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Evolution of a Strategy for the Synthesis of Structurally Complex Batzelladine Alkaloids. Enantioselective Total Synthesis of the Proposed Structure of Batzelladine F and Structural Revision

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Abstract: Stereoselective synthesis of octahydro-5,6,6a-triazaacenaphthalenes **29** and **34** having the anti-relationship of the angular hydrogens flanking the pyrrolidine nitrogen confirmed suspicions that the relative configuration of the left-hand tricyclic guanidine fragment of batzelladine F should be revised to have the syn relationship of these hydrogens. Several strategies were examined for coupling tricyclic guanidine fragments to prepare potential structures for batzelladine F. Eventually, a convergent synthesis strategy was devised, whose central step was a fragment-coupling tethered-Biginelli reaction (Scheme 17). Using this approach we synthesized four potential structures of batzelladine F, **35**−**38**. None of these compounds, nor their enantiomers, were identical to natural batzelladine F. Reinvestigation of mass spectra of natural batzelladine F, and fragments **88** and **89** obtained upon saponification of batzelladine F, demonstrated that the originally proposed connectivity of this alkaloid was also incorrect. The revised connectivity, **90**, of natural batzelladine F depicted in Scheme 21 is proposed.

Introduction

In 1997, Patil and co-workers reported the isolation of batzelladines F−I from a red Jamaican sponge incorrectly identified at the time as *Batzella* sp.;[1,2] the structures **1**−**4** assigned originally to these alkaloids are shown in Figure 1. Each of these natural products contain two tricyclic guanidines and were reported to induce dissociation of the tyrosine kinase $p56^{lck}$ from CD4. It was postulated that disruption of this interaction could be used to treat autoimmune diseases. As part of our larger program in the synthesis of guanidinium alkaloids,[3] we were attracted to batzelladine F for two reasons. First, we were interested in preparing compounds that inhibit specific protein−protein interactions involving large surface areas, and, second, we sought to define totally the relative and absolute configuration of batzelladine F, much of which was unclear at the outset of this work.[4]

The original structural proposal **1** for batzelladine F was based on the following analysis. The relative configuration of the right-hand tricyclic portion (C18 through C28) of batzelladine F was assigned by comparison of its ^{13}C NMR spectra to that of the tricyclic guanidine alkaloid batzelladine D, the structure of

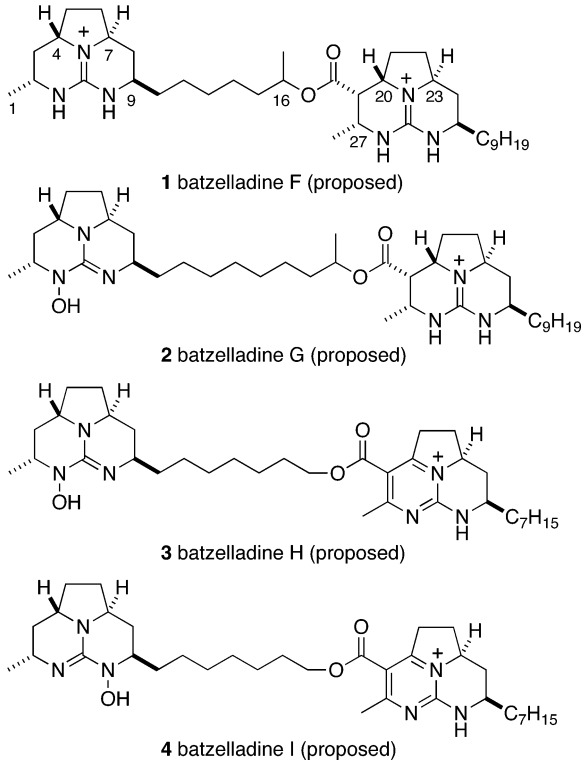

Figure 1. Proposed Structures of Batzelladines F−I.

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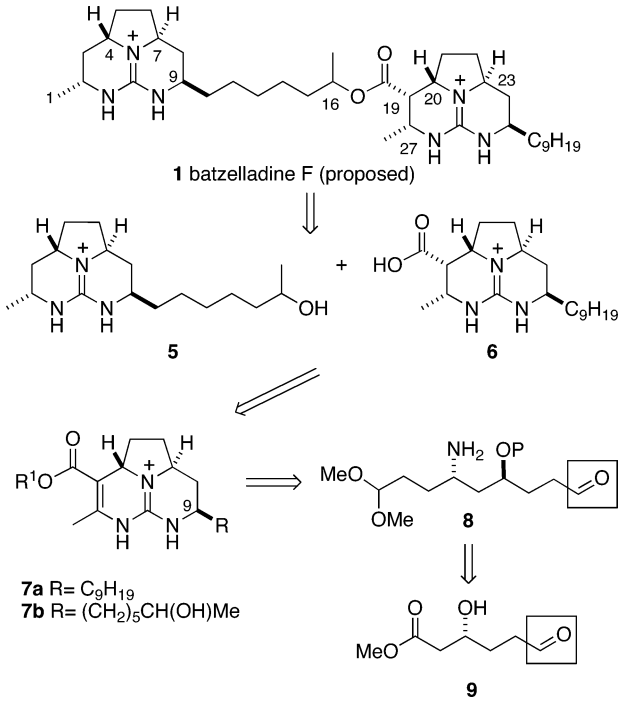
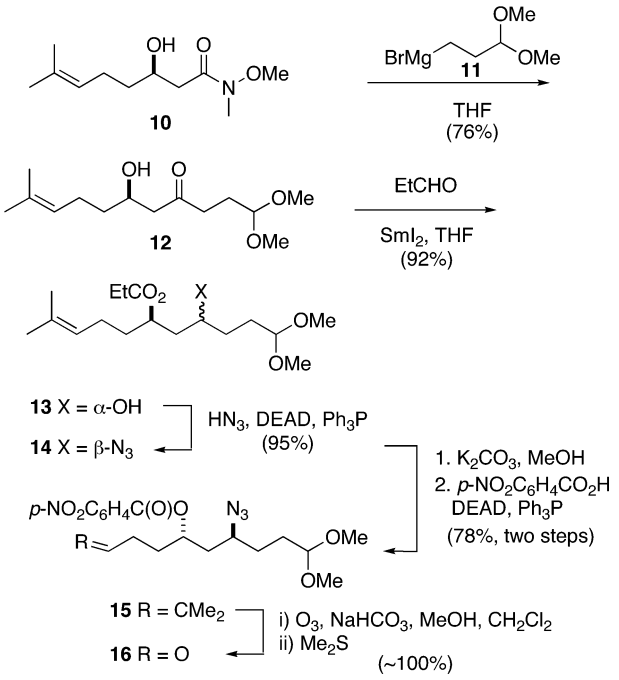
(1) Patil, A. D.; Freyer, A. J.; Taylor, P. B.; Carté, B.; Zuber, G.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem.* **1997**, *62*, 1814−1819.
(2) The identity of the sponge has been revised: Braekman, J. C.; Daloze, D.; Tavares, R.; Hajdu, E.; Van Soest, R. W. M. *J Nat. Prod.* **2000**, *63*, 193−196.
(3) For a recent review, see: Aron, Z. D.; Overman, L. E. *Chem. Commun.* **2004**, 253−265.
(4) (a) For reviews summarizing the isolation, structure and synthesis of batzelladine alkaloids, see: Berlinck, R. G. S.; Kossuga, M. H. *J. Nat. Prod.* **2005**, *22*, 516−550 and earlier reviews in this series. (b) For the recent isolation of a new batzelladine alkaloid, see: Gallimore, W. A.; Kelly, M.; Scheuer, P. J. *J. Nat. Prod.* **2005**, *68*, 1420−1423. (c) For a recent synthetic study, see: Arnold, M. A.; Duron, S. G.; Gin, D. Y. *J. Am. Chem. Soc.* **2005**, *127*, 6924−6925.

10.1021/ja0574320 CCC: $33.50

Scheme 1



Scheme 2



which had been defined by total synthesis.[5,6] The configuration of the left-hand tricyclic portion (C1 through C9) was assigned on the basis of NOE data.[7] The length of the nonyl chain at C25, and the length of the chain connecting the tricyclic guanidines were assigned by MS fragmentation patterns, but this analysis was not discussed, nor were the data presented. There was no information that related the relative configurations of the two tricyclic guanidine fragments, specified the configuration at C16, or defined the absolute configuration.

