Chemical Synthesis of (+)-Brevianamide A Supports a Diels–Alderase-Free Biosynthesis

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The fungal-derived bicyclo[2.2.2]diazaoctane alkaloids are of significant interest to the scientific community for their potent and varied biological activities. Within this large and diverse family of natural products the insecticidal metabolite (+)-brevianamide A is particularly noteworthy for its synthetic intractability and inexplicable biogenesis. Despite five decades of research, this alkaloid has never succumbed to chemical synthesis. It has been suggested that a proposed Diels–Alder reaction in the biosynthesis of (+)-brevianamide A requires a Diels–Alderase enzyme. We herein report the first chemical synthesis of (+)-brevianamide A (7 steps, 8.0% overall yield, 750 mg scale), which provides compelling evidence in support of a Diels–Alderase-free biosynthesis; a significant departure from the established biosynthesis of related alkaloids.

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Chemical synthesis of (+)-brevianamide A supports a Diels–Alderase-free biosynthesis.

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The fungal-derived bicyclo[2.2.2]diazaoctane alkaloids are of significant interest to the scientific community for their potent and varied biological activities. Within this large and diverse family of natural products the insecticidal metabolite (+)-brevianamide A is particularly noteworthy for its synthetic intractability and inexplicable biogenesis. Despite five decades of research, this alkaloid has never succumbed to chemical synthesis. It has been suggested that a proposed Diels–Alderase reaction in the biosynthesis of (+)-brevianamide A requires a Diels–Alderase enzyme. We herein report the first chemical synthesis of (+)-brevianamide A (7 steps, 8.0% overall yield, 750 mg scale), which provides compelling evidence in support of a Diels–Alderase-free biosynthesis; a significant departure from the established biosynthesis of related alkaloids.

The bicyclo[2.2.2]diazaoctane alkaloids are a vast group of natural products isolated from various marine and terrestrial fungi.^{1,2} They are of significant interest to chemists and biologists alike due to their important biological activities, diverse biosynthetic origins, and synthetically daunting structures. There are two distinct families of bicyclo[2.2.2]diazaoctane alkaloids, the monooxopiperazine-type structures, which include the anthelmintic paraherquamides,^{3–5} calmodulin-inhibiting malbrancheamides,⁶ and neuroprotective

chrysogenamides;⁷ and the dioxopiperazine-type structures, which include the anti-tumoral stephacidins,⁸ cytotoxic notoamides,⁹ and insecticidal brevianamides (Figure 1).^{10,11}

Brevianamides A (**1**) and B (**2**) were originally isolated by Birch and Wright in 1969 from the fungus *Penicillium brevicompactum*,¹⁰ and were the first known bicyclo[2.2.2]diazaoctane alkaloids (Figure 1).^{1,2} Brevianamide A (**1**) exhibits potent antifeedant activity against the larvae of the insect pests *Spodoptera frugiperda* (fall armyworm) and *Heliothis virescens* (tobacco budworm),¹¹ and is the major isolated diastereomer (ratio of A to B $\geq$ 90:10).^{12,13} In 1970, Porter and Sammes proposed that the bicyclo[2.2.2]diazaoctane cores of brevianamides A (**1**) and B (**2**) could be biosynthesised through an intramolecular hetero-Diels–Alder cycloaddition (Figure 1),¹⁴ a proposal which has been extended to encompass all bicyclo[2.2.2]diazaoctane alkaloids.^{1,2} Recently, Diels–Alderase enzymes have been identified in the biosynthetic gene clusters responsible for the malbrancheamide and paraherquamide monooxopiperazine-type alkaloids.¹⁵ However, no Diels–Alderase enzyme has yet been identified for the brevianamides, nor any other dioxopiperazine-type alkaloid.¹⁶ Furthermore, despite five decades of research, no chemical synthesis of brevianamide A (**1**) has been achieved, yet several syntheses of the minor diastereomer, brevianamide B (**2**), have been reported (see SI for a full summary).^{17–25} Despite the similar structures of the two natural products, none of these strategies have been successfully applied to the synthesis of brevianamide A (**1**) due to insurmountable issues of stereoselectivity.

