A DIVERSITY-ORIENTED ROUTE TO MICROCOCCINS P1 AND P2 FOR MEDICINAL CHEMISTRY INVESTIGATIONS

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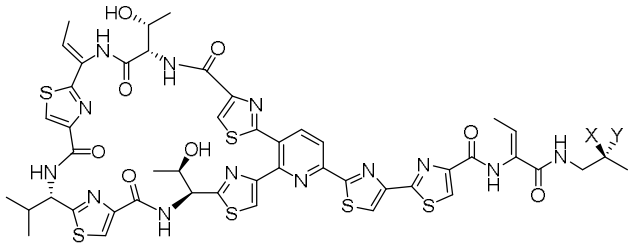
Abstract. *Micrococcins P1 and P2 are thiopeptide natural products with noteworthy antibacterial activity. This renders them of interest as starting points for the development of new antibiotics. Micrococcins and their congeners appear to be especially promising for the treatment of Clostridioides difficile and Mycobacterium tuberculosis infections. The development of antimicrobial agents against such organisms requires a practical, robust, diversity-oriented synthesis serviceable for medicinal chemistry studies. This contribution outlines a synthetic route that satisfies the foregoing requirements.*

Contents

1. Introduction

The thiopeptides are a family of about 90 structurally complex, sulfur-rich natural products possessing a range of noteworthy biological activities.[1] The first thiopeptide ever isolated is "micrococcin P" (MP),[2] an antibiotic complex subsequently[3] shown to consist of a mixture of micrococcin P1 **1** (MP1, major component), and micrococcin P2 **2** (MP2) (Scheme 1).[4]



1 Micrococcin P1 X=H, Y=OH
2 Micrococcin P2 X,Y=O

Scheme 1. Structures of micrococcins P1 **1**, and P2 **2**.

Micrococcins, like virtually all other thiopeptides, exhibit potent antimicrobial activity against Gram-positive organisms, but they are largely ineffective against Gram-negative ones.[5] Still, a number of Gram-positive bacteria are currently of significant concern.[6] An example is drug resistant, hypervirulent *Clostridioides difficile*. This organism causes severe, possibly life-threating, gastrointestinal (GI) tract

infections, which become especially problematic in patients suffering from inflammatory bowel disease (IBD) comorbidity.[7]

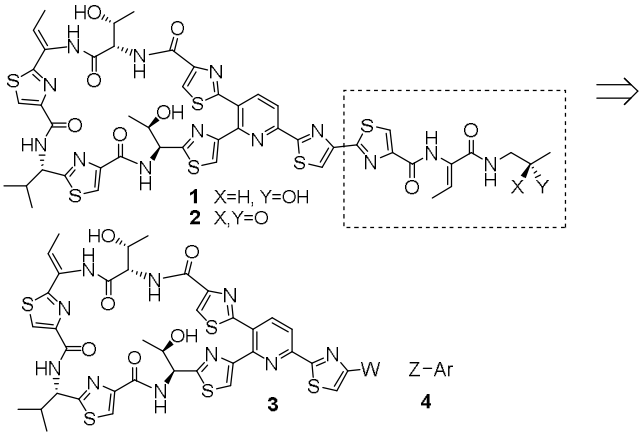
In spite of the ongoing "antibiotic crisis"[8] thiopeptides have remained largely unexploited as sources of new anti-infective agents for use in human medicine.[9,10] This may be partly because of poor aqueous solubility and GI absorption, but it will be seen shortly that such properties may constitute an advantage in particular applications. Other reasons include chemical complexity hampering medicinal chemistry work and persistent structural uncertainties for many members of the family.

In that regard, micrococcin are among the structurally simpler thiopeptides. Their constitution and configuration, uncertain for over 60 years, were unequivocally established by total synthesis in 2009.[11] Their elevated antimicrobial potency against a number of pathogens renders them excellent platforms for the development of new antibiotics. Such an endeavor would be greatly facilitated if a straightforward, economical, scalable, diversity-oriented synthesis might be devised, that would enable the exploration of the structure-activity relationship (SAR) of **1**-**2**. Indeed, it is difficult to modify the natural products in connection with SAR work, leaving total synthesis as a better option.

A visionary pharmaceutical company in the Republic of Korea, A&J Science, recently became interested in micrococcin P2 and its congeners as potential anti-infective agents. It was surmised that the keto group in **2** could provide access to various imino derivatives (oximes, hydrazones *etc.*) with desirable pharmacological properties. However, micrococcin P2 typically constitutes only about 12% of natural MP complex, and its separation from the more abundant MP1 is less than straightforward. A campaign was thus launched to devise a diversity-oriented synthesis of **2** that would provide ample material for antibacterial evaluation, as well as analogues for SAR exploration. A number of routes to MP1 had been recorded in the literature at the onset of such an endeavor,[11,12] but none of MP2.[13] These landmark syntheses were designed to produce MP1 specifically; therefore, they are less than ideal for the conduct of SAR studies. A desire to develop a more suitable avenue in accord with the foregoing principles provided the impetus behind the research described in this article.

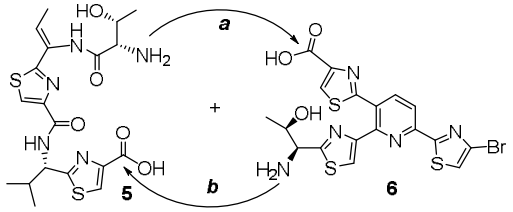
2. Early results and retrosynthetic considerations

Initial experiments with materials obtained by minor modifications of published syntheses revealed that the intact macrocyclic portion of MP1-MP2 is essential for activity, while variability is tolerated at the level of the portion in the dotted box (Scheme 2). It thus was desirable to devise a synthesis involving the coupling of macrocycle **3** with appropriate aryl segments **4** in order to explore the SAR of MP. Substituents W and Z in **3** and **4** would be complementary in the context of a transition metal-mediated coupling reaction. For instance, Suzuki-Miyaura reaction[14] would require W=Br, and Z=$B(OR)_2$, or vice versa. In the latter case, W=$B(OR)_2$ could be introduced by Miyaura borylation[15] of a bromothiazole.



Scheme 2. Retrosynthetic logic for MP1 and MP2.

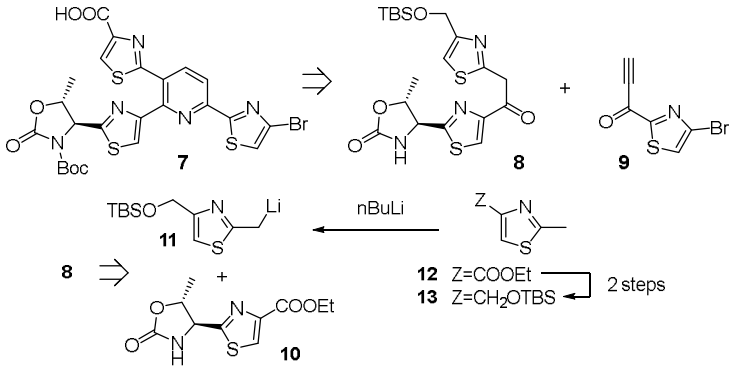
In either case, what was needed was a form of **3** wherein W=Br. Such a compound would be created though the union of fragments **5** ("tripeptide segment") and **6** ("pyridine domain") (Scheme 3). In that respect, past experience[4] had shown that bond ***a*** must be formed first, followed by ***b***, because the reverse order of bond formation leads to inefficient macrocycle formation.



Scheme 3. Fragments required for macrocycle construction.

