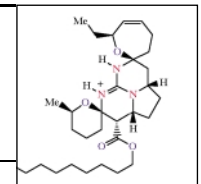
The guanidine metabolites of *Ptilocaulis spiculifer* and related compounds; isolation and synthesis



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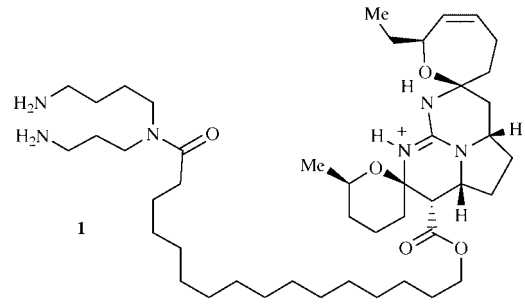
Marine natural products possessing guanidine functionalities display a considerable array of biological activity and not surprisingly have attracted considerable synthetic interest. This review discusses the isolation of several guanidine containing metabolites, primarily from the sponge *Ptilocaulis spiculifer*, but also from other marine organisms. It also explores the synthetic methodologies adopted for their preparation and speculates on the structural similarity of the metabolite ptilomycalin A to abiotic guanidine based anionic receptor molecules.

1 Isolation of ptilomycalin A and related metabolites

Naturally occurring and synthetic compounds containing guanidine are of considerable interest due both to the hydrogen-bond mediated interaction of guanidinium ions and because of the wide range of biological activities these substances display.[1] Recent interest in marine natural products has seen a steady increase in the number of metabolites isolated from these sources and a metabolite of particular interest isolated from the sponge *Ptilocaulis spiculifer*[2] in 1989 is ptilomycalin A **1**. This alkaloid has a unique and fascinating structure consisting of a pentacyclic guanidinium core linked to a spermidine unit *via* a ω-hydroxy acid spacer group. The same compound was also isolated from a Red Sea sponge of the genus *Hemimycale* sp.[3] and subsequently from the sponge *Batzella* sp.[4] and the starfishes *Fromia monilis* and *Celerina heffernani*[5] (Scheme 1).



1

Scheme 1

Ptilomycalin A displays a remarkable range of biological activities including cytotoxicity against the following cell lines; P388 (IC_{50} 0.1 μg mL^{-1}), L1210 (IC_{50} 0.4 μg mL^{-1}), and KB (IC_{50} 1.3 μg mL^{-1}) in addition to antifungal activity against *Candida albicans* (MIC 0.8 μg mL^{-1}) as well as very good antiviral activity (HSV) at a concentration of 0.2 μg mL^{-1}.[2]

Previous to the isolation of ptilomycalin A the related guanidinium alkaloids ptilocaulin **2** and isoptilocaulin **3** had been isolated from the same sponge in 1981[6] (Scheme 2).

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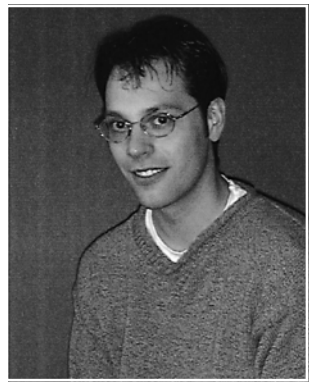
Christopher G. Moore graduated (BSc Hons) from Bangor in 1995 and is currently finishing his postgraduate studies which have focused on the total synthesis of guanidine containing marine metabolites.

Patrick J. Murphy graduated (BSc Hons) from the University of Manchester Institute of Science and Technology in 1983 and went on to complete a PhD entitled 'Synthetic routes towards naturally occurring thiotetronic acids' in 1986 under the supervision of Dr John Brennan at the same institute. After a period of postdoctoral research at the University of Salford under the supervision of Professor Garry Procter (organosilicon chemistry) and Dr Mike Casey (reactions of chiral sulfoxides), he was appointed to the post of Lecturer in Organic Chemistry at the University of Wales, Bangor, gaining promotion to Senior Lecturer in 1999. Since gaining this post he has concentrated his research efforts on the development of new methodology and its applications in synthesis, with two key themes being the synthesis of naturally occurring guanidine containing marine alkaloids and new applications of Wittig chemistry.

Laura Heys



Christopher G. Moore

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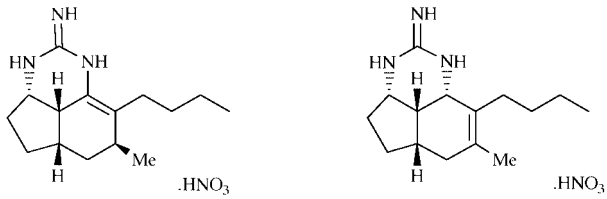


2 **3**

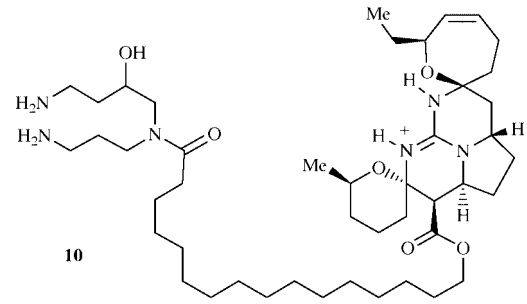
Scheme 2

Several metabolites related to ptilomycalin A have subsequently been isolated including the crambescidins 800, 816, 830 and 844 (**4**–**7**) from the Mediterranean sponge *Crambe crambe*[7] and in the case of **4** also from the sponge *Monanchora arbuscula*.[8] The structures of these closely related guanidines were elucidated in 1991 and differ from ptilomycalin A by the presence of a hydroxyspermidine instead of a spermidine residue, the presence of an extra hydroxy-substituent on C-13 (in the pyrrolidine ring) in the case of crambescidins 816, 830 and 844 and in the variation in length of the ω-hydroxy fatty acid. These polycyclic guanidines were reported to exhibit antiviral activity against *Herpes simplex* virus type 1 (HSV-1) and they were cytotoxic to L1210 murine leukaemia cells[7] and crambescidin 816 has been shown to be a potent calcium channel blocker.[9] In addition two related guanidinium alkaloids were isolated from the New Caledonian starfishes *Celerina heffernani* and *Fromia monilis*, and named celeromycalin **8**, which bears an extra hydroxy function on the ω-hydroxy fatty acid and fromiamycalin **9** which contained a modified spermidine unit (Scheme 3).

Interestingly the polar extracts of the starfish *Fromia monilis* also contained ptilomycalin A and crambescidin 800, which allows us to speculate that these metabolites may have arisen from a symbiotic organism common to both species. Both celeromycalin and fromiamycalin showed antiviral activities against the *Herpes simplex* virus (HSV-1) and were also found to be highly cytotoxic in anti-HIV assays on CEM 4 cells infected with human immunodeficiency virus (HIV-1) with CC-50 (cytotoxic concentration) of 0.11 μg mL^{-1} (ptilomycalin A, crambescidin 800 and fromiamycalin) and 0.32 μg mL^{-1} (celeromycalin).[5]

Possibly one of the most interesting of these metabolites to be isolated is 13,14,15-isocrambescidin 800 **10**, the structure of which was shown to contain a *trans*-ring junction in the guanidine pentacycle.[3,10] Surprisingly this molecule is substantially less cytotoxic to L1210 cells than the other crambescidins, and has no observed antiviral activity against HSV-1. This observation might suggest that the enclosed ionic pocket found in ptilomycalin A and the crambescidins and lacking in **10** might be conferring much of the biological activity found in these compounds (Scheme 4).

