FIRST AYMMETRIC TOTAL STNTHESIS OF (−)-ISOSTEMONAMINE AND KINETIC ANALYSIS OF ITS ISOMERIZATIONS

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1. General Remarks

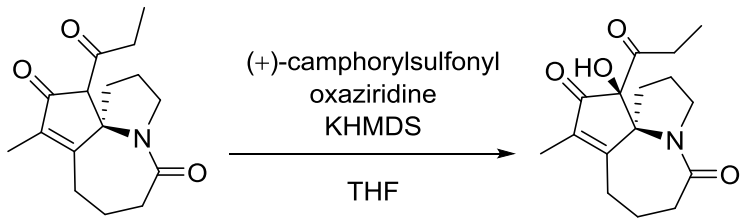
^{1}H NMR spectra were measured in $CDCl_3$ solution containing 0.01% TMS (reference: 0.00 ppm) using JEOL JNM-ECA 600 (600 MHz) spectrometers. ^{13}C-NMR spectra were measured in $CDCl_3$ solution (reference: 77.0 ppm) using JEOL JNM-ECA 600 (150 MHz). ^{1}H NMR and ^{13}C-NMR spectra were measured at room temperature unless otherwise noted. Peak multiplicities are used the following abbreviation: s, singlet; d, doublet; dd, doubledoublet; ddd, doubledoubledoublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded on SHIMADZU IRPrestige-21 FT-IR spectrophotometer using a KBr disk or a NaCl plate. Column chromatography was performed on silica-gel (Kanto Chemical Co.). Thin-layer chromatography was performed on precoated plates (0.25 mm, silica-gel Merck 60 F254). Mass spectra (MS) and High-resolution mass spectra (HRMS) were measured on a JEOL JMS-700 mass spectrometer. The optical rotation was obtained on a JASCO P-2300. Chiral HPLC analysis was performed using 1) a pump (PU-2087, JASCO) equipped with a CD detecter (CD-2095, JASCO) and a UV detector (UV-2075, JASCO) and 2) a pump (LC-10ADvp, SHIMAZU) equipped with a UV detector (SPD-10Avp、SHIMAZU). A CHIRALPAK AD, AD-H, and OD-H column (analytical column, 4.6 mm × 250 mm, DAICEL; preparative column, 20 mm × 250 mm, DAICEL) was used for HPLC analysis and purification.

Reaction mixtures were stirred magnetically unless otherwise noted. Tetrahydrofuran (anhydrous), dichloromethane (anhydrous), diethyl ether (anhydrous) and *n*-butyllithium (1.6 M, hexane solution) were purchased from Kanto Chemical Co., Inc. Lawesson's reagent was purchased from Tokyo Chemical Industry Co., Ltd. Ethyl acetate, hexane, benzene, chloroform, ethanol, methanol, acetonitrile, isopropanol, trimethylamine and ethyl chloroformate were purchased from Wako Pure Chemical Industries, Ltd. 4-Dimethylaminopyridine (DMAP), acetonitrile, (trimethylsilyl)diazomethane solution (2.0 M in diethyl ether), potassium bis(trimethylsilyl)amide solution (0.5 M in toluene) were purchased from Sigma-Aldrich Japan.

n-Butyllithium solution solution were used after titrimetric determination of the concentration by the diphenylacetic acid method. W2-Raney Nickel was prepared according to ref 1. $CDCl_3$ used for measurement of (−)-**2** was used after distillation. HPLC grade solvents were used for all HPLC experiments. All other chemicals and solvents were used without further purification.

2. Procedures

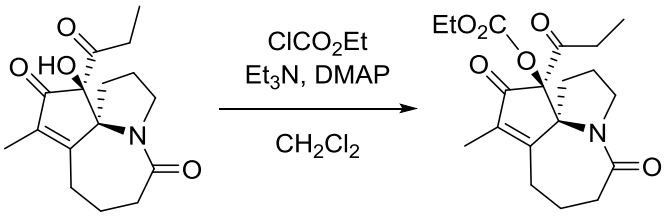
(11*R*,11a*S*)-11-hydroxy-9-methyl-11-propionyl-2,3,7,8-tetrahydro-1*H*-cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-5,10(6*H*,11*H*)-dione (**4**)



