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Total synthesis of brevianamide A. — Source link

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SUPPLEMENTARY INFORMATION

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Total synthesis of brevianamide A

Robert C. Godfrey, Nicholas J. Green, Gary S. Nichol and Andrew L. Lawrence ✉

EaStCHEM School of Chemistry, University of Edinburgh, Edinburgh, UK. ✉e-mail: a.lawrence@ed.ac.uk

SUPPORTING INFORMATION

Total synthesis of brevianamide A.

Robert C. Godfrey, Nicholas J. Green, Gary S. Nichol and Andrew L. Lawrence*

EaStCHEM School of Chemistry, University of Edinburgh, Joseph Black Building, David Brewster Road, Edinburgh, UK, EH9 3FJ. E-mail: a.lawrence@ed.ac.uk

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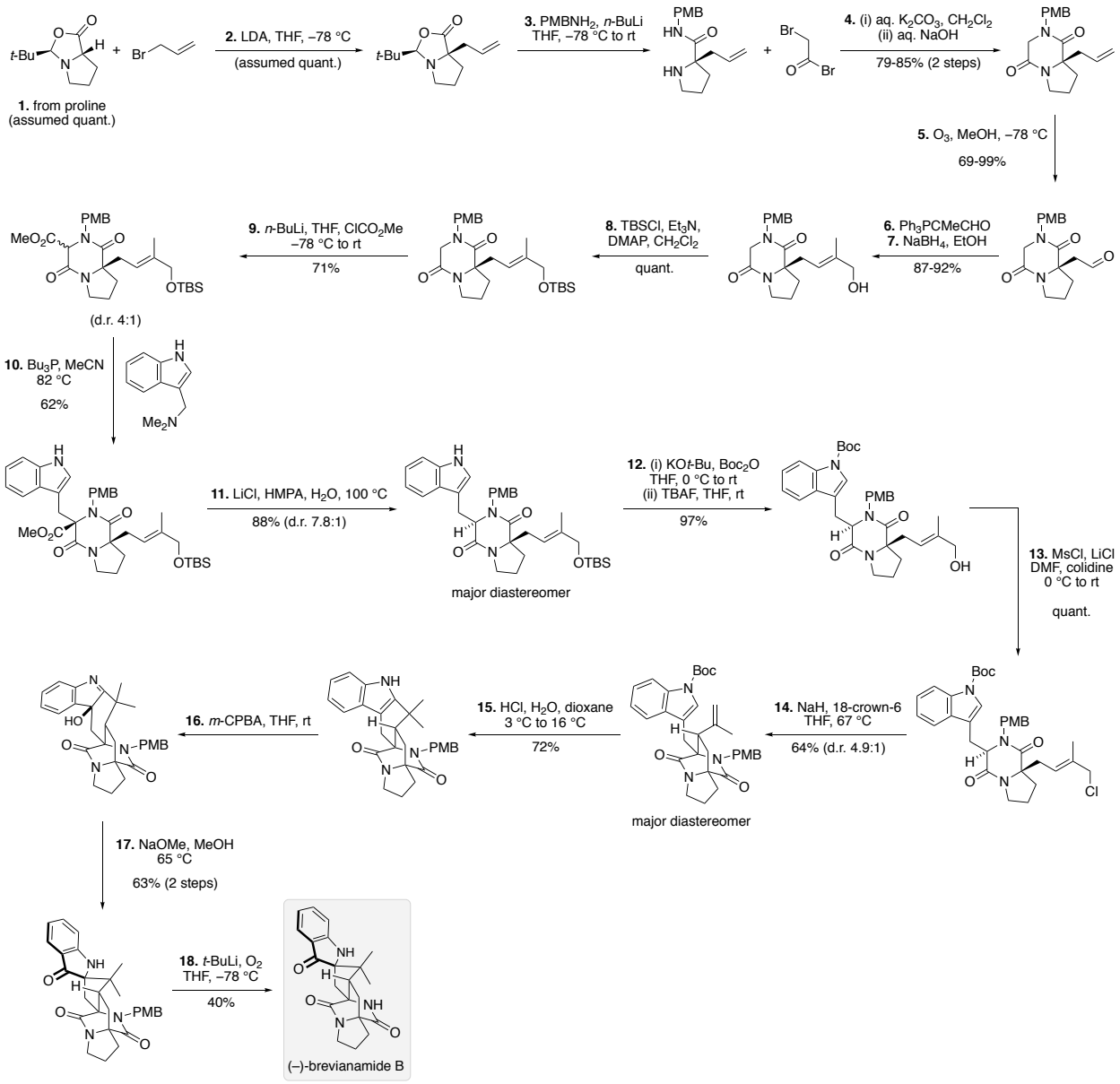
- 2.9% overall yield (6.4% × 45% formal steps)
- 7.1 mg of final intermediate

1.7 Scheerer's 2017 formal synthesis of (±)-brevianamide B (second intermolecular [4+2])

- 15 steps (13 + 2 formal steps)
- 2.1% overall yield (4.6% × 45% formal steps)
- 5.0 mg of final intermediate

1.1 Williams' 1988 synthesis of (−)-brevianamide B (cationic cyclization approach)

- 18 steps
- 1.5-2.5% overall yield
- 0.8 mg prepared



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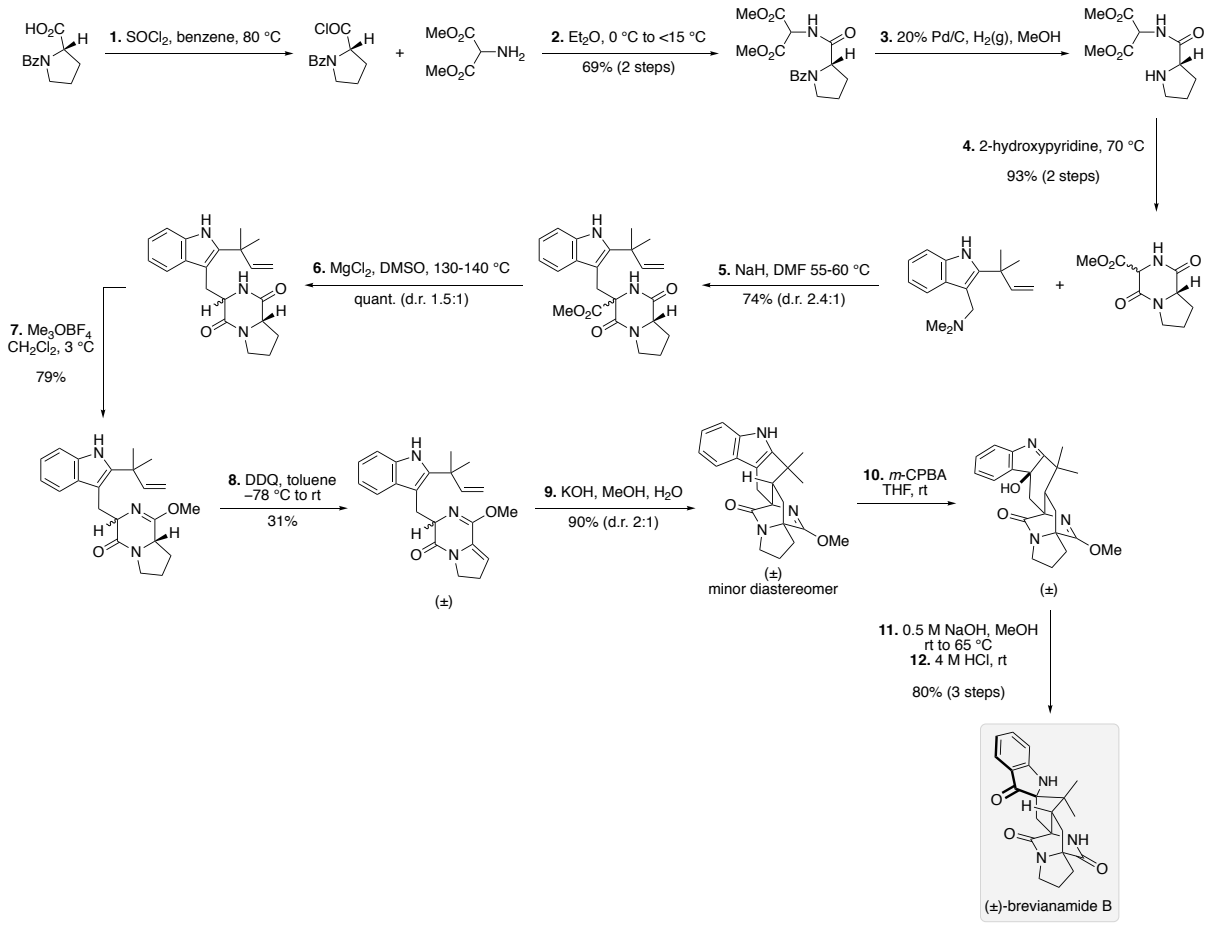
2. Williams, R. M. & Kwast, E. Carbanion-mediated oxidative deprotection of non-enolizable benzylated amides. *Tetrahedron Lett.* **30,** 451–454 (1989)
3. Williams, R. M., Glinka, T., Kwast, E., Coffman, H. & Stille, J. K. Asymmetric, stereocontrolled total synthesis of (−)-brevianamide B. *J. Am. Chem. Soc.* **112,** 808–821 (1990).

1.2 Williams' 1998 synthesis of (±)-brevianamide B (first biomimetic approach)

- 12 steps
- 2.8% overall yield
- 7.7 mg prepared



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1. Williams, R. M., Sanz-Cervera, J. F., Sancenón, F., Marco, J. A. & Halligan, K. Biomimetic Diels−Alder cyclizations for the construction of the brevianamide, paraherquamide sclerotamide, and VM55599 ring systems. *J. Am. Chem. Soc.* **120,** 1090–1091 (1998).
2. Williams, R. M., Sanz-Cervera, J. F., Sancenón, F., Marco, J. A. & Halligan, K. Biomimetic Diels–Alder cyclizations for the construction of the brevianamide, paraherquamide, sclerotamide, asperparaline and VM55599 ring systems. *Bioorg. Med. Chem.* **6,** 1233–1241 (1998).
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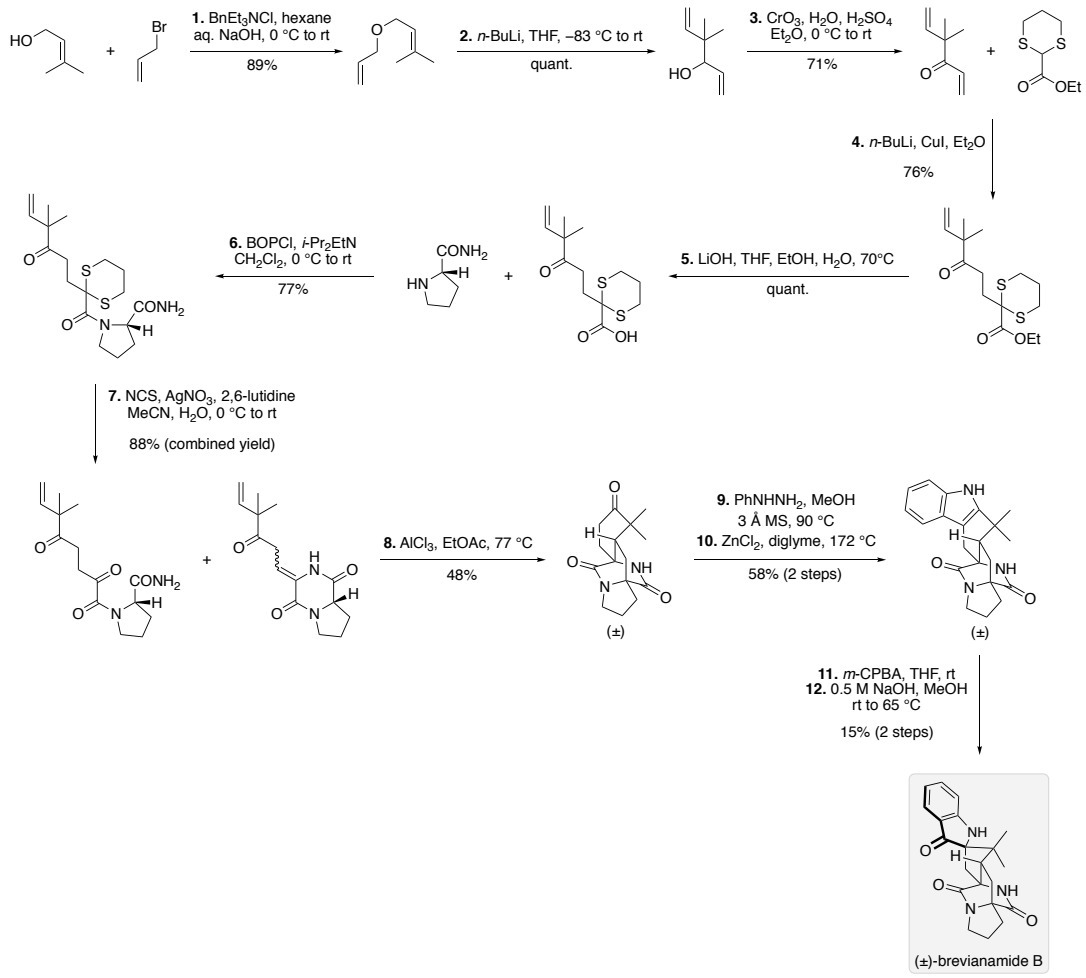
1.3 Williams' 2006 synthesis of (±)-brevianamide B (late-stage indole formation approach)

- 12 steps
- 1.4% overall yield
- 4.0 mg prepared



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2. Steps **1–3** taken from Dauben, W. G., Cogen, J. M., Ganzer, G. A. & Behar, V. Photochemistry of 1,5-hexadien-3-ones: Wavelength-dependent selectivity in intramolecular enone-olefin photoadditions. *J. Am. Chem. Soc.* **113,** 5817–5824 (1991).

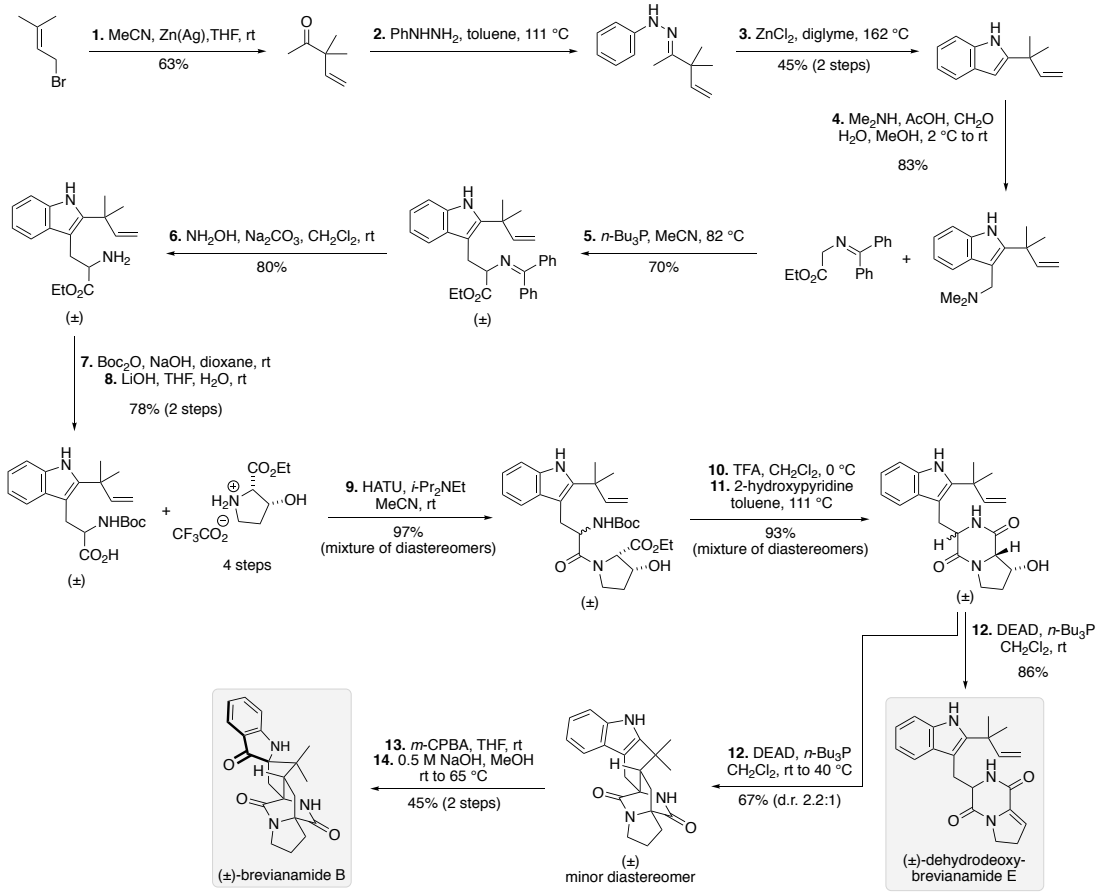
1.4 Williams' 2007 synthesis of (±)-brevianamide B & (±)-dehydrodeoxybreviaminde E (second biomimetic approach)

(±)-brevianamide B

- 14 steps
- 0.9% overall yield
- 4.7 mg prepared

(±)-dehydrodeoxybreviaminde E

- 12 steps
- 8% overall yield
- 98 mg prepared

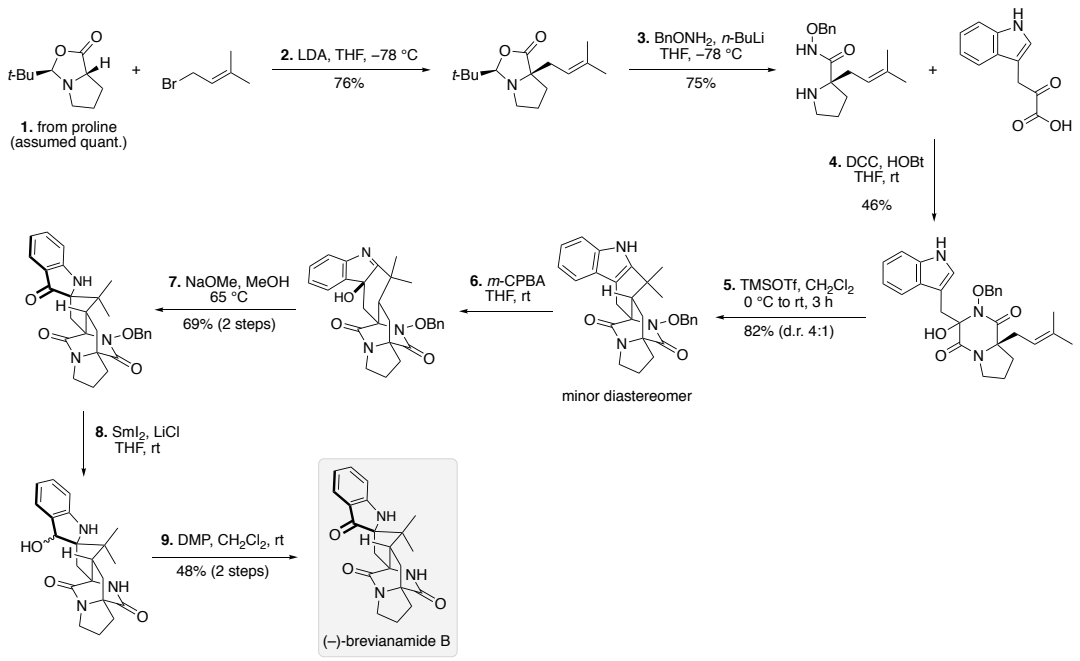


References:

1. Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l-stephacidin A. *Org. Lett.* **9,** 4255–4258 (2007).
2. Steps **1–4** taken from; Sanz-Cervera, J. F., Glinka, T. & Williams, R. M. Biosynthesis of brevianamides A and B: In search of the biosynthetic Diels-Alder construction. *Tetrahedron* **49,** 8471−8482 (1993). (which used $^{3}H_2CO$ in step **4**)
3. Steps **5–8** taken from; Stocking, E. M., Sanz-Cervera, J. F. & Williams, R. M. Total Synthesis of VM55599. Utilization of an Intramolecular Diels–Alder Cycloaddition of Potential Biogenetic Relevance. *J. Am. Chem. Soc.* **122,** 1675–1683 (2000).

1.5 Simpkins' 2009 synthesis of (−)-brevianamide B (cationic double-cyclization approach)

- 9 steps
- 1.4% overall yield
- 6.0 mg prepared



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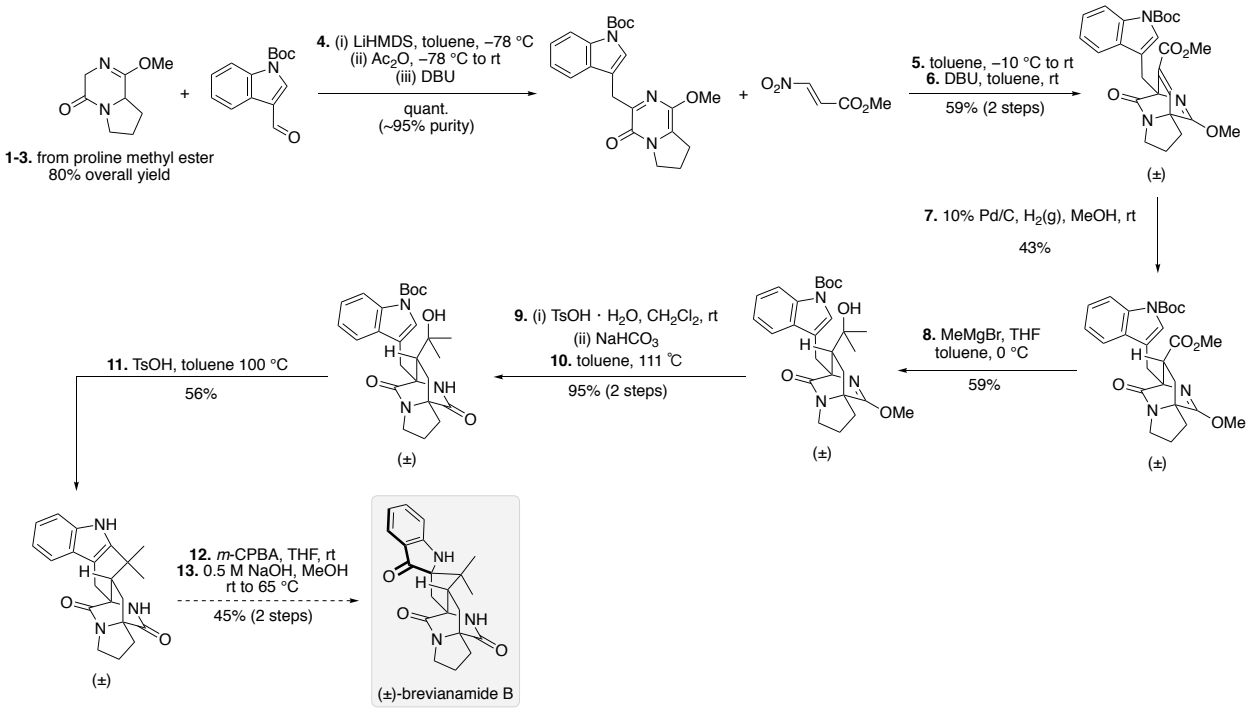
2. Frebault, F. C. & Simpkins, N. S. A cationic cyclisation route to prenylated indole alkaloids: synthesis of malbrancheamide B and brevianamide B, and progress towards stephacidin A. *Tetrahedron* **66,** 6585–6596 (2010).

1.6 Scheerer's 2016 formal synthesis of (±)-brevianamide B (first intermolecular [4+2] approach)

- 13 steps (11 + 2 formal steps)
- 2.9% overall yield (6.4% × 45% formal steps)
- 7.1 mg of final intermediate

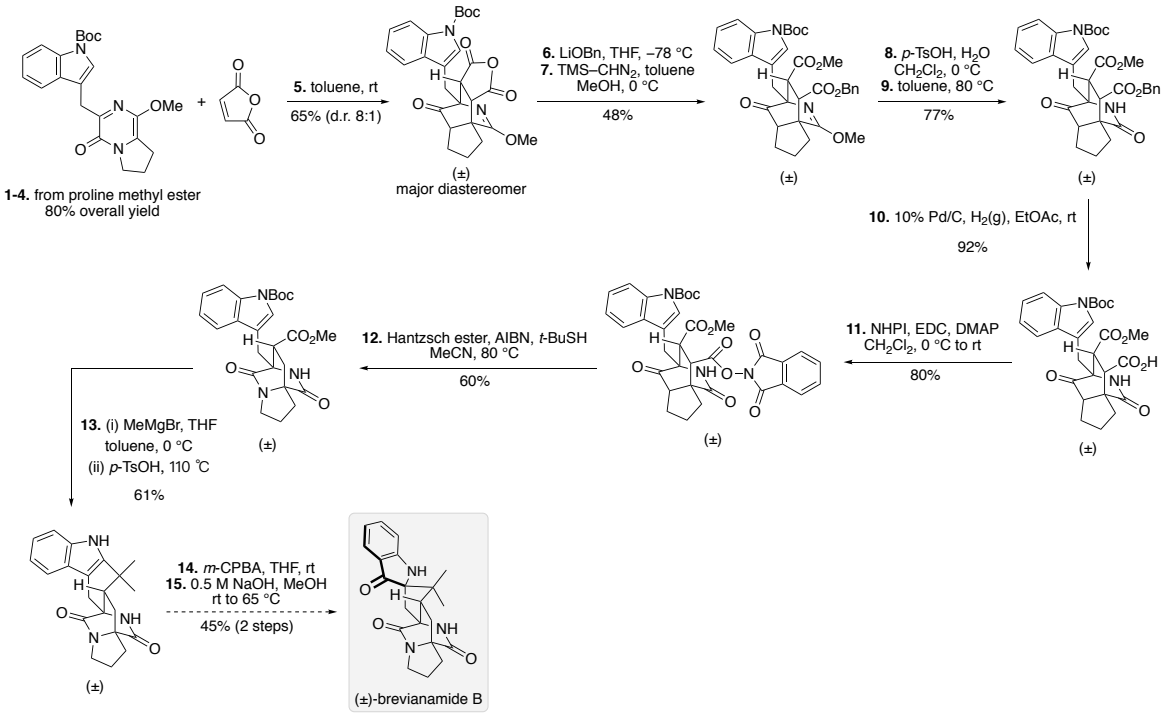


References:

1. Robins, J. G., Kim, K. J., Chinn, A. J., Woo, J. S. & Scheerer, J. R. Intermolecular Diels–Alder Cycloaddition for the Construction of Bicyclo[2.2.2]diazaoctane Structures: Formal Synthesis of Brevianamide B and Premalbrancheamide. *J. Org. Chem.* **81,** 2293–2301 (2016).
2. Steps **1-3** taken from; Laws, S. W. & Scheerer, J. R. Enantioselective Synthesis of (+)-Malbrancheamide B. *J. Org. Chem.* **78,** 2422–2429 (2013).
3. Steps **12-13** taken from; Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l-stephacidin A. *Org. Lett.* **9,** 4255–4258 (2007).

1.7 Scheerer's 2017 formal synthesis of (±)-brevianamide B (second intermolecular [4+2] approach)

- 15 steps (13 + 2 formal steps)
- 2.1% overall yield (4.6% × 45% formal steps)
- 5.0 mg of final intermediate



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1. Perkins, J. C., Wang, X., Pike, R. D. & Scheerer, J. R. Further investigation of the intermolecular Diels–Alder cycloaddition for the synthesis of bicyclo[2.2.2]diazaoctane alkaloids. *J. Org. Chem.* **82,** 13656–13662 (2017).
2. Steps **1-3** taken from; Laws, S. W. & Scheerer, J. R. Enantioselective Synthesis of (+)-Malbrancheamide B. *J. Org. Chem.* **78,** 2422–2429 (2013).
3. Step **4** taken from; Robins, J. G., Kim, K. J., Chinn, A. J., Woo, J. S. & Scheerer, J. R. Intermolecular Diels–Alder Cycloaddition for the Construction of Bicyclo[2.2.2]diazaoctane Structures: Formal Synthesis of Brevianamide B and Premalbrancheamide. *J. Org. Chem.* **81,** 2293–2301 (2016).
4. Steps **14-15** taken from; Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l-stephacidin A. *Org. Lett.* **9,** 4255–4258 (2007).

2. Quantitative analysis of brevianamide A and B in *Penicillium brevicompactum*

The ratio of brevianamide A and B in *Penicillium brevicompactum* cultures was determined to be consistently ≳90:10 by Campbell and co-workers (see tables below).[1]

Supplementary Table 1. Brevianamide A and B production from *P. brevicompactum* colony growth: over culture, inoculum 4.8 x 10^6 spores per plate.

Time after inoculation (h)	Brevianamide A (mg per plate)	Brevianamide B (mg per plate)	A : B
72	0.07	0.01	87.5:12.5
84	0.19	0.02	90.5:9.5
96	0.21	0.02	91.3:8.7
108	0.45	0.03	93.9:6.1
120	0.42	0.04	91.3:8.7
144	0.50	0.04	92.6:7.4
168	0.52	0.04	92.9:7.1
192	0.53	0.05	91.4:8.6
216	0.49	0.04	92.5:7.5

Supplementary Table 2. Brevianamide A and B production from *P. brevicompactum* colony growth: over culture without medium replacement, inoculum 3.6 x 10^6 spores per plate.

Time after inoculation (h)	Brevianamide A (mg per plate)	Brevianamide B (mg per plate)	A : B
72	0.18	0.02	90:10
84	0.28	0.03	90.3:9.7
96	0.14	0.01	93.3:6.7
108	0.19	0.02	90.5:9.5
120	0.67	0.06	91.8:8.2
144	0.66	0.06	91.7:8.3

Supplementary Table 3. Brevianamide A and B production from *P. brevicompactum* colony growth: over culture with continual medium replacement, inoculum 3.6 x 10^6 spores per plate.

Time after inoculation (h)	Brevianamide A (mg per plate)	Brevianamide B (mg per plate)	A : B
72	0.12	0.01	92.3:7.7
84	0.54	0.04	93.1:6.9
96	0.84	0.08	91.3:8.7
108	1.08	0.09	92.3:7.7
120	1.27	0.13	90.7:1.3
144	1.61	0.14	92:8

References:

1. Bird, B. A. & Campbell, I. M. Brevianamides A and B are formed only after conidiation has begun in solid cultures of *Penicillium brevicompactum*. *Appl. Environ. Microbiol.* **42,** 521–525 (1981).

3. Experimental Conditions

NMR Spectroscopy: ^{1}H NMR spectra were recorded at 600 MHz on a Bruker 600 spectrometer with a AVANCE 3HD console, at 500 MHz on Ascend 500 spectrometers with AVANCE 3 and AVANCE 3HD consoles, and at 400 MHz on a Bruker 400 spectrometer with AVANCE 3 console. Residual solvent peaks were used as an internal reference for ^{1}H NMR spectra ($CDCl_3$ δ 7.26 ppm, CD_3OD δ 3.31 ppm, CD_2Cl_2 δ 5.32 ppm, and $(CD_3)_2SO$ δ 2.50 ppm). Coupling constants (*J*) are quoted to the nearest 0.1 Hz. ^{13}C NMR spectra were recorded at 151 MHz on a Bruker 600 spectrometer with an AVANCE 3HD console, 126 MHz on Ascend 500 spectrometers with AVANCE 3 and AVANCE 3HD consoles, and at 101 MHz on a Bruker 400 spectrometer with AVANCE 3 console. Solvent peaks were used as an internal reference for ^{13}C NMR spectra ($CDCl_3$ δ 77.16 ppm, CD_3OD δ 49.00 ppm, CD_2Cl_2 δ 54.00 ppm, and $(CD_3)_2SO$ δ 39.52 ppm). Assignment of ^{1}H and ^{13}C NMR signals was assisted by ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC and ^{1}H-^{1}H NOESY experiments.

IR Spectroscopy: IR spectra were recorded as neat samples on a Shimadzu IR affinity-1 FTIR spectrometer fitted with an ATR attachment.

Mass Spectroscopy: EI mass spectra were recorded on a MAT 900 XP double focussing high resolution sector, run at 70 eV. ESI spectra were recorded on a Bruker microTOF, calibrated with sodium formate clusters, with data analysis using Data Analysis 4.1 (Bruker Daltonics).

Analytical TLC: TLC analyses were performed on Merck silica plates coated with silica gel 60 F254 (0.2mm) and were visualised with UV light and by staining with *p*-anisaldehyde or $KMnO_4$ standard TLC stain solutions, followed by heating.

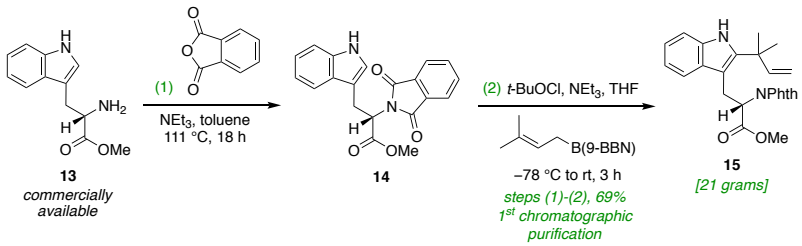
Flash Chromatography: Flash chromatography was performed using Merck silica gel 60 (40–63 μm) for routine separations and Fluorochem silica gel 60 (20–45 μm) for more challenging separations. Neutralised silica refers to columns packed with solvent containing 1% NEt_3 and rinsed with between 1 and 2 column volumes of solvent (free of NEt_3) before use.

Optical Rotation: Optical rotations were recorded using a Bellingham Stanley ADP450 polarimeter with a Bellingham Stanley 0.5 mL cell (l = 0.25 dm). Concentrations (c) are reported in g/100 mL.

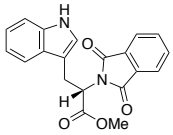
Chiral HPLC: Analytical chiral HPLC was conducted using a modular Shimadzu system using a LC-20AD pump, DGU-20A$_{5R}$ degassing unit, SIL-20A HT autosampler, CTO-20A column oven, SPD-20A UV/Vis detector and CDM-20A communications module.

Experimental Procedures, Reagents and Solvents: Unless stated otherwise, reactions were performed under a positive pressure of dry nitrogen using anhydrous solvents. Commercially available chemicals were used as received, unless specified otherwise. Solvents and reagents dried over 4 Å molecular sieves were dried for at least 24 h before use. Distillates were collected in flasks cooled with a dry ice/acetone bath to avoid loss of material. For reactions performed under anhydrous reaction conditions the reaction vessels were dried with a heat gun under vacuum prior to use. For anhydrous reactions, NEt_3 was dried over activated 4 Å molecular sieves for at least 24 h before use.

3.1 Experimental Procedure for Compound **14**



Step (1) – Based on conditions reported by Vederas and co-workers.[1] To a stirred suspension of L-tryptophan methyl ester hydrochloride (25.0 g, 98.2 mmol) and phthalic anhydride (14.5 g, 98.2 mmol) in toluene (1000 mL) was added NEt_3 (27.4 mL, 196 mmol) and the mixture heated at reflux for 18 h. The reaction was allowed to cool to room temperature and a mixture of saturated aq. NH_4Cl (150 mL) and water (150 mL) was added. The organic phase was separated, washed with brine (300 mL), dried (Na_2SO_4) and concentrated under reduced pressure to yield crude **14** (33.6 g, 98% crude mass recovery) which was used without further purification in the next step. A small sample was purified by flash chromatography (2:3 EtOAc/petroleum spirit) to give compound **14** as a pale-yellow foam suitable for characterisation. All data for compound **14** matched literature values.[1]



14

R_f 0.24 (2:3 EtOAc/petroleum spirit)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (br. s, 1H), 7.74 (dd, *J* = 5.5, 3.1 Hz, 2H), 7.65 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.60 (app. dt, *J* = 7.9, 0.9 Hz, 1H), 7.25 (app. dt, *J* = 7.3, 0.9, 0.9 Hz, 1H), 7.12 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.05 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 6.99 (d, *J* = 2.3 Hz, 1H), 5.28 (dd, *J* = 9.9, 6.1 Hz, 1H), 3.79 (s, 3H), 3.78 – 3.70 (m, 2H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.8, 167.7, 136.2, 134.1, 131.8, 127.3, 123.5, 122.7, 122.2, 119.6, 118.6, 111.2, 111.2, 53.0, 52.7, 24.9 ppm;

IR (film, cm^{-1}) 3404, 3057, 3013, 2953, 2924, 2849, 1775, 1740, 1705, 1612, 1555;

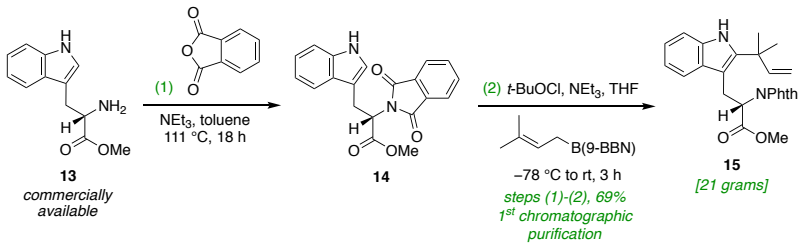
HRMS (ESI^+) *m/z* 349.1211 (calculated $[M + H]^+$ 349.1183), 371.1033 (calculated $[M + Na]^+$ 371.1002)

$[\alpha]_D^{23.3}$ −201.1° (*c* 1.07, $CHCl_3$)

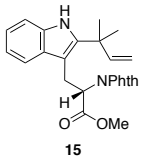
Reference:

1. Liu, H., Pattabiraman, V. R. & Vederas, J. C. Stereoselective Syntheses of 4-Oxa Diaminopimelic Acid and Its Protected Derivatives via Aziridine Ring Opening. *Org. Lett.* **9,** 4211–4214 (**2007**).

