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A General Route to Cyclopeptide Alkaloids: Total Syntheses and Biological Evaluation of Paliurines E and F, Ziziphines N and Q, Abyssenine A, Mucronine E, and Analogues

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Dedicated to Dr. Jean-Paul Mazaleyrat on the occasion of his retirement

Keywords: Alkaloids / Biological activity / Copper catalysis / Cyclopeptide alkaloids / Macrocycles / Total synthesis

A full account of the total syntheses of the cyclopeptide alkaloids paliurine E and F, ziziphine N and Q, abyssenine A, and mucronine E is provided. A key feature of the syntheses involves an intramolecular amidation of a vinyl iodide, which allows us simultaneously to address two synthetic challenges associated with cyclopeptide alkaloids: the formation of the enamide and macrocyclization. We also document the use of other strategies for the macrocyclization step, as well as the evaluation of the antibacterial and cytotoxic properties of the natural products and analogues obtained.

Introduction

Cyclopeptide Alkaloids

Cyclopeptide alkaloids form a group of closely related cyclic polyamide bases of plant origin. Although they were mentioned in the literature as early as 1884,[1] the isolation and structural elucidation of pandamine in 1966[2] marks the beginning of a growing interest in these natural products, which nowadays encompass over 200 compounds. Several characteristic features are common to the majority of these alkaloids. They generally possess a 13- (**1**), 14- (**2**), or 15-membered (**3**) cycle containing an aromatic ring. The remainder of the macrocycle consists of a peptide unit that is connected to the aromatic ring in either a 1,4- or a 1,3-orientation through enamide and alkyl aryl ether (or methylene) linkages (Figure 1).[3]

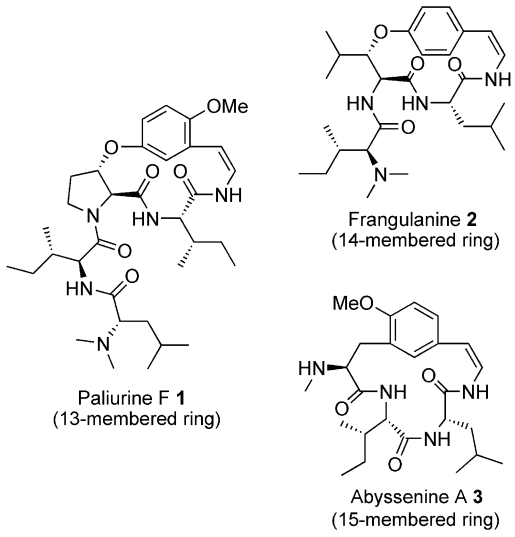

Figure 1. Representative cyclopeptide alkaloids.

Although cyclopeptide alkaloids are found in the roots, leaves, and bark of a number of plants, extraction techniques usually afford complex mixtures, making their isolation an extremely tedious adventure. Yields from dried plants vary from 0.0002–1% depending on the plant source, location, method of isolation, and plant maturity. Their common occurrence has, however, led to a rich history of service in folk medicine, in which sources of cyclopeptide alkaloids have been used as remedies for diarrhea, dysentery, or insomnia.[4] Recently, they have been shown to dis-

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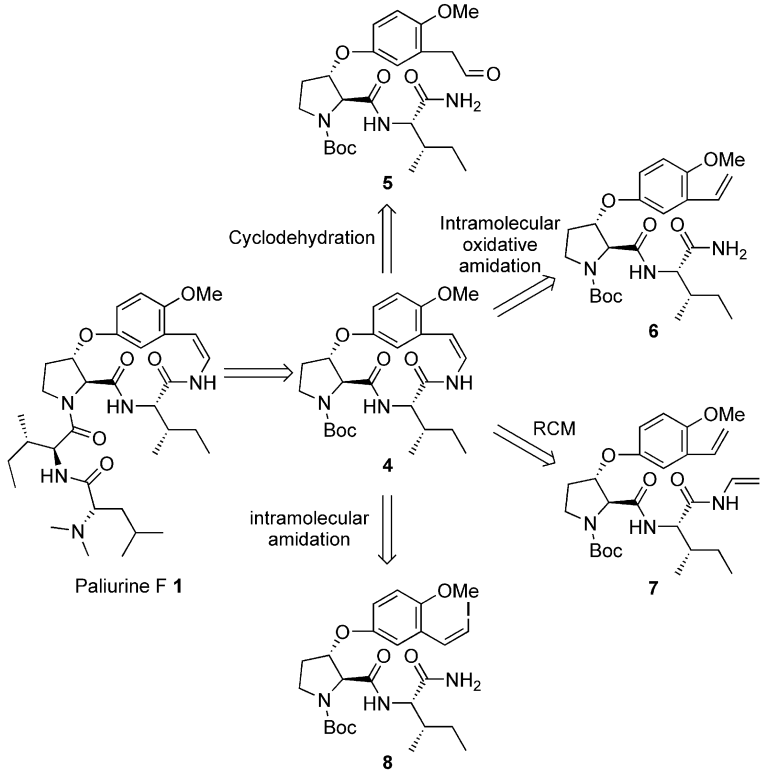
play numerous forms of biological activity, including sedative/stimulant,[5] antibacterial,[6] antifungal,[6a–6c,7] and antiplasmodial.[8] However, the limited supplies of cyclopeptide alkaloids have not been sufficient for extensive pharmacological investigations, and the lack of efficient synthetic strategies has limited structure–activity relationship studies.

Their biological properties, together with their intriguing structures, have resulted in a steady stream of studies directed towards the synthesis of this class of compounds during the past decades.[9] These studies have revealed that the synthetic challenges include elaboration of the aryl alkyl ether, formation of the strained macrocycle, and introduction of the enamide unit. The initial report by Schmidt utilized the macrolactamization of a pentafluorophenyl ester,[10] which was also later used in the total syntheses by Joullié[11] and by Han,[12] whereas Zhu's synthesis incorporated an intramolecular S_NAr reaction as macrocyclization protocol.[13] All these syntheses chose elaboration of the enamide moiety by different stepwise elimination methods after macrocyclization, which rather decreased the overall synthetic efficiency. We have developed an alternative cyclization strategy based on an intramolecular amidation of a vinyl iodide and have successfully implemented it in efficient asymmetric syntheses of paliurine F,[14] abyssenine A,[15] and mucronine E.[16] Besides being convergent, the salient feature of our approach is that two synthetic challenges associated with cyclopeptide alkaloids – the formation of the enamide and macrocyclization – have been reduced to a single operation with high efficiency.[17] In this paper we report in detail on the development of a general route to cyclopeptide alkaloids, as well as on its application to the preparation of two additional natural products: ziziphines N and Q. We also document the use of other strategies for the macrocyclization step, together with evaluation of the antibacterial and cytotoxic properties of the natural products and analogues obtained.

Strategic Considerations

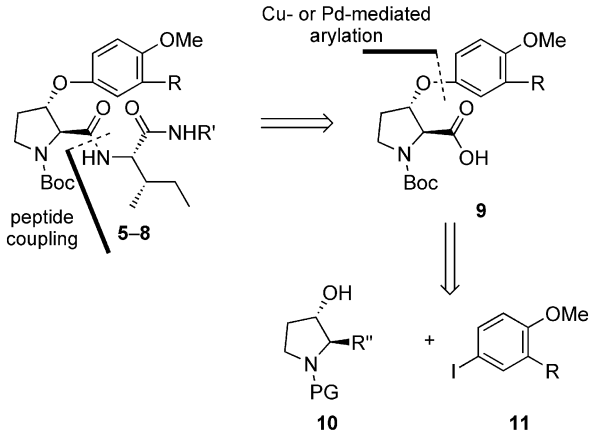
The design of a new synthetic route to cyclopeptide alkaloids has to meet the following criteria: it must be as straightforward as possible, and it should allow the preparation of useful quantities of the target molecules and be flexible enough to permit the efficient construction of analogues. It is also important to consider the generality of the process and its reliability.

Our proposed route, which meets these criteria, is summarized in Scheme 1 and Scheme 2 in a retrosynthetic fashion starting from the sedative cyclopeptide alkaloid paliurine F (**1**),[5c,18] which was chosen as our primary target for this study. From a retrosynthetic perspective, we envisioned the installation of the peptidic side chain late in the synthesis, thereby permitting the construction of paliurine F from the advanced precursor **4**, as depicted in Scheme 1. In the planning of this synthesis, the choice of macrocyclization site and the reaction to be employed to carry out this transformation appeared to be of the utmost importance. In or-



Scheme 1. Key macrocyclization step: the possibilities.

der to minimize the overall number of steps and to avoid a painful stepwise installation of the enamide in **4** after elaboration of the macrocycle, we decided to investigate strategies to allow for their concomitant formation.



Scheme 2. Retrosynthetic analysis of acyclic precursors.

We therefore decided that the macrocycle would be closed at the enamide bond variously through a cyclodehydration reaction starting from α-amido ω-aldehyde **5**, through an intramolecular oxidative amidation starting from acyclic precursor **6**,[19] through a ring-closing metathesis starting from ene-enamide **7**,[20] or through an intramolecular copper-mediated amidation starting from **8** (Scheme 1).[21,22]

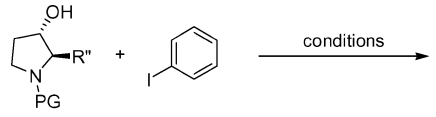
All precursors **5**–**8**, bearing different functionalities, would be derived from the corresponding carboxylic acids of general formula **9** (Scheme 2) by a simple peptide coupling with isoleucine amide or derivatives. Finally, further analysis suggested a disconnection at the aryl ether bond to give the hydroxypyrrolidines **10** and aromatic fragments **11**, which would be coupled in copper-[23] or palladium-mediated[24] arylation reactions.

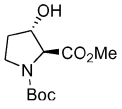
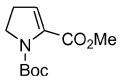
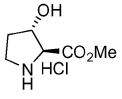
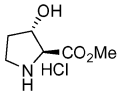
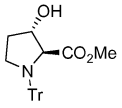
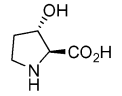
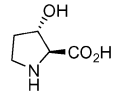
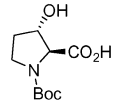
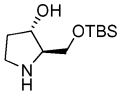
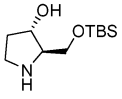
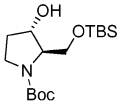
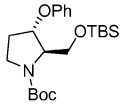
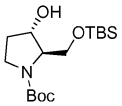
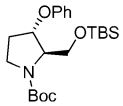
Results and Discussion

Installation of the Aryl Alkyl Ether

Our synthesis started with the optimization of the reaction partners and conditions for the formation of the highly substituted aryl alkyl ether moiety. Initial studies focused on the determination of the structural requirements for the hydroxypyrrolidine fragment **10** with use of iodobenzene (**12**) as a model arylating agent (Table 1). To this end, a set of seven hydroxypyrrolidines **10** were easily prepared from commercially available *trans*-3-hydroxyproline (**10d**)[25] by classical transformations. They were then subjected to the conditions reported by Buchwald for the copper-mediated arylation of alcohols[23] or amino alcohols (Table 1).[26] In an initial experiment, the Boc-protected 3-hydroxyproline methyl ester **10a** was treated with iodobenzene (**12**) in the presence of catalytic amounts of copper(I) iodide, together with phenanthroline and cesium carbonate as a base, in toluene at 110 °C (Table 1, Entry 1). Although all the starting material had been consumed after 12 h, it rapidly turned out that the desired phenyl pyrrolidinyl ether had indeed

Table 1. Optimization of the hydroxypyrrolidine reaction partner for the copper-mediated arylation.

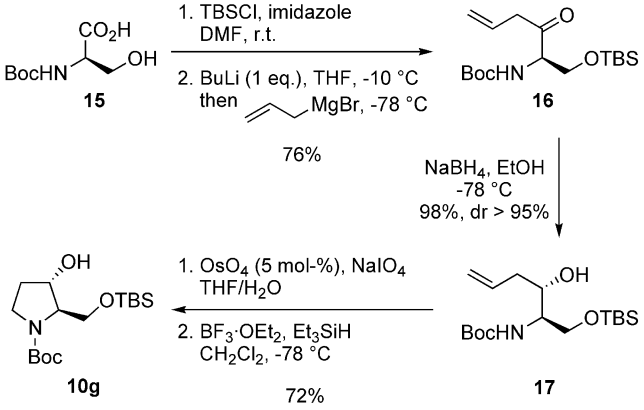


Entry	Hydroxypyrrolidine		Conditions	Results[a]
1		**10a**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	 **13** (73 %)
2		**10b**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	no reaction
3		**10b**	CuI, Cs_2CO_3, butyronitrile, 125 °C	no reaction
4		**10c**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	degradation
5		**10d**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	no reaction
6		**10d**	CuI, Cs_2CO_3, butyronitrile, 125 °C	no reaction
7		**10e**	CuI, Cs_2CO_3, toluene, 125 °C	no reaction
8		**10f**	CuI, Cs_2CO_3, butyronitrile, 125 °C	no reaction
9		**10f**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	no reaction
10		**10g**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 110 °C	 **14** (70%)
11		**10g**	CuI, 1,10-phenanthroline, Cs_2CO_3, toluene, 125 °C	 **14** (75 %)

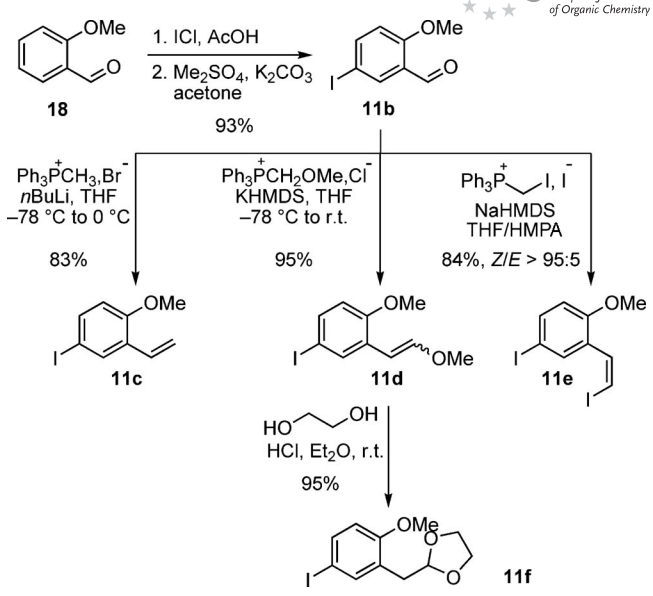
[a] Yields based on pure materials isolated by column chromatography.

been formed in the reaction mixture but that it had immediately undergone β-elimination to yield the endocyclic enamide **13** and phenol. The use of lower temperatures or of other bases such as potassium phosphate or carbonate did not block the undesired elimination. We therefore decided to examine the reactivities of the other substrates **10b**–**e**, variously possessing a free amine (Table 1, Entries 2 and 3), a free carboxylic acid (Table 1, Entry 7), both (Table 1, Entries 5 and 6), or a trityl protecting group on the nitrogen (Table 1, Entry 4) in order to diminish or suppress the acidity of the labile hydrogen involved in the β-elimination reaction. Whereas the trityl group in **10c** did not survive the reaction conditions and extensive degradation was observed, we faced a total lack of reactivity with other substrates **10b**, **10d**, and **10e**, which were fully recovered at the end of the reactions. At this point of screening, we decided to replace the carboxylic acid or ester groups in **10a**–**e** with a protected hydroxymethyl precursor, hoping to obtain products that would be stable under the reaction conditions. Although the absence of a protecting group on the nitrogen again inhibited the reaction, we were delighted to note that substrate **10g** could be smoothly coupled with iodobenzene (**12**), giving the long-awaited phenyl pyrrolidinyl ether **14** in 70% yield (Table 1, Entry 10). This yield could further be improved simply by raising the reaction temperature from 110 to 125 °C (Table 1, Entry 11). A notable feature of this arylation reaction is that, unlike in the originally published procedure, which required an excess of the alcohol, the stoichiometry could be reversed without significantly lowering the yield, provided that the excess of iodobenzene (1.5 equiv.) was added in two portions.