4 **6**

To a solution of **4** (22.0 mg, 0.0800 mmol) in THF (3.0 mL) was added KHMDS in toluene (0.5 M, 320 μL, 0.160 mmol) at −78 °C under argon. After stirring for 30 min, a solution of (+)-(Camphorylsulfonyl)oxaziridine (45.9 mg, 0.200 mmol) in THF (1.0 mL) was added to the mixture. After warmed to 0 °C, the resulting mixture was stirred for another 1 h. The reaction was quenched by the addition of NH_4Cl aq., and the mixture was extracted with CH_2Cl_2, washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (MeOH/$CHCl_3$, 2:98) to give **6** (17.2 mg, 59.0 μmol, 74%) as white solid.; M. p.: 165-166 °C (AcOEt/hexane); $[\alpha]_D^{23}$ = −61 (*c* = 0.22, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.14 (t, *J* = 7.2 Hz, 3H), 1.31-1.40 (m, 1H), 1.83 (s, 3H), 1.86-1.99 (m, 3H), 2.08-2.15 (m, 2H), 2.20 (dd, *J* = 5.2, 13.2 Hz, 1H), 2.33 (ddd, *J* = 10.3, 11.0, 11.0 Hz, 1H), 2.74-2.86 (m, 3H), 2.91 (ddd, *J* = 6.6, 13.2, 13.2 Hz, 1H), 3.51 (dd, *J* = 12.0, 12.0 Hz, 1H), 3.78 (ddd, *J* = 9.2, 9.2, 12.0 Hz, 1H), 5.29 (brs, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 7.4 (q), 8.2 (q), 20.3(t), 22.5 (t), 23.7 (t), 32.0 (t), 34.5 (t), 35.8 (t), 47.0 (t), 76.3 (s), 85.4 (s), 134.7 (s), 171.4 (s), 174.6 (s), 201.0 (s), 212.1 (s); IR (KBr) 1616, 1654, 1697, 1726 cm^{-1}; EI-MS m/z 291 (M^+), HRMS (EI) m/z 291.1471 (M^+), calcd for $C_{16}H_{21}NO_4$ 291.1471. These data matched with the ones presented in our previous report.[2]

ethyl ((11*R*,11a*S*)-9-methyl-5,10-dioxo-11-propionyl-2,3,5,6,7,8,10,11-octahydro-1*H*-cyclopenta[*b*]pyrrolo[1,2-*a*]azepin-11-yl) carbonate (**7**)

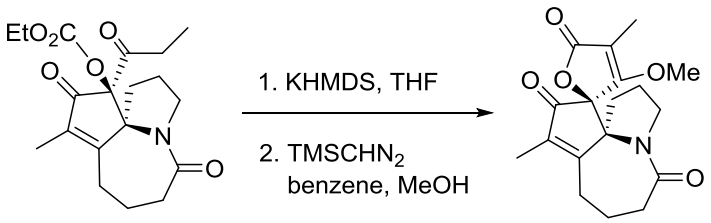


6 **7**

To a solution of **6** (8.5 mg, 29 μmol) in CH_2Cl_2 (320 μL) was added Et_3N (49 μL, 0.35 mmol), DMAP (14.7 mg, 117 μmol) and ethylchloroformate (28 μL, 0.29 mmol) at room temperature under argon atmosphere. After stirring for 3 h, the reaction mixture was concentrated *in vacuo*. The crude product was purified by silica gel column chromatography (MeOH/$CHCl_3$, 2:98) to provide **7** (10.0 mg, 27.5 μmol, 95%) as a white solid; M. p.: 136-137 °C ($CHCl_3$, hexane); $[\alpha]^{23}_D$ = −55.0 (*c* = 0.16, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.11 (t, *J* = 6.9 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H), 1.58-1.67 (m, 1H), 1.85 (s, 3H), 1.86-1.96 (m,

3H), 2.06-2.14 (m, 1H), 2.25-2.87 (m, 1H), 2.30-2.37 (m, 1H), 2.44-2.49 (m, 1H), 2.74-2.80 (m, 3H), 2.86 (dd, *J* = 8.9, 13.1 Hz, 1H), 3.42-3.46 (m, 1H), 3.75-3.82 (m, 1H), 4.12-4.19 (m, 2H); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 7.4 (q), 8.5 (q), 14.1 (q), 20.6 (t), 22.4 (t), 23.8 (t), 32.3 (t), 34.3 (t), 35.7 (t), 47.5 (t), 65.4 (t), 76.2 (s), 88.2 (s), 135.7 (s), 153.2 (s), 170.0 (s), 173.5 (s), 195.8 (s), 206.2 (s); IR (KBr) 1643, 1707, 1724, 1749 cm^{-1}; EI-MS m/z 363 (M^+), HRMS(EI) m/z 363.1682 (M^+), calcd for $C_{19}H_{25}NO_6$ 363.1682.

