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Total Synthesis and Properties of the Crambescidin Core Zwitterionic Acid and Crambescidin 359

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Abstract: The total synthesis of the crambescidin core acid **9**, crambescidins 359 (**8**) and 431 (**7**), and the properties of the crambescidin core are described. A key step of the synthetic route to guanidinium carboxylate **9** is Pd(0) catalyzed cleavage of the ester side chain of pentacyclic cinnamyl ester **15**. This ester is also employed to prepare a small library of crambescidin alkaloid analogues that differ in their C14 side chain. The zwitterionic guanidinium carboxylate **9** was shown to readily decarboxylate to form crambescidin 359 (**8**). Decarboxylation of crambescidin core acid **9** was fastest under basic conditions. In the presence of base, up to eight deuterium atoms can be incorporated into the pentacyclic crambescidin core. Both deuterium incorporation and decarboxylation of crambescidin core acid **9** are the result of facile ring opening of the spirocyclic ether rings of the pentacyclic guanidinium moiety.

Introduction

The crambescidin family of guanidinium alkaloids, isolated from a variety of marine sponges, exemplifies the valuable compounds that have been discovered through recent investigations of marine natural products.[1] The first member of the crambescidin family discovered, ptilomycalin A (**1**), was initially isolated by Kashman, Kakisawa and co-workers from the Red Sea sponge *Hemimycale sp.* and a Caribbean sponge originally identified as *Ptilocaulis spiculifer*.[2,3] Ptilomycalin A (**1**) and many cognate crambescidin alkaloids display nanomolar cytotoxicities against several tumor cell lines.[4−6] Antifungal activity against *Candida albicans*, antiviral activity against herpes simplex virus (HSV),[2,7,8] anti-HIV activity,[9] inhibition of HIV-1 envelope mediated cell fusion,[10] and inhibition of the binding of various proteins to HIV-1 Nef[11] are possibly useful biological activities reported also for members of this family of guanidine alkaloids. The potential for these compounds as therapeutic agents has been recognized, as they are currently undergoing early phase development as anti-cancer agents.[12]

Members of the crambescidin alkaloid family are characterized structurally by a unique pentacyclic guanidinium core (Figure 1). Crambescidins that vary in their substitution at C14 are exemplified by crambescidins 800(**2a**),[4,13] 359 (**8**),[14] 431 (**7**),[14] 657 (**10a**),[5] neofolitispates 1−3 (**11**−**13**),[15] crambescidin core acid **9**,[16] and celeromycalin (**3**).[9] Additional members of this family contain a hydroxyl group at C13: crambescidins 816 (**4**), 830 (**5**), and 844 (**6**).[4] A small group, the 13,14,15-isocrambescidins (not shown), have a different configuration of the pentacyclic guanidinium moiety.[8,17]

Despite the broad range of crambescidin alkaloid structures, there is limited understanding of structure−activity relationships of these natural products. The presence of a side chain is well-established to be important for anticancer activity.[12,18] However, simple spermidine amides of ω-hydroxycarboxylic acids lacking a guanidine core are inactive.[7,9] There is little understanding of how the nature of the C14 side chain impacts biological activity. One reason for the lack of systematic studies in this area has been the inability to remove the C14 side chain from the more available of these marine alkaloids without destroying the

(1) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2004**, *67*, 1216−1238.
(2) Kashman, Y.; Hirsh, S.; McConnell, O. J.; Ohtani, I.; Kusumi, T.; Kakisawa, H. *J. Am. Chem. Soc.* **1989**, *111*, 8925−8926.
(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*, see: Tavares, R.; Daloze, D.; Braekman, J. C.; Hajdu, E.; Van Soest, R. W. M. *J. Nat. Prod.* **1995**, *58*, 1139−1142.
(4) Jares-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712−5715.
(5) Rinehart, K. L.; Jares-Erijman, E. A. U. S. Patent 5,756,734; 1998.
(6) Ptilomycalin A recently was shown to induce differentiation of K562 cells with arrest of the cell cycle at the S-phase, see: Aoki, S.; Kong, D.; Matsui, K.; Kobayashi, M. *Anticancer Res.* **2004**, *24*, 2325−2330.
(7) Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1992**, *114*, 8472−8479.
(8) Jareserijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805−4808.
(9) Palagiano, E.; Demarino, S.; Minale, L.; Riccio, R.; Zollo, F.; Iorizzi, M.; Carre, J. B.; Debitus, C.; Lucarain, L.; Provost, J. *Tetrahedron* **1995**, *51*, 3675−3682.
(10) Chang, L.-C.; Whittaker, N. F.; Bewley, C. A. *J. Nat. Prod.* **2003**, *66*, 1490−1494.
(11) Olszewski, A.; Sata, K.; Aron, Z. D.; Cohen, F.; Harris, A.; McDougall, B. R.; Robinson, W. E. Jr.; Overman, L. E.; Weiss, G. A. *Proc. Natl. Acad. Sci.* USA **2004**, *101*, 14079−14084.
(12) Aron, Z. D.; Pietraszkiewicz, H.; Overman, L. E.; Valeriote, F.; Cuevas, C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3445−3449.
(13) 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.
(14) Braekman, J. C.; Daloze, D.; Tavares, R.; Hajdu, E.; Van Soest, R. W. M. *J. Nat. Prod.* **2000**, *63*, 193−195.
(15) Venkateswarlu, Y.; Reddy, M. V. R.; Ramesh, P.; Rao, *J. V. Indian J. Chem. Sect. B* **1999**, *38*, 254−256.
(16) Meragelman, K. M.; McKee, T. C.; McMahon, J. B. *J. Nat. Prod.* **2004**, *67*, 1165−1167.
(17) Shi, J.-G.; Sun, F.; Rinehart, K. L. Crambescidin Compounds, WO Patent 98/46575; 1998.

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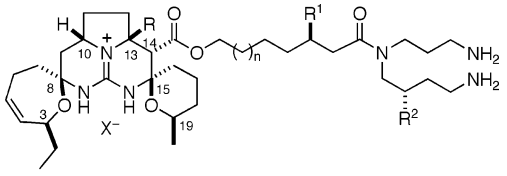
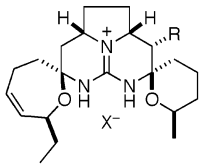


ptilomycalin A (**1**, R = R^1 = R^2 = H, n = 10)
crambescidin 800 (**2a**, R = R^1 = H, R^2 = OH, n = 10)
celeromycalin (**3**, R = H, R^1 = OH, R^2 = H, n = 10)
crambescidin 816 (**4**, R = OH, R^1 = H, R^2 = OH, n = 10)
crambescidin 830 (**5**, R = OH, R^1 = R^2 = H, n = 11)
crambescidin 844 (**6**, R = OH, R^1 = R^2 = H, n = 12)



crambescidin 431 (**7**, R = CO_2Et)
crambescidin 359 (**8**, R = H)
crambescidin core acid (**9**, R = CO_2^-)
crambescidin 657 (**10a**, R = $CO_2(CH_2)_{15}CO_2H$)
neofolitispate 1 (**11**, R = $CO_2(CH_2)_{14}CO_2Me$)
neofolitispate 2 (**12**, R = $CO_2(CH_2)_{15}CO_2Me$)
neofolitispate 3 (**13**, R = $CO_2(CH_2)_{16}CO_2Me$)

Figure 1. Crambescidin natural products.

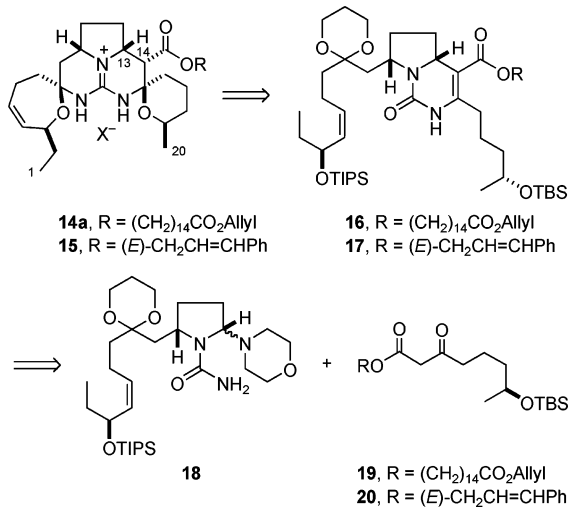
pentacyclic guanidine moiety. For example, Kashman and co-workers reported that subjection of ptilomycalin A (**1**) to acid or base hydrolysis, metal hydride reduction, catalytic hydrogenation or oxidation, resulted in the formation of intractable mixtures.[7,19,20] The inability to access core acid **9** by degradation, and its reported isolation in low yield from natural sources only earlier this year,[16] combine to make chemical synthesis the only viable way to obtain unnatural C14 side chain congeners of the crambescidin alkaloids.

This paper describes the total synthesis, isolation,[21] structural characterization, and derivitization of the crambescidin core acid **9**. Besides providing the first complete description of the physical and chemical properties of this important species, these studies resulted in the synthesis of crambescidins 359 (**8**) and 431 (**7**), and libraries of crambescidin analogues that vary at C14. Evidence concerning the likely biosynthetic origin of crambescidin 359, and a proposal of one potential mode of action of the crambescidin alkaloids is also provided.

