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Asymmetric total syntheses of (+)- and (−)-versicolamide B and biosynthetic implications

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The Diels–Alder reaction is one of the most well-studied, synthetically useful organic transformations. Although it has been postulated that a significant number of naturally occurring substances arise by biosynthetic Diels–Alder reactions, rigorous confirmation of a mechanistically distinct natural Diels–Alderase enzyme remains elusive. Within this context, several related fungi within the *Aspergillus* genus produce a number of metabolites of opposite absolute configuration, including (+)- or (−)-versicolamide B. These alkaloids are hypothesized to arise via biosynthetic Diels–Alder reactions, implying that each *Aspergillus* species possesses enantiomerically distinct Diels–Alderases. In this paper, experimental validation of these biosynthetic proposals via deployment of the intramolecular hetero-Diels–Alder reaction as a key step in the asymmetric total syntheses of (+)- and (−)-versicolamide B is described. Laboratory validation of the proposed biosynthetic Diels–Alder construction, coupled with the secondary metabolite profile of the producing fungi, reveals that each *Aspergillus* species has evolved enantiomerically distinct indole oxidases, as well as enantiomerically distinct Diels–Alderases.

A seemingly limitless array of natural secondary metabolites are produced in nature from plants, microorganisms and animals in both terrestrial and marine environments. In the overwhelming majority of cases, these secondary metabolites, commonly referred to as 'natural products', are produced in optically pure form (when chiral). In the plant kingdom, examples of different species producing enantiomerically opposite metabolites are well documented but rare. Some of the most well-known examples are terpenes, such as carvone, camphor and limonene. These substances arise from an initial enzyme-catalysed cyclization of geranyl pyrophosphate, and the observation of antipodal terpenes in nature has been attributed to species- or genus-specific enantiodivergent cyclases, which cyclize geranyl pyrophosphate to give opposite enantiomers of limonene or borneol[1]. These are further metabolized to give other enantiomerically distinct terpenes.

Although they are much rarer, different genera of marine sponge have also been reported to produce antipodal, manzamine alkaloids, with an *Iricina* species producing ircinal A and B and an *Amphimedon* species producing the antipodal congeners iricinol A and B (ref. 2). This example is particularly notable in that an intramolecular Diels–Alder reaction from an achiral substrate has been invoked in the proposed biosynthesis of these manzamine alkaloids[3]. The above examples reveal that antipodal natural products often result from the action of stereochemically distinct enzymes that can give single and opposite enantiomeric products from achiral substrates.

The diverse family of prenylated indole alkaloids containing a bicyclo[2.2.2]diazaoctane core isolated from both terrestrial and marine fungi have been the subject of intense research efforts owing to their complex molecular structures and range of biological activities[4,5] (Fig. 1). Members of this family of prenylated indole alkaloids have been reported to display insecticidal, antitumor, antihelmintic, calmodulin-inhibitory and antibacterial properties[6–8].

Work from our laboratory[4], as well as that from Sammes[9] and Birch[10], has revealed that these alkaloids are all derived from one or two mevalonate-derived isoprene units, tryptophan, and a cyclic amino acid such as proline, β-methylproline or pipecolic acid. Significant experimental evidence suggests that the bicyclo[2.2.2]diazaoctane core common to all of these natural products arises biosynthetically via an intramolecular hetero-Diels–Alder (IMDA) reaction of a 5-hydroxypyrazin-2(1*H*)-one[4,9]. Indeed, we have applied such IMDA cycloaddition strategies to the total synthesis of several of these prenylated indole alkaloids, including D,L-stephacidin A (refs 11,12), D,L-brevianamide B (refs 13–15), D,L-marcfortine C (ref. 16), D,L-notoamide B (ref. 12), D,L- and (−)-VM55599 (refs 17,18), and, most recently, D,L-malbrancheamide and D,L-malbrancheamide B (ref. 19).

Within the family of prenylated alkaloids containing a bicyclo[2.2.2]diazaoctane core, two distinct stereochemistries have been noted with respect to the relative configuration at the C19 stereogenic centre (sclerotiamide numbering, Fig. 1). Whereas the brevianamides **9** and **10** possess an *anti* relative configuration, all of the members of the paraherquamide and notoamide family, such as **1**, **2**, **5**, **6** and **7**, possess a *syn* relative configuration. To date, all of the stephacidins isolated also possess a *syn* configuration (C6, stephacidin numbering), except for one notable exception, versicolamide B (**8**; ref. 20). Owing in part to this stereochemical anomaly, versicolamide B (**8**) attracted our interest from both a biogenetic and a synthetic perspective.