(11*R*,11a*S*)-3'-methoxy-4',9-dimethyl-2,3,7,8-tetrahydro-1*H*,5*H*,5'*H*-spiro[cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-11,2'-furan]-5,5',10(6*H*)-trione (**8**)

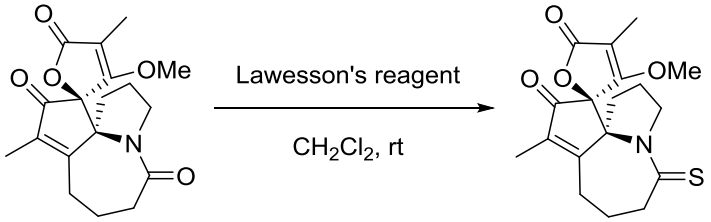


7 **8**

To a solution of **7** (9.8 mg, 27 μmol) in THF (1.9 mL) was added KHMDS (0.50 M in toluene, 10.8 μL, 54 μmol) at −78 °C under argon atmosphere. The reaction mixture was gradually warmed to 10 °C over 4 h. The mixture was quenched with H_2O, acidified with 2M HCl to pH 2, and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude product (5.9 mg, colorless oil) was used without further purification.

The crude product was dissolved in benzene/MeOH (3:1, 1.9 mL) and cooled to 0 °C, and $TMSCHN_2$ (2.0 M in dimethyl ester, 37 μL, 74 μmol) was added to the solution under argon atmosphere. After stirring for 2 h, the reaction mixture was concentrated under reduced pressure. The crude product was purified by HPLC (Mightysil Si60, Kanto chemical, 20 x 250 mm, AcOEt, flow rate: 5.0 mL/min, retention time: 120 min) to afford **8** (1.1 mg, 3.3 μmol, 13% for 2 steps) as a white solid; M. p.: 102-103 °C ($CHCl_3$/hexane, colorless prisms); $[\alpha]^{23}_D$ = +13.3 (*c* = 0.06, MeOH); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.67-1.70 (m, 1H), 1.85 (s, 1H), 1.86- 2.01 (m, 3H), 2.09 (s, 3H), 2.11-2.18 (m, 3H), 2.89-2.35 (m, 3H), 2.83-2.89 (m, 2H), 3.43 (dd, *J* = 11.0, 11.0 Hz, 1H), 3.74-3.79 (m, 1H), 4.17 (s, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 8.3 (q), 9.3 (q), 20.7 (t), 22.7 (t), 23.5 (t), 32.4 (t), 36.5 (t), 47.5 (t), 59.7 (q), 74.1 (s), 86.0 (s), 102.2 (s), 134.7 (s), 169.0 (s), 171.7 (s). 172.1 (s), 173.2 (s), 196.2 (s); IR (KBr) 1645, 1707, 1759 cm^{-1}; EI-MS m/z 331 (M^+), HRMS(EI) m/z 331.1420 (M^+), calcd for $C_{18}H_{21}NO_5$ 331.1420.

(11*R*,11a*S*)-3'-methoxy-4',9-dimethyl-5-thioxo-2,3,5,6,7,8-hexahydro-1*H*,5'*H*,10*H*-spiro[cyclopenta[*b*]pyrrolo[1,2-*a*]azepine-11,2'-furan]-5',10-dione (**9**)

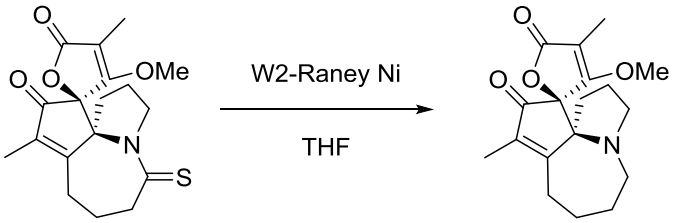


8 **9**

To a solution of **8** (5.0 mg, 15 μmol) in CH_2Cl_2 (720 μL) was added Lawesson's reagent (9.3 mg, 23 μmol) at room temperature. The mixture was stirred for 1.5 h and concentrated *in vacuo*. The crude product