Before we moved on to the optimization of the second reaction partner, we needed a suitable synthetic route to provide useful quantities of the hydroxypyrrolidine fragment **10g** easily. Indeed, the preparation of this fragment from commercially available *trans*-3-hydroxyproline (**10d**), as used for the model studies, was not practical on large scale because of the prohibitive price of this starting material. Our goal was therefore to design a gram-scale, substrate-controlled asymmetric route to **10g** with a limited

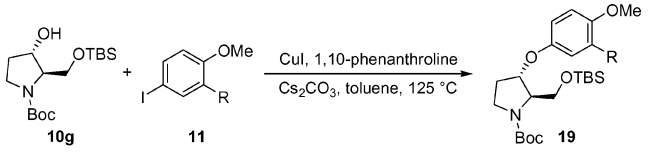


Scheme 3. Gram-scale synthesis of the hydroxypyrrolidine fragment **10g**.



Scheme 4. Synthesis of aromatic fragments.

Table 2. Optimization of the aromatic reaction partner for the copper-mediated arylation.



Entry	R	Aryl iodide	Product	Yield[a]
1		**11a**	**19a**	67 %
2		**11b**	**19b**	75 %
3		**11c**	**19c**	79 %[b]
4		**11d**	**19d**	65 %
5		**11e**	—	competitive arylation and vinylation
6		**11f**	**19f**	47 %

[a] Yields based on pure materials isolated by column chromatography. [b] Reaction performed at 110 °C to avoid polymerization of **11c** and **19c** (the yield drops to 27% when the reaction is run at 125 °C).

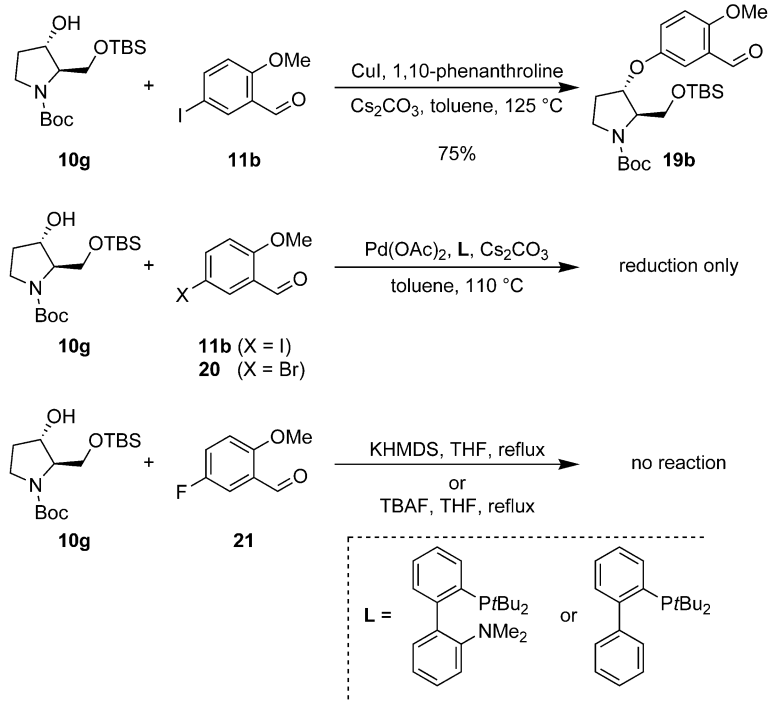
number of steps and protecting groups. After exploring many synthetic routes based on RCM, intramolecular Michael addition, or intramolecular epoxide-opening, we eventually came across the synthesis depicted in Scheme 3, which we later on applied to the efficient preparation of other hydroxypyrrolidines.[27] From the starting protected *N*-Boc-D-serine **15**, the desired fragment could easily be obtained on a multigram scale, in 54% overall yield and by a rather simple sequence. A Tegner reaction allowed for the transformation of the carboxylic acid in **15** into the corresponding unconjugated ketone **16**, which was reduced to **17** with high levels of diastereoselectivity. Two-step formation of the five-membered ring heterocycle finally provided the desired fragment **10g**.[14]

With gram quantities of hydroxypyrrolidine **10g** to hand, screening of different aromatic iodides **11** compatible with our retrosynthetic analysis was initiated. Except for compound **11a**, which was prepared from 5-iodosalicylic acid,[28] the other aromatic iodides **11b–f** were all easily synthesized from salicylaldehyde in excellent yields by iodination/methylation/olefination sequences (Scheme 4).

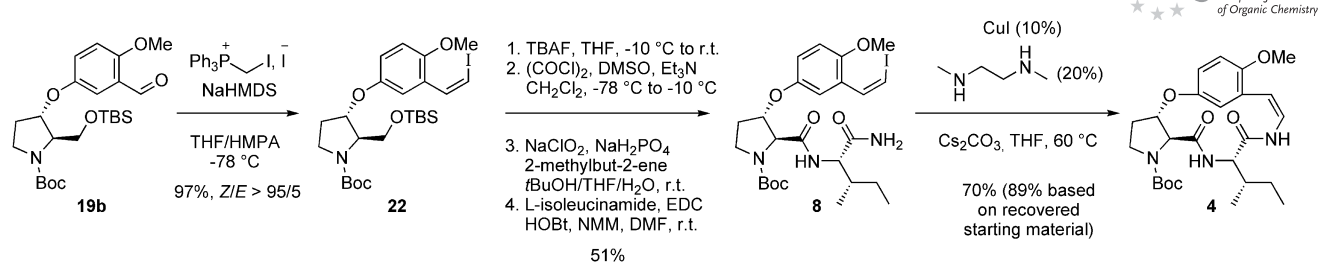
These compounds were then treated under the optimized conditions developed for the coupling of **10g** with iodobenzene: copper(I) iodide (10 mol-%) and 1,10-phenanthroline (20 mol-%) in the presence of cesium carbonate as base in toluene at 125 °C. Results from this screening revealed that all iodides were suitable reaction partners (Table 2), therefore enabling the macrocyclization to be effected, after functionalization, variously through cyclodehydration starting from **19d** or **19f**, through an ene-enamide RCM or an aza-Wacker reaction starting from **19c**, or through intramolecular amidation starting from **19a** or **19b**. Unfortunately, the reaction with bis-iodide **11e**, which would have avoided the installation of the vinyl iodide required for a macrocyclization through intramolecular amidation after the installation of the aryl alkyl ether, turned out to be non-selective because of competing vinylation of the alcohol (Table 2, Entry 5).

Before moving on to the next steps of the synthesis, we briefly evaluated other protocols based on palladium-mediated coupling or nucleophilic aromatic substitution reactions for the arylation of **10g**, to compare their efficiencies with that of the copper-mediated arylation procedure. Therefore, **10g** was treated variously with aromatic iodide **11b** or with bromide **20** in the presence of palladium acetate, cesium carbonate, and phosphane ligands **L**[24b] or with aromatic fluoride **21** in the presence of KHMDS or TBAF (Scheme 5). However, the palladium-mediated reaction mostly led to reduction of the aryl halide and left alcohol **10g** unaffected, which can certainly be attributed to steric crowding at the reacting center.

The nucleophilic aromatic substitution did not afford the desired coupled product either, which is easily understandable due to the low reactivity of **21**. Since the copper-mediated arylation procedure seemed to be the reaction of choice for the formation of **19b**, which could be synthesized in useful quantities, we decided to explore the next important step of the synthesis: the formation of the macrocycle through an intramolecular copper-mediated amidation reaction.



Scheme 5. Attempted fragment coupling by a palladium-mediated arylation or by a S_NAr reaction.



Scheme 6. Elaboration of the acyclic skeleton and copper-mediated macroenamidation.

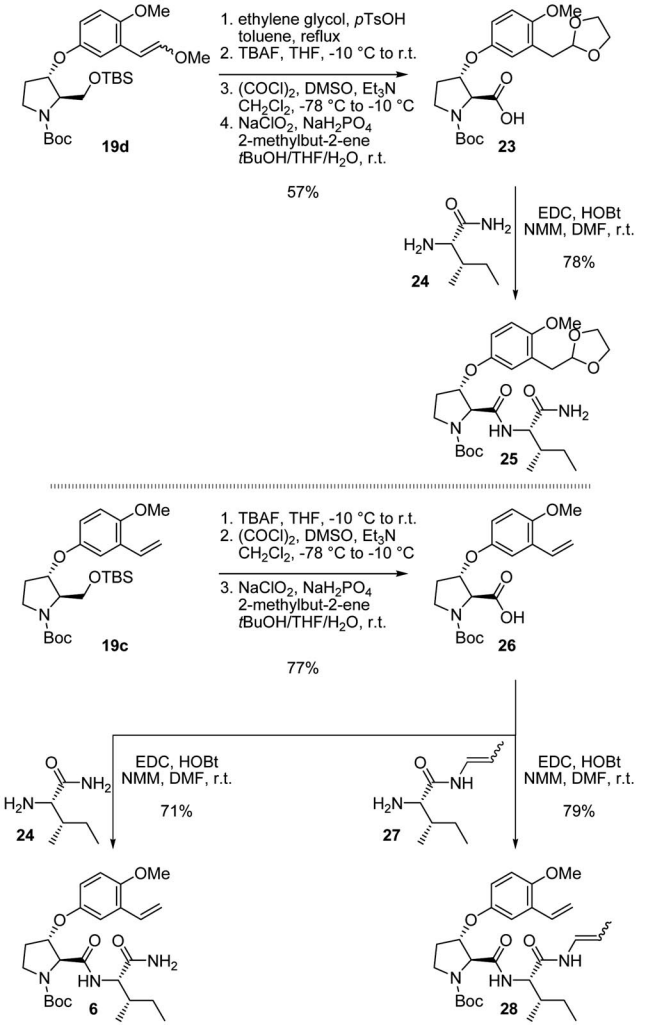
Macrocyclization by Copper-Mediated Macroenamidation

Several chemical manipulations were necessary before macrocyclization. To that end, the *Z* vinyl iodide required for the final macroamidation step was stereoselectively installed by use of Stork–Zhao olefination reagent (97%, *de* > 95%),[29] the TBS ether was deprotected with TBAF in THF, and the resulting primary alcohol was converted into the acid in a two-step sequence (Swern/buffered $NaClO_2$ oxidations) in good overall yield (Scheme 6). Finally, the second constitutive amino acid of the macrocycle, together with the amide group necessary for the intramolecular amidation, were introduced through an EDC/HOBt-mediated coupling with isoleucinamide.

The efficient preparation of the acyclic precursor **8** set the stage for the crucial macrocyclization step. The iodo amide **8** was treated with catalytic copper(I) iodide and *N*,*N*′-dimethylethylenediamine[21c] in THF under high-dilution conditions at 60 °C and smoothly provided the 13-membered ring **4** in 70% yield (89% based on recovered starting material). The most striking feature of this approach is that this mild intramolecular amidation protocol proceeds without any epimerization at the two amino acid stereocenters[30] or isomerization of the *Z* vinyl iodide and without dimerization or formation of higher oligomers (Scheme 6).[14]

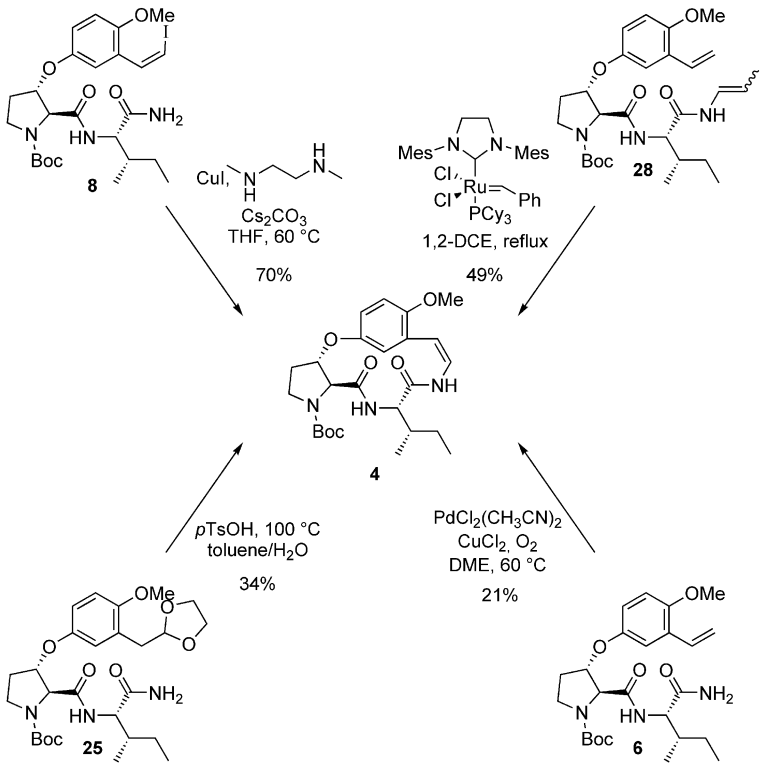
Macrocyclization by Cyclodehydration, Ene-Enamide RCM, or Intramolecular Oxidative Amidation

During the course of our study of the synthesis of paliurine F, we decided also to investigate an alternate route for the preparation of macrocycle **4** by means variously of a cyclodehydration, an ene-enamide RCM, or an intramolecular aza-Wacker reaction. This would have been a good opportunity to test the efficiency of our copper-based macrocyclization procedure and, in the best-case scenario, would have provided more efficient access to the target macrocycle **4**. To this end, the required precursors **25**, **6**, and **28** were prepared from pyrrolidines **19d** and **19c** by the sequence previously established for the preparation of acyclic fragment **8**. Because the enol ether in **19d** did not survive the two consecutive oxidations (Swern/$NaClO_2$), we decided to protect it as a cyclic acetal that could be cleaved in situ during the cyclodehydration (Scheme 7). Subsequent deprotection of the primary alcohol and oxidation gave acid **23**, which was then engaged in a peptidic coupling with isoleucinamide (**24**), yielding acyclic substrate **25**. In a similar way, styrenoxyproline **26** was coupled with isoleucine derivatives **24** and **27**[31] to give the substrates required for the aza-Wacker reaction and for the ene-enamide RCM: **6** and **28**, respectively.



Scheme 7. Elaboration of the acyclic substrates for macrocyclization by cyclodehydration, intramolecular aza-Wacker reaction, or ene-enamide RCM.

The preparation of acyclic fragments finally set the stage for their cyclization (Scheme 8). We began this study with the cyclization of the masked aldehyde **25**. After consider-



Scheme 8. Macrocyclization by cyclodehydration, ene-enamide RCM, or intramolecular oxidative amidation.

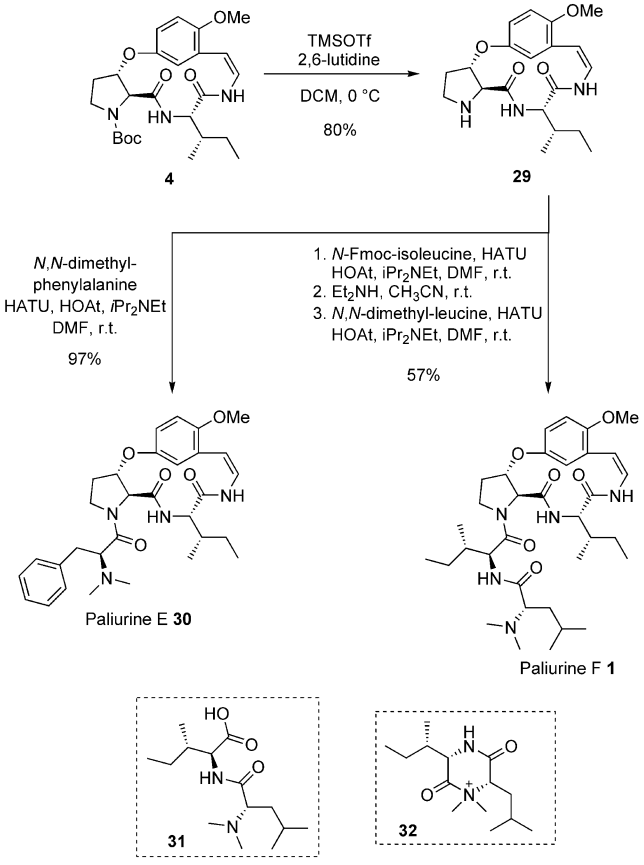
able experimentation with various promoters (TsOH, HCl, oxalic acid, iodine) and conditions for deprotection and/or activation of the acetal and for the cyclization/dehydration sequence, we eventually found that this cyclodehydration was best effected with use of 60 mol-% *p*-toluenesulfonic acid in mixture of toluene and water (8:2) at 100 °C over 7 d.

