

Review

Total Synthesis and Biosynthesis of the Paraherquamides: An Intriguing Story of the Biological Diels–Alder Construction

Robert Michael WILLIAMS

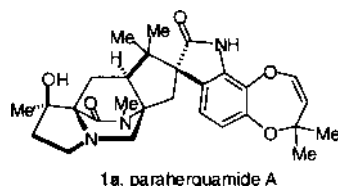
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The syntheses and biosyntheses of the paraherquamide and brevianamide families of prenylated indole-derived alkaloids are reviewed. It has been proposed that the unique bicyclo[2.2.2]diazaoctan ring system that is common to this family of natural products, arises by a biological intramolecular Diels–Alder cycloaddition reaction. Both synthetic approaches and total syntheses of several members of this family of natural products are reviewed. The biosynthesis of these alkaloids has also constituted an active area of research and the current state of knowledge on the biosynthesis of these natural products are reviewed.

Key words paraherquamide; marcfortine; brevianamide; asperparaline; biological Diels–Alder; asymmetric synthesis; biosynthesis

The paraherquamides constitute an unusual family of prenylated indole alkaloids that are secondary metabolites produced by *Penicillium* sp. and *Aspergillus* sp. fungi. This review will cover both the biosynthesis as well as the chemical synthesis of this family of natural products. Particular emphasis will be placed on the provocative hypothesis that the core bicyclo[2.2.2]diazaoctan ring system, which is common to this entire family, is formed by a biological Diels–Alder reaction.



Despite its widespread use in synthetic organic chemistry, the Diels–Alder cycloaddition reaction does not occur frequently in nature.^{1,2)} There are just a few reports in the literature claiming the identification of an enzyme that catalyzes this most ubiquitous synthetic ring-forming reaction.^{3–6)} A possible explanation for this is that, enzymes generally catalyze reactions by stabilizing the structure and charge of the developing transition state. For most reactions subject to this stratagem of catalysis, both the starting substrate and the product differ significantly with respect to structure from the transition state; it is this fundamental difference that allows for turnover; *i.e.*, both the product and the starting substrate must bind to the enzyme less tightly than the transition state structure. In the Diels–Alder reaction, the transition state is highly ordered and closely resembles the structure of the product (see Fig. 1, below). In other words, an enzyme that was designed to stabilize the transition state structure for this reaction would be expected to be inhibited by the product (*via* tight binding) and turnover (thus catalysis) would be precluded. It is from within the excitement of this fundamental question, that this review will hold as a guiding light.

The only examples of *protein*-catalyzed Diels–Alder reactions are those of catalytic antibodies that have recently been shown to catalyze the intermolecular [4+2] cycloaddition reaction.^{7–9)} In these cases, the initial Diels–Alder adducts



Fig. 1. The Diels–Alder [4+2] Cycloaddition Reaction

were high-energy substances that significantly change their structure or conformation after the initial cyclocondensation and were subsequently released from the antibody CDR (allowing for turnover). In addition, ribozymes have been reported to catalyze the Diels–Alder reaction and findings in this area have been reviewed.¹⁰⁾ The *Penicillium* sp. that produces the brevianamides/paraherquamides may be a rare, but vitally important example of the existence of Diels–Alderase. It is further significant to note in the context of the catalytic antibody stratagem that, the brevianamide Diels–Alder adducts (which are proposed to be the brevianamides themselves) are very rigid substances; therefore, a significant change in conformation or structure is either impossible or highly unlikely. Elucidation of the mechanism for turnover in the purported paraherquamide and brevianamide [4+2] cycloaddition reaction should be an extremely interesting and significant finding.

In order to do proper justice to the history of the biosynthetic Diels–Alder proposals dealing specifically with the paraherquamides, the relevant synthetic chemistry and biosynthesis of the brevianamides, structurally simpler cousins of the paraherquamides, will be presented first. Recent interest in the paraherquamides has come from the finding that this class of alkaloids displays potent anthelmintic and antinematodal properties. Intensive semi-synthetic studies on the structural and pharmacological properties of these molecules have been reported and will not be covered extensively here.

The Brevianamides and Austamides

The brevianamides comprise a small but structurally interesting family of indole alkaloids constituted from tryptophan, proline and one isoprene unit (Fig. 2). Brevianamide A was originally isolated from cultures of *Penicillium brevi-*

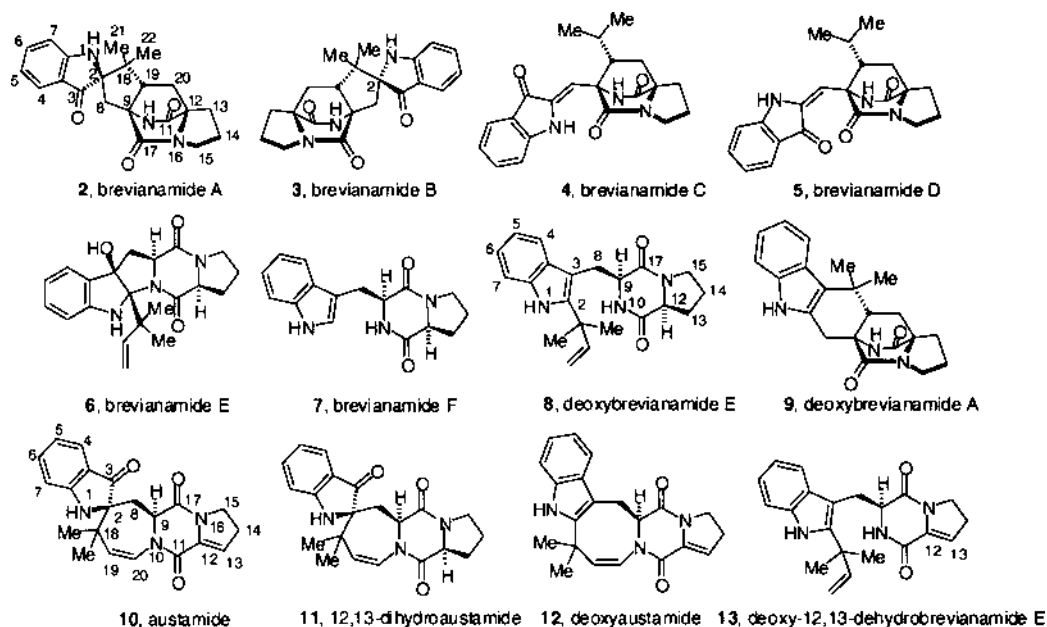


Fig. 2. Structures of the Brevianamides and Austamides

Robert M. Williams was born in New York in 1953 and was raised in Huntington, Long Island by Edith and Valentine Williams. He attended Syracuse University from 1971—1975 and received the B.A. degree in Chemistry in 1975. While at Syracuse, he did undergraduate research with Prof. Ei-ichi Negishi in the area of hydroboration methodology. He then moved to Cambridge, Massachusetts and entered the Ph.D. program at MIT and obtained his Ph.D. degree in 1979 under the supervision of Prof. William H. Rastetter. His doctoral studies were concerned with the total synthesis of two fungal metabolites, gliovictin and hyalodendrin. Following completion of his doctoral studies, he joined the laboratories of the late Prof. R.B. Woodward in 1979 whose postdoctoral group was subsequently managed by Professor Yoshito Kishi. His postdoctoral work was concerned with the completion of the total synthesis of erythromycin A. Upon



Robert M. Williams

completion of his postdoctoral tenure at Harvard, he joined the faculty at Colorado State University in 1980. He was promoted to Associate Professor with tenure in 1985, Full Professor in 1988, and University Distinguished Professor in 2002, his current position. Dr. Williams has received several Honors and Awards including the NIH Research Career Development Award (1984—1989), The Eli Lilly Young Investigator Award (1986), Fellow of the Alfred P. Sloan Foundation (1986); the Merck, Academic Development Award (1991), the Japanese Society for the Promotion of Science Fellowship (1999) and the Arthur C. Cope Scholar Award (2002). He spent a sabbatical at the University of California, Berkeley in 1990 in the laboratories of Professor Peter G. Schultz and was a visiting Professor at Harvard University in 1994 where he spent additional sabbatical time with Professor Stuart Schreiber. He serves on the Editorial Board of the journal *Chemistry & Biology* and was an Editor for the journal *Amino Acids* (1991—1998). He has also recently been appointed as a Series co-Editor for *The Organic Chemistry Series*, published by Pergamon Press/Elsevier with Professor Sir Jack E. Baldwin of Oxford. Dr. Williams was a member of the Scientific Advisory Board of Microcide Pharmaceutical Co. from 1993—1998 located in Mountainview, California and is a founding scientist, Member of the Scientific Advisory Board and Member of the Board of Directors of Xcyte Therapies, located in Seattle, Washington.

Dr. Williams' research results from the interplay of synthetic organic chemistry, microbiology, biochemistry and molecular biology. Dr. Williams research interests have included the total synthesis of natural products, the asymmetric synthesis of amino acids and peptide isosteres, studies on anti-tumor drug-DNA interactions, design and synthesis of antibiotics and DNA-cleaving molecules, combinatorial phage libraries and biosynthetic pathways. He has utilized natural products synthesis to probe and explore biomechanistic and biosynthetic problems with a particular emphasis on antitumor and antimicrobial antibiotics. He has developed technology for the asymmetric synthesis of α -amino acids and peptide isosteres that has been commercialized by Aldrich Chemical Co. and has written a monograph on this subject.

compactum by Birch and Wright in 1969.¹¹⁾ Brevianamide A has also been found in cultures of *Penicillium viridicatum*¹²⁾ and *Penicillium ochraceum*.¹³⁾ Brevianamides A and D have been shown to possess insecticidal activity.^{14,15)} The structure and absolute stereochemistry of brevianamide A was secured through X-ray crystallography by Coetzer in 1974 on the semi-synthetic derivative, 5-bromobrevianamide A.¹⁶⁾ Due to biosynthetic commonalities with the brevianamides to be discussed below, the austamides will also be included in this family. Austamide was first isolated by Steyn from cultures of *Aspergillus ustus* in 1971;¹⁷⁾ subsequently, 12,13-dihydroaustamide and “deoxyaustamide” were isolated from the same fungus by Steyn in 1973.¹⁸⁾ The absolute configuration of austamide was subsequently secured through X-ray crystallography by Coetzer and Steyn in 1973 on the semi-synthetic derivative, 5-bromo-12*S*-tetrahydroaustamide.¹⁹⁾

Following the original isolation, Birch and Wright, reported the isolation and structural elucidation of brevianamides B–F from *Penicillium brevicompactum*.^{20,21)} Brevianamide A is the major fluorescent metabolite produced by this fungus; a minor metabolite named brevianamide B, that also exhibited the characteristic ψ -indoxyl chromophore, was assigned a structure that was epimeric to brevianamide A at the spiro- ψ -indoxyl quaternary center. The structural assignment for brevianamide B was based primarily on the semi-synthetic conversion of natural (+)-brevianamide A into brevianamide B by borohydride reduction and acid dehydration and acid dehydration to deoxybrevianamide A; subsequent air oxidation provided (–)-brevianamide B (Chart 1).²¹⁾

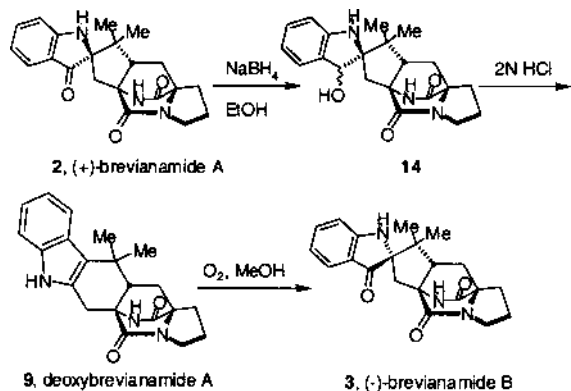


Chart 1. Conversion of (+)-Brevianamide A into (–)-Brevianamide B²¹⁾

Birch and associates did not record optical rotation data for natural or semi-synthetic brevianamide B and this oversight later proved to constitute the basis of an intriguing stereochemical puzzle; the complete stereochemical elucidation of brevianamide B will be discussed below.

Brevianamides C and D, also isolated in minor amounts from these *Penicillium* sp., were postulated by Birch and Russell²¹⁾ to be artifacts of isolation since, visible light irradiation of brevianamide A produces brevianamides C and D in virtually quantitative yield. When cultures of *Penicillium brevicompactum* were grown in the dark, only brevianamides A and B were detected thus verifying the hypothesis that brevianamides C and D are photochemical artifacts²¹⁾ and are not true metabolites of enzymic origin. A mechanism for the photochemical formation of brevianamides C and D from

brevianamide A is provided below in Chart 2.

Interestingly, Birch and Russell reported that during the early stages of photolysis of brevianamide A into brevianamides C and D with white light, traces of brevianamide B could be detected in the mixture.²¹⁾ A mechanism for this transformation was postulated as shown below in Chart 3.

However, the absolute stereochemistry of the brevianamide B produced under these conditions was not determined. As will become clear below, the *absolute* stereochemistry of the brevianamide B produced from brevianamide A, either *via* the photochemical route, or *via* the chemical redox route through deoxybrevianamide A as described above, must be as depicted in Charts 1 and 3 giving (–)-brevianamide B.

The structure and absolute configuration of deoxybrevianamide E was confirmed by a total synthesis reported by Ritchie and Saxton^{22,23)} and the absolute stereochemistry of brevianamide E was similarly secured by a synthesis reported by Kametani *et al.*²⁴⁾

The first significant biosynthetic experiment conducted in this family was reported by Birch and Wright in 1970 wherein, *dl*-[3-¹⁴C]-tryptophan, [2-¹⁴C]-mevalonic acid lactone, Na[2-¹⁴C]acetate and L-[U-¹⁴C]-proline were shown to

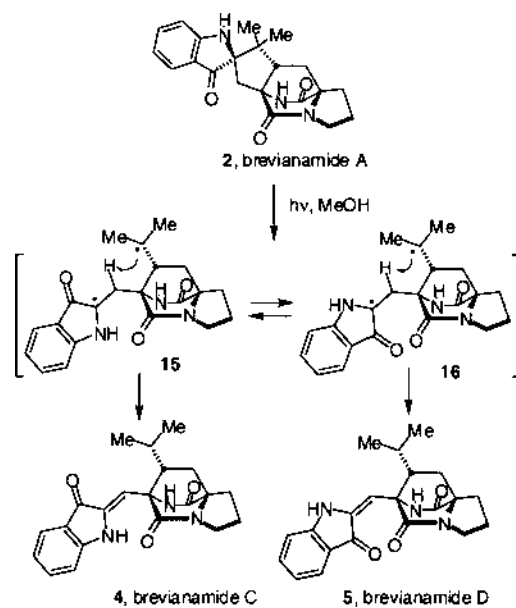


Chart 2. Photochemical Formation of Brevianamides C and D²¹⁾

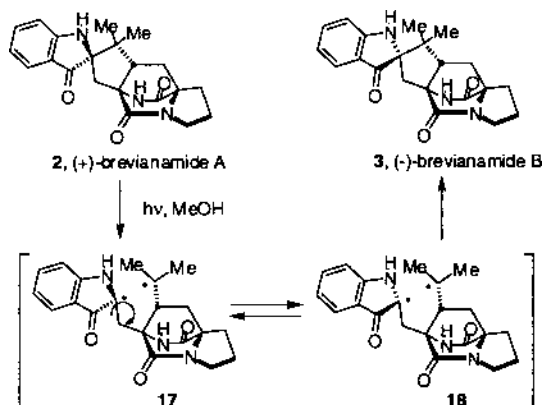


Chart 3. Proposed Mechanism for the Photochemical Formation of Brevianamide B from A²¹⁾

be incorporated into brevianamide A in significant amounts as shown in Table 1, below.²⁰⁾ It was also shown that [¹⁴CH₃]-methionine was not incorporated into brevianamide A. The acetate and mevalonate incorporations also firmly establish the origin of the isoprene unit as being derived from the classical mevalonate pathway.

Table 1. Biosynthetic Feeding Experiments with Radioactive Precursors to *Penicillium brevicompactum*²⁰⁾

Substrate	Activity of brevianamide A (cts/100 s)
<i>dl</i> -[3- ¹⁴ C]-Tryptophan	1430
[2- ¹⁴ C]-Mevalonic lactone	28
Na[2- ¹⁴ C]Acetate	425
[¹⁴ CH ₃]-Methionine	0
L-[U- ¹⁴ C]-Proline	4880

Subsequently, Birch and associates reported the biosynthetic incorporation of doubly radiolabeled brevianamide F (*cyclo*-L-tryptophyl-L-proline) into brevianamide A in *Penicillium brevicompactum*.²⁵⁾ Thus, feeding experiments were conducted with *dl*-[*methylene*-¹⁴C]-tryptophan, L-[5-³H]-proline, *dl*-[2-¹⁴C]-mevalonic acid lactone, and *cyclo*-L-[*methylene*-¹⁴C]-tryptophyl-L-proline as shown by the resulting data in Table 2. The specific molar incorporations were determined by feeding precursors of certain specific activities and recrystallizing the brevianamide A produced under these conditions to constant activity without the addition of cold, unlabeled brevianamide A.

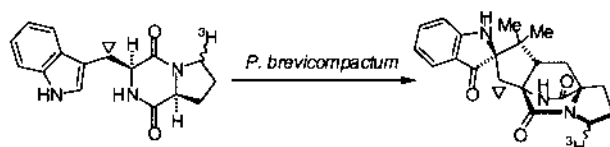
Table 2. Biosynthetic Feeding Experiments with Radioactive Precursors to *Penicillium brevicompactum*²⁵⁾

Precursor	Activity fed (μCi)	% total incorporation
<i>dl</i> -[<i>methylene</i> - ¹⁴ C]-Tryptophan	100	5.1
L-[5- ³ H]-Proline	100	0.40
<i>dl</i> -[2- ¹⁴ C]-Mevalonate	75	0.16
<i>cyclo</i> -L-[<i>methylene</i> - ¹⁴ C]-Tryptophyl-L-proline	12.9	1.8
	12.5	

To demonstrate that brevianamide F was incorporated as an intact unit without any hydrolytic degradation and recombination of the constituent amino acids, *cyclo*-L-[*methylene*-¹⁴C]-tryptophyl-L-[5-³H]-proline was prepared (³H: ¹⁴C ratio = 3.67:1) and fed in two batches of *ca.* 100 mg to *Penicillium brevicompactum*. The brevianamide A isolated from these experiments had ³H: ¹⁴C ratios of 3.82:1 and 3.74:1 and total incorporations of 3.2% and 3.6%, respectively. The high specific incorporation of 14.0% confirms that brevianamide F is a biosynthetic precursor (Chart 4).

Based on the co-occurrence of brevianamide E with austamide in cultures of *Aspergillus ustus* as reported by Steyn,¹⁸⁾ Birch and associates postulated the logical biosynthetic sequence involving: the coupling of L-proline and L-tryptophan → brevianamide F → deoxybrevianamide E → brevianamide A.²⁵⁾

The introduction of the isoprene unit has comprised a subject of much speculation. Birch speculated that: "The re-



7, *cyclo*-L-[*methylene*-¹⁴C]-tryptophyl-L-[5-³H]-proline (brevianamide F, ▽ = ¹⁴C)

2, brevianamide A

Chart 4. Incorporation of Brevianamide F into Brevianamide A²⁵⁾

versed unit could be attached directly through a cation or might be biogenetically transferred in the brevianamides from the dioxopiperazine nitrogen atom, either in an indole or an indoxyl precursor".²⁵⁾ This possibility, shown below in Chart 5, amounts to a formal S_N2' transfer of a "normal" prenylated precursor to the "reverse" prenylated metabolite. Birch further speculated that a second possibility is ring formation leading to austamide by the simultaneous addition of carbon and nitrogen in a carbene-type reaction which was suggested by Birch for the introduction of an isoprene unit into a non-phenolic benzene ring. The fascinating reverse prenylation reaction has also been discussed in the context of the biosynthesis of roquefortine, echinulin and the parherquamides and will be re-addressed below.²⁶⁾

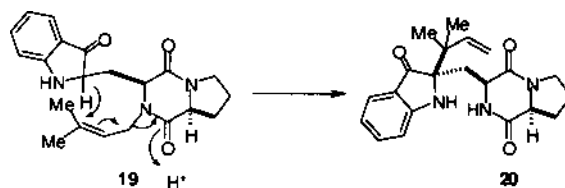


Chart 5. Reverse Prenylation Proposal of Birch *et al.*²⁵⁾

The most intriguing feature of the final assembly of brevianamides A and B is the mechanism of formation of the novel bicyclo[2.2.2] ring system common to this family of metabolites. Porter and Sammes were the first to suggest the involvement of an intramolecular Diels–Alder reaction for the formation of this ring system and drew the following structure to illustrate this provocative idea (Fig. 3).²⁷⁾

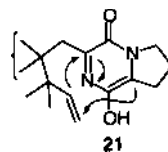
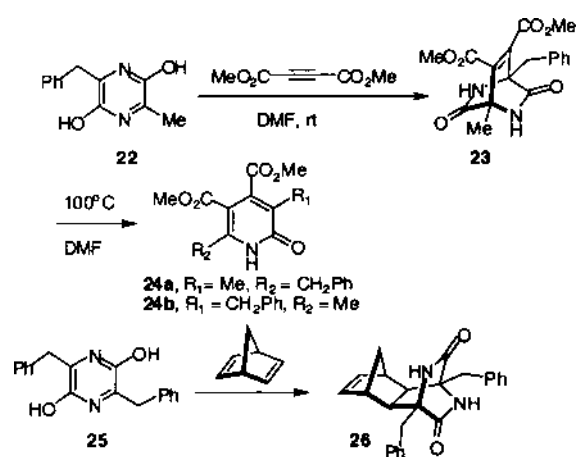


Fig. 3. Porter and Sammes Diels–Alder Proposal²⁷⁾

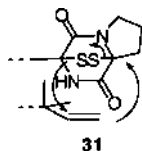
Experimental support for this notion was further presented by the Diels–Alder cycloadditions of dimethyl acetylenedicarboxylate and norbornadiene with the pyrazine derivatives shown below in Chart 6.²⁷⁾

Shortly after the publication of the Diels–Alder hypothesis by Porter and Sammes, Birch speculated on the possible modes of formation of the bicyclo[2.2.2] ring system and stated: "An alternative suggestion (Porter and Sammes 1970) is the occurrence of a Diels–Alder type reaction on a pyrazine, which is perhaps unlikely in view of the unactivated nature of the double bond."²⁸⁾ Birch offered two other mechanisms,

Chart 6. Biomimetic Diels–Alder Cycloadditions²⁷⁾

one involving the intermediacy of an epidithiapiperazinedione as shown below (Fig. 4, although he also admitted that no sulfur compounds could be detected in the mold products) and also alluded to a radical process; no details were given on the latter concept.

