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A synthesis of crambescidin 359

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Abstract—A potentially biomimetic synthesis of the guanidine-containing marine natural product crambescidin 359 via a double Michael addition of guanidine to a suitably functionalised bis-enone is reported.

Crambescidin 359 **1** was recently isolated from the marine sponge *Monanchora unguiculata* by Braekman and co-workers.[1] The structure of **1** consists of a pentacyclic guanidine unit similar to that present in the related natural product, ptilomycalin A **2**.[2] It differs from **2** in that it lacks a pendent spermidine group attached to the pentacyclic core via an ω-hydroxy fatty acid chain and an ester linkage (Fig. 1).

Our general synthetic approach towards the synthesis of **2** and related natural products which encompasses a biomimetic hypothesis has already been reported.[3] For example, addition of guanidine to bis-α,β-unsaturated ketone **3** followed by deprotection and spirocyclisation gave the pentacyclic guanidine **4**, which is structurally similar to the pentacyclic moiety in ptilomycalin A (Scheme 1).

We are prompted to report our synthesis[4] of crambescidin 359 **1** by a publication by Nagasawa and co-workers who prepared **1** using a nitrone cycloaddition approach.[5]

Our approach towards **1** relies upon the preparation of a suitable bis-enone precursor for the guanidine addition step. This initially involved the preparation of ylid **7**, which was obtained in five steps from ethyl-(*R*)-3-hydroxybutyrate **5**. Thus **5** was silyl protected, following which the ester function was reduced to the alcohol and then converted to iodide **6** via the corresponding

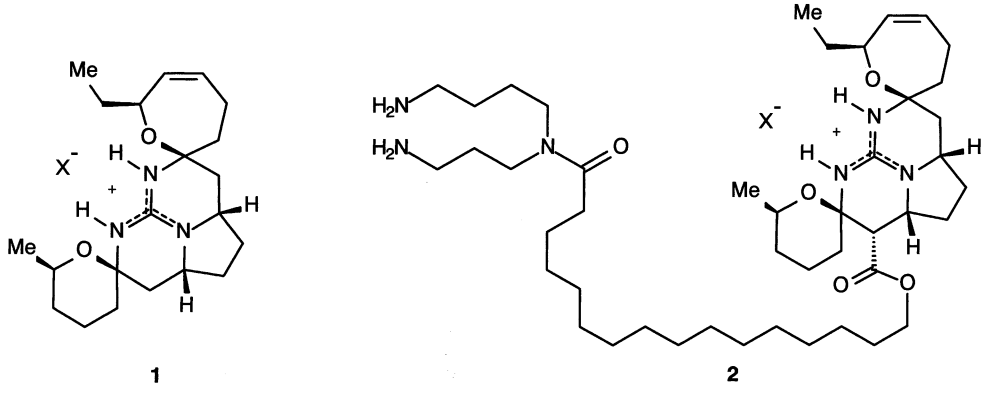

Figure 1. X^- = unspecified.

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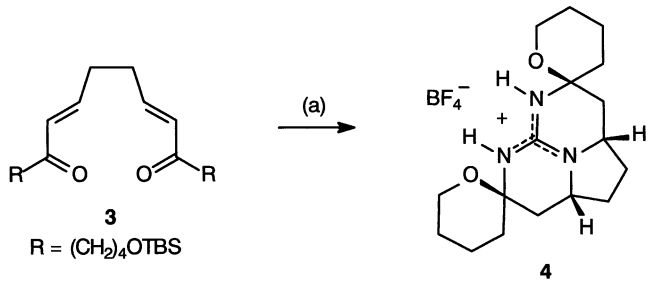
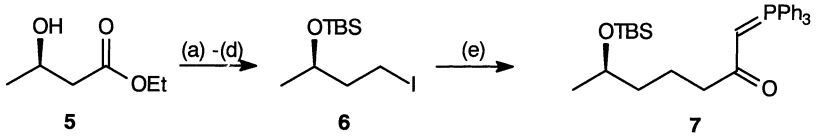


Scheme 1. *Reagents and conditions*: (a) (i) guanidine, DMF, 3 h, (ii) MeOH, HCl, 0°C–rt, 24 h, (iii) $NaBF_4$ (satd, aq.), 25% overall.

