Published in final edited form as:
J Org Chem. 2008 November 21; 73(22): 9065–9074. doi:10.1021/jo801956w.

Synthesis of 7-Epineoptilocaulin, Mirabilin B, and Isoptilocaulin. A Unified Biosynthetic Proposal for the Ptilocaulin and Batzelladine Alkaloids. Synthesis and Structure Revision of Netamines E and G

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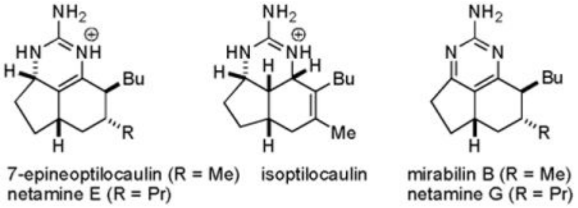
Abstract



Addition of guanidine to a 6-methylhexahydroindenone in MeOH at reflux afforded 7-epineoptilocaulin. A similar reaction with a 6-propylhexahydroindenone afforded netamine E. MnO_2 oxidation of 7-epineoptilocaulin and netamine E afforded mirabilin B and netamine G, respectively. The netamines have the side chains trans, not cis as was initially proposed. A unified biosynthetic scheme for the batzelladines and ptilocaulin family is proposed. Conjugate addition of guanidine to a bis enone followed by an intramolecular Michael reaction of the enolate to the other enone, aldol reaction, dehydration and enamine formation will lead to a tricyclic intermediate at the dehydroptilocaulin oxidation state. 1,4-Hydride addition will lead to ptilocaulin or 7-epineoptilocaulin depending on which face the hydride adds to. 1,2-Hydride addition will lead to isoptilocaulin. The key tricyclic intermediate was prepared from a tetrahydroindenone and guanidine and reduced with $NaBH_4$ to give a mixture rich in ptilocaulin and isoptilocaulin.

Introduction

Ptilocaulin (**1**) and isoptilocaulin (**4**) were isolated by Rinehart from two different samples of *Ptilocaulis* aff. *P. spiculifer* collected at slightly different locations and depths in 1981 (see Chart 1).[1a] The structures were initially determined by NMR experiments. An X-ray structure determination of ptilocaulin (**1**) established that H-5a and H-8b are cis despite the 10 Hz coupling constant between them. Isoptilocaulin (**4**) also has the same stereochemistry at C-5a,[1b] although it was also originally depicted as the trans isomer on the basis of the large coupling constant.[1a] Both ptilocaulin (**1**) and isoptilocaulin (**4**) have been re-isolated from this and related sponges along with the more complex batzelladines, ptilomycalins, and crambescidins.[2] Since 1995, nineteen additional members of this family of tricyclic guanidine alkaloids have been isolated from a variety of other marine sponges. Braekman isolated 8b-hydroptilocaulin (**15**) from *Monanchora arbuscula*.[3] Capon isolated mirabilins A-F (**8**, **9**, **10**, **20**, **21**, and **2**) as their *N*-acetyl derivatives from *Arenochalina mirabilis* in 1996.[4] Patil and Freyer isolated

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mirabilin B (**9**), 8α-hydroxymirabilin B (**13**), and compounds **5** and **6** from *Batzella* sp. in 1997.[5] Compound **5**, which they called 8a,8b-dehydroptilocaulin is epimeric to ptilocaulin at C-7. It is at the same oxidation state as ptilocaulin so the dehydro prefix is incorrect. We have renamed this compound as 7-epineoptilcaulin, with the neo prefix used to indicate a double bond between C-8a and C-8b because the iso prefix was already used to indicate a double bond between C-7 and C-8. Using this system, compound **6** is named 8α-hydroxy-7-epineoptilocaulin. Capon isolated mirabilin G (**3**) from a *Clathria* sp. in 2001.[6] Hamann isolated mirabilin B (**9**) and both 8α- and 8β-hydroxymirabilins (**13** and **14**) from *Monanchora unguifera*.[7] Finally, Kashman isolated netamines A-G (**16-19, 7**, and **11**-**12**) from *Biemna laboutei* in 2006.[8]

Most of these compounds have 7-epi stereochemistry; only mirabilin F (**2**), mirabilin G (**3**), and 8b-hydroxyptilocaulin (**15**) have the same C-7 stereochemistry as ptilocaulin (**1**). Mirabilins A (**8**), B (**9**), and C (**10**), and netamines F (**11**) and G (**12**) are more highly oxidized with an aromatic 2-aminopyrimidine ring. Netamines A-D (**16**-**19**) are more highly reduced with a tetrahydro-2-aminopyrimidine ring. Compounds **6, 15, 20** and **21** are hydroxylated at the dihydro-2-aminopyrimidine oxidation state, while **13** and **14** are hydroxylated at the aromatic 2-aminopyrimidine oxidation state. In the aromatic series or with the double bond between C-8a and C-8b as in the neoptilcaulins, two stereoisomers are possible at C-8. The side chains are trans in 7-epineoptilocaulin (**5**), mirabilin B (**9**), and mirabilin C (**10**). The side chains were assigned to be cis in mirabilin A (**8**) on the basis of a 7-9% NOE enhancement between H-7 and H-8.[4] The cis stereochemistry in all the netamines (**16**-**19**, **7**, **11**, **12**) was also assigned based on an NOE between H-7 and H-8.[8]

Several members of this family have significant biological activity. Ptilocaulin (**1**) and isoptilocaulin (**4**) have ID_{50}s of 0.39 and 1.4 μg/mL, respectively, against L1210 leukemia cells and **1** has a minimum inhibitory concentration of 3.9 μg/mL against *Streptococcus pyogenes*.[1] Ptilocaulin (**1**) showed a fairly broad spectrum of *in vitro* activity against colon and mammary adenocarcinomas, melanomas, leukemias, transformed fibroblasts and normal lymphoid cells with IC_{50}s of 0.05 to >10 μg/mL.[9] Mirabilin B (**9**) exhibited antifungal activity against *Cryptococcus neoformans* with an IC_{50} value of 7.0 μg/mL and antiprotozoal activity against *Leishmania donovani* with an IC_{50} value of 17 μg/mL. A mixture of **13** and **14** is active against the malaria parasite *Plasmodium falciparum* with an IC_{50} value of 3.8 μg/mL.[7]

We reported the first synthesis of ptilocaulin (**1**) in 1983 by the reaction of bicyclic enone **22** with guanidine in benzene at reflux (see Scheme 1).[10] Under equilibrating conditions, conjugate addition occurs from the desired α-face and enamine formation affords the desired double bond between C-8 and C-8a. Addition of nitric acid affords ptilocaulin nitrate identical to the natural product.[10] Roush prepared amino ketone **24** and converted it to a guanidine which led to a mixture of tricyclic products. Heating the mixture in benzene at reflux, as in the reaction of **22** with guanidine, resulted in the equilibration of this tricyclic mixture to give ptilocaulin (**1**).[11] Several more recent ptilocaulin syntheses prepared **22** by alternate methods and completed the synthesis by reaction with guanidine as we described.[12-16]

Although minor stereo- or double bond position isomers are also formed from the reaction of **22** with guanidine, they were not characterized with the exception of **23**. Schmalz found that **23** co-crystallized with ptilocaulin and the structure was determined crystallographically.[16] It is noteworthy that **23** is formed by the presumably kinetic addition of guanidine to the less hindered top convex face of **22**, whereas ptilocaulin is formed by the presumably thermodynamic addition of guanidine to the more hindered bottom face of **22**.

In 1986, Hassner prepared a mixture of **22**, which he converted to ptilocaulin, and **25**, which was treated with guanidine in benzene at reflux to give a compound he characterized as 7-

epiptilocaulin (**26**) (see Scheme 2).[13] The ^{1}H and ^{13}C NMR spectral data of **26** in $CDCl_3$ are very similar to those reported in 1997 for 7-epineoptilocaulin (**5**) in CD_3OD.[5] This suggests that the position of the double bond of Hassner's product **26** has been incorrectly assigned. However, no definitive conclusions can be drawn because the spectra were recorded in different solvents. Heating **25** with guanidine neat at >130 °C resulted in disproportionation to give a mixture of an aromatic compound that is probably mirabilin B (**9**) and a saturated compound **27** as only one of 16 possible stereoisomers.[13b] Unfortunately, the ^{1}H and ^{13}C NMR spectral data of Hassner's aromatic compound were recorded in $CDCl_3$ in 1991, whereas those of natural mirabilin B (**9**) were recorded in CD_3OD in 1997.[5]

Results and Discussion

We decided to develop a practical, efficient, and stereospecific route to optically pure **25** and to unambiguously determine whether reaction with guanidine afforded 7-epiptilocaulin (**26**) or 7-epineoptilocaulin (**5**). We also wanted to explore routes to the aromatic, tetrahydroaromatic and hydroxylated members of this growing family of tricyclic alkaloids. Ptilocaulin (**1**) and mirabilins F (**2**) and G (**3**) with a 7β-methyl group have the double bond between C-8 and C-8a, whereas 7-epineoptilocaulin (**5**) and netamine E (**7**) with a 7α-alkyl group have the double bond between C-8a and C-8b. We therefore started by considering the effect of the stereochemistry of the 7-alkyl group on the stability of the three possible tricyclic enamines. MMX calculations were performed on ureas, which are better paramaterized than guanidinium cations, with side chains truncated to methyl groups to minimize rotational freedom.[17]

The ptilocaulin-like urea **28** is calculated to be 0.11 kcal/mol less stable than the *trans*-neoptilocaulin-like urea **29** (see Chart 2). However the 7-epiptilocaulin-like urea **31** is calculated to be 2.45 kcal/mol less stable than the *trans*-7-epineoptilocaulin-like urea **32**. Both *cis*-neoptilocaulin isomers **30** and **33** are less stable than the corresponding *trans*-neoptilocaulin isomers **29** and **32**. The 0.11 kcal/mol calculated energy difference between **28** and **29** is so small that it is not surprising that **28** is experimentally observed to be more stable. However changing the C-7 methyl group stereochemistry makes the *trans*-7-epineoptilocaulin-like urea **32** much more stable than the 7-epiptilocaulin-like urea **31** suggesting that the change in double bond position in the 7-epi series results from thermodynamic rather than enzymatic control.

