Thiopeptide Antibiotics

Mark C. Bagley,* James W. Dale, Eleanor A. Merritt, and Xin Xiong

School of Chemistry, Main Building, Cardiff University, Park Place, Cardiff, CF10 3AT, Wales, United Kingdom

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1. Introduction

The crisis currently facing antibacterial chemotherapy threatens to return our treatment of bacterial infections to the so-called 'dark age' of a preantibiotic era with the alarming emergence of bacterial strains resistant to conventional treatments.[1] In the face of this medical crisis, many resources have been committed to improving the potency of existing antibiotic classes, discovering new antibacterial agents with novel modes of action, and understanding the mechanisms of resistance that are adopted by different bacterial pathogens to overcome antibacterial action. A discussion of the resistance mechanisms used by antibiotic-producing organisms has been the subject of a number of excellent reviews.[2,3] Antibiotic producers adopt different self-defense mechanisms in order to avoid their own suicide, protecting themselves against extracellular drugs by inactivating their antibiotic products, modifying the antibiotic target sites, such as enzymes or ribosomes, or blocking the entrance of active compounds into the cell. Characterizing the strategies used by either the producers or related bacterial strains to avoid intoxication requires a detailed understanding of how each antibiotic class functions as well as knowledge of the biosynthetic machinery operating in the organism to predict mechanisms of multi-drug resistance (MDR) prior to their clinical emergence in the design of new or strategically modified treatments. This review concentrates upon a single class of antibacterial agent, the thiopeptide antibiotics, the first of which, micrococcin, was isolated in 1948. Our understanding of the biological properties of this agent, the parent of the thiopeptides thiostrepton, discovered later in 1954, and other less well-studied members of this antibiotic class has developed considerably in recent years. Here we draw together structural information on the thiopeptides, defining all of the known members of this family, to discuss the structural basis of their biological properties and its relevance to how producing and nonproducing bacterial strains resist their action. Current synthetic technology for the assembly of these complex targets in the chemical laboratory is also presented along with advances that these studies have made in our understanding of this rapidly expanding family.

The thiopeptide antibiotics are naturally occurring, sulfur-containing, highly modified, macrocyclic peptides, nearly all of which inhibit protein synthesis in bacteria. These complex natural products, grouped as thiazolyl peptides for Bérdy's classification of antibiotics according to chemical structure,[4] share a number of common structural features: a tri- or tetrasubstituted nitrogen heterocycle clustered in a central polyazole domain that is part of a macrocyclic framework consisting of modified heterocyclic residues, including thiazoles, oxazoles, and indoles, and dehydroamino acids. These biologically active substances are secondary metabolites produced by actinomycetes, Gram-positive mycelial sporulating bacteria, largely of the genus *Streptomyces* that can be subdivided into 29 different antibiotic families containing well over 76 structurally distinct entities. Despite their chemical and taxonomical diversity, many of them broadly seem to share a similar biological profile, displaying almost no activity against Gram-negative bacteria, whereas against Gram-positive bacteria they are highly active inhibitors of protein synthesis and are, in many cases, effective against methicillin-resistant *Staphylococcus aureus* (MRSA), a bacterial strain that is resistant to most conventional treatments. This biological property, as well as an increased understanding of their mode of action and the failure of traditional therapies to counter the emergence of bacterial resistance, has led to renewed interest in this antibiotic class.

The development of screening programs to search for new cyclic thiazolylpeptides with a similar bio-

* To whom correspondence should be addressed. Phone: +44 29 2087 4029. Fax: +44 29 2087 4030. E-mail Bagleymc@cardiff.ac.uk.

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Mark C. Bagley, born in 1968 in the United Kingdom, was educated at the University of Oxford (D.Phil. in 1994 at the Dyson Perrins Laboratory, Oxford, under the guidance of Professor L. M. Harwood). He worked with Professor W. Oppolzer at the Université de Genève in Switzerland and Professor C. J. Moody at Loughborough University and the University of Exeter in the United Kingdom before taking an academic position at Cardiff University in 1999, where he is now a Senior Lecturer in Organic Chemistry. His main research interests concern heterocyclic chemistry, the total synthesis of biologically active natural products, and microwave-assisted organic transformations.

James W. Dale, born in 1979 in England, obtained his B.S. degree at Cardiff University, U.K., in 2000. He remained at Cardiff to study his Ph.D. with Dr. Mark C. Bagley on thiopeptide antibiotics, graduating in 2004. He now holds a postdoctoral research fellowship with Professor Richard J. K. Taylor at York University, U.K., where he has been for 10 months working toward the total synthesis of spirocyclic natural products. His research interests include synthetic organic chemistry and natural product chemistry.

logical profile has led to the rapid expansion of the number of structurally distinct thiopeptide antibiotics in recent years. Reinvestigating the chemical identity of many known metabolites using modern methods of structure elucidation has stimulated renewed interest in their biological properties, including their mode of action for the inhibition of bacterial protein synthesis, biosynthetic mechanisms, and the origin of resistance determinants, and has encouraged the development of new and efficient methods for their chemical synthesis and modification. This review draws together all of these findings. Although our technological capability has not yet managed to provide us with facile synthetic access to many of these complex antibiotics, or even verify the structure of many of these agents, the total synthesis of amythiamicin D, promothiocin A, and most recently thiostrepton along with synthetic efforts toward

Eleanor A. Merritt, born in Cardiff, U.K., in 1982, obtained her M.Chem. degree in Chemistry from Cardiff University in 2004. In October 2004 she began postgraduate studies in Dr. Mark C. Bagley's research group in the School of Chemistry, Cardiff University, where she is working toward the total synthesis of a range of thiopeptide antibiotics.

Xin Xiong was born in Liaoning, China, in 1977. He studied at Shenyang Pharmaceutical University, China, where he obtained his B.S. degree in Pharmaceutics in 1999. After receiving his Master's degree in Medicinal Chemistry from Aston University, U.K., he joined Dr. Mark C. Bagley's research group at the School of Chemistry, Cardiff University, for doctoral studies in 2001. His current research interests are focused on synthetic methodologies of heterocyclic compounds, especially pyridine derivatives, and applications to thiopeptide natural product total synthesis.

micrococcin P_1 may facilitate new studies of structurally diverse analogues in the future.

2. Isolation and Structure Elucidation

The individual chemical identity of structurally distinct compounds isolated from natural sources and classified as thiopeptide or thiazolylpeptide antibiotics is relatively diverse. The identification of a plethora of unique structural motifs and unusual functional groups assembled in a macrocyclic array has provided us with a series of tantalizing heterocyclic chemical targets that a number of international synthetic groups have found impossible to resist. Of a more intriguing nature, despite many similarities in biosynthetic origin, these secondary metabolites have been isolated from a number of different strains of actinomycetes, predominantly soil bacteria but also from marine sources, and elicit a wide range of different biological responses. The complexity of the many mechanisms employed by pathogens to avoid intoxication and used by these metabolites in order

Table 1. Thiopeptide Antibiotic Families Classified According to Their Central Heterocyclic Domain

series *a* and *b*	series *c*	series *d*	series *e*
bryamycin (A-8506)[a]	Sch 40832	A10255	glycothiohexide α
Sch 18640 (68-1147)		amythiamicin	MJ347-81F4
siomycin		berninamycin	multhiomycin[b]
thiactin[a]		cyclothiazomycin	nocathiacin
thiopeptin		GE2270	nosiheptide
thiostrepton		GE37468	S-54832
		geninthiocin	
		methylsulfomycin	
		micrococcin	
		promoinducin	
		promothiocin	
		QN3323	
		radamycin	
		sulfomycin	
		thioactin	
		thiocillin	
		thiotipin	
		thioxamycin	
		YM-266183-4	

[a] Shown to be identical to thiostrepton. [b] Shown to be identical to nosiheptide.

to inhibit bacterial protein synthesis is only now coming to light, facilitated by advances in crystallography, NMR spectroscopy, and our understanding of *Streptomyces* transcriptional mechanisms and the dynamic function of the bacterial ribosome. Although further research may reveal that many of these processes are interrelated, at present most can be attributed to certain regions or motifs in metabolite structure particular to certain families or groups of families related by functional-group commonality. For this reason, classifying thiopeptide antibiotics according to structure, in particular, in the nature of the central heterocyclic domain, also categorizes their biological properties and is useful for highlighting structural relationships between the 29 different antibiotic families identified to date. Examining the structure of individual thiopeptides reveals that there are essentially five distinct classes of these natural products, assigned according to the oxidation state of the central heterocyclic domain. Each class can be further subdivided into families that group cyclic peptides with a high degree of structural homology or in some cases that were isolated from the same antibiotic-producing organism (Table 1). It was Hensens who first suggested grouping thiopeptide antibiotics according to the structure and oxidation state of the central heterocyclic domain to distinguish between different constituents of the thiopeptins.[5] Hensens' classification system, in this review, has been extended to describe the five distinct heterocyclic domains. Thus, the parent of the thiopeptide antibiotics, thiostrepton as well as the siomycins are classified as *b* series thiopeptides, whereas some of the thiopeptin factors and Sch 18640 possess the fully reduced *a* series central domain, tetrasubstituted dehydropiperidine or saturated piperidine heterocycles, respectively. Clearly related to the series *a* and *b* thiopeptides, the *c* series, which to date only consists of a single antibiotic, Sch 40832, has an unusual imidazopiperidine core of unique structure. With increasing unsaturation, in line with Hensens' classification, by far the most prolific thiopeptide class is the series *d* antibiotics, possessing a 2,3,6-trisubstituted pyridine domain, which is shared by 19 different families including the first thiopeptide to be isolated and identified, micrococcin. Finally series *e* thiopeptides, such as nosiheptide, exhibit a structurally related central motif, oxidized in comparison with their series *d* counterparts, containing a tetrasubstituted hydroxypyridine.

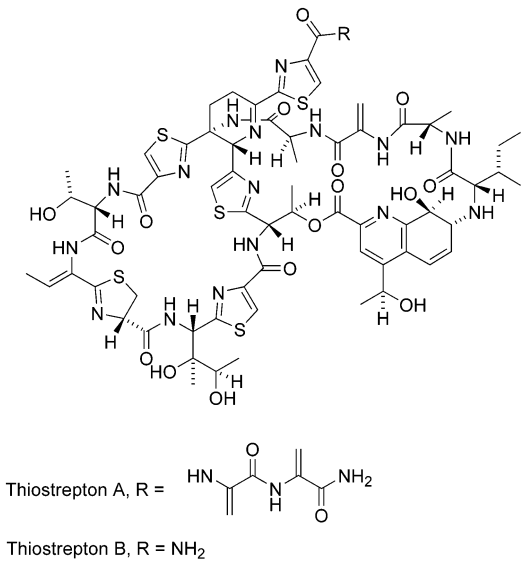


Figure 1. Structure of thiostrepton A and B.

2.1. Piperidines and Dehydropiperidines

All of the series *a* and *b* thiopeptide antibiotics display antibacterial activity and, with a high degree of structural homology, can be identified by their piperidine or dehydropiperidine central heterocyclic domain and bis-macrocyclic peptide backbone, containing quinaldic acid, thiazoline, dehydroalanine, and dehydrodemethylvaline residues as well as a number of thiazole heterocycles. There are four families and 15 structurally distinct entities within these two series, although the structural differences are only very minor. Due to their complex nature, extensive chemical degradation has been used to determine structure as well as multiple NMR spectroscopic techniques and in some cases X-ray crystallographic data.

Thiostrepton ($C_{72}H_{85}N_{19}O_{18}S_5$), sometimes called thiostrepton A or A_1, is often referred to as the parent compound of the thiopeptide antibiotics (Figure 1). First isolated from *Streptomyces azureus* in 1954,[6–8] this secondary metabolite was found to be effective against Gram-positive bacteria with activity comparable to that of the penicillins.[9] However, despite a very promising biological profile, thiostrepton has not been developed for clinical use as resistance by the bacterium develops before a therapeutic dose can be reached, primarily as a consequence of its low aqueous solubility, a problem inherent with most of the thiopeptide antibiotics.

Early developments in the identification of new thiopeptide antibiotics paint a very confusing picture, exemplified by thiostrepton's story. Following isolation of this natural product in 1954, a new thiopep-

tide metabolite called bryamycin was isolated in 1955 from *Streptomyces hawaiiensis*, a strain of soil bacteria discovered in Hawaii.[10] Bryamycin, often referred to by its trade name of A-8506, was shown in 1963 to be identical to both thiostrepton and thiactin by extensive comparison of their hydrolysates, solving just one of the ambiguities in chemical identity inherent in early work on the isolation and identification of new thiopeptide antibiotics.[11] Chemical degradation has also been used to obtain thiazole and quinoline fragments, determining the ratio of a number of amino acid components of thiostrepton.[12] The isolation of residues derived from (−)-alanine, (−)-isoleucine, (−)-threonine, and (+)-cysteine, the latter formed by hydrolysis of a thiazoline, inferred the architecture of a number of structural motifs of the natural product. However, Dorothy Crowfoot Hodgkin made the first real breakthrough in thiopeptide structure determination using X-ray crystallographic methods on monoclinic crystals to confirm previous structural hypotheses and elucidate the absolute stereochemistry and constitution of thiostrepton,[13] with the exception of the identity of the dehydroalanine-containing side chain which was first solved by Tori et al. on the basis of NMR experiments.[14] Thiostrepton, later also isolated from *Streptomyces laurentii*,[15] has been subjected to detailed ^{1}H and ^{13}C NMR spectroscopic analyses by Hensens and Albers-Schönberg,[16] with reinvestigation in 1989 by Floss et al., who used 2D NMR spectroscopic techniques on both unlabeled and biosynthetically multiple ^{13}C-labeled samples to confirm the 1970 structural assignment.[17] Furthermore, a recent investigation by Hunter et al. solved the structure of a tetragonal crystal form of thiostrepton using the anomalous dispersive signal from sulfur collected at the Cu Kα wavelength and placed the coordinates in the public domain.[18] The closely related thiopeptide, known as thiostrepton B (or A_2) ($C_{66}H_{79}N_{17}O_{16}S_5$), isolated from *S. azureus* as a minor component with thiostrepton, has received much less attention. Analysis by ^{13}C NMR spectroscopic methods elucidated the structure, which indicates that thiostrepton B might be an artifact from thiostrepton (Figure 1).[19]

The siomycins are a family of four thiopeptides with structures closely related to that of thiostrepton. The siomycin complex was first isolated from cultures of *Streptomyces sioyaensis* in 1959 and found to be active against Gram-positive bacteria and mycobacteria.[20] Further purification of the crude preparation 10 years later[21] found that this sulfur-containing peptide antibiotic actually consisted of one major constituent, siomycin A, and a number of minor components, siomycin B, C, and D_1, the latter of which was only discovered 21 years after isolation of the original antibiotic complex.[22] Siomycin B (SIM-B, $C_{65}H_{75}N_{17}O_{16}S_5$) is derived from siomycin A (SIM-A, $C_{71}H_{81}N_{19}O_{18}S_5$) during storage, whereas siomycin C (SIM-C, $C_{72}H_{82}N_{18}O_{19}S_5$) and D_1 (SIM-D_1, $C_{70}H_{79}N_{19}O_{18}S_5$) are both true natural products of *S. sioyaensis*. Degradative methods[23] as well as a wide range of spectroscopic experiments, in particular ^{13}C and ^{1}H NMR studies,[14,19,24] were carried out in order to determine the relationship of the siomycin structure

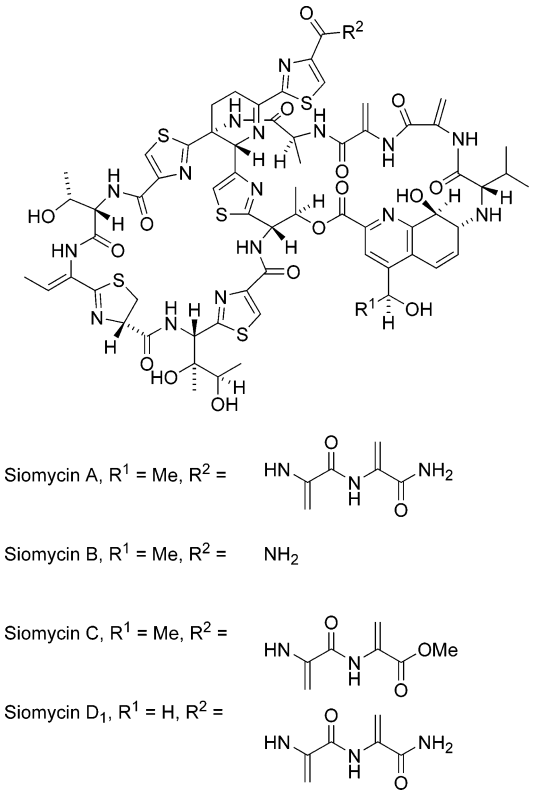


Figure 2. Structure of the siomycins.

with the recently uncovered X-ray crystal structure of thiostrepton[13] and to explain the similar physicochemical and biological properties of all of these antibiotics. The isolation and identification of chemical degradation products, by reduction, oxidation, acidic hydrolysis, and ammonolysis, was supported by extensive ^{1}H, ^{13}C, and nOe NMR spectroscopic data as well as ^{15}N NMR spectroscopy finally to confirm that the siomycins differ from thiostrepton only in a dehydroalanine-valine unit attached to the quinaldic acid in place of an alanine-isoleucine residue (Figure 2).

The thiopeptins, produced by *Streptomyces tateyamensis*, were first characterized by Miyairi et al. and inhibit Gram-positive bacteria with no significant differences in their inhibition of protein synthesis in cell-free *Escherichia coli*.[25] Silica gel chromatography separated the antibiotic complex into its main constituent, thiopeptin B, shown to be useful as a growth-promoting feed additive for pigs and chickens[26] and as a lactic-acidosis preventive in ruminants,[27] and four minor components thiopeptin A_1, A_2, A_3, and A_4. Unable to obtain a crystalline sample suitable for X-ray crystallography, Hensens determined, on the basis of ^{1}H and ^{13}C NMR evidence, that the thiopeptins actually consisted of two distinct series of antibiotics, designated by the subscripts *a* and *b*, but failed to isolate thiopeptin A_2, whose structure still remains undetermined. On the basis of these studies, six distinct compounds were identified, thiopeptin A_{1a} ($C_{72}H_{86}N_{18}O_{18}S_6$), A_{1b} ($C_{72}H_{84}N_{18}O_{18}S_6$), A_{3a} ($C_{65}H_{79}N_{17}O_{15}S_6$), A_{4a} ($C_{68}H_{82}N_{18}O_{16}S_6$), B_a ($C_{71}H_{84}N_{18}O_{18}S_6$), and B_b ($C_{71}H_{82}N_{18}O_{18}S_6$), and two further components that were analyzed as mixtures, thiopeptin A_{3b} ($C_{65}H_{77}N_{17}O_{15}S_6$) and A_{4b} ($C_{68}H_{80}N_{18}O_{16}S_6$) (Figure 3).[5]

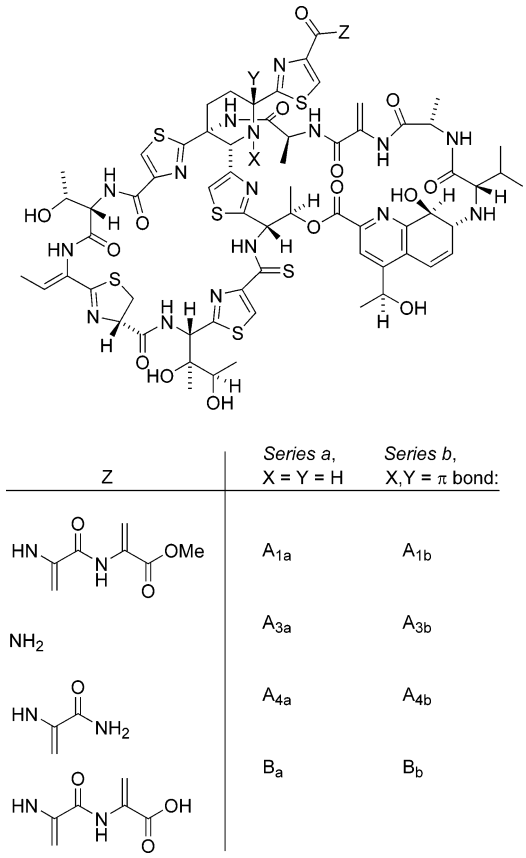


Figure 3. Structure of the thiopeptins.

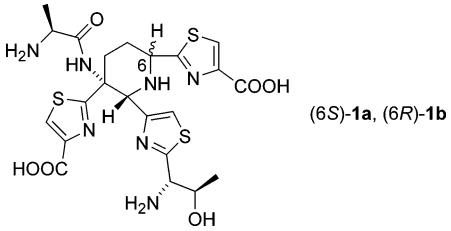


Figure 4. Thiopeptin B_a hydrolysates **1**.

The difference between the two series of thiopeptins has its origin in the nature of, and in particular the oxidation state of, the central heterocyclic domain, the *b* series retaining the Δ^1-piperidine moiety of thiostrepton whereas the *a* series thiopeptin antibiotics (B_a, A_{1a}, A_{3a}, and A_{4a}) possess a fully saturated piperidine core, as confirmed by ^{13}C NMR spectroscopic analysis, with relative and absolute stereochemistry defined by careful analysis of ^1H NMR data and consideration of biogenetic information.[19,28]

Structural assignments were supported by chemical degradation studies, the hydrolysis of the various thiopeptin components yielding 2 mol of alanine and 1 mol of both threonine and valine.[29] Furthermore, although acidic hydrolysis of thiopeptin B_a resulted in the isolation of two diastereoisomeric piperidines **1a,b** (Figure 4) that differed in the configuration at C-6, this was shown to be due to epimerization of the (6*S*)-diastereoisomer (**1a**) during hydrolysis and thus provided further confirmation of structure.[30]

Sch 18640 ($C_{72}H_{87}N_{19}O_{17}S_6$), also referred to as 68-1147 complex, belongs to the series *a* thiopeptides and was isolated as the major constituent of the antibiotic complex produced by *Micromonospora arborensis*.[31] Differentiated from other related natural products known at the time by TLC analysis, the structure of Sch 18640 was established on the basis of degradative and spectroscopic studies, employing 600 MHz ^1H and ^{13}C NMR spectroscopy in combination with plasma desorption mass spectrometry to determine the molecular weight of the natural product. Comparing the ^{13}C NMR spectrum of Sch 18640 with thiostrepton identified a piperidine rather than dehydropiperidine central heterocyclic domain and established that an amide linkage in thiostrepton had been replaced by a thioamide function by observing a characteristic downfield shift $\Delta\delta$ of 28.3 ppm in the quaternary resonance of the thiocarbonyl group. Accounting for these distinctions and supported by amino acid analysis and hydrolysis experiments, the close relationship between the structure of thiostrepton A and Sch 18640 is now firmly established (Figure 5). Although no stereochemical investigation of Sch 18640 has been carried out, the configuration of the 18 stereogenic centers in this metabolite can be inferred tentatively by comparison with other series *a* and *b* thiopeptides.

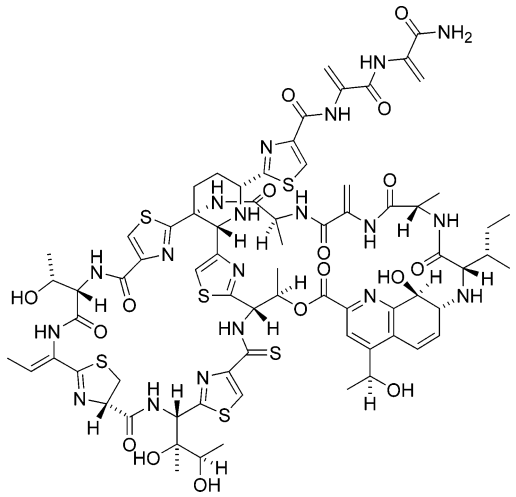


Figure 5. Structure of Sch 18640.