Results

A. Synthetic Strategy. Several syntheses of members and congeners of the crambescidin family of natural products have been reported.[18a,22−27] Of these, only our efforts and those of Snider and co-workers resulted in preparation of the full crambescidin core having a pendant C14 carboxylic ester side chain.[18a,24−26]

Scheme 1. Tethered Bignelli Approach to Crambescidin Alkaloids



14a, R = $(CH_2)_{14}CO_2$Allyl
15, R = (*E*)-CH_2CH=CHPh
16, R = $(CH_2)_{14}CO_2$Allyl
17, R = (*E*)-CH_2CH=CHPh
18
19, R = $(CH_2)_{14}CO_2$Allyl
20, R = (*E*)-CH_2CH=CHPh

Our strategy for the total synthesis of crambescidin alkaloids such as crambescidin 657 (**10a**) employs a tethered Biginelli condensation[28] to combine two fragments: one, **18**, encoding C1−C13 and the urea precursor of the guanidine, and the other, **19**, comprising C14−C20 and the C14 side chain (Scheme 1).[24−27] To access the crambescidin core acid **9**, the elaborate side chain would be replaced with one that could be cleaved under mild conditions to reveal a carboxylic acid substituent at C14. In our earliest investigations,[27,29] we found that an allyl ester could be cleaved efficiently in the presence of a fully constituted pentacyclic guanidine moiety.[30] This strategy was employed in the present investigation. However, rather than using a simple allyl group, we chose to employ a *trans*-cinnamyl ester (**20** → **17** → **15**), because this substituent would both decrease the polarity of late stage intermediates and provide a convenient chromophore.

B. Synthesis of the Crambescidin Core Cinnamyl Ester 15. The starting point for this synthesis was the known enantiopure methyl ester **21**, which is available in five steps and 58% overall yield from commercially available poly-(3-hydroxybutyric) acid.[24,27,29] Initial attempts to convert methyl β-ketooctanoate **21** to its cinnamyl congener **20** by reaction with cinnamyl alcohol in refluxing toluene using 4-(dimethylamino)-pyridine (DMAP) as catalyst[31] were complicated by competitive

(18) (a) Snider, B. B.; Shi, Z. P. *J. Am. Chem. Soc.* **1994**, *116*, 549−557. (b) Black, G. P.; Coles, S. J.; Hizi, A.; Howard-Jones, A. G.; Hursthouse, M. B.; McGown, A. T.; Loya, S.; Moore, C. G.; Murphy, P. J.; Smith, N. K.; Walshe, N. D. A. *Tetrahedron Lett.* **2001**, *42*, 3377−3381. (c) Moore, C. G.; Murphy, P., J.; Williams, Harri L.; McGown, Alan T.; Smith, Nigel K. *Tetrahedron Lett.* **2003**, *44*, 251−254. (d) Georgieva, A.; Hirai, M.; Hashimoto, Y.; Nakata, T.; Ohizumi, Y.; Nagasawa, K. *Synthesis-Stuttgart*, **2003**, 1427−1432.

(19) A patent application claims that the core acid is obtained upon reacting ptilomycalin A with an unspecified mixture of NaOH, MeOH and water at 65 °C.[20] However, no characterization data to support this assertion was provided.

(20) Mai, S. H.; Nagulapalli, V. K.; Patil, A. D.; Truneh, A.; Westly, J. W. Marine Compounds as HIV Inhibitors, WO Patent 93/01193; 1993.

(21) An early version of the synthesis described herein was described in Overman, L. E.; Stappenbeck, F.; McDonald, A. I. Hexahydropyrrolo[1,2-*c*]pyrimidines as Antiviral and/or Antitumor Agents WO Patent 01/00626, January, 2001.

(22) Heys, L.; Moore, C. G.; Murphy, P. J. *Chem. Soc. Rev.* **2000**, *29*, 57−67.

(23) Nagasawa, K.; Georgieva, A.; Koshino, H.; Nakata, T.; Kita, T.; Hashimoto, Y. *Org. Lett.* **2002**, *4*, 177−180.

(24) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **2000**, *122*, 4893−4903.

(25) Coffey, D. S.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **2000**, *122*, 4904−4914.

(26) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc.* **1999**, *121*, 6944−6945.

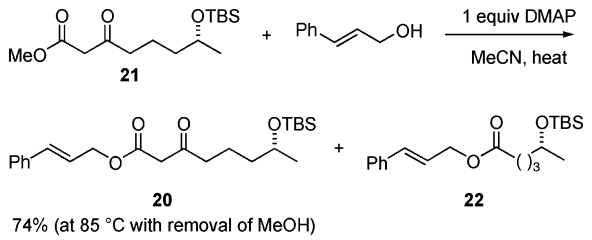
(27) Overman, L. E.; Rabinowitz, M. H.; Renhowe, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 2657−2658.

(28) For a recent review, see: Aron, Z. D.; Overman, L. E. *Chem. Commun.* **2004**, 253−265.

(29) Renhowe, P. A. Ph. D, Dissertation, University of California, Irvine. 1995.

(30) (a) Jeffery, P. D.; McCombie, S., W. *J. Org. Chem.* **1982**, *47*, 587−590. For a review of allylic protecting groups, see: (b) Guibe, F. *Tetrahedron* **1998**, *54*, 2967−3042.

Scheme 2. Preparation of Cinnamyl β-ketoester **20**

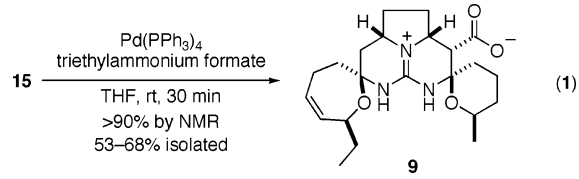


retro-Claisen fragmentation to form ultimately cinnamyl hexanoate **22** (Scheme 2). Lowering the reaction temperature to 85 °C, necessary to avoid the retro-Claisen reaction, resulted in incomplete conversion of **21** to **20**. However, the use of a large excess of cinnamyl alcohol (7 equiv) and azeotropic removal of methanol with acetonitrile resulted in complete consumption of **21**, providing cinnamyl β-ketoester **20** in 74% yield.

Using experimental procedures that had been optimized during our earlier syntheses of crambescidin 800 (**2a**) and congeners,[24,27] the cinnamyl ester derivative **15** of the crambescidin core acid was prepared by the sequence depicted in Scheme 3. Dienyl urea **23**, available in 14 steps from 3-butynol,[24,27] first was converted in two steps to aminal **18**. Tethered Biginelli condensation of this intermediate with β-ketoester **20** provided a 1:6 mixture of pyrrolopyrimidines **24** and **17** in 63% overall yield from urea **23**. Epimers **24** and **17** could be separated by HPLC, but were generally carried forward as a mixture. The configurational assignments of these intermediates were based on nOe studies of later intermediates and ^{1}H NMR correlations of the H13 methine hydrogens (**24**: 4.40 ppm, **17**: 4.26 ppm) with reference compounds.[32] Cleavage of the siloxy protecting groups of **17** with tetrabutylammonium flouride (TBAF) in DMF generated the corresponding diol, which underwent acid-promoted spirocyclization in the presence of 1 equiv of *p*-toluenesulfonic acid at room temperature to form tricyclic urea **25**[33] in 67% overall yield. Limiting the reaction temperature was key to suppressing unproductive side reactions during the spirocyclization step.

The cinnamyl ester **15** of the crambescidin core was prepared in three additional steps from tricyclic intermediate **25**. First the alcohol group of **25** was protected as an α-chloroacetate to avoid methyl ether formation in the subsequent step. At this point, the minor trans tricyclic isomer derived from **24** was easily removed by silica gel chromatography to provide isomerically pure **26** in 74% yield. Exposure of keto urea **26** to excess MeOTf in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) yielded methyl pseudourea **27**. Rigorous exclusion of water was necessary to achieve reproducibly high yields in this step. Finally exposure of crude pseudourea **27** to anhydrous ammonia (18 psi) at 60 °C in allyl alcohol buffered with NH_4Cl for 1.5 days provided a 1.1:1.0 mixture of pentacyclic esters **28** and **15** in 78% yield. This mixture could be separated on silica gel.[34] Our previous studies had shown that the C14 stereocenter would have been equilibrated under these aminolysis conditions.[24,27] Thus, resubjection of **28**, followed by separation of the resulting epimers on silica gel, when repeated two times, provided isomerically pure **15** in 53% overall yield from tricyclic urea **26**.

C. Synthesis, Isolation and Single-Crystal X-ray Analysis of Crambescidin Core Acid 9. A variety of palladium catalysts and reaction conditions[30] were examined for the deprotection of cinnamyl ester **15** to form core acid **9**. (Tetrakistriphenylphosphine)palladium (0.3 equiv) emerged as the preferred catalyst and triethylammonium formate as the reducing agent of choice (eq 1). When deprotection of **15** was carried out using these reagents at room temperature in THF, guanidinium carboxylate **9** was formed in high yield (>90% yield by ^{1}H NMR analysis using an internal standard).



