Total Synthesis

DOI: 10.1002/anie.200900621

Total Synthesis and Stereochemical Assignment of Micrococcin P1**

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This paper describes the total synthesis of the thiopeptide antibiotic micrococcin P1 (MP1, **1**; Figure 1),[1] thereby establishing its constitution and its configuration. Compound **1** is

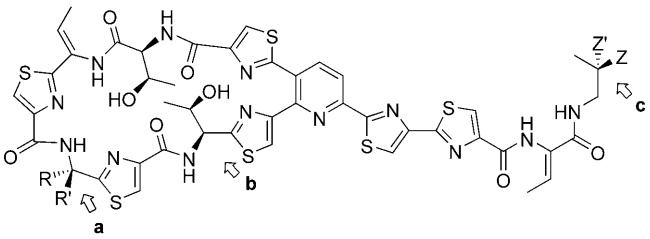

Figure 1. Actual structure of micrococcin P1 (**1**): R = *i*Pr, R′ = H; Z = OH, Z′ = H. Actual structure of micrococcin P2 (**2**): R = *i*Pr, R′ = H; Z, Z′ = O. Bycroft–Gowland structure of MP1 (**4**): R = H, R′ = *i*Pr; Z = OH, Z′ = H. Synthetic "micrococcin P1"[13b,d] (**6**): R = H, R′ = *i*Pr; Z = H, Z′ = OH.

the major component of "micrococcin P", a cytotoxic extract isolated from *Bacillus pumilus* that consists of a roughly 7:1 mixture of **1** and of the corresponding ketone, **2**, which is termed micrococcin P2 (MP2). MP1 binds tightly to ribosomes, thereby disrupting protein synthesis.[2] The compound thus exerts a potent antibiotic activity toward microorganisms,[3] including the malarial parasite *Plasmodium falciparum*.[4]

While MP1[5] is one of the structurally less complex thiopeptides,[6] its precise structure has remained uncertain for over 50 years. The constitution of the central pyridine–thiazole cluster was firmly established by X-ray diffractometry.[7] Important work by Walker and Mijovic ascertained that the 1-amino-2-propanol segment of **1** (see Figure 2, region **c**) has the D-(*R*) configuration[8] and that MP1 incorporates a L-threonine unit.[9] Walker et al. also advanced the hypothesis[10] that the valine-derived thiazole in region **a** of the molecule had the (*R*) configuration, thus implying that the thiazole in question is a formal derivative of D-valine. This would make MP1 unique among thiopeptide antibiotics, all of which incorporate thiazole segments derived from L-amino

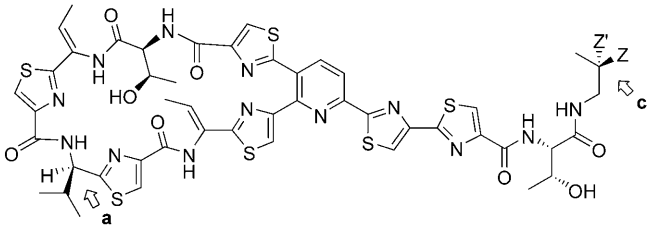

Figure 2. Walker–Lukacs structure of MP1 (**3**): Z = OH, Z′ = H. Synthetic "micrococcin P"[13a,c] (**5**): Z = H, Z′ = OH.

acids. On the basis of these data and of a presumed similarity with other thiopeptides, in 1977 Walker and Lukacs proposed structure **3** for MP1 (Figure 2),[11] but without the benefit of evidence in support of the alleged topography of the macrocycle. Shortly thereafter, new chemical evidence induced Bycroft and Gowland to promulgate the revised structure **4** (Figure 1).[12] The latter authors were unable to assign the configuration of region **b** of the molecule, which, correctly, they left undefined. Moreover, they also left unresolved the issue of macrocycle topography. Errors possibly present in the Walker assignment thus propagated to the revised structure, which nonetheless gained tacit acceptance and gradually came to be consistently represented with the configuration shown.

Remarkably, past synthetic work has been unable to resolve the structural uncertainties surrounding MP1. Indeed, synthetic epimers of the Walker–Lukacs (see compound **5**, Figure 2)[13a,c] and of the Bycroft–Gowland (see **6**, Figure 1)[13b,d] structures have both been stated to be identical to the natural product. Not only the two structures are mutually exclusive: they also possess the (*S*) configuration, instead of the secure (*R*) configuration, at **c**.[14] Even more problematic is the fact that synthetic **4** (Figure 1) is not identical to natural MP1.[15]

Extensive NMR studies ultimately confirmed the Bycroft–Gowland constitution of MP1,[16] and by default that of MP2, ruling out the possibility that MP1 may be **3** or **5**, and implying that the difference between **4** and natural MP1 must be purely stereochemical. While spectroscopic methods failed to unravel the relative configuration of the natural product, incisive work by Bagley and Merritt[17] led to the conclusion that MP1 is likely to be **1**. Total synthesis now confirms this surmise.