In addition, a series of related guanidine alkaloids, the crambescins A, B, C1 and C2 **11**–**14**, were isolated from the same sponge *Crambe crambe* and displayed cytotoxicity



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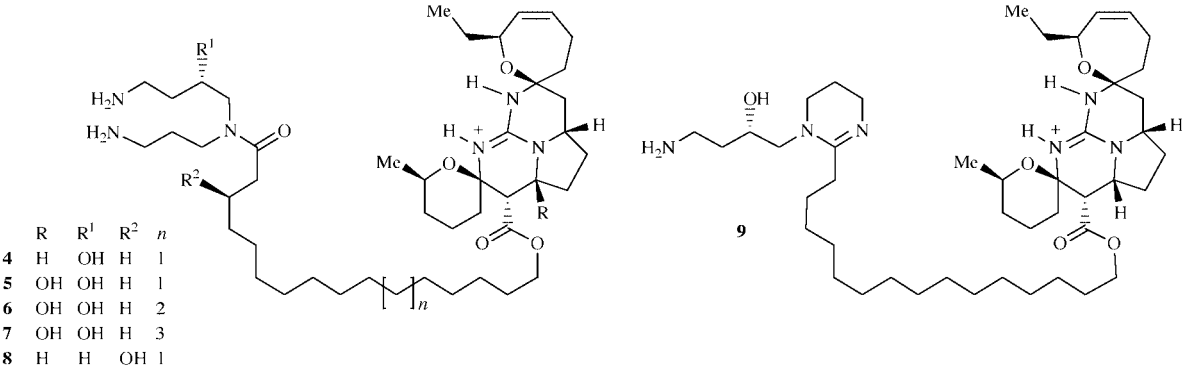
Scheme 4

against L1210 cells (IC_{50} of less than 1 μg mL^{-1}) and were found to be ichthyotoxic (toxic against fish)[11] (Scheme 5).

The batzelladines A–E **15**–**19**, a series of guanidine alkaloids containing a tricyclic guanidine unit, were isolated from the Caribbean sponge *Batzella* sp. which was also found to contain the previously isolated metabolites ptilomycalin A, ptilocaulin, crambescin A, crambescidin 800 and 816.[4,12] Batzelladines A and B were reported to inhibit the binding of HIV-gp120 to the CD4 cell-surface receptor protein on T cells and to be of interest in the treatment of AIDS.[4] The structures of the batzelladines A–E were originally elucidated by interpretation of the spectral data of the natural material and of the methanolysis products. The original stereochemical assignment of the alkaloids had been presumed to be one in which the methine hydrogens in the pyrrolidine ring, and those adjacent to the guanidine were all *cis*-, which was based on a previous literature precedent.[2] Subsequent work by Snider[13] has shown that in the cases of batzelladine A and D the original assignment was incorrect and the relative stereochemistry of these metabolites was in fact *trans*. Both batzelladine A and B contain two guanidinium ring systems connected by a linear hydrocarbon chain, with the left-hand bicyclic guanidine being similar in nature to crambescidin A **11**. It is interesting to note that the carbon skeleton present in the tricyclic guanidine nucleus of batzelladine E, together with the alkene geometry is identical to that found in ptilomycalin A **1** (Scheme 6).

Following the isolation of these alkaloids, a further four batzelladine metabolites, termed batzelladine F–I **20**–**23**, were obtained from the same source.[14] These were interesting in so much as they all contained two tricyclic guanidine units one of which lacks an ester function, together with varying degrees of unsaturation and the presence of *N*-hydroxylation in all but **20**. The relative stereochemical assignment of these metabolites was made by comparison with batzelladines A–E,[4,13] however synthetic work[15] has shown that the original assignment of the left-hand tricycle of batzelladine F is possibly incorrect and that the stereochemistry is in fact an all *cis*-arrangement of the methine hydrogens (Scheme 7).

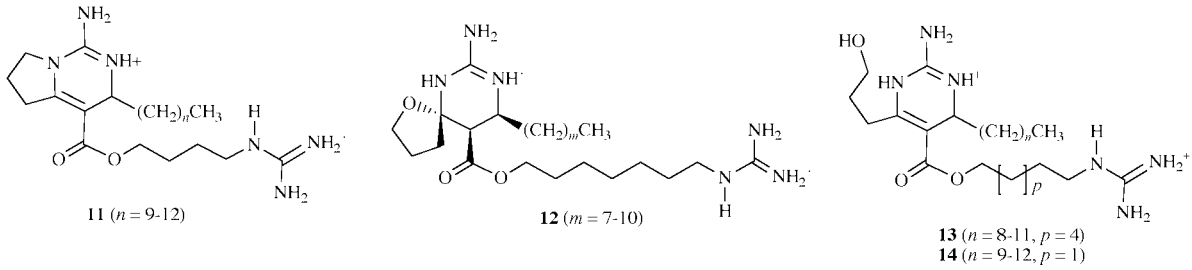
As can be seen, these polycyclic guanidine alkaloids isolated from marine sponges possess a wide range of biological activities as well as intriguing molecular structures. It is thus not surprising that they have attracted considerable interest from



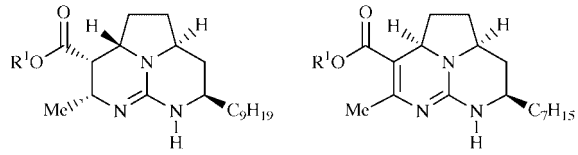
	R	R^1	R^2	*n*
4	H	OH	H	1
5	OH	OH	H	1
6	OH	OH	H	2
7	OH	OH	H	3
8	H	H	OH	1

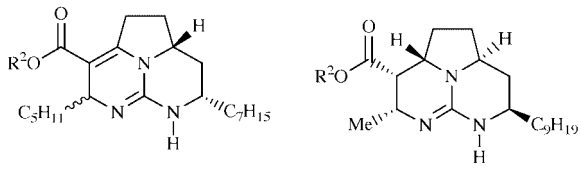
9

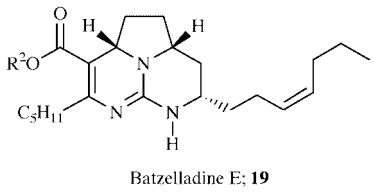
Scheme 3

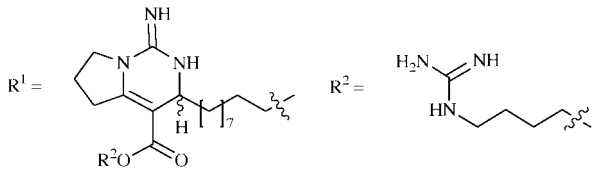


Scheme 5







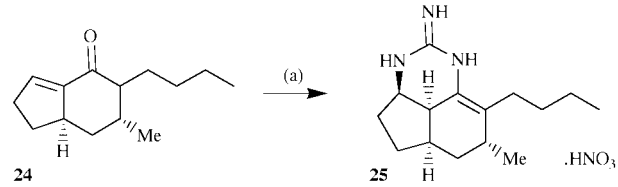


Scheme 6

several synthetic research groups and have also been the topic of some speculation as to the exact biological role and mechanism of action of these metabolites. The remainder of this review will focus on the synthetic routes applied to these metabolites and will comment on work relating to the biological aspects previously mentioned.

2 Synthetic contributions from the Snider group

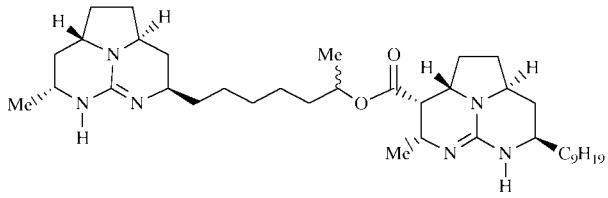
The Snider research group has made several significant contributions to the synthesis of polycyclic guanidine alkaloids beginning with a synthesis, in racemic form, of the metabolite ptilocaulin **2** and the confirmation of its absolute stereochemistry by the synthesis of the unnatural enantiomer **25** *via* addition of guanidine to the enone **24**[16] (Scheme 8).

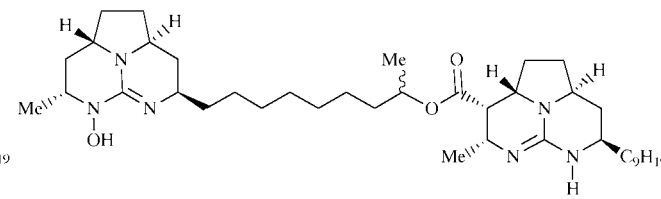


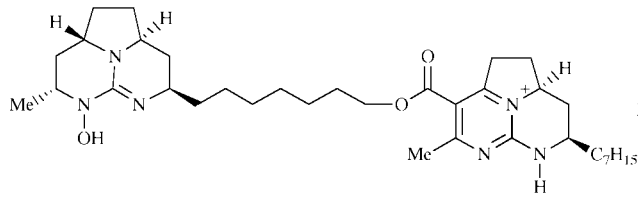
Scheme 8 *Reagents and conditions*: (a) Guanidine, PhH, reflux 25 h, then HNO_3 (1% aq); 40%.

