Thiopeptides: Synthesis and Structure-Activity Relationship Studies

Xavier Just Baringo

Memòria presentada per

Xavier Just Baringo

per optar al grau de doctor per la Universitat de Barcelona

Programa de Química Orgànica

Thiopeptides: Synthesis and Structure-Activity Relationship Studies

Dirigida per:

Dra. Mercedes Álvarez Domingo
(Universitat de Barcelona)

Dr. Fernando Albericio Palomera
(Universitat de Barcelona)

Barcelona, 2013

UNIVERSITAT DE BARCELONA

FACULTAT DE QUÍMICA

DEPARTAMENT DE QUÍMICA ORGÀNICA

THIOPEPTIDES: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Xavier Just Baringo

2013

“The sophistication and diversity of these metabolites points to the ingenuity and flexibility of biosynthetic processes in Nature.”

William Fenical

Com tothom entendrà l'agraïment surt de ben endins i aquest sentiment no té traducció.

En primer lloc vull expressar el meu més sincer agraïment a les dues persones que han fet que aquest projecte pogués existir, la Mercè i el Fernando. El més important és que m'han donat la seva confiança per poder-lo dur a terme. Moltes de les oportunitats que han anat sorgit al llarg del meu camí han estat gràcies al Fernando, passar l'estiu al Parc, l'any al Regne Unit... mai estaré prou agraït. A tots dos moltes gràcies per la llibertat que m'heu donat durant aquests anys i tot el que he pogut aprendre.

A partir d'aquí la cosa es torna molt més complexa. Sou tants els que mereixeu crèdit per haver fet tota aquesta tasca més fàcil que seria pràcticament impossible anomenar-vos a tots. Tothom que ha treballat o treballa als laboratoris de Pharmamar, d'en Rodolfo, de la Míriam, de l'Ernest, la resta del grup del Fernando al 300, als nostres companys sintètics del Toni Riera i el Xavier Verdaguer, la gent del Miquel Pons, etc. Gràcies per haver fet més agradables aquest anys i haver donat un cop de mà sempre que ha fet falta.

Cal una menció especial per a aquells que han donat un suport imprescindible i que sempre han estat disposats a donar un cop de mà. A tots els membres del servei de RMN, especialment al Jesús els hi estic molt agraïts pel seu ajut. Al servei de masses també han estat d'allò més col·laboradors i sobretot molt pacients, l'ajut de la Marta i tot el seu equip ha estat imprescindible.

Mirant a dins del lab 100 hi segueix havent molta gent a la que estar agraït i a la que recordar, tots aquells que ja hi eren quan vaig començar, els que varem començar junts i tots aquells que han arribat després. És impossible no fer menció d'aquells amb qui he compartit moltes hores fins a sumar anys! El Pau, l'Adriana, la Janire i el Paolo, tots excel·lents companys. L'agraïment es fa extensible a tots aquells que han col·laborat d'alguna manera amb el projecte el Lars, el Hossein, l'Ylias i de nou el Paolo, una persona excel·lent i que sorprenentment és capaç de donar-li encara més voltes que jo a les coses més insignificants, però sempre a través de les discussions més interessants. La vitrina ha estat un lloc on compartir moltíssims moments amb les dues companyes que he tingut, la Eva i especialment l'Adriana, amb qui ens hem donat molta guerra fins al punt de semblar un matrimoni i "hem" (bé, he) cantat molt, són moments difícils d'oblidar. La Janire, tot i no estar a la vitrina, també ha estat molt a prop; hem batallat bastant i segur que algun dia arreglarem una mica el món més enllà de les converses; si he aconseguit que passés ella primer, tot és possible.

No em puc oblidar dels companys de la facultat que han arribat a ser bons amics i molts segueixen essent companys del dinar, als que s'hi ha sumat la Isa, un bon i posterior fitxatge que ja no és tan nou. Tots ells mereixen el seu lloc en aquestes línies: Georgina, Toni, Carles, Marçal, Bruix, Àlex, David, Isa, Susana, Laura, Marimon i Mari.

En cap cas podria descuidar-me a dues persones que no tenen cap relació entre elles però que han estat una gran inspiració. Primer de tot la Carina, que a tercer d'ESO va esdevenir la primera i única professora de química que vaig tenir fins a la universitat. A hores d'ara encara tinc seriosos dubtes de si m'hagués decantat per la química tan aviat sense la seva influència. L'Albert Isidro és l'altra persona a qui li dec molt; vaig aprendre moltíssim d'ell en molt poc temps; encara ara penso "com ho faria ell" quan no se com afrontar un problema al laboratori.

La família també ha estat important durant aquests anys, tot i que posar límits al que és o no és família és una tasca que queda massa fora del meu abast. En qualsevol cas, una sola persona va ser testimoni directe de l'obtenció per primer cop de la baringolina al laboratori, la Mª Àngels (Wiki), sense l'ajut de la qual (ejem) mai hagués aconseguit aquesta fita.

No m’oblido de l’Elena, la paciència que ha tingut i segueix tenint és digna de molta admiració. Vull pensar que en el seu moment vaig ser per a ella un suport com l’ha estat ella per a mi. Encara que ella es pensi el contrari, els seus consells sempre mereixen la meva atenció. Afortunadament els nostres camins seguiran junts un cop haguem marxat amb les tesis acabades i això és molt reconfortant.

Per acabar, com queda clar al capítol corresponent de la tesi “the naming of baringolin”, aquest nom té un significat especial. És per aquest motiu que la tesi se la dedico a la meva mare.

The work reported in this doctoral thesis has been carried out at the Institute for Research in Biomedicine of Barcelona (IRB Barcelona), situated in the Barcelona Science Park (PCB).

This project has been possible thanks to financial support by the Spanish Ministerio de Ciencia e Innovación (CTQ2009-07758), Ministerio de Economía y Competitividad (CTQ2012-30930), Generalitat de Catalunya (2009SGR 1024) and Centro de Investigación Biomédica en Red, de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN 0074).

XJB thanks Instituto de Salud Carlos III (ISCIII) for a PFIS grant.

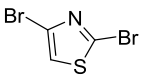
INDEX

PROLOGUE

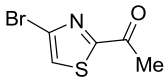
The number of thiopeptides reported to date is considerably large. More than one hundred distinct structures are already known and it would be a complex task to show all of them in a general introduction or wherever else they are mentioned. However, for the sake of rigor and to satisfy the curiosity of readers, as scarce as they might be, it is desirable that as much information as possible can be easily accessed. For this reason, towards the end of this thesis, a section titled *Encyclopædia Thiopeptidum* collects some of the most relevant information and references regarding naturally occurring thiopeptides in an encyclopedic fashion. A section of this kind is expected to provide an easy access to useful data and make the reading of this work a more enriching experience.

As any other art or science, chemistry advances thanks to the work of predecessors and their work can still be an inspiration for all of us. The illustrations that can be found at the beginning of each section were made by three pioneers of the chemical science: Antoine Lavoisier (1743–1794; all illustrations were drawn by her wife and assistant, Marie-Anne Lavoisier), Henry Cavendish (1731–1810) and Antoni de Martí I Franquès (1750–1832).

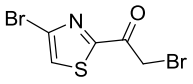
COMPOUND INDEX



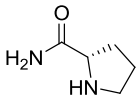
5 (*Chapter 2*)



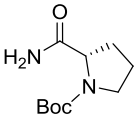
(*Chapter 2*)



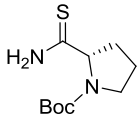
3 (*Chapter 2*)



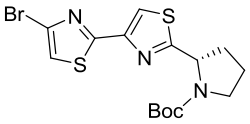
(***S***)-**6** (*Chapter 2*)



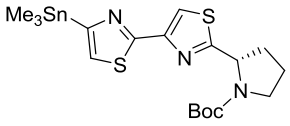
(*Chapter 2*)



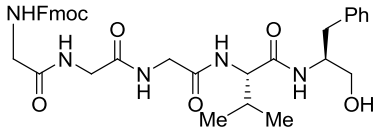
(***S***)-**4** (*Chapter 2*)



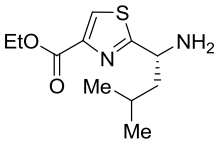
(***S***)-**1** (*Chapter 2*)



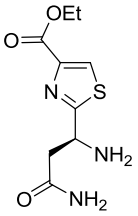
(***S***)-**2** (*Chapter 2*)
11a (*Chapter 4*)
10 (*Chapter 5*)



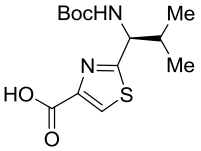
1 (*Chapter 3*)



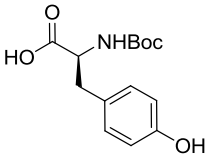
4 (*Chapter 3*)



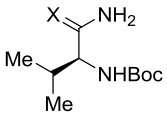
2 (*Chapter 3*)



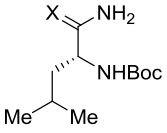
3 (*Chapter 3*)



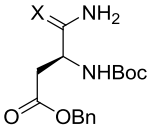
5 (*Chapter 3*)



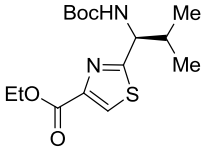
X = O, **6a**
X = S, **6b**
(*Chapter 3*)



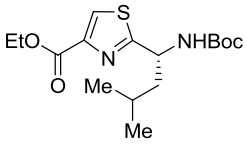
X = O, **7a**
X = S, **7b**
(*Chapter 3*)



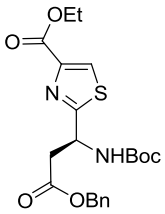
X = O, **8a**
X = S, **8b**
(*Chapter 3*)



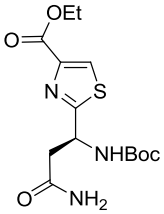
9 (*Chapter 3*)



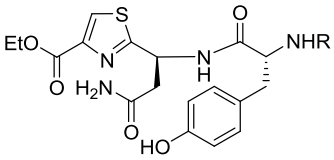
10 (*Chapter 3*)



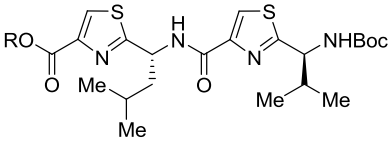
R = Bn, **11**
R = H, **11a**
(*Chapter 3*)



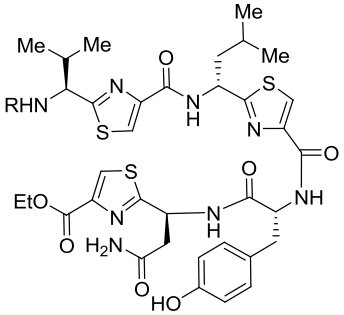
12 (*Chapter 3*)



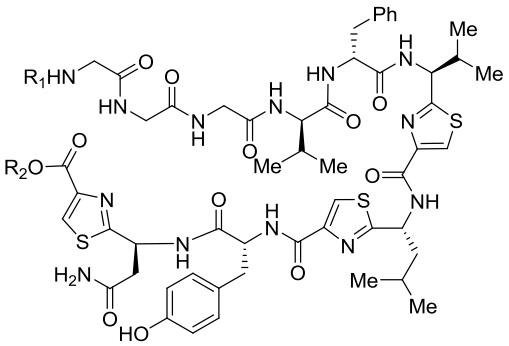
R = Boc, **13**
R = H, **15**
(*Chapter 3*)



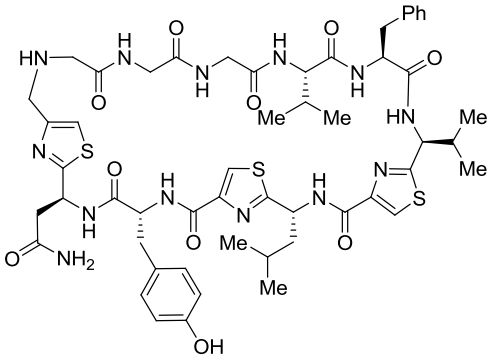
R = Et, **14**
R = H, **16**
(*Chapter 3*)



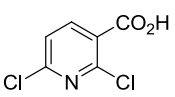
R = Boc, **17**
R = H, **18**
(*Chapter 3*)



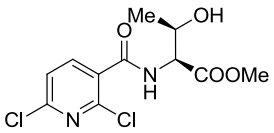
R_1 = Et, R_2 = Fmoc, **19**
R_1 = H, R_2 = H, **20**
(*Chapter 3*)



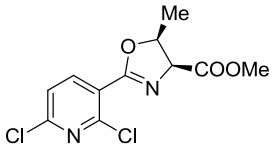
Aeruginazole A (*Chapter* 3)



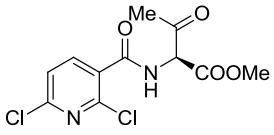
1 (*Chapter* 4)
7a (*Chapter* 5)



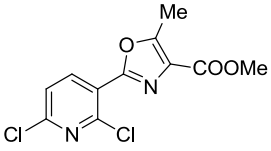
2 (*Chapter* 4)



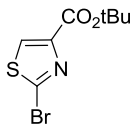
3 (*Chapter* 4)



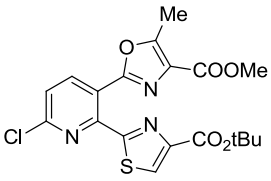
4 (*Chapter* 4)



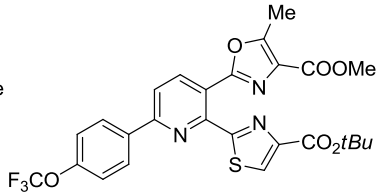
5 (*Chapter* 4)



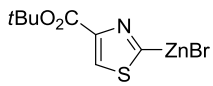
6 (*Chapter* 4)



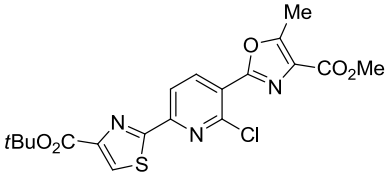
7 (*Chapter* 4)



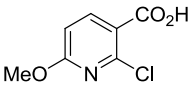
8 (*Chapter* 4)



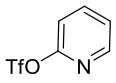
9 (*Chapter* 4)



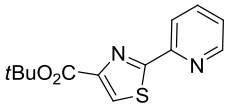
10 (*Chapter* 4)



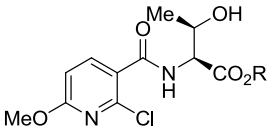
12 (*Chapter* 4)
7b (*Chapter* 5)



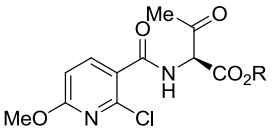
13 (*Chapter* 4)



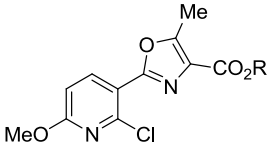
14 (*Chapter* 4)



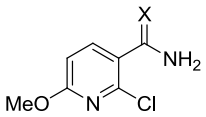
R = Me (*Chapter* 4)
R = Bn, **S1** (*Chapter* 5)



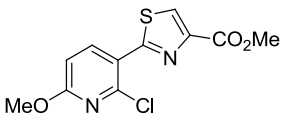
R = Me (*Chapter* 4)
R = Bn, **S2** (*Chapter* 5)



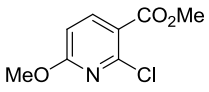
R = Me, **15a** (*Chapter* 4)
R = Bn, **11** (*Chapter* 5)



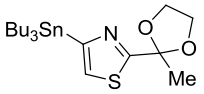
X = O
X = S
(*Chapter* 4)



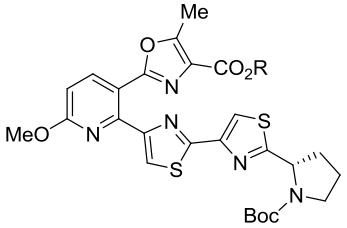
15b (*Chapter* 4)



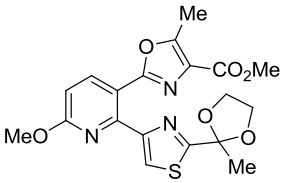
15c (*Chapter* 4)



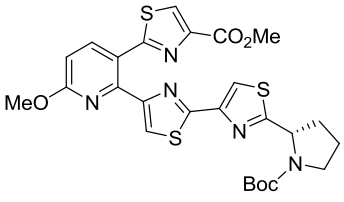
11b (*Chapter* 4)



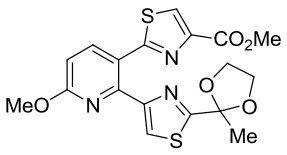
R = Me, **16aa** (*Chapter* 4)
R = Bn, **12** (*Chapter* 5)



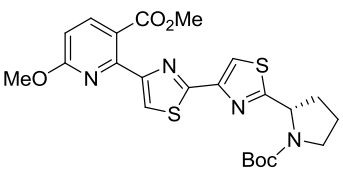
16ab (*Chapter* 4)



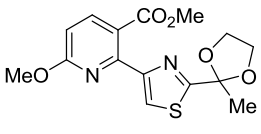
16ba (*Chapter* 4)



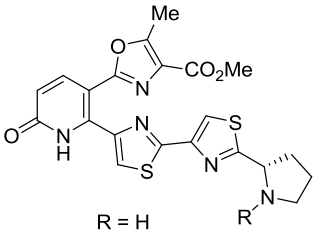
16bb (*Chapter* 4)



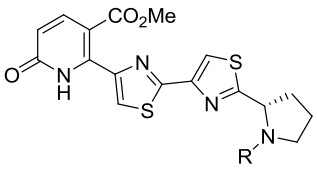
16ca (*Chapter* 4)



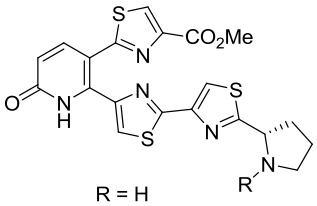
16cb (*Chapter* 4)



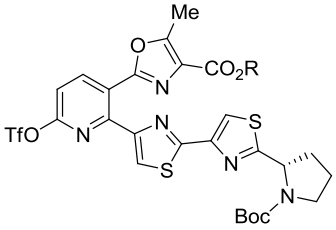
R = H
R = Boc
(*Chapter* 4)



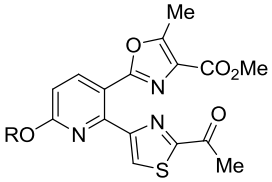
R = H
R = Boc
(*Chapter* 4)



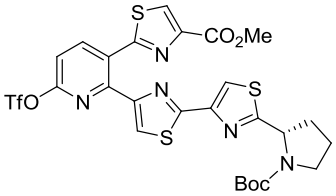
R = H
R = Boc
(*Chapter* 4)



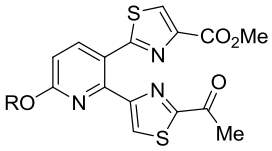
R = Me, **17aa** (*Chapter* 4)
R = Bn, **13** (*Chapter* 5)



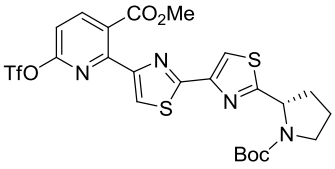
R = H
R = Tf, **17ab**
(*Chapter* 4)



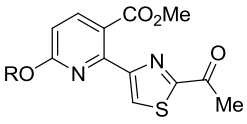
17ba (*Chapter* 4)



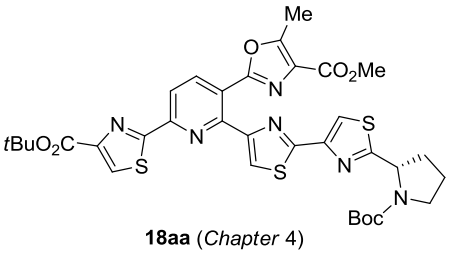
R = H
R = Tf, **17bb**
(*Chapter* 4)



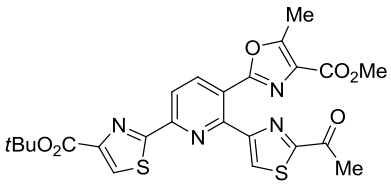
17ca (*Chapter* 4)



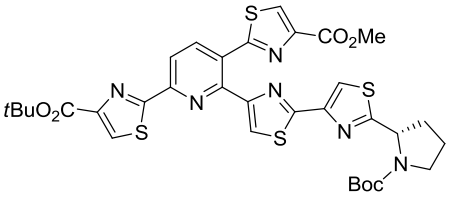
R = H
R = Tf, **17cb**
(*Chapter* 4)



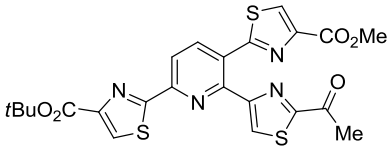
18aa (*Chapter* 4)



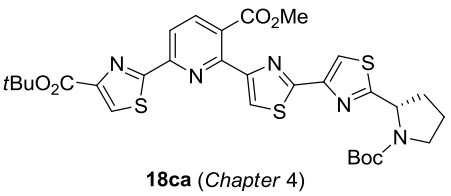
18ab (*Chapter* 4)



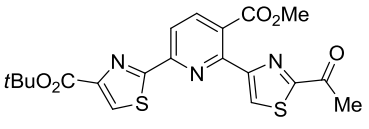
18ba (*Chapter* 4)



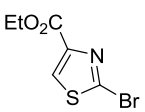
18bb (*Chapter* 4)



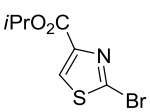
18ca (*Chapter* 4)



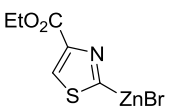
18cb (*Chapter* 4)



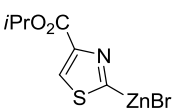
19 (*Chapter* 4)



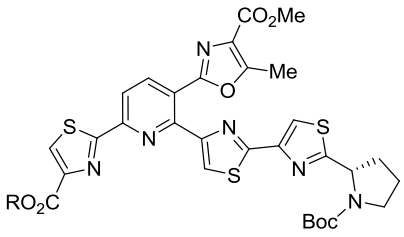
20 (*Chapter* 4)



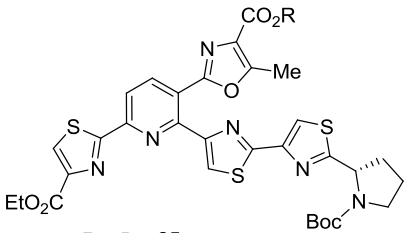
21 (*Chapter* 4)
9 (*Chapter* 5)



22 (*Chapter* 4)

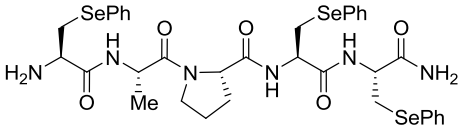


R = Et, **23**
R = *i*Pr, **24**
(*Chapter* 4)

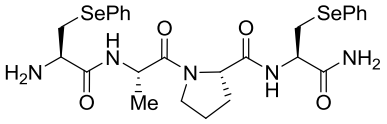


R = Bn, **25**
R = H, **26** (*Chapter* 4)

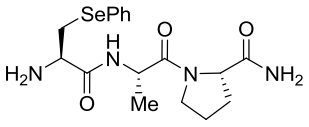
R = Bn, **3**
R = H, **16** (*Chapter* 5)



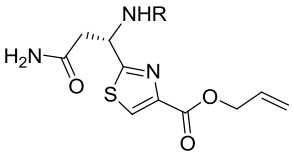
2 (*Chapter* 5)
14 (*Chapter* 7)



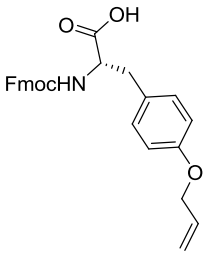
13 (*Chapter* 7)



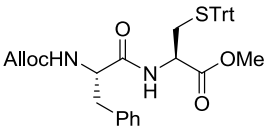
12 (*Chapter* 7)



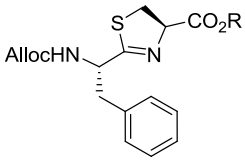
R = Boc, **S5** (*Chapter* 5)
R = H, **4** (*Chapter* 7)



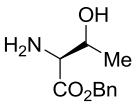
6 (*Chapter* 5)



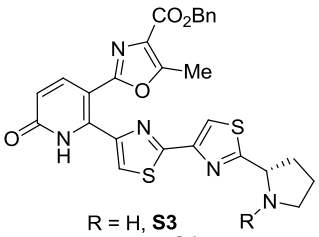
14 (*Chapter* 5)



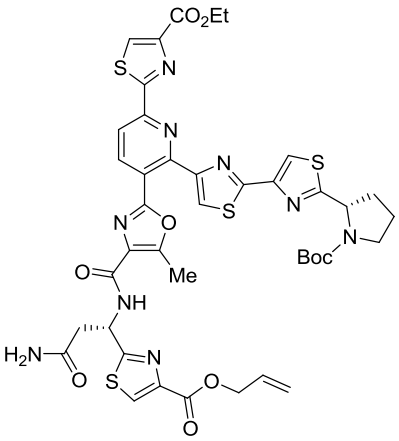
R = Me, **15**
R = H, **5**
(*Chapter* 5)



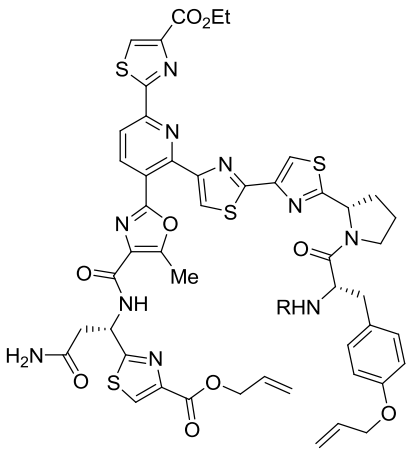
8 (*Chapter* 5)



R = H, **S3**
R = Boc, **S4**
(*Chapter* 5)

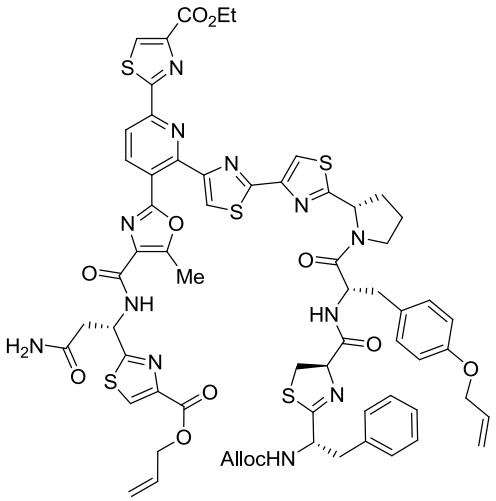


17 (*Chapter* 5)

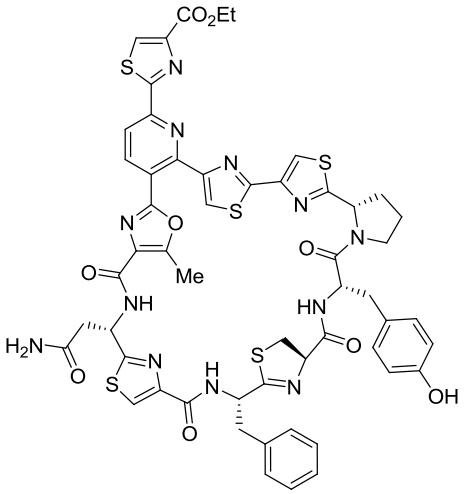


R = Fmoc, **18**
R = H, **S6** (*Chapter* 5)

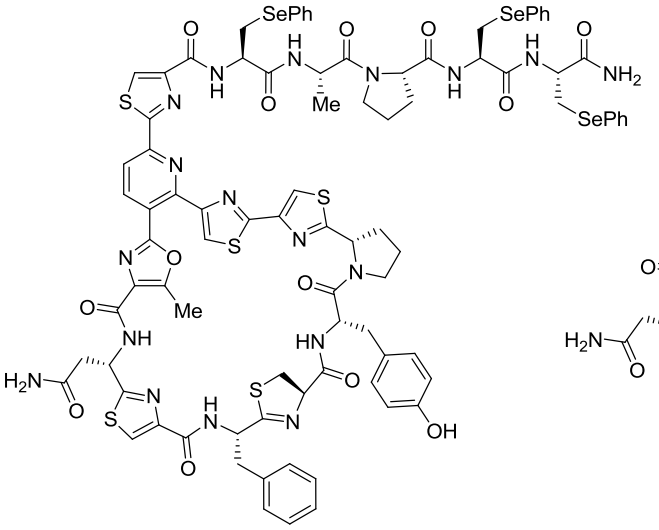
R = H, **15** (*Chapter* 7)



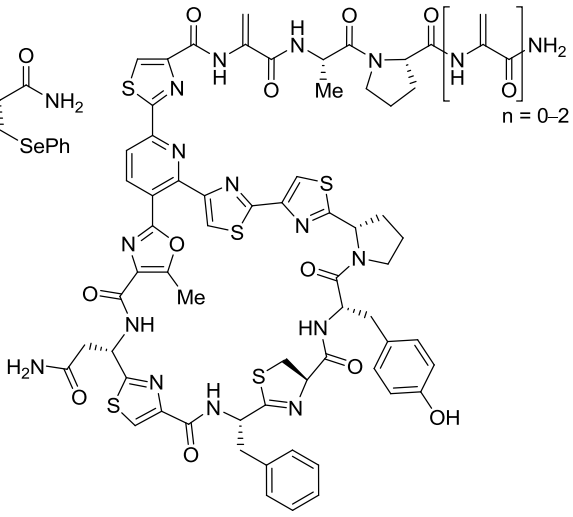
19 (*Chapter* 5)
18 (*Chapter* 7)



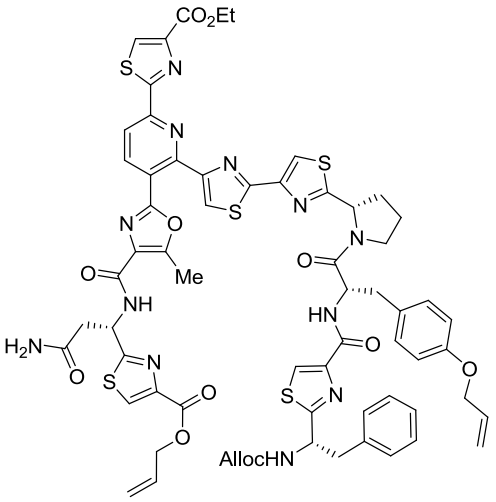
1 (*Chapter* 5)
20 (*Chapter* 7)



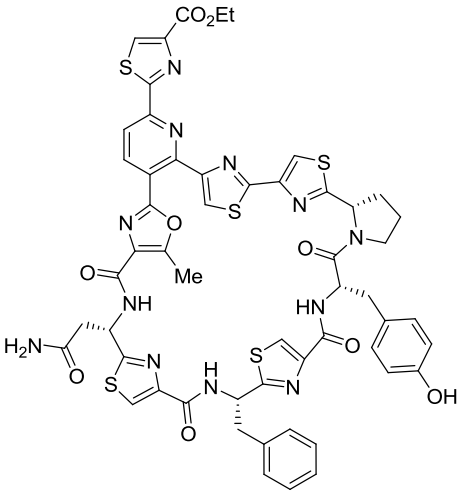
20 (*Chapter* 5)



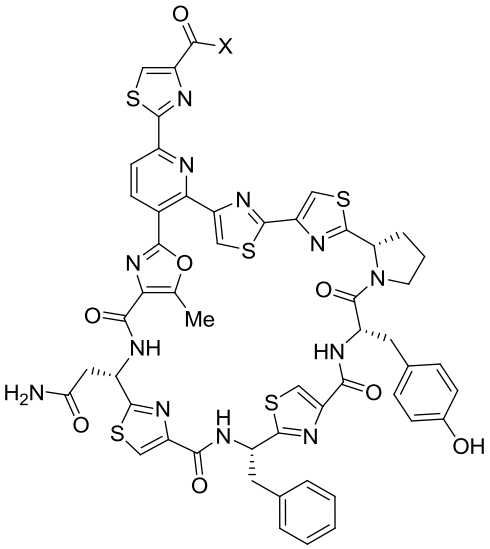
n = 2, **baringolin** (*Chapter* 5)
1 (*Chapter* 7)
n = 1, **28** (*Chapter* 7)
n = 0, **26** (*Chapter* 7)



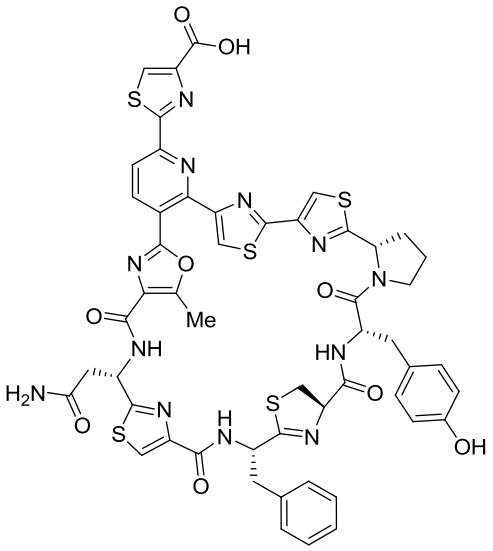
17 (*Chapter* 7)



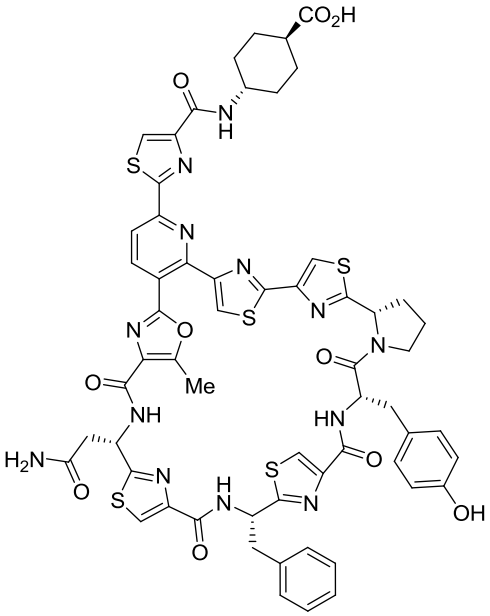
19 (*Chapter* 7)



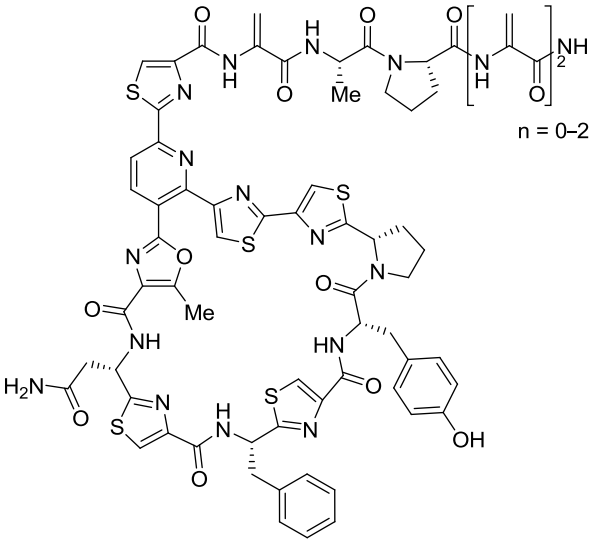
X = OH, **21**
X = NH, **23** (*Chapter* 7)



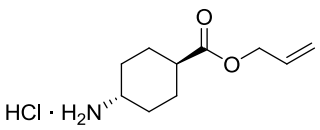
22 (*Chapter* 7)



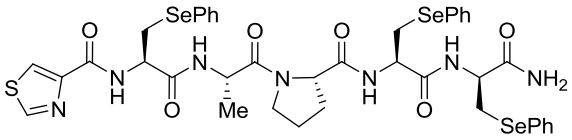
2 (*Chapter* 7)



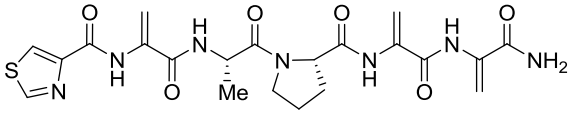
n = 2, **29** (*Chapter* 7)
n = 1, **27** (*Chapter* 7)
n = 0, **25** (*Chapter* 7)



24 (*Chapter* 7)



30 (*Chapter* 7)



31 (*Chapter* 7)

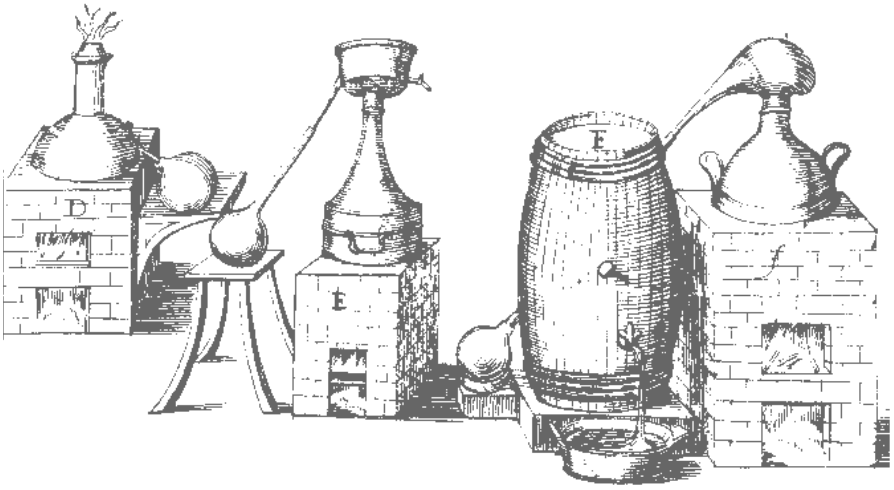
ACRONYMS

$[\alpha]_D$	optical rotation
aa / AA	amino acid
Abu	Aminobutyric acid
Ac	acetyl
Ala	alanine
Alloc	allyloxycarbonyl
aq.	aqueous
Asn	aparagine
Asp	aspartic acid
BLD	below limit of detection
Bn	benzyl
Boc	*tert*-butoxycarbonyl
bs	broad singlet
BTPP	*tert*-butylimino-tri(pyrrolidino)phosphorane
Bu	butyl
CIP	2-chloro-1,3-dimethylimidazolidium hexafluorophosphates
cLogP	calculated partition coefficient logarithm
Cp*	pentamethylcyclopentadienyl
Cy	cyclohexyl
Cys	cysteine
δ	chemical shift
d	doublet
DAST	diethylamino sulfurtrifluoride
dba	dibenzylideneacetone
DBU	1,8-diazabicycloundec-7-ene
dd	doublet of doublet
Dha	dehydroalanine
Dhb	dehydrobutyrine
DIC / DIPCDI	*N*,*N*′-diisopropylcarbodiimide
DIPEA / DIEA	diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
dppp	1,3-bis(diphenylphosphino)propane
EC_{50}	half maximal effective concentration
EDCI / EDC	*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride
EF-G	elongation factor G
EF-Tu	elongation factor thermo unstable
ESI	electrospray ionization
Et	ethyl
Fmoc	9-fluorenylmethoxycarbonyl
FOXM1	transcription factor forkhead box M1
gHSQC	gradient heteronuclear single quantum coherence
GI_{50}	half maximal growth inhibition concentration
Gly	glycine

HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
His	histidine
HOAt	1-hydroxy-7-azabenzotriazole.
HOBt	1-hydroxyben- zotriazole
HPLC	high performance liquid chromatography
HRMS	high ressolution mass spectrometry
*i*Bu	*iso*-butyl
IC_{50}	half maximal inhibitory concentration
Ile	iso-leucine
*i*Pr	*iso*-propyl
IR	infrared
K_D	dissociation constant
KO	knock-out
Leu	leucine
LR	Lawesson's reagent
Lys	lysine
m	multiplet
m/z	mass per charge
MALDI-TOF	matrix-assisted laser desorption/ionization - time of flight
Me	methyl
Met	methionine
MIC	minimum inhibitory concentration
mp	melting point
MRSA	methicillin-resistant *Staphylococcus aureus*
NMR	nuclear magnetic ressonance
nOe	nuclear Overhauser effect
NRPS	non-ribosomal protein / peptide synthetase
Pac	phenacyl
PD_{50}	half maximal protective dose concentration
PDA	photodiode array
Ph	phenyl
Phe	phenylalanine
ppm	parts per million
Pro	proline
PRSP	penicillin-resistant *Streptococcus pneumonia*
PyBOP	(1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate
q	quadruplet
RT / rt	room temperature
s	singlet
SAR	structure-activity relationship
sat.	saturated
SEM	2-(trimethylsilyl)ethoxymethyl
Ser	serine
S_NAr	nucleophilic aromatic substitution
SPPS	solid-phase peptide synthesis
Su	succinyl
t	triplet
TBAF	tetrabutylammonium fluoride
*t*Bu	*tert*-butyl
Tf	trifluoromethansulfonyl

TFA	trifluoroacetic acid
TFAA	thifluoroacetic anhydride
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMS	trimethylsilyl
Trp	tryptophan
Trt	trityl / triphenylmethyl
Tyr	tyrosine
Val	valine
VRE	vancomycin-resistant enterococci

Introduction



INTRODUCTION

1.1. Antibiotics

Since the discovery of the first antibiotics and their golden age in mid 20th century, there has been a dramatic change in the way we face the development of new antimicrobials.[1,2], At first, it seemed that the many classes of naturally occurring antibiotics could be sufficient to fight against bacterial infections, whereas for the last few decades it was thought that semi-synthetic modifications of those natural products would be enough to overcome pathogen resistance. However, we are now facing a new age, where the discovery of novel scaffolds and new modes of action is required to fight against the emergence of resistances and cross-resistances that make previously treatable infections a new threat.

Most of the antibacterial scaffolds known to date were discovered from late 1930s to early 1960s. After that period, almost forty years followed without new bactericide architectures appearing in the market. During those years, semi-synthetic modifications of the already known compounds were used to fight antibacterial resistance. However, with the new century a batch of new antibiotic scaffolds got closer to the clinic (Figure 1). These include oxazolidinones (linezolid, 2000), lipopeptides (daptomycin, 2003) and mutilins (retapamulin, 2007). Parallelly, other types of antibiotics, such as lantibiotics[3] (NVB302) and thiopeptides[4] (LFF571)[5] are under study and some of their members are already in clinical trials for the treatment of human infections.

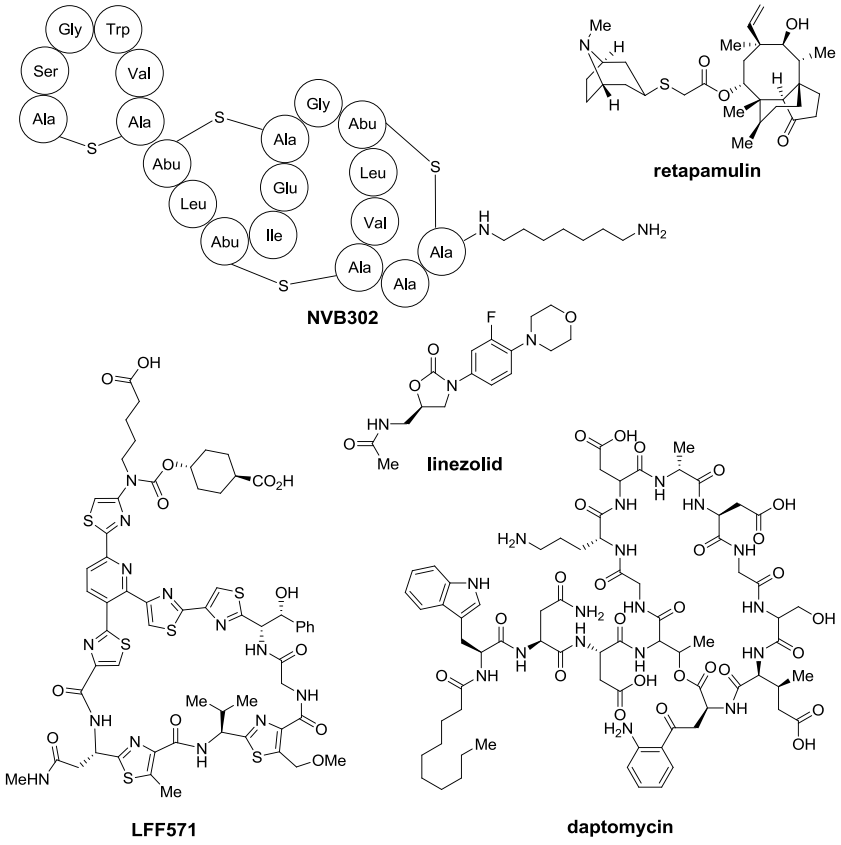


Figure 1. Members of new classes of antibiotics. Abu = aminobutyric acid.

Among these new families of antibiotics, thiopeptides have gathered much attention due to their potent *in vitro* activity against Gram-positive bacteria and their intriguing structures. During the last two decades intensive investigations on known thiopeptides and the discovery of new members of this class of antibiotics have centered the efforts of many research groups.

1.2. Thiopeptides

Thiopeptides, or thiazolyl peptides,[4] are a class of naturally occurring and highly modified sulfur-rich peptides of ribosomal origin. They all share a series of common motifs that differentiate them from other peptide-derived and/or azole-containing natural products. Their most characteristic feature is the central nitrogen-containing six-membered ring, which can be found in many different oxidation states. This central ring serves as scaffold to at least one macrocycle and a tail, and both can be decorated with various dehydroamino acids and azoles, such as thiazoles, oxazoles and thiazolines. Their impressive *in vitro* profile against Gram-positive bacteria and their new mechanisms of action have gathered the attention of many groups, both in academia and industry, as they pose an alternative to other antibiotics presently facing resistance by old pathogens. To date, more than one hundred members of this family of natural products have been identified; however, their huge molecular size and their poor aqueous solubility have been a major drawback to introduce them into the clinic. This has become their major limitation and has restricted their use to topic treatments, and so far only for pet skin infections (thiostrepton, Panolog).

Given the different oxidation state the central ring of thiopeptides can be found in, they have been classified into different series (Figure 2).[4] Thus, the *a* series presents a totally reduced central piperidine, whereas the *b* series is oxidized further and contains a 1,2-dehydropiperidine ring. Only one thiopeptide of the *c* series has been isolated to date and its core moiety is somewhat unexpected, as it displays a piperidine ring fused with imidazoline. All members of series *a*, *b* and *c* have a second macrocycle which contains a quinaldic acid moiety. The *d* series goes further on the oxidation state rank and shows a trisubstituted pyridine ring, which is the landmark of this subgroup, the most numerous among thiopeptides. In a sense, the *e* series is even more oxidized and is easily differentiated for the hydroxyl group in the central pyridine, which is now tetrasubstituted. The *e* series also presents a very characteristic second macrocycle appending from the main one and formed by a modified 3,4-dimethylindolic acid moiety.

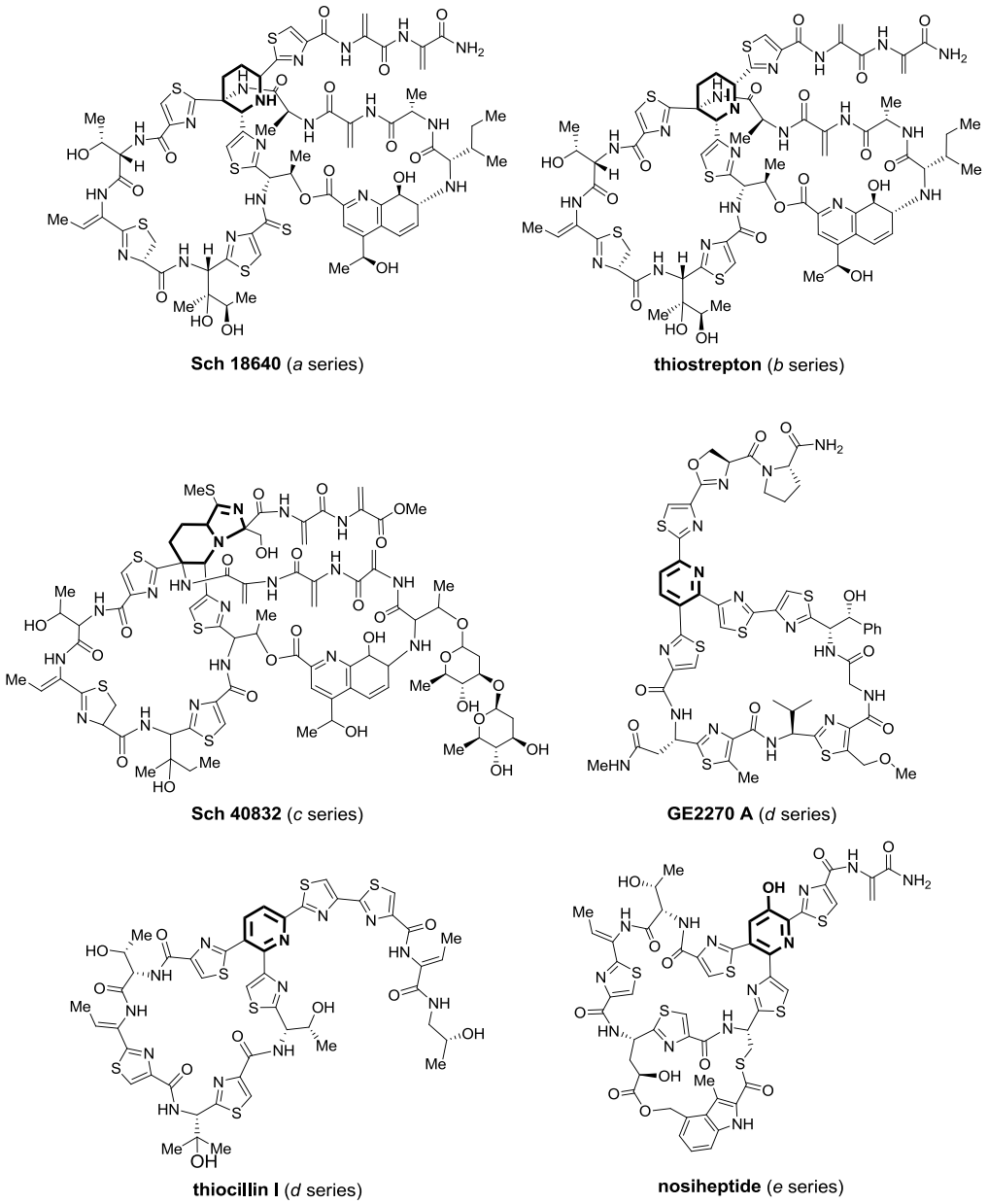


Figure 2. Classification of thiopeptide antibiotics into different series. Their characteristic central six-member ring is highlighted in bold.

1.2.1. Isolation and Structure Elucidation

Thiopeptides have been isolated from diverse sources; in 1948, the first known member of the family, micrococcin, was isolated from a sample of Oxford's sewage waters. Accounting for the highly diverse origin of thiopeptides, micrococcin P1 was more recently isolated from a completely different source, a French cheese.[6] However, more conventional samples, such as soil ones are the main source of most thiopeptides. In fact, thiostrepton, the most famous member among them, has been isolated from different soil samples,[7–9] including one from Hawaii in 1955,[10] short after it was first discovered in 1954.[7–9] Although a few more thiopeptides were isolated during the following years, it was from 1980s, specially during the 1990s, that most of the known members were discovered. Nonetheless, many novel entities have also been described during the last decade. Remarkably, the first thiopeptide antibiotics

isolated from a marine source were YM-266183 and YM-266184, discovered as late as 2003, in Japan.[11] During the last few years some more thiopeptides have been isolated and characterized; these include the thiazomycins (2007)[12–15], philipimycin (2008)[16], thiomuracins (2009),[17] TP-1161 (2010)[18,19] and baringolin (2012)[20] (Figure 3).

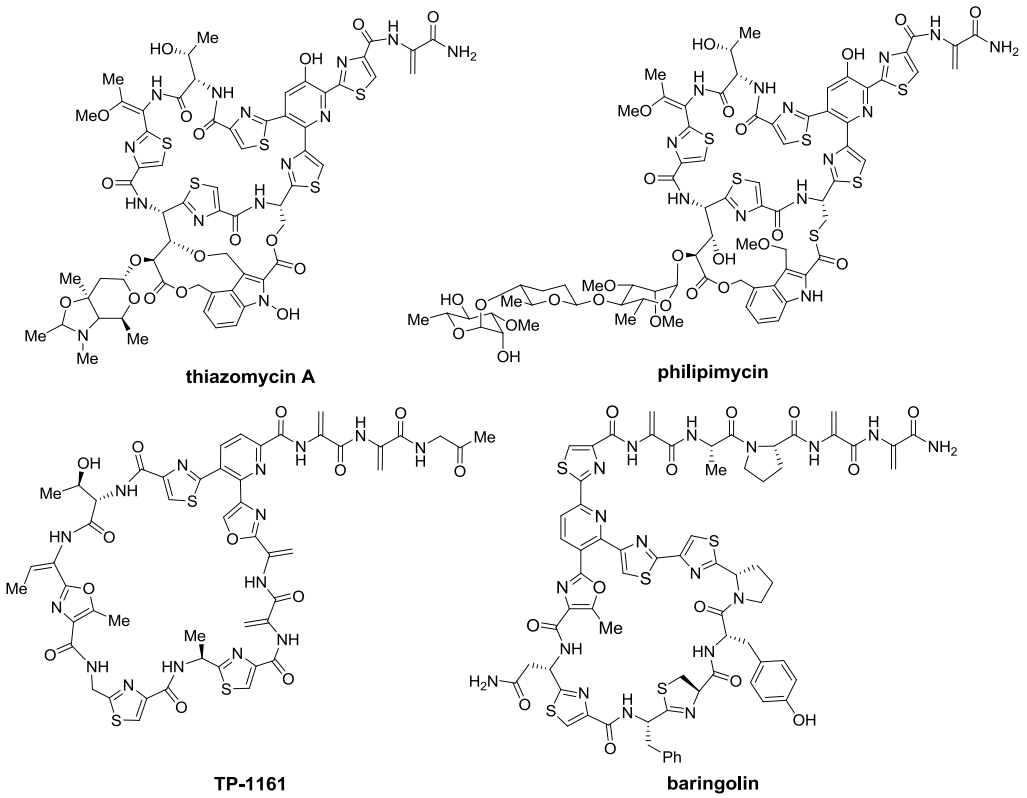


Figure 3. Some of the most recently described thiopeptides.

Assignment of thiopeptide's structure can be a very complex task, as exemplified by thiostrepton, whose structure elucidation was originally faced by degradation studies and structure determination of fragments.[21] However, the later use of X-ray diffraction was essential to elucidate both connectivity and stereochemistry.[22] Although the development of NMR spectroscopy techniques has permitted the elucidation of many thiopeptides' structure, a high degree of uncertainty remains until further evidence is provided. This was clearly the case of micrococcin P1. Early studies on its constitution by hydrolysis[23–25] of the natural extract permitted the identification of most moieties present in micrococcin; however, there was no clear evidence of its connectivity. Later on, NMR studies[26–28] and synthesis of proposed structures[29–32] of the natural compound resulted in better hypotheses for its constitution and stereochemistry, although none of the synthesized products was identical to the natural one. It was not until its total synthesis was achieved by Ciufolini in 2009, 51 years after its discovery, that micrococcin P1 structure and stereochemistry were finally confirmed.[33]

The structure of most thiopeptides has been investigated by a combination of degradation, mass spectrometry and NMR studies. The impossibility to obtain crystals for the vast majority of them prompted the assignment of their structure without a clear evidence of their stereochemistry. In spite of this limitation, in many cases their configuration has been proposed by analogy with similar isolates,[34,35] via amino acid analysis[36–38] or via isotopic

labeling through feeding with labeled amino acids.[39,40] In some cases, less conventional techniques have been chosen. Such are the cases of promoinducin and thiotipin, where chiral-TLC was used to determine the configuration of L-threonine from an acidic hydrolysate.[41,42] Absolute configurations have also been reported after NMR spectroscopy studies and chiral capillary electrophoresis.[43]

As exemplified by micrococcin P1, a synthetic approach to the problem can serve as the ultimate confirmation for both connectivity and stereochemistry. This strategy also includes the comparison of fragments with their synthetic counterparts; such was the case of GE2270A.[44] Synthesis of thiopeptides polyheterocyclic cores has been used to confirm the structure of the corresponding degradation products, while at the same time it has also permitted the development of the necessary synthetic methodology.[45]

1.2.2. Biosynthesis

The biosynthetic pathway of thiopeptides has been very elusive for a long time; however, recent discoveries have put light on the synthesis of these highly modified peptides. Peptide-based natural products can have two distinct origins depending on how its amino acids are condensed together to form the parent peptides. These can be either synthesized on the ribosome as product of mRNA translation or can be assembled by nonribosomal peptide synthetases (NRPSs). Though most highly modified peptide-derived natural products are synthesized by NRPSs, there was no evidence of such origin for thiopeptides. Surprisingly, very recent discoveries by four different groups have demonstrated that thiopeptide's parent pre-peptide in ribosomally synthesized and thus, is genetically encoded.[17,46–48]

Bio-informatics and genome mining have been essential for the identification of genes that encode the precursor peptide and the enzymatic machinery necessary for its subsequent tailoring. [17,19,46–55] The gene encoding the precursor peptide has been identified for many thiopeptides and in all cases there is a perfect agreement with the expected amino acid sequence. This precursor peptide is divided in two different regions, a structural peptide of 12 to 17 residues at the C-terminus, which contains the amino acids that will constitute the thiopeptide itself, and a leading peptide of 34 to 55 residues at the N-terminus, which is cleaved during the bio-synthetic process. In some cases, the C-terminal structural peptide contains one or two extra residues that are cleaved during the tailoring to confer each thiopeptide its characteristic C-terminus.[56,57] All necessary enzymes for pre-peptide tailoring are encoded in genes surrounding that of the precursor peptide, forming a gene cluster (see Figure 4 for an example on GE37468 gene cluster (*get*) and precursor peptide[53]; in the gene cluster, genes appear as arrows and are named systematically from A to M. Each gene (*getX*) codes a gene product, a protein/enzyme (GetX)).

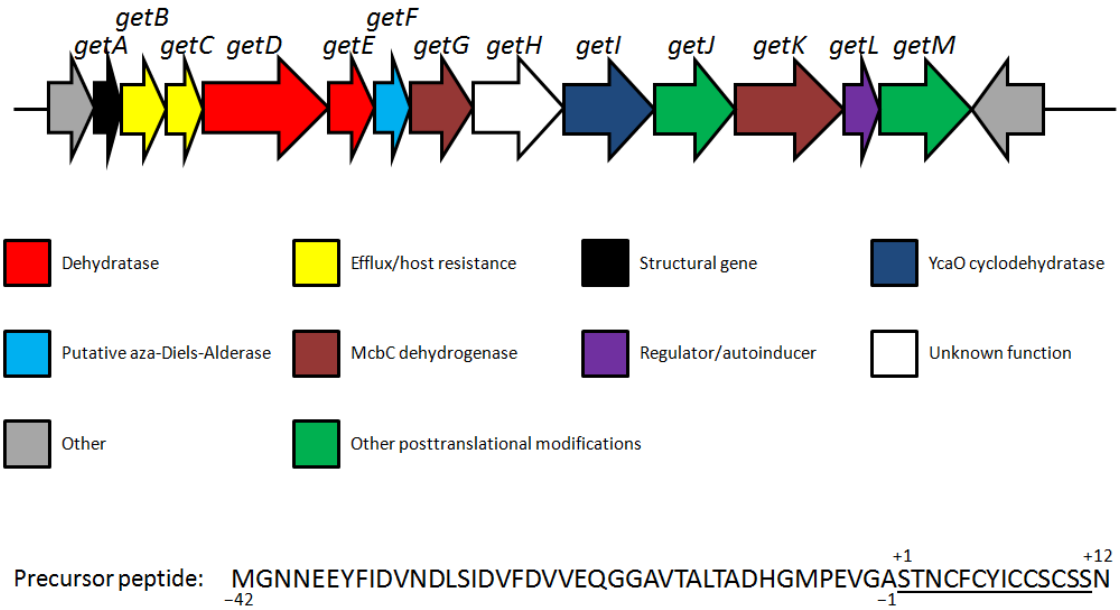
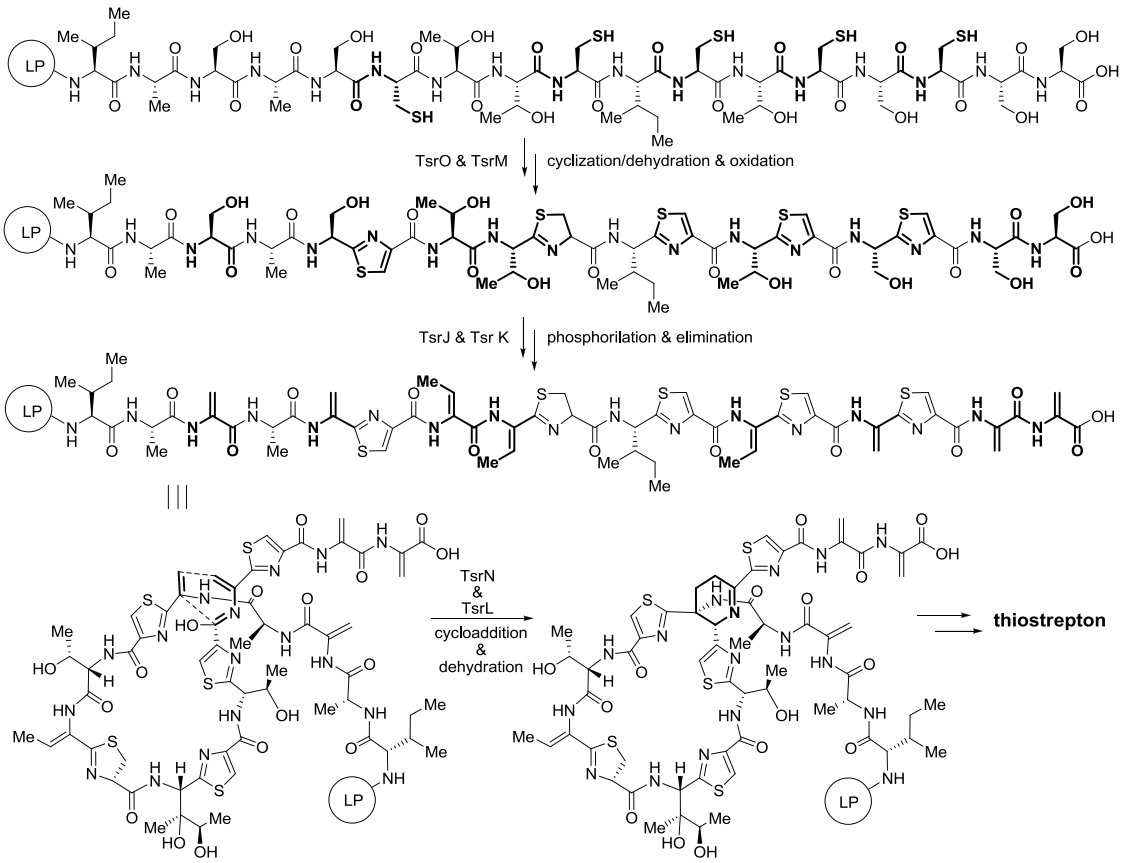


Figure 4. GE37468 biosynthetic gene cluster and its precursor peptide sequence, which is coded in the structural gene. In the precursor peptide sequence, the structural peptide is numbered with positive figures and the leading peptide with negative ones. Residues that appear in the mature thiopeptide are underlined.

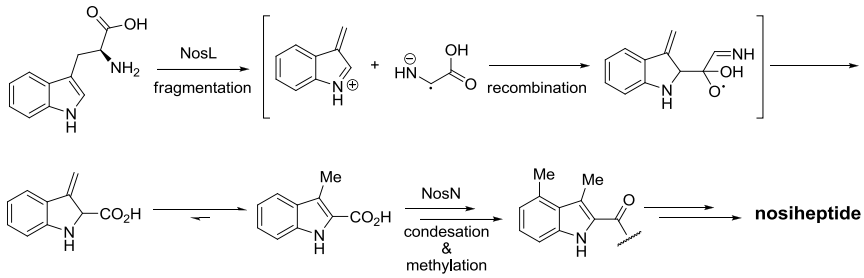
The role of most enzymes present in some thiopeptides gene clusters has been already discovered. Similarity with known enzymes of the same function, gene deletions and characterization of products that result from transformations with isolated enzymes, have permitted to establish which transformations and in which order do these take place. [47,54,55,58–64] Apparently, oxazole, thiazole and thiazoline rings are formed first through cyclization, dehydration and, if required, oxidation of Ser, Thr and Cys residues. In a second step, Ser and Thr phosphorilation and elimination yields the corresponding dehydroalanine (Dha) and dehydrobutyrine (Dhb) residues, respectively. Finally, intramolecular aza-Diels-Alder-like cycloaddition between distant Dha residues occurs, followed by dehydration and, when required, elimination to constitute the central six-membered ring. Further side-chain modifications, such as oxidations, cyclizations, methylations and incorporation of indolic or quinaldic acid moieties seem to occur in later stages of the bio-synthetic pathway (see Scheme 1 for an example on thiostrepton bio-synthesis[47]).



Scheme 1. Biosynthetic pathway of thiostrepton. LP = leading peptide. Enzymes involved in the biosynthetic pathway (TsrX) are named according to their corresponding gene (*tsrX*) in thiostrepton's gene cluster (*tsr*).

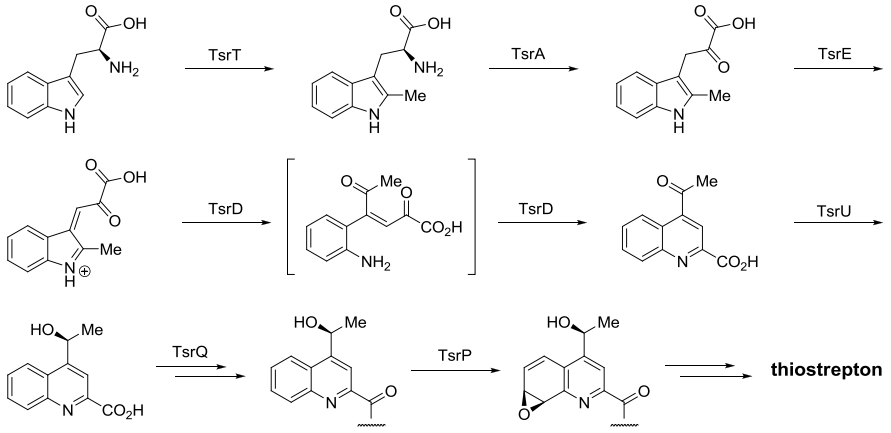
Both quinaldic and indolic acid moieties found in *a*–*c* and *e* series thiopeptides are synthesized from L-tryptophan and are part of the second macrocycle found in these compounds. This was first demonstrated by labeling[65–68] and enzyme function[69,70] experiments and more recently also using genetic engineering methods.[59,61–63]

In the case of indolic acid formation, L-tryptophan undergoes a radical-mediated rearrangement and Cα migrates to position 2 of indole (Scheme 2). Subsequently, *S*-adenosylmethionine-dependent 4-methylation of the aromatic scaffold after condensation with the structural peptide yields an advanced intermediate of the mature thiopeptide.[61,71]



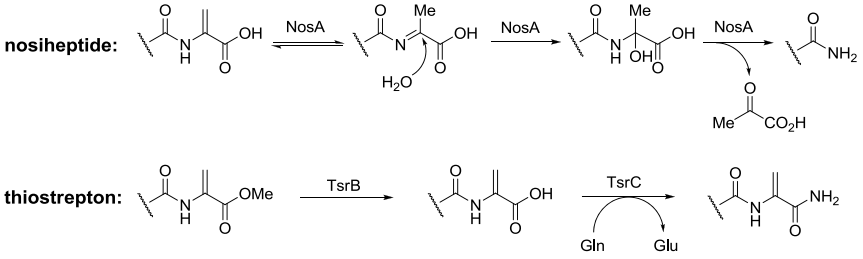
Scheme 2. Biosynthesis of indolic acid moietiy from L-tryptophan and incorporation into nosiheptide. Enzymes involved in the biosynthetic pathway (NosX) are named according to their corresponding gene (*nosX*) in nosiheptide's gene cluster (*nos*).

Alternatively, quinaldic acid synthesis starts with *S*-adenosylmethionine-mediated methylation of Trp (Scheme 3).[48,62] Deamination/oxidation steps follow and after ring opening, recyclization yields the quinaldic acid moiety. This is then reduced, attached to the structural peptide and epoxidized. Upon epoxide opening, the second macrocycle of thiostrepton is formed.



Scheme 3. Biosynthesis of quinaldic acid moietiy from L-tryptophan and incorporation into thiostrepton.

C-terminal tailoring is one of the last steps in thiopeptides maturation. In those cases where a C-terminal amide is present, two distinct mechanisms have been described for their formation (Scheme 4). Nosiheptide structural peptide contains an extra C-terminal Ser residue, which is lost during tail maturation , giving rise to a C-terminal amide.[56] By contrast, thiostrepton's structural peptide does not contain any extra amino acids and its C-terminal Ser residue can be methylated to form the corresponding ester. The C-terminal amide is formed by deesterification and subsequent amidation using Gln as nitrogen donor. [56]



Scheme 4. Proposed mechanisms for C-terminal amide formation during nosiheptide and thiostrepton maturation.

1.2.3. Biological Activity

Thiopeptides are best regarded as antibacterial agents, however, their therapeutic potential is surprisingly broad and have been found to posses anticarcinogenic,[72–78] antiplasmodial,[79–84] immunosuppressive,[85] renin inhibitory,[86] RNA polymerase inhibitory[87] and antifungal[88] activities. This wide variety of biological functions has resulted in a very prolific literature outcome, positioning the macrocyclic scaffold of thiopeptides as a veritable privileged structure.

1.2.3.1. Antibacterial Activity

It is already well established that thiopeptides exert their antibacterial function via the inhibition of ribosomal protein synthesis. However, this is the result of different mechanisms of action that depend on macrocycle size. Thiopeptides exhibit macrocycles of three different sizes, 26-, 29- and 35-membered rings, depending on the number of residues present. Thiopeptides of 26-member macrocycles, such as that of micrococcin P1 and the siomycins (Figure 5) are known to bind the GTPase-associated region of the ribosome/L11 protein complex. By doing so, the thiopeptide blocks the binding region of elongation factor G (EF-G) and does not allow translocation of the growing-peptide/tRNA complex in the ribosome to occur.[89–91] On the contrary, those thiopeptides with a 29-membered ring, in the fashion of GE37468A, bind to elongation factor Tu (EF-Tu), blocking its tRNA/amino acyl complex binding site.[92–94] By doing so, the complex cannot be delivered into the ribosome and peptide elongation does not take place. Compounds with larger macrocycles, those with 35-membered rings, maintain potent antibacterial activity; however, their molecular target still remains unknown.

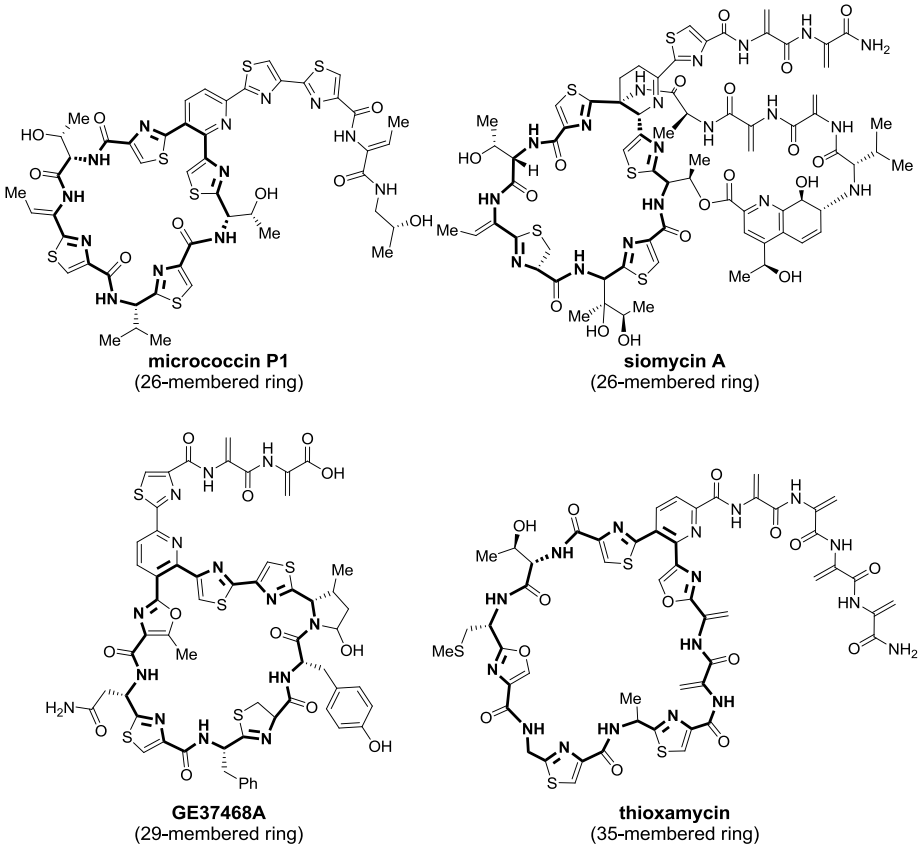


Figure 5. Thiopeptides have macrocycles of different sizes that determine their mode of action.

Somewhat related to antibacterial activity is *tipA* gene promotion, which encodes two thiostrepton-induced proteins (Tip), TipAL and TipAS.[95] The latter, TipAS, serves as a mechanism of defense for bacteria, since it sequesters and covalently binds a thiopeptide molecule, which can no longer inhibit ribosomal protein synthesis. *TipA* promotion has been used to identify thiopeptides in a high throughput screening program, which detected transcription of the promoter of *tipA* (*ptipA*) and led to the discovery of geninthiocin (Figure

6)[96] Other thiopeptides, such as thiotipin[42] and thioxamycin[97] were discovered thanks to its *tipA* promoting activity. Interestingly, the 35-membered thiopeptide radamycin is completely devoid of antibacterial activity, but is a very strong inducer of *tipA* gene expression (Figure 6). Various *tipA* promoting thiopeptides are depicted in Figure 6, where very preserved regions, associated with key interactions for binding with ribosome/L11 complex,[98] are highlighted. Although those residues are different in radamycin, promothiocin B displays those same not preserved residues in a smaller 26-membered macrocycle and retains potent antibacterial activity. Apparently, *tipA* promotion activity is more dependent on the presence of a dehydroalanine-containing tail close to the six-membered central scaffold.[99]

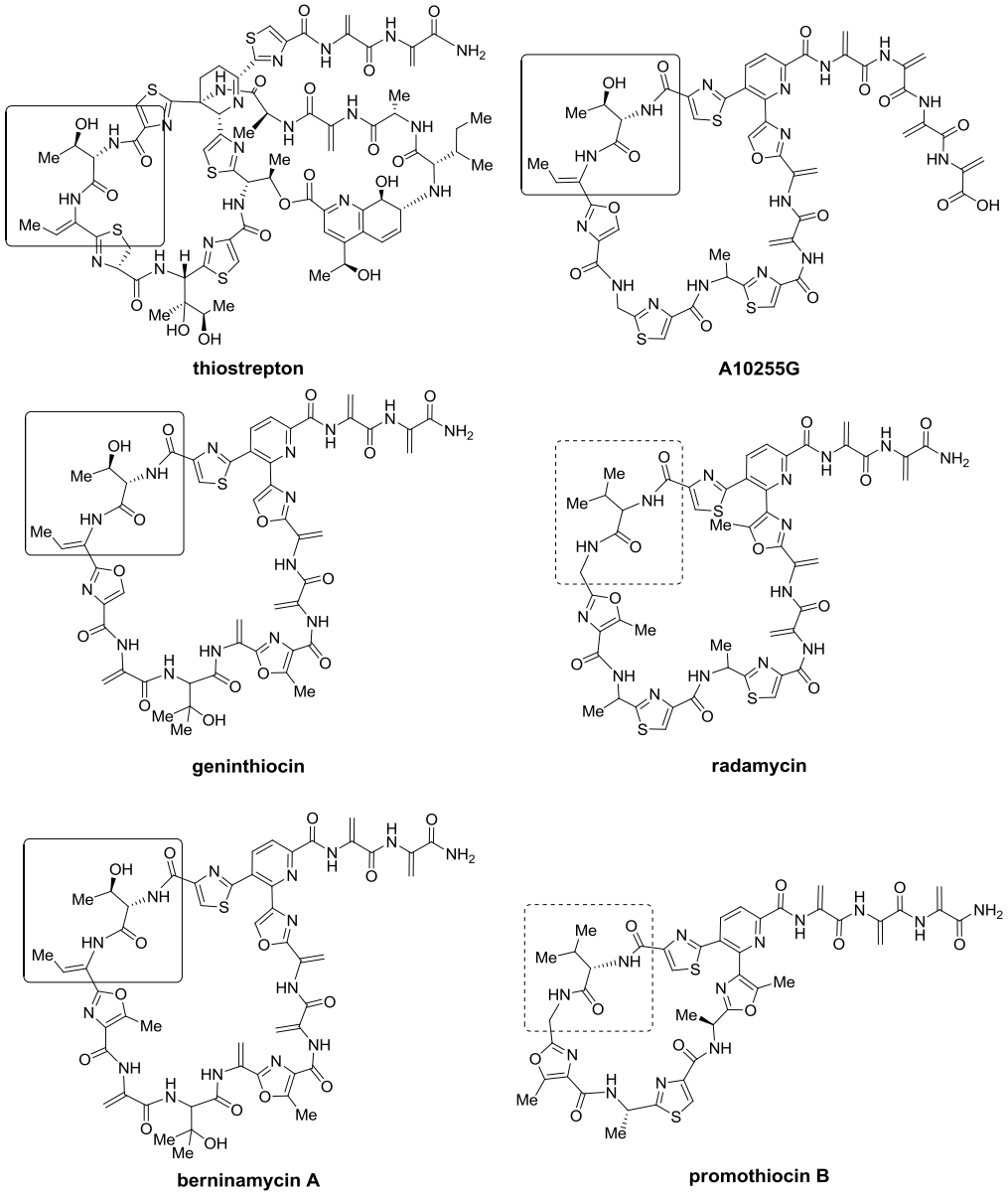


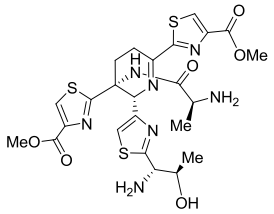
Figure 6. Thiopeptides with *tipA* promoting activity. A very preserved region, which has been shown to interact with the ribosome/L11 complex, is highlighted (solid squares). Radamycin, devoid of antibacterial activity, has a mutated sequence in the previously mentioned region (hashed squares). Promothiocin B posses the same mutated residues, but maintains antibacterial activity, though in a 26-membered macrocycle.

1.2.3.2. Anticarcinogenic Activity

One of the biological properties of thiopeptides of major interest, apart from the antibacterial one, is anticancer activity. In this regard, thiostrepton was found to selectively kill cancer cells without showing any cytotoxicity against healthy tissues.[100] Such promising effect has been demonstrated to arise from selective inhibition of transcription factor forkhead box M1 (FOXM1).[74,77,78] FOXM1 overexpression is associated with the development and progression of cancer and its selective targeting is a huge achievement, since transcription factors have been considered undruggable for a long time.[101,102]

During synthetic efforts of Nicolaou's group, it was discovered that the central core of thiostrepton retained both antibacterial and anticancer activity. Although antibacterial activity was retained, potency decreased. However, the same central fragment displayed increased potency against all cancer cell lines tested (Table 1).[72]

Table 1. Thiostrepton fragment outperforms its parent compound against various cancer cell lines.



	LC_{50} (μM)					IC_{50} (μM)			
compound	NCI-H460	HCT-116	SK-OV-3	MCF-7	K-562	1A9	PTX10	A8	AD10
thiostrepton	1.5	1.6	2.8	3.8	1.7	0.96	1.1	0.9	91.0
central core	0.9	0.6	1.2	0.9	0.8	0.07	0.1	0.2	0.4

1.2.3.3. Antiplasmodial Activity

Many thiopeptides have been found to possess anti-malaria activity.[79–83] Although *Plasmodium falciparum* parasite cell is eukaryote, thiopeptides target apicoplast protein synthesis,[103] which resembles that of prokaryotic organisms. Although it has been demonstrated that thiostrepton binds to the apicoplast 23S rRNA,[79] thiopeptides of the *d* series such as micrococcin P1 and amythiamicin A are much more potent inhibitors of *P. falciparum* growth.[80]

Very recently, the use of thiostrepton semi-synthetic analogues has demonstrated that it targets both the apicoplast ribosomes and the proteasome of *P. falciparum*.[84] This dual mode of action could make thiostrepton and similar thiopeptides less prone to resistance development than single-target drugs.

1.2.3.4. Immunosuppressive Activity

A screening program in search of immunosuppressants identified siomycin as inhibitor of antibody production by murine B-cells.[85] Comparison with thiostrepton showed the superior behavior of the structurally similar siomycin. Both thiopeptides are thought to possess a

different mechanism of action than that of FK506, a common immunosuppressant drug, and would act directly on B-cells.

1.2.3.5. Renin inhibitory activity

Clyclothiazomycin is a very unique thiopeptide that does not possess a tail (Figure 7). In fact, the azole-containing branch that would serve as tail is linked to the macrocycle, forming a second ring, different from those found in thiopeptides of series *a*–*c* or *e*. However, due to its central tri-substituted pyridine ring, it is still considered a member of the *d* series. Maybe because of its peculiar structure, different activities have been found for it. The first one to be described was human plasma renin inhibitory activity.[86] Renin is an enzyme associated with hypertension,[104] diabetes[105] and Alzheimer's disease[106] and is a rate-limiting enzyme in a cascade that starts with the cleavage of angiotensiogen and ends with the formation of angiotensin II. Because of this, renin is regarded as one of the most effective targets to treat hypertension.

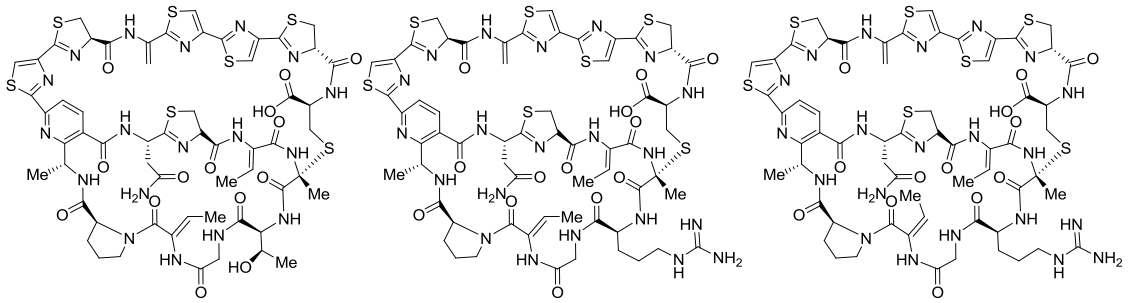


Figure 7. Cyclothiazomycin and its more recently isolated analogues B1 and B2.

1.2.3.6. RNA Polymerase Inhibitory Activity

Thiopeptides with a very similar structure to cyclothioazomycin were recently isolated and characterized and were named after the parent compound (Figure 7).[87] In particular, cyclothiazomycin B1 was found to inhibit transcription by bacteriophage RNA polymerase. Such result might serve to further understand transcription at the molecular level.

1.2.3.7. Antifungal Activity

Cyclothiazomycin B1 (Figure 7) also exhibits antifungal activity and inhibits the growth of various filamentous fungi. It presumably does so by binding to chitin, causing cell wall fragility.[88]

Saramycetin has also been described as an antifungal thiopeptide;[107] however, it was not fully characterized and might not fulfill the structural requirements to fall into this family of natural products.

1.2.4. Conformation and Binding

The structure of thiopeptides has been studied using different techniques, which have also been used to elucidate the active conformation and the key contacts required to exert their

biological activity. Though thiopeptides are relatively large molecules with big macrocycles, they possess many azoles, dehydroamino acids and amide bonds that confer the required rigidity for efficient binding.[108] First conformational studies were performed by NMR and provided solution structures of the promothiocins,[109] nocathiacin I[43] and amythiamicin D.[110] For amythiamicin D, an intramolecular H-bond was detected[110] which seems to favor the bioactive conformation. The same H-bond interaction can be observed between the equivalent positions of active GE2270A and thiomuracin A analogues.[111,112] NMR studies of the thiostrepton/L11/rRNA complex[98] identified key contacts in the 26-membered ring of thiostrepton that led to the design of small analogues that maintained their binding capability.[113]

X-ray analysis of the L11/rRNA complex and superimposition with optimized thiopeptide structures have determined that compounds targeting this complex bind to a cleft between the ribosome and L11 protein.[90,91,114] This region, the so-called GTP associated center (GAC), is also the binding site of elongation factor G (EF-G), which is responsible of translocation during ribosomal protein synthesis.

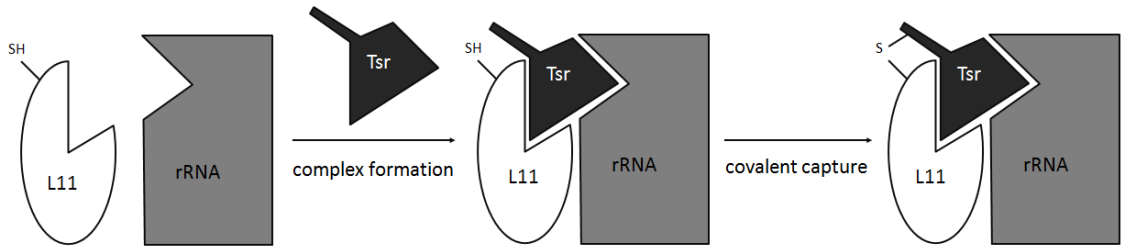


Figure 8. Proximity induced covalent capture. Mutated L11 protein has an external Cys residue in the area where interaction with thiostrepton's tail is expected. Tsr = thiostrepton.

Alternatively, mutation studies have been carried out to study the thiostrepton/L11/rRNA ternary complex. Site-directed mutation of L11 to introduce a Cys residue in a suitable surface position, permitted proximity induced covalent capture (PICC) experiments to be carried out (Figure 8).[89] In a PICC experiment, the newly introduced Cys should perform a 1,4-conjugated addition to a Dha residue of the thiopeptide tail. These experiments suggested a slightly different binding mode for thiostrepton, which would not sit right inside the cleft between L11 and the 23S rRNA, but closer to the ribosome surface. Further mutation studies of either L11 or 23S rRNA were in agreement with this alternative binding mode, demonstrating that mutations on L11 did not avoid thiostrepton binding, whereas mutations on the ribosome diminished affinity between the complex and the thiopeptide.[115]

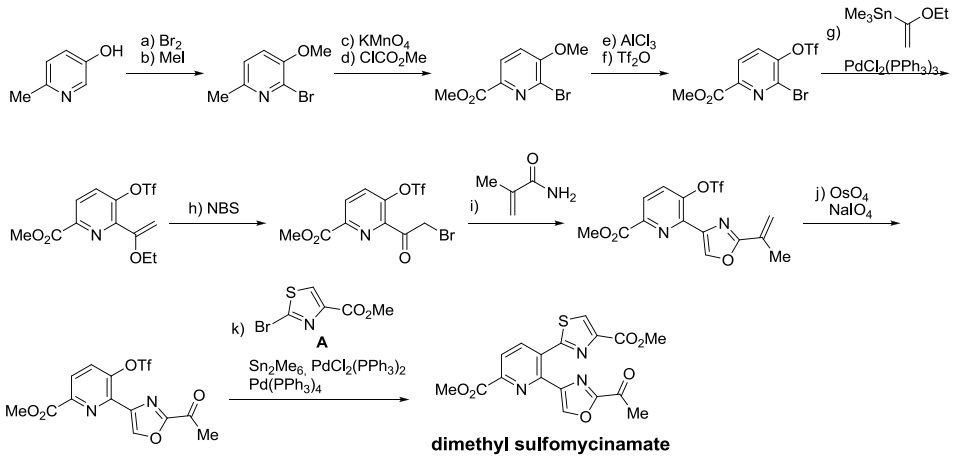
1.2.5. Chemical Synthesis

The complex architectures and challenging structures of thiopeptides have gathered the attention of many groups that have envisioned different strategies to accomplish their syntheses.[45] Pioneering work by the groups of Kelly,[116–119] Shin,[31] Moody,[120,121] Nicolaou,[122,123] Ciufolini[32,33] and Bach[124,125] developed different methodologies that led to the total synthesis of various thiopeptides. However, most efforts have been devoted to the construction of the central polyheterocyclic core,[116–119,126–133] which have been synthesized by two well

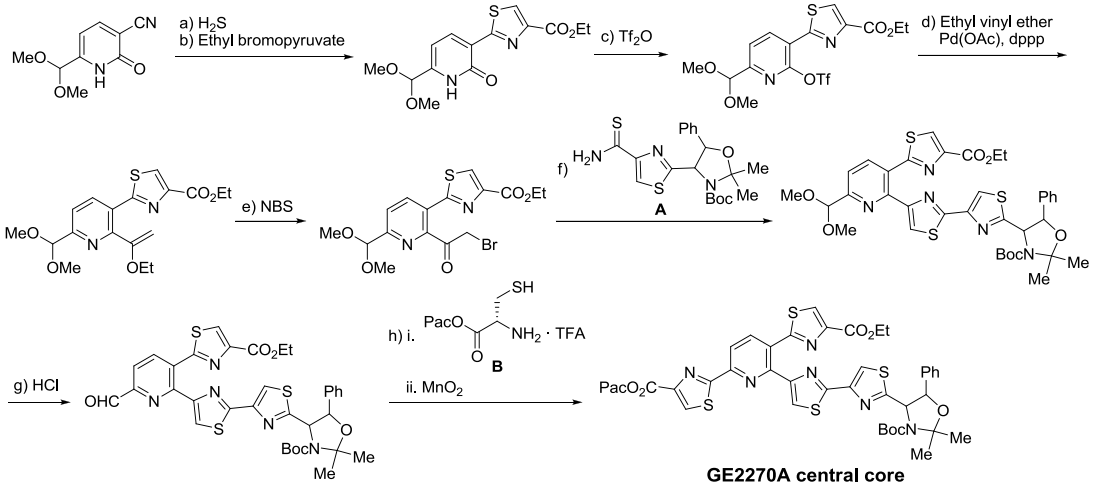
distinguished main strategies: modification of an existing pyridine and construction of the central ring.

Modification of pyridine

Early efforts by the group of Kelly focused on the synthesis of different thiopeptide cores (Scheme 5).[116–119] One of his landmark achievements was the synthesis of dimethyl sulfomycinamate, a product of acidic methanolysis of sulfomycin.[134]



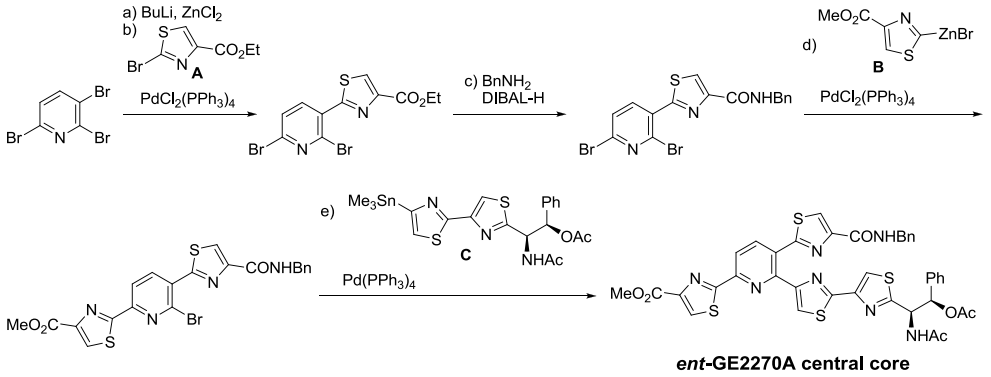
Scheme 5. Kelly's synthesis of dimethyl sulfomycinamate. Reagents and conditions: a) Br_2, pyridine, rt, 77%; b) MeI, K_2CO_3, acetone, reflux, overnight 88%; c) $KMnO_4$, 90 °C, 3 h; d) $ClCO_2Me$, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 2 h, 65%; e) $AlCl_3$, CH_2Cl_2, reflux, 2 days, 93%; f) Tf_2O, 2,6-lutidine, CH_2Cl_2, 0 °C, 5 min, 95%; g) $PdCl_2(PPh_3)_2$, 1,4-dioxane, 100 °C, overnight 97%; h) NBS, THF, H_2O, rt 10 min, 95%; i) THF sealed tube, 100 °C, 3 days, 65%; j) OsO_4, $NaIO_4$, 1,4-dioxane, H_2O, rt 3 h, 85%; k) **A**, Sn_2Bu_6, $Pd(PPh_3)_2Cl_2$, $Pd(PPh_3)_4$, LiCl, 1,4-dioxane, 100 °C, overnight, 35%.



Scheme 6. Shin's synthesis of GE2270A central core. Reagents and conditions: a) H_2S, DMAP, Et_3N, pyridine, rt, 3 days, 90%; b) i. $KHCO_3$, $BrCH_2COCO_2Et$, THF, 0 °C, then rt, overnight; ii. TFAA, pyridine, THF, 0 °C, 1 h, then rt, overnight, 53%; c) Tf_2O, DMAP, pyridine, 0 °C, 1 h, then rt, overnight, 93%; d) ethyl vinyl ether, Et_3N, dppp, $Pd(OAc)_2$, toluene, reflux, overnight, 73%; e) NBS, THF, H_2O, rt, 5 min; f) i. **A**, $KHCO_3$, DME, 0 °C, 1 h, then rt, overnight; ii. TFAA, pyridine, 0 °C, 2 h, 63% (2 steps); g) 2 M HCl in THF, rt, 24 h; h) i. **B**, Et_3N, toluene, rt, 15 min; ii. MnO_2, toluene, rt, 12h, 41% (2 steps). dppp = 1,3-bis(diphenylphosphino)propane; Pac = Phenacyl.

