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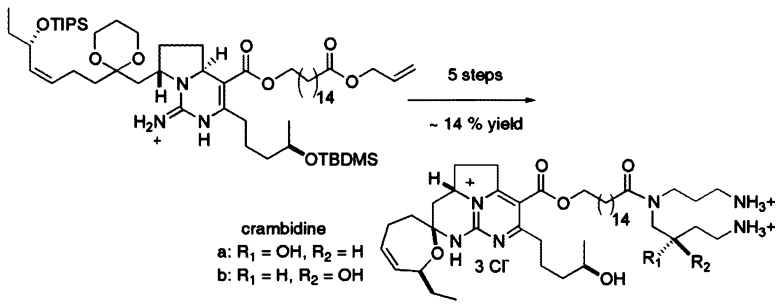

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Total Synthesis of (−)-Crambidine and Definition of the Relative Configuration of Its Unique Tetracyclic Guanidinium Core

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Abstract: Total syntheses of the 3*S*,8*S*,10*S*,19*R*,43*S* isomer **4a** and the 3*S*,8*S*,10*S*,19*R*,43*R* isomer **4b** of the unique crambescidin alkaloid crambidine are reported. These studies confirm the tetracyclic structure proposed by Braekman and co-workers for crambidine, and establish the *rel*-3*R*,8*R*,10*R*,19*S* relative configuration for this moiety. Natural crambidine is most likely the 3*S*,8*S*,10*S*,19*R*,43*S* isomer **4a**. These syntheses were completed in five steps and ~14% overall yield from 1-iminohexahydro[1,2-*c*]pyrimidine carboxylic ester **10**, an intermediate in our earlier total synthesis of 13,14,15-isocrambescidin 800 (**3**). The signature step in the total syntheses of crambidine and several stereoisomers is chemoselective dehydrogenation of the tethered Biginelli adduct **10** or the derived tetracyclic intermediate **17**. Additionally, these studies reveal the unprecedented ring-chain isomerization of the crambidine ring system exemplified by the interconversion of isomers **15a** and **15b**.

Introduction

Many structurally novel alkaloids have been obtained from marine sponges.[2] Among the most noteworthy of these is the crambescidin family of marine alkaloids.[3] The majority of crambescidin alkaloids, exemplified by crambescidins 816 (**1**) and 800 (**2**) and 13,14,15-isocrambescidin 800 (**3**), contain structurally unique pentacyclic guanidinium moieties having two distinctive spiroaminal units (Figure 1). Alkaloids of this type largely differ in the nature of the ester side chain or, in the case of crambescidin 816 (**1**) and congeners, the presence of a hydroxyl substituent at C13.[4] Diverse biological activities have been reported for crambescidin alkaloids, including cytotoxicity toward several cancer cell lines,[4a−f,5,6] antifungal activity,[4a,c] antiviral activities toward herpes simplex virus type 1 (HSV-1)[4a,c,d] and human immunodeficiency virus (HIV),[4f] inhibition of HIV-1 envelope-mediated cell fusion,[4i] induction of differentiation of chronic mylogenous leukemia cells,[6] and inhibition of the binding of various proteins to HIV-1 Nef.[7]

In 1993, Braekman and co-workers reported the isolation of a structurally unique crambescidin alkaloid crambidine (**4**) from the Mediterranean sponge *Crambe crambe*.[4e] Although crambidine itself was not obtained in pure form, a tetraacetylated derivative **5** was purified and employed in the structure elucidation. The molecular formula of peracetylcrambidine (**5**), $C_{53}H_{86}N_6O_{10}$, is consistent with 14 degrees of unsaturation, and maxima in the UV spectra of **5** at 254, 299, 315, and 325 signal the presence of a heteroaromatic chromophore. A novel tetracyclic structure containing a tetrahydro-5,6,6a-triazaacenaphthalene unit was proposed for peracetylcrambidine (**5**), albeit with no suggestion of relative or absolute configuration.[4e] The biological activity of crambidine has not been reported, presumably because of the difficulty encountered in purifying this marine isolate.

Dehydration and opening of the hydropyran ring of crambescidin 816 (**1**) would generate one stereoisomer of the gross structure proposed for crambidine, the 43*S* isomer of structure **4**. This potential relationship, the possibility that the bioactivity of crambescidin 816 (**1**) might derive from such a tetracyclic congener, the inability to isolate crambidine in pure form from nature, and the novelty of the structure proposed for crambidine,

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(2) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2005**, *22*, 15−61 and earlier reviews in this series.
(3) For brief reviews of the isolation and synthesis of crambescidin alkaloids, see: (a) Berlinck, R. G. S. *Prog. Chem. Org. Nat. Prod.* **1995**, *66*, 119−295. (b) Berlinck, R. G. S. *Nat. Prod. Rep.* **1996**, *13*, 377−409. (c) Berlinck, R. G. S. *Nat. Prod. Rep.* **1999**, *16*, 339−365. (d) Heys, L.; Moore, C. G.; Murphy, P. J. *Chem. Soc. Rev.* **2000**, *29*, 57−67. (e) Aron, Z. D.; Overman, L. E. *Chem. Commun.* **2004**, 253−265.
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(5) Aron, A. D.; Pietraszkiewicz, H.; Overman, L. E.; Valeriote, F.; Cuevas, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3445−3449.
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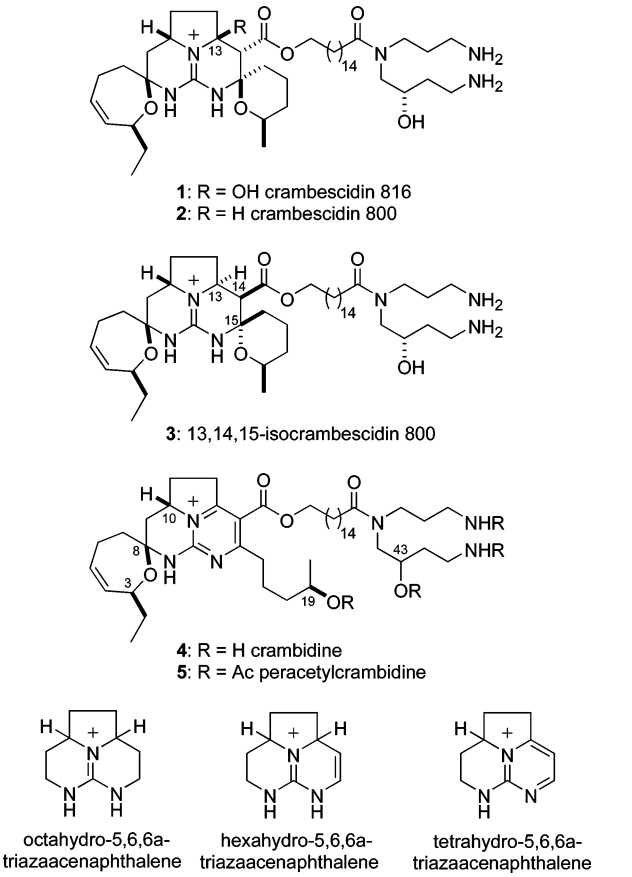

Figure 1. Structures of several crambescidin alkaloids and the tricyclic guanidinium moieties embedded in these marine natural products.

led us to initiate a program to synthesize this rare crambescidin alkaloid. Herein, we disclose the outcome of this study, which culminated in the total synthesis of (−)-crambidine (**4**) and the determination that its three-dimensional structure is as depicted in Figure 1.

