FEATURE ARTICLE

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The tethered Biginelli condensation in natural product synthesis

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Received (in Cambridge, UK) 19th August 2003, Accepted 1st October 2003
First published as an Advance Article on the web 27th October 2003

This review describes the development of the tethered Biginelli condensation and its application to the total synthesis of structurally complex, bioactive guanidine alkaloids.

The discovery of biologically active molecules is critical for both the development of new therapeutics[1] and to provide tools for the elucidation of cellular processes.[2] In the search for these compounds, natural extracts, collections of synthetic compounds archived by pharmaceutical companies, and combinatorial libraries of synthetic compounds are screened. Natural products play a particularly important role in the anticancer and anti-infective arenas as greater than 60% of the drugs in these therapeutic areas approved between the years of 1983 and 1994 were derived from natural products.[3] The continued prominence of natural products as lead structures can be attributed to the greater structural diversity of natural products than typical combinatorial libraries or compound collections.[2]

In recent decades, the ocean has served as a plentiful source of biologically active organic molecules.[4] Marine sponges, in particular, have yielded a diverse range of structurally novel alkaloid natural products.[4,5] Among the most noteworthy of these alkaloids are polycyclic guanidines such as the crambescidins, represented by ptilomycalin A (**1**),[6] crambescidin 800 (**2**)[7] and 13,14,15-isocrambescidin 800 (**3**);[8] the batzelladines, represented by batzelladine B (**5**)[9] and batzelladine F (**6**);[10] and the structurally simpler crambines, represented by crambescin A (**4**)[11] (Fig. 1).

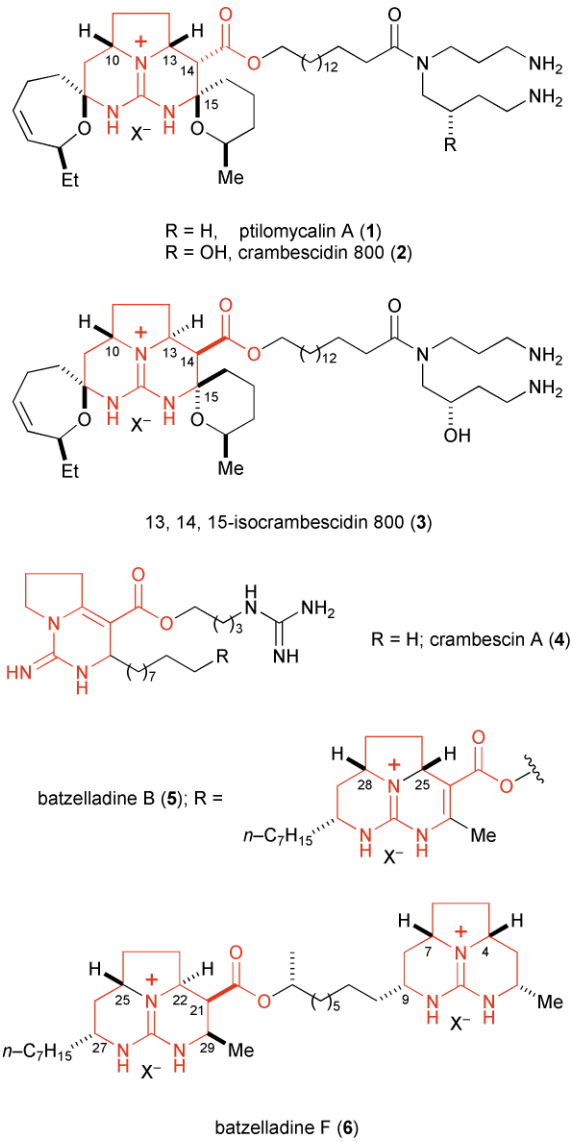

Fig. 1 Guanidine alkaloids from marine sponges.

Molecules containing a guanidine functional group have a high incidence of biological activity,[12] which we attribute to the myriad ways the guanidine functional group can participate in non-covalent interactions. Crambescidin 800 (**2**), and various other crambescidin alkaloids, exhibit low to mid nanomolar activities against several human cancer cell lines and are currently in early phase development as antineoplastic agents.[6,7,13–15] Batzelladines A and B (**5**) were the first low molecular weight natural products reported to inhibit the binding of HIV gp-120 to human CD4, a critical step in the life

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Larry E. Overman was born in Chicago, Illinois, in 1943 and raised in Hammond, Indiana. He obtained a B.A. degree from Earlham College in 1965, and completed his doctoral dissertation in 1969 with Professor Howard W. Whitlock, Jr, at the University of Wisconsin. After a NIH postdoctoral fellowship with Professor Ronald Breslow at Columbia University, he joined the faculty at the University of California, Irvine, in 1971, where he is now Distinguished Professor of Chemistry. Professor Overman's research centers on the invention of new reactions and strategies in organic synthesis and the total synthesis of natural products and their congeners. Using chemical synthesis strategies developed in his laboratory, Professor Overman's group has completed total syntheses of more than 80 structurally diverse natural products.

DOI: 10.1039/b309910e

cycle of the AIDS virus.[9] Batzelladine alkaloids also are described as inhibitors of other protein–protein interactions. For example, batzelladines F (**6**) and G induce dissociation of the protein tyrosine kinase p56 from its complex with CD4,[10] and various batzelladine alkaloids and analogs inhibit HIV envelope-mediated cell fusion.[16]

As marine natural products are typically available in only minute amounts from natural sources, total or partial synthesis is playing an important role in the development of most marine-derived therapeutics. In this review, we summarize recent research in our laboratories that has led to practical methods for preparing guanidine alkaloids of the crambescidin and batzelladine families. The success of these efforts can be attributed to the development of a new chemical transformation, the tethered Biginelli condensation. The origins of this transformation, its current status, and its strategic use in the total synthesis of guanidine alkaloids of the crambescidin and batzelladine families are the topics of this account.

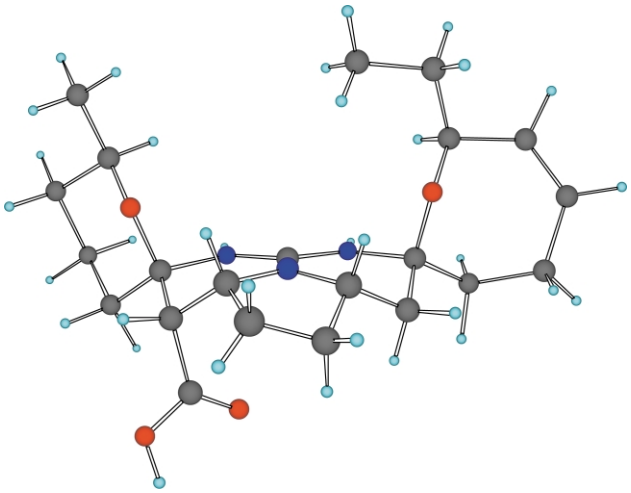


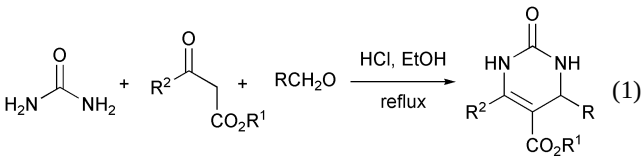
Fig. 2 Model of the pentacyclic crambescidin core.

Synthesis planning

The crambescidins and batzelladines exhibit a common structural feature, the tricyclic hydro-5,6,6a-triazaacenaphthalene moiety, which is shown in red in the structures depicted in Fig. 1. This unit is found with both the *cis* and *trans* stereorelationships of the angular hydrogens flanking the pyrrolidine nitrogen. When our studies began in 1992, there existed no stereocontrolled method for constructing this triazaacenaphthalene ring system.[17] Ptilomycalin A (**1**) and crambescidin 800 (**2**) were the focus of our initial synthesis planning as the batzelladine alkaloids and isocrambescidins had not been reported at the time our investigations in this area were initiated.

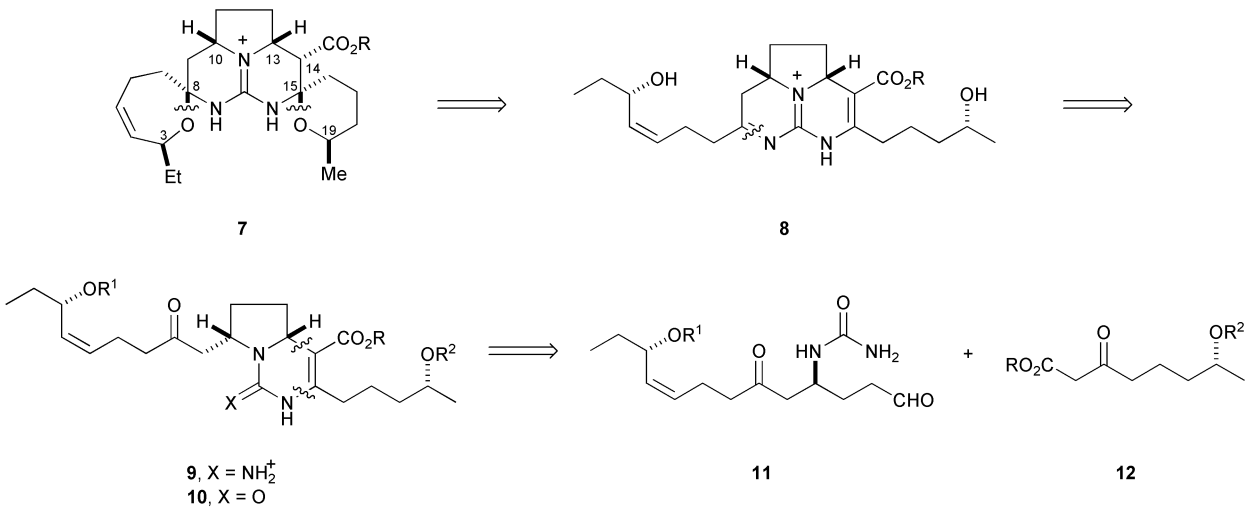
The development of our synthesis strategy was influenced strongly by the topography of the pentacyclic ring system of these alkaloids, depicted in Fig. 2. As seen in this molecular mechanics model,[18] the central decahydro-5,6,6a-triazaacenaphthalene ring system is nearly planar, with the two oxygen atoms that form the distinctive spiroaminal units of these alkaloids oriented in the same direction perpendicular to this plane.