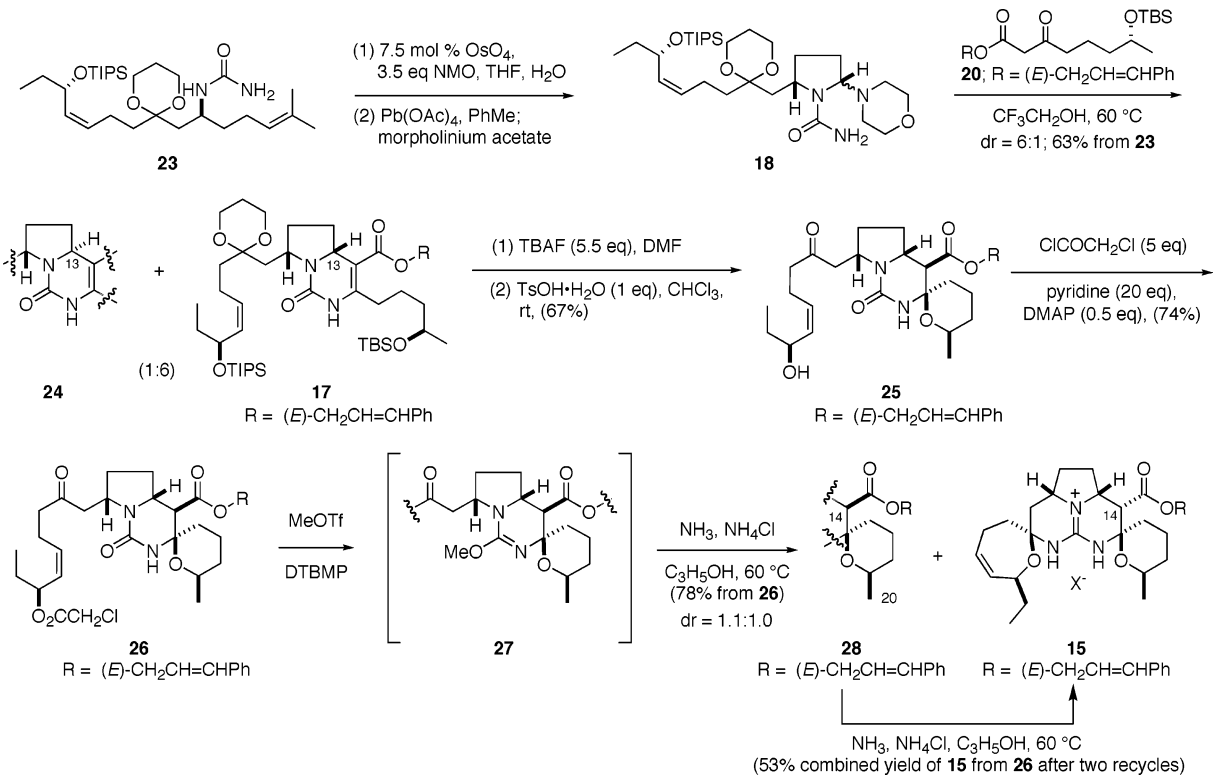
Purification of core acid **9** was challenging as this zwitterion is a polar, highly crystalline solid, which is sparingly soluble in most solvents. When used as an impure oil, acid **9** is slightly soluble (~0.05−0.1 M) in solvents such as benzene, methanol or dichloromethane. However, pure crystalline **9** is soluble only in mixtures of alcoholic and halogenated solvents. In addition to demonstrating poor solubility, acid **9** decarboxylates under a variety of conditions (vide infra). The low solubility and high polarity of **9** effectively prohibited its isolation in pure form by either normal or reverse phase chromatography. However, some purification could be obtained by filtration through Davisil silica gel (20−40% methanol/chloroform) to provide a 60% recovery of a colorless oil that was ~80% pure. Fortunately, core acid **9** precipitates from deallylation reactions when run at high concentrations. Isolation of this material by filtration provided analytically pure **9**, $[\alpha]^{26}_{405}$ −12.4 (*c* 0.1, 1:1 MeOH−$CHCl_3$), in 53−68% yield.[35] Examination of the remaining solution revealed the presence of residual **9**; however, attempts to recover this material from the filtrate led to decomposition and the formation of black solids.

Single crystals of **9** could be grown as its methanol solvate from mixtures of methanol and dichloromethane. X-ray analysis of this material resulted in the first crystal structure of a crambescidin alkaloid (Figure 2).

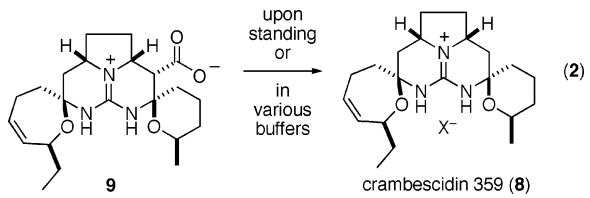
D. Decarboxylation of the Crambescidin Core Acid 9; Total Synthesis of Crambescidin 359 (8). Core acid **9** decarboxylates upon storage at 0 °C or room temperature for several weeks, the product of decarboxylation being consistently observed by electrospray mass spectrometry in samples of **9**. Purification of the decarboxylation product by HPLC provided crambescidin 359 (**8**) (eq 2). The chloride salt of this material

(31) Taber, D. F.; Amedio, J. C., Jr.; Patel, Y. K. *J. Org. Chem.* **1985**, *50*, 3618−3619.
(32) McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520−1528.
(33) This intermediate contained ~15% of the C13 epimer formed during the tethered Biginelli condensation.
(34) Small quantities of other diastereoisomers, possibly epimers at C10, were removed at this point.
(35) (a) The corresponding material isolated from the sponge *Monanchora ungiculata*, $[\alpha]^{25}_D$ −19 (*c* 0.04, MeOH)[16] was apparently an oil as the crystalline core acid is not soluble at that concentration in MeOH. The ^{1}H and ^{13}C NMR spectra reported for natural **9** vary somewhat from what we observe with a crystalline sample. ^{1}H NMR signals for several hydrogens of zwitterion **9** and the corresponding cationic acid vary considerably. The material isolated by McKee and co-workers might have been a mixture of these materials. (b) The absolute configuration of **9** is depicted incorrectly in reference 16.

Scheme 3. Synthesis of Pentacyclic Cinnamyl Ester **15**



(**8**, X = Cl) exhibited spectroscopic properties identical to those reported for natural crambescidin 359 (**8**).[14]



To gain additional insight into the decarboxylation of core acid **9**, it was exposed to several buffers in a 1:1 mixture of $CDCl_3$−CD_3OD (at ∼0.006 M); reaction progress was monitored by the disappearance of the C14 methine hydrogen of **9** using 1,3,5-trimethoxybenzene as an internal standard. At low (formate buffer) or neutral (*p*-nitrophenol buffer) pH, decarboxylation of **9** occurs in a pH independent manner to give ∼20% of crambescidin 359 (**8**) after 2.5 days at room temperature. However under basic conditions, acid **9** undergoes rapid, pH dependent, decarboxylation at room temperature. As summarized in Figure 3, decarboxylation of **9** occurs substantially faster in the presence of a *p*-methoxyphenol buffer (2.5 equiv of both *p*-methoxyphenol and sodium *p*-methoxyphenoxide, pK_a of *p*-methoxyphenol = 10.20 in H_2O) than in a comparable *p*-bromophenol buffer (*p*-bromophenol pK_a = 9.00 in H_2O).

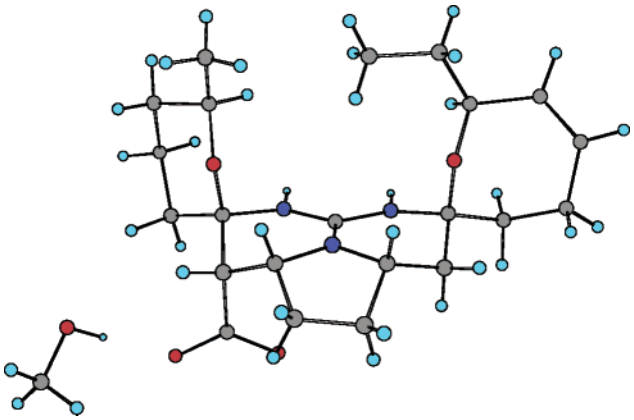


Figure 2. X-ray model of crambescidin core acid **9** methanol solvate.

Table 1. Base Catalyzed Decarboxylation of Crambescidin Acid **9**

time (h)	[buffer] (mM)	buffer ratio (OH:ONa)[a]	% conv.
43	25	1:1	43
43	50	1:1	56
43	75	1:1	59
53	50	2:1	44
53	50	1:1	63
53	50	1:2	73

[a] *p*-methoxyphenol buffers

The mechanism of base catalysis of the decarboxylation of **9** was studied briefly, however the low solubility of **9** and its limited availability prevented a detailed examination. Increasing the concentration of the 1:1 *p*-methoxyphenol/sodium *p*-methoxyphenolate buffer by a factor of 3, increased the rate of decarboxylation slightly (∼35%, see Table 1). Changing the ratio of phenoxide to phenol from 1:2 to 2:1 (equivalent to a change in pH of 0.6 units) increased the rate by 65% (Table 1). These results are consistent with decarboxylation being both specific base and general base (or specific base/general acid) catalyzed.

E. Incorporation of Deuterium into the Crambescidin Core. While studying the decarboxylation of core acid **9** in deuterated solvents, incorporation of deuterium into the product, crambescidin 359 (**8**), was observed. In deuterated solvents at high pH, crambescidin 359 (**8**) incorporated up to eight

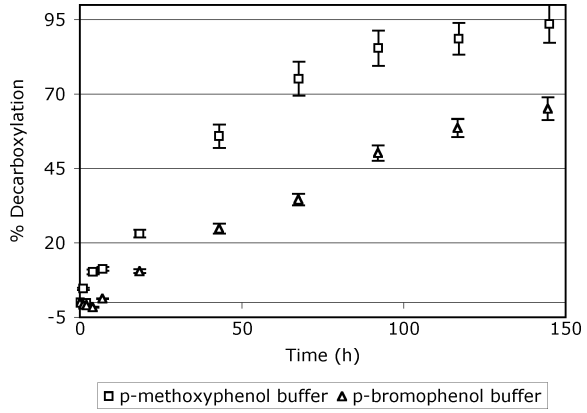


Figure 3. Decarboxylation of core acid **9** in a variety of buffers.

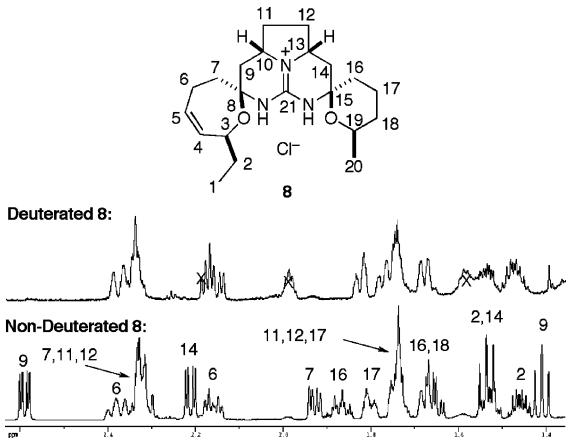


Figure 4. (a) 800 MHz ^{1}H NMR spectrum of crambescidin 359 (**8**) after incorporation of eight nonexchangeable deuterium atoms; the signals marked with an X are from an unknown impurity having an isolated spin system. (b) 800 MHz ^{1}H NMR of unlabeled crambescidin 359 (**8**).

nonexchangeable deuterium atoms. For example, incubating a sample of crambescidin 359 (**8**) with a 1:1 mixture of D_2O and methanol containing a 0.1 M phosphate buffer (pD = 13 in H_2O) at 65 °C for 25 h resulted in the incorporation of 7.9 deuterium atoms. Deuterium incorporation was identified by ^{1}H NMR spectroscopy to have occurred at C7, C9, C14, and C16 (Figure 4).