Results

Synthetic Strategy. In 1995, we reported the first total synthesis of a crambescidin alkaloid.[8] We subsequently completed total syntheses of nine additional crambescidin alkaloids.[9,10] Central to these syntheses is an intramolecular variant of the Biginelli condensation that we developed to provide access to the hexahydro-5,6,6a-triazaacenaphthalene and octahydro-5,6,6a-triazaacenaphthalene moieties found in these alkaloids.[3e,9a] In the context of a total synthesis of the batzelladine alkaloid (−)-dehydrobatzelladine C, we also reported that hexahydro-5,6,6a-triazaacenaphthalenes can be oxidized to give tetrahydro-5,6,6a-triazaacenaphthalenes.[11]

The proposed structural relationship between crambescidin 816 (**1**) and crambidine led us to choose the 3*S*,8*S*,10*S*,19*R*,43*S* stereoisomer of the crambidine core, depicted in structure **4a**, as our initial total synthesis target (Figure 2). Crambidine **4a** should be available from tetracyclic acid **6** by coupling with an appropriately protected hydroxyspermidine fragment. Based on our previous total synthesis experience in the crambescidin and batzelladine areas,[3e,9,11] two approaches to intermediate **6** were envisaged that differ in the stage at which the 2-aminopyrimidinium unit would be revealed. One possibility is late stage oxidation of a tetracyclic precursor **7**, which should in turn be available from tethered Biginelli adducts **9** or **10**.[9b,d] Alternatively, the 2-aminopyrimidinium fragment might be introduced at an earlier stage by direct oxidation of these Biginelli products to give intermediate **8**. At the outset of our studies, it was unclear whether intermediates such as **10**, or the α epimer of **7**, would be viable substrates for the pivotal oxidation step, as our only previous experience involved dehydrogenation of a hexahydro-5,6,6a-triazaacenaphthalene having a cis relationship of the angular hydrogens adjacent to the pyrrolidine nitrogen.[11] As the proposed synthesis of crambidine would be more direct from a tethered Biginelli adduct that already contained the guanidine functional group, 1-iminohexahydro[1,2-*c*]pyrimidine carboxylic ester **10**[9b,d] was chosen for our initial studies.

Synthesis of the 3*S*,8*S*,10*S*,19*R*,43*S* Isomer of Peracetylcrambidine. Our studies began by preparing multigram quantities of tethered Biginelli adduct **10**, which was obtained most conveniently as a 7:1 mixture of trans and cis stereoisomers.[9b,d] Preliminary attempts to dehydrogenate **10** by reaction with 1−3 equiv of cerric ammonium nitrate (CAN) in MeCN[11] provided 1-iminotetrahydro[1,2-*c*]pyrimidine carboxylic ester **12** in trace amounts only (Scheme 1). Monitoring this reaction by electrospray mass spectrometry, and ^{1}H NMR analysis of the crude reaction product, suggested that the major products produced under these conditions were pentacyclic guanidines **11**.[9d] As the formation of **11** most likely was promoted by the acidic nature of CAN oxidations, simply carrying out the oxidation in the presence of excess $NaHCO_3$ (10 equiv) suppressed the formation of **11**, providing tetrahydropyrrolopyrimidine **12** in 75% yield after purification by column chromatography on pH 7-buffered silica gel.[12] Monitoring of the CAN oxidation of **10** by TLC showed the trans epimer to be more reactive than the corresponding cis stereoisomer of the Biginelli product. Oxidation of iminohexahydropyrimidine **10** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), with or without added $NaHCO_3$, gave rise to product **12** in low yield, whereas the reaction of **10** with strong oxidants such as dimethyldioxirane (DMDO) led only to the formation of an intractable mixture of compounds.

With tetrahydropyrrolopyrimidine **12** in hand, we investigated the removal of the alcohol protecting groups to generate diol **13**. We quickly found that this seemingly straightforward transformation was hampered by the highly polar nature of this

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(9) (a) Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 3235−3237. (b) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **1999**, *121*, 6944−6945. (c) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **2000**, *122*, 4893−4903. (d) Coffey, D. S.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **2000**, *122*, 4904−4914. (e) Aron, Z. D.; Overman, L. E. *J. Am. Chem. Soc.* **2005**, *127*, 3380−3390.
(10) Total syntheses of crambescidin 359, which lacks a C14 side chain, have been reported from other laboratories. See: (a) Nagasawa, K.; Georgieva, A.; Koshino, H.; Nakata, T.; Kita, T.; Hashimoto, Y. *Org. Lett.* **2002**, *4*, 177−180. (b) Moore, C. G. M.; Murphy, P. J.; Williams, H. L.; McGown, A. T.; Smith, N. K. *Tetrahedron Lett.* **2003**, *44*, 251−254.
(11) Collins, S. K.; McDonald, A. I.; Overman, L. E.; Rhee, Y. H. *Org. Lett.* **2004**, *6*, 1253−1255.
(12) The spectral data of compound **12** exhibit significant differences depending upon the nature of the counteranion. For example, ^{13}C NMR spectra taken immediately after column chromatography lack peaks corresponding to C13 and C14. Because phosphate-buffered SiO_2 was used, we presume that this sample of **12** was obtained as a phosphate salt. Unlike this salt, the chloride salt of **12**, prepared by washing a $CHCl_3$ solution of **12** with saturated NaCl solution, shows peaks for C13 and C14 in the ^{13}C NMR spectra. All other compounds having a 2-aminopyrimidinium moiety were characterized as chloride salts.