In 1999, Snider and Murphy independently published syntheses of tricyclic guanidines related to the left-hand portion of batzelladine F that have a syn relationship of the methine hydrogens flanking the pyrrolidine nitrogen.[8] The ^{13}C NMR data of these analogues supported a revision of the configuration of the natural product at C4 and C7 from anti to syn. However, rigorous verification of this proposal would require the synthesis of a related anti-fused tricyclic guanidine and the demonstration that its spectra were distinct from those of the natural product.

In this contribution, we first describe our synthesis of 2a,-8a-anti tricyclic guanidines corresponding to the originally proposed relative configuration of these units of batzelladine F and proof that the original configurational assignment of the left-hand guanidine fragment of this alkaloid was incorrect (Section I). We then describe our preparation of analogous 2a,8a-syn tricyclic guanidines (Section II) and the evolution of our strategy for the total synthesis of linked tricyclic guanidines corresponding to the proposed constitution and revised relative configuration of batzelladine F (Sections III−IV). We then demonstrate that the originally reported *connectivity* of the natural product was incorrect, leading to a new proposal for the structure of batzelladine F (Section V). In the following contribution, we detail our synthesis of this new structure, and studies that define all aspects of the structure of batzelladine F.[9]

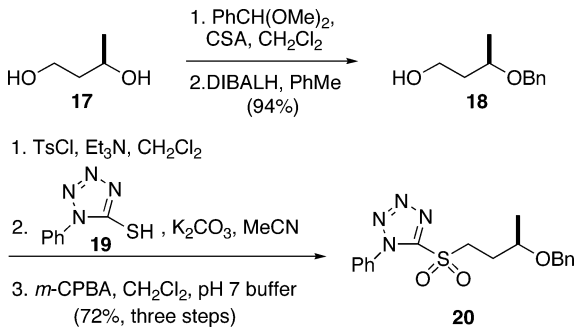
Results and Discussion

I. Synthesis of 2a,8a-Anti-decahydrotriazaacenaphthalenes Corresponding to the Originally Proposed Structure of Batzelladine F. Believing that both tricyclic guanidine fragments of batzelladine F possessed the same anti relationship between the angular hydrogens at carbons 4 and 7, and carbons 20 and 23 (batzelladine numbering), we conceived of a divergent strategy that would allow us to construct each half of the molecule from a common intermediate. We chose the ester bond as a logical first disconnection, thus, generating alcohol **5** and acid **6** as synthetic objectives. Each of these intermediates was conceived as arising from an intermediate such as **7a** or **7b**, which differ in the side chain at C9. Employing a disconnection developed during our total synthesis of batzelladine D,[6] intermediates **7a** and **7b** were simplified to a common precursor **8** bearing a masked aldehyde that would allow for attachment of different side chains by olefination reactions. Anti amino alcohol **8** was seen as arising from β-hydroxy ester **9**.

The preparation of common intermediate **8** began with Weinreb amide **10**,[10] to which Grignard reagent **11**[11] was added to afford β-hydroxy ketone **12** in 76% yield (Scheme 2). This intermediate was subjected to Evans' variant of the Tishchenko reduction to set the 1,3-stereorelationship and differentiate the hydroxyl groups of the resulting 1,3-diol.[12] In this way, monoprotected diol **13** was obtained in 92% yield from ketone precursor **12** as a single stereoisomer. Nitrogen was installed

(5) Snider, B. B.; Chen, J.; Patil, A. D.; Freyer, A. J. *Tetrahedron Lett*. **1996**, *37*, 6977−6980.
(6) Cohen, F.; Overman, L. E.; Sakata, S. K. L. *Org. Lett*. **1999**, *1*, 2169−2172.
(7) These data were not presented in the paper or the Supporting Information.
(8) (a) Snider, B. B.; Busuyek, M. V. *J. Nat. Prod*. **1999**, *62*, 1707−1711. (b) Black, G. P.; Murphy, P. J.; Thornhill, A. J.; Walshe, N. D. A.; Zanetti, C. *Tetrahedron* **1999**, *55*, 6547−6554.
(9) (a) Our synthesis of the natural product has been outlined: Cohen, F.; Overman, L. E. *J. Am. Chem. Soc*. **2001**, *123*, 10782−10783. (b) Cohen, F.; Overman, L. E. *J. Am. Chem. Soc*. **2006**, *128*, 2604−2608.
(10) Coffey, D. S.; McDonald, A. I.; Overman, L. E.; Stappenbeck, F. *J. Am. Chem. Soc*. **1999**, *121*, 6944−6945.
(11) Lee, T. V.; Porter, J. R. *Org. Synth*. **1995**, *72*, 189−195.
(12) Evans, D. A.; Hoveyda, A. H. *J. Am. Chem. Soc*. **1990**, *112*, 6447−6449.

Scheme 3



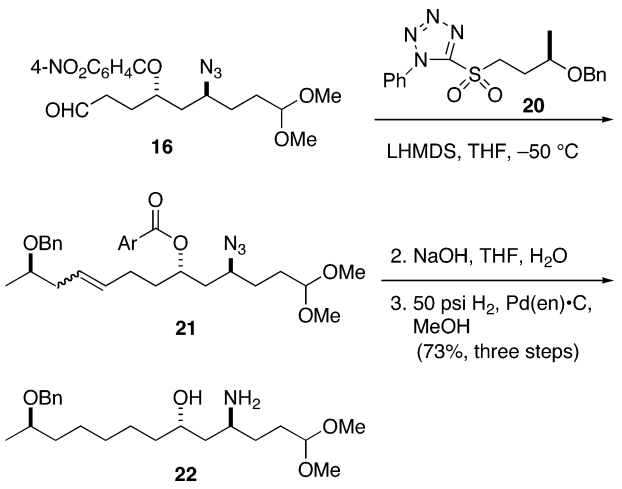
in the form of an azide, by Mitsunobu inversion of alcohol **13** with hydrazoic acid to provide azide **14** in 95% yield.[13,14] The 1,3-anti configuration was then reestablished by methanolysis of propionate **14**, followed by Mitsunobu inversion of the product alcohol with *p*-nitrobenzoic acid[15] to deliver aryl ester **15** in 78% yield for the two steps. Ozonolysis of unsaturated azido ester **15** in a mixture of MeOH, CH_2Cl_2 and $NaHCO_3$ provided aldehyde **16**, setting the stage for attachment of the different tricyclic guanidine side chains.[16]

After aldehyde **16** failed to react cleanly with the ylide derived from a simple hexyl phosphonium salt, we turned to the Kocienski modification of the Julia olefination.[17] This procedure required the synthesis of an appropriate sulfone partner (Scheme 3). Thus, diol **17** was monoprotected by reductive opening of the corresponding benzylidine acetal to provide alcohol **18** in 94% yield and 96% ee.[18,19] Activation of the alcohol as a tosylate allowed displacement with mercaptotetrazole **19**. Oxidation of the resulting sulfide with *m*-chloroperbenzoic acid (*m*-CPBA) in a mixture of CH_2Cl_2 and pH 7 buffer afforded sulfone **20** in 72% yield for the three steps.

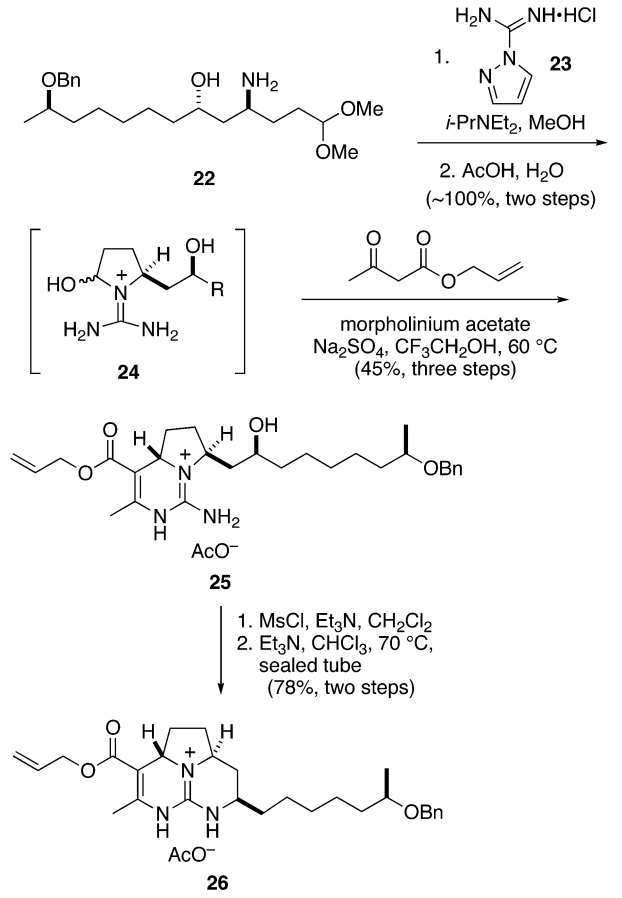
Aldehyde **16** was then elaborated to amino alcohol **22** as summarized in Scheme 4. Union of sulfone **20** with aldehyde **16** was accomplished by addition of a THF solution of the aldehyde to a small excess of the lithium reagent derived from sulfone **20** at −50 °C. Alkene **21** was obtained in ∼75% yield as a 3:2 mixture of stereoisomers. This product was contaminated with residual sulfone **20**, which could be removed more readily after hydrolysis of the ester. The azide and double bond were then reduced concurrently with hydrogen over palladium poisoned with ethylenediamine.[20] As expected, the benzyl ether was stable to this reduction. In this fashion, amino alcohol **22** was obtained in 73% overall yield from aldehyde **16**.

