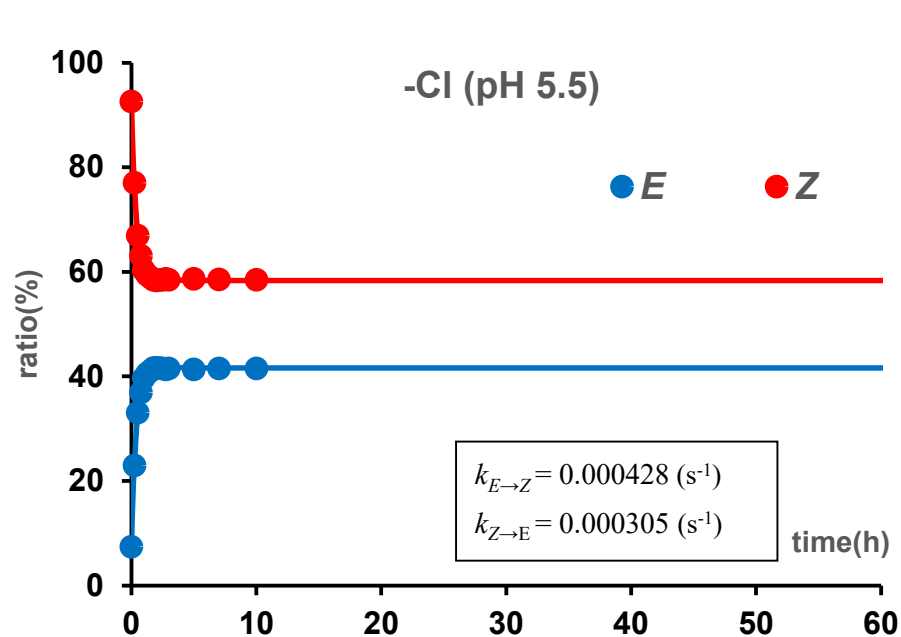
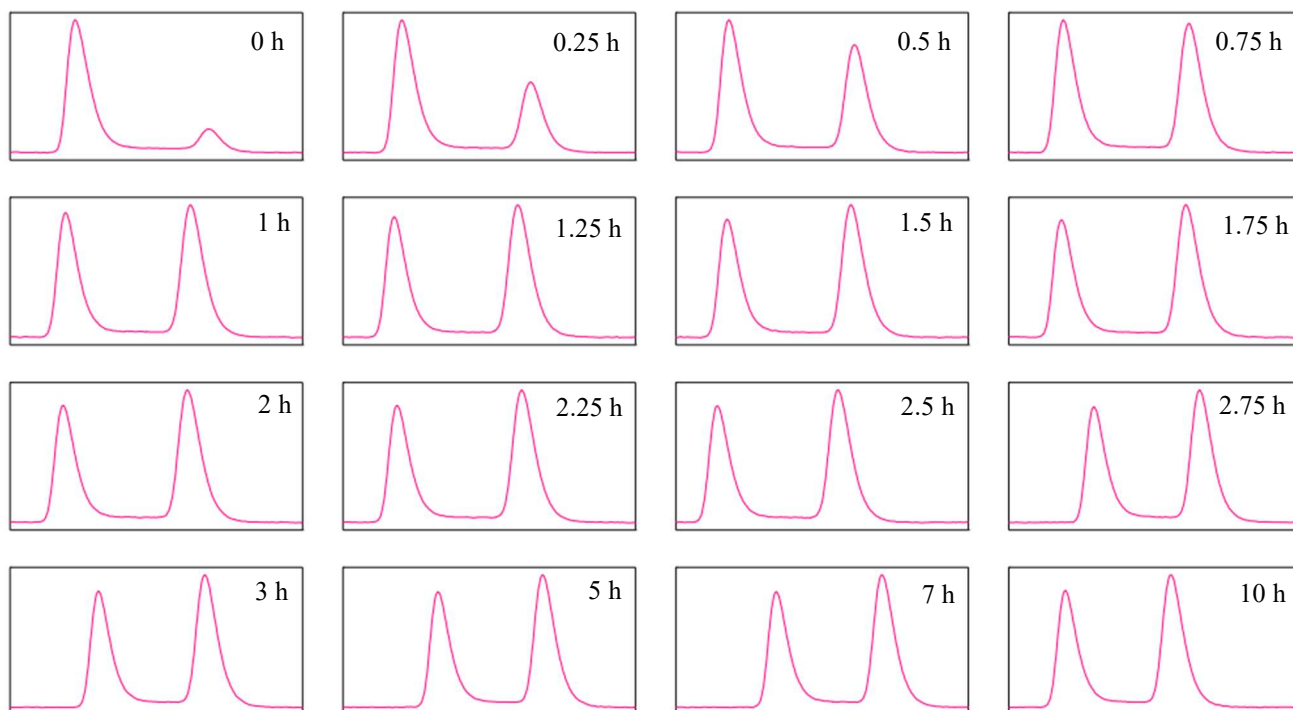
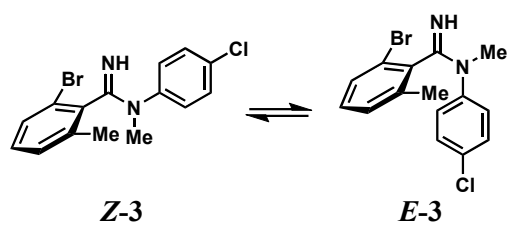
Figure S5.5. Isomerization of compound **3** at pH 4.5



ratio		
time (h)	E	Z
0	5.29	94.71
0.25	11.98	88.02
0.5	18.94	81.06
0.75	24.61	75.39
1	28.68	71.32
1.5	33.65	66.35
2	37.21	62.79
2.5	38.98	61.02
3	40.16	59.84
3.5	40.76	59.24
5	41.41	58.59
10	41.32	58.68

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

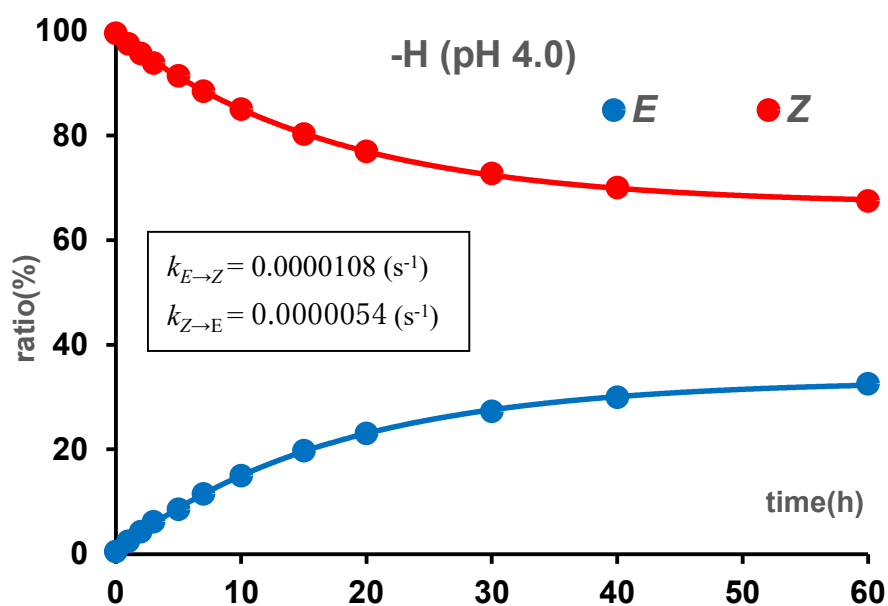
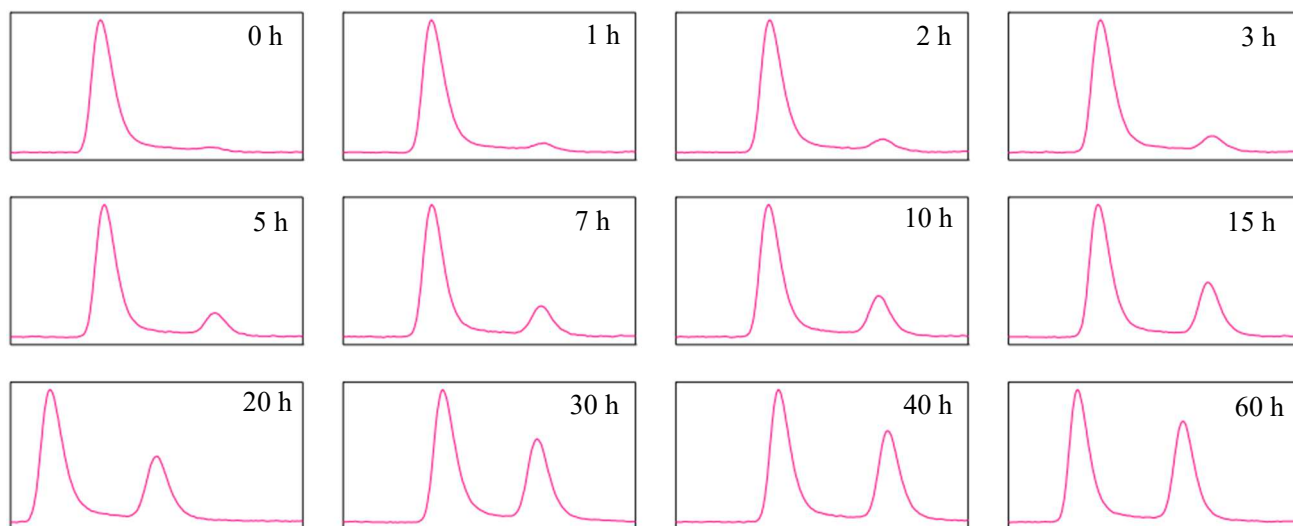
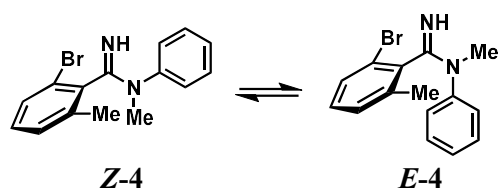
Figure S5.6. Isomerization of compound 3 at pH 5.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	7.40	92.60
0.25	22.96	77.04
0.5	33.07	66.93
0.75	36.93	63.07
1	39.71	60.29
1.25	40.52	59.48
1.5	41.03	58.97
1.75	41.55	58.45
2	41.62	58.38
2.25	41.61	58.39
2.5	41.53	58.47
2.75	41.31	58.69
3	41.55	58.45
5	41.36	58.64
7	41.49	58.51
10	41.52	58.48

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.7. Isomerization of compound **3** at pH 5.5

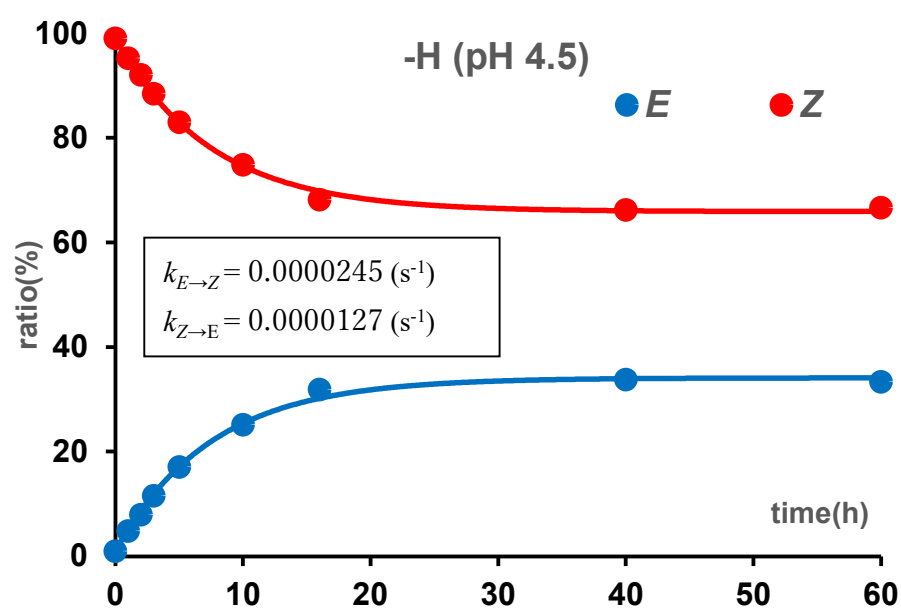
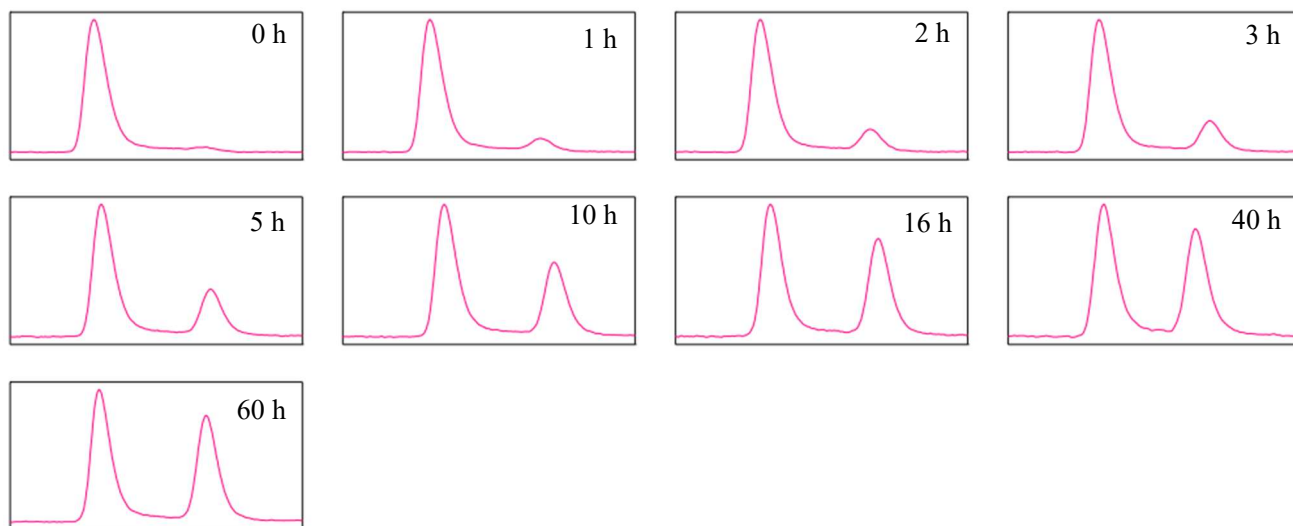
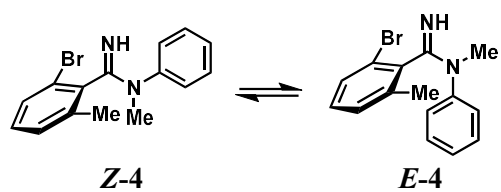


ratio

time (h)	<i>E</i>	<i>Z</i>
0	1.09	98.91
1	2.44	97.56
2	4.29	95.71
3	6.14	93.86
5	8.56	91.44
7	11.48	88.52
10	14.96	85.04
15	19.72	80.28
20	23.04	76.96
30	27.26	72.74
40	29.95	70.05
60	32.51	67.49

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.8. Isomerization of compound 4 at pH 4.0

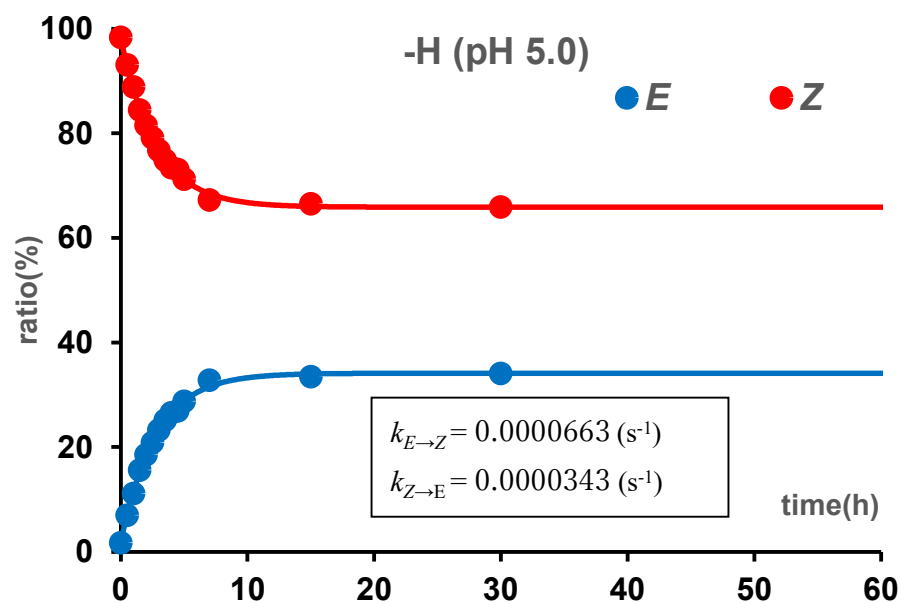
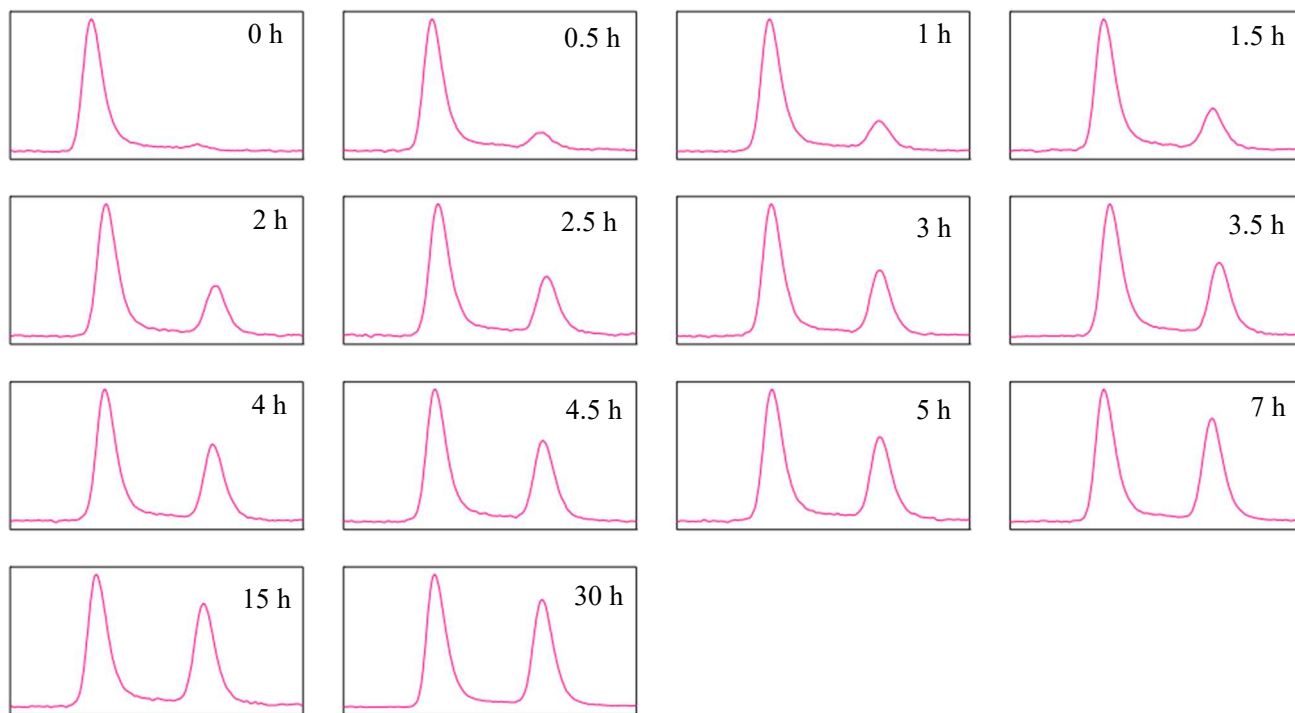
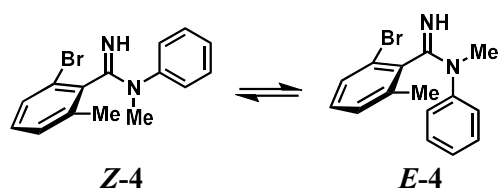


ratio

time (h)	<i>E</i>	<i>Z</i>
0	0.99	99.01
1	4.79	95.21
2	7.93	92.07
3	11.56	88.44
5	17.03	82.97
10	25.17	74.83
16	31.86	68.14
40	33.79	66.21
60	33.34	66.66

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.9. Isomerization of compound 4 at pH 4.5

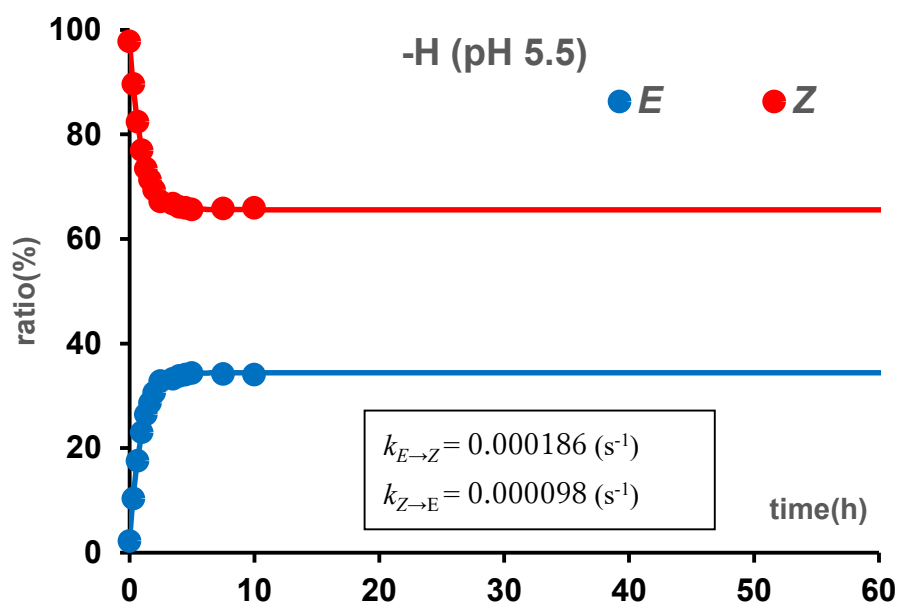
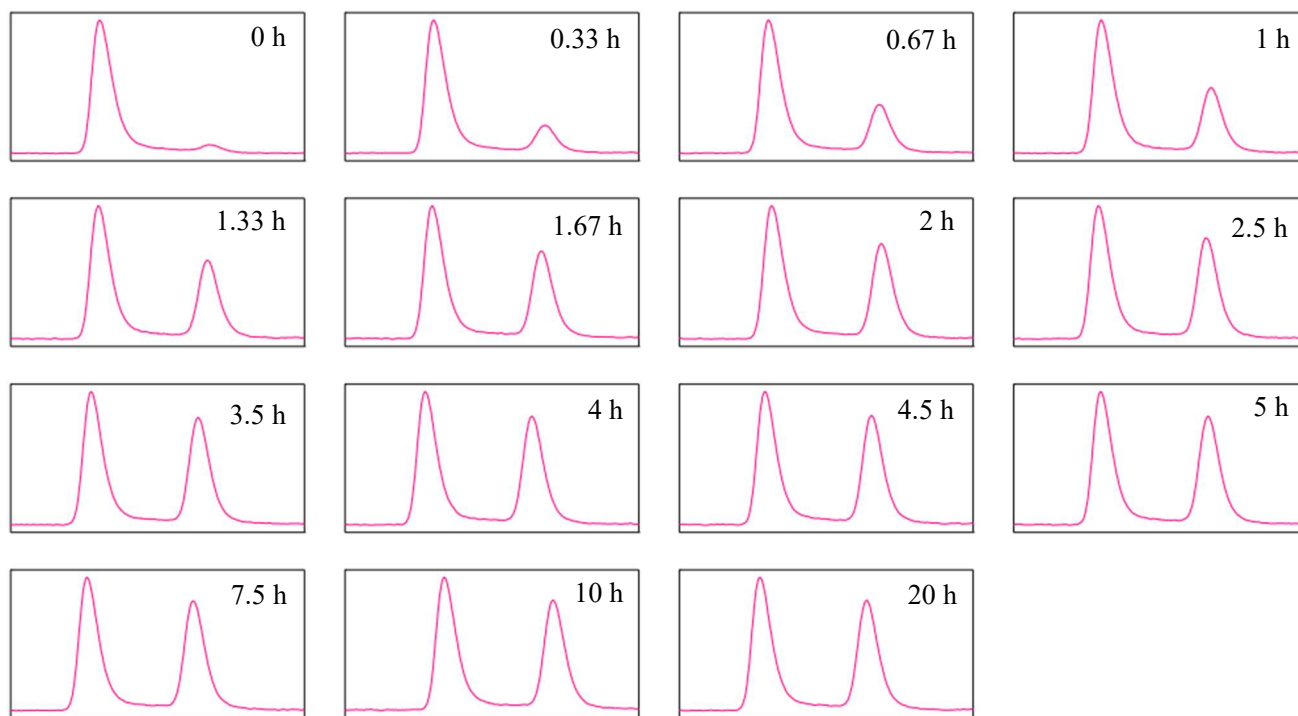
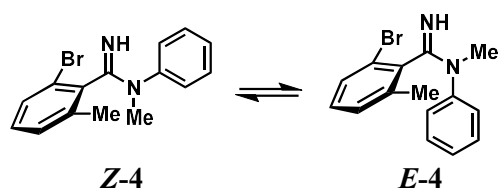


ratio

time (h)	<i>E</i>	<i>Z</i>
0	1.68	98.32
0.5	6.93	93.07
1	11.14	88.86
1.5	15.56	84.44
2	18.51	81.49
2.5	20.85	79.15
3	23.27	76.73
3.5	25.15	74.85
4	26.54	73.46
4.5	26.92	73.08
5	28.75	71.25
7	32.77	67.23
15	33.49	66.51
30	34.08	65.92

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

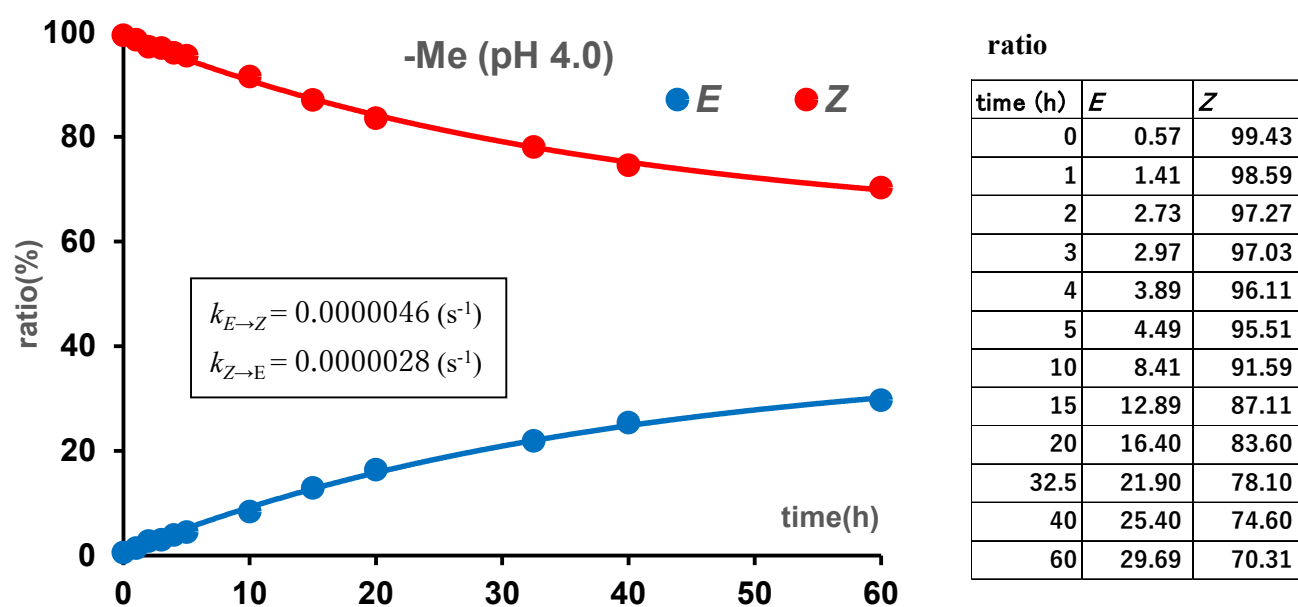
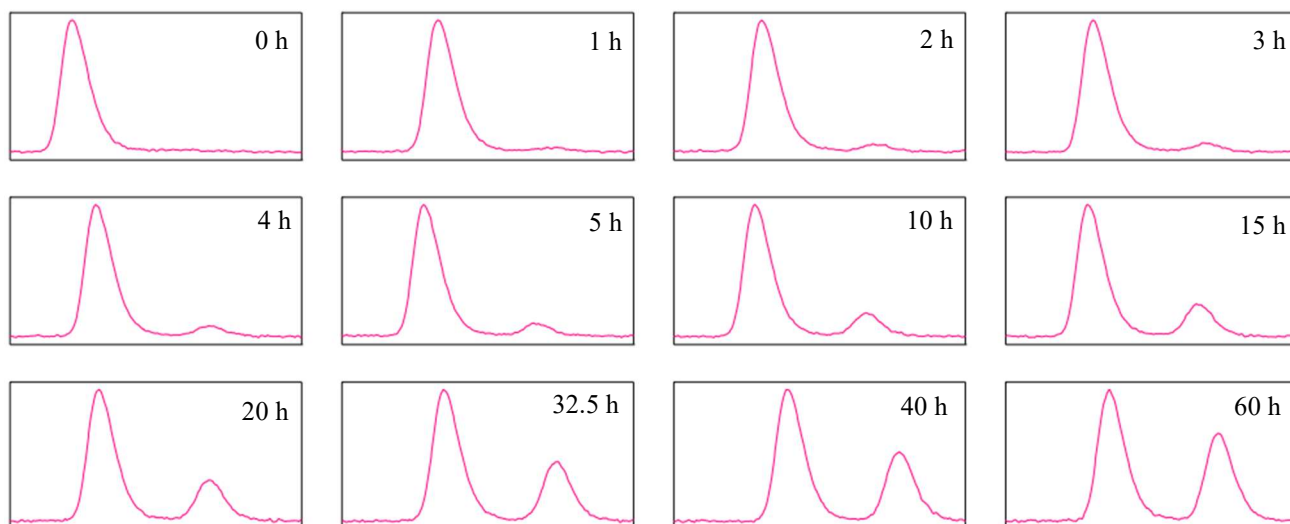
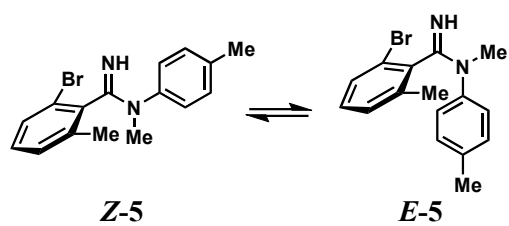
Figure S5.10. Isomerization of compound 4 at pH 5.0



time (h)	E	Z
0	2.21	97.79
0.33	10.35	89.65
0.67	17.57	82.43
1	23.02	76.98
1.33	26.49	73.51
1.67	28.66	71.34
2	30.62	69.38
2.5	32.83	67.17
3.5	33.26	66.74
4	33.84	66.16
4.5	34.04	65.96
5	34.37	65.63
7.5	34.16	65.84
10	34.03	65.97
20	34.20	65.80

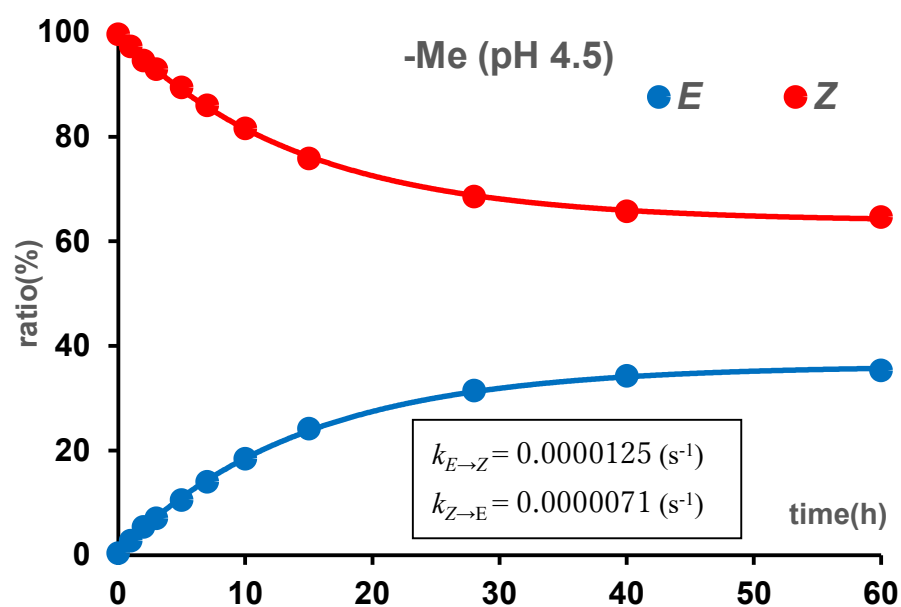
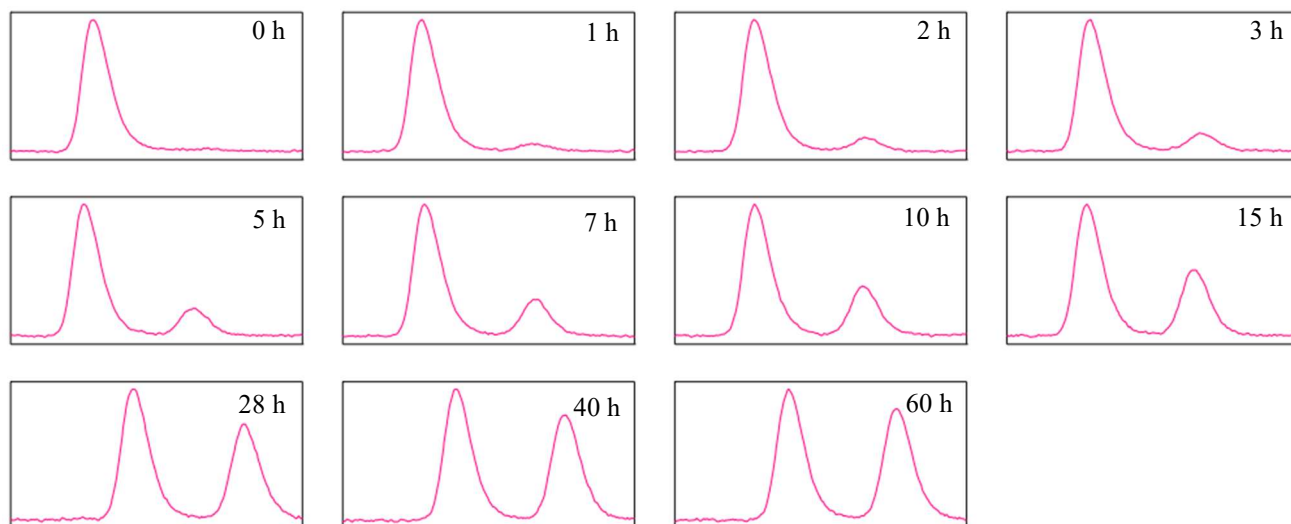
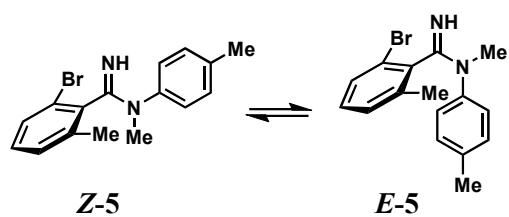
Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.11. Isomerization of compound 4 at pH 5.5



Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

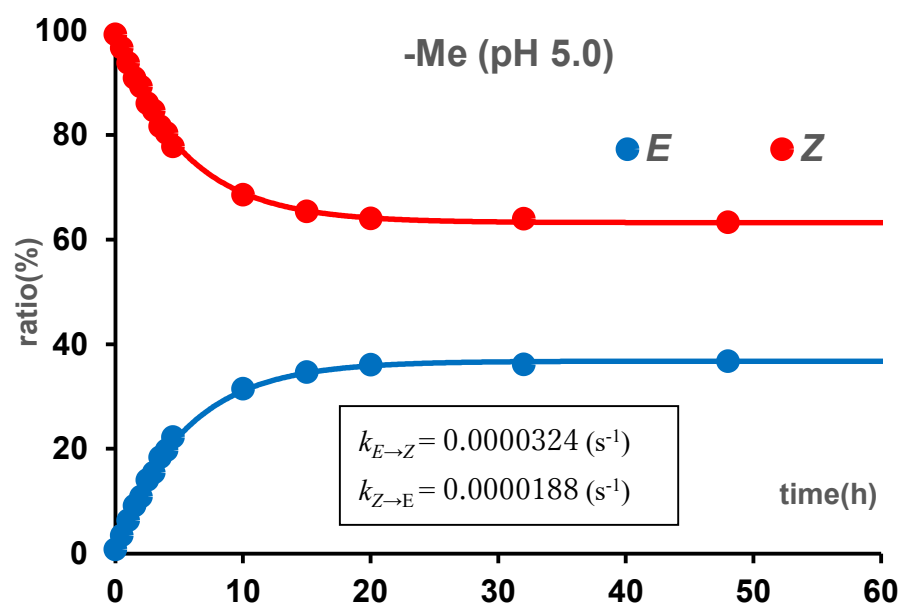
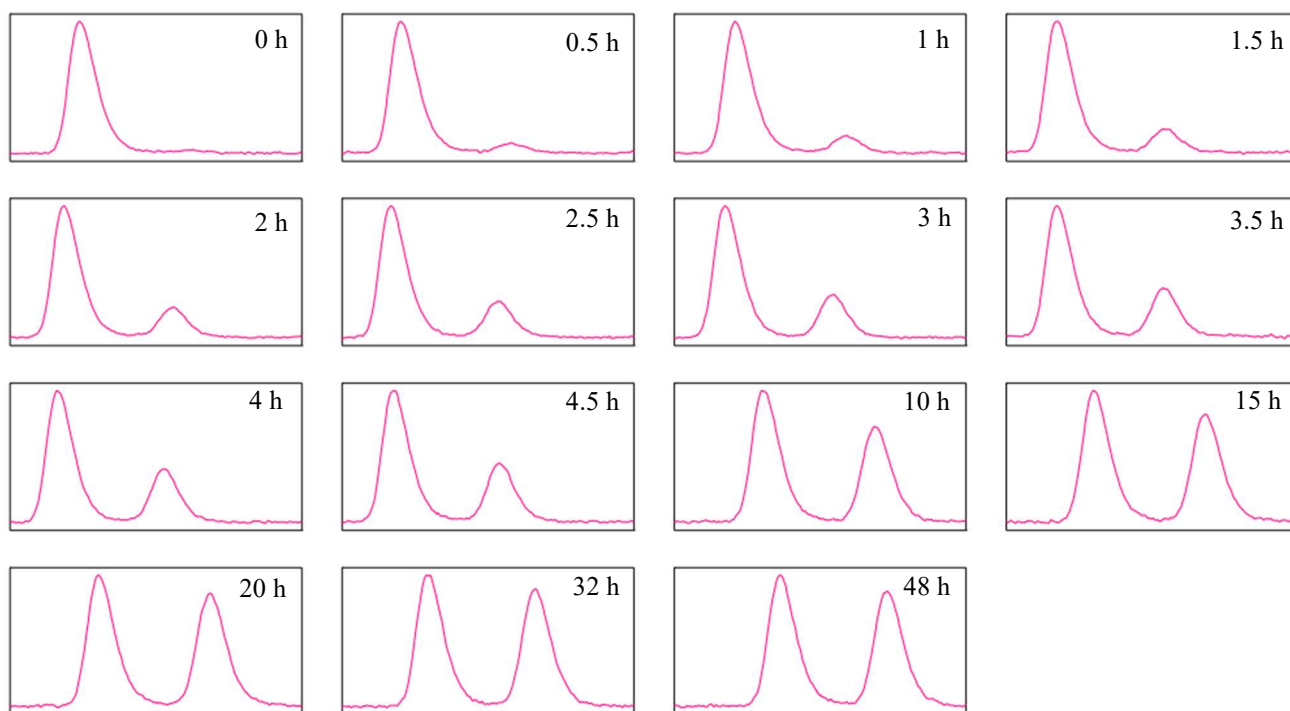
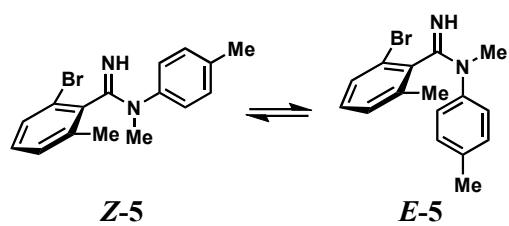
Figure S5.12. Isomerization of compound 5 at pH 4.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	0.39	99.61
1	2.73	97.27
2	5.41	94.59
3	7.07	92.93
5	10.56	89.44
7	14.01	85.99
10	18.43	81.57
15	24.16	75.84
28	31.46	68.54
40	34.28	65.72
60	35.33	64.67

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

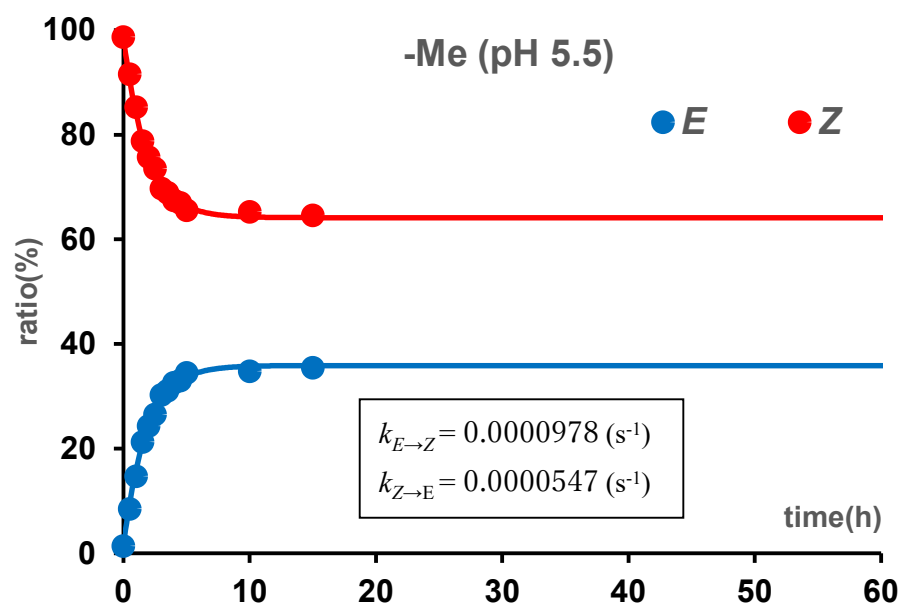
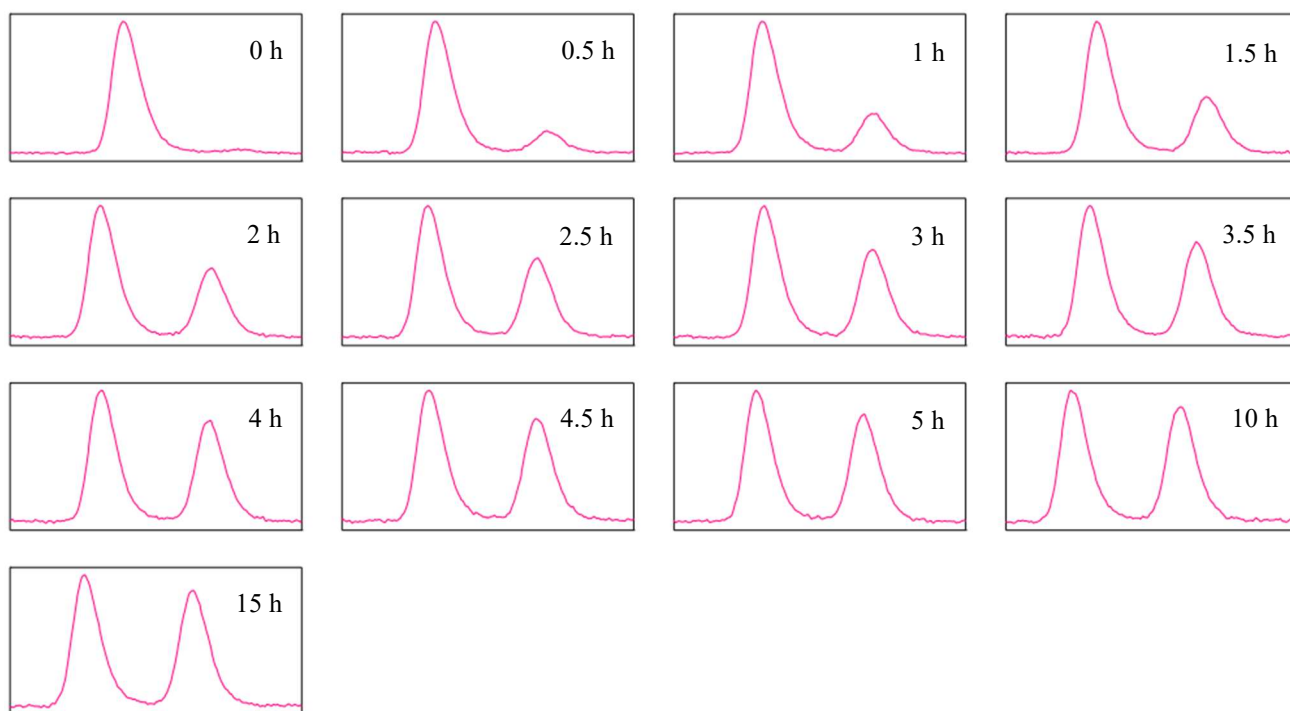
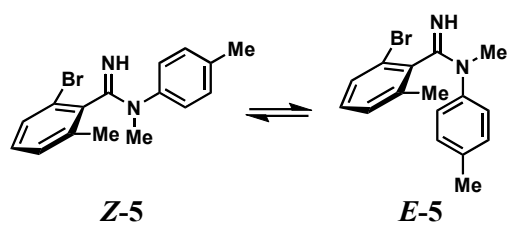
Figure S5.13. Isomerization of compound 5 at pH 4.5



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	0.79	99.21
0.5	3.40	96.60
1	6.26	93.74
1.5	9.10	90.90
2	10.80	89.20
2.5	13.97	86.03
3	15.39	84.61
3.5	18.35	81.65
4	19.67	80.33
4.5	22.21	77.79
10	31.44	68.56
15	34.64	65.36
20	36.00	64.00
32	36.09	63.91
48	36.72	63.28

