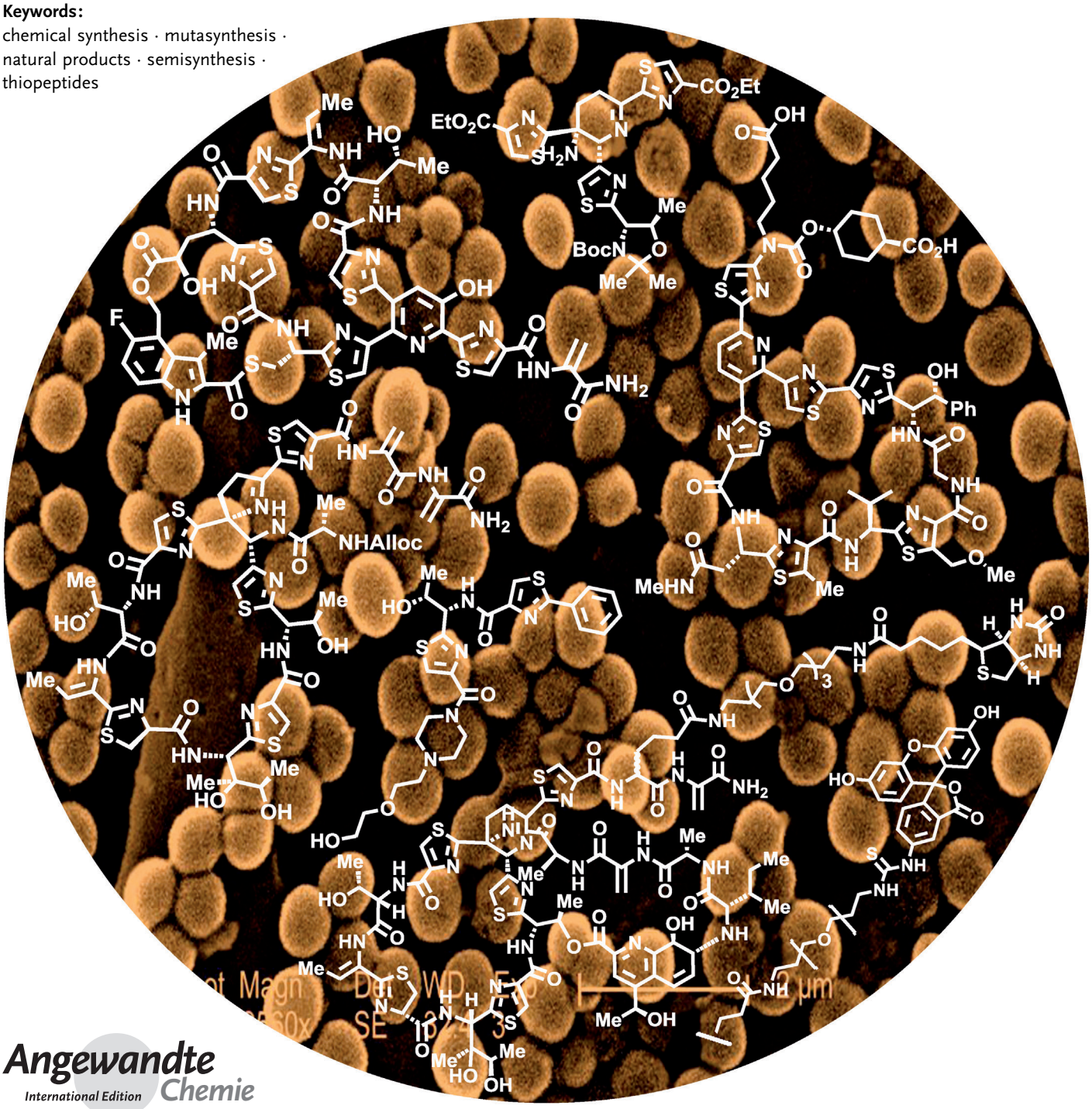
Thiopeptide Antibiotics

DOI: 10.1002/anie.201307288

Thiopeptide Engineering: A Multidisciplinary Effort towards Future Drugs

*Xavier Just-Baringo, Fernando Albericio, and Mercedes Álvarez**

Keywords:
chemical synthesis · mutasynthesis · natural products · semisynthesis · thiopeptides



© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Angewandte International Edition Chemie

The recent development of thiopeptide analogues of antibiotics has allowed some of the limitations inherent to these naturally occurring substances to be overcome. Chemical synthesis, semisynthetic derivatization, and engineering of the biosynthetic pathway have independently led to complementary modifications of various thiopeptides. Some of the new substances have displayed improved profiles, not only as antibiotics, but also as antiplasmodial and anticancer drugs. The design of novel molecules based on the thiopeptide scaffold appears to be the only strategy to exploit the high potential they have shown in vitro. Herein we present the most relevant achievements in the production of thiopeptide analogues and also discuss the way the different approaches might be combined in a multidisciplinary strategy to produce more sophisticated structures.

From the Contents

1. Introduction

Thiazolyl peptides (thiopeptides)[1,2] are an ever-expanding family of antibiotics[3] produced by Gram-positive bacteria that have attracted the interest of many research groups thanks to their outstanding biological profile. They show nanomolar potencies for a variety of Gram-positive bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and penicillin-resistant *Streptococcus pneumonia* (PRSP). They also show other interesting properties such as antiplasmodial and anticancer activities. The members of this family of natural products can be easily identified thanks to their central azole-substituted nitrogen-containing six-membered ring and are classified into different series (Figures 1 and 4). 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 cyclized and/or dehydrated Cys, Ser, and Thr units. The high potential of new modes of action of thiopeptides has gathered the attention of many research groups, who have carried out epic efforts towards their total syntheses[2,4–11] and the study of their structure, biological function, and biosynthetic origin.[12,13] It is now well established that thiopeptides arise from the 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 by blocking protein synthesis on the ribosome (Figure 2). They do so through two different modes of action, which mainly depends on their macrocycle size. Thus, thiopeptides possessing a 26-member macrocycle, such as thiostrepton,[14] nocathiacin I,[15] or the thiocillins,[16] disrupt the ribosome/L11 protein complex and do not permit the correct function of elongation factor G (EF-G), which is no longer able to bind to the 23S rRNA/L11 protein complex. On the other hand, those with a 29-member macrocycle, such as GE2270A[17] or the thiomuracins,[18] bind to elongation factor Tu (EF-Tu) and prevent the amino acyl/*t*RNA complex from being delivered to the ribosome. However, the mode of action of thiopeptides bearing larger 35-member macrocycles, such as those of TP-1161[19] and berninamycin A,[20] is not yet understood. Inhibition of the translation machinery of Gram-negative bacteria has also been reported in in vitro experiments with the isolated molecular targets. However, no growth inhibition has

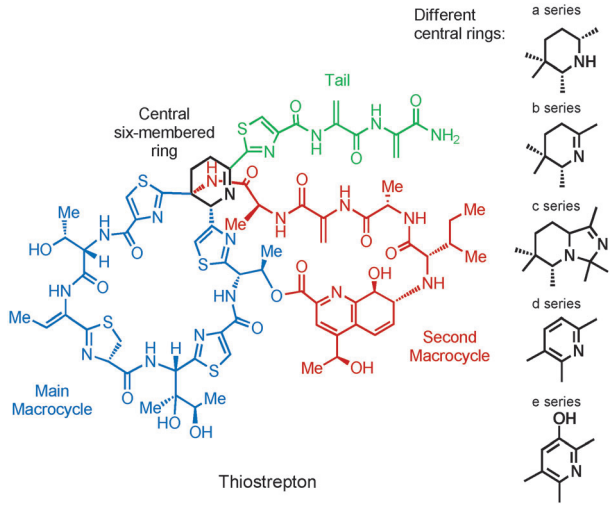


Figure 1. Key regions of thiopeptides and classification into different series depending on their central six-membered ring.

[*] X. Just-Baringo, 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: 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)
and
School of Chemistry and Physics, University of KwaZulu-Natal
4000-Durban (South Africa)
Prof. M. Álvarez
Laboratory of Organic Chemistry, Faculty of Pharmacy
University of Barcelona
08028 Barcelona (Spain)

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

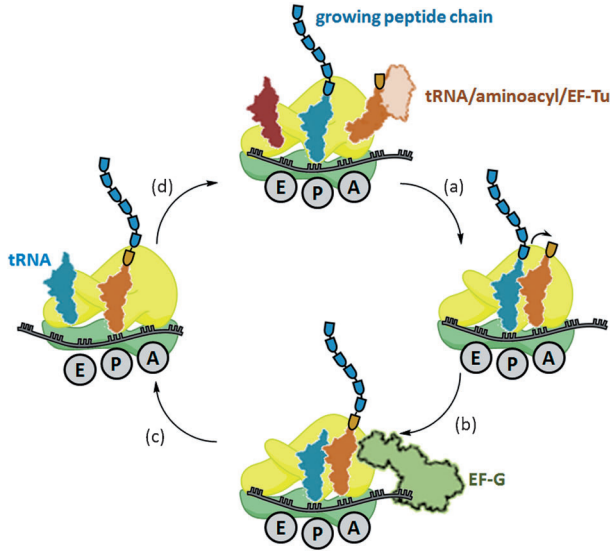


Figure 2. Bacterial protein synthesis at the ribosome: a) EF-Tu delivers the tRNA/aminoacyl complex to the ribosome A site. b) The growing peptide chain is transferred from the P site to the A site and the new peptidic bond is formed. c) EF-G effects translocation and the peptidyl-tRNA moves to the P site and the deacylated tRNA moves to the E site, from where it will exit the ribosome. d) The ribosome is ready to restart the cycle.

been observed in cultures of Gram-negative bacteria, since thiopeptides seem to be unable of crossing their cell wall.[21–24]

Despite their impressive in vitro profile, their 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

To overcome the physicochemical drawbacks of thiopeptides, different approaches have been used to determine which structural features grant them their unique biological profile and to produce analogues with 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 semisynthesis. Given the huge differences that exist between the three methods, the kinds of modifications that can be achieved is equally distinct and have allowed different areas of chemical space to be explored. While engineering of the biosynthetic pathway allows alteration of the enzymatic machinery function to isolate nonmature products and makes residue replacement possible, a semisynthetic 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 used in the other two approaches. Hence, this strategy has a huge potential and is likely to provide deep insights into structure–activity relationships.