The axial nature of the aminal oxygens suggested that the cyclic ethers of ptilomycalin A (**1**) and crambescidin 800 (**2**) would self assemble in the proper configuration about a *cis*-triazaacenaphthalene moiety. This supposition proved correct, as demonstrated by concurrent synthetic efforts at Irvine and in Barry Snider's laboratory at Brandeis.[19] Disconnecting the C8 and C15 spiro centers of **7** leads to the *cis*-hexahydro-5,6,6a-triazaacenaphthalene **8** (Scheme 1). Subsequent ring opening of the imine linkage of this intermediate produces 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester **9**, a logical precursor of which is the congeneric urea **10**. Michael Rabinowitz, the postdoctoral researcher who first took up this problem at Irvine, posited that urea **10** might be generated from the condensation of a ureido aldehyde **11** and a β-ketoester **12**. This postulated reaction would be a novel variant of the venerable three-component Biginelli construction of dihydropyrimidinones, depicted in eqn. (1).[20]



(1)

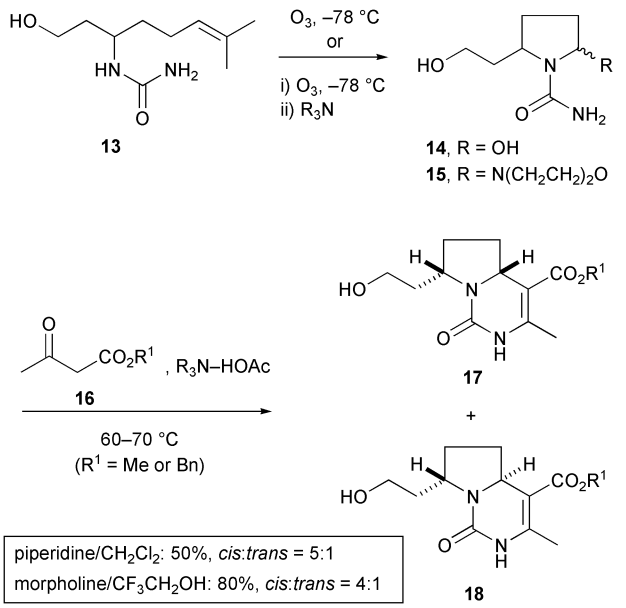
The prospect of assembling intermediate **10** in this way was attractive for at least three reasons. First was the high convergency of this plan as it would divide the crambescidin core into two fragments of similar complexity. Second, no intramolecular variant of the Biginelli condensation had been reported at the time.[21] Third, the proposed Biginelli condensation would examine the possibility that a stereocenter on the tether connecting the urea and aldehyde reactants could control formation of the angular (C13) stereocenter, a prospect that gained additional appeal from the absence of previous uses of the Biginelli condensation in stereocontrolled organic synthesis.



Scheme 1 Retrosynthetic analysis of crambescidin 800 (**2**)/ptilomycalin A (**1**).

Tethered Biginelli condensations

It was not clear at the outset that the tethered Biginelli condensation depicted in Scheme 1 would generate the required *cis* stereoisomer of **10**. We felt that *N*-aminoacyl-2,5-disubstitutedpyrrolidines were plausible intermediates of this reaction, provided that the tethered Biginelli condensation was run under Knoevenagel conditions (see later mechanistic discussion). Our thinking at the time was that the greater thermodynamic stability of the *cis* stereoisomer of such intermediates might translate to a preference for forming the *cis* stereoisomer of the 1-oxohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester product.[22] This stereoselectivity indeed was observed in our initial examination of the tethered Biginelli reaction. In this study, Rabinowitz found that condensation of pyrrolidine derivative **14** and methyl acetoacetate in the presence of piperidinium acetate at elevated temperatures in CH_2Cl_2 resulted in the formation of the crystalline *cis*- and *trans*-hydropyrrolopyrimidinone esters **17** and **18** in a 5 : 1 ratio and 50% overall yield from unsaturated acyclic urea **13**, the precursor of **14** (Scheme 2).[23]

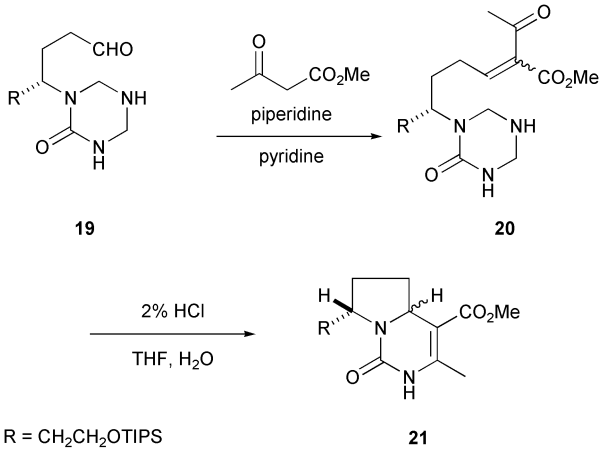


Scheme 2 The tethered Biginelli condensation.

Tethered Biginelli condensations were subsequently studied in more detail by two Irvine graduate students, Paul Renhowe and Andrew McDonald. These workers showed that higher yields, and in some cases higher stereoselectivities, were realized in polar, protic solvents such as methanol or trifluoroethanol.[24] Renhowe also found that the reaction gave higher yields and improved stereoselectivity in the presence of morpholinium acetate (pK_a = 8.3) than with piperidinium acetate (pK_a = 11.1).[25]

Intermediates such as **14** are complex mixtures that contain traces of the acyclic ureido aldehyde, the depicted 2-hydroxypyrrolidine isomer, as well as higher molecular weight oligomers. This heterogeneity was somewhat batch dependent and adversely affected the reproducibility of tethered Biginelli condensations. McDonald discovered that generation of these ureido aldehydes in the presence of morpholinium acetate produced a less heterogeneous intermediate that was largely the morpholine aminal **15**. Use of this intermediate in the tethered Biginelli condensation provided higher yields (up to 80%) of **17** and **18** in more robust reactions.[24]

In the Atwal modification of the Biginelli reaction, the β-ketoester and aldehyde are condensed prior to reaction with a urea derivative.[26] In an effort to avoid highly polar and heterogeneous intermediates such as **14** and **15**, Frank Stappenbeck, a postdoctoral researcher in the laboratory, briefly examined a sequence in which the urea would be masked as a triazone allowing the tethered Biginelli condensation to be carried out in a stepwise fashion (Scheme 3).[22] Knoevenagel

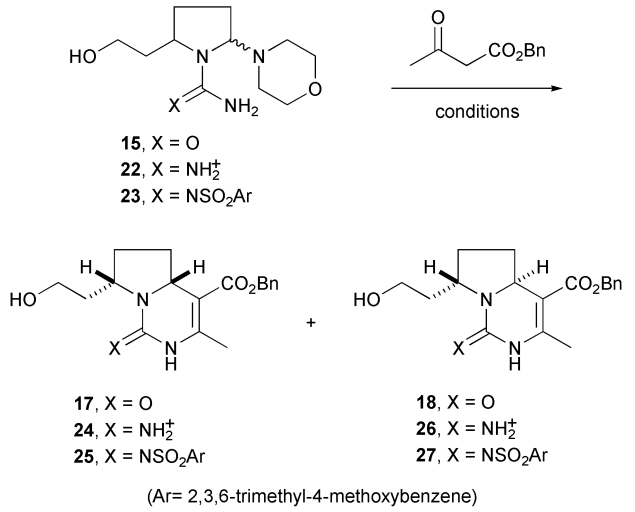


Scheme 3 A stepwise tethered Biginelli condensation.

condensation of triazone aldehyde **19** with methyl acetoacetate efficiently generated **20**. However, subsequent removal of the triazone protecting group under mildly acidic conditions resulted in the formation of the Biginelli adducts **21** in low yields and with poor stereoselectivity. This approach was not explored further.