It should be apparent that such a plan came with a number of unknows. First and foremost, it was far from clear that any form of **3** would be a competent substrate for transition metal-mediated coupling reactions. The numerous ligation sites present in **3** may well sequester the metal catalyst, shutting down the catalytic cycle. In a like vein, the prognosis for the feasibility of the Miyaura borylation of the bromothiazole was uncertain at best. Yet, this is precisely what makes a synthetic route worthy of exploration. The successful resolution of perils, roadblocks, and difficulties not only leads to the desired target, but it also produces new methodology and pushes the boundary of present knowledge beyond the current horizon.

The published route to a suitably protected version of **5** was easily scalable. In contrast, the assembly of appropriate forms of **6** required considerable refinement of past approaches. Observations recorded during the synthesis of MP1[4,11] and the structurally related thiocillin I[16] led to the initial selection of **7** as variant of **6** (Scheme 4). An especially efficient avenue to **7** entails Bohlmann-Rahtz reaction[17] of ketone **8** with ynone **9**, available from commercial 4-bromothiazole-2-carboxaldehyde (*vide infra*). Ketone **8** can be made by reaction of organolithium species **11** with ester **10**. Three equivalents of **11** are necessary in this step: one to act as the nucleophile, one to deprotonate the relatively acidic oxazolidinone, and a third one to deprotonate the nascent **8**, which exhibits a C-H acidity comparable to that of a β-ketoester.



Scheme 4. Early synthetic avenue to fragment **7**.

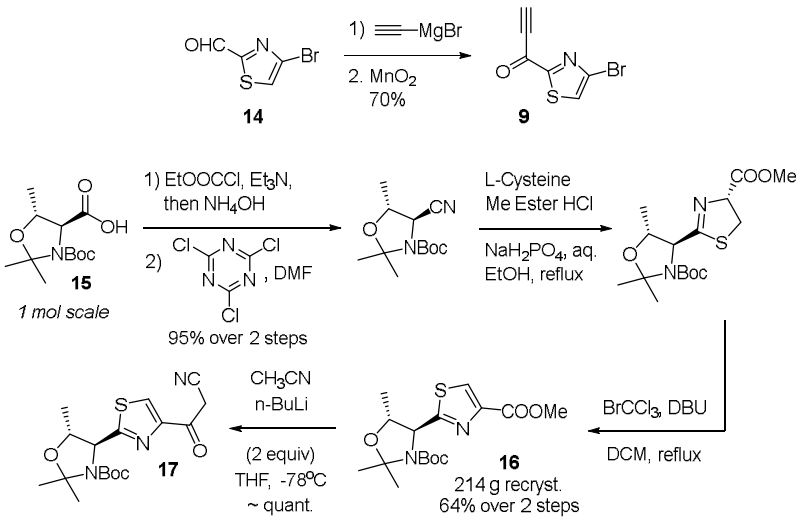
Such a route soon proved to be unsuitable. First, organometallic agent **11** is prepared *in situ* by deprotonation of methylthiazole **13**, which in turn arises from the corresponding ester **12**. It was not possible to generate a well-behaved organometallic directly from **12**, imposing the need for ester reduction and *O*-silylation prior to deprotonation. However, the CH_2OTBS group would later have to be reconverted into a carboxylic functionality. The resulting redox- and protection-deprotection sequences added five steps to the route to **7**. Second, the emerging **8** was accompanied by two equivalents of methylthiazole **13**, which had to be removed by chromatography. Yet, it was desirable to avoid chromatographic operations at such an early

stage. Third, the Bohlman-Rahtz union of **8** with **9** occurs in the presence of glacial acetic acid. In the course of the reaction, the TBS group is released and the liberated OH reacts with AcOH to form the corresponding acetate ester. The acetate form of the Bohlmann-Rahtz pyridine is unsuitable for the conduct of subsequent operations, and it had to be converted back to the silyl ether version in two steps. The use of a sturdier TIPS group in lieu of a TBS diminished the extent of the problem, but it soon became evident that a process that required all such redox, protection-deprotection, and chromatographic operations was utterly unsustainable. A key subgoal was thus the development of a robust synthesis of the pyridine domain.

3. A robust synthesis of the pyridine domain

Several of the problems outlined in Section 2 above would vanish if the "northwestern" thiazole in the pyridine domain were to be installed by condensation of a corresponding cyanopyridine with cysteine, by what may be legitimately termed a White-Siegel thiazole synthesis.[18] This would eliminate five redox and protection-deprotection steps from the sequence leading to the pyridine domain. The Bohlmann-Rahtz reaction would then employ a cyanoketone analogue of **8**, eliminating the problem with silyl group loss and acetylation of the liberated alcohol. Additionally, the requisite cyanoketone can be prepared by reaction of a substrate of the type **10** with the anion of acetonitrile. Unreacted MeCN would then be removed by evaporation, suppressing the need for chromatographic purification. Finally, the use of an analogue of **10** lacking an acidic N–H bond would eliminate the need for of the three equivalents of nucleophile. All such principles were incorporated in the new route to pyridine domain variant **20** outlined herein.

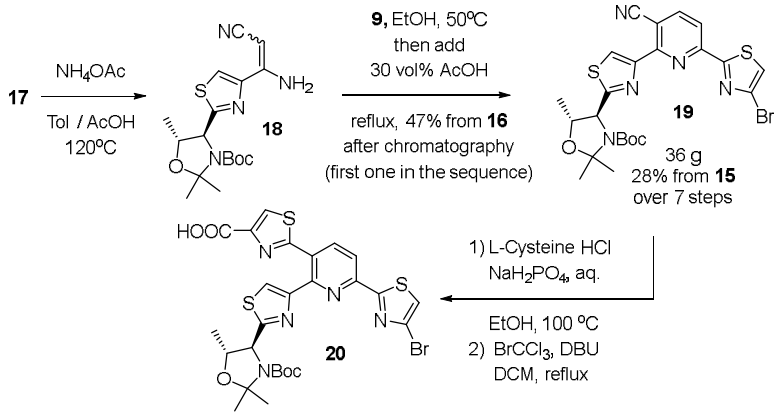
The synthesis of **20** started with the preparation of Bohlmann-Rahtz partners **9** and **17** (Scheme 5). The avenue to **9** from commercial **14** was straightforward and requires no comment. Conversely, the established route to compound **16** by conversion of commercial **15** to the corresponding thioamide, followed by reaction with ethyl bromopyruvate (Hantzsch thiazole synthesis),[19] proved to be poorly scalable. This was due to technical problems associated with the use of Lawesson's reagent[20] for thioamide formation. Fortunately, all such difficulties vanished when a White-Siegel reaction was employed for thiazole formation. The sequence leading to **16** from **15** was readily carried out on a 1 mol scale, and it is currently being scaled up further. As anticipated, the condensation of **16** with 2 equivalents of the anion of MeCN occurred smoothly and in quantitative yield, and the emerging **17** required no chromatographic purification.



Scheme 5. Scalable synthesis of Bohlmann-Rahtz partners **9** and **17**.

The Bohlmann-Rahtz union of the two fragments (Scheme 6) started with the conversion of **17** to vinylogous cyanamide **18** and subsequent condensation thereof with **9**. Such a reaction was best carried out by heating a mixture of **18** and **9** in EtOH to induce 1,4-addition of the enamine to the ynone, followed by

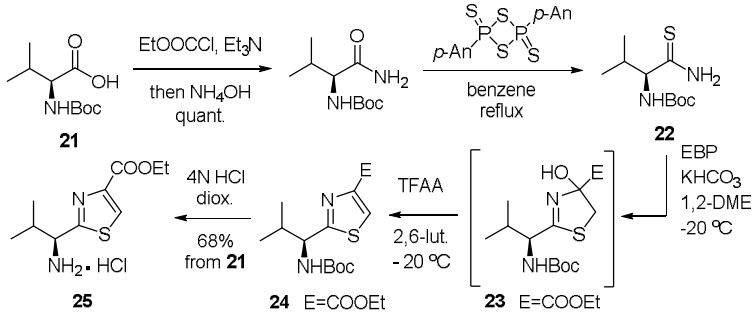
addition of glacial AcOH and further heating to promote cyclization of the adduct to the desired pyridine **19**. The latter was purified to homogeneity by chromatography: the first one in the sequence. A subsequent White-Siegel thiazole synthesis furnished the complete pyridine domain **20**, which required no purification, and that was ready for coupling with the tripeptide segment. The new approach to **20** thus realized significant step- and atom economies.