As mentioned above, it has been hypothesized that the bicyclo[2.2.2]diazaoctane core common to all of these natural products arises biosynthetically via an IMDA reaction, and evidence is mounting that this key transformation is most likely enzyme-mediated. Enzyme-catalysed Diels–Alder reactions have been proposed in the biosynthesis of a large number of natural products[21]; however, rigorous proof that a mechanistically distinct enzyme can indeed catalyse this pericyclic cycloaddition reaction through the requisite concerted transition state is still lacking. With that in mind, a persuasive body of work has accumulated that indirectly supports the existence of these elusive Diels–Alderase enzymes, and biomimetic total syntheses of various natural products have had a major role in providing this evidence. For example, Baldwin and Whitehead initially proposed that the manzamine alkaloids arise by an intramolecular Diels–Alder cycloaddition of an achiral

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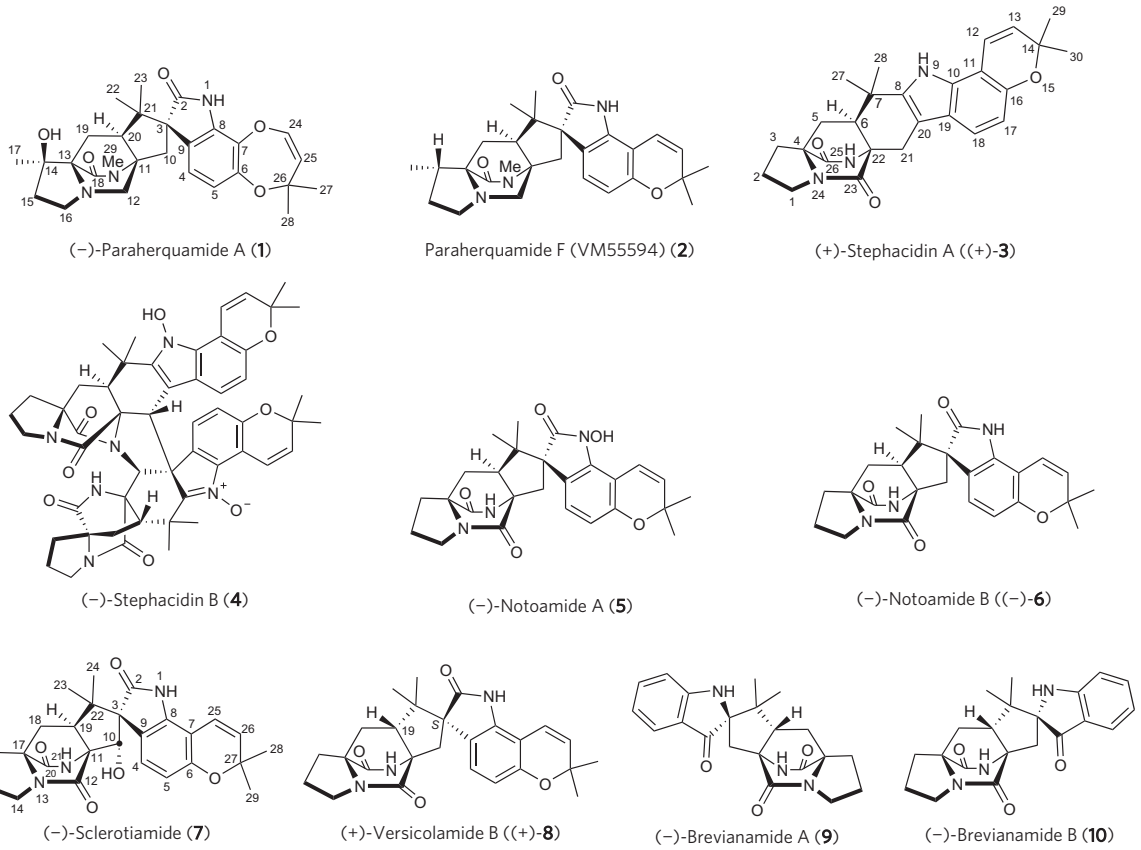

Figure 1 | Several representative prenylated indole alkaloids. In addition to the diverse biological activity exhibited by these natural products, their structural diversity is also notable. Despite this structural diversity, all of these compounds share a bicyclo[2.2.2]diazaoctane core, which is thought to arise biosynthetically through a hetero-Diels–Alder reaction. Two distinct stereochemistries have been observed with respect to the relative configuration at the C19 stereogenic centre (sclerotiamide numbering), either an *anti* configuration (for example **9** and **10**) or a *syn* configuration (for example **1** and **2**). Versicolamide B is the only member of the stephacidin family to possess an *anti* configuration.

