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Biomimetic Diels–Alder Cyclizations for the Construction of the Brevianamide, Paraherquamide, Sclerotamide, Asperparaline and VM55599 Ring Systems

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Abstract—A potentially bio-mimetic Diels–Alder cyclization to construct the bicyclo[2.2.2] ring system common to the paraherquamides, marcfortines, sclerotamides, brevianamides, VM55599, and asperparaline is reported. *Epi*-deoxybrevianamide E (**22**) is converted into the corresponding lactim ether (**23**) and then oxidized with DDQ to provide an azadiene (**24**) which is tautomerized in the presence of base to azadiene **25** which, spontaneously cyclizes to give a 2:1 mixture of cycloadducts **26** and **27**. These cycloadducts are each in turn, converted into D,L-C-19-*epi*-brevianamide A (**20**) and D,L-brevianamide B (**6**). The stereochemical implications of the [4 + 2] cycloaddition is discussed in the context of a working hypothesis on the biosynthesis of this family, particularly VM55599.

Introduction

The paraherquamides (**1**, **2**),[1] marcfortines (**3**),[2] sclerotamides (**4**),[3] brevianamides (**5**, **6**),[4] VM55599 (**7**),[5] and most recently, asperparaline (aspergillimide, **8**)[6] are indolic secondary mold metabolites isolated from various fungi (Fig. 1). This family has attracted considerable attention due to their molecular complexity, intriguing biogenesis[7] and some members, most notably the paraherquamides, display potent anti-parasitic activity.[8] These substances are the result of a mixed biogenesis, being derived from isoprene units oxidatively woven into an ral metabolite). Significantly, the enantiomorphic bicyclo[2.2.2] ring system that has been proposed to arise via the [4 + 2] cycloaddition of the isoprene moiety across the oxidized α-carbons of the amino acid units.[9] A striking stereochemical difference between the brevianamides and all of the other members of this family, is the relative stereochemical relationship at the tertiary carbon at C-19 (brevianamide numbering) which is *anti*-[10] in the brevianamides and *syn*- for all of the others.

Previous work on the biosynthesis of these substances, invoked a facial divergence in the putative Diels–Alder cyclization which sets the relative stereochemical relationship at this stereogenic center.[11] In addition, the brevianamides are the result of oxidation at the indolic 3-position while, the paraherquamides, marcfortines and sclerotamides are the consequence of indole oxidation at the 2-position (indole numbering).

One of the original proposals on the biosynthesis of the brevianamides, postulated the conversion of brevianamide F (**9**) into deoxybrevianamide E (**10**), followed by a 2-electron oxidation and enolization to afford the azadiene **11** (Fig. 2).[7d] Intramolecular Diels–Alder cycloaddition would then furnish *racemic* **12** which, was proposed to be converted into the two optically pure diastereomers **13** and **14** by a pro-(*R*)-selective indole oxidase. Final spiro-rearrangement culminates in brevianamide A (**5**, the major natural metabolite) and brevianamide B (**6**, the minor natural metabolite). Significantly, the enantiomorphic bicyclo[2.2.2] ring systems extant in **5** and **6** were, in principle, accommodated by this scheme. However, subsequent total synthesis of

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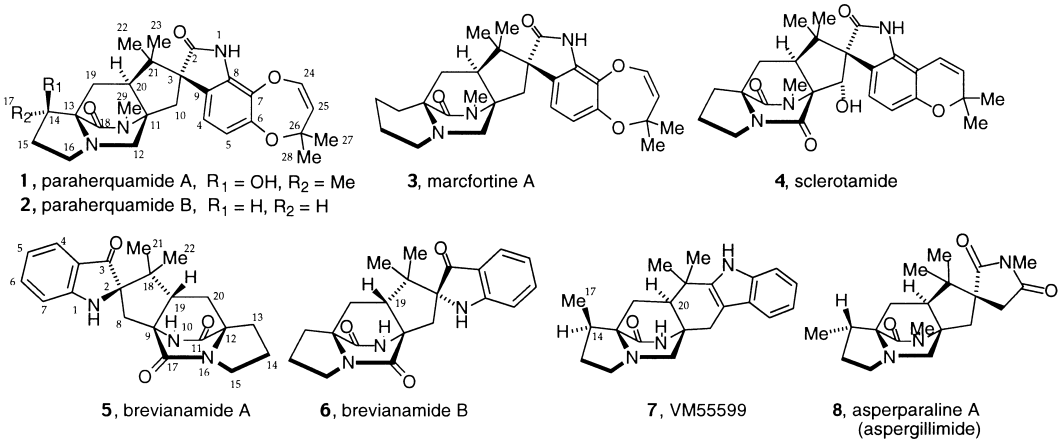

Figure 1.