Scheme 6. Synthesis of pyridine domain **20**.

4. Synthesis of a protected version of tripeptide 5

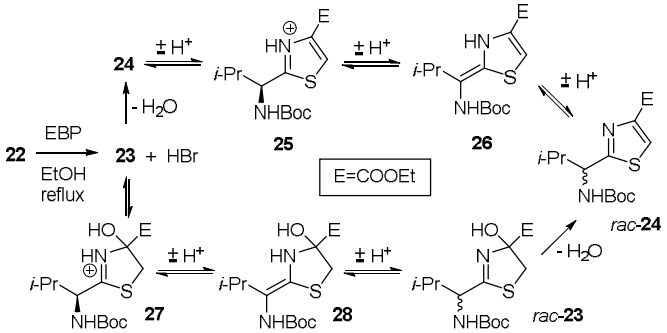
Tripeptide **5** was best employed in the form of the corresponding ethyl ester, the synthesis of which entailed the merger of subunits **15**, **16**, and **25**. Large quantities of the latter were obtained by the Holzapfel-Meyers-Nicolaou ("HMN") variant[21] of the Hantzsch reaction (Scheme 7). Thus, *N*-Boc L-valine **21**, was elaborated to thioamide **22**, which, in crude form, was caused to react with ethyl bromopyruvate (EBP) at –20 °C in the presence of $KHCO_3$ to give the sensitive thiazoline **23**. *In situ* dehydration of **23** with trifluoroacetic anhydride (TFAA) and 2,6-lutidine gave optically pure **24**. In contrast, reaction of **22** with EBP under classical Hantzsch conditions (EtOH, reflux), is known to provide essentially racemic product.[21] Boc group release with 4 M HCl in dry dioxane furnished ammonium salt **25** in quantitative yield. We generally favor HCl in dioxane over the customary trifluoroacetic acid (TFA)/CH_2Cl_2 for Boc group release, in that deprotection completes in less than 10 minutes, and workup is considerably simplified (vacuum removal of volatiles).



Scheme 7. Synthesis of valine-derived thiazole **25**.

A comment is in order at this juncture. Loss of α-configuration upon reaction of an aminoacid-derived thioamide such as **22** with EBP under traditional Hantzsch conditions must be an acid-catalyzed process, because HBr is released as the reaction progresses, and because conduct of the reactions in the presence of

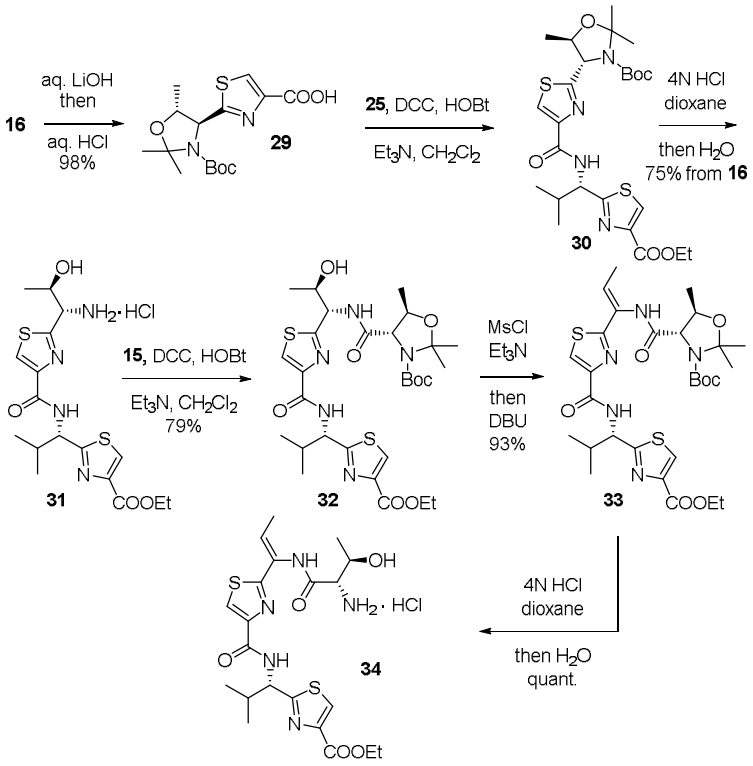
$KHCO_3$ (HMN conditions) suppresses stereochemical ablation. In principle, acid-catalyzed loss of configuration could take place at the stage of either thiazole **24** or thiazoline **23**, through reversible *N*-protonation to **25** and **27**, respectively, followed by prototropic equilibration with **26** or **28** (cf. *rac*-**24** and *rac*-**23**) (Scheme 8). The question of whether racemization occurs primarily at the stage of **23** or **24** appears to be not fully settled. The fact that no erosion of stereochemical integrity was detected upon scrutiny of ^{1}H, ^{13}C and ^{19}F NMR spectra of Mosher amide derivatives[22] of **25** and related thiazoles, all of which arose upon treatment of the corresponding Boc carbamates with the strong acid, HCl/dioxane, lends support to the suggestion that the problem is likely to occur at the stage of the thiazoline.[23] It should also be noted that acid-promoted equilibration of a protonated thiazole such as **25** with **26** incurs loss of aromatic character, whereas the analogous reaction of a protonated thiazoline does not. Acid-promoted racemization of the thiazole may thus be anticipated to be less facile than that of the thiazoline on electronic grounds as well.



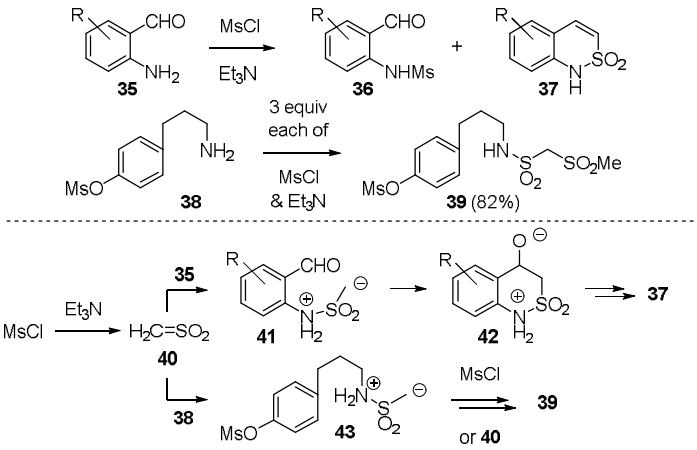
Scheme 8. Loss of configuration during Hatzsch reaction of aminoacid-derived thioamides.

The merger of segments **15**, **16**, and **25** took place as shown in Scheme 9. Amide formation between **25** and acid **29** produced **30**, treatment of which with anhydrous HCl in dioxane, followed by addition of some water (acetonide hydrolysis) yielded **31** in 75% yield overall from **16**. No epimerization of labile nitrogen-bearing stereogenic centers occurred during deprotection, providing additional evidence for the stereochemical solidity of aminoacid-derived thiazoles under acidic conditions. Subsequent coupling of **31** with **15** delivered **32** in 79% yield after rough chromatography. Mesylation of **32** resulted in formation of the expected mesylate, accompanied by traces of **33**, which is the desired end product. Interestingly, conduct of the mesylation reaction in the presence of excess Et_3N, or addition of several equivalents thereof into the reaction mixture containing the mesylate intermediate, promoted no elimination to **33**. However, addition of excess DBU resulted in slow (12 h) but complete conversion of the mesylate into **33**, obtained in 93% yield after chromatography. A final treatment of **33** with anhydrous 4M HCl in dioxane, followed by addition of water, extricated the aminoalcohol segment from its protective webbing, resulting in quantitative formation of **34** with no scrambling of the configuration of epimerizable centers and no adverse effect upon the dehydroamino acid segment.