2.2. Dihydroimidazopiperidines

Sch 40832 ($C_{84}H_{104}N_{18}O_{26}S_5$) is the only example of a series *c* thiopeptide and was isolated as the minor component from the antibiotic complex, referred to as 13-384 complex, produced by *Micromonospora carbonecea* var. africana (ATCC 39149).[32] Purified on reverse-phase silica gel, its potent in vitro activity was determined using a disc-diffusion agar plate assay against Gram-positive bacteria. Analysis by IR spectroscopy confirmed the presence of NH, OH, and amide functional groups, amino acid analysis provided 1 mol of cysteine, 4 mol of threonine, and 1 mol of lysine, FAB mass spectrometry determined the molecular weight and molecular composition, and NMR spectroscopic experiments, using a combination of COSY, HMBC, and ^{13}C techniques, elucidated the connectivity of Sch 40832 (Figure 6) and established its distinctiveness from both thiostrepton and Sch 18640. This thiopeptide possesses a unique and unusual structure with a central domain consisting of a fully saturated piperidine heterocycle fused to

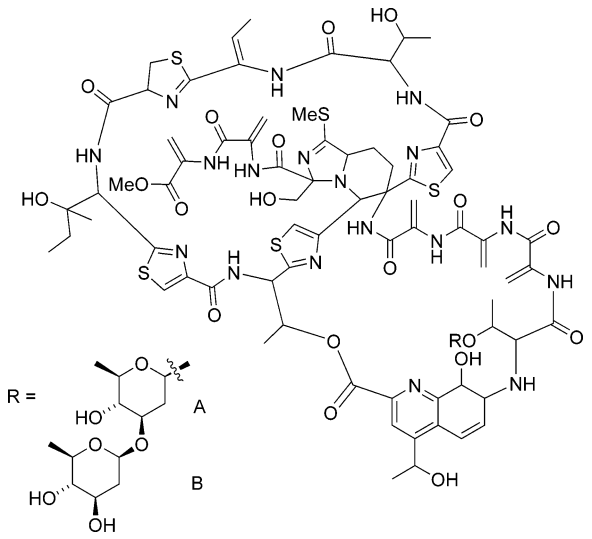


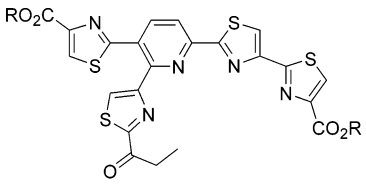
Figure 6. Structure of Sch 40832.

an imidazoline ring derived from a modified thiazoline. In addition to the dihydroimidazo[1,5-*a*]piperidine, Sch 40832 contains a disaccharide moiety attached to a threonine side chain, delineated in a Hartman−Hahn (HOHAHA) experiment and tentatively assigned as β-D-chromose A and B as well as a deoxythiostreptine residue in the peptide backbone.

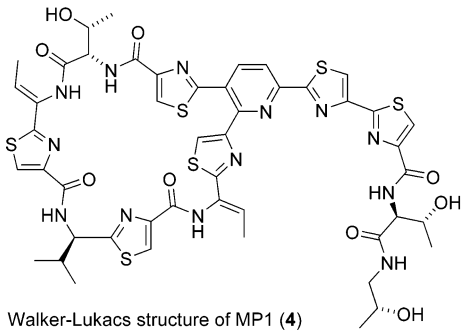
2.3. Trisubstituted Pyridines

Series *d* thiopeptides differ strikingly from the piperidine or dehydropiperidine series *a* and *b* natural products. Predominantly they contain only one macrocyclic peptide loop centered around a 2,3,6-trisubstituted pyridine heteroaromatic domain clustered with thiazole and/or oxazole heterocycles with a peptide side chain consisting of heterocyclic or dehydrated amino acid residues attached at the pyridine 6-position. Numerically, series *d* thiopeptides are the dominant class of thiopeptide antibiotics, with 19 families and over 49 distinct entities, which show diversity in structure as well as biological activity.

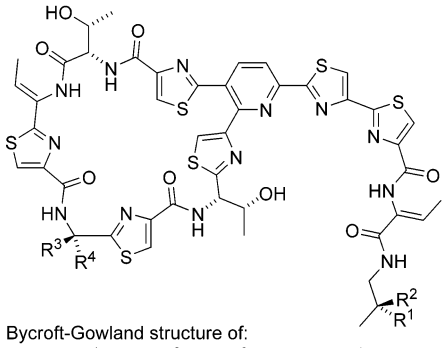
First discovered in 1948 from a strain of *Micrococcus* found in sewage from the city of Oxford and reported in *The British Journal of Experimental Pathology*,[33] the isolate[34] that was to be named micrococcin[35] is recorded as the first example of a thiopeptide antibiotic, although no work to elucidate the chemical structure of that material was ever reported. An antibiotic with therapeutic activity later obtained from the *B. pumilus* group of spore-bearing bacillus, isolated from soil collected in East Africa,[36] demonstrated a considerable degree of identity or near-identity with the antibiotic isolated from *Micrococcus*, perhaps suggesting two members of a closely related family, on the basis of their similar properties and behavior, and so the new complex was named micrococcin P, despite the taxonomic implications. This antibiotic actually consists of two distinct components, present in the complex in ca. a 7:1 ratio and designated micrococcin P_1 and micrococcin P_2, respectively (alternatively written as micrococcin P1 and P2). Gratifyingly, with difficulties experienced in obtaining the complex from these original sources, micrococcin P_1 has recently been obtained from food borne *Staphylococcus equorum* WS2733 isolated from French Raclette cheese,[37] demonstrating how thiopeptide antibiotics can often be discovered from seemingly unrelated sources. Work that spanned the 50-year period following initial isolation to elucidate the structure of micrococcin P_1 tells a remarkable story, amazingly still unresolved, that, despite considerable spectroscopic advances and synthetic achievements, bears witness to a number of oversights and the propagation of unsubstantiated hypotheses, accepted erroneously as the truth. With a lack of structural data, the major isolated component, micrococcin P_1 (also referred to as MP1), was characterized initially purely by examination of its acid hydrolysates, which enabled a provisional molecular weight estimate to be made.[38] Analysis of the acid-soluble fraction gave a laevorotatory hydrochloride that was identified conclusively as L-threonine·HCl by IR spectroscopy, thus confirming the identity and stereochemistry of one of the residues of the natural product. Further analysis of the acid-insoluble fraction established that micrococcin P_1 must contain an extended chromophoric system[39] by isolating two derivatives of the central heterocyclic domain identified as micrococcinic acid (**2**) and methyl micrococcinate (**3**) and separated an amino alcohol from the acid-soluble hydrolysate of the peptide side chain. Although this residue was assigned originally as alaninol (2-aminopropan-1-ol),[40] it was later identified as D-(*R*)-isoalaninol (2-hydroxypropylamine) from ^{13}C NMR spectroscopic data of the natural product.[41] This study culminated in the Walker−Lukacs structure **4** of micrococcin P_1 which, although a considerable advance, had assembled the order of the individual residues without evidence but rather based upon an assumed structural homology with two other thiopeptides, thiostrepton and nosiheptide. Prompted by publication of the Walker−Lukacs structure, Bycroft and Gowland separated micrococcin P_1 ($C_{48}H_{49}N_{13}O_9S_6$) and micrococcin P_2 ($C_{48}H_{47}N_{13}O_9S_6$) and carried out their own NMR spectroscopic studies and analyses of the acid hydrolysates of both micrococcin P_1 and its sodium borohydride derivative.[42] In contrast to Walker's findings, only 1 mol of threonine was produced in each of these hydrolysis experiments, leading to the proposal of the alternative Bycroft−Gowland structure for both micrococcin P_1 (**5**) and P_2 (**6**) that accounted for their hydrolytic behavior (Figure 7). The proposed structure was accepted for over 20 years but was finally shown to be erroneous when, in 1999, Ciufolini completed his landmark total synthesis of MP1 and demonstrated that this architecture did not correspond to that of the natural product.[43] This confusing situation has been compounded further by Shin's synthesis of two epimeric substances of the Walker−Lukacs and Bycroft−Gowland structures, described as micrococcin P[44] and micrococcin P_1 (**7**),[45] respectively, containing in each case an (*S*)-isoalaninol residue in the peptide side chain in place of (*R*)-isoalaninol, established unequivocally by combination of degradation studies[40] and NMR spectroscopy.[41] Ciufolini later drew together all of these findings and validated the 1978



R = H, micrococcinic acid (**2**);
R = Me, methyl micrococcinate (**3**)



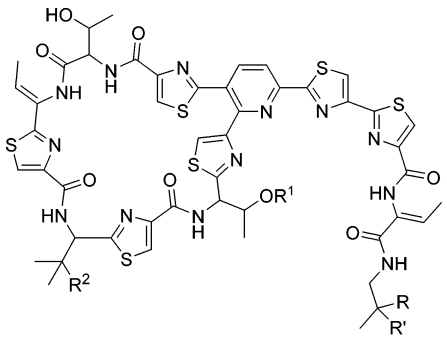
Walker-Lukacs structure of MP1 (**4**)



Bycroft-Gowland structure of:
MP1 (**5**) R^1 = OH, R^2 = H, R^3 = $CHMe_2$, R^4 = H;
MP2 (**6**) R^1,R^2 = O (π-bond), R^3 = $CHMe_2$, R^4 = H;
Shin's totally synthetic epimer of:
MP1 (**7**) R^1 = H, R^2 = OH, R^3 = $CHMe_2$, R^4 = H;
Probable structure of:
MP1 (**8**) R^1 = OH, R^2 = H, R^3 = H, R^4 = $CHMe_2$;
MP2 (**9**) R^1,R^2 = O (π-bond), R^3 = H, R^4 = $CHMe_2$

Figure 7. Proposed structures for the micrococcins and their chemical derivatives.

Bycroft−Gowland hypothesis with extensive NMR studies, confirming that the order of residues in micrococcin P_1 was in accord with their original proposal.[46] It would appear, on the basis of these results, that the difference between synthetic MP1 and the natural material, as examined by Bycroft and Gowland in 1978, is purely stereochemical and therefore must have its origin in either the configuration of the L-threonine-derived thiazole that forms part of the central heterocyclic domain or in the (*R*)-valine-derived thiazole in the peptide macrocycle, both of which were proposed in the absence of reliable experimental evidence. The stereochemical assignment of the latter was derived originally from chemical degradation studies, but the hydrochloride salt of the key hydrolysate, isolated from the natural product, was identified as both (+)- and (−)-2-(1-amino-2-methylpropyl)thiazole-4-carboxylic acid in separate studies,[38] with a specific rotation that varied for the latter between 0° and −20.8°, leading the authors to conclude that the stereochemistry of the



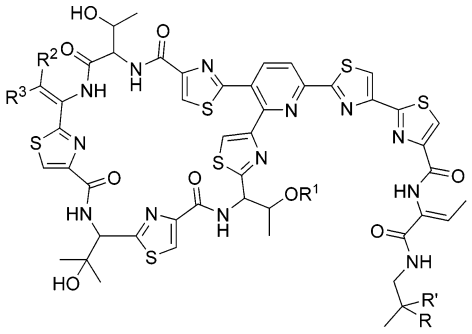
thiocillin I: R^1 = H, R^2 = OH, R = OH, R' = H;
thiocillin II: R^1 = Me, R^2 = OH, R = OH, R' = H;
thiocillin III: R^1 = Me, R^2 = H, R = OH, R' = H;
YM-266183: R^1 = H, R^2 = OH, R,R' = O (π-bond);
YM-266184: R^1 = Me, R^2 = OH, R,R' = O (π-bond)

Figure 8. Structure of the thiocillins and thiopeptides isolated from the marine sponge *H. japonica*.

valine-derived residue "is *probably* of the D-configuration".[47] Considering these instrumental limitations and Ciufolini's findings, it would appear that the most probable structure for MP1 (**8**) and MP2 (**9**) is at variance in the stereochemical assignment of this unit, but it is hoped this longstanding mystery will soon be solved by chemical synthesis.

A number of thiopeptides structurally related to the micrococcins were isolated subsequently from the *Bacillus* genus, including the thiocillins I and II, isolated from the cultured broth of *Bacillus cereus* G-15, and thiocillins II and III, isolated from *Bacillus badius AR-91*.[48] TLC analysis was used to differentiate between these cyclic peptides and both micrococcin P_1 and P_2 and provided a preliminary indication that the thiocillins are in fact produced by other *Bacillus* species, including *Bacillus megatherium* I-13 and strain AR-140, preliminarily identified with *B. pumilus*, although these studies have not been substantiated with any other corroborating data. Structure analysis by ^1H and ^{13}C NMR spectroscopy supported by evidence from chemical degradation studies, including reduction, hydrogenolysis, and hydrolysis, identified the individual structural components of all of these antibiotics and established their connectivity and structural relationship with the micrococcin thiopeptide antibiotics (Figure 8).[49]

Very recently the related cyclic thiazolylpeptides YM-266183 ($C_{48}H_{47}N_{13}O_{10}S_6$) and YM-266184 ($C_{49}H_{49}N_{13}O_{10}S_6$) were found in the cultured broth of *B. cereus* QN3323, isolated from the marine sponge *Halichondria japonica*, representing the first family of thiopeptide natural products to be derived from a marine source.[50] Extensive analytical studies were undertaken to elucidate their structure using high-resolution MALDI-TOF mass spectrometric studies to determine the molecular formulas, a wide range of NMR spectroscopic methods, in particular HMBC experiments, to establish the connectivity of individual residues within these natural products, and ROESY NMR correlations to indicate the *Z*-configuration of propenyl groups in two dehydroamino acid units, one in the macrocycle and one in the peptide side chain (Figure 8).[51] Both factors exhibit potent antibacterial activity against staphylococci and enterococci includ-



QN3323A: R^1 = H, R^2 = Me, R^3 = H, R/R' = O (π-bond);
QN3323B: R^1 = H, R^2 = Me, R^3 = Me, R/R' = O (π-bond);
QN3323Y^1: R^1 = Me, R^2 = H, R^3 = H, R = OH, R' = H

Figure 9. Structure of the QN3323 factors.

ing multiple drug-resistant strains, the MIC for YM-266183 and YM-266184 against *S. aureus* CAY 27701 (MRSA) being 0.78 and 0.39 μg/mL, respectively, but both are inactive against Gram-negative bacteria.

The thiocillins have also been isolated from other cultures of the *Bacillus* genus that in addition yielded the QN3323 compounds, a family of three thiopeptides denoted factors A, B, and Y^1 with antibacterial properties.[52] Although the structure of these natural products seems ambiguous, in particular relating to the configuration of the propenylthiazole in the peptide backbone, and requires the support of additional spectroscopic evidence, clearly these metabolites are closely related to the micrococcins and thiocillins, also produced by species of *Bacillus* (Figure 9).

Following isolation of the micrococcins, two series *d* thiopeptide families, the berninamycins and sulfomycins, were identified as specific inhibitors of bacterial protein synthesis in 1969. The berninamycins are a family of four metabolites, isolated from the fermentation extract of *Streptomyces bernensis*, shown to interfere with amino acid incorporation into peptides.[53] Initial structure determination studies on berninamycin A ($C_{51}H_{51}N_{15}O_{15}S$), which it is proposed corresponds to the purified berninamycin featured in earlier reports, and berninamycin B ($C_{51}H_{51}N_{15}O_{14}S$), carried out in 1975 by Liesch and Rinehart,[54] used NMR spectroscopic analyses of water-soluble sodium and ammonium salt derivatives along with chemical degradation studies by trifluoroacetolysis, sodium borohydride reduction, and catalytic hydrogenolysis.[54,55] The first structural hypothesis likened the heterocyclic core of the berninamycins to that of its acidic hydrolysate, berninamycinic acid (**10**), the structure of which had been elucidated by X-ray crystallographic studies[56] and chemical synthesis.[57] Following further investigation, a revised structure for berninamycin A was postulated by Abe[58] and subsequently confirmed by Rinehart et al. in 1994.[59] Berninamycin B, C ($C_{48}H_{48}N_{14}O_{14}S$), and D ($C_{45}H_{45}N_{13}O_{13}S$) are minor components of the berninamycin complex and were characterized at the same time by ^{13}C NMR spectroscopy and FAB mass spectrometry. The structure of berninamycin B has a valine residue in place of a β-hydroxyvaline unit found in the peptide macrocycle of berninamycin A, whereas berninamycin D has two fewer dehydroalanine units in the pyridine-

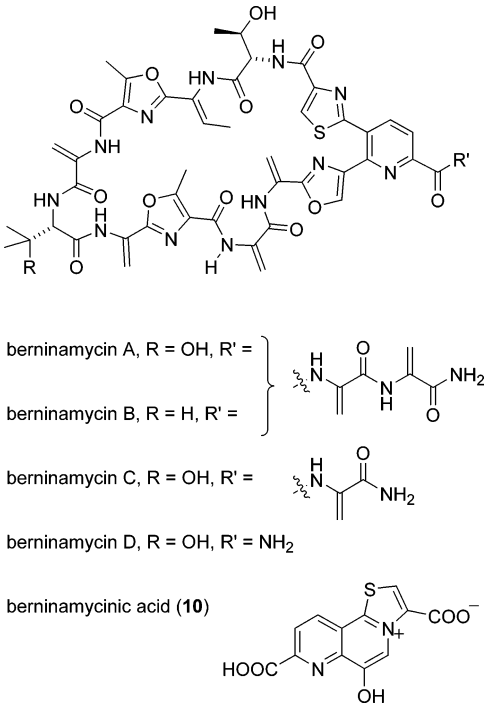
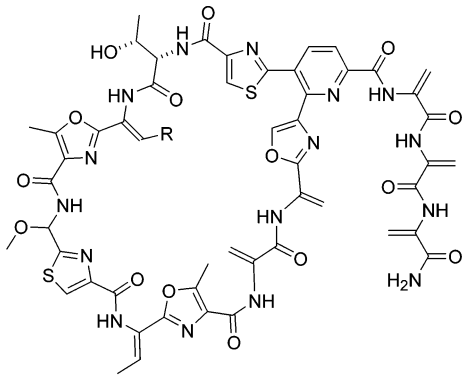


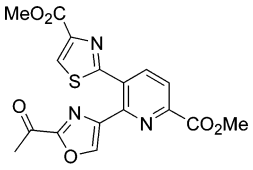
Figure 10. Structure of the berninamycins.

6-carboxamide side chain (Figure 10). On the basis of FAB mass spectrometry, the least abundant berninamycin component C, isolated in a quantity of only about 1 mg from 1 g of the antibiotic complex, is postulated to have a short peptide side chain containing only a single dehydroalanine residue. Stereochemical information can be garnered from biosynthetic studies using ^{13}C-enriched L-valine and threonine.[60] As well as showing antibacterial activity, the berninamycins also show *tipA* promoter activity at nanomolar concentrations as regulators of gene expression.[61] A thiopeptide named neoberninamycin was isolated from *Micrococcus luteus,* and results from ^{1}H NMR and mass spectrometric studies have led to the conclusion that this compound is similar in structure to berninamycin A but not identical,[62] although the complete structure of neoberninamycin has yet to be determined.

The sulfomycins, first isolated in the same year, comprise a family of three cyclic peptides consisting of sulfomycin I ($C_{54}H_{52}N_{16}O_{16}S_2$), II ($C_{54}H_{52}N_{16}O_{15}S_2$), and III ($C_{53}H_{50}N_{16}O_{16}S_2$). Sulfomycin I was obtained from *Streptomyces viridochromogenes* subsp. sulfomycini ATCC 29776 and exhibits strong inhibitory activity against Gram-positive bacteria,[63] whereas all three have been isolated from *S. viridochromogenes* MCRL-0368. The structure of sulfomycin I was determined using a combination of ^{1}H and ^{13}C NMR spectroscopic techniques, FAB mass spectrometric analysis,[53] and chemical evidence. Acidic methanolysis provided a number of different fragments including dimethyl sulfomycinamate (**11**),[64] the structure of which has been confirmed by X-ray crystallographic studies and chemical synthesis (Figure 11).[65,66] Sulfomycin II and III, isolated from subspecies MCRL-0368, have closely related structures that vary only in the nature of the side chain (R^2) located on a 2-(2-aminoalkenyl)oxazole residue in the peptide



sulfomycin I: R = CH(OH)Me; sulfomycin II: R = CH_2Me;
sulfomycin III: R = CH_2OH

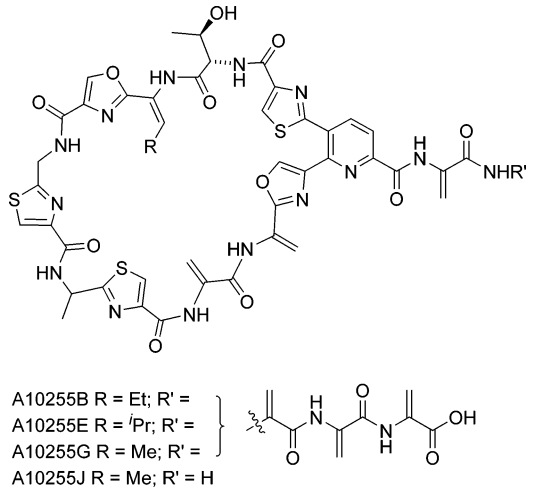


dimethyl sulfomycinamate (**11**)

Figure 11. Structure of the sulfomycins and dimethyl sulfomycinamate (**11**).

backbone.[67] These cyclic peptides share a common oxazole−thiazole−pyridine-type *d* central heterocyclic domain and, as well as a prevalence of dehydroamino acids, contain an unusual alkoxythiazolylmethyl amide unit characteristic of this thiopeptide family. All of the sulfomycins strongly inhibit the growth of Gram-positive bacteria, including methicillin-resistant *S. aureus*, but are not active against Gram-negative bacteria.

A number of related thiopeptide families, discovered subsequently, share the same oxazole−thiazole−pyridine-type *d* domain, including the A10255 factors, geninthiocin, methylsulfomycin, promoinducin, the promothiocins, radamycin, thioactin, thiotipin, and thioxamycin. The thiopeptide antibiotic complex A10255, isolated from *Streptomyces gardneri* NRRL 15537,[68] or cultures of NRRL 18260, a higher producing strain derived from NRRL 15922 by nitrosoguanidine mutagenesis,[69] has been shown to exhibit strong antimicrobial activity against Gram-positive bacteria as well as promote growth and alleviate acidosis in ruminants. This multicomponent complex (designated A10255B, -C, -E, -F, -G, -H, and -J), extracted from the mycelia formed in submerged cultures of the organism, was separated by chromatography to provide four major constituents, isolable in sufficient quantity to elucidate structural data, factors B ($C_{53}H_{48}N_{16}O_{15}S_3$), E ($C_{54}H_{50}N_{16}O_{15}S_3$), G ($C_{52}H_{46}N_{16}O_{15}S_3$), and J ($C_{43}H_{38}N_{14}O_{11}S_3$). The ratio of factors produced by the culture in the A10255 complex was found to be dependent upon the medium, cobalt, or cobalamin supplementation leading to a significant increase in the BE/G ratio, which was consistent with an increase in methionine biosynthesis and provision of a methyl donor for the biosynthetic methylation of A10255G.[70] A combination of collisionally induced dissociation (CID) and FAB mass spectrometry, NMR spectroscopic studies, and selective chemical degradation by reduction, methanolysis, acidic hydrolysis



A10255B R = Et; R' =
A10255E R = ^{i}Pr; R' =
A10255G R = Me; R' =
A10255J R = Me; R' = H

Figure 12. Structure of the A10255 factors.

and trifluoroacetolysis provided the complete structure of the major factors of A10255 (Figure 12).[71] Three of these components are identical except for the extent of methylation on a dehydroamino-acid-derived oxazole residue in the peptide macrocycle, factors G, B, and E being derived from dehydrobutyrine, dehydronorvaline, and dehydronorleucine residues, respectively. A10255J has a similar structure to A10255G, with a masked dehydrobutyrine residue, but differs by a single amidated dehydroalanine unit in the side chain on the central pyridine domain. No stereochemical information has been described for the A10255 thiazolyl peptides regarding the *Z*/*E*-geometry of the 2-(1-prop-1-enyl)oxazole-4-carboxylate residue or the (*R*/*S*)-configuration of the three stereogenic centers, although the successful incorporation of the isotopic label of L-[1-^{13}C]threonine in A10255 factors G, B, and E does indicate the stereochemistry of this residue in all of these metabolites.[72]

The discovery of sensitive and specific thiopeptide screening technology in the 1990s enabled Seto to isolate a number of different but related antibiotic families from microorganisms using a novel *tipA* promoter inducing activity assay. Inserting the promoter (*ptipA*) of the *tipA* gene into a promoter probe vector[73] enabled thiostrepton-like compounds to be identified by their ability to initiate transcription of *ptipA*.[74] In this manner, geninthiocin ($C_{50}H_{49}N_{15}O_{15}S$) was isolated from *Streptomyces* sp. DD84 and its structure determined by a series of spectroscopic experiments using UV, IR, COSY, HMQC, HMBC, and ^{1}H and ^{13}C NMR techniques. High-resolution FAB mass spectrometry identified oxazole and thiazole heterocycles along with several unusual amino acids (Figure 13) and distinguished this antibiotic from neoberninamycin, a cyclic thiazolyl peptide of unknown structure.[62] Further analysis established the L-configuration of the threonine residue in the peptidic macrocycle by chiral-TLC and determined the *Z*-configuration of the 2-(1-aminoprop-1-enyl)-oxazole by nOe ^{1}H NMR spectroscopic experiments.