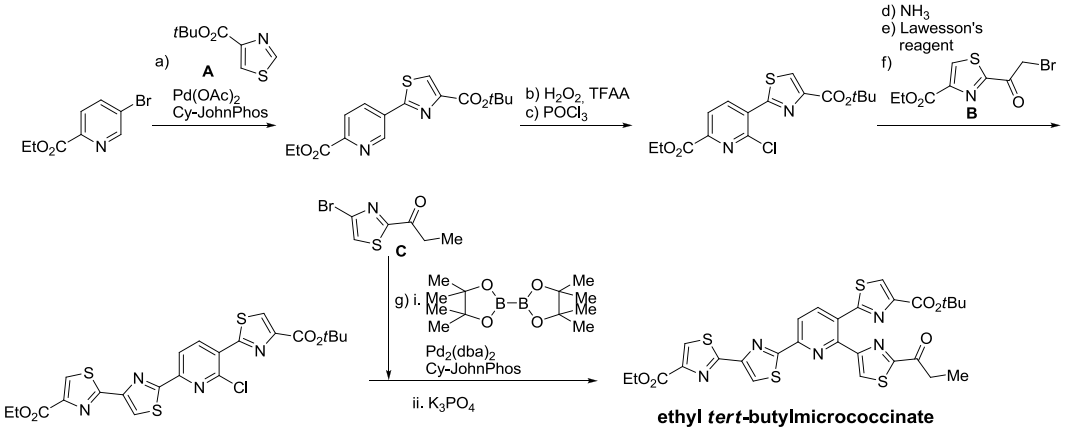
Shin and co-workers developed their own strategies and synthesized many polyheterocyclic cores and fragments.[135–142] Most of their syntheses start from a pre-functionalized pyridine or pyridone (Scheme 6).

The group of Bach described a very convergent approach to the core of GE2270A, which started from 2,3,6-tribromopyridine and was fully based on cross-coupling reactions (Scheme 7).[130] This work served for confirmation of the polyheterocyclic core stereochemistry and also set the methodology for further synthesis of GE2270A[124] and amythiamicins A and D.[143]



Scheme 7. Bach's synthesis of GE2270A's central core. Reagents and conditions: a) BuLi, $ZnCl_2$, THF, ; b) **A**, $PdCl_2(PPh_3)_2$, THF, 81% (2 steps); c) $BnNH_2$, DiBAL-H, THF, CH_2Cl_2, 86%; d) **B**, $PdCl_2(PPh_3)_2$, THF, DMA, 78%; e) **C**, $Pd(PPh_3)_4$, 1,4-dioxane, 80 °C, 61%.

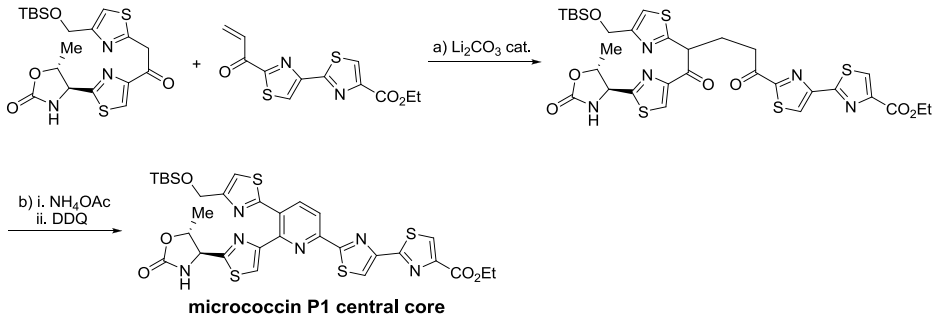
More recently, the group of Hoarau optimized conditions for the cross-coupling of thiazoles with pyridines using direct C-H activation on thiazole position 2[132] and also formation of pinacolboronic acid and *in situ* cross-coupling on thiazole position 4, which is limited to 2-acylthiazoles.[133] Using both methodologies, the synthesis of ethyl *tert*-butylmicrococcinate was achieved (Scheme 8).[133]



Scheme 8. Synthesis of ethyl *tert*-butylmicrococcinate using direct C-H activation and one-pot borylation/cross-coupling of thiazoles. Reagents and conditions: a) **A**, $Pd(OAc)_2$, Cy-JohnPhos, Cs_2CO_3, DMF, 110 °C, 18 h, 74%; b) $CO(NH_2)_2$, H_2O_2, TFAA, MeCN, 0 °C, 30 min; c) $POCl_3$, toluene, DMF, rt, 6 h, 78% (2 steps); d) NH_4OH, THF, rt, 82%; e) Lawesson's reagent, toluene, 2 h, 76%; f) **B**, EtOH/THF (1:1), 65%; g) i. **C**, bispinacolatodiboron, $Pd_2(dba)_2$, Cy-JohnPhos, KOAc, dioxane, 110 °C, 30 min; ii. K_3PO_4, dioxane, H_2O, 110 °C, 12 h, 87%.

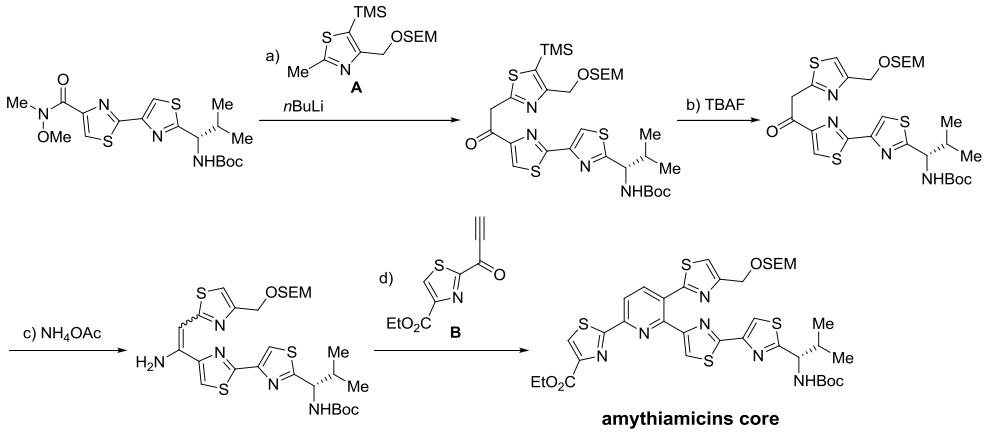
Construction of the central ring

One of the most beautiful contributions and the first one to rely on construction of the pyridine ring was Ciufolini's synthesis of micrococcin's central polyheterocylic core (Scheme 9).[126] With the required thiazole building blocks in hands, the pyridine ring is formed in two steps in almost quantitative yield. This led to the synthesis of the Bycroft-Gowland structure of micrococcin P1, which had been miss-assigned.[32] It took ten more years to finally synthesize the true structure of micrococcin P1 and confirm its stereochemistry.[33] Using the same strategy, thiocillin I has been recently synthesized.[144]



Scheme 9. Ciufolini's synthesis of the polyheterocyclic core of micrococcin P1. Reagents and conditions: a) cat. Li_2CO_3, EtOAc, 92%; b) NH_4OAc, EtOH then DDQ, toluene, 97%.

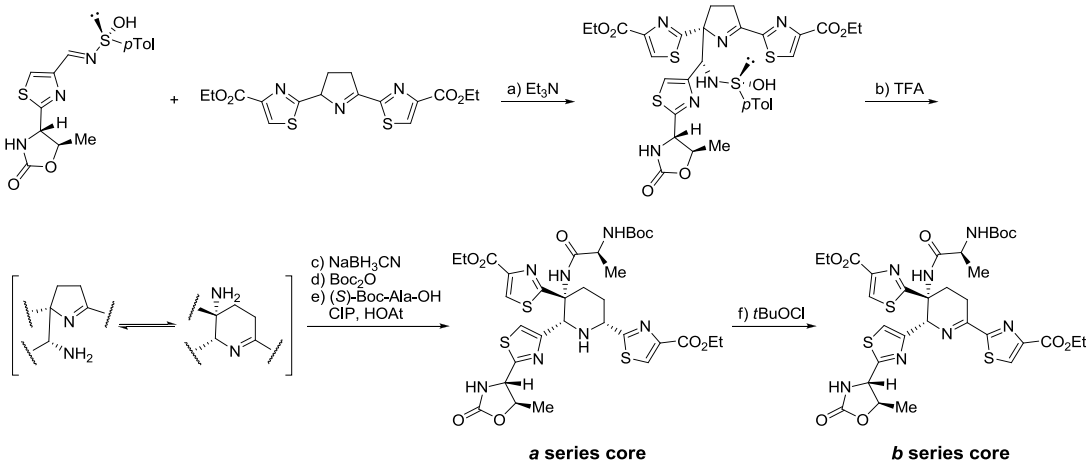
Bagley, first in the group of Moody, and co-workers also described their own syntheses of thiopeptides' cores constructing the pyridine ring with advanced thiazole-containing building blocks. Using Bohlmann-Rahtz pyridine synthesis, various thiopeptide cores were obtained (Scheme 10).[129,145–150] Also, a very early total synthesis of promothiocin A was achieved.[151,152]



Scheme 10. Synthesis of amythiamicins pyridine cluster using Bohlmann-Rahtz pyridine formation. Reagents and conditions: a) **A**, *n*BuLi; H_2O, 91%; b) TBAF, THF, rt, 1 h, 93%; c) NH_4OAc, microwave, 120 °C (100 W), toluene, 30 min, 76%; d) **B**, EtOH, 60 °C; toluene, AcOH, 70 °C, 85% (93% *ee*). SEM = 2-(trimethylsilyl)ethoxymethyl.

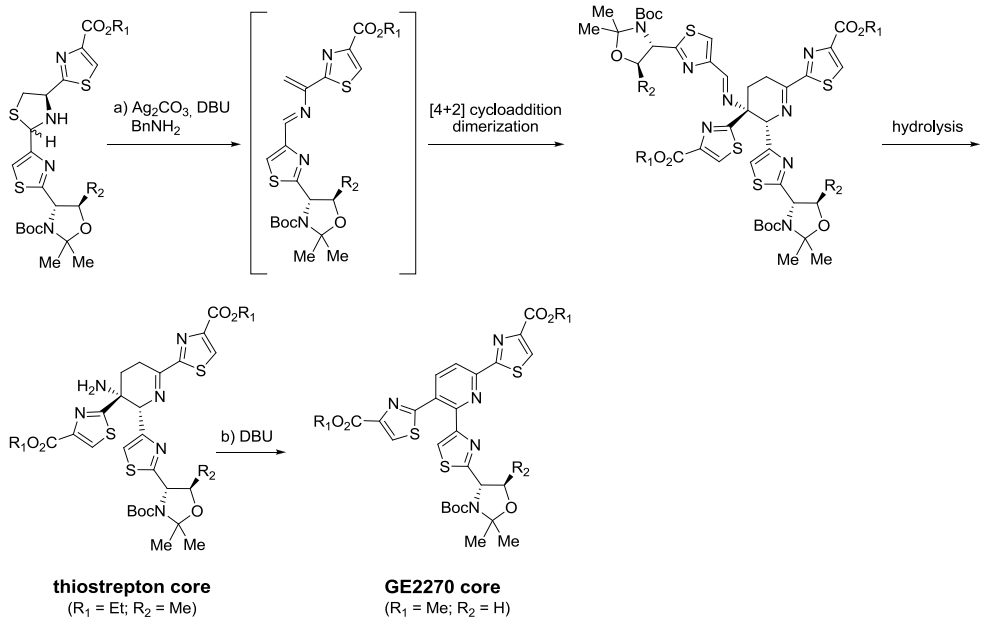
The first synthesis of a polyheterocyclic core from the *a* and *b* series was reported by the group of Hashimoto. Their approach was based on formation of a substituted central 1-pyrroline that underwent ring-expansion to form a piperidine ring (*a* series) that could be selectively oxidized for form the corresponding 2,3,4,5-tetrahydropyridine (*b* series) (Scheme

11).[153] In further reports they described the synthesis of other fragments[154,155] of *b* series thiopeptides and finally achieved the total synthesis of siomycin A.[156,157]



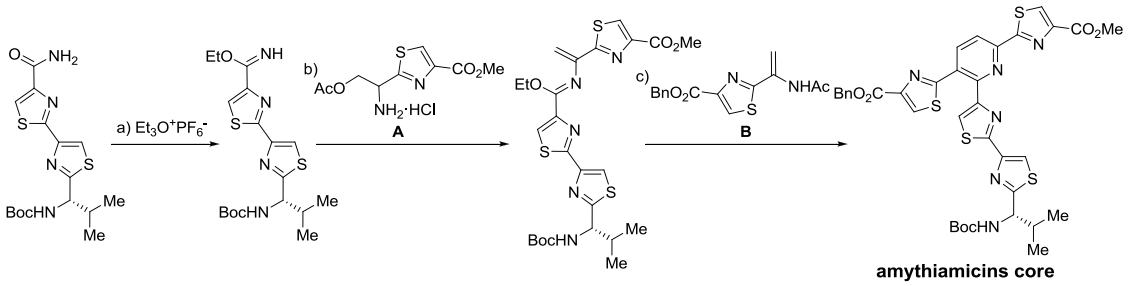
Scheme 11. Synthesis of *a* and *b* series central core by Hashimoto and co-workers. Reagents and conditions: a) Et_3N, THF, −25 °C, 71%; b) TFA, EtOH; c) $NaBH_3CN$, AcOH, EtOH, 52% (2 steps); d) Boc_2O, DMAP, Et_3N, THF, 0 °C, 84%; e) (*S*)-Boc-Ala-OH, CIP, HOAt, DIPEA, CH_2Cl_2, 93%; f) *t*BuOCl, THF, −78 °C, then cat. DMAP, Et_3N, 95%. CIP = 2-chloro-1,3-dimethylimidazolidium hexafluorophosphates.

Cycloadditions have also been used for the synthesis of various central fragments. Almost simultaneously, the groups of Nicolaou[158] and Moody[127] developed two different bio-inspired strategies to obtain their target cores via an aza-Diels-Alder reaction. Nicolaou's strategy was based on the silver-promoted formation of an aza-diene from a precursor thiazolidine, which would dimerize in a [4+2] cycloaddition (Scheme 12). The 2,3,4,5-tetrahydropyridine (*b* series) thus obtained, could be oxidized to form the corresponding fully unsaturated *d* series core.



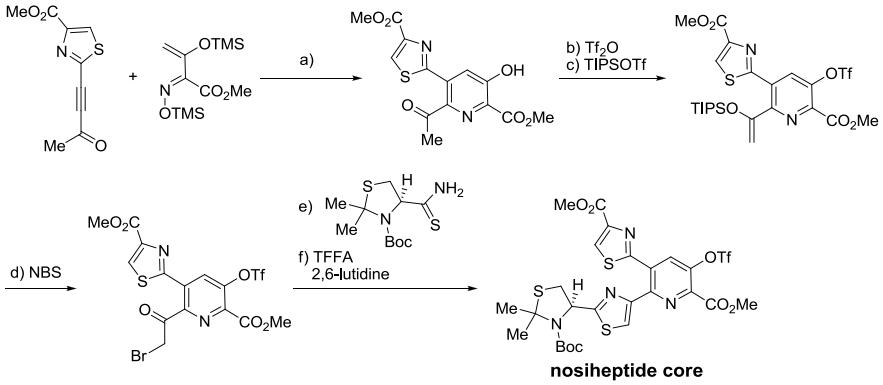
Scheme 12. Bio-inspired synthesis of *b* and *d* series polyheterocyclic cores. Reagents and conditions: a) Ag_2CO_3, $BnNH_2$, DBU, pyridine, −15 °C, 1 h; then H_2O/EtOAc (1:1), 1 h, 60%; b) DBU, EtOAc, reflux, 5 h, 50%.

Using this methodology various total synthesis were achieved, including the challenging thiostrepton,[122,123,159] but also GE2270A and GE2270T[160] and various amythiamicins.[161] On the other hand, Moody's strategy relied on a previously formed aza-diene that could react with a different dienophile, yielding the aromatized cycloaddition product (Scheme 13).[121,162]



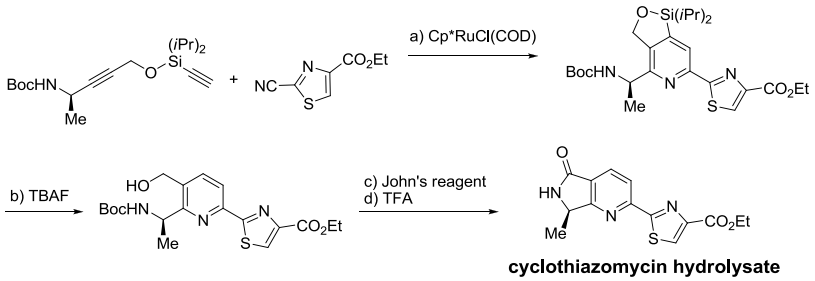
Scheme 13. Bio-mimetic aza-Diels-Alder synthesis of 1,2,3-trisubstituted pyridine cores. Reagents and conditions: a) $Et_3O^+PF_6^-$, CH_2Cl_2, 100%; b) **A**, CH_2Cl_2, then DBU, $CHCl_3$, 63%; c) **B**, toluene, microwave, 120 °C, 33%.

Alternatively, Arndt designed another aza-Diels-Alder strategy that was not based on the biosynthetic pathway, but used an alkyne and a protected α,β-unsaturated oxyme as starting materials, gaving access to 3-hydroxypyridines, including the central core of nosiheptide (Scheme 14).[131] Using this strategy, the main macrocycle of nosiheptide could be synthesized.[163]



Scheme 14. Synthesis of nosiheptide's core via an aza-Diels-Alder cycloaddition. Reagents and conditions: a) toluene, 180 °C, 55%; b) Tf_2O, Et_3N, CH_2Cl_2, 0 °C; c) TIPSOTf, Et_3N, CH_2Cl_2,0 °C, 79% (2 steps); d) NBS, THF/ H_2O, 97%; (e) $KHCO_3$, THF, −40 °C; (f) TFAA, 2,6-lutidine, −20 °C, 60% (2 steps).

Finally, one of the newest and most innovative contributions to the field is the synthesis of cyclothiazomycin's central core hydrolysate through a [2+2+2] ruthenium-catalyzed cyclotrimerization reaction (Scheme 15).[164] This yielded a product identical to that previously described by Bagley, thus confirming its identity.[149]



Scheme 15. Syntehsis of cyclothiazomycin central core hydrolysate via a [2+2+2] ruthenium-catalyzed cycloaddition. Reagents and conditions: a) Cp*RuCl(COD), 1,2-dichloroethane, 60 °C, 82%; b) TBAF, THF, rt, 97%; c) John's reagent, acetone, 0 °C to rt; d) TFA, CH_2Cl_2, rt, 80% (2 steps). Cp* = pentamethylcyclopentadienyl. TBAF = tetrabutylammonium fluoride.

Future advances in the obtention of substituted pyridines will surely facilitate the total synthesis of thiopeptides that still pose a challenge, but most importantly, will give easy access to fragments and analogues for the development of thiopeptide-based drugs with an improved pharmacokinetic profile.

1.2.6. In the Market

To date, only two thiopeptides have hit the market, thiostrepton and nosiheotide. Although both are exclusively devoted to veterinary use, their applications are very different. The lack of thiopeptide formulations for human use stems from their low aqueous solubility, a limitation that also restricts their use in animals.

Thiostrepton is used as one of the ingredient of an ointment for the treatment of cats and dogs skin infections. This ointment always has the same formulation (nystatin, 100,000 units; neomycin sulfate, 2.5 mg; thiostrepton, 2,500 units; triamcinolone acetonide, 1 mg) and is sold by different companies with a variety of brand names: Animax (Dechra), Resortin (Hannah), Panolog (Novartis), Dermalone (Vedco), etc.

Alternatively, nosiheptide, is used as an animal growth promoter. This application was first described short after this thiopeptide was first isolated.[165] Nowadays, tons of pre-mixed animal food with the antibiotic are produced and commercialized.

Given the bad pharmacokinetic profile of thiopeptides, various analogues have been produced, mainly to improve their aqueous solubility. LFF571 (Figure 1), developed by Novartis, is a semi-synthetic analogue of GE2270A currently under clinical trials for the treatment of skin infections in humans.[5,166,167]

When considering the huge therapeutic potential of thiopeptides and the efforts carried out by many research groups, it can be expected that their discoveries will lead to the development of analogues suitable for the treatment of more threatening systemic infections.

References

(1) Fischbach, M. A.; Walsh, C. T. *Science* **2009**, *325*, 1089–1093.

(2) Demain, A. L.; Sanchez, S. *J. Antibiot.* **2009**, *62*, 5–16.

(3) Kumar, N. *Pharma Science Monitor* **2012**, *3*, 2990–3009.

(4) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.

(5) LaMarche, M. J.; Leeds, J. A.; Amaral, A.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Ding, J.; Dzink-fox, J.; Gamber, G.; Jain, A.; Lee, K.; Lee, L.; Lister, T.; Mckenney, D.; Mullin, S.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Trzasko, A.; Whitehead, L.; Yifru, A.; Yu, D.; Yan, W.; Zhu, Q. *J. Med. Chem.* **2012**, *6*, 2376–2387.

(6) Carnio, M. C.; Stachelhaus, T.; Francis, K. P.; Scherer, S. *Eur. J. Biochem.* **2001**, *268*, 6390–6340.

(7) Pagano, J. F.; Weinstein, M. J.; Stout, H. A.; Donovick, R. *Antibiot. Ann.* **1955/56**, 554–559.

(8) Vandeputte, J.; Dutcher, J. D. *Antibiot. Ann.* **1955/56**, 560–561.

(9) Steinberg, B. A.; Jambor, W. P.; Suydam, L. O.; Soriano, A. *Antibiot. Ann.* **1955/56**, 562–565.

(10) Cron, M. J.; Whitehead, D. F.; Hooper, I. R.; Heinemann, B.; Lein, J. *Antibiot. Chemother.* **1956**, *6*, 63–67.

(11) Nagai, K.; Kamigiri, K.; Arao, N.; Suzumura, K.-I.; Kawano, Y.; Yamaoka, M.; Zhang, H.; Watanabe, M.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 123–128.

(12) Jayasuriya, H.; Herath, K.; Ondeyka, J. G.; Zhang, C.; Zink, D. L.; Brower, M.; Gailliot, F. P.; Greene, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Russotti, G.; Walker, A.; Hesse, M.; Seeley, A.; Junker, B.; Connors, N.; Salazar, O.; Genilloud, O.; Liu, K.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *J. Antibiot.* **2007**, *60*, 554–564.

(13) Singh, S. B.; Occi, J.; Jayasuriya, H.; Herath, K.; Motyl, M.; Dorso, K.; Gill, C.; Hickey, E.; Overbye, K. M.; Barrett, J. F. *J. Antibiot.* **2007**, *60*, 565–571.

(14) Zhang, C.; Zink, D. L.; Ushio, M.; Burgess, B.; Onishi, R.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *Bioorg. Med. Chem.* **2008**, *16*, 8818–8823.

(15) Zhang, C.; Herath, K.; Jayasuriya, H.; Ondeyka, J. G.; Zink, D. L.; Occi, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *J. Nat. Prod.* **2009**, *72*, 841–847.

(16) Zhang, C.; Occi, J.; Masurekar, P.; Barrett, J. F.; Zink, D. L.; Smith, S.; Onishi, R.; Ha, S.; Salazar, O.; Genilloud, O.; Basilio, A.; Vicente, F.; Gill, C.; Hickey, E. J.; Dorso, K.; Motyl, M.; Singh, S. B. *J. Am. Chem. Soc.* **2008**, *130*, 12102–12110.

(17) Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

(18) Engelhardt, K.; Degnes, K. F.; Kemmler, M.; Bredholt, H.; Fjaervik, E.; Klinkenberg, G.; Sletta, H.; Ellingsen, T. E.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 4969–4976.

(19) Engelhardt, K.; Degnes, K. F.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 7093–7101.

(20) "Peptides as bioactive compounds": Cañedo Hernández, L. M.; Romero Millan, F.; Fernández Medarde, A.; Fernández Chimeno, R. I.; Hidalgo Villar, J. C. (Instituto Biomar, S.A., Spain)WO2012062906, May 18, **2012**.

(21) Bodanzky, M.; Fried, J.; heehan, J. T.; Williams, N. J.; Alicino, J.; Cohen, A. I.; Keeler, B. T.; Birkhimer, C. A. *J. Am. Chem. Soc.* **1964**, *86*.

(22) Anderson, B.; Hodgkin, D. C.; Viswamitra, M. A. *Nature* **1970**, *225*, 133–235.

(23) Brookes, P.; Fuller, A. T.; Walker, J. *J. Chem. Soc.* **1957**, 689–699.

(24) Mijović, M. P. V.; Walker, J. *J. Chem. Soc.* **1960**, 909.

(25) Brookes, P.; Clark, R. J.; Fuller, A. T.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc.* **1960**, 916.

(26) Walker, J.; Olesker, A.; Valente, L.; Rabanal, R.; Lukacs, G. *J. Chem. Soc., Chem. Commun.* **1977**, 706–708.

(27) Bycroft, B. W.; Gowland, M. S. *J. Chem. Soc., Chem. Commun.* **1978**, 256–258.

(28) Fenet, B.; Pierre, F.; Ciufolini, M. A.; Bernard, C.; Supe, E. *Tetrahedron Lett.* **2002**, *43*, 2367–2370.

(29) Dean, B. M.; Mijović, M. P. V.; Walker, J. *J. Chem. Soc.* **1961**, 3394–3400.

(30) Shin, C.; Okumura, K.; Shigekuni, M.; Nakamura, Y. *Chem. Lett.* **1998**, 139–140.

(31) Okumura, K.; Ito, A.; Yoshioka, D.; Shin, C. *Heterocycles* **1998**, *48*, 1319–1324.

(32) Ciufolini, M. A.; Shen, Y. C. *Org. Lett.* **1999**, *1*, 1843–1846.

(33) Lefranc, D.; Ciufolini, M. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198–4201.

(34) Tori, K.; Tokura, K.; Yoshimura, Y.; Terui, Y.; Okabe, K.; Otsuka, H.; Matsushita, K.; Inagaki, F.; Miyazama, T. *J. Antibiot.* **1981**, *36*, 124–129.

(35) Puar, M. S.; Ganguly, A. K.; Afonso, A.; Brambilla, R.; Mangiaracina, P.; Sarre, O.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1981**, *36*, 5231–5233.

(36) Muramatsu, I.; Motoki, Y.; Aoyama, M. *J. Antibiot.* **1977**, *30*, 383–387.

(37) S. Puar, M.; Chan, T. M.; Hedge, V.; Patel, M.; Bartner, P.; Ng, K. J.; Pramanik, B. N. *J. Antibiot.* **1998**, *51*, 221–224.

(38) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541–1545.

(39) E. Favret, M.; Paschal, J. W.; K. Elzey, T.; D. Boeck, L. *J. Antibiot.* **1992**, *45*, 1499–1511.

(40) Fate, G. D.; Benner, C. P.; Grode, S. H.; Gilbertson, T. J. *J. Am. Chem. Soc.* **1996**, *118*, 11363–11368.

(41) Yun, B.-S.; Seto, H. *Biosci. Biotechol. Biochem.* **1995**, *59*, 876–880.

(42) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *Tetrahedron* **1994**, *50*, 11659–11664.

(43) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284–7285.

(44) Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, *51*, 4867–4890.

(45) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 7930–7954.

(46) Wieland Brown, L. C.; Acker, M. G.; Clardy, J.; Walsh, C. T.; Fischbach, M. A. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2549–2553.

(47) Liao, R.; Duan, L.; Lei, C.; Pan, H.; Ding, Y.; Zhang, Q.; Chen, D.; Shen, B.; Yu, Y.; Liu, W. *Chem. Biol.* **2009**, *16*, 141–147.

(48) Kelly, W. L.; Pan, L.; Li, C. *J. Am. Chem. Soc.* **2009**, *131*, 4327–4334.

(49) Arndt, H.-D.; Schoof, S.; Lu, J.-Y. *Angew. Chem. Int. Ed.* **2009**, *48*, 6770–6773.

(50) Li, C.; Kelly, W. L. *Nat. Prod. Rep.* **2010**, *27*, 153–164.

(51) Walsh, C. T.; Acker, M. G.; Bowers, A. A. *J. Biol. Chem.* **2010**, *285*, 27525–27531.

(52) Wang, J.; Yu, Y.; Tang, K.; Liu, W.; He, X.; Huang, X.; Deng, Z. *Appl. Environ. Microbiol.* **2010**, *76*, 2335–2344.

(53) Young, T. S.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13053–13058.

(54) Yu, Y.; Duan, L.; Zhang, Q.; Liao, R.; Ding, Y.; Pan, H.; Wendt-, E.; Tang, G.; Shen, B.; Liu, W. *ACS Chem Biol.* **2009**, *4*, 855–864.

(55) Ding, Y.; Yu, Y.; Pan, H.; Guo, H.; Li, Y.; Liu, W. *Mol. BioSyst.* **2010**, *6*, 1180–1185.

(56) Yu, Y.; Guo, H.; Zhang, Q.; Duan, L.; Ding, Y.; Liao, R.; Lei, C.; Shen, B.; Liu, W. *J. Am. Chem. Soc.* **2010**, *132*, 16324–16326.

(57) Liao, R.; Liu, W. *J. Am. Chem. Soc.* **2011**, *133*, 2852–2855.

(58) Bowers, A. A.; Walsh, C. T.; Acker, M. G. *J. Am. Chem. Soc.* **2010**, *132*, 12182–12184.

(59) Zhang, Q.; Li, Y.; Chen, D.; Yu, Y.; Duan, L.; Shen, B.; Liu, W. *Nat. Chem. Biol.* **2011**, *7*, 154–160.

(60) Wei, M.; Deng, J.; Wang, S.; Liu, N.; Chen, Y. *Biotechnol. Lett.* **2011**, *33*, 585–591.

(61) Zhang, Q.; Chen, D.; Lin, J.; Liao, R.; Tong, W.; Xu, Z.; Liu, W. *J. Biol. Chem.* **2011**, *286*, 21287–21294.

(62) Pierre, S.; Guillot, A.; Benjdia, A.; Sandström, C.; Langella, P.; Berteau, O. *Nat. Chem. Biol.* **2012**, *8*, 957–959.

(63) Duan, L.; Wang, S.; Liao, R.; Liu, W. *Chem. Biol.* **2012**, *19*, 443–448.

(64) Malcolmson, S. J.; Young, T. S.; Ruby, J. G.; Skewes-Cox, P.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 8483–8488.

(65) Houck, D. R.; Chen, L.; Keller, P. J.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1988**, *110*, 5800–5806.

(66) 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*, 7992–8001.

(67) Mocek, U.; Knaggs, A. R.; Tsuchiya, R.; Nguyen, T.; Beale, J. M.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 7557–7558.

(68) Priestley, N. D.; Smith, T. M.; Shipley, P. R.; Floss, H. G. *Bioorg. Med. Chem.* **1996**, *4*, 1135–1147.

(69) Frenzel, T.; Zhou, P.; Floss, H. G. *Arch. Biochem. Biophys.* **1990**, *278*, 35–40.

(70) Smith, T. M.; Priestley, N. D.; Knaggs, A. R.; Nguyen, T.; Floss, H. G. *J. Chem. Soc., Chem. Commun.* **1993**, 1612–1614.

(71) Zhang, Q. I.; van der Donk, W. A.; Liu, W. *Acc. Chem. Res.* **2012**, *45*, 555–564.

(72) Nicolaou, K. C.; Zak, M.; Rahimipour, S.; Estrada, A. a; Lee, S. H.; O'Brate, A.; Giannakakou, P.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2005**, *127*, 15042–15044.

(73) Radhakrishnan, S. K.; Bhat, U. G.; Hughes, D. E.; Wang, I.-C.; Costa, R. H.; Gartel, A. L. *Cancer Res.* **2006**, *66*, 9731–9735.

(74) Kwok, J. M.-M.; Myatt, S. S.; Marson, C. M.; Coombes, R. C.; Constantinidou, D.; Lam, E. W.-F. *Mol. Cancer Ther.* **2008**, *7*, 2022–2032.

(75) Bowling, B. D.; Doudican, N.; Manga, P.; Orlow, S. J. *Cancer Chemother. Pharmacol.* **2008**, *63*, 37–43.

(76) Bhat, U. G.; Zipfel, P. a; Tyler, D. S.; Gartel, A. L. *Cell Cycle* **2008**, *7*, 1851–1855.

(77) Bhat, U. G.; Halasi, M.; Gartel, A. L. *PloS one* **2009**, *4*, e5592.

(78) Hegde, N. S.; Sanders, D. A.; Rodriguez, R.; Balasubramanian, S. *Nat. Chem.* **2011**, *3*, 725–731.

(79) Clough, B.; Strath, M.; Preiser, P.; Denny, P.; Wilson, I. (R. J. M. . *FEBS Lett.* **1997**, *406*, 123–125.

(80) Clough, B.; Rangachari, K.; Strath, M.; Preiser, P. R.; Iain Wilson, R. J. M. *Protist* **1999**, *150*, 189–195.

(81) Sullivan, M.; Li, J.; Kumar, S.; Rogers, M. J.; McCutchan, T. F. *Mol. Biochem. Parasitol.* **2000**, *109*, 17–23.

(82) Schlitzer, M. *ChemMedChem* **2007**, *2*, 944–986.

(83) Goodman, C. D.; Su, V.; McFadden, G. I. *Mol. Biochem. Parasitol.* **2007**, *152*, 181–191.

(84) Schoof, S.; Pradel, G.; Aminake, M. N.; Ellinger, B.; Baumann, S.; Potowski, M.; Najajreh, Y.; Kirschner, M.; Arndt, H.-D. *Angew. Chem. Int. Ed.* **2010**, *49*, 3317–3321.

(85) Motoi, U.; Furukawa, S.; Abe, F.; Ushioda, M.; Fujine, K.; Johki, S.; Hatori, H.; Ueda, J. *J. Antibiot.* **2004**, *57*, 590–596.

(86) Aoki, M.; Ohtsuka, T.; Yamada, M.; Ohba, Y.; Yoshizaki, H.; Yasuno, H.; Sano, T.; Watanabe, J.; Yokose, K.; Roche, N. *J. Antibiot.* **1991**, *44*, 582–588.

(87) Hashimoto, M.; Murakami, T.; Funahashi, K.; Tokunaga, T.; Nihei, K.; Okuno, T.; Kimura, T.; Naoki, H.; Himeno, H. *Bioorg. Med. Chem.* **2006**, *14*, 8259–8270.

(88) Mizuhara, N.; Kuroda, M.; Ogita, A.; Tanaka, T.; Usuki, Y.; Fujita, K.-I. *Bioorg. Med. Chem.* **2011**, *19*, 5300–5310.

(89) Baumann, S.; Schoof, S.; Harkal, S. D.; Arndt, H.-D. *J. Am. Chem. Soc.* **2008**, *130*, 5664–5666.

(90) Harms, J. M.; Wilson, D. N.; Schluenzen, F.; Connell, S. R.; Stachelhaus, T.; Zaborowska, Z.; Spahn, C. M. T.; Fucini, P. *Mol. Cell* **2008**, *30*, 26–38.

(91) Walter, J. D.; Hunter, M.; Cobb, M.; Traeger, G.; Spiegel, P. C. *Nucleic Acids Res.* **2012**, *40*, 360–370.

(92) Selva, E.; Montanini, N.; Stella, S.; Sofiientini, A.; Gastaldo, L.; Denaro, M. *J. Antibiot.* **1997**, *50*, 22–26.

(93) Heffron, S. E.; Jurnak, F. *Biochemistry* **2000**, *39*, 37–45.

(94) Parmeggiani, A.; Nissen, P. *FEBS Lett.* **2006**, *580*, 4576–81.

(95) Murakami, T.; Holt, T. G.; Thompson, C. J. *J. Bacteriol.* **1989**, *171*, 1459–1466.

(96) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 969–975.

(97) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541–1545.

(98) Lentzen, G.; Klinck, R.; Matassova, N.; Aboul-ela, F.; Murchie, A. I. H. *Chem. Biol.* **2003**, *10*, 769–778.

(99) 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*, 20578–20586.

(100) "Cancer therapeutic agent comprising thiopeptide with multiple thiazole rings": Kim, J. (Korea), WO02066046, August 29, **2002**.

(101) Bauer, R. a; Wurst, J. M.; Tan, D. S. *Curr. Opin. Chem. Biol.* **2010**, *14*, 308–314.

(102) Koehler, A. N. *Curr. Opin. Chem. Biol.* **2010**, *14*, 331–340.

(103) Chaubey, S.; Kumar, A.; Singh, D.; Habib, S. *Mol. Microbiol.* **2005**, *56*, 81–89.

(104) Sleight, P.; Yusuf, S. *J. Hypertens.* **2003**, *21*, 1599–1608.

(105) Prabhakar, S. S. *J. Invest. Med.* **2013**, *61*, 551–557.

(106) Yamazaki, M.; Katayama, Y. *Angiotensin Research* **2013**, *10*, 116–120.

(107) Cooper, R.; Truumees, I.; Barrett, T.; Patel, M.; Schwartz, J.; Puar, M.; Das, P.; Pramanik, B. *J. Antibiot. 43*, 4–7.

(108) Bowers, A. A.; Acker, M. G.; Koglin, A.; Walsh, C. T. *J. Am. Chem. Soc.* **2010**, *132*, 7519–7527.

(109) Yun, B.-S.; Fujita, K.-I.; Furihata, K.; Seto, H. *Tetrahedron* **2001**, *57*, 9683–9687.

(110) Lewis, R. J.; Hughes, R. a; Alcaraz, L.; Thompson, S. P.; Moody, C. J. *Chem. Commun.* **2006**, 4215–4217.

(111) 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.

(112) LaMarche, M. J.; Leeds, J. A.; Dzink-Fox, J.; Gangl, E.; Krastel, P.; Neckermann, G.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Tiamfook, S.; Yu, D. *J. Med. Chem.* **2012**, *55*, 6934–6941.

(113) Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455–2458.

(114) Jonker, H. R. A.; Baumann, S.; Wolf, A.; Schoof, S.; Hiller, F.; Schulte, K. W.; Kirschner, K. N.; Schwalbe, H.; Arndt, H.-D. *Angew. Chem. Int. Ed.* **2011**, *50*, 3308–3312.

(115) Baumann, S.; Schoof, S.; Bolten, M.; Haering, C.; Takagi, M.; Shin-ya, K.; Arndt, H.-D. *J. Am. Chem. Soc.* **2010**, *132*, 6973–6981.

(116) Kelly, T. R.; Echevarren, A.; Chandrakumar, N. S.; Köksal, Y. *Tetrahedron Lett.* **1984**, *25*, 2127–2130.

(117) Kelly, T. R.; T. Jagoe, C.; Gu, Z. *Tetrahedron Lett.* **1991**, *32*, 4263–4266.

(118) Kelly, T. R.; Lang, F. *Tetrahedron Lett.* **1995**, *36*, 5319–5322.

(119) Kelly, T. R.; Lang, F. *J. Org. Chem.* **1996**, *61*, 4623–4633.

(120) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301–3313.

(121) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 15644–15651.

(122) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5087–5092.

(123) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5092–5097.

(124) Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4771–4774.

(125) Delgado, O.; Müller, H. M.; Bach, T. *Chem. Eur. J.* **2008**, *14*, 2322–2339.

(126) Ciufolini, M. A.; Shen, Y. C. *J. Org. Chem.* **1997**, *3263*, 3804–3805.

(127) Moody, C. J.; Hughes, R. a; Thompson, S. P.; Alcaraz, L. *Chem. Commun.* **2002**, 1760–1761.

(128) Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.* **2003**, *5*, 4421–4424.

(129) Bagley, M. C.; Chapaneri, K.; Dale, J. W.; Xiong, X.; Bower, J. *J. Org. Chem.* **2005**, *70*, 1389–1399.

(130) Heckmann, G.; Bach, T. *Angew. Chem. Int. Ed.* **2005**, *44*, 1199–1201.

(131) Lu, J.-Y.; Arndt, H.-D. *J. Org. Chem.* **2007**, *72*, 4205–4212.

(132) Martin, T.; Verrier, C. C.; Hoarau, C.; Marsais, F. *Org. Lett.* **2008**, *10*, 2909–2912.

(133) Martin, T.; Laguerre, C.; Hoarau, C.; Marsais, F. *Org. Lett.* **2009**, *11*, 3690–3693.

(134) Abe, H.; Takaishi, T.; Okuda, T.; Aoe, K.; Date, T. *Tetrahedron Lett.* **1978**, *19*, 2791–2794.

(135) Okumura, K.; Shigekuni, M.; Nakamura, Y.; Shin, C. *Chem. Lett.* **1996**, 1025–1026.

(136) Umemura, K.; Shin, I.; Yoshimura, J.; Okumura, K.; Saito, H.; Shin, C. *Chem. Lett.* **1997**, 1203–1204.

(137) Umemura, K.; Noda, H.; Yoshimura, J.; Konn, A.; Yonezawa, Y.; Shin, C. *Tetrahedron Lett.* **1997**, *38*, 3539–3542.

(138) Okumura, K.; Saito, H.; Shin, C.; Umemura, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1863–1870.

(139) Shin, C.; Okabe, A.; Ito, A.; Ito, A.; Yonezawa, Y. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1583–1596.

(140) Shin, C.; Saito, H.; Yonezawa, Y. *Heterocycles* **2003**, *61*, 45–50.

(141) Endoh, N.; Yonezawa, Y.; Shin, C. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 643–644.

(142) Suzuki, S.; Yonezawa, Y.; Shin, C. *Chem. Lett.* **2004**, *33*, 814–815.

(143) Ammer, C.; Bach, T. *Chem. Eur. J.* **2010**, *16*, 14083–14093.

(144) Aulakh, V. S.; Ciufolini, M. A. *J. Am. Chem. Soc.* **2011**, *133*, 5900–5904.

(145) Moody, C. J.; Bagley, M. C. *Synlett* **1998**, 361–362.

(146) Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.* **2003**, *5*, 4421–4424.

(147) Xiong, X.; Bagley, M. C.; Chapaneri, K. *Tetrahedron Lett.* **2004**, *45*, 6121–6124.

(148) Bagley, M. C.; Dale, J. W.; Jenkins, R. L.; Bower, J. *Chem. Commun.* **2004**, 102–103.

(149) Bagley, M. C.; Xiong, X. *Org. Lett.* **2004**, *6*, 3401–3404.

(150) Merritt, E.; Bagley, M. *Synlett* **2007**, 954–958.

(151) Moody, C. J.; Bagley, M. C. *Chem. Commun.* **1998**, *4*, 2049–2050.

(152) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301–3313.

(153) Higashibayashi, S.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2002**, *43*, 105–110.

(154) Higashibayashi, S.; Kohno, M.; Goto, T.; Suzuki, K.; Mori, T.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2004**, *45*, 3707–3712.

(155) Mori, T.; Tohmiya, H.; Satouchi, Y.; Higashibayashi, S.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2005**, *46*, 6423–6427.

(156) Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2007**, *48*, 1331–1335.

(157) Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Chem. Asian. J.* **2008**, *3*, 984–1012.

(158) Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem. Int. Ed.* **2002**, 1941–1945.

(159) Nicolaou, K. C. *Angew. Chem. Int. Ed.* **2012**, *51*, 12414–12436.

(160) Nicolaou, K. C.; Zou, B.; Dethe, D. H.; Li, D. B.; Chen, D. Y.-K. *Angew. Chem. Int. Ed.* **2006**, *45*, 7786–7792.

(161) Nicolaou, K. C.; Dethe, D. H.; Chen, D. Y.-K. *Chem. Commun.* **2008**, 2632–2634.

(162) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *Chem. Commun.* **2004**, 946–948.

(163) Lu, J.-Y.; Riedrich, M.; Mikyna, M.; Arndt, H.-D. *Angew. Chem. Int. Ed.* **2009**, *48*, 8137–8140.

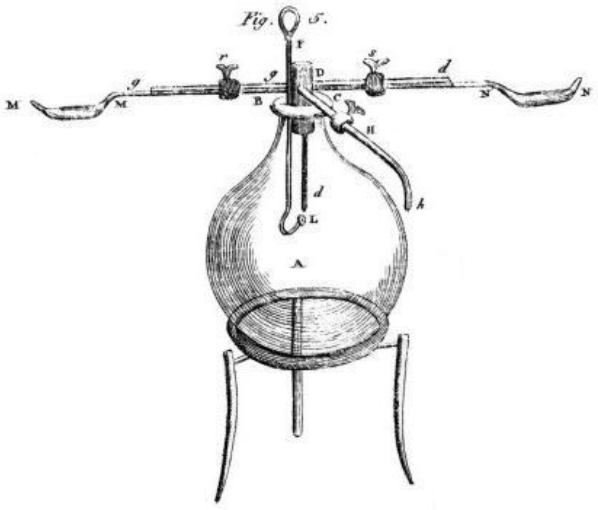
(164) Zou, Y.; Liu, Q.; Deiters, A. *Org. Lett.* **2011**, *13*, 4352–4355.

(165) Keppens, L.; Groote, G. De *Revue de l'Agriculture* **1979**, *32*, 159–177.

(166) Trzasko, A.; Leeds, J. A.; Praestgaard, J.; LaMarche, M. J.; McKenney, D. *Antimicrob. Agents Chemother.* **2012**, *56*, 4459–4462.

(167) Leeds, J. A.; Sachdeva, M.; Mullin, S.; Dzink-Fox, J.; LaMarche, M. J. *Antimicrob. Agents Chemother.* **2012**, *56*, 4463–4465.

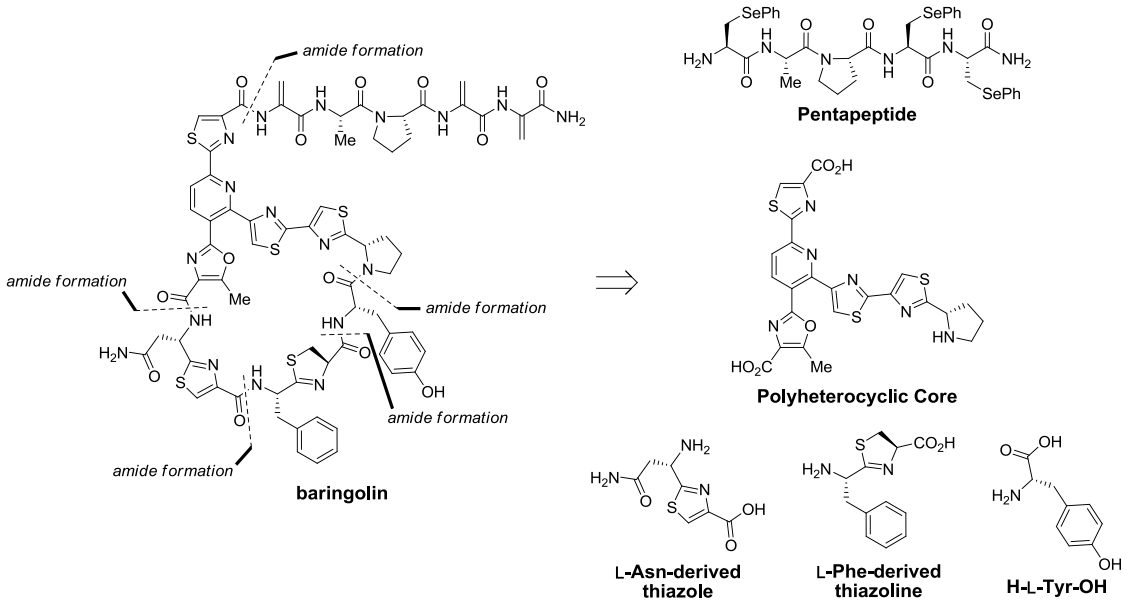
Synthetic Target and Objectives



SYNTHETIC TARGET: BARINGOLIN

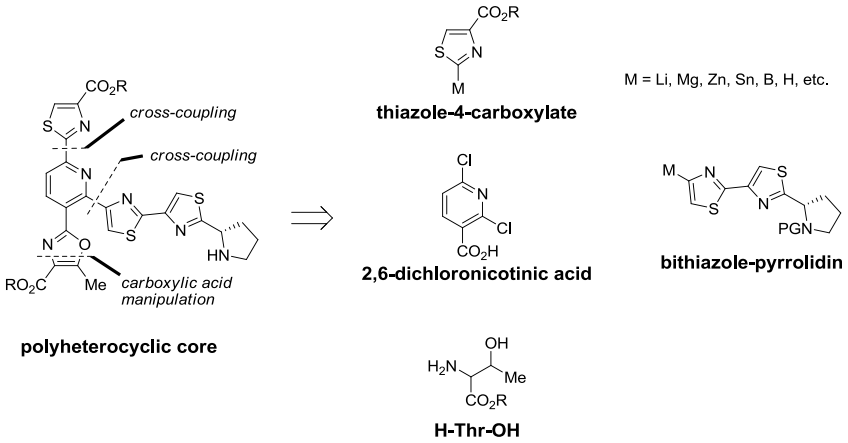
During their exploration of the marine potential as a source of therapeutic agents, the Spanish pharmaceutical company Instituto Biomar discovered a new thiopeptide antibiotic produced by *Kocuria sp.* MI-67-EC3-038 strain. It was isolated from Alicante's coast and displayed an excellent activity profile against Gram-positive bacteria.[1] Using NMR spectroscopy its structure was proposed, although originally, no stereochemistry was assigned (Scheme 1).

Continuing our collaboration with Instituto Biomar,[2–4] we started a synthetic program devoted to the total synthesis of baringolin to confirm its structure, assign its stereochemistry and produce analogues to assess its structure-activity relationship. In order to do so, a retrosynthetic disconnection was envisioned where the thiopeptide was divided into five fragments, its polyheterocyclic core, the pentapeptidic tail and three more fragments, including two amino acid-derived azoles, to complete the macrocycle (Scheme 1). Although no stereochemistry had been previously reported, the ribosomal origin of thiopeptides suggested that all stereocenters could be introduced from the corresponding L-amino acids. Dehydroalanine residues of the peptidic tail could be introduced from phenylselenocysteine precursors.[5]



Scheme 1. Retrosynthetic analysis of baringolin.

Regarding the polyheterocyclic core, a strategy based on cross-coupling reactions on 2,6-dichloronicotinic acid was proposed due to the suitable substitution pattern of this pyridine derivative (Scheme 2). Formation of the oxazole and regioselective introduction of thiazoles required investigation to achieve a modular and convergent strategy.



Scheme 2. Retrosynthetic analysis of baringolin's polyheterocyclic core.

Once a successful strategy is developed, it should facilitate the synthesis of analogues to explore structure-activity relationships of baringolin.

References

(1) "Peptides as bioactive compounds": Cañedo Hernández, L. M.; Romero Millan, F.; Fernández Medarde, A.; Fernández Chimeno, R. I.; Hidalgo Villar, J. C. (Biomer, S.A., Spain) WO2012062906, May 18, **2012**.

(2) Cruz, L. J.; Cuevas, C.; Cañedo, L. M.; Giralt, E.; Albericio, F. *J. Org. Chem.* **2006**, *71*, 3339–3344.

(3) Hernández, D.; Vilar, G.; Riego, E.; Cañedo, L. M.; Cuevas, C.; Albericio, F.; Alvarez, M. *Org. Lett.* **2007**, *9*, 809–811.

(4) Lorente, A.; Pla, D.; Cañedo, L. M.; Albericio, F.; Alvarez, M. *J. Org. Chem.* **2010**, *75*, 8508–8515.

(5) Levengood, M. R.; van der Donk, W. A. *Nat. protoc.* **2006**, *1*, 3001–3010.

OBJECTIVES

The main objective of the present thesis is to develop a robust and convergent methodology for the total synthesis of the new thiopeptide antibiotic baringolin. However, other objectives will follow once it has been achieved. The procedure must be flexible for the preparation of analogues and derivatives. In order to trace a clear work plan that will eventually lead to the reproduction of baringolin in the laboratory, its synthesis has been divided in various objectives:

- **Total synthesis of baringolin:**

    - **Synthesis of enantiomerically pure amino acid-derived azole building blocks.** A suitable strategy for the construction of a bithizole-pyrrolidine fragment amenable for a cross-coupling approach must be developed. The synthesis of other amino acid-derived azoles must also be addressed.

    - **Synthesis of baringolin's central polyheterocyclic core.** The utility of 2,6-dichloronicotinic acid as a suitably pre-functionalized scaffold for the synthesis of 2,3,6-trisubstituted pyridines will be.

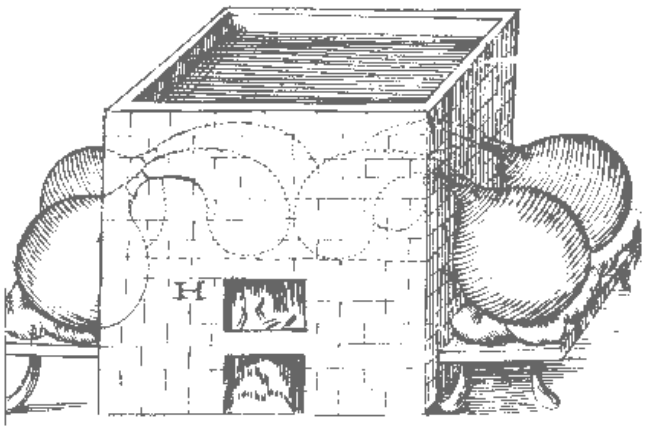
    - **Synthesis of pentapeptidic tail, assembling of fragments and final steps of baringolin's total synthesis.** Phenylselenocysteine-containing pentapeptide building block is a perfect candidate for solid phase peptide synthesis (SPPS). With a suitable methodology for the synthesis of all building blocks, selection of protecting groups, macrocyclization and oxidation to obtain dehydroalanine residues are the major challenges.

- **Synthesis of baringolin analogues and study of their structure-activity relationship.** With an efficient strategy for the synthesis of baringolin in hands, analogues can be synthesized to assess the impact of the most characteristic moieties in this thiopeptide structure. Substitution of thiazoline for thiazole and shortening of the peptidic will be evaluated.

- **Use of building blocks and methodology developed in baringolin's total synthesis for the construction of other thiazole-containing natural products.** With a reliable method for the obtention of amino acid-derived thiazoles in hands, the total synthesis of other natural products that contain this moiety can be addressed.

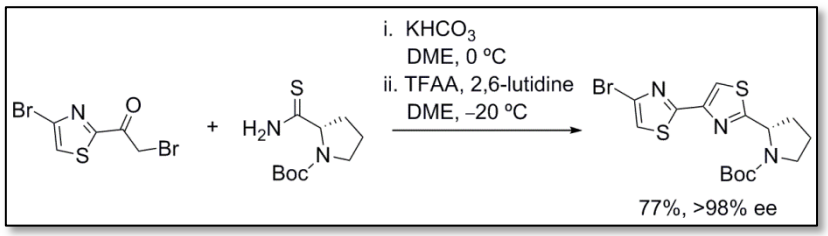
Chapter 2:

Synthesis of an Enantiopure Bithiazole-Pyrrolidine Building Block



SYNTHESIS OF AN ENANTIOPURE BITHIAZOLE-PYRROLIDINE BUILDING BLOCK

Highly efficient, multigram and enantiopure synthesis of 2-(2,4'-bithiazol-2'-yl)pyrrolidine.



Xavier Just-Baringo,[a,b] Paolo Bruno,[a,b] Fernando Albericio[a,b,c] and Mercedes Álvarez[a,c,d]

Tetrahedron Letters **2011**, *52*, 5435-5437.

[a] *Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain.*

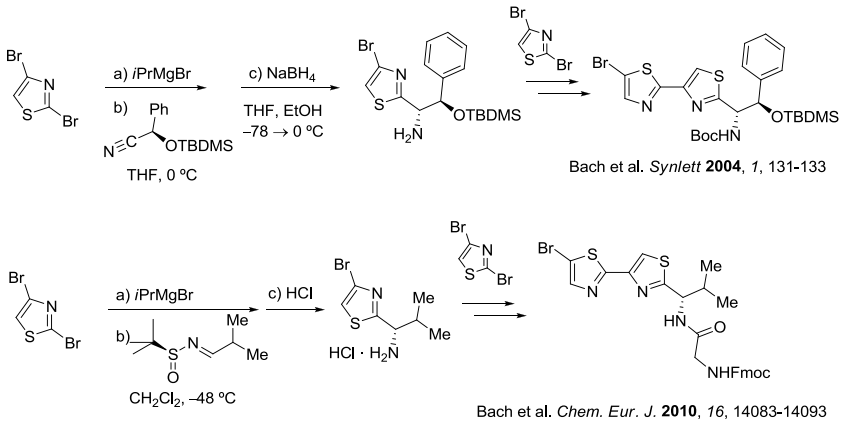
[b] *CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028-Barcelona, Spain*

[c] *Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona.*

[d] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona.*

Conspectus

The efforts towards the synthesis of a 4-bromobithiazole-pyrrolidine building block suitably functionalized for its subsequent use in a cross-coupling-based strategy are described in this chapter. Previously reported methods for the synthesis of similar chiral building blocks required the use of a nitrile or a chiral sulfinylimine as precursors of a primary amine. Because of this limitation, these methodologies could not be applied to our proline-derived scaffold (Scheme 1).



Scheme 1. Precedents of chiral amine bithiazole derivatives.

Our early attempts focused on a strategy based in the Hunsdiecker reaction. However, the negative results obtained prompted us to move to an alternative approach. Hence, the central ring of the bithiazole-pyrrolidine fragment could be formed using a modified 2-step Hantzsch thiazole synthesis reaction starting from suitable building blocks. The product thus obtained could be converted into the corresponding trimethyltin derivative for subsequent use in a Stille cross-coupling reaction.

Tetrahedron Letters 52 (2011) 5435–5437

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Highly efficient, multigram and enantiopure synthesis of (*S*)-2-(2,4′-bithiazol-2-yl)pyrrolidine

Xavier Just-Baringo [a,b], Paolo Bruno [a,b], Fernando Albericio [a,b,c], Mercedes Álvarez [a,c,d,*]

[a] *Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, E-08028 Barcelona, Spain*
[b] *CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, E-08028 Barcelona, Spain*
[c] *Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain*
[d] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, E-08028 Barcelona, Spain*

ARTICLE INFO

Article history:
Received 20 June 2011
Revised 8 July 2011
Accepted 27 July 2011
Available online 16 August 2011

Keywords:
Hantzsch cyclization
Thiazole
Thiopeptides
Proline

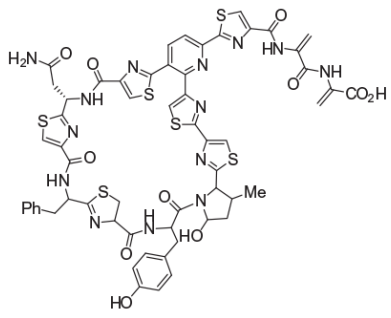
ABSTRACT

(*S*)-2-(4-Bromo-2,4′-bithiazole)-1-(*tert*-butoxycarbonyl)pyrrolidine ((***S*)-1**) was obtained as a single enantiomer and in high yield by means of a two-step modified Hantzsch thiazole synthesis reaction when bromoketone **3** and thioamide (***S*)-4** were used. Further conversion of (***S*)-1** into trimethyltin derivative (***S*)-2** broadens the scope for further cross-coupling reactions.

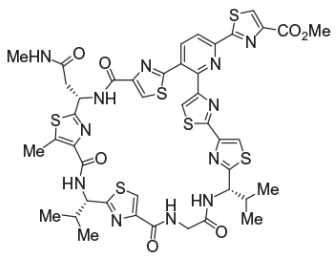
Polyheterocyclic scaffolds containing thiazole rings are common features of numerous biologically active natural products.[1] Chiral 2-(2,4′-bithiazole)amines fragments can be found attached to the pyridine ring at the heterocyclic core of many thiopeptides (Fig. 1).[2,3] The syntheses of a handful of these interesting natural antibiotics has been achieved using a range of different strategies.[2b] Those based on cross-coupling reactions have the need of pyridines and azoles properly functionalized as halides and/or organometallic derivatives. To date, only primary amines substituted with a suitable halogenated 2,4′-bithiazole have been described.[4] However, the methodology used so far cannot be applied when cyclic amines are pursued.[5] In this note we present the synthesis of enantiopure (***S*)-2-(4-bromo-2,4′-bithiazol-2′-yl)pyrrolidine (*S*)-1**, which is easily converted into its trimethyltin derivative (***S*)-2** for use in subsequent Stille cross-couplings (Scheme 1).

In order to avoid any stereoselective steps, the use of starting materials directly derived from the chiral pool and therefore available in Kg scale should be mandatory.[6] Thus, racemisation can be avoided throughout the course of the synthesis.

During our investigations we found out that a 4-halo-2,4′-bithiazole fragment could not be accessed if the strategy relied on a key Hunsdiecker halodecarboxylation.[7,8] A more convergent approach involving the construction of the middle ring of (***S*)-1**,[9] by means of a modified two-step Hantzsch thiazole synthesis[10]



GE37468A

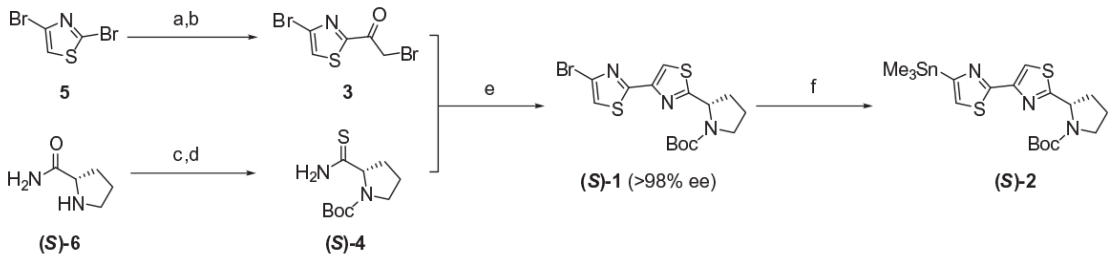


amythiamicin D

Figure 1. Thiopeptides containing chiral bithiazole amine moieties.

* Corresponding author. Tel.: +34 93 4037086; fax: +34 93 403 71 26.
E-mail address: mercedes.alvarez@irbbarcelona.org (M. Álvarez).

0040-4039/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2011.07.128

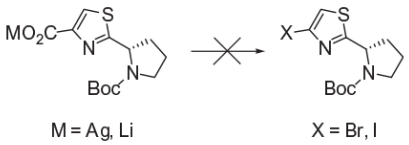


Scheme 1. Synthesis of 2-(4-bromo-2,4′-bithiazol-2-yl)pyrrolidine (***S***)-**1** and trimethyltin derivative (***S***)-**2**. Reagents and conditions: (a) (i) *n*BuLi, THF, −78 °C, (ii) *N*-acetylmorpholine, −78 °C (67%); (b) Br_2, HBr, AcOH, rt (80%); (c) $(Boc)_2O$, H_2O, 1,4-dioxane, rt (quant.); (d) Lawesson's reagent, THF, rt (quant.); (e) (i) $KHCO_3$, DME, 0 °C, (ii) TFAA, 2,6-lutidine, DME, −20 °C (77%); (f) $Me_3SnSnMe_3$, $[Pd(PPh_3)_4]$, toluene, 100 °C (89%).

between **3**[11] and (***S***)-**4**,[12] afforded the optically pure product[13] in 77% yield (Scheme 1).[14] Bromoketone **3** was obtained after consecutive bromine/lithium exchange and acylation of **5**[15] and subsequent bromination under acidic conditions of the resulting 2-acylthiazole. Protection of prolylamide ((***S***)-**6**) with the Boc group[16] and further treatment with Lawesson's reagent gave thioamide (***S***)-**4** in excellent yield. The reaction was satisfactorily scaled-up without any loss of either optical purity or chemical yield and provided 6 g of the desired biaryl (***S***)-**1**, which was subsequently converted into trimethyltin derivative (***S***)-**2** in high yield.[17]

In summary, an improved, convergent and high yield preparation of a 2-(2,4′-bithiazol-2′-yl)pyrrolidine fragment suitably functionalized in its thiazole-4-position either as bromide ((***S***)-**1**) or tin derivative ((***S***)-**2**) for use in cross-coupling reactions has been described. The products have been obtained in multigram scale without any loss of their optical integrity.

These building blocks will allow the preparation of those thiopeptide central cores bearing such moieties and that could not be accessed by previously reported methods. Further studies with these structures and their use in total synthesis of natural products are underway.



Acknowledgments

We gratefully acknowledge support for this research from Ministerio de Ciencia e Innovación, CICYT (CTQ2009-07758) and *Generalitat de Catalunya* (2009SGR 1024). X.J.B. thanks ISCIII for a PFIS grant. P.B. thanks Ministerio de Ciencia e Innovación for a Torres Quevedo grant.

References and notes

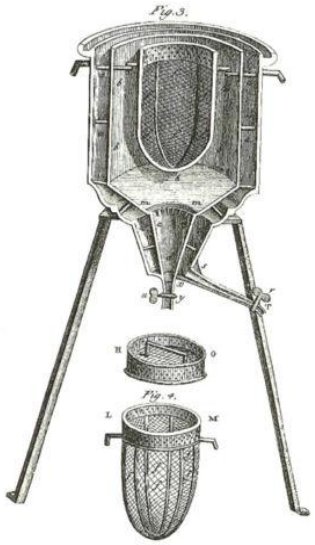
1. For a review about natural polyazoles see: Riego, E.; Hernández, D.; Albericio, F.; Álvarez, M. *Synthesis* **2005**, *12*, 1907–1922.
2. General review about thiopeptides: (a) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685; Review about thiopeptide synthesis: (b) Hughes, R. A.; Moody, C. J. *Angew. Chem., Int. Ed.* **2007**, *46*, 7930–7954; Review about thiopeptide biosynthesis: (c) Li, C.; Kelly, W. L. *Nat. Prod. Rep.* **2010**, *27*, 153–164.
3. Examples of thiopeptides with a 2-(2,4′-bithiazol-2-yl)pyrrolidine fragment: (a) Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304–1311; (b) Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K.; Krastel, P. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.
4. (a) Spieß, A.; Heckmann, G.; Bach, T. *Synlett* **2004**, 131–133; (b) Schultz-Fademrecht, C.; Kinzel, O.; Markó, I. E.; Pospisil, T.; Pesci, S.; Rowley, M.; Jones, P. *Tetrahedron* **2009**, *65*, 9487–9493.
5. Previously described methodologies rely on the attack of an organometallic heteroarene to either a nitrile (see Ref. 4a) or a sulfinylimine (see Ref. 4b).
6. Some of these can be found in the pool of Boc-protected amino acids, which are available in a great diversity at a very reasonable price.
7. After extensive investigations on the Hunsdiecker halodecarboxylation process of thiazole-4-carboxylates of various metals, the desired 4-halothiazole was never obtained. The corresponding 5-halogenated thiazole-4-carboxylate was the only observed product when the starting material was not recovered.
8. Johnson, R. G.; Ingham, R. K. *Chem. Rev.* **1956**, *56*, 219–269.
9. Classical Hantzsch conditions yielded the partially racemized product (42% ee). Prodecure: To a solution of thioamide (***S***)-**4** (58 mg, 0.25 mmol) and bromoketone **3** (60 mg, 0.21 mmol) in dry EtOH (3 mL) was added pyridine (25 μL, 0.32 mmol) and the resulting mixture was heated under reflux. After 4 h the solvent was removed under reduced pressure and the crude product was purified by silica flash chromatography (hex/EtOAc, 8:2) to yield the title compound as a tan solid (67 mg, 77%).
10. (a) Bredenkamp, M. W.; Holzapfel, C. W.; van Zyl, W. J. *Synth. Commun.* **1990**, *20*, 2235–2249; (b) Aguilar, E.; Meyers, A. I. *Tetrahedron Lett.* **1994**, *35*, 2473; (c) Boden, C. D. J.; Pattenden, G.; Ye, T. *Synlett* **1995**, 417–419; (d) Merritt, E. A.; Bagley, M. C. *Synthesis* **2007**, *22*, 3535–3541. Nicolaou et al. revisited previous works on two-step Hantzsch cyclization and used those investigations for his landmark synthesis of thiostrepton; (e) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Lee, S. H.; Nevalainen, M.; Bella, M.; Estrada, A. A.; Funke, C.; Zécri, F. J.; Bulat, S. *J. Am. Chem. Soc.* **2005**, *127*, 11159; (f) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Estrada, A. A.; Lee, S. H.; Nevalainen, M. *J. Am. Chem. Soc.* **2005**, *127*, 11176.
11. Preparation of 4-Bromo-2-(bromoacetyl)thiazole (**3**): A 33% HBr solution in AcOH (680 μL, 3.88 mmol), and bromine (200 μL, 3.88 mmol) were added to a solution of 2-acetyl-4-bromothiazole (800 mg, 3.88 mmol) in AcOH (43 mL) and the mixture was stirred at rt under N_2. After 5 h the mixture was poured over a suspension of ice in EtOAc (100 mL) and then solid Na_2CO_3 was added stepwise until basic pH was reached. The aqueous phase was extracted with EtOAc (2 × 100 mL) and the combined organic extracts dried over Na_2SO_4 and then concentrated in vacuo. The crude product was purified by automated silica column chromatography (0–40% CH_2Cl_2 in hexanes), which yielded the product as a yellowish solid (887 mg, 80%). IR (KBr) 3113, 1704, 1454, 1379, 1191, 954, 640 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 4.69 (s, 2 H), 7.67 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 30.6 (t), 126.4 (d), 127.8 (s), 163.8 (s), 183.9 (s) ppm. HRMS(ESI) *m*/*z* calcd for $C_5H_4Br_2NOS$ (M+H) 283.8375, found 283.8375.
12. Preparation of (*S*)-1-(*tert*-Butoxycarbonyl)prolinthioamide ((***S***)-**4**): A solution of (***S***)-1-(*tert*-butoxycarbonyl)prolinamide (9.0 g, 42.0 mmol) and Lawesson's reagent (8.5 g, 21.0 mmol) in dry THF (55 mL) was stirred at rt under N_2 for 3.5 h. The solvent was removed under reduced pressure, saturated aq. $NaHCO_3$ (100 mL) was added and the mixture stirred for 1 h. The aqueous suspension was extracted with EtOAc/CH_2Cl_2 (1:1) (400 mL), the organic fraction was washed with saturated aq $NaHCO_3$ (2 × 100 mL) and the combined aqueous fractions were extracted with EtOAc/CH_2Cl_2 (200 mL). The combined organics extracts were dried over Na_2SO_4 and then concentrated in vacuo to obtain the title compound as a white solid (8.74 g, 90%), mp (EtOAc) 190–192 °C. $[\alpha]_D$ −103.4 (*c* 1.00, $CHCl_3$). IR (KBr) 3380, 3202, 2981, 2881, 1671, 1411, 1166 cm^{-1}.^1H NMR (400 MHz, $CDCl_3$) δ = 1.41–1.50 (m, 9 H), 1.65–2.75 (m, 4 H), 3.22–3.74 (m, 2 H), 4.67 (dd, *J* = 8.0 and 3.6 Hz, 1 H) ppm. HRMS(ESI) *m*/*z* calcd for $C_{10}H_{19}N_2O_2S$ (M+H) 231.1162, found 231.1161.
13. The ee was determined by HPLC on a chiral stationary phase CHIRALPAK IA 250 × 4.6 mm, 5 μm Analytical Column, flow rate 1 mL min^{-1}; H_2O (0.045% TFA)/MeOH, 20:80; detected at 254 nm.
14. Preparation of (*S*)-*tert*-Butyl 2-(4-bromo-2,4′-bithiazol-2′-yl)pyrrolidine-1-carboxylate ((***S***)-**1**): A mixture of thioamide (***S***)-**4** (6.51 g, 28.27 mmol) and $KHCO_3$ (15.09 g, 150.72 mmol) in dry DME (23 mL) under N_2 was stirred at rt. After 15 min the mixture was placed in an ice bath. A solution of bromoketone **3** (5.37 g, 18.84 mmol) in dry DME (22 mL) was added dropwise and the resulting mixture was stirred at 0 °C. After 23 h the mixture was allowed to

reach rt, filtered through celite and washed with Et_2O. After removing the volatiles the crude hydroxythiazoline was redissolved in dry DME (47 mL) and cooled to −20 °C. A mixture of trifluoroacetic anhydride (15.5 mL, 75.36 mmol) and 2,6-lutidine (18.7 mL, 160.14 mmol) was added dropwise and the resulting solution was stirred at −20 °C. After 4 h the mixture was diluted with CH_2Cl_2 (200 mL), washed with 1 N HCl (200 mL) and saturated aq $NaHCO_3$ (200 mL), dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hex/EtOAc, 8:2). The title product was obtained as a yellowish solid (6.04 g, 77%), mp (EtOAc) 134–137 °C. $[\alpha]_D$ −72.3 (*c* 1.00, CH_2Cl_2). IR (KBr) 3120, 2972, 2868, 1688, 1395 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.29–1.57 (m, 9 H), 1.85–2.10 (m, 2 H), 2.15–2.50 (m, 2 H), 3.30–3.70 (m, 2 H), 5.10–5.30 (m, 1 H), 7.23 (s, 1 H), 7.91 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 23.4 and 24.2 (t), 28.5 and 28.6 (q), 32.9 and 34.3 (t), 46.8 and 47.1 (t), 59.1 and 59.5 (d), 80.6 (s), 116.5 and 116.8 (d), 117.5 (d), 126.2 (s), 148.2 (s), 154.3 and 155.0 (s), 163.8 (s), 176.8 (s) ppm. HRMS(ESI) *m*/*z* calcd for $C_{15}H_{19}BrN_3O_2S_2$ (M+H) 416.0097, found 416.0096.

15. Acylation of **5** was performed as descrived in: Gebauer, J.; Arseniyadis, S.; Cossy, J. *Org. Lett.* **2007**, *9*, 3425.
16. (*S*)-1-(*tert*-Butoxycarbonyl)prolinamide was either purchased or prepared as described in Scheme 1. The spectroscopic data of the protected product prepared in our labs was identical to the commercially available compound.
17. Preparation of (*S*)-*tert*-Butyl 2-[4-(trimethyltin)-2,4′-bithiazol-2′-yl]pirolidine-1-carboxylate ((***S***)-**2**): To a stirred solution of bithiazole (***S***)-**1** (1.97 g, 4.73 mmol) and hexamethyldistannane (8.3 mL, 40.22 mmol) in degassed dry toluene (160 mL) was added $[Pd(PPh_3)_4]$ (543 mg, 0.47 mmol) and the mixture was stirred at 100 °C under argon. After 2 h the mixture was allowed to reach rt and the solvent was removed under reduced pressure. The crude product was purified by neutral alumina column chromatography column (hex/EtOAc, 95:5 to 90:10) to yield the title compound as a white solid (2.11 g, 89%), mp (hexanes) 111–113 °C. $[\alpha]_D$ −63.8 (*c* 0.99, CH_2Cl_2). IR (KBr) 3131, 3094, 2977, 1699, 1386 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.39 (s, 9 H), 1.28–1.54 (m, 9 H), 1.88–2.04 (m, 2 H), 2.16–2.45 (m, 2 H), 3.36–3.70 (m, 2 H), 5.10–5.30 (m, 1 H), 7.38 (s, 1 H), 7.88 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = −8.6 (q), 23.4 and 24.1 (t), 28.5 (q), 33.0 and 34.4 (t), 46.8 and 47.1 (t), 59.2–59.6 (d), 80.5 (s), 115.3 and 115.7 (d), 126.3 (d), 149.9 (s), 154.4 (s) 161.3 (s), 163.7 (s), 176.3 (s) ppm. HRMS(ESI) *m*/*z* calcd for $C_{18}H_{28}N_3O_2S_2Sn$ (M+H) 502.0639, found 502.0639.

Chapter 3:

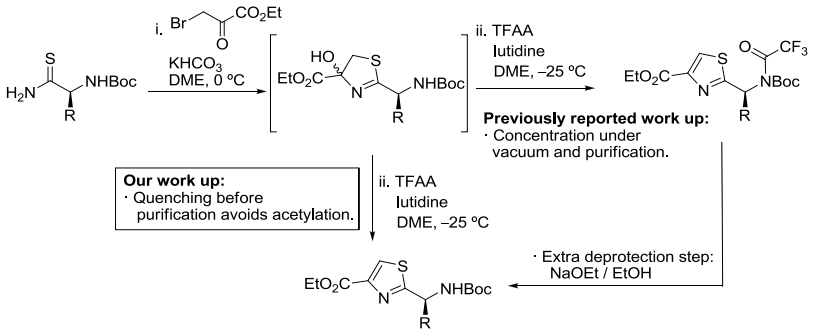
Synthesis of Amino Acid-Derived Thiazole Building Blocks



SYNTHESIS OF AMINO ACID-DERIVED THIAZOLE BUILDING BLOCKS

Conspectus

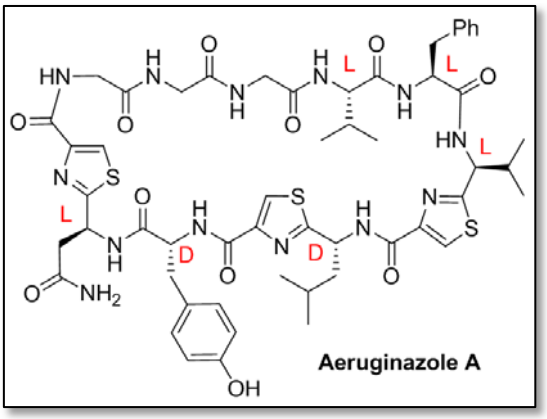
Synthesis of an L-Asn-derived thiazole was required for the synthesis of Baringolin. Such fragments can be obtained in excellent enantiomeric excesses if a modified two-step Hantzsch cyclization reaction is used. However, precedents in the literature reported the undesired trifluoroacetylation of protected primary amino groups. Detrifluoroacetylation was also reported, but the harsh conditions required limit the scope of substrates that can be used and lengthen the synthesis.



Modification of work-up conditions avoided the undesired side-reaction and saved the extra step. Once the methodology was set up, L-Asn-derived thiazole was synthesized and similar building blocks could be easily prepared.

Aeruginazole A, a thiazole-containing macrocyclic peptide with antibiotic properties contains three of these building blocks, L-Asn-, L-Val- and D-Leu-derived thiazoles. Hence, Aeruginazole A was an amenable target to exploit the facile production of such moieties.

Total synthesis of aeruginazole A.



Paolo Bruno,[†,‡] Stella Peña,[†,§] Xavier Just-Baringo,[†,‡] Fernando Albericio,[†,‡,ǁ] and Mercedes Álvarez[†,‡,⊥]

Organic Letters **2011**, *13*, 4648-4651.

† Institute for Research in Biomedicine, Barcelona Science Park, Carrer Baldiri Reixac 10, 08028 Barcelona, Spain.

‡CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine.

§ Present address: Cátedra de Química Farmacéutica (DQO), Facultad de Química, UdelaR, Montevideo, Uruguay.

ǁ *Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain.*

⊥ *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.*

ORGANIC LETTERS

2011
Vol. 13, No. 17
4648–4651

Total Synthesis of Aeruginazole A

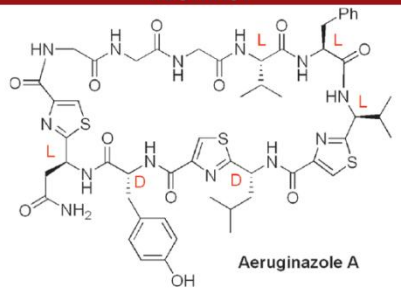
Paolo Bruno,[*,†,‡] Stella Peña,[†,§] Xavier Just-Baringo,[†,‡] Fernando Albericio,[†,‡,∥] and Mercedes Álvarez[*,†,‡,⊥]

Institute for Research in Biomedicine, Barcelona Science Park, Carrer Baldiri Reixac 10, 08028 Barcelona, Spain, CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine, Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain, and Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

**pbruno@pcb.ub.es; mercedes.alvarez@irbbarcelona.org*

Received July 11, 2011

ABSTRACT



Aeruginazole A

The first total synthesis of Aeruginazole A, prepared via a convergent strategy that involved both solid-phase peptide synthesis and solution phase chemistry and that enabled conservation of the stereochemistry of the intermediates, is reported.

Aeruginazole A is a macrocyclic dodecapeptide that was recently isolated from the cyanobacterium *Microcystis* sp. strain (IL-323) and that exhibits inhibitory activity, toward *Bacillus subtilis*.[1] It is an interesting example of the numerous macrocyclic thiazole-containing compounds that have been isolated from natural sources over the past few decades and subsequently tested for biological activity, and whose extremely varied structures have been the targets of total syntheses.[2]

The diverse combination of structural motifs in Aeruginazole A is interesting: its macrocycle comprises a pentapeptide of L-amino acids (known as the *northern region*) and a group of three thiazole moieties combined with a D-Tyr residue (the *southern region*).

The presence of L- and D-amino acids, and of amino acid-derived moieties, in the compound is interesting, as is their location: the L-amino acids are located in the *western* and *eastern regions* only, whereas there are two consecutive D-amino acids in the southern region. The stereochemistry of the target molecule is therefore an important feature and a critical synthetic challenge. Intrigued by its peculiar structure and seeking to further explore its biological activity, we decided to undertake the total synthesis of Aeruginazole A.

Work began with the retrosynthetic analysis represented in Figure 1: in addition to the *northern pentapeptide* **1**, tyrosine[3] **5** and the optically active thiazole-building blocks **2**–**4** were identified through disconnections of the southern region of Aeruginazole A (Figure 1).

The planned route to the stereodefined thiazole building blocks **2**–**4** was to subject precursor thioamides to Hantzsch thiazole synthesis. These precursors were readily accessed from Boc-L-Asp(OBzl)-OH, Boc-L-Val-OH, and

[†] Institute for Research in Biomedicine.
[‡] CIBER-BBN Networking Centre on Bioengineering, Biomaterials, and Nanomedicine.
[§] Present address: Cátedra de Química Farmacéutica (DQO), Facultad de Química, UdelaR, Montevideo, Uruguay.
[∥] Department of Organic Chemistry, University of Barcelona.
[⊥] Faculty of Pharmacy, University of Barcelona.

(1) Raveh, A.; Carmeli, S. *Org. Lett.* **2010**, *12*, 3536.

(2) For recent reviews on thiazole-containing natural products, see: (a) Davyt, D.; Serra, G. *Mar. Drugs* **2010**, *8*, 2755. (b) Jin, Z. *Nat. Prod. Rep.* **2009**, *26*, 382.

(3) The absence of a protecting group on the Tyr phenol (for which a *t*Bu group is normally used) enabled use of Boc for temporary protection of the α-amino function in the southern region, thereby facilitating the synthesis.

10.1021/ol2018592
Published on Web 08/03/2011

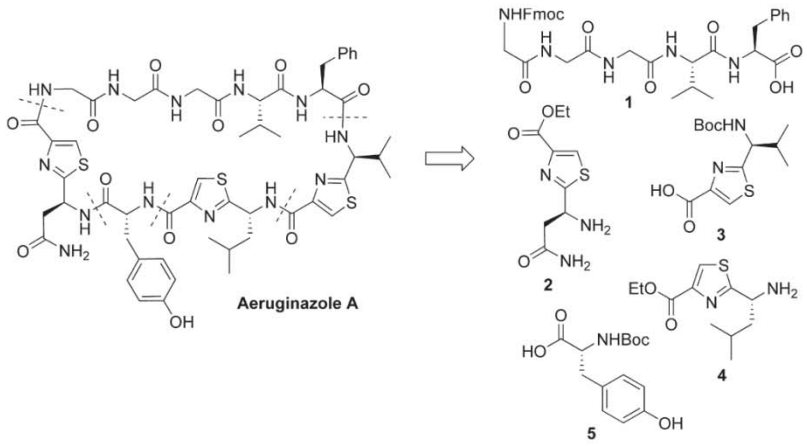


Figure 1. Structure of Aeruginazole A and retrosynthetic analysis.

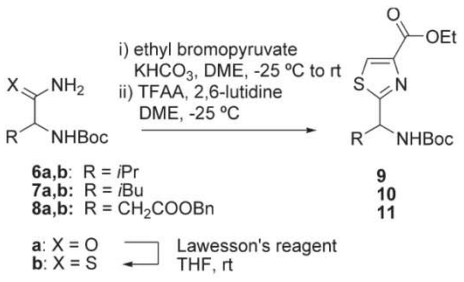
Boc-D-Leu-OH. Commercially available *N*-protected amino acids were converted into the corresponding primary amides **6a**–**8a** (Scheme 1) through activation and subsequent treatment with concentrated aqueous ammonia.

The Leu was activated for nucleophilic substitution through transformation into the corresponding methyl ester.[4] Unfortunately, this easy protocol gave poor yields with Val and was incompatible with the benzyl ester group of protected Asp. Alternatively, these residues were activated by treatment with 2,2,2-trichloroethyl chloroformate. Each of the amides **6a**–**8a** was then converted into its corresponding thioamide **6b**–**8b**, respectively, using Lawesson's reagent (LR).[5]

The three thioamides were then subjected to Hantzsch thiazole syntheses, a critical point in the total synthesis, given the need to preserve the stereochemical information present in the thioamides themselves. First, classical conditions for this reaction were tested: the thioamides were treated with ethyl bromopyruvate and pyridine in refluxing ethanol.[6] Optical purity was checked in the case of the known compound Val-thiazole **9** (Scheme 1) by comparison of its rotatory power with published data.[7]

However, racemization of Leu-thiazole **10** and Asp-thiazole **11** was detected further in the synthetic sequence: NMR spectra revealed formation of diastereomeric mixtures of products when **10** and **11** were converted into the more advanced synthetic intermediates **13** and **14**, respectively (Scheme 3), each of which bears two stereocenters.[8] Once a chiral HPLC analysis method for all three Hantzsch synthesis products had been established, racemization was definitively confirmed (see Experimental Procedures for details, Supporting Information). Switching to Merritt and Bagley's protocol[9] (Scheme 1) for Hantzsch synthesis of stereodefined thiazoles provided the building blocks **9**–**11** in 75–98% yields with conservation of optical purity (ee ranging from 91 to 94%). It should be noted that special care had to be paid to the dehydration step (Scheme 1, step ii). Lower-than-expected ee's were observed when the reaction temperature was increased before the dehydration was complete.

Scheme 1. Synthesis of Thiazole Moieties



(4) Xu, Z.; DiCesare, J. C.; Baures, P. W. *J. Comb. Chem.* **2010**, *12*, 248.

(5) Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 4771.

(6) Rzasa, R. M.; Kaller, M. R.; Liu, G.; Magal, E.; Nguyen, T. T.; Osslund, T. D.; Powers, D.; Santora, V. J.; Viswanadhan, V. N.; Wang, H.-L.; Xiong, X.; Zhonga, W.; Norman, M. H. *Bioorg. Med. Chem.* **2007**, *15*, 6574.

(7) Downing, S. V.; Aguilar, E.; Meyers, A. I. *J. Org. Chem.* **1999**, *64*, 826.

(8) ^{1}H NMR spectra of compounds **13** and **14** were analyzed: singlets falling in the region from δ 8.00 to 8.15 ppm, relative to the protons in position 5 of each thiazole ring, were used as diagnostic signals. Observation of a second set of these signals revealed that substrates of low optical purity were coupled, thus forming mixtures of diastereomeric products.

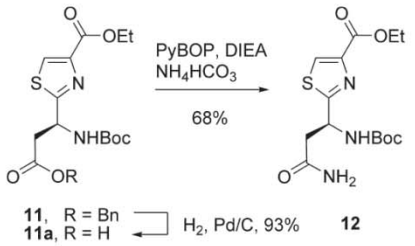
(9) Merritt, E. A; Bagley, M. C. *Synthesis* **2007**, *22*, 3535.

(10) The original paper described direct concentration of the reaction mixtures with consequent trifluoroacetylation of Boc-protected amino groups; an additional transformation was therefore required to remove the trifluoroacetyl groups and provide access to the desired products. This problem was solved by quenching excess TFAA with saturated aqueous $NaHCO_3$, providing thiazoles **9**–**11** directly.

Therefore, termination of the step at −25 °C had to be carefully confirmed before the work up. Furthermore, the previously published work-up procedures had to be slightly modified to enable direct access to the desired compounds.[10]

Once the Hantzsch products **9**–**11** were obtained in suitable optical purity, Asp-thiazole **11** was converted into the desired building block **12** (Scheme 2).

Scheme 2. Synthesis of Asn-thiazole **12**



The benzyl ester protecting group was cleanly removed by hydrogenolysis; the reaction was first attempted in MeOH but, unexpectedly, partial conversion of the acid to the corresponding methyl ester was observed (from trace amounts up to 53%). This problem was solved by running the reaction in *i*PrOH, which is more sterically hindered. Acid **11a** was then converted into the corresponding amide **12**. The conditions for this transformation had to be extensively investigated, since the initially tested activation strategies and NH_3 sources gave low yields. Actually, activation as mixed anhydrides using either Boc_2O[11] or 2,2,2-trichloroethyl chloroformate and use of alternative NH_3 sources (conc. aq. NH_3 or NH_4Cl) never gave yields superior to 55%. However, better yields (68%) were obtained when benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP)[12] was used as activating agent and ammonium bicarbonate was used as NH_3 source.

The building blocks **9**, **10**, and **12** were then deprotected. The ethyl ester of **9** was hydrolyzed with 2 N LiOH to give compound **3**; the Boc-protecting groups from **12** and **10** were removed with 4 M HCl to afford their corresponding hydrochloride salts **2**·**HCl** and **4**·**HCl**, respectively (Scheme 3). At this stage, all of the building blocks required for the southern peptide had been synthesized; the preparation of northern peptide was therefore addressed.

(11) (a) Pozdnev, V. F. *Tetrahedron Lett.* **1995**, *36*, 7115. (b) Ardá, A.; Soengas, R. G.; M. Nieto, M. I.; Jiménez, C.; Rodríguez, J. *Org. Lett.* **2008**, *10*, 2175. (c) Trotter, N. S.; Brimble, M. A.; Harris, P. W. R.; Callis, D. J.; Sieg, F. *Bioorg. Med. Chem.* **2005**, *13*, 501.

(12) (a) Kahlon, D. K.; Lansdell, T. A.; Fisk, J. S.; Hupp, C. D.; Friebe, T. L.; Hovde, S.; Jones, A. D.; Dyer, R. D.; Henry, R. W.; Tepe, J. J. *J. Med. Chem.* **2009**, *52*, 1302. (b) Arienti, K. L.; Brunmark, A.; Axe, F. U.; McClure, K.; Lee, A.; Blevitt, J.; Neff, D. K.; Huang, L.; Crawford, S.; Pandit, C. R.; Lars Karlsson, L.; Breitenbucher, J. G. *J. Med. Chem.* **2005**, *48*, 1873.

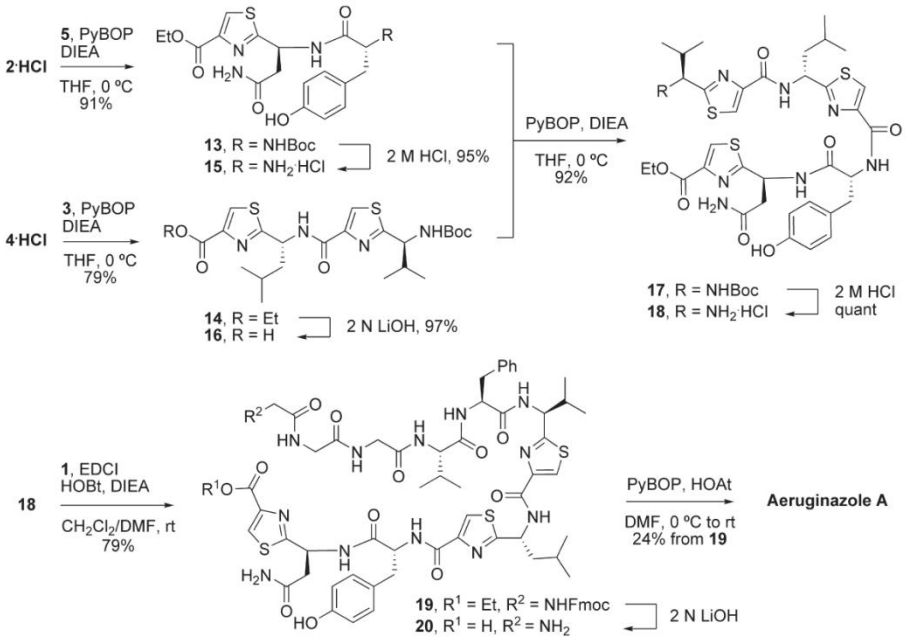
Fmoc-protected pentapeptide **1** was smoothly synthesized by solid-phase peptide synthesis (SPPS) on 2-chlorotritylchloride resin, first manually, through activation with diisopropyl carbodiimide and 1-hydroxybenzotriazole (HOBt), then by adapting the procedure to automated microwave-assisted synthesis using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as coupling agent.

Since the building blocks **1**–**4** (Figure 1) had been prepared, condensation of the individual synthetic intermediates was then undertaken. The coupling pattern (Scheme 3) was chosen according to the highest possible degree of convergence: Asn-thiazole **2**·**HCl** was coupled to Boc-D-Tyr-OH, giving rise to building block **13** (91% yield), whereas D-Leu-thiazole **4**·**HCl** was condensed with Val-thiazole **3** to form building block **14** (79% yield). Both couplings proceeded uneventfully (3 h at 0 °C) through activation by PyBOP.

The amine **15** was then coupled to the acid **16** to form the tris-thiazole peptide **17**. Interestingly, the yield of this reaction was strongly dictated by the ratio of amine to acid: whereas an excess (1.2 equiv) of acid **16** gave a disappointingly low yield (58%), an excess (1.6 equiv) of amine hydrochloride **15** allowed an increase the yield to 92%. The Boc-protecting group was then removed from compound **17** (Scheme 3) to afford the southern peptide **18**, which was directly coupled to northern peptide **1**. The protocol (PyBOP in THF) used in all previous couplings was again tested but proved unsatisfactory in this case (yields of roughly 10%). Observed solubility issues, as well as a desire to minimize the amount of *N*,*N*-diisopropylethylamine (DIEA) used, in consideration of the presence of the Fmoc group, were therefore addressed by switching to a different system, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI)/HOBt in CH_2Cl_2/DMF, which gave a gratifying yield of 79%. At this stage, the entire linear skeleton of Aeruginazole A had been prepared and thiazole dodecapeptide **19** (Scheme 3) was ready for final hydrolysis of its ethyl ester group, cleavage of its Fmoc group and subsequent macrocyclization. Thus, compound **19** was treated with LiOH in THF/H_2O in a one-pot removal of the protecting groups on both ends of the peptide to give the acid **20**. Macrocyclization of crude **20** was then performed in high dilution conditions (2.5 mM in DMF) by activation with PyBOP and HOAt, affording the desired product in a 24% yield over the two steps of deprotection and cyclization.