No other publications on the biogenesis of the breviranamides appeared in the literature until the late 80's when a total synthesis of (–)-breviramide B was reported by Williams *et al.*^{29,30)}

Fig. 4. Epidithiapiperazinedione Proposal of Birch²⁸⁾

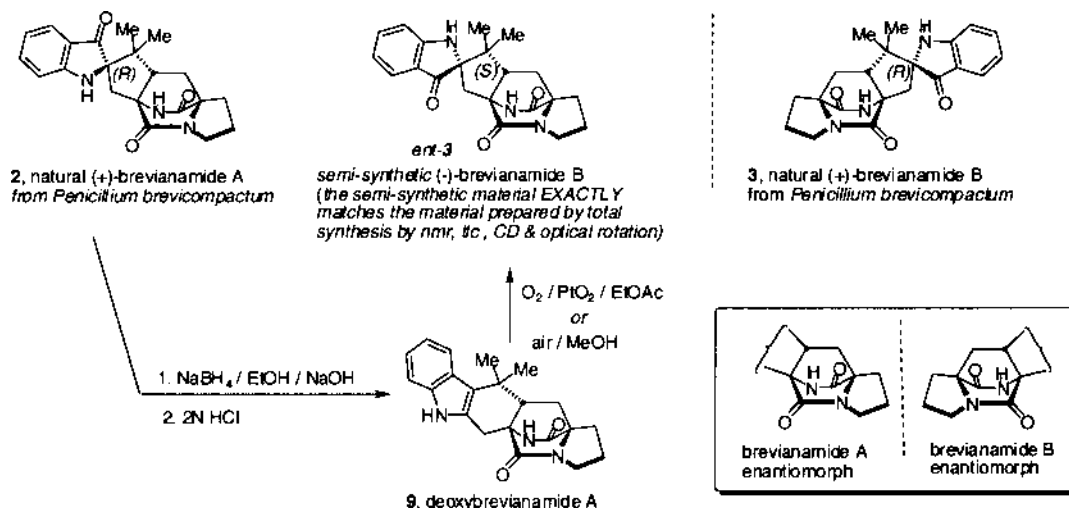
Synthesis of (–)-Breviramide B Reveals Unusual Biogenesis

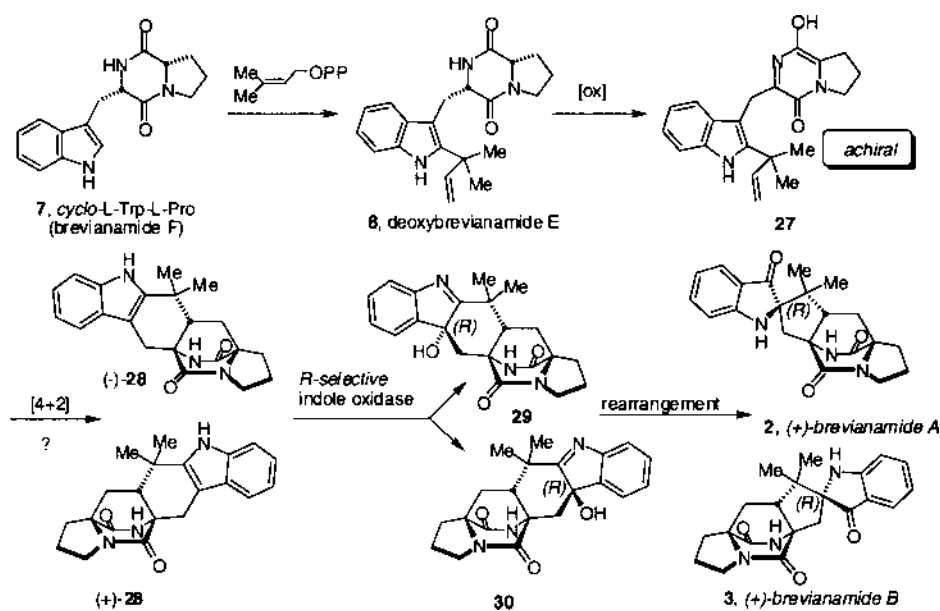
Williams *et al.*, accomplished the first total synthesis of breviramide B which also served to elucidate the absolute configuration of this substance.^{29,30)} The synthesis is illustrated in the next section in Chart 19.

Due to the very low levels of breviramide B produced by *Penicillium brevircompactum* relative to breviramide A, the authentication of the synthetic (–)-breviramide B was secured through the semi-synthetic conversion of natural (+)-breviramide A into (–)-breviramide B as originally described by Birch and Russell.²¹⁾

The semi-synthetic and totally synthetic breviramide B samples both exhibited the same specific optical rotation ($[\alpha]_D^{25} = -124^\circ$) and circular dichroism (CD) spectra. These workers found that *natural* breviramide B obtained directly from cultures of *Penicillium brevircompactum* had a specific optical rotation $[\alpha]_D^{25} = +124^\circ$, and a CD spectrum with Cotton effects of *exactly opposite sign* to that of the synthetic and semi-synthetic materials. The fact that the optical rotation of natural (+)-breviramide B was of equal magnitude and opposite sign to that of semi-synthetic and totally synthetic (–)-breviramide B suggests that the natural metabolite is optically pure; the significance of this will be discussed in more detail below. This rather unexpected finding mandates that the absolute configuration of breviramide A and breviramide B are *enantiomorph* with respect to the bicyclo[2.2.2] moiety but that each metabolite has the (*R*)-absolute configuration at the spiro- ψ -indoxyl stereogenic center; these relationships are summarized in Chart 7.

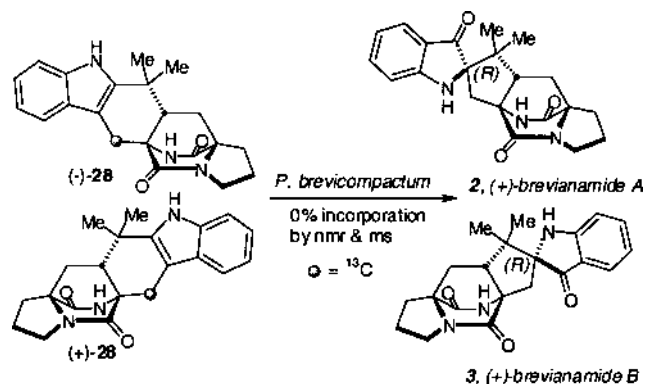
Based on this unusual finding, Williams *et al.*, proposed a biogenetic pathway that could accommodate the formation of the two enantiomorph bicyclo[2.2.2] ring systems in the respective natural metabolites as shown in Chart 8.³¹⁾ Reverse prenylation of breviramide F as outlined by Birch to deoxybreviramide E followed by two-electron oxidation of the tryptophyl moiety and enolization of the proline residue would provide an *achiral* azadiene. Cycloaddition could, in principle, occur from either face of the azadiene system thus yielding the *racemic* (or partially *racemic*) hexacyclic indole cycloaddition products. Since both natural breviramides A and B have the (*R*)-absolute stereochemistry at the indoxyl spiro-center, the involvement of an (*R*)-selective indole oxidase which, only recognizes the binding orientation of the 2,3-disubstituted indole, was invoked to effect the “resolution” of the hypothetical *racemic* Diels–Alder adduct into two optically pure, diastereomeric hydroxyindolenines. Subsequent pinacol-type ring contraction in a stereospecific

Chart 7. Stereochemical Relationships of the Breviramides as Determined by Williams *et al.*^{29,30)}

Chart 8. Early Proposal for Brevianamide Biosynthesis, Williams *et al.*³¹⁾

manner would thus furnish the respective optically pure (+)-brevianamides.

To test this hypothesis, Williams *et al.*, synthesized the hypothetical Diels–Alder cycloadduct in racemic form bearing a ^{13}C label at the tryptophyl methylene carbon.³²⁾ Biosynthetic feeding of this potential precursor suspended in dimethyl sulfoxide (DMSO) to cultures of *Penicillium brevicompactum* yielded both brevianamides A and B but, within the limits of experimental error, there was no evidence for the incorporation of the labeled precursor into either metabolite by nmr and/or mass spectral analysis (see Chart 9). In addition, culture filtrates and mycelia from *Penicillium brevicompactum* were extracted and examined for the presence of the hypothetical Diels–Alder cycloaddition product, yet evidence for the existence of this substance was not obtained.

Chart 9. Biosynthetic Feeding of the Hexacyclic Indole³²⁾

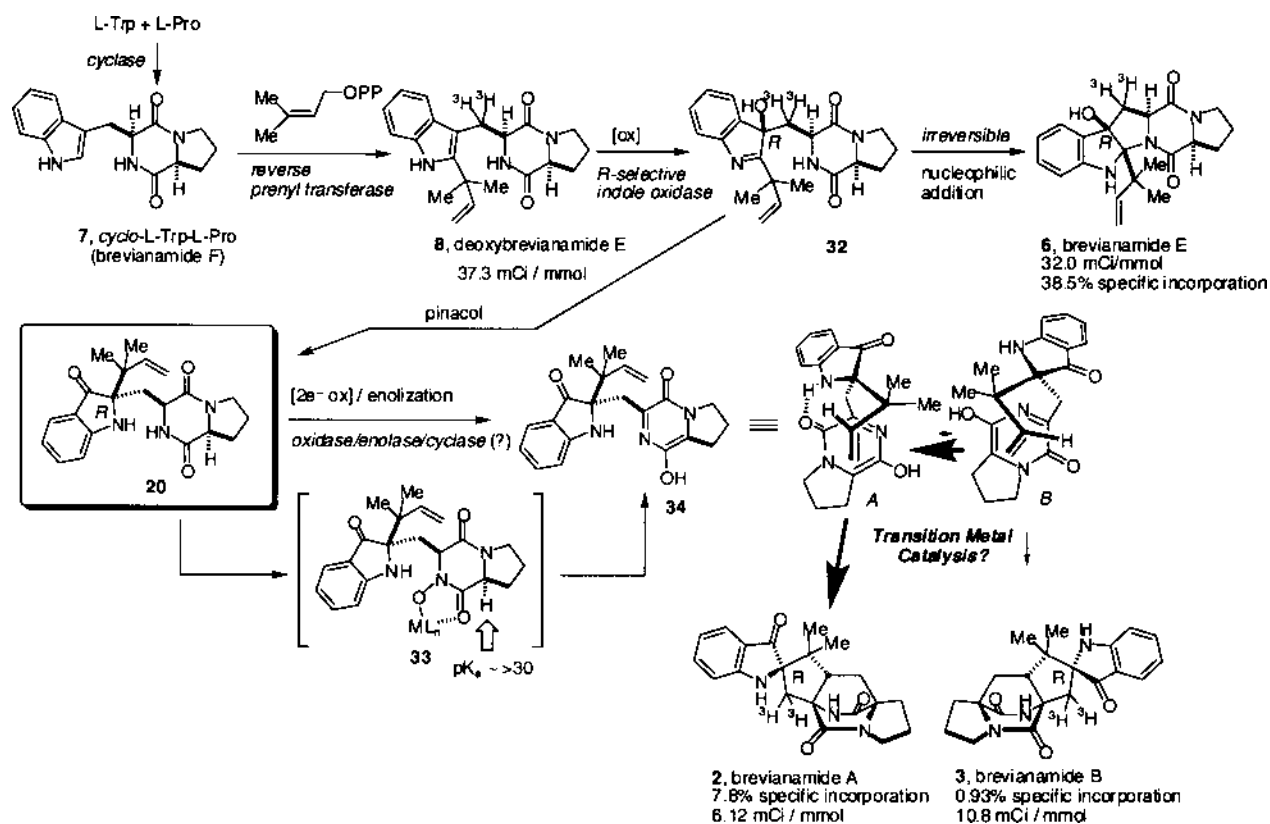
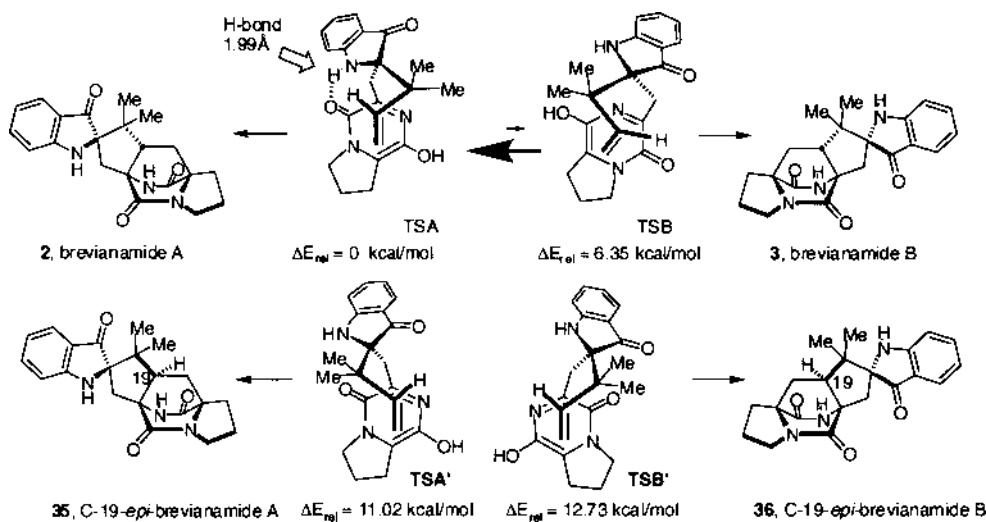
Although these results do not rigorously exclude the possibility of the hexacyclic indole as a biosynthetic precursor to the brevianamides (poor solubility, cell penetration, uptake, k_D 's, *etc.* may limit the access of the labeled, synthetic precursor to the biosynthetic machinery in the cells) enough doubt had been cast on the pathway depicted in Chart 8 such that alternatives were pursued.

The synthesis of [8-methylene- $^3\text{H}_2$]deoxybrevianamide E was carried out by Williams *et al.*, to probe the intermediacy of deoxybrevianamide E in the biosynthesis of brevianamides A, B and E.³²⁾ It was found that this substance, when fed to cultures of *Penicillium brevicompactum*, was very efficiently incorporated into brevianamide E, brevianamide A and brevianamide B. The data are reproduced in Table 3.

Table 3. Incorporation of [8-methylene- $^3\text{H}_2$]Deoxybrevianamide E into Brevianamides A, B and E³²⁾

Metabolite	% specific incorporation
Brevianamide A	12.1
Brevianamide B	1.4
Brevianamide E	38.5

When radioactive brevianamide E was re-fed to the cultures, there was no significant incorporation into either brevianamide A or B clearly indicating that brevianamide E is a dead-end, shunt metabolite. Based on the available data, these workers proposed the biosynthetic pathway illustrated in Chart 10 that can accommodate the formation and stereochemistry of all of the relevant metabolites. Thus, reverse prenylation of brevianamide F (*cyclo-L-Trp-L-Pro*) to deoxybrevianamide E followed by an (*R*)-selective oxidation at the 3-position of the indole gives an (*R*)-hydroxyindolenine that suffers one of two fates: (1) irreversible nucleophilic addition of the tryptophyl amide nitrogen forming brevianamide E or, (2) a stereospecific pinacol-type rearrangement to an as yet, unidentified ψ -indoxyl (**20**, Chart 10, box). This hypothetical substance was envisioned to constitute the penultimate intermediate that must undergo three final reactions to the brevianamides: (1) two-electron oxidation of the tryptophyl α -carbon, (2) enolization of the prolyl methine and, (3) intramolecular Diels–Alder cycloaddition from two of the four possible diastereomeric transition states to yield brevianamides A and B. Based on the existence of numerous

Chart 10. Brevianamide Biosynthesis Proposal of Williams *et al.*³²⁾Chart 11. *Ab Initio* Calculations on the Putative Diels–Alder Cycloadditions by Domingo *et al.*³³⁾

hydroxamate-containing secondary metabolites such as mycelianamide, it can be further proposed that the tryptophyl oxidation proceeds through a hydroxamic acid intermediate (shown in Chart 10). This functionality, an excellent chelator of (a non-heme) Fe(III), for instance, could further function to assist the elimination of water to form the imino portion of the azadiene. Since the prolyl methine ($pK_a > 30$) must also be removed at the active site of an enzyme, a metal-coordinated amide might also be essential to lower the activation barrier for the deprotonation step. Finally, the intramolecular Diels–Alder cycloaddition might enjoy a catalytic effect of azadiene-metal coordination.

In order to gain some insight into the putative biosynthetic Diels–Alder cycloaddition, Domingo *et al.*, carried out *ab initio* calculations on the four possible transition structures from the azadiene as summarized above in Chart 11.³³⁾ The calculations were performed with analytical gradients at *ab initio* 3-21G and 6-31G* basis sets within Hartree-Fock procedures. This Diels–Alder reaction appears to be controlled by frontier molecular orbitals $HOMO_{(dieneophile)}/LUMO_{(azadiene)}$. All four diastereomeric transition structures were examined and it was found that the lowest_(rel.) energy transition structure is TSA which leads to brevianamide A (see Table 4). The next highest energy transition structure is

TSB, which lies approximately 6–7 kcal/mol above TSA; this transition structure would produce brevianamide B. An intramolecular H-bond was found in TSA between the indoxyl N–H and the tryptophyl carbonyl group which can account for a large fraction of the relative stabilization energy of this transition structure. After TSB, the next highest energy transition structures are TSA' and TSB', which would lead to C-19-*epi*-brevianamide A and C-19-*epi*-brevianamide B, respectively. Williams *et al.*, have previously described the total synthesis of C-19-*epi*-brevianamide A^{29,30,34}) and using this authentic specimen, have been unable to detect this substance as a metabolite of *Penicillium brevicompactum*.

Table 4. Calculated Potential Energy Barriers and Relative Energies (kcal/mol) from TSA, TSB, TSA' and TSB'³³⁾

	3-21G/3-21G		6-31G*/3-21G	
	ΔE_a	ΔE_{rel}	ΔE_a	ΔE_{rel}
TSA	31.81	0.00	38.68	0.00
TSB	39.42	7.61	45.03	6.35
TSA'	44.17	12.36	49.71	11.02
TSB'	47.07	15.26	51.41	12.73

This theoretical study therefore predicts that the relative distribution of brevianamides A and B from *Penicillium brevicompactum* is a direct manifestation of the relative energies of the four diastereomeric transition structures: brevianamide A is the major metabolite, brevianamide B is a minor metabolite and neither of the C-19-*epi*-structures are found.

Domingo *et al.*, also examined the effect of a Lewis acid (BH₃) on the simpler model system shown in Chart 12. It was found that Lewis acid complexation lowered the potential energy barrier by 3–5 kcal/mol relative to the parent system. The speculation presented above, regarding the complexation of the azadiene system to a non-heme metal, such as Fe(III), appears to be supported by this theoretical insight.

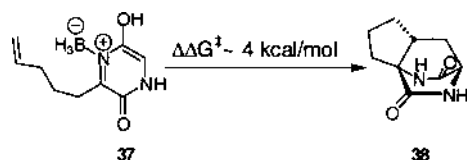


Chart 12. Lewis Acid Activation of the Proposed Biosynthetic Intramolecular [4+2] Cycloaddition³³⁾

Further work needs to be done to confirm the pathway outlined in Chart 11 and in particular, to elucidate the possible involvement of protein organization and/or catalysis of the biosynthetic reactions culminating in the formation of brevianamides A and B. Extensive effort in the author's laboratory to prepare key intermediate **20** has met with failure. From the stability of precursors to **20**, it appears that this substance is unstable, leading to a retro Michael reaction giving a dehydro alanine fragment of the diketopiperazine and the reverse prenylated indoxyl.³⁵⁾ It also appears possible that the oxidation of **32** to an azadiene and cycloaddition may precede the pinacol-type rearrangement to the indoxyl. Further work is needed to explore this possibility.