Further screening experiments by Seto on cultures of *Streptomyces* sp. SF2741 harvested two thiopeptides from the mycelial cake, promothiocin A ($C_{36}H_{37}N_{11}O_8S_2$) and promothiocin B ($C_{42}H_{43}N_{13}O_{10}S_2$).[75] A combina-

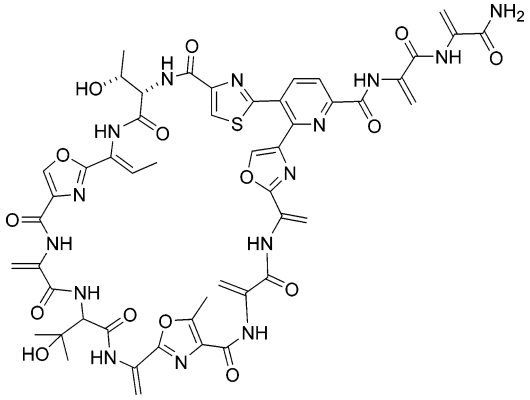


Figure 13. Structure of geninthiocin.

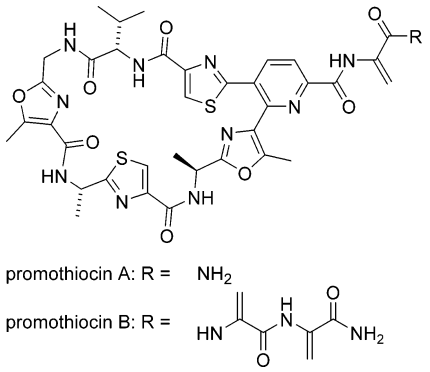


Figure 14. Structure of the promothiocins.

tion of IR spectroscopy, high-resolution FAB mass spectrometry, 2D NMR experiments, and amino acid analyses, which provided 1 mol each of alanine, glycine, and valine, elucidated the constitution of both of the promothiocin factors but did not provide any insight into the stereochemical assignment of these antibiotics. However, the chemical synthesis of promothiocin A by Moody and Bagley[76,77] established unequivocally the (*S*)-configuration of the three stereogenic centers in the natural product, and this was later supported by degradation studies and molecular modeling (Figure 14).[78] Chiral-HPLC analysis of the acid hydrolysates confirmed the L-configuration of both alanine and valine, whereas the (*S*)-2-(1-amino-1-ethyl)thiazole-4-carboxylate residue provided the best calculated fit in a DADAS90 conformational study. Minimum *tipA* promoter induction concentrations of promothiocins A and B were reported as 0.2 and 0.1 μg/mL, respectively.[75]

Further investigation of the metabolites produced by *Streptomyces* sp. SF2741, the producing strain of the promothiocin antibiotics, isolated the thiazolyl peptide promoinducin ($C_{57}H_{54}N_{16}O_{18}S_2$) from the mycelial extract. Its structure was elucidated by ^{1}H, ^{13}C, COSY, NOESY, HSQC, and ^{13}C-decoupled HMBC spectroscopic experiments as well as high-resolution FAB mass spectrometry and contains a dehydroalanine tetrapeptide side chain and oxazole−thiazole−pyridine central domain (Figure 15).[79] The configuration of the L-threonine residue in the macrocycle was established by chiral-TLC analysis of the acid hydrolysate (6 N HCl, 110 °C, 20 h), whereas the geometrical configuration of the β-methine and propenyl groups was established as *Z* in both cases from

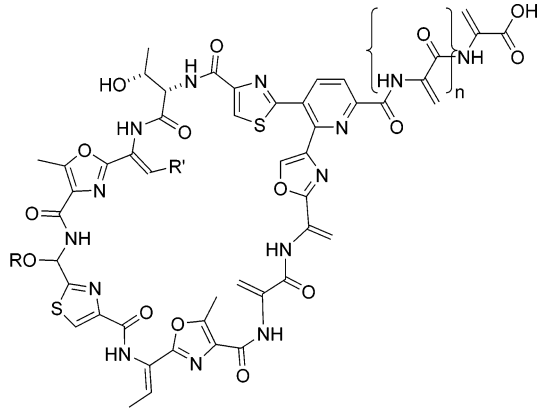


Figure 15. Structure of Promoinducin and Thiotipin.

NOESY data. This thiopeptide, related to the sulfomycins, promothiocins, and geninthiocin, shows activity against Gram-positive bacteria including *M. luteus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes,* and methicillin-resistant *S. aureus* at minimum inhibitory concentrations of 0.39, 0.1, 0.1, and 1.56 μg/mL, respectively, and acts as a *tipA* promoter at 40 ng/mL.

Thiotipin ($C_{55}H_{50}N_{16}O_{17}S_2$), a series *d* thiopeptide that is structurally related to promoinducin, was also isolated by Seto from the mycelium of *Streptomyces* sp. DT31 as a *tipA* promoter inducing substance.[80] Structure elucidation using a combination of high-resolution FAB mass spectrometry and ^{1}H, ^{13}C, COSY, HMQC, and nOe NMR spectroscopic techniques, the latter of which assigned the *Z*-configuration of the propenyloxazole units, showed considerable structural homology with promoinducin with only three points of variance, including the length of the polydehydroalanine peptide side chain (Figure 15). The configuration of the L-threonine residue was established by chiral TLC analysis of the acid hydrolysate, but these studies could not assign the stereochemistry of the remaining unusual hydroxyaminoamide. Thiotipin was reported to show antibacterial activity against *S. pneumoniae*, *S. pyogenes,* and *M. luteus* at the level of 3−6 μg/mL and a minimum induction concentration of 80 ng/mL for *tipA* promoter inducing activity.

Two closely related thiopeptides, thioxamycin ($C_{52}H_{48}N_{16}O_{15}S_4$) and its simpler derivative thioactin ($C_{43}H_{40}N_{14}O_{11}S_4$), were both isolated from the mycelium cake of *Streptomyces* sp. DP94 screening for *tipA* promoter inducing activity,[81] although the former has also been found in the cultured broth of another strain PA-46025.[82] The distinct nature of thioxamycin was apparent from its acidity, the free carboxylic acid in the dehydroalanine side chain distinguishing the structure of this metabolite from the related sulfomycins. Furthermore, treatment with aqueous acid and analysis of the hydrolysates determined that thioxamycin contained 1 mol of L-threonine, an (*R*)-2-[1-amino-2-(methylthio)ethyl]oxazole unit, and two other thiazole residues. Although no stereochemical investigation has been carried out on thioactin, tentatively it can be assumed to possess the same

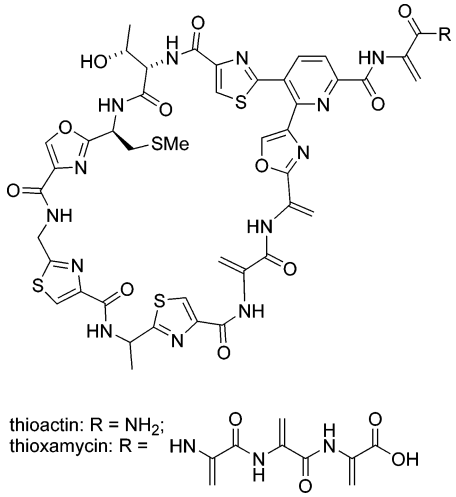


Figure 16. Structure of thioactin and thioxamycin.

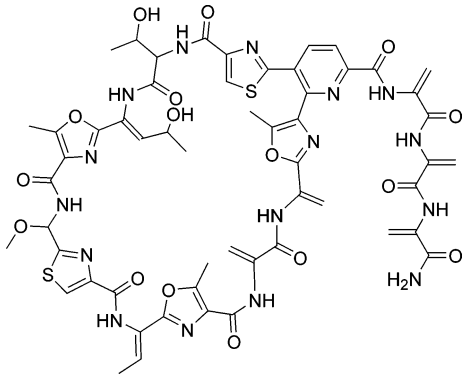


Figure 17. Structure of methylsulfomycin.

stereochemistry as ^{1}H and ^{13}C NMR spectroscopic analyses of these metabolites connected their structures and confirmed the constitution of both natural products (Figure 16). Minimum induction concentrations of thioxamycin and thioactin for *tipA* promoter activity were 80 and 40 ng/mL, respectively, whereas activity against aerobic Gram-positive bacteria including *S. pyogenes* C-203 and *S. pneumoniae* Type I as well as against anaerobic bacteria such as *Streptococcus constellatus* ATCC 27823 were 0.39, 0.78, and 6.25 μg/mL, respectively.

Methylsulfomycin ($C_{55}H_{54}N_{16}O_{16}S_2$), also sometimes referred to as methylsulfomycin I,[83] was first isolated from *Streptomyces* sp. HIL Y-9420704. Mass spectrometry, chemical analysis, and COSY, ^{1}H, and ^{13}C NMR spectroscopic experiments determined that this thiopeptide differed structurally from sulfomycin by only a single 5-methyl group in the oxazole of the central domain (Figure 17).[84] Curiously, this change would appear to make methylsulfomycin much more sensitive to oxygen, although its stability was improved sufficiently in structural analyses by the use of degassed solvents. Further nOe NMR spectroscopic experiments assigned the *Z*-configuration of both double bonds in the dehydroamino acid residues of the macrocyclic peptide.

Methylsulfomycin has also recently been isolated from the fermentation broth of *Streptomyces* sp. RSP9, where it was identified by comparison of its NMR and mass spectra with previously reported data, along with an unusual cyclic peptide, radamycin ($C_{48}H_{47}N_{15}O_{11}S_3$), a very strong inducer of the *tipA* gene that possesses no antibacterial activity.[85] The structure of radamycin was elucidated by IR spectroscopy, high-resolution FAB mass spectrometry, and 1D and 2D NMR spectroscopic experiments and is closely related to that of methylsulfomycin, with an oxazole−thiazole−pyridine central domain (Figure 18).[86] The ability of radamycin to induce the *tipA* promoter without itself having any antibacterial activity, while being a curious feature, may provide a number of future research opportunities for the construction of inducible *tipAP* vectors lacking a thiostrepton resistance gene (*tsr*) or in the industrial-scale production of proteins, where the addition of antibiotics may cause the selection of thiopeptide-resistant strains.

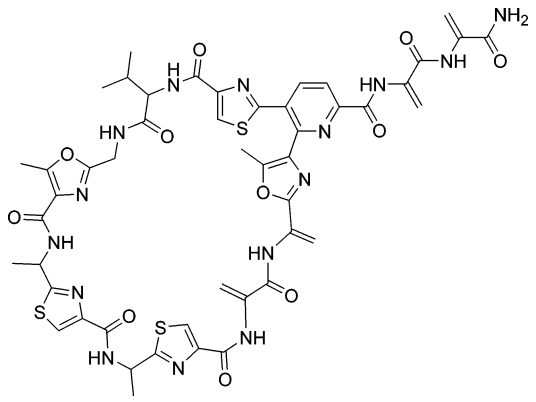
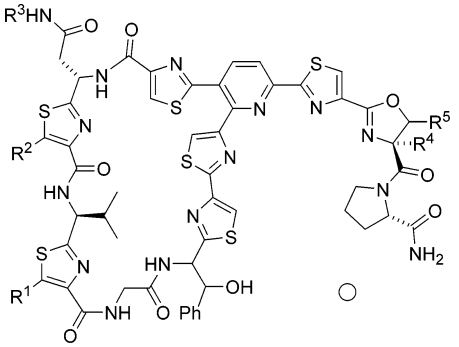


Figure 18. Structure of radamycin.

A number of novel pyridine-containing cyclic peptide antibiotics were discovered in the 1990s from screening programs designed to detect inhibitors of protein synthesis. The GE2270 (MDL 62,879) class of series *d* thiopeptides is the largest family of these natural products with over 12 structurally related components (Figure 19).[87] The main factor of this complex, GE2270A ($C_{56}H_{55}N_{15}O_{10}S_6$), isolated in a screening program designed to detect inhibitors of protein synthesis from the fermentation broth of *Planobispora rosea* ATCC 53773, was extracted from the mycelium and purified by column chromatography on silica gel.[88] The original structure, proposed for this factor on the basis of chemical degradation, UV, IR, and NMR spectroscopic studies[89] and mass spectrometric techniques,[90] was revised upon further experimentation, correcting the sequence of thiazole amino acids in the cyclic peptide through analysis of the hydrolysates[91] with some assignment of absolute stereochemistry.[92] The isolation and characterization of nine of the minor components, separated from the GE2270 antibiotic complex by HPLC, was reported in 1995 and facilitated by altering the fermentation conditions, a study that showed that *P. rosea* modifies the GE2270 backbone by introducing a variable number of methylene units.[93] The structures of the individual factors, E, D1, D2, C1, C2a and C2b (not to be confused with subscripts *a* and *b* used to denote differences in the central domain in the thiopeptin factors), B1, B2, and T were determined by 2D NMR spectroscopy and differ from GE2270A in the nature of the 5-substituent on two thiazole residues in the peptide backbone, the asparagine *N*′-substituent, and



Factor	R^1	R^2	R^3	R^4, R^5
A	CH_2OMe	CH_3	CH_3	H
B1	CH_2OMe	CH_3	H	H
B2	CH_3	CH_3	CH_3	H
C1	H	CH_3	CH_3	H
C2a	CH_2OMe	CH_2OH	CH_3	H
C2b	CH_2OMe	H	CH_3	H
D1	H	CH_3	H	H
D2	CH_2OH	CH_3	CH_3	H
E	CH_2OH	CH_3	H	H
T	CH_2OMe	CH_3	CH_3	π-bond

Figure 19. Structure of the GE2270 factors.

the oxidation state of the azole in the 6-pyridine side chain. All of the GE2270 factors were found to inhibit protein synthesis in Gram-positive microorganisms and anaerobes,[93] with particular activity noted against *Propionibacterium acnes* (MIC for GE2270A against L1014 ATCC 6919 < 0.004 μg/mL) and *Mycobacterium tuberculosis* (MIC for GE2270A of 1 μg/mL),[88] as well as being quite active against some Gram-negative bacteria. These antibiotics inhibit bacterial protein synthesis by acting specifically on the GTP-bound form of Ef-Tu,[94] the elongation factor required for the binding of aminoacyl-tRNA to the ribosomal A site, and so function in a fashion similar to kirromycin-like antibiotics and pulvomycin, although their spectrum of antibacterial activity is quite different.

The inhibition of bacterial protein synthesis through binding to elongation factor Tu was subsequently incorporated in a screening program to isolate antibiotics with a similar structure and mode of action to GE2270A. In this fashion the thiazolylpeptide GE37468A ($C_{59}H_{52}N_{14}O_{12}S_5$) was obtained from the fermentation broth of *Streptomyces* sp. ATCC 55365 isolated from a soil sample collected in Italy, although the productivity and reproducibility of the isolate was poor (typically < 10 mg/L).[95] The selection of morphochromatic spontaneous phenotypes led to the isolation of a stable high-yielding variant ATCC 55365/O/5, increasing antibiotic production dramatically with respect to the parent strain.[96] Structure elucidation by ^1H and ^{13}C NMR spectroscopy and FAB mass spectrometric analysis on both the parent compound and its hydrolysates showed that this thiopeptide was related to both the amythiamicins

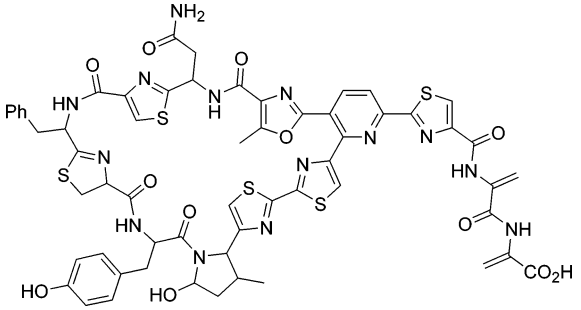


Figure 20. Structure of GE37468A.

and the GE2270 factors, although the 3-thiazolyl substituent on the pyridine in GE2270A is replaced in GE37468A with a 3-(4-methyloxazolyl) group and two extra dehydroalanine units are found in the 6-pyridine side chain along with an unusual 5-hydroxyproline residue in the peptide macrocycle (Figure 20).[97] Two other thiopeptide factors have been attributed to this family, GE37468B and -C; however, no structural data have been published to date.[98] GE37468A is highly active in vitro against Gram-positive bacteria (MIC against *P. acnes* ATCC 6919 and *S. aureus* Smith is 2 and 16 μg/L, respectively), selective for prokaryotic protein synthesis by acting on Ef-Tu in comparative studies in cell-free *E. coli* and rat liver systems, and protects mice against *S. aureus* infection.[95]

The amythiamicins were detected by a paper disk diffusion screen based upon their in vitro antibacterial activity against *S. aureus* Smith and were obtained from the fermentation broth of *Amycolatopsis* sp. MI481-42F4, isolated from soil samples collected in Tokyo, Japan.[99] These antibiotics, although inactive against most Gram-negative bacteria and fungi, inhibit the growth of Gram-positive bacteria, including multi-drug-resistant strains such as *S. aureus* MS9610 and methicillin-resistant *S. aureus* (MRSA),[100] and show no signs of toxicity when administered intraperitoneally to mice at a dose of 100 mg/kg.[99] The structure of all of the components was elucidated by a combination of NMR spectroscopic techniques, chemical degradation, and FAB mass spectrometry.[101,102] Amino acid analyses of amythiamicin A ($C_{50}H_{51}N_{15}O_8S_6$), B ($C_{50}H_{53}N_{15}O_9S_6$), and C ($C_{50}H_{50}N_{14}O_9S_6$) found them to contain 1 mol of glycine and 1 mol extra of both L-proline and L-serine, determined by chiral HPLC, with respect to amythiamicin D ($C_{43}H_{42}N_{12}O_7S_6$) (Figure 21). As the composition of the macrocyclic loop was determined to be the same for all of these thiopeptides, differences in amino acid analyses were attributed to variations in the peptide side chain. Although the absolute configuration of the two valine-derived and one aspartate-derived thiazole residues in the amythiamicin macrocycle could not be determined from the isolated natural product, due to racemization under acid-catalyzed hydrolysis conditions, the chemical synthesis of amythiamicin D by Moody et al. has since verified the structure and absolute stereochemistry of this thiopeptide family, substantiated by X-ray crystallographic data, confirming the L-stereochemistry of all constituent amino acids.[103] Interest-

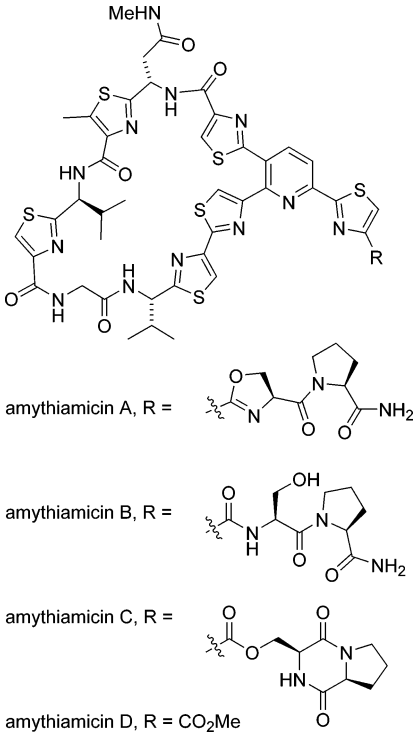


Figure 21. Structure of amythiamicin A−D.

ingly the amythiamicins are one of the few thiopeptides in this class that do not contain any dehydroalanine residues (along with GE2270 and the thiocillins) and show an unusual mode of action for the inhibition of bacterial protein synthesis, in common with the GE2270 family, binding to protein elongation factor Tu (Ef-Tu).

Cyclothiazomycin ($C_{59}H_{64}N_{18}O_{14}S_7$) is an unusual series *d* thiazolylpeptide that possesses a number of unique structural features. Isolated from the fermentation broth of *Streptomyces* sp. NR0516 from a soil sample collected at Kanagawa, Japan, and purified first by column chromatography and then by preparative HPLC,[104] initial structure determination, using high-resolution FAB mass spectrometry, elemental analysis, and ^{13}C and ^{1}H NMR spectroscopic data, was supported by chemical degradation studies, acidic hydrolysis generating an unusual pyridine-containing γ-amino acid as lactam **12**, the identity of which has been verified by synthesis,[105] and saramycetic acid I (**13**).[106] NOESY experimental data elucidated both the structure and stereochemistry of cyclothiazomycin, the latter supported by amino acid analyses, and showed this unique series *d* thiazolylpeptide lacked the characteristic 2- and 3-azole substituents on the central domain, containing instead an alanine-derived heterocyclic residue of (*R*)-configuration, quaternary sulfide, and two macrocyclic peptide loops (Figure 22).[107] Although no antibacterial data has been associated with cyclothiazomycin, which also lacks the characteristic polydehydroalanine side chain implicated in *tipA* promoter activity, this thiopeptide is still very worthy of note as a novel and selective inhibitor of human plasma renin with an IC_{50} of 1.7 μM.[104]

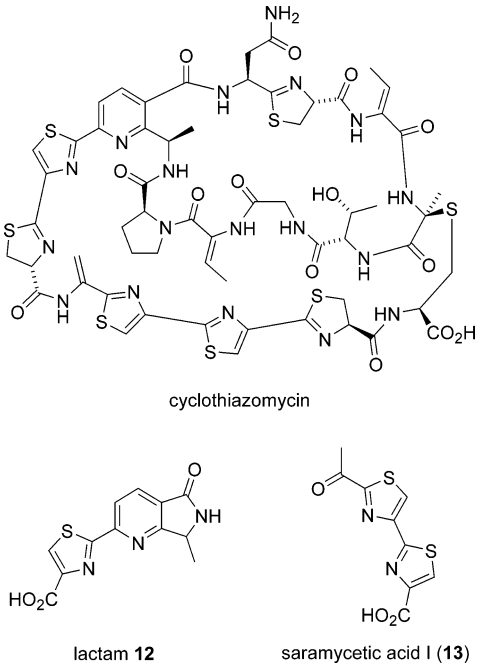


Figure 22. Cyclothiazomycin and its hydrolysates.