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

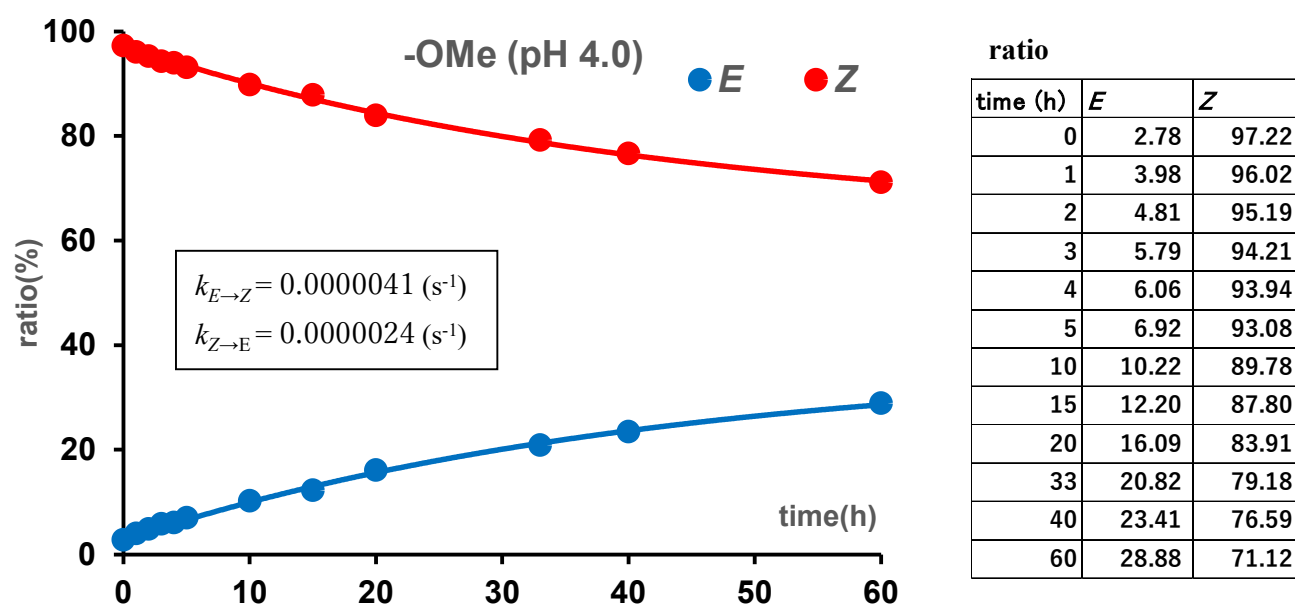
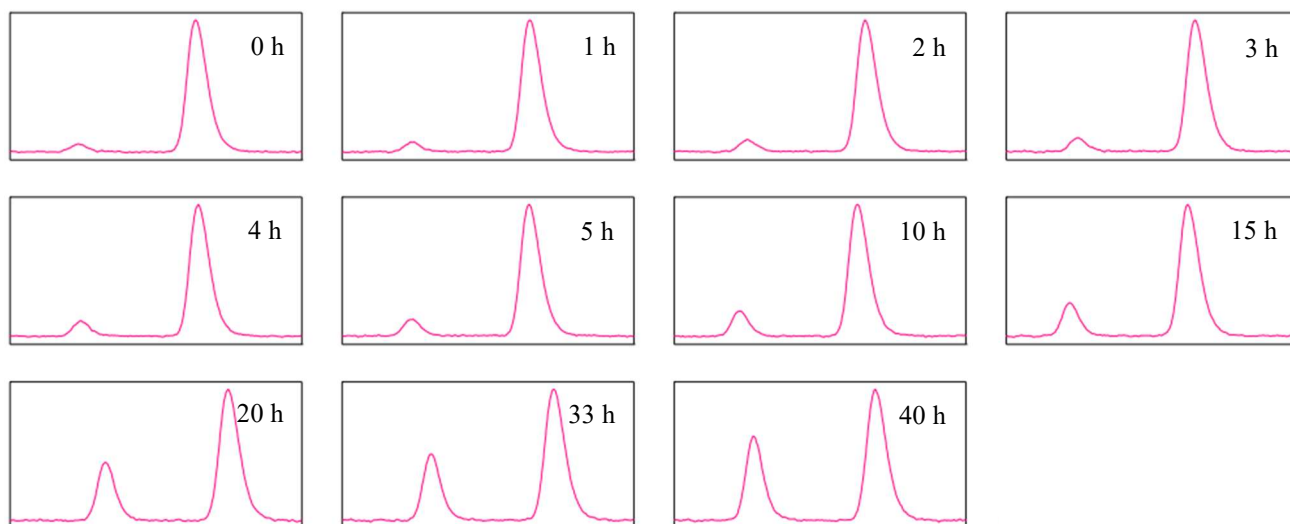
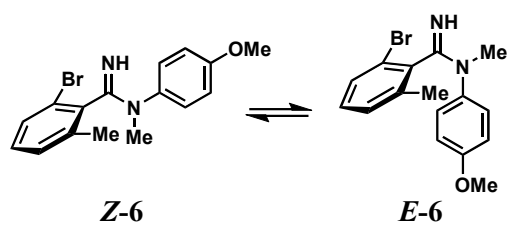
Figure S5.14. Isomerization of compound 5 at pH 5.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	1.33	98.67
0.5	8.45	91.55
1	14.71	85.29
1.5	21.24	78.76
2	24.31	75.69
2.5	26.51	73.49
3	30.25	69.75
3.5	31.06	68.94
4	32.55	67.45
4.5	33.01	66.99
5	34.47	65.53
10	34.78	65.22
15	35.43	64.57

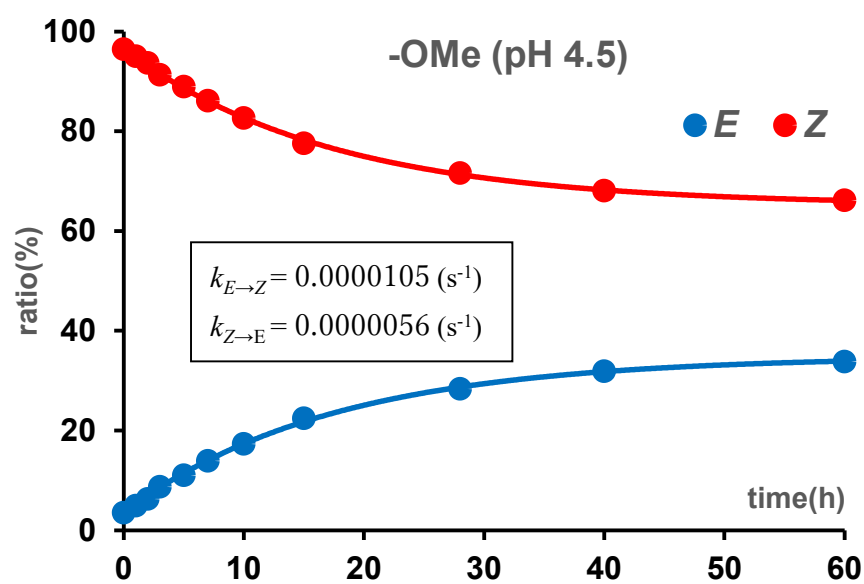
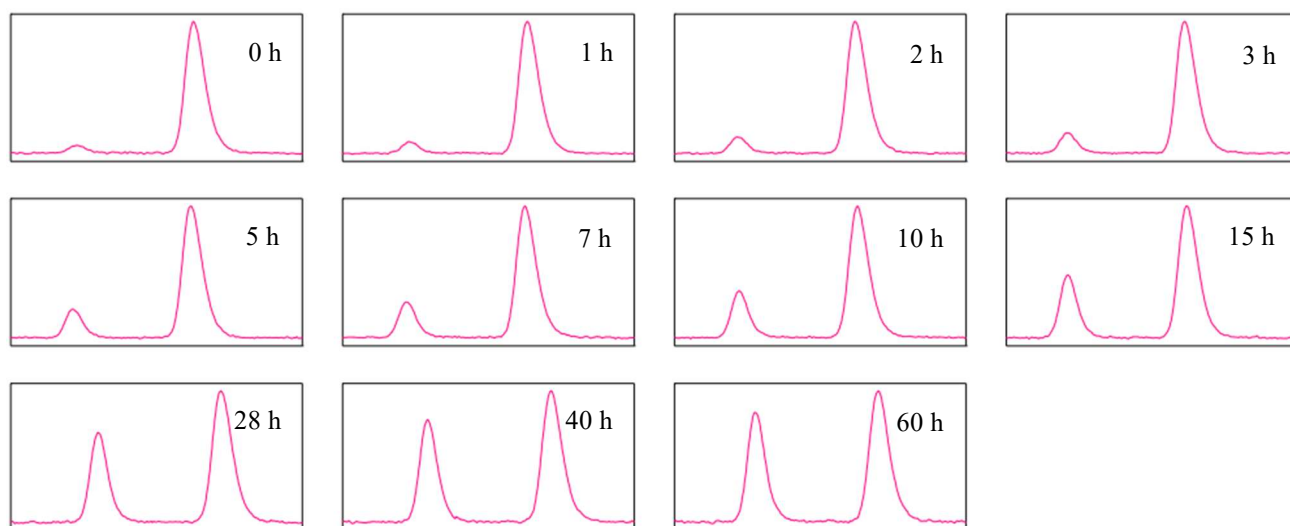
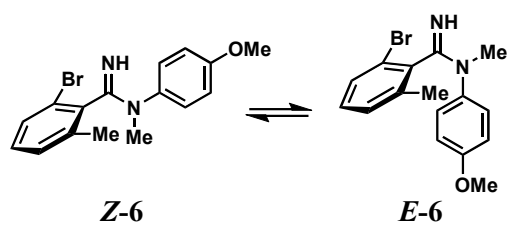
Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.15. Isomerization of compound 5 at pH 5.5



Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

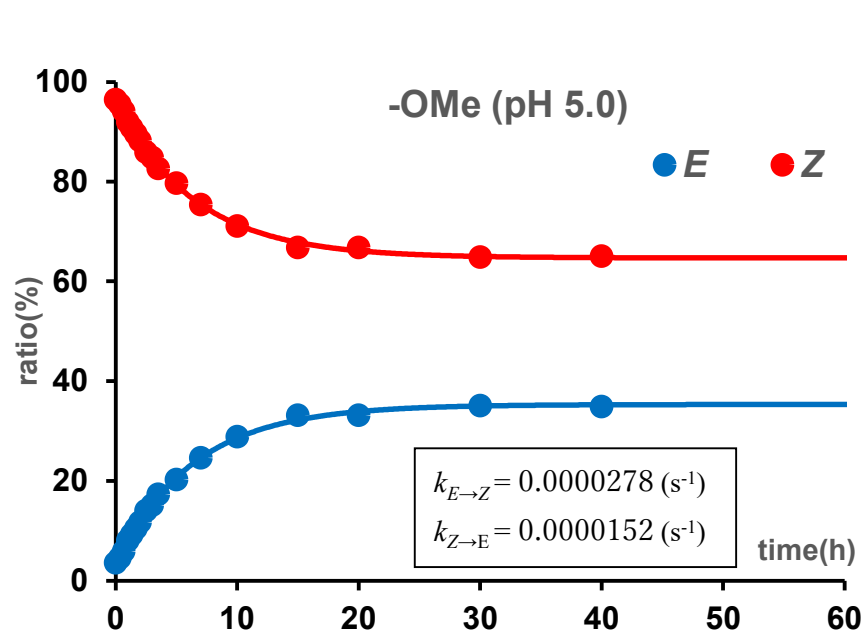
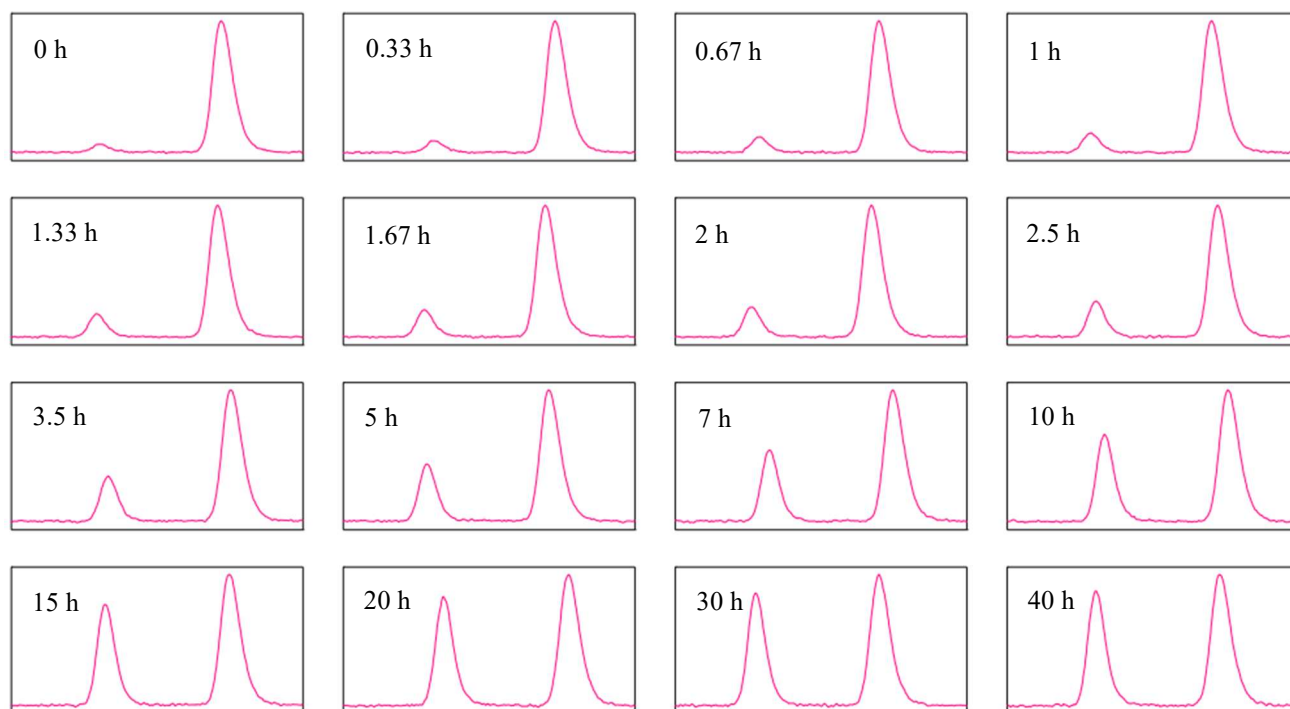
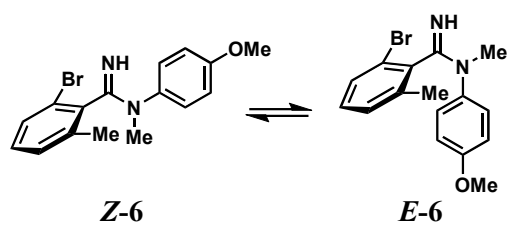
Figure S5.16. Isomerization of compound **6** at pH 4.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	3.52	96.48
1	4.94	95.06
2	6.29	93.71
3	8.69	91.31
5	11.03	88.97
7	13.91	86.09
10	17.35	82.65
15	22.43	77.57
28	28.37	71.63
40	31.90	68.10
60	33.84	66.16

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

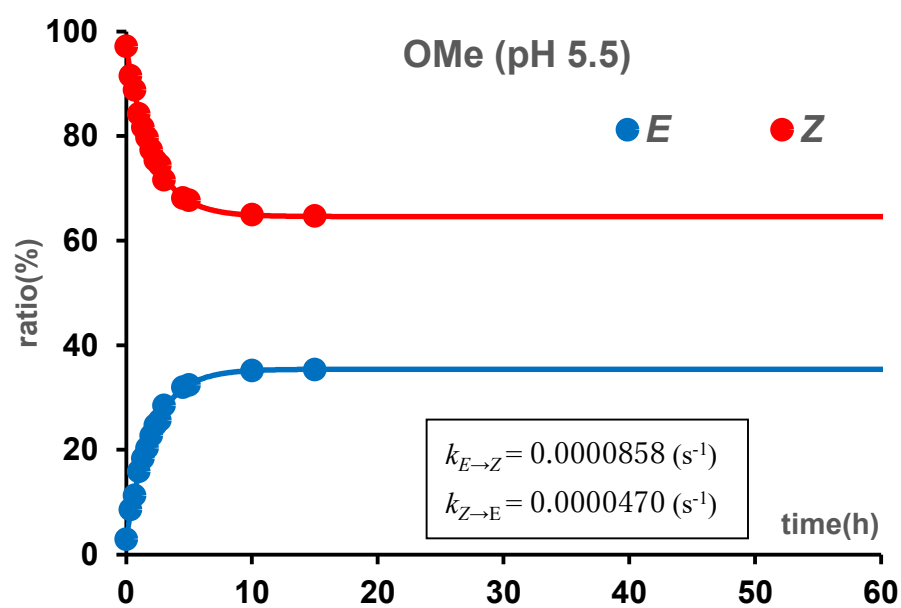
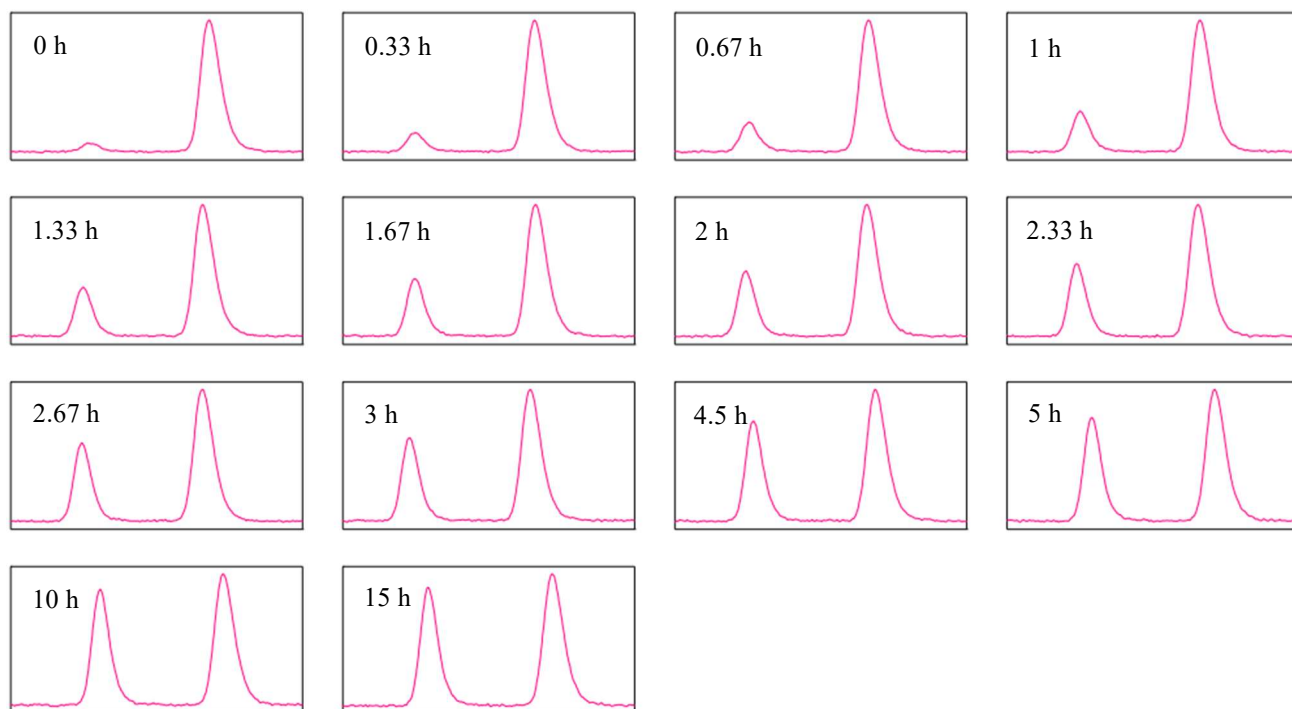
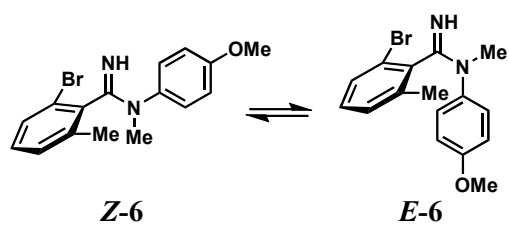
Figure S5.17. Isomerization of compound **6** at pH 4.5



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	3.57	96.43
0.33	4.53	95.47
0.67	5.89	94.11
1	8.06	91.94
1.33	9.23	90.77
1.67	10.45	89.55
2	11.75	88.25
2.5	14.08	85.92
3.5	17.35	82.65
5	20.30	79.70
7	24.63	75.37
10	28.88	71.12
15	33.18	66.82
20	33.20	66.80
30	35.11	64.89
40	34.93	65.07

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.18. Isomerization of compound 6 at pH 5.0

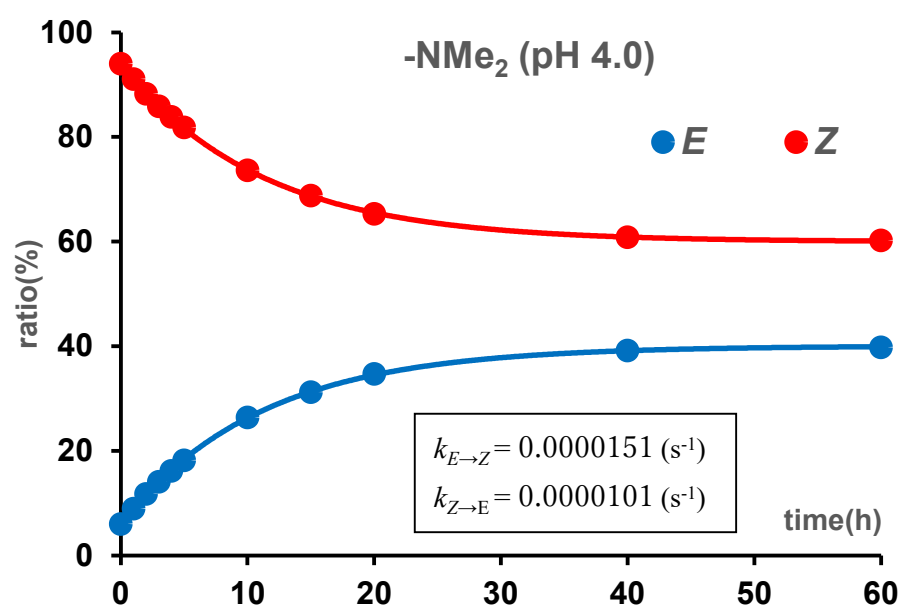
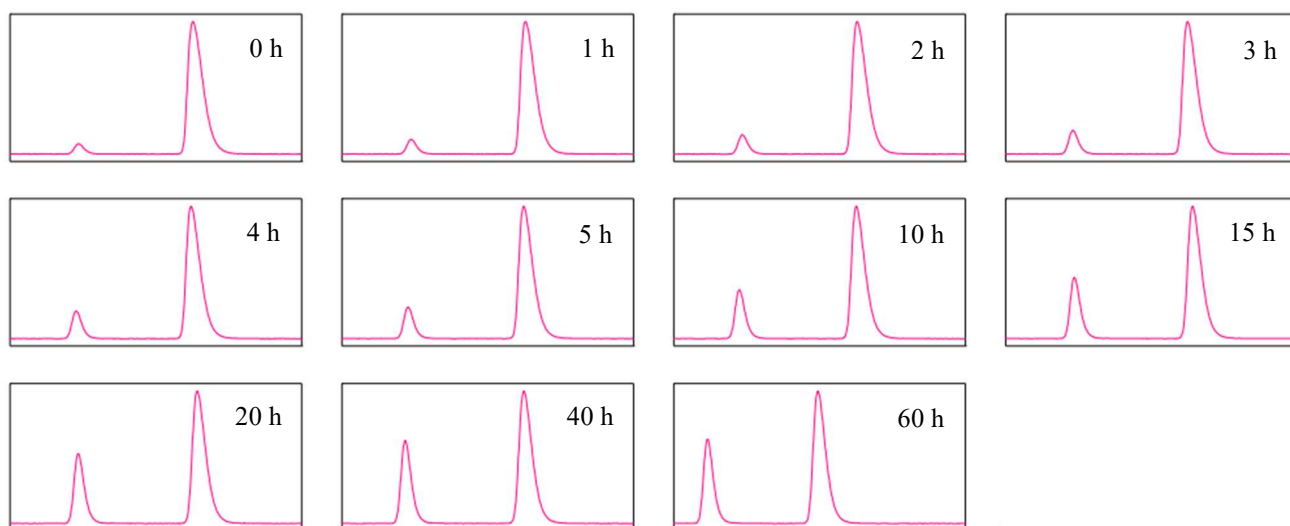
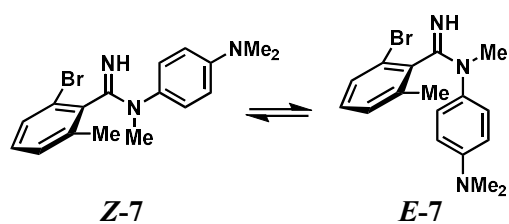


ratio

time (h)	<i>E</i>	<i>Z</i>
0	2.90	97.10
0.33	8.51	91.49
0.67	11.24	88.76
1	15.80	84.20
1.33	18.35	81.65
1.67	20.32	79.68
2	22.67	77.33
2.33	24.71	75.29
2.67	25.60	74.40
3	28.41	71.59
4.5	31.92	68.08
5	32.33	67.67
10	35.11	64.89
15	35.31	64.69

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

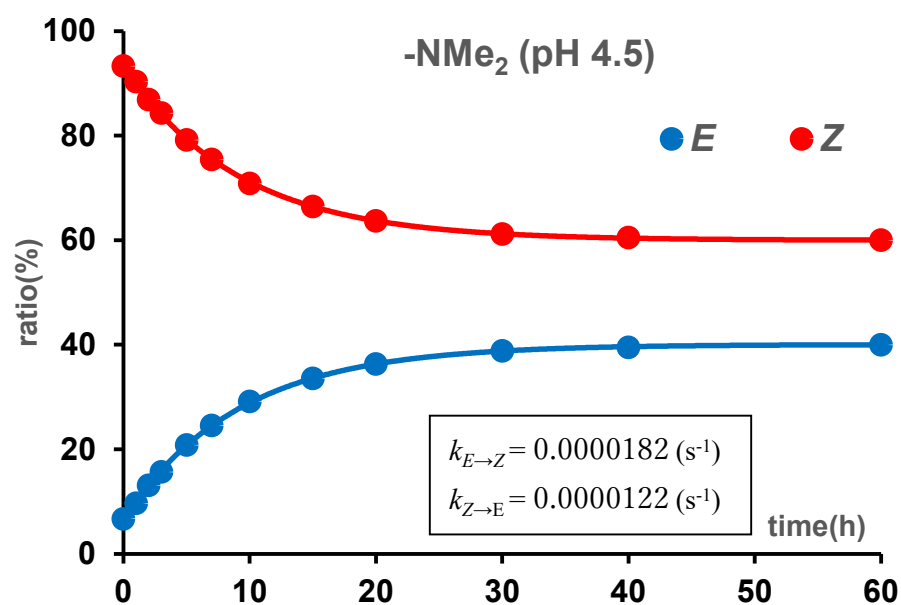
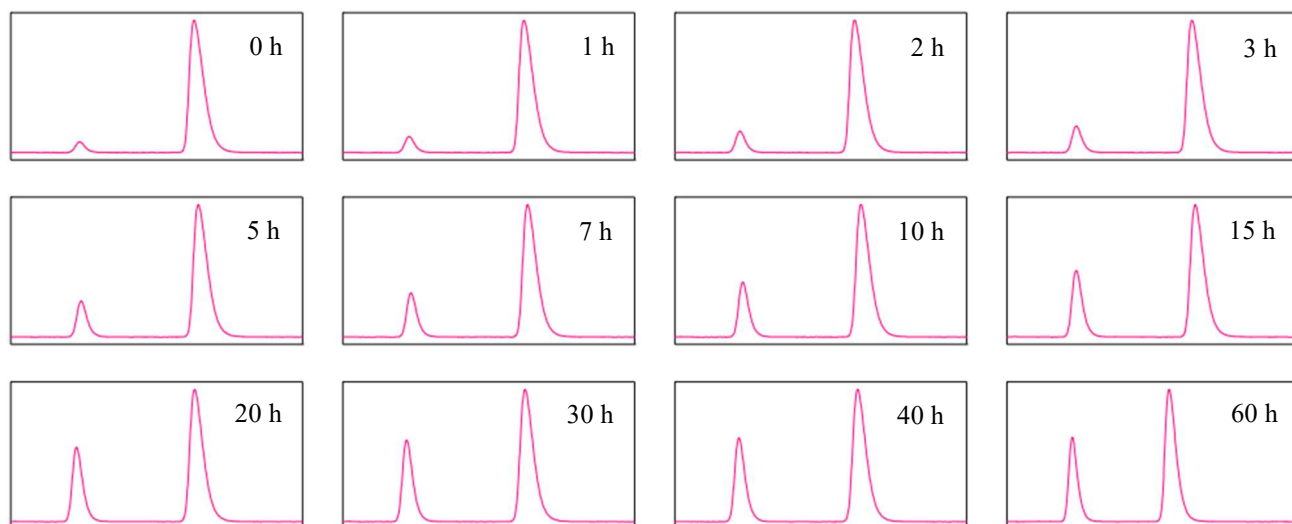
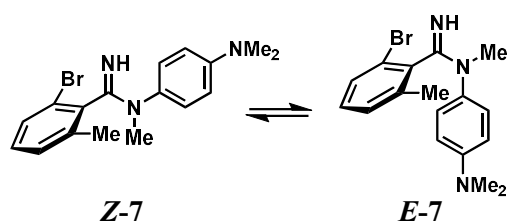
Figure S5.19. Isomerization of compound 6 at pH 5.5



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	5.96	94.04
1	8.94	91.06
2	11.72	88.28
3	14.10	85.90
4	16.16	83.84
5	18.19	81.81
10	26.36	73.64
15	31.22	68.78
20	34.71	65.29
40	39.15	60.85
60	39.78	60.22

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

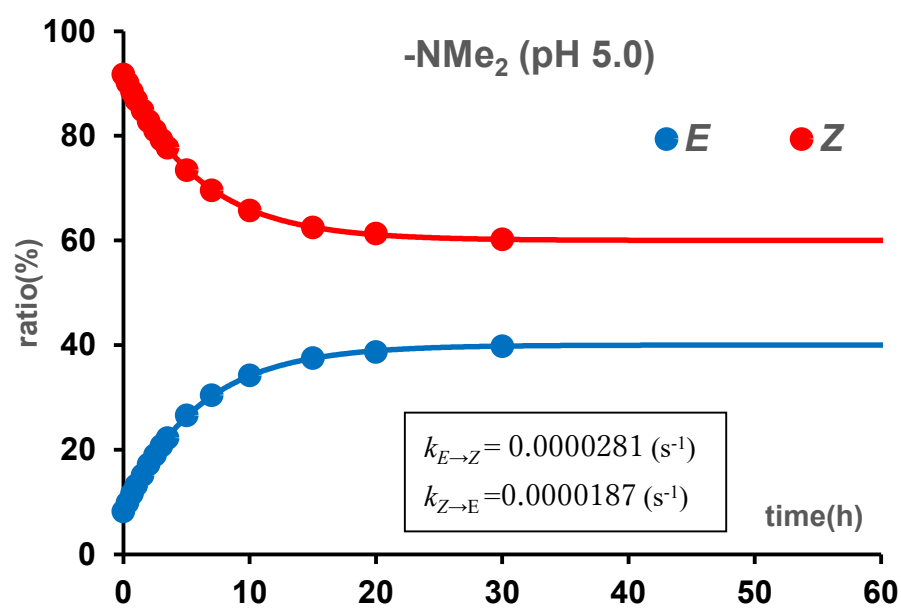
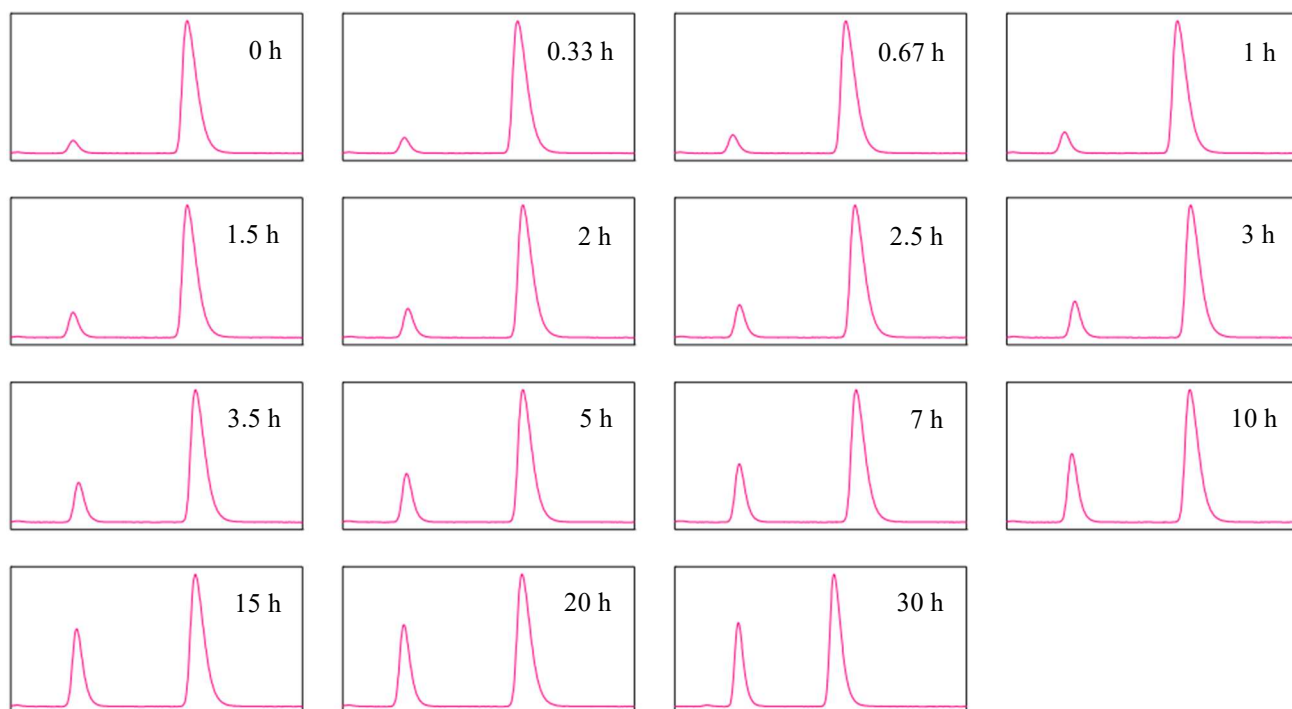
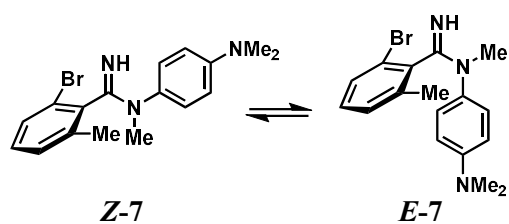
Figure S5.20. Isomerization of compound 7 at pH 4.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	6.67	93.33
1	9.69	90.31
2	13.11	86.89
3	15.67	84.33
5	20.85	79.15
7	24.59	75.41
10	29.13	70.87
15	33.57	66.43
20	36.32	63.68
30	38.85	61.15
40	39.51	60.49
60	39.97	60.03

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

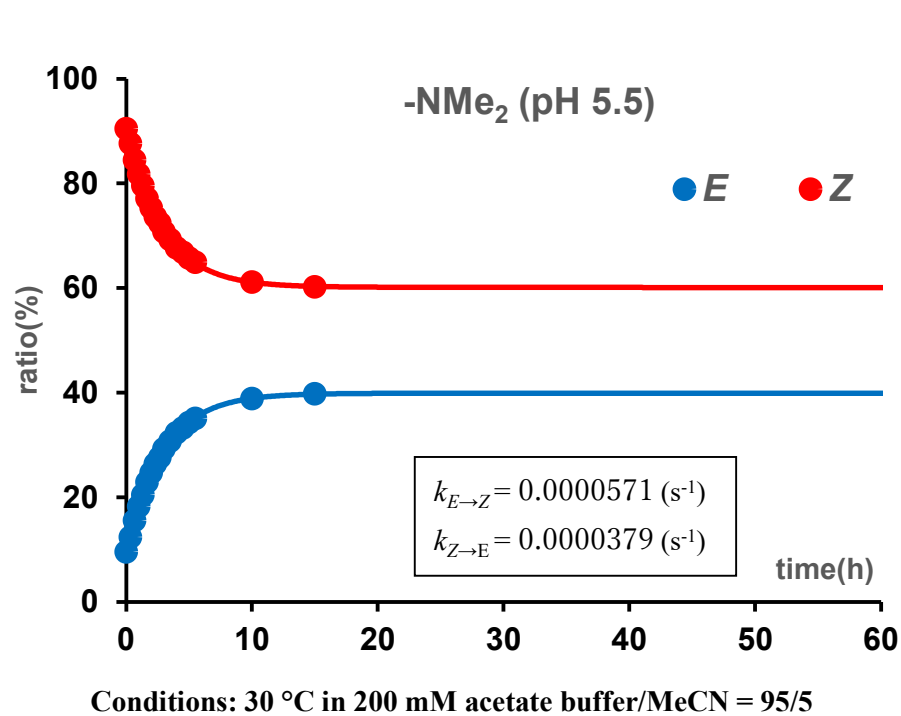
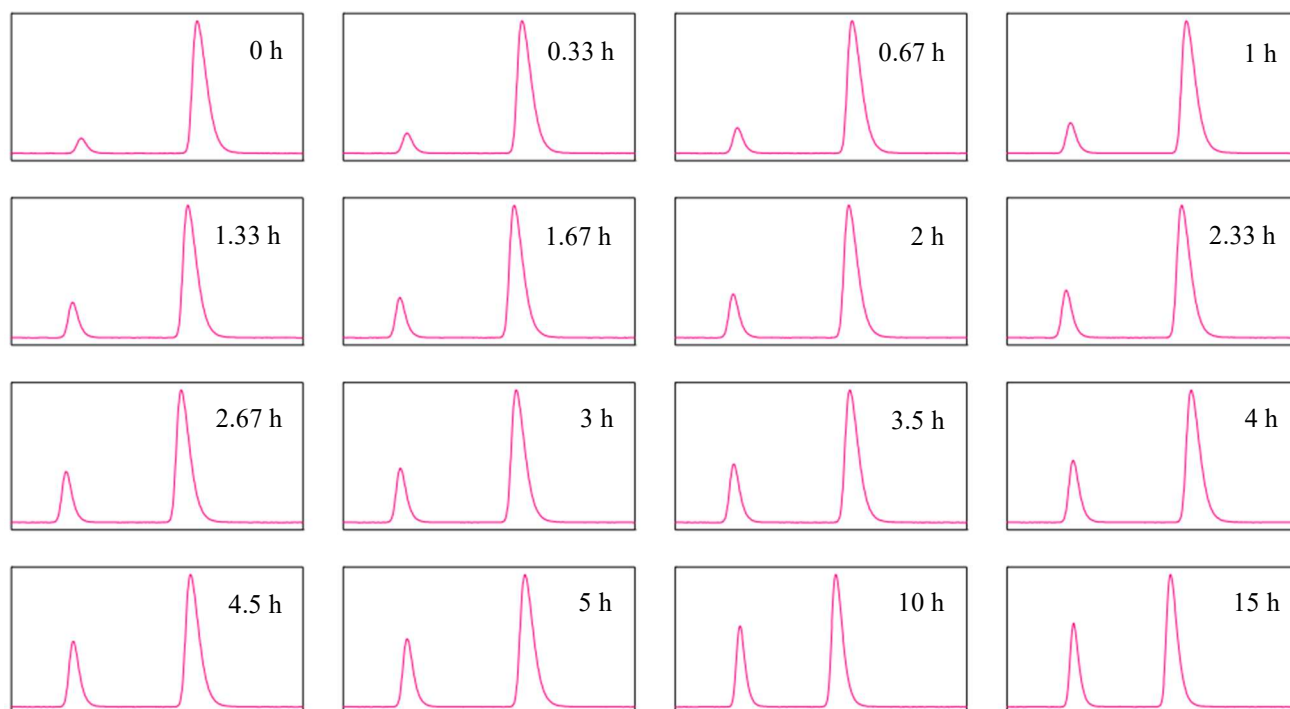
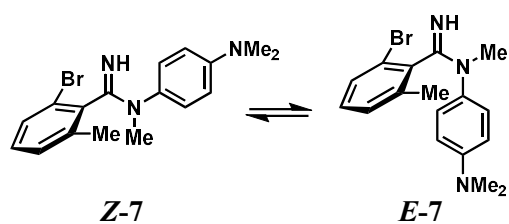
Figure S5.21. Isomerization of compound 7 at pH 4.5



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	8.23	91.77
0.33	9.83	90.17
0.67	11.53	88.47
1	13.16	86.84
1.5	15.11	84.89
2	17.18	82.82
2.5	18.95	81.05
3	20.74	79.26
3.5	22.24	77.76
5	26.58	73.42
7	30.42	69.58
10	34.24	65.76
15	37.50	62.50
20	38.69	61.31
30	39.79	60.21

Conditions: 30 °C in 200 mM acetate buffer/MeCN = 95/5

Figure S5.22. Isomerization of compound 7 at pH 5.0



ratio		
time (h)	<i>E</i>	<i>Z</i>
0	9.52	90.48
0.33	12.36	87.64
0.67	15.54	84.46
1	18.22	81.78
1.33	20.46	79.54
1.67	22.86	77.14
2	24.66	75.34
2.33	26.39	73.61
2.67	27.59	72.41
3	29.27	70.73
3.5	30.70	69.30
4	32.32	67.68
4.5	33.20	66.80
5	34.28	65.72
10	38.85	61.15
15	39.79	60.21

Figure S5.23. Isomerization of compound 7 at pH 5.5

6. Monitoring of the racemization

Prior to monitoring of the racemization, racemic amidine **1** was purified by chiral RP-HPLC to afford each enantiomer, which was used to the following investigation (Figure S6.1-6.3).

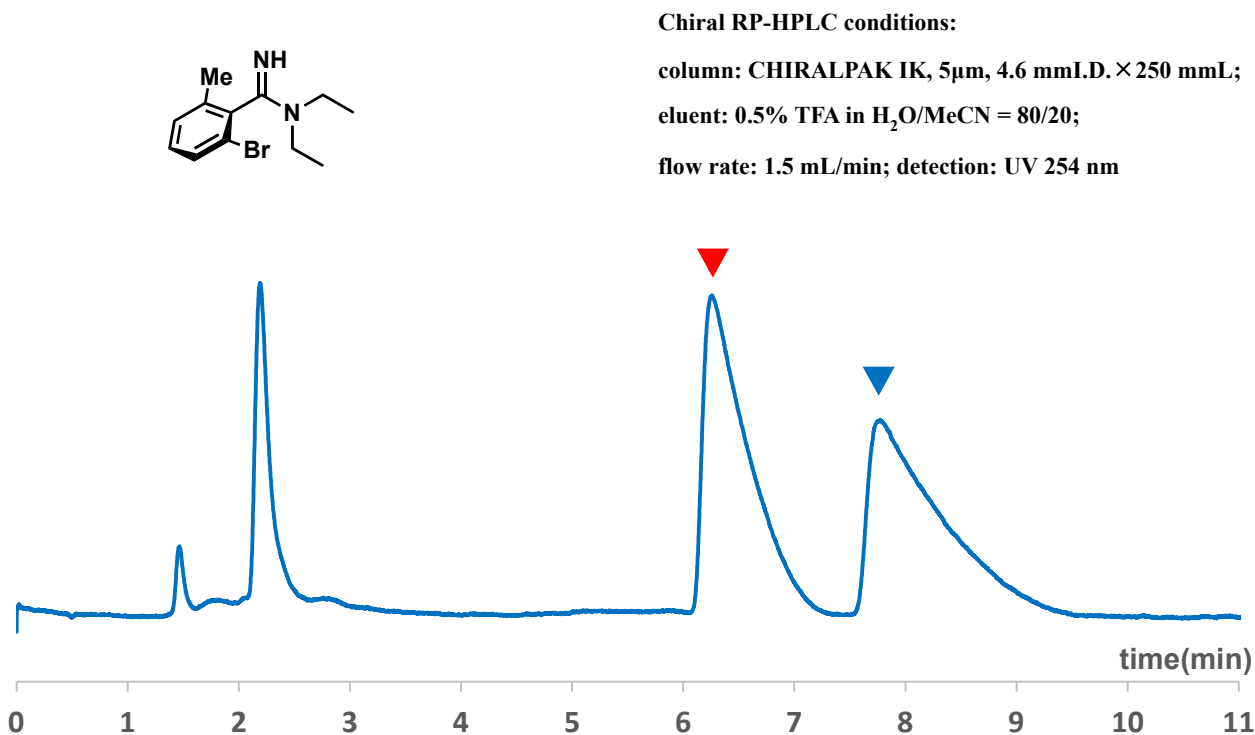


Figure S6.1. Chiral HPLC chart of racemic compound **1**

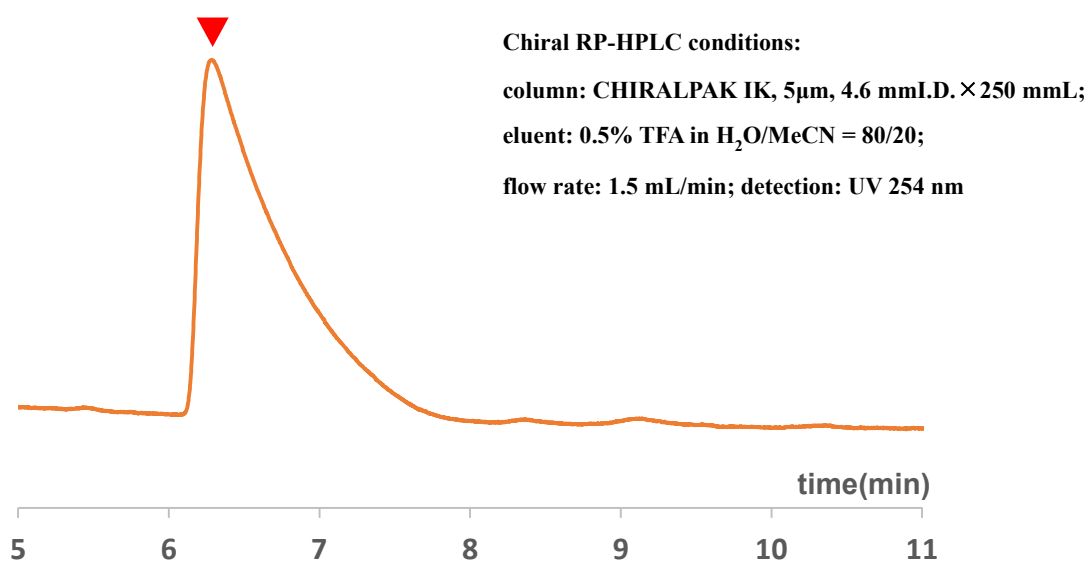


Figure S6.2. Reanalysis of the 1st eluted enantiomer of compound **1**

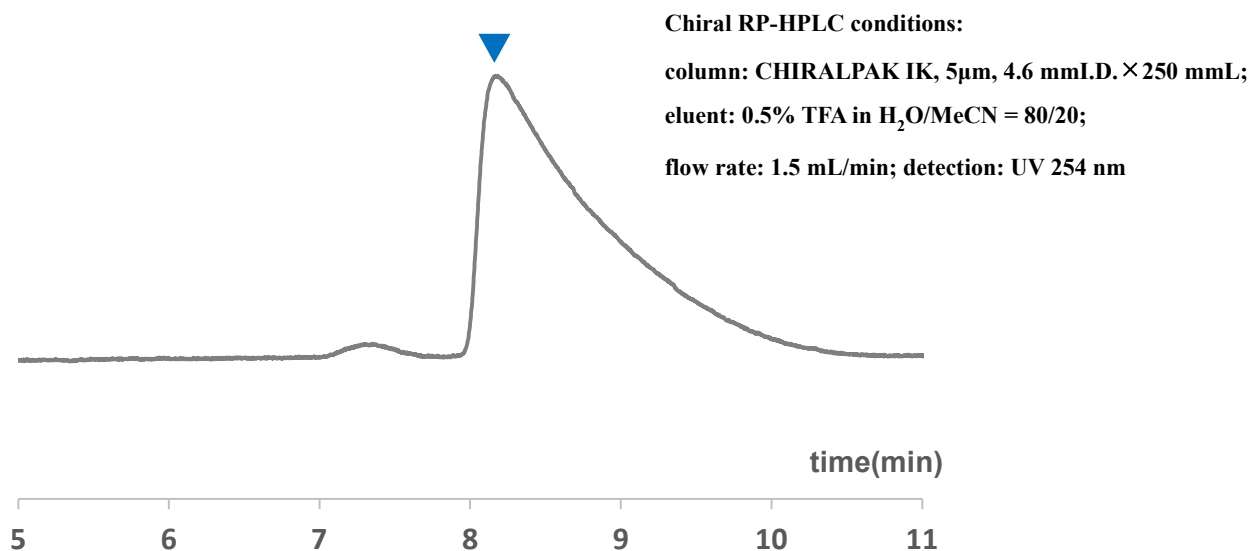


Figure S6.3. Reanalysis of the 2nd eluted enantiomer of compound 1

After the separation of each enantiomer, a solution of the enantiomer that elutes first was concentrated in *vacuo*, and diluted with a buffer (pH =9.2, 10.3, 11.7 or 12.7) and MeCN (final ratio: buffer/MeCN = 9/1). The resulting solution was maintained at 70 °C in an incubator. The progress of the racemization was monitored by chiral RP-HPLC analysis (detailed conditions were described below).