Fig 1: Representative bicyclo[2.2.2]diazaoctane alkaloids (bicyclo[2.2.2]diazaoctane cores highlighted).

Following decades of detailed chemical and biochemical studies, the biosynthetic origins of (+)-brevianamide A (**1**) and B (**2**) still remain largely unknown. We herein report a new biosynthetic hypothesis, which builds upon the pioneering work of several research groups.^{1,2} To appreciate the origins of our new biosynthetic proposal it is important to first outline key details of the elegant work of Williams and co-workers.¹⁹⁻²² Their biomimetic synthetic studies have mainly focused upon an early proposed pathway (Figure 2, Pathway 1), which was informed by the seminal work of Birch and Sammes.^{14,27-29} The pathway begins with a stereoablative oxidation of (+)-deoxybrevianamide E (**3**) to give achiral azadiene **4**, which then undergoes enantioselective Diels–Alder cycloaddition to give a scalemic mixture of bicyclo[2.2.2]diazaoctane enantiomers, **5** and *ent*-**5**. Both enantiomers then undergo (*R*)-selective indole oxidation and a [1,2]-alkyl shift, so that the major enantiomer (**5**) gives brevianamide A (**1**) and the minor enantiomer (*ent*-**5**) gives brevianamide B (**2**). Williams’ synthetic studies, however, revealed that this proposed Diels–Alder reaction (**4** to **5/ent-5**) actually produces an unwanted diastereomer as the major product.²² Therefore, a Diels–Alderase enzyme would be required to reverse this inherent diastereoselectivity and yet provide only modest enantioselectivity. Further evidence against this proposed pathway was provided through biosynthetic feeding experiments with isotopically labelled **5**, which failed to show any significant incorporation into (+)-brevianamide A (**1**).³⁰ Williams, therefore, proposed an alternative biosynthetic pathway (Figure 2, Pathway 2), which begins with a diastereoselective indole oxidation of (+)-deoxybrevianamide E (**3**) to give hydroxyindolenine **6**.^{30,31} A stereospecific [1,2]-alkyl shift then gives indoxyl **7**, which undergoes a diketopiperazine oxidation and Diels–Alder cycloaddition to give brevianamides A (**1**) and B (**2**).^{30,31} Williams and co-workers reasoned that “[t]he preponderance of **1** over **2** would be due either to the relative activities of two different [Diels–Alderase] enzymes or the affinity of a single enzyme active site for the individual conformers”.³⁰ All efforts to substantiate this second-generation pathway *in vitro*,

however, have failed, largely due to the instability of indoxyl **7**.¹ Furthermore, hydroxyindolenine **6** is likely to undergo rapid 5-*exo-trig* cyclisation to give the known shunt-metabolite brevianamide E (**8**),^{10,27} which would represent a biosynthetic dead-end.³⁰

Our new biosynthetic hypothesis for (+)-brevianamide A (**1**) involves an alternative biosynthetic precursor, (+)-dehydrodeoxybrevianamide E (**9**), which is a known natural product isolated from various *Penicillium* and *Aspergillus* species (Figure 2, Pathway 3).³²⁻³⁴ Crucially, the diketopiperazine ring in (+)-dehydrodeoxybrevianamide E (**9**) is already at the oxidation level required for a later Diels–Alder reaction. Our pathway involves a point-to-point chirality transfer *via* a sequential diastereoselective indole oxidation, stereospecific [1,2]-alkyl shift, and tautomerisation, to give enantiopure azadiene **10**. Diels–Alder cycloaddition of azadiene **10** would then give brevianamides A (**1**) and B (**2**).^{30,31} Although our proposed intermediate **11** is likely to undergo reversible 5-*exo-trig* cyclisation to give pentacycle **12**, this is not a known natural product and is therefore unlikely to represent a biosynthetic dead-end (cf. brevianamide E (**8**) in Pathway 2). We herein report our synthetic investigation into the chemical feasibility of this new biosynthetic proposal, which has resulted in the first total synthesis of this infamous natural product.