2.4. Hydroxypyridine Thiopeptides

The series *e* thiopeptides all possess very closely related structures, characterized by a 2,3,5,6-tetrasubstituted pyridine central heterocyclic domain containing a 5-alkoxy or 5-hydroxy substituent. The peptide backbone is divided into at least two macrocyclic loops and contains an indole or 1-hydroxyindole, connected in some cases by an *S*-thioester linkage, as well as a glycosidic unit attached through the anomeric position to a γ-amino acid residue, or glutamate derivative, in the macrocycle. This series consists of at least five thiopeptide families, although considerable structural homology or near identity exists between them, and contains over 12 structurally distinct compounds.

Nosiheptide (also known as RP9671, $C_{51}H_{43}N_{13}O_{12}S_6$) is one of the oldest known thiopeptide antibiotics isolated from *Streptomyces actuosus* 40037 (NRRL 2954).[108] Its general formula and its structural relationship to thiostrepton[109] was first suggested by combustion analyses and NMR spectroscopic experiments[110,111] and improved subsequently by modification of the HSQC and HMBC pulse sequences[112] and chemical hydrolysis, which isolated and analyzed a number of key fragments,[113] although it was X-ray crystallography that finally elucidated the structure[114] and stereochemistry.[115] Multhiomycin, isolated from *Streptomyces antibioticus* 8446-CC_1,[116] has been shown by ^{13}C NMR and IR spectroscopy as well as thin-layer chromatography to be structurally identical with nosiheptide,[117] with a characteristic thioester linkage at the macrocyclic bridgehead, 3-methylindole unit, hydroxyglutamate residue, and dehydroalanine side chain (Figure 23). Nosiheptide has been used as a feed additive to promote growth in pigs and poultry[118] and can be monitored in meat and egg samples by liquid chromatography with fluorescence detection.[119] This antibiotic is very active in vitro against Gram-positive bacteria (MIC 0.9 ng/

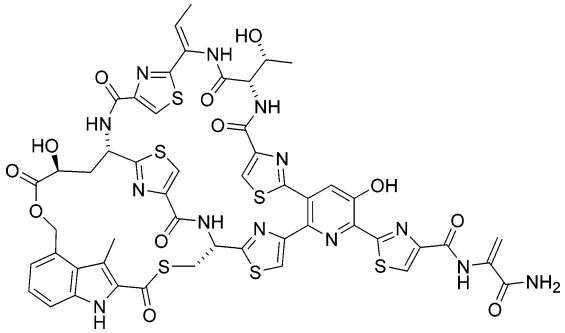


Figure 23. Structure of nosiheptide.

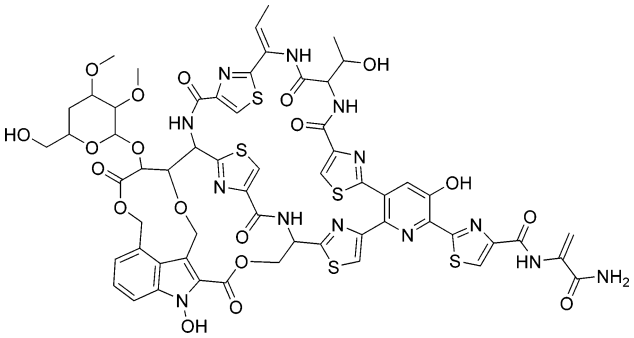
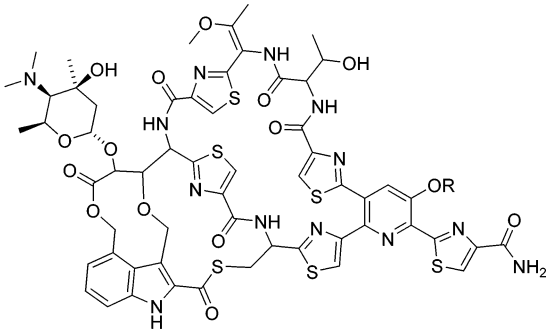


Figure 24. Structure of S 54832 A−I.

mL against *S. aureus* ATCC 6538 P) but inactive in vivo in experimentally infected mice[108] and selectively inhibits protein synthesis in whole cells of *Bacillus subtilis* and in *E. coli* lamelloplast by binding directly to the ribosomes.[120]

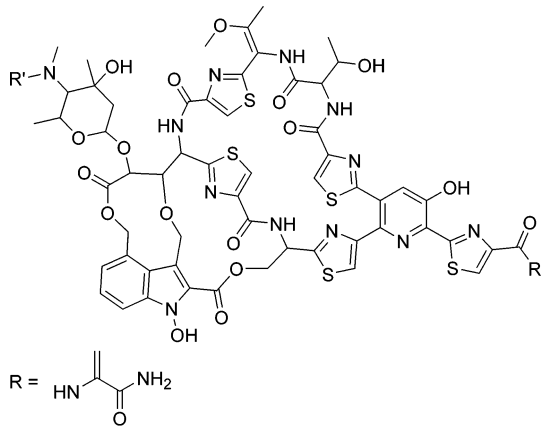
Shortly thereafter the antibiotics S 54832 A-I ($C_{59}H_{55}N_{13}O_{19}S_5$), A-II, A-III, and A-IV ($C_{59}H_{57}N_{13}O_{19}S_5$) were isolated from a strain of Micromonosporaceae in a Spanish soil sample, *Micromonospora globosa*, and shown to be structurally distinct from nosiheptide on the basis of chromatographic evidence, amino acid analyses, and UV and IR spectroscopic data.[121] Although the data for S 54832 A-II and A-III proved inconclusive, a structure for S 54832 A-I has been proposed (Figure 24) which closely resembles that of another recently discovered thiopeptide family, the nocathiacins, varying only in the composition of the glycosidic residue and one dehydrothreonine amino acid in place of a methoxydehydrothreonine in the peptide macrocycle. All of the members of the S 54832 family exhibit a growth-inhibiting effect toward Gram-positive bacteria, including Staphylococci, Streptococci, Corynebacteria, and Mycobacteria in vitro and against *S. pyogenes* and *pneumoniae* and *S. aureus* in vivo.

The taxonomy, fermentation, and biological evaluation of the series *e* thiopeptide glycothiohexide α, consisting of two structurally distinct components LL-14E605*β* and *O*-methyl-LL-14E605*β* (Figure 25) isolated from the fermentation broth of *Sebekia benihana* (NRRL 21083),[122] have been described.[123] The chemical structure of glycothiohexide α ($C_{58}H_{57}N_{13}O_{15}S_6$) was determined by extensive 2D NMR studies as well as high-resolution FAB mass spectrometry and IR spectroscopy. Closely related to the structure of nosiheptide, glycothiohexide α in contrast possesses a methoxydehydrothreonine unit, glycosidic aminodideoxypyranose moiety, carbinol methylene-substituted indole, and modified glutamate residue with additional 3-hydroxylation but lacks the dehydroalanine side chain often characteristic of the thiopeptide antibiotics.[124]



glycothiohexide α: R = H;
O-methyl-glycothiohexide α: R = Me

Figure 25. Structure of glycothiohexide α.



MJ347-81F4 A (nocathiacin I): R' = Me;
MJ347-81F4 B: R' = H,

Figure 26. Structure of MJ347-81F4 thiopeptides.

Amycolatopsis sp. MJ347-81F4, isolated from soil collected in Japan, produces two cyclic thiazolyl peptide antibiotics, components A and B, the former of which, as the major component, shows in vitro activity against Gram-positive bacteria including MRSA and *Enterococcus faecalis* with MICs typically in the range from 0.006 to 0.1 *μ*g/mL.[125] Furthermore, component A showed poor antibacterial activity against a thiostrepton-resistant mutant (L11) of *B. subtilis* B-558 but was active against an amythiamicin-resistant mutant (V228A) of the same strain, with a MIC of >100 and <0.19 *μ*g/mL, respectively, indicating that the molecular target of this antibiotic may well be the bacterial 50S ribosomal subunit. The structures of both components A ($C_{61}H_{60}N_{14}O_{18}S_5$) and B ($C_{60}H_{58}N_{14}O_{18}S_5$) were elucidated by chemical degradation and spectroscopic analyses and are reported to differ from glycothiohexide α by the presence of a dehydroalanine side chain and an ester linkage instead of an *S*-thioester as a result of replacing the modified cysteine residue at the macrocyclic bridgehead with a serine (Figure 26).

The nocathiacins are cyclic thiazolyl peptide antibiotics isolated by fermentation of ATCC-202099 of the genus *Nocardia* or the fungus *Amicolaptosis* sp. Nocathiacin I ($C_{61}H_{60}N_{14}O_{18}S_5$) is structurally identical to MJ347-81F4 component A (Figure 27) and

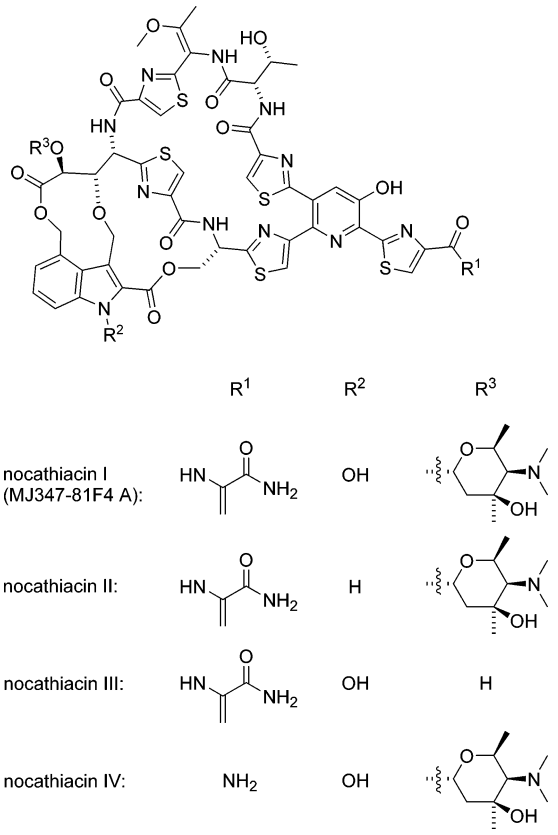


Figure 27. Structure of nocathiacins I–IV.

displays potent activity in vitro and in vivo against Gram-positive bacteria, including a number of antibiotic-resistant strains, by interacting directly with the L11 protein and 23S RNA complex of the bacterial ribosome.[126] In addition, nocathiacin I has been reported to be more soluble at low pH than other thiopeptide antibiotics. Although the structure and stereochemistry of the nocathiacins could not be determined by X-ray crystallography, extensive 2D NMR studies have been carried out, including NOESY, HMBC and HMQC experiments on both ^{13}C- and ^{15}N-labeled and unlabeled samples in combination with metal chelate chiral capillary electrophoresis. These studies established that nocathiacin I (and hence MJ347-81F4 A) contains L-threonine and determined the conformation of the antibiotic in solution in order to computationally model the interaction between thiazolylpeptides and the bacterial ribosome. The structures of three other factors, nocathiacin II ($C_{61}H_{60}N_{14}O_{17}S_5$), III ($C_{52}H_{43}N_{13}O_{16}S_5$), and IV ($C_{58}H_{57}N_{13}O_{17}S_5$), have been reported (Figure 27), the latter of which can be derived chemically from nocathiacin I by dehydroalanine cleavage under mild conditions using iodomethane and hydroiodic acid in THF at 45 °C.[127] Nocathiacin I and its structural surrogate nocathiacin IV have been used as leads for the development of a parenterally administered broad-spectrum antibiotic through chemical modification of the natural material.[128] The direct incorporation of 2-hydroxy- or 2-(dialkylamino)ethyl groups in the amide side chain (R^1) of synthetic analogues by condensing nocathiacin IV with glycolaldehyde followed by reduction of the Amadori-rearranged intermediate improved the water solubility profile of the nocathiacins while retaining good antibacterial activity.[129]

2.5. Unidentified Thiopeptides

Despite the considerable structural information that is known about many thiopeptide antibiotics, the notorious difficulty in obtaining X-ray crystallographic data on individual factors has meant that, above and beyond the minor structural and stereochemical ambiguities and connectivity discrepancies, there are a number of metabolites that have been isolated that would appear to belong to the thiazolyl peptide class but have not been fully characterized. Many of these 'unidentified' thiopeptide antibiotics have only been subjected to preliminary analyses using techniques such as IR and UV spectroscopy, mass spectrometry, and combustion and have not been examined in NMR spectroscopic experiments or by X-ray crystallography. The biological properties of these compounds suggest that they belong to the thiopeptide class, but the lack of sufficient experimental data means that even a tentative proposal of structure is not possible. These compounds were understood to be structurally distinct from other known thiopeptide antibiotics, although with the rapid expansion of isolated and identified metabolites in recent years it cannot be known for certain that named compounds, isolated in early studies, represent structurally distinct factors or new thiopeptide families without the benefit of further experimentation that in view of the difficulty in obtaining the original organism seems unlikely to be carried out.

The kimorexins (90-GT-302), isolated from *Kitasatosporia kimorexae*,[130,131] pepthiomycin A and B isolated from the culture broth of *Streptomyces roseospinus*,[132] the antifungal antibiotic saramycetin (also referred to as X-5079C or Sch 43057)[133,134] active against systemic mycoses,[135] and sporangiomycin, isolated from a soil sample collected in Argentina (strain B987) containing *Planomonospora parontospora* var. antibiotica,[136] all fall into the category of structurally ambiguous or unidentified thiopeptide antibiotics isolated from actinomycetes. Although the classification of these compounds as thiazolyl peptides was made on the basis of good experimental evidence, in particular their high sulfur content as indicated by combustion and mass spectrometric analyses, no structures have been proposed for any of these metabolites. The ^1H NMR spectrum of saramycetin indicated that this metabolite contains dehydroalanine residues and a number of thiazoline heterocycles, and chemical degradation studies suggest a considerable degree of structural identity with cyclothiazomycin. Biological data would indicate that some of these unidentified antibiotics belong to the series *a* or *b* thiopeptide class, with similar spectra of activity for the inhibition of bacterial protein synthesis being notably inactive against thiostrepton-resistant strains. However, in the absence of 2D NMR studies or X-ray crystallographic data, the structural classification of many of these compounds will remain largely unsupported.

The structurally ambiguous metabolite neoberninamycin, produced by *M. luteus*,[62] is active against

Gram-positive and Gram-negative anaerobes, with a spectrum of activity that is similar to the berninamycins. Analytical data, from mass spectrometry and ^{1}H NMR studies, suggested that this antibiotic shares a considerable degree of structural homology with the berninamycins but also demonstrated that this thiopeptide is distinct from the known members of the berninamycin family (neoberninamycin M_r 1131; berninamycin M_r 1146).

Multhiomycin was first isolated in 1970 from *S. antibioticus* 8444-CC$_1$ and found to have a M_r of 1043 by isothermal distillation. Its empirical formula, $C_{44}H_{45}N_{11}O_{11}S_5$, was confirmed by elemental analysis and clearly indicated its thiopeptide nature.[116] The mode of action of multhiomycin is similar to other thiopeptide antibiotics, blocking bacterial protein synthesis by inhibiting the transfer of amino acyl-tRNA to the A site of the bacterial 50S ribosomal subunit. Later in 1977 the same group claimed that multhiomycin and nosiheptide were identical. Degradation of multhiomycin provided 1 mol each of dehydroalanine, cysteine, and threonine, thiazole heterocycles, and a weakly acidic fragment. The empirical formula of multhiomycin was later reported as $C_{51}H_{43}N_{13}O_{12}S_6$, without any comment on its discrepancy with earlier reports.[117] On the basis of the available evidence, multhiomycin and nosiheptide would appear to be identical, although no known thiopeptide fits the original description of multhiomycin, indicating that the material first isolated in 1970 may yet prove to be a unique metabolite of the thiopeptide class.

3. Biosynthesis

Antibiotic-producing organisms can adopt a number or combination of different strategies to defend themselves against extracellular drugs and thus avoid self-intoxication, including modification of the drug binding site, drug inactivation/sequestration, or establishing membrane permeability barriers, with an efficient efflux and exclusion mechanism.[2,3] In actinomycetes resistance determinants are commonly linked to antibiotic production genes, with coregulation involving divergent promoters, overlapping transcripts, and/or polycistronic transcripts. The regulation of antibiotic production can be linked to the regulation of resistance, the downregulation of an enzyme-modifying gene product achieved by the appropriate use of a weak promoter. However, for many of the thiopeptide producers it is not clear how genes encoding resistance and antibiotic biosynthesis enzymes came to congregate in the same cell. A detailed understanding of the biosynthesis of these antibiotics and its genetic origin could help uncover actinomycetes resistance determinants and allow the prediction of novel resistance mechanisms prior to their emergence.

The biosynthesis of a number of thiopeptides has been investigated by following the incorporation of isotopically labeled amino acids in order to determine the origin of many of the unusual heterocyclic structural motifs inherent in these antibiotics. Replacing some of the atoms of amino acid precursors with ^{13}C, ^{14}C, deuterium, or tritium and examining the incorporation of these labels in the metabolite has been used not only to indicate the biosynthetic pathways operating in the producing organism but also to validate stereochemical hypotheses, confirm structural identity, and suggest biomimetic routes for their chemical synthesis, the latter of which has been put to good use in separate studies by Nicolaou[137] and Moody[138] for the laboratory synthesis of the central heterocyclic domain of series *a*/*b* and *d* thiopeptide targets, respectively. The various components all originate from amino acids heavily modified by the organism to elaborate the complex heterocyclic structural arrays, although notable biosynthetic differences have been found in different bacterial strains. Distinct structural similarities exist between the thiopeptide antibiotics and a number of other oxazoline and thiazoline peptide natural products for which the nature and function of the molecular machinery responsible for the biosynthesis of heterocyclic components from amino acid precursors is well described,[139] and so one might expect that the biogenesis of cyclic thiazolylpeptides is well understood also. Indeed, as a result of a number of key biosynthetic studies, much is known about the origin of many of the components, but the mechanisms of these multistep processes and the mode of assembly of the various modified components, poorly characterized in the past, remain salient points for discussion that are only now being unraveled.

The biosynthesis of thiostrepton was investigated by administering isotopically labeled precursors,[140] including cysteine, serine, isoleucine, threonine, methionine, and tryptophan, to cultures of *S. azureus* ATCC 14921 or *S. laurentii* ATCC 31255, the latter of which gave better antibiotic yields. The amino acid origin of all components was demonstrated, the threonine and butyrine residues were both formed from threonine, whereas isoleucine was the precursor to thiostreptine, a dihydroxylated derivative further elaborated at the *C*-terminus to a thiazole heterocycle. Experiments with (*S*)-[1,2-$^{13}C_2$]- and (*S*)-[2,3-$^{13}C_2$]serine demonstrated the incorporation of this amino acid into thiazole, thiazoline, alanine, and dehydroalanine residues, the origin of the *Z*-hydrogens in the latter being the *pro-R* β-hydrogens of serine according to feeding experiments with (2*S*,3*S*)-[3-^{13}C,^{2}H$_1$]serine (Figure 28). The 2,3,4,5-carbons of the tetrahydropyridine core also originate from serine in a tail-to-tail condensation that probably proceeds via the corresponding dehydroalanine moieties, suprafacial addition to the terminal dehydroalanine being followed by anti addition of two hydrogens, one to the *Si* face at C3 and the second to the *Re* face at C6, which is provided by an adjacent cysteine (Scheme 1).

The quinaldic acid residue was shown to originate from tryptophan and methionine.[140] The first step in this sequence was shown to be the formation of 2-methyltryptophan from tryptophan and (*S*)-adenosylmethionine (AdoMet)[141] by a methyl transfer that proceeds surprisingly with retention.[142] Ring expansion by cleavage of the N1/C2 bond and cyclization onto the tryptophan α-position was confirmed by labeling studies with (*S*)-[1′,2′-^{13}C,*indole*-^{15}N]tryp-

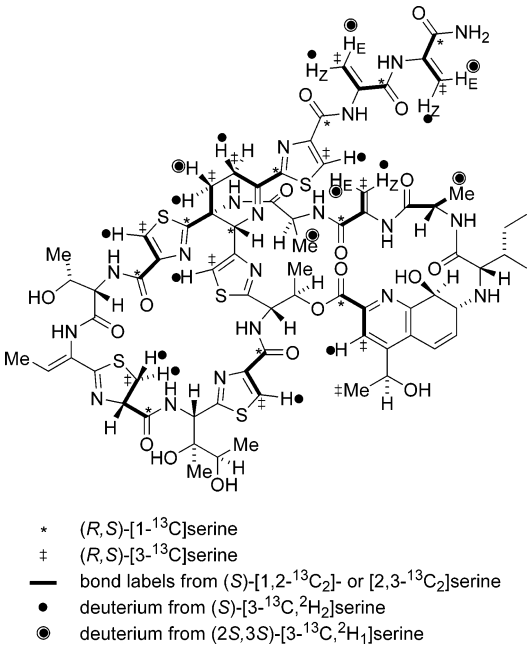
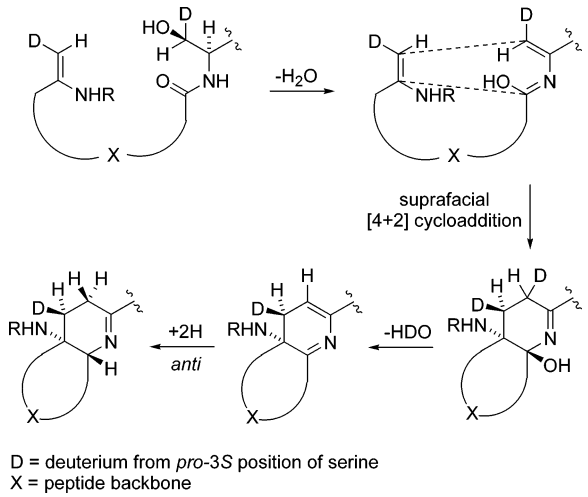


Figure 28. Serine labeling studies on thiostrepton.

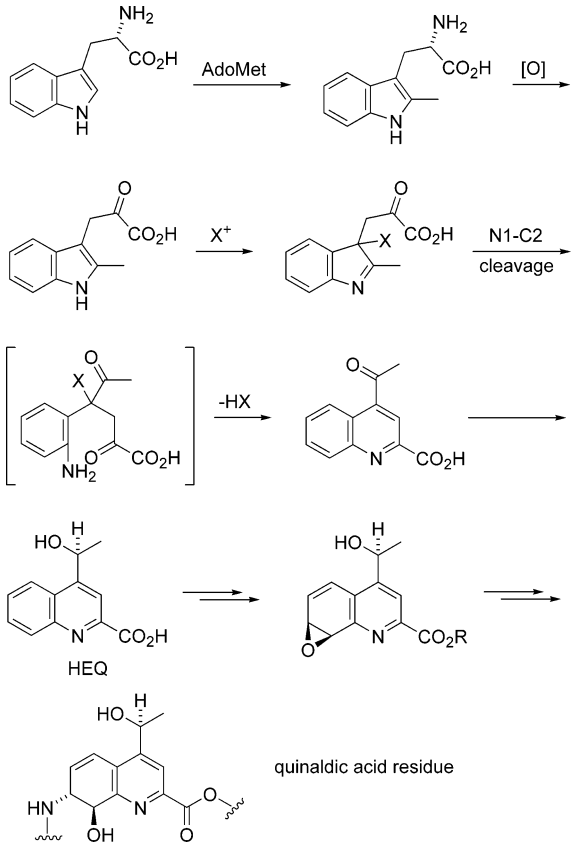
Scheme 1. Biosynthesis of the Dehydropiperidine Domain of Thiostrepton



tophan that generated thiostrepton with ^{13}C enrichment in the quinaldic acid carboxyl group (Scheme 2).[140a] The intermediacy of 4-(1-hydroxyethyl)quinaldic acid (HEQ) was demonstrated by the albeit low incorporation of tritiated HEQ, adding further support for a ring-expansion mechanism in the biosynthesis of the quinaldic acid residue.[140]

Consideration of this evidence indicates that thiostrepton is generated by the modification of a linear peptide, containing at least one residue, (*S*)-cysteine, and possibly more of unnatural configuration, presumably generated in a postsynthetic modification. In these biosynthetic operations the peptide chain must fold back upon itself to facilitate the cycloaddition that generates the series *b* domain and establishes the large macrocycle. The amide nitrogen of the side chain probably arises from an additional carboxy-terminal serine removed in an oxidative process, although this has not been shown experimentally. Subsequent attachment of the quinaldic

Scheme 2. Biosynthesis of the Quinaldic Acid Residue of Thiostrepton

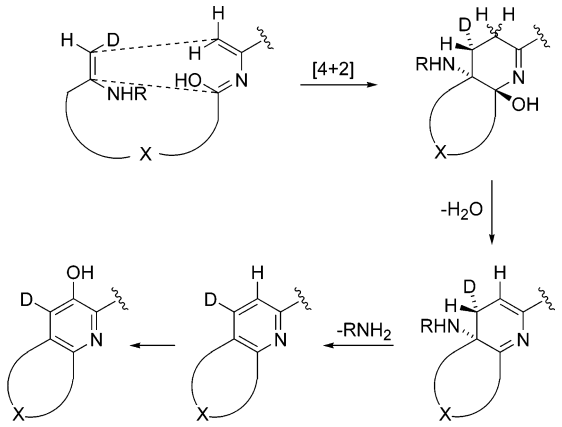


acid[140c] and epoxide ring opening with the *N*-terminus would establish the second macrocycle and complete the skeletal assembly of the antibiotic.