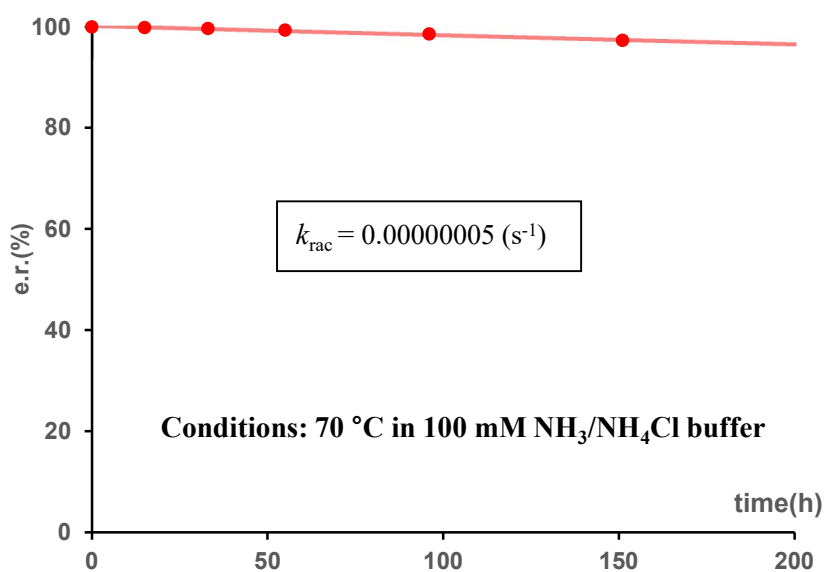
The ratio of each isomer was plotted as a function of time, and curve-fitting analysis of the experimental results using differential equations describing the isomerization (eq. 4, 5), to calculate kinetic constant k (Figure S6.4-6.7).

$$\frac{d[1st]}{dt} = k_{rac}[2nd] - k_{rac}[1st] \text{ (eq. 4)}$$

$$\frac{d([1st]+[2nd])}{dt} = 0 \text{ (eq. 5)}$$

Chiral RP-HPLC conditions for the racemization experiments

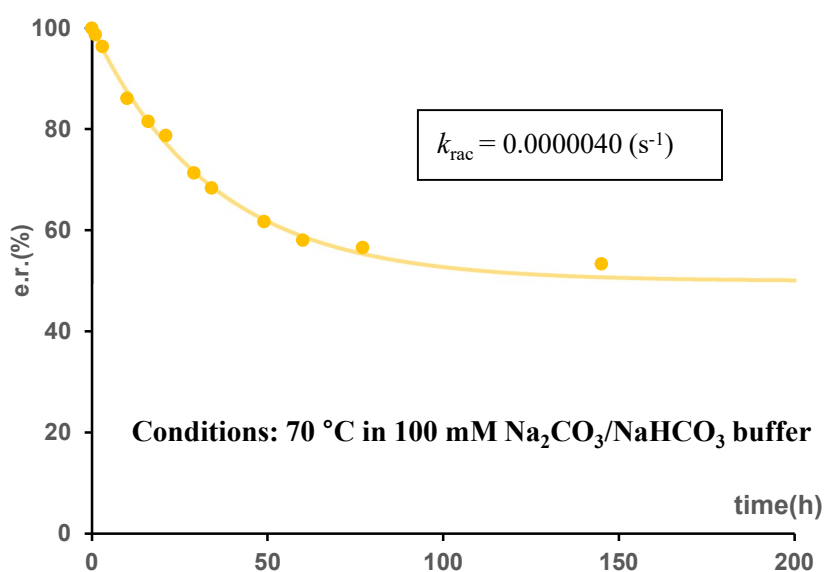
column: CHIRALPAK IK, 5µm, 4.6 mmI.D. × 250 mmL, flow rate: 1.2 mL/min, eluent: A; 0.5% TFA in H₂O, B; MeCN, 30% B in A isocratic elusion, detection: UV (254 nm)



ratio

time(h)	1st	2nd
0	100.00	0.00
15	99.84	0.16
33	99.64	0.36
55	99.33	0.67
96	98.60	1.40
151	97.32	2.68
220	95.87	4.13
342	94.80	5.21

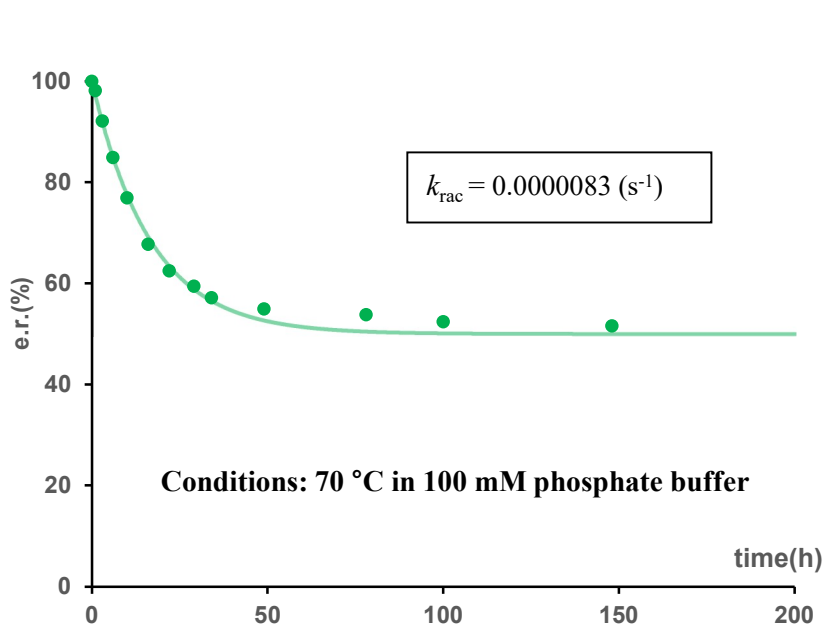
Figure S6.4. Racemization of compound **1** at pH 9.2



ratio

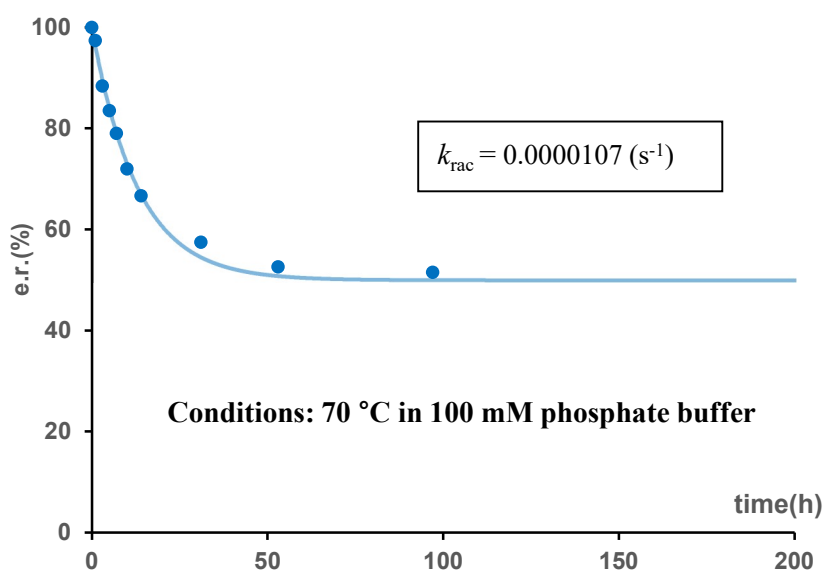
time(h)	1st	2nd
0	100.00	0.00
1	98.70	1.30
3	96.36	3.64
10	86.09	13.91
16	81.57	18.43
21	78.76	21.24
29	71.37	28.63
34	68.37	31.63
49	61.69	38.31
60	58.07	41.93
77	56.59	43.41
145	53.33	46.67

Figure S6.5. Racemization of compound **1** at pH 10.3



ratio		
time(h)	1st	2nd
0	100.00	0.00
1	98.16	1.84
3	92.15	7.85
6	84.93	15.07
10	76.97	23.03
16	67.80	32.21
22	62.52	37.49
29	59.47	40.53
34	57.22	42.79
49	54.97	45.03
78	53.85	46.15
100	52.46	47.54
148	51.63	48.37

Figure S6.6. Racemization of compound **1** at pH 11.7



ratio		
time(h)	1st	2nd
0	100.00	0.00
1	97.45	2.55
3	88.40	11.60
5	83.52	16.48
7	79.07	20.93
10	72.02	27.98
14	66.68	33.32
31	57.50	42.50
53	52.65	47.35
97	51.57	48.44

Figure S6.7. Racemization of compound **1** at pH 12.7

Chiral RP-HPLC charts at each time point

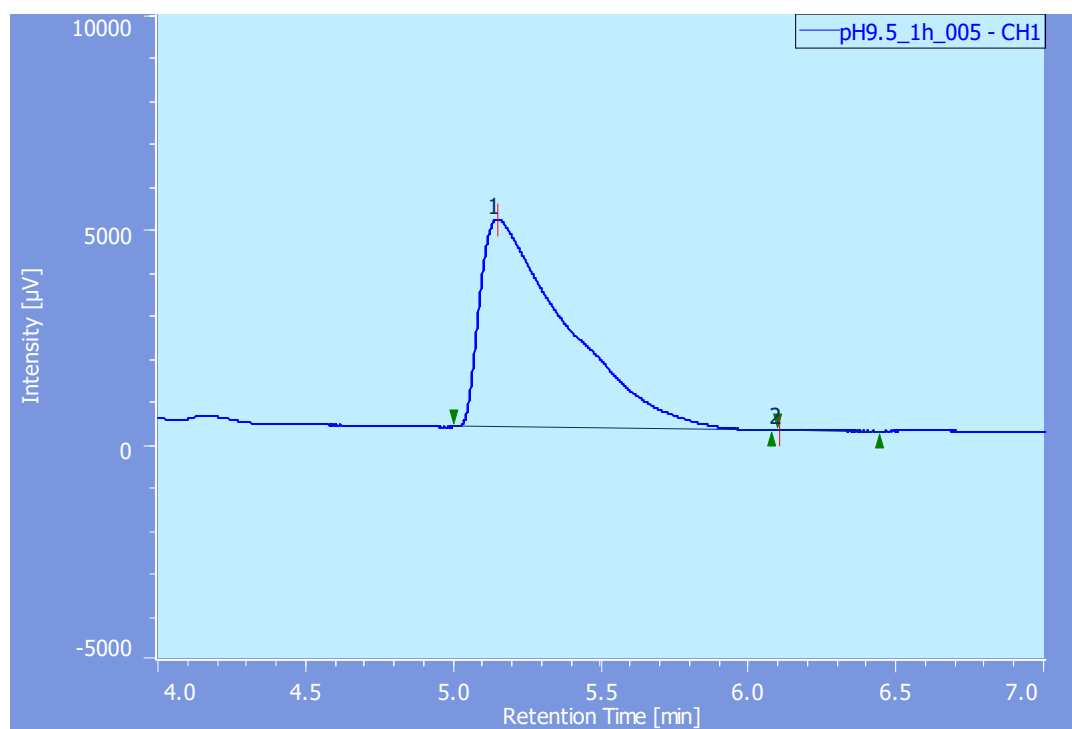


Figure S6.8. HPLC chart of **1** at pH 9.2 (0 h after heating)

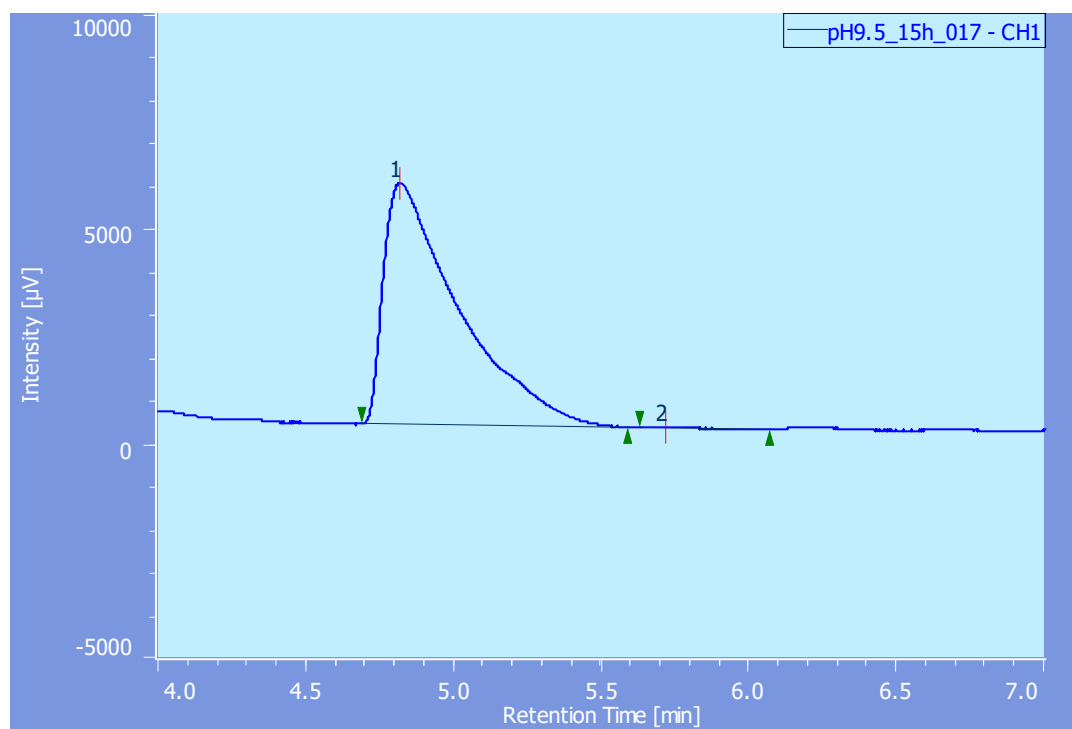


Figure S6.9. HPLC chart of **1** at pH 9.2 (15 h after heating)

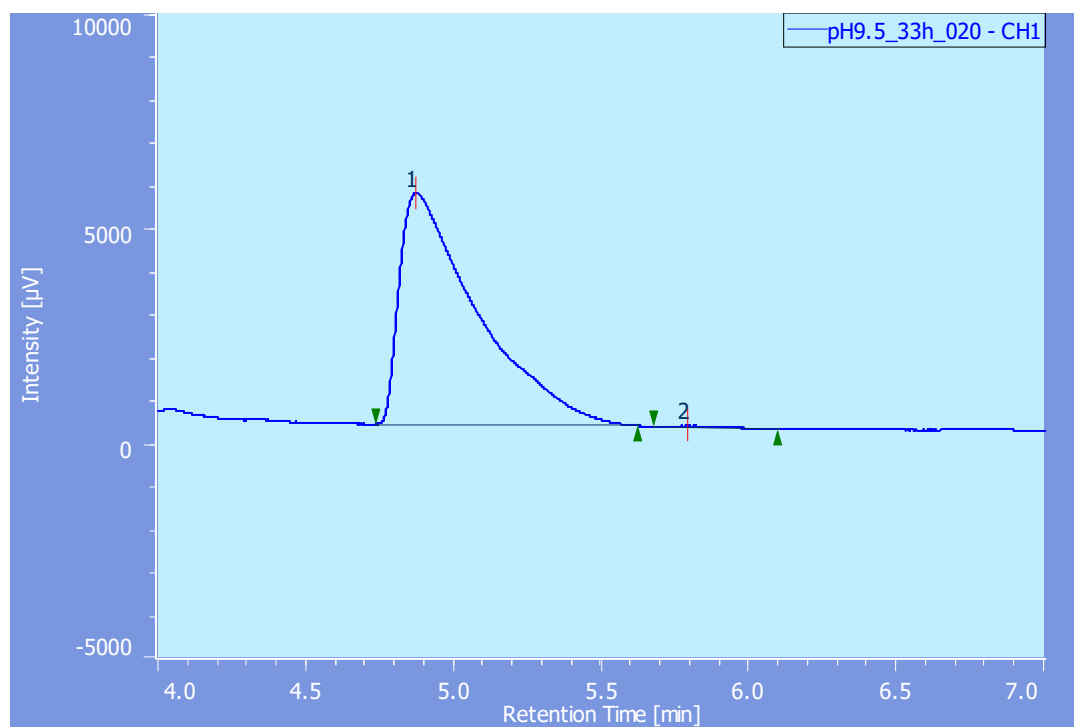


Figure S6.10. HPLC chart of **1** at pH 9.2 (33 h after heating)

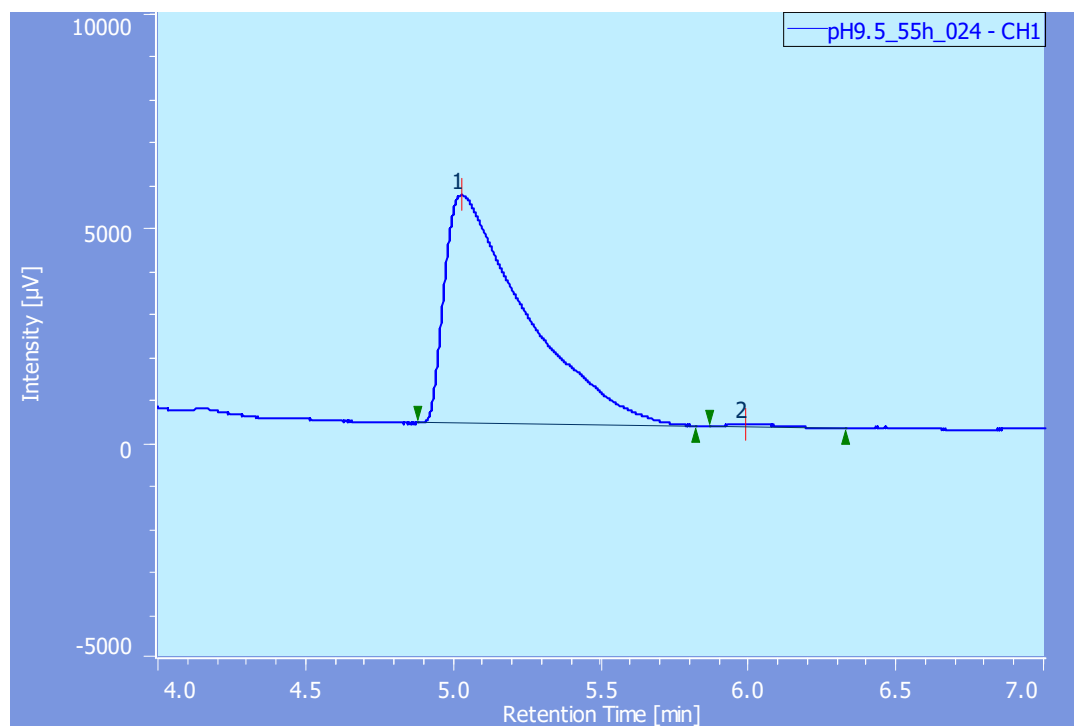


Figure S6.11. HPLC chart of **1** at pH 9.2 (55 h after heating)

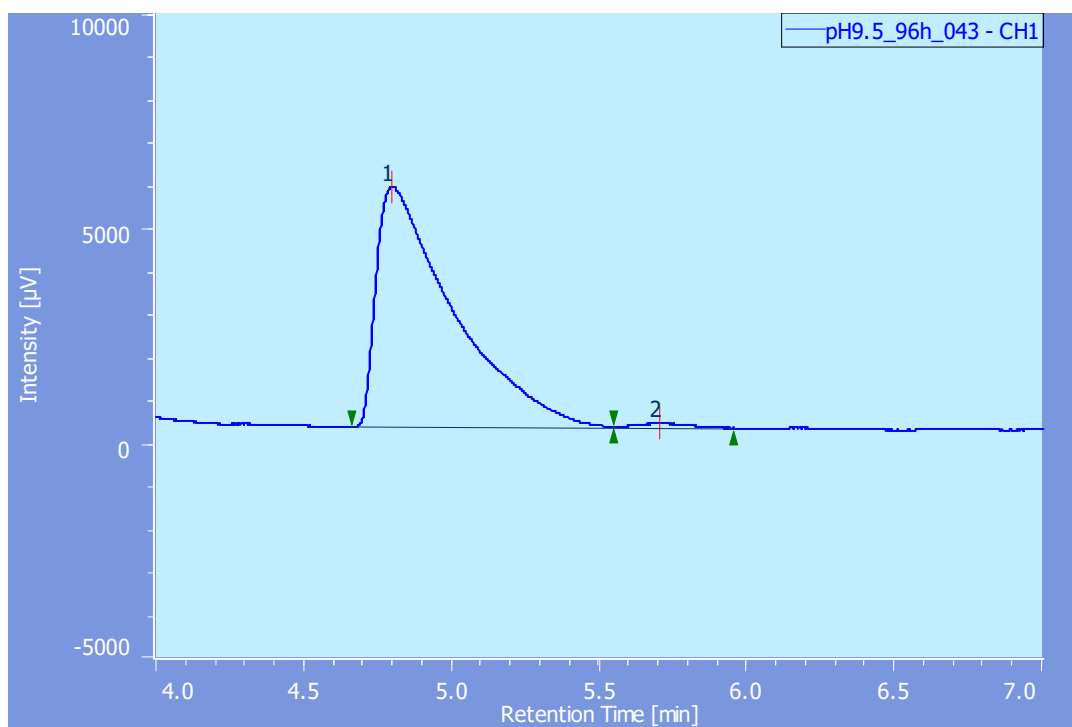


Figure S6.12. HPLC chart of **1** at pH 9.2 (96 h after heating)

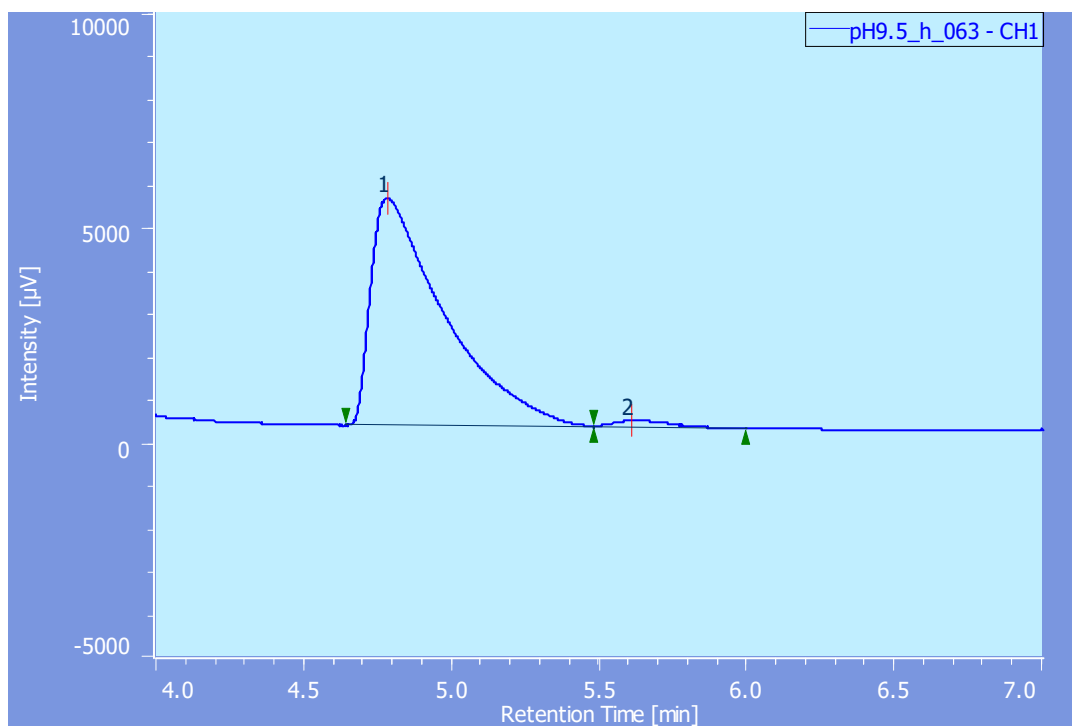


Figure S6.13. HPLC chart of **1** at pH 9.2 (151 h after heating)

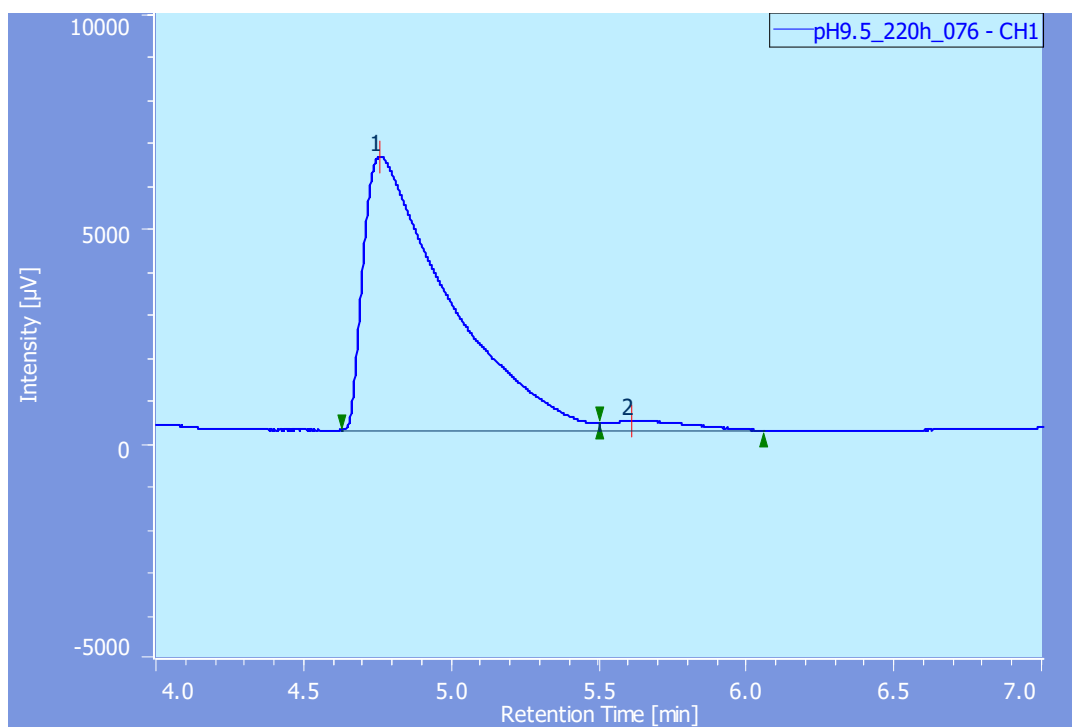


Figure S6.14. HPLC chart of **1** at pH 9.2 (220 h after heating)

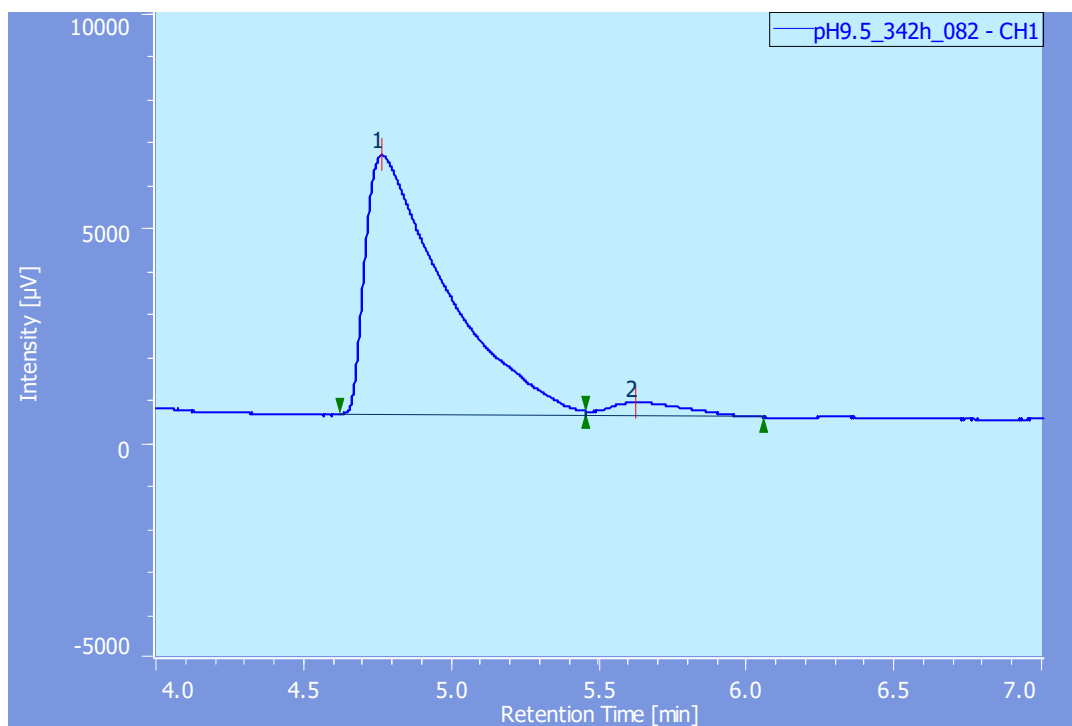


Figure S6.15. HPLC chart of **1** at pH 9.2 (342 h after heating)

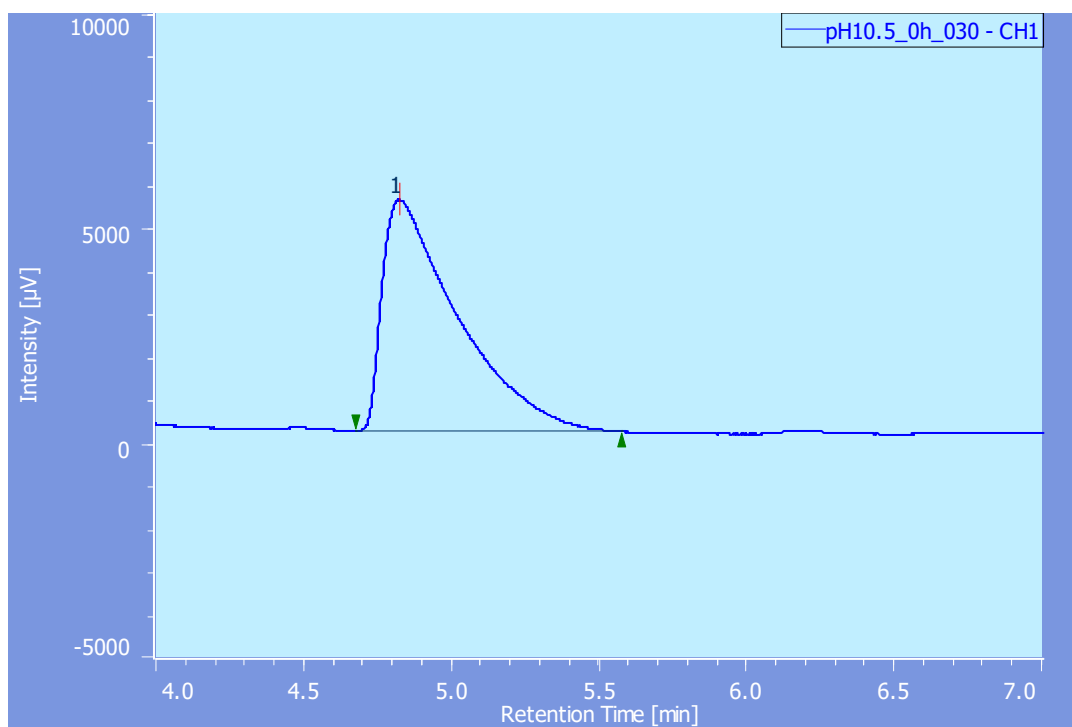


Figure S6.16. HPLC chart of **1** at pH 10.3 (0 h after heating)

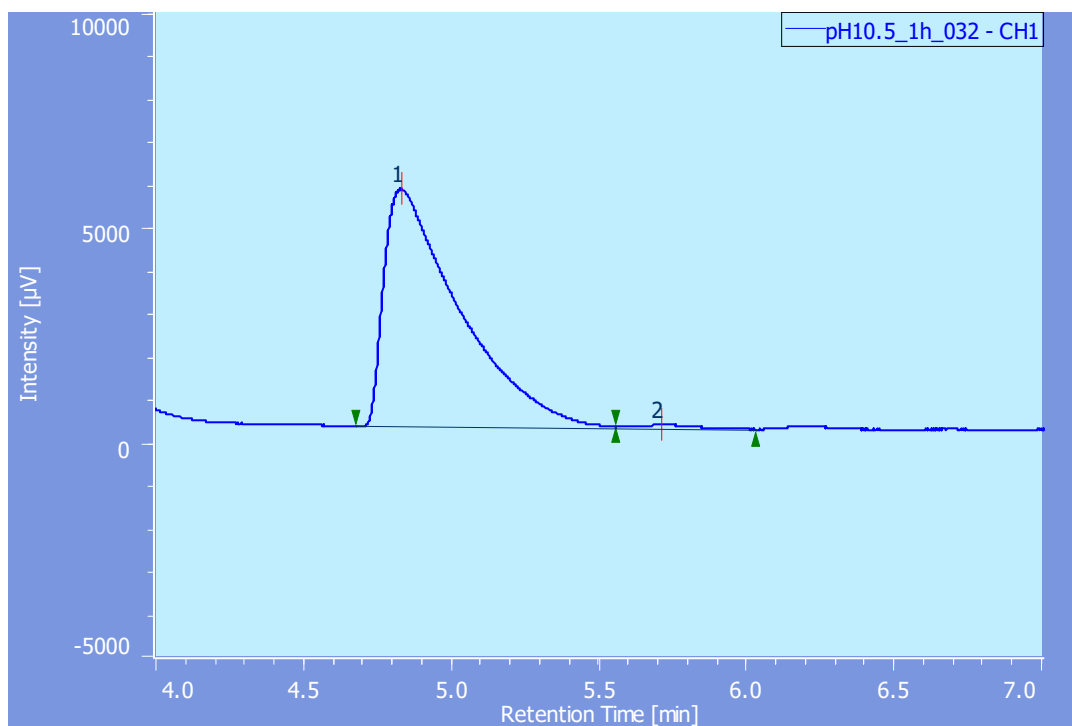


Figure S6.17. HPLC chart of **1** at pH 10.3 (1 h after heating)

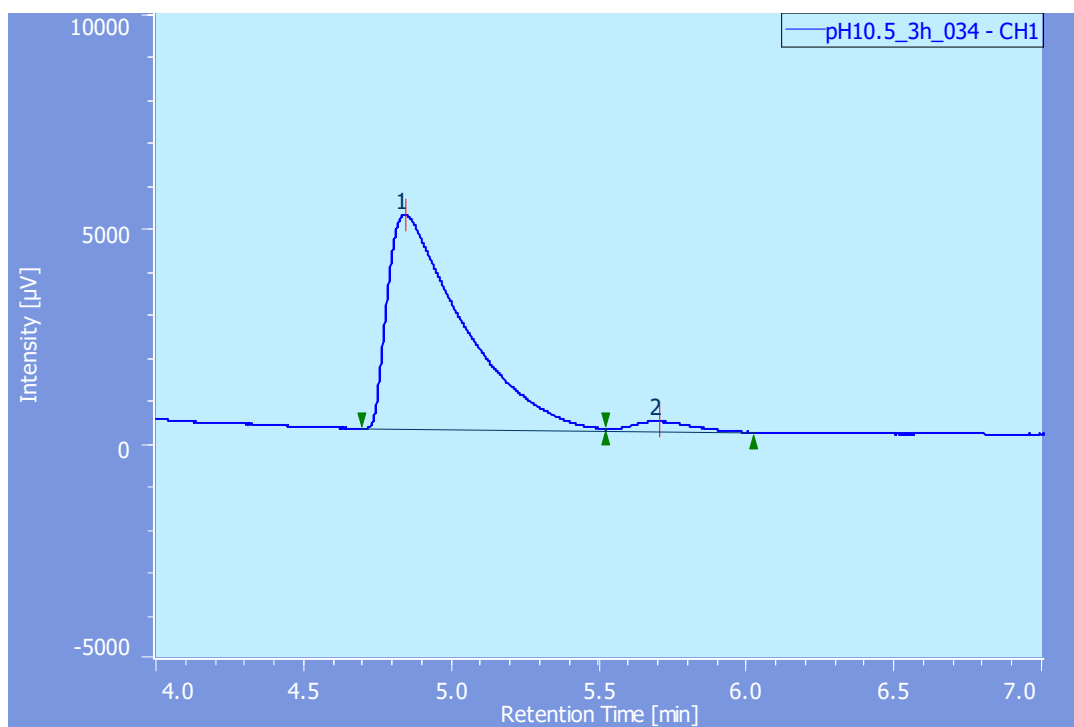


Figure S6.18. HPLC chart of **1** at pH 10.3 (3 h after heating)

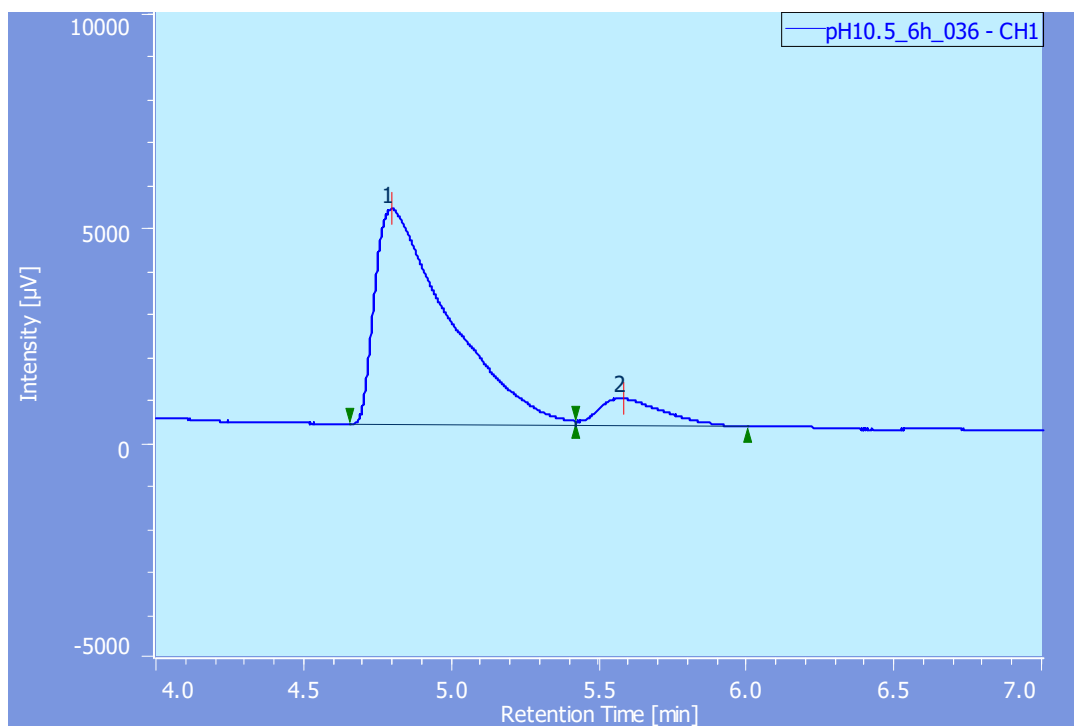


Figure S6.19. HPLC chart of **1** at pH 10.3 (6 h after heating)

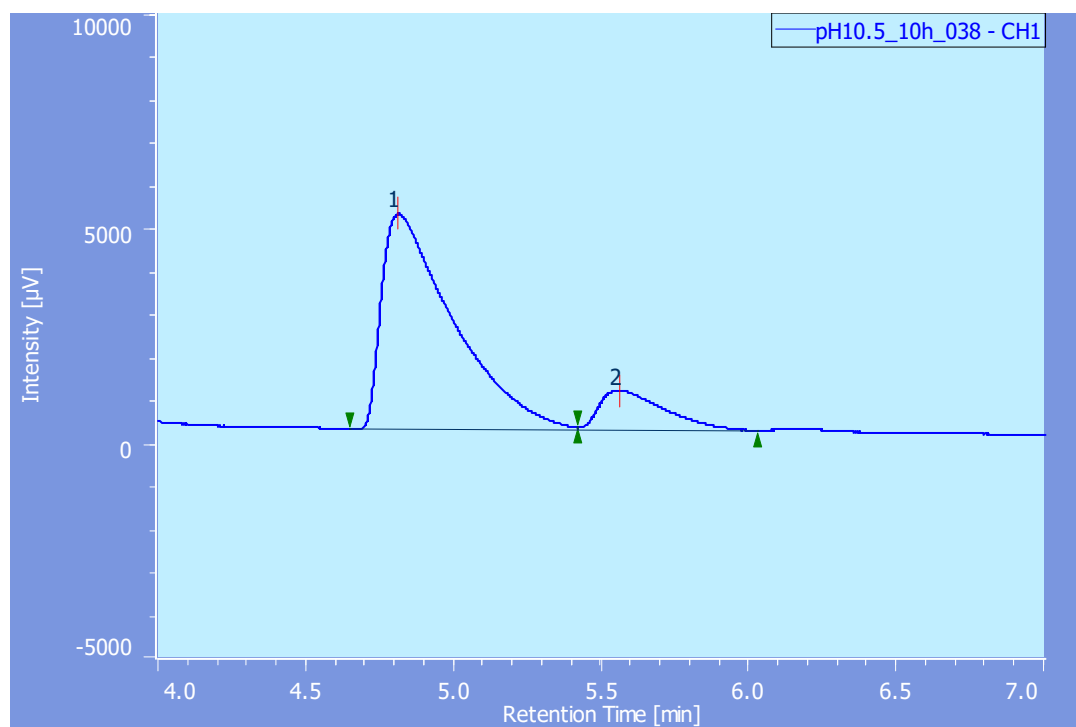


Figure S6.20. HPLC chart of **1** at pH 10.3 (10 h after heating)

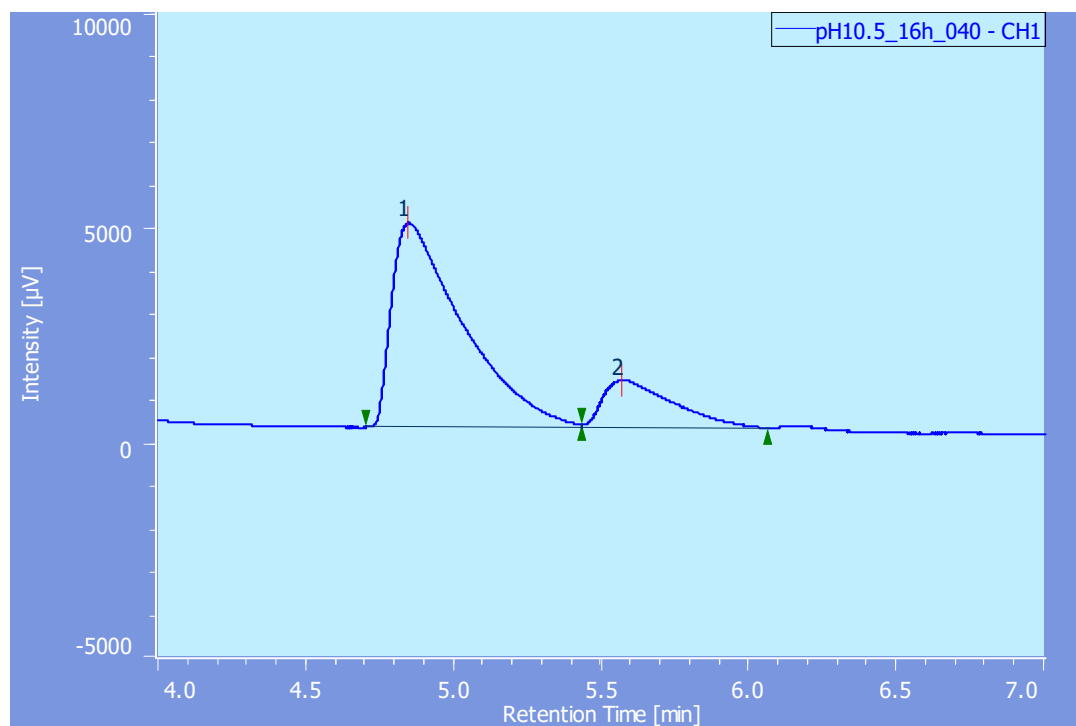


Figure S6.21. HPLC chart of **1** at pH 10.3 (16 h after heating)

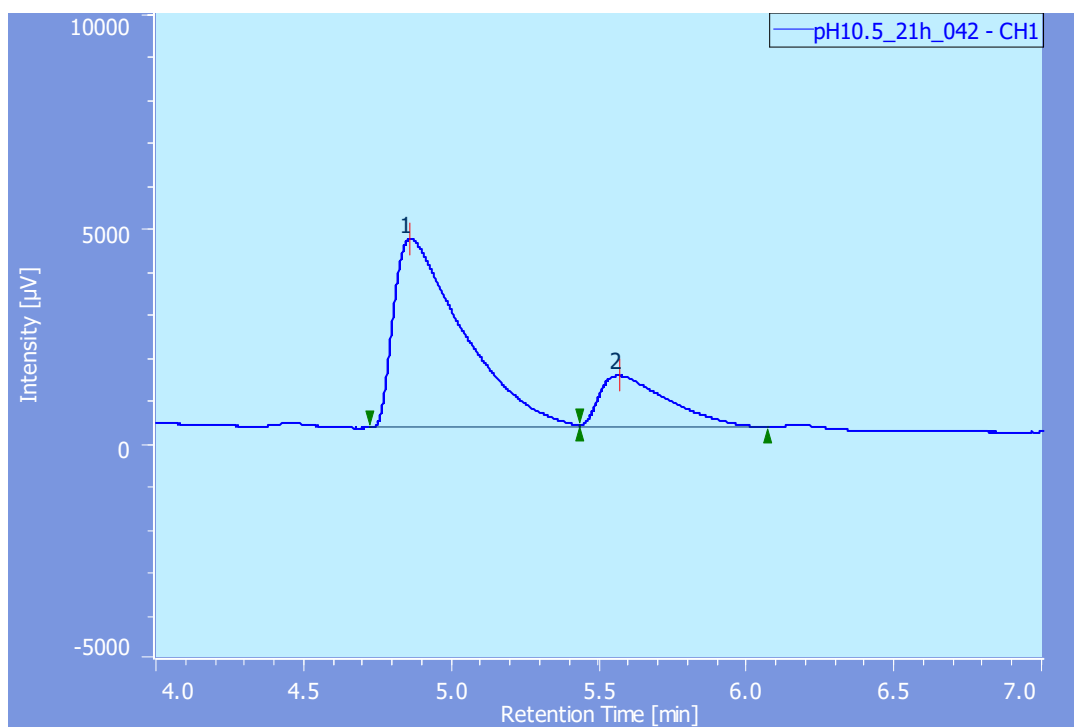


Figure S6.22. HPLC chart of **1** at pH 10.3 (21 h after heating)

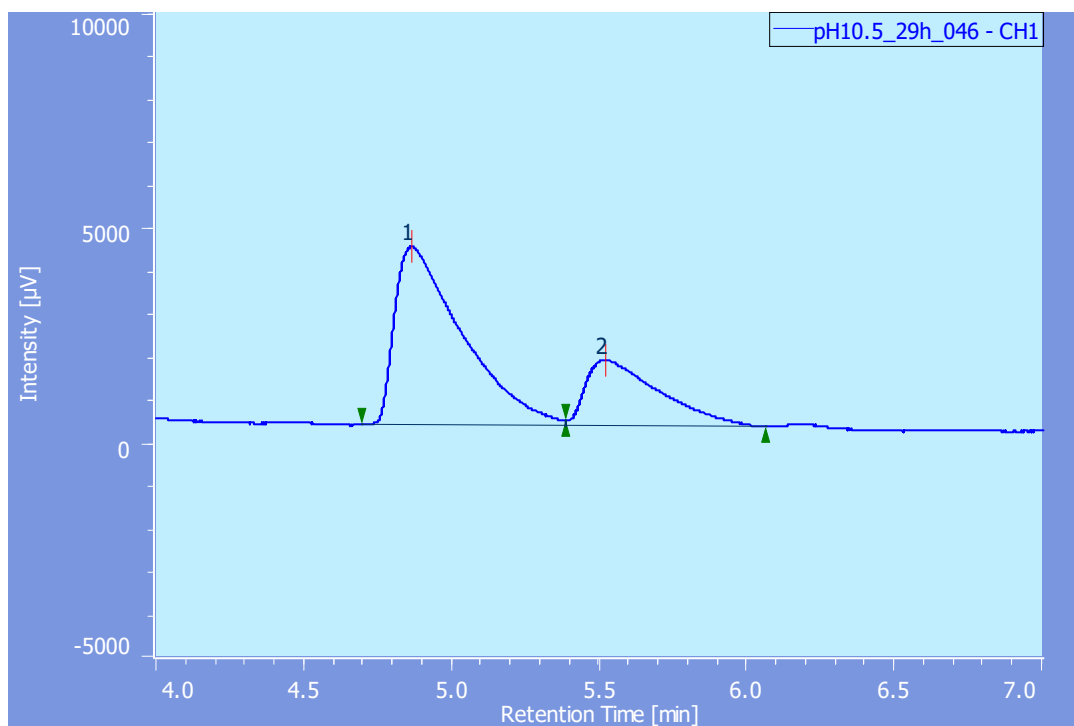


Figure S6.23. HPLC chart of **1** at pH 10.3 (29 h after heating)

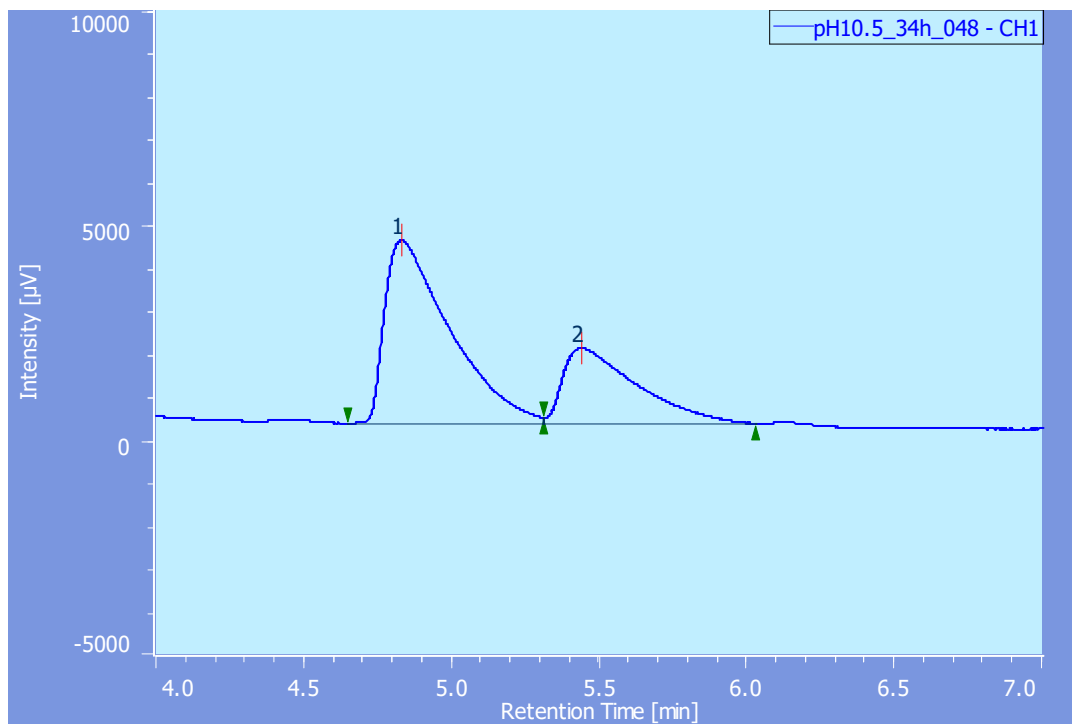


Figure S6.24. HPLC chart of **1** at pH 10.3 (34 h after heating)