The spectroscopic data for this product fully agreed with those for a sample of natural product, thereby enabling confirmation of the structure proposed for Aeruginazole A by Raveh and Carmeli.[1] In summary, Aeruginazole A was obtained in an overall yield of 4.3% through a convergent synthesis combining solution- and solid-phase procedures. Special care was paid to conserve the integrity of the stereocenters in the intermediates, via strict control of Hantzsch thiazole syntheses and through use of various peptide-coupling reagents.

Scheme 3. Total Synthesis of Aeruginazole A



Acknowledgment. This work was funded by CICYT (Grant CTQ 2009-07758), *Generalitat de Catalunya* (Grant 2009SGR 1024), Barcelona Science Park, the Spanish Ministry of Science and Innovation (MICINN-PTQ 2009), CSIC (UdelaR, Uruguay) and the Chemistry Faculty (UdelaR, Uruguay). We also gratefully thank Prof. Carmeli (Raymond and Beverly Sackler School of Chemistry and Faculty of Exact Sciences, Tel-Aviv University, Ramat Aviv, Tel-Aviv 69978, Israel) for precious advice and for sending us original NMR spectra as well as an authentic sample of Aeruginazole A.

Supporting Information Available. Experimental procedures and characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

Supporting Information

Total synthesis of Aeruginazole A

Paolo Bruno, Stella Peña, Xavier Just-Baringo, Fernando Albericio and Mercedes Álvarez**

Institute for Research in Biomedicine, Barcelona Science Park, Carrer Baldiri Reixac 10, 08028 Barcelona, Spain

pbruno@pcb.ub.es, mercedes.alvarez@irbbarcelona.org

Table of Contents

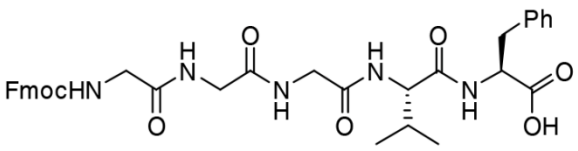
General Information

Flash chromatography was performed on Merck silica gel (60A 35-70 μm) as stationary phase. Analytical thin layer chromatography was performed using aluminium-backed plates coated with Merck Kieselgel 60 F_{254}; compounds were visualized under a UV lamp (254 nm) or by staining, using either a saturated solution of $KMnO_4$ in acetone, or 6% (w/v) vanillin in acidic ethanol (1% H_2SO_4). Melting points were determined in open capillaries using a Büchi B-540 melting point device. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer. Chemical shifts are quoted in parts per million (ppm) relative to the solvent indicated for each case (as internal standard). Multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and bs, broad singlet. HPLC-MS analyses were performed on a Waters 2795 Separations Module equipped with a SunFire C_{18} column (100 x 4.6 mm, 3.5 μm particle size). Detection was performed using a Waters 2487 Dual λ Absorbance Detector and a Waters Micromass ZQ using electrospray ionization (ESI). Acetonitrile (0.07% formic acid) and H_2O (0.1% formic acid) were used as mobile phases. High-resolution mass spectroscopy (HRMS) analyses were performed on a LTQ-FT Ultra (Thermo Scientific) mass spectrometer using nanoelectrospray ionization (nanoESI). IR spectra were recorded as thin film on a Thermo Nicolet Nexus FT-IR spectrometer. Analytical HPLC was performed on a Waters 2695 Separations Module equipped with a Waters XBridge C_{18} column (75 x 4.6 mm, 2.5 μm particle size) or a ChiralPak IA column (250 x 4.6 mm, 5 μm particle size) when determining ee values. Compounds were detected using a Waters 996 photodiode array detector. Acetonitrile (0.036% TFA) and H_2O (0.045% TFA) were used as mobile phases.

Experimental Procedures

Compounds **6a**[1] and **8a**[2] were obtained following described procedures and with the same yields as those reported.

Fmoc-peptide (1)



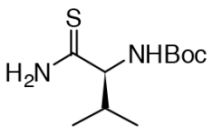
Manual synthesis. The synthesis was done in a plastic syringe attached to a vacuum manifold to enable rapid removal of reagents and solvents. The 2-chlorotritylchloride resin (loading = 1.0 mmol/g; 100 mg) was swelled in CH_2Cl_2 (3 × 5 mL), then acylated with a solution of Fmoc-Phe-OH (0.1 mmol) and DIEA (0.15 mmol) in CH_2Cl_2 (2 mL). The reaction mixture was gently shaken for 1 h then an extra 3 mmol of DIPEA was added and shaking was continued for 5 min. MeOH (0.08 mL) was then added in order to cap

[1] Merritt, E. A.; Bagley, M. C. *Synthesis* **2007**, *22*, 3535–3541.
[2] Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 15644-15651.

unreacted functional groups on the resin; the mixture was then shaken for 20 min. The resin was filtered, and then washed with CH_2Cl_2 and DMF (5 × 5 mL each). The *N*-terminus was deprotected using 20% piperidine in DMF (5 mL × 5 min x 2, 5 mL × 10 min). The resin was then washed with CH_2Cl_2 and DMF (5 × 5 mL each). Fmoc-AA-OH (0.3 mmol), preactivated by vigorous shaking for 4 min in the presence of *N*,*N*'-diisopropylcarbodiimide (0.3 mmol) and HOBt (0.3 mmol) in DMF (2 mL), was poured onto the resin and the resulting mixture was gently shaken for 1 h. Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the pentapeptide. The peptide was cleaved from the resin by treatment with 2% TFA in CH_2Cl_2 (10 mL) for 10 sec at rt followed by filtration and collection of the filtrate in water (10 mL). The treatment was repeated three times. Solvents were removed *in vacuo* and the crude peptide was precipitated with cold diethyl ether. The precipitate was collected and washed twice with diethyl ether. Lyophilization yielded 68 mg (98% yield) of the title compound as a white solid. The purity (96.3%) was determined by HPLC (linear gradient: 50 to 100% acetonitrile in H_2O over 8 min; flow rate = 1.0 mL/min; t_R = 1.95 min).

Automated synthesis. 2-chlorotritylchloride resin (1 g) was manually acylated with Fmoc-Phe-OH according to the previously described procedure, and then treated for peptide elongation in a CEM Discover microwave peptide synthesizer. Peptide couplings were performed in DMF with the appropriate Fmoc-amino acid (2 equiv), using HBTU (2 equiv) as coupling agent. The peptide chain was cleaved from the resin following the procedure described above. Fmoc-peptide **1** (560 mg; 85% yield; 96% pure) was obtained as a white solid. m.p 195 – 206 ºC (decomp). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.76 (d, *J* = 6.7, 3H), 0.80 (d, *J* = 6.7, 3H), 1.94 (m, 1H), 2.90 (dd, *J* = 13.9 and 9.0 Hz, 1H), 3.03 (dd, *J* = 13.9 and 5.4 Hz, 1H), 3.66 (d, *J* = 6.0 Hz, 2H), 3.74 (m, 4H), 4.22 (m, 2H), 4.28 (m, 2H), 4.40 (ddd, *J* = 9.0, 7.8 and 5.4 Hz, 1H), 7.18-7.27 (m, 5H), 7.33 (dd, *J* = 7.9 and 7.5 Hz, 2H), 7.41 (dd, *J* = 7.5 and 7.5 Hz, 2H), 7.56 (t, *J* = 5.9 Hz, 1H), 7.70-7.74 (m, 3H), 7.89 (d, *J* = 7.5 Hz, 2H), 8.08 (t, *J* = 5.8 Hz, 1H), 8.15 (t, *J* = 5.7 Hz, 1H), 8.29 (d, *J* = 7.8 Hz, 1H). ^{13}C-NMR (DMSO-d_6, 400 MHz) δ 17.8, 19.1, 30.7, 36.5, 41.8, 42.0, 43.5, 46.6, 53.5, 57.2, 65.7, 120.1 (2C), 125.2 (2C), 126.4, 127.1 (2C), 127.6 (2C), 128.1 (2C), 129.1 (2C), 137.5, 140.7 (2C), 143.8 (2C), 156.5, 168.4, 169.0, 169.5, 170.8, 172.7. HRMS (m/z): calcd for $C_{35}H_{39}N_5O_8$ $[M+H]^+$: 658.2871; found: 658.2873.

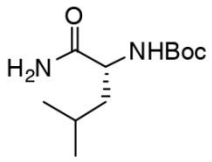
(*S*)-*N*-*tert*-(Butoxycarbonyl)thiovalinamide (6b)



Compound **6a** (2.95 g, 13.6 mmol) was dissolved in dry THF (40 mL) under N_2. Lawesson's reagent was added (3.26 g, 8.06 mmol) and the resulting suspension was stirred at rt for 4 h. At this point, more dry THF was added (10 mL) and the mixture was stirred at rt for 1 h. The reaction was quenched with sat. aq. $NaHCO_3$ (100 mL) and subsequently diluted with EtOAc (100 mL), and the resulting mixture was stirred at rt for 1 h. The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$ (100 mL) and brine (100 mL). The aqueous layers were combined and subsequently extracted with EtOAc (50 mL). Combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column

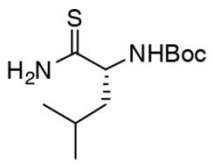
chromatography (hexane/EtOAc 70:30) yielded 2.97 g (94%)[3] of the title compound as a pale yellow oil. Spectroscopic data were identical in all respects to those previously reported.[1] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.97 (m, 6H), 1.43 (s, 9H), 2.12 (bs, 1H), 4.17 (m, 1H), 5.10 (bs, 1H), 7.75 (bs, 1H), 8.13 (bs, 1H).

(*R*)-*N*-*tert*-(Butoxycarbonyl)leucinamide (7a)



Boc-D-Leu-OH · H_2O (3.84 g, 15.4 mmol) was dissolved in CH_2Cl_2/MeOH 3:1 (40 mL). The solution was cooled to 0 ºC then $TMSCHN_2$ (2.0 M in hexane, 9.2 mL, 18.4 mmol), was added dropwise while stirring. Upon completion of the addition, the solution turned pale yellow and stirring was continued at rt for 5 min. The crude mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (20 mL), the resulting solution was cooled to 0 ºC, and then treated with concentrated aqueous NH_3 (15 mL). The mixture was stirred overnight and allowed to gradually reach rt, then it was concentrated *in vacuo* and the residue was partitioned between EtOAc (100 mL) and water (100 mL). The aqueous layer was further extracted with EtOAc. Combined organic layers were washed with sat. aq. $NaHCO_3$, water and brine, then dried over Na_2SO_4, filtered and finally concentrated *in vacuo*. Purification by flash column chromatography (hexane/EtOAc 90:10 to 40:60) yielded 2.94 g (83%) of the title compound as a white solid. m. p. 147 – 149 ºC. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.93 (d, J = 5.0 Hz, 3H), 0.94 (d, J = 5.1 Hz, 3H), 1.43 (s, 9H), 1.45-1.55 (m, 1H), 1.60-1.76 (m, 2H), 4.15 (bs, 1H), 4.98 (bs, 1H), 5.71 (bs, 1H), 6.31 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1, 23.1, 24.9, 28.5 (3C), 41.3, 52.7, 80.3, 156.0, 175.4. FTIR (neat) cm^{-1}: 3389, 2958, 1674, 1521, 1393, 1367, 1327, 1253, 1169, 1048, 1020, 665. HRMS (*m/z*): calcd for $C_{11}H_{23}N_2O_3$, $[M+H]^+$: 231.1703; found: 231.1705.

(*R*)-*N*-*tert*-(Butoxycarbonyl)thioleucinamide (7b)

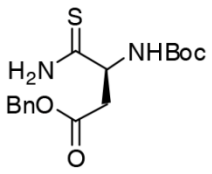


Lawesson's reagent (2.39 g, 5.91 mmol) was added to a solution of **7a** (2.18 g, 9.47 mmol) in dry THF (40 mL) under N_2 and the resulting suspension was stirred at rt for 4 h. The reaction was quenched with sat. aq. $NaHCO_3$ and subsequently diluted with EtOAc; the resulting mixture was stirred at rt for 1 h. The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (hexane/EtOAc 70:30) yielded 2.30 g (quantitative yield) of the title compound as a white solid. m. p. 84.1 – 86.6 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.95 (m, 6H), 1.43 (s, 9H), 1.58-1.74 (m, 3H), 4.48 (m, 1H), 5.17 (bs, 1H), 7.62 (bs, 1H), 8.11 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.1, 23.1, 25.1, 28.5 (3C), 44.6, 58.2, 80.6, 156.1, 211.0. FTIR (neat) cm^{-1}: 3311, 3199, 2959, 1683, 1635, 1506, 1436, 1368, 1253, 1164, 1047, 1024, 875, 738. HRMS (*m/z*): calcd for $C_{11}H_{23}N_2O_2S$, $[M+H]^+$: 247.1475; found: 247.1475.

---

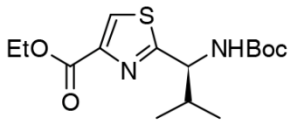
[3] In the previously published synthesis of compound **6a** (performed in refluxing CH_2Cl_2), the reported yield was 62% (ref 1). However, for the work reported here, THF was used instead of CH_2Cl_2, which enabled a lower reaction temperature (rt vs 40 ºC) and shorter reaction time (1 h vs overnight) and provided a better yield (94% vs 62%).

(*S*)-benzyl 4-amino-3-(*tert*-Butoxycarbonylamino)-4-thioxobutanoate (8b)



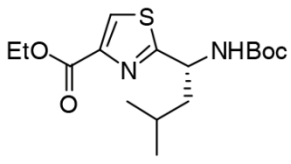
To a suspension of **8a** (6.25 g, 19.4 mmol) in dry THF (120 mL) under N_2 was added Lawesson's reagent (4.81 g, 11.9 mmol) and the resulting suspension was stirred at rt for 4 h. The reaction was quenched with sat. aq. $NaHCO_3$ and the resulting mixture was stirred at rt for 1 h, then diluted with water and EtOAc until two clear layers were obtained. The layers were separated and the aqueous layer was extracted with EtOAc. Combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (hexane/EtOAc 70:30 to 60:40) yielded 6.34 g (97%) of the title compound as a pale yellow powder. m. p. 75.6 – 82.1 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.44 (s, 9H), 2.91 (dd, *J* = 16.9 and 6.5 Hz, 1H), 3.21 (dd, *J* = 16.9 and 4.9, 1H), 4.83 (bs, 1H), 5.13 (s, 2H), 5.77 (d, *J* = 8.6 Hz, 1H), 7.34 (m, 5H), 7.64 (br s, 1H), 8.02 (br s, 1H). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 28.4 (3C), 39.5, 55.6, 67.1, 80.9, 128.4 (2C), 128.6, 128.8 (2C), 135.4, 155.4, 171.6, 207.1. FTIR (neat) cm^{-1}: 3396, 2978, 2931, 1699, 1628, 1498, 1456, 1392, 1368, 1251, 1164, 1051, 737, 698. HRMS (*m/z*): calcd for $C_{16}H_{23}N_2O_4S$, $[M+H]^+$: 339.1373; found: 339.1374.

Boc-Val-Thz (9)



To a solution of **6b** (2.60 g, 11.2 mmol) in dry DME (29 mL) under N_2 was added $KHCO_3$ (12.4 g, 124 mmol) in one portion and the resulting suspension was stirred at rt for 10 min, then cooled to -35 ºC. Ethyl bromopyruvate (4.20 mL, 33.5 mmol) was added dropwise and the mixture was stirred overnight and allowed to gradually reach rt. The mixture was filtered through a short pad of Celite and the filtrate was concentrated *in vacuo*. The residue was dissolved in dry DME (20 mL) under N_2 and the resulting solution was cooled to -35 ºC. A solution of 2,6-lutidine (11.9 mL, 102 mmol) and TFAA (6.4 mL, 44 mmol) in dry DME (1 mL) was then added dropwise over a period of 40 min. The solution was stirred at -25 ºC for 3 h, then quenched with sat. aq. $NaHCO_3$. The resulting mixture was extracted with Et_2O and the organic layer was washed with sat. aq. $NaHCO_3$, 0.01 N HCl, sat. aq. NaH_2PO_4 and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (CH_2Cl_2/hexane 50:50 to 100:0, followed by CH_2Cl_2/EtOAc 97:3) yielded 3.19 g (87%) of the title compound as an orange oil. *e.e.* 94% – Determined by HPLC on a ChiralPak IA column (250 x 4.6 mm, 5 μm particle size) eluting with an isocratic mobile phase of 40% acetonitrile (0.036% TFA) and 60% H_2O (0.045% TFA); flow rate = 1.0 mL/min; t_R (L-enantiomer) = 17.17 min, t_R (R-enantiomer) = 18.67 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.8 Hz, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.43 (s, 9H), 2.42 (m, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 4.88 (dd, *J* = 8.1 and 5.8 Hz, 1H), 5.29 (d, *J* = 8.1 Hz, 1H), 8.06 (s, 1H). The spectroscopic data were identical in all respects to those previously reported.[1]

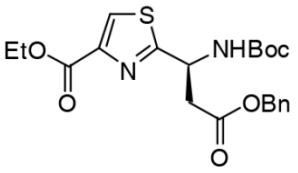
Boc-Leu-Thz (10)



To a solution of **7b** (1.84 g, 7.47 mmol) in dry DME (19 mL) under N_2 was added $KHCO_3$ (8.29 g, 82.8 mmol) in one

portion. The resulting suspension was stirred at rt for 10 min, then cooled to -35 ºC. Ethyl bromopyruvate (2.80 mL, 22.3 mmol) was added dropwise and the mixture was stirred overnight and allowed to gradually reach rt. The mixture was filtered through a short pad of Celite and the filtrate was concentrated *in vacuo*. The residue was dissolved in dry DME (13 mL) under N_2 and the solution was cooled to -35 ºC. A solution of 2,6-lutidine (7.90 mL, 67.8 mmol) and TFAA (4.25 mL, 30.1 mmol) in dry DME (0.5 mL) was then added dropwise over 20 min. The solution was stirred at -25 ºC for 3 h, then diluted with Et_2O (80 mL) and washed with sat. aq. $NaHCO_3$ (2 × 80 mL), 1 N HCl (5 × 100 mL), sat. aq. NaH_2PO_4 (3 × 40 mL) and brine (100 mL). Combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (CH_2Cl_2/hexane 10:90 to 100:0, followed by CH_2Cl_2/EtOAc 97:3) afforded 2.55 g (quantitative yield) of the title compound a pale yellow powder. m. p. 87.2 – 95.4 °C. *e.e.* 91% – Determined by HPLC on a ChiralPak IA column (250 x 4.6 mm, 5 µm particle size) eluting with an isocratic mobile phase of 40% acetonitrile (0.036% TFA) and 60% H_2O (0.045% TFA); flow rate = 1.0 mL/min; t_R (L-enantiomer) = 29.97 min, t_R (R-enantiomer) = 31.43 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.95 (d, *J* = 2.6 Hz, 3H), 0.97 (d, *J* = 2.7 Hz, 3H), 1.38 (t, *J* = 7.1 Hz, 3H), 1.43 (s, 9H), 1.65-1.80 (m, 2H), 1.85-2.00 (m, 1H), 4.40 (q, *J* = 7.1 Hz, 2H), 4.95-5.10 (m, 1H), 5.10-5.20 (m, 1H), 8.05 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 21.9, 23.1, 25.1, 28.4 (3C), 44.6, 51.5, 61.5, 80.3, 127.0, 147.4, 155.2, 161.5, 174.6. FTIR (neat) cm^{-1}: 3354, 2959, 1716, 1520, 1368, 1248, 1210, 1170, 1096, 1022, 754. HRMS (*m/z*): calcd for $C_{16}H_{27}N_2O_4S$, $[M+H]^+$: 343.1686; found: 343.1687.

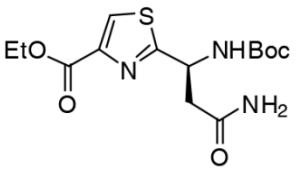
Boc-Asp(OBzl)-Thz (11)



To a solution of **8b** (3.88 g, 11.5 mmol) in dry DME (30 mL) under N_2 was added $KHCO_3$ (12.8 g, 128 mmol) in one portion. The resulting suspension was stirred at rt for 10 min, then cooled to -20 ºC. Ethyl bromopyruvate (4.3 mL, 34.3 mmol) was added dropwise and the mixture was stirred at -20 ºC for 24 h, then filtered through a short pad of Celite. The filtrate was concentrated *in vacuo*, the residue was dissolved in dry DME (20 mL) under N_2 and the resulting solution was cooled to -20 ºC. A solution of TFAA (6.5 mL, 46.0 mmol) and 2,6-lutidine (12 mL, 103 mmol) in dry DME (10 mL) was then added dropwise over 1 h. After 4 h of stirring at -20 ºC, the reaction mixture was diluted with EtOAc (300 mL) and quenched with sat. aq. NaH_2PO_4. The organic layer was further washed with sat. aq. NaH_2PO_4, 0.01 N HCl, H_2O and brine, and then dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (hexane/EtOAc 95:5 to 80:20) yielded 3.74 g (75%) of the title compound as a yellow oil. *e.e.* 91% – Determined by HPLC on a ChiralPak IA column (250 x 4.6 mm, 5 µm particle size) eluting with an isocratic mobile phase of 45% acetonitrile (0.036% TFA) and 55% H_2O (0.045% TFA); flow rate = 1.0 mL/min; t_R (L-enantiomer) = 25.07 min, t_R (R-enantiomer) = 29.96 min. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.38 (t, *J* = 7.1 Hz, 3H), 1.46 (s, 9H), 3.06 (dd, *J* = 16.8 and 5.1 Hz, 1H), 3.39 (dd, *J* = 16.8 and 4.2 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 5.08 (s, 2H), 5.38 (bs, 1H), 5.98 (d, *J* = 8.7 Hz, 1H), 7.31 (m, 5H), 8.06 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 28.4 (3C), 38.7, 49.6, 61.5, 66.9, 80.7, 127.8, 128.3 (2C), 128.4, 128.7 (2C), 135.5, 147.4, 155.1, 161.3, 171.0, 173.1. FTIR (neat) cm^{-1}:

3349, 2979, 2934, 1716, 1498, 1369, 1238, 1170, 1097, 1022, 753, 699. HRMS (*m/z*): calcd for $C_{21}H_{27}N_2O_6S$, $[M+H]^+$: 435.1584; found: 435.1594.

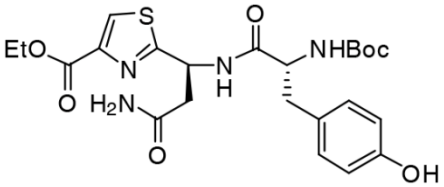
Boc-Asn-Thz (12)



A suspension of **11** (2.02 g, 4.65 mmol) and Pd/C (10% wt, 525 mg) in *i*PrOH (50 mL) was saturated with H_2 and vigorously stirred overnight at rt. Then N_2 atmosphere was restored and the mixture was filtered through a short pad of Celite. The filtrate was concentrated *in vacuo* and the residual *i*PrOH was removed by coevaporation, first with EtOAc, then with CH_2Cl_2. The crude acid **11a** (1.49 g, 93%) was obtained as a light yellow powder which was used in the next step without further purification.

To a solution of the crude acid **11a** (523 mg, 1.52 mmol) in dry 1,4-dioxane (15 mL) under N_2 was added NH_4HCO_3 (369 mg, 4.67 mmol) and the resulting suspension was cooled in an ice-water bath. PyBOP (960 mg, 1.84 mmol) and DIPEA (1 mL, 5.84 mmol) were then added. A thick precipitate formed, so dry DMF (5 mL) was added to facilitate stirring. After 4 h of stirring at 0 ºC, the reaction was quenched with 0.01 N HCl. The mixture was extracted with EtOAc. Combined organic layers were washed with sat. aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (EtOAc/hexane 90:10) yielded 354 mg (68%) of pure amide **12** as a white powder. m.p. 149 – 152 °C. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.38 (t, *J* = 7.1 Hz, 3H), 1.46 (s, 9H), 2.86 (dd, *J* = 15.3 and 4.6 Hz, 1H), 3.27 (dd, *J* = 15.3 and 4.0 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 2H), 5.32 (m, 1H), 5.60 (bs, 1H), 6.05 (bs, 1H), 6.58 (d, *J* = 8.2 Hz, 1H), 8.07 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 28.5 (3C), 38.8, 50.2, 61.6, 80.5, 127.9, 147.1, 155.5, 161.5, 172.8, 174.4. FTIR (neat) cm^{-1}: 3405, 2980, 1669, 1506, 1368, 1238, 1167, 1907, 1050, 1021, 755. HRMS (*m/z*): calcd for $C_{14}H_{22}N_3O_5S$, $[M+H]^+$: 344.1275; found: 344.1275.

Asn-Thz-Tyr peptide (13)

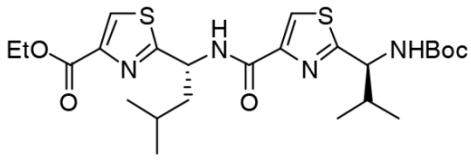


To a solution of compound **12** (281 mg, 0.818 mmol) in 1,4-dioxane (8 mL) was added HCl (4 M in 1,4-dioxane, 8 mL, 32 mmol) and the resulting solution was stirred at rt for 1 h 15 min. The mixture was diluted with PhMe (50 mL), then concentrated *in vacuo*. Residual HCl and solvents were removed by repeated coevaporation with Et_2O. Digestion with Et_2O and filtration yielded 210 mg (92%) of the crude amine hydrochloride **2·HCl** as a light yellow powder.

A mixture of **2·HCl** (198 mg, 0.708) and Boc-L-Tyr-OH (218 mg, 0.775 mmol) in dry THF (9 mL) under N_2 was cooled in an ice-water bath. To the stirring solution were added DIPEA (480 µL, 2.80 mmol) and PyBOP (442 mg, 0.849 mmol). The suspension was stirred at 0 ºC for 3 h, and then diluted with EtOAc (50 mL) and washed with sat. aq. NaH_2PO_4, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (CH_2Cl_2/hexane 50:50 to 100:0 then CH_2Cl_2/MeOH 98:2 to 95:5) yielded 326 mg (91%) of the title compound as a white powder. m. p. 187 – 191 °C. ^{1}H NMR (CD_3OD, 400 MHz): δ 1.38 (m, 12H), 2.80 (dd, *J* = 13.8 and 8.4 Hz, 1H), 2.91 (d, *J* = 5.9 Hz, 2H), 3.00 (dd, *J* = 13.8

and 6.0 Hz, 1H), 4.25 (dd, *J* = 8.4, 6.0 Hz, 1H), 4.37 (q, *J* = 7.1 Hz, 2H), 5.58 (t, J = 5.9 Hz, 1H), 6.69 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 8.3 Hz, 2H), 8.29 (s, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 14.7, 28.8 (3C), 38.4, 39.8, 49.8, 58.1, 62.6, 80.6, 116.4 (2C), 129.2, 129.7, 131.5, (2C), 147.8, 157.5, 157.8, 162.9, 174.0, 174.4, 174.7. FTIR (nujol) cm^{-1}: 3338, 3054, 2475, 1652, 1263, 1168, 1101, 1045, 1021, 956, 896, 826, 743. HRMS (*m/z*): calcd for $C_{23}H_{31}N_4O_7S$, $[M+H]^+$: 507.1908; found: 507.1911.

Bis-thiazole peptide ethyl ester (14)

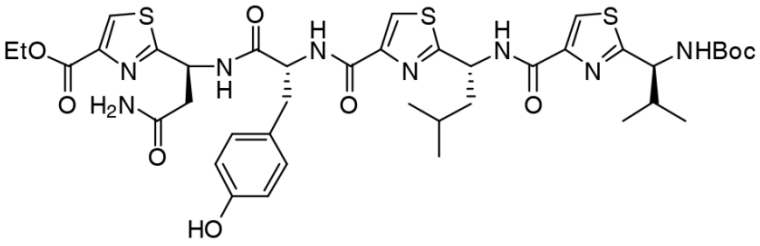


To a solution of **10** (2.52 g, 7.36 mmol) in 1,4-dioxane (15 mL) was added HCl (4 M in 1,4-dioxane, 20 mL, 80 mmol) in 5 mL portions over 2 h. After 30 min of additional stirring, the reaction was concentrated *in vacuo*. Residual HCl and 1,4-dioxane were removed by repeated coevaporation with hexane, CH_2Cl_2 and Et_2O. Crude amine hydrochloride **4·HCl** (2.06 g, quantitative yield) was obtained as a white powder and was used in the next step without further purification.

To a solution of **9** (2.60 g, 7.92 mmol) in THF (26 mL) cooled in an ice-water bath was added dropwise 2 N LiOH (17.2 mL, 34.4 mmol), the cooling bath was removed and the mixture was stirred at rt for 5 h. The mixture was then transferred to a separatory funnel and diluted with sat. aq. $NaHCO_3$ (20 mL) and Et_2O (20 mL). After vigorous shaking the layers were separated and the organic layer was extracted with sat. aq. $NaHCO_3$. Combined aqueous layers were further washed with Et_2O (20 mL). All Et_2O layers were discarded. The aqueous layer was acidified to pH ~ 2 with 2N HCl, and then extracted, first with EtOAc, and then with CH_2Cl_2. Combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude acid **3** (1.58 g, 66%) was obtained as a white foam and was used in the next step without further purification. The spectroscopic data were identical in all respects to those previously reported.[2 rep ref jacs 2005]

DIPEA (3.50 mL, 20.4 mmol) was added at 0 ºC to a mixture of **4·HCl** (1.44 g, 5.16 mmol) and crude acid **3** (1.55 g, 5.16 mmol) in dry THF (40 mL) under N_2. After 5 min of stirring at 0 ºC, the mixture was treated with PyBOP (3.08 g, 5.92 mmol) and then stirred at 0 ºC for 3 h. The reaction mixture was then quenched with sat. aq. $NaHCO_3$ (40 mL) and stirring was continued at 0 ºC for 1 h. The mixture was then diluted with Et_2O (40 mL), EtOAc (40 mL) and water (80 mL). The layers were separated and the organic layer was washed with sat. aq. $NaHCO_3$, sat. aq. NaH_2PO_4 and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo*. Purification by flash column chromatography (hexane/EtOAc 95:5 slowly increasing to 60:40) yielded 2.13 g (79%) of the title compound as a white powder. m. p. 54 – 57 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.92 (d, *J* = 6.8 Hz, 3H), 0.95-1.03 (m, 9H), 1.39 (t, *J* = 7.1 Hz, 3H), 1.46 (s, 9H), 1.67-1.75 (m, 1H), 1.95-2.10 (m, 1H), 2.12 (ddd, J_1 = 14.2, 10.3 and 6.0 Hz, 1H), 2.35 (m, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 4.86 (m, 1H), 5.17 (d, *J* = 8.4 Hz, 1H), 5.56 (m, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 8.02 (s, 1H), 8.07 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.4, 17.4, 19.4, 22.0, 23.0, 25.2, 28.4 (3C), 33.3, 43.8, 49.6, 58.1, 61.5, 80.3, 123.6, 127.3, 147.4, 149.3, 155.5, 160.7, 161.4, 172.7, 173.2. FTIR (cm^{-1}): 3324, 2962, 1714, 1666, 1537, 1486, 1367, 1238, 1210, 1170, 1096, 1019, 913, 874. HRMS (*m/z*): calcd for $C_{24}H_{37}N_4O_5S_2$, $[M+H]^+$: 525.2200; found: 525.2203.

Boc-southern peptide (17)

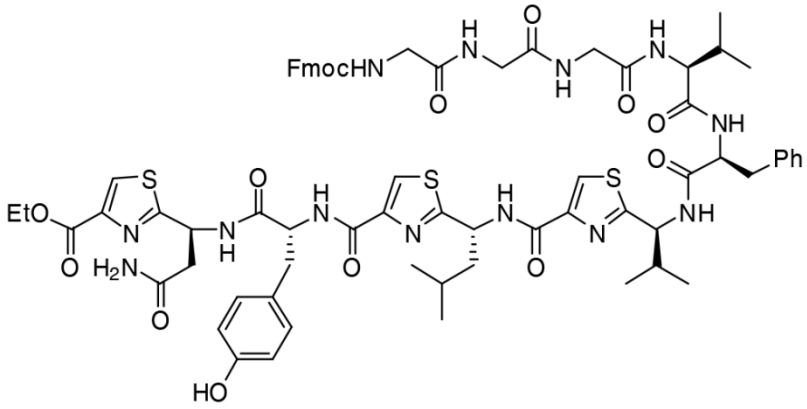


To a solution of **14** (1.08 g, 2.06 mmol) in THF (20 mL) cooled in an ice-bath was added 2N LiOH (1.2 mL, 2.4 mmol). The resulting solution was stirred overnight and allowed to gradually reach rt. Three portions of 2 N LiOH (1.5 mL total, 3.0 mmol) were then added at 6 h intervals; each of the additions was performed at 0 ºC and stirring in between additions was performed at rt. After a total reaction time of 48 h, the reaction was quenched with sat. aq. NaH_2PO_4 (150 mL). The resulting mixture was extracted with EtOAc/CH_2Cl_2 (1:1). Combined organic layers were dried over Na_2SO_4, filtered and concentrated *in vacuo* to give 996 mg (97%) of the crude acid **16** as a white powder.

Compound **13** (316 mg, 0.624 mmol) was dissolved in 1,4-dioxane-MeOH 2:1 (8 mL) then treated with HCl (4M in 1,4-dioxane, 2 mL, 8 mmol). The resulting solution was stirred at rt for 2 h, then diluted with PhMe (10 mL) and concentrated *in vacuo*. Residual HCl and solvents were removed by sequential coevaporation with MeOH, CH_2Cl_2, and Et_2O to give 261 mg (95%) of the crude amine hydrochloride **15** as a pale yellow powder. The crude product was used in the next step without further purification.

The crude acid **16** (169 mg, 340 µmol), the crude amine hydrochloride **15** (234 mg, 528 µmol) and DIPEA (240 µL, 1.40 mmol) were dissolved in dry DMF/THF 2:1 (6 mL), and the resulting solution was cooled in an ice-water bath. After 5 min of stirring at 0 ºC, the solution was treated with PyBOP (232 mg, 446 µmol), and then stirred at 0 ºC for 3 h. The mixture was then diluted with EtOAc/CH_2Cl_2 (8:2) and washed with sat. aq. NaH_2PO_4, sat. aq. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (CH_2Cl_2/MeOH 96:4) yielded 276 mg (92%) of the title compound as a white powder. m. p. 126 – 131 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.99 (m, 6H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.44 (s, 9H), 1.74 (m, 1H), 1.91 (m, 1H), 2.02 (m, 1H), 2.34 (m, 1H), 2.47 (bs, 1H), 2.61 (dd, *J* = 15.2 and 3.9 Hz, 1H), 2.98-3.18 (m, 3H), 4.29 (q, *J* = 7.1 Hz, 2H), 4.86 (bs, 2H), 5.29 (d, *J* = 8.4 Hz, 1H), 5.48-5.53 (m, 2H), 6.32 (bs, 1H), 6.57 (bs, 1H), 6.68 (d, *J* = 7.8 Hz, 2H), 6.98 (d, *J* = 7.8 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.96 (m, 2H), 8.07 (m, 2H), 8.20 (d, *J* = 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.4, 17.5, 19.5, 22.1, 23.1, 25.2, 28.5 (3C), 33.2, 38.3, 38.5, 43.9, 48.5, 49.7, 55.3, 58.3, 61.6, 80.6, 116.0 (2C), 123.9, 124.3, 127.4, 128.2, 130.5 (2C), 146.7, 149.1, 149.3, 155.7, 155.9, 161.0, 161.1, 161.5, 171.2, 172.6, 172.7, 172.9, 173.6. FTIR (nujol) cm^{-1}: 3303, 1651, 1234, 1166, 1097, 1015, 753, 665. HRMS (*m/z*): calcd for $C_{40}H_{53}N_8O_9S_3$, $[M+H]^+$: 885.3092; found: 885.3110.

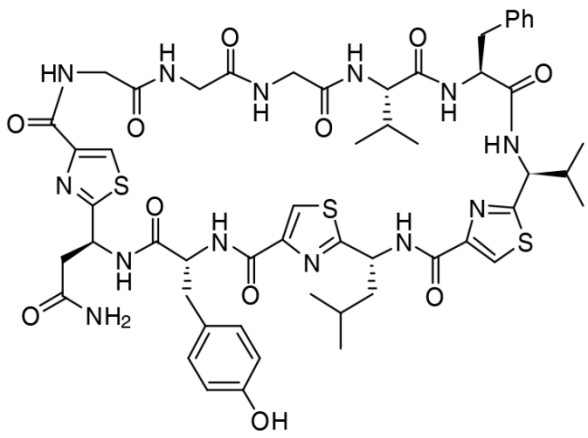
Protected open-chain Aeruginazole A (19)



To a stirring solution of **17** (261 mg, 0.295 mmol) in 1,4-dioxane/THF 3:2 (5 mL) was slowly added HCl (4 M in 1,4-dioxane, 2.7 mL, 10.8 mmol). After 3 h of stirring at rt, the solution was treated with PhMe (10 mL) then the volatiles were removed *in vacuo*. Residual HCl and solvents were removed by sequential coevaporation with MeOH, CH_2Cl_2 and Et_2O. The crude amine hydrochloride **18** (246 mg, quantitative yield) was obtained as a pale yellow powder and was used in the next step without further purification.

A solution of Fmoc-peptide **1** (131 mg, 223 µmol), HOBt·H_2O (39 mg, 255 µmol) and EDCI (43 mg, 224 µmol) in dry CH_2Cl_2/DMF 4:6 (3 mL) was stirred at rt for 4 min. In another flask, a solution of **18** (140 mg, 170 µmol) and DIPEA (30 µL, 175 µmol) in dry CH_2Cl_2/DMF 8:2 (3 mL) was stirred at rt for 4 min. The solution of the amine was poured into the solution of the activated Fmoc-peptide, and the resulting mixture was stirred at rt for 5 h. The mixture was diluted with EtOAc (100 mL), and then quenched with sat. aq. NaH_2PO_4 (100 mL). The organic layer was washed with brine, and then dried over Na_2SO_4, filtered, and concentrated *in vacuo*. Purification by flash column chromatography (CH_2Cl_2/MeOH 100:0 slowly increasing to 95:5) yielded 191 mg (79%) of the title compound as a white powder. m. p. 177 – 185 °C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.72 (d, *J* = 6.8 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.89 (d, *J* = 6.9 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 1.29 (t, *J* = 7.1 Hz, 3H), 1.60-1.72 (m, 1H), 1.92 (m, 2H), 2.05 (m, 1H), 2.25-2.30 (m, 1H), 2.60-2.75 (m, 1H), 2.82 (dd, *J* = 14.1 and 9.8 Hz, 1H), 2.85-3.05 (m, 4H), 3.67 (d, *J* = 6.0 Hz, 2H), 3.75 (bs, 4H), 4.15-4.30 (m, 6H), 4.64-4.73 (m, 2H), 5.01 (dd, *J* = 8.4 and 6.6 Hz, 1H), 5.35 (m, 1H), 5.46 (bdd, 1H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.98 (bs, 1H), 7.03 (d, *J* = 8.4 Hz, 2H), 7.13 (t, *J* = 7.0 Hz, 1H), 7.15-7.25 (m, 4H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.47 (bs, 1H), 7.55 (t, *J* = 6.0 Hz, 1H), 7.71 (d, *J* = 7.4 Hz, 2H), 7.78 (d, *J* = 8.7 Hz, 1H), 7.89 (d, *J* = 7.3 Hz, 2H), 7.94 (d, *J* = 8.4 Hz, 1H), 8.06-8.10 (m, 1H), 8.12 (s, 1H), 8.12-8.20 (m, 2H), 8.22 (s, 1H), 8.40 (s, 1H), 8.48 (d, *J* = 8.6 Hz, 1H), 8.97 (d, *J* = 8.5 Hz, 1H), 9.09 (d, *J* = 8.0 Hz, 1H), 9.19 (bs, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 14.2, 17.8 (2C), 19.0, 19.3, 21.4, 22.9, 24.6, 30.5, 32.5, 37.0, 37.2, 39.1, 42.0 (2C), 42.1, 43.6, 46.6, 48.3, 49.5, 53.8, 54.2, 56.4, 57.7, 60.7, 65.8, 115.0 (2C), 120.1 (2C), 124.0, 124.5, 125.2 (2C), 126.2, 127.0, 127.1 (2C), 127.6 (2C), 128.0 (2C), 129.1 (2C), 129.3, 130.2 (2C), 137.4, 140.7 (2C), 143.8 (2C), 145.5, 148.6, 148.8, 156.0, 156.5, 159.8, 160.6, 160.7, 168.7, 169.1, 169.6, 170.7, 170.7 (2C), 171.3, 172.7, 173.0, 174.2. FTIR (nujol) cm^{-1}: 3404, 1652, 1539, 1234, 1170, 1102, 1023, 740, 665. HRMS (*m/z*): calcd for $C_{70}H_{82}N_{13}O_{14}S_3$, $[M+H]^+$: 1424.5261; found: 1424.5250.

Aeruginazole A



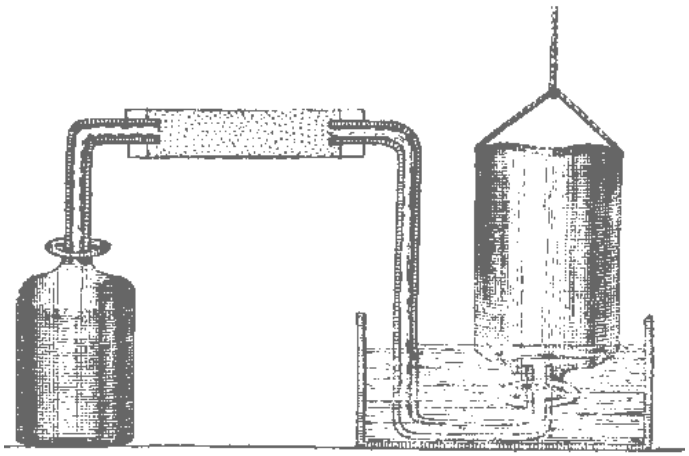
To a solution of **19** (137 mg, 96.2 µmol) in THF/H_2O 4:1 (15 mL) cooled in an ice-water bath was added dropwise 2N LiOH (300 µL, 600 µmol) over 15 min. The cold bath was removed, the solution was stirred at rt for 4 h, and then the pH was lowered to ~2 with 2N HCl. The reaction mixture was concentrated *in vacuo*. Residual water was removed by coevaporation with MeOH. The crude deprotected product **20** (together with NaCl) was obtained as a white powder and was used in the next step without further purification.

A solution of crude compound **20** (96.2 µmol), HOAt (85.3 mg, 0.627 mmol), DIPEA (165 µL, 0.964 mmol) and PyBOP (301 mg, 0.578 mmol) in dry DMF (40 mL) was cooled in an ice-water bath and stirred at rt for 60 h. The reaction was then quenched with sat. aq. NaH_2PO_4 and extracted with CH_2Cl_2/*i*PrOH. Combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. After purification by flash column chromatography (CH_2Cl_2/MeOH 95:5 to 80:20), residual traces of HOAt were removed by dissolving the mixture in CH_2Cl_2/*i*PrOH 8:2 and washing with sat. aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to yield 26.3 mg (24% over two steps) of Aeruginazole A as a light yellow powder. m. p. 171 – 179 °C (decomposes). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.58 (d, *J* = 6.6 Hz, 6H), 0.84 (d, *J* = 6.7 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 6H), 1.60-2.18 (m, 5H), 2.69 (dd, *J* = 15.3 and 7.9 Hz, 1H), 2.72-3.10 (m, 5H), 3.63 (dd, *J* = 15.9 and 4.8 Hz, 1H), 3.70-3.95 (m, 5H), 4.07 (dd, *J* = 16.6 and 6.0 Hz, 1H), 4.57 (m, 1H), 4.77 (bdd, 1H),4.99 (dd, *J* = 8.4 and 6.1 Hz, 1H), 5.42 (bdd, 1H), 5.53 (bdd, 1H), 6.63 (d, *J* = 8.5 Hz, 2H), 7.03 (bs, 1H), 7.04 (d, *J* = 8.5 Hz, 2H), 7.09 (t, *J* = 7.1 Hz, 1H), 7.16 (dd, *J* = 7.1 and 7.1 Hz, 2H), 7.22 (d, *J* = 7.1 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 1H), 7.48 (bs, 1H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.8 Hz, 1H), 8.15 (s, 1H), 8.17 (s, 1H),8.19 (m, 1H), 8.24 (s, 1H), 8.24 (t, *J* = 6.0 Hz, 1H), 8.44 (m, 2H), 8.69 (d, *J* = 8.9 Hz, 1H), 9.06 (d, *J* = 7.9 Hz, 1H), 9.19 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 17.4, 17.7, 18.8, 19.2, 21.7, 22.8, 24.5, 29.9, 33.1, 37.0, 37.2, 40.0, 41.2, 42.4 (2C), 42.6, 48.1, 48.5, 53.5, 54.2, 56.2, 58.2, 114.9 (2C), 124.4, 124.6, 124.7, 126.2, 126.9, 128.0 (2C), 129.0 (2C), 130.3 (2C), 137.5, 147.9, 148.5, 148.7, 156.0, 159.7, 160.0, 160.8, 169.1, 169.6, 169.7, 170.5 (2C), 170.8, 171.1, 171.3, 171.9, 172.7. FTIR (nujol) cm^{-1}: 3281, 1651, 1537, 1247, 1024, 665. HRMS (*m/z*): calcd for $C_{53}H_{66}N_{13}O_{11}S_3$, $[M+H]^+$: 1156.4161; found: 1156.4187.

^{1}H-NMR and ^{13}C-NMR spectra

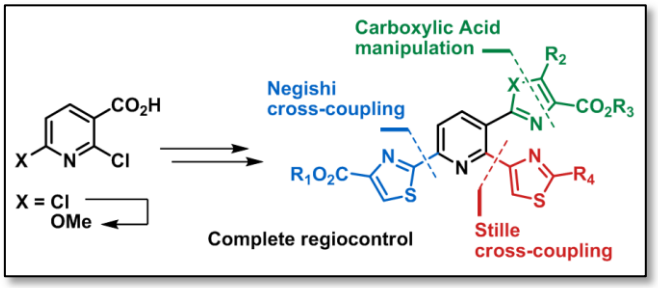
NMR spectra images are available in the Supporting Information in electronic format.

Chapter 4:
Synthesis of Azole-Substituted Pyridines



SYNTHESIS OF AZOLE-SUBSTITUTED PYRIDINES

From 2,6-dichloronicotinic acid to thiopeptide cores.



Xavier Just-Baringo,[a,b] Fernando Albericio[a,b,c,d] and Mercedes Álvarez[a,b,e]

European Journal of Organic Chemistry **2013**, ACCEPTED.

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.

[b] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.

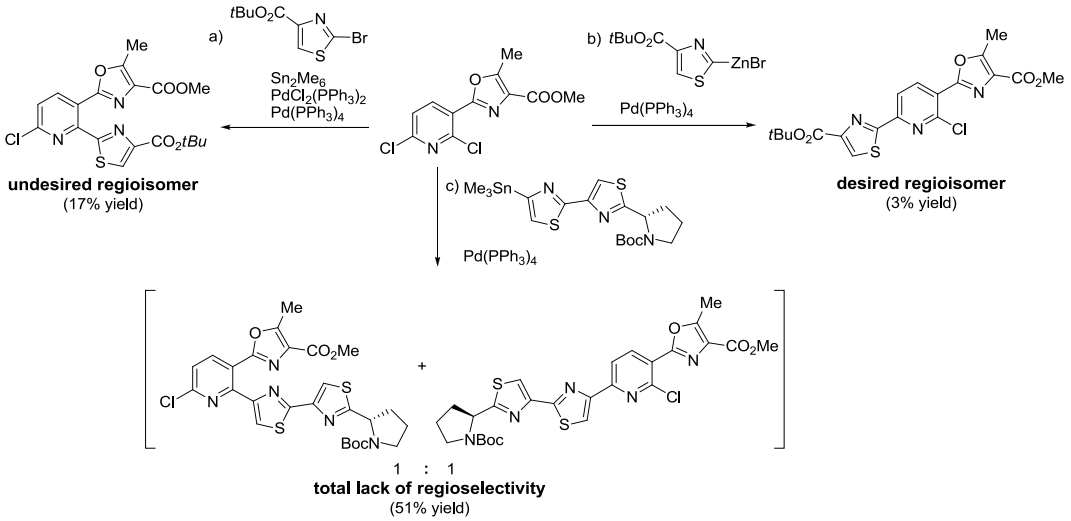
[c] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

[d] School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.

[e] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

Conspectus

When facing the synthesis of thiopeptide antibiotics, their polyheterocyclic core usually poses the biggest synthetic challenge. In this chapter, the results of our investigations towards a fully regioselective and modular approach strategy to obtain 2,3,6-azole-substituted pyridines are addressed. Screening of cross-coupling methodologies and conditions on 2,6-dichloropyridines failed in providing either good conversions or acceptable regioselectivities. Stille and Negishi cross-coupling reactions using thiazole-4-carboxylates displayed opposite regioselectivity, but equally disappointing conversions.



Selective nucleophilic aromatic substitution at position 6 of 2,6-dichloronicotinic acid with methoxide provided a masked reactive site, an ideal substrate to achieve excellent yields in subsequent cross-coupling reactions and avoid further regioselectivity problems. Using this strategy, the central core of baringolin and five more analogues were obtained. Moreover, baringolin core was prepared with different sets of protecting groups to assess their selective deprotection.

Job/Unit: O30877 /KAP1 Date: 22-08-13 10:17:20 Pages: 17

EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201300877

From 2,6-Dichloronicotinic Acid to Thiopeptide Cores

Xavier Just-Baringo,[a,b] Fernando Albericio,[a,b,c,d] and Mercedes Álvarez*[a,b,e]

Keywords: Synthetic methods / Cross-coupling / Nitrogen heterocycles / Peptides

The scope of 2,6-dichloronicotinic acid as a precursor of thiopeptide polyheterocyclic cores has been extensively studied in a cross-coupling-based approach. Differentiation of the two chlorinated positions under S_NAr conditions and versatility of the carboxylic acid are key for the preparation of 2,3,6-trisubstituted pyridines with complete regiocontrol. With the present strategy, nine different azole-substituted pyridines were synthesized. Studies towards the selective deprotection of their functionalities resulted in a set of fully orthogonal protecting groups that permits the elongation of all three pyridine substituents.

Introduction

Polyheterocyclic scaffolds containing thiazole and oxazole rings are common to numerous biologically active natural products,[1] such as thiopeptide antibiotics,[2,3] which have garnered much attention during the past few years owing to their therapeutic utility and challenging synthesis.[2b,3] Thiopeptide antibiotics are classified according to structure,[2a] and those bearing a fully unsaturated 2,3,6-trisubstituted pyridine outnumber those with different polyheterocyclic cores (Figure 1).

Over the past few decades, various research groups have endeavored to synthesize the pyridine core of thiopeptides by developing two general procedures: modification of prefunctionalized pyridines, and late-stage construction of the pyridine ring.[2b] Current advances in cross-coupling methodologies have enabled the facile preparation of aryl-substituted thiazoles.[4] However, during our investigations towards the total synthesis of baringolin,[5] we found that more alternative cross-coupling methodologies and strategies are in demand to broaden the scope of starting materials and functionalities that can be used to assemble the polyheterocyclic cores of thiopeptides with the double aim to synthesize natural compounds as well as to develop analogue programs for structure-activity relationship studies.

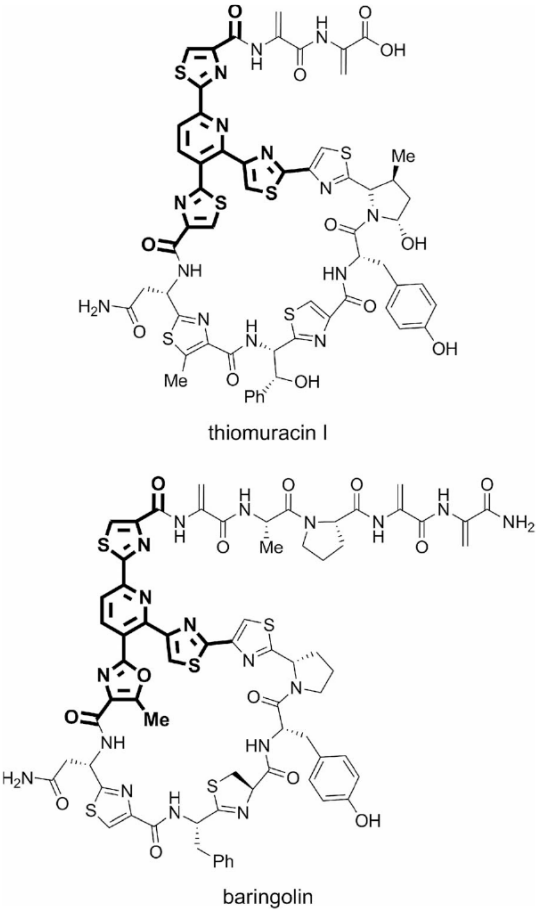
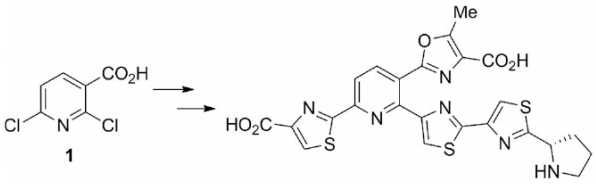


Figure 1. Examples of thiopeptides containing a 2,3,6-trisubstituted pyridine core (highlighted in bold).

[a] Institute for Research in Biomedicine, Barcelona Science Park – University of Barcelona,
Baldiri Reixac 10, 08028 Barcelona, Spain
E-mail: mercedes.alvarez@irbbarcelona.org
Homepage: http://www.pcb.ub.edu/fama
[b] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine,
08028 Barcelona, Spain
[c] Department of Organic Chemistry, University of Barcelona,
08028 Barcelona, Spain
[d] School of Chemistry, University of KwaZulu-Natal,
4001 Durban, South Africa
[e] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona,
08028 Barcelona, Spain

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201300877.

2,6-Dichloronicotinic acid (**1**) has been widely used as the starting material for the synthesis of many drugs.[6] Pyridine **1** already has the required substitution pattern, which should permit the formation of the oxazole ring in its position 3 and also serve for the subsequent cross-coupling reactions with the appropriate thiazole fragments in positions 2 and 6 (Scheme 1).

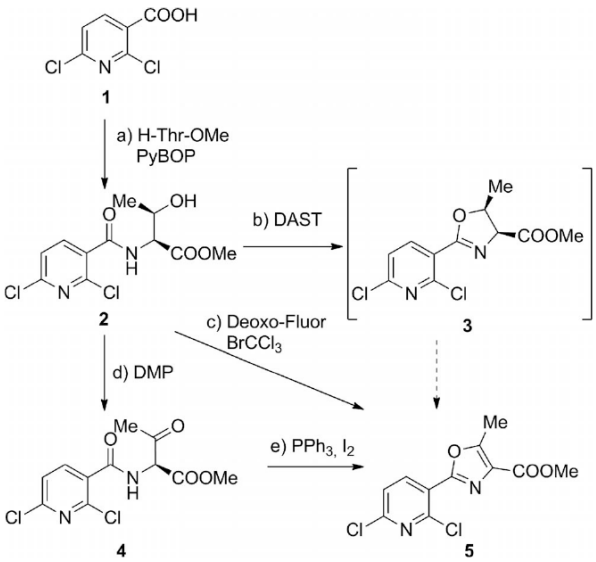


Scheme 1. Synthesis of baringolin's polyheterocyclic core from **1**.

The scope, limitations and applications of this strategy to the synthesis of the tri-azole-substituted pyridine core of thiopeptides are discussed in this paper. With the total synthesis of thiopeptides in mind, special attention was paid to the preparation of their central cores with a suitable set of protecting groups, which permits the elongation of the thiazole chains. This modular approach was applied for the syntheses of several core analogues.

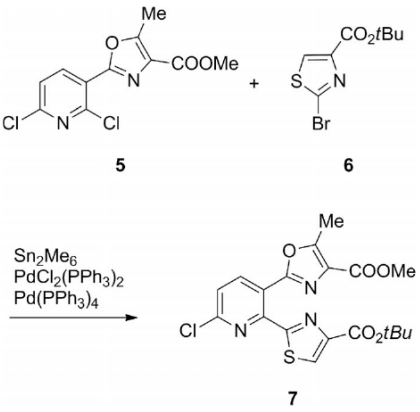
Results and Discussion

To study the required cross-coupling reactions on a suitable substrate, formation of the methyloxazole ring in position 3 of pyridine **1** was carried out first, following a biomimetic strategy (Scheme 2). After condensation of **1** with threonine methyl ester (H-Thr-OMe) to yield amide **2**, a two-step cyclization/oxidation approach was attempted. However, the resulting oxazoline **3** was never isolated as a pure substance, but always in combination with varying amounts of the starting material, pointing out the reversibility of the cyclization. Hence, an alternative one-step route by using deoxo-fluor and bromotrichloromethane was taken into consideration.[7] Despite the convenience of a one-step procedure, the low yield obtained thereby moved us to a third sequence based on a two-step oxidation/cyclization formation of the oxazole. Oxidation of the Thr residue to the corresponding methyl ketone **4** and cyclization in the presence of triphenylphosphane and iodine rendered the desired methyloxazole **5** in excellent overall yield.[8]



Scheme 2. Synthesis of oxazolylpyridine **5**. Reagents and conditions: (a) (L)-H-Thr-OMe, PyBOP, DIPEA, THF, 0 °C, 5 h, 94%. (b) Diethylaminosulfur trifluoride (DAST), K_2CO_3, CH_2Cl_2, –78 °C to 0 °C, 7 h, 61%. (c) Deoxo-Fluor, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 3.5 h, 39%. (d) Dess–Martin periodinane, CH_2Cl_2, room temp., 6 h, 87%. (e) PPh_3, I_2, Et_3N, CH_2Cl_2, 0 °C to room temp., 16 h, 94%.

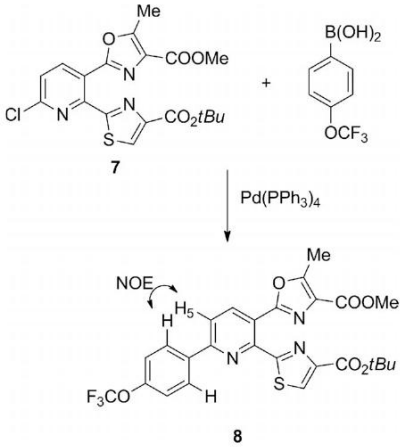
With **5** in hand, introduction of a thiazol-2-yl substituent was assessed first. Given the difficulty in preparing 2-stannylthiazole-4-carboxylate reagents,[9] alternatives to these reagents have been found through the use of a one-pot stannation-coupling protocols to obtain the desired cross-coupling product.[10] This methodology and its regioselectivity was assessed by using bromothiazole **6** as coupling partner and different distannanes (Scheme 3). After screening several variables, including the use of additives and different distannanes, the best conditions, as outlined in Scheme 3, only yielded small amounts of product **7**. NMR and MS spectroscopic data correspond to structure **7**; however with this data it was impossible to determine which regioisomer had been obtained.



Scheme 3. One-pot stannation-coupling reaction between **5** and **6**. Reagents and conditions: Sn_2Me_6, $PdCl_2(PPh_3)_2$, $Pd(PPh_3)_4$, 1,4-dioxane, 100 °C, 17%.

Despite the low yields obtained with the cross-coupling procedure, **7** was further converted into derivative **8** to determine the regioselectivity of the reaction. The NOE correlation observed between the proton in position 5 of the pyridine and the *ortho* protons of the phenyl substituent revealed that the thiazole was linked to position 2 of the pyridine (Scheme 4).

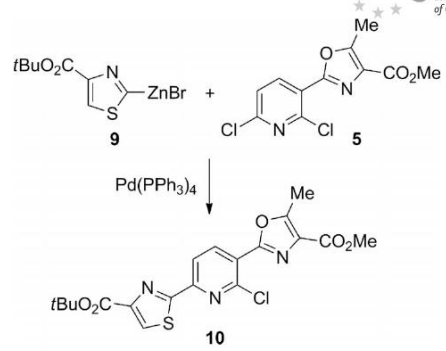
The next attempt focused on the use of thiazolezinc derivative **9**, which has been reported to selectively react with 3-substituted 2,6-dibromopyridines in position 6 under palladium(II) catalysis.[3f] Very small conversions were obtained with pyridine **5** and only when palladium(0) was used (Scheme 5). ^1H NMR spectroscopic analysis[11] of the



Scheme 4. Derivatization of **7**. Reagents and conditions: 4-(CF_3O)-C_6H_4-$B(OH)_2$, $Pd(PPh_3)_4$, 2 M $NaHCO_3$, *i*PrOH, 90 °C MW, 20 min, 65%.

product obtained with this methodology showed a different regioisomer (**10**) to the one obtained with the previous stannation-coupling approach (see Supporting Information).

Although the desired isomer did form, the low conversion and complex crude mixtures obtained with this approach did not allow reliable development of a robust methodology. At this point, Stille cross-coupling with trimethyltinbithiazole **11a**[12] was attempted to evaluate whether higher yields and selectivity could be achieved (Scheme 6). However, although conversions were higher, no regioselectivity was observed and only a 1:1 mixture of both possible regioisomers was obtained.

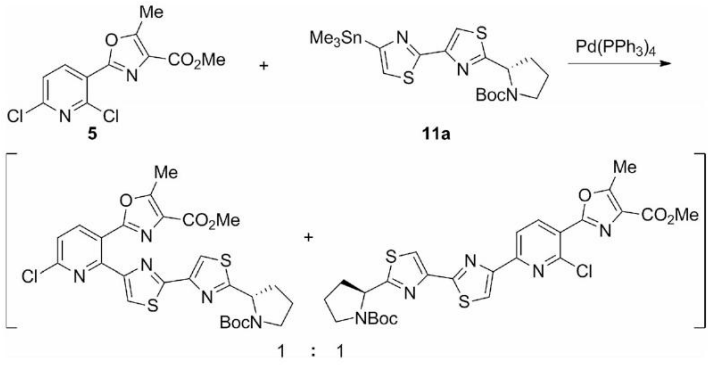


Scheme 5. Negishi cross-coupling between **9** and **5**. Reagents and conditions: $Pd(PPh_3)_4$, DMA, 60 °C, 5 d, 3%.

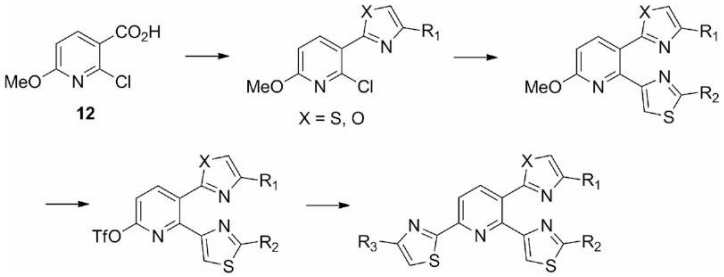
To overcome the previous regioselectivity problems, early differentiation of the two alpha positions of 2,6-dichloronicotinc acid (**1**) might be the answer. Nucleophilic aromatic substitution (S_NAr) with in situ generated methoxide gives 2-chloro-6-methoxynicotinic acid (**12**) in good yield and regioselectivity.[6h]

Starting from **12**, a general strategy to construct an azole from a carboxylic acid moiety, in the same fashion as described for the synthesis of **5**, was envisioned. Next, cross coupling at the chlorinated position and subsequent conversion of the methoxy group into a triflate would render the required leaving group for a final cross-coupling step (Scheme 7). Such a sequential route would avoid any possible regioselectivity problems.

The cornerstone of this synthetic route was the Negishi cross coupling between a triflate and a thiazolylzinc brom-

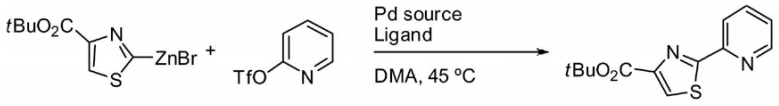


Scheme 6. Cross-coupling of **5** with bithiazole **11a**. Reagents and conditions: $Pd(PPh_3)_4$, 1,4-dioxane, 80 °C, 5 h, 51%.

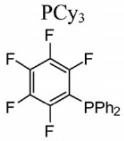
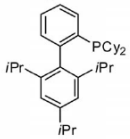
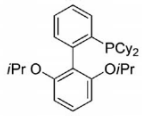
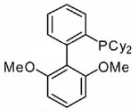
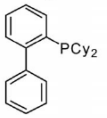


Scheme 7. Synthetic route towards polyazole-substituted pyridines from nicotinic acid **12**.

Table 1. Cross-coupling of thiazolylzinc bromide **9** with triflate **13.** Conditions: **9** (1.0 mmol, 2 equiv.), **13** (0.5 mmol, 1 equiv.), palladium source (20 mol-%), ligand (40 mol-%), DMA, 45 °C.



9 **13** **14**

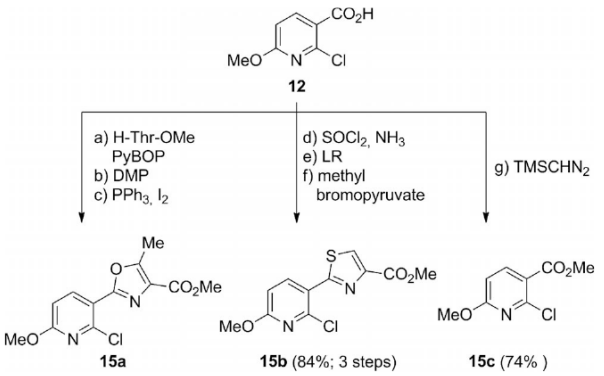
Entry	Pd source	Ligand	Pd (mol-%)	Time (h)	Yield[a] (%)
1	$PdCl_2(PPh_3)_2$	-	20	1.5	0
2	$Pd(PPh_3)_4$	-	1	17	12
3	$Pd(PPh_3)_4$	-	5	3	76
4	$Pd(PPh_3)_4$	-	10	3	84
5	$Pd(PPh_3)_4$	-	20	1.5	100
6	$Pd(dba)_2$	PCy_3	20	24	20
7	$Pd(dba)_2$		20	17	46
8	$Pd(dba)_2$		20	17	33
9	$Pd(dba)_2$		20	17	44
10	$Pd(dba)_2$		20	17	67
11	$Pd(dba)_2$		20	17	63
12	$Pd(dba)_2$	PPh_3	20	1.5	80

[a] Yield determined by HPLC analysis of the reaction mixture.

ide. This last step was previously studied with compound **9** and pyridyl triflate **13** as model system (Table 1). Whereas $PdCl_2(PPh_3)_2$ did not work (Table 1, Entry 1), the use of increasing amounts of $Pd(PPh_3)_4$ did give progressively higher yields of **14** (Table 1, Entries 2–5). Because quantitative conversion was only observed when 20 mol-% of $Pd(PPh_3)_4$ was used, other ligands were tested to find one with a comparable outcome (Table 1, Entries 6–11). Interestingly, triphenylphosphane (Table 1, Entry 12) performed better than any of the other ligands (Table 1, Entries 6–11).

With a reliable methodology for cross coupling of thiazolylzinc bromide **9** with pyridin-2-yl triflates in hand, the construction of the desired fragments was addressed. Starting from 2-chloro-6-methoxynicotinc acid (**12**) it was easy to reproduce the construction of the oxazole ring by using the carboxylic acid in the same two-step oxidation/cyclization sequence to obtain **15a** (Scheme 8). In parallel, thiazolyl-pyridine **15b** was prepared in excellent yield through classical conversion of acid **12** into the corresponding thioamide, followed by Hantzsch thiazole formation with ethyl bromopyruvate. Esterification of **12** with (trimethylsilyl)diazomethane gave **15c**. With these different scaffolds **15a**–**15c** the scope of the strategy was tested.

First, cross coupling between 4-trialkyltinthiazoles **11a**–**11b** and 2-chloro-6-methoxypyridines **15a**–**15c** was evaluated (Table 2). Moderate to excellent yields of methoxypyridines **16aa**–**16cb** were obtained depending on the partner combination. The yields obtained with tributyltinthiazole **11b**[13] (Table 2, Entries 2, 4 and 6) were lower than those obtained with trimethyltinbithiazole **11a** (Table 2, Entries 1, 3 and 5). This discrimination caused either by the different bulkiness of alkyltin groups or the different electronic nature of the substituents in position 2 of the thiazole ring. Nicotinate **15c** (Table 2, Entries 5 and 6) performed slightly better than the other pyridines, owing to the electro-withdrawing effect of the carboxylate. The yields obtained with



Scheme 8. Derivatization of 2-chloro-6-methoxynicotinic acid (**12**). Reagents and conditions: (a) H-Thr-OMe (1 equiv.), PyBOP (1.2 equiv.), DIPEA (3.5 equiv.), THF, 0 °C, 5 h, 82%. (b) Dess–Martin periodinane (1.2 equiv.), CH_2Cl_2, room temp., 7 h, 86%. (c) PPh_3 (2 equiv.), I_2 (2 equiv.), NEt_3 (4 equiv.), CH_2Cl_2, 0 °C to room temp., 15 h, 85% (d) (i) $SOCl_2$ (10 equiv.), reflux, 1 h; (ii) NH_4OH, THF, 0 °C, 1 h, 96% (2 steps). (e) Lawesson's Reagent (LR; 1 equiv.), THF, 70 °C, 2 h, 88%. (f) Methyl bromopyruvate (2 equiv.), pyridine (1.5 equiv.), EtOH, 80 °C, 3.5 h, quant. (g) $TMSCHN_2$ (1.8 equiv.), CH_2Cl_2/MeOH (1:1), 0 °C to room temp., 20 min, 74%.

oxazolylpyridine **15a** (Table 2, Entries 1 and 2) were higher than those obtained with thiazolylpyridine **15b** (Table 2, Entries 3 and 4).

All methoxypyridines **16aa**–**16cb** obtained were demethylated with HBr to yield the corresponding pyridones. This treatment also caused acetal hydrolysis or *tert*-butyloxycarbonyl (Boc) removal. In the latter case further pyrrolidine protection was required. All pyridone analogues were easily converted into corresponding triflates **17aa**–**17cb** by using triflic anhydride under 4-(dimethylamino)pyridine (DMAP) catalysis.[14] Lastly, palladium(0) catalyzed cross-coupling of **17aa**–**17cb** with thiazolezinc bromide **9** yielded expected polyazolepyridines **18aa**–**18cb** (Table 3). The presence of the methyl ketone (Table 3, Entries 2, 4 and 6) was detrimental for the reaction outcome. For the synthesis of baringolin's core **18aa**, the use of higher amounts of **9** rendered the desired polyheterocycle in excellent yield (Table 3, Entry 1).

After developing a useful strategy to synthesize azole-substituted pyridines, the next goal was selective removal of protecting groups to develop a reliable method for the total synthesis of thiopeptides. Compound **18aa** was the substrate of choice because it was part of our studies towards the total synthesis of baringolin.[5b] Our first attempts of Boc deprotection in **18aa** in the presence of a *tert*-butyl ester yielded the desired amine in excellent yield when literature protocols were used.[15] However, scaling up of this reaction resulted in reduced selectivities and therefore switching to alternative protecting groups patterns was required.

To circumvent the compatibility of protecting groups, the ester at the thiazole ring was changed. To do so, ethyl ester **19**[10] was converted into isopropyl ester **20** (Scheme 9). Both **19** and **20** could be used as precursors of organometallic thiazol-2-yl nucleophiles and appropriately converted into corresponding zinc bromides **21** and **22**, respectively, for subsequent use in the final cross coupling with pyridyl triflate **17aa** to yield **23** and **24** (Scheme 10). All our attempts to selectively deprotect the methyl ester in either **23** or **24** failed with trimethyltin hydroxide.[16] In both cases, mixtures of carboxylic acids were obtained even when equimolar amounts of reagent were used.

At this point, and given the previous bad results obtained for the selective removal of the esters, the need for a fully orthogonal pattern of protecting groups became evident. To this end, the polyheterocyclic core was synthesized again with a benzyl ester on the oxazole and an ethyl ester on the thiazole by using appropriately protected starting materials, giving rise to **25**.[5b]

Many attempts of benzyl ester hydrogenolysis of **25** with varying amounts of palladium on charcoal at different temperatures and increasing H_2 pressures only yielded traces of the acid. Only when palladium black was used, higher conversions were observed (Scheme 11). However, 100 wt.-% of this reagent was required for quantitative conversion to occur. Acid **26** was used in further transformations, which yielded the expected products.[5b] The use of palladium black with a similar substrate has also been described by Moody and co-workers,[3d] however, no details of previously performed tests are provided.

Conclusions

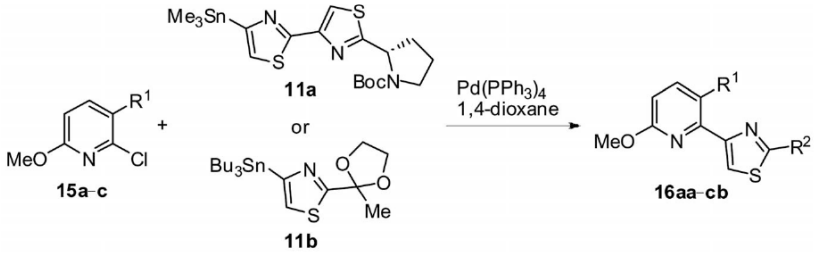
In summary, studies towards the synthesis of various azole-substituted pyridines have been carried out by using 2,6-dicholoronicotinic acid (**1**) and 2-chloro-6-methoxynicotinic acid (**12**) as starting points. The best results were obtained with **12** owing to the differentiated α-positions and the higher reactivity of thiazolzinc bromides with pyridyl triflates. With the present strategy, a series of polyazole-substituted pyridines **18aa**–**18cb** were synthesized, which demonstrated the scope of the approach, which could also be used for the construction of various thiopeptides analogues (GE2270 A and T, thiomuracins, GE37468A, baringolin, etc.).[5a,17] This approach also permitted the synthesis of the polyheterocyclic core of baringolin, which has been synthesized with different combinations of protecting groups. Subsequent compatibility studies have facilitated the assessment of different chemistries. Finally, a set of orthogonal protecting groups was used to further modify baringolin's core in a fully selective and reliable manner. This combination of protecting groups could be useful for the total syntheses of other thiopeptides containing a fully unsaturated 2,3,6-trisubstituted pyridine core.

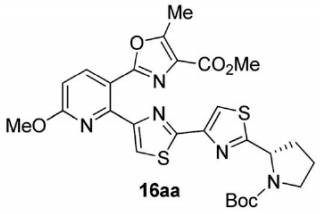
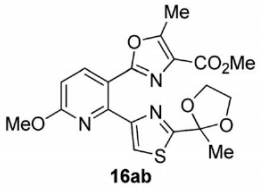
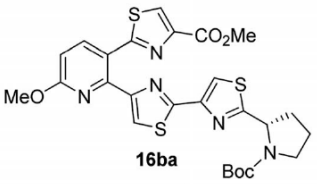
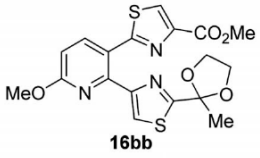
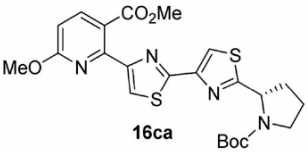
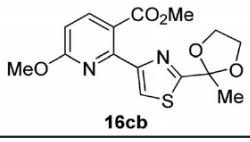
Experimental Section

See Supporting Information for general procedures.

(2*S*,3*R*)-*N*-[(2,6-Dichloropyridin-3-yl)carbonyl]threonine Methyl Ester (2): *N*,*N*-Diisopropylethylamine (DIPEA; 2.66 mL, 15.51 mmol) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP; 2.65 g, 5.09 mmol) were added to a

Table 2. Cross-coupling between pyridines **15a**–**15c** and alkyltin thiazoles **11a** and **11b**. Conditions: Chloropyridine **15** (1 equiv.), alkyltin-thiazole **11** (1.1 equiv.), $Pd(PPh_3)_4$ (10 mol-%), 1,4-dioxane, 80 °C.

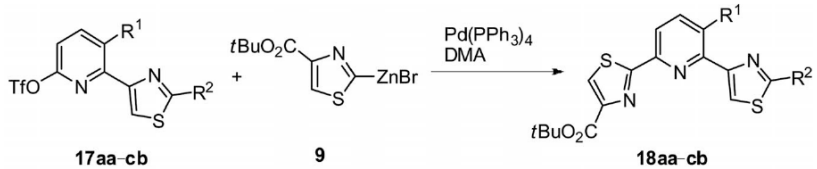


Entry	15	11	Product	Yield (%)[a]
1	**15a**	**11a**	 **16aa**	85
2		**11b**	 **16ab**	53
3	**15b**	**11a**	 **16ba**	56
4		**11b**	 **16bb**	44
5	**15c**	**11a**	 **16ca**	94
6		**11b**	 **16cb**	57

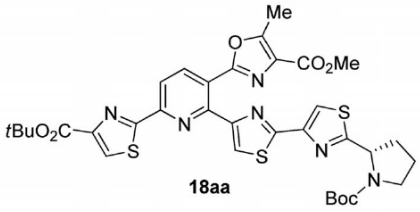
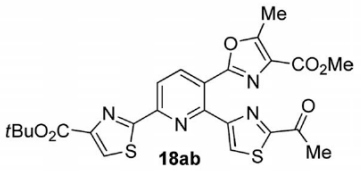
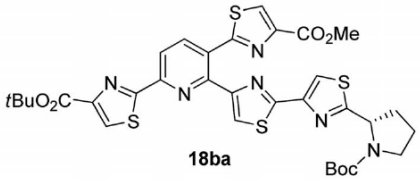
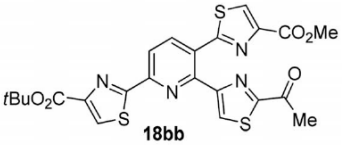
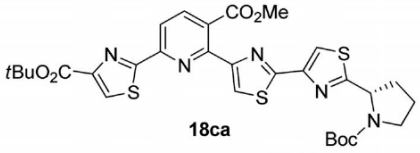
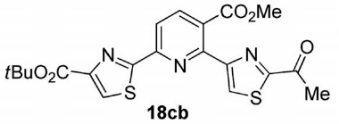
[a] Isolated yield.

solution of 2,6-dichloronicotinic acid (**1**; 850 mg, 4.43 mmol) and H-L-Thr-OMe (751 mg, 4.43 mmol) in dry tetrahydrofuran (THF; 22 mL) at 0 °C under argon. The mixture was then stirred at this temperature for 5 h. EtOAc and saturated aq. $NaHCO_3$ were added to the mixture and part of the solvent was evaporated under reduced pressure. More EtOAc (100 mL) was added and the organic layer was then washed with saturated aq. $NaHCO_3$ (50 mL) and saturated aq. NH_4Cl (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1) to yield the title compound as a pale solid (1.28 g, 94%), m.p. (EtOAc) 128–130 °C. $[\alpha]_D$ = 20.6 (c = 0.50, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3433, 3296, 3073, 2985, 2920, 1744, 1643, 1577, 844 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.35 (d, J = 6.4 Hz, 3

Table 3. Cross-coupling between pyridines **17aa–17cb** and thiazole **9**. Conditions: triflate **17** (1 equiv.), thiazolezinc bromide **9** (2 equiv.), $Pd(PPh_3)_4$ (20 mol-%), DMA, 45 °C.



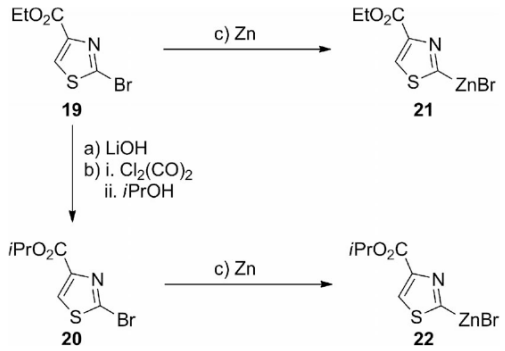
17aa–cb **9** **18aa–cb**

Entry	17	Product	Yield (%)[a]
1	**17aa**	 **18aa**	37 (92)[b]
2	**17ab**	 **18ab**	43
3	**17ba**	 **18ba**	54
4	**17bb**	 **18bb**	41
5	**17ca**	 **18ca**	66
6	**17cb**	 **18cb**	39

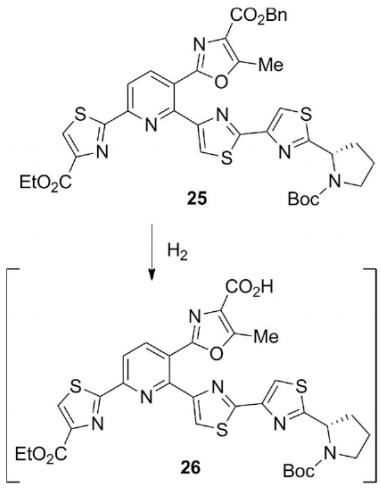
[a] Isolated yield. [b] Triflate **17aa** (1 equiv.), thiazolezinc bromide **9** (8 equiv.), $Pd(PPh_3)_4$ (20 mol-%), DMA, 45 °C.

H), 3.82 (s, 3 H), 4.50 (dq, J = 6.4 and 2.3 Hz, 1 H), 4.80 (dd, J = 8.6 and 2.3 Hz, 1 H), 7.39 (d, J = 8.0 Hz, 1 H), 8.12 (d, J = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.5 (q), 53.1 (q), 58.2 (d), 68.1 (d), 123.7 (d), 129.5 (s), 142.3 (d), 146.8 (s), 152.3 (s), 164.5 (s), 171.0 (s) ppm. HRMS: calcd. for $C_{11}H_{13}Cl_2N_2O_4$ [M + H] 307.0247; found 307.0247.

Methyl (*S*)-2-(2,6-Dichloronicotamido)-3-oxobutanoate (4): Dess–Martin periodinane (1.91 g, 4.50 mmol) was added to a solution of pyridine **2** (1.15 g, 3.75 mmol) in dry CH_2Cl_2 (125 mL). The mixture was then stirred at room temp. under argon. After 7 h the reaction mixture was poured onto a mixture of saturated aq. $NaHCO_3$ (100 mL) and saturated aq. $Na_2S_2O_3$ (100 mL) and extracted with CH_2Cl_2 (2× 100 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1) to yield the title compound as a white solid (0.99 g, 87%), m.p. (EtOAc) 112–113 °C. $[\alpha]_D$ = –0.3 (c = 1.01, $CHCl_3$). IR (KBr): $\tilde{\nu}$ = 3290, 3072, 2958, 1740, 1721, 1642, 1578 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 2.48 (s, 3 H), 3.89 (s, 3 H), 5.43 (d, J = 6.2 Hz, 1 H), 7.40 (d, J = 7.8 Hz, 1 H), 7.90 (d, J = 6.2 Hz, 1 H), 8.16 (d, J = 7.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 (q), 53.9 (q), 64.1 (d), 123.7 (d), 128.4 (s), 142.7 (d), 147.1 (s), 152.7 (s), 163.3 (s), 166.1 (s), 197.5 (s) ppm.



Scheme 9. Synthesis of bromothiazole **20** and preparation of organozinc reagents **21** and **22**. Reagents and conditions: (a) **19** (1 equiv.), LiOH (2 equiv.), THF/H_2O (10:1), room temp., 18 h, quant. (b) (i) Oxalyl chloride (2 equiv.), DMF (cat.); (ii) *i*PrOH/CH_2Cl_2 (10:1), 81%. (c) zinc dust, 1,2-dibromoethane, TMSCl, DMA, room temp.



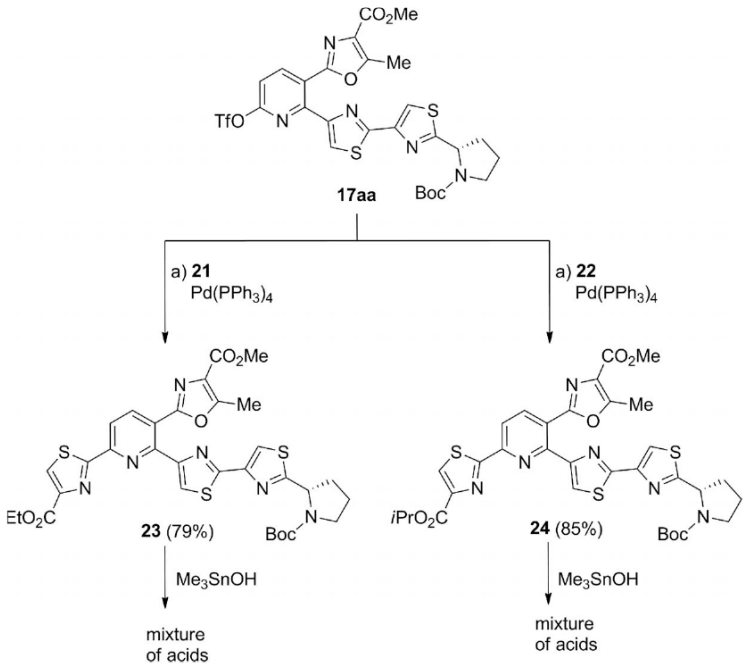
Scheme 11. Hydrogenolysis of **25**. Reagents and conditions: H_2 (1 atm), Pd black (100 wt.-%), CH_2Cl_2/EtOH (1:1), room temp., 4 h, quant.

HRMS: calcd. for $C_{11}H_{11}Cl_2N_2O_4$ [M + H] 305.0090; found 305.0090.

Synthesis of 2,6-Dichloro-3-[4-(methoxycarbonyl)-5-methyloxazole-2-yl]pyridine (5)

From Pyridine 2: Deoxo-Fluor 50% in THF (76 µL, 0.18 mmol) was added to a stirring solution of pyridine **2** (50 mg, 0.16 mmol) in dry CH_2Cl_2 (1.4 mL) at –20 °C under N_2. After 30 min, $BrCCl_3$ (58 µL, 0.58 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 87 µL, 0.58 mmol) were added dropwise and then the mixture stirred for 3.5 h at 0 °C. Saturated aq. $NaHCO_3$ (25 mL) was added and the aqueous layer extracted with CH_2Cl_2 (3× 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by silica flash column chromatography (hex/EtOAc, 8:2) to yield the title compound as a white solid (24 mg, 50%).

From Pyridine 4: A solution of pyridine **4** (0.97 g, 3.16 mmol) in dry CH_2Cl_2 (38 mL) was added to a stirring solution of PPh_3 (1.65 g, 6.31 mmol) and I_2 (1.6 g, 6.31 mmol) in dry CH_2Cl_2 (58 mL) at 0 °C under an argon atmosphere. The resulting mixture was allowed to reach room temp. and stirred for 16 h. The solvent was removed under reduced pressure and the crude product purified by silica flash column chromatography (hexanes/EtOAc, 7:3) to yield the title compound as a white solid (0.85 g, 94%), m.p.



Scheme 10. Negishi cross-couplings of **17aa**. Reagents and conditions: (a) thiazolezinc bromide (8 equiv.), $Pd(PPh_3)_4$ (20 mol-%), DMA, 45 °C.

(EtOAc) 135–136 °C. IR (KBr): $\tilde{\nu}$ = 3087, 2957, 1715, 1609, 1562, 1427, 1351 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 2.75 (s, 3 H), 3.96 (s, 3 H), 7.39 (d, J = 8.2 Hz, 1 H), 8.36 (d, J = 8.2 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.5 (q), 52.4 (q), 121.8 (s), 123.3 (d), 129.2 (s), 141.9 (d), 148.2 (s), 151.9 (s), 155.5 (s), 157.9 (s), 162.5 (s) ppm. HRMS: calcd. for $C_{11}H_9Cl_2N_2O_3$ [M + H] 286.9985; found 286.9985.

2-[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]-6-chloro-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (7): Hexamethyldistannane (195 µL, 0.95 mmol) was added to a stirring solution of bromothiazole **6**[3f] (166 mg, 0.63 mmol), dichloropyridine **5** (273 mg, 0.95 mmol), $PdCl_2(PPh_3)_2$ (13 mg, 0.03 mmol) and $Pd(PPh_3)_4$ (37 mg, 0.03 mmol) in dry 1,4-dioxane (7.6 mL) under N_2 in a Schlenk tube. The tube was sealed with a glass stopper and the mixture stirred at 100 °C. After 4 h the mixture was allowed to reach room temp., filtered through a pad of Celite, washed with EtOAc, dried (Na_2SO_4) and concentrated in vacuo. The resulting crude was purified by silica flash column chromatography (hexane/EtOAc, 7:3) to yield the title product as a pale solid (46 mg, 17%), m.p. (EtOAc) 175–180 °C. IR (KBr): $\tilde{\nu}$ = 3111, 2978, 1707, 1620, 1437, 1352, 1163, 1102, 1007 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.52 (s, 9 H), 2.67 (s, 3 H), 3.93 (s, 3 H), 7.46 (d, J = 8.0 Hz, 1 H), 8.05 (d, J = 8.0 Hz, 1 H), 8.20 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.3 (q), 28.2 (q), 52.1 (q), 82.1 (s), 120.2 (s), 124.8 (d), 128.7 (s), 130.0 (d), 141.0 (s), 142.3 (d), 150.1 (s), 152.6 (s), 156.8 (s), 157.7 (s), 160.4 (s), 162.9 (s), 165.4 (s) ppm. HRMS: calcd. for $C_{19}H_{19}ClN_3O_5S$ [M + H] 436.0729; found 436.0727.

2-[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-[4-(trifluoromethoxy)phenyl]pyridine (8): A solution of pyridine **7** (30 mg, 0.07 mmol), 4-(trifluoromethoxy)-phenylboronic acid (17 mg, 0.08 mmol) and $Pd(PPh_3)_4$ (4 mg, 0.003 mmol) in *i*PrOH (0.35 mL) and $NaHCO_3$ (2 M, 0.1 mL) was stirred at 90 °C for 20 min under microwave irradiation. The reaction mixture was diluted with EtOAc, filtered through Celite, dried (Na_2SO_4) and concentrated in vacuo. Silica flash column chromatography (hexane/EtOAc, 7:3) yielded the title compound as a yellowish solid (25 mg, 65%), m.p. (EtOAc) 160–161 °C. IR (KBr): $\tilde{\nu}$ = 3123, 2974, 1724, 1699, 1254, 1169 cm^{-1}. ^1H NMR (400 MHz, [D_6]acetone): δ = 1.52 (s, 9 H), 2.66 (s, 3 H), 3.88 (s, 3 H), 7.55 (m, 2 H), 8.25 (d, J = 8.0 Hz, 1 H), 8.27 (d, J = 8.0 Hz, 1 H), 8.42 (m, 2 H), 8.46 (s, 1 H) ppm. ^{13}C NMR (100 MHz, [D_6]-acetone): δ = 11.6 (q), 27.5 (q), 51.1 (q), 81.2 (s), 120.3 (s), 121.0 (d), 121.5 (d), 128.7 (s), 129.3 (d), 130.3 (d), 136.3 (s), 141.4 (d), 148.9 (s), 149.8 (s), 150.8 (s), 156.6 (s), 157.2 (s), 157.3 (s), 160.2 (s), 162.7 (s), 167.2 (s) ppm. HRMS: calcd. for $C_{26}H_{23}F_3N_3O_6S$ [M + H] 562.1254; found 562.1251.

General Procedure for the Preparation of (Thiazol-2-yl)zinc Bromides: These reagents were prepared by using the method described by Bach and co-workers.[3f] Dry *N*,*N*-dimethylacetamide (DMA) and 1,2-dibromoethane (0.36 equiv.) were added to an oven-dried flask charged with zinc dust (3.12 equiv.) under an inert atmosphere. The mixture was heated with a heat gun until bubbling was observed and then cooled to room temp. This procedure was repeated twice and then chlorotrimethylsilane (0.66 equiv.) was added and the suspension stirred at room temp. After 5 min a solution of the alkyl 2-bromothiazole-4-carboxylate (1.00 equiv.) in dry DMA was added. After 30 min the suspension was allowed to settle for at least 30 min. The reagent solution was freshly prepared before each use.

[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]zinc Bromide (9): This reaction was performed according to the general procedure for the preparation of (thiazol-2-yl)zinc bromides by using zinc dust (920 mg, 14.00 mmol), 1,2-dibromoethane (140 µL, 1.60 mmol) and chlorotrimethylsilane (380 µL, 3.00 mmol) in DMA (14.0 mL) and a solution of *tert*-butyl 2-bromothiazole-4-carboxylate[3f] (1.20 g, 4.50 mmol) in DMA (7.2 mL) This procedure gave a 0.21 M solution of the reagent.

[4-(Ethoxycarbonyl)thiazol-2-yl]zinc Bromide (21): This reaction was performed according to the general procedure for the preparation of (thiazol-2-yl)zinc bromides by using zinc dust (4.04 g, 61.77 mmol), 1,2-dibromoethane (615 µL, 7.13 mmol) and chlorotrimethylsilane (1.7 mL, 13.07 mmol) in DMA (62 mL) and a solution of ethyl 2-bromothiazole-4-carboxylate (**19**)[10] (4.67 g, 19.80 mmol) in DMA (32 mL) This procedure gave a 0.21 M solution of the reagent.

[4-(Isopropoxycarbonyl)thiazol-2-yl]zinc Bromide (22): This reaction was performed according to the general procedure for the preparation of (thiazol-2-yl)zinc bromides by using zinc dust (734 mg, 11.23 mmol), 1,2-dibromoethane (112 µL, 1.30 mmol) and chlorotrimethylsilane (300 µL, 2.38 mmol) in DMA (11.4 mL) and a solution of **20** (900 mg, 3.60 mmol) in DMA (5.7 mL) This procedure gave a 0.21 M solution of the reagent.