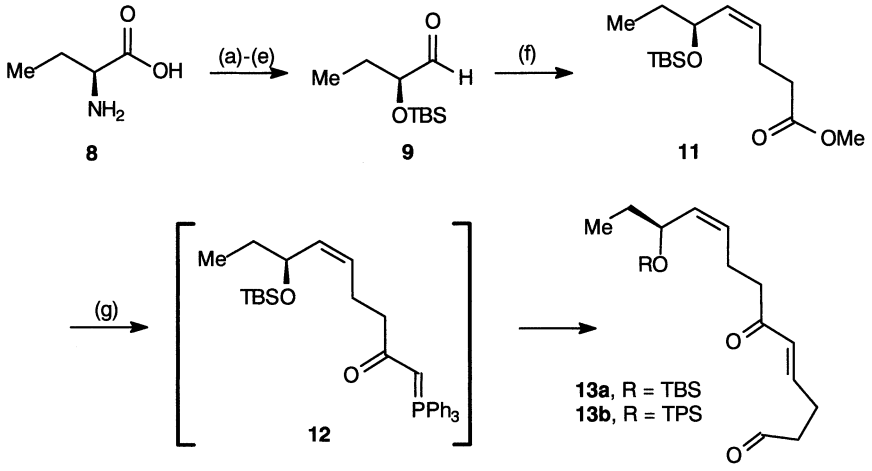
tosylate. This was then treated with the lithium anion of acetylmethylene triphenylphosphorane to afford **7** in 51% yield (Scheme 2).

The preparation of aldehyde **13a** proved to be a more significant challenge. Starting from (*S*)-2-aminobutyric acid **8**, aldehyde **9** was prepared in five steps. Thus **8** was diazotised in the presence of sodium nitrite and 1 M H_2SO_4 to give the corresponding α-hydroxy acid[6] which was then esterified using HCl in methanol (23% over two steps). Silyl protection of the alcohol function (47%) was followed by DIBAL-H reduction (72%) and Swern oxidation of the resulting alcohol to give aldehyde **9** in 93% yield. Wittig reaction of **9** with the ylid generated from 3-carboxypropyltriphenylphosphonium bromide **10**, followed by esterification with diazomethane gave the *cis*-alkene **11** together with the *trans*-isomer (minor) in 45% yield and in a 4:1 ratio.[7] After separation from the *trans*-isomer, *cis*-**11** was treated with 2 equiv. of methylenetriphenylphosphorane to give the stabilised phosphorane **12** which on reaction with an excess of freshly prepared succinaldehyde gave the desired aldehyde **13a** in 42% yield. Whilst this route was convenient in terms of the number of steps, the low yields in several of the reactions together with the separation of the *E*/*Z* isomers in the preparation of **11** were problematical. We also prepared the known compound **13b** via an alternative route[8] as part of a study on the Knoevenagel condensation[4] and it was this compound which proved to be the best precursor for the final transformations (Scheme 3).

