The first total synthesis of aeruginosamide†

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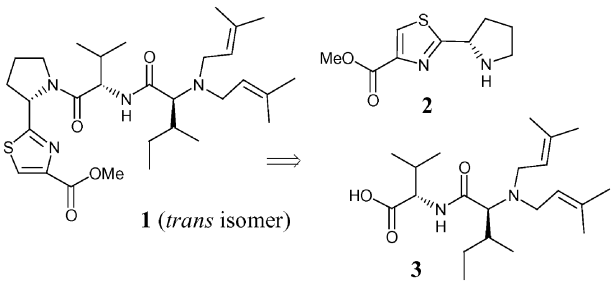
Synthesis of aeruginosamide, a metabolite of the cyanobacterium *Microcystis aeruginosa*, required overcoming difficulties encountered in a convergent route and an interesting change in conformation of the product governed by the conditions for the final step.

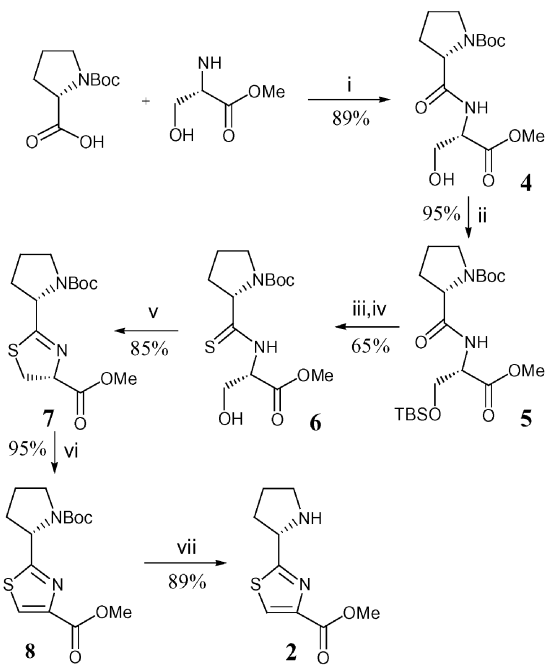
There has been considerable activity recently in the areas of isolation and synthesis of natural products originating from marine cyanobacteria.[1–6] Such organisms produce the biologically potent compounds hectochlorin,[7] dolastatin 10,[8] onchidin[9] and apratoxins.[10,11] Since it is difficult to obtain significant amounts of these structurally-interesting moieties, we[12] and other groups[13] have undertaken extensive programmes towards their syntheses. Here we present our work on the preparation of aeruginosamide **1**, a secondary metabolite of the cyanobacterium *Microcystis aeruginosa*, which was isolated from a sample taken from Rutland Water, UK.[14] Aeruginosamide has shown impressive cytotoxicity to human ovarian tumour and leukaemia cells and as such is an interesting target for our group. Aeruginosamide possesses a relatively simple structure compared to many of the compounds extracted from cyanobacteria. This makes it an attractive target for medicinal chemists, as it has a structure which is readily accessible and modifications based on the general structure of **1** would allow quick entry to a series of analogues. Our initial retrosynthesis (Scheme 1) was based on a convergent route to **1**, employing the disconnections shown. The two fragments **2** and **3** would be prepared from commercially available amino acids, and assembled using standard peptide coupling reagents. Preparation of the thiazole moiety **2** would require a route which avoided racemization of the adjacent stereogenic centre.[15]

Scheme 2 details the preparation of fragment **2**. (*S*)-*N*-Boc-proline was coupled with (*S*)-serine methyl ester to produce dipeptide **4** in 89% yield, with DCC and HOBt used as activating agents. Protection of the alcohol as its TBS ether, **5**, allowed thionation of the amide to proceed using Lawesson's reagent,[16] followed by removal of the protecting group upon treatment with TBAF, to give thioamide **6**. This was readily converted (85% yield) into thiazole **7** using Burgess's reagent,[17] and aromatization was accomplished by stirring **7** with manganese(IV) oxide,[18] to give intermediate **8** in 95% yield. This route has proven to be racemization-free in our hands previously, and later couplings showed no detectable amounts of diastereoisomer formation. The Boc group on **8** was readily removed by treating it with trifluoroacetic acid in dichloromethane to give target synthon **2**.



Scheme 1 Retrosynthetic analysis.

With compound **2** in hand, we turned our attention to fragment **3** (Scheme 3). It was envisaged that this could be secured *via* coupling of the suitably protected valine and isoleucine fragments. (*S*)-Boc-isoleucine was reacted with valine methyl ester to give dipeptide **9** in 90% yield. Removal of



Scheme 2 Preparation of fragment **2**. *Reagents and conditions*: i, HOBt, DCC, Et_3N, THF; ii, TBSCl, DMAP, Et_3N; iii, Lawesson's reagent, PhH, 80 °C; iv, TBAF, THF; v, Burgess's reagent, THF, 65 °C; vi, MnO_2, CH_2Cl_2; vii, TFA, 0 °C.