Very little has appeared in the literature concerning the biosynthesis of austamide. The occurrence of deoxybre-

vianamide E and deoxyaustamide in culture extracts of *Aspergillus ustus*, suggests the following biosynthetic pathway (Chart 13).

Reverse prenylation of *cyclo*-L-Trp-L-Pro (brevianamide F) furnishes deoxybrevianamide E. Oxidative ring closure of the tryptophyl amide nitrogen across the isoprene-derived olefinic moiety and desaturation of the proline ring would yield the eight-membered ring metabolite deoxyaustamide. Finally, oxidation at the 3-position of the 2,3-disubstituted indole followed by a pinacol-type rearrangement would provide the spiro ψ -indoxyl austamide.

Harrison has suggested that fumitremorgin B and austamide may arise biosynthetically from an isoprene-derived imine such as that shown in Chart 14.³⁶⁾ Cyclization of the imine either *via* path A or path B result in the formation of the fumitremorgin B and deoxyaustamide ring systems, respectively.

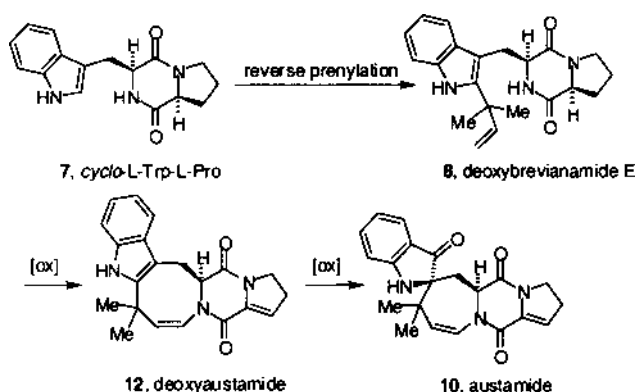


Chart 13. Possible Biosynthetic Pathway to Austamide

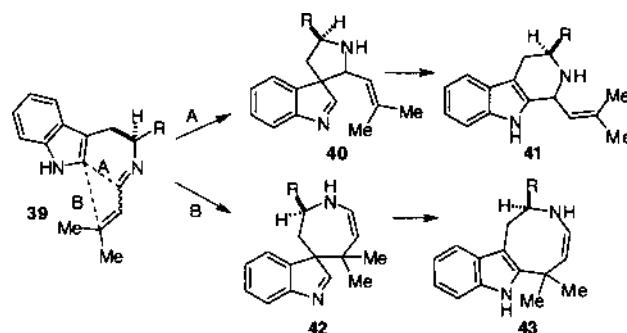
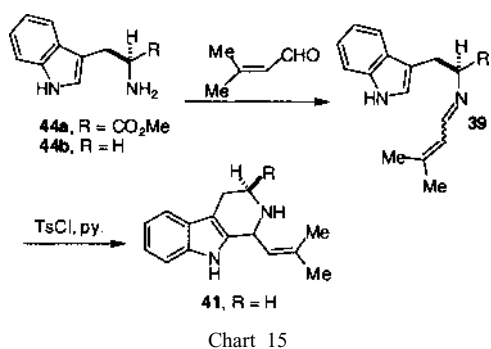


Chart 14. Possible Biosynthetic Modes of Ring Closure to Austamide and Fumitremorgin³⁶⁾

A simple experimental system designed to test this hypothesis was conducted as shown in Chart 15.³⁶⁾ Condensation of L-tryptophan methyl ester and tryptamine with 3-methylbut-2-enal gave the corresponding imines which were recalcitrant to cyclization under acidic or neutral conditions. The tryptamine-derived imine was induced to undergo the Pictet–Spengler cyclization in the presence of *p*-toluenesulfonyl chloride in pyridine to the tetrahydro- β -carboline derivative. In neither case, were products derived by pathway B (Chart 14) detected.

It might also be parenthetically noted that, the absolute stereochemistry of austamide was determined by X-ray analysis on 5-bromo-12*S*-tetrahydroaustamide¹⁹⁾ (obtained from austamide by hydrogenation followed by bromination)



and that the absolute configuration at the spiroindoxyl stereogenic center (C-2) was determined to be *S*. This assignment was based on the hydrolytic liberation of *S*-proline from 12,13-dihydroaustamide; the remaining stereogenic centers at C-9 and C-2 (the spiro center) were assigned relative to that for C-12. This is in marked contrast to breviramides A and B which both possess the *R*-absolute configuration at the corresponding spiroindoxyl stereogenic center. Assuming that this assignment is correct, it is interesting that *Aspergillus ustus* must therefore have a pro-*S*-selective indole oxidase and that *Penicillium brevicompactum* utilizes a pro-*R*-selective indole oxidase.

Very recent work on the biosynthesis of austamide will be discussed below in conjunction with work on the biosynthesis of paraherquamide A.

Synthetic Studies on the Brevianamides

The first synthetic studies on breviramides were reported by Sammes as described above in Chart 6. Nothing more appeared in the literature until 1986, when Williams and Glinka reported an intramolecular Michael-type cyclization reaction to give the bicyclo[2.2.2]diazaoctan ring system.³⁷⁾ This synthetic model study is illustrated in Chart 16. The α -alkylated proline residue was built up from *D,L*-homoserine and alkylation with 1,3-dibromopropane. Oxidative manipulation to the aldehyde and Horner–Emmons–Wadsworth olefination gave the incipient unsaturated ethyl ester that suffered enolate formation and cyclization to give a 3 : 2 mixture of two diastereomeric pairs favoring the undesired relative configuration of

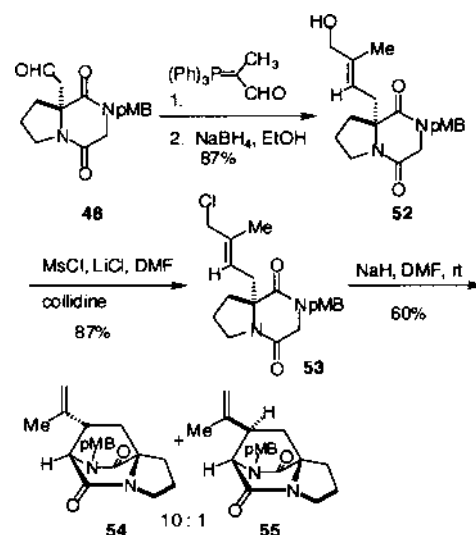
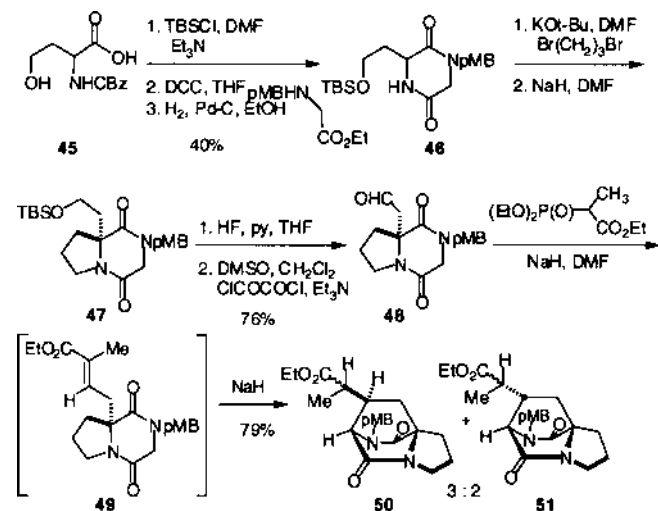


Chart 17. Intramolecular S_N2' Cyclization³⁷⁾

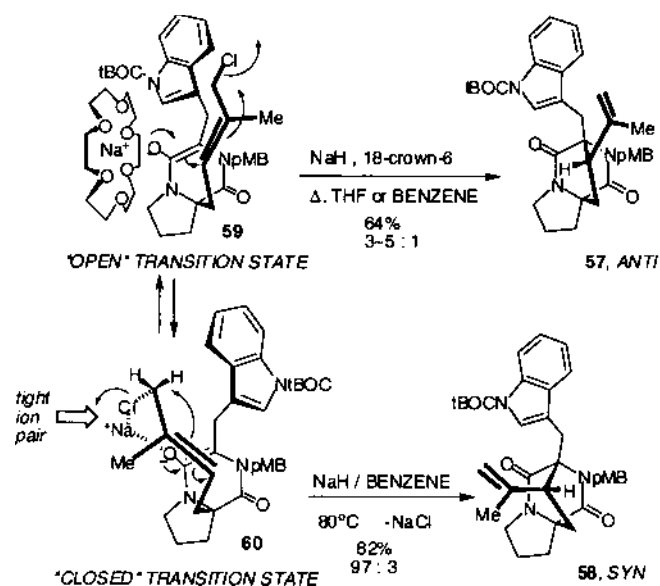


Chart 18. Transition State Models for the S_N2' Cyclization Proposed by Williams *et al.*^{29,30)}

the newly created stereogenic center. The structure of the major isomer from this reaction was secured by single-crystal X-ray analysis.

An alternative approach that explored the utility of an intramolecular S_N2' cyclization is illustrated in Chart 17.³⁷⁾ Wittig homologation of aldehyde **48** followed by borohydride reduction gave the *E*-allylic alcohol (**52**). Allylic chloride formation proceeded with retention of stereochemistry of the olefin. Subjecting this species to NaH in *N,N*-dimethylformamide (DMF) resulted in the formation of two tricyclic products in 60% yield and in a 10 : 1 ratio favoring the desired (brevianamide) *anti*-relative stereochemistry.

The diastereoselectivity of the intramolecular S_N2' cyclization was rationalized by these workers to proceed through an open transition state^{38,39)} as shown in Chart 18 and Table 5.^{29,30)}

Three substrates were examined and the effect of the metal counterion, solvent, temperature and additives varied to ob-

serve the effect on the facial selectivity of this reaction. It was found that simply replacing the solvent benzene for DMF using NaH as a base completely reversed the diastereoselectivity to favor the *syn*-relative stereochemistry (Table 5). These workers reasoned that the polar solvent DMF, formed a solvent ligation sphere surrounding the enolate metal counterion that exerted steric encumbrance in the region proximal to the enolate oxygen atom. This would preclude cyclization from a conformation that would result in the *syn*-isomer (“*endo*” transition state, Chart 18) thus forcing the allylic chloride to populate the “*exo*” conformer re-

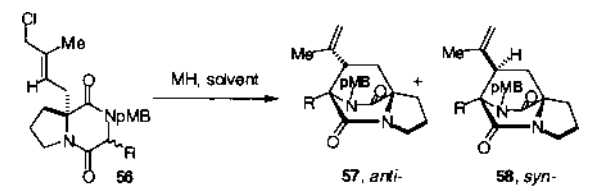
sulting in the *anti*-relative stereochemistry.


Based on this rationalization, several metal ligands were screened that might exert a similar steric effect to that observed with DMF with the aim of improving the diastereoselectivity. It was found that the combination of 18-crown-6 with NaH as the base in benzene or THF gave satisfactory results with the indolyl side chain which was required for the brevianamide B total synthesis. For this substrate, a 3–5 : 1 *anti* : *syn* ratio was realized in synthetically useful yields. Curiously, when the crown ether and enolate cations were “matched”, such as the K/18-crown-6 or Na/15-crown-5 pairings, poor facial selectivity was observed. These workers interpreted these observations to indicate that “mis-matched” 18-crown-6 is a weak ligand for sodium but one that resides in the vicinity of the enolate oxygen to exert the desired steric influence on the conformational population of the pre-cyclization substrate. The “matched” crown ether–cation pairings apparently rapidly separate the metal cation from the enolate anion at a rate faster than the *S_N2'* cyclization leaving a “naked” enolate anion with no apparent influence on the conformational disposition of the allylic chloride resulting in poor facial selectivity.

In the “mis-matched” Na/18-crown-6 pairing, these workers reasoned that rate of cyclization is faster than the rate at which the crown-ligated sodium cation can be solvent-separated from the enolate oxygen atom. The crown-ligated enolate must therefore have sufficient residence time in the vicinity of the enolate to influence the pre-cyclization population of allylic chloride conformers favoring the “*exo*”, open transition state.

This work culminated an asymmetric total synthesis of (–)-brevianamide B by Williams and co-workers as shown in Chart 19.^{29,30} This synthesis served to establish the relative stereochemistry originally assigned by Birch and also served

Table 5. Diastereoselectivity of Williams' *S_N2'* Cyclization^{29,30}



R	Solvent	Temp. (°C)	Base	Ratio <i>anti</i> : <i>syn</i>	Yield (%)
H	DMF	25	NaH	10 : 1	60
	Benzene	80	NaH	ca. 0 : 1	10
Bn	DMF	25	NaH	2 : 3	65
	Benzene	80	NaH	ca. 0 : 1	20
	DMF	25	NaH	2 : 1	63
	Benzene	80	NaH	3 : 97	82
	Benzene	80	NaH/18-Cr-6	3.9 : 1	56
	Benzene	80	KH/18-Cr-6	1.1 : 1	38
	Benzene	80	NaH/15-Cr-5	1.42 : 1	40
	Benzene	25	NaH/18-Cr-6	6 : 1	14
	THF	67	NaH/18-Cr-6	4.9 : 1	64

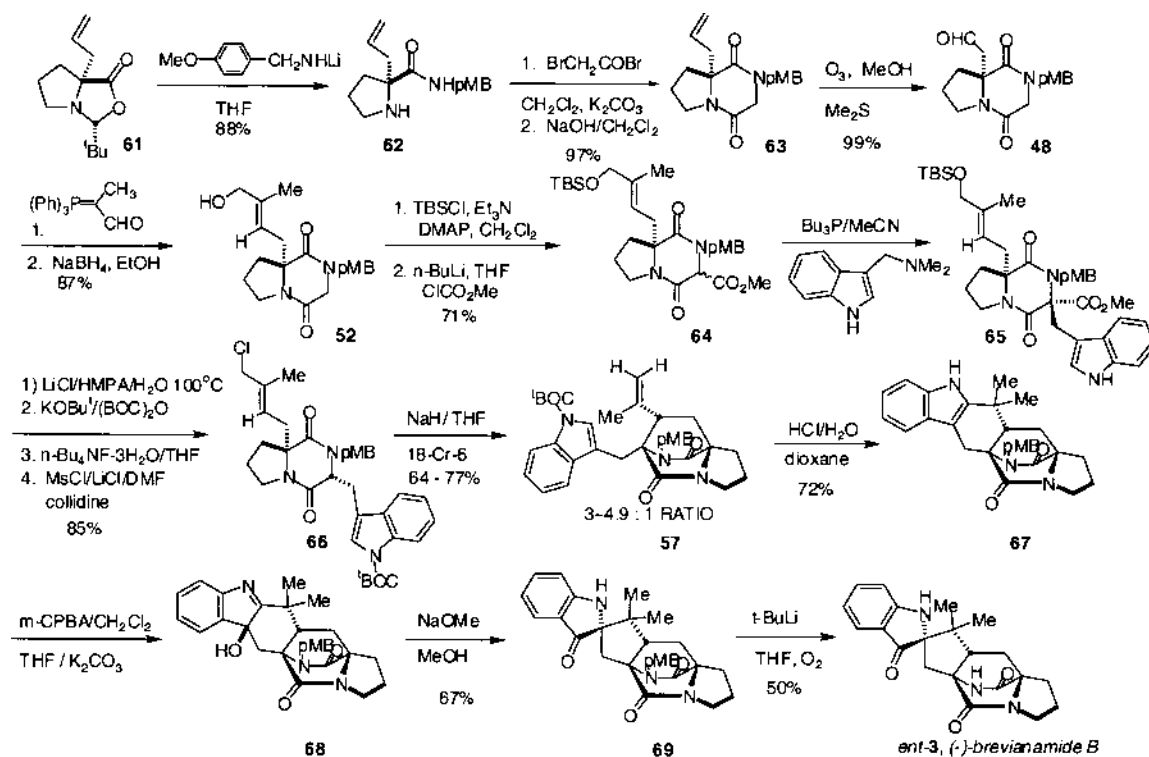


Chart 19. Asymmetric Synthesis of (–)-Brevianamide B by Williams *et al.*^{29,30}

to assign the absolute stereochemistry that was previously mis-assigned. The key feature of this synthesis exploited the discoveries discussed above, to control the facial selectivity of an intramolecular S_N2' cyclization.

The synthesis began with an enantioselective alkylation of L-proline. Formation of the pivaldehyde acetal from L-proline followed by enolate alkylation with allyl bromide according to Seebach, furnished the known⁴⁰ allylated heterocycle **61**. Reaction of this substance with the lithium salt of *para*-methoxybenzylamine cleanly provided the corresponding amide (~quant.). Acylation with bromoacetyl bromide followed by ring closure furnished the enantiomerically pure piperazinedione (79–85%). Ozonolysis of the olefin in methanol followed by quenching with dimethyl sulfide furnished the corresponding optically pure aldehyde. Protection of the allylic alcohol as the corresponding *tert*-butyldimethylsilyl ether furnished an appropriate substrate for examining introduction of the indole nucleus.

Direct introduction of the indole nucleus was carried out *via* the methodology devised by Kametani and Somei for the synthesis of brevianamide E.^{41,42} Thus, carbomethoxylation of the silyl ether derived from **52** (*n*-BuLi/tetrahydrofuran (THF)/ClCO₂Me) afforded a 4 : 1 diastereomeric mixture of **64** which was directly treated with gramine in the presence of tri-*n*-butylphosphine in acetonitrile to furnish a single diastereoisomer **65**. The relative configuration was quite easy to assign based on ¹H-NMR behavior. Conversion of the carbomethoxy group, to the corresponding carboxylic acid with concomitant thermal decarboxylation was realized with LiCl in wet hexamethylphosphoramide (HMPA) at 100 °C. Protection of the indole nitrogen as the *t*-BOC derivative and removal of the silyl group furnished the allylic alcohol precursor to **66** in good overall yield. Conversion to the key allylic chloride (**66**) was achieved by treatment with mesyl chloride in DMF containing collidine and excess lithium chloride.⁴³ Reaction of **66** with 10 equivalents of NaH in warm THF containing 5 equivalents of 18-crown-6 result in a 3–4.9 : 1 ratio of *anti* : *syn* S_N2' cyclization products **57** and **58** in 64–77% combined yield.

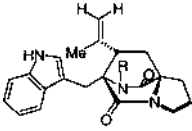
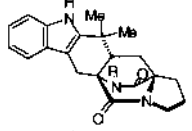
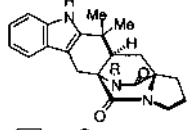
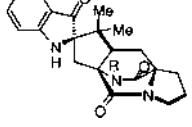
With the moderately stereoselective obtention of **57**, the stage was now set to examine the final, crucial carbon–carbon bond construction *via* an olefin–cation cyclization approach.⁴⁴ Isomer **57** underwent clean, regiospecific cyclization and concomitant *t*-BOC deprotection to afford the crystalline hexacyclic compound **67** in the presence of aqueous HCl in dioxane. Oxidation of the 2,3-disubstituted indole with *m*-chloroperbenzoic acid (CPBA) in dichloromethane^{45–48} provided the stable hydroxy indolenine **68** as a single isomer. Treatment of this material with NaOMe in MeOH resulted in a clean, stereospecific pinacol-type rearrangement to give the expected yellow, crystalline indoxyl **69**. The relative stereochemistry of this substance was unambiguously established by single crystal X-ray analysis. This structure elucidation served both to confirm the stereochemical outcome of the S_N2' cyclization reactions as well as that of the indole oxidation. As expected (*vide supra*), the facial selectivity of the indole oxidation is controlled by the stereogenic center at C-19 of the bicyclo[2.2.2] nucleus; the peracid is directed to the least hindered face of the indole.⁴⁹

The final step of the synthesis required removal of the amide protecting group and took extensive experimentation.

It was found that removal of the *N*-*para*-methoxybenzyl protecting group proved extremely difficult under the standard oxidative conditions employing ceric ammonium nitrate.^{50,51} While these conditions were found to effect deprotection on the simple, unsubstituted compound **54**, the presence of the electron-rich indoxyl apparently reacted much faster with the oxidizing reagent than did the *N*-*para*-methoxybenzyl group. Additional attempts to remove the *N*-*para*-methoxybenzyl protecting group by dissolving metal reduction; catalytic hydrogenation; acidic solvolysis; boron tribromide; boron trichloride; 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) oxidation and photooxidation all met with complete failure to provide even a trace of brevianamide B. Eventually it was found that treatment of **69** with excess *t*-butyllithium in THF at low temperature, followed by quenching with MoOPH⁵² or more conveniently, O₂ and aqueous extractive isolation afforded (–)-brevianamide B in 40% yield.^{53,54–57} This compound was identical to an authentic sample of brevianamide B obtained from harvesting cultures of *Penicillium brevicompactum* by ¹H-NMR, IR, TLC and UV. However, the specific rotation of the natural and synthetic samples were of opposite sign and of equal magnitude indicating that the synthetic material was the *enantiomorph* of the natural product. This was further corroborated by examination of the mirror image CD curves of both samples. These data have been further corroborated by comparison of synthetic brevianamide B and semi-synthetic brevianamide B derived from **2** by the redox scheme developed by Birch involving deoxybrevianamide A. The conversion of natural brevianamide A (from *Penicillium brevicompactum*) to deoxybrevianamide A and then into (–)-brevianamide B proceeded as exactly as described by Birch (Chart 7). The brevianamide B obtained from natural brevianamide A was identical in every respect to the synthetic substance including the sign and magnitude of the specific optical rotation and CD spectra; these materials have the absolute configuration depicted above in Fig. 2 and Chart 7.