We developed a six-step route to indenone **25** starting from 2*E*,6*Z*-nonadienal (**34**), which contains a double bond that serves as a latent aldehyde and is readily available because of its valuable fragrance properties (see Scheme 3).[18] Michael addition[19] of *tert*-butyl 3-oxooctanoate (**35**)[20] to dienal **34** followed by aldol reaction with 0.25 equiv of KO-*t*-Bu in *t*-BuOH at 0 °C to reflux afforded a keto ester that was hydrolyzed and decarboxylated with TsOH in toluene at 80 °C to provide cyclohexenone **36** in 77% yield. Addition of excess MeLi and $CeCl_3$[21] afforded the tertiary allylic alcohol, which was treated with PCC in CH_2Cl_2 containing 0.8 equiv of NaOAc to give 79% of cyclohexenone **37**.[22] House reported that Birch reduction of 3,5-dimethyl-2-cyclohexenone afforded *cis*-3,5-dimethylcyclohexanone containing 6-12% of the trans isomer.[23] We therefore expected that Birch reduction of **37** would control the stereochemistry at C-3. Reduction of **37** with Li in NH_3 containing 9 equiv of *t*-BuOH at −33 °C afforded **38** in 73% yield as an irrelevant 10:1 mixture of stereoisomers at the butyl group. Minor amounts of the two isomers with a β-methyl group at C-3 are probably also formed, but analysis is complicated by the mixture of isomers at C-2. Ozonolysis of the side chain double bond followed by reduction of the ozonide with Ph_3P provided aldehyde **39** in 93% yield. Intramolecular aldol reaction was most effectively carried out in a 30:1 mixture of DME/6 M aqueous HCl at 55 °C under microwave irradiation for 10 min to give **25** in 76% yield as a 4:1 mixture of isomers.[10] Poorer results were obtained in THF, which has been widely used in intramolecular aldol reactions that form **22**.[15,24]

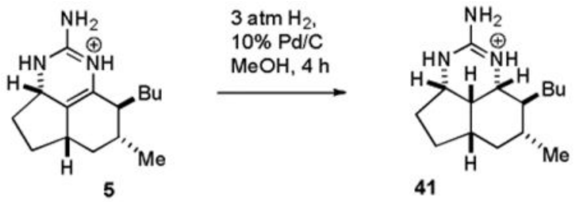
Treatment of **25** with guanidine in benzene at reflux gave mixtures containing 7-epineoptilocaulin (**5**). Better results were obtained with guanidine in MeOH[25] at 85 °C in a sealed tube for 24 h followed by workup with 1% nitric acid, which led to 7-epineoptilocaulin (**5**) in ~50% yield, 3a,7-bisepiptilocaulin (**40**) in 10% yield, and minor isomers with absorptions for H-3a at δ 4.01 to 3.66 that were not characterized (see Scheme 4). The ^{1}H and ^{13}C NMR spectral data of synthetic **5** in CD_3OD are identical to those reported by Patil and Freyer[5,26] and the data in $CDCl_3$ are identical to those reported by Hassner for 7-epitilocaulin (**26**)[13] whose structure should therefore be revised to 7-epineoptilocaulin (**5**). H-3a of **5** absorbs at δ 4.25, considerably downfield from δ 3.77 of ptilocaulin (**1**), as expected for an allylic hydrogen. H-3a of a minor isomer that was not obtained in pure form absorbs at δ 3.15 (ddd, J = 11.6, 11.6, 5.2 Hz). The structure is tentatively assigned as **40**, based on the very similar chemical shift and coupling pattern for H-3a of **23** at δ 3.13 (ddd, J = 11.6, 11.6, 5.3 Hz).[16,27]

Oxidation of 7-epineoptilocaulin (**5**) with activated MnO_2[28] in CH_2Cl_2 at 55 °C in a sealed tube for 1 d afforded mirabilin B (**9**) in 80% yield. Heating **25** with guanidine neat for 4 h at 130-140 °C followed by workup with 1% HNO_3 afforded mirabilin B (**9**) in 39% yield and **27** in 31% yield as described by Hassner.[13b] The NMR spectral data of synthetic **9** in $CDCl_3$ are identical to those reported by Hassner[13b] and the data in CD_3OD are identical to those reported for natural mirabilin B[5] establishing that synthetic **9** is mirabilin B.

Remarkably, tricycle **27** is formed as one of sixteen possible stereoisomers. The spectral data for **27** are quite different from those of saturated netamines A-D (**16-19**) indicating that the stereochemistry is different. H-3a absorbs at δ 3.79 (br dd, J = 5.6, 5.6 Hz) and H-8a absorbs at δ 3.77 (br dd, J = 2.7, 2.7 Hz). H-3a, H-8a, H-8b are on the same face of the ring because a large coupling constant would be observed if either H-3a or H-8a was trans to and therefore in a diaxial orientation relative to H-8b. This restricts the sixteen possible isomers to only four. These three hydrogens can be all up or all down and the butyl group can be either cis or trans to the methyl group. Protons and carbons in $CDCl_3$ were assigned on the basis of COSY, HMQC, and HMBC experiments at 800 MHz (see Table S1 in the supporting material). A ROESY experiment showed NOEs between H-8b and both H-3a and H-8a confirming that they are all on the same face of the molecule as expected from the analysis of coupling constants. NOEs between H-8 and both H-6α and H-8b establish that all these hydrogens are on the α-face. NOEs were also observed between NH-1 at δ 7.63 and the side chain hydrogens H-1′ at δ 1.52 and 1.70 and between NH-3 at δ 8.2 and H-4β at δ 1.72 confirming the stereochemical assignment shown in Figure 1.

Compounds **27** and mirabilin B (**9**) are presumably formed by disproportionation of intermediates at the oxidation state of 7-epineoptilocaulin (**5**) as suggested by Hassner. It is quite surprising that the stereochemistry of **27** at C-3a is the opposite of that of **5** and identical to that of the minor byproduct **40**. 7-Epineoptilocaulin (**5**) is clearly formed under thermodynamic conditions. A kinetically formed intermediate with the C-3a stereochemistry of **40** must undergo reduction in the disproportionation reaction more readily than intermediates with the C-3a stereochemistry of **5**.

Kashman reported that hydrogenation of netamine E (**7**) over Pd/C afforded a compound with the same stereochemistry as netamines A-D (**16-19**).[8] We therefore hydrogenated 7-epineoptilocaulin (**5**) over Pd/C under 3 atm of H_2 for 4 h to give saturated tricyclic guanidine **41** in ~90% yield (see equation 1). As expected, the spectral data for **41** were quite different from those of the stereoisomer **27** obtained in the disproportionation reaction. We therefore assigned all the protons and carbons in **41** in $CDCl_3$ as shown in Table S1 in the supporting material on the basis of COSY, HMQC, and HMBC experiments at 800 MHz. H-3a absorbs at δ 3.89 (br dd, J = 6, 4.2 Hz) and H-8a absorbs at δ 3.54 (br dd, J = 4.9, 1.1 Hz). As in **27**, H-3a, H-8a, H-8b are on the same face of the ring because a large coupling constant would be observed if either H-3a or H-8a was trans to and therefore in a diaxial arrangement with H-8b. A ROESY experiment showed NOEs between H-8b and both H-3a and H-8a confirming that they are all on the same face of the molecule as expected from the analysis of coupling constants. The cyclohexane ring is calculated to be a boat (see Figure 2) making H-6α and H-8 3.52 Å apart so that no NOE is observed. However, the guanidinium nitrogens are observed as three separate peaks in $CDCl_3$. The NOE observed between NH-1 at δ 7.64 and H-8 at δ 1.46 confirms that side chains in **41** are trans.



(1)

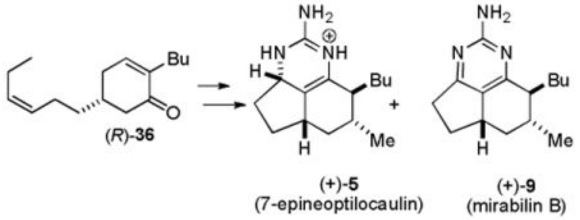
The spectral data of saturated tricyclic guanidine **41** are remarkably similar to those of netamines A-D (**16**-**19**) in which the side chains are reported to be cis rather than trans as in **41**. The spectral data of **41** are virtually identical to those of netamine C (**18**), which has a hexyl rather than a butyl group on C-8 (see Table S1 in the supporting material). The stereochemistry of netamines A (**16**), C (**18**), E (**7**), and G (**12**) was therefore reexamined and established to be trans as discussed below.

We now turned our attention to the preparation of hydroxylated members of this family. Oxidation of 7-epineoptilocaulin (**5**) with $K_3Fe(CN)_6$[29] in aqueous KOH and benzene afforded only mirabilin B (**9**). However treatment of **25** with guanidine in MeOH in a sealed tube in an 85 °C oil bath and oxidation of the crude product mixture with $K_3Fe(CN)_6$ as described above afforded mirabilin B (**9**) in 25% yield and a 1:1 mixture of 8-hydroxymirabilins B (**13** and **14**) in 5% yield (see Scheme 5). The spectral data of this 1:1 mixture are identical to those of the 1:1 mixture isolated by Hamann.[7,30] We suspect that **13** and **14** are formed by hydroxylation and oxidation of minor components of the mixture with the enamine double bond between H-8 and H-8a as in ptilocaulin, such as **40**, to give **42** and then **13** and **14**. Neither **5** nor **9** can be intermediates in the formation of **13** and **14** because oxidation of **5** affords only mirabilin B (**9**). Dioxiranes have been reported to effect benzylic hydroxylations.[31] Unfortunately treatment of **9** with dimethyldioxirane afforded no **13** and **14** and a complex mixture that appeared to contain *N*-oxides, which are known to be readily formed from 2-aminopyrimidines.[32]

Synthesis of (+)-7-epineoptliocaulin (5) and (+)-mirabilin B (9)

The syntheses described above were carried out in the racemic series because the Michael reaction of **34** and **35** afforded cyclohexenone **36** as a racemic mixture. Jørgensen recently reported an asymmetric organocatalytic protocol for such Michael reactions.[33] Reaction of **34** and **35** neat with 10 mol% catalyst **43** and then treatment with 20 mol% *p*-toluenesulfonic acid in toluene at 80 °C afforded (*R*)-**36** in 55% yield and 90% ee (see Scheme 6). The absolute stereochemistry was assigned by analogy to Jørgensen's examples.[33] Reduction of (*R*)-**36** with $NaBH_4$ and $CeCl_3$ afforded cis allylic alcohol **44** which was converted to the Mosher esters **45** and **46**. This established that the ee was 90% as observed by Jørgensen in related examples. The upfield shifts of the protons proximate to the phenyl group confirmed the assignment of absolute stereochemistry.[34]

The same series of reactions as in the racemic series afforded (+)-7-epineoptliocaulin (**5**) and (+)-mirabilin B (**9**) (see equation 2). The optical rotation for synthetic **5**, $[\alpha]_D^{25}$ + 45.9, has the same sign, but is much larger than that of natural **5**, $[\alpha]_D$ + 13.3.[5] The optical rotation for synthetic **9**, $[\alpha]_D^{25}$ + 123.6 (MeOH), has the same sign, but is much larger than that initially reported for **9**, $[\alpha]_D$ + 41.6 (MeOH).[5] However the value for synthetic **9**, $[\alpha]_D^{25}$ + 145 ($CHCl_3$), compares favorably with that of the mirabilin B (**9**) that was re-isolated in 2004, $[\alpha]_D^{22}$ + 129 ($CHCl_3$).[35] The absolute stereochemical assignment of synthetic **5** and **9** is based on the expected product of the Jørgensen asymmetric Michael reaction and analysis of the NMR spectra of the Mosher esters. Therefore the absolute stereochemistry of natural **5** and **9** is as shown.



(2)

A proposal for a unified biosynthesis of the ptilocaulin and batzelladine family of alkaloids

It is possible that ptilocaulin (**1**) and 7-epineoptilocaulin (**5**) are biosynthesized by addition of guanidine to enones **22** and **25**, respectively, as can be easily achieved in one pot in ~50% yield in the laboratory. However, it is not clear how nature could adapt this route to the biosynthesis of isoptilocaulin (**4**), which has an allylic guanidine. Furthermore, this would require the presence of two different precursor enones in the sponge. A unified biosynthetic proposal that would lead to ptilocaulin (**1**), isoptilocaulin (**4**), 7-epineoptilocaulin (**5**) and the batzelladines such as batzelladine K (**53**) is shown in Scheme 7.

