A Practical Entry to the Crambescidin Family of Guanidine Alkaloids. Enantioselective Total Syntheses of Ptilomycalin A, Crambescidin 657 and Its Methyl Ester (Neofolitispates 2), and Crambescidin 800

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Abstract: Among the most structurally remarkable guanidine natural products are the crambescidin/ptilomycalin A family of marine alkaloids. The evolution of a practical strategy for preparing pharmacologically significant crambescidin/ptilomycalin A alkaloids that lack oxidation at C13 is described. The first total syntheses of crambescidin 800 (**2**), crambescidin 657 (**6**), and neofolitispate 2 (**7**) are reported in full detail. The central strategic step in these convergent total syntheses is tethered Biginelli condensation of β-keto ester **24** with ureido aminal **61** to combine all carbons of the guanidine nucleus and set the pivotal C10−C13 stereorelationship. The total synthesis of crambescidin 800 (**2**) was accomplished in 3% overall yield from commercially available 3-butyn-1-ol by way of 16 isolated and purified intermediates. Full details of our earlier total synthesis of ptilomycalin A (**1**) are also presented. The total syntheses described in this disclosure confirm the stereochemical assignments of **1**, **2**, **6**, and **7** and rigorously establish that the absolute configuration of the hydroxyspermidine side chain of crambescidin 800 (**2**) is *S*.

Introduction

A striking variety of structurally novel guanidines have been isolated from marine organisms.[1] Diverse biological activities are associated with many of these alkaloids, likely reflecting the multiple ways that a guanidinium cation can participate in noncovalent interactions. Among the most remarkable marine guanidine natural products are the family of alkaloids depicted in Figure 1 that have a rigid pentacyclic guanidine carboxylic acid core linked to an ω-hydroxycarboxylic acid, ester, or polyamine amide. The first member of this group to be isolated was ptilomycalin A (**1**), which was originally obtained by Kusumi, Kashman, and co-workers from a red sea sponge, *Hemimycale* sp., and a sponge found in the Caribbean.[2,3] The groups of Rinehart,[4] Braekman,[5] and patent applications from Rinehart and Pharma Mar[6] describe a large series of cognate alkaloids, the crambescidins (e.g., **2**−**4** and **6**). These latter alkaloids, of which crambescidin 816 (**3**) is the most abundant, were isolated from *Crambe crambe*, a bright red conspicuous species of sponge found at shallow depths along the rocky coast of the Mediterranean. Ptilomycalin A (**1**), several of the crambescidins,[5b] and neofolitispates 2 (**7**, crambescidin 657 methyl ester)[7] have been reported from extracts of other warm-water sponges, whereas **1**, crambescidin 800 (**2**), cereromycalin (**5**), and fromiamycalin (**8**) have been isolated also from starfishes collected off New Caledonia.[8] *C. crambe* is likewise the source of 13,14,15-isocrambescidin 800 (**9**), which has a different stereochemistry of the pentacyclic guanidine unit.[4b,5a]

With the exception of **5**, **8**, and **9**, members of this guanidine alkaloid family differ from each other either in the length of the ω-hydroxycarboxylic acid unit, the presence or absence of a hydroxyl group at C13 of the guanidine moiety, or in the nature of the side chain terminus (carboxylic acid, ester, or polyamine amide). Structural assignments for the pentacyclic guanidine fragment derive almost exclusively from spectroscopic and mass spectrometric data. Extensive NMR studies demonstrated that the relative stereochemistry of the pentacyclic guanidine cores of ptilomycalin A (**1**) and crambescidins 800 (**2**), 816 (**3**), and

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(1) For reviews, see: (a) Berlinck, R. G. S. *Nat. Prod. Rep.* **1999**, *16*, 339−365. (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.

(2) (a) Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925−8926. (b) Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1992**, *114*, 8472−8479. (c) Ohtani, I.; Kusumi, T.; Kakisawa, H. *Tetrahedron Lett.* **1992**, *33*, 2525−2528.

(3) The Caribbean sponge was originally identified as *Ptilocaulis* aff. *Spiculifer*. A reexamination of the voucher specimen has led to this sponge being characterized as belonging to the genus Batzella Topsent, 1891, which is closely related to the genera *Crambe* and *Monanchora*.[5]

(4) (a) Jares-Erijman, E. A.; Sakai, R. Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712−5715. (b) Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805−4808. (c) Rinehart, K. L. *Abstracts of Papers*, 213th National Meeting of the American Chemical Society, San Franciso, CA, April 13−17, 1997; American Chemical Society: Washington, DC, 1997; ORG 348.

(5) (a) 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. (b) Tavares, R.; Daloze, D.; Braekman, J. C.; Hajdu, E.; Muricy, G.; Van Soest, R. W. M. *Biochem. Syst. Ecol.* **1994**, *22*, 645−646.

(6) (a) Shi, J.-G.; Sun, F.; Rinehart, K. L. WO Patent 3,756,734, 1998. (b) Rinehart, K. L.; Jares-Erijman, E. A. U.S. Patent 5,756,734, 1998.

(7) Venkateswarlu, Y.; Reddy, M. V. R.; Rao, P. R.; Rao, J. V. *Indian J. Chem., Sect. B* **1999**, *38*, 254−256.

(8) Palagiano, E.; De Marino, S.; Minale, L.; Riccio, R.; Zollo, F.; Iorizzi, M.; Carre, J. B.; Debitus, C.; Lucarain, L.; Provost, J. *Tetrahedron* **1995**, *51*, 3675−3682.

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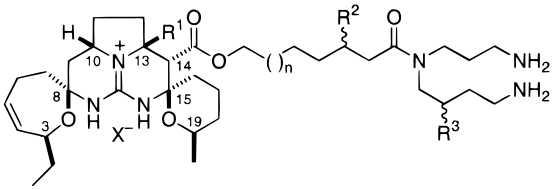
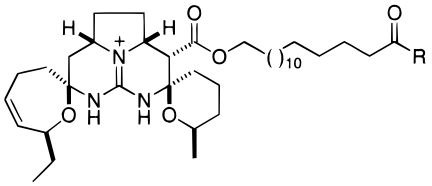
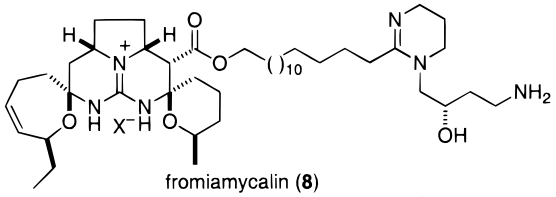


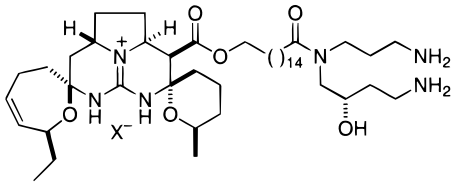
ptilomycalin A (**1**, $R^1 = R^2 = R^3 = H$; n = 10)
crambescidin 800 (**2**, $R^1 = R^2 = H$, $R^3 = \alpha$-OH; n = 10)
crambescidin 816 (**3**, $R^1 = OH$, $R^2 = H$, $R^3 = \alpha$-OH; n = 10)
crambescidin 844 (**4**, $R^1 = R^3 = OH$, $R^2 = H$; n = 13)
celeromycalin (**5**, $R^1 = R^3 = H$, $R^2 = \beta$-OH; n = 10)



crambescidin 657 (**6**, R = O^-)
neofolitispates 2 (**7**, R = OMe)



fromiamycalin (**8**)



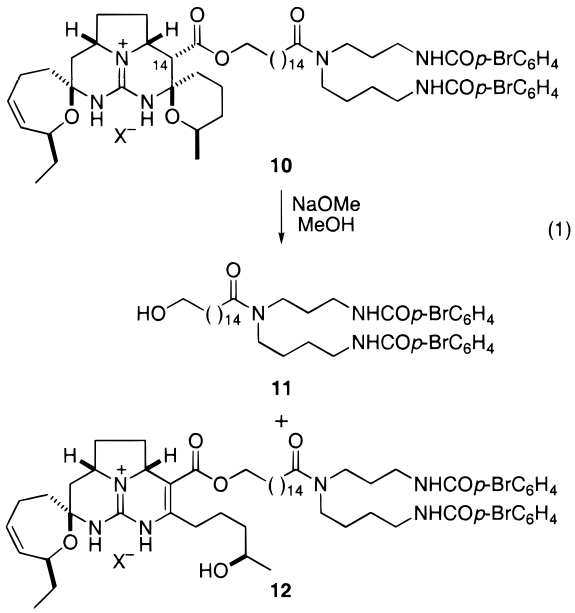
13,14,15-isocrambescidin 800 (**9**)

Figure 1. Pentacyclic marine guanidine alkaloids.

657 (**6**) are identical, whereas 13,14,15-isocrambescidin 800 (**9**) is epimeric at C13, C14, and C15.[2,4−6] The absolute configuration of the guanidine moieties of **3** and **9** was established by oxidative degradation of the oxepene rings of these alkaloids to yield (*S*)-2-hydroxybutanoic acid,[4a,b] whereas the absolute configuration of the hydroxyspermidine unit of **3** was assigned using Mosher's method.[5a,9] Because ^{1}H NMR and ^{13}C NMR chemical shifts of the hydroxyspermidine fragment of **3** are nearly identical to those of **2** and **9**, it has been assumed that the stereochemistry of the hydroxyspermidine is the same for all crambescidins containing this unit.

Little chemistry of these natural products has been described. Ptilomycalin A (**1**) was reported to give intractable mixtures upon attempted hydrolysis under both acidic and basic conditions or upon metal hydride reduction. Methanolysis of di-*p*-bromobenzoate derivative **10** was reported to yield side-chain fragment **11** and tetracyclic vinylogous urethane **12** (eq 1).[2b] In no instance has degradation of these alkaloids provided an intact pentacyclic guanidine carboxylic acid.

Ptilomycalin A and the crambescidin alkaloids exhibit a variety of pharmacological activities. Antiviral activity against *Herpes simplex* virus, type 1, antifungal activity against *Candida albicans*, and anti-HIV activities have been described.[2b,4a] Ptilomycalin A is reported to be the first nonnucleotide analogue that inhibits Na^+, K^+- and Ca^{2+}-ATPases by interaction with



10

NaOMe
MeOH (1)

11 + **12**

ATP at its binding site.[10] Ptilomycalin A (**1**) and crambescidins 800 (**2**), 816 (**3**), 844 (**4**), and 657 (**6**) are reported to exhibit low- to mid-nanomolar activities in vitro against L1210 murine leukemia cells,[4c,6] and similar levels of cytotoxicity against several human cancer cell lines including lung carcinoma A-549, colon carcinoma HT-29, and melanoma MEL-28.[2,5,6,11] Synthetic ptilomycalin A (NSC 700559) exhibits low- to mid-nanomolar activity against many human tumors in the National Cancer Institute (NCI) in vitro panel, with particular selectivity realized against nonsmall cell lung cancer (HOP-62) and melanoma (M14).[12] Crambescidin 816 is also reported to be a powerful in vitro Ca^{2+}-channel blocker ($IC_{50} = 0.15$ nM; nifedipine = 1.2 μM in this assay).[5a]

Synthesis Plan. A molecular mechanics model of the methyl ester of the crambescidin/ptilomycalin A pentacyclic guanidine core is shown in Figure 2. The central triazaacenaphthalene ring system of these alkaloids is nearly planar with the seven- and six-membered cyclic ethers being oriented on one face. Because the two C−O bonds are axial, we surmised that the C8 and C15 spirocenters would assemble with the required stereochemistry if the central triazaacenaphthalene unit had the proper cis stereochemistry. At the outset of our endeavors, we envisaged that setting the cis stereorelationship of the angular hydrogens at C10 and C13 and relating the chirality of this unit to the C3 and C19 stereogenic centers of the oxepene and hydropyran rings would be critical elements in evolving a stereocontrolled strategy for preparing the crambescidin/ptilomycalin A class of guanidine alkaloids.