Employing chemistry developed during our earlier synthesis of the guanidine core of batzelladine D,[6] the conversion of amino alcohol **22** to the corresponding tricyclic guanidine **26** took place smoothly (Scheme 5). Guanylation of amine **22** with reagent **23**,[22] followed by hydrolysis of the acetal provided guanidine

Scheme 4



Scheme 5

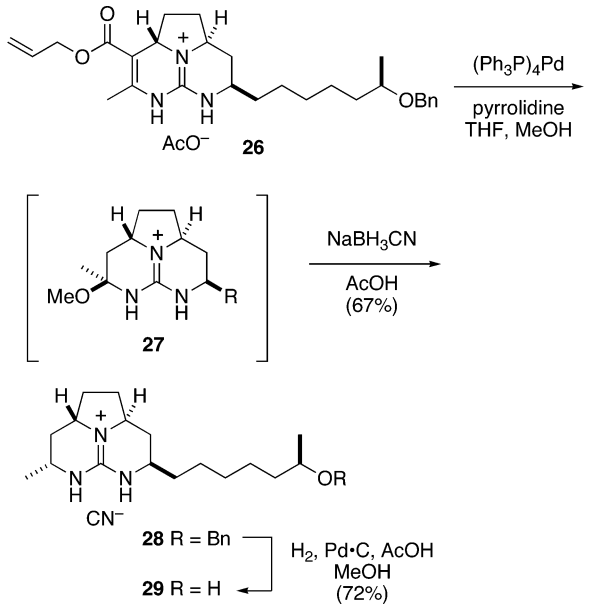


hemi aminal **24**. This labile intermediate was immediately condensed with allyl acetoacetate to provide Biginelli product **25** in 45% yield as an 8:1 mixture of stereoisomers.[23] After separation of the minor syn stereoisomer by chromatography, mesylation of the anti product and ring closure provided tricyclic guanidine **26** in 78% yield.

With allyl ester **26** in hand, we sought to investigate its decarboxylation. Such transformations of simple vinylogous

(13) Mitsunobu, O. *Synthesis* **1981**, 1−32.
(14) Hassner, A.; Fibiger, R.; Andisik, D. J. *J. Org. Chem.* **1984**, *49*, 4237−4244.
(15) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017−3020.
(16) Without this basic additive, the bis-dimethyl acetal was isolated in quantitative yield. See: Woodward, R. B. et al. *J. Am. Chem. Soc.* **1981**, *103*, 3210−3213.
(17) Blakemore, P. R.; Cole, W. J.; Kocienski, P. J.; Morley, A. *Synlett* **1998**, 26−28.
(18) Seebach, D.; Zuger, M. *Helv. Chem. Acta*. **1982**, *65*, 495−503.
(19) Volkov, A. A.; Zlotski, S. S.; Kravets, E. K.; Spirknin, L. V. *Chem. Heterocycl. Compd. (Engl. Trans.)* **1985**, *21*, 861−863.
(20) Sjiki, H.; Hattori, K.; Hirota, K. *J. Org. Chem.* **1998**, *63*, 7990−7992.
(21) Franklin, A. S.; Ly, S. K.; Mackin, G. H.; Overman, L. E.; Shaka, A. J. *J. Org. Chem.* **1999**, *64*, 1512−1519.
(22) Bernatowicz, M. Z.; Wu, Y.; Matsueda, G. R. *J. Org. Chem.* **1992**, *57*, 2497−2502.
(23) Stereoselection in the tethered Biginelli condensation has been thoroughly investigated: McDonald, A. I.; Overman, L. E. *J. Org. Chem.* **1999**, *64*, 1520−1528.

Scheme 6



carbamates were precedented, typically involving heating the free acid to >100 °C in the presence of an acid or catalyst such as cyanide.[24] We were delighted to find that reaction of ester **26** with catalytic $(PPh_3)_4Pd$ and pyrrolidine in a mixture of THF and MeOH resulted within an hour in cleavage of the allyl ester and concomitant decarboxylation.[25] The intermediate enamine absorbed one equivalent of MeOH to yield hemi aminal **27**. Without isolation, this intermediate was reduced with $NaBH_3CN$ in AcOH to afford saturated tricyclic guanidine **28** in 67% yield. Stereoselection in this reduction results from axial delivery of hydride to the *N*-amidinyl iminium ion derived from **27**.[26] Finally, the benzyl ether of **28** was removed by hydrogenolysis to provide alcohol **29**.

The facility with which guanidine ester **26** decarboxylates after deallylation can be explained by postulating an intermediate such as **31**, which would arise from tautomerization of **30** (Scheme 7). The breaking bond in guanidnium carboxylate **31** would be aligned well with the iminium π-system.

We were now in a position to compare the NMR spectra of our tricyclic guanidine products, batzelladine F, and Murphy's syn-fused model compound **33** (Table 1).[27] The ^{13}C NMR spectra of the two anti-fused tricyclic guanidines **29** and **34**[28] match each other almost perfectly yet differ somewhat from those of the natural product. In contrast, the ^{13}C NMR data for **33** match those of the natural product closely. It was clear from this comparison that the proposed anti relationship of the angular hydrogens of the left-hand guanidine fragment of batzelladine F was incorrect: the actual configurational relationship is indeed syn.

II. Synthesis of 2a,8a-Syn-decahydrotriazaacenaphthalenes. Although the relative configuration of the left-hand tricyclic portion of batzelladine F was now firmly established as syn, no information was available regarding the relative configuration of the two guanidine fragments, the relative configuration of the methyl substituent of the linker, nor the absolute configuration. Thus, there were eight compounds **35**−**38** (four pairs of enantiomers) that fit all of the available data for batzelladine F (Figure 2).

We turned next to develop a convenient synthesis of the left-hand tricyclic guanidine portions of **35**−**38**. The approach we chose was founded on the chemistry we had developed earlier to synthesize the tricyclic moiety of batzelladine B,[21] in this case a tethered Biginelli reaction would be employed to combine fragments **41** and **42** (Scheme 8). Both of these intermediates we saw being available from the same starting material, methyl (*R*)-3-hydroxybutarate (**44**).

The synthesis of β-keto ester **52** began with (*R*)-iodide **45**, which was prepared in three routine steps from ester **44** (Scheme 9).[29] Alkylation of this iodide with the lithium enolate of *tert*-butyl acetate proceeded well on a small scale.[30] On larger scale, the desired product **46** was contaminated with varying amounts of the dialkylation product **47**, which was difficult to remove. Reduction of this mixture with $LiAlH_4$ provided a mixture of

Scheme 7

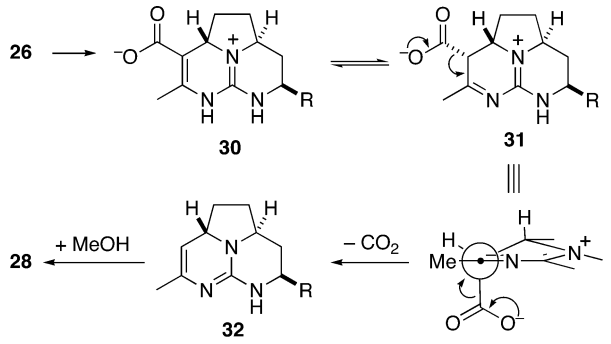
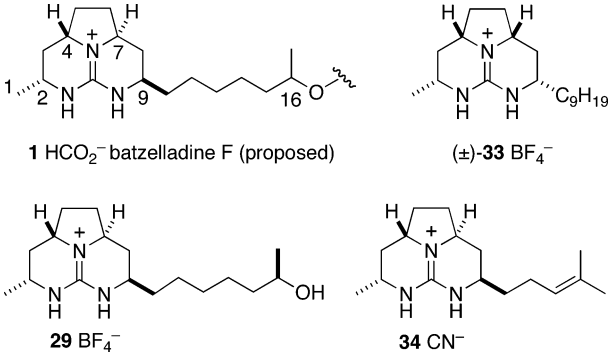


Table 1. Comparison of ^{13}C Chemical Shifts of Batzelladine F and Tricyclic Model Compounds[a]



carbon no.	**29**	**34**	**1**	**33**
1	21.7	21.7	20.7	20.7
2	48.9	48.6	47.2	47.3
4	56.5	56.5	57.5	57.5
7	56.4	56.3	57.4	57.5
9	53.1	52.8	51.6	51.6

[a] ^{13}C NMR in CD_3OD (500 MHz).