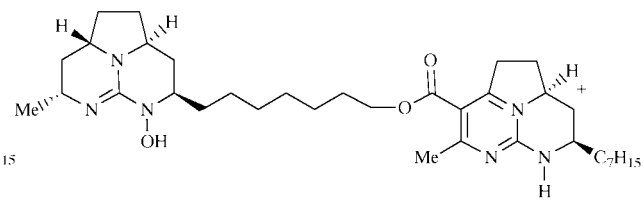
A conceptually similar strategy has been adopted by Snider in his synthetic approach to the crambescins, ptilomycalin A and the batzelladine alkaloids. The first report in this area detailed the addition of *O*-methylisourea to the enones **26** leading to the formation of the dihydropyrimidines **27** after desilylation, which were then converted into the guanidines **28** by treatment with methanolic ammonia. These key intermediates were then converted into crambescins C1 **13** and C2 **14** by hydrogenolysis, crambescin A **11** by mesylation, cyclisation and hydrogenolysis and to crambescin B **12** by cyclisation under basic conditions and hydrogenolysis[17] (Scheme 9).

This work set the scene for a similar synthetic approach to ptilomycalin A and preliminary model studies[18] on the bis-

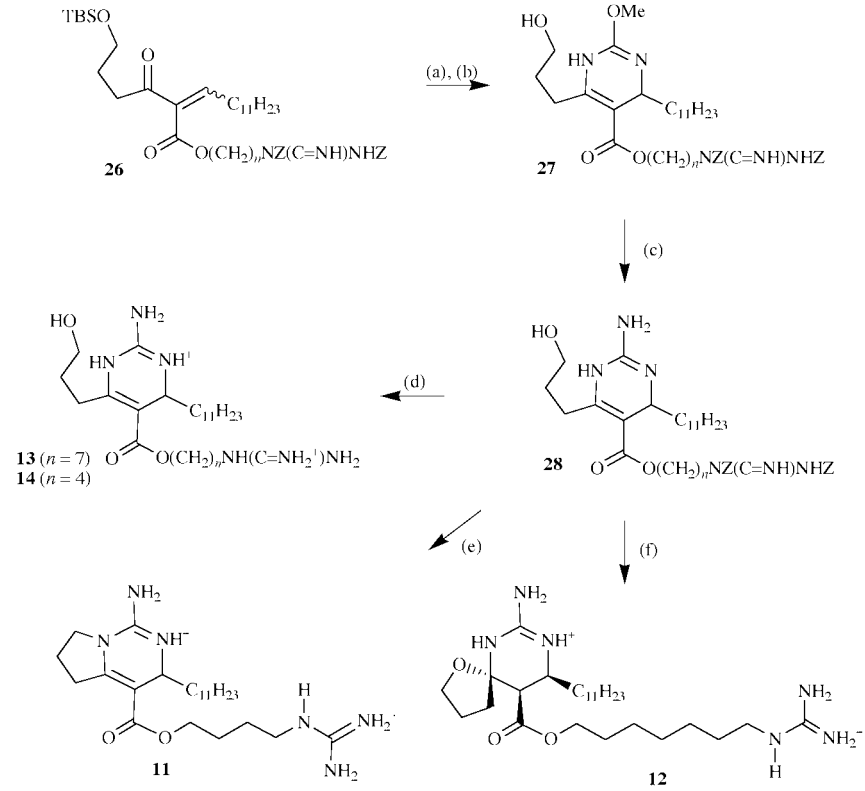








Scheme 7

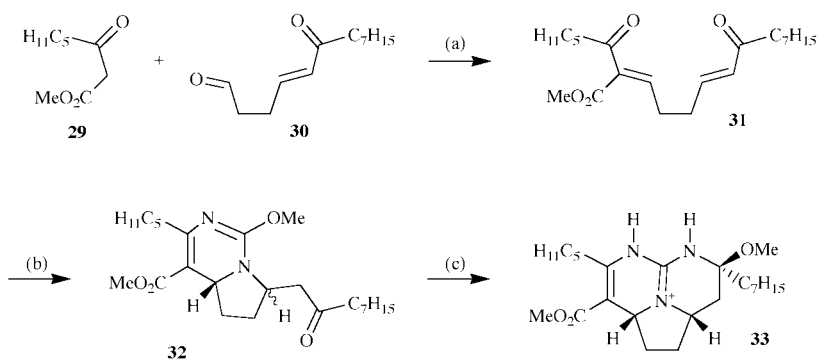


Scheme 9 *Reagents and conditions*: (a) 2 equiv. *O*-methylisoureido sulfate, $NaHCO_3$, DMF, 55 °C, 3 h, 94%; (b) HF, CH_3CN, rt, 1.5 h, 88%; (c) NH_3, NH_4OH, *t*-BuOH, MeOH, 60 °C, 3 days, (94% and 81% loss of one of the two Z-groups is observed); (d) H_2/Pd/C, HCl, $CHCl_3$ (88% and 91%); (e) MsCl, Et_3N, CH_2Cl_2, then Et_3N, $CHCl_3$, Δ, 24 h, (78%) then H_2/Pd/C, HCl, $CHCl_3$, 3 h (93%); (f) Et_3N, Δ, $CHCl_3$, 12 h, then H_2/Pd/C, HCl, $CHCl_3$, rt, 3 h, (89% and 91%).

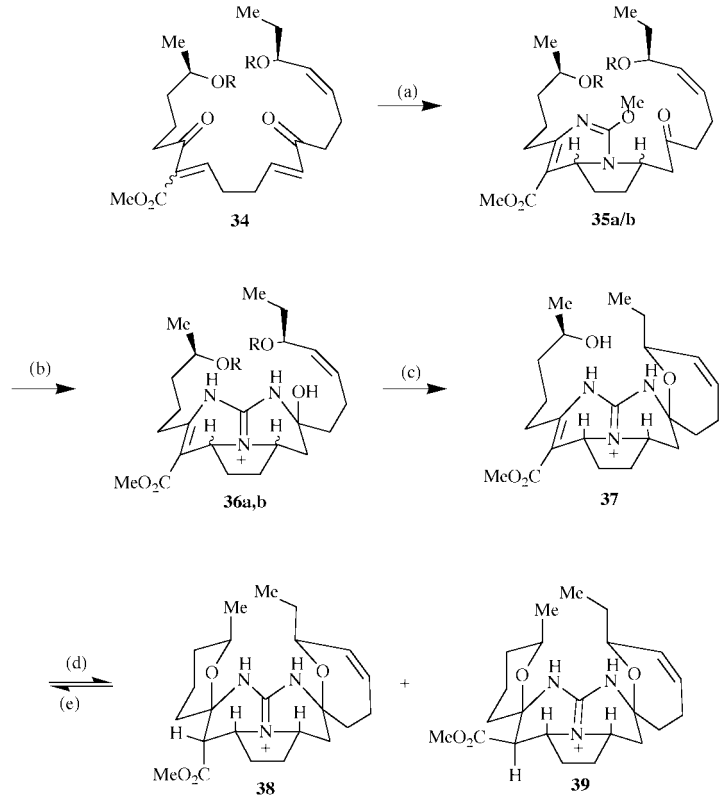
enone **31** prepared by Knoevenagel condensation of β-ketoester **29** and aldehyde **30**, illustrated that the addition of *O*-methylisourea was a feasible process leading to the bicyclic compound **32** as a 3:1 mixture of *trans*- and *cis*-isomers. This reaction appears to lead to the incorrect stereochemistry at the pyrrolidine ring junction, however on reaction with methanolic ammonia, which generates the guanidine functionality, *trans*-**32** undergoes isomerisation, presumably by an elimination and readdition process, with concomitant cyclisation to form the tricyclic model **33** of ptilomycalin A (Scheme 10).