In connection with the above work on the total synthesis of brevianamide B, Williams and Kwast reported the removal of

Table 6. Oxidative Removal of *N*-pMB Groups⁵³

Substrate R=pMB	Conditions	Yield (%) (R=H)
	<i>n</i> -BuLi, MoOPH	21
	<i>n</i> -BuLi, MoOPH	35
	<i>n</i> -BuLi, MoOPH	30
	<i>t</i> -BuLi, O ₂	40

the *N*-*para*-methoxybenzyl group from a series of compounds generated from this work.⁵³ As shown in Table 6, C-10-*epi*-brevianamide A along with several indoles, prepared in conjunction with the total synthesis effort, withstood the conditions of benzylic carbanion formation and oxidation. While the yields are modest, this method offers an alternative to the oxidative conditions that are typically used to remove *N*-*para*-methoxybenzyl residues, such as DDQ or ceric ammonium nitrate (CAN).

Dunkerton and co-workers, reported a synthetic approach to brevianamides A and B that was inspired by the original intramolecular Diels–Alder approach of Sammes.⁵⁸ This constitutes the first attempt to approach the intramolecular Diels–Alder cycloaddition reaction to construct the bicyclo[2.2.2]diazaoctan ring system.

As shown in Chart 20, an Ireland ester enolate Claisen rearrangement was utilized to prepare the reverse-prenylated dihydroindole aldehyde **74**. This compound was condensed with the *N*-*para*-methoxybenzyl-protected *cyclo*-Gly-L-Pro diketopiperazine (**75**) to give the α,β -unsaturated species **76**, which was the final product reported in this study. No further work from this laboratory was reported using this approach and attempts to conduct Diels–Alder reactions were not described. As will be seen below, it now appears feasible that this approach might have been viable for a biomimetic approach to the brevianamides.

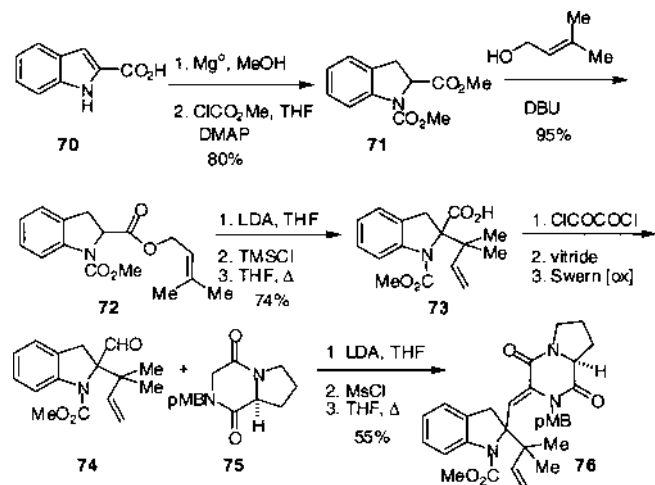


Chart 20. Dunkerton's Synthetic Approach⁵⁸

In 1998, Williams, *et al.*, reported the first successful biomimetic intramolecular Diels–Alder cycloaddition reaction as shown in Chart 21.^{59,60} This approach culminated in a total synthesis of racemic brevianamide B and C-19-*epi*-brevianamide A. 9-*Epi*-deoxybrevianamide E (**77**) was synthesized according to the procedure of Kametani. Conversion of this substance to the lactim ether (**78**) was accomplished with Me_3OBF_4 in CH_2Cl_2 in the presence of Na_2CO_3 . Next, oxidation with DDQ gave the unsaturated substance **79**. Treatment of **79** with aqueous methanolic KOH at room temperature, cleanly produced the labile azadiene **80** which cyclized to give a mixture of **81** and **82** (2 : 1, 68% 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. Conversion of **81** to C-

19-*epi*-brevianamide A (**84**), a non-natural isomer of brevianamide A previously synthesized in this laboratory,⁵³ was accomplished by diastereoselective *m*-CPBA oxidation to the corresponding hydroxyindolenine (**83**, ~quant.) and pinacol rearrangement (1 M NaOMe, MeOH, reflux) to the corresponding *spiro*-indoxyl; subsequent removal of the lactim ether with HCl afforded the corresponding ring-opened aminoester which was cyclized in hot toluene containing 2-hydroxypyridine furnishing *d,l*-**85** in 46% overall yield from **83**. This material was identical to the authentic material in every respect (except for being racemic). Conversion of **82** to *d,l*-brevianamide B was accomplished in like manner in 65% overall yield from **82** securing the relative stereochemistry of each respective isomer **81** and **82**. However, in this case the cleavage of the lactim ether with HCl in aqueous methanol led directly to brevianamide B without any intermediate ring-opened amino ester detectable.

This study demonstrated that the core bicyclo[2.2.2]diazaoctan ring system common to this family might indeed arise by an intramolecular Diels–Alder cyclization from a pre-formed dioxopiperazine that subsequently undergoes oxidation to an azadiene species. It is significant that the diastereofacial bias of the Diels–Alder cyclization of **80** was not strongly affected by solvent. The same ratio of **81** : **82** (*ca.* 2 : 1) was obtained in THF as in aqueous methanol. We have also shown that, C-19-*epi*-metabolites (corresponding to **85**) are not produced by *Penicillium brevicompactum* and there

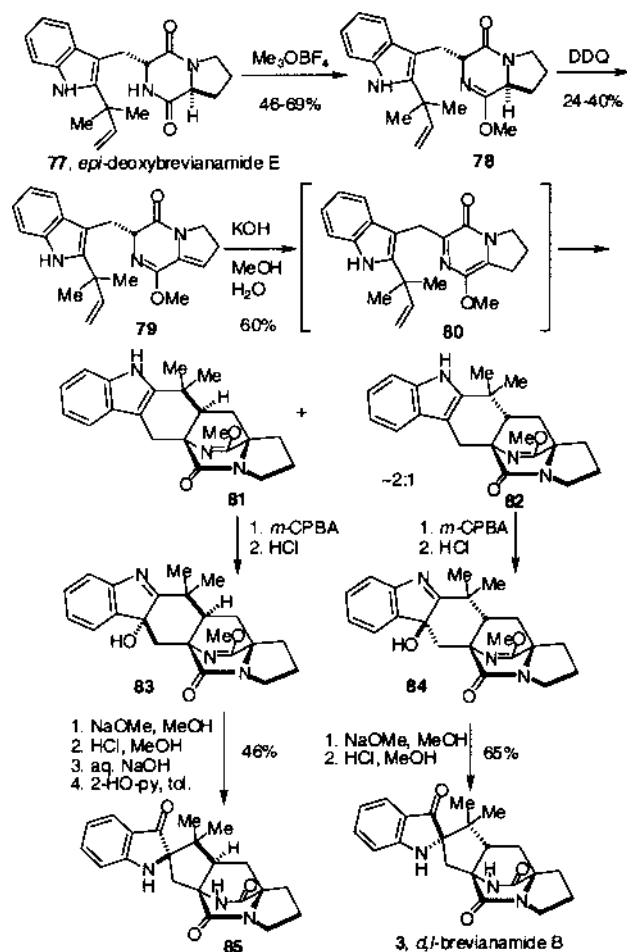


Chart 21. Williams' Biomimetic Total Synthesis of *d,l*-Brevianamide B^{59,60}

have been no reports on the isolation of similarly epimeric metabolites from paraherquamide- or sclerotamide-producing organisms. Thus, in each biosynthetic system, there appears to be complete facial exclusivity in the construction of the bicyclo[2.2.2] ring nucleus; such was not the case for the laboratory cycloaddition reported in Chart 21. The utility of this approach will be discussed below in the context of both racemic and asymmetric syntheses of VM55599.

Another study that impacts on the Diels–Alder cycloaddition reactions described in Chart 21, was a report by a French group as illustrated in Chart 22.⁶¹

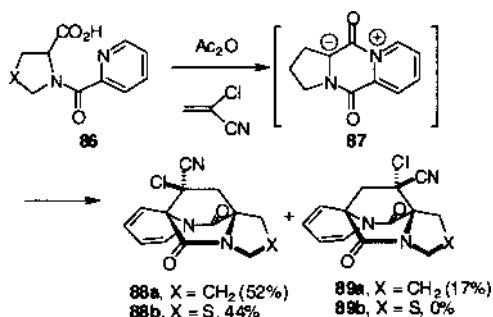


Chart 22. Dipolar Cycloaddition Reported by Fabre *et al.*⁶¹

Cyclization of *N*-(2-pyridinecarbonyl)proline (**86**) with acetic anhydride generated the incipient dipolar species that underwent cycloaddition with 2-chloroacrylonitrile. While this reaction is formally a dipolar cycloaddition reaction, it bears electronic homology to the proposed Diels–Alder constructions of the bicyclo[2.2.2]diazaoctan ring system.

Sanz-Cervera and co-workers investigated a series of intermolecular Diels–Alder cycloaddition reactions of *O*-acyl lactim ethers as shown in Chart 23 and Table 7.⁶² It was observed that electron-deficient dienophiles, such as dimethyl acetylenedicarboxylate (DMAD) and DEAD, reacted smoothly with the azadiene without a Lewis acid catalyst to give the bicyclic cycloadducts in high yields. With neutral dienophiles, such as cyclopentene and cyclohexene, the pres-

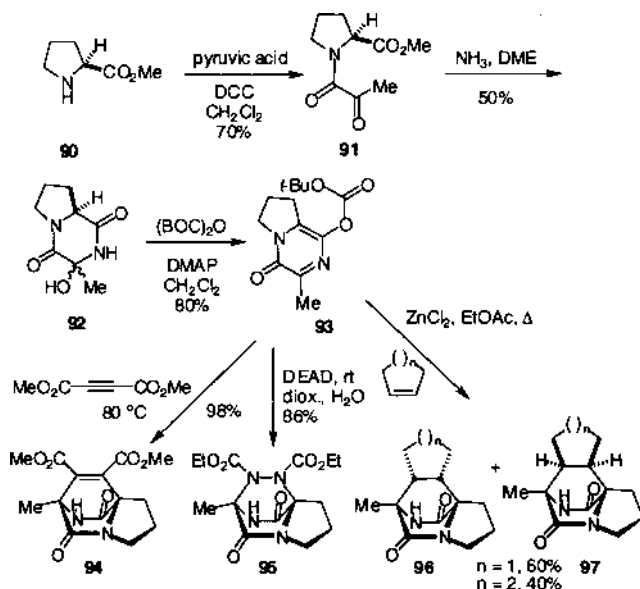


Chart 23. Intermolecular Diels–Alder Reactions Reported by Sanz-Cervera *et al.*⁶²

Table 7. Cycloadditions of Azadiene **93**⁶²

Dienophile	Solvent (°C)	Catalyst	Yield (%)
DMAD	Neat, 80	None	98
DMAD	Neat, rt	ZnCl ₂	75
DEAD	Neat, rt	None	50
DEAD	Diox., H ₂ O, rt	None	86
Cyclopentene	EtOAc, Δ	None	0
Cyclopentene	EtOAc, Δ	AlCl ₃	62
Cyclopentene	EtOAc, Δ	ZnCl ₂	60
Cyclohexene	EtOAc, Δ	None	0
Cyclohexene	EtOAc, 150	AlCl ₃	42

ence of Lewis acids or elevated temperatures was required.

These workers also examined some model intramolecular Diels–Alder reactions as shown in Chart 24. Alkylation of **98** with either 1-bromo-4-pentene or 1-bromo-5-hexene, afforded diastereomeric mixtures of the alkenes **99**. Decarbomethoxylation followed by lactim ether formation and DDQ oxidation afforded the azadienes **102**. Tautomerization with methanolic aqueous KOH afforded the azadienes **103a** and **103b**. Spontaneous intramolecular [4+2] cycloaddition ensued to give the corresponding tetracyclic compounds **104a** and **104b**. Both systems gave the corresponding *anti*-stereochemistry at C-19 (brevianamide A numbering). This is in sharp contrast to the related cycloaddition reaction described in Chart 21 and also to that discussed below in Chart 25 which displayed a preference for the alternative *syn*-diastereomeric series.

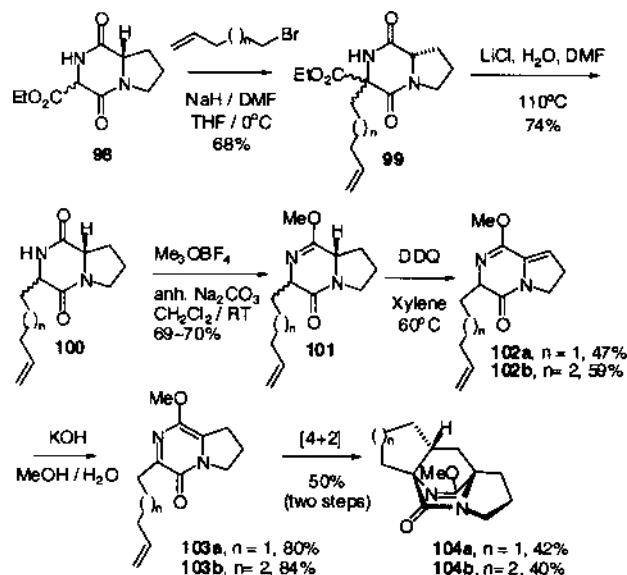
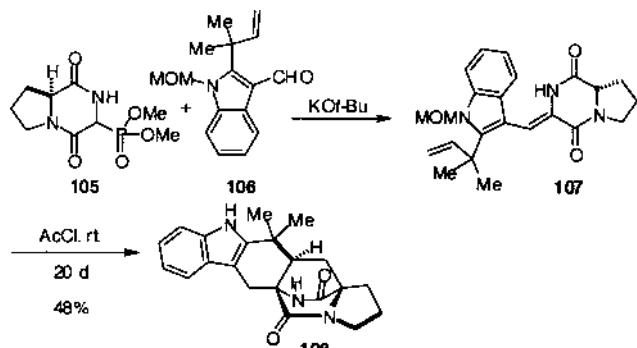


Chart 24. Model Intramolecular Diels–Alder Cycloadditions Reported by Sanz-Cervera *et al.*⁶²

Very recently, Liebscher *et al.*, reported a series of both intermolecular and intramolecular Diels–Alder cycloaddition reactions on 3-ylidenepiperazine-2,5-diones giving a series of bicyclo[2.2.2]diazaoctan systems. The most interesting example from this study is illustrated in Chart 25.⁶³

Horner–Wadsworth–Emmons condensation of phosphonate **105** with the known aldehyde **106** provided the *Z*-ylidene piperazinedione (**107**) in unspecified yield. Treatment of this substance with acetyl chloride as a solvent at room

Chart 25. Diels–Alder Cycloaddition Reported by Liebscher *et al.*⁶³⁾

temperature for 20 d afforded the hexacyclic substance **108** previously prepared by Williams and Kwast in optically pure form.⁵³⁾ These reactions are very similar to those described above by Williams *et al.* (Chart 21) and differ by introducing the unsaturation in the tryptophanyl residue as opposed to the proline residue (*vide supra*). It is interesting to note that the diastereoselectivity of the cycloaddition reaction reported by Liebscher gave only the *syn*-relative stereochemistry (at C-19) corresponding to the paraherquamide relative stereochemistry whereas, the related cycloaddition reported by Williams in Chart 21, gave a *ca.* 2 : 1 mixture of *syn* : *anti*.⁶⁴⁾ The reasons for these distinctions at present remain unclear.

The Paraherquamides, Marcfortines and Related Alkaloids

The paraherquamides are a structurally complex family of alkaloids comprised of two isoprene units, tryptophan and

variously substituted proline derivatives (Fig. 5). Many members of this family display potent anthelmintic and antinematodal activities and have been under intensive investigation for use in veterinary medicine to treat various intestinal parasites.^{65–69)} The parent and most potent member, paraherquamide A, was isolated from cultures of *Penicillium paraherquei* as first described by Yamazaki in 1980.^{70,71)} The simplest member, paraherquamide C, plus five other structurally related paraherquamides C–G were isolated from *Penicillium charlesii* (*fellutanum*) (ATCC 20841) in 1990 at Merck & Co.^{72–78)} and concomitantly at SmithKline Beecham.⁷⁹⁾ Subsequently, three additional related compounds, VM55596, VM55597 and VM55599 were discovered by the same group at SmithKline Beecham from *Penicillium* strain IMI 332995.⁸⁰⁾ Very recently, a Pfizer group⁸¹⁾ reported the isolation of anthelmintic metabolites VM54159, SB203105, SB200437 along with the non-tryptophan-derived metabolites possessing the common bicyclo[2.2.2] nucleus aspergillimide (identical to asperparaline A,^{82,83)} Fig. 6) and 16-keto-aspergillimide from *Aspergillus* strain IMI 337664.

This report, and a recent paper by Whyte and Gloer,⁸⁴⁾ describing the isolation of sclerotamide from *Aspergillus sclerotiorum* constitute the first examples of paraherquamide derivatives isolated outside of the *Penicillia*.

The structurally related alkaloid marcfortine, first described by Polonsky *et al.*, in 1980 is rendered from two isoprene units, tryptophan and pipercolic acid.^{85,86)} The structures of the members of this family of mold metabolites are presented in Fig. 5. The paraherquamides differ with respect to substitution and oxygenation in the proline ring and the prenylated oxindole ring; paraherquamide B is the simplest

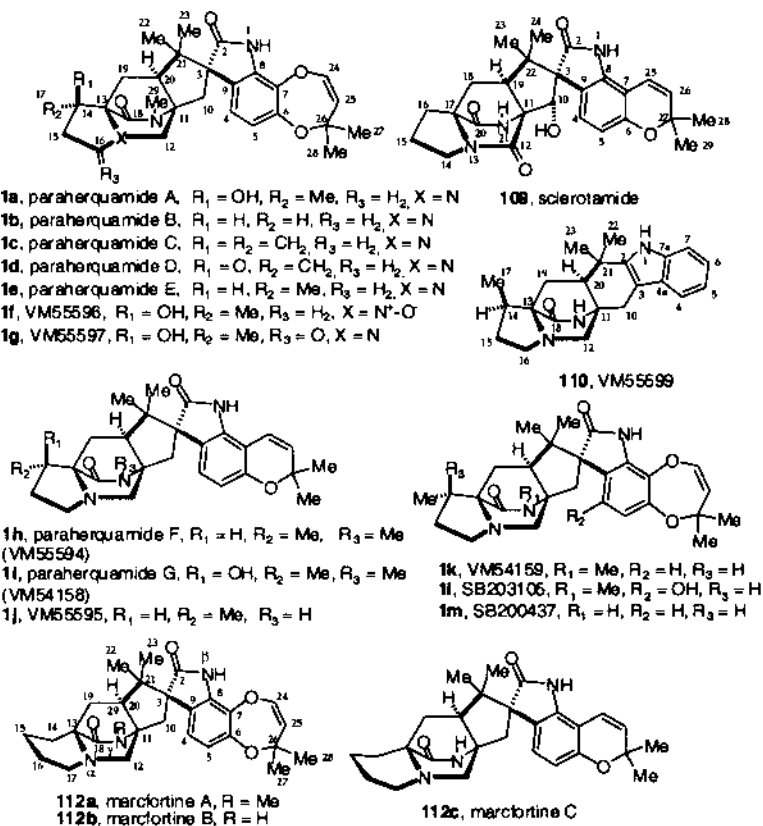
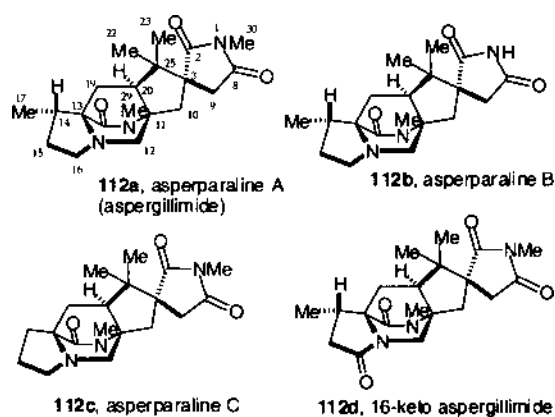
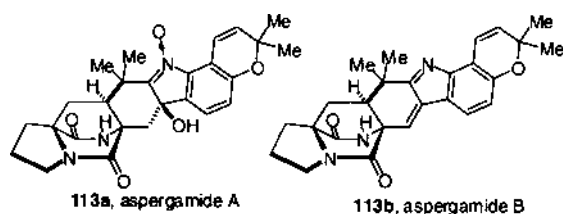


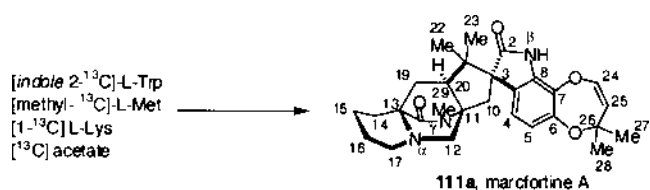
Fig. 5. Structures of the Paraherquamides and Related Alkaloids

Fig. 6. Structures of the Asperparalines and the Aspergillimides^{81–83}Fig. 7. Structures of the Aspergamides A and B^{87,88}

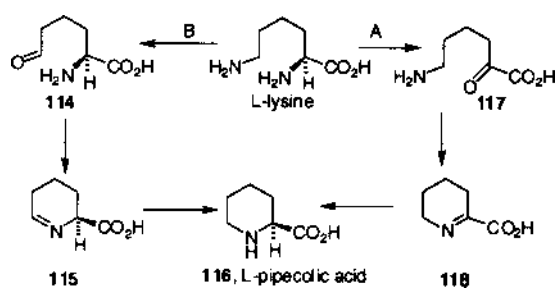
member of the paraherquamide family being comprised of the amino acids proline, tryptophan and two isoprene units.

