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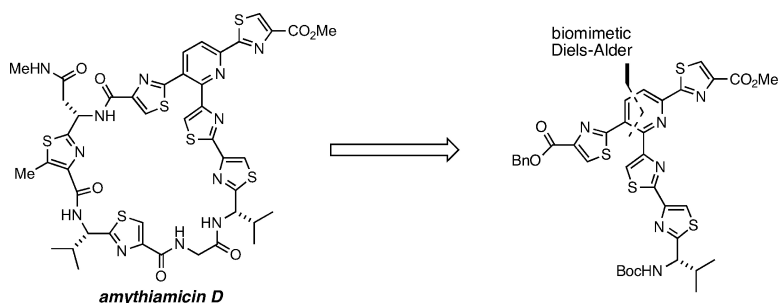# Total Synthesis of the Thiopeptide Antibiotic Amythiamicin D

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Total Synthesis of the Thiopeptide Antibiotic Amythiamicin D

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Abstract: The thiopeptide (or thiostrepton) antibiotics are a class of sulfur containing highly modified cyclic peptides with interesting biological properties, including reported activity against MRSA and malaria. Described herein is the total synthesis of the thiopeptide natural product amythiamicin D, which utilizes a biosynthesis-inspired hetero-Diels−Alder route to the pyridine core of the antibiotic as a key step. Preliminary studies using a range of serine-derived 1-ethoxy-2-azadienes established that hetero-Diels−Alder reaction with *N*-acetylenamines proceeded efficiently under microwave irradiation to give 2,3,6-trisubstituted pyridines. The thiazole building blocks of the antibiotic were obtained by either classical Hantzsch reactions or by dirhodium(II)-catalyzed chemoselective carbene N−H insertion followed by thionation, and were combined to give the bis-thiazole that forms the left-hand fragment of the antibiotic. The key Diels−Alder reaction of a tris-thiazolyl azadiene with benzyl 2-(1-acetylaminoethenyl)thiazole-4-carboxylate gave the core tetrathiazolyl pyridine, which was elaborated into the natural product by successive incorporation of glycine and bis-thiazole fragments followed by macrocyclization.

Introduction

The thiopeptide (or thiostrepton) antibiotics are a class of sulfur containing highly modified cyclic peptides characterized by several common structural features: the presence of thiazole and, in some cases, oxazole rings, unusual and dehydro amino acids, and a heterocyclic centerpiece consisting of a tri- or tetra-substituted pyridine all in a macrocyclic array.[1] The five-membered heterocycles are derived from amino acids by nonribosomal peptide synthesis,[2−5] followed by cyclization of serine, threonine, or cysteine side chains. Many of the compounds such as the micrococcins (e.g., micrococcin P1 **1**) and thiostrepton **2** itself (Figure 1) have been known for over 50 years, although in many cases, the structures have only been fully elucidated more recently, and even now some structural uncertainties remain (see below).

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 fac-tors.[6,7] Alternatively, other thiopeptides such as GE2270A act directly on the elongation factor proteins, inhibiting their action.[8] Despite the fascinating biological activity of the thiopeptide antibiotics, relatively few synthetic studies have been carried out to date, and only two members of this series of natural products, which numbers over 75 in total, have succumbed to total synthesis to date, although, the syntheses of various fragments of other thiopeptides have been reported; for example the pyridine clusters of dimethyl sulfomycinamate,[9,10] nosihep-tide,[11] A10255,[12] and GE2270A.[13,14]

The structure of micrococcin P1 was originally assigned by Walker et al. in 1977,[15] but was corrected by Bycroft and Gowland the following year.[16] Initially, synthesis served to confuse rather than clarify the structure of micrococcin P1, since the first reported success actually described a compound that is epimeric with the Bycroft-Gowland structure **1** in the isoalaninol