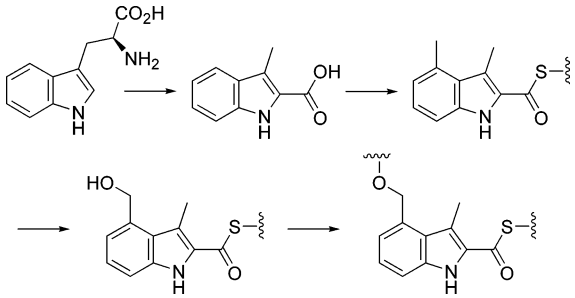
One of the first thiopeptides to be studied biosynthetically was nosiheptide.[143] In a similar fashion, by feeding radioactive and stable-isotope-labeled amino acid precursors to cultures of the producing organism, the origin of many of its unusual components was verified. Dehydroamino acid residues are formed by the anti elimination of water from either serine or threonine, thiazole heterocycles are produced from cysteine with loss of the *pro*-3*R* hydrogen in the oxidation step, and the terminal amide nitrogen in the side chain is derived from an additional serine residue, removed except for its nitrogen during processing. The central hydroxypyridine domain is produced by the tail-to-tail condensation of two serine residues, situated nine amino acids apart in the peptide chain, and incorporates the carboxyl group of an adjacent cysteine in an overall process that formally can be represented as a cycloaddition, a mechanism which was proposed originally by Bycroft and Gowland.[42] Related to the corresponding biosynthesis of the dehydropiperidine domain of thiostrepton (Scheme 1), loss of water from the vinylogous carbinolamine, aromatization by elimination of ammonia or some additional amino-terminal residue, and subsequent hydroxylation would complete the biosynthesis of the central domain (Scheme 3).

The indolic acid moiety is derived from tryptophan, although the mechanism of its production remains unclear. This residue is apparently attached to the peptide backbone at a late stage in the biosynthesis

Scheme 3. Biosynthesis of the Hydroxypyridine Domain of Nosiheptide



Scheme 4. Biosynthesis of Indolic Acid Moiety of Nosiheptide



and subsequently hydroxylated on the 4-methyl substituent to complete the macrocyclic lactone, although whether the attachment of the indolecarboxylic acid occurs before or after 4-methylation is not apparent. Evidence for the order of events comes from feeding experiments, which show the efficient incorporation of 3-methylindole-2-carboxylic acid and 3,4-dimethylindole-3-carboxylic acid but not 4-(hydroxymethyl)-3-methylindole-3-carboxylic acid (Scheme 4).[140c,143]

The biosynthetic origin of A10255G, -B, and -E has been investigated in *S. gardneri* and confirms the amino acid origin of all of the components.[72] The incorporation of (*R,S*)-[1-^{13}C]serine and [2-^{13}C]glycine was found in 15 of the 17 amino acid residues, suggesting the conversion of glycine to [2,3-^{13}C]serine. Biosynthetic studies on berninamycin A, using first ^{14}C-labeled[144] and then ^{13}C-enriched amino acids,[60] confirmed many of these findings, such as indicating that dehydroalanine residues are formed by the dehydration of serine, oxazoles are generated by the cyclodehydration of serine- or threonine-containing dipeptide units, and the thiazole is derived by the cyclodehydration of a cysteine residue onto the carboxyl group of a neighboring serine. The origin of the central heterocyclic domain was confirmed in both of these studies as a tail-to-tail condensation of two serine residues, feeding experiments with (*R,S*)-[3-^{13}C]- and (*R,S*)-[1-^{13}C]serine in the biosynthesis of berninamycin providing additional support for the original Bycroft−Gowland proposal of micrococcin domain biogenesis.[42] The isolation of minor metabolites containing dehydroalanine side chains of varying length provide some indication as to the origin of the terminal carboxamide nitrogen in berninamycin, suggesting that the cleavage of a serine or dehydroalanine unit is responsible for the biosynthesis of this group in many of the thiopeptides. Incorporation of (*S*)-[1-^{13}C]valine and the observation that berninamycin with no significant radioactivity is produced by the growth medium of *S. bernensis* administered with (*R,S*)-[3-^{14}C]-β-hydroxyvaline verifies that the hydroxyvaline residue in the macrocyclic backbone originates from valine and is hydroxylated after the peptide is assembled.

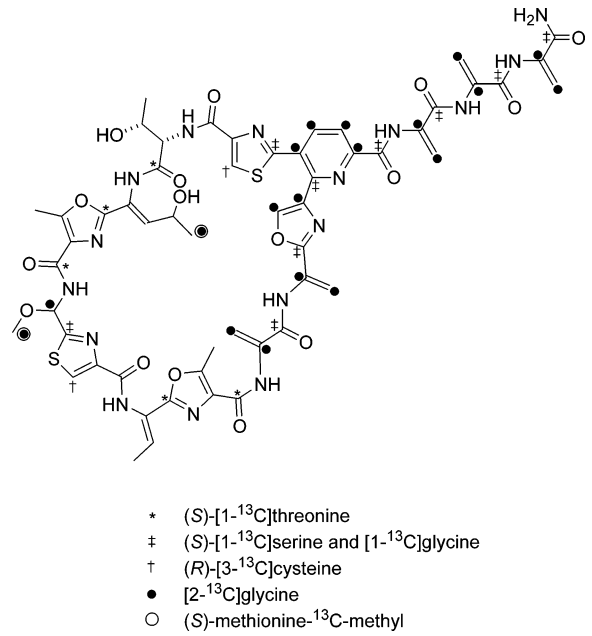


Figure 29. ^{13}C incorporation into sulfomycin I using isotopically labeled precursors.

Isotopic labeling studies on the biogenesis of sulfomycin I (U-102408) by *Streptomyces arginensis* demonstrated a number of unusual features not observed in the biosynthesis of other thiopeptides.[145] In accordance with related studies, fortifying the fermentation medium of *S. arginensis* with ^{13}C- or ^{2}H-labeled threonine, serine, glycine, or methionine indicates that thiazoles and oxazoles are derived by the cyclocondensation of the corresponding amino acid with an adjacent carbonyl group and that dehydroalanine residues originate from the dehydration of serine (Figure 29). Additionally, [2-^{13}C]glycine was found to be incorporated into both the 2- and 3-positions of dehydroalanine residues, indicating that glycine is used as a precursor for the biosynthesis of serine, mediated by serine hydroxymethyltransferase with a tetrahydrofolate cofactor. On this basis [2-^{13}C]glycine incorporation was observed at four of the positions in the pyridine motif, supporting a tail-to-tail condensation route to the central domain of these antibiotics, although glycine-serine interconversion might also have been responsible for the lack of incorporation of [3-^{2}H]- or [3-^{3}H]serine into sulfomycin I. Unusually, in a number of experiments, threonine was incorporated into sites labeled by serine, although the converse incorporation was not observed. The 2-amino-4-hydroxy-2-pentenoic acid

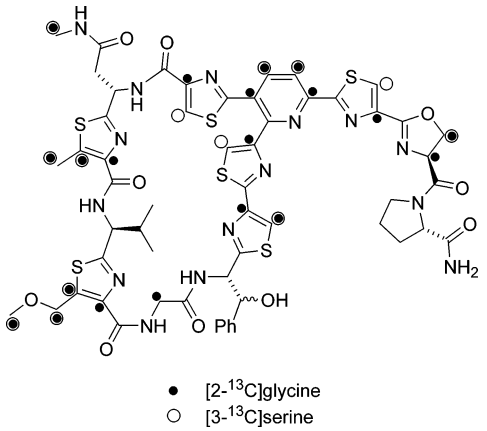


Figure 30. ^{13}C incorporation into GE2270A using isotopically labeled glycine or serine.

and methoxyglycine residues were found to originate from threonine and glycine, respectively, by the appendage of an additional one-carbon unit with incorporation from [2-^{13}C]glycine or methionine-*methyl*-^{13}C in both cases.

Further evidence that the methylene group of glycine is an effective source of methylating equivalents in other actinomycete strains has been obtained in a biosynthetic study of GE2270A by *P. rosea*.[146] Incorporation of [2-^{13}C]glycine, with enrichment at C-2 and C-3 of serine-derived residues, at C-4 and C-5 of the oxazoline, as well as C-2 through C-5 of the pyridine and to *C*-, *N*-, and *O*-methyl groups (Figure 30), supports the efficient conversion of glycine into serine and the use of a cellular methyl donor by the organism. Furthermore, it was noted that the two 5-substituted thiazole residues were derived from cysteine with subsequent methylation and not by incorporation of an *S*-analogue of threonine. Addition of [2-^{13}C]acetate did show enrichment at all of the positions of the asparagine residue, consistent with its conversion into oxaloacetate and transamination to the amino acid, but also demonstrated the poor utilization of this carbon source under these experimental conditions.

The manner that these antibiotics are assembled in the organism has been a matter of some conjecture, although since broadly speaking they all share a similar architecture it could be anticipated that the biosynthetic machinery responsible for biogenesis in different strains has many similarities, and thus the blueprint which specifies the amino acid sequence and requisite postsynthetic modifications should be highly conserved. The construction of the peptide from its amino acid precursors may occur either by a ribosomal process or by a template-directed nonribosomal enzymatic process, the latter of which would seem the most likely.[140a] Either the peptide synthetase generates the requisite linear peptide which is subsequently modified by individual separate enzymes or the whole operation proceeds upon one or several multienzyme complexes that modify the individual amino acid components as the peptide chain or fragments thereof are assembled, both of which have been shown to operate in the biogenesis of other oxazole or thiazole peptide natural products.[139] The number and organization of iterated modules would dictate the size and structural composition of the final antibiotic, each module activating a certain amino acid in closely coupled domains in nonribosomal peptide synthetase (NRPS) multimodular templates. A putative NRPS gene fragment that probably encodes a module of the micrococcin P_1 synthetase complex has been identified in the producing strain *S. equorum* WS2733, representing an adenylation (A) domain for generation of the corresponding amino acyl adenylate organized into a 'condensation−adenylation−thiolation−condensation' module that was selective for threonine.[37b] This finding supports the hypothesis that the biosynthesis of the thiopeptide antibiotics occurs nonribosomally and may provide the basis for the characterization of thiopeptide gene clusters and the future manipulation of NRPS templates for the targeted engineering of new antibiotics.

4. Biological Properties

To interpret the biological relevance of an antibiotic purely in terms of its ability to inhibit the growth of competing organisms would constitute a considerable oversight. The biological challenge is only initiated by the discovery of a new antibacterial agent, although the importance of many targeted screening programs developed to identify novel metabolites with specific binding properties should not be understated. However, in order to develop agents of clinical importance, an in depth understanding on the mode of action of microbial products must be gained, starting with identification of the biological target, followed by the site and nature of binding in order to establish which essential cellular function is being inhibited, a challenging problem in itself in the past with ribosomal inhibitors, and most importantly a determination of how the target organism can counter the designed purpose of an antibiotic and so become resistant to its action. Clues to processes employed by emerging resistant bacterial strains and indeed insights into mechanisms that may develop in the future can be gained by studying the survival strategies adopted by the antibiotic-producing organisms themselves to avoid self-intoxication for it may well be the case that these organisms are the source of some resistance determinants, a hypothesis with far-reaching consequences for the characterization of novel resistance mechanisms prior to their clinical emergence and in the rational design of advantageous agents.

The mode of action of an antibiotic involves its interaction with a specific receptor either within the cell or associated with the cell surface. By modifying these antibiotic target sites, the organism can weaken or even prevent drug−receptor interactions and achieve very high levels of resistance. Recent experimentation has led to a number of discoveries on the origin of the biological properties of antibiotics of the thiopeptide class and has increased our understanding of the organisms that produce them considerably. With advancements in our structural knowledge of the bacterial ribosome[147] and new insights into its function,[148] along with the ready availability of many bacterial genomes[149] and evermore sophisticated

computational methods, strategies for the modification of known antibiotics, development of existing or novel antibacterial targets, or discovery of new classes of agent by structure-based drug design have never been so well developed.[150]

The thiopeptide antibiotics largely inhibit the growth of Gram-positive bacteria, although the activity of some of these metabolites as antifungal or anticancer agents, against Gram-negative bacteria, as renin inhibitors, or against *Plasmodium falciparum*, the malaria parasite, has also been reported. Despite considerable structural homology the site and mode of action for these antibiotics actually varies in different thiopeptide families and can be categorized, broadly, into two classes: those that bind to a region of the 23S ribosomal RNA (rRNA) known as the L11 binding domain (L11BD) and those that bind to a protein (Ef-Tu) complex involved in the elongation cycle.

The antibacterial activity of the thiopeptide antibiotics in vitro is comparable to that of the penicillins, with little or no adverse toxicological effects in mammalian cells, disrupting protein synthesis in the bacterial cell's protein factory, the ribosome. Prokaryotic and eukaryotic ribosomes interpret the information in messenger RNA (mRNA) and use it to assemble the corresponding sequence of amino acids in a protein.[151] Although bacterial and mammalian ribosomes do exhibit many structural similarities, they differ considerably in size, the latter being about 30% larger and containing so-called expansion sequences in the rRNA as well as a number of additional ribosomal proteins. The job of the ribosome is translation, that is to read each codon of the mRNA in turn and match it with the anticodon of the corresponding transfer RNA (tRNA) bound amino acid, assembled by the respective synthetase, and thus build up the protein, residue by residue, that it encodes. All ribosomes are composed of two subunits of unequal size: the bacterial ribosome, with a relative sedimentary rate of 70S, consisting of a large 50S and a small 30S subunit. These two subunits are associated through noncovalent interactions and organize to give a ribonucleoprotein particle 2.6−2.8 MDa in size, with a diameter of 200−250 Å, that functions as a platform for bacterial protein synthesis. In the eubacteria *E. coli* each subunit consists of proteins and rRNA fragments: the small 30S subunit containing 21 proteins (S1−S21) and a 16S rRNA strand, whereas the 50S subunit comprises 34 proteins (L1-L34) and two strands of rRNA, the 23S and 5S (Figure 31).

The sites on the ribosome involved in the sequential construction of the nascent protein from the individual amino acid components are denoted as the A site, where aminoacyl-tRNA (aa-tRNA) containing the next amino acid residue docks on instruction from the codon of its corresponding mRNA, the P site, where the growing peptide chain waits in readiness to form the next peptide bond, and the E site, which receives the tRNA for its exit at the end of the sequence (Scheme 5). Once bacterial protein synthesis has been initiated by interaction of the 3′ end of the 16S rRNA in the 30S subunit with a complementary sequence on mRNA,[148] the initiator tRNA binds directly to the P site (Scheme 5a) and each aa-tRNA in accordance with its corresponding codon is delivered to the A site as a ternary complex (Scheme 5b) formed by combination with the elongation factor Tu (Ef-Tu) and GTP. GTP hydrolysis causes a conformational change in the ternary complex that releases Ef-Tu·GDP from the ribosome to be recycled back to Ef-Tu·GTP, leaving the aa-tRNA bound in the A site (Scheme 5c). The next peptide bond is then formed on the large ribosomal subunit by the transfer of the peptide to the A site, generating a peptidyl-tRNA while leaving behind its own tRNA in the P site (Scheme 5d). Translocation of the peptidyl-tRNA from the A site back to the P site is then mediated by a different elongation factor, Ef-G (Scheme 5e), which vacates the A site and moves the deacylated tRNA to the E site ready for exit (Scheme 5f). When the next aa-tRNA-containing ternary complex binds (Scheme 5g), the tRNA docked in the E site is released and the protein elongation cycle repeats itself (Scheme 5c) until protein termination factors liberate the finished peptide and dissociate the ribosome.

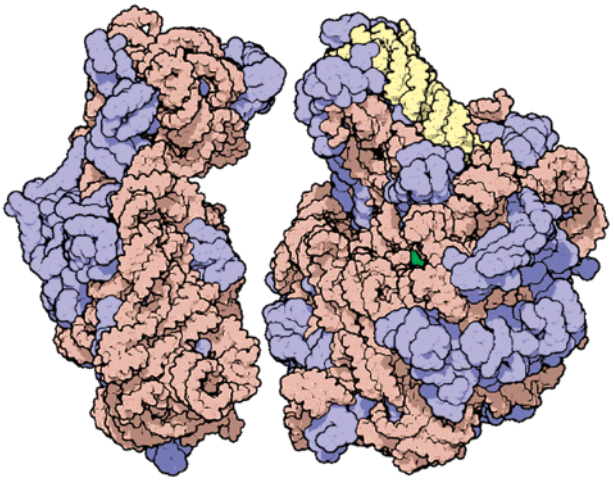


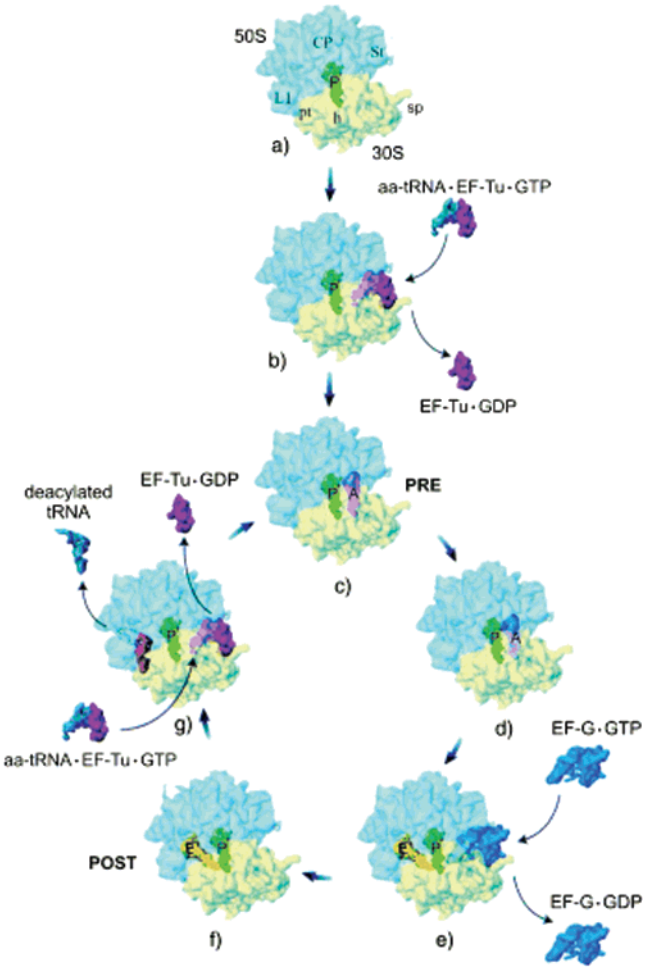
Figure 31. Composition of the 70S bacterial ribosome.[147,152] Color key: (left) small 30S subunit, proteins S1−S21 (blue) and 16S rRNA strand (pink); (right) large 50S subunit, proteins L1−L34 (blue), 23S rRNA (pink), and 5S rRNA (yellow). (Credit to David S. Goodsell of The Scripps Research Institute.)

4.1. Ribosomal Inhibitors

Seven different classes of antibiotics in clinical practice target the bacterial ribosome, including the aminoglycosides, tetracyclines, macrolides, and streptogramins. Ribosomal inhibitors that interact with rRNA may exhibit a favorable resistance profile in the clinic as most pathogens have multiple copies of the *rrn* operons that encode rRNA; thus, resistance-inducing mutations are rarely dominant.[150]

Many thiopeptide antibiotics interfere with bacterial protein synthesis on the ribosome, although the precise inhibitory mechanism operating for many of these agents has not been established. The most closely studied mode of action of all of the thiopeptides is that of thiostrepton, which has been applied in rational structure-based drug design in an attempt

Scheme 5. Overview of Protein Translation[a]



[a] The small 30S subunit is depicted in yellow, and the 50S subunit is depicted in blue. A, P, and E denote sites on the ribosome that can be occupied by tRNA. The A site is where aa-tRNA binds, the P site is where peptidyl-tRNA binds before peptide bond formation, and the E site is the exit site for deacylated tRNA. Translation cycle consists of (a) initiator tRNA binds in the P site; (b) aa-tRNA·Ef-Tu·GTP is delivered to the A site; (c) aa-tRNA is bound in the A site; (d) peptide is transferred to the A site with formation of next peptide bond; (e) translocation of peptidyl-tRNA from the A site back to the P site is mediated by Ef-G·GTP; (f) deacylated tRNA waits in the E site; (g) aa-tRNA·Ef-Tu·GTP is delivered to the A site, releasing the deacylated tRNA from the E site. (Reprinted with permission from ref 151. Copyright 2003 Wiley-VCH Verlag GmbH & Co. KgaA.)

to address problems with this antibiotic's low solubility and poor bioavailability.[153] In vivo thiostrepton inhibits the binding of the aminoacyl-tRNA-containing ternary complex to the ribosomal A site.[154] The energy for protein translation is provided by the action of the elongation factors Ef-Tu and Ef-G, these hydrolysis reactions taking place on the large ribosomal subunit at a GTPase center located on a double-hairpin structure within domain II of 23S rRNA,[155] where ribosomal protein L11 and the pentameric complex L10·$(L12)_4$ assemble cooperatively, and on a ribotoxin hairpin loop within domain VI of 23S rRNA.[156] The action of thiostrepton inhibits peptide elongation, probably by impeding a conformational change within protein L11, when bound in a region of the 23S rRNA known as the L11 binding domain (L11BD).[157] The RNA-binding domain of protein L11 recognizes an rRNA tertiary structure that is stabilized by thiostrepton,[158] the antibiotic preventing one or more conformational transitions critical for stimulating the GTPase action of the elongation factors,[159] necessary to drive the directional movement of transfer and messenger RNA on the ribosome.[160]

Both thiostrepton and micrococcin inhibit the growth of the malaria parasite *P. falciparum*, inhibiting organellar protein synthesis by targeting the large subunit encoded by a 35-kb organelle, which is one of two extrachromosomal DNAs possessed by the parasite.[161] Thiostrepton has been shown to bind to malarial plastid rRNA[162] and has a 50% inhibitory concentration (IC_{50}) of 3.2 nM, whereas growth inhibition by micrococcin has an IC_{50} of 35 nM.[163]