Another diversion is necessary at this point. It is interesting to speculate as to why some elimination occurred during mesylation of **32**, even though Et_3N had virtually no effect on the mesylate. Evidently, elimination must occur through a mechanism other than Et_3N-promoted E2 or E1cb reaction. Two observations recorded earlier in this group provide a hint. Thus, reaction of 2-aminobenzaldehydes **43** with MsCl/Et_3N produced variable amounts of cyclic sulfonamides **37** besides the expected **36**,[24] while reaction of **38** with excess MsCl and Et_3N afforded **39** in 82% yield[25] (Scheme 10). A plausible mechanism for both reactions starts with the consensus[26] Et_3N-mediated dehydrochlorination of MsCl to sulfene **40**, followed by nucleophilic capture of the latter. In the case of **35**, the resulting **41** may cyclize rapidly to **42**, which can advance to **37** in a number of ways. It is worthy of note that treatment of **36** with *t*-BuOK or NaH failed to produce **37**, none of which was observed when the mesylation of **35** was carried out with MsCl/pyridine, consistent with the fact that this second reaction probably involves Ms group transfer from an *N*-sulfonylpyridinium ion.[26] In the case of **38**, reaction of intermediate **43** may react rapidly with intact MsCl or another molecule of **40**, leading to the ultimate **39**.



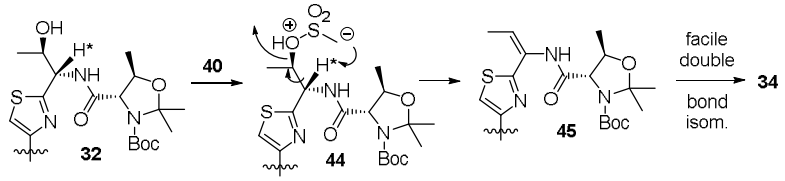
Scheme 9. Synthesis of tripeptide **34**.



Scheme 10. Mechanistic hypothesis for the unusual course of the mesylation of **35** and **38**.

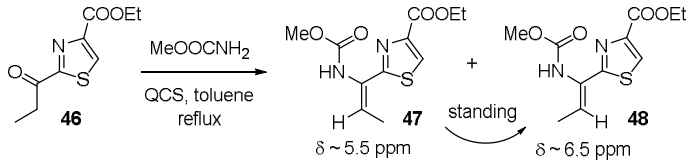
The foregoing suggests that the formation of some **33** upon mesylation of **32** may occur as shown in Scheme 11. Addition of the OH group to sulfene **40** produces zwitterionic intermediate **44**, which is primed to undergo elimination by a pericyclic mechanism. *Syn*-elimination is likely to be especially facile in the present case, because the thiazole ring greatly enhances the acidity of the starred proton. Such a process

would yield the thermodynamically disfavored *E*-isomer **45** of the alkene, isomerization of which to the more stable *E*-isomer **34** occurs readily.



Scheme 11. Mechanistic hypothesis for the formation of **34**.

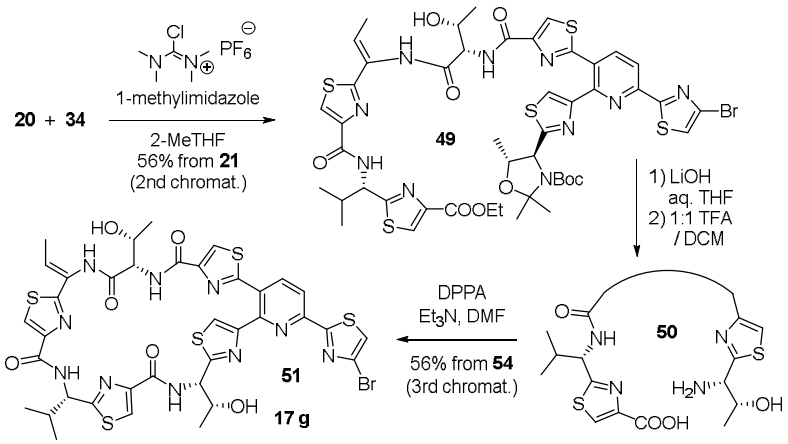
The ease of double bond isomerization was uncovered during early stages of our efforts toward micrococcin.[27] It was unclear at that point whether the dehydroaminoacid-like features of the natural products were of *E* or *Z* geometry. The following experiment determined that the configuration of the double bonds was the thermodynamic one, and that the incorrect isomer isomerized readily to the good one. Thus, treatment of ketone **46** with methyl carbamate in refluxing toluene in presence of quinolinium *p*-toluenesulfonate (QCS) afforded a mixture of enecarbamates **47** and **48** (Scheme 12). A notable difference between the two isomers was the NMR chemical shift (DMSO-d_6) of the broad quartet corresponding to the olefinic proton. In one of the products, that H appeared at ca. 6.5 ppm, very close to the signals of the olefinic H's in MP1, while in the other, it resonated at ca. 5.5 ppm. On standing, the signal of the upfield proton became less intense, and ultimately it disappeared, leaving only the peak at 6.5 ppm.



Scheme 12. Facile isomerization of *E*-enecarbamate **47** to the *Z*-isomer **48**.

5. Assembly of the macrocycle and Suzuki-Miyaura reactions thereof

The merger of fragments **20** and **34** produced macrocycle **51**: a version of **3** where W=Br (Scheme 13).

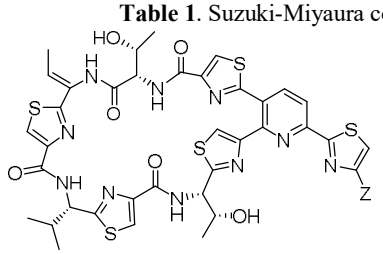


Scheme 13. Synthesis of macrocycle **51**.

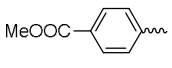
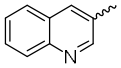
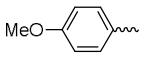
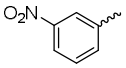
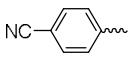
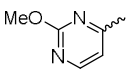
The first amide bond, established in the past using BOP-Cl as the condensing agent,[28] was more efficiently formed in the presence of tetramethyl chloroformamidinium hexafluorophosphate (TCFH).[29] This coupling agent resulted in a much faster reaction that was complete in under 15 minutes. Mono-seco intermediate **49** thus emerged in 56% yield after chromatography (the second one in the sequence) over 3 steps from cyanopyridine **19**. Full deprotection and cyclization of crude **50** in the presence of diphenylphosphorylazidate (DPPA),[30] the best reagent yet identified for such a transformation, afforded **51** in 56% yield after chromatography (the third one in the sequence) over 3 steps from **49**. Thus, the route to macrocycle **51** involved 13 linear steps from **15**, and it proceeded with an overall yield of just over 10%. More than 17 grams of pure end-product emerged from the first run of the sequence, which is currently being scaled up (Scheme 13).

Trepidation regarding the competence of **51** as a substrate for Suzuki-Miyaura reaction was rapidly allayed. Indeed, coupling with arylboronic acids to furnish products **52** proceeded quite well, as apparent from the representative results of Table 1. It is stressed that these are the first examples of Suzuki coupling with a fully formed thiopeptide macrocycle. On a different note, The reactions of Table **1** employed [bis(di-*tert*-butylphosphino)ferrocene]palladium dichloride [Pd(dtbpf)Cl_2] as the catalyst. This complex afforded slightly higher yields (5-8%) relative to $(Ph_3P)_4Pd$. However, the more economical $(Ph_3P)_4Pd$ may be more attractive for routine work.

