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Total synthesis of brevianamide A

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

The fungal-derived bicyclo[2.2.2]diazaoctane alkaloids are of 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 remained an elusive target for chemical synthesis due to insurmountable issues of reactivity and selectivity associated with all previously explored strategies. We herein report the chemical synthesis of (+)-brevianamide A (seven steps, 7.2% overall yield, 750 mg scale), which involves a bioinspired cascade transformation of the linearly fused (−)-dehydrobrevianamide E into the topologically complex bridged-spiro-fused structure of (+)-brevianamide A.

The Diels–Alder cycloaddition is one of the most important reactions in synthetic organic chemistry, allowing the stereoselective construction of six-membered rings through the concerted formation of two new bonds (Fig. 1a)[1]. The limited appearance of Diels–Alder reactions in biosynthetic pathways has always fascinated chemists[2–6]. The bicyclo[2.2.2]diazaoctane alkaloids, which are a vast group of natural products isolated from various marine and terrestrial fungi, have played a key role in the development of our understanding of biosynthetic Diels–Alder reactions[7,8]. They are also of broader interest to both 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 (Fig. 1b), which include the anthelmintic paraherquamides[9–11], calmodulin-inhibiting malbrancheamides[12] and neuroprotective chrysogenamides[13]; and the dioxopiperazine-type structures (Fig. 1c), which include the cytotoxic stephacidins and notoamides[14,15], and the insecticidal brevianamides[16,17].

Brevianamides A (**1**) and B (**2**) were originally isolated by Birch and Wright in 1969 from the fungus *Penicillium brevicompactum*[16], and were the first known bicyclo[2.2.2]diazaoctane alkaloids (Fig. 1c)[7,8]. Brevianamide A (**1**) exhibits potent antifeedant activity against the larvae of the insect pests *Spodoptera frugiperda* (fall armyworm) and *Heliothis virescens* (tobacco budworm)[17], and is the major isolated diastereomer (d.r. ≳90:10)[18,19]. In 1970, Porter and Sammes proposed that the bicyclo[2.2.2]diazaoctane cores of brevianamides A (**1**) and B (**2**) could be biosynthesized through an intramolecular hetero-Diels–Alder cycloaddition (Fig. 1d)[20], a proposal that has been extended to encompass all bicyclo[2.2.2]diazaoctane alkaloids[7,8]. Recently, Diels–Alderase enzymes have been identified in the biosynthetic gene clusters responsible for the malbrancheamide and paraherquamide monooxopiperazine-type alkaloids[21]. However, no Diels–Alderase enzyme has yet been identified for the brevianamides, or for any other dioxopiperazine-type alkaloid[22,23]. Furthermore, despite five decades of research, the chemical synthesis of brevianamide A (**1**) has remained an elusive target, although several syntheses of the minor diastereomer, brevianamide B (**2**), have been reported (for a full summary, see Supplementary Section 1)[24–32]. 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 reactivity and selectivity.

Despite decades of detailed chemical and biochemical studies, the biosynthetic origins of (+)-brevianamide A (**1**) and B (**2**) remain unknown. We herein propose a modified biosynthetic hypothesis that builds on the pioneering work of several research groups[7,8]. To appreciate the origins of this modified biosynthetic proposal it is important first to outline key details of the elegant work of Williams and co-workers[26–29]. Their biomimetic synthetic studies have mainly focused on an early proposed pathway (Fig. 2, pathway 1)[33], which was informed by the seminal work of Birch and Sammes[20,34–36]. 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's synthetic studies, however, revealed that this proposed Diels–Alder reaction (**4** to **5**/***ent*-5**) actually produces an unwanted diastereomer as the major product[29]. Furthermore, biosynthetic feeding experiments with isotopically labelled **5** failed to show notable incorporation into (+)-brevianamide A (**1**)[37]. Williams, therefore, proposed an alternative biosynthetic pathway (Fig. 2, pathway 2), which begins with a diastereoselective indole oxidation of (+)-deoxybrevianamide E (**3**) to give hydroxyindolenine **6**[37,38]. 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**)[37,38]. 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"[37]. All efforts to substantiate this second-generation pathway in vitro, however, have failed, largely due to the instability of indoxyl **7**[7]. Furthermore, hydroxyindolenine **6** is likely to undergo rapid 5-*exo*-*trig* cyclization to give the known shunt-metabolite brevianamide E (**8**)[16,34], which is proposed to be a biosynthetic dead-end[37].