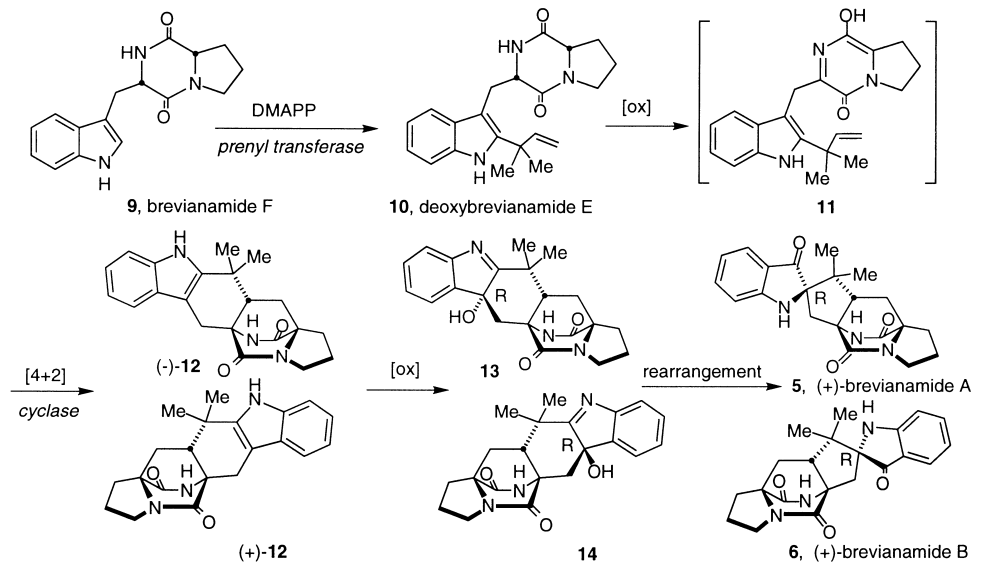

Figure 2. Early biosynthetic proposal for brevianamides A and B.

^{13}C-labeled, racemic **12** and feeding experiments with the producing organism *Penicillium brevicompactum*, failed to provide experimental evidence for the intermediacy of and/or the production of structures **12** in *Penicillium brevicompactum*.[7d]

Subsequent work from this laboratory, based primarily on the metabolite co-occurence of brevianamide E (**16**), gave rise to an alternate biogenetic proposal shown in Fig. 3.[7c,7d] Tritium-labeled deoxybrevianamide E (**10**) was synthesized and found to incorporate efficiently into brevianamide A (**5**), brevianamide B (**6**) and brevianamide E (**16**) in *Penicillium brevicompactum*.[7d] When tritium-labeled brevianamide E (**16**) was fed to *Penicillium brevicompactum*, no incorporation was observed into either **5** or **6** indicating that, the conversion of **10** into **16** is an irreversible, dead-end shunt pathway. Thus, our working hypothesis envisions an (*R*)-selective indole oxidase that converts **10** into the hydroxyindolenine **15** which can suffer either of two fates: (1) irreversible nucleophilic ring closure to **16** or; (2) spiro-rearrangement to the (*R*)-indoxyl **17**. Subsequent 2-electron oxidation and enolization provides azadiene **19** that can suffer intramolecular [4+2] cycloaddition to directly furnish brevianamide A (**5**) and brevianamide B (**6**). Whether this reaction occurs spontaneously or involves the agency of the enzyme active site to organize and select from the four possible diastereomeric transition states, is not known and constitutes the focus of our program.

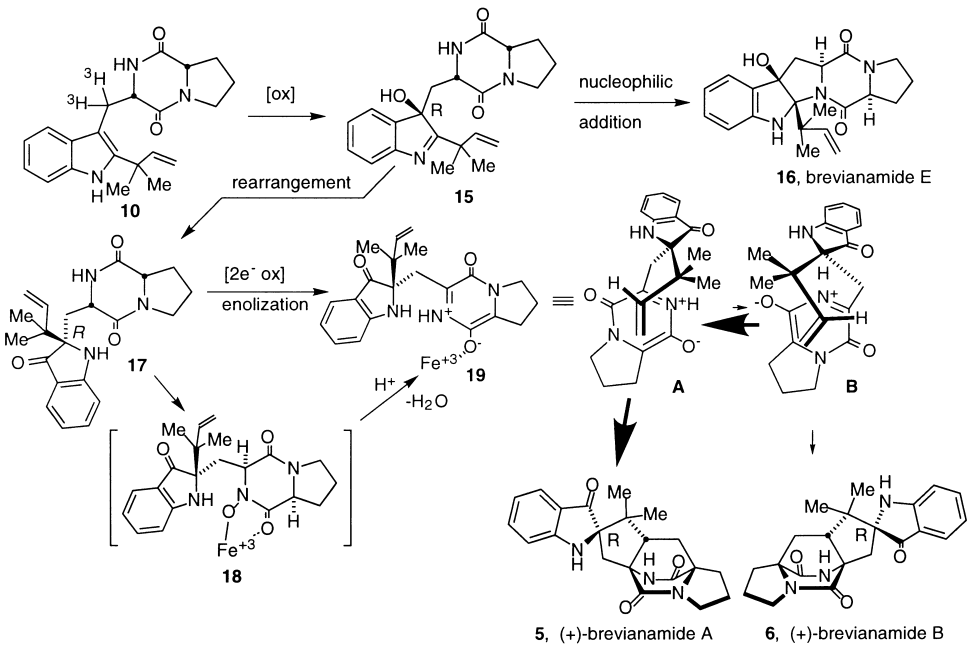

Figure 3.

