Enantioselective Total Syntheses of 13,14,15-Isocrambescidin 800 and 13,14,15-Isocrambescidin 657

D. Scott Coffey,[†] Larry E. Overman,* and Frank Stappenbeck[‡]

Contribution from the Department of Chemistry, 516 Rowland Hall, University of California, Irvine, CA 92697-2025
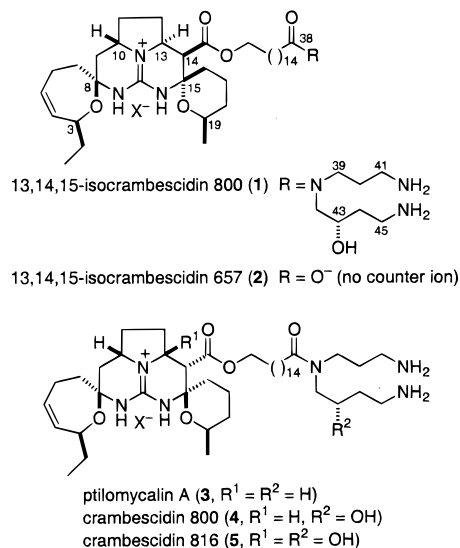
Received January 21, 2000

Abstract: The first total syntheses of 13,14,15-isocrambescidin 800 (**1**) and 13,14,15-isocrambescidin 657 (**2**) were accomplished in convergent fashion. The central strategic step was tethered Biginelli condensation of guanidine aminal **14** and β-ketoester **15** to give 1-iminohexahydropyrrolo[1,2-c]pyrimidine carboxylic ester **16**. This step united all the heavy atoms of the pentacyclic guanidine nucleus and set the critical trans C10–C13 stereorelationship. Acidic treatment of derivative **18** triggered tricyclization to generate pentacyclic guanidine **19b** in high yield. After cleavage of the allyl ester, the derived acid underwent coordinated epimerization at C14 and C15 in the presence of triethylamine to form the pentacyclic isocrambescidin nucleus. The synthesis of **1** was achieved in 11% overall yield from amine **12** by a sequence involving five isolated intermediates. As detailed in the preceding account, **12** can be accessed from commercially available 3-butyn-1-ol in 30% overall yield by way of nine isolated and purified intermediates. Mosher derivatives were prepared from (S)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid and natural **1**, synthetic **1**, and synthetic C43 epimer **31**. Analysis by ¹⁹F NMR showed that the Mosher derivatives of natural and synthetic **1** were identical, thus establishing for the first time that the stereochemistry of 13,14,15-isocrambescidin 800 (**1**) at C43 is S. The mechanism of the tricyclization and epimerization steps is discussed, as are the relative energies of the 13,14,15-isocrambescidin, 13,15-epicrambescidin, and 13-epicrambescidin guanidine moieties.

Introduction

Crambe crambe is a bright red marine sponge that is the most widespread species of littoral sponge found in the Northwestern Mediterranean.[1] For years, extracts of *C. crambe* have been known to be ichthyotoxic and show various pharmacological activities.[2] As discussed in more detail in the preceding paper,[3] a structurally remarkable group of complex guanidines, the crambescidins, have been isolated from *C. crambe*.[4] Although the pentacyclic guanidine cores of nearly all the crambescidins have the stereochemistry exemplified by ptilomycalin A (**3**), crambescidin 800 (**4**), and crambescidin 816 (**5**),[4,5] the Rinehart and Braekman groups described two rare crambescidin alkaloids,

13,14,15-isocrambescidin 800 (**1**)[5f,6] and 13,14,15-isocrambescidin 657 (**2**),[7] having a quite different topography.

13,14,15-isocrambescidin 800 (**1**) R = N...

13,14,15-isocrambescidin 657 (**2**) R = O⁻ (no counter ion)

ptilomycalin A (**3**, R¹ = R² = H)
crambescidin 800 (**4**, R¹ = H, R² = OH)
crambescidin 816 (**5**, R¹ = R² = OH)

Extensive NMR studies revealed that 13,14,15-isocrambescidin 800 (**1**) is epimeric at C13, C14, and C15 to other members of the crambescidin family.[5f,6] Particularly diagnostic were ¹H NMR nuclear Overhauser effects (NOEs) observed for **1** between H19 and H13 and H14, and the absence of NOEs between H10 and H13 and H19 and N2H that are seen in **3**–**5**

† Current address: Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN 46285.

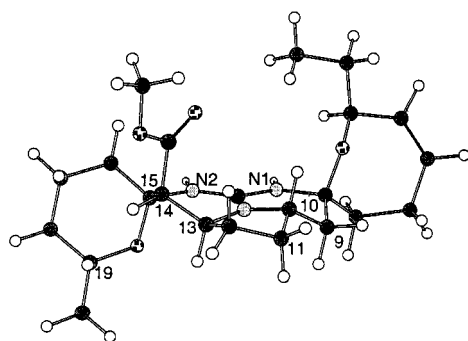‡ Current address: Elan Pharmaceuticals, 800 Gateway Boulevard, South San Francisco, CA 94080.

(1) Becerro, M. A.; Uriz, M. J.; Turon, X. *Mar. Biol.* **1994**, *121*, 301–307.

(2) For a brief review of the biology and ecology of *C. crambe*, see: Berlinck, R. G. S. *Nat. Prod. Rep.* **1999**, *16*, 339–365.
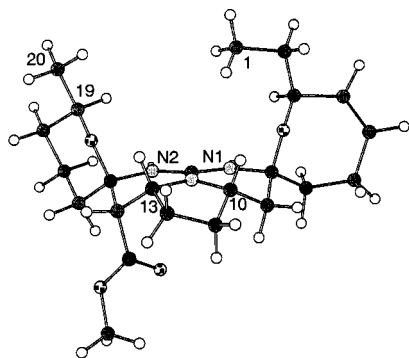
(3) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A., preceding paper in this issue.

(4) For reviews, see: (a) ref 3. (b) Berlinck, R. G. S. *Nat. Prod. Rep.* **1996**, *13*, 377–409. (c) Berlinck, R. G. S. *Prog. Chem. Org. Nat. Prod.* **1995**, *66*, 119–295. (d) Faulkner, D. J. *Nat. Prod. Rep.* **1999**, *16*, 155–198, and earlier reviews in this series.

(5) (a) Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925–8926. (b) Jares-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712–5715. (c) Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1992**, *114*, 8472–8479. (d) Ohtani, I.; Kusumi, T.; Kakisawa, H. *Tetrahedron Lett.* **1992**, *33*, 2525–2528. (e) Tavares, R.; Daloze, D.; Braekman, J. C.; Hajdu, E.; Muricy, G.; Van Soest, R. W. M. *Biochem. Syst. Ecol.* **1994**, *22*, 645–646. (f) Berlinck, R. G. S.; Braekman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015.

(6) Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808.

(7) Shi, J.-G.; Sun, F.; Rinehart, K. L. WO Patent 3,756,734, 1998.

Syntheses of Two Crambescidin Alkaloids

J. Am. Chem. Soc., Vol. 122, No. 20, 2000 4905

13,14,15-isocrambescidin core
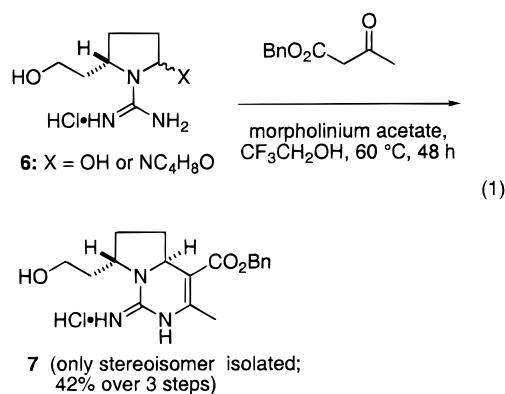

crambescidin/ptilomycalin A core

Figure 1. Models of the methyl ester analogs of 13,14,15-isocrambescidin and crambescidin/ptilomycalin A.[10]

(Figure 1).[5f,6] Moreover, the long-range NOE between the C1 methyl group and H19 that signals the stereochemistry of the other crambescidins was not seen in **1**.[6] The absolute configuration of the guanidine core of **1** was established by ozonolytic excision of C1−C4 to yield methyl (*S*)-2-hydroxybutanoate.[6] Prior to the total synthesis recorded herein,[8] the absolute configuration at C43 of the hydroxyspermidine unit of **1** had not been established, although it had been assumed to be *S* in analogy with crambescidin 816 (**5**).[5f,9] The similarity of ^{1}H and ^{13}C NMR data of **1** and **2**, and limited NOESY data, support the structure assignment of 13,14,15-isocrambescidin 657 (**2**).[7] The pharmacological properties of 13,14,15-isocrambescidin 800 (**1**) and 13,14,15-isocrambescidin 657 (**2**) have received only scant attention due to the low abundance of these isocrambescidin alkaloids. Isocrambescidins **1** and **2** are reported to be less cytotoxic to L1210 murine leukemia cells than other crambescidins.[6,7]

Synthesis Plan. The structural differences and similarities between the two crambescidin families are apparent in molecular mechanics models of the methyl esters of the 13,14,15-isocrambescidin and crambescidin/ptilomycalin A pentacyclic guanidine moieties (Figure 1).[10,11] For instance, the C10 and C13 angular hydrogens are trans in the isocrambescidin core

and cis in the corresponding crambescidin/ptilomycalin A unit, whereas the stereochemical relationship between the substituents at C13, C14, and C15 is the same in both structures. For both alkaloid families, the C−O bonds of the hydropyran and oxepene units are axial. Thus, as in the crambescidin/ptilomycalin A series,[12,13] we anticipated that the C8 and C15 spirocenters of the isocrambescidins would evolve with the desired stereochemistry if the central triazaacenaphthalene ring system was constructed with the proper trans stereochemistry.

An intramolecular variant of the venerable Biginelli condensation that we introduced several years ago[14] has proven to be highly useful in the design of concise strategies for synthesizing complex guanidine alkaloids. As detailed in the accompanying account,[3] tethered Biginelli condensation of a ureido aldehyde and a β-ketoester can be employed to combine all the carbons of the crambescidin/ptilomycalin A pentacyclic core and set the pivotal cis relationship of the H10 and H13 hydrogens.[15] Recent exploratory studies of stereoselection in tethered Biginelli condensations were critical in our planning on how to synthesize the isocrambescidin alkaloids.[16] These investigations revealed that the stereochemical outcome of tethered Biginelli condensations could be reversed if the urea component was replaced with a basic guanidine. Thus, Biginelli condensation of guanidine aldehyde (or aminal) **6** with benzyl acetoacetate provided *trans*-1-iminohexahydropyrrolo[1,2-*c*]pyrimidine **7** with high selectivity (eq 1).[16]

(1)

6: X = OH or NC$_4$H$_8$O

morpholinium acetate,
CF$_3$CH$_2$OH, 60 °C, 48 h

7 (only stereoisomer isolated; 42% over 3 steps)

On the basis of these exploratory studies and our experience in the crambescidin/ptilomycalin A series, a convergent plan for preparing 13,14,15-isocrambescidin 800 (**1**) readily emerged (Scheme 1). Tethered Biginelli condensation of guanidine aldehyde **10** and β-ketoester **11** would be employed to set the critical trans C10−C13 stereorelationship and unite *all* the heavy atoms of the pentacyclic guanidine moiety. It was hoped that acid-promoted dehydration of **9** would then generate the remaining three heterocyclic rings of **8** in a single step. We were mindful from the outset of one challenge posed by this strategy: the guanidine functional group would be introduced early in the synthesis. Unless we wanted to add protection and

(8) For a preliminary communication, see: Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **1999**, *121*, 6944−6945.