Table 1. Suzuki-Miyaura coupling reactions of **51**.



51 Z=Br
52 Z=Ar
Ar-B(OR)$_2$, K_2CO_3
10 mol % Pd(dtbpf)Cl_2
aq. THF, 60 °C, 12h

Ar				
Entry	**a**	**b**	**c**	**d**
% yield[a]	70	67	65	70
Ar				
Entry	**e**	**f**	**g**	**h**
% yield[a]	68	64	62	61

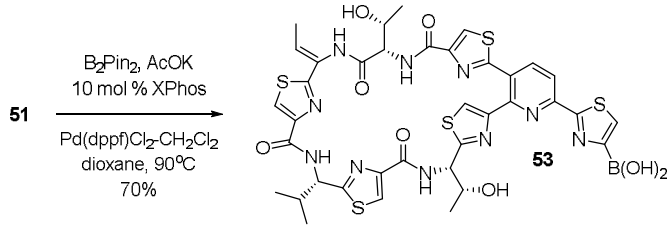
[a]After chromatography

Initial attempts to translate the above results into a synthesis of **1**-**2** focused on the preparation of suitable 2-borylthiazoles. Unfortunately, such organometallics are elusive species,[31] and indeed, numerous approaches to their preparation consistently met with failure.[32] Turning then to a reversal of Suzuki roles, it was found that **51** undergoes efficient Miyaura borylation to **53** (Scheme 14), opening the door to coupling reactions with diverse aryl halides. Compound **53** is rather sensitive and it is expedient to employ it in crude form: purification is best postponed until after subsequent Suzuki steps.

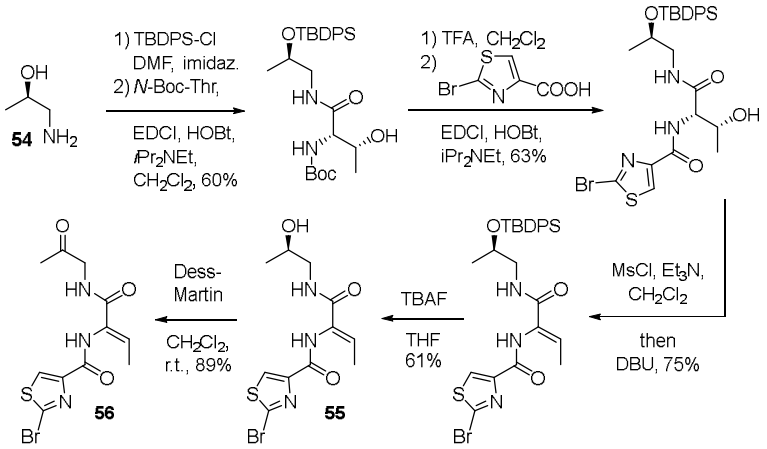
6. Total synthesis of MP1, MP2, and congeners

The final offensive toward **1**-**2** commenced with the preparation of compounds **55** and **56** from commercial (*R*)-isoalaninol **54**, *N*-Boc threonine, and 2-bromothiazole-4-carboxylic acid (Scheme 15). The route to **55**-**56** was straighforward and requires no discussion, beyond the fact that optimization of the

various reactions (some yields are on the low side) was postponed to a more opportune time. It is recognized that **55** carries the entire side chain portion of MP1, and **56** of MP2.



Scheme 14. Miyaura borylation of macrocycle **51**.



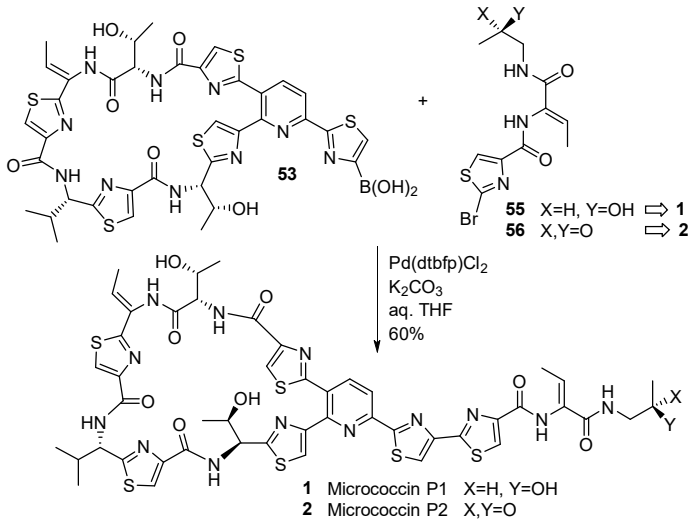
Scheme 15. Synthesis of bromothiazoles **55**-**56**.

Crude boronic acid **53** combined smoothly with **55** and **56**, leading to MP1 and MP2, respectively, in 60% chromatographed yield in either case (Scheme 16). Synthetic **1** was spectroscopically identical to the natural product. However, spectra of pure, natural **2** are not available. While little doubt existed about the identity of MP2 thus obtained, the following experiment laid all remaining concerns to rest. Treatment of synthetic **2** with $NaBH_4$ in MeOH returned a 1:1 mixture of two diastereomeric alcohols. Interestingly, the NMR spectra of this mixture in DMSO-d_6, in which micrococcins are highly soluble, failed to reveal the presence of two diastereomers, due to overlap of all signals. Fortunately, relevant resonances were well separated in spectra recorded in $CDCl_3$, which, however, is a poor solvent for the natural products. The two diastereomeric alcohols were not chromatographically separable, even by HPLC, but one set of resonances coincided with those of pure MP1 in $CDCl_3$, while the other was attributed to (unnatural) *epi*-MP1. This confirmed that synthetic **2** was indeed micrococcin P2.

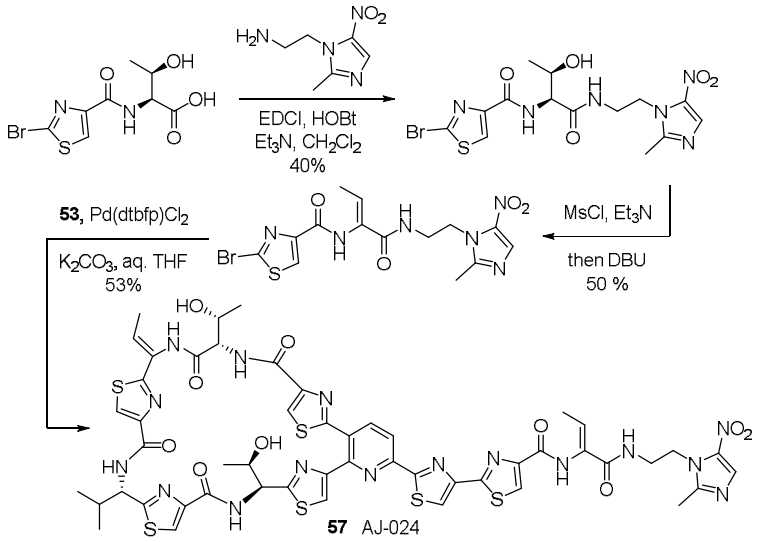
The present avenue is more efficient than other routes yet devised to micrococcins, and it provided ample quantities of **2** for biological evaluation. Such an exercise revealed that MP2 is especially effective against drug-resitant strains of the Gram-positive organism, *C. difficile*, and of the mycobacterium, *M. tuberculosis*.[13,33] Of course, the synthesis just described can be easily modified to produce analogues of micrococcins for medicinal chemistry studies. In that respect, a substance even more potent than the natural product emerged in the form of AJ-024 **57**, quantities of which were made as detailed in Scheme 17. The new antibiotic was superior to vancomycin against numerous clinical isolates of drug-resistant *C. difficile* and of other Gram+pathogens.[34] Recent studies have unveiled analogues with higher potency still.