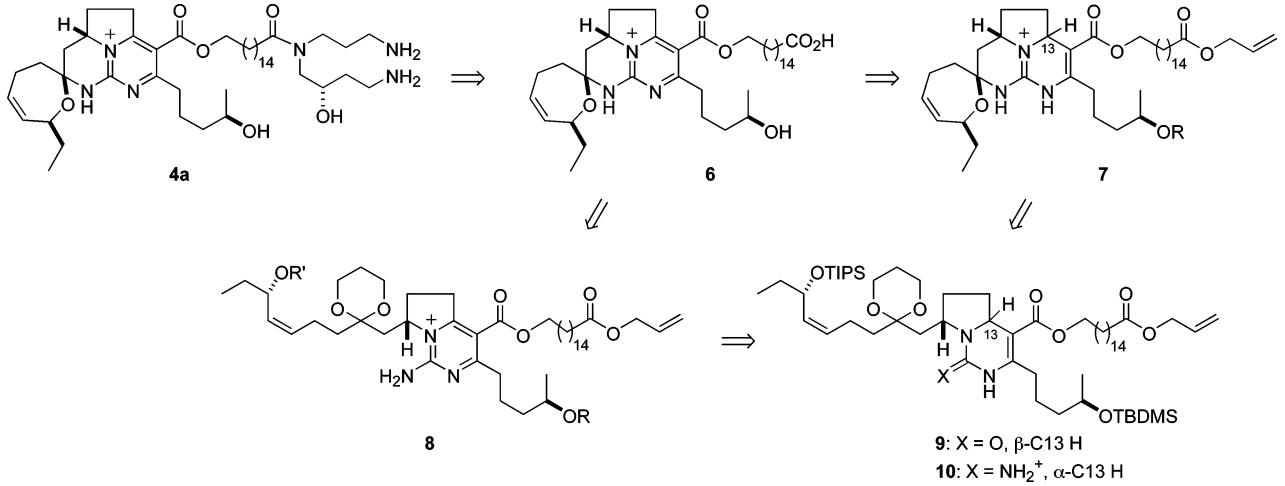
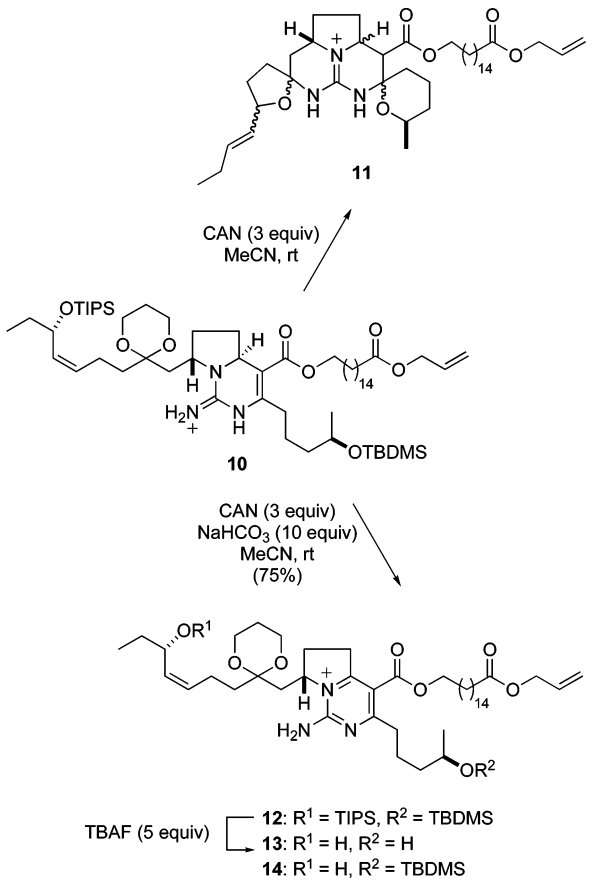


Figure 2. Two potential strategies for the total synthesis of the 3*S*,8*S*,10*S*,19*R*,43*S* isomer **4a** of crambidine.

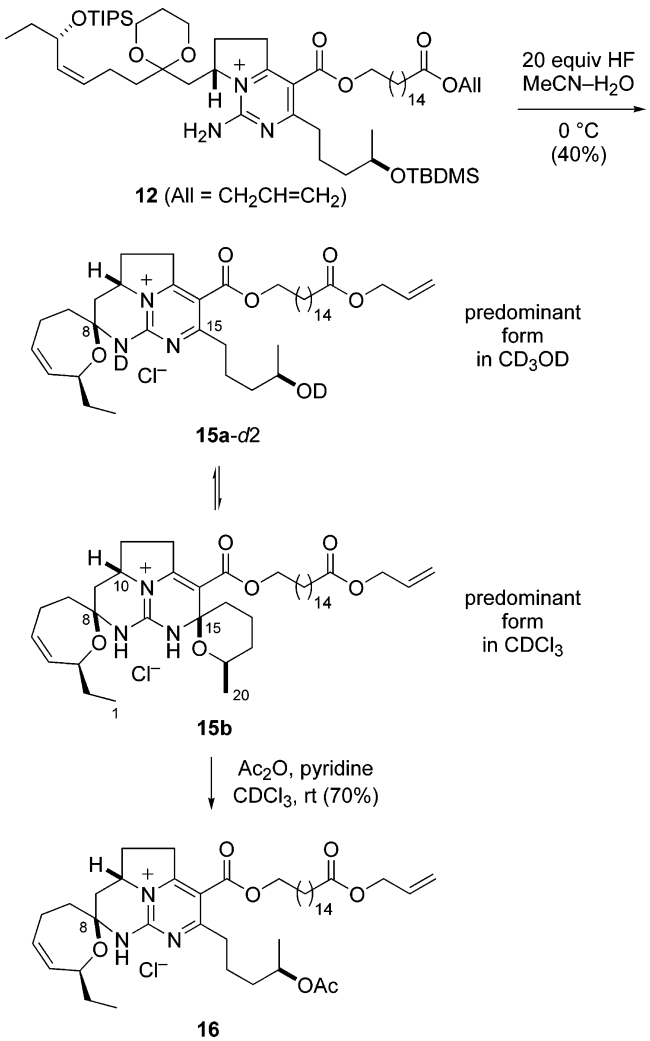
Scheme 1



guanidine diol and the relative robustness of the *tert*-butyldimethylsilyl (TBDMS) group. To our surprise, reaction of intermediate **12** with 2−3 equiv of tetrabutylammonium flouride (TBAF) at room temperature resulted in preferential cleavage of the triisopropylsilyl (TIPS) group to give derivative **14** as the major product. Both silyl protective groups could be removed by reaction of **12** with a large excess of TBAF in THF;[13] however, separation of the partially water-soluble guanidine diol **13** from excess Bu_4NF was problematic. Using CsF or KF in this deprotection resulted in removal of the TIPS group, but only partial cleavage of the TBDMS group.

(13) Using less than 5 equiv of TBAF resulted in incomplete removal of the TBDMS group.