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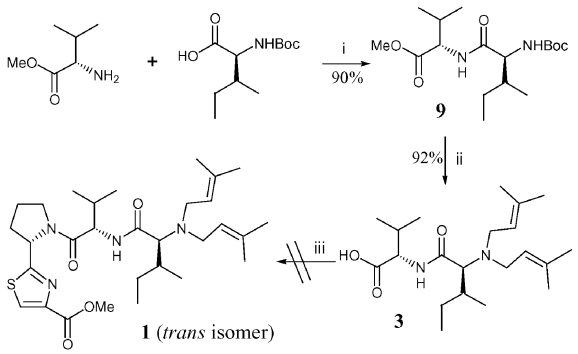
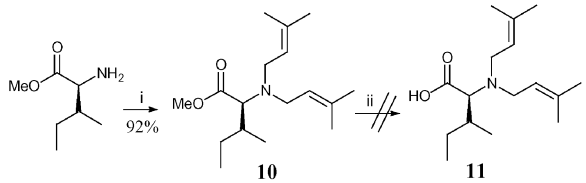


Scheme 3 Attempted coupling of fragment **3**. *Reagents and conditions*: i, EDCI, HOBt, DIPEA, THF; ii, (a) TFA, 0 °C, (b) prenyl bromide, $NaHCO_3$, TBAI, DMF, 70 °C; (c) LiOH, THF, H_2O, MeOH; iii, **2**, EDCI, HOBt, DIPEA, THF; or PyBop, TEA, THF.

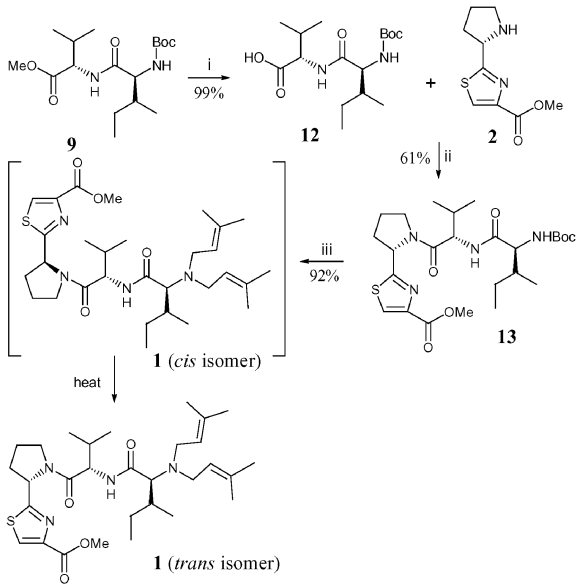
the Boc group, double alkylation of the nitrogen with prenyl bromide and hydrolysis of methyl ester gave **3** in high yield. However, all attempts to couple units **2** and acid **3** failed, with no desired product detected at all. The reason for this lack of reactivity is not fully understood. The steric hindrance caused by the prenyl groups was not expected to be too great, but it appears to have had some influence on the outcome of this step.

The next approach involved reacting isoleucine methyl ester with prenyl bromide to effect alkylation and this proceeded cleanly, with compound **10** secured in 92% yield (Scheme 4). Astonishingly, it now proved impossible to hydrolyze the methyl ester on this fragment, upon treatment with lithium hydroxide in THF. From this result it seemed reasonable to assume that the prenyl groups attached to the nitrogen of the isoleucine residue were having a marked effect on the behaviour of these compounds. The next route was designed to circumvent the need for any transformations to be performed with the prenyl groups present.

Scheme 5 depicts the final route that was undertaken. Starting from compound **9**, an intermediate in an earlier route, it was decided to build the peptide chain before alkylating the nitrogen. Thus, saponification of **9** under the previously employed conditions afforded the free acid **12** in high yield. This was readily coupled with fragment **2** under standard conditions, to give compound **13** (61%), which had the necessary peptide chain in place. Alkylation now proceeded relatively smoothly to yield **1** (*trans* isomer)[19] and thus complete the synthesis of aeruginosamide.† The identity of the synthesized aeruginosamide was confirmed by comparison of ^{1}H NMR and TLC data with that of an authentic sample of the naturally occurring material, and by comparison of the ^{13}C



Scheme 4 Preparation of fragment **11**. *Reagents and conditions*: i, prenyl bromide, $NaHCO_3$, TBAI, DMF, 70 °C; ii, LiOH, THF, H_2O, MeOH.



Scheme 5 Completion of the total synthesis. *Reagents and conditions*: i, LiOH, THF, MeOH, H_2O; ii, EDCI, HOBt, DIPEA, THF; iii, (a) TFA, 0 °C, (b) prenyl bromide, $NaHCO_3$, TBAI, DMF, 70 °C.

NMR spectrum and optical rotation with published data.[14] The observed optical rotation $[\alpha]_D$ −81.6 (*c* 8.7, $CHCl_3$) was identical in sign, but slightly higher than the reported value $[\alpha]_D$ −71.4 (*c* 0.01, $CHCl_3$), therefore establishing the absolute stereochemistry.

However, to complicate matters, it was found in subsequent preparations of aeruginosamide that production of the correct material was dependent on the time taken for the alkylation step. After a short reaction time (30 minutes), the major product isolated is the *cis* isomer of aeruginosamide **1**, and the data for this material did not match that published for the natural product.† Only on extended heating (18 hours) did the NMR data of the synthetic compound match the natural product, even though the alkylation proceeded very rapidly. This led us to believe that aeruginosamide can readily exist in both the *cis* and *trans* forms with respect to the peptide bond at the nitrogen of proline. The results suggest that the *cis* product is kinetically favoured and on heating, the thermodynamically more stable *trans* isomer predominates. Such a result is not unique, as it supports the isolation and synthesis of ceratospongamide.[20] It is probable that this phenomenon is occurring at the time the proline residue is coupled, but since the intermediates were not being identified relative to any known compounds, it was not apparent. The explanation for how this interconversion between the *cis* and *trans* forms of the peptides arises has been given.[21]

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