(9) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519.

(10) The lowest energy conformation found from Monte Carlo searches using Macromodel version 5.5 and the optimized potentials for liquid simulations OPLS force field is depicted.[11] Ten thousand starting conformations were examined; in all cases, several conformations that differ only in the spatial orientation of the methyl ester fragment were within a few kilocalories of the global minimum. As discussed later in the text, the conformation of the 13,15-isocrambescidin core depicted in Figure 2 is undoubtedly not the lowest energy conformation.

(11) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379−4386.

(12) (a) Snider, B. B.; Shi, Z. *Tetrahedron Lett.* **1993**, *34*, 2099−2102. (b) Snider, B. B.; Shi, Z. *J. Am. Chem. Soc.* **1994**, *116*, 549−557.
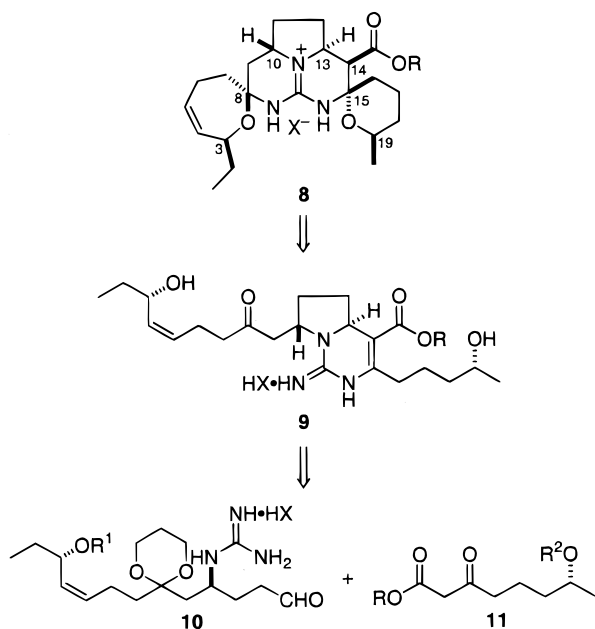
(13) Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657−2658.

(14) Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 3235−3237.
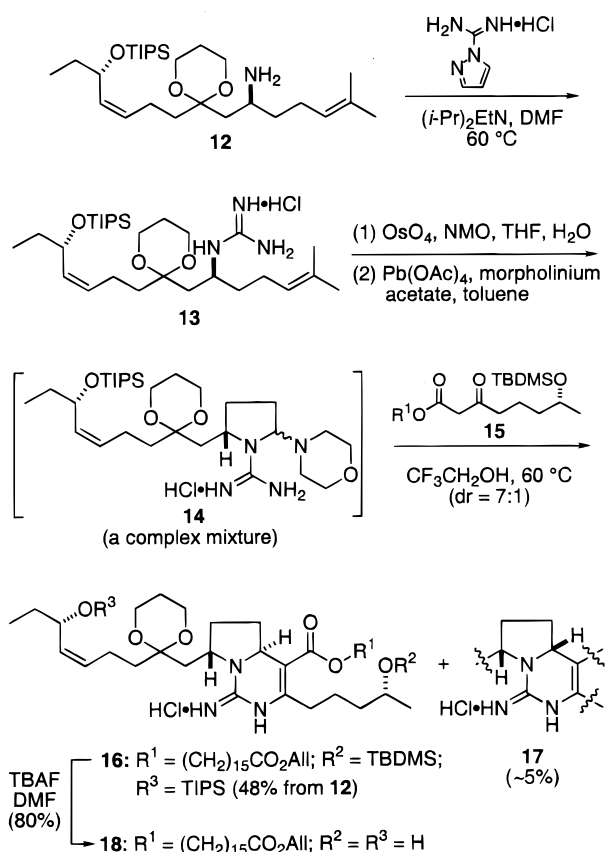
(15) For a review of the Biginelli reaction, see: Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937−6963.

(16) McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520−1528.

Scheme 1

Scheme 2

deprotection steps, we would be forced to carry this highly polar functionality through several stages of the synthesis.

Results and Discussion

Synthesis of *trans*-1-Iminohexahydropyrrolo[1,2-*c*]pyrimidine 18. The total syntheses of **1** and **2** began with diene amine **12**, which we also utilized in our synthesis of (−)-crambescidin 800 (Scheme 2).[3] Treatment of **12** with 1-*H*-

pyrazole-1-carboxamidine hydrochloride[17] and diisopropylethylamine at 60 °C generated guanidine **13**, which was utilized directly without purification. The trisubstituted double bond of this intermediate next had to be cleaved to liberate the electrophilic component of the Biginelli condensation. Fortunately, the oxidation strategy that we had employed to realize this degradation in the related urea series was compatible with the guanidine functionality. Thus, selective dihydroxylation of the trisubstituted double bond of **13** with catalytic osmium tetroxide (OsO$_4$) and *N*-methylmorpholine-*N*-oxide (NMO),[18] followed by cleavage of the resulting diol with Pb(OAc)$_4$ in the presence of morpholinium acetate, provided **14**. This intermediate was purified only by filtration to remove PbO$_2$ and was a mixture of several components as judged by ^{1}H and ^{13}C NMR analysis.[19]

Biginelli condensation of crude **14** and β-ketoester **15**[3] in EtOH at 60 °C proceeded with modest trans selectivity (3:1). Fortunately, we found that heating **14** with 1.5 equiv of **15** in 2,2,2-trifluoroethanol at 60 °C for 20 h improved diastereoselection to 7:1. After purification of the crude products on silica gel deactivated with pH 7.0 buffer,[20] the desired trans adduct **16** was isolated in 48% yield and cis adduct **17** was isolated in ca. 5% yield.[21] The stereochemistry of **16** was provisionally assigned based on our earlier exploratory studies.[16] As we will see shortly, this assignment could be confirmed rigorously at a latter stage. Deprotection of **16** with tetra-*n*-butylammonium fluoride (TBAF) in *N,N*-dimethylformamide (DMF) at room temperature for 36 h gave rise to diol **18** in 80% yield. In some runs, this reaction did not go to completion and intermediates in which only the triisopropylsilyl (TIPS) group had been removed were isolated in 10−15% yield. Heating the reaction mixture at 60 °C avoided this complication, however, other unidentified products were formed and the isolated yield of **18** was not improved.

Cyclization to Form Pentacycle 19. We were now positioned to examine formation of the central triazaacenaphthalene ring and the two spiro aminal units. Initially guanidine diol **18** was exposed at room temperature to 3 equiv of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H$_2$O) in CHCl$_3$ for 24 h (Scheme 3). After the reaction mixture was washed with aqueous HCO$_2$Na, a 1:1 mixture of a pentacyclic product, subsequently shown to be **19a**, and tetrahydrofuryl isomer **20a** were isolated in ca. 50% yield.[22]

The constitution of these pentacyclic products was ascertained as follows. The gross structure of pentacycle **20a**, a ∼1:1 mixture of stereoisomers at the center carrying the 1-butenyl side chain, was secured by ^{1}H NMR correlation spectroscopy (COSY) and ^{13}C NMR studies. The stereochemistry of **20a** at C15[23] followed from the chemical shift of the C14 methine hydrogen (δ 2.88),[24] whereas the stereochemistry at C8 was not determined and is assigned on the basis of analogy only.

(17) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497−2502.

(18) Sharpless, K. B.; Williams, D. R. *Tetrahedron Lett.* **1975**, 3045−3046.

(19) Multiple signals were observed for many carbon atoms in ^{13}C NMR spectra of **14** and ^{1}H NMR spectra showed several broad peaks; no aldehyde signal was apparent.

(20) Deactivated silica was prepared by adding 10% (by weight) pH 7 phosphate buffer to Merck silica gel (0.040−0.063 μm) and mixing until homogeneous.

(21) Because the cis adduct **17** was slower moving on silica gel than **16**, it was difficult to isolate pure **17**.

(22) Exchange of the tosylate counter ion for formate required several washings with aqueous sodium formate, which led to some erosion in yield.

(23) The crambescidin numbering system is employed in the discussion of synthetic intermediates; correct IUPAC names and numbering can be found in the Experimental Section.

Scheme 3
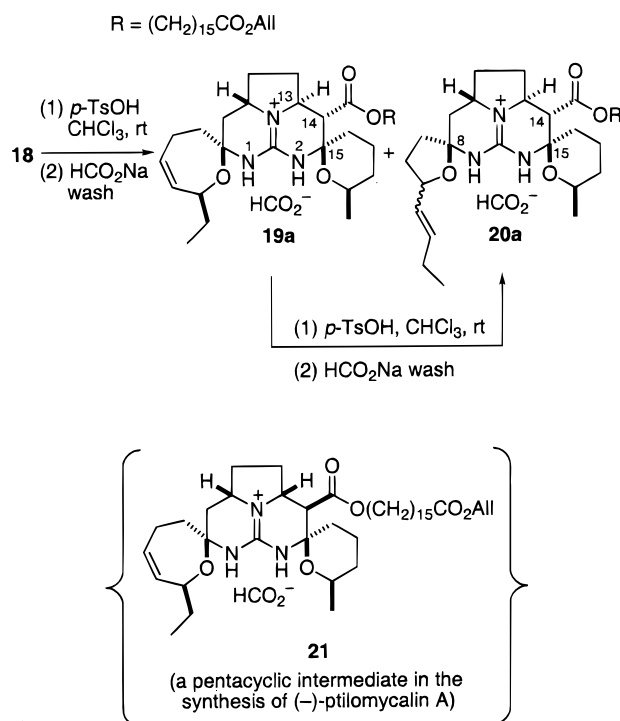
Pentacyclic guanidines **19a** and **20a** were isolated as their formate salts to allow direct comparisons with pentacycle **21**, an intermediate in our original synthesis of (−)-ptilomycalin A.[3,13] That **19a** was epimeric to **21** at C13 was signaled by the absence of an ^{1}H NMR NOE between H10 and H13 in the former, while the 11.7 Hz coupling constant of the C14 methine hydrogen of **19a** showed that the ester side chain was equatorial. Inexplicably, the ^{1}H NMR signal for N2H was not apparent in formate salt **19a**, which led to us originally misassign the stereochemistry of this intermediate at C15.[25]

Because none of the pentacyclic guanidine intermediates or products prepared during our investigations were crystalline, ^{1}H NMR NOE studies proved indispensable in assigning stereochemistry. A molecular mechanics model of the guanidine moiety of **19a** (an intermediate having the 13-epicrambescidin core), which helped in analysis of critical NOE enhancements, is provided in Figure 2.[10,26] Also provided in this figure are models of the two additional guanidine pentacycles (13,14,15-isocrambescidin and 13,15-epicrambescidin ring systems) we will soon encounter in our discussion, and, for reference, a model of the crambescidin/ptilomycalin A pentacyclic guanidine moiety.