Under these conditions, the cyclized product **4** could be isolated in 34% yield, a low yield that could be attributed to extensive degradation of both starting material and macrocyclic enamide under the reaction conditions. We next focused on the intramolecular aza-Wacker reaction, using a procedure that had been shown to provide β-aminostyrenes regioselectively by intermolecular amidation.[19] Upon treatment with a mixture of $PdCl_2(CH_3CN)_2$ and $CuCl_2$ under O_2 in DME at 60 °C, amidostyrene **6** indeed furnished the desired macrocycle **4**, but in a disappointing 21% yield. The ene-enamide ring-closing metathesis proved to be a bit more efficient but turned out to be especially substrate-dependent and required extensive optimization of both reaction conditions and substitution pattern of the enamide.[31] In the best case, the methyl-substituted enamide **28** could be cyclized in 49% yield in the presence of the second-generation Grubbs catalyst in 1,2-dichloroethane at reflux. Attempts to optimize this yield by use of additives that should prevent either degradation of the catalyst or chelation with the substrate or by use of other metathesis promoters failed to give better results. Again it was clear that the copper-mediated intramolecular amidation reaction was the most viable option for formation of the macrocycle together with the installation of the enamide. We therefore decided to use this option to complete the synthesis of the target molecule, paliurine F, as well as the related alkaloid paliurine E, possessing a different side-chain.

Synthesis of Paliurines E and F

To install the side chains of paliurines E and F, the Boc group was first carefully removed in 80% yield by use of TMSOTf and 2,6-lutidine (Scheme 9). Next, a HATU/HOAt-mediated coupling of the resulting free amine **29** with *N*,*N*-dimethyl-L-phenylalanine gave the desired paliurine E (**30**) in 97% yield. The synthetic (–)-paliurine E exhibited physical, spectroscopic, and spectrometric characteristics (^{1}H NMR, ^{13}C NMR, IR, $[a]_D$, UV, and MS) identical to those reported for the natural product.[18]

Elaboration of paliurine F (**1**) proved to be more challenging than originally anticipated, and an extensive review of conditions to promote the coupling of **29** with dipeptide **31** was undertaken. Among the conditions surveyed were the use of HATU/K_2CO_3, HATU/*i*Pr_2NEt, DCC/HOBt/*i*Pr_2NEt, or EDC/HOAt/*i*Pr_2NEt. All of these proved ineffective, giving back starting material and an impressive number of side-products among which paliurine F could not even be detected. This might be attributed to the presence of the dimethylamino group in **31**, which probably interferes with the activated carboxylic acid under the coupling conditions to give highly nucleophilic and epimerizable cyclic species such as **32**. A more predictable method



Scheme 9. Synthesis of paliurines E and F.

was then used to attach the side-chain by a stepwise procedure: coupling of **29** with *N*-Fmoc-L-isoleucine, followed by removal of the Fmoc group by treatment with diethylamine in acetonitrile and subsequent coupling with *N*,*N*-dimethyl-L-leucine, gave the desired paliurine F (**1**) in 57% yield over the three final steps.[14]

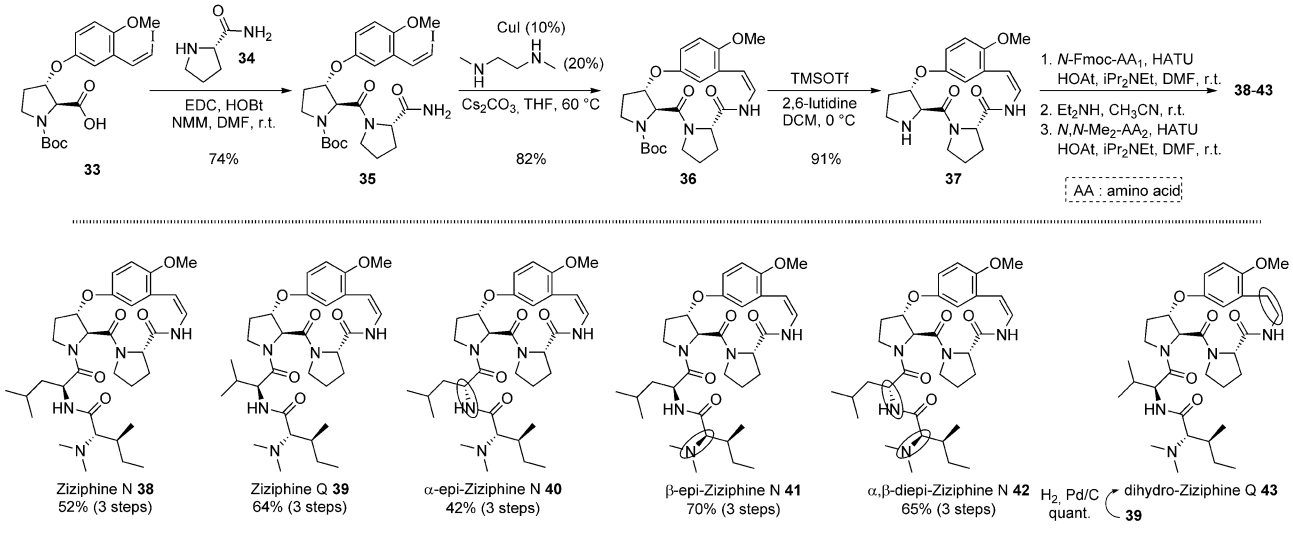
Synthesis of Ziziphines N and Q and Analogues

The success met with for the synthesis of the paliurines prompted us to apply our synthesis to the preparation of other cyclopeptide alkaloids. We therefore targeted ziziphines N (**38**) and Q (**39**), members of the ziziphine family isolated from *Ziziphus oenoplia* that display significant antiplasmodial activity.[8,32] The synthesis started from the common intermediate **33** (Scheme 10). Treatment with L-prolinamide (**34**) in the presence of EDC and HOBt gave the acyclic precursor **35**, which was smoothly cyclized, by use of catalytic copper iodide and *N*,*N*′-dimethylethylenediamine, in 82% yield. Removal of the Boc group finally gave the fully elaborated macrocycle **37**, which served as a platform for the synthesis of ziziphines N (**38**) and Q (**39**), obtained in 52 and 64% yields, respectively, after installation of the peptidic side chains.[33]

Following an observation by Han and co-workers, who demonstrated that epimerization of the side chains could have a deep impact on the biological activities of related compounds, we next turned our attention to stereochemical variations at the side-chain residues. Macrocyclic compound **37** was therefore engaged in peptidic coupling with all combinations of *N*-Fmoc-L-leucine or *N*-Fmoc-D-leucine ("AA_1" in Scheme 10) and *N*,*N*-dimethyl-L-isoleucine or *N*,*N*-dimethyl-D-*allo*-isoleucine ("AA_2" in Scheme 10), giving the ziziphine N diastereoisomers **40**, **41**, and **42**. In order to allow assessment of the role of the enamide, ziziphine Q was reduced by treatment with hydrogen in the presence of palladium on carbon in methanol to furnish dihydroziziphine Q (**43**) in quantitative yield. Biological evaluation of all these compounds is discussed at the end of this article.

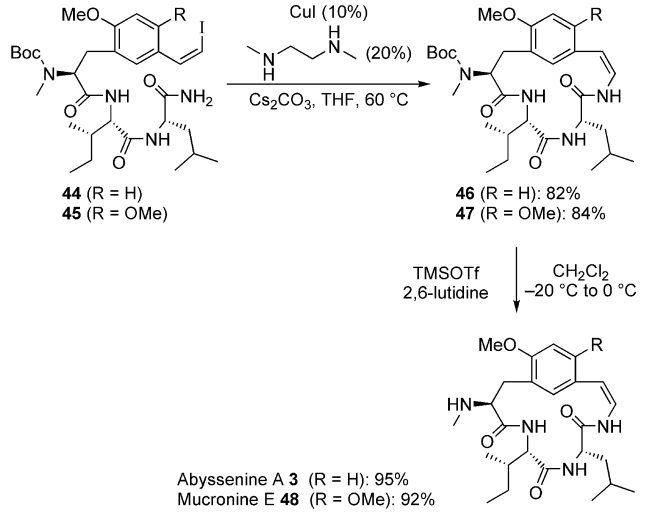
Synthesis of the 15-Membered Ring Cyclopeptide Alkaloids Abyssenine A and Mucronine E

When we started this project, only slight attention had been paid to the synthesis of 15-membered ring alkaloids.



Scheme 10. Synthesis of ziziphines N, Q, and analogues.

Their structures are slightly different from those of their 13-membered ring homologues because they incorporate a methylene group in place of the ether bond and possess three amino acids within the macrocycle. Whereas much work has been devoted to the synthesis of the 13- or 14-membered ring cyclopeptide alkaloids,[9–15] a single synthesis of a 15-membered ring compound, mucronine B,[10b,10c] featuring a macrolactamization reaction and a stepwise installation of the enamide, had been reported.[34] We therefore initiated studies directed towards the extension of our synthetic route to the preparation of this subclass of cyclopeptide alkaloids and chose abyssenine A[6c,35] (**3**) and mucronine E[6c] (**48**) as target molecules (Scheme 11). Acyclic precursors **44**[15] and **45**[16] were therefore prepared and subjected to the copper-mediated "macroenamidation" procedure. Cyclization proceeded smoothly in both cases, yielding the 15-membered ring macrocycles **46** and **47** in excellent yields, and therefore expending the scope of this protocol for the preparation of macrocyclic enamides. Careful removal of the Boc groups in **46** and **47** by treatment with TMSOTf and 2,6-lutidine provided synthetic abyssenine A (**3**) and mucronine E (**48**), respectively and completed our synthetic studies on cyclopeptide alkaloids. Biological evaluation of all these compounds, with a focus on their antibacterial and cytotoxic properties, is discussed below.



Scheme 11. Synthesis of the 15-membered ring cyclopeptide alkaloids abyssenine A and mucronine E.

Cytotoxicity Assays

The human HT1080 tumoral cell line was used to test the cytotoxicities of the ten compounds. These cells are able to undergo physiological cell death, a process termed apoptosis,[36] and are sensitive to a wide variety of cytotoxic drugs such as etoposide, cisplatin, staurosporine, or TNF-*α*. The ten synthetic cyclopeptides **1**, **3**, **30**, **38**–**43**, and **48** were first tested at high concentration (1 mM) over 48 h on HT1080 cells, and the percentages of dead cells were determined by flow cytometry analysis. In addition, we thought it would be interesting to distinguish between necrotic and non-necrotic cell death within global cell death, because necrosis is considered to be basically harmful to the organism, unlike non-necrotic cell death. Results from flow cytometry analysis are given in Figure 2 and show that paliurine F (**1**), abyssenine A (**3**), and mucronine E (**48**) are cytotoxic, whereas the other compounds are no different from controls.[37] Of the active compounds, mucronine E (**48**) is the most efficient in triggering cell death and clearly is the most interesting because it mostly induces non-necrotic cell death. Cell morphology is shown in Figure 2: floating dead cells can be observed for treatments with **1**, **3**, and **48** whereas living cells reach confluence in controls and other treatments, except in the case of paliurine E (**30**). For this compound it can be noted that the cells do not reach confluence although no significant cell death was indicated, which suggests that **30** specifically inhibits cellular proliferation.

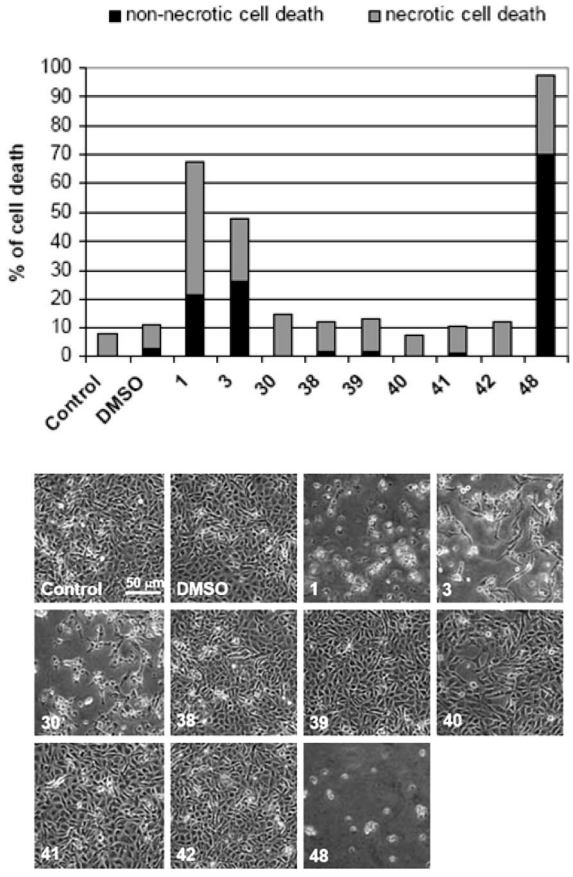


Figure 2. Cellular viability (top) and cell morphology (bottom) after 48 h treatment with synthetic cyclopeptides at 1 mM.

We next determined the IC_{50} values after incubation of cells with various concentrations of the three cytotoxic compounds (Table 3). These values confirmed that mucronine E (**48**) was the most cytotoxic and globally showed that compounds **1**, **3**, and **48** were indeed cytotoxic, although at relatively high concentrations. These results suggest that whereas the size of the macrocycle does not seem to have a crucial influence, the constitutive amino acids do, because

the paliurines and ziziphines show dramatically different cytotoxicities. Comparison of the effects of paliurines F (**1**) and E (**30**) demonstrates that the nature of the peptidic side chain also seems to be of great importance and provides the first insights into SARs of cyclopeptide alkaloids.

Table 3. Concentrations inducing 50% cellular viability (IC_{50}) in HT1080 cells after 48 h treatment.

Entry	Synthetic cyclopeptide	IC_{50} [mM]
1	paliurine F (**1**)	0.82
2	abyssenine A (**3**)	1.03
3	mucronine E (**48**)	0.68

Antimicrobial Assays

A selection of test compounds including paliurines E (**30**) and F (**1**), ziziphines N (**38**) and Q (**39**), α-*epi*-ziziphine N (**40**), β-*epi*-ziziphine N (**41**), α,β-*diepi*-ziziphine N (**42**), and the deprotected macrocycle **29** were also evaluated for in vitro antimicrobial activity, to assess whether the human cytotoxicity would also be observed in prokaryotic cells. These compounds were individually subjected to in vitro susceptibility assays on agar against three representative test microbes: a methicillin-resistant strain of *Staphylococcus aureus* (MRSA), *Bacillus anthracis*, and *Escherichia coli*. First we ran Kirby–Bauer well diffusion experiments on agar plates to look for visible signs of growth inhibition appearing around the wells. We evaluated each of the compounds at three different amounts (20, 50, and 100 μg) in each well. None of the compounds produced completely cleared inhibition zones at these quantities, whereas the control antibiotic, penicillin G, produced boldly visible growth inhibition zones even against the MRSA. Nevertheless, close inspection of the plates indicated that *B. anthracis* was somewhat more susceptible to the inhibitory effects of the compounds than either MRSA or *E. coli*. The strongest bioactivity of all the compounds screened appeared to be for paliurine F, whereas the rest of the analogues were all about equivalent in activity against *B. anthracis*, and were all completely inactive against MRSA and *E. coli*. Despite having weak bioactivity against *B. anthracis*, all of the compounds gave only very pale zones that were largely filled in with bacterial colonies, indicating partial growth inhibition, but the clearing became more pronounced as the drug amounts were increased from 20 μg to 50 μg and 100 μg. For paliurine F, the zone size also increased from 12 mm to 18 mm to 22 mm, but the zones were again still largely opaque, indicating that bacterial growth was only partially inhibited within these zones even at 50 μg well loading. In summary, the Kirby–Bauer data indicate that the compounds have only very weak antibacterial properties, with *B. anthracis* being the most vulnerable of the three microbes examined and paliurine F having the strongest potency of all the ring analogues.

To investigate this further, we conducted additional in vitro bacterial susceptibility experiments by measuring the minimum inhibitory concentration (MIC) values of the above compounds against *B. anthracis* and MRSA. This was done by mixing different amounts of the test compound into agar to afford final compound concentrations ranging from 512 μg mL^{-1} down to 4 μg mL^{-1}. The agar was then inoculated with the microbe, and incubation was carried out for 24 h at 37 °C to determine whether the compounds could inhibit bacterial replication on the surface of the agar. The MIC value was determined as being the lowest concentration of test compound capable of completely preventing opaqueness or growth of bacteria colonies on the surface of the agar. None of the compounds, including paliurine F, had a MIC value below 512 μg mL^{-1}, demonstrating the lack of significant antibacterial activity.