was purified by silica gel column chromatography (MeOH/CH_3Cl_3, 30:70) to afford **9** (4.3 mg, 12.4 μmol, 83%) as a white solid; M. p.: 212 ºC ($CHCl_3$, hexane, colorless prisms); $[\alpha]^{23}_D$ = +63.3 (*c* = 0.06, benzene); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.73-1.82 (m, 1H), 1.85 (s, 3H), 1.87-1.95 (m, 1H), 2.04-2.09 (m, 1H), 2.10 (s, 3H), 2.11-2.16 (m, 1H), 2.22-2.29 (m, 1H), 2.31-2.42 (m, 2H), 2.87 (dd, *J* = 8.9, 12.4 Hz, 1H), 3.08 (ddd, *J* = 2.1, 4.8, 13.7 Hz, 1H), 3.34 (ddd, *J* = 6.2, 13.7, 13.7 Hz, 1H), 3.71-3.76 (m, 1H), 4.18 (s, 3H), 4.19-4.24 (m, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 8.3 (q), 9.4 (q), 20.7 (t), 22.6 (t), 25.6 (t), 36.5 (t), 42.0 (t), 55.5 (t), 59.9 (q), 78.2 (s), 85.5 (s), 102.3 (s), 134.4 (s), 168.9 (s), 171.2 (s), 171.7 (s), 195.4 (s), 205.0 (s); IR (KBr) 1655, 1721, 1765 cm^{-1}; EI-MS m/z 347 (M^+), HRMS (EI) m/z 347.1192 (M^+), calcd for $C_{18}H_{21}NO_4S$ 347.1191.

(−)- isostemonamine (2)