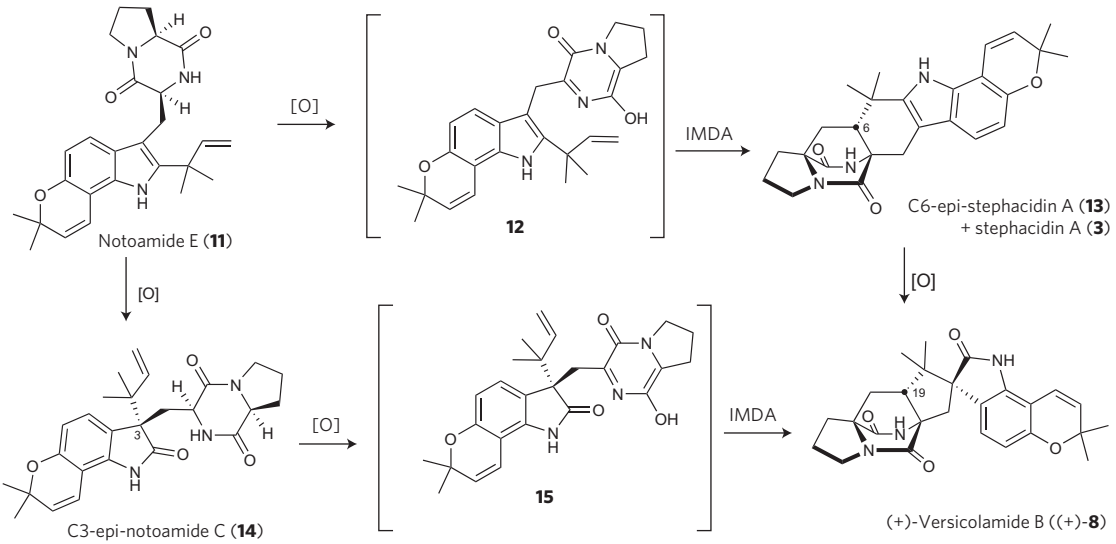

Figure 2 | Two proposed biosynthetic pathways to (+)-versicolamide B. One pathway is oxidation of notoamide E (**11**) to the corresponding azadiene **12**, followed by IMDA and indole oxidation (top pathway). Alternatively, indole oxidation could occur first to give the oxindole **14**, followed by a subsequent oxidation to provide the azadiene **15** and IMDA (bottom pathway).

bispyridinium macrocyclic species[3]. Subsequent isolation of these bispyridinium alkaloids from the producing organism provided support for this biosynthetic proposal[22], and elegant synthetic work further confirmed that these alkaloids do indeed undergo Diels–Alder reaction in the laboratory to provide keramaphidin B (ref. 23), a precursor to the manzamide alkaloids, albeit in very low yield. Although their synthesis produced racemic keramaphidin B, the fact that this and other manzamine alkaloids are isolated as

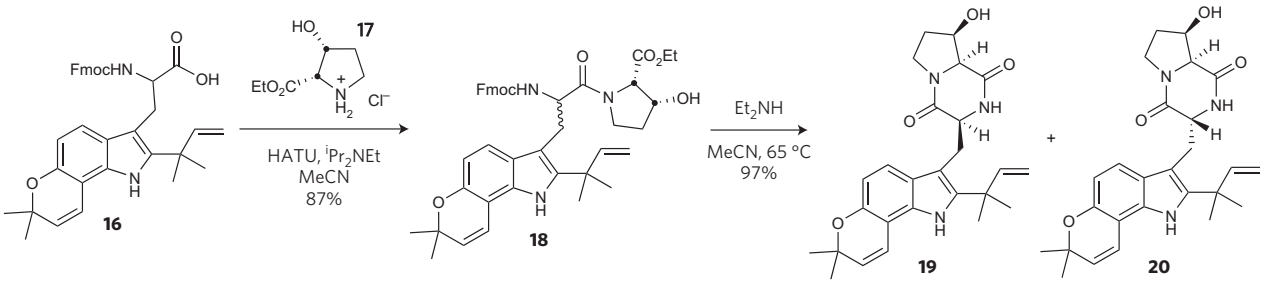

Figure 3 | Preparation of the dioxopiperazines 19 and 20. Straightforward amino acid coupling followed by deprotection and cyclization gave a mixture of *cis*- and *trans*-dioxopiperazines **19** and **20**, respectively. HATU = *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate.