The retrosynthetic logic that directed the construction of **1** is delineated in Scheme 1. Experience had revealed the necessity of minimizing chemical operations after macrocycle formation. Accordingly, MP1 would emerge upon the union of a pair of suitably COOH- and NH_2-protected segments, **A** and **B**. Past experience had also shown that macrocyclization was facile only if the order of bond formation was (a) first, then (b). In turn, each segment was accessible by means of the

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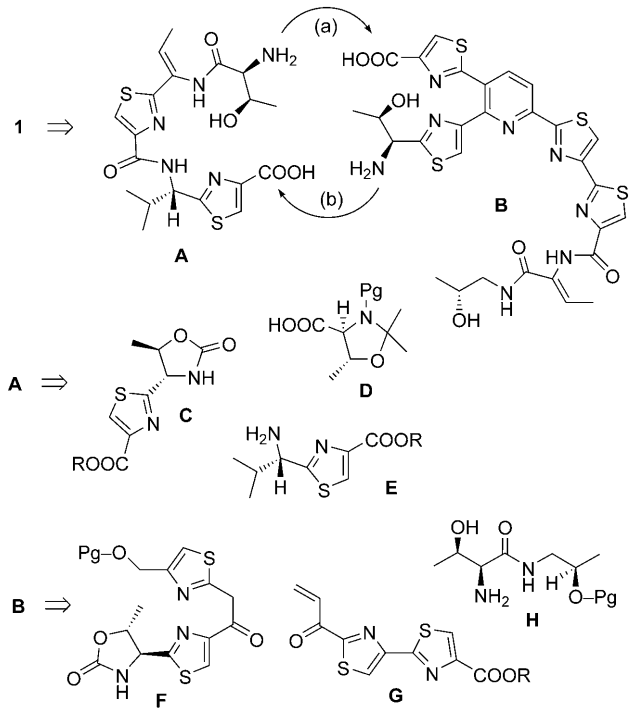
[**] We gratefully acknowledge support for this research from the University of British Columbia, the Canada Research Chair program, NSERC, CIHR, and MerckFrosst Canada.

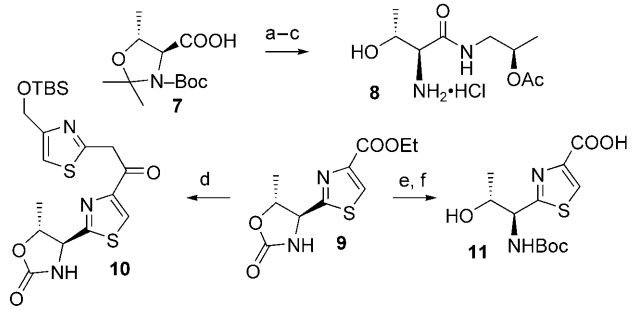
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200900621.



Scheme 1. Retrosynthetic disconnection of micrococcin P1 (**1**) into fragments **C**–**H**. Pg = protecting group.

fusion of a triad of appropriately protected subunits: **C**–**E** for **A**; **F**–**H** for **B**.