(24) Reuman, M.; Eissenstat, M. A.; Weaver, J. D., III *Tetrahedron Lett.* **1994**, *35*, 8303−8306, and references therein.
(25) A model compound similar to **26** bearing a methyl ester was completely resistant to both basic and acidic hydrolysis.
(26) Snider, B. B.; Chen, J.; Patil, A. D.; Freyer, A. J. *Tetrahedron Lett.* **1996**, *37*, 6977−6980.
(27) Black, G. P.; Murphy, P. J.; Walshe, N. D. A. *Tetrahedron* **1998**, *54*, 9481−9488.
(28) Synthesized from unsaturated azido benzoate **15** in a fashion analogous to **29**; see Supporting Information.
(29) Overman, L. E.; Rabinowitz, M. H. *J. Org. Chem.* **1993**, *58*, 2335−3237.
(30) Heathcock, C. H.; Pietter, S.; Ruggeri, R. B.; Ragan, J. A.; Kath, J. C. *J. Org. Chem.* **1992**, *57*, 2554−2566.

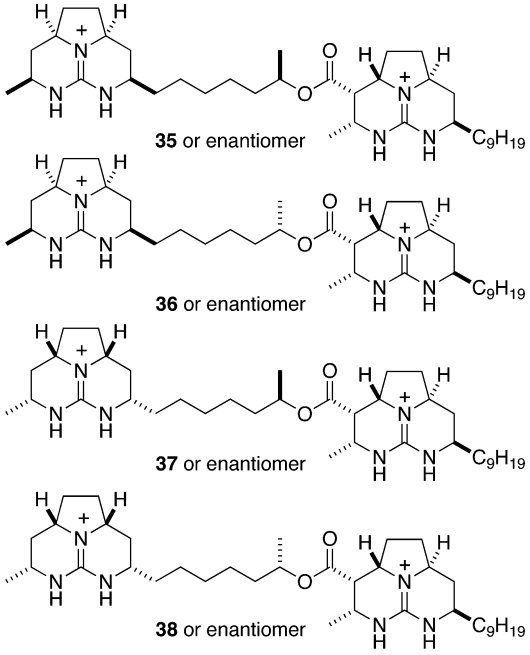
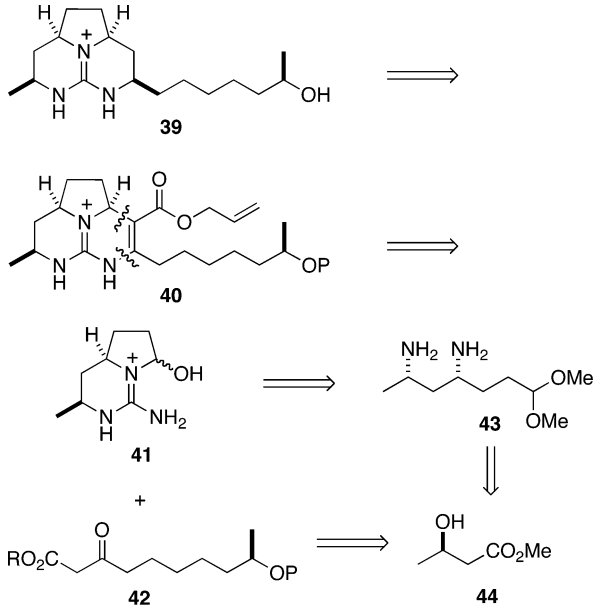


Figure 2. Proposed revised constitution of batzelladine F (**90**) and the configurational uncertainties yet to be resolved. Plausible Structures for Batzelladine F as of 1999.

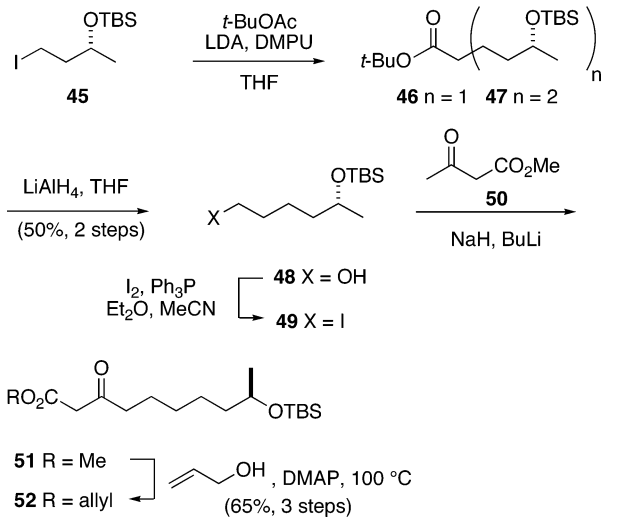
Scheme 8



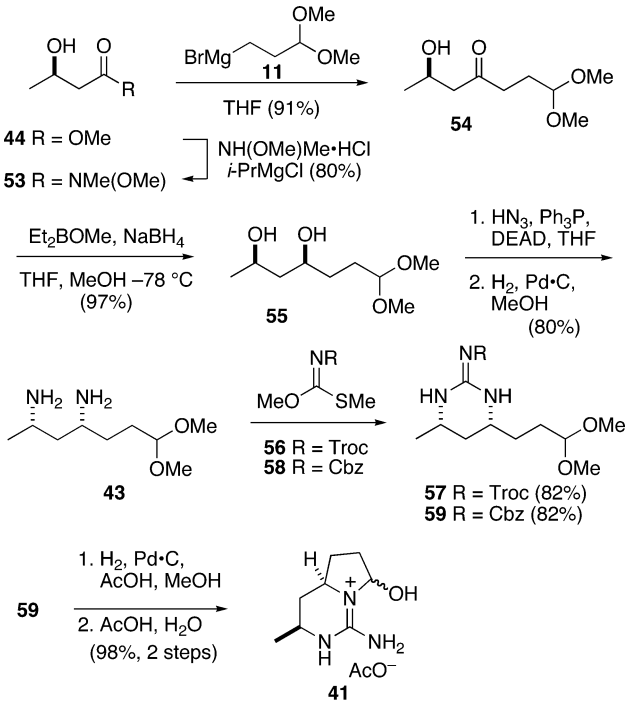
primary alcohols, from which the desired product **48** could be isolated cleanly.[31] Conversion of alcohol **48** to iodide **49** set the stage for elaboration to a β-keto ester. Thus, addition of the dianion of methyl acetoacetate (**50**) to iodide **49** proceeded cleanly, delivering **51** in 68% yield.[32] Transesterification of this product with allyl alcohol gave allyl ester **52** in nearly quantitative yield.[33]

Guanidine hemi aminal **41** was assembled by the sequence summarized in Scheme 10. Hydroxybutyrate **44** was converted

Scheme 9



Scheme 10

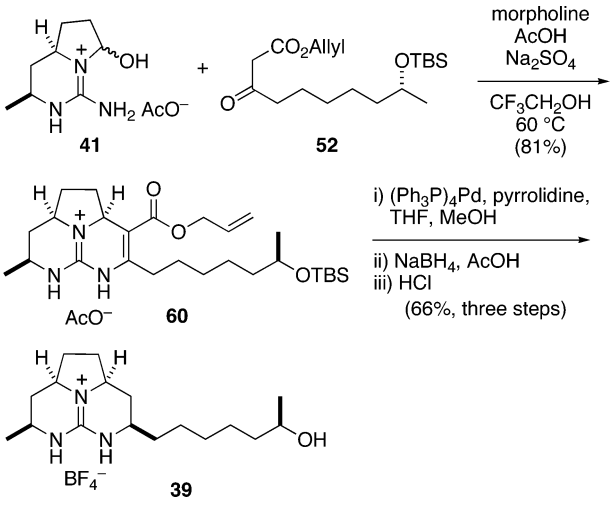


first to Weinreb amide **53** in 80% yield using the Merck procedure (Scheme 10).[34] Addition of Grignard reagent **11** to this amide provided hydroxy ketone **54**, which was reduced with Et_2BOMe and $NaBH_4$ to provide syn-diol **55** as a single stereoisomer.[35] Double Mitsunobu reaction of this intermediate with hydrazoic acid, followed by hydrogenation of the diazide product over Pd•C provided diamine **43** in 80% yield. Conversion of this intermediate to Troc-protected guanidine **57** was accomplished with reagent **56** in 82% yield.[36]

To set the stage for the Biginelli condensation, the Troc group and the dimethyl acetal were removed from guanidine **57** simultaneously by reaction with Zn dust in aqueous AcOH.