6-[4-(*tert*-Butoxycarbonyl)thiazol-2-yl]-2-chloro-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (10): A solution of thiazole **9** in dry DMA (0.21 M, 0.92 mL, 0.19 mmol) was slowly added to a solution of pyridine **5** (50 mg, 0.17 mmol) and $Pd(PPh_3)_4$ (10 mg, 0.009 mmol) in dry DMA (0.5 mL) under nitrogen in a Schlenk tube. The tube was sealed and the mixture stirred at 60 °C for 5 d. The mixture was filtered through a pad of Celite, dried ($MgSO_4$) and concentrated in vacuo. The resulting crude product was purified by using a C18 chromatography column. A gradient of H_2O (0.1% TFA)/MeCN (0.1% TFA) from 8:2 to 1:9 yielded the product as a white solid (2 mg, 3%). ^1H NMR (400 MHz, $CDCl_3$): δ = 1.64 (s, 9 H), 2.77 (s, 3 H), 3.97 (s, 3 H), 8.21 (s, 1 H), 8.36 (d, J = 8.0 Hz, 1 H), 8.51 (d, J = 8.0 Hz, 1 H) ppm. HRMS: calcd. for $C_{19}H_{19}ClN_3O_5S$ [M + H] 436.0729; found 436.0738.

***tert*-Butyl 2-(Pyridin-2-yl)thiazole-4-carboxylate (14):** A solution of **9** (0.21 M, 4.8 mL, 1.0 mmol, 2 equiv.) was added to a Schlenk tube charged with the palladium species (20 mol-%) and the indicated phosphane (40 mol-%) under a nitrogen atmosphere. The mixture was pre-stirred at 45 °C and then treated with a solution of pyridin-2-yl trifluromethanesulfonate (**13**;[18] 114 mg, 0.5 mmol, 1 equiv.) in DMA (6 mL). The tube was sealed and the mixture stirred at 45 °C. The reaction mixture was analyzed by HPLC to determine the conversion of **13** to **14** according to previously prepared standards of these two compounds. Pure **14** was obtained after running the reaction with $Pd(PPh_3)_4$ (20 mol-%). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 8:2) to yield the title compound as a white solid (99 mg, 75%), m.p. (Et_2O) 89–93 °C. IR (KBr): $\tilde{\nu}$ = 2978, 2931, 1726, 1161 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.64 (s, 9 H), 7.35 (ddd, J = 7.5, 4.8 and 1.2 Hz, 1 H), 7.82 (ddd, J = 8.0, 7.5 and 1.7 Hz, 1 H), 8.13 (s, 1 H), 8.34 (d, J = 8.0 Hz, 1 H), 8.61 (dm, J = 4.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (q), 82.3 (s), 120.5 (d), 125.2 (d), 128.8 (d), 137.4 (d), 149.5 (d), 149.8 (s), 150.8 (s), 160.7 (s), 169.7 (s) ppm. HRMS: calcd. for $C_{13}H_{15}O_2N_2S$ [M + H] 263.0849; found 263.0852. The product obtained matched that described in the literature.[13]

Synthesis of 2-Chloro-6-methoxy-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (15a):

(2*S*,3*R*)-*N*-[(2-Chloro-6-methoxypyridin-3-yl)carbonyl]threonine Methyl Ester: DIPEA (3.2 mL, 18.66 mmol) and PyBOP (3.19 g,

6.13 mmol) were added to a solution of 2-chloro-6-methoxynicotinic acid **12**[6h] (1.00 g, 5.33 mmol) and H-L-Thr-OMe (904 mg, 5.33 mmol) in dry THF (27 mL) at 0 °C under argon, and the mixture was stirred for 5 h. EtOAc and saturated aq. $NaHCO_3$ were then added to the mixture, which was partially concentrated under reduced pressure. The resulting concentrate was poured onto saturated aq. $NaHCO_3$ (100 mL) and extracted with EtOAc (3× 100 mL). The combined organic extracts were washed with saturated aq. NH_4Cl (2× 50 mL), dried (Na_2SO_4) and then concentrated in vacuo. The crude product was purified by flash column chromatography (hexanes/EtOAc, 4:6) to yield the title compound as a white wax (1.33 g, 82%). $[\alpha]_D$ = +20.9 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3411, 2978, 2954, 1745, 1645, 1599, 1479, 1353, 1310 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.28 (d, *J* = 6.4 Hz, 3 H), 3.78 (s, 3 H), 3.96 (s, 3 H), 4.38 (qd, *J* = 6.4 and 3.2 Hz, 1 H), 4.64 (d, *J* = 3.2 Hz, 1 H), 6.82 (d, *J* = 8.4 Hz, 1 H), 7.88 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4 (q), 53.0 (q), 54.8 (q), 58.1 (d), 68.3 (d), 110.2 (d), 122.6 (s), 143.0 (d), 145.4 (s), 164.6 (s), 165.2 (s), 171.4 (s) ppm. HRMS: calcd. for $C_{12}H_{16}N_2O_5Cl$ [M + H] 303.0784; found 303.0742.

Methyl (*S*)-2-(2-Chloro-6-methoxynicotamido)-3-oxobutanoate: Dess–Martin periodinane (2.50 g, 5.90 mmol) was added to a solution of (2*S*,3*R*)-*N*-[(2-chloro-6-methoxypyridin-3-yl)carbonyl]threonine methyl ester (1.48 g, 4.92 mmol) in dry CH_2Cl_2 (80 mL). The mixture was then stirred at room temp. under argon. After 7 h the reaction mixture was poured onto a mixture of saturated aq. $NaHCO_3$ (100 mL) and saturated aq. $Na_2S_2O_3$ (100 mL) and then extracted with CH_2Cl_2 (2× 100 mL). The combined organic extracts were dried (Na_2SO_4) and then concentrated under reduced pressure. The crude product was purified by flash column chromatography (hexanes/EtOAc, 7:3) to yield the title compound as a white solid (1.27 g, 86%), m.p. (EtOAc) 92–96 °C. IR (KBr): $\tilde{\nu}$ = 3322, 2957, 1742, 1727, 1632, 1600, 1539, 1485, 1365, 1317, 1268 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.46 (s, 3 H), 3.87 (s, 3 H), 3.99 (s, 3 H), 5.42 (d, *J* = 5.8 Hz, 1 H), 6.75 (d, *J* = 8.4 Hz, 1 H), 8.02 (d, *J* = 5.8 Hz, 1 H), 8.14 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 (q), 53.7 (q), 54.8 (q), 64.2 (d), 110.2 (d), 121.5 (s), 143.1 (d), 145.8 (s), 164.1 (s), 164.8 (s), 166.5 (s), 198.1 (s) ppm. HRMS: calcd. for $C_{12}H_{14}ClN_2O_5$ [M + H] 301.0586; found 301.0585.

2-Chloro-6-methoxy-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-pyridine (15a): A solution of methyl (*S*)-2-(2-chloro-6-methoxynicotamido)-3-oxobutanoate (400 mg, 1.33 mmol) in dry CH_2Cl_2 (10 mL) was added to a stirring solution of PPh_3 (700 mg, 2.67 mmol) and I_2 (675 mg, 2.67 mmol) in dry CH_2Cl_2 (17 mL) at 0 °C under argon. The resulting mixture was allowed to reach room temp. and stirred for 15 h. The solvent was removed under reduced pressure and the crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3) to yield the title compound as a white solid (318 mg, 85%), m.p. (EtOAc) 131–133 °C. IR (KBr): $\tilde{\nu}$ = 2990, 2957, 1716, 1618, 1603, 1473, 1354 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.73 (s, 3 H), 3.95 (s, 3 H), 4.01 (s, 3 H), 6.76 (d, *J* = 8.4 Hz, 1 H), 8.22 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.4 (q), 52.3 (q), 54.7 (q), 109.9 (d), 115.6 (s), 128.7 (s), 142.1 (d), 157.1 (s), 162.9 (s), 164.3 (s) ppm. HRMS: calcd. for $C_{12}H_{12}ClN_2O_4$ [M + H] 283.0480; found 283.0479.

Synthesis of 2-Chloro-6-methoxy-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine (15b):

2-Chloro-6-methoxynicotinamide: A mixture of 2-chloro-6-methoxynicotinic acid **12**[6h] (1.00 g, 5.33 mmol) and thionyl chloride (3.9 mL, 53.31 mmol) was heated at reflux temperatures under nitrogen atmosphere for 5 h. After the mixture reached room temp., the excess thionyl chloride was removed under reduced pressure and the residue was dissolved in toluene. The volatiles were evaporated, more toluene was added and then the mixture was concentrated again. The resulting solid was dissolved in THF (10 mL) and cooled to 0 °C in an ice bath. An aq. solution of NH_3 (32%, 10 mL) was added and the mixture was stirred for 1 h. The THF was evaporated under reduced pressure and saturated aq. $NaHCO_3$ (40 mL) was added. The solution was extracted with CH_2Cl_2 (3× 50 mL), and the combined organic extracts were dried (Na_2SO_4) and then concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2). The title product was obtained as a white solid (863 mg, 96%), m.p. (CH_2Cl_2) 172–173 °C. IR (KBr): $\tilde{\nu}$ = 3350, 3179, 1656, 1598 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.99 (s, 3 H), 6.14 (br. s, 1 H), 6.76 (d, *J* = 8.4 Hz, 1 H), 6.82 (br. s, 1 H), 8.22 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 54.8 (q), 110.3 (d), 121.9 (s), 143.5 (d), 145.5 (s), 164.7 (s), 166.2 (s) ppm. HRMS: calcd. for $C_7H_8ClN_2O_2$ [M + H] 187.0274; found 187.0262.

2-Chloro-6-methoxynicotinthioamide: A solution of 2-chloro-6-methoxynicotinamide (794 mg, 4.26 mmol) and Lawesson's reagent (860 mg, 2.13 mmol) in dry THF (85 mL) was stirred at reflux temperatures for 2 h and then allowed to reach room temp. The volatiles were evaporated under reduced pressure and saturated aq. $NaHCO_3$ (150 mL) was added. The aqueous layer was extracted with CH_2Cl_2 (3× 100 mL), and the combined organic extracts were dried (Na_2SO_4), and then concentrated in vacuo. The crude product was purified by silica flash column chromatography (CH_2Cl_2). The title product was obtained as a pale solid (760 mg, 88%), m.p. (CH_2Cl_2) 158–161 °C. IR (KBr): $\tilde{\nu}$ = 3310, 3143, 1642, 1593, 1477, 1299 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.98 (s, 3 H), 6.71 (d, *J* = 8.4 Hz, 1 H), 7.52 (br. s, 1 H), 7.98 (br. s, 1 H), 8.22 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 54.8 (q), 110.1 (d), 128.4 (s), 142.0 (s), 144.1 (d), 164.3 (s), 199.3 (s) ppm. HRMS: calcd. for $C_7H_8ClN_2OS$ [M + H] 203.0046; found 203.0036.

2-Chloro-6-methoxy-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine (15b): Methyl bromopyruvate (750 µL, 7.05 mmol), and pyridine (430 µL, 5.28 mmol) were added to a solution of 2-chloro-6-methoxy-nicotinthioamide (714 mg, 3.52 mmol) in dry EtOH under a nitrogen atmosphere. The mixture was stirred at 80 °C for 3.5 h and then cooled down. Volatiles were evaporated under reduced pressure, and the resulting crude product was purified by silica flash column chromatography (CH_2Cl_2). The title product was obtained as a white solid (1.03 g, quant.), m.p. (CH_2Cl_2) 156–159 °C. IR (KBr): $\tilde{\nu}$ = 3120, 3021, 2955, 1749, 1600, 1351, 1268, 1218 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.98 (s, 3 H), 4.01 (s, 3 H), 6.81 (d, *J* = 8.8 Hz, 1 H), 8.27 (s, 1 H), 8.62 (d, *J* = 8.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 52.7 (q), 54.7 (q), 110.5 (d), 121.3 (s), 128.5 (d), 142.3 (d), 146.0 (s), 146.6 (s), 162.1 (s), 163.1 (s), 164.0 (s) ppm. HRMS: calcd. for $C_{11}H_{10}ClN_2O_3S$ [M + H] 285.0107; found 285.0101.

Methyl 2-Chloro-6-methoxynicotinate (15c): A solution of trimethylsilyldiazomethane ($TMSCHN_2$) in diethyl ether (2.0 M, 2.2 mL, 4.32 mmol) was added to a stirring solution of 2-chloro-6-methoxynicotinic acid **12**[6h] (450 mg, 2.40 mmol) in CH_2Cl_2 (12 mL) and MeOH (12 mL) at 0 °C. After 20 min the reaction was allowed to reach room temp. and then stirred for another 20 min. Solvents were removed under reduced pressure and the crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1). The title product was obtained as a white solid (359 mg, 74%), m.p. (EtOAc) 59–62 °C. IR (KBr): $\tilde{\nu}$ = 3087, 3006, 2953, 1725,

1594, 1480, 1248 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.91 (s, 3 H), 4.00 (s, 3 H), 6.70 (d, J = 8.8 Hz, 1 H), 8.13 (d, J = 8.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 52.6 (q), 54.8 (q), 109.4 (d), 118.6 (s), 143.1 (d), 149.5 (s), 165.0 (s), 165.1 (s) ppm. HRMS: calcd. for $C_8H_9NO_3Cl$ [M + H] 202.0271; found 202.0269.

General Procedure for the Stille Cross-Coupling: A Schlenk tube was charged under argon with degassed dry 1,4-dioxane, pyridine **15** (1 equiv.), $Pd(PPh_3)_4$ (10 mol-%) and organotin **11** (1.1 equiv.). The tube was sealed and the mixture was stirred at 80 °C for 23 h, except for the indicated reaction. After this time the mixture was allowed to reach room temp., filtered through Celite with EtOAc, dried (Na_2SO_4) and then concentrated in vacuo. The crude product was purified by silica flash column chromatography.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-methoxy-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (16aa): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15a** (771 mg, 2.73 mmol), $Pd(PPh_3)_4$ (315 mg, 0.27 mmol) and organotin **11a**[12] (1.50 g, 3.00) in 1,4-dioxane (55 mL) for 30 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3 to 1:1). The title product was obtained as a white wax (1.35 g, 85%). $[\alpha]_D$ = –43.1 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2952, 2928, 1734, 1700, 1385 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.54 (m, 9 H), 1.92–2.08 (m, 2 H), 2.20–2.44 (m, 2 H), 2.54 (s, 3 H), 3.38–3.70 (m, 2 H), 3.95 (s, 3 H), 4.07 (s, 3 H), 5.10–5.30 (m, 1 H), 6.79 (d, J = 8.4 Hz, 1 H), 7.39 (s, 1 H), 7.94 (d, J = 8.4 Hz, 1 H), 8.07 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1 (q), 23.4 and 24.1 (t), 28.5 (q), 32.9 and 34.3 (t), 46.8 and 47.1 (t), 52.2 (q), 53.9 (q), 59.1 and 59.5 (d), 80.6 (s), 110.2 (d), 114.9 (d), 115.2 (s), 120.7 (d), 128.3 (s), 142.0 (d), 149.6 (s), 154.9 (s), 156.6 (s), 159.8 (s), 163.3 (s), 164.5 (s), 176.5 (s) ppm. HRMS: calcd. for $C_{27}H_{30}O_6N_5S_2$ [M + H] 584.1632; found 584.1628.

6-Methoxy-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-2-[2-(2-methyl-1,3-dioxolan-2-yl)thiazol-4-yl]pyridine (16ab): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15a** (100 mg, 0.35 mmol), $Pd(PPh_3)_4$ (40 mg, 0.04 mmol) and organotin **11b**[13] (174 mg, 0.38 mmol) in 1,4-dioxane (7 mL) for 47 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The title product was obtained as a white solid (77 mg, 53%), m.p. (Et_2O) 127–130 °C. IR (KBr): $\tilde{\nu}$ = 2991, 2952, 2893, 1732, 1716, 1615, 1470, 1386, 1326, 1197, 1104, 1022 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.61 (s, 3 H), 2.60 (s, 3 H), 3.92 (s, 3 H), 3.97–4.07 (m, 7 H), 6.77 (d, J = 8.6 Hz, 1 H), 7.93 (d, J = 8.6 Hz, 1 H), 7.97 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 24.8 (q), 52.0 (q), 53.9 (q), 65.6 (t), 107.0 (s), 110.1 (d), 115.1 (s), 120.6 (d), 128.2 (s), 141.8 (d), 149.7 (s), 154.8 (s), 156.8 (s), 159.3 (s), 163.3 (s), 164.4 (s), 172.2 (s) ppm. HRMS: calcd. for $C_{19}H_{20}O_6N_3S$ [M + H] 418.1067; found 418.1065.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-methoxy-3-[4-(methoxycarbonyl)thiazole-2-yl]pyridine (16ba): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15b** (142 mg, 0.50 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and organotin **11a**[12] (275 mg, 0.55 mmol) in 1,4-dioxane (10 mL) for 23 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (164 mg, 56%), m.p. (CH_2Cl_2) 87–91 °C. $[\alpha]_D$ = –73.8 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3111, 2975, 1739, 1696, 1596, 1381 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28–1.57 (m, 9 H), 1.91–2.04 (m, 2 H), 2.21–2.45 (m, 2 H), 3.39–3.68 (m, 2 H), 3.95 (s, 3 H), 4.05 (s, 3 H), 5.11–5.28 (m, 1 H), 6.83 (d, J = 8.4 Hz, 1 H), 7.42 (s, 1 H), 7.88 (br. s, 1 H), 8.02 (d, J = 8.4 Hz, 1 H), 8.24 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4 and 24.1 (t), 28.5 (q), 34.3 (t), 46.8 and 47.1 (t), 52.7 (q), 54.0 (q), 59.5 (d), 80.6 (s), 110.5 (d), 115.5 and 115.8 (d), 121.2 (d), 129.1 (d), 141.9 (d), 149.1 (s), 154.5 (s), 162.3 (s), 164.2 (s), 167.5 (s), 207.2 (s) ppm. HRMS: calcd. for $C_{26}H_{28}N_5O_5S_3$ [M + H] 586.1247; found 586.1243.

6-Methoxy-3-[4-(methoxycarbonyl)thiazol-2-yl]-2-[2-(2-methyl-1,3-dioxolan-2-yl)thiazol-4-yl]pyridine (16bb): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15b** (100 mg, 0.35 mmol), $Pd(PPh_3)_4$ (40 mg, 0.04 mmol) and organotin **11b**[13] (177 mg, 0.38 mmol) in 1,4-dioxane (7 mL) for 47 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The title product was obtained as a white solid (65 mg, 44%), m.p. (Et_2O) 114–118 °C. IR (KBr): $\tilde{\nu}$ = 3112, 2990, 2952, 2895, 2854, 1736, 1722, 1596, 1209 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.59 (s, 3 H), 3.93 (s, 3 H), 3.94–4.06 (m, 7 H), 6.80 (d, J = 8.6 Hz, 1 H), 7.84 (s, 1 H), 7.96 (d, J = 8.6 Hz, 1 H), 8.22 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.0 (q), 52.6 (q), 54.0 (q), 65.5 (t), 107.0 (s), 110.3 (d), 121.3 (d), 121.4 (s), 129.0 (d), 141.8 (d), 146.5 (s), 149.2 (s), 154.5 (s), 162.3 (s), 164.2 (s), 167.2 (s), 171.5 (s) ppm. HRMS: calcd. for $C_{18}H_{18}O_5N_3S_2$ [M + H] 420.0682; found 420.0684.

Methyl (*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-methoxynicotinate (16ca): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15c** (100 mg, 0.50 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and organotin **11a**[12] (275 mg, 0.55 mmol) in 1,4-dioxane (10 mL) for 23 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 8:2). The title product was obtained as a white solid (237 mg, 94%), m.p. (CH_2Cl_2) 69–73 °C. $[\alpha]_D$ = –59.5 (c = 0.50, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3119, 2976, 1730, 1698, 1594, 1386 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.54 (m, 9 H), 1.92–2.10 (m, 2 H), 2.24–2.46 (m, 2 H), 3.40–3.70 (m, 2 H), 3.79 (s, 3 H), 4.03 (s, 3 H), 5.12–5.32 (m, 1 H), 6.74 (d, J = 8.4 Hz, 1 H), 7.80–7.85 (m, 2 H), 7.99 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5 and 24.2 (t), 28.5 (q), 32.9 and 34.3 (t), 46.8 and 47.2 (t), 52.4 and 52.6 (q), 53.9 (q), 59.2 and 59.6 (d), 80.6 (s), 110.0 (d), 113.6 (s), 115.4 (d), 119.7 (d), 119.9 (s), 121.2 (s), 139.8 (d), 142.8 (s), 148.8 (s), 155.1 (s), 164.2 (s) ppm. HRMS: calcd. for $C_{23}H_{27}N_4O_5S_2$ [M + H] 503.1417; found 503.1415.

Methyl 6-Methoxy-2-[2-(2-methyl-1,3-dioxolan-2-yl)thiazol-4-yl]-nicotinate (16cb): The reaction was performed according to the general procedure for the Stille cross-coupling by using pyridine **15c** (100 mg, 0.50 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and organotin **11b**[13] (253 mg, 0.55 mmol) in 1,4-dioxane (10 mL) for 17 h. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 8:2). The title product was obtained as a white solid (96 mg, 57%), m.p. (Et_2O) 95–99 °C. IR (KBr): $\tilde{\nu}$ = 2992, 2951, 2898, 1730, 1596, 1023 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.85 (s, 3 H), 3.83 (s, 3 H), 4.01 (s, 3 H), 4.05–4.12 (m, 4 H), 6.72 (d, J = 8.4 Hz, 1 H), 7.80 (d, J = 8.4 Hz, 1 H), 7.93 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.4 (q), 52.5 (q), 53.9 (q), 65.6 (t), 107.1 (s), 109.9 (d), 119.7 (d), 121.2 (s), 139.8 (d), 148.7 (s), 154.9 (s), 164.1 (s), 169.5 (s), 171.4 (s) ppm. HRMS: calcd. for $C_{15}H_{17}O_5N_2S$ [M + H] 337.0853; found 337.0854.

General Procedure for the Demethylation of Methoxypyridines: A solution of the starting methoxypyridine in HBr in AcOH (33%) was stirred at 90 °C under nitrogen for 30 min. The mixture was allowed to reach room temp., poured onto saturated aq. $NaHCO_3$, extracted with CH_2Cl_2 (×3), dried ($NaSO_4$) and then concentrated

in vacuo. If required the crude product was purified by silica flash column chromatography.

General Procedure for Boc Protection of Bithiazolylpyrrolidines: *tert*-Butyl dicarbonate (1 equiv.) and NEt_3 (2 equiv.) were added to a flask charged with a solution of free bithiazolylpyrrolidine (1 equiv.) in dry CH_2Cl_2 at 0 °C for 4 h under nitrogen. The resulting mixture was stirred at 0 °C for the indicated time. The reaction mixture was poured into brine, extracted with CH_2Cl_2 (×3), dried ($Na2SO_4$) and then concentrated in vacuo. The crude product was purified by silica flash column chromatography.

General Procedure for the Preparation of Pyridyl Triflates: 2,6-Lutidine (1.4 equiv.) and trifluoromethanesulfonic anhydride (1.2 equiv.) were added to a solution of the pyridone and DMAP (20 mol-%) in dry CH_2Cl_2 at 0 °C under a nitrogen atmosphere. The mixture was stirred at 0 °C for 2.5 h and then at room temp. for 2 h. The mixture was diluted with CH_2Cl_2, washed with water (× 2), dried (Na_2SO_4) and then concentrated in vacuo. The crude product was purified by silica flash column chromatography.

Synthesis of (*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-3-[-4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17aa):

(*S*)-5-[4-(Methoxycarbonyl)-5-methyloxazol-2-yl]-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16aa** (590 mg, 1.01 mmol). The title compound was obtained as a yellowish solid (420 mg, 89%), m.p. decomp. (CH_2Cl_2) 188 °C. $[\alpha]_D$ = –64.3 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3420, 3088, 2955, 2918, 2849, 1653 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.80–1.96 (m, 2 H), 2.00–2.10 (m, 1 H), 2.29–2.40 (m, 1 H), 2.63 (s, 3 H), 3.07–3.21 (m, 2 H), 3.94 (s, 3 H), 4.62 (dd, *J* = 8.2 and 5.4 Hz, 1 H), 6.61 (dd, *J* = 9.6 Hz, 1 H), 7.86 (dd, *J* = 9.6 Hz, 1 H), 7.89 (s, 1 H), 8.37 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 25.8 (t), 34.3 (t), 47.3 (t), 52.3 (q), 59.6 (d), 104.4 (s), 117.6 (d), 120.2 (d), 123.8 (d), 128.5 (s), 139.7 (s), 142.3 (d), 145.8 (s), 148.5 (s), 156.6 (s), 157.1 (s), 162.3 (s), 162.7 (s), 162.9 (s), 180.8 (s) ppm. HRMS: calcd. for $C_{21}H_{20}N_5O_4S_2$[M + H] 470.0957; found 470.0966.

(*S*)-6-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for the Boc protection of bithiazolylpyrrolidines by using (*S*)-5-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one (500 mg, 1.06 mmol) in CH_2Cl_2 (21 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 2:8). The title product was obtained as a yellowish solid (593 mg, 98%), m.p. (CH_2Cl_2) 143–147 °C. $[\alpha]_D$ = –59.5 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3091, 2975, 1699, 1657, 1385, 1110 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.56 (m, 9 H), 1.93–2.10 (m, 2 H), 2.20–2.48 (m, 2 H), 2.66 (s, 3 H), 3.40–3.72 (m, 2 H), 3.96 (s, 3 H), 5.12–5.30 (m, 1 H), 6.62 (d, *J* = 9.6 Hz, 1 H), 7.86 (d, *J* = 9.6 Hz, 1 H), 7.94 (br. s, 1 H), 8.38–8.47 (m, 1 H), 10.92 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 23.5 and 24.2 (t), 28.5 (q), 33.0 and 34.3 (t), 46.8 and 47.2 (t), 52.3 (q), 59.1 and 59.5 (d), 80.7 (s), 104.5 (q), 117.0 (d), 120.2 (d), 124.0 (d), 128.5 (s), 139.6 (s), 142.3 (d), 145.9 (s), 148.1 (s), 154.4 and 154.9 (s), 156.6 (s), 157.0 (s), 162.4 (s), 162.7 (s), 176.9 (s) ppm. HRMS: calcd. for $C_{26}H_{28}O_6N_5S_2$ [M + H] 570.1476; found 570.1474.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-3-[-4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17aa): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using (*S*)-6-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridin-2(1*H*)-one (568 mg, 1.03 mmol), trifluoromethanesulfonic anhydride (207 µL, 1.23 mmol), 2,6-lutidine (168 µL, 1.44 mmol) and DMAP (27 mg, 0.21 mmol) in CH_2Cl_2 (10 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1). The title product was obtained as a white solid (587 mg, 81%), m.p. (EtOAc) 77–81 °C. $[\alpha]_D$ = –42.3 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3118, 2976, 1699, 1426, 1388, 1212 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.25–1.55 (m, 9 H), 1.92–2.05 (m, 2 H), 2.20–2.46 (m, 2 H), 2.57 (s, 3 H), 3.38–3.70 (m, 2 H), 3.97 (s, 3 H), 5.10–5.30 (m, 1 H), 7.21 (d, *J* = 8.2 Hz, 1 H), 7.34 (br. s, 1 H), 8.18 (s, 1 H), 8.26 (d, *J* = 8.2 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1 (q), 23.5 and 24.2 (t), 28.5 and 28.7 (q), 32.9 and 34.2 (t), 46.8 and 47.1 (t), 52.4 (q), 59.2 and 59.5 (d), 80.6 (s), 113.2 (d), 115.1 and 115.5 (d), 118.9 (q), 121.8 (s), 123.2 (d), 128.7 (s), 144.9 (d), 148.8 (s), 151.1 (s), 152.6 (s), 155.9 (s), 157.3 (s), 157.7 (s), 162.4 (s), 162.9 (s), 176.8 (s) ppm. HRMS: calcd. for $C_{27}H_{27}N_5O_8F_3S_3$ [M + H] 702.0974; found 702.0988.

Synthesis of 2-(2-Acetylthiazol-4-yl)-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17ab):

6-(2-Acetylthiazol-4-yl)-5-[4-(methoxycarbonyl)-5-methyloxazol-2-yl-]pyridine-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16ab** (70 mg, 0.17 mmol). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 96:4). The title product was obtained as a pale solid (41 mg, 67%), m.p. (Et_2O) 194–198 °C. IR (KBr): $\tilde{\nu}$ = 3083, 2921, 2851, 1719, 1674, 1441, 1112 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.62 (s, 3 H), 2.65 (s, 3 H), 3.93 (s, 3 H), 6.66 (d, *J* = 9.6 Hz, 1 H), 7.90 (d, *J* = 9.6 Hz, 1 H), 8.81 (s, 1 H), 12.04 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 26.1 (q), 52.3 (q), 106.0 (s), 120.6 (d), 128.5 (s), 130.0 (d), 139.6 (s), 142.5 (d), 147.5 (s), 156.6 (s), 157.0 (s), 162.6 (s), 163.4 (s), 166.5 (s), 191.2 (s) ppm. HRMS: calcd. for C16H14O5N3S [M + H] 360.0649; found 360.0647.

2-(2-Acetylthiazol-4-yl)-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17ab): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using 6-(2-acetylthiazol-4-yl)-5-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine-2(1*H*)-one (38 mg, 0.11 mmol), trifluoromethanesulfonic anhydride (22 µL, 0.13 mmol), 2,6-lutidine (17 µL, 0.15 mmol) and DMAP (2 mg, 0.02 mmol) in CH_2Cl_2 (1.1 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (54 mg, quant.). ^1H NMR (400 MHz, $CDCl_3$): δ = 2.38 (s, 3 H), 2.64 (s, 3 H), 3.96 (s, 3 H), 7.26 (d, *J* = 8.4 Hz, 1 H), 8.27 (d, *J* = 8.4 Hz, 1 H), 8.48 (s, 1 H) ppm.

Synthesis of (*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-3-[4-(methoxycarbonyl)thiazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17ba):

(*S*)-5-[4-(Methoxycarbonyl)thiazole-2-yl]-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16ba** (149 mg, 0.25 mmol). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a white solid (80 mg, 68%), m.p. (CH_2Cl_2) 119–123 °C. $[\alpha]_D$ = –35.2 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2920, 1732, 1652 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.80–1.96 (m, 2 H), 1.99–2.09 (m, 1 H), 2.27–2.39 (m, 1 H), 3.06–3.21 (m, 2 H), 3.99 (s, 3 H), 4.62 (dd, *J*

= 8.4 and 5.4 Hz, 1 H), 6.62 (d, J = 9.4 Hz, 1 H), 7.41 (s, 1 H), 7.66 (d, J = 9.4 Hz, 1 H), 7.92 (s, 1 H), 8.31 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.8 (t), 34.3 (t), 47.2 (t), 52.9 (q), 59.6 (d), 110.6 (s), 117.8 (d), 120.3 (d), 122.8 (d), 129.8 (d), 139.1 (s), 143.2 (d), 145.9 (s), 147.4 (s), 148.5 (s), 161.8 (s), 162.5 (s), 163.5 (s), 164.9 (s), 180.5 (s) ppm. HRMS: calcd. for $C_{20}H_{18}O_3N_5S_3$ [M + H] 472.0566; found 472.0564.

(*S*)-6-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-[4-(methoxycarbonyl)thiazole-2-yl]pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for Boc protection of bithiazolylpyrrolidines by using (*S*)-5-[4-(methoxycarbonyl)thiazole-2-yl]-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one (75 mg, 0.16 mmol) in CH_2Cl_2 (3 mL). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a pale solid (91 mg, quant.), m.p. (CH_2Cl_2) 118–122 °C. $[\alpha]_D$ = –35.7 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3094, 2975, 1732, 1695, 1656, 1388 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32–1.53 (m, 9 H), 1.92–2.06 (m, 2 H), 2.20–2.46 (m, 2 H), 3.40–3.70 (m, 2 H), 3.99 (s, 3 H), 5.11–5.28 (m, 1 H), 6.62 (d, J = 9.4 Hz, 1 H), 7.44 (br. s, 1 H), 7.65 (d, J = 9.4 Hz, 1 H), 7.95 (br. s, 1 H), 8.32 (s, 1 H), 10.77 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5 and 24.2 (t), 28.5 (q), 33.0 and 34.3 (t), 46.8 and 47.2 (t), 52.9 (q), 59.2 and 59.5 (d), 80.7 (s), 110.5 (s), 117.2 and 117.5 (d), 120.3 (d), 123.0 (d), 129.9 (d), 139.0 (s), 143.3 (d), 145.9 (s), 147.4 (s), 148.1 (s), 161.8 (s), 162.4 (s), 164.8 (s), 176.9 (s) ppm. HRMS: calcd. for $C_{25}H_{26}N_5O_5S_3$ [M + H] 572.1091; found 572.1093.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-3-[4-(methoxycarbonyl)thiazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17ba): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using (*S*)-6-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-[4-(methoxycarbonyl)thiazole-2-yl]pyridin-2(1*H*)-one (65 mg, 0.11 mmol), trifluoromethanesulfonic anhydride (22 µL, 0.13 mmol), 2,6-lutidine (18 µL, 0.15 mmol) and DMAP (3 mg, 0.02 mmol) in CH_2Cl_2 (1.1 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The title product was obtained as a white solid (60 mg, 78%), m.p. (EtOAc) 153–157 °C. $[\alpha]_D$ = –37.6 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2976, 1733, 1696, 1426, 1386, 1212 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.52 (m, 9 H), 1.92–2.01 (m, 2 H), 2.19–2.43 (m, 2 H), 3.38–3.68 (m, 2 H), 3.97 (s, 3 H), 5.08–5.27 (m, 1 H), 7.23 (d, J = 8.4 Hz, 1 H), 7.28 (s, 1 H), 8.08 (br. s, 1 H), 8.26 (d, J = 8.4 Hz, 1 H), 8.37 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4 and 24.2 (t), 28.5 (q), 33.0 and 34.2 (t), 46.8 and 47.2 (t), 52.8 (q), 59.1 and 59.5 (d), 80.6 (s), 113.5 (d), 115.7 and 116.1 (d), 117.3 (s), 120.5 (s), 123.5 (d), 127.9 (s), 129.7 (d), 144.9 (d), 146.9 (s), 150.5 (s), 152.5 (s), 155.6 (s), 162.0 (s), 165.2 (s), 176.6 (s) ppm. HRMS: calcd. for $C_{26}H_{25}O_7N_5F_3S_4$ [M + H] 704.0583; found 704.0593.

Synthesis of 2-(2-Acetylthiazol-4-yl)-3-[4-(methoxycarbonyl)thiazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17bb):

6-(2-Acetylthiazol-4-yl)-5-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16bb** (111 mg, 0.26 mmol). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 96:4). The title product was obtained as a yellowish solid (68 mg, 72%), m.p. (Et_2O) 210–214 °C. IR (KBr): $\tilde{\nu}$ = 3093, 2921, 2850, 1731, 1660, 1239, 1214 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.69 (s, 3 H), 3.98 (s, 3 H), 6.67 (d, J = 9.2 Hz, 1 H), 7.70 (d, J = 9.2 Hz, 1 H), 8.02 (s, 1 H), 8.30 (s, 1 H), 11.20 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.3 (q), 52.9 (q), 111.3 (s), 121.1 (d), 129.5 (d), 129.7 (d), 138.2 (s), 143.3 (d), 147.1 (s), 147.5 (s), 161.6 (s), 162.4 (s), 164.5 (s), 167.0 (s), 191.1 (s) ppm. HRMS: calcd. for $C_{15}H_{12}O_4N_3S_2$ [M + H] 362.0264; found 362.0265.

2-(2-Acetylthiazol-4-yl)-3-[4-(methoxycarbonyl)thiazol-2-yl]-6-(trifluoromethylsulfonyloxy)pyridine (17bb): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using 6-(2-acetylthiazol-4-yl)-5-[4-(methoxycarbonyl)thiazol-2-yl]pyridine-2(1*H*)-one (25 mg, 0.07 mmol), trifluoromethanesulfonic anhydride (14 µL, 0.08 mmol), 2,6-lutidine (11 µL, 0.10 mmol) and DMAP (2 mg, 0.014 mmol) in CH_2Cl_2 (0.7 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (27 mg, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.27 (s, 3 H), 3.97 (s, 3 H), 7.27 (d, J = 8.2 Hz, 1 H), 8.18 (d, J = 8.2 Hz, 1 H), 8.38 (s, 1 H), 8.43 (s, 1 H) ppm.

Synthesis of methyl (*S*)-2-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-(trifluoromethylsulfonyloxy)nicotinate (17ca):

Methyl (*S*)-5-(methoxycarbonyl)-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16ca** (225 mg, 0.45 mmol). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a white solid (152 mg, 87%), m.p. (CH_2Cl_2) 84–88 °C. $[\alpha]_D$ = –51.4 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2919, 1717, 1652 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.80–1.96 (m, 2 H), 1.99–2.10 (m, 1 H), 2.28–2.40 (m, 1 H), 3.07–3.21 (m, 2 H), 3.86 (s, 3 H), 4.63 (dd, J = 8.2 and 5.4 Hz, 1 H), 6.53 (d, J = 9.6 Hz, 1 H), 7.94 (s, 1 H), 7.96 (d, J = 9.6 Hz, 1 H), 8.55 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.8 (t), 34.3 (t), 47.2 (t), 52.5 (q), 59.6 (d), 108.4 (s), 117.5 (d), 119.3 (d), 125.4 (d), 142.5 (d), 142.9 (s), 145.6 (s), 148.5 (s), 162.4 (s), 162.7 (s), 166.0 (s), 180.6 (s) ppm. HRMS: calcd. for $C_{17}H_{17}O_3N_4S_2$ [M + H] 389.0737; found 389.0735.

Methyl (*S*)-6-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-(methoxycarbonyl)pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for Boc protection of bithiazolylpyrrolidines by using methyl (*S*)-5-(methoxycarbonyl)-6-[2′-(pyrrolidin-2-yl)-2,4′-bithiazol-4-yl]pyridin-2(1*H*)-one (140 mg, 0.36 mmol) in CH_2Cl_2 (7.2 mL). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a pale solid (170 mg, 97%), m.p. (CH_2Cl_2) 96–100 °C. $[\alpha]_D$ = –55.7 (c = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3088, 2975, 1699, 1653, 1386 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.55 (m, 9 H), 1.92–2.07 (m, 2 H), 2.23–2.46 (m, 2 H), 3.41–3.71 (m, 2 H), 3.87 (s, 3 H), 5.12–5.29 (m, 1 H), 6.53 (d, J = 9.6 Hz, 1 H), 7.94–8.00 (m, 2 H), 8.58 (s, 1 H), 10.96 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5 and 24.2 (t), 28.5 (q), 33.0 and 34.3 (t), 46.8 and 47.2 (t), 52.6 (q), 59.1 and 59.5 (d), 80.7 (s), 108.4 (s), 116.9 and 117.3 (d), 119.3 (d), 125.7 (d), 140.6 (s), 142.5 (d), 142.8 (s), 145.6 (s), 148.1 (s), 154.3 (s), 162.6 (s), 166.0 (s), 176.9 (s) ppm. HRMS: calcd. for $C_{22}H_{25}N_4O_5S_2$ [M + H] 489.1261; found 489.1263.

Methyl (*S*)-2-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-(trifluoromethylsulfonyloxy)nicotinate (17ca): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using methyl (*S*)-6-{2′-[*N*-(*tert*-butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-5-(methoxycarbonyl)pyridin-2(1*H*)-one (137 mg, 0.28 mmol), trifluoromethanesulfonic anhydride (57 µL, 0.34 mmol), 2,6-lutidine (45 µL, 0.39 mmol) and DMAP (7 mg, 0.06 mmol) in CH_2Cl_2 (2.8 mL).

The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (103 mg, 59%), m.p. (EtOAc) 59–62 °C. $[\alpha]_D$ = –48.1 (*c* = 0.50, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2977, 1734, 1699, 1427, 1388, 1216, 1172 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.28–1.56 (m, 9 H), 1.92–2.09 (m, 2 H), 2.22–2.46 (m, 2 H), 3.38–3.72 (m, 2 H), 3.88 (br. s, 3 H), 3.11–3.31 (m, 1 H), 7.15 (d, *J* = 8.2 Hz, 1 H), 7.82 (s, 1 H), 8.04 (d, *J* = 8.2 Hz, 1 H), 8.12 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5 and 24.2 (t), 28.5 (q), 32.9 and 34.3 (t), 46.8 and 47.2 (t), 53.2 (q), 59.1 and 59.3 (d), 80.6 (s), 113.3 (d), 115.8 and 116.2 (d), 117.3 (s), 120.5 (s), 122.2 (d), 128.1 (s), 141.9 (d), 149.0 (s), 149.5 (s), 152.6 (s), 155.3 (s), 168.3 (s), 176.8 (s) ppm. HRMS: calcd. for $C_{23}H_{24}O_7N_4F_3S_3$ [M + H] 621.0754; found 621.0756.

Synthesis of Methyl 2-(2-Acetylthiazol-4-yl)-6-(trifluoromethylsulfonyloxy)nicotinate (17cb):

6-(2-Acetylthiazol-4-yl)-5-(methoxycarbonyl)pyridin-2(1*H*)-one: The reaction was performed according to the general procedure for the demethylation of methoxypyridines by using **16cb** (89 mg, 0.26 mmol). The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2). The title product was obtained as a white solid (49 mg, 64%), m.p. (Et_2O) 184–188 °C. IR (KBr): $\tilde{\nu}$ = 3440, 3094, 1710, 1687, 1298 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.75 (s, 3 H), 3.83 (s, 3 H), 6.55 (d, *J* = 9.8 Hz, 1 H), 7.97 (d, *J* = 9.8 Hz, 1 H), 8.66 (s, 1 H), 11.58 (br. s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.3 (q), 52.6 (q), 109.7 (s), 119.9 (d), 130.9 (d), 142.3 (d), 142.5 (s), 147.2 (s), 163.3 (s), 165.8 (s), 166.3 (s), 191.2 (s) ppm. HRMS: calcd. for $C_{12}H_{11}O_4N_2S$[M + H] 279.0434; found 279.0436.

Methyl 2-(2-Acetylthiazol-4-yl)-6-(trifluoromethylsulfonyloxy)nicotinate (17cb): The reaction was performed according to the general procedure for the preparation of pyridyl triflates by using 6-(2-acetylthiazol-4-yl)-5-(methoxycarbonyl)pyridin-2(1*H*)-one (45 mg, 0.15 mmol), trifluoromethanesulfonic anhydride (30 µL, 0.18 mmol), 2,6-lutidine (26 µL, 0.22 mmol) and DMAP (4 mg, 0.03 mmol) in CH_2Cl_2 (1.5 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (61 mg, 99%). ^1H NMR (400 MHz, $CDCl_3$): δ = 2.71 (s, 3 H), 3.93 (s, 3 H), 7.21 (d, *J* = 8.0 Hz, 1 H), 8.08 (d, *J* = 8.0 Hz, 1 H), 8.42 (s, 1 H) ppm.

General Procedure for Negishi Cross-Coupling: A solution of **9** in DMA (0.21 M, 2 equiv.) was added to a Schlenk tube charged with $[Pd(PPh_3)_4]$ (20 mol-%) under nitrogen atmosphere. The mixture was stirred at 45 °C and then treated with a solution of pyridine-2-yl triflate (**3**; 1 equiv.) in DMA. The tube was sealed and the mixture was stirred at 45 °C for 18 h. After the indicated time the solution was allowed to reach room temp., filtered through Celite, washed with EtOAc, dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (18aa): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (27 mL, 5.70 mmol), $Pd(PPh_3)_4$ (164 mg, 0.14 mmol) and **17aa** (500 mg, 0.71 mmol) in DMA (8.9 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The desired product was obtained as a yellowish solid (481 mg, 92%), m.p. (EtOAc) 186–190 °C. $[\alpha]_D$ = –40.9 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3088, 2976, 1698, 1390, 1165, 1110 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.28–1.54 (m, 9 H), 1.65 (s, 9 H), 1.92–2.06 (m, 2 H), 2.21–2.46 (m, 2 H), 2.55 (s, 3 H), 3.38–3.70 (m, 2 H), 3.97 (s, 3 H), 5.10–5.30 (m, 1 H), 7.36–7.43 (m, 1 H), 8.21–8.23 (m, 2 H), 8.25 (d, *J* = 8.0 Hz, 1 H), 8.38 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1 (q), 23.5 (t), 28.4 (q), 28.5 (q), 32.9 and 34.3 (t), 46.8 and 47.1 (t), 52.3 (q), 59.5 (d), 80.6 (s), 82.5 (s), 115.0 and 115.3 (d), 118.7 (d), 121.5 (d), 122.6 (s), 128.6 (s), 129.6 (d), 132.3 and 132.4 (s), 140.9 (d), 150.2 (s), 151.2 (s), 151.5 (s), 154.1 (s), 157.0 (s), 158.9 (s), 160.6 (s), 163.1 (s), 168.6 (s) ppm. HRMS: calcd. for $C_{34}H_{37}N_6O_7S_3$ [M + H] 737.1886; found 737.1894.

2-(2-Acetylthiazol-4-yl)-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]-3-[4-(methoxycarbonyl)-5-methyloxazol-2-yl]pyridine (18ab): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (1.0 mL, 0.22 mmol), $Pd(PPh_3)_4$ (25 mg, 0.022 mmol) and **17ab** (54 mg, 0.11 mmol) in DMA (1.4 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (25 mg, 43%), m.p. (Et_2O) 178–182 °C. IR (KBr): $\tilde{\nu}$ = 3113, 2924, 2852, 1719, 1690, 1161 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.65 (s, 9 H), 2.41 (s, 3 H), 2.62 (s, 3 H), 3.96 (s, 3 H), 8.22 (s, 1 H), 8.25 (d, *J* = 8.0 Hz, 1 H), 8.42 (d, *J* = 8.0 Hz, 1 H), 8.54 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 25.7 (q), 28.4 (q), 52.3 (q), 82.6 (s), 119.3 (d), 122.8 (s), 128.1 (d), 128.8 (s), 129.6 (d), 141.0 (d), 150.3 (s), 150.4 (s), 151.7 (s), 155.2 (s), 157.0 (s), 158.4 (s), 160.5 (s), 162.8 (s), 166.3 (s), 168.2 (s), 191.4 (s) ppm. HRMS: calcd. for $C_{24}H_{23}O_6N_4S_2$ [M + H] 527.1054; found 527.1059.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]-3-[4-(methoxycarbonyl)-thiazole-2-yl]pyridine (18ba): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (0.8 mL, 0.16 mmol), $Pd(PPh_3)_4$ (19 mg, 0.016 mmol) and **17ba** (58 mg, 0.08 mmol) in DMA (1.0 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The title product was obtained as a white solid (32 mg, 54%), m.p. (Et_2O) 110–114 °C. $[\alpha]_D$ = –30.0 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2975, 1699, 1385, 1162 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.54 (m, 9 H), 1.65 (s, 9 H), 1.92–2.05 (m, 2 H), 2.21–2.45 (m, 2 H), 3.39–3.69 (m, 2 H), 3.97 (s, 3 H), 5.11–5.30 (m, 1 H), 7.44 (s, 1 H), 8.04 (br. s, 1 H), 8.20 (s, 1 H), 8.28–8.35 (m, 2 H), 8.41 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4 and 24.2 (t), 28.4 (q), 28.5 (q), 33.0 and 34.2 (t), 46.8 (t), 52.8 (q), 59.2 and 59.5 (d), 80.6 (s), 82.5 (s), 115.8 and 116.2 (d), 119.2 (d), 122.0 (d), 129.1 (d), 129.5 (d), 140.5 (d), 146.8 (s), 149.0 (s), 150.1 (s), 150.9 (s), 151.1 (s), 153.8 (s), 160.6 (s), 162.1 (s), 166.3 (s), 168.7 (s), 176.5 (s) ppm. HRMS: calcd. for $C_{33}H_{35}O_6N_6S_4$ [M + H] 739.1495; found 739.1503.

2-(2-Acetylthiazol-4-yl)-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]-3-[4-(methoxycarbonyl)thiazol-2-yl]pyridine (18bb): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (0.44 mL, 0.09 mmol), $Pd(PPh_3)_4$ (11 mg, 0.009 mmol) and **17bb** (23 mg, 0.05 mmol) in DMA (0.6 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3). The title product was obtained as a white solid (10 mg, 41%), m.p. (Et_2O) 185–187 °C. IR (KBr): $\tilde{\nu}$ = 3111, 2928, 1724, 1690, 1213, 1161 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.65 (s, 9 H), 2.33 (s, 3 H), 3.97 (s, 3 H), 8.19 (d, *J* = 8.2 Hz, 1 H), 8.21 (s, 1 H), 8.34 (s, 1 H), 8.43 (d, *J* = 8.2 Hz, 1 H), 8.46 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.0 (q), 28.4 (q), 52.8 (q), 82.6 (s), 119.4 (d), 128.6 (d), 128.8 (s), 129.4 (d), 129.6 (d), 141.0 (d), 145.1 (s), 147.2 (s), 150.1 (s), 150.2 (s), 151.4 (s), 155.0 (s), 160.6 (s), 161.9 (s), 166.2 (s), 168.3 (s), 191.6 (s) ppm. HRMS: calcd. for $C_{23}H_{21}O_5N_4S_3$ [M + H] 529.0669; found 529.0676.

Methyl (*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]nicotinate (18ca): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (1.6 mL, 0.33 mmol), $Pd(PPh_3)_4$ (38 mg, 0.03 mmol) and **17ca** (101 mg, 0.16 mmol) in DMA (1.9 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 6:4). The title product was obtained as a white solid (69 mg, 66%), m.p. (Et_2O) 107–110 °C. $[\alpha]_D$ = –38.3 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 2975, 1732, 1699, 1385, 1162 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.30–1.56 (m, 9 H), 1.65 (s, 9 H), 1.92–2.11 (m, 2 H), 2.24–2.49 (m, 2 H), 3.41–3.71 (m, 2 H), 3.89 (br. s, 3 H), 5.13–5.33 (m, 1 H), 7.86 (s, 1 H), 8.02 (d, *J* = 8.0 Hz, 1 H), 8.18–8.21 (m, 2 H), 8.32 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.5 and 24.2 (t), 28.4 (q), 28.5 (q), 33.0 and 34.3 (t), 46.8 (t), 53.0 (q), 59.2 and 59.6 (d), 80.6 (s), 82.5 (s), 115.7 and 116.0 (d), 118.7 (d), 120.5 (d), 128.9 (s), 129.5 (d), 138.1 (d), 149.8 (s), 150.1 (s), 151.1 (s), 154.1 (s), 154.1 (s), 160.6 (s), 162.3 (s), 168.7 (s), 169.2 (s) ppm. HRMS: calcd. for $C_{30}H_{34}O_6N_5S_3$ [M + H] 656.1666; found 656.1666.

Methyl 2-(2-Acetylthiazol-4-yl)-6-[4-(*tert*-butoxycarbonyl)thiazol-2-yl]nicotinate (18cb): The reaction was performed according to the general procedure for Negishi cross-coupling by using **9** (1.4 mL, 0.30 mmol), $Pd(PPh_3)_4$ (34 mg, 0.03 mmol) and **17cb** (61 mg, 0.15 mmol) in DMA (1.9 mL). The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 8:2). The title product was obtained as a yellowish solid (26 mg, 39%), m.p. (Et_2O) 129–133 °C. IR (KBr): $\tilde{\nu}$ = 2977, 2929, 1730, 1690 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.65 (s, 9 H), 2.72 (s, 3 H), 3.92 (s, 3 H), 8.05 (d, *J* = 8.2 Hz, 1 H), 8.20 (s, 1 H), 8.37 (d, *J* = 8.2 Hz, 1 H), 8.50 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 26.1 (q), 28.4 (q), 52.8 (q), 82.6 (s), 119.2 (d), 127.2 (d), 128.7 (s), 129.6 (d), 138.5 (d), 149.1 (s), 150.2 (s), 151.4 (s), 155.2 (s), 160.5 (s), 166.5 (s), 168.3 (s), 168.6 (s), 191.5 (s) ppm. HRMS: calcd. for $C_{20}H_{20}O_5N_3S_2$ [M + H] 446.0839; found 446.0843.

Isopropyl 2-Bromothiazole-4-carboxylate (20): A solution of lithium hydroxide (1.16 g, 27.62 mmol) in H_2O (13 mL) was added to a stirring solution of ethyl 2-bromothiazole-4-carboxylate[10] (3.26 g, 13.81 mmol) in THF (130 mL). The mixture was stirred at room temp. for 18 h and then concentrated under vacuum before aqueous HCl (2 M, 150 mL) was then added. The resulting mixture was extracted with EtOAc (3 × 200 mL), dried (Na_2SO_4) and concentrated in vacuo. The resulting white solid was dissolved in dry CH_2Cl_2 (28 mL) and cooled in an ice/water bath. Oxalyl chloride (2.3 mL, 27.68 mmol) and dimethylformamide (DMF; 3 drops) were added dropwise and the resulting mixture was stirred allowing it to reach room temp. After 1 h all volatiles were evaporated under reduced pressure. The residue was dissolved in *i*PrOH (100 mL) and stirred for 2 h. Volatiles were evaporated and the crude product purified by silica flash column chromatography (hexanes/EtOAc, 95:5). The title product was obtained as a white solid (2.80 g, 81%), m.p. (CH_2Cl_2) 58–59 °C. IR (KBr): $\tilde{\nu}$ = 3084, 2975, 1713, 1431, 1226, 1111, 1015 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.38 (d, *J* = 6.2 Hz, 6 H), 5.27 (h, *J* = 6.2 Hz, 1 H), 8.08 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.8 (q), 69.6 (d), 130.5 (d), 136.6 (s), 147.7 (s), 159.7 (s) ppm. HRMS: calcd. for $C_7H_8BrNO_2S$ [M + H] 249.9531; found 249.9532.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-6-[4-(ethoxycarbonyl)thiazol-2-yl]-3-[5-methyl-4-(methoxycarbonyl)oxazol-2-yl]pyridine (23): A solution of **21** in DMA (0.21 M, 2.2 mL, 0.456 mmol) was added to a Schlenk tube charged with **17aa** (40 mg, 0.057 mmol) and $Pd(PPh_3)_4$ (13 mg, 0.011 mmol). The mixture was stirred at 45 °C. After 2 h EtOAc (20 mL) the mixture was added and washed with H_2O (2 × 5 mL), dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 9:1 to 1:1). The desired product was obtained as a yellowish solid (32 mg, 79%), m.p. (CH_2Cl_2) decomp. above 106 °C. $[\alpha]_D$ = –34.7 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3122, 2975, 2924, 1694, 1386, 1239, 1201, 1105 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.27–1.59 (m, 12 H), 1.91–2.07 (m, 2 H), 2.17–2.46 (m, 2 H), 2.53 (s, 3 H); 3.36–3.70 (m, 2 H), 3.96 (s, 3 H), 4.46 (q, *J* = 7.2 Hz, 2 H), 5.09–5.29 (m, 1 H), 7.39 (br. s, 1 H), 8.20 (s, 1 H), 8.24 (d, *J* = 8.0 Hz, 1 H), 8.32 (s, 1 H), 8.37 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 14.7 (q), 23.6 and 24.3 (t), 28.6 and 28.8 (q), 33.1 and 34.4 (t), 46.9 and 47.3 (t), 52.4 (q), 59.3 and 59.6 (d), 62.0 (t), 80.7 (s), 115.1 and 115.5 (d), 118.9 (d), 121.6 (d), 122.8 (s), 128.7 (s), 130.5 (d), 141.0 (d), 149.0 (s), 149.1 (s) 151.4 and 151.5 (s), 154.2 (s) and 154.4 (s), 157.1 (s), 159.0 (s), 161.6 (s); 162.1 (s), 163.2 (s), 169.1 (s), 176.7 (s) ppm. HRMS: calcd. for $C_{32}H_{33}N_6O_7S_3$ [M + H] 709.1590; found 709.1567.

(*S*)-2-{2′-[*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl]-2,4′-bithiazol-4-yl}-3-[5-methyl-4-(methoxycarbonyl)oxazol-2-yl]-6-[4-(isopropoxycarbonyl)thiazol-2-yl]pyridine (24): A solution of **22** in DMA (0.21 M, 9.2 mL, 1.94 mmol) was added to a Schlenk tube charged with **17aa** (170 mg, 0.24 mmol) and $Pd(PPh_3)_4$ (55 mg, 0.05 mmol). The mixture was stirred at 45 °C. After 16 h EtOAc (50 mL) the mixture was added and washed with H_2O (4 × 50 mL), dried (Na_2SO_4) and concentrated in vacuo. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 7:3 to 6:4). The desired product was obtained as a pale solid (148 mg, 85%), m.p. (CH_2Cl_2) decomp. above 130 °C. $[\alpha]_D$ = –37.2 (*c* = 1.00, CH_2Cl_2). IR (KBr): $\tilde{\nu}$ = 3122, 2975, 1700, 1386, 1239, 1214, 1105 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.27–1.53 (m, 15 H), 1.89–2.03 (m, 2 H), 2.18–2.45 (m, 2 H), 2.53 (s, 3 H), 3.36–3.68 (m, 2 H), 3.96 (s, 3 H), 5.10–5.36 (m, 2 H), 7.39 (br. s, 1 H), 8.20 (s, 1 H), 8.24 (d, *J* = 8.0 Hz, 1 H), 8.28 (s, 1 H), 8.38 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.2 (q), 22.3 (q), 23.6 and 24.3 (t), 28.6 and 28.8 (q), 33.1 and 34.4 (t), 46.9 and 47.3 (t), 52.4 (q), 59.3 and 59.7 (d), 69.6 (d), 80.7 (s), 115.1 and 115.5 (d), 118.9 (d), 121.6 (d), 122.8 (s), 128.7 and 128.9 (s), 130.2 (d), 141.0 (d), 149.1 (s), 149.4 (s), 151.4 and 151.6 (s), 154.2 and 154.5 (s), 157.2 (s), 159.0 (s), 161.2 (s), 162.1 (s), 163.2 (s), 169.0 (s), 176.7 (s) ppm. HRMS: calcd. for $C_{33}H_{35}O_7N_6S_3$ [M + H] 723.1735; found 723.1724.

Supporting Information (see footnote on the first page of this article): General procedures and NMR spectra of compounds **2**, **4**, **5**, **7**, **8**, **10**, **14**, **15a**–**15c**, **16aa**–**16cb**, **17aa**–**17cb**, **18aa**–**18cb**, **20**, **23** and **24** as well as those of non-numbered intermediates.

Acknowledgments

The authors gratefully acknowledge financial support from the Spanish Science and Innovation Ministry (CICYT) (CTQ2009-07758 and CTQ2012-30930) and the Generalitat de Catalunya (2009SGR 1024). X. J. thanks the Instituto de Salud Carlos III (ISCIII) for a PFIS grant.

[1] a) E. Riego, D. Hernández, F. Albericio, M. Álvarez, *Synthesis* **2005**, *12*, 1907; b) F. Gianfranco, *Curr. Bioactive Compd.* **2010**, *6*, 284; c) Z. Jin, *Nat. Prod. Rep.* **2013**, *30*, 869.

[2] For reviews on thiopeptide syntheses, see: a) R. S. Roy, A. M. Gehring, J. C. Milne, P. J. Belshaw, C. T. Walsh, *Nat. Prod. Rep.* **1999**, *16*, 249; b) M. C. Bagley, J. W. Dale, E. A. Merritt, X. Xiong, *Chem. Rev.* **2005**, *105*, 685; c) R. A. Hughes, C. J.

Moody, *Angew. Chem.* **2007**, *119*, 8076; *Angew. Chem. Int. Ed.* **2007**, *46*, 7930; d) C. Li, W. L. Kelly, *Nat. Prod. Rep.* **2010**, *27*, 153.

[3] As a selection of landmark total syntheses of thiopeptides, see: a) M. C. Bagley, K. E. Bashford, C. L. Hesketh, C. J. Moody, *J. Am. Chem. Soc.* **2000**, *122*, 3301; b) K. C. Nicolaou, B. S. Safina, M. Zak, A. A. Estrada, S. H. Lee, *Angew. Chem.* **2004**, *116*, 5197; *Angew. Chem. Int. Ed.* **2004**, *43*, 5087; c) R. A. Hughes, S. P. Thompson, L. Alcaraz, C. J. Moody, *J. Am. Chem. Soc.* **2005**, *127*, 15644; d) H. M. Müller, O. Delgado, T. Bach, *Angew. Chem.* **2007**, *119*, 4855; *Angew. Chem. Int. Ed.* **2007**, *46*, 4771; e) O. Delgado, H. M. Müller, T. Bach, *Chem. Eur. J.* **2008**, *14*, 2322; f) D. Lefranc, M. A. Ciufolini, *Angew. Chem.* **2009**, *121*, 4262; *Angew. Chem. Int. Ed.* **2009**, *48*, 4198.

[4] For a review on cross-coupling reactions of heteroarenes, see: a) S. Schröter, C. Stock, T. Bach, *Tetrahedron* **2005**, *61*, 2245; b) V. F. Slagt, A. H. M. de Vries, J. G. de Vries, R. M. Kellogg, *Org. Process Res. Dev.* **2010**, *14*, 30.

[5] a) L. M. Canedo Hernandez, F. Romero Millan, A. Fernandez Medarle, R. I. Fernandez Chimeno, J. C. Hidalgo Villar (Instituto Biomar, S. A., Spain). Peptides as bioactive compounds. WO 2012062906, May 18, **2012**; b) Total Synthesis and Stereochemical Assignment of Baringolin: X. Just-Baringo, P. Bruno, L. K. Ottesen, F. Albericio, M. Álvarez, *Angew. Chem. Int. Ed.* (in press).

[6] Selected references: a) Y. Hirokawa, T. Horikawa, S. Kato, *Chem. Pharm. Bull.* **2000**, *48*, 1847; b) Y. Hirokawa, I. Fujiwara, K. Suzuki, H. Harada, T. Yoshikawa, N. Yoshida, S. Kato, *J. Med. Chem.* **2003**, *46*, 702; c) Y. Tsuzuki, K. Tomita, K. Shibamori, Y. Sato, S. Kashimoto, K. Chiba, *J. Med. Chem.* **2004**, *47*, 2097; d) I. N. Houpis, R. Liu, Y. Wu, Y. Yuan, Y. Wang, U. Nettekoven, *J. Org. Chem.* **2010**, *75*, 6965; e) C. Boos, D. M. Bowles, C. Cai, A. Casimiro-Garcia, X. Chen, C. A. Hulford, S. M. Jennings, E. J. Kiser, D. W. Piotrowski, M. Sammons, R. A. Wade, *Tetrahedron Lett.* **2011**, *52*, 7025; f) J. B. Baell, I. P. Street, B. E. Sleebs, *Preparation of pyrido[3',2':4,5]-thieno[3,2-d]pyrimidin-4-ylamine derivatives as LIM kinase inhibitors*, Australian patent, WO 2012131297, October 4, **2012**; g) J. S. Scott, A. L. Gill, L. Godfrey, S. D. Groombridge, A. Rees, J. Revill, P. Schofield, P. Sorme, A. Stocker, J. G. Swales, P. R. O. Whittamore, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6756; h) P. J. Conn, C. W. Lindsley, S. R. Stauffer, J. Manka, J. Jacobs, Y. S. Zhou, J. M. Bartolome-Nebreda, G. MacDonald, S. Conde-Ceide, C. K. Jones, Vanderbilt University, USA, *Preparation of naphthyridone analogues as nGluR5 positive allosteric modulators*, US patent, WO 2012092530, July 5, **2012**; i) J. S. Scott, S. S. Bowker, J. deSchoolmeester, S. Gerhardt, D. Hargreaves, E. Kilgour, A. Lloyd, R. M. Mayers, W. McCoull, N. J. Newcombe, D. Ogg, M. J. Packer, A. Rees, J. Revill, P. Schofield, N. Selmi, J. G. Swales, P. R. O. Whittamore, *J. Med. Chem.* **2012**, *55*, 5951.

[7] G. S. Lal, G. P. Pez, R. J. Pesaresi, F. M. Prozonic, H. Cheng, *J. Org. Chem.* **1999**, *64*, 7048.

[8] P. Wipf, C. P. Miller, *J. Org. Chem.* **1993**, *58*, 3604.

[9] T. R. Kelly, F. Lang, *Tetrahedron Lett.* **1995**, *36*, 9293.

[10] T. R. Kelly, F. Lang, *J. Org. Chem.* **1996**, *61*, 4623.

[11] A huge chemical shift difference for pyridine H atoms was observed for **7** and **10**; for **7**: δ = 7.46 (d, J = 8.0 Hz, 1 H) and 8.05 (d, J = 8.0 Hz, 1 H) ppm; for **10**: δ = 8.36 (d, J = 8.0 Hz, 1 H) and 8.51 (d, J = 8.0 Hz, 1 H) ppm.

[12] X. Just-Baringo, P. Bruno, F. Albericio, M. Álvarez, *Tetrahedron Lett.* **2011**, *52*, 5435.

[13] T. Martin, C. Verrier, C. Hoarau, F. Marsais, *Org. Lett.* **2008**, *10*, 2909.

[14] See Experimental Section for conditions and further details of all steps.

[15] F. S. Gibson, S. C. Bergmeier, H. Rapoport, *J. Org. Chem.* **1994**, *59*, 3216.

[16] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem.* **2005**, *44*, 1378.

[17] GE2270 A: a) A. Selva, G. Beretta, N. Montanini, G. S. Saddler, L. Gastaldo, P. Ferrari, R. Lorenzetti, P. Landini, F. Ripamonti, B. P. Goldstein, M. Berti, L. Montanaro, M. Denaro, *J. Antibiot.* **1991**, *44*, 693; b) Thiomuracin I: R. P. Morris, J. A. Leeds, H. U. Naegeli, L. Oberer, K. Memmert, E. Weber, M. J. LaMarche, C. N. Parker, N. Burrer, S. Esterow, A. E. Hein, E. K. Schmitt, P. Krastel, *J. Am. Chem. Soc.* **2009**, *131*, 5946; Thiomuracin derivatives: c) M. J. LaMarche, J. A. Leeds, J. Dzink-Fox, E. Gangl, P. Krastel, G. Neckermann, D. Palestrant, M. A. Patane, E. M. Rann, S. Tiamfook, D. Yu, *J. Med. Chem.* **2012**, *55*, 6934; GE37468A: d) S. Lociuro, P. Tavecchia, E. Marzorati, P. Landini, B. P. Goldstein, M. Denarod, R. Ciabatti, *J. Antibiot.* **1995**, *48*, 780; e) P. Ferrari, L. Colombo, S. Stella, E. Selva, L. F. Zerilli, *J. Antibiot.* **1995**, *48*, 1304; f) F. Marinelli, L. Gastaldo, G. Toppo, C. Quarta, *J. Antibiot.* **1996**, *49*, 880.

[18] Pyridin-2-yl trifluromethanesulfonate (**13**) was prepared according to the procedure described in: W. M. Seganish, P. DeShong, *J. Org. Chem.* **2004**, *69*, 1137.

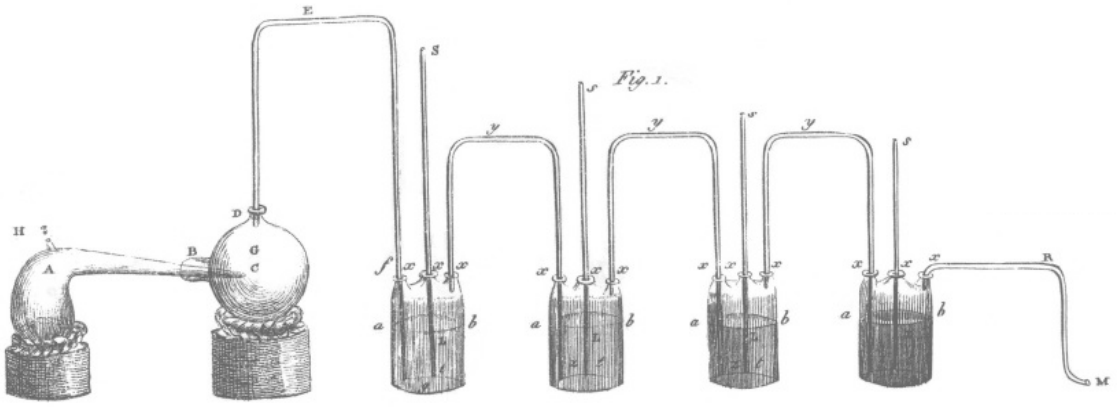
Received: June 14, 2013
Published Online: ■

^{1}H-NMR and ^{13}C-NMR spectra

NMR spectra images are available in the Supporting Information in electronic format.

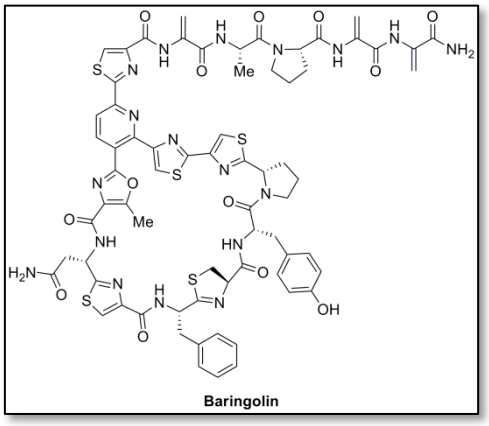
Chapter 5:

Total Synthesis of Baringolin: Assembling of Fragments and Final Steps



TOTAL SYNTHESIS OF BARINGOLIN: ASSEMBLING OF FRAGMENTS AND FINAL STEPS

Total synthesis and stereochemical assignment of baringolin.



Xavier Just-Baringo,[a,b] Paolo Bruno,[a,b] Lars K. Ottesen,[a,b] Librada M. Cañedo,[c] Fernando Albericio,[a,b,d] and Mercedes Álvarez[a,b,e]

Angewandte Chemie Internation Edition **2013**, *52*, 7818-7821.

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.

[b] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.

[c] Instituto Biomar, S.A. Parque Tecnológico de Leon-M10.4 24009 Armunia, León, Spain

[d] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

[e] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

Conspectus

In order to accomplish the total synthesis of baringolin, synthesis of the remaining building blocks, condensation of the different fragments and final steps are required. All these are described in this chapter. With bithiazole-pyrrolidine, Asn-thiazole and central core fragments in hands, the only building blocks that must to be synthesized are the peptidic tail and Phe-derived thiazoline.

The phenylselenocysteine (SecPh)-containing pentapeptide was obtained by means of solid phase peptide synthesis (SPPS) using a Fmoc strategy. Amino acids used were both proteinogenic (Fmoc-L-Pro-OH and Fmoc-L-Ala-OH) and synthetic (Fmoc-L-SecPh-OH). The use of ChemMatrix/Rinkamide resin and OxymaPure as coupling additive, both developed in our group, resulted in high yield and excellent purity of the product with a C-terminal amide. The remaining fragment, Phe-derived thiazoline, was produced by cyclization of Phe-Cys(Trt) dipeptide. Further saponification with Me_3SnOH was crucial to avoid epimerization of the partially reduced azole.

It was of paramount importance to avoid palladium-labile protecting groups for construction of the polyheterocyclic core, whose synthesis is based on palladium-catalyzed reactions. Once the central fragment had been synthesized it could be elongated and different protecting groups were introduced along with the new building blocks. It was key for an efficient strategy to use three allyl-based protecting groups that could be removed at once under palladium catalysis prior to macrocyclization. Final steps included a second saponification with Me_3SnOH, condensation with the pentapeptide and a final oxidation followed by *in situ* elimination to produce the desired dehydroalanine (Dha) residues.

Comparison of both spectroscopic and biological data demonstrated that natural and synthetic baringolin were identical, thus confirming its structure and stereocheminstry.

Natural Products

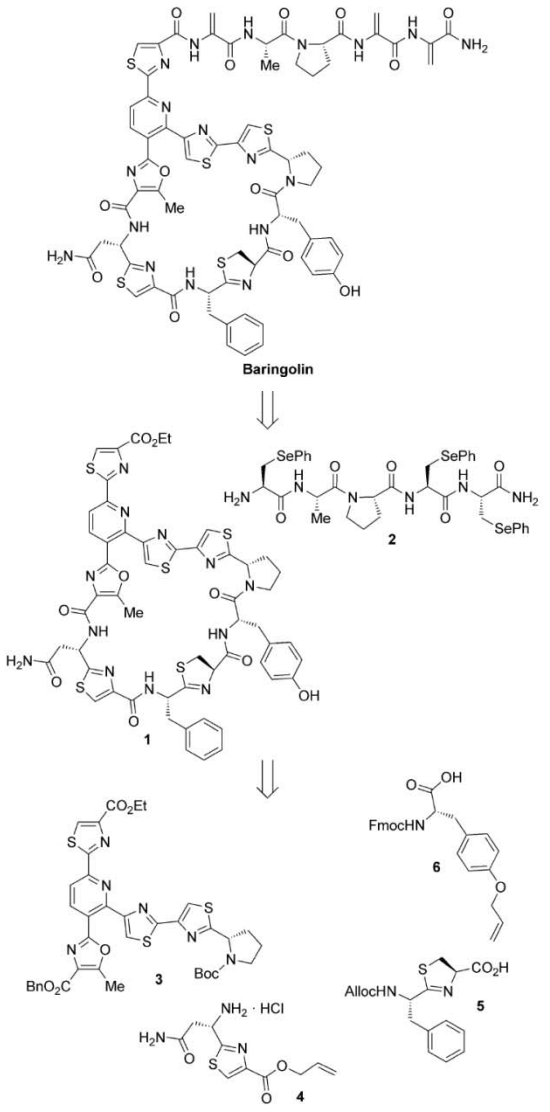
DOI: 10.1002/anie.201302372

Total Synthesis and Stereochemical Assignment of Baringolin**

Xavier Just-Baringo, Paolo Bruno, Lars K. Ottesen, Librada M. Cañedo, Fernando Albericio, and Mercedes Álvarez**

Thiopeptides are a family of naturally occurring, peptide-derived molecules with high sulfur content formed by a central nitrogen-containing six-membered heterocycle decorated with many azoles in a macrocyclic array.[1,2] These natural products have drawn the attention of many research groups mainly owing to their interesting antibiotic activities[3] and their challenging structures.[4] One member of this family, thiostrepton, which is an ingredient of Panolog, has reached the market.

Baringolin is a novel thiopeptide of the *d* series,[1] and thus contains a central 2,3,6-trisubstituted pyridine (for structure see Scheme 1). It was isolated by Biomar SA from fermentation of the marine-derived bacterium *Kucuria sp* MI-67-EC3-038 strain of the Micrococcaceae family, found at the coast of Alicante (southern Spain). Important antibacterial activity at nanomolar concentrations was found in several strains, such as *Staphylococcus aureus*, *Micrococcus luteus*, *Propionibacterium acnes*, and *Bacillus subtilis*.[5] The structure of baringolin was established by spectroscopic methods.[6] The macrocycle in baringolin contains, in addition to three natural amino acids (Tyr, Phe, and Asn), a pyridine, three thiazoles, a methyloxazole ring, and also some motifs not present in other thiopeptides at the same time, such as a thiazoline with an α-chiral center and a pyrrolidine motif derived from a Pro residue. A long peptidic tail is attached to the pyridine through a fourth thiazole. This tail is a pentapeptide containing three methylidenes resulting from dehydration of Ser.[7] Surprisingly and to our knowledge, this is the longest tail reported to date for this family of antibiotics. Baringolin



Scheme 1. Retrosynthesis of baringolin. Boc = *tert*-butyloxycarbonyl, Alloc = allyloxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl.

[*] X. Just-Baringo, Dr. P. Bruno, Dr. L. K. Ottesen, Prof. F. Albericio, Prof. M. Álvarez
Institute for Research in Biomedicine
Barcelona Science Park–University of Barcelona
Baldiri Reixac 10, 08028 Barcelona (Spain)
and
CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona (Spain)
E-mail: fernando.albericio@irbbarcelona.org
mercedes.alvarez@irbbarcelona.org
Homepage: http://www.pcb.ub.edu/fama/htm/home.htm
Prof. F. Albericio
Department of Organic Chemistry, University of Barcelona
08028 Barcelona (Spain)
Prof. M. Álvarez
Laboratory of Organic Chemistry, Faculty of Pharmacy
University of Barcelona, 08028 Barcelona (Spain)
Dr. L. M. Cañedo
Instituto Biomar, S.A. Parque Tecnológico de Leon-M10.4
24009 Armunia, León (Spain)

[**] We gratefully acknowledge support from the Spanish Science and Innovation Ministry, CICYT (CTQ2012-30930) and the Generalitat de Catalunya (2009SGR 1024). X.J. thanks ISCIII for a PFIS grant. We also thank Dr. Antonio Fernandez from Biomar SA for supplying a sample of the natural product for comparison.

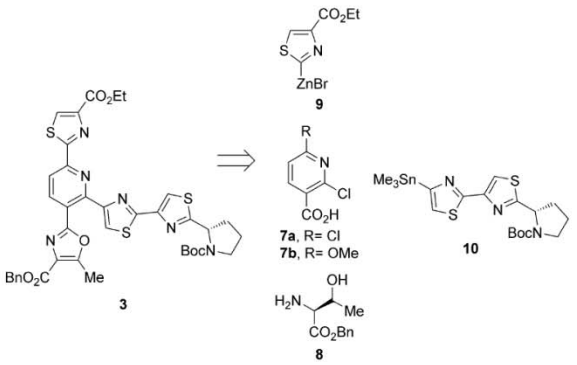
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201302372.

contains seven stereocenters, the configuration of which was considered to be that of natural L-amino acids. A thorough review of literature precedents shows that all thiopeptides of which the stereochemistry has been confirmed to date are made out of L-amino acids; this finding is consistent with their ribosomal origin.[7]

The aim of this work was not only to synthesize this new entity, but also to develop a synthetic strategy that would fulfill our aspiration for an easy construction of closely related new entities to evaluate the structure-activity relationships (SAR) of this interesting family of antibiotics. Moreover, this first synthesis should also serve as the ultimate confirmation of the structure and stereochemical assignment of baringolin. With this premise in mind, the total synthesis of baringolin was designed using only commercially available L-amino acids as the sole source of chirality to confirm if the previous hypothesis was correct.

The retrosynthetic analysis started with the disconnection of the peptidic tail (Scheme 1) to give two synthetic fragments, macrocycle **1** and pentapeptide **2**. In turn, macrocycle **1** could be obtained from trisubstituted pyridine **3** and building blocks **4**–**6**. The concourse of orthogonal protecting groups was key to the success of the synthesis of these complex molecules. This was clearly evidenced in the structure of **3**.

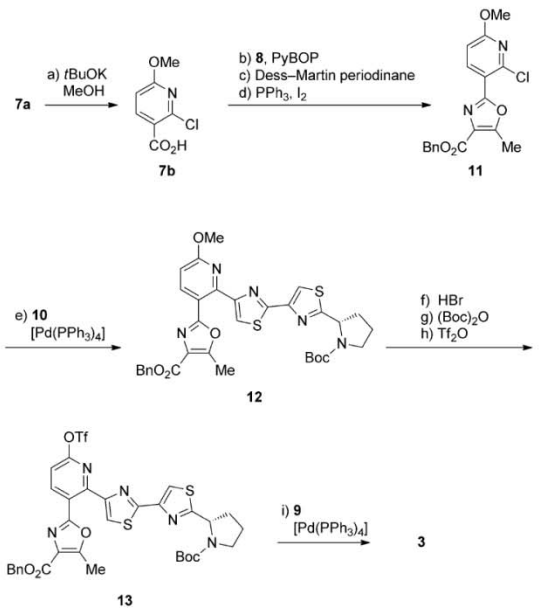
First of all, the synthesis of the central polyheterocyclic core **3** was attempted. A cross-coupling-based strategy[2,8] was chosen, since it would offer a modular approach to the target structure (Scheme 2). The synthetic approach was based on the chemoselective derivatization of commercial 2,6-dichloronicotinic acid (**7a**), which can be easily converted into 2-chloro-6-methoxynicotinic acid (**7b**),[9] which contains two differentiated α-positions along with a carboxylic acid that serves as a precursor of the methyloxazole motif.



Scheme 2. Retrosynthesis of pyridine building block **3**.

The other building blocks for the construction of **3** were benzyl-protected Thr **8**, zinc thiazole **9**,[10] and bithiazole pyrrolidine **10**. The synthesis of the later has been recently reported by us,[11] and it was prepared as a suitable building block for a cross-coupling-based strategy.

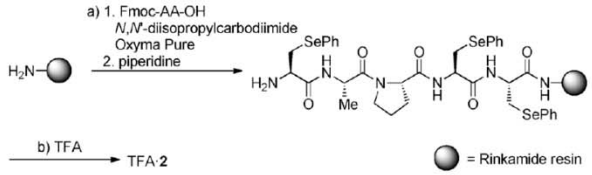
Transformation of pyridine carboxylic acid **7b** into pyridine oxazole **11** (Scheme 3) was performed by condensation with Thr **8**, followed by Dess–Martin oxidation of the side chain into the corresponding methyl ketone and subsequent cyclization to yield the desired biaryl **11**.[12] Stille cross-coupling between chloropyridine **11** and enantiopure trimethyltin derivative **10** rendered methoxypyridine **12**, which could be converted into triflate **13** after acidolysis of the methoxy group. Lastly, **13** was subjected to Negishi cross-coupling conditions with thiazole zinc bromide **9** to render quantitatively the desired central polyheterocyclic core **3**, which was suitably functionalized for subsequent orthogonal deprotections.



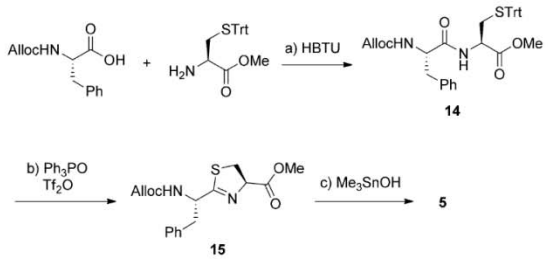
Scheme 3. Synthesis of pyridine building block **3**. Reagents and conditions: a) *t*BuOK, MeOH, 65 °C, 4 days, 85 %; b) **8**, PyBOP, DIPEA, THF, 0 °C, 3 h, 89 %; c) Dess–Martin periodinane, CH_2Cl_2, RT, 6 h, 95 %; d) PPh_3, I_2, NEt_3, CH_2Cl_2, 0 °C to RT, 15 h, 78 %; e) **10**, [Pd(PPh_3)$_4$], 1,4-dioxane, 80 °C, 48 h, 88 %; f) HBr, AcOH, RT, 28 h, 73 %; g) $(Boc)_2O$, NEt_3, CH_2Cl_2, 0 °C, 4 h, 94 %; h) Tf_2O, 2,6-lutidine, DMAP, CH_2Cl_2, 0 °C to RT, 3 h, 88 %; i) **9**, [Pd(PPh_3)$_4$], DMA, 45 °C, 1 h, quant. PyBOP = (1*H*-benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate, DIPEA = diisopropylethylamine, DMAP = 4-(dimethylamino)pyridine, DMA = dimethylacetamide.

Construction of the pentapeptide tail **2** was carried out by solid-phase peptide synthesis (SPPS) using Fmoc chemistry and Rinkamide ChemMatrix resin,[13] using L-alanine and L-proline, as well as Fmoc-L-phenylselenocysteine[14] as precursor of dehydroalanine residues (Scheme 4). Condensation of the different Fmoc-protected amino acids (Fmoc-AA-OH) was carried out with *N*,*N*′-diisopropylcarbodiimide and Oxyma Pure[15] as coupling agents. Deprotection before the introduction of a new Fmoc-AA-OH was achieved with piperidine. The final cleavage with trifluoroacetic acid (TFA) afforded pentapeptide **2** with the free amine and a C-terminal amide ready for condensation with the carboxylic acid of the macrocycle in the last steps of the synthetic process.

Thiazole **4** was prepared by protecting-group manipulation of a previously described synthon (see the Supporting



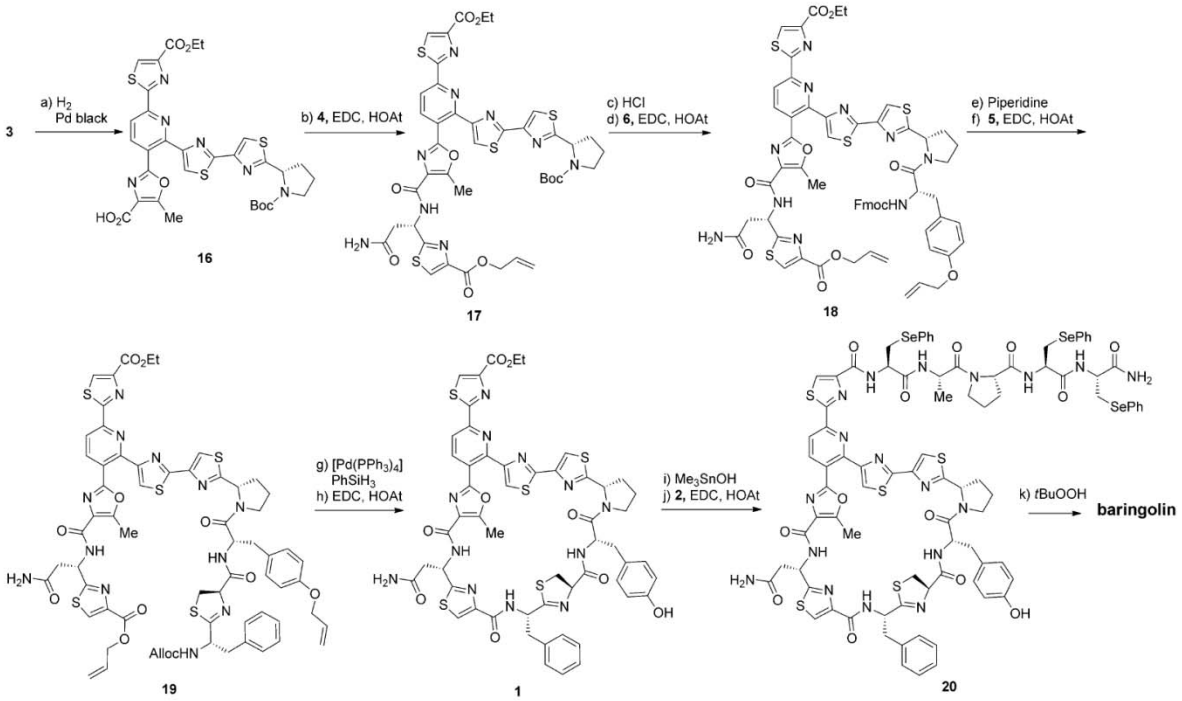
Scheme 4. Synthesis of pentapeptide **2**. Reagents and conditions: a) 1. Fmoc-AA-OH, *N*,*N*′-diisopropylcarbodiimide, Oxyma Pure, DMF, RT, 1.5 h; 2. 20% piperidine in DMF, RT (4 treatments); b) 95% TFA in CH_2Cl_2, RT (4 treatments), 89%.



Scheme 5. Synthesis of thiazoline **5**. Reagents and conditions: a) HBTU, DIPEA, CH_2Cl_2, RT, 1 h, 94%; b) Ph_3PO, Tf_2O, CH_2Cl_2, −20°C, 2 h, 86%; c) Me_3SnOH, CH_2Cl_2, 60°C, 4 h. HBTU = *O*-(1*H*-benzotriazoyl-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, Trt = trityl.

Information).[16] The last building block was the Phe-derived thiazoline **5**, the synthesis of which was addressed by cyclization of the corresponding dipeptide **14** (Scheme 5).[17] Both the condensation and the cyclization steps yielded products **14** and **15** in excellent yields. Further saponification of the methyl ester with trimethyltin hydroxide[18] afforded acid **5** in an excellent diastereomeric ratio (d.r. 96:4).

Next, it was taken into consideration that the thiazoline moiety is prone to epimerization under both basic and acidic conditions, and its manipulation should be limited to as few steps as possible. Deprotection of the carboxylic acid of **3** by hydrogenolysis of the Bn ester was performed with excellent conversion by using Pd black (Scheme 6).[4j,19] Acid **16** was condensed with Asn-derived thiazole **4** to yield **17** by using EDC and HOAt as coupling agents, which would become the reagents of choice for further amide formations. Fmoc-Tyr-OH **6** was introduced next after elimination of the Boc group at the pyrrolidine ring in **17**. Fmoc removal under standard conditions and subsequent condensation with thiazoline **5** rendered **19**, the protected open form of the macrocycle. All allyl-based protecting groups of **19** were simultaneously removed by using catalytic $[Pd(PPh_3)_4]$ and the crude was subjected to macrocyclization conditions in the absence of base, yielding the desired product **1**. Ethyl ester hydrolysis of **1** was carried out by using trimethyltin hydroxide to avoid epimerization of the thiazoline moiety under more common



Scheme 6. Total synthesis of baringolin. Reagents and conditions: a) H_2 (1 atm), Pd black, CH_2Cl_2/EtOH (1:1), RT, 4 h, quant.; b) **4**, EDC, HOAt, DIPEA, DMF, 0°C, 18 h, 82%; c) HCl, dioxane, RT, 7 h; d) **6**, EDC, HOAt, DIPEA, DMF, 0°C, 7 h, 71% (2 steps); e) piperidine, CH_2Cl_2, RT, 3 h, 87%; f) **5**, EDC, HOAt, DIPEA, DMF, 0°C, 3 h, 68% (2 steps); g) $[Pd(PPh_3)_4]$, $PhSiH_3$, CH_2Cl_2, RT, 7 h; h) EDC, HOAt, DMF (1 mM), 0°C to RT, 21 h, 30% (2 steps); i) Me_3SnOH, $ClCH_2CH_2Cl$, 60°C, 19 h; j) **2**, EDC, HOAt, DIPEA, DMF, 0°C, 3 h, 81% (2 steps); k) *t*BuOOH, CH_2Cl_2, RT, 12 h, 66%. EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride, HOAt = 1-hydroxy-7-azabenzotriazole.

and drastic basic aqueous conditions.[18] Condensation of the resulting acid with pentapeptide **2** yielded **20** in excellent yield. Finally, oxidation with *tert*-butyl hydroperoxide and in situ elimination of the resulting phenylselenide oxide groups at room temperature rendered baringolin.

Coelution with a natural product sample of baringolin and comparison of their NMR spectra showed that both compounds are identical (see the Supporting Information). Biological assessment against different strains of methicillin-resistant *S. aureus* (MRSA) showed a minimum inhibitory concentration (MIC) in the nanomolar range for both the natural and synthetic compounds. These results confirm that the structure of baringolin is based only on L-amino acids, as other precedent thiopeptides are.

In conclusion, the total synthesis of baringolin was achieved by a convergent strategy with a good overall yield. The developed convergent synthetic procedure is especially suitable for the preparation of baringolin analogues for SAR-studies, which are currently ongoing. Furthermore, it could also be applied to the preparation of other complex peptides of the same family.

Received: March 20, 2013
Published online: June 18, 2013

Keywords: antibiotics · cross-couplings · heterocycles · natural products · thiopeptides

[1] M. C. Bagley, J. W. Dale, E. Merritt, X. Xiong, *Chem. Rev.* **2005**, *105*, 685 – 714.

[2] R. A. Hughes, C. J. Moody, *Angew. Chem.* **2007**, *119*, 8076 – 8101; *Angew. Chem. Int. Ed.* **2007**, *46*, 7930 – 7954.

[3] a) F. von Nussbaum, M. Brands, B. Hinzen, S. Weigand, D. Häbich, *Angew. Chem.* **2006**, *118*, 5194 – 5254; *Angew. Chem. Int. Ed.* **2006**, *45*, 5072 – 5129; b) A. L. Demain, *Med. Res. Rev.* **2009**, *29*, 821 – 842.

[4] For a selection of landmark thiopeptides central core constructions and total syntheses, see: a) T. R. Kelly, A. Echevarren, N. S. Chandrakumar, Y. Köksal, *Tetrahedron Lett.* **1984**, *25*, 2127 – 2130; b) T. R. Kelly, C. T. Jagoe, Z. Gu, *Tetrahedron Lett.* **1991**, *32*, 4263 – 4266; c) T. R. Kelly, F. Lang, *Tetrahedron Lett.* **1995**, *36*, 5319 – 5322; d) M. Ciufolini, Y.-C. Shen, *J. Org. Chem.* **1997**, *62*, 3804 – 3805; e) M. C. Bagley, K. E. Bashford, C. L. Hesketh, C. J. Moody, *J. Am. Chem. Soc.* **2000**, *122*, 3301 – 3313; f) C. J. Moody, R. A. Hughes, S. P. Thompson, L. Alcaraz, *Chem. Commun.* **2002**, 1760 – 1761; g) K. C. Nicolaou, B. S. Safina, M. Zak, A. A. Estrada, S. H. Lee, *Angew. Chem.* **2004**, *116*, 5197 – 5202; *Angew. Chem. Int. Ed.* **2004**, *43*, 5087 – 5092; h) K. C. Nicolaou, M. Zak, B. S. Safina, S. H. Lee, A. A. Estrada, *Angew. Chem.* **2004**, *116*, 5202 – 5207; *Angew. Chem. Int. Ed.* **2004**, *43*, 5092 – 5097; i) G. Heckmann, T. Bach, *Angew. Chem.* **2005**, *117*, 1223 – 1226; *Angew. Chem. Int. Ed.* **2005**, *44*, 1199 – 1201; j) R. A. Hughes, S. P. Thompson, L. Alcaraz, C. J. Moody, *J. Am. Chem. Soc.* **2005**, *127*, 15644 – 15651; k) M. C. Bagley, K. Chapaneri, J. W. Dale, X. Xiong, J. Bower, *J. Org. Chem.* **2005**, *70*, 1389 – 1399; l) J.-Y. Lu, H.-D. Arndt, *J. Org. Chem.* **2007**, *72*, 4205 – 4212; m) H. M. Müller, O. Delgado, T. Bach, *Angew. Chem.* **2007**, *119*, 4855 – 4858; *Angew. Chem. Int. Ed.* **2007**, *46*, 4771 – 4774; n) D. Lefranc, M. Ciufolini, *Angew. Chem.* **2009**, *121*, 4262 – 4265; *Angew. Chem. Int. Ed.* **2009**, *48*, 4198 – 4201.

[5] "Peptides as bioactive compounds": L. M. Canedo Hernandez, F. Romero Millan, A. Fernandez Medarle, R. I. Fernandez Chimeno, J. C. Hidalgo Villar (Instituto Biomar, S.A., Spain), WO 2012062906, May 18, **2012**.