Two types of resistance mechanisms have been observed to thiostrepton in Gram-positive bacteria, whereas Gram-negative organisms are completely resistant to its action, as thiostrepton cannot penetrate the bacterial cell. In Gram-positive organisms one resistance mechanism is defined by the absence of a protein homologous with L11 in *E. coli* and designated BM-L11[164] in *Bacillus megaterium* and BS-L11[165] in *B. subtilis*, giving rise to protein-deficient ribosomes with a reduced affinity for thiostrepton in vitro but that retain substantial protein synthetic activity. Mutants of *B. subtilis* were found to be resistant to both thiostrepton and sporangiomycin, further substantiating the hypothesis that these antibiotics inhibit protein synthesis by an identical mechanism and that the mutation alters the site on the 50S ribosomal subunit that is responsible for antibiotic binding.[166] In a similar fashion a mutant strain of *B. megaterium* possessed an altered form of protein BM-L11, causing the strain to be resistant to the action of micrococcin.[167] However, the tRNA uncoupled hydrolysis reaction catalyzed jointly by the ribosome and the protein factor Ef-G, which is inhibited by thiostrepton, is markedly stimulated by micrococcin upon organisms sensitive to this drug,[168] a disparity worthy of note in considering the seemingly common mode of action of thiopeptide ribosomal inhibitors. Both thiostrepton and micrococcin bind to the GTPase region in domain II of 23S rRNA, and in so doing they alter the accessibility of adenosine (A)-1067 and A1095 in the 23S rRNA toward chemical reagents, indicating that thiopeptide ribosomal inhibitors interact directly with these nucleotides.[169] These two drugs had different effects on the chemical reactivity of A1067 in a terminal loop of *E. coli* ribosomes in vitro: micrococcin enhanced its reactivity, whereas thiostrepton protected the N-1 position reducing reactivity, a difference that correlates with the opposite effects of the two antibiotics on GTPase activity.[170] This can be rationalized in a model for the tRNA uncoupled system where the dissociation of Ef-G·GDP from the ribosome is the rate-limiting step of Ef-G-dependent GTP hydrolysis.[171] Thiostrepton and micrococcin act by increasing the dissociation rates of both Ef-G·GTP and Ef-G·GDP, although micrococcin with lesser potency has a weaker dissociating effect on Ef-G·GTP than thiostrepton. In this model the action of micrococcin

would increase the turnover of Ef-G, increasing the rate of GTP hydrolysis, whereas Ef-G·GTP dissociation induced by thiostrepton would be too rapid to allow for GTP hydrolysis, reducing the rate, thus explaining the apparently different inhibitory effects of the two drugs. The structural basis for the contrasting activities of ribosome-binding thiazole antibiotics has been studied using NMR and a thiostrepton-resistance methyltransferase assay, which rationalized the different binding profiles of thiostrepton, nosiheptide, siomycin, and micrococcin based upon the interaction of the quinaldic acid residue in thiostrepton, or an equivalent aromatic group, with the A1067 residue located in the L11BD.[172] However, it may well be the case that the key step in protein synthesis inhibition differs from organism to organism and depends on cellular growth conditions, although clearly the conformational constraint of protein L11 perturbs the function of the ribosomal factor−guanosine nucleotide complexes and thus inhibits cell growth.[171]

Actinomycetes antibiotic producers have to adopt a resistance mechanism to defend themselves against their own self-intoxication.[2] In general, *Streptomyces* are quite sensitive to thiostrepton and yet the producing organism, *S. azureus*, is totally unaffected by the drug.[3] An RNA-pentose methylase enzyme[173] produced constitutively from its own promoter is responsible for the autoimmunity in thiostrepton producers,[174] and this has been demonstrated in vitro upon ribosomal RNA from other bacteria. The methylase enzyme, thiostrepton-resistance methylase, introduces a single methyl group into the A1067 residue of the 23S rRNA in *E. coli* to give a modified 2′-*O*-methyladenosine-containing ribosome that is completely resistant to the antibiotic,[175] a phenomenon that has been observed in 23S rRNA mutants of *Halobacterium halobium*.[176] Base changes at position 1159 in halobacteria, which corresponds to A1067 of the *E. coli* rRNA, from A to U or G as well as base methylation causes high-level resistance to thiostrepton.[177] The thiostrepton-resistance (*tsr*) gene hybridizes with a sulfomycin resistance determinant from *S. viridochromogenes* ATCC 29776,[178] encodes the 23S rRNA A1067 methyltransferase in *S. laurentii,* and is located within a cluster of ribosomal protein operons not clustered with genes that encode the biosynthetic enzymes.[179] The *tsr* gene product has been overexpressed from *S. azureus* in *E. coli* to characterize the enzymic reaction and establish that recognition is dependent upon the secondary structure of the ribosomal hairpin loop that contains nucleotide 1067.[180] The study of thiostrepton-resistant mutants is thus not only of relevance to account for the origin and determinants of antibiotic resistance, but also as a valuable tool in establishing the complex function and specific dynamic processes in operation during bacterial protein synthesis to provide new leads and focus for structure-based drug design.

RNA-pentose methylation confers resistance in other actinomycetes, including *S. laurentii*, another thiostrepton producer, *P. parontospora*, from which sporangiomycin was isolated, and *S. sioyaensis*, the producer of the siomycins.[181] Radiographical studies on the inhibition of bacterial protein synthesis by siomycin supports many of the mechanistic findings for thiostrepton and lends credence to a common, or very closely related, mode of action that operates for all thiopeptide ribosomal inhibitors. The incorporation of radioactive amino acids and base pairs into the nascent peptide and mRNA, respectively, was studied in the presence of siomycin.[182] Although the action of the antibiotic prevented the incorporation of radioactive amino acids into *B. subtilis* cells, ^{14}C base pairs were incorporated into the mRNA, indicating that the agent acted as a ribosomal rather than transcription inhibitor, which was selective for Gram-positive and mycobacteria. Antibiotic binding at the ribosomal G site could cause a distortion of the A site, which would explain why siomycin inhibits translocation of the peptidyl-tRNA from the A to the P site, and prevents the binding of both Ef-G·GTP and the ternary complex aa-tRNA·Ef-Tu·GTP to the ribosome at the G and A sites, respectively.[183]

Actinomycetes that produce thiopeptide metabolites with very different chemical structures have been shown to adopt the same resistance mechanism and give rise to antibiotics that function by a common mode of action. The growth of *S. bernensis*, the producer of the berninamycins, is totally resistant to the action of thiostrepton, even though structurally these two antibiotics are grouped in a different thiopeptide series.[53] Similarly, nosiheptide has been shown to partially inhibit both the enzymatic binding of aminoacyl-tRNA to the ribosome and the simultaneous hydrolysis of GTP but only when present at quite a high molar excess over the ribosomes.[184] Despite differences in antibiotic structure, all of these compounds bind to the complex of 23S RNA and protein L11 and affect various functions of the ribosomal A site, although thiostrepton is considerably more potent in its action than nosiheptide.[185] The specific pentose-methylation of the 23S rRNA renders the ribosomes of *S. bernensis* and *S. actuosus* totally refractory to berninamycin and nosiheptide, respectively, and prevents the binding of thiostrepton,[186] indicating both a common mode of action of these drugs and a common mechanism of self-defense employed by the respective producing organisms.

4.2. Inhibition of Elongation Factor Tu

Elongation factor Tu (Ef-Tu) is the most abundant protein of the bacterial cell and participates in peptide elongation by mediating the recognition and delivery of noninitiator aminoacyl-tRNA, as a ternary complex with GTP, to the mRNA codon in the acceptor site of the bacterial ribosome. When its GTPase action is triggered, Ef-Tu·GDP, which does not bind aa-tRNA, dissociates from the ribosomal complex, leaving behind the aa-tRNA in the ribosomal A site in readiness for peptide translocation. Many types of antibiotics act by binding to Ef-Tu, which either prevents the dissociation of Ef-Tu·GDP from the ribosomal complex, as is the case for the polyketide kirromycin, or inhibits the binding of aa-tRNA to Ef-Tu·GTP, exemplified by pulvomycin.

The thiopeptide GE2270A (MDL 62,879) inhibits bacterial protein synthesis at an IC_{50} of 0.4 μM by

binding to Ef-Tu at a distinct site from kirromycin and so differs in its mode of action from thiopeptide ribosomal inhibitors.[187] GE2270A interacts directly with the GTP-bound form of Ef-Tu, and this inhibits the formation of a stable ternary complex by preventing the binding of aa-tRNA to Ef-Tu·GTP.[94] Although it does not change the GTPase activity of Ef-Tu, antibiotic binding does prevent the ribosomal catalysis of this process and slows down the dissociation of the Ef-Tu·GTP complex to even a greater degree than aa-tRNA. GE2270A forms a strongly bound 1:1 molar complex with Ef-Tu, which can be observed by a mobility shift in an electric field, only reversed by protein denaturation. The strong affinity between *E. coli* Ef-Tu·GDP and GE2270A has been explained by X-ray diffraction studies of the complex at a resolution of 2.35 Å, which showed, in addition to 18 protein-antibiotic van der Waals interactions (<3.5 Å), a highly unusual salt bridge between Arg 223 and Glu 259 that folds over the GE2270A side chain when bound in a pocket formed by three segments of amino acids, Glu 215-Arg 230, Thr 256-Leu 264, and Asn 273-Leu 277, located in the second domain of Ef-Tu·GTP.[188] The exchange of guanine nucleotides induces conformational changes in Ef-Tu complexes, as confirmed by proteolytic cleavage experiments.[187] The superposition of the Ef-Tu·GDP·GE2270A and Ef-Tu·GTP structures suggests that antibiotic binding causes steric constraints that prevent complete formation of the GTP conformation. Thus, it seems likely that on binding to domain II GE2270A impedes the GDP to GTP conformational change and prevents the formation of an Ef-Tu·GTP·aa-tRNA ternary complex by blocking the aa-tRNA binding site in the antibiotic-bound complex.[188]

This mode of action has been shown to operate for the GE2270 complex, GE37468, and the amythiamicins and has been used to develop a screening program for thiopeptide antibiotics with similar binding properties by selecting activities antagonized by exogenous Ef-Tu.[100,189] Inhibitors of this GTP-dependent translation factor have been shown to possess antimalarial activity in blood cultures of *P. falciparum*, the most active being amythiamicin A with an IC_{50} of 0.01 μM, and to bind to recombinant *P. falciparum* plastid Ef-Tu, indicating that endogenous plastid protein synthesis is a potential target for thiopeptides that inhibit prokaryotic protein synthesis by this mechanism.[190]

Not much is known about how the producers of Ef-Tu binding thiopeptides avoid self-intoxication, although resistance mechanisms are complicated by the fact that some actinomycetes possess more than one *tuf* gene to encode Ef-Tu translation factors. In *P. rosea*, the GE2270A producer, both the *tuf1* and *tuf3* genes encode Ef-Tu-like proteins, the former encoding the regular elongation factor EF-Tu1.[191] This bacterial protein is totally resistant to GE2270A, 10 times more resistant to kirromycin than Ef-Tu1 of *Streptomyces coelicolor,* and not at all resistant to pulvomycin, an antibiotic that also inhibits the formation of the aa-tRNA·EF-Tu·GTP complex. Kirromycin resistance is accounted for by the replacement of Tyr 160 with a Gln at the kirromycin binding site, the interface of domains 1 and 3 of Ef-Tu in its GTP-bound conformation. The mutations that confer GE2270A resistance would appear to map to a number of amino acids in domain 2, located at the GE2270A binding pocket,[188] in close proximity to residue 226 at the domain 1-2 interface, which is part of the binding site of the 3′-end of aa-tRNA. Ef-Tu mutants of *B. subtilis* that were resistant to Ef-Tu-binding thiopeptide antibiotics also showed significant changes of conserved amino acids, which may account for their similar resistance behavior.[100,192]

4.3. *TipA* Promotion

Actinomycetes multi-drug-resistance mechanisms often rely upon transport proteins or modifying enzymes and transcriptional regulatory proteins to recognize multiple drugs and respond to stress−response signals. *Streptomyces lividans* 1326, a strain that is not known as a thiazolylpeptide producer, reacts to thiostrepton by inducing resistance to a number of structurally heterogeneous antibiotics with the accumulation of thiostrepton-induced proteins (Tip), two of which, TipAL (253 amino acids) and TipAS (144 amino acids), the latter corresponding to the *C*-terminal region of TipAL, are in-frame translation products of the same gene, *tipA*.[73] TipAS, the independently translated thiopeptide binding domain present in vast molar excess (>20:1) over TipAL, renders the organism resistant by sequestering the drug in the cytosol and modulating a drug-dependent positive feedback loop that controls its own expression.[193] Their transcription is induced by a number of thiopeptide antibiotics, including thiostrepton, promothiocin, and nosiheptide, by complex formation with TipAL in the absence of added cofactors, which activates transcription of a monocistronic mRNA from the tipA promoter (*ptipA*) and so elicits autogenous expression of its own promoter.[193] The TipAL protein is a dimer in solution with an *N*-terminal domain (residues 1−109), containing both a helix−turn−helix that binds DNA (residues 5−25) and a long coiled-coil dimerization region (residues 74−109) and a *C*-terminal domain (residues 110−253), represented by TipAS, for thiopeptide recognition (Figure 32A). The formation of a covalent bond between cysteine 214 (not cysteine 207)[61] and a dehydroalanine residue in the thiopeptide irreversibly generates a ligand·TipAL complex (Figure 32B) and enhances the affinity of the bound protein for its operator site, inducing the recruitment of RNA polymerase (RNAP) to the promoter *ptipA*[194] and activating its transcription at least 200-fold.[73] On binding thiostrepton, conformational changes within TipAL enhance its association with *ptipA* and lower the rate of dissociation from the binding site, increasing the affinity of RNAP for *ptipA* in an alternative mechanism of transcriptional activation.[195] By analogy with the mercury-resistance regulator (MerR) protein, the ligand-bound TipAL dimer activates transcription and increases the affinity of the ligand-bound TipAL to the *tipA* promoter by inducing folding of the unstructured *N*-terminal part of apo TipAS, a globin-like α-helical fold with a deep surface cleft, and an unfolded *N*-terminal region that is the linker to

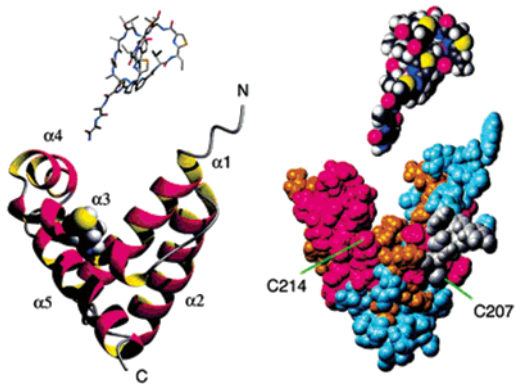
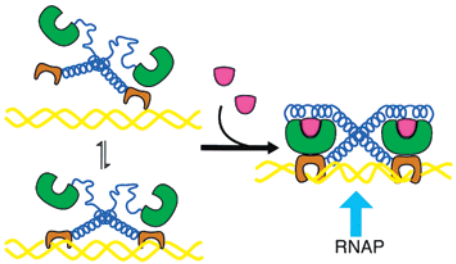


Figure 32. Structure of the folded part of apo TipAS. (Left) Ribbon representation showing α-helices, *N*- and *C*-termini, and ligand binding residue C214 as a space-filling model. (Right) Color map of ligand-induced chemical shift changes on TipAS−thiostrepton complex formation. Red indicates strongly affected residues, orange indicates moderately affected residues, and blue indicates weakly affected residues. The thiopeptide antibiotic shown alongside the TipAS-ligand binding site is thiostrepton. (Reprinted with permission from ref 196. Copyright 2003 European Molecular Biology Organization.)

Scheme 6. Suggested Mechanism of *TipA*-Induction by TipAL·Thiopeptide Complex Formation[a]



[a] Cartoon color coding: thiopeptide antibiotic, red; DNA, yellow; TipAL, green, blue, and brown. (Reprinted with permission from ref 196. Copyright 2003 European Molecular Biology Organization.)

the DNA-binding domain of TipAL.[196] TipAL and other MerR regulators bind as dimers to an inverted repeat sequence located within the spacer region of their promoters. In contrast to the MerR regulatory proteins, TipAL can bind to its target site and activate transcription in the absence of the ligand, elevated external osmolarity causing an increase in intracellular negative DNA supercoiling that enhances *ptipA* expression.[197] On binding thiopeptide antibiotics the *N*-terminal coiled-coil linker parts of the TipAL complex become rigid. This conformational change clamps the DNA-binding domains of the dimer and twists the DNA helix of the promoter, bringing the two consensus recognition sequences into alignment to allow RNAP to initiate transcription (Scheme 6).

A number of novel thiopeptide antibiotics have been identified from actinomycete metabolite libraries by screening for *ptipA*-inducing activities in a specific microbiological disk assay, including geninthiocin,[74] promoinducin,[79] the promothiocins,[75] thioxamycin,[81] thioactin, and thiotipin.[80] Minimum induction concentrations (C_{min}s) for *ptipA* induction activity were found to vary depending upon the structure of the thiopeptide ligand (Table 2) with the series *d* thiopeptides promothiocin B, geninthiocin, and berninamycin A being the most active.[61] Promothiocin derivatives without dehydroalanine residues (promothiocin MO: methyl ester) as well as thiostrepton B, which does not possess the characteristic polydehydroamino-acid-containing side chain, did not form TipAS·antibiotic complexes but nevertheless were found to induce both the promoter and the synthesis of TipAS protein, confirming that covalent attachment was not required for stable interaction in vitro or in vivo and that the macrocyclic thiopeptide contained a TipA recognition motif. This autogenously controlled antibiotic resistance system responds to these antibiotics by synthesizing a single mRNA that includes TipAS to sequester the antibiotic and thus limit activation of the TipAL-dependent promoter. It may well be the case that these proteins, in addition to providing a low-level antibiotic-resistance system, regulate resistance to other stress−response signals, such as those induced by heavy metals or changes in redox potentials, indicating a biological relevance far beyond growth inhibition in competing organisms.

Table 2. Thiopeptide *ptipA* Induction Activity

thiopeptide	C_{min}/nM
promothiocin B	0.63
geninthiocin	1.0
berninamycin A	1.0
thiostrepton A	1.4
promothiocin A	24
promoinducin	30
thiotipin	32
thioactin	38
thioxamycin	63
A10255G	66
thiostrepton B	67
cyclothiazomycin[a]	>700
amythiamicin A[a]	>800
GE2270A[a]	>1000
promothiocin MO[a]	3300

[a] Contains no dehydroalanine residues.

5. Total Synthesis

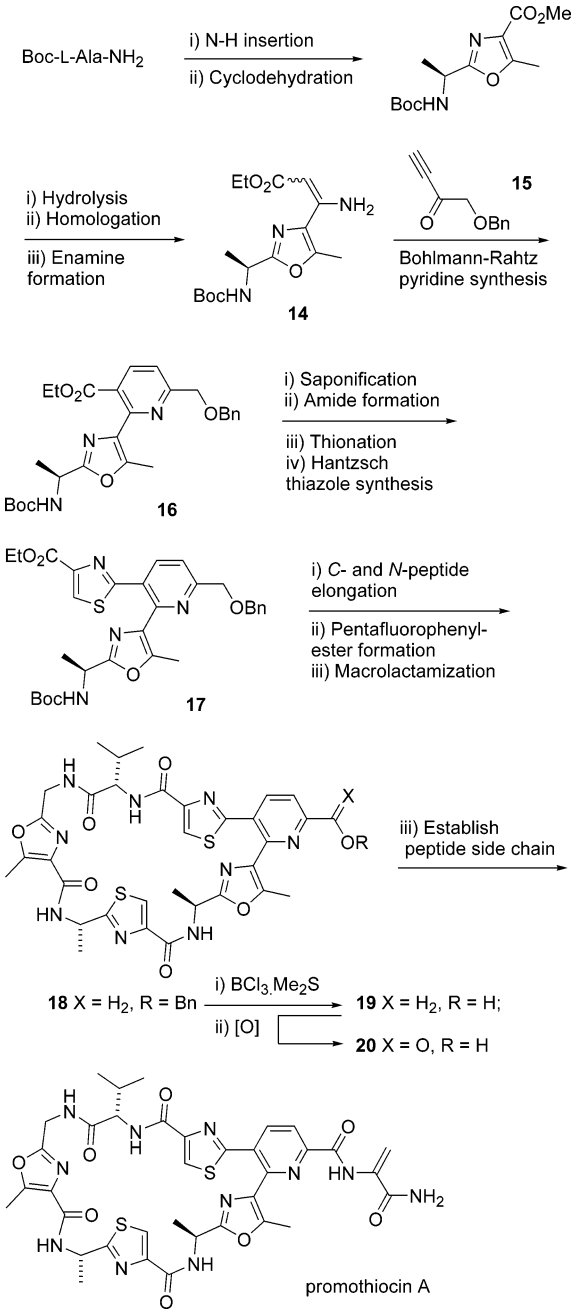
In recent years significant advances toward the synthesis of many of the thiopeptide antibiotics and their unusual heterocyclic or heavily modified constituent components have been made. The structural complexity of many of these antibiotics means that efforts toward their total synthesis rarely result in success. Despite, or perhaps because of, the significant challenge, considerable effort has been directed toward a number of thiopeptide families in recent years, culminating in the total synthesis of promothiocin A and more recently amythiamicin D and thiostrepton. Considerable progress has been made toward the acidic hydrolysates of many thiopeptide antibiotics, including dimethyl sulfomycinamate,[65,66] berninamycinic acid,[57] and micrococcinic acid,[198] as well as useful building blocks for the synthesis of heterocyclic components of, among others, thiostrepton,[199] nosiheptide,[200−202] glycothiohexide α,[203] the promothiocins,[204] sulfomycins,[66,205] amythiamicins,[206] berninamycins,[207] cyclothiazomycin,[105,208,209] A10255,[210]

GE2270A,[211] and the micrococcins,[212] the latter being perhaps the most unusual thiopeptide targets that have witnessed three separate total syntheses, none of which have yielded a natural product.[43−45] However, with significant advances made in this decade toward the expedient preparation of some of the more complex members of this antibiotic family, it can be presumed with some degree of certainty that major synthetic achievements will be made in this area soon.

The first chemical synthesis of a thiopeptide natural product was the preparation of promothiocin A by Moody.[76,77] This landmark synthesis featured the little-used Bohlmann−Rahtz heteroannulation reaction to establish the central oxazole−thiazole−pyridine domain **17** by saponification, amide formation, thionation, and Hantzsch thiazole synthesis. The Bohlmann−Rahtz synthesis of pyridine **16** from ethyl 3-amino-3-(4-oxazolyl)propenoate **14**, prepared from (*S*)-*N*-*tert*-butoxycarbonylalaninamide by dirhodium(II)-catalyzed carbenoid insertion into the amide N−H followed by cyclodehydration with triphenylphosphine−iodine under basic conditions, saponification, homologation using the magnesium enolate of ethyl potassium malonate and enamine formation, and 1-benzyloxybut-3-yn-2-one (**15**), obtained by Grignard addition and propargylic oxidation, proceeds in two steps by initial Michael addition at 50 °C and subsequent double-bond isomerization−cyclization at 140 °C in the absence of solvent (Scheme 7). The elongation of the linear peptide by *N*- and *C*-terminus functionalization followed by macrolactamization under basic conditions via the pentafluorophenylester gave macrocycle **18**. Benzyl ether cleavage with boron trichloride dimethyl sulfide complex gave alcohol **19**, which was oxidized to carboxylic acid **20** using *o*-iodoxybenzoic acid (IBX) in DMSO followed by treatment with sodium chlorite and then coupled with an *O*-protected serinamide derivative using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI). Protodesilylation and dehydration by mesylate formation followed by treatment with triethylamine installed the dehydroalanine side chain, established the first total synthesis of one of the thiopeptide antibiotics, and verified the (*S*)-stereochemistry of all three stereogenic centers in the metabolite isolated from *Streptomyces* sp. SF2741.