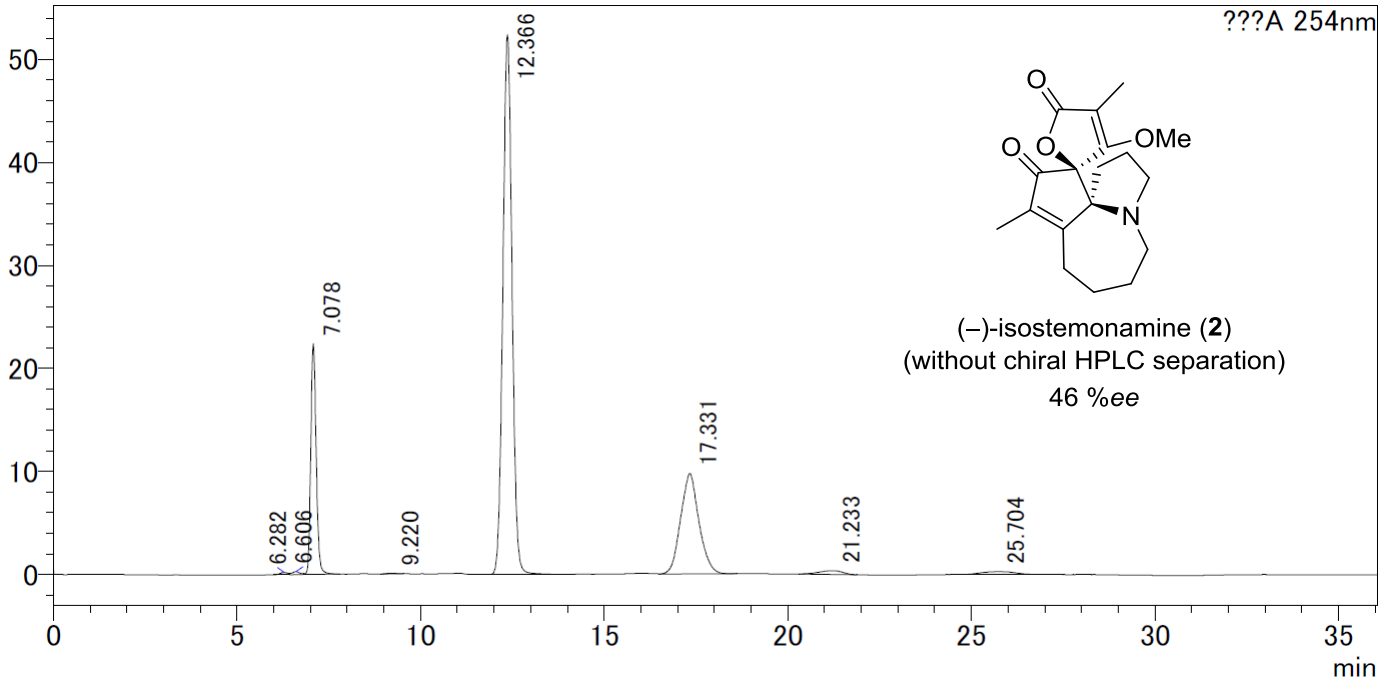
9 **(–)-2**

To a solution of **9** (4.3 mg, 12 μmol) in THF (780 μL) was added freshly prepared W2-Raney Ni (in THF, 5 drops) at room temperature. After stirring for 30 min, the reaction mixture was filtered. The residue was washed with cold toluene, and the filtrate, which was kept at 0 °C during filtration, was evaporated at 0 °C under reduced pressure. The obtained product (white solid) were immediately measured optical purity and optical rotation; 46% ee, $[\alpha]^{23}_D$ = −42.8 (*c* = 0.18, benzene). The solutions for these measurements were evaporated under reduced pressure at 0 °C. Immediately after concentration, the product was purified by chiral HPLC (CHIRALPAK OD-H, 20 mm × 250 mm, 10% ethanol/hexane, flow rate: 8.0 mL/min). Retention times for (−)-isostemonamine, (−)-stemonamine, (+)-isostemonamine and (+)-stemonamine were 26.5 min, 30.4 min, 34.3 min and 38.1 min, respectively. The obtained eluates were kept under ice bath during the purification, and concentrated at 0 °C under reduced pressure, which provided (−)-isostemonamine (1.2 mg, 31%), (+)-isostemonamine (0.4 mg, 10%), (−)-stemonamine (0.1 mg, 3%), and (+)-stemonamine (0.1 mg, 3%) all as a white solid (total yield: 1.8 mg, 46%). Optical purity and optical rotation of (−)-isostemonamine was measured immediately after concentration. After this, NMR spectra of (−)-isostemonamine was measured using deuterated chloroform distilled immediately prior to use.; (−)-**2**: 97% ee; $[\alpha]^{23}_D$ = −70.8 (*c* = 0.12 ,benzene); ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.14-1.21 (m, 1H), 1.38-1.40 (m, 1H), 1.50-1.56 (m, 1H), 1.68-1.81 (m, 4H), 1.76 (s, 3H), 2.00-2.06 (m, 2H), 2.09 (s, 3H), 2.37 (dd, *J* = 6.6, 13.2 Hz, 1H), 2.84-2.87 (m, 2H), 3.09 (dd, *J* = 13.2, 15.1 Hz, 1H), 3.17-3.22 (m, 2H), 4.13 (s, 3H); EI-MS m/z 317 (M^+); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 8.0, 9.3, 24.26, 24.31, 27.3, 27.8, 35.6, 49.1, 51.0, 59.3, 75.3, 89.2, 102.3, 134.5, 169.5, 173.5, 176.4, 199.0; HRMS (EI) m/z 317.1629 (M^+), calcd for $C_{18}H_{21}NO_4S$ 317.1627.

3. Chiral HPLC analysis

3-1. (−)-isostemonamine (**2**) without chiral HPLC separation
Conditions: column: CHIRALPAK AD-H, eluent: 20% isopropanol/hexane, flow: 0.5 mL / min

Chromatogram with a UV detector (254 nm)