[6] 1D- and 2D- ^{1}H and ^{13}C NMR spectra, as well HRMS of natural sample.

[7] a) H.-D. Arndt, S. Schoof, J. Y. Lu, *Angew. Chem.* **2009**, *121*, 6900 – 6904; *Angew. Chem. Int. Ed.* **2009**, *48*, 6770 – 6773; b) C. Li, W. L. Kelly, *Nat. Prod. Rep.* **2010**, *27*, 153 – 164.

[8] a) T. Bach, S. Heuser, *Angew. Chem.* **2001**, *113*, 3283 – 3284; *Angew. Chem. Int. Ed.* **2001**, *40*, 3184 – 3185; b) T. Bach, S. Heuser, *J. Org. Chem.* **2002**, *67*, 5789 – 5795; c) S. Schröter, C. Stock, T. Bach, *Tetrahedron* **2005**, *61*, 2245 – 2267.

[9] Y. Hirokawa, I. Fujiwara, K. Suzuki, H. Harada, T. Yoshikawa, N. Yoshida, S. Kato, *J. Med. Chem.* **2003**, *46*, 702 – 715.

[10] **9** was prepared following the procedure described in: O. Delgado, H. M. Müller, T. Bach, *Chem. Eur. J.* **2008**, *14*, 2322 – 2329.

[11] X. Just-Baringo, P. Bruno, F. Albericio, M. Álvarez, *Tetrahedron Lett.* **2011**, *52*, 5435 – 5437.

[12] P. Wipf, C. P. Miller, *J. Org. Chem.* **1993**, *58*, 3604 – 3606.

[13] F. García-Martín, M. Quintanar-Audelo, Y. García-Ramos, L. J. Cruz, C. Gravel, R. Furic, S. Côté, J. Tulla-Puche, F. Albericio, *J. Comb. Chem.* **2006**, *8*, 213 – 220.

[14] N. M. Okeley, Y. Zhu, W. A. van der Donk, *Org. Lett.* **2000**, *2*, 3603 – 3606.

[15] R. Subirós-Funosas, R. Prohens, R. Barbas, A. El-Faham, F. Albericio, *Chem. Eur. J.* **2009**, *15*, 9394 – 9403.

[16] P. Bruno, S. Peña, X. Just-Baringo, F. Albericio, M. Álvarez, *Org. Lett.* **2011**, *13*, 4648 – 4651.

[17] S.-L. You, H. Razavi, J. W. Kelly, *Angew. Chem.* **2003**, *115*, 87 – 89; *Angew. Chem. Int. Ed.* **2003**, *42*, 83 – 85.

[18] K. C. Nicolaou, A. A. Estrada, M. Zak, S. H. Lee, B. S. Safina, *Angew. Chem.* **2005**, *117*, 1402 – 1406; *Angew. Chem. Int. Ed.* **2005**, *44*, 1378 – 1382.

[19] Attempts of hydrogenolysis over palladium on charcoal under different conditions, either heating, working at high pressures or both, only yielded traces of the desired acid.

Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Total Synthesis and Stereochemical Assignment of Baringolin**

Xavier Just-Baringo, Paolo Bruno, Lars K. Ottesen, Librada M. Cañedo, Fernando Albericio, and Mercedes Álvarez**

anie_201302372_sm_miscellaneous_information.pdf

Table of Contents

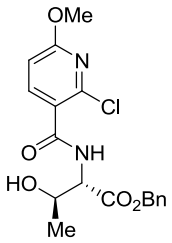
1. General Information

Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were dried using a PureSolv solvent purification system. All other solvents and reagents were used as purchased without further purification. Flash column chromatography was performed on SDS silica gel (60A 35-70 μm) as stationary phase. Analytical thin layer chromatography was performed using aluminium-backed plates coated with Merck Kieselgel 60 F_{254}; compounds were visualized under a UV lamp (254 nm). Melting points were determined in a Buchi Melting Point B540 apparatus in open capillaries. Reverse-phase analytical HPLC was performed on a Waters Alliance separation module 2695 equipped with a Waters XBridge C18 column (4.6×75 mm, 2.5 μm) and a Waters 996 PDA with a photodiode array detector, using MeCN (0.036% TFA) and H_2O (0.045% TFA) as mobile phases. Polarimetry studies were performed on a Perkin–Elmer 241 or Jasco P-2000 polarimeter. IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz or Bruker 600MHz spectrometer. Multiplicity of the carbons was assigned with gHSQC experiments. Stardard abbreviations for off-resonance decoupling were employed: (s) singlet, (d) doublet, (t) triplet, and (q) quartet. The same abbreviations were also used for the multiplicity of signals in ^{1}H NMR, plus: (m) multiplet, (dd) double doublet, (ddd) double doublet of doublets, (dq) double quartet and (bs) broad singlet. Spectra were referenced to appropriate residual solvent peaks ($CDCl_3$, DMSO-d_6 or pyridine-d_5). High-Resolution Mass Spectroscopy (HRMS) was performed on either a LTQ-FT Ultra (Thermo Scientific) or an LCT-Premier (Waters) high resolution mass

spectrometer by the Mass Spectrometry Service of the Institute for Research in Biomedicine (IRB).

2. Experimental Procedures and Characterization

(2*S*,3*R*)-*N*-[(2-Chloro-6-methoxypyridin-3-yl)carbonyl]threonine benzyl ester (S1). DIPEA (15.6 mL, 91.25 mmol) and pyBOP (13.56 g, 26.07 mmol) were added to a solution of 2-chloro-6-methoxynicotinic acid (**7b**)[1] (4.89 g, 26.06 mmol) and L-threonine benzyl ester oxalate salt (8.58 g, 28.68 mmol) in dry THF (130 mL) at 0 °C, and the mixture was stirred for 3 h at the same temperature. EtOAc and saturated aq. $NaHCO_3$ were added to the mixture, which was partially concentrated under reduced pressure. EtOAc (500 mL) was added and then washed with saturated aq. $NaHCO_3$ (250 mL), 1 M HCl (250 mL) and saturated aq. NaCl (250 mL). The organic fraction was dried (Na_2SO_4) and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 6:4 to 1:1 gradient) to yield the title compound as a colorless oil (8.82 g, 89%). $[\alpha]_D$ +18.1 (c = 1.00, CH_2Cl_2). IR (KBr) 3405, 2977, 1743, 1646, 1598, 1478, 1353, 1310, 1172, 1018, 906, 698 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (d, *J* = 6.4 Hz, 3H), 3.98 (s, 3 H), 4.47 (qd, *J* = 6.4 and 2.4 Hz, 1 H), 4.84 (dd, *J* = 8.8 and 2.4 Hz, 1 H), 5.20 (d, *J* = 12.4 Hz, 1 H), 5.26 (d, *J* = 12.4 Hz, 1 H), 6.72 (d, *J* = 8.4 Hz, 1 H), 7.32−7.38 (m, 5 H), 8.05 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 20.5 (q), 54.9 (q), 58.4 (d), 67.8 (t), 68.5 (d), 110.2 (d), 122.8 (s), 128.6 (d), 128.9 (d), 129.0 (d), 135.5 (s), 142.9 (d), 145.5 (s), 164.7 (s), 165.4 (s), 170.8 (s) ppm. HRMS m/z calcd for $C_{18}H_{20}O_5N_2Cl$ (M+H) 379.1055, found 379.1055.



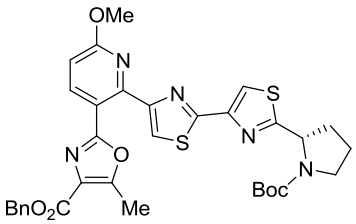
[1] Y. Hirokawa, I. Fujiwara, K. Suzuki, H. Harada, T. Yoshikawa, N. Yoshida, S. Kato, *J. Med. Chem*, **2003**, *46*, 702−715.

Benzyl (*S*)-2-(2-chloro-6-methoxynicotamido)-3-oxobutanoate (S2). Dess-Martin periodinane (10.08 g, 23.76 mmol) was added to a solution of **S1** (7.50 g, 19.80 mmol) in dry CH_2Cl_2 (283 mL). The mixture was then stirred at rt under argon. After 6 h the reaction mixture was poured into a 1:1 mixture of saturated aq. $NaHCO_3$ and saturated aq. $Na_2S_2O_3$ (300 mL) and then extracted with CH_2Cl_2 (2 × 300 mL). The combined organic extracts were dried (Na_2SO_4) and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 7:3) to give the title compound as a colorless oil (7.11 g, 95%). $[\alpha]_D$ 0.0 (c = 2.00, CH_2Cl_2). IR (KBr) 3396, 3033, 2955, 1753, 1728, 1655, 1599, 1476, 1353, 1312, 1268, 1054, 1018 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 2.34 (s, 3 H), 3.99 (s, 3 H), 5.24 (d, *J* = 12.0, 1 H), 5.32 (d, *J* = 12.0, 1 H), 5.44 (d, *J* = 6.0, 1 H), 6.73 (d, *J* = 8.4 Hz, 1 H), 7.34−7.39 (m, 5 H), 8.01 (d, *J* = 6.0 Hz, 1 H), 8.11 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.0 (q), 54.6 (q), 64.1 (d), 68.4 (t), 109.9 (d), 121.4 (s), 128.5 (d), 128.7 (d), 128.8 (d), 134.5 (s), 142.8 (d), 145.5 (s), 163.9 (s), 164.5 (s), 165.6 (s), 197.7 (s) ppm. HRMS m/z calcd for $C_{18}H_{18}O_5N_2Cl$ (M+H) 377.0899, found 377.0899.

2-Chloro-6-methoxy-3-[4-(benzyloxycarbonyl)-5-methyloxazol-2-yl]pyridine (11) NEt_3 (10.4 mL, 74.42 mmol) was added to a stirring solution of PPh_3 (9.76g, 37.21 mmol) and I_2 (9.41 g, 37.21 mmol) in dry CH_2Cl_2 (300 mL) at 0 °C. Subsequently, a solution of **S2** (7.01 g, 18.60 mmol) in dry CH_2Cl_2 (70 mL) was added. The resulting mixture was allowed to reach rt and stirred for 15 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (hexanes/EtOAc, 8:3 to 6:4) to give the title compound as a tan solid (5.18 g, 78%), mp (CH_2Cl_2) 85−89 °C. IR (KBr) 2954, 1735, 1714, 1609, 1475, 1362, 1317, 1091, 108, 756, 698 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 2.69 (s, 3 H), 4.00 (s, 3 H), 5.40 (s, 2 H), 6.75 (d, *J* = 8.6 Hz, 1 H), 7.30−7.41 (m, 3 H), 7.43−7.49 (m, 2 H), 8.22 (d, *J* = 8.6 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.4 (q), 54.5 (q), 66.6 (t), 109.7 (d), 115.4 (s),

128.3 (d), 128.4 (d), 128.5 (s), 128.6 (d), 135.7 (s), 142.0 (d), 146.6 (s), 156.6 (s), 156.8 (s), 162.0 (s), 164.1 (s) ppm. HRMS m/z calcd for $C_{18}H_{16}O_4N_2Cl$ (M+H) 359.0793, found 359.0793.

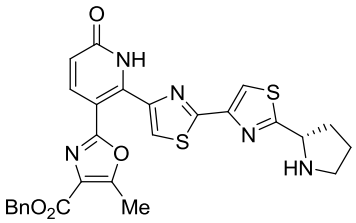
(*S*)-2-[2'-(*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2,4'-bithiazol-4-yl]-6-methoxy-3-[4-(benzyloxycarbonyl)-5-methyloxazol-2-yl]pyridine (12). **11**



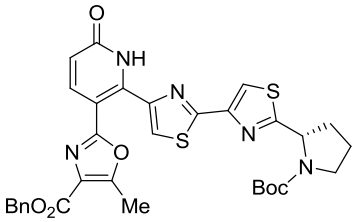
(100 mg, 0.28 mmol), **10**[2] (153 mg, 0.31 mmol) and $Pd(PPh_3)_4$ (32 mg, 0.028 mmol) were added to a flask containing degassed 1,4-dioxane (5.6 mL) and the mixture was stirred at 80 °C for 48 h. The reaction mixture was allowed to reach rt and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 7:3) to yield the title product as a white solid (163 mg, 88%), mp (Et_2O) 65−68 °C. $[\alpha]_D$ −42.0(c = 1.00, CH_2Cl_2). IR (KBr) 3117, 2976, 2883, 1733, 1699, 1615, 1470, 1385, 1325, 1265, 1172, 1100, 1020, 829, 737, 697 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.27−1.55 (m, 9 H), 1.91−2.06 (m, 2 H), 2.18−2.45 (m, 2 H), 2.50 (s, 3 H), 3.38−3.70 (m, 2 H), 4.06 (s, 3 H), 5.10−5.28 (m, 1 H), 5.41 (s, 2 H), 6.79 (d, *J* = 8.6 Hz, 1 H), 7.29−7.39 (m, 4 H), 7.44−7.48 (m, 2 H), 7.94 (d, *J* = 8.6 Hz, 1 H), 8.06 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.0 (q), 23.2 (t), 28.2 and 28.5 (q), 34.0 (t), 46.6 (t), 53.7 (q), 59.3 (d), 66.5 (t), 80.3 (s), 109.9 (d), 114.7 (d), 120.4 (d), 128.2 (s), 128.3 (d), 128.4 (d), 128.6 (d), 135.9 (s), 141.7 (d), 149.4 (s), 154.6 (s), 159.5 (s), 162.4 (s), 164.2 (s) ppm. HRMS m/z calcd for $C_{33}H_{34}O_6N_5S_2$ (M+H) 660.1945, found 660.1940.

[2] X. Just-Baringo, P. Bruno, F. Albericio, M. Álvarez, *Tetrahedron Lett.* **2011**, *52*, 5435–5437.

(*S*)-5-[4-(Benzyloxycarbonyl)-5-methyloxazol-2-yl]-6-[2'-(pyrrolidin-2-yl)-2,4'-bithiazol-4-yl]pyridin-2(1*H*)-one (S3). 33% HBr in acetic acid (13.2 mL, 72.93 mmol) was added to a stirring solution of **12** (4.01 g, 6.08 mmol) in acetic acid (235 mL). After 28 h of stirring at rt the mixture was concentrated *in vacuo*. The residue was partitioned between CH_2Cl_2 (400 mL) and saturated aq. $NaHCO_3$ (400 mL). Solid Na_2CO_3 was added until pH = 8 was reached and the aq. layer was extracted with more CH_2Cl_2 (2 × 400 mL). The combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 94:6). The title compound was obtained as a yellow solid (2.43 g, 73%), mp (CH_2Cl_2) 96−100 °C. $[\alpha]_D$ −55.8 (c = 1.00, CH_2Cl_2). IR (KBr) 3439, 3091, 2964, 2870, 1715, 1656, 1622, 1343, 1183, 1107 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.83−1.96 (m, 2 H), 2.02−2.11 (m, 1 H), 2.31−2.41 (m, 1 H), 2.65 (s, 3 H), 3.09−3.22 (m, 2 H), 4.65 (dd, *J* = 8.2 and 5.4 Hz, 1 H), 5.40 (s, 2 H), 6.62 (d, *J* = 9.6 Hz, 1 H), 7.34−7.44 (m, 3 H), 7.45−7.50 (m, 2 H), 7.87 (d, *J* = 9.6 Hz, 1 H), 7.94 (s, 1 H), 8.66 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.1 (q), 25.5 (t), 34.0 (t), 46.9 (t), 59.4 (d), 66.7 (t), 104.1 (s), 117.5 (d), 120.0 (d), 124.4 (d), 128.3 (d), 128.4 (d), 128.7 (d), 135.6 (s), 139.3 (s), 142.0 (d), 145.5 (s), 148.3 (s), 156.4 (s), 156.6 (s), 161.8 (s), 162.0 (s), 162.5 (s), 179.9 ppm. HRMS m/z calcd for $C_{27}H_{24}O_4N_5S_2$ (M+H) 546.1264, found 546.1261.

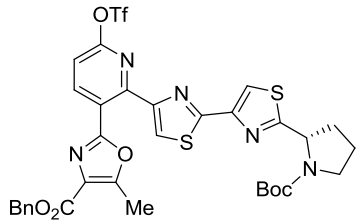


(*S*)-6-[2'-(*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2,4'-bithiazol-4-yl]-5-[4-(benzyloxycarbonyl)-5-methyloxazol-2-yl]pyridin-2(1*H*)-one (S4). *tert*-Butyl dicarbonate (565 μL, 2.46 mmol) and NEt_3 (685 μL, 4.92 mmol) were added to a flask charged with a solution of the free **S3** (1.34 g, 2.46 mmol) in dry CH_2Cl_2 (50 mL) at 0 °C and the mixture was stirred for 4 h at the same temperature. The reaction was poured into brine, extracted with CH_2Cl_2 (3 × 100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2) to



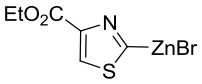
yield the title product as a yellowish solid (1.50 g, 94%), mp (CH_2Cl_2) 100−105 °C. $[\alpha]_D$ −65.4 (c = 0.50, CH_2Cl_2). IR (KBr) 3090, 2975, 2881, 1698, 1657, 1390, 1174, 1108 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.28−1.54 (m, 9 H), 1.92−2.08 (m, 2 H), 2.22−2.45 (m, 2 H), 2.65 (s, 3 H), 3.40−3.70 (m, 2 H), 5.11−5.29 (m, 1 H), 5.40 (s, 2 H), 6.62 (d, *J* = 9.4 Hz, 1 H), 7.32−7.42 (m, 3 H), 7.43−7.48 (m, 2 H), 7.87 (d, *J* = 9.4 Hz, 1 H), 7.94 (s, 1 H), 8.69 (m, 1 H), 10.78 (brs, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.1 (q), 23.3 and 23.9 (t), 28.3 and 28.4 (q), 32.7 and 34.0 (t), 46.6 and 46.9 (t), 59.0 and 59.3 (d), 66.7 (q), 80.5 (s), 104.1 (s), 116.7 and 117.1 (d), 120.0 (d), 124.4 (d), 128.3 (d), 128.4 (d), 128.7 (d), 135.6 (s), 139.3 (s), 142.0 (d), 145.6 (s), 147.9 (s), 154.1 (s), 156.4 (s), 156.7 (s), 161.8 (s), 162.0 (s), 176.7 (s) ppm. HRMS m/z calcd for $C_{32}H_{32}O_6N_5S_2$ (M+H) 646.1789, found 646.1786.

(*S*)-2-[2'-(*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2,4'-bithiazol-4-yl]-3-[4-(benzyloxycarbonyl)-5-methyloxazol-2-yl]-6-(trifluoromethanesulfonyloxy) pyridine (13). 2,6-lutidine (358 μL, 3.07 mmol) and trifluoromethanesulfonic anhydride (443 μL, 2.63 mmol) were added to a stirring solution of pyridone **S4** (1.42 g, 2.20 mmol) and DMAP (54 mg, 0.44 mmol) in dry CH_2Cl_2 (22 mL) at 0 °C. After 2 h the mixture was allowed to reach rt and was then stirred at this temperature for 2.5 h. The reaction mixture was diluted with CH_2Cl_2 (150 mL), washed with H_2O (150 mL), dried (Na_2SO_4), and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 7:3) to yield the title product as a white solid (1.50 g, 88%), mp (CH_2Cl_2) 60−63 °C. $[\alpha]_D$ −43.5 (c = 1.00, CH_2Cl_2). IR (KBr) 3117, 2976, 2883, 1734, 1699, 1425, 1389, 1213, 1175, 1100, 962, 888, 857, 813, 602 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.27−1.56 (m, 9 H), 1.93−2.02 (m, 2 H), 2.18−2.44 (m, 2 H), 2.53 (s, 3 H), 3.38−3.70 (m, 2 H), 5.08−5.27 (m, 1 H), 5.43 (s, 2 H), 7.19 (d, *J* = 8.3 Hz, 1 H), 7.29 (s, 1 H), 7.32−7.40 (m, 3 H), 7.44−7.49 (m, 2 H), 8.16 (s, 1 H), 8.25 (d, *J* = 8.3 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.0 (q), 23.2 and 23.9 (t), 28.3 and 28.4 (q), 32.6 and 34.0 (t), 46.6 and 46.9 (t), 58.9 and 59.3 (d), 66.7



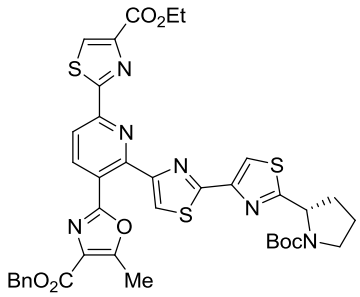
(t), 80.4 (s), 113.0 (d), 114.9 (d), 117.0 (s), 120.2 (s), 121.6 (s), 122.9 (d), 128.4 (d), 128.6 (2d), 135.7 (s), 144.6 (d), 152.3 (s), 155.6 (s), 157.4 (s), 162.1 (s), 184.8 (s) ppm. HRMS m/z calcd for $C_{33}H_{31}O_8N_5F_3S_3$ (M+H) 778.1281, found 778.1290.

4-(Ethoxycarbonyl)thiazol-2-yl zinc bromide (9). This reagent was prepared



using the method described by Bach and coworkers.[3] To an oven-dried flask charged with zinc dust (4.04 g, 61.77 mmol) under inert atmosphere were added dry *N*,*N*-dimethylacetamide (DMA) (62 mL) and 1,2-dibromoethane (615 μL, 7.13 mmol). The mixture was heated with a heat gun until bubbling was observed and then allowed to cool down to rt. This procedure was repeated twice and then chlorotrimethylsilane (1.7 mL, 13.07 mmol) was added and the suspension was stirred at rt. After 5 min a solution of ethyl 2-bromothiazole-4-carboxylate (4.67 g, 19.80 mmol) in dry DMA (32 mL) was added. After 30 min the suspension was allowed to settle for at least 30 min. This procedure gave a 0.21 M solution of the title compound. The reagent solution was freshly prepared before use.

(*S*)-2-[2'-(*N*-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)-2,4'-bithiazol-4-yl]-6-[4-(ethoxycarbonyl)thiazol-2-yl]-3-[4-(benzyloxycarbonyl)-5-methyloxazol-2-yl]pyridine (3). A 0.21 M solution of **9** (89 mL, 18.64 mmol) in DMA was added

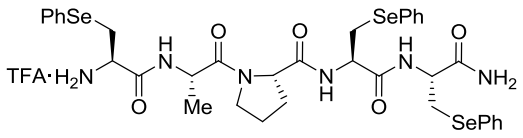


to a flask charged with pyridyl triflate **13** (1.45 g, 1.86 mmol) and $Pd(PPh_3)_4$ (430 mg, 0.37 mmol). The resulting mixture was stirred at 45 °C for 1 h. The reaction mixture was filtered through a pad of Celite 545 with the aid of CH_2Cl_2. The organic solution was washed with H_2O (2 × 250 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Upon addition of EtOAc, a precipitate was formed. After filtration under vacuum the title product was obtained as a tan solid (1.46 g, quant.), mp (EtOAc) 214−218 °C. $[\alpha]_D$ −38.9 (c = 1.00, CH_2Cl_2). IR

[3] O. Delgado, H. M. Müller, T. Bach, *Chemistry Eur. J.* **2008**, *14*, 2322

(KBr) 3114, 2976, 1732, 1702, 1385, 1211, 1109, 1078, 761, 703 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.27−1.58 (m, 12 H), 1.91−2.05 (m, 2 H), 2.20−2.44 (m, 2 H), 2.52 (s, 3 H), 3.39−3.69 (m, 2 H), 4.48 (q, *J* = 7.2 Hz, 2 H), 5.10−5.29 (m, 1 H), 5.43 (s, 2 H), 7.30−7.41 (m, 4 H), 7.45−7.50 (m, 2 H), 8.20 (s, 1 H), 8.26 (d, *J* = 8.0, 1 H), 8.33 (s, 1 H), 8.38 (d, *J* = 8.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.0 (q), 14.4 (q), 23.2 and 23.9 (t), 28.3 and 28.4 (q), 32.7 and 34.0 (t), 46.6 and 46.9 (t), 58.9 and 59.3 (d), 61.2 (t), 66.6 (t), 80.3 (s), 114.8 and 115.1 (d), 118.5 (d), 121.2 (d), 122.6 (s), 128.3 (d), 128.4 (d), 128.5 (s), 128.6 (d), 130.2 (d), 132.0 and 132.1 (s), 135.8 (s), 140.6 (d), 148.6 (s), 151.1 (s), 153.8 (s), 156.8 (s), 158.6 (s), 161.3 (s), 162.2 (s), 168.8 (s), 175.4 (s), 176.3 (s) ppm. HRMS m/z calcd for $C_{38}H_{37}O_7N_6S_3$ (M+H) 785.1880, found 785.1883.

Northern Peptide 2. The Rink-Amide Chem-Matrix resin (900 mg, loading = 0.52 mmol/g;) was swellen in MeOH, then in DMF and finally in CH_2Cl_2. Fmoc-Sec(Ph)-OH[4] (655 mg, 1.4 mmol), preactivated by vigorous shaking for 4 min with DIPCDI (240 μL, 1.54 mmol) and Oxyma Pure (219 mg, 1.54 mmol) in DMF (10 mL), was poured onto the resin and the resulting mixture was gently shaken for 1.5 h. The resin was then washed with DMF and CH_2Cl_2 (5 × 10 mL). The *N*-terminus was deprotected using 20% piperidine in DMF (treatments of 2 × 1 min × 10 mL then 2 × 5 min × 10 mL). The resin was then washed with CH_2Cl_2 and DMF (5 × 10 mL each). Loading onto the resin (0.249 mmol/g, 48%) was determined through measuring dibenzofulvene absorbance at 290 nm of cleavage solutions and washings.

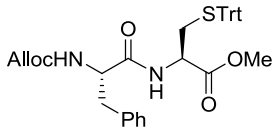


Elongation of the peptide proceeded as follows. Fmoc-AA-OH (1.08 mmol), was preactivated by vigorous shaking for 4 min in the presence of DIPCDI (184 μL, 1.19 mmol) and Oxyma Pure (169 mg, 1.19 mmol) in DMF (7 mL), and was then poured onto the resin. The resulting mixture was gently shaken for 1 h. Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the pentapeptide. The peptide was cleaved from the resin by

[4] N. M. Okeley, Y. Zhu, W. A. van der Donk, *Org. Lett.* **2000**, *2*, 3603-3606

treatment with 95% TFA in CH_2Cl_2 (4 × 20 min × 10 mL) at rt followed by filtration and collection of the filtrate. Next, washing of the resin with CH_2Cl_2 (6 × 10 mL) was performed. Most TFA was removed under vacuum and the resulting concentrated solution was poured into cold Et_2O. Centrifugation and pouring off the solvent yielded the crude northern peptide as a white powder (196 mg, 89% based on loading of the resin). Purity (100%) was determined by HPLC (linear gradient: 0 to 100% acetonitrile in H_2O over 8 min; flow rate = 1.0 mL/min; t_R = 5.98 min). mp (EtOAc) 102−105 ºC. $[\alpha]_D$ = −130.2 (c = 1.00, CH_2Cl_2). IR (neat): 3289, 3052, 1668, 1630, 1197, 1130 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.24 (d, *J* = 6.7 Hz, 3H), 1.76-1.98 (m, 3H), 1.98-2.12 (m, 1H), 3.05-3.15 (m, 2H), 3.15-3.35 (m, 4H), 3.52-3.62 (m, 2H), 4.07 (m, 1H), 4.26-4.44 (m, 3H), 4.46-4.60 (m, 1H), 7.20-7.37 (m, 9H), 7.42-7.57 (m, 6H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.25 (d, *J* = 7.5 Hz, 1H), 8.39 (bs, 4H), 8.84 (7.6, 1H). ^{13}C NMR (100 MHz DMSO-d_6): δ 18.1(q), 25.4 (t), 28.1 (t), 29.5 (t), 29.7 (t), 29.9 (t), 47.5 (d), 47.8 (t), 52.6 (d), 53.8 (d), 54.1 (d), 60.8 (d), 127.7 (d), 127.8 (d), 128.2 (d), 129.9 (s), 130.2 (d), 130.9 (s), 131.1 (s), 132.4 (d), 132.7 (d), 132.9 (d), 167.2 (s), 170.8 (s), 170.9 (s), 172.4 (s), 172.6 (s) ppm. HRMS m/z calcd for $C_{35}H_{43}O_5N_6Se_3$ (M+H) 867.0791, found 867.0793.

Alloc-L-Phe-L-Cys(Trt)-OMe (14). A solution of H-Cys(Trt)-OMe (5.37 g, 14.23 mmol) in dry CH_2Cl_2 (40 mL) was added *via cannula* over a solution of crude Alloc-L-Phe-OH (4.93 g, 19.78 mmol) in dry CH_2Cl_2 (40 mL) under N_2 atmosphere. HBTU (10.6 g, 27.95 mmol) was then added in one portion while stirring. Slow addition of DIPEA (9.6 mL, 56.08 mol) followed. The reaction mixture was stirred at rt for 1 h, and was then diluted with ethyl acetate (200 mL) and washed with saturated aqueous $NaHCO_3$ (150 mL), H_2O (150 mL), saturated aqueous NH_4Cl (2 × 150 mL), H_2O again (150 mL) and finally brine (150 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash column chromatography (hexanes/EtOAc 80:20 to 60:40) yielded the title compound as a white solid (8.12 g, 94%), mp (EtOAc) 111–115 °C. $[\alpha]_D$ = −2.99 (c = 0.92, CH_2Cl_2). IR (neat): 3314, 3059, 2918, 1744, 1725,

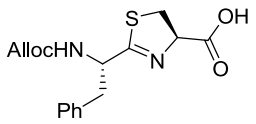


1666, 1536, 1494, 1443, 1213, 1034, 743, 700 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.56 (dd, *J* = 12.5, 5.0 Hz, 1H), 2.64 (dd, *J* = 12.5, 5.8 Hz, 1H), 3.02 (dd, *J* = 13.9, 7.1 Hz, 1H), 3.08 (dd, *J* = 13.9, 6.3 Hz, 1H), 3.69 (s, 3H), 4.37 (m, 1H), 4.44 (ddd, J = 7.6, 5.8, 5.0 Hz, 1H), 4.54 (d, *J* = 5.6 Hz, 2H), 5.16 – 5.32 (m, 3H), 5.79 – 5.95 (m, 1H), 6.11 (d, *J* = 7.6 Hz, 1H), 7.10 – 7.46 (m, 20H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 33.8 (t), 39.0 (t), 51.7 (d), 53.0 (q), 56.2 (d), 66.3 (t), 67.3 (d), 118.3 (t), 127.3 (d), 127.4 (d), 128.4 (d), 129.1 (d), 129.8 (d), 129.8 (d), 132.9 (s), 136.5 (s), 144.6 (s), 156.0 (s), 170.6 (s), 170.8 (s) ppm. HRMS m/z calcd for $C_{36}H_{36}O_5N_2NaS$ (M+Na) 631.2237, found 631.2239.

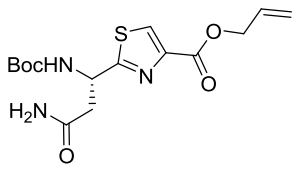
Methyl 2-((*S*)-1-(allyloxycarbonylamino)-2-phenylethyl)-(*R*)-4,5-dihydro-thiazole-4-carboxylate (15). Tf_2O (1.56 mL, 9.27 mmol) was added dropwise over a cooled (ice-H_2O bath) solution of PPh_3O (5.18 g, 18.59 mmol) in dry CH_2Cl_2 (60 mL) under N_2 atmosphere. Stirring was maintained at 0 °C for 15 min and a white precipitated was formed. The mixture was then cooled down to −20 °C and a solution of **14** (3.77 g, 6.19 mmol) in dry CH_2Cl_2 (60 mL) was added over 30 min. The resulting mixture was stirred at −20 °C for 2 h, then the reaction was quenched by the addition of saturated aqueous $NaHCO_3$ (120 mL). The mixture was allowed to warm to rt while stirring. The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 70 mL). Combined organic layers were washed with brine (350 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the crude product by flash column chromatography (hexanes/EtOAc 90:10 to 60:40) yielded the title compound as a white solid (1.86 g, 86%), mp (EtOAc) 53–55 °C. $[\alpha]_D$ = −30.1 (c = 0.04, $CHCl_3$). IR (neat) 3315, 3026, 2949, 2924, 1726, 1232, 1047 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.16 (dd, *J* = 13.6, 6.4 Hz, 1H), 3.30 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.59 (dd, *J* = 11.2, 10.0 Hz, 1H), 3.69 (dd, *J* = 11.2, 8.8 Hz, 1H), 3.85 (s, 3H), 4.61–4.60 (m, 2H), 4.97–4.92 (m, 1H), 5.36–5.14 (m, 3H), 5.50 (d, *J* = 7.6 Hz, 1H), 5.99–5.89 (m, 1H), 7.36–7.24 (m, 5H), ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.8 (t), 40.4 (t), 53.0 (q), 54.7 (d), 66.1 (t), 78.1 (d), 118.0 (t), 127.2

(d), 128.7 (d), 129.9 (d), 132.9 (d), 136.1 (s), 155.6 (s), 171.0 (s), 175.6 (s) ppm. HRMS m/z calcd for $C_{17}H_{21}O_4N_2S_1$ (M+H) 349.1216, found 349.1217.

(*S*)-Phe-(4*R*)-Thiazoline-OH 5. Trimethyltin hydroxide (488 mg, 2.87 mg) was added over a solution of **15** (500 mg, 1.44 mmol) in 1,2-dichloroethane (7.2 mL) charged into a Schlenck tube. The tube was sealed and the mixture stirred at 60 °C. After 4 h the mixture was allowed to reach rt, diluted with CH_2Cl_2 (100 mL), washed with 5% HCl (100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. According to ^{1}H NMR analysis of the crude acid, it is obtained in approximately 50% purity (contains trimethyltin residues). HPLC analysis (Waters XBridge C18 2.5 μm 4.6 × 75 mm analytical column, flow rate 1 mL min^{-1}; H_2O (0.045% TFA) : MeCN (0.036% TFA), 30% to 40% MeCN, 8 min (detected at 220 nm) showed a dr of 96:4. The crude product was used in further reactions.



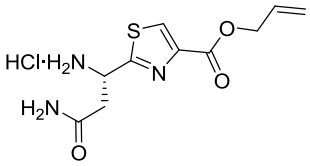
(*S*)-allyl 2-(3-amino-1-(*tert*-butoxycarbonylamino)-3-oxopropyl)thiazole-4-carboxylate (S5). $NaHCO_3$ (2.14 g, 25.57 mmol) was added in one portion over a solution of Boc-Asn-Thz-OH[5] (1.12 g, 3.55 mmol) in dry DMF (20 mL) and the resulting suspension was stirred at rt for 10 min. Allyl bromide (6.5 mL, 74.58 mmol) was then added and the suspension was stirred at rt for 20 h. The reaction mixture was diluted with EtOAc (200 mL) and H_2O (200 mL) and the layers were separated. The organic layer was washed with brine (3 × 100 mL) then dried over Na_2SO_4, filtered and concentrated. Purification by flash column chromatography (EtOAc) yielded the title compound as a white powder (1.04 g, 83%), mp (EtOAc) 146–153 °C (the compound turned waxy above 98 °C). $[\alpha]_D$ = −14.5 (c = 0.99, CH_2Cl_2). IR (neat): 3341, 2978, 2928, 1712, 1672, 1485, 1367, 1211, 1166, 1097 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H), 2.85 (dd, *J* = 4.5, 15.3 Hz, 1H), 3.27 (dd, *J* = 4.1, 15.3 Hz, 1H), 4.81 (d, *J* = 7.1 Hz, 1H), 5.24 – 5.45 (m, 3H), 5.65 (bs, 1H), 6.00



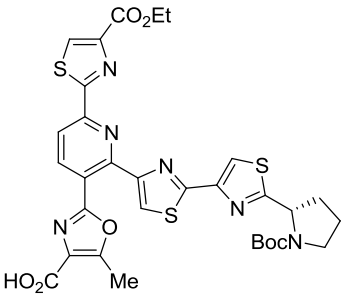
[5] P. Bruno, S. Peña, X. Just-Baringo, F. Albericio, M. Álvarez, *Org. Lett.* **2011**, *17*, 4648-4651.

(m, 1H), 6.16 (bs, 1H), 6.60 (d, J = 8.6 Hz, 1H), 8.09 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3 (q), 38.6 (t), 50.0 (d), 65.9 (t), 80.4 (s), 118.9 (t), 128.1 (d), 131.8 (d), 146.6 (s), 155.3 (s), 160.9 (s), 172.7 (s), 174.4 (s). HRMS m/z calcd for $C_{15}H_{22}O_5N_3S$ (M+H) 356.1275, found 356.1278.

(*S*)-allyl 2-(3-amino-1-(tert-butoxycarbonylamino)-3-oxopropyl)thiazole-4-carboxylate hydrochloride (4). 4 N HCl in 1,4-dioxane (19.4 mL, 77.40 mmol) was added to a stirring solution of **S5** (1.38 g, 3.87 mmol) in 1,4-dioxane (20 mL). The mixture was stirred at rt for 4 h. Toluene was added and volatiles were evaporated under reduced pressure. The title compound was obtained as a pale solid (1.15g, quant.). It was used without further purification.



Polyheterocyclic core carboxylic acid 16. Pd black (205 mg, 0.26 mmol) was added to a stirring solution of central fragment **3** (205 mg, 0.26 mmol) in CH_2Cl_2/EtOH (1:1, 14 mL). The resulting mixture was stirred under H_2 (1 bar) at rt for 3.5 h. The mixture was filtered through a pad of Celite 545 with the aid of CH_2Cl_2/MeOH (1:1) and then concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/*i*PrOH, 8:2) to obtain the title compound as a pale solid (180 mg, quant.).



Allyl ester 17. DIPEA (61 μL, 354 μmol), EDC·HCl (37 mg, 195 μmol) and HOAt (27 mg, 195 μmol) were added to a stirring solution of polyheterocyclic core carboxylic acid **16** (123 mg, 177 μmol)) and thiazole hydrochloride **4** (50 mg, 195 μmol) in dry DMF (1.6 mL) at 0 °C. Next, the reaction mixture was allowed to reach rt and was stirred for 18 h. The mixture was diluted with EtOAc (50 mL), washed with saturated aq. NH_4Cl (25 mL), saturated aq. $NaHCO_3$ (25 mL) and H_2O (2 × 25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (hexanes/EtOAc, 2:8) to yield the title product as a white solid (135 mg, 82%), mp (CH_2Cl_2) 133−135 °C. $[\alpha]_D$ +53.3 (c = 1.00, CH_2Cl_2). IR (KBr) 3406, 2975, 2925, 1693, 1678, 1626, 1501, 1386, 1234, 1207, 763 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.23−1.51 (m, 12 H), 1.84−2.03 (m, 2 H), 2.12−2.43 (m, 2 H), 2.45−3.33 (m, 5 H), 3.36−3.64 (m, 2 H), 4.42 (q, *J* = 7.1 Hz, 2 H), 4.76 (d, *J* = 5.2 Hz, 2 H), 5.05−5.18 (m, 1 H), 5.22 (d, *J* = 6.0 Hz, 1 H), 5.33 (d, *J* = 17.6 Hz, 1 H), 5.66−5.88 (m, 1 H), 5.89−6.02 (m, 1 H), 6.30−6.71 (m, 1 H), 7.34−7.45 (m, 1 H), 7.98−8.16 (m, 2 H), 8.20 (d, *J* = 8.2 Hz, 1 H), 8.27 (s, 1 H), 8.30 (d, *J* = 8.2 Hz, 1 H), 8.51−8.82 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 11.6 and 11.8 (q), 14.3 (q), 23.2 and 23.8 (t), 28.2 and 28.4 (q), 33.1 and 33.9 (t), 38.0 and 38.6 (t), 46.6 and 47.1 (t), 47.5 and 47.9 (d), 59.2 (d), 61.6 (t), 65.8 (t), 80.3 and 80.6 (s), 114.7 and 115.0 (d), 118.6 (d), 118.7 and 118.8 (t), 121.2 and 121.6 (d), 122.6 (s), 128.0 (d), 128.4 and 128.5 (s), 129.7 (s), 130.2 (d), 131.8 (d), 131.9 (s), 132.0 and 132.1 (s), 139.6 and 140.3 (d), 146.5 (s), 148.5 (s), 149.3 (s), 150.7 and 151.1 (s), 153.8 (s), 154.2 and 154.8 (s), 156.7 and 157.6 (s), 160.8 (s), 161.3 (s), 161.6 (s), 168.7 (s), 171.9 and 172.3 (s), 172.9 and 173.6 (s), 176.0 and 176.2 (s) ppm. HRMS m/z calcd for $C_{41}H_{42}O_9N_9S_4$ (M+H) 932.1983, found 932.1996.

Tyrosine-coupled allyl ester 18. 4 M HCl in 1,4-dioxane (0.75 mL) was added over a stirring solution of allyl ester **17** (135 mg, 145 μmol) in 1,4-dioxane (0.75 mL) at rt and the mixture was stirred for 6.5 h. The reaction mixture was concentrated under reduced pressure to obtain the hydrochloride of the Boc-deprotected product (133 mg, quant.). DIPEA (53 μL, 308 μmol), EDC·HCl (32 mg, 169 μmol) and HOAt (23 mg, 169 μmol) were added to a stirring solution of the Boc-deprotected product hydrochloride (133 mg, 154 μmol) and **6** (75 mg, 169 μmol) in dry DMF (1.4 mL) at 0 °C. The reaction mixture was then allowed to reach rt and was stirred for 7 h. The mixture was diluted with EtOAc (50 mL), washed with saturated aq. NH_4Cl (50 mL), saturated aq. $NaHCO_3$ (2 × 50 mL) and H_2O (50 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/EtOAc, 1:1 to 7:3) to yield the title product as a white solid (138 mg, 71%), mp (CH_2Cl_2) 137−140 °C. $[\alpha]_D$ +21.5 (c = 1.00, CH_2Cl_2). IR (KBr) 3344, 3116, 2980, 1720, 1669, 1510, 1446, 1425, 1321, 1237, 1209, 1101, 1079, 1030, 759, 740 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.43−1.49 (m, 3 H), 1.94−2.32 (m, 4 H), 2.50−3.38 (m, 7 H), 3.39−3.85 (m, 2 H), 4.05−4.20 (m, 1 H), 4.22−4.57 (6 H), 4.68−4.84 (m, 3 H), 5.16−5.50 (m, 5 H), 5.58 (s, 1 H), 5.77−6.10 (m, 2 H), 6.23 (d, *J* = 8.4 Hz, 1 H), 6.56 (bs, 2 H), 6.65−6.92 (m, 2 H), 6.97−7.19 (m, 2 H), 7.23−7.47 (m, 5H), 7.49−7.60 (m, 2 H), 7.61−7.80 (m, 2 H), 8.05−8.10 (m, 1 H), 8.11−8.18 (m, 1 H), 8.24 (d, *J* = 8.2, 1 H), 8.30−8.34 (m, 1 H), 8.36 (d, *J* = 8.2 Hz, 1 H), 8.65 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 11.8 (q), 14.4 (q), 24.5 (t), 31.8 (t), 38.1 (t), 38.3 (t), 47.0 (d), 47.4 (t), 47.7 (d), 53.9 (d), 58.7 (d), 61.6 (t), 65.9 (t), 67.1 (t), 68.7 (t), 114.8 (d), 115.1 (d), 115.8 (d) 117.5 (t), 118.6 (d), 118.8 (t), 119.9 (d), 121.6 (d), 122.6 (s), 125.2 (d), 127.0 (d), 127.6 (d), 128.0 (s), 128.2 (d),130.2 (d), 130.4 (d), 131.8 (s), 133.2 (d), 139.8 (d), 141.2 (s), 143.8 (s), 146.5 (s), 148.6 (s), 148.9 (s), 150.9 (s), 151.1 (s), 153.9 (s), 154.2 (s), 155.9 (s), 157.0 (s), 157.4 (s), 157.6 (s), 157.7 (s), 160.8 (s), 161.3 (s), 161.3 (s); 161.7 (s), 168.7

(s), 171.4 (s), 172.0 (s), 172.4 (s); 173.2 (s) ppm. HRMS m/z calcd for $C_{63}H_{57}O_{11}N_{10}S_4$ (M+H) 1257.3086, found 1257.3114.

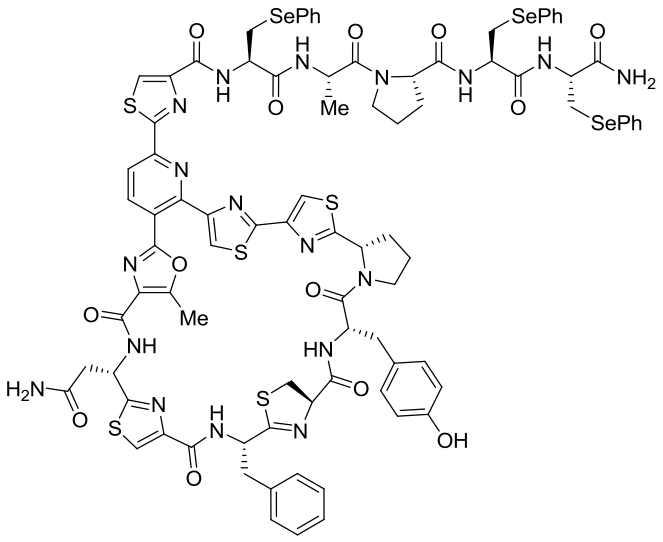
Free amino tyrosine adduct (S6) Piperidine (275 μL, 2.75 mmol) was added to a stirring solution of **18** (138 mg, 0.11 mmol) in CH_2Cl_2 (0.54 mL) at rt during 3 h. Toluene was added and the mixture was then concentrated to dryness under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 95:5). The title product was obtained as a pale solid (101 mg, 87%), mp (CH_2Cl_2) 120−124 °C. $[\alpha]_D$ +58.3 (c = 1.00, CH_2Cl_2). IR (KBr) 3347, 3116, 2981, 2917, 2238, 1720, 1668, 1623, 1520, 1233, 1207 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.43 (d, *J* = 7.2 Hz, 3 H), 1.67−1.83 (m, 1 H), 1.99−2.31 (m, 3 H), 2.50−3.34 (m, 6 H), 3.35−3.84 (m, 3 H), 4.38−4.56 (m, 5 H), 4.75−4.82 (m, 2 H), 5.18−5.29 (m, 2 H), 5.30−5.40 (m, 2 H), 5.40−5.49 (m, 1H), 5.53 (bs, 1H), 5.71−5.87 (m, 1 H), 5.90−6.09 (m, 2 H), 6.32−6.47 (m, 1 H), 6.63 (bs, 1 H), 6.72−6.88 (m, 2 H), 7.00−7.13 (m, 2 H), 7.38−7.47 (m, 1 H), 8.02−8.08 (m, 1 H), 8.09−8.18 (m, 1 H), 8.18−8.27 (m, 1 H), 8.30 (s, 1 H), 8.31−8.37 (m, 1 H), 8.59−8.67 (m, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.1 (q), 14.7 (q), 22.0 and 34.5 (t), 24.7 and 32.1 (t), 38.5 and 38.9 (t), 41.7 and 42.3 (t), 46.3 and 47.3 (t), 47.9 and 48.2 (d), 55.3 and 55.9 (d), 58.9 (d), 59.1 (d), 61.9 (t), 66.1 and 66.2 (t), 69.0 and 69.1 (t), 115.2 (d), 115.2 and 116.0 (d), 117.9 and 118.0 (t), 118.9 and 119.0 (d), 119.1 and 119.2 (t), 121.8 and 121.9 (d), 122.9 and 123.0 (s), 128.4 (d), 129.8 and 129.9 (s), 130.1 (s), 130.5 (d), 130.6 (d), 132.1 (s), 133.5 (d), 133.5 (d), 140.0 and 140.4 (d), 146.8 and 146.9 (s), 148.9 (s), 149.2 (s), 151.1 and 151.2 (s), 151.3 and 151.4 (s), 154.3 and 154.4 (s), 154.5 and 154.6 (s), 157.2 and 157.6 (s), 157.8 (s), 161.1 and 161.3 (s), 161.6 (s), 161.9 (s), 169.0 (s), 172.3 (s), 173.4 (s), 173.6 (s), 174.4 (s) ppm. HRMS m/z calcd for $C_{48}H_{47}O_9N_{10}S_4$ (M+H) 1035.2405, found 1035.2400.

Peptide 19. DIPEA (18 μL, 0.10 mmol), EDC·HCl (20 mg, 0.10 mmol) and HOAt (14 mg, 0.10 mmol) were added to a stirring solution of **5** (69 mg, 50% purity, 0.10 mmol), and **S6** (97 mg, 0.09 mmol) in DMF at 0 °C. After 6 h, more EDC·HCl (10 mg, 0.05 mmol) was added and the mixture was then stirred for 2.5 h at the same temperature. The mixture was diluted in EtOAc (40 mL), washed with 6% HCl (40 mL), saturated aq. $NaHCO_3$ (40 mL) and H_2O (40 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/EtOAc, 2:8 to CH_2Cl_2/MeOH 95:5). The title product was obtained as a pale solid (86 mg, 68%) mp (CH_2Cl_2) 127−131 °C. $[\alpha]_D$ +37.0 (c = 1.00, CH_2Cl_2). IR (KBr) 3427, 2924, 1717, 1652, 1509, 1425, 1237, 1024 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.45 (t, *J* = 7.2 Hz, 3 H), 2.00−2.34 (m, 4 H), 2.55−2.76 (m, 4 H), 2.80−3.89 (m, 9 H), 4.32−4.62 (m, 6 H), 4.74−4.87 (m, 3 H), 4.88−5.01 (m, 2H), 5.08−5.47 (m, 7 H), 5.50−5.60 (m, 2 H), 5.75−6.10 (3 H), 6.49 (bs, 1 H), 6.65−6.92 (m, 2 H), 7.00−7.33 (m, 8 H), 7.41−7.49 (m, 1 H), 8.05−8.12 (m, 1 H), 8.12−8.18 (m, 1 H), 8.20−8.30 (m, 1 H), 8.32−8.34 (m, 1 H), 8.35−8.41 (m, 1 H), 8.57 (d, *J* = 9.2 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 11.8 (q), 14.4 (q), 24.5 (t), 31.9 (t), 36.0 (t), 37.5 (t), 38.4 (t), 39.3 (t), 47.4 (t), 47.7 (d), 52.2 (d), 54.7 (d), 58.8 (d), 61.6 (t), 65.9 (t), 65.9 (t), 68.7 (t), 78.4 (d), 114.7 (d), 115.1 (d); 117.5 (t), 117.9 (t), 118.7 (d), 118.8 (t), 121.5 (d), 122.7 (s), 127.1 (d), 128.2 (d), 128.6 (d), 129.3 (d), 129.4(d), 129.4 (s), 129.8 (s), 130.2 (d), 130.4 (d), 131.8 (s), 132.6 (d), 133.2 (d), 135.8 (s), 139.8 (d), 146.5 (s), 148.6 (s), 148.8 (s), 150.9 (s), 151.2 (s), 153.9 (s), 154.2 (s), 155.7 (s), 157.0 (s), 157.6 (s), 160.8 (s), 161.3 (s), 161.4 (s), 161.6 (s), 161.7 (s), 168.8 (s), 170.6 (s), 170.7 (s), 172.0 (s), 172.9 (s)176.3 (s) ppm. HRMS m/z calcd for $C_{64}H_{63}O_{12}N_{12}S_5$ (M+H) 1351.3287, found 1351.3342.

Macrocycle 1. A solution of $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) in CH_2Cl_2 (3.3 mL) and phenylsilane (31 μL, 0.348 mmol) were added over **19** (67 mg, 0.050 mmol) and the mixture was stirred for 5 h at rt. More $Pd(PPh_3)_4$ (6 mg, 0.005 mmol) in CH_2Cl_2 (3.3 mL) was added, and the the mixture was stirred for 2 h and then dried under reduced pressure. The crude was dissolved in DMF (50 mL) and cooled to 0 °C. EDC·HCl (12 mg, 0.060 mmol) and HOAt (8 mg, 0.060 mmol) were added, the mixture was allowed to reach rt and stirred for 21 h. The mixture was diluted with CH_2Cl_2 (50 mL), washed with H_2O (2 × 50 mL), dried (Na_2SO_3) and concentrated *in vacuo* with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 99:1 to 95:5). The title product was obtained as a pale solid (20 mg, 30%), mp (toluene) 103 °C (decomp.). $[\alpha]_D$ −12.3 (c = 0.20, CH_2Cl_2). IR (neat) 2954, 2924, 2851, 1652, 1200, 1127, 1020 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ = 1.40 (t, *J* = 4.8 Hz, 3 H), 1.92–2.24 (m, 3 H), 2.25−2.34 (m, 1 H), 2.42−2.49 (m, 1 H) 2.69−2.76 (m, 4 H), 2.84−3.06 (m, 2 H) , 3.22−3.39 (m, 3 H), 3.61−3.70 (m, 1 H), 3.79−3.86 m, 2 H), 4.42 (q, *J* = 4.8 Hz, 2 H), 4.82−4.89 (m, 1 H), 4.91−5.02 (m, 2 H), 5.26−5.32 (m, 1 H), 5.34−5.40 (m, 1 H), 6.66 (d, *J* = 8.1 Hz, 2 H), 7.10 (d, *J* = 8.1 Hz, 2 H), 7.18−7.45 (m, 5 H), 7.76 (s, 1 H), 7. 96 (s, 1 H), 8.34 (s, 1 H), 8.37 (d, *J* = 8.4 Hz, 1 H), 8.49 (d, *J* = 8.4 Hz, 1 H), 8.61 (d, *J* = 7.2 Hz, 1 H), 8.76−8.84 (m, 3 H), 8.94 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ = 12.5 (q), 15.1 (q), 25.6 (t), 33.6 (t), 36.7 (t), 37.4 (t), 38.7 (t), 38.8 (t), 48.2 (t), 49.3 (d), 53.1 (d), 54.9 (d), 60.4 (d), 61.9 (t), 78.2 (d), 115.9 (d), 117.4 (d), 119.5 (d), 123.2 (d), 123.8 (s), 124.8 (d), 127.5 (d), 129.1 (d), 130.0 (d), 130.5 (s), 131.4 (d), 133.2 (d), 138.1 (s), 141.3 (d), 148.6 (s), 150.2 (s), 151.5 (s), 152.4 (s), 153.5 (s), 153.9 (s), 154.6 (s), 156.7 (s), 156.9 (s), 158.6 (s), 158.8 (s), 159.0 (s), 161.7 (s), 163.2 (s), 168.4 (s), 170.1 (s), 170.8 (s), 172.4

(s), 174.0 (s), 174.5 (s), 175.4 (s) ppm. HRMS m/z calcd for $C_{54}H_{49}O_9N_{12}S_5$ (M+H) 1169.2344, found 1169.2330.

Tris-selenocysteine macrocycle 20. Trimethyltin hydroxide (6 mg, 0.034 mmol) was added to a solution of **1** (20 mg, 0.017 mmol) in 1,2-dichloroethane (170 μL) and the reaction mixture was then stirred at 60 °C for 2 h. After this time, more trimethyltin hydroxide (6 mg, 0.034 mmol) and 1,2-dichloroethane (50 μL) were added. After 17 h the mixture was diluted in CH_2Cl_2 (10 mL), washed with 6% HCl (5 mL), dried (Na_2SO_3), and concentrated *in vacuo*.



EDC·HCl (4 mg, 19.3 μmol), HOAt (3 mg, 19.3 μmol) and DIPEA (3 μL, 19.3 μmol) were added to a stirring solution of the crude and **2** (20 mg, 19.3 μmol) in DMF (0.35 mL) at 0 °C. After 3 h the mixture was diluted in CH_2Cl_2 (15 mL), washed with H_2O (10 mL), dried (Na_2SO_3) and concentrated *in vacuo* with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 94:6). The title product was obtained as a white solid (28 mg, 81%), mp (toluene) 115 °C (decomp.). $[\alpha]_D$ −53.5 (c = 0.33, CH_2Cl_2). IR (neat) 2952, 2921, 2849, 1648, 1167, 1019 cm^{-1}. Some NMR signals split into different peaks due to the coexistence of rotamers. Only the major peaks are listed : ^{1}H NMR (600 MHz, DMSO-d_6) δ = 1.20−1.32 (m, 5 H), 1.86−2.18 (m, 7 H), 2.24−2.51 (m, 2 H), 2.75 (s, 3 H), 2.79−3.07 (m, 3 H), 3.10−3.89 (m, 12 H), 4.30−4.54 (m, 3 H), 4.64 (t, *J* = 6.9 Hz, 1 H), 4.75−5.10 (m, 4 H), 5.25−5.51 (m, 2 H), 6.66 (d, *J* = 7.8 Hz, 2 H), 7.11 (d, *J* = 7.8 Hz, 2 H), 7.18−7.42 (m, 14 H), 7.47−7.62 (m, 6 H), 7.76 (s, 1 H), 7.95 (s, 1 H), 8.14 (d, *J* = 8.4 Hz, 1 H), 8.23 (d, *J* = 7.2 Hz, 1 H), 8.35 (s, 1 H), 8.42 (d, *J* = 7.8 Hz, 1 H), 8.50−8.69 (m, 4 H), 8.84 (d, *J* = 7.2 Hz, 1 H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ = 12.5 (q), 17.9 (q), 25.4 (t), 25.6 (t), 29.4 (t), 29.7 (t), 29.9 (2t), 30.3 (t),

33.5 (t), 37.1 (t), 37.4 (t), 39.1 (t), 47.7 (d), 47.8 (t), 48.2 (t), 49.3 (d), 53.2 (d), 53.7 (d), 53.8 (d), 54.2 (d), 54.9 (d), 60.4 (d), 61.0 (d), 78.2 (d), 115.9 (d), 117.4 (d), 119.5 (d), 123.2 (d), 123.7 (s), 124.8 (d), 127.5 (d), 127.6 (2d), 127.8 (d), 128.8 (d), 129.2 (2d), 130.1 (d), 130.2 (2d), 130.6 (s), 130.8 (s), 131.0 (s), 131.4 (d), 132.3 (s), 132.4 (d), 132.6 (d), 132.7 (d), 138.1 (s), 141.1 (d), 148.4 (s), 150.2 (s), 151.5 (s), 151.6 (s), 152.5 (s), 153.9 (s), 154.6 (s), 156.8 (s), 156.9 (s), 159.0 (s), 159.3 (s), 160.9 (s), 161.8 (s), 162.0 (s), 167.8 (s), 170.1 (s), 170.8 (s), 171.7 (s), 172.4 (2s), 172.8 (s), 174.0 (s), 174.6 (s), 175.4 (s) ppm. HRMS m/z calcd for $C_{87}H_{85}N_{18}O_{13}S_5Se_3$ (M+H) 1987.2645, found 1987.2634.

Baringolin. 5.5 M *t*BuOOH in decane (51 μL, 0.28 mmol) was added to a stiring solution of **20** (28 mg, 14.1 μmol) in CH_2Cl_2 (4.7 mL) at 0 °C. After 5 min the mixture was allowed to reach rt and was then stirred for 1 h. A second portion of 5.5 M *t*BuOOH in decane (51 μL, 0.28 mmol) was added. After 2 hours more 5.5 M *t*BuOOH in decane was added (102 μL, 0.56 mmol). The reaction mixture was stirred for a total of 12 h. After this time a mixture of saturated aq. $NaHCO_3/Na_2S_2O_3$ (1:1, 6 mL) was added. The solution was extracted with CH_2Cl_2 (3 × 10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 100:0 to 94:6). The title compound was obtained as a pale solid (14 mg, 66%), mp (pyridine) 166 °C (decomp.). $[\alpha]_D$ +23.4 (c = 0.13, CH_2Cl_2). IR (KBr) 3308, 2930, 2847, 1675, 1194, 1130 cm^{-1}. ^1H NMR (600 MHz, pyridine-d_5) δ = 1.34−1.41 (m, 1 H), 1.44 (d, *J* = 7.0 Hz, 3 H), 1.49−1.86 (m, 6 H), 2.02−2.12 (m, 2 H), 2.58 (s, 3 H), 2.83 (dd, J = 17.2, 2.9 Hz, 1 H), 3.00 (d, *J* = 4.4 Hz, 2 H), 3.20 (dd, *J* = 14.0, 3.9 Hz, 1 H), 3.43 (dd, *J* = 14.0, 5.7 Hz, 1 H), 3,48−3.55 (m, 3 H), 3.56−3.60 (m, 2 H), 3.63−3.71 (m, 1 H), 4.74−4.81 (m, 1 H),

4.84−4.89 (m, 1 H), 5.00 (q, *J* = 7.0, 1 H), 5.05−5.10 (m, 1 H), 5.16−5.29 (m, 2 H), 5.46 (s, 1 H), 5.51 (s, 1 H), 5.66−5.72 (m, 1 H), 5.83 (s, 1 H), 5.96 (s, 1 H), 6.28 (s, 1 H), 6.51 (s, 1 H), 6.83−6.91 (m, 5 H), 6.93−7.02 (m, 3 H), 7.16 (d, *J* = 7.3 Hz, 2 H), 7.53 (d, *J* = 9.1 Hz, 1 H), 7.72 (s, 1 H), 7.79 (s, 1 H), 7.97 (s, 1 H), 8.00−8.04 (m, 3 H), 8.36 (s, 1 H), 8.42 (s, 1 H), 8.56 (d, J = 6.5 Hz, 1 H), 8.66 (s, 1 H), 8.83 (bs, 1 H), 9.15 (d, *J* = 8.7 Hz, 1 H), 9.56 (d, *J* = 7.1 Hz, 1 H), 9.60 (s, 1 H), 9.98 (s, 1 H), 10.41 (s, 1 H) ppm. ^{13}C NMR (150 MHz, pyridine-d_5) δ = 11.8 (q), 17.3 (q), 25.3 (t), 25.5 (t), 28.7 (t), 34.3 (t), 36.2 (t), 36.8 (t), 38.3 (t), 39.9 (t), 47.4 (t), 48.1 (t), 48.5 (d), 49.2 (d), 52.3 (d), 54.1 (d), 61.3 (d), 61.9 (d), 78.8 (d), 103.2 (t), 103.8 (t), 104.9 (t), 115.8 (d), 116.8 (d), 118.6 (d), 123.1 (d), 123.4 (d), 125.7 (s), 127.2 (d), 127.9 (d), 128.7 (d), 130.2 (d), 131.0 (s), 131.6 (d), 135.1 (s), 135.7 (s), 136.9 (2s), 139.3 (d), 148.8 (s), 150.2 (s), 150.9 (s), 152.0 (s), 152.2 (s), 153.9 (s), 154.7 (s), 155.8 (s), 157.8 (s), 159.7 (s), 160.5 (s), 161.2 (s), 162.7 (s), 163.1 (s), 164.7 (s), 166.7 (s), 168.3 (s), 169.0 (s), 171.2 (s),171.5 (s), 172.6 (s), 172.7 (s), 173.2 (s), 174.7 (s), 175.1 (s) ppm. HRMS m/z calcd for $C_{69}H_{66}O_{13}N_{18}S_5$ (M) 1514.3660, found 1514.3675.

3. NMR Spectra

NMR spectra images are available in the Supporting Information in electronic format.

4. NMR Spectra Comparison for Natural and Synthetic baringolin.

Table S1. Comparison of ^{1}H and ^{13}C NMR chemical shifts of natural and synthetic baringolin**.**

Position	Natural		Synthetic	
	^{13}C (δ)	**^{1}H (δ)**	**^{13}C (δ)**	**^{1}H (δ)**
Methyloxazole				
2-C	155.9		155.8	
4-C	130.9		131.0	
5-C	153.9		153.9	

CO	162.7		162.7	
5-CH_3	11.8	2.58 (s)	11.8	2.58 (s)
Asparagine				
NH		9.16 (d, 8.5)		9.15 (d, 8.7)
αCH_2	38.3	2.81 (d, 17.2) 1.35 (m)	38.3	2.83 (dd, 2.9, 17.2) 1.34−1.41 (m)
βCH	49.2	5.68 (m)	49.2	5.66−5.72 (m)
CO-NH_2	173.3		173.2	
Thiazole (1)				
2-C	175.2		175.1	
4-C	150.2		150.2	
5-CH	123.2	7.99 (s)	123.1	7.97 (s)
CO	160.5		160.5	
Phenylalanine				
NH		8.56 (d, 6.6)		8.56 (d, 6.5)
αCH	54.1	5.08 (m)	54.1	5.05−5.10 (m)
βCH_2	39.9	3.43 (dd, 5.1, 14.0) 3.19 (dd, 4.0, 14.0)	39.9	3.43 (dd, 5.7, 14.0) 3.20 (dd, 3.9, 14.0)
1-C	137.0		136.9	
2,6-CH	130.3	7.17 (d, 7.5)	130.2	7.16 (d, 7.3)
3,5-CH	128.7	7.02 (m)	128.7	6.93−7.02 (m)
4-CH	127.3	6.94 (m)	127.2	6.93−7.02 (m)
Thiazoline (2)				
2-C	172.6		172.7	
4-CH	78.8	4.87 (t, 10.0)	78.8	4.84−4.89 (m)
5-CH_2	36.2	3.59 (d, 10.0)	36.2	3.56−3.60 (m)
CO	169.1		169.0	
Tyrosine				
NH		7.53 (d, 9.1)		7.53 (d, 9.1)
αCH	52.3	5.22 (m)	52.3	5.16−5.29 (m)
βCH_2	36.8	2.99 (d, 4.4)	36.8	3.00 (d, 4.4)
1-C	125.7		125.7	
2,6-CH	131.7	6.89 (m)	131.6	6.83−6.91 (m)
3,5-CH	116.8	6.87 (m)	116.8	6.83−6.91 (m)
4-C-OH	157.9	11.05 (s)	157.8	8.66 (s)[a]
Proline				
αCH	62.0	-[b]	61.9	5.16−5.29 (m)
βCH_2	34.4	2.06 (m)	34.3	2.02−2.12 (m)

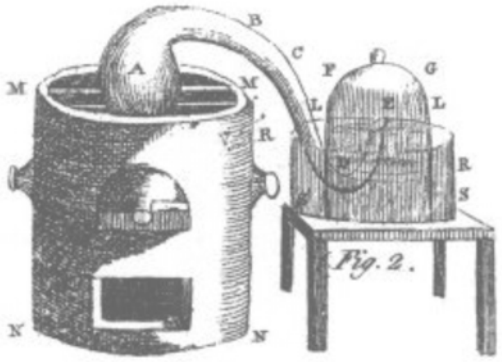
		1.62 (m)		1.49−1.86 (m)
γCH_2	25.5	1.80 (m) 1.56 (m)	25.5	1.49−1.86 (m)
δCH_2	47.5	3.68 (m)	47.4	3.63−3.71 (m) 3.48−3.55 (m)
Thiazole (3)				
2-C	174.8		174.7	
4-C	148.8		148.8	
5-CH	115.9	7.82 (s)	115.8	7.79 (s)
Thiazole (4)				
2-C	161.2		161.2	
4-C	154.7		154.7	
5-CH	123.6	8.02 (s)	123.4	8.00−8.04 (m)
Pyridine				
2-C	152.1		152.2	
3-C	122.9		-[c]	
4-CH	139.4	8.04 (bs)	139.3	8.00−8.04 (m)
5-CH	118.9	8.04 (bs)	118.6	8.00−8.04 (m)
6-C	150.8		150.9	
Thiazole (5)				
2-C	168.4		168.3	
4-C	152.0		152.0	
5-CH	128.0	8.43 (s)	127.9	8.42 (s)
CO	159.7		159.7	
Dehydroalanine (1)				
NH		8.40 (s)		8.36 (s)
αC	135.2		135.1	
βCH_2	103.3	6.92 (s) 6.00 (s)	103.2	6.83−6.91 (m) 5.96 (s)
CO	164.8		164.7	
Alanine				
NH		9.60 (s)		9.56 (d, 7.1)
CH	48.2	3.52 (m)[d]	48.5	5.00 (q, 7.0)
CH_3	17.3	1.44 (d, 7.0)	17.3	1.44 (d, 7.0)
CO	172.7		172.6	
Proline				
αCH	61.3	-[b]	61.3	4.74−4.81 (m)
βCH_2	28.8	2.08 (m)	28.7	2.02−2.12 (m)

		1.70 (m)		1.49−1.86 (m)
γCH_2	25.3	1.80 (m) 1.56 (m)	25.3	1.49−1.86 (m)
δCH_2	48.5	3.52 (m)	48.2	3.48−3.55 (m)
CO	171.6		171.5	
Dehydroalanine (3)				
NH		10.02 (s)		9.98 (s)
αC	136.9		136.9	
βCH_2	105.1	6.00 (s)[d] 5.53 (s)	104.9	6.28 (s) 5.51 (s)
CO	163.1		163.1	
Dehydroalanine (4)				
NH		9.60 (s)		9.60 (s)
αC	135.6		135.7	
βCH_2	103.9	6.54 (s) 5.87 (s)	103.8	6.51 (s) 5.83 (s)
$CO\text{-}NH_2$	166.7		166.7	

[a] The phenolic proton has the most different chemical shift due to its lability. [b] Not listed in the patent. [c] Not observed due to solvent overlap. [d] Typing error in the patent.

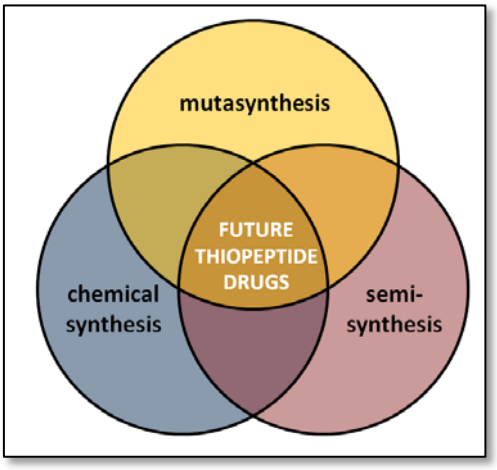
Chapter 6:

Production of Thiopeptide Analogues



PRODUCTION OF THIOPEPTIDE ANALOGUES

Thiopeptide engineering: a multidisciplinary effort towards future drugs



Xavier Just-Baringo,[a,b] Fernando Albericio,[a,b,c,d] and Mercedes Álvarez [a,b,e]

SUBMITTED

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.

[b] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.

[c] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

[d] School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.

[e] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

Conspectus

In this chapter, different strategies used to produce thiopeptide analogues are described. The low success rate of thiopeptides to enter the clinic is mainly a consequence of their big molecular size and their poor solubility. These drawbacks have been addressed by three different strategies, chemical synthesis, semi-synthesis and mutasynthesis. These studies have also provided insights into structure-activity relationships and biosynthetic pathways of thiopeptides.

All three strategies used for the obtention of analogues are complementary. A semi-synthetic approach is limited to the modification of the most reactive functionalities of the natural product, while mutasynthesis can alter the backbone of thiopeptides, though it cannot overcome the many restrictions of the enzymatic machinery. On the contrary, chemical synthesis offers a greater freedom to design new molecules but is much more expensive and time consuming. Due to the different modifications that can be performed depending on the approach used, a multidisciplinary strategy has been proposed to achieve a grater molecular diversity that can overcome the limitations of all three methods as stand-alone disciplines.

Thiopeptide Engineering: A Multidisciplinary Effort Towards Future Drugs

Xavier Just-Baringo,[1,2] Fernando Albericio, [1,2,3,4] Mercedes Álvarez[1,2,5]*

[1] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.

[2] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.

[3] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

[4] School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.

[5] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

1. Introduction

Thiazolyl peptides, or thiopeptides,[1,2] are an ever-expanding family of antibiotics produced by Gram-positive bacteria that have attracted the interest of many research groups thanks to their outstanding biological profile, displaying nanomolar potencies for a variety of Gram-positive bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), penicillin-resistant Streptococcus pneumonia (PRSP) among others, along with other interesting properties such as antiplasmodial and anticarcinogenic activities. The members of this family of natural products are easily spotted thanks to their central azole-substituted nitrogen-containing six-membered ring. Other characteristic features of these natural products are the presence of at least one macrocycle and a tail of variable length, both decorated with highly modified amino acid residues, mainly cyclised and/or dehydrated Cys, Ser and Thr units. The high potential of new modes of action of thiopeptides has gathered the attention of many groups, which have carried out epic efforts towards their total syntheses[2–8] and the study of their structure, biological function and biosynthetic origin.[9,10] It is now well established that thiopeptides arise from the thorough modification of a ribosomally synthesized linear pre-peptide by a variety of post-translational modification enzymes, all of them encoded in the same gene cluster.

Thiopeptides have been found to inhibit the growth of Gram-positive bacteria through two different modes of action, mainly depending in their macrocycle size. Thus, thiopeptides possessing a 26-member macrocycle, such as thiostrepton,[11] nocathiacin I[12] or the thiocillins,[13] disrupt the ribosome/L11 protein complex and do not permit the correct function of elongation factor G (EF-G). On the other hand, those with a 29-member macrocycle, such as GE2270A[14] or the thiomuracins,[15] bind to elongation factor Tu (EF-TU) and prevent the amino acyl/*t*RNA complex to be delivered to the ribosome. However, the mode of action of thiopeptides bearing larger 35-member macrocycles such as those of TP-1161[16] and berninamycin A[17] is not yet understood.

Despite their impressive *in vitro* profile, poor pharmacokinetic properties, especially low aqueous solubility, have limited their use as therapeutic agents, which to date has been restricted to topical use and only for the treatment of animal infections.

2. Different Approaches to Thiopeptide Analogues

In order to overcome the physicochemical drawbacks of thiopeptides, different approaches have been used to determine which structural features grant them their unique biological profile and produce analogues of improved solubility that can retain both activity and potency. Three main routes have been explored to achieve such a goal: chemical synthesis, biosynthetic pathway engineering and semi-synthesis. Given the huge differences existing between all three methods, the kinds of modifications that can be achieved is equally distinct and have served to explore different areas of the chemical space. While engineering of the biosynthetic pathway grants the alteration of the enzymatic machinery function to isolate non-mature products and makes residue replacement possible, a semi-synthetic approach facilitates the introduction of new fragments or degradation at the most reactive sites of the natural product. On the other hand, chemical synthesis allows the introduction of modifications that are not possible when relying on the *in vivo* production that the other two approaches are taking advantage of. However, this strategy is the most tedious and time consuming one because of the large number of steps it usually involves.

2.1. Chemical Synthesis

Huge synthetic efforts during the last decade have led to the total synthesis of many thiopeptide antibiotics. Such epic achievements have implied the development of powerful synthetic methodologies and strategies that are also amenable for the production of analogues. These syntheses are inherently lengthy, costly and time-consuming and this has led to a limited use of this approach. Nonetheless, insights into the activity of analogues otherwise impossible to produce by alternative methods and the activity of fragments obtained as synthetic building blocks have helped to determine key structure-activity relationships (SAR). If drug-like compounds were required to obtain new medicines, chemical synthesis would appear as a competitive option for the production of such small molecules.

Pioneering work by the group of Nicolaou permitted the first total synthesis of thiostrepton.[3,4] During their synthetic studies an analogue of the natural product lacking the second macrocycle and bearing a central five-membered ring instead of the naturally occurring six-membered one was produced, analogue **1** (Figure 1).[18] This product lacked any significant antibacterial activity, but highlighted the important role of the central six-membered ring as scaffold. Further investigations on the central polyheterocyclic core revealed that fragment **2**, used for the total synthesis of thiostrepton retained some *in vivo* activity and could be the starting point for the development of a low molecular weight antibacterial lead with improved pharmacological profile.[19] The *in vitro* ability of **2** to target the translational machinery was assessed along with that of other building blocks such as **3** and **4** and many of their analogues and stereoisomers. Central polyheterocycle **4** is a fully unsaturated version of the core fragment, such as the one found in GE2270 A and T.[20] Surprisingly, the *in vitro* results point to an

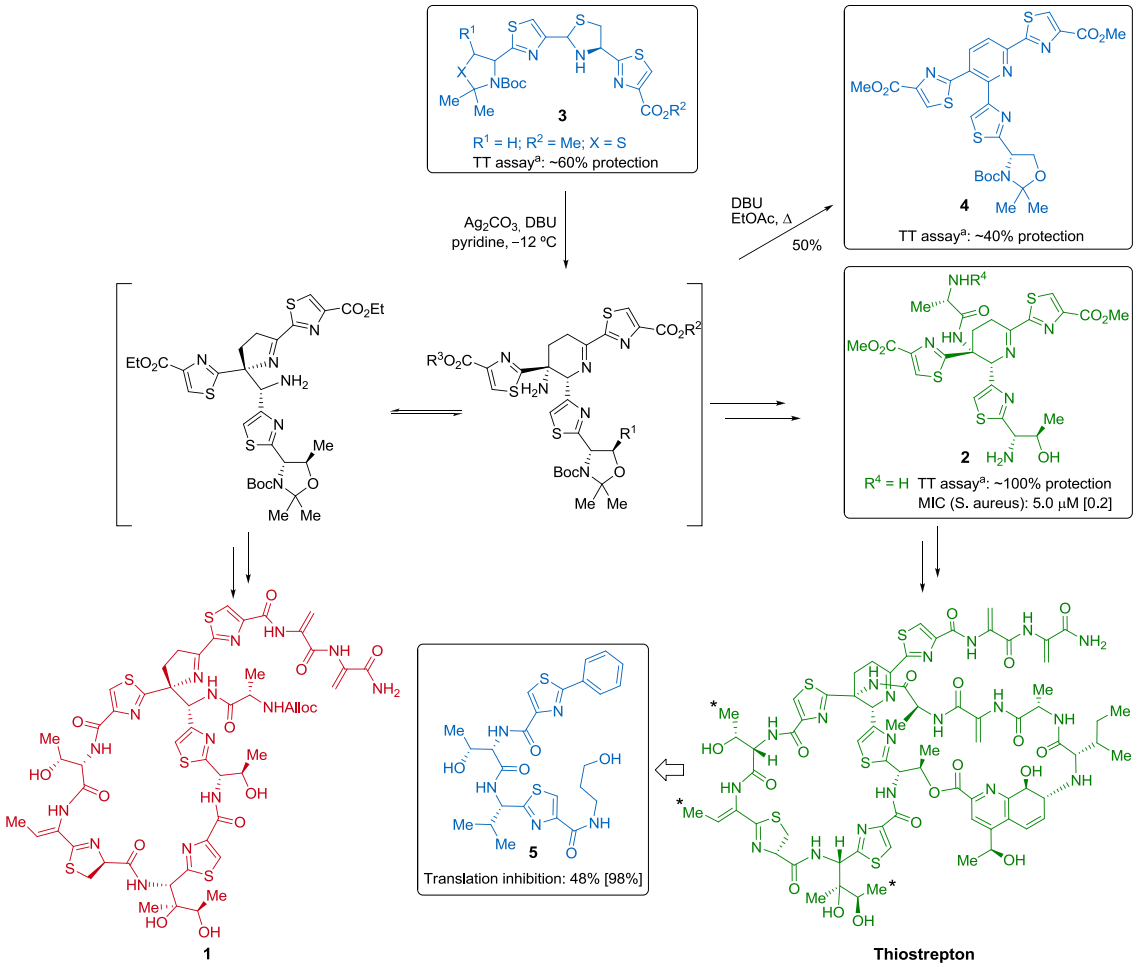


Figure 1. Relationship between different thiopeptides intermediates and analogues produced during total syntheses and fragment screening. Compounds displaying *in vivo* or *in vitro* activity are highlighted in green. Products that were found to interact only with molecular targets in *in vitro* assays are highlighted in blue. Non-active molecules are highlighted in red. Activity and solubility values of the natural product appear in brackets next to that of the analogue. [a]Inhibition of GFP synthesis using a transcription-translation (TT) assay was carried out in the presence of thiostrepton.

alternative mode of action for the fragments other than inhibition of the translational machinery.[21] Some of the tested compounds were also found to restore formation of the 70S initiation complex (70SIC) in the presence of thiostrepton, indicating that those fragments might be competing with the natural product even if they do not strongly bind to the ribosome.

Identification of the key contacts between thiostrepton and protein L11 binding domain of the 23S ribosomal RNA, highlighted with stars in figure 1, led to the design and synthesis of a library of analogues of the like of **5**, bearing those moieties involved in such interactions.[22] Although some of the compounds displayed some residual *in vitro* inhibition of protein translation, they all failed to inhibit bacterial growth. This may stem from the increased flexibility of the fragments outside of their original macrocyclic frame. However, the fragments synthesized only included two out of the three interacting residues mentioned and the thiazoline ring was substituted for a more robust thiazole. Such simplifications might have also caused the decrease of *in vitro* inhibition and the lack of *in vivo* activity.

2.2. Semi-Synthesis

The most widely used strategy for the synthesis of thiopeptide libraries of analogues has been the chemical modification of the natural extract, or semi-synthesis. The relatively large amounts of natural product than can be obtained using fermentation tanks provide enough material for subsequent derivatization, characterization and screening. This approach is mainly limited by the inherent reactivity of the thiopeptide that is used. Consequently, although very selective methods have been developed, all modifications are necessarily carried out at the most reactive sites of the substrate and result in products of a molecular weight comparable to that of the extract. Nonetheless, the fewer chemical transformations required when compared to *de novo* synthesis and the large amount of analogues that can be readily obtained, have granted access to the most promising derivatives to date.

Nocathiacin I (Figure 2A) has been one of the most thoroughly modified thiopeptides and is a perfect example of the different transformations than can be performed at distinct sites of the thiopeptide scaffold. The vast majority of derivatives have been obtained through alteration of the peptidic tail (Figure 3). When considering the configurational restrictions of the macrocycle, a fact that supports its high specificity towards its biological target, the dehydroalanine (Dha) residue present in the tail appears as a much more flexible moiety of enhanced reactivity. One of the most common modifications is the Michael addition of nucleophiles to Dha,[23] leading to amines[24] and thioethers,[25] being the latter amenable for further conversion into the corresponding sulfones under mild oxidative conditions, which should increase their solubility profile (Figure 3C).[26] Alternatively, the Dha residue can be diastereoselectively hydrogenated to obtain the corresponding Ala side chain, which can be further modified (Figure 3 A).[27] Degradation of the tail has been broadly used as well and grants access to two different products depending on the conditions that are used, leading to either the thiazole-4-carboxilic acid derivative (nocathiacin acid), or the corresponding amide (nocathiacin IV).[28,29] Nocathiacin acid can be left unmodified for solubility enhancement or can be further modified for subsequent condensation with amines.[30,31] Nocathiacin IV, has been used in one-pot *N*-alkylation/reductive amination protocols to introduce long alkyl tails (Figure 3B).[32] Such modifications have led to the discovery of nocathiacins analogues with improved solubility and similar potencies to that of nocathiacin I against various pathogens, including vancomycin resistant strains.[33]

Other nocathiacin I modifications reported include the selective substitution of one of the two most reactive hydroxyl groups of the molecule, 2-hydroxypyridine and *N*-hydroxyindole, leading to regioselective alkylations and also formation of carbamates and phosphonates.[34,35] Mono-*O*-substitution or bis-*O*-substituted compounds with alkyl chains bearing a phosphoric acid group in many cases maintained excellent *in vitro* and *in vivo* activities while improving aqueous solubility (Figure 4A).[34] Such derivatives might be suitable pro-drugs of nocathiacin I since phosphonooxymethyl ethers are known substrates of phosphatases, resulting in hydrolysis and hence, the release of nocathiacin I.[36] Even if the development of thiopeptide pro-drugs might appear as a very conservative strategy, it allows the potency to be retained, circumventing the natural product's lack of solubility. Similarly, regioselective substitution at one of the threonine residues of QN3323A, and either oxyme formation or reductive amination at the methyl ketone moiety produced derivatives of improved *in vitro* profile (Figure 4B).[37,38]

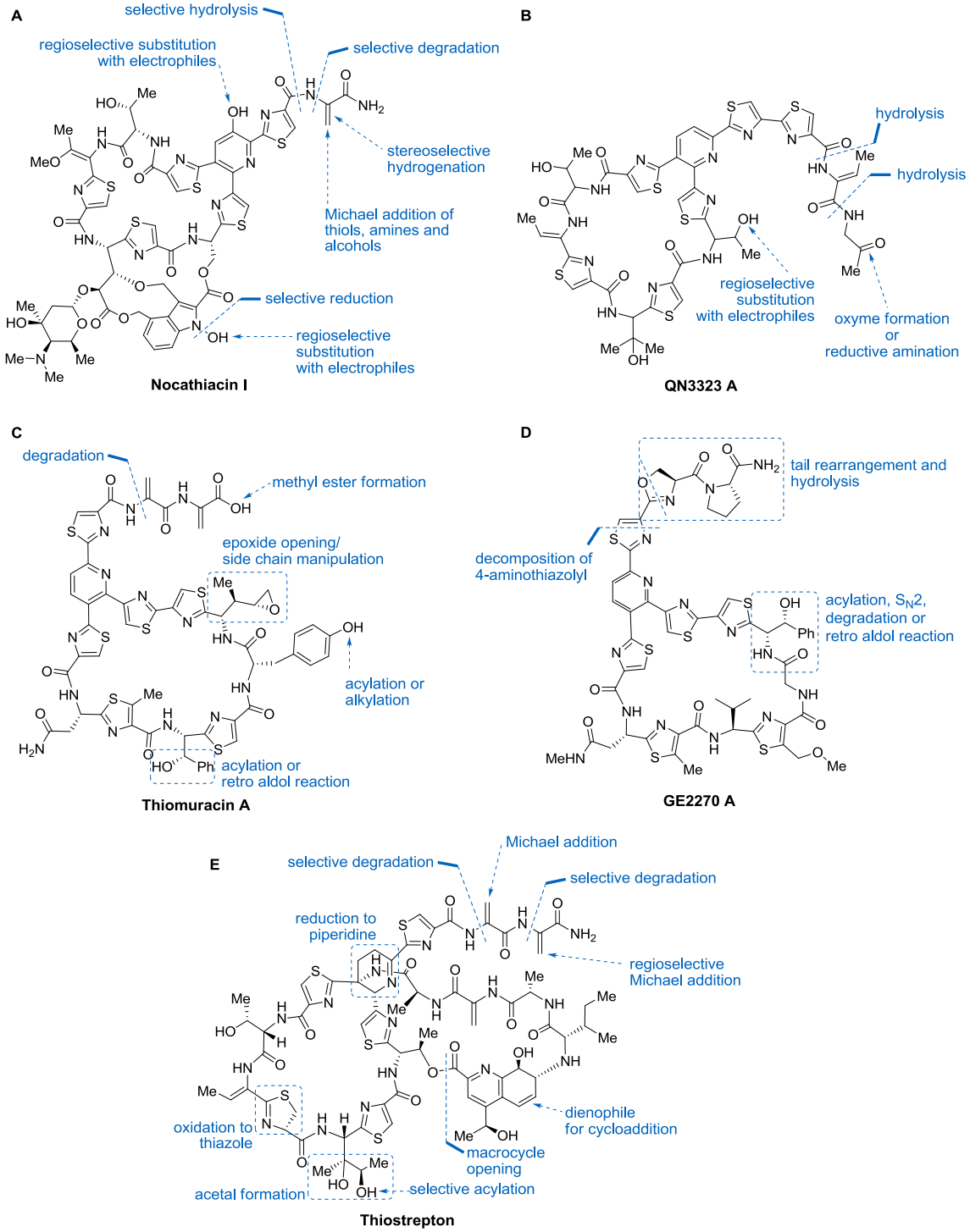


Figure 2. Reactivity map of thiopeptides during semi-synthetic modifications: nocathiacin I (A); QN3323 A (B); thiomuracin A (C); GE2270 A (D); thiostrepton (E).

A series of different transformations were performed on thiomuracin A.[39] The presence of a naturally occurring epoxide became an excellent playground for thorough derivatization of this appendix and permitted the formation of the corresponding substituted proline residue. This pyrrolidine ring bore methyl and hydroxyl groups, however, its substitution differed from than of the naturally occurring residue present in Thiomuracin I[15] and GE37468.[40] Removal of the tail was also carried out, resulting in even more robust and simplified products. One of them maintained activity and displayed a generally increased potency *in vitro*, while *in vivo*

performance had higher strain dependence (Figure 4C). Such simplified analogues were also intended to facilitate further drug development.

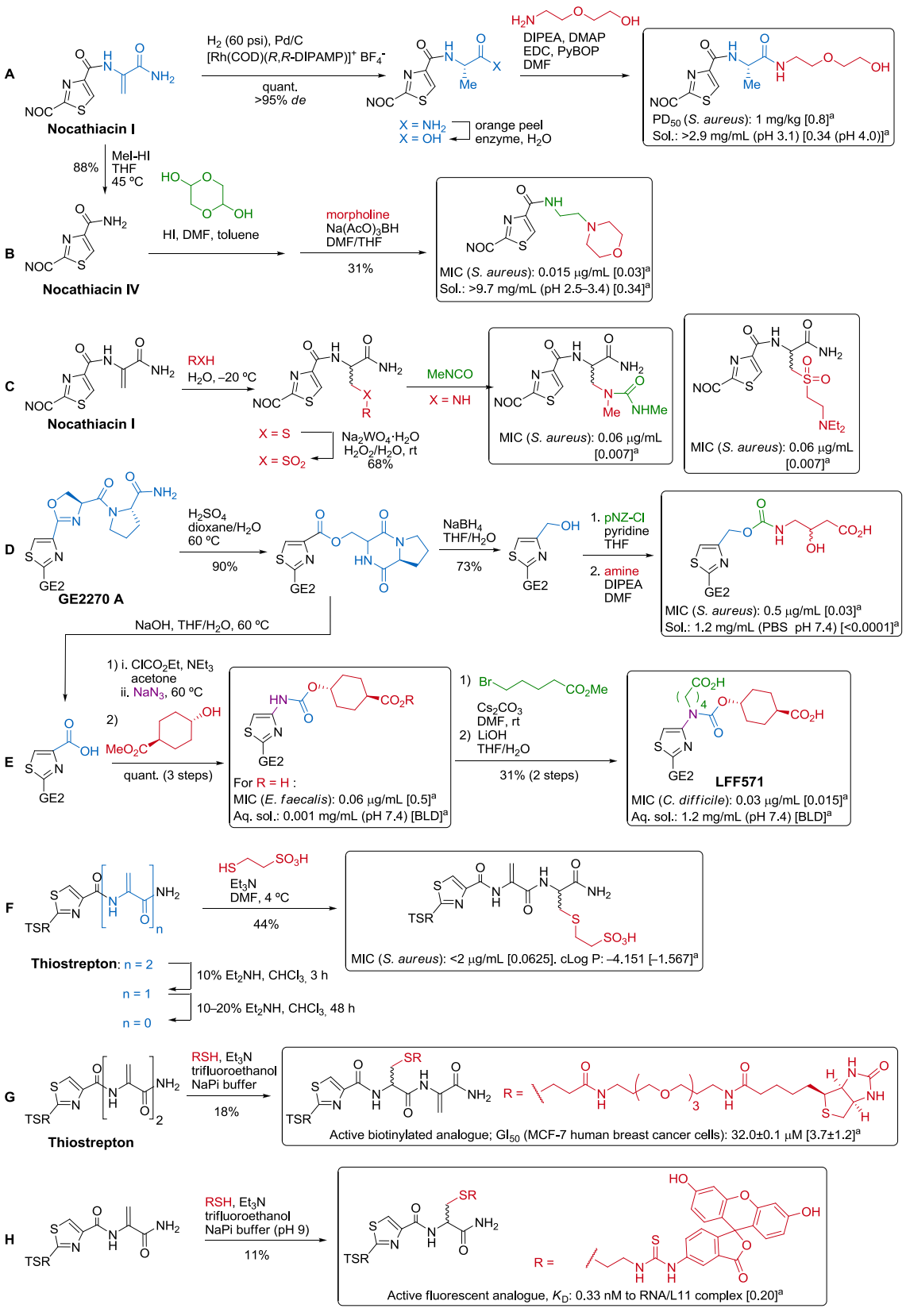


Figure 3. Examples of semi-synthetic modifications of thiopeptide tails. Macrocycle codes: nocathiacin (NOC), GE2270A (GE2) and thiostrepton (TSR). PD_{50} (dose required to cure 50% of the animals infected); MIC (minimum inhibitory concentration); cLogP (calculated partition coefficient between octanol and water); GI_{50} (concentration of drug required for 50% growth inhibition); K_D (dissociation constant). [a]Activity and solubility values of the natural product appear in brackets next to that of the analogue. BLD (below limit of detection).

Along with the amythiamicins, GE2270 A, lacks any dehydroamino acids. However, it has been one of the most thoroughly studied and derivatized thiopeptides by semi-synthetic methods.[41] Despite not having any Michael acceptors for the addition of nucleophiles, modification and degradation of its peculiar tail has been addressed in many reports and has produced a large number of analogues (Figure 3D). Another reactive point of GE2270 A is the phenylserine side chain,[42,43] which can be modified or even removed to convert it into a glycine residue in the same fashion that this transformation can be performed in thiomuracin A.[39] Since the macrocycle modifications did not provide any compounds of interest, most of the efforts have focused on tail replacement.[44] Acidic treatment of GE2270 A causes a rearrangement of the tail and provides a thiazole-4-carboxylate that can be hydrolyzed under basic conditions to yield the corresponding acid.[43] Using this moiety as a starting point, a myriad of transformations became possible. The acid can be either condensed with amines or peptides or it can be reduced to the alcohol, which can also be used in many different reactions (Figure 3D).[45] Although about 500 compounds were obtained in this manner, only a few retained activity while displaying good solubility. Interestingly, all good performers shared a common motif, a carboxylic acid at least five atoms apart from the thiazole ring.