To gain a preliminary understanding of deuterium incorporation into the crambescidin pentacyclic guanidinium moiety, samples of crambescidin 359 (**8**), core acid **9** and cinnamyl ester **15** were dissolved in a 1:1 mixture of $CDCl_3$–CD_3OD in the presence of a 0.015 M *p*-methoxyphenol/sodium *p*-methoxyphenolate (1:1) buffer. These reactions then were monitored as a function of time by quenching aliquots into acidic methanol, which also washed out any exchangeable deuterium atoms. The average amount of deuterium incorporated at a given time was calculated from isotope patterns.[36,37] After 55 h at room temperature, crambescidin 359 (**8**) had incorporated an average of 2.8 deuterium atoms, whereas cinnamyl ester **15** had incorporated an average of 1.2 deuterium atoms. As was expected, core acid **9** underwent extensive decarboxylation under these reaction conditions to form crambescidin 359 (**8**). To our surprise, **9** did not incorporate deuterium, despite the concurrent incorporation of deuterium into crambescidin 359 (**8**).

(36) Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1993**, *115*, 6317−6321.
(37) Seto, H.; Mizukai, K.; Fujioka, S.; Koshino, H.; Yoshida, S. *J. Chem. Soc. Perkin 1.* **2002**, *21*, 2395−2399.

Table 2. Deuterium Incorporation into Crambescidin 359 (**8**) at Various PD and Buffer Concentrations

time (h)	pD	[buffer] (mM)	average no. of D incorporated
62	12.0	40	1.0
62	12.5	40	2.8
62	13.0	40	3.8
62	13.0	20	2.2
62	13.0	10	1.1

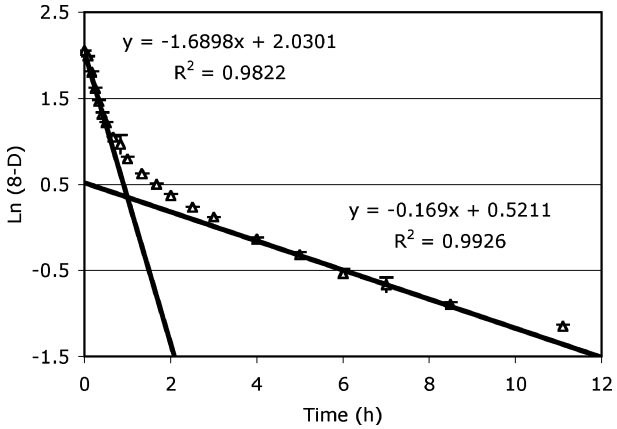


Figure 5. Deuterium incorporation into crambescidin 359 (**8**) as a function of time at 63 °C and pD = 13.

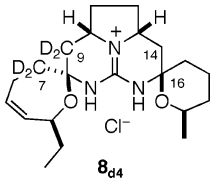
Deuterium incorporation into the crambescidin core was studied also in D_2O.[38] The low solubility of cinnamyl ester **15** in D_2O prohibited the collection of meaningful data for this substrate. Accordingly, only core acid **9** and crambescidin 359 (**8**) were examined. Studies were carried out over the pD range 4−12.5; however, deuterium incorporation at room temperature was only seen at high pH. As had been observed in organic solvents, core acid **9** underwent extensive decarboxylation at pD > 12.[39] Although core acid **9** did not incorporate deuterium in organic solvents, deuterium incorporation into both residual core acid **9** and crambescidin 359 (**8**) occurred with similar rates in D_2O in the pD range 9−12.5.[40]

Crambescidin 359 (**8**) was chosen for detailed study in aqueous medium, with deuterium incorporation being examined in various phosphate buffers. The rate of decarboxylation increased with both increasing pD and buffer concentration (Table 2). These variations in rate indicate that deuterium incorporation into crambescidin 359 (**8**), similar to decarboxylation of core acid **9**, is both specific base and general base (or specific base/general acid) catalyzed.

The amount of deuterium incorporated into crambescidin 359 (**8**) was studied also as a function of time at 63 °C and pD = 13 (Figure 5). Two features are apparent in the data obtained from the experiment summarized in Figure 5: deuterium incorporation exhibits a small induction period[40] and a sudden decrease in rate after the incorporation of approximately four deuterium atoms. Data collected between 10 min and 8.5 h is consistent with the presence of two parallel pseudo-first-order processes, with approximate values for k_{obs} of 1.7 and 0.17 h^{-1}.[41]

(38) In experiments performed in water, the ionic strength of buffer solutions was maintained constant by the addition of cesium iodide.
(39) Accurate quantification of the rate of decarboxylation of **9** in aqueous media was prohibited by both the low solubility of **9** in D_2O and substantially different ionization potentials of core acid **9** and crambescidin 359 (**8**).
(40) This is best seen in the tabulated data, see Supporting Information.

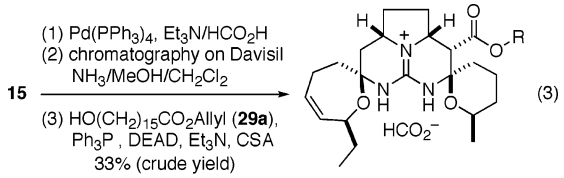
To identify the site of the initial, rapid deuterium incorporation, a sample of crambescidin 359 (**8**) was exposed to a 1:1 *p*-methoxyphenol/sodium *p*-methoxyphenolate buffer in a 1:1 mixture of $CDCl_3$ and CD_3OD at room temperature. This reaction was halted after 7 days, at which time approximately 4 nonexchangeable deuterium atoms had been introduced. ^{1}H NMR analysis revealed that deuterium incorporation had occurred predominantly at C7 and C9.



8_{d4}

F. Reaction of Crambescidin 359 (8) with Basic Ethanethiol. The behavior of crambescidin 359 (**8**) in basic ethanethiol was examined to determine whether the unveiling of an *N*-amidinyl iminium ion (vide infra) under basic conditions would lead to reaction with incipient nucleophiles. Incubation of crambescidin 359 (**8**) with ethanethiol in the presence of a 1:1 *p*-methoxyphenol/sodium *p*-methoxyphenolate buffer for 3 days at room-temperature resulted in partial conversion of **8** to an adduct of **8** and ethanethiol. This adduct was observed by both high-resolution electrospray mass spectrometry (HRESMS) and HPLC-MS analysis.[42] Unfortunately, all attempts to isolate this species were unsuccessful as it reverted to the starting materials upon removal of the solvent.

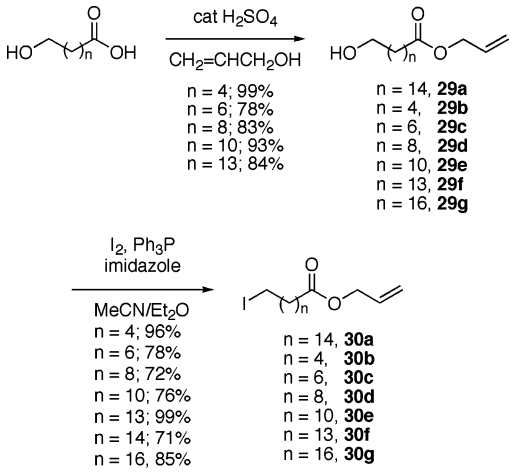
G. Esterification of Core Acid 9. Total Synthesis of Crambescidin 431 (7) and Preparation of a Small Library of Crambescidin Analogues. Early scouting studies indicated that esters of core acid **9** could not be prepared by acyl transfer,[43] undoubtedly a result of a high degree of steric congestion around the C14 carboxylate carbon (see Figure 2). Accordingly, the conversion of core acid **9** to ester derivatives was pursued using the carboxylate as a nucleophile. We initially examined Mitsunobu coupling. However, coupling of allyl 16-hydroxyhexadecanoic acid (**29a**) with a sample of partially purified core acid **9**[44] in the presence of triethylammonium camphorsulfonate[45] provided ester **14a** in low yield (eq 3).

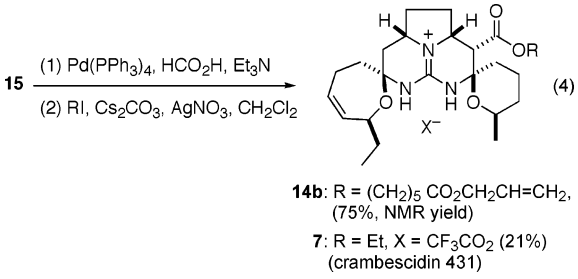


14a: R = $(CH_2)_{15}CO_2CH_2CH{=}CH_2$

We turned to the coupling of the crude zwitterionic core acid **9** with alkyl iodides. The direct reaction of carboxylate **9** with ethyl iodide, carried out in the presence, or absence, of added $CsCO_3$ provided the corresponding ester **7** in trace amounts only. Larock has reported that silver salts promote the esterification of carboxylic acids with alkyl halides.[46] Accordingly, the coupling of carboxylate **9** with allyl 6-iodohexanoate (**30b**) was examined in the presence of a range of bases and silver additives. Carrying out this reaction in CH_2Cl_2 at room temperature with 7 equiv of iodide **30b**, 1.5 equiv of $CsCO_3$ and 3 equiv of $AgNO_3$ proved optimal, providing ester **14b** in good yield from cinnamyl ester **15** (eq 4).[47] In a similar fashion, crambescidin 431 (**7**) was isolated as its trifluoroacetate salt in 21% overall yield from cinnamyl ester **15**.

