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Studies on paraherquamide biosynthesis: synthesis of deuterium-labeled 7-hydroxy-pre-paraherquamide, a putative precursor of paraherquamides A, E, and F

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ABSTRACT

The stereocontrolled, asymmetric synthesis of triply deuterium-labeled 7-hydroxy-pre-paraherquamide (**27**) was accomplished, employing a diastereoselective intramolecular S_N2' cyclization strategy. The deuterium-labeled substrate was interrogated in a precursor incorporation experiment in the paraherquamide-producing organism *Penicillium fellutanum*. The isolated sample of paraherquamide A revealed incorporation of one of the two geminal deuterons of the CD_2-group at C-12 exclusively. The lack of signals for the second deuteron of the CD_2-group at C-12 and for the CH_2D-group (C-22/C-23) suggests that this substrate suffered an unexpectedly selective catabolic degradation and metabolic re-incorporation of deuterium thus casting doubt on the proposed biosynthetic intermediacy of **27**. Consideration of alternative biosynthetic pathways, including oxidation of the indole C-6 position prior to hydroxylation at C-7 or oxidative spiro-contraction of pre-paraherquamide prior to construction of the dioxepin is discussed. The synthesis of **27** also provides for a concise, asymmetric stereocontrolled synthesis of an advanced intermediate that will be potentially useful in the synthesis of paraherquamides E and F.

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1. Introduction

The paraherquamides constitute part of an unusual family of prenylated indolic natural products derived from various genera of fungi, which contain a common bicyclo[2.2.2]diazaoctane core structure, a spiro-oxindole, and a substituted proline moiety. The parent member, paraherquamide A **1**, was first isolated from cultures of *Penicillium paraherquei* by Yamazaki and co-workers in 1981.[1] Since then, paraherquamides B–I,[2] VM55595, VM55596, and VM55597,[3] SB203105 and SB200437,[4] and sclerotiamide[5] have been isolated from various *Penicillium* and *Aspergillus* species. Marcfortines A–C are structurally similar, containing a pipecolic acid unit in place of proline.[6] In addition, VM55599,[3] aspergamides A and B,[7] avrainvillamide (CJ-17,665),[8] and the most recently isolated members of this family, stephacidin A (**10**), stephacidin B (**11**),[9] and malbrancheamide[10] are closely related members of this rapidly expanding family of prenylated indole alkaloids. This last group of compounds contains a 2,3-disubstituted indole in place of the spiro-oxindole, spiro-indoxyl, or spiro-succinimide. Brevianamides A and B (**9**),[11] which contain a spiro-indoxyl rather than a spiro-oxindole, and the asperparalines, which contain a spiro-succinimide,[12] are also structurally related (Fig. 1).

The members of this family of prenylated alkaloids have attracted considerable attention due to their molecular complexity, intriguing biogenesis, and biological activities.[13] Some members, most notably paraherquamide A, display potent anthelmintic activity and antinematodal properties.[14] In this context, since the paraherquamides are unique both structurally and in their mode of action,[15] they represent a potentially promising new class of anthelmintics. Accordingly, modifications of paraherquamide A led to the discovery of 2-deoxoparaherquamide A (PNU-141962), which exhibits comparable biological activities to that as paraherquamide A, but has an improved safety profile and is currently undergoing evaluation in clinical studies.[16] Stephacidins A (**10**) and B (**11**)[9] have also displayed promising anti-cancer activity through a unique mechanism of action that is just beginning to emerge.[17] Of particular interest was the report that stephacidin B exhibited selective, antitumor activity against a testosterone-sensitive prostrate LNCaP cell line with an IC_{50} value of 0.06 μM.[9]

From a biogenetic perspective, the paraherquamides along with the brevianamides, asperparalines, marcfortines, the malbrancheamides, notoamides[18] and sclerotiamide comprise an

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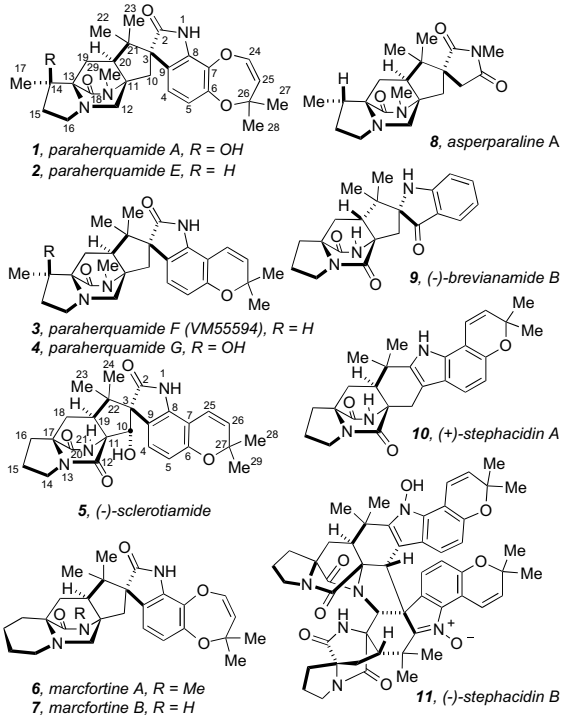
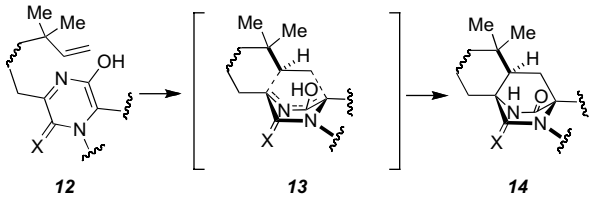


Figure 1. Structures of selected paraherquamides and several related prenylated indole alkaloids.

interesting class of structurally related secondary metabolites containing a bicyclo[2.2.2]diazaoctane core.

An emerging body of evidence supports the notion that this structural motif is formed by a biosynthetic intramolecular [4+2] cycloaddition of the isoprene-derived olefin across a preformed azadiene moiety derived from an oxidized piperazinedione (**12**/**13**) as shown in Scheme 1.[13,19,20]



Scheme 1. Proposed Diels–Alder reaction forming the core structure of brevianamides, paraherquamides, and asperparalines.

In this context, Everett and co-workers described in 1993 the isolation of VM55599 (**21**, Scheme 2), a minor metabolite from culture extracts of a *Penicillium* sp. (IMI332995) that also produces paraherquamide A (**1**), among other paraherquamides.[3] Taking into account the structural similarities between these co-occurring metabolites, these authors proposed that VM55599 might indeed be a biosynthetic precursor of paraherquamide A.[3] However, recent experimental observations from this laboratory brought into question the capacity of VM55599 to serve as a biosynthetic precursor to the paraherquamides and we proposed a distinct, unified biogenesis of the paraherquamides and VM55599.[21] In this 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. This hypothesis was recently experimentally tested through feeding experiments of racemic, doubly ^{13}C-labeled compounds **20**–**23**.[22] These experiments revealed that only intermediate **20** was incorporated into paraherquamide A, while racemic, doubly ^{13}C-labeled VM55599 (**22**), **23**, and **21** were not incorporated. Significantly, pre-paraherquamide has been detected as a natural metabolite of the paraherquamide-producing organism *P. fellutanum* and the asperparaline-producing organism *Aspergillus japonicus*.[23] The lack of incorporation of the dioxopiperazine **21** raises interesting questions concerning the timing of the reduction of the tryptophan-derived carbonyl group. The incorporation of **20** indicates that formation of the bicyclo[2.2.2]diazaoctane occurs at the stage of the non-oxidized tryptophyl moiety. This mandates that oxidations of the indole ring to form both the catechol-derived dioxepin and spiro-oxindole must occur *after* the formation of pre-paraherquamide. The dioxepin-derived isoprenylation and the *S*-adenosylmethionine-mediated N-methylation reactions probably occur late in the pathway.

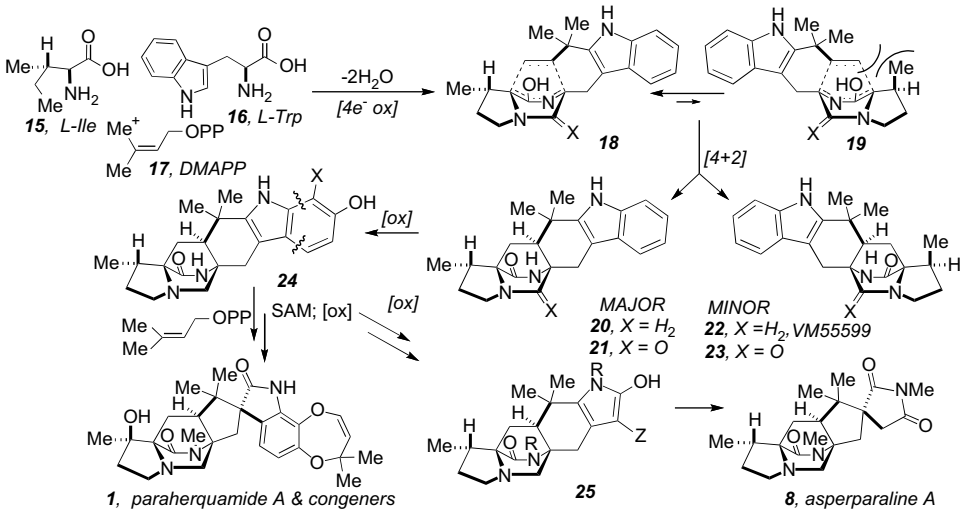
For asperparaline A, in addition sharing the bicyclo[2.2.2]diazaoctane core, the orientation of the spiro-succinimide ring is consistent with the spiro-ring configuration of the paraherquamides. It has been demonstrated that in paraherquamide A biosynthesis, L-tryptophan, L-β-methylproline (which is derived from L-isoleucine), and an isoprene moiety are coupled together to provide the hexacyclic indole **20**.[24] Based on the experimental observation that the same three primary biosynthetic building blocks (L-tryptophan, L-isoleucine, and dimethylallyl pyrophosphate) constitute asperparaline A, we proposed a unified biogenesis of the paraherquamides and the asperparalines as shown in Scheme 2.[25] As previously reported, coupling of L-tryptophan, L-β-methylproline, and dimethylallyl pyrophosphate (DMAPP) gives compound **20** via intramolecular Diels–Alder cycloaddition, which can be oxidized to the hypothetical intermediate **24**. Compound **24** is considered to be a key branch-point species for the two respective pathways to paraherquamide A and asperparaline A. In paraherquamide biosynthesis, **24** would suffer prenylation and conversion to the dioxepin. In asperparaline biosynthesis, this compound would undergo further oxidation ultimately removing four carbon atoms from the benzenoid ring of the tryptophan moiety.

In order to test this hypothesis experimentally and to further penetrate the biogenesis of paraherquamide A and asperparaline A, we have initiated studies on the synthesis of ^{2}H- and/or ^{13}C-labeled hexacyclic putative biosynthetic intermediates **26**–**29** that will be useful to investigate the intermediate stages in the biosynthesis of paraherquamide A and asperparaline A.

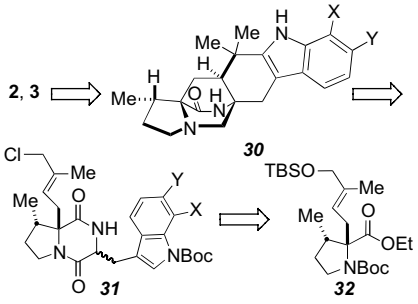
We are especially interested in the exact sequence of hydroxylations and prenylation of the indole nucleus to fashion the unique dioxepin moiety of paraherquamide A and the related dihydropyran system of paraherquamides F and G. On the other hand, the oxidative construction of the unique spiro-succinimide ring system of asperparaline A from the tryptophan residue provides a challenging and mechanistically provocative biosynthetic puzzle.

Recent interest in identifying new structural classes of anthelmintics is due to the appearance of drug resistance, and thus, the unique mode of action of the paraherquamides and their considerable biological activity have provided the motivation for us to contemplate total syntheses of paraherquamides E (**2**), F (**3**) and the asperparalines that would be readily adaptable to the preparation of analogues.

Our synthetic strategy to access these β-methylproline-containing natural products entailed an oxidative spiro-ring contraction of the generic hexacycle **30** that would be constructed via a face-selective intramolecular S_N2' cyclization reaction (Scheme 3).[26] The 3-hydroxy analogue of compound **32** has been prepared for the asymmetric total synthesis of paraherquamide A and has been deployed for the synthesis of a hexacyclic species analogous to

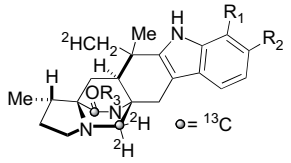


Scheme 2. Proposed unified biogenesis of paraherquamides and asperparalines.



Scheme 3. Retrosynthetic approach to paraherquamides E and F.

30.[27] Our plan thus required the synthesis of the critical 2-substituted, 3-methylproline derivative (**32**) with the desired absolute and relative stereochemistry. Conversion of **32** to species **31** has been well documented in our laboratory as well as the subsequent intramolecular S_N2' cyclization, and Wacker-type construction of the 2,3-disubstituted indole (**30**).[28] Oxidative spiro-ring contraction would then be expected to provide entry to both paraherquamides E and F.[29] The major advantage of this strategy is that at the same time all four candidate precursors **26**–**29** (Fig. 2) are accessible from a single synthesis. In addition, both of the ^{13}C labels in these compounds would be derived from relatively inexpensive ^{13}C-glycine and 1-^{13}C-ethyl bromo-acetate.



26: $R^1 = OCH_2CH{=}CMe_2$, $R^2 = OH$, $R^3 = Me$
27: $R^1 = OH$, $R^2 = H$, $R^3 = H$
28: $R^1 = H$, $R^2 = OH$, $R^3 = H$
29: $R^1 = H$, $R^2 = H$, $R^3 = Me$

Figure 2. Synthesis targets for biosynthesis studies.