Additional investigation revealed that formation of tetrahydrofuran isomer **20a** from **18** could be controlled by varying reaction time and equivalents of *p*-TsOH·H$_2$O. Larger amounts of acid and longer reaction times favored the formation of **20a**. Exposing **19a** to *p*-TsOH·H$_2$O at room temperature for extended periods also led to **20a**. The best conditions found for generating **19a** involved exposing **18** to 2 equiv of *p*-TsOH·H$_2$O in CHCl$_3$ for 7 h at room temperature; a 5:1 mixture of **19a** and **20a** was

produced. Because these isomers were difficult to separate, the isolated yield of **19a** produced in this way was never greater than 50%.

We next examined using pyridinium *p*-toluenesulfonate (PPTS) to cleave the 1,3-dioxane protecting group of **18** and promote cyclization of the resulting keto guanidine diol. With this weaker acid, higher reaction temperatures were required and mixtures of **19a**, tetracyclic vinylogous carbamate **22a**, and several unidentified minor byproducts were produced (Scheme 4). When **18** was heated with 2 equiv of PPTS at 60 °C in CHCl$_3$ for 5 h and the crude product was washed with aqueous HCO$_2$Na, **19a** and **22a** were generated in a 1:5 ratio. Increasing the reaction temperature to 90 °C (sealed tube) for 24 h provided **19a** and **22a** in a 2:1 ratio.[27] Separation of these products on silica gel, followed by resubjection of **22a** to PPTS at 90 °C gave **19a** in 75% combined yield.

Initially, **19a** and **22a** were converted to their formate salts prior to chromatography and were eluted from deactivated silica gel using 95:5:0.1 EtOAc−2-propanol−formic acid. We later found that the hydrochloride salts, **19b** and **22b**, were easier to separate on silica gel. These salts were prepared by washing the reaction mixture with 0.1 M HCl or saturated aqueous sodium chloride; several washings were required to completely exchange the tosylate counterion.

Because both NH hydrogens were readily apparent in ^{1}H NMR spectra of **19b**, extensive NMR studies (^{1}H heteronuclear multiple bond coherence spectroscopy (HMBC), heteronuclear multiple quantum coherence spectroscopy (HMQC), and nuclear Overhauser enhancement spectroscopy (NOESY)) eventually revealed that **19b** had the 13-epicrambescidin stereochemistry (i.e., the spiro hydropyran and ester side chain are both epimeric to those of **1** and **2**). Key findings were diagnostic ^{1}H NMR NOEs observed between N2H and H19, N2H and H17 (axial), and H13 and H16 (axial); see the model of the 13-epicrambescidin core in Figure 2.[28] As we will soon see, the stereochemistry of both the spiro hydropyran and ester side chain can be readily inverted, allowing **19b** to be a viable intermediate for accessing isocrambescidins **1** and **2**.

Although the procedures just described provided pentacyclic guanidine salts **19** in synthetically useful yields, these sequences were cumbersome. Ideally, we needed to find acidic conditions for cyclizing **18** that would not promote allylic rearrangement of the C3 alcohol, yet would irreversibly transform tetracyclic vinylogous carbamate intermediate **22** to a pentacyclic guanidine isomer. We eventually discovered that treatment of **18** with 3 equiv of HCl in EtOAc at room-temperature delivered **19b** in 78% yield (Scheme 5). Careful purification of the crude cyclization product by reversed-phase HPLC (9:1 MeOH−0.1 M NaCl) afforded, in addition to **19b**, 5−7% of pentacyclic guanidine **23**.

That **23** was epimeric to the isocrambescidins only at C14 (ester side chain) was apparent from ^{1}H NMR COSY, HMQC, HMBC, and NOESY experiments.[28] The stereochemistry at C15 followed directly from diagnostic ^{1}H NMR NOEs observed between N2H and the H17 (axial) and N2H and H20, and the lack of NOE between N2H and H19. These NOE data are consistent with the hydropyran ring of **23** preferentially adopting a chair conformation having the methyl substituent axial (Figure 3, conformation A). This conformational preference undoubtedly

(24) The C14 methine hydrogen of **19** is observed at δ 2.91, whereas this hydrogen of **23** occurs at δ 2.30. The C15 stereochemistry of these products was rigorously determined (vide infra).

(25) The stereochemistry at C15 of this intermediate (**17** of ref 8) is depicted incorrectly in our preliminary communication.[8]

(26) Relative molecular mechanics energies of guanidine isomers are not reported, because numerous low and medium quality parameters were involved in these calculations.[10]

(27) A small amount, ∼10% relative to **22a**, of the formate analogue of **23** was also produced. This product is the unidentified byproduct described in the Supporting Information that accompanies ref 8. When **22a** was heated with PPTS at 90 °C, **19a** and **22a** were formed also in a ∼2:1 ratio.

(28) Complete assignments of ^{1}H and ^{13}C chemical shifts of this intermediate are provided in Supporting Information.
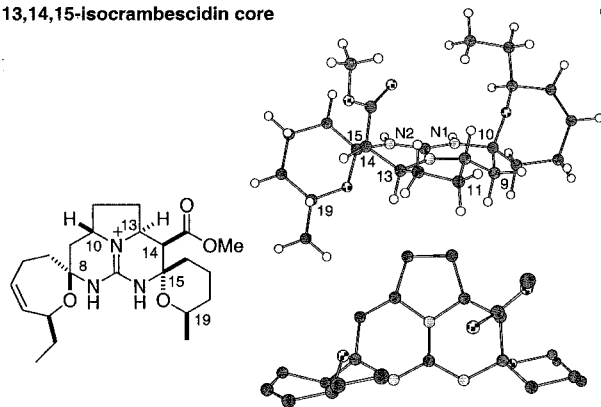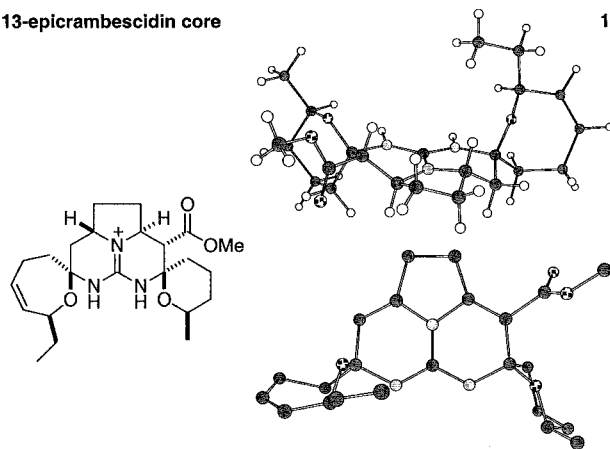
13,14,15-isocrambescidin core   **crambescidin/ptilomycalin A core**
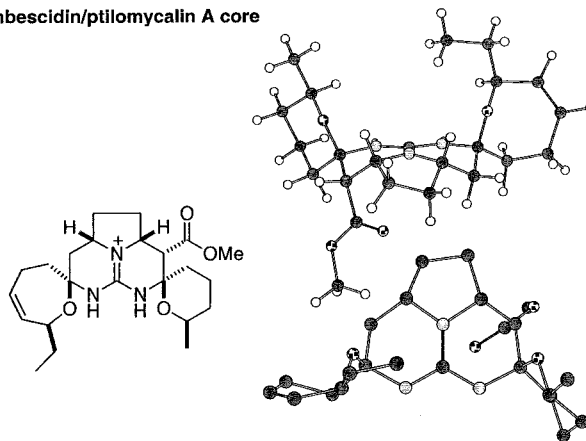
13-epicrambescidin core   **13,15-epicrambescidin core**
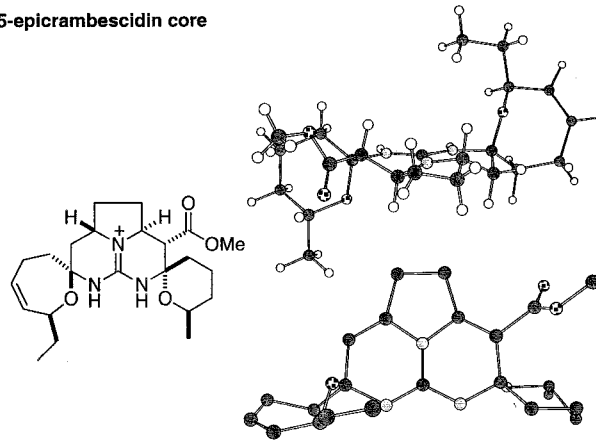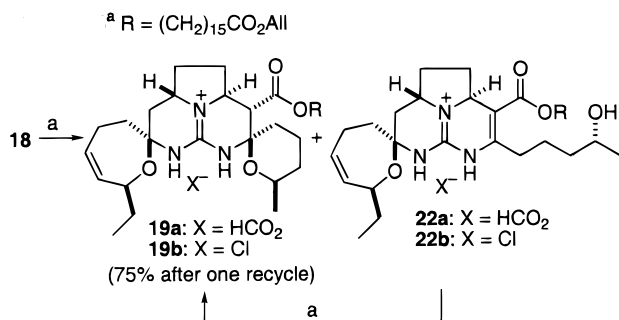

Figure 2. Three-dimensional models of methyl ester analogues of four pentacyclic guanidine units.[10] Models depicting only heavy atoms are oriented identical to the line drawings; models also showing hydrogen atoms are oriented with the guanidine unit projecting back.