(31) Several lengthier synthesis of this compound are reported: (a) Nokami, J.; Taniguchi, T.; Ogawa, Y. *Chem. Lett.* **1995**, 43−4. (b) Solladié, G.; Lohse, O. *J. Org. Chem.* **1993**, *58*, 4555−4563. (c) Ernst, B.; Wagner, B. *Helv. Chim. Acta*. **1989**, *72*, 165−171.
(32) Huckin, S. N.; Weiler, L. *J. Am. Chem. Soc.* **1974**, *96*, 1082−1087.
(33) Taber, D. F.; Amedio, J. C.; Patel, Y. K. *J. Org. Chem.* **1985**, *50*, 3618−1619.
(34) Williams, J. M.; Jobson, R. B.; Yasuda, N.; Marchesini, G.; Dolling, U.-H.; Grabowski, E. J. J. *Tetrahedron Lett.* **1995**, *36*, 5461−5464.
(35) Chen, K. M.; Hardmann, G. E.; Prasad, K.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155−8.
(36) Atkins, P. R.; Glue, S. E. J.; Kay, I. T. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2644−2646.

Scheme 11

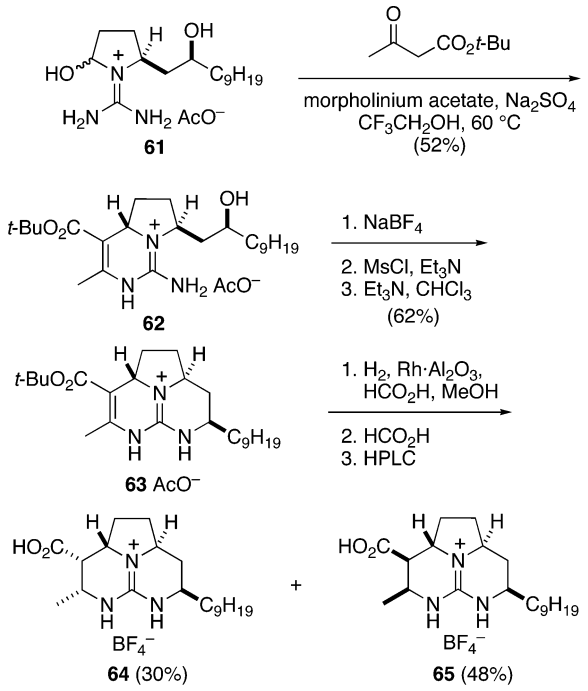


However, removal of residual zinc salts from the resulting crude guanidine hemi aminal **41** proved to be problematic. For example, saturation of the reaction with H_2S formed a thick precipitate, presumably ZnS, which was difficult to filter. Moreover, the polar nature of guanidine salt **41** caused it to adhere to these salts. At most, 40% of the desired product could be recovered from this reaction. Therefore, we prepared the benzyl imino thiocarbamate **58** (see Supporting Information for details), and condensed it with diamine **43** to form the Cbz-protected guanidine **59** in 82% yield. Hydrogenolysis of this intermediate removed the Cbz group. Hydrolysis of the resulting guanidinium acetal proceeded cleanly, albeit slowly, at room temperature in aqueous acetic acid to provide bicyclic hemi aminal **41** in nearly quantitative yield over the two steps.

With the two tethered Biginelli reaction partners in hand, the construction of 2a,8a-syn-decahydrotriazaacenaphthalene **39** proceeded uneventfully (Scheme 11). Guanidine **41** condensed smoothly with an excess of β-keto ester **52**, under previously optimized conditions,[21] to provide tricyclic guanidine **60** in 81% yield as a 5:1 mixture of syn and anti stereoisomers. As in the anti series, the unnecessary ester appendage was removed by brief treatment of Biginelli product **60** with $(Ph_3P)_4Pd$ and pyrrolidine, followed by reduction of the intermediate hemi aminal with $NaBH_4$ in AcOH. Workup of this reaction with 1 N HCl then removed the silyl group. After chromatography on SiO_2, the product was treated with aqueous $NaBF_4$ to provide guanidine alcohol **39** having a BF_4^- counterion in 66% overall yield from ester **60**. As expected, the diagnostic methine resonances in the ^{13}C NMR spectrum of syn-decahydrotriazaacenaphthalene **39** matched almost exactly those signals of batzelladine F and Murphy's model compounds (see Supporting Information).

III. Synthesis of the Revised Proposed Structure of Batzelladine F: α-Bromo Acid Strategy. The preparation of the right-hand 2a,8a-anti-decahydrotriazaacenaphthalene-carboxylate is summarized in Scheme 12. This synthesis began with hemi aminal **61**,[6] which was condensed with *tert*-butyl acetoacetate under conditions optimized for anti stereoselection. This reaction provided anti bicyclic guanidine **62**, which was isolated as the acetate salt after chromatography on silica gel. Conversion of this intermediate to the corresponding mesylate was complicated by *N*-sulfonylation of the guanidine. Eventually, we found that exchanging the acetate counterion of guanidine **62** for tetrafluoroborate allowed for clean mesylation of the alcohol upon reaction with MsCl and Et_3N in CH_2Cl_2. This mesylate cyclized in hot $CHCl_3$ in the presence of excess Et_3N to provide tricyclic guanidine ester **63** in 62% overall yield. As expected,[6] hydrogenation of **63** over $Rh{\cdot}Al_2O_3$ proceeded with little stereoselection. The resulting mixture of esters was immediately deprotected with HCO_2H to provide the corresponding acids. These isomers were separated by reverse-phase HPLC to provide stereoisomer **64**, having the same relative configuration as batzelladine D and the right-hand guanidine fragment of batzelladine F, in 30% yield along with 48% of stereoisomer **65**.

Scheme 12

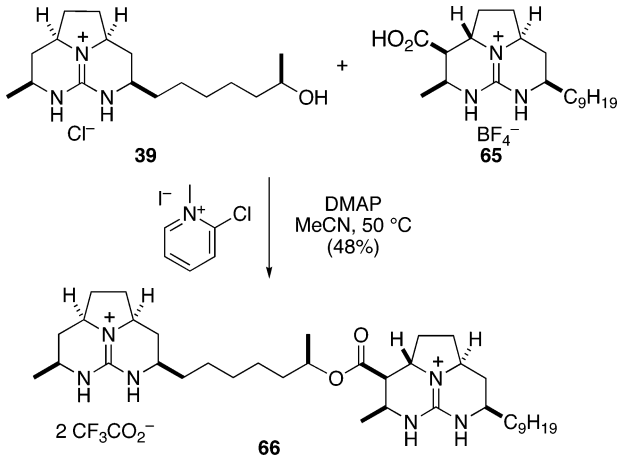


With the two tricyclic guanidine fragments available, their union was investigated. Using the more abundant isomer **65** as a model, a variety of coupling reagents were evaluated, including diimides, DEAD-Ph_3P, and activated esters.[37] The only conditions that showed promise involved the use of 2-chloro-1-methylpyridinium iodide (Mukaiyama's salt) and DMAP in MeCN.[38] Coupling of acid **65** and alcohol **39** in this way provided ester **66** in 48% yield after purification by HPLC (Scheme 13).