First of all, extensive testing revealed that MP2 is nontoxic to mammalian cells, even at concentrations many-fold higher than the anticipated therapeutic doses.[33] The data in Table 2 indicate that MP2 exhibits

clinically useful potency against *M. tuberculosis* strains that have become insensitive to common anti-TB drugs.[35] The poor oral bioavailability of micrococcins may not constitute an obstacle in this context, in that direct pulmonary delivery *via* inhalation of a nebulized aqueous solution is a viable administration route. Noteworthy is also the activity of **2** against non-tuberculotic mycobacteria, such as *M. avium*, which constitute yet another group of agents responsible for emerging infectious diseases.[36]



Scheme 16. Total synthesis of MP1 and MP2 by late-stage Suzuki coupling.



Scheme 17. Synthetic route to AJ-024 **57**.

Equally significant is the activity of MP2 and of the generally more potent AJ-024 against representative Gram-positive pathogens. Table 3 lists relevant data vs. the activity of established antibiotics such as ciprofloxacin, linezolid, and, especially, vancomycin. Once again, AJ-024 appears to be superior to MP2 and vancomycin across the board.

Table 2. *In vitro* activity of MP2 against mycobacteria.[a,b]

Species and type		MIC_{50}
Mycobacterium tuberculosis	H37Rv	89 nM
	pan-susceptible isolate # 2	199 nM
	multidrug resistant[c] # 3	73 nM
	multidrug resistant[c] # 11	51 nM
	multidrug resistant[c] # 21	44 nM
	extensively drug resistant[d] # 3	375 nM
	extensively drug resistant[d] # 4	94 nM
	extensively drug resistant[d] # 5	114 nM
Mycobacterium avium		28 nM[e]

[a]Data from Dr. Dakyum Lee at Masan National Tuberculosis Hospital. Clinical isolates were obtained from that hospital's TB specimen Biobank. [b]Additional data in ref. 13. [c]Resistant to isoniazid, rifampicin. [d]Resistant to isoniazid, rifampicin, moxifloxacin, amikacin. [e]In comparison, the MIC_{50} of clarithromycin is 76 nM

Table 3. *In vitro* activity (MIC_{50} in μg/mL) against some Gram+ pathogens.[a,b]

Strain	Details[c]	MP2[d]	AJ-024[e]	CIP[d]	VAN[d]	LIZ[d]
S. aureus	ATCC 25923[MS]	0.125	0.25	0.25	0.5	2
	ATCC 43300[MR]	1	0.25	1	0.5	4
	Clinical isolate[VR]	1	0.5	> 32	> 32	8
S. epidermidis	ATCC 12228	1		0.25	2	4
	Clinical isolate[MS]	0.125		> 32	1	1
	Clinical isolate[MR]	0.125		> 32	0.5	1
E faecalis	ATCC 19433	0.25		1	1	2
	Clinical isolate[VR]	0.125		> 32	> 32	2
	Clinical isolate[LR]	0.125		32	1	> 32
E faecium	ATCC 19434	0.5	0.25	8	0.5	4
	Clinical isolate[VR]	1	0.5	32	> 32	2
	Clinical isolate[LR]	0.125		16	0.5	32

[a]Data from Mr. Young-Jin Son, A&J Science. [b]Additional data in ref. 13. [c]MS=methicillin sensitive; MR=methicillin resistant; VR=vancomycin resistant, LR=linezolid resistant. [d]MP2=micrococcin P2, CIP=ciprofloxacin, VAN=vancomycin, LIZ=linezolid. [e]Additional data in ref. 36.

Among Gram+ organisms, *Clostridioides difficile* is of increasing concern.[37] This microbe is responsible for a mounting number of life-threatening nosocomial infections that become extremely problematic in patients also suffering from inflammatory bowel disease. Micrococcin P2 and its congeners are especially promising for the treatment of such conditions,[35] as apparent from the data of Table 4. Notice again the superiority of AJ-024 to MP2 and to vancomycin against clinical isolates of drug-resistant *C. difficile*. Interestingly, poor gastrointestinal bioavailability is desirable in anti-*C. difficile* drugs,[33] turning a potential difficulty with micrococcins and thiopeptides in general to advantage.

8. A noteworthy chemical property of MP2

Medicinal chemistry studies soon revealed that all clinically interesting analogues of micrococcins are amide derivatives of acid **58**, which may be termed micrococcin P acid (MP acid) (Scheme 18). Clearly, it would be useful to establish a biosynthetic avenue to this compound, either through direct fermentation or by selective hydrolysis of microbially produced **1**-**2**. New MP congeners could then be rapidly obtained from **58** by coupling with appropriate amines.

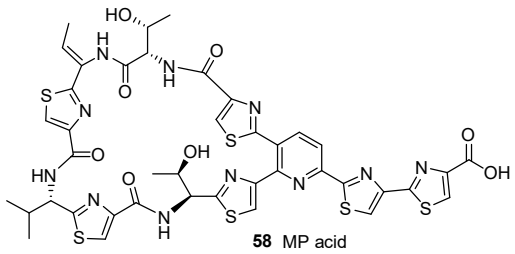
A bold, if risky, surmise provided the incentive to study the base hydrolysis of **2** under controlled conditionsas a means to obtain **58**. A carbonyl at a position adjacent to the ring N atom in pyridines, oxazoles, thiazoles, and related heterocycles is especially electrophilic, being inductively activated by the

neighboring heteroatom. It thus undergoes nucleophilic attack much more readily than an ordinary C=O group. In some cases, this enhanced reactivity can become a problem. To illustrate, addition of reagent **60** to aldehyde **59**, followed by a customary aqueous workup, consistently returned the desired alcohol **61** in less than 50% yield, plus about half of the starting aldehyde, regardless of mode and rate of addition. consistently produced alcohol **76** is less than 50% yield, regardless of mode and rate of addition. Quenching of the reaction mixture with TMS-Cl led to the isolation of **64** as a mixture of diastereomers. Evidently, initially formed alkoxide **62** added to intact **59** faster than the latter could combine with **60**, leading to a complex that probably existed as chelate **63**. Reaction of the latter with TMS-Cl then furnished **64**, which upon TBAF treatment yielded an equimolar amount of **61** and **59** (Scheme 19).[38]

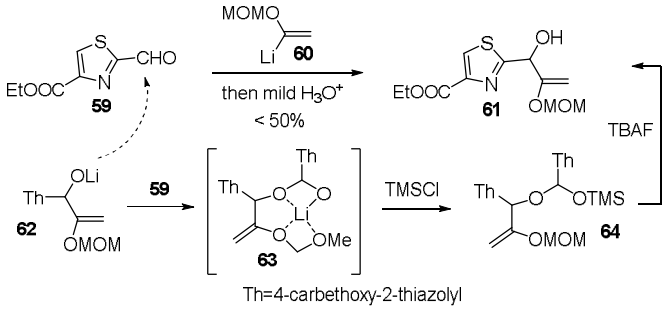
Table 4. *In vitro* activity of MP2 (MIC_{50} in μg/mL) against *Clostridiodes difficile* clinical isolates.[a]

Clade	Ribotype	MLST[b]	VAN[c,d]	MP2[c,d]	AJ-024[c,e]
1	RT018 (10)	ST17	1	0.5	0.25
1	RT001 (4)	ST3	2	1	0.5
1	RT002 (4)	ST8	1	1	0.25
1	RT012 (3)	ST54	2	1	0.25
1	RT014 (3)	ST14	1	1	0.5
1	RT015 (3)	ST35	1	1	0.5
1	RT293 (2)	ST129	1	1	0.5
2	RT027 (2)	ST1	1	0.5	0.5
3	RT130 (5)	ST5	1	0.5	0.25
3	Unclassified (3)	ST221	2	1	0.25
4	RT017 (10)	ST37	1	0.5	0.25
5	RT078 (3)	ST11	1	1	0.25

[a]Data from Profs. Hyunjoo Pai, Hanyang Medical School, and Jin-Hwan Kwak, Handong Global University. [b]Multi Locus Sequence Typing. [c]VAN=vancomycin, MP2=micrococcin P2. [d]Additional data in ref. 35. [e]Additional data in ref. 36.