Scheme 4[a]   **Scheme 5**


[a] Reagents: (a) PPTS, CHCl₃, 90 °C, 24 h; HCO₂Na wash or 0.1 N HCl wash.

derives from two factors: (1) In the alternate hydropyran chair conformer, destabilizing syn pentane interactions would exist between C17 and C19 of the hydropyran ring and the carbonyl carbon of the ester group; for two views of this conformation, see Figure 3, conformation B and the model of the 13,15-epicrambescidin core in Figure 2. (2) Conformer A would be stabilized by an anomeric interaction between the hydropyran oxygen and the C15−N2 bond.[29]

To gain more insight into the mechanism of hydropyran formation, pure **19b** was resubjected to the cyclization conditions

(29) (a) Kirby, A. J. *Stereoelectronic Effects*; Oxford University Press: Oxford, 1995; pp 3−24. (b) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer: Berlin, 1983. (c) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: Oxford, 1983.
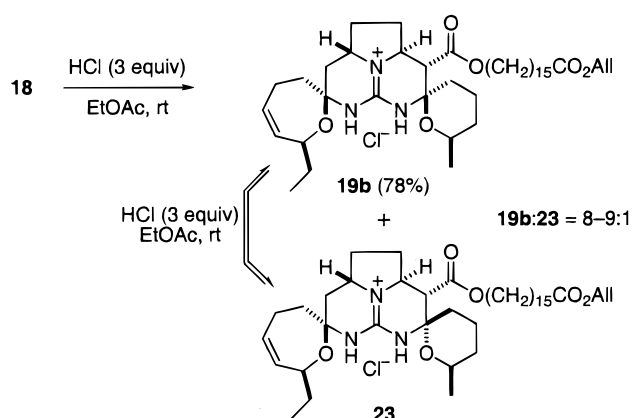
(3 equiv HCl in EtOAc at room temperature) to yield an approximate 8−9:1 mixture of **19b** and **23** (Scheme 5). That this represents an equilibrium ratio of the C15 epimers under these conditions was established by (a) demonstrating that the 8−9:1 mixture of **19b** and **23** was unchanged when resubjected to the reaction conditions for an additional 24 h, and (b) showing that pure **23** gives an identical ratio of epimers when exposed for 24 h to 3 equiv HCl in EtOAc. Because no intermediates or byproducts having the ester side chain on the β face were detected in HCl-promoted cyclization of **18**, or HCl-promoted equilibrations of the spiro hydropyran epimers, we surmised that the equilibration of **19b** and **23** did not involve tetracyclic intermediates such as **22**. Consistent with this hypothesis, exposure of **23** to DCl in EtOAc gave an approximate 8−9:1
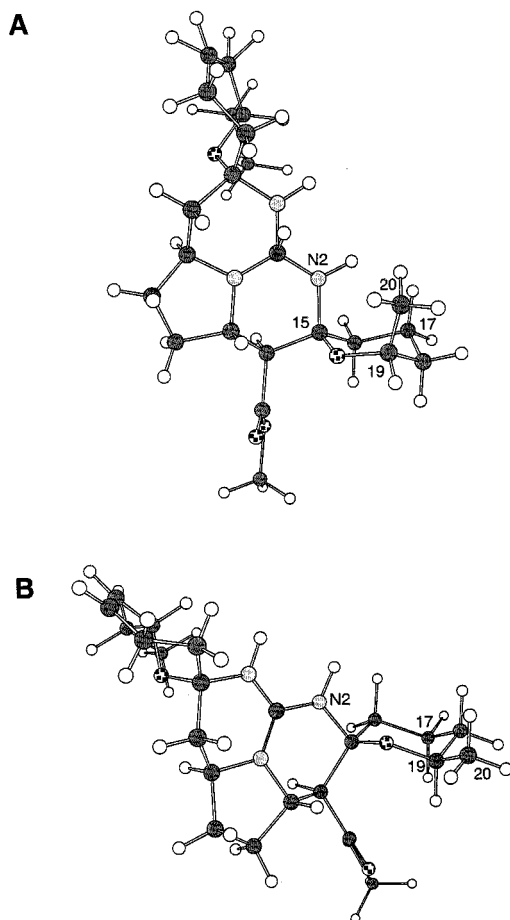
Syntheses of Two Crambescidin Alkaloids

J. Am. Chem. Soc., Vol. 122, No. 20, 2000 4909

A

B

Figure 3. Models of the methyl ester analog of **23** showing the two chair conformations of the hydropyran ring. In conformation A the methyl group is axial and in conformation B it is equatorial.

mixture of **19b** and **23** without incorporation of deuterium into **19b** (Scheme 6). Iminium cation **24** is the likely intermediate in the equilibration of the spiro hydropyran epimers.[30] We conclude from these studies that formation of **19b** as the major product from HCl-promoted cyclization of **18** arises from kinetically controlled axial protonation of the vinylogous carbamate unit of **18** to generate the protio equivalent of **24**, which undergoes thermodynamically controlled spirocyclization to generate **19b** preferentially.[31]

Epimerization of 19b at C14 and C15 to Give Pentacyclic Guanidine Acid 25 and Total Synthesis of 13,14,15-Isocrambescidin 657 (2). Not long after we had first prepared **19a**, we were able to establish that exposure of this intermediate to Et$_3$N in hot methanol provided a pentacyclic guanidine whose stereochemistry was identical to that of 13,14,15-isocrambescidin 800 (**1**). Although we did not initially appreciate this fact, epimerization at C14 and C15 is a coupled event. This reorganization was best accomplished after removal of the allyl group of the hexadecanoate ester. To this end, the 8–9:1 mixture of **19b** and **23** resulting from HCl-promoted cyclization of **18** was deprotected with (Ph$_3$P)$_4$Pd and morpholine (Scheme 7).

(30) Although not rigorously precluded, we consider the alternate possibility that epimerization at C15 occurs by cleavage of the N2–C15 bond to form a six-membered oxocarbenium ion intermediate to be less likely.

(31) In our syntheses of ptilomycalin A and crambescidin 800, the only spirohydrofuran products formed from acid-promoted cyclization of related vinylogous carbamates have the oxygen axial.[3] In those cases, equilibration of hydropyran epimers by a pathway related to that depicted in Scheme 6 would occur at slower rates because less stable *N*-acyliminium cations would be involved.

Scheme 6

Scheme 7

[**25**:**26**:**27** = 10–14:1:1]
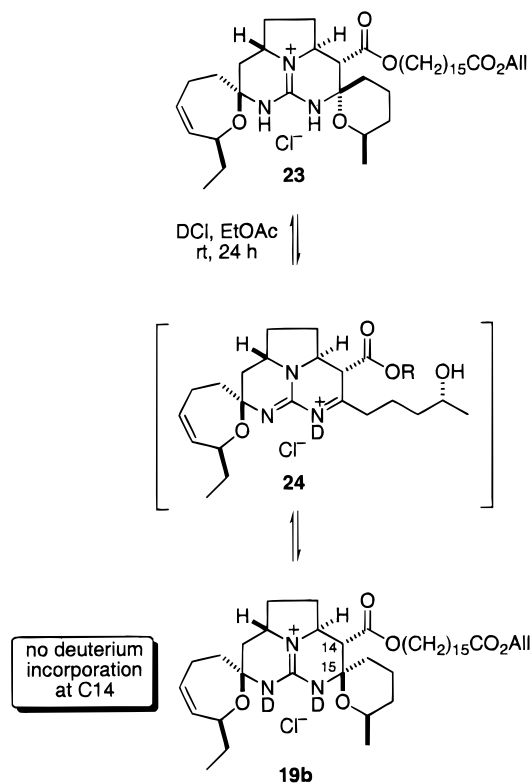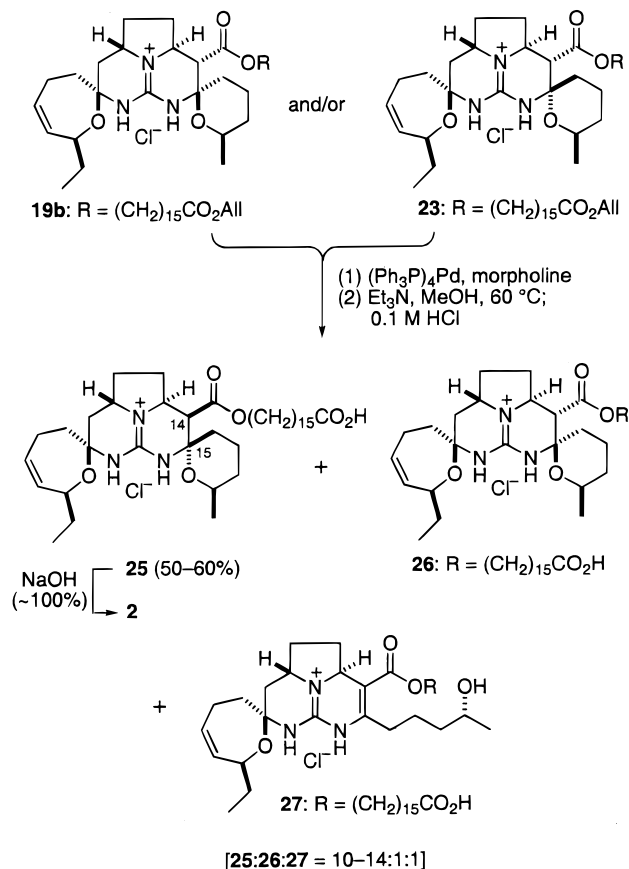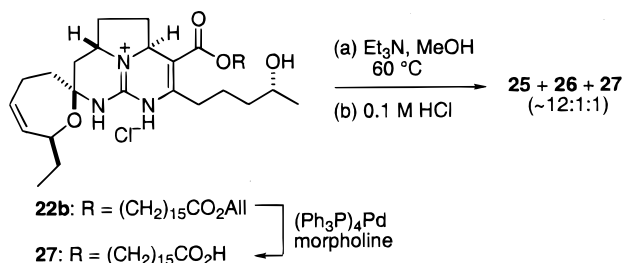
The resulting mixture of acids was then epimerized by heating in MeOH at 60 °C in the presence of 10 equiv of Et$_3$N. Acidification of this product with 0.1 M HCl yielded a mixture of pentacyclic guanidine acids **25** and **26** and tetracyclic

Scheme 8

22b: R = (CH$_2$)$_{15}$CO$_2$All
**27:** R = (CH$_2$)$_{15}$CO$_2$H   (Ph$_3$P)$_4$Pd morpholine

Scheme 9

guanidine **27** in an approximate ratio of 10−14:1:1.[32] The pentacyclic guanidine acid resulting from deallylation of **23** was not detected. After purification by flash chromatography on silica gel, **25**, which exhibits a diagnostic 3.3 Hz coupling constant for the equatorial C14 methine hydrogen, was isolated in 50−60% yield for the two steps. A similar mixture of products was obtained when pure samples of **19b** or **23** were individually deallylated and heated with Et$_3$N in MeOH. In contrast to precursors of (−)-ptilomycalin A (**3**) and crambescidin 800 (**4**),[13] the axial ester is highly favored in the isocrambescidin series.

The structure of **25** was secured by extensive ^{1}H NMR COSY, HMQC, HMBC, and NOESY experiments. The stereochemistry of **25** at C15 followed from diagnostic ^{1}H NMR NOEs observed between H19 and H14 and between H19 and H13 (weaker), and the absence of NOEs between N2H and H19 (see the 3-dimensional model of the 13,14,15-isocrambescidin core in Figure 2). Carboxylic acid **25** was quantitatively converted to the corresponding inner salt by washing with dilute NaOH. This product showed ^{1}H and ^{13}C NMR data fully consistent with those reported[7a] for 13,14,15-isocrambescidin 657 (**2**).[33] The specific rotation of synthetic **2** was [α]$^{23}_D$ −35.4 (*c* 0.8 MeOH), which agrees well with the specific rotation, [α]$^{23}_D$ −32.7 (*c* 0.3 MeOH), reported[7a] for natural 13,14,15-isocrambescidin 657 (**2**). Complete assignments of the ^{1}H and ^{13}C chemical shifts of **2** and **25** are provided in Supporting Information.