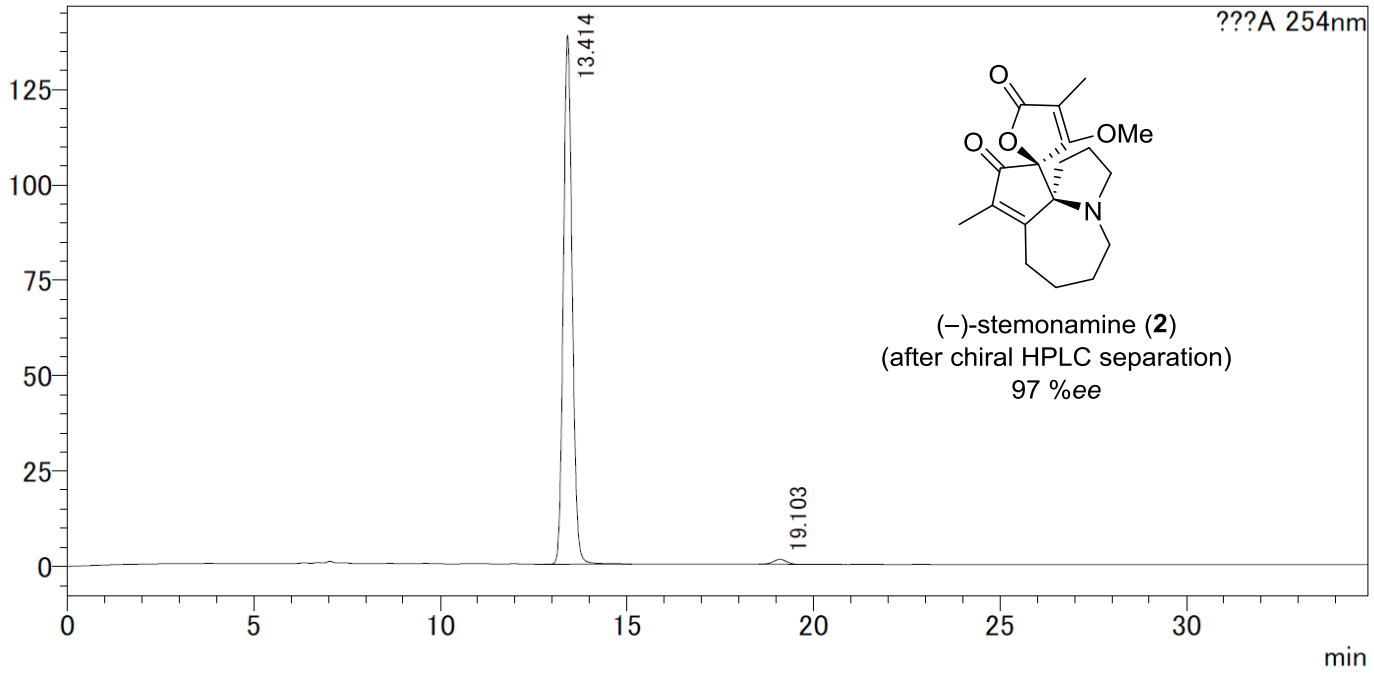


Peak	compound	Retention time (min)	ratio (%)
1	(−)-isostemonamine	12.4	71.0
2	(+)-isostemonamine	17.3	26.3
3	(−)-stemonamine	21.2	1.3
4	(+)-stemonamine	25.7	1.4

3-2. isostemonamine (**2**) after chiral HPLC separation

Conditions; column: CHIRALPAK AD-H, eluent: 20% isopropanol/hexane, flow: 0.5 mL / min

Chromatogram with a UV detector (254 nm)



Peak	compound	Retention time (min)	ratio (%)
1	(−)-isostemonamine	13.4	98.6
2	(+)-isostemonamine	19.1	1.4

4. Determination of rate constants and half-lives.

Kinetic study of (−)-isostemonamine was performed with the temperature controlled using a thermoblock (NISSIN, ND-M11 or NDC-100) as shown in Figure S4-1. Rate constants of racemization and epimerization were determined by chiral HPLC measurements of enantiomeric excess and diastereomeric excess at proper time intervals in several solvents. Plot of ln *a* (equation (1, 2)) versus time (hour) showed linear line which provided rate constants (*k*) and half-lives ($t_{1/2}$, equation (3)).

$$a = \frac{|\,[\text{(-)-stemonamine}] - [\text{(+)-stemonamine}]\,|}{[\text{(-)-stemonamine}] + [\text{(+)-stemonamine}]} \qquad (1)$$

$$a = \frac{|\,[(\pm)\text{-stemonamine}] - [(\pm)\text{-isostemonamine}]\,|}{[(\pm)\text{-stemonamine}] + [(\pm)\text{-isostemonamine}]} \qquad (2)$$