Finally, Zeeck *et al.*, isolated the interesting substances aspergamide A and B (Fig. 7) from *Aspergillus ochraceus*.^{87,88} The *N*-oxide of aspergamide B (named CJ-17,665) has recently been independently isolated from the same fungus by Sugie *et al.*⁸⁸ These metabolites, including VM55599 are the only natural members of the paraherquamide family that do not have a spiro-ring system but rather, a 2,3-disubstituted indole-derived system.

The biosynthesis of marcfortine has been investigated at the Pharmacia and Upjohn company.^{89,90} Industrial interest in the biosynthesis of marcfortine A, which does not display the potency that paraherquamide A possesses, is presumably due to the report from the Pharmacia and Upjohn group demonstrating that marcfortine A can be semi-synthetically converted into paraherquamide A.⁹¹ Kuo *et al.*, found that marcfortine is derived from L-tryptophan (oxindole moiety), L-methionine (*via* SAM methylation at the γ -N; C29), L-lysine (pipercolic acid residue) and acetate (isoprene units) (Chart 26).^{82,83}

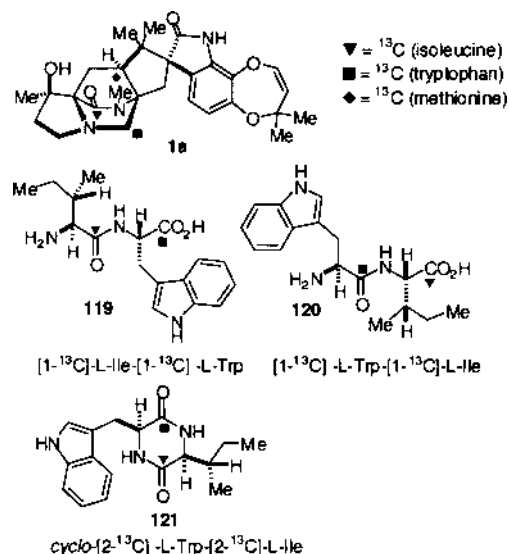
Chart 26. Biosynthetic Constitution of Marcfortine A^{89,90}

These results clearly demonstrate that the isoprene moieties are of mevalonate origin. The pipercolic acid moiety, being derived from L-lysine, can arise by two biochemical pathways as shown in Chart 27.

Chart 27. Possible Biosynthetic Pathways from L-Lysine to Pipercolic Acid⁸⁹

In order to discriminate between these pathways (A and B), Kuo *et al.*, fed individually [α -¹⁵N]- or [ϵ -¹⁵N]-L-lysine to a marcfortine A-producing *Penicillium* strain UC7780 and found that the α -amino group (pathway A) is lost in the biosynthetic production of the pipercolic acid moiety.^{89,90} Specifically, it was found that [α -¹⁵N]-L-lysine gave incorporation at the α -, β -, and γ -nitrogen atoms with a rate of 1.8, 13 and 9%, respectively. Incorporation of [ϵ -¹⁵N]-L-lysine at the α -, β -, and γ -nitrogen atoms gave incorporation rates of 54, 2.7 and 3.9% respectively. The enrichments were measured by FAB-MS, ¹H-NMR and heteronuclear multiple bond correlation (HMBC) experiments. The authors state that this is the first example of the HMBC technique being successfully employed to quantitate the enrichment of an isotope. The low levels of ¹⁵N-enrichment at the other positions (β - and γ -) was attributed to lysine catabolism and reconstitution.

The origin of the β -methylproline moiety present in paraherquamide A and several congeners has recently been investigated. Examination of the absolute stereochemistry of paraherquamide A, which possesses the (*S*)-absolute stereochemistry at C-14, led Williams *et al.*, to speculate that the methylated proline may be derived from L-isoleucine as opposed to proline and S-adenosylmethionine (SAM) and this possibility was experimentally tested in *Penicillium fellutanum* (ATCC: 20841), as shown in Fig. 8.⁹² The position of ¹³C incorporation in paraherquamide A was determined using ¹³C-

Fig. 8. Biosynthetic Derivation of the β -Methylproline Moiety of Paraherquamide A⁹²

NMR and the percentage of the labeled amino acid incorporated was also determined using ^{13}C -NMR.

These workers found that 1- ^{13}C -L-tryptophan was incorporated, as expected, (2.5%) with the label at C-12. The methyl- ^{13}C -methionine was not incorporated in the β -methylproline ring, but rather, only at C-29, the N-methyl position of the monoketopiperazine ring (0.6%). Feeding of 1- ^{13}C -L-isoleucine to *Penicillium fellutanum* (ATCC: 20841), followed by harvesting the cells and isolation of paraherquamide A revealed that the labeled L-isoleucine was incorporated into the monoketopiperazine ring system in high isotopic yield (3.3–3.7%) with the label at C-18.

Since L-isoleucine forms the β -methylproline ring of paraherquamide A, *cyclo*-L-Trp-L- β -methylproline or *cyclo*-L-Trp-L-Ile are plausible precursors. There are numerous possible sequences of events that might occur in the formation of the final β -methylproline ring system. Formation of the dipeptides NH_2 -L-Ile-L-Trp-COOH or NH_2 -L-Trp-L-Ile-COOH and dehydration to *cyclo*-L-Trp-L-Ile followed by oxidation of the terminal carbon of L-Ile and cyclization to form the β -methylproline moiety would result in *cyclo*-L-Trp-L- β -methylproline. Another possibility involves oxidation of the L-Ile followed by cyclization and reduction to afford β -methylproline followed by coupling to L-Trp to give *cyclo*-L-Trp-L- β -methylproline. Many other possibilities exist, that would involve formation of the β -methylproline ring at a later stage.

Williams *et al.*, investigated the simplest of these possibilities by synthesizing and feeding doubly labeled NH_2 -1- ^{13}C -L-Ile-1- ^{13}C -L-Trp-COOH; NH_2 -1- ^{13}C -L-Trp-1- ^{13}C -L-Ile-COOH and 2,5- $^{13}\text{C}_2$ -*cyclo*-L-Trp-L-Ile to *P. fellutanum*.⁹² After growing and harvesting the cells, 1.2–1.8% incorporation at C-18 and 0.4–0.9% incorporation at C-12 was evidenced by ^{13}C -NMR. The ^{13}C -NMR spectra of the paraherquamide A so-produced, did not provide compelling evidence for site-specific incorporation of both labels from the intact dipeptides and there was no significant incorporation of 2,5- $^{13}\text{C}_2$ -*cyclo*-L-Trp-L-Ile. This low level of incorporation is more consistent with dipeptide hydrolysis, re-incorporation of the individual amino acids presumably coupled with additional metabolic degradation and reconstitution of ^{13}C -enriched building blocks. Based on these observations, it seems likely that β -methylproline is formed prior to conjugation to tryptophan, although other possibilities exist.

Oxidative cyclization of the nitrogen atom onto the C-5-methyl group of isoleucine appears to be a unique biosynthetic transformation. Two reasonable pathways, depicted in Chart 28, would involve 4-electron oxidation of the distal side-chain methyl group to an aldehyde followed by cyclization and loss of water to produce an iminium species; subsequent reduction (or in the case of VM55597, oxidation) furnishes the β -methylproline derivative. Alternatively, direct functionalization of the C-5 methyl group for example, by chlorination and closure can also be envisioned to lead to β -methylproline; a related example of this type of reaction has been observed by Arigoni and Looser in the biosynthesis of victorin C.⁹³

Williams *et al.*, have elucidated the mechanism of the oxidative cyclization of L-Ile to β -methylproline as illustrated in Chart 27.⁹⁴ These workers prepared L-[5- ^{13}C , 5- $^2\text{H}_3$]isoleucine using the procedure developed by Oppolzer

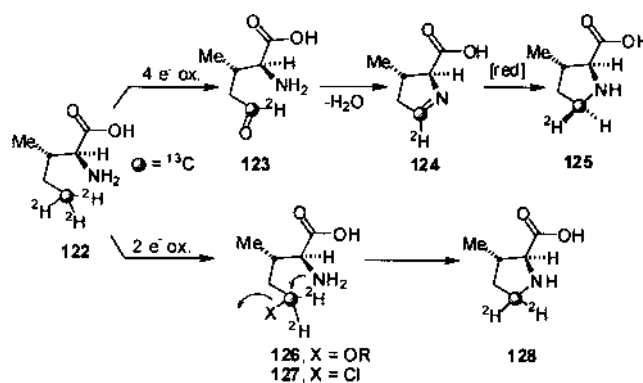


Chart 28. Mechanism of the Oxidative Cyclization of L-Ile to β -Methylproline as Elucidated by Williams *et al.*⁹⁴

and co-workers for the synthesis of unlabeled L-isoleucine.^{95,96} A feeding experiment in *Penicillium fellutanum* using L-[5- ^{13}C , 5- $^2\text{H}_3$]isoleucine followed by isolation and purification of the paraherquamide A produced revealed 0.34% incorporation of the labeled amino acid. The ^{13}C -spectrum revealed a triplet at 51.6 ppm, which would indicate that, in the labeled compound, C-16 is coupled to a single deuterium atom. It was determined that H-16a (3.21 ppm) is the deuterium, H-16b (2.22 ppm) is the proton and the *pro-S* hydrogen is retained in the oxidative cyclization. This result implies that reduction occurs on the same face of the proline ring as the methyl group, C-17. From these experiments, it was determined that cyclization of L-isoleucine occurs through a 4-electron oxidation of the terminal methyl group such as via the putative intermediate **123** followed by cyclization and diastereoselective 2-electron reduction to give **125**.

It is also interesting to note that β -methylproline residues can be found in several peptide antibiotics such as bottromycin, scytonemin A and roseotoxin B in addition to several members of the paraherquamide family. Arigoni and Kellenberger have recently shown that the methyl group in the β -methylproline in bottromycin, a metabolite of *Streptomyces bottropensis*, is derived from S-adenosylmethionine via methylation of proline (Fig. 9) and have proposed a radical mechanism for this reaction.⁹⁷

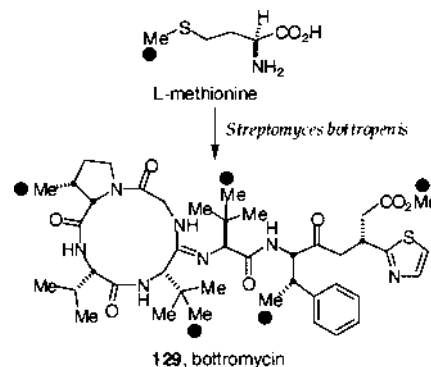


Fig. 9. Biosynthesis of β -Methylated Amino Acids in Bottromycin⁹⁷

The identification of L-isoleucine as the biosynthetic building block of paraherquamide A has posed an interesting stereochemical paradox with respect to the origin and stereo-

chemistry of the β -methylproline in the natural metabolite VM55599 which is produced by a paraherquamide-producing fungus, *Penicillium* sp. strain IMI332995. The relative stereochemistry of VM55599 was determined by $^1\text{H-NMR}$ nuclear Overhauser effect (nOe) experiments by a Smith-Kline Beecham group but the absolute stereochemistry was not determined.⁸⁰⁾ Significantly, the methyl group in the β -methylproline ring of VM55599 is disposed *syn*- to the bridging isoprene moiety whereas, in paraherquamides A, E, F, G, VM55596, VM55597, VM55595, VM54159, SB203105, SB200437 and the aspergillimides, the methyl group of the β -methylproline ring is disposed *anti*- to the bridging isoprene moiety of the bicyclo[2.2.2] ring system.

Using the assumption that the absolute stereochemistry at C-20 of VM55599 is *S* (the same as that found in the paraherquamides), the stereochemistry at C-14 was assigned as *R*, which is the *opposite* to that found in paraherquamides.

The side-chain stereochemistry of *L*-isoleucine is preserved in the biosynthesis of paraherquamide A with hydroxylation at C-14 proceeding with net *retention* of configuration. If *L*-isoleucine was also the precursor to the β -methylproline ring of VM55599, it must follow that the bicyclo[2.2.2]diazaoctan ring system of this compound must be *enantiomorphic* to that of the paraherquamides (see Fig. 10).

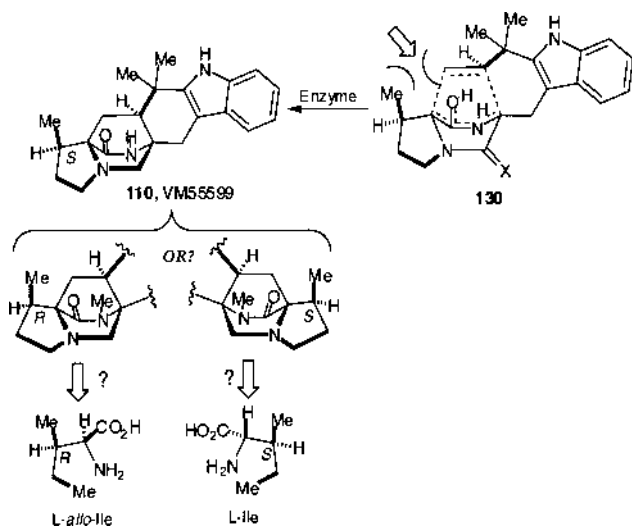


Fig. 10. The VM55599 Stereochemical Paradox⁹²⁾

Alternatively, VM55599 may be derived from *L*-allo-isoleucine; this would result in the (*R*)-stereochemistry at C-14 and would accommodate the same bicyclo[2.2.2]diazaoctan ring system absolute stereochemistry as paraherquamide A. Finally, since the methyl group of the β -methylproline ring of VM55599 is *syn*- to the isoprene unit comprising the bicyclo[2.2.2]diazaoctan ring system, this implies that the putative biosynthetic Diels–Alder cyclization to form this system occurs from the more hindered face of the azadiene system. The stereochemical paradox posed by VM55599 raises numerous interesting questions concerning the biogenesis of these substances: is VM55599 a biosynthetic precursor to various paraherquamide family members? or, is VM55599 a minor shunt metabolite with the opposite absolute stereochemistry of the bicyclo[2.2.2]diazaoctan ring system? The mechanism of formation of the bicyclo[2.2.2]diazaoctan ring system in both series posed an interesting stereochemical and enzymological puzzle that has been recently solved by Williams *et al.* as discussed below in Chart 41.

In the course of isotopic labeling studies aimed at examining the origin of the isoprene units in the paraherquamide structure, Williams *et al.*, discovered an unexpected stereochemical distribution of the geminal methyl groups derived from dimethylallylpyrophosphate (DMAPP) (Fig. 11).⁹⁸⁾

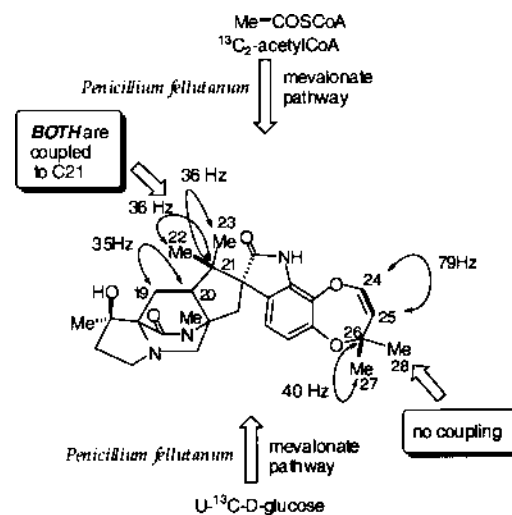


Fig. 11. ^{13}C Labeling in Paraherquamide A Corresponding to a Feeding Experiment with $[\text{U-}^{13}\text{C}_6]\text{-D-Glucose}$ and $[\text{C}_2\text{-}^{13}\text{C}_2]\text{-Acetate}$ via the Mevalonate and 1-Deoxy-D-xylulose Pathways

Thick lines represent intact acetate units and arrows represent couplings expected and observed in the $^{13}\text{C-NMR}$ spectrum.⁹⁸⁾

These workers carried out feeding experiments in *Penicillium fellutanum* with $[\text{U-}^{13}\text{C}_6]\text{-glucose}$ and $[\text{C}_2\text{-}^{13}\text{C}_2]\text{-acetate}$ which aid in distinguishing between the classical mevalonate and deoxyxylulose pathways. Specific incorporation of intact C_2 units was observed, in agreement with the mevalonic acid pathway. However, in the first C_5 fragment (C19 to C23), the observed couplings mean that C19 is coupled to C20, while C21 is coupled to C22 or C23, but not to both simultaneously. For the second C_5 unit (C24 to C28), the coupling constants show that C24 and C25 are coupled, while C26 is coupled to C27. In this case, although C-28 shows ^{13}C enhancement, C28 shows no coupling with C26, while C27 does. This means that the methyl groups in DMAPP are not equivalent in the biosynthesis of the geminal methyl groups at C-21 of this metabolite. In contrast, in the other C_5 fragment, formed by carbons C19 to C23, both methyl groups show coupling with C20, although not simultaneously. The stereochemical integrity of DMAPP in forming the dioxepin ring system is thus left intact.

This unexpected result was interpreted to mean that the *reverse* prenyl transferase presents the olefinic π -system of DMAPP in a manner in which both faces of the π -system are susceptible to attack by the 2-position of the indole moiety. The simplest explanation is to invoke binding of the DMAPP in an “upside down” orientation relative to “normal” prenyl transferases which permits a facially non-selective S_N' attack

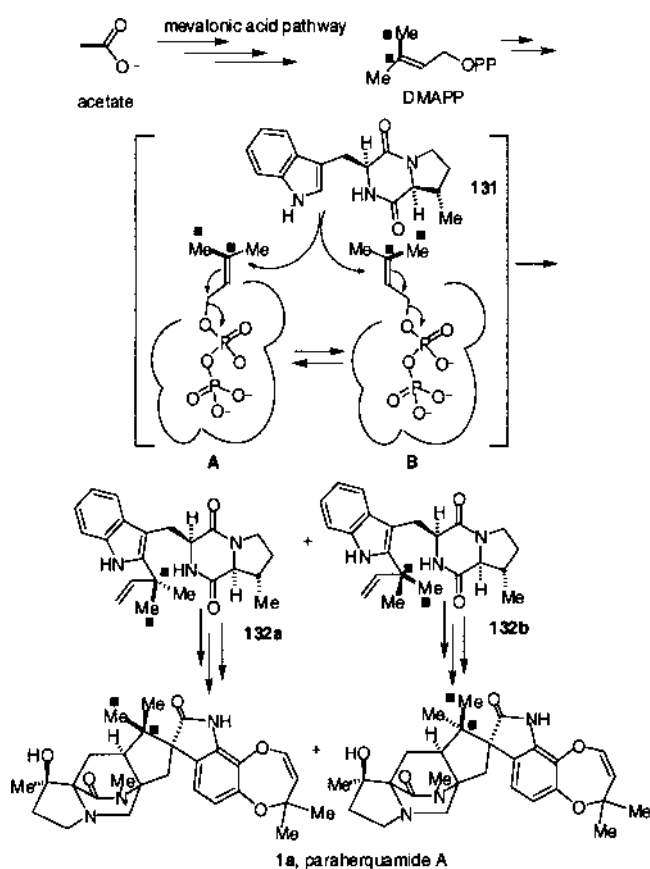


Chart 29. A Possible Biosynthetic Sequence That May Explain Why C22 and C23 Are Rendered Equivalent in the Biosynthesis of Paraherquamide A

Thick bonds [with the black square] represent one intact C2 unit from acetate, incorporated in C3/C5 of individual DMAPP molecules, and in C21, C22, and C23 of paraherquamide A.⁹⁸

on the π -system as shown in Chart 29. It was speculated that, in this situation, the pyrophosphate group is likely to be anchored in the enzyme active site with the hydrophobic isopropenyl moiety being presented in a conformationally flexible ($A \rightleftharpoons B$) disposition with respect to the tryptophan-derived substrate (Chart 29). This is in contrast to the normal mode of prenyl transfer where, the nucleophilic displacement at the pyrophosphate-bearing methylene carbon occurs with inversion of stereochemistry at carbon with the hydrophobic tail of DMAPP buried in the enzyme active site.