3.2 Experimental Procedure for Compound **15**



Step (2) – Based on a procedure reported by Danishefsky and co-workers.[1] Reaction performed under anhydrous reaction conditions. A solution of crude compound **14** (25.0 g) and dry NEt_3 (12.0 mL, 86.1 mmol) in THF (250 mL) was cooled to −78 °C, and the lights in the vicinity of the reaction were turned off. *t*-BuOCl (9.7 mL, 86.1 mmol, see page 30 for preparation) was added dropwise and the reaction stirred for 30 minutes at −78 °C. A solution of *B*-Prenyl-9-BBN (360 mL, 180 mmol, 0.5 M in THF, see pages 28-29 for preparation) was added dropwise at −78 °C, over 50 min. After stirring for 1 h at −78 °C the cooling bath was removed and the reaction slowly allowed to warm to room temperature. Reaction progress was monitored by TLC analysis, following the disappearance of starting material **14**, R_f 0.24 (1:1 EtOAc/petroleum spirit) and appearance of an intermediate with R_f 0.63 (1:1 EtOAc/petroleum spirit). After stirring for 2 h saturated aq. K_2CO_3 (75 mL) was added. The layers were separated and the aqueous layer extracted with EtOAc (3 × 125 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The crude product was filtered through silica with CH_2Cl_2, concentrated and purified by flash chromatography (1:9 to 1:3 EtOAc/petroleum spirit) to give compound **15** (21.1 g, 50.7 mmol, 69% over 2 steps) as a pale-yellow foam. All spectroscopic data matched literature values.[1]



R_f 0.55 (1:1 EtOAc/petroleum spirit), 0.24 (1:4 EtOAc/petroleum spirit);

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.86 (br. s, 1H), 7.69 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.62 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.28 (app. dt, *J* = 7.9, 0.9 Hz, 1H), 7.13 (app. dt, *J* = 8.0, 0.8 Hz, 1H), 6.90 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 6.71 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.19 (dd, *J* = 17.5, 10.5 Hz, 1H), 5.23 – 5.17 (m, 2H), 5.15 (dd, *J* = 10.5, 1.0 Hz, 1H), 3.86 (dd, *J* = 15.4, 3.9 Hz, 1H), 3.78 (s, 3H), 3.67 (dd, *J* = 15.4, 11.3 Hz, 1H), 1.58 (s, 3H), 1.57 (s, 3H) ppm;

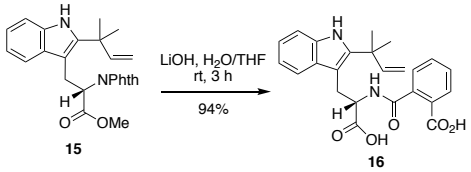
^{13}C NMR (151 MHz, $CDCl_3$) δ 169.7, 167.8, 146.0, 140.3, 134.0, 134.0, 132.0, 129.9, 123.3, 121.3, 119.3, 117.9, 112.3, 110.3, 106.4, 53.6, 52.9, 39.3, 27.8, 27.7, 24.6 ppm;

$[\alpha]_D^{23.7}$ −221.6° (*c* 3.97, $CHCl_3$), literature: $[\alpha]_D^{25}$ −180.8° (*c* 3.9, $CHCl_3$).[1]

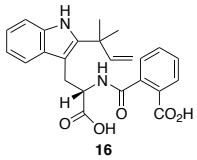
Reference:

1. Schkeryantz, J. M., Woo, J. C. G., Siliphaivanh, P., Depew, K. M. & Danishefsky, S. J. Total synthesis of gypsetin, deoxybrevianamide E, brevianamide E, and tryprostatin B: Novel constructions of 2,3-disubstituted indoles. *J. Am. Chem. Soc.* **121,** 11964–11975 (1999).

3.3 Experimental Procedure for Compound **16**



Compound **16** was prepared based on conditions reported by Ley and co-workers.[1] To a solution of compound **15** (67 mg, 0.16 mmol) in 1:1 THF/H_2O (2 mL) was added solid LiOH (19 mg, 0.79 mmol). The solution was stirred at rt for 3 h before being neutralised with 1 M aq. HCl and the aqueous layer extracted with Et_2O (3 × 5 mL). The combined organics were washed with H_2O (2 × 2.5 mL), brine (2.5 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give crude compound **16** (62 mg, 0.15 mmol, 94% crude mass recovery) as a yellow foam, which was sufficiently pure for characterisation purposes.



^{1}H NMR (500 MHz, CD_3OD) δ 7.91 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.46 (app. td, *J* = 7.6, 1.6 Hz, 1H), 7.42 (app. td, *J* = 7.5, 1.6 Hz, 1H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.04 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.98 – 6.93 (m, 1H), 6.89 (dd, *J* = 7.4, 1.5 Hz, 1H), 6.26 (dd, *J* = 17.5, 10.6 Hz, 1H), 5.17 (dd, *J* = 17.4, 1.3 Hz, 1H), 5.11 (dd, *J* = 10.5, 1.2 Hz, 1H), 4.98 (app. t, *J* = 7.8 Hz, 1H), 3.51 (dd, *J* = 14.5, 7.7 Hz, 1H), 3.27 (dd, *J* = 14.5, 7.9 Hz, 1H), 1.59 (s, 3H), 1.59 (s, 3H) ppm;

^{13}C NMR (126 MHz, CD_3OD) δ 175.3, 172.2, 169.2, 147.6, 142.1, 139.4, 136.4, 132.9, 131.2, 131.1, 130.5, 130.4, 129.0, 121.7, 119.6, 119.3, 112.1, 111.6, 106.6, 55.6, 40.4, 28.9, 28.5, 28.4 ppm;

IR (film, cm^{-1}) 3000, 1771, 1694, 1597, 1525, 1462, 1389;

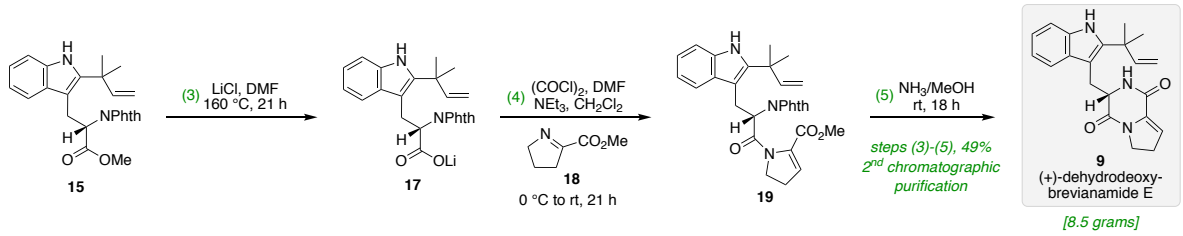
HRMS (EI^+) *m/z* 420.16655 (calculated $[M]^+$: 420.16797);

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{23.4}}$ −28.3° (*c* 0.10, MeOH).

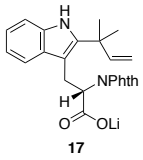
Reference:

1. Hewitt, P. R., Cleator, E. & Ley, S. V. A concise total synthesis of (+)-okaramine C. *Org. Biomol. Chem.* **2,** 2415–2417 (2004).

3.4 Experimental Procedure for Compound **17**



Step (3) – Inspired by the work of Hell,[1,2] and Fisher.[3] Compound **15** (24.6 g, 59.0 mmol) and LiCl (50.0 g, 1180 mmol) were suspended in DMF (125 mL) and the resulting mixture heated at 160 °C for 21 h under a flow of nitrogen. The thick brown suspension was diluted with CH_2Cl_2 (300 mL), sonicated for 30 minutes and the solid removed by filtration. The residual solid was broken up, suspended in CH_2Cl_2 (300 mL), stirred for 30 minutes and filtered. The combined filtrates were concentrated under reduced pressure, then dried at 160 °C under reduced pressure. The crude product was re-dissolved in CH_2Cl_2, filtered and concentrated twice to remove any residual LiCl solid, before being dried at 160 °C under reduced pressure to give crude lithium carboxylate **17** (23.6 g), which was used without further purification in the next step.



^{1}H NMR (500 MHz, CD_3OD) δ 7.62 (app. s, 4H), 7.21 (app. dt, *J* = 8.1, 0.9 Hz, 1H), 7.11 (app. t, *J* = 8.0, 0.9 Hz, 1H), 6.75 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 6.53 (ddd, *J* = 8.0, 7.0, 1.0 Hz 1H), 6.26 (dd, *J* = 17.4, 10.6 Hz, 1H), 5.13 (dd, *J* = 17.4, 1.2 Hz, 1H), 5.04 (dd, *J* = 10.6, 1.2 Hz, 1H), 4.95 (dd, *J* = 12.0, 3.3 Hz, 1H), 3.84 (dd, *J* = 15.3, 3.3 Hz, 1H), 3.58 (dd, *J* = 15.2, 12.0 Hz, 1H), 1.59 (s, 3H), 1.54 (s, 3H) ppm;

^{13}C NMR (126 MHz, CD_3OD) δ 176.1, 170.1, 147.8, 141.3, 136.2, 134.6, 133.7, 131.1, 123.5, 121.2, 118.9, 118.6, 111.7, 111.2, 108.5, 57.8, 40.4, 28.6, 28.3, 26.0 ppm;

IR (film, cm^{-1}) 2924, 2365, 1773, 1701, 1611, 1466, 1396, 1350;

HRMS (ESI^-) *m/z* 401.1476 (calculated $[M - Li]^-$ 401.1507);

$[\alpha]_D^{22.9}$ −226.0° (*c* 0.20, MeOH).

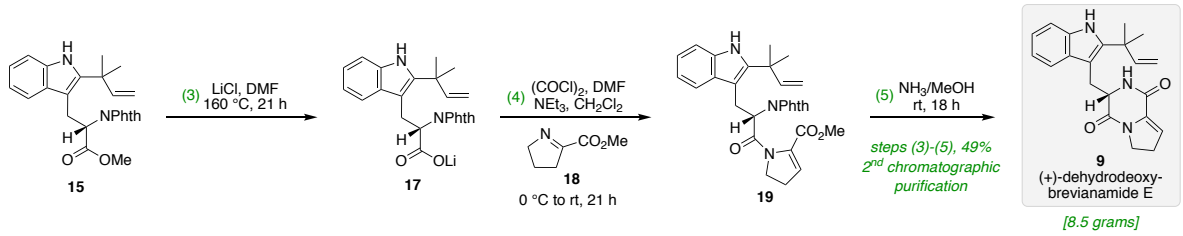
References:

1. Fölling, J., Belov, V., Kunetsky, R., Medda, R., Schönle, A., Egner, A., Eggeling, C., Bossi, M. & Hell, S. W. Photochromic rhodamines provide nanoscopy with optical sectioning. *Angew. Chem. Int. Ed.* **46,** 6266–6270 (2007).

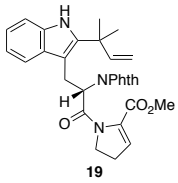
2. Belov, V. N., Bossi, M. L., Fölling, J., Boyarskiy, V. P. & Hell, S. W. Rhodamine spiroamides for multicolor single-molecule switching fluorescent nanoscopy. *Chem. Eur. J.* **15,** 10762-10776 (2009).

3. Fisher, J. W. & Trinkle, K. L. Iodide dealkylation of benzyl, PMB, PNB, and t-Butyl N-acyl amino acid esters via lithium ion coordination. *Tetrahedron Lett.* **35,** 2505-2508 (1994).

3.5 Experimental Procedure for Compound **19**



Step (4) – Inspired by the work of Schmalz,[1] and Soai.[2] Reaction performed under anhydrous reaction conditions. DMF (1.90 mL, 24.6 mmol) was added dropwise to a solution of oxalyl chloride (6.24 mL, 73.8 mmol) in CH_2Cl_2 (200 mL) at 0 °C. A suspension of crude compound **17** from **Step (3)** (20.1 g) in CH_2Cl_2 (200 mL) was then added dropwise over 30 min at 0 °C and the residual **17** was washed into the reaction with CH_2Cl_2 (2 × 50 mL) and the resulting mixture was stirred at 0 °C for 30 min. A solution of compound **18** (5.84 mL, 49.2 mmol, see page 27 for preparation) and NEt_3 (10.3 mL, 73.8 mmol) in CH_2Cl_2 was added dropwise over 20 min at 0 °C. The cooling bath was then removed and the reaction stirred for 21 h at rt. The reaction was quenched by the addition of 1 M aq. HCl (80 mL), the phases separated and the aqueous phase extracted with CH_2Cl_2 (3 × 50 mL). The combined organics were washed with saturated aq. $NaHCO_3$ (80 mL), and the aqueous layer then back extracted with CH_2Cl_2 (50 mL). The combined organics were washed with brine (120 mL), dried (Na_2SO_4) and concentrated under reduced pressure to give crude compound **19** (23.9 g) which was used without further purification in the next step. A small sample could be purified by flash chromatography (1:1 EtOAc/petroleum spirit) to give compound **19** as a lemon-yellow foam suitable for characterisation.



R_f 0.28 (1:1 EtOAc/petroleum spirit);

^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.95 (s, 1H), 7.64 (app. s, 4H), 7.17 – 7.13 (m, 2H), 6.86 (dd, *J* = 8.2, 7.1 Hz, 1H), 6.61 (app. t, *J* = 7.4 Hz, 1H), 6.20 (dd, *J* = 17.4, 10.5 Hz, 1H), 5.87 (dd, *J* = 3.5, 2.5 Hz, 1H), 5.24 (dd, *J* = 10.4, 4.4 Hz, 1H), 5.20 (dd, *J* = 17.5, 1.1 Hz, 1H), 5.13 (dd, *J* = 10.6, 1.1 Hz, 1H), 3.94 (br. s, 1H), 3.83 (dd, *J* = 15.4, 4.4 Hz, 1H), 3.72 (s, 3H), 3.62 (app. q, *J* = 10.5 Hz, 1H), 3.46 (dd, *J* = 15.4, 10.4 Hz, 1H), 2.62 (app. dtd, *J* = 17.6, 10.0, 2.5 Hz, 1H), 2.39 (app. ddt, *J* = 17.5, 10.6, 3.4 Hz, 1H), 1.55 (s, 3H), 1.55 (s, 3H);

^{13}C NMR (151 MHz, CD_2Cl_2) δ 167.8, 167.7, 162.1, 146.6, 140.9, 137.0, 134.5, 134.5, 131.9, 130.3, 123.6, 123.5, 121.5, 119.4, 118.1, 112.3, 110.6, 106.6, 54.2 (beneath solvent peak), 52.6, 50.4 (br.), 39.7, 29.6, 27.9, 27.9, 25.4;

IR (film, cm^{-1}) 1775, 1713, 1655, 1639, 1611, 1437, 1381;

HRMS (ESI^+) *m/z* 512.2202 (calculated $[M + H]^+$ 512.2180), 534.2016 (calculated $[M + Na]^+$ 534.1999);

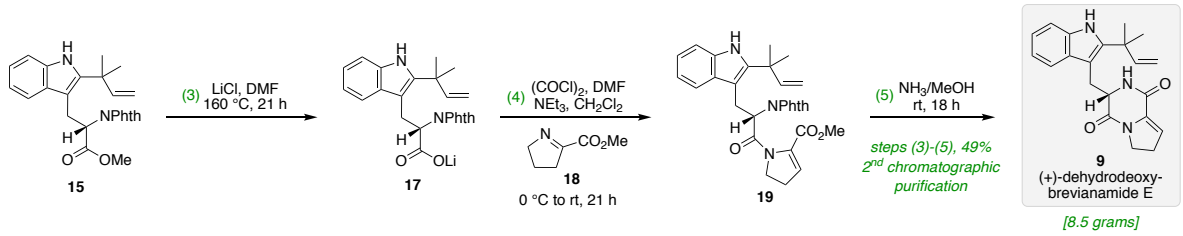
$[\alpha]_D^{23.0}$ −130.1° (*c* 0.38, MeOH).

References:

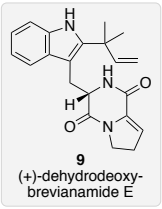
1. Huy, P., Neudörfl, J.-M. & Schmalz, H.-G. A practical synthesis of trans-3-substituted proline derivatives through 1,4-addition. *Org. Lett.* **13,** 216-219 (2011).

2. Ookawa, A. & Soai, K. Asymmetric synthesis of optically active threo- and erythro-pyrrolidinylbenzyl alcohol by the highly stereospecific arylation of (*S*)-proline and the subsequent highly diastereoselective reduction of the α-amino ketone. *J. Chem. Soc., Perkin Trans. 1* 1465-1465 (1987).

3.6 Experimental Procedure for Compound **9**



Step (5) – The crude product **19** (23.9 g) from **Step (4)** was dissolved in a solution of NH_3 in MeOH (7 M, 1200 mL) and stirred at room temperature for 18 h. The crude reaction mixture was concentrated under reduced pressure. Flash chromatography on neutralised silica (7:3 to 8:2 EtOAc/petroleum spirit) gave (+)-dehydrodeoxybrevianamide E (**9**) (8.51 g, 24.4 mmol, 49% over 3 steps) as a pale-yellow foam. All spectroscopic data matched literature values.[1]



R_f 0.19 (8:2 EtOAc/petroleum spirit);

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.08 (br. s, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.32 (app. dt, *J* = 8.1, 0.9 Hz, 1H), 7.17 (ddd, *J* = 8.1, 7.1, 1.2 Hz, 1H), 7.11 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 6.14 (app. t, *J* = 3.1 Hz, 1H), 6.12 (dd, *J* = 17.2, 10.4 Hz, 1H), 5.66 (br. s, 1H), 5.20 – 5.15 (m, 2H), 4.52 (ddd, *J* = 11.3, 3.5, 1.8 Hz, 1H), 4.14 – 4.02 (m, 2H), 3.73 (dd, *J* = 14.7, 3.7 Hz, 1H), 3.23 (dd, *J* = 14.6, 11.3 Hz, 1H), 2.78 (app. td, *J* = 9.1, 3.1 Hz, 2H), 1.55 (s, 3H), 1.55 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 162.7, 156.6, 145.8, 141.8, 134.4, 133.2, 128.9, 122.3, 120.2, 118.9, 118.3, 112.6, 110.9, 104.6, 57.6, 45.7, 39.2, 30.9, 28.1, 28.0, 27.9 ppm;

IR (film, cm^{-1}) 3331 br., 2967, 2926, 1672, 1639, 1435;

HRMS (ESI^+) *m/z* 350.1875 (calculated $[M + H]^+$ 350.1863), 372.1689 (calculated $[M + Na]^+$ 372.1682);

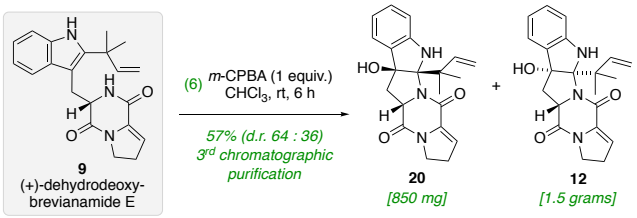
$[\alpha]_D^{23.1}$ −33.2° (*c* 1.30, $CHCl_3$), literature: $[\alpha]_D^{22}$ −38° (*c* 1.3, $CHCl_3$).[2]

References:

1. Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l-stephacidin A. *Org. Lett.* **9,** 4255–4258 (2007).

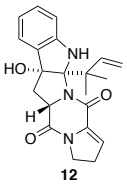
2. Steyn, P. S. The structures of five diketopiperazines from *Aspergillus ustus*. *Tetrahedron* **29,** 107–120 (1973).

3.7 Experimental Procedure for Compounds **12** and **20**



Step (6) – Inspired by the work of Kametani[1] and Wolff.[2] To a rapidly stirred solution of dehydrodeoxy-brevianamide E (**9**) (4.00 g, 11.5 mmol) in $CHCl_3$ (80 mL) at room temperature was added dropwise over 4 h a solution of *m*-CPBA in $CHCl_3$ (0.29 M, 40 mL, 11.7 mmol).* The reaction was stirred for 2 h at room temperature and quenched by the addition of saturated aq. $Na_2S_2O_3$ (20 mL) and saturated aq. $NaHCO_3$ (20 mL). The reaction mixture was diluted with EtOAc (200 mL) and $CHCl_3$ (150 mL) and the phases separated. The aqueous phase was back-extracted with $CHCl_3$ (50 mL) and the combined organics washed with saturated aq. $NaHCO_3$ (80 mL), brine (80 mL), dried (Na_2SO_4) and concentrated under reduced pressure. A diastereomeric ratio of 63:37 for **12**:**20** was determined by analysis of the ^{1}H NMR spectrum of this crude reaction product. Flash column chromatography (1.5:7:3 petroleum spirit/CH_2Cl_2/*i*-Pr_2O to 7:3 CH_2Cl_2/*i*-Pr_2O to EtOAc to *i*-PrOH) of the crude reaction product gave dehydro-brevianamide E (**12**) (1.43 g, 3.91 mmol, 34%) as a white foam, a fraction containing mixed dehydro-brevianamide E (**12**) and compound **20** (1.37 g, 18:82 **12**/**20**, 3.75 mmol, 33%) as a cream foam, alongside recovered starting material dehydrodeoxy-brevianamide E (**9**) (0.270 g, 0.774 mmol, 7%) as a cream foam. A second round of chromatography on the mixed **12**/**20** fraction gave additional pure dehydro-brevianamide E (**12**) (0.108 g, 0.296 mmol, 3%) as a cream glass, pure minor diastereomer **20** (0.454 g, 1.24 mmol, 11%) as a white foam, and a fraction containing mixed dehydro-brevianamide E (**12**) and compound **20** (0.638 g, 9:91 **12**/**20**, 1.75 mmol, 15%). A third round of chromatography on this mixed **12**/**20** fraction gave additional pure minor diastereomer **20** (0.405 g, 1.11 mmol, 10%) as a white foam. Overall, purification by column chromatography gave dehydro-brevianamide E (**12**) (1.54 g, 4.21 mmol, 37%), compound **20** (0.859 g, 2.35 mmol, 20%), and recovered dehydrodeoxy-brevianamide E (**9**) (0.270 g, 0.774 mmol, 7%).

* *m*-CPBA (69% w/w by iodometric titration, 4.38 g, 17.5 mmol) was added to $CHCl_3$ (60 mL) and dried over Na_2SO_4 (27.5 g) with stirring for 40 mins.



R_f 0.15 (7:3 CH_2Cl_2/*i*-Pr_2O);

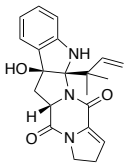
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.18 (app. td, *J* = 7.7, 1.3 Hz, 1H), 6.81 (td, *J* = 7.4, 1.0 Hz, 1H), 6.74 (app. dt, *J* = 7.9, 0.8 Hz, 1H), 6.40 – 6.34 (m, 2H), 6.16 (app. t, *J* = 3.1 Hz, 1H), 5.15 (dd, *J* = 17.7, 1.4 Hz, 1H), 5.07 (dd, *J* = 10.9, 1.3 Hz, 1H), 4.06 (ddd, *J* = 12.0, 8.7, 7.3 Hz, 1H), 3.93 – 3.83 (m, 1H), 3.81 (dd, *J* = 11.5, 7.3 Hz, 1H), 2.79 – 2.69 (m, 4H), 2.43 (br. s, 1H), 1.34 (s, 3H), 1.27 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 163.7, 162.1, 149.4, 144.7, 136.4, 131.0, 129.8, 123.8, 120.6, 120.2, 113.4, 111.2, 91.5, 89.3, 60.3, 45.8, 45.0, 36.7, 29.0, 27.6, 23.2 ppm;

IR (film, cm^{-1}) 3362, 2967, 2926, 1670, 1634, 1609, 1485, 1468, 1437, 1393, 1360;

HRMS (ESI^+) *m/z* 366.1814 (calculated $[M + H]^+$ 366.1812), 388.1629 (calculated $[M + Na]^+$ 388.1632);

$[\alpha]_D^{20.9}$ −208° (*c* 0.23, EtOH).



20

R_f 0.09 (7:3 CH_2Cl_2/*i*-Pr_2O);

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.22 (d, *J* = 7.5 Hz, 1H), 7.13 (app. td, *J* = 7.7, 1.3 Hz, 1H), 6.76 (app. td, *J* = 7.4, 0.9 Hz, 1H), 6.62 (app. dt, *J* = 7.9, 0.8 Hz, 1H), 6.47 (br. s, 1H), 6.34 (dd, *J* = 17.7, 10.8 Hz, 1H), 6.06 (app. t, *J* = 3.0 Hz, 1H), 5.22 (dd, *J* = 17.7, 1.3 Hz, 1H), 5.10 (dd, *J* = 10.9, 1.3 Hz, 1H), 4.73 (app. t, *J* = 8.8 Hz, 1H), 4.00 (ddd, *J* = 12.4, 11.1, 6.1 Hz 1H), 3.82 (dddd, *J* = 12.3, 11.4, 8.3, 0.8 Hz, 1H), 2.86 (ddd, *J* = 13.6, 8.6, 0.7 Hz, 1H), 2.80 – 2.62 (m, 3H), 2.57 (br. s 1H), 1.45 (s, 3H), 1.35 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 161.9, 156.2, 147.0, 144.8, 134.7, 131.5, 130.4, 123.8, 119.5, 119.3, 114.1, 109.9, 95.3, 89.6, 60.5, 46.6, 45.4, 43.4, 28.2, 27.2, 22.5 ppm;

IR (film, cm^{-1}) 3360, 2965, 2924, 2854, 1665, 1632, 1611, 1487, 1466, 1439, 1414, 1371;

HRMS (ESI^+) *m/z* 366.1807 (calculated $[M + H]^+$ 366.1812);

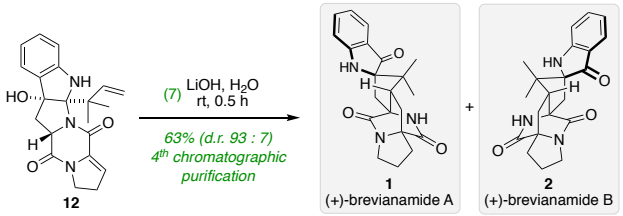
$[\alpha]_D^{22.3}$ +86.3° (*c* 0.19, EtOH).

References:

1. Kametani, T., Kanaya, N. & Ihara, M. Asymmetric total synthesis of brevianamide E. *J. Am. Chem. Soc.* **1980,** *102* (11), 3974-3975.

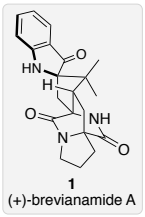
2. Adam, W., Bosio, S. G. & Wolff, B. T. Chiral-Auxiliary-Controlled Diastereoselectivity in the Epoxidation of Enecarbamates with DMD and *m*CPBA. *Org. Lett.* **2003,** *5* (6), 819-822.

3.8 Experimental Procedure for Compounds **1** and **2**



Step (7) – The synthesis of brevianamide A (**1**) was carried out under aerobic conditions, inspired by the work of Kishi[1] and Williams.[2] Effort was made to limit the exposure of the material to light once the reaction was complete, to prevent photolysis of brevianamide A (**1**) to brevianamide C and D.[3] To a solution of dehydro-brevianamide E (**12**) (1.30 g, 3.56 mmol) was added a 1 M solution of aq. LiOH (450 mL) and the reaction stirred rapidly, with vigorous manual shaking every 2–3 minutes to facilitate gradual dissolution of the starting material. After 30 minutes the reaction mixture was extracted with CH_2Cl_2 (2 × 450 mL, 2 × 200 mL), and the combined organics dried (Na_2SO_4). Flash column chromatography (2:8 to 3:7 to 1:1 THF/$CHCl_3$ to 25:25:1 THF/$CHCl_3$/*i*-PrOH) gave (+)-brevianamide A (**1**) (905 mg, 16.2% $CHCl_3$ w/w, 2.07 mmol, 58%)* as a yellow amorphous solid and (+)-brevianamide B (**2**) (59.8 mg, 0.164 mmol, 5%) as a yellow crystalline solid. A sample of (+)-brevianamide A (**1**) was crystallised from $CHCl_3$ by slow vapour diffusion with Et_2O. See pages 58-68 for X-ray crystal structure of brevianamide A·$CHCl_3$.

*In Birch's original isolation paper brevianamide A '*crystallized from $CHCl_3$ in needles containing one molecule of solvent of crystallisation*'.[4] The reported crystal structure for 5-bromo-brevianamide A also showed co-crystallisation with one molecule of acetone.[5] Consistent with these observations, we found that a certain portion of solvent could not be removed from brevianamide A under high vacuum. Proteo-solvent was replaced with deutero-solvent to record NMR spectra for characterisation.



Spectroscopic data matched literature values reported by Zhu Weiming and co-workers.[6] Subtle differences in ^{1}H NMR data are attributed to concentration effects in $CDCl_3$ (see page 42).

R_f 0.21 (3:7 THF/$CHCl_3$);

MP 173–179 °C, literature: 175–180 °C '*with loss of solvent*';[4]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (dd, *J* = 7.7, 1.2 Hz, 1H), 7.44 (ddd, *J* = 8.3, 7.1, 1.4 Hz, 1H), 6.84 – 6.78 (m, 2H), 6.62 (br. s, 1H), 4.98 (br. s, 1H), 3.52 – 3.41 (m, 2H), 2.81 – 2.74 (m, 2H), 2.40 (ddd, *J* = 9.8, 7.3, 1.1 Hz, 1H), 2.35 (d, *J* = 15.6 Hz, 1H), 2.04 (app. pd, *J* = 6.8, 1.6 Hz, 2H), 1.96 – 1.81 (m, 3H), 1.12 (s, 3H), 0.93 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 202.2, 172.4, 169.8, 160.2, 137.8, 124.8, 121.2, 119.3, 112.1, 78.9, 69.5, 67.8, 55.7, 48.4, 44.1, 37.4, 29.2, 29.0, 25.1, 24.1, 19.9 ppm;

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.65 (br. s, 1H), 7.84 (br. s, 1H), 7.41 (ddd, *J* = 8.3, 7.0, 1.4 Hz, 1H), 7.37 (d, *J* = 7.7 Hz, 1H), 6.81 (dd, *J* = 8.2, 0.8 Hz, 1H), 6.64 (ddd, *J* = 7.8, 7.0, 0.8 Hz, 1H), 3.34 – 3.23 (m, 2H), 2.55 (ddd, *J* = 10.1, 7.5, 1.1 Hz, 1H), 2.49 – 2.44 (m, 2H), 2.39 (d, *J* = 15.1 Hz, 1H), 2.01 – 1.91 (m, 1H), 1.90 – 1.77 (m, 3H), 1.68 (dd, *J* = 13.1, 7.6 Hz, 1H), 0.98 (s, 3H), 0.68 (s, 3H) ppm;

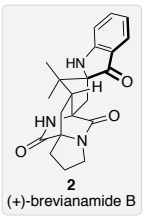
^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 199.9, 172.1, 169.5, 160.5, 137.2, 123.6, 119.4, 116.7, 111.1, 79.0, 68.5, 66.4, 53.1, 48.3, 43.2, 39.5, 28.4, 27.2, 24.6, 21.2, 17.7 ppm;

IR (film, cm^{-1}) 3314, 3229, 2938, 2876, 1667, 1618, 1491, 1466, 1395;

HRMS (ESI^+) *m/z* 366.1818 (calculated $[M + H]^+$ 366.1812);

$[\alpha]_D^{23.5}$ +316° (*c* 0.12, EtOH), literature: $[\alpha]_D^{25}$ +413° (EtOH);[4]

e.r. 93:7, after crystallisation 99:1 (Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm) t_{Rminor} = 5.41 min, t_{Rmajor} = 6.72 min. See Pages 54-55 for chiral HPLC traces.



2
(+)-brevianamide B

Brevianamide B (**2**) was only sparingly soluble in $CDCl_3$, consistent with the observation in Birch's original isolation paper that brevianamide B '*was insoluble in most solvents except hot DMSO and CF_3CO_2H, the latter causing decomposition*'.[3] ^{1}H and ^{13}C NMR spectra could be recorded using a saturated sample in $CDCl_3$, to enable comparison to literature data,[7,8] however 2D NMR spectra were unobtainable. High quality 1D and 2D NMR spectra were instead recorded in $(CD_3)_2SO$.

R_f 0.08 (3:7 THF/$CHCl_3$);

MP 286–290 °C (decomposition), literature: 324–328 °C (decomposition);[3]

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.56 (app. ddt, *J* = 7.6, 1.3, 0.7 Hz, 1H), 7.43 (ddd, *J* = 8.4, 7.1, 1.4 Hz, 1H), 6.84 – 6.78 (m, 2H), 5.78 (br. s, 1H), 4.73 (br. s, 1H), 3.50 – 3.44 (m, 2H), 3.31 (ddd, *J* = 10.3, 7.4, 1.3 Hz, 1H), 3.27 (d, *J* = 15.6 Hz, 1H), 2.74 (ddd, *J* = 13.1, 7.0, 6.1 Hz, 1H), 2.06 – 1.93 (m, 3H), 1.90 – 1.77 (m, 2H), 1.70 (d, *J* = 15.7, 1H), 1.14 (s, 3H), 0.83 (s, 3H) ppm;

^{13}C NMR (126 MHz, $CDCl_3$) δ 203.9, 173.4, 169.2, 160.3, 137.5, 125.2, 120.1, 119.2, 111.5, 77.7, 68.9, 66.5, 49.7, 46.6, 44.0, 36.8, 29.3, 28.6, 25.1, 22.6, 20.5 ppm;

^{1}H NMR (500 MHz, $(CD_3)_2SO$) δ 8.64 (br. s, 1H), 7.41 (ddd, *J* = 8.3, 7.1, 1.4 Hz, 1H), 7.33 (d, *J* = 7.7 Hz, 1H), 7.27 (br. s, 1H), 6.89 (app. dt, *J* = 8.3, 0.9 Hz, 1H), 6.64 (ddd, *J* = 7.8, 7.0, 0.9 Hz, 1H), 3.35 – 3.24 (m, 2H), 3.01 (ddd, *J* = 10.3, 7.5, 1.1 Hz, 1H), 2.67 (d, *J* = 15.1 Hz, 1H), 2.46 (dd, *J* = 11.9, 6.1 Hz, 1H), 2.02 – 1.90 (m, 3H), 1.85 – 1.74 (m, 2H), 1.63 (dd, *J* = 13.0, 7.5 Hz, 1H), 1.05 (s, 3H), 0.63 (s, 3H) ppm;

^{13}C NMR (126 MHz, $(CD_3)_2SO$) δ 204.7, 172.6, 169.2, 161.3, 137.2, 123.8, 118.1, 116.9, 111.4, 77.5, 68.1, 65.2, 48.8, 46.0, 43.3, 33.4, 28.4, 27.5, 24.4, 21.9, 19.7 ppm;

IR (film, cm^{-1}) 3348, 3231, 2949, 2878, 1705, 1664, 1653, 1616, 1587, 1495, 1468;

HRMS (ESI^+) *m/z* 366.1796 (calculated $[M + H]^+$ 366.1812);

$[\alpha]_D^{24.7}$ +136° (*c* 0.23, 2.5% HCO_2H in CH_2Cl_2), literature (enantiomer): $[\alpha]_D^{25}$ −147° (*c* 0.23, 2.5% HCO_2H in CH_2Cl_2),[8] $[\alpha]_D^{25}$ −124° (*c* 0.81, 2.5% HCO_2H in CH_2Cl_2);[7]

e.r. 93:7 (Chiralpak IA, 1:3 EtOH/hexane, 1 mL min^{-1}, λ 254 nm) t_{Rminor} = 13.99 min, t_{Rmajor} = 26.10 min. See Pages 56-57 for chiral HPLC traces.

References:

1. Hutchison, A. J. & Kishi, Y. Stereospecific total synthesis of dl-austamide. *J. Am. Chem. Soc.* **101,** 6786–6788 (1979).

2. Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l- stephacidin A. *Org. Lett.* **9,** 4255–4258 (2007).

3. Birch, A. J. & Russell, R. A. Studies in relation to biosynthesis—XLIV: Structural elucidations of brevianamides-B, -C, -D and -F. *Tetrahedron* **28,** 2999–3008 (1972).