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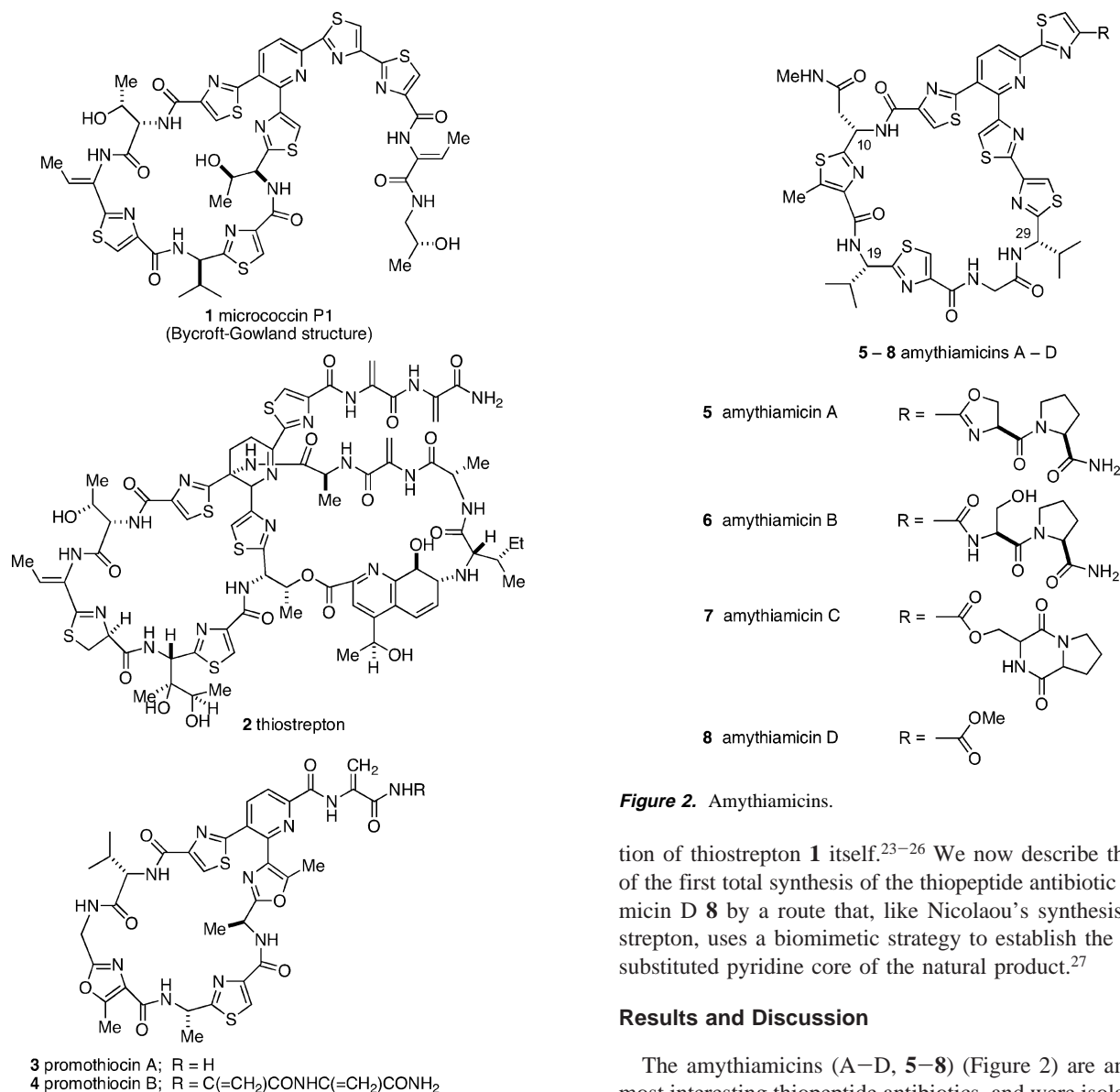(16) Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc. Chem. Commun.* **1978**, 256−258.

1 micrococcin P1
(Bycroft-Gowland structure)

2 thiostrepton

3 promothiocin A; R = H
4 promothiocin B; R = C(=CH₂)CONHC(=CH₂)CONH₂

Figure 1. Structures of the thiopeptide antibiotics micrococcin P1, thiostrepton, and the promothiocins.

side-chain.[17,18] Subsequently, Ciufolini's elegant synthesis of structure **1**,[19] together with his detailed NMR studies,[20] clarified the situation: the Bycroft-Gowland structure **1** for micrococcin P1 is correct in terms of connectivity of residues, but contains a stereochemical misassignment at one or more centers. Hence, in view of the structural uncertainty associated with micrococcin P1, the first definitive synthesis of a thiopeptide natural product was the preparation of promothiocin A **3**, carried out in our own laboratory in 1998.[21,22] Very recently, in a landmark synthesis, Nicolaou and co-workers have reported the prepara-
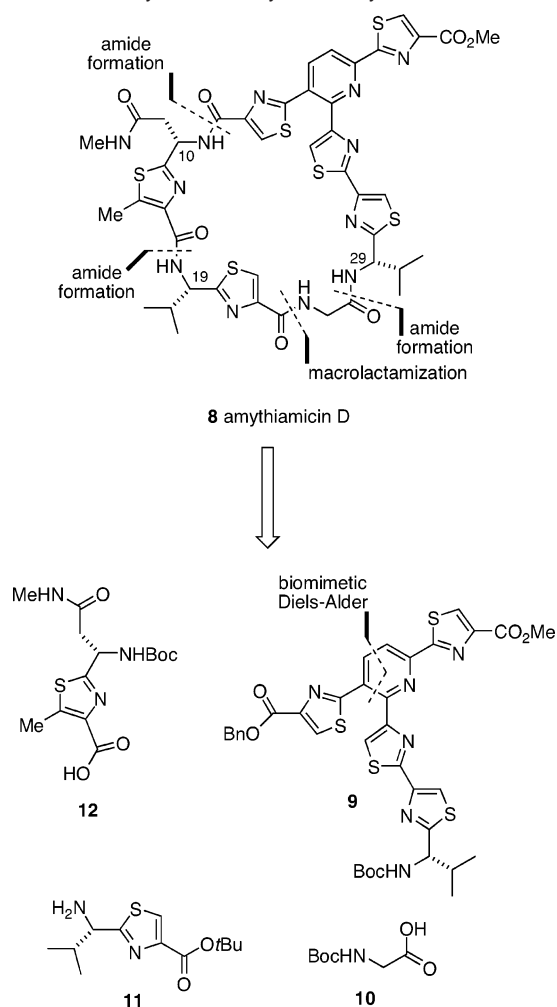
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5 – 8 amythiamicins A – D