Conclusions

In conclusion, we have achieved the total syntheses of the cyclopeptide alkaloids paliurines E and F, ziziphines N and Q, abyssenine A, and mucronine E in average overall yields of about 10%. Our approach featured a key intramolecular cycloenamidation reaction for the construction of the paracyclophane ring. This original macrocyclization procedure allowed us simultaneously to address two synthetic challenges associated with cyclopeptide alkaloids: the formation of the enamide and of the macrocycle, which were reduced to a single operation. We have also documented the use of other strategies for the macrocyclization step, which highlighted the high efficiency of the intramolecular copper-mediated vinylation reaction that allowed us to develop a unified strategy for these natural products. Preliminary results of the biological screening showed that none of the synthetic cyclopeptides displayed significant antibacterial activity, whereas paliurine F and mucronine E turned out to be moderately cytotoxic. Further studies on the use of copper-mediated cyclization reactions[38] for the preparation of other alkaloids are underway and will be reported in due time.

Experimental Section

General: All reactions were carried out in oven- or flame-dried glassware under argon with use of standard techniques for handling air-sensitive materials.

All solvents were reagent grade. Tetrahydrofuran (THF) and toluene were freshly distilled from sodium/benzophenone under argon immediately prior to use. Dichloromethane, 1,2-dimethoxyethane, DMSO, and DMF were freshly distilled from calcium hydride. Methanol was distilled from magnesium turnings and iodine.

Diethylamine, diisopropylethylamine, *N*-methylmorpholine, and triethylamine were distilled from calcium hydride. Copper(I) iodide (99.999% purity) was purchased from Aldrich and used as supplied. Finely powdered potassium carbonate (325 mesh) and cesium carbonate were used for copper-mediated coupling reactions. All other reagents were used as supplied.

Unless otherwise noted, reaction mixtures were magnetically stirred and reactions were monitored by thin layer chromatography (Merck Kieselgel 60F_{254} plates). Chromatography was performed

with silica gel 60 (particle size 35–70 μm) supplied by SDS. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise noted.

Proton NMR spectra were recorded with use of an internal deuterium lock at ambient temperature with a Bruker 300 MHz spectrometer. Internal references of δ_H = 7.26 ppm and δ_H = 2.50 ppm were used for residual $CHCl_3$ in $CDCl_3$ and residual $[D_5]$DMSO in $[D_6]$DMSO, respectively. Data are presented as follows: chemical shift (in ppm on the δ scale relative to δ_{TMS} = 0 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint. = quintuplet, m = multiplet, br = broad, app = apparent), coupling constant (*J*/Hz) and integration. Resonances that are either partially or fully obscured are denoted obscured (obs.). Carbon-13 NMR spectra were recorded at 75 MHz. Internal references of δ_C = 77.16 ppm and δ_C = 39.52 ppm were used for $CDCl_3$ and $[D_6]$DMSO, respectively.

Infrared spectra were recorded with a Nicolet OPUS IR (impact 400D) spectrophotometer. Optical rotations were recorded with a Perkin–Elmer 341 polarimeter at 589 nm and are reported as follows: $[\alpha]_D^{20}$, concentration (*c* in g/100 mL), and solvent. Melting points were recorded with a Büchi B-545 instrument. UV spectra were recorded in methanol with a Shimadzu UV-160A instrument. Mass spectra were obtained with a GCMS Hewlett–Packard 5989B spectrometer (EIMS: 70eV, CIMS: 230 eV, ESIMS: analytica Brandford ionization interface, capex 180 V, skimmer 30 V).

Because of the presence of complex rotameric mixtures, it was necessary to record ^1H and ^{13}C NMR spectra of some compounds in $[D_6]$DMSO at 345–355 K, provided that they were stable enough at these temperatures. Even at those temperatures, some ^{13}C peaks were poorly resolved and are denoted "broad" (br).

(2*S*,3*S*)-3-Hydroxyproline Methyl Ester Hydrochloride (10b): Thionyl chloride (780 μL, 10.7 mmol) was slowly added at 0 °C to a suspension of commercially available (2*S*,3*S*)-3-hydroxyproline (**10d**, 1.0 g, 7.6 mmol) in methanol (10 mL). The resulting mixture was allowed to warm to room temp., heated at reflux for 2 h, and concentrated under vacuum to yield the desired methyl ester as a white solid (1.4 g, quantitative crude yield), which was used in the next step without further purification. $[\alpha]_D^{20}$ = +11 (*c* = 1.5, MeOH). ^1H NMR (300 MHz, D_2O): δ = 4.66–4.69 (obs. m, 1 H), 4.29 (d, *J* = 2.7 Hz, 1 H), 3.76 (s, 3 H), 3.46–3.51 (m, 2 H), 1.96–2.17 (m, 2 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 168.4, 72.9, 66.4, 54.0, 44.4, 31.4 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3288, 1741, 1588, 1444, 1235 cm^{-1}. CIMS (NH_3 gas): 146, 86. HRMS (CI, NH_3 gas): *m*/*z* calcd. for $C_6H_{12}NO_3$ $[M + H]^+$ 146.0817; found 146.0812.

(2*S*,3*S*)-1-(*tert*-Butoxycarbonyl)-3-hydroxyproline (10a): Triethylamine (3.0 mL, 21.5 mmol) and di-*tert*-butyl dicarbonate (1.9 g, 8.6 mmol) were successively added to a solution of **10b** (1.3 g, 7.2 mmol) in dichloromethane (30 mL). The resulting mixture was stirred at room temp. overnight, quenched with water, and extracted with dichloromethane. The combined organic layers were washed with HCl solution (1 M) and brine, dried with $MgSO_4$, filtered, and concentrated to yield the desired carbamate as a colorless oil (1.8 g, quantitative crude yield), which was used in the next step without further purification. $[\alpha]_D^{20}$ = –21 (*c* = 1.4, $CHCl_3$). ^1H NMR (300 MHz, $[D_6]$DMSO): δ = 4.42 (br. s, 1 H), 4.28 and 4.16 (rotamers, 2 br. s, 1 H), 3.72 (s, 3 H), 3.54–3.66 (m, 2 H), 2.80 (dd, *J* = 13.0, 3.3 Hz, 1 H), 2.03–2.16 (m, 1 H), 1.85–1.93 (m, 1 H), 1.45 and 1.39 (rotamers, s, s, 9 H) ppm. ^{13}C NMR (75 MHz, $[D_6]$-DMSO): δ = 171.9 and 171.5 (rotamers), 154.4 and 154.1 (rotamers), 80.4, 75.3, 68.1 and 67.9 (rotamers), 52.5 and 52.3 (rotamers), 44.7 and 44.3 (rotamers), 32.7 and 32.4 (rotamers), 28.5 and 28.4 (rotamers); CIMS (NH_3 gas): 246, 207, 190, 186, 146. ESIHRMS: *m*/*z* calcd. for $C_{11}H_{19}NO_5Na$ $[M + Na]^+$ 268.1161; found 268.1150.

(2*R*,3*S*)-1-(Triphenylmethyl)-3-hydroxyproline Methyl Ester (10c): Triethylamine (845 μL, 6.0 mmol) and trityl chloride (613 mg, 2.2 mmol) were added to a solution of **10b** (363 mg, 2.0 mmol) in dichloromethane (10 mL). The resulting mixture was stirred at room temp. for 5 h, quenched with water, and extracted with dichloromethane. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc/PE 1:1) to yield the desired protected pyrrolidine **10c** as a white solid (512 mg, 1.32 mmol, 66%); m.p. 107 °C. $[\alpha]_D^{20}$ = –54 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.63 (d, *J* = 7.7 Hz, 6 H), 7.33 (t, *J* = 7.7 Hz, 6 H), 7.23 (t, *J* = 7.3 Hz, 3 H), 4.05 (br. s, 1 H), 3.80 (s, 1 H), 3.70 (s, 3 H), 3.50 (td, *J* = 10.7, 3.3 Hz, 1 H), 2.79–2.88 (m, 1 H), 2.05–2.17 (m, 1 H), 1.21–1.33 (m, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.7, 143.8, 129.6, 127.9, 126.7, 70.2, 51.9, 47.0, 33.3 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3488, 2971, 2863, 1721, 1440, 1258, 1163, 968, 877, 733, 657 cm^{-1}. ESIMS (positive mode): *m*/*z* = 410.3, 243.2, 166.1. ESIHRMS: *m*/*z* calcd. for $C_{25}H_{25}NO_3Na$ $[M + Na]^+$ 410.1732; found 410.1738.

(2*S*,3*S*)-1-(*tert*-Butoxycarbonyl)-3-hydroxyproline (10e): A solution of commercially available (2*S*,3*S*)-3-hydroxyproline (**10d**, 400 mg, 3.0 mmol) in a mixture of THF (7 mL) and aqueous NaOH (1 M, 7 mL) was treated with di-*tert*-butyl dicarbonate (800 mg, 3.7 mmol). The resulting mixture was vigorously stirred at room temp. for 2 h, acidified to pH 1 with HCl (1 M), and extracted with diethyl ether. The combined organic layers were dried with $MgSO_4$, filtered, and concentrated to yield the desired carbamate as a white solid (708 mg, quantitative crude yield), which was used in the next step without further purification; m.p. 157 °C. $[\alpha]_D^{20}$ = –9 (*c* = 1.4, MeOH). ^1H NMR (300 MHz, MeOD): δ = 4.88 (br. s, 2 H), 4.37 (app. t, *J* = 1.9 Hz, 1 H), 4.14 and 4.07 (rotamers, s, s, 1 H), 3.44–3.57 (m, 2 H), 1.96–2.08 (m, 1 H), 1.83–1.90 (m, 1 H), 1.45 and 1.40 (rotamers, s, s, 1 H) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 175.7 and 175.4 (rotamers), 157.8 and 157.5 (rotamers), 82.9 and 82.7 (rotamers), 77.2 and 76.4 (rotamers), 70.8 and 70.5 (rotamers), 47.2 and 46.8 (rotamers), 34.8 and 34.2 (rotamers), 30.1 and 30.0 (rotamers) ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3207, 1755, 1661, 1434, 1252 cm^{-1}. CIMS (NH_3 gas): *m*/*z* = 249, 232, 193, 176, 132, 86. ESIHRMS: *m*/*z* calcd. for $C_{10}H_{17}NO_5Na$ $[M + Na]^+$ 254.1004; found 254.1009.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)methyl]-3-hydroxypyrrolidine (10g): Lithium borohydride (44 mg, 2.0 mmol) was added at 0 °C to a solution of **10a** (246 mg, 1.0 mmol) in THF (5 mL). The resulting mixture was stirred overnight at room temp., quenched by slow addition of a saturated solution of NH_4Cl, basified with NaOH (1 M) and extracted with diethyl ether. The combined organic layers were washed with HCl solution (1 M) and brine, dried with $MgSO_4$, filtered, and concentrated to yield the intermediate diol as a white solid (220 mg, quantitative crude yield). This solid was dissolved in THF (1 mL) and treated with imidazole (82 mg, 1.20 mmol) and *tert*-butyldimethylsilyl chloride (146 mg, 0.97 mmol). The resulting white slurry was vigorously stirred at 0 °C for 2 h and quenched by addition of water. The organic layer was separated and the aqueous layer was extracted with diethyl ether. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated under vacuum. The crude residue was purified by flash chromatography over silica gel (petroleum ether/EtOAc 8:2) to yield the protected product **10g** as a white solid (303 mg, 0.92 mmol, 92%); m.p. 110 °C. $[\alpha]_D^{20}$ = –38 (*c* = 2.3, $CHCl_3$). ^1H NMR (300 MHz, $[D_6]$-

DMSO, 343 K): δ = 4.66 (s, 1 H), 4.18 (br. s, 1 H), 3.67 (app. q, J = 6.8 Hz, 1 H), 3.53 (br. s, 2 H), 3.39 (td, J = 9.8, 7.3 Hz, 1 H), 3.23 (td, J = 9.8, 2.6 Hz, 1 H), 1.92–2.04 (m, 1 H), 1.62–1.73 (m, 1 H), 1.42 (s, 9 H), 0.88 (s, 9 H), 0.05, 0.03 (s, s, 3 H, 3 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 343 K): δ = 153.4, 77.7, 71.7 (br), 66.6, 61.8 (br), 44.4, 31.4 (br), 27.8, 25.3, 17.4, –5.9, –6.0 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3375, 2925, 2756, 2592, 1675, 1424, 1096, 835 cm^{-1}. ESIMS (positive mode): m/z = 354.3, 298.3, 254.2. HRMS (CI, NH$_3$): m/z calcd. for $C_{16}H_{34}NO_4Si$ [M + H]$^+$ 332.2257; found 332.2252.

(2*R*,3*S*)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-hydroxypyrrolidine (10f): Anhydrous zinc bromide (1.0 g, 4.5 mmol) was added to a solution of **10g** (331 mg, 1.0 mmol) in dichloromethane (30 mL). The resulting slurry was stirred overnight at room temp., concentrated, and purified by flash chromatography over silica gel (EtOAc) to yield the desired deprotected pyrrolidine **10f** as a white solid (152 mg, 0.66 mmol, 66%); m.p. 110 °C. $[\alpha]_D^{20}$ = –38 (c = 2.3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.07–4.16 (m, 1 H), 3.69 (A of ABX syst., J = 10.0, 5.1 Hz, 1 H), 3.56 (B of ABX syst., J = 10.0, 6.2 Hz, 1 H), 3.0–3.12 (m, 3 H), 2.33 (s, 2 H), 1.95–2.06 (m, 1 H), 1.70–1.80 (m, 1 H), 0.89 (s, 9 H), 0.1 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 74.9, 67.8, 64.4, 44.9, 35.1, 26.1, 18.4, –5.3 ppm. ESIHRMS: m/z calcd. for $C_{11}H_{25}NO_2SiNa$ [M + Na]$^+$ 254.1552; found 254.1544.

Methyl 1-(*tert*-Butoxycarbonyl)-4,5-dihydropyrrole-2-carboxylate (13): A 15 mL pressure tube was charged with **10a** (245 mg, 1.0 mmol), iodobenzene (135 μL, 1.2 mmol), cesium carbonate (650 mg, 2.0 mmol), 1,10-phenanthroline (38 mg, 0.2 mmol), and copper(I) iodide (19 mg, 0.1 mmol). Toluene (0.5 mL) was added, the pressure tube was closed, and the brownish suspension was heated to 110 °C for 24 h. The crude reaction mixture was filtered through a plug of silica gel (washed with EtOAc), concentrated, and purified by flash chromatography over silica gel (EtOAc/petroleum ether 2:8) to yield elimination product **13** as a pale yellow oil (227 mg, 0.73 mmol, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.78 (t, J = 3.0 Hz, 1 H), 3.93 (t, J = 8.8 Hz, 2 H), 3.80 (s, 3 H), 2.62 (td, J = 8.8, 3.0 Hz, 2 H), 1.44 (s, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.8, 152.9, 136.7, 119.4, 81.1, 52.1, 48.4, 28.6, 28.2 ppm. ESIHRMS: m/z calcd. for $C_{11}H_{17}NO_4Na$ [M + Na]$^+$ 250.1055; found 250.1039.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-phenoxypyrrolidine (14): A 15 mL pressure tube was charged with **10g** (330 mg, 1.0 mmol), iodobenzene (110 μL, 1.0 mmol), cesium carbonate (650 mg, 2.0 mmol), 1,10-phenanthroline (38 mg, 0.2 mmol), and copper(I) iodide (19 mg, 0.1 mmol). Toluene (0.5 mL) was added, the pressure tube was closed, and the brownish suspension was heated to 125 °C for 24 h. Another portion of iodobenzene (110 μL, 1.0 mmol) was then added and the reaction mixture was heated for an additional 24 h and allowed to cool to room temp. The crude reaction mixture was finally filtered through a plug of silica gel (washed with EtOAc), concentrated, and purified by flash chromatography over silica gel (EtOAc/petroleum ether 3:7) to yield ether **14** a pale yellow sticky oil (306 mg, 0.75 mmol, 75%). $[\alpha]_D^{20}$ = –13 (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.13–7.20 (m, 2 H), 6.80–6.92 (m, 3 H), 4.79 (dd, J = 14.1, 3.5 Hz, 1 H), 3.95–3.98 and 3.76–3.83 (rotamers, m, m, 0.3, 1.7 H), 3.33–3.58 (m, 3 H), 1.97–2.21 (m, 2 H), 1.36 (s, 9 H), 0.84 (s, 9 H), 0.00 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.5, 154.6, 129.5, 120.9, 115.6, 79.7 and 79.3 (rotamers), 79.0 and 78.5 (rotamers), 64.1, 63.1, and 62.5 (rotamers), 45.4 and 45.1 (rotamers), 30.1 and 29.0 (rotamers), 28.6, 26.0, 18.3, –5.4 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2955, 1695, 1600, 1393 cm^{-1}. CIMS (NH$_3$ gas): m/z = 408, 352, 308, 294. ESIHRMS: m/z calcd. for $C_{22}H_{37}NO_4SiNa$ [M + Na]$^+$ 430.2390; found 430.2402.