As illustrated in Scheme 1, disconnection of the C8 aminal and retrosynthetic cleavage of the C15−O bond of **13** leads to 1-oxohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester (X = O) or 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester (X = NH_2) intermediates **14**. The 5-alkoxycarbonyl-1,2,3,4-tetrahydropyrimidine part structure of **14** (X = O) suggested to us that this bicyclic intermediate might be prepared by a modification of the three-component Biginelli condensation in which the urea and aldehyde reactants would be linked as

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

(10) Ohizumi, Y.; Sasaki, S.; Kusumi, T.; Ohtani, I. I. *Eur. J. Pharmacol.* **1996**, *310*, 95−98.

(11) Greater activity against L1210 (5−10 ×) has been reported for chlorospermidine analogues of crambescidin 800 and 816.[4c,6a]

(12) National Cancer Institute Developmental Therapeutics Program testing to L.E.O., dated March 12, 1998.

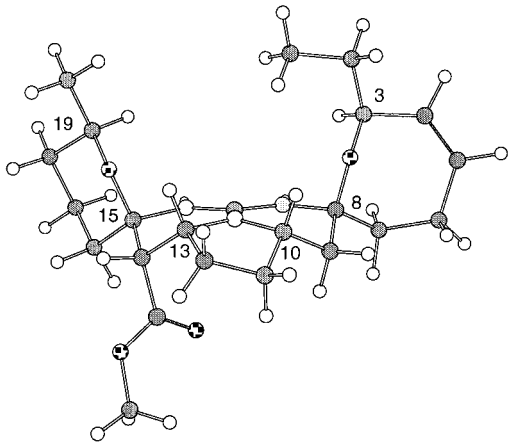
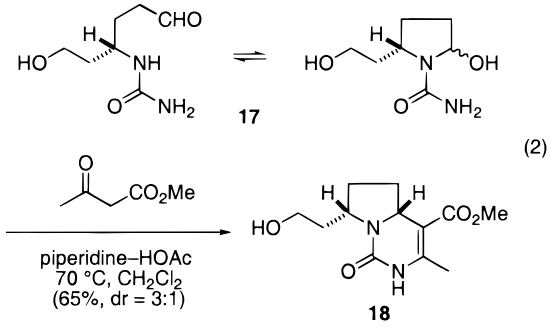


Figure 2. Molecular mechanics model of the pentacyclic core of the crambescidin/ptilomycalin A alkaloids.

depicted in **15**.[13] This analysis had the appeal of high convergence, because the left-hand three rings of **13** would derive from acyclic fragment **15**, while the right two rings and the ester side chain would be incorporated as the simple β-keto ester unit **16**.

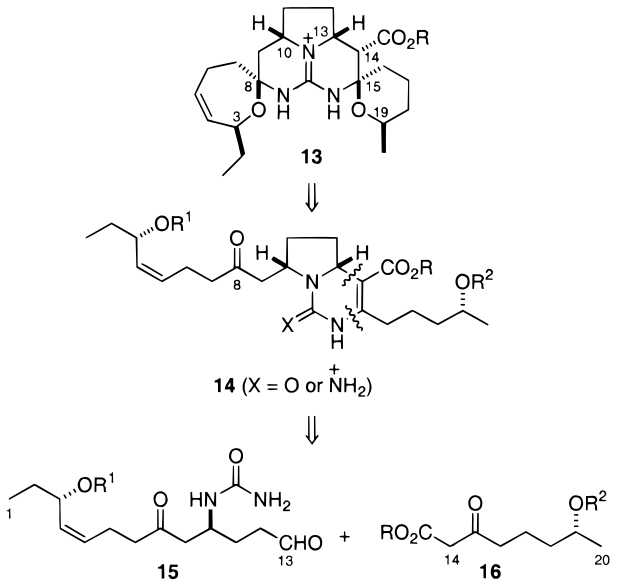
In 1993, we reported initial model studies that established the viability of "tethered-Biginelli" condensations (eq 2) and verified that the cis orientation of the angular methine hydrogens could be preferentially realized when this dehydrative condensation was promoted under Knoevenagel conditions.[14] A more thorough study of stereoselection in tethered Biginelli condensations was recently published and revealed that either bicyclic stereoisomer can be formed preferentially using the proper acyl substituent and reaction conditions.[15]



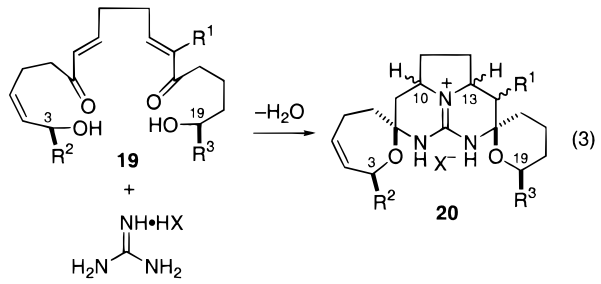
(2)

Before describing our investigations that led to the first total syntheses of members of the crambescidin/ptilomycalin A alkaloid group,[16] the accomplishments registered by the groups of Snider and Murphy in assembling the basic guanidinium units of these alkaloids from acyclic precursors must be mentioned.[17−19] The presumed biomimetic strategy illustrated in eq 3 has the appeal that pentacycles such as **20** would be assembled using

Scheme 1



guanidine (or a guanidine precursor) to stitch together an acyclic dihydroxy dienedione. The ranking accomplishment of these studies is Snider's early construction of the pentacyclic nucleus of ptilomycalin A from **19** ($R^1 = CO_2Me$, $R^2 = Et$, $R^3 = Me$).[18b] The appeal of this approach to the ptilomycalin A/crambescidin alkaloids is compromised in part by the inability of the remote secondary alcohol stereocenters C3 and C19 to control in any way the orientation of the cis C10 and C13 angular hydrogens. The biomimetic approach has proven particularly powerful for the preparation of simple pentacyclic congeners from the direct reaction of guanidinium salts with achiral dienediones (dihydro analogues of **19** with $R^1 = R^2 = R^3 = H$).[19]



(3)

Results and Discussion

Enantioselective Total Synthesis of Ptilomycalin A (1). At the time our investigations began, ptilomycalin A was the sole member of this alkaloid group to have been reported. In light of the difficulty experienced during degradation studies in removing the ester side-chain of **1**,[2] we chose to incorporate the 16-hydroxyhexadecanoic acid fragment from the outset. The synthesis of β-ketoester **24**, which incorporates this unit, is summarized in Scheme 2. Alkylation of the dianion of methyl acetoacetate (**21**)[20] with enantiopure (*R*)-siloxy iodide **22** provided **23** in 73% yield. Iodide **22** is available in high yield from methyl (*R*)-2-hydroxybutanoate.[21a] Selective transesterification of **23** with allyl 16-hydroxyhexadecanoate using 4-(dimethylamino)pyridine (DMAP) as catalyst gave **24** in 64% overall yield from **21**.[22]

(13) (a) Biginelli, P. *Gazz. Chim. Ital.* **1893**, *23*, 360. (b) For a recent review, see: Kappe, C. O. *Tetrahedron* **1993**, *49*, 6937−6963.

(14) Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 3235−3237.

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

(16) Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657−2658.

(17) For brief reviews of synthetic work in this area, see refs 1a and 1b.

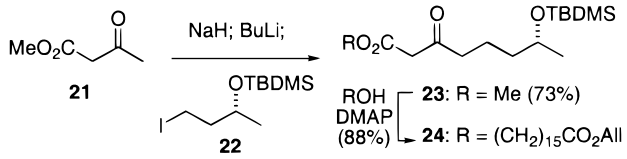
(18) (a) Snider, B. B.; Shi, Z. *Tetrahedron Lett.* **1993**, *34*, 2099−2102. (b) Snider, B. B.; Shi, Z. *J. Am. Chem. Soc.* **1994**, *116*, 549−557.

(19) (a) Murphy, P. J.; Williams, H. L.; Hursthouse, M. B.; Abdul Malik, K. M. *J. Chem. Soc., Chem. Commun.* **1994**, 119−120. (b) Murphy, P. J.; Williams, H. L. *J. Chem. Soc., Chem. Commun.* **1994**, 819−820. (c) Murphy, P. J.; Williams, H. L.; Hibbs, D. E.; Hursthouse, M. B.; Abdul Malik, K. M. *Tetrahedron* **1996**, *52*, 8315−8332. See also, Nagasawa, K.; Georgieva, A.; Nakata, T. *Tetrahedron* **2000**, *56*, 187−192.

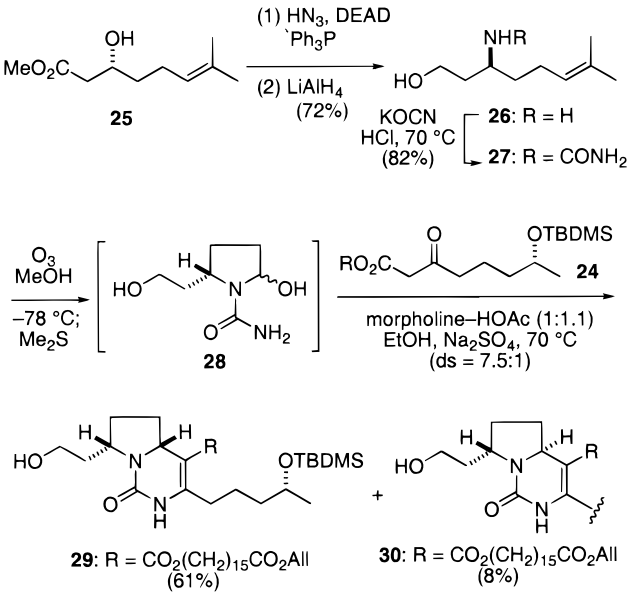
(20) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082−1087.

(21) (a) Kitamura, M.; Tokunaga, M.; Ohkuma, T.; Noyori, R. *Org. Synth., Coll. Vol. 9* **1998**, 589−595. (b) Taber, D. F.; Silverberg, L. J. *Tetrahedron Lett.* **1991**, *32*, 4227−4230.

Scheme 2



Scheme 3

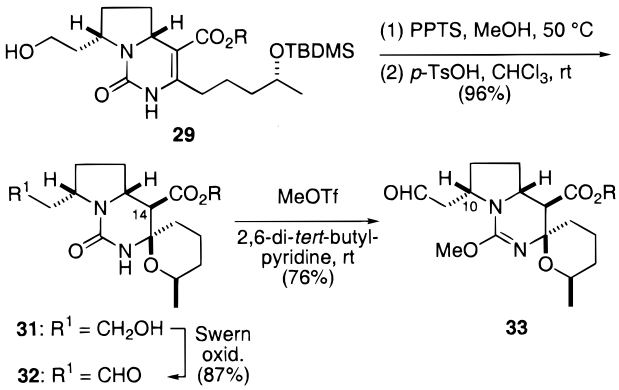


Because the tethered Biginelli condensation had just been developed,[14] we elected in this first generation effort to arrive at the central condensation reaction as early as possible in the synthetic sequence. For this reason, the electrophilic component of the Biginelli condensation was simplified from that depicted in Scheme 1 by deletion of the C1−C7 fragment. The precursor of this less elaborate intermediate, unsaturated urea **27**, was prepared in three steps from enantiopure methyl (*R*)-3-hydroxy-7-methyloct-6-enoate (**25**)[21] as summarized in Scheme 3. Mitsunobu displacement of alcohol **25** with hydrazoic acid followed by reduction of the crude β-azido ester with $LiAlH_4$ gave *S* amino alcohol **26** in 72% yield and in >98% enantiomeric excess (ee).[23] Use of other nitrogen nucleophiles such as phthalimide in the Mitsunobu reaction led to significant amounts of the corresponding α,β-unsaturated ester. Reaction of **26** with potassium cyanate and HCl under standard conditions provided unsaturated urea **27** in 82% yield after recrystallization. Ozonolysis of **27** in MeOH at −78 °C followed by reduction of the intermediate hydroperoxide with Me_2S and concentration furnished a viscous yellow oil. Further concentration of this product at 0.1 Torr for 5 days at 50 °C to remove residual Me_2SO led to a nearly colorless amorphous powder. This crude intermediate is more complex than formulation **28** implies. Multiple signals were observed for many carbon atoms in the ^{13}C NMR spectra and the ^1H NMR spectrum was broad. No aldehyde signal was apparent, and mass spectral data indicated the presence of higher molecular weight materials derived from dehydrative oligomerization. Because all attempts to enhance the purity of **28** by chromatography were unsuccessful, we proceeded with this crude intermediate.