In contrast, the methyl groups in the other C₅ unit (dioxepin moiety) are clearly differentiated; therefore, it is quite likely that this C₅ group (carbons 24 to 28) is introduced in the molecule *via* direct alkylation with DMAPP by a normal prenyl transferase followed by a stereospecific net oxidative addition to the olefinic system.

A plausible mechanism, of several possibilities, for the formation of the dioxepin ring system, is *via* face-selective epoxidation of the isoprene unit followed by ring-opening and dehydration. Since this isoprene unit is introduced without loss of stereochemical integrity, it was deemed unlikely that the *Penicillium* sp. has a mechanism for scrambling the DMAPP *via* a dimethyl vinyl carbinol-type intermediate which would necessarily provide stereochemically scrambled isoprene equivalents to the cell's cytosolic pool of DMAPP.

These experiments clearly indicate that the C₅ units in paraherquamide A are introduced in stereofacially distinct

manners. Since it has been established that prenylation of the indole moiety in the biosynthesis of the structurally related brevianamides occurs in an analogous fashion to that postulated for paraherquamide A,³² the prenyl transferase that installs this C₅ unit must display DMAPP to the 2-position of the indole in a π -facially indiscriminate manner. This work demonstrates the first case where *both* a non face-selective *and* a face-selective addition to the tri-substituted olefinic portion of DMAPP has occurred within the same molecule.

These workers also observed a similar phenomenon with respect to the corresponding reverse prenyl groups in brevianamide A and austamide (Fig. 12).⁹⁹

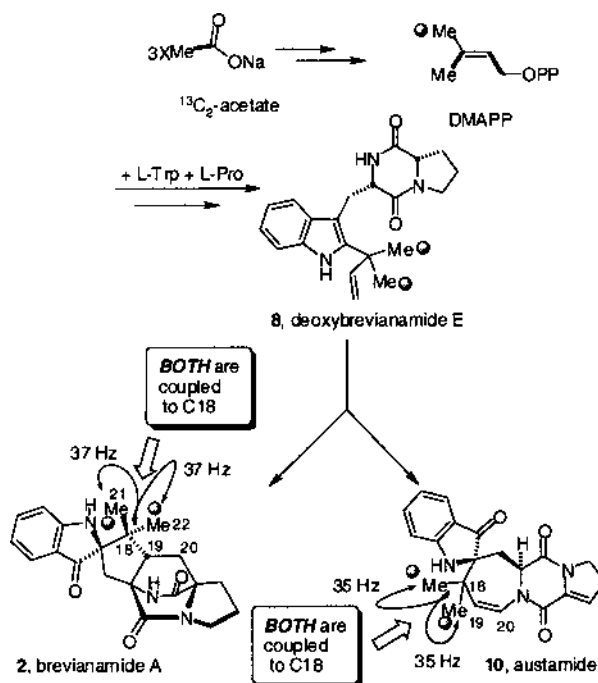


Fig. 12. Incorporation of ¹³C-Acetate into Brevianamide A and Austamide Reveals a Lack of Stereospecificity in the Reverse Prenyl Transferase Reaction That Constructs Deoxybrevianamide E, a Common Precursor⁹⁹

In summary, isotopic enrichment experiments with [¹³C₂]-acetate have shown that the isoprene units in the secondary metabolites paraherquamide A (**1**), brevianamide A (**2**) and austamide (**10**) all arise *via* the classical mevalonate pathway. In all three systems, a loss of stereochemical integrity at the isoprene-derived quaternary center attached to the 2-position of the indole ring was observed. In the biosynthesis of paraherquamide A (**1**), the geminal methyl groups from the isoprene unit which antecedes the bicyclo[2.2.2] ring system shows approximately equal incorporation of ¹³C from intact C-2 units, which indicates that these methyl groups become essentially equivalent at some point in the biosynthesis.

The isoprene unit that constitutes the dioxepin ring, displays retention of stereochemical integrity and infers a completely net face-selective biosynthetic addition reaction to the olefinic π -system derived from DMAPP *via* the tryptophyl ring hydroxyl group. Two possible mechanisms for the formation of this novel ring system have been proposed by Williams *et al.* (Chart 30).^{98,99}

In the biosynthesis of austamide (**10**), there are also approximately equal levels of specific ¹³C enrichment from in-

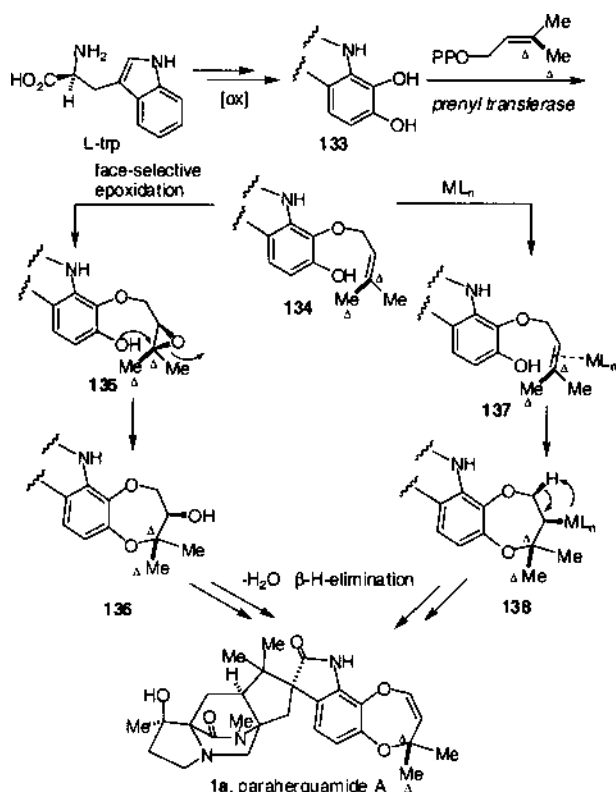


Chart 30. Possible Mechanisms for Construction of the Dioxepin in Paraherquamide Biosynthesis^{98,99}

tact C2 units at the isoprene derived geminal methyl groups. Brevianamide A (**2**), on the other hand, exhibits significant but incomplete loss of stereochemical integrity in the construction of the reverse prenyl unit. In this regard, it is most interesting to note that both brevianamide A (**2**) and austamide (**10**) are apparently fashioned from the *same* reverse-prenylated precursor namely, deoxybrevianamide E (**8**). While *Penicillium* sp. and *Aspergillus* sp. are very similar genera of fungi genetically, it would appear that the reverse prenylases in each organism that construct deoxybrevianamide E display distinct levels of facial discrimination in the transfer of DMAPP to the 2-position of the indole.

Much work remains to more fully establish the sequence of events involved in the biosynthesis of the paraherquamides. Recent studies by our laboratory will be discussed following the next section on the total synthesis of paraherquamides A, B and VM55599.

The Total Synthesis of Paraherquamides A and B

The first synthetic model study on the paraherquamides was reported by Williams *et al.*¹⁰⁰ Utilizing a hexacyclic indole (**139**) generated during the course of their work on brevianamide B, it was found that treatment of this substance with *t*-butyl hypochlorite generated a diastereomeric mixture of 3-chloroindolenines (**140**). Without isolation, these materials were subjected to hydration and pinacol-type rearrangement to give a 3.86 : 1 mixture of diastereomeric *spiro*-oxindoles **142** : **143**, respectively (Chart 31). The major isomer (**142**) was found to possess the desired (paraherquamide) relative stereochemistry.

With this result as an entrée, Williams and co-workers set out to complete asymmetric syntheses of both para-

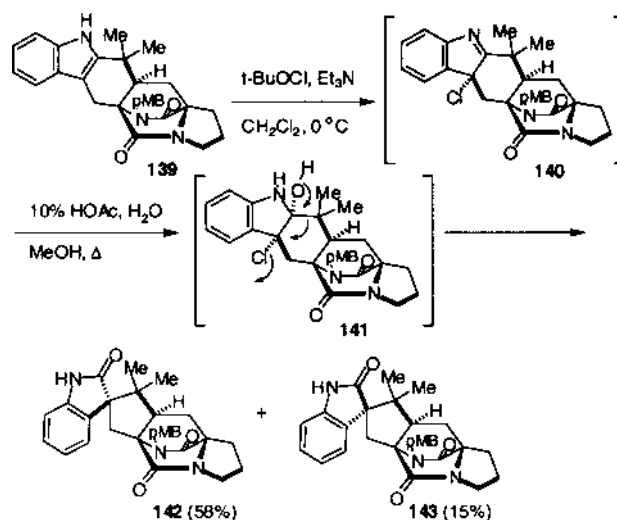


Chart 31. Oxindole Model Study of Williams¹⁰⁰

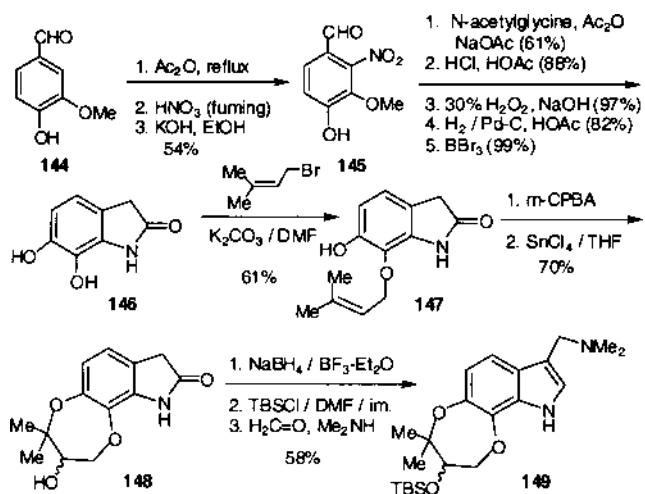


Chart 32. Construction of the Dioxepin Gramine Derivative¹⁰¹

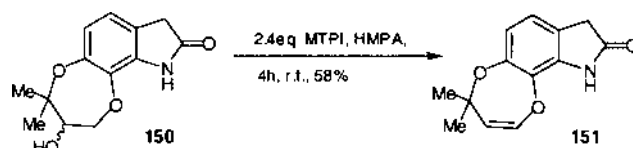
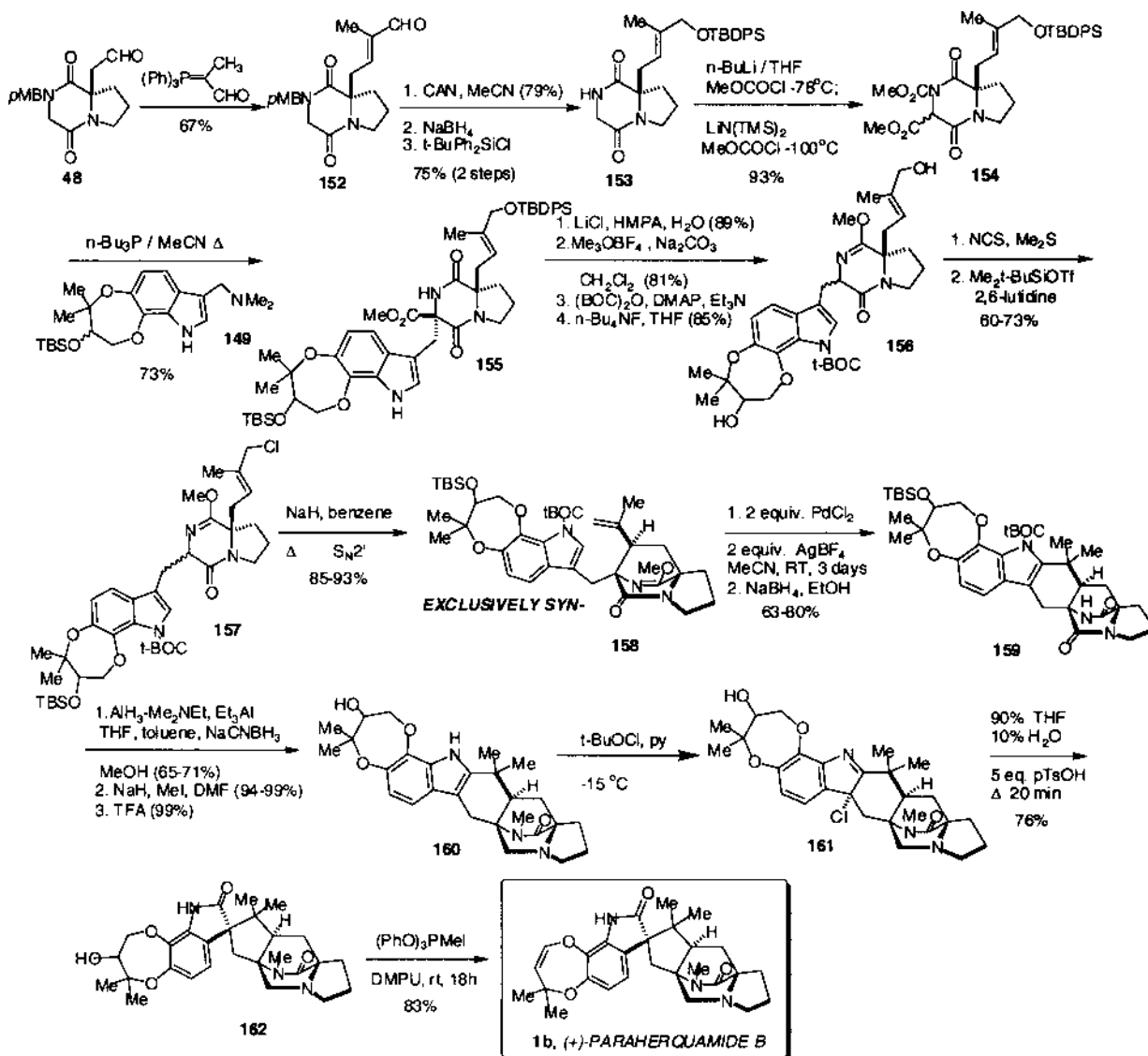


Chart 33. Synthesis of a Model Dioxepin Oxindole¹⁰¹

herquamide A and B. The construction of the dioxepin-containing gramine derivative required for the synthesis of both paraherquamides A and B was described separately as shown in Chart 32.¹⁰¹

Vanillin (**144**) was acetylated and nitrated followed by hydrolysis to furnish 2-nitrovanillin **145** in 54% yield for three steps. Conversion of **145** into the known catechol **146** proceeded in five steps in good overall yield. Selective prenylation of **146** furnished the desired regioisomer **147** as the major product in 61% yield. Epoxidation of **147** followed by SnCl₄-mediated epoxide ring-opening afforded the desired seven-membered ring oxindole **148** in 70% overall yield from **147**. Sodium borohydride reduction of the oxindole carbonyl group furnished the corresponding indole derivative that was silylated and converted into the key gramine deriva-

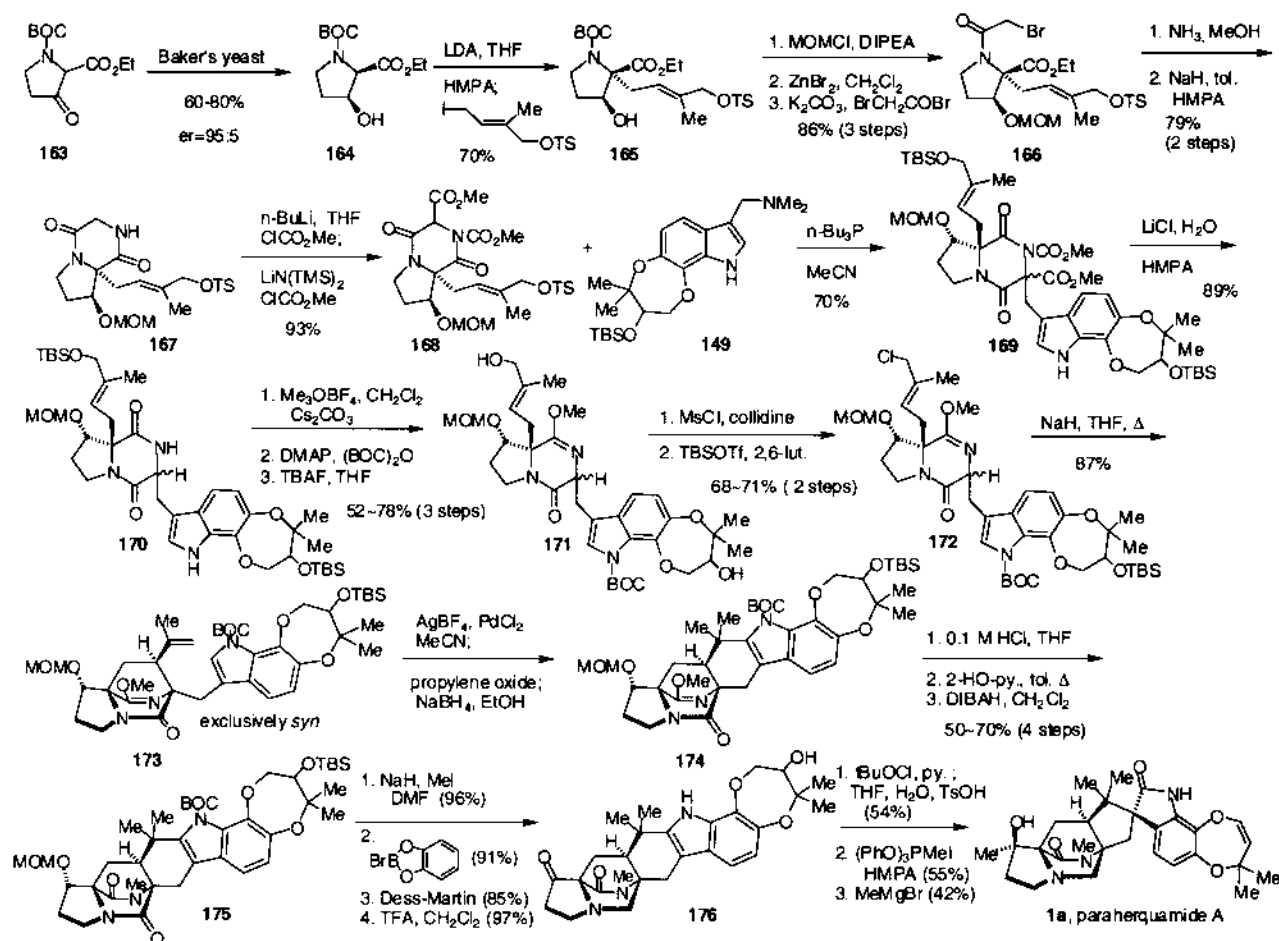
Chart 34. Williams Total Synthesis of Paraherquamide B^{102,103}

tive **149** in 58% overall yield for the three-step sequence.

To demonstrate that the secondary alcohol of such a system could be converted to the dioxepin moiety of the paraherquamides, intermediate **150** was treated with methyltriphenoxyphosphonium iodide (MTPI) in HMPA to afford the dioxepin oxindole **151** in 58% yield (Chart 33).

The first asymmetric total synthesis of paraherquamide B commenced with enal **152** (Chart 34) obtained as described above for the brevianamide B synthesis (Chart 19).^{102,103} Enal **152** was treated with CAN to remove the *para*-methoxybenzyl group (79%). The resulting amide was reduced to the corresponding alcohol with NaBH_4 and protected with *t*-butyldiphenylsilyl chloride, to give **153**. This compound was treated in a two-step one-pot procedure with methyl chloroformate yielding the desired imidocarbamate **154** as a mixture of epimers in variable ratios (epimerization was observed to occur on silica gel). When **154** and **149** were refluxed with 0.5 eq of $n\text{-Bu}_3\text{P}$ in acetonitrile,^{41,42} the desired alkylation occurred together with a fortuitous decarbomethoxylation of the imidocarbamate. Indole **155** was treated with LiCl in wet HMPA at 100°C effecting decar-

bomethoxylation (obtained as a 3:1 *syn:anti* mixture that proved easy to separate by chromatography); and both diastereomers were separately treated to the next series of manipulations. Lactim ether formation was accomplished by treating the amides with Me_3OBF_4 in CH_2Cl_2 in the presence of Na_2CO_3 . The lactim ethers were then treated with $(\text{BOC})_2\text{O}$ followed by $n\text{-Bu}_4\text{NF}$ to give the corresponding diols **156**. The sensitive allylic chlorides were prepared according to the protocol reported by Corey and Kim (*N*-chlorosuccinimide (NCS) and Me_2S),¹⁰⁴ and the secondary alcohols were carefully re-protected with *tert*-butyldimethylsilyl (TBS)-triflate to furnish the key $\text{S}_{\text{N}}2'$ cyclization substrates **157**. Individual treatment of the diastereomers of **157** with 20 equivalents of NaH in hot benzene effected stereoselective intramolecular $\text{S}_{\text{N}}2'$ cyclization giving the desired bicyclo[2.2.2] product **158** (93% from **157a**; 85% from **157b**). Treatment of **158** with Pd(II) according to the procedure developed by Trost¹⁰⁵ furnished the desired heptacycle **159** with concomitant loss of the lactim ether in 63–80% yield. Regioselective reduction of the tertiary amide of **159** proceeded cleanly with $\text{AlH}_3\text{-Me}_2\text{NEt}$ complex and Et_3Al , fol-

Chart 35. Williams Synthesis of Paraherquamide A¹⁰⁸

lowed by a NaCNBH_3 work-up to give the tertiary amine in 64–71% yield.¹⁰⁶ *N*-Methylation of the secondary amide (NaH/MeI in DMF, 94–98%) followed by removal of both protecting groups, (80 eq. trifluoroacetic acid (TFA) in CH_2Cl_2) gave the indole **160** in 59% overall yield from **159**. The final oxidative spirooxidation was accomplished by treatment of **160** with *t*-BuOCl and pyridine, followed by hydration of the incipient chloroindolenine with 90% THF–10% H_2O and 5 eq. TsOH furnishing the desired *spiro*-oxindole (**162**, 76% plus 4% of the unnatural *spiro*-epimer). Finally, dehydration was effected with MTPI in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU) to give (+)-paraherquamide B (the enantiomer of the natural product) in 83% yield.