Scheme 2



We explored next removal of the silyl protecting groups of tetrahydropyrrolopyrimidine **12** under acidic conditions, whereupon we quickly learned that the reactivity pattern of the two silyl groups was reversed (Scheme 2). For example, reaction of **12** at 0 °C with 5 equiv of 50% aqueous HF in acetonitrile led to rapid cleavage of the TBDMS group. Under these conditions, the TIPS group was cleaved at about the same rate

as the ketal protective group. Therefore, we turned to explore the possibility of directly converting 1-iminotetrahydro[1,2-*c*]-pyrimidine carboxylic ester **12** to the tetracyclic crambidine ring system. Increasing the amount of 50% aqueous HF to 20 equiv resulted in the clean transformation of **12** to two tetracyclic products, formed in a ~4:1 ratio.[14] Purification of this product mixture by reverse-phase HPLC provided the major isomer **15** as its hydrochloride salt in 40% yield.[15]

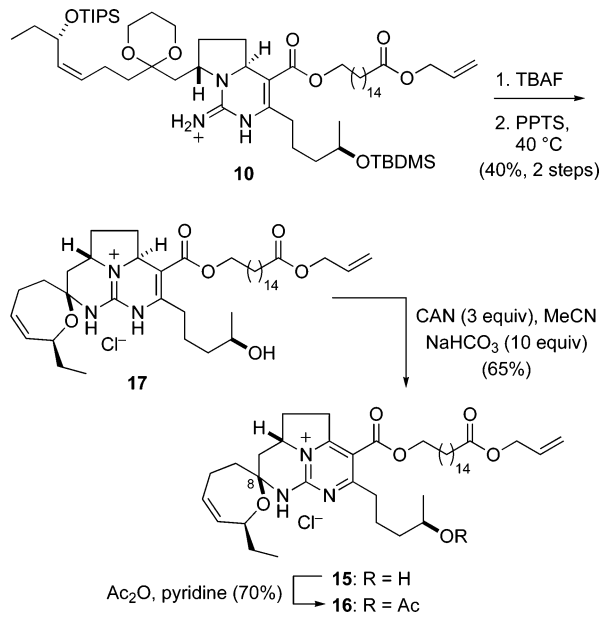
The ^{13}C NMR and UV spectra of product **15** taken in CD_3OD were in close agreement with those reported for peracetylcrambidine,[4e] giving us confidence that the 2-aminopyrimidinium unit was present. However, spectral data recorded for **15** in $CDCl_3$ were quite different. The most dramatic change was seen in the ^{13}C NMR signal for C15, which appears at 82.9 ppm in $CDCl_3$ and 181.9 ppm in CD_3OD solution. The UV spectrum also showed significant deviation depending upon the solvent: $\lambda_{max} = 254$ nm, $\epsilon = 19\,000$ in CD_3OD and 271 nm, $\epsilon = 16\,000$ in $CDCl_3$. From these observations, we conclude that product **15** exists as tetracyclic isomer **15a**-d_2 in CD_3OD and as pentacyclic isomer **15b** in $CDCl_3$ solution.[16] In other polar solvents such as CD_3CN and THF-d_8, tetracyclic isomer **15a** predominates.

Although preliminary efforts to open pentacyclic intermediate **15b** to give tetracyclic isomer **15a** by reaction with amine bases (1−10 equiv of pyridine or Et_3N) in $CDCl_3$ failed, acetylation of the former compound in $CDCl_3$ proceeded slowly to give tetracyclic acetate **16** in 70% yield.[17] Switching to the more polar solvent MeCN, or performing the reaction in pyridine, significantly shortened the reaction time, providing tetracyclic acetate **16** in comparable yield.

At this stage, the configuration of isomers **15a** and **15b** at the C8 spiroaminal stereocenter was assumed based on an axial trajectory in the formation of the dihydrooxepin ring.[9c] However, we were unsure of the relative orientation of the dihydrooxepin and tetrahydropyran rings in pentacyclic isomer **15b**, as both the cis and the trans relationships of these rings are observed in crambescidin natural products.[4] To determine the relative configuration the oxacyclic rings in pentacyclic isomer **15b**, extensive ^1H NMR NOE studies were performed at 800 MHz. Diagnostic NOEs were observed between the methyl hydrogens at C1 and C20, and between the C20 methyl and both C18 methylene hydrogens.[18,19] These data are consistent only with a cis relationship of the two oxacyclic rings, as found in crambescidin 816 (**1**) and crambescidin 800 (**2**). Furthermore, these NOEs show that the tetrahydropyran ring of **15b** preferentially adopts a chair conformation having the methyl substituent equatorial.

To further confirm the relative configuration of potential crambidine precursors **15** and **16** at the spiroaminal stereocenter C8, we examined the possibility of assembling these intermediates from pentacyclic precursor **17**, an intermediate of established relative and absolute configuration (Scheme 3).[9d] Following previously defined procedures, an isomerically pure sample of trans Biginelli adduct **10** was converted in two steps to tetracyclic alcohol **17**.[9d] Chemoselective dehydrogenation of this intermediate proceeded smoothly upon reaction with 3 equiv of buffered CAN to provide aminopyrimidine **15** in 65% yield. Subsequent acetylation gave rise to tetracyclic acetate **16**. Both of these products were identical to the samples prepared using the route summarized in Scheme 2, thus rigorously establishing the relative and absolute configuration at C8.

Scheme 3

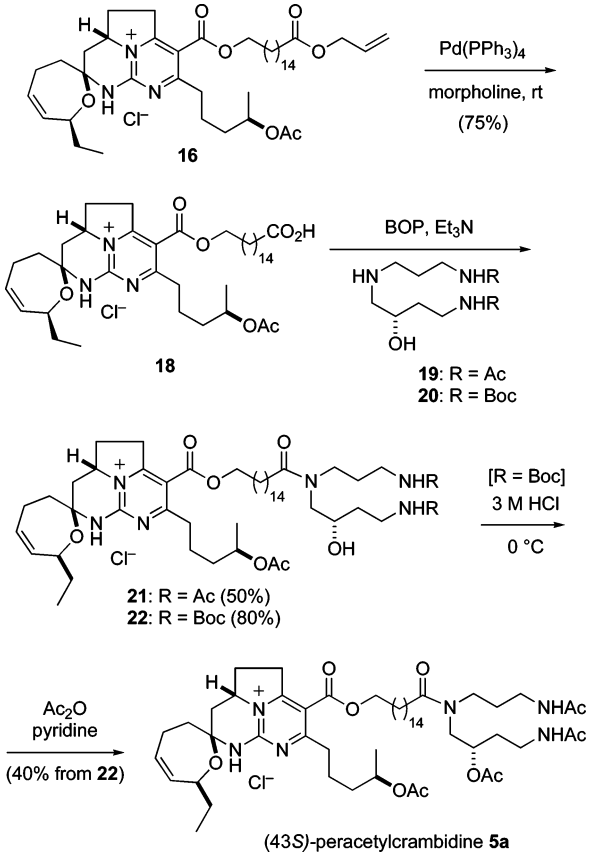


With the tetracyclic core of crambidine assembled, we focused on elaborating the hydroxyspermidine side chain. After removing the allyl protecting group from **16** to provide carboxylic acid **18**, we investigated the coupling of this acid with hydroxyspermidine derivatives **19**[20] and **20**.[21] Amino alcohol **19** successfully coupled with acid **18** in DMF in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) to form the triacetyl derivative **21** of crambidine in 50% yield. Acetylation of **21** provided (43*S*)-peracetylcrambidine **5a** in 70% yield. However, this sequence was not optimized as it would lead only to peracetylcrambidine. Rather we chose to couple **18** with hydroxyspermidine derivative **20**,[21] which under optimized conditions delivered amide **22** in 80% yield. Removal of the *tert*-butoxycarbonyl (Boc) group from this product using anhydrous HCl at 0 °C, followed by reaction of the crude diamine product with Ac_2O in pyridine, provided (43*S*)-peracetylcrambidine (**5a**) in 40% yield over two steps (Scheme 4). Spectral data for the synthetic product (^1H NMR, ^{13}C NMR, UV spectra) agreed well with those reported for the peracetyl derivative of crambidine.[4e]