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 involved the development of powerful synthetic methods and strategies that are also amenable for the production of analogues. Thanks to this progress, insight into the activity of analogues impossible to produce by alternative methods and the activity of fragments obtained as synthetic building blocks has helped to determine key structure–activity relationships (SARs). Given the huge potential of chemical synthesis, it is the method of choice to produce new compounds that contain large modifications from the natural product structure, especially for the discovery of antibacterial drugs.[25]

Pioneering work by the research group of Nicolaou permitted the first total synthesis of thiostrepton.[4,5] During their synthetic studies, an analogue of the natural product that lacked the second macrocycle and bore a central five-membered ring instead of the naturally occurring six-membered one was produced: analogue **1** (Figure 3).[26] This product lacked any significant antibacterial activity, but highlighted the important role of the central six-membered ring in the 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 an improved pharmacological profile.[27] The in vitro ability of **2** to target the translational machinery was assessed along with that of other

Xavier Just-Baringo received his BSc in chemistry from the University of Barcelona (2008). After a one-year industrial placement at GlaxoSmithKline (Stevenage, UK), he joined the group of Fernando Albericio and Mercedes Álvarez at the Institute for Research in Biomedicine (Barcelona, Spain), where he earned his PhD working on the total synthesis and structure–activity relationship studies of thiopeptides.

Fernando Albericio received his PhD in chemistry at the University of Barcelona (1981), which was followed by postdoctoral research. During 1992 and 1994, he was Director of Peptide Research with Millipore/Waters at Boston. He rejoined the University of Barcelona, where he was promoted to professor in 1995. He participated in the foundation of the Barcelona Science Park, where he was General Director (2005–2012). His research interests include peptide synthesis and combinatorial chemistry methods, as well as synthesis of peptides and small molecules with therapeutic activities.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

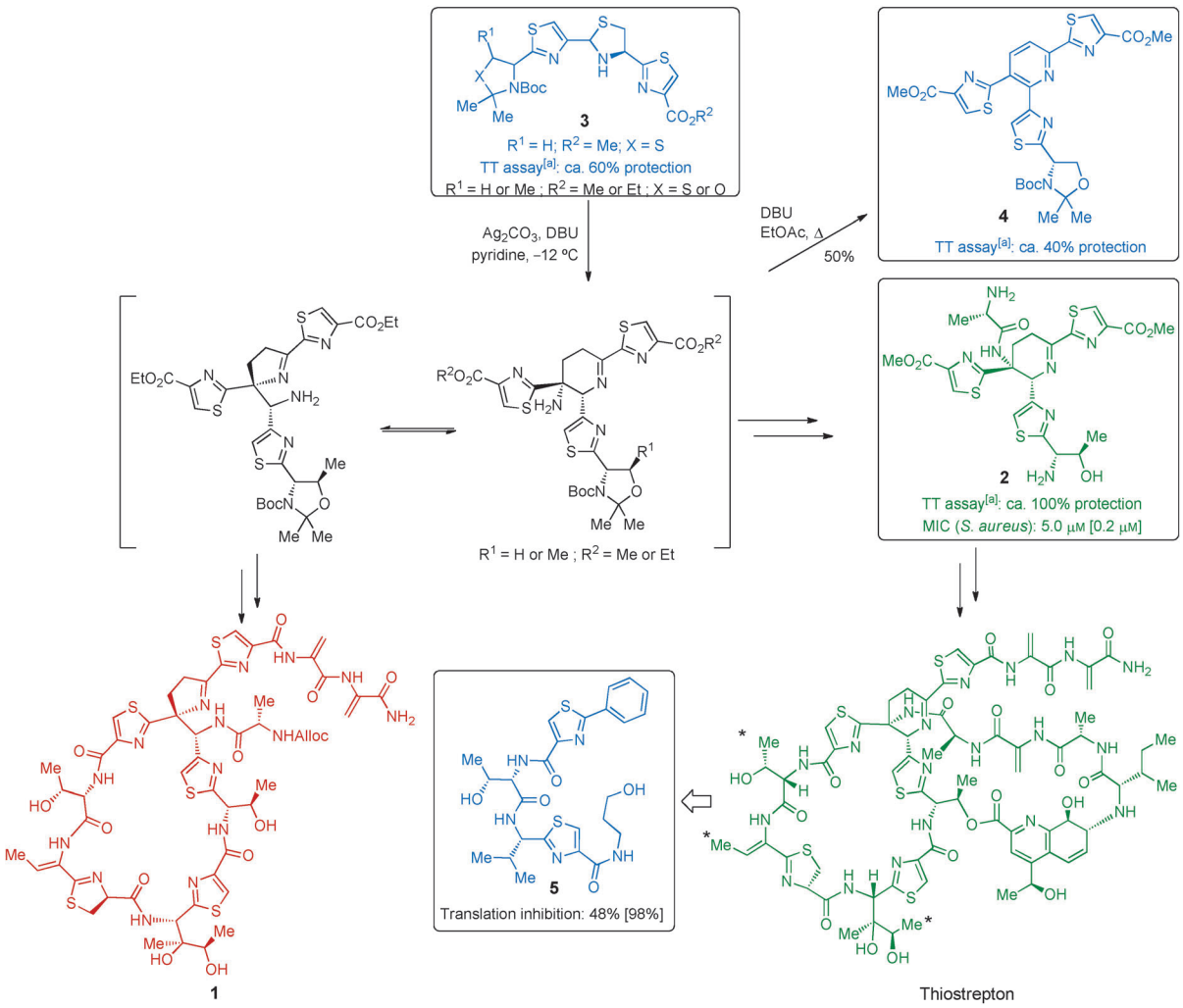


Figure 3. Relationship between different thiopeptide intermediates and analogues produced during total syntheses and fragment screening. Compounds displaying in vivo or in vitro activity are highlighted in green. Products that were found to interact only with molecular targets in in vitro assays are highlighted in blue. Non-active molecules are highlighted in red. The activity and solubility values of the natural product appear in brackets next to that of the analogue. [a] Inhibition of green fluorescent protein (GFP) synthesis through a transcription-translation (TT) assay was carried out in the presence of thiostrepton.

building blocks, such as **3** and **4**, as well as many of their analogues and stereoisomers. The central polyheterocycle **4** is a fully unsaturated version of the core fragment, such as the one found in GE2270 A and T.[28] The in vitro results point to an alternative mode of action for the fragments other than inhibition of the translational machinery.[29] Some of the tested compounds were also found to restore formation of the 70S initiation complex (70SIC) in the presence of thiostrepton, thus indicating that those fragments might compete with the natural product even if they do not strongly bind to the ribosome.

Identification of the key contacts between thiostrepton and the protein L11 binding domain of the 23S ribosomal RNA (highlighted with stars in Figure 3) led to the design and synthesis of a library of analogues such as **5**, which bear those moieties involved in such interactions.[30] 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 by a more robust thiazole. Such simplifications might have also caused the decrease of the in vitro inhibition

Mercedes Álvarez is currently Professor at the University of Barcelona and Researcher at the Barcelona Science Park's Institute for Research in Biomedicine. She earned her PhD in Chemistry from the University of Barcelona under the supervision of Prof. Ricardo Granados. She spent a sabbatical year at Manchester University working with Prof. John A. Joule. In 2002 she moved her research group to the Barcelona Science Park. Her research interests comprise the synthesis of natural products, heterocyclic chemistry, combinatorial chemistry, and solid-phase synthesis.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

and the lack of in vivo activity, which suggests that small molecules might not be able to provide all the key contacts that thiopeptides do.

2.2. Semisynthesis

The most widely used strategy for the synthesis of libraries of thiopeptide analogues has been the chemical modification of the natural products, or semisynthesis. The relatively large amounts of natural product that can be obtained using fermentation tanks is sufficient for subsequent derivatization, characterization, and screening. This approach is mainly limited by the inherent reactivity of the thiopeptide 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 with molecular weights comparable to that of the natural product.