Conjugate addition of guanidine to bis enone **47** will give guanidinium enolate **48**, which could undergo an intramolecular Michael addition to give cyclopentane dione **49**. There are ample precedents for tandem conjugate additions leading to *trans*,*trans*-cyclopentanes analogous to **49**. For example, addition of a thiophenoxide anion to bis enone **54a** gave **55a** in 58% yield (see Scheme 8).[36] Addition of an amide anion to bis enoate **54b** provided **55b** in 80% yield.[37] Dione **49** would have to undergo an intramolecular aldol and dehydration reaction, epimerization of H-8b, and iminium ion formation in unspecified order to give the key tricyclic intermediate **52**. There is precedent for these steps in the work of Roush who reported that addition of PMe_3 to bis enone **56** gives **57**, which undergoes an intramolecular aldol and dehydration reaction to give bicyclic dienone **58** after elimination of PMe_3.[38]

The key step in this biosynthetic scheme is the reduction of **52** which can form ptilocaulin (**1**), isoptilocaulin (**4**), and 7-epineoptilocaulin (**5**). 1,2-Reduction of **52** from the less hindered β-face will give isoptilocaulin (**4**). 1,4-Reduction from the α-face will give ptilocaulin (**1**). 1,4-Reduction from the β-face and isomerization to the more stable enamine in the 7-epi series will give 7-epineoptilocaulin (**5**).

A proton shift will convert guanidinium enolate **48** to guanidine ketone **50**. An intramolecular conjugate addition of the guanidine can form pyrrolidine **51**. Iminium ion formation and 1,2-reduction will give rise to batzelladine K (**53**). Numerous batzelladines are known with different side chains;[2a] batzelladine K (**53**) which would be formed from the same precursor as the ptilocaulin natural products was isolated in 2007.[39] Reaction of guanidine with bis enones, differing from **47** only in the alkyl side chains, followed by reduction affords batzelladines analogous to **53** in excellent yields.[40] It is therefore not straightforward to develop the reaction of guanidine with bis enones as a laboratory route to the ptilocaulin family of natural products.

Fortunately it is fairly easy to develop an alternate route to the key intermediate **52** and explore whether reduction can lead to ptilocaulin (**1**), isoptilocaulin (**4**), and 7-epineoptilocaulin (**5**). The cyclohexene double bond of **52** was easily installed simply by using enone **37** without the Birch reduction that afforded **38**. Selective ozonolysis of the more electron rich double bond of **37** to give **59** in 66% yield was easily accomplished in the presence of pyridine,[41] which also served to reduce the ozonide (see Scheme 9). A complex mixture of products was obtained in the absence of pyridine. Intramolecular aldol reaction with 30:1 DME/6 M aqueous HCl gave bicyclic dienone **60** in 70% yield. Reaction of **60** with guanidine in MeOH in a sealed tube in an 85 °C oil bath for 24 h presumably afforded tricyclic iminium ion **52**. Cooling the reaction to room temperature and addition of $NaBH_4$ afforded an inseparable 1:1 mixture of ptilocaulin (**1**) and isoptilocaulin (**4**) and various minor byproducts completing the first synthesis of isoptilocaulin (**4**) and establishing the plausibility of the proposed biosynthetic route. The presence of isoptilocaulin in this mixture was established by comparison with data for the natural product.[1b,2c] We were frustrated by our inability to separate ptilocaulin and isoptilocaulin, but note that isoptilocaulin has only been obtained pure when it has been isolated from a source that does not also produce ptilocaulin.[1,2b,2c]

Stereochemistry of the Netamines

The close similarity of the ^{1}H NMR spectra of hydrogenation product **41** and netamine C (**18**), which has a hexyl rather than a butyl side chain, suggested that the side chains in the netamines might be trans, not cis. The stereochemistry of the netamines was assigned on the basis of an NOE between H-8 and both H-7 and H-8a in the saturated netamines A-D. However, the MMX-calculated distances[17] between H-8 and both H-7 and H-8a in saturated netamines A-D (**16**-**19**) are 2.37 Å and 2.25 Å, respectively, for the cis isomer and 2.87 Å and 2.51 Å, respectively, for the trans isomer so these NOEs are inconclusive. Mirabilin A (**8**) was also assigned to have side chains cis based on an NOE between H-7 and H-8. In the aromatic series, the calculated distances[17] are 2.28 Å for the cis isomer mirabilin A (**8**) and 3.08 Å for the trans isomer mirabilin C (**10**), which was also isolated, so this assignment seems secure. The ^{1}H and ^{13}C NMR spectra of aromatic netamines F (**11**) and G (**12**) correspond closely to those of mirabilin B (**9**) and *N*-acetyl mirabilin C with trans side chains and differ considerably from *N*-acetyl mirabilin A with cis side chains (see supporting material) suggesting that these netamines also have the side chains trans.

The trans isomers of netamines E (**65**) and G (**66**) should be readily accessible by a slight modification of the route used to prepare 7-epineoptilocaulin (**5**) and mirabilin B (**9**). Addition of PrMgCl and $CeCl_3$ to (*R*)-**36** followed by PCC oxidation afforded cyclohexenone **61** in 78% yield (see Scheme 10). Birch reduction afforded **62** in 78% yield as a 7:1 mixture of isomers at C-2. This Birch reduction is not as selective as that of **37** for the required 3,5-cis isomer, probably because of steric interactions between the larger butyl and propyl substituents. We unsuccessfully explored a variety of copper hydride conjugate reductions of **61**.[42] Apparently the double bond of **61** is too hindered. Ozonolysis of **62** afforded keto aldehyde **63** in 91% yield, which was treated with HCl in DME in a microwave oven at 55 °C for 10 min to give bicyclic enone **64** in 78% yield as an 8:4:2:1 mixture of *trans*-**64**, *cis*-**64**, and the two isomers with a β-propyl group, respectively. The selectivity for the trans isomer of **64** (2:1) is much lower than for the trans isomer of **25** (4:1). MMX calculations[17] indicate that *trans*-**64** is only 0.5 kcal/mol more stable than the cis isomer, whereas *trans*-**25** is 1.2 kcal/mol more stable than the cis isomer. Bicyclic enone **64** may also contain some of the two isomers with the propyl group on the top face resulting from the less selective Birch reduction.

Heating **64** with guanidine in MeOH in a sealed tube in an 85 °C oil bath for 24 h followed by neutralization with 1% HNO_3 afforded a more complex mixture of products than the analogous reaction of **25**. Repeated careful chromatography gave **65** that was >90% pure in 38% yield. Oxidation of the crude reaction mixture with MnO_2 in CH_2Cl_2 in a sealed tube in a 55 °C oil bath for 48 h afforded **66** in 25% overall yield from **64**.

The spectral data of **66** in $CDCl_3$ vary significantly as a function of the amount of acid in solution as is well known for 2-aminopyrimidines.[43] We titrated **66** with a solution of TFA in $CDCl_3$ to obtain a complete set of partially protonated spectra. The spectrum of **66** in $CDCl_3$ containing 9% TFA matched the published spectra of netamine G very well.[8,44] At this degree of protonation, H-5 (δ 2.38) and H-8 (δ 2.30) are well resolved and H-8 absorbs as a ddd, J = 8.8, 4.4, 4.4 Hz, as calculated[17] and observed in trans isomers mirabilin B (**9**) and *N*-acetyl mirabilin C (**10**).[4,5] The 8.8 Hz coupling constant between H-7 and H-8 establishes that these two hydrogens are trans. With more TFA, H-5 and H-8 appear together as a broad multiplet at δ 2.40. H-8 absorbs as a ddd, J = 6, 6, 5 Hz, as calculated,[17] in the cis isomer *N*-acetyl mirabilin A (**8**).[4] This analysis establishes that the side chains of natural netamine G are trans and the structure should therefore be revised to **66**. The rotation of synthetic netamine G (**66**), $[\alpha]_D^{25}$ + 26.3, is identical to that of natural netamine G, $[\alpha]_D^{21}$ + 27.0, establishing that the absolute stereochemistry of the natural product is as shown for **66**.

The trans stereochemistry of the side chains of **65** was established by a 1D NOESY experiment in CD_3OD with irradiation of axial H-6α at δ 0.77 which showed an NOE to both H-8 at δ 1.95 and the propyl CH_2 group at δ 1.20 and 1.60. This analysis establishes that the side chains of natural netamine E are trans and the structure should therefore be revised to **65**. The rotation of synthetic netamine E (**65**), $[\alpha]_D^{25}$ + 15.6, has the same sign as that of natural netamine E, $[\alpha]_D^{25}$ + 35.0, establishing that the absolute stereochemistry of the natural product is as shown for **65**.

The stereochemistry of the side chains of saturated tricycle **41** was assigned by analysis of the NOEs to the NH protons, which are separately observable in $CDCl_3$. We carried out a ROESY experiment at 800 MHz on a sample of natural netamine A (**16**) kindly provided by Prof. Kashman.[44] NOEs between NH-1 at δ 7.76 and H-8a at δ 3.57 and H-8 at δ 1.51, but not the hexyl group H-1′ at δ 1.27, established that H-8 is down and the hexyl group is up and therefore trans to the propyl group at C-7 (see Figure 3). The structure of netamine A should therefore be revised to **67**.

The structures of netamines E and G have been revised to **65** and **66**, respectively, on the basis of the identity of their spectra to those of the natural products. The NOE between NH-1 and H-8 of natural netamine A establishes that the side chains are trans and the structure should be revised from **16** to **67**. The similarity of the spectra of **41** and netamine C, which has a hexyl rather than a butyl side chain, establishes that structure of netamine C should be revised to **68**. This suggests that netamines B, D and F may also have the side chains trans with a β-alkyl group at C-8.

In conclusion, we have prepared optically pure indenone **25** in six steps in 31% overall yield and converted it to 7-epineoptilocaulin (**5**, ~50%), mirabilin B (**9**, 39%), and 8-hydroxymirabilins (**13** and **14**, 5%). Optically pure indenone **64** was prepared analogously and converted to netamine E (**65**, 38%) and netamine G (**66**, 25%). These studies establish that the structures of netamines A (**67**), C (**68**), E (**65**), and G (**66**) should be revised so that the 8-alkyl group is trans to the alkyl substituent at C-7. A unified proposal for ptilocaulin, isoptilocaulin and 7-epineoptilocaulin family of alkaloids suggests that all of these natural products can be obtained by 1,2- or 1-4 reduction of tricyclic iminium salt **52**, which could be formed by addition of guanidine to bis enone **47**. Tricyclic iminium salt was generated in the laboratory by addition of guanidine to dienone **60**. Reduction with $NaBH_4$ generated a mixture rich in ptilocaulin (**1**) and isoptilocaulin (**4**) providing experimental support for this hypothesis.