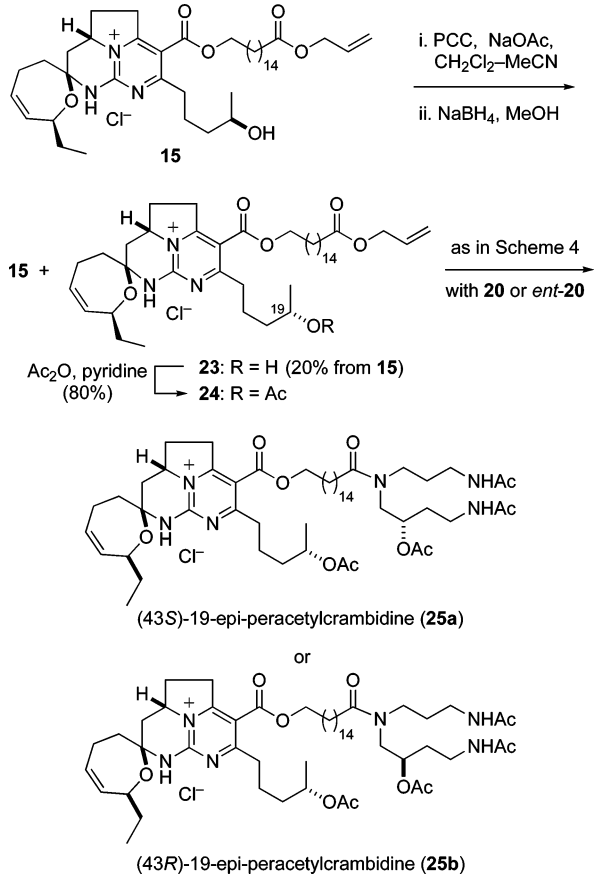
Preparation of Peracetylcrambidine Stereoisomers. As the C19 and C43 stereocenters are far removed from the tetracyclic 2-aminopyriminidine moiety, we were concerned that epimers at these stereogenic centers might not be distinguishable from

(14) The use of HF/pyridine effected the same conversion very slowly.
(15) Efforts to separate the minor stereoisomer in pure form proved fruitless.
(16) Formation of a pentacyclic isomer in $CDCl_3$ was observed for all of the intermediates having a *R* hydroxyl group at C19. Crambidine is insoluble in $CDCl_3$.
(17) This result stands in sharp contrast to our earlier observations with pentacyclic guanidines of the crambescidin 800 series, where the tetrahydropyran ring proved stable under identical conditions.[9c,d]
(18) Examination of mechanical and computational models shows that introduction of a C13−C14 double bond results in significant tilting of the spirotetrahydrofuran ring towards the spirodihydroxepin ring, bringing the terminal methyl and ethyl substituents in close proximity.
(19) The diagnostic NOE seen between the C19 and C1 hydrogens observed in crambescidin alkaloids such as **1** and **2**[4b,c] was not seen in **15b**.
(20) Prepared from hydroxyspermidine derivative **20**.[21] Full details can be found in the Supporting Information.
(21) Coffey, D. S.; McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 8741−8742.

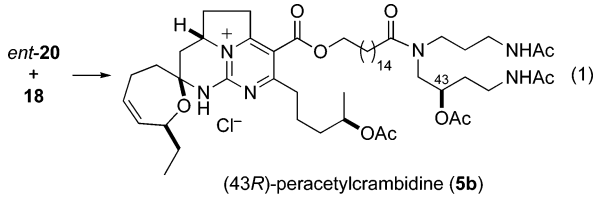
Scheme 4



Scheme 5



the 43*S* isomer of peracetylcrambidine (**5a**).[22] To pursue this issue, these epimers were synthesized. (43*R*)-Peracetylcrambidine (**5b**) was prepared readily from tetracyclic carboxylic acid **18** and hydroxyspermidine derivative *ent*-**20** (eq 1). As we had feared, we were unable to distinguish the C43 epimers of peracetylcrambidine by ^{1}H or ^{13}C NMR analysis.



We went on to generate both C43 epimers of 19-epi-peracetylcrambidine and 19-epi-crambidine. Possible Mitsunobu inversion of tetracyclic hydroxy allyl ester **15** was explored initially. However, these efforts proved fruitless as numerous variations of carboxylic acids, diazo compounds, and counteranions failed to generate C19 ester derivatives of **15**. Therefore, we resorted to a nonstereoselective synthesis of the desired epimers by an oxidation−reduction sequence. As summarized in Scheme 5, reaction of hydroxy allyl ester **15** with pyridinium chlorochromate (PCC) under buffered conditions provided an unstable ketone, which was immediately reduced with $NaBH_4$ to give a ∼1:1 mixture of **15** and epimer **23**. These isomers could be separated by column chromatography, providing the pure 19*S* isomer **23** in ∼20% overall yield. Unlike the 19*R* isomer **15**, epimer **23** exists exclusively in the tetracyclic form even in nonpolar solvents such as $CDCl_3$.[23] Acetylation of the secondary alcohol of **23** proceeded smoothly to generate acetate **24**. The ^{1}H NMR spectrum of **24** differs in subtle ways from that of C19 epimer **16**. In particular, the C19 methine hydrogen appears at 4.88 ppm in isomer **24**, whereas this signal for **16** is seen at 4.80 ppm.[24]

Using the sequence developed for the synthesis of (43*S*)-peracetylcrambidine (**5a**), the 19S isomer **24** was deprotected and coupled with bis-Boc hydroxyspermidine fragments **20** or *ent*-**20** to provide (43*S*)-19-epi-peracetylcrambidine (**25a**) and (43*R*)-19-epi-peracetylcrambidine (**25b**). Although ^{1}H and ^{13}C NMR spectra of these products are identical, they both differ from the corresponding spectra of the C19 epimers **5a** and **5b**. Again particularly revealing are the signals for the C19 methine hydrogens: 4.87 ppm for the 19*S* isomers and 4.80 for the 19*R* isomers.