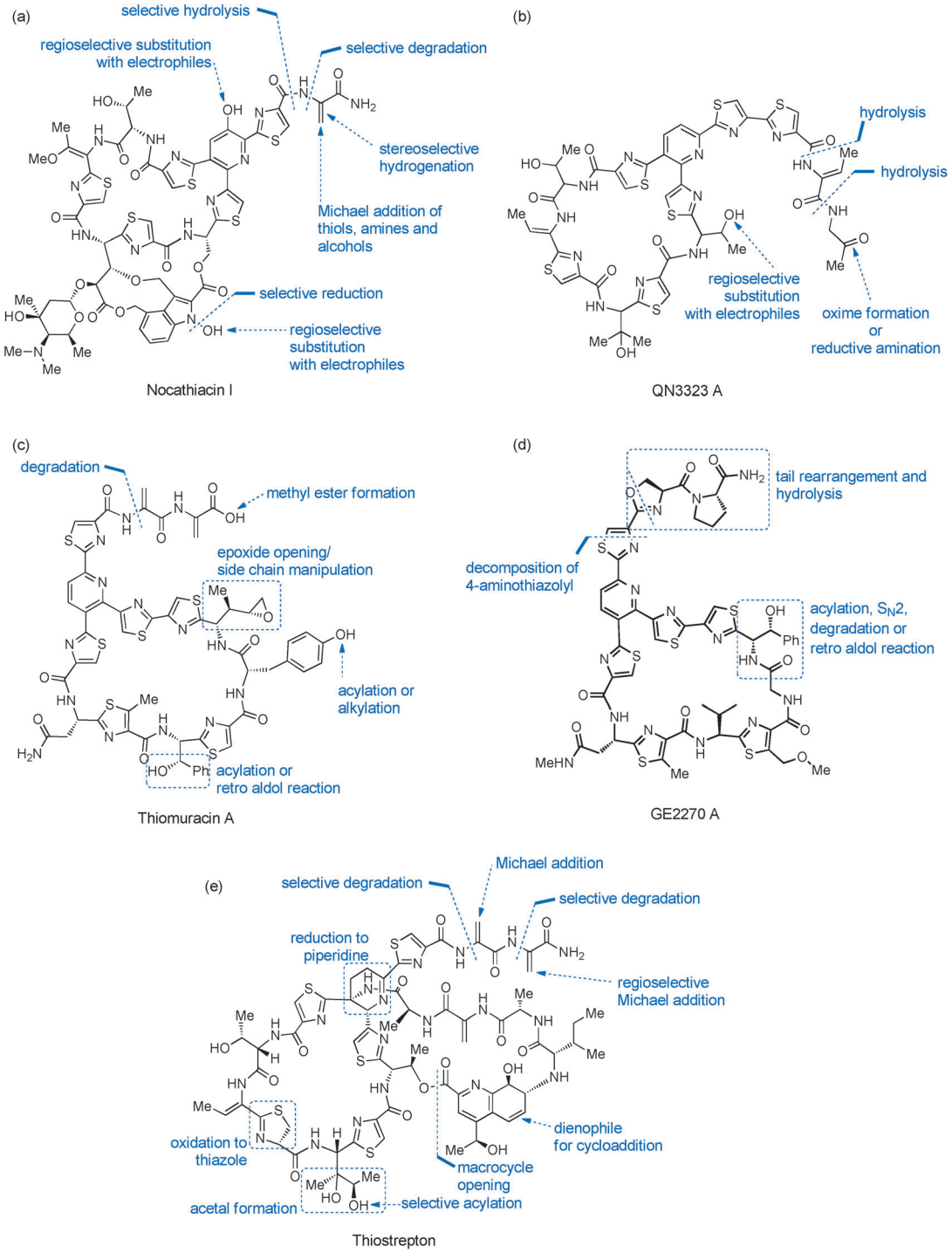


Figure 4. Graphical representation of the reactivity of thiopeptides during semisynthetic modifications of nocathiacin I, QN3323 A, thiomuracin A, GE2270 A, and thiostrepton.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Nonetheless, the fewer chemical transformations required when compared to de novo synthesis and the large number of analogues that can be readily obtained have granted access to the most promising derivatives to date.

Nocathiacin I (Figure 4a) 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 (Figures 5–7). 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 to be a much more flexible moiety with enhanced reactivity. One of the most common modifications is the Michael addition of nucleophiles to Dha,[31] which leads to amines (**6**)[32] and thioethers (**7**).[33] The former can be transformed into ureas (**8**) and amides,[34] whereas the latter are amenable for further conversion into the corresponding sulfones (**9**) under mild oxidative conditions, which should increase their solubility profile (Figure 5a).[34] Alternatively, the Dha residue can be hydrogenated diastereoselectively to obtain the corresponding Ala side chain (**10**), which can be further modified: it can be converted enzymatically into acid **11** to introduce solubilizing groups, such as in **12**, which should facilitate their formulation without losing potency (Figure 5b).[35] Degrada-

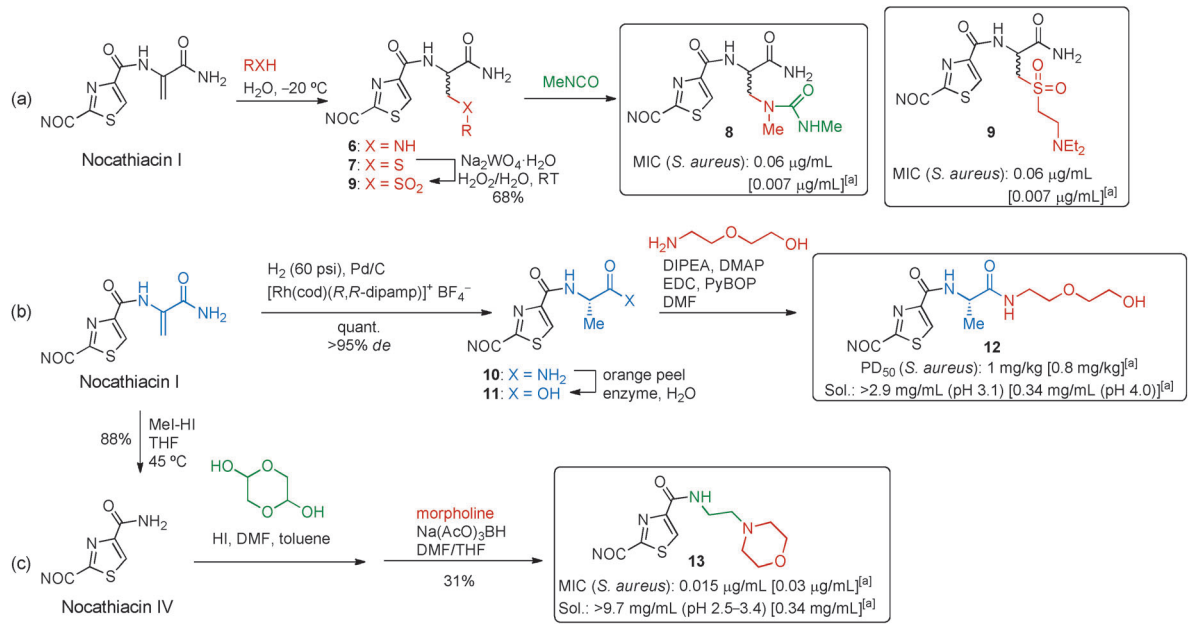


Figure 5. Examples of semisynthetic modifications of nocathiacins tails. NOC = nocathiacin macrocycle; PD_{50}: dose required to cure 50% of the animals infected; MIC: minimum inhibitory concentration. [a] Activity and solubility values of the natural product appear in brackets next to that of the analogue.

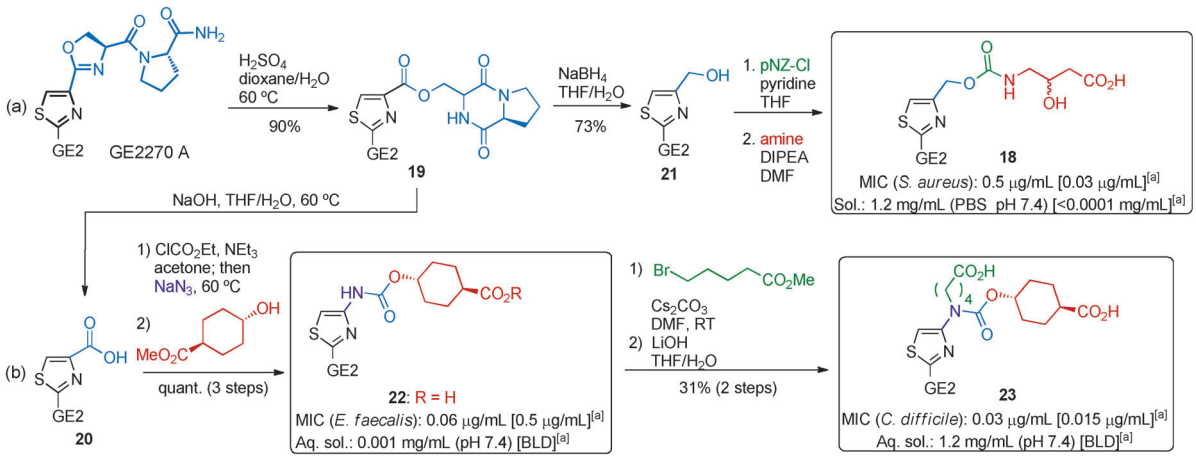


Figure 6. Examples of semisynthetic modifications of the GE2270 A tail. GE2 = GE2270 A macrocycle; MIC: minimum inhibitory concentration. [a] Activity and solubility values of the natural product appear in brackets next to that of the analogue. BLD: below limit of detection. pNZ-Cl = *para*-nitrobenzyloxycarbonyl chloride.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

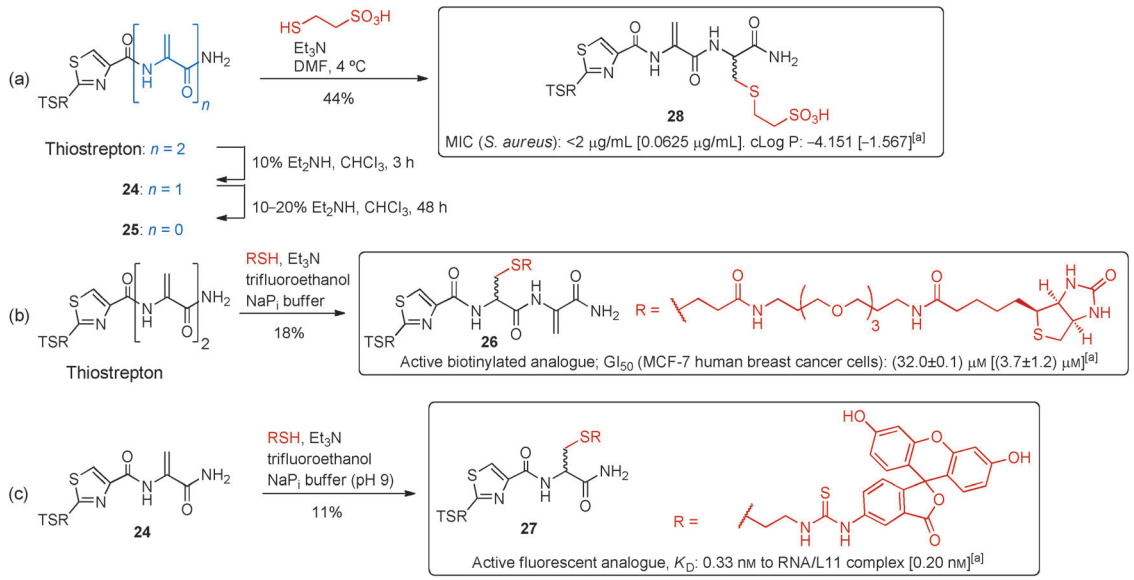


Figure 7. Examples of semisynthetic modifications of the thiostrepton tail. TSR = thiostrepton macrocycle; MIC: minimum inhibitory concentration; cLogP: calculated partition coefficient between octanol and water; GI_{50}: concentration of drug required for 50% growth inhibition; K_D: dissociation constant. [a] Activity and solubility values of the natural product appear in brackets next to that of the analogue.