Both Shin and Ciufolini made considerable progress toward the synthesis of the micrococcins, with the preparation of micrococcin isomers by the former[44,45] and the synthesis of the Bycroft−Gowland structure **5** of micrococcin P_1 by the latter.[43] In Ciufolini's approach the central domain **21** was established by the heteroannulation of 1,5-diketone **22** (Figure 33), generated by Michael addition and cyclized in two steps by treatment with ammonium acetate followed by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ).[212] Subsequent incorporation of the (*R*)-isoalaninol side chain to give tris(thiazolyl)-pyridine **24**, coupling with the *N*-terminus of modified pentapeptide **23,** and finally macrolactamization by hydrolysis and acid-catalyzed deprotection of the *C*- and *N*-terminal protecting groups, respectively, followed by treatment with diphenylphosphoryl azide (DPPA) gave the Bycroft−Gowland structure **5** in 23 steps and an overall yield of 5.7%. However, although the spectra of the synthetic material and natural micrococcin P were very similar, they were not identical, their diastereoisomeric relationship being shown subsequently in NMR and computational studies.[46]

Scheme 7. Total Synthesis of Promothiocin A



An alternative route to the series *d* domain by Moody used a biomimetic strategy for the synthesis of the 2,3,6-tris(thiazolyl)pyridine **27** of the amythiamicins, elaborated to amythiamicin D to establish the (*S*)-stereochemistry of the three stereogenic centers of the natural product (Scheme 8).[103] The formal aza-Diels−Alder cycloaddition of dehydroalanine dienophile **25** and 2-azadiene **26** proceeded in modest yield by microwave irradiation[138] in toluene at 120 °C for 12 h. Elongation of the central pyridine domain **27** gave linear peptide **28**, which was cyclized by libera-

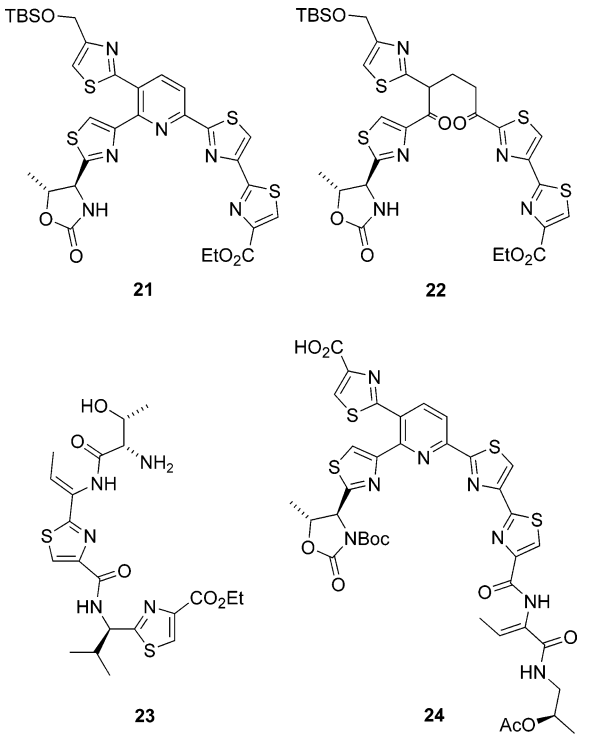
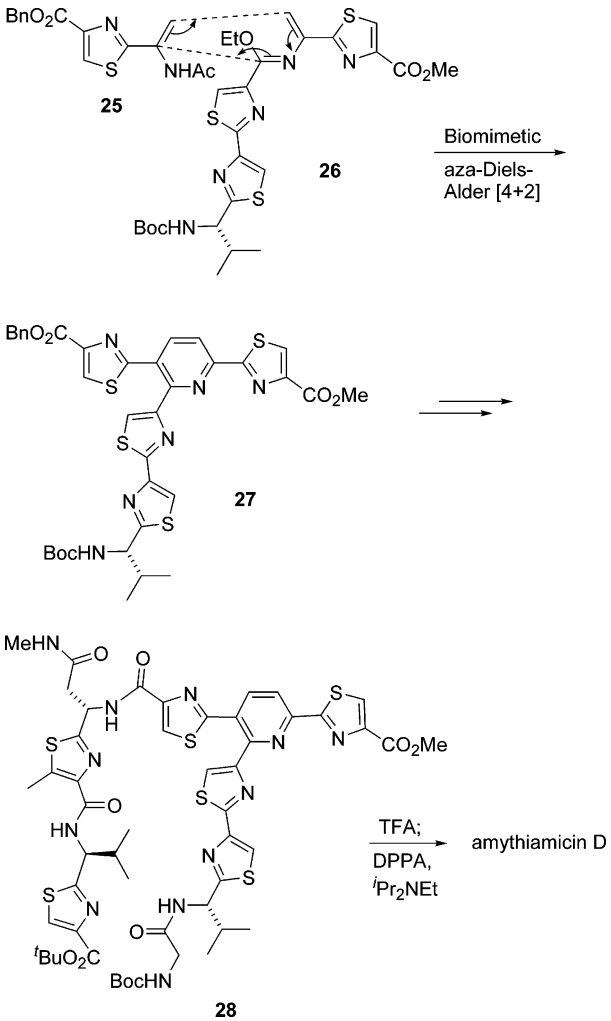
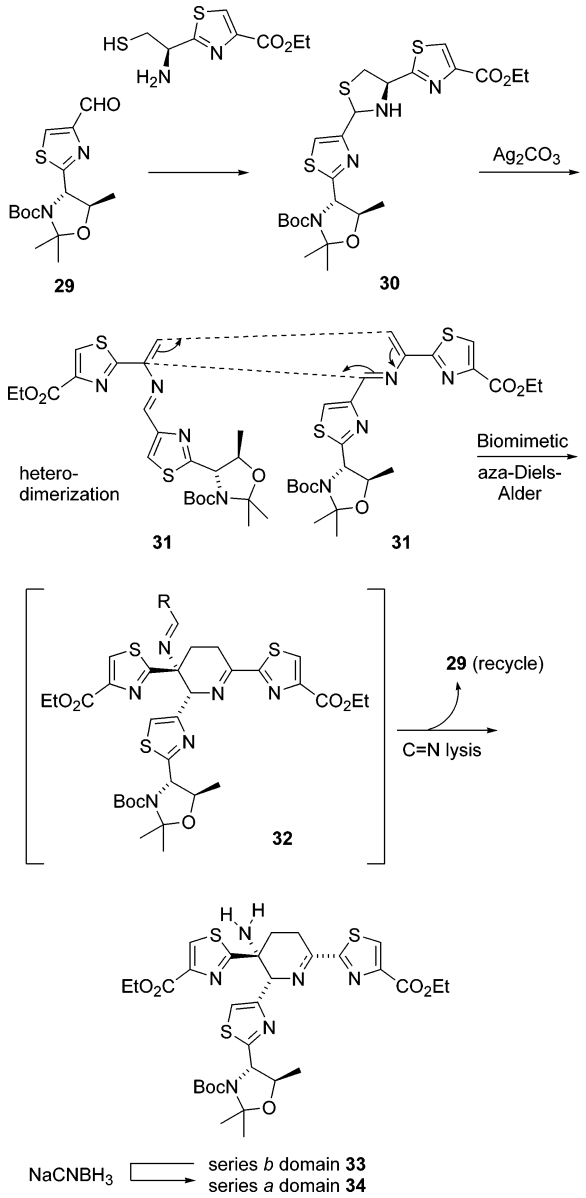


Figure 33. Intermediates in the synthesis of the Bycroft–Gowland structure **5** of micrococcin P_1.

Scheme 8. Total Synthesis of Amythiamicin D



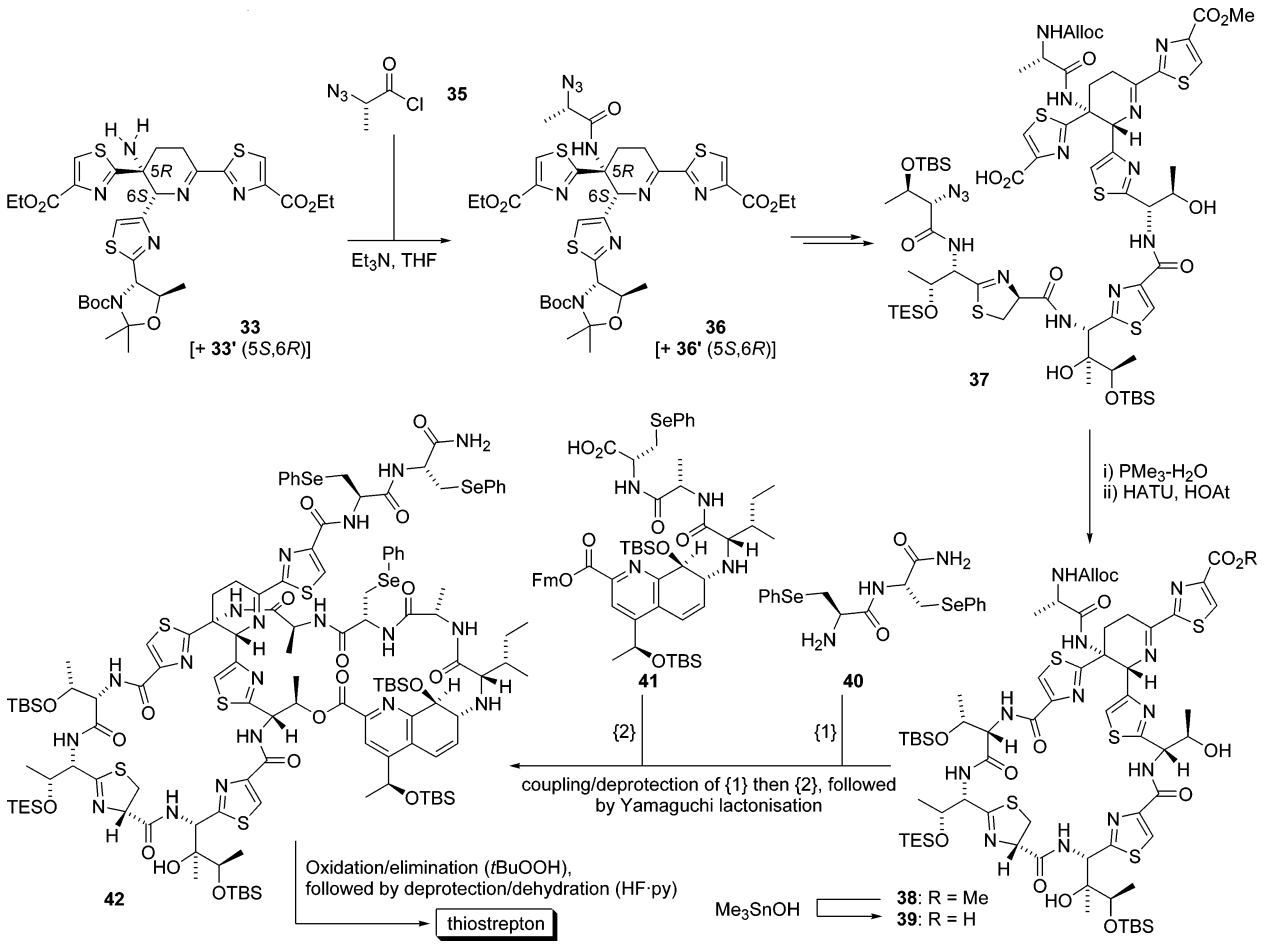
Scheme 9. Biomimetic Synthesis of Series *a* or *b* Piperidine Domain



tion of both *C*- and *N*-termini using trifluoroacetic acid (TFA) followed by treatment with diphenylphosphoryl azide (DPPA) and Hünig's base in DMF to give the macrocyclic natural product amythiamicin D.

An elegant biomimetic strategy has been used by Nicolaou to establish the dehydropiperidine domain of thiostrepton, and this, with the stereoselective synthesis of the quinaldic acid-containing macrocycle[213] and construction of all requisite components,[214,215] led to the highly convergent total synthesis of this complex antibiotic.[216] The regioselective and *endo*-selective hetero-Diels–Alder dimerization of 2-azadiene **31**, obtained from thiazolidine **30** by treatment with silver carbonate, proceeded without facial selectivity to give dehydropiperidine **33** as a 1:1 mixture of diastereomers in a cascade sequence with in situ lysis of imine intermediate **32** and release of aldehyde **29** to be recycled to dimerization precursor **30** (Scheme 9).[137] Stereospecific reduction of cycloadduct **33** using sodium cyanoborohydride generated piperidine **34** and demonstrated that a bio-

Scheme 10. Total Synthesis of Thiostrepton



mimetic heterodimerization approach can provide expedient access to the central domain of either series *a* (**34**) or *b* (**33**) thiopeptide antibiotics.

The application of this work in the total synthesis of thiostrepton was realized by capturing the free amino group of the dehydropiperidine intermediate with the acyl chloride of an azide derivative of alanine (**35**) to produce imine **36** exclusively as a diastereomeric mixture (Scheme 10).[215] After peptide elaboration, closure of the thiazoline-containing macrocycle was successful for only one (**37**) of two monoacids, formed by the action of Me_3SnOH in 1,2-dichloroethane, to give the desired macrocycle **38** following reduction with PMe_3-H_2O and treatment with HATU−HOAt−iPr$_2$NEt. The construction of the two macrocyclic domains was effected by attachment of a phenylseleno-disubstituted peptide **40**,[217] as a precursor for the dehydroalanine subunits in the side chain, to acid **39**, followed by *N*-terminal deprotection, coupling with quinaldic acid linear peptide **41**, and macrolactonization under Yamaguchi conditions with 2,4,6-trichlorobenzoyl chloride.[216] The synthesis was completed by *t*BuOOH-mediated oxidation of all three phenylseleno groups in bis-macrocycle **42**, which brought about spontaneous selenoxide syn elimination, followed by silyl ether deprotection with hydrogen fluoride−pyridine, with concomitant elimination to form the thiazoline-conjugated *Z* double bond. This landmark route gave synthetic thiostrepton with identical physical properties to an authentic sample, constituting a highly convergent and stereoselective synthesis of a complex thiopeptide antibiotic that may pave the way for related synthetic studies in the future.

6. Future Perspectives

Recent years have seen many developments in our understanding of the chemistry and biology of the thiopeptide antibiotics. Targeted screening programs have isolated an ever-increasing number of actinomycete thiazolylpeptide metabolites obtained from various sources. Alongside this increase in diversity, analytical methods, in particular X-ray crystallography and NMR techniques, well suited to these macrocyclic natural products have evolved to elucidate thiopeptide structure and stereochemistry with much greater certainty, removing many of the structural ambiguities inherent in earlier work in the area. Considerable advances have been made in our understanding of the dynamic function of the bacterial ribosome, the mode of action and site of binding of thiopeptide ribosomal inhibitors, the inhibition of organellar protein synthesis by these agents in *P. falciparum,* and the manner in which these metabolites are assembled in the organism, and it is suspected that many more revelations in these areas will be forthcoming. New insights into multi-drug-resistance systems in bacteria have revealed the stress responses of actinomycetes to thiopeptide antibiotics and their role and its structural basis in regulating

gene expression. Furthermore, significant progress in the chemical synthesis of complex molecular architectures found in this family of antibiotics has been made, with the total synthesis of promothiocin A, amythiamicin D, and the stunningly complex thiostrepton, making it appear likely that further success will be enjoyed in the synthesis of similarly challenging metabolites and derivatives thereof in the future to optimize their biological function in the computer-assisted design of analogue structures. With bacterial evolution threatening to overthrow our current antibiotic regime, we can be certain that despite 50 years of discoveries the thiopeptide antibiotics will continue to enjoy increasing attention from a wide variety of scientific consortia whose work will continue to surprise us with its innovation, tenacity, ambition, and strategic relevance.

7. Acknowledgment

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8. References

(1) Niccolai, D.; Tarsi, L.; Thomas, R. J. *Chem. Commun.* **1997**, 2333.
(2) Cundliffe, E. *Annu. Rev. Microbiol.* **1989**, *43*, 207.
(3) Cundliffe, E. *Br. Med. Bull.* **1984**, *40*, 61.
(4) Bérdy, J. *Adv. Appl. Microbiol.* **1974**, *18*, 309.
(5) Hensens, O. D.; Albers-Schönberg, G. *Tetrahedron Lett.* **1978**, 3649.
(6) Pagano, J. F.; Weinstein, M. J.; Stout, H. A.; Donovick, R. *Antibiot. Ann.* **1955/56**, 554.
(7) Vandeputte, J.; Dutcher, J. D. *Antibiot. Ann.* **1955/56**, 560.
(8) Steinberg, B. A.; Jambor, W. P.; Suydam, L. O.; Soriano, A. *Antibiot. Ann.* **1955/56**, 562.
(9) (a) Jones, W. F., Jr.; Finland, M. *Antibiot. Chemother.* **1958**, *8*, 387. (b) Kutscher, A. H.; Seguin, L.; Rankow, R. M.; Piro, J. D. *Antibiot. Chemother.* **1958**, *8*, 576.
(10) Cron, M. J.; Whitehead, D. F.; Hooper, I. R.; Heinemann, B.; Lein, J. *Antibiot. Chemother.* **1956**, *6*, 63.
(11) Bodanszky, M.; Dutcher, J. D.; Williams, N. J. *J. Antibiot., Ser. A* **1963**, *16*, 76.
(12) Bodanszky, M.; Fried, J.; Sheehan, J. T.; Williams, N. J.; Alicino, J.; Cohen, A. I.; Keeler, B. T.; Birkhimer, C. A. *J. Am. Chem. Soc.* **1964**, *86*, 2478.
(13) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 233.
(14) Tori, K.; Tokura, K.; Okabe, K.; Ebata, M.; Otsuka, H. *Tetrahedron Lett.* **1976**, 185.
(15) Trejo, W. H.; Dean, L. D.; Pluscec, J.; Meyers, E.; Brown, W. E. *J. Antibiot.* **1977**, *30*, 639.
(16) Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 832.
(17) Mocek, U.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1649.
(18) Bond, C. S.; Shaw, M. P.; Alphey, M. S.; Hunter, W. N. *Acta Crystallogr.* **2001**, *D57*, 755.
(19) Tori, K.; Tokura, K.; Yoshimura, Y.; Terui, Y.; Okabe, K.; Otsuka, H.; Matsushita, K.; Inagaki, F.; Miyazawa, T. *J. Antibiot.* **1981**, *34*, 124.
(20) Nishimura, H.; Okamoto, S.; Mayama, M.; Otsuka, H.; Nakajima, K.; Tawara, K.; Shimohira, M.; Shimaoka, N. *J. Antibiot., Ser. A.* **1961**, *14*, 255.
(21) Ebata, M.; Miyazaki, K.; Otsuka, H. *J. Antibiot.* **1969**, *22*, 364.
(22) Tokura, K.; Tori, K.; Yoshimura, Y.; Okabe, K.; Otsuka, H.; Matsushita, K.; Inagaki, F.; Miyazawa, T. *J. Antibiot.* **1980**, *33*, 1563.
(23) Ebata, M.; Miyazaki, K.; Otsuka, H. *J. Antibiot.* **1969**, *22*, 434.
(24) Tori, K.; Tokura, K.; Yoshimura, Y.; Okabe, K.; Otsuka, H.; Inagaki, F.; Miyazawa, T. *J. Antibiot.* **1979**, *32*, 1072.
(25) Miyairi, N.; Miyoshi, T.; Aoki, H.; Kohsaka, M.; Ikushima, H.; Kunugita, K.; Sakai, H.; Imanaka, H. *Antimicrob. Agents Chemother.* **1972**, *1*, 192.
(26) Mine, K.; Miyairi, N.; Takano, N.; Mori, S.; Watanabe, N. *Antimicrob. Agents Chemother.* **1972**, *1*, 496.
(27) Muir, L. A.; Barreto, A. U.S. Patent 4,061,732,1977; *Chem. Abstr.* **1978**, *88*, 58574y.
(28) Hensens, O. D.; Albers-Schönberg, G. *J. Antibiot.* **1983**, *36*, 814.
(29) Muramatsu, I.; Motoki, Y.; Aoyama, M.; Suzuki, H. *J. Antibiot.* **1977**, *30*, 383.
(30) Motoki, Y.; Muramatsu, I. *Pept. Chem.* **1979** (Pub. **1980**), 13; *Chem. Abstr.* **1981**, *94*, 103798m.
(31) Puar, M. S.; Ganguly, A. K.; Afonso, A.; Brambilla, R.; Mangiaracina, P.; Sarre, O.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1981**, *103*, 5231.
(32) Puar, M. S.; Chan, T. M.; Hegde, V.; Patel, M.; Bartner, P.; Ng, K. J.; Pramanik, B. N.; MacFarlane, R. D. *J. Antibiot.* **1998**, *51*, 221.
(33) Su, T. L. *Br. J. Exp. Path.* **1948**, *29*, 473.
(34) Kelly, B. K.; Miller, G. A.; Whitmarsh, J. M. Brit. Patent 711,-593, 1954; *Chem. Abstr.* **1955**, *49*, 573.
(35) Heatley, N. G.; Doery, H. M. *Biochem. J.* **1951**, *50*, 247.
(36) Fuller, A. T. *Nature* **1955**, *175*, 722.
(37) (a) Carnio, M. C.; Holtzel, A.; Rudolf, M.; Henle, T.; Jung, G.; Scherer, S. *Appl. Environ. Microbiol.* **2000**, *66*, 2378. (b) Carnio, M. C.; Stachelhaus, T.; Francis, K. P.; Scherer, S. *Eur. J. Biochem.* **2001**, *268*, 6390.
(38) Brookes, P.; Fuller, A. T.; Walker, J. *J. Chem. Soc.* **1957**, 689.
(39) Brookes, P.; Clark, R. J.; Fuller, A. T.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc. C* **1960**, 916.
(40) Mijović, M. P. V.; Walker, J. *J. Chem. Soc. C* **1960**, 909.
(41) Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706.
(42) Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem. Commun.* **1978**, 256.
(43) Ciufolini, M. A.; Shen, Y.-C. *Org. Lett.* **1999**, *1*, 1843.
(44) Shin, C.; Okumura, K.; Shigekuni, M.; Nakamura, Y. *Chem. Lett.* **1998**, 139.
(45) Okumura, K.; Ito, A.; Yoshioka, D.; Shin, C. *Heterocycles* **1998**, *48*, 1319.
(46) Fenet, B.; Pierre, F.; Cundliffe, E.; Ciufolini, M. A. *Tetrahedron Lett.* **2002**, *43*, 2367.
(47) Dean, B. M.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394.
(48) Shoji, J.; Hinoo, H.; Wakisaka, Y.; Koizumi, K.; Mayama, M.; Matsuura, S.; Matsumoto, K. *J. Antibiot.* **1976**, *29*, 366.
(49) Shoji, J.; Kato, T.; Yoshimura, Y.; Tori, K. *J. Antibiot.* **1981**, *34*, 1126.
(50) Nagai, K.; Kamigiri, K.; Arao, N.; Suzumura, K.-I.; Kawano, Y.; Yamaoka, M.; Zhang, H.; Watanabe, M.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 123.
(51) Suzumura, K.-I.; Yokoi, T.; Funatsu, M.; Nagai, K.; Tanaka, K.; Zhang, H.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 129.
(52) Kamigiri, K.; Watanabe, M.; Nagai, K.; Arao, N.; Suzumura, K.; Suzuki, K.; Kurane, R.; Yamaoka, M.; Kawano, Y. PCT Int. Appl. 072617, 2002; *Chem. Abstr.* **2002**, *137*, 246602.
(53) Reusser, F. *Biochemistry* **1969**, *8*, 3303.
(54) Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1976**, *98*, 8237.
(55) Liesch, J. M.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1977**, *99*, 1645.
(56) Liesch, J. M.; McMillan, J. A.; Pandey, R. C.; Paul, I. C.; Rinehart, K. L., Jr.; Reusser, F. *J. Am. Chem. Soc.* **1979**, *98*, 299.
(57) Kelly, T. R.; Echavarren, A.; Chandrakumar, N. S.; Köksal, Y. *Tetrahedron Lett.* **1984**, *25*, 2127.
(58) Abe, H.; Kushida, K.; Shiobara, Y.; Kodama, M. *Tetrahedron Lett.* **1988**, *29*, 1401.
(59) Lau, R. C. M.; Rinehart, K. L. *J. Antibiot.* **1994**, *47*, 1466.
(60) Lau, R. C. M.; Rinehart, K. L. *J. Am. Chem. Soc.* **1995**, *117*, 7606.
(61) Chiu, M. L.; Folcher, M.; Katoh, T.; Puglia, A. M.; Vohradsky, J.; Yun, B.-S.; Seto, H.; Thompson, C. J. *J. Biol. Chem.* **1999**, *274*, 20578.
(62) Biskupiak, J. E.; Meyers, E.; Gillum, A. M.; Dean, L.; Trejo, W. H.; Kirsch, D. R. *J. Antibiot.* **1988**, *41*, 684.
(63) Egawa, Y.; Umino, K.; Tamura, Y.; Shimizu, M.; Kaneko, K.; Sakurazawa, M.; Awataguchi, S.; Okuda, T. *J. Antibiot.* **1969**, *22*, 12.
(64) Abe, H.; Takaishi, T.; Okuda, T.; Aoe, K.; Date, T. *Tetrahedron Lett.* **1978**, 2791.
(65) Kelly, T. R.; Lang, F. *J. Org. Chem.* **1996**, *61*, 4623.
(66) Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.* **2003**, *5*, 4421.
(67) Kohno, J.; Kameda, N.; Nishio, M.; Kinumaki, A.; Komatsubara, S. *J. Antibiot.* **1996**, *49*, 1063.
(68) Boeck, L. D.; Berry, D. M.; Mertz, F. P.; Wetzel, R. W. *J. Antibiot.* **1992**, *45*, 1222.
(69) Boeck, L. D.; Favret, M. E.; Wetzel, R. W. *J. Antibiot.* **1992**, *45*, 1278.
(70) Favret, M. E.; Boeck, L. D. *J. Antibiot.* **1992**, *45*, 1809.