(22) Taber, D. F.; Amedio, J. C., Jr.; Patel, Y. K. *J. Org. Chem.* **1985**, *50*, 3618−3619.

(23) Enantiomeric excess was determined by evaluation of the ^{19}F NMR spectra of the corresponding (*R*)- and (*S*)-Mosher amides,[9] see Supporting Information.

Scheme 4



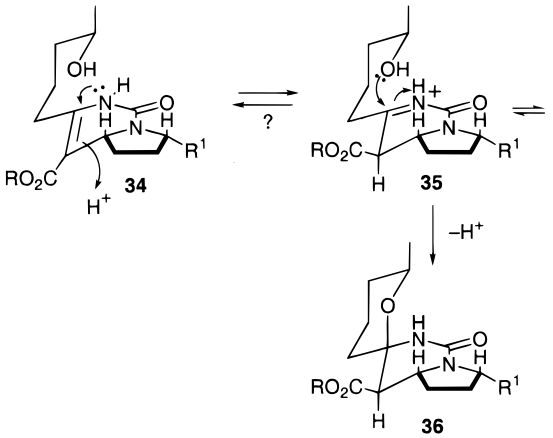
Under the conditions developed during our original model study,[14] Biginelli condensation of crude **28** and β-keto ester **24** took place in low yield. A number of reaction parameters were then surveyed and it was soon found that the efficiency of the Biginelli reaction improved considerably in polar solvents. Best results were achieved by heating a mixture of crude **28**, 1.5 equiv of β-keto ester **24**, 1 equiv of morpholinium acetate, a catalytic amount of acetic acid, and excess Na_2SO_4 at 70 °C in EtOH. Purification of the resulting product on silica gel provided cis adduct **29** in 61% yield and trans adduct **30** in 8% yield. Stereochemical assignments for these hexahydropyrrolo[1,2-*c*]-pyrimidines followed from the similarity of their angular methine hydrogen signals (**29**: 4.25 and 4.11 ppm; **30**: 4.44 and 4.09 ppm) with those of **18** and its trans epimer, the latter of which had earlier been analyzed by single-crystal X-ray diffraction analysis.[14] The condensation reported in Scheme 3 was carried out on a large scale (17 g of **24** and 4 g of **28**) and the yield obtained in this experiment appears to be reliable. However, high efficiency under these conditions was not reproducible, presumably due to the indeterminate nature of **28**. In a recent detailed examination of stereoselection in related Biginelli condensations, a reliable procedure for generating the electrophilic reaction component and for carrying out the Biginelli condensation was developed; these conditions consistently provide cis adducts such as **29** in yields of 60−65%.[15]

Although **29** could be converted in one step to spirotricyclic intermediate **31** by exposure to a slight excess of *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O), the reaction was more reproducible on a large scale if the *tert*-butyldimethylsilyl (TBDMS) group was first discharged with pyridinium *p*-toluenesulfonate (PPTS) in MeOH and the resulting alcohol was then cyclized at room temperature in $CHCl_3$ with a catalytic amount of *p*-TsOH·H_2O (Scheme 4). This sequence provided a single tricyclic product **31** in nearly quantitative yield. The 11.5 Hz diaxial-coupling constant of the C14 methine hydrogen of **31** signaled that this intermediate was epimeric to ptilomycalin A at C14.[24]

That diastereoselection would be high in forming the spirohydropyran had been established in our earliest model study[14] and can be rationalized as outlined in Scheme 5. Axial protonation of the vinylogous carbamate **34** would generate *N*-acyliminium cation **35**. Spirocyclization of this intermediate from the β-face to generate **36** would be favored to maximize a staggered conformation with respect to the forming bond.[25,26]

(24) The crambescidin numbering system is employed in the discussion of all synthetic intermediates. Proper IUPAC designations of intermediates can be found in the Experimental Section or Supporting Information.

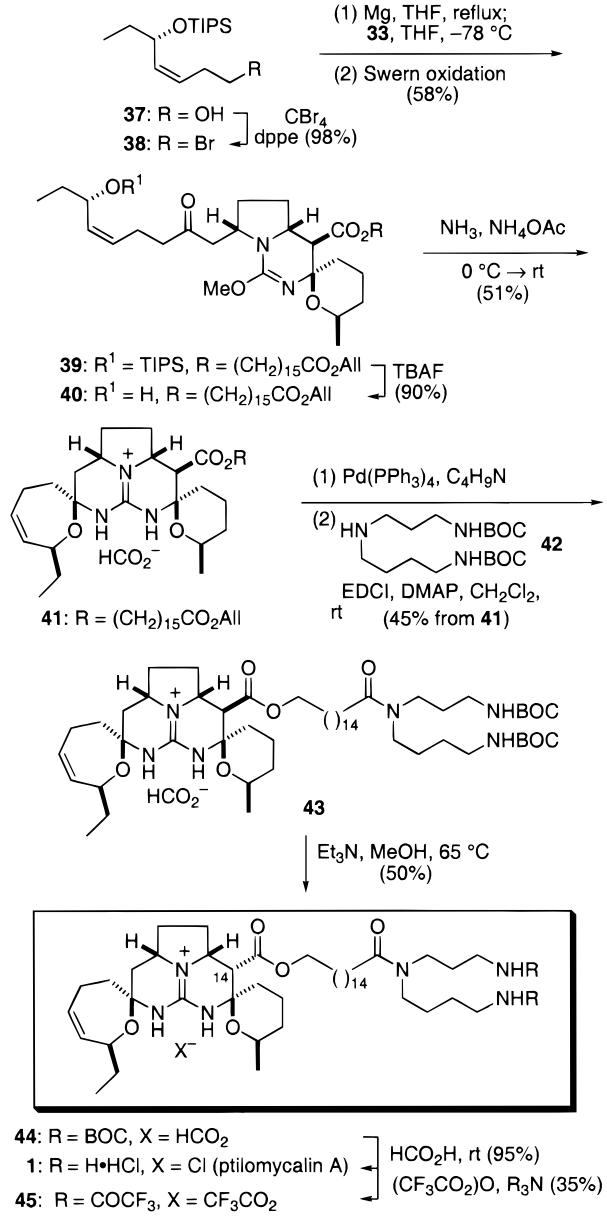
Scheme 5



Although epimerization of **31** to the axial ester might have been possible at this point, we chose to defer this adjustment to the final stage of the synthesis, hoping to benefit from a presumed (incorrect as it turned out) thermodynamic preference for this group to be axial in the natural product. To prepare for the addition of the remaining carbons of the guanidine core, **31** was oxidized with the Swern reagent[27] to provide **32** and the urea functional group was protected and activated for subsequent guanidine formation by *O*-methylation (Scheme 4). This delicate methylation had to be performed under carefully prescribed conditions, and pseudourea product **33** had to be purified rapidly on Et_3N-treated silica gel, or else significant epimerization at C10 was observed. Reversible β-elimination of the pseudourea group is undoubtedly responsible for the erosion of C10 stereochemistry.

At this juncture, the remaining seven carbons of the pentacyclic guanidine nucleus needed to be appended, an elaboration that proved to be extremely challenging. Bromide **38** was available in high yield from alcohol **37** (86% ee) by reaction with CBr_4 and 1,2-bis(diphenylphosphino)ethane (dppe) (Scheme 6).[28] In early scouting studies, we were unsuccessful in efficiently coupling lithium or cerium reagents derived from bromide **38** with a benzyl ester congener of aldehyde **33**. Epimerization of **33** at C10 in the presence of Lewis acidic reagents emerged as a critical complicating issue. We eventually found that the Grignard reagent derived from **38** could be joined to **33** in acceptable yield at −78 °C in tetrahydrofuran (THF). Quenching this reaction at low temperature with morpholinium acetate and immediate filtration of the reaction mixture to remove magnesium salts provided the adduct as a mixture of alcohol epimers. Direct oxidation of this intermediate under Swern conditions[27] then provided **39** in 58% yield from **33**. Approximately 5% of a diastereomer arising from the minor enantiomer of alcohol **37** was removed at this point. This sequence was extremely delicate and yields were markedly eroded if magnesium salts resulting from the Grignard step were not removed.

Scheme 6



Cleavage of the silyl protecting group of **39** with tetrabutylammonium fluoride (TBAF) furnished alcohol **40**, which was exposed to ammonia and ammonium acetate under conditions similar to those originally reported by Snider.[18b] After purification of the crude product on silica gel using an eluent containing formic acid, **41** was isolated in 51% as its formate salt (^{1}H NMR: δ 8.23; ^{13}C NMR: δ 165.8). Formation of the second spiroaminal took place exclusively by axial C−O bond formation, because only a single pentacyclic guanidine was detected.

A model of the tetracyclic cation **46**, which is the likely direct precursor of pentacyclic guanidine **41**, is shown in Figure 3; the C1−C7 side chain was replaced with a methyl group to generate this model. It is apparent from examining the model that torsional interactions would be minimized by axial addition of the oxygen nucleophile to the electron-deficient carbon.[25]

The total synthesis of (−)-ptilomycalin A was readily completed from **41**. The allyl ester of this intermediate was cleaved using palladium(0) catalysis[29] and the resulting acid was

(25) See, inter alia: (a) Cherest, M.; Felkin, H. *Tetrahedron Lett.* **1968**, 2199−2204, 2205−2208. (b) Lucero, M. J.; Houk, K. N. *J. Org. Chem.* **1998**, *63*, 6973−6977.

(26) Torsional steering as a potential control element in spirocyclization is developed more fully later in the text in the discussion of forming the spirooxepene.

(27) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480−2482.

(28) The alcohol precursor **37** of bromide **38** used in these experiments was prepared in 86% ee by asymmetric reduction of an ynone precursor.[16] A better synthesis of alcohol **37**, which provides material of high enantiopurity, is shown in Scheme 7.

(29) Deziel, R. *Tetrahedron Lett.* **1987**, *28*, 4371−4372.