4-Iodo-1-methoxy-2-vinylbenzene (11c): *n*-BuLi (1.6 M in hexanes, 32.0 mL, 51.2 mmol) was added at –78 °C to a suspension of methyltriphenylphosphonium bromide (20.5 g, 57.3 mmol) in THF (200 mL). The resulting orange solution was stirred at –78 °C for 1 h and a solution of 5-iodo-2-methoxy-benzaldehyde (**11b**,[14] 5.0 g, 19.1 mmol) in THF (50 mL) was added by cannula. The reaction mixture was warmed to 0 °C over 2 h and quenched with saturated aqueous NH_4Cl solution (100 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (Et_2O/petroleum ether 2:8) to give the desired substituted styrene **11c** (4.1 g, 15.8 mmol, 83%) as a pale yellow liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65 (d, J = 2.2 Hz, 1 H), 7.42 (dd, J = 8.6, 2.2 Hz, 1 H), 6.84 (dd, J = 17.7, 11.1 Hz, 1 H), 6.54 (d, J = 8.6 Hz, 1 H), 5.63 (d, J = 17.7 Hz, 1 H), 5.21 (d, J = 11.1 Hz, 1 H), 3.74 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.6, 137.4, 135.2, 130.4, 129.4, 115.8, 113.2, 83.2, 55.7 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2991, 1480, 1240, 1122, 1025 cm^{-1}. ESIMS (positive mode): m/z = 283.1 ppm. ESIHRMS: m/z calcd. for C_9H_9IONa [M + Na]$^+$ 282.9596; found 282.9603.

4-Iodo-1-methoxy-2-(2-methoxyvinyl)benzene (11d): Potassium bis-(trimethylsilyl)amide (0.5 M solution in toluene, 115 mL, 58 mmol) was added over 30 min to a cooled solution (–78 °C) of methoxymethyltriphenylphosphonium chloride (24.5 g, 71.5 mmol) in dry THF (200 mL). The resulting red solution was stirred at –78 °C for 30 min and at 0 °C for 20 min, and was cooled back to –78 °C. 5-Iodo-2-methoxybenzaldehyde (**11b**,[14] 4 g, 15.3 mmol) in dry THF (60 mL) was then added dropwise at –78 °C by cannula and the resulting orange-red solution was allowed to warm to room temp. and to stir overnight. The mixture was quenched at 0 °C with water and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether, and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was finally purified by flash chromatography over silica gel (EtOAc/EP 5:95) to give the desired 2-methoxyvinyl product as a pale yellow, oily solid (4.2 g, 14.5 mmol, 95%) and as a 1:1 mixture of *Z* and *E* isomers; m.p. 41 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.30 and 7.53 (stereoisomers, d, J = 2.3 Hz, d, J = 2.2 Hz, 1 H), 7.40 and 7.37 (stereoisomers, dd, J = 8.6, 2.3 Hz, dd, J = 8.6, 2.2 Hz, 1 H), 7.12 and 6.19 (stereoisomers, d, J = 13.0 Hz, d, J = 7.2 Hz, 1 H), 6.58 and 6.57 (stereoisomers, d, J = 8.6 Hz, d, J = 8.6 Hz, 1 H), 5.91 and 5.54 (stereoisomers, d, J = 13.0 Hz, d, J = 7.2 Hz, 1 H), 3.80 and 3.69 (stereoisomers, s, s, 3 H), 3.78 and 3.77 (stereoisomers, s, s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.7 and 155.4 (stereoisomers), 150.4 and 148.8 (stereoisomers), 137.9 and 135.3 (stereoisomers), 135.0 and 134.3 (stereoisomers), 127.2 and 128.0 (stereoisomers), 112.9 and 112.4. (stereoisomers), 99.4 and 97.4 (stereoisomers), 83.3, 60.9, 56.5 and 55.6 (stereoisomers) ppm. IR (neat): $\tilde{\nu}_{max}$ = 3068, 2997, 2930, 2843, 1639, 1573, 1481, 1245, 1219, 1097, 933, 790 cm^{-1}. CIMS (NH$_3$ gas): m/z = 290, 289, 232, 148, 133, 90, 77. ESIHRMS: m/z calcd. for $C_{10}H_{11}IO_2Na$ [M + Na]$^+$ 312.9701; found 312.9708.

(*Z*)-4-Iodo-2-(2-iodovinyl)-1-methoxybenzene (11e): A solution of NaHMDS (2.0 M solution in THF, 7.6 mL, 15.2 mmol) was added dropwise at room temp. to a suspension of iodomethyltriphenylphosphonium iodide (8.6 g, 16.2 mmol) in THF (90 mL). The resulting red-orange solution was stirred at room temp. for 20 min and cooled to –78 °C before addition of HMPA (10 mL) and a solution of **11b**[14] (2.5 g, 9.5 mmol) in THF (60 mL). The reaction

mixture was stirred at –85 °C for three hours and quenched at –78 °C by addition of saturated aqueous $NaHCO_3$. The mixture was allowed to warm to room temp., diluted with Et_2O, and filtered through a plug of Celite®, which was thoroughly washed with diethyl ether. The biphasic filtrate was separated and the organic layer was dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (Et_2O/petroleum ether 3:97) to give the desired vinyl iodide **11e** as a pale yellow solid (3.1 g, 8.0 mmol, 76%). The diastereoisomeric excess was determined by analysis of ^1H NMR spectra of the crude reaction mixture and was found to be higher than 95%; m.p. 67 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.91 (d, J = 2.2 Hz, 1 H), 7.50 (dd, J = 8.7, 2.0 Hz, 1 H), 7.19 (d, J = 8.5 Hz, 1 H), 6.56 (d, J = 8.6 Hz, 2 H), 3.73 (s, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.7, 138.2, 137.3, 133.7, 128.4, 122.9, 82.5, 82.0, 55.7 ppm. ESIHRMS: m/z calcd. for $C_9H_8I_2ONa$ $[M + Na]^+$ 408.8562; found 408.8570.

2-(5-Iodo-2-methoxybenzyl)-1,3-dioxolane (11f): Ethylene glycol (3 mL) and concentrated HCl (35 wt.-% in H_2O, 4 drops) were added dropwise to a solution of enol ether **11d** (200 mg, 0.69 mmol) in Et_2O (3 mL). The resulting mixture was stirred at room temp. for 3 d, carefully quenched with aqueous Na_2CO_3 (7 wt.-% in H_2O), and diluted with diethyl ether. The aqueous layer was extracted with Et_2O and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude product was purified by flash chromatography over silica gel (EtOAc/petroleum ether/Et_3N 10:89.5:0.5) to give the desired dioxolane **11f** (211 mg, 0.66 mmol, 96%) as a white solid; m.p. 64 °C. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.44 (d, J = 2.0 Hz, 1 H), 7.40 (dd, J = 8.5, 2.1 Hz, 1 H), 6.52 (d, J = 8.6 Hz, 1 H), 5.01 (t, J = 5.0 Hz, 1 H), 3.86–3.91 (m, 2 H), 3.74–3.77 (m, 2 H), 3.70 (s, 3 H), 2.83 (d, J = 5.0 Hz, 2 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.5, 139.7, 136.7, 127.5, 112.7, 103.2, 82.8, 64.9, 55.6, 34.7 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 2935, 2765, 1490, 1244, 1122, 1072, 805 cm^{-1}. EIMS: m/z = 320, 247, 73. ESIHRMS: m/z calcd. for $C_{11}H_{13}IO_3Na$ $[M + Na]^+$ 342.9807; found 342.9801.

Typical Procedure for Copper-Mediated Arylation of Hydroxypyrrolidine (10g): A 15 mL pressure tube was charged with **10g** (330 mg, 1.0 mmol), aryl iodide **11** (1.0 mmol), cesium carbonate (652 mg, 2.0 mmol), 1,10-phenanthroline (36 mg, 0.2 mmol), and copper(I) iodide (19 mg, 0.1 mmol). Toluene (0.5 mL) was added, the pressure tube was closed, and the brownish suspension was heated to 125 °C (110 °C in the case of styrene derivative **11c**) for 24 h. Another portion of aryl iodide **11** (0.5 mmol) was then added and the reaction mixture was heated for an additional 24 h and allowed to cool to room temp. The crude reaction mixture was finally filtered through a plug of silica gel (washed with EtOAc), concentrated, and purified by flash chromatography over silica gel to give the desired ethers **19**.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-(4-methoxy-3-methoxycarbonylphenoxy)pyrrolidine (19a): Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/petroleum ether 3:7; pale sticky oil (scale: 332 mg of compound obtained; yield 67%). $[\alpha]_D^{20}$ = +4 (c = 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.92 (d, J = 3.6 Hz, 1 H), 7.65 (dd, J = 7.6, 1.0 Hz, 1 H), 6.67 (dd, J = 8.8, 3.3 Hz, 1 H), 5.38 (t, J = 4.2 Hz, 1 H), 3.66–3.97 (m, 2 H), 3.81 (s, 3 H), 3.78 (s, 3 H), 3.64 (dd, J = 10.8, 4.9 Hz, 1 H), 3.39–3.54 (m, 2 H), 2.24–2.33 (m, 1 H), 1.97–2.0 (m, 1 H), 1.40 (s, 9 H), 0.84 (s, 9 H), 0.0 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.0, 164.2, and 164.4 (rotamers), 159.0, 154.4, 142.0, and 142.2 (rotamers), 139.8 and 140.0 (rotamers), 122.1 and 122.4 (rotamers), 114.4, 81.7, 79.4, and 79.7 (rotamers), 65.0, 62.0, and 62.7 (rotamers), 56.1, 45.2, and 45.5 (rotamers), 29.7 and 30.6 (rotamers), 28.6, 25.8, 18.2, –5.5 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2958, 1731, 1693, 1588, 1485, 1397, 1118 cm^{-1}. ESIMS (positive mode): m/z = 630.1, 614.2, 518.3, 492.1. HRMS (CI, NH_3) m/z calcd. for $C_{25}H_{41}NO_7Si$ $[M]^+$ 495.2652; found 495.2639.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-(3-formyl-4-methoxyphenoxy)pyrrolidine (19b): Solvent system for purification by flash chromatography over silica gel, eluent gradient from CH_2Cl_2 to CH_2Cl_2/EtOH 95:5; pale yellow sticky oil (scale: 1.9 g of compound obtained; yield 75%). $[\alpha]_D^{20}$ = –5 (c = 1.2, $CHCl_3$). ^1H NMR (300 MHz, [D$_6$]DMSO, 345 K): δ = 10.33 (s, 1 H), 7.27 (d, J = 3.1 Hz, 1 H), 7.22–7.25 (m, 1 H), 7.13 (d, J = 8.8 Hz, 1 H), 4.82 (d, J = 4.1 Hz, 1 H), 3.78–3.93 (m, 2 H), 3.88 (s, 3 H), 3.58–3.63 (m, 1 H), 3.34–3.51 (m, 2 H), 2.23 (app. qt, J = 9.8, 4.7 Hz, 1 H), 2.10 (app. dd, J = 13.4, 6.2 Hz, 1 H), 1.42 (s, 9 H), 0.88 (s, 9 H), 0.06 (s, 6 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]-DMSO, 345 K): δ = 186.9, 155.2, 152.2, 149.4, 123.8, 123.0, 113.0, 112.5, 78.8 (br), 77.2, 62.6, 60.7 (br), 55.0, 43.3, 27.0 (br), 26.7, 24.2, 16.4, –7.1 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2883, 2755, 1694, 1490, 1398 cm^{-1}. ESIMS (positive mode): m/z = 970.0, 953.7, 599.5, 583.5, 488.4. HRMS (CI, NH_3) m/z calcd. for $C_{24}H_{39}NO_6Si$ $[M]^+$ 465.2547; found 465.2552.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-(4-methoxy-3-vinylphenoxy)pyrrolidine (19c): Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/petroleum ether 15:85; colorless oil (scale: 1.1 g of compound obtained; yield 79%). $[\alpha]_D^{20}$ = –2 (c = 1.5, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 6.96–7.06 (m, 2 H), 6.85–6.91 (m, 1 H), 6.78 and 6.77 (rotamers, d, J = 8.8 Hz, d, J = 8.8 Hz, 1 H), 5.72 and 5.71 (rotamers, d, J = 17.7 Hz, d, J = 17.7 Hz, 1 H), 5.28 and 5.27 (rotamers, d, J = 11.1 Hz, d, J = 11.1 Hz, 1 H), 4.84 and 4.80 (rotamers, d, J = 3.0 Hz, d, J = 4.1 Hz, 1 H), 3.80–4.07 (m, 2 H), 3.81 (s, 3 H), 3.46–3.73 (m, 3 H), 2.05–2.29 (m, 2 H), 1.49 (s, 9 H), 0.92 (s, 9 H), 0.08 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.7, 151.6, and 151.3 (rotamers), 151.4, 131.5, and 131.4 (rotamers), 128.0, 115.5, 115.1 and 114.6 (rotamers), 115.0, 112.2, and 112.1 (rotamers), 80.0 and 79.8 (rotamers), 79.6 and 79.4 (rotamers), 64.2, 63.1, and 62.5 (rotamers), 56.3 and 56.2 (rotamers), 45.4 and 45.1 (rotamers), 30.1 and 28.9 (rotamers), 28.7, 26.0, 18.4, –5.3, –5.4 ppm. IR (neat): $\tilde{\nu}_{max}$ = 1694, 1682, 1494, 1393, 1180 cm^{-1}. CIMS (NH_3 gas): m/z (%) = 464 (37), 408 (31), 364 (26), 350 (100), 306 (12). ESIHRMS: m/z calcd. for $C_{25}H_{41}NO_5SiNa$ $[M + Na]^+$ 486.2652, found 486.2638.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-[3-(2-methoxyvinyl)-4-methoxyphenoxy]pyrrolidine (19d): Solvent system for purification by flash chromatography over silica gel, eluent gradient from EtOAc/petroleum ether 1:9 to 2:8; colorless oil (scale: 321 mg of compound obtained; yield 65%). This compound was obtained as a 7:3 mixture of isomers and each isomer exists as a mixture of rotamers. $[\alpha]_D^{20}$ = –2 (c = 1.1, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.60 (app. s, 0.3 H), 7.12 (dd, J = 13.0 and 3.1 Hz, 0.7 H), 6.82 (br. s, 0.7 H), 6.70–6.78 (m, 2 H), 6.09 (dd, J = 7.1 and 4.1 Hz, 0.4 H), 5.89 (d, J = 11.9 Hz, 0.6 H), 5.52 (d, J = 7.2 Hz, 0.3 H), 4.71 (dd, J = 14.2 and 2.7 Hz, 1 H), 4.03 (br. s, 0.5 H), 3.75–3.91 (m, 5.5 H), 3.67 (s, 3 H), 3.44–3.60 (m, 2 H), 1.97–2.18 (m, 2 H), 1.38 (s, 9 H), 0.84 (s, 9 H), 0.00 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.6, 151.4, 151.3, 151.1, 150.9, 150.8, 150.5, 150.4, 150.1, 150.0, 148.3, 148.2, 126.6, 125.8, 125.7, 118.1, 117.4, 114.5, 114.3, 113.6, 113.3, 112.9, 100.3, 98.7, 98.6, 80.0, 79.9, 79.7, 79.6, 79.4, 79.3, 79.2, 64.3, 64.2, 63.0, 62.4, 62.3, 60.7, 56.4, 56.3, 56.2, 56.1, 56.0, 45.5, 45.4, 45.2, 45.0, 30.3, 30.1, 29.8, 29.0, 28.8, 28.6, 26.0, 18.3, –5.3 ppm. IR (neat): $\tilde{\nu}_{max}$ = 3000, 1705, 1694, 1681, 1650, 1494, 1392, 1224, 1096,