(71) Debono, M.; Molloy, R. M.; Occolowitz, J. L.; Paschal, J. W.; Hunt, A. H.; Michel, K. H.; Martin, J. W. *J. Org. Chem.* **1992**, *57*, 5200.
(72) Favret, M. E.; Paschal, J. W.; Elzey, T. K.; Boeck, L. D. *J. Antibiot.* **1992**, *45*, 1499.
(73) Murakami, T.; Holt, T. G.; Thompson, C. J. *J. Bacteriol.* **1989**, *171*, 1459.
(74) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 969.
(75) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 510.
(76) Moody, C. J.; Bagley, M. C. *Chem. Commun.* **1998**, 2049.
(77) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301.
(78) Yun, B.-S.; Fujita, K.-i.; Furihata, K.; Seto, H. *Tetrahedron* **2001**, *57*, 9683.
(79) Yun, B.-S.; Seto, H. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 876.
(80) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *Tetrahedron* **1994**, *50*, 11659.
(81) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541.
(82) Matsumoto, M.; Kawamura, Y.; Yasuda, Y.; Tanimoto, T.; Matsumoto, K.; Yoshida, T.; Shoji, J. *J. Antibiot.* **1989**, *42*, 1465.
(83) Nadkarni, S. R.; Mukhopadhyay, T.; Jayvanti, K.; Vijaya Kumar, E. K. Eur. Patent 96111156,4, 1998; *Chem. Abstr.* **1998**, *128*, 153208.
(84) Vijaya Kumar, E. K. S.; Kenia, J.; Mukhopadhyay, T.; Nadkarni, S. R. *J. Nat. Prod.* **1999**, *62*, 1562.
(85) Holgado, G. G.; Rodríguez, J. C.; Hernández, L. M. C.; Díaz, M.; Fernández-Abalos, J. M.; Trujillano, I.; Santamaría, R. I. *J. Antibiot.* **2002**, *55*, 383.
(86) Rodríguez, J. C.; Holgado, G. G.; Sánchez, R. I. S.; Cañedo, L. M. *J. Antibiot.* **2002**, *55*, 391.
(87) Selva, E.; Beretta, G.; Montanini, N.; Goldstein, B. P.; Denaro, M. Eur. Patent 359,062, 1990; *Chem. Abstr.* **1990**, *113*, 38919w.
(88) Selva, E.; Beretta, G.; Montanini, N.; Saddler, G. S.; Gastaldo, L.; Ferrari, P.; Lorenzetti, R.; Landini, P.; Ripamonti, F.; Goldstein, B. P.; Berti, M.; Montanaro, L.; Denaro, M. *J. Antibiot.* **1991**, *44*, 693.
(89) Kettenring, J.; Colombo, L.; Ferrari, P.; Tavecchia, P.; Nebuloni, M.; Vékey, K.; Gallo, G. G.; Selva, E. *J. Antibiot.* **1991**, *44*, 702.
(90) Colombo, L.; Tavecchia, P.; Selva, E.; Gallo, G. G.; Zerilli, L. F. *Org. Mass Spectrom.* **1992**, *27*, 219.
(91) Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Lociuro, S.; Selva, E. *J. Antibiot.* **1994**, *47*, 1564.
(92) Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, *51*, 4867.
(93) Selva, E.; Ferrari, P.; Kurz, M.; Tavecchia, P.; Colombo, L.; Stella, S.; Restelli, E.; Goldstein, B. P.; Ripamonti, F.; Denaro, M. *J. Antibiot.* **1995**, *48*, 1039.
(94) Anborgh, P. H.; Parmeggiani, A. *EMBO J.* **1991**, *10*, 779.
(95) Stella, S.; Montanini, N.; Le Monnier, F.; Ferrari, P.; Colombo, L.; Landini, P.; Ciciliato, I.; Goldstein, B. P.; Selva, E.; Denaro, M. *J. Antibiot.* **1995**, *48*, 780.
(96) Marinelli, F.; Gastaldo, L.; Toppo, G.; Quarta, C. *J. Antibiot.* **1996**, *49*, 880.
(97) Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304.
(98) Stella, S.; Montanini, N.; Le Monnier, F. J.; Colombo, L.; Selva, E.; Denaro, M. PCT Int. Appl. WO 94 14,838, 1994; *Chem. Abstr.* **1995**, *122*, 128491r.
(99) Shimanaka, K.; Kinoshita, N.; Iinuma, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 668.
(100) Shimanaka, K.; Iinuma, H.; Hamada, M.; Ikeno, S.; S.-Tsuchiya, K.; Arita, M.; Hori, M. *J. Antibiot.* **1995**, *48*, 182.
(101) Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1153.
(102) Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1145.
(103) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *Chem. Commun.* **2004**, 946.
(104) Aoki, M.; Ohtsuka, T.; Yamada, M.; Ohba, Y.; Yoshizaki, H.; Yasuno, H.; Sano, T.; Watanabe, J.; Yokose, K. *J. Antibiot.* **1991**, *44*, 582.
(105) Bagley, M. C.; Xiong, X. *Org. Lett.* **2004**, *6*, 3401.
(106) Aoki, M.; Ohtsuka, T.; Itezono, Y.; Yokose, K.; Furihata, K.; Seto, H. *Tetrahedron Lett.* **1991**, *32*, 217.
(107) Aoki, M.; Ohtsuka, T.; Itezono, Y.; Yokose, K.; Furihata, K.; Seto, H. *Tetrahedron Lett.* **1991**, *32*, 221.
(108) Benazet, F.; Cartier, M.; Florent, J.; Godard, C.; Jung, G.; Lunel, J.; Mancy, D.; Pascal, C.; Renaut, J.; Tarridec, P.; Theilleux, J.; Tissier, R.; Dubost, M.; Ninet, L. *Experientia* **1980**, *36*, 414.
(109) Depaire, H.; Thomas, J.-P.; Brun, A.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1403.
(110) Depaire, H.; Thomas, J.-P.; Brun, A.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1397.
(111) Depaire, H.; Thomas, J.-P.; Brun, A.; Hull, W. P.; Olesker, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1401.
(112) Gasmi, G.; Massiot, G.; Nuzillard, J. M. *Magn. Reson. Chem.* **1996**, *34*, 185.
(113) Depaire, H.; Thomas, J.-P.; Brun, A.; Lukacs, G. *Tetrahedron Lett.* **1977**, 1395.
(114) Prange, T.; Ducruix, A.; Pascard, C.; Lunel, J. *Nature* **1977**, *265*, 189.
(115) Pascard, C.; Ducruix, A.; Lunel, J.; Prangé, T. *J. Am. Chem. Soc.* **1977**, *99*, 6418.
(116) Tanaka, T.; Endō, T.; Shimazu, A.; Yoshida, R.; Suzuki, Y.; Ōtake, N.; Yonehara, H. *J. Antibiot.* **1970**, *23*, 231.
(117) Endō, T.; Yonehara, H. *J. Antibiot.* **1978**, *31*, 623.
(118) Casteels, M.; Bekaert, H.; Buysse, F. X. *Rev. Agric.* (*Brussels*) **1980**, *33*, 1069; *Chem. Abstr.* **1981**, *94*, 101869m.
(119) Horii, S.; Oku, N. *J. AOAC Int.* **2000**, *83*, 17.
(120) Tanaka, T.; Sakaguchi, K.; Yonehara, H. *J. Antibiot.* **1970**, *23*, 401.
(121) Keller-Juslen, C.; Kuhn, M.; DeLisle King, H. Ger. Offen. 2,-921,148, 1979; *Chem. Abstr.* **1980**, *93*, 6124k.
(122) Northcote, P. T.; Williams, D.; Manning, J. K.; Borders, D. B.; Maiese, W. M.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 894.
(123) Steinberg, D. A.; Bernan, V. S.; Montenegro, D. A.; Abbanat, D. R.; Pearce, C. J.; Korshalla, J. D.; Jacobus, N. V.; Petersen, P. J.; Mroczenski-Wildey, M. J.; Maiese, W. M.; Greenstein, M. *J. Antibiot.* **1994**, *47*, 887.
(124) Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 901.
(125) Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715.
(126) (a) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284. (b) Li, W.; Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-Tomc, J.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 226. (c) Leet, J. E.; Li, W.; Ax, H. A.; Matson, J. A.; Huang, S.; Huang, R.; Cantone, J. L.; Drexler, D.; Dalterio, R. A.; Lam, K. S. *J. Antibiot.* **2003**, *56*, 232.
(127) Regueiro-Ren, A.; Ueda, Y. *J. Org. Chem.* **2002**, *67*, 8699.
(128) Naidu, B. N.; Li, W.; Sorenson, M. E.; Connolly, T. P.; Wichtowski, J. A.; Zhang, Y.; Kim, O. K.; Matiskella, J. D.; Lam, K. S.; Bronson, J. J.; Ueda, Y. *Tetrahedron Lett.* **2004**, *45*, 1059.
(129) Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789.
(130) Yeo, W.-H.; Kim, S.-K.; Kim, S.-S.; Yu, S.-H.; Park, E. K. *J. Microbiol. Biotechnol.* **1994**, *4*, 349.
(131) Yeo, W.-H.; Kim, S.-K.; Kim, S.-S.; Yu, S.-H.; Park, E. K. *J. Microbiol. Biotechnol.* **1994**, *4*, 354.
(132) Mizuno, K.; Hamada, M.; Maeda, K.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 429.
(133) Cooper, R.; Truumees, I.; Barrett, T.; Patel, M.; Schwartz, J.; Puar, M.; Das, P.; Pramanik, B. *J. Antibiot.* **1990**, *43*, 897.
(134) Baudet, P.; Cherbuliez, E. *Helv. Chim. Acta* **1964**, *47*, 661.
(135) Berger, J.; Sternbach, L. H.; Muller, M.; Lasala, E. R.; Grunberg, E.; Goldberg, M. W. *Antimicrob. Agents Chemother.* **1962**, 436.
(136) Thiemann, J. E.; Coronelli, C.; Pagani, H.; Beretta, G.; Tamoni, G.; Arioli, V. *J. Antibiot.* **1968**, *21*, 525.
(137) Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem., Int. Ed.* **2002**, *41*, 1941.
(138) Moody, C. J.; Hughes, R. A.; Thompson, S. P.; Alcaraz, L. *Chem. Commun.* **2002**, 1760.
(139) Roy, R. S.; Gehring, A. M.; Milne, J. C.; Belshaw, P. J.; Walsh, C. T. *Nat. Prod. Rep.* **1999**, *16*, 249.
(140) (a) Mocek, U.; Zeng, Z.; O'Hagan, D.; Zhou, P.; Fan, L.-D. G.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7992. (b) Priestley, N. D.; Smith, T. M.; Shipley, P. R.; Floss, H. G. *Biorg. Med. Chem.* **1996**, *4*, 1135. (c) Smith, T. M.; Priestley, N. D.; Knaggs, A. R.; Nguyen, T.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1993**, 1612.
(141) Frenzel, T.; Zhou, P.; Floss, H. G. *Arch. Biochem. Biophys.* **1990**, *278*, 35.
(142) Floss, H. G.; Lee, S. *Acc. Chem. Res.* **1993**, *26*, 116.
(143) Mocek, U.; Knaggs, A. R.; Tsuchiya, R.; Nguyen, T.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7557.
(144) Pearce, C. J.; Rinehart, K. L., Jr. *J. Am. Chem. Soc.* **1979**, *101*, 5069.
(145) Fate, G. D.; Benner, C. P.; Grode, S. H.; Gilbertson, T. J. *J. Am. Chem. Soc.* **1996**, *118*, 11363.
(146) De Pietro, M. T.; Marazzi, A.; Sosio, M.; Donadio, S.; Lancini, G. *J. Antibiot.* **2001**, *54*, 1066.
(147) Ban, N.; Nissen, P.; Hansen, J.; Moore, P. B.; Steitz, T. A. *Science* **2000**, *289*, 905.
(148) Ramakrishnan, V. *Cell* **2002**, *108*, 557.
(149) Payne, D. J.; Holmes, D. J.; Rosenberg, M. *Curr. Opin. Invest. Drugs* **2001**, *2*, 1028.
(150) Knowles, D. J. C.; Foloppe, N.; Matassova, N. B.; Murchie, A. I. H. *Curr. Opin. Pharmacol.* **2002**, *2*, 501.
(151) Wilson, D. N.; Nierhaus, K. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 3464.
(152) (a) Carter, A. P.; Clemons, W. M.; Broderson, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V. *Nature* **2000**, *407*, 340. (b) Schluenzen, F.; Tocilj, A.; Zarivach, R.; Harms, J.;

Gluehmann, M.; Janell, D.; Bashan, A.; Bartels, H.; Agmon, I.; Franceschi, F.; Yonath, A. *Cell* **2000**, *102*, 615. (c) Wimberly, B. T.; Broderson, D. E.; Clemons, W. M., Jr.; Morgan-Warren, R. J.; Carter, A. P.; Vonrhein, C.; Hartsch, T.; Ramakrishnan, V. *Nature* **2000**, *407*, 327. (d) Protein Data Bank (PDB) ID: 1FFK, 1FKA, and 1FLG. Credit also to David S. Goodsell, The Scripps Research Institute for diagrams.
(153) Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455.
(154) Cundliffe, E. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 912.
(155) (a) Ryan, P. C.; Lu, M.; Draper, D. E. *J. Mol. Biol.* **1991**, *221*, 1257. (b) Conn, G. L.; Draper, D. E.; Lattman, E. E.; Gittis, A. G. *Science* **1999**, *284*, 1171.
(156) Egebjerg, J.; Douthwaite, S. R.; Liljas, A.; Garrett, R. A. *J. Mol. Biol.* **1990**, *213*, 275.
(157) Xing, Y.; Draper, D. E. *Biochemistry* **1996**, *35*, 1581.
(158) (a) Blyn, L. B.; Risen, L. M.; Griffey, R. H.; Draper, D. E. *Nucleic Acids Res.* **2000**, *28*, 1778. (b) Wimberly, B. T.; Guymon, R.; McCutcheon, J. P.; White, S. W.; Ramakrishnan, V. *Cell* **1999**, *97*, 491.
(159) Porse, B. T.; Leviev, I.; Mankin, A. S.; Garrett, R. A. *J. Mol. Biol.* **1998**, *276*, 391.
(160) Rodnina, M. V.; Savelsbergh, A.; Katunin, V. I.; Wintermeyer, W. *Nature* **1997**, *385*, 37.
(161) McConkey, G. A.; Rogers, M. J.; McCutchan, T. F. *J. Biol. Chem.* **1997**, *272*, 2046.
(162) Clough, B.; Strath, M.; Preiser, P.; Denny, P.; Wilson, I. R. J. M. *FEBS Lett.* **1997**, *406*, 123.
(163) Rogers, M. J.; Cundliffe, E.; McCutchan, T. F. *Antimicrob. Agents Chemother.* **1998**, *42*, 715.
(164) Cundliffe, E.; Dixon, P.; Stark, M.; Stöffler, G.; Ehrlich, R.; Stöffler-Meilicke, M.; Cannon, M. *J. Mol. Biol.* **1979**, *132*, 235.
(165) Wienen, B.; Ehrlich, R.; Stöffler-Meilicke, M.; Stöffler, G.; Smith, I.; Weiss, D.; Vince, R.; Pestka, S. *J. Biol. Chem.* **1979**, *254*, 8031.
(166) Bazzicalupo, M.; Parisi, B.; Pirali, G.; Polsinelli, M.; Sala, F. *Antimicrob. Agents Chemother.* **1975**, *8*, 651.
(167) Spedding, G.; Cundliffe, E. *Eur. J. Biochem.* **1984**, *140*, 453.
(168) Cundliffe, E.; Thompson, J. *Eur. J. Biochem.* **1981**, *118*, 47.
(169) Rosendahl, G.; Douthwaite, S. *Nucleic Acids Res.* **1994**, *22*, 357.
(170) Egebjerg, J.; Douthwaite, S.; Garrett, R. A. *EMBO J.* **1989**, *8*, 607.
(171) Porse, B. T.; Cundliffe, E.; Garrett, R. A. *J. Mol. Biol.* **1999**, *287*, 33.
(172) Lentzen, G.; Klinck, R.; Matassova, N.; Aboul-ela, F.; Murchie, A. I. H. *Chem. Biol.* **2003**, *10*, 769.
(173) Cundliffe, E.; Thompson, J. *Nature* **1979**, *278*, 859.
(174) Cundliffe, E. *Nature* **1978**, *272*, 792.
(175) Thompson, J.; Schmidt, F.; Cundliffe, E. *J. Biol. Chem.* **1982**, *257*, 7915.
(176) Mankin, A. S.; Leviev, I.; Garrett, R. A. *J. Mol. Biol.* **1994**, *244*, 151.
(177) Hummel, H.; Böck, A. *Biochimie* **1987**, *69*, 857.
(178) Nakanishi, N.; Oshida, T.; Yano, S.; Takeda, K.; Yamaguchi, T.; Ito, Y. *Plasmid* **1986**, *15*, 217.
(179) Smith, T. M.; Jiang, Y.-F.; Shipley, P.; Floss, H. G. *Gene* **1995**, *164*, 137.
(180) Bechthold, A.; Floss, H. G. *Eur. J. Biochem.* **1994**, *224*, 431.
(181) Thompson, J.; Cundliffe, E. *J. Bacteriol.* **1980**, *142*, 455.
(182) Tanaka, K.; Watanabe, S.; Tamaki, M. *J. Antibiot.* **1970**, *23*, 13.
(183) Watanabe, S. *J. Mol. Biol.* **1972**, *67*, 443.
(184) Tanaka, T.; Sakaguchi, K.; Yonehara, H. *J. Biochem.* **1971**, *69*, 1127.
(185) Cundliffe, E.; Thompson, J. *J. Gen. Microbiol.* **1981**, *126*, 185.
(186) Thompson, J.; Cundliffe, E.; Stark, M. J. R. *J. Gen. Microbiol.* **1982**, *128*, 875.
(187) Landini, P.; Soffientini, A.; Monti, F.; Lociuro, S.; Marzorati, E.; Islam, K. *Biochemistry* **1996**, *35*, 15288.
(188) Heffron, S. E.; Jurnak, F. *Biochemistry* **2000**, *39*, 37.
(189) Selva, E.; Montanini, N.; Stella, S.; Soffientini, A.; Gastaldo, L.; Denaro, M. *J. Antibiot.* **1997**, *50*, 22.
(190) Clough, B.; Rangachari, K.; Strath, M.; Preiser, P. R.; Wilson, R. J. M. *Protist* **1999**, *150*, 189.
(191) Möhrle, V. G.; Tieleman, L. N.; Kraal, B. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 320.
(192) Sosio, M.; Amati, G.; Capellano, C.; Sarubbi, E.; Monti, F.; Donadio, S. *Mol. Microbiol.* **1996**, *22*, 43.
(193) Holmes, D. J.; Caso, J. L.; Thompson, C. J. *EMBO J.* **1993**, *12*, 3183.
(194) Chiu, M. L.; Folcher, M.; Griffin, P.; Holt, T.; Klatt, T.; Thompson, C. J. *Biochemistry* **1996**, *35*, 2332.
(195) Chiu, M. L.; Viollier, P. H.; Katoh, T.; Ramsden, J. J.; Thompson, C. J. *Biochemistry* **2001**, *40*, 12950.
(196) Kahmann, J. D.; Sass, H.-J.; Allan, M. G.; Seto, H.; Thompson, C. J.; Grzesiek, S. *EMBO J.* **2003**, *22*, 1824.
(197) Ali, N.; Herron, P. R.; Evans, M. C.; Dyson, P. J. *Microbiology* **2002**, *148*, 381.
(198) Kelly, T. R.; Jagoe, C. T.; Gu, Z. *Tetrahedron Lett.* **1991**, *32*, 4263.
(199) (a) Higashibayashi, S.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2002**, *43*, 105. (b) Higashibayashi, S.; Kohno, M.; Goto, T.; Suzuki, K.; Mori, T.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2004**, *45*, 3707.
(200) Koerber-Plé, K.; Massiot, G. *Synlett* **1994**, 759.
(201) Umemura, K.; Tate, T.; Yamaura, M.; Yoshimura, J.; Yonezawa, Y.; Shin, C. *Synthesis* **1995**, 1423.
(202) Umemura, K.; Noda, H.; Yoshimura, J.; Konn, A.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1391.
(203) Bentley, D. J.; Fairhurst, J.; Gallagher, P. T.; Manteuffel, A. K.; Moody, C. J.; Pinder, J. L. *Org. Biomol. Chem.* **2004**, *2*, 701.
(204) Moody, C. J.; Bagley, M. C. *Synlett* **1998**, 361.
(205) Kayano, T.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2004**, *33*, 72.
(206) Bagley, M. C.; Dale, J. W.; Jenkins, R. L.; Bower, J. *Chem. Commun.* **2004**, 102.
(207) Yamada, T.; Okumura, K.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2001**, 102.
(208) Shin, C.; Okabe, A.; Ito, A.; Ito, A.; Yonezawa, Y. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1583.
(209) Endoh, N.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 643.
(210) Umemura, K.; Ikeda, S.; Yoshimura, J.; Okumura, K.; Saito, H.; Shin, C. *Chem. Lett.* **1997**, 1203.
(211) Okumura, K.; Saito, H.; Shin, C.; Umemura, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1863.
(212) (a) Ciufolini, M. A.; Shen, Y. C. *J. Org. Chem.* **1997**, *62*, 3804. (b) Suzuki, S.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2004**, *33*, 814.
(213) Nicolaou, K. C.; Safina, B. S.; Funke, C.; Zak, M.; Zécri, F. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 1937.
(214) Nicolaou, K. C.; Nevalainen, M.; Zak, M.; Bulat, S.; Bella, M.; Safina, B. S. *Angew. Chem., Int. Ed.* **2003**, *42*, 3418.
(215) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem., Int. Ed.* **2004**, *43*, 5087.
(216) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 5092.
(217) Nicolaou, K. C. Personal communication. The stereochemistry of Boc-Sec(Ph) residues, derived from L-serine, is (*R*) as shown in Scheme 10.

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