5 amythiamicin A   R =

6 amythiamicin B   R =

7 amythiamicin C   R =

8 amythiamicin D   R =

Figure 2. Amythiamicins.

tion of thiostrepton **1** itself.[23−26] We now describe the details of the first total synthesis of the thiopeptide antibiotic amythiamicin D **8** by a route that, like Nicolaou's synthesis of thiostrepton, uses a biomimetic strategy to establish the 2,3,6-trisubstituted pyridine core of the natural product.[27]

Results and Discussion

The amythiamicins (A−D, **5−8**) (Figure 2) are among the most interesting thiopeptide antibiotics, and were isolated from a strain of *Amycolatopsis* sp. MI481−42F4, and their structures determined by degradative and spectroscopic techniques.[28−30] They are among the very few thiopeptides that do *not* contain a dehydroalanine residue,[1] and they are also some of the most biologically active. Not only are they reported to inhibit the growth of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA),[28] they have also been shown to inhibit the action of elongation factor Tu (EF-Tu), a GTP-dependent translation factor. Such inhibitors exhibit antimalarial
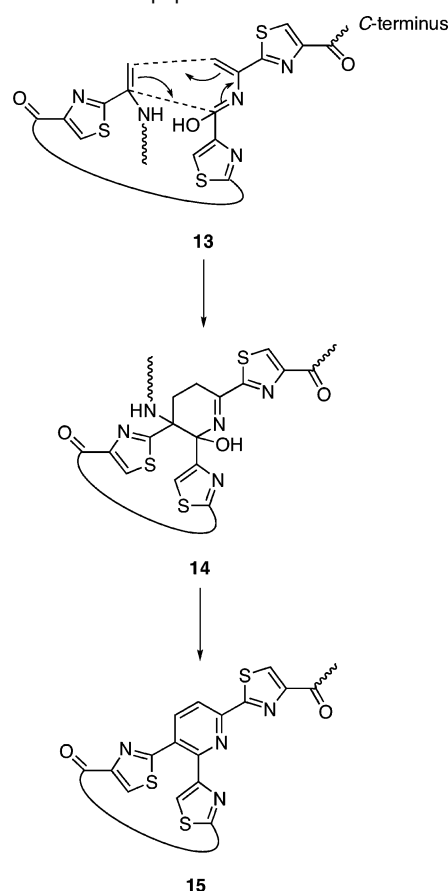
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Scheme 1. Retrosynthetic Analysis of Amythiamicin D **8**.

Scheme 2. Bycroft−Floss Hypothesis for the Biosynthesis of the Pyridine Core of the Thiopeptide Antibiotics.

activity against *Plasmodium falciparum*,[31] the parasite that causes the majority of malarial infections in humans, the most potent being amythiamicin A (IC$_{50}$ 0.01 μM). It was shown that the thiopeptide binds to *P. falciparum* EF-Tu, thereby blocking protein synthesis in the parasite, and suggesting that drugs which target this mechanism might be useful in the treatment of this disease.

Although the detailed structural assignment of amythiamicin D **8** was reported,[29] the stereochemistry of the three chiral centers was not disclosed. Therefore, we assumed that they derive from natural L-amino acids, an assumption supported by the structure of the very closely related antibiotic GE2270A.[8,32] Our synthetic strategy is indicated in Scheme 1, and "obvious" disconnections at the amide bonds reveal the building blocks as the core pyridine **9**, a simple glycine derivative **10**, and two thiazoles **11** and **12**. However, it is the synthesis of the core pyridine **9** that lies at the center of the problem, and in view of the difficulties associated with the synthesis of poly-substituted pyridines, we decided at the outset that we would construct the pyridine fragment by a ring synthesis rather than by an approach involving sequential modification and substitution of a preformed pyridine ring. In the thiopeptide arena, the latter approach is by far the most common, although there are some exceptions.[33,34] Indeed in our synthesis of promothiocin

A **3**,[21,22] we also used a ring synthesis approach, employing the little known Bohlmann-Rahtz reaction[35] as a route to the pyridine core of the natural product.[36] On this occasion, however, we elected to draw our inspiration from Nature, and use a biosynthesis-mimicking Diels−Alder route to the pyridine core of the antibiotic as a key step.

In 1978, Bycroft and Gowland, in addition to reporting the structure of micrococcin P1,[16] 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, who showed that serine, the precursor of dehydroalanine, was incorporated into the pyridine as predicted by such a proposal.[37,38] Floss also viewed the Bycroft proposal for the biosynthesis of the pyridine ring **15** as a formal Diels−Alder cycloaddition (i.e. **13**, but not necessarily concerted) followed by aromatization as shown in Scheme 2, the intermediate tetrahydropyridine **14** being clearly related to the corresponding structural unit in thiostrepton.