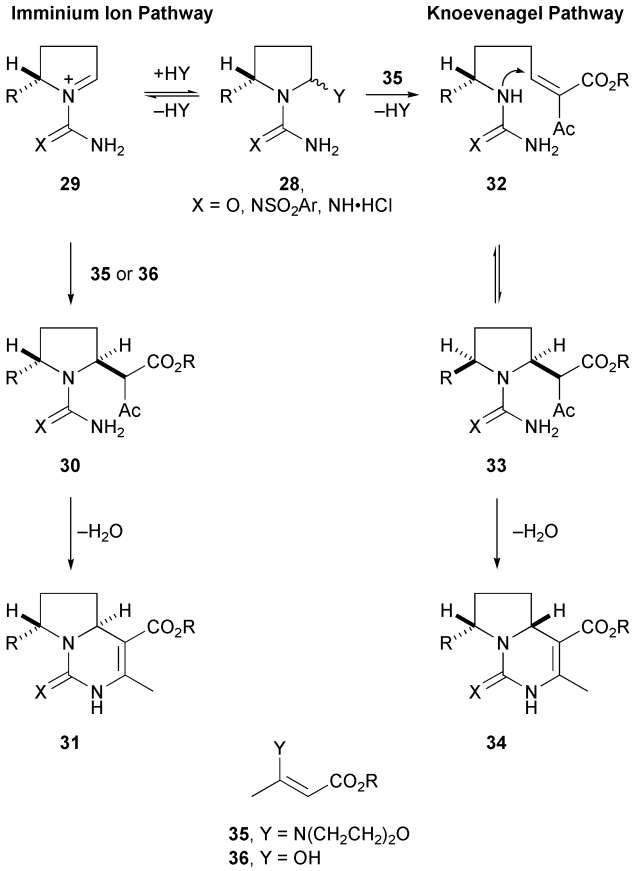
In 1993, isocrambescidin 800 (**3**)[8] was reported, and a few years later batzelladines A and D.[9] As the decahydro-5,6,6a-triazaacenaphthalene moieties of these alkaloids have the *trans* orientation of the angular hydrogens of the 5-membered ring, we became interested in the possibility that tethered Biginelli condensations carried out under other conditions might selectively form *trans*-hexahydropyrrolo[1,2-*c*]pyrimidine esters. McDonald quickly found that carrying out the condensation of aminal **15** in the presence of the mild dehydrating agent polyphosphate ester (PPE) resulted in a reversal of stereoselectivity giving a 4 : 1 mixture of the *trans* and *cis* products **18** and **17** in 60% overall yield from the acyclic urea precursor **13** of pyrrolidine aminal **15** (Scheme 4).[24] He also discovered that tethered Biginelli condensations of guanidine precursors accomplished under Knoevenagel conditions generated the *trans*-1-iminohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester product **26** with excellent (> 15 : 1) stereoselectivity.

To explore the origins of the observed reversals of stereoselectivity, Biginelli condensations of the *N*-arylsulfonylguanidine electrophile **23** were examined.[24] This precursor was chosen to probe electronic effects because the acidity of *N*-sulfonylguanidinium salts is similar to that of protonated ureas (pK_a ~ 1) and quite different from guanidinium salts (pK_a ~ 15). Biginelli reaction of **23** under standard Knoevenagel conditions (morpholinium acetate, trifluoroethanol) gave a 6 : 1 mixture of *cis* and *trans* products in good yield, whereas this reaction promoted by polyphosphate ester (PPE) generated the *trans*-1-iminohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester **27** in high stereoselectivity (20 : 1). To summarize, pyrrolidine aminal substrates containing a urea (X = O) or a *N*-arylsulfonylguanidine (X = NSO_2Ar) unit yield tethered Biginelli products having *cis* stereochemistry around the pyrrolidine ring when the cyclocondensation is promoted with morpholinium acetate, whereas substrates containing an unprotected guanidine (X = NH_2^+) provide the *trans* stereoisomer under identical conditions. Under acidic dehydrating conditions, urea and *N*-arylsulfonylguanidine precursors preferentially form the *trans* product.



Conditions	X = O	X = NH_2^+	X = NSO_2Ar
CF_3CH_2OH, 60 °C morpholine-HOAc	80%, 4:1 (**17**:**18**)	42%, (**26**)	61%, 6:1 (**25**:**27**)
PPE, CH_2Cl_2, 23 °C	60%, 1:4 (**17**:**18**)	N/A	61%, 1:20 (**25**:**27**)

Scheme 4 Tuning stereoselectivity.



Scheme 5 Stereorationale of tethered Biginelli condensations.

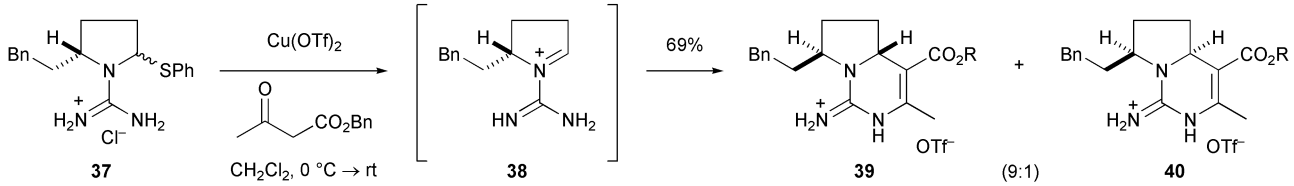
A working hypothesis that rationalizes stereoselection in tethered Biginelli condensations of these substrates under Knoevenagel and acidic conditions is outlined in Scheme 5. We postulate that *cis* stereoselectivity arises from a Knoevenagel pathway, whereas *trans* stereoselectivity originates through the intermediacy of imminium ion **29**. Under Knoevenagel conditions, the urea and *N*-sulfonylguanidine precursors are proposed to follow the Knoevenagel pathway. Molecular mechanics calculations on the malonate congener of *N*-aminoacyl-2,5-disubstitutedpyrrolidine intermediate **33** (R = Me, X = O) show that the *cis* stereoisomer is 1.9 kcal mol^{-1} more stable than the *trans* isomer. Accordingly, cyclization of **32** by a late transition state, or even reversible formation of **33** prior to dehydration, would explain the observed *cis* stereoselection. In contrast, tethered Biginelli condensations of the urea and *N*-sulfonylguanidine precursors in the presence of PPE are proposed to take place by the iminium ion pathway, similar to the mechanism believed to be followed in the classical three-component Biginelli condensation promoted by mineral acids.[22] In acid promoted reactions, stereoselectivity is thought to derive from addition of the β-ketoester-derived nucleophile **35** or **36** to iminium ion **29** from the face opposite the side chain. Tethered Biginelli condensations of the guanidinium substrate under Knoevenagel conditions are also proposed to follow the iminium ion pathway in this case, because loss of HY from the guanidinium precursor to form *N*-amidinyliminium ion **29** (X = NH) should be much more favorable than the generation of related species from the corresponding electron-deficient urea or sulfonylguanidine precursors.

To further explore the mechanism of tethered Biginelli condensations, John Wolfe, when he was a postdoctoral researcher at Irvine, developed an unambiguous method for generating the *N*-amidinyliminium ion intermediate **38** (Scheme 6).[27] In this method, 2-thiophenyl precursor **37** is allowed to react with the thiophilic Lewis acid $Cu(OTf)_2$. When this reaction is carried out in the presence of benzyl acetoacetate, the *trans* tethered Biginelli adduct **39** is produced with 9 : 1 stereoselectivity. As formation of this product by a Knoevenagel pathway is most unlikely, this result leads credence to the proposal that *N*-amidinyliminium ions are intermediates in tethered Biginelli condensations of guanidine precursors.

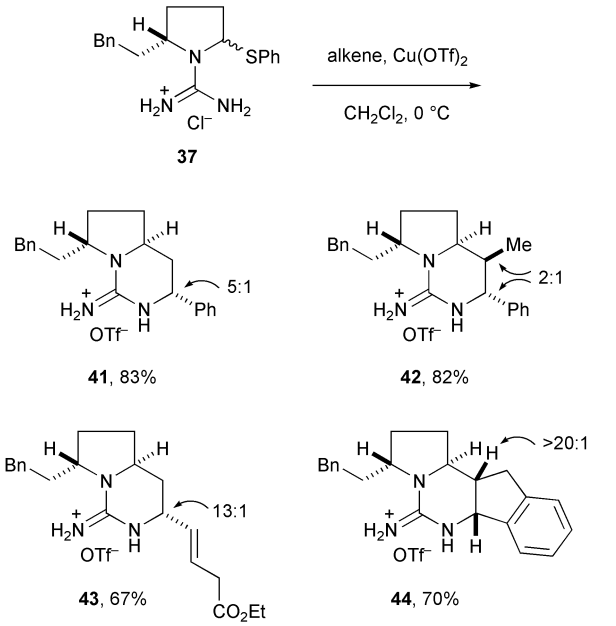
Wolfe also showed that guanidine sulfide **37** reacts with nucleophilic alkenes in the presence of $Cu(OTf)_2$ to provide a variety of 1-iminohexahydropyrrolo[1,2-*c*]pyrimidines (Scheme 7).[27] In all cases, addition of the alkene occurred from the face opposite the phenethyl side chain. Only conjugated alkenes, which could form delocalized carbenium ion intermediates, participate in this reaction. This requirement, and the observation that *cis*- and *trans*-β-methylstyrene both give predominantly adduct **42**, point to a stepwise mechanism for these cyclocondensation reactions.

Total synthesis of crambescidin and batzelladine alkaloids

The utility of the tethered Biginelli reaction for stereocontrolled synthesis of crambescidin and batzelladine alkaloids is now well established, with this chemistry being employed in our



Scheme 6 Copper-initiated tethered Biginelli condensation.



Scheme 7 Cyclocondensations of *N*-amidinyliminium ions

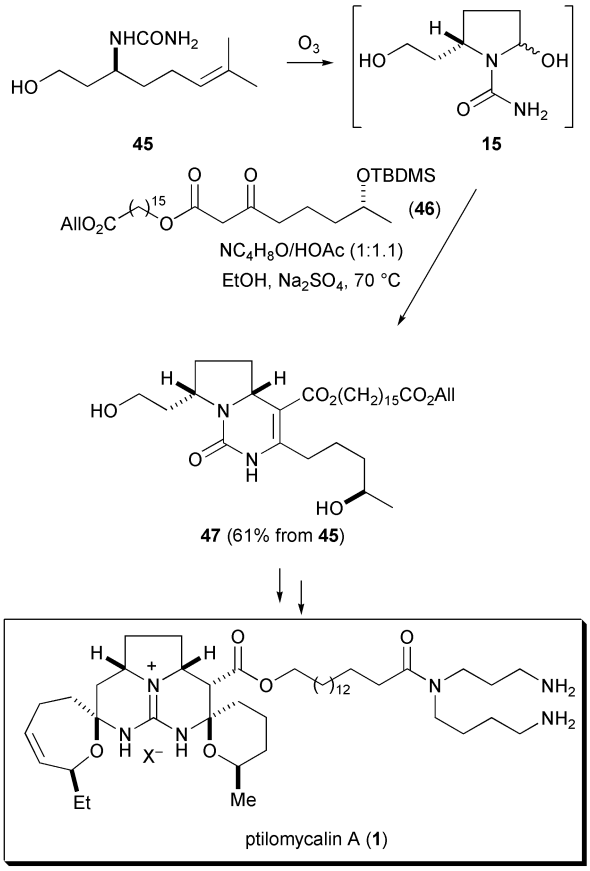
laboratories to accomplish enantioselective total syntheses of ten sponge alkaloids: ptilomycalin A (**1**),[28,29] crambescidins 359,[30] 431,[31] 657,[29] and 800 (**2**),[29] neopfolitispate 2,[29] isocrambescidins 657[32] and 800 (**3**)[32,33] and batzelladines D[34] and F (**6**).[35] Representative total syntheses will be discussed in detail to illustrate different strategic implementations of tethered Biginelli condensations.