tion of the tail has been widely used as well and grants access to two different products depending on the conditions used, which leads to either the thiazole-4-carboxilic acid derivative (nocathiacin acid) or the corresponding amide (nocathiacin IV).[36,37] Nocathiacin acid does not have to be modified for solubility enhancement, but can be further modified for subsequent condensation with amines.[38,39] Nocathiacin IV has been used in one-pot N-alkylation/reductive amination procedures to introduce long alkyl tails, similar to that in **13** (Figure 5c).[40] Such modifications have led to the discovery of nocathiacin analogues with improved solubility and similar potencies to that of nocathiacin I against various pathogens, including vancomycin-resistant strains.[41]

Other reported modifications of nocathiacin I include the selective substitution of one of the two most reactive hydroxy groups of the molecule, 2-hydroxypyridine and *N*-hydroxyindole, to result in regioselective alkylations and also the formation of carbamates and phosphonates.[42,43] In many cases singly (**14**) or doubly O-substituted (**15**) compounds with alkyl chains bearing a phosphoric acid group maintained excellent in vitro and in vivo activities while improving the aqueous solubility (Figure 8a).[42] Such derivatives might be suitable prodrugs of nocathiacin I, since phosphonooxymethyl ethers are known substrates of phosphatases and result in hydrolysis and, hence, the release of nocathiacin I.[44] Even if the development of thiopeptide prodrugs might appear to be a very conservative strategy, it allows the potency to be retained, thus circumventing the poor solubility of the natural product. Similarly, regioselective substitution at one of the threonine residues of QN3323 A (Figure 4b) and either oxime formation or reductive amination at the methyl ketone moiety produced derivatives (**16**) with an improved in vitro profile (Figure 8b).[45,46] Generally, modifications of the nocathiacins and QN3323 A, both of which target the ribosome/L11 protein complex, have focused on tail modifications and functionalization of the "right-hand half" of the macrocycle, which have been broadly tolerated. This region of their macrocycle is far away from the highly preserved "left-hand half" that they share with thiostrepton and that had been identified as part of the pharmacophore (see Figure 3).

A series of different transformations were performed on thiomuracin A (Figure 4c).[47] The presence of a naturally occurring epoxide became an excellent playground for derivatization of this appendix and permitted the formation of the corresponding substituted proline residue **17** (Figure 8c). This pyrrolidine ring bore methyl and hydroxy groups; however, its substitution differed from than of the naturally occurring residue present in thiomuracin I[18] and GE37468.[48] Removal of the tail was also carried out, which resulted in even more robust and simplified products. Compound **17** maintained its activity and displayed a generally increased potency in vitro, while the in vivo performance had higher strain dependence (Figure 8c). Such simplified analogues were also intended to facilitate further drug development.

Along with the amythiamicins, GE2270 A (Figure 4d) lacks any dehydroamino acids. However, it has been one of the thiopeptides most thoroughly studied and derivatized by semisynthetic methods.[49] Despite not having any Michael acceptors for the addition of nucleophiles, the modification and degradation of its peculiar tail has been addressed in many reports and has resulted in a large number of analogues with improved solubility, such as **18** (Figure 6a). Another reactive point of GE2270 A is the phenylserine side chain,[50,51] which can be modified or even removed to convert it into a glycine residue in the same fashion as for thiomuracin A (Figure 4c,d).[47] Since the modifications of the macrocycle did not provide any compounds of interest, most of the efforts have focused on tail replacement (Figure 6).[52] Acidic treatment of GE2270 A causes a rearrangement of the tail

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

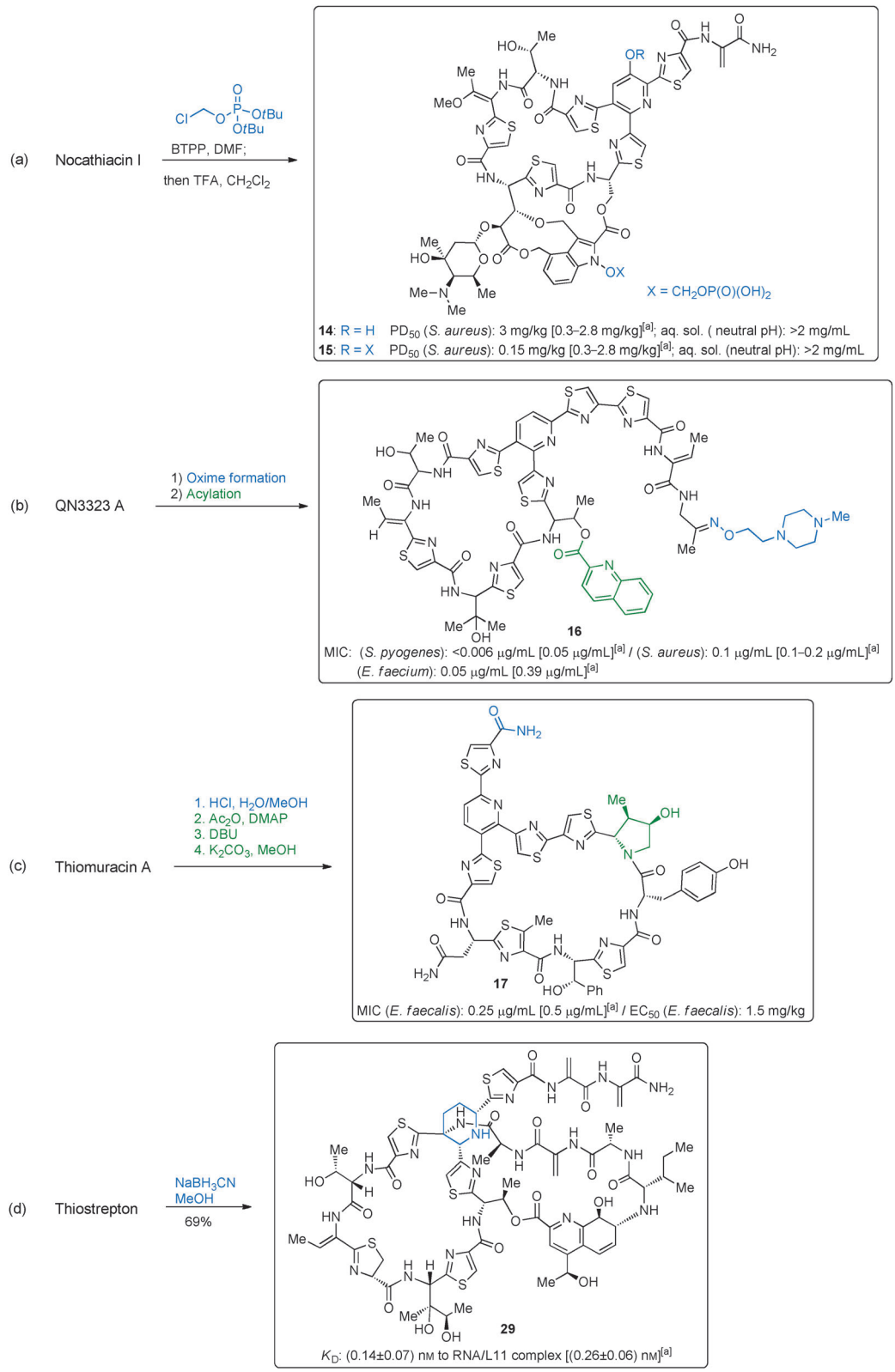


Figure 8. Examples of semisynthetic modifications of the thiopeptide macrocycles. PD_{50}: dose required to cure 50% of the animals infected; MIC: minimum inhibitory concentration; K_D: dissociation constant. [a] Activity and solubility values of the natural product appear in brackets next to that of the analogue.

and provides thiazole-4-carboxylate **19**, which can be hydrolyzed under basic conditions to yield the corresponding acid (**20**) or reduced to alcohol **21**.[51] Acid **20** can be either condensed with amines or peptides, and alcohol **21** can also be used in many different reactions (Figure 6a).[53] Although about 500 compounds were obtained in this manner, only

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

a few retained the activity while displaying good solubility. Interestingly, all the good performers shared a common motif, a carboxylic acid at least five atoms away from the thiazole ring, which suggests a new key contact between this functional group and EF-Tu, its biological target.

More recently, outstanding results have been achieved by LaMarche et al., who based their modifications on the substitution of the 4-aminothiazolyl analogue obtained after Curtius rearrangement of tailless GE2270 A (Figure 6b). Although the 4-aminothiazolyl analogue could be further modified to obtain the corresponding picolinamide derivative, it was devoid of activity.[54] The 4-aminothiazolyl moiety permitted the formation of an imidazole ring, which was used as a substitute for the original oxazoline. However, this and other imidazole-containing analogues showed poor in vitro behavior.[55] Many other substituted 4-aminothiazolyl analogues were also synthesized, thus leading to the discovery of the cyclohexanoic acid series (**22**; Figure 6b),[56,57] which were linked to the thiazole through either an amide or a carbamate linkage. In both cases, these compounds contained a carboxylic acid, which again was at least five atoms away from the thiazole moiety. Cocrystal structures of EF-Tu-bound analogues bearing the cyclohexanoic acid fragment revealed the existence of the previously suggested key contact between this moiety and the elongation factor.[56] These compounds displayed similar in vitro potencies as the parent natural compound, but their improved solubility made them amenable for in vivo testing, thereby resulting in an excellent profiling.[58] Further derivatization of the cyclohexanoic acid series led to the discovery of LFF571 (**23**; Figure 6b), which contained an alkyl chain with an extra carboxylic acid at the carbamate moiety to further improve solubility.[59] This new analogue, the first one to enter clinical trials, is being evaluated for the treatment of intestinal infections caused by *Clostridium difficile* in humans. Despite its improved solubility, **23** is not found at high concentrations in the bloodstream because of its low membrane-permeating ability, which causes its accumulation in feces when dosed orally. This fact makes it an ideal candidate for the treatment of intestinal infections caused by *C. difficile*. It has shown an excellent behavior during in vivo testing[60,61] and it has been demonstrated that it still targets elongation factor Tu.[62]