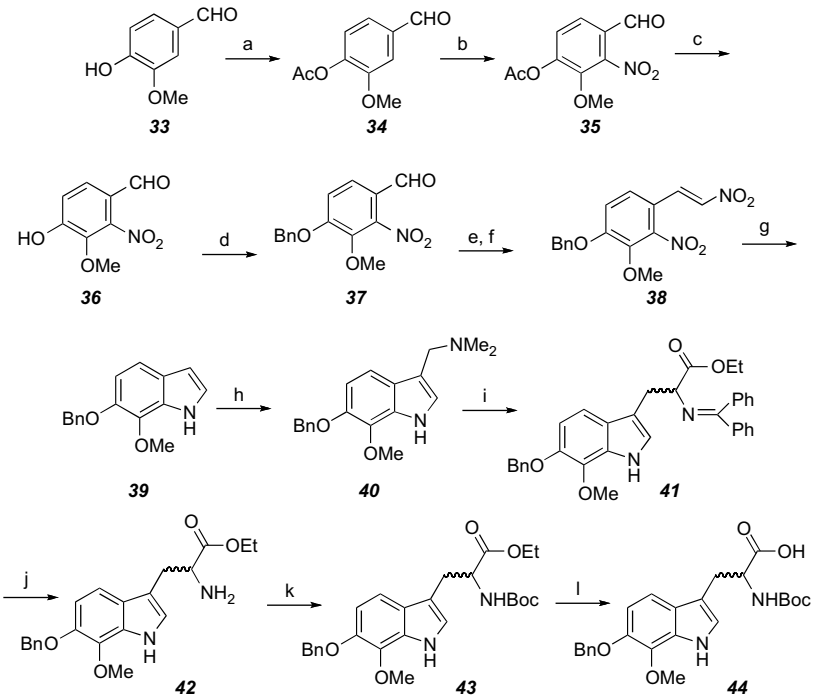
We report here, a preparative synthesis of D_3-7-hydroxy-preparaherquamide (**27**) that has subsequently been used in a feeding experiment with *P. fellutanum*. Significantly, this synthetic strategy offers a rapid, versatile, and inexpensive access to a range of further putative biosynthesis intermediates and inherently permits the incorporation of ^{13}C-labeling strategies as well. Moreover, this approach should prove to be a useful strategy by which paraherquamides E and F as well as asperparaline A might be constructed.

2. Results and discussion

We recently described two practical methods for the synthesis of the optically active α-alkylated-β-methylproline species **32**. With this key substance in hand and in light of the required regioselective transformations of the C-6/C-7-dihydroxyindole moiety at the late stage of the synthesis of **26**–**29**, we desired a convenient synthesis of the differentially protected tryptophan derivative **44** (Scheme 4). Accordingly, starting from vanillin (**33**), acetylation followed by nitration[30] gave the 3-nitro-isomer exclusively, which upon basic hydrolysis of the acetate and subsequent O-benzylation afforded **37**. Henry reaction and subsequent dehydration yielded the dinitrostyrene **38**.[31] Reductive cyclization of **38** provided the expected indole **39**. Functionalization of the indole 3-position was carried out by treatment of **39** with formaldehyde/dimethylamine to give the corresponding gramine derivative **40**, which was then utilized in a Somei–Kametani-type reaction[32] to access tryptophan derivative **41**. Finally, cleavage of the benzophenone imine group and subsequent Boc-protection followed by basic hydrolysis afforded the desired tryptophan derivative **44**.

In our previous work on the total synthesis of paraherquamides A and B, the synthetic strategy deployed to reach substrates analogous to **46** included a stepwise formation of the dioxopiperazine ring, two methoxy carbonylations followed by a Somei–Kametani coupling and decarboxylation.[33] In an effort to improve upon this protocol, a new and more efficient strategy that exploited two successive peptide couplings was investigated.[34,35]

Accordingly, coupling of the tryptophan derivative **44** with methylproline fragment **32** was accomplished with the pyBOP (or BOP) reagent (Scheme 5). The subsequent selective removal of the Boc protecting group in the presence of the acid-labile *O*-TBS protecting group afforded a ~2:1 mixture of *syn*/*anti* diastereomers, which were separated by column chromatography. Cyclization assisted by 2-hydroxypyridine gave the dioxopiperazine **46**. Starting from the *anti*-dioxopiperazine **46b**, protection of the secondary amide as the corresponding lactim ether was accomplished



Scheme 4. (a) Ac_2O, DMAP, Et_2O, 20 h, room temperature, 96%; (b) HNO_3 fuming, −20 °C, 3 h; (c) NaOH, H_2O, room temperature, 20 min, 72–76% (two steps); (d) BnBr, K_2CO_3, DMF, room temperature, 24 h, 97%; (e) $MeNO_2$, KF, *i*-PrOH, NMM, room temperature, 48 h; (f) Ac_2O, NaOAc, room temperature, 48 h, 72% (two steps); (g) Fe, SiO_2, AcOH, toluene, 30 min, reflux, 92%; (h) $HNMe_2$, CH_2O, AcOH, EtOH, 0 °C→room temperature, 15 h, 97–99%; (i) $(Ph)_2C{=}NCH_2CO_2Et$, *n*-BuP_3, MeCN, reflux, 6 h, 82%; (j) HCl, H_2O, THF, 4 h, room temperature 100%; (k) Boc_2O, NaOH, H_2O, THF, room temperature, 24 h, 100%; (l) LiOH, H_2O, THF, 21 h, room temperature, 100% (three steps).

efficiently by a treatment with trimethyloxonium tetrafluoroborate and Cs_2CO_3 in dichloromethane to give **47b**. Next, the indole nitrogen was protected as the corresponding *N*-*tert*-Boc derivative, and then the silyl ether was removed with tetra-*n*-butylammonium fluoride to provide **49b**. Standard conditions for allylic chloride formation provided **50b** in good yield. The analogous reaction sequence was carried out for the *syn*-dioxopiperazine **46a**.

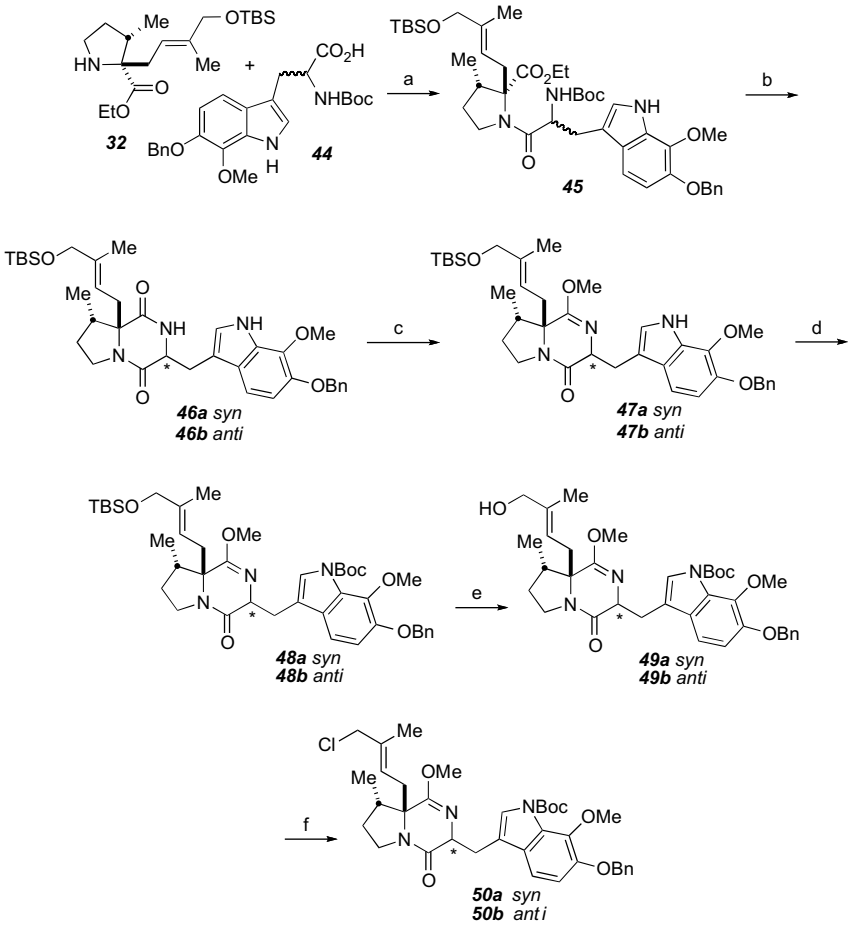
The stage was now set for the critical intramolecular S_N2' cyclization that sets the relative stereochemistry at C-20 during formation of the bicyclo[2.2.2]diazaoctane ring nucleus. Based on precedent from our paraherquamide A and B syntheses, *anti*-**50b** was treated with NaH in refluxing benzene. However, no S_N2' reaction took place and only epimerization of the tryptophan residue was observed. We have previously observed in our paraherquamide B synthesis that the S_N2' reaction is highly temperature dependent, and we speculated that higher temperatures might allow the present substrate to reach a reactive conformation for the S_N2' reaction. Indeed, treatment of *anti*-**50b** with NaH in refluxing xylene afforded the desired S_N2' reaction product **51** in 77% yield exclusively as the desired *syn*-isomer (Scheme 5). Compound *syn*-**50a** also underwent the same transformation to give **51** in 56% yield. In practice, we found it convenient to employ the *anti*/*syn*-diastereomeric mixture of **50**, which was carried through the subsequent steps leading to **51**.

In our previously recorded paraherquamide A synthesis, Wacker-type cyclizations[26c,d] were utilized to form the sixth ring using $PdCl_2$, $AgBF_4$, and propylene oxide in acetonitrile, however, in the current case, these reaction conditions led to several by-products. Eventually, it was found, that the conversion of **52** to hexacycle **53** could be carried out successfully when $Pd(CH_3CN)_4(BF_4)_2$ was utilized or preferably, $Pd(TFA)_2$ in acetonitrile. As a result, closure of the sixth ring was carried out using $Pd(TFA)_2$ in acetonitrile/propylene oxide followed by $NaBH_4$ (5 equiv) in EtOH to reduce the incipient hexacyclic σ-palladium adduct (Scheme 6). Under these conditions, the main product observed following the $NaBH_4$ quench was the desired product, which was missing the *O*-benzyl residue. Nonetheless, the large excess of $NaBH_4$ resulted in a virtually quantitative reduction of the σ-palladium hexacyclic intermediate, and as a result, after re-benzylation, **55** was obtained in an excellent yield of 94% over two steps. The analogous transformation was carried out using 10 equiv $NaBD_4$ (99% atom D) in EtOD and provided the deuterium-labeled analogue **56** in 89% yield as a single diastereomer (note that a new stereogenic center is created in this process due to deuteration of the diastereotopic methyl group).

The incorporation of the deuterium in **56** was determined to be 93% atom D, most likely a result of the reaction of traces of the σ-palladium intermediate with EtOH used during the work-up and/or Pd-mediated H/D exchange on the glass reaction vessel surface.

In the paraherquamide A synthesis, conditions could not be found, which would allow direct and high-yielding conversion of the analogous lactim ether **55** to the amide **59**. However, 0.1 N aqueous HCl in THF gave the corresponding ring-opened amine methyl ester, which was cyclized with a catalytic amount of 2-hydroxypyridine in hot toluene. Employing the same procedure here, the conversion of the lactim ether **55** to the secondary amide **59** was effected using 0.1 N aqueous HCl in THF to provide in this case, a mixture of the secondary amide and the respective amine methyl ester, which was directly cyclized to the bicyclo[2.2.2]diazaoctane by treatment with 2-hydroxypyridine in refluxing toluene. The analogous transformation was carried out with **56** to provide the deuterium-labeled analogue **60** in 86% over the two steps.

Next, the chemoselective reduction of the tertiary amide in the presence of the secondary amide to give **61** was investigated. Treatment of **59** with 4–5 equiv DIBAL-H in toluene at room temperature provided **61**. Since the analogous deuterated reagent DIBAL-D is neither commercially available nor reported as a solution in toluene, we prepared this important reagent based on the procedure developed for the synthesis of dineopentylaluminum hydride in toluene.[36] Accordingly, triisobutylaluminum was



Scheme 5. (a) pyBOP, DIEA, CH_2Cl_2, room temperature, 4 days, 84–89% (*anti*/*syn*-DKP); (b) TMSOTf, 2,6-lutidine, CH_2Cl_2, 1 h, room temperature, then NH_4Cl, H_2O, 50 min, room temperature; then 2-HO-py, toluene, reflux, 12 days, 98% (*anti*-DKP), 67% (*syn*-DKP); (c) Me_2OBF_4, Cs_2CO_3, CH_2Cl_2, room temperature, 20 h, 99% (*anti*-DKP), 71% (*syn*-DKP); (d) $(Boc)_2O$, NEt_3, DMAP, CH_2Cl_2, room temperature, 25 h, 92% (*anti*-DKP), 79% (*syn*-DKP); (e) *n*-Bu_4NF, THF, room temperature, 24 h, 94% (*anti*-DKP), 92% (*syn*-DKP); (f) MsCl, LiCl, 2,4,6-collidine, DMF, 0 °C→room temperature, 5 days, 86% (*anti*-DKP), 86% (*syn*-DKP) or MsCl, 2,4,6-collidine, CH_2Cl_2, 0 °C→room temperature, 24 h, then $Bn(Bu)_3NCl$, DMF, 0 °C→room temperature, 22 h, 95% (*anti*-DKP).

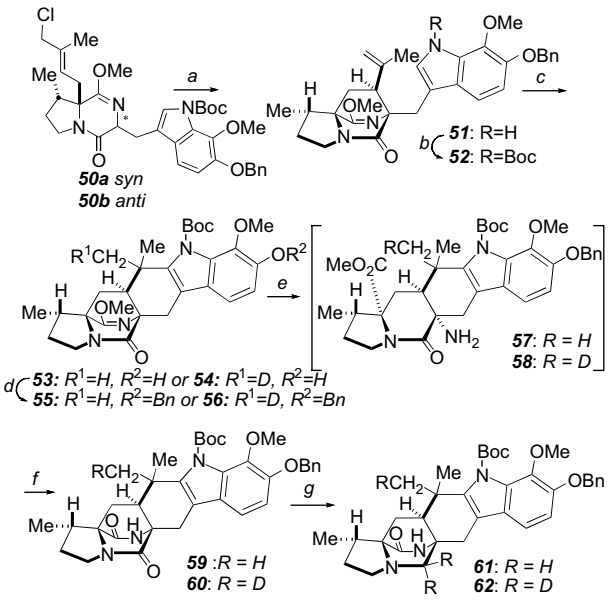
reduced with $LiAlD_4$ in heptanes under refluxing conditions and the solvent replaced with toluene to provide the required reagent. Freshly prepared toluene solutions of DIBAL-D effected the chemoselective reduction of the tertiary amide in the presence of the secondary amide furnishing hexacycle **62**. After an initial reduction with 4 equiv DIBAL-D yielded a mixture of the desired product and the starting material, successive reductions of the crude material with additional DIBAL-D gave the triply deuterated material in 52% yield as a 5.7:1 inseparable mixture of Boc-protected product **62** and the corresponding product that had suffered loss of the *N*-*tert*-Boc residue.

To reach the triply deuterium-labeled 7-hydroxy-pre-paraherquamide, the benzyl protecting group of **62** was removed via catalytic hydrogenation in the presence of Pd/C and the resulting crude phenol was converted into the corresponding 6-mesylate **64** (Scheme 7). The reductive removal of the oxygen atom at the C6-indole position proved troublesome. The C-6-mesylate **64** displayed only limited reactivity toward catalytic hydrogenation and was reduced only when exposed to a large molar excess of $Pd(OH)_2/C$. Not surprisingly, these conditions resulted in the concomitant reduction of the indole moiety to the corresponding dihydroindole species **65**. The Boc protecting group of **65** was removed under standard reaction conditions to give **66**. Cleavage of the methyl ether was affected with BBr_3 in CH_2Cl_2 and resulted in a mixture of air sensitive compound **67** (major) and the oxidation product, namely, the desired deuterium-labeled 7-hydroxy-pre-paraherquamide **27** (minor). Finally, when exposed to air in a biphasic EtOAc/satd $NaHCO_3$ solution, the mixture was readily oxidized to give **27** exclusively.