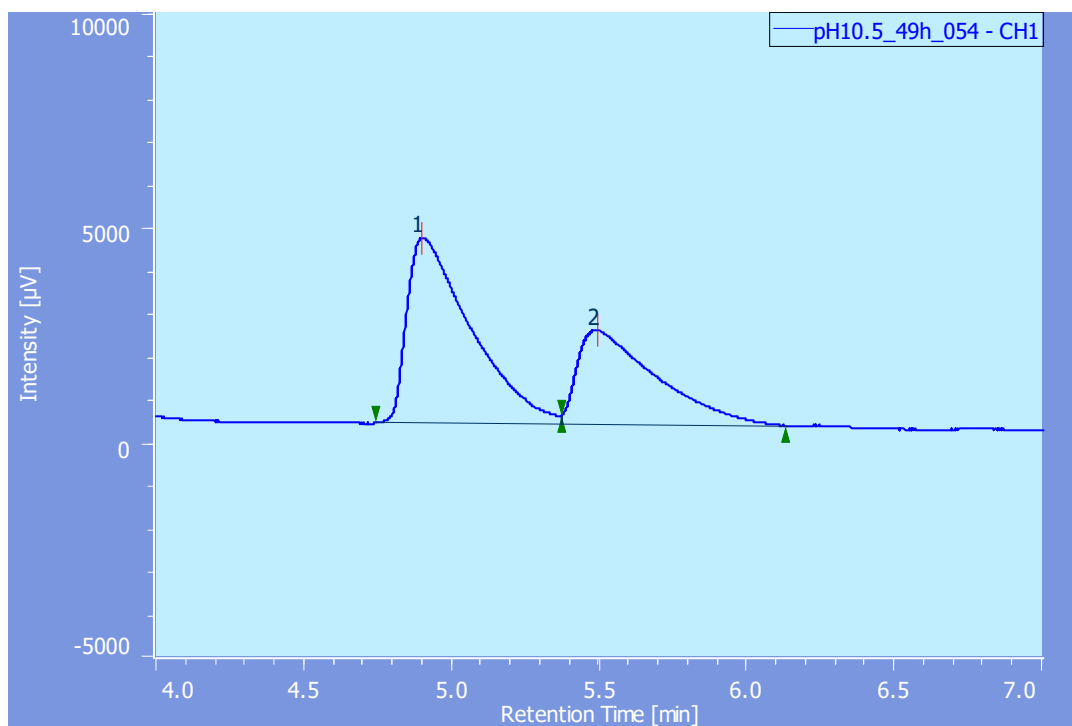


Figure S6.25. HPLC chart of **1** at pH 10.3 (49 h after heating)

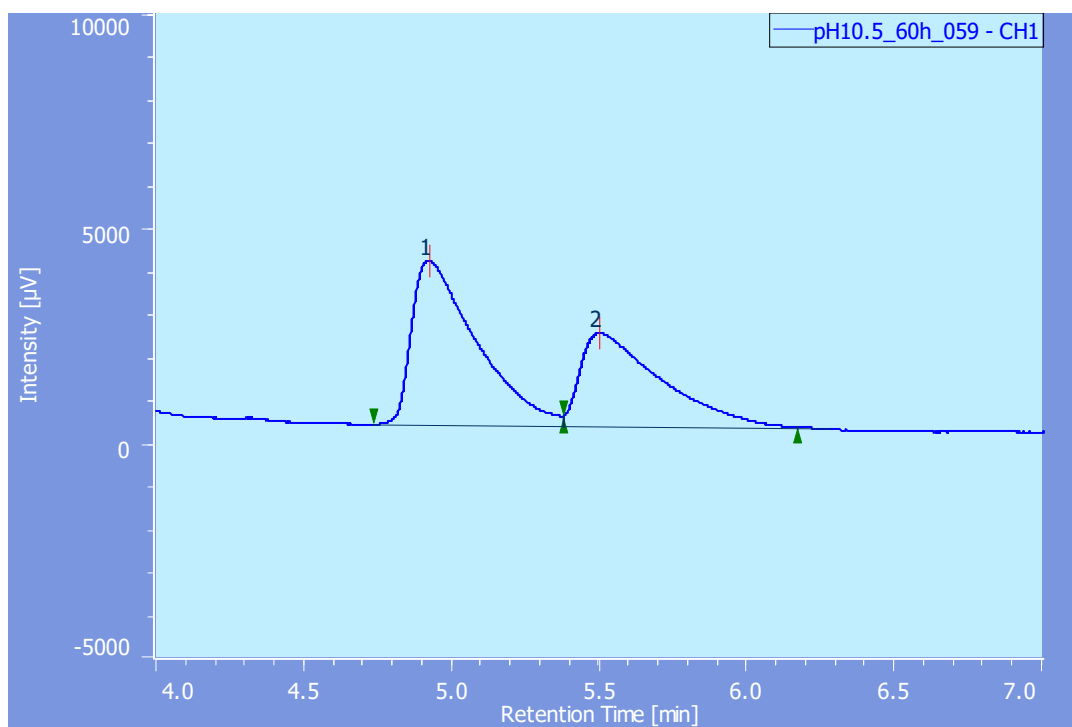


Figure S6.26. HPLC chart of **1** at pH 10.3 (60 h after heating)

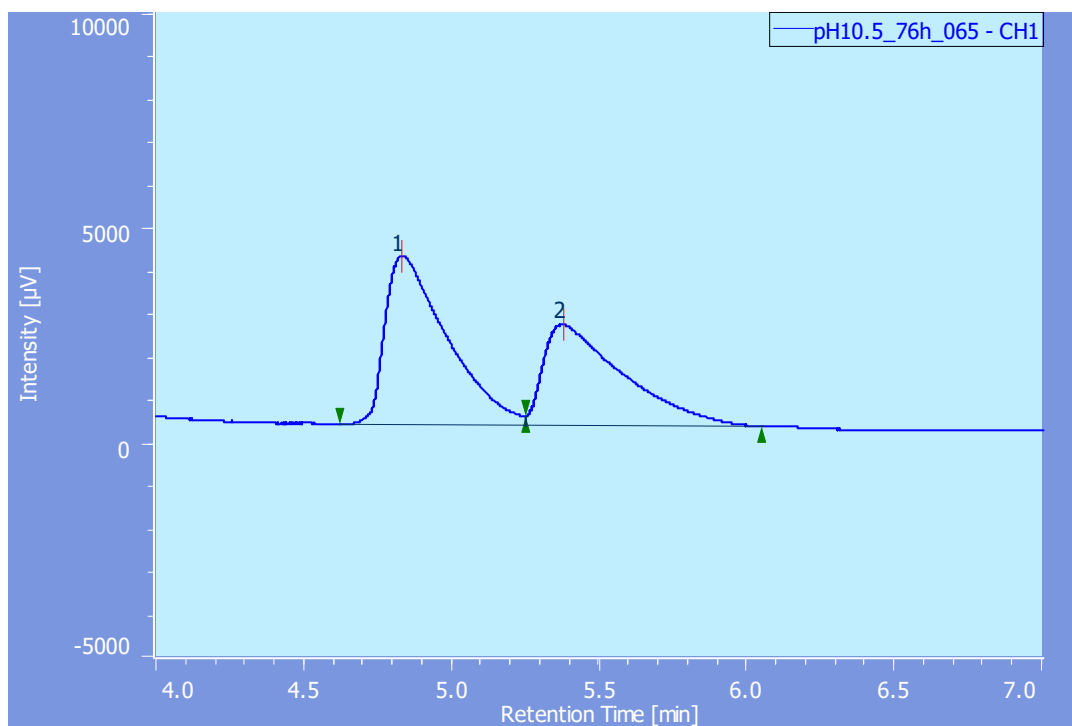


Figure S6.27. HPLC chart of **1** at pH 10.3 (76 h after heating)

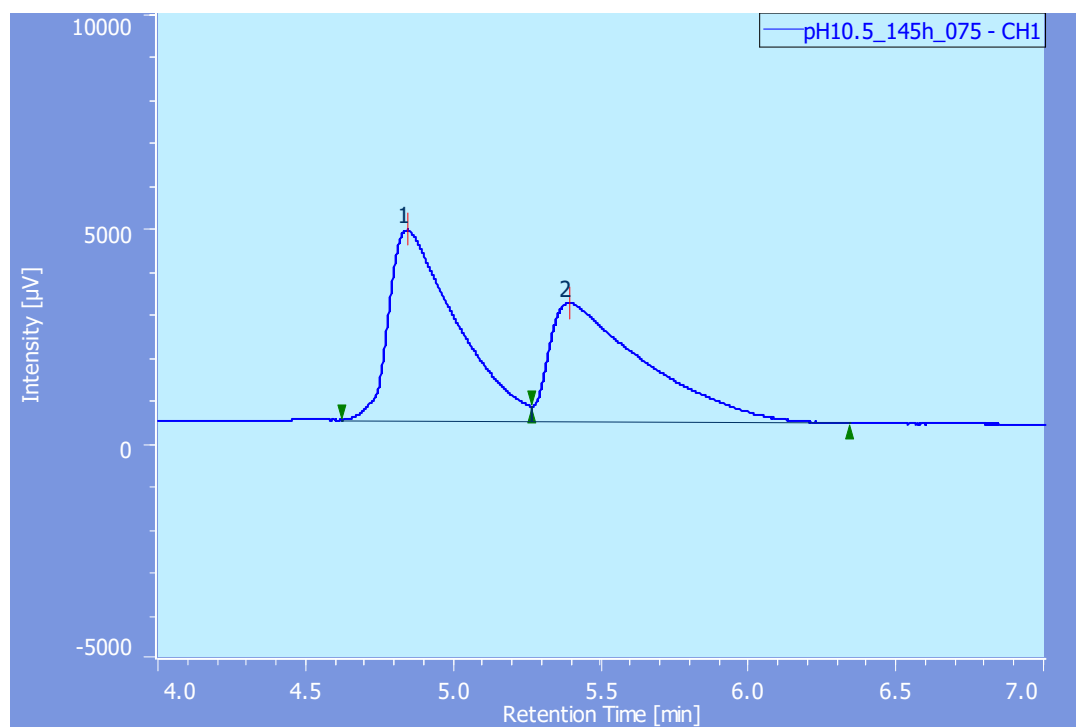


Figure S6.28. HPLC chart of **1** at pH 10.3 (145 h after heating)

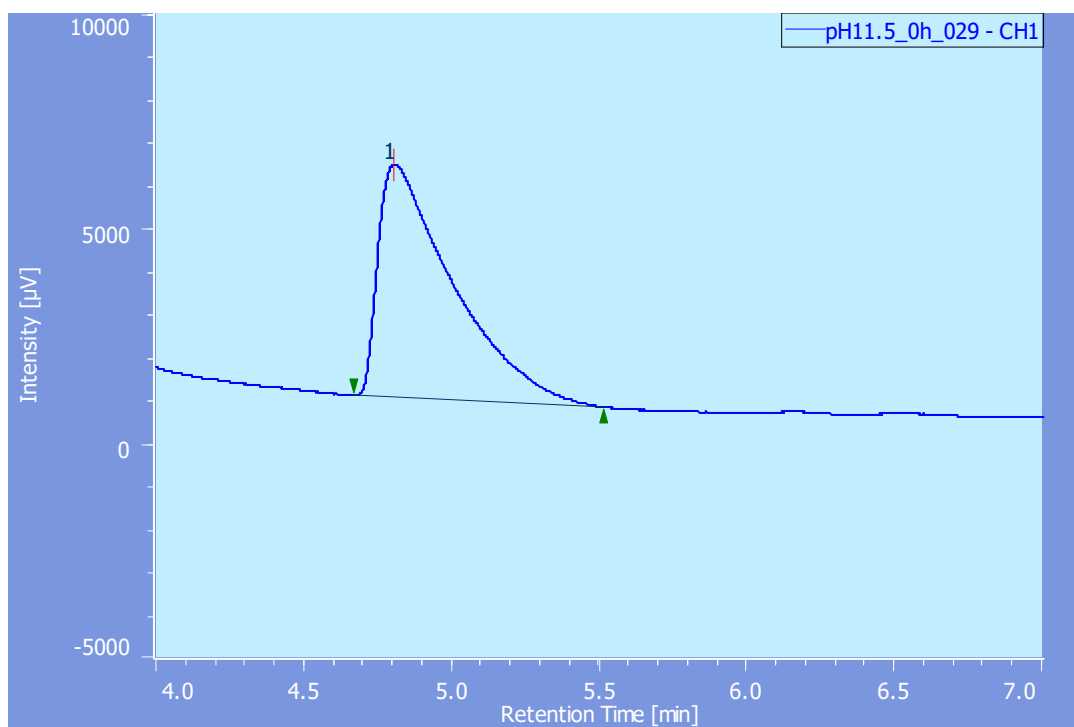


Figure S6.29. HPLC chart of **1** at pH 11.7 (0 h after heating)

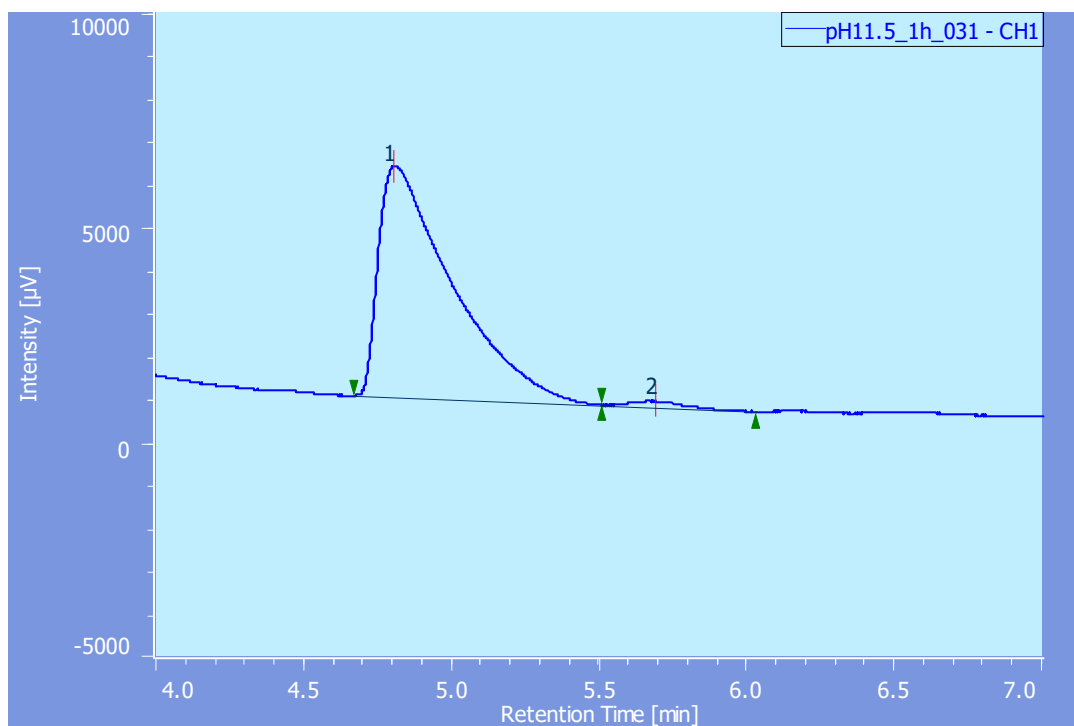


Figure S6.30. HPLC chart of **1** at pH 11.7 (1 h after heating)

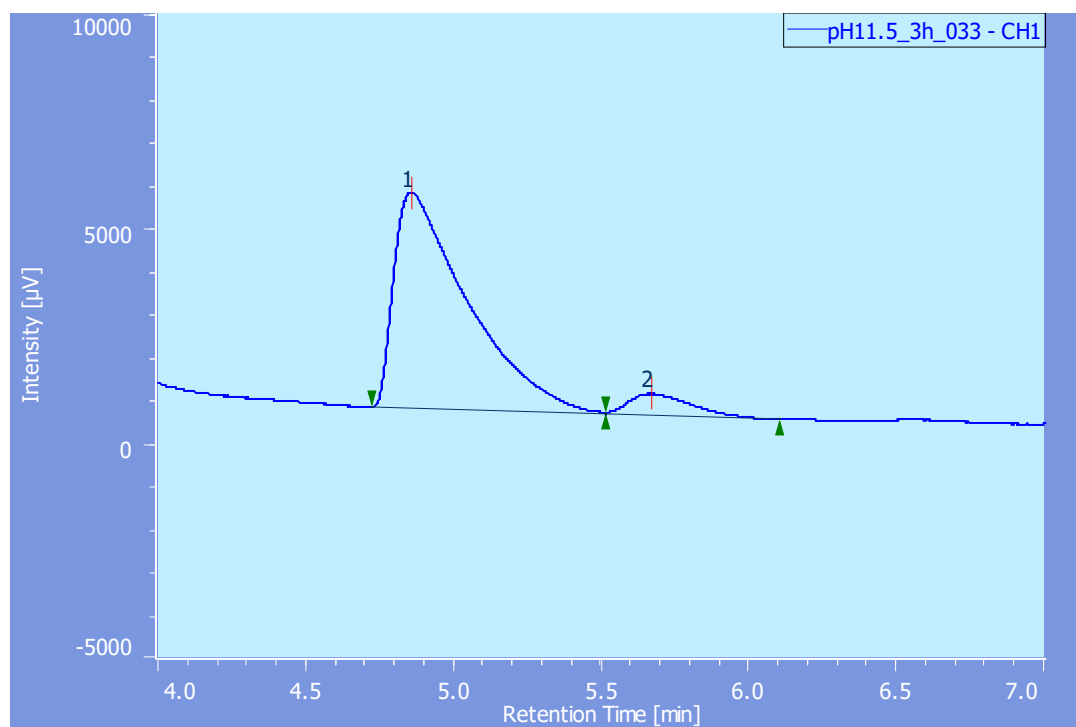


Figure S6.31. HPLC chart of **1** at pH 11.7 (3 h after heating)

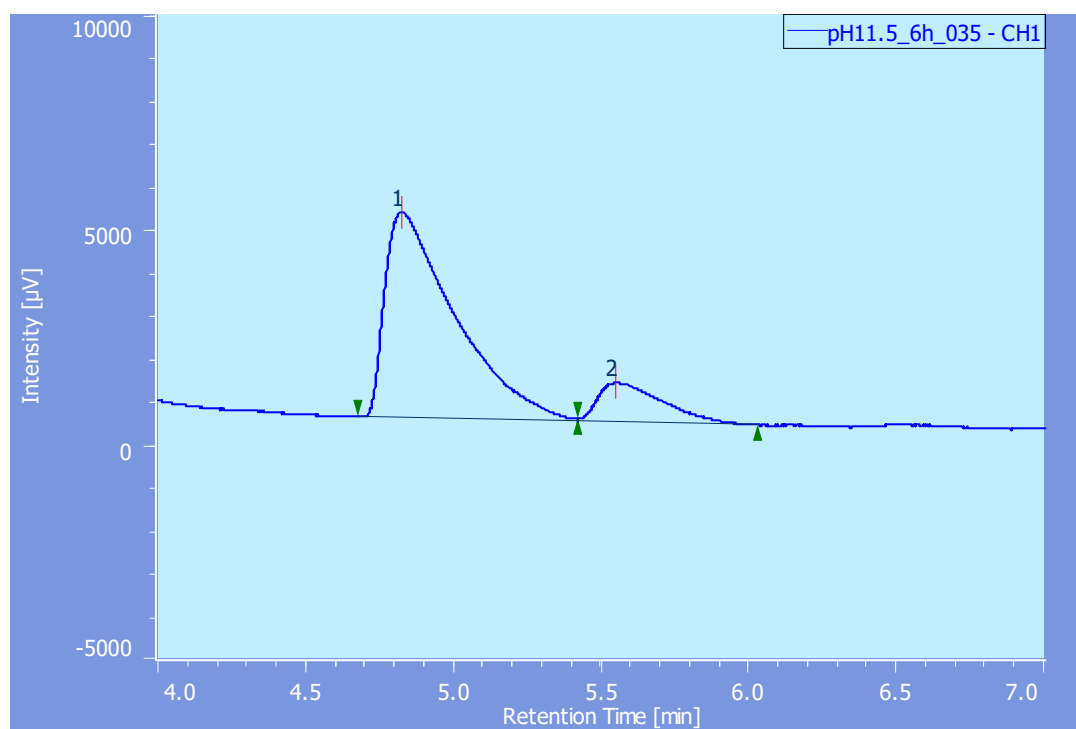


Figure S6.32. HPLC chart of **1** at pH 11.7 (6 h after heating)

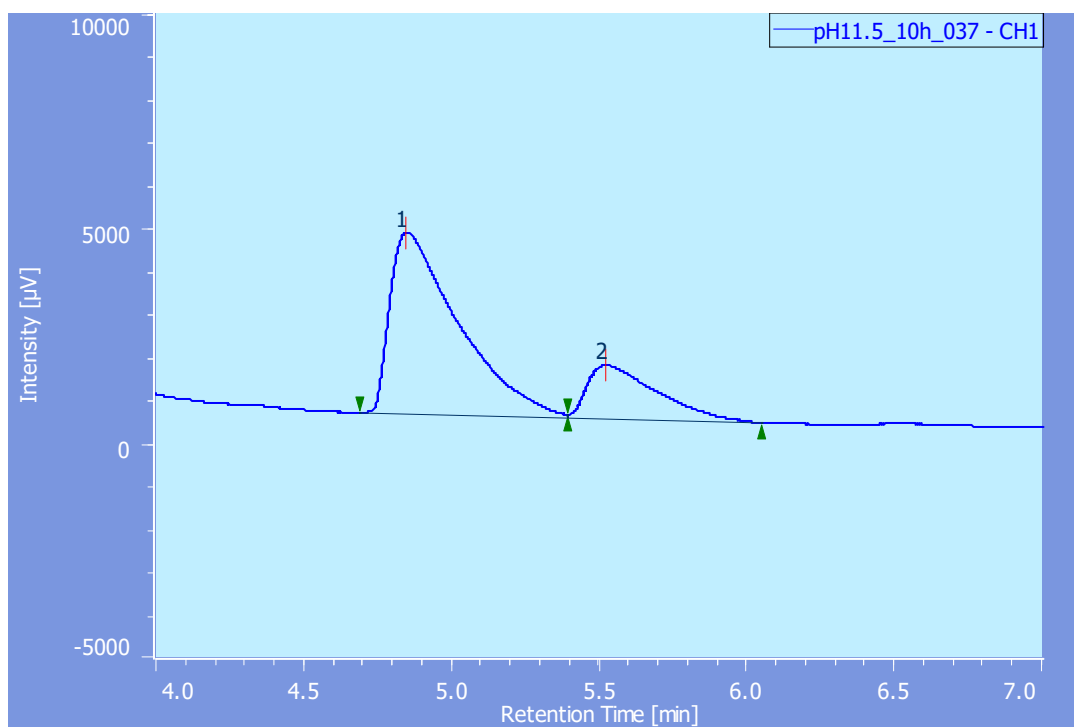


Figure S6.33. HPLC chart of **1** at pH 11.7 (10 h after heating)

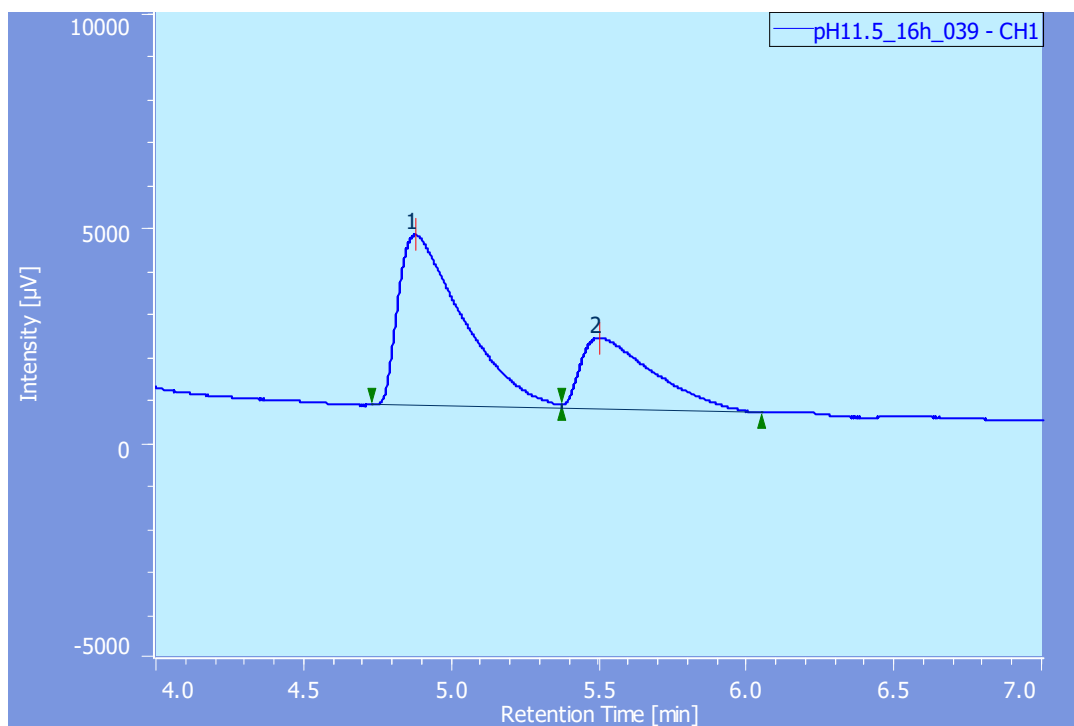


Figure S6.34. HPLC chart of **1** at pH 11.7 (16 h after heating)

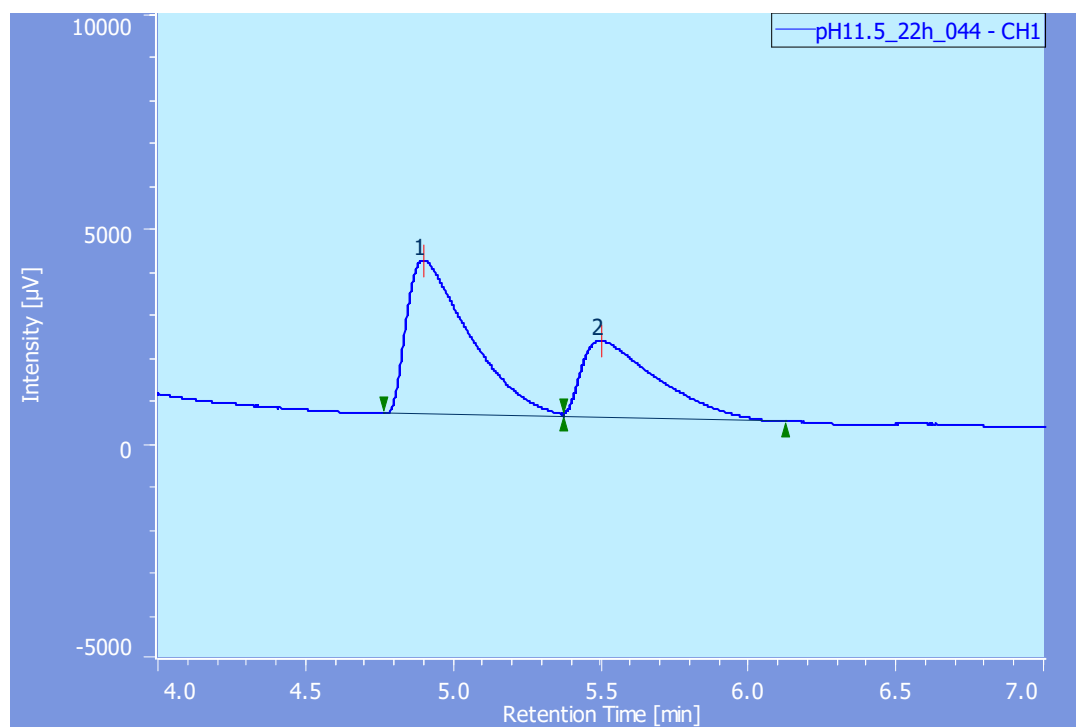


Figure S6.35. HPLC chart of **1** at pH 11.7 (22 h after heating)

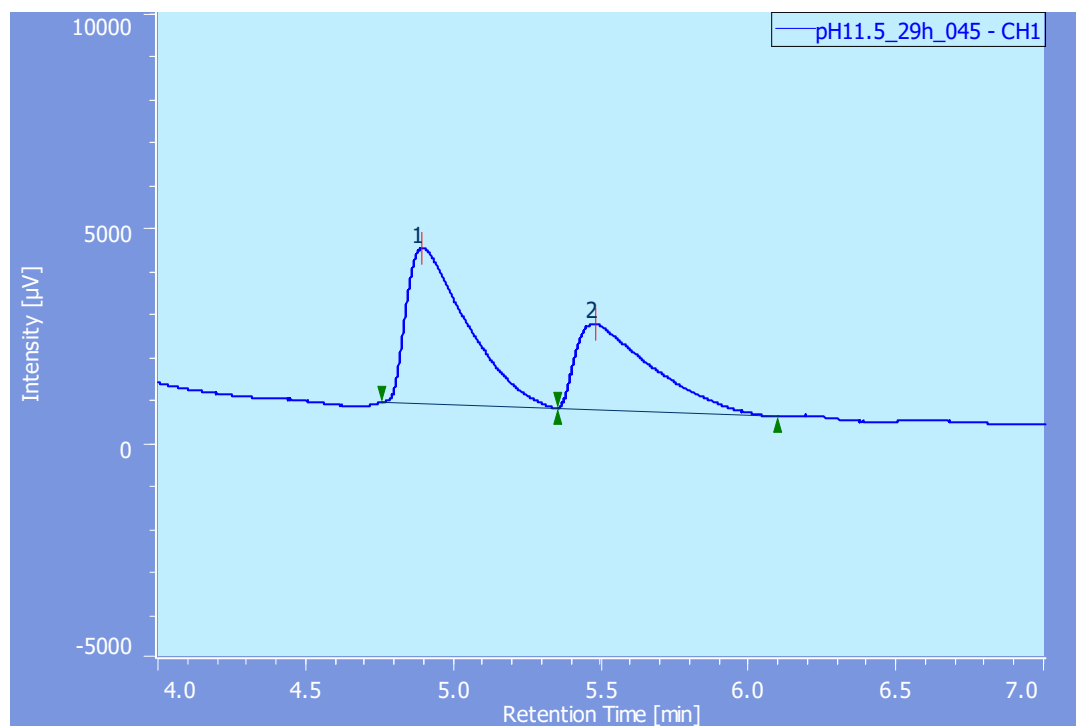


Figure S6.36. HPLC chart of **1** at pH 11.7 (29 h after heating)

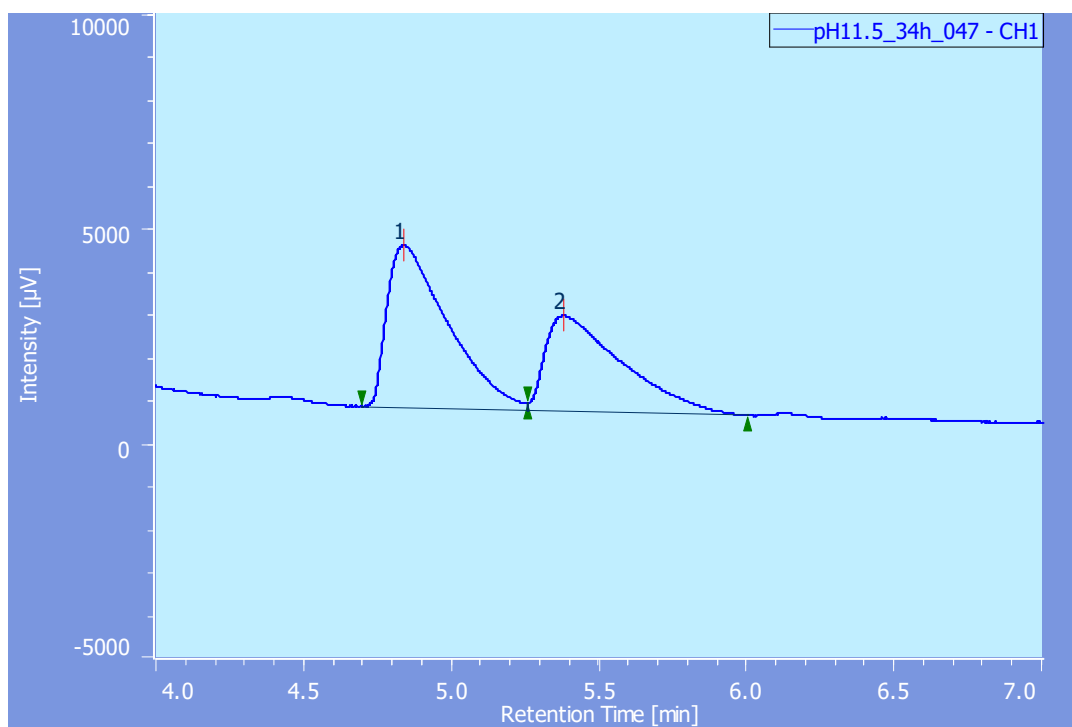


Figure S6.37. HPLC chart of **1** at pH 11.7 (34 h after heating)

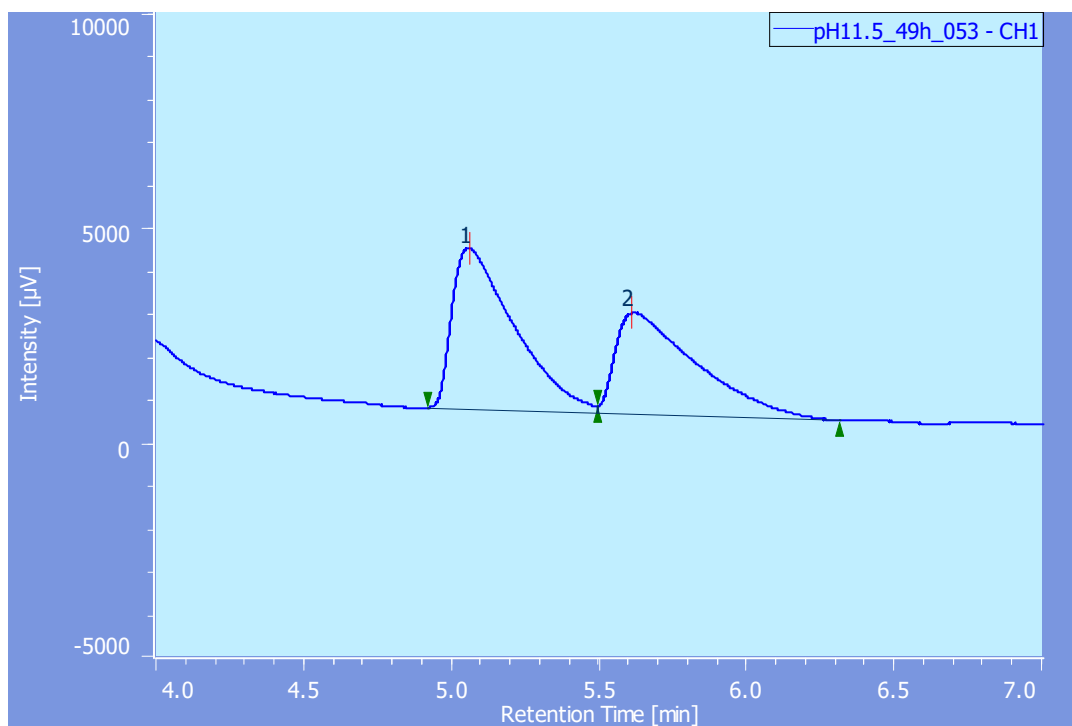


Figure S6.38. HPLC chart of **1** at pH 11.7 (49 h after heating)

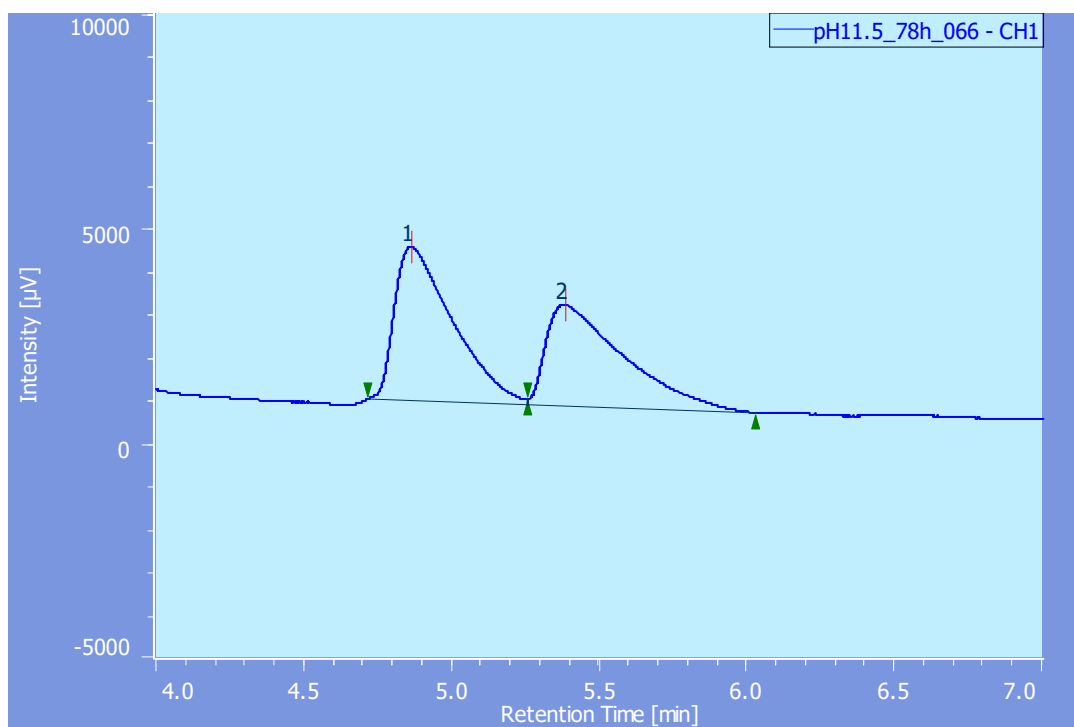


Figure S6.39. HPLC chart of **1** at pH 11.7 (78 h after heating)

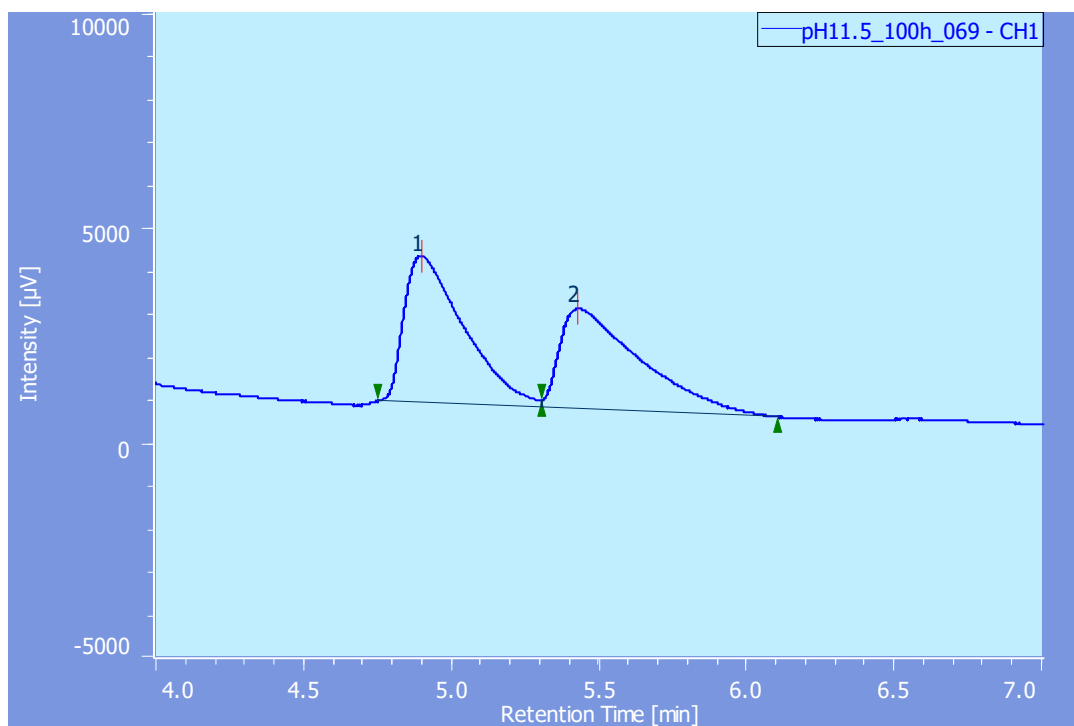


Figure S6.40. HPLC chart of **1** at pH 11.7 (100 h after heating)

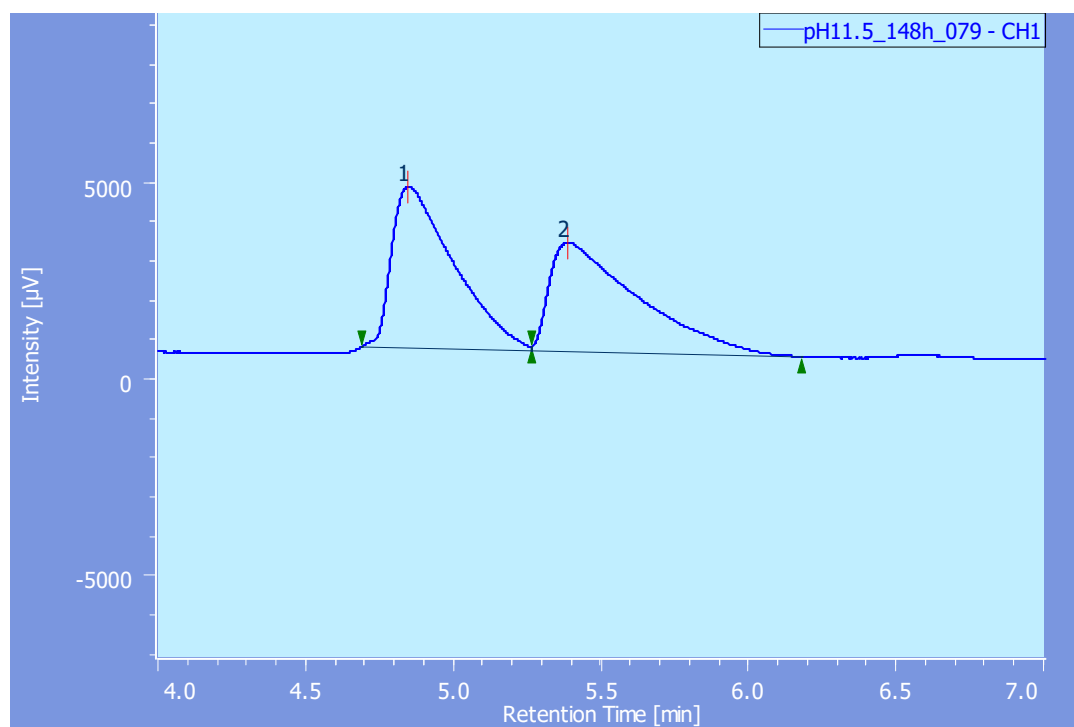


Figure S6.41. HPLC chart of **1** at pH 11.7 (148 h after heating)

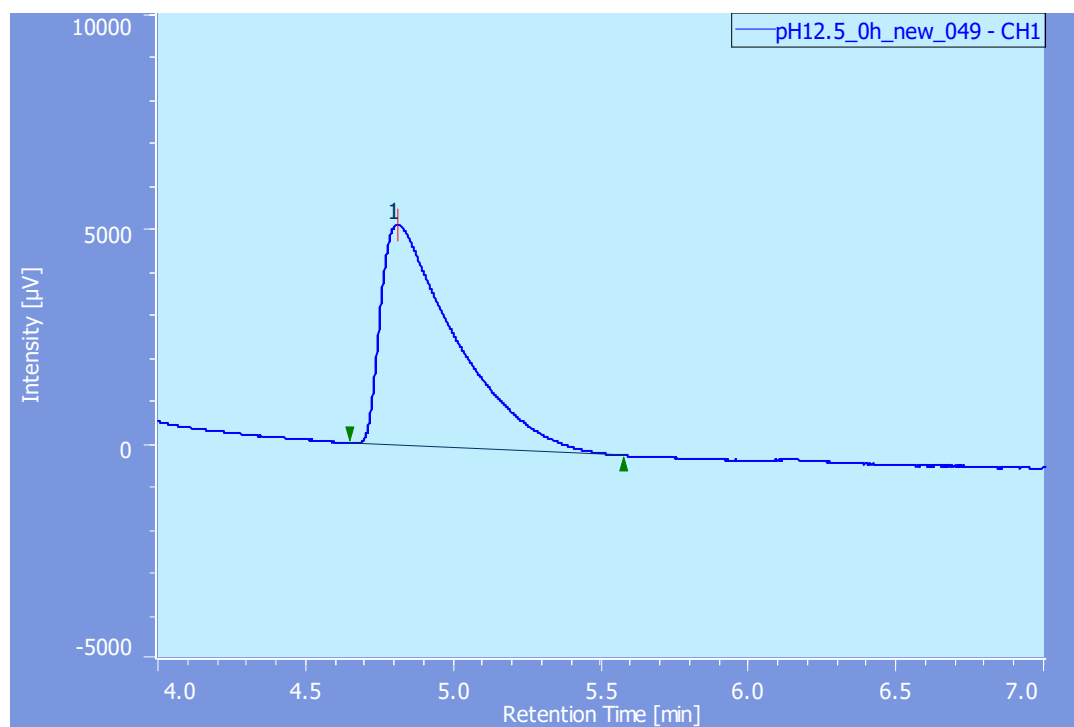


Figure S6.42. HPLC chart of **1** at pH 12.7 (0 h after heating)

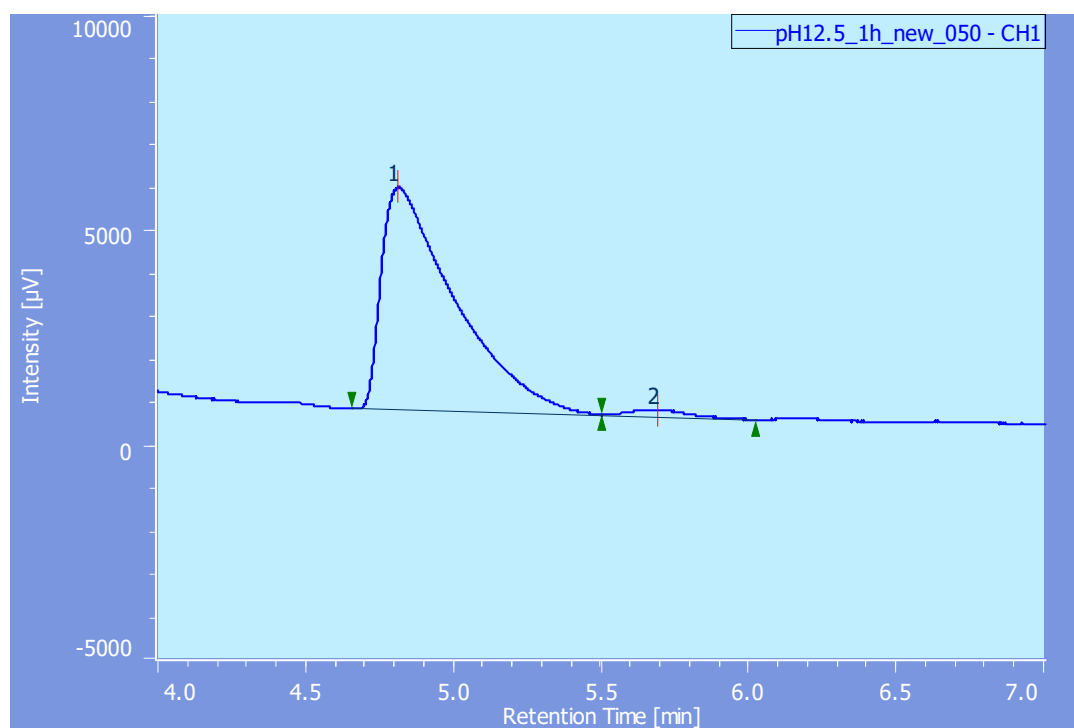


Figure S6.43. HPLC chart of **1** at pH 12.7 (1 h after heating)