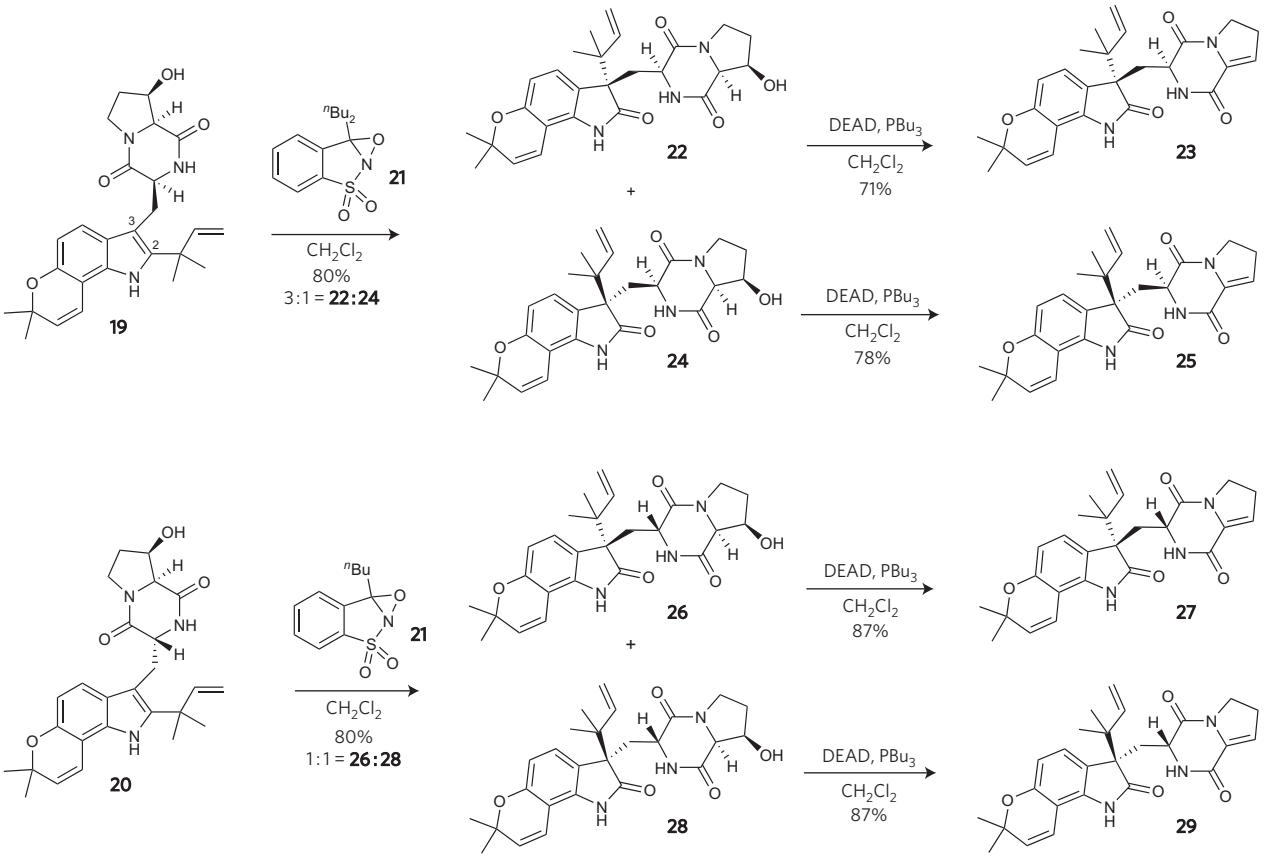

Figure 4 | Preparation of the Diels–Alder precursors 23, 25, 27 and 29. Both dioxopiperazine substrates **19** and **20** were individually oxidized to give mixtures of oxindoles, each of which was dehydrated. As a result, all four possible stereoisomeric enamide Diels–Alder precursors could be obtained: **23**, **25**, **27** and **29**. DEAD = diethyl azodicarboxylate.

single enantiomers suggests that the putative biosynthetic Diels–Alder reaction takes place in the chiral environment of an enzyme.

Ichihara and co-workers provided particularly compelling evidence of a possible enzyme-catalysed Diels–Alder reaction in nature by studying the biosynthesis of the solanapyrones[24]. By carrying out feeding studies, they observed incorporation of isotopically labelled, achiral Diels–Alder precursors into solanapyrone A, and they also showed that the isotopically labelled solanapyrone A was enantiomerically enriched, thus convincingly eliminating the possibility of a spontaneous cycloaddition. It should also be noted that alternative, stepwise (non-concerted) pathways can be invoked to rationalize the construction of molecular skeletons that otherwise seem to be Diels–Alder constructions[25]. As the number of secondary metabolites that can be rationalized as arising from a key, biosynthetic Diels–Alder reaction accumulates, especially in cases where the candidate achiral Diels–Alder substrates lead to enantiomerically pure products, the case for the existence of Diels–Alderase enzymes in nature has become increasingly compelling.

From a biogenetic perspective, we envisioned that versicolamide B could plausibly arise via two distinct pathways, as illustrated in Fig. 2. Oxidation of notoamide E (**11**) could lead to the azadiene **12**, which undergoes an IMDA cycloaddition directly providing stephacidin A (**3**) and C6-epi-stephacidin A (**13**). From compound **13**, oxidation of the indole and subsequent pinacol rearrangement would give rise to versicolamide B. Notably, we have demonstrated that the laboratory oxidation and pinacol rearrangement of C6-epi-stephacidin A (**13**) does indeed provide versicolamide B (**8**), and identical reaction conditions provide notoamide B (**6**) from stephacidin A. However, further complicating this biogenetic proposal was the recent and provocative discovery that the (−)-stephacidin A ((−)-**3**) and (+)-notoamide B ((+)-**6**) isolated from the producing organism, *Aspergillus versicolor* NRRL 35600, were the enantiomers