Fig 2: Biosynthetic proposals for brevianamides A and B.

Results and Discussion

The synthesis of our proposed biosynthetic precursor, (+)-dehydrodeoxybrevianamide E (**9**), commenced with phthaloyl protection of commercially available L-tryptophan methyl ester **13** (Figure 3).³⁵ The crude product from this reaction, ester **14**, was subjected directly to Danishefsky's reverse prenylation conditions using *B*-prenyl-9-BBN to give more than 20 grams of intermediate **15**.³⁶ Hydrolysis of methyl ester **15** was accompanied by ring opening of the phthaloyl group to give diacid **16**, which presumably explains why a protecting group switch from phthaloyl to *tert*-butoxycarbonyl or trityl has been undertaken in related synthetic endeavours.^{36,37} To avoid these extraneous two steps (deprotection/re-protection) we developed a quantitative S_N2-type demethylation of methyl ester **15** using LiCl in DMF.³⁸ Lithium carboxylate **17** was then subjected to a one-pot acyl chloride formation and imine acylation reaction with dehydropyrolone **18** to give *N*-acyl

enamine **19**.³⁹ Attempts to deprotect the primary amine in **19** using conventional phthaloyl deprotection reagents, such as hydrazine, resulted in unwanted cleavage of the amide bond. Our search for less nucleophilic reagents eventually led us to ammonia in methanol, which not only deprotected the primary amine but also resulted in spontaneous cyclisation to give more than eight grams of (+)-dehydrodeoxybrevianamide E (**9**) in 49% yield over the three steps from methyl ester **15**. Thus, by developing a new imine acylation reaction and avoiding unnecessary protecting group manipulations, the shortest total synthesis of (+)-dehydrodeoxybrevianamide E (**9**) has been achieved, proceeding in a longest linear sequence of 5 steps, 34% overall yield, and requiring just two chromatographic purifications (cf. previous synthesis: 12 steps, 8% overall yield).²²

Fig 3: Total synthesis of (+)-brevianamides A and B. 9-BBN, 9-borabicyclo(3.3.1)nonane; Phth, phthaloyl; DMF, dimethylformamide; NCS, *N*-chlorosuccinimide; *m*-CPBA, *meta*-chloroperoxybenzoic acid.

Oxidation of (+)-dehydrodeoxybrevianamide E (**9**) with *m*-CPBA gave our proposed dehydro-analogue of brevianamide E, **12**, and a minor diastereomer *dia*-**12** in 67% combined yield (d.r. **12**:*dia*-**12**, 3:2). Exposure of intermediate **12** to LiOH in water at ambient

temperature successfully gave (+)-brevianamide A (**1**) and (+)-brevianamide B (**2**) in a combined 63% yield, presumably *via* the domino retro-5-*exo-trig*/[1,2]-alkyl shift/Diels–Alder reaction sequence as outlined in Figure 2 (Pathway 3). Following purification by column chromatography, which is only the fourth purification in the entire seven-step synthesis, 750 milligrams of (+)-brevianamide A (**1**) and 60 milligrams of (+)-brevianamide B (**2**) were isolated.⁴⁰ The unnatural (–)-enantiomers of brevianamides A (**1**) and B (**2**) could also be accessed by subjecting the minor diastereomer, *dia*-**12**, to the same reaction conditions (see SI for full details). The biomimetic Diels–Alder reaction produces (+)-brevianamide A (**1**) and (+)-brevianamide B (**2**) in a 93:7 diastereomeric ratio (Figure 3), which closely matches the ratio observed when they are isolated from *Penicillium brevicompactum* (see SI for further details).¹² This result demonstrates that Diels–Alderase catalysis is not necessary to achieve stereocontrol in the biosynthesis of the brevianamides.³⁰ Indeed, if the biosynthetic Diels–Alder reaction is enzyme catalysed, the putative Diels–Alderase enzyme must lower the energy barrier without exerting any stereocontrol (within experimental error).^{41–43} To the best of our knowledge, this would represent an unprecedented discovery for Diels–Alderase enzymes.^{44–46} Thus, we suggest that this Diels–Alder reaction is instead a spontaneous process exploited by Nature without direct enzyme participation. This is in stark contrast to the requirement of Diels–Alderase enzymes in the stereoselective biogenesis of the closely related malbrancheamide and paraherquamide alkaloids.¹⁵

