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Biosynthesis inspired Diels–Alder route to pyridines: synthesis of the 2,3-dithiazolylpyridine core of the thiopeptide antibiotics

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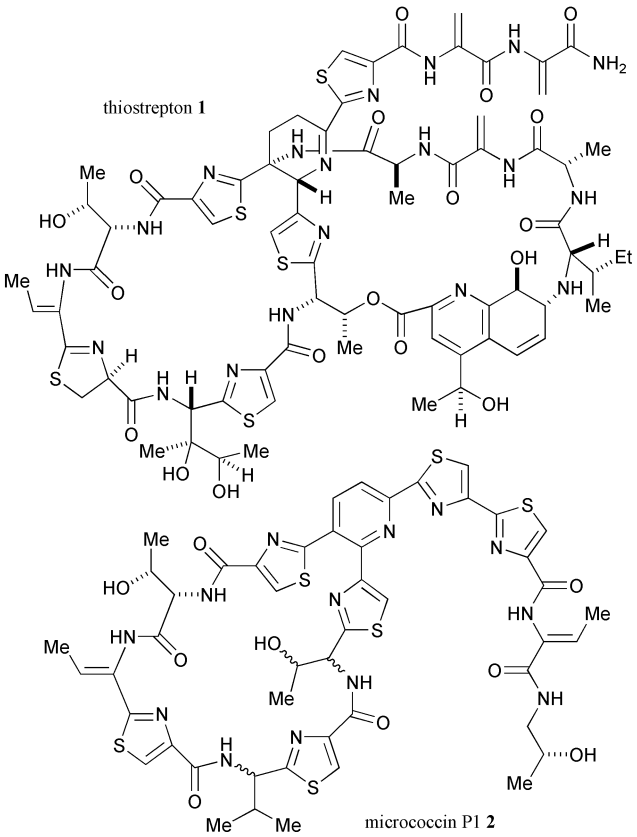
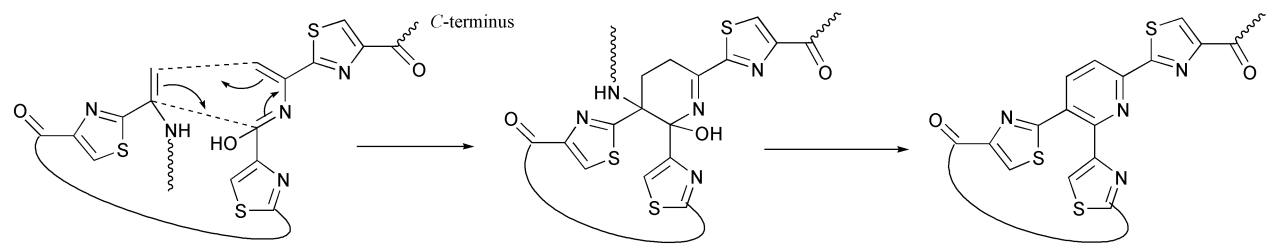
Reaction of serine derived 1-alkoxy-2-azadienes with dehydroalanine derived dienophiles results in Diels–Alder reaction and aromatisation to give 2,3,6-trisubstituted pyridines, thereby establishing the viability of the proposed biosynthetic route to the pyridine ring of the thiopeptide antibiotics originally proposed by Bycroft and Gowland.

The thiopeptide (or thiostrepton) antibiotics are a class of sulfur containing highly modified cyclic peptides characterised by several common structural features: the presence of thiazole and, in some cases, oxazole rings, unusual and dehydro amino acids, and a heterocyclic centrepiece of a tri- or tetra-substituted pyridine all in a macrocyclic array. Many of the compounds such as thiostrepton **1** itself,[1] nosiheptide,[2] and the micrococcins[3] have been known for some time. Most of the thiopeptide antibiotics inhibit protein synthesis in bacteria, and share a common mode of action. They act by binding to the complex of 23SrRNA with ribosomal protein L11, inhibiting the action of GTP-dependent elongation factors.[4] 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 **2**,[5,6] and our own work on promothiocin A.[7] However, the syntheses of various fragments of other thiopeptides have been reported, for example the pyridine fragments of sulfomycin I,[8] nosiheptide,[9] A10255,[10] and GE2270A.[11]

In a 1978 *Chemical Communication* Bycroft and Gowland, as well as reporting the structure of micrococcin P1 **2**,[3b,5] suggested that its pyridine ring (as well as the tetrahydropyridine in thiostrepton **1**) could be biogenetically derived 'from the interaction of two dehydroalanine units.' This interesting proposal for the biosynthesis of the pyridine ring in thiopeptides was subsequently supported by isotopic labelling experiments by Floss and coworkers.[12,13] Floss also viewed the Bycroft proposal for the biosynthesis of the pyridine ring as a formal cycloaddition (not necessarily concerted) followed by aromatisation as shown in Scheme 1, the intermediate tetrahydropyridine being clearly related to the corresponding structural unit in thiostrepton. We now report the realisation of Bycroft's original 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 derivatives.