Experimental Section

(±)-(3a*S*,5a*S*,7*R*,8*S*)-8-Butyl-1,3a,4,5,5a,6,7,8-octahydro-7-methylcyclopenta[*de*]quinazolin-2-amine (7-Epineoptilocaulin, 5)

A 0.18 M solution of guanidine in MeOH was prepared by adding guanidinium hydrochloride (86 mg, 0.90 mmol) to a solution of NaOMe in MeOH prepared by adding Na (21 mg, 0.90 mmol) to anhydrous MeOH (5.0 mL) under nitrogen.

Guanidine in MeOH (0.18 M, 2.5 mL, 0.45 mmol) was transferred to a resealable tube containing indenone **25** (41 mg, 0.20 mmol) in 2.5 mL of MeOH under nitrogen. The tube was sealed and heated in an 85 °C oil bath for 24 h. The mixture was cooled, quenched with 1% HNO_3 (3.0 mL, 0.33 mmol) and diluted with $CHCl_3$ (20 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to yield 82 mg of crude **5** as a brown oil. Repeated flash chromatography on silica gel (20:1 $CHCl_3$/MeOH) gave 13 mg (21%) of >95% pure **5** as a colorless oil and 23 mg (37%) of 80% pure **5** as a light yellow oil: ^1H NMR ($CDCl_3$) 8.99 (br s, 1), 8.21 (br s, 1), 7.60 (br s, 2), 4.25 (br dd, 1, J = 7.0, 6.4), 2.45-2.32 (m, 1), 2.19-2.05 (m, 1), 1.98-1.83 (m, 3), 1.82-1.51 (m, 4), 1.41-1.20 (m, 3), 1.20-1.05 (m, 2), 1.00 (d, 3, J = 6.4), 0.89 (t, 3, J = 7.2), 0.86 (ddd, 1, J = 12, 12, 12); (CD_3OD) 4.27 (br dd, 1, J = 7.0, 6.4), 2.51-2.40 (m, 1), 2.25-2.12 (m, 1), 2.04-1.92 (m, 2), 1.92-1.81 (m, 1), 1.79-1.54 (m, 4), 1.40-1.20 (m, 4), 1.20-1.10 (m, 1), 1.06 (d, 3, J = 6.8), 0.93 (t, 3, J = 7.2), 0.90 (ddd, 1, J = 12, 12, 12); ^{13}C NMR ($CDCl_3$) 153.6, 127.9, 117.1, 52.3, 42.5, 38.9, 36.6, 32.9, 32.0, 29.7, 27.0, 25.9, 23.1, 20.0, 14.2; ^{13}C NMR (CD_3OD) 154.7, 128.7, 119.5, 53.9, 44.1, 40.1, 37.8, 33.9, 33.7, 30.6, 28.4, 27.4, 24.4, 20.4, 14.6; IR (neat) 3223, 1674, 1576, 1455, 1380; HRMS (EI) calcd for $C_{15}H_{25}N_3$ (M^+) 247.2048, found 247.2050.

Our ^1H NMR (CD_3OD) data are 0.01 to 0.02 ppm downfield and our ^{13}C NMR (CD_3OD) data are shifted by up to 0.1 to 0.2 ppm from those reported.[5] The appearance of the ^1H NMR spectrum in CD_3OD corresponds well with an authentic spectrum provided by Dr. Alan J. Freyer.[26] The spectral data in ^1H NMR data in $CDCl_3$ are identical to those reported for “7-epiptilocaulin” by Hassner. The ^{13}C NMR data are shifted upfield by 0 to 0.2 ppm.[13]

The ^1H NMR spectrum of the crude mixture in $CDCl_3$ showed the presence of **40** [~10%, δ 3.15 (ddd, 1, J = 11.6, 11.6, 5.2)] and uncharacterized isomers with peaks at δ 4.01 (br dd, 1, J = 6.4, 5.6), 3.89 (ddd, 1, J = 6.8, 5.6, 1.1), and 3.82-3.66 (m).

An identical reaction with (7a*S*)-**25** afforded (5a*S*)-**5**: $[\alpha]_D^{25}$ + 45.9 (c 0.135, MeOH); [lit.[5] $[\alpha]_D$ + 13.3 (c 1.2, MeOH)].

(±)-(5a*S*,7*R*,8*S*)-8-Butyl-4,5,5a,6,7,8-hexahydro-7-methylcyclopenta[*de*]quinazolin-2-amine (Mirabilin B, 9)

A mixture of **5** (7 mg, 0.023 mmol) and activated MnO_2 (13.1 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) was stirred in a sealed tube in a 55 °C oil bath for 24 h. The mixture was cooled and filtered through Celite. The residue was washed with CH_2Cl_2 (3 × 4 mL). The combined organic layers were concentrated to yield 7 mg of crude **9** as a yellow oil. Flash chromatography on silica gel (70:1 CH_2Cl_2/MeOH) gave 5.7 mg (80%) of **9** as a light yellow oil: ^1H NMR ($CDCl_3$) 4.90 (br s, 2), 2.97-2.83 (m, 2), 2.58 (dd, 1, J = 16.4, 8.8), 2.35 (ddd, 1, J = 12.4, 7.2, 7.2), 2.20 (br ddd, 1, J = 10.2, 4.4, 4.4), 2.09-1.96 (m, 2), 1.91-1.80 (m, 1), 1.80-1.69 (m, 1), 1.59-1.45 (m, 1), 1.38-1.19 (m, 3), 1.09 (d, 3, J = 6.8), 1.16-1.05 (m, 1), 0.91 (ddd, 1, J = 11.6, 11.6, 12.4), 0.87 (t, 3, J = 6.8); (CD_3OD) 2.99-2.83 (m, 2), 2.55 (dd, 1, J = 16.4, 8.4), 2.38 (ddd, 1, J =

12.4, 7.2, 7.2), 2.19 (br dt, 1, *J* = 9.6, 4,4), 2.12-1.96 (m, 2), 1.95-1.70 (m, 2), 1.60-1.44 (m, 1), 1.38-1.19 (m, 3), 1.11 (d, 3, *J* = 6.8), 1.14-1.01 (m, 1), 0.91 (ddd, 1, *J* = 12, 12, 12), 0.88 (t, 3, *J* = 7.2); ^{13}C NMR ($CDCl_3$) 174.6, 166.1, 163.1, 126.1, 47.0, 39.7, 37.7, 34.1, 33.6, 33.1, 30.3, 27.7, 23.3, 21.0, 14.1; ^{13}C NMR (CD_3OD) 176.3, 167.6, 164.8, 126.9, 48.4, 41.0, 39.1, 35.3, 34.3, 34.3, 31.2, 28.7, 24.5, 21.4, 14.5; IR (neat) 3317, 3184, 1622, 1587, 1456, 1386; HRMS (EI) calcd for $C_{15}H_{23}N_3$ (M^+) 245.1893, found 245.1892.

The ^{1}H NMR (CD_3OD) data and ^{13}C NMR (CD_3OD) correspond to the literature data,[5] except that our ^{1}H NMR (CD_3OD) data are 0.01 to 0.02 ppm downfield and our ^{13}C NMR (CD_3OD) data are 0.1 to 0.2 ppm downfield. The ^{1}H NMR data in $CDCl_3$ are identical and the ^{13}C NMR data are within 0.1 ppm to those reported.[13b]

An identical reaction with (5a*S*)-**5** afforded (5a*S*)-**9**: $[\alpha]_D^{25}$ + 123.6 (*c* 0.09, MeOH), $[\alpha]_D^{25}$ + 145 (*c* 0.06, $CHCl_3$); [lit.[5] $[\alpha]_D$ + 41.6 (*c* 0.48, MeOH)], [lit.[35] $[\alpha]_D^{22}$ + 129 (*c* 0.1, $CHCl_3$)].

(±)-Mirabilin B (9) and (±)-(3a*R*,5a*S*,7*R*,8*S*,8a*S*,8b*R*)-8-Butyl-1,3a,4,5,5a,6,7,8,8a,8b-decahydro-7-methylcyclopenta[*de*]quinazoline-2-amine (27)

Guanidine in MeOH (0.18 M, 1.2 mL, 0.22 mmol) was transferred to a 50 mL flask containing indenone **25** (31 mg, 0.15 mmol). The MeOH was evaporated and the reaction mixture was heated in a 130-140 °C oil bath under nitrogen for 4 h. The solution was cooled, treated with benzene (15 mL) and 1% HNO_3 (2 mL), and stirred for 30 min. The organic layer was separated and the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to yield 53 mg of crude **9** and **27** as a brown oil. Flash chromatography on silica gel (70:1 CH_2Cl_2/MeOH) gave 18 mg (39%) of **9** as a yellow oil. Flushing the silica gel with MeOH gave 14 mg (31%) of **27**.

Compound **27**: ^{1}H NMR (800 MHz, $CDCl_3$) 8.20 (br s, 1), 7.63 (br s, 1), 7.32 (br s, 2), 3.79 (br dd, 1, *J* = 5.6, 5.6), 3.77 (br dd, 1, *J* = 2.7, 2,7), 2.10 (m, 1), 1.98-1.85 (m, 2), 1.78-1.65 (m, 3), 1.55-1.42 (m, 4), 1.42-1.22 (m, 2), 1.22-1.10 (m, 3), 0.96 (d, 3, *J* = 7.2), 0.94 (t, 3, *J* = 8), 0.86 (ddd, 1, *J* = 12, 12, 12); ^{13}C NMR 154.2, 51.6, 47.0, 46.2, 46.1, 39.6, 36.3, 32.4, 31.7, 29.1, 29.0, 27.4, 22.7, 20.1, 14.2; IR (neat) 3225, 3087, 1667, 1621, 1463, 1380; HRMS (EI) calcd for $C_{15}H_{27}N_3$ (M^+) 249.2205, found 249.2202.

(±)-Mirabilin B (9) and (±)-(5a*S*,7*R*,8*R*)- and (±)-(5a*S*,7*R*,8*S*)-2-Amino-8-butyl-4,5,5a,6,7,8-hexahydro-7-methylcyclopenta[*de*]quinazolin-8-ol ((±)-8α-hydroxymirabilin B, 13 and (±)-8β-hydroxymirabilin B, 14)

Crude **5**, containing isomers (47 mg) was prepared as described above from indenone **25** (33.5 mg, 0.16 mmol). This material was taken up in water (1.9 mL) and benzene (1.9 mL). KOH (17.1 mg, 0.30 mmol) and $K_3[Fe(CN)_6]$ (105 mg, 0.32 mmol) were added and the mixture was stirred at 25 °C for 16 h. The organic layer was separated and the aqueous layer was extracted with benzene (2 × 15 mL). The combined organic layers were washed with water, dried over $MgSO_4$ and concentrated to yield 26 mg of crude **9**, **13**, and **14** as a yellow oil. Flash chromatography on silica gel (70:1 ~ 20:1 CH_2Cl_2/MeOH) gave 12.5mg (25%) of **9** followed by 2.8 mg (5%) of a 1:1 mixture of **13** and **14**: ^{1}H NMR 4.96 (br s, 2), 3.05-2.83 (m, 2), 2.66 (dd, 0.5 × 1, *J* = 17.2, 8), 2.60 (dd, 0.5 × 1, *J* = 17.2, 8.4), 2.44-2.27 (m, 1), 2.27-2.16 (m, 0.5 × 1), 2.10 (ddd, 0.5 × 1, *J* = 12.4, 12.4, 4.4), 2.08-1.98 (m, 0.5 × 1), 1.97-1.81 (m, 2), 1.76 (ddd, 0.5 × 1, *J* = 12.4, 12.4, 4.4), 1.71-1.47 (m, 1), 1.40 (ddd, 0.5 × 1, *J* = 11.6, 11.6, 12.4), 1.34-1.11 (m, 3.5), 1.17 (d, 0.5 × 3, *J* = 6.8), 1.06 (d, 0.5 × 3, *J* = 6.8), 1.01-0.72 (m, 1), 0.85 (t, 0.5 × 3, *J* = 7.2), 0.82 (t, 0.5 × 3, *J* = 7.2); ^{13}C NMR (500 MHz) (**13**) 176.3, 164.7, 125.5, 75.3, 38.3, 37.2, 36.7, 35.2, 34.1, 33.5, 27.1, 23.5, 15.0, 14.0 (the peak at 163.5 was not observed); (**14**) 125.5, 74.1, 42.0, 37.8, 37.4, 36.8, 33.8, 33.3, 26.9, 23.1, 15.6, 13.8 (the peaks at 175.7, 163.7

and 163.2 were not observed); IR (neat) 3416, 3314, 3184, 1607, 1584, 1464, 1385; HRMS (EI) calcd for $C_{15}H_{23}ON_3$ (M^+) 261.1841, found 261.1842.