More recently, outstanding results have been achieved by LaMarche *et al.*, who have based their modifications on the substitution of the 4-aminothiazolyl analogue obtained after Curtius rearrangement of tail-less GE2270 A (Figure 3E). Although the 4-aminothiazolyl analogue could be further eroded to obtain the corresponding picolinamide derivative, it was devoid of activity. [46] The 4-aminothiazolyl moiety permitted the formation of an imidazole ring that was used to substitute the original oxazoline. However, this and other imidazole-containing analogues showed a poor in vitro behavior.[47] Many other substituted 4-aminothiazolyl analogues were also synthesized, leading to the discovery of the cyclohexanoic acid series (Figure 3E),[48,49] which were linked to the thiazole through either an amide or a carbamate linkage. In both cases, these compounds exhibited a carboxylic acid, which again was at least five atoms apart from the thiazole moiety. These compounds displayed similar *in vitro* potencies to the parent natural compound, but their improved solubility made them amenable for *in vivo* testing, resulting in an excellent profiling.[50] Further derivatization of the cyclohexanoic acid series led to the discovery of LFF571, which incorporated an extra carboxylic acid-containing alkyl chain at the carbamate moiety.[51] This new analogue, the first one to enter clinical trials for the treatment of human infections, showed an excellent behavior during in vivo testing[52] and has been demonstrated that it still targets elongation factor Tu.[53]

These results demonstrate that a semi-synthetic approach is an excellent strategy for the assessment of structure-activity relationships and high throughput screening of such big molecules. The fact that only a few chemical transformations are required validates this method for the obtention of highly complex molecules in relatively large amounts.

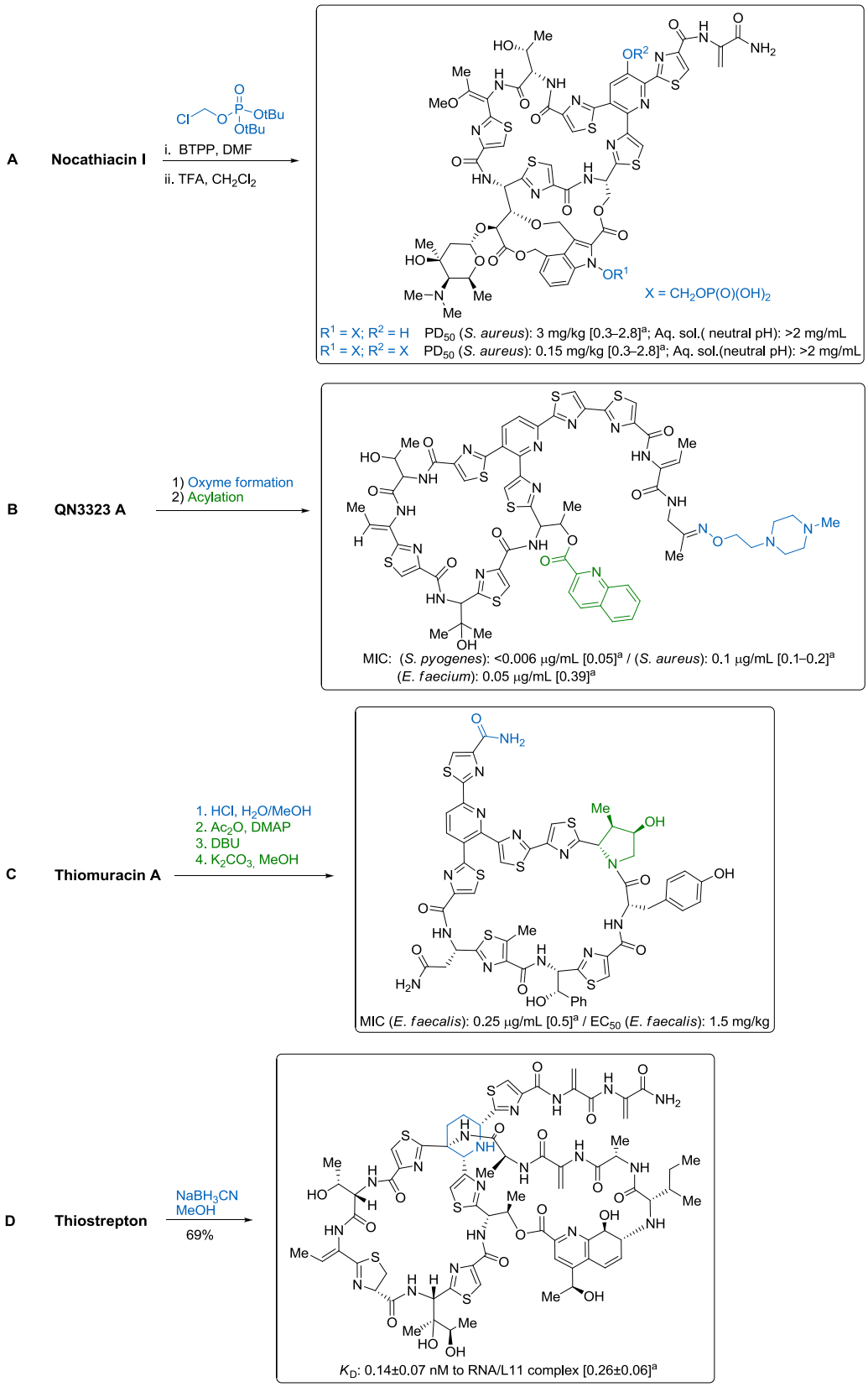


Figure 4. Examples of semi-synthetic modifications of the thiopeptides macrocycles. PD_{50} (dose required to cure 50% of the animals infected); MIC (minimum inhibitory concentration); K_D (dissociation constant). [a]Activity and solubility values of the natural product appear in brackets next to that of the analogue.

Despite its more complex architecture, thiostrepton has been selectively modified at many different regions of its structure using the unique moieties it possesses. Apart from the common Dha residues, other fragments susceptible of modification are also present. During their investigations on FOXM1[54] transcription factor inhibition with thiostrepton, Balasubramanian *et al.* prepared various surprising analogues.[55] First, ketal formation using the dihydroxylated Ile residue was achieved, although it was stated that the transformation proved quite irreproducible. However, one of the most surprising transformations reported is the cycloaddition of Danishefsky's diene with the double bond of the quinaldic acid moiety (Figure 2E). Unluckily inhibitory activity of FOXM1 was completely lost after this modification. Another outstanding modification is the selective degradation of the Dha tail reported by the group of Arndt, yielding products with a single Dha or with no tail at all (Figure 3F), depending on reaction conditions.[56] Either thiostrepton or their mono-Dha analogues were amenable for selective Michael additions with various thiols, leading to products that have been used for a variety of studies. These include functionalization with a biotin tag to assesses the previously mentioned inhibition of FOXM1 (Figure 3G), SAR studies of thiostrepton analogues as antiplasmodial drugs,[57] derivatization with fluorescent probes to study their binding to the ribosome (Figure 3H)[56,58] and assessment of thiostrepton's tail region impact on protein translation inhibition, including Gram-negative bacteria translation machinery.[59]

Alternative modifications of thiostrepton include the selective oxidation of its thiazoline ring to furnish the corresponding thiazole in high yield. This, along with the selective elimination of a single Dha residue, gave access to a series of Michael addition products of improved antiplasmodial efficiency.[57] During the same study, solvolysis of the quinaldic acid-containing ring yielded a mono-macrocyclic analogue completely devoid of activity.[60,61] Selective reduction of thiostrepton's central dehydropiperidine ring into its fully saturated analogue was also achieved with good conversions and displayed an increased affinity for the ribosome (Figure 4D).[62]

Although the derivatization of thiostrepton has not been as extensive as that of other thiopeptides, its higher diversity of reactive sites and its various biological activities have resulted in more diverse results. The use of different tags and the ever-surprising imagination of researchers have allowed to merge many fields using just a few thiostrepton analogues. This demonstrates that thiopeptides are not only good candidates for the development of new drugs, but can also serve as an excellent platform for chemical biology investigations.

2.3. *In Vivo* Production of Analogues

The structure of thiopeptides results from extensive modification of a ribosomally synthesized pre-peptide, opposite to the more common peptide-derived natural products produced by nonribosomal peptide synthetases (NRPS).[63] Its ribosomal origin implies that the peptide sequence is genetically encoded, and in fact, the structural gene of many thiopeptides has been found in the same gene cluster that encodes the enzymatic machinery necessary for post-translational tailoring.[9,10] Mutation studies of both the pre-peptide and surrounding enzyme-coding genes have offered insights into thiopeptide biosynthetic pathways,[64] structure-activity relationships and enzymatic machinery tolerance for modifications.

Although this approach has a great potential for the easy generation of analogues, some inconveniences must be circumvented in order to efficiently produce sophisticated derivatives. First, culture yields are affected after the introduction of even just one conservative residue replacement mutation, requiring thorough optimization to achieve an efficient production.[65] Moreover, many pre-peptide mutations are not accepted by the tailoring enzymes, resulting in a huge area of the chemical space that cannot be studied by this method. Similarly, the use of non-natural amino acids is also subjected to the preferences of this machinery and must compete with the natural substrate during translation, leading to mixtures and even lower yields. Finally, the use of knock-out (KO) mutants can provide non-mature analogues of great interest; however, the sequential nature of the biosynthetic pathway does not allow performing most of the transformations that should take place afterwards due to the lack of substrate recognition. Therefore, only KO of the enzymes responsible for the latest transformations during the maturation process will produce sufficiently mature analogues.

The groups of Walsh and Kelly have reported single residue-replacement mutation studies on the pre-peptides of the thiocillins,[66,67] GE37468[68] and thiostrepton[69,70] (Figure 5). Characterization of products produced in this manner provides very valuable information, such as whether the replacement is accepted by the tailoring enzymes and if it is not, at which step is the residue vital for recognition. Production of such analogues requires fermentation in large enough quantities of liquid media that can produce the necessary amounts of product for further structure elucidation. Despite all the information that can be obtained, this process can be tedious and is not amenable for the production of a large number of analogues. Walsh and collaborators recently reported the use of codon randomization for similar investigations with GE37468 pre-peptide mutants (Figure 5A),[71] allowing the replacement of the selected residues for all the other proteinogenic amino acids, thus demonstrating that this approximation results in a much more rapid evaluation of the analogues produced by colonies in solid media. High throughput MALDI-TOF analysis of colony samples led to the detection of 29 samples capable of producing enough quantities of mature analogues. Liquid cultures of these colonies were further analyzed and sequenced to identify their particular mutation. These colonies were found to produce mixtures of compounds formed by the expected fully matured analogues and partially or alternatively processed products. Remarkably, a Thr2Cys replacement resulted in slightly improved *in vitro* potency and Thr2Ser maintained it. It is also notable that Ile8 tolerated many substitutions and in the case of Ile8Leu, products of varying oxidation level were present. Some mutations of Asn3 were allowed; however, none of the products obtained was active, pointing to the crucial role of this residue for binding to EF-Tu.

Modifications of the pre-peptide aiming at the obtention of thiocillin analogues of varying macrocycle sizes were also studied in the Walsh's group.[72] Two different strategies were used to obtain such analogues. On one hand, deletion of Thr3 or the introduction of up to three extra Gly residues between Thr3 and Thr4 gave rise to analogues of 23, 29, 32 and 35-atom macrocycles. Although there are thiopeptides of different macrocycle sizes, 23 and 32-membered rings are not found in any naturally occurring members of this family. On the other hand, in order to produce alternative macrocycles, new Ser residues were incorporated to the pre-peptide, either replacing an already existing amino acid or inserting it between two of those

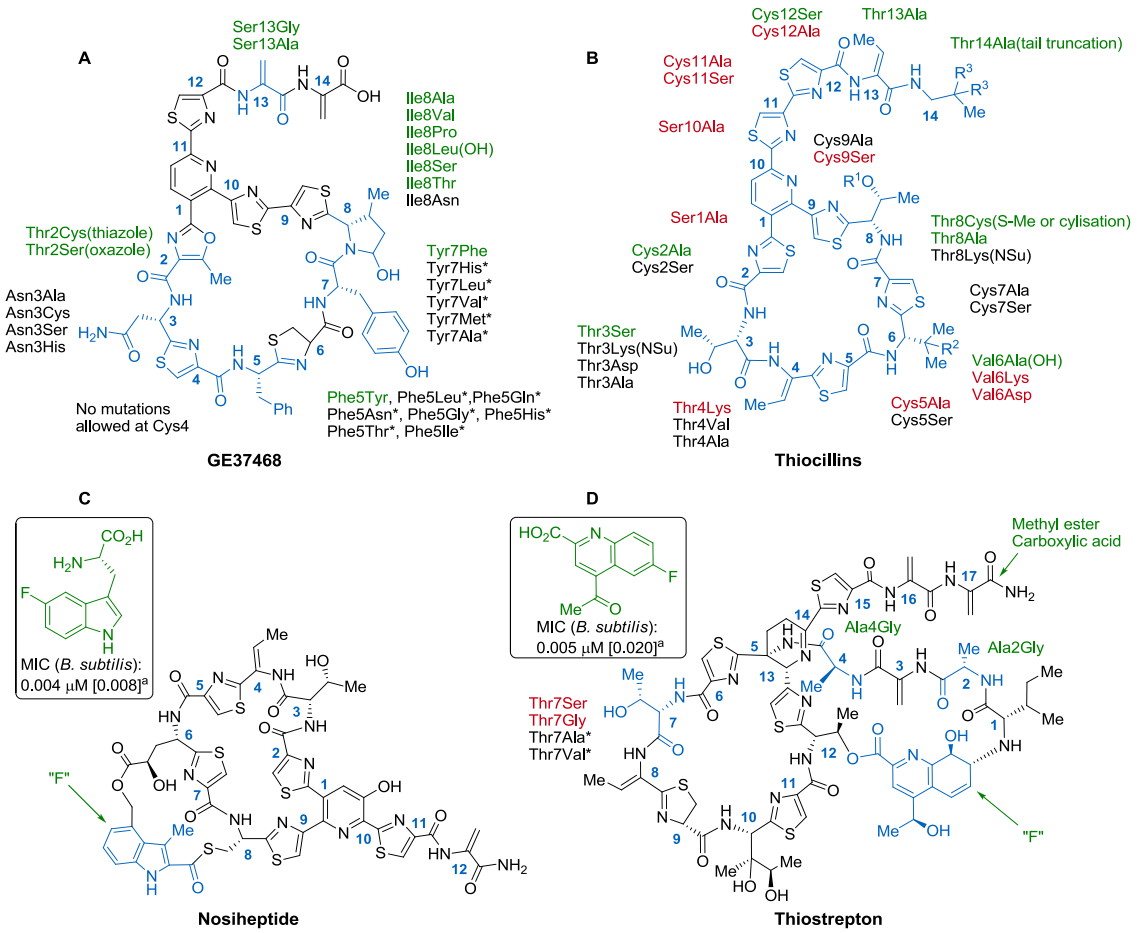


Figure 5. Production of analogues by pre-peptide mutagenesis, feeding with modified building blocks or gene deletion: GE37469 (A), thiocillins (B), nosiheptide (C) and thiostrepton (D). Modifications that produced active analogues are colored in green. Residue replacements that resulted in non-active mature products are colored in black and those that did not produce the mature analogue or did not provide high enough yields for testing are colored in red. A star highlights those mutations that resulted in altered downstream processing. [a]Compounds used for feeding experiments are in boxes; activity values correspond to mature analogues and the activity of the natural product appears in brackets.

found in the natural sequence. By doing so, once dehydration of Ser had occured, competition between Dha residues took place and new compounds of different ring sizes or mixtures of them were produced. All this series of analogues lacked antibiotic activity; however, these results demonstrate that TclM, the enzyme responsible for the putative cycloaddition, is largely promiscuous and could be used for the production of many analogues effective against targets other than the ribosome or elongation factors.

Investigations on the role of different enzymes present in the thiopeptide gene clusters have provided analogues during inactivation experiments. For example, the above mentioned TclM can be inactivated by *tclM* deletion to produce linear thiocillin precursors.[73] Inactivation of enzymes involved in tail maturation has provided active macrocyclic analogues. These enzymes act after cycloaddition, which leads to the obtention of very mature products containing most of thiopeptide's characteristic structural features. Thiostrepton terminal amide can be replaced for its corresponding methyl ester or carboxylic acid through inactivation of TsrS or TsrT, respectively (Figure 5D).[74,75] Thiostrepton methyl ester was found to be one order of magnitude more potent than the natural amide; however, its solubility was substantially reduced. On the

other hand, thiostrepton acid profile was completely opposite and despite its improved solubility, it displayed reduced *in vitro* potency.

The different existing pathways of amide formation in thiopeptide tails provide a chance for the isolation of distinct intermediates other than those observed for thiostrepton. Such is the case of nosiheptide, whose C-terminal amide is formed after cleavage of the last Dha residue of the leading peptide by NosA. Fermentation of a *nosA* KO yields nosiheptide with the extra Dha amino acid, which was not detrimental for *in vitro* potency.[76] Inhibition of the methyltransferase NosN results in the production of an analogue displaying the same extended tail; however, in this case, the second macrocycle of nosiheptide is not formed, demonstrating that NosN is responsible for 4'-methylation of the indolic acid moiety, which is required for subsequent cyclization.[77] With these experiments, both the bio-synthetic pathway and the *in vivo* production of analogues, can be explored synergistically.

Indolic and quinaldic acids are present in the second macrocycle of various thiopeptides. During investigation on these moieties bio-synthesis, Liu and collaborators disclosed the role of various enzymes accounting for the conversion of Trp into those fragments.[78–80] Given that the starting Trp residue is not present in the pre-peptide sequence and that it is incorporated into the maturating thiopeptide after its own processing, feeding experiments with Trp derivatives are possible. To date, analogues of indolic acid and quinaldic acid containing thiopeptides have been obtained, both resulting from incorporation of the fluorinated precursor into the bio-synthetic pathway. Feeding of *S. actuosus* cultures with 5-fluoro-DL-tryptophan resulted in incorporation of the modified L-amino acid into Nosiheptide structure, giving rise to 5'-fluoro-nosiheptide along with the natural product (Figure 5C).[78] In order to obtain a 6'-fluorinated thiostrepton analogue, a 6-flurinated quinaldic acid derivative was fed to a culture of mutated *S. laurentii* lacking *tsrT* gene. TsrT is a methyltransferase required in the initial steps of Trp conversion into quinaldic acid and, by using this strategy, competition of unmodified Trp was completely avoided and only the desired 6'-fluoro-thiostrepton was obtained (Figure 5D).[80] In both cases, fluorination resulted in increased *in vitro* potencies, highlighting the huge potential of mutasynthetic methods.

Alternatively, modified thiopeptides can also be obtained from cultures of the wild-type producing strain if the activity of enzymes is tuned by modification of different factors, such as pH, metal ions and other additives. In this regard, *in situ* conversion of nocathiacin I into nocathiacin acid can be achieved without relying on semi-synthetic methods.[81]

In spite of some of the highly modified products produced by *in vivo* methods, only thiopeptides with very conservative alterations and still bearing their characteristic macrocycles, have displayed interesting profiles. Actually, the enzymatic machinery devoted to thiopeptides bio-synthesis cannot produce products much more different than those it has developed to work with. Moreover, although residue replacement is possible thanks to the ribosomal origin of the pre-peptide, to date it has limited the residues than can be introduced to the set of 20 naturally occurring amino acids. Despite these limitations, the use of non-natural tryptophan or its corresponding processed metabolites has permitted the introduction of altered moieties derived from it, widening the chemical space that can be explored.

3. Perspective

Most of the literature regarding the modification of thiopeptides is very recent and new advances are expected to appear during the following years. Along with information gathered from other approaches, further modification of the natural products may reveal more of the features that grant thiopeptides their huge potencies and that could serve to improve them as well as their solubility. However, the different disciplines to which this review is devoted might not be able to produce the required diversity as standalone strategies. As previously stated, they all have some limitations, including the kind of modifications that they allow. Although, in principle, chemical synthesis should be able to provide any analogue that can be designed, the lengthy and costly routes it requires do not make it feasible for neither preparation of libraries nor large-scale production. Thus, a combination of all strategies should give rise to more sophisticated analogues with the characteristic modifications of every approach introduced at different stages (Figure 6).[82] First, a pre-peptide including both the structural and leading peptides could be synthesized by chemical methods (Figure 6B), such as solid-phase peptide synthesis (SPPS) for ease of preparation. At this point, many modifications can be included,

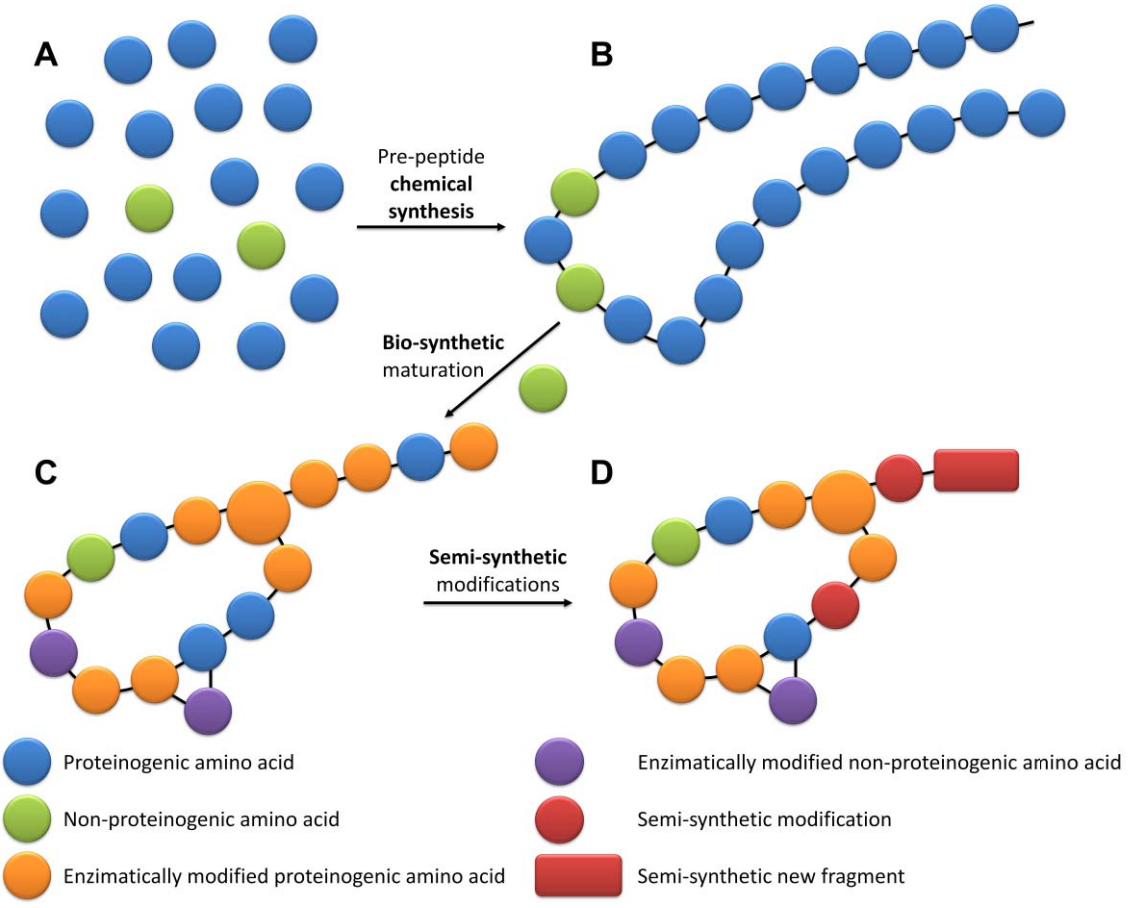


Figure 6. Proposed sequence for the future production of highly modified thiopeptide analogues: Using both proteinogenic and modified amino acids (A), altered synthetic pre-peptides could be produced (B). *In vivo* maturation should provide thiopeptides with their characteristic structural features and other modified building blocks could be introduced (C). Semi-synthetic derivatization of the isolated product could introduce further modifications (D).

such as amino acid replacement or the introduction of non-proteinogenic amino acidss, otherwise forbidden due to the ribosomal origin of thiopeptides. Next, maturation of the peptide by the enzymatic machinery of an adequate strain, which can be genetically engineered, would perform its thorough tailoring to install the unique features of thiopeptides. Moreover, feeding with modified indole would introduce more diversity (Figure 6C). After isolation, semi-synthetic modifications could be made in order to perform further improvements, such as attaching moieties for solubility enhancement (Figure 6D).

Developing new highly altered thiopeptides will require epic efforts if libraries of semi-synthetically modified bio-engineered products have to be prepared. However, once a product of interest has been identified as a candidate, fermentation of the mutant and its semi-synthetic modifications should grant access to it in a fast and affordable manner. Genetic engineering and chemical post-fermentation modifications appear as ideal partners for the discovery and development of future thiopeptide-based drugs. Upcoming developments in all the areas herein discussed will surely overcome many of the limitations they all possess and will provide us with new compounds of increased sophistication.

4. References

(1) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.

(2) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 7930–7954.

(3) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5087–5092.

(4) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5092–5097.

(5) Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4771–4774.

(6) Lefranc, D.; Ciufolini, M. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198–4201.

(7) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 15644–15651.

(8) Just-Baringo, X.; Bruno, P.; Ottesen, L. K.; Cañedo, L. M.; Albericio, F.; Alvarez, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 7818–7821.

(9) Arndt, H.-D.; Schoof, S.; Lu, J.-Y. *Angew. Chem. Int. Ed.* **2009**, *48*, 6770–6773.

(10) Li, C.; Kelly, W. L. *Nat. Prod. Rep.* **2010**, *27*, 153–164.

(11) Vandeputte, J.; Dutcher, J. D. *Antibiot. Ann.* **1955**, *3*, 560–561.

(12) Leet, J. E.; Ax, H. A.; Gustavson, D. R.; Brown, D. M.; Turner, L.; Brown, K.; Li, W.; Lam, K. S. Nocathiacin antibiotics **2001**.

(13) Shoji, J.; Hinoo, H.; Wakisaka, Y.; Koizumi, K.; Mayama, M.; Matsuura, S.; Matsumoto, K. *J. Antibiot.* **1976**, *29*, 366–374.

(14) Selva, E.; Beretta, G.; Montanini, N.; Saddler, G. S.; Gastaldo, L.; Ferrari, P.; Lorenzzetti, R.; Landini, P.; Ripamonti, F.; Goldstein, B. P.; Berti, M.; Montanaro, L.; Denaro, M. *J. Antibiot.* **1991**, *44*, 693–701.

(15) Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

(16) Engelhardt, K.; Degnes, K. F.; Kemmler, M.; Bredholt, H.; Fjaervik, E.; Klinkenberg, G.; Sletta, H.; Ellingsen, T. E.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 4969–4976.

(17) Abe, H.; Katsuhiko, K.; Yoshinori, S.; Mitsuaki, K. *Tetrahedron Lett.* **1988**, *29*, 1401–1404.

(18) Nicolaou, K. C.; Nevalainen, M.; Zak, M.; Bulat, S.; Bella, M.; Safina, B. S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3418–3424.

(19) Nicolaou, K. C.; Zak, M.; Rahimipour, S.; Estrada, A. a; Lee, S. H.; O'Brate, A.; Giannakakou, P.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2005**, *127*, 15042–15044.

(20) Nicolaou, K. C.; Zou, B.; Dethe, D. H.; Li, D. B.; Chen, D. Y.-K. *Angew. Chem. Int. Ed.* **2006**, *45*, 7786–7792.

(21) Starosta, A. L.; Qin, H.; Mikolajka, A.; Leung, G. Y. C.; Schwinghammer, K.; Nicolaou, K. C.; Chen, D. Y.-K.; Cooperman, B. S.; Wilson, D. N. *Chem. Biol.* **2009**, *16*, 1087–1096.

(22) Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455–2458.

(23) 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*, 1059–1063.

(24) Naidu, B. N.; Sorenson, M. E.; Zhang, Y.; Kim, O. K.; Matiskella, J. D.; Wichtowski, J. a; Connolly, T. P.; Li, W.; Lam, K. S.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5573–5577.

(25) Naidu, B. N.; Sorenson, M. E.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2069–2072.

(26) Naidu, B. N.; Li, W.; Lam, K. S.; Sorenson, M. E.; Wichtowski, J. A.; Connolly, T. P.; Ueda, Y.; Bronson, J. J.; Zhang, Y.; Kim, O. K. Water Soluble Thiazolyl Peptide Derivatives **2002**.

(27) Naidu, B. N.; Sorenson, M. E.; Matiskella, J. D.; Li, W.; Sausker, J. B.; Zhang, Y.; Connolly, T. P.; Lam, K. S.; Bronson, J. J.; Pucci, M. J.; Yang, H.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3545–9.

(28) Regueiro-Ren, A.; Ueda, Y. *J. Org. Chem.* **2002**, *67*, 8699–702.

(29) Connolly, T. P.; Regueiro-Ren, A.; Leet, J. E.; Springer, D. M.; Goodrich, J.; Huang, X. S.; Pucci, M. J.; Clark, J. M.; Bronson, J. J.; Ueda, Y. *J. Nat. Prod.* **2005**, *68*, 550–3.

(30) Xu, L.; Farthing, A. K.; Shi, Y.-J.; Meinke, P. T.; Liu, K. *J. Org. Chem.* **2007**, *72*, 7447–7450.

(31) Xu, L.; Farthing, A. K.; Dropinski, J. F.; Meinke, P. T.; McCallum, C.; Leavitt, P. S.; Hickey, E. J.; Colwell, L.; Barrett, J.; Liu, K. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3531–3535.

(32) Hrnciar, P.; Ueda, Y.; Huang, S.; Leet, J. E.; Bronson, J. J. *J. Org. Chem.* **2002**, *67*, 8789–8793.

(33) Pucci, M. J.; Bronson, J. J.; Barrett, J. F.; Denbleyker, K. L.; Discotto, L. F.; Joan, C.; Fung-tomc, J. C.; Ueda, Y. *Antimicrob. Agents Chemother.* **2004**, *48*, 3697–3701.

(34) Regueiro-Ren, A.; Naidu, B. N.; Zheng, X.; Hudyma, T. W.; Connolly, T. P.; Matiskella, J. D.; Zhang, Y.; Kim, O. K.; Sorenson, M. E.; Pucci, M.; Clark, J.; Bronson, J. J.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 171–175.

(35) Naidu, B. N.; Sorenson, M. E.; Hudyma, T.; Zheng, X.; Zhang, Y.; Bronson, J. J.; Pucci, M. J.; Clark, J. M.; Ueda, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3743–3746.

(36) Golik, J.; Wong, H. S. L.; Chcn, S. H.; Doyle, T. W.; Wright, J. J. K.; Knipe, J.; Rose, W. C.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1837–1842.

(37) Tojo, T.; Hanadate, T.; Okada, A.; Kazami, J.; Takeda, S.; Shibazaki, M. Preparation of cyclic thiopeptides (QN3323-A derivatives) and their pharmaceutical compositions for multidrug-resistant bacteria **2008**.

(38) Junichi, K.; Takashi, O.; Masato, W.; Kazuma, K.; Takashi, Y.; Kaniaki, T. Preparation of O-acyl-QN3323-A oxime thiopeptide compounds as antibacterial agents **2009**.

(39) LaMarche, M. J.; Leeds, J. A.; Dzink-Fox, J.; Gangl, E.; Krastel, P.; Neckermann, G.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Tiamfook, S.; Yu, D. *J. Med. Chem.* **2012**, *55*, 6934–6941.

(40) Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304–1311.

(41) Tavecchia, P.; Gentili, P.; Kurz, M.; Sottani, C.; Bonfichi, R.; Selva, E.; Lociuro, S.; Restelli, E.; Ciabatti, R. *Tetrahedron* **1995**, *51*, 4867–4890.

(42) Tavecchia, P.; Kurz, M.; Colombo, L.; Bonfichi, R.; Selva, E.; Lociuro, S.; Marzorati, E.; Ciabatti, R. *Tetrahedron* **1996**, *52*, 8763–8774.

(43) Lociuro, S.; Tavecchia, P.; Marzorati, E.; Landini, P.; Goldstein, B. P.; Denarod, M.; Ciabatti, R. *J. Antibiot.* **1997**, *50*, 344–349.

(44) Malabarba, A.; Cavaleri, M.; Mosconi, G.; Jabes, D.; Romano, G. Use of amide derivative of GE 2270 factor a3 for the treatment of acne **2007**.

(45) Clough, J.; Chen, S.; Gordon, E. M.; Hackbarth, C.; Lam, S.; Trias, J.; White, R. J.; Candiani, G.; Donadio, S.; Romanò, G.; Ciabatti, R.; Jacobs, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3409–3414.

(46) LaMarche, M. J.; Leeds, J. a; Dzink-Fox, J.; Gunderson, K.; Krastel, P.; Memmert, K.; Patane, M. a; Rann, E. M.; Schmitt, E.; Tiamfook, S.; Wang, B. *J. Med. Chem.* **2011**, *54*, 2517–2521.

(47) LaMarche, M. J.; Leeds, J. a; Dzink-Fox, J.; Mullin, S.; Patane, M. a; Rann, E. M.; Tiamfook, S. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3210–3215.

(48) LaMarche, M. J.; Leeds, J.; Amaral, K.; Brewer, J.; Bushell, S.; Dewhurst, J.; Dzink-Fox, J.; Gangl, E.; Goldovitz, J.; Jain, A.; Mullin, S.; Neckermann, G.; Osborn, C.; Palestrant, D.; Patane, M.; Rann, E.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Whitehead, L.; Yu, D. *J. Med. Chem.* **2011**, *54*, 8099–8109.

(49) LaMarche, M. J.; Bushell, S.; Patane, M. A.; Whitehead, L. Aminothiazoles and their Uses **2010**.

(50) Leeds, J. A.; LaMarche, M. J.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Dzink-Fox, J.; Gangl, E.; Jain, A.; Lee, L.; Lilly, M.; Manni, K.; Mullin, S.; Neckermann, G.; Osborne, C.; Palestrant, D.; Patane, M. a; Raimondi, A.; Ranjitkar, S.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Whitehead, L.; Yu, D. *Antimicrob. Agents Chemother.* **2011**, *55*, 5277–5283.

(51) LaMarche, M. J.; Leeds, J. A.; Amaral, A.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Ding, J.; Dzink-fox, J.; Gamber, G.; Jain, A.; Lee, K.; Lee, L.; Lister, T.; Mckenney, D.; Mullin, S.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Trzasko, A.; Whitehead, L.; Yifru, A.; Yu, D.; Yan, W.; Zhu, Q. *J. Med. Chem.* **2012**, *6*, 2376–2387.

(52) Trzasko, A.; Leeds, J. A.; Praestgaard, J.; LaMarche, M. J.; McKenney, D. *Antimicrob. Agents Chemother.* **2012**, *56*, 4459–4462.

(53) Leeds, J. A.; Sachdeva, M.; Mullin, S.; Dzink-Fox, J.; LaMarche, M. J. *Antimicrob. Agents Chemother.* **2012**, *56*, 4463–4465.

(54) Myatt, S. S.; Lam, E. W.-F. *Nat. Rev. Cancer* **2007**, *7*, 847–859.

(55) Hegde, N. S.; Sanders, D. A.; Rodriguez, R.; Balasubramanian, S. *Nat. Chem.* **2011**, *3*, 725–731.

(56) Schoof, S.; Baumann, S.; Ellinger, B.; Arndt, H.-D. *ChemBioChem* **2009**, *10*, 242–245.

(57) Schoof, S.; Pradel, G.; Aminake, M. N.; Ellinger, B.; Baumann, S.; Potowski, M.; Najajreh, Y.; Kirschner, M.; Arndt, H.-D. *Angew. Chem. Int. Ed.* **2010**, *49*, 3317–3321.

(58) Baumann, S.; Schoof, S.; Bolten, M.; Haering, C.; Takagi, M.; Shin-ya, K.; Arndt, H.-D. *J. Am. Chem. Soc.* **2010**, *132*, 6973–6981.

(59) Myers, C. L.; Hang, P. C.; Ng, G.; Yuen, J.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **2010**, *18*, 4231–4237.

(60) Schoof, S.; Arndt, H.-D. *Chem. Commun.* **2009**, *46*, 7113–7115.

(61) Pandit, B.; Bhat, U.; Gartel, A. L. *Cancer Biol. Ther.* **2011**, *11*, 43–47.

(62) Jonker, H. R. A.; Baumann, S.; Wolf, A.; Schoof, S.; Hiller, F.; Schulte, K. W.; Kirschner, K. N.; Schwalbe, H.; Arndt, H.-D. *Angew. Chem. Int. Ed.* **2011**, *50*, 3308–3312.

(63) Schofield, C. J.; Baldwin, J. E.; Byford, M. F.; Clifton, I.; Hajdu, J.; Hensgens, C.; Roach, P. *Curr. Opin. Struct. Biol.* **1997**, *7*, 857–864.

(64) Zhang, Q.; Liu, W. *Nat. Prod. Rep.* **2013**, *30*, 218–226.

(65) Zhang, F.; Kelly, W. L. *In vivo production of thiopeptide variants*; 1st ed.; Elsevier Inc., 2012; Vol. 516, pp. 3–24.

(66) Acker, M. G.; Bowers, A. A.; Walsh, C. T. *J. Am. Chem. Soc.* **2009**, *131*, 17563–17565.

(67) Bowers, A. A.; Acker, M. G.; Koglin, A.; Walsh, C. T. *J. Am. Chem. Soc.* **2010**, *132*, 7519–7527.

(68) Young, T. S.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13053–13058.

(69) Li, C.; Zhang, F.; Kelly, W. L. *Mol. BioSyst.* **2011**, *7*, 82–90.

(70) Li, C.; Zhang, F.; Kelly, W. L. *Chem. Commun.* **2012**, *48*, 558–560.

(71) Young, T. S.; Dorrestein, P. C.; Walsh, C. T. *Chem. Biol.* **2012**, *19*, 1600–1610.

(72) Bowers, A. A.; Acker, M. G.; Young, T. S.; Walsh, C. T. *J. Am. Chem. Soc.* **2012**, *134*, 10313–10316.

(73) Bowers, A. A.; Walsh, C. T.; Acker, M. G. *J. Am. Chem. Soc.* **2010**, *132*, 12182–12184.

(74) Kelly, W. L.; Pan, L.; Li, C. *J. Am. Chem. Soc.* **2009**, *131*, 4327–4334.

(75) Liao, R.; Liu, W. *J. Am. Chem. Soc.* **2011**, *133*, 2852–2855.

(76) Yu, Y.; Guo, H.; Zhang, Q.; Duan, L.; Ding, Y.; Liao, R.; Lei, C.; Shen, B.; Liu, W. *J. Am. Chem. Soc.* **2010**, *132*, 16324–16326.

(77) Yu, Y.; Duan, L.; Zhang, Q.; Liao, R.; Ding, Y.; Pan, H.; Wendt-, E.; Tang, G.; Shen, B.; Liu, W. *ACS Chem Biol.* **2009**, *4*, 855–864.

(78) Zhang, Q.; Li, Y.; Chen, D.; Yu, Y.; Duan, L.; Shen, B.; Liu, W. *Nat. Chem. Biol.* **2011**, *7*, 154–160.

(79) Zhang, Q.; Chen, D.; Lin, J.; Liao, R.; Tong, W.; Xu, Z.; Liu, W. *J. Biol. Chem.* **2011**, *286*, 21287–21294.

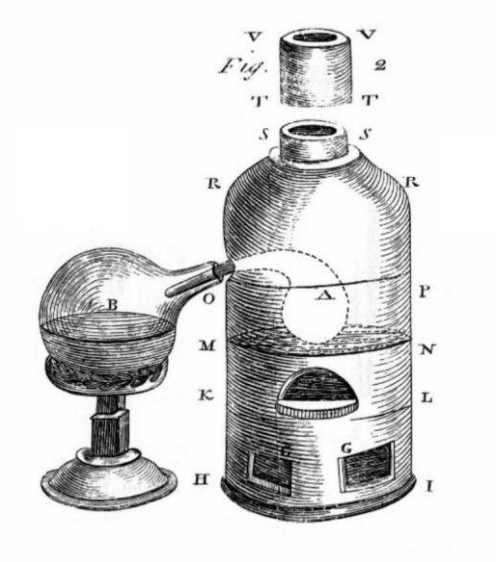
(80) Duan, L.; Wang, S.; Liao, R.; Liu, W. *Chem. Biol.* **2012**, *19*, 443–448.

(81) Wei, M.; Wang, S.; Fang, Y.; Chen, Y. *Bioresour. Technol.* **2010**, *101*, 3617–3622.

(82) Kirschning, A.; Hahn, F. *Angew. Chem. Int. Ed.* **2012**, *3*, 4012–4022.

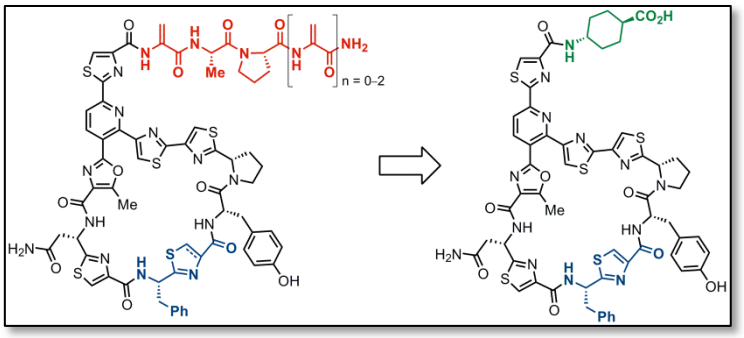
Chapter 7:

Synthesis of Baringolin Analogues and Structure-Activity Relationship Studies



SYNTHESIS OF BARINGOLIN ANALOGUES AND STRUCTURE-ACTIVITY RELATIONSHIP STUDIES

Dissecting the structure of thiopeptides: assessment of thiazoline and tail moieties of baringolin and antibacterial activity optimization



Xavier Just-Baringo,[a,b] Paolo Bruno,[a,b] Cristina Pitart,[c] Jordi Vila,[c] Fernando Albericio[a,b,d,e] and Mercedes Álvarez [a,b,f]

SUBMITTED

[a] Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.

[b] CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.

[c] Center for International Health Research, CRESIB, Hospital Clínic, University of Barcelona, 08036 Barcelona, Spain.

[d] Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

[e] School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.

[f] Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.

Conspectus

With a concise and modular strategy for the synthesis of baringolin in hands, it was now possible to generate analogues with modifications at key regions. The two of the most characteristic moieties of baringolin, its surprisingly long tail and the Phe-derived thiazoline ring, were substituted to assess their structure-activity profile. Thiazoline was substituted by its aromatic counterpart using the corresponding Phe-derived thiazole. Peptidic tail variants were obtained using the same solid-phase strategy that had been previously used for pentapeptidic tail synthesis. Various thiazole-4-carboxylic acid derivatives were also assessed in the absence of a peptidic tail. Solid-phase synthesis of the original pentapeptidic tail capped with a thiazole-4-carboxylate moiety also permitted to evaluate the antibacterial activity of this appendix *per se*.

Testing of all analogues against various Gram-positive bacterial strains revealed the key impact of thiazoline in broadening the activity profile of baringolin. By contrast, its tail did not appear as a crucial moiety. Substitution of the pentapeptide for a *trans*-4-aminocyclohexanoic acid moiety improved the antibacterial potency against most strains and overcame activity restrictions of the thiazole series of analogues.

Remarkably, this is the first fully synthetic library of thiopeptide analogues ever reported. This fact, combined with the good activity results obtained is an excellent evidence to validate our synthetic strategy as a suitable one for the assessment of structure-activity relationships of such complex molecules.

Dissecting the Structure of Thiopeptides: Assessment of Thiazoline and Tail Moieties of Baringolin and Antibacterial Activity Optimization.

Xavier Just-Baringo,[1,2] Paolo Bruno,[1,2] Cristina Pitart,[3] Jordi Vila,[3] Fernando Albericio, [1,2,4,5] Mercedes Álvarez[1,2,6]*

[1] *Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.*

[2] *CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.*

[3] Center for International Health Research, CRESIB, Hospital Clínic, University of Barcelona, 08036 Barcelona, Spain.

[4] *Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.*

[5] *School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.*

[6] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.*

KEYWORDS

Thiopeptides / Thiazoline / Thiazole / Antibiotics / Synthesis / Structure-Activity Relationships / Solid-Phase Peptide Synthesis

ABSTRACT

Baringolin (**1**) analogues were prepared to evaluate the role of its characteristic thiazoline ring and pentapeptidic tail. These modifications helped to define structure-activity relationships for these regions. The thiazoline ring appeared as a crucial moiety to maintain a broad scope of activities against different Gram positive bacteria strains. Further modifications were performed to simplify the structure of the natural product and assess the role of its tail, resulting in an enhanced *in vitro* performance. Analogue **2**, with the thiazole-containing macrocycle and a 4-aminocyclohexanoic acid moiety in place of the pentapeptidic tail, was identified as a much more potent analogue, capable of overcoming the absence of the thiazoline ring and performing extraordinarily well against all strains tested. This is the first library of thiopeptide analogues produced by chemical synthesis alone, which demonstrates the robustness and convenience of the synthetic strategy used.

INTRODUCTION

Antibiotic resistance to marketed drugs is an increasing concern in the clinic and requires the development of new compounds that can overcome this phenomenon.[1] The discovery of new molecules with new modes of action is key to avoid cross resistances. In this context, thiopeptide antibiotics have arisen as promising candidates due to their good performing in *in vitro* assays against various microorganisms. Despite the good activities reported, their lack of aqueous solubility has limited their use to the treatment of skin infections, regardless of the huge efforts carried out for the synthesis of more soluble analogues.[2,3,4]

The complex architecture of thiopeptides[5] has prompted many groups to develop sophisticated and robust synthetic strategies to achieve the total synthesis of many members of this family of antibiotics.[6] However, these syntheses are inherently lengthy and time consuming and have scarcely been applied to the synthesis of analogues, with most of the reports focused on fragment and synthetic intermediates screening.[7,8,9,10] Thus, alternative approaches to the

preparation of modified thiopeptides have been explored, all taking advantage of the biosynthetic pathway that produces the parent natural products.[11] On one hand, engineering of the biosynthetic pathway has shown its potential for the obtention of analogues arising from different kinds of modifications: residue replacement, enzyme knock out and feeding with non-natural precursors.[12] Replacement of putative Ile, which is oxidized to form 2-hydroxy-4-methylpyrrolidine, in the parent peptide sequence of GE37468A (**3**)[13] with Pro, produced the mutasynthetic analogue **4**,[14] which has a macrocycle identical to that of baringolin (**1**) (Figure 1). On the other hand, semi-synthesis permits chemical modification of the product at its most reactive sites, giving rise to a wide variety of transformations, such as the conversion of thiomuracin A (**5**)[15] into its derivative **6**.[16]

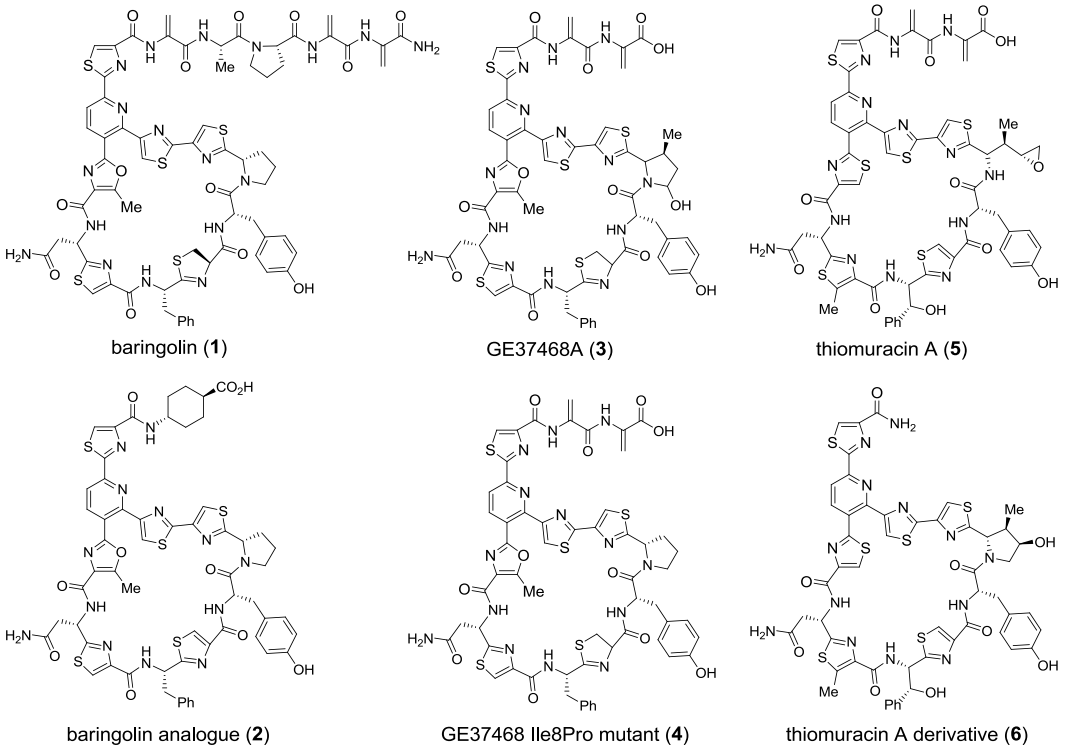


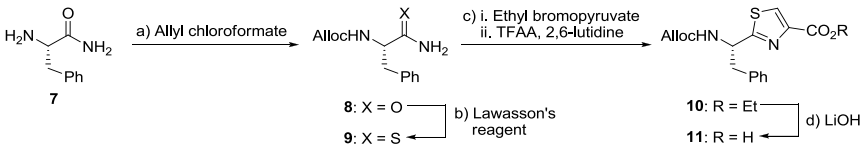
Figure 1. Thiopeptide antibiotics of the *d* series (top) and analogues of diverse origin derived from them (bottom). Baringolin analogue **2**, reported herein, was produced by chemical synthesis alone; **4** was produced by mutasynthesis; and **6** was obtained by semi-synthesis.

Despite the very limited presence of fully synthetic thiopeptide derivatives in the literature, our recent studies on **1** total synthesis aimed at the development of a modular and convergent strategy that should facilitate the preparation of its analogues.[17] Our goal was to assess the role of both the thiazoline ring and the pentapeptidic tail of **1**. In order to do so, we first aimed at the synthesis of a macrocycle analogue introducing a thiazole moiety in place of the naturally occurring thiazoline. Secondly, shorter peptidic tails were to be introduced to assess their impact in antibacterial activity. Once these modifications were performed, substitution of the peptidic tail by other moieties should help us identify compounds of improved performance. Herein we present the first library of thiopeptide analogues obtained solely by chemical synthesis.

RESULTS AND DISCUSSION

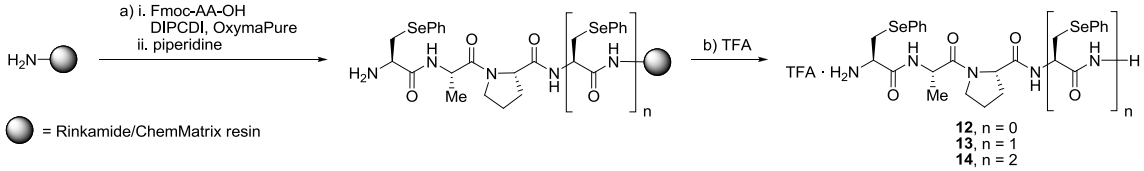
Substitution of the thiazoline moiety of the macrocycle in **1** for the corresponding thiazole was regarded as a modification likely to introduce rigidity and stability to the macrocyclic scaffold.[14] Furthermore, this modification is more favorable from a synthetic point of view and should also enhance the stability of the final compound. Such a modification was expected to retain activity activity against *S. aureus*, since the thiomuracins display the same fully

unsaturated thiazole on the equivalent position of their similar macrocycle.[15] In order to obtain the desired analogue, a suitable building block was synthesized (Scheme 1). Phenylalaninamide (**7**) was protected with the Alloc group, yielding **8**, which was then converted into the corresponding thiomaide **9** and subsequently transformed into the desired thiazole **10** by means of a two-step Hantzsch cyclization. Ester hydrolysis produced carboxylic acid **11**, a suitably functionalized fragment for further condensation. The use of Alloc-protected fragment **11** will facilitate the deprotection step prior to macrocyclization in subsequent stages of the synthesis.



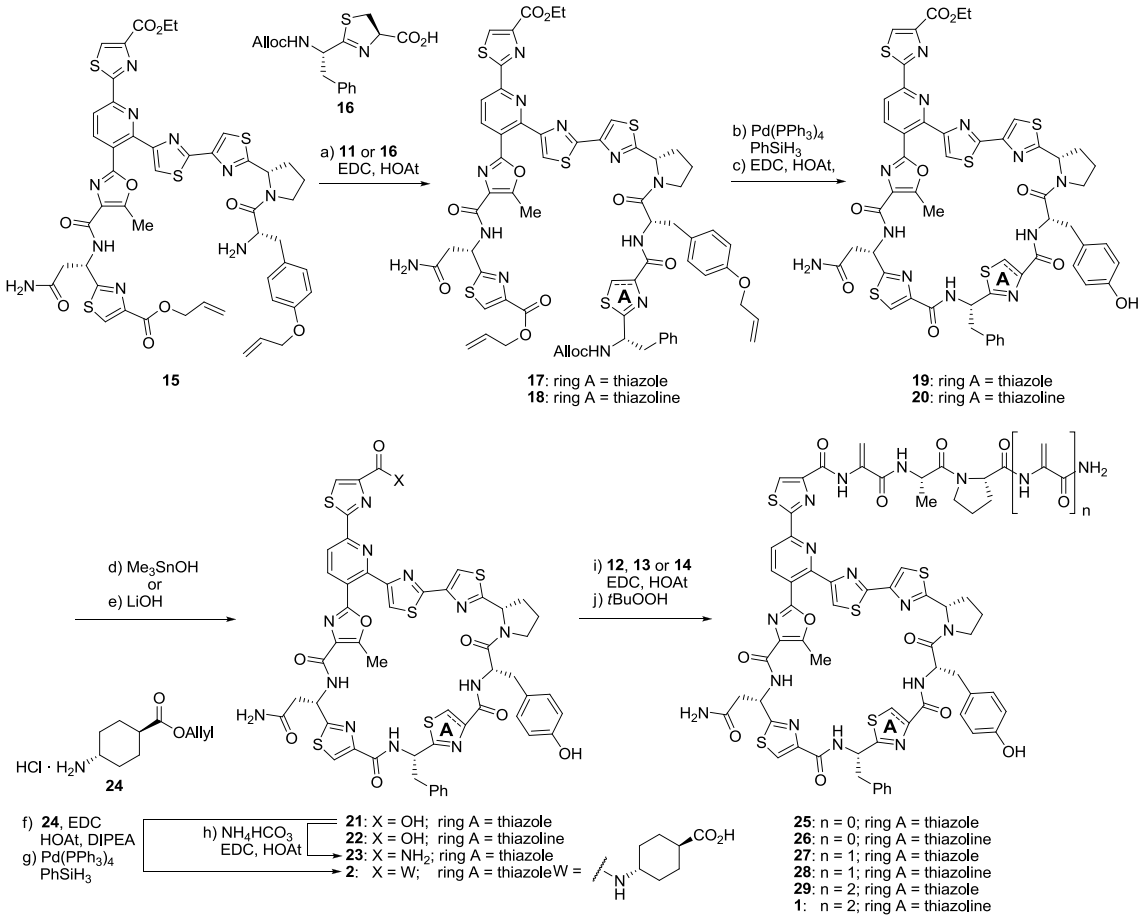
Scheme 1. Synthesis of phenylalanine-derived thiazole **11**. Reagents and conditions: Allyl chloroformate, NEt_3, CH_2Cl_2, 0 ºC, 2 h, 86%; b) Lawesson's reagent, THF, rt, 4.5 h, 88%; c) i. ethyl bromopyruvate, $KHCO_3$, DME, 0 ºC, 2.5 h; ii. TFAA, 2,6-lutidine, DME, −20 ºC, 2.5 h, 99% (95% ee); d) LiOH, H_2O/THF, rt, 15 h, 86%. DME = dimethoxyethane, TFAA = trifluoroacetic anhydride.

To evaluate the role of baringolin's tail, the corresponding tri- and tetra- precursor peptides were synthesized to be used in the preparation of analogues of various tail lengths. The phenylselenocysteine-containing peptides, **12** and **13**,[18] were synthesized by solid phase peptide synthesis (SPPS) on a Rinkamide/ChemMatrix resin[19] to obtain the corresponding C-terminal amides using the same methodology described for the preparation of baringolin's pentapeptide precursor **14**[17,20] (Scheme 2).

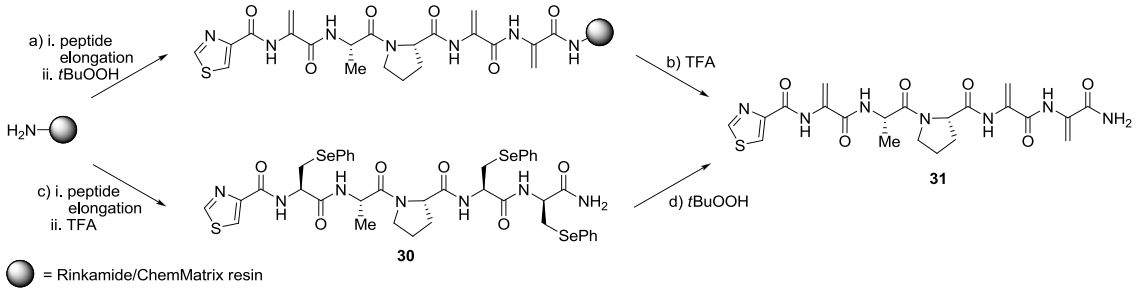


Scheme 2. Solid phase synthesis of tri-, tetra- and pentapeptide precursors, **12**, **13** and **14**, respectively. Reagents and conditions: a) i. Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, rt, 1.5 h; ii. 20% pipepridine in DMF, rt (4 treatments); b) 95% TFA in CH_2Cl_2, rt (4 treatments). Yields: **12** (quant.), **13** (quant.), **14** (89%). DIPCDI = *N*,*N*'-diisopropylcarbodiimide.

Preparation of the macrocycle-containing analogues started from **15**, a common intermediate to the total synthesis of baringolin, which could be condensed with either thiazole building block **11** or the corresponding thiazoline **16**[17] to furnish the protected macrocycle precursors **17** and **18**, respectively (Scheme 3). Next, removal of all allyl-based protecting groups and macrocyclization under diluted conditions (1 mM) rendered the desired macrocycles **19** and **20**. At this point, different conditions were required for hydrolysis of the ethyl ester of the two macrocycles. While **19** was hydrolyzed under more conventional basic conditions to obtain carboxylic acid **21**, **20** contains a thiazoline ring and necessitated the use of trimethyltin hydroxide to grant a mild and epimerization-free saponification to yield **22**.[21] With both **21** and **22** in hands, manipulation of the carboxylic acids was performed to introduce a series of substituents. The amide of the thiazole-macrocycle analogue was also synthesized in an analogous manner, giving rise to **23**. Moreover, allyl *trans*-4-aminocyclohexanoate (**24**) was also condensed with **21** to generate the corresponding cyclohexanoic acid derivative **2** after deprotection. The cyclohexanoic acid moiety has been previously installed into other thiopeptides through different linkers with satisfactory results.[22] Peptides **12**−**14** were condensed with acids **21** and **22** to obtain analogues **25**−**29** and **1**[17] in order to assess the impact of both thiazoline and thiazole rings, as well as the role of the different peptidic tails.



Scheme 3. Synthesis of analogues **2**, **19**–**21**, **23** and **25**–**29**. Reagents and conditions: a) **11** or **16**, EDC, HOAt, DIPEA, DMF, 0 ºC to rt, 3 h, 85% (**17**), 68% (**18**); b) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt; c) EDC, HOAt, DMF (1 mM), rt, 61% (**19**), 30% (**20**); d) Me_3SnOH, $ClCH_2CH_2Cl$, 60 ºC, 19 h; e) LiOH, H_2O/THF, rt, 17 h, 99% (**21**); f) **24**, EDC, HOAt, DIPEA, DMF, 0 ºC to rt, 5 h; g) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, rt, 2 h, 39% (2 steps); h) NH_4CO_3, EDC, HOAt, DIPEA, DMF, 0 ºC to rt, 28 h, 68%; i) **12**, **13** or **14**, EDC, HOAt, DIPEA, DMF, 0 ºC to rt; j) *t*BuOOH, CH_2Cl_2, rt, 39% (**25**, 2 steps), 63% (**26**, 3 steps), 33% (**27**, 2 steps), 55% (**28**, 3 steps), 50% (**29**, 2 steps), 53% (**1**, 3 steps).



Scheme 4. Synthesis of peptidic tail analogue **31**. Reagents and conditions: a) Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, rt, 1.5 h; 20% pipepridine in DMF, rt (4 treatments); ii. *t*BuOOH, CH_2Cl_2, rt; b) 95% TFA in CH_2Cl_2, rt (4 treatments), 36% (2 steps); c) Fmoc-AA-OH, *N*,*N*'-diisopropylcarbodiimide, OxymaPure, DMF, rt, 1.5 h; 20% pipepridine in DMF, rt (4 treatments); ii. 95% TFA in CH_2Cl_2, rt (4 treatments), 47%; d) *t*BuOOH, CH_2Cl_2, rt, 72%. DIPCDI = *N*,*N*'-diisopropylcarbodiimide.

Apart from the macrocyclic analogues, fragment **30**, formed by baringolin's pentapeptide bearing a C-terminal 4-thiazolecarboxylic acid, was synthesized using the same solid phase strategy described above (Scheme 4) in order to assess whether the peptidic tail possess antibiotic activity in the absence of the macrocyclic scaffold. Two alternative approaches were used for the obtention of **30**, either an on-resin or an in-solution oxidation/elimination protocol.

In both cases, the outcome was very similar, showing the robustness of the solid-phase approach, and **30** was obtained in 34−36% overall yield.

Once all analogues had been synthesized, their antibacterial activity was evaluated *in vitro* against different strains of Gram positive bacteria, *Staphylococcus aureus*, *Propionibacterium acnes*, *Bacillus subtilis* and *Micrococcus luteus*. Baringolin (**1**) was active against all strains (Table 1), displaying a good potency, while analogues with shorter tails, **26** and **28**, performed similarly to **1**, indicating that the unusually long tail of baringolin is not essential for its activity. Interestingly, analogues with the same peptidic tails of variable length, but incorporating a thiazole ring in place of thiazoline, **25**, **27** and **29**, showed a reduced scope of activities and overall only maintained the same potency levels against *S. aureus*, whereas activity against *P. acnes* was clearly affected. Such results point out to the higher flexibility of the thiazoline ring, which might facilitate accommodation of the compound to its biological target, presumably elongation factor Tu (EF-Tu).[23] The presence of the thiazoline moiety in baringolin's macrocycle might have been selected towards its less saturated counterpart in order to maintain a broader palette of activities. While ethyl esters **19** and **20** were devoid of any remarkable activity, carboxylic acid **21** and amide **23** retained theirs against *S. aureus*. Such behavior was in accordance with the results obtained for the thiazole-series analogues mentioned above. Surprisingly, and to our delight, analogue **2** showed an improved profile when compared to baringolin. Despite the presence of the thiazole ring in place of thiazoline, **2** remained active towards all tested strains and showed higher potencies against *S. aureus*, *P. acnes* and *B. subtilis*. These results point out to the key interactions of the newly introduced carboxylic acid,[3,2] which were able to overcome the presumably increased rigidity of the non-natural macrocycle. Despite the higher solubility of **31** (Table 1), its poor biological profile reinforces the hypothesis of the limited impact of the peptidic tail and the otherwise key role of the macrocyclic scaffold to exert its antibacterial activity.

Table 1. Antibacterial activity and solubility of baringolin analogues.

Compound	MIC[a] (μg/mL)				Solubility[b] (mg/mL)	
	S. aureus	*P. acnes*	*B.subtilis*	*M. luteus*	H_2O	PB 0.1 M
Baringolin (**1**)	0.25	0.125	0.25	0.5	BLD[c]	BLD[c]
26	0.25	0.125	0.25	0.5	BLD[c]	BLD[c]
28	0.5	0.5	0.5	1	BLD[c]	BLD[c]
25	0.5	4	8	2	BLD[c]	BLD[c]
27	0.5	8	0.5	2	BLD[c]	BLD[c]
29	0.5	8	1	2	BLD[c]	BLD[c]
19	>8	4	>8	2	BLD[c]	BLD[c]
20	>8	4	8	4	BLD[c]	BLD[c]
21	2	2	8	>8	BLD[c]	0,023
23	1	8	8	>8	BLD[c]	0,007
2	0.03	0.06	0.03	0.5	BLD[c]	0,018
31	>8	8	>8	2	4.661	6.654

[a]MIC = minimum inhibitory concentration.
[b]Solubility was determined by measuring the concentration of a saturated solution of compounds.
[c]BLD = below limit of detection.

CONCLUSION

A robust and convergent strategy by a combination of solution and solid-phase modes has facilitated the construction of the first fully synthetic library of thiopeptide analogues. The modifications introduced have helped us identify the thiazoline moiety as responsible of the broader activity profile of baringolin when compared to its less saturated analogue **29**. Moreover, the role of the tail region has also been evaluated, showing a very limited impact of tail length in activity and potency. Using the thiazole-containing macrocycle analogue as a more robust and accessible platform, *trans*-4-aminocyclohexanoic acid moiety was introduced to furnish **2**; this modification restored the activity profile and highly improved the potency of baringolin towards most strains. The use of a fully synthetic approach such as the one presented herein could be used to further assess the role of other regions of thiopeptides not easily modified by alternative methods for the production of analogues.

EXPERIMENTAL SECTION

Synthesis and Characterization of Compounds. Synthetic procedures, characterization and ^{1}H and ^{13}C NMR spectra of compounds are found in the Supporting Information. Purity of tested compounds was assessed by HPLC to be >95%.

MIC Assays. MIC assays were performed using *Staphylococcus aureus* and *Propionibacterium acnes* from our collection, isolated from clinical samples, *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633. Isolates were taken from the freezer and transferred at least twice on supplemented Brucella agar for anaerobes and on sheep blood agar for aerobes to ensure purity and good growth. Anaerobes were incubated for 48 h and aerobes for 24 h prior to testing. Inocula were prepared by direct suspensions of cells into saline solution to achieve the turbidity of the 0.5 McFarland standard. For facultative and aerobic bacteria (*S.aureus*, *B.subtilis* and *M.luteus*) MIC was performed by microdilution method in Mueller Hinton broth according to CLSI guideleness (M7-A9) (Clinical and Laboratory Standards Institute. 2012. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard-eighth edition. CLSI document M11-A8. CLSI, Wayne, PA.) incubated at 35ºC for 24h. For anerobic bacteria (*P. acnes*) MIC was performed in Brucella broth supplemented with hemin (5 mcg/mL), vitamin K1 (1mcg/mL) and lysed horse blood (5%) as described in CLSI-M11-A8 (Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility testing of bacteria that grow aerobically. Approved standard, 8th ed. CLSI document M7-A9. Clinical and Laboratory Standards Institute, Wayne, PA.) incubated at anaerobic conditions, at 35ºC for 24h.

Solubility Determination. An amount of approximately 1 mg of compound was weighted and a known volume of the solvent was added to ensure a saturated solution would result. Vigorous vortexing for 1 min and shaking for 48 h followed. After centrifugation at 10,000 rpm during 3 min, the supernatant was analyzed using a spectrophotometer at a reading wavelength of 304 nm.

ASSOCIATED CONTENT

Supporting Information. Detailed synthetic procedures and compound characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author. *e-mail: mercedes.alvarez@irbbarcelona.org.

Current address of PB: Aptuit Verona, Srl, Via Alessandro Fleming 4, 37135 Verona, Italy.

ACKNOWLEDGEMENTS

We gratefully acknowledge support from the Spanish Science and Innovation Ministry, CICYT (CTQ2012-30930) and the Generalitat de Catalunya (2009SGR 1024). XJ thanks ISCIII for a PFIS grant.

ABBREVIATIONS

TFAA, trifluoroacetic anhydride; DME, dimethoxyethane; DIPCDI, *N*,*N*'-diisopropylcarbodiimide; EDC, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride; HOAt, 1-hydroxy-7-azabenzotriazole; DIPEA, diisopropylethylamine; TFA, trifluoroacetic acid; MIC, mínimum inhibitory concentration; BLD, below limit of detection.

REFERENCES

(1) Poulakou, G.; Giamarellou, H. *Expert Opin. Invest. Drugs* **2007**, *16*, 137–155.

(2) Clough, J.; Chen, S.; Gordon, E. M.; Hackbarth, C.; Lam, S.; Trias, J.; White, R. J.; Candiani, G.; Donadio, S.; Romanò, G.; Ciabatti, R.; Jacobs, J. W. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3409–3414.

(3) 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.

(4) LaMarche, M. J.; Leeds, J. A.; Amaral, A.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Ding, J.; Dzink-fox, J.; Gamber, G.; Jain, A.; Lee, K.; Lee, L.; Lister, T.; Mckenney, D.; Mullin, S.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Trzasko, A.; Whitehead, L.; Yifru, A.; Yu, D.; Yan, W.; Zhu, Q. *J. Med. Chem.* **2012**, *6*, 2376–2387.

(5) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.

(6) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 7930–7954.

(7) Bower, J.; Drysdale, M.; Hebdon, R.; Jordan, A.; Lentzen, G.; Matassova, N.; Murchie, A.; Powles, J.; Roughley, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455–2458.

(8) Nicolaou, K. C.; Nevalainen, M.; Zak, M.; Bulat, S.; Bella, M.; Safina, B. S. *Angew. Chem. Int. Ed.* **2003**, *42*, 3418–3424.

(9) Nicolaou, K. C.; Zak, M.; Rahimipour, S.; Estrada, A. a; Lee, S. H.; O'Brate, A.; Giannakakou, P.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2005**, *127*, 15042–15044.

(10) Starosta, A. L.; Qin, H.; Mikolajka, A.; Leung, G. Y. C.; Schwinghammer, K.; Nicolaou, K. C.; Chen, D. Y.-K.; Cooperman, B. S.; Wilson, D. N. *Chem. Biol.* **2009**, *16*, 1087–1096.

(11) Kirschning, A.; Hahn, F. *Angew. Chem. Int. Ed.* **2012**, *3*, 4012–4022.

(12) Zhang, Q.; Liu, W. *Nat. Prod. Rep.* **2013**, *30*, 218–226.

(13) Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304–1311.

(14) Young, T. S.; Dorrestein, P. C.; Walsh, C. T. *Chem. Biol.* **2012**, *19*, 1600–1610.

(15) Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

(16) LaMarche, M. J.; Leeds, J. A.; Dzink-Fox, J.; Gangl, E.; Krastel, P.; Neckermann, G.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Tiamfook, S.; Yu, D. *J. Med. Chem.* **2012**, *55*, 6934–6941.

(17) Just-Baringo, X.; Bruno, P.; Ottesen, L. K.; Cañedo, L. M.; Albericio, F.; Alvarez, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 7818–7821.

(18) Okeley, N. M.; Zhu, Y.; Donk, W. A. van Der *Org. Lett.* **2000**, *2*, 3603–3606.

(19) García-Martín, F.; Quintanar-Audelo, M.; García-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Côté, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213–220.

(20) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem. Eur. J.* **2009**, *15*, 9394–9403.

(21) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem. Int. Ed.* **2005**, *44*, 1378–1382.

(22) LaMarche, M. J.; Leeds, J.; Amaral, K.; Brewer, J.; Bushell, S.; Dewhurst, J.; Dzink-Fox, J.; Gangl, E.; Goldovitz, J.; Jain, A.; Mullin, S.; Neckermann, G.; Osborn, C.; Palestrant, D.; Patane, M.; Rann, E.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Whitehead, L.; Yu, D. *J. Med. Chem.* **2011**, *54*, 8099–8109.

(23) Parmeggiani, A.; Nissen, P. *FEBS Lett.* **2006**, *580*, 4576–81.

Dissecting the Structure of Thiopeptides: Assessment of Thiazoline and Tail Moieties of Baringolin and Antibacterial Activity Optimization.

Xavier Just-Baringo,[1,2] Paolo Bruno,[1,2] Cristina Pitart,[3] Jordi Vila,[3] Fernando Albericio, [1,2,4,5] Mercedes Álvarez[1,2,6]*

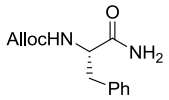
[1] *Institute for Research in Biomedicine, Barcelona Science Park-University of Barcelona, Baldiri Reixac 10, 08028 Barcelona, Spain.* [2] *CIBER-BBN, Networking Centre on Bioengineering Biomaterials and Nanomedicine, 08028 Barcelona, Spain.* [3] Center for International Health Research, CRESIB, Hospital Clínic, University of Barcelona, 08036 Barcelona, Spain. [4] *Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.* [5] *School of Chemistry, University of KwaZulu-Natal, 4001-Durban, South Africa.* [6] *Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain.*

1. General Information

Tetrahydrofuran (THF) and *N*,*N*-dimethylformamide (DMF) were dried using a PureSolv solvent purification system. All other solvents and reagents were used as purchased without further purification. Flash column chromatography was performed on SDS silica gel (60A 35-70 μm) as stationary phase. Analytical thin layer chromatography was performed using aluminium-backed plates coated with Merck Kieselgel 60 F_{254}; compounds were visualized under a UV lamp (254 nm). Melting points were determined in a Buchi Melting Point B540 apparatus in open capillaries. Reverse-phase analytical HPLC was performed on a Waters Alliance separation module 2695 equipped with a Waters XBridge C18 column (4.6×75 mm, 2.5 μm) and a Waters 996 PDA with a photodiode array detector, using MeCN (0.036% TFA) and H_2O (0.045% TFA) as mobile phases for runs of 8 min. The enantiomeric excess (ee) was determined by HPLC on the same separation module with a chiral stationary phase CHIRALPAK IA 250 × 4.6 mm 5 μm analytical column, flow rate 1 mL min^{-1} in 40 min runs. Polarimetry studies were performed on a Perkin–Elmer 241 or Jasco P-2000 polarimeter. IR spectra were recorded on a Thermo Nicolet FT-IR Nexus spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz or Bruker 600MHz spectrometer. Multiplicity of the carbons was assigned with gHSQC experiments. Stardard abbreviations for off-resonance decoupling were employed: (s) singlet, (d) doublet, (t) triplet, and (q) quartet. The same abbreviations were also used for the multiplicity of signals in ^{1}H NMR, plus: (m) multiplet, (dd) double doublet, (ddd) double doublet of doublets, (dq) double quartet and (bs) broad singlet. Spectra were referenced to appropriate residual solvent peaks ($CDCl_3$, DMSO-d_6, acetone-d_6 or pyridine-d_5). High-Resolution Mass Spectroscopy (HRMS) was performed on either a LTQ-FT Ultra (Thermo Scientific) or an LCT-Premier (Waters) high resolution mass spectrometer by the Mass Spectrometry Service of the Institute for Research in Biomedicine (IRB).

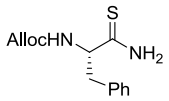
2. Experimental Procedures

(*S*) *N*-(allyloxycarbonyl)phenylalaninamide (8).



Allyl chloroformate (1.2 mL, 10.96 mmol) was added slowly to a stirring suspension of phenylalaninamide (**7**) hydrochloride (2.0 g, 9.97 mmol) in dry CH_2Cl_2 (200 mL) cooled in an ice bath. Next, NEt_3 (3.1 mL, 21.93 mmol) was added drop-wise. After stirring for 2 h at 0 ºC, the reaction mixture was poured over brine (200 mL), fractions were separated and the aqueous layer extracted with CH_2Cl_2 (2 × 100 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 3:7). The title product was obtained as a white solid (2.13 g, 86%). The product obtained in this manner was identical to the one described in the literature.[1] ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.06 (dd, *J* = 14.0, 6.8 Hz, 1 H), 3.12 (dd, *J* = 13.6, 6.8 Hz, 1 H), 4.38−4.48 (m, 1 H), 4.53−4.57 (m, 2 H), 5.21 (ddd, *J* = 10.4, 2.8, 1.2 Hz, 1 H), 5.27 (ddd, *J* = 17.2, 2.8, 1.6 Hz, 1 H), 5.36 (bs, 1 H), 5.53 (bs, 1 H), 5.77 (bs, 1 H), 5.82−5.93 (m, 1 H), 7.20−7.28 (m, 3 H), 7.29−7.34 (m, 2 H) ppm. HRMS m/z calcd for $C_{13}H_{17}O_3N_2$ (M+H) 249.1234, found 249.1234.

(*S*) *N*-(allyloxycarbonyl)phenylalanine thioamide (9).

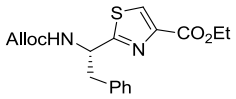


A solution of **8** (1.83 g, 7.37 mmol) and Lawesson's reagent (1.49 g, 3.69 mmol) in dry THF (10 mL) was stirred at rt. After 4.5 h saturated aq. $NaHCO_3$ (50 mL) was added to the reaction vessel. After 1 h the reaction mixture was poured into saturated aq. $NaHCO_3$ (100 mL). Layers were separated and the aquoeus phase was extracted with CH_2Cl_2 (3 × 150 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (hexanes/EtOAc, 1:1). The title product was obtained as a colorless oil (1.71 g, 88%). $[\alpha]_D$ +30.6 (c = 1.00, CH_2Cl_2). IR (film) 3302, 3206, 2943, 1700, 1623, 1502, 1438, 1152, 1041 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 3.12 (dd, *J* = 13.6, 8.2 Hz, 1 H), 3.21 (dd, *J* = 13.6, 8.2 Hz, 1 H), 4.50−4.56 (m, 2 H), 4.62−4.70 (m, 1 H), 5.21 (ddd, *J* = 10.4, 2.8, 1.2 Hz, 1 H), 5.28 (ddd, *J* = 17.2, 2.8, 1.6 Hz, 1 H), 5.58 (bs, 1 H), 5.82−5.93 (m, 1 H), 7.14 (bs, 1 H), 7.22−7.38 (m, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ =

[1] North, M.; Pattenden, G. *Tetrahedron*, **1990**, *46*, 8267.

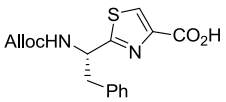
42.2 (t), 61.6 (d), 66.4 (t), 118.3 (t), 127.4 (d), 128.9 (d), 129.6 (d), 132.5 (d), 136.5 (s), 156.2 (s) ppm. HRMS m/z calcd for $C_{13}H_{17}O_2N_2S$ (M+H) 265.1005, found 265.1018.

(*S*)-Ethyl 2-(1-(allyloxycarbonylamino)-2-phenylethyl)thiazole-4-carboxylate (10).



A mixture of **9** (1.41 g, 5.35 mmol) and $KHCO_3$ (5.9 g, 58.85 mmol) in dry DME (13.4 mL) was stirred at rt. Ater 15 min the mixture was placed in an ice bath and ethyl bromopyruvate (2.0 mL, 16.05 mmol) was added drop-wise and the resulting mixture was stirred at 0 ºC. After 20 h the mixture was allowed to reach rt, filtered through celite and washed with Et_2O. After removing the volatiles the crude hydroxythiazoline was redissolved in dry DME (13.4 mL) and cooled at −20 ºC. A preformed mixture of trifluoroacetic anhydride (3.0 mL, 21.4 mmol) and 2,6-lutidine (5.6 mL, 48.15 mmol) was added drop-wise to the stirring solution. After 2.5 h the mixture was diluted with CH_2Cl_2 (250 mL), washed with 1 N HCl (250 mL) and saturated aq. $NaHCO_3$ (300 mL), dried (Na_2SO_4) and concentrated in *vacuo*. The crude product was purified by silica flash column chromatography (hexanes/*t*BuOMe, 1:1). The title product was obtained as a yellowish oil (1.91 g, 99%). 95% ee; H_2O (0.045% TFA):MeCN (0.036% TFA), 50% MeCN (t_R = 10.30 min); detected at 254 nm. $[\alpha]_D$ −15.4 (c = 1.00, CH_2Cl_2). IR (KBr) 3327, 2982, 2933, 1715, 1237, 1212 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.42 (t, *J* = 7.6 Hz, 3 H), 3.34 (d, *J* = 6.4 Hz, 2 H), 4.44 (q, *J* = 7.6 Hz, 2 H), 4.54 (d, *J* = 5.6 Hz, 2 H), 5.19 (d, *J* = 10.4 Hz, 1 H), 5.26 (d, *J* = 16.8 Hz, 1 H), 5.30−5.38 (m, 1 H), 5.51 (bs, 1 H), 5.77−5.94 (m, 1 H), 7.07−7.12 (m, 2 H), 7.19−7.29 (m, 3 H), 8.04 (s, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 16.7 (q), 43.9 (t), 56.6 (d), 63.8 (t), 68.3 (t), 120.2 (t), 129.5 (d), 129.6 (d), 131.0 (d), 131.7 (d), 134.8 (d), 138.3 (s), 149.7 (s), 157.8 (s), 163.6 (s)174.5 (s) ppm. HRMS m/z calcd for $C_{18}H_{21}O_4N_2S$ (M+H) 361.1217, found 361.1218.

(*S*)-2-(1-(Allyloxycarbonylamino)-2-phenylethyl)thiazole-4-carboxylic acid (11).



Aqueous 2 N LiOH (5 mL, 10.18 mmol) was added to a stirring solution of **10** (1.84 g, 5.09 mmol) in THF (57 mL). The mixture was stirred at rt under air. After 15 h, EtOAc (250 mL) and H_2O (250 mL) were added and layers were separated. Starting material was recoverd from the organic fraction (257 mg, 14%). Aq. 2N HCl was added to the aqueous layer until it reached pH 2−3 and it was extracted with EtOAc (3 × 250 mL). The title product was obtained as a

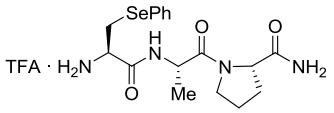
white solid (1.46 g, 86%). The crude product was used in following reactions without further purification or characterization.

General method for solid-phase peptide synthesis.

The Rink-Amide Chem-Matrix resin (loading = 0.52 mmol/g;) was swellen in MeOH, then in DMF and finally in CH_2Cl_2. Fmoc-AA-OH (3 equiv), preactivated by vigorous shaking for 4 min with DIPCDI (3.3 equiv) and Oxyma Pure (3.3 equiv) in DMF, was poured onto the resin and the resulting mixture was gently shaken for 1 h. The resin was then washed with DMF and CH_2Cl_2 (5 × each). The *N*-terminus was deprotected using 20% piperidine in DMF (treatments of 2 × 1 min, then 2 × 5 min). The resin was then washed with CH_2Cl_2 and DMF (5 × each). Loading onto the resin (0.249 mmol/g, 48%) was determined through measuring dibenzofulvene absorbance at 290 nm of cleavage solutions and washings.

Elongation of the peptide proceeded as follows; the number of equiv refers to the original functionalization, not the loading. Fmoc-AA-OH (2.3 equiv), was preactivated by vigorous shaking for 4 min in the presence of DIPCDI (2.5 equiv) and Oxyma Pure (2.5 equiv) in DMF, and was then poured onto the resin. The resulting mixture was gently shaken for 1.5 h. Deprotection and coupling cycles were repeated with the appropriate amino acids to provide the desired peptide. It was cleaved from the resin by treatment with 95% TFA in CH_2Cl_2 (4 × 20 min) at rt followed by filtration and collection of the filtrate. Next, washing of the resin with CH_2Cl_2 (× 6) was performed. Most TFA was removed under vacuum and the resulting concentrated solution was poured into cold Et_2O. Centrifugation and pouring off the solvent yielded the desired peptideas TFA salt. Purity was determined by HPLC (linear gradient: 0 to 100% MeCN in H_2O over 8 min; flow rate = 1.0 mL/min).

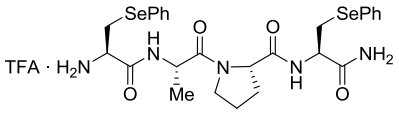
H-Sec(Ph)-Ala-Pro-NH_2 tripeptide 12.



Prepared according to the general method for solid-phase peptide synthesis, starting from 1.0 g of resin. The title product was obtained as a pale powder (131 mg, quant. based on calculated loading of the resin). HPLC purity: 100%; H_2O (0.045% TFA):MeCN (0.036% TFA), 0 to 100% MeCN (t_R = 3.91 min); detected at 254 nm. mp (Et_2O) decomposes above 125 °C. $[\alpha]_D$ −22.2 (c = 0.50, CH_2Cl_2). IR (KBr) 1675, 1630, 1207, 1130 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.26 (d, *J* = 7.2 Hz, 3 H), 1.78−2.11 (m, 4 H), 3.23−3.37 (m, 2 H), 3.55−3.64 (m, 2 H), 4.08−4.16 (m, 1 H), 4.25 (dd, *J* = 8.4, 3.6 Hz, 1 H), 4.47−4.58 (m, 1 H), 6.94 (bs, 2 H),

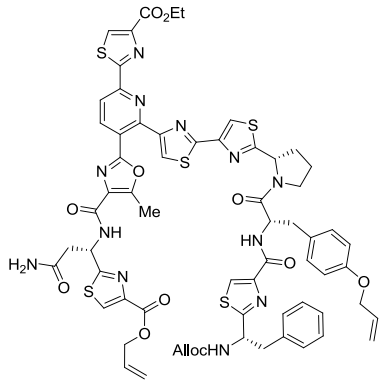
7.29−7.36 (m, 3 H), 7.51−7.57 (m, 2 H), 7.58−7.62 (m, 1 H), 8.51 (bs, 3 H), 8.85 (d, *J* = 7.6 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 18.1 (q), 25.3 (t), 28.1 (t), 30.3 (t), 47.5 (d), 47.6 (t), 52.7 (d), 60.5 (d), 128.2 (d), 130.0 (s), 130.2 (d), 132.9 (d), 167.3 (s), 170.4 (s), 174.4 (s) ppm. HRMS m/z calcd for $C_{17}H_{25}N_4O_3Se$ (M+H) 413.1086, found 413.1087

H-Sec(Ph)-Ala-Pro-Sec(Ph)-NH$_2$ tetrapeptide 13.



Prepared according to the general method for solid-phase peptide synthesis, starting from 1.0 g of resin. The title product was obtained as a white powder (187 mg, quant. based on calculated loading of the resin). HPLC purity: 100%; H_2O (0.045% TFA):MeCN (0.036% TFA), 0 to 100% MeCN (t_R = 5.21 min); detected at 254 nm. mp (Et_2O) 87−90 °C. $[\alpha]_D$ −26.8 (c = 1.00, CH_2Cl_2). IR (KBr) 1662, 1201, 1130cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.28 (d, *J* = 7.2 Hz, 3 H), 1.78−2.15 (m, 4 H), 3.12−3.40 (m, 4 H), 3.50−3.69 (m, 2 H), 4.07−4.16 (m, 1 H), 4.27−4.35 (m, 1 H), 4.36−4.45 (m, 1 H), 4.52−4.63 (m, 1 H), 7.23−7.39 (m, 8 H), 7.49−7.59 (m, 4 H), 8.01−8.09 (m, 1 H), 8.51 (bs, 3 H), 8.90−8.97 (m, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 18.1 (q), 25.4 (t), 28.1 (t), 29.8 (t), 29.9 (t), 47.6 (d), 47.8 (t), 52.7 (d), 53.4 (d), 61.1 (d), 127.6 (d), 128.2 (d), 129.9 (s), 130.2 (d), 130.2 (d), 131.2 (s), 132.4 (d), 132.9 (d), 167.3 (s), 171.1 (s), 172.2 (s); 172.8 (s) ppm. HRMS m/z calcd for $C_{26}H_{34}N_5O_4Se_2$ (M+H) 640.0936, found 640.0951.

Protected open macrocycle thiazole analogue (17).

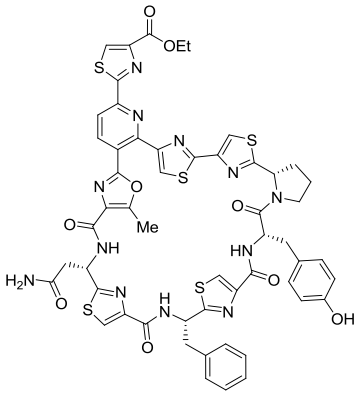


EDC·HCl (69 mg, 0.359 mmol), HOAt (49 mg, 0.359 mmol) and DIPEA (60 μL, 0.359 mmol) were added to a solution of **15**[2] (310 mg, 0.299 mmol) and **11** (119 mg, 0.359 mmol) in dry

[2] Just-Baringo, X.; Bruno, P.; Ottesen, L. K.; Cañedo, L. M.; Albericio, F.; Alvarez, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 7818–7821.

DMF (6 mL) cooled in an ice bath. The resulting solution was stirred at 0 ºC for 2.5 h, then allowed to reach rt and stirred for another 2 h. The reaction mixture was diluted with EtOAc (150 mL), washed with saturated aq. NH_4Cl (100 mL), $NaHCO_3$ (100 mL) and H_2O (100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/EtOAc, 2:8 to EtOAc). The title compound was obtained as a white solid (343 mg, 85%), mp (EtOAc) 135−138 °C. $[\alpha]_D$ +9.6 (c = 0.33, CH_2Cl_2). IR (KBr) 3440, 2922, 1719, 1643, 1510, 1424, 1239 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.44 (t, *J* = 7.0 Hz, 3 H), 1.98−2.32 (m, 4 H), 2.36−2.60 (m, 3 H), 2.69−3.41 (m, 6 H), 3.46−4.06 (m, 2 H), 4.26−4.62 (m, 6 H), 4.72−4.84 (m, 2 H), 4.91−5.42 (m, 8 H), 5.43−5.50 (m, 1 H), 5.58 (bs, 1 H), 5.68−6.10 (m, 4 H), 6.62−6.94 (m, 2 H), 6.98−7.25 (m, 7 H), 7.41 (s, 1 H), 7.81 (bs, 1 H), 8.02−8.08 (m, 1 H), 8.11−8.15 (m, 1 H), 8.25 (d, *J* = 8.2 Hz, 1 H), 8.28−8.33 (m, 1 H), 8.37 (d, *J* = 8.2 Hz, 1 H), 8.59−8.68 (m, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.0 (q), 14.7 (q), 24.8 (t), 32.2 (t), 37.5 (t), 38.9 (t), 40.6 (t), 47.8 (t), 48.1 (d), 53.1 (d), 54.2 (d), 59.2 (d), 62.0 (t), 66.2 (t), 66.2 (t), 69.0 (t), 115.2 (d), 115.4 (d), 117.8 (t), 118.3 (t), 119.0 (d), 119.2 (t), 121.8 (d), 123.0 (s), 124.1 (d), 127.2 (d), 128.5 (d), 128.9 (d), 129.7 (s), 129.9 (d), 130.2 (s), 130.6 (d), 130.6 (d), 130.8 (s), 132.1 (d), 132.9 (d), 133.6 (d), 136.6 (s), 140.3 (d), 146.8 (s), 149.0 (s), 149.1 (s), 149.3 (s), 149.6 (s), 151.3 (s), 151.4 (s), 154.1 (s), 154.3 (s), 154.6 (s), 155.9 (s), 157.5 (s), 157.9 (s), 161.2 (s), 161.6 (s), 161.8 (s), 161.9 (s), 162.1(s), 169.1 (s), 172.4 (s), 173.3 (s) ppm. HRMS m/z calcd for $C_{64}H_{61}O_{12}N_{12}S_5$ (M+H) 1349.3130, found 1349.3196.

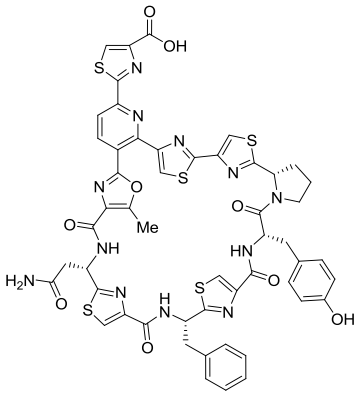
Thiazole analogue ethyl ester (19).



A solution of $Pd(PPh_3)_4$ (64 mg, 0.055 mmol) in dry CH_2Cl_2 (7 mL) was added to a stirring solution of **17** (745 mg, 0.552 mmol) in dry CH_2Cl_2 (30 mL). $PhSiH_3$ (340 μL, 2.760 mmol) was subsequently added. The resulting mixture was stirred at rt. After 7 h more $(PPh_3)_4$ (32 mg, 0.028 mmol) in dry CH_2Cl_2 (3.5 mL) was added and the reaction mixture stirred for another 2 h. Upon consumption of all starting material, volatiles were evaporated under reduced pressure. The flask was filled with N_2 and the crude dissolved in dry DMF (550 mL). Addition of EDC·HCl (131 mg, 0.662 mmol) and HOAt (90 mg, 0.662 mmol) followed. After 3 days, more

EDC·HCl (131 mg, 0.662 mmol) and HOAt (90 mg, 0.662 mmol) were added. After 3 more days all starting material was consumed and the volume of DMF was reduced to approximately 100 mL under reduced pressure. H_2O (250 mL) was added and the mixture extracted with CH_2Cl_2 (3 × 250 mL). Combined organics were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 95:5). The title product was obtained as a white solid (392 mg, 61%). HPLC: 30 to 80% MeCN (t_R = 7.70 min). mp (CH_2Cl_2) decomposes above 120 °C. $[\alpha]_D$ +117.8 (c = 1.00, CH_2Cl_2). IR (KBr) 3383, 3118, 2924, 2852, 1781, 1728, 1655, 1546, 1497, 1211, 1168 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 0.65 (d, *J* = 16.8, 1 H), 1.44 (t, *J* = 7.1 Hz, 3 H), 1.97−2.08 (m, 1 H), 2.10−2.22 (m, 1 H), 2.26−2.40 (m, 2 H), 2.45−2.58 (m, 1 H), 2.83 (s, 3 H), 3.09 (dd, *J* = 14.0, 4.8 Hz, 1 H), 3.18 (dd, *J* = 14.0, 3.0 Hz, 1 H), 3.35 (d, *J* = 5.2 Hz, 2 H), 3.95−4.05 (m, 2 H), 4.47 (q, *J* = 7.1 Hz, 2 H), 5.02−5.10 (m, 1 H), 5.29−5.44 (m, 3 H), 5.60 −5.67 (m, 1 H), 6.60 (d, *J* = 8.4 Hz, 2 H), 6.82−6.97 (m, 4 H), 7.18−7.24 (m, 3 H), 7.57 (s, 1 H), 7.73 (d, *J* = 10.0 Hz, 1 H), 7.86 (s, 1 H), 7.93 (s, 1 H), 8.09 (s, 1 H), 8.27−8.33 (m, 2 H), 8.41 (d, *J* = 8.0 Hz, 1 H), 8.62 (d, *J* = 7.2 Hz, 1 H), 8.70 (d, *J* = 9.6 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 12.4 (q), 14.7 (q), 25.8 (t), 34.2 (t), 37.2 (t), 38.5 (t), 43.5 (t), 48.2 (d), 48.5 (t), 51.0 (d), 53.3 (d), 61.7 (d), 62.0 (t), 114.3 (d), 117.2 (d), 119.2 (d), 122.7 (d), 122.8 (d), 122.9 (s); 124.7 (d), 125.5 (s), 127.4 (d), 128.6 (d), 130.2 (d), 130.3 (s), 130.6 (d), 131.6 (d), 135.6 (s), 138.8 (d), 148.6 (s), 148.9 (s), 148.9 (s), 149.2 (s), 151.0 (s), 151.9 (s), 154.5 (s), 154.6 (s), 156.0 (s), 156.3 (s), 160.0 (s), 160.3 (s), 160.7 (s), 161.6 (s), 163.3 (s), 169.0 (s), 169.2 (s), 171.0 (s), 172.4 (s), 173.1 (s); 173.8 (s) ppm. HRMS m/z calcd for $C_{54}H_{47}O_9N_{12}S_5$ (M+H) 1167.2187, found 1167.2190.