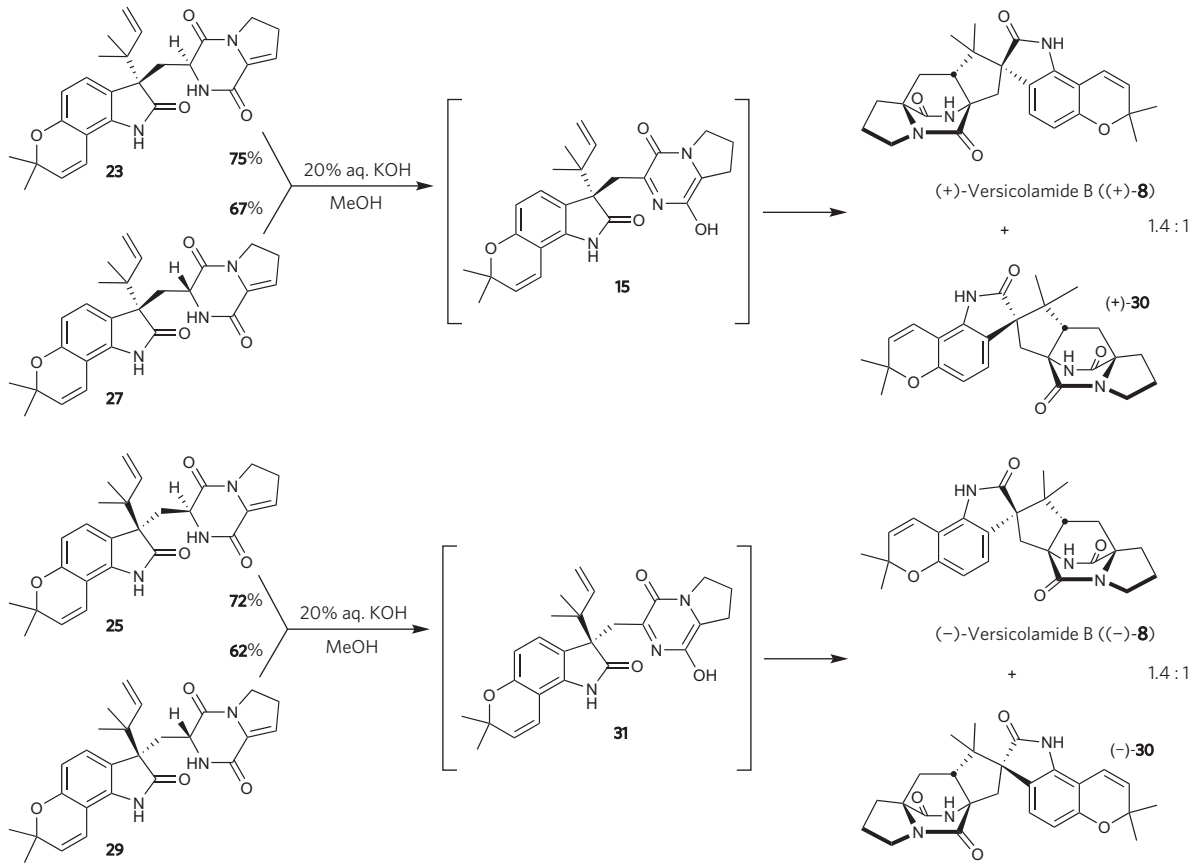

Figure 5 | Biomimetic Diels–Alder cycloaddition reactions. Tautomerization of either enamide, **23** or **27**, under basic conditions led to the azadiene **15**, which efficiently and spontaneously underwent IMDA reaction to give (+)-versicolamide B and the other *anti* diastereomer (+)-**30**. Starting with either enamide **25** or **29**, identical reaction conditions gave (−)-versicolamide B and the *anti* diastereomer (−)-**30** after IMDA reaction.

of (+)-stephacidin A and (−)-notoamide B isolated by Tsukamoto and co-workers from a marine-derived *Aspergillus* species obtained from the common mussel harvested off the Noto peninsula in the Sea of Japan. The Tsukamoto *Aspergillus* species produces (+)-stephacidin A with the same absolute stereochemistry as that originally described by Bristol-Myers Squibb from *A. ochraceus*. Moreover, Tsukamoto and co-workers have recently identified (−)-versicolamide B from their marine-derived *Aspergillus* species. Thus, both enantiomers of all three natural products, stephacidin A, notoamide B and versicolamide B, exist in nature as secondary metabolites of distinct species of the *Aspergillus* genus. To date, C6-epi-stephacidin A (**13**) has yet to be detected as a natural metabolite from *Aspergillus* species, and that has raised questions concerning the validity of this chemically feasible pathway with respect to the biogenesis of versicolamide B (**8**).

Here, we wish to suggest an alternative pathway wherein oxidation and pinacol rearrangement of notoamide E (**11**) might occur first, furnishing the oxindole C3-epi-notoamide C (**14**), which has recently been detected by Tsukamoto and co-workers as a natural metabolite of the marine-derived *Aspergillus* species. Subsequent oxidation of **14** could provide the azadiene **15**, which, on Diels–Alder reaction, would provide **8**. *Ab initio* calculations[26,27], as well as previous synthetic studies, suggest that the oxindole Diels–Alder substrate **15** would undergo cycloaddition to provide the *anti* stereochemistry at C19 in a highly selective, if not exclusive, fashion. Notably, the Diels–Alder reaction of **12** takes place via an achiral azadiene, raising questions concerning the mechanism by which the paraherquamides, notoamides and stephacidins are biosynthesized in enantiomerically pure form[20]. We have endeavoured to secure experimental support to validate these biogenetic hypotheses, and the corroborating enantioselective total syntheses of (+)- and (−)-versicolamide B (**8**) are reported herein.

The discovery of distinct *Aspergillus* species that produce at least three secondary metabolites with the opposite absolute configurations is striking and demands both a genetic and a mechanistic explanation. The possibility that individual species of the *Aspergillus* genus have evolved enantiomerically distinct genes that direct the biosynthesis of either (+)- or (−)-versicolamide B as well as the corresponding enantiomeric pairs of stephacidin A and notoamide B was unexpected and, to us, fascinating. We have endeavoured to accumulate experimental support for the biosynthetic hypotheses illustrated in Fig. 2 by undertaking biomimetic total syntheses of (+)- and (−)-versicolamide B through the putative azadiene **15** that would, at the molecular level, validate the postulated pathways.

Results

The syntheses began with the known protected amino acid **16**, which has been used as a synthetic intermediate for the total syntheses of notoamides B, C and D and of stephacidin A (Fig. 3)[12]. Coupling of **16** with (*R*)-*cis*-3-hydroxy-L-proline (**17**) gave the amide **18** as a 1 : 1 mixture of diastereomers, which underwent fluorenylmethyloxycarbonyl (Fmoc) deprotection on treatment with diethylamine, and concomitant cyclization to provide the readily separable dioxopiperazines **19** and **20**.