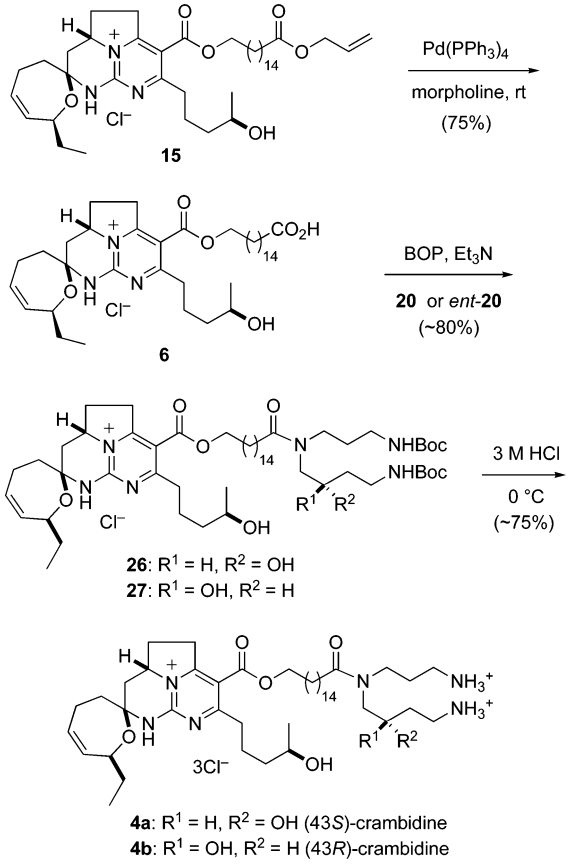
Synthesis of Crambidine. Having established the relative configuration of the tetracyclic core of crambidine, we turned to prepare both C43 epimers in the 3*S*,8*S*,10*S*,19*R* series. The marine isolate is most likely the 43*S* isomer, as this configuration of the hydroxyspermidine unit is found in crambescidins 816 (**1**)[4e] and 800 (**2**),[4c] 13,14,15-isocrambescidin 800 (**3**),[4d] and celeromycalin.[4f] Nonetheless, our studies revealed no rigorous evidence to rule out the 43*R* epimer as a potential candidate for the structure of natural crambidine.[25] Preparation of both C43 epimers of crambidine (**4a** and **4b**) was accomplished

(22) For example, the 43*R* and 43*S* epimers of 13,14,15-isocrambescidin cannot be distinguished by ^{1}H or ^{13}C NMR analysis.

(23) This result can be rationalized by the unfavorable steric environment of the C20 methyl in the pentacyclic isomer.

(24) The ^{13}C NMR spectra of **24** and **16** were indistinguishable (±0.2 ppm).

Scheme 6



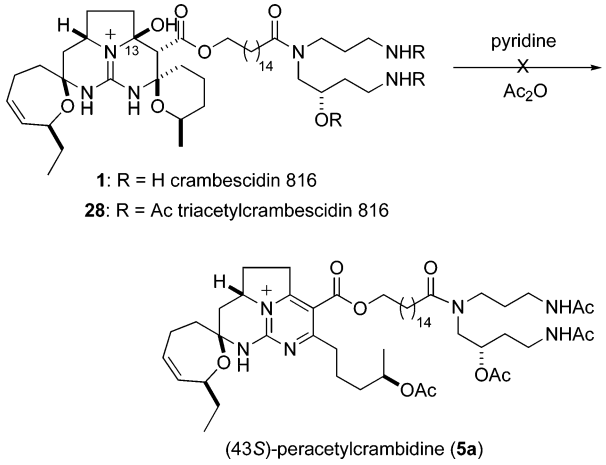
uneventfully, as summarized in Scheme 6. Removal of allyl group of **15** proceeded under Pd(0) catalysis to give carboxylic acid **6** in 75% yield. Coupling of acid **6** with the *S* enantiomer of Boc-protected hydroxyspermidine **20** gave amide **26** in 80% yield. Finally, removal of the Boc groups of **26** with 3 M HCl at 0 °C provided the (43*S*)-crambidine (**4a**), $[\alpha]_D$ −18.3 (*c* 1.4 CH_3OH), in 70% yield. Using the analogous procedure, acid **6** and *ent*-**20** were converted in comparable yield to (43*R*)-crambidine (**4b**), $[\alpha]_D$ −24.8 (*c* 0.25 CH_3OH). As with their peracetyl derivatives, ^1H and ^{13}C NMR spectra of these epimers were identical.

Unfortunately, optical rotations of natural crambidine or its peracetylated derivative were not reported.[4e] Thus, the absolute configuration of crambidine is not rigorously established by our total syntheses; nonetheless, it is undoubtedly the 3*S*,8*S*,10*S*,19*R* isomer as depicted in **4a** and **4b**, because this absolute configuration of the guanidine core is common to the crambescidin alkaloids.[3,4]

Attempted Hydration of the Crambidine Ring System and Dehydration of Crambescidin 816. After the synthesis of crambidine was completed, we investigated briefly the feasibility of adding water to the 2-aminopyrimidinium unit of the crambidine ring system to generate analogues of crambescidin 816 (**1**). Incubation of a methanol or acetonitrile solution of tetracyclic allyl ester **15** with an equal volume of aqueous pH 4, 7, or 10 buffer solutions at temperatures ranging from 25 to

(25) As a sample of authentic peracetylcrambidine was not available, we were unable to employ the method we used to define the absolute configuration of the hydroxyspermidine fragment of crambescidin 800 (**2**) and 13,14,15-isocrambescidin 800 (**3**).[9c,d]

Scheme 7



55 °C led to no reaction.[26] When the pH was reduced below 3, significant changes in the vinylic region were observed in ^1H NMR spectra, suggesting that the tetrahydrooxepin ring is unstable under acidic conditions. Attempted hydration under strongly basic conditions led to ester hydrolysis and extensive decomposition.[27]

We briefly explored the potential conversion of crambescidin 816 (**1**) to (43*S*)-crambidine **4a** with a sample of the natural product. However, **4a** was not detected when **1** was incubated under various acidic or basic conditions.[28] In addition, triacetylcrambescidin 816 (**28**) was prepared to examine its potential conversion to (43*S*)-peracetylcrambidine **5a** (Scheme 7). However, exposing **28** to Ac_2O in pyridine or triethylamine (25−70 °C) did not lead to the formation of detectable amounts of peracetylcrambidine.[26] Moreover, treatment of triacetylcrambescidin 816 (**28**) with bases such as pyridine and triethylamine in the absence of acetic anhydride did not lead to the elimination of the C13-hydroxyl group. Although our efforts to establish a chemical relationship between crambescidin 816 and crambidine were unsuccessful, these studies strongly suggest that crambidine and peracetylcrambidine are not artifacts that were generated from crambescidin 816 during the isolation process.[4e]

Summary

Total syntheses of the 3*S*,8*S*,10*S*,19*R*,43*S* isomer **4a** and the 3*S*,8*S*,10*S*,19*R*,43*R* isomer **4b** of the unique crambescidin alkaloid crambidine have been accomplished. These studies confirm the tetracyclic structure proposed by Braekman and coworkers for crambidine[4e] and establish the *rel*-3*R*,8*R*,10*R*,19*S* configuration for this moiety. Natural crambidine is most likely the 3*S*,8*S*,10*S*,19*R*,43*S* isomer **4a**. These syntheses were completed in five steps and ∼14% overall yield from 1-iminohexahydro[1,2-*c*]pyrimidine carboxylic ester **10**, an intermediate in our earlier total synthesis of 13,14,15-isocrambescidin 800 (**3**).[9b,d] The signature step in the total syntheses of crambidine and several stereoisomers is chemoselective dehydrogenation of the tethered Biginelli adduct **10** or the derived tetracyclic