Total synthesis of ptilomycalin A and crambescidin 800

Our first total synthesis in this area, initiated by Michael Rabinowitz and completed in 1995 by Paul Renhowe, targeted ptilomycalin A (**1**).[28] In this inaugural effort, we decided to arrive at the pivotal tethered Biginelli condensation as early as possible in the synthetic sequence. Thus, the Biginelli condensation in this synthesis employed the relatively simple intermediate **15** as the electrophilic component (Scheme 8). We also chose to incorporate the 16-hydroxyhexadecanoic acid fragment from the outset. This decision traces back to Kakisawa and co-workers' report that attempted base promoted removal of the ester side chain of ptilomycalin A (**1**) resulted in decomposition of the pentacyclic guanidine core,[6] which suggested to us that late stage ester formation might be problematic.

The opening stages of the total synthesis of ptilomycalin A (**1**) are outlined in Scheme 8. When carried out in ethanol in the presence of morpholine and a slight excess of acetic acid, tethered Biginelli condensation of **15** and β-ketoester **46** proceeded with 9 : 1 stereoselectivity to deliver the *cis* product **47** in 61% overall yield from acyclic unsaturated urea **45**. Hydropyrrolopyrimidine ester **47** was then elaborated in 12 steps to ptilomycalin A (**1**), thereby completing the first total synthesis of a crambescidin alkaloid.[25] As several of the steps in this sequence, notably those required to append carbons 1–7, were quite delicate, we turned to develop the more convergent approach outlined in Scheme 1 in which all atoms of the pentacyclic guanidine core would be incorporated in the two components of the tethered Biginelli reaction.

The enantioselective total synthesis of crambescidin 800 (**2**), following this more convergent strategy, was accomplished by Andrew McDonald (Scheme 9).[29] The first key step of this synthesis was the coupling of two enantioenriched building blocks, the lithium reagent derived from enantiopure alkyl iodide **49** and the Weinreb amide **50**.[36] Iodide **49** was available

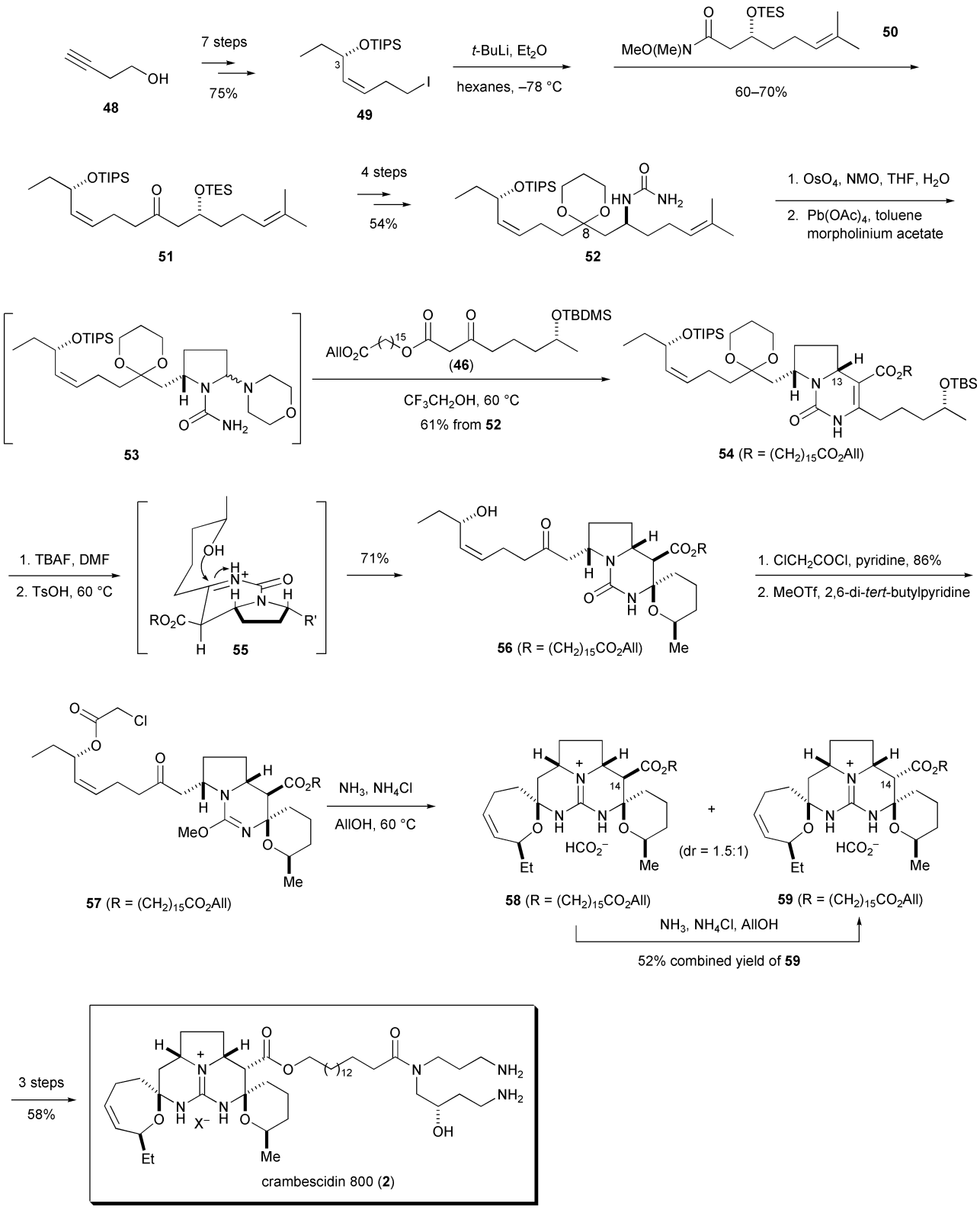


Scheme 8 Total synthesis of ptilomycalin A (**1**).

in 7 steps and 75% yield from 3-butyn-1-ol (**48**) using the catalytic asymmetric addition of diethylzinc to a propargyl aldehyde intermediate to introduce the C3 stereogenic center.[37] The (*R*)-β-siloxy Weinreb amide **50** was also accessed using asymmetric catalysis, in this case ruthenium catalyzed hydrogenation of a β-ketoester intermediate.[38] Lithium–halogen exchange by reaction of iodide **49** with *tert*-butyllithium and subsequent coupling of the derived heptenyllithium with amide **50** gave ketone **51** in 60–70% yield. This β-siloxy ketone was converted in 4 steps to ketal urea **52**. The choice of a 1,3-dioxane to protect the C8 ketone deserves brief comment. In our first examination of this convergent sequence, a 1,3-dioxolane was used to mask the C8 carbonyl group. Difficulties in removing this protecting group at a late stage in the synthesis led to our eventual choice of the more readily cleaved 1,3-dioxane.

To prepare for the tethered Biginelli condensation, unsaturated urea **52** was elaborated in two steps to the ureido aminal **53**. Selective dihydroxylation of the trisubstituted double bond of **52** with osmium tetroxide was critical to the success of this sequence. Biginelli condensation of **53** with β-ketoester **46** generated hydropyrrolopyrimidine ester **54** and the corresponding C13 epimer in a 6–7 : 1 ratio and 61% yield. Although these epimers could be separated, it was more convenient to process this mixture to a later stage where isomer separation was easy.

Hydropyrrolopyrimidine ester **54** was transformed to the pentacyclic ester **59** by a five step sequence. Removal of the silyl protecting groups of **54**, followed by exposure of the resulting diol to warm *p*-toluenesulfonic acid generated the spirocyclic tetrahydropyran with concomitant discharge of the ketal protecting group. The high stereoselectivity observed in this transformation is the result of kinetic control and undoubtedly derives from two stereoelectronic factors: preferential axial protonation of the endocyclic enamine unit of **54** to



Scheme 9 Total synthesis of crambescidin 800 (**2**).

generate *N*-acyliminium intermediate **55** and axial addition of the tethered secondary alcohol nucleophile. After protecting the side chain allylic alcohol of **56** as a chloroacetate, sequential reaction of this intermediate with methyl triflate and ammonia delivered a mixture of epimeric pentacyclic esters **58** and **59** in high yield. The high stereoselectivity observed in forming the 7-membered spirocyclic ether is attributed once again to a stereoelectronic preference for axial incorporation of the alcohol side chain. The 1.5 : 1 mixture of C14 epimers produced in this reaction represents the equilibrium mixture, which is established under the basic aminolysis conditions. Separation of the desired axial epimer **59** by chromatography and recycling the equatorial epimer **58** allowed pentacyclic ester **59** to be obtained in 52% overall yield from the tricyclic chloroacetate intermediate. The hydroxyspermidine end group was then appended in three steps to deliver enantiopure crambescidin 800 (**2**). As the configuration of the hydroxyspermidine fragment of crambescidin 800 (**2**) had not been established, the hydrox-

yspermidine epimer of **2** was also prepared to resolve this uncertainty.

