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Synthesis of the Bycroft–Gowland Structure of Micrococcin P1

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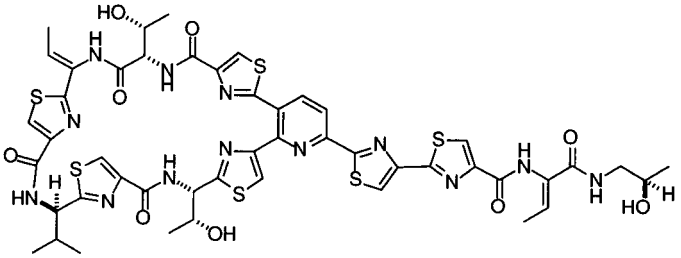
ABSTRACT



We describe the chemical synthesis of the accepted structure of micrococcin P1, a member of the thiostrepton group of antibiotics, and we show that this architecture does not correspond to that of the natural product. Methods developed during the present study should greatly facilitate ongoing efforts centering on the determination of the actual structure of microccin P1, in addition to being applicable to the synthesis of more complex thiostrepton congeners.

Micrococcin P[1] is a cytotoxic agent that expresses its activity by strongly inhibiting protein synthesis.[2] This substance is one of the structurally simpler members of the thiostrepton group of antibiotics,[3] and it is obtained as a mixture of two components termed micrococcins P1 (MP1) and P2 (MP2). These differ in that a D-(*R*)-(−)-1-amino-2-propanol (D-isoalaninol)[4,5] segment present in in the side chain of MP1 (major component) becomes an aminoacetone unit in MP2 (minor).

The structure of micrococcins P was originally assigned by Walker, Lukacs, and collaborators as **1** (Figure 1).[6] However, this architecture proved to be untenable and it was revised to **2** by Bycroft and Gowland[7] on the basis of extensive degradation studies. Bycroft and Gowland were unable to assign the stereochemistry of the threonine-derived thiazole bound to the pyridine. Nonetheless, other researchers have often represented that molecular subunit as having an L-threonine-like stereochemistry, as apparent, e.g., from recent papers describing synthetic studies on micrococcins.[8] Tacit acceptance of this assignment appears to have ensued.

(1) Isolation from *Bacillus pumilus*: Fuller, A. T. *Nature* **1955**, *175*, 722. Micrococcin P is very similar, possibly identical, to an antibiotic isolated from a *Micrococcus* species and named "micrococcin" by: Su, T. L. *Brit. J. Exp. Path.* **1948**, *29*, 473. No structural work on the Su micrococcin appears to have been described in the literature.

(2) (a) Rosendahl, G.; Douthwaite, S. *Nucl. Acids Res.* **1994**, *22*, 357. (b) Cundliffe, E.; Thompson, C. J. *Eur. J. Biochem.* **1981**, *118*, 47.

(3) Review: Pestka, S. In *Antibiotics*; Corcoran, J. W.; Hahn, F. E., Eds.: Springer-Verlag: New York, 1975; Vol. 3, p 480 ff.

(4) Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1960**, 909.

(5) The side chain amino alcohol was originally assigned as alaninol (ref 4) and later ascertained to be isoalaninol (ref 6). In either case, the *levo* isomer is the one of D-(*R*)-configuration: Klyne, W. Buckingham, J. *Atlas of Stereochemistry: Absolute Configurations of Organic Molecules*, 2nd ed.; Oxford University Press: New York, 1978.

(6) (a) Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706. (b) Reference 6a, p 708, top of the page.

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

(8) (a) Nakamura, Y.; Shin, C.-G.; Umemura, K.; Yoshimura, J. *Chem. Lett.* **1992**, 1005. (b) Okumura, K.; Shigekuni, M.; Nakamura, Y.; Shin, C.-G. *Chem. Lett.* **1996**, 1025.

(9) (a) Murakami, T.; Holt, T. G.; Thompson, C. T. *J. Bacteriol.* **1989**, *171*, 1459. (b) Holmes, D. G.; Caso, J. L.; Thompson, C. T. *EMBO J.* **1993**, *8*, 3183. (c) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 510.

