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Synthesis of saramycetic acid

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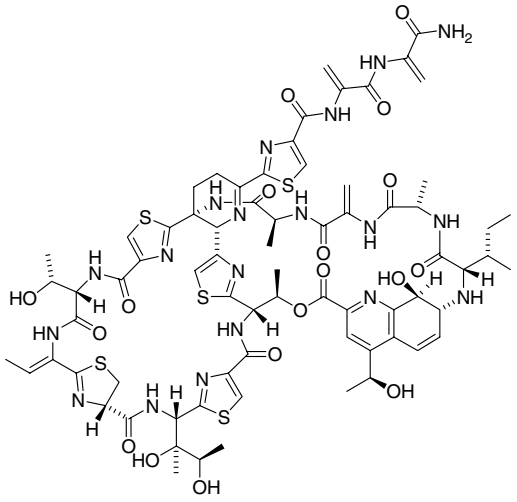
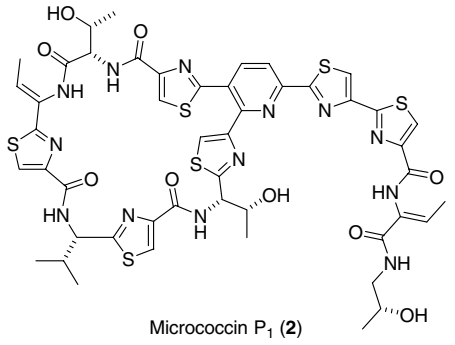
Abstract—The first reported synthesis of saramycetic acid, a degradation product of the complex thiopeptide antibiotic cyclothiazomycin, is achieved in nine steps and 11% overall yield from diethoxyacetonitrile by a strategy, which incorporates two Hantzsch thiazole syntheses using thioamides prepared from the corresponding nitriles without the use of gaseous H_2S. The synthetic material was transformed to methyl saramycetate, which had spectroscopic properties in excellent agreement with the literature data.

Thiopeptide antibiotics are a class of naturally occurring sulfur-containing, highly modified, macrocyclic peptides, nearly all of which inhibit protein synthesis in bacteria. These biologically active substances, exemplified by thiostrepton (**1**) and micrococcin P_1 (**2**) (Fig. 1),[1] 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. Due to their biological properties, including bacterial protein synthesis inhibition, antimalarial activity, renin inhibition and *tip*A promotion, they have attracted a number of biological and chemical studies.[1] Classification of the thiopeptide antibiotic families is according to their structure and, in particular, in the nature of the central heterocyclic domain. Essentially there are five distinct classes (series a–e), assigned based upon the oxidation state of the central pyridine/piperidine heterocyclic core. This domain is embedded in a macrocyclic framework of modified heterocyclic residues, including thiazoles, oxazoles, indoles and dehydroamino acids.

Micrococcin was the first example of a thiopeptide antibiotic to be recorded in 1948.[2] Despite work spanning the last 50 years, the correct stereochemistry of this thio-



Thiostrepton (**1**)



Micrococcin P_1 (**2**)

Figure 1. Thiostrepton (**1**) and micrococcin P_1 (**2**).

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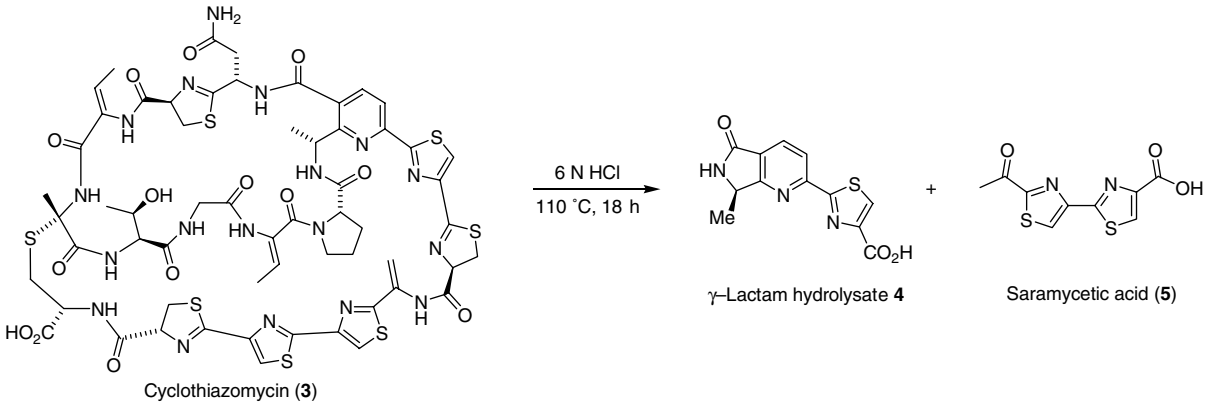
peptide remains unresolved.[3–9] However, the total synthesis of other thiopeptide antibiotics has been achieved, including promothiocin A[10,11] and amythiamicin D.[12] More recently, the remarkable laboratory preparation of the parent thiopeptide antibiotic thiostrepton was reported,[13,14] demonstrating the potential of chemical synthesis as a viable means of access and its potential for structure elucidation.