With a successful coupling procedure in hand, we turned to prepare the hexacyclic diguanidine **35** depicted in Figure 2 (Scheme 14). The desired coupling of guanidine alcohol **39** and guanidinium carboxylate **64** did not take place at 50 °C or 75 °C.[37] However, when this coupling reaction was conducted at 100 °C, ester **68** was formed in 57% yield. Unfortunately, upon characterization, it became apparent that **68** had undergone epimerization at C19. Particularly diagnostic was the ^1H NMR signal of C19, which appeared as an apparent triplet at δ 2.38 (t, J = 10.3 Hz, CD_3OD); whereas authentic batzelladine F

(37) These reactions were conducted on a small scale (<1 mg) and analyzed by HPLC−MS.
(38) Mukayama, T.; Usui, M.; Shimada, E.; Saigo, K. *Chem. Lett*. **1975**, 1045−1048.

Scheme 13

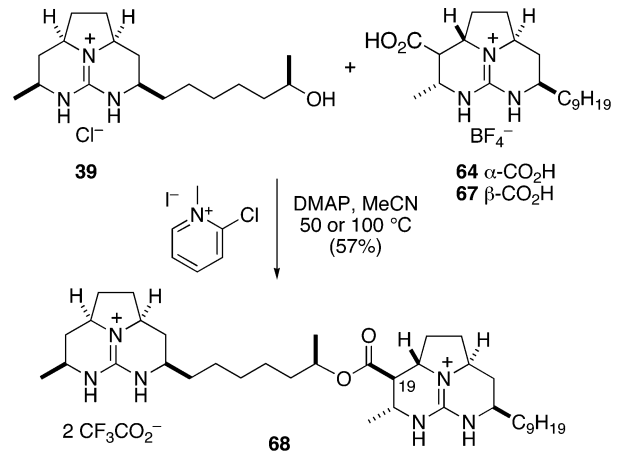


shows the corresponding resonance at δ 3.06 (dd, J = 4.6, 3.3 Hz, CD_3OD). It was subsequently discovered that acid **64** rapidly epimerizes under these reaction conditions, and the resulting epimeric acid, **67**, couples to alcohol **39** rapidly at 50 °C. These results lead us to believe that epimerization preceded coupling. The failure of **64** to couple under these conditions and its epimerization under more forcing conditions is readily attributed to the hindered axial nature of the carboxylic acid substituent.

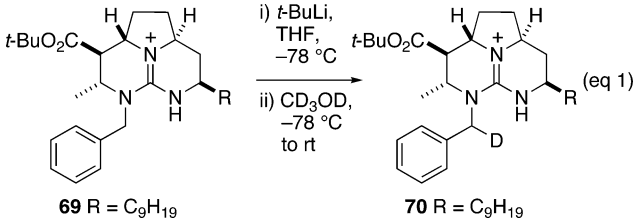
We turned to explore whether the ester could be epimerized after coupling. In our synthesis of batzelladine D, an attempt was made to epimerize a related ester by kinetic protonation of an enolate intermediate.[6] Although yields and selectivities were low in this earlier study, we felt that this strategy warranted reinvestigation. The desired configuration of the diguanidine ester would arise if the enolate of ester **68** protonated from the less-hindered β face.

Before embarking on such an ambitious undertaking, we sought to explore the epimerization step in a model system. We anticipated that success would require that at least some of the NH hydrogens of the guanidine groups be masked. Thus, the tricyclic guanidine ester **69** depicted in eq 1 was assembled by reduction of α,β-unsaturated ester **63** with $NaBH_3CN$ in AcOH, followed by *N*-benzylation by reaction with KHMDS and benzyl bromide. We were disappointed to find that reaction of this ester with a variety of bases (LDA, $LiNEt_2$, KHMDS, BuLi), followed by quenching with a deuterium source resulted

Scheme 14

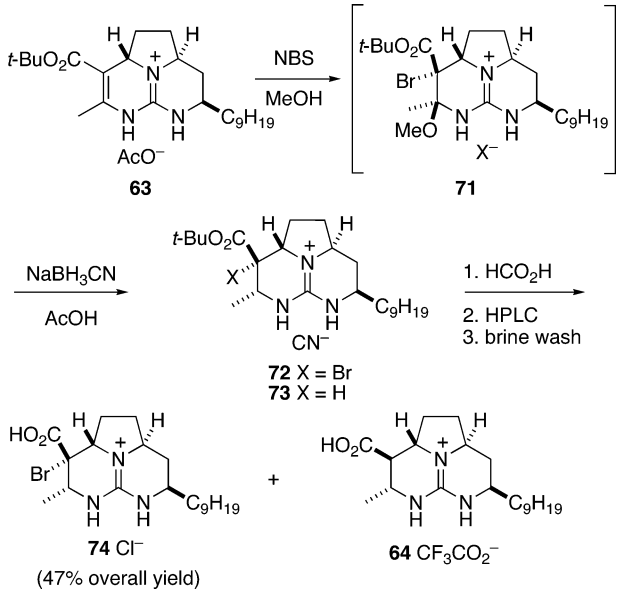


in no deuterium incorporation (eq 1). In contrast, reaction of guanidine ester **69** with excess *tert*-butyllithium at −78 °C in THF, followed by quenching the reaction with CD_3OD resulted in 100% deuteration. However, ^1H NMR showed to our surprise that deuterium had been introduced at the benzylic methylene group.[39]



Unable to generate an enolate by deprotonation, we sought to access this intermediate in a slightly more circuitous manner. We anticipated that a bromine substituent α to the ester would allow the enolate required for epimerization to be generated by reduction. The synthesis of the bromo acid required to investigate this strategy began with unsaturated ester **63**, which was treated with *N*-bromosuccinimide (NBS) in MeOH to generate bromo ether **71** in quantitative yield (Scheme 15). This

Scheme 15

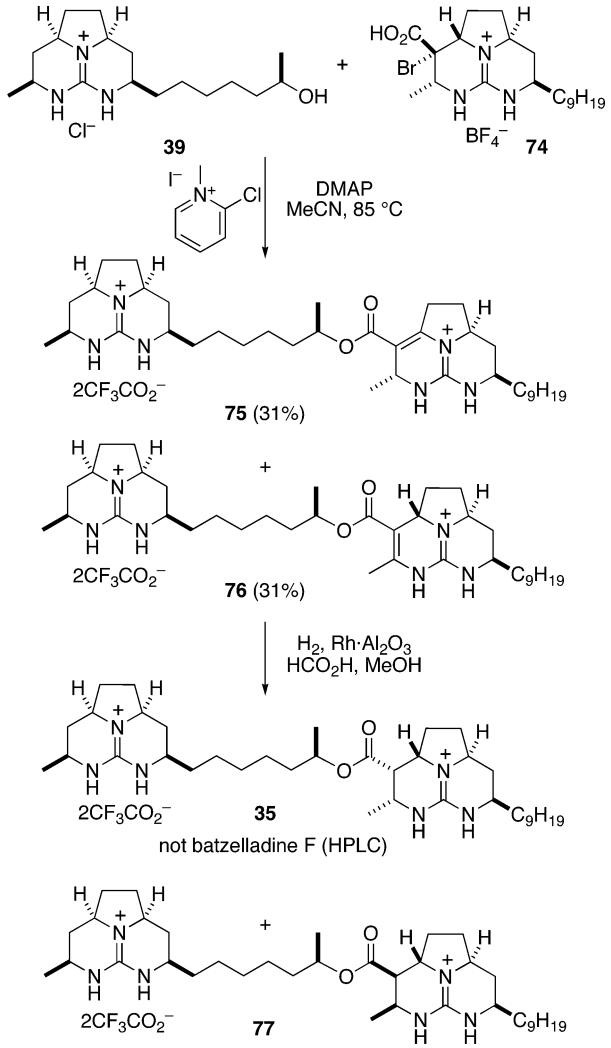


intermediate was reduced immediately with $NaBH_3CN$ in AcOH to provide α-bromo ester **72**. This reduction was plagued by competing elimination of "BrOMe" to return unsaturated ester **63**, which under the reaction conditions was reduced to ester **73**.[40] Cleavage of the *tert*-butyl group of ester **72** with 98% HCO_2H, and purification of the product by HPLC furnished the desired acid **74** in 47% overall yield. Although not established rigorously, the configuration indicated for **74** would arise from diaxial addition of MeOBr to **63** and axial reduction of the *N*-amidinyl iminium ion generated from **71**.

(39) Molecular mechanics modeling shows that the ester group of intermediates of this type exists preferntially in a conformation in which the methine hydrogen and the carbonyl π-system are nearly orthogonal. This feature is likely responsible for the failure to generate the ester enolate of **69**.

