Total synthesis of the thiopeptide amythiamicin D⁺

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The first total synthesis of the thiopeptide antibiotic amythiamicin D is described.

The amythiamicins are members of the thiopeptide family of antibiotics, a class of sulfur-containing highly modified cyclic peptides characterized by several common structural features such as the presence of thiazole and, in some cases, oxazole rings, unusual and dehydro amino acids, and a heterocyclic centerpiece of a tri- or tetra-substituted pyridine all in a macrocyclic array. Most of the thiopeptide antibiotics inhibit protein synthesis in bacteria, and share common modes of action. They act by binding to the complex of 23SrRNA with ribosomal protein L11, inhibiting the action of GTP-dependent elongation factors.^{1,2} Alternatively, other thiopeptides such as GE2270A, a compound structurally related to the amythiamicins, act directly on the elongation factor proteins inhibiting their action.³

The amythiamicins, isolated from a strain of Amycolatopsis sp. MI481-42F4, and their structures determined by degradative and spectroscopic techniques,^{4–6} were originally reported to inhibit the growth of Gram-positive bacteria including MRSA,4 but more recently, in common with other thiopeptides such as thiostrepton and micrococcin,^{7,8} they have been shown to exhibit antimalarial activity against Plasmodium falciparum,9 the parasite that causes the majority of malarial infections in humans. Despite the fascinating biological activity of the thiopeptide antibiotics, relatively little synthetic work has been carried out to date, and the only reported total syntheses are that of micrococcin P1,10-12 and our own work on promothiocin A.13 However, the syntheses of various fragments of other thiopeptides have been reported, for example the pyridine fragments of dimethyl sulfomycinamate,14,15 nosiheptide,16 A10255,17 and GE2270A,18 and Nicolaou has recently reported substantial progress towards the synthesis of thiostrepton.^{19,20} We now report the first synthesis of one of the amythiamicins, amythiamicin D 1, using a biosynthesis inspired Diels-Alder route to the pyridine core of the antibiotic as the key step.21

Although the detailed structural assignment of amythiamicin D $\mathbf{1}$ was reported,⁵ the stereochemistry of the three chiral centres was

† Electronic supplementary information (ESI) available: Spectroscopic data for synthetic 1. See http://www.rsc.org/suppdata/cc/b4/b401580k/

not disclosed. Therefore, we assume that they derive from natural Lamino acids, an assumption supported by the structure of the closely related antibiotic GE2270A.^{3,22} Our synthetic strategy is indicated in Scheme 1, and disconnections at the amide bonds reveal the building blocks as two thiazoles 2 and 3, a simple glycine derivative 4, and the pyridine 5. In 1978 Bycroft and Gowland, as well as reporting the structure of micrococcin P1,23 suggested that its pyridine ring (as well as the tetrahydropyridine in thiostrepton) could result biogenetically "from the interaction of two dehydroalanine units," themselves derived from serine residues. This interesting proposal for the biosynthesis of the pyridine ring in thiopeptides was subsequently supported by isotopic labeling experiments by Floss and co-workers.^{24,25} Floss also viewed the Bycroft proposal for the biosynthesis of the pyridine ring as a cycloaddition (not necessarily concerted) followed by aromatisation. We have recently reported the realisation of Bycroft's original



Scheme 2 Synthesis of thiazole 2. Reagents and conditions: (i) (a) EtO₂CCl (1 eq), Et₃N (1 eq), THF, 0 °C; (b) aq. NH₃ (35%), THF, 0 °C, 96%; (ii) methyl 2-diazo-3-oxobutanoate (1.2 eq), Rh₂(Oct)₄ (0.025 eq), CH₂Cl₂, reflux, 74%; (iii) Lawesson's reagent (2 eq), THF, reflux, 65%; (iv) H₂ (60 psi), 10% Pd/C, MeOH, reflux, 80%; (v) (a) EtO₂CCl (1 eq), Et₃N (1 eq), THF, 0 °C; (b) 2 M MeNH₂ in THF (3 eq), 0 °C, 73%; (vi) 1 M aq. LiOH, MeOH, rt, 79%. Oct = octanoate.



biosynthesis proposal in a biomimetic cycloaddition route to 2,3,6-trisubstituted pyridines, involving the Diels–Alder reaction of serine-derived 1-alkoxy-2-azadienes with dehydroalanine deriva-tives,²⁶ and therefore a cycloaddition approach to the pyridine **5** remained the keystone of our overall strategy.²⁷

Our synthesis started with the construction of the thiazole **2** from commercially available *N*-Boc-L-aspartic acid 4-benzyl ester (Scheme 2). Thus the corresponding amide **6** underwent an N–H insertion reaction with the rhodium carbene derived from methyl 2-diazo-3-oxobutanoate to give the 1,4-dicarbonyl compound **7** in good yield, a reaction we have used previously as a key step in a route to oxazole building blocks of nostocyclamide and promothiocin A.^{13,28} However, on this occasion rather than dehydrating the ketoamide **7** to give an oxazole, it was treated with Lawesson's reagent to give the corresponding thiazole **8**.²⁹ It thus remained to install the correct side-chain and this was achieved by hydrogenolysis of the benzyl ester and amide formation to give **9**; this was followed by alkaline hydrolysis to reveal the free thiazole-4-carboxylic acid **2** for subsequent coupling reaction.