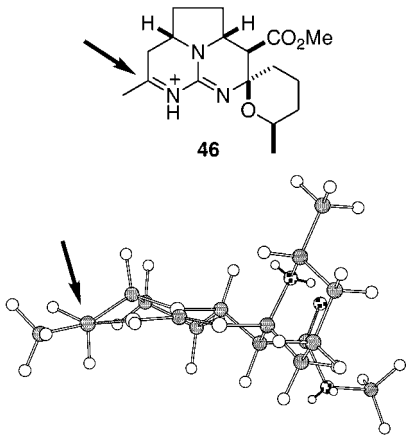


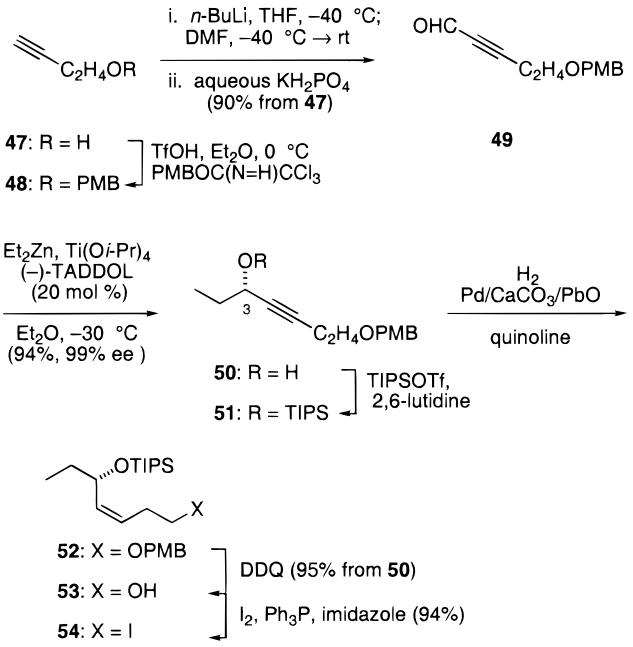
Figure 3. Model showing the expected preference for axial addition in forming the oxepene ring.

coupled with di-*tert*-butoxycarbonyl (BOC)-protected spermidine **42**[30] to generate amide **43**. The ester was then epimerized by heating in MeOH in the presence of excess Et_3N. Epimerization under these conditions, however, favored the β epimer to the extent of 2−3:1. Consequently, three recycles were required to obtain axial ester **44** in 50% yield. The equatorial C14 methine hydrogen of **44** showed a diagnostic doublet (J = 4.8 Hz) at δ 2.93 in the ^{1}H NMR spectrum. Finally, cleavage of the BOC protecting groups of **44** with HCO_2H, followed by concentration and washing with aqueous NaOH−NaCl provided (−)-ptilomycalin A trihydrochloride (**1**) in good yield. Synthetic **1** showed ^{1}H and ^{13}C NMR spectra consistent with those reported for (−)-ptilomycalin A[2a,b] and was indistinguishable from an authentic sample by thin-layer chromatography (TLC) comparisons on three adsorbents. Synthetic **1** was converted to ditrifluoroacetate derivative **45**, which also exhibited ^{1}H and ^{13}C NMR spectra indistinguishable from those reported.[2b] The specific rotation of synthetic **45**, $[\alpha]^{23}_D$ −15.9 (*c* 0.8, $CHCl_3$), was identical within experimental precision to the rotation, $[\alpha]^{23}_D$ −15.8 (*c* 0.7, $CHCl_3$), reported for this well-characterized derivative of the natural product.[2b]

Second-Generation Synthesis Plan. Motivated by the therapeutic potential of the crambescidin/ptilomycalin A class of guanidine alkaloids and the opportunity to use the tools of organic synthesis to probe the molecular origins of antitumor activity in this series, a second-generation synthesis approach to these targets was developed, which we hoped would provide practical synthetic access to these structures. A weakness of the strategy we employed to prepare ptilomycalin A was the series of delicate transformations that had to be carried out on advanced intermediates to introduce C1−C7. This elaboration would be avoided, and the overall convergency of the synthesis would be enhanced, if *all* carbons of the pentacyclic core could be joined in the pivotal Biginelli condensation. This optimally convergent approach is outlined in Scheme 1 and was initially implemented to achieve the inaugural total syntheses of crambescidin 800 (**2**), crambescidin 657 (**6**), and its methyl ester neofolitispates 2 (**7**).

Total Synthesis of Crambescidins 800 (2) and 657 (6) and Neofolitispates 2 (7). Developing a rapid enantioselective construction of the common C1−C13 fragment (**15** of Scheme 1) was critical to evolving an efficient route for the total synthesis of the ptilomycalin A/crambescidin alkaloids.[31] The synthesis of this unit began with 3-butyn-1-ol (**47**), which was

Scheme 7



initially protected to give *p*-methoxybenzyl (PMB) ether **48** (Scheme 7).[32] Deprotonation of **48** with *n*-butyllithium at −40 °C, trapping the acetylide intermediate with anhydrous dimethylformamide (DMF), and quenching the intermediate α-aminoalkoxide into aqueous phosphate buffer[33]provided ynal **49** in 90% overall yield. The C3 stereocenter was introduced by the method of Weber and Seebach[34] by reaction of **49** with Et_2Zn in the presence of 20 mol % of (4*R*,5*R*)-2,2-dimethyl-α,α,α′,α′-tetra(naphth-2-yl)-1,3-dioxolan-4,5-dimethanol [(−)-TADDOL] and Ti(O*i*-Pr)$_4$ to give **50** in 94% yield. This excellent asymmetric transformation could be performed reliably on scales as large as 50 g to provide **50** in 99% ee.[9] A triisopropylsilyl (TIPS) group was next introduced to protect the C3 hydroxyl group, because this ether was expected to survive the conditions of the Biginelli condensation. Semi-hydrogenation of **51** using Lindlar's catalyst yielded *cis*-alkene **52** and removal of the *p*-methoxybenzyl group from this intermediate with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone DDQ provided alcohol **53**. By purifying only intermediates **49** and **50**, the synthesis of **53** could be realized in 80% overall yield from commercially available 3-butyn-1-ol. Conversion of alcohol **53** to iodide **54** was accomplished in standard fashion in high yield.[35]

The remaining carbons of the C1−C13 fragment came from methyl (*R*)-3-hydroxy-7-methyloct-6-enoate (**25**) (Scheme 8). This intermediate, which is readily available in 97% ee,[21] was transformed to amide **55** in 88% yield by reaction with *N*,*O*-dimethylhydroxylamine hydrochloride,[36] followed by protection of the secondary alcohol as a triethylsilyl (TES) ether. The lithium reagent derived from iodide **54** was then coupled with **55** at −78 °C to generate dienone **56** in 60−70% yield. Masking

(30) Cohen, G. M.; Cullis, P. M.; Hartley, J. A.; Mather, A.; Symons, M. C. R.; Wheelhouse, R. T. *J. Chem. Soc., Chem. Commun.* **1992**, 298−300.

(31) A preliminary report of the synthesis of **59** has appeared, see: Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **1999**, *121*, 6944−6945.

(32) (a) Takaku, H.; Ueda, S.; Ito, T. *Tetrahedron Lett.* **1983**, *24*, 5363−5366. (b) Nakajima, N.; Horita, K.; Abe, R.; Yonemitsu, O. *Tetrahedron Lett.* **1988**, *29*, 4139−4142.

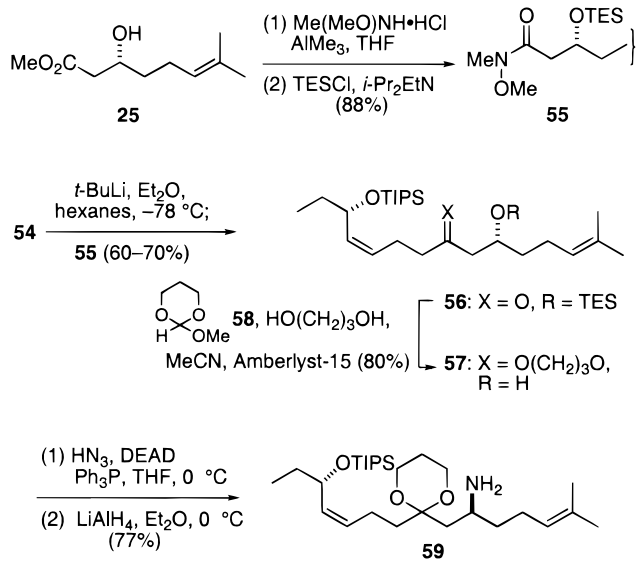
(33) Journet, M.; Cai, D.; DiMichele, L. M.; Larsen, R. D. *Tetrahedron Lett.* **1998**, *39*, 6427−6428.

(34) Weber, B.; Seebach, D. *Tetrahedron* **1994**, *50*, 7473−7484.

(35) Singh, A. K.; Bakshi, R. K.; Corey, E. J. *J. Am. Chem. Soc.* **1987**, *109*, 6187−6189.

(36) Garigipati, R. S.; Tschaen, D. M.; Weinreb, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 7790−7792.

Scheme 8

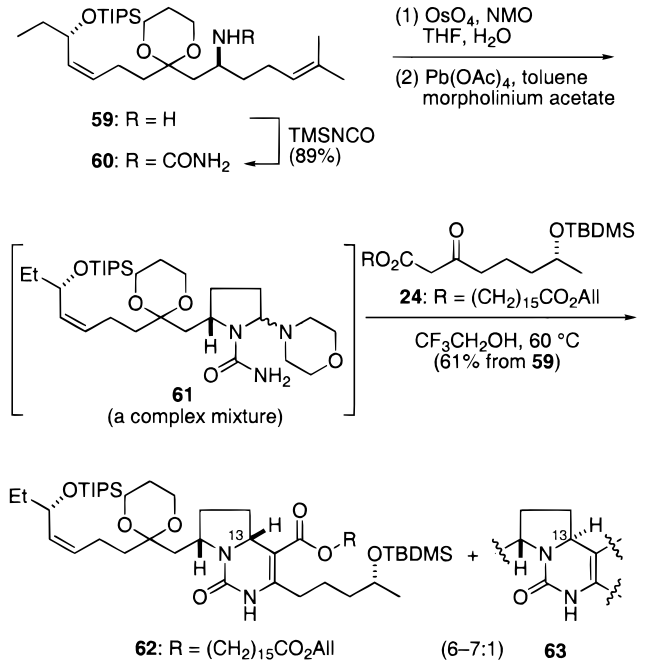


the C8 carbonyl of **54** as a ketal became necessary to prevent dehydration of the β-hydroxy ketone under the Mitsunobu conditions employed in a subsequent step to install the C10 amino group. However, ketalization of **56** was quite sluggish. We eventually discovered that when the β-hydroxy group of this intermediate was not protected, ketalization was much easier. Ultimately, conditions were developed that cleaved the TES group of **56**, did not promote dehydration of the intermediate β-hydroxy ketone, and effected ketalization. Thus, reaction of **56** with ortho ester **58**[37] and 1,3-propanediol in the presence of Amberlyst-15 at room temperature provided hydroxy ketal **57** in 80% yield. Mitsunobu displacement of the secondary alcohol of **57** with azide followed by reduction to the amine delivered **59** in 77% yield from **57**. The sequence summarized in Schemes 7 and 8 is readily scaled and provided amine **59** on multigram scales in 11 steps and ~30% overall yield from commercially available 3-butyn-1-ol.