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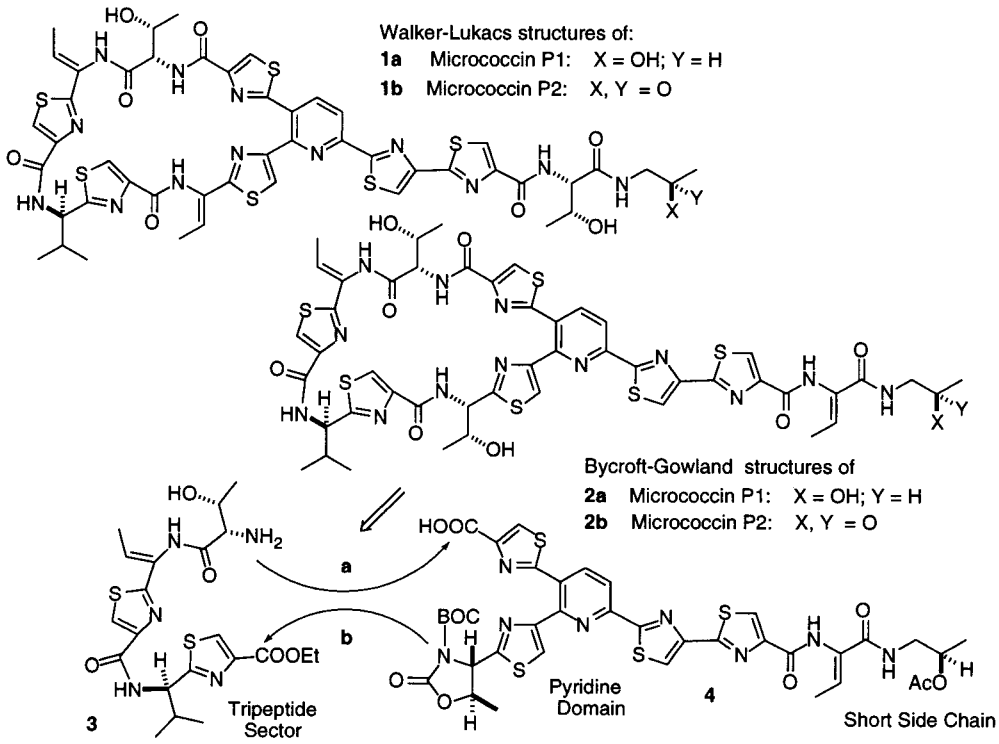
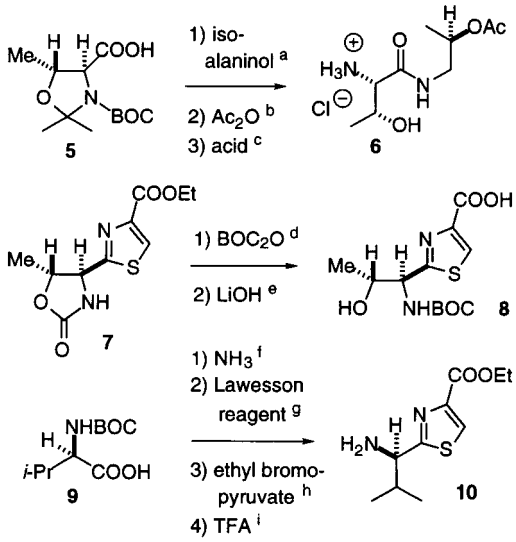


Figure 1. Proposed structures of micrococcins P1 and P2 and retrosynthetic dissection thereof.