Scheme 4. Synthesis of Iodides **30a**−**g**





14b: R = $(CH_2)_5\ CO_2CH_2CH{=}CH_2$, (75%, NMR yield)

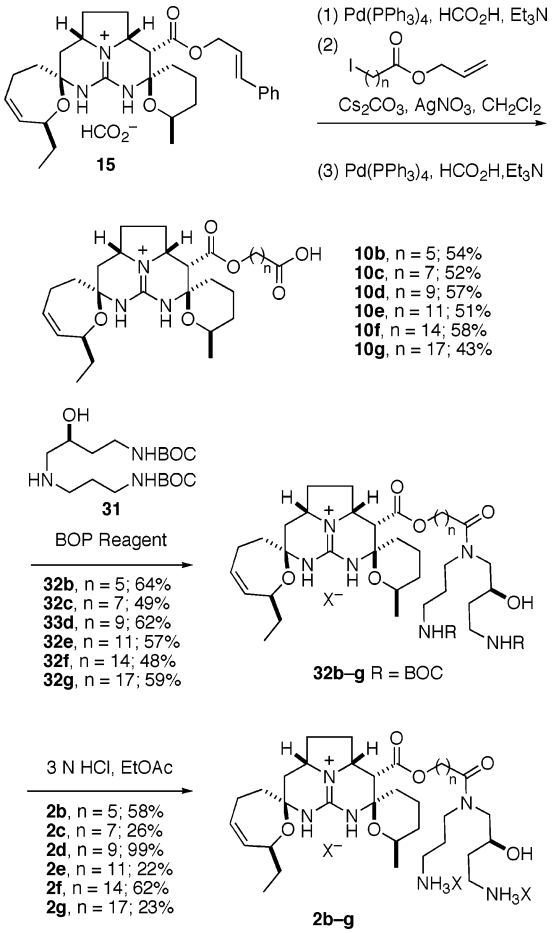
7: R = Et, X = CF_3CO_2 (21%) (crambescidin 431)

Crambescidin 431 (**7**) was isolated initially as the trifluoroacetate salt, the ^{13}C NMR spectra of which correlated well with data reported for the natural product.[14] However, the ^{1}H NMR spectrum of the trifluoroacetate salt showed significant discrepancies with the ^{1}H NMR data reported for crambescidin 431. NMR spectra of crambescidin alkaloids is known to vary somewhat depending upon the counterion.[24] Thus, the trifluoroacetate salt of synthetic crambescidin 431 (**7**) was washed with saturated ammonium chloride to form the chloride salt. The ^{1}H NMR spectrum of this material matched closely with that reported for natural **7**.[14,40]

To access side chain analogues of crambescidin 657 (**10a**) and 800 (**2a**) that varied in the length of the ω-hydroxyalkanoic acid unit, a series of straight chain allyl ω-iodocarboxylates was required. These compounds, **30a**−**g**, were obtained by the straightforward two step sequence summarized in Scheme 4.

(41) For a description of the interpretation of data of this type, see Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism; A Study of Homogeneous Chemical Reactions*; John Wiley & Sons: New York, 1961, p 160−164.
(42) That an adduct between crambescidin 359 (**8**) and ethanethiol was formed was further confirmed by the repetition of this experiment using partially deuterated crambescidin 359; deuterium was observed in the adduct.
(43) McDonald, A. I. Ph.D. Dissertation, University of California, Irvine, 2000.
(44) For these reactions, core acid **9** was purified on Davisil resulting in a 60% yield of material that was ~80% pure.
(45) Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J. *J. Am. Chem. Soc.* **1988**, *110*, 6487−6491.
(46) Larock, R. C. *J. Org. Chem.* **1974**, *39*, 3721−3727.
(47) Unfortunately, ester **14a** could not be separated from triphenylphosphine oxide. Conversions were obtained by isolating material contaminated with triphenylphosphine oxide and calculating the yield by ^{1}H NMR calibrated with an internal standard.

Scheme 5. Synthesis of Crambescidin 657 and 800 Analogues **2b-g** and **10a−g**



With iodides **30b**−**g** in hand, we turned to the synthesis of analogues of crambescidin 657 (**10a**) and crambescidin 800 (**2a)** (Scheme 5). Using the optimized carboxylate alkylation conditions developed for preparing **14b**, crambescidin 657 analogues **10b**−**g** were generated from pentacyclic cinnamyl ester **15** in 3 steps and 43−58% overall yield. Using procedures developed during our total synthesis of crambescidin 800 (**2a**),[24] these analogues were coupled with protected hydroxyspermidine **31**[48] and the resulting amide was deprotected by reaction with anhydrous HCl in ethyl acetate to provide crambescidin 800 analogues **2b**−**g**.

Purification of analogues **2b**−**g** was typically difficult. Although analogues **2d** and **2f** could be obtained in greater than 98% purity by C18 reverse phase preparative HPLC, analogues **2b**, **2c**, **2e**, and **2g** were not completely pure after preparative reverse phase HPLC. Fortunately, it was found that material that had been purified by reverse phase HPLC could be further purified by silica gel chromatography. In this way, analogues **2b**, **2c**, **2e**, and **2g** were obtained in greater than 98% purity.[49]

A series of crambescidin analogues containing simple hydrocarbon ester side chains was prepared also (Scheme 6). The low yields realized in the synthesis of **33**−**36** reflect losses during reverse phase preparative HPLC purification of the chloride salts, rather than poor yields in the alkylation reactions.

(48) Coffey, D. S.; McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *74*, 8741−8742.
(49) The yields obtained reflect largely the ease or difficulty of purification.

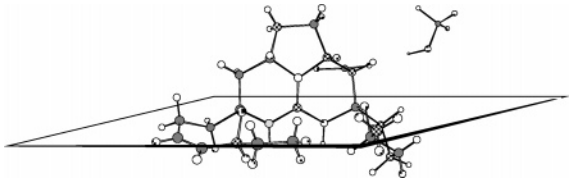
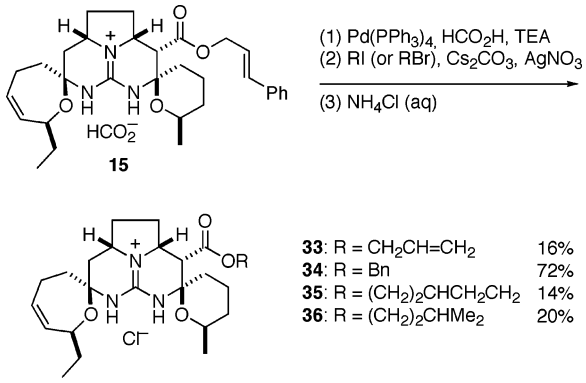


Figure 6. X-ray model of crambescidin core acid **9**. The plane is drawn orthogonal to the guanidinium π-system and passing through the two disubstituted guanidinium nitrogen atoms.

Scheme 6. Synthesis of Crambescidin Analogs Having Short Hydrocarbon Side Chains



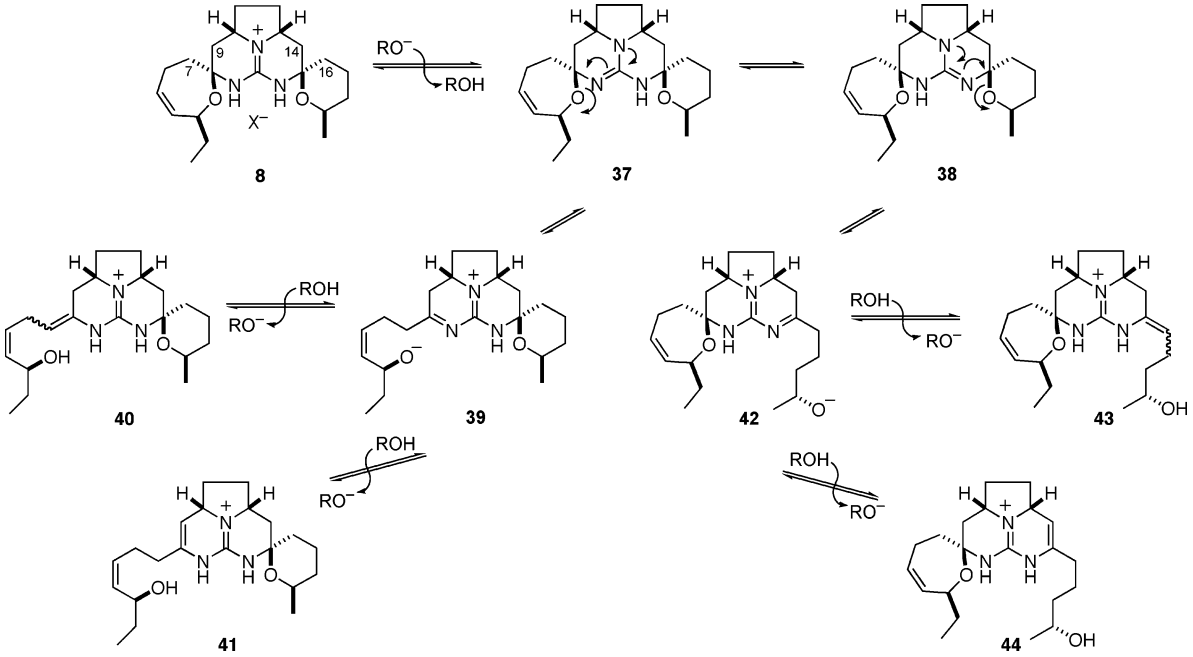
Discussion

A. Conformation of Crambescidin Core Acid 9. Crambescidin alkaloids are known to bind anions through hydrogen bonding interactions with their guanidinium functional group.[7,50] The selectivity of these interactions undoubtedly is defined by the absolute and relative configuration of the pentacyclic ring system and the conformations of the spiroaminal rings that frame the guanidinium hydrogens. A second perspective of the X-ray model of crambescidin core acid **9** is shown in Figure 6. Whereas both oxygens of the spiroaminal rings are disposed axially, an observation consistent with earlier ^{1}H NMR studies,[50] the orientation of these two rings with respect to the guanidinium nitrogens is notably different.The chair tetrahydropyran ring of **9** projects 1.2 Å past a plane passing through the disubstituted guanidinium nitrogen atoms (Figure 6), whereas the 2,3,4,5-tetrahydrooxepine ring exists in a flatter conformation in which few atoms of this ring project past this perpindicular plane. Thus, nonbonded steric interactions between anions hydrogen-bonding to the guanidinium hydrogens and the pentacyclic crambescidin core would be most pronounced with the hydropyran ring of these alkaloids.