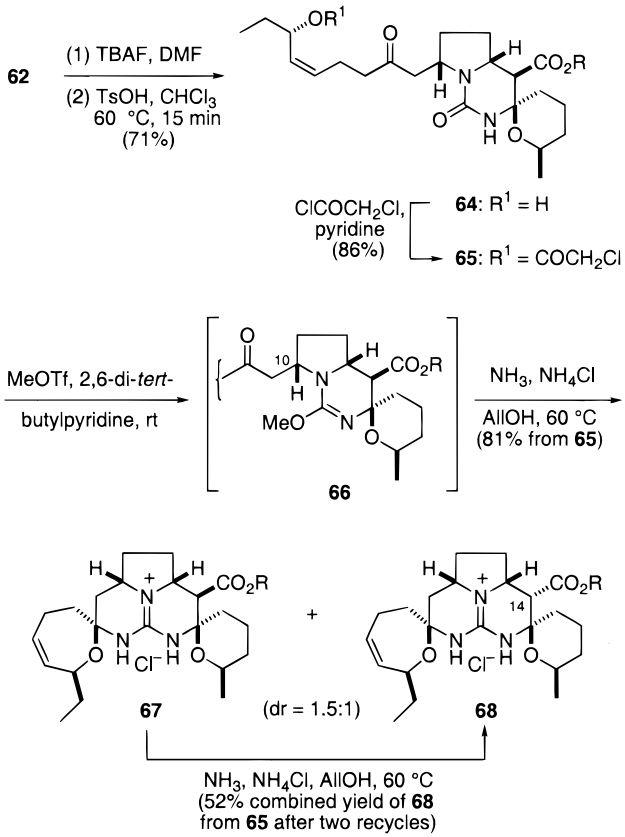
The critical tethered Biginelli condensation was next realized with little difficulty. Condensation of amine **59** with trimethylsilyl isocyanate provided urea **60** in high yield (Scheme 9). Selective dihydroxylation of the trisubstituted double bond of **60**,[38] followed by cleavage of the vicinal diol with $Pb(OAc)_4$ in toluene and addition of morpholinium acetate yielded intermediate **61**, which was used without purification in the critical Biginelli condensation step. Using conditions that we had previously optimized with simpler urea aminal intermediates,[15] crude **61** was condensed with 2.8 equiv of β-ketoester **24** at 60 °C in 2,2,2-trifluoroethanol to give a 6−7:1 mixture of cis and trans hexahydropyrrolopyrimidines **62** and **63** in 61% overall yield from **60**. Although these stereoisomers could be separated by HPLC, they were difficult to resolve on a preparative scale. Therefore, this mixture was carried on to a tricyclic intermediate where isomer separation was straightforward. Stereochemical assignments for the hexahydropyrrolo-[1,2-*c*]pyrimidines **62** and **63** again followed from the similarity of ^{1}H NMR signals for their H13 methine hydrogens (**62**: 4.22 ppm; and **63**: 4.44 ppm) with those of **18** and its trans epimer.[14]

The remaining three rings of the pentacyclic guanidine nucleus were generated as follows. First, the silyl protecting

Scheme 9



Scheme 10



groups of **62** were removed with TBAF to provide the corresponding urea diol (Scheme 10). Brief exposure of this intermediate to 1 equiv of p-TsOH·H_2O induced formation of the spirohydropyran and discharge of the ketal to generate **64** in 71% yield for the two steps. As in our earlier synthesis of ptilomycalin A, cyclization to form the spirocyclic hydropyran took place with untarnished stereoselectivity.

Initial survey experiments showed that it would not be easy to selectively activate the urea functional group of **64**. Conse-

(37) (a) Roush, W. R.; Gillis, H. R. *J. Org. Chem.* **1980**, *45*, 4283−4287. (b) Baganz, H.; Domaschke, L. *Chem. Ber*. **1958**, *91*, 650−653.

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

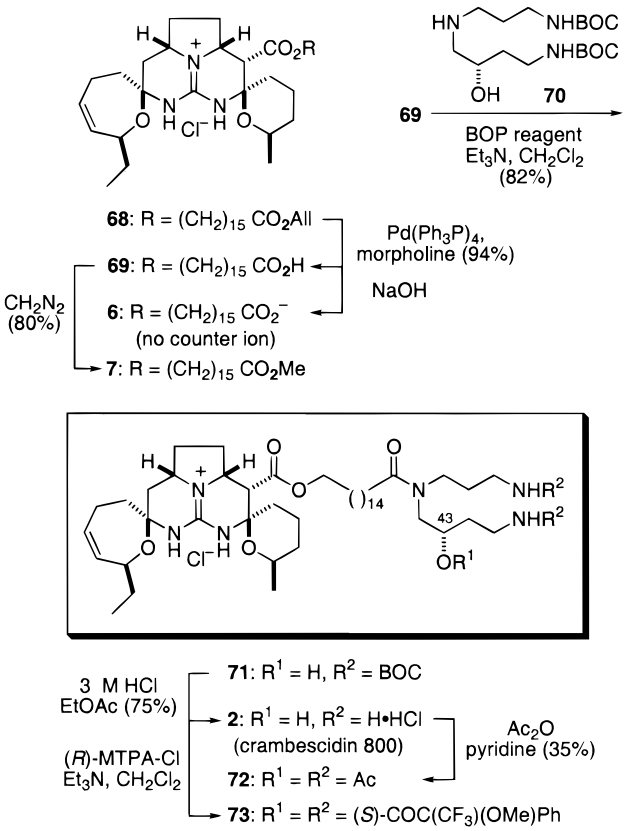
quently, the secondary alcohol of **64** was protected as its chloroacetate derivative, this protecting group being chosen because it would be removed upon exposure to ammonia. At this stage, the minor trans isomer (~12%) resulting from the Biginelli condensation was removed by silica gel chromatography and isomerically pure **65** was obtained in 86% yield. Exposure of **65** to excess MeOTf in the presence of 2,6-di-*tert*-butylpyridine delivered methyl pseudourea **66**, a labile intermediate that was directly cyclized to the pentacyclic guanidine. It was critical that **66** not be exposed to silica gel, because epimerization at C10 and some decomposition resulted under typical chromatographic purification conditions. After considerable experimentation, it was found that saturating an allyl alcohol solution of crude **66** (buffered with 2 equiv of NH_4Cl) with anhydrous ammonia at room temperature, followed by heating the resulting solution in a resealable tube at 60 °C for 1 day provided a 1.5:1 mixture of pentacyclic guanidines **67** and **68** in 81% yield. That 1.5:1 represented the equilibrium ratio of the C14 epimers under these conditions was readily established by resubmission of **67** and **68** to the reaction conditions. Separation of these isomers on silica gel and two recycles of **67** (NH_3-NH_4Cl, allyl alcohol, 60 °C) provided **68** (^{1}H NMR of H14: $J = 4.8$ Hz) in 52% overall yield from urea **65**.

The sequence summarized in Scheme 10 for assembling pentacyclic guanidine **68** represents a significant improvement over that employed in our first-generation synthesis of ptilomycalin A. Not only is the overall yield higher, but material throughput is increased because the pentacyclic guanidine nucleus is assembled under conditions that equilibrate the C14 ester epimers. Moreover, the guanidine products are obtained directly with chloride counterions, obviating the need for detrimental aqueous washes. The use of allyl alcohol as solvent allowed higher concentrations of ammonia to be employed without complicating transesterification of the allyl hexadecanoate side chain.

The total syntheses of crambescidin 657 (**6**), neofolitispates 2 (**7**), and crambescidin 800 (**2**) were culminated as summarized in Scheme 11. Initially the allyl protecting group of **68** was cleaved with $Pd(PPh_3)_4$ and morpholine[29] and the product was washed with dilute HCl and purified on silica gel to provide acid **69** in 94% yield (Scheme 11). This acid was quantitatively converted to the carboxylate inner salt **6** by washing with dilute NaOH. The ^{13}C NMR spectrum of this latter product in $CDCl_3$ was identical (mean difference $< \pm 0.1$ ppm) to that reported for natural crambescidin 657.[39] The specific rotation of synthetic **6** was $[\alpha]^{23}_D$ −13.6 (*c* 0.45, MeOH), whereas $[\alpha]^{23}_D$ −12.1 (*c* 0.34, MeOH) is reported for the natural isolate.[6a] Reaction of synthetic **69** with diazomethane provided methyl ester **7**,[6a] $[\alpha]^{23}_D$ −17 (*c* 0.2, $CHCl_3$), whose ^{13}C NMR spectrum agreed in all respects (mean difference ±0.13 ppm) with that reported for neofolitispates 2, $[\alpha]^{23}_D$ −18 (*c* 1, $CHCl_3$), obtained from the sponge *Neofolitispa dianchora* collected at the Andaman Islands, India.[7]

Coupling of carboxylic acid **69** with (*S*)-7-hydroxyspermidine **70**[40] using benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent)[41] provided the corresponding amide **71** in 82% yield. Removal of the BOC groups with 3 M HCl in ethyl acetate[42] followed by reversed-phase HPLC

Scheme 11



purification of the crude product provided the trihydrochloride salt of crambescidin 800 (**2**) in 75% yield. ^{1}H and ^{13}C NMR data for synthetic **2** were in accord with those reported for natural **2**,[43] and synthetic **2** was indistinguishable from a natural specimen by HPLC comparisons using three eluents.[4a,5a] Synthetic **2** was also converted to the triacetate derivative **72**. ^{1}H and ^{13}C NMR data for synthetic **72** were in perfect accord with those reported for naturally derived **72**.[4a,5a] The specific rotation of synthetic **72** was $[\alpha]^{25}_D$ −37 (*c* 0.20, $CHCl_3$), whereas $[\alpha]^{25}_D$ −43 (*c* 0.15, $CHCl_3$) was reported for this derivative of the natural isolate.[5a]

No criteria had suggested that synthetic **2** might differ from natural crambescidin 800. Nonetheless, because the configuration of crambescidin 800 at C43 had not been established, we thought it prudent to vouchsafe that we could distinguish a C43 epimer. If the guanidine and hydroxy spermidine domains did not interact appreciably, differences between these two permutations might be difficult to discern. To this end, we prepared the C43 epimer of crambescidin 800 from **69** and the *R* enantiomer of **70**.[40,44] ^{19}F NMR studies demonstrated that the Mosher derivative **73** prepared from synthetic **2** was indistinguishable from this derivative prepared from a 150 μg sample of natural **2**, yet different from the identical derivative of the C43 epimer of synthetic **2**.

Conclusion. The first total syntheses of crambescidin 800 (**2**), crambescidin 657 (**6**), and neofolitispates 2 (**7**) were accomplished in enantioselective fashion using a convergent strategy. The central strategic step is tethered Biginelli conden-

(39) Natural crambescidin 657 is reported as an inner salt.[6a]

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

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

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

(43) Table 2 of ref 4a lists MeOD as the solvent for NMR spectra of natural crambescidin 800. This designation is apparenly in error; the solvent should be $CDCl_3$.

(44) A more detailed discussion of the challenge in distinguishing C43 epimers in the crambescidin series is found in the accompanying paper: Coffey, D. S.; Overman, L. E.; Stappenbeck, F., following paper in this issue.

sation of β-keto ester **24** with ureido aminal **61** to combine all carbons of the guanidine nucleus and set the pivotal C10−C13 stereorelationship. The total synthesis of crambescidin 800 (**2**) was accomplished in 3% overall yield from commercially available 3-butyn-1-ol by way of 16 isolated and purified intermediates. Full details of our earlier total synthesis of ptilomycalin A (**1**) are also provided. These total syntheses confirm the stereochemical assignments of **1**, **2**, **6**, and **7** and rigorously establish that the absolute configuration of the hydroxyspermidine side chain of crambescidin 800 (**2**) is *S*. The fully convergent synthesis route detailed herein constitutes a viable way to prepare the major group of crambescidin alkaloids that lack oxidation at C13 on multigram scales.

Experimental Section[45]

(6*R*,11*Z*,13*S*)-2-Methyl-6-triethylsilyloxy-13-triisopropylsiloxypentadeca-2,11-dien-8-one (56). A pentane solution of *t*-BuLi (23.5 mL, 40 mmol, 1.7 M) was added dropwise to a −78 °C solution of iodide **54** (6.67 g, 16.8 mmol) and Et_2O−hexanes (1:1, 100 mL). The solution was maintained at −78 °C for 30 min and a solution of amide **55** (6.10 g, 18.5 mmol) and Et_2O−hexanes (1:1, 40 mL) was added dropwise. The resulting solution was maintained at −78 °C for 30 min, allowed to warm to 0 °C, and maintained at 0 °C for 2 h. The solution was then added to saturated aqueous NH_4Cl (150 mL), the phases were separated, and the aqueous phase was extracted with Et_2O (2 × 150 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated. Purification of the residue by flash chromatography (98:2 hexanes−Et_2O) gave 5.93 g (65%) of **56** as a clear oil. The product was ca. 95% pure and was used without further purification in the next step.