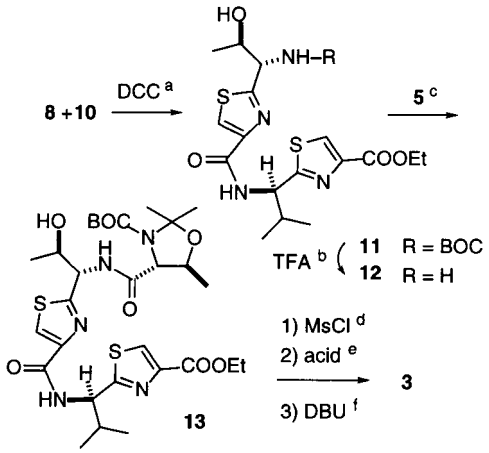
Several members of the thiostrepton family induce expression of genes of unknown function in *Streptomyces* species,[9] an observation that has stimulated much interest in the molecular biology arena.[10] This behavior has not yet been reported for **2**, but because all thiostrepton substances display similar biological properties, it seems likely that MP1/MP2 may also be gene inducers. Despite these exciting observations, little synthetic work has been recorded in the thiostrepton area.[11,12] Recently, Shin has announced the synthesis of two substances described as micrococcin P[13] and micrococcin P1.[14] However, these synthetic compounds are *epimers* of the Walker−Lukacs structure **1a** and of the Bycroft−Gowland structure **2a**, respectively, in that L-(*S*)-

Scheme 1[a]



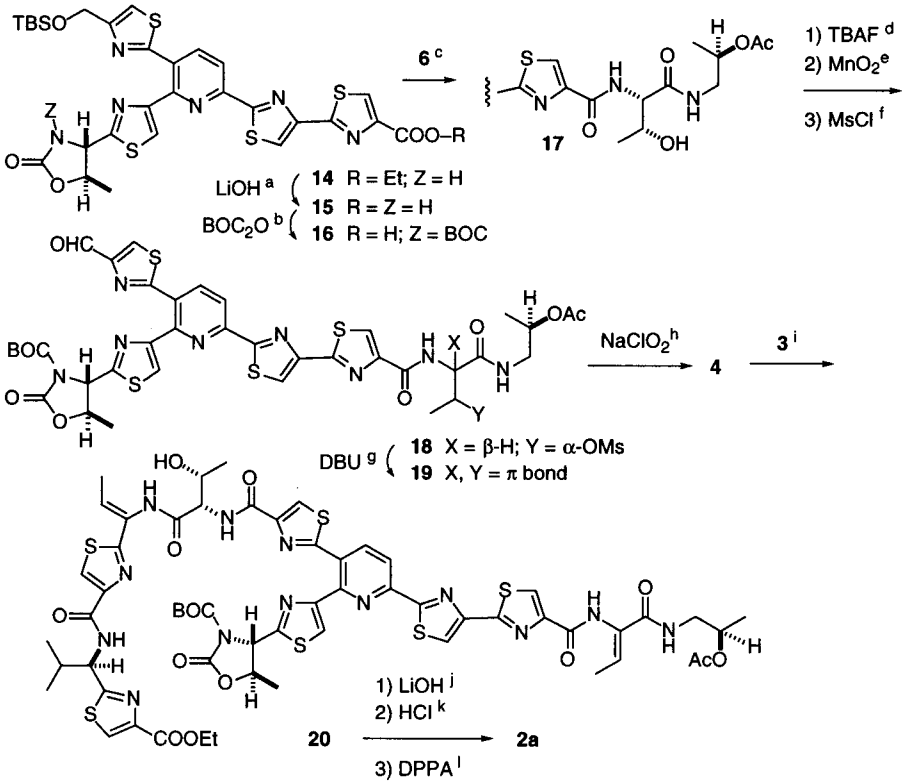
[a] (a) (*R*)-isomer, DCC, CH_2Cl_2; (b) pyridine, 96% a−b; (c) TFA, CH_2Cl_2, then aqueous HCl, 93%; (d) catalytic DMAP, Et_3N, CH_2Cl_2, 99%; (e) aqueous THF, 99%; (f) EtOOCCl, Et_3N, CH_2Cl_2, then aqueous NH_3, 94%; (g) PhH, reflux; (h) $KHCO_3$, DME, then TFAA, pyridine, 0 °C, 72% f−h; (i) CH_2Cl_2, 99%.

Scheme 2[a]



[a] (a) (a) CH_2Cl_2, 91%; (b) CH_2Cl_2, 99%; (c) DCC, CH_2Cl_2, 97%; (d) Et_3N, CH_2Cl_2, (e) first TFA, CH_2Cl_2 (−BOC), then 0.2 N aqueous HCl, THF; (f) $CHCl_3$, 88% d−f.