B. Deuterium Incorporation Into the Crambescidin Pentacyclic Core. In the presence of base, crambescidin 359 (**8**) incorporates deuterium at C7, C9, C14, and C16, positions adjacent to the two spiroaminal carbons, C8 and C15. A plausible mechanism for this process is depicted in Scheme 7. Reversible opening of the spiroaminal rings of the crambescidin core generates *N*-amidinyl iminium ion intermediates **39** and **42**,[51] which incorporate deuterium by virtue of equilibrium with their respective enamonium ion congeners **40**/**41** and **43**/**44**.

(50) Anion binding has also been studied with simpler crambescidin analogues, see: Murphy, P. J.; Williams, H. L.; Hibbs, D. E.; Hursthouse, M. B.; Malik, K. M. A. *Chem. Commun.* **1996**, 445−447.
(51) For simplicity, the conjugate acids of **39** and **40**, which would be present also, are not shown.

Scheme 7. Specific Base Catalyzed Mechanism for Incorporation of Deuterium at C7, C9, C14, and C16 of Crambescidin 359 (**8**)



That deuterium is incorporated fastest at C7 and C9 demonstrates that equilibration of pentacyclic guanidine **37** with tetracyclic guanidinium ions **39**, **40**, and **41** is faster than the related equilibration of pentacyclic guanidine **38** with tetracyclic guanidinium ions **42**, **43**, and **44**.[52] Most likely this rate difference resides in the ring-opening step, which would be expected to be faster for the spiro 2,3,4,5-tetrahydrooxepine ring.[53]

Deuterium incorporation into crambescidin 359 (**8**) occurs at room temperature at an appreciable rate only at pD $\geq$ 12. If the pK_a of the guanidinium functional group of the pentacyclic crambescidin core is approximated by that of tetramethylguanidinium (pK_a = 13.6 in water),[54] ~3% would be deprotonated in D_2O at pD = 12 (phosphate buffer). In nonaqueous solvents, the extent of deprotonation could be greater.[54] Base catalysis of deuterium incorporation would be expected if spiroaminal ring-opening were rate-determining. Deprotonation of the guanidinium ion generates a more electron-rich guanidine functional group, which by virture of donation of electron density into the σ^* orbital of the spirocyclic carbon−oxygen bond, would facilitate ring-opening.[55] Specific base catalysis is depicted in Scheme 5, however, the observed small increase in rate with increasing buffer concentration implies the existence of a related general base (or specific base-general acid) catalyzed process.

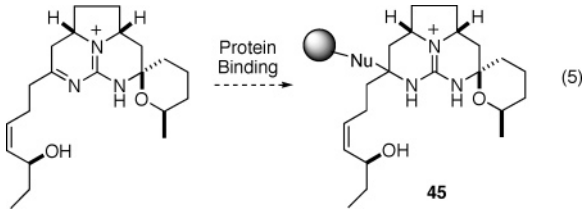
(52) Another manifestation of this rate difference is the slight epimerization that is observed at C14 of cinnamyl ester **15** after the incorporation of four deuterium atoms.

(53) Typically, seven-membered rings possess greater ring strain (3−6 kcal/mol) than their six-membered counterparts, e.g., see: Dudev, T.; Lim, C. *J. Am. Chem. Soc.* **1998**, *120*, 4450−4458.

(54) Under the nonaqueous conditions used to examine deuterium incorporation into crambescidin core acid **9** and crambescidin 359 (*p*-methoxyphenol buffers in 1:1 CD_3OD-$CHCl_3$), the guanidinium acid might be more extensively ionized. The pK_a of tetramethylguanidine increases by 10 going from water to acetonitrile (pK_a = 13.6 in water; 23.3 in acetonitrile), whereas the pK_a of phenol increases by 17 with a similar solvent transition (pK_a = 10.0 in water; 27.2 in acetonitrile), see: Rodima, T.; Kaljurand, I.; Pihl, A.; Mäemets, V.; Leito, I.; Koppel, I. A. *J. Org. Chem.* **2002**, *67*, 1873−1881.

(55) Kirby, A. J. *Stereoelectronic Effects*; Oxford University Press: Oxford; New York, 1996.

C. A Possible Role for Spiroaminal Ring Opening in the Biological Activity of Crambescidin Alkaloids. The proposal that deprotonation of the guanidinium cation of a crambescidin alkaloid initiates spirocyclic ring opening suggests a potential mechanism for at least some of the biological activity of these alkaloids. Base-promoted opening of a spiroaminal ring upon binding of a crambescidin alkaloid to a receptor[56] would reveal an *N*-amidinyl iminium ion electrophile,[57] which could lead to covalent binding to form an adduct such as **45** (eq 5). The observation that an adduct is formed when crambescidin 359 (**8**) is exposed to ethanethiol in a *p*-methoxyphenol/*p*-methoxyphenolate buffer is consistent with this proposal.



(5)

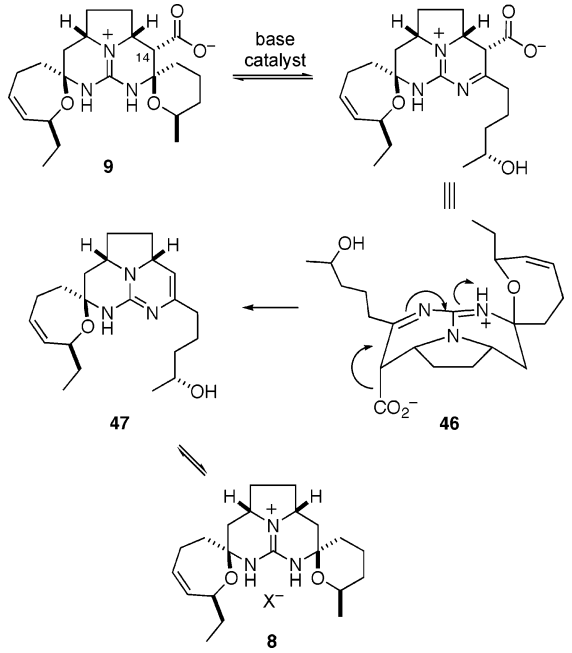
D. Mechanism of Decarboxylation of Crambescidin Core Acid 9. Base-catalyzed decarboxylation of crambescidin core acid **9** likely occurs by a sequence of steps closely related to those involved in deuterium incorporation. Catalyzed (specific and/or general base) opening of the hydropyran ring of zwitterionic guanidinium carboxylate **9** generates *N*-amidinyl iminium ion **46** (Scheme 8). The axial disposition of the carboxylate functionality in this intermediate provides ideal orbital overlap for decarboxylation with concomitant formation of neutral *N*-amidinyl enamine **47**. Protonation of this intermediate at C14, followed by spirocyclization would yield crambescidin 359 (**8**).

The mechanism of the slower pH independent decarboxylation reaction is undoubtedly similar. Because of the excellent

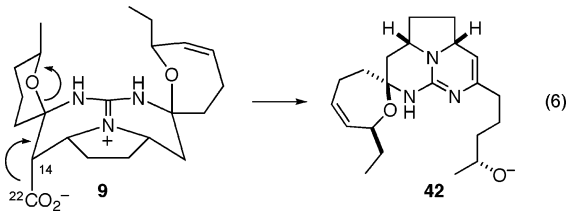
(56) Zhou, G.; Somasundaram, T.; Blanc, E.; Parthasarathy, G.; Ellington, W. R.; Chapman, M. S. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95*, 8449−8454.

(57) Overman, L. E.; Wolfe, J. P. *J. Org. Chem.* **2001**, *66*, 3167−3175.

Scheme 8. Mechanism of Base Catalyzed Decarboxylation



overlap of the C14−C22 and C−O σ-bonds of guanidinium carboxylate **9**, concerted decarboxylation with concomitant opening of the C14 spiroaminal is plausible (eq 6).



As noted earlier, under basic conditions deuterium was not incorporated into core acid **9** in organic solvents. In aqueous media in contrast, **9** incorporated deuterium at a rate similar to that of decarboxylation product crambescidin 359 (**8**). These observations are readily accounted for by the closely related mechanisms we have proposed for base catalyzed decarboxylation and deuterium incorporation. Apparently in less polar solvents, intermediate **46** decarboxylates more rapidly than it tautomerizes. This scenario is reasonable as decarboxylation transforms zwitterionic **46** to a neutral product, a process that would be dramatically faster in less polar solvents.[58] Tautomeric equilibration of **46** with enamine isomers would not involve similar charge dissipation.

Decarboxylation of crambescidin acid **9** to form crambescidin 359 (**8**) is a probable step in the biosynthesis of **8**. We speculate that guanidinium carboxylate **9** arises from esterase cleavage of more abundant crambescidin alkaloids, such as crambescidin 800 (**2a**). There is evidence that the spiroaminal rings of ptilomycalin A (**1**) protect the carboxylic ester from cleavage by the pendant spermidine moiety.[59] Perhaps cleavage of the ester side chain is facilitated by prior opening of the tetrahydropyran ring.[60]

(58) (a) Kemp, D. S.; Paul, K. G. *J. Am. Chem. Soc.* **1975**, *97*, 7305−7312. (b) For a recent study, see: Catalán, J. Días, C.; Garcia-Blanco, F. *J. Org. Chem.* **2000**, *65*, 3409−3415.
(59) Grillot, A. L.; Hart, D. J. *Tetrahedron* **1995**, *51*, 11377−11392.