The ^{1}H NMR spectral data match those of an authentic sample provided by Prof. Hamann.[30] The ^{13}C NMR spectral data match the literature data,[7] except that some aromatic carbons were not observed due to the low sample concentration.

(±)-(3a*S*,5a*S*,7*R*,8*S*,8a*R*,8b*S*)-8-Butyl-1,3a,4,5,5a,6,7,8,8a,8b-decahydro-7-methylcyclopenta[*de*]quinazoline-2-amine (41)

7-Epineoptilocaulin (**5**) (4.5 mg, 0.0145 mmol) in methanol (5 mL) was hydrogenated over 10% Pd/C (5 mg) for 4 h at 3 atm. The solution was filtered through Celite and concentrated to afford 5 mg (111%) of 80 % pure **41**: ^{1}H NMR (800 MHz) 7.64 (br s, 1), 7.61 (br s, 1), 7.05 (br s, 2), 3.89 (dd, 1, *J* = 6, 4.2), 3.54 (dd, 1, *J* = 4.9, 1.1), 2.34 (ddd, 1, *J* = 11.4, 5.7, 5.7), 2.14-2.06 (m, 1), 1.97 (ddd, 1, *J* = 12.9, 7.1, 7.1), 1.93 (dd, 1, *J* = 13.2, 5.8), 1.71-1.58 (m, 2), 1.48-1.45 (m, 1), 1.40-1.20 (m, 8), 1.13 (ddd, 1, *J* = 13, 13, 13), 1.09 (d, 3, *J* = 6.7), 0.92 (t, 3, *J* = 6.7); ^{13}C NMR (800 MHz) 154.8, 53.8, 50.0, 44.7, 35.8, 35.2, 34.8, 34.6, 34.5, 33.4, 30.5, 29.8, 23.4, 22.9, 14.0; IR (neat) 3268, 1668, 1634, 1456, 1373; HRMS (Qtof) calcd for $C_{15}H_{28}N_3$ (MH^+) 250.2283, found 250.2281. Attempted purification by chromatography on silica gel gave even less pure **41**.

(±)-3a*S*,5a*S*,7*S*,8b*R*)-8-Butyl-1,3a,4,5,5a,6,7,8b-octahydro-7-methylcyclopenta[*de*]quinazolin-2-amine (Ptilocaulin, 1) and (±)-3a*S*,5a*S*,8a*S*,8b*R*)-8-Butyl-1,3a,4,5,5a,6,8a,8b-octahydro-7-methylcyclopenta[*de*]quinazolin-2-amine (Isoptilocaulin, 4)

A 0.36 M solution of guanidine in MeOH was prepared by adding guanidinium hydrochloride (170 mg, 1.8 mmol) to a solution of NaOMe in MeOH prepared by adding Na (42 mg, 1.8 mmol) to anhydrous MeOH (5.0 mL) under nitrogen.

Guanidine in MeOH (0.36 M, 0.84 mL, 0.30 mmol) was transferred to a resealable tube containing indenone **60** (35 mg, 0.17 mmol) in 2.5 mL methanol under nitrogen. The tube was sealed and heated in an 85 °C oil bath for 24 h. The mixture was cooled, treated with water (1 mL) and $NaBH_4$ (13 mg, 0.34 mmol), stirred overnight at 25 °C, quenched with 1% HNO_3 (2.5 mL, 0.28 mmol) and diluted with $CHCl_3$ (30 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to yield 50 mg of crude **1** and **4** as a brown oil. Flash chromatography on silica gel (18:1 $CHCl_3$/MeOH) gave 20.6 mg (39%) of an inseparable mixture of **1** (~30%), **4** (~30%) and numerous other isomers.

Data for ptilocaulin (**1**) were determined from the mixture : ^{1}H NMR ($CDCl_3$) 8.97 (br s, 1), 8.35 (br s, 1), 7.44 (br s, 2), 3.80-3.68 (m, 1), 2.52-1.81 (m, 6), 1.81-1.52 (m, 2), 1.52-1.18 (m, 7), 1.04 (d, 3, *J* = 6.8), 0.89 (m, 3); (CD_3OD) 3.84 (br d, 1, *J* = 10), 2.58-2.33 (m, 3), 2.33-2.05 (m, 3), 1.89-1.56 (m, 2), 1.56-1.21 (m, 7), 1.09 (d, 3, *J* = 6.8), 0.94 (t, 3, *J* = 7.2); ^{13}C NMR ($CDCl_3$) 151.7, 126.9, 121.0, 53.2, 36.5, 33.9, 32.2, 31.5, 29.7, 29.6, 27.7, 24.6, 22.4, 19.5, 14.0; (CD_3OD) 152.9, 128.1, 122.9, 51.3, 37.8, 35.5, 34.2, 33.3, 30.9, 29.2, 28.1, 25.6, 23.7, 20.1, 14.5. The ^{1}H NMR ($CDCl_3$ and CD_3OD) data and ^{13}C NMR ($CDCl_3$ and CD_3OD) correspond to the literature data,[1,10] except that our ^{1}H NMR ($CDCl_3$ and CD_3OD) data are 0.01 to 0.04 ppm downfield, our ^{13}C NMR ($CDCl_3$) data are 0.1 to 0.4 ppm upfield, and our ^{13}C NMR (CD_3OD) data are all about 0.1 to 0.3 ppm shifted.

Data for isoptilocaulin (**4**) were determined from the mixture: ^{1}H NMR ($CDCl_3$) 7.73 (br s, 1), 7.33 (br s, 1), 7.09 (br s, 2), 3.96 (d, 1, *J* = 4.8), 3.90-3.80 (m, 1), 2.52-1.81 (m, 7), 1.81-1.52 (m, 1), 1.74 (s, 3), 1.52-1.18 (m, 6), 0.91 (m, 3); (CD_3OD) 4.02 (br d, 1, *J* = 4.8), 3.93-3.84 (m, 1), 2.58-2.33 (m, 1), 2.33-2.05 (m, 4), 2.05-1.88 (m, 2), 1.89-1.56 (m, 1), 1.78 (s, 3), 1.56-1.21 (m, 6), 0.95 (t, 3, *J* = 7.2); ^{13}C NMR (CD_3OD) 156.5, 136.4, 133.1, 54.6, 50.8, 41.0, 37.6, 37.2, 35.5, 32.9, 31.9, 31.8, 23.9, 19.9, 14.4; IR (neat); HRMS (EI, from mixture) calcd for $C_{15}H_{25}N_3$ (M^+) 247.2048, found 247.2049. The ^{1}H NMR ($CDCl_3$ and CD_3OD) data and ^{13}C NMR (CD_3OD) correspond to the literature data,[1,2c] except that our ^{1}H NMR (CD_3OD) data are 0.04 to 0.05 ppm downfield and our ^{13}C NMR (CD_3OD) data are 0.1 to 0.2 ppm downfield.

Partial data for minor isomers: ^{1}H NMR ($CDCl_3$) 3.76-3.64 (m, 1), 3.53 (br d, 1, *J* = 4.4), 1.15 (d, 3, *J* = 7.2), 0.97 (d, 3, *J* = 7.2); (CD_3OD) 3.77 (d, 1, *J* = 5.6), 3,59 (br d, 1, *J* = 4.8), 1.19 (d, 3, *J*= 7.2), 1.06 (d, 3, *J* = 6.8), 1.03 (d, 3, *J* = 6.8).

(3a*S*,5a*S*,7*R*,8*S*)-8-Butyl-1,3a,4,5,5a,6,7,8-octahydro-7-propylcyclopenta[*de*]quinazolin-2-amine (Netamine E, 65)

A 0.22 M solution of guanidine in MeOH was prepared by adding guanidinium hydrochloride (104 mg, 1.1 mmol) to a solution of NaOMe in MeOH prepared by adding Na (25 mg, 1.1 mmol) to anhydrous MeOH (5.0 mL) under nitrogen.

Guanidine in MeOH (0.22 M, 2.0 mL, 0.44 mmol) was transferred to a resealable tube containing the 8:4:2:1 mixture of indenone **64** and stereoisomers (58 mg, 0.25 mmol) in 3.0 mL of MeOH under nitrogen. The tube was sealed and heated in an 85 °C oil bath for 24 h. The mixture was cooled, quenched with 1% HNO_3 (5.0 mL, 0.55 mmol) and diluted with $CHCl_3$ (30 mL). The layers were separated and the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated to yield 82 mg of crude **65** as a yellow oil. Repeated flash chromatography on silica gel (25:1 $CHCl_3$/MeOH) gave 31.5 mg (37.5%) of 90% pure **65** as a yellow oil: $[\alpha]_D^{25}$ + 15.6 (*c* 0.09, CH_2Cl_2); [lit.[8] $[\alpha]_D^{21}$ + 35.0 (*c* 0.80, CH_2Cl_2)]; ^{1}H NMR ($CDCl_3$) 8.85 (br s, 1), 8.26 (br s, 1), 7.63 (br s, 2), 4.26 (br t, 1, *J* = 6.8), 2.51-2.21 (m, 1), 2.14 (br dd, 1, *J* = 13.6, 6.8), 2.10-2.00 (m, 1), 2.00-1.87 (m, 2), 1.77-1.03 (m, 13), 0.90 (t, 3, *J* = 6.8), 0.89 (t, 3, *J* = 6.8), 0.72 (ddd, 1, *J* = 11.2, 11.2, 11.2); (CD_3OD) 4.27 (br t, 1, *J* = 6.8), 2.46-2.34 (m, 1), 2.19 (br dd, 1, *J* = 12.8, 6.8), 2.16-2.05 (m, 1), 2.05-1.80 (m, 2), 1.78-1.10 (m, 13), 0.94 (t, 3, *J* = 7.2), 0.93 (t, 3, *J* = 7.2), 0.77 (ddd, 1, *J* = 12, 12, 12); ^{13}C NMR (CD_3OD) 128.8, 119.8, 54.1, 42.3, 38.4, 37.7, 37.3, 36.5, 34.0, 30.4, 28.7, 27.5, 24.4, 21.4, 14.8, 14.6 (the peak at 155 was not observed); IR (neat) 3205, 1674; HRMS (EI) calcd for $C_{17}H_{29}N_3$ (M^+) 275.2361, found 275.2363.