A small sample was further purified by flash chromatography (98:2 hexanes−Et_2O) to obtain an analytical specimen: $[\alpha]^{25}_D$ +4.1, $[\alpha]^{25}_{577}$ +4.8, $[\alpha]^{25}_{546}$ +4.9, $[\alpha]^{25}_{435}$ +11.0, $[\alpha]^{25}_{405}$ +14.3 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.41−5.36 (m, 1 H), 5.29−5.24 (m, 1 H), 5.08 (tt, *J* = 7.1, 1.3 Hz, 1 H), 4.45 (app q, *J* = 6.7 Hz, 1 H), 4.18 (quintet, *J* = 6.0 Hz, 1 H), 2.60 (A of ABX, J_{AB} = 15.3, J_{AX} = 7.2 Hz, 1 H), 2.48−2.43 (m, 3 H), 2.30−2.24 (m, 2 H), 2.05−1.93 (m, 2 H), 1.68 (s, 3 H), 1.64−1.40 (m, 4 H), 1.59 (s, 3 H), 1.04 (s, 21 H), 0.94 (t, *J* = 7.9 Hz, 9 H), 0.85 (t, *J* = 7.5 Hz, 3 H), 0.58 (q, *J* = 7.9 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 135.1, 131.8, 126.7, 123.8, 69.8, 68.7, 50.2, 44.1, 37.9, 31.6, 25.7, 23.8, 21.9, 18.1, 18.0, 17.6, 12.3, 9.3, 6.9, 4.9 ppm; IR (film) 1717 cm^{-1}; high-resolution mass spectroscopy (HRMS) fast atom bombardment (FAB) *m*/*z* 537.4141 (M − H, 537.4159 calcd for $C_{31}H_{61}O_3Si_2$).

(6*R*,11*Z*,13*S*)-8-(1′,3′-Dioxan-2′-yl)-6-hydroxy-2-methyl-13-triisopropylsiloxypentadeca-2,11-diene (57). A mixture of crude ketone **56** (3.74 g, 6.94 mmol), ortho ester **58**[37b] (4.10 g, 34.7 mmol), 1,3-propanediol (12.6 mL, 174 mmol), Amberlyst-15 resin (278 mg), and MeCN (70 mL) was maintained at room temperature for 7 h. The mixture was then filtered through Celite and the filtrate was partitioned between Et_2O (150 mL) and H_2O (50 mL). The organic phase was washed with H_2O (2 × 50 mL), dried ($MgSO_4$), filtered, and the filtrate was concentrated. Purification of the residue by flash chromatography (85:15 hexanes−Et_2O) gave 2.68 g (80%) of ketal **57** as clear oil: $[\alpha]^{25}_D$ +13.3, $[\alpha]^{25}_{577}$ +14.2, $[\alpha]^{25}_{546}$ +16.8, $[\alpha]^{25}_{435}$ +30.1, $[\alpha]^{25}_{405}$ +37.4 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.42−5.29 (m, 2 H), 5.14 (broad t, *J* = 7.1 Hz, 1 H), 4.45 (app q, *J* = 7.5 Hz, 1 H), 4.11−4.08 (m, 1 H), 4.02−3.85 (m, 4 H), 3.80 (s, 1 H), 2.16−1.96 (m, 6 H), 1.84−1.76 (m, 2 H), 1.68 (s, 3 H), 1.65−1.36 (m, 6 H), 1.61 (s, 3 H), 1.05 (s, 21 H), 0.86 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.6, 131.5, 127.5, 124.3, 101.1, 69.9, 67.0, 59.5, 59.5, 43.7, 37.5, 31.7, 31.3, 25.7, 25.2, 24.1, 22.3, 18.1, 18.0, 17.6, 12.4, 9.3 ppm; IR (film) 3532 cm^{-1}; HRMS (FAB) *m*/*z* 505.3683 (M + Na, 505.3691 calcd for $C_{28}H_{54}O_4SiNa$). Anal. Calcd for $C_{28}H_{54}O_4Si$: C, 69.65; H, 11.27. Found: C, 69.40; H, 11.28.

(6*S*,11*Z*,13*S*)-6-Amino-8-(1′,3′-dioxan-2′-yl)-2-methyl-13-triisopropylsiloxypentadeca-2,11-diene (59). Triphenylphosphine (2.89 g, 11.0 mmol) and hydrazoic acid (5.8 mL, 12 mmol, 2.1 M in toluene)[46] were added to a 0 °C solution of alcohol **57** (2.65 g, 5.49 mmol) and THF (55 mL). Diethyl azodicarboxylate (DEAD, 2.6 mL, 16 mmol) was then added dropwise over a period of 15 min. The resulting solution was maintained at 0 °C for 1.5 h, then approximately half of the solvent was removed in vacuo. The resulting solution was diluted with hexanes (30 mL) and filtered through a plug of silica gel using 97:3 hexanes−Et_2O. The eluent was concentrated, and the crude product was purified by flash chromatography (97:3 hexanes−Et_2O) to give 2.45 g (88%) of the corresponding azide as a clear oil: $[\alpha]^{25}_D$ +9.5, $[\alpha]^{25}_{577}$ +10.3, $[\alpha]^{25}_{546}$ +12.1, $[\alpha]^{25}_{435}$ +24.1, $[\alpha]^{25}_{405}$ +31.2 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.41−5.29 (m, 2 H), 5.10 (broad t, *J* = 7.1 Hz, 1 H), 4.47 (app q, *J* = 7.4 Hz, 1 H), 3.96−3.86 (m, 4 H), 3.71−3.66 (m, 1 H), 2.12−2.07 (m, 3 H), 2.00−1.72 (m, 6 H), 1.70 (s, 3 H), 1.64 (s, 3 H), 1.63−1.42 (m, 5 H), 1.05 (s, 21 H), 0.87 (t, *J* = 7.4 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.6, 132.4, 127.7, 123.3, 99.1, 69.9, 59.6, 59.6, 58.3, 42.2, 36.1, 32.2, 31.7, 25.7, 25.1, 24.7, 22.2, 18.1, 18.1, 17.6, 12.4, 9.4 ppm; IR (film) 2101 cm^{-1}; HRMS (FAB) *m*/*z* 506.3776 (M − H, 506.3781 calcd for $C_{28}H_{52}N_3O_3Si$). Anal. Calcd for $C_{28}H_{53}N_3O_3Si$: C, 66.22; H, 10.52. Found: C, 66.27; H, 10.50.

A solution of this azide (2.45 g, 4.82 mmol) and Et_2O (18 mL) was added dropwise to a 0 °C solution of $LiAlH_4$ (12 mL, 12 mmol, 1.0 M in Et_2O) and Et_2O (18 mL). The ice bath was removed, and the solution was allowed to warm to room temperature. After 1 h, the reaction was quenched by sequential addition of H_2O (600 μL), NaOH (600 μL, 3 N), and H_2O (1.8 mL). The resulting mixture was stirred for 1 h, $MgSO_4$ was added, the mixture was filtered through Celite, and the eluent was concentrated to afford a brown oil. Purification of this oil by flash chromatography (10:1:0.1 $CHCl_3$−MeOH−concentrated NH_4OH) gave 2.05 g (88%) of amine **59** as a light yellow oil: $[\alpha]^{25}_D$ +21.2, $[\alpha]^{25}_{577}$ +22.7, $[\alpha]^{25}_{546}$ +26.1, $[\alpha]^{25}_{435}$ +47.2, $[\alpha]^{25}_{405}$ +58.1 (*c* 1.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.39−5.29 (m, 2 H), 5.11 (br t, *J* = 7.1 Hz, 1 H), 4.46 (app q, *J* = 7.4 Hz, 1 H), 3.95−3.84 (m, 4 H), 3.15−3.11 (m, 1 H), 2.10−1.96 (m, 4 H), 1.83−1.69 (m, 4 H), 1.68 (s, 3 H), 1.63−1.31 (m, 6 H), 1.61 (s, 3 H), 1.05 (s, 21 H), 0.86 (t, *J* = 7.5 Hz, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 134.3, 131.4, 127.9, 124.1, 100.4, 69.8, 59.4, 59.2, 46.7, 43.1, 38.8, 32.7, 31.6, 25.6, 25.3, 24.6, 22.1, 18.0, 18.0, 17.6, 12.3, 9.3 ppm; IR (film) 3387, 3310 cm^{-1}; HRMS (FAB) *m*/*z* 482.4011 (M + H, 482.4029 calcd for $C_{28}H_{56}NO_3Si$). Anal. Calcd for $C_{28}H_{55}NO_3Si$: C, 69.80; H, 11.51. Found: C, 69.85; H, 11.56.

(6*S*,11*Z*,13*S*)-8-(1′,3′-Dioxan-2′-yl)-2-methyl-13-triisopropylsiloxy-6-ureidopentadeca-2,11-diene (60). Trimethylsilyl isocyanate (0.55 mL, 4.1 mmol) was added to a room temperature solution of **59** (1.61 g, 3.35 mmol), CH_2Cl_2 (6.8 mL), and *i*-PrOH (0.31 mL). After 15 h, additional *i*-PrOH (3 mL) was added and the solution was maintained for 1 h and then concentrated. The resulting oil was purified on silica gel (100% EtOAc) to provide 1.57 g (89%) of **60** as a colorless oil: $[\alpha]^{25}_D$ +7.0, $[\alpha]^{25}_{577}$ +12.0, $[\alpha]^{25}_{546}$ +17.3, $[\alpha]^{25}_{435}$ +20.7, $[\alpha]^{25}_{405}$ +25.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24−5.36 (m, 2H), 5.03−5.15 (m, 4H), 4.41 (dd, *J* = 13.2, 7.1 Hz, 1H), 3.80−3.91 (m, 4H), 3.64 (m, 1H), 1.71−2.03 (m, 8H), 1.63 (s, 3H), 1.55 (s, 3H), 1.36−1.63 (m, 6H), 1.00 (s, 21H), 0.82 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 159.3, 134.4, 131.8, 127.5, 123.7, 99.9, 69.7, 59.4, 59.2, 46.7, 36.9, 31.5, 31.1, 25.6, 25.0, 24.5, 22.1, 17.9, 17.8, 17.5, 12.2, 9.2 ppm; IR (film) 3354, 1660, 1600 cm^{-1}. Anal. Calcd for $C_{29}H_{56}N_2O_4Si$: C, 66.36; H, 10.75; N, 5.34. Found: C, 66.31; H, 10.70; N 5.41.

(4a*R*,7*S*)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6,7-hexahydro-3-[(4*S*)-*tert*-butyldimethylsiloxypentyl)]-7-[(7*S*,5*Z*)-2-(1′,3′-dioxan-2′-yl)-7-triisopropylsiloxynon-5-enyl)]-1-oxo-pyrrolo-[1,2-*c*]pyrimidine (62). Osmium tetroxide (0.75 mL, 0.1 M in *t*-BuOH) was added to a solution of **60** (524 mg, 1.00 mmol), *N*-methylmorpholine-*N*-oxide (NMO, 406 mg, 3.46 mmol), and 10:1 THF−H_2O (25 mL). After 1.5 h, Florisil (3 g), $NaHSO_3$ (3 g), and EtOAc (50 mL) were added and the reaction mixture was stirred vigorously. After 30 min, this mixture was filtered, and the filtrate was concentrated to

(45) General experimental details have been described: Metais, E.; Overman, L. E.; Rodriguez, M. I.; Stearns, B. A. *J. Org. Chem.* **1997**, *62*, 9210−9216. Additional details are presented in Supporting Information.