4. Birch, A. J. & Wright, J. J. Studies in relation to biosynthesis—XLII: The structural elucidation and some aspects of the biosynthesis of the brevianamides-A and -E. *Tetrahedron* **26,** 2329–2344 (1970).

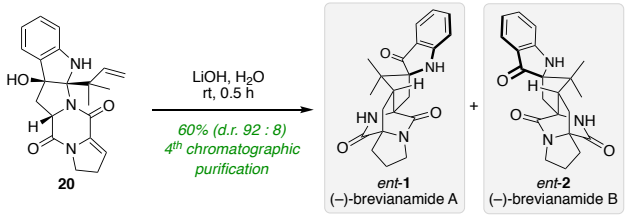
5. Coetzer, J. The structure and absolute configuration of 5-bromobrevianamide A. *Acta Crystallographica Section B* **30,** 2254–2256 (1974).

6. Lingling, C., Tonghan, Z., Guoliang, Z., Yunlong, L., Cong, W., Pawinee, P., Arthit, C. & Weiming, Z. Bioactive Natural Products from the Marine-Derived *Penicillium brevicompactum* OUCMDZ-4920. *Chin. J. Org. Chem.* **37,** 2752–2762 (2017).

7. Williams, R. M., Glinka, T., Kwast, E., Coffman, H. & Stille, J. K. Asymmetric, stereocontrolled total synthesis of (-)-brevianamide B. *J. Am. Chem. Soc.* **112,** 808–821 (1990).

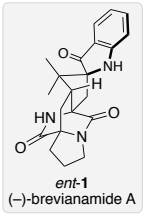
8. Frebault, F. C. & Simpkins, N. S. A cationic cyclisation route to prenylated indole alkaloids: synthesis of malbrancheamide B and brevianamide B, and progress towards stephacidin A. *Tetrahedron* **66,** 6585–6596 (2010).

3.9 Experimental Procedure for Compounds ***ent*-1** and ***ent*-2**



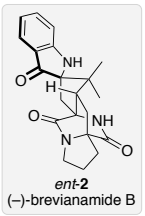
To a solution of compound **20** (234 mg, 0.640 mmol) was added a 1 M solution of aq. LiOH (80 mL) and the reaction stirred rapidly, with vigorous manual shaking every 2–3 minutes to facilitate gradual dissolution of the starting material. After 30 minutes the reaction mixture was extracted with CH_2Cl_2 (4 × 100 mL), and the combined organics dried (Na_2SO_4). Flash column chromatography (2:8 to 3:7 to 1:1 THF/$CHCl_3$ to 25:25:1 THF/$CHCl_3$/*i*-PrOH) gave (–)-brevianamide A (***ent*-1**) (155 mg, 17.5% $CHCl_3$ w/w, 0.350 mmol, 55%)* as a yellow amorphous solid and (–)-brevianamide B (***ent*-2**) (12 mg, 0.032 mmol, 5%) as a yellow crystalline solid.

*In Birch's original isolation paper brevianamide A '*crystallized from $CHCl_3$ in needles containing one molecule of solvent of crystallisation*'.[1] The reported crystal structure for 5-bromo-brevianamide A also showed co-crystallisation with one molecule of acetone.[2] Consistent with these observations, we found that a certain portion of solvent could not be removed from brevianamide A under high vacuum. Proteo-solvent was replaced with deutero-solvent to record NMR spectra for characterisation.



$[\alpha]_D^{24.7}$ −281° (*c* 0.12, EtOH), literature (enantiomer): $[\alpha]_D^{25}$ +413° (EtOH);[1]

e.r. 95:5 (Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm) t_{Rmajor} = 5.38 min, t_{Rminor} = 6.74 min. See Pages 54-55 for chiral HPLC traces.



$[\alpha]_D^{25.1}$ −119° (*c* 0.23, 2.5% HCO_2H in CH_2Cl_2), literature: $[\alpha]_D^{25}$ −147° (*c* 0.23, 2.5% HCO_2H in CH_2Cl_2),[3] $[\alpha]_D^{25}$ −124° (*c* 0.81, 2.5% HCO_2H in CH_2Cl_2);[4]

e.r. 92:8 (Chiralpak IA, 1:3 EtOH/hexane, 1 mL min^{-1}, λ 254 nm) t_{Rmajor} = 13.86 min, t_{Rminor} = 26.14 min. See Pages 56-57 for chiral HPLC traces.

References:

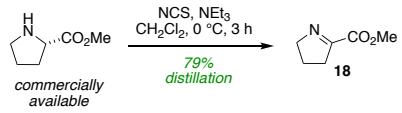
1. Birch, A. J., & Wright, J. J. Studies in relation to biosynthesis—XLII: The structural elucidation and some aspects of the biosynthesis of the brevianamides-A and -E. *Tetrahedron* **1970,** *26* (10), 2329-2344.

2. Coetzer, J. The structure and absolute configuration of 5-bromobrevianamide A. *Acta Crystallographica Section B* **30,** 2254–2256 (1974).

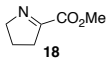
3. Frebault, F. C. & Simpkins, N. S. A cationic cyclisation route to prenylated indole alkaloids: synthesis of malbrancheamide B and brevianamide B, and progress towards stephacidin A. *Tetrahedron* **66,** 6585–6596 (2010).

4. Williams, R. M., Glinka, T., Kwast, E., Coffman, H. & Stille, J. K. Asymmetric, stereocontrolled total synthesis of (-)-brevianamide B. *J. Am. Chem. Soc.* **112,** 808–821 (1990).

3.10 Experimental Procedure for Compound **18**



Compound **18** was prepared under anhydrous conditions, based on a procedure reported by Schmalz and co-workers.[1] To a rapidly stirred suspension of L-proline methyl ester hydrochloride (25.0 g, 151 mmol) and NEt_3 (48.3 mL, 347 mmol) in CH_2Cl_2 (450 mL) at 0 °C was added slowly, portion-wise, solid NCS (22.2 g, 166 mmol). The mixture was stirred at room temperature for 3 h and then diluted with Et_2O (350 mL). A mixture of saturated aq. NH_4Cl (150 mL) and water (150 mL) was added, the organic phase was separated and the aqueous phase was back-extracted with CH_2Cl_2 (2 × 150 mL). The combined organics were washed with a mixture of saturated aq. NH_4Cl (100 mL) and water (100 mL), saturated aq. $NaHCO_3$ (150 mL), brine (150 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Vacuum distillation under reduced pressure (5 mbar) gave imine **18** (15.1 g, 119 mmol, 79%) as a pale-yellow liquid. All spectroscopic data matched literature values.[2]



BP 60–64 °C (5 mbar);

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.04 (app. tt, *J* = 7.6, 2.6 Hz, 2H), 3.80 (s, 3H), 2.76 (app. ddt, *J* = 8.5, 7.6, 2.6 Hz, 2H), 1.96 – 1.89 (m, 2H) ppm;

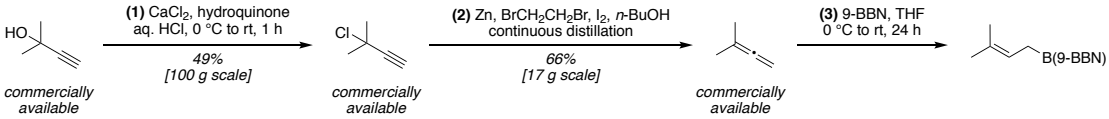
^{13}C NMR (126 MHz, $CDCl_3$) δ 168.2, 163.2, 62.5, 52.5, 35.3, 22.1 ppm.

References:

1. Huy, P., Neudörfl, J.-M. & Schmalz, H.-G. A practical synthesis of *trans*-3- substituted proline derivatives through 1,4-addition. *Org. Lett.* **13,** 216–219 (2011).

2. Sezen, B. & Sames, D. Oxidative C-Arylation of Free (NH)-Heterocycles via Direct (sp^3) C−H Bond Functionalization. *J. Am. Chem. Soc.* **126,** 13244–13246 (2004).

3.11 Experimental Procedure for *B*-Prenyl-9-BBN



1,1-Dimethylallene is a commercially available reagent, but was prepared on large scale based on a procedure reported by Pfeffer and co-workers.[1]

Step (1) – Anhydrous $CaCl_2$ (229 g, 2.06 mol) was added in portions to a stirred mixture of concentrated hydrochloric acid (800 mL), 2-methyl-3-butyn-2-ol (200 mL, 2.06 mol) and hydroquinone (1.82 g, 16.5 mmol) at 0 °C over 7 minutes and the reaction was stirred for 1 h at room temperature. The layers were seperated and solid K_2CO_3 added cautiously to the organic phase until effervescence ceased. The organic phase was then distilled under reduced pressure, collected up to 50 °C at 147 mbar. The crude distilate was redistilled under reduced pressure (147 mbar) to give 3-chloro-3-methyl-1-butyne (103 g, 1.00 mol, 49%) as a colourless oil. All spectroscopic data matched a comercially available sample.

BP 32–36 °C (147 mbar);

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.62 (s, 1H), 1.87 (s, 6H) ppm;

^{13}C NMR (101 MHz, $CDCl_3$) δ 86.7, 72.0, 57.1, 34.7 ppm.

Step (2) – Zinc powder (52.0 g, 760 mmol) was washed with 3% aq. HCl (4 × 40 mL), 2% aq. $CuSO_4$ (2 × 60 mL), ethanol (2 × 60 mL) and *n*-butanol (2 × 60 mL). The treated zinc was suspended in *n*-butanol (110 mL) in a 3 neck RBF with a dropping funnel, magnetic stirrer bar and a distillation head fitted with a 30 cm Vigreux column. 3-Chloro-3-methyl-1-butyne (44.4 mL, 390 mmol) was placed in the dropping funnel along with 1,2-dibromoethane (2.50 mL, 29.0 mmol). A portion of the alkyne mixture (3 mL) was added to the zinc butanol slurry along with a few crystals of iodine and 1,2-dibromoethane (2.50 mL, 29.0 mmol). The mixture was then heated cautiously (using a heat gun) with stirring until the reaction had clearly commenced. Slow addition of the remaining alkyne mixture followed, with external heating applied to maintain the reaction. The crude product was collected by distillation from the reaction mixture during the course of the reaction, and external heat applied to continue collection up to a temperature of 44 °C. The crude distillate was redistilled to give 1,1-dimethylallene (17.5 g, 257 mmol, 66%) as a colourless liquid. All spectroscopic data matched literature values.[1]

BP 40–42 °C;

^{1}H NMR (500 MHz, $CDCl_3$) δ 4.52 (hept, J = 3.1 Hz, 2H), 1.69 (t, J = 3.2 Hz, 6H) ppm;

^{13}C NMR (151 MHz, $CDCl_3$) δ 206.8, 94.2, 72.7, 20.3 ppm.

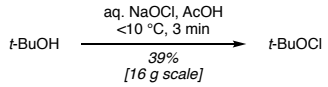
Step (3) – A solution of *B*-Prenyl-9-BBN was prepared under anhydrous conditions, based on a procedure reported by Trauner and co-workers.[66] A solution of 9-BBN in THF (360 mL, 179 mmol, 0.5 M in THF) was cooled to 0 °C. Once the solution was observed to go cloudy, 1,1-dimethylallene (20.5 mL, 208 mmol) was added dropwise over 30 minutes with stirring. The reaction was warmed to room temperature and stirred for 18 h before being used directly in the reverse prenylation procedure described on page 15.

References:

1. Chengebroyen, J., Linke, M., Robitzer, M., Sirlin, C. & Pfeffer, M. Palladium-mediated intramolecular C–N bond formation involving allyl substituted pyridines. Application to a novel strategy for the synthesis of the skeleton of berberinium derivatives. *J. Organomet. Chem.* **687,** 313–321 (2003).

2. Kuttruff, C. A., Zipse, H. & Trauner, D. Concise Total Syntheses of Variecolortides A and B through an Unusual Hetero-Diels–Alder Reaction. *Angew. Chem. Int. Ed.* **50,** 1402–1405 (2011).

3.12 Experimental Procedure for *t*-BuOCl



t-BuOCl was prepared based on a procedure reported by Mintz and co-workers.[1] For safety reasons exposure of the reaction and product to light was minimised.

An aqueous solution of bleach (500 mL, 0.767 M, 384 mmol)* was cooled below 10 °C with stirring. A solution of *t*-BuOH (43.3 mL, 383 mmol) and glacial acetic acid (24.1 mL, 421 mmol) was added in a single portion to the rapidly stirred bleach and the stirring continued for 3 min. The entire reaction mixture was transferred to a separating funnel, the aqueous layer discarded, the yellow organic layer washed with 10% aq. Na_2CO_3 (50 mL) and water (50 mL). The product was dried ($CaCl_2$ approximately 3 g) and decanted into its final container via pipette. The *t*-BuOCl (16.4 g, 151 mmol, 39%) was stored in a fridge over $CaCl_2$ in an amber glass bottle as a yellow liquid.

*Bleach solution was titrated to a concentration of 0.767 M by iodometric titration using sodium thiosulfate prior to use.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 9H) ppm;

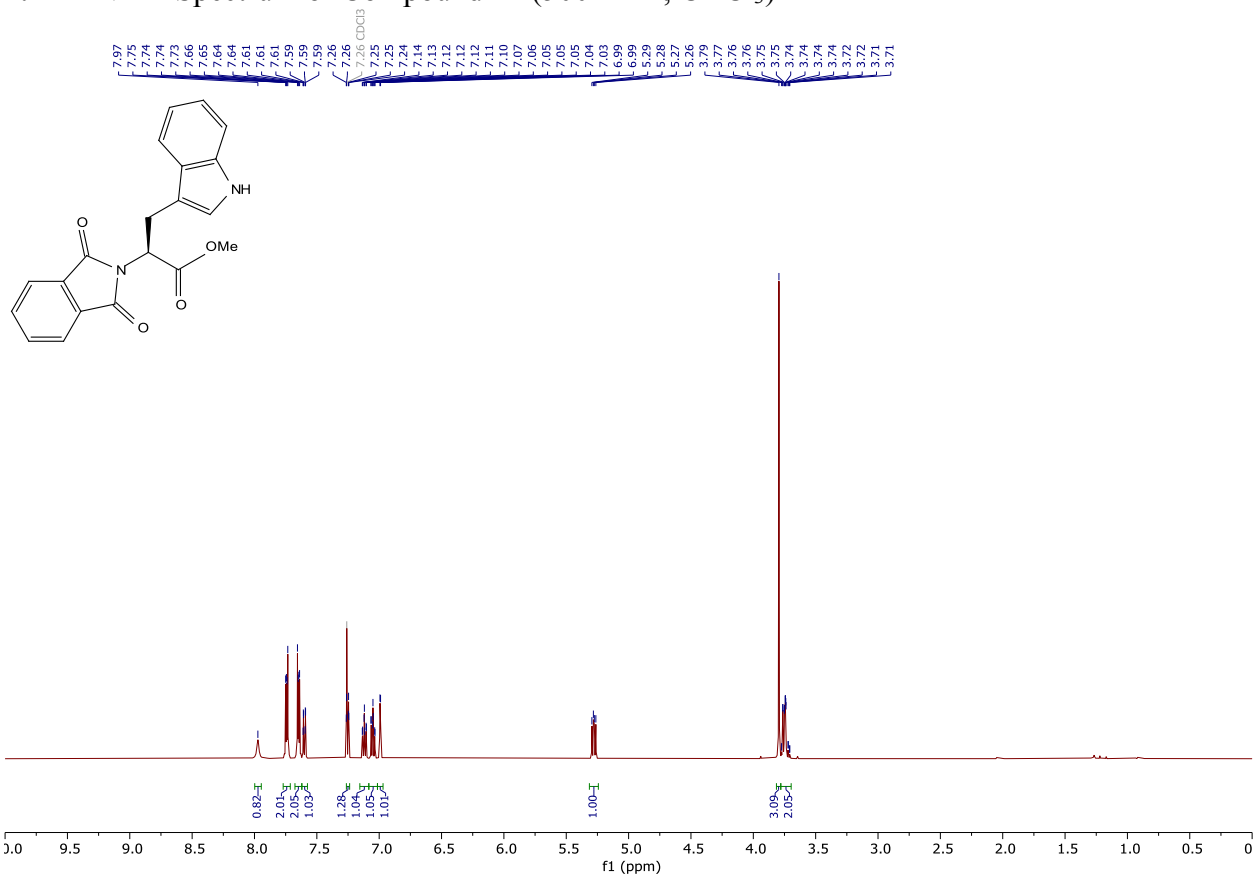
^{13}C NMR (126 MHz, $CDCl_3$) δ 84.1, 27.0 ppm.

References:

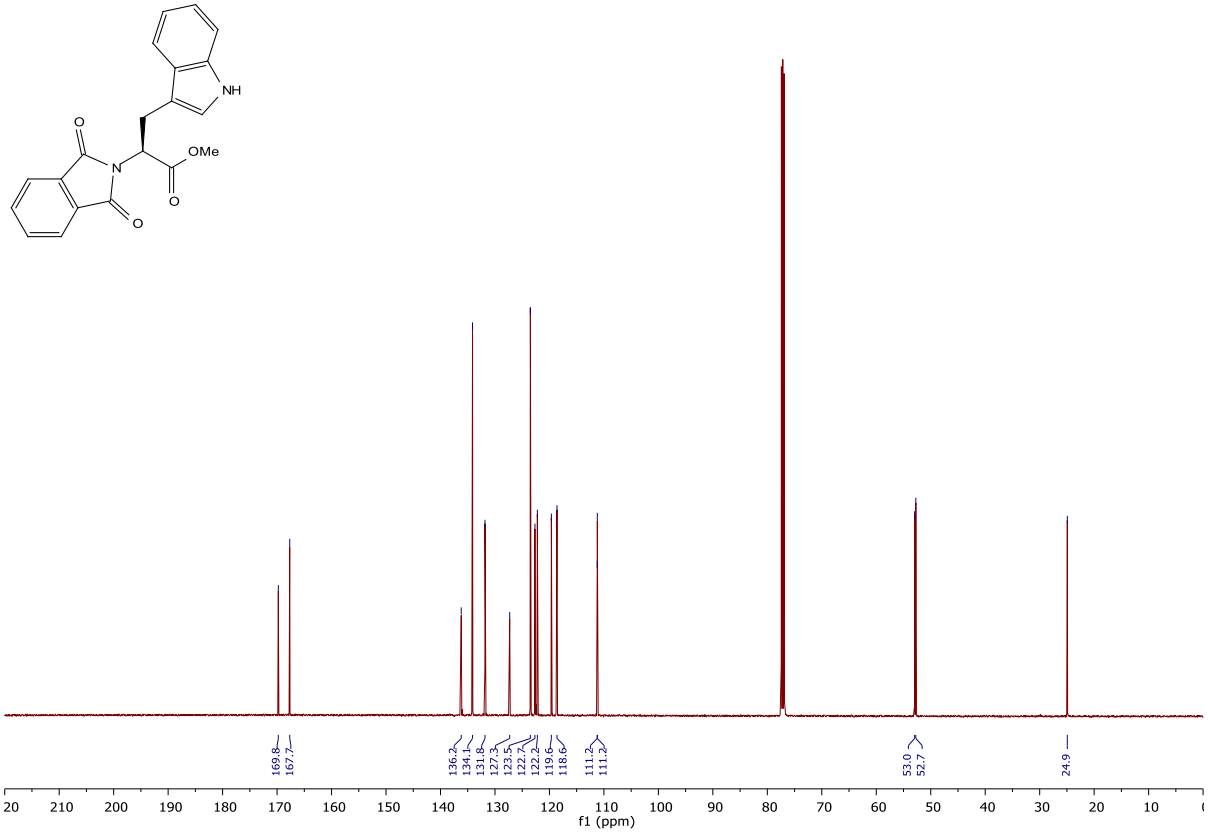
1. Mintz, M. J. & Walling, C. *t*-BUTYL HYPOCHLORITE. *Org. Synth.* **49**, 9 (1969).

4 NMR Spectra

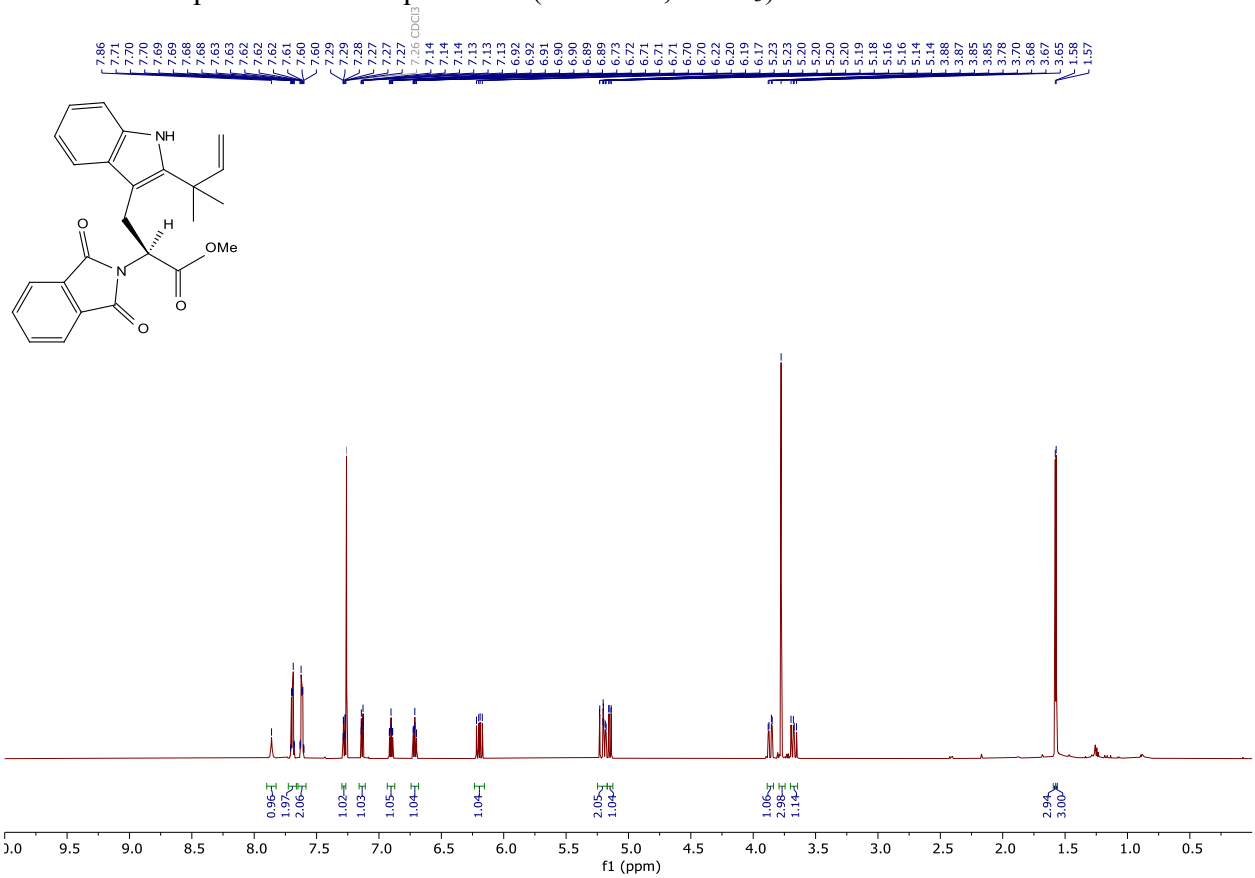
4.1 ^{1}H NMR Spectrum of Compound **14** (500 MHz, $CDCl_3$)



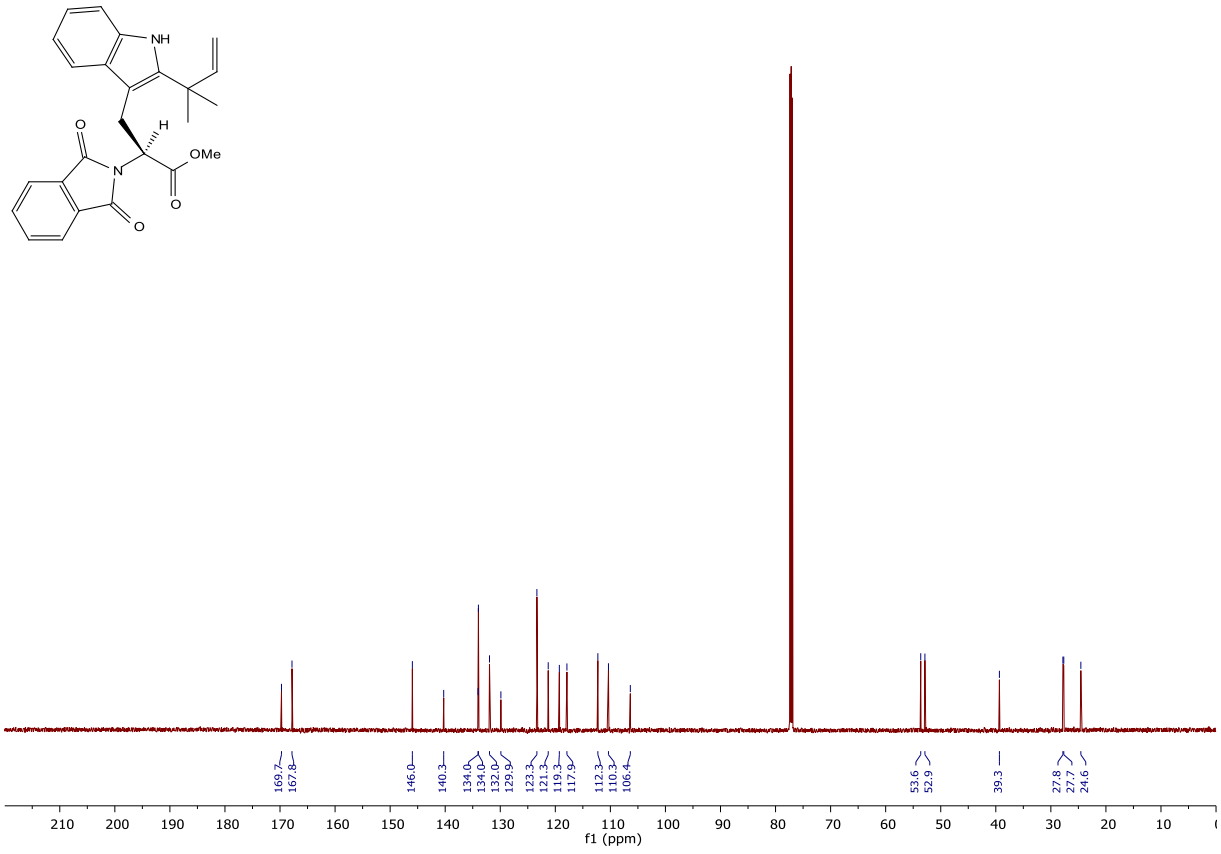
4.2 ^{13}C NMR Spectrum of Compound **14** (126 MHz, $CDCl_3$)



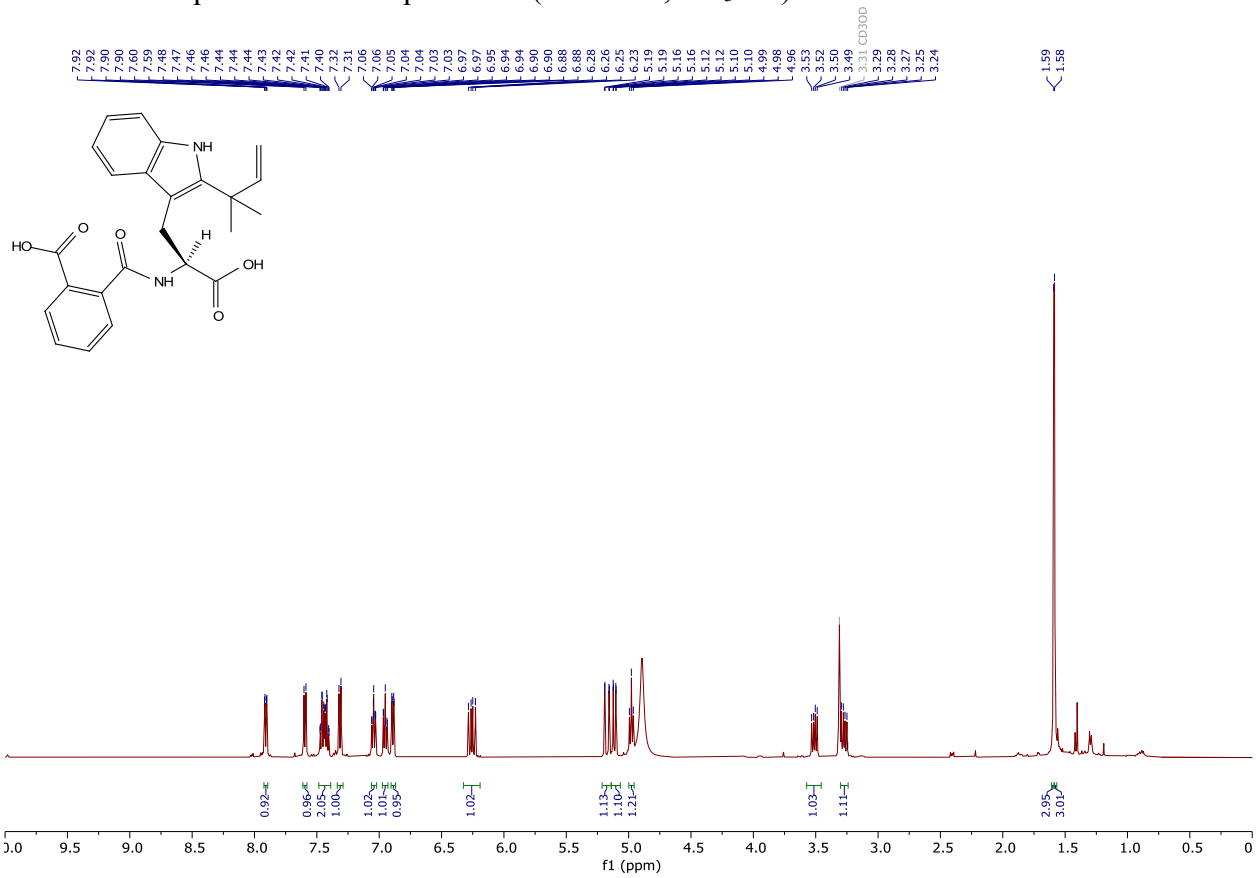
4.3 ^{1}H NMR Spectrum of Compound **15** (600 MHz, $CDCl_3$)



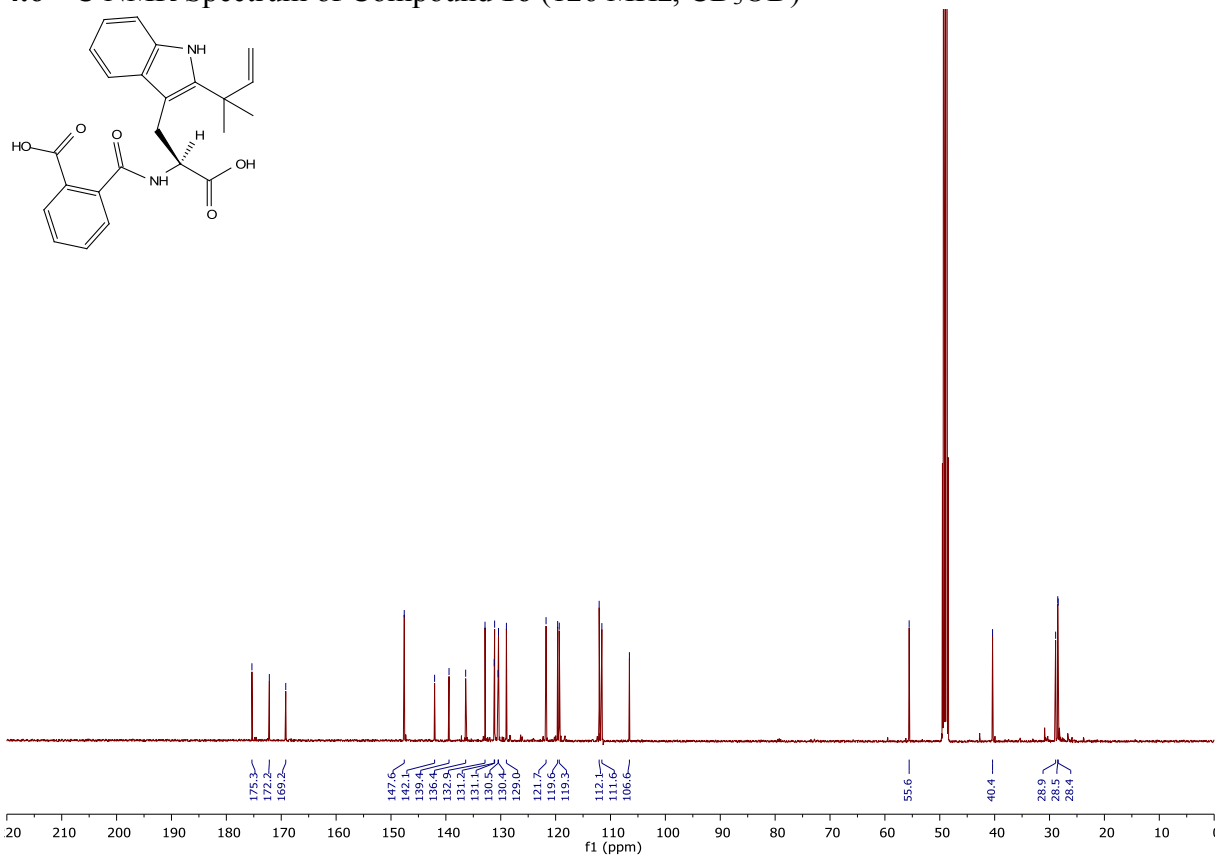
4.4 ^{13}C NMR Spectrum of Compound **15** (151 MHz, $CDCl_3$)



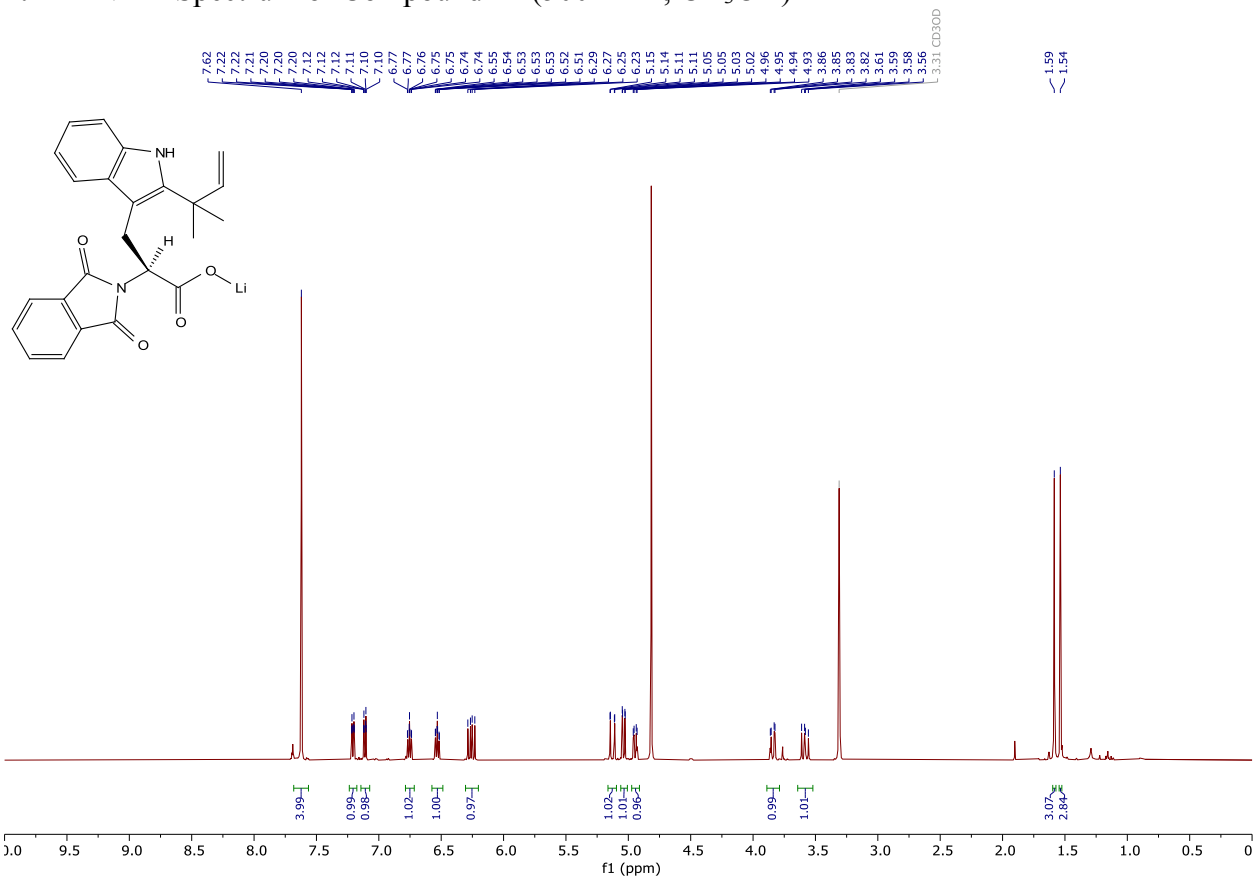
4.5 ^{1}H NMR Spectrum of Compound **16** (500 MHz, CD_3OD)