A 1D NOESY experiment with irradiation of H-6α at δ 0.77 showed NOE enhancements of H-6β at δ 2.15 (very strong), H-5α at δ 1.30 (strong), H-8 at δ 1.95 (medium strong), H-1″ at δ 1.60 and δ 1.20 (medium strong), and H-5a at δ 2.43 (weak).

The ^{1}H NMR (CD_3OD) and ^{13}C NMR (CD_3OD) data correspond to the literature data,[8] except that our ^{1}H NMR (CD_3OD) data are 0.01 to 0.03 ppm upfield and our ^{13}C NMR (CD_3OD) data are all about 0.1 to 0.4 ppm upfield.

(5a*S*,7*R*,8*S*)-8-Butyl-4,5,5a,6,7,8-hexahydro-7-propylcyclopenta[*de*]quinazolin-2-amine (Netamine G, 66)

Crude **65**, containing isomers, (105 mg) was prepared as described above from indenone **64** (63 mg, 0.27 mmol). CH_2Cl_2 (6 mL) and activated MnO_2 (103 mg, 1.2 mmol) was added and the mixture was stirred in a sealed tube in a 55°C oil bath for 48 h. The mixture was cooled, filtered through Celite. The residue was washed with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with 10% K_2CO_3 solution (10 mL), dried over $MgSO_4$ and concentrated to yield 45 mg of crude **66** as a yellow oil. Flash chromatography on silica gel (70:1 CH_2Cl_2/MeOH) gave 22.5 mg (25%) of **66** as a yellow oil: $[\alpha]_D^{25}$ + 26.3 (*c* 0.255, CH_2Cl_2); [lit.[8] $[\alpha]_D^{21}$ + 27.0 (*c* 0.20, CH_2Cl_2)]; ^{1}H NMR ($CDCl_3$ 9% protonated by TFA) 5.19

(br s, 2), 3.00-2.80 (m, 2), 2.60 (br dd, 1, *J* = 16.4, 8.4), 2.38 (ddd, 1, *J* = 12.0, 7.2, 7.2), 2.30 (ddd, 1, *J* = 8.8, 4.4, 4.4, H-8), 2.17 (ddd, 1, *J* = 12.4, 3.8, 3.8), 2.09-1.91 (m, 1), 1.83-1.64 (m, 2), 1.64-1.41 (m, 3), 1.41-1.15 (m, 5), 1.15-1.02 (m, 1), 0.93 (t, 3, *J* = 6.8), 0.87 (t, 3, *J* = 6.8), 0.78 (ddd, 1, *J* = 11.6, 11.6, 11.6); ^{13}C NMR ($CDCl_3$ 9% protonated by TFA) 174.5, 166.4, 163.1, 126.3, 45.2, 38.8, 37.5, 37.1, 36.2, 33.6, 33.0, 31.0, 27.7, 23.2, 20.2, 14.4, 14.0; IR (neat) 3334, 3193, 1588; HRMS (EI) calcd for $C_{17}H_{27}N_3$ (M^+) 273.2205, found 273.2203.

The ^{1}H NMR data and ^{13}C NMR correspond to the literature data,[8,44] except that our ^{1}H NMR data are 0.02 to 0.05 ppm upfield and our ^{13}C NMR data are all about 0.1 to 0.5 ppm shifted, possibly because of a slightly different extent of protonation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are grateful to the National Institutes of Health (GM-50151) for support of this work. The 800 MHz spectrometer in the Landsman Research Facility, Brandeis University was purchased under NIH RR High-End Instrumentation program, 1S10RR017269-01. We thank Dr. Alan J. Freyer, GlaxoSmithKline Pharmaceuticals, for a copy of the ^{1}H NMR spectrum of **5**. We thank Prof. Hans-Guenther Schmalz, University of Cologne, for a copy of the NMR spectrum of the mixture of **1** and **23**. We thank Prof. Mark T. Hamann, University of Mississippi, for copies of the ^{1}H NMR spectra of **9** and the mixture of **13** and **14**, a sample of the mixture of **13** and **14**, and for determining the $[\alpha]_D$ of mirabilin B. We thank Prof. Yoel Kashman, Tel-Aviv University, for helpful discussions, copies of the proton spectra of netamine G, and samples of netamines A and F.

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27. This proton was reported to absorb as a dt, J = 11.6, 5.3 Hz. It actually absorbs as a dt, J = 5.3, 11.6 Hz. Prof. Hans-Guenther Schmalz, University of Cologne, personal communication, January 8, 2008. We thank Prof Schmalz for a copy of the ^{1}H NMR spectra of the mixture of **1** and **23**.
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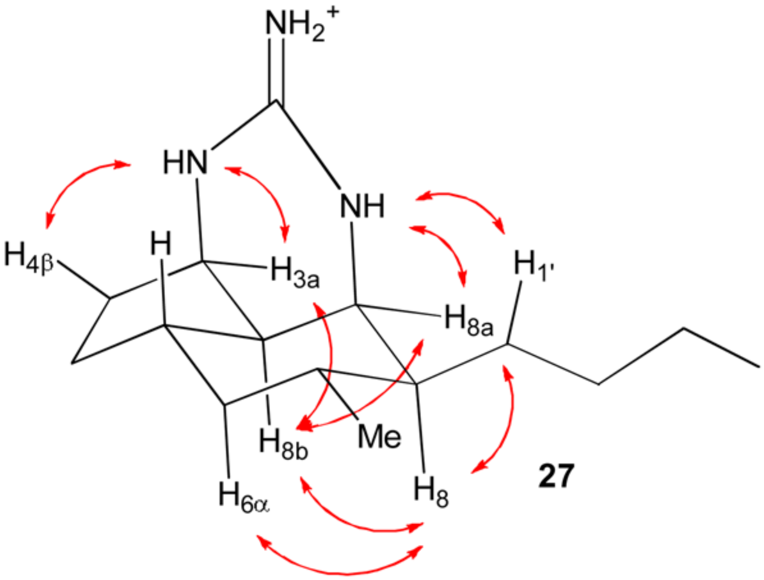
44. We thank Prof. Yoel Kashman, Tel-Aviv University, for helpful discussions, copies of the proton spectra of netamine G, and samples of netamines A and F.



27

Figure 1.
MMX-calculated structure of **27** with NOEs shown that establish the stereochemistry.

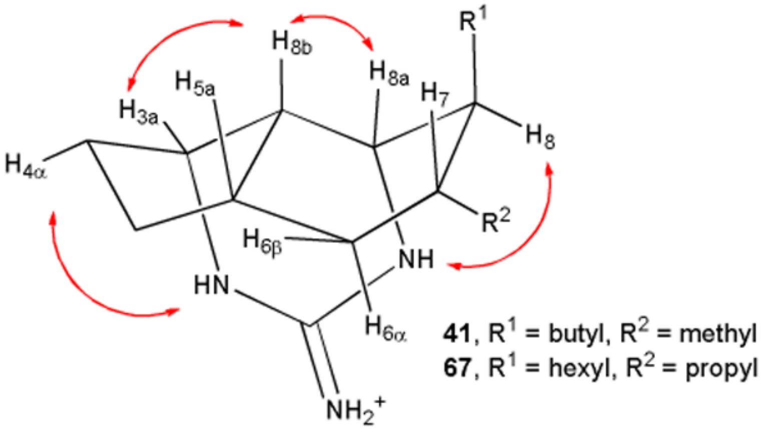
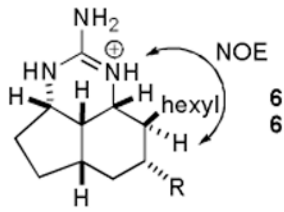
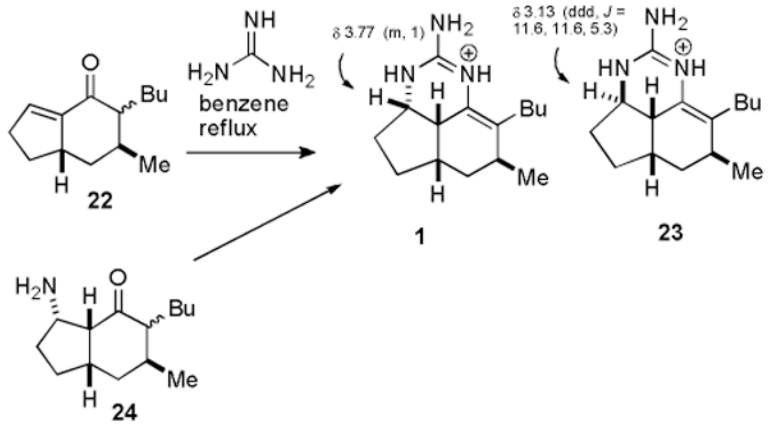


Figure 2.
MMX-calculated structures of **41** and **67** with NOEs shown that establish the stereochemistry.

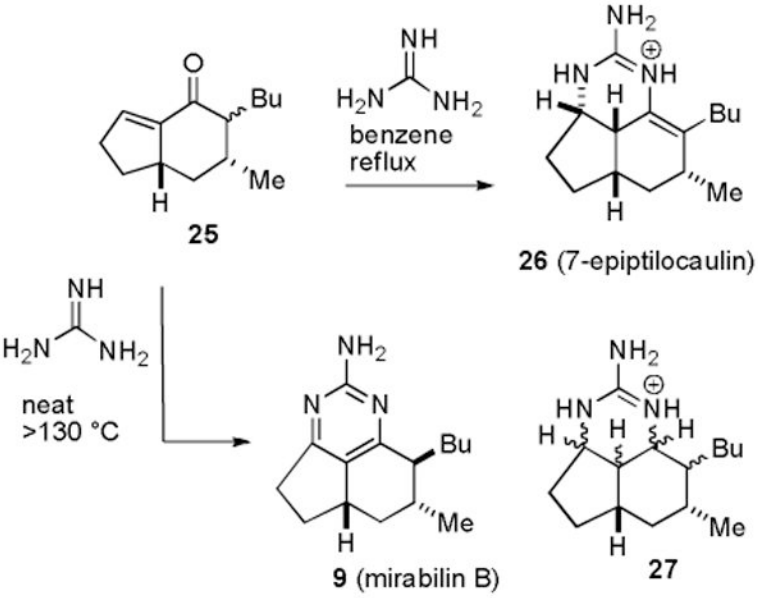


67, R = Pr (revised structure of netamine A)
68, R = Me (revised structure of netamine C)

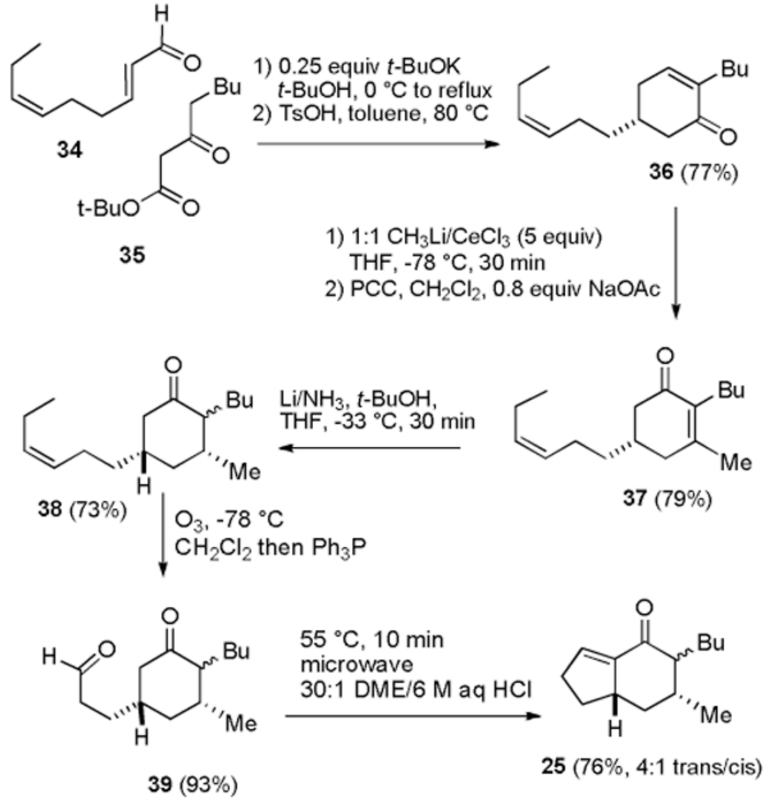
Figure 3.
Revised Structures of Netamines A and C.