Our modified biosynthetic hypothesis for (+)-brevianamide A (**1**) involves an alternative biosynthetic precursor, (+)-dehydro deoxybrevianamide E (**9**), which is a known natural product isolated from various *Penicillium* and *Aspergillus* species (Fig. 3)[39–41]. Crucially, the diketopiperazine ring in (+)-dehydrodeoxybrevianamide E (**9**) is already at the oxidation level required for a subsequent Diels–Alder reaction. Our pathway involves a point-to-point chirality

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

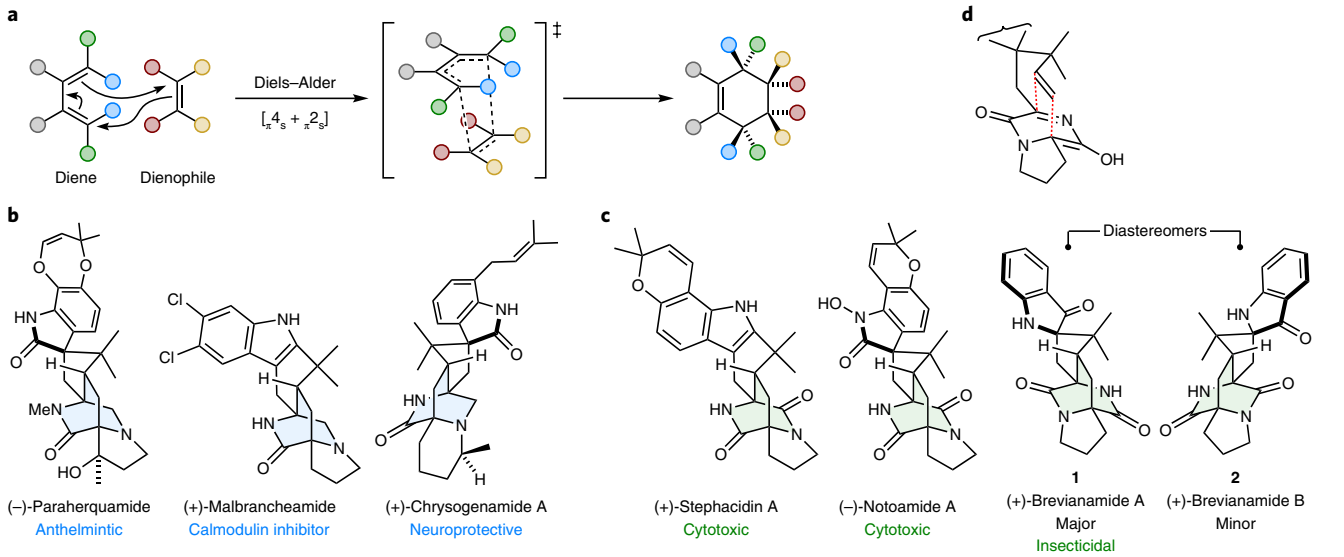

Fig. 1 | Diels–Alder cycloaddition and representative examples of bicyclo[2.2.2]diazaoctane alkaloids, which are proposed to be biosynthesized via intramolecular hetero-Diels–Alder reactions. a, Diels–Alder cycloaddition. **b**, Representative monooxopiperazine-type bicyclo[2.2.2]diazaoctane alkaloids (bicyclo[2.2.2]diazaoctane cores highlighted in blue). **c**, Representative dioxopiperazine-type bicyclo[2.2.2]diazaoctane alkaloids (bicyclo[2.2.2]diazaoctane cores highlighted in green). **d**, Sammes' proposed biosynthetic hetero-Diels–Alder reaction. Coloured circles indicate general substituents for the Diels–Alder reaction components.