Because a pure sample of **22b** was available from our earlier studies of the cyclization of **18** with PPTS, this tetracyclic guanidine was deallylated to form **27** (Scheme 8). Exposure of **27** to Et$_3$N and MeOH at 60 °C provided a product mixture containing **25**, **26**, and **27** in an approximate 12:1:1 ratio. As in the related conversions of **19b** and **23**, the acid congener of **23** was not detected. The experiment summarized in Scheme 8 provides permissive evidence for the intermediacy of **27** in the epimerization of **19b** at C14 and C15 to provide **25**.

Total Synthesis of 13,14,15-Isocrambescidin 800 (1). The (*S*)-7-hydroxyspermidine fragment **28**, which is available from (*R*)-epichlorohydrin,[34] was coupled to pentacyclic acid **25** using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[35] to provide **29** in 71% yield (Scheme 9). Removal of the *tert*-butoxycarbonyl (BOC) protecting groups with 2 M HCl in ethyl acetate[36] and purification of the crude
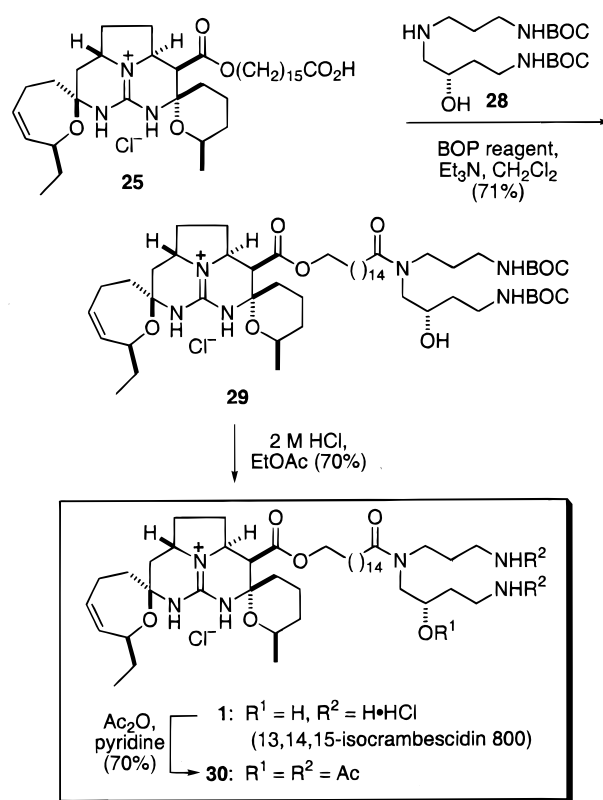
product by reversed-phase HPLC gave the trihydrochloride salt of 13,14,15-isocrambescidin 800 (**1**), [α]$^{23}_D$ −67.7 (*c* 0.7 MeOH), in 70% yield. A specific rotation of [α]$^{23}_D$ −48 (*c* 0.5 MeOH) is reported for natural 13,14,15-isocrambescidin 800 (**1**).[6] Because the counterion of natural **1** was not described, the significance, if any, of this discrepancy in rotation magnitude is unknown. NMR data for the trihydrochloride salt of synthetic **1** were in good agreement with those reported for natural **1**,[6,37,38] and synthetic **1** was indistinguishable from a natural sample of **1** by HPLC comparisons using three eluents. To provide one additional point of comparison, synthetic **1** was converted to triacetylated derivative **30**. Data for this product agreed perfectly with ^{1}H and ^{13}C NMR data reported for this derivative of natural **1**.[5f]

Proof that the C43 Stereocenter of 13,14,15-Isocrambescidin 800 (1) is *S*. As noted earlier, the *S* configuration of the C43 stereocenter of 13,14,15-isocrambescidin 800 (**1**) had been proposed solely by analogy with crambescidin 816.[5f,6] On the surface, our total synthesis of **1** appeared to confirm this assignment. However, because the C43 stereocenter is far removed from stereocenters of the pentacyclic guanidine moiety, we were not confident that epimers at this sterogenic center would be readily distinguished. To pursue this issue further, (43*R*)-13,14,15-isocrambescidin 800 (**31**) was prepared from **25** and *ent*-**28** (Scheme 10).[39] As we had feared, **31** was indistinguishable from synthetic **1** and natural **1** by ^{1}H and ^{13}C NMR comparisons as well as by HPLC analysis.

(32) The ratio of **25** to **26** and **27** was determined from the crude product mixture by ^{1}H NMR analysis at 500 MHz. Because of the complexity of this spectrum and the presence of minor impurities, we estimate that this ratio is only accurate to ±20%. The ratio of **26**:**27** was more difficult to ascertain, although these products appeared to be formed in similar amounts. Attempts to resolve this mixture by HPLC were unsuccessful.

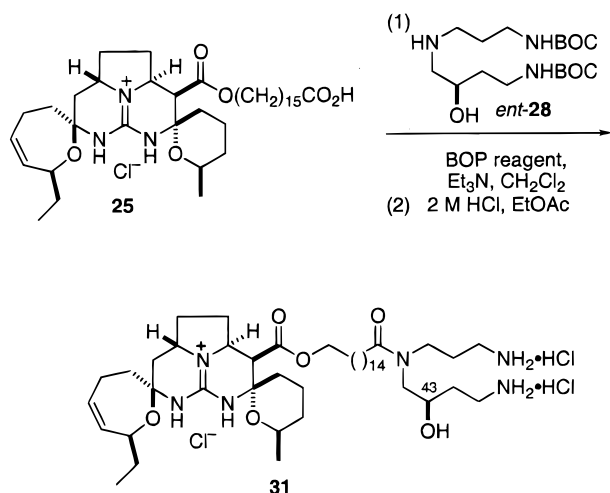(33) The mean deviation between the ^{13}C NMR signals for synthetic and natural **2** was ±0.13 ppm.

(34) Coffey, D. S.; McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 8741−8742.

(35) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219−1222.

(36) Stahl, G. L.; Walter, R.; Smith C. W. *J. Org. Chem.* **1978**, *43*, 2285−2286.

(37) The ^{1}H NMR spectrum (500 MHz, CD$_3$OD) of synthetic **1** trihydrochloride is identical to the spectrum of natural **1** published in the Supporting Information of ref 6 (spectrum S-3); spectrum S-3 is apparently also of the tricationic salt. There are minor errors in the tabulated data in Table 1 of ref 6, because there are discrepancies between the tabulated data and spectrum S-3. The ^{13}C NMR spectrum of synthetic **1** trihydrochloride is also identical to the spectrum of natural **1** published in the Supporting Information of ref 6 (spectrum S-8a). Again, there are minor errors in the tabulated data in Table 1 of ref 6, because there are discrepancies between the tabulated data and spectrum S-8a.

Syntheses of Two Crambescidin Alkaloids

J. Am. Chem. Soc., Vol. 122, No. 20, 2000 4911

Scheme 10

25

(1) *ent-28*

BOP reagent,
Et$_3$N, CH$_2$Cl$_2$

(2) 2 M HCl, EtOAc

31

Table 1. ^{19}F NMR Data for Mosher Derivatives of **1** and **31**

32: 43*S*
33: 43*R*

entry	starting material	product	^{19}F NMR (CDCl$_3$),[a] δ ppm
1	synthetic **1**	**32**	−68.77, −68.82 (2 peaks), −68.9, −70.5, −70.9
2	**31**	**33**	−68.6, −68.7, −68.8, −68.9, −71.0, −71.1
3	natural **1**	**32**	−68.77, −68.82 (2 peaks), −68.9, −70.5, −70.9

[a] Because of rotamers about the C38 amide bond on the NMR time scale, six peaks are observed in the ^{19}F NMR spectra.

To unambiguously differentiate the C43 epimers of 13,14,-15-isocrambescidin 800, we decided to prepare and compare a common derivative of natural **1**, synthetic **1**, and **31**. Because only 200 mg of natural **1** was available,[40] we chose to employ Mosher derivatives and do the analysis by ^{19}F NMR spectroscopy.[9] The tris Mosher derivatives **32** (43*S*) and **33** (43*R*) were prepared from (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenyl-acetic acid (MTPA), synthetic **1**, and **31** according to the method developed by Ward,[41] and their ^{19}F NMR spectra were recorded. Because these products were mixtures of two rotamers on the NMR time scale, six ^{19}F signals were observed. Fortunately, several of the signals were substantially different in diastereomers **32** and **33** (Table 1). The (*S*)-MTPA derivatives of natural and synthetic **1** were identical, thus unambiguously establishing that the stereochemistry of 13,14,15-isocrambescidin 800 (**1**) at C43 is *S*.

Relative Energies of Pentacyclic Guanidine Stereoisomers. In contrast to our studies in the ptilomycalin A/crambescidin

(38) We were certain that we had obtained the trihydrochloride salt of **1**, because a basic workup was not performed after the removal of the BOC groups. However, natural **1** has been depicted with the spermidine nitrogens in the free base form,[5f,6] yet the ^{1}H and ^{13}C NMR spectra of synthetic **1** and natural **1** were indistinguishable. Treatment of synthetic **1** with 0.1 M NaOH saturated with NaCl resulted in downfield shifts of the C41 and C45 hydrogens. To investigate this issue further, *i* was prepared to model the hydroxyspermidine unit of 13,14,15-isocrambescidin 800. Chemical shifts of the hydrogens of the hydroxyspermidine units of *i* and synthetic **1** were nearly identical; the absence of the guanidine unit made assignments for *i* straightforward. Treatment of *i* with 0.1 M NaOH gave *ii* as the free base. As summarized in the table, there were significant upfield shifts of the C41 and C45 hydrogens in *ii* upon deprotonation. From this study, and related experiments with synthetic **1**, we are confident that natural 13,14,15-isocrambescidin 800 (**1**) was isolated as the trihydrochloride salt.
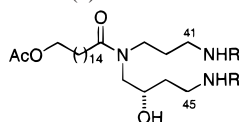
i R = H•HCl
ii R = H

^{1}H NMR shifts of the C41 and C45 hydrogens.[a]

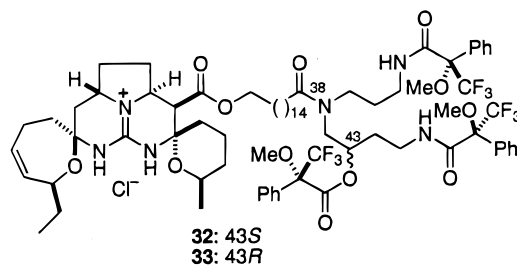| position	δ (ppm), mult	
	i	*ii*
41	2.99–2.84, m	2.66–2.60, m
45	3.14–3.08, m	2.86–2.78, m

[a] In CD$_3$OD at 500 MHz.