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Table 1. Preparation of 1-alkoxy-2-azabutadienes **19**

	R^2	**17** yield/%	R	**19** yield/%
a	Ph	a	Me	48
b	$4\text{-Cl}-C_6H_4$	b	Me	55^c
c	2-thienyl	b	Me	65^c
d	2-pyridyl	b	Me	38^c
e	2-Me-4-thiazolyl	b	Me	72^c
f	Ph	a	PNB^d	64
g	A^e	b	Me	31^c
h	B^f	~100	PNB^d	50

^{a} Commercial ethyl benzimidate hydrochloride used. ^{b} Imidate used directly in next step. ^{c} Yield is over the two steps. ^{d} PNB = 4-nitrobenzyl. ^{e} A = (S)-2-(2-*tert*-butoxycarbonylamino-3-methyl)propylthiazol-4-yl. ^{f} B = (S)-2-[(2-*tert*-butoxycarbonylamino-3-methyl)propylthiazol-4-yl]thiazol-4-yl.

Although the case for a Diels−Alderase enzyme remains the subject of debate,[39−42] it does not invalidate biosynthesis-inspired Diels−Alder approaches to target molecules. Our first task was, therefore, to realize 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 (cf. **13**).[43] The dienes chosen for study were the 1-alkoxy-2-azadienes **19**, which mimic the dehydroalanine dipeptide diene proposed by Bycroft and Floss, by fixing it in the required 'enol' form (cf. Scheme 2).[44,45] The dienes were prepared from *O*-acetylserine esters **18** by reaction with the imidates **17**, which are commercially available (R^2 = Ph) or obtained by reaction of the corresponding carboxamide **16** with triethyloxonium hexafluorophosphate,[46] using a procedure based on a literature route to 2-azadiene **19a**.[47] As summarized in Table 1, a range of 2-azadienes **19** incorporating aryl (**19a**, **19b**, **19f**) and heteroaryl (**19c** − **e**) groups was prepared. The 1-ethoxy-1-phenyl-2-azadiene system was prepared with both methyl (**19a**) and 4-nitrobenzyl (PNB) (**19f**) esters groups, the latter being chosen to offer the possibility of selective ester deprotection at a subsequent stage.

Also, two model thiopeptide dienes (**19g**, **19h**), incorporating a more complex thiazole and a bis-thiazole at the 1-position, were prepared from the (*S*)-thiazole-4-carboxamides **16g** and **16h**, obtained from the known thiazole-4-carboxylate **20**[48] as shown in Scheme 3. The chiral thiazole **20** was readily obtained from *N*-Boc-valine on a 5−20 g-scale (~65% yield over three

Scheme 3. LR = Lawesson's Reagent.
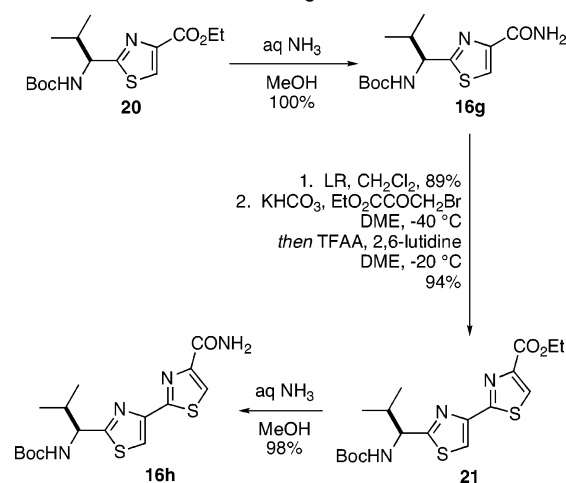
steps) using the modified Hantzsch procedure to avoid racemization.[49] Conversion into the amide **16g** was quantitative, and thionation and a second Hantzsch reaction, again under Holzapfel's modified conditions, gave the bis-thiazole **21** in excellent yield. To check the stereochemical integrity of the bis-thiazole **21**, it was converted into α-methoxy-α-trifluoromethyl phenylacetic acid (Mosher) amides. Removal of the *N*-Boc-group with TFA was followed by separate coupling reactions to (*R*)- and (*S*)-Mosher's acid to give the corresponding amides. Analysis by ^{19}F NMR spectroscopy established the purity of each diastereomeric amide, confirming that no racemization had occurred in the synthesis of the thiazole rings. The ester group in **21** was finally converted into the corresponding amide **16h** by treatment with ammonia (Scheme 3).

In keeping with the desire to mimic Nature, the first dienophiles investigated were the serine derived *N*-acetyldehydroalanine esters **24a** and **24b**, the NHAc group mimicking the *N*-terminus peptide chain in the proposed biosynthetic route (cf. Scheme 2). Although not commonly used as dienophiles because of their poor reactivity, dehydroalanine derivatives do participate in Diels−Alder reactions.[50−57] On the other hand, there is no precedent for simple enamides such as the phenyl substituted enamide **24c** acting as a dienophile, and therefore this compound, along with the more relevant 2-thiazolyl substituted derivatives **24d**−**24f**, formed part of our early feasibility studies. Methyl 2-acetamidoacrylate **24a** is commercially available, and its ethyl analogue **24b** was prepared from the corresponding acid. Dienophiles **24c**−**24f** were prepared by reduction of the corresponding oximes **23** using iron with acetic anhydride−acetic acid in toluene.[58] The two thiazolyl ketones **22e** and **22f** were prepared as shown in Scheme