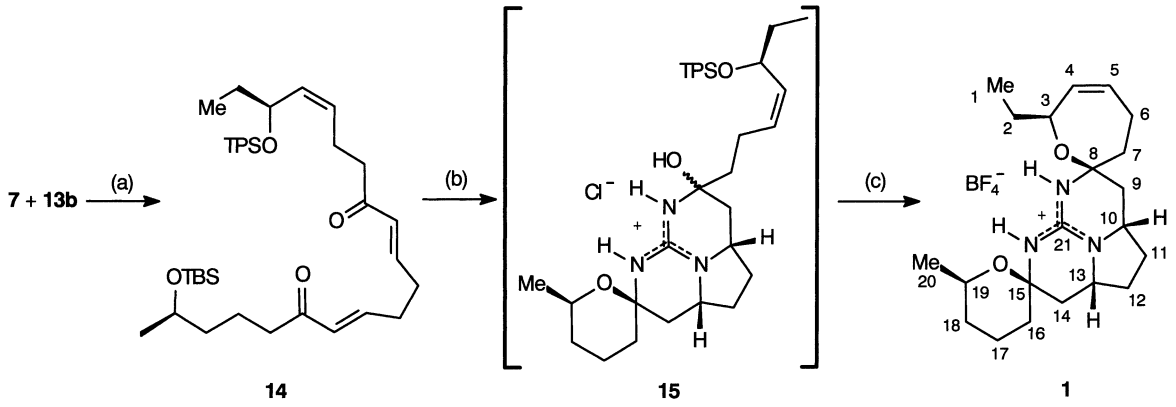
The synthesis of the required bis-enone **14** was accomplished in 73% yield by reaction of ylid **7** with aldehyde **13b** in dichloromethane. Addition of a DMF solution of guanidine to **14**, also in DMF, at 0°C followed by stirring at this temperature for 6 h resulted in the complete consumption of the starting bis-enone. After dilution with water and addition of a solution of methanolic HCl we obtained, after work-up, a mixture of compounds in which the TBS protecting group had been removed, but the TPS group remained; NMR evidence strongly suggested the presence of the tetracycle **15**.[9] This mixture was therefore treated with 5



Scheme 2. *Reagents and conditions*: (a) TBSCl, DMF, imid., 95%; (b) DIBAL-H, hexane, –78–0°C, 8 h, 79%; (c) TosCl, py., 0°C–rt, 16 h; 78%; (d) NaI, acetone, reflux, 4 h, 95%; (e) $CH_3COCHPPh_3$, *n*BuLi, –78°C, then **6**, warm to rt, 51%.



Scheme 3. *Reagents and conditions*: (a) $NaNO_2$, H_2SO_4 0°C–rt, 24 h; (b) MeOH, HCl, 48 h (23%, two steps); (c) TBSCl, imid., DMF, 0°C–rt, 48 h, 47%; (d) DIBAL-H, –78°C–rt, 5 h, 72%; (e) Swern ox., 93%; (f) (i) $MeO_2CCH_2CH_2CH_2PPh_3^+Br^-$ **10**, NaHMDS, THF, reflux, 30 min, (ii) **9**, rt, 30 min, (iii) CH_2N_2, Et_2O (45%, 4:1, *Z*:*E*); (g) (i) 2 equiv. $CH_2{=}PPh_3$, THF, –78°C–rt, 2 h, (ii) succinaldehyde, THF, 42 h (42%, two steps).



Scheme 4. *Reagents and conditions*: (a) DCM, 0°C–rt, 36 h, 73%; (b) (i) guanidine, DMF, 0°C, 6 h, (ii) H_2O, MeOH, HCl, 0°C–rt, 16 h; (c) (i) THF, TBAF, 25–30°C, 3 h, then rt, 64 h, (ii) MeOH, HCl, 0°C, 4 h, then $NaBF_4$ (satd, aq.), CH_2Cl_2, 18% overall.

equiv. of TBAF in THF at 25–30°C for 3 h and then at ambient temperature for a further 64 h. Finally, acid-mediated cyclisation followed by counterion exchange and purification by column chromatography on silica gel gave the desired pentacycle **1**·BF_4 in 18% yield from **14**[†] (Scheme 4).

The structure and stereochemistry of **1**·BF_4 were confirmed by detailed 2D NMR and NOE spectroscopy. The analytical data[11] for **1**·BF_4 was found to be consistent with that reported.[1,5] In particular the optical rotation of −8.2 (*c* 0.2, CH_2Cl_2) compares favourably with that of the synthetic material[5] (−8.0, *c* 0.2, CH_2Cl_2) and the natural product[1] (−9.0, *c* 0.2, CH_2Cl_2) isolated as the chloride salt. We also investigated the biological activity of synthetic crambescidin 359 **1**·BF_4, which was tested against four cancer cell lines in comparison with ptilomycalin A[12] (Table 1). As can be seen **1**·BF_4 is active against all the cell lines tested, however, the levels of activity are all significantly lower than those reported for ptilomycalin A. This is in line with our previous studies in this area which suggest that the fatty acid chain and spermidine residue are essential for high levels of activity in this series of compounds.[12]

Table 1.