(39) Hydroxyspermidine derivative *ent-28* was prepared from (*S*)-epichlorohydrin.[34]

(40) Kindly provided by Professor K. Rinehart.

(41) Ward, D. E.; Rhee, C. K. *Tetrahedron Lett.* **1991**, *32*, 7165–7166.

area,[3] our investigations in the isocrambescidin series provided access to several pentacyclic guanidine stereoisomers. The relative energies of the 13,15-epicrambescidin and 13-epicramb-escidin pentacyclic guanidine moieties are readily discerned, because **23** and **19** equilibrate at room temperature in the presence of HCl (see Scheme 5). No similarly clean equilibration allows us to precisely specify the relative energy of the 13,14,15-isocrambescidin ring system. Nonetheless, that the 13,14,15-isocrambescidin ring system is considerably more stable than the 13-epicrambescidin guanidine moiety was signaled early in our studies when we observed that the 13-epicrambescidin ester **19a** was converted in good yield to the allyl ester analogue of the 13,14,15-isocrambescidin acid **25** upon treatment with Et$_3$N in hot methanol. Moreover, exposure of **26**, **27**, or the acid derived from **23** to methanolic Et$_3$N at 60 °C provided the 13,14,15-isocrambescidin acid **25** and the 13-epicrambescidin acid **26** in an approximate ratio of 12:1 (see Scheme 7). Although the complexity of this reaction mixture, our inability to isolate **26** in pure form, and analytical difficulties[32] prevent unambiguous specification that this ratio of **25** and **26** accurately represents thermodynamic equilibrium at 60 °C, this ratio is a reasonable estimate. Using this estimate, the energetic ordering of the 13-epicrambescidin, 13,15-epicramb-escidin, and 13,14,15-isocrambescidin pentacyclic guanidine ring systems depicted in Figure 4 is obtained.

That epimerization of the 13,15-epicrambescidin guanidine moiety at C14 would be highly favored is apparent in the molecular models shown in Figures 2 and 3. In one hydropyran chair conformer of the 13,15-epicrambescidin ring system the ester substituent is thrust over the hydropyran ring (conformer B of Figure 3 and alternate views shown in Figure 2) and in the other chair conformer, which relieves this interaction, the methyl group is axial (conformer A of Figure 3). No such destabilizing interactions exist in the 13,14,15-isocrambescidin ring system.

Conclusion

The first total syntheses of 13,14,15-isocrambescidin 800 (**1**) and 13,14,15-isocrambescidin 657 (**2**) were accomplished in convergent fashion. The synthesis of **1** was achieved in 11% overall yield from amine **12** by a sequence involving five isolated intermediates. As detailed in the preceding account, **12** can be accessed from commercially available 3-butyn-1-ol in
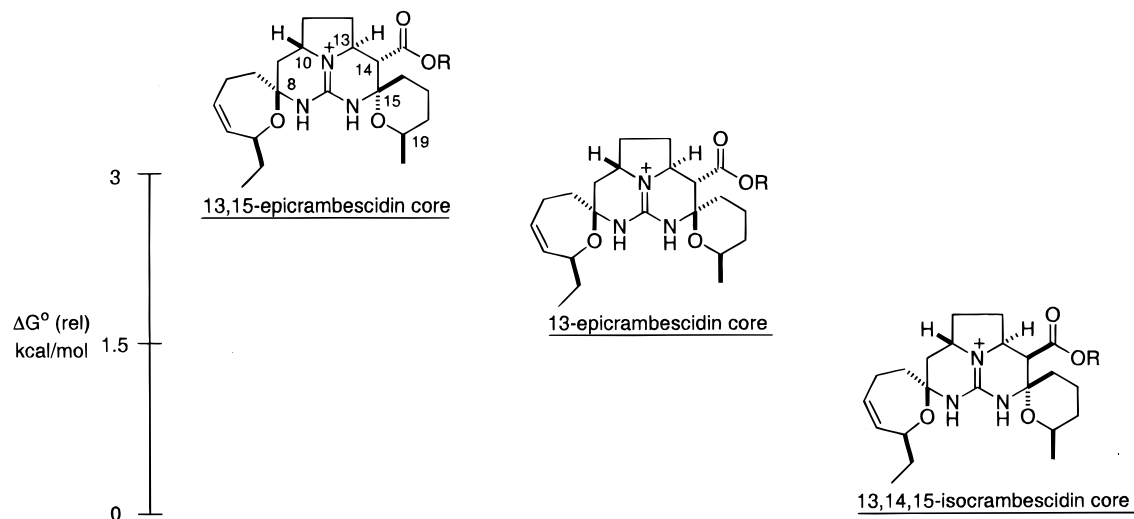
Figure 4. Relative energy of pentacyclic guanidine isomers.

30% overall yield by way of nine isolated and purified intermediates.[3] Thus, the approach to the isocrambescidins recorded here is capable of providing these guanidine alkaloids on meaningful scales.

The total syntheses detailed herein confirm the stereochemical assignments of **1** and **2** and rigorously establish that the absolute configuration of the hydroxyspermidine side chain of **1** is *S*. Moreover, this investigation demonstrated for the first time that our tethered Biginelli strategy for preparing crambescidin alkaloids can be extended to guanidine intermediates and that the key Biginelli condensation can be accomplished under sufficiently mild conditions that fragments containing the full functionality of the crambescidin core can be employed.

Experimental Section[42]

(6*S*,11*Z*,13*S*)-6-Amino-*N*-carboxamidine-8-(1′,3′-dioxan-2′-yl)-2-methyl-13-triisopropylsiloxypentadeca-2,11-diene (13). A solution of amine **12** (2.95 g, 6.12 mmol), 1-*H*-pyrazole-1-carboxamidine hydrochloride (2.70 g, 18.4 mmol), *i*-Pr$_2$EtN (4.4 mL, 24 mmol), and DMF (6 mL) was maintained at room temperature for 16 h and then at 60 °C for 4 h. The solution was cooled to room temperature and partitioned between CHCl$_3$ (300 mL) and 0.1 M HCl (75 mL). The organic phase was washed with 0.1 M HCl (75 mL) and H$_2$O (75 mL), dried (Na$_2$SO$_4$), filtered, and concentrated to give a 2:1 mixture of guanidine **13** and amine **12**. This mixture was dissolved in DMF (6 mL) and again allowed to react (room temperature for 16 h and 60 °C for 4 h) with 1-*H*-pyrazole-1-carboxamidine hydrochloride (1.35 g, 9.2 mmol) and *i*-Pr$_2$EtN base (2.2 mL, 12 mmol). The reaction was worked up as previously described; residual DMF was removed by evacuation for several hours at 0.1 mm to provide 3.20 g (~99%) of crude guanidine **13** as a light yellow oil. This intermediate was used without further purification: ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.82 (app d, *J* = 6.7 Hz, 1H), 7.24 (br s, 1H), 5.43−5.39 (m, 1H), 5.29−5.24 (m, 1H), 5.09 (br t, *J* = 7.0 Hz, 1H), 4.45 (app q, *J* = 7.3 Hz, 1H), 3.98−3.76 (m, 4H), 3.62−3.59 (m, 1H), 2.20−2.13 (m, 2H), 2.02−1.74 (overlapping m, 6H), 1.74−1.67 (m, 2H), 1.69 (s, 3H), 1.64−1.58 (overlapping m, 2H), 1.62 (s, 3H), 1.51−1.38 (m, 2H), 1.05 (m, 21H), 0.87 (t, *J* = 7.4 Hz, 3H);[43] ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.6, 135.0, 132.7, 126.9, 123.1, 100.5, 69.8, 59.8, 59.3, 46.6, 45.0, 36.5, 31.7, 30.5, 25.7, 25.0, 24.8, 22.2, 18.1, 18.0, 17.6, 12.3, 9.3 ppm; IR (film) 2961, 2865, 1651, 1463, 1383, 1246, 1109 cm^{-1}; high-resolution mass spectroscopy (HRMS) fast atom bombardment (FAB) *m/z* 524.4225 (524.4250

calcd for C$_{27}$H$_{58}$N$_3$O$_3$Si, M − Cl); [α]$^{25}_D$ +1.7, [α]$^{25}_{577}$ +2.7, [α]$^{25}_{546}$ +3.2, [α]$^{25}_{435}$ +7.3, [α]$^{25}_{405}$ +9.3 (*c* 1.3, CHCl$_3$).

(4a*S*,7*S*)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-3-[(4*S*)-4-*t*-butyldimethylsiloxypentyl]-7-[(5*Z*,7*S*)-2-(1′,3′-dioxan-2′-yl)-7-triisopropylsiloxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-1-imino-pyrrolo-[1,2-*c*]-pyrimidine Hydrochloride (16). *N*-Methylmorpholine-*N*-oxide (2.16 g, 18.4 mmol) and OsO$_4$ (3.1 mL, 0.24 mmol, 2% in *tert*-butyl alcohol) were added to a solution of guanidine **13** (3.2 g, ~6.1 mmol), tetrahydrofuran (THF) (105 mL), and H$_2$O (15 mL). The mixture was stirred at room temperature for 8 h, Florisil (1.5 g) and NaHSO$_3$ (1.5 g) were added, and the resulting mixture was stirred for an additional 10 h. Celite and MgSO$_4$ then were added, the mixture was filtered, and the eluent was concentrated to give the corresponding crude diol as a brown oil.