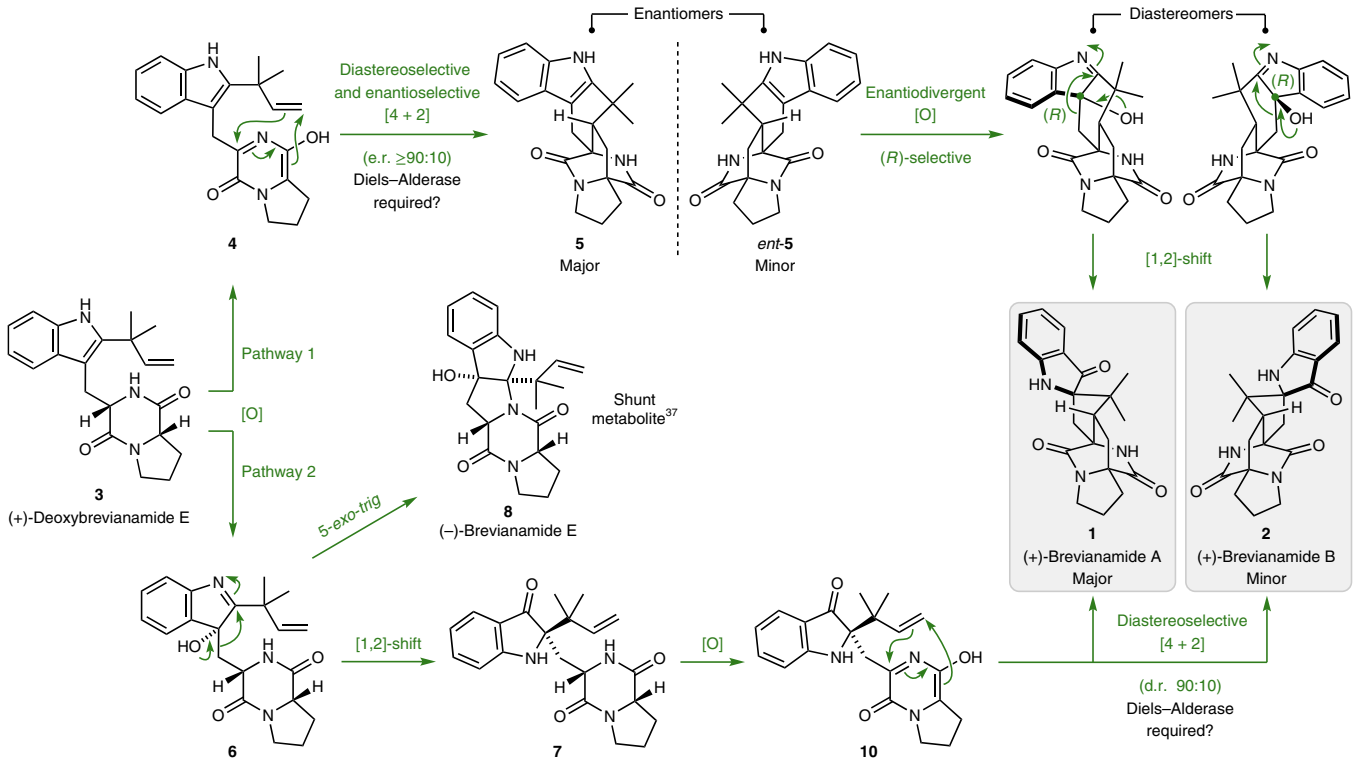

Fig. 2 | Previous biosynthetic proposals for brevianamides A and B. In 1989, building on the earlier work of Birch and Sammes, Williams and co-workers proposed pathway 1 as a plausible biosynthetic pathway towards brevianamides A and B[33]. In 1993, following their synthetic and biosynthetic studies, Williams and co-workers proposed an alternative order of biosynthetic transformations, as shown in pathway 2[37]. Biosynthetic feeding experiments with isotopically labelled **5** and (–)-brevianamide E (**8**), however, show no notable incorporation into **1** or **2**.

transfer via a sequential diastereoselective indole oxidation, a stereospecific [1,2]-alkyl shift and tautomerization, to give enantiopure azadiene **10**. Diels–Alder cycloaddition of azadiene **10** would then give brevianamides A (**1**) and B (**2**)[37,38]. Although our proposed intermediate **11** is likely to undergo reversible 5-*exo-trig* cyclization to give pentacycle **12**, this is not a known natural product and is therefore unlikely to represent a dead-end (cf. brevianamide E (**8**) in pathway 2, Fig. 2). We herein report the total synthesis of