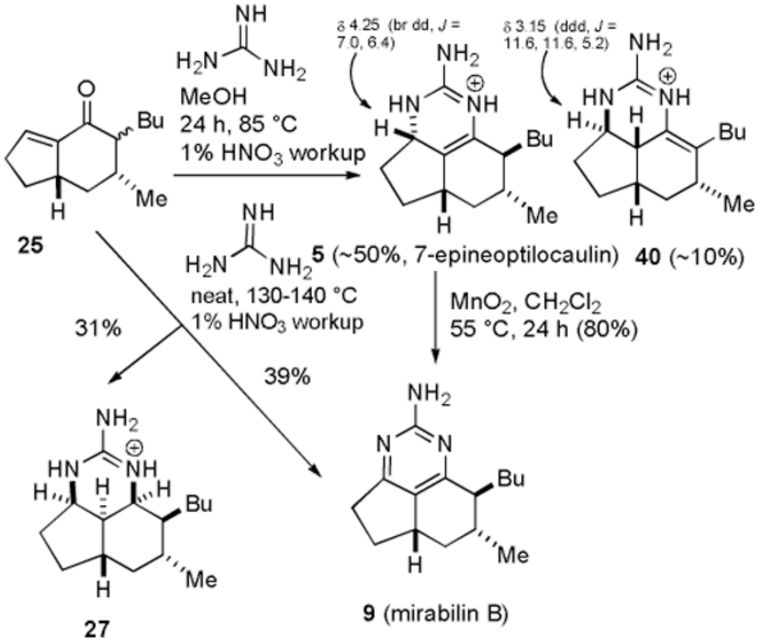
Scheme 1. Syntheses of Ptilocaulin (1)



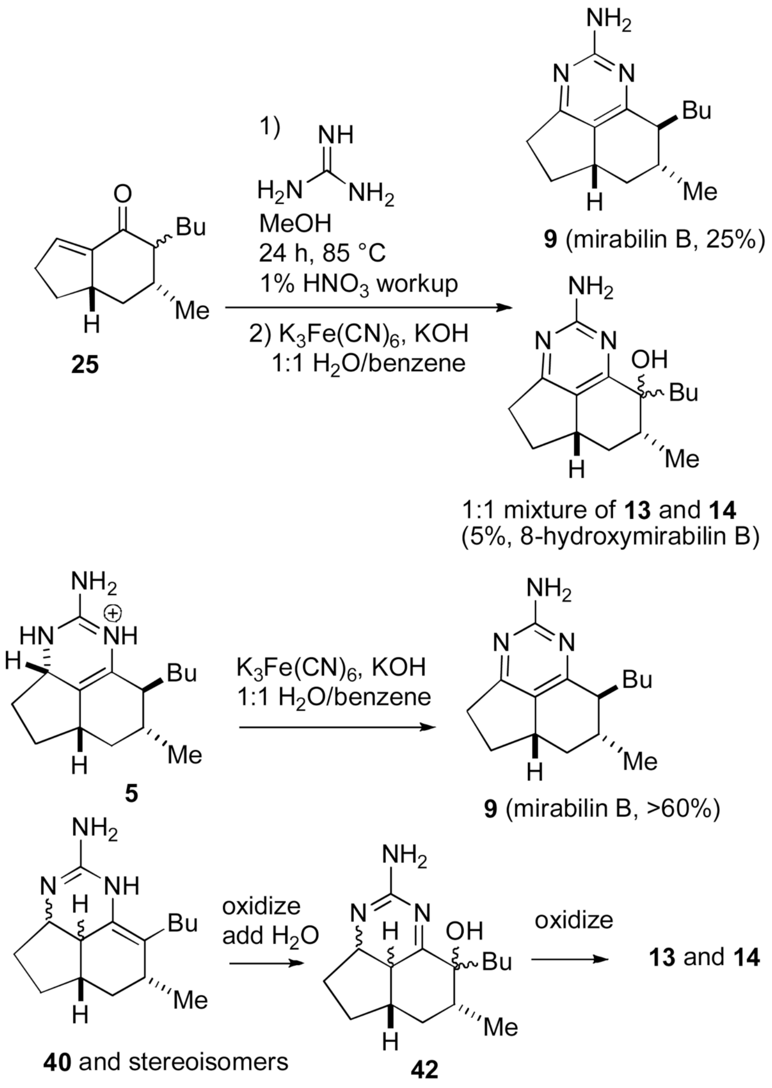
Scheme 2. Hassner's Studies with Hexahydroindenone 25



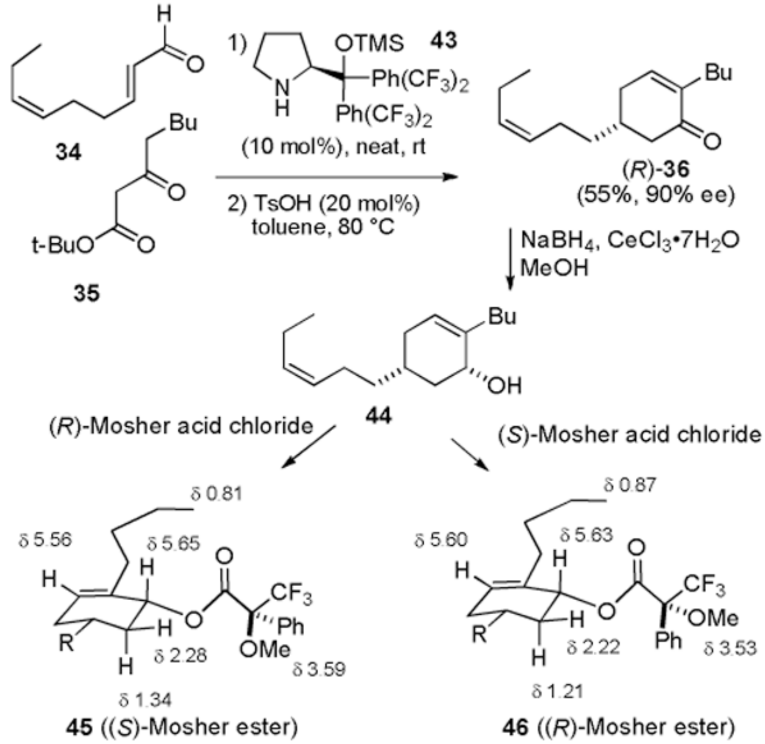
Scheme 3. Synthesis of Hexahydroindenone 25



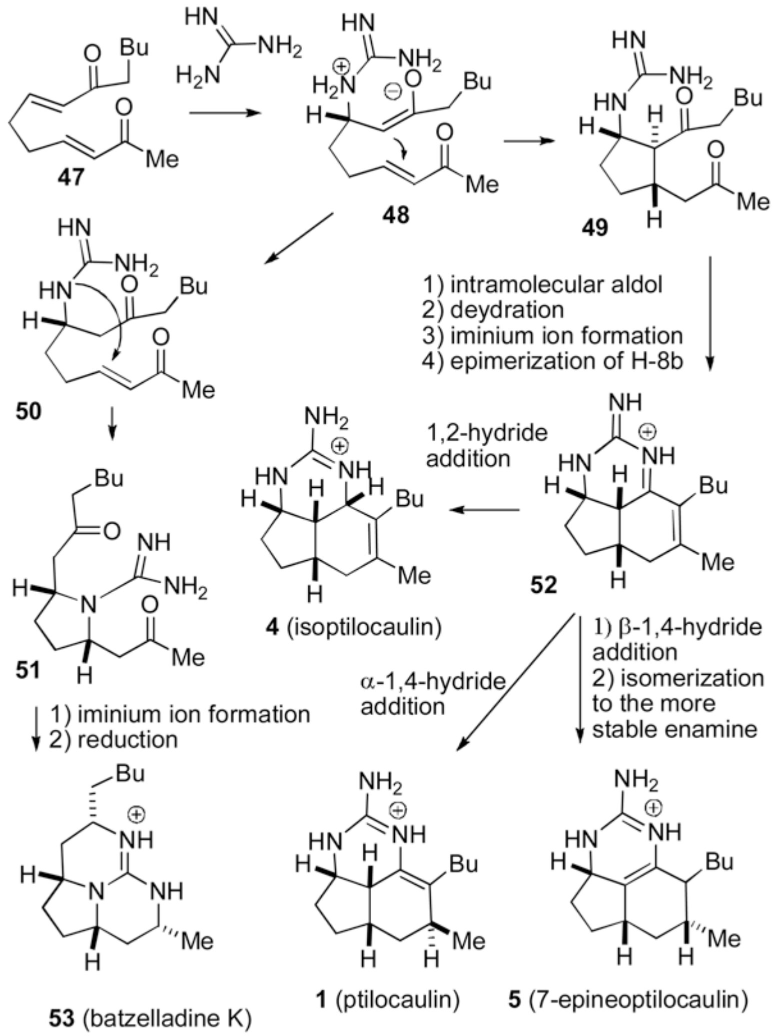
Scheme 4. Synthesis of 7-Epineoptilocaulin and Mirabilin B



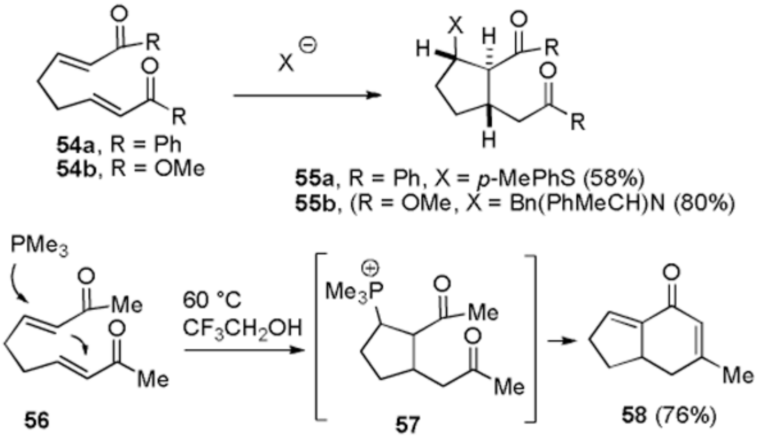
Scheme 5. Synthesis of Hydroxymirablins (13 and 14)