Recent theoretical work on an indoxyl-based Diels–Alder cyclization pathway, supported the observed isomer production of the brevianamides in *Penicillium brevicompactum* which produces brevianamide A as the major metabolite and brevianamide B as the minor metabolite (Fig. 4).[12] Of interest in the biosynthetic process, is the fact that cycloaddition appears to occur primarily from transition state/conformer **A**, with a small amount occurring from transition state/conformer **B** with no detectable metabolite production (of **20**/**21**) from either transition state/conformer **A′** and/or transition state/conformer **B′**.

The relatively recent isolation of VM55599 (**7**) forces somewhat of a resurrection of the original biosynthetic [4+2] cycloaddition pathway (Fig. 2) that we have already probed in the brevianamide pathway via substance **12**. In particular, the existence of **7** begs the question of the timing of the indole and amino acid oxidation reactions in each biosynthetic system.

Our laboratory has been concerned with elucidating the biosynthetic mechanism of formation of the unique bicyclo[2.2.2] ring system, particularly with respect to the question of possible enzymatic catalysis of this reaction. In this paper, we provide a full account of a possibly biomimetic intramolecular Diels–Alder cyclization reaction that constructs the core framework of this class of fungal metabolites. The stereochemical implications of our findings bear on the possible biosynthetic construction of this ring system.

Results and Discussion

The starting material for this investigation, 9-*epi*-deoxybrevianamide E (**22**, Fig. 5) was synthesized according to a slightly modified procedure originally reported by Kametani.[13] Conversion of this substance to the lactim ether (**23**) was accomplished with Me_3OBF_4 in CH_2Cl_2 (79% yield). Next, oxidation of **23** with DDQ gave the unsaturated substance **24** in moderate yield (24–40%). Treatment of **24** with aqueous methanolic KOH at room temperature, cleanly produced the labile azadiene **25** which could be isolated by silica gel chromatography and characterized spectroscopically. Upon standing however, **25** spontaneously cyclized to give a mixture of the cycloadducts **26** and **27** (2:1, 90% combined yield). The structures of the cyclization products were secured through analysis of their respective NMR spectra as well as by chemical correlation to known substances as described below. Alternatively, azadiene **24** could be tautomerized and cyclized to **26** (37%) and **27** (28%) by treatment with DBU in THF at room temperature.

Conversion of **26** to C-19-*epi*-brevianamide A (**20**), a non-natural isomer of brevianamide A previously synthesized in this laboratory,[14] was accomplished by diastereoselective *m*-CPBA oxidation to the corresponding hydroxyindolenine (**28**, ~quant.) and pinacol-type rearrangement (0.5 M NaOH, MeOH, rt, reflux) to the corresponding *spiro*-indoxyl; subsequent removal of the lactim ether with HCl afforded D,L-**20** in 70% overall yield from **26**.

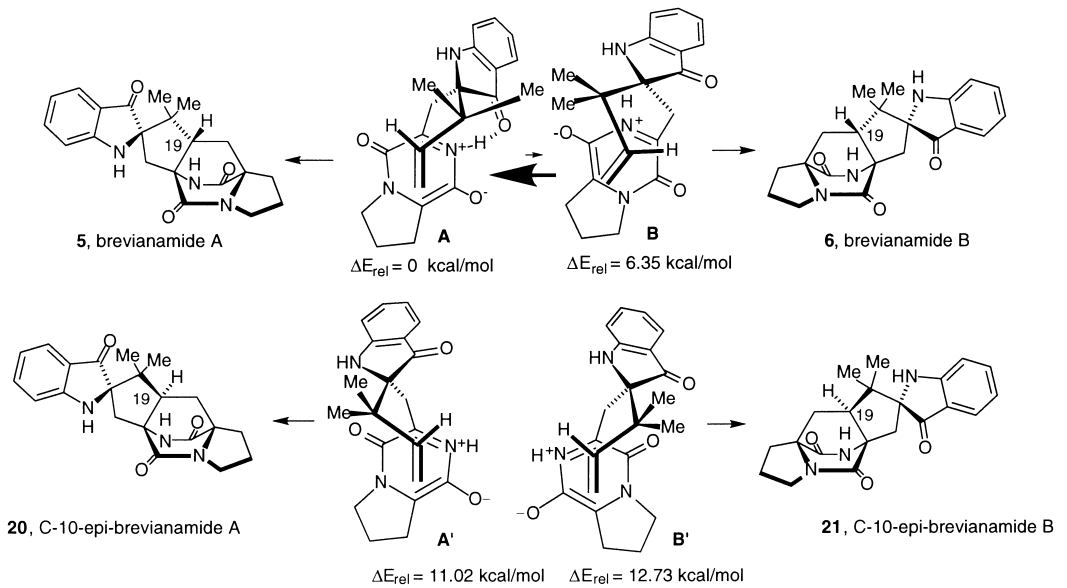

Figure 4. Calculated relative energies for transition state structures for intramolecular [4 + 2] cycloadditions.[12]