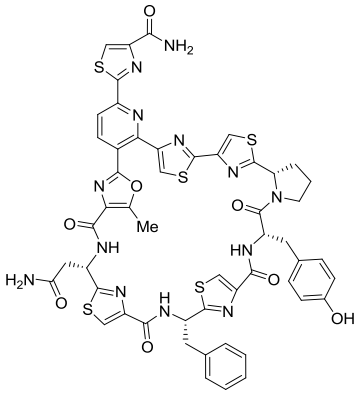
Thiazole analogue carboxylic acid (21).



Aqueous 3 N LiOH (340 μL, 1.028 mmol) was added to a stirring solution of **19** (200 mg, 0.171 mmol) in THF (1.7 mL). The resulting mixture was stirred at rt under air. After 17 h the reaction was diluted with THF/CH_2Cl_2 (25 mL, 1:1) and 2 M HCl (25 mL) was added. Layers were

separated and the aqueous phase was extracted with THF/CH_2Cl_2 (2 × 25 mL, 1:1). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The title product was obtained as a white solid (193 mg, 99%). HPLC: 50 to 100% MeCN (t_R = 2.68 min). mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +119.5 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2847, 1649, 1549, 1489, 1425, 1201 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.15−1.42 (m, 1 H), 1.66−2.28 (m, 5 H), 2.81 (s, 3 H), 3.17−3.85 (m, 6 H), 5.38−5.54 (m, 1 H), 5.63−5.73 (m, 1 H), 5.76−5.85 (m, 1 H), 5.88−6.07 (m, 1 H), 7.04−7.13 (m, 2 H), 7.15−7.22 (m, 5 H), 7.39 (d, *J* = 8.4 Hz, 2 H), 7.64 (bs, 1 H), 7.98 (bs, 1 H), 8.04 (s, 1 H), 8.22 (s, 1 H), 8.28 (s, 1 H), 8.32 (d, *J* = 8.0 Hz, 1 H), 8.37 (s, 1 H), 8.38−8.42 (m, 1 H), 8.52 (d, *J* = 8.0 Hz, 1 H), 8.76 (s, 1 H), 9.08 (d, *J* = 7.2 Hz, 1 H), 9.46 (d, *J* = 8.4 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.9 (q), 25.2 (t), 33.6 (t), 37.7 (t), 39.0 (t), 43.5 (t), 48.0 (t), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 115.6 (d), 116.6 (d), 118.9 (d), 122.9 (d), 123.0 (d), 124.8 (d), 126.4 (s), 127.3 (d), 128.7 (d), 130.2 (d), 131.0 (s), 131.5 (d), 136.7 (d), 139.3 (d), 149.3 (s), 149.3 (s), 151.3 (s), 151.6 (s), 152.2 (s), 154.0 (s), 154.9 (s), 156.0 (s), 158.1 (s), 160.1 (s), 160.5 (s), 161.3 (s), 162.8 (s), 164.0 (s), 168.4 (s), 169.2 (s), 171.7 (s), 173.1 (s), 175.0 (s), 175.1 (s) ppm. HRMS m/z calcd for $C_{52}H_{43}O_9N_{12}S_5$ (M+H) 1139.1874, found 1139.1888.

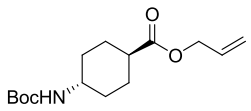
Thiazole Amide Analogue (23).



NH_4HCO_3 (10 mg, 132.0 μmol), EDC·HCl (10 mg, 52.7 μmol), HOAt (7 mg, 52.7 μmol) and DIPEA (24 μL, 132.0 μmol) were added to a stirring solution of **21** (50 mg, 43.9 μmol) in dry DMF (0.9 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 28 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq. NH_4Cl (25 mL) and saturated aq. $NaHCO_3$ (25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 99:1 to 96:4). The title product was obtained as a white solid (34 mg, 68%). HPLC: 50 to 100% MeCN (t_R = 2.60 min). mp (CH_2Cl_2) decomposes above 180 °C. $[\alpha]_D$ +114.4 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2847, 1649, 1534, 1489, 1419 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ =

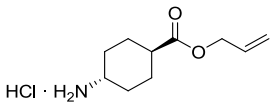
1.92−2.27 (m, 5 H), 2.39−2.51 (m, 1 H), 2.78 (s, 3 H), 2.82−2.97 (m, 2 H), 3.10−3.26 (m, 2 H), 3.71−3.92 (m, 2 H), 5.03−5.13 (m, 1 H), 5.30−5.42 (m, 2 H), 5.63−5.72 (m, 1 H), 6.68 (d, *J* = 8.4 Hz, 2 H), 6.83 (bs, 1 H), 7.12 (d, *J* = 8.4 Hz, 2 H), 7.17−7.38 (6 H), 7.74−7.81 (m, 2 H), 8.00 (d, *J* = 9.2 Hz, 1 H), 8.10 (bs, 1 H), 8.13 (s, 1 H), 8.23 (s, 1 H), 8.37 (s, 1 H), 8.50 (d, *J* = 8.2 Hz, 1 H), 8.52 (s, 1 H), 8.56 (d, *J* = 8.2 Hz, 1 H), 8.70−8.80 (m, 2 H), 9.25 (s, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 12.6 (q), 25.5 (t), 33.6 (t), 38.0 (t), 39.0 (t), 42.6 (t), 48.2 (t), 49.4 (d), 52. 7 (d), 53.4 (d), 60.4 (d), 116.1 (d), 117.2 (d), 119.6 (d); 123.2 (d), 123.3 (s), 124.5 (d), 125.8 (d), 127.4 (d), 127.7 (s), 128.3 (d), 129.2 (d), 130.4 (d), 131.3 (d), 137.4 (s), 140.9 (d), 148.5 (s), 149.8 (s), 151.8 (s), 152.1 (s), 152.7 (s), 154.0 (s), 154.6 (s), 156.6 (s), 157.0 (s), 160.0 (s), 161.1 (s), 161.7 (s), 162.0 (s), 163.0 (s), 167.6 (s), 170.3 (s), 171.2 (s), 172.6 (s), 174.5 (s), 175.3 (s) ppm. HRMS m/z calcd for $C_{52}H_{44}O_8N_{13}S_5$ (M+H) 1138.2034, found 1138.2043.

***trans*-Allyl-4-(*tert*-butoxycarbonylamino)cyclohexanoate (S1).**



Dry DMF (23 mL) was added to a flask charged with 4-*trans*-(*tert*-butoxycarbonylamino)cyclohexanoic acid (1.00 g, 4.11 mmol) and $NaHCO_3$ (2.49 g, 29.59 mmol). The mixture was stirred at rt under inert atmosphere. After 10 min allyl bromide (7.5 mL, 86.31 mmol) was added and the mixture stirred for another 24 h at rt. The reaction mixture was then diluted with EtOAc (250 mL), washed with H_2O (250 mL) and brine (2 × 100 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The desired product was obtained as a colorless oil (1.13 g, 97%). IR (film) 3378, 2976, 2936, 2862, 1733, 1713m 1519, 1173 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.05−1.17 (m, 2 H), 1.43 (s, 9 H), 1.47−1.63 (m, 2 H), 1.98−2.12 (m, 4 H), 2.25 (tt, *J* = 12.0, 3.6 Hz, 1 H), 3.41 (bs, 1 H), 4.37 (bs, 1 H), 4.56 (dt, *J* = 5.6, 1.2 Hz, 2 H), 5.20−5.33 (m, 2 H), 5.84−5.96 (m, 1 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 28.2 (t), 28.8 (q), 32.9 (t), 42.8 (d), 49.3 (d), 65.3 (t), 79.6 (s), 118.4 (t), 132.6 (d), 155.5 (s), 175.4 (s) ppm. HRMS m/z calcd for $C_{15}H_{26}O_4N$ (M+H) 284.1856, found 2841858.

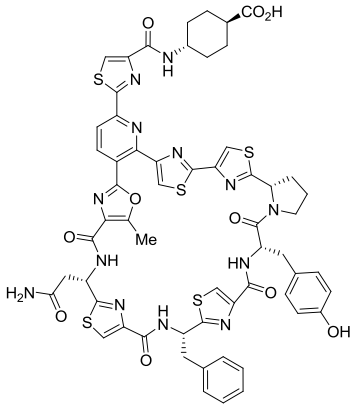
Allyl *trans*-4-aminocyclohexanoate hydrochloride (24).



A solution of 4 M HCl in 1,4-dioxane (18 mL, 70.2 mmol) was added to a stirring solution of **S1** (996 mg, 3.51 mmol) in dry 1,4-dioxane (18 mL). After 4 h the reaction mixture was diluted

with CH_2Cl_2 and then concentrated *in vacuo*. The title product was obtained as a white solid (747 mg, 97%). The crude product was used in following reactions without further purification or characterization.

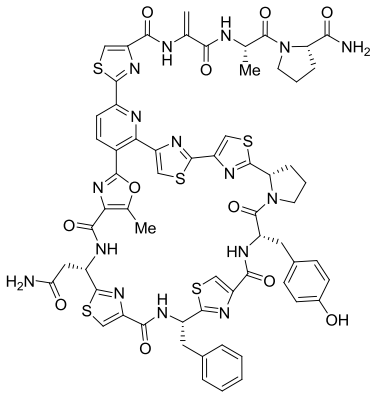
Thiazole Cyclohexanoic Acid Analogue (2).



24 (14 mg, 65.8 μmol), EDC·HCl (10 mg, 52.7 μmol), HOAt (7 mg, 52.7 μmol) and DIPEA (12 μL, 65.8 μmol) were added to a stirring solution of **21** (50 mg, 43.9 μmol) in dry DMF (0.9 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 5 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq. NH_4Cl (25 mL) and saturated aq. $NaHCO_3$ (25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 96:4). The condensation product was obtained as a white solid (36 mg, 63%). A solution of $Pd(PPh_3)_4$ (3 mg, 2.8 μmol) in dry CH_2Cl_2 (1.9 mL) and $PhSiH_3$ (17 μL, 0.138 mmol) were added to a flask charged with the condensation product (36 mg, 27.6 μmol). The resulting solution was stirred at rt for 2 h. Volatiles were removed under reduced pressure and the crude product purified by preparative reverse-phase column (C18, 30% to 70% MeCN in H_2O). The title product was obtained as a white solid (21 mg, 39% for two steps). HPLC: 30 to 80% MeCN (t_R = 6.26 min). mp (CH_2Cl_2) decomposes above 140 °C. $[\alpha]_D$ +92.7 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3385, 2917, 2841, 1656, 1534, 1495, 1419, 1194 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.18−2.05 (m, 8 H), 2.12−2.36 (m, 5 H), 2.42−2.52 (m, 1 H), 2.79 (s, 3 H), 3.19−3.62 (m, 5 H), 3.66−3.84 (m, 2 H), 4.33−4.46 (m, 1 H), 5.38−5.46 (m, 1 H), 5.63−5.72 (m, 1 H), 5.74−5.83 (m, 1 H), 5.98−6.06 (m, 1 H), 7.04−7.10 (m, 2 H), 7.16−7.22 (m, 5 H), 7.38 (d, *J* = 8.4 Hz, 2 H), 7.64 (bs, 1 H), 7.95−8.03 (m, 3 H), 8.21 (s, 1 H), 8.23−8.28 (m, 2 H), 8.36 (s, 1 H), 8.39 (d, *J* = 10.0 Hz, 1 H), 8.66 (s, 1 H), 8.71−8.76 (d, *J* = 8.8 Hz, 1 H), 9.08 (d, *J* = 6.8Hz, 1 H), 9.46 (d, *J* = 8.8 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 25.2 (t); 28.9 (t), 32.4 (t), 33.7 (t), 37.7 (t); 39.1 (t), 43.2 (d), 43.5 (t), 48.0 (t), 48.6 (d), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 115.6 (d), 116.6 (d), 118.7 (d), 122.9 (d), 123.0 (d), 124.8 (d), 126.4 (s), 127.0 (s), 127.3

(d), 128.7 (d), 130.2 (d), 131.0 (s), 131.5 (d), 136.7 (d), 139.1 (d), 149.2 (s), 149.4 (s), 151.4 (s), 152.2 (s), 153.1 (s), 153.9 (s), 154.8 (s), 156.0 (s), 158.1 (s), 160.1 (s), 160.5 (s), 160.6 (s), 161.3 (s), 162.8 (s), 168.0 (s), 169.2 (s), 171.7 (s), 173.1 (s), 175.1 (s), 175.1 (s), 177.8 (s) ppm. HRMS m/z calcd for $C_{59}H_{54}O_{10}N_{13}S_5$ (M+H) 1264.2715, found 1264.2741.

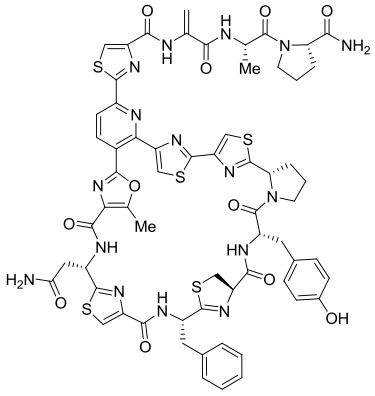
Thiazole Tripeptide Analogue (25).



12 (25 mg, 0.047 mmol), EDC·HCl (9 mg, 0.047 mmol), HOAt (6 mg, 0.047 mmol) and DIPEA (8 μL, 0.047 mmol) were added to a stirring solution of **21** (45 mg, 0.039 mmol) in dry DMF (0.8 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 5 h, CH_2Cl_2 (15 mL) was added and the resulting solution was washed with saturated aq. NH_4Cl (10 mL), saturated aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10).The condensation product was obtained as white solid (32 mg, 53%). 5.5 M *t*BuOOH in decane (38 μL, 0.209 mmol) was added to a solution of the condensation product (32 mg, 20.9 μmol) in dry CH_2Cl_2 (7 mL) and the resulting solution was stirred at rt. 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 h and 22 h after the first addition. Upon consumption of the starting material after 48 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq. $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (3 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10). The title product was obtained as a white solid (21 mg, 73%). HPLC: 40 to 60% MeCN (t_R = 5.11 min). mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +42.0 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3340, 2911, 2847, 1681, 1643, 1515, 1201, 1124 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.14−1.31 (m, 2 H), 1.59 (d, *J* = 6.8 Hz, 3 H), 1.64−2.25 (m, 7 H), 2.80 (s, 3 H), 3.19−3.31 (m, 2 H), 3.36 (dd, *J* = 14.6, 4.6 Hz, 1 H), 3.47 (dd, *J* = 16.6, 3.0 Hz, 1 H), 3.56 (dd, *J* = 13.4, 4.2 Hz, 1 H), 3.64−3.94 (m, 4 H), 4.91 (dd, *J* = 8.2, 3.4 Hz, 1 H), 5.10−5.25 (m, 1 H), 5.38−5.47 (m, 1 H), 5.37−5.72 (m, 1 H),

5.76−5.83 (m, 1 H), 5.97−6.04 (m, 1 H), 6.13 (s, 1 H), 7.03−7.11 (m, 3 H), 7.13−7.22 (m, 5 H), 7.38 (d, *J* = 8.0 Hz, 2 H), 7.64 (bs, 1 H), 7.89−8.00 (m, 2 H), 8.03 (s, 1 H), 8.15−8.30 (m, 5 H), 8.33−8.42 (m, 2 H), 8.63 (s, 1 H), 9.08 (d, J = 6.8 Hz, 1 H), 9.45 (d, J = 8.4 Hz, 1 H), 9.70 (d, J = 7.2 Hz, 1 H), 10.64 (s, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 17.3 (q), 25.2 (t), 29.4 (t), 30.0 (t), 33.7 (t), 37.6 (t), 39.0 (t), 43.5 (t), 47.4 (t), 48.0 (t), 48.5 (d), 49.5 (d), 52.2 (d), 53.6 (d), 60.5 (d), 61.3 (d), 103.2 (t), 115.6 (d), 116.6 (d), 118.8 (d), 123.0 (s), 123.3 (d), 123.5 (d), 124.9 (d), 126.4 (s), 127.3 (d), 128.0 (d), 128.7 (d), 130.2 (d), 130.9 (s), 131.5 (d), 136.7 (s), 139.4 (d), 149.2 (s), 149.3 (s), 150.8 (s), 152.0 (s), 152.1 (s), 154.0 (s), 154.7 (s), 155.9 (s), 158.1 (s), 159.7 (s), 160.1 (s), 160.4 (s), 161.2 (s), 162.7 (s), 164.7 (s), 168.3 (s), 169.2 (s), 171.7 (s), 171.8 (s), 173.1(s), 174.5 (s), 175.0 (s), 175.1 (s) ppm. HRMS m/z calcd for $C_{63}H_{59}O_{11}N_{16}S_5$ (M+H) 1375.3147, found 1375.3247.

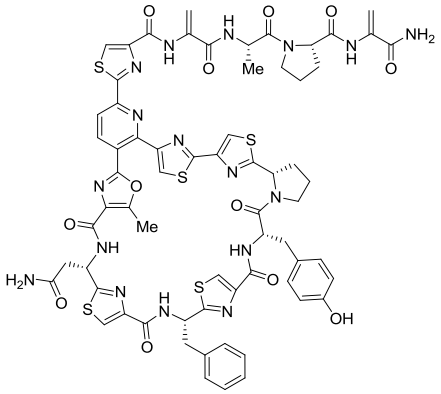
Thiazoline Tripeptide Analogue (26).



Trimethyltin hydroxide (47 mg, 0.256 mmol) was added to a solution of **20**[2] (50 mg, 42.8 μmol) in dry 1,2-dichloroethane (0.85 mL) and the reaction mixture was then stirred at 60 °C. After 15 h the mixture was diluted in CH_2Cl_2 (40 mL), washed with 6% HCl (30 mL), dried (Na_2SO_3), and concentrated *in vacuo*. EDC·HCl (10 mg, 51.4 μmol), HOAt (7 mg, 51.4 μmol) and DIPEA (9 μL, 51.4 μmol) were added to a stirring solution of the crude carboxylic acid (**22**)[2] and **12** (27 mg, 51.4 μmol) in DMF (0.9 mL) at 0 °C. After 17 h the mixture was diluted in CH_2Cl_2 (20 mL), washed with 1 M $NaHCO_3$ (20 mL), dried (Na_2SO_3) and concentrated *in vacuo* with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 98:2 to 90:10). The condensation product was obtained as a white solid (47 mg, 72%). 5.5 M *t*BuOOH in decane (56 μL, 0.306 mmol) was added to a solution of the condensation product (47 mg, 30.6 μmol) in dry CH_2Cl_2 (10 mL) and the resulting solution was stirred at rt. 5.5 M *t*BuOOH in decane (70 μL, 0.384 mmol) was added twice more after 5 h and 22 h after the first addition. Upon consumption of the starting material after 46 h, CH_2Cl_2 (10 mL) and a mixture of saturated aq. $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were

added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (2 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product purified by preparative reverse-phase column (C18, 40% to 50% MeCN in H_2O). The title product was obtained as a white solid (37 mg, 88%). HPLC: 40 to 60% MeCN (t_R = 5.23 min). mp (CH_2Cl_2) decomposes above 130 °C. $[\alpha]_D$ +3.2 (c = 0.5, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3327, 2917, 2841, 1649, 1502, 1457 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6) δ = 1.23–1.32 (m, 2 H), 1.38 (d, *J* = 6.6 Hz, 3 H), 1.81–2.34 (m, 7 H), 2.39–2.50 (m, 1 H), 2.74 (s, 3 H), 2.79–3.06 (m, 2 H), 3.09–3.45 (m, 4 H), 3.48–3.72 (m, 2 H), 4.26–4.31 (m, 1 H), 4.72–4.89 (m, 2 H), 4.90–5.11 (m, 2 H), 5.27–5.50 (m, 2 H), 5.97 (s, 1 H), 6.48–6.68 (m, 3 H), 6.83–7.15 (m, 3H), 7.16–7.46 (m, 5 H), 7.62–7.77 (m, 1 H), 7.86–8.08 (m, 1 H), 8.31–8.75 (m, 4 H), 8.76–8.87 (m, 4 H), 8.98 (d, *J* = 9.0 Hz, 1 H), 9.13 (d, *J* = 8.4 Hz, 1 H), 10.04–10.11 (m, 2 H) ppm. ^{13}C NMR (150 MHz, DMSO-d_6) δ = 12.5 (q), 17.4 (q), 25.4 (t), 25.6 (t), 29.9 (t), 30.1 (t), 33.5 (t), 37.1 (t), 37.4 (t), 39.1 (t), 39.1 (t), 47.5 (t), 48.3 (d), 49.3 (d), 53.2 (d), 54.9 (d), 60.3 (d), 60.6 (d), 78.2 (d), 104.3 (t), 115.9 (d), 117.4 (d), 119.1 (d), 123.3 (d), 124.8 (d), 127.5 (d), 129.2 (d), 129.6 (d), 130.2 (d), 130.5 (s), 131.4 (d), 134.5 (s), 138.1 (s), 141.8 (d), 148.8 (s), 150.2 (s), 150.8 (s), 151.5 (s), 152.5 (s), 153.8 (s), 154.6 (s), 156.9 (s), 157.2 (s), 158.9 (s), 159.5 (s), 160.2 (s), 163.7 (s), 168.4 (s), 170.1 (s), 171.0 (s), 172.3 (s), 174.0 (s), 174.4 (s), 175.3 (s), 176.0 (s) ppm. HRMS m/z calcd for $C_{63}H_{61}O_{11}N_{16}S_5$ (M+H) 1377.3304, found 1377.3327.

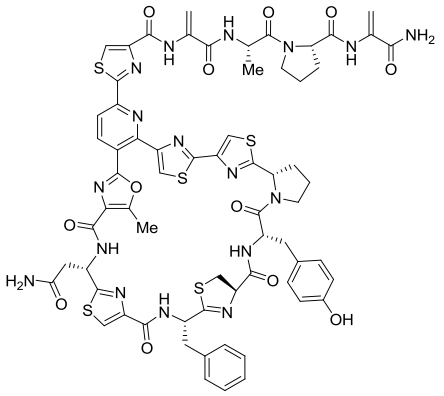
Thiazole Tetrapeptide Analogue (27).



13 (35 mg, 0.047 mmol), EDC·HCl (9 mg, 0.047 mmol), HOAt (6 mg, 0.047 mmol) and DIPEA (8 μL, 0.047 mmol) were added to a stirring solution of **21** (45 mg, 0.039 mmol) in dry DMF (0.8 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 4 h, CH_2Cl_2 (15 mL) was added and the resulting solution was washed with saturated aq. NH_4Cl (10 mL), saturated aq. $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10).The condensation product was obtained as white solid (36 mg, 52%). 5.5 M *t*BuOOH in decane (56 μL, 0.306 mmol) was added to a solution of the condensation product

(36 mg, 20.4 μmol) in dry CH_2Cl_2 (7 mL) and the resulting solution was stirred at rt. 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 h and 22 h after the first addition. Upon consumption of the starting material after 47 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq. $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (3 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 90:10). The title product was obtained as a white solid (19 mg, 64%). HPLC: 40 to 60% MeCN (t_R = 5.93 min). mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +63.7 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3334, 2917, 2847, 1643, 1515, 1419 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.67 (d, *J* = 6.8 Hz, 3 H), 1.70−2.02 (m, 7 H), 2.14−2.26 (m, 2 H), 2.80 (s, 3 H), 3.23−3.32 (m, 2 H), 3.37 (dd, *J* = 14.6, 4.6 Hz, 1 H), 3.48 (dd, *J* = 16.4, 3.2 Hz, 1 H), 3.56 (dd, *J* = 13.2, 4.0 Hz, 1 H), 3.65−3.84 (m, 3 H), 3.85−3.94 (m, 1 H), 4.94−5.00 (m, 1 H), 5.15−5.25 (m, 1 H), 5.40−5.47 (m, 1 H), 5.64−5.74 (m, 1 H), 5.77−5.85 (m, 1 H), 5.96 (s, 1 H), 5.97−6.05 (m, 1 H), 6.15 (s, 1 H), 6.85 (s, 1 H), 7.04−7.12 (m, 3 H), 7.14−7.22 (m, 5 H), 7.38 (d, *J* = 8.4 Hz, 2 H), 7.63 (bs, 1 H), 7.97 (bs, 1 H), 8.03 (s, 1 H), 8.21 (s, 1 H), 8.24 (s, 1 H), 8.27 (d, *J* = 8.0 Hz, 1 H), 8.30 (d, *J* = 8.0 Hz, 1 H), 8.37 (s, 1 H), 8.49 (bs, 1 H), 8.63 (s, 1 H), 8.86−8.94 (m, 1 H), 9.09 (d, *J* = 6.8 Hz, 1 H), 9.46 (d, *J* = 8.8 Hz, 1 H), 9.77 (d, *J* = 6.8 Hz, 1 H), 9.85 (s, 1 H), 10.63 (s, 1 H) ppm. ^{13}C NMR (100 MHz, pyridine-d_5) δ = 11.8 (q), 17.5 (q), 25.3 (t), 25.3 (t), 28.8 (t), 33.7 (t), 37.7 (t), 39.1 (t), 43.5 (t), 47.5 (t), 48.1 (t), 48.5 (d), 49.5 (d), 52.2 (d), 53.6 (d), 61.3 (d), 61.7 (d), 102.8 (t), 103.3 (t), 115.6 (d), 116.6 (d), 118.9 (d), 123.0 (s), 123.4 (d), 123.6 (d), 124.0 (s), 124.9 (d), 126.4 (s), 127.3 (d), 128.0 (d), 128.8 (d), 130.2 (d), 130.9 (s), 131.6 (d), 135.0 (s), 136.7 (s), 139.4 (d), 149.2 (s), 149.3 (s), 149.3 (s), 150.8 (s), 152.0 (s), 152.1 (s), 154.0 (s); 154.8 (s), 155.9 (s), 158.1 (s), 159.7 (s), 160.1 (s), 160.5 (s), 161.2 (s), 162.8 (s), 164.8 (s), 166.8 (s), 168.4 (s), 169.2 (s), 171.0 (s), 171.8 (s), 172.9 (s), 173.1 (s), 175.0 (s), 175.2 (s) ppm. HRMS m/z calcd for $C_{66}H_{62}O_{12}N_{17}S_5$ (M+H) 1444.3362, found 1444.3399.

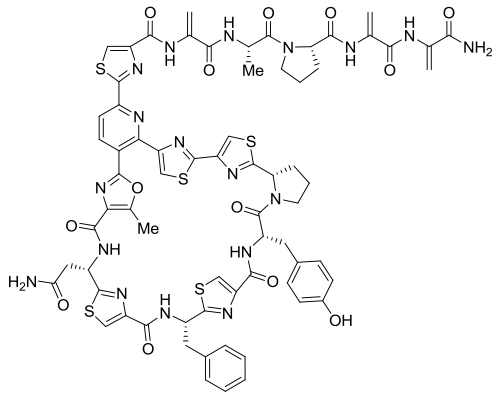
Thiazoline Tetrapeptide Analogue (28).



Trimethyltin hydroxide (47 mg, 0.256 mmol) was added to a solution of **20**[2] (50 mg, 42.8 μmol) in dry 1,2-dichloroethane (0.85 mL) and the reaction mixture was then stirred at 60 °C. After 15 h the mixture was diluted in CH_2Cl_2 (40 mL), washed with 6% HCl (30 mL), dried (Na_2SO_3), and concentrated *in vacuo*. EDC·HCl (10 mg, 51.4 μmol), HOAt (7 mg, 51.4 μmol) and DIPEA (9 μL, 51.4 μmol) were added to a stirring solution of the crude carboxylic acid (**22**)[2] and **13** (39 mg, 51.4 μmol) in DMF (0.9 mL) at 0 °C. After 17 h the mixture was diluted in CH_2Cl_2 (20 mL), washed with 1 M $NaHCO_3$ (20 mL), dried (Na_2SO_3) and concentrated *in vacuo* with the aid of toluene to remove DMF traces. The crude product was purified by flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10). The condensation product was obtained as a white solid (45 mg, 60%). 5.5 M *t*BuOOH in decane (70 μL, 0.384 mmol) was added to a solution of the condensation product (45 mg, 25.6 μmol) in dry CH_2Cl_2 (8.5 mL) and the resulting solution was stirred at rt. 5.5 M *t*BuOOH in decane (70 μL, 0.384 mmol) was added twice more after 5 h and 22 h after the first addition. Upon consumption of the starting material after 45 h, CH_2Cl_2 (10 mL) and a mixture of saturated aq. $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (2 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product purified by preparative reverse-phase column (C18, 40% to 50% MeCN in H_2O). The title product was obtained as a white solid (34 mg, 92%). HPLC: 40 to 60% MeCN (t_R = 5.47 min). mp (CH_2Cl_2) decomposes above 200 °C. $[\alpha]_D$ +12.4 (c = 0.50, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3430, 2917, 2841, 1739, 1649, 1444, 1041 cm^{-1}. ^1H NMR (400 MHz, pyridine-d_5) δ = 1.58−1.70 (m, 2 H), 1.88−2.06 (m, 4 H), 2.08−2.41 8m, 6 H), 2.52−2.66 (m, 2 H), 3.11−3.20 (m, 3 H), 3.36−3.43 (m, 1 H), 3.53−3.58 (m, 1 H), 3.73−3.78 (m, 1 H), 3.96−4.17 (m, 5 H), 4.22−4.30 (m, 1 H), 5.31−5.46 (m, 2 H), 5.50−5.66 (m, 2 H), 5.73−5.80 (m, 2 H), 6.23−6.28 (m, 1 H), 6.33 (s, 1 H), 6.52 (s, 1 H), 7.22 (s, 1 H), 7.40−7.58 (m, 8 H), 7.64−7.77 (m, 2 H), 7.97 (bs, 1 H), 8.09 (d, *J* = 9.0 Hz, 1 H), 8.27 (bs, 1 H), 8.35 (s, 1 H), 8.52−8.62 (m, 4 H), 8.86 (bs, 1 H), 8.99 (s, 1 H), 9.12 (d, *J* = 6.6 Hz, 1 H), 9.29 (bs, 1 H), 9.70 (d, *J* = 9.0 Hz, 1 H), 10.12 (d, *J*

= 6.6 Hz, 1 H), 10.23 (d, *J* = 6.6 Hz, 1 H),10.94−11.00 (m, 1 H) ppm. ^{13}C NMR (150 MHz, pyridine-d_5) δ = 11.8 (q), 17.5 (q), 25.2 (t), 25.5 (t), 28.8 (t), 30.0 (t), 34.3 (t), 36.2 (t), 36.8 (t), 38.4 (t), 39.9 (t), 47.5 (t), 48.5 (d), 49.2 (d), 52.3 (d), 54.1 (d), 61.7 (d), 61.9 (d), 78.8 (d), 102.8 (t), 103.3 (t), 116.2 (d), 116.8 (d), 118.6 (d), 123.0 (d), 123.1 (d), 125.8 (s), 127.3 (d), 127.9 (d), 128.7 (d), 130.2 (s), 130.2 (d), 131.0 (s), 131.7 (d), 136.9 (s), 139.2 (d), 148.9 (s), 150.8 (s), 150.9 (s), 152.0 (s), 153.9 (s), 154.7 (s), 155.9 (s), 157.9 (s), 159.7 (s), 160.5 (s), 161.2 (s), 162.7 (s), 164.8 (s), 166.8 (s), 168.3 (s), 169.1 (s), 171.0 (s), 171.2 (s), 172.6 (s), 173.0 (s), 173.2 (s), 174.8 (s), 175.1 (s) ppm. HRMS m/z calcd for $C_{66}H_{64}O_{12}N_{17}S_5$ (M+H) 1446.3518, found 1446.3603.

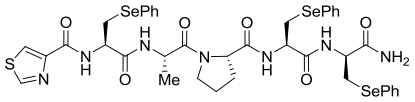
Thiazole Pentapeptide Analogue (29).



14[2] (36 mg, 42.1 μmol), EDC·HCl (8 mg, 42.1 μmol), HOAt (6 mg, 42.1 μmol) and DIPEA (8 μL, 42.1 μmol) were added to a stirring solution of **21** (40 mg, 35.1 μmol) in dry DMF (0.7 mL) cooled in an ice bath. The reaction mixture was allowed to reach rt. After 4 h, CH_2Cl_2 (25 mL) was added and the resulting solution was washed with saturated aq. NH_4Cl (25 mL), saturated aq. $NaHCO_3$ (25 mL) and brine (25 mL), dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5 to 90:10).The condensation product was obtained as white solid (49 mg, 70%). 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added to a solution of the condensation product (47 mg, 23.7 μmol) in dry CH_2Cl_2 (8 mL) and the resulting solution was stirred at rt. 5.5 M *t*BuOOH in decane (86 μL, 0.474 mmol) was added twice more after 5 h and 26 h after the first addition. Upon consumption of the starting material after 32 h, CH_2Cl_2 (5 mL) and a mixture of saturated aq. $Na_2S_2O_3$/$NaHCO_3$ (1:1, 10 mL) were added to the reaction mixture and the aqueous layer was extracted with more CH_2Cl_2 (4 × 10 mL). Combined organic fractions were dried (Na_2SO_4) and concentrated *in vacuo*. The crude product was purified by silica flash column chromatography (CH_2Cl_2/MeOH, 99:1 to 90:10). The title product was obtained as a white solid (26 mg, 72%). HPLC: 40 to 60% MeCN (t_R = 5.67 min). mp (CH_2Cl_2) decomposes above 120

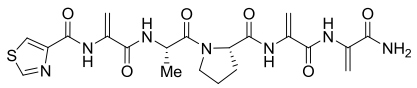
°C. $[\alpha]_D$ +20.6 (c = 1.00, CH_2Cl_2/MeOH, 96:4). IR (KBr) 3443, 2917, 2853, 1649, 1489, 1419 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.24−1.31 (m, 1 H), 1.40 (d, *J* = 6.8 Hz, 3 H), 1.85−2.26 (m, 8 H), 2.40−2.51 (m, 1 H), 2.78 (s, 3 H), 2.83−2.97 (m, 2 H), 3.09−3.27 (m, 2 H), 3.34−3.44 (m, 1 H), 3.62−3.91 (m, 3 H), 4.52−4.60 (m, 1 H), 4.71−4.82 (m, 1 H), 5.02−5.13 (m, 1 H), 5.30−5.43 (m, 2 H), 5.58 (s, 1 H), 5.62−5.72 (m, 2 H), 5.90 (s, 1 H), 5.98 (s, 1 H), 6.14 (s, 1 H), 6.56 (s, 1 H), 6.68 (d, *J* = 8.6 Hz, 2 H), 6.83 (bs, 1 H), 7.11 (d, *J* = 8.6 Hz, 2 H), 7.20 (d, *J* = 6.8 Hz, 2 H), 7.24−7.39 (m, 4 H), 7.57 (bs, 1 H), 7.76 (s, 1 H), 7.96−8.04 (m, 2 H), 8.13 (s, 1 H), 8.23 (s, 1 H), 8.32−8.40 (m, 2 H), 8.58 (d, *J* = 8.4 Hz, 1 H), 8.71 (s, 1 H), 8.76 (d, *J* = 7.6 Hz, 2 H), 8.85 (d, *J* = 6.4 Hz, 1 H), 9.13 (s, 1 H), 9.52 (s, 1 H), 10.09 (s, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 12.6 (q), 17.3 (q), 25.5 (t), 25.6 (t), 29.9 (t), 29.9 (t), 33.5 (t), 37.9 (t), 42.6 (t), 47.7 (t), 48.3 (t), 48.3 (d), 49.4 (d), 52.7 (d), 53.4 (d), 60.4 (d), 61.0 (d), 104.3 (t), 105.3 (t), 107.9 (t), 116.1 (d), 117.2 (d), 119.3 (d), 123.4 (d), 123.5 (s), 124.5 (d), 125.8 (d), 127.4 (s), 127.7 (d), 129.1 (s), 129.2 (d), 129.6 (d), 130.1 (s); 130.4 (d); 131.3 (d), 134.4 (s), 135.6 (s), 137.3 (s), 137.4 (s), 141.3 (d), 148.5 (s), 149.8 (s), 151.2 (s), 151.5 (s), 152.2 (s), 153.9 (s), 154.7 (s), 156.5 (s), 157.0 (s), 159.5 (s), 160.0 (s), 161.1 (s), 161.8 (s), 162.0 (s); 163.2 (s), 163.8 (s), 166.0 (s), 168.4 (s), 170.3 (s), 171.2 (s), 171.5 (s), 172.2 (s), 172.6 (s), 174.6 (s), 175.3 (s) ppm. HRMS m/z calcd for $C_{69}H_{65}O_{13}N_{18}S_5$ (M+H) 1513.3577, found 1513.3673.

Thz-Sec(Ph)-Ala-Pro-Sec(Ph)-Sec(Ph)-NH_2 (30).



Prepared according to the general method for solid-phase peptide synthesis, starting from 1.35 g of resin. The crude product was further purified by silica flash column chromatography (CH_2Cl_2/MeOH, 95:5). The title product was obtained as a pale solid (330 mg, 47% based on functionalization of the resin). HPLC: 0 to 100% MeCN (t_R = 7.29 min). mp (CH_2Cl_2) 185−188 °C. $[\alpha]_D$ −116.6 (c = 0.33, CH_2Cl_2). IR (KBr) 3436, 3295, 2924, 1668, 1623, 1041 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6) δ = 1.26 (d, *J* = 6.8 Hz, 3 H), 1.77−2.12 (m, 4 H), 3.08−3.19 (m, 2 H), 3.24−3.50 (m, 4 H), 3.54−3.68 (m, 2 H), 4.28−4.47 (m, 3 H), 4.56−4.65 (m, 1 H), 4.76−4.86 (m, 1 H), 7.21−7.36 (m, 9 H), 7.46−7.56 (m, 6 H), 8.15 (d, *J* = 8.4 Hz, 1 H), 8.22 (d, *J* = 7.2 Hz, 1 H), 8.39 (d, *J* = 2.0 Hz, 1 H), 8.48−8.58 (m, 2 H), 9.22 (d, *J* = 2.0 Hz, 1 H) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 17.9 (q), 25.4 (t), 29.4 (t), 29.6 (t), 29.9 (t), 30.4 (t), 47.5(d), 47.7 (t), 53.4 (d); 53.8 (d), 54.1 (d), 60.9 (d), 125.7 (d), 127.6 (d), 127.6 (d), 127.8 (d), 130.0 (d), 130.2 (d), 130.2 (d), 130.7 (s), 130.8 (s), 131.0 (s), 132.4 (d), 132.7 (d), 132.7 (d), 150.1 (s), 155.9 (d), 161.0 (s), 170.0 (s), 170.8 (s), 171.5 (s), 172.4 (s), 172.7 (s) ppm. HRMS m/z calcd for $C_{39}H_{44}N_7O_6SSe_3$ (M+H) 998.0392, found 998.0454.

Thz-Dha-Ala-Pro-Dha-Dha-NH_2 (31).



The title peptide could be obtained by two different methods: (A) On-resin oxidation/elimination; (B) Oxidation/elimination after cleavage.

Method A:

Prepared according to the general method for solid-phase peptide synthesis, starting from 1.06 g of resin. Prior to cleavage from the resin, an overnight treatment with 3 M *t*BuOOH in isooctane (6 mL, 18 mmol, 32 equiv) in CH_2Cl_2 (3 mL) at rt was performed. After cleavage the crude product was further purified by silica flash column chromatography (EtOAc/THF, 9:1 to 8:2). The title product was obtained as a white solid (102 mg, 36% based on functionalization of the resin).

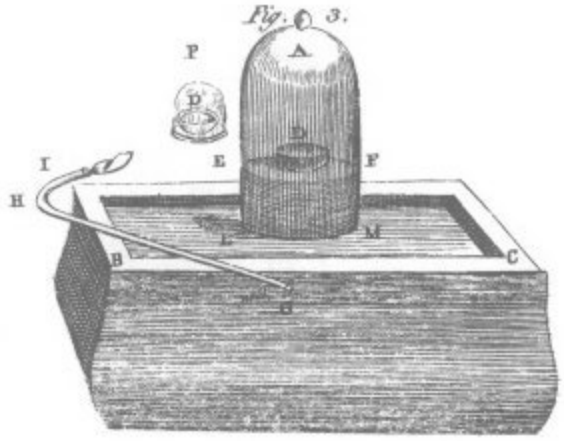
Method B:

3 M *t*BuOOH in isooctane (3.5 mL, 10.5 mmol, 34 equiv) was added to a solution of **30** (304 mg, 0.311 mmol) in dry CH_2Cl_2 (9 mL). The resulting mixture was stirred at rt and after 7 h . The crude product was purified by silica flash column chromatography (EtOAc/THF, 9:1 to 8:2). The title product was obtained as a white solid (112 mg, 72%). HPLC: 50 to 100% MeCN (t_R = 1.18 min). mp (EtOAc) decomposes above 100 °C. $[\alpha]_D$ −85.9 (c = 1.00, CH_2Cl_2). IR (KBr) 3334, 2981, 2911, 2873, 1630, 1515 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6) δ = 1.56 (d, *J* = 6.8 Hz, 3 H), 2.12−2.30 (m, 4 H), 3.77−3.85 (m, 1 H), 3.88−3.96 (m, 1 H), 4.62−4.69 (m, 1 H), 4.90 (q, *J* = 3.0, 1 H), 5.61 (bs, 1 H), 5.68 (bs, 1 H), 5.72 (bs, 1 H),6.24 (bs, 1 H), 6.31 (d, *J* = 1.2 Hz, 1 H), 6.40 (d, *J* = 1.2 Hz, 1 H), 6.68 (d, *J* = 1.6 Hz, 1 H), 6.96 (bs, 1 H), 7.66 (d, *J* = 6.4 Hz, 1 H), 8.37 (d, *J* = 2.0 Hz, 1 H), 8.79 (bs, 1 H), 8.94 (bs, 1 H), 9.10 (d, *J* = 2.0 Hz, 1 H), 10.05 (bs, 1 H) ppm. ^{13}C NMR (100 MHz, acetone-d_6) δ = 16.9 (q), 25.2 (t), 28.6 (t), 47.6 (t), 48.1 (d), 61.3 (d), 102.0 (t), 104.5 (t), 105.0 (t), 124.7 (d), 134.4 (s), 134.4 (s), 136.0 (s), 151.2 (s), 154.8 (d), 159.5 (s), 162.6 (s), 163.5 (s), 165.7 (s), 171.3 (s), 172.4 (s) ppm. HRMS m/z calcd for $C_{21}H_{26}O_6N_7S$ (M+H) 504.1660, found 504.1663.

3. NMR Spectra

NMR spectra images are available in the Supporting Information in electronic format.

Conclusions



CONCLUSIONS

The work carried out during this doctoral thesis has been devoted to different aspects of the study of a novel natural product with antibacterial activity, baringolin. First, a general retrosynthetic analysis was designed and the study of synthetic strategies and methodologies necessary to obtain the required building blocks followed. With a mind to develop an analogues program, the order of fragments condensation was decided to best suit that purpose; protecting groups were chosen accordingly. Upon completion of its total synthesis, structure and stereochemistry of baringolin were confirmed. With a useful synthetic strategy in hands, various analogues were designed and synthesized to evaluate the impact of different moieties in the activity profile of the thiopeptidic scaffold. The successful preparation of the first fully synthetic library of thiopeptide antibiotics accounts for the versatility and convergence of the synthetic approach developed herein.

Moving from a general overview and focusing into more succinct points of the present work, several conclusions can be drawn:

- An approach to synthesize a 4-bromo-bithiazole-pyrrolidine fragment was developed to overcome the lack of literature precedents regarding the preparation of such moieties. Hantzsch cyclization offered a convergent and effective way of preparing the fragment.

- A previously described protocol for the synthesis of amino acid derived thiazoles via a two-step modified Hantzsch cyclization was improved to avoid trifluoroacetylation and the corresponding extra deprotection step.

- The ease of synthesis of amino acid-derived thiazoles prompted us to synthesize a recently described thiazole-containing macrocyclic peptide, aeruginazole A.

- Studies towards a palladium-catalyzed synthesis of baringolin polyheterocyclic core led to a fully regioselective strategy that allowed the synthesis of various central core analogues. Evaluation of different sets of protecting groups led to a fully orthogonal combination that permitted selective elongation of the central fragment.

- Synthesis of the thiazoline moiety was achieved according to reported procedures. Obtention of the required free carboxylic acid avoiding epimerization during saponification was possible thanks to the use of Me_3SnOH.

- Synthesis of the phenylselenocysteine-containing pentapeptide with a C-terminal amide was easily achived using ChemMatrix/Rinkamide resin and OxymaPure as coupling additive in a solid-phase approach.

- Assembling of fragments was carried out successfully. Selective elongation of the central fragment and condensation with building blocks from the macrocycle moiety bearing allyl-based protecting groups, set the stage for a one-step deprotection and subsequent macrocyclization. Lastly, saponification, condensation

of the peptide and oxidation/elimination of the phenylselenide groups yielded synthetic baringolin.

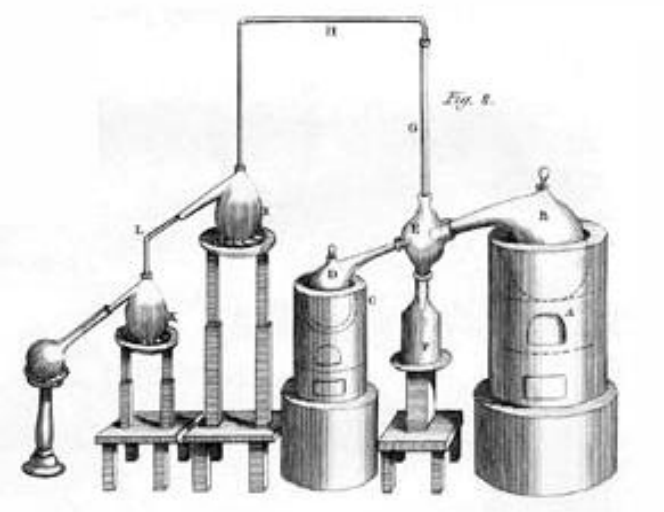
- Comparison of spectroscopic data and biological activity of natural and synthetic baringolin confirmed its structure and stereochemistry.

- A library of fully synthetic analogues was produced using the same synthetic strategy that served for the total synthesis of baringolin. The desired modifications could be easily introduced and provided sufficient material for full characterization and biological assays to be carried out. These results demonstrate the usefulness of the synthetic strategy developed during this thesis.

- Assessment of the analogues ability to inhibit Gram-positive bacterial growth delimited the structure-activity relationship of the modified moieties. The thiazoline ring was identified as crucial for a broad scope of antibacterial activity. Its substitution for the corresponding thiazole reduced potency against some strains, but in general maintained it against *Staphylococcus aureus*. Substitution of baringolin's peptidic tail with a cyclohexanoic acid moiety in the thiazole series of analogues restored activity towards all strains and in most cases improved potency.

During the years the present work has been carried out, many discoveries and advances have completely changed the scenario of thiopeptides' science. The discovery of new members of this family and reports on various novel total syntheses, especially that of micrococcin P1, have engrossed the already rich variety of structures and methodologies available for their synthesis. However, identification of the genetic cluster that encodes the precursor peptide and all the enzymatic machinery required for its maturation has been a major revolution to the field. Disclosure of the biosynthetic pathways has confirmed suspicions regarding the ribosomal origin of thiopeptides, but more importantly, has opened the field to genetic engineering and mutasynthesis. Our own research is a consequence of its context and might have been completely different if started either a few years earlier or later. The constant development of new synthetic methodologies determines the tools available for the synthetic chemist and makes new strategies possible. The newest reports would have offered a different perspective and alternative methodologies might have been tested during our investigations, probably outlining a different outcome. Nonetheless, this thesis responds to its own context and our own needs of both natural product and analogues synthesis. The positive results obtained are good evidence for the usefulness of the strategy developed herein.

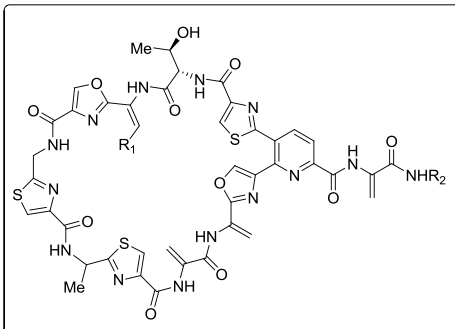
Encyclopædia Thiopeptidum



ENCYCLOPÆDIA THIOPEPTIDUM

Behold! You are at the library. Here you will find a compilation of all thiopeptides of yore and new. Enter and you shall find the knowledge you are seeking.

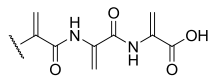
The following pages contain information and references regarding all thiopeptides reported to date (to the best of my knowledge), which are listed alphabetically. This thiopeptide encyclopedia includes the first reported place of isolation, the kind of sample and the original reference or references where the thiopeptide was reported. In those cases where the genetic cluster has been identified, the sequence of the structural peptide and the original reference are indicated. Reports on total syntheses have also been added. No references or information regarding analogues have been added for the sake of space and clarity. Thus, only data concerning naturally occurring thiopeptides is reported herein.



A10255 B R_1 = Et; R_2 =

A10255 E R_1 = *i*-Pr; R_2 =

A10255 G R_1 = Me; R_2 =



A10255 J R_1 = Me; R_2 = H

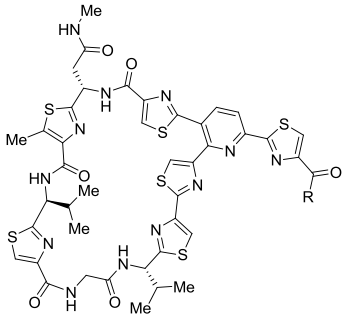
Series: d

First found in: Huerfand County, Colorado, USA

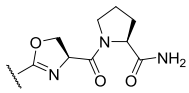
Kind of sample: soil

Producing strain: *Streptomyces gardneri*

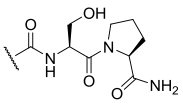
Original report: a) Boeck, L. D.; Berry, D. M.; P. Mertz, F.; Wetzel, R. W. *J. Antibiot.* **1992**, *45*, 1222–1230; b) Debono, M.; Molloy, R. M.; Occolowitz, J. L.; Paschal, J. W.; Hunt, A. H.; Michel, K. H.; Martin, J. W. *J. Org. Chem.* **1992**, *68*, 5200–5208.



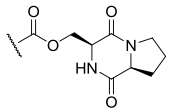
Amythiamicin A R =



Amythiamicin B R =



Amythiamicin C R =



Amythiamicin D R = CO_2Me

Series: d

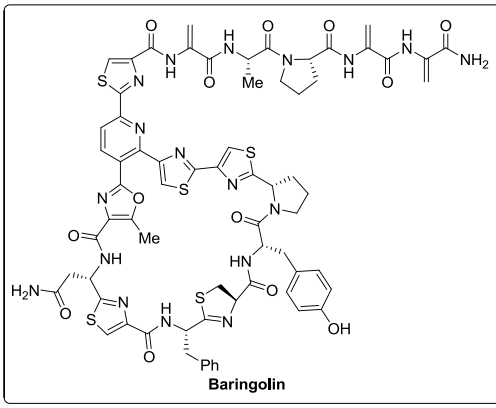
First found in: Nerima-ku, Tokyo, Japan

Kind of sample: soil

Producing strain: *Amycolatopsis sp.*

Original report: a) Shimanaka, K.; Kinoshita, N.; Iinuma, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 668–674; b) Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1145–1152; c) Shimanaka, K.; Takahashi, Y.; Iinuma, H.; Naganawa, H.; Takeuchi, T. *J. Antibiot.* **1994**, *47*, 1153–1159.

Total syntheses: a) Hughes, R. a; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *Chem. Commun.* **2004**, 946–948. b) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 15644–15651. c) Nicolaou, K. C.; Dethe, D. H.; Chen, D. Y.-K. *Chem. Commun.* **2008**, 2632–2634. d) Ammer, C.; Bach, T. *Chem. Eur. J.* **2010**, *16*, 14083–14093.



Baringolin

Series: d

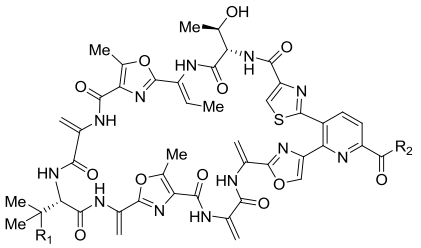
First found in: Almeria, Spain

Kind of sample: marine

Producing strain: *Kocuria sp.* MI-67-EC3-038

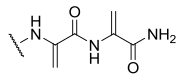
Original report: Cañedo Hernández, L. M.; Romero Millan, F.; Fernández Medarde, A.; Fernández Chimeno, R. I.; Hidalgo Villar, J. C. New peptides as bioactive compounds (Biomar, S.A. 2012). WO2012062906A1.

Total synthesis: Just-Baringo, X.; Bruno, P.; Ottesen, L. K.; Cañedo, L. M.; Albericio, F.; Alvarez, M. *Angew. Chem. Int. Ed.* **2013**, *52*, 7818–7821.

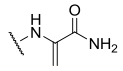


Berninamycin A R_1 = OH; R_2 =

Berninamycin B R_1 = H; R_2 =



Berninamycin C R_1 = OH; R_2 =



Berninamycin D R_1 = OH; R_2 = NH_2

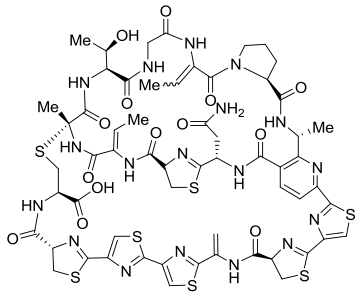
Series: d

First found in: not reported

Kind of sample: soil

Producing strain: *Streptomyces bernensis*

Original reports: a) Reusser, F. *Biochem.* **1969**, *8*, 3303–3308; b) Liesch, J. M.; Rinehart, Kenneth L, J. *J. Am. Chem. Soc.* **1977**, *99*, 1645–1646.; c) Lau, R. C. M.; Rinehart, K. L.; Adamslaboratory, R. *J. Antibiot.* **1994**, *47*, 1466–1472.



Cyclothiazomycin B1 "*Z*" isomer

Cyclothiazomycin B2 "*E*" isomer

Series: d

First found in: Kamakura, Kanagawa, Japan

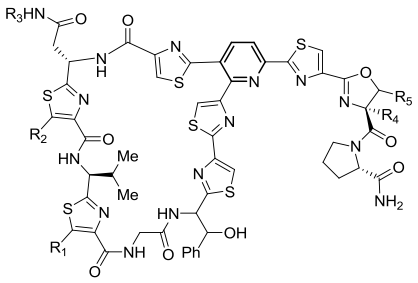
Kind of sample: soil

Producing strain: *Streptomyces sp.* NR0516 / *Streptomyces hygroscopicus* 10-22

Original report: Aoki, M.; Ohtsuka, T.; Yamada, M.; Ohba, Y.; Yoshizaki, H.; Yasuno, H.; Sano, T.; Watanabe, J.; Yokose, K.; Roche, N. *J. Antibiot.* **1991**, *44*, 582–588.

Gene cluster identification: Wang, J.; Yu, Y.; Tang, K.; Liu, W.; He, X.; Huang, X.; Deng, Z. *Appl. Environ. Microbiol.* **2010**, *76*, 2335–2344.

Structural peptide: SNCTSTGTPASCCSCCCC



GE2270 A R_1 = CH_2OMe; R_2 = CH_3; R_3= CH_3; R_4,R_5 = H

GE2270 B1 R_1 = CH_2OMe; R_2 = CH_3; R_3= H; R_4,R_5 = H

GE2270 B2 R_1 = CH_3; R_2 = CH_3; R_3= CH_3; R_4,R_5 = H

GE2270 C1 R_1 = H; R_2 = CH_3; R_3= CH_3; R_4,R_5 = H

GE2270 C2a R_1 = CH_2OMe; R_2 = CH_3OH; R_3= CH_3; R_4,R_5 = H

GE2270 C2b R_1 = CH_2OMe; R_2 = H; R_3= CH_3; R_4,R_5 = H

GE2270 D1 R_1 = H; R_2 = CH_3; R_3= H; R_4,R_5 = H

GE2270 D2 R_1 = CH_2OH; R_2 = CH_3; R_3= CH_3; R_4,R_5 = H

GE2270 E R_1 = CH_2OH; R_2 = CH_3; R_3= H; R_4,R_5 = H

GE2270 T R_1 = CH_2OMe; R_2 = CH_3; R_3= CH_3; R_4,R_5 = π-bond

Series: d

First found in: Lamole, Italy

Kind of sample: soil

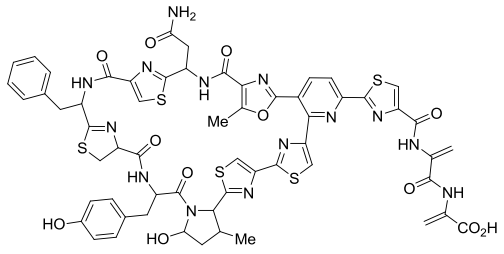
Producing strain: *Planobispora rosea*

Original reports: a) Selva, E.; Beretta, G.; Montanini, N.; Saddler, G. S.; Gastaldo, L.; Ferrari, P.; Lorenzzetti, R.; Landini, P.; Ripamonti, F.; Goldstein, B. P.; Berti, M.; Montanaro, L.; Denaro, M. *J. Antibiot.* **1991**, *44*, 693–701.; b) Nebuloni, M.; Vekey, K.; Gallo, G. G.; Selva, E. *J. Antibiot.* **1991**, *44*, 702–715.

Gene cluster identification: Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

Structural peptide: SCNCVCGFCCSCSPSA

Total syntheses: a) Nicolaou, K. C.; Zou, B.; Dethe, D. H.; Li, D. B.; Chen, D. Y.-K. *Angew. Chem. Int. Ed.* **2006**, *45*, 7786–7792; b) Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4771–4774; c) Delgado, O.; Müller, H. M.; Bach, T. *Chem. Eur. J.* **2008**, *14*, 2322–2339; d) Nicolaou, K. C.; Dethe, D. H.; Leung, G. Y. C.; Zou, B.; Chen, D. Y.-K. *Chem. Asian. J.* **2008**, *3*, 413–429.



GE37468A

Series: d

First found in: Lamole, Italy

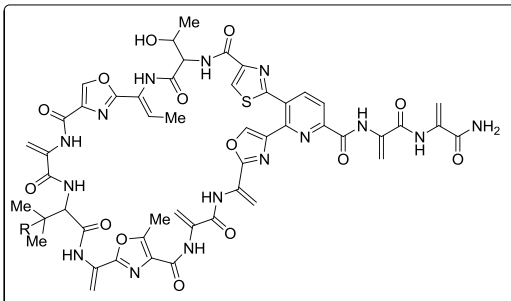
Kind of sample: soil

Producing strain: *Streptomyces sp.* ATCC 55365

Original reports: a) Stella, S.; Montanni, N.; Monnier, F. Le; Ferrari, P.; Colombo, L.; Landini, P.; Ciciliato, I.; Goldstein, B. P.; Selva, E.; Denaro, M. *J. Antibiot.* **1995**, *48*, 780–786; b) Ferrari, P.; Colombo, L.; Stella, S.; Selva, E.; Zerilli, L. F. *J. Antibiot.* **1995**, *48*, 1304–1311.

Gene cluster identification: Young, T. S.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13053–13058.

Structural peptide: STNCFCYICCSCSSN



Geninthiocin R = OH
Val-geninthiocin R = H

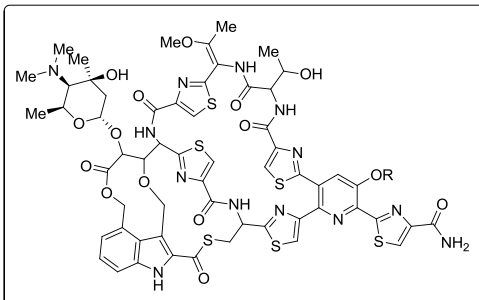
Series: d

First found in: Izu-Oshima, Japan

Kind of sample: soil

Producing strain: *Streptomyces sp.* DD84 / *Streptomyces sp.* RSF18

Original reports: a) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 969–975. b) Sajid, I.; Shaaban, K. A.; Frauendorf, H.; Hasnain, S.; Laatsch, H. *Z. Naturforsch. B* **2008**, *63*, 1223-1230.



Glycothiohexide α R = H

O-methyl-glycothiohexide α R = Me

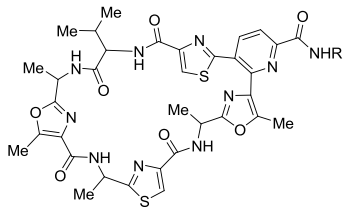
Series: e

First found in: Egypt

Kind of sample: soil

Producing strain: "*Sebekia*" *sp.* NRRL 21083

Original reports: a) 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*, 887–893; b) Northcote, P. T.; Williams, D.; Manning, J. K.; Borders, D. B.; Maiese, W. M.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 894–900. c) Northcote, P. T.; Siegel, M.; Borders, D. B.; Lee, M. D. *J. Antibiot.* **1994**, *47*, 901–908.



JBIR-83 R = H

JBIR-84 R =

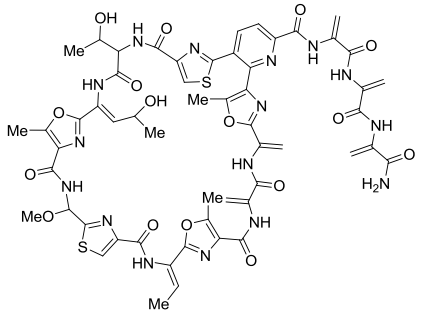
Series: d

First found in: Shuri, Okinawa Prefecture, Japan

Kind of sample: soil

Producing strain: *Streptomyces sp.* RI19

Original report: Takagi, M.; Motohashi, K.; Nagai, A.; Hashimoto, J.; Shin-Ya, K. *J. Antibiot.* **2010**, *63*, 405–408.



Methylsulfomycin

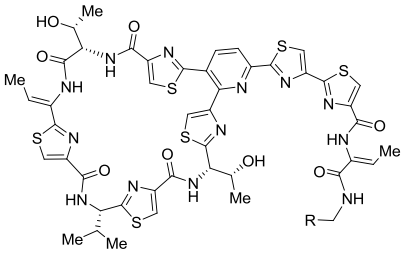
Series: d

First found in: Greece

Kind of sample: soil

Producing strain: Streptomyces sp. HIL Y-9420704

Original report: Kumar, E. K. S. V.; Kenia, J.; Mukhopadhyay, T.; Nadkarni, S. R. *J. Nat. Prod.* **1999**, *62*, 1562–1564.



Micrococcin P1 R = 

Micrococcin P2 R = 

Series: d

First found in: East Africa

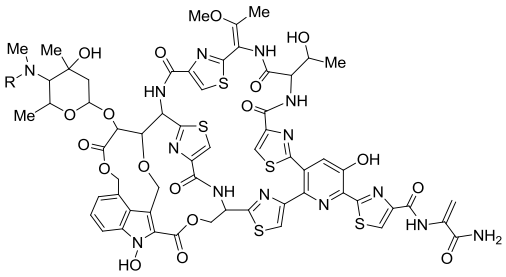
Kind of sample: soil

Producing strain: *Bacillus pumilus*

Original report: Fuller, A. T. *Nature* **1955**, *175*, 722.

Total synthesis: Lefranc, D.; Ciufolini, M. A *Angew. Chem. Int. Ed.* **2009**, *48*, 4198–4201.

Micrococcin was first isolated from sewage waters in Oxford in 1948 from a strain of *Micrococcus*; however, no structural data was obtained. Later, in 1955, a compound of almost identical properties was isolated from *B. pumilus*, found in East Africa, and was thus named micrococcin P.



MJ347-81F4 A R = Me

MJ347-81F4 B R = H

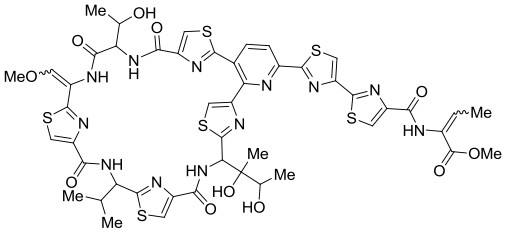
Series: e

First found in: Hamochi-machi, Sado-gun, Niigata prefecture, Japan

Kind of sample: soil

Producing strain: *Amycolatopsis sp.* MJ347-81F4

Original report: Sasaki, T.; Otani, T.; Matsumoto, H.; Unemi, N.; Hamada, M.; Takeuchi, T.; Hori, M. *J. Antibiot.* **1998**, *51*, 715-721.



Nocardithiocin

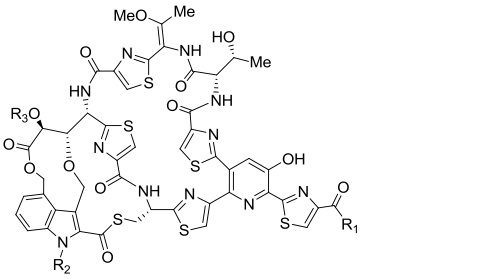
Series: d

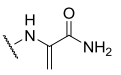
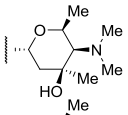
First found in: patient at the Health Center, University of Texas, USA.

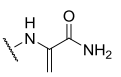
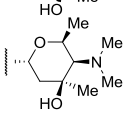
Kind of sample: clinical material

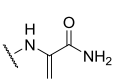
Producing strain: *Nocardia pseudobrasiliensis* IFM 0757

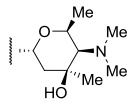
Original report: Mukai, A.; Fukai, T.; Hoshino, Y.; Yazawa, K.; Harada, K.; Mikami, Y. *J. Antibiot.* **2009**, *62*, 613–619.



Nocathiacin I R_1 =  R_2 = OH; R_3 = 

Nocathiacin II R_1 =  R_2 = H; R_3 = 

Nocathiacin III R_1 =  R_2 = OH; R_3 = H

Nocathiacin IV $R_1 = NH_2$ R_2 = OH; R_3 = 

Series: e

First found in: New Mexico, USA

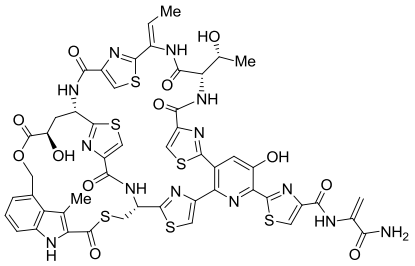
Kind of sample: soil

Producing strains: *Nocardia sp.* WW-12651 / *Amycolatopsis sp.*

Original reports: a) Constantine, K. L.; Mueller, L.; Huang, S.; Abid, S.; Lam, K. S.; Li, W.; Leet, J. E. *J. Am. Chem. Soc.* **2002**, *124*, 7284–7285; b) Li, W.; E. Leet, J.; A. Ax., H.; R. Gustavson, D.; M. Brown, D.; Turner, L.; Brown, K.; Clark, J.; Yang, H.; Fung-tomc, J.; S. Lam, K. *J. Antibiot.* **2003**, *56*, 226–231; 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*, 232–242.

Gene cluster identification: Ding, Y.; Yu, Y.; Pan, H.; Guo, H.; Li, Y.; Liu, W. *Mol. BioSyst.* **2010**, *6*, 1180–1185.

Structural peptide: SCTTCECSCSCSS



Nosiheptide

Series: e

First found in: Kagamihara, Gifu prefecture, Japan

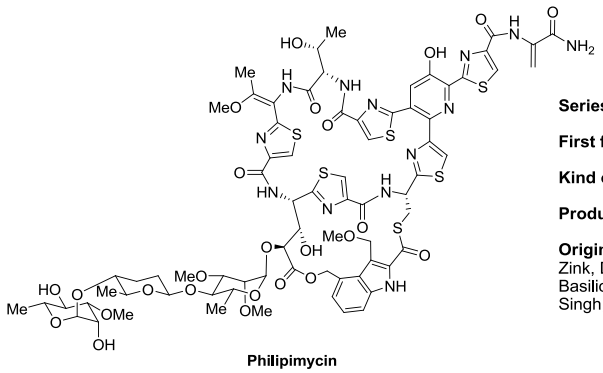
Kind of sample: soil

Producing strain: *Streptomyces antibioticus* 8446-CCI and *Streptomyces actuosus*.

Original reports: a) Tanaka, T.; Endo, T.; Shimazu, A.; Yoshida, R.; Suzuki, Y.; Otake, N.; Yonehara, H. *J. Antibiot.* **1970**, *23*, 231–237; b) 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*, 414–416.

Gene cluster identification: Yu, Y.; Duan, L.; Zhang, Q.; Liao, R.; Ding, Y.; Pan, H.; Wendt-, E.; Tang, G.; Shen, B.; Liu, W. *ACS Chem Biol.* **2009**, *4*, 855–864.

Structural peptide: SCTTCECCCSCSS



Philipimycin

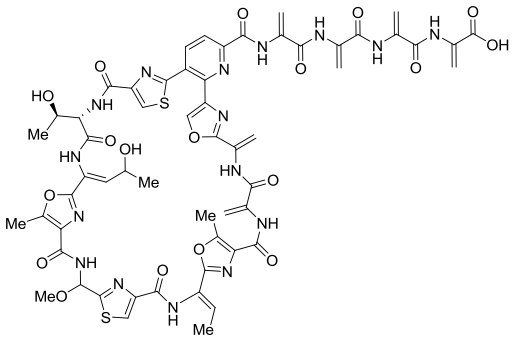
Series: e

First found in: Namaqualand, South Africa

Kind of sample: soil

Producing strain: *Actinoplanes philippinensis*

Original report: Zhang, C.; Occi, J.; Masurekar, P.; Barrett, J. F.; Zink, D. L.; Smith, S.; Onishi, R.; Ha, S.; Salazar, O.; Genilloud, O.; Basilio, A.; Vicente, F.; Gill, C.; Hickey, E. J.; Dorso, K.; Motyl, M.; Singh, S. B. *J. Am. Chem. Soc.* **2008**, *130*, 12102–12110.



Promoinducin

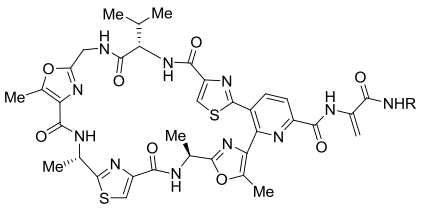
Series: d

First found in: Sakurasi, Japan

Kind of sample: soil

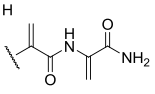
Producing strain: *Streptomyces sp.* SF2741

Original report: Yun, B.-S.; Seto, H. *Biosci. Biotechol. Biochem.* **1995**, *59*, 876–880.



Promothiocin A R = H

Promothiocin B R =



Series: d

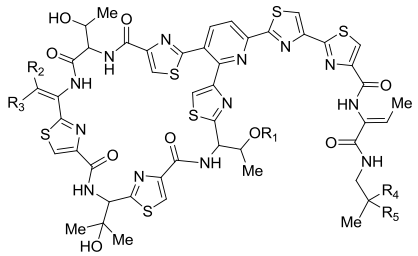
First found in: Sakurasi, Japan

Kind of sample: soil

Producing strain: *Streptomyces sp.* SF2741

Original report: Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 510-514.

Total synthesis: a) Moody, C. J.; Bagley, M. C. *Chem. Commun.* **1998**, *4*, 2049–2050; b) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301–3313.



QN3323 A R_1 = H; R_2 = Me; R_3 = H; R_4/R_5 = O (π-bond)

QN3323 B R_1 = H; R_2 = Me; R_3 = Me; R_4/R_5 = O (π-bond)

QN3323 Y R_1 = Me; R_2 = H; R_3 = H; R_4 = H; R_5 = OH

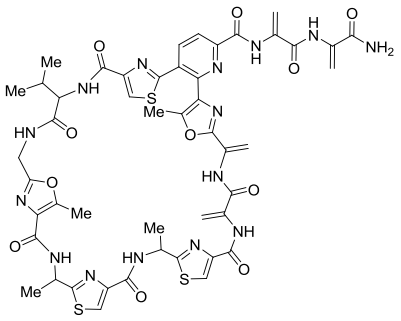
Series: d

First found in: not reported

Kind of sample: not reported

Producing strain: *Bacillus sp.*

Original report: Kamigiri, K.; Watanabe, M.; Nagai, K.; Arao, N.; Suzumura, K.; Suzuki, K.; Kurane, R.; Yamaoka, M.; Kawano, Y. Thiopeptide compounds suitable for treatment of multidrug resistant bacteria infection (2002) US2004097702A1.



Radamycin

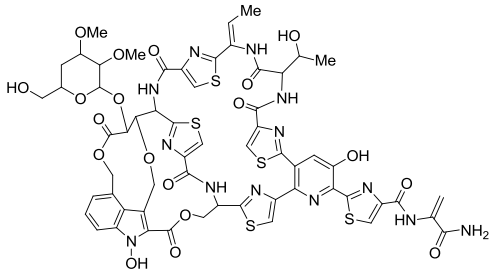
Series: d

First found in: not reported

Kind of sample: not reported

Producing strain: *Streptomyces sp.* RSP9

Original report: a) Holgado, G. G.; Rodríguez, J. C.; Cañedo Hernández, L. M.; Diaz, M.; Fernandez-Abalos, J. M.; Trujillano, I.; Santamaria, R. I. *J. Antibiot.* **2002**, *55*, 383–390; b) Castro Rodríguez, J.; Holgado, G. G.; Santamaria Sánchez, R. I.; Cañedo, L. M. *J. Antibiot.* **2002**, *55*, 391–395.



S 54832 A-I

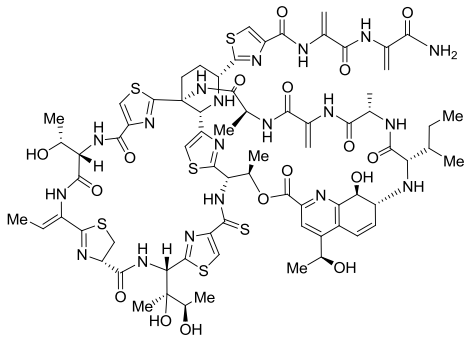
Series: e

First found in: Spain

Kind of sample: soil

Producing strain: Micromonospora globosa

Original report: Keller-Juslen, C.; Kuhn, M.; D. King, H. Antibiotics, pharmaceutical compositions and their use (Sandoz, Ltd. Basel, Switzerland, 1984). US4478831A



Sch 18640

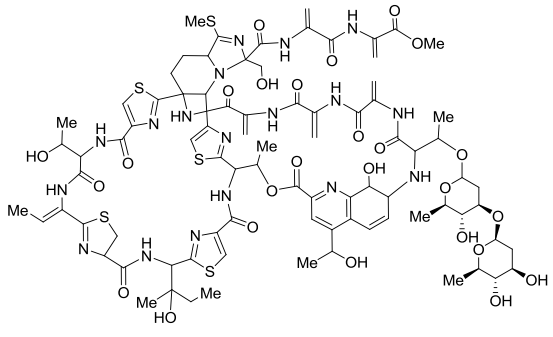
Series: a

First found in: not reported

Kind of sample: not reported

Producing strain: *Micromonospora arborensis*

Original report: Puar, M. S.; Ganguly, A. K.; Afonso, A.; Brambilla, R.; Mangiaracina, P.; Sarre, O.; MacFarlane, R. D. *J. Am. Chem. Soc.* **1981**, *36*, 5231–5233.



Sch 40832

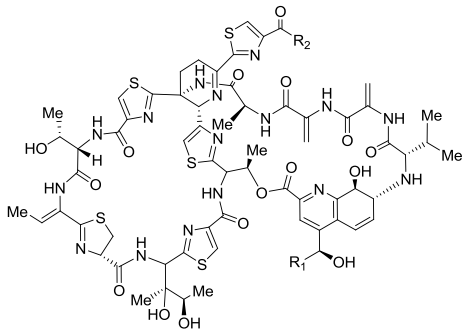
Series: c

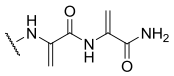
First found in: Africa

Kind of sample: soil

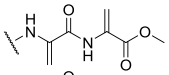
Producing strain: *Micromonospora carbonacea var africana*

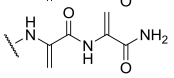
Original report: S. Puar, M.; Chan, T. M.; Hedge, V.; Patel, M.; Bartner, P.; Ng, K. J.; Pramanik, B. N. *J. Antibiot.* **1998**, *51*, 221–224.



Siomycin A R_1 = Me; R_2 = 

Siomycin B R_1 = Me; R_2 = NH_2

Siomycin C R_1 = Me; R_2 = 

Siomycin D R_1 = H; R_2 = 

Series: b

First found in: not reported

Kind of sample: not reported

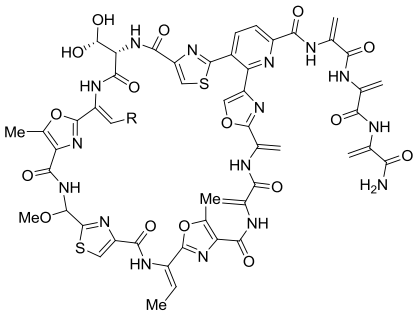
Producing strain: *Streptomyces sioyaensis*

Original reports: a) Nishimura, H.; Othsuka, H.; Nakajima, K.; Tawara, K.; Mayama, M.; Shimohira, M.; Shimaoka;, N.; Okamoto, S. *J. Antibiot., Ser. A* **1961**, *14*, 255–263; b) Ebata, M.; Miyazaki, K.; Otsuka, H. *J. Antibiot.* **1969**, *22*, 364–368; c) Tokura, K.; Tori, K.; Yoshimura, Y.; Yoshimura, K.; Otsuka, H.; Matsushita, K.; Inagaki, F.; Miyazama, T. *J. Antibiot.* **1980**, *33*, 1563–1567.

Gene cluster identification: Liao, R.; Duan, L.; Lei, C.; Pan, H.; Ding, Y.; Zhang, Q.; Chen, D.; Shen, B.; Yu, Y.; Liu, W. *Chem. Biol.* **2009**, *16*, 141–147.

Structural peptide: VSSASCTTCICTCSCSS

Total synthesis: a) Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2007**, *48*, 1331–1335; b) Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Chem. Asian. J.* **2008**, *3*, 984–1012.



Sulfomycin I R = CH(OH)Me

Sulfomycin II R = CH_2Me

Sulfomycin III R = CH_2OH

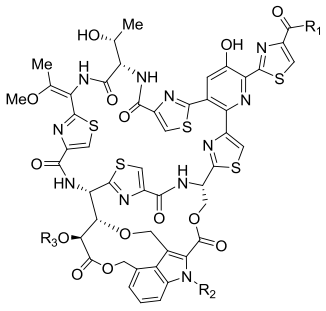
Series: d

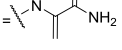
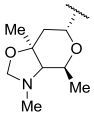
First found in: not reported

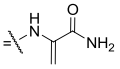
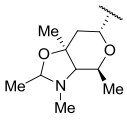
Kind of sample: soil

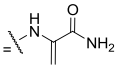
Producing strain: *Streptomyces viridochromogenes*

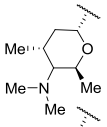
Original report: Egawa, Y.; Umino, K.; Tamura, Y.; Shimizu, M.; Kaneko, K.; Sakurazawa, M.; Awataguchi, S.; Okuda, T. *J. Antibiot.* **1969**, *22*, 12–17.

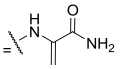
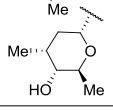


Thiazomycin R_1 =  ; R_2 = OH; R_3 = 

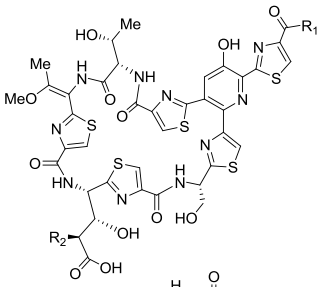
Thiazomycin A R_1 =  ; R_2 = OH; R_3 = 

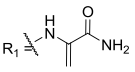
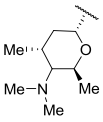
Thiazomycin B R_1 =  ; R_2 = H; R_3 = H

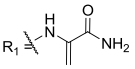
Thiazomycin C R_1 = NH_2 ; R_2 = H; R_3 = 

Thiazomycin D R_1 =  ; R_2 = OH; R_3 = 

Continued on next page...



Thiazomycin E_1 R_1  ; R_2 = 

Thiazomycin E_2 R_1  ; R_2 = H

Thiazomycin E_3 $R_1 = NH_2$; R_2 = H

... continued from previous page.

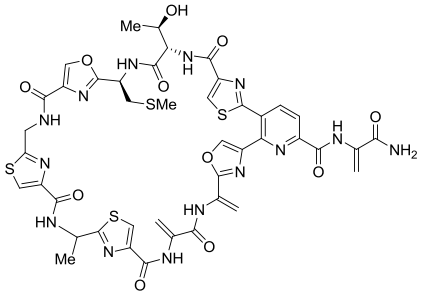
Series: e

First found in: Nocathiacins producing cultures of *Amycolatopsis fastidiosa*. However, thiazomycins are produced in very small amounts.

Kind of sample: soil

Producing strain: *Amycolatopsis fastidiosa*

Original reports: Zhang, C.; Herath, K.; Jayasuriya, H.; Ondeyka, J. G.; Zink, D. L.; Occi, J.; Birdsall, G.; Venugopal, J.; Ushio, M.; Burgess, B.; Masurekar, P.; Barrett, J. F.; Singh, S. B. *J. Nat. Prod.* **2009**, *72*, 841–847.



Thioactin

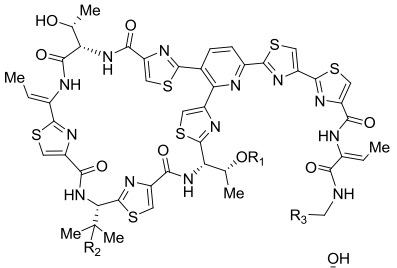
Series: d

First found in: not reported

Kind of sample: not reported

Producing strain: *Streptomyces sp.* DP94

Original report: Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541–1545.



Thiocillin I R_1 = H; R_2 = OH; R_3 = 

Thiocillin II $R_1 = CH_3$; R_2 = OH; R_3 = 

Thiocillin III $R_1 = CH_3$; R_2 = H; R_3 = 

Thiocillin IV $R_1 = CH_3$; R_2 = H; R_3 = 

Series: d

First found in: not reported

Kind of sample: not reported

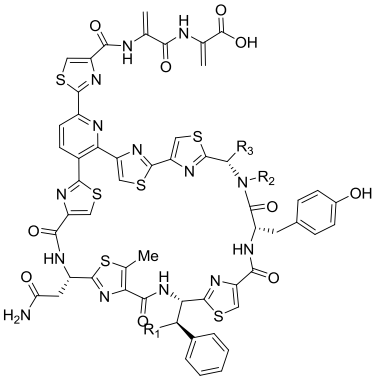
Producing strain: *Bacillus sp.*

Original report: a) Shoji, J.; Hinoo, H.; Wakisaka, Y.; Koizumi, K.; Mayama, M.; Matsuura, S.; Matsumoto, K. *J. Antibiot.* **1976**, *29*, 366–374; b) Shoji, J.; Kato, T.; Yoshimura, Y.; Tori, K. *J. Antibiot.* **1981**, *34*, 1126–1136.

Gene cluster identification: Wieland Brown, L. C.; Acker, M. G.; Clardy, J.; Walsh, C. T.; Fischbach, M. A. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2549–2553.

Structural peptide: SCTTCVCTCSCCTT

Total synthesis: Aulakh, V. S.; Ciufolini, M. A. *J. Am. Chem. Soc.* **2011**, *133*, 5900–5904.



Series: d

First found in: not reported

Kind of sample: nor reported

Producing strain: *Nonomuraea sp.* Bp3714-39

Original report: Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

Gene cluster identification: Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

Structural peptide: SCNCFCYICCSCSSA

Thiomuracin A R_1 = OH; R_2 = H; R_3 = 

Thiomuracin B R_1 = OH; R_2 = H; R_3 = 

Thiomuracin C R_1 = OH; R_2 = H; R_3 = 

Thiomuracin D R_1 = H; R_2 = H; R_3 = 

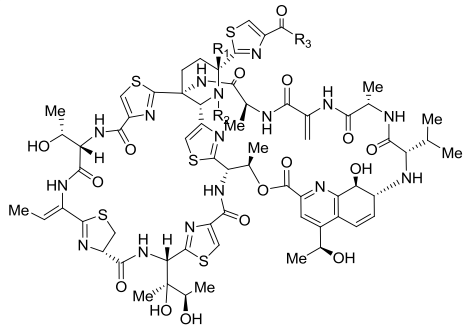
Thiomuracin E R_1 = OH; R_2 = H; R_3 = 

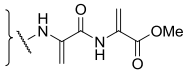
Thiomuracin F R_1 = OH; R_2 = H; R_3 = 

Thiomuracin G R_1 = OH; R_2 = H; R_3 = 

Thiomuracin H R_1 = OH; R_2 = H; R_3 = 

Thiomuracin I R_1 = OH; R_2 =  R_3 = 

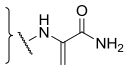


Thiopeptin A1a $R_1 = R_2 = H$; $R_3 =$ 

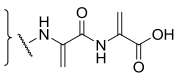
Thiopeptin A1b $R_1/R_2 = \pi$-bond; $R_3 =$

Thiopeptin A3a $R_1 = R_2 = H$; $R_3 = NH_3$

Thiopeptin A3b $R_1/R_2 = \pi$-bond; $R_3 = NH_3$

Thiopeptin A4a $R_1 = R_2 = H$; $R_3 =$ 

Thiopeptin A4b $R_1/R_2 = \pi$-bond; $R_3 =$

Thiopeptin Ba $R_1 = R_2 = H$; $R_3 =$ 

Thiopeptin Bb $R_1/R_2 = \pi$-bond; $R_3 =$

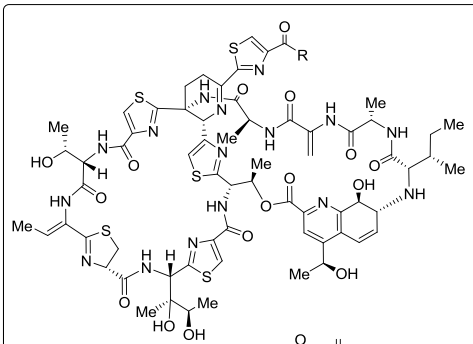
Series: a and b

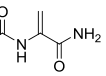
First found in: Tateyama, Toyama Prefecture, Japan

Kind of sample: soil

Producing strain: *Strptomyces tateyamensis*

Original report: a) Miyairi, N.; Miyoshi, T.; Aoki, H.; Kohsaka, M.; Ikushima, H.; Kunugita, K.; Sakai, H.; Imanaka, H. *J. Antibiot.* **1970**, *23*, 113–119, b) Miyairi, N.; Miyoshi, T.; Aoki, H.; Kohsaka, M.; Ikushima, H.; Kunugita, K.; Sakai, H.; Imanaka, H. *Antimicrob. Agents Chemother.* **1972**, *1*, 192–196; c) Mine, K.; Miyairi, N.; Takano, N.; Mori, S.; Watanabe, N. *Antimicrob. Agents Chemother.* **1972**, *1*, 496–503.



Thiostrepton A R = 

Thiostrepton B R = NH_2

Series: b

First found in: not reported

Kind of sample: soil

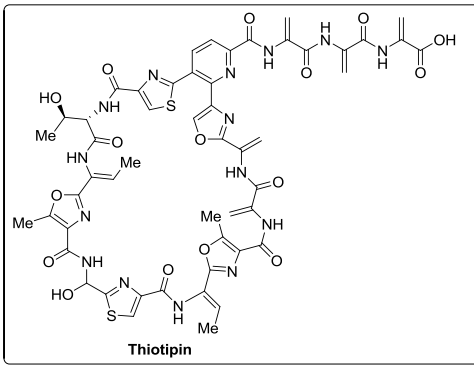
Producing strains: *Streptomyces azureus* / *S. laurentii* / *S. hawaiiensis*

Original report: a) Pagano, J. F.; Weinstein, M. J.; Stout, H. A.; Donovick, R. *Antibiot. Ann.* **1955**, *3*, 554-559; b) Vandeputte, J.; Dutcher, J. D. *Antibiot. Ann.* **1955**, *3*, 560–561; c) Steinberg, B. A.; Jambor, W. P.; Suydam, L. O.; Soriano, A. *Antibiot. Ann.* **1955**, *3*, 562–565.

Gene cluster identification: Kelly, W. L.; Pan, L.; Li, C. J. Am. Chem. Soc. 2009, 131, 4327–4334.

Structural peptide: IASASCTTCICTCSCSS

Total synthesis: a) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5087–5092; b) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5092–5097; c) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Lee, S. H.; Nevalainen, M.; Bella, M.; Estrada, A. a; Funke, C.; Zécri, F. J.; Bulat, S. *J. Am. Chem. Soc.* **2005**, *127*, 11159–11175; d) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Estrada, A. a; Lee, S. H.; Nevalainen, M. *J. Am. Chem. Soc.* **2005**, *127*, 11176–11183; e) Nicolaou, K. C. *Angew. Chem. Int. Ed.* **2012**, *51*, 12414–12436.



Thiotipin

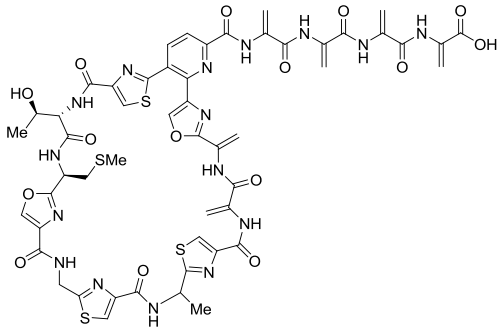
Series: d

First found in: mycelium of *Streptomyces sp.* DT31

Kind of sample: mycelium

Producing strain: *Streptomyces sp.* DT31

Original report: Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *Tetrahedron* **1994**, *50*, 11659–11664.



Thioxamycin

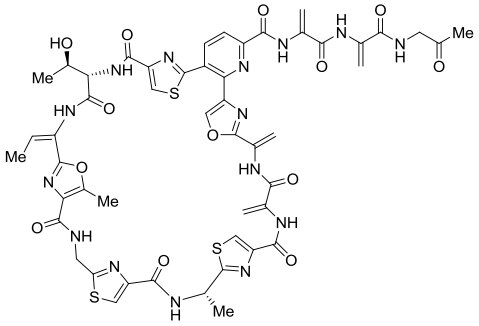
Series: d

First found in: not reported

Kind of sample: not reported

Producing strain: *Streptomyces sp.* PA-46025 / DP94

Original reports: a) Matsumoto, M.; Kawamura, Y.; Yasuda, Y.; Tanimoto, T.; Matsumoto, K.; Yoshida, T.; Shoji, J. *J. Antibiot.* **1989**, *42*, 1465–1469; b) Yun, B.-S.; Hidaka, T.; Furihata, K.; Seto, H. *J. Antibiot.* **1994**, *47*, 1541–1545.



TP-1161

Series: d

First found in: Trondheim Fjord, Norway

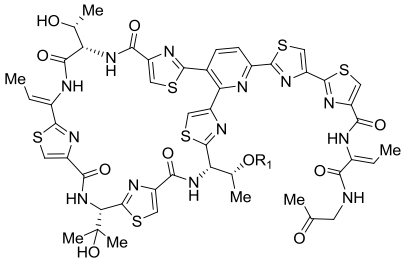
Kind of sample: marine sediment

Producing strain: *Nocardiopsis sp.*

Original report: Engelhardt, K.; Degnes, K. F.; Kemmler, M.; Bredholt, H.; Fjaervik, E.; Klinkenberg, G.; Sletta, H.; Ellingsen, T. E.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 4969–4976.

Gene cluster identification: Engelhardt, K.; Degnes, K. F.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 7093–7101.

Structural peptide: SCTTSGCACSSSSSS



YM-266183 $R_1 = H$

YM-266184 $R_1 = CH_3$

Series: d

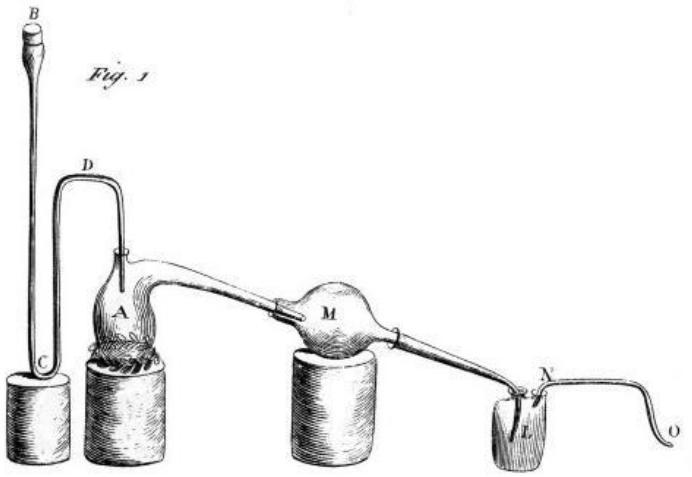
First found in: Hoshisuna Beach, Iriomote Island, Okinawa Prefecture, Japan

Kind of sample: marine sponge

Producing strain: *Bacillus cereus* QN3323

Original report: a) Nagai, K.; Kamigiri, K.; Arao, N.; Suzumura, K.-I.; Kawano, Y.; Yamaoka, M.; Zhang, H.; Watanabe, M.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 123–128; b) Suzumura, K.-I.; Yokoi, T.; Fanatsu, M.; Nagai, K.; Tanaka, K.; Zhang, H.; Suzuki, K. *J. Antibiot.* **2003**, *56*, 129–134.