The convergent total synthesis of crambescidin 800 (**2**) summarized in Scheme 9 was accomplished in 3% overall yield from commercially available 3-butyn-1-ol by way of 16 isolated and purified intermediates. This sequence was subsequently scaled up and streamlined by PharmaMar and is being used to provide preclinical supplies of crambescidin 800 (**2**).[39] The flexibility available in this synthetic route has allowed a range of crambescidin alkaloids and analogs to be prepared in ongoing studies at Irvine to develop structure activity relationships (SAR) and to search for the molecular targets of these anticancer agents.

Total synthesis of 13,14,15-isocrambescidin 800 (3)

To illustrate the use of tethered Biginelli condensations of guanidine precursors, we will briefly consider the total synthesis of 13,14,15-isocrambescidin 800 (**3**) initiated by Frank Stappenbeck and completed by Scott Coffey, another postdoctoral associate.[32,33] In 1991, Rinehart and co-workers described the isolation of isocrambescidin 800 from the same sponge, *Crambe crambe*, that provided crambescidin 800.[8] A molecular mechanics model of the 13,14,15-isocrambescidin 800 core acid is shown in Fig. 3. The C10 and C13 angular hydrogens are *trans* in the isocrambescidin core rather than *cis* as they are in the corresponding fragment of crambescidin 800. Moreover, in this iso series, the spirocyclic ethers are oriented on opposite faces of the central tricyclic decahydro-5,6,6a-triazaacenaphthalene moiety. However, as the C–O bonds of

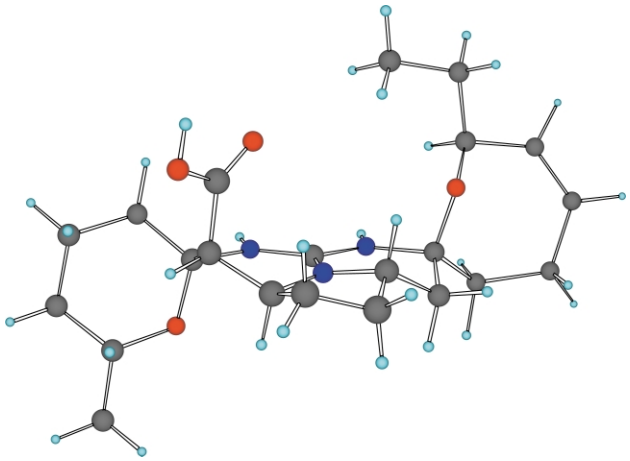
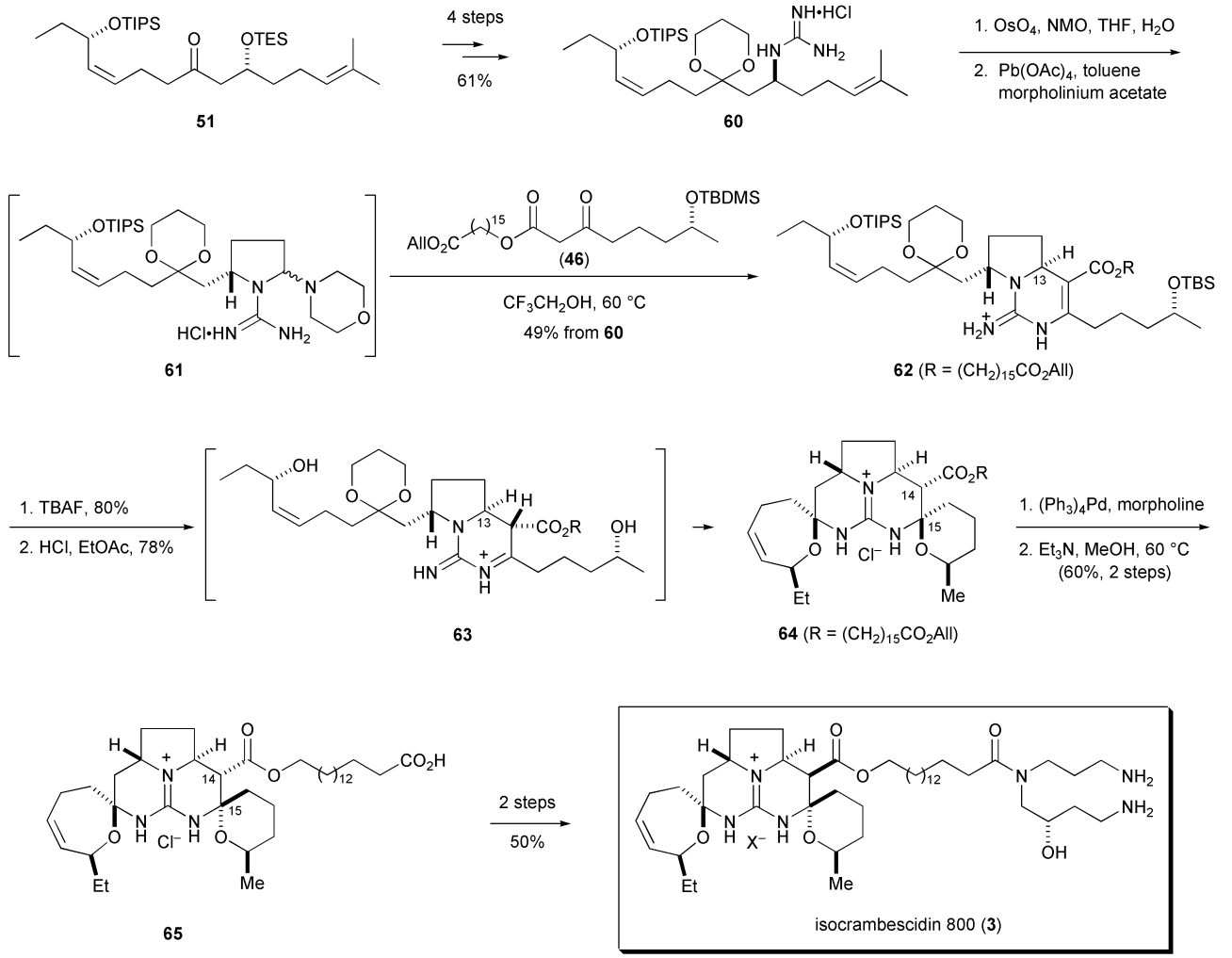


Fig. 3 Model of the isocrambescidin core.

both of these fragments are axial, much like in the crambescidin/ptilomycalin A series, we anticipated that the C8 and C15 spirocenters of 13,14,15-isocrambescidin 800 would evolve directly with the desired three-dimensional orientation if the C10 and C13 hydrogens of a triazaacenaphthalene precursor were *trans*.

The starting point for this synthesis was enantiopure ketone **51**, which was converted to guanidine acetal **60** in four steps and 61% yield (Scheme 10). Selective dihydroxylation of **60** using osmium tetroxide, followed by lead tetraacetate cleavage of the resulting diol generated *N*-amidinylpyrrolidine aminal **61** as a



Scheme 10 Total synthesis of 13,14,15-isocrambescidin 800 (**3**).

complex mixture of species. Tethered Biginelli condensation of this intermediate with β-ketoester **46** in trifluoroethanol proceeded with 7 : 1 diastereoselectivity to provide *trans*-1-iminohexahydropyrrolo[1,2-*c*]pyrimidine ester **62** in 49% yield. Following deprotection of the silyl ethers of **62** with TBAF, the resulting diol was transformed in one step to pentacyclic guanidine **64**. After some initial confusion,[33] we ultimately realized that although the 7-membered spirocyclic ether had evolved as desired, the spiro hydropyran unit of this product was epimeric to that found in 13,14,15-isocrambescidin 800 (**3**). Careful mechanistic examination of this cyclization by Scott Coffey eventually revealed that **64** resulted from kinetically-controlled axial protonation of the endocyclic enamine functionality of **62** to give **63**, followed by thermodynamically-controlled spirocyclization.[32]

Fortunately in this series the most stable of the four possible C14 and C15 stereoisomers is the desired one. Thus, following palladium mediated deprotection of the terminal allyl ester, the pentacyclic core could be epimerized at both C14 and C15 by heating this guanidinium carboxylate in methanol in the presence of triethylamine to provide pentacyclic acid **65** in 60% overall yield. The synthesis of 13,14,15-isocrambescidin 800 (**3**) was then completed in two additional steps. The total synthesis of 13,14,15-isocrambescidin 800 (**3**) was accomplished in 3.3% overall yield from 3-butyn-1-ol by way of 14 isolated intermediates. This total synthesis, besides establishing the configuration of the hydroxyspermidine unit of **3**, was suitably efficient to provide for the first time sufficient amounts of this rare alkaloid for biological evaluation.