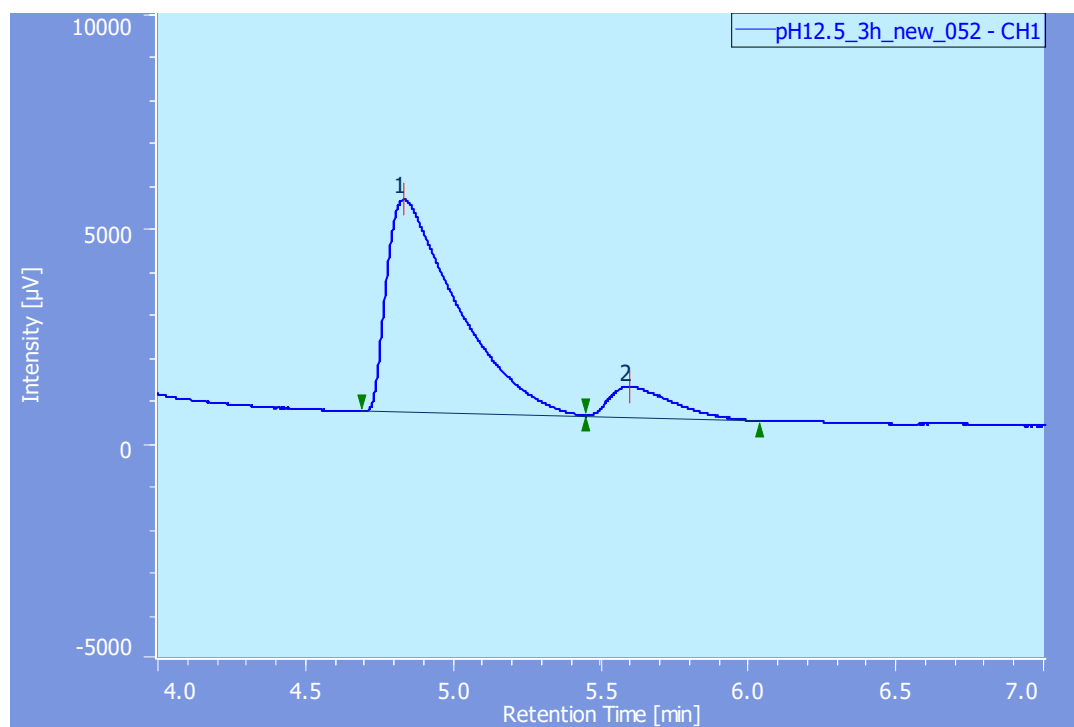


Figure S6.44. HPLC chart of **1** at pH 12.7 (3 h after heating)

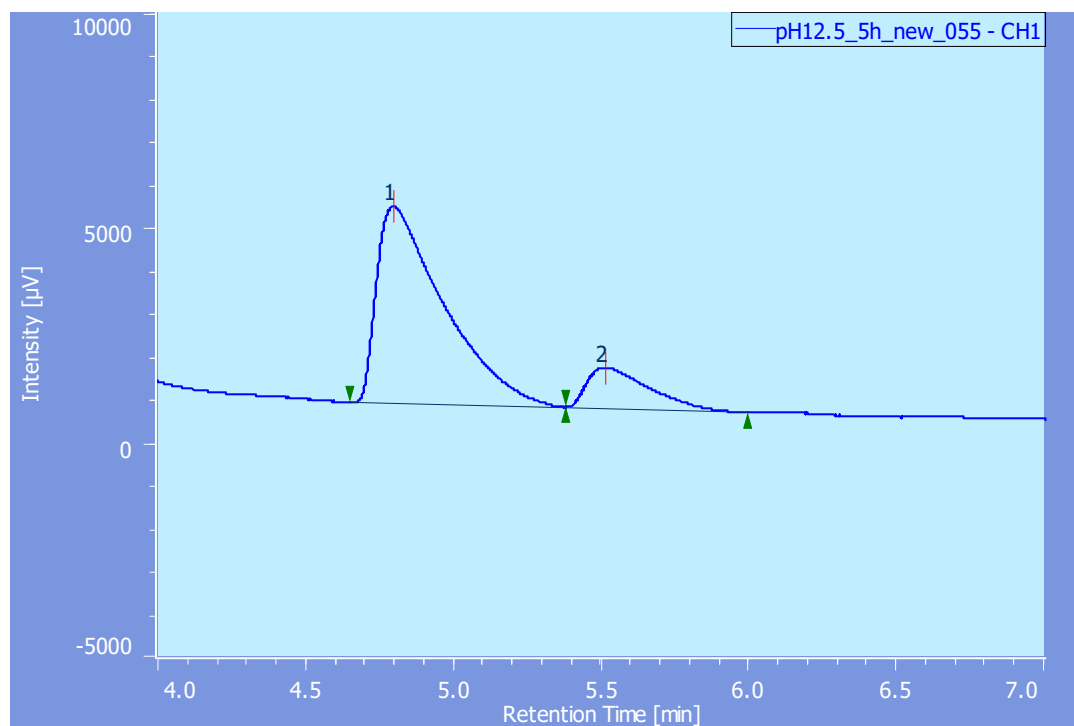


Figure S6.45. HPLC chart of **1** at pH 12.7 (5 h after heating)

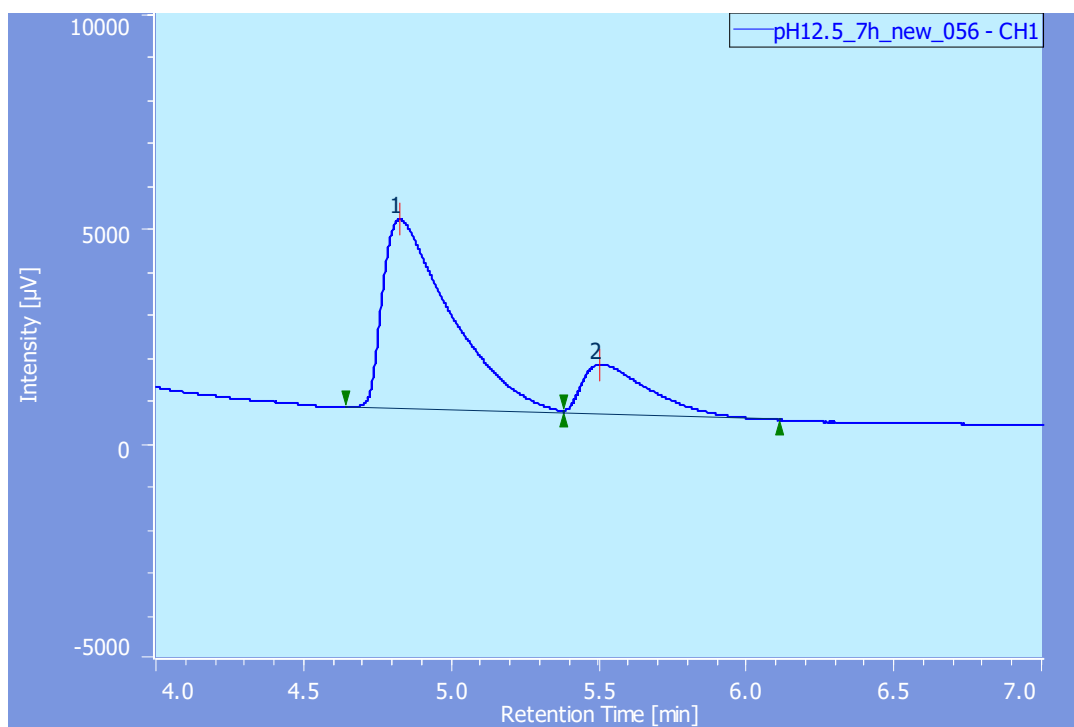


Figure S6.46. HPLC chart of **1** at pH 12.7 (7 h after heating)

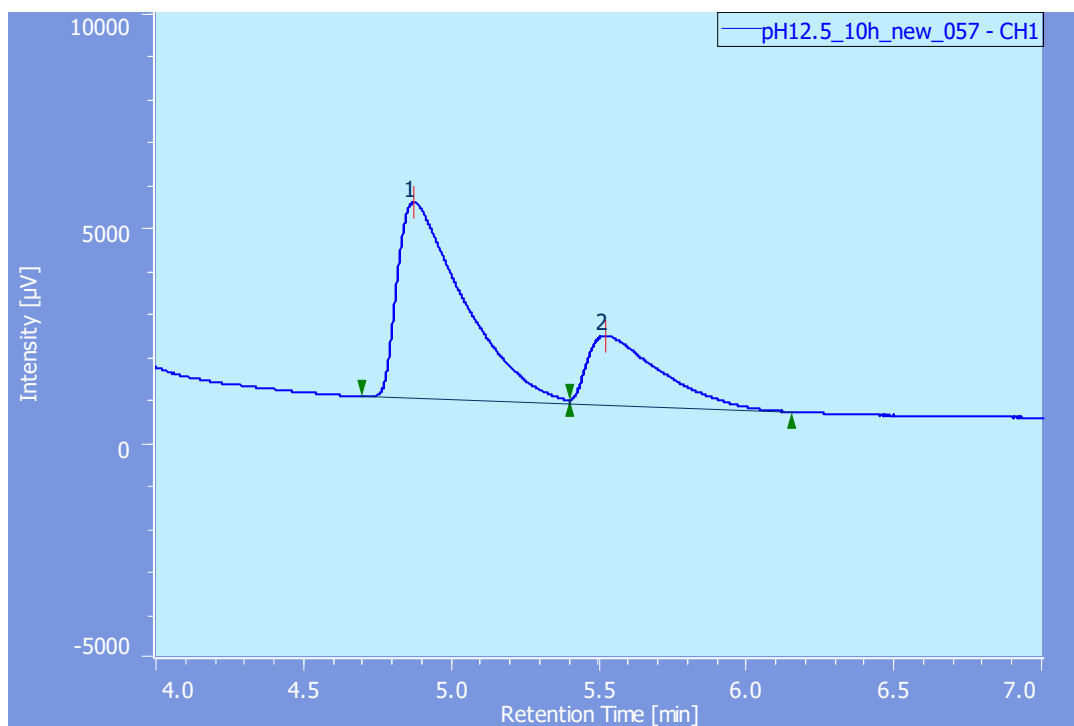


Figure S6.47. HPLC chart of **1** at pH 12.7 (10 h after heating)

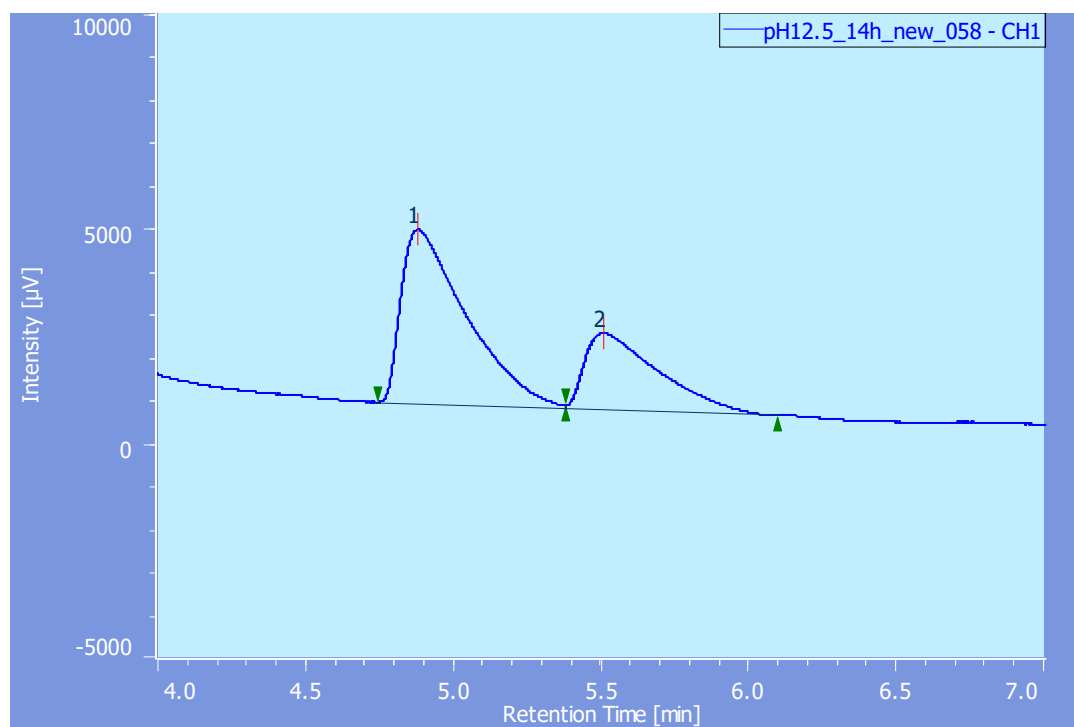


Figure S6.48. HPLC chart of **1** at pH 12.7 (14 h after heating)

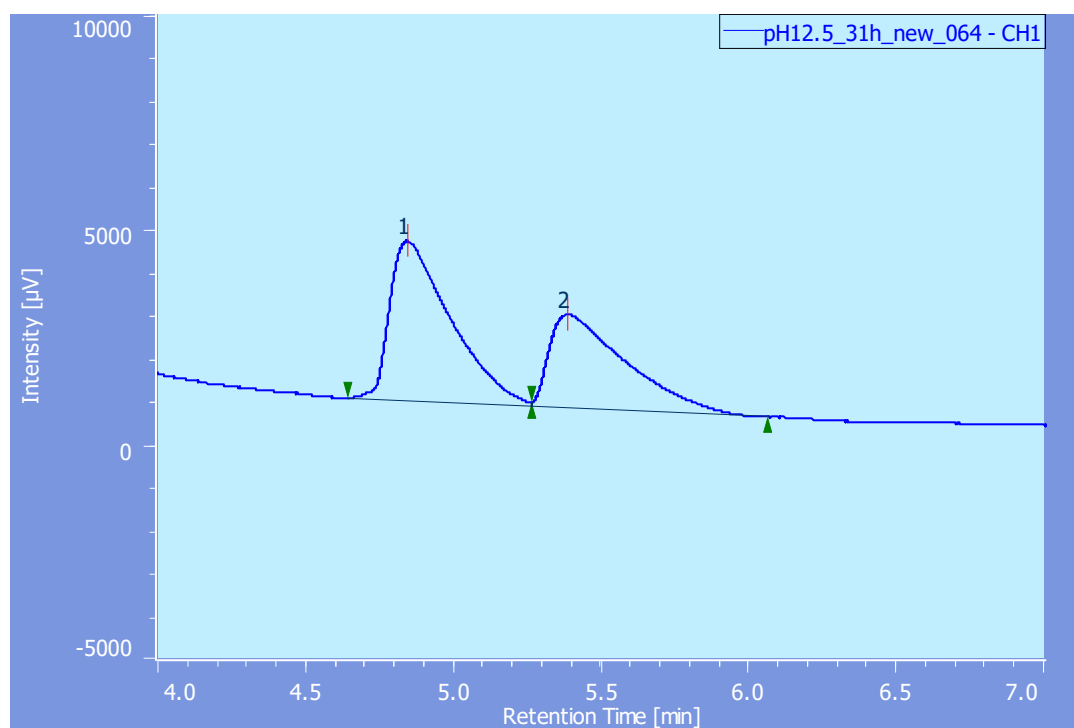


Figure S6.49. HPLC chart of **1** at pH 12.7 (31 h after heating)

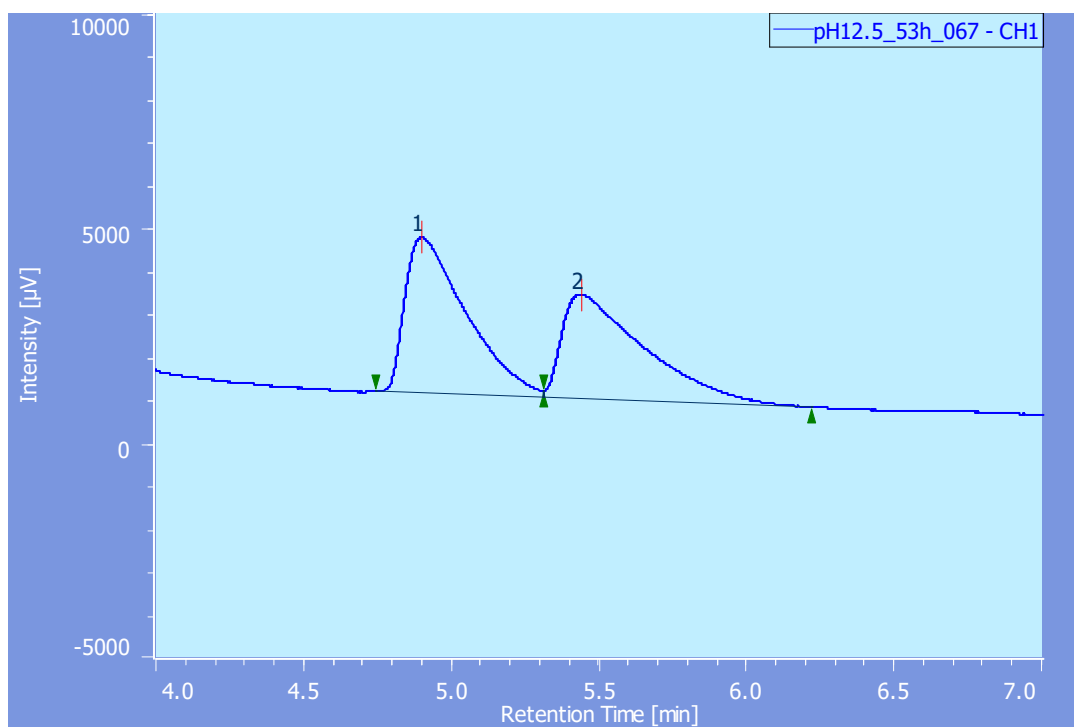


Figure S6.50. HPLC chart of **1** at pH 12.7 (53 h after heating)

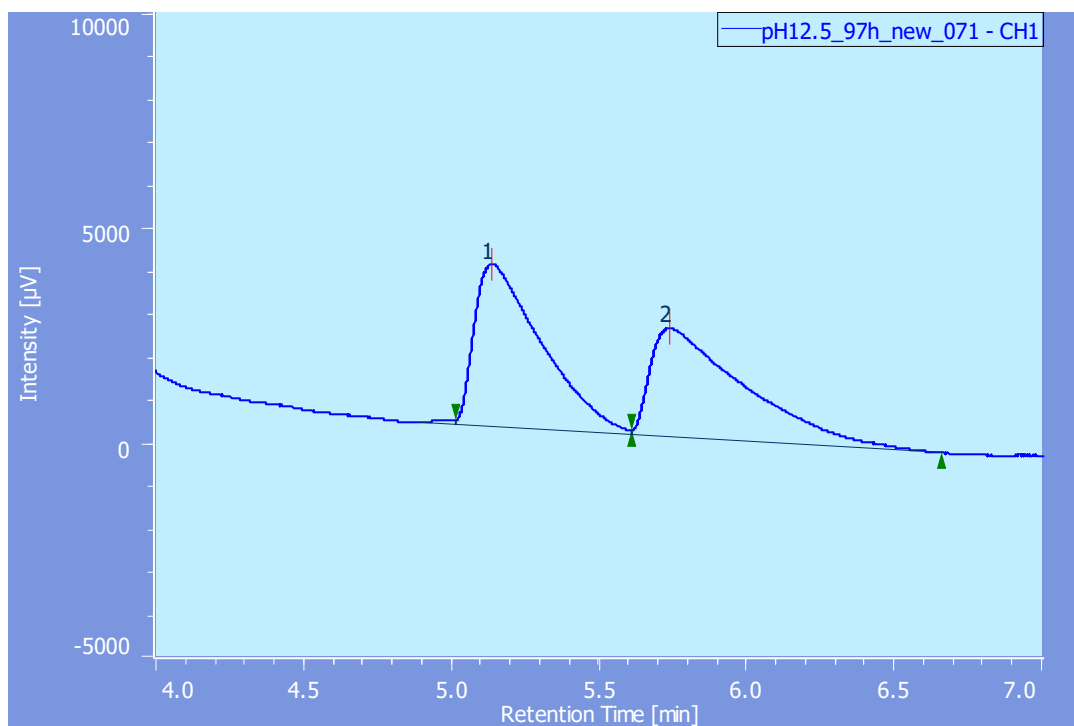


Figure S6.51. HPLC chart of **1** at pH 12.7 (97 h after heating)

7. Assignment of the *E/Z* configuration of benzamidines

E or *Z* configuration of compounds **1-7** were determined by ^1H NMR and NOE experiments. The *E* and *Z* isomers can be clearly distinguished by NOE correlation from the *N*-Me groups, as shown in Figure S7.1.

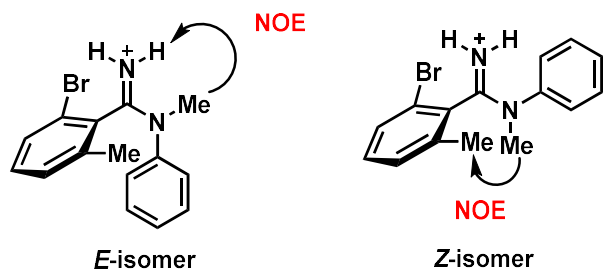


Figure S7.1. Determination of *E/Z* configuration by the NOE experiment

8. Quantum chemical calculations

Rotational barriers of the thermal isomerization calculated by DFT method.

The structures of equilibrium or transition state in a singlet state were optimized at M06-2X/Def2-SVP[SMD-water] level of theory. Vibrational calculations were performed at the same level to confirm that no imaginary frequencies (equilibrium structures) or one imaginary frequency (transition structure) exist. We also performed IRC calculation to confirm the desired connection from the transition state. Calculated equilibrium or transition state structures were shown in Figure S7.1. Gibbs free energies were calculated as the sum of the electronic energy (M06-2X/Def2-TZVP[SMD-water]) and the thermal correction term from the vibrational calculation at the same level to the optimization. All calculated energy were summarized in Table S8.1. All the DFT calculations were performed using Gaussian 16².

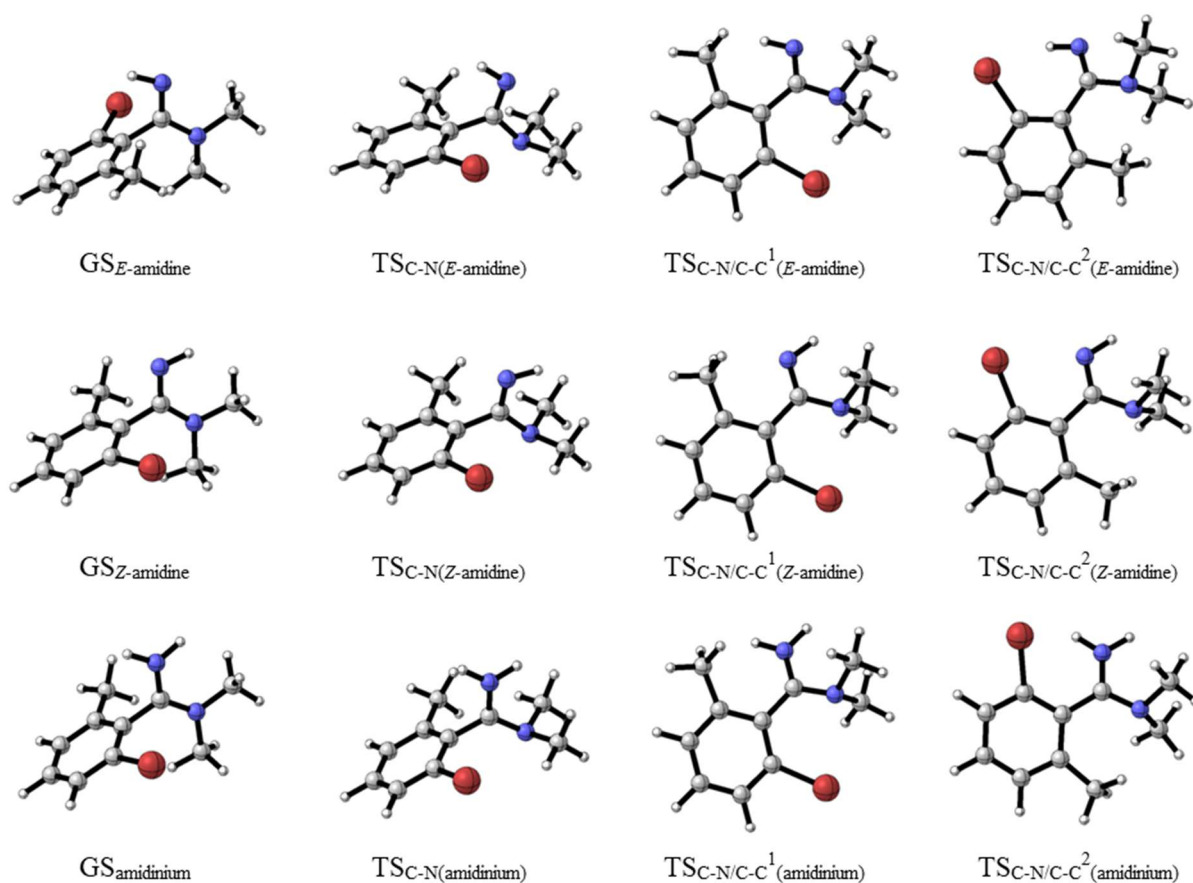


Figure S8.1. Equilibrium or transition state structures

Table S8.1. Calculated energy values of each structure in the singlet state

structure	E (hartrees)	ZPE (hartrees)	G (hartrees)	ΔG (kJ/mol)
GS_E-amidine	-3072.55772	0.172658	-3072.385062	0.0
TS _{C-N} (<i>E</i> -amidine)	-3072.53395	0.176065	-3072.357885	71.4
TS _{C-N/C-C} ¹ (<i>E</i> -amidine)	-3072.51716	0.177958	-3072.339202	120.4
TS _{C-N/C-C} ² (<i>E</i> -amidine)	-3072.55772	0.172658	-3072.385062	131.0
GS_Z-amidine	-3072.53362	0.176455	-3072.357168	68.3
TS _{C-N} (<i>Z</i> -amidine)	-3072.55637	0.173198	-3072.383175	0.0
TS _{C-N/C-C} ¹ (<i>Z</i> -amidine)	-3072.51873	0.177179	-3072.341549	109.3
TS _{C-N/C-C} ² (<i>Z</i> -amidine)	-3072.51532	0.176789	-3072.338529	117.2
GS_{amidinium}	-3073.0286	0.187634	-3072.840962	0.0
TS _{C-N} (amidinium)	-3072.97991	0.189216	-3072.790693	132.0
TS _{C-N/C-C} ¹ (amidinium)	-3072.97274	0.190261	-3072.782483	153.5
TS _{C-N/C-C} ² (amidinium)	-3072.97017	0.190144	-3072.780021	160.0

Cartesian coordinates for the thermal rotational barrier

GS_{E-amidine}

C	-2.17671	-1.17197	0.239962
C	-0.8659	-0.78532	-0.02982
C	-0.50948	0.554875	-0.1987
C	-1.50269	1.546983	-0.08733
C	-2.81772	1.157534	0.184042
C	-3.15398	-0.18572	0.344502
C	-1.1422	3.000066	-0.23666
Br	0.469563	-2.13388	-0.15057
C	0.898699	0.941266	-0.55865
N	1.767827	1.153537	0.473918
C	1.494542	0.709753	1.827797
N	1.265509	1.093662	-1.78163
C	3.163274	1.344123	0.133421
H	0.459762	0.933907	-2.38826
H	-2.42714	-2.22529	0.366218
H	-3.58867	1.925398	0.270855
H	-4.18579	-0.47108	0.554188
H	-2.04126	3.628243	-0.21998
H	-0.48098	3.320592	0.58239
H	-0.603	3.175982	-1.17761
H	0.420996	0.755112	2.044243
H	1.847672	-0.32082	2.007728
H	2.007744	1.374177	2.536168
H	3.268626	2.124033	-0.63027
H	3.712223	1.647025	1.033047
H	3.614736	0.4187	-0.26606

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.172658 hartrees

E (M06-2X/def2-TZVP[SMD-water]): -3072.55772 hartrees

TS_{C-N(E-amidine)}

C	0.303301	2.104754	0.080357
C	0.084623	0.712561	0.146655
C	-0.773141	2.949528	-0.212663
H	-0.597358	4.026012	-0.256123
C	-1.2144	0.232141	-0.064791
C	-2.050325	2.44623	-0.440636
H	-2.876143	3.121159	-0.669356
C	-2.281219	1.076197	-0.36261
Br	-1.595034	-1.627334	0.081088
H	-3.278239	0.665981	-0.522754
C	1.665877	2.704232	0.306389
C	1.218918	-0.225199	0.474717
N	1.495925	-0.591454	1.658142
N	2.000979	-0.693602	-0.640881
C	1.98804	-2.153989	-0.726288
H	2.457401	-2.630329	0.153805
H	0.960643	-2.524902	-0.817209
H	2.544474	-2.459038	-1.622796
C	3.386952	-0.234909	-0.540528
H	3.932556	-0.557271	-1.437624
H	3.434372	0.858402	-0.488531

H	3.899413	-0.650086	0.346905
H	1.571831	3.749124	0.628513
H	2.251347	2.697061	-0.62574
H	2.235922	2.153871	1.066358
H	0.839873	-0.167165	2.322049

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.176065 hartrees

E (M06-2X/def2-TZVP[SMD-water]): -3072.53395 hartrees

TS_{C-N/C-C¹(E-amidine)}

C	2.184712	0.70149	-0.040586
C	0.784337	0.408387	0.025238
C	3.127616	-0.328677	0.037979
H	4.185399	-0.066255	-0.008229
C	0.443775	-0.971892	0.029445
C	2.756088	-1.658853	0.156401
H	3.506369	-2.446678	0.232857
C	1.408804	-1.978084	0.124801
Br	-1.29972	-1.737435	-0.166289
H	1.091434	-3.019644	0.153723
C	2.790213	2.072441	-0.257613
C	-0.220683	1.548032	0.081295
N	0.063382	2.771837	0.298753
N	-1.59131	1.231475	-0.112199
C	-2.341377	2.264154	-0.808362
H	-2.457526	3.188972	-0.215741
H	-1.846287	2.519898	-1.75446
H	-3.343483	1.872426	-1.034529
C	-2.223477	0.9357	1.170228
H	-3.202907	0.462441	1.01119
H	-1.606672	0.252137	1.767282
H	-2.365818	1.863323	1.758989
H	3.822431	1.949259	-0.605967
H	2.841492	2.663947	0.670018
H	2.249081	2.657059	-1.012745
H	1.052146	2.933955	0.438912

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.177958 hartrees

E (M06-2X/def2-TZVP[SMD-water]): -3072.51716 hartrees

TS_{C-N/C-C²(E-amidine)}

C	0.810875	1.744	-0.127645
C	0.220549	0.445785	0.009409
C	-0.003446	2.88402	-0.043175
H	0.472379	3.861841	-0.125049
C	-1.198872	0.422372	0.067425
C	-1.377614	2.815408	0.117161
H	-1.979819	3.722045	0.184587
C	-1.984697	1.569246	0.137422
Br	-2.29228	-1.151946	-0.076732
H	-3.069142	1.481789	0.191541
C	2.263302	2.058986	-0.422104

C	1.057118	-0.819674	0.114057
N	0.654293	-1.977451	0.45799
N	2.450205	-0.683158	-0.161736
C	3.010901	-1.807824	-0.895669
H	3.026692	-2.741826	-0.306509
H	2.434245	-1.985407	-1.813358
H	4.04437	-1.560394	-1.177705
C	3.181407	-0.479671	1.085587
H	4.207159	-0.142847	0.875415
H	2.68547	0.27382	1.711986
H	3.230746	-1.417937	1.672366
H	2.34952	3.135896	-0.611848
H	2.622279	1.51641	-1.301638
H	2.938351	1.816889	0.405591
H	-0.341061	-2.013784	0.651653

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.177308 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3092.528427 hartrees

GS_{Z-amidine}

C	2.154731	-1.222588	0.222919
C	0.853866	-0.800135	-0.042433
C	0.531229	0.54883	-0.203239
C	1.549854	1.513333	-0.089305
C	2.855095	1.089627	0.179012
C	3.157311	-0.262598	0.331465
C	1.227758	2.974789	-0.24198
Br	-0.515729	-2.114221	-0.167324
C	-0.869502	0.971694	-0.551721
N	-1.739259	1.146756	0.490543
C	-1.450597	0.702762	1.842974
N	-1.124986	1.154646	-1.796468
H	-2.102305	1.422676	-1.931836
C	-3.135129	1.372925	0.170988
H	2.377792	-2.282816	0.342498
H	3.645477	1.837191	0.268876
H	4.181711	-0.575502	0.538373
H	2.131431	3.586903	-0.132534
H	0.780768	3.172068	-1.226069
H	0.497355	3.29356	0.516345
H	-1.816596	-0.321327	2.030767
H	-0.373099	0.731868	2.039839
H	-1.939722	1.377978	2.558091
H	-3.260714	2.271773	-0.451003
H	-3.575806	0.516004	-0.368572
H	-3.693745	1.52573	1.100971

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.173198 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.556373 hartrees

TS_{C-N(Z-amidine)}

C	0.220189	2.118653	0.090502
C	0.05302	0.719747	0.158573
C	-0.885279	2.924488	-0.204622
H	-0.748117	4.006557	-0.249205

C	-1.22701	0.19305	-0.05398
C	-2.14323	2.375177	-0.432847
H	-2.99298	3.019185	-0.66328
C	-2.323681	0.997707	-0.35358
Br	-1.541737	-1.678846	0.090014
H	-3.304963	0.551445	-0.514083
C	1.56063	2.766262	0.315885
C	1.223852	-0.172208	0.479861
N	1.460616	-0.45211	1.695829
N	2.005063	-0.623581	-0.648086
C	2.03624	-2.08342	-0.739269
H	2.54192	-2.554096	0.126302
H	1.020393	-2.487091	-0.813481
H	2.588148	-2.367253	-1.645236
C	3.377123	-0.118813	-0.574047
H	3.915171	-0.422324	-1.481969
H	3.388272	0.975276	-0.519515
H	3.927927	-0.519527	0.299021
H	1.430345	3.809994	0.629116
H	2.149875	2.771402	-0.613855
H	2.144649	2.2404	1.082451
H	2.289297	-1.058384	1.760181

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.176455 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.51248 hartrees

TS_{C-N/C-C¹(Z-amidine)}

C	2.16804	0.783556	0.006408
C	0.782874	0.420654	0.037877
C	3.151265	-0.211638	-0.030636
H	4.197814	0.094921	-0.057143
C	0.496449	-0.968169	-0.011411
C	2.83513	-1.562105	-0.040683
H	3.618914	-2.320332	-0.064234
C	1.502056	-1.937753	-0.03697
Br	-1.233003	-1.785227	-0.050814
H	1.227536	-2.991487	-0.060701
C	2.699267	2.19656	-0.022714
C	-0.261699	1.512112	0.094089
N	0.087434	2.709779	0.357724
N	-1.617825	1.164144	-0.176728
C	-2.307676	2.105874	-1.043417
H	-2.515661	3.082723	-0.562991
H	-1.722726	2.281105	-1.956302
H	-3.276304	1.673004	-1.330582
C	-2.358928	0.964343	1.063175
H	-3.320872	0.475459	0.851347
H	-1.795545	0.324613	1.754407
H	-2.556031	1.929186	1.572154
H	3.782243	2.164124	-0.196525
H	2.503881	2.726336	0.916913
H	2.226511	2.793793	-0.811422
H	-0.742583	3.311439	0.362286

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.177179 hartrees

E (M06-2X/def2-TZVP[SMD-water]):

-3072.518728 hartrees

TS_{C-N/C-C²}(Z-amidine)

C	0.78041	1.776271	-0.083989
C	0.20433	0.469984	0.011149
C	-0.055459	2.90196	-0.057234
H	0.405753	3.888598	-0.114779
C	-1.214908	0.405943	0.037087
C	-1.43584	2.804105	0.02672
H	-2.058896	3.699171	0.047308
C	-2.017881	1.546688	0.053633
Br	-2.264011	-1.186939	-0.032203
H	-3.101523	1.438689	0.078154
C	2.249387	2.10824	-0.246761
C	1.046454	-0.782268	0.092104
N	0.523648	-1.905228	0.384195
N	2.450407	-0.64224	-0.154556
C	2.995966	-1.669889	-1.0274
H	3.021531	-2.67624	-0.563741
H	2.413344	-1.726421	-1.956834
H	4.031134	-1.40295	-1.283226
C	3.185184	-0.58433	1.103943
H	4.218412	-0.252568	0.923843
H	2.706707	0.119106	1.798901
H	3.217548	-1.57563	1.599386
H	2.351577	3.197247	-0.333143
H	2.673361	1.644572	-1.143452
H	2.861129	1.78442	0.602187
H	1.256055	-2.623089	0.40951

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.176789 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.515318 hartrees

GS_{amidine}

C	2.500285	-0.178791	0.226808
C	1.147685	-0.393855	-0.017876
C	0.26681	0.676452	-0.195074
C	0.733643	2.000605	-0.119921
C	2.094139	2.206093	0.12578
C	2.965887	1.132435	0.293804
C	-0.219988	3.153974	-0.261361
Br	0.495379	-2.173593	-0.087746
C	-1.174981	0.428807	-0.491959
N	-2.051503	0.273231	0.473616
C	-1.701037	0.324754	1.890014
N	-1.522577	0.376752	-1.765334
C	-3.447355	0.001237	0.14209
H	-2.481946	0.227901	-2.067595
H	-0.80956	0.507154	-2.476742
H	3.177371	-1.021813	0.362918
H	2.469504	3.228663	0.187731
H	4.024438	1.31429	0.484001
H	0.323465	4.106214	-0.264268
H	-0.802013	3.07925	-1.190703
H	-0.933441	3.16542	0.577137

H	-1.873414	-0.660038	2.345439
H	-0.653634	0.612256	2.019863
H	-2.341192	1.064488	2.387489
H	-3.522078	-0.90858	-0.470911
H	-4.004051	-0.148313	1.071352
H	-3.883678	0.848716	-0.406411

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.187634 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3073.028596 hartrees

TS_{C-N(amidine)}

C	0.14046	2.137094	0.064593
C	0.005391	0.736814	0.129441
C	-1.00247	2.89867	-0.201892
H	-0.905277	3.984601	-0.245471
C	-1.252882	0.149843	-0.060913
C	-2.242737	2.30333	-0.404027
H	-3.118922	2.918433	-0.612231
C	-2.378478	0.919547	-0.331418
Br	-1.47502	-1.731686	0.06969
H	-3.348172	0.44323	-0.475342
C	1.46413	2.825641	0.251635
C	1.203331	-0.118667	0.400994
N	1.464362	-0.435757	1.617141
N	2.021935	-0.550308	-0.656207
C	2.086905	-2.018866	-0.771667
H	2.58188	-2.490271	0.095874
H	1.084304	-2.439548	-0.894597
H	2.670831	-2.248061	-1.670521
C	3.387125	0.001757	-0.551124
H	3.923978	-0.275077	-1.465774
H	3.359041	1.093178	-0.48127
H	3.932689	-0.405282	0.318202
H	1.304822	3.866836	0.557418
H	2.024465	2.838456	-0.695487
H	2.088581	2.33554	1.010934
H	2.278382	-1.016688	1.848248
H	0.88038	-0.104861	2.390614

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.189216 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.979909 hartrees

TS_{C-N/C-C¹}(amidine)

C	-2.168424	0.712628	0.010406
C	-0.781867	0.358663	-0.07141
C	-3.133751	-0.296377	0.053101
H	-4.18275	-0.007374	0.122099
C	-0.460213	-1.02749	-0.013905
C	-2.79215	-1.639498	0.026726
H	-3.564213	-2.409462	0.049867
C	-1.453187	-2.002998	0.008855
Br	1.279243	-1.788629	0.05206
H	-1.170967	-3.054727	0.027466
C	-2.716835	2.115607	0.130405
C	0.244484	1.427081	-0.151305

N	-0.064711	2.618228	-0.562018
N	1.566391	1.218703	0.236517
C	2.122961	2.272288	1.087707
H	2.351379	3.204347	0.539795
H	1.434841	2.48942	1.913867
H	3.064646	1.895042	1.506043
C	2.436887	0.96096	-0.921852
H	3.361956	0.479983	-0.579375
H	1.94481	0.304664	-1.647948
H	2.683308	1.911913	-1.427783
H	-3.736967	2.059716	0.525254
H	-2.794609	2.619026	-0.84726
H	-2.129857	2.746116	0.810818
H	0.658841	3.338108	-0.608282
H	-0.993095	2.881528	-0.880522

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.190261 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.972744 hartrees

TS_{C-N/C-C²(amidinium)}

C	0.743622	1.843449	-0.099666
C	0.207941	0.533036	0.082982
C	-0.127704	2.942321	-0.048786
H	0.300619	3.939358	-0.156353
C	-1.215699	0.420314	0.104653
C	-1.494711	2.802836	0.10801
H	-2.142637	3.678922	0.150002
C	-2.049212	1.526919	0.136841
Br	-2.184469	-1.214809	-0.135116
H	-3.130443	1.395286	0.152267
C	2.18205	2.205262	-0.373466
C	1.069044	-0.672417	0.238686
N	0.624741	-1.722023	0.862451
N	2.353785	-0.74257	-0.277415
C	2.727412	-2.037521	-0.852952
H	2.931722	-2.809483	-0.089474
H	1.94361	-2.385583	-1.536649
H	3.651686	-1.886202	-1.424258
C	3.382001	-0.280108	0.675405
H	4.247127	0.116224	0.129599
H	2.98957	0.490075	1.345559
H	3.698611	-1.132363	1.300212
H	2.195011	3.17369	-0.887416
H	2.689278	1.469889	-1.001603
H	2.754914	2.329388	0.555218
H	1.227688	-2.537525	0.9826
H	-0.298498	-1.775748	1.283872

Free energy correction (M06-2X/def2-SVP[SMD-water]): 0.190144 hartrees

E (M06-2X/def2-TZVP[SMD-water]):
-3072.970165 hartrees

9. NMR spectra of the synthesized compounds

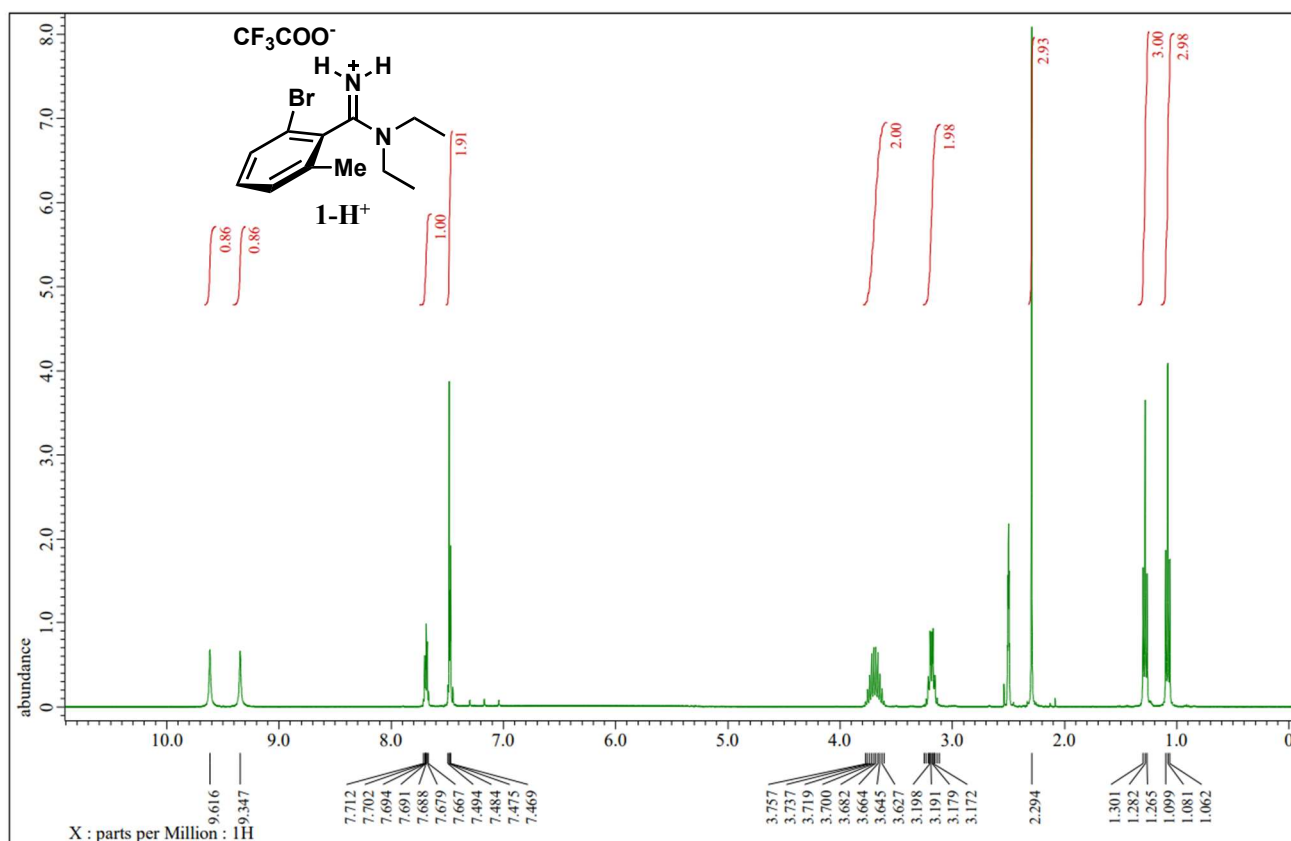


Figure S9.1. ¹H NMR spectrum of **1** (DMSO-*d*₆, 400 MHz, 25 °C)

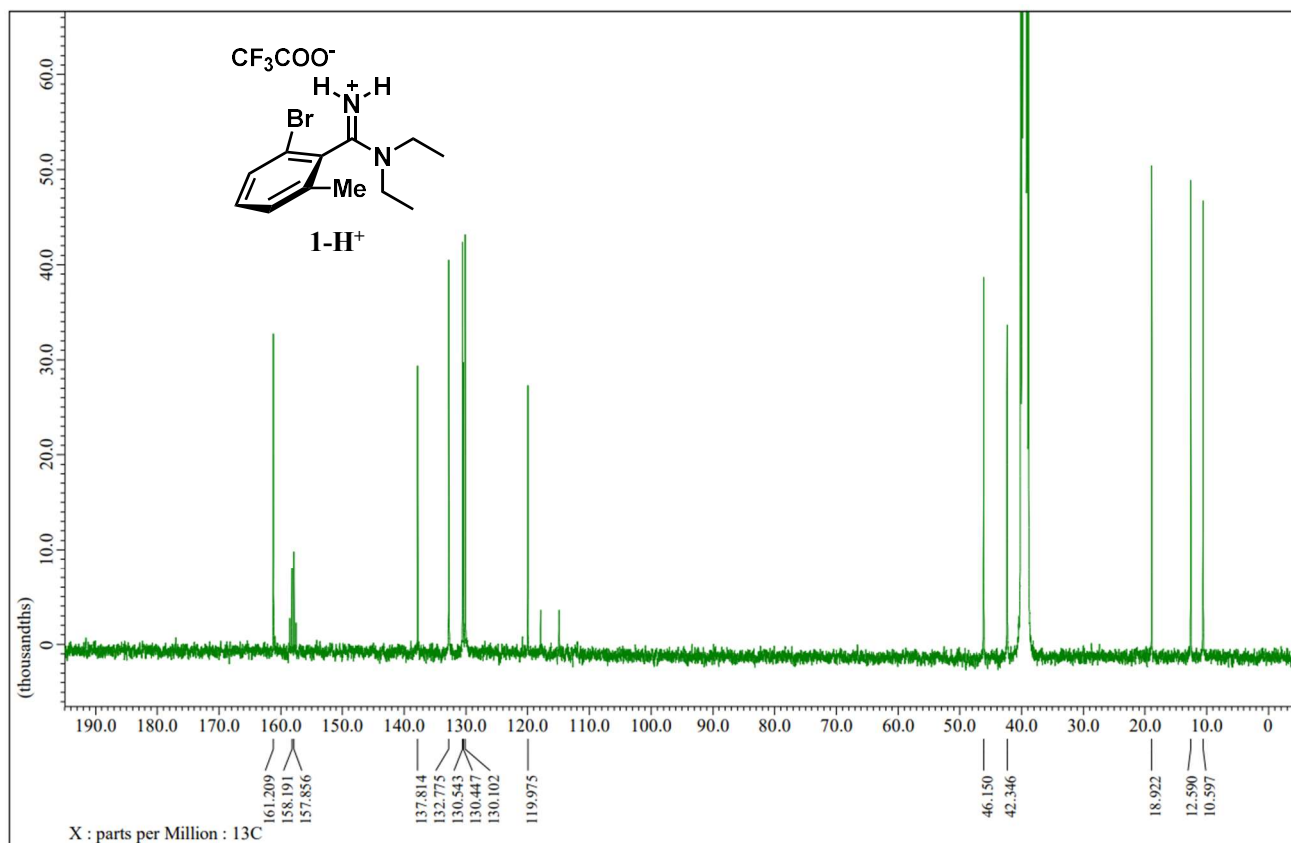


Figure S9.2. ¹³C NMR spectrum of **1** (DMSO-*d*₆, 100 MHz, 25 °C)