Contribution to Publications



CONTRIBUTION TO PUBLICATIONS

- "*Highly efficient, multigram and enantiopure synthesis of 2-(2,4'-bithiazol-2'-yl)pyrrolidine*". Xavier Just-Baringo, Paolo Bruno, Fernando Albericio and Mercedes Álvarez (*Tetrahedron Lett.* **2011**, *52*, 5435-5437).

  Design of synthetic plan. Testing of alternative strategies. Synthetic development and scale-up of products described herein. Characterization of products. Writing of the manuscript.

- "*Total synthesis of aeruginazole A*". Paolo Bruno, Stella Peña, Xavier Just-Baringo, Fernando Albericio and Mercedes Álvarez (*Org. Lett.* **2011**, *13*, 4648-4651).

  Design of synthetic plan. Development of methodology used for the synthesis of amino acid-derived building blocks.

- "*From 2,6-dichloronicotinic acid to thiopeptide cores*". Xavier Just-Baringo, Fernando Albericio and Mercedes Álvarez (*Eur. J. Org. Chem.* **2013**, ACCEPTED).

  Design of synthetic plan. Testing of all attempted strategies. Synthesis and characterization of all compounds reported. Writing of the manuscript.

- "*Total synthesis and stereochemical assignment of baringolin*". Xavier Just-Baringo, Paolo Bruno, Lars K. Ottesen, Librada M. Cañedo, Fernando Albericio, and Mercedes Álvarez (*Angew. Chem. Int. Ed.* **2013**, *52*, 7818-7821).

  Design of synthetic plan. Development of central fragment synthesis. Scale-up of the synthesis of all fragments. Assembling of fragments, macrocyclization studies and final steps. Characterization of compounds. *In vitro* testing of synthetic baringolin. Writing of the manuscript.

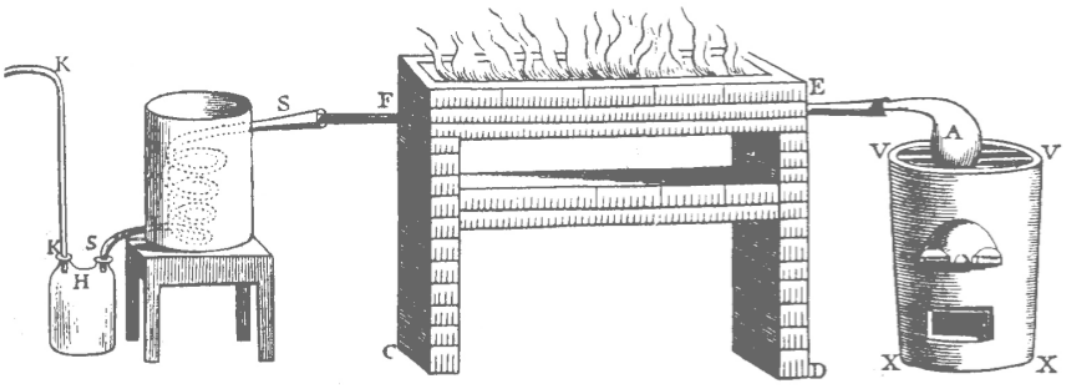
- "*Thiopeptide engineering: a multidisciplinary effort towards future drugs*". Xavier Just-Baringo, Fernando Albericio, and Mercedes Álvarez (**2013**, SUBMITTED).

  Choosing of the topic. Writing of the review.

- "*Dissecting the structure of thiopeptides: assessment of thiazoline and tail moieties of baringolin and antibacterial activity optimization*". Xavier Just-Baringo, Paolo Bruno, Cristina Pitart, Jordi Vila, Fernando Albericio and Mercedes Álvarez (**2013**, SUBMITTED).

  Design of analogues and synthetic plan. Synthesis of all analogues but the peptidic tail capped with thiazole. Solubility determination. Characterization of compounds. Writing of the manuscript.

The Naming of Baringolin



THE NAMING OF BARINGOLIN

Giving a chemical compound its name can be done in many ways. They can be company codes, but also endless systematic scary combinations of numbers, prefixes and suffixes. Sometimes they are functionality-based catchy names that remind us of their biological activity and in some other cases they are curious names that tell us about their origin, a plant, a microorganism, etc. Each name has its use and often molecules have too many of the former.

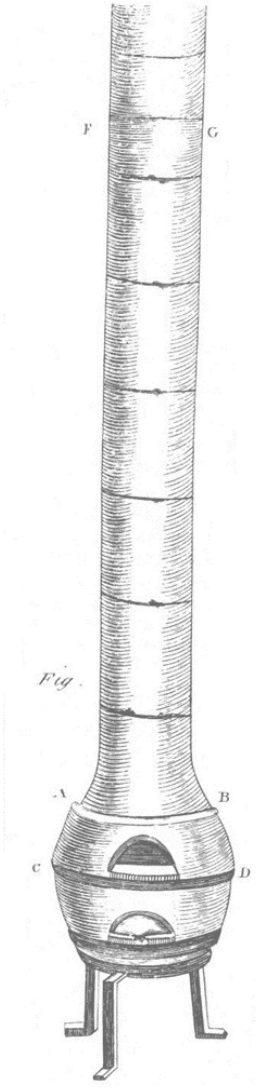
The naming of a new molecule, the one this entire thesis spins around, does not fit much to any of the above mentioned cases. It was nothing but quick. In less than the blink of an eye a specific array of atoms in space could be referred to with a name, baringolin. The memory of that precise moment comes back easily to my mind, is one of those moments that deserve a privileged place in one's thoughts. Back then, all synthetic work had been already done, all intermediates had been characterized; however, we still referred to the compound with its original company code "AD0037". I was writing the manuscript, much in the same way that I do write this text now, in front of my laptop, at the office. But then Mercedes came, she told me about many things, too fast for me to understand, still awakening from my own thoughts. She was asking me about the manuscript; there were many things to be arranged. Then she said "Some journals do not accept company codes for compounds, have you thought of a name?" At that precise moment time stopped. All my five senses focused back into my own thoughts, a much deeper dream than that I had just awaken from…

My thoughts settled, but a long time back in time, almost ten years and a half ago. Back then my mind was in a much worst savage turmoil. It was August 2002, I was about to start a degree in chemistry at university, only a few weeks were left and some paperwork still had to be done. Nonetheless, it was nothing when compared to the real burden. Just a few weeks ago, my mother, Maribel Baringo, had passed away after long years of fight against cancer. So many thoughts were spinning around my mind those days. Some chaotic, others clearer. One thing I was certain about, I would pay her homage if possible. In my still innocent thoughts of an 18-year old teen I decided that whenever my future chemistry education brought me a chance to name a molecule, whatever it might be, I would name it "baringolina", in my mother's memory. Years later that thought was almost forgotten; the realization of how chemistry and the world worked convinced me of the ingenuity of that idea. However, all this had come back to my mind as fast as lightning after Mercede's question "Have you thought of a name?"

Of course I had. I blushed, I looked down and I said "Yes", then felt even smaller. She asked for the name and all that came out was "baringolina" with a thin and timid voice. Surprisingly, she liked the name and so she told me. I never told her about my reasons for choosing it, but when I asked, she made clear that that would end up being its final name. She was right and I find it hard to think of the words necessary to say how much grateful I am.

Sometimes true dreams, those that are dreamed awake, become true.

Resum en Català



RESUM EN CATALÀ

1. La crisi dels antibiòtics

Des de fa dècades el perill de no disposar d'antibiòtics eficients per combatre infeccions que eren tractables en el passat ha anat creixent per culpa de la creació de resistències. Després de l'aparició dels primers antibiòtics, les estratègies utilitzades per tal de combatre aquest problema s'han anat modificant al llarg del temps. En l'època daurada dels antibiòtics, entre els anys quaranta i seixanta, el descobriment de nous antibiòtics semblava una font inesgotable de nous fàrmacs, però posteriorment s'hagué de recórrer a la síntesi d'anàlegs per tal de combatre la ineficiència dels vells compostos. Aquesta estratègia però, va començar a mostrar les seves mancances durant les dècades més recents, quan semblava que es perdia la batalla. Aquest fet va promoure la cerca de nous antibiòtics, els quals devien actuar mitjançant nous mecanismes per tal d'evitar resistències creuades i poder eliminar l'amenaça d'aquelles infeccions que havien estat tractades eficaçment amb anterioritat.

Des de l'any 2000 s'han descobert nous tipus d'antibiòtics que compleixen la condició d'actuar sobre noves dianes i que han fomentat un renovat interès en el desenvolupament de nous agents antimicrobians (Figura 1).[1] Entre les noves famílies d'antibiòtics hi podem trobar les oxazolidinones (linezolid, 2000), els lipopèptids (daptomicina, 2003) i les mutilines (retapamulina, 2007). A més, tot i que encara no han arribat a ser comercialitzats, estan en fases avançades de desenvolupament els lantibiòtics[2] (NVB302) i els tiopèptids[3] (LFF571)[4].

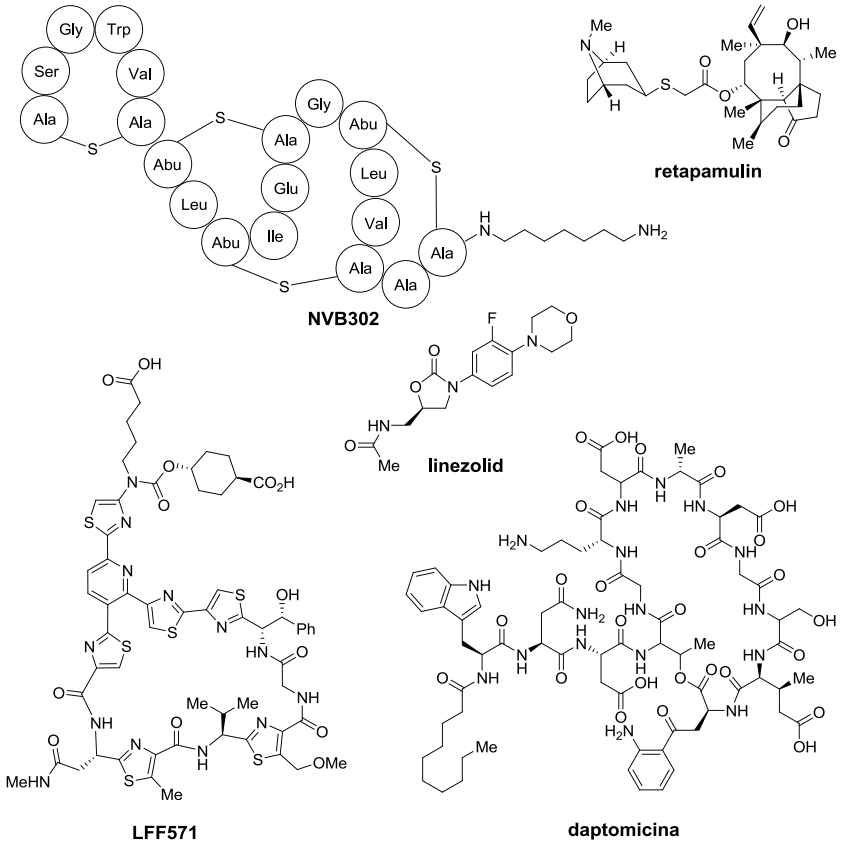


Figura 1. Membres de les noves classes d'antibiòtics. Abu = àcid dehidrobutíric.

Recentment, els avenços assolits en l'estudi dels tiopèptids han despertat un gran interès per aquests antibiòtics. La consecució de la síntesi total d'alguns dels seus membres,[5] la identificació del clúster de gens que en codifica l'estructura i regula la seva biosíntesi[6] i la obtenció d'anàlegs que superen alguns dels inconvenients dels productes naturals són fruit dels grans esforços destinats al seu estudi.

2. Tiopèptids

2.1. Estructura i biosíntesi

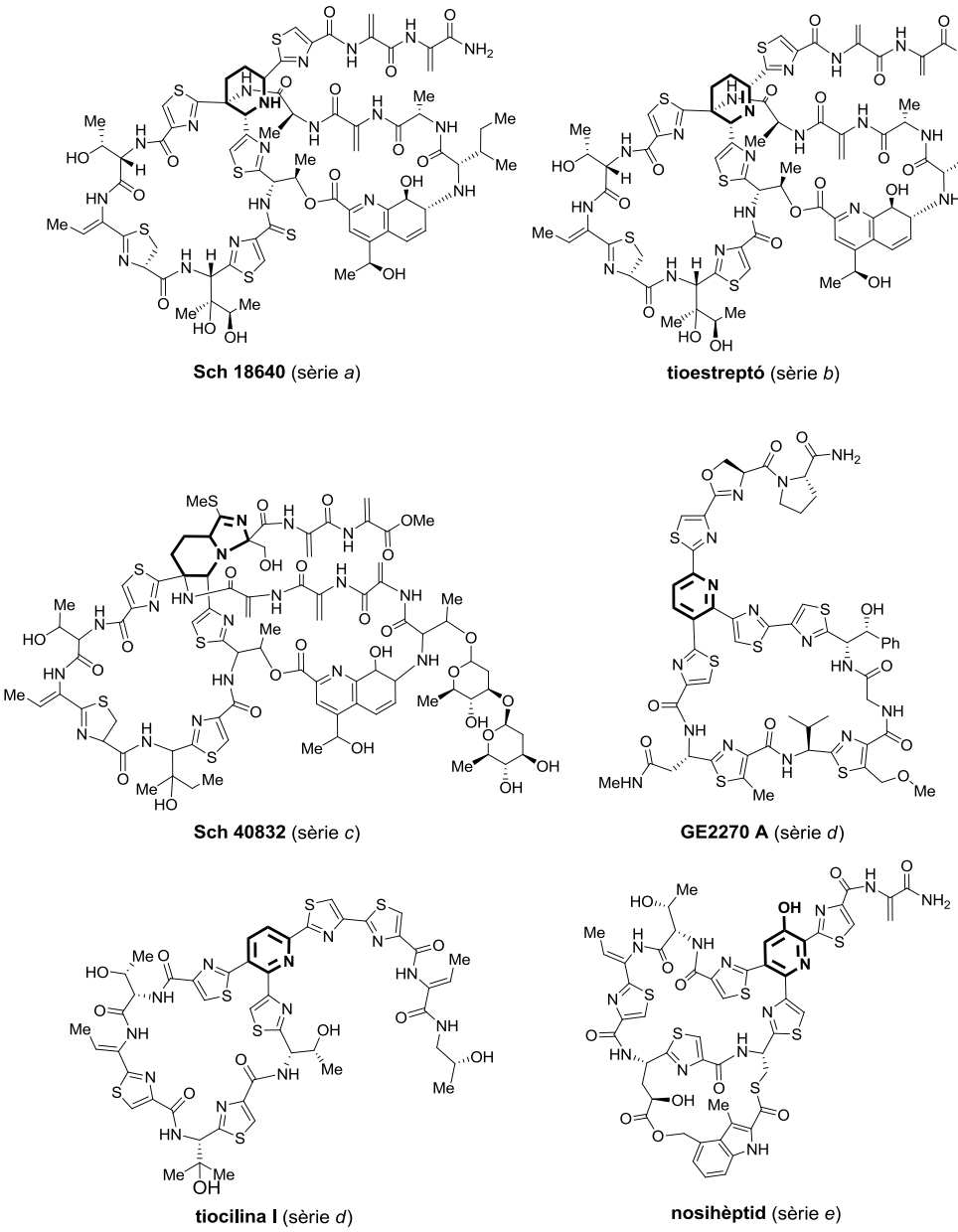
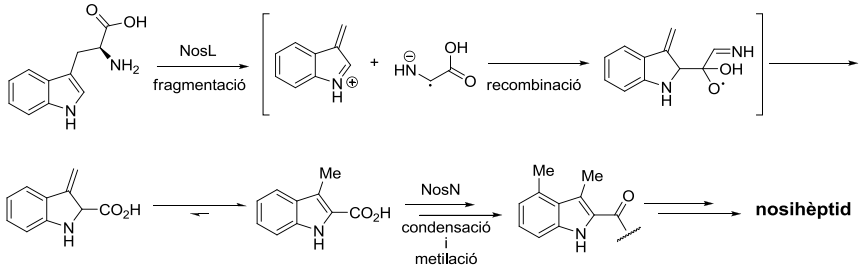


Figura 2. Classificació dels antibiòtics ens diferents sèries.

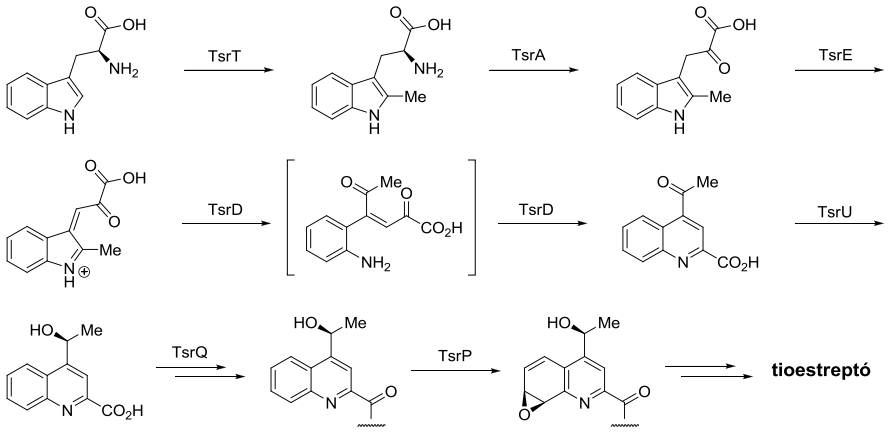
Els tiopèptids són compostos derivats de la modificació exhaustiva d'un pèptid precursor amb alt contingut de sofre en forma de residus de cisteïna.[3] La principal característica de la seva estructura és un anell central nitrogenat de sis membres que serveix de punt d'ancoratge per a almenys un macrocícle i una cua. A més s'hi troben diversos motius recurrents, com oxazols i

tiazols i dehidroamino àcids. En funció del grau d'oxidació de l'anell central els tiopèptids es classifiquen en diferent sèries (Figura 2); així, aquells amb un anell de piperidina pertanyen a la sèrie *a*, mentre que aquells amb un anell de 1,2-dehidropiperidina cauen en la sèrie *b*; en la sèrie *c* hi trobem un sol membre, Sch 40832, el qual té un motiu central de hexahidro-imidazo[1,5-*a*]piridina. La sèrie *d* és la més nombrosa i la formen els tiopèptids amb una pirididina central trisubstituida, mentre que els de la sèrie *e* tenen una hidroxipiridina tetrasubstituida.



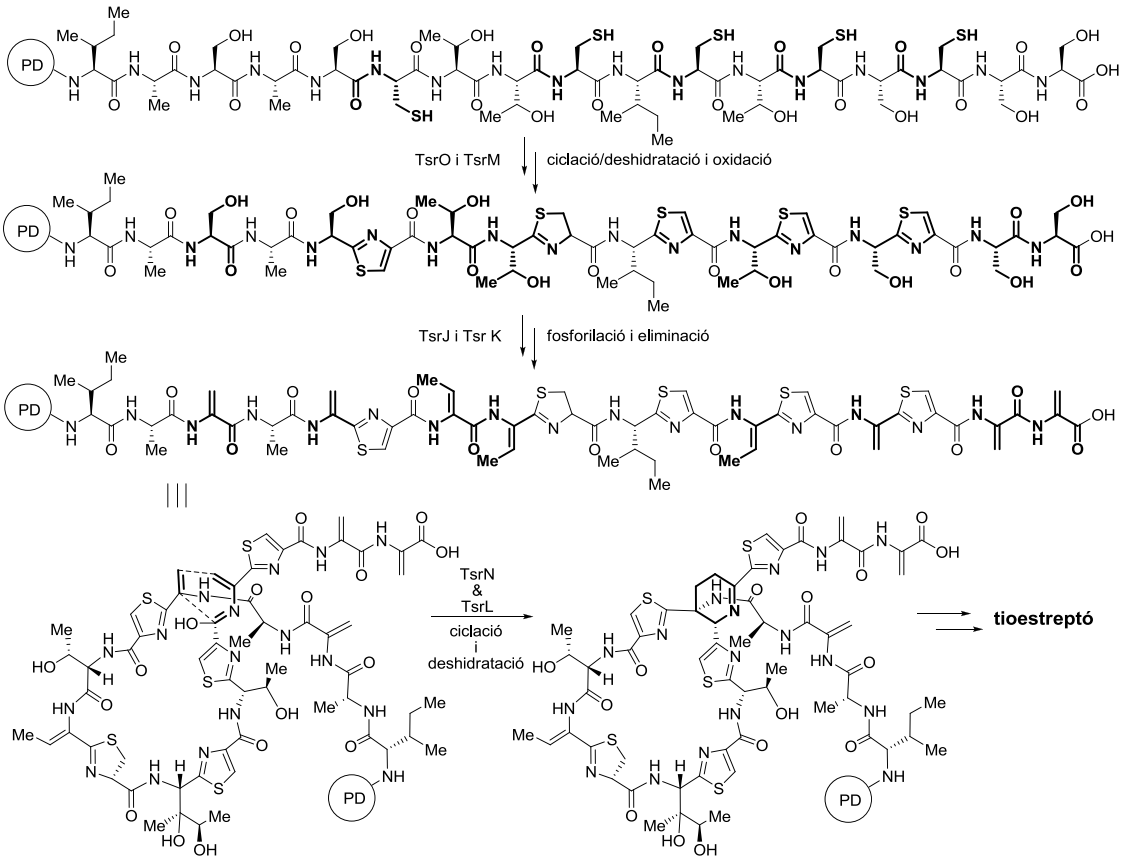
Esquema 1. Biosíntesi de l'àcid indòlic a partir de L-triptòfan i incorporació en el nosihèptid.

Pel que fa als macrocícles, totes les sèries en tenen dos, menys la *d*, els membres de la quan en tenen un de sol. Els tiopèptids de les sèries *a*, *b* i *c* contenen una unitat d'àcid quinàldic en el segon macrocícle, mentre que els de la sèrie *e* el tenen format per una unitat d'àcid 4-metilindòlic. En tots dos casos, tant l'àcid quinàldic, com l'acid indòlic es formen a partir de la modificació del triptòfan (Esquemes 1 i 2).[7,8]

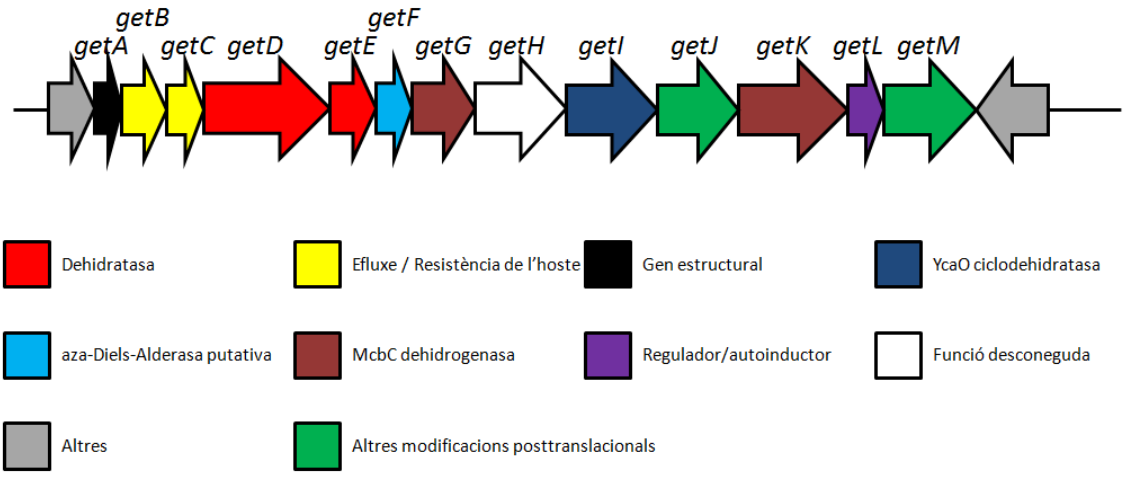


Esquema 2. Biosíntesi de l'àcid quinàldic a partir de L-tryptophan i incorporació en el tioestreptó.

Tot i les diferències que apareixen entre membres de les diferents sèries, els tiopèptids, segons es pot concloure dels resultats presentats en diferents estudis,[6,9,10] comparteixen una ruta biosintètica comú. Així, un pèptid precursor que es troba codificat genèticament és sintetitzat en el ribosoma i és posteriorment modificat fins a formar el tiopèptid (Esquema 3). En primer lloc, es formen els anells de oxazol i tiazol o tiazolina mitjançant un procés de ciclació/eliminació/oxidació o de ciclació/eliminació, en el cas dels anells de tiazolina. Posteriorment, la deshidratació de serines i treonines forma els característics dehidroamino àcids. L'últim punt comú per a tots els tiopèptids és la formació de l'anell cenral, la qual té lloc via una cicloaddició entre diferents dehidroalanines.



Esquema 3. Ruta biosintètica del tioestreptó. PD = pèptid director.



Pèptid prescursor: MGNNEEYFIDVNDLSIDVFDVVEQGGAVTALTADHGMPEVGASTNCFCYICCSCSSN

−42 +1 −1 +12

Figura 3. Clúster genètic de GE37468 i seqüència del pèptid precursor. El pèptid estructural apareix numerat en positiu i el pèptid director en negatiu. Els residus que acaben formant part del tiopèptid aparixen subratllats.

L'esmentat clúster genètic codifica tant el pèptid precursor, com la maquinària enzimàtica necessària per a la seva modificació. Aquest pèptid precursor està clarament dividit en dues regions, el pèptid estructural, el qual acaba formant part del tiopèptid, i el pèptid director, que

es va perdent al llarg del procés biosintètic. Diversos clústers genètics han estat identificats fins a dia d'avui.[11–19] Per tal d'exemplificar-los, a la figura 3 s'hi pot veure l'esquema del clúster genètic corresponent al tiopèptid GE37468A.

2.2. Activitat biològica

L'activitat biològica principal dels tiopèptids és l'antibiòtica, la qual exerceixen mitjançant la inhibició de la síntesi proteica en bacteris Gram positius. En funció de la mida del macrocícle s'han identificat diferents dianes. Així, els tiopèptids amb macrocícles de 26 membres inhibeixen el factor d'elongació G, mentre que els que tenen macrocícles amb un residu més, és a dir, de 29 membres, inhibeixen el factor d'elongació Tu. També existeixen tiopèptids amb macrocícles de 35 membres, però en aquest cas la seva diana biològica és desconeguda.

Malgrat el gran interès per la seva activitat antibiòtica, en alguns casos se n'han mostrat d'altres molt variades i de gran interès. Així, el tioestreptó ha estat estudiat extensivament per la seva activitat antitumoral, la qual exerceix mitjançant la inhibició del factor de transcripció FOXM1, el qual se sobreexpressa en diverses cèl·lules tumorals.[20–25] A més, diferents tiopèptids han estat identificats amb d'altres activitats, tals com antimalàrica,[26,27] inhibició de la renina,[28] immunosupressora,[29] antifúngica,[30,31] inhibidora de RNA polimerasa,[32] etc.

2.3. Síntesi química

Durant les últimes dues dècades, diversos grups han fet grans esforços per aconseguir la síntesi total d'alguns tiopèptids. La mida i les arquitectures d'aquests compostos han estat un repte formidable gràcies al qual s'han desenvolupat diverses metodologies i estratègies sintètiques. Les parts més estudiades i que han estat el focus de més atenció són els fragments poliheterocíclics centrals. La síntesi d'aquests ha estat afrontada mitjançant dues estratègies bàsiques: la modificació d'un anell de piridina ja existent i la construcció de l'anell nitrogenat de sis membres.[5]

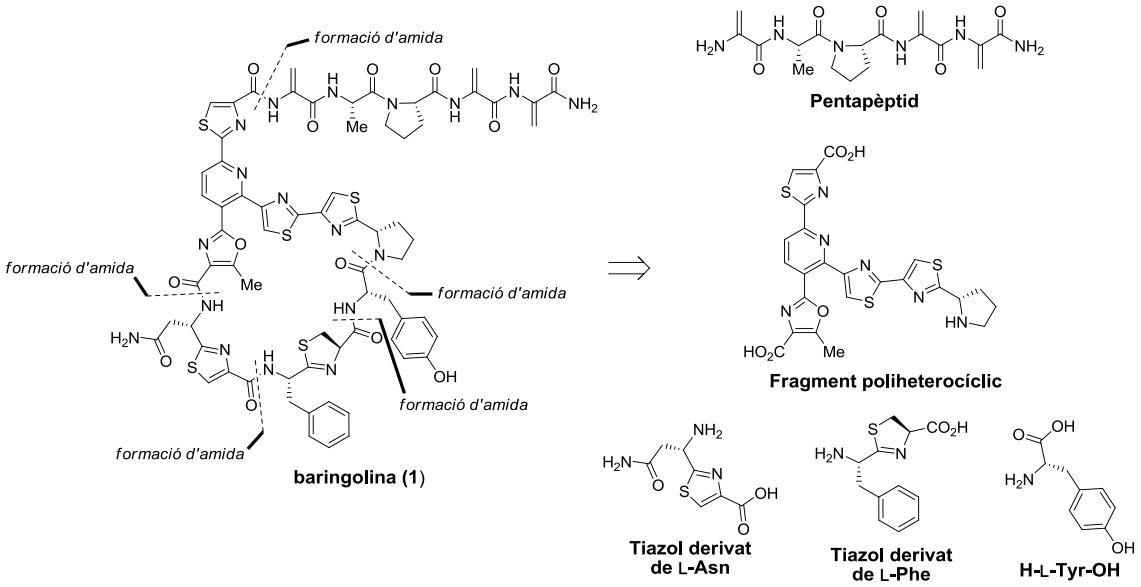
Les estratègies basades en la modificació d'un anell de piridina prèviament funcionalitzat han estat, en la gran majoria de casos, estretament vinculades a la utilització de reaccions d'acoblament creuat catalitzades per pal·ladi. Així, han estat rellevants les aportacions de Kelly,[33–35] Shin,[36–38] Bach[39,40] i Hoarau.[41,42] D'altra banda, la formació *de novo* de l'anell central ha permès el desenvolupament d'una major varietat d'aproximacions. Les més destacades són la utilització de diferents reaccions biomimètiques desenvolupades paral·lelament pels grups de Moody[43,44] i Nicolaou,[45–47] la ciclació desenvolupada per Ciufolini,[48,49] la reacció de Bohlmann-Rahtz,[50–53] la utilització de una reacció de aza-Diels-Alder amb una desconnexió alternativa a la biomimètica,[54] la formació de una piperidina central per una reacció d'expansió d'anell[55,56] o la més recent, una cicloaddició [2+2+2] catalitzada per ruteni.[57]

A més de ser l'excusa perfecte per al desenvolupament de metodologia sintètica, la síntesi total de tiopèptids ha estat important per a l'assignació definitiva de l'estructura d'alguns d'aquests, ja que en la majoria de casos no ha estat possible la obtenció de cristalls.

3. Objectius de la present tesi

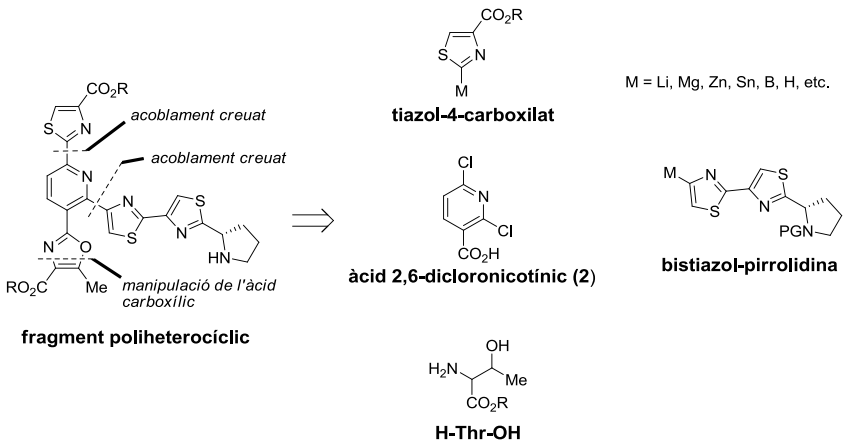
En el context presentat anteriorment, la empresa Biomar S.A. va aïllar un nou membre de la família dels tiopèptids, la baringolina (**1**), amb interessant activitat antibiòtica front a bacteris Gram positiu, la quan va ser caracteritzada únicament per estudis de ressonància magnètica,

sense cap mena d'assignació de l'estereoquímica (Esquema 4).[58] Per tal de confirmar la seva estructura i dur a terme un programa d'anàlegs ens varem proposar de desenvolupar una nova estratègia sintètica que permetés la seva síntesi total, alhora que facilités la introducció de diferents modificacions. Així, **1** es dividí en cinc fragments, el poliheterocicle central, el pentapèptid amb residus de dehidroalanina, i tres fragments més per completar el macrocícle, per una banda un tiazol derivat d'asparagina, una tiazolina derivada de fenilalanina i, per últim, tirosina (Esquema 4).



Esquema 4. Anàlisi retrosintètic de la baringolina.

D'altra banda, el fragment poliheterocíclic central també es dividí en d'altres sintons que permetessin la utilització d'una estratègia basada en reaccions d'acoblament creuat amb pal·ladi. Com a piridina de partida ens varem inclinar per l'àcid 2,6-dicloronicotínic, que té una funcionalització adequada per a la introducció dels tres azols necessaris (Esquema 5). Així, a part de l'àcid 2,6-dicloronicotínic són necessaris també un tiazol-4-carboxilat, un fragment de bistiazol-pirrolidina i treonina per a construir l'anell d'oxazol.



Esquema 5. Anàlisi retrosintètic del fragment poliheterocíclic central de la baringolina.

Per tal de poder dur a terme la síntesi total i accedir a la posterior síntesi d'anàlegs, primer cal posar a punt la síntesi dels fragments necessaris. Tot plegat dóna lloc a una sèrie

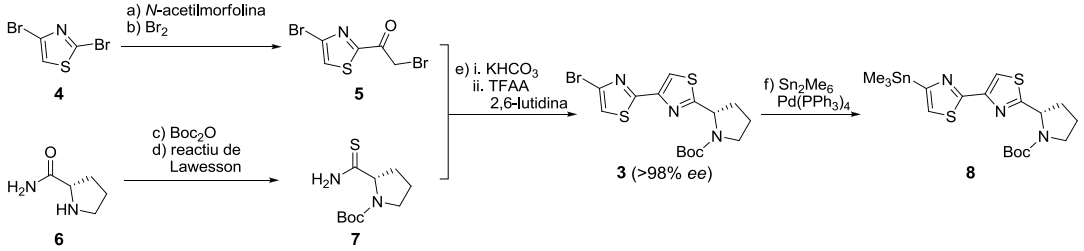
d'objectius: La síntesi dels tiazols i la tiazolina derivats d'amino àcids, la posada a punt de la metodologia necessària per a la construcció del fragment poliheterocíclic, la síntesi del pentapèptid amb dehidroalanines i l'ensamblatge de tots els fragments i dissenyar i sintetitzar una llibreria d'anàlegs accessibles mitjançant la mateixa estratègia sintètica.

4. Síntesi de tiazols i tiazolina derivats d'amino àcids

La primera tasca a afrontar és la síntesi dels tiazols i la tiazolina derivats d'amino àcids. En total aquests fragments en són tres: el de bistiazol-pirrolidina, tiazol-asparagina i tiazolina-fenilalanina. Donades les diferències entre els tres fragments, cadascún requereix un mètode diferent de preparació.

4.1. Síntesi d'un fragment de bistiazol-pirrolidina

Per tal de compatibilitzar la síntesi del fragment de bistiazol-pirrolidina present en l'estructura de **1** amb una síntesi del fragment poliheterocíclic central basada en reaccions d'acoblament creuat amb pal·ladi, es fa necessària la correcta funcionalització del bisaril. Així, el primer objectiu era sintetitzar el bistiazol **3**, adequadament bromat en la posició 4 i amb l'amina protegida amb Boc (Esquema 6). Davant la impossibilitat d'aplicar els mètodes descrits en publicacions prèvies,[59,60] per tractar-se el nostre compost objectiu d'un derivat d'una amina secundària, va ser necessari buscar una alternativa. Així, per tal d'obtenir **3** es varen utilitzar condicions modificades de la síntesi de tiazols de Hantzsch[61] per tal de formar l'anell central (Esquema 6).[62] Per tal d'obtenir els productes necessaris es va partir de 2,4-dibromotiazol (**4**), el qual es va acetilar i bromar per obtenir la bromocetona **5**. D'altra banda, la prolinamida (**6**) es va protegir i convertir en la corresponent tioamida **7**. Amb tots dos compostos, **5** i **7**, preparats, es dugué a terme la reacció de Hantzsch en dues etapes per tal d'obtenir **3** sense epimerització del centre quiral. Un cop obtingut, es pogué transformar en el corresponent derivat de trimetilestany **8**, permetent així la utilització posterior en un l'acoblament creuat de Stille.

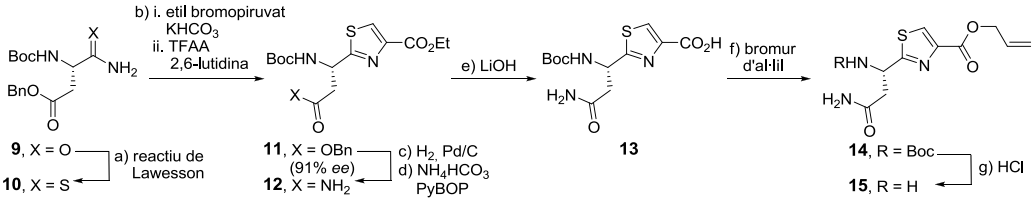


Esquema 6. Síntesi dels fragments de bistiazol-pirrolidina **3** i **8**. Reactius i condicions: a) i. *n*BuLi, THF, −78 °C. ii. *N*-acetilmorfolina, −78 °C, 67%; b) Br_2, HBr, AcOH, t.a., 80%; c) $(Boc)_2O$, H_2O, 1,4-dioxà, t.a., quant.; d) reactiu de Lawesson, THF, t.a., quant.; e) i. $KHCO_3$, DME, 0 °C; ii. TFAA, 2,6-lutidina, DME, −20 °C, 77%; f) Sn_2Me_6, $Pd(PPh_3)_4$, toluè, 100 °C, 89%.

4.2. Síntesi de tiazol-4-carboxilats derivats d'amino àcids

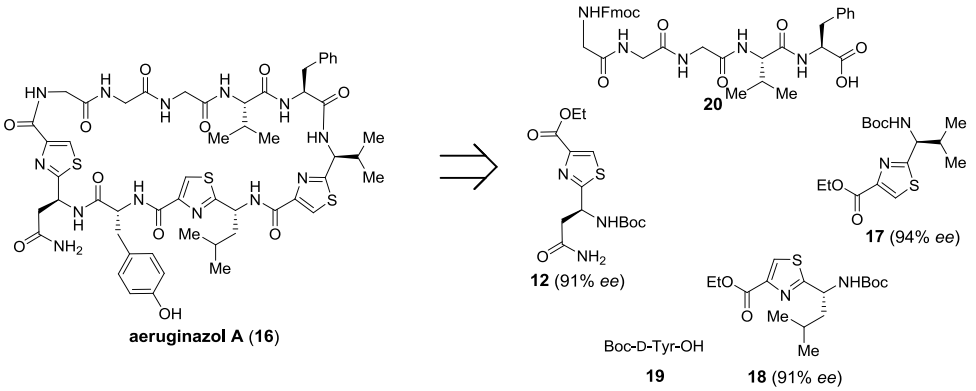
El segon tipus de fragment necessari per a la síntesi de la baringolina és el tiazol-4-carboxilat derivat d'asparagina. En aquest cas també es pot utilitzar la reacció de Hantzsch modificada per a evitar l'epimerització del centre quiral. A més, per tal d'evitar reaccions no desitjades en l'amida de la cadena lateral, es partí de l'àcid aspàrtic degudament protegit com a èster benzílic (**9**), el qual es podrà convertir en la corresponent amida primària més endavant. **9** es convertí fàcilment en **10** utilitzant el reactiu de Lawesson. En aquest punt es pogué dur a terme la reacció de Hantzsch modificada, tot i que amb una petita variació

respecte al procediment descrit.[61] Per tal d'evitar la trifluoroacetilació descrita en amines primàries protegides com a carbamats, sobre la mescla de reacció s'hi addicionà una solució aquosa en lloc de concentrar directament sota buit. D'aquesta manera s'evita una etapa extra de desprotecció, la qual hagués allargat la síntesi i hagués limitat els grups funcionals que s'haguessin pogut utilitzar. Així doncs, s'obtingué **11** amb un 91% d'excés enantiomèric i seguidament es convertí en **12** mitjançant la conversió de l'èster benzílic de la cadena lateral en l'amida primària desitjada. A continuació s'hidrolitzà l'èster etílic per obtenir l'àcid **13**, el qual es convertí en el corresponent èster al·lílic **14**. La desprotecció en medi àcid de **14** donà lloc a l'amina **15**.



Esquema 7. Síntesi del fragment de tiazol derivat d'asparagina **15**. Reactius i condicions: a) Reactiu de Lawesson, THF, t.a., 4 h, 97%; b) i. Etil bromopiruvat, $KHCO_3$, DME, 0 °C, 24 h; ii. TFAA, 2,6-lutidina, DME, −20 °C, 4 h, 75%; c) H_2, Pd/C, *i*PrOH, rt, 93%; d)NH_4CO_3, PyBOP, DIPEA, 1,4-dioxà, DMF, 0 °C, 68%; e) LiOH, THF, H_2O, t.a., quant.; f) bromur d'al·lil, $NaHCO_3$, DMF, t.a., 20 h, 83%; g) HCl, 1,4-dioxà, t.a., 4 h, quant.

Aprofitant el mètode millorat posat a punt i que evita la trifluoroacetilació durant la síntesi de tiazols de Hantzsch en dues etapes, es va decidir aplicar-lo per a la síntesi de aeruginazol A (**16**), un producte natural aïllat de fonts aquàtiques amb propietats antibiòtiques que conté tres unitats diferents de tiazol derivades d'amino àcids **12**, **17** i **18** (Esquema 8).[63] A més, **16** es pot dividir en altres fragments, tirosina (**19**) i un pentapèptid (**20**). **17** i **18** es van sintetitzar utilitzant el mateix procés descrit per a **12** i el pèptid **20** es va obtenir fàcilment per síntesi en fase sòlida. La condensació de tots els fragments **12** i **17**–**20** en solució va permetre l'obtenció d'aeruginazol A sintètic,[64] idèntic al natural, demostrant que l'assignació estructural original era correcta i que **16** conté amino àcids tant L com D.

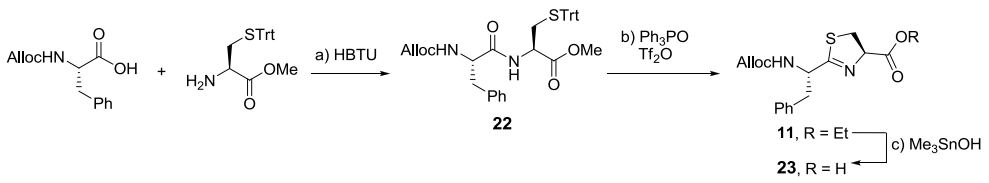


Esquema 8. Anàlisi retrosintètic d'aeruginazol A (**16**). L'excés enantiomèric dels productes obtinguts per síntesi de Hantzsch en dues etapes s'indica entre parètesi.

4.3. Síntesi d'un fragment de tiazolina derivada de fenilalanina

El fragment de tiazolina **21**, com és evident, no pot sintetitzar-se pel mètode de Hantzsch, però utilitzant metodologia descrita recentment és possible obtenir el producte desitjat a partir d'un dipèptid precursor **22** de fàcil preparació (Esquema 9).[65] Per tal d'obtenir l'àcid

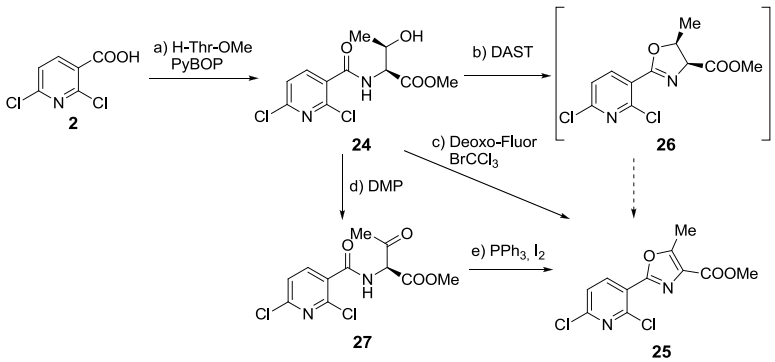
carboxílic lliure **23** sense afectar l'estereoquímica s'utilitzà hidròxid de trimetilestany.[66] Això és necessari donada la tendència a epimeritzar de les tiazolines en condicions tant àcides com bàsiques.



Esquema 9. Síntesi de la tiazolina **21**. Reactius i condicions: a) HBTU, DIPEA, CH_2Cl_2, t.a., 1 h, 94%; b) Ph_3PO, Tf_2O, CH_2Cl_2, −20 °C, 2 h, 86%; c) Me_3SnOH, CH_2Cl_2, 60 °C, 4 h.

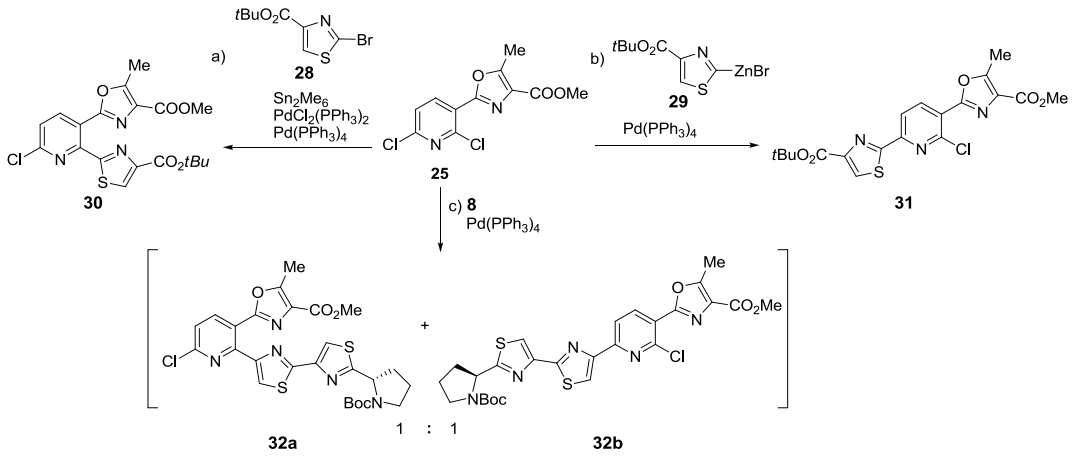
5. Síntesi del fragment poliheterocíclic central

Com s'ha indicat anteriorment, per a la síntesi del poliheterocícle central és va optar per començar des de l'àcid 2,6-dicloronicotínic (**2**). En primer lloc es va dur a terme la construcció de l'anell d'oxazol a partir de l'àcid carboxílic present en **2** (Esquema 10). En primer lloc, **2** es condensà amb l'èster metílic de treonina per formar **24**, el qual es va utilitzar per trobar la ruta més adient per la formació de l'oxazol **25**. En primer lloc es va optar per una via en dues etapes, començant per la ciclació de la treonina per formar la oxazolina **26** i posteriorment oxidar. Malauradament, **26** resultà poc estable i s'hi observà la formació de **24** tant bon punt s'havia purificat. Per tal de no requerir l'aïllament de **26**, s'optà per una ciclació/oxidació en una sola etapa, però d'aquesta manera s'obtingueren rendiments massa baixos per als nostres interessos. Així, s'optà per una tercera via, en la qual es dugué a terme en primer lloc la oxidació amb el periodinà de Dass-Martin per obtenir **27** i posteriorment es ciclà,[67] obtenint-se així **25** en un rendiment global excel·lent.



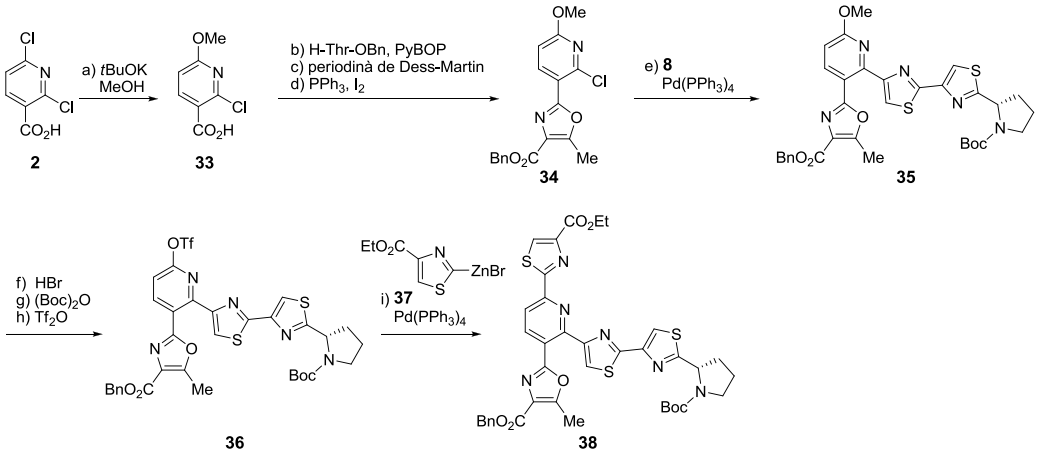
Esquema 10. Síntesi de l'oxazol **25**. Reactius i condicions: a) (L)-H-Thr-OMe, PyBOP, DIPEA, THF, 0 °C, 5 h, 94%; b) DAST, K_2CO_3, CH_2Cl_2, −78 °C to 0 °C, 7h, 61%; c) Deoxo-Fluor, $BrCCl_3$, DBU, CH_2Cl_2, 0 °C, 3.5 h, 39%; d) Periodinà de Dess-Martin, CH_2Cl_2, t.a., 6 h, 87%; e) PPh_3, I_2, Et_3N, CH_2Cl_2, 0 °C fins a t.a., 16 h, 94%.

Un cop s'obtingué **25**, es dugueren a terme diferent proves d'acoblament creuat per tal de trobar la millor estratègia possible. Així, es provaren diferents acoblaments de **25** amb **8**, **28** i **29** (Esquema 11). Tot i el gran nombre de proves realitzades, en cap cas s'obtingueren resultats satisfactoris. L'acoblament amb **28**, utilitzant el protocol d'estannació i acoblament *in situ* desenvolupat per Kelly,[35] donà baixos rendiments del regioisòmer no desitjat (**30**). D'altra banda, l'acoblament equivalent en condicions de Negishi, tot i donar lloc al regioisòmer desitjat (**31**), només va permetre obtenir el producte en quantitats irrisòries. L'acoblament amb **8**, tot i ser clarament un sistema molt més reactiu, no va presentar cap selectivitat i s'obtingueren els dos regioisòmers possibles (**32a** i **b**) en quantitats iguals.



Esquema 11. Proves d'acoblament creuat sobre **25**. Reactius i condicions: a) **28**, Sn_2Me_6, $PdCl_2(PPh_3)_2$, $Pd(PPh_3)_4$, 1,4-dioxà, 100 °C, 17%; **29**, $Pd(PPh_3)_4$, DMA, 60 °C, 5 dies, 3%; c) **8**, $Pd(PPh_3)_4$, 1,4-dioxà, 80 °C, 5 h, 51%.

Veient la manca de reactivitat i/o selectivitat, segons les condicions, obtinguda amb **25**, es va decidir d'utilitzar una via alternativa per tal d'obtenir una ruta sintètica eficient. Així, per tal d'evitar el problema de regioselectivitat es va optar per diferenciar les dues posicions α i α' de **2**.[68] La posició 6 de l'àcid 2,6-dicloronicotínic (**2**) es pot substituir selectivament amb metòxid per obtenir **33** i posteriorment formar l'oxazol **34** a partir de l'àcid carboxílic de forma totalment anàloga a la descrita amb anterioritat (Esquema 12). A partir d'aquest punt s'utilitzaren fragments iguals o anàlegs als presentats anteriorment per dur a terme reaccions d'acoblament creuat. En primer lloc s'acoblà **34** amb **8** mitjançant una reacció de Stille, i a continuació, la metoxipiridina obtinguda (**35**) es convertí en el corresponent triflat **36**. Per últim, l'acoblament de Negishi amb l'organozinc **37** donà lloc al fragment central **38** amb rendiment excel·lent.



Esquema 12. Síntesi del fragment central **38**. Reactius i condicions: a) *t*BuOK, MeOH, 65 °C, 4 dies, 85%; b) H-Thr-OBn, PyBOP, DIPEA, THF, 0 °C, 3 h, 89%; c) periodinà de Dess-Martin, CH_2Cl_2, t.a., 6 h, 95%; d) PPh_3, I_2, NEt_3, CH_2Cl_2, 0 °C to t.a., 15 h, 78%; e) **8**, $Pd(PPh_3)_4$, 1,4-dioxà, 80 °C, 48 h, 88%; f) HBr, AcOH, rt, 28 h, 73%; g) $(Boc)_2O$, NEt_3, CH_2Cl_2, 0 °C, 4 h, 94%; h) Tf_2O, 2,6-lutidina, DMAP, CH_2Cl_2, 0 °C to t.a., 3 h, 88%; i) **37**, $Pd(PPh_3)_4$, DMA, 45 °C, 1 h, quant.

Fent servir la mateixa estratègia utilitzada per a l'obtenció de **38**, el fragment que finalment servirà per a la síntesi de la baringolina, se sintetitzaren diversos anàlegs (**39**–**43**), incloent variacions dels grups protectors en el fragment central de la baringolina per fer proves de

desprotecció (**44**–**46**) (Figura 4). Amb cap dels fragments centrals amb variacions dels grups protectors s'aconseguí un resultat satisfactori de desprotecció selectiva i per aquest motiu s'acabà utilitzant **38** per a les etapes posteriors de la síntesi total.

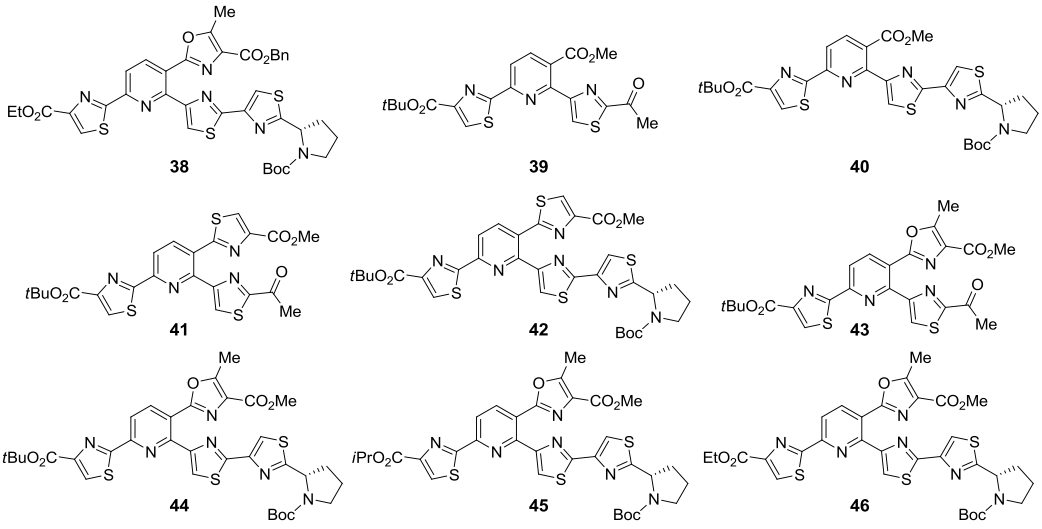
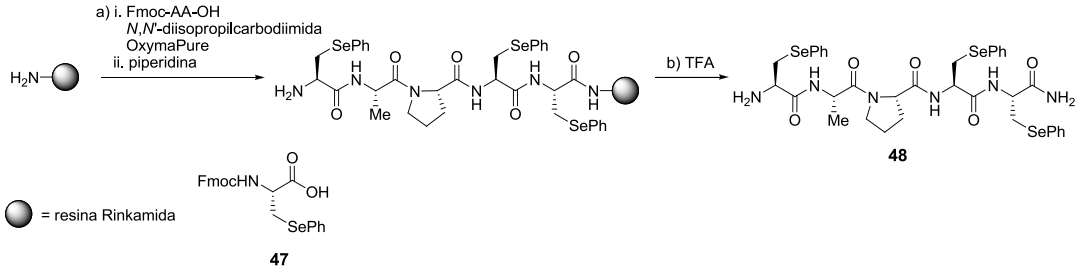


Figura 4. Fragment central de la baringolina i anàlegs sintetitzats (**38**), anàlegs sintetitzats (**39**–**43**) i variacions dels grups protectors (**44**–**46**).

6. Síntesi del pentapèptid de la cua

L'últim dels fragments necessaris per poder afrontar les últimes etapes de la construcció de la baringolina és la síntesi del pèptid de la cua. Per tal d'obtenir-lo de la forma més eficient possible i aprofitar l'experiència del grup, es va sintetitzar en fase sòlida. Per tal de poder introduir el pèptid a la molècula i generar els residus de dehidroalanina, s'utilitzà fenilselenocisteïna adequadament protegida (**47**) com a precursora d'aquests residus insaturats.[69] Utilitzant OxymaPure[70] com a additiu pels acoblaments i la resina Rinkamida ChemMatrix[71] s'obtinguè el pèptid **48** amb l'amida C-terminal amb excel·lents rendiment i puresa (Esquema 13).

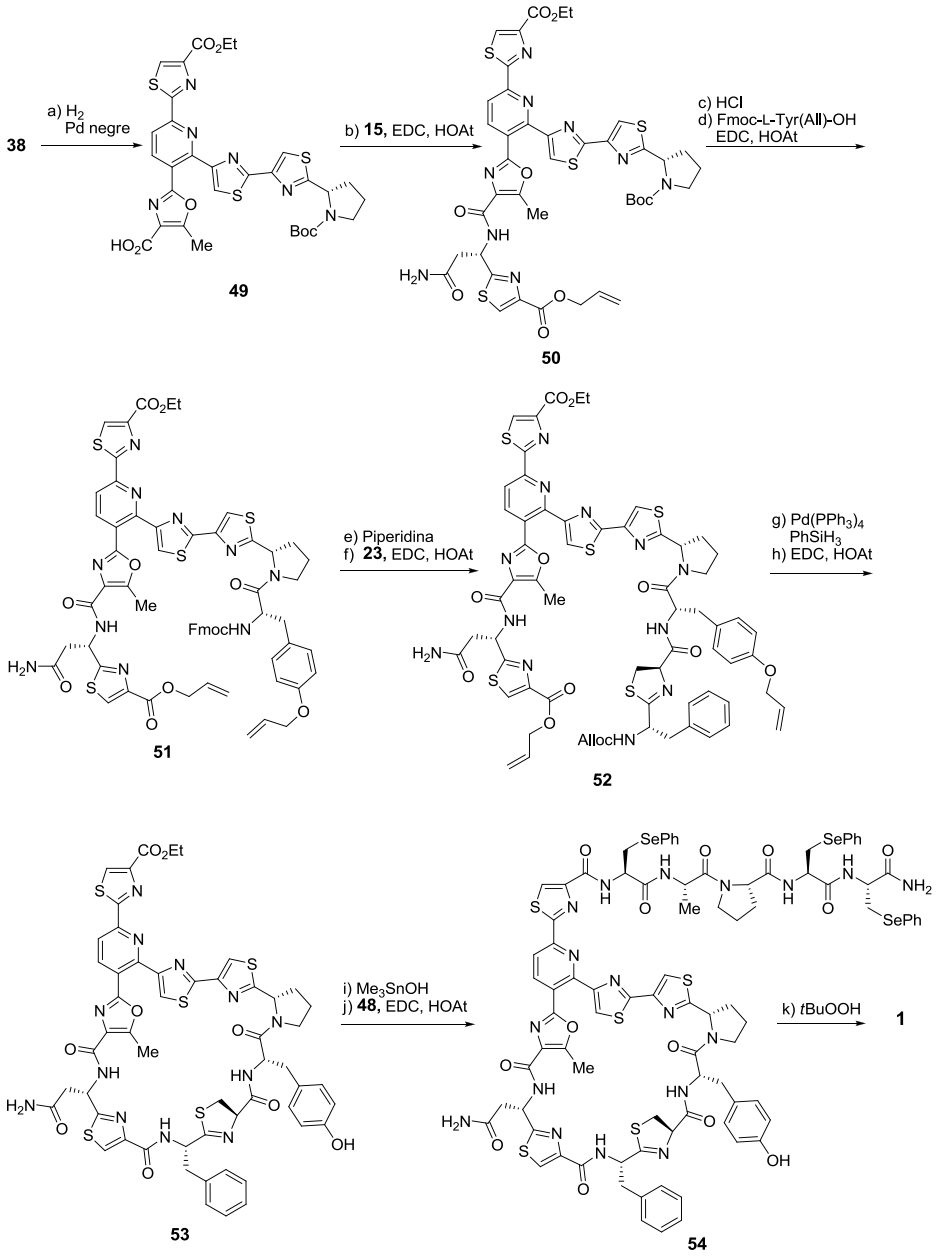


Esquema 13. Síntesi del pentapèptid **48**. Reactius i condicions: a) i. Fmoc-AA-OH, *N*,*N*'-diisopropilcarbodiimida, OxymaPure, DMF, t.a., 1.5 h; ii. 20% Piperidina en DMF, t.a. (4 tractaments); b) 95% TFA en CH_2Cl_2, rt (4 tractaments), 89%.

7. Unió dels fragments i últimes etapes

Un cop es varen sintetitzar tots els fragments necessaris, el procedí a la seva unió, començant per la desprotecció del fragment central **38**, la qual requerí hidrogenar sobre pal·ladi negre (Esquema 14). A partir d'aquí, es procedí a la construcció del macrocicle; en primer lloc es condensà **49** amb **15** per obtenir **50**, el qual es va desprotegir en medi àcid per

condensar-lo amb Fmoc-L-Tyr(All)-OH i formar **51**. Seguidament es desprotegí amb piperidina per condensar amb la tiazolina **23** i obtenir el precursor del macrocicle totalment protegit (**52**). En aquest punt, a **52** hi ha tres grups protectors diferents basats en el grup al·lil: un èster al·lílic, un al·lil carbamat i un èter al·lílic. Així, es procedí a la desprotecció de **52** amb Pd catalític i es dugué a terme la macrociclació amb el cru de desprotecció, obtenint-se **53**. A continuació, per tal de no afectar la tiazolina, se saponificà l'èster etílic amb hidròxid de trimetilestany i seguidament es condensà amb **48**, donant lloc a **54**. Finalment, un tractament oxidant amb hidroperòxid de *tert*-butil induí la oxidació i la posterior eliminació espontània dels grups de fenilselenur, formant els residus de dehidroalanina present a la baringolina (**1**).



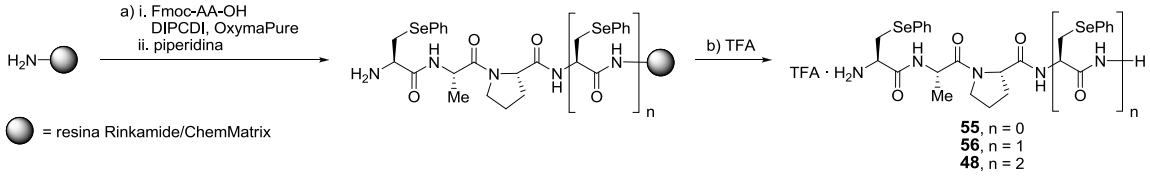
Esquema 14. Síntesi total de la baringolina (**1**). Reactius i condicions: a) H_2 (1 atm), Pd negre, CH_2Cl_2/EtOH (1:1), t.a., 4 h, quant.; b) **15**, EDC, HOAt, DIPEA, DMF, 0 °C, 18 h, 82%; c) HCl, 1,4-dioxà, rt, 7 h; d) Fmoc-L-Tyr(All)-OH, EDC, HOAt, DIPEA, DMF, 0 °C, 7 h, 71% (2 etapes); e) piperidina, CH_2Cl_2, t.a., 3 h, 87%; f) **23**, EDC, HOAt, DIPEA, DMF, 0 °C, 3 h, 68% (2 etapes); g) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, t.a., 7 h; h) EDC, HOAt, DMF (1 mM), 0 °C to rt, 21 h, 30% (2 etapes); i) Me_3SnOH, $ClCH_2CH_2Cl$, 60 °C, 19 h; j) **2**, EDC, HOAt, DIPEA, DMF, 0 °C, 3 h, 81% (2 etapes); k) *t*BuOOH, CH_2Cl_2, t.a., 12 h, 66%.

Quan s'obtingué la baringolina sintètica, els espectres de ressonància d'aquesta es compararen amb els d'una mostra autèntica, revelant que tots dos compostos són idèntics. A més, per tal de tenir una confirmació addicional, la seva activitat antibiòtica es va avaluar contra *Staphylococcus aureus* mitjançant una prova de CMI (concentració mínima inhibitòria), donant els mateixos resultats. Així, mitjançant la síntesi total de la baringolina es va ser capaç de confirmar la seva estructura i d'assignar-ne l'estereoquímica.

8. Síntesi d'anàlegs de baringolina i estudi de les relacions estructura-activitat

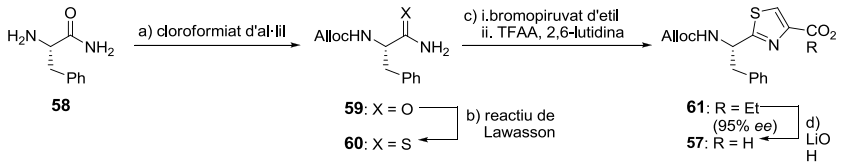
Un cop demostrada la utilitat de l'estratègia sintètica desenvolupada per a la síntesi total de la baringolina, aquesta s'utilitzà per a l'obtenció d'anàlegs que permetessin estudiar el paper de certes regions de la molècula a l'hora d'exercir la seva activitat antibiòtica. D'aquesta manera, es decidir d'estudiar el impacte de la llargada del pentapèptid de la cua i de la tiazolina.

En primer lloc se sintetitzaren els corresponents tri- i tetrapeptid, **55** i **56** respectivament, usant exactament la mateixa aproximació descrita per a **48** (Esquema 15). En tots els casos s'obtingueren resultats similars, amb rendiments excel·lents i productes d'elevada puresa sense necessitat de cap purificació addicional després de l'escissió de la resina.



Esquema 15. Síntesi dels pèptids **55**, **56** i **48**. Reactius i condicions: a) i. Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, t.a., 1.5 h; ii. 20% pipepridina en DMF, t.a. (4 tractaments); b) 95% TFA en CH_2Cl_2, t.a. (4 tractaments). Rendiments: **55** (quant.), **56** (quant.), **48** (89%).

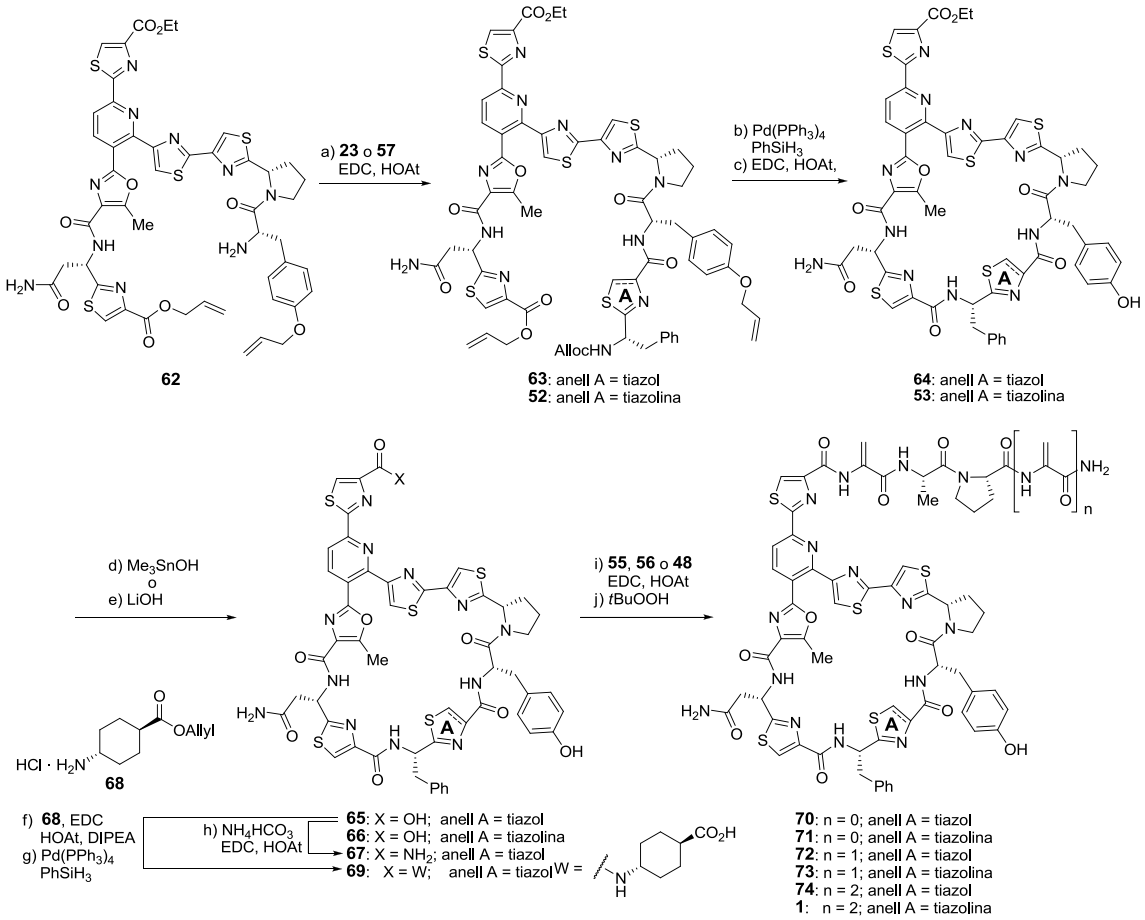
A continuació se sintetitzà un fragment de tiazol derivat de Phe anàleg al de la tiazolina **23** utilitzada en la síntesi total de la baringolina. Emprant la mateixa metodologia descrita prèviamenent per a tiazols derivats d'amino àcids s'obtinguè **57** amb elevat excés enantiomèric (Esquema 16). Partint de l'amino amida **58**, es dugué a terme la seva protecció com a carbamat (**59**) i posteriorment es transformà en la corresponent tioamida **60**. Una nova reacció de Hantzsch modificada en dues etapes permeté obtenir el producte esperat (**57**).



Esquema 16. Síntesi del tiazol derivat de Phe **11**. Reactius i condicions: cloroformiat d'al·lil, NEt_3, CH_2Cl_2, 0 °C, 2 h, 86%; b) reactiu de Lawesson, THF, t.a., 4.5 h, 88%; c) i. Bromopiruvat d'etil, $KHCO_3$, DME, 0 °C, 2.5 h; ii. TFAA, 2,6-lutidina, DME, −20 °C, 2.5 h, 99% (95% *ee*); d) LiOH, H_2O/THF, t.a., 15 h, 86%.

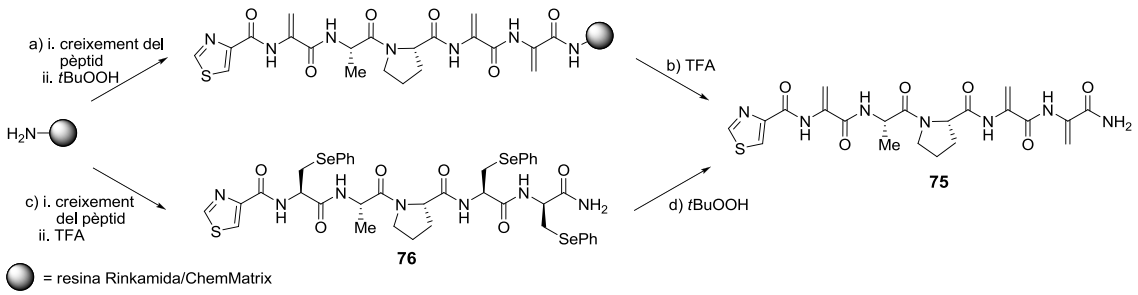
Amb els fragments necessaris preparats, es pogueren començar a construir els diferents anàlegs (Esquema 17). Amb **62**, un intermedi de la síntesi de la baringolina, s'hi condensaren tant **23** com **57**, obtenint-se els precursors dels macrocícles, **52** i **63**, respectivament. A partir d'aquests, es dugué a terme la desprotecció amb pal·ladi i a continuació la macrociclació per obtenir els productes **53** i **64**. Seguidament, se saponificaren els èsters etílics respectius per obtenir els àcids **65** i **66**. L'àcid **65** es transformà en l'amida **67** i també s'acoblà amb **68**, que

donà lloc a **69** un cop es desprotegí l'èster al·lílic. L'acoblament de **65** i **66** amb els tres pèptids obtinguts per fase sòlida, **48**, **55** i **56**, i la posterior oxidació/eliminació donà lloc als anàlegs **70**–**74**.



Esquema 17. Síntesi de **1** i dels anàlegs **53**, **64**–**67** i **69**–**74**. Reactius i condicions: a) **23** o **57**, EDC, HOAt, DIPEA, DMF, 0 °C fins a t.a., 3 h, 85% (**63**), 68% (**52**); b) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, t.a.; c) EDC, HOAt, DMF (1 mM), t.a., 61% (**64**), 30% (**53**); d) Me_3SnOH, $ClCH_2CH_2Cl$, 60 °C, 19 h; e) LiOH, H_2O/THF, t.a., 17 h, 99% (**65**); f) **68**, EDC, HOAt, DIPEA, DMF, 0 °C fins a t.a., 5 h; g) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2, t.a., 2 h, 39% (2 steps); h) NH_4CO_3, EDC, HOAt, DIPEA, DMF, 0 °C fins a t.a., 28 h, 68%; i) **55**, **56** o **48**, EDC, HOAt, DIPEA, DMF, 0 °C fins a rt; j) *t*BuOOH, CH_2Cl_2, t.a., 39% (**70**, 2 steps), 63% (**71**, 3 steps), 33% (**72**, 2 steps), 55% (**73**, 3 steps), 50% (**74**, 2 etapes), 53% (**1**, 3 etapes).

A part dels anàlegs macrocíclics, també es va voler sintetitzar un anàleg que presentés només la cua peptídica per tal d'avaluar si aquesta té alguna mena d'activitat antibiòtica per si sola. Així, es va sintetitzar, de nou per fase sòlida, l'anàleg **75**, el qual es va obtenir per dues rutes diferents per tal de comparar-ne l'eficàcia (Esquema 18). Per una banda, es van dur a terme tots els acoblaments, però també la oxidació/eliminació, en fase sòlida i finalment es va escindir de la resina. Alternativament, es va escindir el pèptid **76** abans del tractament amb hidroperòxid de *tert*-butil, però en tots dos casos els rendiments globals obtinguts foren molt similars, demostrant la versatilitat d'una estratègia basada en la síntesi en fase sòlida.



Esquema 18. Síntesi de l'anàleg de la cua peptídica **75**. Reactius i condicions: a) Fmoc-AA-OH, DIPCDI, OxymaPure, DMF, t.a., 1.5 h; 20% pipepridina en DMF, t.a. (4 tractaments); ii. *t*BuOOH, CH_2Cl_2, t.a.; b) 95% TFA en CH_2Cl_2, t.a. (4 tractaments), 36% (2 etapes); c) Fmoc-AA-OH, *N*,*N*'-diisopropilcarbodiimida, OxymaPure, DMF, t.a., 1.5 h; 20% pipepridina en DMF, rt (4 tractaments); ii. 95% TFA en CH_2Cl_2, t.a. (4 tractaments), 47%; d) *t*BuOOH, CH_2Cl_2, t.a., 72%.

La preparació d'aquesta petita llibreria de compostos ja és una fita important, doncs es tracta de la primera llibreria de tiopèptids sintetitzada únicament per mètodes químics. Per tal de poder observar quin és el impacte de les modificacions realitzades, s'avaluà l'activitat antibiòtica dels anàlegs, juntament amb la baringolina, contra quatre soques diferents de bacteris Gram positius: *Staphylococcus aureus*, *Propionibacterium acnes*, *Bacillus subtilis* i *Micrococcus luteus*. A més, la solubilitat dels compostos es determinà tant en aigua com en tampó fosfat (PB) 0.1 M.

Taula 1. Activitat antibacteriana i solubilitat de **1** i els seus anàlegs.

Entrada	Compost	CMI[a] (μg/mL)				Solubilitat[b] (mg/mL)	
		S. aureus	*P. acnes*	*B.subtilis*	*M. luteus*	H_2O	PB 0.1 M
1	Baringolina (**1**)	0.25	0.125	0.25	0.5	SLD[c]	SLD[c]
2	**71**	0.25	0.125	0.25	0.5	SLD[c]	SLD[c]
3	**73**	0.5	0.5	0.5	1	SLD[c]	SLD[c]
4	**70**	0.5	4	8	2	SLD[c]	SLD[c]
5	**72**	0.5	8	0.5	2	SLD[c]	SLD[c]
6	**74**	0.5	8	1	2	SLD[c]	SLD[c]
7	**64**	>8	4	>8	2	SLD[c]	SLD[c]
8	**53**	>8	4	8	4	SLD[c]	SLD[c]
9	**65**	2	2	8	>8	SLD[c]	0,023
10	**67**	1	8	8	>8	SLD[c]	0,007
11	**69**	0.03	0.06	0.03	0.5	SLD[c]	0,018
12	**75**	>8	8	>8	2	4.661	6.654

[a]CMI = concentració mínima inhibitòria.
[b]La solubilitat es determinà messurant la concentració d'una solució saturada dels compostos.
[c]SLD = per sota del límit de detecció.

A partir dels resultats obtinguts es poden extreure diverses conclusions. En primer lloc, es fa evident que la cua o la seva llargada no té un gran impacte sobre l'activitat antibiòtica, com es pot veure en comparar entre elles les entrades 1–3 o 4–6. A més, el compost **75** pràcticament no va resultar actiu. Els dos èsters, **64** i **53**, entrades 7 i 8, respectivament, tampoc van mostrar activitats destacables. D'altra banda, la substitució de la tiazolina per tiazol (entrades 1–6) ha permès extreure conclusions molt interessants. Es pot observar clarament que aquest substitució fa que pràcticament només es mantingui l'activitat contra *S. aureus*, fet que fa pensar que l'anell de tiazol, més rigid, no permet un bon acomodament dels

anàlegs en la seva diana molecular, presumiblement el factor d'elongació Tu,[72] el qual presentarà mutacions d'una espècie a l'altra. Per al nostre delit, l'anàleg **69**, que presenta un motiu d'àcid ciclohexanoic[4,73] en lloc de la cua peptídica, tot i tenir la tiazolina substituïda pel corresponent tiazol, va mostrar potències excel·lents contra totes les soques testades, millorant amb escreix al producte natural contra tres d'elles, *S. aureus*, *P. acnes* i *B. subtilis*. Aquest potencia millorada de **69** i la restauració de l'activitat contra totes les soques és molt probablement conseqüència de l'aparició d'un nou contacte amb la diana molecular, afavorint la interacció tot i la rigidesa del tiazol. Malauradament, la solubilitat de tots els anàlegs macrocíclics segueix essent marginal.

9. Conclusions

En resum, s'ha desenvolupat una nova estratègia per a la síntesi de tiopèptids i en concret s'ha utilitzat per a la síntesi total de la baringolina, un nou antibiòtic d'origen marí. La síntesi duta a terme ha permès confirmar l'estructura del producte natural i al mateix temps assignar-ne l'estereoquímica. Per tal de sintetitzar la baringolina ha calgut preparar una sèrie de fragments derivats d'amino àcids, un fragment de bistiazol-pirrolidina degudament funcionalitzat per ser utilitzat en reaccions d'acoblament creuat, un fragment de tiazol-4-carboxilat i una tiazolina. A més, ha calgut posar a punt una estratègia per a la construcció del fragment central, el qual ha estat possible d'obtenir de forma totalment regioselectiva a partir de l'àcid 2,6-dicloronicotínic. Utilitzant aquesta mateixa estratègia s'han obtingut diversos anàlegs del fragment central, els quals han servit tant per demostrar la versatilitat d'aquesta aproximació, com per fer proves de diferents grups protectors. Un cop seleccionats els grups protectors adequats es va poder procedir a la unió de tots els fragments en que s'havia dividit la baringolina, incloent el pentapèpid, el qual es va obtenir de forma molt eficient utilitzant la síntesi en fase sòlida. Un cop completada la síntesi total, es va afrontar la preparació de la primera llibreria d'anàlegs de tiopèptids obtinguda per mètodes purament químics. Gràcies als resultats obtinguts en avaluar els compostos front diverses soques de bacteris Gram positius es va poder determinar la importància relativa que té la cua peptídica i, per contra, el paper crucial que juga el motiu de tiazolina per mantenir un ampli ventall d'activitat contra totes les soques testades. A més, la introducció d'un motiu d'àcid ciclohexanoic en el macrocícle modificat amb tiazol va restaurar l'activitat front totes les soques i en va augmentar la potència contra tres d'elles respecte del producte natural. Així, els resultats presentats demostren la gran utilitat de la síntesi en diversos aspectes de la ciència dels productes naturals, permetent determinar-ne l'estructura i l'estereoquímica, estudiar-ne les relacions d'estructura-activitat i, a més, millorar-ne la potència.

Referències

(1) Fischbach, M. A.; Walsh, C. T. *Science* **2009**, *325*, 1089–1093.

(2) Kumar, N. *Pharma Science Monitor* **2012**, *3*, 2990–3009.

(3) Bagley, M. C.; Dale, J. W.; Merritt, E. A.; Xiong, X. *Chem. Rev.* **2005**, *105*, 685–714.

(4) LaMarche, M. J.; Leeds, J. A.; Amaral, A.; Brewer, J. T.; Bushell, S. M.; Deng, G.; Dewhurst, J. M.; Ding, J.; Dzink-fox, J.; Gamber, G.; Jain, A.; Lee, K.; Lee, L.; Lister, T.; Mckenney, D.; Mullin, S.; Osborne, C.; Palestrant, D.; Patane, M. A.; Rann, E. M.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Trzasko, A.; Whitehead, L.; Yifru, A.; Yu, D.; Yan, W.; Zhu, Q. *J. Med. Chem.* **2012**, *6*, 2376–2387.

(5) Hughes, R. A.; Moody, C. J. *Angew. Chem. Int. Ed.* **2007**, *46*, 7930–7954.

(6) Zhang, Q.; Liu, W. *Nat. Prod. Rep.* **2013**, *30*, 218–226.

(7) Duan, L.; Wang, S.; Liao, R.; Liu, W. *Chem. Biol.* **2012**, *19*, 443–448.

(8) Zhang, Q.; Chen, D.; Lin, J.; Liao, R.; Tong, W.; Xu, Z.; Liu, W. *J. Biol. Chem.* **2011**, *286*, 21287–21294.

(9) Li, C.; Kelly, W. L. *Nat. Prod. Rep.* **2010**, *27*, 153–164.

(10) Arndt, H.-D.; Schoof, S.; Lu, J.-Y. *Angew. Chem. Int. Ed.* **2009**, *48*, 6770–6773.

(11) Wang, J.; Yu, Y.; Tang, K.; Liu, W.; He, X.; Huang, X.; Deng, Z. *Appl. Environ. Microbiol.* **2010**, *76*, 2335–2344.

(12) Morris, R. P.; Leeds, J. A.; Naegeli, H. U.; Oberer, L.; Memmert, K.; Weber, E.; LaMarche, M. J.; Parker, C. N.; Burrer, N.; Esterow, S.; Hein, A. E.; Schmitt, E. K. *J. Am. Chem. Soc.* **2009**, *131*, 5946–5955.

(13) Young, T. S.; Walsh, C. T. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13053–13058.

(14) Ding, Y.; Yu, Y.; Pan, H.; Guo, H.; Li, Y.; Liu, W. *Mol. BioSyst.* **2010**, *6*, 1180–1185.

(15) Yu, Y.; Duan, L.; Zhang, Q.; Liao, R.; Ding, Y.; Pan, H.; Wendt-, E.; Tang, G.; Shen, B.; Liu, W. *ACS Chem Biol.* **2009**, *4*, 855–864.

(16) Liao, R.; Duan, L.; Lei, C.; Pan, H.; Ding, Y.; Zhang, Q.; Chen, D.; Shen, B.; Yu, Y.; Liu, W. *Chem. Biol.* **2009**, *16*, 141–147.

(17) Wieland Brown, L. C.; Acker, M. G.; Clardy, J.; Walsh, C. T.; Fischbach, M. A. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 2549–2553.

(18) Kelly, W. L.; Pan, L.; Li, C. *J. Am. Chem. Soc.* **2009**, *131*, 4327–4334.

(19) Engelhardt, K.; Degnes, K. F.; Zotchev, S. B. *Appl. Environ. Microbiol.* **2010**, *76*, 7093–7101.

(20) Bhat, U. G.; Halasi, M.; Gartel, A. L. *PloS one* **2009**, *4*, e5592.

(21) Radhakrishnan, S. K.; Bhat, U. G.; Hughes, D. E.; Wang, I.-C.; Costa, R. H.; Gartel, A. L. *Cancer Res.* **2006**, *66*, 9731–9735.

(22) Kwok, J. M.-M.; Myatt, S. S.; Marson, C. M.; Coombes, R. C.; Constantinidou, D.; Lam, E. W.-F. *Mol. Cancer Ther.* **2008**, *7*, 2022–2032.

(23) Bhat, U. G.; Zipfel, P. a; Tyler, D. S.; Gartel, A. L. *Cell Cycle* **2008**, *7*, 1851–1855.

(24) Pandit, B.; Bhat, U.; Gartel, A. L. *Cancer Biol. Ther.* **2011**, *11*, 43–47.

(25) Hegde, N. S.; Sanders, D. A.; Rodriguez, R.; Balasubramanian, S. *Nat. Chem.* **2011**, *3*, 725–731.

(26) Clough, B.; Strath, M.; Preiser, P.; Denny, P.; Wilson, I. (R. J. M. . *FEBS Lett.* **1997**, *406*, 123–125.

(27) Goodman, C. D.; Su, V.; McFadden, G. I. *Mol. Biochem. Parasitol.* **2007**, *152*, 181–191.

(28) Aoki, M.; Ohtsuka, T.; Yamada, M.; Ohba, Y.; Yoshizaki, H.; Yasuno, H.; Sano, T.; Watanabe, J.; Yokose, K.; Roche, N. *J. Antibiot.* **1991**, *44*, 582–588.

(29) Motoi, U.; Furukawa, S.; Abe, F.; Ushioda, M.; Fujine, K.; Johki, S.; Hatori, H.; Ueda, J. *J. Antibiot.* **2004**, *57*, 590–596.

(30) Mizuhara, N.; Kuroda, M.; Ogita, A.; Tanaka, T.; Usuki, Y.; Fujita, K.-I. *Bioorg. Med. Chem.* **2011**, *19*, 5300–5310.

(31) Cooper, R.; Truumees, I.; Barrett, T.; Patel, M.; Schwartz, J.; Puar, M.; Das, P.; Pramanik, B. *J. Antibiot. 43*, 4–7.

(32) Hashimoto, M.; Murakami, T.; Funahashi, K.; Tokunaga, T.; Nihei, K.; Okuno, T.; Kimura, T.; Naoki, H.; Himeno, H. *Bioorg. Med. Chem.* **2006**, *14*, 8259–8270.

(33) Kelly, T. R.; Echevarren, A.; Chandrakumar, N. S.; Köksal, Y. *Tetrahedron Lett.* **1984**, *25*, 2127–2130.

(34) Kelly, T. R.; Lang, F. *Tetrahedron Lett.* **1995**, *36*, 5319–5322.

(35) Kelly, T. R.; Lang, F. *J. Org. Chem.* **1996**, *61*, 4623–4633.

(36) Okumura, K.; Saito, H.; Shin, C.; Umemura, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1863–1870.

(37) Shin, C.; Okabe, A.; Ito, A.; Ito, A.; Yonezawa, Y. *Bull. Chem. Soc. Jpn.* **2002**, *75*, 1583–1596.

(38) Shin, C.; Saito, H.; Yonezawa, Y. *Heterocycles* **2003**, *61*, 45–50.

(39) Heckmann, G.; Bach, T. *Angew. Chem. Int. Ed.* **2005**, *44*, 1199–1201.

(40) Müller, H. M.; Delgado, O.; Bach, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4771–4774.

(41) Martin, T.; Verrier, C. C.; Hoarau, C.; Marsais, F. *Org. Lett.* **2008**, *10*, 2909–2912.

(42) Martin, T.; Laguerre, C.; Hoarau, C.; Marsais, F. *Org. Lett.* **2009**, *11*, 3690–3693.

(43) Moody, C. J.; Hughes, R. a; Thompson, S. P.; Alcaraz, L. *Chemical communications (Cambridge, England)* **2002**, 1760–1.

(44) Hughes, R. A.; Thompson, S. P.; Alcaraz, L.; Moody, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 15644–15651.

(45) Nicolaou, K. C.; Nevalainen, M.; Safina, B. S.; Zak, M.; Bulat, S. *Angew. Chem. Int. Ed.* **2002**, 1941–1945.

(46) Nicolaou, K. C.; Safina, B. S.; Zak, M.; Estrada, A. A.; Lee, S. H. *Angew. Chem. Int. Ed.* **2004**, *43*, 5087–5092.

(47) Nicolaou, K. C.; Zak, M.; Safina, B. S.; Lee, S. H.; Estrada, A. A. *Angew. Chem. Int. Ed.* **2004**, *43*, 5092–5097.

(48) Ciufolini, M. A.; Shen, Y. C. *Org. Lett.* **1999**, *1*, 1843–1846.

(49) Lefranc, D.; Ciufolini, M. A. *Angew. Chem. Int. Ed.* **2009**, *48*, 4198–4201.

(50) Bagley, M. C.; Bashford, K. E.; Hesketh, C. L.; Moody, C. J. *J. Am. Chem. Soc.* **2000**, *122*, 3301–3313.

(51) Bagley, M. C.; Dale, J. W.; Xiong, X.; Bower, J. *Org. Lett.* **2003**, *5*, 4421–4424.

(52) Bagley, M. C.; Dale, J. W.; Jenkins, R. L.; Bower, J. *Chem. Commun.* **2004**, 102–103.

(53) Xiong, X.; Bagley, M. C.; Chapaneri, K. *Tetrahedron Lett.* **2004**, *45*, 6121–6124.

(54) Lu, J.-Y.; Arndt, H.-D. *J. Org. Chem.* **2007**, *72*, 4205–4212.

(55) Higashibayashi, S.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2002**, *43*, 105–110.

(56) Mori, T.; Higashibayashi, S.; Goto, T.; Kohno, M.; Satouchi, Y.; Shinko, K.; Suzuki, K.; Suzuki, S.; Tohmiya, H.; Hashimoto, K.; Nakata, M. *Tetrahedron Lett.* **2007**, *48*, 1331–1335.

(57) Zou, Y.; Liu, Q.; Deiters, A. *Org. Lett.* **2011**, *13*, 4352–4355.

(58) Cañedo Hernández, L. M.; Romero Millan, F.; Fernández Medarde, A.; Fernández Chimeno, R. I.; Hidalgo Villar, J. C. New peptides as bioactive compounds **2012**.

(59) Spieβ, A.; Heckmann, G.; Bach, T. *Synlett* **2004**, *1*, 131–133.

(60) Ammer, C.; Bach, T. *Chem. Eur. J.* **2010**, *16*, 14083–14093.

(61) Merritt, E.; Bagley, M. *Synthesis* **2007**, *2007*, 3535–3541.

(62) Just-Baringo, X.; Bruno, P.; Albericio, F.; Álvarez, M. *Tetrahedron Lett.* **2011**, *52*, 5435–5437.

(63) Raveh, A.; Carmeli, S. *Org. Lett.* **2010**, *12*, 3536–3539.

(64) Bruno, P.; Pe, S.; Just-baringo, X.; Albericio, F.; Alvarez, M.; Peña, S.; Álvarez, M.; Peña, S.; Álvarez, M. *Org. Lett.* **2011**, *13*, 4648–4651.

(65) You, S.; Razavi, H.; Kelly, J. W. *Angew. Chem. Int. Ed.* **2003**, *44*, 83–85.

(66) Nicolaou, K. C.; Estrada, A. A.; Zak, M.; Lee, S. H.; Safina, B. S. *Angew. Chem. Int. Ed.* **2005**, *44*, 1378–1382.

(67) Wipf, P.; Miller, C. P. *J. Org. Chem.* **1993**, *58*, 3604–3606.

(68) Hirokawa, Y.; Fujiwara, I.; Suzuki, K.; Harada, H.; Yoshikawa, T. *J. Med. Chem.* **2003**, *46*, 702–715.

(69) Okeley, N. M.; Zhu, Y.; Donk, W. A. van Der *Org. Lett.* **2000**, *2*, 3603–3606.

(70) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem. Eur. J.* **2009**, *15*, 9394–9403.

(71) García-Martín, F.; Quintanar-Audelo, M.; García-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Côté, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213–220.

(72) Parmeggiani, A.; Nissen, P. *FEBS Lett.* **2006**, *580*, 4576–81.

(73) LaMarche, M. J.; Leeds, J.; Amaral, K.; Brewer, J.; Bushell, S.; Dewhurst, J.; Dzink-Fox, J.; Gangl, E.; Goldovitz, J.; Jain, A.; Mullin, S.; Neckermann, G.; Osborn, C.; Palestrant, D.; Patane, M.; Rann, E.; Sachdeva, M.; Shao, J.; Tiamfook, S.; Whitehead, L.; Yu, D. *J. Med. Chem.* **2011**, *54*, 8099–8109.