(26) Reactions were monitored by electrospray mass spectrometry.
(27) Decarboxylation was not observed.
(28) (a) Attempted dehydration under aqueous or anhydrous conditions was unsuccessful. The following acidic conditions were examined: *p*-TsOH in $CHCl_3$ at 23−60 °C, 1 M dry HCl in EtOAc at 23 °C, H_2O (pH 4−9) at 23−60 °C, 1 M aqueous HCl at 23 °C (rapid decomposition). (b) The decomposition pathways observed in the attempted hydration of crambidine at high or low pH were seen here also.

intermediate **17**. Additionally these studies are the first to reveal the unprecedented ring-chain isomerization of the crambidine ring system, exemplified by the interconversion of isomers **15a** and **15b**, with pentacyclic isomer **15b** being favored in nonpolar solvents.

Experimental Section[29]

Pyrrolopyrimidine 12. Sodium bicarbonate (197 mg, 1.43 mmol) and cerric ammonium nitrate (235 mg, 0.431 mmol) were added sequentially to a solution of Biginelli product **10** (154 mg, 0.143 mmol, a 7:1 trans:cis mixture)[9d] and CH_3CN (1.4 mL, 0.1 M). This mixture was stirred for 7 h at room temperature, diluted with CH_3CN (20 mL), and filtered through a pad of Celite. The filtrate was concentrated, and the residue was purified by flash chromatography (silica gel buffered at pH 7, eluted with 1−2% MeOH in $CHCl_3$) to give a colorless oil. This product was partitioned between $CHCl_3$ (100 mL) and 0.1 N HCl (15 mL). The organic layer was washed with 0.1 N HCl (15 mL) and brine (2 × 15 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated to give chloride salt **12** as a colorless oil (115 mg, 0.107 mmol, 75% yield): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (br s, 1H), 8.25 (br s, 1H), 5.88−5.95 (m, 1H), 5.49 (app t, J = 4.4 Hz, 1H), 5.42 (app t, J = 2.5 Hz, 1 H), 5.21−5.33 (m, 3H), 4.57 (br d, J = 5.7 Hz, 2H), 4.42 (m, 1H), 4.31 (br t, J = 6.3 Hz, 2H), 3.95 (m, 1H), 3.92 (m, 1H), 3.88 (m, 1H), 3.79 (m, 1H), 3.42−3.60 (m, 2H), 3.02 (m, 2H), 2.60 (m, 1H), 2.34 (m, 1H), 2.32 (t, J = 6.1 Hz, 3H), 1.75−1.99 (m, H), 1.24−1.73 (m, H), 1.11 (d, J = Hz, H), 1.03 (s, 21H), 0.86 (t, J = Hz, 3H), 0.037 (s, 3H), 0.024 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 179.1, 173.5, 167.0, 163.5, 153.1, 135.1, 132.3, 126.8, 118.0, 111.9, 100.7, 69.9, 68.1, 66.5, 64.9, 63.0, 59.4, 59.1, 39.3, 38.2, 37.5, 34.3, 34.2, 33.4, 31.7, 30.3, 29.7, 29.6(3), 29.5, 29.4, 29.2(2), 29.1, 28.6, 27.2, 26.0, 25.9, 25.8, 24.9, 24.5, 24.2, 23.8, 22.1, 18.1(2), 18.0, 12.4, 12.33, 12.36, 9.3, −4.4, −4.7; IR (KBr) 2927, 2856, 2177, 2158, 2034, 2011, 1972, 1741, 1714, 1683, 1540, 1463, 1254 cm^{-1}; $[\alpha]^{27}_{589}$ −90.3, $[\alpha]^{27}_{577}$ −119, $[\alpha]^{27}_{546}$ −118, $[\alpha]^{27}_{435}$ −245, $[\alpha]^{27}_{405}$ −300 (*c* 0.10, MeOH); HRMS (ESI$^+$) *m*/*z* 1042.8 (1042.6 calculated for $C_{59}H_{107}N_3O_8Si_2$ [M$^+$]).

Preparation Crambidine Congener 15 from Pyrrolopyrimidine 12. A solution of pyrrolopyrimidine **12** (200 mg, 0.186 mmol), HF (50% aqueous solution, 100 mg, 1.86 mmol), and CH_3CN (1.8 mL, 0.1 M) was maintained at 0 °C. After 6 h, this solution was partitioned between $CHCl_3$ (50 mL) and water (10 mL), and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and concentrated. The residual oil was purified by reverse-phase preparative HPLC (Phenomenex C-18, 10 mL/min, 10% MeCN:0.1% HCl in H_2O to 90% MeCN:0.1% HCl in H_2O over 20 min, t_R = 22 min) to give chloride salt **15** as a clear oil (54 mg, 0.074 mmol, 40% yield): ^{1}H NMR (800 MHz, $CDCl_3$) δ 11.7 (br s, 1H), 10.5 (br s, 1H), 5.87−5.95 (m, 1H), 5.65 (m, 1H), 5.50 (br d, J = 10.9 Hz, 1H), 5.31 (dq, J = 17.2, 1.5 Hz, 1H), 5.22 (dq, J = 10.4, 1. 4 Hz, 1H), 4.57 (d, J = 7.2 Hz, 2H), 4.56 (m, 1H), 4.32 (m, 1H), 4.17 (m, 3H), 3.60 (dd, J = 19, 8 Hz, 1H), 2.95 (m, 1H), 2.80 (m, 1H), 2.66 (m, 1H), 2.58 (m, 1H), 2.42 (m, 1H), 2.34−2.28 (m, 3H), 2.32 (t, J = 7.5 Hz, 2H), 2.24−2.18 (m, 1H), 1.95 (m, 1H), 1.78 (m, 1H), 1.75−1.72 (m, 3H), 1.68−1.65 (m, 2H), 1.61−1.58 (m, 2H), 1.47 (m, 1H), 1.42 (m, 1H), 1.20−1.45 (m, 18H), 1.08 (d, J = 5.6 Hz, 3H), 0.85 (t, J = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 164.5, 149.0, 147.2, 133.3, 132.3, 129.7, 118.0, 107.3, 84.2, 82.9, 71.5, 66.8, 65.0, 64.9, 54.7, 37.2, 36.9, 34.2, 31.6, 31.3, 30.4, 29.58, 29.57, 29.52, 29.47, 29.4, 29.2, 29.1, 29.0, 28.5, 26.2, 24.9, 23.5, 21.7, 18.6, 10.4; ^{1}H NMR (500 MHz, CD_3OD) δ 5.97−5.89 (m, 1H), 5.76 (m, 1H), 5.55 (br d, J = 11.2 Hz, 1H), 5.30 (br d, J = 17.1 Hz, 1H), 5.20 (br d, J = 10.5 Hz, 1H), 4.86 (m, 1H), 4.56 (m, 3H), 4.37 (t, J = 6.6 Hz, 2H), 3.77−3.70 (m, 1H), 3.63 (dd, J = 19, 8 Hz, 1H), 3.60−3.50 (m, 1H), 3.30−3.09 (m, 2H), 2.85 (dd, J = 13.4, 3.6 Hz, 1H), 2.75−2.70 (m, 1H), 2.64 (app t, J = 13 Hz, 1H), 2.48 (app t, J = 14 Hz, 1H), 2.34 (t, J = 7.4 Hz, 2H), 2.28−2.20 (m, 1H), 2.16−2.09 (m, 1H), 2.08−2.02 (m, 1H), 1.92−1.72 (m, 4H), 1.70 (app t, J = 13 Hz, 1H), 1.54−1.60 (m, 3H), 1.52−1.47 (m, 2H), 1.45−1.40 (m, 2H), 1.38−1.28 (m, 22H), 1.15 (d, J = 6.2 Hz, 3H), 0.83 (t, J = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 181.9, 175.2, 167.6, 164.7, 151.8, 133.9, 133.8, 131.6, 118.3, 114.6, 87.0, 72.7, 68.3, 67.5, 66.1, 61.0, 39.7, 38.7, 37.7, 35.7, 35.1, 34.7, 30.89, 30.87, 30.84, 30.82, 30.80, 30.71, 30.5, 30.4, 30.3, 29.8, 27.4, 26.2, 25.7, 25.0, 23.8, 10.7; UV($CHCl_3$) λ_{max} 271 (16 000); UV(MeOH) λ_{max} 254 nm (19 000), 300 (5500); IR (neat) 3558, 2927, 2856, 1725, 1629, 1583, 1455, 1258, 1119 cm^{-1}; $[\alpha]^{27}_{589}$ −31.9, $[\alpha]^{27}_{577}$ −35.3, $[\alpha]^{27}_{546}$ −45.4, $[\alpha]^{27}_{435}$ −120, $[\alpha]^{27}_{405}$ −151 (*c* 0.22, MeOH); HRMS (ESI$^+$) *m*/*z* 696.4954 (696.4952 calculated for $C_{41}H_{66}N_3O_6$ [M$^+$]).