777 cm^{-1}. CIMS (NH_3 gas): *m*/*z* = 493, 438, 380. ESIHRMS: *m*/*z* calcd. for $C_{26}H_{43}NO_6SiNa$ $[M + Na]^+$ 516.2757, found 516.2773.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]pyrrolidine (19f): Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/ petroleum ether/Et_3N 14.5:85:0.5; orange oil (scale: 126 mg of compound obtained; yield 47%). $[\alpha]_D^{20}$ = –0.3 (*c* = 1.4, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 6.78–6.86 (m, 2 H), 6.75 and 6.72 (rotamers, s, s, 1 H), 5.11 (dd, *J* = 10.4, 5.1 Hz, 1 H), 4.78 (dd, *J* = 13.2, 2.9 Hz, 1 H), 3.98–4.03 (m, 1 H), 3.96–3.98 (m, 2 H), 3.86–3.91 (m, 1 H), 3.83–3.85 (m, 2 H), 3.78–3.81 (m, 1 H), 3.77 (s, 3 H), 3.44–3.57 (m, 2 H), 2.94–2.97 (m, 2 H), 2.01–2.26 (m, 2 H), 1.46 (s, 9 H), 0.91 (s, 9 H), 0.01 (s, 6 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.7, 152.2, 151.2 and 151.1 (rotamers), 126.3 and 126.2 (rotamers), 120.0 and 119.7 (rotamers), 113.9 and 113.7 (rotamers), 111.2 and 111.4 (rotamers), 103.7 (C_3), 79.7 and 79.3 (rotamers), 65.0, 64.2, 63.1, 56.1 and 56.0 (rotamers), 45.5 and 45.1 (rotamers), 35.3 and 35.2 (rotamers), 30.2 and 29.8 (rotamers), 28.9 and 28.6 (rotamers), 26.0, 18.4, –6.3 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2966, 1731, 1690, 1501, 1383, 1117 cm^{-1}. CIMS (NH_3 gas): *m*/*z* = 524, 468, 424, 410.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-[(*tert*-butyldimethylsilyloxy)-methyl]-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy)pyrrolidine (19f): Preparation from enol ether **19d**. *p*-Toluenesulfonic acid monohydrate (50 mg, 0.25 mmol) and ethylene glycol (840 μL, 15.1 mmol) were added to a solution of **19d** (150 mg, 0.30 mmol) in toluene (7 mL). The solution was heated at reflux under a Dean–Stark apparatus, monitored by TLC until all starting material was consumed, concentrated, and diluted with water. The aqueous solution was extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was finally purified by flash chromatography over silica gel (EtOAc/petroleum ether/Et_3N 14.5:85:0.5) to give acetal **19f** (110 mg, 0.21 mmol, 70%) as an orange oil.

(2*R*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-(hydroxymethyl)-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]pyrrolidine: A solution of **19f** (440 mg, 0.84 mmol) in THF (9 mL) was treated at –10 °C with a solution of TBAF (1 M solution in THF, 1.25 mL, 1.25 mmol). The resulting light orange mixture was allowed to warm to room temp. over 60 min and quenched with water. The aqueous layer was extracted with Et_2O and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was finally purified by flash chromatography over silica gel (gradient from EtOAc/petroleum ether/Et_3N 34.5:65:0.5 to EtOAc/petroleum ether/Et_3N 44.5:55:0.5) to give the unprotected alcohol (344 mg, 0.84 mmol, quant) as an orange oil. $[\alpha]_D^{20}$ = –16 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 6.85 (br. s, 1 H), 6.74 (br. s, 2 H), 5.07 (t, *J* = 4.9 Hz, 1 H), 4.80 and 4.60 (rotamers, 2 br. s, 1 H), 3.87–4.13 (m, 2 H), 3.81–3.95 (m, 4 H), 3.81–3.83 (m, 1 H), 3.74 (s, 3 H), 3.43–3.58 (m, 2 H), 2.97 (A of ABX syst., *J* = 13.7, 4.8 Hz, 1 H), 2.89 (B of ABX syst., *J* = 13.8, 5.1 Hz, 1 H), 2.08 (br. s, 2 H), 1.44 (s, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 156.2, 152.3, 150.6, 126.0, 119.3, 114.3, 111.4, 103.6, 80.2 and 80.0 (rotamers), 79.6, 65.0, 64.9, 64.3, 56.0, 45.4, 35.0, 29.9, 28.5 ppm. IR (neat): $\tilde{\nu}_{max}$ = 3455, 3037, 1711, 1667, 1514, 1503, 1454, 1416, 1123, 944, 875 cm^{-1}. ESIMS (positive mode): *m*/*z* = 841.6, 432.3, 376.3. ESIHRMS: *m*/*z* calcd. for $C_{21}H_{31}NNaO_7$ $[M + Na]^+$ 432.1998, found 432.2014.

(2*S*,3*S*)-1-(*tert*-Butoxycarbonyl)-2-formyl-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]pyrrolidine: DMSO (220 μL, 3.1 mmol) was added at –78 °C to a solution of oxalyl chloride (230 μL, 2.6 mmol) in dichloromethane (7 mL). The resulting solution was stirred at –78 °C for 30 min and a solution of (2*R*,3*S*)-1-(*tert*-butoxycarbonyl)-2-(hydroxymethyl)-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]pyrrolidine (500 mg, 1.22 mmol) in dichloromethane (6.5 mL) was added dropwise by cannula. The reaction mixture was stirred for 40 min at –78 °C before dropwise addition of triethylamine (740 μL, 5.2 mmol), and the mixture was warmed to –10 °C over 2 h. The reaction was next quenched at –10 °C with water and diluted with CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated to give the desired aldehyde as pale yellow oil (497 mg, 1.22 mmol, quant.), which was used without purification in the next step. An analytical sample was purified by flash chromatography over silica gel (EtOAc/petroleum ether 1:1) to give a pale yellow oil. $[\alpha]_D^{20}$ = –25 (*c* = 0.8, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 9.69 and 9.63 (rotamers, s, s, 1 H), 6.83 (br. s, 1 H), 6.76 (br. s, 2 H), 5.11 (dd, *J* = 10.1, 5.1 Hz, 1 H), 4.84 (br. s, 1 H), 4.50 and 4.32 (rotamers, s, s, 1 H), 3.82–4.01 (m, 4 H), 3.78 (s, 3 H), 3.60–3.76 (m, 2 H), 2.95 (d, *J* = 5.0 Hz, 2 H), 2.20 (dd, *J* = 13.5, 5.8 Hz, 1 H), 1.88–2.00 (m, 1 H), 1.43 and 1.49 (rotamers, s, s, 9 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 200.1 and 200.0 (rotamers), 155.0, 150.3, and 150.2 (rotamers), 150.6, 126.5, 119.8, and 119.7 (rotamers), 114.0 and 113.9 (rotamers), 111.5 and 111.4 (rotamers), 103.6, 80.6–80.0 (rotamers), 78.8, 70.9, and 70.6 (rotamers), 65.0, 56.0, 45.3, and 45.0 (rotamers), 35.3 and 35.2, 31.1 and 30.5 (rotamers), 28.5 and 28.4 ppm. IR (neat): $\tilde{\nu}_{max}$ = 2991, 1731, 1685, 1650, 1511, 1496, 1424 cm^{-1}. CIMS (CH_4 gas): *m*/*z* = 407, 352, 308, 290, 246, 211, 142, 114. ESIHRMS: *m*/*z* calcd. for $C_{21}H_{29}NNaO_7$ $[M + Na]^+$ 430.1842, found 430,1823.

(2*S*,3*S*)-1-(*tert*-Butoxycarbonyl)-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]proline (23): 2-Methylbut-2-ene (90%, 1.3 mL, 11.0 mmol), followed by a solution of sodium chlorite (80%, 335 mg, 2.96 mmol) and sodium dihydrogen phosphate dihydrate (400 mg, 2.6 mmol) in water (8 mL), was added to a solution of (2*S*,3*S*)-1-(*tert*-butoxycarbonyl)-2-formyl-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]pyrrolidine (497 mg, 1.22 mmol) in a mixture of THF (4 mL) and *tert*-butyl alcohol (12 mL). The yellow reaction mixture was then stirred for 1 h, carefully quenched with HCl solution (1 M), and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether, and the combined organic layers were dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOH/DCM 5:95) to give the free carboxylic acid as a yellow, oily solid (423 mg, 1.0 mmol, 82% over two steps). $[\alpha]_D^{20}$ = –32 (*c* = 1.0, $CHCl_3$). ^1H NMR (300 MHz, [D_6]DMSO, 350 K): δ = 6.84–6.93 (m, 3 H), 5.03 (t, *J* = 3.9 Hz, 1 H), 4.86 (app. d, *J* = 3.2 Hz, 1 H), 4.24 (s, 1 H), 3.88–3.93 (m, 2 H), 3.74–3.79 (m, 2 H), 3.75 (s, 3 H), 3.44–3.60 (m, 2 H), 2.84 (d, *J* = 5.1 Hz, 2 H), 2.00–2.20 (m, 2 H), 1.39 (br. s, 9 H) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO, 350 K): δ = 170.8, 153.1 (br. s), 152.1, 149.7, 125.9, 119.1, 113.9, 111.8, 102.6, 80.2 (br), 78.7, 64.6 (br), 63.7, 55.8, 44.0, 34.0, 29.0 (br), 27.6 ppm. IR (neat): $\tilde{\nu}_{max}$ = 3441, 2930, 1757, 1629, 1424 cm^{-1}. ESIMS (positive mode): *m*/*z* = 462.2, 446.2, 390.2, 362.2, 346.2. ESIHRMS: *m*/*z* calcd. for $C_{21}H_{29}NNaO_8$ $[M + Na]^+$ 446.1791, found 446.1805.

(2*S*,3*S*)-{1-(*tert*-Butoxycarbonyl)-3-[3-(1,3-dioxolan-2-ylmethyl)-4-methoxyphenoxy]prolyl}isoleucinamide (25): 1-Hydroxybenzotriazole (HOBt, 103 mg, 0.76 mmol) was added to a solution of **23** (305 mg, 0.72 mmol) and isoleucinamide acetic acid salt[39] (138 mg, 0.72 mmol) in DMF (6.5 mL). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 152 mg, 0.79 mmol) and *N*-methylmorpholine (200 μL, 1.80 mmol) were next added at 0 °C and the solution was stirred for 16 h while allowed to warm progressively to room temp. The yellow reaction mixture was

quenched with water and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were successively washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, and brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc) to give the desired peptide (300 mg, 0.56 mmol, 78%) as a pale yellow solid; m.p. 72 °C. $[\alpha]_D^{20} = -43$ (c = 0.8, $CHCl_3$). ^1H NMR (300 MHz, [D$_6$]DMSO, 350 K): δ = 7.18 (d, J = 8.5 Hz, 1 H), 7.15 (br. s, 1 H), 6.86–6.91 (m, 4 H), 5.02 (t, J = 5.0 Hz, 1 H), 4.77 (br. s, 1 H), 4.37 (s, 1 H), 4.20 (t, J = 8.1 Hz, 1 H), 3.84–3.92 (m, 2 H), 3.82–3.74 (m, 5 H), 3.41–3.55 (m, 2 H), 2.84 (d, J = 4.9 Hz, 2 H), 2.06 (app. d, J = 5.8 Hz, 2 H), 1.72–1.85 (m, 1 H), 1.41 (s, 9 H), 1.03–1.19 (m, 2 H), 0.83–0.90 (m, 6 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 360 K): δ = 172.1, 168.7, 153.6 (br), 152.0, 149.8, 125.8, 119.2, 114.1, 111.9, 102.6, 80.2 (br), 79.1, 65.8 (br), 63.7, 56.6, 55.8, 44.4, 36.4, 34.0, 28.9 (br), 27.6, 24.0, 15.1, 10.6 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3406, 3196, 2960, 1675, 1501, 1404, 1219, 1173, 1122, 1035 cm^{-1}. CIMS (NH_3 gas): m/z = 535, 436, 278, 211, 86. ESIHRMS: m/z calcd. for $C_{27}H_{41}N_3O_8Na$ [M + Na]$^+$ 558.2791, found 558.2805.

(2*S*,3*S*)-[1-*tert*-Butoxycarbonyl-3-(4-methoxy-3-vinylphenoxy)prolyl]-isoleucinamide (6): 1-Hydroxybenzotriazole (HOBt, 59 mg, 0.43 mmol) was added to a solution of **26**[31] (150 mg, 0.41 mmol) and isoleucinamide acetate[39] (79 mg, 0.41 mmol) in DMF (4 mL). 1-(3-Dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC, 86 mg, 0.45 mmol) and *N*-methylmorpholine (110 µL, 1.02 mmol) were next added at 0 °C, and the solution was stirred for 16 h while being allowed to warm progressively to room temp. The yellow reaction mixture was quenched with water and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were successively washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, and brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc/petroleum ether 8:2) to give the desired peptide (138 mg, 0.29 mmol, 71%) as a pale yellow solid; m.p. 85 °C. $[\alpha]_D^{20} = -25$ (c = 0.7, $CHCl_3$). ^1H NMR (300 MHz, [D$_6$]DMSO, 345 K): δ = 7.61 (d, J = 8.7 Hz, 1 H), 7.16 (d, J = 2.7 Hz, 1 H), 6.88–7.12 (m, 4 H), 5.82 and 5.26 (rotamers, dd, J = 17.7, 1.5 Hz, dd, J = 11.2, 1.5 Hz, 1 H), 4.84 (br. s, 1 H), 4.39 (app. s, 1 H), 4.21 (dd, J = 8.5, 6.6 Hz, 1 H), 3.78 (s, 3 H), 3.57 (app. tt, J = 10.6, 2.9 Hz, 1 H), 3.42–3.51 (m, 1 H), 2.07–2.10 (br. m, 2 H), 1.76–1.85 (m, 1 H), 1.40–1.54 (m, 1 H), 1.41 (s, 9 H), 1.04–1.18 (m, 1 H), 0.89 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 7.4 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO, 345 K): δ = 172.1, 168.7, 153.5, 151.2, 150.3, 130.8, 127.5, 116.1, 114.8, 113.7, 112.9, 80.4 (br), 79.0, 65.9, 56.6, 55.9, 44.4, 36.4, 29.0 (br), 27.7, 24.0, 15.1, 10.6 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3319, 2966, 1690, 1486, 1388, 1219, 1173 cm^{-1}. ESIMS (positive mode): m/z = 499.3, 498.3. ESIHRMS: m/z calcd. for $C_{25}H_{37}N_3O_6Na$ [M + Na]$^+$ 498.2580; found 498.2589.

Paliurine Core 4 by Cyclodehydration from 25: *p*-Toluenesulfonic acid monohydrate (1.2 mg, 5.6 µmol) was added to a solution of **25** (10 mg, 18.6 µmol) in mixture of toluene (2.5 mL) and water (250 µL). The mixture was progressively heated from room temp. to 80 °C during five days. After five days, water (250 µL) and *p*-toluenesulfonic acid monohydrate (1.2 mg, 5.6 µmol) were added and the solution was heated to 100 °C for 2 d. The light orange mixture was then hydrolyzed with saturated aqueous $NaHCO_3$ and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (Et_2O/EtOH 99:1) to give the desired cyclized product **4**[14] (3 mg, 6.3 µmol, 34%) as a white solid.

Paliurine Core 4 by Oxidative Amidation from 6: $PdCl_2(CH_3CN)_2$ (2.6 mg, 0.01 mmol) and copper(II) chloride (1.3 mg, 0.01 mmol) were successively added to a solution of **6** (47 mg, 0.10 mmol) in 1,2-dimethoxyethane (10 mL). The resulting mixture was stirred at 60 °C for 3 d under O_2, filtered through a plug of silica gel, and concentrated. The crude residue was purified by flash chromatography over silica gel (Et_2O/EtOH 99:1) to give the desired cyclized product **4**[14] (10 mg, 0.021 mol, 21%) as a white solid.