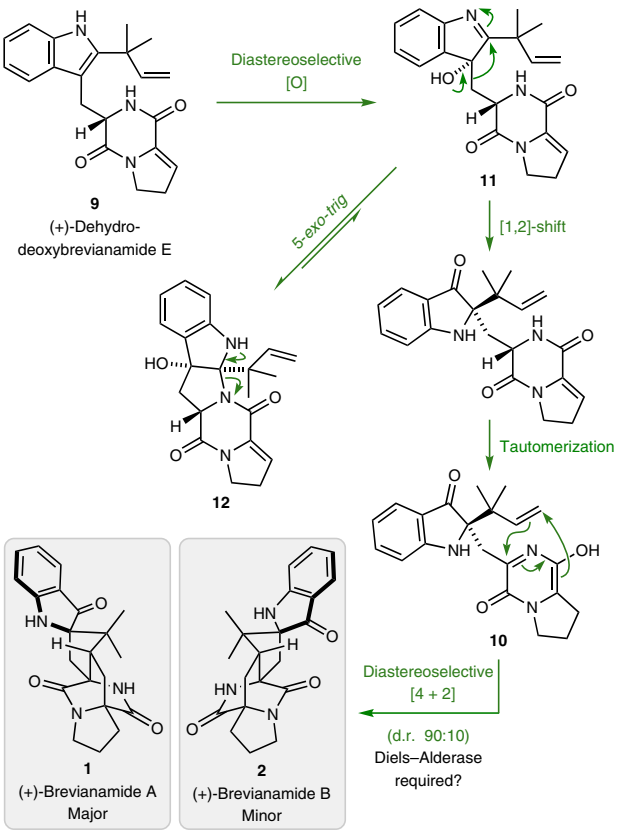

Fig. 3 | A modified biosynthetic proposal for brevianamides A and B. The known natural product (+)-dehydrodeoxybrevianamide E (**9**), which has the diketopiperazine ring at the oxidation level required for a subsequent Diels–Alder reaction, is invoked as an alternative biosynthetic precursor towards brevianamides A (**1**) and B (**2**). This avoids the intermediacy of indoxyl **7** (Fig. 2, pathway 2), the instability of which has thwarted previous biomimetic approaches. Although oxidation of (+)-dehydrodeoxybrevianamide E (**9**) is expected to give compound **12**, akin to the oxidative ring closure of **3** to give **8** (Fig. 2, pathway 2), (−)-dehydrobrevianamide E (**12**) is not a known natural product and is therefore less likely to represent a biosynthetic dead-end.

brevianamide A (**1**), following a strategy inspired by our modified biosynthetic proposal.

Results and discussion

The synthesis of (+)-dehydrodeoxybrevianamide E (**9**) commenced with phthaloyl protection of commercially available L-tryptophan methyl ester **13** (Fig. 4)[42]. The crude product from this reaction, ester **14**, was subjected directly to Danishefsky's reverse prenylation conditions using B-prenyl-9-borabicyclo[3.3.1]nonane to give intermediate **15** in 69% yield over the two steps[43]. 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*-butyloxycarbonyl or trityl has been undertaken in related synthetic endeavours[43,44]. To avoid these extraneous two steps (deprotection/reprotection), we explored the possibility of an S_N2-type demethylation of methyl ester **15**. Although LiI in EtOAc, as reported by Fisher and Trinkle[45], gave the desired product **17**, we found LiCl in DMF gave a cleaner reaction. Lithium carboxylate **17** was then subjected to a one-pot acyl chloride formation and imine acylation reaction with dehydroproline **18** to give *N*-acyl enamine **19**[46]. Attempts to deprotect the primary amine in **19** using conventional phthaloyl deprotection reagents, such as hydrazine, ethylene diamine, methylamine, hydroxylamine, ethanolamine and phenylhydrazine, resulted in unwanted cleavage of the amide bond. Our search for less nucleophilic reagents eventually led us to ammonia in methanol[47], which not only deprotected the primary amine but also resulted in spontaneous cyclization to give (+)-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 total synthesis of (+)-dehydrodeoxybrevianamide E (**9**) has been achieved, proceeding in a longest linear sequence of five steps, in 34% overall yield, and requiring just two chromatographic purifications (cf. previous synthesis: 12 steps, 8% overall yield)[29].