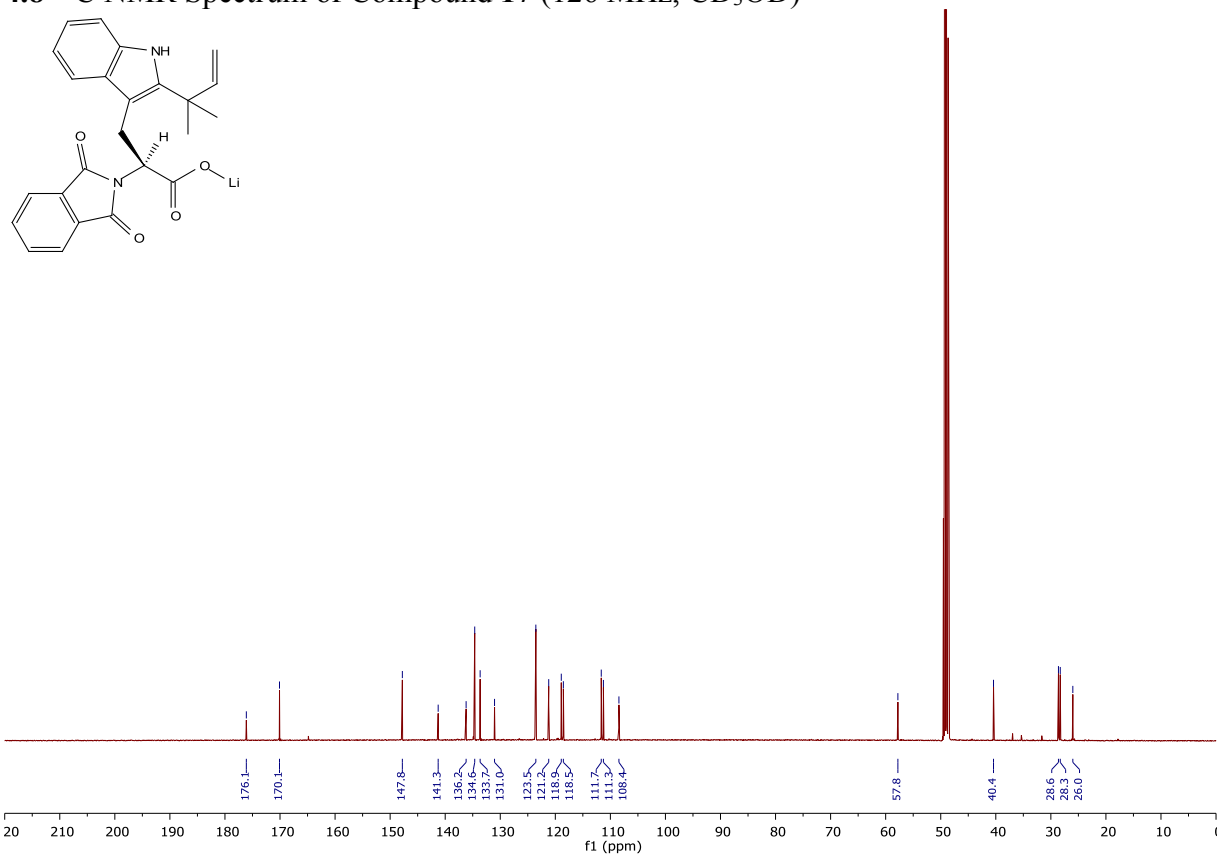
4.6 ^{13}C NMR Spectrum of Compound **16** (126 MHz, CD_3OD)



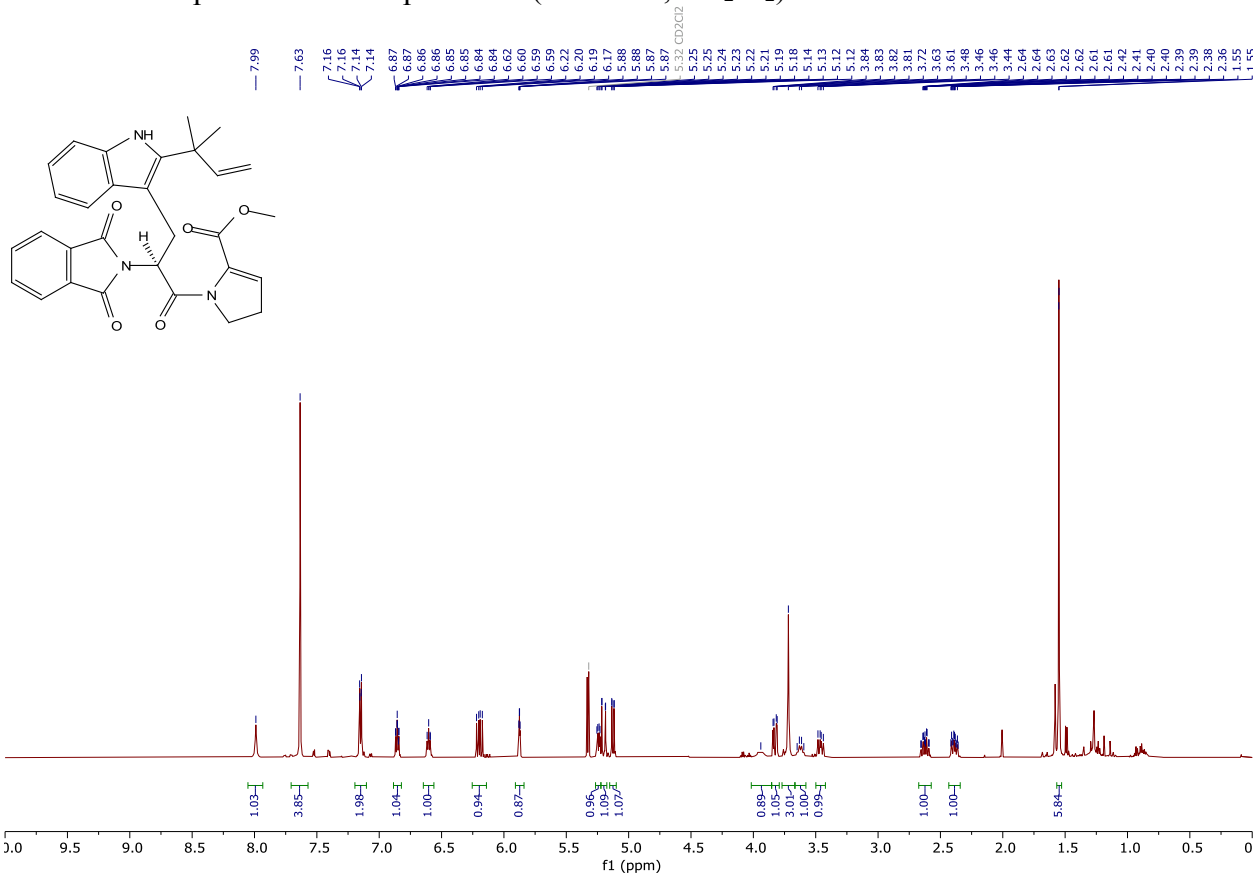
4.7 ^{1}H NMR Spectrum of Compound **17** (500 MHz, CD_3OD)



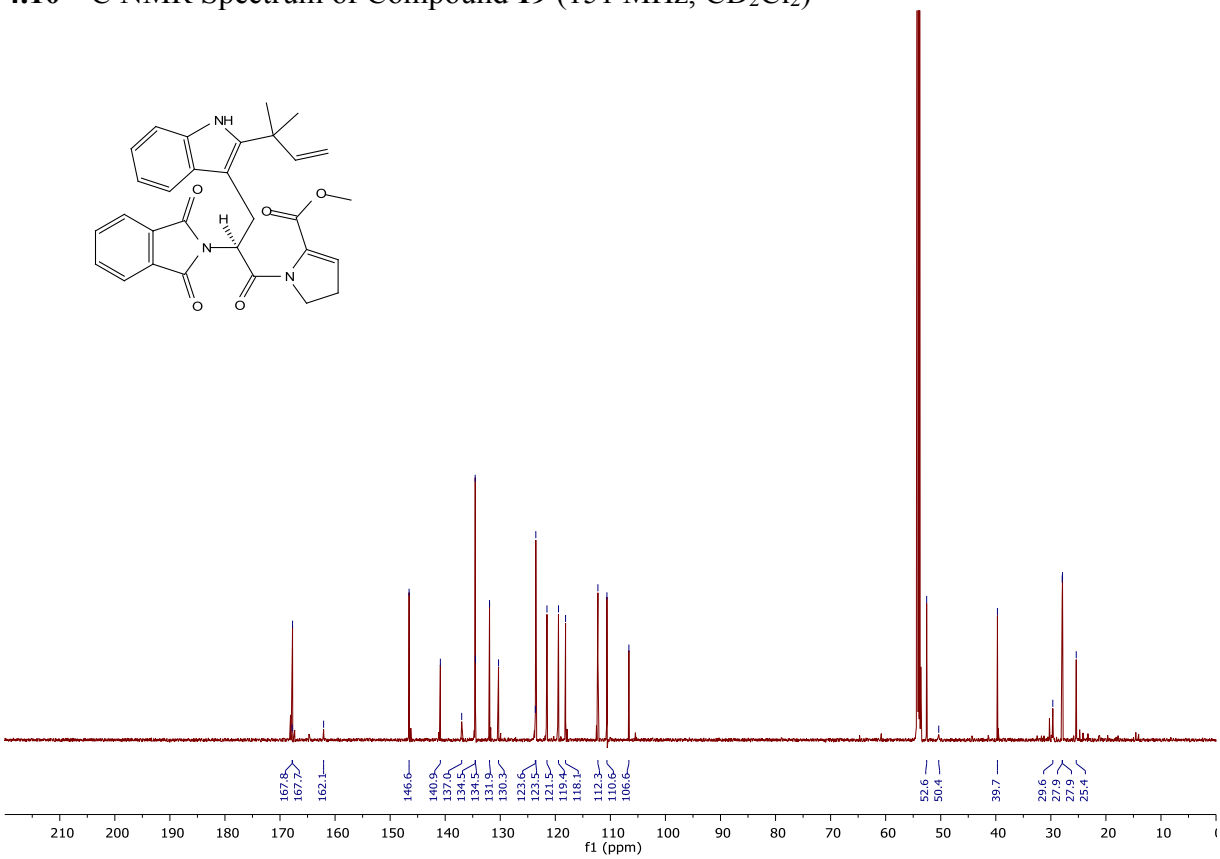
4.8 ^{13}C NMR Spectrum of Compound **17** (126 MHz, CD_3OD)



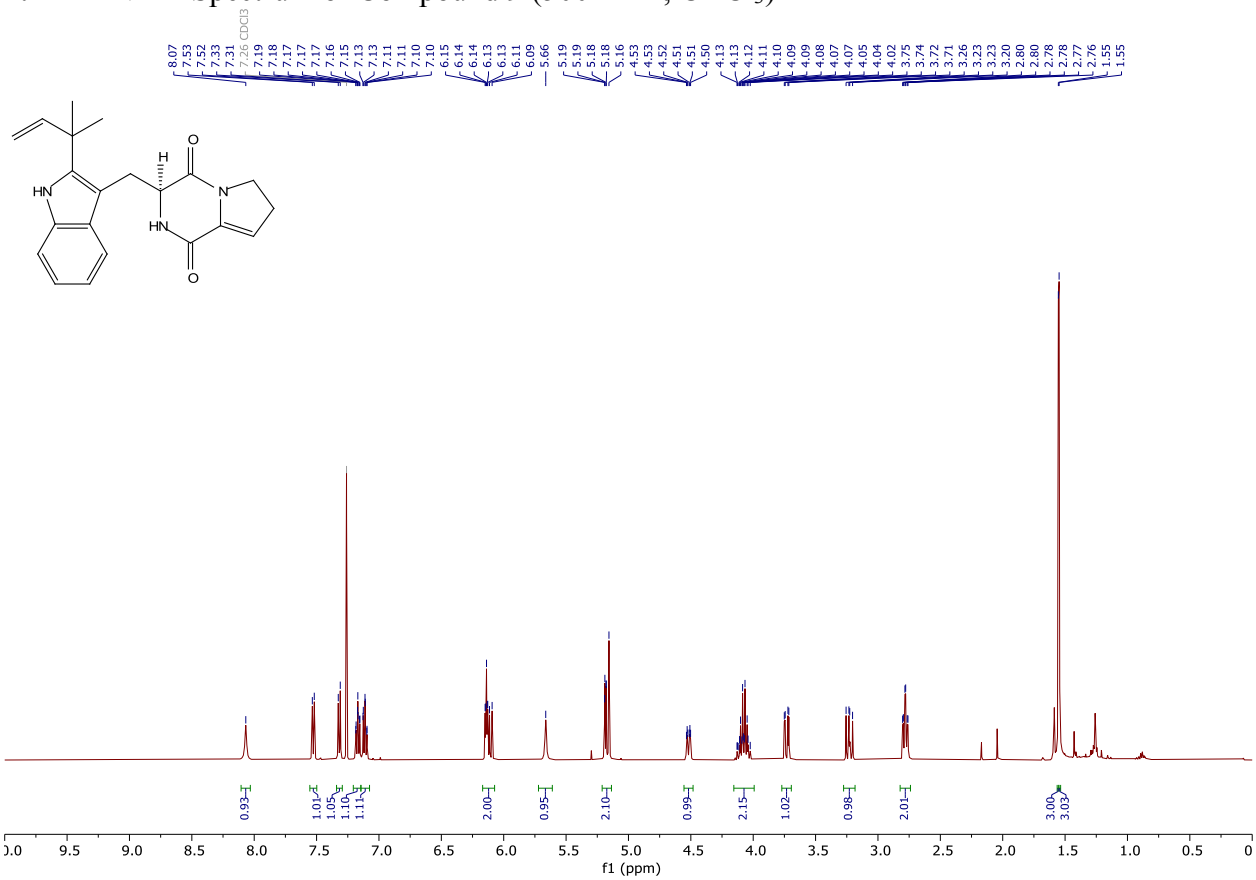
4.9 ^{1}H NMR Spectrum of Compound **19** (500 MHz, CD_2Cl_2)



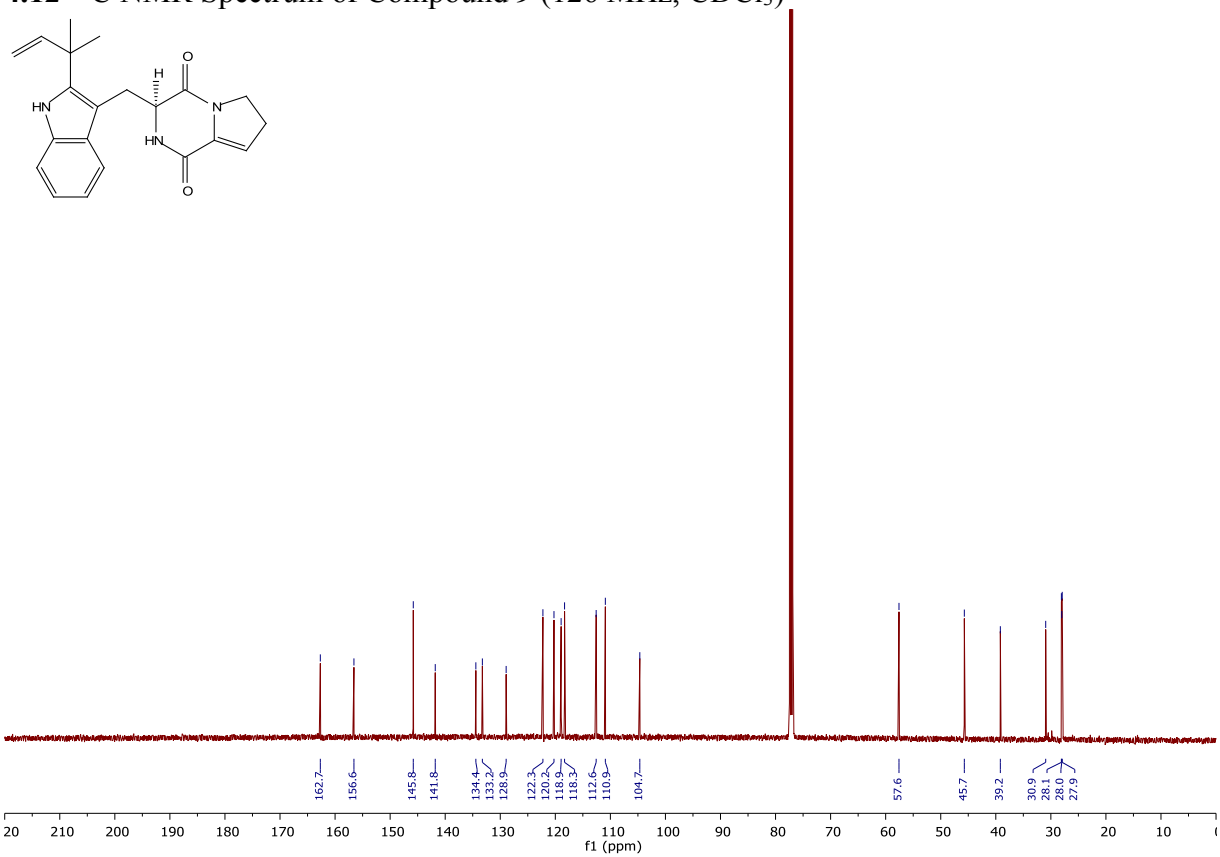
4.10 ^{13}C NMR Spectrum of Compound **19** (151 MHz, CD_2Cl_2)



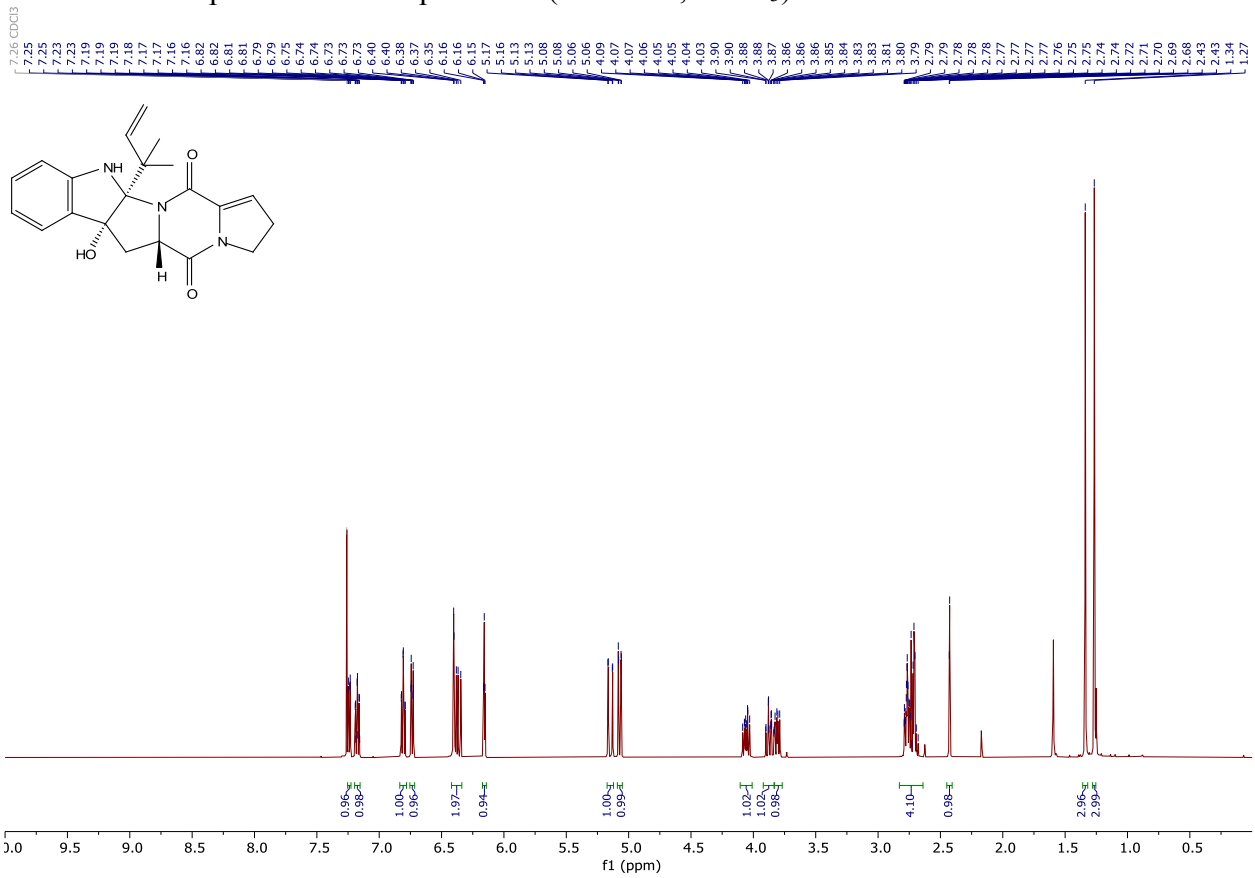
4.11 ^{1}H NMR Spectrum of Compound **9** (500 MHz, $CDCl_3$)



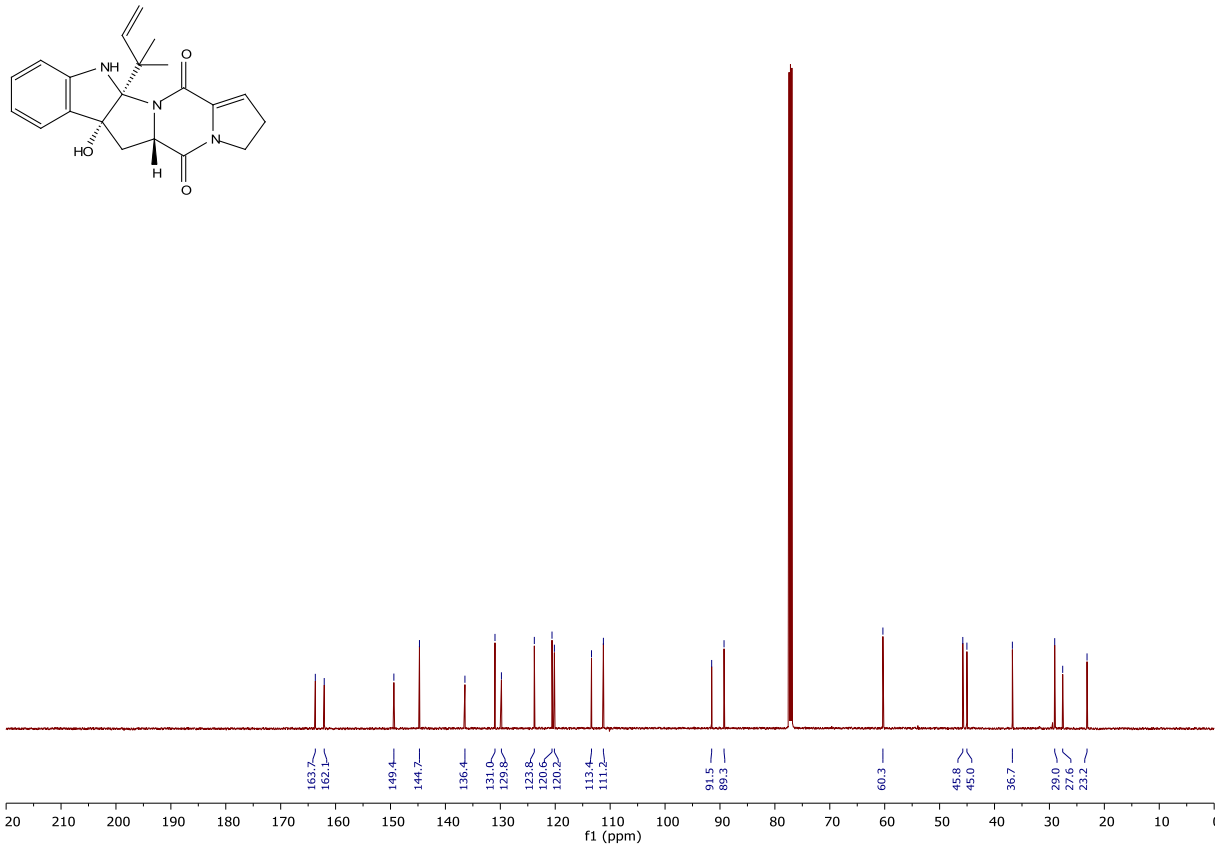
4.12 ^{13}C NMR Spectrum of Compound **9** (126 MHz, $CDCl_3$)



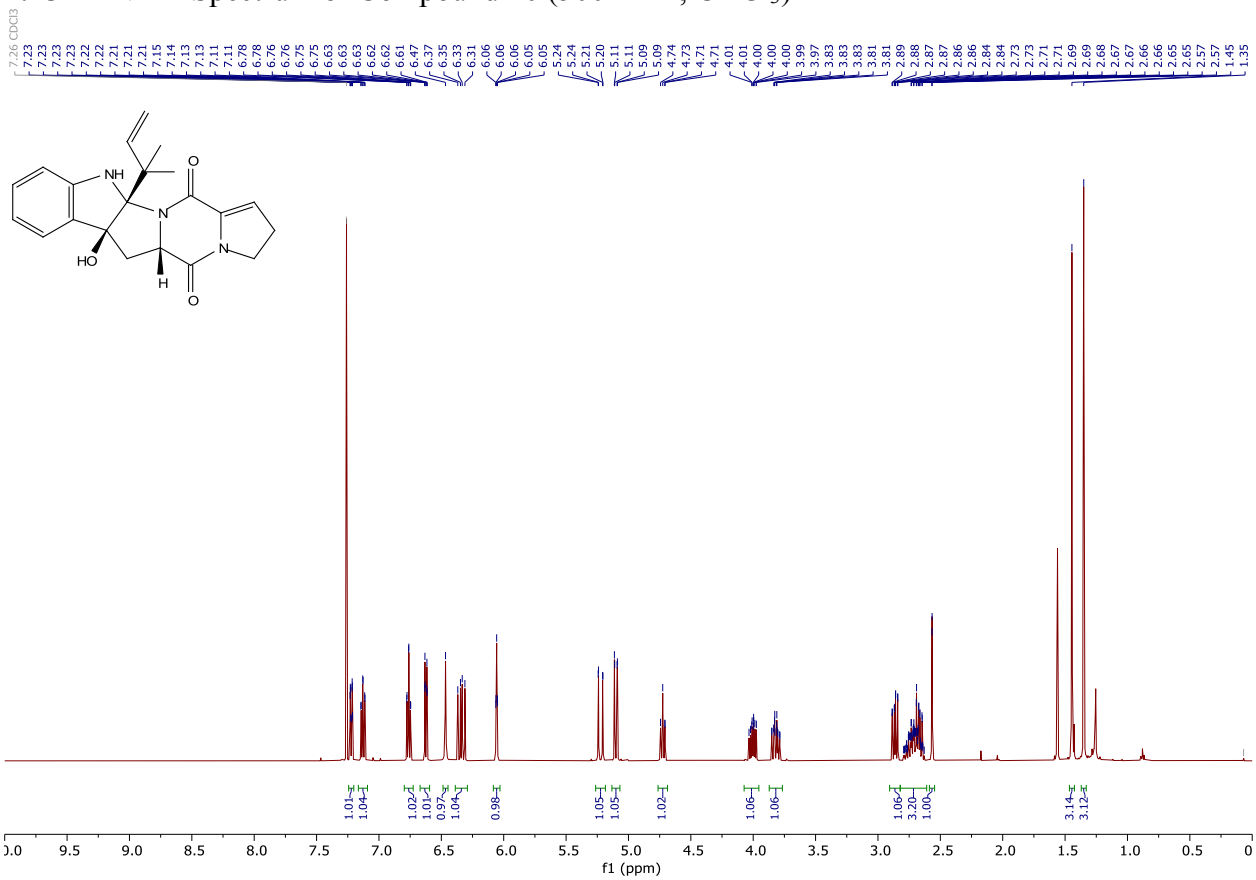
4.13 ^{1}H NMR Spectrum of Compound **12** (500 MHz, $CDCl_3$)



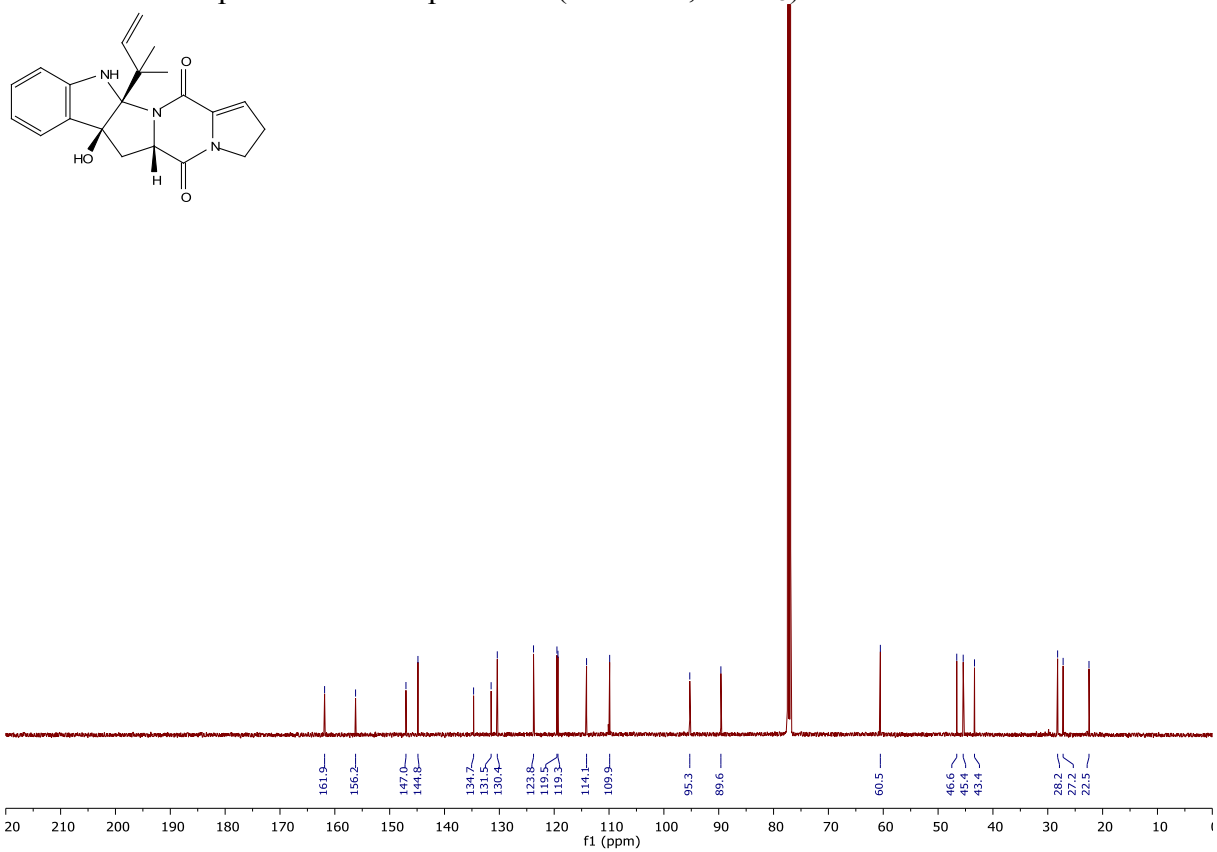
4.14 ^{13}C NMR Spectrum of Compound **12** (126 MHz, $CDCl_3$)



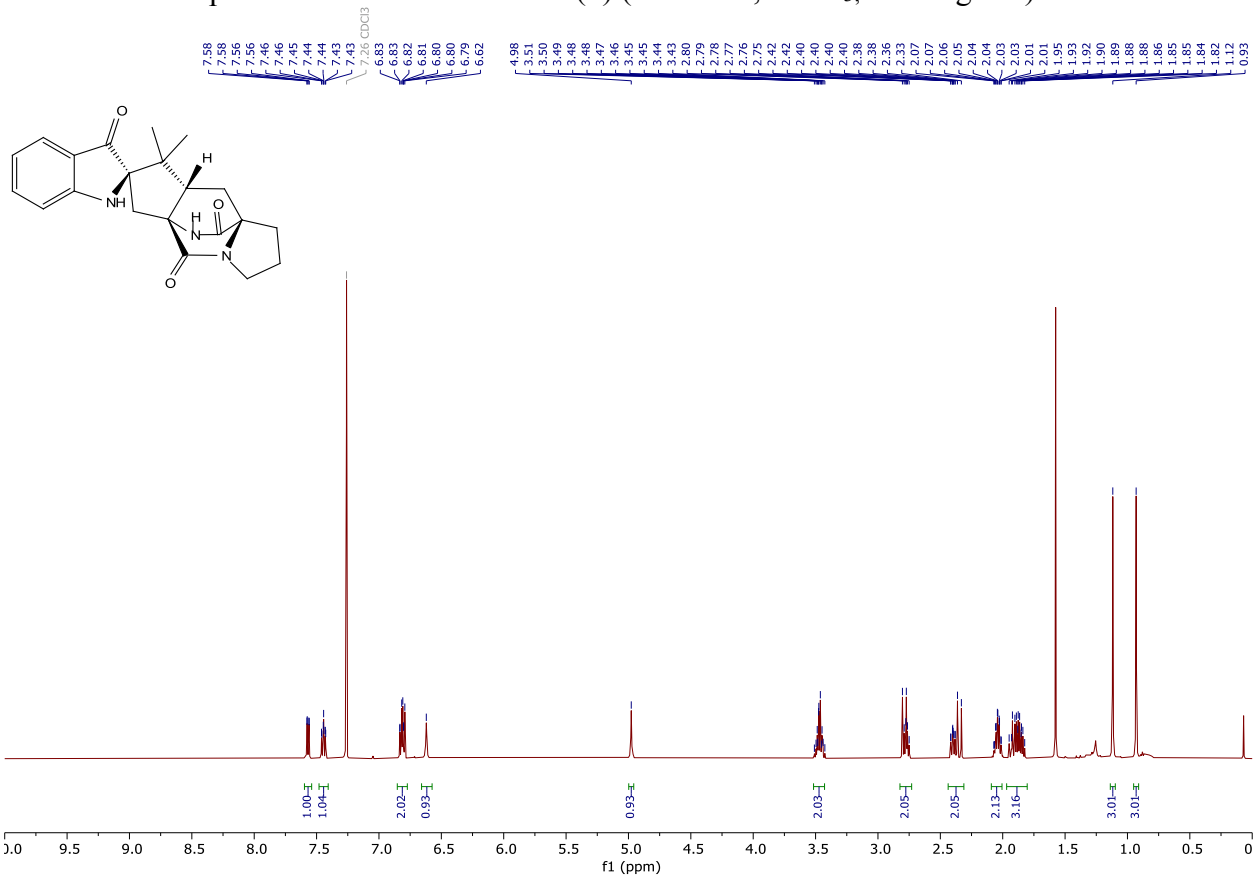
4.15 ^{1}H NMR Spectrum of Compound **20** (500 MHz, $CDCl_3$)