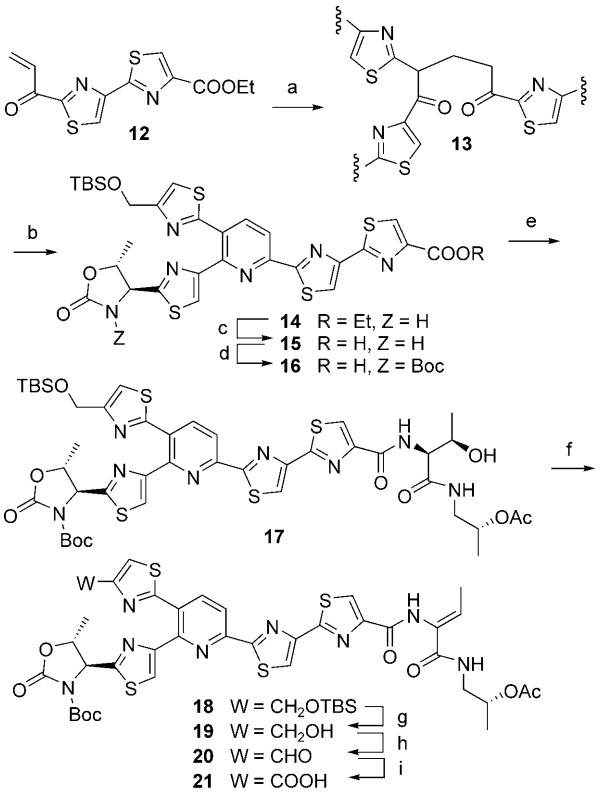
Building blocks **8**, **10**, and **11** were thus prepared from the known **7**[5a,15] and **9**[18] as previously described (Scheme 2).[15] A challenging aspect of the synthesis of **1** was the assembly of the central pyridine–thiazole cluster, an objective that is best attained through a Hantsch-type pyridine construction proceeding through the merger of **10** with **12**.[19] The proclivity of **12** to undergo base-promoted polymerization precluded the implementation of traditional procedures for the initial Michael reaction leading to intermediate **13** (Scheme 3).



Scheme 2. Synthesis of fragments **8**, **10**, and **11**. a) DCC, (*R*)-isoalaninol, CH_2Cl_2, RT, overnight; b) Ac_2O, DMAP, pyridine, 2 h, 85% a–b; c) 4 N HCl in dioxane, 20 min, then addition of H_2O, 15 min, 100%; d) 3 equiv of 2-(lithiomethyl)-4-(*tert*-butyldimethylsilyloxy)methylthiazole, THF, −78 °C, 81%; e) Boc_2O, Et_3N, DMAP, 99% (crude); f) LiOH, 50% aq. THF, then acidification to pH 3 with NaH_2PO_4 sol., 95% (crude). TBS = *tert*-butyldimethylsilyl, DCC = *N*,*N*′-dicyclohexylcarbodiimide, DMAP = 4-dimethylaminopyridine, Boc = *tert*-butoxycarbonyl.

Thus, the union of **10** and **12** could be achieved only through the use of a heterogeneous catalytic system comprising powdered Li_2CO_3 in EtOAc (Scheme 3).[18] The resultant **13** was converted into **14**, and the latter was then advanced to the complete pyridine core of MP1, **21**.

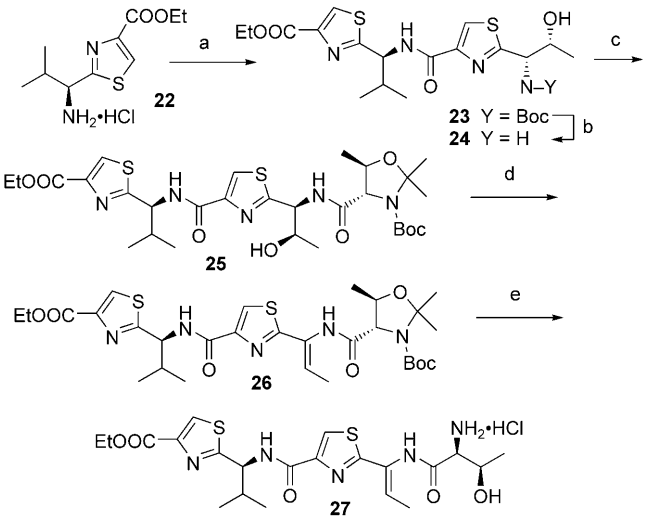
Parallel work reached **27** through the sequence outlined in Scheme 4. Owing to the propensity of valine-derived thiazole