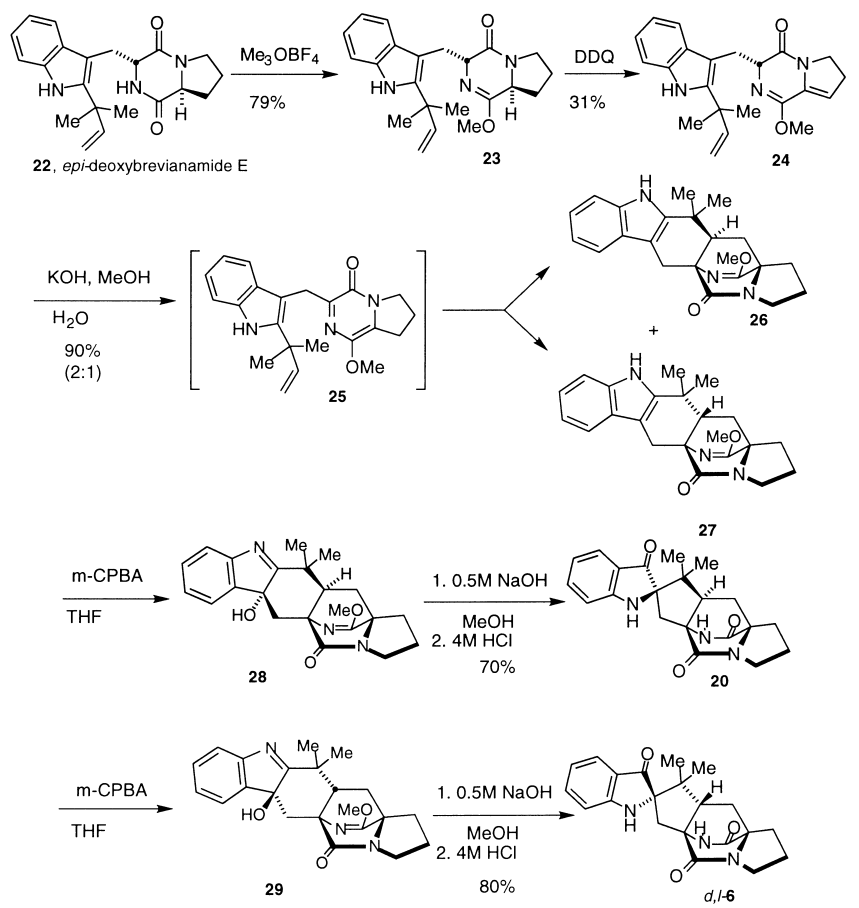

Figure 5.

Initially, the labile ring-opened aminoester (**30**, Fig. 6) was produced from this sequence but could be cyclized in hot toluene containing 2-hydroxypyridine furnishing D,L-**20**. Careful control of the reaction time and temperature obviated the formation of **30**. Curiously, we have never observed a related ring-opened amino ester derived from **29** (see below). The C-19-*epi*-brevianamide A (**20**), was identical to the authentic material[14] in every respect (except for being racemic).

Conversion of the minor cycloaddition product **27** to D,L-brevianamide B (**6**) was accomplished in like manner in 80% overall yield from **27** securing the relative stereochemistry of each respective cycloadduct **26** and **27**. This represents an alternative (albeit racemic) total synthesis of brevianamide B (six steps from **22**). In addition, the construction of **26** constitutes a model study for the synthesis of VM55599.

A significant implication of these observations concerns the biogenesis and stereochemistry of the related metabolite VM55599 (**7**) isolated from the paraherquamide-producing mold *Penicillium* sp. IMI332995. Since Paraherquamide A and VM55599 both possess the bicyclo[2.2.2] monoketopiperazine ring system, it seems plausible that these substances arise via a related or, more provocatively, a common [4+2] cycloaddition intermediate. The relative stereochemistry of VM55599 was originally assigned by extensive ^{1}H NMR NOE studies where the methyl group in the β-methylproline moiety was assigned as being *syn*- to the bridging isoprene unit.[5] Thus, if a similar Diels–Alder cyclization, whether it be un-catalyzed or enzyme-catalyzed, is operating in the biosynthetic construction of these metabolites, the isoprene unit must approach the azadiene from the same face as the methyl group in the proline ring (**31b**, Fig. 7), whereas in paraherquamide A, which has been shown by this laboratory[15] to be derived from L-isoleucine, Diels–Alder cyclization must occur from the face *opposite* to the methyl group (**31a**).

The absolute stereochemical implications of the *relationship* of VM55599 to the biosynthesis of the paraherquamides has been previously discussed[15] and is an issue that is currently under study. It is important to note that, the absolute stereochemistry of VM55599 has not been determined and constitutes an important, missing piece of this puzzle. Efforts are underway to determine the intrinsic facial bias of related Diels–Alder cyclizations on β-methylproline-containing substrates (i.e., **31**) to address these and related issues.

Conclusion

This study provides experimental validity to the hypothesis that, the core bicyclo[2.2.2] ring system

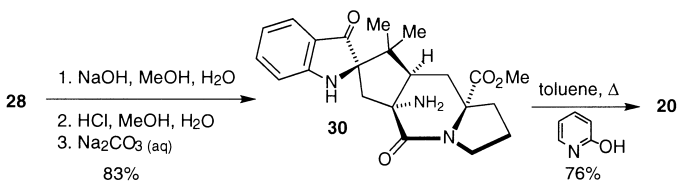

Figure 6.