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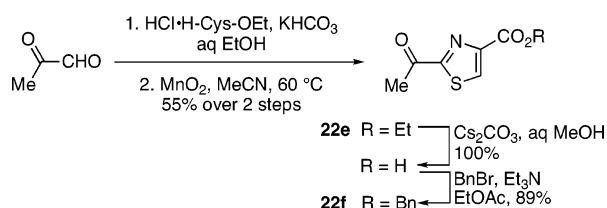(54) Burkett, B. A.; Chai, C. L. L. *Tetrahedron Lett.* **1999**, *40*, 7035−7038.
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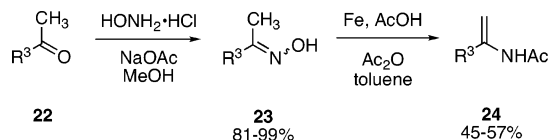(57) Avenoza, A.; Barriobero, J. I.; Cativiela, C.; Fernandez-Recio, M. A.; Peregrina, J. M.; Rodriguez, F. *Tetrahedron* **2001**, *57*, 2745−2755.
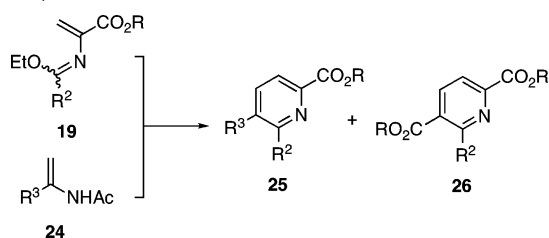(58) Burk, M. J.; Casy, G.; Johnson, N. B. *J. Org. Chem.* **1998**, *63*, 6084−6085.

Scheme 4

22e R = Et ⎤ Cs$_2$CO$_3$, aq MeOH
            ⎥ 100%
   R = H ⎢
            ⎥ BnBr, Et$_3$N
22f R = Bn ⎦ EtOAc, 89%

Scheme 5. **24a**, R^3 = CO$_2$Me; **24b**, R^3 = CO$_2$Et; **24c**, R^3 = Ph; **24d**, R^3 = 2-thiazolyl; **24e**, R^3 = 4-EtO$_2$C-2-thiazolyl; **24f**, R^3 = 4-BnO$_2$C-2-thiazolyl.

22

23
81-99%

24
45-57%

Scheme 6. **25a**, R = Me, R^2 = Ph, R^3 = CO$_2$Me; **25b**, R = Me, R^2 = Ph, R^3 = CO$_2$Et.

19

24

25

26

4, and the preparation of the enamide dienophiles **24** is summarized in Scheme 5.

Initial experiments on the Diels−Alder reactions of the 2-azadienes were carried out with the 1-phenyl diene **19a** and methyl acetamidoacrylate **24a** as dienophile. Prolonged heating of the two components in xylene resulted in a 42% yield of the pyridine **25a** after chromatography (Scheme 6), thereby establishing for the first time the viability of the 'biomimetic' Diels−Alder-aromatization sequence, albeit under thermal rather than 'biological' conditions. Interestingly, the pyridine **25a** was also formed slowly in 79% yield on heating the diene **19a** alone. That the dehydroalanine dienophile ester **24a** was actually involved in the cycloaddition was shown by the use of the corresponding ethyl ester **24b** that gave pyridine **25b**, together with **25a**, formed by competing 'dimerization' of the 2-azadiene. The reaction was also investigated under microwave irradiation,[59] and these conditions were applied to a range of other 1-ethoxy-2-azadienes **19** and enamide dienophiles **24** to give the corresponding pyridines **25** in modest yield (Table 2, Supporting Information). Crucially, in terms of our planned synthesis of a thiopeptide pyridine core, thiazole rings could be incorporated into both diene and dienophile components, resulting in the synthesis of a series of 2,3-dithiazolyl-pyridines (Table 2, Supporting Information). In particular, pyridine **25o** is fully functionalized, with orthogonal protecting groups, for further elaboration although in the event, it was not used in the final synthesis of amythiamicin D, since the selective deprotection could not be carried out satisfactorily. The Diels−Alder reactions were regiospecific and gave the 2,3,6-trisubstituted pyridines **25** as evidenced by their ^{1}H NMR spectra, which showed two doublets ($J \approx 8$ Hz) for the two adjacent pyridine ring protons. There was no evidence for the formation of the alternative 2,4,6-substituted regioisomers.

Most of the Diels−Alder reactions described in Table 2 suffer from competing 'dimerization' of the 2-azadiene to give pyridines **26**. For completeness, a series of separate 'dimerization' experiments were carried out as summarized in Table 3 (Supporting Information). With the exception of diene **19h**, all azadienes investigated gave pyridines **26**, presumably by way of a Diels−Alder dimerization−aromatization sequence with the loss of ethanol and the imidate. Similar 'dimerizations' of 2-azadienes to give pyridines have been noted previously.[60]

The yields of the desired 2,3,6-trisubstituted pyridines are somewhat variable, although no attempts were made to optimize the reactions, and probably reflect the fact that the diene−dienophile components are not ideally matched in terms of their electronic properties for an efficient Diels−Alder process. Additionally, although initial Diels−Alder adducts were never observed, the subsequent aromatization sequence may not be trivial given the relatively poor natures of the acetamide anion and ethoxide as potential leaving groups. Nevertheless, that is what the 'biomimetic' Diels−Alder-aromatization sequence requires, albeit under 'biological' conditions, rather than the simple thermal conditions described here.