In summary, 50 years after its discovery by Birch,¹⁰ the first chemical synthesis of brevianamide A (**1**) has finally been achieved. This synthesis provides valuable insights into a previously enigmatic biosynthetic pathway and demonstrates the utility of the biomimetic approach in the synthesis of complex natural products.

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Author Contributions

R.C.G., N.J.G. and A.L.L. conceived, designed and carried out the synthetic experiments. G.S.N. performed the crystallographic studies. All authors discussed and co-wrote the manuscript.

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SUPPORTING INFORMATION

Chemical synthesis of (+)-brevianamide A supports a Diels–Alderase-free biosynthesis.

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1. Overview of previous total and formal syntheses of brevianamide B.

NOTE: No synthesis of brevianamide A has been reported.

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

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

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

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

1.3 Williams' 2006 synthesis of (±)-brevianamide B (late-stage indole formation)

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

1.4 Williams' 2007 synthesis of (±)-brevianamide B (second biomimetic)

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

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

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

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

- 13 steps (11 + 2 formal steps)
- 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

References:

1. Williams, R. M., Glinka, T. & Kwast, E. Facial selectivity of the intramolecular S_N2' cyclization: stereocontrolled total synthesis of brevianamide B. *J. Am. Chem. Soc.* **110**, 5927–5929 (1988).
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

References:

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).
3. Steps 1–6 taken from; Kametani, T., Kanaya, N. & Ihara, M. Studies on the syntheses of heterocyclic compounds. Part 876. The chiral total synthesis of brevianamide E and deoxybrevianamide E. *J. Chem. Soc. Perkin Trans. I* 959–963 (1981).

1.3 Williams' 2006 synthesis of (±)-brevianamide B (late-stage indole formation approach)

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

References:

1. Adams, L. A., Valente, M. W. N. & Williams, R. M. A concise synthesis of d,l-brevianamide B via a biomimetically-inspired IMDA construction. *Tetrahedron* **62**, 5195–5200 (2006).
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 & (±)-dehydrodeoxybreviamine 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 $^3\text{H}_2\text{CO}$ 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

References:

1. Frebault, F. C., Simpkins, N. S. & Fenwick, A. A concise enantioselective synthesis of ent-malbrancheamide B. *J. Am. Chem. Soc.* **131**, 4214–4215 (2009).
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

References:

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 $\geq 90:10$ by Campbell and co-workers (see tables below).¹

Table 1. Brevianamide A and B production from *P. brevicompactum* colony growth: over culture, inoculum 4.8×10^6 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

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

Table 3. Brevianamide A and B production from *P. brevicompactum* colony growth: over culture with continual medium replacement, inoculum 3.6×10^6 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: ^1H 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 ^1H NMR spectra (CDCl_3 δ 7.26 ppm, CD_3OD δ 3.31 ppm, CD_2Cl_2 δ 5.32 ppm, and $(\text{CD}_3)_2\text{SO}$ 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 $(\text{CD}_3)_2\text{SO}$ δ 39.52 ppm). Assignment of ^1H and ^{13}C NMR signals was assisted by ^1H - ^1H COSY, ^1H - ^{13}C HSQC, ^1H - ^{13}C HMBC and ^1H - ^1H 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