Oxidation of (+)-dehydrodeoxybrevianamide E (**9**) using a variety of oxidants (for example, dioxiranes[43], oxaziridines[23], singlet oxygen[48] and peroxy acids) gave dehydrobrevianamide E (**12**), alongside diastereomer **20** (Fig. 4). Use of *m*-CPBA gave the highest diastereoselectivity (d.r. 64:36, 57% yield), with no appreciable improvement observed at lower temperatures. The stereoselectivity of related biosynthetic indole oxidations is known to be controlled by flavin-dependent monooxygenase enzymes[8]. Synthetic access to both diastereomers **12** and **20**, however, enabled us to prepare both the natural-(+) and unnatural-(−) enantiomers of brevianamides A (**1**) and B (**2**) (see below). Exposure of dehydrobrevianamide E (**12**) to LiOH in water at ambient temperature for 30 min 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 outlined in Fig. 3. Keeping the reaction time to a minimum and avoiding elevated temperatures was essential to prevent alkaline hydrolysis of the products, as previously observed by Birch and co-workers[34]. Following purification by column chromatography, which is only the fourth purification in the entire seven-step synthesis, 750 mg of (+)-brevianamide A (**1**) and 60 mg of (+)-brevianamide B (**2**) were isolated. Chiral-HPLC analysis revealed both products (**1** and **2**) were isolated in a 93:7 enantiomeric ratio, which is not surprising given many of our synthetic intermediates may have undergone partial racemization; recrystallization gave (+)-brevianamide A (**1**) in an enantiomeric ratio of 99:1. The unnatural (−)-enantiomers of brevianamides A (**1**) and B (**2**) were similarly accessed by subjecting the minor diastereomer, compound **20**, to the same reaction conditions (Fig. 4).

The Diels–Alder reaction produces (+)-brevianamide A (**1**) and (+)-brevianamide B (**2**) in a 93:7 diastereomeric ratio (Fig. 4), which closely matches the ratio observed when they are isolated from *Penicillium brevicompactum* (for further details, see Supplementary Section 2)[18]. Thus, we suggest that this Diels–Alder reaction might be a spontaneous process that occurs naturally without direct enzyme participation[37]. This is in contrast to the requirement of Diels–Alderase enzymes in the stereoselective biogenesis of the closely related malbrancheamide and paraherquamide alkaloids[21]. During the review process for this manuscript, Williams, Sherman, Li and co-workers reported their identification of the brevianamide A biosynthetic gene cluster[49]. Their detailed biosynthetic studies, which included targeted gene disruption, heterologous expression, precursor incorporation studies and in vitro biochemical analysis also support a spontaneous, non-enzyme-mediated, biosynthetic Diels–Alder reaction[49].

In summary, 50 years after its discovery by Birch[16], the chemical synthesis of brevianamide A (**1**) has been achieved (seven steps, 7.2% overall yield). Key to the success of this synthesis is a bioinspired one-step cascade transformation of the linearly fused pentacyclic dehydrobrevianamide E (**12**) into the far more complex hexacyclic structure of brevianamide A (**1**). This complexity-generating sequence would probably not have been constructed from a purely retrosynthetic analysis of the target, demonstrating the unique utility of the biomimetic approach in natural product synthesis.