The above feasibility study has 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. Thus, it has not only resulted in the realization of Bycroft's original idea, but also allowed us to adopt such a cycloaddition approach to the core pyridine **9** of the amythiamicins as the keystone of our overall strategy.

With the key methodology established for the synthesis of 2,3,6-trisubstituted pyridines relevant to the core pyridine **9** of the amythiamicins, attention turned to the preparation of the thiazoles **11** and **12** that were also required by the retrosynthetic analysis (Scheme 1). Although the Hantzsch reaction discovered in 1889 remains one of the most reliable routes to thiazoles, and has been used by others for the synthesis of the thiazole building block **12** common to GE2270A and the amythiamicins,[61] we elected to use the versatile rhodium carbene N−H insertion method developed in our own laboratory.[62] The substrate for the rhodium reaction was the aspartate-derived amide **28**, readily obtained from commercially available (*S*)-*N*-Boc-aspartic acid benzyl ester **27** using the mixed anhydride method. The amide **28** underwent chemoselective N−H insertion with the rhodium carbene derived from methyl 2-diazo-3-oxobutanoate by dirhodium tetraoctanoate catalyzed reaction. The resulting 1,4-dicarbonyl compound, ketoamide **29**, formed as a mixture of diastereoisomers, was readily converted into the thiazole **30** upon treatment with two equivalents of Lawesson's reagent in boiling THF.[63] Use of less reagent or lower temperatures resulted in poorer yields. It thus remained to install the correct side-chain and this was achieved by hydrogenolysis of the benzyl ester followed by amide formation to give **31**, although the first step was not without difficulty: fairly forcing condi-
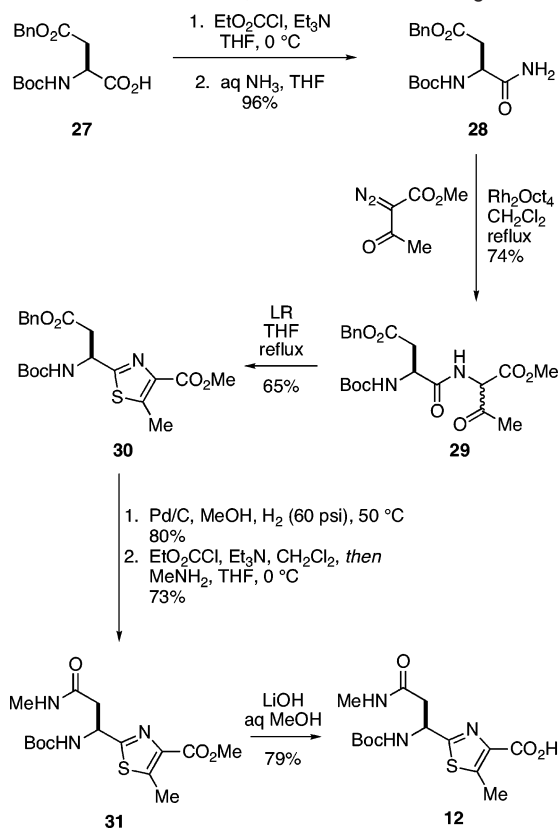
(59) *Microwave Assisted Organic Synthesis*; Tierney, J. P., Lidström, P., Eds.; Blackwell Publishing Ltd: Oxford, 2005.
(60) Palacios, F.; Alonso, C.; Rubiales, G.; Ezpeleta, J. M. *Eur. J. Org. Chem.* **2001**, 2115−2122.
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(62) Davies, J. R.; Kane, P. D.; Moody, C. J. *Tetrahedron* **2004**, *60*, 3967−3977.
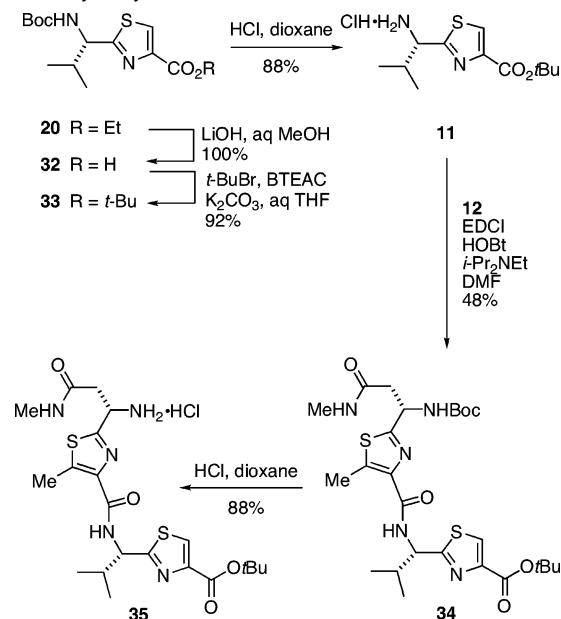(63) Gordon, T. D.; Singh, J.; Hansen, P. E.; Morgan, B. A. *Tetrahedron Lett.* **1993**, *34*, 1901−1904.

Scheme 7. Oct = Octanoate; LR = Lawesson's Reagent.

Scheme 8. BTEAC = Benzyltriethylammonium Chloride: EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride; HOBt = 1-hydroxybenzotriazole.