R_f 0.24 (2:3 EtOAc/petroleum spirit)

¹H 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;

¹³C 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 $[\text{M} + \text{H}]^+$ 349.1183), 371.1033 (calculated $[\text{M} + \text{Na}]^+$ 371.1002)

$[\alpha]_D^{23.3} -201.1^\circ$ (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 NEt₃ (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. K₂CO₃ (75 mL) was added. The layers were separated and the aqueous layer extracted with EtOAc (3 × 125 mL). The combined organic layers were dried (MgSO₄) and concentrated under reduced pressure. The crude product was filtered through silica with CH₂Cl₂, 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);

¹H NMR (600 MHz, CDCl₃) 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;

¹³C NMR (151 MHz, CDCl₃) 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;

[α]_D^{23.7} -221.6° (*c* 3.97, CHCl₃), literature: [α]_D²⁵ -180.8° (*c* 3.9, CHCl₃).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/H₂O (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 Et₂O (3 × 5 mL). The combined organics were washed with H₂O (2 × 2.5 mL), brine (2.5 mL), dried (Na₂SO₄) 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.

¹H NMR (500 MHz, CD₃OD) 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;

¹³C NMR (126 MHz, CD₃OD) 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);

[α]_D^{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 CH₂Cl₂ (300 mL), sonicated for 30 minutes and the solid removed by filtration. The residual solid was broken up, suspended in CH₂Cl₂ (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 CH₂Cl₂, 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.

¹H NMR (500 MHz, CD₃OD) 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;

¹³C NMR (126 MHz, CD₃OD) 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);

[α]_D^{22.9} –226.0° (*c* 0.20, MeOH).

References:

- 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).
- 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).
- 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 CH₂Cl₂ (200 mL) at 0 °C. A suspension of crude compound **17** from **Step (3)** (20.1 g) in CH₂Cl₂ (200 mL) was then added dropwise over 30 min at 0 °C and the residual **17** was washed into the reaction with CH₂Cl₂ (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 NEt₃ (10.3 mL, 73.8 mmol) in CH₂Cl₂ 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 CH₂Cl₂ (3 × 50 mL). The combined organics were washed with saturated aq. NaHCO₃ (80 mL), and the aqueous layer then back extracted with CH₂Cl₂ (50 mL). The combined organics were washed with brine (120 mL), dried (Na₂SO₄) 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);

¹H NMR (500 MHz, CD₂Cl₂) 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);

¹³C NMR (151 MHz, CD₂Cl₂) 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 residual 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);

[α]_D^{23.0} –130.1° (*c* 0.38, MeOH).

References:

- 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).
- Ookawa, A. & Soai, K. Asymmetric synthesis of optically active threo- and erythro-pyrrolidinybenzyl 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 NH₃ 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);

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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);

[α]_D^{23.1} –33.2° (*c* 1.30, CHCl₃), literature: [α]_D²² –38° (*c* 1.3, CHCl₃).²

References:

- Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of d,l-stephacidin A. *Org. Lett.* **9**, 4255–4258 (2007).
- Steyn, P. S. The structures of five diketopiperazines from *Aspergillus ustus*. *Tetrahedron* **29**, 107–120 (1973).