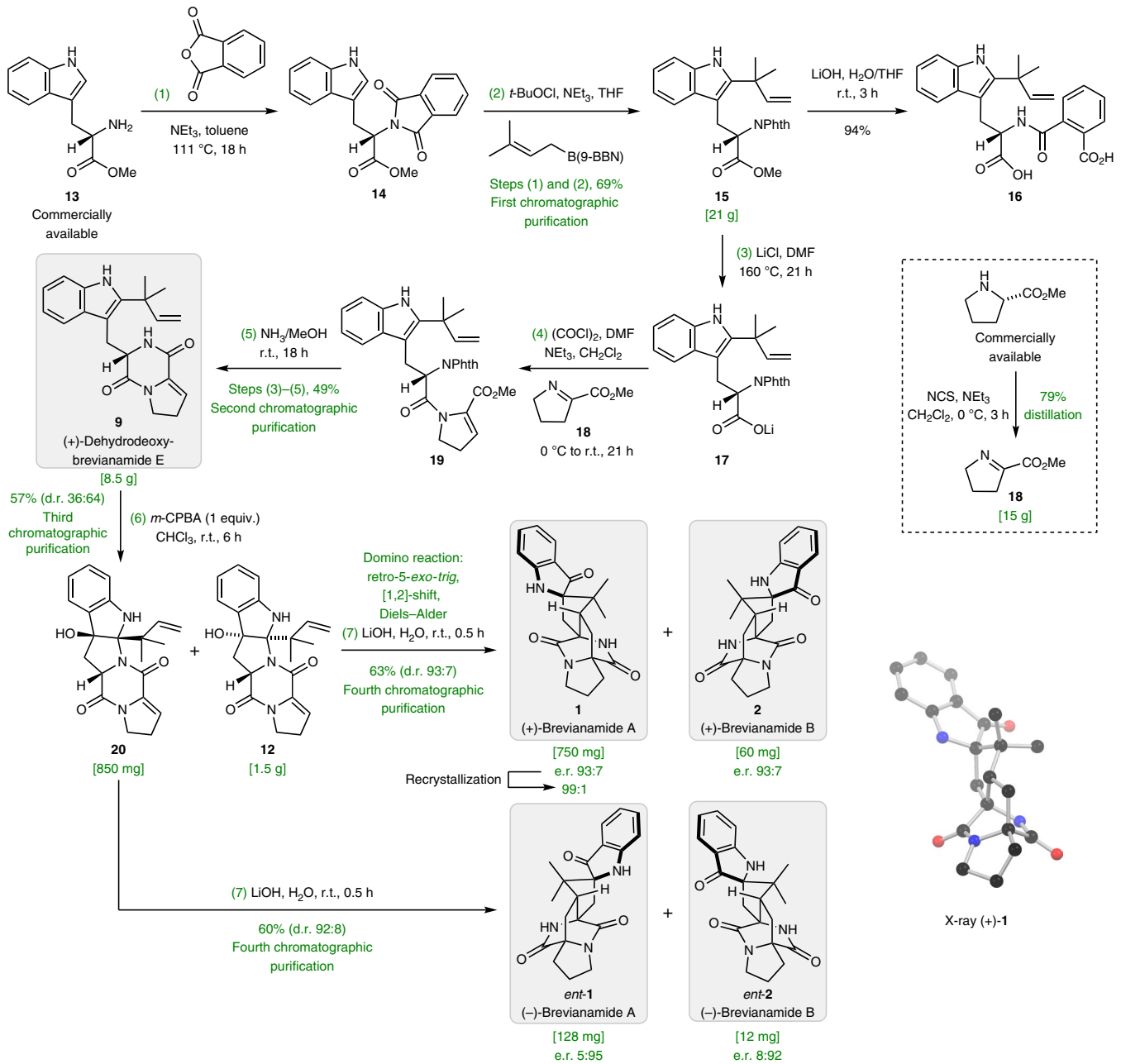

Fig. 4 | Total synthesis of brevianamides A and B. The synthesis begins with the shortest reported total synthesis of (+)-dehydrodeoxybrevianamide E (**9**) (five steps, 34% overall yield, 8.5 g scale). Oxidation of (+)-dehydrodeoxybrevianamide E (**9**) gives a diastereomeric mixture of dehydrobrevianamide E (**12**) and **20**. Exposure of **12** and **20** to LiOH in water gives the natural and unnatural enantiomers of brevianamides A (**1**) and B (**2**), respectively. 9-BBN, 9-borabicyclo[3.3.1]nonane; *m*-CPBA, *meta*-chloroperoxybenzoic acid; DMF, dimethylformamide; NCS, *N*-chlorosuccinimide; Phth, phthaloyl. The black, blue and red spheres represent carbon, nitrogen and oxygen, respectively.