Scheme 9. LR = Lawesson's Reagent.

tions and high catalyst loadings were required to achieve good yields. Finally, lithium hydroxide hydrolysis revealed the free thiazole-4-carboxylic acid **12** for a subsequent coupling reaction (Scheme 7).

The synthesis of the second thiazole **11** was readily achieved from the known (*S*)-ethyl thiazole-4-carboxylate **20** that, to facilitate subsequent deprotection and coupling reactions, was converted into the corresponding *tert*-butyl ester **33**. Hydrolysis of the ethyl ester to the acid **32** was followed by re-esterification using phase-transfer catalyzed alkylation with *tert*-butyl bromide.[64] Selective removal of the *N*-Boc-group[65] by treatment with HCl in dioxane gave the required amine **11** isolated as its hydrochloride salt. This was coupled to thiazolecarboxylic acid **12** using carbodiimide methodology to give the bis-thiazole **34** in modest yield. Bis-thiazole **34** was readied 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 bis-thiazole amine hydrochloride **35** (Scheme 8).

With the thiazole building blocks prepared and already co-joined, the stage was set to exploit the biosynthesis inspired azadiene Diels−Alder reaction. Both diene and dienophile components are required to contain a thiazole-4-carboxylate, and therefore the ester groups need to be differentiated. Since amy-thiamicin D bears a thiazole methyl ester at the pyridine-6-position, this fixes the corresponding 3-substituent of the proposed 2-azadiene component, thereby defining the thiazole **24f** containing an orthogonally protected carboxyl, as the dienophile. The 2-azadiene component **42** that contains the remaining three
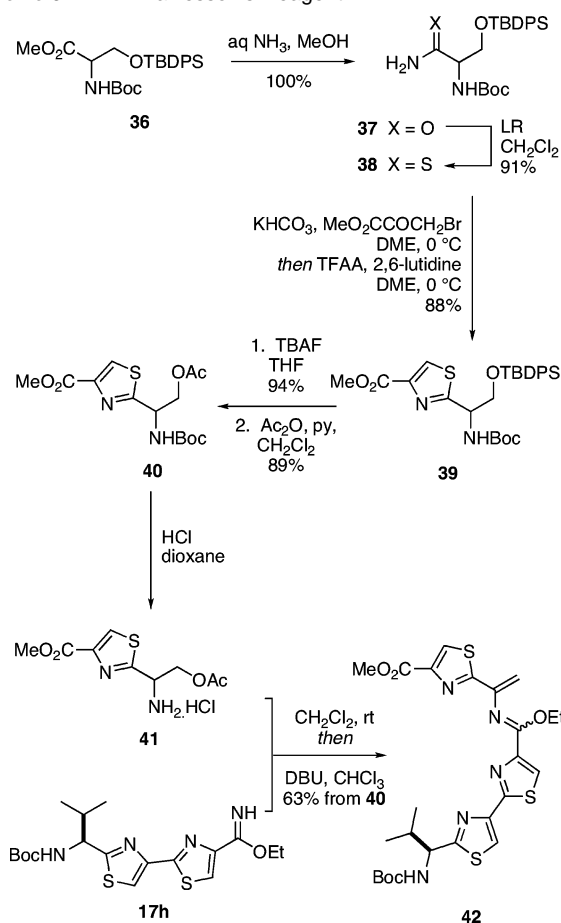
thiazole rings was constructed from the valine-derived bis-thiazole imidate **17h** described earlier and the serine-derived thiazole **41**. *N*-Boc-Serine methyl ester was converted into the known silyl derivative **36**; subsequent reaction with ammonia gave the amide **37** and hence the thioamide **38** (Scheme 9). Hantzsch reaction with methyl bromopyruvate established the

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(65) Gibson, F. S.; Bergmeier, S. C.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 3216−3218.