With the substrates **19** and **20** in hand, we next directed our attention to oxidation of the indole C2–C3 double bond and Diels–Alder cycloaddition to complete the synthesis (Fig. 4). Treatment of the *cis*-dioxopiperazine **19** with the oxaziridine reagent **21** resulted in oxidation of the indole C2–C3 double bond followed by pinacol rearrangement to afford the oxindoles **22** and

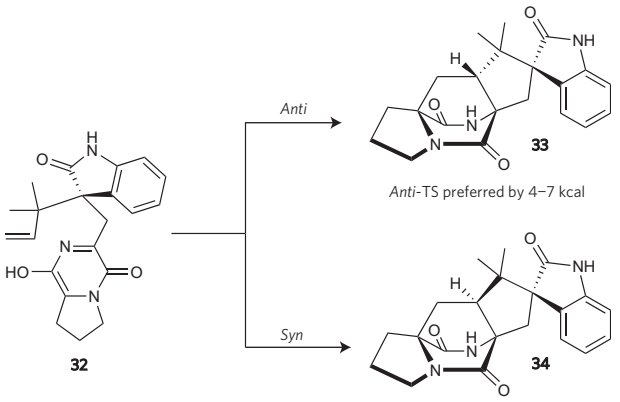

Figure 6 | Molecular modelling in support of the observed Diels–Alder stereochemical preference. *Ab inito* calculations by Domingo and co-workers[26,27] reveal a strong *anti* selectivity for the Diels–Alder reaction of the simplified model system **32**. TS = transition state.

24 in a 3:1 ratio. The absolute stereochemical configuration of these oxindoles was confirmed by circular dichroism (CD) spectroscopy[28] (see Supplementary Information), as well as by comparison of the optical rotation and CD spectrum of the synthetic **8** derived from **22** and **24** with that of the natural product. Mitsunobu dehydration of **22** and **24** gave the enamides **23** and **25** respectively, which would serve as Diels–Alder precursors. The same series of transformations were carried out starting with the *trans*-dioxopiperazine substrate **20** to arrive at the enamide Diels–Alder precursors **27** and **29**. In this case, indole oxidation and pinacol rearrangement provided the oxindoles **26** and **28** in a 1:1 ratio. In such a fashion, the preparation of all four possible Diels–Alder stereoisomer precursors was realized.

Treatment of the enamide **23** or **27** with KOH resulted in tautomerization to provide the azadiene **15**, which spontaneously underwent IMDA cycloaddition reaction to afford a mixture of (+)-versicolamide B ((+)-**8**) and the diastereomer (+)-**30** in a 1.4:1 ratio. The enamide **25** or **29**, under identical reaction conditions, provided a mixture of (−)-versicolamide B ((−)-**8**) and (−)-**30**, also in a 1.4 : 1 ratio (Fig. 5). As predicted by *ab initio* calculations[26,27], only the *anti* cycloadducts were isolated from the hetero-Diels–Alder reactions of the oxindolic azadienes **15** and **31**, and no *syn* cycloadducts were detected. The exclusive preference for the *anti* cycloadducts is particularly striking, considering that all previous cycloadditions using indole-based azadienes, such as **12**, had modest *syn* selectivity (typically about 2.5 : 1 *syn* : *anti*). The current work complements previous experimental support[29,30] for the theoretical calculations of Domingo and co-workers, who investigated the slightly simpler model system **32** (Fig. 6). These calculations predicted a 4–7 kcal mol^{-1} preference for the formation of the *anti* cycloadduct **33**. The absolute configurations of the cycloadducts were confirmed by CD spectroscopy and comparison with that of natural (+)-versicolamide B ((+)-**8**). Working backwards, we could corroborate the absolute configurations of all of the oxindole intermediates **22**–**29**.

Discussion

Confirmation by experiment that the Diels–Alder reaction of **23** or **27** yields (+)-versicolamide B ((+)-**8**) leads to a number of intriguing biosynthetic possibilities, which could help to explain an unresolved stereochemical paradox with regard to the biosynthetic relationships between (+)-versicolamide B ((+)-**8**), (−)-stephacidin A ((−)-**3**) and (+)-notoamide B ((+)-**6**). Previous work from our laboratory revealed that the fungus *Aspergillus versicolor* NRRL 35600, from which (+)-versicolamide B ((+)-**8**) was isolated, produces the opposite enantiomers of stephacidin A (**3**) and notoamide B (**6**) to those obtained from the related fungus *A. ochraceus* WC76466 (ref. 7) and from the marine-derived *Aspergillus* species, studied by Tsukamoto and co-workers[31]. As mentioned above (see Fig. 2), versicolamide B ((+)-**8**) can arise biogenetically from oxidation and pinacol rearrangement of C6-epi-stephacidin A (**13**) or from Diels–Alder reaction of the oxindolic azadiene **15**. The observation that (+)-versicolamide B ((+)-**8**) is pseudoenantiomeric to both (−)-stephacidin A ((−)-**3**) and (+)-notoamide B ((+)-**6**), all of which are isolated from the same organism, suggests that (+)-versicolamide B ((+)-**8**) might plausibly arise from the latter pathway involving IMDA reaction of the oxindolic azadiene **15** derived from the natural metabolite C3-epi-notoamide C (**14**). If such a pathway is operative, we anticipate that perhaps the versicolamide diastereomer (+)-**30** is an as-yet undetected minor metabolite, and efforts to identify this compound are underway. Alternatively, perhaps each species of *Aspergillus* possesses a distinct and enantiomerically opposite Diels–Alderase enzyme that pre-organizes the azadiene substrates such that only one enantiomeric cycloadduct is produced.