The deuterated 7-hydroxy-pre-paraherquamide (**27**) possessed the unfortunate property of being insoluble in water thus rendering the planned feeding experiments challenging. To circumvent this problem, the detergent TWEEN 80 was added and was found to increase the miscibility of the substances in the culture broth without inhibiting the production of paraherquamide A.

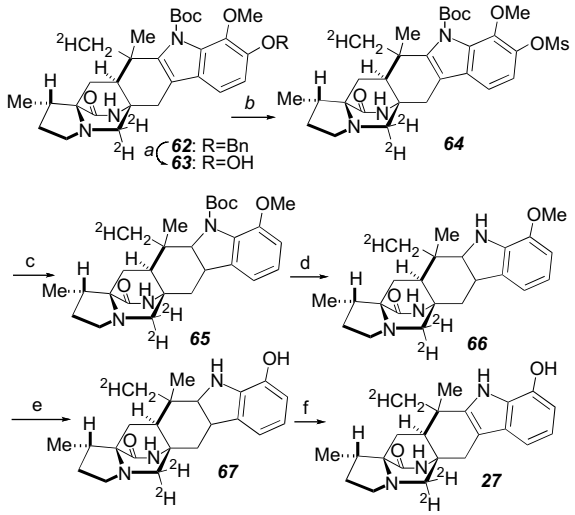
Feeding experiments were performed on *P. fellutanum* (ATCC20841) followed by isolation and purification of paraherquamide A. Within the limits of detection by ^2H NMR spectroscopy no incorporation was observed for the intact triply labeled material **27**. However, the isolated paraherquamide A sample (14 mg) revealed the incorporation of one of the two geminal deuterons of the CD_2-group (^2H NMR, 500 MHz, $CHCl_3$/CH_3OH: 2.4 ppm; see Fig. 3). The lack of signals for the second deuteron of the CD_2-group (~3.6 ppm) and for the CH_2D-group (~1.2 ppm) suggests that **27** suffered catabolic degradation and metabolic re-uptake of the deuteron. A rationalization for how deuterium atoms from triply labeled II could be removed, and then selectively re-incorporated at C–X of paraherquamide A is currently lacking and more experimental data will need to be secured to address this puzzling observation.

The implications of these observations are considerable. Since the deuterated 7-hydroxy-pre-paraherquamide (**27**) was not



Scheme 6. (a) NaH, xylenes, 135 °C, 60 min; (b) Boc_2O, DMAP, NEt_3, CH_2Cl_2, room temperature, 2 days, 77% (from the *anti*-diastereomer), 56% (from the *syn*-diastereomer); (c) $Pd(OTFA)_2$, MeCN, propylene oxide, room temperature, 18–24 h, then EtOH/$NaBH_4$ or EtOD/$NaBD_4$, room temperature, 3–8 h; (d) BnBr, K_2CO_3, DMF, room temperature, 2–3 days, 63–94% (two steps); (e) 0.1 N HCl, THF, 0 °C→room temperature, 80–90 min; (f) 2-HO-py, toluene, reflux, 3–5 h, 81–86% (two steps); (g) DIBAL-H or DIBAL-D/toluene, −78 °C→room temperature, 2–5 days, 52–77%.

incorporated intact, this raises numerous provocative questions concerning the timing and the regioselectivity of the hydroxylation of the tryptophan-derived indole moiety for ultimate construction of the dioxepin (for paraherquamide A and congeners) and the pyran system (for paraherquamides F and G). The lack of incorporation of the intact triply labeled species **27** may indicate that hydroxylation of the indole C-6 position (see Scheme 8, **66**) likely precedes hydroxylation of the indole C-7 position. It is also plausible that oxidative processing of pre-paraherquamide to the oxindole species **68** precedes hydroxylation at either C-6 or C-7 of



Scheme 7. (a) H_2, Pd/C (10%), EtOH, room temperature, 19 h; (b) MsCl, py, CH_2Cl_2, 0 °C to room temperature, 12–48 h, 64–89% (two steps); (c) H_2, $Pd(OH)_2$/C, MeOH, room temperature, 3 days, 47%; (d) TFA, CH_2Cl_2, 0 °C, 3 h, 98%; (e) (1) BBr_3, CH_2Cl_2, −78 °C to room temperature, 13 h; (2) MeOH, −78 °C to room temperature, 1 h; 3) $NaHCO_3$, H_2O, room temperature, 30 min, >99% (BRSM), 87% conversion; (f) EtOAc, satd $NaHCO_3$, air, 48 h, >99%.

the indole nucleus. The syntheses of the regioisomeric 6-hydroxy-pre-paraherquamide (**66**) and the corresponding spiro-oxindole (**68**) derived from pre-paraherquamide itself, as well as their corresponding *N*-methyl derivatives (**67** and **69**) are currently under study to evaluate these alternatives.

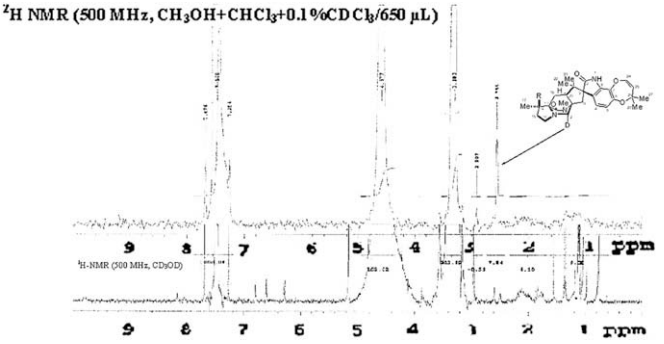
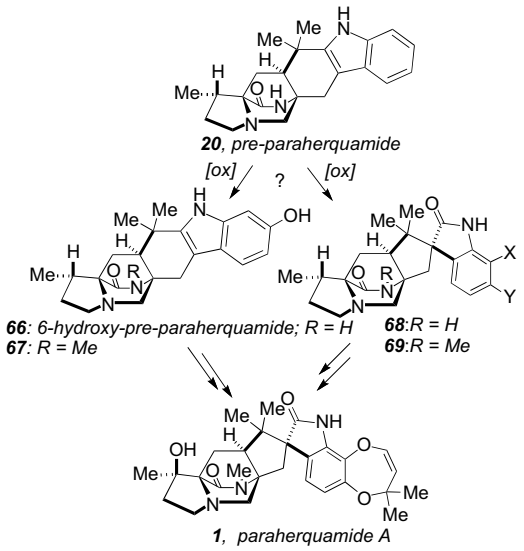


Figure 3. ^2H NMR (500 MHz, CH_3OH+$CHCl_3$+0.1%$CDCl_3$/650 µL) spectrum of the isolated sample of paraherquamide A from feeding experiment with *P. fellutanum*.

The apparent transfer of the single deuterium atom from triply labeled substrate **27** to paraherquamide A might be rationalized by considering that this species serves as a non-endogenous substrate for a reductase that employs NADH as a co-factor. Deuterium transfer from **27** to the oxidized NAD^+ co-factor and subsequent reduction of the endogenous substrate en route to paraherquamide A might be one of many reasonable explanations for this unexpected deuterium incorporation.



Scheme 8. Some alternative biosynthetic transformations from pre-paraherquamide (**17**) to paraherquamide A.

3. Conclusion

In summary, we successfully prepared the putative biosynthetic intermediate 7-hydroxy-pre-paraherquamide (**27**) in deuterium-labeled form. This substance was used in a feeding experiment in the paraherquamide-producing organism *P. fellutanum*. The isolated sample of paraherquamide A indicated incorporation of only one of the two geminal deuterons of the CD_2-group (C-12). The lack of signals for the second deuteron of the CD_2-group at C-12 and for the CH_2D-group (C-22/C-23) suggests an unusual catabolic processing of this substrate.

The synthetic technology developed herein, also provides advanced synthetic intermediates that should be useful in the asymmetric total synthesis of 6-hydroxy-pre-paraherquamide as well as potential intermediates for the synthesis of paraherquamides E and F. Efforts along these lines are currently in progress in our laboratory and will be reported on in due course.

4. Experimental

4.1. General methods

Unless otherwise noted, materials were obtained from commercial sources and utilized without purification. All reactions requiring anhydrous conditions were performed under argon using flame-dried glassware. Tetrahydrofuran, dimethylformamide, and toluene were degassed with argon and passed through a solvent purification system (J.C Meyer of Glass Contour) containing alumina or molecular sieves. Dichloromethane was distilled from CaH_2 prior to use. Column chromatography was performed on Merck silica gel Kieselgel 60 (230–400 mesh). Mass spectra were obtained on Fisons VG Autospec. HPLC data were obtained on a Waters 600 HPLC. ^{1}H NMR, ^{13}C NMR, and NOE experiments were recorded on a Varian 300 or 400 MHz spectrometer. Chemical shifts were given in parts per million and were recorded relative to the residual solvent peak unless otherwise noted. ^{1}H NMR signals were tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant in hertz, and number of protons. When a signal was deemed 'broad' it was noted as such. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. Optical rotations were determined with a Rudolph Research Autopol III automatic polarimeter referenced to the D-line of sodium.

4.2. Acetic acid 4-formyl-2-methoxy-phenyl ester (34)

To a stirred suspension of vanillin (40.0 g, 263 mmol) in dry ethyl ether (100 mL) under argon was added slowly acetic anhydride (39.1 mL, 408 mmol) and then DMAP (10 mg). The clarified reaction mixture was stirred at room temperature for 20 h. The crystallized white solid was filtered off and washed with water (500 mL) to give 32.6 g of the title product. The aqueous mother liquid was extracted with ethyl ether (3×200 mL), the combined organic extracts were washed with water (3×250), and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo to give further 16.6 g of acetic acid 4-formyl-2-methoxy-phenyl ester **34** (49.2 g overall, 96%) as white solid.

TLC (SiO_2, hexanes/EtOAc: 3:2): R_f=0.52.

IR (thin film): 2966, 2846, 1750, 1686, 1277, 1204 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.34 (s, 3H), 3.90 (s, 3H), 7.22 (d, J=7.9 Hz, 1H), 7.48 (dd, J=9.3, 1.5 Hz, 2H), 9.94 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.2, 168.5, 152.1, 145.0, 135.4, 124.8, 123.6, 111.0, 56.2, 20.8. HRMS (FAB$^+$) calcd for $C_{10}H_{10}O_4$ (m/z) 195.0657 [M$^+$+1]; found (m/z) 195.0649.

4.3. 4-Hydroxy-3-methoxy-2-nitro-benzaldehyde (36)

To a stirred and cooled (−20 °C) fuming nitric acid (240 mL) was added slowly **34** (48.7 g, 251 mmol) over 1.5 h. The dark brown reaction mixture was stirred for 3 h at −20 °C and then poured into 2000 g of crushed ice. The yellow precipitate was filtered off and washed with cold water (2000 mL). The resulting solid was dissolved in a solution of NaOH (15 g) in water (300 mL) and the dark red solution stirred for 20 min. The solution was acidified (pH=1) with diluted hydrochloric acid (4 N, 250 mL), the precipitate filtered off, washed with water, and dried in vacuo to give **36** (37.4 g, 76%) as yellow solid.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.13.

IR (thin film): 3269, 2957, 2844, 1669, 1578, 1508, 1353, 1321, 1268, 1193 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 3H), 6.45 (s, 1H), 7.22 (d, J=8.4 Hz, 1H), 7.67 (d, J=8.6 Hz, 1H), 9.81 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): 188.2, 159.0, 141.3, 130.8, 123.2, 120.8, 62.6. HRMS (FAB$^+$) calcd for $C_8H_7NO_5$ (m/z) 198.0402 [M$^+$+1]; found (m/z) 198.0399.

4.4. 4-Benzyloxy-3-methoxy-2-nitro-benzaldehyde (37)

To a degassed solution of **36** (37.2 g, 189 mmol) in dry DMF (350 mL) under argon were added anhydrous K_2CO_2 (39.1 g, 283 mmol) and benzyl bromide (38.7 g, 226 mmol). The reaction mixture was stirred at room temperature for 24 h, poured into water (4000 mL), the precipitate filtered off, and washed with water (2000 mL). The resulting solid was dried under vacuum and crystallized from ethyl acetate to give 4-benzyloxy-3-methoxy-2-nitro-benzaldehyde (**37**) (52.4 g, 97%) as pale yellow crystals.

TLC (SiO_2, hexanes/CH_2Cl_2: 1:4): R_f=0.53.

IR (thin film): 3111, 3031, 2949, 2887, 1525, 1367, 1019, 953 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.99 (s, 3H), 5.27 (s, 2H), 7.18 (d, J=8.6 Hz, 1H), 7.35–7.50 (m, 5H), 7.63 (d, J=8.6 Hz, 1H), 9.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 186.0, 157.7, 141.5, 135.0, 129.1 (2C), 128.9, 128.2 (2C), 127.6 (2C), 120.7, 114.5, 71.7, 62.5. HRMS (FAB$^+$) calcd for $C_{15}H_{13}NO_5$ (m/z) 288.0872 [M$^+$+1]; found (m/z) 288.0866.

4.5. 1-Benzyloxy-2-methoxy-3-nitro-4-(2-nitro-vinyl)-benzene (38)

To a cooled (0 °C) solution of **37** (40.0 g, 139 mmol) in a mixture of DMF (100 mL), isopropanol (150 mL), and *N*-methylmorpholine (140 mL) were added KF (4.05 g, 69.7 mmol) and nitromethane (25.5 g, 418 mmol). The reaction mixture was stirred at room temperature for 48 h. The solvent was removed in vacuo and the resulting oil partitioned between water (2000 mL) and ethyl ether (500 mL). The layers were separated and the aqueous layer extracted with further ethyl ether (2×500 mL). The combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the solid residue was re-dissolved in acetic anhydride (190 mL). Sodium acetate (4.00 g) was added and the resulting suspension stirred at room temperature under argon for 2 days. The reaction mixture was poured into ice (2000 g) and the resulting slurry was stirred for 2 h until a fine suspension emerged. The precipitate was filtered off and washed with water (2000 mL). The crude material was dried under vacuum and crystallized from ethyl acetate/hexane to give 19.5 g of the title compound. The remaining mother liquid was concentrated in vacuo and purified by flash chromatography (silica, 6:4 hexane/methylene dichloride then 5:5 hexane/methylene dichloride) to yield further 13.6 g of **38** (33.1 g overall yield, 72%) as a pale yellow solid.

TLC (SiO_2, hexanes/CH_2Cl_2: 1:4): R_f=0.33.