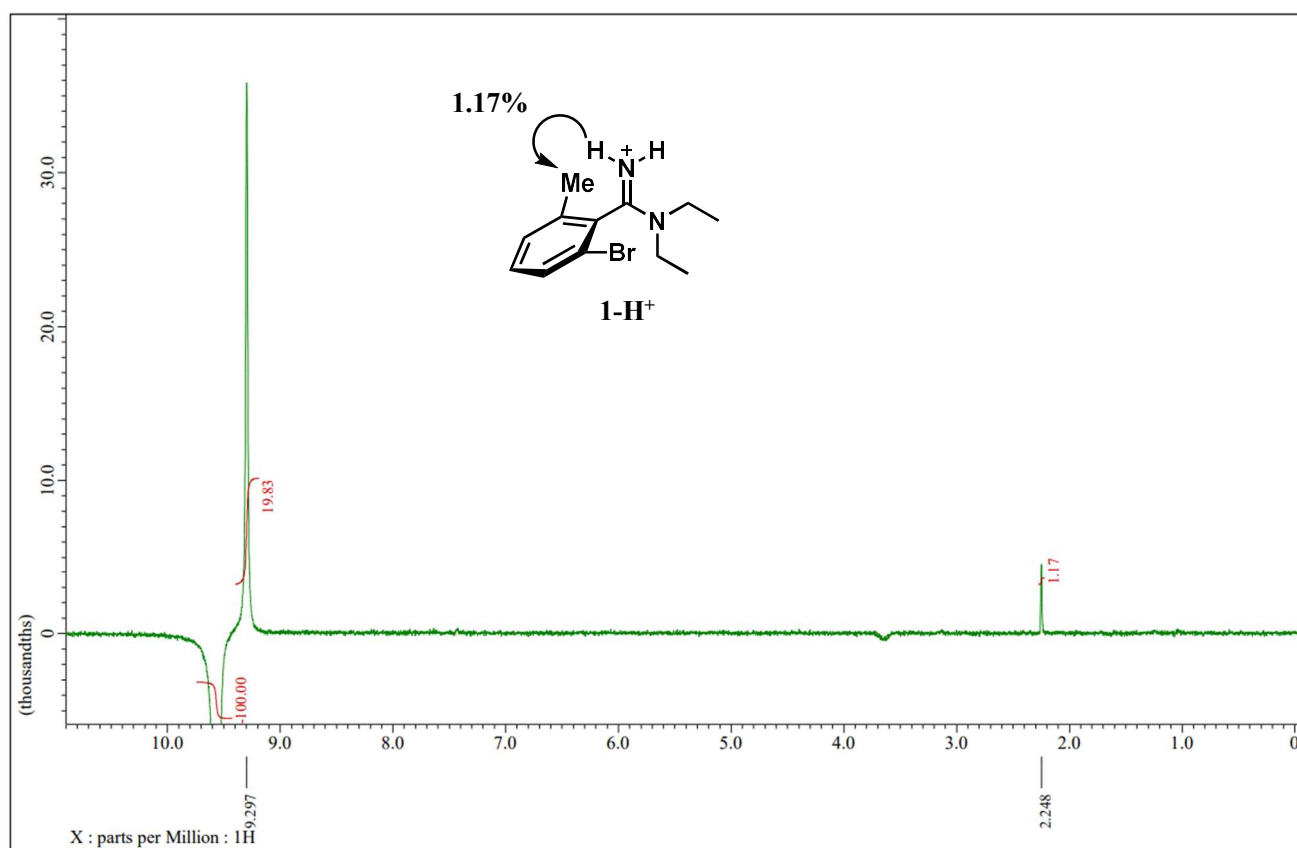


Figure S9.3. NOE spectrum of **1** (DMSO-*d*₆, 400 MHz, 25 °C)

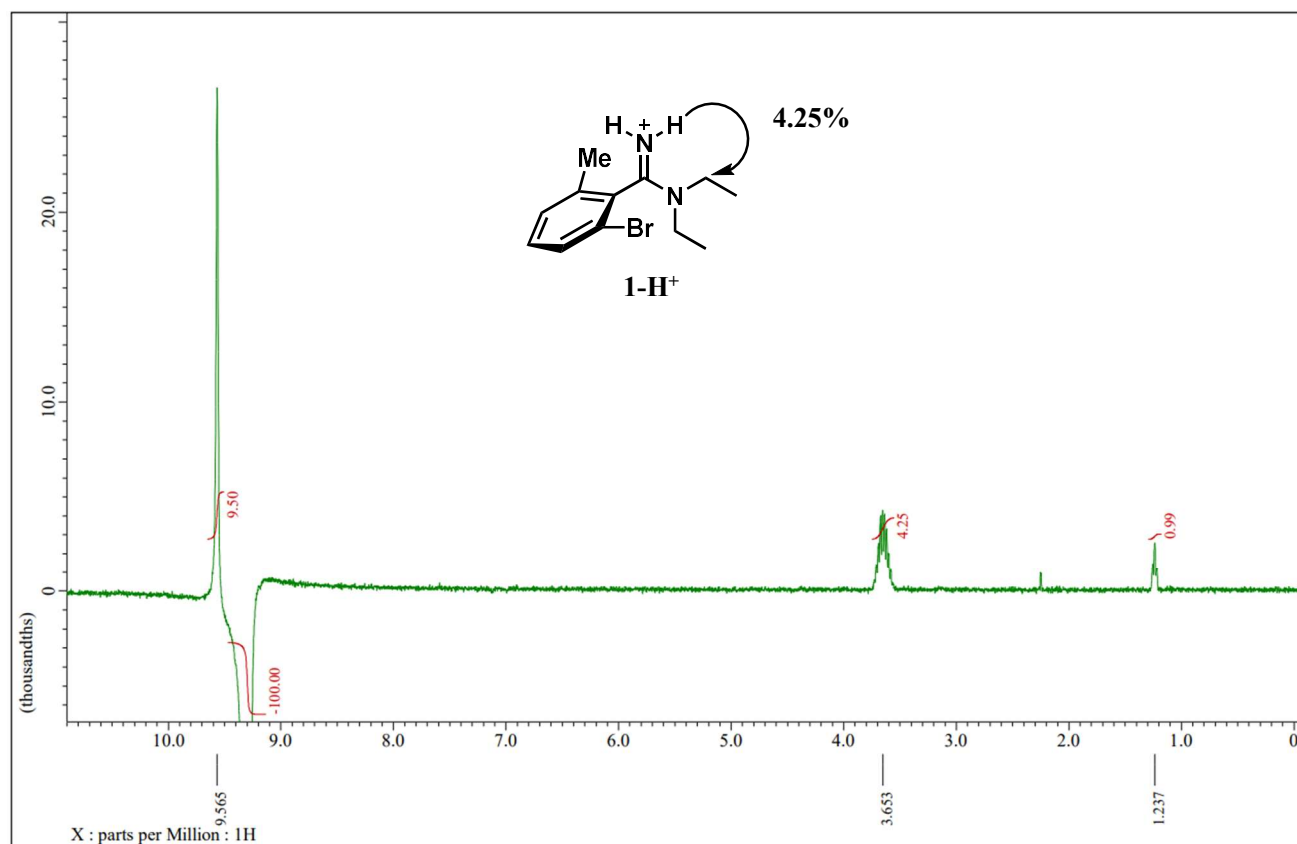


Figure S9.4. NOE spectrum of **1** (DMSO-*d*₆, 400 MHz, 25 °C)

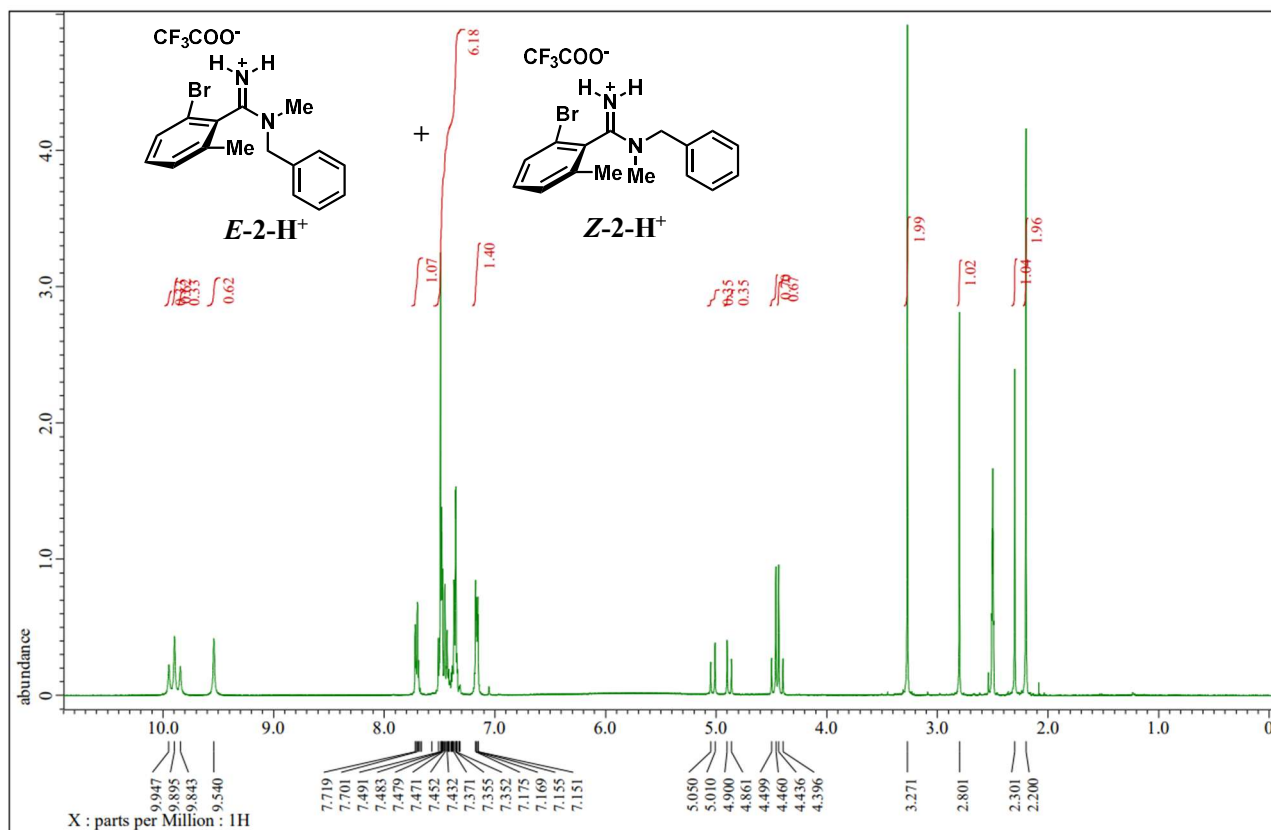


Figure S9.5. ¹H NMR spectrum of **2** (DMSO-*d*₆, 400 MHz, 25 °C)

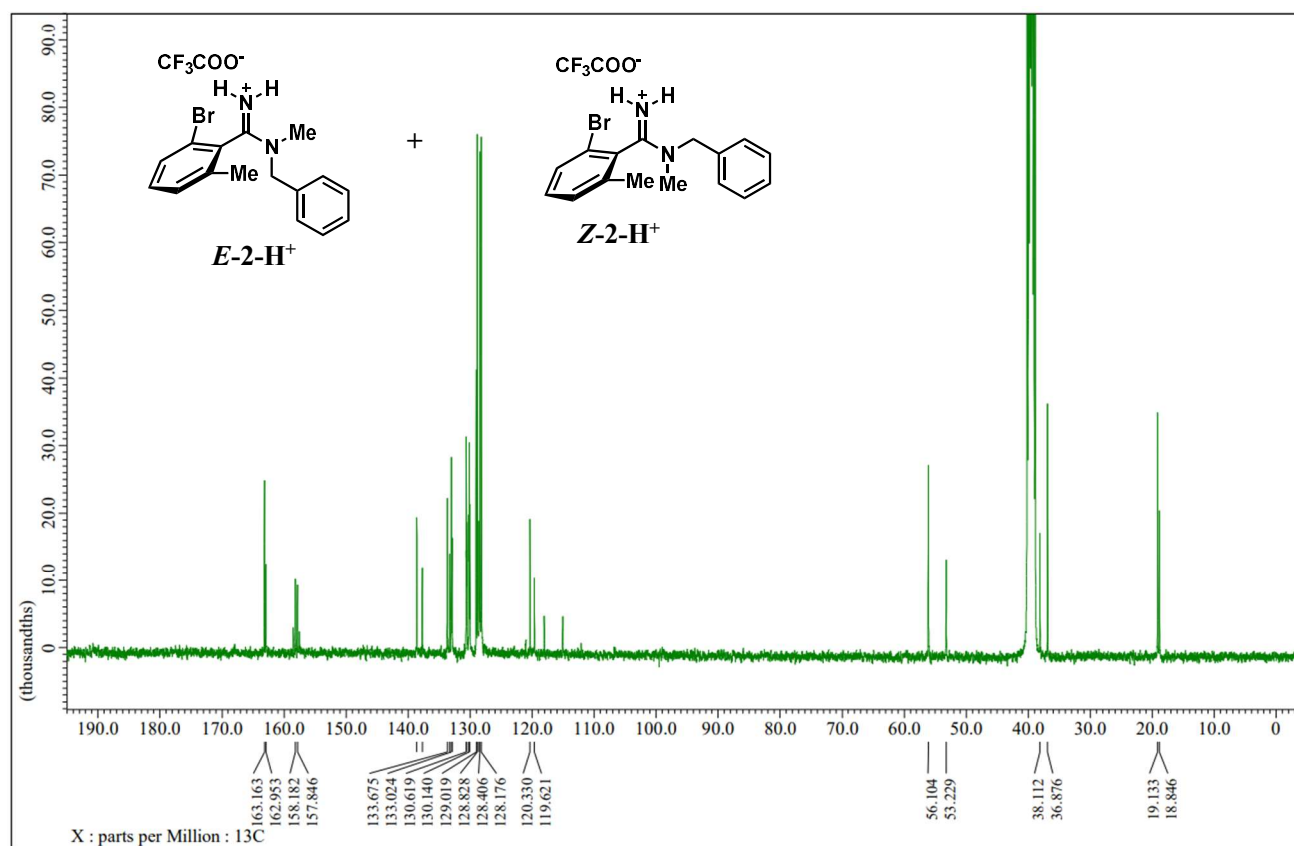


Figure S9.6. ¹³C NMR spectrum of **2** (DMSO-*d*₆, 100 MHz, 25 °C)

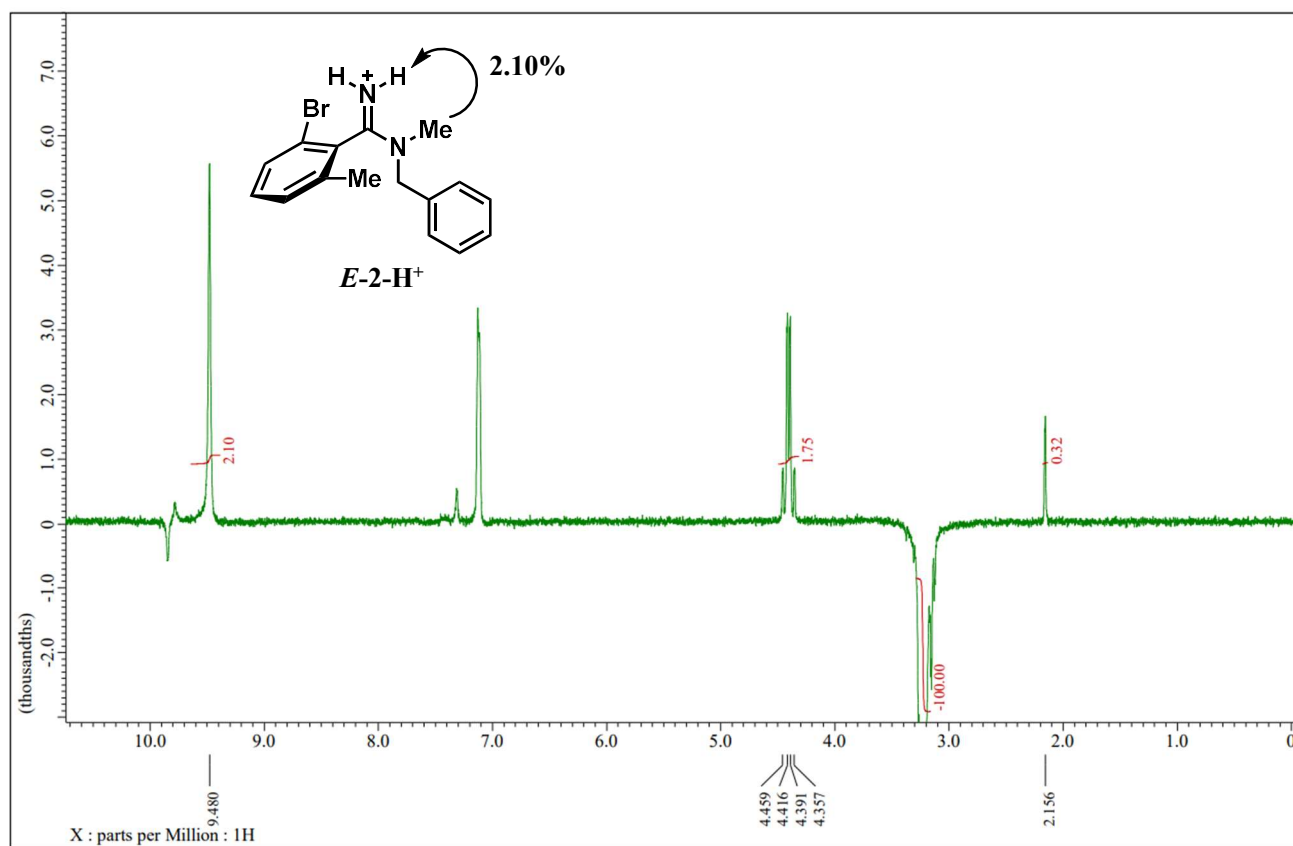


Figure S9.7. NOE spectrum of *E*-2 (DMSO- d_6 , 400 MHz, 25 °C)

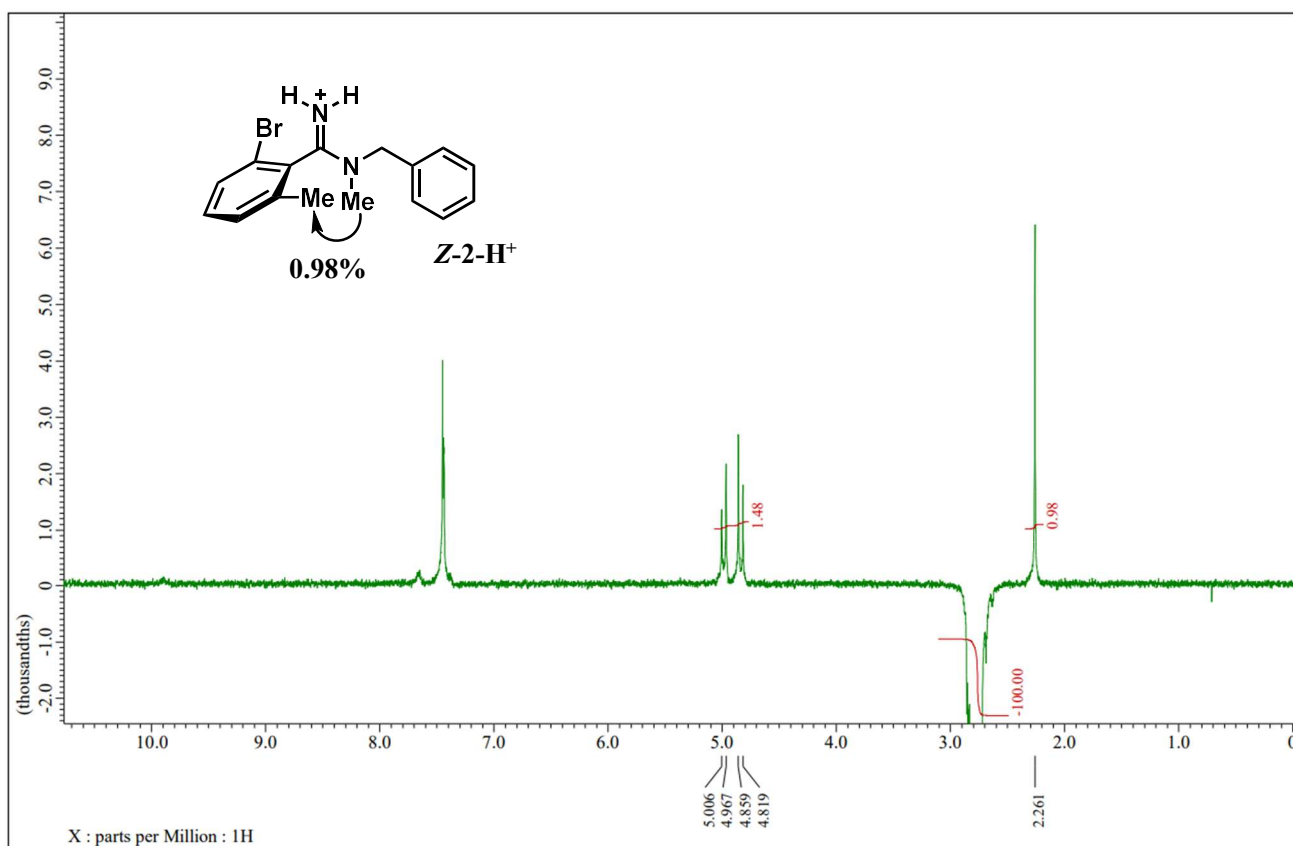


Figure S9.8. NOE spectrum of *Z*-2 (DMSO- d_6 , 400 MHz, 25 °C)

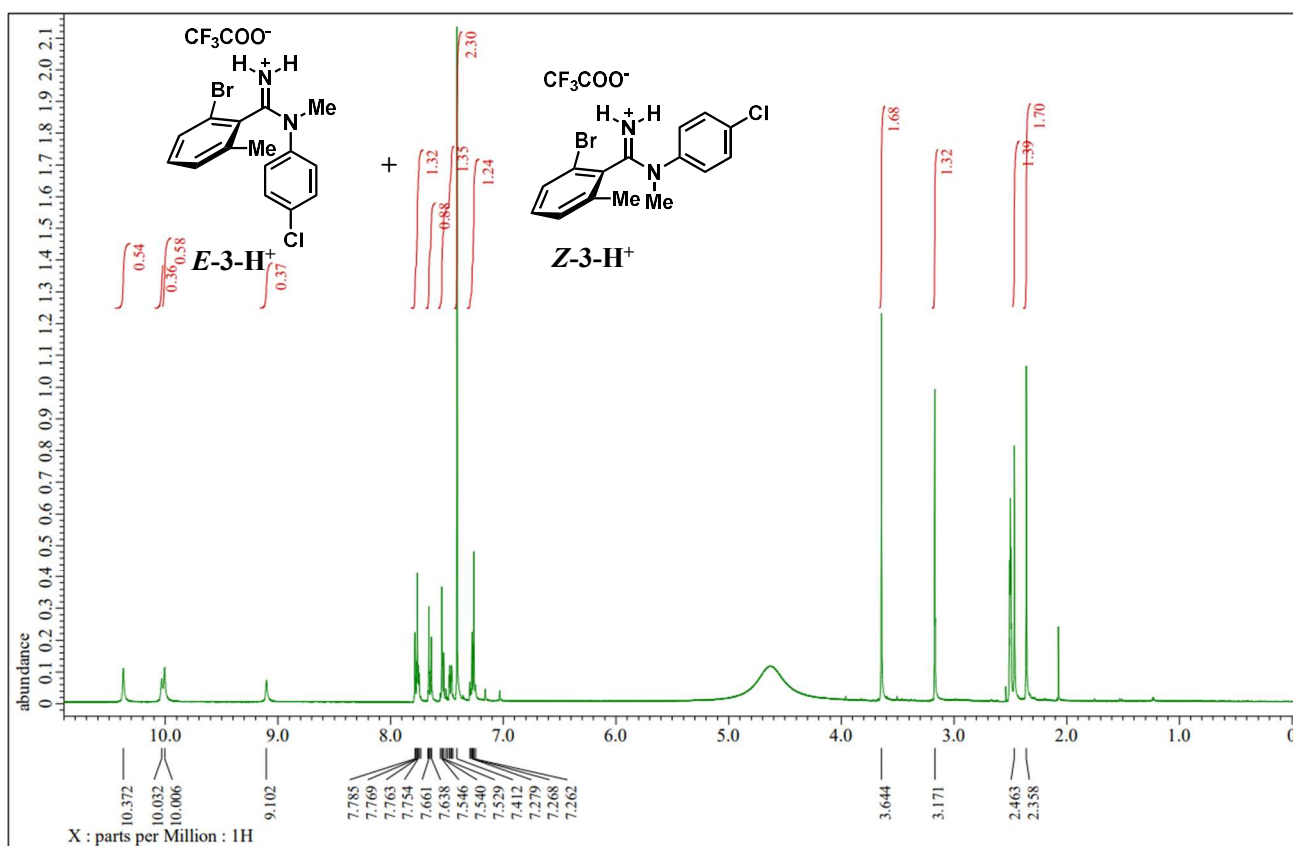


Figure S9.9. ¹H NMR spectrum of **3** (DMSO-*d*₆, 400 MHz, 25 °C)

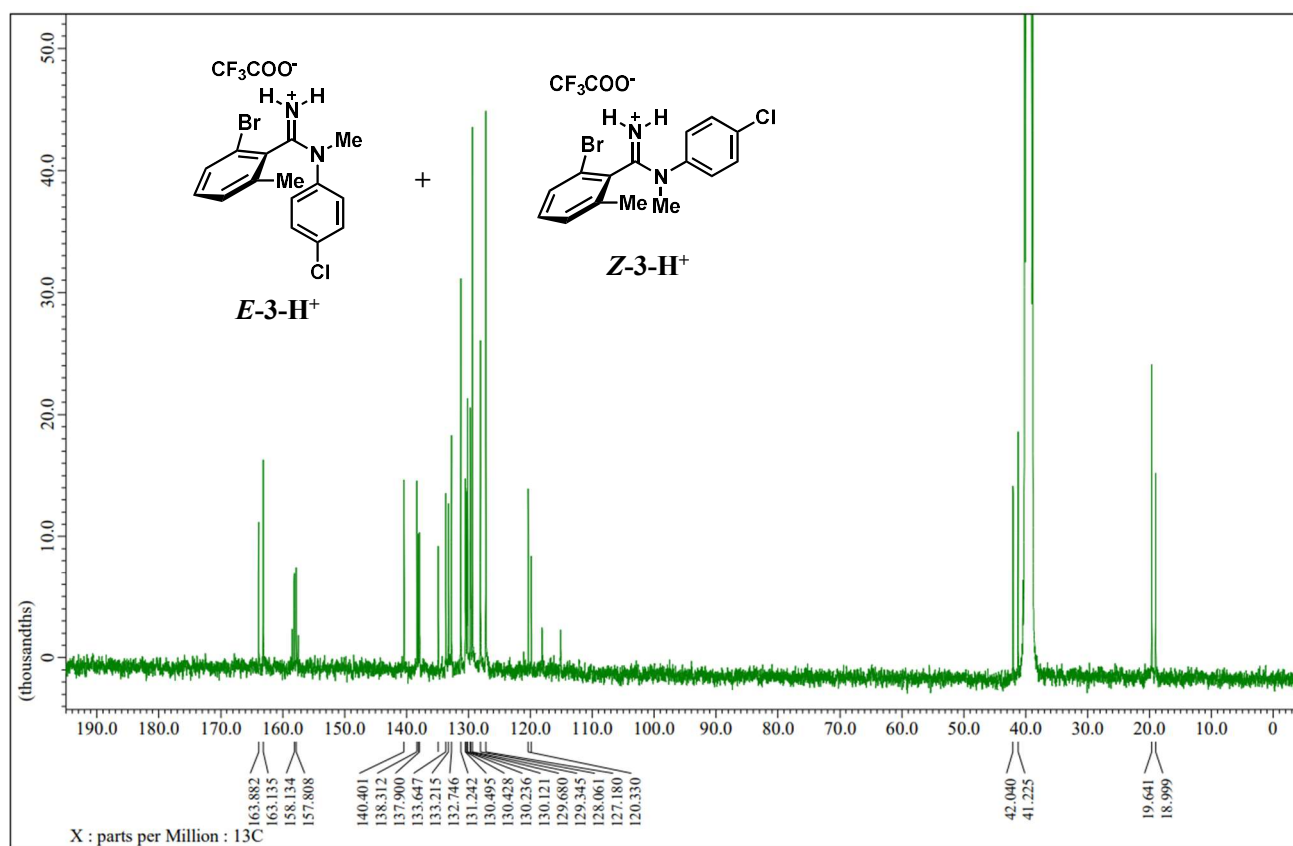


Figure S9.10. ¹³C NMR spectrum of **3** (DMSO-*d*₆, 100 MHz, 25 °C)

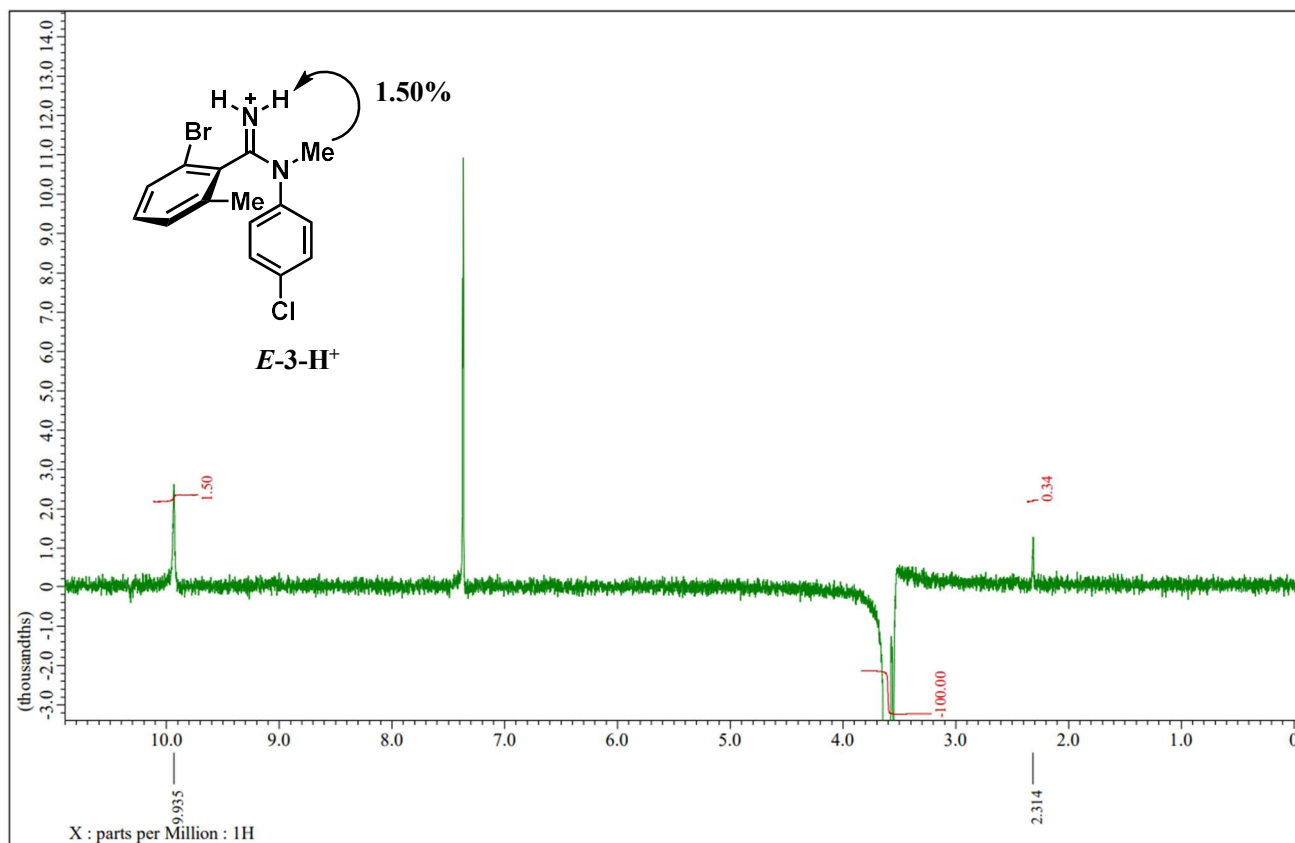


Figure S9.11. NOE spectrum of *E*-3 (DMSO- d_6 , 400 MHz, 25 °C)

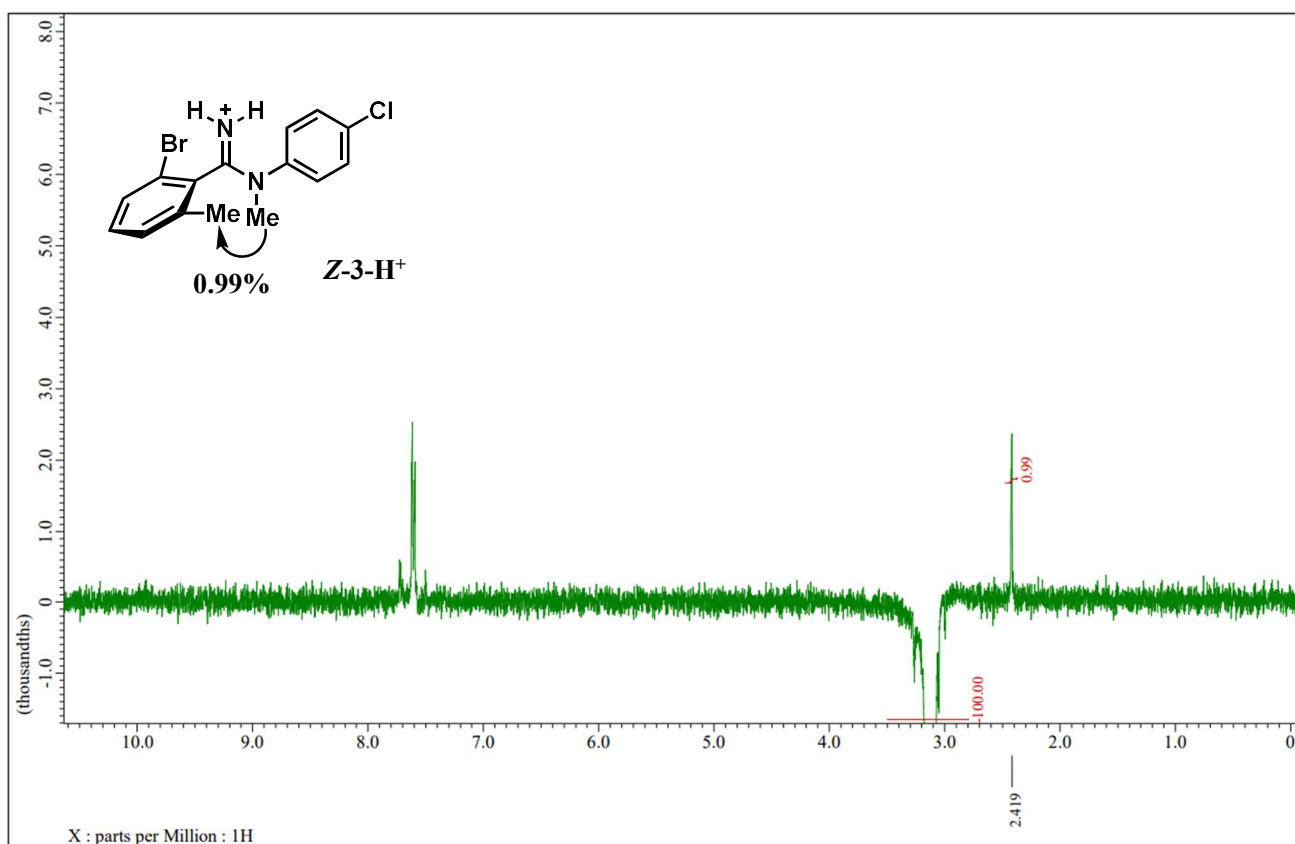


Figure S9.12. NOE spectrum of *Z*-3 (DMSO- d_6 , 400 MHz, 25 °C)

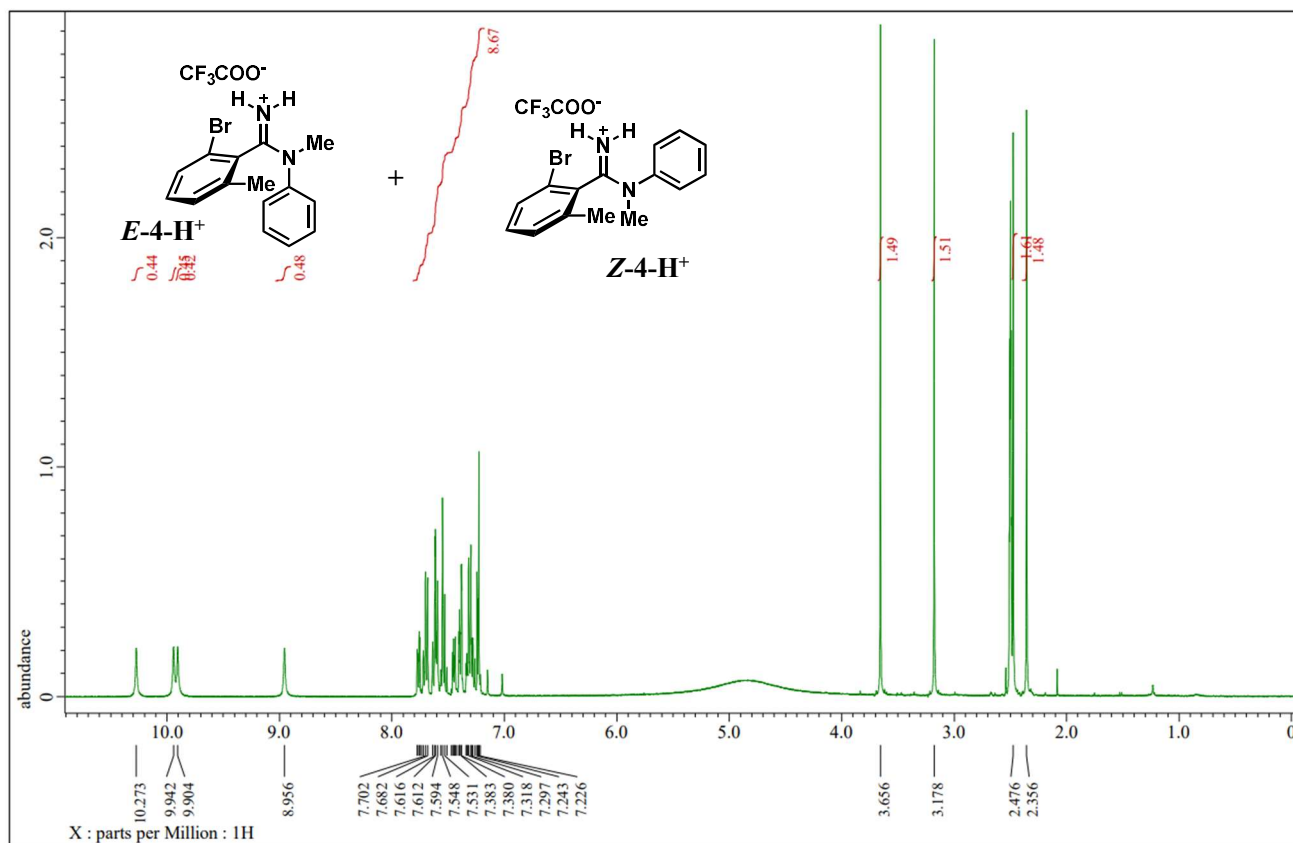


Figure S9.13. ¹H NMR spectrum of **4** (DMSO-*d*₆, 400 MHz, 25 °C)

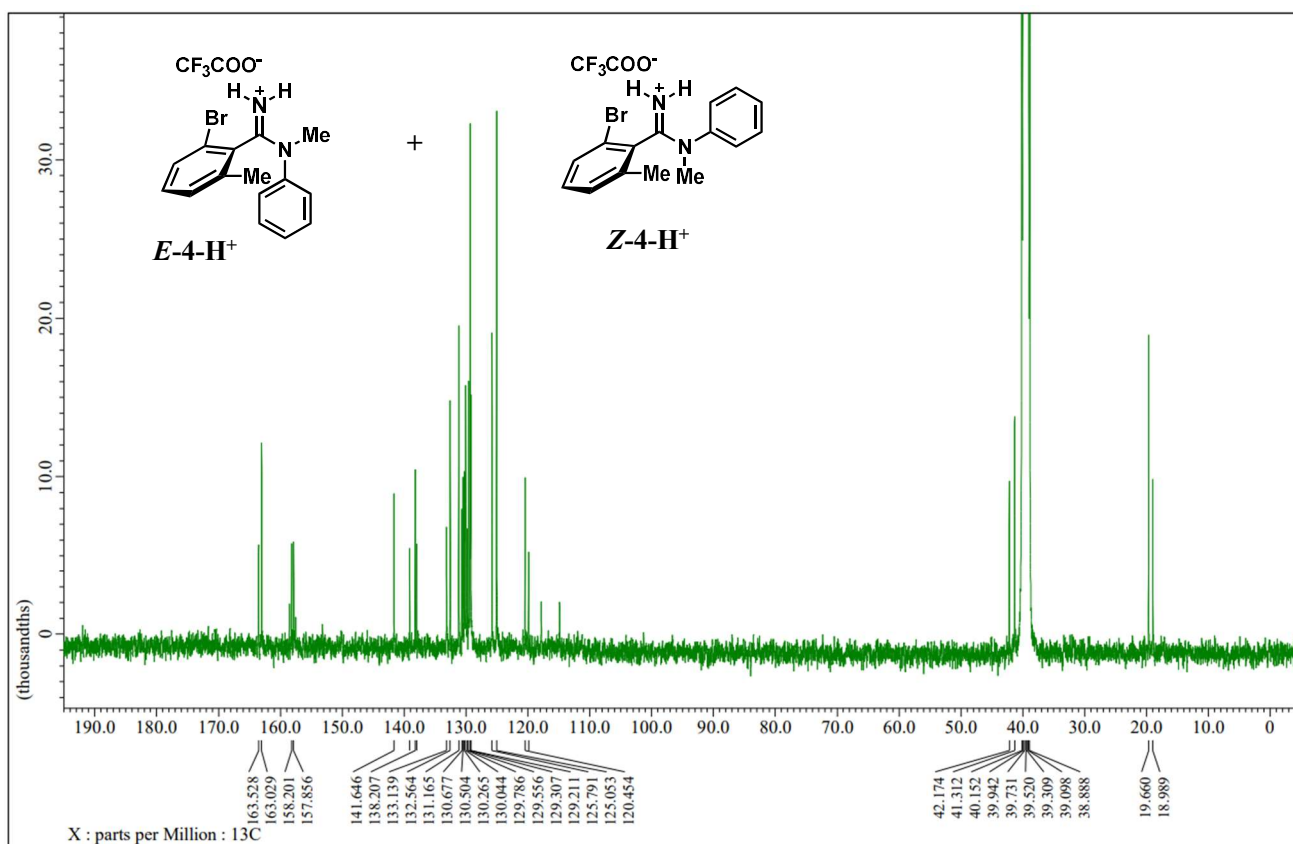


Figure S9.14. ¹³C NMR spectrum of **4** (DMSO-*d*₆, 100 MHz, 25 °C)

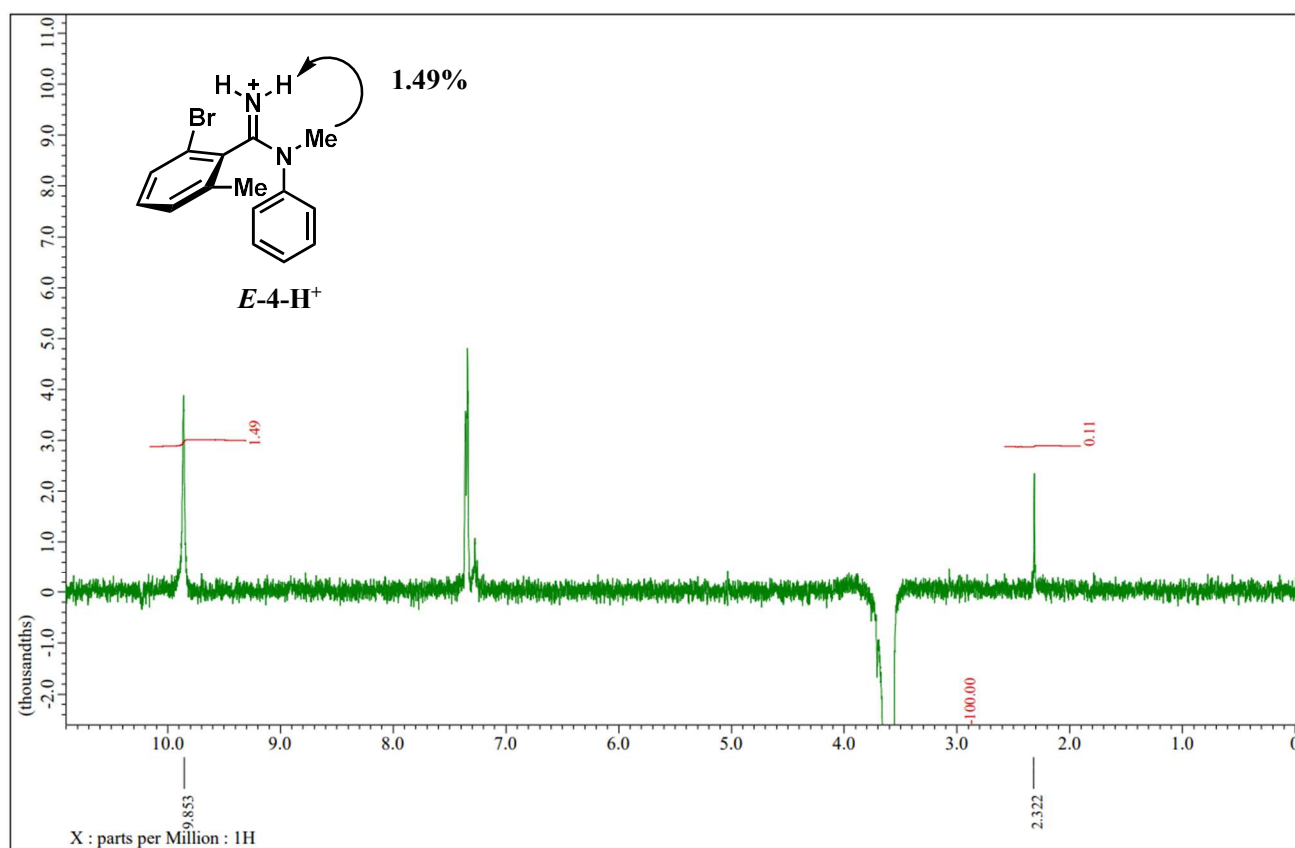


Figure S9.15. NOE spectrum of *E*-4 (DMSO- d_6 , 400 MHz, 25 °C)

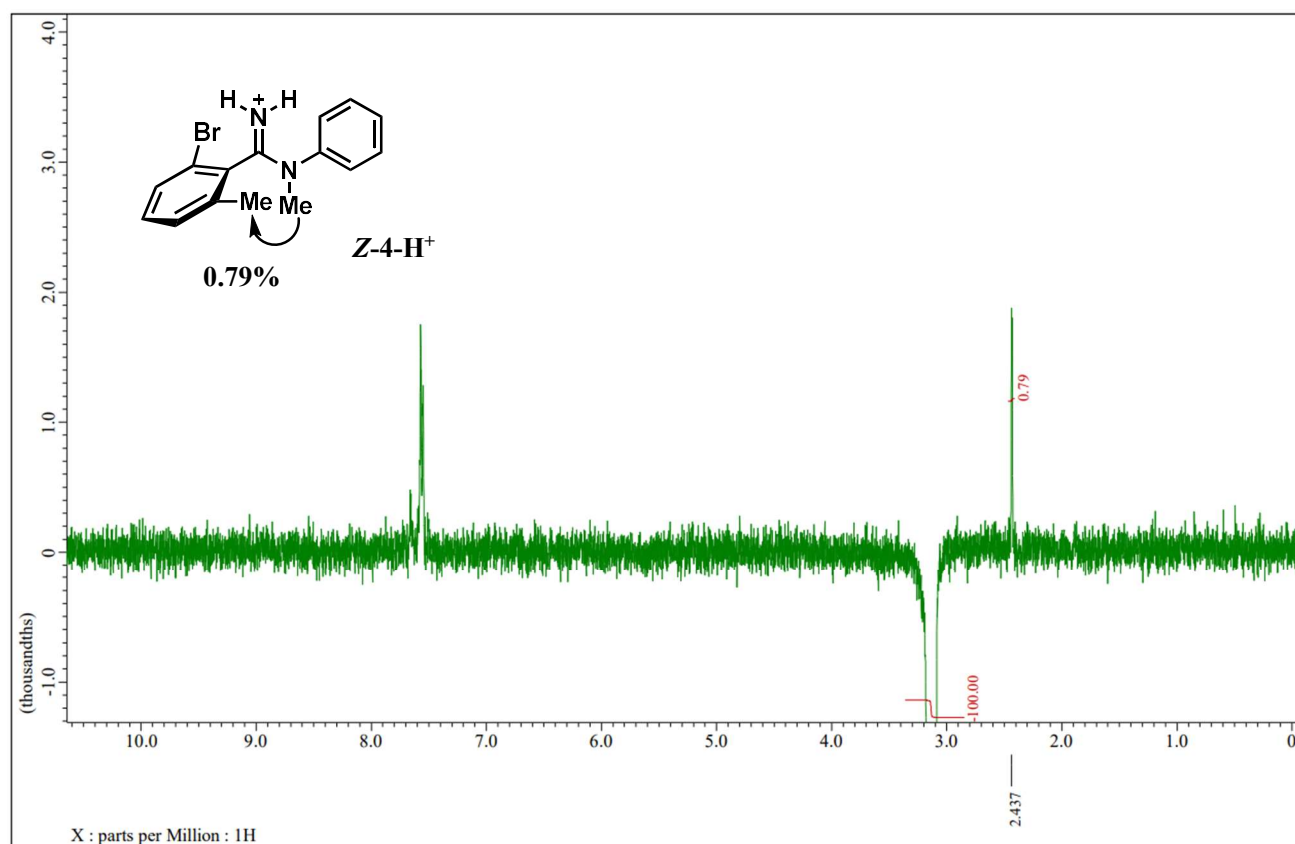


Figure S9.16. NOE spectrum of *Z*-4 (DMSO- d_6 , 400 MHz, 25 °C)