Conclusion

The first practical method to obtain the crambescidin core acid **9** has been developed. This enantioselective total synthesis was accomplished in 29 total steps and 2.8% overall yield from commercially available 3-butyn-1-ol by way of 15 isolated intermediates. A key step in this synthesis was palladium mediated deprotection of cinnamyl ester **15**, an intermediate that should be useful for the synthesis of various crambescidin alkaloid analogues. In the present study, this ester was employed to prepare crambescidins 431 (**7**) and 359 (**8**), and a library of crambescidin analogues.[61]

Crambescidin core acid **9** provided single crystals, allowing the first X-ray structure of the fully constituted crambescidin core to be accomplished. Zwitterionic guanidinium carboxylate **9** was shown to readily decarboxylate to form crambescidin 359 (**8**), which was fastest under basic conditions. In the presence of base, up to eight deuterium atoms can be incorporated into the crambescidin core. Deuterium incorporation and decarboxylation of crambescidin core acid **9** are the result of ring opening of the spirocyclic ether rings of the pentacyclic crambescidin moiety. This facile ring opening generates electrophilic *N*-amidinyl iminium ion intermediates, which in vivo could result in covalent binding.

Experimental Section.

Preparation of Pentacyclic Guanidine Esters 28 and 15. A solution of tricyclic urea **26** (0.29 g, 0.46 mmol), methyl triflate (1.4 mL, 9.1 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (0.50 mL, 2.3 mmol) and CH_2Cl_2 (23 mL) was maintained at room temperature for 9 h. This solution was diluted with Et_2O (150 mL) and washed with 1 N NaOH (2 × 15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to give pseudourea **27**. This intermediate was used without further purification.

Ammonia was bubbled through a solution of this sample of crude **27**, NH_4Cl (0.055 g, 1.0 mmol), and allyl alcohol (5 mL) for 20 min at room temperature. The reaction vessel then was sealed and heated to 60 °C. After 20 min, the pressure typically varied, depending on reaction scale, from 18 to 25 psi. If the pressure was higher than 18 psi, the reaction vessel was vented at this stage to adjust the pressure to 18 psi. The sealed reaction vessel was maintained at 60 °C and 18 psi for 36 h. The reaction then was cooled to room temperature, diatomaceous earth (1 g) was added and the mixture was concentrated to give a fine powder. Purification of this powder by silica gel MPLC (100:0.3:0.1−100:0.6:0.1 $CHCl_3$:*i*-PrOH:CF_3CO_2H) provided 95 mg (0.15 mmol) of **15**, 110 mg (0.17 mmol) of **28** and 21 mg (0.033 mmol) of a 1.5:1 mixture of **28**:**15** (78% overall yield). Resubjection of **28** and the 1.5:1 mixture of **28**:**15** to these reaction conditions (twice) provided, after MPLC purification, an additional 60 mg (53% combined yield) of pure **15** trifluoroacetate salt: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.02 (br s, 1H), 9.82 (br s, 1H), 7.38−7.42 (m, 2H), 7.32−7.36 (m, 2H), 7.23−7.30 (m, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.26 (dt, J = 15.9, 6.6 Hz, 1H), 5.63−5.67 (m, 1H), 5.47 (d, J = 10.8 Hz, 1H), 4.73 (d, J = 6.6 Hz, 2H), 4.50−4.52 (m, 1H), 4.31 (quint, J = 4.9 Hz, 1H), 3.96−4.10 (m, 2H), 2.99 (d, J = 4.7 Hz, 1H), 2.42−2.51 (m, 2H), 2.22−2.37 (m,

(60) Incubating ptilomycalin A (**1**) at room temperature in a pH 13.0 phosphate buffer and monitoring of the reaction by LCMS identified crambescidin 359 (**8**) as a major product. Trace quantities of the crambescidin core acid (**9**) were observed by ESMS.
(61) Using this library, in vitro cytotoxicity against several cancer cell lines was shown to increase with increasing length of the ω-hydroxyalkanoic acid side chain fragment.[12] Moreover, tumor selectivity was shown to vary widely with side chain structure, with analogues having short, nonpolar side chains being both potent and showing good selectivity for some solid tumors.[12] Members of this library were also used in recent studies that identified the first small molecular inhibitors of the HIV-1 protein Nef.[11]

3H), 2.07−2.21 (m, 2H), 1.50−1.89 (m, 6H), 1.35−1.48 (m, 2H), 1.15−1.35 (m, 3H), 1.05 (d, J = 6.10 Hz, 3H), 0.83 (t, J = 7.16 Hz, 3H); ^{13}C (125 MHz, $CDCl_3$)[62] 168.0, 149.0, 135.9, 135.7, 133.7, 129.8, 128.7, 128.5, 126.7, 121.9, 83.6, 80.7, 71.0, 67.3, 65.9, 53.9, 51.9, 50.1, 36.9, 32.1, 32.0, 30.6, 29.7, 29.1, 26.8, 23.5, 21.5, 18.4, 10.0 ppm; IR (film) 3212, 3088, 2941, 2864, 1679, 1625 cm^{-1}; $[\alpha]^{26}_{405}$ −17.5, $[\alpha]^{26}_{435}$ −12.2, $[\alpha]^{26}_{546}$ −4.75, $[\alpha]^{26}_{577}$ −4.73, $[\alpha]^{26}_{589}$ −3.74 (*c* 0.3, CH_2Cl_2); HRMS (ES) calculated for $C_{33}H_{42}N_3O_6$ (M^+): 520.3175, found: 520.3154.

28 trifluoroacetate salt: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.48 (s, 1H), 10.17 (s, 1H), 7.38−7.39 (m, 2H), 7.32−7.36 (m, 2H), 7.26−7.30 (m, 1H), 6.68 (d, J = 16.0 Hz, 1H), 6.27 (dt, J = 15.9, 6.5 Hz, 1H), 5.62−5.69 (m, 1H), 5.48 (d, J = 10.6 Hz, 1H), 4.79−4.88 (m, 2H), 4.42−4.50 (m, 1H), 4.35 (dt, J = 11.6, 7.7 Hz, 1H), 4.08−4.17 (m, 1H), 3.77−3.82 (m, 1H), 2.55−2.64 (m, 2H), 2.45 (d, J = 11.5 Hz, 1H), 2.29−2.34 (m, 3H), 2.13−2.16 (m, 2H), 1.94−1.97 (m, 1H), 1.80−1.90 (m, 1H), 1.59−1.77 (m, 4H), 1.50−1.59 (m, 1H), 1.39−1.49 (m, 1H), 1.23−1.34 (m, 2H), 1.01−1.11 (m, 4H), 0.82 (t, J = 7.2 Hz, 3H); ^{13}C (125 MHz, $CDCl_3$)[62] 167.4, 147.7, 135.7, 134.9, 133.4, 129.6, 128.5, 128.2, 126.4, 122.0, 83.7, 81.6, 70.8, 67.6, 66.0, 53.7, 53.5, 53.3, 37.1, 36.2, 32.1, 30.9, 30.0, 29.7, 29.3, 23.9, 21.5, 18.2, 10.5 ppm; IR (film) 3231, 3111, 3026, 2968, 2937, 1737, 1675, 1613 cm^{-1}; $[\alpha]^{26}_{405}$ +6.5, $[\alpha]^{26}_{435}$ +16.8, $[\alpha]^{26}_{546}$ +6.7, $[\alpha]^{26}_{577}$ +2.2, $[\alpha]^{26}_{589}$ +1.7 (*c* 0.3, CH_2Cl_2); HRMS (ES) calculated for $C_{33}H_{42}N_3O_6$ (M^+): 520.3175, found: 520.3154.

Alternatively, **15** and **28** could be isolated as their formate salts by MPLC (100:0.3:0.2−100:0.6:0.2 $CHCl_3$:*i*-PrOH:HCO_2H) purification. Formate salts show a diagnostic ^{1}H NMR signal at 8.67 ppm. Subsequent transformations of cinnamyl ester **15** were little effected by the nature of its counterion.

Preparation of Crambescidin Core Acid 9. A vial containing the formate salt of ester **15** (23 mg, 0.041 mmol), palladium tetrakis(triphenylphosphine) (4.5 mg, 0.0041 mmol) and a solution of triethylammonium formate in THF (0.21 mL, 1 M) was maintained at room temperature for 14 h, diluted with freshly distilled acetone (3 mL) and filtered through a medium porosity sintered glass frit. The filtrate was washed with 10 mL of freshly distilled acetone to give 7.9 mg of zwiterionic **9** as a colorless crystalline solid (0.020 mmol, 47%): ^{1}H NMR (500 MHz, 1:1 $CDCl_3$:CD_3OD − calibrated to $CDCl_3$): δ 5.38−5.42 (m, 1H), 5.19 (d, J = 10.9 Hz, 1H), 3.97−4.02 (m, 1H), 3.88 (dt, J = 8.6, 4.8 Hz, 1H), 3.62−3.70 (m, 1H), 3.42−3.53 (m, 1H), 2.38 (d, J = 4.7 Hz, 1H), 2.16 (dd, J = 12.7, 4.6 Hz, 1H), 2.08 (br t, J = 15.2 Hz, 1H), 1.73−1.96 (m, 5H), 1.63 (dd, J = 13.9, 5.8 Hz, 1H), 1.30−1.53 (m, 6H), 1.13−1.28 (m, 3H), 0.92 (q, J = 12.0 Hz, 1H), 0.76 (d J = 6.2 Hz, 3H) 0.52 (t, J = 7.2 Hz, 3H); ^{13}C (125 MHz, 1:1 $CDCl_3$:CD_3OD − calibrated to $CDCl_3$) 173.1, 148.8, 132.7, 129.8, 83.1, 80.8, 70.2, 65.9, 53.6, 53.1, 52.1, 36.8, 36.0, 31.6, 30.8, 29.6, 28.5, 26.3, 22.9, 20.7 17.9, 9.3 ppm; IR (film) 2961, 2926, 1637 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 5.70−5.75 (m, 1H), 5.52 (br d, J = 11.1 Hz, 1H), 4.31−4.38 (m, 1H), 4.22 (dt, J = 8.7, 5.5 Hz, 1H), 3.96−4.04 (m, 1H), 3.78−3.86 (m, 1H), 2.69 (d, J = 5.0 Hz, 1H), 2.53 (dd, J = 12.8, 4.8 Hz, 1H), 2.43 (br t, J = 15.5 Hz, 1H), 2.11−2.30 (m, 3H), 1.99−2.10 (m, 2H), 1.80−1.90 (m, 2H), 1.70−1.79 (m, 3H), 1.67 (br d, J = 13.9 Hz, 1H), 1.42−1.58 (m, 3H), 1.22−1.38 (m, 2H), 1.08 (d, J = 6.2 Hz, 3H), 0.89 (t, J = 7.3 Hz, 3H); $[\alpha]^{26}_{405}$ −12.4, $[\alpha]^{26}_{435}$ −2.8, $[\alpha]^{26}_{546}$ −5.4, $[\alpha]^{26}_{577}$ −5.1, $[\alpha]^{26}_{589}$ −2.9 (*c* 0.1, 1:1 CH_2Cl_2:MeOH); $[\alpha]^{24}_{405}$ −17, $[\alpha]^{24}_{435}$ −54, $[\alpha]^{24}_{546}$ −120, $[\alpha]^{24}_{577}$ −102, $[\alpha]^{24}_{589}$ −88 (*c* 0.006, MeOH); HRMS (ES): calculated for $C_{22}H_{33}N_3O_4$ ($M + H^+$): 404.2549, found: 404.2555.