Scheme 10. PyBOP = Benzotriazol-1-yloxy-tris(pyrrolidino)Phosphonium Hexafluorophosphate; DPPA = Diphenylphosphoryl Azide.
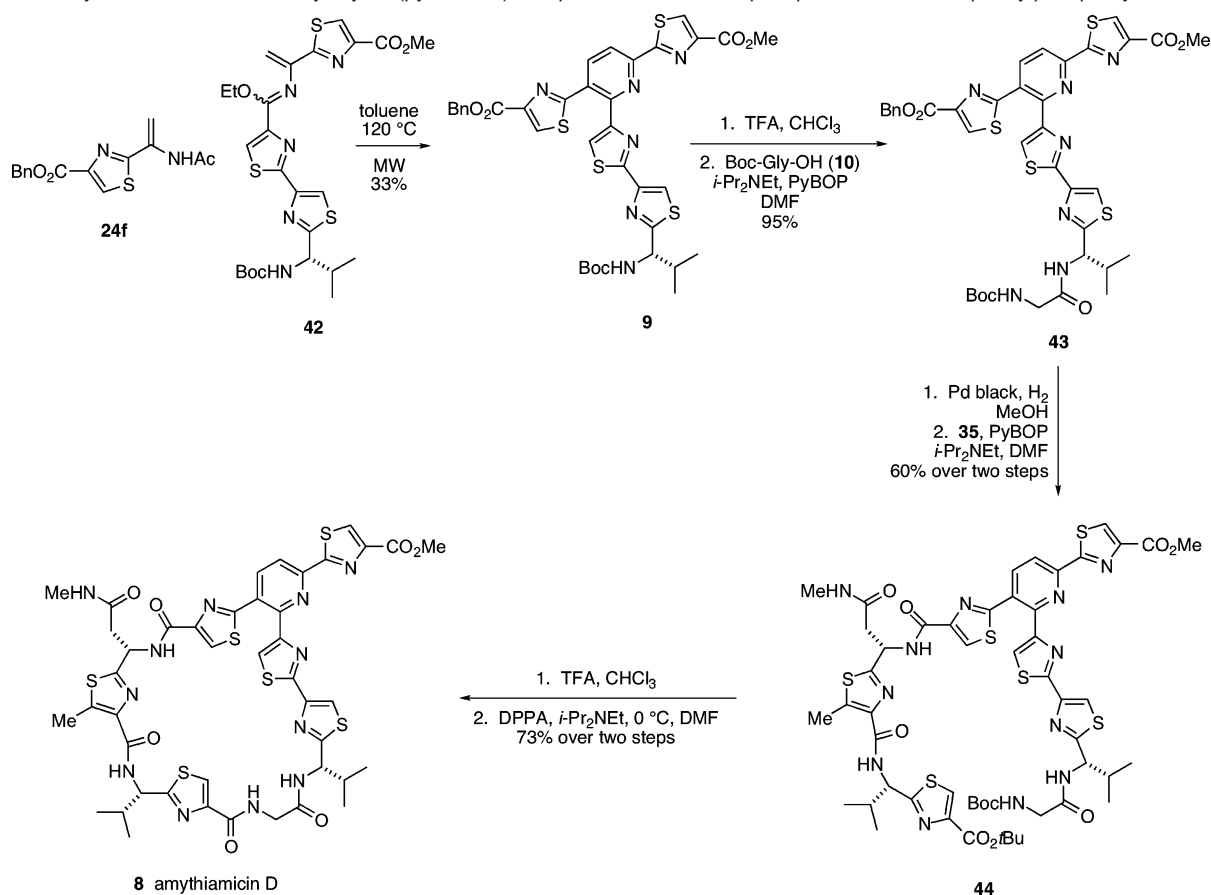
thiazole **39** that was prepared for azadiene formation by de-silylation and acetylation to give the thiazole **40**. Building on the earlier examples, the key azadiene **42** was accessed by reaction of amine hydrochloride **41**, obtained by simple HCl-mediated deprotection of **40**, with imidate **17h**, followed by elimination of the acetate using DBU as base (Scheme 9).

The synthesis had now reached the critical Diels−Alder reaction, and heating the azadiene **42** with enamide dienophile **24f** under microwave irradiation in toluene at 120 °C for 12 h gave the required 2,3,6-tris(thiazolyl)pyridine **9**, albeit in a modest 33% yield. Given that pyridine **9** forms the core of the natural product, it was essential to establish its stereochemical integrity, and this was again achieved by formation of Mosher amides. Removal of the *N*-Boc-group with TFA was followed by separate coupling reactions to (*R*)- and (*S*)-Mosher's acid to give the corresponding amides. Analysis by ^{19}F NMR spectroscopy established the purity of each diastereomeric amide, confirming that no racemization had occurred during the synthesis of diene **42** and hence pyridine **9**.

With all the components now in hand, the assembly of the natural product could be addressed. Deprotection of the terminal *N*-Boc-group on the bis-thiazole moiety of pyridine **9** was followed by a PyBOP-mediated coupling to *N*-Boc-glycine **10** to give the complete right-hand fragment of amythiamicin D **43** (Scheme 10). After much experimentation, it was found that the benzyl ester in compound **43** could be removed by hydrogenolysis over palladium black to give the corresponding carboxylic acid. The use of other palladium catalysts resulted in little or no debenzylation, presumably as a result of catalyst poisoning by the poly-sulfur system; under more

forcing conditions, reduction of the pyridine ring occurred. A second PyBOP-mediated reaction successfully coupled the left-hand bis(thiazole) **35** to provide the cyclization precursor **44**. The terminal *N*-Boc and *tert*-butyl ester groups in **44** were simultaneously cleaved using TFA, and the resulting amino acid treated with diphenylphosphoryl azide (DPPA)[66−69] and Hünig's base in DMF. This resulted in macrolactamization in a yield of 73% (from **44**) to give amythiamicin D **8** (Scheme 10).

Our synthetic material had properties consistent with those reported for the natural product,[29] suggesting that our original assumption about the stereochemistry of the three chiral centers was correct. Subsequent correspondence with the original authors revealed that unpublished *X*-ray crystallographic data substantiates 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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Supporting Information Available: Experimental details and characterization data for all compounds described herein; copies of ^{1}H and ^{13}C NMR spectra of compounds **16h**, **18b**, **19b–h**, **23f**, **24e**, **24f**, **25i–l**, **25n**, **25o**, **26b**, **26c**, **26e**, **29**, **30**, **11**, **34**, **37–39**, **42–44**, and **8**; ^{19}F NMR analyses of Mosher amides of compounds **21** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.