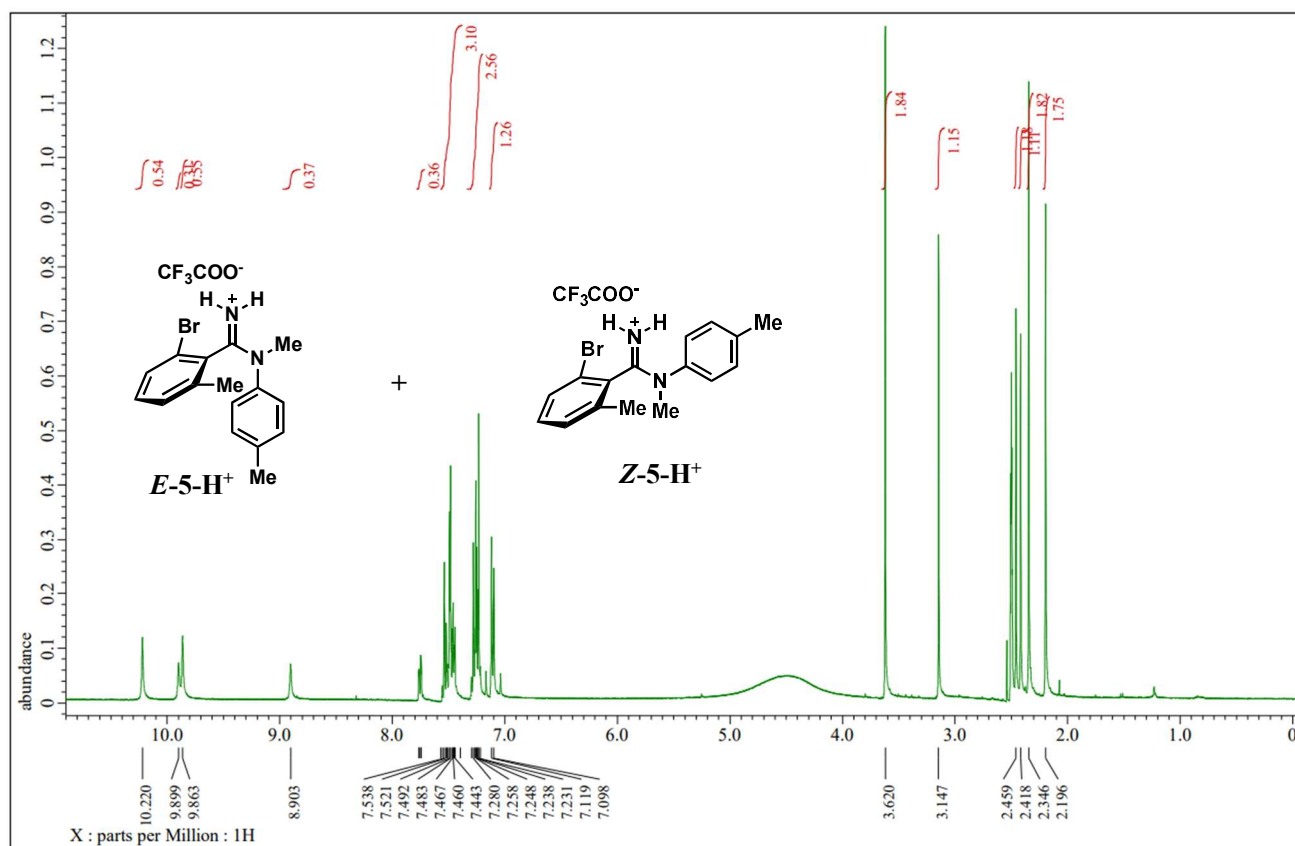


Figure S9.17. 1H NMR spectrum of **5** (DMSO- d_6 , 400 MHz, 25 °C)

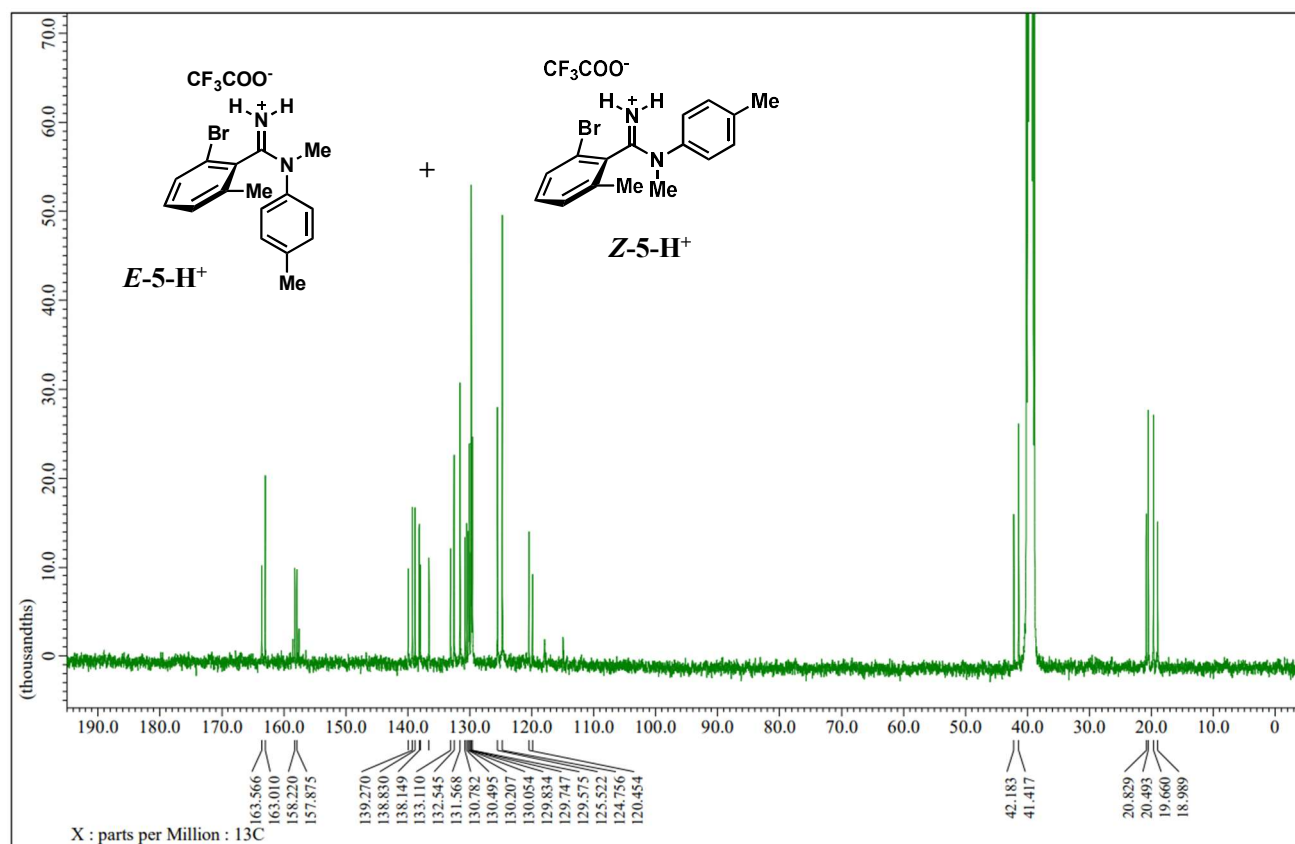


Figure S9.18. ^{13}C NMR spectrum of **5** (DMSO- d_6 , 100 MHz, 25 °C)

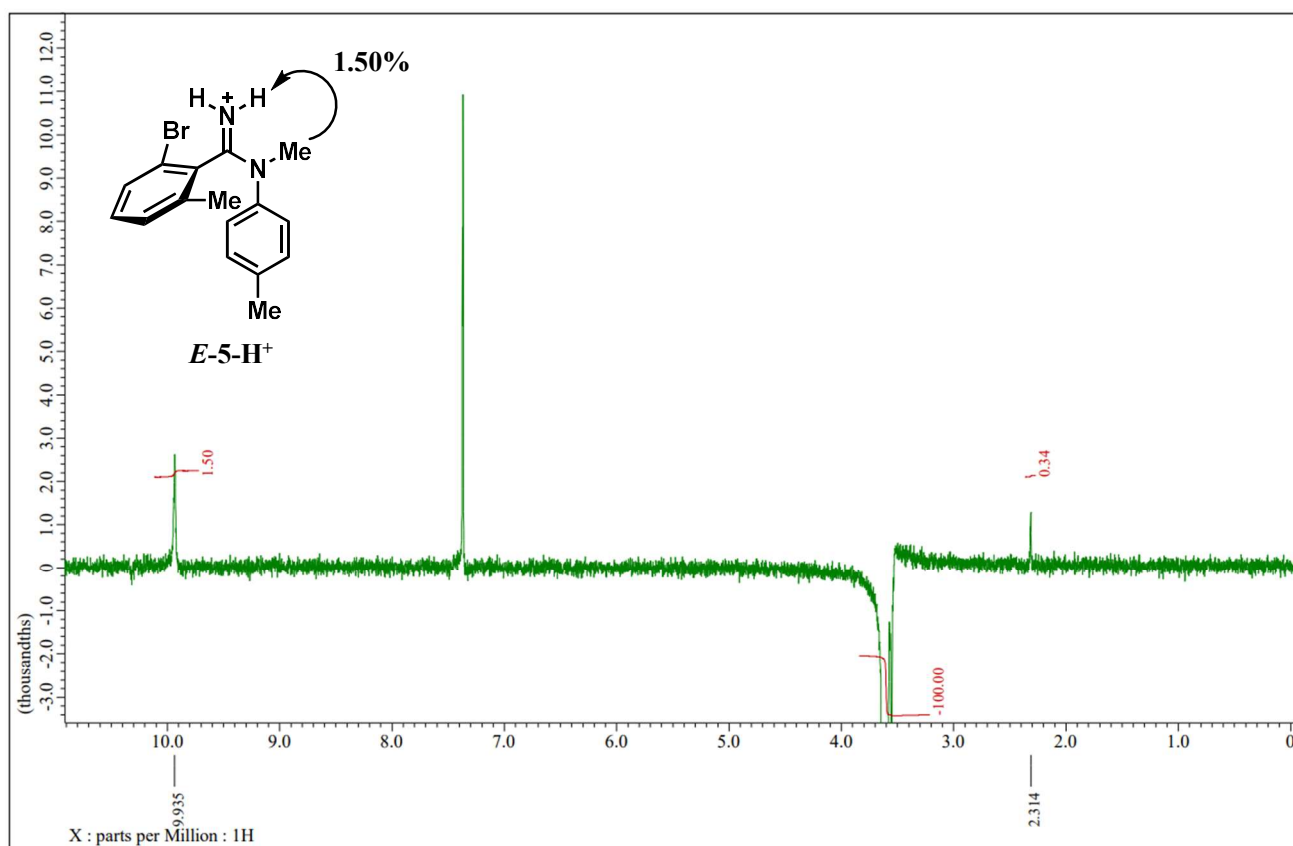


Figure S9.19. NOE spectrum of *E*-5 (DMSO- d_6 , 400 MHz, 25 °C)

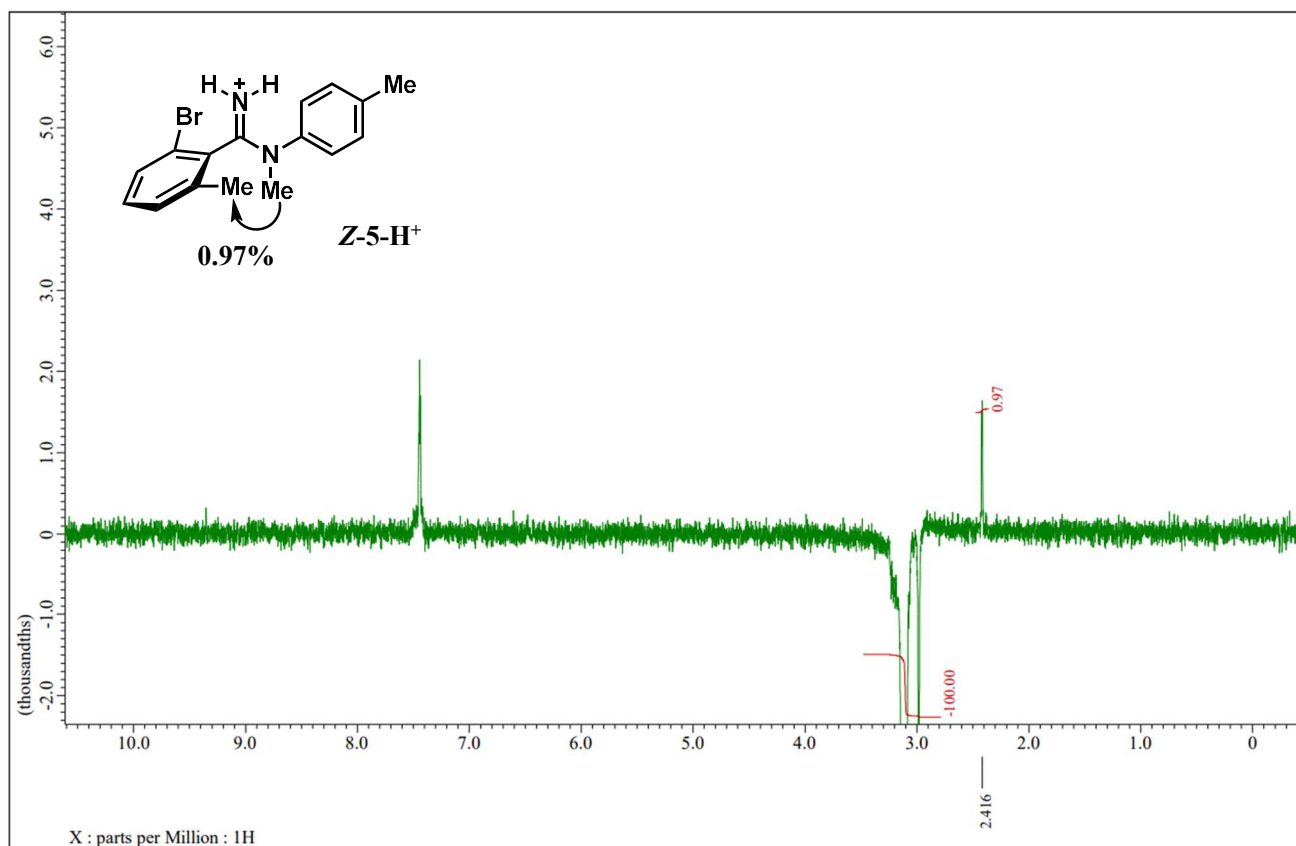


Figure S9.20. NOE spectrum of *Z*-5 (DMSO- d_6 , 400 MHz, 25 °C)

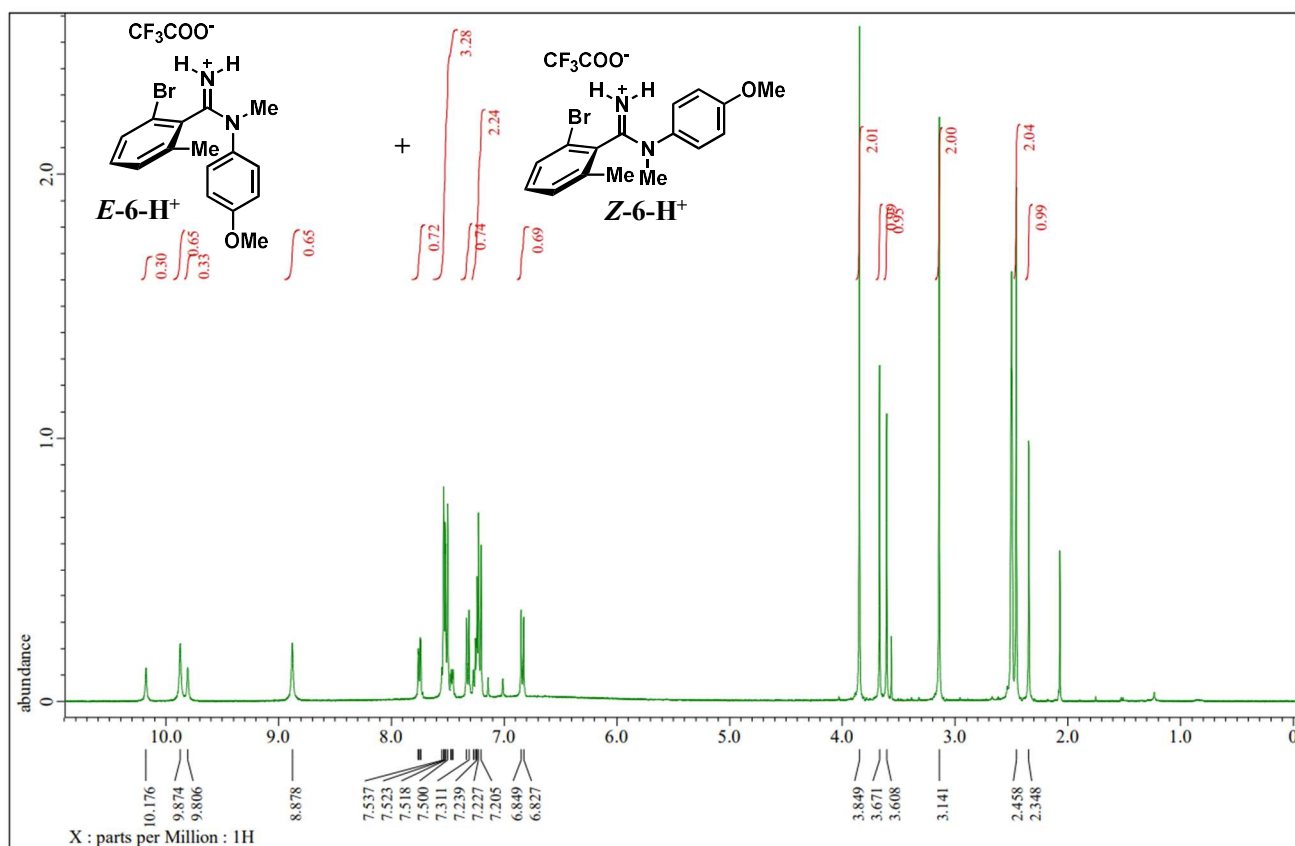


Figure S9.21. ¹H NMR spectrum of **6** (DMSO-*d*₆, 400 MHz, 25 °C)

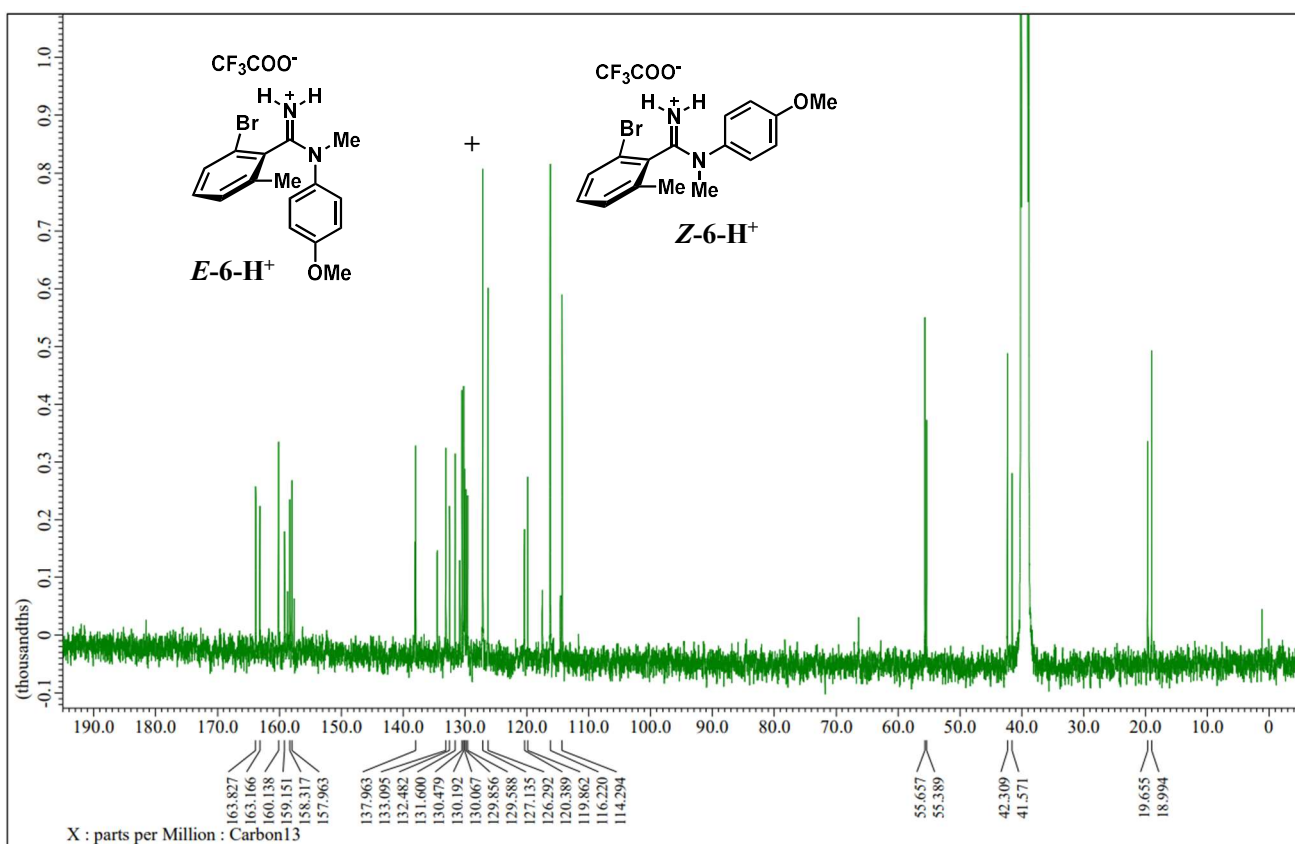


Figure S9.22. ¹³C NMR spectrum of **6** (DMSO-*d*₆, 100 MHz, 25 °C)

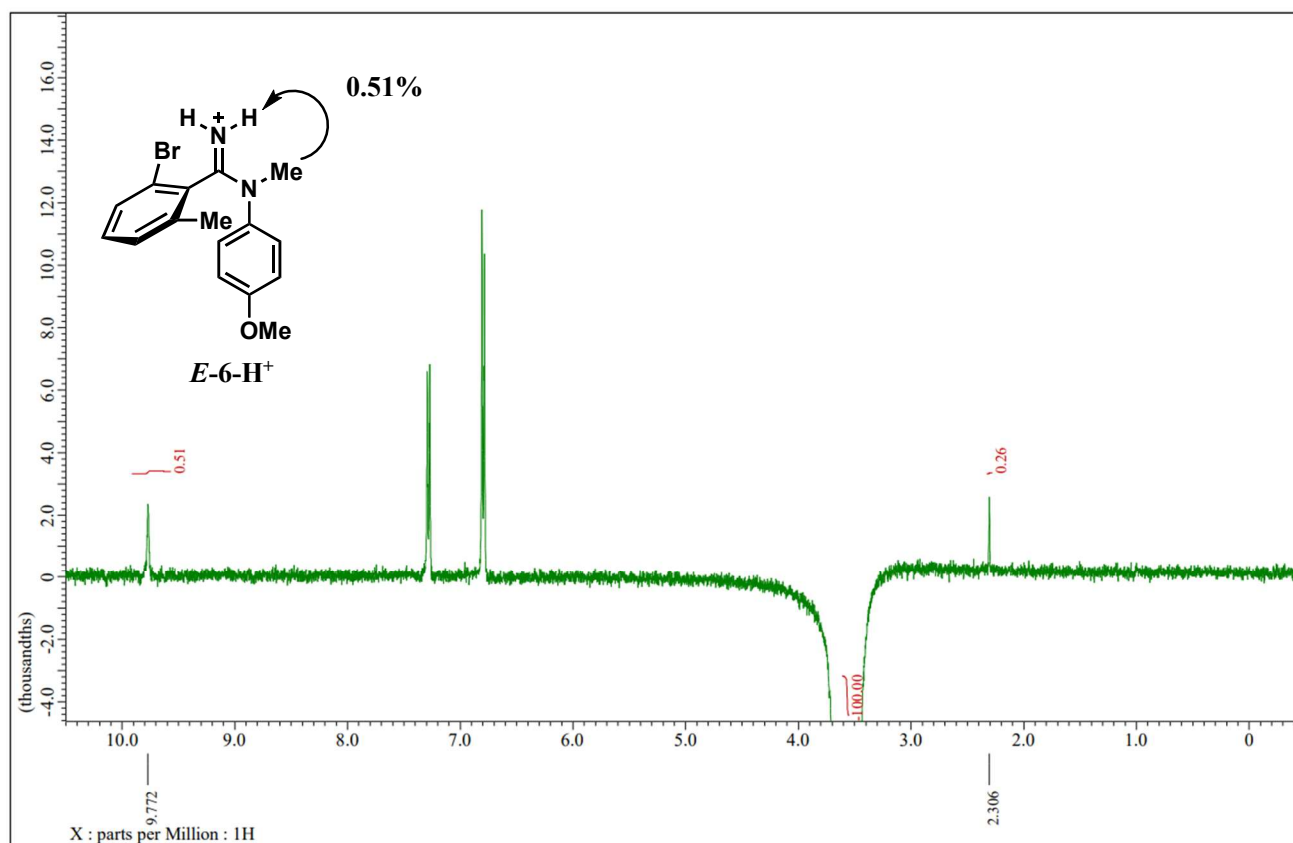


Figure S9.23. NOE spectrum of *E*-6 (DMSO-*d*₆, 400 MHz, 25 °C)

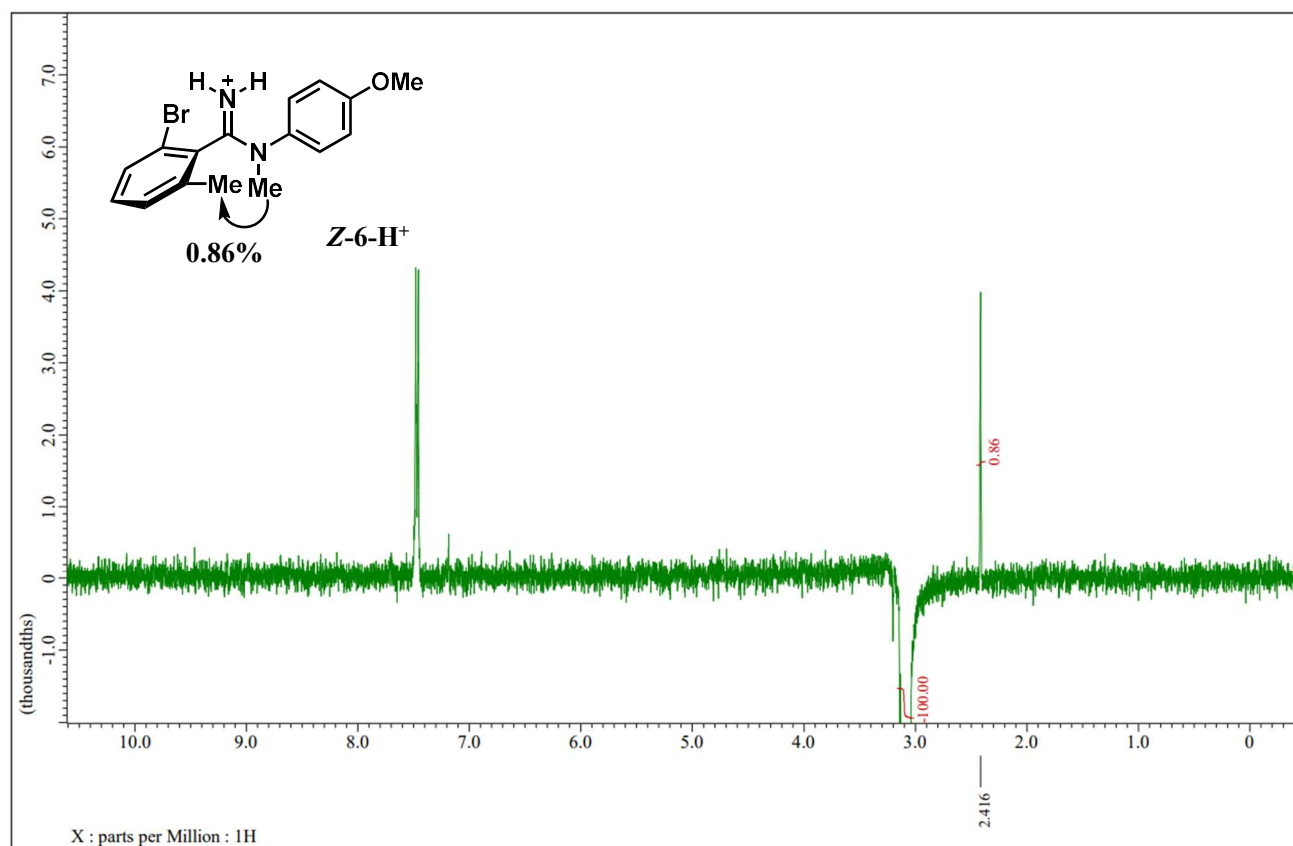


Figure S9.24. NOE spectrum of *Z*-6 (DMSO-*d*₆, 400 MHz, 25 °C)

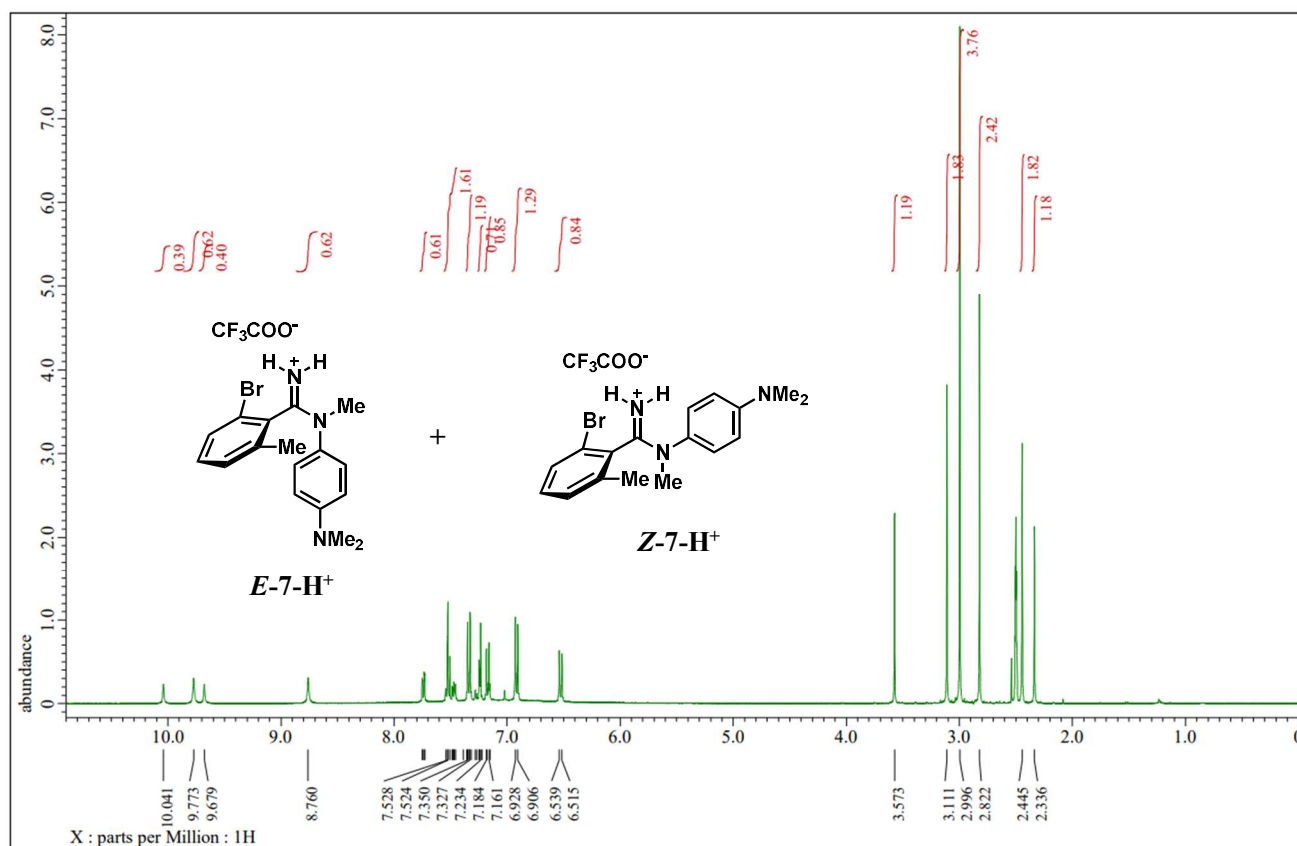


Figure S9.25. ^1H NMR spectrum of 7 (DMSO- d_6 , 400 MHz, 25 °C)

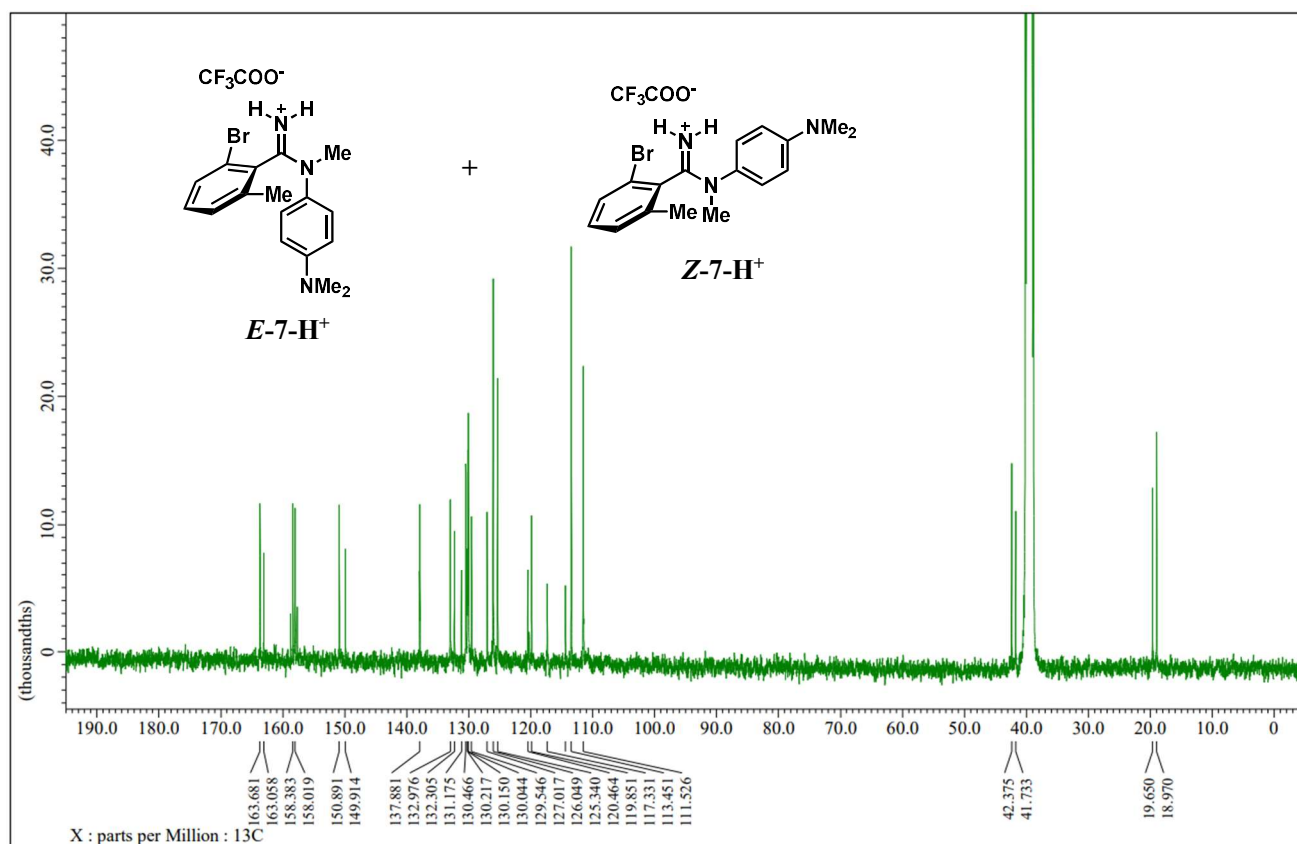


Figure S9.26. ^{13}C NMR spectrum of 7 (DMSO- d_6 , 100 MHz, 25 °C)

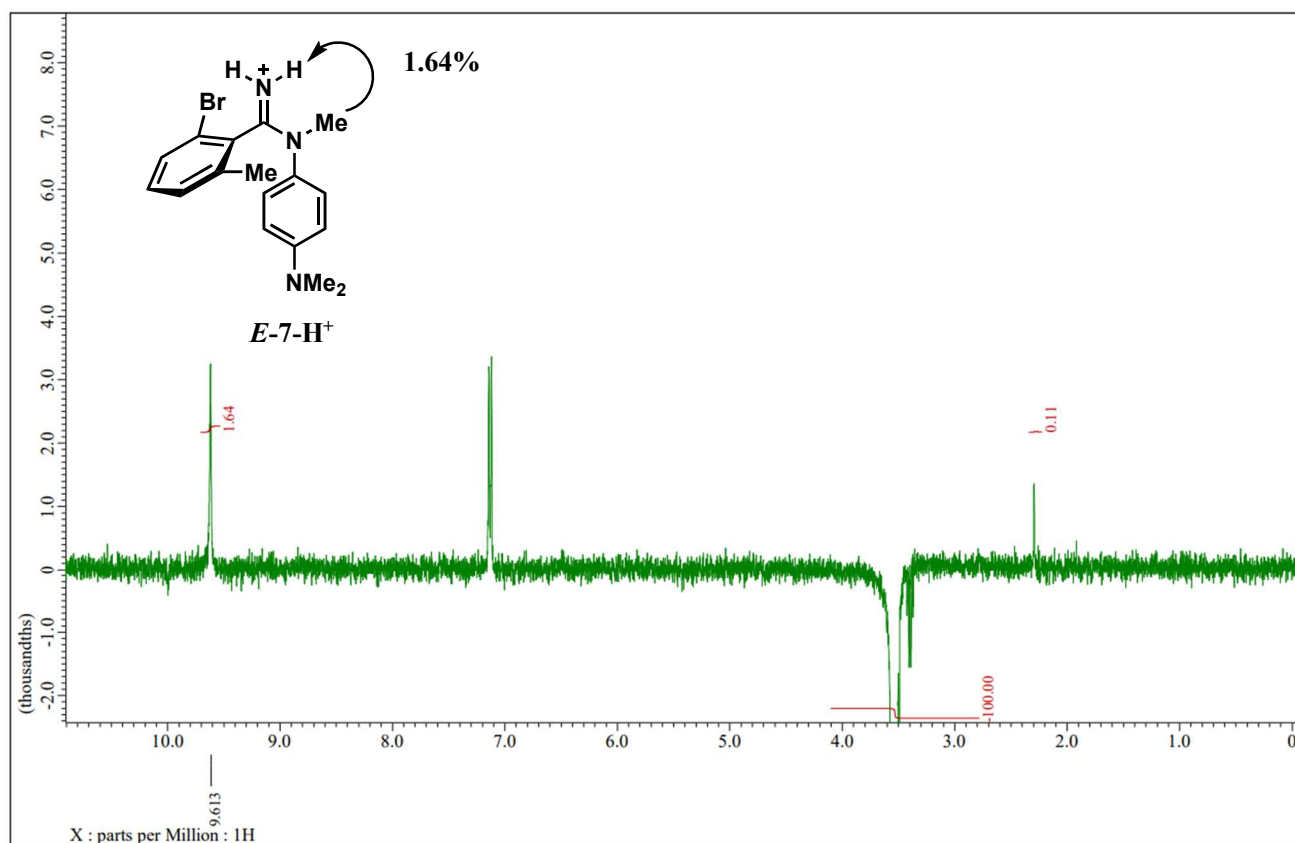


Figure S9.27. NOE spectrum of *E*-7 (DMSO- d_6 , 400 MHz, 25 °C)

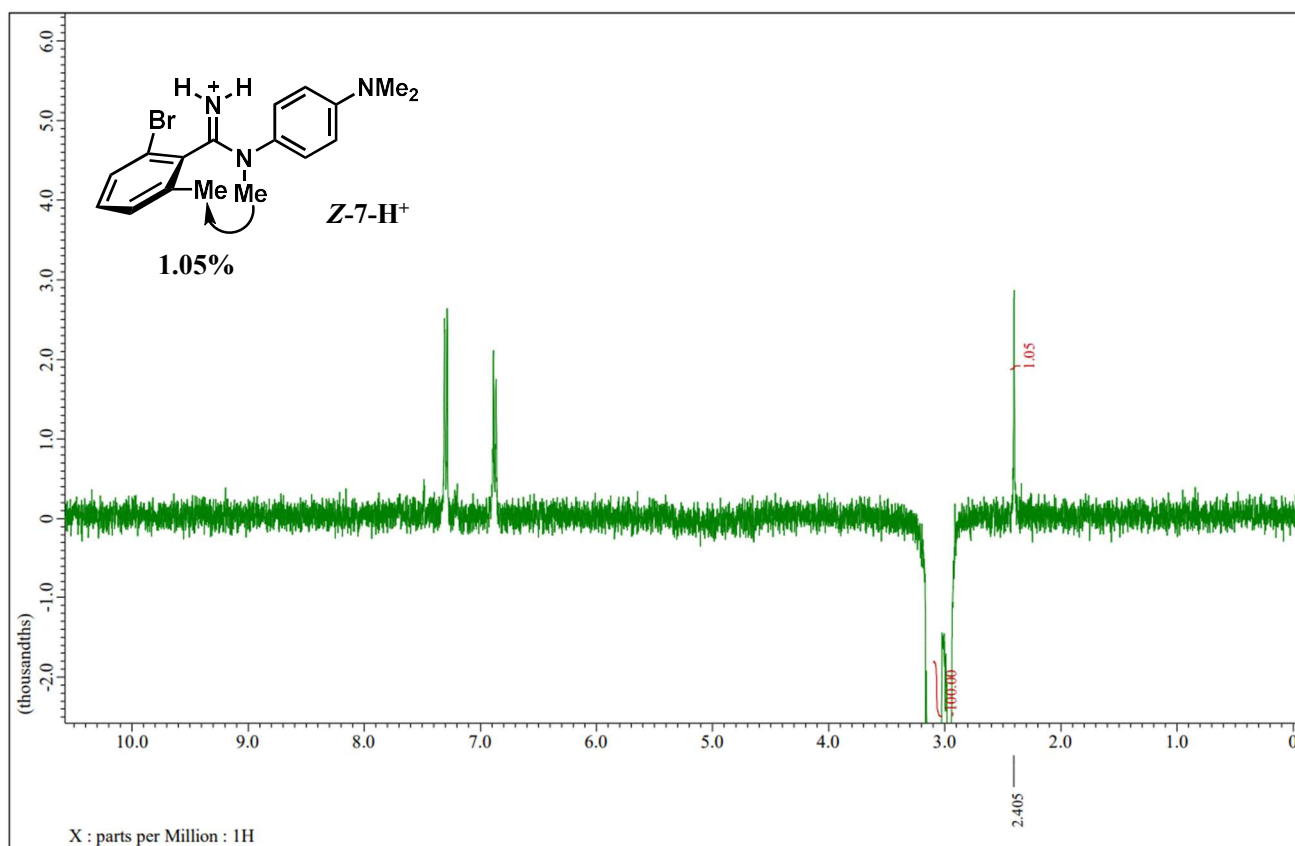
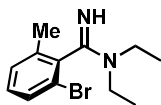


Figure S9.28. NOE spectrum of *Z*-7 (DMSO- d_6 , 400 MHz, 25 °C)

10. LR-MS and HR-MS charts of the synthesized compounds

2-Bromo-*N,N*-diethyl-6-methylbenzimidamide (1)



Sample No. : C:\Xcalibur\...0418\250078_23KIM1-84_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 13:17:42

Instrumental method : C:\Xcalibur\methods\ESI_100u\MS60_100ul_mz100_1500pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

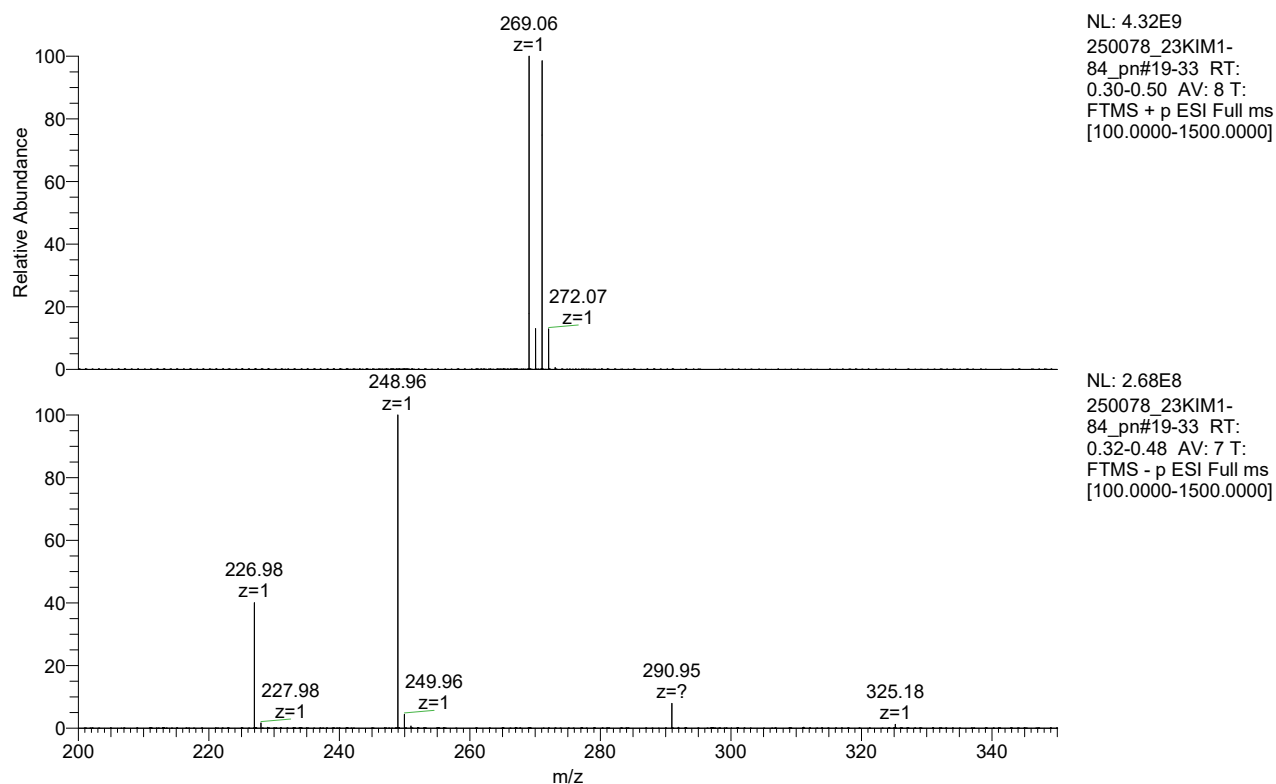


Figure S10.1. LR-MS spectra of compound 1

Sample No. : C:\Xcalibur\...\0418\250078_23KIM1-84_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

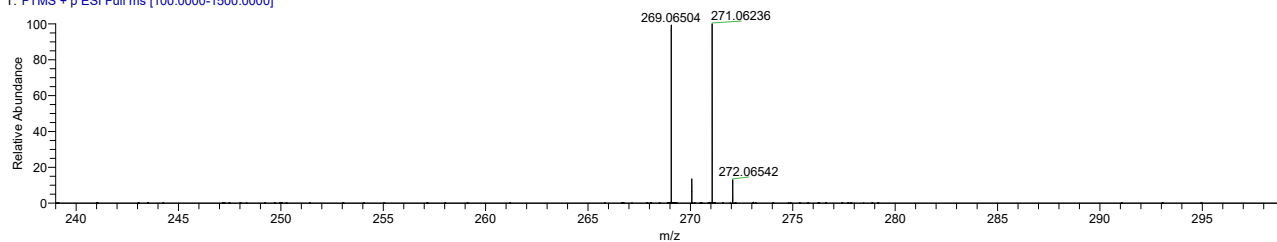
Date : 04/18/25 13:17:42

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz100_1500pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