Following on from these preliminary studies Snider reported a synthesis of the methyl ester of the pentacyclic nucleus of ptilomycalin A **38** *via* a convergent and biomimetic 14-step route.[19] The key steps of this route involved the conversion of the bis-enone **34** to **38** in four steps. The addition of *O*-methylisourea to **34** gave a 1:4 mixture of the two *cis* diastereomers **35a** and the two *trans* diastereomers **35b** in 52% yield. Ammonolysis converted this mixture of isoureas into a 1:1 mixture of the two *cis* diastereomeric guanidines **36a** (*cis*, β) and **36b** (*cis*, α) in 72% yield. Deprotection of the silyl ethers was accomplished by reaction with a 3:7 mixture of 50% aqueous HF and acetonitrile. Treatment of the resulting crude mixture (containing intermediate **37**) with triethylamine in methanol gave a 60% yield of an approximately 65% pure 1.3:1 mixture of **38** and the diastereomer **39** with an *equatorial* methyl ester. The remaining material was thought to consist of tri- and tetracyclic compounds from the undesired diastereomer **36b**. Flash chromatography succeeded in separating **38** and **39** but gave only 80–85% pure material. Purification of this mixture, therefore, was best accomplished by treating the 1.3:1 mixture of **38** and **39** with triethylamine in 1:1 MeOH–H_2O to give tetracyclic alcohol **37**. This was purified by flash chromatography and recycled with triethylamine in methanol to again give a 1.3:1 mixture of **38** and **39**, which were separated to give pure **38** (34% from **36a**) and **39** (26% from **36a**) (Scheme 11).

Snider has also reported the first unequivocal synthesis (in racemic form) of one of the batzelladine alkaloids, batzelladine E, and in doing so was able to correct the structural misassignment of this metabolite which was initially given an *E*-stereochemistry in the unsaturated side chain.[13b] The synthesis begins with the aldehyde **40** which was condensed with



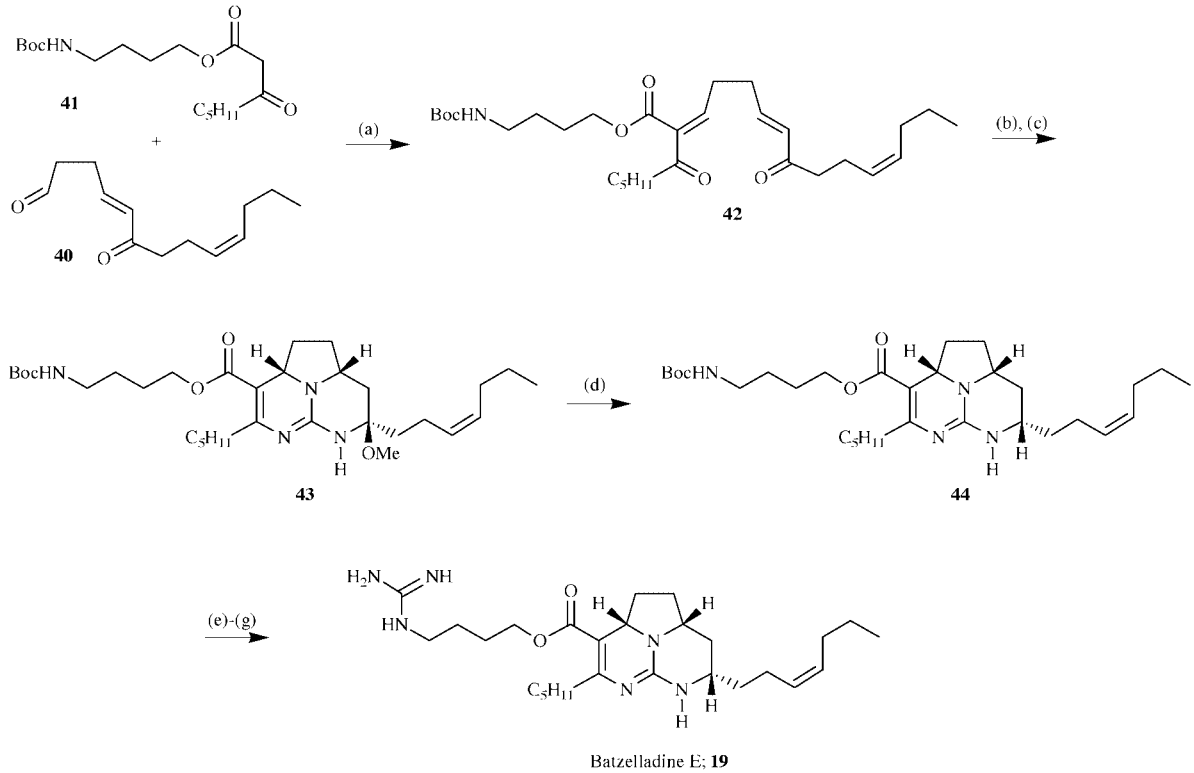
Scheme 10 *Reagents and conditions*: (a) Piperidine, CH_2Cl_2, −20 °C, 2 days, 61%; (b) 2 equiv. *O*-methylisoureido sulfate, $NaHCO_3$, DMF, 50 °C, 2 h, 56%; (c) NH_3, NH_4OAc, MeOH, 60 °C, 4 days, 60%.



Scheme 11 *Reagents and conditions*: (a) *O*-methylisoureido sulfate, *i*-Pr_2EtN, DMSO, 80 °C, 1.5 h, 52%; (b) NH_3, NH_4OAc, *t*-BuOH, 60 °C, 40 h, 72%; (c) 3:7 HF–CH_3CN, −30 °C, 3 days; (d) Et_3N, MeOH, 60 °C, 20 h (78%); (e) Et_3N, MeOH–H_2O (1:1), 60 °C, 16 h, then (d), **38**; 34% and **39**; 26%.

β-ketoester **41** under Knoevenagel conditions to give the enone **42**. Reaction of this enone with *O*-methylisourea followed by ammonolysis gave the product **43** which on careful reduction led to the tricyclic guanidine **44**. Deprotection of the amine function was followed by conversion to the terminal guanidine group, thus completing a synthesis of batzelladine E in 9 steps and in 3% overall yield from a commercially available starting material (Scheme 12).

As mentioned previously, Snider had also reported[13a] the reassignment of the relative stereochemistry of batzelladines A **15** and D **18**. This was accomplished by reaction of enone **45** with *O*-methylisourea under standard conditions leading to a



Batzelladine E; **19**

Scheme 12 *Reagents and conditions*: (a) 0.33 equiv. piperidine, 0.30 equiv. AcOH, CH_2Cl_2, −20 °C, 2 days; (b) *O*-methylisourea, *i*-Pr_2EtN, DMSO, 55 °C, 4 h; (c) NH_3, NH_4OAc, *t*-BuOH, 60 °C, 1 day (14% from **40**); (d) $NaCNBH_3$, NaH_2PO_4, MeOH, 25 °C, 88%; (e) 1:4 TFA–CH_2Cl_2, 25 °C, 5 min, 93%; (f) $(BocNH)_2C{=}S$, 2-chloro-*N*-methylpyridinium chloride, CH_2Cl_2, NEt_3, 25 °C, 1 h, 64%; (g) 1:1 TFA–CH_2Cl_2, 25 °C, 2 h, 90%.

35% yield of a 6:1 mixture of the dihydropyrimidine **46** and its *cis*-isomer. At this point in the synthesis the ketone function was 'protected' by reduction to the alcohol **47**, which also serves to lock the pyrrolidine in a *cis*-configuration, which was then converted to the guanidine **48**. Regeneration of the ketone function led to the formation of the tricyclic guanidine **49** which was reduced and saponified to give the carboxylic acid **50**, a known degradation product of batzelladines A and D, thus leading to the conclusion that the stereochemistry in these metabolites was indeed *trans*- across the pyrrolidine ring system (Scheme 13).