IR (thin film): 3111, 3031, 2949, 2887, 1525, 1367, 1019, 953 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.00 (s, 3H), 5.25 (s, 2H), 7.12 (d, J=8.8 Hz, 1H), 7.33 (d, J=8.8 Hz, 1H), 7.35–7.50 (m, 6H), 7.81 (d, J=13.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 155.5, 142.1, 138.5, 135.1, 131.6, 129.1 (2C), 128.9 (2C), 127.5 (2C), 123.9, 115.6, 115.1, 71.7, 62.5. HRMS (FAB$^+$) calcd for $C_{16}H_{14}N_2O_6$ (m/z) 331.0930 [M$^+$+1]; found (m/z) 331.0942.

4.6. 6-Benzyloxy-7-methoxy-1*H*-indole (39)

1-Benzyloxy-2-methoxy-3-nitro-4-(2-nitro-vinyl)-benzene (**38**) (10.8 g, 32.6 mmol), silica gel (70 g, 40–63 μm), and reduced iron powder (31 g) were suspended in a degassed mixture of glacial acetic acid (167 mL) and toluene (300 mL) under argon. The reaction

mixture was heated under reflux for 45 min (pre-heated heating cap) with efficient stirring. The fast cooled mixture (ice bath) was filtered through a pad of silica and the solid material was washed with ethyl ether (300 mL). Water (500 mL) was added and solid $NaHCO_3$ was carefully added in small portions until the aqueous phase pH=8.5. The organic phase was separated, the aqueous phase was extracted with ethyl ether (300 mL), and the combined organic phases were filtered through a pad of Na_2SO_4. The solvent was removed in vacuo to yield **39** (7.79 g, 92%) as a yellow solid of sufficient purity.

TLC (SiO_2, hexanes/Et_2O: 1:1): R_f=0.40.

IR (thin film): 3428, 3063, 3031, 2934, 2864, 1504, 1443, 1378, 1293, 1228, 734 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 4.06 (s, 3H), 5.19 (s, 2H), 6.50 (dd, J=3.3, 2.2 Hz, 1H), 6.90 (d, J=8.4 Hz, 1H), 7.16 (dd, J=3.1, 2.2 Hz, 1H), 7.27 (dd, J=8.4, 0.5 Hz, 1H), 7.30–7.45 (m, 3H), 7.51 (d, J=7.3 Hz, 2H), 8.30 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.1, 137.9, 135.6, 130.6, 128.7 (2C), 128.0, 127.7 (2C), 125.0, 124.1, 115.6, 111.2, 102.9, 73.1, 61.2. HRMS (FAB^+) calcd for $C_{16}H_{15}NO_2$ (m/z) 254.1181 [M^++1]; found (m/z) 254.1177.

4.7. (6-Benzyloxy-7-methoxy-1*H*-indol-3-ylmethyl)-dimethylamine (40)

To a cooled (0 °C) and stirred solution of 37% aqueous formaldehyde (4.47 mL, 0.060 mmol) and 40% aqueous dimethylamine (7.54 mL, 0.060 mmol) in a mixture of glacial acetic acid (184 mL) and ethanol (106 mL) was added a solution of **39** (6.08 g, 0.024 mmol) in ethanol (76 mL) and the reaction mixture stirred for 15 min at 0 °C and then at room temperature for 15 h. The mixture was diluted with water (300 mL) and made strongly basic with a solution of NaOH (160 g) in water (1500 mL) while cooling in an ice/water bath. The aqueous solution was extracted with ethyl acetate (4×300 mL) and the combined organic extracts were dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by flash chromatography (silica, 6:4 hexane/ethyl acetate then 4:6 hexane/ethyl acetate) to yield **40** (7.26 g, 97%) as a yellow solid.

TLC (SiO_2, hexanes/EtOAc: 1:1): R_f=0.60.

IR (thin film): 3467, 3367, 3063, 3030, 2936, 2858, 1508, 1454, 1347, 1267, 1229, 1027 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 2.29 (s, 6H), 3.59 (s, 2H), 4.04 (3s, 3H), 5.17 (s, 2H), 6.90 (d, J=8.8 Hz, 1H), 7.08 (d, J=1.6 Hz, 1H), 7.20–7.45 (m, 4H), 7.49 (d, J=7.3 Hz, 2H), 8.21 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.2, 137.9, 135.4, 130.9, 128.6 (2C), 127.9, 127.7 (2C), 125.2, 123.6, 114.3, 113.7, 110.7, 73.0, 61.1, 54.8, 45.5 (2C). HRMS (FAB^+) calcd for $C_{19}H_{22}N_2O_2$ (m/z) 311.1760 [M^++1]; found (m/z) 311.1768.

4.8. 2-(Benzhydrylidene-amino)-3-(6-benzyloxy-7-methoxy-1*H*-indol-3-yl)-propionic acid ethyl ester (41)

To a suspension of **40** (100 mg, 0.322 mmol) and (benzhydrylidene-amino)-acetic acid ethyl ester (85.9 mg, 0.322 mmol) in degassed and dry acetonitrile (2.5 mL) under argon was added tri-*n*-butylphosphane (19.5 mg, 0.096 mmol), and the reaction mixture was refluxed under argon for 6 h. After cooling to room temperature, the solvent was removed in vacuo and the crude product was purified by flash silica gel column chromatography to yield **41** (141 mg, 82%) as an oil.

TLC (SiO_2, hexanes/EtOAc: 3:2): R_f=0.41.

IR (thin film): 3368, 3060, 2977, 2932, 2864, 1733, 1623, 1576, 1508, 1228, 1027 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.24 (t, J=7.0 Hz, 3H), 3.20 (1/2 ABX, J=8.8 Hz, J_{AB}=14.3 Hz, 1H), 3.40 (1/2 ABX, J=4.4 Hz, J_{AB}=13.9 Hz, 1H), 4.01 (s, 3H), 4.10–4.25 (m, 2H), 4.34 (dd, J=4.8, 8.8 Hz, 1H), 5.15 (s, 2H), 6.61 (d, J=6.6 Hz, 2H), 6.71 (d, J=8.8 Hz, 1H), 6.85 (d, J=8.4 Hz, 1H), 6.90 (d, J=2.2 Hz, 1H), 7.13 (t, J=7.7 Hz, 2H), 7.22 (d, J=7.5 Hz, 1H), 7.30–7.45 (m, 6H), 7.49 (d, J=8.5 Hz, 2H), 7.59 (d, J=7.6 Hz, 2H), 8.00 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.3, 146.0, 139.6, 138.0, 136.1, 135.4, 130.6, 130.4, 129.0, 128.7 (2C), 128.3 (2C), 128.2 (2C), 128.1 (2C), 128.0 (2C), 127.7 (2C), 127.7 (2C), 125.0, 122.9, 114.0, 112.6, 110.5, 73.0, 66.2, 61.2, 61.1, 29.5, 14.4. HRMS (FAB^+) calcd for $C_{34}H_{32}N_2O_4$ (m/z) 533.2440 [M^++1]; found (m/z) 533.2431.

4.9. 2-Amino-3-(6-benzyloxy-7-methoxy-1*H*-indol-3-yl)-propionic acid ethyl ester (42)

To a solution of **41** (1.00 g, 1.88 mmol) in THF (5 mL) was added 1 N HCl solution (18.8 mL, 18.8 mmol) and the reaction mixture was stirred for 4 h at room temperature. THF was removed in vacuo and the aqueous solution was extracted with diethyl ether (2×20 mL). The aqueous phase was basified to pH=8.5 with $NaHCO_3$ and extracted with methylene chloride (3×20 mL). Concentration under reduced pressure gave **42** (699 mg, >99%) as an oil.

TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.32.

IR (thin film): 3367, 3165, 3065, 2980, 2933, 2865, 1732, 1629, 1510, 1452, 1195, 1027 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.25 (t, J=7.3 Hz, 3H), 1.83 (br s, 2H), 3.01 (1/2 ABX, J=7.7 Hz, J_{AB}=14.3 Hz, 1H), 3.22 (1H, 1/2 ABX, J=4.8 Hz, J_{AB}=14.3 Hz, 1H), 3.80 (dd, J=4.4 Hz, 7.3 Hz, 1H), 4.03 (s, 3H), 4.17 (q, J=7.3 Hz, 2H), 5.17 (s, 2H), 6.89 (d, J=8.4 Hz, 1H), 7.01 (d, J=2.2 Hz, 1H), 7.23 (d, J=8.4 Hz, 1H), 7.30–7.45 (m, 3H), 7.49 (d, J=7.3 Hz, 2H), 8.24 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 146.3, 137.9, 135.5, 131.1, 128.7 (2C), 128.0 (2C), 127.7 (2C), 124.8, 122.9, 113.8, 111.7, 110.7, 73.0, 61.1, 55.1, 31.0, 14.4. HRMS (FAB^+) calcd for $C_{21}H_{24}N_2O_4$ (m/z) 369.1814 [M^++1]; found (m/z) 369.1816.

4.10. 3-(6-Benzyloxy-7-methoxy-1*H*-indol-3-yl)-2-*tert*-butoxycarbonylamino-propionic acid ethyl ester (43)

To a degassed solution of **42** (686 mg, 1.86 mmol) in dioxane (10 mL) was added solid di-*tert*-butyl dicarbonate (448 mg, 2.05 mmol) followed by degassed aqueous 0.5 M NaOH solution (3.73 mL, 1.86 mmol) and the reaction mixture was stirred for 14 h at room temperature. The solvent was removed under reduced pressure, the residue taken up in water (30 mL), and the suspension acidified with 10% KHSO4 to pH 2. The mixture was extracted with ethyl acetate (3×50 mL), the combined organic phases dried over anhydrous Na_2SO_4, and the solvent removed under reduced pressure to yield **43** (863 mg, >99%).

TLC (SiO_2, hexanes/EtOAc: 3:2): R_f=0.34.

IR (thin film): 3359, 3304, 2971, 2929, 2829, 1735, 1691, 1520, 1229, 1159, 1024 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) (rotamers) δ 1.21 (t, J=7.3 Hz, 3H), 1.33, 1.43 (2s, 9H), 3.23 (d, J=5.5 Hz, 2H), 4.03 (s, 3H), 4.13 (q, J=7.3 Hz, 2H), 4.40, 4.61 (2dd, J=5.6, 7.7 Hz, 1H), 5.09 (d, J=8.1 Hz, 1H), 5.17 (s, 2H), 6.88 (d, J=8.4 Hz, 1H), 6.95 (d, J=1.8 Hz, 1H), 7.17 (d, J=8.4 Hz, 1H), 7.30–7.45 (m, 3H), 7.49 (d, J=7.7 Hz, 2H), 8.16 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.5, 155.4, 146.3, 137.9, 135.5, 130.9, 128.7 (2C), 128.0 (2C), 127.7 (2C), 125.1, 122.6, 113.9, 110.9, 79.9, 73.0, 61.5, 61.2, 54.4, 28.5 (3C), 28.3, 14.3. HRMS (FAB^+) calcd for $C_{26}H_{32}N_2O_6$ (m/z) 468.2260; found (m/z) 468.2243.

4.11. 3-(6-Benzyloxy-7-methoxy-1*H*-indol-3-yl)-2-*tert*-butoxycarbonylamino-propionic acid (44)

To a solution of **43** (852 mg, 1.82 mmol) in a degassed mixture of THF and water (60 mL, 2:1) was added solid LiOH (218 mg, 9.10 mmol) at room temperature under argon. The reaction mixture was stirred at room temperature for 6 h and the solvent removed under reduced pressure. The resulting slurry was taken in water (50 mL), acidified with 10% KHSO4 to pH 2, and extracted with methylene dichloride (3×50 mL) and ethyl acetate

(1×50 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to afford **44** (805 mg, >99%).

TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.20.

IR (thin film): 3341, 2977, 2933, 1700, 1509, 1256, 1229, 1067, 1027 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) (rotamers) δ 1.31, 1.45 (2s, 9H), 3.08, 3.29 (2s, 2H), 4.01 (s, 3H), 4.47, 4.71 (2s, 1H), 5.16 (s, 2H), 6.85 (s, 1H), 6.88 (d, J=8.8 Hz, 1H), 7.23 (d, J=8.8 Hz, 1H), 7.30–7.45 (m, 3H), 7.48 (d, J=7.3 Hz, 2H), 8.58 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.5, 157.9, 147.2, 139.5, 137.0, 132.2, 129.6 (2C), 128.9, 128.9, 126.8, 124.5, 114.9, 111.9, 111.4, 80.6, 74.2, 61.5, 56.2, 29.0 (2C), 28.8, 28.5. HRMS (FAB^+) calcd for $C_{24}H_{28}N_2O_6$ (m/z) 440.1947; found (m/z) 440.1948.

4.12. 1-[3-(6-Benzyloxy-7-methoxy-1*H*-indol-3-yl)-2-*tert*-butoxycarbonylamino-propionyl]-2-[4-(*tert*-butyl-dimethyl-silanyloxy)-3-methyl-but-2-enyl]-3-methyl-pyrrolidine-2-carboxylic acid ethyl ester (45 *syn*/*anti*)

To a solution of BOP (402 mg, 951 μmol), **44** (419 mg, 951 μmol), and **32** (307 mg, 864 μmol) in dry CH_2Cl_2 (1.5 mL) was added DIEA (223 mg, 1.73 mmol) at room temperature and the reaction mixture was stirred for 44 h. The solvent was removed in vacuo and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 3:7 then 2:8 then 0:10) to yield **45** as a mixture of diastereomers (474 mg, 71%) as an oil.

TLC (SiO_2, hexanes/EtOAc: 3:2): R_f=0.32.

IR (thin film): 3330, 2955, 2931, 2852, 1726, 1711, 1643, 1445, 1365, 1250, 1170, 1068, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) (mixture of two diastereomers and two rotamers) δ 0.04 (s, 6H), 0.89 (s, 9H), 0.89–0.98 (m, 3H), 1.20–1.32 (m, 3H), 1.37, 1.39 (2s, 9H), 1.62 (s, 3H), 1.50–1.65 (m, 1H), 1.68–1.82 (m, 1H), 2.10–2.30 (m, 1H), 2.65 (dd, J=8.0, 15.2 Hz, 1H), 2.86–3.46 (m, 5H), 3.85–4.00 (m, 2H), 4.02 (s, 3H), 4.06–4.28 (m, 2H), 4.62–4.74, 4.82–4.92 (2 m, 1H), 5.17 (s, 2H), 5.24 (t, J=9.2 Hz, 1H), 6.88 (t, J=8.4 Hz, 1H), 6.95, 7.14 (2s, 1H), 7.24–7.42 (m, 4H), 7.49 (d, J=7.0 Hz, 2H), 8.14, 8.37 (2br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): (mixture of two diastereomers and two rotamers) δ 171.9, 170.8, 170.4, 155.3, 155.2, 146.1, 146.0, 138.7, 138.7, 137.8, 137.8, 135.4, 135.4, 130.7, 130.7, 128.6, 128.6, 127.9, 127.9, 127.6, 127.6, 125.1, 125.0, 123.1, 123.1, 122.9, 122.9, 119.7, 119.6, 118.9, 118.9, 113.8, 111.2, 111.1, 110.7, 110.5, 79.5, 79.5, 73.0, 72.9, 72.2, 72.0, 69.0, 61.2, 61.0, 60.9, 52.6, 52.5, 48.4, 47.8, 40.1, 39.8, 31.5, 31.4, 29.6, 29.5, 8.8, 28.6, 26.2, 26.2, 18.7, 14.7, 14.6, 14.4, 14.2, 14.2, −4.85, −4.91. HRMS (FAB^+) calcd for $C_{43}H_{63}N_3O_8Si$ (m/z) 777.4385; found (m/z) 777.4399.