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

Despite its more complex architecture, thiostrepton has been selectively modified at many different regions of its structure (Figure 4e). Apart from the common Dha residues, other fragments susceptible to modification are also present. During their investigations on the inhibition of transcription factor forkhead box M1 (FOXM1)[63] with thiostrepton, Balasubramanian and co-workers prepared various surprising analogues.[64] First, ketal formation using the dihydroxylated Ile residue was achieved (Figure 4e), 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 4e). Unfortunately, the inhibitory activity of FOXM1 was completely lost after this modification. Another outstanding modification is the selective degradation of the Dha tail reported by the research group of Arndt, which yielded products with a single Dha (**24**) or with no tail at all (**25**; Figure 7a), depending on the reaction conditions.[65] Either thiostrepton or their mono-Dha analogues were amenable to selective Michael additions with various thiols, which led to products that have been used for a variety of studies (Figure 7). These include functionalization with a biotin tag (**26**) to assess the previously mentioned inhibition of FOXM1 (Figure 7b), SAR studies of thiostrepton analogues as antiplasmodial drugs,[66] derivatization with fluorescent probes (**27**) to study their binding to the ribosome (Figure 7c),[65,67] and assessment of the impact of the thiostrepton tail region on the inhibition of protein translation, including Gram-negative bacteria translation machinery.[24]

In contrast to what is generally observed in bacterial growth inhibition experiments, FOXM1 inhibition did not tolerate certain subtle tail modifications.[64] Thus, tail removal produced an inactive analogue, and although biotinylated thiostrepton (**26**) maintained activity (Figure 7b), the unaltered Dha residue was required to retain its biological function. These results seem to indicate a more crucial role of the tail in FOXM1 inhibition than the disruption of bacterial protein synthesis.

Alternative modifications of thiostrepton include the selective oxidation of its thiazoline ring to furnish the corresponding thiazole in high yield (Figure 4e). This, along with the selective elimination of a single Dha residue, gave access to a series of Michael addition products with improved antiplasmodial efficiency that contained various alkyl and hydroxyalkyl chains in the tail region.[66] During the same study, solvolysis of the ring containing the quinaldic acid yielded a mono-macrocyclic analogue completely devoid of activity, which again points to the importance of a macrocyclic array.[68,69] The introduction of a sulfonic acid moiety in the thiostrepton tail produced an analogue (**28**) that inhibited both Gram-positive and Gram-negative translation machineries (Figure 7a).[24] This further demonstrated that the lack of inhibitory activity in Gram-negative bacteria cultures is due to differences in their cell membranes. The fact that both antiplasmodial and antibacterial activities are similarly maintained after modification of thiostrepton's tail is easily rationalized because of the close relationship between the bacterial and the apicoplasts ribosomes. Moreover, thiostrepton derivatives were shown to also target the 20S proteasome, thus providing a dual mode of action that could lead to more effective antiplasmodial drugs.[66]

Focusing on the interaction of thiostrepton with the bacterial ribosome, functionalization of the tail with a fluorescent tag (**27**; Figure 7c)[65] allowed the study of which molecular determinants are critical for the appearance of resistances and also to gain a deeper insight into the binding mode.[67] This study revealed that mutations in the ribosome diminished the affinity between the thiopeptide and the ribosome/L11 protein complex, whereas mutation of L11 did not. This finding suggests that thiostrepton has a more

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

intimate contact with the ribosome and does not sit exactly in the middle of the crevice of the complex. In a separate study, selective reduction of the central dehydropiperidine ring of thiostrepton to its fully saturated analogue **29** was also achieved with good conversions. This compound displayed an increased affinity for the ribosome (Figure 8d), thus suggesting that thiopeptides do not fit perfectly with their targets, which have a great substrate tolerance.[70]

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 the merging of many fields with 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. The overall picture of the semisynthetic modification of various thiopeptides tells us about the great promiscuity of their biological targets, mainly in the tail region. Whereas removal of the tail or attachment of a large variety of functional groups to it generally preserves activity, functionalization or removal of various moieties in the macrocyclic scaffolds usually results in inactive analogues. This leads to the conclusion that interactions between the various targets and the macrocycle are more specific and need to be preserved.

2.3. In Vivo Production of Analogues

In contrast to the more common peptide-derived natural products produced by nonribosomal peptide synthetases (NRPS), the structure of the thiopeptides results from extensive modification of a ribosomally synthesized pre-peptide.[71] 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 (Figure 9a).[12,13] The structural gene of thiopeptides encodes a pre-peptide with two main regions: the C-terminal sequence, or structural peptide, that will constitute the mature thiopeptide, and the N-terminal sequence, the so-called leader peptide, that is lost during the biosynthetic pathway (Figure 9b). The main posttranslational modifications of the pre-peptide are general for all thiopeptides (Figure 9c). First, dehydration of Ser and Thr, along with cyclodehydration/oxidation of Ser, Thr, and Cys in pre-peptide **30**, form the characteristic dehydroamino acids and azoles. Cycloaddition between two different dehydroalanine residues in **31** furnishes the macrocycle and the central six-membered ring in **32**. Further tailoring can introduce other more specific features such as a second macrocycle, methylations, hydroxylations, indolic and quinaldic acid moieties, etc. Mutation studies of both the pre-peptide and surrounding enzyme-coding genes have offered insight into thiopeptide biosynthetic pathways,[3,72] structure–activity relationships, and tolerance of the enzymatic machinery for modifications.

Although this approach has a great potential for the easy generation of analogues, some inconveniences must be circumvented to produce sophisticated derivatives efficiently. First, culture yields are often substantially reduced when even just one conservative residue is replaced through mutation, and requires thorough optimization to achieve an efficient production.[73] Moreover, many pre-peptide mutations are not accepted by the tailoring enzymes, which results in a huge area of the chemical space that cannot be studied by this

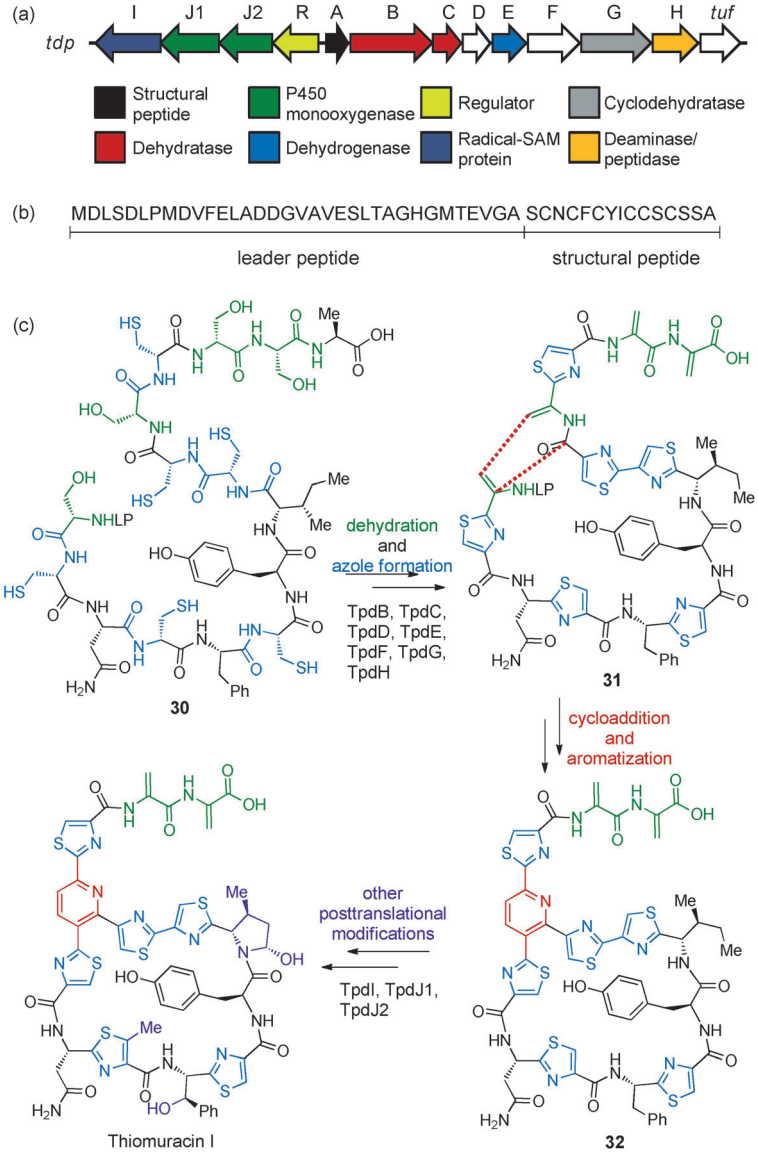


Figure 9. Biosynthesis of thiopeptides. a) Thiomuracin genetic cluster; each gene "*tdpX*" codes the corresponding protein/enzyme "TdpX". b) Thiomuracin pre-peptide. c) Proposed route for thiomuracin I biosynthetic pathway. LP = leading peptide.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

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, thus 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 most of the transformations that should take place afterwards to be performed because of 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 research groups of Walsh and Kelly have reported single residue replacement mutation studies on the pre-peptides of the thiocillins,[74,75] GE37468,[76] and thiostrepton[77,78] (Figure 10). Characterization of the 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 sufficiently large quantities of liquid media to 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 co-workers recently reported the use of codon randomization for similar investigations with GE37468 pre-peptide mutants (Figure 10a).[79] This allowed 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 sufficient quantities of mature analogues. Liquid cultures of these colonies were further analyzed and sequenced to identify their particular mutation. These