3 Synthetic contributions from the Overman group

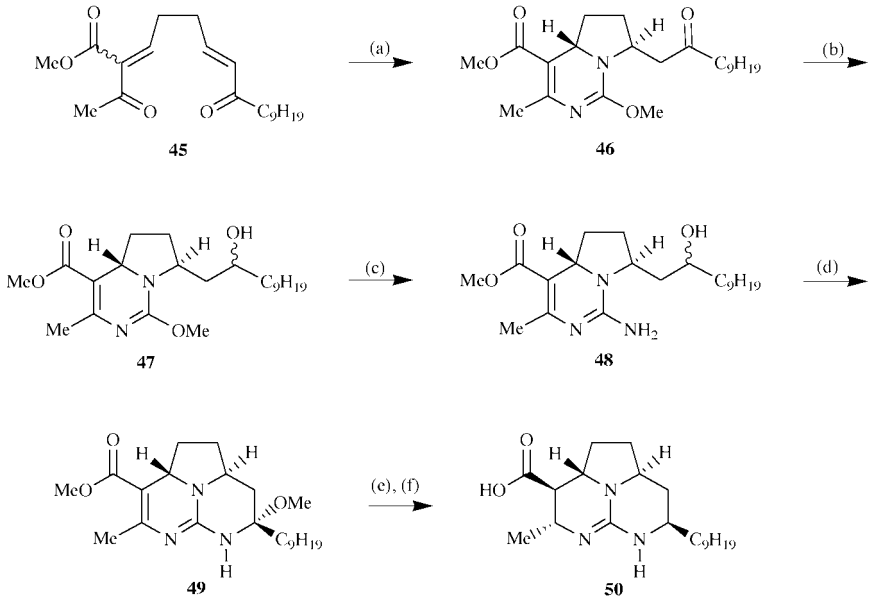
The Overman research group has also been actively pursuing the synthesis of these polycyclic guanidine alkaloids, indeed, they have reported the first total synthesis of ptilomycalin A **1** using a tethered intramolecular Biginelli reaction.[20,21] Preliminary studies on this reaction,[20] which classically involves the condensation of an aldehyde, a β-ketoester and a urea to give a 3,4-dihydro-1*H*-pyrimidin-2-one, had indicated its potential as a synthetic methodology. A subsequent report[21] illustrated that reaction of **51**, which represents the urea and masked aldehyde portions of the reaction, with the β-ketoester **52** in the presence of morpholinium acetate (typical Knoevenagel conditions), led to the formation of the bicyclic pyrimidin-2-one **53**. This product was cyclised to give the lower half of the pentacyclic guanidine core **54** by treatment under acidic conditions and converted to the aldehyde **55** *via* Swern oxidation and *O*-methylation with MeOTf. These two intermediates differ from the corresponding portion in ptilomycalin A only by the stereochemistry at the ester position. Introduction of the upper half of the pentacycle was effected by reaction with the Grignard reagent **58** followed by Swern oxidation to the ketone **56**; this was followed by deprotection of the silyl protecting group and treatment with a mixture of ammonia and ammonium acetate to give **57**. Routine modification of the ester function to a protected spermidine unit was followed by epimerisation of the ester stereocentre by treatment with triethylamine and finally deprotection, leading to the first total synthesis of ptilomycalin A **1** (Scheme 14).

Overman has also applied the tethered intramolecular Biginelli reaction to the synthesis of tricyclic systems similar to those observed in the batzelladines and has reported that reaction of bicyclic guanidine **59**, prepared in 32% yield from nonan-2-one, with methyl acetoacetate led to the formation of the tricycle **60** as a 10:1 mixture of *cis*- and *trans*-isomers in 94% yield.[13c] This structure is identical to a reported[4] degradation product of batzelladine B in all respects, including the sign of rotation, thus confirming the absolute stereochemistry of the batzelladines to be that illustrated (Scheme 15).

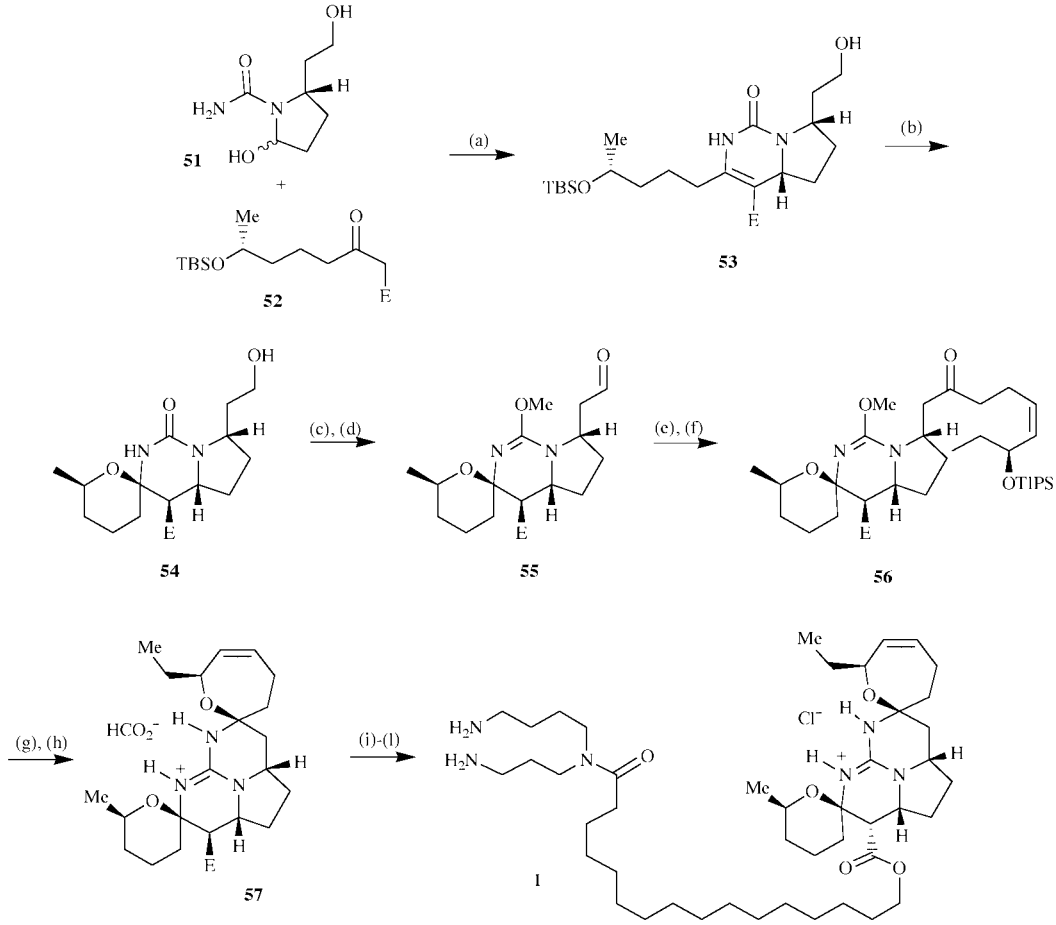
Interestingly Overman has also reported that by judicious choice of reaction conditions it is possible to tune the selectivity of this reaction to obtain either the *cis*- or *trans*-pyrrolidine geometry.[22] Thus treatment of either the urea derivative **61** (X = O, R = H or Bn) with benzyl acetoacetate under Knoevenagel conditions leads to the formation of *cis*-**62** and *trans*-**62** (X = O, R = H or Bn) in a 4:1 ratio (80 and 81% yield respectively). However on reaction of **61** (X = O, R = Bn) and benzyl acetoacetate with the mild dehydrating agent polyphosphate ester (PPE) the stereoselectivity was reversed leading to a 4:1 ratio of products with the *trans*-isomer predominating. Interestingly, treatment of the guanidine **61** (X = NH·HCl, R = OH) under identical Knoevenagel conditions led to the formation of *trans*- **62** (X = NH·HCl, R = OH) exclusively in 42% yield, which is in complete contrast to the stereoselection observed with ureas. Further work with the *N*-sulfonylguanidine **61** (X = MtrN, R = H, Bn) led to the formation of *cis*-**62** and *trans*-**62** in a 6–7:1 ratio (X = MtrN, R = H, Bn; 61 and 84% yield respectively) under Knoevenagel conditions, but again reaction of **61** (X = MtrN, R = Bn) with PPE led to a reversal of selectivity with the *trans*-**62** (X = MtrN, R = Bn) predominating in a 20:1 ratio (61% yield) (Scheme 16).