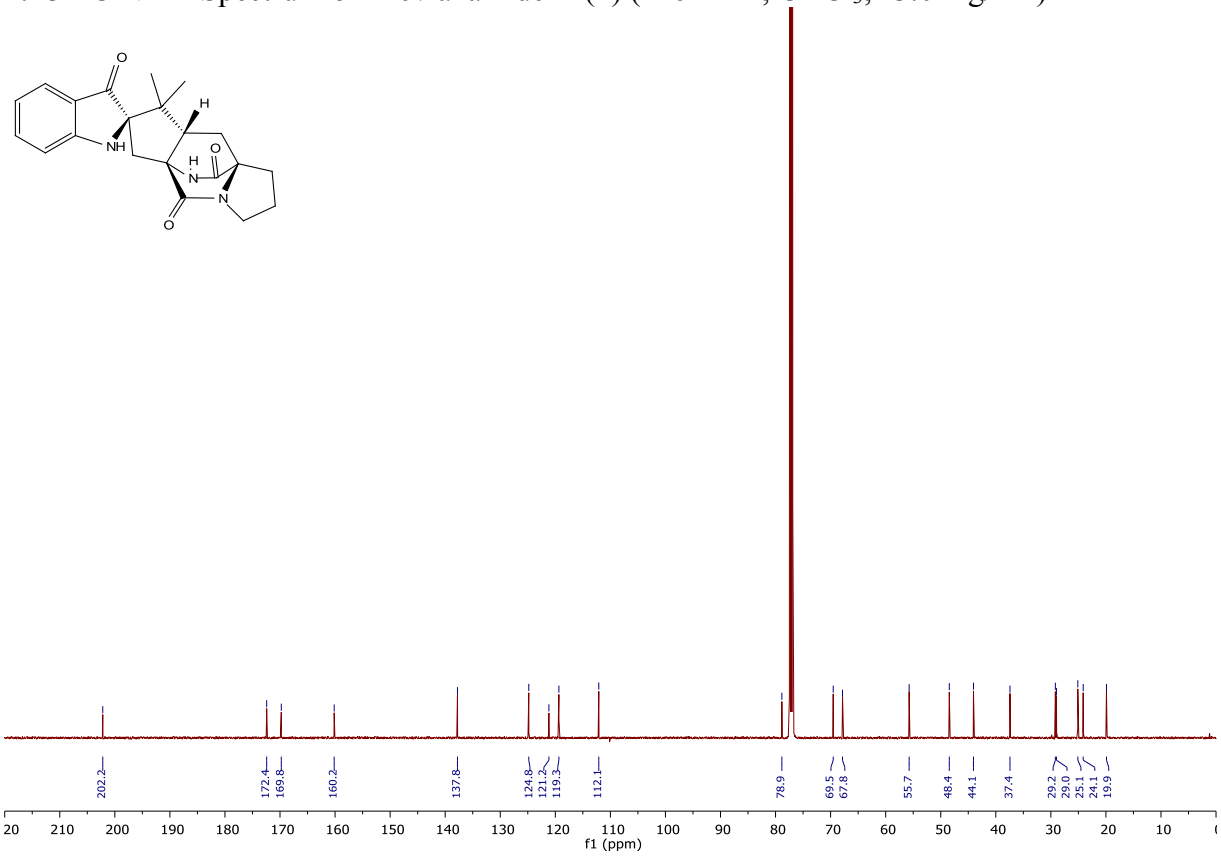
4.16 ^{13}C NMR Spectrum of Compound **20** (126 MHz, $CDCl_3$)



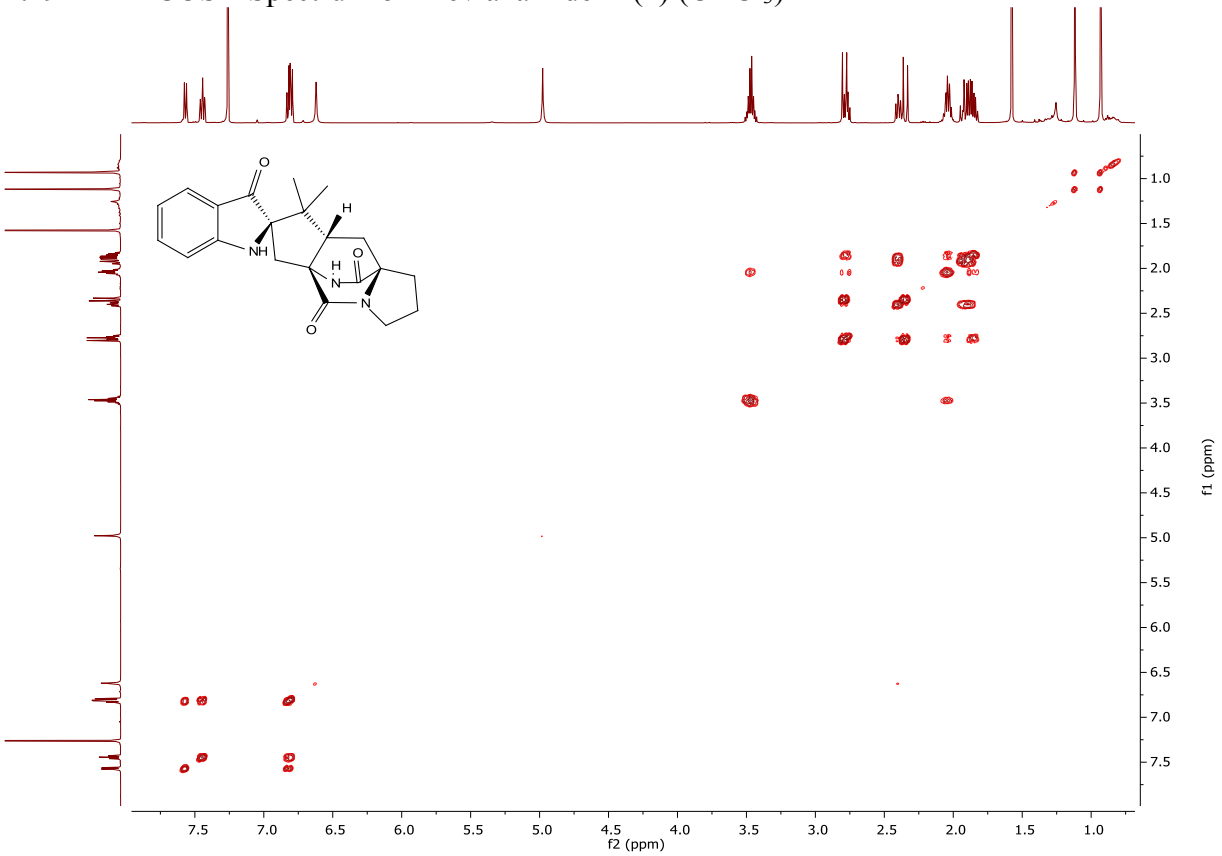
4.17 ^{1}H NMR Spectrum of Brevianamide A (**1**) (500 MHz, $CDCl_3$, ~3.0 mg/mL)



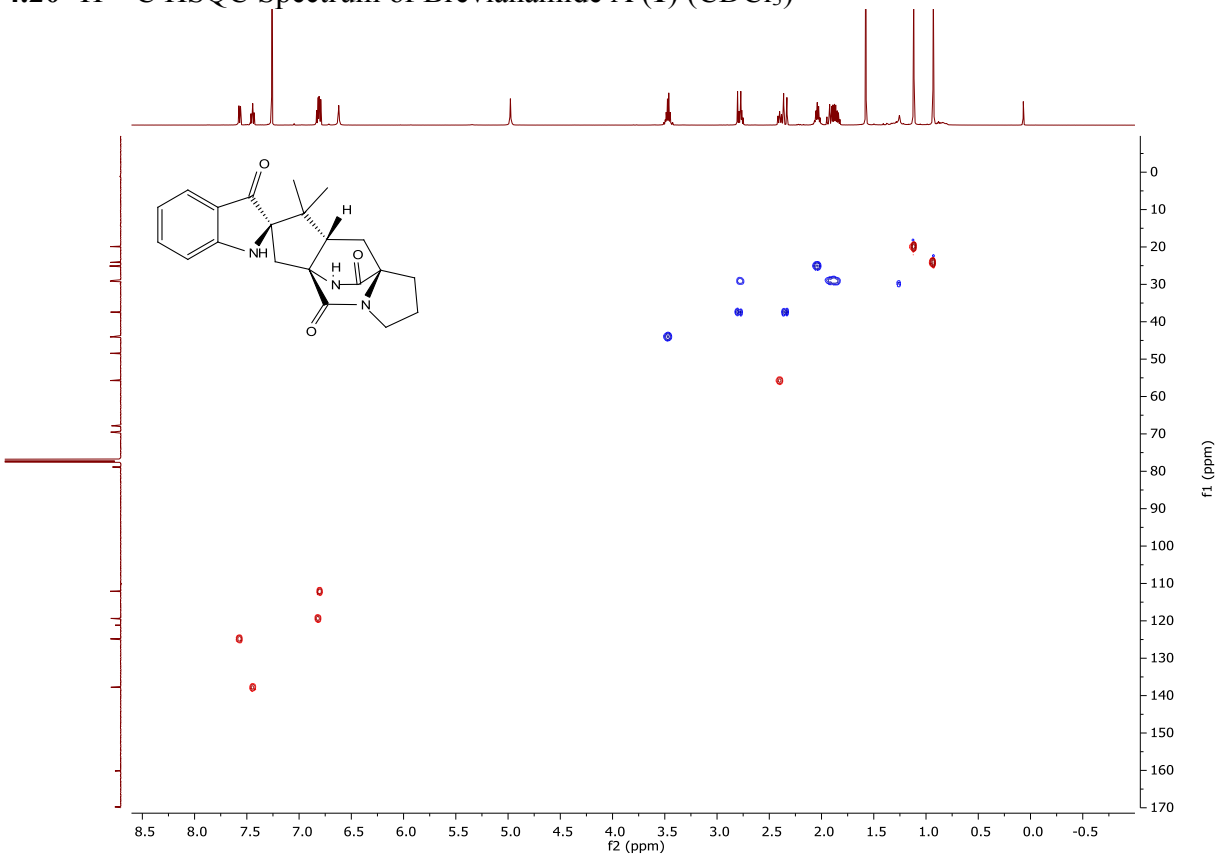
4.18 ^{13}C NMR Spectrum of Brevianamide A (**1**) (126 MHz, $CDCl_3$, ~3.0 mg/mL)



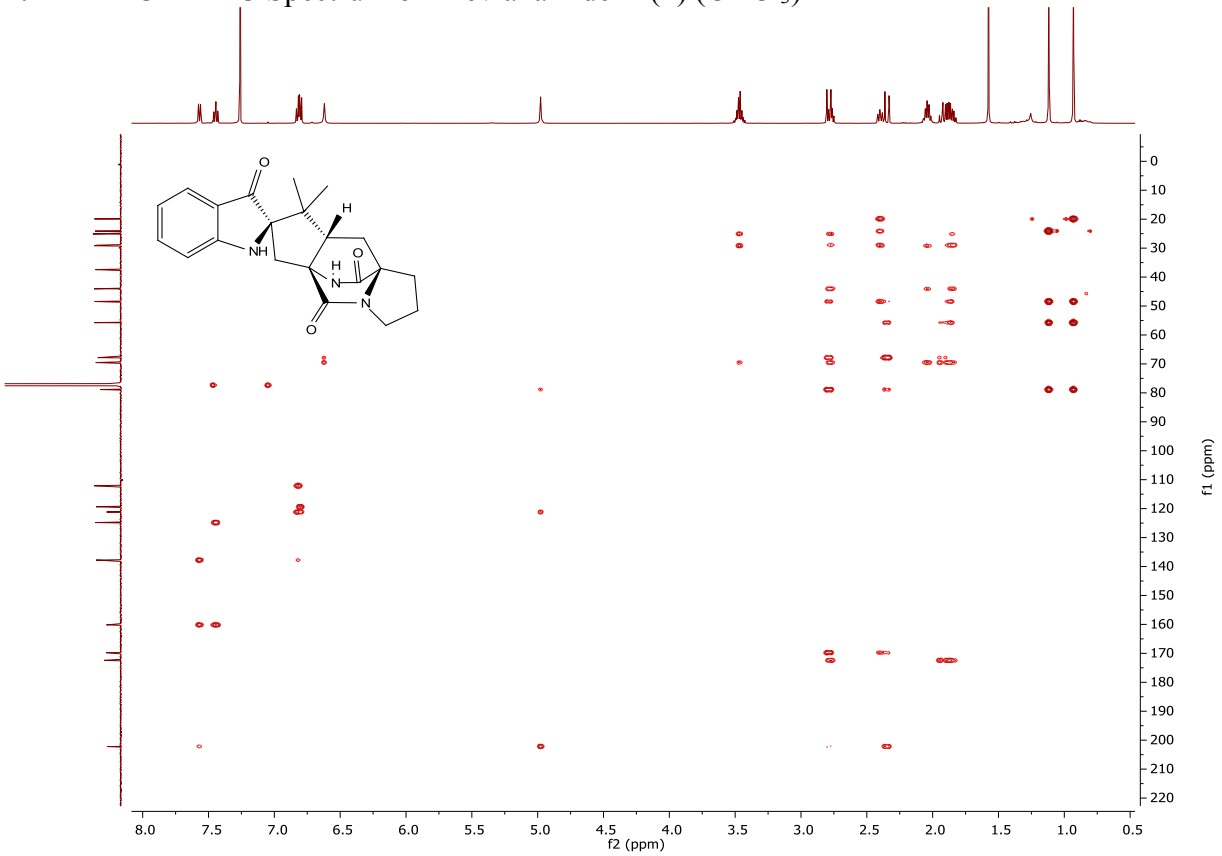
4.19 ^{1}H-^{1}H COSY Spectrum of Brevianamide A (**1**) ($CDCl_3$)



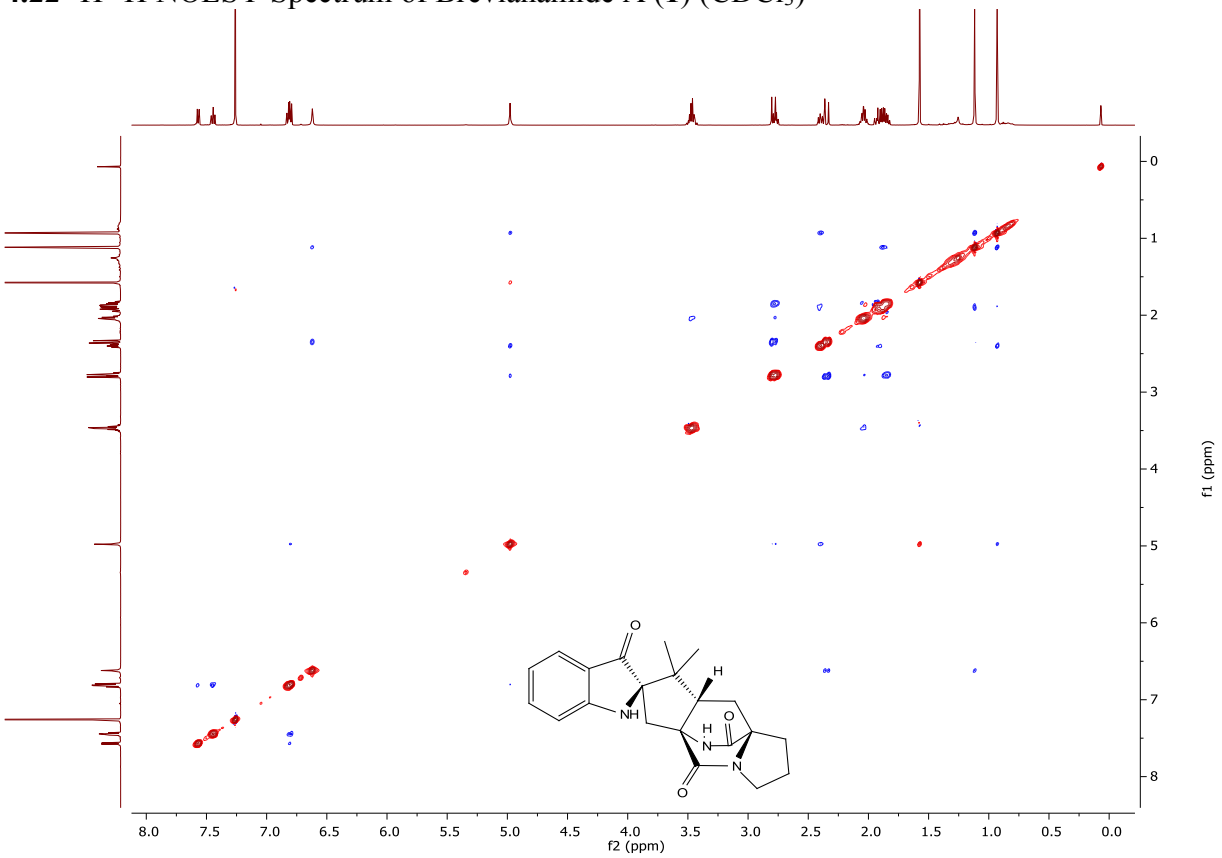
4.20 ^{1}H-^{13}C HSQC Spectrum of Brevianamide A (**1**) ($CDCl_3$)



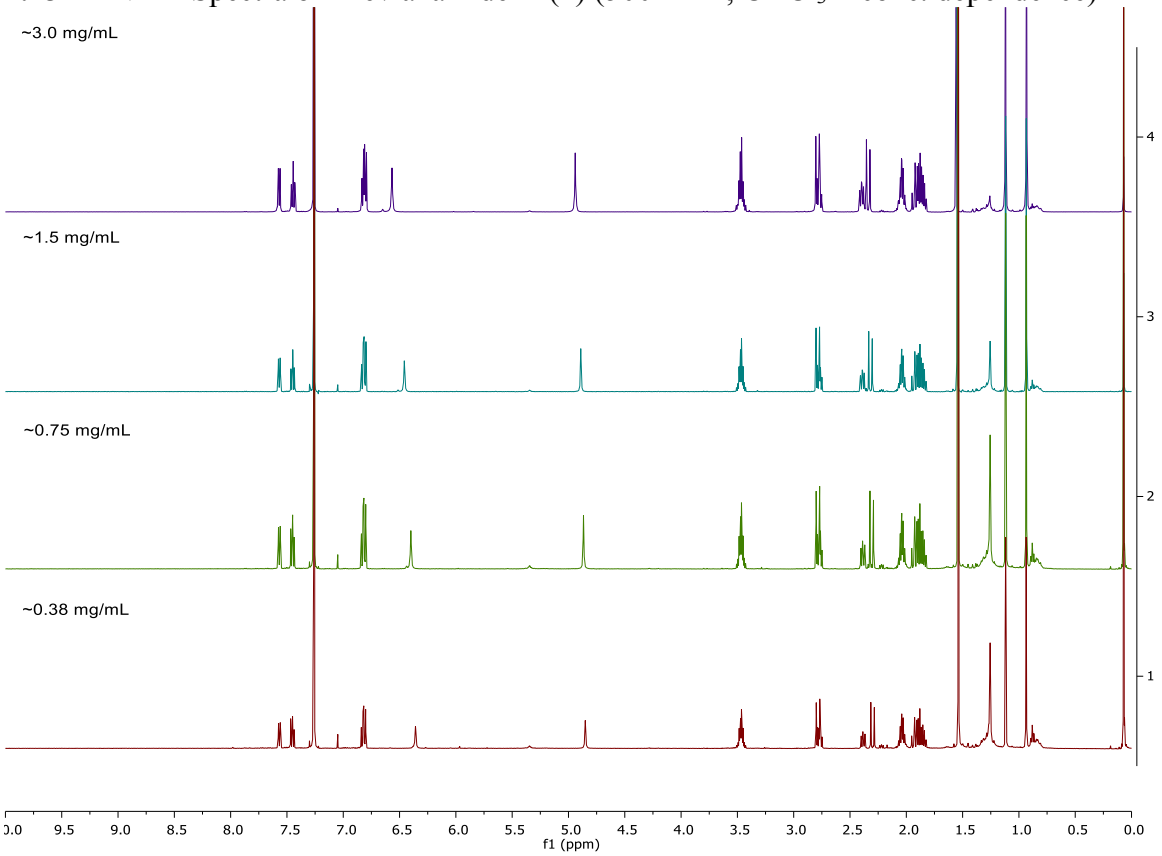
4.21 ^{1}H-^{13}C HMBC Spectrum of Brevianamide A (**1**) ($CDCl_3$)

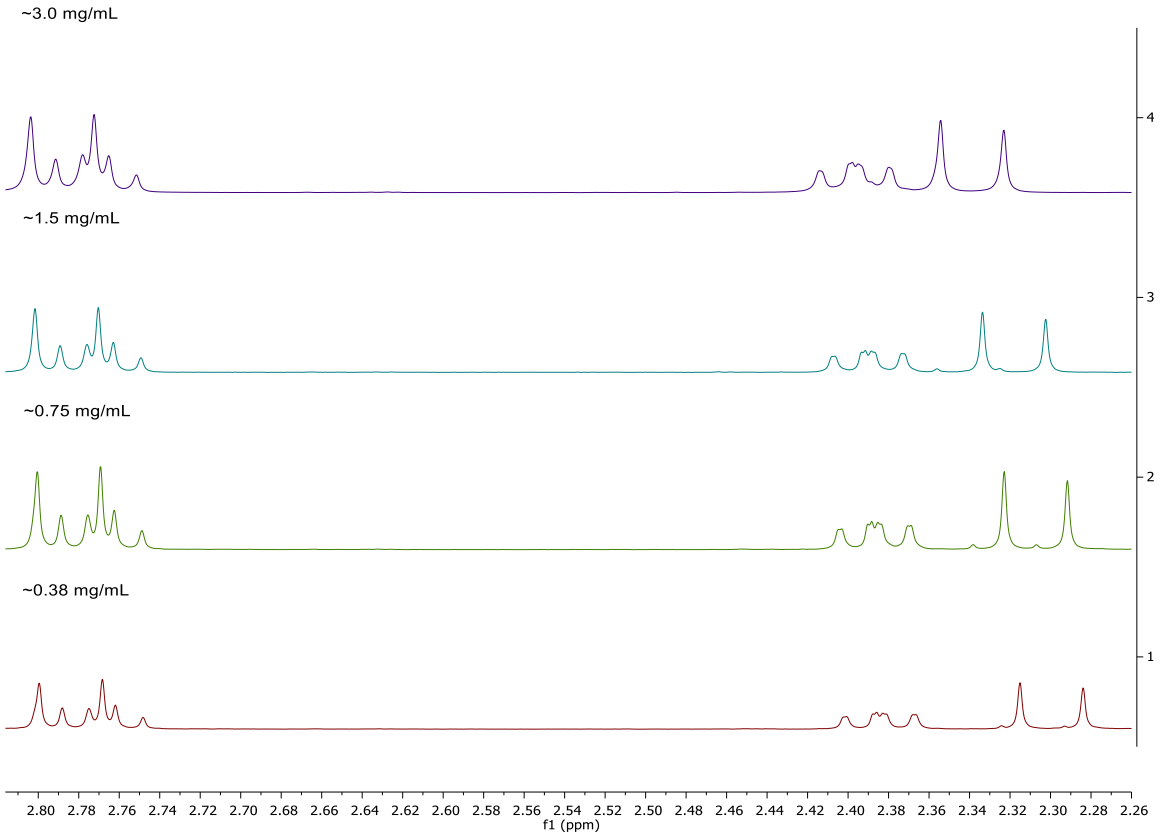


4.22 ^{1}H-^{1}H NOESY Spectrum of Brevianamide A (**1**) ($CDCl_3$)

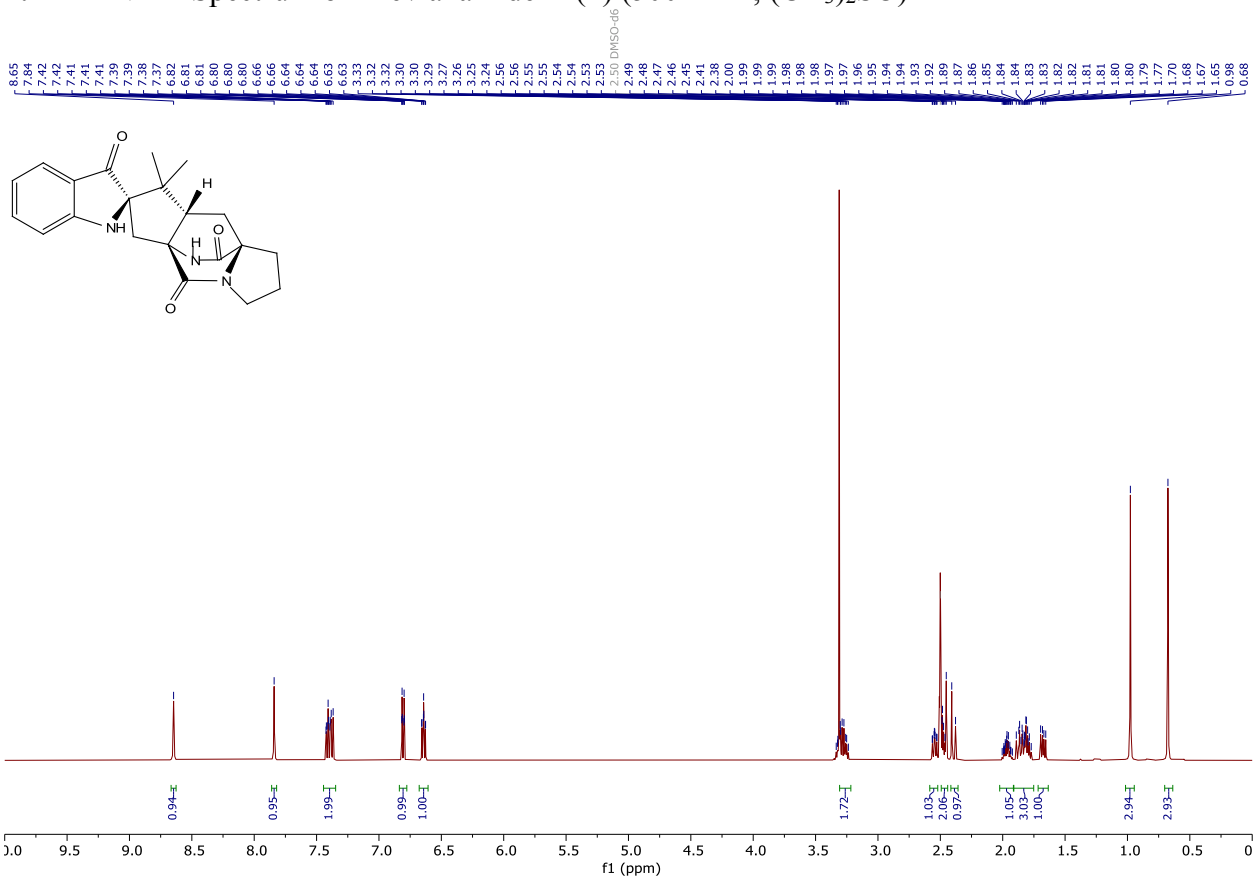


4.23 ^{1}H NMR Spectra of Brevianamide A (**1**) (500 MHz, $CDCl_3$ – conc. dependence)

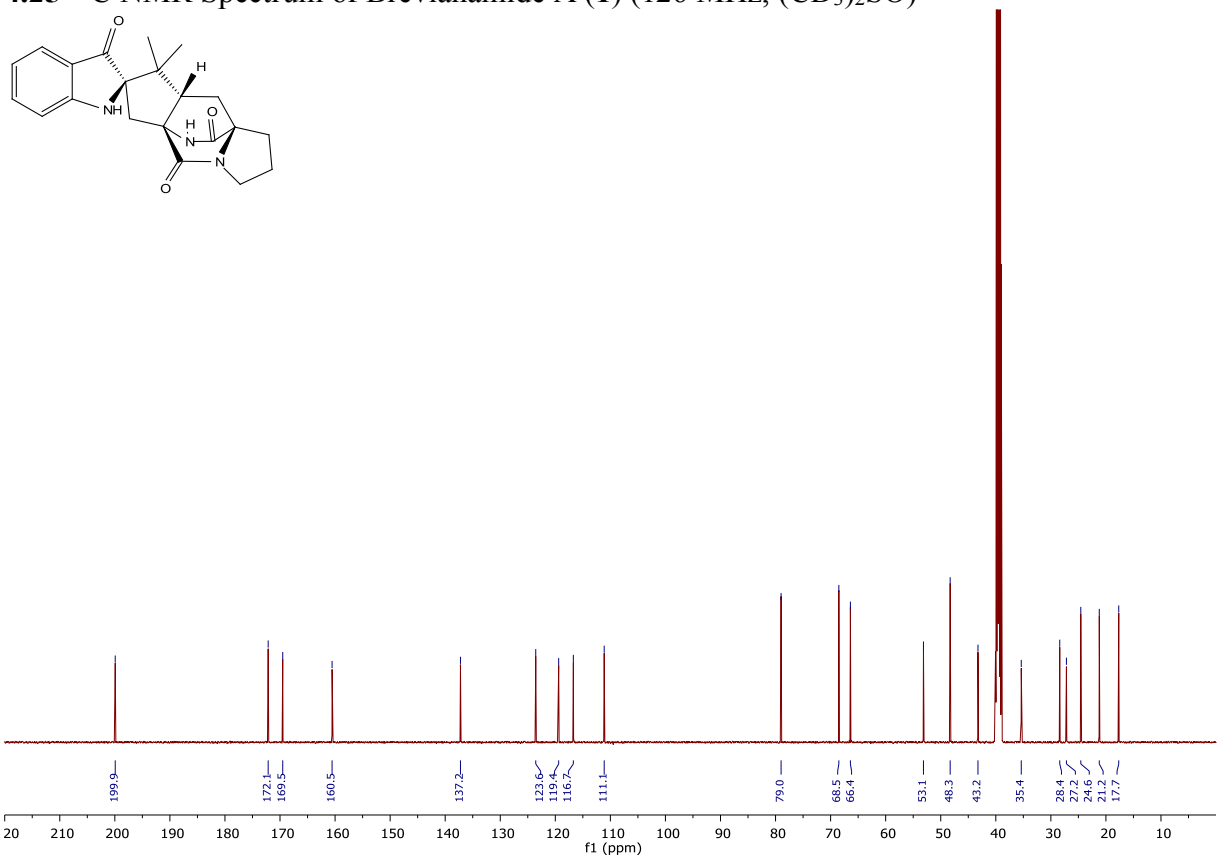




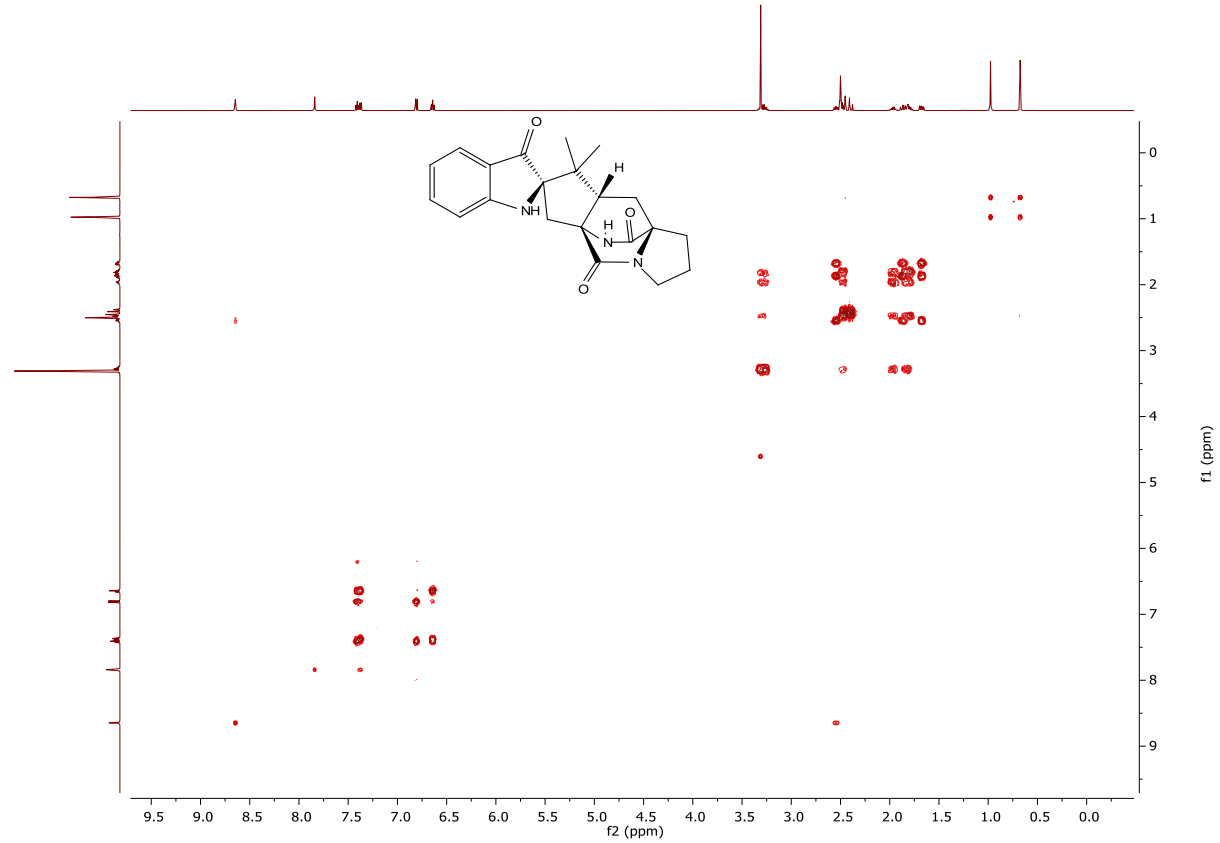
4.24 ^{1}H NMR Spectrum of Brevianamide A (**1**) (500 MHz, $(CD_3)_2SO$)



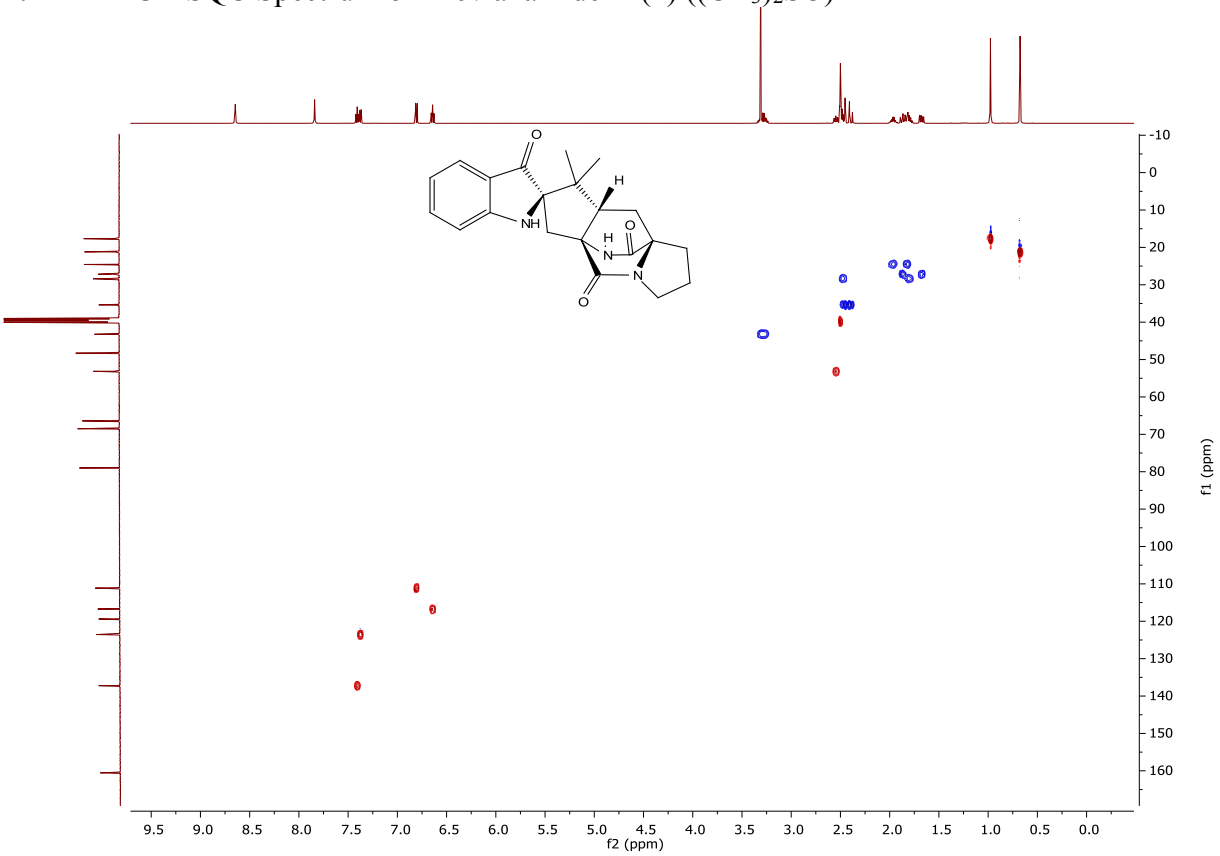
4.25 ^{13}C NMR Spectrum of Brevianamide A (**1**) (126 MHz, $(CD_3)_2SO$)