***N*,*N*-Dimethyl-L-leucinyl-L-isoleucine Benzyl Ester:** *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 913 mg, 2.4 mmol) was added at 0 °C to a cooled solution of *N*,*N*-dimethyl-L-leucine (500 mg, 2.6 mmol) in DMF (20 mL). The resulting beige solution was stirred at 0 °C for 20 min and a mixture of L-isoleucine benzyl ester *p*-toluenesulfonate (787 mg, 2 mmol) and diisopropylethylamine (1.45 mL, 8.2 mmol) in DMF (15 mL) was added dropwise by cannula at 0 °C. The resulting yellow solution was allowed to warm to room temp. and stirred overnight. The mixture was quenched at 0 °C with saturated aqueous NH_4Cl and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc/EtOH 96:4) to give the desired dipeptide (580 mg, 1.6 mmol, 80%) as an orange oil. $[\alpha]_D^{20} = +0.5$ (c = 1.0, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.31 (d, J = 9.1 Hz, 1 H), 7.20–7.26 (m, 5 H), 5.12 (A of AB syst., J = 12.2 Hz, 1 H), 5.01 (B of AB syst., J = 12.2 Hz, 1 H), 4.52 (dd, J = 9.1, 4.8 Hz, 1 H), 2.79 (dd, J = 8.8, 5.0 Hz, 1 H), 2.18 (s, 6 H), 1.82–1.90 (m, 1 H), 1.56–1.67 (m, 1 H), 1.42–1.51 (m, 1 H), 1.23–1.37 (m, 2 H), 0.92–1.12 (m, 1 H), 0.76–0.85 (m, 12 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.7, 172.0, 135.5, 128.6, 128.4, 67.8, 66.9, 56.1, 42.5, 37.8, 37.5, 25.9, 25.1, 23.6, 22.1, 15.8, 11.6 ppm. IR (neat): $\tilde{\nu}_{max}$ = 3350, 2955, 1742, 1675, 1496, 1189, 738, 691 cm^{-1}. ESIMS (positive mode): m/z = 385.4, 363.4, 114.1. ESIHRMS: m/z calcd. for $C_{21}H_{35}N_2O_3$ [M + H]$^+$ 363.2648; found 363.2662.

***N*,*N*-Dimethyl-L-leucinyl-L-isoleucine (31):** Palladium on carbon (10 wt.-% on activated carbon, 55 mg) was added to a solution of *N*,*N*-dimethyl-L-leucinyl-L-isoleucine benzyl ester (510 mg, 1.41 mmol) in methanol (80 mL). The system was purged three times under vacuum and stirred under hydrogen for 90 min. The mixture was filtered through a plug of Celite® and concentrated under vacuum to give the deprotected dipeptide (391 mg, 1.41 mmol, quant.) as a yellow solid, which was used without purification for the next step; m.p. 74 °C. $[\alpha]_D^{20} = +10$ (c = 1.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.10 (br. s, 1 H), 4.50 (dd, J = 7.7, 3.8 Hz, 1 H), 3.82 (br. s, 1 H), 2.64 (s, 6 H), 1.81–1.96 (m, 2 H), 1.42–1.62 (m, 3 H), 1.18–1.32 (m, 1 H), 0.87–0.98 (m, 12 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 175.6 (br), 168.0 (br), 66.9 (br), 58.2, 41.0 (br), 38.4, 37.4, 25.9, 25.4, 23.1, 21.6, 15.4, 12.1 ppm. IR (neat): $\tilde{\nu}_{max}$ = 3432, 2966, 2935, 1665, 1593, 1470, 1388 cm^{-1}. CIMS (NH_3 gas): m/z = 273, 114. ESIHRMS: m/z calcd. for $C_{14}H_{29}N_2O_3$ [M + Na]$^+$ 273.2178; found 273.2185.

(2*S*,3*S*,*Z*)-{1-(*tert*-Butoxycarbonyl)-3-[3-(2-iodovinyl)-4-methoxyphenoxy]prolyl}prolinamide (35): 1-Hydroxybenzotriazole (HOBt, 265 mg, 1.96 mmol) was added to a solution of **33**[14] (600 mg, 1.23 mmol) and prolinamide (183 mg, 1.60 mmol) in DMF (12 mL). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 330 mg, 1.72 mmol) and *N*-methylmorpholine (405 µL, 3.68 mmol) were next added at 0 °C and the solution was stirred for 16 h while being allowed to warm progressively to room

temp. The yellow reaction mixture was quenched with water and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were successively washed with aqueous HCl (1 M), saturated aqueous $NaHCO_3$, and brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc/EtOH 88:12) to give the desired peptide **35** (530 mg, 0.91 mmol, 74%) as a pale yellow solid; m.p. 93 °C. $[\alpha]_D^{20}$ = –11 (*c* = 0.53, $CHCl_3$). ^1H NMR (300 MHz, DMSO, 355 K): δ = 7.39 (d, *J* = 8.5 Hz, 1 H), 7.33 (d, *J* = 2.6 Hz, 1 H), 7.06 (dd, *J* = 8.5, 2.5 Hz, 1 H), 7.01 (br. s, 1 H), 6.72–6.96 (br. s, 2 H), 6.78 (d, *J* = 8.5 Hz, 1 H), 4.86 (br. d, *J* = 3.5 Hz, 1 H), 4.57 (br. s, 1 H), 4.33 (br. d, *J* = 6.2 Hz, 1 H), 3.78 (s, 3 H), 3.40–3.60 (m, 4 H), 1.85–2.28 (m, 6 H), 1.39 (s, 9 H) ppm. ^{13}C NMR (75 MHz, DMSO, 355 K): δ = 172.6, 167.6, 151.6, 149.2, 133.9, 126.4, 118.0, 117.2, 112.5, 82.5, 81.2, 78.5, 63.6, 59.2, 55.9, 46.2, 44.3, 28.7, 27.7, 24.1 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3426, 3109, 2981, 1690, 1650, 1496, 1404, 1280, 1255, 1219, 1163 cm^{-1}. ESIMS (positive mode): *m*/*z* = 608.3. ESIHRMS: *m*/*z* calcd. for $C_{24}H_{32}IN_3O_6Na$ $[M + Na]^+$ 608.1234; found 608.1245.

Ziziphine Core 36: A 100 mL flask was charged with iodo-amide **35** (400 mg, 0.68 mmol), copper(I) iodide (39 mg, 0.21 mmol), and cesium carbonate (334 mg, 1.03 mmol). The flask was evacuated under high vacuum, backfilled with argon, and closed with a rubber septum. Dry and degassed THF (92 mL) and *N*,*N*′-dimethylethylene-1,2-diamine (45 μL, 0.41 mmol) were next added, the rubber septum was replaced by a glass stopper, and the light green suspension was heated to 60 °C for 64 h. The reaction mixture was allowed to cool to room temp., filtered through a plug of silica gel (washed with EtOAc), and concentrated. The crude residue was purified by flash chromatography over silica gel (Et_2O/EtOH 99:1) to give the desired cyclized product **35** (255 mg, 0.56 mmol, 82%) as a white solid; m.p. 94 °C. $[\alpha]_D^{20}$ = –363 (*c* = 0.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.40 (d, *J* = 11.6 Hz, 1 H), 6.80–6.97 (m, 4 H), 5.94 (d, *J* = 8.3 Hz, 1 H), 5.25–5.32 (m, 1 H), 4.64 (dd, *J* = 9.1, 4.2 Hz, 1 H), 4.29 (d, *J* = 4.8 Hz, 1 H), 4.28–4.36 (m, 1 H), 3.79 (s, 3 H), 3.73–3.86 (m, 1 H), 3.39–3.48 (m, 1 H), 3.24–3.33 (m, 1 H), 2.33–2.41 (m, 1 H), 2.15–2.26 (m, 2 H), 1.93–2.04 (m, 2 H), 1.71–1.86 (m, 1 H), 1.43 (s, 9 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 172.7, 168.0, 154.1, 151.4, 151.2, 124.3, 121.8, 117.2, 114.0, 110.9, 106.7, 80.4, 79.9, 62.5, 62.0, 56.1, 48.0, 45.4, 32.6, 29.2, 28.5, 25.0 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3411, 3155, 2981, 1690, 1634, 1506, 1475, 1399, 1316, 1260, 1219, 1168, 1132, 1061, 902, 769 cm^{-1}. ESIMS (positive mode): *m*/*z* = 937.7, 480.4. ESIHRMS: *m*/*z* calcd. for $C_{24}H_{31}N_3O_6Na$ $[M + Na]^+$ 480.2111; found 480.2931.

Deprotected Ziziphine Core 37: 2,6-Lutidine (66 μL, 0.56 mmol) and a solution of trimethylsilyl trifluoromethanesulfonate (1.4 M solution in dichloromethane, 1.6 mL, 2.24 mmol) were added at –10 °C to a solution of **36** (255 mg, 0.56 mmol) in dichloromethane (12.5 mL). The resulting light pink solution was stirred for 1 h while progressively warming to 0 °C. The mixture was next hydrolyzed at 0 °C by addition of saturated aqueous $NaHCO_3$ and diluted with dichloromethane. The aqueous layer was extracted with dichloromethane and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel (EtOAc/EtOH/NH_3 85:10:5) to give the desired *N*-deprotected macrocycle **37** (182 mg, 0.51 mmol, 91%) as a white solid; m.p. 196 °C. $[\alpha]_D^{20}$ = –351 (*c* = 0.54, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ = 8.24 (d, *J* = 11.4 Hz, 1 H), 6.68–6.91 (m, 4 H), 5.91 (d, *J* = 9.0 Hz, 1 H), 5.20–5.25 (m, 1 H), 4.55 (dd, *J* = 8.4, 5.1 Hz, 1 H), 3.76 (s, 3 H), 3.72–3.84 (m, 1 H), 3.61 (d, *J* = 3.7 Hz, 1 H), 3.44–3.5 (m, 1 H), 3.03–3.21 (m, 2 H), 2.70 (br. s, 1 H), 2.19–2.34 (m, 2 H), 2.07–2.15 (m, 1 H), 1.81–2.03 (m, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6, 167.4, 154.1, 151.4, 151.1, 124.0, 121.3, 117.6, 113.8, 111.2, 107.1, 80.6, 67.5, 61.2, 56.0, 47.8, 35.9, 29.3, 25.0 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3395, 3309, 3150, 3114, 3068, 2940, 2873, 1696, 1644, 1624, 1511, 1419, 1306, 1255, 1219, 1184, 1086, 1025, 815, 799, 784, 730, 661 cm^{-1}. ESIMS (positive mode): *m*/*z* = 737.5, 380.4, 358.4. ESIHRMS: *m*/*z* calcd. for $C_{19}H_{24}N_3O_6N_4$ $[M + H]^+$ 358.1767; found 358.1760.

Typical Procedure for the Synthesis of Ziziphines N (38) and Q (39) and Stereoisomers 40–42: *O*-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 134 mg, 0.35 mmol) and diisopropylethylamine (185 μL, 1.05 mmol) were added at 0 °C to a cooled solution of *N*-Fmoc-protected amino acid ("*N*-Fmoc-AA_1" in Scheme 10, 0.38 mmol) and 1-hydroxyazabenzotriazole (HOAt, 62 mg, 0.45 mmol) in DMF (3 mL). The resulting yellow solution was stirred at 0 °C for 20 min and added dropwise by cannula at 0 °C to a cooled solution of **37** (90 mg, 0.25 mmol) in DMF (4 mL). The flask containing the activated acid was rinsed with an additional portion of DMF (2.5 mL), which was cannulated into the solution of the amine. The resulting yellow solution was allowed to warm to room temp. and stirred overnight. The mixture was quenched at 0 °C with saturated aqueous NH_4Cl and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated to give an orange oil that was used in the next step without further purification.

This oil was dissolved in CH_3CN (4.5 mL) and diethylamine was added (950 μL, 9.2 mmol) at room temp. After 50 min, the crude yellow mixture was concentrated under vacuum and residue was immediately engaged in the next step without further purification.

O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU, 134 mg, 0.35 mmol) and diisopropylethylamine (185 μL, 0.72 mmol) were added at 0 °C to a cooled solution of *N*,*N*-dimethylamino acid ("*N*,*N*-Me_2-AA_2" in Scheme 10, 0.38 mmol) and 1-hydroxyazabenzotriazole (HOAt, 62 mg, 0.45 mmol) in DMF (3 mL). The resulting yellow solution was stirred at 0 °C for 20 min and added dropwise by cannula at 0 °C to a solution of the previously deprotected amine in DMF (4 mL). The flask containing the activated acid was rinsed with an additional portion of DMF (2.5 mL), which was cannulated into the solution of the amine. The yellow solution was allowed to warm to room temp. and stirred overnight. The mixture was hydrolyzed at 0 °C with saturated aqueous NH_4Cl and diluted with diethyl ether. The aqueous layer was extracted with diethyl ether and the combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by flash chromatography over silica gel to give natural cyclopeptides and stereoisomers **38**–**42**.

Ziziphine N (38): Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/EtOH 96:4; white solid (scale: 79 mg of compound obtained; yield 52% over three steps). R_f = 0.28 (Merck Kieselgel 60F_{254} TLC plates, EtOAc/EtOH 95:5); m.p. 82 °C {ref. 117–119 °C}.[8] $[\alpha]_D^{20}$ = –127 (*c* = 0.68, $CHCl_3$) {ref. natural ziziphine N: $[\alpha]_D^{20}$ = –327 (*c* = 0.18, $CHCl_3$)};[8,33] ^1H NMR (300 MHz, $CDCl_3$):[40] δ = 8.35 (d, *J* = 11.5 Hz, 1 H), 6.94 (d, *J* = 9.0 Hz, 1 H), 6.81–6.92 (m, 3 H), 6.79 (br. s, 1 H), 5.94 (d, *J* = 8.7 Hz, 1 H), 5.21–5.27 (m, 1 H), 4.78 (app. q, *J* = 7.5 Hz, 1 H), 4.54 (dd, *J* = 9.1, 4.0 Hz, 1 H), 4.38 (d, *J* = 5.6 Hz, 1 H), 4.21–4.29 (m, 2 H), 3.79 (s, 3 H), 3.57–3.66 (m, 1 H), 3.26–3.33 (m, 1 H), 2.57 (br. s, 1 H), 2.43–2.49 (m, 1 H),

2.35–2.39 (m, 1 H), 2.25 (s, 6 H), 2.20–2.30 (m, 1 H), 1.91–2.04 (m, 2 H), 1.76–1.86 (m, 2 H), 1.42–1.67 (m, 4 H), 1.11–1.21 (m, 1 H), 0.92 (d, J = 6.3 Hz, 6 H), 0.92 (t, J = 6.3 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$):[40] δ = 171.7, 171.4, 167.9, 151.4, 151.1, 124.2, 121.8, 117.1, 114.0, 110.9, 106.8, 78.8, 74.5, 62.9, 62.2, 56.1, 48.0 (2C), 45.5, 43.3, 41.0, 34.5, 32.9, 29.2, 27.1, 25.1, 24.8, 23.3, 21.9, 14.8, 12.1 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3400, 2955, 2925, 2868, 2781, 1690, 1649, 1506, 1224, 1050, 767 cm^{-1}. UV: λ_{max} (log ε) = 273 (4.42), 324 (4.31) nm. CIMS (NH_3 gas): m/z = 612. ESIHRMS: m/z calcd. for $C_{33}H_{50}N_5O_6$ $[M + H]^+$ 612.3761, found 612.3735.