$$t_{1/2} = \frac{\text{Ln } 2}{k} \qquad (3)$$

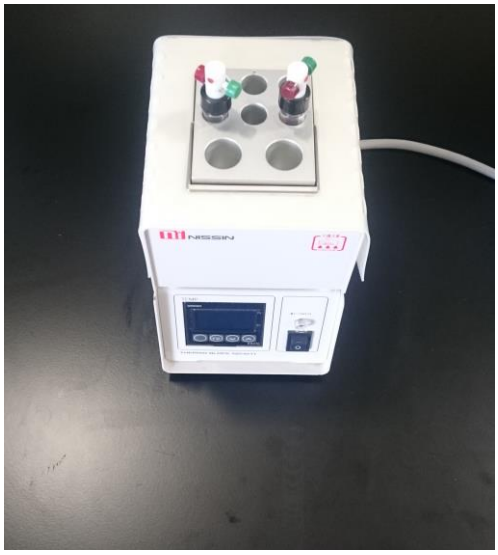
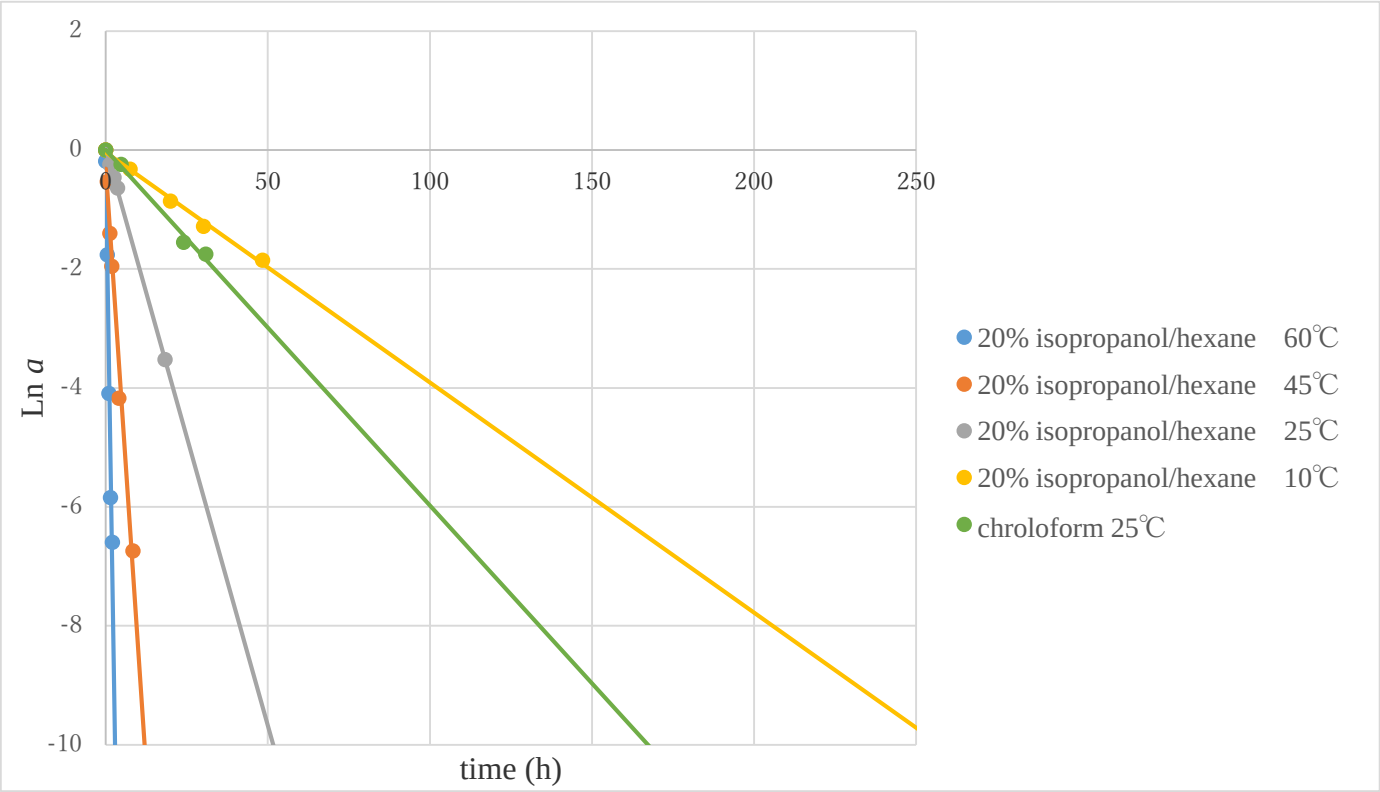


Figure S4-1. Thermoblock ND-M11

4-1. Racemization of (–)-stemonamine to (+)-stemonamine.