Total synthesis of batzelladine alkaloids

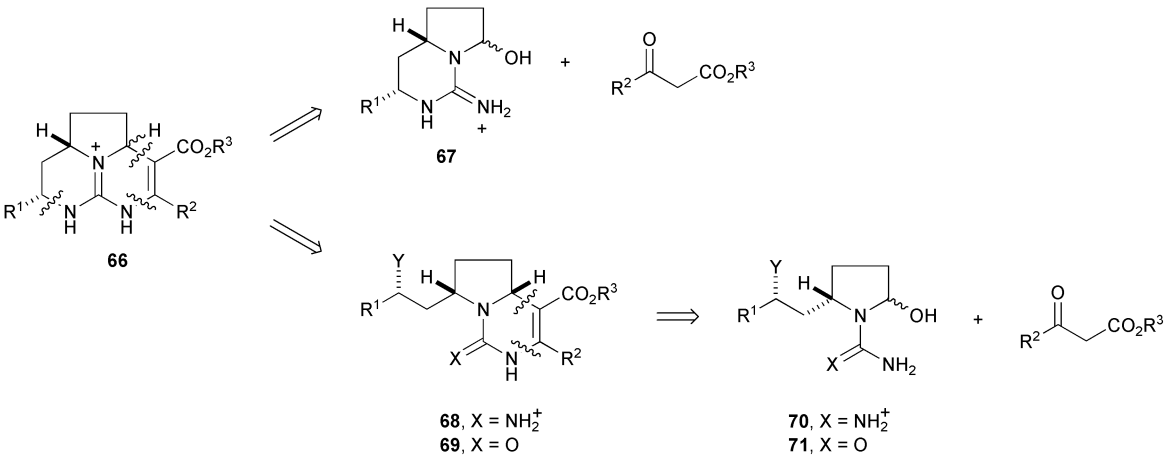
Members of the batzelladine family of guanidine alkaloids are rare examples of small molecules that inhibit protein–protein interactions.[9,10] These guanidine alkaloids are related in structure to the crambescidin alkaloids as they also contain hydro-5,6,6a-triazaacenaphthalene moieties (Fig. 1). This ring system is found in the batzelladine alkaloids with both the *cis* and *trans* configuration of the angular hydrogens, and in various degrees of saturation, for example, an octahydro derivative in batzelladine B (**5**) and decahydro congeners in batzelladine F (**6**). To further develop tethered Biginelli reactions as well as provide these alkaloids and analogues for biological studies, Alison Franklin, a postdoctoral researcher in the group, started in 1995 to define practical synthetic routes to batzelladine alkaloids.[40,41]

As depicted in Scheme 11, tethered Biginelli reactions could be orchestrated in at least two different ways to build a tricyclic octahydro-5,6,6a-triazaacenaphthalene such as **66**. Most directly, tethered Biginelli condensation of a bicyclic precursor such as **67** with a β-ketoester could deliver octahydro-5,6,6a-triazaacenaphthalene **66**. The stereoselectivity of this condensation was unclear at the outset because the bicyclic ring system of guanidine **67** would undoubtedly influence stereoselection. Alternatively, in a sequence akin to that employed in our crambescidin alkaloid total syntheses, 1-iminohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester **68** or 1-oxohexahydropyrrolo[1,2-*c*]pyrimidine carboxylic ester **69** could be assembled from pyrrolidine derivatives **70** or **71**. In this case, condensations carried out under Knoevenagel conditions would deliver the *trans* stereoisomer in the guanidine series and the *cis* stereoisomer in the urea series. As depicted in Scheme 11, if the side chain of **68** or **69** possessed a leaving group at C2, one should be able fashion the third ring by an intramolecular S_N2 displacement.

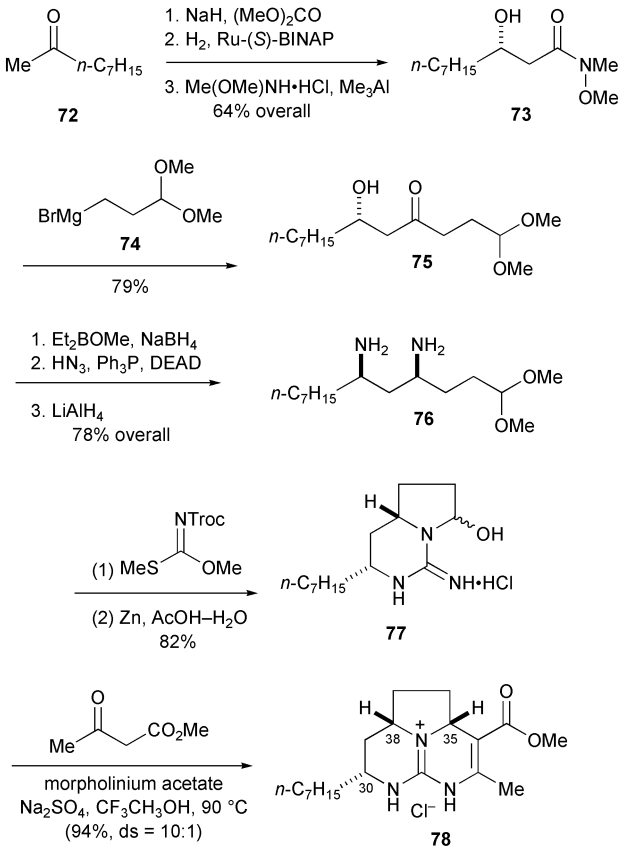
Enantioselective synthesis of the tricyclic guanidine fragment of batzelladine B

At the outset of our efforts in this area, the more convergent of the sequences outlined in Scheme 11 was developed by Alison Franklin to prepare the methyl ester derivative **78** of the octahydro-5,6,6a-triazaacenaphthalene fragment of batzelladine B (Scheme 12).[41,42] This synthesis began with Claisen condensation of dimethyl carbonate with 2-nonanone (**72**) to generate the corresponding β-ketoester in good yield. Asymmetric Noyori reduction[38] of this intermediate, followed by conversion of the (*S*)-β-hydroxyester product to the corresponding Weinreb amide gave **73** in 64% yield and 95% ee. Condensation of this product with Grignard reagent **74** provided β-hydroxy ketone **75**, which was stereoselectively reduced to the corresponding *syn* 1,3-diol.[43] This diol was elaborated in 4 steps to bicyclic guanidine **77** by way of the *syn*-1,3-diamine derivative **76**.

Tethered Biginelli condensation of a two carbon side chain homolog of **77** with allyl or methyl acetoacetate was studied under a variety of reaction conditions.[41] These reactions gave a mixture of the three possible stereoisomeric octahydro-5,6,6a-triazaacenaphthalene esters in moderate to excellent yield. In general, *cis* stereoselection predominated, with the degree of selectivity increasing with the polarity of the solvent. For example, Biginelli condensation of **67** (R^1 = *n*-C_9H_{19}) with methyl acetoacetate in the presence of morpholinium acetate at room temperature in trifluoroethanol provided the *cis*- and *trans*-octahydro-5,6,6a-triazaacenaphthalene esters **66** (R^1 = *n*-C_9H_{19}, R^2 = R^3 = Me) in a 97 : 3 ratio, however more than 1 week was required for complete consumption of the starting material. At 60 °C, this condensation took place at a reasonable rate with only a moderate loss of stereoselectivity. When these conditions were employed in the tethered Biginelli condensa-



Scheme 11 Strategies for triazaacenapthalene synthesis.



Scheme 12 Synthesis of batzelladine B methyl ester (**78**).

tion of **77** with methyl acetoacetate the reaction proceeded in 10 : 1 diastereoselectivity to afford **78**, $[\alpha]^{25}_{D}$ = +97, in 82% yield. As the dextrorotatory methyl ester **78** had been generated by methanolysis of batzelladine B,[9] this enantioselective synthesis established, for the first time, the absolute configuration of the tricyclic guanidine portion of batzelladine B (**5**). Notable is the efficiency of this sequence, the enantiopure batzelladine B methanolysis product **78** being accessed in 10 steps and 25% overall yield from 2-nonanone and methyl acetoacetate.

cis Stereoselection in tethered Biginelli reactions of bicyclic guanidine intermediates such as **77** is believed to arise from a torsional effect. As illustrated in Fig. 4 for the methyl analog **79**

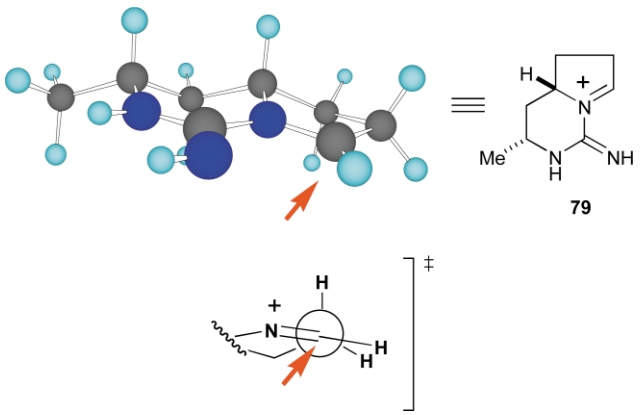
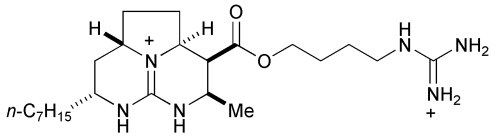


Fig. 4 Rationale for stereoselectivity.

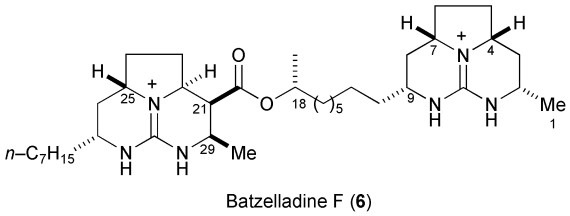
of the iminium cation that would be generated from **77**, attack of the β-ketoester-derived nucleophile from the face opposite the angular hydrogen would be favored. Because no conditions were found in this inaugural study to cleanly deliver the *trans* stereoisomer of octahydro-5,6,6a-triazaacenaphthalene esters **66** from bicyclic guanidine precursors, the more lengthy alternative sequence outlined in Scheme 11 was developed by graduate student Sylvie Ly Sakata to prepare batzelladine D (**80**).[34] As we will next discuss, this chemistry was further refined by Frederick Cohen, also a graduate student at the time, to prepare batzelladine F (**6**).