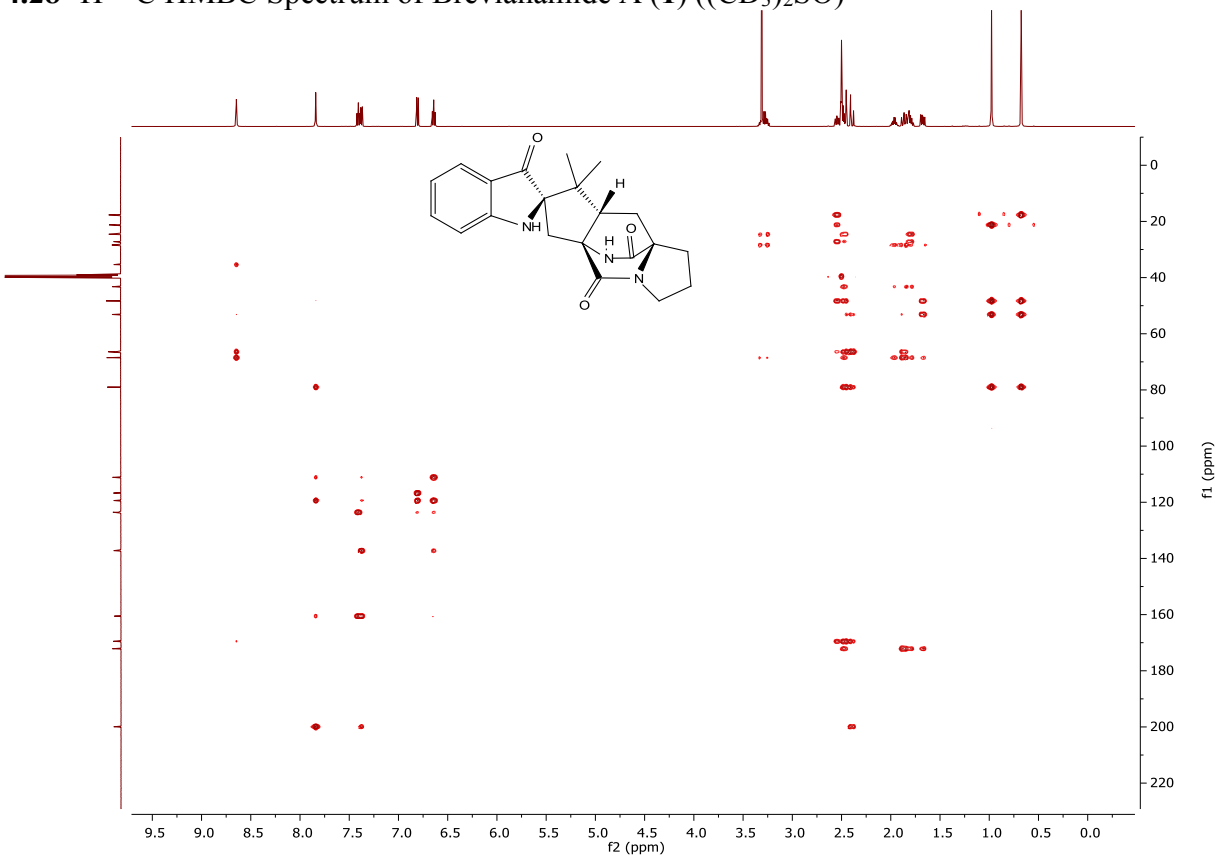
4.26 ^{1}H-^{1}H COSY Spectrum of Brevianamide A (**1**) ($(CD_3)_2SO$)



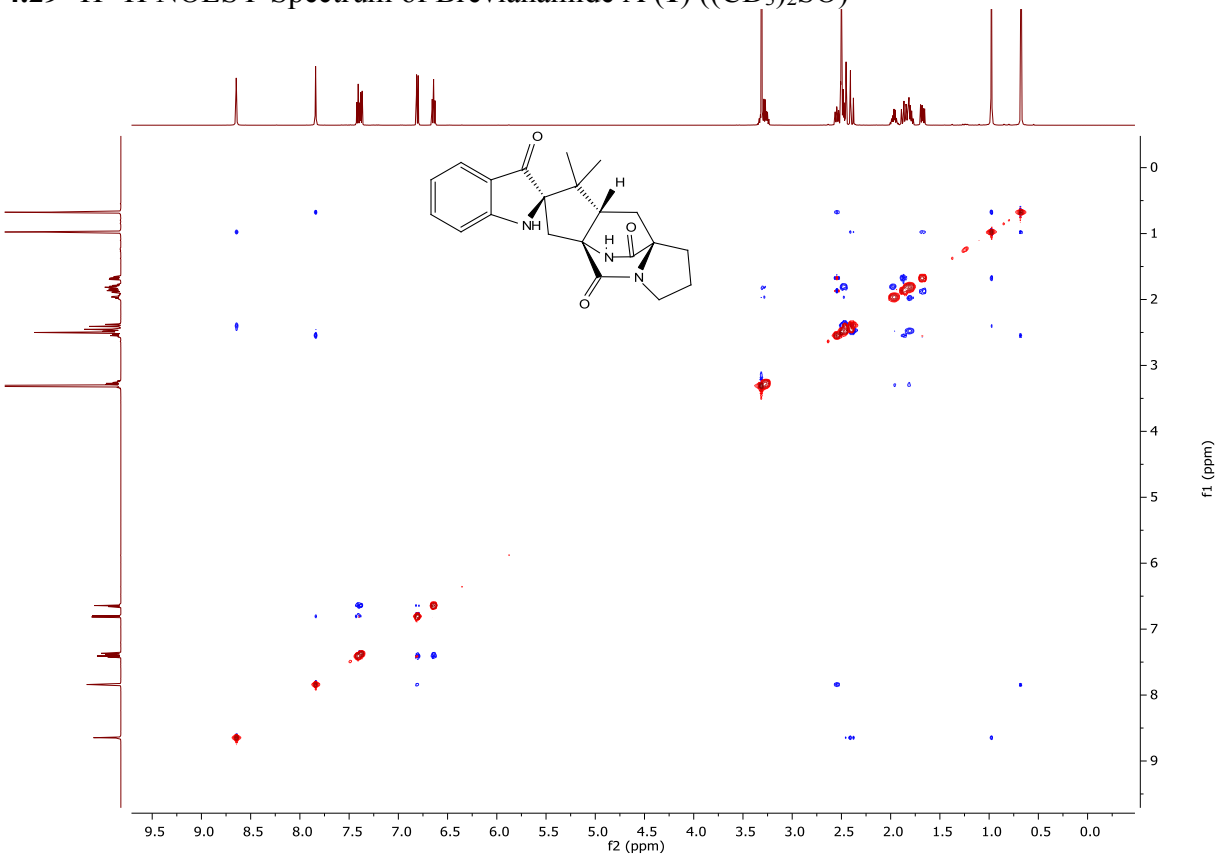
4.27 ^{1}H-^{13}C HSQC Spectrum of Brevianamide A (**1**) ($(CD_3)_2SO$)



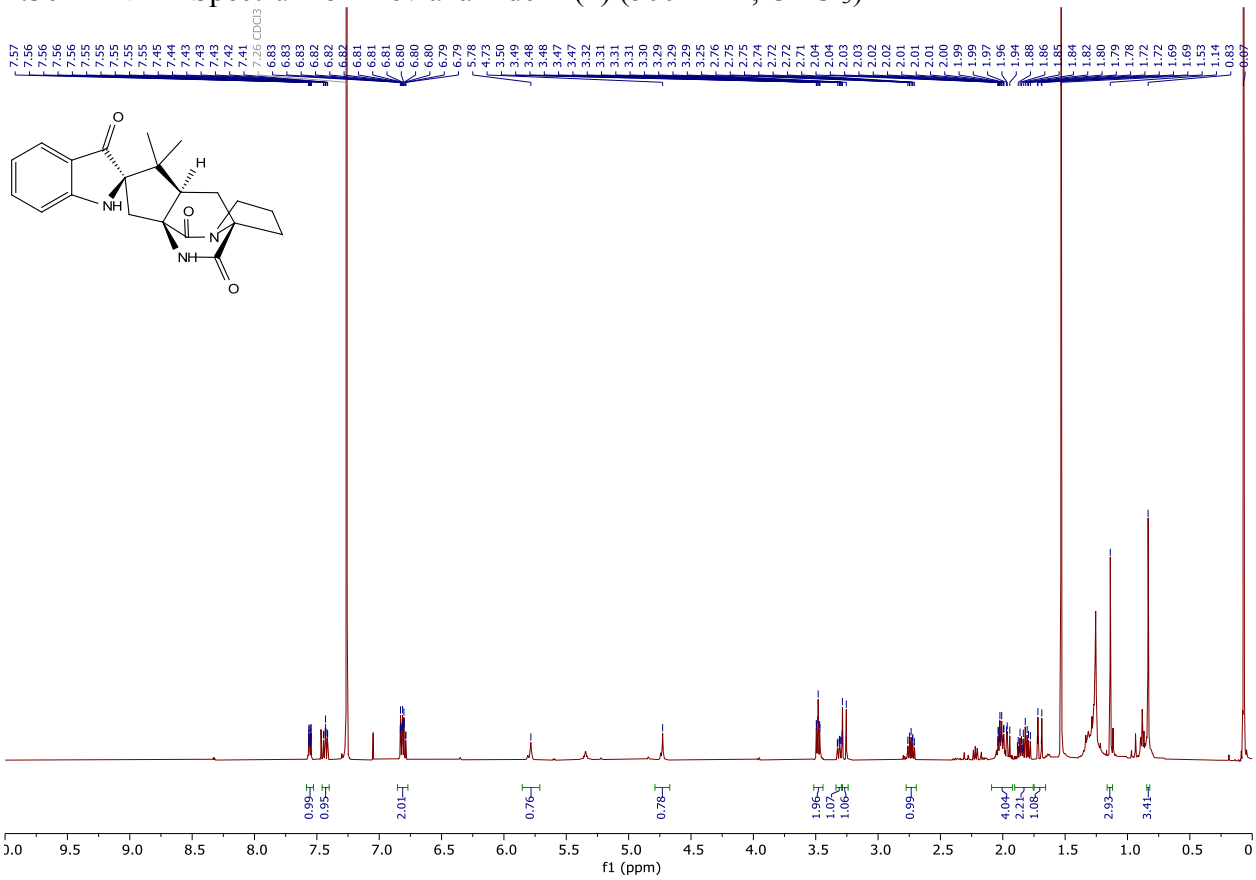
4.28 ^{1}H-^{13}C HMBC Spectrum of Brevianamide A (**1**) ($(CD_3)_2SO$)



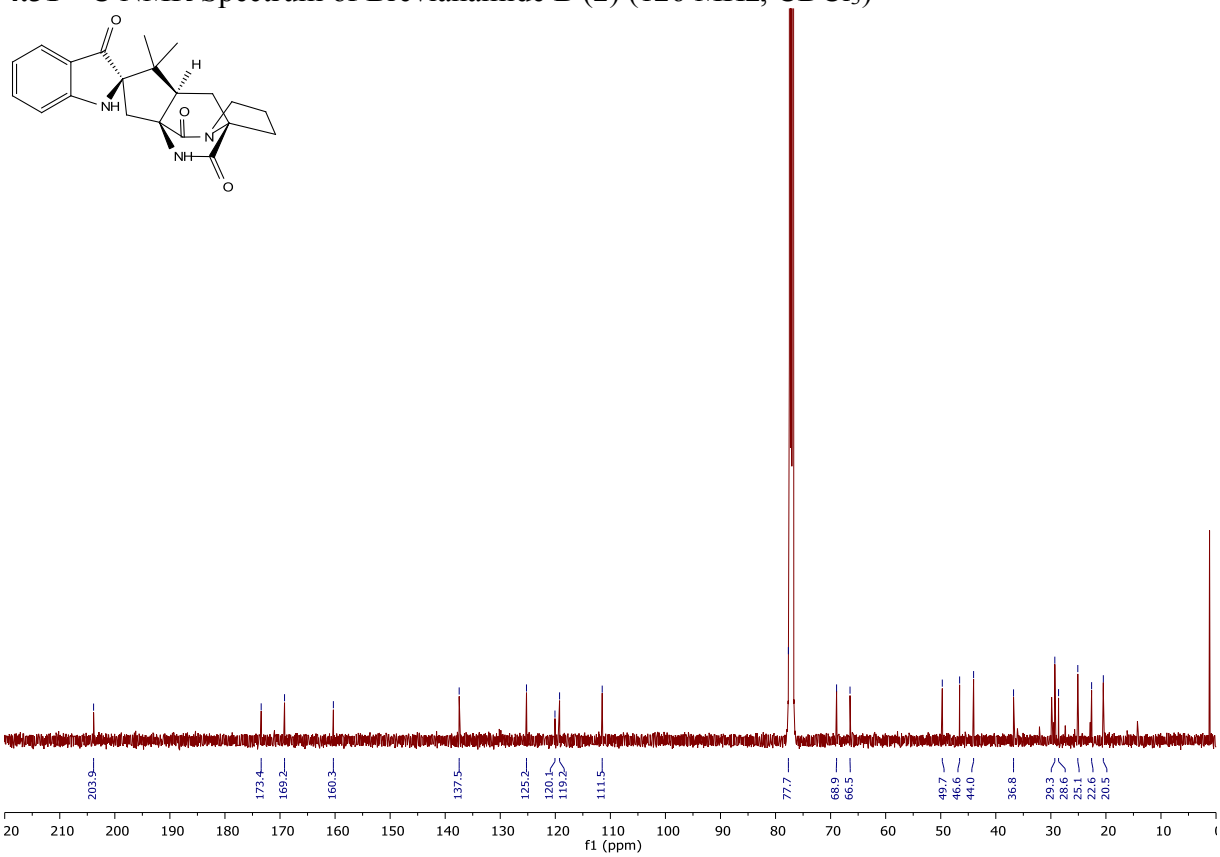
4.29 ^{1}H-^{1}H NOESY Spectrum of Brevianamide A (**1**) ($(CD_3)_2SO$)



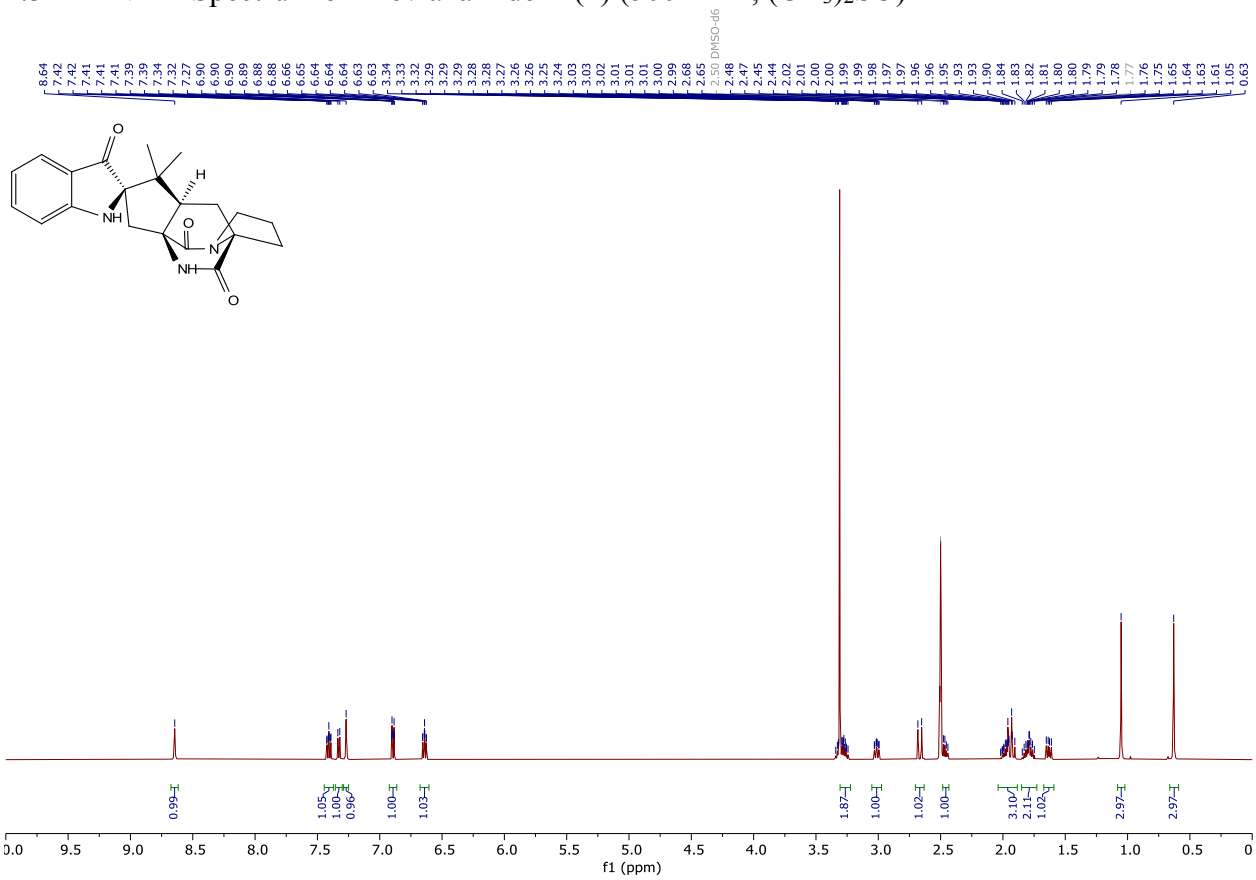
4.30 ^{1}H NMR Spectrum of Brevianamide B (**2**) (500 MHz, $CDCl_3$)



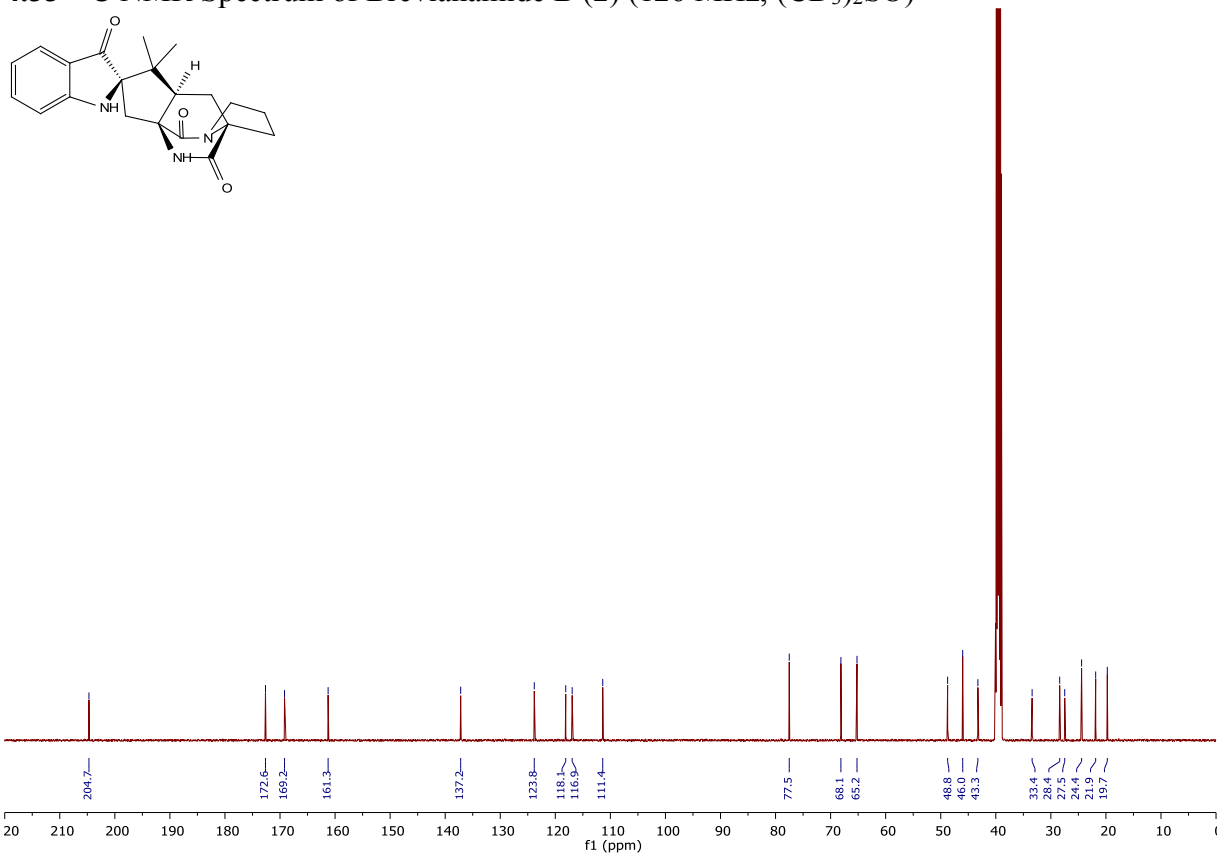
4.31 ^{13}C NMR Spectrum of Brevianamide B (**2**) (126 MHz, $CDCl_3$)



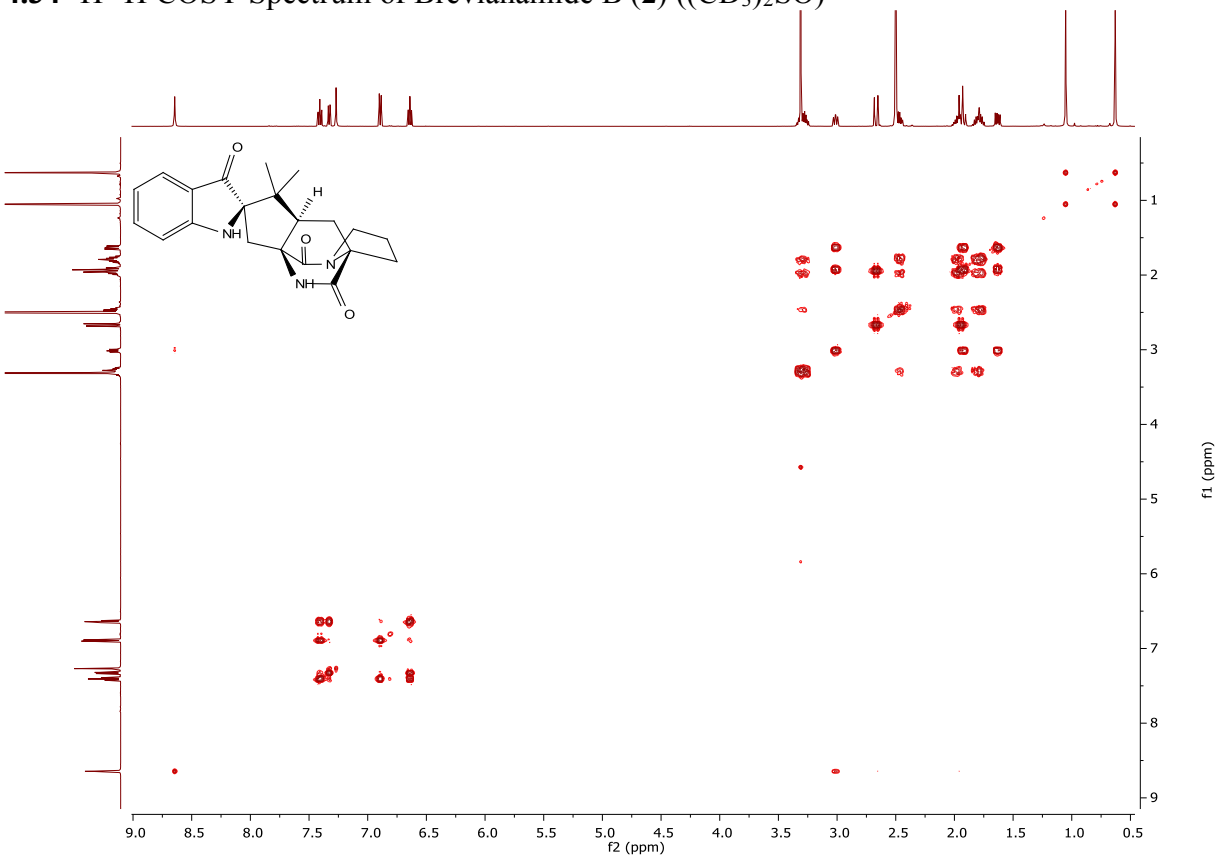
4.32 ^{1}H NMR Spectrum of Brevianamide B (**2**) (500 MHz, $(CD_3)_2SO$)



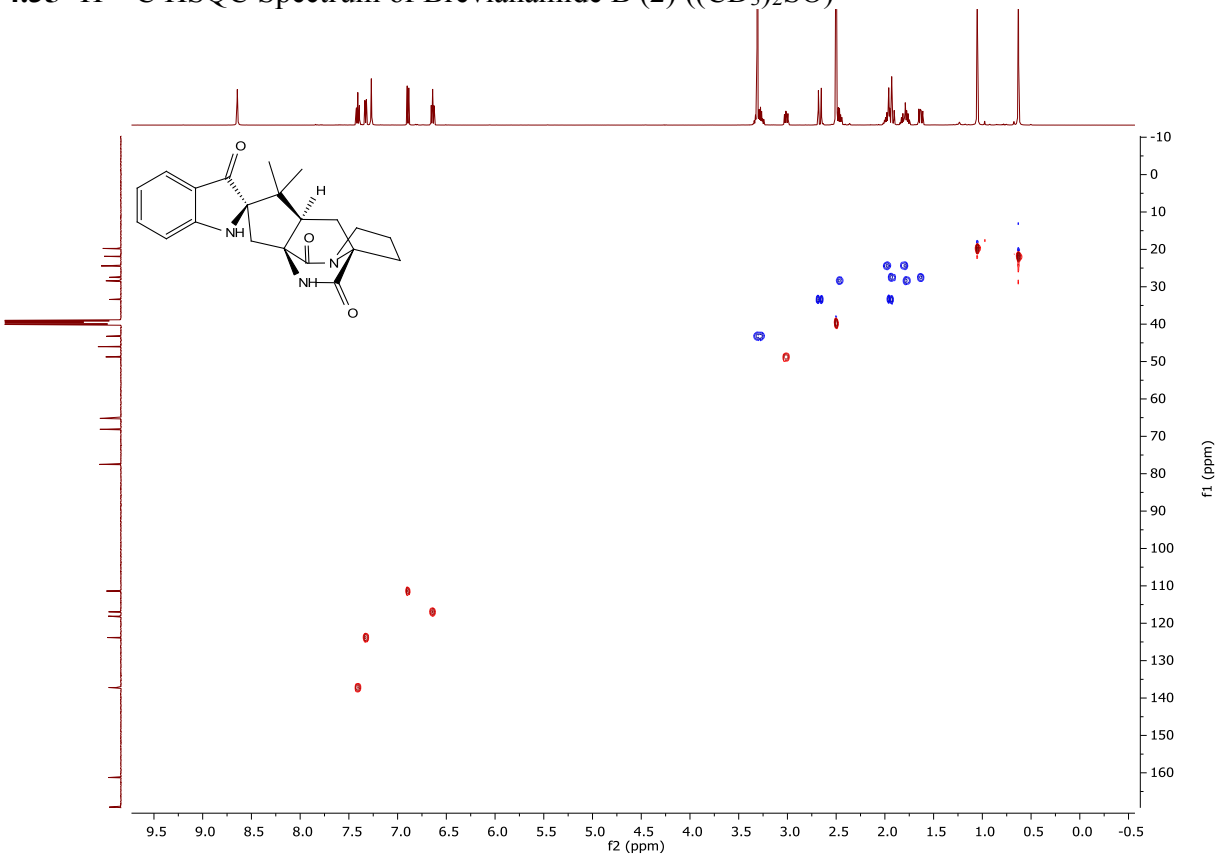
4.33 ^{13}C NMR Spectrum of Brevianamide B (**2**) (126 MHz, $(CD_3)_2SO$)



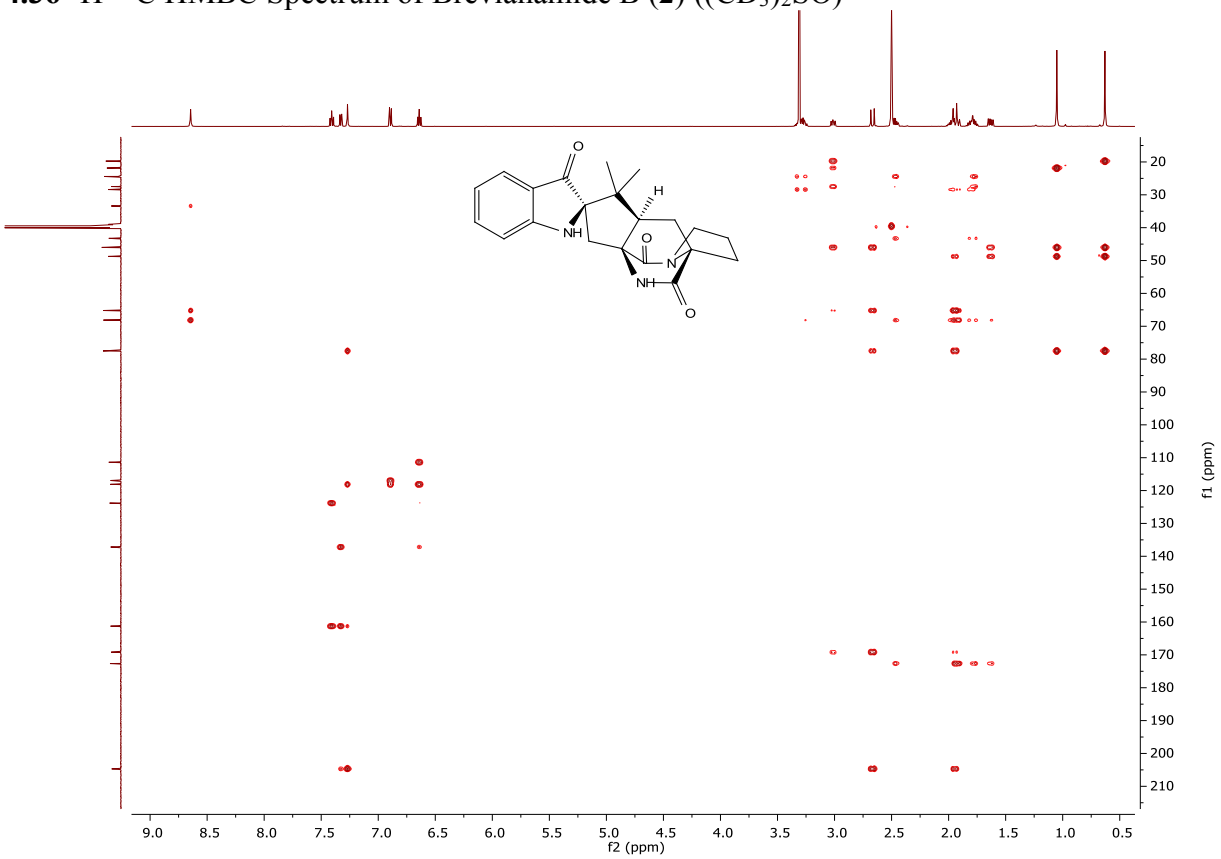
4.34 ^{1}H-^{1}H COSY Spectrum of Brevianamide B (**2**) ($(CD_3)_2SO$)



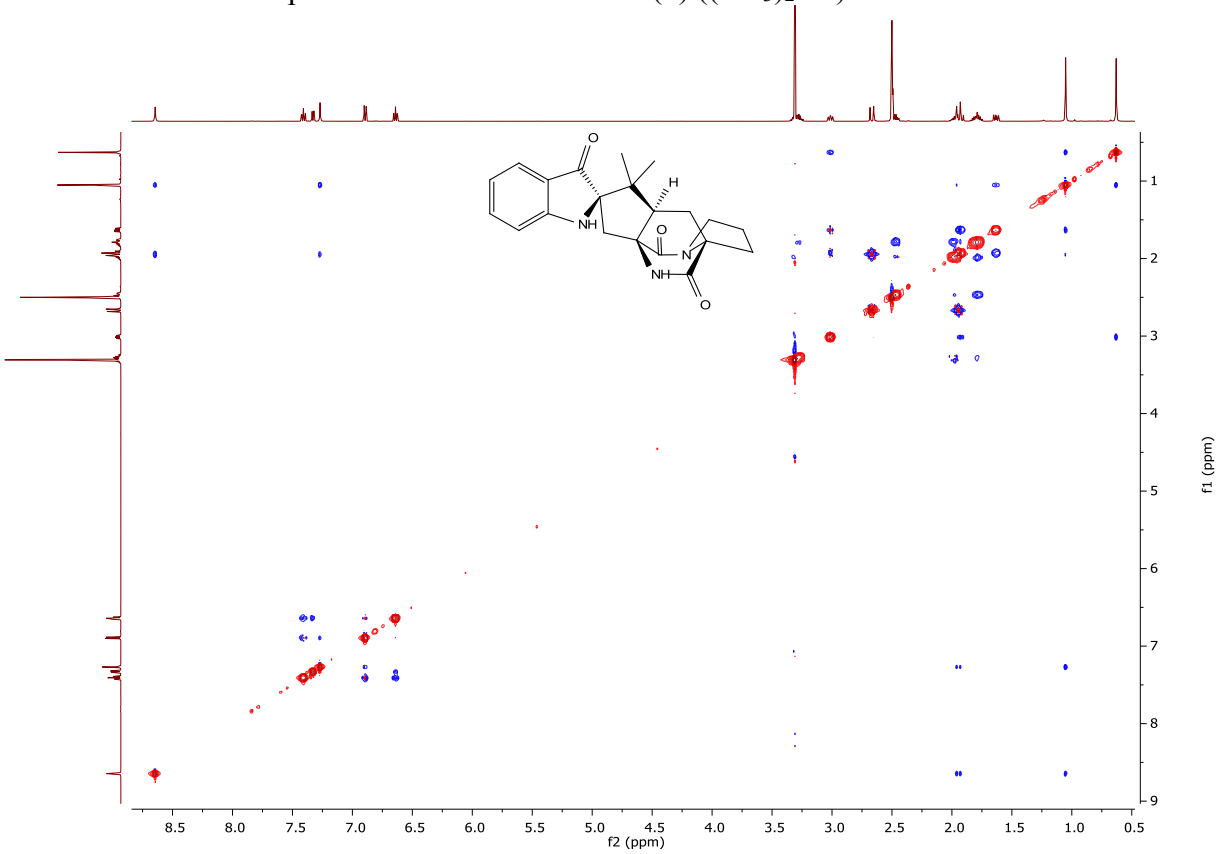
4.35 ^{1}H-^{13}C HSQC Spectrum of Brevianamide B (**2**) ($(CD_3)_2SO$)



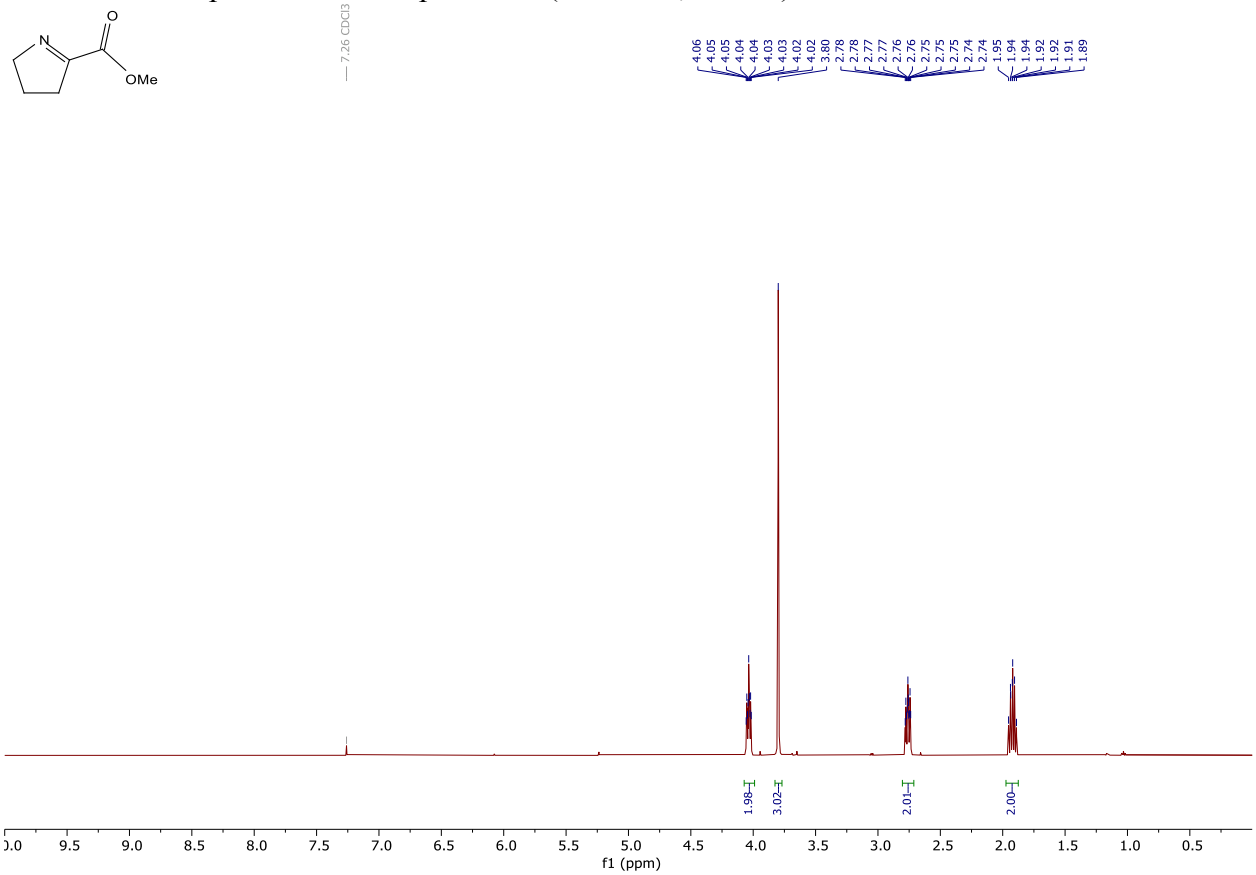
4.36 ^{1}H-^{13}C HMBC Spectrum of Brevianamide B (**2**) ($(CD_3)_2SO$)