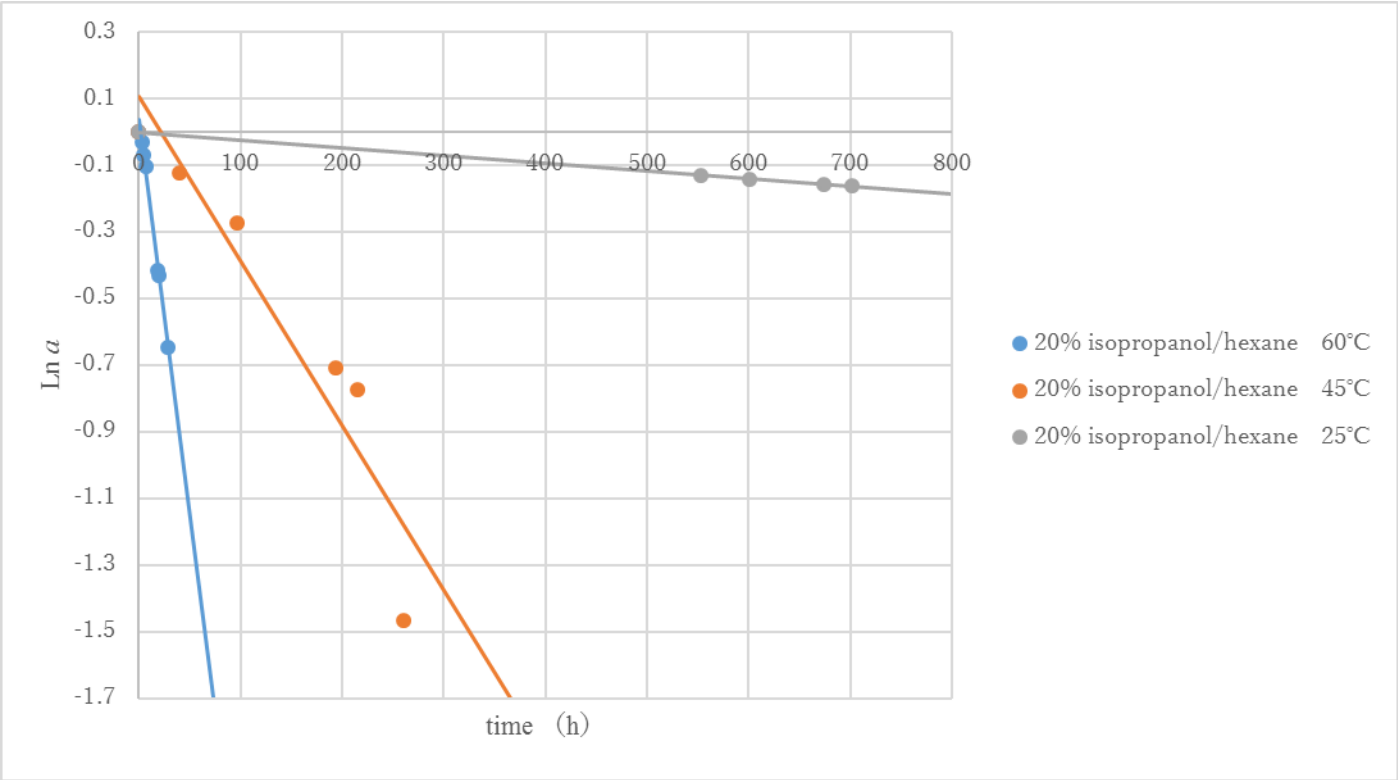
The plot of ln *a* versus time



entry	solvent	temperature	k (10^{-4} s^{-1})	half time
1	20% isopropanol/hexane	60 ºC	11	0.18
2	20% isopropanol/hexane	45 ºC	2.9	0.67
3	20% isopropanol/hexane	25 ºC	0.53	3.6
4	20% isopropanol/hexane	10 ºC	0.11	18
5	$CHCl_3$	25 ºC	0.16	12

4-2. Epimerization of (−)-stemonamine to (±)-isostemonamine.

The plot of ln *a* versus time



entry	solvent	temperature	k (10^{-4} s^{-1})	half time
1	20% isopropanol/hexane	60 °C	0.066	29
2	20% isopropanol/hexane	45 °C	0.010	190
3	20% isopropanol/hexane	25 °C	<0.001	>1000

5. References

[1] R. Mozingo, *Org. Synth*. 1941, **21**, 15; 1955, **Coll. Vol. 3**, 181.
[2] S. Fujita, K. Nishikawa, T. Iwata, T. Tomiyama, H. Ikenaga, K. Matsumoto and M. Shindo, *Chem. Eur. J.*, 2018, **24**, 1539.