4 Synthetic contributions from the Murphy group

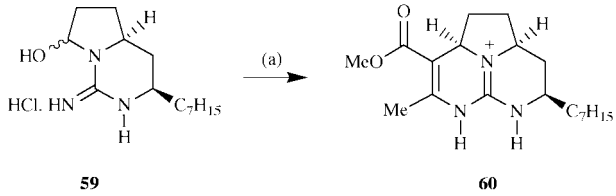
Within our own research group we have been pursuing the synthesis of both ptilomycalin A and the batzelladine alkaloids utilising a strategy based upon the double 1,4-addition of guanidine to bis-enones. Preliminary studies[23] illustrated that the formation of the pentacyclic guanidines **66** and **69** was possible using this strategy. Synthesis of the substrate for these model studies was straightforward in that reaction of lactones



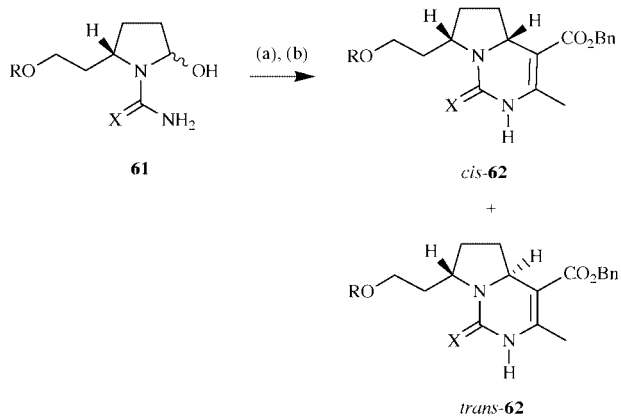
Scheme 13 *Reagents and conditions*: (a) *O*-methylisourea hydrogen sulfate, *i*-Pr_2EtN, DMSO, 75 °C, 5 h; (b) $NaBH_4$, *i*-PrOH, 25 °C; (c) NH_3, NH_4OAc, MeOH, 60 °C, 2 days; (d) Dess–Martin, CH_2Cl_2, 25 °C, then MeOH, 25 °C, 12 h; (e) $NaCNBH_3$, NaH_2PO_4, MeOH, 25 °C, 16 h then 65 °C, 5 h; (f) NaOH, MeOH 25 °C, 18 h.



Scheme 14 *Reagents and conditions*: (a) Morpholine, AcOH, EtOH, Na_2SO_4, 70 °C, 61%; (b) PPTS, MeOH, 50 °C, then *p*-TosOH, $CHCl_3$, 23 °C, 96%; (c) Swern oxidation; (d) MeOTf, 2,6-di-*tert*-butylpyridine, CH_2Cl_2, 23 °C, 67%; (e) (*S*)-*Z*-EtCH(OTIPS)CH=$CHCH_2CH_2MgBr$ (**58**), THF, −78 °C; (f) Swern oxidation, 58% (2 steps); (g) TBAF; (h) NH_3, NH_4OAc, *t*-BuOH, 60 °C, 51% (2 steps); (i) $Pd(Ph_3P)_4$, pyrrolidine, MeCN, 23 °C, 75%; (j) $BocHN(CH_2)_3NH(CH_2)_4NHBoc$, EDCl, DMAP, CH_2Cl_2, 23 °C, 60%; (k) NEt_3, MeOH, 65 °C, 50%; (l) HCO_2H, 23 °C, 100%. E = $CO_2(CH_2)_{15}CO_2Allyl$.



Scheme 15 *Reagents and conditions*: (a) Methyl acetoacetate, morpholinium acetate (1 equiv.), CF_3CH_2OH, Na_2SO_4, 90 °C, 36 h, 94%.



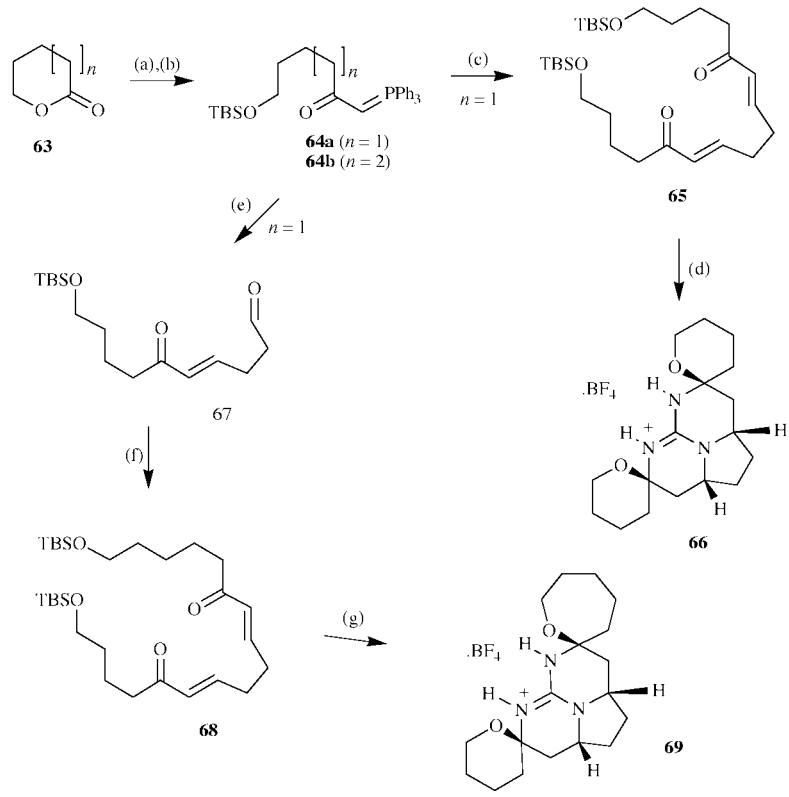
Scheme 16 *Reagents and conditions*: (a) Benzyl acetoacetate (1.5 equiv.), morpholinium acetate (1.5 equiv), CF_3CH_2OH, Na_2SO_4, 60 °C, 48 h; (b) PPE, CH_2Cl_2, 23 °C, 48 h.

63 with two equivalents of methylenetriphenylphosphorane followed by silyl protection of the intermediate phosphonium alkoxide gave the phosphoranes **64**. Wittig reaction of **64a** with 0.4 equivalents of succinaldehyde gave the symmetrical bis-enone **65** in 54% yield. Further reaction of this with one equivalent of guanidine, followed by removal of solvent, deprotection/cyclisation with methanolic HCl and counter ion exchange, afforded two products identified as the *cis*-product **66** and the corresponding *trans*-pentacycle in an approximate 4:1 ratio, from which the major product could be isolated in 25% yield by recrystallisation.

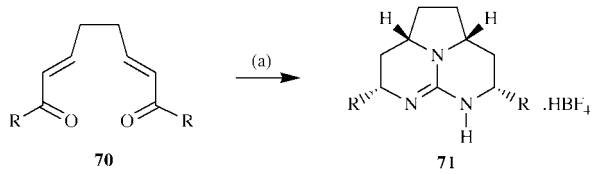
Similarly reaction of the phosphorane **64a** with an excess of succinaldehyde led to the formation of the aldehyde **67** in 43% yield from the lactone starting material; reaction of this with phosphorane **64b** gave the unsymmetrical bis-enone **68**. Reaction of **68** with guanidine under identical conditions to those previously employed, led to the formation of two pentacyclic guanidines in an approximate 4:1 ratio and from which the major isomer **69** could be obtained by crystallisation in 20% yield (Scheme 17).

Following on from this work we also reported that the addition of guanidine to a series of bis-enones **70** followed by reduction with sodium borohydride led to the formation of the tricyclic guanidines **71**, which, before the structural reassignments reported by Snider,[13] were thought to be models of the saturated batzelladine alkaloids A and D[24] (Scheme 18).

With the isolation of batzelladine F, it became apparent that there was a strong correlation in structure between the left-hand portion of this molecule and the model compounds previously prepared by us;[15a] we thus embarked upon a synthesis of the



Scheme 17 *Reagents and conditions*: (a) 2 equiv. CH_2=PPh_3, THF, −78 °C; (b) TBDMSCl, imidazole, DMF; (c) 0.4 equiv. succinaldehyde, THF, 48 h; 54% overall; (d) (i) guanidine, DMF, 3 h, (ii) MeOH, HCl, 0 °C–rt, 24 h; (iii) aq. $NaBF_4$ (sat.), (iv) trituration and crystallisation; 25% overall; (e) steps (a), (b) then 10 equiv. succinaldehyde, THF, 43%; (f) **64b**, THF, 48 h; 37%; (g) as (d) 20%.



Scheme 18 *Reagents and conditions*: (a) (i) Guanidine, DMF, 0 °C–rt, 5–8 h, (ii) 3 : 1 : 3 DMF–H_2O–MeOH, then $NaBH_4$, 16 h, (iii) HCl (aq), (iv) aq. $NaBF_4$ (sat.). R = Me, C_5H_{11}, C_9H_{19}, Ph, (22–33% yield).

left-hand sub unit of this metabolite employing this methodology.[15b] Reaction of the iodide **72** with the anion generated from deprotonation of phosphorane **73** led to the phosphorane **74**, which on reaction with excess succinaldehyde gave the aldehyde **75**. Subsequent reaction of this with a further equivalent of phosphorane **73** led to the formation of bis-enone **76**, which on reaction with guanidine under standard conditions gave the tricycle **77**. Comparison of the spectral data of **77** or the corresponding deprotected analogue **78** or acetate **79**, illustrated a strong correlation between these materials and the naturally occurring batzelladine F thus leading to the conclusion that the reported stereochemistry is incorrect and is actually as illustrated[25] (Scheme 19).