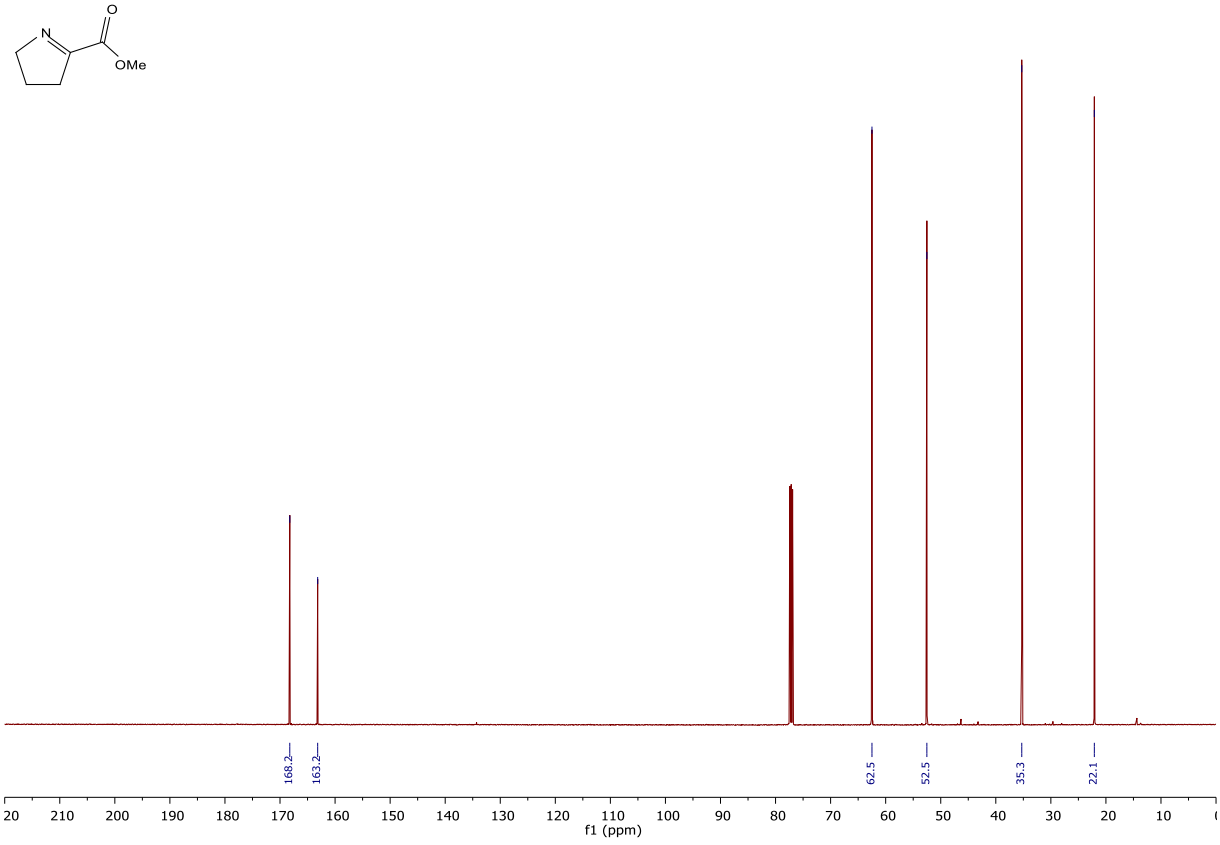
4.37 ^{1}H-^{1}H NOESY Spectrum of Brevianamide B (**2**) ($(CD_3)_2SO$)



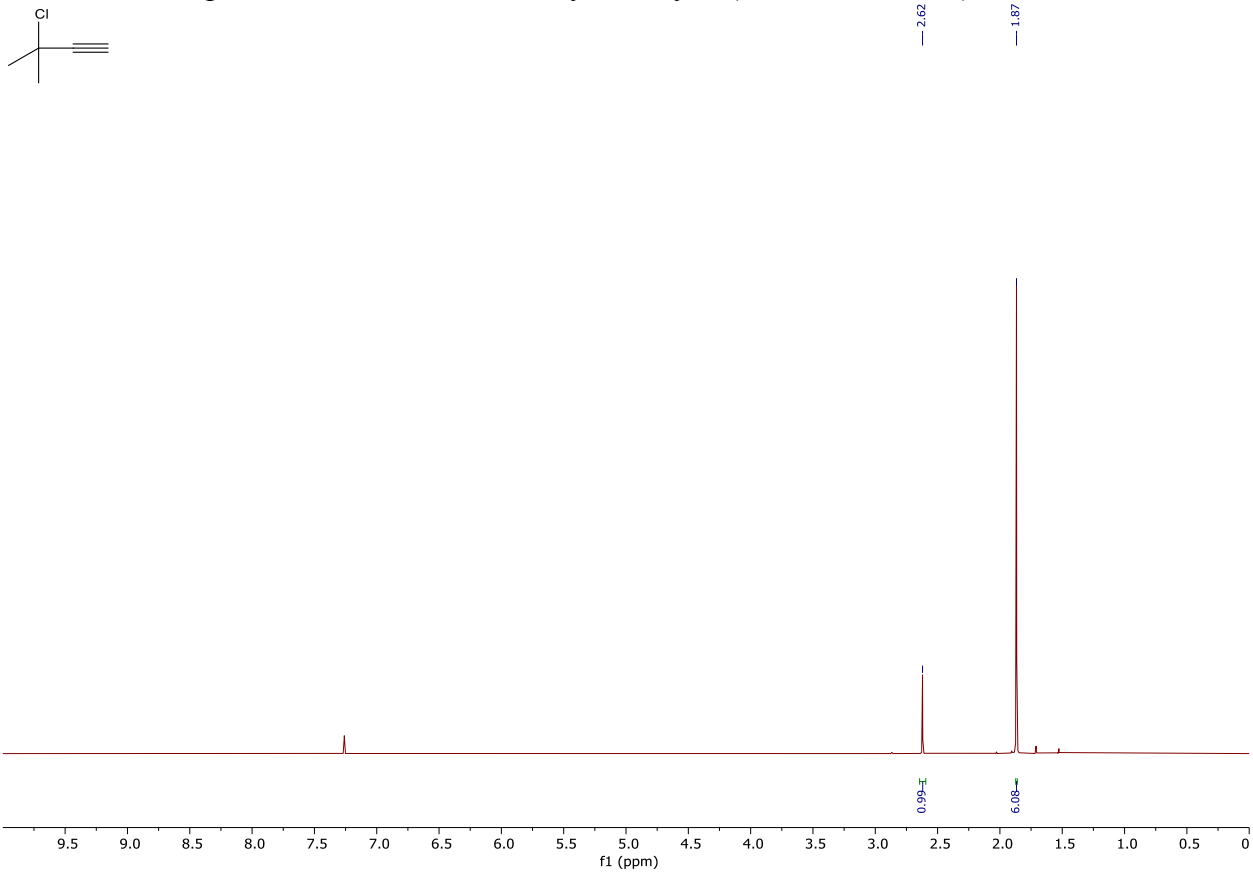
4.38 ^{1}H NMR Spectrum of Compound **18** (500 MHz, $CDCl_3$)



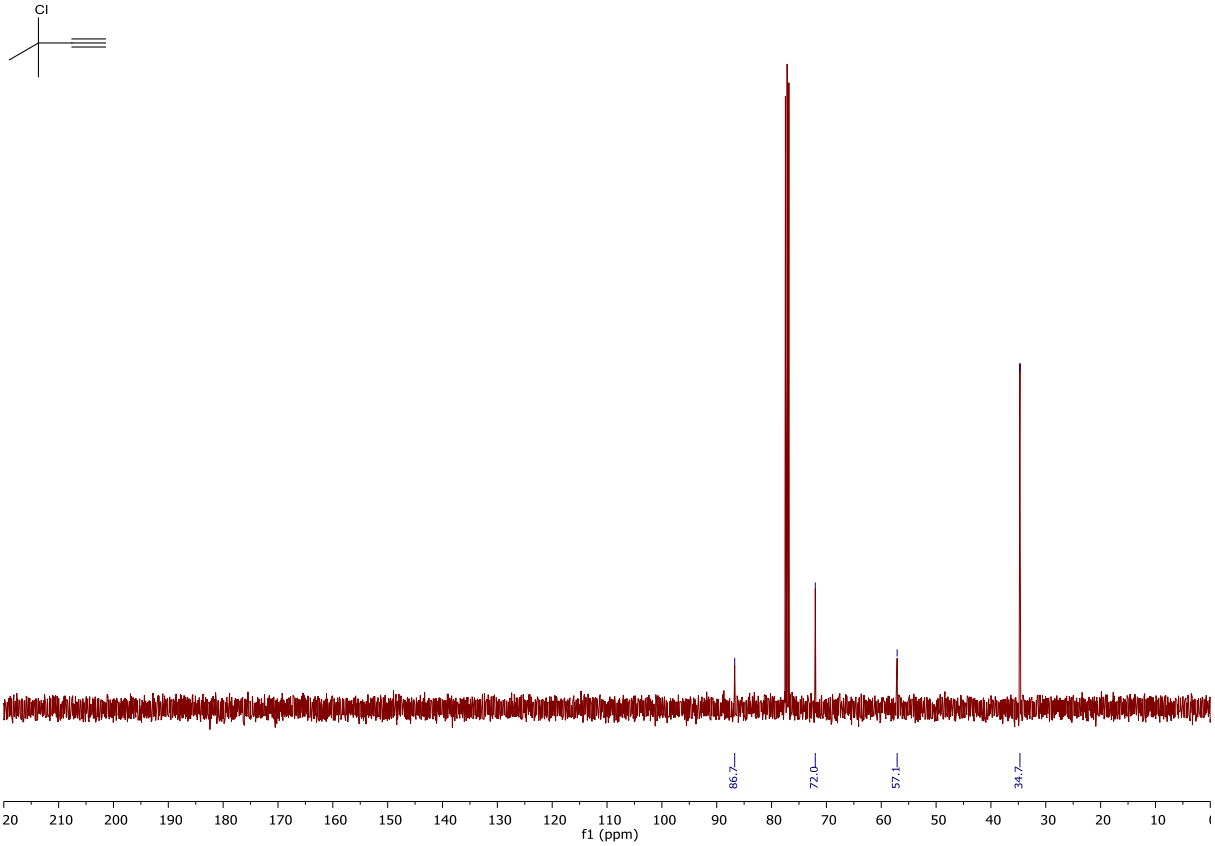
4.39 ^{13}C NMR Spectrum of Compound **18** (126 MHz, $CDCl_3$)



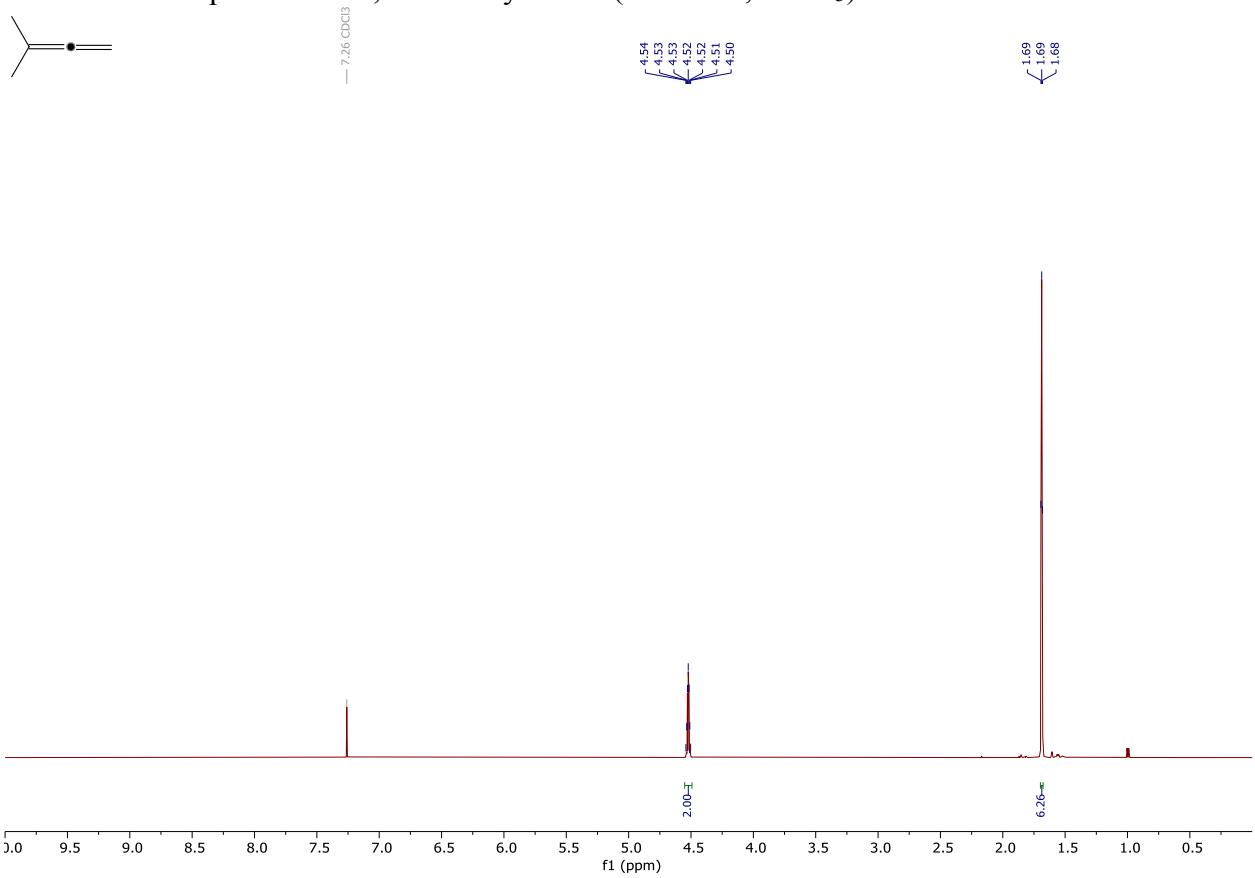
4.40 ^{1}H NMR Spectrum of 3-Chloro-3-methyl-1-butyne (500 MHz, $CDCl_3$)



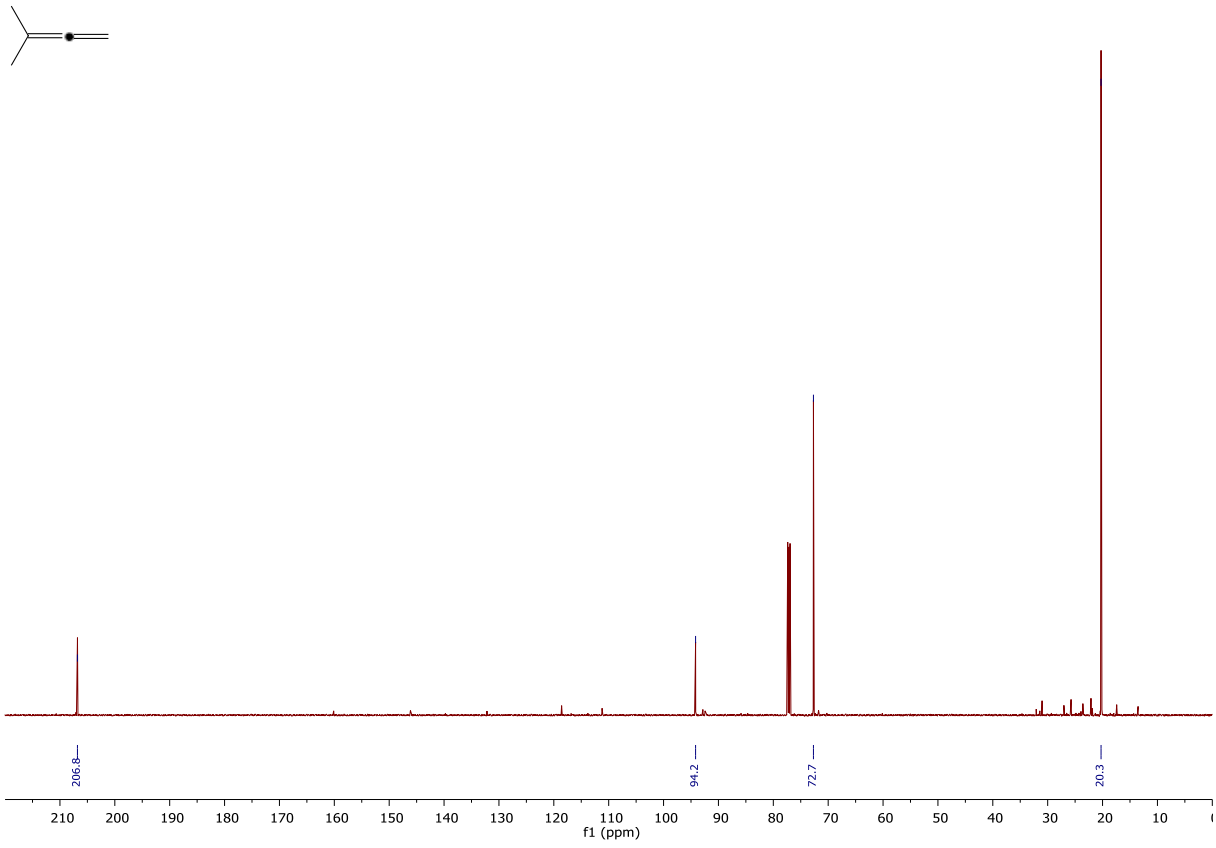
4.41 ^{13}C NMR Spectrum of 3-Chloro-3-methyl-1-butyne (101 MHz, $CDCl_3$)



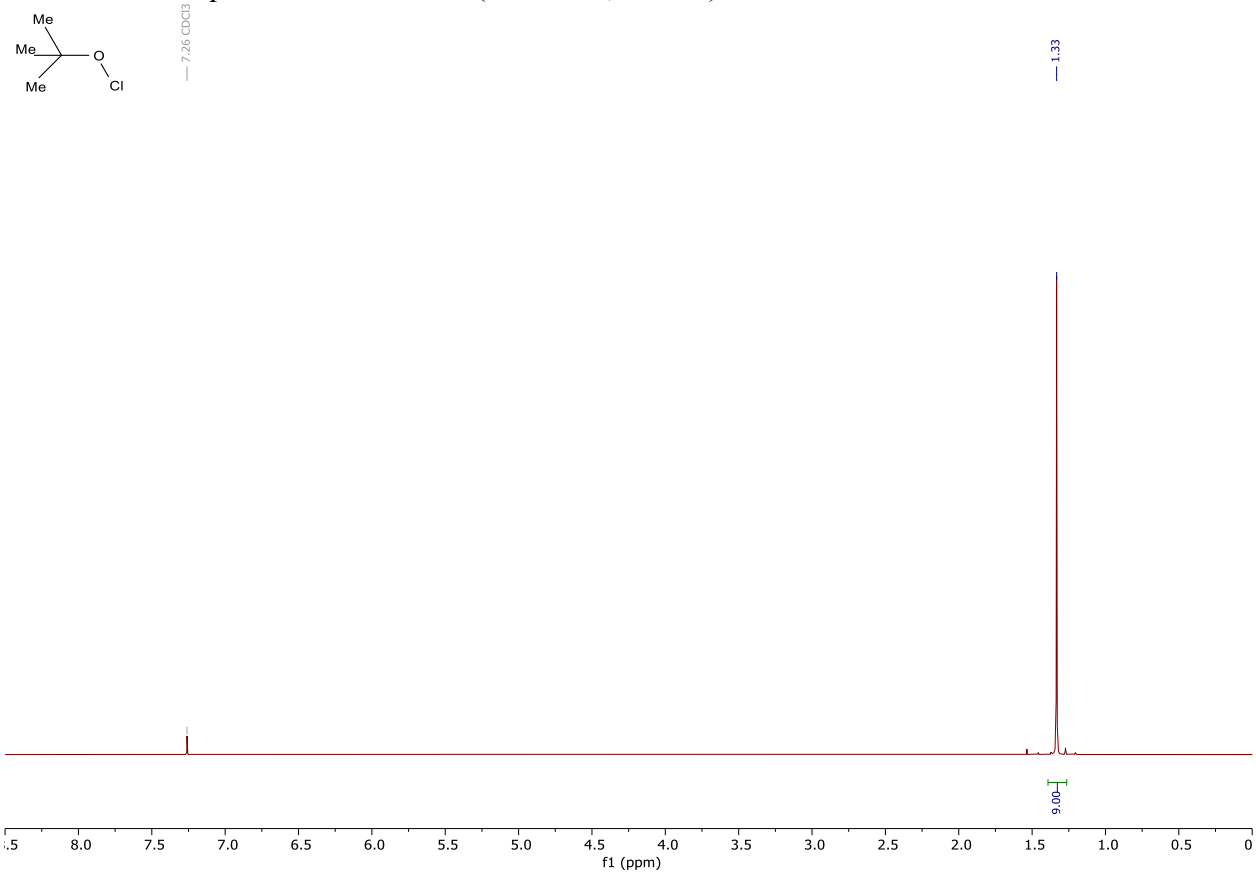
4.42 ^{1}H NMR Spectrum of 1,1-Dimethylallene (500 MHz, $CDCl_3$)



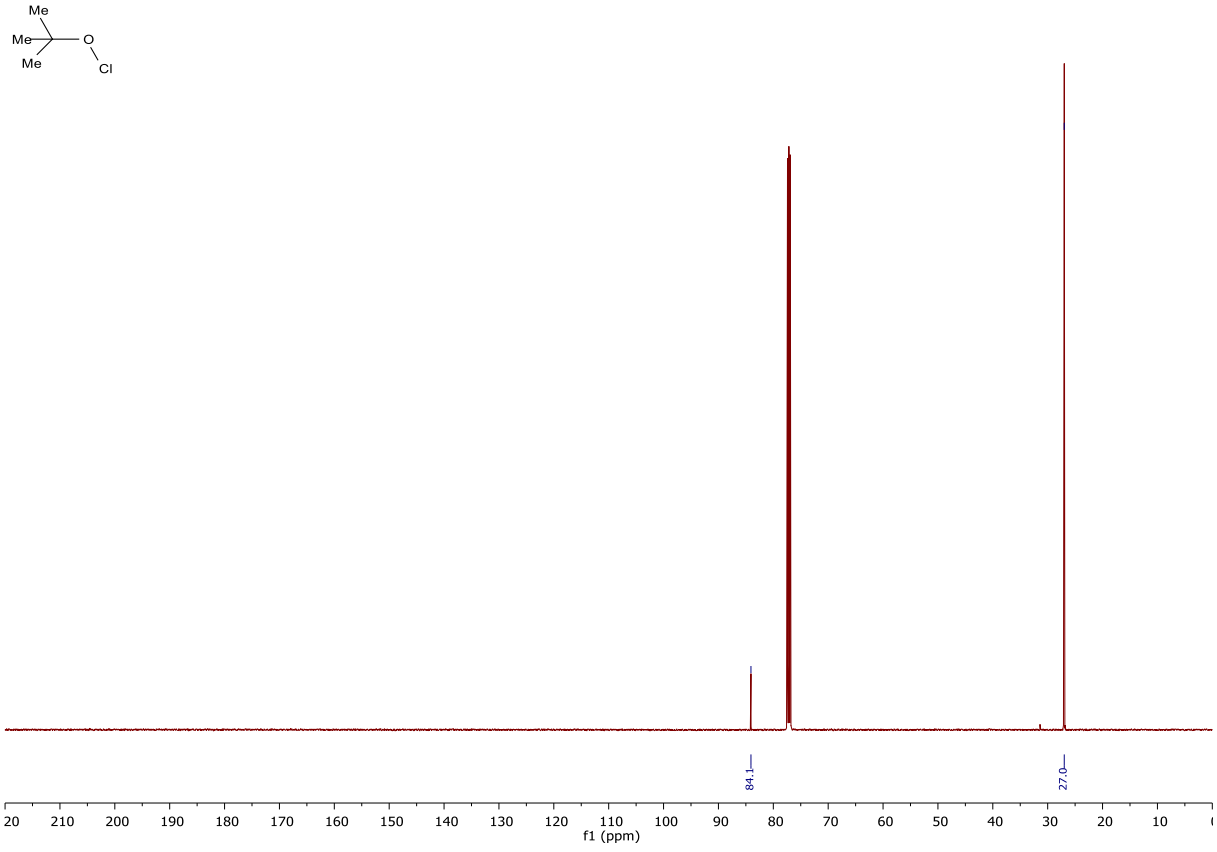
4.43 ^{13}C NMR Spectrum of 1,1-Dimethylallene (151 MHz, $CDCl_3$)



4.44 ^{1}H NMR Spectrum of *t*-BuOCl (500 MHz, $CDCl_3$)



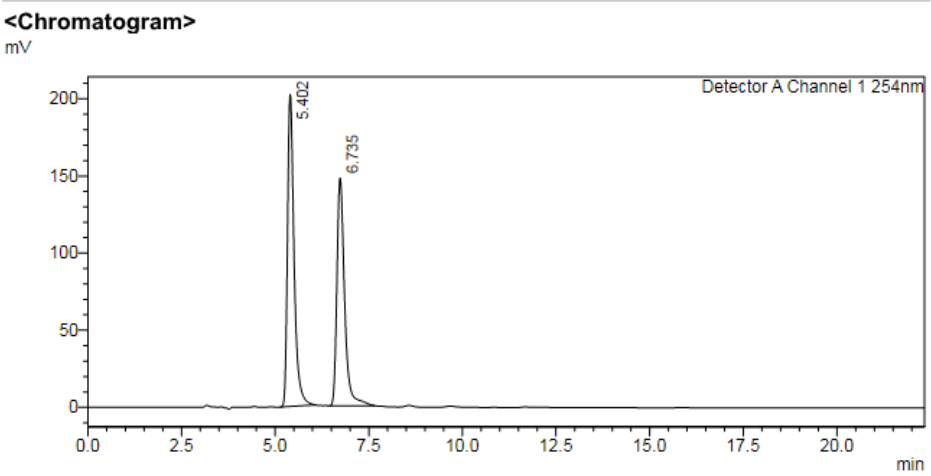
4.45 ^{13}C NMR Spectrum of *t*-BuOCl (126 MHz, $CDCl_3$)



5 Chiral HPLC Chromatograms

5.1 Chiral HPLC Chromatograms of Brevianamide A

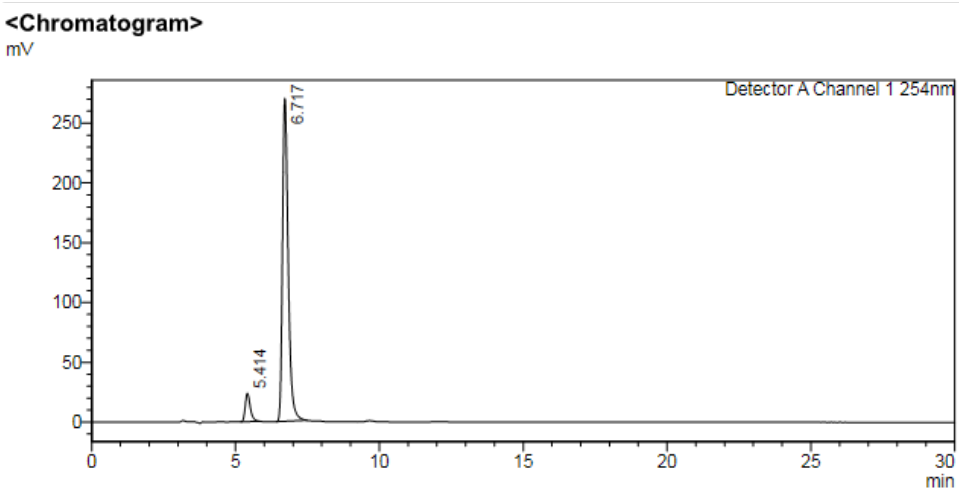
(+)/(–)-brevianamide A (**1**)
(Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{R1} = 5.40 min, t_{R2} = 6.74 min.



<Peak Table>
Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.402	2456350	202003	53.925			
2	6.735	2098773	147492	46.075			
Total		4555123	349495				

(+)-brevianamide A (**1**) **e.r.** 93:7
(Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{Rminor} = 5.41 min, t_{Rmajor} = 6.72 min.

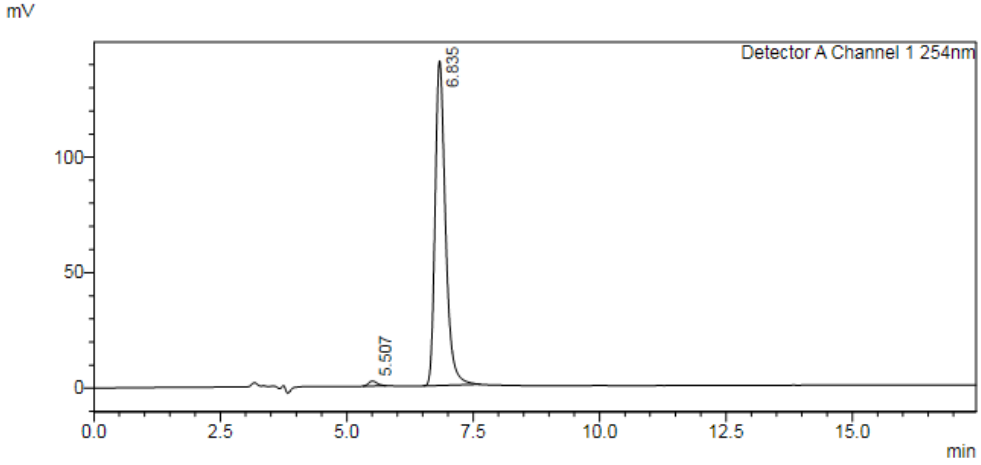


<Peak Table>
Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.414	289622	23737	7.232			
2	6.717	3715018	269840	92.768			
Total		4004641	293577				

(+)-brevianamide A (**1**) **e.r.** 99:1 *after crystallisation*
(Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{Rminor} = 5.51 min, t_{Rmajor} = 6.84 min.

<Chromatogram>



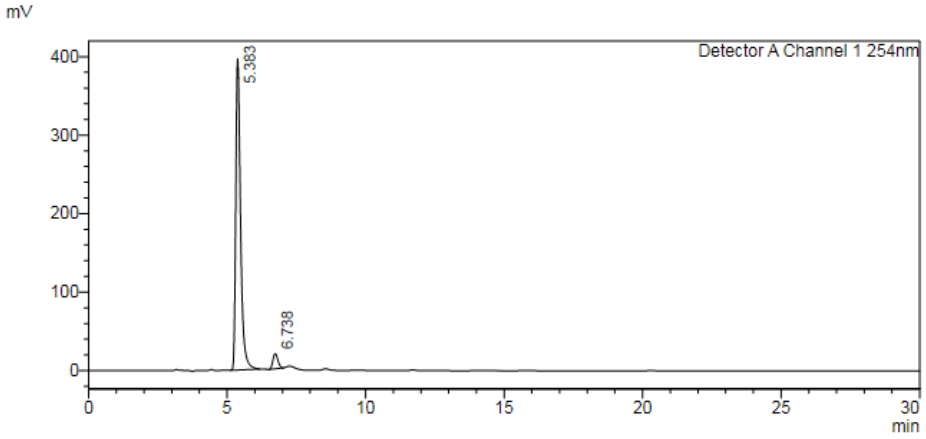
<Peak Table>

Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.507	24842	2113	1.220			
2	6.835	2010703	140355	98.780			
Total		2035545	142469				

(–)-brevianamide A (**1**) **e.r.** 95:5
(Chiralpak IA, 1:1 *i*-PrOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{Rmajor} = 5.38 min, t_{Rminor} = 6.74 min.

<Chromatogram>



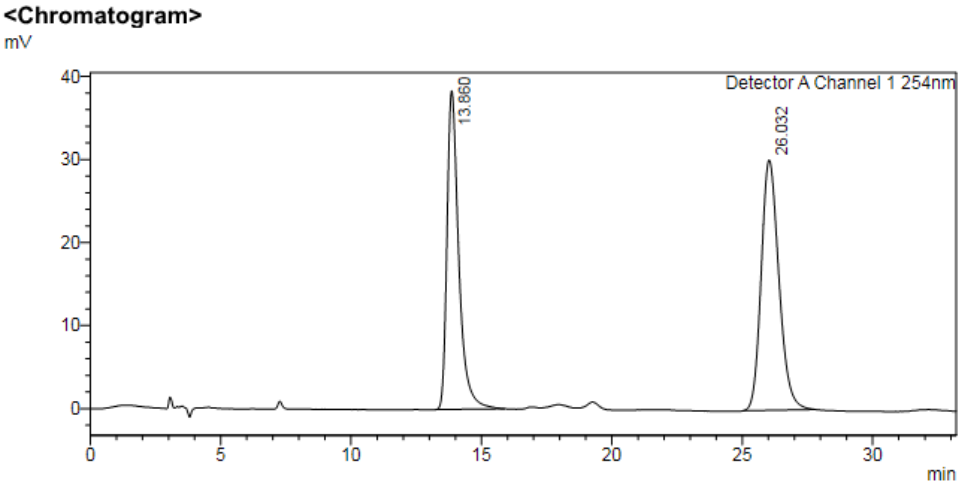
<Peak Table>

Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	5.383	4758681	396107	95.170			
2	6.738	241511	19400	4.830			
Total		5000192	415507				

5.2 Chiral HPLC Chromatograms of Brevianamide B

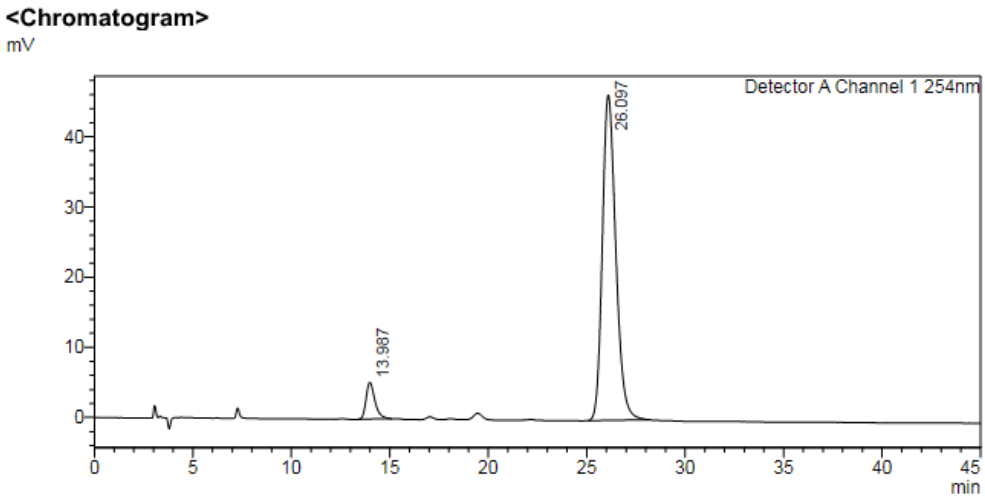
(+)/(–)-Brevianamide B (**2**)
(Chiralpak IA, 1:3 EtOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{R1} = 13.86 min, t_{R2} = 26.03 min.



<Peak Table>
Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	13.860	1202069	38310	45.782			
2	26.032	1423551	30114	54.218			
Total		2625620	68424				

(+)-Brevianamide B (**2**) **e.r.** 93:7
(Chiralpak IA, 1:3 EtOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{Rminor} = 13.99 min, t_{Rmajor} = 26.10 min.

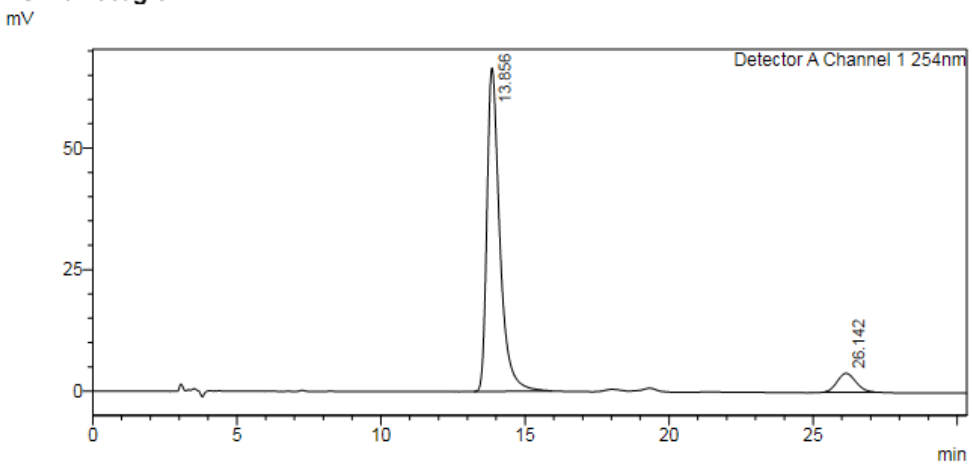


<Peak Table>
Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	13.987	162378	5219	6.875			
2	26.097	2199609	46337	93.125			
Total		2361987	51556				

(–)-Brevianamide B (**2**) **e.r.** 92:8
(Chiralpak IA, 1:3 EtOH/hexane, 1 mL min^{-1}, λ 254 nm)
t_{Rmajor} = 13.86 min, t_{Rminor} = 26.14 min.