Preparation Crambidine Congener 15 from Tricyclic Guanidine 17. Sodium bicarbonate (70 mg, 0.51 mmol) and finally ground cerric ammonium nitrate (86 mg, 0.16 mmol) were added sequentially to a solution of **17** (41 mg, 0.051 mmol) and CH_3CN (0.5 mL, 0.1 M). This mixture was stirred for 7 h at room temperature, diluted with CH_3CN (20 mL), filtered through a pad of Celite, and concentrated. The residual oil was purified by reverse-phase preparative HPLC (Phenomenex C-18, 10 mL/min, 10% MeCN:0.1% HCl in H_2O to 90% MeCN:0.1% HCl in H_2O over 20 min, t_R = 22 min) to give chloride salt **15** as a clear oil (24 mg, 0.033 mmol, 65% yield). Analytical and spectral data for this product were identical to those of the sample prepared from **12**.

(43*S*)-Crambidine (4a). A solution of amide **26** (26 mg, 0.025 mmol) and 3 M HCl in ethyl acetate (500 μL) was maintained at 0 °C for 2 h. After concentration, the residual polar oil was purified by reverse-phase HPLC (Phenomenex C-18, 10 mL/min, 10% MeCN:0.1% HCl in H_2O to 90% MeCN:0.1% HCl in H_2O over 20 min, t_R = 11 min) to give trichloride salt **4a** as a colorless oil (18 mg, 0.019 mmol, 77% yield): ^{1}H NMR (500 MHz, CD_3OD) δ 5.76 (m, 1H), 5.55 (br d, J = 11.0 Hz, 1H), 4.84 (m, 1H), 4.56 (m, 1H), 4.37 (t, J = 6.7 Hz, 2H), 3.96 (m, 1H), 3.74 (m, 1H), 3.67 (m, 1H), 3.65−3.52 (m, 3H), 3.46−3.42 (m, 2H), 3.20−3.12 (m, 5H), 2.98−2.82 (m, 2H), 2.78−2.72 (m, 1H), 2.66−2.62 (m, 1H), 2.55−2.45 (m, 3H), 2.32−2.20 (m, 1H), 2.15−1.98 (m, 3H), 1.95−1.75 (m, 6H), 1.75−1.65 (m, 2H), 1.62−1.42 (m, 9H), 1.41−1.31 (m, 23H), 1.16 (d, J = 6.2 Hz, 3H), 0.83 (t, J = 7.3 HHH Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 181.8, 177.6, 172.6, 164.7, 151.8, 133.9, 131.6, 114.7, 87.0, 72.7, 71.9, 68.6, 68.3, 67.5, 61.1, 55.1, 44.2, 39.70, 39.68, 38.7, 37.7, 35.7, 34.3, 33.1, 30.92, 30.87, 30.84, 30.82, 30.80, 30.6, 30.5, 30.4, 29.8, 27.4, 26.8, 25.7, 25.0, 23.8, 10.7; IR (neat) 3385, 2927, 2856, 1722, 1629, 1586, 1459, 1293, 1262, 1162, 1127 cm^{-1}; $[\alpha]^{27}_{589}$ −18.3, $[\alpha]^{27}_{577}$ −19.6, $[\alpha]^{27}_{546}$ −24.3, $[\alpha]^{27}_{435}$ −108, $[\alpha]^{27}_{405}$ −120 (*c* 1.41, MeOH); HRMS (ESI$^+$) *m*/*z* 801.6360 (801.6122 calculated for $C_{45}H_{81}N_6O_6$ [M$^+$]).

(43*R*)-Crambidine (4b). Using the analogous procedure, the Boc groups of amide **27** were removed to provide trihydrochloride salt **4b** (8 mg, 0.008 mmol, 71% yield) as a colorless oil: ^{1}H NMR and ^{13}C NMR spectral data of this compound are identical to those for **4a**; $[\alpha]^{27}_{589}$ −24.8, $[\alpha]^{27}_{577}$ −30.1, $[\alpha]^{27}_{546}$ −41.1, $[\alpha]^{27}_{435}$ −131, $[\alpha]^{27}_{405}$ −164 (*c* 0.25, MeOH); HRMS (ESI$^+$) *m*/*z* 801.6220 (801.6122 calculated for $C_{45}H_{81}N_6O_6$ [M$^+$]).

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Supporting Information Available: Experimental procedures and characterization data for new compounds not reported in the Experimental Section, and copies of ^{1}H and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(29) General experimental details have been described: Ando, S.; Minor, K. P.; Overman, L. E. *J. Org. Chem.* **1997**, *62*, 6379−6387.