3.7 Experimental Procedure for Compounds **12** and *dia*-**12**

Step (6) – Inspired by the work of Kametani¹ and Wolff.² To a rapidly stirred solution of dehydrodeoxy-brevianamide E (**9**) (4.00 g, 11.5 mmol) in CHCl₃ (80 mL) at room temperature was added dropwise over 4 h a solution of *m*-CPBA in CHCl₃ (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. Na₂S₂O₃ (20 mL) and saturated aq. NaHCO₃ (20 mL). The reaction mixture was diluted with EtOAc (200 mL) and CHCl₃ (150 mL) and the phases separated. The aqueous phase was back-extracted with CHCl₃ (50 mL) and the combined organics washed with saturated aq. NaHCO₃ (80 mL), brine (80 mL), dried (Na₂SO₄) and concentrated under reduced pressure. Flash column chromatography (1.5:7:3 petroleum spirit/CH₂Cl₂/*i*-Pr₂O to 7:3 CH₂Cl₂/*i*-Pr₂O 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 *dia*-**12** (1.37 g, 18:82 **12**/*dia*-**12**, 3.75 mmol, 33%) as a cream foam, alongside recovered starting material dehydrodeoxy-brevianamide E (**9**) (270 mg, 0.774 mmol, 7%) as a cream foam. Overall, **12**/*dia*-**12** (2.80 g, 60:40, 7.67 mmol, 67%). A second round of chromatography on the mixed dehydro-brevianamide E (**12**) and compound *dia*-**12** fraction gave additional pure dehydro-brevianamide E (**12**) (0.108 g, 0.296 mmol, 3%) as a cream glass and pure minor diastereomer *dia*-**12** (0.454 g, 1.24 mmol, 11%) as a white foam. At this point, further chromatography was deemed unnecessary since 92% of the major diastereomer dehydro-brevianamide E (**12**) had been isolated in pure form.

^{*} *m*-CPBA (69% w/w by iodometric titration, 4.38 g, 17.5 mmol) was added to CHCl₃ (60 mL) and dried over Na₂SO₄ (27.5 g) with stirring for 40 mins.

R_f 0.15 (7:3 CH₂Cl₂/*i*-Pr₂O);

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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);

[α]_D^{20.9} –208° (*c* 0.23, EtOH).

R_f 0.09 (7:3 CH₂Cl₂/*i*-Pr₂O);

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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);

[α]_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 Kishi¹ 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 CH₂Cl₂ (2 × 450 mL, 2 × 200 mL), and the combined organics dried (Na₂SO₄). Flash column chromatography (2:8 to 3:7 to 1:1 THF/CHCl₃ to 25:25:1 THF/CHCl₃/*i*-PrOH) gave (+)-brevianamide A (**1**) (905 mg, 16.2% CHCl₃ 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 CHCl₃ by slow vapour diffusion with Et₂O. See pages 58-59 for X-ray crystal structure of brevianamide A·CHCl₃.

*In Birch's original isolation paper brevianamide A '*crystallized from CHCl₃ 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 deuterio-solvent to record NMR spectra for characterisation.

Spectroscopic data matched literature values reported by Zhu Weiming and co-workers.⁶ Subtle differences in ¹H NMR data are attributed to concentration effects in CDCl₃ (see page 42).

R_f 0.21 (3:7 THF/CHCl₃);

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

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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;

¹H NMR (500 MHz, (CD₃)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;

¹³C NMR (126 MHz, (CD₃)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);

[α]_D^{23.5} +316° (*c* 0.12, EtOH), literature: [α]_D²⁵ +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.

Brevianamide B (**2**) was only sparingly soluble in CDCl₃, consistent with the observation in Birch's original isolation paper that brevianamide B '*was insoluble in most solvents except hot DMSO and CF₃CO₂H, the latter causing decomposition*'.³ ¹H and ¹³C NMR spectra could be recorded using a saturated sample in CDCl₃, 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 (CD₃)₂SO.