Compound	K562[a]	A2780[a]	H-460[a]	P388[a]
1·BF_4	12.30	24.44	10.36	2.93
Ptilomycalin A **2**	0.35	0.27	0.35	0.11[b]

[a] Cytotoxic activity (IC_{50}/μg ml^{-1}); K562, human chronic myelogenous leukaemia; A2780, human, ovarian carcinoma; H-460, human large cell carcinoma, lung. High DT-Diaphorase; P388: mouse, lymphoid neoplasm.

[b] Data obtained from Ref. 2a.

[†] This yield is comparable with our previous work in this area, and as the guanidine addition is not diastereoselective, half of the tricyclic bis-silylated guanidine adduct does not undergo cyclisation to **1**.

In conclusion, we have reported a convergent and potentially biomimetic synthesis of crambescidin 359 **1** and have shown that it displays lower levels of biological activity than ptilomycalin A, another member of this group of natural products.

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11. Selected data for **1**·BF_4: IR ν_{max}: 3224 (w), 2975 (m), 2933 (s), 2868 (w), 1656 (s), 1604 (s), 1445 (w), 1342 (w), 1236 (w), 1066 (s), 1023 (s) cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.89 (1H, br s, NH), 7.83 (1H, br s, NH), 5.66 (1H, ddt, $J=11.4$, 7.7, 2.4 Hz, CH-5), 5.49 (1H, dt, $J=11.0$, 2.8, CH-4), 4.47 (1H, br d, $J=9.8$ Hz, CH-3), 4.06 (2H, m, CH-10, CH-13), 3.81 (1H, ddq, $J=12.6$, 6.5, 2.8 Hz, CH-19), 2.59 (1H, dd, $J=12.7$, 5.7 Hz, CH-9β), 2.55 (1H, br t, $J=13.7$ Hz, CH-7α), 2.32 (2H, m, CH-6β, CH-12β) 2.28 (1H, m, CH-14β), 2.19 (1H, dd, $J=14.0$, 5.7 Hz, CH-11β), 2.16 (1H, m, CH-6α), 2.06 (1H, m, CH-17α), 1.87 (1H, dd, $J=14.3$, 5.7 Hz, CH-7β), 1.72 (3H, m, CH-16α, CH-16β, CH-17β), 1.70 (1H, m, CH-12α), 1.69 (1H, m, CH-11α), 1.62 (1H, m, CH-18α), 1.52 (1H, m, CH-2α), 1.49 (1H, t, $J=12.7$ Hz, CH-14α), 1.42 (1H, m, CH-2β), 1.36 (1H, t, $J=12.4$ Hz, CH-9α), 1.20 (1H, m, CH-18β), 1.06 (3H, d, $J=6.4$ Hz, CH_3-20), 0.83 (3H, t, $J=7.2$ Hz, CH_3-1) ppm. ^{13}C NMR: δ 147.68 (C-21), 133.61 (CH-4), 129.77 (CH-5), 84.06 (C-8), 80.46 (C-15), 70.94 (C-3), 67.17 (C-19), 53.50 (CH-10), 51.97 (CH-13), 39.80 (CH_2-14), 37.08 (CH_2-9), 36.46 (CH_2-7), 33.56 (CH_2-16), 32.19 (CH_2-18), 30.04 (CH_2-11), 29.84 (CH_2-12), 29.21 (CH_2-2), 23.72 (CH_2-6), 21.65 (CH_3-20), 17.91 (CH_2-17), 10.21 (CH_3-1) ppm. MS (CI) m/z: 360 (5% $[M+H]^+$). HRMS (EI) m/z: found: 359.2566, $C_{21}H_{33}N_3O_2$ ($[M]^+$) requires: 359.2573. $[\alpha]_D^{26}=-8.2$ (c 0.2, CH_2Cl_2).
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