Scheme 3[a]



[a] Reagents and conditions: (a) aqueous THF; (b) DMAP, Et_3N, CH_2Cl_2; (c) BOP-Cl, Et_3N, CH_3CN, 84% a−c; (d) THF; (e) EtOAc; (f) CH_2Cl_2, Et_3N; (g) $CHCl_3$, 68% d−g; (h) 2-Me-2-butene, NaH_2PO_4, THF, H_2O; (i) BOP-Cl, Et_3N, CH_3CN, 76% h−i; (j) aqueous THF; (k) gas, dioxane; (l) Et_3N, 0.02 M in DMF, 0 °C, 36 h, 65% j−l.

(+)-isoalaninol, and not the D-(*R*)-(−)-isomer, is present as a subunit of the side chains. In addition, the work appears to rest on the assumption that micrococcin P is **1a** and micrococcin P1 is **2a**. This would be inaccurate in light of refs 1−7. Until this confusion is resolved, and until synthetic materials are compared with natural **2a**, Shin's important work probably should not be regarded as embodying the total synthesis of any known natural products.

Herein, we report the total synthesis of the Bycroft−Gowland structure of micrococcin P1, **2a**. The target molecule may be considered as the result of fusion of a tripeptide-like sector, **3**, with subunit **4**. A number of key observations[15] directed us to manufacture segments **6**, **8**, and **10** as shown in Scheme 1. Precursor **6** to the short side chain was made from the known[16] threonine derivative **5** and commercial (*R*)-(−)-isoalaninol. The previously described **7**,[12] a building block for **14**, was subjected to simultaneous hydrolysis of oxazolone[17] and ester units, leading to acid **8**. Amine **10** was obtained from L-valine by thiazole formation under Meyers conditions.[18] The merger of **8** and **10** was effected with DCC, and the resulting **11** was N-deblocked and condensed with **5**. Mesylation of the emerging **13**,

(10) E.g.: (a) McConkey, G. A.; Gogers, M. J.; McCutchan, T. F. *J. Biol. Chem.* **1997**, *272*, 2046. (b) Clough, B.; Strath, M.; Preiser, P.; Denny, P.; Wilson, I. *FEBS Lett.* **1997**, *406*, 123. (c) Chiu, M. L.; Folcher, M.; Griffin, P.; Holt, T.; Klatt, T.; Thompson, C. J. *Biochemistry* **1996**, *35*, 2332. (d) Lu, M.; Draper, D. E. *Nucleic Acids Res.* **1995**, *23*, 3426. (e) Rosendahl, G.; Douthwaite, S. *J. Mol. Biol.* **1993**, *234*, 1013. (f) Egebjerg, J.; Douthwaite, S. R.; Liljas, A.; Garrett, R. A. *J. Mol. Biol.* **1990**, *213*, 275 and references therein.

(11) For pioneering work in this area, see: Kelly, T. R.; Jagoe, C. T.; Gu, Z. *Tetrahedron Lett.* **1991**, *32*, 4263 as well as refs 8 and 13.

(12) Ciufolini, M. A.; Shen, Y.-C. *J. Org. Chem.* **1997**, *62*, 3804.

(13) Shin, C.-G.; Okamura, K.; Shigekuni, M.; Nakamura, Y. *Chem. Lett.* **1998**, 139.

(14) Okamura, K.; Ito, A.; Yoshioka, D.; Shin, C.-G. *Heterocycles* **1998**, *48*, 1319.

(15) A route to an intermediate differing from **3** only at the level of protecting groups has been described (ref 8a), but the present objectives proved to be better served by an alternative avenue to that fragment. The merger of **3** and **4** was best conducted by contributing the pyridine−thiazole cluster as compound **14** (ref 13), by having the short side chain already present on the pyridine−thiazole array during macrocyclization; by forming the bonds between **3** and **4** in the order **a** first and then **b**, by introducing dehydroamino acid units as threonines to be dehydrated at a well-defined stage; and by performing the macrocyclization via an acyl azide.