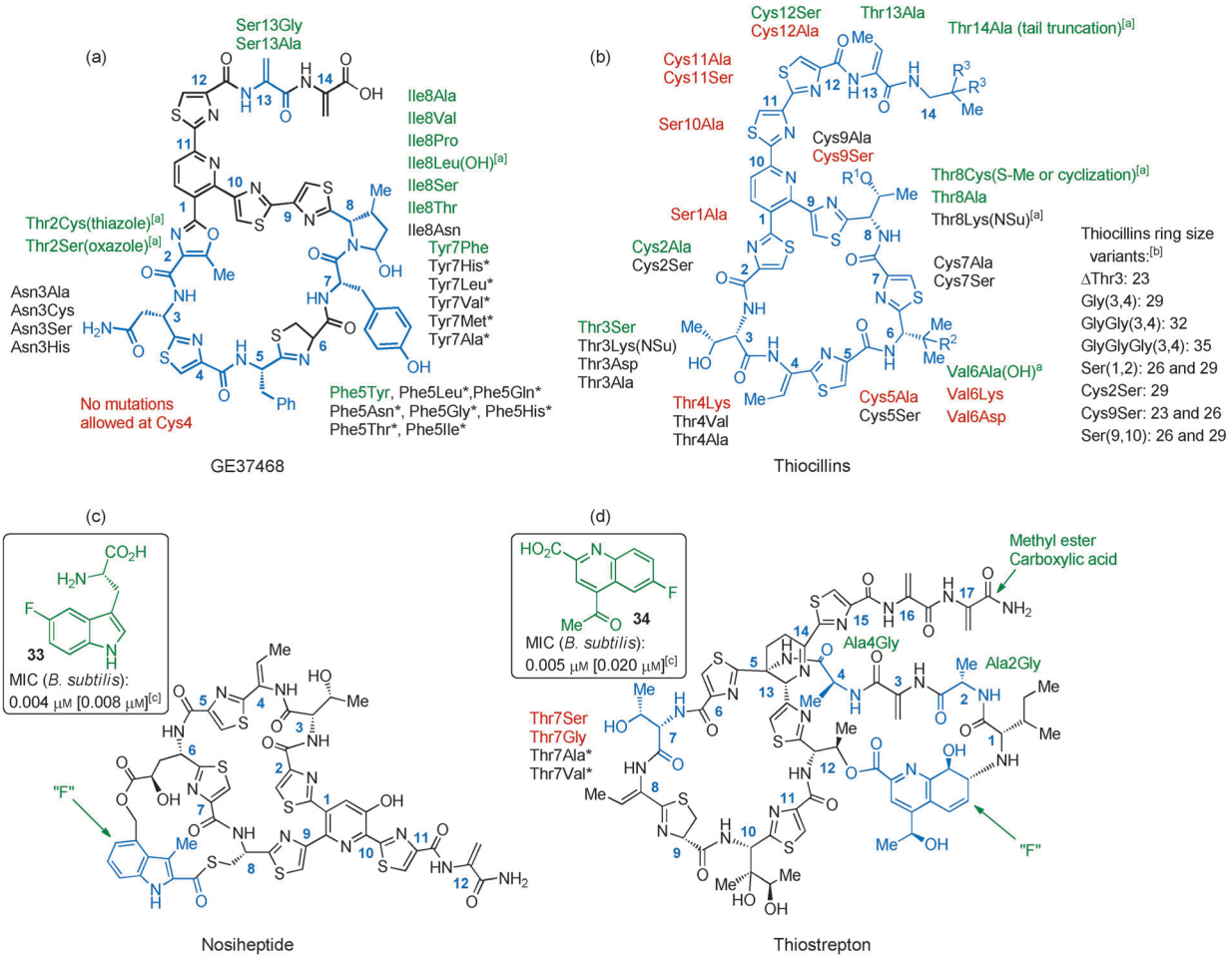


Figure 10. Production of analogues by pre-peptide mutagenesis by feeding with modified building blocks or gene deletion: a) GE37469; b) thiocillins; c) nosiheptide; d) thiostrepton. Modifications that produced active analogues are in green. Residue replacements that resulted in non-active mature products are in black and those that did not produce the mature analogue or did not provide high enough yields for testing are in red. A star highlights those mutations that resulted in altered downstream processing. [a] Post-translational modifications of mutated residues, such as hydroxylation (OH), azole formation, N-succinylation (NSu), etc., are in parenthesis. [b] The specified mutations led to analogues with different sized macrocycles, indicated next to the mutation; Δ = deletion; numbers in parenthesis indicate that new residues were introduced between those positions of the peptidic sequence. [c] Compounds **33** and **34** were used for feeding experiments; fluorinated positions obtained with this method are highlighted with "F" in the thiopeptide structure; activity values correspond to mature analogues and the activity of the natural product appears in brackets.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

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. Although these mutations are quite conservative, since they lead to the formation of other azoles (Figure 10a), the possibility of generating such analogues demonstrates a promising substrate tolerance of the thiopeptide biosynthetic machinery, as well as new areas of the chemical space that can be explored for improved potencies. It is also notable that Ile8 tolerated many substitutions, and in the case of Ile8Leu, products of varying oxidation level were present, which means that the enzyme responsible for Ile8 oxidation tolerates a Leu residue in that position. Other mutations of Ile8, mainly by amino acids with alkyl side chains, were tolerated and produced analogues with antibacterial activity, thus suggesting that this residue might not provide key contacts with EF-Tu, its molecular target. Some mutations of Asn3 were allowed (Figure 10a); however, none of the products obtained was active, which points to the crucial role of this residue for binding to EF-Tu. When examining the structure of the other 29-membered thiopeptides, the amythiamicins, baringolin, GE2270 A, GE37468 A, and the thiomuracins, it can be observed that Asn3 is preserved in all of them, thus confirming its importance.

Modifications of the pre-peptide aiming at the obtention of thiocillin analogues of various macrocycle sizes were also studied by the Walsh research group (Figure 10b).[80] 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 containing 23, 29, 32, and 35-atom macrocycles. Although thiopeptides exist with different macrocycle sizes, 23 and 32-membered rings are not found in any naturally occurring members of this family. On the other hand, to produce alternative macrocycles, new Ser residues were incorporated into the pre-peptide, by either replacing an already existing amino acid or inserting it between two of those found in the natural sequence. By doing so, once dehydration of Ser had occurred, competition between Dha residues took place and new compounds with different ring sizes or mixtures of them were produced. All the analogues of this series 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.

Inactivation experiments to study the role of different enzymes present in the thiopeptide gene clusters have provided analogues. For example, the above-mentioned TclM can be inactivated by deletion of the *tclM* gene to produce linear thiocillin precursors.[81] Although these linear analogues were completely inactive, this demonstrated that TclM is the actual enzyme responsible for the cycloaddition step. Inactivation of enzymes involved in tail maturation has provided active macrocyclic analogues, thus indicating again the great tolerance of modifications in this region. These enzymes act after cycloaddition, which leads to the obtention of very mature products containing most of the characteristic structural features of thiopeptides. The terminal amide of thiostrepton can be replaced by its corresponding methyl ester or carboxylic acid through inactivation of TsrS or TsrT, respectively (Figure 10d).[82,83] 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, the profile of the thiostrepton acid was completely opposite and, despite its improved solubility, it displayed reduced in vitro potency.

The different existing pathways for amide formation in the thiopeptide tails provide a chance for the isolation of distinct intermediates other than those observed for thiostrepton. Such is the case for nosiheptide, whose C-terminal amide is formed after cleavage of the last Dha residue of the structural peptide by NosA. Fermentation of a *nosA* KO yields nosiheptide with the extra Dha amino acid, which was not detrimental for in vitro potency.[84] 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, thus demonstrating that NosN is responsible for the 4′-methylation of the indolic acid moiety, which is required for subsequent cyclization.[85] Moreover, the order in which these transformations occur along the biosynthetic pathway is also revealed. With these experiments, both the biosynthetic 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 investigations on the biosynthesis of these moieties, Liu and co-workers disclosed the role of various enzymes that account for the conversion of Trp into those fragments.[86–88] 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 thiopeptides containing indolic acid and quinaldic acid moieties have been obtained, both resulting from incorporation of the fluorinated precursor into the biosynthetic pathway. Feeding of *S. actuosus* cultures with 5-fluoro-D,L-tryptophan (**33**) resulted in the incorporation of the modified L-amino acid into the nosiheptide structure, thereby giving rise to 5′-fluoronosiheptide along with the natural product (Figure 10c).[86] Attempts to obtain other indolic acid derivatives from Trp with substituents other than fluorine failed when using isolated NosL. This enzyme is responsible for the transformation of Trp into 3-methylindolic acid,[87] but these results account for its high substrate specificity, greatly limiting the number of analogues that could be produced by using this strategy. To obtain a 6′-fluorinated thiostrepton analogue, a 6-fluorinated quinaldic acid derivative **34** was fed to a culture of mutated *S. laurentii* lacking the *tsrT* gene. TsrT is a methyltransferase required in the initial steps of the conversion of Trp into quinaldic acid. By using this strategy, competition of unmodified Trp was completely avoided and only the desired 6′-fluorothiostrepton was obtained (Figure 10d).[88] In both cases, fluorination resulted in increased in vitro potencies (Figure 10c,d), thereby highlighting the huge potential of mutasynthetic methods.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

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 value, metal ions, and other additives. In this regard, in situ conversion of nocathiacin I into nocathiacin acid can be achieved without relying on semisynthetic methods.[89]

In general, most single point mutations are not tolerated and do not produce the expected mature analogues. This is especially evident in the codon-randomization experiment carried out with GE37468 A, where only 29 analogues, out of the 133 possible ones, were obtained.[79] A quick look to Figure 10 reveals that most active analogues are obtained from modifications of the tail and the "right-hand half" of thiopeptides. Similarly to what was concluded from the assessment of semisynthetic analogues, the "left-hand half" of these molecules, which contains the most preserved residues, must provide key contacts with their biological targets.

Despite some highly modified products being 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 the biosynthesis of thiopeptides cannot produce products that are very different from 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 derivatives or its corresponding processed metabolites has permitted the introduction of altered moieties derived from it, thus widening the chemical space that can be explored.

3. Summary and Outlook

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 stand-alone 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 either the preparation of libraries or 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 11).[90] First, a pre-peptide including both the structural and leading peptides could be synthesized by chemical methods (Figure 11 b), such as solid-phase peptide synthesis (SPPS), for ease of preparation. At this point, many modifications can be included, such as amino acid replacement or the introduction of non-proteinogenic amino acids, otherwise forbidden because of 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 the thiopeptides. Feeding with modified indole would introduce more diversity (Figure 11 c). After isolation, semisynthetic modifications could be made to make further improvements, such as attaching moieties for solubility enhancement (Figure 11 d).