Utilizing the paraherquamide B synthesis as a solid platform, the Williams group also completed an asymmetric total synthesis of paraherquamide A as shown in Chart 35.¹⁰⁷ In approaching the synthesis of paraherquamide A, that contains the unusual β -hydroxy- β -methyl proline residue, new methodology was developed to access a suitably functionalized α -alkylated- β -hydroxyproline moiety that could be conscripted for the multi-step task of constructing paraherquamide A. Despite the apparent similarity in the structures of paraherquamide A and B, the synthesis of the former turned out to be a significantly more challenging endeavor. Williams and Cao described a potentially general method to construct α -alkylated- β -hydroxyproline residues by the dian-

ion alkylation of the readily available *N*-*t*-BOC- β -hydroxyproline ethyl ester derivative **164** with net retention of stereochemistry. These workers then successfully applied this methodology to a concise asymmetric and stereocontrolled total synthesis of paraherquamide A in 27 steps from the racemic β -keto ester **163** as shown in Chart 35.¹⁰⁸

The β -ketoester **163** was subjected to Baker's yeast reduction to afford the optically active β -hydroxyester **164** (60–80%, *ca.* 95 : 5 *er*). Dianion alkylation of this substance with *E*-3-methyl-4-(*O*-*tert*-butyldimethylsilyl)-2-butene afforded the desired α -alkylated product **165** in 58–70% isolated yield without attendant *O*-alkylation.¹⁰⁹ Protection of the secondary alcohol as the corresponding methoxy methyl ether (MOM ether) followed by removal of the *N*-*t*-BOC group with ZnBr_2 in dichloromethane and acylation of the incipient secondary amine with bromoacetyl bromide in the presence of potassium carbonate afforded the bromoacetamide **166** in 86% overall yield from **165**. Next, treatment of **166** with methanolic ammonia afforded the corresponding glycinamide derivative that was directly subjected to cyclization in the presence of sodium hydride in a solvent mixture of toluene/HMPA to afford the bicyclic substance **167** in 79% overall yield from **166**. This product was subjected to a one-pot double carbomethoxylation reaction that was performed by the sequential addition of *n*-BuLi in THF, followed by addition of methylchloroformate, that carbomethoxylates the amide nitrogen. Subsequent addition of

four equivalents of methylchloroformate followed by the addition of five equivalents of $\text{LiN}(\text{TMS})_2$ afforded **168** as a mixture of diastereomers in 93% yield that were taken on directly without separation.

Somei-Kametani coupling^{41,42} of **168** with the gramine derivative **149**, prepared as described above in Chart 32¹⁰¹ in the presence of tri-*n*-butylphosphine gave the tryptophan derivative **169** as a 3 : 1 mixture of diastereomers (epimeric at the newly created stereogenic center) in 70% combined yield. Decarbomethoxylation of **169** was accomplished by treatment of **169** with LiCl in hot, aqueous HMPA at 105 °C providing **170** as a mixture of diastereomers that were separated by chromatography and carried forward individually. Protection of the secondary amide as the corresponding methyl lactim ether was accomplished by treating **170** with Meerwein's reagent (trimethyloxonium tetrafluoroborate in dichloromethane containing cesium carbonate). Next, the indole nitrogen was protected as the corresponding *N*-*t*-BOC derivative by treatment with di-*tert*-butyl dicarbonate in the presence of DMAP and the silyl ether was removed with tetrabutylammonium fluoride to provide the corresponding diol **171** in 52–78% overall yield from **170**. Selective conversion of the allylic alcohol to the corresponding allylic chloride was accomplished by mesylation in the presence of collidine. Silylation of the secondary alcohol with *tert*-butyldimethylsilyl triflate in the presence of 2,6-lutidine afforded the key allylic chloride **172** in 68–71% yield over two steps.

The critical intramolecular S_N2' cyclization that sets the relative stereochemistry at C-20 during formation of the bicyclo[2.2.2]octan ring nucleus was next examined. Based on solid precedent from the paraherquamide B synthesis,^{102,103} it was found that treatment of **172** with NaH in hot THF afforded the desired S_N2' cyclization product **173** in 87% exclusively as the desired *syn*-isomer.⁶⁴ This remarkably *syn*-selective intramolecular S_N2' cyclization reaction proceeds via a tight, intramolecular ion-pair driven cyclization ("closed" transition state) as discussed above and shown in Chart 18. Closure of the seventh ring was effected by treatment of **173** with 5.0 equivalents of PdCl_2 and 3.2 equivalents of AgBF_4 ¹⁰⁵ in acetonitrile containing propylene oxide as an acid scavenger. The authors note that, without propylene oxide to buffer this reaction, the MOM ether suffered extensive cleavage. The incipient heptacyclic σ -palladium adduct was immediately reduced by the addition of ethanol and sodium borohydride to afford the desired 2,3-disubstituted indole **174**.

Cleavage of the lactim ether of **174** was effected with 0.1 M HCl to give the corresponding ring-opened amine methyl ester that was re-cyclized to the heptacyclic piperazinedione by treatment of this material with 2-hydroxypyridine in hot toluene (63% overall from **174**). Chemoselective reduction of the secondary amide in the presence of the tertiary amide was effected by treatment of the product obtained above with excess diisobutylaluminum hydride (DIBALH) in dichloromethane (50–72%) furnishing **175**.¹¹⁰ Methylation of the secondary amide (NaH , MeI , DMF) of **175** proceeded in 96% yield. Cleavage of the MOM ether with bromocatecholborane¹¹¹ (91%) followed by oxidation of the secondary alcohol with Dess–Martin periodinane (85%) and cleavage of the *N*-*t*-BOC group and TBS ether with TFA (97%) gave ketone **176**.

The final critical oxidative spirocyclization of the 2,3-disubstituted indole to the *spiro*-oxindole was accomplished by treatment of **176** with *tert*-butyl hypochlorite in pyridine providing a labile 3-chloroindolenine. The authors note that, it was found to be necessary to rigorously remove all of the pyridine solvent prior to the pinacol-type rearrangement that was conducted by treating the incipient 3-chloroindolenine with *p*-TsOH in THF/ H_2O that provided the desired *spiro*-oxindole. It was reasoned that the chlorination of **176** proceeds from the least hindered face of the indole giving the corresponding α -chloroindolenine **177** (Chart 36). The hydration of the imine functionality interestingly, must also occur from the same α -face that is *syn*- to the relatively large chlorine atom furnishing the *syn*-chlorohydrin (**178**) that subsequently rearranges stereospecifically to the desired *spiro*-oxindole **179**.

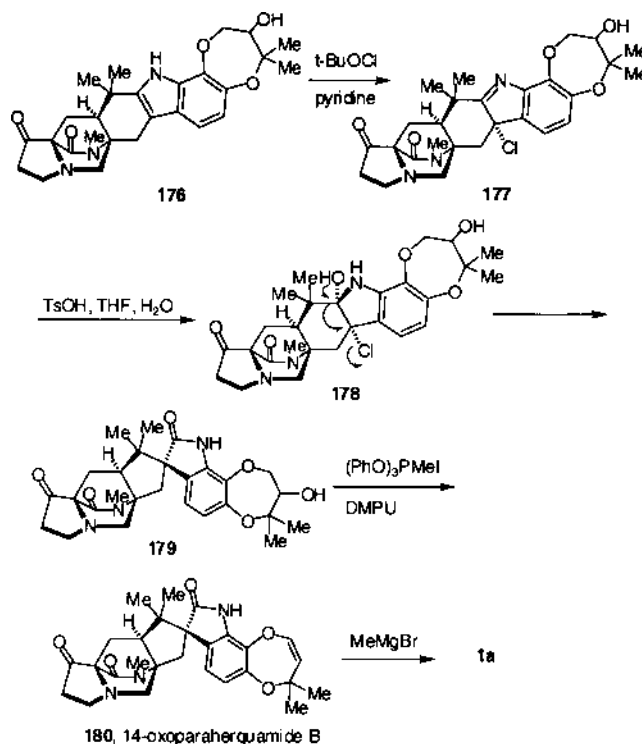
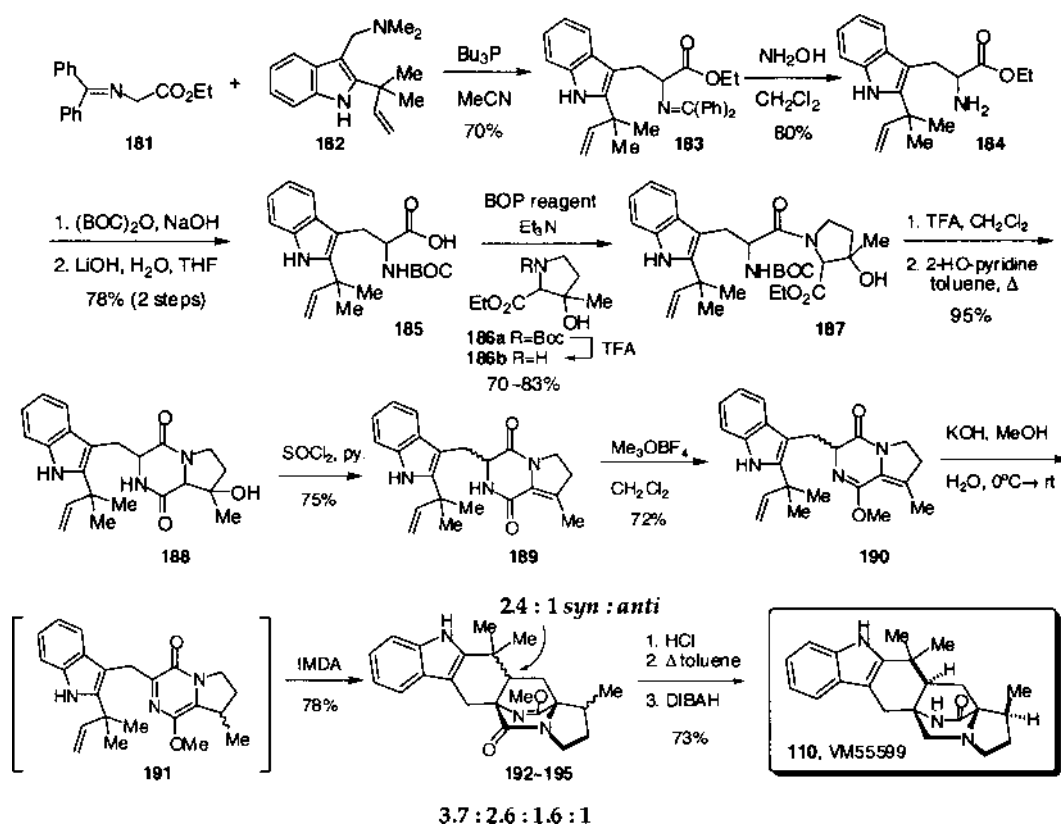


Chart 36. Final Transformations to Paraherquamide A¹⁰⁸

The dioxepin ring was then formed by dehydration of the secondary alcohol of **180** with MTPI in DMPU to afford 14-oxoparaherquamide B **29**, (55%).^{112,113} This intermediate has been previously described by a Pharmacia-Upjohn group, obtained semi-synthetically from marcfortine A. The final step, methyl Grignard addition to the ketone group of **180** has been previously described to give paraherquamide A along with a trace of the corresponding C-14 epimer in *ca.* 50% yield when MeMgBr was employed. Employment of the published protocol using MeMgBr with the synthetic ketone (**180**) gave (–)-paraherquamide A (**1a**) as the exclusive product (the C14 epimer was not detected) in 42% yield that was identical in all respects to natural paraherquamide A.

Biomimetic Total Syntheses of VM55599

The stereochemical paradox posed by VM55599, as discussed above, first required devising a method to unambigu-

Chart 37. Williams Biomimetic Total Synthesis of VM55599¹¹⁴⁾

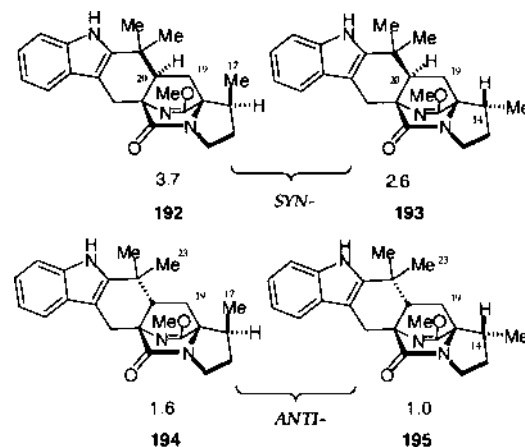
ously establish the absolute configuration of this substance. Williams and co-workers tackled this problem in two phases. First, due to the scarcity of the natural product, it became necessary to re-isolate a small quantity of this substance from *Penicillium* sp. strain IMI332995. Due to the complex mixture of secondary metabolites produced by this organism, a total synthesis of racemic VM55599 was achieved and the synthetic sample was utilized to guide re-isolation of VM55599.¹¹⁴⁾ From 121 of solid media, 0.4 mg of natural VM55599 was isolated. The synthesis, illustrated in Chart 37, helped to secure the relative configuration that was correctly assigned by Everett and co-workers.

The benzophenone imine of glycine ethyl ester (**181**) was condensed with the dimethylallylated gramine derivative **182** in the presence of tri-*n*-butylphosphine^{41,42)} furnishing the tryptophan derivative **183** (70% yield). Cleavage of the benzophenone imine with hydroxylamine provided the amine ethylester **184** in high yield. Subsequent *t*-BOC protection of the primary amine and basic hydrolysis of the ethyl ester furnished the acid **185** in 78% yield (two steps). Coupling of acid **185** with racemic β -methyl- β -hydroxyproline ethyl ester with the BOP reagent provided the desired dipeptide **187** in 70–83% yield. The BOC group was removed with TFA and the resulting amino ethyl ester was cyclized to the corresponding piperazinedione (**188**) in the presence of 2-hydroxypyridine in excellent yield.

Treatment of **188** with thionyl chloride in pyridine furnished the unsaturated compound **189** in 75% yield. Next, treatment of **189** with trimethyloxonium tetrafluoroborate in dichloromethane provided the labile azadiene **190** in 72% yield. This substance could either be isolated and purified or taken on directly for the Diels–Alder cycloaddition step.

Treatment of azadiene **190** with KOH in aqueous methanol effected tautomerization to the labile incipient azadiene **191** which spontaneously suffered intramolecular Diels–Alder cycloaddition at room temperature to give a mixture of all four possible racemic cycloadducts **192–195** in 78% combined yield in a 3.7 : 2.6 : 1.6 : 1 ratio, respectively.

The Diels–Alder cycloadducts (**192–195**) were found to be separable by silica gel chromatography and their relative stereochemistry was assigned by ¹H-NMR nOe studies. The structures of all four cycloadducts (**192–195**) depicting their relative stereochemistries are shown in Fig. 13. Of significant interest, was the unexpected observation that the major products (**192** and **194**) in each diastereomeric subset displayed the methyl group in the β -methylproline ring *syn*- to the bridging isoprene unit. The diastereoselectivity in this regard

Fig. 13. Structures of the Diels–Alder Cycloadducts¹¹⁴⁾

was 1.47:1 favoring the methyl group disposed *syn*- to the bridging isoprene moiety. The authors speculate that, although it is reasonable to expect modest diastereoselectivity for this Diels–Alder cycloaddition, based purely on the slight steric bias expected to be exerted by the methyl group in the proline ring, a modest preference for cycloadducts that displayed the methyl group *anti*- to the bridging isoprene moiety was anticipated.

Secure confirmation of the structure for cycloadduct **192** was secured through conversion into racemic VM55599. Thus, treatment of **192** with dilute HCl effected cleavage of the lactim ether to the corresponding secondary amide **196** in 85% yield (Chart 38). Selective reduction with excess DIBAH (20 equivalents) provided VM55599 in 86% yield whose ¹H- and ¹³C-NMR spectral characteristics matched the published data. The synthetic material was subsequently utilized to guide re-isolation of natural VM55599 from cultures of *Penicillium* sp. IMI332995 (obtained from the International Mycological Institute) grown in our laboratory. The synthetic and natural specimens were found to have identical ¹H-NMR spectra and TLC mobility thereby confirming the assignment.

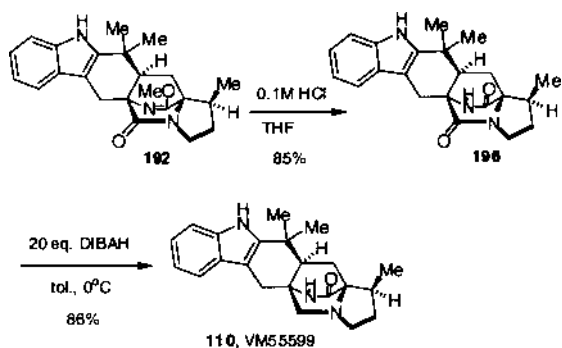


Chart 38. Final Conversion to *d,l*-VM55599¹¹⁴⁾

To further confirm this assignment, the three other cycloadducts **193**–**195** were similarly converted into the corresponding C-14 and/or C-20 epimers of VM55599. It was interesting to observe that, in the case of cycloadducts **193** and **195**, cleavage of the lactim ether with dilute HCl led to the production of the corresponding ring-opened amino esters (*i.e.*, **197** from **193**, Chart 39). These were readily cyclized to the bicyclo[2.2.2]-containing secondary amides (**198** from **197**) by simply heating in toluene at reflux temperature overnight. In contrast, the lactim ethers of both cycloadducts **192** and **194** could be cleaved to the corresponding bicyclo[2.2.2]-containing substrates without attendant ring-opening to the corresponding amino esters. The authors rationalized this difference in reactivity by considering that there is A^(1,3)-type strain in compounds **193** and **195** caused by compression between the methyl group disposed on the β -face of the proline ring and the lactim ether methoxy group that is relieved upon ring-opening. In substrates **192** and **194**, where the methyl group in the proline ring is on the α -face, the opportunity for A^(1,3)-type strain is obviated by the *anti*-relationship between the lactim ether group and the methyl group.