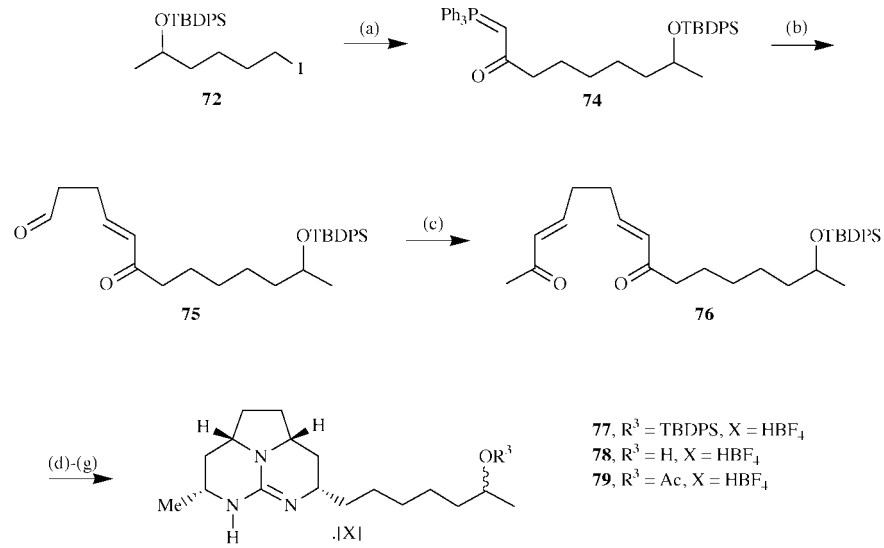
5 Synthetic contributions from the Hiemstra group

A recent publication from the Hiemstra group describes the preparation of tricyclic guanidines from substituted pyrrolidin-2-ones utilising an *N*-acyliminium ion coupling reaction with silyl enol ethers and a direct guanylation with bis-Boc-thiourea and mercury(II) chloride.[26] They reported that reaction of the silyl enol ether **80** with lactam **81** led to the formation of the substituted lactam **82** in 63% yield. After conversion of **82** into the corresponding thiolactam, an Eschenmoser sulfide contraction procedure led to the formation of the vinylogous amide **83**. Reduction and *N*-Boc protection of **83** gave the substrate **84** which was subjected to a three stage procedure which included protected guanidine **85** as an intermediate. This was cyclised under acidic conditions to give the tricyclic guanidine **86** in 33% overall yield, together with several other *trans*-substituted guanidines which could be recycled to give further **86** by treatment with ammonia and ammonium acetate in methanol at 60 °C[26] (Scheme 20).

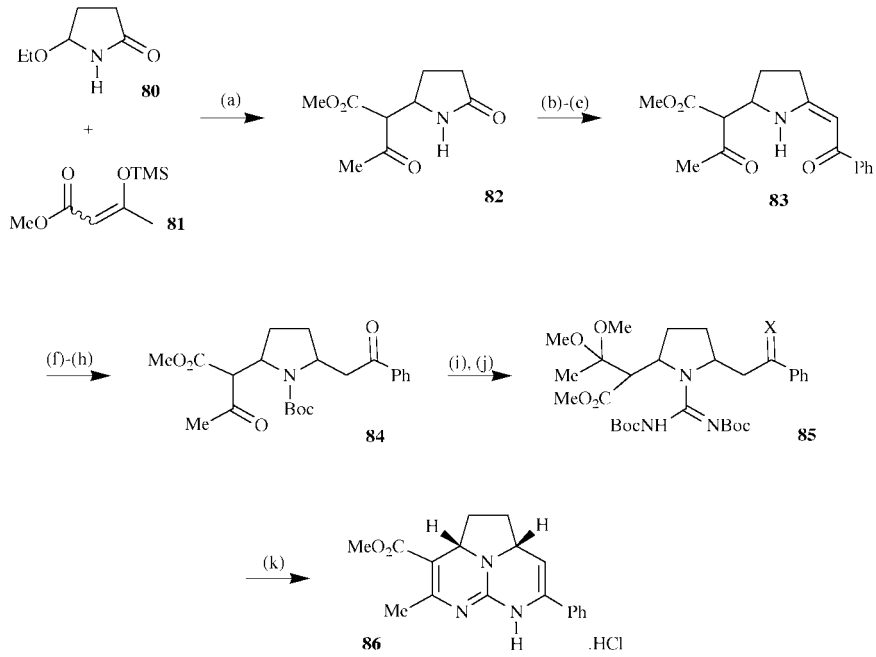
This synthesis is obviously applicable to the preparation of both ptilomycalin A **1** and the batzelladine alkaloids. Indeed Hiemstra has further reported[27] that bis-acetoxylactam **87** reacts with the silyl enol ether **88** in a stereocontrolled fashion to give **89**, which is a potential precursor of **1** (Scheme 21).

6 Synthetic contributions from the Rama Rao group

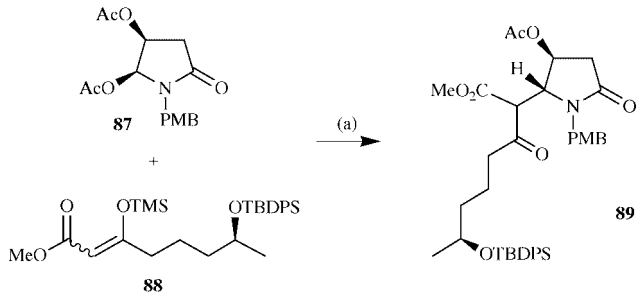
The Rama Rao research group has also reported[28] an enantiospecific synthesis of the tricyclic guanidine portion of batzelladine A, which was based upon the original stereochemical assignment made by Patil,[4] and thus targeted an all *cis*-arrangement of hydrogens in the final product. In common with Hiemstra, the synthesis proceeds *via* a lactam, **91** prepared in eight steps from the azetidinone derivative **90**, and introduction of the side chain was effected using an Eschenmoser sulfide contraction reaction, leading to the α,β-unsaturated ketone **92**. After reduction of the alkene and ketone functions and *N*-Boc protection of the pyrrolidine nitrogen, the introduction of the remaining nitrogen containing groups was accomplished by sequential nucleophilic displacement of the secondary alcohols present on each side chain with azide (under Mitsunobu conditions), reduction and *N*-boc protection, ultimately leading to azide **93**. This was converted to the cyclic urea **94** using 1,1′-carbonyldiimidazole, which in turn was cyclised by treatment with dimethylsulfate and hydrogenation to give the guanidine **95**, after removal of the silyl protecting group (Scheme 22).



Scheme 19 *Reagents and conditions*: (a) $CH_3COCHPPh_3$ (**73**), *n*-BuLi, THF, −78 °C–rt; (b) succinaldehyde, CH_2Cl_2, 24 h, 54% for 2 steps; (c) **73**, CH_2Cl_2, 24 h, 91%; (d) (i) guanidine, DMF, 0 °C, 5 h; (ii) 3 : 1 : 3 DMF–H_2O–MeOH, then $NaBH_4$, 16 h; (iii) HCl (aq), (iv) aq. $NaBF_4$ (sat); **77**, 29% overall; (e) (i) MeOH–HCl, (ii) aq. $NaBF_4$ (sat.), **78**, 91%; (f) Ac_2O–Py, then HCl (2 M), 41%; (g) aq. $NaBF_4$ (sat), **79**, 100%.