4.13. 3-(6-Benzyloxy-7-methoxy-1*H*-indol-3-ylmethyl)-8a-[4-(*tert*-butyl-dimethyl-silanyloxy)-3-methyl-but-2-enyl]-8-methyl-hexahydro-pyrrolo[1,2-*a*]pyrazine-1,4-dione (46a and 46b)

To a solution of **45** (457 mg, 588 μmol) in dry CH_2Cl_2 (7 mL) was added 2,6-lutidine (173 mg, 1.62 mmol) followed by TMSOTf (261 mg, 1.18 mmol) and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was quenched with satd aqueous solution of NH_4Cl (10 mL), stirred for 45 min, and 10% aqueous $NaHCO_3$ solution (20 mL) was added. The organic phase was separated and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over anhydrous Na_2SO_4, the solvent was removed in vacuo, and the residue was dissolved in dry toluene and 2-hydroxypyridine (53.5 mg, 562 μmol) was added. The reaction mixture was heated under reflux for 12 days. The solvent was removed in vacuo and the residue was purified by flash column chromatography (silica, CH_2Cl_2/MeOH: 100:0 then 98:2 then 96:4) to yield **46b** (116 mg, 49%) as an oil and **46a** (78 mg, 34%) as an oil.

Compound **46a**: TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.48.

$[\alpha]_D^{25}$ −77.0 (c 0.40, $CHCl_3$). IR (thin film): 3275, 3064, 2957, 2929, 2855, 1653, 1436, 1323, 1253, 1068, 1029, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.03 (s, 6H), 0.88 (s, 9H), 0.82–0.88 (m, 3H), 1.66 (s, 3H), 1.55–1.70 (m, 2H), 2.15–2.40 (m, 2H), 2.40–2.55 (m, 1H), 2.74 (1/2 ADX, J=8.9 Hz, J_{AB}=14.6 Hz, 1H), 2.95 (1/2 ABX, J=11.5 Hz, J_{AB}=14.9 Hz, 1H), 3.45 (1/2 ABX, J=2.9 Hz, J_{AB}=12.5 Hz, 1H), 3.56 (1/2 ABX, J=3.3 Hz, J_{AB}=14.6 Hz, 1H), 4.03 (s, 3H), 4.09 (s, 2H), 4.19 (dt, J=3.2, 11.3 Hz, 1H), 5.17 (s, 2H), 5.52 (t, J=8.2 Hz, 1H), 5.75 (d, J=2.1 Hz, 1H), 6.90 (d, J=8.9 Hz, 1H), 7.01 (d, J=2.1 Hz, 1H), 7.24 (d, J=8.4 Hz, 1H), 7.30–7.45 (m, 3H), 7.49 (d, J=7.6 Hz, 2H), 8.23 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 165.4, 146.4, 139.7, 137.6, 135.5, 131.3, 128.6, 127.9, 127.6, 123.8, 123.4, 117.2, 113.8, 110.9, 110.7, 72.9, 72.4, 68.3, 61.2, 57.3, 43.2, 41.0, 35.6, 32.6, 27.5, 26.2, 26.2, 18.7, 16.9, 14.0, −4.98, −5.07. HRMS (FAB^+) calcd for $C_{36}H_{49}N_3O_5Si$ (m/z) 631.3442; found (m/z) 631.3442.

Compound **46b**: TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.27.

$[\alpha]_D^{25}$ +64.6 (c 0.57, $CHCl_3$). IR (thin film): 3272, 3064, 2955, 2929, 2855, 1676, 1655, 1446, 1256, 1069, 1029, 838 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.02, 0.04 (2s, 6H), 0.87 (s, 9H), 0.82–0.88 (m, 3H), 1.55 (s, 3H), 1.56–1.68 (m, 2H), 2.18–2.34 (m, 1H), 2.36–2.48 (m, 2H), 2.63 (1/2 ADX, J=7.7 Hz, J_{AB}=13.9 Hz, 1H), 2.88 (1/2 ABX, J=10.2 Hz, J_{AB}=14.7 Hz, 1H), 3.52 (1/2 ABX, J_{AB}=11.5 Hz, 1H), 3.61 (1/2 ABX, J=3.3 Hz, J_{AB}=14.7 Hz, 1H), 3.78–3.88 (m, 1H), 3.93 (s, 2H), 4.03 (s, 3H), 4.28 (dd, J=3.3, 9.9 Hz, 1H), 5.17 (s, 2H), 5.40 (t, J=7.7 Hz, 1H), 5.73 (s, 1H), 6.88 (d, J=8.4 Hz, 1H), 7.01 (s, 1H), 7.19 (d, J=8.4 Hz, 1H), 7.30–7.44 (m, 3H), 7.46 (d, J=7.7 Hz, 2H), 8.21 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 165.3, 146.3, 140.1, 137.6, 135.4, 131.3, 128.6 (2C), 127.9, 127.6 (2C), 124.1, 123.5, 116.3, 113.8, 110.8, 110.3, 72.9, 72.7, 68.0, 61.1, 55.1, 43.1, 40.4, 36.4, 29.2, 27.8, 26.1 (3C), 18.6, 16.3, 13.8, −5.03, −5.07. HRMS (FAB^+) calcd for $C_{36}H_{49}N_3O_5Si$ (m/z) 631.3442; found (m/z) 631.3437.

4.14. *syn*-3*S*-(6-Benzyloxy-7-methoxy-1*H*-indol-3-ylmethyl)-8a*R*-[4-(*tert*-butyl-dimethyl-silanyloxy)-3-methyl-but-2*E*-enyl]-1-methoxy-8*S*-methyl-6,7,8,8a-tetrahydro-3*H*-pyrrolo[1,2-*a*]pyrazin-4-one (47a and 47b)

To a solution of **46a** and **46b** (2.36 g, 3.73 mmol) in dry CH_2Cl_2 (100 mL) was added Cs_2CO_3 (24.3 g, 74.7 mmol) and the reaction mixture was stirred for 30 min at room temperature. Me_3OBF_4 (2.76 g, 18.7 mmol) was added in one portion and the stirring continued for 20 h. The reaction mixture was poured into satd $NaHCO_3$ solution (150 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3) to yield **47a** (1.16 g, 48%) and **47b** (560 mg, 23%) both as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:4): R_f=0.30 (*syn*).

TLC (SiO_2, hexanes/EtOAc: 1:4): R_f=0.55 (*anti*).

Compound **47a**: $[\alpha]_D^{25}$ +8.33 (c 0.42, $CHCl_3$). IR (thin film): 3287, 2947, 2929, 2855, 1692, 1645, 1447, 1253, 1069, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.73 (d, J=6.8 Hz, 3H), 0.87 (s, 9H), 1.45–1.60 (m, 1H), 1.51 (s, 3H), 1.82 (dd, J=7.1, 16.0 Hz, 1H), 2.00–2.20 (m, 2H), 2.20–2.40 (m, 1H), 2.98 (dd, J=8.0, 14.6 Hz, 1H), 3.27 (1/2 ABX, J_{AB}=11.9 Hz, 1H), 3.40 (1/2 ABX, J=4.2 Hz, J_{AB}=14.1 Hz, 1H), 3.65 (s, 3H), 3.80–3.20 (m, 3H), 3.98 (s, 3H), 4.38 (dd, J=3.0, 8.4 Hz, 1H), 5.15 (s, 2H), 5.29 (t, J=7.8 Hz, 1H), 6.83 (d, J=8.2 Hz, 1H), 7.06 (d, J=1.5 Hz, 1H), 7.30–7.45 (m, 4H), 7.47 (d, J=8.1 Hz, 2H), 8.20 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4, 159.7, 145.9, 138.2, 137.9, 135.4, 130.6, 128.5 (2C), 127.8, 127.6 (2C), 125.4, 122.7, 118.0, 114.7, 113.7, 110.6, 73.1, 70.3, 68.2, 62.8, 61.1, 52.8, 42.0, 39.6, 35.0, 32.0, 27.8, 26.2 (3C), 18.6, 16.9, 13.7, −5.06, −5.09. HRMS (FAB^+) calcd for $C_{37}H_{51}N_3O_5Si$ (m/z) 646.3654 [M^++1]; found (m/z) 646.3654.

Compound **47b**: $[\alpha]_D^{25}$ +97.6 (*c* 0.52, $CHCl_3$). IR (thin film): 3291, 2953, 2929, 2855, 1698, 1635, 1452, 1252, 1068, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ −0.12 (d, *J*=7.0 Hz, 3H), 0.05 (s, 6H), 0.90 (s, 9H), 1.25–1.35 (m, 1H), 1.53 (s, 3H), 2.00–2.15 (m, 2H), 2.23 (1/2 ADX, *J*=8.5 Hz, J_{AB}=14.9 Hz, 1H), 2.33 (1/2 ABX, *J*=8.2 Hz, J_{AB}=14.9 Hz, 1H), 3.20–3.40 (m, 2H), 3.49 (1/2 ABX, *J*=5.1 Hz, J_{AB}=14.7 Hz, 1H), 3.62 (s, 3H), 3.55–3.70 (m, 1H), 3.95 (s, 2H), 3.96 (s, 3H), 4.26 (t, *J*=4.4 Hz, 1H), 5.15 (s, 2H), 5.21 (dt, *J*=1.5, 8.2 Hz, 1H), 6.80 (d, *J*=8.2 Hz, 1H), 6.97 (d, *J*=2.1 Hz, 1H), 7.30–7.45 (m, 4H), 7.46 (d, *J*=7.5 Hz, 2H), 7.98 (br s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.6, 159.1, 145.5, 139.3, 137.9, 135.1, 130.2, 128.5 (2C), 127.8, 127.7 (2C), 126.1, 123.2, 117.0, 115.6, 113.0, 110.2, 73.1, 70.5, 68.2, 61.3, 61.1, 52.6, 41.6, 39.7, 35.3, 29.3, 28.3, 26.2 (3C), 18.6, 15.0, 13.7, −5.05 (2C). HRMS (FAB^+) calcd for $C_{37}H_{51}N_3O_5Si$ (*m*/*z*) 645.3598; found (*m*/*z*) 645.3601.

4.15. *syn*-6-Benzyloxy-3-{8a*R*-[4-(*tert*-butyl-dimethyl-silanyloxy)-3-methyl-but-2*E*-enyl]-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*S*-ylmethyl}-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (48a)

To a solution of **47a** (1.09 g, 1.69 mmol) in dry CH_2Cl_2 (15 mL) was added triethyl amine (188 mg, 1.86 mmol) followed by DMAP (206 mg, 1.69 mmol) and the reaction mixture was stirred for 10 min at room temperature. A solution of Boc_2O (1.10 g, 5.06 mmol) in dry CH_2Cl_2 (5 mL) was added and the stirring continued for 72 h. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6) to yield **48a** (990 mg, 79%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.30.

$[\alpha]_D^{25}$ −17.8 (*c* 0.46, $CHCl_3$). IR (thin film): 2950, 2931, 2855, 1753, 1695, 1652, 1436, 1354, 1257, 1157, 1043, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ −0.04, −0.03 (2s, 6H), 0.76 (d, *J*=6.4 Hz, 3H), 0.84 (s, 9H), 1.56 (s, 3H), 1.64 (s, 9H), 2.05–2.25 (m, 2H), 2.30–2.45 (m, 2H), 2.75 (dd, *J*=10.1, 14.6 Hz, 1H), 3.25–3.45 (m, 2H), 3.64 (s, 3H), 3.92 (s, 3H), 3.98 (s, 2H), 3.90–4.20 (m, 1H), 4.31 (dd, *J*=3.0, 8.9 Hz, 1H), 5.17 (s, 2H), 5.36 (t, *J*=7.8 Hz, 1H), 6.94 (d, *J*=8.2 Hz, 1H), 7.19 (d, *J*=8.9 Hz, 1H), 7.30–7.45 (m, 3H), 7.48 (d, *J*=8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.1, 159.5, 150.0, 148.8, 139.5, 138.8, 137.8, 129.2, 129.0, 128.5 (2C), 127.8, 127.6 (2C), 126.3, 117.5, 117.4, 114.7, 113.8, 82.8, 73.4, 70.6, 68.0, 62.1, 61.5, 52.8, 42.0, 40.1, 34.8, 31.7, 28.3 (3C), 27.9, 26.1 (3C), 18.6, 17.0, 13.7, −5.13, −5.18. HRMS (FAB^+) calcd for $C_{42}H_{59}N_3O_7Si$ (*m*/*z*) 746.4201 [M^++1]; found (*m*/*z*) 746.4187.

4.16. *anti*-6-Benzyloxy-3-{8a*R*-[4-(*tert*-butyl-dimethyl-silanyloxy)-3-methyl-but-2*E*-enyl]-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*R*-ylmethyl}-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (48b)

To a solution of **47b** (1.54 g, 2.38 mmol) in dry CH_2Cl_2 (20 mL) was added triethyl amine (265 mg, 2.62 mmol) followed by DMAP (291 mg, 2.38 mmol) and the reaction mixture was stirred for 10 min at room temperature. A solution of Boc_2O (1.56 g, 7.15 mmol) in dry CH_2Cl_2 (5 mL) was added and stirring continued for 25 h. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 9:1 then 8:2 then 7:3 then 6:4) to yield **48b** (1.64 g, 92%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.65.

$[\alpha]_D^{25}$ +5.95 (*c* 0.42, $CHCl_3$). IR (thin film): 2933, 2856, 1753, 1695, 1652, 1436, 1352, 1235, 1157, 1043, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 6H), 0.09 (d, *J*=6.3 Hz, 3H), 0.86 (s, 9H), 1.25–1.40 (m, 1H), 1.49 (s, 3H), 1.59 (s, 9H), 2.00–2.17 (m, 2H), 2.22 (1/2 ADX, *J*=8.4 Hz, J_{AB}=14.2 Hz, 1H), 2.33 (1/2 ABX, *J*=8.3 Hz, J_{AB}=14.6 Hz, 1H), 3.22 (1/2 ABX, *J*=4.1 Hz, J_{AB}=14.7 Hz, 1H), 3.33 (1/2 ABX, *J*=5.1 Hz, J_{AB}=14.7 Hz, 1H), 3.57 (s, 3H), 3.55–3.70 (m, 1H), 3.85 (s, 3H), 3.90 (s, 2H), 4.21 (t, *J*=4.4 Hz, 1H), 5.13 (s, 2H), 5.19 (t, *J*=8.2 Hz, 1H), 6.90 (d, *J*=8.8 Hz, 1H), 7.20–7.40 (m, 4H), 7.44 (d, *J*=7.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.5, 159.5, 149.6, 148.8, 139.4, 139.1, 137.9, 129.7, 128.7 (2C), 128.5, 127.7, 127.7 (2C), 126.7, 116.7, 116.7, 115.5, 113.5, 82.7, 73.3, 70.7, 68.1, 61.3, 60.5, 52.6, 41.7, 39.7, 35.3, 28.5, 28.4 (3C), 26.2 (3C), 18.6, 15.6, 13.7, −5.07 (2C). HRMS (FAB^+) calcd for $C_{42}H_{59}N_3O_7Si$ (*m*/*z*) 746.4201 [M^++1]; found (*m*/*z*) 746.4202.