Data for the trifluoroacetate salt formed from **9** and excess trifluoroacetic acid: ^{1}H NMR (500 MHz, CD_3OD) δ 5.70−5.75 (m, 1H), 5.52 (br d, J = 11.0 Hz, 1H), 4.34−4.42 (m, 2H), 4.02−4.10 (m, 1H), 3.80−3.88 (m, 1H), 3.01 (d, J = 5.0 Hz, 1H), 2.64 (dd, J = 13.0, 4.8 Hz, 1H), 2.40−2.49 (m, 1H), 2.28−2.40 (m, 2H), 2.12−2.22 (m, 1H), 1.90−2.04 (m, 2H), 1.85−1.88 (m, 1H), 1.76−1.82 (m, 3H), 1.63−1.74 (m, 2H), 1.51−1.62 (m, 1H), 1.38−1.50 (m, 2H), 1.25−1.35 (m, 2H), 1.11 (d, J = 6.2 Hz, 3H), 0.85 (t, J = 7.2 Hz, 3H); $[\alpha]^{24}_{405}$ −45, $[\alpha]^{24}_{435}$ −6, $[\alpha]^{24}_{546}$ −88, $[\alpha]^{24}_{577}$ −109, $[\alpha]^{24}_{589}$ −81 (*c* 0.006, MeOH).

A sample of **9** (6 mg) was dissolved in a 1:1 mixture of CH_2Cl_2 and MeOH (5 mL); slow evaporation of this solution at room temperature over 7 d resulted in colorless needles that were suitable for X-ray analysis (CCDC 253224).

Preparation of Crambescidin 359 Chloride Salt (8). A mixture of cinnamyl ester **15** formate salt (15 mg, 0.026 mmol), triethylammonium formate (0.13 mL, 1 M in THF), palladium tetrakis(triphenylphosphine) (8.6 mg, 0.0079 mmol) and THF (2.6 mL) was maintained at room temperature for 12 h. The reaction then was concentrated and the residue was dried at 0.5 Torr for 12 h. The crude core acid **9** was used without further purification.

A solution of this sample of acid **9**, *p*-methoxyphenol (32 mg, 0.26 mmol), sodium methoxide (0.13 mL, 1 M in MeOH), MeOH (2.1 mL) and chloroform (2.1 mL) was maintained at room temperature. After 4 days, the reaction solution was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (2 mL) and MeOH (2 mL). This solution was heated to 75 °C and allowed to concentrate to near dryness over 1 day. The residue was dissolved in MeOH and purified by C18 reverse phase preparative HPLC (1:9−4:6 MeOH:0.1% trifluoroacetic acid in water) to give 7.0 mg (0.015 mmol, 58%) of the triflouroacetate salt of crambescidin 359 (**8**) as a pale yellow oil:^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (br s, 1H), 10.04 (br s, 1H), 5.65 (br t, J = 10.4 Hz, 1H), 5.48 (br d, J = 10.8 Hz, 1H), 4.47 (d, J = 10.3 Hz, 1H), 3.98−4.08 (m, 2H), 3.76−3.85 (m, 1H), 2.63 (t, J = 13.4, 1H), 2.56 (dd, J = 12.7, 4.6 Hz, 1H), 2.18−2.38 (m, 6H), 1.78−1.82 (m, 2H), 1.61−1.75 (m, 12H),[63] 1.38−1.59 (m, 5H), 1.12−1.34 (m, 14H), 1.20 (d, J = 6.2 Hz, 3H), 0.81 (t, J = 1.2 Hz, 3H); ^{13}C (125 MHz, $CDCl_3$)[62] 148.5, 133.7, 129.8, 83.7, 80.2, 70.7, 66.8, 53.1, 40.0, 37.1, 36.1, 33.6, 32.3, 30.1, 30.0, 29.7, 29.1, 23.7, 21.6, 18.1, 10.2 ppm; IR (film) 3231, 3115, 2972, 2880, 1675, 1652, 1606 cm^{-1}; HRMS (ES) calculated for $C_{21}H_{34}N_3O_2$ (M^+): 360.2651; found: 360.2643.

The chloride salt of crambescidin 359 (**19**) was generated by dissolving a sample of its triflouroacetate salt (7 mg, 0.015 mmol) in a mixture of CH_2Cl_2 (3 mL) and saturated aqueous NH_4Cl (3 mL). This mixture was vigorously stirred for 6 h, partitioned and the aqueous layer was extracted with CH_2Cl_2 (5 × 3 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated and the residue was filtered through a plug of silica gel (1:20 MeOH: CH_2Cl_2) to give 5.9 mg (0.015 mmol, 99%) of crambescidin 359 chloride salt (**8**) as a colorless oil: HRMS (ES) calculated for $C_{21}H_{34}N_3O_2$ (M^+): 360.2651, found: 360.2638; ^{1}H and ^{13}C NMR spectra for this sample agreed well with data reported for the natural product.[14]

Representative Procedure for the Synthesis of Crambescidin 657 Analogues. Preparation Crambescidin 657 Analogue 10b. A mixture of the formate salt of ester **15** (22 mg, 0.039 mmol), palladium tetrakis(triphenylphosphine) (13 mg, 0.012 mmol), a solution of triethylammonium formate (1 M in THF, 0.20 mL) and THF (3.9 mL) was maintained at room temperature for 16 h, concentrated and the residue was dried (0.05 Torr, 6 h) to give the crude core acid **9** as a yellow oil. This material was used without further purification.

Cesium carbonate (19 mg, 0.06 mmol) was added to a solution of this sample of core acid **9**, allyl 6-iodohexanoate (**30b**, 77 mg, 0.27 mmol) and CH_2Cl_2 (0.39 mL). After 5 min, $AgNO_3$ (20 mg, 0.12 mmol) was added and the reaction was stirred at room temperature. After 16 h, this mixture was filtered through diatomaceous earth (∼300 mg), which was washed with CH_2Cl_2 (∼8 mL). The combined filtrates were concentrated and the residue was purified on silica gel (10:1:1000 HCO_2H:*i*-Pr:$CHCl_3$; 1:9 MeOH:$CHCl_3$) to give a 1:0.5 mixture of ester

(62) The carbon signals of the trifluoroacetate counterion were typically not observed because of coupling with the fluorine atoms and line broadening resulting from exchange.

(63) Excess signals are attributed to water.

14b and triphenylphosphine oxide (as determined by ^{1}H NMR analysis). This sample was carried forward without further purification.

A solution of triethylammonium formate (1 M in THF, 0.080 mL, 0.080 mmol) was added to a solution of this sample of ester **14b**, palladium tetrakis(triphenylphosphine) (9.0 mg, 0.008 mmol) and THF (0.80 mL). This solution was maintained at room temperature for 10 h. The reaction then was concentrated onto diatomaceous earth and the residue was purified on silica gel (1000:5:10 $CHCl_3$:*i*-PrOH:HCO_2H; 1:9 MeOH:$CHCl_3$) to give 11 mg (0.021 mmol, 54%) of crambescidin 657 derivative **10b** as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.35 (br s, 1H), 10.02 (br s, 1H), 5.64−5.70 (m, 1H), 5.48 (d, J = 10.2 Hz, 1H), 4.51 (d, J = 8.7 Hz, 1H), 4.29 (dt, J = 9.2, 5.13 Hz, 1H), 4.14−4.21 (m, 1H), 4.06−4.13 (m, 1H), 3.96−4.04 (m, 1H), 3.90−3.96 (m, 1H), 2.95 (d, J = 5.0 Hz, 1H), 2.54−2.64 (m, 2H), 2.14−2.39 (m, 7H), 1.50−1.92 (m, 13H),[63] 1.30−1.47 (m, 5H), 1.14−1.27 (m, 2H), 1.04 (d, J = 6.2 Hz, 3H), 0.83 (t, J = 7.2 Hz, 3H); ^{13}C (125 MHz, $CDCl_3$) 177.0, 168.2, 148.8, 133.6, 129.9, 83.7, 80.7, 70.9, 67.1, 65.0, 53.9, 53.4, 51.9, 50.5, 37.0, 36.3, 35.2, 32.0, 29.7, 29.1, 28.2, 26.7, 25.2, 24.8, 23.6, 21.5, 18.2, 14.1, 10.1 ppm; IR (film) 2926, 2856, 2362, 2339, 1733, 1656, 1613 cm^{-1}; HRMS (ES) calculated for $C_{28}H_{43}N_3O_6$ ($M + H^+$): 518.3230, found: 518.3224.

Other crambescidin 657 derivatives described in Scheme 5 were prepared similarly. Samples for biological testing were purified by both silica gel chromatography (as described) and C18 reverse phase preparative HPLC (30:70−95:5 MeCN:0.1% CF_3CO_2H (v/v) in H_2O).

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Supporting Information Available: A. General experimental details; experimental procedures and characterization data for all new compounds not reported in Experimental Section of the paper; Tabular NMR data for crambescidin 359 (**9**); descriptions of the deuterium incorporation and decarboxylation experiments as well as tables of raw data; CIF file for X-ray structure of **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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