(40) Other reductive conditions ($NaBH(OAc)_3$, AcOH.; $NaBH_4$, EtOH, 98% HCO_2H, Et_3SiH, TFA) either failed to reduce **71** or led to decomposition.

Scheme 16

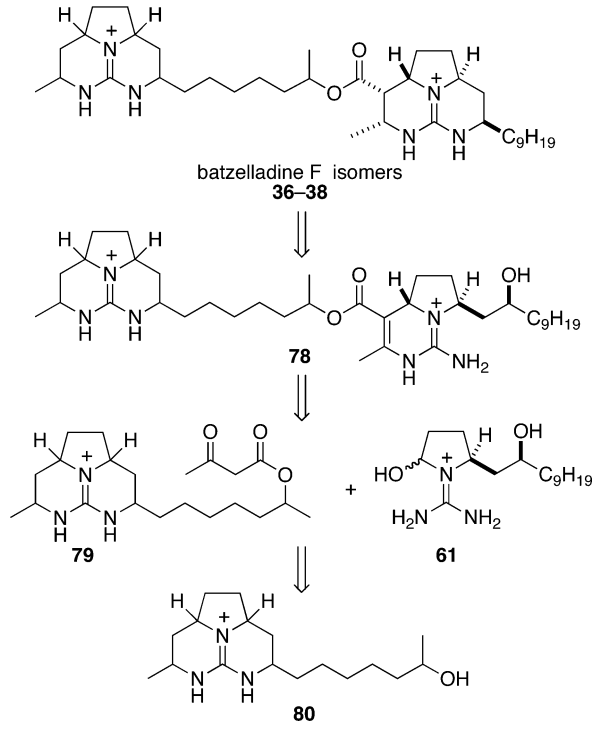


With guanidine α-bromo acid **74** available, its coupling to guanidine alcohol **39** was investigated (Scheme 16). Using Mukaiyama's salt, coupling proceeded to a small extent (~20%, HPLC−MS analysis) after 24 h at 50 °C. Attempts to force the reaction by increasing the temperature to 85 °C provided a mixture of diguanidines **75** and **76** in which both elimination of HBr and coupling had taken place. These double bond isomers could be separated by HPLC, and were readily distinguished by ^{1}H NMR spectroscopy.[41]

The formation of **76** finally suggested a potentially straightforward route for accessing batzelladine F: couple the guanidine fragments prior to saturating the right-hand tricyclic guanidine fragment. We proceeded to test the last stage of this strategy with diguanidine **76**, which was now available (Scheme 17). Hydrogenation of **76** over Rh·Al_2O_3 provided stereoisomeric diguanidines **35** and **77**, which could be separated by HPLC. Although compound **35** showed ^{1}H and ^{13}C NMR spectra consistent with those of natural batzelladine F, it was substantially different from the natural product by HPLC analysis.

(41) The signals in the ^{1}H NMR spectrum of **76** corresponding to the left-hand tricyclic portion were nearly identical to that of **68**. The two most diagnostic peaks were the vinyl methyl singlet at δ 2.26 and the allylic methine at δ 4.25 (dd, J = 11.7, 5.2 Hz). Both of these resonances were absent from the spectrum of **75**, and were replaced with an allylic methine at δ 4.49 (q, J = 6.3 Hz) and a multiplet at δ 2.45−2.30 corresponding to the allylic methylene.

Scheme 17



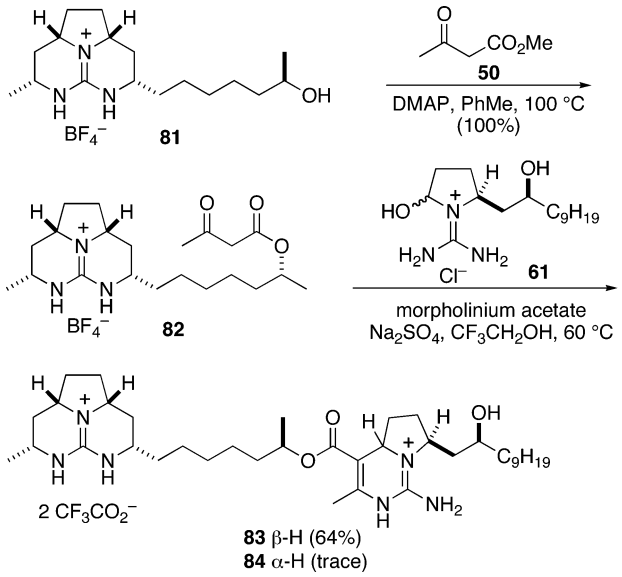
Given that we had only a 25% chance of making the correct relative stereoisomer at the outset, it was not surprising that **35** was not batzelladine F. At this point, we were faced with two issues. First was the problem of efficiently linking the guanidine fragments and installing the desired configuration at C19. Second was the identity of the natural product. Although it was not immediately clear how the former could be solved, a late stage catalytic hydrogenation strategy should permit us to prepare the remaining three stereoisomers, allowing for elucidation of the relative and absolute configuration of the natural product.

IV. Fragment Coupling Biginelli Strategy for the Synthesis of the Revised Proposed Structure of Batelladine F and Stereoisomers. Having decided to pursue a synthesis strategy with saturation of the C19−C27 double bond as the final step, we realized that there was a shorter route to such late-stage intermediates than the α-bromo acid coupling strategy. This approach is outlined retrosynthetically in Scheme 17. As in our synthesis of batzelladine D, we envisaged the right-hand tricyclic guanidine fragment of **1** evolving from pentacyclic diguanidine **78** by ring closure followed by hydrogenation. This intermediate would be the product of a highly convergent tethered Biginelli condensation between β-keto ester **79** and guanidine hemiaminal **61**. Ester **79** would arise from alcohol **80**.

To pursue the strategy outlined in Scheme 17, we began with guanidine alcohol **81** whose tricyclic guanidine moiety is enantiomeric to the guanidine fragment of alcohol **39**. Alcohol **81**, which was prepared analogously to **39**,[42] was acylated with methyl acetoacetate in the presence of DMAP to provide β-keto ester **82** in quantitative yield (Scheme 18).[33] This material was combined with an excess of guanidine aminal **61** and morpholinium acetate in 2,2,2-trifluoroethanol and heated at 60 °C for 48 h. Pentacyclic diguanidine **83** was obtained in 64% yield after separation from residual **61** and minor amounts (≤10%) of isomer **84**.

(42) Details are provided in the Supporting Information.

Scheme 18



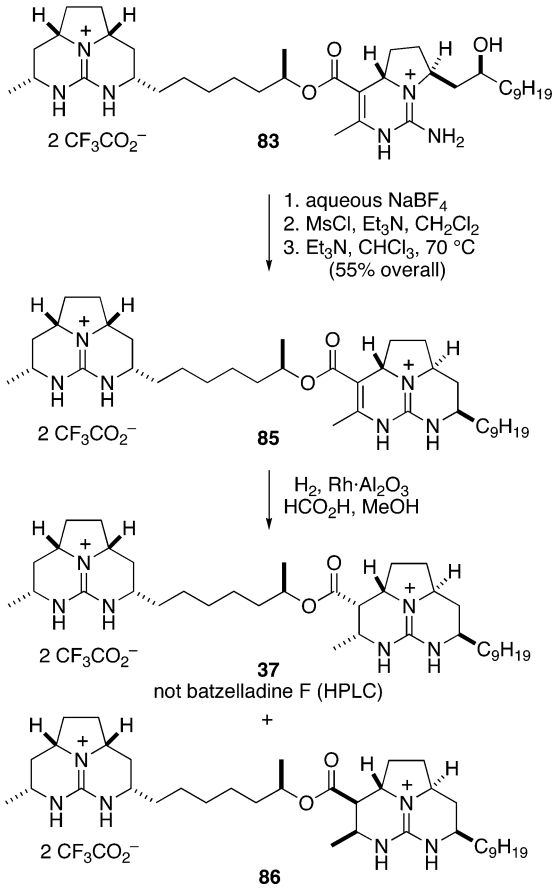
To complete the synthesis of the hexacyclic diguandine, we needed to close the final ring and saturate the C19−C27 double bond (Scheme 19). To this end, the triflouroacetate counterions of intermediate **83** were exchanged for tetrafluoroborate by washing a $CHCl_3$ solution of **83** with aqueous $NaBF_4$. This diguanidine salt was converted to its mesylate derivative by reaction with MsCl and Et_3N in CH_2Cl_2, and the mesylate intermediate was cyclized in hot $CHCl_3$ in the presence of excess Et_3N to deliver **85** in 55% overall yield. Finally, hydrogenation of hexacyclic diguanidine **85** over $Rh{\cdot}Al_2O_3$ in acidic MeOH provided batzelladine F isomers **37** and **86**, neither of which was identical to the natural product by HPLC comparison.