R_f 0.08 (3:7 THF/CHCl₃);

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

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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;

¹H NMR (500 MHz, (CD₃)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;

¹³C NMR (126 MHz, (CD₃)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);

[α]_D^{24.7} +136° (*c* 0.23, 2.5% HCO₂H in CH₂Cl₂), literature (enantiomer): [α]_D²⁵ –147° (*c* 0.23, 2.5% HCO₂H in CH₂Cl₂),⁸ [α]_D²⁵ –124° (*c* 0.81, 2.5% HCO₂H in CH₂Cl₂);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 **dia-12** (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 CH₂Cl₂ (4 × 100 mL), and the combined organics dried (Na₂SO₄). Flash column chromatography (2:8 to 3:7 to 1:1 THF/CHCl₃ to 25:25:1 THF/CHCl₃/*i*-PrOH) gave (–)-brevianamide A (**ent-1**) (155 mg, 17.5% CHCl₃ 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 CHCl₃ 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 deuterio-solvent to record NMR spectra for characterisation.

$[\alpha]_D^{24.7} -281^\circ$ (*c* 0.12, EtOH), literature (enantiomer): $[\alpha]_D^{25} +413^\circ$ (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.

$[\alpha]_D^{25.1} -119^\circ$ (*c* 0.23, 2.5% HCO₂H in CH₂Cl₂), literature: $[\alpha]_D^{25} -147^\circ$ (*c* 0.23, 2.5% HCO₂H in CH₂Cl₂),³ $[\alpha]_D^{25} -124^\circ$ (*c* 0.81, 2.5% HCO₂H in CH₂Cl₂);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).

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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 NEt₃ (48.3 mL, 347 mmol) in CH₂Cl₂ (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 Et₂O (350 mL). A mixture of saturated aq. NH₄Cl (150 mL) and water (150 mL) was added, the organic phase was separated and the aqueous phase was back-extracted with CH₂Cl₂ (2 × 150 mL). The combined organics were washed with a mixture of saturated aq. NH₄Cl (100 mL) and water (100 mL), saturated aq. NaHCO₃ (150 mL), brine (150 mL), dried (Na₂SO₄) 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);

¹H NMR (500 MHz, CDCl₃) 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;

¹³C NMR (126 MHz, CDCl₃) 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 (sp³) 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 separated 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 distillate 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 commercially available sample.

BP 32–36 °C (147 mbar);

^1H 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;

^1H 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. Na₂CO₃ (50 mL) and water (50 mL). The product was dried (CaCl₂ 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 CaCl₂ 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.

¹H NMR (500 MHz, CDCl₃) δ 1.33 (s, 9H) ppm;

¹³C NMR (126 MHz, CDCl₃) δ 84.1, 27.0 ppm.

References:

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

4.1 ¹H NMR Spectrum of Compound **14** (500 MHz, CDCl₃)

4.2 ¹³C NMR Spectrum of Compound **14** (126 MHz, CDCl₃)

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

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

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

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

4.9 ^1H 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 ^1H NMR Spectrum of Compound **9** (500 MHz, CDCl_3)

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

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

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

4.15 ^1H NMR Spectrum of Compound *dia*-12 (500 MHz, CDCl_3)

4.16 ^{13}C NMR Spectrum of Compound *dia*-12 (126 MHz, CDCl_3)

4.17 ^1H 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 ^1H - ^1H COSY Spectrum of Brevianamide A (**1**) (CDCl_3)

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

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

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

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

4.24 ^1H NMR Spectrum of Brevianamide A (**1**) (500 MHz, $(\text{CD}_3)_2\text{SO}$)

4.25 ^{13}C NMR Spectrum of Brevianamide A (**1**) (126 MHz, $(\text{CD}_3)_2\text{SO}$)

4.26 ^1H - ^1H COSY Spectrum of Brevianamide A (**1**) ($(\text{CD}_3)_2\text{SO}$)

4.27 ^1H - ^{13}C HSQC Spectrum of Brevianamide A (**1**) ($(\text{CD}_3)_2\text{SO}$)

4.28 ^1H - ^{13}C HMBC Spectrum of Brevianamide A (**1**) ($(\text{CD}_3)_2\text{SO}$)

4.29 ^1H - ^1H NOESY Spectrum of Brevianamide A (**1**) ($(\text{CD}_3)_2\text{SO}$)