As will be noted below, compound **199** proved to be a key biosynthetic precursor to paraherquamide A and the synthe-

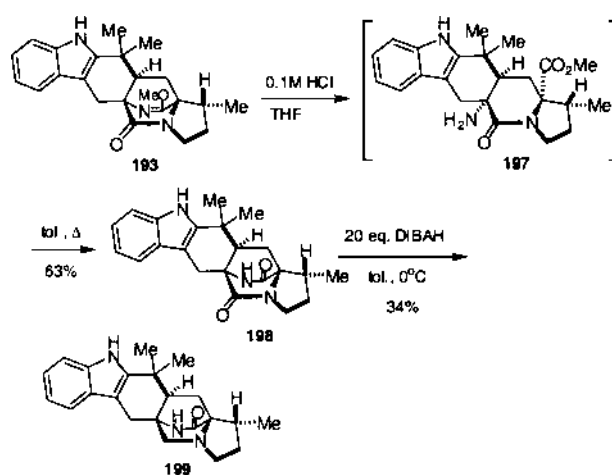
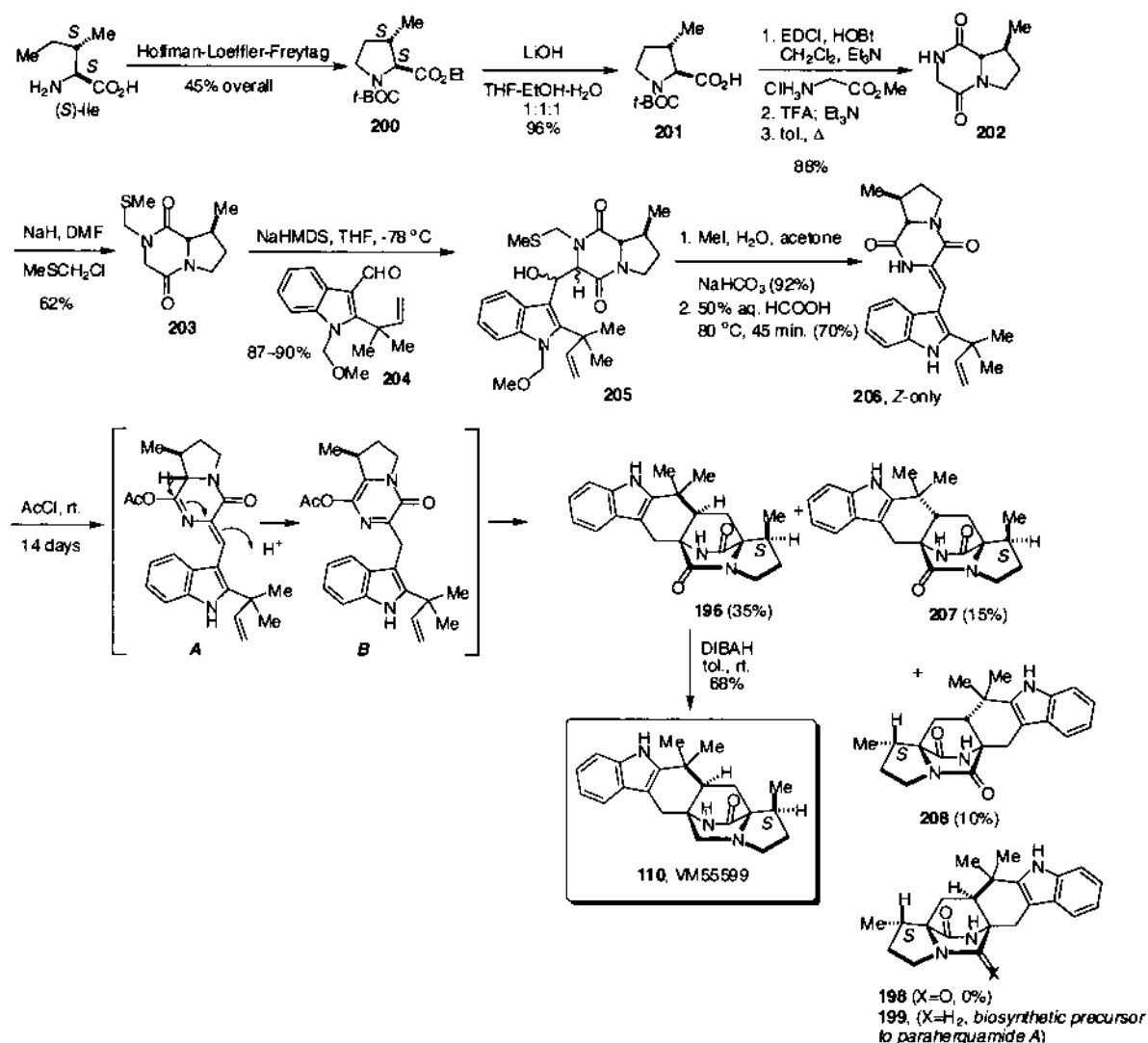


Chart 39. Synthesis of a Paraherquamide Precursor¹¹⁴⁾

sis of this substance was undertaken in doubly-¹³C-labeled form according to the protocol described in Charts 37 and 39.

The asymmetric synthesis of VM55599 was next undertaken to firmly establish the absolute configuration of this material as illustrated in Chart 40.¹¹⁵⁾ The configuration of the carbon that supports the methyl group in L-Ile is *S*, and it can be transformed conveniently in five steps and 47% overall yield into the optically pure β -methylproline derivative **200** in multigram amounts using a Hoffman–Loeffler–Freitag sequence.^{116,117)} Compound **200** was hydrolyzed to acid **201** in 96% yield and was then coupled to glycine methyl ester hydrochloride with EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) in the presence of 3-hydroxybenzotriazole and triethylamine in dichloromethane to give the corresponding dipeptide in 96% yield. The resulting crude compound was deprotected with TFA at room temperature and the crude deprotected dipeptide was treated with triethylamine to neutralize the excess TFA, and the mixture of salts thus prepared was heated in refluxing toluene to give the diketopiperazine **202** in 88% yield over 3 steps.

The bicyclic compound **202** was protected as its *N*-methylthiomethyl (MTM) derivative through formation of the sodium salt with NaH in DMF at 0 °C, followed by reaction with chloromethyl methyl thioether at room temperature.¹¹⁸⁾ This procedure furnished a mixture of the MTM-protected diketopiperazine **203** (61.5% yield) and an epimer (10% yield), which were separated by silica gel chromatography. Although both compounds would have been potentially useful as substrates for the synthesis of VM55599, only the major diastereomer was carried forward. The enolate of **203** was generated with sodium bis(trimethylsilyl)amide in THF at –78 °C, and condensation with aldehyde **204**¹¹⁹⁾ furnished a mixture of three diastereomers **205** in 87–90% combined yield. The relative stereochemistry of these compounds was not determined, and these were carried forward in the next series of transformations. The MTM group in the mixture of diastereomers **205** was removed by treatment with excess iodomethane in acetone in the presence of an aqueous solution of NaHCO₃ to give a mixture of three diastereomers in 92% yield. Treatment of the diastereomeric mixture with 50% aqueous formic acid at 80 °C for 45 min resulted in removal of the MTM group and dehydration of the alcohol giving exclusively compound **206** in 70% yield; no trace of the

Chart 40. Williams' Asymmetric Synthesis of (-)-VM55599¹¹⁵⁾

corresponding *E*-isomer was detected.

Compound **206** was treated with acetyl chloride at room temperature for 14 d according to conditions described by Liebscher and coworkers,⁶³⁾ to furnish a mixture of three diastereomeric cycloadducts **196** (35% yield), **207** (15% yield), and **208** (10% yield). It was assumed that acetylation of **206** yields the *O*-acyl lactim (**A**) that tautomerizes to azadiene **B**, that immediately suffers intramolecular Diels–Alder cycloaddition from three of the four possible diastereomeric transition states, followed by loss of acetate. The cycloadducts obtained had identical spectroscopic properties to the racemic compounds previously prepared in our laboratories¹¹⁴⁾ except for being optically pure.

Finally, reduction of the cycloadduct **196** with an excess of DIBALH in toluene at room temperature for 24 h gave synthetic (-)-VM55599 (**110**) that was identical in all respects to natural (-)-VM55599, including optical rotation, CD and retention time on chiral HPLC. Thus, natural VM55599 (**110**) has the absolute configuration depicted (and predicted) in Chart 1.

This result rigorously confirms the predicted absolute stereochemistry of VM55599, and provides additional experimental support for the unified biogenesis proposed for this

compound and the paraherquamides (Chart 41). It was quite surprising to observe that cycloadduct **198**, which contains the relative and absolute stereochemistry of the paraherquamides, was not detected from the cycloaddition reaction. The cycloadducts obtained in Williams' racemic synthesis¹¹⁴⁾ gave (as *O*-methyl lactim ethers), compounds stereochemically corresponding to **196**:**207**:**208**:**198** in a ratio 3.7:1.6:1:2.6. In the present case, the ratio is 3.5:1.5:1:0. Thus, both laboratory cycloaddition reactions display a proclivity for the formation of the VM55599 relative stereochemistry with differing proportions of the other three diastereomeric relationships.

It is interesting to compare the *syn*:*anti*-ratios with respect to the relative stereochemistry at C-20. In the optically active case, the *syn*:*anti*-ratio was 1.4:1. In the previously reported racemic system, the *syn*:*anti*-ratio was 2.4:1. The ratio of cycloaddition of the isoprene-derived dienophile across the azadiene with respect to the methyl group in the proline ring was 5:1, in the optically active case, favoring approach of the dienophile from the *same face* of the azadiene as the methyl group and 1.47:1 in the racemic *O*-methyl lactim ether system. While structural differences in the laboratory azadienes (see **B**, Chart 40 and **191**, Chart 37) and that for

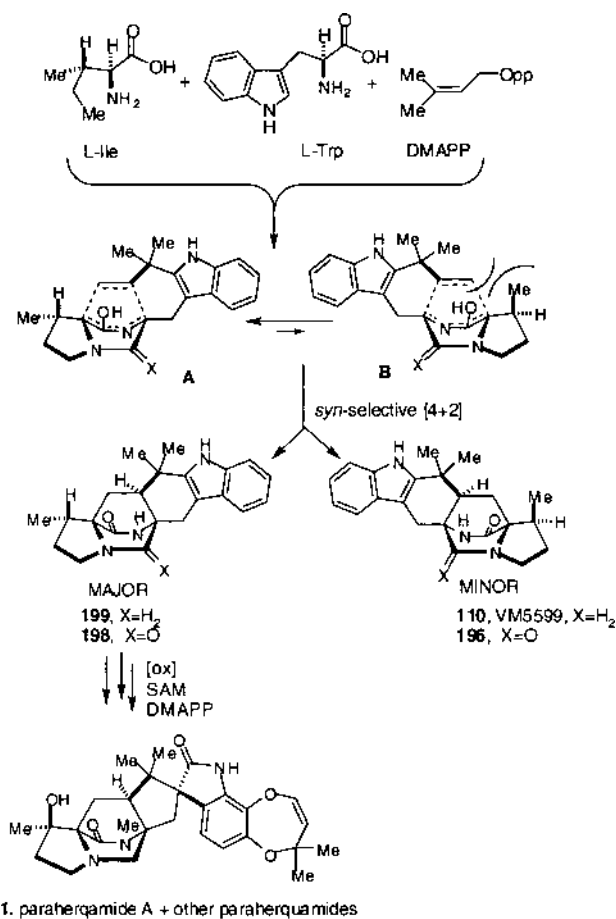


Chart 41. Williams' Unified Biogenesis of the Paraherquamides and VM55599

the biological system (see A, Chart 41) indeed exist, the intrinsic facial selectivity of this cycloaddition appears not to be greatly biased towards the paraherquamide stereochemistry. In the biological system, the diastereochemical distribution is expressed as $>600:1$ ¹²⁰ (corresponding to **198** or **199**:**196** or **110**) as evidenced by the complete lack of natural metabolites that would arise from substances containing the *anti*-stereochemistry imbedded in either **207** or **208**.

These experimental observations led Williams and co-workers to propose a unified biosynthesis of the paraherquamides and VM55599 (**110**), as shown in Chart 41. In their proposal, the biosynthetic precursors of the paraherquamides and that of VM55599 would arise as diastereomeric products of the Diels-Alder cycloaddition of a common azadiene through two of four possible diastereomeric transition states. The minor product of this cycloaddition culminating in VM55599 would constitute approach of the dienophile *syn*- to the methyl group (as in structure B, Chart 41). The major product of the cycloaddition would be compound **198** or **199**, wherein the dienophile attacks the opposite face of the azadiene, *anti*- to the methyl group, which would give rise to the various paraherquamides.

This hypothesis was recently experimentally tested through feeding experiments of racemic, doubly ¹³C-labeled compounds **110**, **196**, **198**, and **199** (Chart 42).¹²¹ Feeding experiments were performed on *Penicillium fellutanum* (ATCC: 20841) using all four potential precursors followed by isolation and purification of paraherquamide A. Within

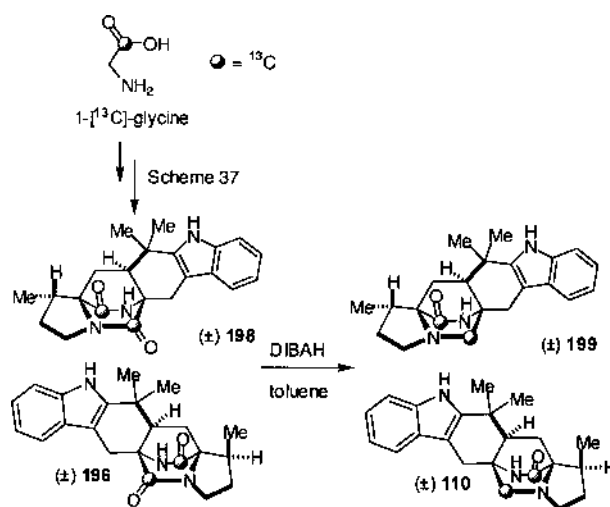


Chart 42. Synthesis of Doubly-¹³C-Labeled Substrates¹²¹

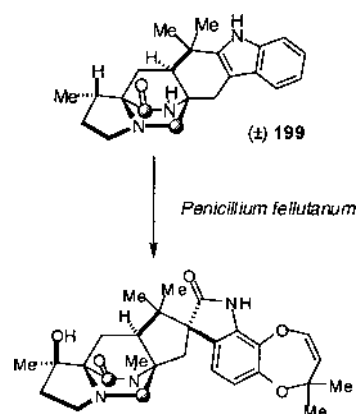


Chart 43. Biosynthetic Incorporation of **199** into Paraherquamide A

the limits of detection by ¹³C-NMR and mass spec, no incorporation was observed for VM55599 ((±)-**110**) nor its oxidized counterpart (±)-**196**. In addition, no incorporation was observed for the diketopiperazine (±)-**198**. However, for the C14 epimer of VM55599 ((±)-**199**), significant incorporation was observed by ¹³C-NMR at C-12 and C-18 of paraherquamide A. From analysis of the electrospray mass spectrum, incorporation was determined to be 0.72% for the intact doubly labeled material. Mono-¹³C-labeled paraherquamide A, from catabolism of (±)-**199**, was not detected in the mass spectrum. The implications of these observations are considerable.

Since the diketopiperazine (±)-**198**, was not incorporated, this raises interesting questions concerning the timing of the reduction of prolyl-derived carbonyl group. The incorporation of compound (±)-**199** in significant isotopic yield (Chart 43), indicates that the formation of the bicyclo[2.2.2]-diazaoctan occurs at the stage of the non-oxidized tryptophyl moiety (*i.e.*, indolyl). This mandates that oxidations of the indole ring to form both the catechol-derived dioxepin and spirooxindole, occur *after* the formation of this intermediate (**199**). It thus follows that the dioxepin-derived isoprenylation and the S-adenosylmethionine-mediated *N*-methylation reactions also must occur late in the pathway.

These results also cast considerable doubt on the agency of

VM55599 and its oxidized precursor **196**, as being intermediates in paraherquamide biosynthesis and provide additional circumstantial evidence that VM55599 is a minor shunt metabolite. Finally, this work documents the intermediacy of an advanced metabolite (**199**) containing the core structural elements of the paraherquamide framework prior to a series of oxygenation reactions. Additional work needs to be done to elucidate the exact sequence of biosynthetic reactions immediately preceding and following the formation of **199**.

These results lend strong experimental support to the biogenesis of paraherquamide A proposed by Williams *et al.*, and also provided the basis for the prediction as to the correct stereostructure of VM55599 which has now been verified. The absolute stereochemistry of VM55599 and the failure of this substance to undergo biotransformation to paraherquamide thus abrogates the connection of this metabolite to the paraherquamide pathway.

Synthetic Approach to Asperparaline

The first synthetic model study toward asperparaline A was reported by Williams as shown in Chart 44.¹²²⁾ Their approach to the spirosuccinimide ring system begins with commercially available 3,3,5,5-tetramethyl cyclohexanone **209** which was transformed into oxime **210** using hydroxylamine hydrochloride. The pyrrole **211** was obtained from oxime **210** through the employment of a Trofimov reaction.¹²³⁾ This interesting transformation, which has not found extensive implementation in synthetic organic chemistry, was found to be a very useful reaction to construct 2,3-disubstituted pyrroles from the corresponding oxime using acetylene in a superbasic media. Pyrrole **211** was then *N*-methylated with iodomethane to afford **212** in high yield. It was found that the oxidation of the *N*-methylpyrrole **212** could be realized through a photooxygenation reaction using Rose Bengal as a photosensitizer under UV light irradiation to afford hydroxy pyrrolidinone **213**. The key spiro-rearrangement reaction required for the transformation of compound **213** into **214**, was accomplished by treatment of pyrrolidinone **213** with sodium hydride in DMSO at 180 °C to furnish the desired succinimide **214**.

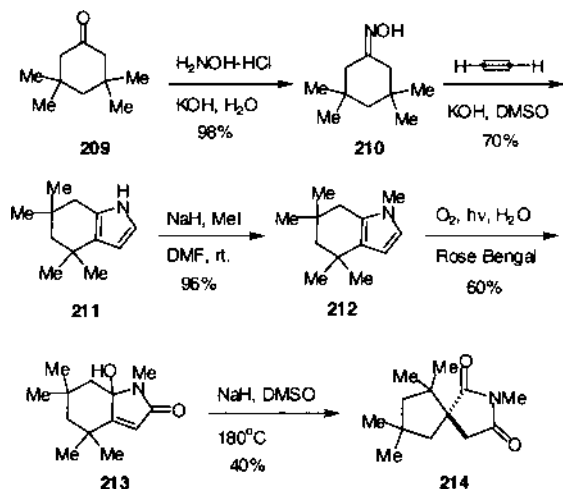


Chart 44. Gonzalez' and Williams' Model Study on the Spirosuccinimide Ring System of Asperparaline A

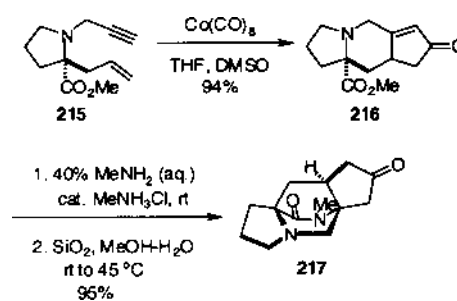


Chart 45. Tanimori's Approach to Asperparaline C¹²⁴⁾

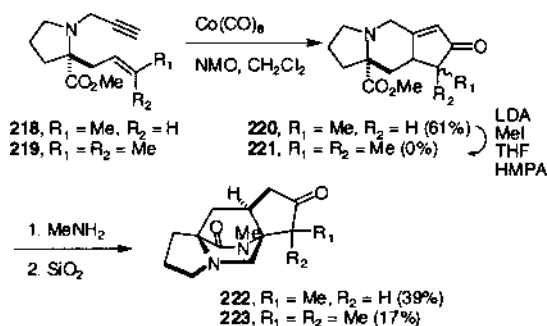


Chart 46. Tanimori's Approach to Asperparaline C¹²⁴⁾

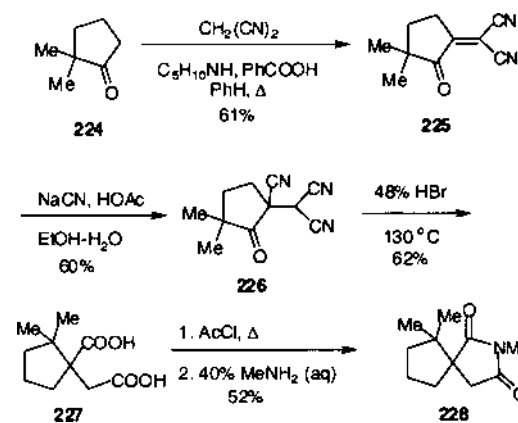


Chart 47. Tanimori's Approach to the Spirosuccinimide Ring System of Asperparaline A

Tanimori and co-workers have recently described a Pauson-Khand cyclization reaction to construct the tetracyclic indolidine core of asperparaline C as shown in Charts 45 and 46.¹²⁴⁾ Reaction of enyne **215** with $\text{Co}(\text{CO})_8$ furnished the tricyclic compound **216** in high yield. Condensation of this substance with MeNH_2 resulted in conjugate addition of the amine to the enone moiety followed by ring closure providing **217** in 95% overall yield.

These workers also attempted to install the *gem*-dimethyl group but found that the Pauson-Khand cyclization proved more difficult with one methyl group (substrate **218**) and did not proceed at all with the *gem*-dimethyl substrate **219** (Chart 46). Substrate **221** was prepared by enolate methylation of **220** (58% yield). Subsequent amination of **220** and **221** gave the tetracyclic compounds **222** and **223** in 39% and 17% yields, respectively. This approach promises to provide a concise and stereocontrolled method to access the asperpara-

lines that is distinct from all other published approaches to construct natural products containing the bicyclo[2.2.2] ring system common to the paraherquamides.

Tanimori and co-workers have also recently published an approach to the spirosuccinimide ring system of the asperparalines utilizing a model cyclopentanone (**224**, Chart 47).¹²⁵⁾

Conclusion

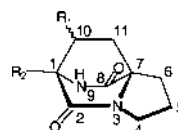
The paraherquamide family of alkaloids has provided a rich array of synthetic and biosynthetic challenges.¹²⁶⁾ Many unusual and unexpected aspects concerning the stereochemistry and biogenesis of these natural products have been revealed. Total synthesis has played a significant role in penetrating Nature's secrets with respect to biosynthetic constructions but much additional work remains. It is hoped that this review will stimulate others in the field of natural products synthesis to consider harnessing the potential of their own complex synthetic work, to probe biosynthetic pathways in other systems. A huge bounty of unclaimed knowledge exists in this respect. The biosynthetic origin of the unusual spirosuccinimide ring system in the asperparalines poses an interesting challenge and is currently being studied in these laboratories. In addition, other conceptual approaches to the bicyclo[2.2.2] ring system shared by all members of this family stands as an area ripe for development. Finally, the strong implication of protein organization of the transition state conformer distribution in the proposed biosynthetic Diels–Alder cyclization reactions invoked in the biogenesis of the paraherquamides, earmarks these metabolites and their respective producing organisms for genetic studies. Ongoing work in these laboratories and others is anticipated to seek answers to the most provocative issues raised in this review.

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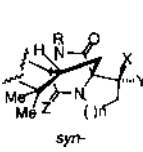
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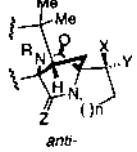


Throughout this manuscript, the numbering system adopted reflects

that shown on brevianamide A in Fig. 2.

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syn-



anti-
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