4.17. *syn*-6-Benzyloxy-3-[8a*R*-(4-hydroxy-3-methyl-but-2*E*-enyl)-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*S*-ylmethyl]-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (49a)

To a solution of **48a** (980 mg, 1.31 mmol) in dry THF (13 mL) was added a 1 M solution of TBAF (4.33 mL, 4.33 mmol) in THF and the reaction mixture was stirred for 24 h at room temperature. Most of the solvent was removed in vacuo and satd $NaHCO_3$ solution (50 mL) was added, and then the reaction mixture was extracted with ethyl acetate (3×30 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, methylene dichloride/methanol: 10:0 then 98:2 then 96:4) to yield **49a** (780 mg, 92%) as a foam.

TLC (SiO_2, CH_2Cl_2/MeOH: 95:5): R_f=0.35.

$[\alpha]_D^{25}$ −11.3 (*c* 0.39, $CHCl_3$). IR (thin film): 3406, 2974, 2942, 1751, 1695, 1635, 1456, 1354, 1261, 1157, 1026 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 0.74 (d, *J*=6.7 Hz, 3H), 1.59 (s, 3H), 1.63 (s, 9H), 1.50–1.70 (m, 2H), 1.75–1.85 (br s, 1H), 2.00–2.20 (m, 2H), 2.25–2.40 (m, 2H), 2.82 (dd, *J*=9.9, 15.1 Hz, 1H), 3.27 (1/2 ADX, *J*=3.0 Hz, J_{AB}=13.9 Hz), 3.35 (1/2 ADX, *J*=3.9 Hz, J_{AB}=15.6 Hz), 3.64 (s, 3H), 3.91 (s, 3H), 3.95 (s, 2H), 3.90–4.10 (m, 1H), 4.34 (dd, *J*=3.9, 8.9 Hz, 1H), 5.16 (s, 2H), 5.22 (t, *J*=7.8 Hz, 1H), 6.95 (d, *J*=9.2 Hz, 1H), 7.22 (d, *J*=8.9 Hz, 1H), 7.30–7.45 (m, 3H), 7.47 (d, *J*=8.1 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.1, 159.6, 150.0, 148.9, 139.4, 138.8, 137.8, 129.1, 129.0, 128.5 (2C), 127.8, 127.6 (2C), 126.4, 118.7, 117.2, 114.6, 113.7, 83.0, 73.3, 70.5, 68.1, 62.0, 61.4, 52.8, 42.2, 40.2, 35.1, 31.5, 28.3 (3C), 27.9, 17.0, 13.9. HRMS (FAB^+) calcd for $C_{36}H_{45}N_3O_7$ (*m*/*z*) 632.3336 [M^++1]; found (*m*/*z*) 632.3304.

4.18. *anti*-6-Benzyloxy-3-[8a*R*-(4-hydroxy-3-methyl-but-2*E*-enyl)-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*R*-ylmethyl]-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (49b)

To a solution of **48b** (1.86 g, 2.49 mmol) in dry THF (25 mL) was added a 1 M solution of TBAF (8.21 mL, 8.21 mmol) in THF and the reaction mixture was stirred for 24 h at room temperature. The most of solvent was removed in vacuo and satd $NaHCO_3$ solution (100 mL) was added, and then the reaction mixture was extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, methylene dichloride/methanol: 10:0 then 98:2 then 96:4) to yield **49b** (1.47 g, 94%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.68.

$[\alpha]_D^{25}$ +66.5 (*c* 0.32, $CHCl_3$). IR (thin film): 3407, 2975, 2941, 2887, 1752, 1695, 1646, 1436, 1352, 1266, 1156, 1025 cm^{-1}. ^1H NMR

(300 MHz, $CDCl_3$) δ 0.07 (d, J=6.3 Hz, 3H), 1.25–1.45 (m, 1H), 0.86 (s, 9H), 1.59 (s, 3H), 1.62 (s, 9H), 1.92 (br s, 1H), 2.00–2.20 (m, 2H), 2.24 (1/2 ADX, J=7.9 Hz, J_{AB}=14.2 Hz, 1H), 2.37 (1/2 ABX, J=8.3 Hz, J_{AB}=14.6 Hz, 1H), 3.24 (1/2 ABX, J=4.1 Hz, J_{AB}=14.7 Hz, 1H), 3.38 (1/2 ABX, J=5.1 Hz, J_{AB}=14.7 Hz, 1H), 3.20–3.40 (m, 1H), 3.59 (s, 3H), 3.55–3.70 (m, 1H), 3.87 (s, 3H), 3.95 (s, 2H), 4.28 (t, J=5.1 Hz, 1H), 5.16 (s, 2H), 5.22 (t, J=8.4 Hz, 1H), 6.92 (d, J=8.8 Hz, 1H), 7.20–7.40 (m, 4H), 7.46 (d, J=7.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.5, 159.5, 149.6, 148.8, 140.1, 139.1, 137.8, 129.6, 128.6 (2C), 128.5, 127.8, 127.6 (2C), 126.7, 117.7, 116.5, 115.4, 113.4, 82.8, 73.2, 70.6, 68.2, 61.3, 60.6, 52.7, 41.7, 39.7, 35.4, 28.6, 28.3 (3C), 15.4, 13.9. HRMS (FAB^+) calcd for $C_{36}H_{45}N_3O_7$ (m/z) 631.3258; found (m/z) 631.3264.

4.19. *syn*-6-Benzyloxy-3-[8a*R*-(4-chloro-3-methyl-but-2*E*-enyl)-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*S*-ylmethyl]-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (50a)

To a solution of **49a** (770 mg, 1.22 mmol) in dry DMF (12 mL) was added 2,4,6-collidine (1.48 g, 12.2 mmol). After 10 min LiCl (258 mg, 6.09 mmol) was added, after another 10 min MsCl (419 mg, 3.66 mmol) was added at 0 °C, and the reaction mixture was stirred for 5 days at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (200 mL), and the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 6:4 then 5:5 then 4:6 then 2:8) to yield **50a** (679 mg, 86%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:4): R_f=0.37.

$[\alpha]_D^{25}$ −4.6 (c 0.32, $CHCl_3$). IR (thin film): 2975, 2942, 2894, 1751, 1695, 1652, 1436, 1353, 1266, 1236, 1156, 1026 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.75 (d, J=7.2 Hz, 3H), 1.63 (s, 9H), 1.69 (s, 3H), 1.50–1.70 (m, 2H), 1.95–2.20 (m, 2H), 2.25–2.40 (m, 2H), 2.81 (dd, J=9.2, 14.5 Hz, 1H), 3.28 (1/2 ADX, J=3.0 Hz, J_{AB}=11.9 Hz), 3.38 (1/2 ADX, J=3.9 Hz, J_{AB}=14.6 Hz), 3.66 (s, 3H), 3.91 (s, 3H), 3.95 (s, 2H), 3.90–4.20 (m, 1H), 4.37 (dd, J=3.9, 8.6 Hz, 1H), 5.17 (s, 2H), 5.34 (t, J=7.8 Hz, 1H), 6.97 (d, J=8.6 Hz, 1H), 7.22 (d, J=8.8 Hz, 1H), 7.30–7.45 (m, 3H), 7.48 (d, J=8.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 159.6, 150.2, 149.0, 139.5, 137.9, 135.8, 129.1, 129.1, 128.6 (2C), 127.9, 127.8 (2C), 126.6, 124.1, 117.0, 114.7, 113.8, 83.0, 73.3, 70.3, 61.8, 61.5, 53.0, 51.7, 42.1, 40.0, 35.4, 31.7, 28.3 (3C), 27.7, 16.9, 14.5. HRMS (FAB^+) calcd for $C_{36}H_{44}ClN_3O_6$ (m/z) 649.2919; found (m/z) 649.2932.

4.20. *anti*-6-Benzyloxy-3-[8a*R*-(4-chloro-3-methyl-but-2*E*-enyl)-1-methoxy-8*S*-methyl-4-oxo-3,4,6,7,8,8a-hexahydro-pyrrolo[1,2-*a*]pyrazin-3*R*-ylmethyl]-7-methoxy-indole-1-carboxylic acid *tert*-butyl ester (50b)

To a solution of **49b** (1.26 g, 1.99 mmol) in dry DMF (20 mL) was added 2,4,6-collidine (2.42 g, 19.9 mmol). After 10 min LiCl (423 mg, 9.97 mmol) was added, after another 10 min MsCl (685 mg, 5.98 mmol) was added at 0 °C, and the reaction mixture was stirred for 5 days at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (200 mL), and the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 10:0 then 9:1 then 8:2 then 7:3 then 6:4) to yield **50b** (1.12 g, 86%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:1): R_f=0.34.

$[\alpha]_D^{25}$ +65.6 (c 0.82, $CHCl_3$). IR (thin film): 2974, 2941, 2894, 1752, 1695, 1650, 1436, 1351, 1266, 1235, 1157, 1026 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.06 (d, J=6.3 Hz, 3H), 1.25–1.45 (m, 1H), 0.86 (s, 9H), 1.60 (s, 3H), 1.65 (s, 9H), 2.00–2.15 (m, 2H), 2.22 (1/2 ADX, J=7.9 Hz, J_{AB}=14.2 Hz, 1H), 2.36 (1/2 ABX, J=8.3 Hz, J_{AB}=14.6 Hz, 1H), 3.24 (1/2 ABX, J=4.1 Hz, J_{AB}=14.7 Hz, 1H), 3.36 (1/2 ABX, J=5.1 Hz, J_{AB}=14.7 Hz, 1H), 3.20–3.40 (m, 1H), 3.57 (s, 3H), 3.55–3.70 (m, 1H), 3.86 (s, 3H), 3.91 (s, 2H), 4.30 (t, J=4.6 Hz, 1H), 5.13 (s, 2H), 5.30 (t, J=7.4 Hz, 1H), 6.90 (d, J=8.84 Hz, 1H), 7.20–7.40 (m, 4H), 7.44 (d, J=7.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.3, 159.1, 149.6, 148.8, 139.1, 137.8, 136.6, 129.6, 128.6 (2C), 128.4, 127.7, 127.6 (2C), 126.7, 123.2, 116.5, 115.4, 113.4, 82.7, 73.2, 70.4, 61.3, 60.5, 52.7, 51.6, 41.8, 39.8, 35.8, 28.6, 28.3 (3C), 15.4, 14.4. HRMS (FAB^+) calcd for $C_{36}H_{44}ClN_3O_6$ (m/z) 649.2919; found (m/z) 649.2933.

4.21. Preparation of compound 52

To NaH (372 mg, 9.29 mmol, 60% dispersion in mineral oil, freshly washed in pentane) was added a solution of **50a** (302 mg, 464 μmol) in dry xylenes (40 mL). This mixture was placed in pre-heated oil bath (150 °C) and stirred at reflux (135 °C internal temperature) for 60 min. The reaction mixture (red now) was cooled in an ice/water bath and was poured into satd $NaHCO_3$ solution (200 mL). After 10 min, the organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. As the ^{1}H NMR of the crude material indicated 10–15% of Boc-deprotected material, the crude reaction mixture was re-dissolved in CH_2Cl_2 (5 mL) and triethyl amine (51.7 mg, 510 μmol) followed by DMAP (56.7 mg, 464 μmol) was added and the reaction mixture was stirred for 10 min at room temperature. A solution of Boc_2O (304 mg, 1.39 mmol) in dry CH_2Cl_2 (1 mL) was added and the stirring continued for 48 h. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 9:1 then 8:2 then 7:3 then 6:4 then 5:5) to yield **52** (159 mg, 56%) as a foam. Employing the above conditions starting with **50b** (376 mg, 578 μmol) yielded **52** (273 mg, 77%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:1): R_f=0.31.

$[\alpha]_D^{25}$ +21.8 (c 0.45, $CHCl_3$). IR (thin film): 2973, 2940, 2880, 1751, 1682, 1634, 1497, 1417, 1350, 1267, 1235, 1157, 1027, 906 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (d, J=7.2 Hz, 3H), 1.35–1.45 (m, 1H), 1.63 (s, 9H), 1.66 (s, 3H), 2.05–2.20 (m, 2H), 2.20–2.35 (m, 1H), 2.50 (dd, J=5.2, 10.5 Hz, 1H), 3.09 (1/2 ADX, J_{AB}=15.9 Hz), 3.30–3.40 (m, 1H), 3.37 (1/2 ADX, J_{AB}=15.6 Hz), 3.63 (s, 3H), 3.60–3.70 (m, 1H), 3.94 (s, 3H), 4.73 (s, 1H), 4.86 (s, 1H), 5.17 (s, 2H), 6.92 (d, J=8.5 Hz, 1H), 7.30–7.40 (m, 3H), 7.43 (d, J=8.4 Hz, 1H), 7.47–7.55 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.1, 172.2, 149.6, 149.0, 144.3, 139.0, 138.0, 130.5, 128.7 (2C), 128.5, 127.8, 127.7, 127.7 (2C), 116.4, 116.4, 115.2, 113.4, 82.6, 73.4, 68.7, 66.1, 61.5, 54.3, 48.6, 43.1, 40.9, 37.9, 33.1, 28.4 (3C), 27.0, 19.8, 14.2. HRMS (FAB^+) calcd for $C_{36}H_{43}N_3O_6$ (m/z) 614.3230 [M^++1]; found (m/z) 614.3222.

4.22. Hexacycle (54)

To $Pd(TFA)_2$ (260 mg, 0.782 mmol) was added acetonitrile (3.0 mL) and the reaction mixture was stirred for 30 min at room temperature. Propylene oxide (1.42 g, 24.4 mmol) was added and the reaction mixture was stirred for 10 min at room temperature and then this solution was added to a solution of **52** (300 mg, 0.488 mmol) in acetonitrile (2.0 mL). The reaction mixture was stirred for 24 h at room temperature, and EtOH (5 mL) was added, followed by $NaBH_4$ (92.3 mg, 2.44 mmol) at room temperature. After 3 h, the black reaction mixture was filtered through Celite to remove palladium black and solvent was removed in vacuo. The residue was taken in a phosphate buffer solution (pH=7) and extracted with

EtOAc (3×100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. As the ^1H NMR of the crude material (320 mg) indicated a partly Bn-deprotected product, the crude reaction mixture was re-dissolved in DMF (5 mL) and K_2CO_3 (226 mg, 1.64 mmol) followed by BnBr (224 mg, 1.31 mmol) was added and the reaction mixture was stirred for 3 days at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 2:8) to yield **54** (280 mg, 94%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.32.