This oil was dissolved in toluene (120 mL), and morpholinium acetate (3.6 g, 24 mmol) and Pb(OAc)$_4$ (3.3 g, 7.3 mmol) were added. The resulting mixture was maintained at room temperature for 45 min and Celite was added. This mixture was filtered through a plug of Celite, the eluent was diluted with toluene (200 mL), and the solution was concentrated to give an oil. This oil was azeotroped to dryness with toluene (200 mL) and the residue was combined with β-ketoester **15** (5.3 g, 9.2 mmol) and 2,2,2-trifluoroethanol (9 mL). The resulting solution was maintained at 60 °C for 20 h and then partitioned between CHCl$_3$ (250 mL) and 0.1 M HCl (50 mL). The organic phase was washed with 0.1 M HCl (50 mL) and brine (50 mL), dried (Na$_2$SO$_4$), filtered, and concentrated. Analysis by ^{1}H NMR revealed a 7:1 ratio of trans/cis Biginelli adducts. Purification of the crude mixture by flash chromatography (CHCl$_3$ → 99:1 CHCl$_3$−MeOH → 98:2 CHCl$_3$−MeOH) on silica gel deactivated with pH 7.0 buffer[16] provided 3.22 g (48% from **12**) of the desired anti adduct **16** as a light brown oil and 331 mg (5% from **12**) of syn adduct **17**. Data for **16**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 9.06 (s, 1H), 7.33 (s, 1H), 5.95−5.88 (m, 1H), 5.43 (app t, *J* = 9.8 Hz, 1H), 5.31 (app dq, *J* = 17.2, 1.5 Hz, 1H), 5.27−5.25 (m, 1H), 5.23 (app dq, *J* = 10.4, 1.3 Hz, 1H), 4.57 (br d, *J* = 5.7, 2H), 4.46−4.41 (m, 2H), 4.27−4.24 (m, 1H), 4.17−4.07 (m, 2H), 4.01−3.95 (m, 2H), 3.91−3.78 (m, 3H), 2.77−2.71 (m, 2H), 2.65−2.59 (m, 1H), 2.45−2.40 (m, 1H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.07−1.88 (m, 6H), 1.79−1.55 (m, 11H), 1.53−1.43 (m, 4H), 1.31−1.25 (m, 21H), 1.13 (d, *J* = 6.1 Hz, 3H), 1.05 (s, 21H), 0.87 (t, *J* = 7.4 Hz, 3H), 0.86 (s, 9H), 0.037 (s, 3H), 0.032 (s, 3H);[43] ^{13}C NMR (100 MHz, CDCl$_3$) δ 173.4, 165.0, 149.9, 147.3, 135.3, 132.2, 126.4, 117.9, 100.9, 100.3, 69.8, 68.3, 64.8, 64.7, 59.9, 59.4, 57.5, 54.1, 46.1, 39.0, 34.8, 34.2, 33.3, 31.6, 30.9, 30.3, 29.6, 29.52, 29.48, 29.42, 29.3, 29.1, 29.0, 28.5, 26.0, 25.8, 24.83, 24.76, 24.4, 23.6, 22.1, 18.01, 17.98, 12.3, 9.2, −4.5, −4.7 ppm;[44] IR (film) 2926, 2856, 1738, 1713, 1681, 1538, 1462, 1382,

---

(42) Experimental details are the same as those described in the preceding paper.[3]

(43) At least one of the hydrogens attached to a heteroatom was not detected in the ^{1}H NMR spectrum of this intermediate.

(44) ^{13}C NMR signals of many of the methylene carbons of the hexadecanoate side chain overlap.

1256, 1086 cm^{-1}; HRMS (FAB) *m/z* 1044.6 (1044.8 calcd for C$_{59}$H$_{110}$N$_3$O$_8$Si$_2$ M − Cl); [α]^{25}$_D$ −21.2, [α]^{25}$_{577}$ −21.3, [α]^{25}$_{546}$ −23.3, [α]^{25}$_{435}$ −28.8, [α]^{25}$_{405}$ −25.1 (*c* 1.9, CHCl$_3$).

(4a*S*,7*S*)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-7-[(5*Z*,7*S*)-2-(1′,3′-dioxan-2′-yl)-7-hydroxy-5-nonenyl]-1,2,4a,5,6,7-hexahydro-3-[(4*S*)-4-hydroxypentyl]-1-iminopyrrolo[1,2-*c*]pyrimidine Hydrochloride (18). A solution of **16** (2.80 g, 2.59 mmol), TBAF (13 mL, 13 mmol, 1.0 M), and DMF (26 mL) was maintained at room temperature for 24 h, then more TBAF (6 mL, 6 mmol, 1.0 M) was added. The solution was maintained at room temperature for 6 h then partitioned between CHCl$_3$ (200 mL) and 0.1 M HCl (75 mL). The organic phase was washed with saturated aqueous HCO$_2$Na (2 × 50 mL), dried (Na$_2$SO$_4$), filtered, and the filtrate was concentrated. The crude product was purified by flash chromatography (95:5:0.1 EtOAc−2-propanol−formic acid → 90:10:0.1 EtOAc−2-propanol−formic acid → 85:15:0.1 EtOAc−2-propanol−formic acid) on silica gel deactivated with pH 7.0 buffer[20] to give the formate salt of the diol 1.68 g (80%) as a light brown oil.

The formate salt was easier to purify, but the chloride salt was more stable. Therefore, after purification, the formate salt was converted quantitatively to chloride salt **18** by partitioning the formate salt between CHCl$_3$ (150 mL) and 0.1 M HCl (25 mL) and washing the organic layer with 0.1 M HCl (25 mL) and brine (25 mL). The organic phase was dried (Na$_2$SO$_4$), filtered, and concentrated to give diol **18**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 8.63 (s, 1H), 7.43 (s, 1H), 5.95−5.87 (m, 1H), 5.51−5.42 (m, 2H), 5.31 (ddd, *J* = 17.2, 3.0, 1.5 Hz, 1H), 5.22 (ddd, *J* = 9.2, 3.0, 1.3 Hz, 1H), 4.57 (dt, *J* = 5.7, 1.3 Hz, 2H), 4.43 (dd, *J* = 9.9, 4.3 Hz, 1H), 4.32 (app q, *J* = 7.1 Hz, 1H), 4.28−4.25 (m, 1H), 4.17−4.08 (m, 2H), 4.05−3.92 (m, 3H), 3.89−3.82 (m, 2H), 2.91−2.86 (m, 1H), 2.62−2.58 (m, 1H), 2.52 (dt, *J* = 11.8, 4.6 Hz, 1H), 2.42−2.39 (m, 1H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.16−1.96 (m, 6H), 1.86−1.72 (m, 3H), 1.70−1.44 (m, 11H), 1.30−1.24 (m, 22H), 1.19 (d, *J* = 6.2 Hz, 3H), 0.91 (t, *J* = 7.4 Hz, 3H);[43] ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.5, 165.0, 149.7, 147.5, 133.5, 132.3, 130.4, 118.0, 101.0, 100.5, 68.7, 65.4, 64.85, 64.76, 60.1, 59.6, 57.6, 54.2, 45.8, 38.1, 34.7, 34.2, 33.1, 30.4, 30.2, 29.6, 29.51, 29.46, 29.37, 29.2, 29.1, 28.6, 26.0, 24.9, 24.7, 24.0, 23.5, 22.2, 9.7 ppm;[44] IR (film) 3344, 2925, 2854, 1736, 1685, 1542, 1462, 1384, 1259, 1170, 1084, 1001 cm^{-1}; MS: HRMS (FAB) *m/z* 774.5615 (774.5632 calcd for C$_{44}$H$_{76}$N$_3$O$_8$, M − Cl); [α]^{25}$_D$ −39.4, [α]^{25}$_{577}$ −40.2, [α]^{25}$_{546}$ −44.8, [α]^{25}$_{435}$ −66.0, [α]^{25}$_{405}$ −70.0 (*c* 1.2, CHCl$_3$).

Formation of Pentacycle 19b from 18 by Reaction with Methanolic HCl. Acetyl chloride (320 μL, 4.5 mmol) was added to a 0 °C solution of MeOH (200 mL, 5.0 mmol) and EtOAc (30 mL) to give a 0.15 M solution of HCl in EtOAc. Diol **18** (1.10 g, 1.36 mmol) was then dissolved in 27 mL of this solution. This solution (containing 4.1 mmol of HCl) was maintained at room temperature for 6 h, then partitioned between CHCl$_3$ (250 mL) and brine (50 mL). The organic phase was dried (Na$_2$SO$_4$), filtered, and concentrated. Purification of the residue by flash chromatography (CHCl$_3$ → 99:1 CHCl$_3$−MeOH → 98:2 CHCl$_3$−MeOH) gave 780 mg (78%) of an approximate 8−9:1 mixture of pentacycles **19b** and **23** as a light yellow oil.[45] This mixture was used without further purification in the next step.

For characterization purposes, a sample of this mixture was purified by reversed-phase HPLC (9:1 MeOH−0.1 M NaCl). To ensure that the counterions of **19b** and **23** were uniquely chloride, pure samples of **19b** and **23** were dissolved in CHCl$_3$ (50 mL), washed with 0.1 M HCl (10 mL), and the organic phases were dried (Na$_2$SO$_4$), filtered, and concentrated.[46]

Data for **19b**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 10.37 (s, 1H), 9.81 (s, 1H), 5.95−5.87 (m, 1H), 5.69−5.65 (m, 1H), 5.48 (br d, *J* = 10.9 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.22 (dq, *J* = 10.4, 1.3 Hz, 1H), 4.57 (dt, *J* = 5.7, 1.4 Hz, 2H), 4.50 (br d, *J* = 8.1 Hz, 1H), 4.31−

(45) It was difficult to measure accurately the ratio of **19b** and **23**, because many peaks in the ^{1}H NMR spectra overlapped.

(46) There are small differences in the ^{1}H NMR and ^{13}C NMR spectra of **19b**, **23**, and **2** before and after washing with 0.1 M HCl. In our preliminary communication, **19b** and **2** were not washed with 0.1 M HCl after purification;[8] as a result, there are slight differences in some of the ^{1}H NMR and ^{13}C NMR chemical shifts reported for **19b** and **2** in the Supporting Information that accompanied our preliminary communication.

4.27 (m, 1H), 4.26−4.21 (m, 1H), 4.12−4.07 (m, 1H), 3.98−3.95 (m, 1H), 3.77−3.72 (m, 1H), 2.91 (d, *J* = 11.7 Hz, 1H), 2.58−2.53 (m, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 2.31−2.28 (m, 3H), 2.21−2.17 (m, 2H), 1.93 (dd, *J* = 14.5, 5.3 Hz, 1H), 1.86−1.72 (m, 3H), 1.69−1.60 (m, 7H), 1.57−1.36 (m, 6H), 1.32−1.20 (m, 19H), 1.17−1.12 (m, 1H), 1.13 (d, *J* = 6.0 Hz, 3H), 0.87 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.4, 169.0, 150.9, 133.3, 132.3, 129.8, 118.0, 85.6, 84.7, 70.8, 68.8, 65.5, 64.8, 58.5, 55.1, 52.2, 37.5, 37.2, 34.2, 33.0, 32.1, 30.9, 30.0, 29.56, 29.53, 29.46, 29.38, 29.2, 29.11, 29.09, 28.5, 25.9, 24.9, 23.8, 22.0, 18.0, 10.2 ppm;[44] IR (film) 2926, 2853, 1732, 1659, 1615, 1462, 1349, 1202, 1022 cm^{-1}; HRMS (FAB) *m/z* 698.5117 (698.5108 calcd for C$_{41}$H$_{68}$N$_3$O$_6$, M − Cl); [α]^{25}$_D$ −54.6, [α]^{25}$_{577}$ −55.6, [α]^{25}$_{546}$ −64.2, [α]^{25}$_{435}$ −115, [α]^{25}$_{405}$ −141 (*c* 1.25, CHCl$_3$).