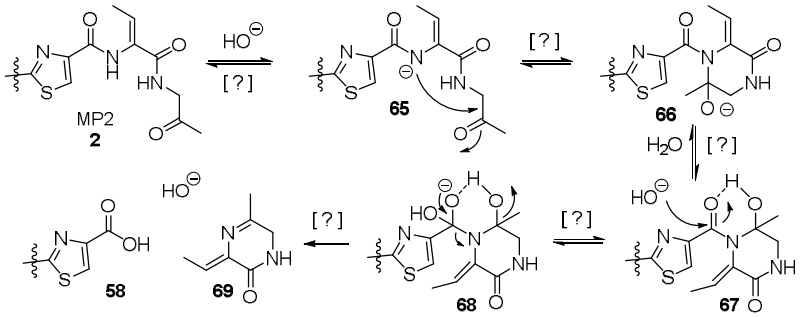


Scheme 18. Structure of micrococcin P acid **58**.



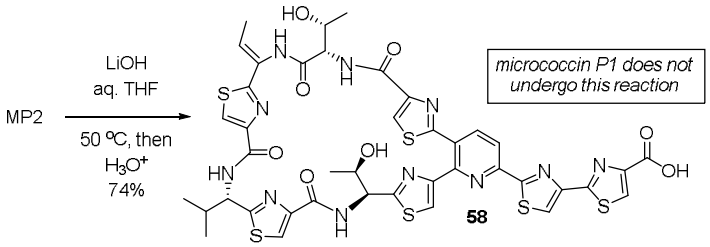
Scheme 19. Problematic electrophilic character of C=O groups adjacent to a thiazole N.

There are 4 thiazolyl carbonyls in **1**-**2**, all of which are part of amide bonds. In MP2, however, the side-chain thiazolyl amide can undergo a reaction that is unavailable to the other 3. Thus, reversible deprotonation of the amide could promote equilibration with **65** with **66** and **67** (Scheme 20). Internal hydrogen bonding with the hemiamidal OH would activate the C=O in **67** even further toward nucleophilic attack. This may well cause HO^- to add faster to that carbonyl relative to the others. Moreover, tetrahedral intermediate **68** seemed to be well poised to undergo fragmentation into **58**, **69**, and hydroxide ion, thus avoiding the expulsion of a more basic, ergo more energetic, N-anion. Acid **58**, which would be rapidly deprotonated under basic conditions, would be retrieved upon acidification of the reaction mixture, while **69** may well tautomerize to an aromatic 2-pyrazinone.



Scheme 20. Mechanistic hypothesis for the hydrolysis of micrococcin P2.

In the event, it transpired that treatment of MP2 with LiOH in aqueous THF at 50 °C, followed by acidification, resulted in rapid, clean formation of highly polar MP acid in 74% yield (Scheme 21).[13] No attempt was made to retrieve products arising from the excised portion of the side chain. Under similar conditions, MP1 was slowly converted into a complex mixture of hydrolysis products containing only some of the desired **58**. The difference in hydrolytic behavior between **1** and **2** is nothing short of remarkable.



Scheme 21. Selective hydrolysis of micrococcin P2 to MP acid.

The foregoing result added urgency to the establishment of a biosynthetic avenue to **58**. Consultation with molecular biologists indicated that it would be easier to engineer an organism to produce MP2, rather than MP acid. Indeed, appropriate manipulation of *Bacillus subtilis* rapidly generated a strain that yielded only MP2 and no MP1.[13] It is worthy of note that the initial fermentation yield of **2** was a modest 0.07 mg/L, while the current yield is above 3 mg/L. Ongoing work aims to improve the yield to about 30 mg/L. The availability of significant quantities of biosynthetic **2**, and consequently of **58**, will permit the preparation of new MP analogues and of GMP drug material by semisynthesis, greatly facilitating medicinal chemistry research and CMC operations, and realizing considerable economies at various levels.

9. Conclusion

The work summarized herein is well on the way to producing valuable new resources to address unmet medical needs. It should be apparent that such essential medicines could not have come about without the

benefit and the support of target-oriented synthetic organic chemistry, which, oddly, has fallen out of favor in the academic world. It is our hope that this reader will appreciate the message contained in this chapter, that organic synthesis is as essential and as central as ever, when it comes to providing solutions to pressing medical problems.