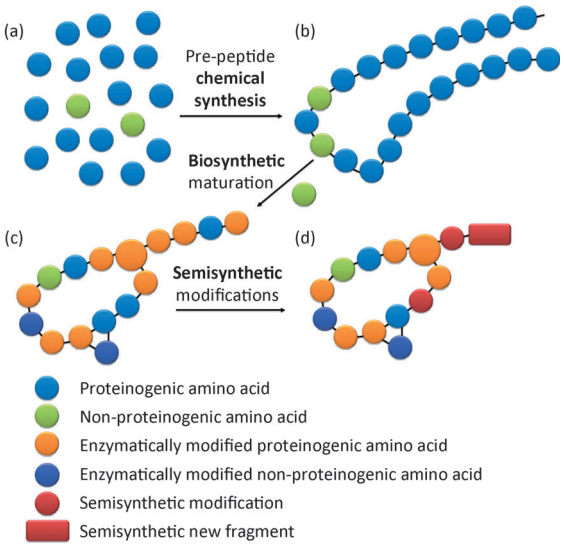


Figure 11. Proposed sequence for the future production of highly modified thiopeptide analogues: By using both proteinogenic and modified amino acids (a), altered synthetic pre-peptides could be produced (b). In vivo maturation should provide thiopeptides with their characteristic structural features, and other modified building blocks could be introduced (c). Semisynthetic modifications of the isolated product could introduce further alterations (d).

During the upcoming years we are likely to see new reports on thiopeptide analogues designed and obtained in all the ways described in this Review. The new results to come will surely provide a deeper understanding of the chemistry and biology of these fascinating natural products, putting to the test the versatility of synthetic strategies and methods, the selectivity of semisynthetic approaches, and the tolerance of biosynthetic machineries.[91]

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.

Received: August 19, 2013
Published online: May 23, 2014

[1] M. C. Bagley, J. W. Dale, E. A. 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] H. A. Kirst, *Expert Opin. Drug Discovery* **2013**, *8*, 479 – 493.

© 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[4] 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.
[5] 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.
[6] H. M. Müller, O. Delgado, T. Bach, *Angew. Chem.* **2007**, *119*, 4855 – 4858; *Angew. Chem. Int. Ed.* **2007**, *46*, 4771 – 4774.
[7] D. Lefranc, M. A. Ciufolini, *Angew. Chem.* **2009**, *121*, 4262 – 4265; *Angew. Chem. Int. Ed.* **2009**, *48*, 4198 – 4201.
[8] R. A. Hughes, S. P. Thompson, L. Alcaraz, C. J. Moody, *J. Am. Chem. Soc.* **2005**, *127*, 15644 – 15651.
[9] V. S. Aulakh, M. A. Ciufolini, *J. Am. Chem. Soc.* **2011**, *133*, 5900 – 5904.
[10] X. Just-Baringo, P. Bruno, L. K. Ottesen, L. M. Cañedo, F. Albericio, M. Álvarez, *Angew. Chem.* **2013**, *125*, 7972 – 7975; *Angew. Chem. Int. Ed.* **2013**, *52*, 7818 – 7821.
[11] J.-Y. Lu, M. Riedrich, K. P. Wojtas, H.-D. Arndt, *Synthesis* **2013**, *45*, 1300 – 1311.
[12] H.-D. Arndt, S. Schoof, J.-Y. Lu, *Angew. Chem.* **2009**, *121*, 6900 – 6904; *Angew. Chem. Int. Ed.* **2009**, *48*, 6770 – 6773.
[13] C. Li, W. L. Kelly, *Nat. Prod. Rep.* **2010**, *27*, 153 – 164.
[14] J. Vandeputte, J. D. Dutcher, *Antibiot. Ann.* **1955**, *3*, 560 – 561.
[15] J. E. Leet, H. A. Ax, D. R. Gustavson, D. M. Brown, L. Turner, K. Brown, W. Li, K. S. Lam, *Nocathiacin Antibiotics*, U.S. Patent US 6218398 B1, **2001**.
[16] J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi, M. Mayama, S. Matsuura, K. Matsumoto, *J. Antibiot.* **1976**, *29*, 366 – 374.
[17] E. Selva, G. Beretta, N. Montanini, G. S. Saddler, L. Gastaldo, P. Ferrari, R. Lorenzzetti, P. Landini, F. Ripamonti, B. P. Goldstein, M. Berti, L. Montanaro, M. Denaro, *J. Antibiot.* **1991**, *44*, 693 – 701.
[18] 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 – 5955.
[19] K. Engelhardt, K. F. Degnes, M. Kemmler, H. Bredholt, E. Fjaervik, G. Klinkenberg, H. Sletta, T. E. Ellingsen, S. B. Zotchev, *Appl. Environ. Microbiol.* **2010**, *76*, 4969 – 4976.
[20] H. Abe, K. Katsuhiko, S. Yoshinori, K. Mitsuaki, *Tetrahedron Lett.* **1988**, *29*, 1401 – 1404.
[21] J. Thompson, E. Cundliffe, M. Stark, *Eur. J. Biochem.* **1979**, *98*, 261 – 265.
[22] J. Thompson, E. Cundliffe, *Biochimie* **1991**, *73*, 1131 – 1135.
[23] B. T. Porse, I. Leviev, A. S. Mankin, R. A. Garrett, *J. Mol. Biol.* **1998**, *276*, 391 – 404.
[24] C. L. Myers, P. C. Hang, G. Ng, J. Yuen, J. F. Honek, *Bioorg. Med. Chem. Lett.* **2010**, *18*, 4231 – 4237.
[25] 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.
[26] K. C. Nicolaou, M. Nevalainen, M. Zak, S. Bulat, M. Bella, B. S. Safina, *Angew. Chem.* **2003**, *115*, 3540 – 3546; *Angew. Chem. Int. Ed.* **2003**, *42*, 3418 – 3424.
[27] K. C. Nicolaou, M. Zak, S. Rahimipour, A. A. Estrada, S. H. Lee, A. O'Brate, P. Giannakakou, M. R. Ghadiri, *J. Am. Chem. Soc.* **2005**, *127*, 15042 – 15044.
[28] K. C. Nicolaou, B. Zou, D. H. Dethe, D. B. Li, D. Y.-K. Chen, *Angew. Chem.* **2006**, *118*, 7950 – 7956; *Angew. Chem. Int. Ed.* **2006**, *45*, 7786 – 7792.
[29] A. L. Starosta, H. Qin, A. Mikolajka, G. Y. C. Leung, K. Schwinghammer, K. C. Nicolaou, D. Y.-K. Chen, B. S. Cooperman, D. N. Wilson, *Chem. Biol.* **2009**, *16*, 1087 – 1096.
[30] J. Bower, M. Drysdale, R. Hebdon, A. Jordan, G. Lentzen, N. Matassova, A. Murchie, J. Powles, S. Roughley, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2455 – 2458.
[31] B. N. Naidu, W. Li, M. E. Sorenson, T. P. Connolly, J. A. Wichtowski, Y. Zhang, O. K. Kim, J. D. Matiskella, K. S. Lam, J. J. Bronson, Y. Ueda, *Tetrahedron Lett.* **2004**, *45*, 1059 – 1063.
[32] B. N. Naidu, M. E. Sorenson, Y. Zhang, O. K. Kim, J. D. Matiskella, J. A. Wichtowski, T. P. Connolly, W. Li, K. S. Lam, J. J. Bronson, M. J. Pucci, J. M. Clark, Y. Ueda, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5573 – 5577.
[33] B. N. Naidu, M. E. Sorenson, J. J. Bronson, M. J. Pucci, J. M. Clark, Y. Ueda, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2069 – 2072.
[34] B. N. Naidu, W. Li, K. S. Lam, M. E. Sorenson, J. A. Wichtowski, T. P. Connolly, Y. Ueda, J. J. Bronson, Y. Zhang, O. K. Kim, *Water Soluble Thiazolyl Peptide Derivatives*, U.S. Patent US2002065219A1, **2002**.
[35] B. N. Naidu, M. E. Sorenson, J. D. Matiskella, W. Li, J. B. Sausker, Y. Zhang, T. P. Connolly, K. S. Lam, J. J. Bronson, M. J. Pucci, H. Yang, Y. Ueda, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3545 – 3549.
[36] A. Regueiro-Ren, Y. Ueda, *J. Org. Chem.* **2002**, *67*, 8699 – 8702.
[37] T. P. Connolly, A. Regueiro-Ren, J. E. Leet, D. M. Springer, J. Goodrich, X. S. Huang, M. J. Pucci, J. M. Clark, J. J. Bronson, Y. Ueda, *J. Nat. Prod.* **2005**, *68*, 550 – 553.
[38] L. Xu, A. K. Farthing, Y.-J. Shi, P. T. Meinke, K. Liu, *J. Org. Chem.* **2007**, *72*, 7447 – 7450.
[39] L. Xu, A. K. Farthing, J. F. Dropinski, P. T. Meinke, C. McCallum, P. S. Leavitt, E. J. Hickey, L. Colwell, J. Barrett, K. Liu, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3531 – 3535.
[40] P. Hrnciar, Y. Ueda, S. Huang, J. E. Leet, J. J. Bronson, *J. Org. Chem.* **2002**, *67*, 8789 – 8793.
[41] M. J. Pucci, J. J. Bronson, J. F. Barrett, K. L. Denbleyker, L. F. Discotto, C. Joan, J. C. Fung-Tomc, Y. Ueda, *Antimicrob. Agents Chemother.* **2004**, *48*, 3697 – 3701.
[42] A. Regueiro-Ren, B. N. Naidu, X. Zheng, T. W. Hudyma, T. P. Connolly, J. D. Matiskella, Y. Zhang, O. K. Kim, M. E. Sorenson, M. Pucci, J. Clark, J. J. Bronson, Y. Ueda, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 171 – 175.
[43] B. N. Naidu, M. E. Sorenson, T. Hudyma, X. Zheng, Y. Zhang, J. J. Bronson, M. J. Pucci, J. M. Clark, Y. Ueda, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3743 – 3746.
[44] J. Golik, H. S. L. Wong, S. H. Chcn, T. W. Doyle, J. J. K. Wright, J. Knipe, W. C. Rose, A. M. Casazza, D. M. Vyas, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1837 – 1842.
[45] T. Tojo, T. Hanadate, A. Okada, J. Kazami, S. Takeda, M. Shibazaki, *Preparation of Cyclic Thiopeptides (QN3323-A Derivatives) and Their Pharmaceutical Compositions for Multidrug-Resistant Bacteria*, U.S. Patent J2008115165A, **2008**.
[46] K. Junichi, O. Takashi, W. Masato, K. Kazuma, Y. Takashi, T. Kaniaki, *Preparation of O-Acyl-QN3323-A Oxime Thiopeptide Compounds as Antibacterial Agents*, U.S. Patent JP2009018991A, **2009**.
[47] 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. H. Yu, *J. Med. Chem.* **2012**, *55*, 6934 – 6941.
[48] P. Ferrari, L. Colombo, S. Stella, E. Selva, L. F. Zerilli, *J. Antibiot.* **1995**, *48*, 1304 – 1311.
[49] P. Tavecchia, P. Gentili, M. Kurz, C. Sottani, R. Bonfichi, E. Selva, S. Lociuro, E. Restelli, R. Ciabatti, *Tetrahedron* **1995**, *51*, 4867 – 4890.
[50] P. Tavecchia, M. Kurz, L. Colombo, R. Bonfichi, E. Selva, S. Lociuro, E. Marzorati, R. Ciabatti, *Tetrahedron* **1996**, *52*, 8763 – 8774.
[51] S. Lociuro, P. Tavecchia, E. Marzorati, P. Landini, B. P. Goldstein, M. Denarod, R. Ciabatti, *J. Antibiot.* **1997**, *50*, 344 – 349.
[52] A. Malabarba, M. Cavaleri, G. Mosconi, D. Jabes, G. Romano, *Use of Amide Derivative of GE 2270 Factor a3 for the Treatment of Acne*, U.S. Patent WO 03105881, **2007**.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