(16) E.g.: (a) Favara, D.; Omodei-Sale, A.; Consonni, P.; Depaoli, A. *Tetrahedron Lett.* **1982**, *23*, 3105. (b) Garner, P. *Tetrahedron Lett.* **1984**, *25*, 5855.

(17) (a) Flynn, D. L.; Zelle, R. E.; Grieco, P. A. *J. Org. Chem.* **1983**, *48*, 2424. (b) Ishizuka, T.; Kunieda, T. *Tetrahedron Lett.* **1987**, *28*, 4185.

(18) Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2473. Application of this protocol was essential to suppress racemization of **10**.

(19) Attenburrow, J.; Cameron, A. F. B.; Chapman, J. H.; Evans, R. M.; Hems, B. A.; Jansen, A. B. A.; Walker, T. *J. Chem. Soc.* **1952**, 1094.

(20) Cf. Kraus, G. A.; Taschner, M. J. *J. Org. Chem.* **1980**, *45*, 1175.

(21) It is noteworthy that the elimination succeeded only when the terminal threonine was fully deblocked (oxazoline formation occurred with the blocked compound) and that the mesylate intermediate showed little propensity to undergo intramolecular S_N2 reaction with the free amino group. Presumably, the rigidly defined *s-trans* conformation of the threonine amide greatly disfavored approach of the NH_2 group to the mesylate backside.

deprotection of the threonine subunit, and DBU treatment gave **3** (Scheme 2).[21]

Base hydrolysis of **14**, introduction of an *N*-BOC group on the oxazolone, and coupling of free acid **16** with **6** resulted in installation of the short side chain. The diol obtained by *O*-desilylation of **17** underwent chemoselective oxidation of the benzylic-type carbinol to an aldehyde with Attenburrow MnO_2.[19] Mesylation of the sole expressed alcohol gave **18**, DBU elimination of which provided **19**. Oxidation of the aldehyde to the free acid was effected with $NaClO_2$,[20] and the complete segment **4** thus obtained was condensed with **3** under the influence of BOP-Cl. The emerging **20** was subjected to deblocking and macrocyclization with DPPA to afford the target structure in 65% chromatographed yield (Scheme 3). The longest linear sequence in our synthesis of **2a** thus requires 23 steps from glycolonitrile, and the overall yield along this pathway is 5.7%.

Spectra of our synthetic material were very similar, but not identical, to those obtained from natural micrococcin P. The accepted structure of the natural product must thus be incorrect. The location of individual heterocycles in the pyridine−thiazole cluster [22] and the absolute configuration of fragment **10**,[23] of the isoalaninol,[4−6] and of the L-threonine[24] components of micrococcin are secure. However, attentive scrutiny of the micrococcin literature unravels uncertainties regarding not only the stereochemistry of the threonine-derived thiazole in **14**/**4** but also the order in which **8**, **10**, and L-threonine are arranged within the macrocycle. Indeed, Walker and Lukacs proposed the arrangement of **8**, **10**, and L-threonine as depicted in **1** on the basis of a presumed structural similarity between micrococcin P, thiostrepton, and nosiheptide, but alternative permutations of the three subunits could not be ruled out.[6b] The Bycroft correction focused primarily on the structure of the side chain, but it assumed that the topography of the macrocycle was correct. Any error in the Walker structure would thus have been propagated to the Bycroft−Gowland one.

Work directed toward the resolution of structural issues is currently underway in our laboratory. These efforts are hampered by the extreme rarity of micrococcin P1, but furtunately, the chemical methodology developed in the course of the present study should greatly assist us in these endeavors. It also seems plausible that principles and techniques demonstrated herein should be applicable to the synthesis of more complex thiostrepton substances and of chemically modified variants thereof. Hopefully, this will stimulate additional interest in these remarkable natural products.

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Supporting Information Available: Experimental procedures and spectral data for selected compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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(22) Ascertained by X-ray crystallography: James, M. N. G.; Watson, K. J. *J. Chem. Soc. C* **1966**, 1361.

(23) Dean, B. M.; Mijovic, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394.

(24) Brookes, P.; Fuller, A. T.; Walker, J. *J. Chem. Soc.* **1957**, 689.