$[\alpha]_D^{25}$ +93.8 (*c* 0.45, $CHCl_3$). IR (thin film): 2963, 2931, 2874, 1743, 1683, 1629, 1501, 1444, 1322, 1246, 1154, 1058, 994 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.37 (s, 3H), 1.42 (d, *J*=7.2 Hz, 3H), 1.60 (s, 9H), 1.65–1.80 (m, 2H), 2.05–2.20 (m, 2H), 2.30–2.50 (m, 2H), 3.08 (1/2 ADX, J_{AB}=17.9 Hz, 1H), 3.20–3.35 (m, 1H), 3.61 (dt, *J*=2.4, 5.9 Hz, 1H), 3.85 (s, 3H), 3.88 (s, 3H), 3.90 (1/2 ADX, J_{AB}=16.6 Hz), 5.18 (s, 2H), 6.88 (d, *J*=8.9 Hz, 1H), 7.13 (d, *J*=8.1 Hz, 1H), 7.30–7.45 (m, 3H), 7.47 (d, *J*=7.4 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 172.5, 153.7, 147.8, 139.8, 137.7, 136.9, 131.1, 128.5 (2C), 127.8, 127.6 (2C), 124.7, 113.6, 111.7, 110.6, 84.3, 72.9, 66.5, 65.1, 60.8, 54.3, 49.2, 43.0, 41.2, 37.1, 33.1, 33.0, 28.1, 27.6 (3C), 27.0, 21.0, 14.2. HRMS (FAB^+) calcd for $C_{36}H_{43}N_3O_6$ (*m*/*z*) 613.3152; found (*m*/*z*) 613.3147.

4.23. Deuterium-labeled hexacycle (56)

To $Pd(TFA)_2$ (203 mg, 0.611 mmol) was added acetonitrile (2.0 mL) and the reaction mixture was stirred for 15 min at room temperature. Propylene oxide (1.18 g, 20.4 mmol) was added and the reaction mixture was stirred for 10 min at room temperature and then this solution was added to a solution of **52** (250 mg, 0.407 mmol) in acetonitrile (2.0 mL). The reaction mixture was stirred for 18 h at room temperature, and EtOD (4 mL) was added, followed by $NaBD_4$ (171 mg, 4.07 mmol) at room temperature. After 8 h, the black reaction mixture was filtered through Celite to remove palladium black and the solvent was removed in vacuo. The residue was taken in satd $NaHCO_3$ solution (100 mL), and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. As the ^1H NMR of the crude material indicated a partly Bn-deprotected product, the crude reaction mixture was re-dissolved in DMF (4 mL) and K_2CO_3 (169 mg, 1.22 mmol) followed by BnBr (167 mg, 0.98 mmol) was added and the reaction mixture was stirred for 2 days at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL), the organic phase was separated, and the aqueous phase extracted with ethyl acetate (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 2:8) to yield **56** (157 mg, 63%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 2:3): R_f=0.34.

$[\alpha]_D^{25}$ +117.3 (*c* 0.15, $CHCl_3$). IR (thin film): 2973, 2936, 2877, 1742, 1683, 1634, 1505, 1456, 1324, 1245, 1152, 1056 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.36 (d, *J*=8.1 Hz, 2H), 1.42 (d, *J*=7.2 Hz, 3H), 1.56 (s, 9H), 1.55–1.65 (m, 2H), 1.65–1.70 (m, 2H), 2.05–2.15 (m, 2H), 2.29 (dd, *J*=5.2, 11.1 Hz), 2.30–2.45 (m, 1H), 3.00 (1/2 ADX, J_{AB}=16.4 Hz, 1H), 3.20–3.35 (m, 1H), 3.61 (dt, *J*=2.2, 7.9 Hz, 1H), 3.78 (s, 3H), 3.88 (s, 3H), 3.89 (1/2 ADX, J_{AB}=16.2 Hz), 5.18 (s, 2H), 6.88 (d, *J*=8.6 Hz, 1H), 7.13 (d, *J*=8.5 Hz, 1H), 7.32 (t, *J*=7.5 Hz, 1H), 7.39 (t, *J*=7.1 Hz, 2H), 7.47 (d, *J*=6.8 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.8, 172.7, 153.9, 148.0, 140.0, 137.9, 137.0, 131.3, 128.7 (2C), 128.0, 127.7 (2C), 124.8, 113.7, 111.8, 110.8, 84.4, 72.9, 66.5, 65.2, 60.9, 54.4, 49.2, 43.0, 41.3, 37.1, 33.1, 33.0, 28.1, 27.6 (3C), 20.0, 14.2. HRMS (FAB^+) calcd for $C_{36}H_{42}DN_3O_6$ (*m*/*z*) 614.3215; found (*m*/*z*) 614.3212.

4.24. Diketopiperazine (59)

To compound **54** (280 mg, 0.456 mmol) in THF (50 mL) was added aqueous HCl solution (4.55 mL, 0.1 N) at 0 °C and the reaction mixture was stirred for 80 min at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL) and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. The crude reaction product was dissolved in toluene (20 mL), 2-hydroxypyridine (47.6 mg, 0.501 mmol) was added, and the reaction mixture was heated under reflux for 3.5 h. The solvent was removed in vacuo and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 2:8) to yield **59** (222 mg, 81%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:4): R_f=0.40.

$[\alpha]_D^{25}$ +97.8 (*c* 0.5, $CHCl_3$). IR (thin film): 3221, 2970, 2933, 2874, 1744, 1692, 1502, 1444, 1329, 1247, 1056 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.27 (s, 3H), 1.39 (s, 3H), 1.54 (d, *J*=7.2 Hz, 3H), 1.60 (s, 9H), 1.80–1.95 (m, 2H), 2.05–2.20 (m, 1H), 2.25–2.35 (m, 1H), 2.42 (1/2 ADX, *J*=11.1 Hz, J_{AB}=13.9 Hz), 2.54 (1/2 ADX, J_{AB}=16.8 Hz), 2.59 (1/2 ADX, *J*=4.1 Hz, J_{AB}=11.2 Hz), 3.25–3.35 (m, 1H), 3.60–3.70 (m, 1H), 3.74 (1/2 ADX, J_{AB}=15.8 Hz), 3.89 (s, 3H), 6.02 (s, 1H), 6.90 (d, *J*=8.5 Hz, 1H), 7.06 (d, *J*=8.2 Hz, 1H), 7.30–7.45 (m, 3H), 7.47 (d, *J*=7.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 169.6, 153.4, 148.0, 139.8, 137.6, 136.9, 131.1, 128.6 (2C), 127.9, 127.6 (2C), 124.0, 113.3, 111.8, 108.3, 84.7, 72.8, 67.1, 60.9, 59.5, 51.1, 43.5, 41.9, 36.7, 32.8, 31.8, 27.7, 26.7 (3C), 25.1, 20.6, 13.4. HRMS (FAB^+) calcd for $C_{35}H_{41}N_3O_6$ (*m*/*z*) 599.2995; found (*m*/*z*) 599.3005.

4.25. Diketopiperazine (60)

To compound **56** (160 mg, 0.260 mmol) in THF (24 mL) was added aqueous HCl solution (2.60 mL, 0.1 N) at 0 °C and the reaction mixture was stirred for 85 min at room temperature. The reaction mixture was poured into satd $NaHCO_3$ solution (100 mL) and extracted with EtOAc (3×100 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. The crude reaction product was dissolved in toluene (5 mL), 2-HO-py (27.2 mg, 0.286 mmol) was added, and the reaction mixture was heated under reflux for 5 h. The solvent was removed in vacuo and the residue was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 2:8) to yield **60** (135 mg, 86%) as a foam.

TLC (SiO_2, hexanes/EtOAc: 1:4): R_f=0.41.

$[\alpha]_D^{25}$ +104 (*c* 0.55, $CHCl_3$). IR (thin film): 3228, 2973, 2934, 2877, 1744, 1692, 1502, 1455, 1369, 1246, 1153, 1055 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (s, 3H), 1.38 (d, *J*=5.9 Hz, 2H), 1.54 (d, *J*=7.1 Hz, 3H), 1.60 (s, 9H), 1.80–1.95 (m, 2H), 2.05–2.20 (m, 1H), 2.25–2.35 (m, 1H), 2.42 (1/2 ADX, *J*=10.4 Hz, J_{AB}=13.5 Hz), 2.54 (1/2 ADX, J_{AB}=15.8 Hz), 2.60 (1/2 ADX, *J*=4.4 Hz, J_{AB}=10.3 Hz), 3.25–3.35 (m, 1H), 3.60–3.70 (m, 1H), 3.73 (1/2 ADX, J_{AB}=15.6 Hz, 1H), 3.89 (s, 3H), 5.17 (s, 2H), 5.97 (s, 1H), 6.90 (d, *J*=8.5 Hz, 1H), 7.06 (d, *J*=8.2 Hz, 1H), 7.30–7.45 (m, 3H), 7.47 (d, *J*=6.8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.0, 169.6, 153.4, 148.0, 139.8, 137.6, 136.9, 131.1, 128.6 (2C), 127.9, 127.6 (2C), 124.0, 113.3, 111.9, 108.3, 84.7, 72.9, 67.2, 60.9, 59.5, 51.1, 43.5, 41.9, 36.6, 32.8, 31.8, 27.7 (3C), 26.7, 25.1, 20.6, 13.4. HRMS (FAB^+) calcd for $C_{35}H_{40}DN_3O_6$ (*m*/*z*) 600.3058; found (*m*/*z*) 600.3070.

4.26. Deuterium-labeled monoketopiperzine (62)

To a suspension of $LiAlD_4$ (2.77 g, 66.0 mmol) in dry heptanes was added triisobutylaluminum (11.9 g, 60.6 mmol) at room temperature under argon. The reaction mixture was heated at

100 °C for 6 h. After cooling to room temperature, the resulting suspension was filtered and the insoluble gray solid was washed with heptanes. The solvent was removed in vacuo and the liquid residue was re-dissolved in dry toluene to give 1.3 M DIBAL-D solution in toluene. Compound **60** (342 mg, 0.569 mmol) was dissolved in toluene (11.0 mL) and cooled to −78 °C. At this temperature DIBAL-D solution in toluene (1.75 mL, 1.3 M) was added dropwise. The reaction mixture was allowed to warm to room temperature slowly and was stirred for 4 days at room temperature. Satd $NaHCO_3$ solution (100 mL), followed by satd Na–K-tartrate solution (100 mL) was added and the reaction mixture was stirred for 1 h. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×200 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. The residue (mixture of 70% product and 30% substrate, 0.35 g) was re-dissolved in toluene (11.0 mL) and cooled to −78 °C. At this temperature, DIBAL-D solution in toluene (1.33 mL, 1.3 M) was added dropwise. The reaction mixture was allowed to warm to room temperature slowly and was stirred for 4 days at room temperature. Additional DIBAL-D solution in toluene (0.3 mL, 1.3 M) was added dropwise, and the reaction mixture was stirred for 2 days at room temperature. Satd $NaHCO_3$ solution (100 mL), followed by satd Na–K-tartrate solution (100 mL) was added, and the reaction mixture was stirred for 1 h. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3×200 mL). The combined organic phases were dried over Na_2SO_4 and the solvent was removed in vacuo. The residue (0.35 g) was purified by flash column chromatography (silica, hexane/ethyl acetate: 8:2 then 7:3 then 6:4 then 5:5 then 4:6 then 2:8) and (silica, CH_2Cl_2/MeOH: 10:0 then 99:1 then 98:2 then 97:3 then 96:4 then 95:5) to give **62** together with 15% of the Boc-deprotected analogue as a non-separable mixture: (189 mg, 52%).

TLC (SiO_2, hexanes/EtOAc: 3:7): R_f=0.41.

$[\alpha]_D^{25}$ +41.2 (*c* 0.50, $CHCl_3$). IR (thin film): 3196, 2947, 2930, 2870, 1746, 1674, 1501, 1455, 1369, 1245, 1153, 1055 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, *J*=10.3 Hz, 2H), 1.42 (d, *J*=6.4 Hz, 3H), 1.56 (s, 3H), 1.62 (s, 9H), 1.60–1.75 (m, 2H), 1.85–1.95 (m, 1H), 1.95–2.10 (m, 2H), 2.10–2.25 (m, 2H), 2.30 (dt, *J*=4.5, 13.9 Hz, 1H), 2.74 (1/2 ADX, J_{AB}=17.4 Hz, 1H), 2.81 (1/2 ADX, J_{AB}=16.1 Hz), 3.15–3.30 (m, 1H), 3.92 (s, 3H), 5.17 (s, 2H), 6.09 (s, 1H), 6.90 (d, *J*=7.5 Hz, 1H), 7.03 (d, *J*=8.4 Hz, 1H), 7.33 (t, *J*=7.0 Hz, 1H), 7.39 (t, *J*=7.3 Hz, 2H), 7.47 (d, *J*=7.2 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.4, 153.6, 148.0, 141.4, 137.7, 137.0, 131.4, 128.7 (2C), 128.0, 127.6 (2C), 124.1, 112.7, 111.9, 108.1, 84.8, 72.8, 65.6, 60.8, 55.8, 53.8, 47.9, 40.5, 35.6, 30.7, 30.2, 30.0, 28.7, 28.4, 27.6 (3C), 22.0, 13.3. HRMS (FAB^+) calcd for $C_{35}H_{40}D_3N_3O_5$ (*m*/*z*) 588.3391; found (*m*/*z*) 588.3412.

4.27. Mesylate (64)

A solution of **62** (100 mg, 170 μmol) in dry ethanol (6 mL) was stirred under H_2 (1 atm) for 19 h at room temperature in the presence of Pd/C (10 wt %) (50 mg). The catalyst was removed by filtration through a pad of Celite, which was washed with ethanol (30 mL), and the solvent was removed in vacuo to yield the deprotected intermediate (85 mg, 100%) as a white solid, which was used further without purification. TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.46. To a solution of the deprotected intermediate (85 mg, 170 μmol) in CH_2Cl_2 (2.0 mL) was added pyridine (2.0 mL) followed by MsCl (195 mg, 1.70 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 12 h. The solvent was removed in vacuo and the residue taken in $NaHCO_3$/EtOAc (100 mL), the organic phase was separated and the aqueous (pH=8.5) was extracted with EtOAc (3×50 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by preparative thin layer chromatography (2 mm/silica, CH_2Cl_2/MeOH: 95:5) to give **64**, together with 15% of the Boc-deprotected analogue as a non-separable mixture: (63 mg, 64%).