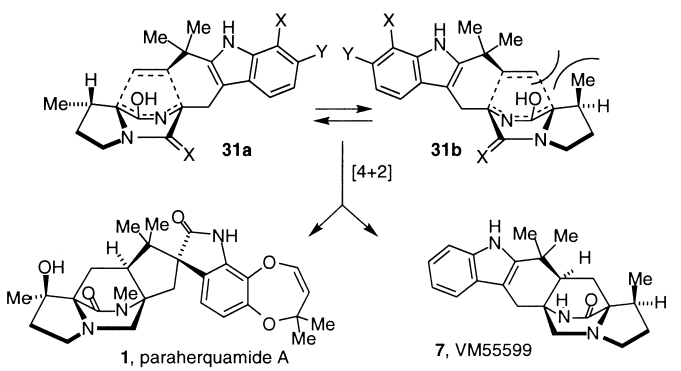

Figure 7.

common to this family of secondary metabolites very likely arises by an intramolecular Diels–Alder cyclization from a pre-formed dioxopiperazine[16] that subsequently undergoes oxidation to an azadiene species. The diastereofacial bias of the Diels–Alder cyclization of **25** is significant in that, the diastereoselectivity is not strongly affected by solvent. A comparable ratio of **26**:**27** was observed in THF as in aqueous methanol (~1.3–2:1).[17] We have also shown that, C-19- *epi*-metabolites (corresponding to **26** and **20**) are not produced by *Penicillium brevicompactum* and there have been no reports on the isolation of similarly epimeric metabolites from paraherquamide-, sclerotamide- or asperparaline-producing organisms. *Thus, in each biosynthetic system, there appears to be complete facial exclusivity with respect to the isoprene unit in the construction of the bicyclo[2.2.2] ring nucleus*. Since the laboratory cycloaddition reported here does not show a strong bias for either the *syn*- or the *anti*-relationship, it would appear plausible that there is some organization of the transition state structures in the putative biosynthetic cycloadditions. Uncertainties as to the oxidation state of the indole moiety as being either oxindole (for the paraherquamides, marcfortine and sclerotamide), or indoxyl (for the brevianamides) as opposed to the non-oxidized indole (for VM55599) at the [4 + 2] cyclization phase as well as the question of possible protein organization of the transition state structures still exists and are the subject of ongoing investigations in these laboratories.

Experimental

Lactim ether of deoxybrevianamide E (23)

A solution of *epi*-deoxybrevianamide E (**22**) (3.41 g, 9.71 mmol) in dry CH_2Cl_2 (88 mL) was stirred at 0 °C under argon. After 10 min, BF_4OMe_3 (4.31 g, 29.15 mmol) was added. The mixture was stirred in the cold room (3 °C) with an argon balloon and drying tube for 16 h. The crude material was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq). It was extracted with CH_2Cl_2 (3×100 mL) and washed with brine. The organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was purified by flash chromatography (silica, 2:1 CH_2Cl_2/ether) to afford 2.8 g (79%) of lactim ether (**23**) as a yellow foam. IR (neat, cm^{-1}) 3320, 3078, 3053, 2970, 1681, 1644, 1462, 1431, 1322, 1257, 1027, 999, 917, 744; ^1H NMR (300 MHz, $CDCl_3$) δ 7.96 (brs, 1H), 7.53 (dd, J = 7.3, 1.2 Hz, 1H), 7.19 (ddd, J = 7.2, 0.7, 1.3 Hz, 1H), 7.02 (m, 2H), 6.15 (dd, J = 17.5, 10.6 Hz, 1H), 5.13 (dd, J = 17.5, 1.0 Hz, 1H), 5.08 (dd, J = 10.6, 1.0 Hz, 1H), 4.53 (ddd, J = 5.7, 5.0, 1.6 Hz, 1H), 3.65 (s, 3H), 3.53 (m, 1H), 3.50 (dd, J = 14.7, 5.0 Hz, 1H), 3.33 (dd, J = 14.3, 6.4 Hz, 1H), 3.18 (m, 2H), 2.02 (m, 1H), 1.84 (m, 1H), 1.41–1.63 (m, 2H), 1.54 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) (169.44, 161.54, 146.38, 140.40, 134.23, 129.92, 106.59, 121.29, 119.34, 118.68, 109.97, 111.55, 63.82, 56.18, 53.11, 44.33, 39.49, 29.29, 29.14, 27.83 (2 C), 21.81; MS m/z (M^+) calcd 366.2182, obsd 366.2168.

Azadiene (24)

Lactim ether (**23**) (128 mg, 0.3507 mmol) was dissolved in dry toluene (9.6 mL) and stirred under argon at −78 °C. Once cooled, DDQ (95 mg, 0.4208 mmol) was dissolved in toluene (3.2 mL) and added via syringe to the mixture. The mixture stirred for 34 h and was allowed to warm from −78 °C to room temperature. The mixture was then brought to reflux temperature for 8 h. The crude reaction was filtered through alumina (CH_2Cl_2/MeOH, 50:1). Purification by preparative TLC (CH_2Cl_2/EtOAc, 25:1) provided 22 mg (31%) of the oxidized product (**24**) as a yellow foam. IR (neat, cm^{-1}) 3337, 3078, 3043, 2960, 2913, 2878, 2854, 1680, 1644, 1633, 1627, 1622, 1454, 1335, 1245, 1049, 914, 744; ^1H NMR (300 MHz, $CDCl_3$) δ 7.85 (brs, 1H), 7.62 (d, J = 8 Hz, 1H), 7.22 (d, J = 8 Hz, 1H), 7.05 (m, 2H), 6.12 (dd, J = 17.0, 10.0 Hz, 1H), 5.50 (dd, J = 3.3, 3.3 Hz, 1H), 5.16 (dd, J = 17.0, 1.0 Hz, 1H), 5.12 (dd, J = 10.0, 1.0 Hz, 1H), 4.61 (br dd, J = 3.5, 9.5 Hz, 1H), 3.85–3.95 (m, 2H), 3.77 (dd, J = 14.0, 3.5 Hz, 1H), 3.64 (s, 3H), 3.08 (dd, J = 9.5, 14.5 Hz, 1H), 2.63 (m, 2H), 1.59 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 166.9, 151.3, 146.1, 140.1, 134.1, 130.3, 121.2, 119.7, 118.7, 111.9, 110.5, 110.0, 108.1, 64.5, 52.9, 44.3, 39.3, 31.4, 29.6, 27.7, 25.9; MS m/z (M^+) calcd 364.2025, obsd 364.2023.