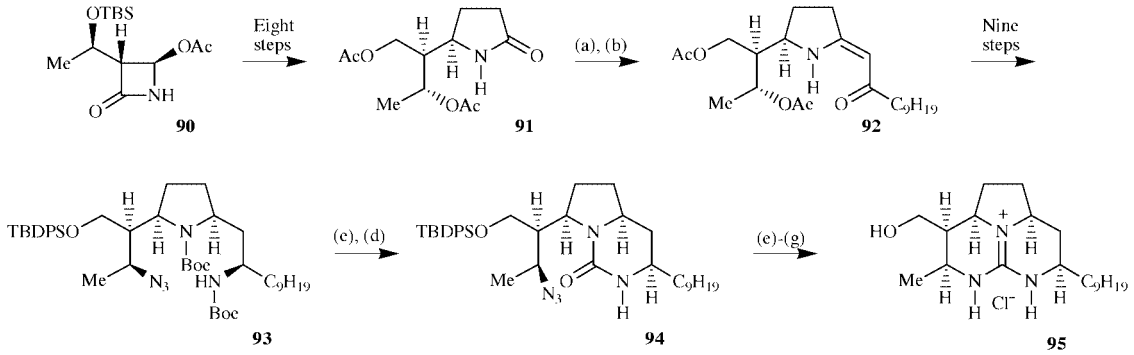
Scheme 20 *Reagents and conditions*: (a) TMSOTf, −78 °C–rt, CH_2Cl_2, 18 h, 63%; (b) Lawesson's reagent, $PhCH_3$, 80 °C, 10 min, 91%; (c) $PhCOCH_2Br$, Et_2O, rt, 18 h; (d) Et_3N, CH_2Cl_2, rt, 2 h, 83%; (e) PPh_3, $CHCl_3$, 60 °C, 18 h, 82%; (f) $NaBH_3CN$, 3 : 1 AcOH–THF, 0 °C, 40 min, 99%; (g) Boc_2O, DIPEA, THF, rt, 18 h, 91%; (h) PCC, CH_2Cl_2, mol. sieves (4 Å), rt, 3 h, 91%; (i) $CH(OMe)_3$, H_2SO_4 (cat), MeOH, 50 °C, 5 h; (j) $SC(NHBoc)_2$, $HgCl_2$, Et_3N, DMF, 0 °C–rt, 18 h; X = O, $(OMe)_2$; (k) HCl, MeOH, rt, 3 h, 33% for three steps.



Scheme 21 *Reagents and conditions*: (a) TMSOTf, −78 °C–rt, CH_2Cl_2, DIPEA, 1 h, 75%.

7 Ptilomycalin A as a potential host molecule

One important feature of the chemistry of guanidinium compounds is their ability to interact with anionic species, indeed, this is a key feature in the function of the amino acid arginine whose guanidine group serves as a key binding site for carboxylate and phosphate containing substrates in a wide range of biological applications.[29] It is apparent that ptilomycalin A **1** has an extraordinary molecular architecture in which the pentacyclic core (Fig. 1) can be considered to have a 'cage' like structure capable of binding strongly to an anion and possibly encapsulating it, indeed, Kashman referred to this core as the 'vessel' part of the molecule linked *via* the aliphatic 'chain' to a spermidine 'anchor'.[2b] In fact, one interesting feature of this metabolite is its structural similarity to abiotic guanidine based anionic receptor molecules.[30] Based on this hypothesis, Kashman[2b] investigated the complexing ability of a trifluoroacetate derivative of **1** with various organic carboxylates and determined a scale of binding ability for *N*-acetylamino acids which was estimated to be as follows: L-*N*-acetylmethionate ≈ L-*N*-acetylvalinate > L-*N*-acetylalanate ≈ L-*N*-acetylisoleucinate >> L-*N*-acetylglycinate. In addition, there is evidence suggest-



Scheme 22 *Reagents and conditions*: (a) $C_9H_{19}COCH_2Br$, CH_2Cl_2, rt, 30 min $KHCO_3$; (b) PPh_3, *t*-BuOK, *t*-BuOH, C_6H_6, heat, 65%; (c) TFA, CH_2Cl_2, 0 °C–rt, 30 min; (d) $(imid)_2C{=}O$, THF, 0 °C–rt, 65%; (e) Me_2SO_4, C_6H_6, heat, 16 h; (f) H_2, $Pd/BaSO_4$, MeOH, 12 h, 65%; (g) HCl (1 M), MeOH, 50 °C, 2 h, 90%.

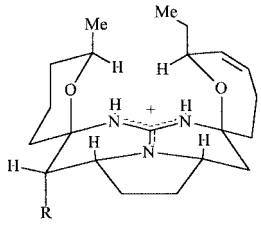


Fig. 1

ing that the spermidine unit may also be involved in the binding of anionic species to ptilomycalin A.[31] It is also interesting to note that ptilomycalin A, despite containing several polar functional groups, is a relatively non-polar molecule which is freely soluble in organic solvents such as chloroform.[2a] These properties suggest an anionic binding capability possibly linked to strong lipophillic behaviour.[21]

It has been speculated[32] that the enclosed ionic 'cage' at the central guanidine sub-unit might indeed be acting as a specific anion recognition site thereby conferring much of the biological activity found in these compounds. In relation to this, it is interesting to note that the subsequently isolated 13,14,15-iso-crambescidin 800 **10** is substantially less cytotoxic to L1210 cells than other crambescidins and has no observed antiviral activity against HSV-1;[10] this reduced activity may be due to the lack of this structural feature (Fig. 2).

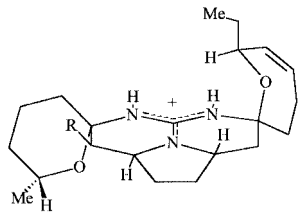
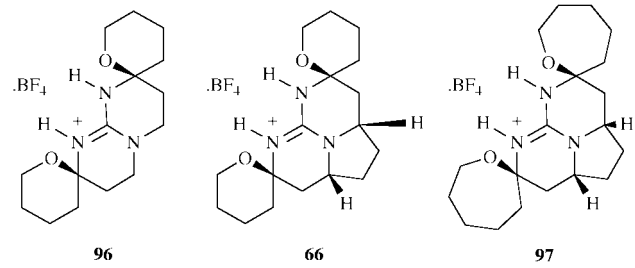


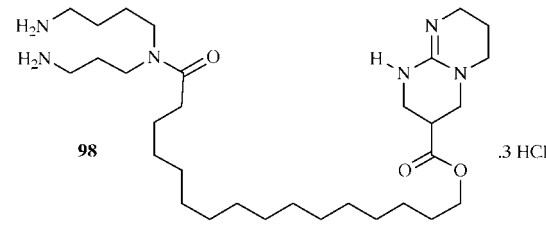
Fig. 2

We have reported[32] an interesting behaviour pattern in the interaction between the guanidinium and fluoroborate ions in the model compounds **96**, **66** and **97**. Fluoroborate can undergo a similar interaction with a guanidinium ion to that of the bidentate ligating interaction that is observed with a carboxylate or a phosphate. It was thus surprising to find that in compound **96** only one of the fluorine atoms of the fluoroborate anion was involved in a strong hydrogen bonding interaction with the guanidinium cation (to both N–H bonds). This observation led us to suppose that the guanidinium cavity of **96** was not of sufficient size to accommodate the fluoroborate anion. This behaviour was however not observed in the pentacyclic 6,6,5,6,6 model compound **66** which corresponds more closely to the structure of ptilomycalin A. In this case, the fluoroborate anion was involved in two separate non-symmetrical hydrogen bonding interactions, but was unable to achieve co-planarity with the guanidinium ion. However, near co-planarity was achieved in the case of the pentacyclic 7,6,5,6,7 model **97** which undergoes an identical hydrogen bonding pattern to **66** (Scheme 23). These observations offer some support to the theory of the 'cage' portion of ptilomycalin A being involved in a recognition process and that the structure of **1** represents an optimum host design for an as yet undetermined guest molecule.



Scheme 23

A further report from Hart and Grillot[33] describes the synthesis of an analogue **98** (Scheme 24), of ptilomycalin A **1**, which models the guanidine core, chain and spermidine sub-



Scheme 24

units of the natural material and was prepared in a 12 step convergent sequence from acyclic precursors *via* a cyclic thiourea derivative. This molecule was prepared in an attempt to mimic the biological activity found in the natural system, however, it was found to be unstable and underwent an unidentified decomposition process over a period of a few weeks which precluded biological evaluation. It was suggested that this process involved cleavage of the ester linkage and the authors speculated that the role of the spiro-*N,O*-acetals present in ptilomycalin A **1** might be to protect the ester linkages from hydrolysis or aminolysis.

8 Conclusion

Ptilomycalin A and related molecules are obviously of considerable interest from both a synthetic and biological perspective. The current state of the synthetic work offers several strategies

for the synthesis of these metabolites and analogues thereof, with several total syntheses having been reported. The biological activities of these metabolites ranges over a broad spectrum, however, little is known about the exact mechanism of these activities despite much speculation. We believe that in the near future this area of investigation, together with continued synthetic efforts, will offer a valuable insight into these intriguing metabolites.

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