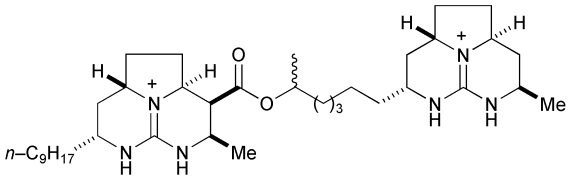
Batzelladine D (**80**)

Enantioselective total synthesis and definition of structure of batzelladine F (6)

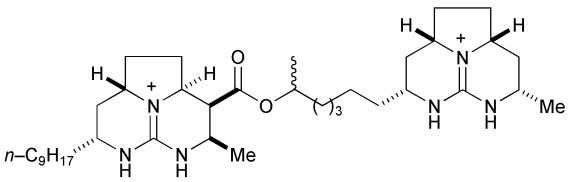
Patil and co-workers reported in 1997 the isolation of batzelladine F from a red Jamaican sponge incorrectly identified at the time as *Batzella* sp.[10] What ultimately proved in 2001 to be the correct structure of batzelladine F (**6**) is depicted in Fig. 5, together with the structure originally proposed in 1997, **81**,



Batzelladine F (**6**)



81
structure originally proposed in 1997



82
one of several possible structures circa 1999

Fig. 5 Batzelladine F (**6**) and incorrect earlier proposed structures.

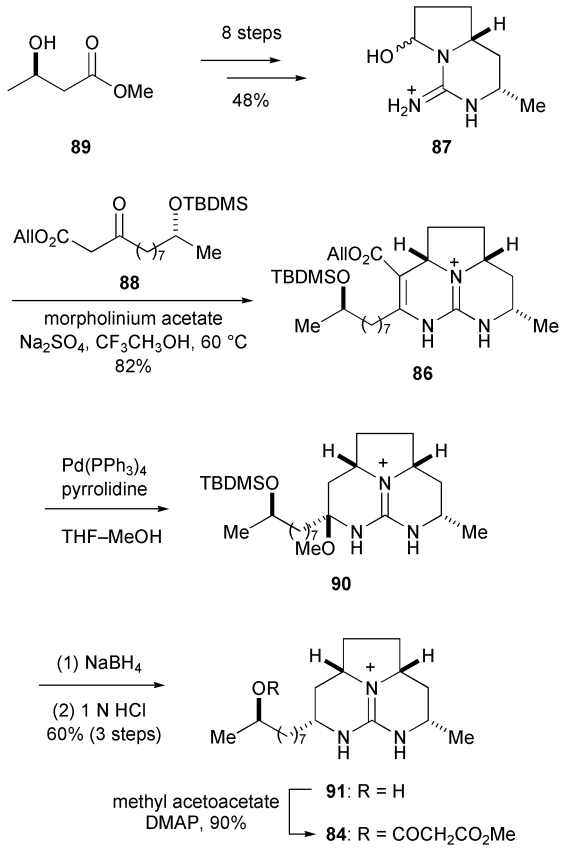
and a revised formulation **82** that the Murphy group, the Snider group, and our group arrived at nearly simultaneously in early 1999 from our respective syntheses of tricyclic model compounds.[40b,d,44] In **82**, the right hand tricyclic decahydro-5,6,6a-triazaacenaphthalene fragments is correctly posited to have a *cis* stereorelationship between the angular hydrogens at C4 and C7, whereas the right hand tricyclic guanidine moiety has a *trans* relationship of the comparable hydrogens.

Our discussion will begin at the time structure **82** emerged as one of several possible three-dimensional formulations for batzelladine F. Structure **82** depicts two of the eight stereoisomers that were believed at the time could be batzelladine F, the ambiguity arising because no information was available concerning the absolute configuration of this alkaloid, its relative configuration at C18, or the relative configurations of the two tricyclic guanidine fragments. Besides these configurational uncertainties, our total synthesis of batzelladine F (**6**) had

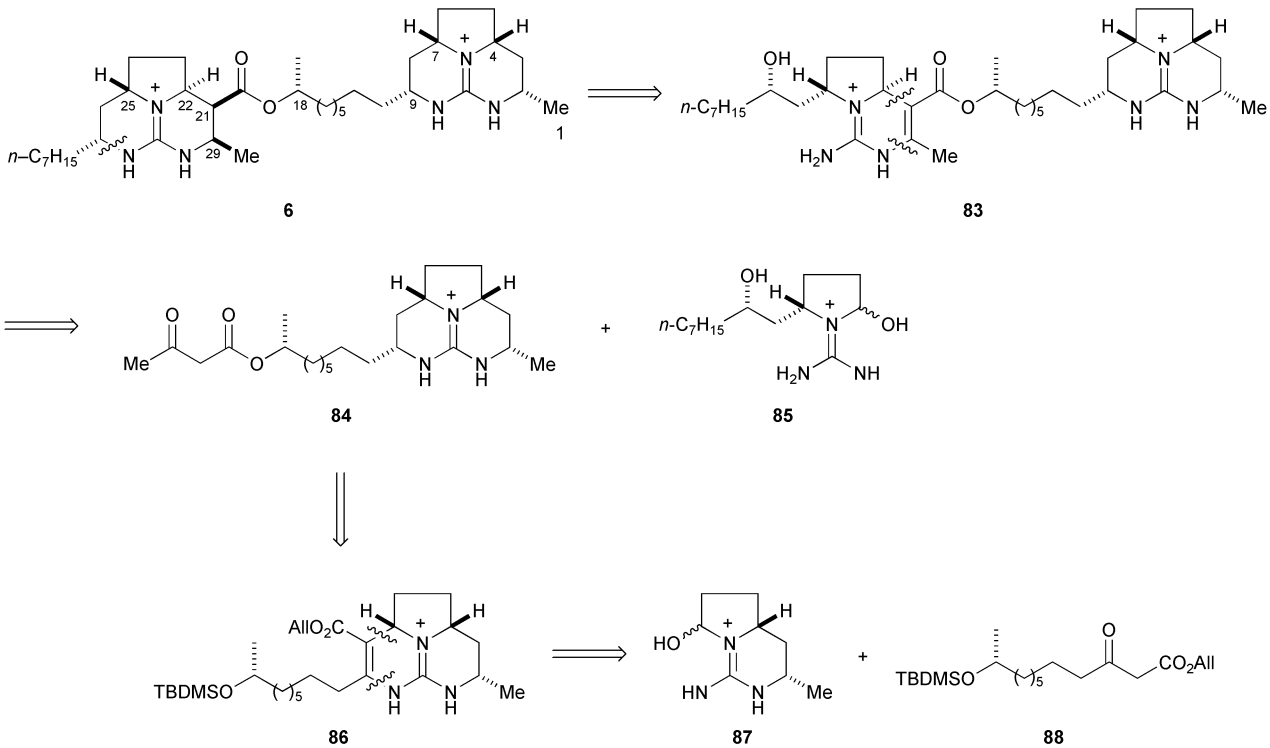
to confront several additional synthetic challenges. Foremost was the degree of modification required to transform tethered Biginelli products to the left and right hand tricyclic units of batzelladine F. As these elaborations would undoubtedly each involve several steps, it was essential that the strategy be as convergent as possible. The approach we ultimately settled upon is summarized in Scheme 13 in the context of preparing **6**. We envisaged the right hand tricyclic guanidine as evolving from pentacyclic bisguanidine **83** by ring closure and reduction of the C21–C29 double bond. In the central step of this plan, **83** was to be assembled by tethered Biginelli condensation of guanidine β-ketoester **84** and tethered guanidine aldehyde **85**. This pivotal step would both combine the two guanidine fragments and set the stereorelationship between C22 and C25. Decahydrotriazaacenaphthalene **84** was seen as deriving from the tethered Biginelli adduct **86** of guanidine aldehyde **87** and β-ketoester **88** by decarboxylation and reduction.

The chemistry eventually employed by Fred Cohen to prepare batzelladine F was worked out during initial total syntheses of the proposed structures of batzelladine F in which a 7 carbon chain connects the guanidine units and the right hand tricyclic guanidine has a nine-carbon side chain.[44] Hexacyclic guanidine **82** and one enantiomer of the three additional possible relative stereoisomers having this connectivity were synthesized and each shown to be different from batzelladine F by HPLC and mass spectrometric comparisons. Re-analysis of MS fragmentation patterns of batzelladine F (**6**) ultimately convinced us that the natural product has 9 carbons in the chain connecting the guanidines and 7 carbons in the right-hand side chain. Fortunately, by this time the chemistry was sufficiently well worked out that during a 4½ month period, Fred Cohen was able to prepare batzelladine F (**6**) and one enantiomer of the three other possible relative stereoisomers.

The synthesis of batzelladine F (**6**) began by preparing the right hand tricyclic guanidine unit (Scheme 14). Bicyclic guanidine hemiaminal **87** was fashioned in 48% overall yield from methyl (*R*)-3-hydroxybutanoate (**89**) using the route we developed during our earlier synthesis of batzelladine B methyl ester **78**. Biginelli condensation of **87** with β-ketoester **88** generated octahydro-5,6,6a-triazaacenaphthalene **86** in 82% yield as a 5 : 1 mixture of *cis* and *trans* stereoisomers. The superfluous ester and C–C double bond functionalities of **86** were removed next by exposing this intermediate at room temperature to typical deallylation conditions, catalytic $(Ph_3P)_4Pd$ and pyrrolidine, which delivered **90**. Reduction of this product with sodium borohydride, removal of the silyl



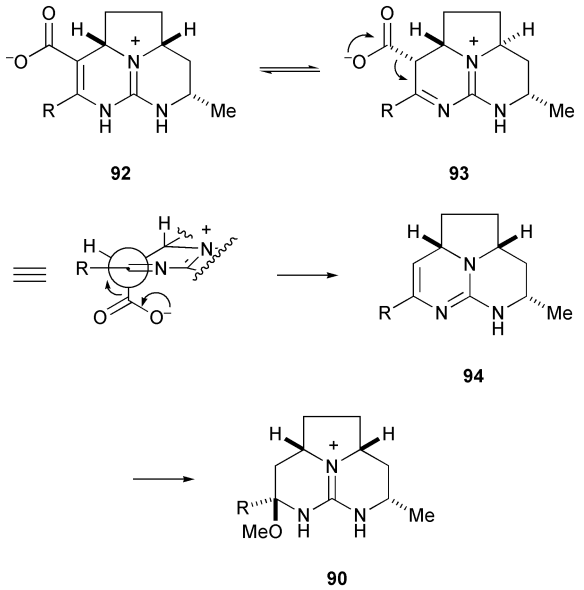
Scheme 14 Synthesis of the right hand portion of batzelladine F (**6**).