Cyclothiazomycin (**3**) is an unusual thiopeptide that possesses a number of unique structural features. It lacks the 2- and 3-azole substituents on the central domain, and instead contains an alanine derived heterocyclic residue, reportedly of (*R*)-configuration, a quaternary sulfide, and two macrocyclic polypeptide loops. Although no antibacterial data have been associated with cyclothiazomycin, which also lacks the characteristic polydehydroalanine side chain, this thiopeptide is still worthy of note as a novel and selective inhibitor of human plasma renin with an IC_{50} of 1.7 μM.[15] First 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,[16] initial structure elucidation using HR-FAB-MS, elemental analysis, and ^{1}H and ^{13}C NMR spectroscopic data, was supported by chemical degradation studies, acidic hydrolysis generating an unusual pyridine-containing γ-amino acid, lactam **4**,[17] the constitution of which has been verified by synthesis, and a curious bisthiazole motif named as saramycetic acid (**5**) (Scheme 1).[16b] Interestingly, the more recently discovered congeners cyclothiazomycin B1 and B2, isolated from *Streptomyces* sp. A307, have been shown to act as RNA polymerase inhibitors and also yield saramycetic acid (**5**) on chemical degradation.[18] It is the synthesis of bisthiazole **5** and confirmation of its structure that is the subject of this work.

Over the last two decades, a number of unique natural products containing directly linked thiazoles have been isolated from natural sources. Many of these compounds are interesting leads for drug development and have therefore drawn the attention of a number of research groups. For example, bisthiazoles can be found within the side chain of a large number of other thiopeptide antibiotics, including micrococcin **2**, all of the thiocillins I, YM-266183, YM-266184, QN3323A, QN3323B and QN3323Y, and in the macrocyclic loops of Ef–Tu binders such as the amythiamicins, GE2270 factors (A, Fig. 2, **6**) and GE37468A.[1] Furthermore, isolation of a family of antifungal cystothiazoles including the potent cystothiazole A (Fig. 2, **7**) from the culture broth of the myxobacterium *Cystobacter fuscus* has been reported.[19] Structurally related to the cystothiazoles are the myxothiazoles[20] and melithiazoles,[21] along with a group of glycopeptide antibiotics isolated from the microorganism *Streptomyces verticillus*.[22] In particular, bleomycin A_2 (Fig. 2, **8**) is the major component of the anticancer drug bleonoxane, which has found clinical use in combination chemotherapy for the treatment of a range of cancer related illnesses.[23]

Saramycetic acid (**5**), also described as 2-(2-acetylthiazol-4-yl)-4-thiazolecarboxylic acid, was isolated as long ago as 1967 from saramycetin, an unidentified antifungal antibiotic.[24] In order to consolidate our previously reported method for constructing thioamides[25] from the corresponding nitriles without the use of gaseous H_2S and in order to validate an approach towards cyclothiazomycin (**3**), as part of our interest in the total synthesis[17,26] of thiopeptide antibiotics, we set out to design and implement a facile route to saramycetic acid (**5**), the acid hydrolysate of the cyclothiazomycins.

Our linear strategy assembled each heterocyclic component in consecutive transformations with subsequent elaboration of the acetyl group following acetal deprotection and subsequent ester hydrolysis to provide the functionalized bisthiazole unit of **5**. Starting with 2,2-diethoxyacetonitrile (**9**), treatment with ammonium sulfide in methanol at room temperature according to our recently reported procedure gave thioamide **10** in excellent yield.[25] Thiazole **11** was prepared by reaction with ethyl bromopyruvate in ethanol under Hantzsch conditions. Saponification using lithium hydroxide in methanol–water gave carboxylic acid **12**, which was converted by ammonolysis in tetrahydrofuran, following derivatization with ethyl chloroformate in the presence of triethylamine, using saturated ammonia solution to give amide **13**. In a more direct approach, amide **13** was gen-



Scheme 1. Degradation products of cyclothiazomycin (**3**).