(46) Hydrazoic acid was generated by the procedure reported in: *Org. React.* **1946**, *3*, 327.

provide the corresponding diol, a colorless oil which was used without further purification.

A solution of this crude diol, $Pb(OAc)_4$ (532 mg, 1.20 mmol), and toluene (60 mL) was maintained at room temperature for 30 min. The reaction mixture was filtered through a plug of Celite, morpholinium acetate (300 mg, 2.0 mmol) was added, and the solution was concentrated to provide the crude aminal **61** as a slightly yellow oil.

A solution of this crude aminal, **24** (1.95 g, 3.36 mmol), and 2,2,2-trifluoroethanol (1.0 mL) was maintained at 60 °C for 2 days. The reaction was then quenched by adding Et_2O (20 mL) and 50% aqueous NH_4Cl (5 mL). The layers were separated, the organic layer was dried ($MgSO_4$), concentrated, and the resulting oil was purified on silica gel (10:1 hexanes−EtOAc; 7:1 hexanes−EtOAc; 3:1 hexanes−EtOAc) to provide 1.5 g of **24** and 638 mg (61%) of a ∼6.5:1 mixture of **62** and **63**, respectively, which was carried forward without separation.

For characterization purposes, a 50 mg sample of this mixture was separated by HPLC (7:1 hexanes−EtOAc; Altima 5 μm silica) to give a pure sample of **62**: $[\alpha]^{25}_{D}$ −4.5, $[\alpha]^{25}_{577}$ −4.9, $[\alpha]^{25}_{546}$ −5.7, $[\alpha]^{25}_{435}$ −15.5, $[\alpha]^{25}_{405}$ −22.7 (*c* 0.75, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (s, 1H), 5.87−5.95 (m, 1H), 5.21−5.37 (m, 4H), 4.56 (d, J = 5.7 Hz, 2H), 4.51 (dd, J = 12.7, 7.1 Hz, 1H), 4.22 (dd, J = 11.0, 4.6 Hz, 1H), 4.06−4.13 (m, 3H), 3.97−3.98 (m, 1H), 3.76−3.88 (m, 4H), 2.47−2.58 (m, 3H), 2.39 (d, J = 13.6 Hz, 1H), 2.32 (t, J = 7.5 Hz, 2H), 2.26−2.32 (m, 1H), 2.15 (dd, J = 13.0, 6.0 Hz, 1H), 1.99−2.03 (m, 1H), 1.50−1.90 (m, 13H), 1.41−1.48 (m, 3H), 1.11−1.40 (m, 23H), 1.10 (d, J = 6.1 Hz, 3H), 0.91−1.07 (m, 21H), 0.82−0.91 (m, 12H), 0.03 (s, 3H), 0.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 166.0, 151.9, 151.2, 134.3, 132.2, 128.2, 118.0, 102.1, 99.2, 69.9, 68.3, 64.9, 64.2, 59.3, 57.7, 52.7, 39.0, 37.4, 34.5, 34.2, 31.8, 31.3, 30.4, 29.6, 29.57, 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, 26.1, 25.9, 25.3, 24.9, 24.4, 23.6, 21.8, 18.1, 12.3, 9.3, −4.4, −4.7 ppm;[47] IR (film) 3211, 1741, 1682, 1627 cm^{-1}. Anal. Calcd for $C_{59}H_{108}N_2O_9Si_2$: C, 67.77; H, 10.41; N, 2.68. Found: C, 67.68; H, 10.27; N 2.65.

(3*R*,4*R*,4a*R*,6′*R*,7*S*)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6,7-hexahydro-1-oxo-7-[(7*S*,5*Z*)-7-hydroxy-2-oxo-5-nonenyl]-pyrrolo[1,2-*c*]pyrimidine-3-spiro-6′-(2′-methyl)-3′,4′,5′,6′-tetrahydro-2H-pyran (64). A solution of the **62**/**63** mixture (1.30 g, 1.24 mmol), TBAF (6.2 mL, 1.0 M solution in Et_2O), and DMF (31 mL) was maintained at room temperature for 5 h, the solution was diluted with Et_2O (150 mL), and washed with H_2O (50 mL) and brine (2 × 50 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated to give a residue that was used without further purification.

A solution of this crude diol, $TsOH\cdot H_2O$ (236 mg, 1.24 mmol), and $CHCl_3$ (180 mL) was maintained at 60 °C for 15 min. The reaction was quenched by adding saturated aqueous $NaHCO_3$ (20 mL), the layers were separated, and the organic layer was washed with brine (20 mL). The organic layer was dried ($MgSO_4$), concentrated, and the resulting oil was purified on silica gel (1:3 hexanes−EtOAc; 100% EtOAc) to provide 630 mg (71%) of **64** as a ∼6.5:1 mixture of epimers. This mixture of stereoisomers was not separated, but directly used in the next step.

A pure sample of **64** was obtained by HPLC (7:1 hexanes−EtOAc; Altima 5 μm silica): $[\alpha]^{25}_{D}$ +42.2, $[\alpha]^{25}_{577}$ +42.7, $[\alpha]^{25}_{546}$ +49.8, $[\alpha]^{25}_{435}$ +91.0, $[\alpha]^{25}_{405}$ +114 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.87−5.95 (m, 1H), 5.56 (s, 1H), 5.34−5.43 (m, 2H), 5.31 (dd, J = 17.2, 1.5 Hz, 1H), 5.22 (dd, J = 10.6, 1.3 Hz, 1H), 4.57 (dd, J = 4.3, 1.3 Hz, 2H), 4.38 (dd, J = 14.5, 6.8 Hz, 1H), 4.29−4.31 (m, 1H), 4.08−4.18 (m, 2H), 4.02 (dt, J = 11.1, 4.8 Hz, 1H), 3.77−3.80 (m, 1H), 3.37 (d, J = 16.8 Hz, 1H), 2.52−2.60 (m, 2H), 2.43−2.50 (m, 1H), 2.32 (t, J = 7.5 Hz, 2H), 2.22−2.27 (m, 2H), 2.04−2.20 (m, 4H), 1.69−1.76 (m, 4H), 1.56−1.65 (m, 7H), 1.42−1.48 (m, 3H), 1.24−1.28 (m, 21H), 1.06−1.09 (m, 1H), 1.05 (d, J = 6.0 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 209.0, 173.5, 168.9, 153.0, 134.1, 132.3, 129.8, 118.1, 82.2, 68.4, 66.2, 65.1, 64.9, 55.0, 54.0, 53.2, 46.2, 42.7, 34.3, 32.2, 32.1, 30.3, 30.0, 29.6, 29.57, 29.5, 29.4, 29.3, 29.2, 29.1, 28.7, 26.0, 24.9, 22.0, 21.7, 18.8 ppm;[47] IR (film) 3450, 3231, 3081, 2927, 2855, 1732, 1715, 1659, 1651 cm^{-1}. Anal. Calcd for $C_{41}H_{68}N_2O_8$: C, 68.68; H, 9.56; N, 3.91. Found: C, 68.71; H, 9.51; N 3.84.

(3*R*,4*R*,4a*R*,6′*R*,7*S*)-4-[15-(Allyloxycarbonyl)pentadecyloxycarbonyl]-1,2,4a,5,6,7-hexahydro-1-oxo-7-[(7*S*,5*Z*)-7-chloroacetoxy-2-oxo-5-nonenyl]-pyrrolo[1,2-*c*]pyrimidine-3-spiro-6′-(2′-methyl)-3′,4′,5′,6′-tetrahydro-2H-pyran (65). Chloroacetyl chloride (0.34 mL, 0.46 mmol) was added dropwise to a 0 °C solution of **64** (0.63 g, 0.88 mmol, containing ∼12% of the C4a *S* epimer), pyridine (1.4 mL, 18 mmol), and CH_2Cl_2 (50 mL). The solution was allowed to warm to room temperature, and after 1 h, was quenched by adding Et_2O (200 mL). This solution was washed with 1 N NaOH (25 mL), $CuSO_4$ (2 × 25 mL), and brine (25 mL), dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (2:1 hexanes−EtOAc; 1:1 hexanes−EtOAc; 1:2 hexanes−EtOAc) to yield 600 mg (86%) of isomerically pure **65** as a colorless oil, and ∼85 mg (∼12%) of the C4a *S* isomer that was derived from **61**. **65**: $[\alpha]^{25}_{D}$ +42.7, $[\alpha]^{25}_{577}$ +47.0, $[\alpha]^{25}_{546}$ +52.6, $[\alpha]^{25}_{435}$ +96.1, $[\alpha]^{25}_{405}$ +120, (*c* 1.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.34 (s, 1H), 5.87−5.94 (m, 1H), 5.48−5.56 (m, 2H), 5.27−5.32 (m, 2H), 5.22 (d, J = 10.4 Hz, 1H), 4.56 (d, J = 5.7 Hz, 2H), 4.31−4.33 (m, 1H), 4.09−4.19 (m, 2H), 4.03 (s, 2H), 4.00−4.06 (m, 1H), 3.77−3.81 (m, 1H), 3.34 (d, J = 16.6 Hz, 1H), 2.40−2.48 (m, 3H), 2.25−2.38 (m, 5H), 2.05−2.17 (m, 3H), 1.69−1.74 (m, 4H), 1.55−1.62 (m, 7H), 1.42−1.50 (m, 1H), 1.24−1.31 (m, 22H), 1.06−1.15 (m, 1H), 1.05 (d, J = 6.0 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 207.9, 173.4, 168.8, 166.6, 153.4, 133.0, 132.3, 127.8, 118.0, 82.1, 73.7, 66.1, 64.9, 64.8, 54.9, 53.9, 53,1, 46.3, 42.3, 41.1, 34.2, 32.2, 32.0, 29.5, 29.49, 29,4, 29.3, 29.2, 29.1, 29.07, 29.0, 28.6, 27.4, 25.9, 21.8, 21.6, 18.5, 9.3 ppm;[47] IR (film) 3296, 2928, 2855, 1732, 1652 cm^{-1}. Anal. Calcd for $C_{43}H_{69}N_2O_9Cl$: C, 65.09; H, 8.77; N, 3.53. Found: C, 65.16; H, 8.79; N 3.57.

Pentacycles 67 and 68. A solution of **65** (327 mg, 0.412 mmol), MeOTf (1.29 mL, 8.21 mmol), 2,6-di-*tert*-butylpyridine (0.46 mL, 2.1 mmol), and CH_2Cl_2 (20 mL) was maintained at room temperature for 8 h. The solution was then poured into Et_2O (100 mL) and washed with 1 N NaOH (2 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate concentrated. The resulting crude pseudourea **66** was used without further purification.

Ammonia was bubbled through a solution of this sample of **66**, NH_4Cl (50 mg, 0.93 mmol), and allyl alcohol (5 mL) at room temperature until the solution was saturated (∼20 min). The reaction vessel was sealed and heated to 60 ° C for 28 h. The reaction was then cooled to room temperature, concentrated, and the resulting oil was purified by silica gel medium-pressure liquid chromatography (MPLC) (100:0.6 $CHCl_3$-*i*-PrOH) to provide 147 mg of **67** and 98 mg of **68**. Resubjecting **67** to these reaction conditions (2×), followed by chromatographic separation provided an additional 60 mg of **68** (52% combined yield of **68**).