Scheme 13 Fragment coupling tethered Biginelli strategy for preparing batzelladine F (**6**).

protecting group, and transesterification with methyl acetoacetate then provided **84** in 54% overall yield. The high stereoselection in the reduction step arises from well-precedented axial delivery of hydride to such *N*-amidinyliminium ion intermediates.[19]

Although somewhat related decarboxylations of vinylogous carbamates were known,[45] the facility of the transformation of **86** to **90** was striking. A likely mechanism for this conversion is outlined in Scheme 15; the excellent overlap of the breaking C–C bond and the *N*-amidinyliminium ion π system undoubtedly facilitates decarboxylation of **93**.

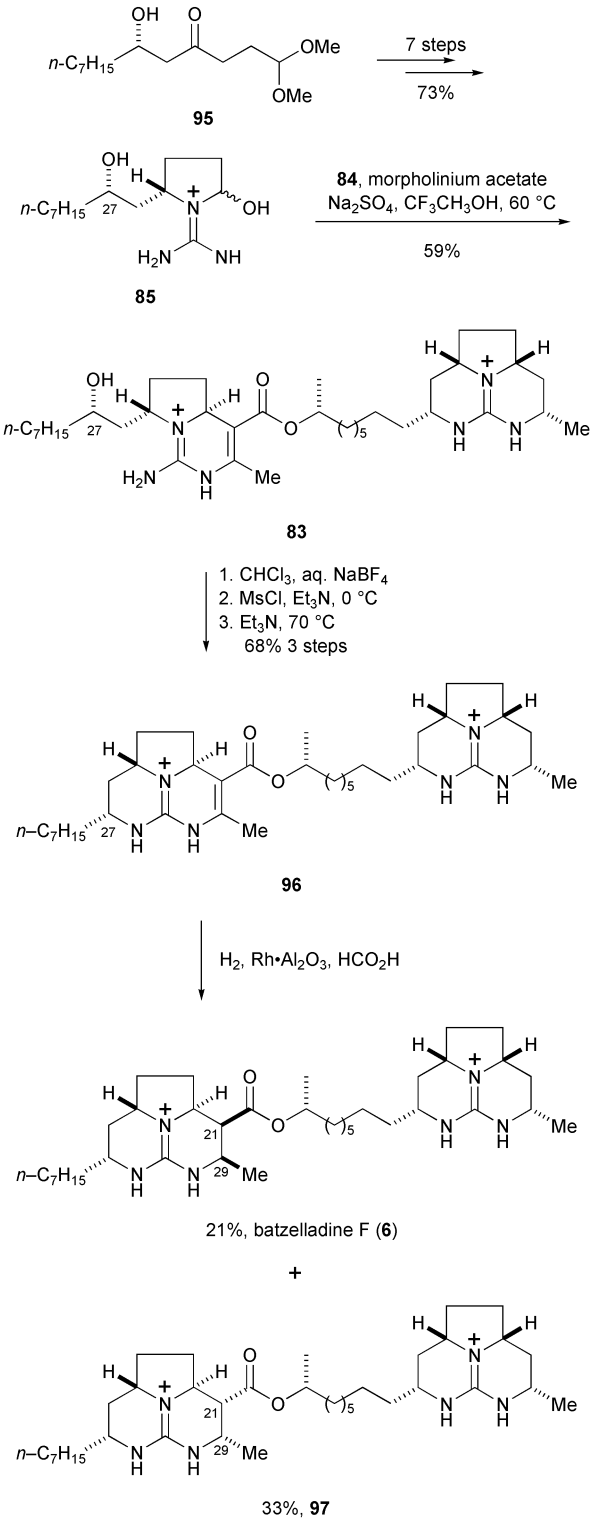


Scheme 15 Proposed decarboxylation mechanism.

To prepare for coupling the guanidine fragments, guanidine aldehyde **85** was synthesized from (*R*)-β-hydroxy ketone **95** using a 7-step sequence identical to the one we employed to assemble the nonyl analog during our earlier total synthesis of batzelladine D (**80**).[34] The pivotal tethered Biginelli union was accomplished by morpholinium acetate promoted condensation of guanidine β-ketoester **84** with 3 equiv. of **85** in 2,2,2-trifluoroethanol to provide the required *trans* C22,C25 stereoisomer **83** in 59% yield (Scheme 16); only trace amounts (< 10%) of the *cis* epimer were observed. At this point, all the carbon and nitrogen atoms of batzelladine F (**6**) had been installed. Following counterion exchange, the final ring of the left-hand tricyclic guanidine fragment was fashioned by preparing the C27 mesylate, which cyclized upon heating in $CHCl_3$ in the presence of excess Et_3N to deliver hexacyclic bisguanidine **96** in good yield. All that remained was to saturate the C21–C29 double bond. A variety of catalysts and reduction conditions were examined for this transformation, however, we failed to identify conditions that accomplished this reduction in a stereoselective fashion. The synthesis was finally completed by hydrogenation of **96** over $Rh{\cdot}Al_2O_3$ in acidic MeOH. Separation of the resulting products by HPLC then provided batzelladine F (**6**) in 21% yield and diastereomer **97** in 33% yield.

The uncertainty regarding the structure of batzelladine F was resolved by synthesizing stereoisomers **98**, **99** and **100** (Fig. 6) by similar sequences. Employing a combination of HPLC and CD comparisons, these isomers were shown to be different from batzelladine F, whereas **6** showed identical HPLC mobility as the marine isolate and was also indistinguishable from it by CD, ^1H NMR and ^{13}C NMR comparisons.[35]

The synthesis of batzelladine F (**6**), stereoisomers **98**–**100** and the four related stereoisomers having the constitution of **82**



Scheme 16 Completion of the total synthesis of batzelladine F (**6**).

demonstrates that tethered Biginelli reactions can successfully join fragments of considerable complexity. Moreover, the fragment coupling tethered Biginelli chemistry developed during this total synthesis endeavor is allowing libraries of bisguanidine batzelladine analogs to be readily synthesized in ongoing efforts to identify novel inhibitors of protein–protein interactions.[46]

Conclusion

The tethered variant of the Biginelli condensation provides new opportunities for exploiting this venerable transformation in

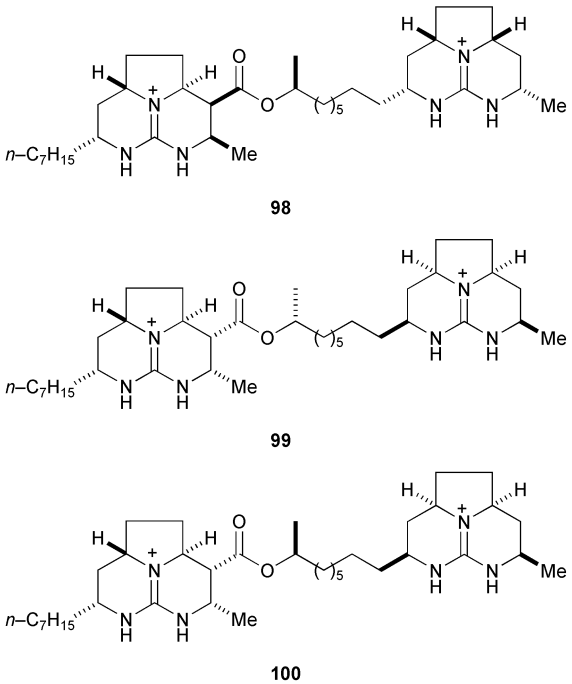


Fig. 6 Synthetic analogs of batzelladine F (**6**).

organic synthesis. With the ability to tune the stereoselectivity of the tethered Biginelli reaction by changing either the reaction conditions or the electrophilic component, this transformation is of outstanding utility for the stereocontrolled synthesis of polycyclic guanidines. Using the tethered Biginelli reaction as the central strategic step, total syntheses of ten crambescidin and batzelladine alkaloids have been accomplished. These syntheses defined the stereochemistry of the side chains of crambescidins 800 (**2**) and 13,14,15-isocrambescidin 800 (**3**), established the absolute configuration of batzelladines D and F (**6**) as well as the tricyclic guanidine moiety of batzelladine B (**5**), and established the constitution and configuration of batzelladine F (**6**). The synthetic routes we have developed are generally sufficiently concise that synthesis can be employed to provide initial supplies of these complex guanidine natural products for biological evaluation and study. This chemistry, which is well suited for the synthesis of analog structures, is currently being employed to develop structure–activity relationships and explore the molecular targets of the crambescidin and batzelladine alkaloids.

Acknowledgement

The accomplishments summarized in this article were made possible by the creativity, dedication, and experimental skill of the members of the Overman group who have worked in the demanding area of guanidine alkaloid total synthesis. We thank the National Institutes of Health for providing the majority of financial support for these investigations. Financial assistance from Merck, Pfizer, Pharma Mar and Roche Palo Alto, and graduate fellowship support to Z.A. from Lilly, are gratefully acknowledged.

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