TLC (SiO_2, CH_2Cl_2/MeOH: 95:5): R_f=0.38.

IR (thin film): 3354, 3195, 2933, 2873, 2841, 1748, 1673, 1494, 1447, 1370, 1249, 1153, 1023 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.39 (d, *J*=8.7 Hz, 2H), 1.41 (s, 3H), 1.57 (d, *J*=9.4 Hz, 3H), 1.59 (s, 9H), 1.50–1.65 (m, 2H), 1.70 (d, *J*=10.2 Hz, 1H), 1.85–2.10 (m, 3H), 2.10–2.25 (m, 2H), 2.22 (dt, *J*=4.4, 10.9 Hz, 1H), 2.79 (1/2 ADX, J_{AB}=15.8 Hz, 1H), 2.83 (1/2 ADX, J_{AB}=15.1 Hz), 3.17 (s, 3H), 3.15–3.30 (m, 1H), 3.93 (s, 3H), 6.71 (s, 1H), 7.12 (d, *J*=8.5 Hz, 1H), 7.16 (d, *J*=8.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.3, 152.8, 143.6, 139.0, 138.4, 130.5, 128.5, 118.2, 113.7, 108.8, 85.5, 65.6, 61.9, 55.8, 55.7, 53.9, 53.8, 47.9, 40.5, 37.4, 35.7, 30.7, 30.3, 29.9, 27.6 (3C), 21.9, 13.2. HRMS (FAB^+) calcd for $C_{29}H_{36}D_3N_3O_7S$ (*m*/*z*) 577.2775 [M^++1]; found (*m*/*z*) 577.2762.

4.28. Indoline (65)

A solution of **64** (63 mg, 109 μmol) in dry methanol (6 mL) was stirred under H_2 (1 atm) for 3 days at room temperature in the presence of $Pd(OH)_2$/C (20 wt %) (1.2 g). The catalyst was removed by filtration through a pad of Celite, which was washed with methanol (300 mL), and the solvent was removed in vacuo to yield **65** (25 mg, 47%) as a white solid.

TLC (SiO_2, CH_2Cl_2/MeOH: 95:5): R_f=0.21.

IR (thin film): 3180, 3057, 2950, 2929, 2874, 1690, 1673, 1485, 1454, 1368, 1276, 1167 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 0.48 (s, 3H), 1.02 (d, *J*=7.3 Hz, 2H), 1.29 (d, *J*=6.8 Hz, 3H), 1.25–1.35 (m, 2H), 1.37 (d, *J*=5.9, 13.2 Hz, 1H), 1.48 (s, 9H), 1.64 (dd, *J*=5.6, 11.1 Hz, 1H), 1.72 (dt, *J*=4.4, 10.4 Hz, 1H), 1.75–1.85 (m, 1H), 1.85–1.95 (m, 1H), 1.98 (1/2 ADX, J_{AB}=11.5 Hz, 1H), 2.00–2.15 (m, 3H), 2.38 (1/2 ADX, J_{AB}=14.7 Hz), 3.03 (dt, *J*=4.0, 9.7 Hz, 1H), 3.78 (t, *J*=7.4 Hz, 1H), 3.84 (s, 3H), 4.30 (d, *J*=5.5 Hz, 1H), 6.75 (d, *J*=7.2 Hz, 1H), 6.85 (d, *J*=8.4 Hz, 1H), 7.08 (t, *J*=8.3 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.1, 156.2, 151.7, 140.3, 133.7, 127.4, 114.9, 112.5, 82.0, 74.2, 67.2, 56.4, 56.1, 54.8, 47.1, 41.8, 41.4, 38.8, 31.5, 31.3, 30.9, 28.7 (3C), 17.3, 13.5. HRMS (FAB^+) calcd for $C_{28}H_{36}D_3N_3O_4$ (*m*/*z*) 484.3129; found (*m*/*z*) 484.3112.

4.29. Deprotected indoline (66)

To **65** (32 mg, 66.3 μmol) in CH_2Cl_2 (1.5 mL) was added TFA (605 mg, 5.30 mmol) at 0 °C and the reaction mixture was stirred for 3 h at 0 °C. The solvent was removed in vacuo and the residue taken in $NaHCO_3$/EtOAc (10 mL), the organic phase was separated, and the aqueous (pH=8.5) was extracted with EtOAc (3×5 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by preparative thin layer chromatography (2 mm/silica, CH_2Cl_2/MeOH: 9:1) to give **66** (15.5 mg, 98%).

TLC (SiO_2, CH_2Cl_2/MeOH: 9:1): R_f=0.52.

IR (thin film): 3283, 3179, 3049, 2945, 2930, 2874, 1668, 1490, 1463, 1369, 1261, 1117 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 0.81 (s, 3H), 0.96 (d, *J*=7.2 Hz, 2H), 1.32 (d, *J*=6.8 Hz, 3H), 1.38 (dd, *J*=7.9, 13.0 Hz, 2H), 1.63 (dd, *J*=7.9, 10.9 Hz, 1H),1.72 (dq, *J*=4.3, 10.9 Hz, 1H), 1.70–1.80 (m, 1H), 1.90–2.05 (m, 4H), 2.10–2.25 (m, 2H), 3.07 (dt, *J*=4.3, 9.2 Hz, 1H), 3.49 (dd, *J*=7.5, 8.3 Hz, 1H), 3.60 (d, *J*=8.7 Hz, 1H), 3.79 (s, 3H), 6.55–6.75 (m, 3H). ^{13}C NMR (100 MHz, CD_3OD): δ 175.4, 146.5, 141.5, 133.8, 120.3, 116.5, 111.1, 71.0, 67.8, 56.8, 56.7, 56.0, 54.3, 41.3, 41.3, 37.4, 32.7, 31.3, 30.8, 17.8, 13.6. HRMS (FAB^+) calcd for $C_{23}H_{28}D_3N_3O_2$ (*m*/*z*) 384.2605; found (*m*/*z*) 384.2636.

4.30. 7-HO-Pre-paraherquamide (27)

To **66** (15.5 mg, 40 μmol) in CH_2Cl_2 (0.5 mL) was added BBr_3 (150 μL, 1 M in CH_2Cl_2) at −78 °C and the reaction mixture was allowed to warm to room temperature slowly over 13 h. The reaction mixture was cooled to −78 °C and MeOH (0.5 mL) was

added. The reaction mixture was warmed to room temperature and the solvent was removed in vacuo. The HBr-salt of (**20**) was taken in $NaHCO_3$/EtOAc (10 mL), the organic phase was separated, and the aqueous (pH=8.5) was extracted with EtOAc (3×5 mL). The combined organic phases were dried over Na_2SO_4, the solvent was removed in vacuo, and the residue was purified by preparative thin layer chromatography (2 mm/silica, CH_2Cl_2/MeOH: 9:1) to give a mixture of **67** (15 mg, 87%) and **27** (4:1). The mixture was dissolved in EtOAc, satd $NaHCO_3$ solution was added, and the biphasic mixture was stirred under air atmosphere and at room temperature for 48 h. The organic phase was separated, the aqueous phase was extracted with EtOAc, and the combined organic phases were dried over Na_2SO_4. The solvent was removed in vacuo and the residue was purified by preparative thin layer chromatography (2 mm/silica, CH_2Cl_2/MeOH: 95:5) to give **27** (14.5 mg, >99%).

TLC (SiO_2, CH_2Cl_2/MeOH: 95:5): R_f=0.20.

$[\alpha]_D^{25}$ −26.7 (*c* 0.038, MeOH/CH_2Cl_2: 1:1). IR (thin film): 3355, 3196, 2950, 2921, 2850, 1662, 1456, 1374, 1260, 1113 cm^{-1}. ^1H NMR (400 MHz, CD_3OD/$CDCl_3$: 5:1) δ 1.36 (d, *J*=7.1 Hz, 3H), 1.41 (d, *J*=6.8 Hz, 2H), 1.42 (s, 3H), 1.74 (1/2 ADX, *J*=10.7 Hz, J_{AB}=18.8 Hz, 1H), 1.86 (dt, *J*=4.7, 10.2 Hz, 1H), 1.95–2.10 (m, 2H), 2.17 (1/2 ADX, *J*=11.9 Hz, J_{AB}=17.5 Hz, 1H), 2.10–2.25 (m, 1H), 2.29 (dt, *J*=4.9, 9.4 Hz, 1H), 2.87 (s, 2H), 3.20 (m, 1H), 6.54 (d, *J*=7.3 Hz, 1H), 6.84 (t, *J*=7.7 Hz, 1H), 6.90 (d, *J*=6.7 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD/$CDCl_3$: 5:1): δ 175.9, 143.8, 141.6, 129.9, 127.5, 120.3, 110.2, 107.0, 105.1, 66.4, 57.6, 57.5, 54.7, 47.4, 41.3, 35.1, 31.3, 30.6, 30.6, 24.4, 13.6. HRMS (FAB$^+$) calcd for $C_{22}H_{24}D_3N_3O_2$ (*m*/*z*) 369.2370 [M^++1]; found (*m*/*z*) 369.2370.

4.31. Feeding experiment with 7-HO-pre-paraherquamide in *P. fellutanum*

Each malt extract agar slant was streaked with *P. fellutanum* spores (50 μL of a 10% glycerol/water suspension). Malt extract agar (500 mL) is prepared by combining malt extract (10 g), dextrose (10 g), peptone (0.5 g), and agar (10 g) in 500 mL distilled water and heating gently until all solids dissolve. The slants were then sterilized in an autoclave at 250 °C for 45 min. After inoculation, the slants were incubated in the dark at 25 °C for 7–14 days. The spores of two slants were shaken into a flask containing 400 mL sterile corn steep liquor broth. One liter corn steep liquor broth is prepared by combining corn steep liquor (22 g) with dextrose (40.0 g) and heating gently until all solids dissolve. The broth was then sterilized in an autoclave at 250 °C for 45 min. The inoculated flask was incubated in the dark at 25 °C for 7 days. The broth was decanted from the flask leaving a disk of *P. fellutanum*. A sterile solution of 7-HO-pre-paraherquamide (**27**) in trace element solution (7 mg in 100 mL) was added by dripping it down the side of the flask and under the mycelia using a sterile syringe. A detergent had to be used to dissolve the water-insoluble proposed precursor. The compound was dissolved in a 10% solution of absolute ethanol/chloroform. TWEEN 80 (0.1) was added to the dissolved precursor. The solvent was removed in vacuo, trace element solution was added, and the solution filtered through sterile antibacterial filter. The flask was left in the incubator another 14 days. The flasks were swirled daily to allow even distribution of the labeled compound. The aqueous solution containing the precursor was decanted off. Methylene chloride (1–2 mL) was added to the solution, which was then covered and stored at 4 °C. The mycelia were pureed with 500 mL 1:1 MeOH/$CHCl_3$ in an *Oster* blender. The contents of the blender were poured into a 2 L Erlenmeyer flask. The blender was rinsed three times with 1:1 MeOH/$CHCl_3$ and the suspension of mycelia cells was diluted to a volume of 1.2 L with 1:1 MeOH/$CHCl_3$. The mycelia cells were placed in a shaker at room temperature for 24 h. Celite (30 g) was added to the flask. The suspension was filtered through *Whatman* #2 paper. The filtrate was stored at 4 °C. The residual Celite and mycelia was suspended in 1.2 L 1:1 MeOH/$CHCl_3$ and shaken at room temperature for an additional 24 h. The Celite and mycelia suspension was filtered through *Whatman* #2 paper. The organic solvent from the combined filtrates was concentrated by rotary evaporation. The aqueous solution from the feeding experiment was combined with aqueous residue and the mixture was acidified to pH 3 with glacial acetic acid. The acidic solution was filtered through a pad of Celite and extracted with ethyl acetate (400 mL×3). The aqueous layer was made basic, pH 12, by the addition of 10% aqueous Na_2CO_3. The aqueous layer was extracted with ethyl acetate (450 mL×4). The combined organic extracts from the basic extraction were washed with brine (800 mL), dried (Na_2SO_4), filtered, and concentrated by rotary evaporation (34 mg). The paraherquamide A was purified by preparative thin layer chromatography with 95:5 CH_2Cl_2/MeOH to give 14.5 mg of the natural product.

4.32. Examination of percent deuterium incorporation in the isolated sample of paraherquamide A by ^2H NMR

Examination of percent deuterium incorporation in the isolated sample of paraherquamide A (14 mg) was accomplished by comparing the signal intensities from the partially enriched sample with the signal intensity from the solvent, $CHCl_3$, that was spiked with a known amount of 99.8% $CDCl_3$. The spiked solvent was made up as follows. $CDCl_3$ (100 μL, 99.8%) was added to 4.90 mL of $CHCl_3$. This produced a solvent with 2% by volume of $CDCl_3$. MeOH (5 mL) and 90 mL $CHCl_3$ were added. This produced a solvent with 0.1% by volume of $CDCl_3$. Several small aliquots of this thoroughly mixed solvent were added to a round bottom flask containing the sample, swirled around and then decanted into a new Wilmad 535pp NMR tube. The sample volume in the NMR tube was brought finally to 675 μL by adding the mixed solvent dropwise until the volume mark on the NMR tube was reached. The NMR signal averaging transients were acquired at a rate that allowed the steady-state $CDCl_3$ magnetization to recover to 91% of its value. This was done to acquire more transients for better signal averaging of the deuterium that are incorporated in the very dilute sample since their T1s are almost certainly much smaller than those of $CDCl_3$. This means that the signal intensity from the $CDCl_3$ is slightly underestimated and should be corrected by this factor before comparing directly with that from the deuterium incorporated in the sample. There are several sources of error in this method. It is estimated that the error in the volumetric measurements is about 1% overall. It is assumed that T1s for the sample are much shorter than that for the $CDCl_3$ necessitating that the sample was dissolved although it is assumed that nearly all of the sample was dissolved in the mixed solvent. The $CHCl_3$ used as the base for the mixed solvent has an unknown amount of deuterium incorporated although it is likely no more than what is naturally abundant at a level of 0.012%. This could be tested by NMR but is a very small source of error. The spectral integrations plotted assume 20,000 ppm by volume, with no correction for T1 applied, of deuterium for the $CDCl_3$ signal in the mixed solvent. The values that are finally reported will need to first take the steady-state value of the $CDCl_3$ integral into account. The NMR data was acquired on Varian Inova-500 NMR equipped with a 5 mm broadband tunable probe. The probe frequency was set to 76.78 MHz and the lock channel was determined. The sample was shimmed in the magnet with the lock off (free drift) and by using 1H PFG shimming. The NMR signal was acquired with the following parameters: 25 °C, 8.8 μs RF pulse (90° tip angle), 1500 Hz spectral window, 2K complex data points, total recycle time of 2.8 s, Gaussian smoothing, spectral complex transform 16K. The main magnet field drifts naturally less than 0.1 Hz per hour. Total acquisition time: 40 h.

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