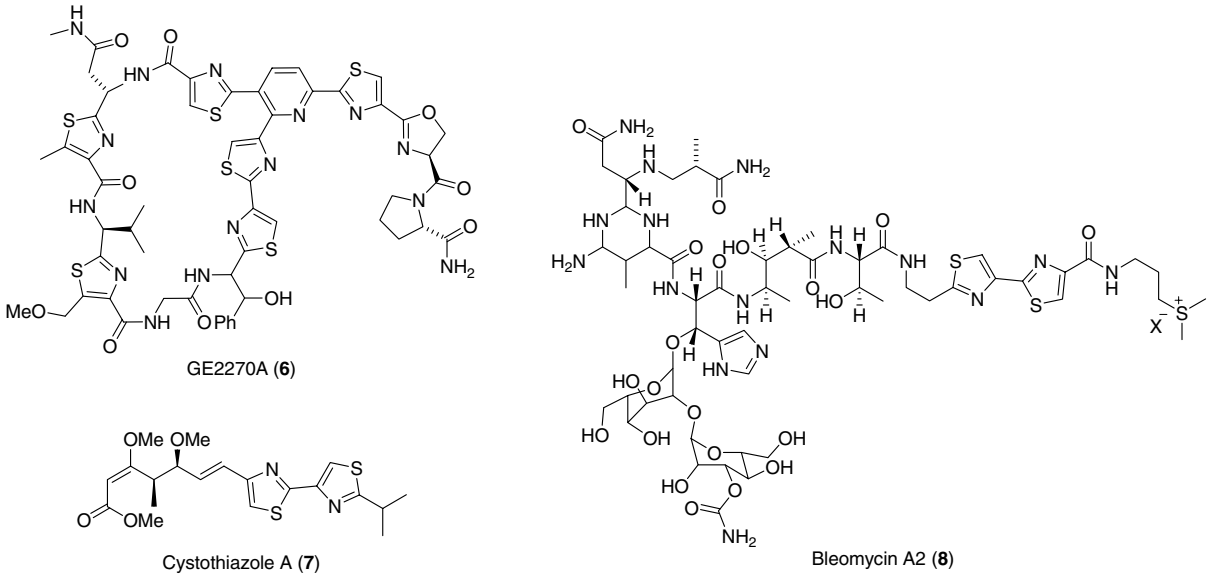
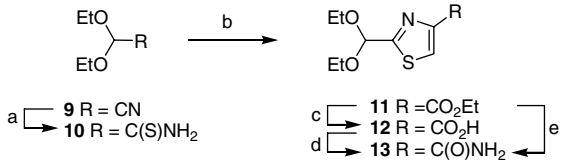


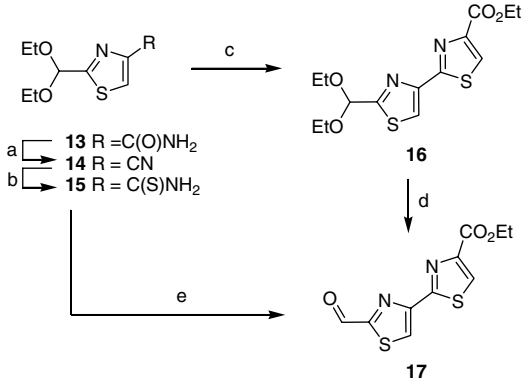
Figure 2. Bisthiazole-containing natural products.



Scheme 2. Hantzsch thiazole synthesis. Reagents and conditions: (a) $(NH_4)_2S$, MeOH, rt, 18 h (100%); (b) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (c) LiOH, MeOH–H_2O, rt, 18 h (64%); (d) ethyl chloroformate, Et_3N, THF, 0 °C, 1 h; then NH_4OH, 0 °C, 1 h (85%); (e) MeOH–NH_3, 45 °C, 7 days (65%).

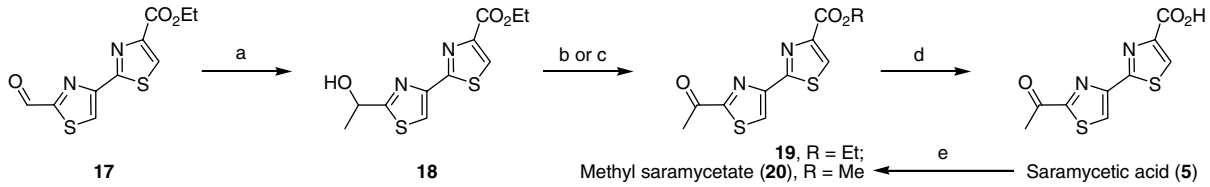
erated in one step from thiazole **11** by treatment with saturated methanolic ammonia solution, improving the overall yield and optimizing the route (Scheme 2).