Data for minor pentacycle **23**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 10.23 (s, 1H), 9.59 (s, 1H), 5.96−5.88 (m, 1H), 5.68−5.64 (m, 1H), 5.48 (br d, *J* = 11.0 Hz, 1H), 5.31 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.23 (dq, *J* = 10.4, 1.3 Hz, 1H), 4.57 (dt, *J* = 5.7, 1.3 Hz, 2H), 4.56 (br s, 1H), 4.16 (t, *J* = 6.7 Hz, 2H), 4.08 (dt, *J* = 11.0, 5.4 Hz, 1H), 3.97−3.92 (m, 1H), 3.91−3.88 (m, 1H), 2.57−2.52 (m, 2H), 2.46−2.43 (m, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.30 (d, *J* = 11.1 Hz, 1H), 2.30−2.26 (m, 1H), 2.25−2.17 (m, 2H), 1.92 (dd, *J* = 14.2, 5.8 Hz, 1H), 1.77−1.42 (m, 16H), 1.36 (t, *J* = 12.3 Hz, 1H), 1.33 (d, *J* = 6.7 Hz, 3H), 1.32−1.24 (m, 19H), 0.85 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 173.5, 167.9, 148.9, 133.3, 132.3, 129.8, 118.1, 84.7, 82.9, 70.7, 70.1, 65.6, 64.9, 54.6, 53.0, 52.5, 37.8, 36.8, 34.2, 31.1, 30.33, 30.31, 29.61, 26.56, 29.49, 29.42, 29.23, 29.15, 29.11, 28.6, 28.4, 25.9, 24.9, 23.9, 21.8, 14.1, 10.3 ppm;[44] IR (film) 2926, 2853, 1732, 1662, 1620 cm^{-1}; low-resolution mass spectroscopy (LRMS) (FAB) *m/z* 698.51 (698.5108 calcd for C$_{41}$H$_{68}$N$_3$O$_6$, M − Cl); [α]^{25}$_D$ −73.2, [α]^{25}$_{577}$ −67.3, [α]^{25}$_{546}$ −81.5, [α]^{25}$_{435}$ −149, [α]^{25}$_{405}$ −184 (*c* 0.3, CHCl$_3$).

Carboxylic Acid 25 and 13,14,15-Isocrambescidin 657 (2). A solution of the 8−9:1 mixture of **19b** and **23** (50 mg, 0.068 mmol), morpholine (24 μL, 0.27 mmol), (Ph$_3$P)$_4$Pd (16 mg, 0.014 mmol), and MeCN (5 mL) was maintained at room temperature for 2 h. Additional morpholine (12 μL, 0.13 mmol) and (Ph$_3$P)$_4$Pd (8 mg, 0.007 mmol) were added and the solution was maintained at room temperature for an additional 2 h. The solution was then partitioned between CHCl$_3$ (50 mL) and 0.1 M HCl (10 mL). The organic phase was washed with 0.1 M HCl (10 mL), dried (Na$_2$SO$_4$), filtered, and concentrated to give a brown oil. The brown oil was filtered through a plug of silica gel (99:1 CHCl$_3$−MeOH → 98:2 CHCl$_3$−MeOH), concentrated, and the residue was dissolved in Et$_3$N (95 μL, 0.68 mmol) and MeOH (7 mL). The resulting solution was maintained at 60 °C for 36 h and then partitioned between CHCl$_3$ (50 mL) and 0.1 M HCl (8 mL). The organic phase was washed with 0.1 M HCl (8 mL), dried (Na$_2$SO$_4$), filtered, and concentrated. Purification of the residue by flash chromatography (99:1 CHCl$_3$−MeOH → 98:2 CHCl$_3$−MeOH → 95:5 CHCl$_3$−MeOH) provided 28 mg (60%) of **25** as a light yellow oil. To ensure that the counterion was uniquely chloride, **25** was dissolved in CHCl$_3$ (50 mL) and washed with 0.1 M HCl (10 mL). The organic phase was dried (Na$_2$SO$_4$), filtered, and concentrated.[46] Data for **25**: ^{1}H NMR (500 MHz, CDCl$_3$) δ 10.00 (s, 1H), 9.23 (s, 1H), 5.64 (app t, *J* = 8.1 Hz, 1H), 5.50 (br d, *J* = 11.0 Hz, 1H), 4.57 (br s, 1H), 4.16−4.11 (m, 1H), 4.03−3.99 (m, 1H), 4.00−3.97 (m, 1H), 3.92−3.88 (m, 1H), 3.72−3.68 (m, 1H), 3.45 (d, *J* = 3.3 Hz, 1H), 2.59−2.51 (m, 2H), 2.33 (t, *J* = 7.5 Hz, 2H), 2.29−2.24 (m, 1H), 2.24−2.17 (m, 3H), 1.89−1.80 (m, 4H), 1.75−1.45 (m, 10H), 1.39 (t, *J* = 12.3 Hz, 1H), 1.30−1.24 (m, 23H), 1.18 (d, *J* = 6.0 Hz, 3H), 0.95 (t, *J* = 7.3 Hz, 3H);[43] ^{13}C NMR (125 MHz, CDCl$_3$) δ 178.4, 167.7, 149.3, 133.6, 129.6, 85.0, 82.9, 70.8, 69.1, 65.3, 52.8, 52.0, 41.7, 38.1, 37.4, 33.9, 32.7, 31.4, 30.2, 29.5, 29.43, 29.37, 29.35, 29.2, 29.1, 29.0, 28.5, 27.9, 25.8, 24.7, 24.0, 22.1, 20.0, 10.2 ppm;[44] IR (film) 3200, 2924, 2852, 1732, 1660, 1621, 1189, 1167, 1027 cm^{-1}; HRMS (FAB) *m/z* 658.4789, (658.4795 calcd for C$_{38}$H$_{64}$N$_3$O$_6$, M − Cl); [α]^{25}$_D$ −47.3, [α]^{25}$_{577}$ −49.5, [α]^{25}$_{546}$ −55.9, [α]^{25}$_{435}$ −99.8, [α]^{25}$_{405}$ −122 (*c* 1.2, CHCl$_3$).

Carboxylic acid **25** was quantitatively converted to the carboxylate inner salt by washing a CHCl$_3$ (5 mL) solution of the acid (5 mg) with 1 M NaOH (1 mL) and brine (1 mL). The organic layer was dried (Na$_2$SO$_4$) and then concentrated to provide **2** as a colorless oil: [α]^{25}$_D$ −35.4 (*c* 0.8, MeOH). Spectroscopic and mass spectrometric data for this sample were consistent with data published for natural **2**.[7a,33]

41,45-Di-*tert*-butoxycarbonyl-13,14,15-isocrambescidin 800 (29).
A solution of carboxylic acid **25** (30 mg, 0.043 mmol), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (28 mg, 0.064 mmol), (*S*)-hydroxyspermidine derivative **28**[34] (23 mg, 0.064 mmol), Et$_3$N (29 μL, 0.22 mmol), and CH$_2$Cl$_2$ (2.0 mL) was maintained at room temperature for 1 h and then partitioned between Et$_2$O (40 mL) and 0.1 M HCl (10 mL). The organic phase was washed with brine (2 × 10 mL), dried (MgSO$_4$), filtered, and concentrated. Purification of this residue by flash chromatography (99:1 CHCl$_3$−MeOH → 97:3 CHCl$_3$−MeOH) gave 32 mg (71%) of **29** as a colorless foam: ^{1}H NMR (500 MHz, CD$_3$OD) δ 5.70 (br t, J = 8.8 Hz, 1H), 5.51 (d, J = 11.1 Hz, 1H), 4.45 (br s, 1H), 4.19−4.06 (m, 3H), 3.92−3.78 (m, 3H), 3.84 (d J = 3.4 Hz, 1H), 3.59−3.23 (m, 3H), 3.19−3.12 (m, 3H), 3.06−2.97 (m, 2H), 2.58 (dd, J = 12.8, 2.3 Hz, 1H), 2.45−2.32 (m, 4H), 2.31−2.24 (m, 2H), 2.18−2.12 (m, 1H), 1.96 (dd, J = 13.1, 6.1 Hz, 1H), 1.81−1.44 (m, 18H), 1.43 (s, 18H), 1.38−1.17 (m, 23H), 1.16 (d, J = 6.0 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H);[43] ^{13}C NMR (125 MHz, CD$_3$OD)[47] δ (176.6/176.2), 169.8, 158.6, 158.4, 150.2, 134.1, 131.3, 86.7, 84.6, 80.02, 79.95, 72.0, 70.1, (69.0/68.3), 66.2, (55.0/53.4), 54.8, 54.3, 45.0, 42.6, 39.1, (38.9/38.7), 38.1, 36.2, 34.3, 34.1, 33.7, 32.9, 31.0, 30.78, 30.75, 30.67, 30.64, 30.57, 30.54, 30.50, 30.24, 30.16, 29.7, 28.9, 28.8, 28.7, 27.0, (26.7/26.6), 25.0, 22.4, 21.0, 10.8 ppm;[44] IR (film) 3385, 2927, 2854, 1731, 1668 (br), 1614, 1449, 1366, 1253, 1167, 1028 cm^{-1}; HRMS (FAB) *m/z* 1001.7 (1001.7 calcd for C$_{55}$H$_{97}$N$_6$O$_{10}$, M − Cl); [α]$^{22}_D$ −68.7, [α]$^{22}_{577}$ −72.9, [α]$^{22}_{546}$ −83.3, [α]$^{22}_{435}$ −148 (*c* 0.6, CHCl$_3$).

13,14,15-Isocrambescidin 800 Trihydrochloride (1). A solution of **29** (30 mg, 0.029 mmol) and 2.9 mL of a 2.0 M solution of HCl in

---

(47) The C38 amide exists on the NMR time scale as an approximate 1:1 mixture of rotamers. Some of the signals of carbons in close proximity to C38, including the carbons of the hydroxyspermidine unit, are doubled. In cases where the rotamers can be distinguished, these signals are listed in parentheses.

EtOAc was maintained at room temperature for 30 min and then concentrated. Purification of the residue by reversed-phase HPLC (3.5:1 MeOH−0.1 M NaCl, 5 μm Altima C18 column) gave 18 mg (70%) of 13,14,15-isocrambescidin 800 (**1**), a light yellow oil, as its trihydrochloride salt: [α]$^{22}_D$ −67.7, [α]$^{22}_{577}$ −70.9, [α]$^{22}_{546}$ −80.6 (*c* 0.73, MeOH). NMR data for this sample were consistent with data published for natural **1**, and synthetic **1** was indistinguishable from a natural sample of **1** by HPLC comparisons using three eluents.[6,37]

Supporting Information Available: Experimental procedure for preparing **30**; characterization data for **17**, **22b**, *i*, and *ii*; tables of assigned ^{1}H and ^{13}C NMR data for **19b** (Table 2), **23** (Table 3), **25** (Table 4), and **2** (Table 5); copies of ^{1}H NMR spectra of **1**, **20b**, and **30**; and copies of ^{19}F NMR spectra of **32** prepared from natural and synthetic **1** and **33** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA000235A