Cycloaddition of 24 (formation of 26 and 27) with DBU

To a stirred solution of azadiene (**24**) (22 mg, 0.0606 mmol) in dry THF (0.6 mL) was added DBU (4.6 mL, 0.0303 mmol) via syringe. The mixture was stirred at room temperature under argon for 18 h. The crude mixture was partitioned between CH_2Cl_2 and $NaHCO_3$ (aq), extracted with CH_2Cl_2 (3×10 mL) and washed with brine. The organic extracts were combined and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (silica gel, CH_2Cl_2/MeOH, 25:1) afforded **26** (8.2 mg, 37%) and **27** (6.2 mg, 28%) as yellow foams.

Cycloaddition of 24 (formation of 26 and 27) with KOH

Azadiene (**24**) (10 mg, 0.0275 mmol) was stirred in MeOH (2 mL) and 20% KOH (aq) (0.5 mL) under argon at 0 °C in an ice bath. The ice bath was allowed to warm to room temperature and after 4 h, the pH of the crude mixture was adjusted to 7 with phosphate buffer.

The mixture was extracted with CH_2Cl_2 (3×10 mL) and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. Purification by preparative TLC (silica gel, CH_2Cl_2/MeOH, 25:1) afforded **26** (6.0 mg, 60%) and **27** (3.0 mg, 30%) as yellow foams.

Major cycloaddition product (26)

IR (neat, cm^{-1}) 3306, 3118, 3046, 2925, 1662, 1445, 1353, 1313, 1262, 1190, 1144, 1108, 1005, 990, 742, 703; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (brs, 1H), 7.56 (d, J=7.0 Hz, 1H), 7.25 (d, J=7.0 Hz, 1H), 7.09 (m, 2H), 4.01 (d, J=16.0 Hz, 1H), 3.79 (s, 3H), 3.50 (ddd, J=5.5, 6.3, 11.8 Hz, 1H), 3.33 (ddd, J=7.1, 7.1, 11.3 Hz, 1H), 3.10 (d, J=16.0 Hz, 1H), 2.66 (m, 1H), 2.27 (dd, J=5.0, 10.2 Hz, 1H), 1.88–2.04 (m, 4H), 1.78 (dd, J=5.0, 12.6 Hz, 1H), 1.26 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 171.6, 136.4, 133.4, 127.5, 121.4, 119.1, 118.8, 110.3, 107.0, 66.3, 64.2, 54.5, 47.3, 43.4, 35.1, 32.2, 29.3, 28.6, 27.8, 24.8, 22.8; MS m/z (M^+) calcd 363.1947, obsd 363.1940.

Minor cycloaddition product (27)

IR (neat, cm^{-1}) 3306, 3046, 2952, 1662, 1456, 1428, 1351, 1310, 1256, 1200, 1108, 1005, 990, 741, 697; ^1H NMR (300 MHz, $CDCl_3$) δ 7.69 (brs, 1H), 7.59 (d, J=7.0, Hz, 1H), 7.27 (d, J=7.0 Hz, 1H), 7.10 (m, 2H), 3.91 (d, J=17.0 Hz, 1H), 3.69 (s, 3H), 3.48 (m, 2H), 3.30 (d, J=17.0 Hz, 1H), 2.66 (m, 1H), 2.36 (dd, J=4.0, 9.5 Hz, 1H), 1.88–2–08 (m, 4H), 1.82 (dd, J=4.0, 13.0 Hz, 1H), 1.25 (s, 3H), 1.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) ppm 172.3, 170.5, 139.7, 136.3, 128.0, 121.5, 119.2, 118.9, 110.3, 106.3, 67.3, 64.2, 54.3, 45.8, 43.6, 34.9, 33.6, 29.2, 28.5, 26.1, 25.3, 24.7; MS m/z (M^+) calcd 363.1947, obsd 363.1942.