Ziziphine Q (39): Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/EtOH 96:4; white solid (scale: 96 mg of compound obtained; yield 64% over three steps). R_f = 0.25 (Merck Kieselgel 60F_{254} TLC plates, EtOAc/EtOH 95:5); m.p. 96 °C {ref. 140–142 °C}.[8] $[\alpha]_D^{20}$ = –234 (c = 0.54, $CHCl_3$) {lit. natural ziziphine Q: $[\alpha]_D^{20}$ = –325 (c = 0.16, $CHCl_3$)};[8,33] ^{1}H NMR (300 MHz, $CDCl_3$):[41] δ = 8.33 (d, J = 11.5 Hz, 1 H), 6.94 (dd, J = 8.8, 11.7 Hz, 1 H), 6.89–6.95 (obs. m, 1 H), 6.79–6.88 (m, 3 H), 5.94 (d, J = 8.8 Hz, 1 H), 5.24 (dt, J = 9.6, 6.6 Hz, 1 H), 4.47–4.54 (m, 2 H), 4.38 (d, J = 6.0 Hz, 1 H), 4.20–4.31 (m, 2 H), 3.78 (s, 3 H), 3.59–3.68 (m, 1 H), 3.27–3.34 (m, 1 H), 2.58 (d, J = 5.1 Hz, 1 H), 2.41–2.49 (m, 1 H), 2.30–2.38 (m, 1 H), 2.24 (s, 6 H), 2.21–2.26 (m, 1 H), 1.90–2.03 (m, 3 H), 1.77–1.86 (m, 2 H), 1.48–1.62 (m, 1 H), 1.15–1.22 (m, 1 H), 0.94 (d, J = 7.3 Hz, 3 H), 0.92 (t, J = 6.7 Hz, 3 H), 0.92 (d, J = 6.4 Hz, 3 H), 0.90 (d, J = 6.5 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$):[41] δ = 172.1, 171.5, 170.8, 167.7, 151.2, 150.9, 124.0, 121.6, 116.9, 113.5, 110.7, 106.6, 78.5, 74.5, 62.6, 62.1, 55.9, 54.9, 47.8, 45.5, 43.0, 34.3, 32.6, 30.8, 29.0, 26.9, 24.9, 19.0, 18.5, 14.7, 11.9 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3401, 2966, 2925, 2878, 2776, 1639, 1506, 1412, 1225, 1051 cm^{-1}. UV: λ_{max} (log ε) = 270 (3.60), 318 (3.48) nm. CIMS (NH_3 gas): m/z = 598, 358. ESIHRMS: m/z calcd. for $C_{32}H_{48}N_5O_6$ $[M + H]^+$ 598.3605, found 598.3594.

***α*-Epiziziphine N (40):** Solvent system for purification by flash chromatography over silica gel, eluent EtOAc/EtOH 96:4; white solid (scale: 46 mg of compound obtained; yield 42% over three steps); m.p. 59 °C. $[\alpha]_D^{20}$ = –418 (c = 0.53, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, J = 11.5 Hz, 1 H), 6.92 (d, J = 9.0 Hz, 1 H), 6.91 (dd, J = 11.5, 9.0 Hz, 1 H), 6.87 (br. s, 1 H), 6.81 (app. d, J = 9.5 Hz, 2 H), 5.93 (d, J = 8.8 Hz, 1 H), 5.29 (app. ddd, J = 11.8, 9.5, 6.8 Hz, 1 H), 4.89 (dt, J = 9.3, 5.1 Hz, 1 H), 4.52 (dd, J = 8.5, 3.5 Hz, 1 H), 4.33 (d, J = 5.2 Hz, 1 H), 4.27–4.32 (m, 1 H), 3.83–3.90 (m, 2 H), 3.79 (s, 3 H), 3.23–3.31 (m, 1 H), 2.64 (br. s, 1 H), 2.49–2.57 (m, 1 H), 2.27–2.37 (m, 1 H), 2.25 (s, 6 H), 2.08–2.18 (m, 1 H), 1.90–2.05 (m, 2 H), 1.73–1.85 (m, 2 H), 1.53–1.64 (m, 2 H), 1.39–1.51 (m, 2 H), 1.06–1.21 (m, 1 H), 0.99 (d, J = 6.3 Hz, 6 H), 0.93 (t, J = 6.4 Hz, 3 H), 0.80 (d, J = 6.7 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 171.3, 170.8, 167.9, 151.5, 151.0, 124.3, 121.7, 117.1, 114.0, 110.8, 106.6, 78.7, 74.1, 63.1, 62.0, 56.1, 47.9, 47.4, 45.9, 43.0, 42.0, 34.8, 32.8, 29.1, 26.7, 25.1, 25.0, 23.5, 22.0, 14.6, 12.1 ppm. IR (KBr): $\tilde{\nu}_{max}$ = 3503, 2955, 2920, 2873, 1701, 1644, 1516, 1455, 1419, 1260, 1219, 1178, 1045 cm^{-1}. ESIMS (positive mode): m/z = 634.2, 612.3. ESIHRMS: m/z calcd. for $C_{33}H_{50}N_5O_6$ $[M + H]^+$ 612.3761, found 612.3739.

β-Epiziziphine N (41): Solvent system for purification by flash chromatography over silica gel, eluent CH_2Cl_2/EtOH/30 wt.-% aqueous ammonia 95:4.5:0.5; white solid (scale: 98 mg of compound obtained; yield 70% over three steps); m.p. 103 °C. $[\alpha]_D^{20}$ = –478 (c = 0.57, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): 8.32 (d, J = 11.6 Hz, 1 H), 6.89 (dd, J = 11.5, 8.8 Hz, 1 H), 6.85 (app. d, J = 4.0 Hz, 1 H), 6.80 (app. d, J = 3.0 Hz, 1 H), 6.77 (br. s, 1 H), 6.65 (d, J = 8.5 Hz, 1 H), 5.91 (d, J = 8.8 Hz, 1 H), 5.22 (app. ddd, J = 12.2, 10.2, 6.9 Hz, 1 H), 4.78 (app. dt, J = 9.3, 5.1 Hz, 1 H), 4.50 (dd, J = 9.1, 4.2 Hz, 1 H), 4.34 (d, J = 5.8 Hz, 1 H), 4.19–4.27 (m, 2 H), 3.76 (s, 3 H), 3.58 (ddd, J = 10.4, 5.7, 2.1 Hz, 1 H, H_{21b}), 3.24–3.31 (m, 1 H), 2.50 (d, J = 6.9 Hz, 1 H), 2.37–2.47 (m, 1 H), 2.29–2.37 (m, 1 H), 2.23 (s, 6 H), 2.15–2.28 (m, 1 H), 1.89–2.0 (m, 2 H), 1.72–1.84 (m, 3 H), 1.49–1.62 (m, 2 H), 1.41–1.48 (m, 2 H), 0.99 (d, J = 6.3 Hz, 6 H), 0.93 (t, J = 6.4 Hz, 3 H), 0.80 (d, J = 6.7 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.6, 171.4, 171.2, 167.8, 151.4, 151.0, 124.1, 121.7, 117.0, 113.9, 110.8, 106.7, 78.7, 75.3, 62.9, 62.1, 56.0, 48.0, 47.8, 45.1, 42.7, 41.2, 34.6, 32.8, 29.1, 25.0, 24.9, 24.8, 23.1, 22.0, 16.2, 11.9 ppm. ESIMS (positive mode): m/z = 634.5, 612.5. ESIHRMS: m/z calcd. for $C_{33}H_{50}N_5O_6$ $[M + H]^+$ 612.3761, found 612.3741.

α,β-Diepiziziphine N (42): Solvent system for purification by flash chromatography over silica gel, eluent CH_2Cl_2/EtOH/30 wt.-% aqueous ammonia 95:4.5:0.5; white solid (scale: 35 mg of compound obtained; yield 65% over three steps); m.p. 99 °C. $[\alpha]_D^{20}$ = –209 (c = 0.53, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.42 (d, J = 11.6 Hz, 1 H), 6.89 (dd, J = 11.5, 8.9 Hz, 1 H), 6.87 (app. d, J = 9.1 Hz, 1 H), 6.78 (br. s, 1 H), 6.65 (d, J = 8.5 Hz, 1 H), 5.92 (d, J = 8.8 Hz, 1 H), 5.25 (app. ddd, J = 12.7, 9.6, 6.9 Hz, 1 H), 4.89 (app. dt, J = 9.4, 4.9 Hz, 1 H), 4.49 (dd, J = 8.8, 3.7 Hz, 1 H), 4.30 (d, J = 5.5 Hz, 1 H), 4.20–4.26 (m, 1 H), 3.80–3.92 (m, 2 H), 3.83 (s, 3 H), 3.24–3.31 (m, 1 H), 2.48–2.55 (m, 1 H), 2.46 (d, J = 6.0 Hz, 1 H), 2.23–2.35 (m, 1 H), 2.22 (s, 6 H), 2.09–2.18 (m, 1 H), 1.88–2.01 (m, 1 H), 1.70–1.83 (m, 3 H), 1.50–1.68 (m, 2 H), 1.29–1.48 (m, 2 H), 1.06–1.21 (m, 1 H), 0.98 (d, J = 6.4 Hz, 6 H), 0.94 (t, J = 6.7 Hz, 3 H), 0.84 (d, J = 6.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.43, 171.38, 171.3, 167.8, 151.5, 151.0, 124.3, 121.7, 117.1, 114.0, 110.8, 106.6, 78.7, 75.8, 63.1, 62.0, 56.1, 48.0, 47.4, 45.8, 43.1, 42.2, 34.8, 32.8, 29.1, 25.0, 24.9, 24.8, 23.1, 22.0, 16.5, 12.3. ESIMS (positive mode): m/z = 634.4, 612.3. ESIHRMS: m/z calcd. for $C_{33}H_{50}N_5O_6$ $[M + H]^+$ 612.3761, found 612.3744.

Dihydroziziphine Q (43): Pd/C (10% wt, 10 mg) was added to a solution of ziziphine Q (**39**, 9.9 mg, 0.0165 mmol) in dry methanol (7 mL). The solution was purged three times, placed under hydrogen, stirred for 1 h, filtered through a pad of Celite®, and concentrated in vacuo to give dihydroziziphine Q (10.0 mg, 0.0165 mmol, quant) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.89 (d, J = 9.0 Hz, 1 H), 6.76 (br. s, 1 H), 6.68–6.74 (m, 2 H), 6.43 (app. d, J = 4.9 Hz, 1 H), 5.37 (app. dt, J = 11.1, 7.1 Hz, 1 H), 4.59 (d, J = 7.3 Hz, 1 H), 4.45 (t, J = 8.5 Hz, 1 H), 4.30 (t, J = 9.3 Hz, 1 H), 4.18 (d, J = 7.6 Hz, 1 H), 3.84–3.93 (m, 2 H), 3.74–3.83 (m, 1 H), 3.77 (s, 3 H), 3.64–3.72 (m, 1 H), 3.13–3.20 (m, 1 H), 3.07 (app. t, J = 7.8 Hz, 2 H), 2.38–2.48 (m, 1 H), 2.30–2.36 (m, 1 H), 2.22 (s, 6 H), 2.17–2.27 (m, 1 H), 1.92–2.02 (m, 1 H), 1.67–1.87 (m, 5 H), 1.47–1.62 (m, 1 H), 1.09–1.33 (m, 1 H), 0.98 (d, J = 6.7 Hz, 6 H), 0.95 (d, J = 6.6 Hz, 3 H), 0.90 (t, J = 7.9 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.5, 171.3, 171.1, 170.4, 152.6, 150.0, 127.5, 117.3, 116.3, 112.3, 78.5, 74.7, 62.6, 60.0, 56.1, 47.8, 45.5, 43.3, 36.7, 34.5, 31.6, 31.2, 30.8, 27.2, 26.6, 25.1, 19.2, 19.0, 14.9, 12.1 ppm. ESIMS (positive mode): m/z = 600.4, 408.3, 361.0, 359.0, 183.0. ESIHRMS: m/z calcd. for $C_{32}H_{50}N_5O_6$ $[M + H]^+$ 600.3761; found 600.3785.

Cellular Viability: The human HT1080 fibrosarcoma adherent cell line was cultured at 37 °C under a humidified atmosphere containing CO_2 (5%) in Dulbecco's modified Eagle's medium (DMEM/F12) supplemented with fetal bovine serum (10%) together with penicillin (100 μg mL^{-1}), streptomycin (100 U mL^{-1}), and glutamax (1% v/v) from Invitrogen. Stock solutions of the nine compounds in DMSO (100 mM) were prepared and stored at –20 °C. Cells were

seeded in 12-well plates (5×10^4 cells per well). After 24 h, the medium was replaced in each well by complete medium (1 mL) with the appropriate concentrations of the tested drugs and cells were incubated for 48 h. Corresponding controls with analogous concentrations of DMSO were carried out in parallel. After drug treatment, the media from each well were kept in centrifuge tubes. The adherent cells were detached (trypsin), pooled with the corresponding media, centrifuged, and resuspended in complete medium. Cells were then loaded with fluorescein diacetate (FDA, 1 μg mL^{-1}) 30 min and propidium iodide (PI 5 μg mL^{-1}) 5 min prior to analysis. FDA (Polysciences) is a nonfluorescent compound that becomes fluorescent (free fluorescein) when cleaved by esterases in living cells, and PI (Sigma) specifically penetrates in necrotic cells after loss of their plasma membrane integrity.[42] Percentages of cell deaths were determined with a XL3C flow cytometer (Beckman–Coulter, France). Pictures of cells were taken in culture wells before trypsinization under a Nikon TMS microscope equipped fitted with a Nikon F601 camera.

Antimicrobial Susceptibility Testing: MRSA ATCC 43300, *B. anthracis* Sterne strain, and *E. coli* ATCC23590 were purchased from ATCC sources. A culture of each microorganism from a freezer stock in tryptic soy broth (Difco Laboratories, Detroit, MI) and glycerol (20%) was grown on tryptic soy agar (TSA) plates (Becton–Dickinson Laboratories, Cockeysville, MD) at 37 °C for 24 h. A 10^8 suspension was then made in sterile phosphate buffered saline (pH 7.4) and swabbed across fresh TSA plates. Four equidistantly-spaced circular wells (6 mm in diameter) were cut into the inoculated plates, and into each well was pipetted 20, 50, or 100 μL volumes of a 1 mg mL^{-1} stock solution of the test compound in dimethylsulfoxide (DMSO), whereas 10 μL of a 1 mg mL^{-1} solution of penicillin G in sterile phosphate buffered saline was added to the fourth well (as a control). The plates were covered and then incubated for 24 h at 37 °C. Antimicrobial susceptibilities were determined by looking for cleared circular zones of growth inhibition appearing around each well, and the diameters were recorded in mm.

Agar Dilution Assays: The minimum inhibitory concentration (MIC) values of the test compounds were determined by agar dilution by NCCLS protocols.[43] The test media was prepared in 24-well plates (Costar 3524, Cambridge, MA) by addition of a known concentration of the test drug in DMSO together with a solution of Mueller–Hinton II agar (Becton–Dickinson Laboratories, Cockeysville, MD) for a total volume of 1 mL in each well. Calculations of the overall concentrations of antibiotics in the wells were standardized by measuring from a 1 mg mL^{-1} stock solution of the test drug, starting with 512 μL of stock solution, and cutting that amount in half for each successive well. The final concentrations of test compound in each line of wells were thus 512, 256, 128, 64, 32, 16, 8, and 4 μg mL^{-1}. Following preparation of the well plates, the medium was allowed to solidify at room temperature for 24 h before inoculation. Inoculation: From a 24-hour culture of each microorganism (*S. aureus* 849, *B. anthracis* 848, and *E. coli*) on TSA plates (Becton–Dickinson Laboratories, Cockeysville, MD), the test bacterial strains were grown overnight in tryptic soy broth (Difco Laboratories, Detroit, MI, 5 mL) at 37 °C to confluence at 10^7 CFU mL^{-1}, as monitored by optical density measurement at 600 nm on a Bio-Tek Biomate 3 spectrophotometer (Thermo Fisher). One microliter of each culture was then applied to the appropriate well of agar and incubated at 37 °C overnight. After 24 h, the wells were examined for growth. The MIC value is the lowest concentration (in ug mL^{-1}) at which there was no visible growth of bacteria in the wells.

Supporting Information (see also the footnote on the first page of this article): Copies of ^{1}H and ^{13}C NMR spectra for all new compounds and synthetic ziziphines. Ziziphines N and Q spectroscopic data and comparison with reported data.

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[33] Synthetic materials displayed spectral properties (^{1}H NMR, ^{13}C NMR, IR, UV, MS) that matched those of the natural products. However, the observed optical rotations of synthetic **38** {$[\alpha]_D^{20}$ = –127 (*c* = 0.68, $CHCl_3$)} and **39** {$[\alpha]_D^{20}$ = –234 (*c* = 0.54, $CHCl_3$)} were found to be lower than the reported values {$[\alpha]_D^{20}$ = –327 (*c* = 0.18, $CHCl_3$) and $[\alpha]_D^{20}$ = –325 (*c* = 0.16, $CHCl_3$), respectively, according to ref. 8}. The reason for this discrepancy in the optical rotations is unclear at present, although it might be attributable to contamination by small amounts of impurities in the natural samples.
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