The synthesis of the second thiazole **3** was readily achieved from the known (*S*)-thiazole **10**³⁰ as shown in Scheme 3. Hydrolysis of the ethyl ester was followed by re-esterification using phasetransfer catalyzed alkylation with *tert*-butyl bromide,³¹ and then selective removal of the Boc-group.³² The resulting amine **3** was coupled to the thiazolecarboxylic acid **2** using carbodiimide methodology to give the bis-thiazole **11**, which was prepared for a further coupling reaction by a second selective removal of an *N*-Boc-group in the presence of a *tert*-butyl ester to give the bisthiazole amine hydrochloride **12**.

In order to set up the key Diels–Alder reaction we required both diene and dienophile components to contain a thiazole-4-carboxylate, the ester groups of which needed to be differentiated. Since the target molecule **1** bears a thiazole methyl ester at the pyridine-6-position, this fixes the corresponding substituent at the 3-position of the proposed 2-azadiene component, and therefore a dienophile containing an orthogonally protected carboxyl was prepared (Scheme 4). Starting from cysteine ethyl ester hydrochloride, benzyl 2-acetylthiazole-4-carboxylate **13** was built up by reaction with pyruvic aldehyde, manganese(rv) oxide oxidation of the resulting thiazoline, and ester exchange. Conversion into the oxime **14** (*E/Z* ~ 1 : 2) was followed by reduction to the required 'dehydroalanine' dienophile, the *N*-acetylenamine **15**, using Burk's iron/acetic anhydride/acetic acid protocol.³³

The 2-azadiene component **21** which contains the remaining three thiazole rings was constructed from the serine-derived thiazole **18** and the valine-derived bis-thiazole **20** using the imidate-based methodology developed in our preliminary studies.²⁶ *N*-Boc-Serine methyl ester was converted into the thiazole-4-ester **16** in four straightforward steps, and this was followed by desilylation and acetylation to give **17**, and final deprotection of the Boc-group to deliver the amine hydrochloride **18**. The lower half of the azadiene was formed from the valine-derived thiazole **10** that was



Scheme 3 Synthesis of bis-thiazole fragment 12. *Reagents and conditions:* (i) 1 M aq. LiOH, MeOH, rt, 100%; (ii) BTEAC (1 eq), 'BuBr (48 eq), K₂CO₃ (24 eq), THF, reflux, 92%; (iii) 4 M HCl in dioxane (8 eq), rt, 1 h, 88%; (iv) 2, EDCI (1 eq), HOBt (1 eq), DMF, 0 °C, 1 h, rt, overnight, 48%; (v) 4 M HCl in dioxane (8 eq), 88%. BTEAC = benzyltriethylammonium chloride; EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro-chloride; HOBt = 1-hydroxybenzotriazole.

converted into the bis-thiazole **19** by a standard ester to amide to thioamide and Hantzsch reaction sequence. The carboxamide **19** was then reacted with triethyloxonium hexafluorophosphate to give the imidate **20**, reaction of which with the serine derivative **18** gave the complex 2-azadiene **21** after elimination of acetate using DBU as base (Scheme 5).

The stage was now set for the key 'biomimetic' hetero-Diels– Alder-aromatisation sequence which was carried out under our previously developed thermal conditions,²⁶ rather than 'biological' conditions. Thus heating the two components **15** and **21** under microwave irradiation in toluene at 120 °C for 12 hours gave the required 2,3,6-tris(thiazolyl)pyridine **5**, albeit in modest yield (Scheme 6). With all the components **2–5** now in hand, the assembly of the natural product could be addressed. Deprotection











Scheme 6 Synthesis of amythiamicin D 1. *Reagents and conditions:* (i) toluene, 120 °C, microwave (CEM DiscoverTM Focused Synthesizer), 150 W, 12 h, 33%; (ii) TFA, CHCl₃, rt; (iii) Boc-Gly-OH 4 (1.5 eq), PyBOP (1.3 eq), iPr_2NEt (4 eq), CH_2Cl_2 , rt, 95% over 2 steps; (iv) H_2 (1 atm), Pd black, MeOH, rt; (v) 12, PyBOP (1.3 eq), iPr_2NEt (10 eq), DMF, 60% over 2 steps; (vi) TFA, CHCl₃, rt; (vii) DPPA (2 eq), iPr_2NEt (10 eq), DMF (1 mM), 0 °C, 73% over 2 steps. PyBOP = benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; DPPA = diphenylphosphoryl azide.

of the terminal *N*-Boc-group on the bis-thiazole moiety **5** was followed by a PyBOP-mediated coupling to *N*-Boc-glycine **4** to give the complete right-hand fragment of amythiamicin D **22** (Scheme 6). After much experimentation, it was found that the benzyl ester in compound **22** could be removed by hydrogenolysis over palladium black to give the corresponding carboxylic acid. A second PyBOP-mediated reaction successfully coupled the lefthand bis(thiazole) **12** to provide the cyclization precursor **23**. The terminal *N*-Boc and *tert*-butyl ester groups in **23** were simultaneously cleaved using TFA, and the resulting amino acid treated with DPPA and Hünig's base in DMF. This resulted in macrolactamization in a yield of 73% (from **23**) to give amythiamicin D **1**.

Our synthetic material had properties consistent with those reported for the natural product,^{5,+} suggesting that our original assumptions about the stereochemistry of the three chiral centers was correct. Subsequent correspondence with the original authors revealed that unpublished X-ray crystallographic data substantiate the (10*S*,19*S*,29*S*)-stereochemistry thereby providing final confirmation that we had completed the first synthesis of the natural product amythiamicin D, and paving the way for syntheses of other thiopeptide natural products.

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