In conclusion, the first asymmetric total synthesis of each enantiomer of versicolamide B (**8**) has been achieved. This work provides the first experimental support for the biogenetic hypothesis that versicolamide B (**8**) possibly arises from IMDA reaction of an oxindolic substrate such as **15**. The preference for exclusive formation of the *anti* cycloadducts in this work stands in stark contrast to the modest *syn* selectivity exhibited by all of the previous examples of hetero-Diels–Alder reactions on indole-based azadienes[4–8]. The total synthesis recorded herein will enable the preparation of a number of ^{13}C-labelled biosynthetic intermediates that will be useful for biosynthetic precursor incorporation experiments that are planned. That work is the subject of current investigation, and will be reported in due course.

Methods

^{1}H- and ^{13}C-NMR spectra were obtained using 300 or 400 MHz spectrometers. The chemical shifts are given in parts per million (ppm) relative to tetramethylsilane at δ 0.00 ppm or $CDCl_3$ at δ 7.27 ppm for proton spectra and relative to $CDCl_3$ at δ 77.23 ppm for carbon spectra. Infrared spectra were recorded on a Fourier-transform infrared (FTIR) spectrometer as thin films. Mass spectra were obtained using a high/low-resolution magnetic sector mass spectrometer. Flash column chromatography was performed with silica gel grade 60 (230–400 mesh). Unless otherwise noted, materials were obtained from commercially available sources and used without further purification. Dichloromethane (CH_2Cl_2), tetrahydrofuran (THF), toluene (PhMe), benzene (PhH), *N,N*-dimethylformamide (DMF), acetonitrile (CH_3CN), triethylamine (Et_3N), *N,N*-diisopropylamine and methanol (MeOH) were all degassed with argon and passed through a solvent purification system containing alumina or molecular sieves.

Representative procedure for Mitsunobu dehydration. (*S*)-3-(((*R*)-7,7-dimethyl-3-(2-methylbut-3-en-2-yl)-2-oxo-1,2,3,7-tetrahydropyrano[2,3-*g*]indol-3-yl)methyl)-2,3,6,7-tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**23**). A solution of diethyl azodicarboxylate (DEAD; 161 mg solution, 168 µl, 40% in toluene, 0.369 mmol) was added to a solution of **22** (57 mg, 0.123 mmol) in CH_2Cl_2 (5 ml) at room temperature. The reaction was stirred for 5 min, and then PBu_3 (75 mg, 92 µl, 0.369 mmol) was added. The reaction was stirred at room temperature for 12 h, after which time the entire contents were concentrated under reduced pressure. The residue was purified by flash chromatography eluting with EtOAc/hexanes (1:1–1:0) to give 39 mg (71%) of **23** as a colourless oil; $[\alpha]_D^{25} = +81.3$ ($c = 0.07$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.27 (s, 1H), 8.09 (d, $J = 4.1$ Hz, 1H), 6.96 (d, $J = 8.1$ Hz, 1H), 6.52 (d, $J = 10.0$ Hz, 1H), 6.48 (d, $J = 8.2$ Hz, 1H), 6.05 (dd, $J = 17.4$, 10.8 Hz, 1H), 5.98 (t, $J = 3.0$ Hz, 1H), 5.62 (d, $J = 9.9$ Hz, 1H), 5.10 (d, $J = 10.8$ Hz, 1H), 5.01 (d, $J = 17.4$ Hz, 1H), 4.05 (dt, $J = 10.9$, 5.4 Hz, 1H), 3.84–3.57 (comp, 2H), 2.84–2.27 (comp, 4H), 1.50 (s, 3H), 1.42 (s, 3H), 1.15 (s, 3H), 1.04 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.0, 164.0, 158.5, 153.2, 143.1, 138.5, 133.2, 129.6, 126.2, 119.7, 119.4, 118.5, 114.3, 109.6, 107.0, 76.1, 56.2, 55.9, 45.6, 42.7, 39.6, 28.9, 27.9, 27.3, 22.1, 21.9; IR (neat) 3184, 1704, 1681, 1645, 1447, 912, 732 cm^{-1}; HRMS (TOF +) calculated for $C_{26}H_{30}N_3O_4$ (M + H) 448.2231, found 448.2239.

(*S*)-3-(((*S*)-7,7-dimethyl-3-(2-methylbut-3-en-2-yl)-2-oxo-1,2,3,7-tetrahydropyrano[2,3-*g*]indol-3-yl)methyl)-2,3,6,7-tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**25**). The enamide **25** was obtained in 78% yield (0.077 mmol scale) according to the representative procedure as a clear, colourless oil after flash

chromatography eluting with EtOAc/hexanes (1 : 1–1 : 0); $[\alpha]_D^{25} = +103.9$ ($c = 0.1$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.84 (s, 1H), 8.79 (s, 1H), 6.90 (d, $J = 8.2$ Hz, 1H), 6.58 (d, $J = 9.9$ Hz, 1H), 6.29 (d, $J = 8.2$ Hz, 1H), 6.12 (dd, $J = 17.4$, 10.8 Hz, 1H), 5.69–5.66 (comp, 2H), 5.10 (d, $J = 10.8$ Hz, 1H), 5.00 (d, $J = 17.4$ Hz, 1H) 4.26 (d, $J = 7.1$ Hz, 1H), 3.88–3.64 (comp, 2H), 2.94 (d, $J = 14.3$ Hz, 1H), 2.73–2.44 (comp, 3H), 1.47 (s, 3H), 1.40 (s, 3H), 1.13 (s, 3H), 1.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 184.4, 162.8, 157.6, 153.2, 143.3, 139.4, 132.3, 129.9, 128.1, 119.2, 117.4, 117.2, 114.0, 108.3, 106.4, 76.2, 56.1, 55.8, 45.6, 42.5, 34.1, 29.3, 27.6, 27.4, 22.2, 21.7; IR (neat) 3191, 1703, 1680, 1645, 1453, 909, 731 cm^{-1}; HRMS (TOF +) calculated for $C_{26}H_{30}N_3O_4$ (M + H) 448.2231, found 448.2228.