3-Hydroxyindolenine (28)

Compound (**26**) (38 mg, 0.1079 mmol) was dissolved in dry THF (5 mL). *m*-CPBA (24.5 mg, 0.14 mmol) was added and the mixture was stirred at room temperature under argon. After 12 h, the crude reaction was quenched with one drop of DMS. The hydroxyindolenine **28** was not purified, but taken on crude for the next step. IR (neat, cm^{-1}) 3222, 3055, 2948, 2872, 1682, 1668, 1652, 1636, 1581, 1456, 1418, 1359, 1206, 1108, 755, 734; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (ddd, J=7.0, 1.5, 0.5 Hz, 1H), 7.44 (ddd, J=8.0, 1.5, 0.5 Hz, 1H), 7.29 (ddd, J=8.0, 8.0, 1.5 Hz, 1H), 7.18 (ddd, J=8.0, 7.0, 1.5 Hz, 1H), 3.66 (s, 3H), 3.2–3.5 (m, 2H), 2.60 (m, 1H), 1.99 (dd, J=13.0 Hz, 1H), 1.93 (d, J=13.0 Hz, 1H), 1.92–2.17 (m, 5H), 1.60 (dd, J=7.0, 12.5 Hz, 1H), 1.30 (s, 3H), 1.18 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.68, 173.21, 170.55, 153.08, 140.44, 129.24, 126.11, 122.29, 120.22, 82.62, 65.91, 65.47, 54.25, 43.76, 43.69, 39.37, 37.66, 32.04, 28.70, 27.96, 24.63, 23.49.

3-Hydroxyindolenine (29)

Indole (**27**) (20 mg, 0.0568 mmol) was dissolved in dry THF (2.7 mL). *m*-CPBA (12.9 mg, 0.0742 mmol) was added and the mixture was stirred at room temperature under argon. After 12 h, the crude reaction was quenched with one drop of DMS. The hydroxyindolenine **29** was not purified, but taken on crude for the next step. IR (neat, cm^{-1}) 3324, 3052, 2950, 2876, 1668, 1652, 1634, 1580, 1428, 1361, 1337, 1264, 1227, 1195, 1180, 1145, 1115, 1088, 1007, 988, 762, 735; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.41 (m, 2H), 7.24 (ddd, J=7.5, 7.0, 1.5 Hz, 1H), 7.18 (ddd, J=7.5, 7.0, 0.5 Hz, 1H), 3.69 (s, 3H), 3.23–3.36 (m, 2H), 2.57 (m, 1H), 1.92–2.17 (m, 5H), 1.99 (dd, J=13.0 Hz, 1H), 1.93 (d, J=13.0 Hz, 1H), 1.60 (dd, J=7.0, 12.5 Hz, 1H), 1.24 (s, 3H), 1.07 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.81, 171.57, 170.69, 153.33, 140.52, 129.44, 126.05, 122.15, 120.42, 83.86, 65.81, 64.32, 54.62, 46.05, 43.61, 38.76, 36.14, 31.86, 29.29, 28.75, 24.66, 21.33.

D,L-19-*epi*-Brevianamide A (20)

Crude compound **28**, prepared as described above (10 mg, 0.0264 mmol) was dissolved in 0.5 M NaOH (2 mL) and MeOH (1 mL) and stirred at room temperature for 12 h then refluxed for 2 h. After cooling to room temperature, most of the MeOH was removed in vacuo. At this point, the lactim ether of **20** could be isolated and purified, but was in practice taken on crude (purified by TLC, silica gel, CH_2Cl_2/ether, 5:1). Data for the lactim ether: IR (neat, cm^{-1}) 3353, 3030, 2942, 2871, 1694, 1660, 1613, 1488, 1470, 1428, 1320, 1295, 1179, 920, 768; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, J=7.5 Hz, 1H), 7.37 (ddd, J=8.0, 7.5, 1.0 Hz, 1H), 6.75 (d, J=8.0 Hz, 1H), 6.70 (dd, J=7.5, 7.5 Hz, 1H), 5.43 (brs, 1H), 3.66 (s, 3H), 3.28–3.48 (m, 2H), 2.91 (d, J=14.0 Hz, 1H), 2.61 (dd, J=11.5, 6.0 Hz, 1H), 2.57 (dd, J=10.0, 7.0 Hz, 1H), 2.49 (d, J=14.0 Hz, 1H), 1.82–2.09 (m, 3H), 1.81 (dd, J=12.5, 10.0 Hz, 1H), 1.54 (dd, J=12.5, 7.0 Hz, 1H), 0.87 (s, 3H), 0.86 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.88, 172.18, 172.05, 160.59, 137.04, 124.62, 119.47, 117.78, 111.32, 77.15, 72.91, 66.06, 54.71, 54.45, 44.93, 43.16, 39.67, 31.19, 29.33, 24.98, 24.92, 20.97; MS m/z (M^+) calcd 380.1974, obsd 380.1980. The crude mixture was treated with 4 M HCl until pH 4 and then 2 M NaOH was added to adjust the pH to 7. The mixture was extracted with CH_2Cl_2 (3×5 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 25:1) furnished **20** (6.7 mg, 70%) as a fluorescent yellow oil. IR