The stereoisomers of the proposed structure of batzelladine F that remained to be synthesized were **36** and **38**, which were epimeric at C16 to the isomers prepared thusfar (Figure 2). The synthesis of these compounds, which paralleled that of **37**, is detailed in the Supporting Information. To our chagrin, these isomers were also distinct from an authentic sample of batzelladine F by HPLC comparisons.

V. What is Batzelladine F? We had made one enantiomer of what we believed to be the four "possible" structures of batzelladine F that differed in relative configuration, however none were identical by HPLC comparisons to the natural product. Attention next turned to the remote possibility that the guanidinium counterions were complicating these comparisons. Batzelladine F was isolated as its diformate salt, and retains these counterions strongly. Thus, we converted synthetic stereoisomer **37**, a ditrifluoroacetate salt, to its diformate salt by washing a $CHCl_3$ solution of **37** with aqueous formic acid-sodium formate solution (0.5 M each). Complete conversion to the formate salt was confirmed by ^{19}F NMR analysis by observing disappearance of the signal at −76.8 ppm. As expected this diformate salt had the same HPLC retention time as the corresponding ditrifluoroacetate salt.

Having confirmed that the counterion would be unlikely to interfere with HPLC comparisons, our focus turned to a careful examination of the small sample of authentic batzelladine F that we had obtained from the SKB sample collection. Although some impurities were apparent, it was clear from its

Scheme 19

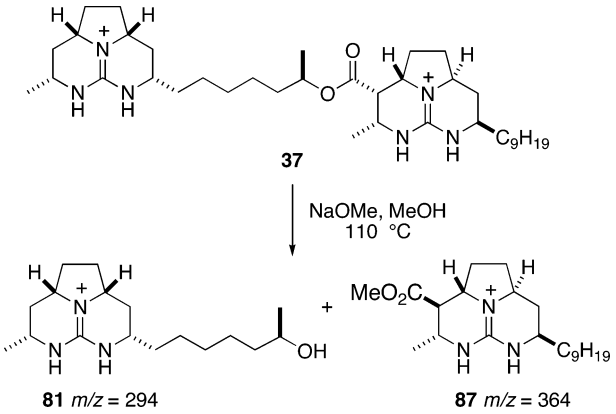


^1H NMR spectrum that this sample had not decomposed appreciably. In particular, the major component retained the original axial orientation of the ester as evidenced by the chemical shift and coupling constants of the methine hydrogen adjacent to the ester: δ 3.06 (dd, J = 4.6, 3.3 Hz, CD_3OD). As expected, the major peak in the HPLC chromatogram of the authentic sample possessed the expected exact mass by electrospray ionization (m/z 625.5161; 625.5169 calcd for $C_{37}H_{65}N_6O_2$). However, the mass spectrometry (MS) fragmentation pattern of authentic batzelladine F differed from those of the isomers we had prepared. Our samples showed peaks at m/z = 304 and 322 resulting from the expected McLafferty fragmentation of the ester bond, whereas the synthetic sample lacked these fragments and showed peaks at m/z = 276 and 350.[43]

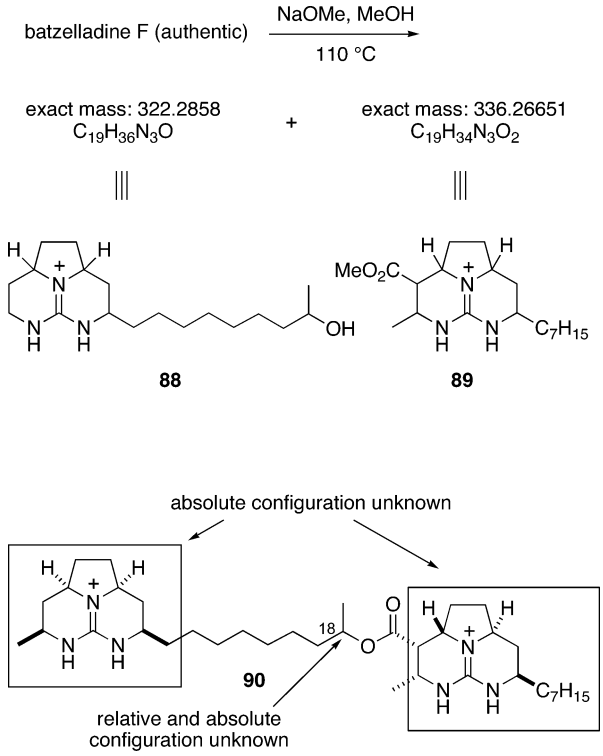
Now suspicious of the constitution of natural batezelladine F, we subjected both synthetic isomer **37** and authentic batzelladine F to basic methanolysis followed by MS analysis. Synthetic **37** yielded the expected peaks at m/z = 294 and 364, corresponding to alcohol **81** and ester **87** (Scheme 20). Yet the authentic sample yielded peaks at m/z = 322 and 336 (Scheme 21). As one product was 28 amu heavier than expected, whereas the other was 28 amu lighter, it became apparent that the lengths of the guanidine side chains in the originally proposed structure

(43) For a discussion of the McLafferty rearrangement in other batzelladine alkaloids see (a) Patil, A. D.; Kumar, N. V.; Kokke, W. C.; Bean, M. F.; Freyer, A. J.; De Brosse, C.; Mai, S.; Truneh, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Potts, B. C. M. *J. Org. Chem.* **1995**, *60*, 1182−1188. (b) Braekman, J. C.; Daloze, D.; Tavares, R.; Hajdu, E.; Van Soest, R. W. M. *J. Nat. Prod.* **2000**, *63*, 193−196.

Scheme 20



Scheme 21



were incorrect. It seemed likely that the methanolysis products of batzelladine F are **88** and **89**. Union of these fragments yields structure **90** (Figure 2), which fits all of the NMR and mass spectrometric data for batzelladine F.

Conclusion

Stereoselective synthesis of octahydro-5,6,6a-triazaacenaphthalenes having the anti relationship of the angular hydrogens flanking the pyrrolidine nitrogen confirmed suspicions[8] that the relative configuration of the left-hand tricyclic guanidine fragment of batzelladine F had been assigned incorrectly;[1] this unit should be revised to have the syn relationship of these hydrogens. A convergent synthesis strategy was devised, whose central step was a fragment-coupling tethered-Biginelli reaction, that allowed one enantiomer of the eight hexacyclic diguanidines depicted in Figure 2 to be prepared. These synthetic products differed from batzelladine F, therefore the mass spectrum of natural batzelladine F was reinvestigated. This analysis established that the originally proposed connectivity of this alkaloid also was incorrect, leading us to propose the revised costitution **90** of natural batzelladine F.

As the absolute configuration of the two tricyclic guanidine fragments of batzelladine F was still unknown and the relative configuration at C18 (Figure 2), eight possibilities (four enantiomer pairs) remained for the structure of batzelladine F. The total synthesis of one enantiomer of these four diastereomers and the comparisons of these products with the natural marine isolate that firmly established **90** as the correct constitution and revealed the full three-dimensional structure of batzelladine F are described in the accompanying article.[9b]

Acknowledgment. We thank NIH (HL-25854) for financial support, Pharmacia and the ACS Organic Division for fellowship support (F. C.), Dr. John Greaves for mass spectrometric analyses, and Dr. J. A. Chan of SmithKline Beecham for providing an authentic sample of batzelladine F, without which the structural revision would not have been possible. NMR and mass spectra were determined at UC Irvine with instruments purchased with the assistance of NSF and NIH.

Supporting Information Available: Reaction schemes for the preparation of compounds reported only in Supporting Information, experimental details and tabulated characterization data for new compounds, HPLC chromatograms of repuified natural batzelladine F and synthetic batzelladine analogues **36**, **37**, **38**, **86**, **S14**, and **S16**, ESI mass spectrometric analysis of synthetic batzelladine analogue **37** and authentic batzelladine F, copies of ^{1}H and ^{13}C NMR spectra of new compounds, and complete ref 16 (125 pages, print/PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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