Online content

Any Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41557-020-0442-3.

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References

1. Nicolaou, K. C., Snyder, S. A., Montagnon, T. & Vassilikogiannakis, G. The Diels–Alder reaction in total synthesis. *Angew. Chem. Int. Ed.* **41**, 1668–1198 (2002).
2. Stocking, E. M. & Williams, R. M. Chemistry and biology of biosynthetic Diels–Alder reactions. *Angew. Chem. Int. Ed.* **42**, 3078–3115 (2003).
3. Oikawa, H. & Tokiwano, T. Enzymatic catalysis of the Diels–Alder reaction in the biosynthesis of natural products. *Nat. Prod. Rep.* **21**, 321–352 (2004).
4. Klas, K., Tsukamoto, S., Sherman, D. H. & Williams, R. M. Natural Diels–Alderases: elusive and irresistable. *J. Org. Chem.* **80**, 11672–11685 (2015).
5. Minami, A. & Oikawa, H. Recent advances of Diels–Alderases involved in natural product biosynthesis. *J. Antibiot.* **69**, 500–506 (2016).
6. Jamieson, C. S., Ohashi, M., Liu, F., Tang, Y. & Houk, K. N. The expanding world of biosynthetic pericyclases: cooperation of experiment and theory for discovery. *Nat. Prod. Rep.* **36**, 698–713 (2019).
7. Finefield, J. M., Frisvad, J. C., Sherman, D. H. & Williams, R. M. Fungal origins of the bicyclo[2.2.2]diazaoctane ring system of prenylated indole alkaloids. *J. Nat. Prod.* **75**, 812–833 (2012).