4.30 ^1H 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 ^1H NMR Spectrum of Brevianamide B (**2**) (500 MHz, $(\text{CD}_3)_2\text{SO}$)

4.33 ^{13}C NMR Spectrum of Brevianamide B (**2**) (126 MHz, $(\text{CD}_3)_2\text{SO}$)

4.34 ^1H - ^1H COSY Spectrum of Brevianamide B (**2**) ($(\text{CD}_3)_2\text{SO}$)

4.35 ^1H - ^{13}C HSQC Spectrum of Brevianamide B (**2**) ($(\text{CD}_3)_2\text{SO}$)

4.36 ^1H - ^{13}C HMBC Spectrum of Brevianamide B (**2**) ($(\text{CD}_3)_2\text{SO}$)

4.37 ^1H - ^1H NOESY Spectrum of Brevianamide B (**2**) ($(\text{CD}_3)_2\text{SO}$)

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

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

4.40 ^1H 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 ^1H 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 ^1H 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.

<Chromatogram>

<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)

$t_{R\text{minor}} = 5.41$ min, $t_{R\text{major}} = 6.72$ min.

<Chromatogram>

<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.

<Chromatogram>

<Peak Table>

Detector A Channel 1 254nm						
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark
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.

<Chromatogram>

<Peak Table>

Detector A Channel 1 254nm						
Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark
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)

$t_{R\text{major}}$ = 13.86 min, $t_{R\text{minor}}$ = 26.14 min.

<Chromatogram>

mV

<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)

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 $0.24 \times 0.09 \times 0.05 \text{ mm}^3$ 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 $T = 120.0 \text{ K}$ 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. $\text{C}_{22}\text{H}_{24}\text{Cl}_3\text{N}_3\text{O}_3$, $M_r = 484.79$, monoclinic, $I2$ (No. 5), $a = 13.2441(2) \text{ \AA}$, $b = 8.65980(10) \text{ \AA}$, $c = 20.3280(2) \text{ \AA}$, $\beta = 104.2140(10)^\circ$, $\alpha = \gamma = 90^\circ$, $V = 2260.07(5) \text{ \AA}^3$, $T = 120.0 \text{ K}$, $Z = 4$, $Z' = 1$, $\mu(\text{CuK}\alpha) = 3.919$, 22429 reflections measured, 4696 unique ($R_{\text{int}} = 0.0715$) 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	$\text{C}_{22}\text{H}_{24}\text{Cl}_3\text{N}_3\text{O}_3$
$D_{\text{calc.}} / \text{g cm}^{-3}$	1.425
μ / mm^{-1}	3.919
Formula Weight	484.79
Colour	yellow
Shape	plate
Size/ mm^3	$0.24 \times 0.09 \times 0.05$
T / K	120.0
Crystal System	monoclinic
Flack Parameter	-0.007(8)
Hooft Parameter	-0.004(5)
Space Group	$I2$
$a / \text{\AA}$	13.2441(2)
$b / \text{\AA}$	8.65980(10)
$c / \text{\AA}$	20.3280(2)
$\alpha / ^\circ$	90
$\beta / ^\circ$	104.2140(10)
$\gamma / ^\circ$	90
$V / \text{\AA}^3$	2260.07(5)
Z	4
Z'	1
Wavelength/ $\text{\AA}$	1.54184
Radiation type	$\text{CuK}\alpha$
$\theta_{\text{min}} / ^\circ$	3.618
$\theta_{\text{max}} / ^\circ$	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 -0.007(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 α 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 $\Theta = 76.076^\circ$ (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 harmonics as implemented in SCALE3 ABSPACK.. The absorption coefficient μ of this material is 3.919 mm⁻¹ at this wavelength ($\lambda = 1.542$ Å) and the minimum and maximum transmissions are 0.732 and 1.000.

The structure was solved and the space group $I2$ (# 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.

Brevianamide A - SI.pdf (5.49 MiB)

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