(neat, cm^{-1}) 3282, 3077, 2925, 2851, 1682, 1615, 1467, 1395, 1323, 1251, 1189, 1149, 1097, 913, 754, 728; ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (d, $J=7.5$ Hz, 1H), 7.37 (ddd, $J=8.0$, 7.5, 1.0 Hz, 1H), 6.75 (d, $J=8.0$ Hz, 1H), 6.63 (dd, $J=8.0$, 7.5 Hz, 1H), 3.30–3.50 (m, 2H), 3.09 (dd, $J=10.0$, 8.0 Hz, 1H), 2.76 (d, $J=14.5$ Hz, 1H), 2.67 (ddd, $J=12.5$, 7.0, 5.2 Hz, 1H), 2.08 (d, $J=14.5$ Hz, 1H), 2.00 (dd, $J=14.0$, 10.0 Hz, 1H), 1.85–2.00 (m, 2H), 1.77 (dd, $J=13.0$, 8.0 Hz, 1H), 1.54 (dd, $J=13.0$, 8.0 Hz, 1H), 0.81 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 206.10, 173.95, 169.92, 161.22, 137.69, 124.50, 118.53, 117.92, 111.27, 76.57, 68.83, 65.27, 55.80, 45.28, 43.86, 34.85, 30.42, 29.53, 24.58, 23.02, 19.18; MS m/z (M^+) calcd 366.1818, obsd 366.1818.

D,L-Brevianamide B (6)

Crude compound **29**, prepared as described above (10 mg, 0.0264 mmol) was dissolved in 0.5 M NaOH (2 mL) and MeOH (1 mL) and stirred at room temperature for 12 h then refluxed for 2 h. After cooling to room temperature, most of the MeOH was removed in vacuo. At this point, the lactim ether of **6** could be isolated and purified, but was in practice taken on crude (purified by TLC, silica gel CH_2Cl_2/MeOH, 15:1, silica gel then CH_2Cl_2/EtOAc, 3:1). Data for the lactim ether: IR (neat, cm^{-1}) 3322, 3024, 2946, 2873, 1694, 1682, 1668, 1652, 1621, 1470, 1416, 1360, 1324, 1259, 1199, 990, 924, 752, 730; ^1H NMR (300 MHz, $CDCl_3$) δ 7.51 (ddd, $J=7.8$, 1.2, 0.6 Hz, 1H), 7.37 (ddd, $J=8.4$, 7.2, 1.5 Hz, 1H), 6.69–6.78 (m, 2H), 4.95 (brs, 1H), 3.79 (s, 3H), 3.37 (dd, $J=6.6$, 6.6 Hz, 2H), 3.20 (d, $J=14.5$ Hz, 1H), 3.15 (dd, $J=10.0$, 6.6 Hz, 1H), 2.51–2.61 (m, 1H), 2.28 (d, $J=14.5$ Hz, 1H), 1.78–2.05 (m, 4H), 1.48 (dd, $J=12.5$, 7.0 Hz, 1H), 0.95 (s, 3H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.63, 171.60, 171.30, 160.21, 136.89, 124.81, 119.96, 118.14, 111.16, 78.12, 72.74, 66.93, 54.34, 51.11, 46.40, 43.17, 39.08, 30.16, 28.85, 25.00, 23.27, 21.36; MS m/z (M^+) calcd 380.1974, obsd 380.1982. The crude mixture containing the lactim ether of brevianamide B was treated with 4 M HCl until pH 4 and then 2 M NaOH was added to adjust the pH to 7. The mixture was extracted with CH_2Cl_2 (3×5 mL), washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, CH_2Cl_2/MeOH, 25:1) furnished D,L-**6** (7.7 mg, 80%) as a fluorescent yellow oil. This material was identical to natural brevianamide B by TLC, and ^1H NMR. IR (neat, cm^{-1}) 3279, 3070, 2927, 1683, 1616, 1490, 1466, 1389, 1324, 1304, 1258, 1139, 1119, 1098, 1031, 980, 924, 751, 694; ^1H NMR (300 MHz, $CDCl_3$) δ 7.67 (brs, 1H), 7.50 (d, $J=7.5$ Hz, 1H), 7.37 (br dd, $J=8.0$, 7.5 Hz, 1H), 6.75 (d, $J=8.0$ Hz, 1H), 6.63 (dd, $J=7.5$, 7.5 Hz, 1H), 3.37–3.47 (m, 2H), 3.20 (dd, $J=10.0$, 8.0 Hz, 1H), 3.09 (d, $J=15.0$ Hz, 1H), 3.09 (d, $J=15.0$ Hz, 1H), 2.67 (ddd, $J=12.3$, 6.5, 6.5 Hz, 1H), 1.90–2.02 (m, 3H), 1.82 (dd, $J=13.0$, 7.0 Hz, 1H), 1.72 (dd, $J=13.0$, 8.0 Hz, 1H), 0.81 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.72, 173.42, 169.92, 161.12, 137.22, 123.89, 118.20, 117.39, 110.77, 77.29, 68.57, 65.87, 49.33, 45.94, 43.30, 33.64, 29.06, 28.29, 24.24, 21.50, 19.11; MS m/z (M^+) calcd 366.1818, obsd 366.1820.

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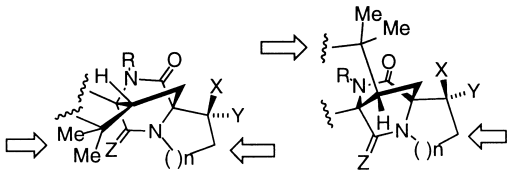
10. The *syn*-/*anti*-relationship refers to the relative stereochemistry between the C-19 stereogenic center (brevianamide numbering) and the cyclic amino acid residue (proline, β-methylproline, or pipecolic acid):



syn- *anti-*

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