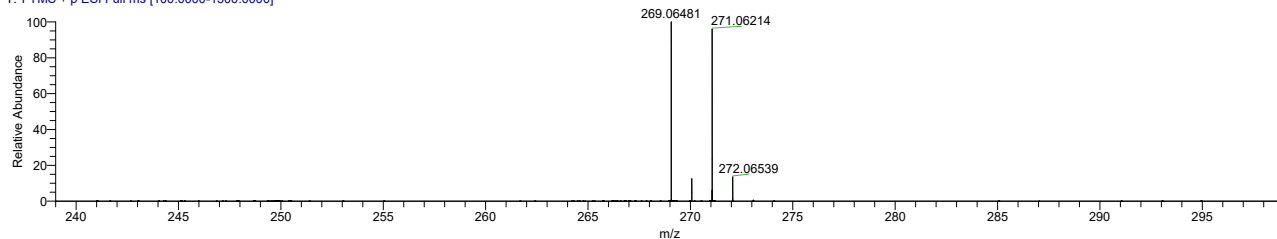
250078_23KIM1-84_pn #20-24 RT: 0.33-0.36 AV: 2 NL: 1.71E9

T: FTMS + p ESI Full ms [100.0000-1500.0000]



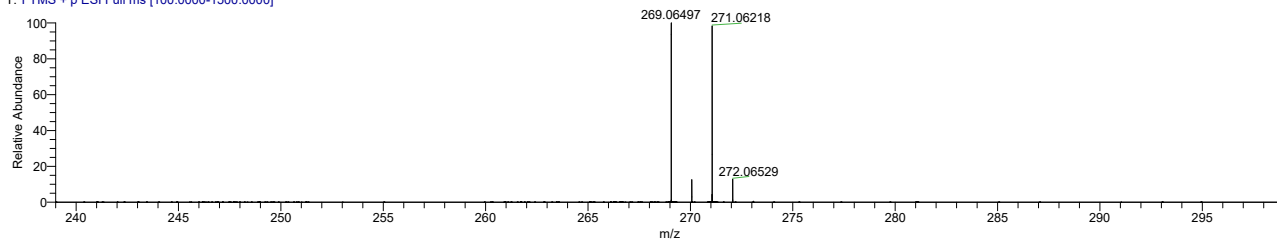
250078_23KIM1-84_pn #26-30 RT: 0.42-0.44 AV: 2 NL: 6.69E9

T: FTMS + p ESI Full ms [100.0000-1500.0000]



250078_23KIM1-84_pn #31-37 RT: 0.47-0.55 AV: 4 NL: 5.18E9

T: FTMS + p ESI Full ms [100.0000-1500.0000]



Elemental composition search on mass 269.06

m/z= 264.06-274.06

Isotope Min Max
N-14 0 3
O-16 0 3
C-12 0 20
H-1 0 25
Na-23 0 0
Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

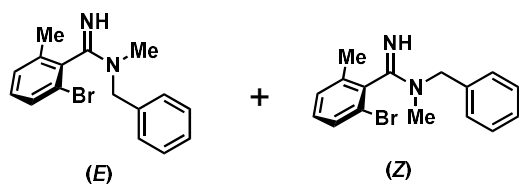
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
269.06481	269.06479	0.08	4.5	C ₁₂ H ₁₈ N ₂ Br

Figure S10.2. HR-MS spectra of compound 1

***N*-Benzyl-2-bromo-*N*,6-dimethylbenzimidamide (2)**



Sample No. : C:\Xcalibur\BG_250072_23KIM1-7_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 14:47:51

Instrumental method : C:\Xcalibur\methods\ESI_100u\lS60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

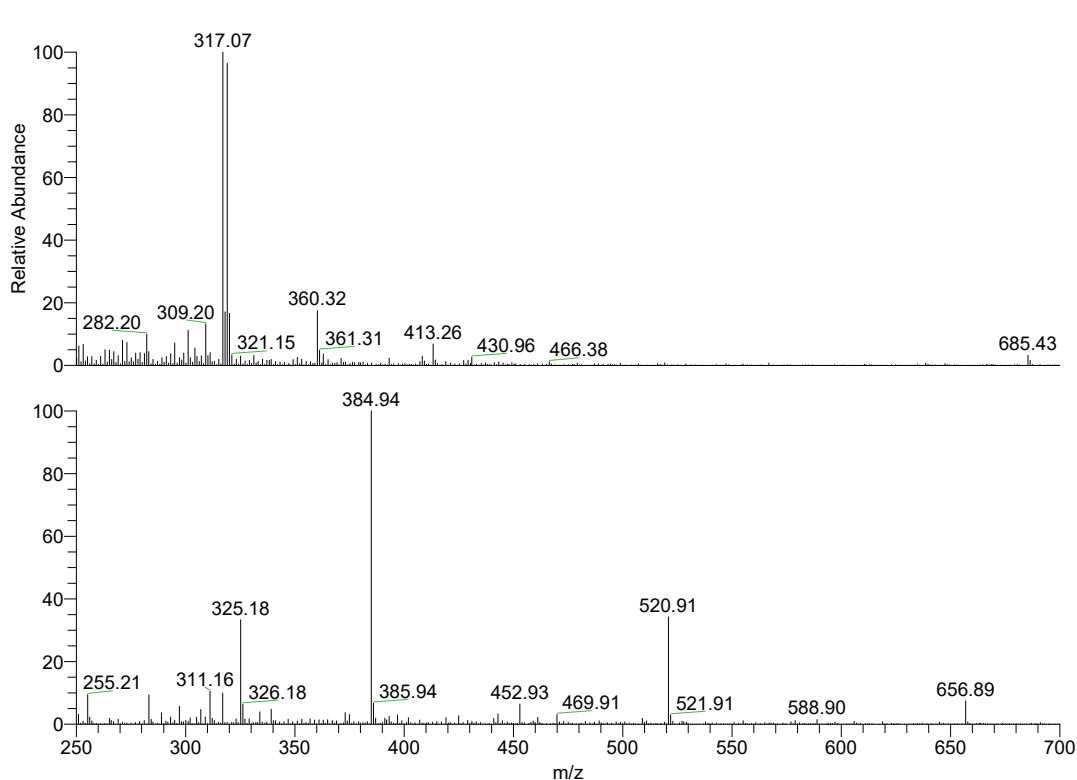


Figure S10.3. LR-MS spectra of compound **2**

Sample No. : C:\Xcalibur\...\0418\250072_23KIM1-7_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

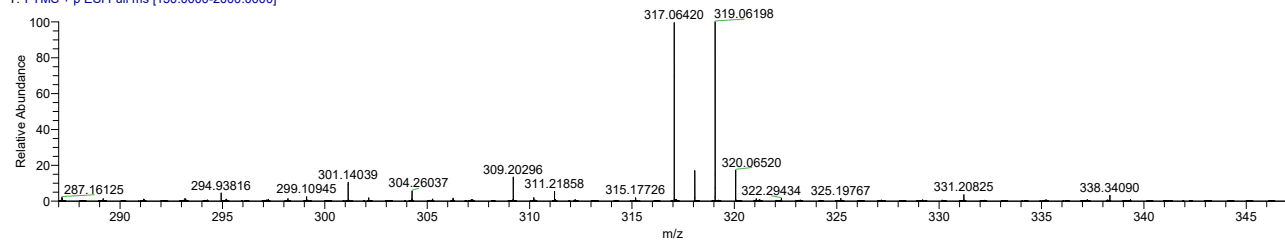
Date : 04/18/25 12:47:05

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

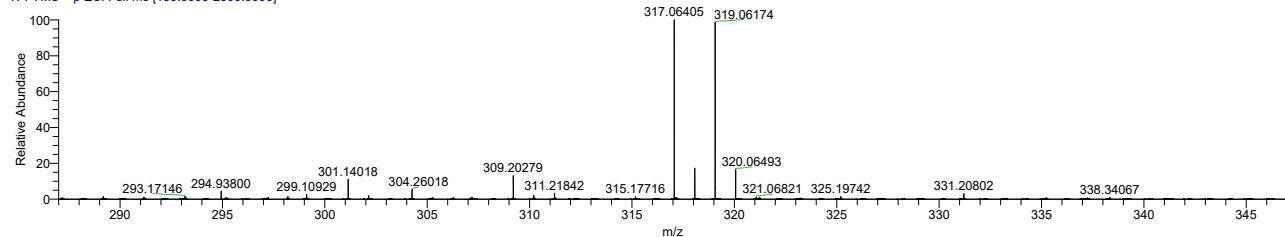
250072_23KIM1-7_pn #19-25 RT: 0.30-0.39 AV: 4 NL: 7.46E6

T: FTMS + p ESI Full ms [150.0000-2000.0000]



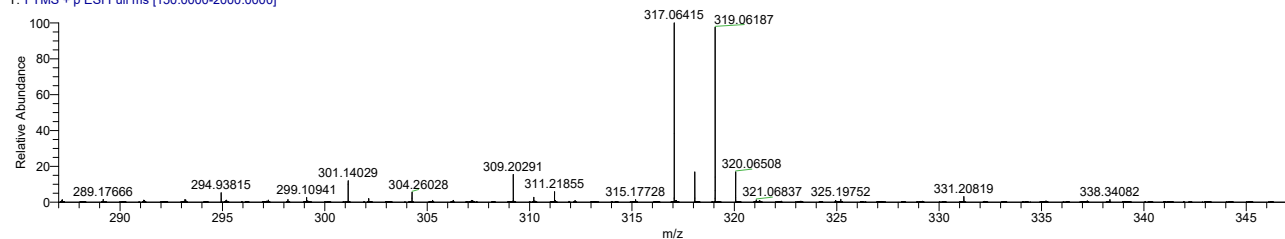
250072_23KIM1-7_pn #25-32 RT: 0.39-0.48 AV: 4 NL: 3.43E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250072_23KIM1-7_pn #32-39 RT: 0.51-0.60 AV: 4 NL: 1.71E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 317.06

m/z= 312.06-322.06

Isotope Min Max
N-14 0 3
O-16 0 0
C-12 0 20
H-1 0 20
Na-23 0 1
Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

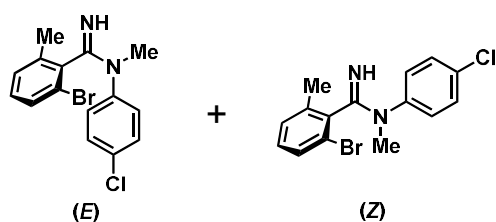
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
317.06405	317.06479	-2.33	8.5	C ₁₆ H ₁₈ N ₂ Br

Figure S10.4. HR-MS spectra of compound 2

2-Bromo-*N*-(4-chlorophenyl)-*N*,6-dimethylbenzimidamide (3)



Sample No. : C:\Xcalibur\...BG_250074_23KIM1-38_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 15:03:42

Instrumental method : C:\Xcalibur\methods\ESI_100u\MS60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

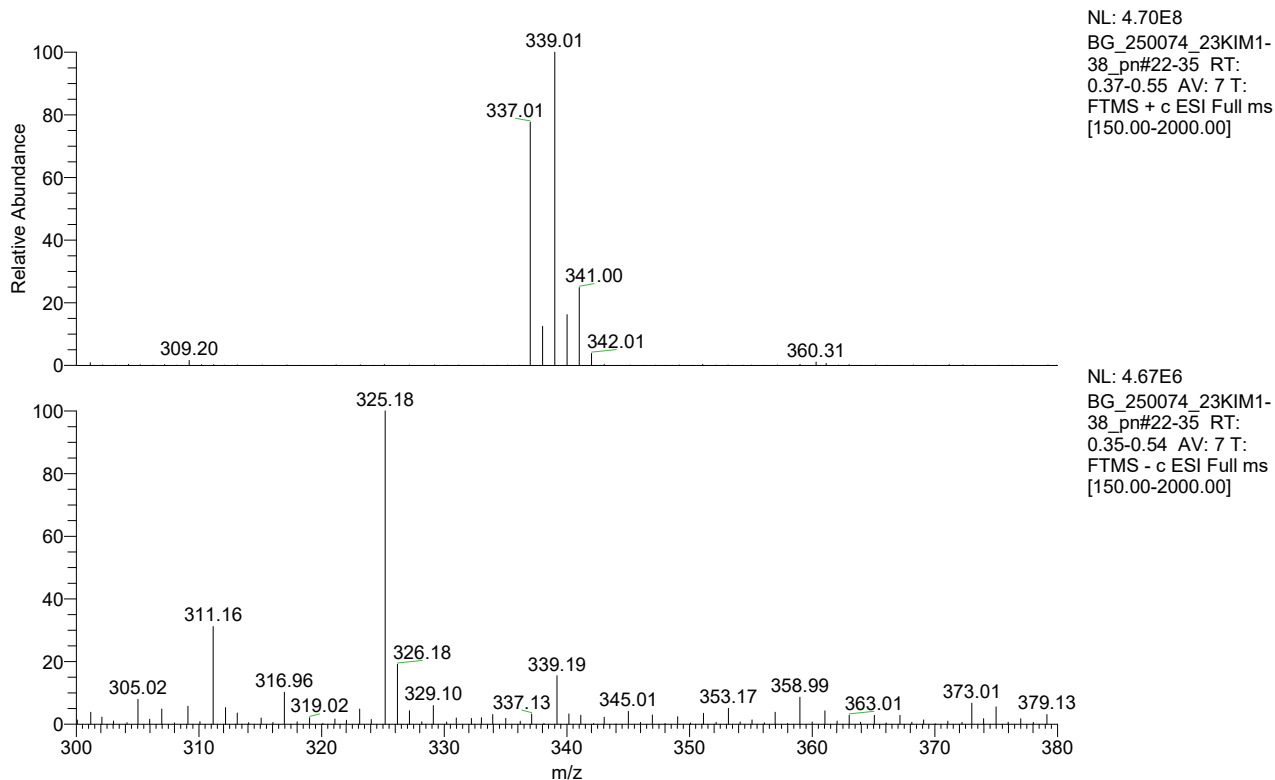


Figure S10.5. LR-MS spectra of compound **3**

Sample No. : C:\Xcalibur\...\0418\250074_23KIM1-38_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

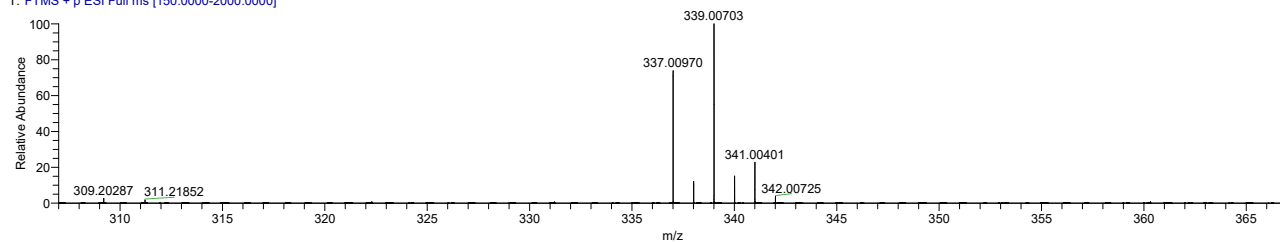
Date : 04/18/25 12:57:07

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

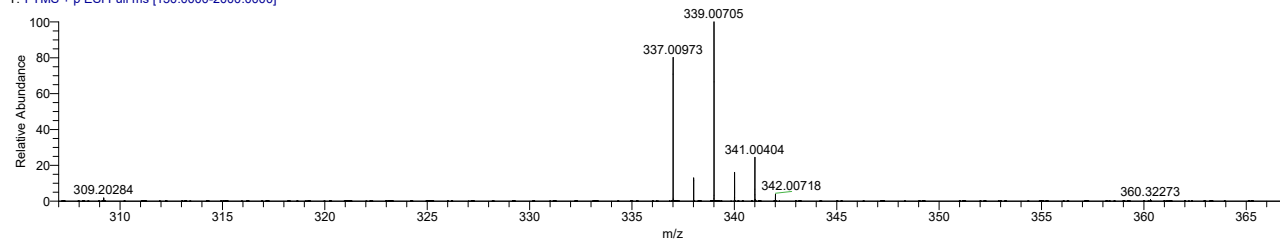
250074_23KIM1-38_pn #19-24 RT: 0.30-0.37 AV: 3 NL: 1.11E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



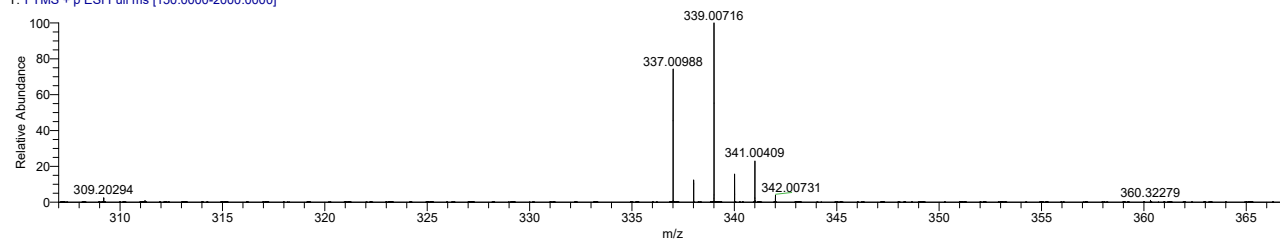
250074_23KIM1-38_pn #25-31 RT: 0.40-0.49 AV: 4 NL: 1.42E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250074_23KIM1-38_pn #32-38 RT: 0.52-0.58 AV: 3 NL: 6.62E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 337.01

m/z= 332.01-342.01

Isotope Min Max
N-14 0 3
O-16 0 0
C-12 0 20
H-1 0 20
Na-23 0 1
Br-79 1 1
Cl-35 0 3

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

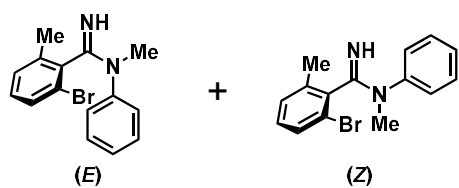
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
337.00973	337.01017	-1.29	8.5	C ₁₅ H ₁₅ N ₂ BrCl

Figure S10.6. HR-MS spectra of compound 3

2-Bromo-*N*,6-dimethyl-*N*-phenylbenzimidamide (4)



Sample No. : C:\Xcalibur\...BG_250073_23KIM1-28_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

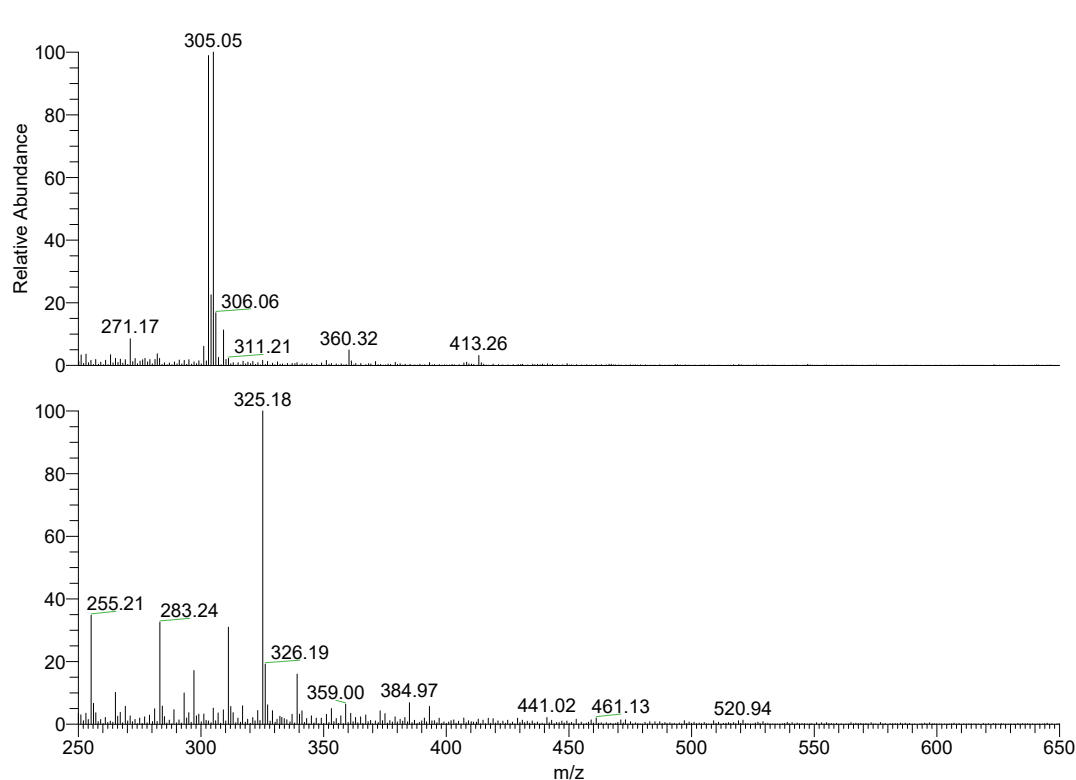
Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 14:57:24

Instrumental method : C:\Xcalibur\methods\ESI_100u\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University



NL: 1.52E8
BG_250073_23KIM1-28_pn#22-35 RT: 0.37-0.55 AV: 7 T: FTMS + c ESI Full ms [150.00-2000.00]

NL: 7.65E6
BG_250073_23KIM1-28_pn#22-35 RT: 0.35-0.54 AV: 7 T: FTMS - c ESI Full ms [150.00-2000.00]

Figure S10.7. LR-MS spectra of compound 4

Sample No. : C:\Xcalibur\...\0418\250073_23KIM1-28_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

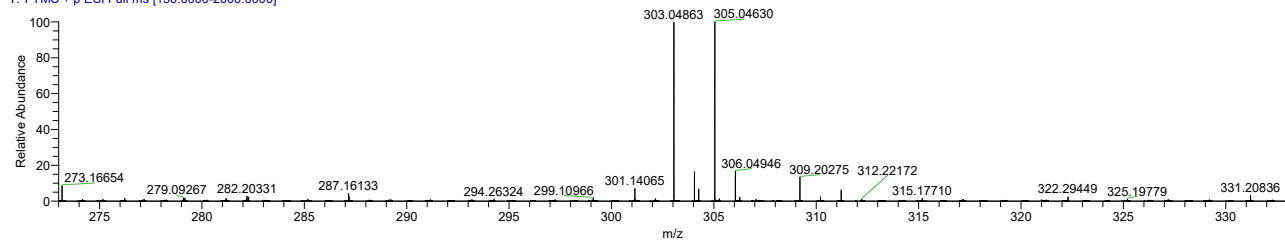
Date : 04/18/25 12:52:06

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

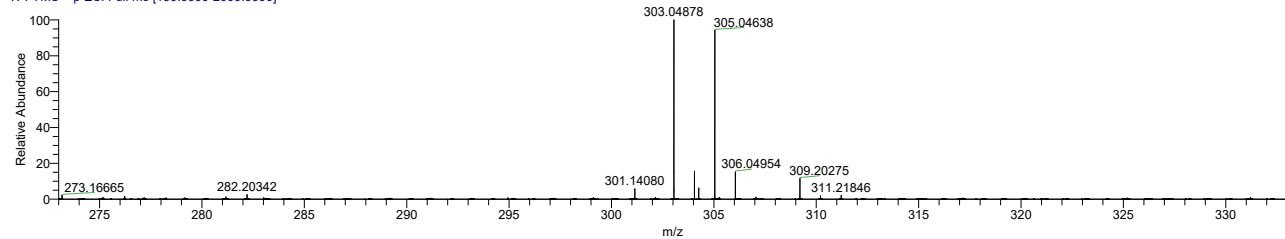
250073_23KIM1-28_pn #19-24 RT: 0.30-0.37 AV: 3 NL: 6.20E6

T: FTMS + p ESI Full ms [150.0000-2000.0000]



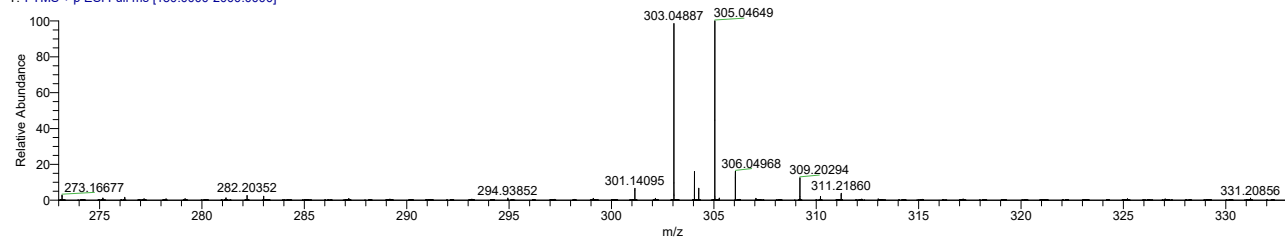
250073_23KIM1-28_pn #25-31 RT: 0.40-0.49 AV: 4 NL: 4.65E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250073_23KIM1-28_pn #32-38 RT: 0.52-0.58 AV: 3 NL: 2.20E7

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 303.05

m/z= 298.05-308.05

Isotope Min Max

N-14 0 3

O-16 0 0

C-12 0 20

H-1 0 20

Na-23 0 1

Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

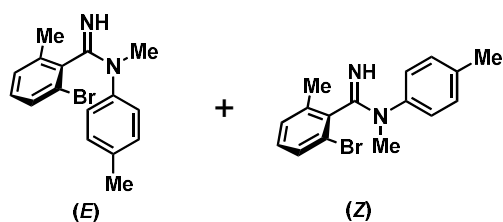
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
303.04878	303.04914	-1.18	8.5	C ₁₅ H ₁₆ N ₂ Br

Figure S10.8. HR-MS spectra of compound 4

2-Bromo-*N*,6-dimethyl-*N*-(4-tolyl)benzimidamide (5)



Sample No. : C:\Xcalibur\...BG_250075_23KIM1-46_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 15:11:30

Instrumental method : C:\Xcalibur\methods\ESI_100u\MS60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

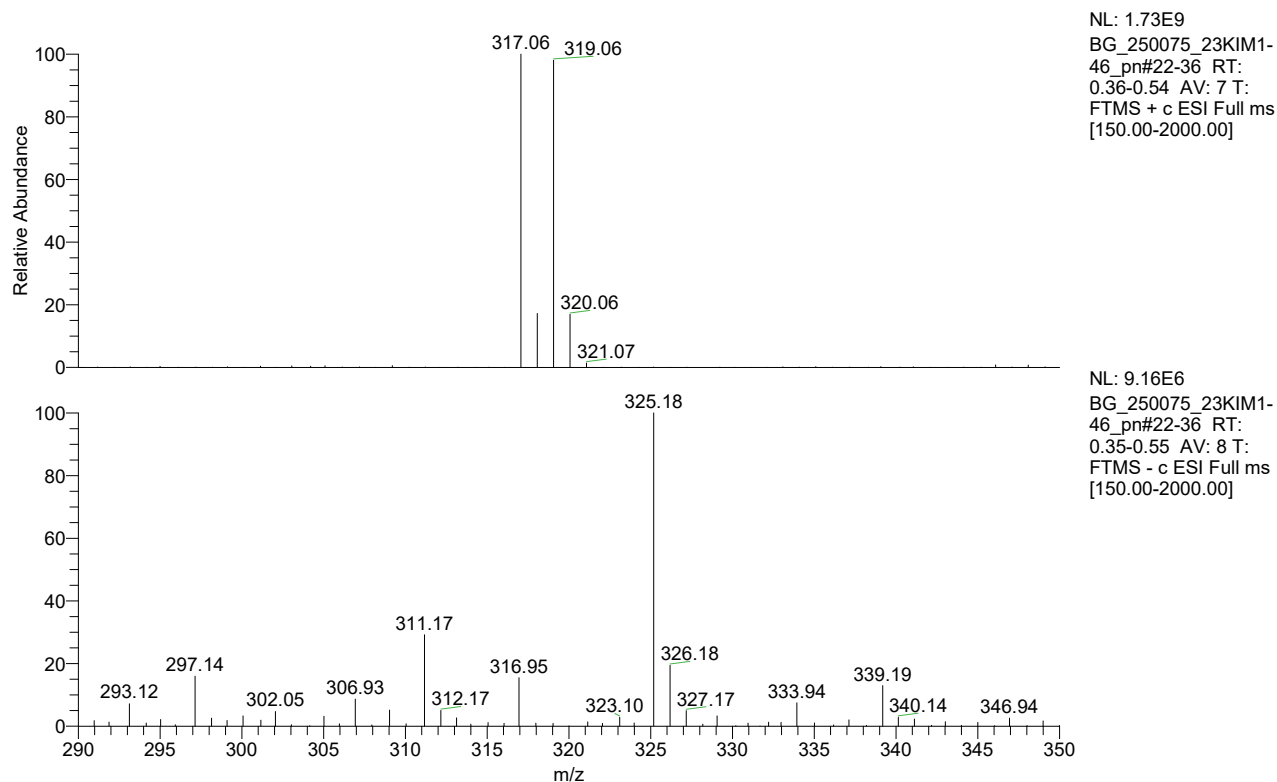


Figure S10.9. LR-MS spectra of compound **5**

Sample No. : C:\Xcalibur\...\0418\250075_23KIM1-46_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

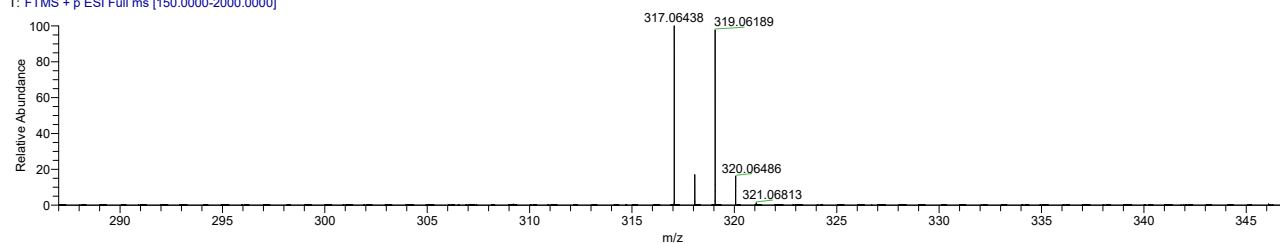
Date : 04/18/25 13:02:08

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

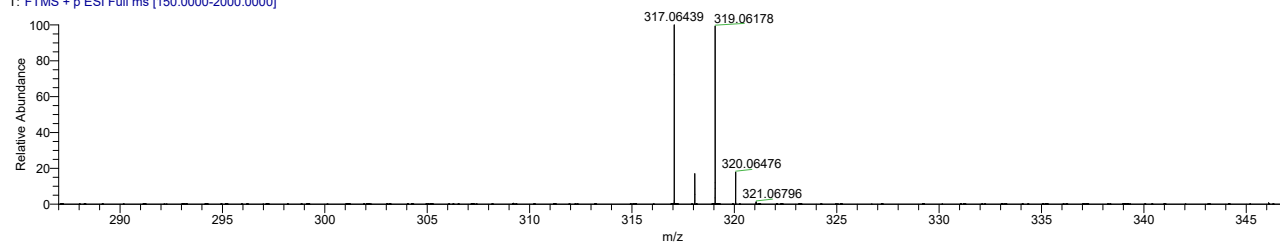
250075_23KIM1-46_pn #19-25 RT: 0.30-0.39 AV: 4 NL: 1.01E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



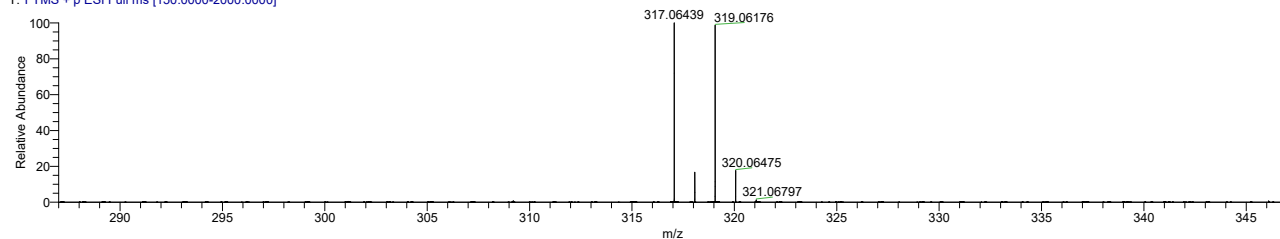
250075_23KIM1-46_pn #25-32 RT: 0.39-0.48 AV: 4 NL: 5.19E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250075_23KIM1-46_pn #32-39 RT: 0.51-0.60 AV: 4 NL: 2.29E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 317.06

m/z= 312.06-322.06

Isotope Min Max

N-14 0 3

O-16 0 0

C-12 0 20

H-1 0 20

Na-23 0 1

Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

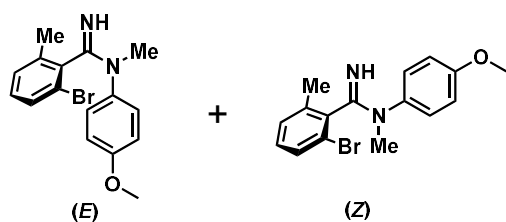
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
317.06439	317.06479	-1.25	8.5	C ₁₆ H ₁₈ N ₂ Br

Figure S10.10. HR-MS spectra of compound **5**

2-Bromo-N-(4-methoxyphenyl)-N,6-dimethylbenzimidamide (6)



Sample No. : C:\Xcalibur\...BG_250076_23KIM1-47_pn

Operator name : yamamoto hiroshi

Date : 04/18/25 15:18:45

Instrumental method : C:\Xcalibur\methods\ESI_100u\MS60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Sample solvent : MeOH

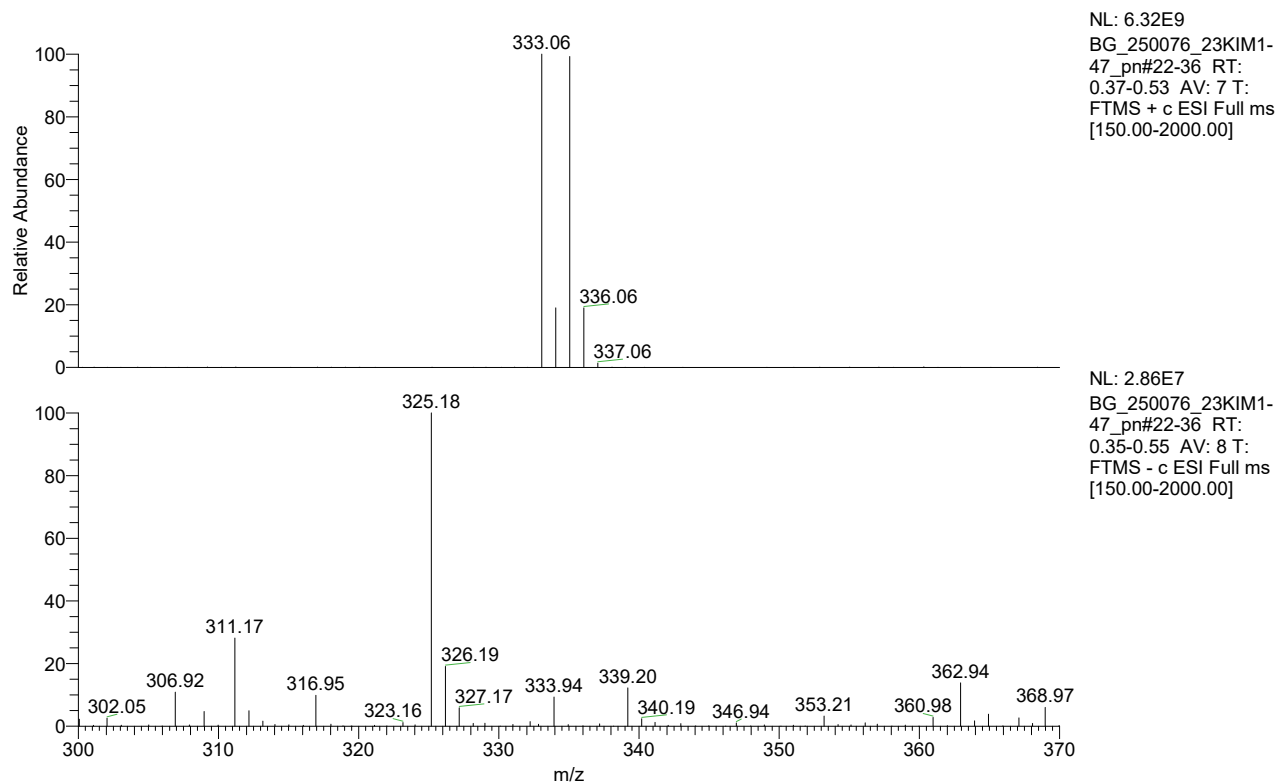


Figure S10.11. LR-MS spectra of compound 6

Sample No. : C:\Xcalibur\...\0418\250076_23KIM1-47_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

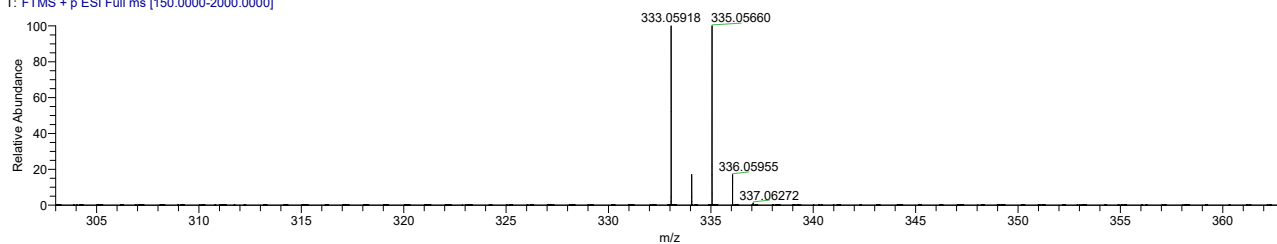
Date : 04/18/25 13:07:12

Instrumental method : C:\Xcalibur\methods\ESI_100ul\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

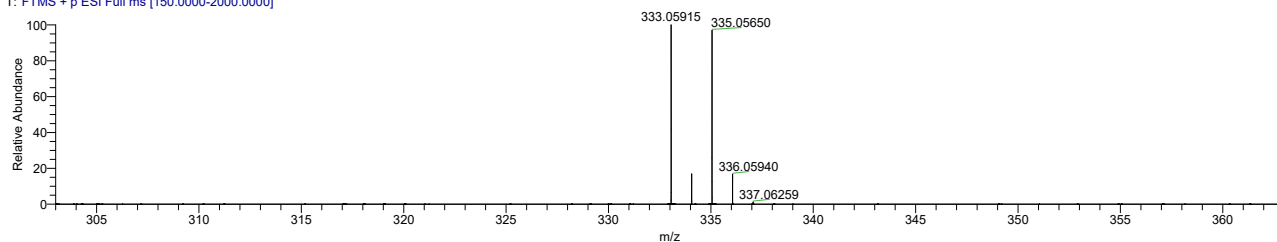
250076_23KIM1-47_pn #19-25 RT: 0.30-0.39 AV: 4 NL: 5.33E8

T: FTMS + p ESI Full ms [150.0000-2000.0000]



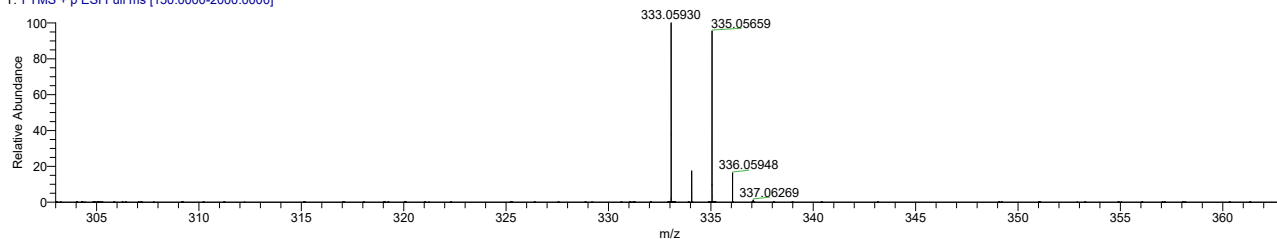
250076_23KIM1-47_pn #25-32 RT: 0.39-0.48 AV: 4 NL: 2.11E9

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250076_23KIM1-47_pn #33-40 RT: 0.50-0.59 AV: 4 NL: 1.06E9

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 333.06

m/z= 328.06-338.06

Isotope Min Max

N-14 0 3

O-16 0 3

C-12 0 20

H-1 0 20

Na-23 0 1

Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

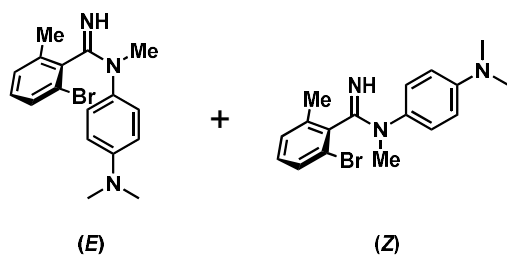
RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
333.05915	333.05970	-1.66	8.5	C ₁₆ H ₁₈ ON ₂ Br

Figure S10.12. HR-MS spectra of compound 6

2-Bromo-*N*-(4-(dimethylamino)phenyl)-*N*,6-dimethylbenzimidamide (7)



Sample No. : C:\Xcalibur\...BG_250077_23KIM1-76_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH

Date : 04/18/25 15:25:04

Instrumental method : C:\Xcalibur\methods\ESI_100u\S60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

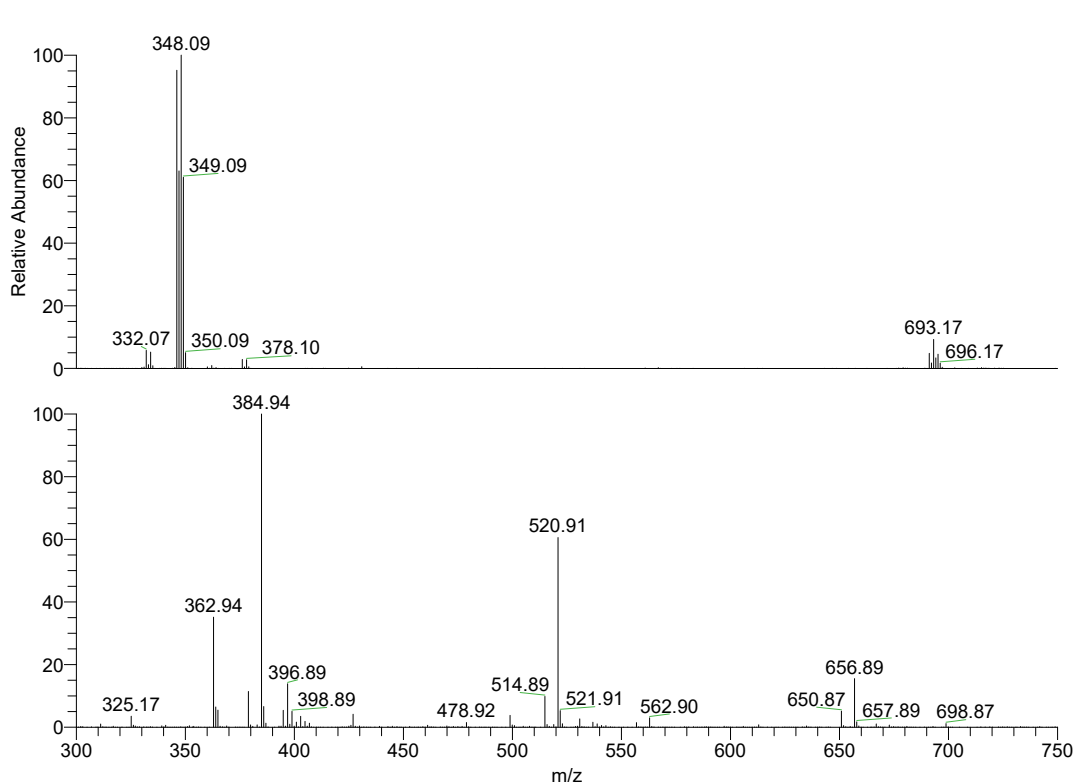


Figure S10.13. LR-MS spectra of compound **7**

Sample No. : C:\Xcalibur\...\0418\250077_23KIM1-76_pn

Instrument : Exactive Plus

Mobile phase solvent : MeOH

Operator name : yamamoto hiroshi

Sample solvent : MeOH MeOH

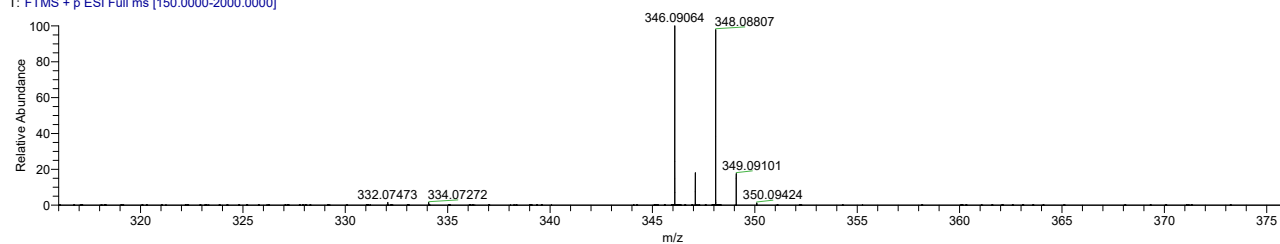
Date : 04/18/25 13:12:14

Instrumental method : C:\Xcalibur\methods\ESI_100u\lS60_100ul_mz150_2000pn.meth

Instrumental Analysis Division, Global Facility Center, Creative Research Institution, Hokkaido University

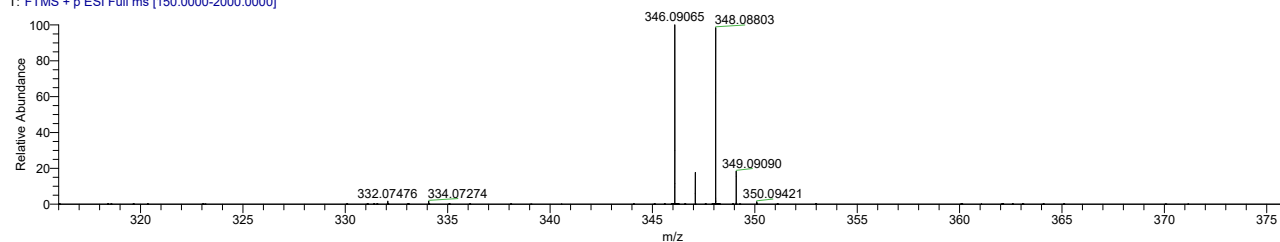
250077_23KIM1-76_pn #19-25 RT: 0.30-0.39 AV: 4 NL: 1.67E9

T: FTMS + p ESI Full ms [150.0000-2000.0000]



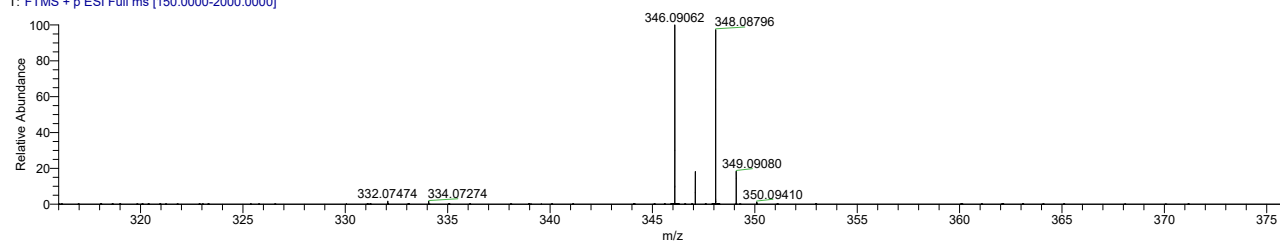
250077_23KIM1-76_pn #26-32 RT: 0.42-0.47 AV: 3 NL: 4.97E9

T: FTMS + p ESI Full ms [150.0000-2000.0000]



250077_23KIM1-76_pn #33-40 RT: 0.50-0.58 AV: 4 NL: 3.22E9

T: FTMS + p ESI Full ms [150.0000-2000.0000]



Elemental composition search on mass 346.09

m/z= 341.09-351.09

Isotope Min Max

N-14 0 3

O-16 0 3

C-12 0 20

H-1 0 25

Na-23 0 0

Br-79 1 1

Charge 1

Mass tolerance 5.00 ppm

Nitrogen rule not used

RDB equiv -1.00-100.00

max results 100

m/z	Theo. Mass	Delta (ppm)	RDB equiv.	Composition
346.09065	346.09134	-1.98	8.5	C ₁₇ H ₂₁ N ₃ Br

Figure S10.14. HR-MS spectra of compound 7

11. Reference

1. Du, B.; Jiang, X.; Sun, P. J. Org. Chem. 2013, 78, 2786–2791.
2. Gaussian 16, Revision C.01, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Petersson, G. A.; Nakatsuji, H.; Li, X.; Caricato, M.; Marenich, A. V.; Bloino, J.; Janesko, B. G.; Gomperts, R.; Mennucci, B.; Hratchian, H. P.; Ortiz, J. V.; Izmaylov, A. F.; Sonnenberg, J. L.; Williams-Young, D.; Ding, F.; Lipparini, F.; Egidi, F.; Goings, J.; Peng, B.; Petrone, A.; Henderson, T.; Ranasinghe, D.; Zakrzewski, V. G.; Gao, J.; Rega, N.; Zheng, G.; Liang, W.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Throssell, K.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M. J.; Heyd, J. J.; Brothers, E. N.; Kudin, K. N.; Staroverov, V. N.; Keith, T. A.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A. P.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Millam, J. M.; Klene, M.; Adamo, C.; Cammi, R.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Farkas, O.; Foresman, J. B.; Fox, D. J. Gaussian, Inc., Wallingford CT (2016).