Acknowledgements

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References

1. Leading reviews: a) Chan, D. C. K.; Burrows, L. L. *J. Antibiot.* **2021**, *74*, 161-175. b) Vinogradov, A. A.; Suga, H. *Cell Chem. Biol.* **2020**, *27*, 1032-1051. c) Bird, K. E.; Bowers, A. A. *Comprehensive Natural Products III*; Liu, H.-W.; Begley, T. B., Eds.; Elsevier: Amsterdam, The Netherlands, 2020; vol. 2, 166-191. d) Shen, X.; Mustafa, M.; Chen, Y.; Cao, Y.; Gao, J. *Med. Chem. Res.* **2019**, *28*, 1063-1098. e) Just-Baringo, X,; Albericio, F.; Alvarez, M. *Angew. Chem. Int. Ed.* **2014**, *53*, 6602-6616. f) Just-Baringo, X,; Albericio, F.; Alvarez, M. *Marine Drugs* **2014**, *12*, 317-351. g) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685-714.
2. Su, T. L. *Br. J. Exp. Pathol.* **1948**, *29*, 473-481.
3. Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem Commun.* **1978**, 256-258.
4. Ciufolini, M. A.; Lefranc, D. *Nat. Prod. Rep.* **2010**, *27*, 330-342.
5. See discussion of biological activity in refs. 1.
6. For details see: https://www.cdc.gov/drugresistance/biggest-threats.html.
7. See *e.g.*, Issa, M.; Ananthakrishnan, A. N.; Binion, D. G. *Inflamm. Bowel. Dis.* **2008**, 14, 1432-1442.
8. Murray, J. L., Ikuta, K. S.; Sharara, F.; Swetschinski, L.; Aguilar, G. R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; Johnson, S. C.; Browne, A. J.; Chipeta, M. G.;; Fell, F.; Hackett, S.; Haines-Woodhouse, G.; Hamadani, B. H. K.; Kumaran, E. A. P.; McManigal, B.; Agarwal, R.; Akech, S.; Albertson, S.; Amuasi, J.; Andrews, J.; Aravkin, A.; Ashley, E.; Bailey, F.; Baker, S.; Basnyat, B.; Bekker, A.; Bender, R.; Bethou, A.; Bielicki, J.; Boonkasidecha, S.; Bukosia, J.; Carvalheiro, C.; Castaneda-Orjuela, C.; Chansamouth, V.; Chaurasia, S.; Chiurchiù, S.; Chowdhury, F.; Cook, A. J.; Cooper, B.; Cressey, T. R.; Criollo-Mora, E.; Cunningham, M.; Darboe, S.; Day, N. P. J.; De Luca, M.; Dokova, K.; Dramowski, A.; Dunachie, S. J.; Eckmanns, T.; Eibach, D.; Emami, A.; Feasey, N.; Fisher-Pearson, N.; Forrest, K.; Garrett, D.; Gastmeier, P.; Giref, A. Z.; Greer, R. C.; Gupta, V.; Haller, S.; Haselbeck, A.; Hay, S. I.; Holm, M.; Hopkins, S.; Iregbu, K. C.; Jacobs, J.; Jarowsky, D.; Javanmardi, F.; Khorana, M.; Kissoon, N.; Kobeissi, T. K.; Krapp, F.; Krumkamp, R.; Kumar, A.; Kyu, H. H.; Lim, C.; Limmathurotsakul, D.; Loftus, M. J.; Lunn, M.; Ma, J.; Mturi, N.; Munera-Huertas, T.; Musicha, P.; Mussi-Pinhata, M. M.; Nakamura, T.; Nanavati, R.; Nangia, S.; Newton, P.; Ngoun, C.; Novotney, A.; Nwakanma, D.; Obiero, C. W.; Olivas-Martinez, A.; Olliaro, P.; Ooko, E.; Ortiz-Brizuela, E.; Peleg, A. Y.; Perrone, C.; Plakkal, N.; Raad, A. P.-d.-L. M.; Ramdin, T.; Riddell, A.; Roberts, T.; Robotham, J. V.; Roca, A.; Rudd, K. E.; Russell, N.; Schnall, J.; Scott, J. A. G.; Shivamallappa, M.; Sifuentes-Osornio, J.; Steenkeste, N.; Stewardson, A. J.; Stoeva, T.; Tasak, N.; Thaiprakong, A.; Thwaites, G.; Turner, C.; Turner, P.; van Doorn, R.; Velaphi, S.; Vongpradith, A.; Vu, H.; Walsh, T.; Warner, S.; Wangrangsimakul, T.; Wozniak, T.; Zheng, P.; Sartorius, B.; Lopez, A. D.; Stergachis, A.; Moore, C.; Dolocek, C.; Naghavi, M. *Lancet* **2022**, *399*, 629-655.
9. Some thiopeptides find application in veterinary practice *e.g.*: a) Bailly, C. *Eur. J. Pharmacol.* **2022**, *914*, 174661-174675 (thiostrepton). b) Cao, G.; Yin, H.; Guo, Z. *Guowai Yiyao Kangshengsu Fence* **2011**, *32*, 198-204 (nosiheptide).
10. An early 2010's Novartis program in human medicine centered on LFF571, a derivative of a thiopeptide known as GE2270: a) LaMarche, M. J.; Leeds, J. A.; Amaral, K.; Brewer, J. T.; Bushell, S. M.; Dewhurst, J. M.; Dzink-Fox, J.; Gangl, E.; Goldovitz, J.; Jain, A.; Mullin, S.; Neckermann, G.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Whitehead, L.; Yu, D. *J. Med. Chem*. **2011**, *54*, 8099-8109. b) Ting, L. S. L.; Praestgaard, J.; Grunenberg, N.; Yang, J. C.; Leeds, J. A.; Pertel, P. *Antimicrob. Agents Chemother.* **2012**, *56*, 5946-5951. The authors possess no information about the current status of this program.
11. Lefranc, D.; Ciufolini, M. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198-4201.

12. a) MP1: Akasapu, S.; Hinds, A. B.; Powell, W. C.; Walczak, M. A. *Chem. Sci.* **2019**, *10*, 1971-1975. b) Christy, M. P.; Johnson, T.; McNerlin, C. D.; Woodard, J.; Nelson, A. T.; Lim, B.; Hamilton, T.; Freiberg, K. M.; Siegel, D. *Org. Lett.* **2020**, *22*, 2365-2670.
13. MP2 fell to total synthesis only in 2022: Hwang, H.-J.; Son, Y.-J.; Kim, D.; Lee, J.; Shin, Y.-J.; Kwon, Y.; Ciufolini, M. A. *Org. Biomol. Chem.* **2022**, *20*, 1893-1899.
14. a) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457-2483. b) Suzuki, A. *Angew. Chem. Int. Ed.* **2011**, *50*, 6723-6737.
15. Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508-7510.
16. Aulakh, V. S.; Ciufolini, M. A. *J. Am. Chem. Soc.* **2011**, *133*, 5900-5904. See also ref. 12b.
17. Review: Bagley, M. C.; Glover, C.; Merritt, E. A. *Synlett* **2007**, *2007*, 2559-2482.
18. a) White, E. H.; Field, G. F.; McCapra, F. *J. Am. Chem. Soc.* **1963**, *85*, 337-343. b) Johnson, T. C.; Christy, M. P.; Siegel, D. *Synthesis* **2021**, *53*, 498-508.
19. Hantzsch, A.; Weber, J. H. *Chem. Ber.* **1887**, *20*, 3118-3132.
20. Leading reviews on the chemistry of this valuable reagent: a) Khatoon, H.; Abdulmalek, E. *Molecules* **2021**, *26*, 6937-6980. b) Magalhaes de Moreira, D. R. *Synlett* **2008**, *2008*, 463-464. c) Jesberger, M.; Davis, T. P.; Barner, L. *Synthesis* **2003**, *2003*, 1929-1958.
21. Merritt, E. A.; Bagley, M. C. *Synthesis* **2007**, *2007*, 3535-3541.
22. a) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143-2147. b) Seco, J. M.; Latypov, S. K.; Quinoa, E.; Riguera, R. *J. Org. Chem.* **1997**, *62*, 7569-7574.
23. Bredenkamp, M. W.; Holzapfel, C. W.; van Zylm W. J. *Synth. Commun.* **1990**, *20*, 2235-2249.
24. Bennabi, S. *Dissertation*, University Claude Bernard Lyon I, 2000.
25. Liang, H.; Ciufolini, M. A. *Chem. Eur. J.* **2010**, *16*, 13262-13270.
26. Smith, M. B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 5th Ed.*, John Wiley and Sons, Inc., New York, NY, **2001**, 575.
27. Shen, Y.-C. *Dissertation*, Rice University, 1997.
28. Ciufolini, M. A.; Shen, Y. C. *Org. Lett*. **1999**, *1*, 1843-1846.
29. Beutner, G. L.; Young, I. S.; Davies, M. L.; Hickey, M. R.; Park, H.; Stevens, J. M.; Ye, Q. *Org. Lett.* **2018**, *20,* 4218-4222.
30. Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203-6205.
31. a) Cox, P. A.; Leach, A. G.; Campbell, A. D.; Lloyd-Jones, G. C. *J. Am. Chem. Soc.* **2016**, *138,* 9145-9157. b) Karmel, C.; Rubel, C. Z.; Kharitonova, E. V.; Hartwig, J. F. *Angew. Chem. Int. Ed.* **2020**, *59,* 6074-6081. c) Primas, N.; Bouillon, A.; Rault, S. *Tetrahedron* **2010**, *6*, 8121-8136.
32. Unpublished results from these laboratories.
33. Son, Y.-J.; Kim, Y.-R.; Oh, S.-H. Jung, S.-J.; Ciufolini, M. A.; Hwang, H.-J.; Kwak, J.-H.; Pai, H. *J. Nat. Prod.* **2022**, *85*, DOI:10.1021/acs.jnatprod.2c00120.
34. Kim, D.; Kim, Y.-R.; Hwang, H.-J.; Ciufolini, M. A.; Lee, J.; Lee, H.; Clovis, S.; Jung, S.; Oh, S.-H.; Son, Y.-J.; Kwak, J.-H. *Pharmaceuticals* **2022**, *15*, 623-635.
35. TB is a resurgent threat: https://www.cdc.gov/tb/default.htm.
36. For a description of the problem see: https://www.cdc.gov/HAI/organisms/mycobacterium.html.
37. For a description of the problem see: https://www.cdc.gov/cdiff/index.html.
38. Hwang, H.-J.; Ciufolini, M. A. *J. Org. Chem.* **2015**, *80*, 4184-4188.