Although this represented a useful building block towards the synthesis of saramycetic acid (**5**), the conversion of amide **13** to the corresponding thioamide using Lawesson's reagent was unsuccessful giving only a complex mixture of products. In order to circumvent these problems with the electrophilic thionation of amide **13**, and to explore the versatility of our reported procedure for thionation of nitriles,[25] amide **13** was reacted with phosphorus oxychloride by stirring in pyridine at room temperature for 2 h to give nitrile **14** in good yield. Gratifyingly, when nitrile **14** was treated with ammonium sulfide in methanol at room temperature for 18 h, thioamide **15** was isolated in quantitative yield, with no need for purification by column chromatography. Furthermore, when thioamide **15** was reacted according to our Hantzsch thiazole procedure,[27] bisthiazole **16** was obtained after simple extraction in quantitative yield. Deprotection of acetal **16** was achieved in good yield to give aldehyde **17** (Scheme 3). Following this success, a one pot, two step procedure was investigated for the transformation of thioamide **15** to the corresponding aldehyde. Again thioamide **15** was reacted according to our Hantzsch thiazole procedure and after evaporation in vacuo the residue was dissolved in a solution of acetone–aqueous hydrochloric acid (2 M) and stirred at reflux for a further 1.5 h. Agreeably, after a simple aqueous work up in dichloromethane, aldehyde **17** was isolated in excellent yield, providing a more direct route to saramycetic acid (**5**) without compromising the yield (Scheme 3).



Scheme 3. Synthesis of bisthiazole **17**. Reagents and conditions: (a) $POCl_3$, pyridine, 0 °C to rt, 2 h (83%); (b) $(NH_4)_2S$, MeOH, rt, 18 h (100%); (c) ethyl bromopyruvate, EtOH, reflux, 1 h (100%); (d) 2 M HCl (aq), acetone, reflux, 1 h (87%); (e) ethyl bromopyruvate, EtOH, reflux, 1 h, then 2 M HCl (aq), acetone, reflux, 1.5 h (100%).

Grignard addition with methylmagnesium bromide in dichloromethane at 0 °C for 18 h gave the desired methyl alcohol **18** in modest yield. Secondary alcohol **18** was oxidized to the corresponding ketone **19** using either manganese dioxide in dichloromethane or *o*-iodoxybenzoic acid (IBX) in dimethylsulfoxide. Hydrolysis of **19** by stirring with a slight excess of lithium hydroxide in a solution of methanol–water for 18 h, gave saramycetic acid (**5**) in a total of nine steps and 11% overall yield (Scheme 4). The spectroscopic data of the synthetic material [^{1}H NMR (DMSO-d_6) δ 8.85



Scheme 4. Synthesis of saramycetic acid (**5**). Reagents and conditions: (a) MeMgBr, CH_2Cl_2, 0 °C to rt, 18 h (41%); (b) MnO_2, CH_2Cl_2, rt, 40 min (52%); (c) IBX, DMSO, rt, 1 h (50%); (d) LiOH, MeOH–H_2O, 18 h (100%); (e) MeOH, AcCl, 80 °C, 6 h (67%). rt = room temperature.

(0.18H, s, 5′-H), 8.82 (0.82H, s, 5′-H), 8.71 (0.18H, s, 5-H), 8.63 (0.82H, s, 5-H), 2.77 (3H, s, Me); ^{13}C NMR (125 MHz; DMSO-d_6) δ 191.4 (C), 167.9 (C), 162.4 (C), 161.7 (C), 149.8 (C), 148.8 (C), 130.1 (CH), 125.8 (CH), 26.3 (Me); *m*/*z* (APCI) 255 (MH^+, 40%); UV (MeOH)/nm λ_{max} 220 (logε 4.29), 291 (logε 4.14)] were in reasonable agreement with the literature data from the degradation product [^{1}H NMR (DMSO-d_6) δ 8.97 (1H, s, 5-H), 7.92 (1H, s, 5-H), 2.70 (3H, s, Me); ^{13}C NMR (DMSO-d_6) δ 189.6, 165.4, 162.4, 158.4, 157.5, 122.7, 120.7, 24.4; UV (MeOH)/nm λ_{max} 218 (logε 4.32), 291 (log ε 4.16)][15,18] with notable differences in ^{1}H NMR spectroscopic analyses in line with previous observations.[18] To resolve these discrepancies, **5** was esterified according to the reported procedure to give methyl saramycetate (**20**) with identical spectroscopic properties to those previously reported.[18] This comparison confirmed the outcome of the degradation experiments[15,18,24] and the structure of saramycetic acid (**5**) and provides a viable route to bisthiazoles for application in the synthesis of a range of natural products in the future.

Acknowledgements

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