(*R*)-3-(((*R*)-7,7-dimethyl-3-(2-methylbut-3-en-2-yl)-2-oxo-1,2,3,7-tetrahydropyrano[2,3-*g*]indol-3-yl)methyl)-2,3,6,7-tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**27**). The enamide **27** was obtained in 87% yield (0.47 mmol scale) according to the representative procedure as a clear, colourless oil after flash chromatography eluting with EtOAc/hexanes (1 : 1–1 : 0); $[\alpha]_D^{25} = -97.6$ ($c = 0.1$, $CHCl_3$); all other spectral data (^{1}H NMR, ^{13}C NMR, IR and HRMS) matched those of **25**.

(*R*)-3-(((*S*)-7,7-dimethyl-3-(2-methylbut-3-en-2-yl)-2-oxo-1,2,3,7-tetrahydropyrano[2,3-*g*]indol-3-yl)methyl)-2,3,6,7-tetrahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (**29**). The enamide **29** was obtained in 78% yield (0.18 mmol scale) according to the representative procedure as a clear, colourless oil after flash chromatography eluting with EtOAc/hexanes (1 : 1–1 : 0); $[\alpha]_D^{25} = -81.8$ ($c = 0.1$, $CHCl_3$); all other spectral data (^{1}H NMR, ^{13}C NMR, IR and HRMS) matched those of **23**.

Representative procedure for the hetero-Diels–Alder reaction of enamides. (+)-Versicolamide B ((+)-**8**) and cycloadduct (+)-**30**. To a solution of **23** (55 mg, 0.12 mmol) in MeOH (10 ml) at 0 °C was added 20% aqueous KOH (3 ml). The reaction was warmed to room temperature and was stirred for 12 h. The reaction was quenched with saturated NH_4Cl (30 ml) and extracted with CH_2Cl_2 (3 × 30 ml). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography eluting with MeOH/CH_2Cl_2 (3 : 97) to give 41 mg (75%) of the mixture of (+)-**8** and (+)-**30** as a white film. The mixture of diastereomers was separated by preparative thin-layer chromatography (EtOAc/hexanes, 3 : 1) for characterization purposes; data for the higher-R_f diastereomer (+)-**30**: $[\alpha]_D^{25} = +53$ ($c = 0.1$, acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (s, 1H), 7.42 (s, 1H), 6.90 (d, $J = 8.2$ Hz, 1H), 6.44 (s, 1H), 6.41 (d, $J = 3.1$ Hz, 1H), 5.73 (d, $J = 9.9$ Hz, 1H), 3.49 (t, $J = 6.7$ Hz, 2H), 2.92 (d, $J = 15.4$ Hz, 1H), 2.78 (m, 1H), 2.60 (m, 1H), 2.41 (d, $J = 15.4$ Hz, 1H), 2.06–1.82 (comp, 5H), 1.44 (s, 3H), 1.41 (s, 3H), 1.18 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 183.1, 172.9, 169.5 152.9, 136.4, 131.5, 125.7, 125.0, 116.3, 110.0, 105.7, 76.4, 69.6, 69.1, 63.2, 55.0, 48.1, 44.0, 35.7, 29.7, 29.2, 28.0, 27.8, 27.1, 25.0, 22.1; IR (neat) 3233, 2973, 1708, 1643, 1458, 909, 731 cm^{-1}; HRMS (TOF+) calculated for $C_{26}H_{30}N_3O_4$ (M + H) 448.2231, found 448.2228. All spectral data for (+)-versicolamide B ((+)-**8**) were identical to those previously reported.

(−)-Versicolamide B ((−)-**8**) and cycloadduct (−)-**30**. A mixture of (−)-**8** and (−)-**30** was obtained in 72% yield (0.31 mmol scale) according to the representative procedure as a white film after flash chromatography eluting with MeOH/CH_2Cl_2 (3 : 97); data for the higher-R_f diastereomer (−)-**30**: $[\alpha]_D^{25} = -51$ ($c = 0.1$, acetone); all other spectral data (^{1}H NMR, ^{13}C NMR, IR and HRMS) matched those of (+)-**30**; data for the lower-R_f diastereomer (−)-**8**: $[\alpha]_D^{25} = -34$ ($c = 0.1$, acetone); all other spectral data (^{1}H NMR, ^{13}C NMR, IR and HRMS) matched those of (+)-**8**.

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Author contributions

R.M.W and K.A.M conceived the experiments. K.A.M performed the laboratory experiments and analysed the results. S.T. discovered (−)-versicolamide B as a natural metabolite of a marine-derived *Aspergillus* species. K.A.M and R.M.W wrote the paper.

Additional information

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