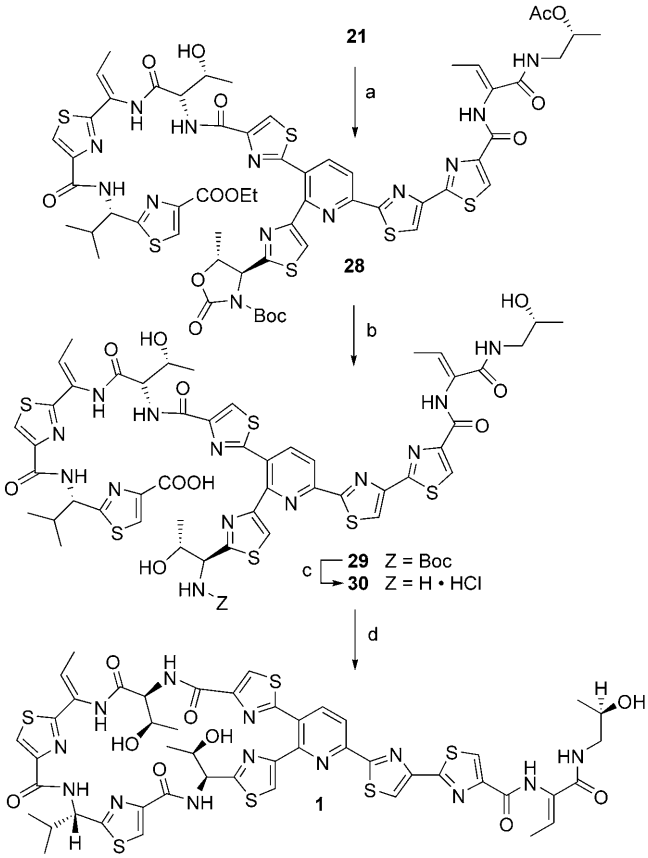
Scheme 3. Construction of the pyridine–thiazole cluster of MP1. a) **10**, cat. Li_2CO_3, EtOAc, 92%; b) NH_4OAc, EtOH then DDQ, toluene, 97%; c) LiOH, H_2O, THF; d) Boc_2O, DMAP, Et_3N, DCM; e) **8**, BOP-Cl, Et_3N, CH_3CN, 77% over 3 steps (c–e); f) MsCl, Et_3N, then DBU, DCM; g) TBAF, THF; h) Dess–Martin periodinane, $NaHCO_3$, DCM, 88% over three steps (f–h); i) $NaClO_2$, 2-methyl-2-butene, NaH_2PO_4, THF, H_2O, 84%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DCM = dichloromethane, BOP-Cl = bis(2-oxo-3-oxazolindinyl)phosphinic chloride, MsCl = mesityl chloride, DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TBAF = tetrabutylammonium fluoride.

22[20] and of its derivatives to racemize/epimerize,[21] the stereochemical integrity of each intermediate in this sequence was ascertained by ^{13}C and ^{19}F NMR scrutiny of Mosher derivatives. No racemization/epimerization occurred during subsequent transformations. This was also apparent from the ^1H NMR spectra of intermediates **23**, **25**, and **26**, wherein a single diastereomer was discernible.

The final sequence of the synthesis (Scheme 5) commenced with the coupling of **21** and **27** to furnish **28**, which subsequently underwent deblocking and macrocyclization (reaction with DPPA).[22] This produced compound **1** contaminated with a byproduct of unknown structure and with similar chromatographic characteristics. This contaminant appeared to be present also in an aged sample of natural



Scheme 4. Synthesis of segment **27** of MP1. a) **11**, HOBt, DCC, CH_2Cl_2, 84%; b) 4 M HCl in dioxane, 100%; c) **7**, DCC, CH_2Cl_2, 81%; d) MsCl, Et_3N, CH_2Cl_2, then DBU, 93%; e) 4 N HCl in dioxane, then H_2O, THF, 100%. HOBt = 1-hydroxy-1*H*-benzotriazole.



Scheme 5. Total synthesis of micrococcin P1. a) BOP-Cl, Et_3N, **27**, MeCN, 73%; b) LiOH, THF/H_2O (1:1); c) 4 N HCl in dioxane; d) DPPA, Et_3N, DMF, 24 h, 41% over 3 steps (b–d). DPPA = diphenylphosphoryl azide.

micrococcin P1.[23] At this time, we believe that the unknown material is likely to be a product of dehydration of the threonine-derived thiazole segment comprising region **b** of the molecule (see Scheme 2). In any event, purification of synthetic **1** was accomplished by HPLC. Purified **1** was chromatographically (HPLC, TLC) indistinguishable from authentic micrococcin P1, and its optical rotation {$[\alpha]_D^{25} = +68°$ (90% aq. EtOH, $c = 0.45\ g\,cm^{-3}$; lit. $[\alpha]_D^{21} = +63.7°$ ($c = 1.19\ g\,cm^{-3}$, 90% aq. EtOH)[24]} and ^1H and ^{13}C NMR spectra are coincident with those of authentic MP1. This established the identity of **1** to the natural product.

In summary, chemical synthesis has now settled the structural ambiguities that have surrounded micrococcin P1 during more than fifty years since its discovery. The methods detailed herein are applicable to a number of other synthetically appealing thiopeptide antibiotics, and developments in this domain will be the subject of future reports.

Received: February 2, 2009
Published online: April 30, 2009

Keywords: antibiotics · heterocycles · micrococcin P1 · thiopeptides

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