<Chromatogram>



<Peak Table>

Detector A Channel 1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	13.856	2058078	66555	91.955			
2	26.142	180065	3929	8.045			
Total		2238144	70484				

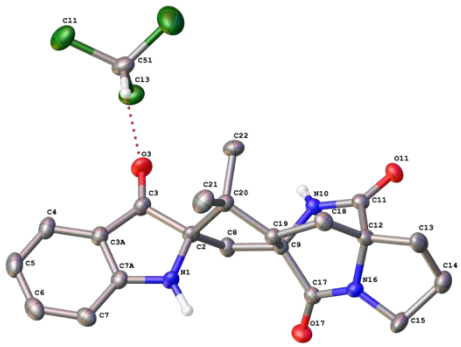
6 X-ray Crystal Structure of (+)-Brevianamide A (**1**) [CCDC 1918446 (**1**)]

Submitted by: **Robert Godfrey**
The University of Edinburgh
Solved by: **Gary S Nichol**

Sample ID: **RG-G-96**

Compound RG-G-96 was provided as orange prism/plate-shaped crystals suitable for single crystal X-ray diffraction, yielding structure AL19001.

Crystal Data and Experimental



Experimental. Single yellow plate-shaped crystals of **AL19001** were recrystallised from a mixture of chloroform and diethyl ether by vapour diffusion. A suitable crystal 0.24×0.09×0.05 mm³ was selected and mounted on a MITIGEN holder in Paratone oil on an Rigaku Oxford Diffraction SuperNova diffractometer. The crystal was kept at a steady T = 120.0 K during data collection. The structure was solved with the **ShelXT** (Sheldrick, 2015) structure solution program using the Intrinsic Phasing solution method and by using **Olex2** (Dolomanov et al., 2009) as the graphical interface. The model was refined with version 2018/3 of **ShelXL** (Sheldrick, 2015) using Least Squares minimisation.

Crystal Data. $C_{22}H_{24}Cl_3N_3O_3$, M_r = 484.79, monoclinic, $I2$ (No. 5), a = 13.2441(2) Å, b = 8.65980(10) Å, c = 20.3280(2) Å, β = 104.2140(10)°, α = γ = 90°, V = 2260.07(5) Å³, T = 120.0 K, Z = 4, Z' = 1, μ(CuK$_\alpha$) = 3.919, 22429 reflections measured, 4696 unique (R_{int} = 0.0715) which were used in all calculations. The final wR_2 was 0.1135 (all data) and R_1 was 0.0434 (I > 2(I)).

Compound	**AL19001**
Formula	$C_{22}H_{24}Cl_3N_3O_3$
$D_{calc.}$/ g cm^{-3}	1.425
μ/mm^{-1}	3.919
Formula Weight	484.79
Colour	yellow
Shape	plate
Size/mm³	0.24×0.09×0.05
T/K	120.0
Crystal System	monoclinic
Flack Parameter	-0.007(8)
Hooft Parameter	-0.004(5)
Space Group	$I2$
a/Å	13.2441(2)
b/Å	8.65980(10)
c/Å	20.3280(2)
α/°	90
β/°	104.2140(10)
γ/°	90
V/Å³	2260.07(5)
Z	4
Z'	1
Wavelength/Å	1.54184
Radiation type	CuK$_\alpha$
Θ_{min}/°	3.618
Θ_{max}/°	76.076
Measured Refl.	22429
Independent Refl.	4696
Reflections with I > 2(I)	4622
R_{int}	0.0715
Parameters	301
Restraints	12
Largest Peak	0.283
Deepest Hole	-0.377
GooF	1.040
wR_2 (all data)	0.1135
wR_2	0.1130
R_1 (all data)	0.0437
R_1	0.0434

Structure Quality Indicators

Reflections:	d min (Cu)	0.79	I/σ	29.7	Rint	7.15%	complete 100% (IUCr)	100%	
Refinement:	Shift	0.000	Max Peak	0.3	Min Peak	-0.4	GooF	1.040	Flack	.007(8)

A yellow plate-shaped crystal with dimensions 0.24×0.09×0.05 mm³ was mounted on a MITIGEN holder in Paratone oil. Data were collected using an Rigaku Oxford Diffraction SuperNova diffractometer equipped with an Oxford Cryosystems Cryostream 700+ low-temperature device operating at T = 120.0 K.

Data were measured using ω scans using CuK$_\alpha$ radiation. The total number of runs and images was based on the strategy calculation from the program **CrysAlisPro** (Rigaku, V1.171.39.46, 2018) The maximum resolution that was achieved was Θ = 76.076° (0.79 Å).

The diffraction pattern was indexed The total number of runs and images was based on the strategy calculation from the program **CrysAlisPro** (Rigaku, V1.171.39.46, 2018) and the unit cell was refined using **CrysAlisPro** (Rigaku, V1.171.39.46, 2018) on 18841 reflections, 84% of the observed reflections.

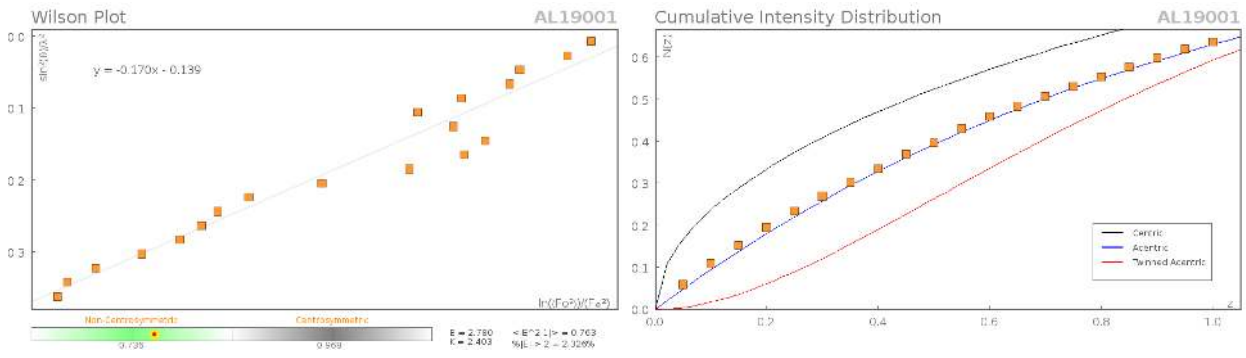
Data reduction, scaling and absorption corrections were performed using **CrysAlisPro** (Rigaku, V1.171.39.46, 2018). The final completeness is 100.00 % out to 76.076° in Θ. A multi-scan absorption correction was performed using CrysAlisPro 1.171.39.46 (Rigaku Oxford Diffraction, 2018) using spherical harmonicsas implemented in SCALE3 ABSPACK.. The absorption coefficient μ of this material is 3.919 mm^{-1} at this wavelength (λ = 1.542Å) and the minimum and maximum transmissions are 0.732 and 1.000.

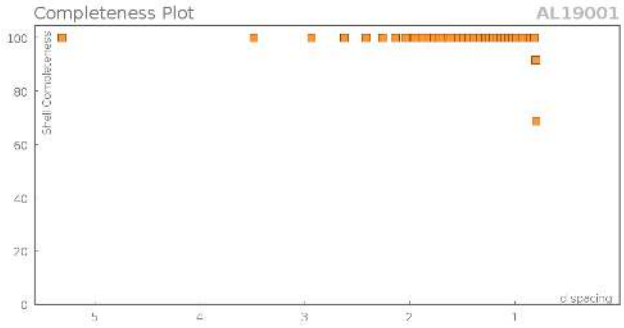
The structure was solved and the space group *I*2 (# 5) determined by the **ShelXT** (Sheldrick, 2015) structure solution program using Intrinsic Phasing and refined by Least Squares using version 2018/3 of **ShelXL** (Sheldrick, 2015). All non-hydrogen atoms were refined anisotropically. Hydrogen atom positions were calculated geometrically and refined using the riding model. Most hydrogen atom positions were calculated geometrically and refined using the riding model, but some hydrogen atoms were refined freely.

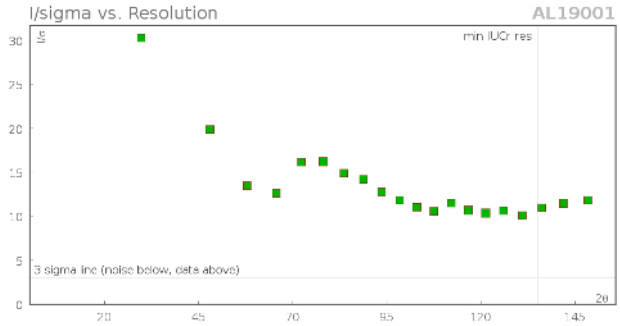
_*refine_special_details*: N-bound H atoms were identified from a difference Fourier map and freely refined. C14 was modelled as disordered over two sites consistent with a residual peak in a difference map. Geometric and displacement ellipsoid restraints were used

The Flack parameter was refined to -0.007(8). Determination of absolute structure using Bayesian statistics on Bijvoet differences using the Olex2 results in -0.004(5). Note: The Flack parameter is used to determine chirality of the crystal studied, the value should be near 0, a value of 1 means that the stereochemistry is wrong and the model should be inverted. A value of 0.5 means that the crystal consists of a racemic mixture of the two enantiomers.

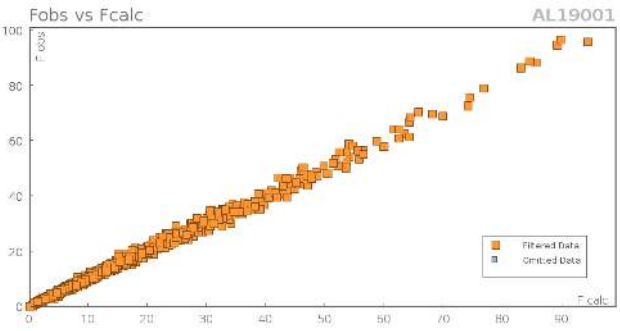
Data Plots: Diffraction Data

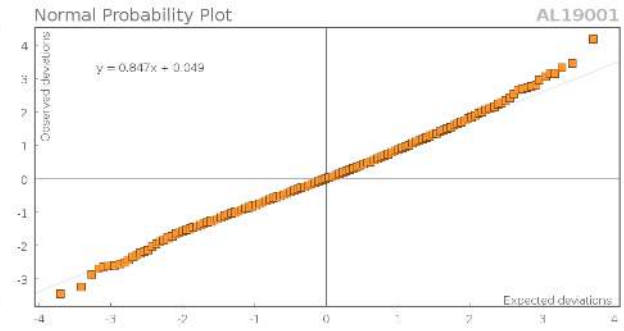






Data Plots: Refinement and Data





Reflection Statistics

Total reflections (after filtering)	22429	Unique reflections	4696
Completeness	0.992	Mean I/σ	22.37
hkl_{max} collected	(16, 10, 24)	hkl_{min} collected	(-16, -10, -25)
hkl_{max} used	(16, 10, 25)	hkl_{min} used	(-16, -10, 0)
Lim d_{max} collected	100.0	Lim d_{min} collected	0.77
d_{max} used	12.22	d_{min} used	0.79
Friedel pairs	3373	Friedel pairs merged	0
Inconsistent equivalents	39	R_{int}	0.0715
R_{sigma}	0.0337	Intensity transformed	0
Omitted reflections	0	Omitted by user (OMIT hkl)	0
Multiplicity	(2018, 2488, 1509, 896, 509, 293, 113, 66, 60, 54, 32, 17, 4, 1)	Maximum multiplicity	16
Removed systematic absences	0	Filtered off (Shel/OMIT)	0

Table 1: Fractional Atomic Coordinates (×10⁴) and Equivalent Isotropic Displacement Parameters (Å²×10³) for **AL19001**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
Cl1	5403.6(6)	10543.2(12)	957.1(4)	45.8(2)
Cl2	4150.5(11)	7793.6(15)	635.0(6)	69.2(4)

Atom	x	y	z	U_{eq}
Cl3	3665.4(6)	10071.0(11)	1550.5(4)	41.5(2)
C51	4662(2)	9167(4)	1266.6(15)	34.0(6)
O3	5568.8(19)	7723(3)	2669.1(11)	36.9(5)
O11	2503.3(16)	1574(2)	2500.9(11)	26.4(4)
O17	3852.8(16)	4056(3)	4699.3(10)	29.8(4)
N1	6559.5(18)	5817(3)	4225.4(11)	26.7(5)
N10	3504.9(17)	3609(2)	3018.7(11)	20.9(4)
N16	3939.4(18)	1792(3)	4142.0(11)	23.1(5)
C2	5805(2)	5759(3)	3567.3(12)	23.4(5)
C3	6069(2)	7250(3)	3219.7(14)	26.5(5)
C3A	6966(2)	7942(3)	3680.1(14)	24.9(5)
C4	7527(2)	9290(3)	3620.3(16)	29.1(6)
C5	8325(2)	9736(4)	4167.3(18)	35.3(6)
C6	8552(2)	8836(4)	4760.7(16)	32.2(6)
C7	8008(2)	7506(4)	4827.0(14)	27.3(5)
C7A	7192(2)	7061(3)	4272.6(14)	23.5(5)
C8	4665.0(19)	5717(3)	3638.1(13)	23.4(5)
C9	4365.4(19)	4010(3)	3612.1(12)	19.6(5)
C11	3239(2)	2112(3)	2940.0(12)	21.5(5)
C12	4003(2)	1148(3)	3472.1(13)	23.0(5)
C13	3702(2)	-526(3)	3521.8(14)	30.2(6)
C14	2973(4)	-500(5)	3972(2)	36.3(11)
C15	3492(3)	696(4)	4537.2(17)	36.7(7)
C17	4024(2)	3337(3)	4222.6(13)	22.0(5)
C18	5096(2)	1458(3)	3355.3(13)	24.6(5)
C19	5368.9(19)	3125(3)	3569.7(12)	20.5(5)
C20	5928(2)	4224(3)	3178.2(13)	23.3(5)
C21	7064(2)	3757(4)	3255.3(17)	34.7(6)
C22	5389(2)	4348(4)	2418.1(13)	30.5(6)
C14A	3891(9)	-875(12)	4304(5)	26(3)

Table 2: Anisotropic Displacement Parameters ($\times 10^4$) **AL19001**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2a^{*2} \times U_{11} + \ldots + 2hka^* \times b^* \times U_{12}]$

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
Cl1	29.5(3)	62.4(6)	46.8(4)	16.5(4)	11.7(3)	-3.2(3)
Cl2	85.4(8)	62.6(7)	67.8(6)	-27.0(6)	34.6(6)	-18.1(6)
Cl3	36.2(4)	58.8(5)	33.6(3)	-0.8(3)	16.4(3)	0.7(3)
C51	34.4(14)	41.9(16)	27.0(13)	5.8(12)	10.3(11)	0.8(13)
O3	42.3(12)	35.4(12)	27.3(9)	13.2(9)	-2.4(9)	-4.3(9)
O11	27.0(9)	21.8(8)	27.5(8)	-3.7(7)	1.4(7)	-1.0(7)
O17	34.1(10)	30.3(10)	27.5(9)	-7.2(8)	12.3(8)	-0.5(8)
N1	26.3(10)	29.0(12)	21.1(10)	8.0(9)	-1.0(8)	-5.9(9)
N10	22.4(10)	18.5(10)	20.3(9)	-0.8(8)	2.2(8)	0.6(8)
N16	28.7(10)	22.2(11)	19.0(10)	0.6(8)	6.8(8)	-2.4(8)
C2	25.3(12)	23.1(12)	19.8(11)	4.3(9)	1.4(9)	-1.3(10)
C3	30.4(13)	22.2(12)	26.8(12)	5.4(10)	7.0(10)	-1.6(10)
C3A	24.5(12)	23.1(12)	27.9(12)	1.0(10)	7.9(10)	-1.2(10)
C4	29.1(13)	23.0(12)	38.9(14)	1.3(11)	15.3(11)	-2.8(11)
C5	31.0(13)	29.2(14)	49.8(17)	-5.9(12)	17.9(13)	-8.5(11)
C6	22.5(12)	35.8(15)	38.6(15)	-11.0(12)	7.7(11)	-2.5(11)
C7	23.6(12)	31.1(13)	26.0(12)	-3.6(10)	4.0(10)	1.5(10)
C7A	21.5(11)	23.6(12)	27.2(12)	0.9(10)	9.6(10)	-0.2(9)
C8	23.1(11)	17.7(11)	28.1(12)	0.3(10)	3.8(9)	-0.5(9)
C9	21.3(11)	17.1(10)	19.8(11)	-0.9(8)	3.9(9)	0.0(9)
C11	25.8(12)	19.7(11)	18.9(11)	-2.1(9)	5.4(9)	1.2(9)
C12	29.4(13)	18.6(11)	20.6(11)	-1.7(9)	5.0(9)	0.4(9)
C13	40.7(15)	17.1(11)	29.7(13)	0.6(10)	2.6(12)	-1.1(10)
C14	44(2)	27.3(18)	40(2)	0.8(16)	14.8(17)	-9.9(16)
C15	52.0(17)	29.4(14)	36.7(14)	-0.8(13)	25.9(13)	-11.7(14)
C17	21.3(11)	22.2(12)	22.0(11)	-1.8(9)	4.2(9)	-0.4(9)
C18	26.5(12)	19.4(12)	27.5(12)	-2.7(10)	6.0(9)	4.7(10)

Atom	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C19	21.0(11)	19.5(11)	20.9(10)	0.5(9)	5.1(8)	1.4(9)
C20	24.2(12)	23.3(12)	22.7(11)	2.3(9)	6.2(9)	0.0(9)
C21	26.3(13)	37.4(16)	42.9(16)	7.0(13)	13.3(11)	3.6(11)
C22	35.8(14)	35.4(14)	21.0(12)	1.7(11)	8.1(10)	-1.2(12)
C14A	32(6)	21(5)	27(5)	3(4)	8(4)	-6(4)

Table 3: Bond Lengths in Å for **AL19001**.

Atom	Atom	Length/Å
Cl1	C51	1.756(3)
Cl2	C51	1.759(3)
Cl3	C51	1.750(3)
O3	C3	1.223(4)
O11	C11	1.240(3)
O17	C17	1.219(3)
N1	C2	1.461(3)
N1	C7A	1.354(4)
N10	C9	1.483(3)
N10	C11	1.342(3)
N16	C12	1.493(3)
N16	C15	1.460(4)
N16	C17	1.349(4)
C2	C3	1.552(4)
C2	C8	1.552(3)
C2	C20	1.575(4)
C3	C3A	1.449(4)
C3A	C4	1.406(4)
C3A	C7A	1.394(4)

Atom	Atom	Length/Å
C4	C5	1.387(5)
C5	C6	1.405(5)
C6	C7	1.383(4)
C7	C7A	1.410(4)
C8	C9	1.528(3)
C9	C17	1.537(4)
C9	C19	1.555(3)
C11	C12	1.534(3)
C12	C13	1.513(4)
C12	C18	1.546(4)
C13	C14	1.485(5)
C13	C14A	1.577(11)
C14	C15	1.573(5)
C15	C14A	1.574(11)
C18	C19	1.525(3)
C19	C20	1.542(3)
C20	C21	1.529(4)
C20	C22	1.538(3)

Table 4: Bond Angles in ° for **AL19001**.

Atom	Atom	Atom	Angle/°
Cl1	C51	Cl2	110.26(16)
Cl3	C51	Cl1	110.35(19)
Cl3	C51	Cl2	110.90(18)
C7A	N1	C2	111.4(2)
C11	N10	C9	116.7(2)
C15	N16	C12	112.4(2)
C17	N16	C12	117.4(2)
C17	N16	C15	127.6(2)
N1	C2	C3	102.0(2)
N1	C2	C8	112.2(2)
N1	C2	C20	110.9(2)
C3	C2	C20	114.2(2)
C8	C2	C3	113.2(2)
C8	C2	C20	104.4(2)
O3	C3	C2	124.3(3)
O3	C3	C3A	128.8(3)
C3A	C3	C2	106.9(2)
C4	C3A	C3	130.9(3)
C7A	C3A	C3	107.5(2)
C7A	C3A	C4	121.5(3)
C5	C4	C3A	118.5(3)
C4	C5	C6	119.6(3)
C7	C6	C5	122.6(3)
C6	C7	C7A	117.7(3)
N1	C7A	C3A	112.1(2)
N1	C7A	C7	127.9(3)
C3A	C7A	C7	120.1(3)
C9	C8	C2	105.7(2)

Atom	Atom	Atom	Angle/°
N10	C9	C8	113.3(2)
N10	C9	C17	104.4(2)
N10	C9	C19	109.62(19)
C8	C9	C17	117.7(2)
C8	C9	C19	105.1(2)
C17	C9	C19	106.4(2)
O11	C11	N10	125.8(2)
O11	C11	C12	124.7(2)
N10	C11	C12	109.5(2)
N16	C12	C11	105.3(2)
N16	C12	C13	102.8(2)
N16	C12	C18	109.9(2)
C11	C12	C18	106.1(2)
C13	C12	C11	115.4(2)
C13	C12	C18	116.7(2)
C12	C13	C14A	105.8(4)
C14	C13	C12	104.4(3)
C13	C14	C15	103.1(3)
N16	C15	C14	100.9(2)
N16	C15	C14A	100.6(4)
O17	C17	N16	125.3(3)
O17	C17	C9	126.6(3)
N16	C17	C9	108.1(2)
C19	C18	C12	106.6(2)
C18	C19	C9	109.7(2)
C18	C19	C20	123.2(2)
C20	C19	C9	104.42(19)
C19	C20	C2	98.06(19)

Atom	Atom	Atom	Angle/°
C21	C20	C2	113.1(2)
C21	C20	C19	111.6(2)
C21	C20	C22	108.9(2)
C22	C20	C2	111.5(2)
C22	C20	C19	113.5(2)
C15	C14A	C13	99.0(6)

Table 5: Torsion Angles in ° for **AL19001**.

Atom	Atom	Atom	Atom	Angle/°
O3	C3	C3A	C4	-2.4(5)
O3	C3	C3A	C7A	173.7(3)
O11	C11	C12	N16	-123.4(3)
O11	C11	C12	C13	-10.7(4)
O11	C11	C12	C18	120.2(3)
N1	C2	C3	O3	-174.4(3)
N1	C2	C3	C3A	3.6(3)
N1	C2	C8	C9	-94.9(3)
N1	C2	C20	C19	77.9(2)
N1	C2	C20	C21	-39.9(3)
N1	C2	C20	C22	-162.9(2)
N10	C9	C17	O17	-117.8(3)
N10	C9	C17	N16	62.0(3)
N10	C9	C19	C18	-43.0(3)
N10	C9	C19	C20	90.7(2)
N10	C11	C12	N16	57.7(3)
N10	C11	C12	C13	170.4(2)
N10	C11	C12	C18	-58.8(3)
N16	C12	C13	C14	30.4(3)
N16	C12	C13	C14A	-20.7(5)
N16	C12	C18	C19	-44.0(3)
N16	C15	C14A	C13	-41.9(6)
C2	N1	C7A	C3A	-0.7(3)
C2	N1	C7A	C7	179.7(3)
C2	C3	C3A	C4	179.7(3)
C2	C3	C3A	C7A	-4.2(3)
C2	C8	C9	N10	-116.5(2)
C2	C8	C9	C17	121.4(2)
C2	C8	C9	C19	3.2(2)
C3	C2	C8	C9	150.2(2)
C3	C2	C20	C19	-167.5(2)
C3	C2	C20	C21	74.8(3)
C3	C2	C20	C22	-48.3(3)
C3	C3A	C4	C5	175.9(3)
C3	C3A	C7A	N1	3.2(3)
C3	C3A	C7A	C7	-177.2(2)
C3A	C4	C5	C6	0.2(4)
C4	C3A	C7A	N1	179.8(3)
C4	C3A	C7A	C7	-0.7(4)
C4	C5	C6	C7	-0.1(5)
C5	C6	C7	C7A	-0.3(4)
C6	C7	C7A	N1	-179.8(3)
C6	C7	C7A	C3A	0.7(4)
C7A	N1	C2	C3	-1.8(3)
C7A	N1	C2	C8	-123.3(2)
C7A	N1	C2	C20	120.2(2)
C7A	C3A	C4	C5	0.2(4)
C8	C2	C3	O3	-53.6(4)
C8	C2	C3	C3A	124.4(2)
C8	C2	C20	C19	-43.2(2)
C8	C2	C20	C21	-161.0(2)
C8	C2	C20	C22	76.0(3)
C8	C9	C17	O17	8.8(4)

Atom	Atom	Atom	Atom	Angle/°
C8	C9	C17	N16	-171.4(2)
C8	C9	C19	C18	-165.2(2)
C8	C9	C19	C20	-31.4(2)
C9	N10	C11	O11	175.4(2)
C9	N10	C11	C12	-5.7(3)
C9	C19	C20	C2	45.4(2)
C9	C19	C20	C21	164.2(2)
C9	C19	C20	C22	-72.3(3)
C11	N10	C9	C8	176.2(2)
C11	N10	C9	C17	-54.5(3)
C11	N10	C9	C19	59.2(3)
C11	C12	C13	C14	-83.7(3)
C11	C12	C13	C14A	-134.8(5)
C11	C12	C18	C19	69.4(2)
C12	N16	C15	C14	-16.3(3)
C12	N16	C15	C14A	32.6(5)
C12	N16	C17	O17	169.8(3)
C12	N16	C17	C9	-10.0(3)
C12	C13	C14	C15	-40.8(3)
C12	C13	C14A	C15	39.3(6)
C12	C18	C19	C9	-16.8(3)
C12	C18	C19	C20	-140.2(2)
C13	C12	C18	C19	-160.4(2)
C13	C14	C15	N16	34.7(4)
C15	N16	C12	C11	113.4(3)
C15	N16	C12	C13	-7.8(3)
C15	N16	C12	C18	-132.7(3)
C15	N16	C17	O17	9.8(5)
C15	N16	C17	C9	-170.1(3)
C17	N16	C12	C11	-49.6(3)
C17	N16	C12	C13	-170.8(2)
C17	N16	C12	C18	64.3(3)
C17	N16	C15	C14	144.6(3)
C17	N16	C15	C14A	-166.6(5)
C17	C9	C19	C18	69.3(2)
C17	C9	C19	C20	-156.95(19)
C18	C12	C13	C14	150.7(3)
C18	C12	C13	C14A	99.6(5)
C18	C19	C20	C2	171.1(2)
C18	C19	C20	C21	-70.1(3)
C18	C19	C20	C22	53.4(3)
C19	C9	C17	O17	126.3(3)
C19	C9	C17	N16	-53.9(3)
C20	C2	C3	O3	65.8(4)
C20	C2	C3	C3A	-116.2(2)
C20	C2	C8	C9	25.3(2)

Table 6: Hydrogen Fractional Atomic Coordinates ($\times 10^4$) and Equivalent Isotropic Displacement Parameters ($Å^2 \times 10^3$) for **AL19001**. U_{eq} is defined as 1/3 of the trace of the orthogonalised U_{ij}.

Atom	x	y	z	U_{eq}
H51	5128.69	8622.02	1659.55	41
H1	6570(30)	5250(50)	4627(18)	24(8)
H10	3110(30)	4460(50)	2772(19)	28(9)
H4	7364.24	9882.45	3214.65	35
H5	8714.89	10643.93	4140.78	42
H6	9101.52	9154.41	5130.9	39
H7	8177.38	6912.99	5232.33	33
H8A	4616.39	6182.66	4074.01	28

Atom	x	y	z	U_{eq}
H8B	4199.62	6290.87	3262.1	28
H13A	3355.24	-941.82	3068.99	36
H13B	4321.47	-1164.53	3720.26	36
H13C	4137.02	-1207.49	3313.56	36
H13D	2960.9	-689.35	3287.26	36
H14A	2918.46	-1531.99	4170.17	44
H14B	2270.92	-154.12	3723.55	44
H15A	2968.09	1194.96	4741.07	44
H15B	4035.38	206.61	4899.29	44
H15C	2721.33	749.45	4418.23	44
H15D	3759.98	864.76	5030.98	44
H18A	5613.77	741.95	3630.73	29
H18B	5087.98	1312.67	2871.04	29
H19	5815.11	3076.07	4043.76	25
H21A	7443.49	4612.39	3110.21	52
H21B	7094.76	2848.39	2973.46	52
H21C	7383.25	3510.98	3731.58	52
H22A	4703.81	4829.65	2362.55	46
H22B	5304.54	3313.25	2216.75	46
H22C	5816.08	4979.4	2190.88	46
H14C	3473.8	-1764.57	4392.32	32
H14D	4636.59	-1055.16	4521.23	32

Table 7: Hydrogen Bond information for **AL19001**.

D	H	A	d(D-H)/Å	d(H-A)/Å	d(D-A)/Å	D-H-A/deg
N1	H1	O17[1]	0.95(4)	1.91(4)	2.826(3)	162(3)

[1]1-x,+y,1-z

Table 8: Atomic Occupancies for all atoms that are not fully occupied in **AL19001**.

Atom	Occupancy
H13A	0.759(11)
H13B	0.759(11)
H13C	0.241(11)
H13D	0.241(11)
C14	0.759(11)
H14A	0.759(11)
H14B	0.759(11)
H15A	0.759(11)
H15B	0.759(11)
H15C	0.241(11)
H15D	0.241(11)
C14A	0.241(11)
H14C	0.241(11)
H14D	0.241(11)

Citations

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O.V. Dolomanov and L.J. Bourhis and R.J. Gildea and J.A.K. Howard and H. Puschmann, Olex2: A complete structure solution, refinement and analysis program, *J. Appl. Cryst.*, (2009), **42**, 339-341.

Sheldrick, G.M., Crystal structure refinement with ShelXL, *Acta Cryst.*, (2015), **C27**, 3-8.

Sheldrick, G.M., ShelXT-Integrated space-group and crystal-structure determination, *Acta Cryst.*, (2015), **A71**, 3-8.

```
#===============================================================================
# PLATON/CHECK-(220119) versus check.def version 190121, Entry: al19001
# Data: AL19001.cif - Type: CIF                  Bond Precision   C-C = 0.0041 A
# Refl: AL19001.fcf - Type: LIST4                                   Temp = 120 K
# Audit:OLEX2 1.2-BETA (COMPILED 2018.05.29 SVN.R3508 FOR OLEXSYS, GUI SVN.R5506
# Refin:SHELXL (SHELDRICK, 2015)
# X-ray CuKa              R(int) = 0.072,  wR2/R(int) =  1.6,  Nref/Npar =   8.4
# Cell  13.2441(2)   8.6598(1)  20.3280(2)          90   104.214(1)          90
# Wavelength 1.54184   Volume Reported     2260.07(5)  Calculated     2260.07(5)
# SpaceGroup from Symmetry I 2          Hall: I 2y                 monoclinic
#                 Reported I 1 2 1            I 2y                 monoclinic
# MoietyFormula C21 H23 N3 O3, C H Cl3
#      Reported C H Cl3, C21 H23 N3 O3
#    SumFormula C22 H24 Cl3 N3 O3
#      Reported C22 H24 Cl3 N3 O3
# Mr        =    484.79[Calc],    484.79[Rep]            Volume/NonHatoms = 18.2
# Dx,gcm-3  =     1.425[Calc],     1.425[Rep]
# Z         =         4[Calc],         4[Rep]
# Mu (mm-1) =     3.919[Calc],     3.919[Rep]  Xtal Size =  0.051x0.091x0.238 mm
# F000      =    1008.0[Calc],    1008.0[Rep]  or F000' =   1014.83[Calc]
# Reported   T Limits: Tmin=0.732              Tmax=1.000  AbsCorr = MULTI-SCAN
# Calculated T Limits: Tmin=0.684 Tmin'=0.375  Tmax=0.819  Exti    =     0.00130
# Reported   Hmax= 16, Kmax= 10, Lmax= 25, Nref=   4696        , Th(max)= 76.076
# Obs in FCF Hmax= 16, Kmax= 10, Lmax= 25, Nref=   4696[  2523], Th(max)= 76.076
# Calculated Hmax= 16, Kmax= 10, Lmax= 25, Nref=   4735[  2530], Ratio=1.86/0.99
# Reported   Rho(min) = -0.38, Rho(max) =  0.28 e/Ang**3 (From CIF)
# Calculated Rho(min) = -0.40, Rho(max) =  0.28 e/Ang**3 (From CIF+FCF data)
# w=1/[sigma**2(Fo**2)+(0.0887P)**2+  0.4125P], P=(Fo**2+2*Fc**2)/3
# R= 0.0434(  4622), wR2= 0.1135(  4696), S = 1.040      (From CIF+FCF data)
# R= 0.0434(  4622), wR2= 0.1135(  4696), S = 1.040      (From FCF data only)
# R= 0.0434(  4622), wR2= 0.1135(  4696), S = 1.040, Npar=  301, Flack -0.007(8)
# Number Bijvoet Pairs = 2173 ( 99%),      2108 Selected for:  Parsons -0.004(5)
# P2(tr) 1.000, P3(tr) 1.000, P3(tw) 0.000, Student-T Nu =  20,  Hooft -0.005(7)
#===============================================================================
For Documentation: http://http://www.platonsoft.nl/CIF-VALIDATION.pdf
#===============================================================================

#===============================================================================
>>> The Following Improvement and Query ALERTS were generated - (Acta-Mode) <<<
#===============================================================================
Format: alert-number_ALERT_alert-type_alert-level text

244_ALERT_4_C Low    'Solvent' Ueq as Compared to Neighbors of        C51 Check
340_ALERT_3_C Low Bond Precision on  C-C Bonds ...............     0.0041 Ang.
#===============================================================================
002_ALERT_2_G Number of Distance or Angle Restraints on AtSite          4 Note
003_ALERT_2_G Number of Uiso or Uij Restrained non-H Atoms ...          3 Report
042_ALERT_1_G Calc. and Reported MoietyFormula Strings  Differ     Please Check
143_ALERT_4_G s.u. on c - Axis Small or Missing ..............    0.00020 Ang.
176_ALERT_4_G The CIF-Embedded .res File Contains SADI Records          2 Report
187_ALERT_4_G The CIF-Embedded .res File Contains RIGU Records          1 Report
301_ALERT_3_G Main Residue  Disorder ..............(Resd  1  )         4% Note
431_ALERT_2_G Short Inter HL..A Contact  Cl3      ..O11      .       3.04 Ang.
x,1+y,z  =      1_565 Check
791_ALERT_4_G Model has Chirality at C2          (Chiral SPGR)          R Verify
791_ALERT_4_G Model has Chirality at C9          (Chiral SPGR)          R Verify
791_ALERT_4_G Model has Chirality at C12         (Chiral SPGR)          R Verify
791_ALERT_4_G Model has Chirality at C19         (Chiral SPGR)          S Verify
802_ALERT_4_G CIF Input Record(s) with more than 80 Characters          1 Info
860_ALERT_3_G Number of Least-Squares Restraints .............         12 Note
912_ALERT_4_G Missing # of FCF Reflections Above STh/L=  0.600          7 Note
978_ALERT_2_G Number C-C Bonds with Positive Residual Density.          3 Info
#===============================================================================

ALERT_Level and ALERT_Type Summary
==================================
2 ALERT_Level_C = Check. Ensure it is Not caused by an Omission or Oversight
16 ALERT_Level_G = General Info/Check that it is not Something Unexpected
```

```
1 ALERT_Type_1 CIF Construction/Syntax Error, Inconsistent or Missing Data.
4 ALERT_Type_2 Indicator that the Structure Model may be Wrong or Deficient.
3 ALERT_Type_3 Indicator that the Structure Quality may be Low.
10 ALERT_Type_4 Improvement, Methodology, Query or Suggestion.
#===============================================================================

1 Missing  Experimental  Info Issue(s) (Out of  60 Tests) -   98 % Satisfied
0 Experimental  Data  Related Issue(s) (Out of  30 Tests) -  100 % Satisfied
7 Structural  Model   Related Issue(s) (Out of 131 Tests) -   95 % Satisfied
10 Unresolved or to be Checked Issue(s) (Out of 257 Tests) -   96 % Satisfied

#===============================================
```