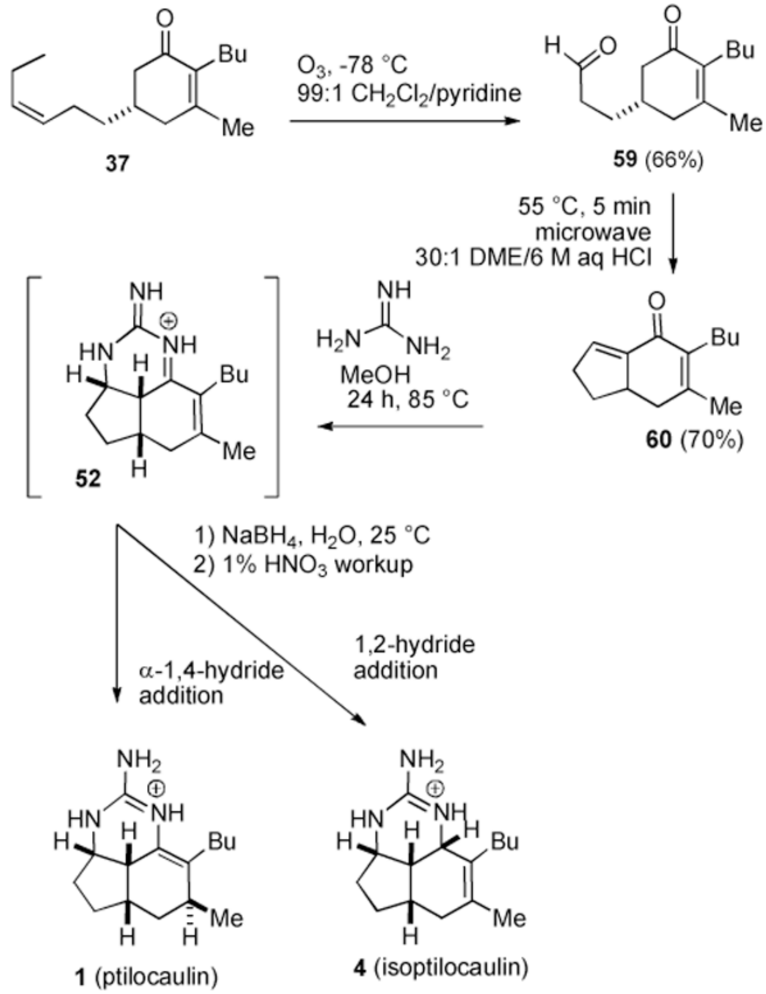
Scheme 6. Synthesis of (-)-(*R*)-36



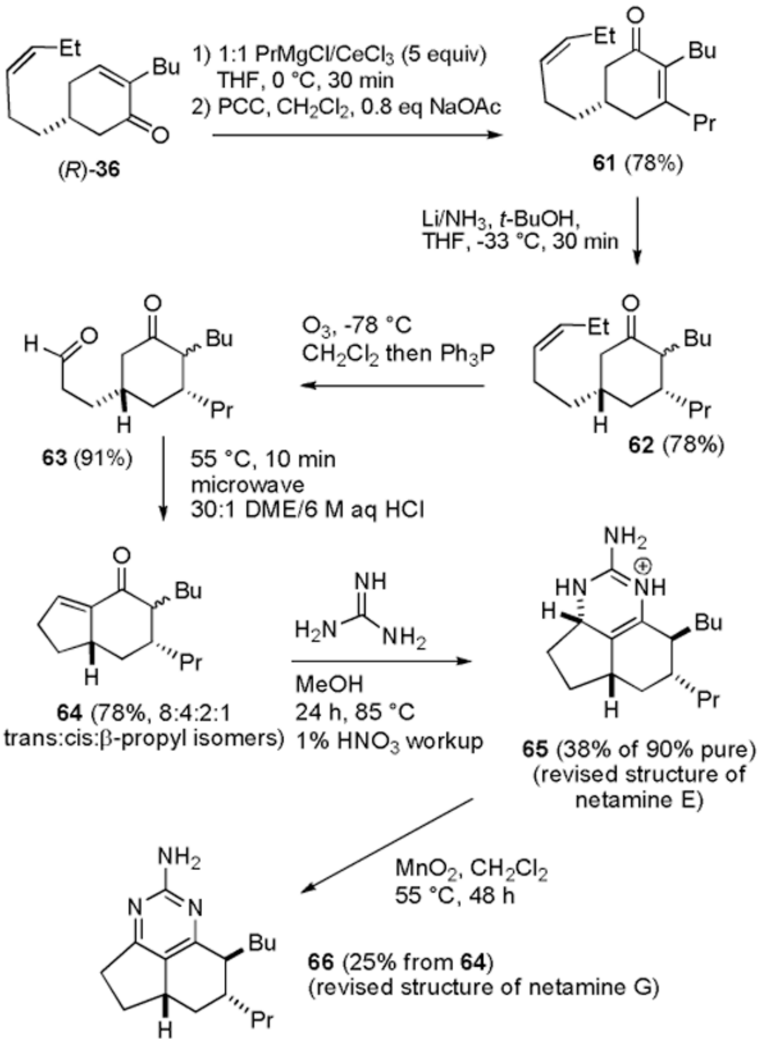
Scheme 7. Unified Biosynthetic Proposal for Ptilocaulin, Isoptilocaulin, 7-Epineoptilocaulin, and Batzelladine K



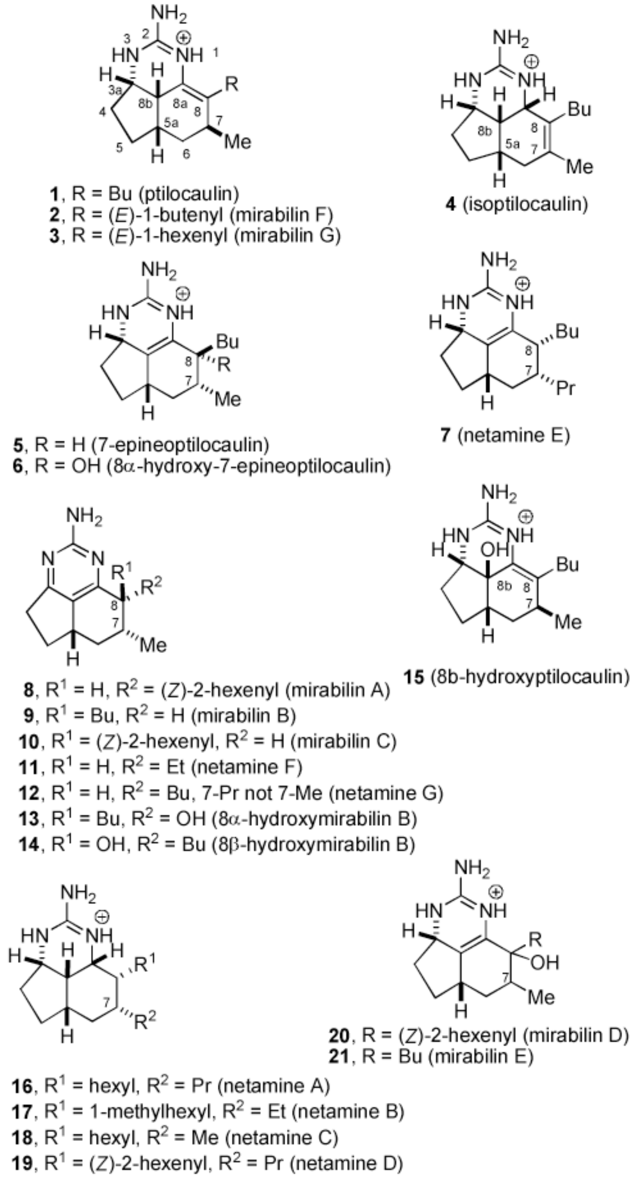
Scheme 8. Precedents for the Proposed Biosynthesis



Scheme 9. Biomimetic Synthesis of Ptilocaulin and Isoptilocaulin



Scheme 10. Synthesis of Netamines E (65) and G (66)



1, R = Bu (ptilocaulin)
2, R = (*E*)-1-butenyl (mirabilin F)
3, R = (*E*)-1-hexenyl (mirabilin G)

4 (isoptilocaulin)

5, R = H (7-epineoptilocaulin)
6, R = OH (8α-hydroxy-7-epineoptilocaulin)

7 (netamine E)

8, R^1 = H, R^2 = (*Z*)-2-hexenyl (mirabilin A)
9, R^1 = Bu, R^2 = H (mirabilin B)
10, R^1 = (*Z*)-2-hexenyl, R^2 = H (mirabilin C)
11, R^1 = H, R^2 = Et (netamine F)
12, R^1 = H, R^2 = Bu, 7-Pr not 7-Me (netamine G)
13, R^1 = Bu, R^2 = OH (8α-hydroxymirabilin B)
14, R^1 = OH, R^2 = Bu (8β-hydroxymirabilin B)

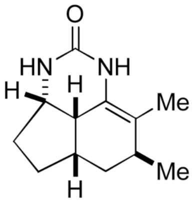
15 (8b-hydroxyptilocaulin)

16, R^1 = hexyl, R^2 = Pr (netamine A)
17, R^1 = 1-methylhexyl, R^2 = Et (netamine B)
18, R^1 = hexyl, R^2 = Me (netamine C)
19, R^1 = (*Z*)-2-hexenyl, R^2 = Pr (netamine D)

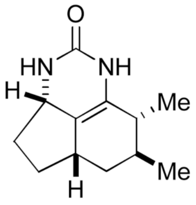
20, R = (*Z*)-2-hexenyl (mirabilin D)
21, R = Bu (mirabilin E)

Chart 1.

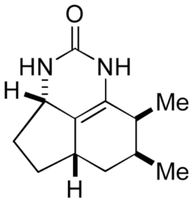
Structures of the ptilocaulin (**1**) family.



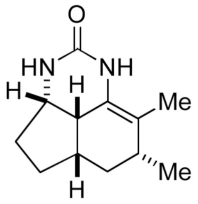
28 (ptilocaulin)
11.33 kcal/mol



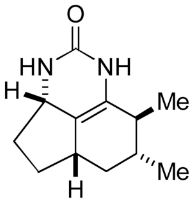
29 (*trans*-neoptilocaulin)
11.22 kcal/mol



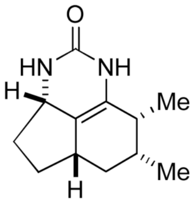
30 (*cis*-neoptilocaulin)
11.82 kcal/mol



31 (7-epiptilocaulin)
12.54 kcal/mol



32 (*trans*-7-epineoptilocaulin)
10.09 kcal/mol



33 (*cis*-7-epineoptilocaulin)
11.94 kcal/mol

Chart 2.

Calculated energies of ptilocaulin- and neoptilocaulin-like ureas.