67: $[\alpha]^{25}_{D}$ +12.2, $[\alpha]^{25}_{577}$ +13.1, $[\alpha]^{25}_{546}$ +14.1 (*c* 2.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.68 (s, 1H), 8.56 (s, 1H), 5.88−5.95 (m, 1H), 5.64−5.67 (m, 1H), 5.48 (d, J = 10.9 Hz, 1H), 5.33 (dd, J = 17.2, 1.5 Hz, 1H), 5.25 (dd, J = 10.4, 1.2 Hz, 1H), 4.57 (d, J = 5.7 Hz, 2H), 4.48 (d, J = 10.3 Hz, 1H), 4.32−4.38 (m, 1H), 4.10−4.24 (m, 3H), 3.78−3.81 (m, 1H), 2.56−2.61 (m, 2H), 2.45 (d, J = 11.6 Hz, 1H), 2.32 (t, J = 7.6 Hz, 2H), 2.26−2.36 (m, 3H), 2.15−2.18 (m, 2H), 2.00 (dt, J = 13.8, 4.7 Hz, 1H), 1.87 (dd, J = 14.6, 5.4 Hz, 1H), 1.61−1.78 (m, 10H), 1.53−1.58 (m, 1H), 1.42−1.49 (m, 1H), 1.23−1.35 (m, 22H), 1.05−1.15 (m, 1H), 1.05 (d, J = 6.1 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 167.6, 147.3, 133.4, 132.3, 129.7, 118.0, 83.9, 81.7, 70.9, 67.6, 65.7, 64.9, 53.4, 53.3, 36.8, 36.2, 34.2, 31.8, 30.6, 29.7, 29.6, 29.5, 29.46, 29.4, 29.2, 29.1, 29.08, 29.0, 28.5, 25.9, 24.9, 23.6, 21.3, 17.9, 10.1 ppm;[47] IR (film) 3268, 1732, 1660, 1608 cm^{-1}; HRMS (FAB) *m*/*z* 698.5096 (M − Cl, 698.5108 calcd for $C_{41}H_{68}N_3O_6$).

68: $[\alpha]^{25}_{D}$ −9.6, $[\alpha]^{25}_{577}$ −10.5, $[\alpha]^{25}_{546}$ −9.5, $[\alpha]^{25}_{435}$ −16.5, $[\alpha]^{25}_{405}$ −17.2 (*c* 0.75, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.43 (s, 1H), 5.88−5.95 (m, 1H), 5.64−5.67 (m, 1H), 5.48 (d, J = 10.9 Hz, 1H), 5.31 (dd, J = 17.2, 1.5 Hz, 1H), 5.22 (dd, J = 10.4, 1.2 Hz, 1H), 4.57 (dd, J = 5.7, 1.2 Hz, 2H), 4.48 (d, J = 9.7 Hz, 1H), 4.29−4.33 (m, 1H), 4.08 (t, J = 6.8 Hz, 2H), 3.99−4.05 (m, 1H), 3.84−3.87 (m, 1H), 2.93 (d, J = 4.8 Hz, 1H), 2.55−2.63 (m, 2H), 2.32 (t, J

(47) ^{13}C NMR signals of many of the methylene carbons of the hexadecanoate side chain overlap.

= 7.6 Hz, 2H), 2.26−2.36 (m, 2H), 2.13−2.24 (m, 3H), 1.98 (dd, *J* = 14.7, 5.3 Hz, 1H), 1.78−1.84 (m, 1H), 1.51−1.76 (m, 10H), 1.38−1.48 (m, 2H), 1.21−1.30 (m, 22H), 1.07−1.20 (m, 1H), 1.05 (d, *J* = 6.1 Hz, 3H), 0.81 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) 173.5, 168.3, 148.4, 133.5, 132.3, 129.7, 118.0, 84.0, 80.8, 70.9, 67.2, 65.5, 64.9, 54.1, 52.2, 49.9, 36.7, 36.1, 34.4, 31.8, 31.7, 30.5, 29.6, 29.56, 29.5, 29.45, 29.4, 29.2, 29.1, 29.06, 28.4, 26.7, 25.8, 24.9, 23.6, 21.5, 17.7, 10.0 ppm;[47] IR (film) 3263, 1732, 1652, 1614, cm^{-1}; HRMS (FAB) *m*/*z* 698.5106 (M − Cl, 698.5108 calcd for $C_{41}H_{68}N_3O_6$).

Carboxylic Acid Hydrochloride 69 and Crambescidin 657 (6): A solution of **68** (27 mg, 37 μmol), $Pd(PPh_3)_4$ (21 mg, 18 μmol), morpholine (13 μL, 0.15 mmol), and MeCN (1.0 mL) was maintained at room temperature for 5 h. The solution was diluted with Et_2O (30 mL), and washed with 0.1 N HCl (2 × 5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (100:1 $CHCl_3$−MeOH; 33:1 $CHCl_3$−MeOH) to yield 24 mg (94%) of **69** as a colorless oil: $[\alpha]^{25}_D$ 15.2 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.63−5.66 (m, 1H), 5.46−5.49 (m, 1H), 4.48 (br d, *J* = 10.2 Hz, 1H), 4.27−4.31 (m, 1H), 4.04−4.12 (m, 2H), 3.96−4.03 (m, 1H), 3.85−3.88 (m, 1H), 2.92 (d, *J* = 4.9 Hz, 1H), 2.62 (t, *J* = 13.8 Hz, 1H), 2.55 (dd, *J* = 12.7, 4.7 Hz, 1H), 2.12−2.32 (m, 7H), 1.86 (dd, *J* = 14.8, 5.3 Hz, 1H), 1.77−1.81 (m, 1H), 1.60−1.73 (m, 9H), 1.51−1.59 (m, 1H), 1.37−1.45 (m, 2H), 1.20−1.30 (m, 22H), 1.16−1.20 (m, 1H), 1.04 (d, *J* = 6.1 Hz, 3H), 0.80 (t, *J* = 7.2 Hz, 3H), the NH and OH signals were not observed; ^{13}C NMR (125 MHz, $CDCl_3$) 179.1, 168.4, 148.7, 133.6, 129.8, 83.9, 80.8, 70.8, 67.0, 65.4, 54.0, 52.0, 50.0, 36.7, 36.0, 31.9, 31.8, 30.5, 29.4, 29.3, 29.2, 29.1, 29.0, 28.4, 26.7, 25.8, 25.5, 23.7, 21.5, 17.8, 10.0 ppm;[47] IR (film) 3261, 3138, 2919, 2849, 1728, 1658 cm^{-1}; HRMS (FAB) *m*/*z* 658.4791 (M − Cl, 658.4795 calcd for $C_{38}H_{64}N_3O_6$).

Carboxylic acid **69** was quantitatively converted to the carboxylate inner salt by washing a $CHCl_3$ (5 mL) solution of the acid (5 mg) with 1 N NaOH (1 mL) and brine (1 mL). The organic layer was dried (Na_2SO_4) and then concentrated to provide **6** as a colorless oil: $[\alpha]^{25}_D$ −13.6 (*c* 0.45, MeOH). Spectroscopic and mass spectrometric data for this sample were consistent with data published for natural **6**.[6a]

41,45-di-(*tert*-Butoxycarbonyl)crambescidin 800 (71). Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (22 mg, 50 μmol) was added to a room temperature solution of carboxylic acid **69** (23 mg, 33 μmol), amine **70** (18 mg, 50 μmol),[40] Et_3N (0.15 mL, 1.1 mmol), and CH_2Cl_2 (5 mL). After 4 h, the reaction was diluted with Et_2O (20 mL), and washed with saturated aqueous NH_4Cl (5 mL) and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (50:1 $CHCl_3$−MeOH) to yield 28 mg (82%) of **71** as a colorless oil: $[\alpha]^{25}_D$ −3.0, $[\alpha]^{25}_{577}$ −2.2, $[\alpha]^{25}_{546}$ −2.8, $[\alpha]^{25}_{435}$ −3.5, $[\alpha]^{25}_{405}$ −3.6, (*c* 0.75, $CHCl_3$); ^{1}H NMR (500 MHz, d^4-MeOD) δ 5.70−5.73 (m, 1H), 5.47−5.52 (m, 1H), 4.40 (br d, *J* = 10.3 Hz, 1H), 4.33−4.37 (m, 1H), 4.10−4.16 (m, 2H), 4.02−4.09 (m, 1H), 3.75−3.85 (m, 2H), 3.34−3.59 (m, 2H), 3.23−3.29 (m, 2H), 3.12−3.20 (m, 2H), 3.07 (d, *J* = 4.8 Hz, 1H), 2.94−3.06 (m, 2H), 2.64 (dd, *J* = 13.0, 4.7 Hz, 1H), 2.26−2.46 (m, 6H), 2.10−2.20 (m, 1H), 2.00 (dd, *J* = 13.9, 5.8 Hz, 1H), 1.79−1.85 (m, 3H), 1.50−1.77 (m, 11H), 1.36−1.47 (m, 20H), 1.22−1.35 (m, 25H), 1.09 (d, *J* = 6.1 Hz, 3H), 0.85 (t, *J* = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, d^4-MeOD) 176.6/176.2, 170.2, 158.5, 150.2, 134.3, 131.3, 85.1, 82.2, 80.0, 79.96, 72.3, 69.1, 68.4, 68.37, 66.5, 55.6, 55.0, 54.2, 53.5, 50.7, 45.1, 38.9, 38.7, 38.3, 38.1, 37.9, 36.2, 34.3, 34.1, 33.0, 32.6, 31.5, 30.8, 30.7, 30.6, 30.5, 30.3, 30.2, 29.6, 28.8, 28.7, 27.6, 27.0, 26.7, 26.6, 24.4, 21.8, 19.5, 10.8 ppm;[47] IR (film) 3356, 1732, 1706, 1657, 1613 cm^{-1}; HRMS (FAB) *m*/*z* 1001.7 (M − Cl, 1001.7 calcd for $C_{55}H_{97}N_6O_{10}$).

Crambescidin 800 Trihydrochloride (2). A solution of **71** (13 mg, 13 μmol) and 1.3 mL of a 3.0 M solution of HCl in EtOAc was maintained at room temperature for 20 min and then concentrated. Purification of the residue by reversed-phase HPLC (4:1 MeOH−0.1 M NaCl, Altima C18, 5 μm column) gave 11 mg (75%) of crambescidin 800 (**2**) as its trihydrochloride salt (a light yellow oil): $[\alpha]^{25}_D$ −4.4, $[\alpha]^{25}_{577}$ −5.0, $[\alpha]^{25}_{546}$ −4.0, $[\alpha]^{25}_{435}$ −6.3, $[\alpha]^{25}_{405}$ −6.2, (*c* 0.7, $CHCl_3$). Spectroscopic and mass spectrometric data for this sample were consistent with data published for natural **2**.[4a,5a,43]

Peracetylcrambescidin 800 Chloride (72). A solution of crambescidin 800 (**2**) (5.0 mg, 5.5 μg), Ac_2O (0.5 mL), and pyridine (1 mL) was maintained at room temperature for 23 h and then concentrated (0.9 mm, 23 °C).[5a] The residue was diluted with $CHCl_3$ (20 mL) and washed with 0.1 M HCl (5 mL), and brine (5 mL). The organic layer was dried ($MgSO_4$), filtered, and the filtrate was concentrated. The resulting residue was purified on silica gel (20:1 $CHCl_3$−MeOH; 10:1 $CHCl_3$−MeOH) to yield 2 mg (35%) of peracetylcrambescidin 800 (**72**) as a colorless wax: $[\alpha]^{25}_D$ −37 (*c* 0.2, $CHCl_3$). NMR and mass spectrometric data for this sample were consistent with published data.[5a]

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Supporting Information Available: Characterization data and detailed experimental procedures for the preparation of ptilomycalin A (**1**), neofolitispates 2 (**7**), **22**−**33**, **38**−**41**, **43**−**45** and **48**−**55**. ^{1}H and ^{13}C NMR spectra for **1**, **2**, **6**, **7**, **45**, and **72**; ^{19}F NMR spectra for synthetic **73** and natural **73**. This material is available free of charge via the Internet at http://pubs.acs.org.

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