8. Klas, K. R. et al. Structural and stereochemical diversity in prenylated indole alkaloids containing the bicyclo[2.2.2]diazaoctane ring system from marine and terrestrial fungi. *Nat. Prod. Rep.* **35**, 532–558 (2018).
9. Robertson, A. P. et al. Paraherquamide and 2-deoxy-paraherquamide distinguish cholinergic receptor subtypes in *Ascaris* muscle. *J. Pharmacol. Exp. Ther.* **303**, 853–860 (2002).
10. Little, P. R. et al. Efficacy of a combined oral formulation of derquantel-abamectin against the adult and larval stages of nematodes in sheep, including anthelmintic-resistant strains. *Vet. Parasitol.* **181**, 180–193 (2011).
11. Buxton, S. K. et al. Investigation of acetylcholine receptor diversity in a nematode parasite leads to characterization of tribendimidine- and derquantel-sensitive nAChRs. *PLoS Pathog.* **10**, e1003870 (2014).
12. Martínez-Luisa, S. et al. Malbrancheamide, a new calmodulin inhibitor from the fungus *Malbranchea aurantiaca*. *Tetrahedron* **62**, 1817–1822 (2006).
13. Lin, Z. et al. Chrysogenamide A from an endophytic fungus associated with *Cistanche deserticola* and its neuroprotective effect on SH-SY5Y cells. *J. Antibiot.* **61**, 81–85 (2008).
14. Qian-Cutrone, J. et al. Stephacidin A and B: two structurally novel, selective inhibitors of the testosterone-dependent prostate LNCaP cells. *J. Am. Chem. Soc.* **124**, 14556–14557 (2002).
15. Kato, H. et al. Notoamides A–D: prenylated indole alkaloids isolated from a marine-derived fungus, *Aspergillus* sp. *Angew. Chem. Int. Ed.* **46**, 2254–2256 (2007).
16. Birch, A. J. & Wright, J. J. The brevianamides: a new class of fungal alkaloid. *J. Chem. Soc. D* 644–645 (1969).
17. Paterson, R. R. M., Simmonds, M. J. S., Kemmelmeier, C. & Blaney, W. M. Effects of brevianamide A, its photolysis product brevianamide D, and ochratoxin A from two *Penicillium* strains on the insect pests *Spodoptera frugiperda* and *Heliothis virescens*. *Mycol. Res.* **94**, 538–542 (1990).
18. 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).
19. Bird, B. A., Remaley, A. T. & Campbell, I. M. Disposition of mycophenolic acid, brevianamide A, asperphenamate, and ergosterol in solid cultures of *Penicillium brevicompactum*. *Appl. Environ. Microbiol.* **43**, 345–348 (1982).
20. Porter, A. E. A. & Sammes, P. G. A Diels–Alder reaction of possible biosynthetic importance. *J. Chem. Soc. D* 1103a–1103a (1970).
21. Dan, Q. et al. Fungal indole alkaloid biogenesis through evolution of a bifunctional reductase/Diels–Alderase. *Nat. Chem.* **11**, 972–980 (2019).
22. Li, S. et al. Comparative analysis of the biosynthetic systems for fungal bicyclo[2.2.2]diazaoctane indole alkaloids: the (+)/(−)-notoamide, paraherquamide and malbrancheamide pathways. *Med. Chem. Commun.* **3**, 987–996 (2012).
23. Miller, K. A., Tsukamoto, S. & Williams, R. M. Asymmetric total syntheses of (+)- and (−)-versicolamide B and biosynthetic implications. *Nat. Chem.* **1**, 68–69 (2009).
24. 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).
25. 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).
26. 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).
27. 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).
28. 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).
29. Greshock, T. J. & Williams, R. M. Improved biomimetic total synthesis of D,L-stephacidin A. *Org. Lett.* **9**, 4255–4258 (2007).
30. 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).
31. 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).
32. 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).
33. Williams, R. M., Kwast, E., Coffman, H. & Glinka, T. Remarkable, enantiodivergent biogenesis of brevianamide A and B. *J. Am. Chem. Soc.* **111**, 3064–3065 (1989).
34. 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).
35. 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).
36. Baldas, J., Birch, A. J. & Russell, R. A. Studies in relation to biosynthesis. Part XLVI. Incorporation of *cyclo*-L-tryptophyl-L-proline into brevianamide A. *J. Chem. Soc. Perkin Trans. 1*, 50–52 (1974).
37. 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).
38. Domingo, L. R., Sanz-Cervera, J. F., Williams, R. M., Picher, M. T. & Marco, J. A. Biosynthesis of the brevianamides. An ab initio study of the biosynthetic intramolecular Diels–Alder cycloaddition. *J. Org. Chem.* **62**, 1662–1667 (1997).
39. Steyn, P. S. The structures of five diketopiperazines from *Aspergillus ustus*. *Tetrahedron* **29**, 107–120 (1973).
40. Scott, P. M., Kennedy, B. P. C., Harwig, J. & Chen, Y.-K. Formation of diketopiperazines by *Penicillium italicum* isolated from oranges. *Appl. Microbiol* **28**, 892–894 (1974).
41. Kaur, A., Raja, H. A., Deep, G., Agarwal, R. & Oberlies, N. H. Pannorin B, a new naphthopyrone from an endophytic fungal isolate of *Penicillium* sp. *Magn. Reson. Chem.* **54**, 164–167 (2016).
42. 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).
43. 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).
44. Zhao, L., May, J. P., Huang, J. & Perrin, D. M. Stereoselective synthesis of brevianamide E. *Org. Lett.* **14**, 90–93 (2012).
45. 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).
46. 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).
47. Nigst, T. A., Antipova, A. & Mayr, H. Nucleophilic reactivities of hydrazines and amines: the futile search for the α-effect in hydrazine reactivities. *J. Org. Chem.* **77**, 8142–8155 (2012).
48. Kametani, T., Kanaya, N. & Ihara, M. Asymmetric total synthesis of brevianamide E. *J. Am. Chem. Soc.* **102**, 3974–3975 (1980).
49. Ye, Y. et al. Cofactor-independent pinacolase directs non-Diels–Alderase biogenesis of the brevianamides. Preprint at https://doi.org/10.26434/chemrxiv.9122009.v1 (2019).

Data availability

All the characterization data and experimental protocols are provided in this article and its Supplementary Information. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre, under deposition number CCDC 1918446 (compound **1**). Copies of the data can be obtained free of charge via https://www.ccdc.cam.ac.uk/structures/.

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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.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to A.L.L.

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