The dienes chosen for study were the 1-alkoxy-2-azadienes **5** which mimic the dehydroalanine dipeptide diene proposed by Bycroft and Floss, by fixing it in the required 'enol' form (*cf.* Scheme 1).[14] The dienes were prepared from *O*-acetylserine methyl ester **3** by reaction with the corresponding imidate **4**, commercially available (R^2 = Ph) or obtained by reaction of the carboxamide with triethyloxonium hexafluorophosphate,[15] using a procedure based on the literature route to 2-azadiene **5** (R^2 = Ph).[16] Initial Diels–Alder reactions of the 2-azadiene **5** (R^2 = Ph) were carried out using the serine derived *N*-acetyldehydroalanine methyl ester **6** (R^3 = CO_2Me) as dienophile,[17] the NHAc group mimicking the *N*-terminus peptide chain in the proposed biosynthetic route (*cf.* Scheme 1). Prolonged heating of the two components in xylene resulted in a 42% yield of the pyridine **7a** after chromatography (Scheme 2), thereby establishing for the first time the viability of the





Scheme 1

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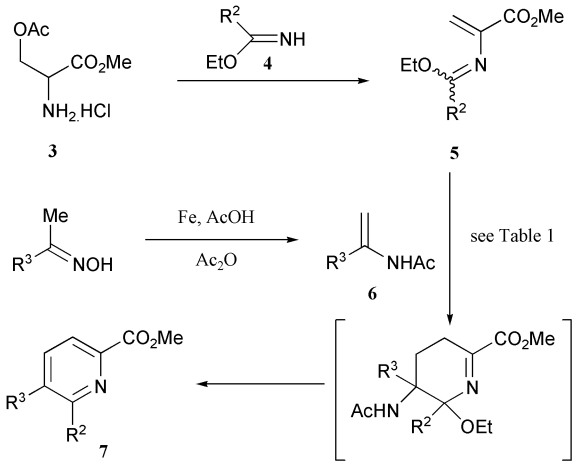


Scheme 2

'biomimetic' Diels–Alder-aromatisation sequence, albeit under thermal rather than 'biological' conditions. Interestingly, the pyridine **7a** (79%) was also formed slowly on heating the diene **5** (R^2 = Ph) alone. That the dehydroalanine derivative ester **6** (R^3 = CO_2Me) was actually involved in the cycloaddition was shown by the use of the corresponding ethyl ester **6** (R^3 = CO_2Et) that gave pyridine **7b**, together with **7a**, formed by competing 'dimerisation' of the azadiene. The reaction was also investigated under microwave irradiation, and these conditions were applied to a range of other 1-ethoxy-2-azadienes **5** (R^2 = 4-chlorophenyl, 2-thienyl, 2-pyridyl prepared in 38, 65 and 38% yield respectively from **3**). These dienes gave the corresponding pyridines **7c**–**e** upon heating with *N*-acetyldehydroalanine ethyl ester **6** (R^3 = CO_2Et) as dienophile (Table 1).

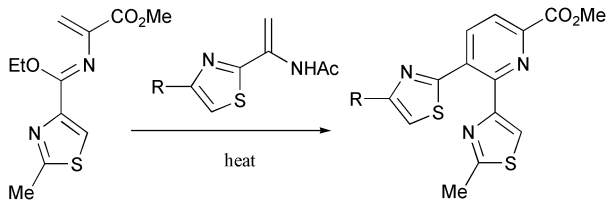
Table 1 Preparation of 2,3,6-trisubsituted pyridines **7** by Diels–Alder reaction of 1-alkoxy-2-azadienes **5** with dienophiles **6**

7	R^2	R^3	Conditions [a]	Yield[b] (%)
a	Ph	CO_2Me	Xylene, 48 h	42
b	Ph	CO_2Et	Xylene, 48 h	25
c	4-ClC_6H_4	CO_2Et	DCB, MW, 180 °C, 4 h	60
d	2-Thienyl	CO_2Et	DCB, MW, 180 °C, 2.5 h	58
e	2-Pyridyl	CO_2Et	DCB, MW, 180 °C, 4 h	28
f	Ph	2-Thiazolyl	DCB, 48 h	64
f	Ph	2-Thiazolyl	DCB, MW, 180 °C, 4 h	50

[a] Reactions carried out in the specified solvent at reflux. DCB = 1,2-dichlorobenzene; MW = microwave (all reactions carried out in a CEM Focused Synthesiser at the temperature specified). [b] In some cases, the pyridine formed by competing 'dimerisation' of the azadiene **5** is also isolated.

Given the presence of a 2-thiazolyl substituent at the 3-position of the pyridine ring in all thiopeptide antibiotics, the 2-thiazolyl dienophile **6** (R^3 = 2-thiazolyl), prepared in 49% yield by reduction of 2-acetylthiazole oxime using iron with acetic anhydride–acetic acid in toluene,[18] was investigated next. Diels–Alder reaction under conventional or microwave heating gave the pyridine **7f** in reasonable yield,† together with pyridine **7a** (26 or 50%) formed by competing 'dimerisation' of the azadiene. The reaction was then extended to the thiazole containing 2-azadiene **8**, prepared from 2-methylthiazole-4-carboxamide *via* the corresponding imidate in 72% yield from serine derivative **3**. Diels–Alder reaction (microwave heating, neat, 180 °C, 15 min) with the 2-thiazolyl dienophile **6** (R^3 = 2-thiazolyl) gave the 2,3-dithiazolylpyridine **9a** in 29% yield. Reaction of the corresponding 4-ethoxycarbonylthiazole dienophile gave the 2,3-dithiazolylpyridine **9b** (64%) containing an additional ester group for further elaboration (Scheme 3).

Thus we have established for the first time the viability of the long-standing proposal for the biosynthesis of the pyridine ring in the thiopeptide antibiotics involving a cycloaddition of two serine derived dehydroalanine type fragments.[19]



Scheme 3

We thank the EPSRC and AstraZeneca for financial support.

Notes and references

† The structure of methyl 2-phenyl-3-(2-thiazolyl)pyridine-6-carboxylate **7f** was confirmed by X-ray crystallography; we thank Dr Alex Slawin (University of St Andrews) for carrying out this structure determination, details of which will be published separately.

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