[53] J. Clough, S. Chen, E. M. Gordon, C. Hackbarth, S. Lam, J. Trias, R. J. White, G. Candiani, S. Donadio, G. Romanò, R. Ciabatti, J. W. Jacobs, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3409 – 3414.
[54] M. J. LaMarche, J. A. Leeds, J. Dzink-Fox, K. Gunderson, P. Krastel, K. Memmert, M. A. Patane, E. M. Rann, E. Schmitt, S. Tiamfook, B. Wang, *J. Med. Chem.* **2011**, *54*, 2517 – 2521.
[55] M. J. LaMarche, J. A. Leeds, J. Dzink-Fox, S. Mullin, M. A. Patane, E. M. Rann, S. Tiamfook, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3210 – 3215.
[56] M. J. LaMarche, J. Leeds, K. Amaral, J. Brewer, S. Bushell, J. Dewhurst, J. Dzink-Fox, E. Gangl, J. Goldovitz, A. Jain, S. Mullin, G. Neckermann, C. Osborne, D. Palestrant, M. A. Patane, E. M. Rann, M. Sachdeva, J. Shao, S. Tiamfbok, L. Whitehead, D. H. Yu, *J. Med. Chem.* **2011**, *54*, 8099 – 8109.
[57] M. J. LaMarche, S. Bushell, M. A. Patane, L. Whitehead, *Aminothiazoles and Their Uses*, U.S. Patent WO2007142986, **2010**.
[58] J. A. Leeds, M. J. LaMarche, J. T. Brewer, S. M. Bushell, G. Deng, J. M. Dewhurst, J. Dzink-Fox, E. Gangl, A. Jain, L. Lee, M. Lilly, K. Manni, S. Mullin, G. Neckermann, C. Osborne, D. Palestrant, M. A. Patane, A. Raimondi, S. Ranjitkar, E. M. Rann, M. Sachdeva, J. Shao, S. Tiamfook, L. Whitehead, D. Yu, *Antimicrob. Agents Chemother.* **2011**, *55*, 5277 – 5283.
[59] M. J. LaMarche, J. A. Leeds, A. Amaral, J. T. Brewer, S. M. Bushell, G. Deng, J. M. Dewhurst, J. Ding, J. Dzink-fox, G. Gamber, A. Jain, K. Lee, L. Lee, T. Lister, D. McKenney, S. Mullin, C. Osborne, D. Palestrant, M. A. Patane, E. M. Rann, M. Sachdeva, J. Shao, S. Tiamfook, A. Trzasko, L. Whitehead, A. Yifru, D. H. Yu, W. L. Yan, Q. M. Zhu, *J. Med. Chem.* **2012**, *55*, 2376 – 2387.
[60] A. Trzasko, J. A. Leeds, J. Praestgaard, M. J. LaMarche, D. McKenney, *Antimicrob. Agents Chemother.* **2012**, *56*, 4459 – 4462.
[61] L. S. L. Ting, J. Praestgaard, N. Grunenberg, J. C. Yang, J. A. Leeds, P. Pertel, *Antimicrob. Agents Chemother.* **2012**, *56*, 5946 – 5951.
[62] J. A. Leeds, M. Sachdeva, S. Mullin, J. Dzink-Fox, M. J. LaMarche, *Antimicrob. Agents Chemother.* **2012**, *56*, 4463 – 4465.
[63] S. S. Myatt, E. W.-F. Lam, *Nat. Rev. Cancer* **2007**, *7*, 847 – 859.
[64] N. S. Hegde, D. A. Sanders, R. Rodriguez, S. Balasubramanian, *Nat. Chem.* **2011**, *3*, 725 – 731.
[65] S. Schoof, S. Baumann, B. Ellinger, H.-D. Arndt, *ChemBioChem* **2009**, *10*, 242 – 245.
[66] S. Schoof, G. Pradel, M. N. Aminake, B. Ellinger, S. Baumann, M. Potowski, Y. Najajreh, M. Kirschner, H.-D. Arndt, *Angew. Chem.* **2010**, *122*, 3389 – 3393; *Angew. Chem. Int. Ed.* **2010**, *49*, 3317 – 3321.
[67] S. Baumann, S. Schoof, M. Bolten, C. Haering, M. Takagi, K. Shin-ya, H.-D. Arndt, *J. Am. Chem. Soc.* **2010**, *132*, 6973 – 6981.
[68] S. Schoof, H.-D. Arndt, *Chem. Commun.* **2009**, 7113 – 7115.
[69] B. Pandit, U. Bhat, A. L. Gartel, *Cancer Biol. Ther.* **2011**, *11*, 43 – 47.
[70] H. R. A. Jonker, S. Baumann, A. Wolf, S. Schoof, F. Hiller, K. W. Schulte, K. N. Kirschner, H. Schwalbe, H.-D. Arndt, *Angew. Chem.* **2011**, *123*, 3366 – 3370; *Angew. Chem. Int. Ed.* **2011**, *50*, 3308 – 3312.
[71] C. J. Schofield, J. E. Baldwin, M. F. Byford, I. Clifton, J. Hajdu, C. Hensgens, P. Roach, *Curr. Opin. Struct. Biol.* **1997**, *7*, 857 – 864.
[72] Q. Zhang, W. Liu, *Nat. Prod. Rep.* **2013**, *30*, 218 – 226.
[73] F. Zhang, W. L. Kelly, *In Vivo Production of Thiopeptide Variants*, Elsevier, San Diego, **2012**.
[74] M. G. Acker, A. A. Bowers, C. T. Walsh, *J. Am. Chem. Soc.* **2009**, *131*, 17563 – 17565.
[75] A. A. Bowers, M. G. Acker, A. Koglin, C. T. Walsh, *J. Am. Chem. Soc.* **2010**, *132*, 7519 – 7527.
[76] T. S. Young, C. T. Walsh, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 13053 – 13058.
[77] C. Li, F. Zhang, W. L. Kelly, *Mol. BioSyst.* **2011**, *7*, 82 – 90.
[78] C. Li, F. Zhang, W. L. Kelly, *Chem. Commun.* **2012**, *48*, 558 – 560.
[79] T. S. Young, P. C. Dorrestein, C. T. Walsh, *Chem. Biol.* **2012**, *19*, 1600 – 1610.
[80] A. A. Bowers, M. G. Acker, T. S. Young, C. T. Walsh, *J. Am. Chem. Soc.* **2012**, *134*, 10313 – 10316.
[81] A. A. Bowers, C. T. Walsh, M. G. Acker, *J. Am. Chem. Soc.* **2010**, *132*, 12182 – 12184.
[82] W. L. Kelly, L. Pan, C. Li, *J. Am. Chem. Soc.* **2009**, *131*, 4327 – 4334.
[83] R. Liao, W. Liu, *J. Am. Chem. Soc.* **2011**, *133*, 2852 – 2855.
[84] Y. Yu, H. Guo, Q. Zhang, L. Duan, Y. Ding, R. Liao, C. Lei, B. Shen, W. Liu, *J. Am. Chem. Soc.* **2010**, *132*, 16324 – 16326.
[85] Y. Yu, L. Duan, Q. Zhang, R. Liao, Y. Ding, H. Pan, E. Wendt, G. Tang, B. Shen, W. Liu, *ACS Chem. Biol.* **2009**, *4*, 855 – 864.
[86] Q. Zhang, Y. Li, D. Chen, Y. Yu, L. Duan, B. Shen, W. Liu, *Nat. Chem. Biol.* **2011**, *7*, 154 – 160.
[87] Q. Zhang, D. Chen, J. Lin, R. Liao, W. Tong, Z. Xu, W. Liu, *J. Biol. Chem.* **2011**, *286*, 21287 – 21294.
[88] L. Duan, S. Wang, R. Liao, W. Liu, *Chem. Biol.* **2012**, *19*, 443 – 448.
[89] M. Wei, S. Wang, Y. Fang, Y. Chen, *Bioresour. Technol.* **2010**, *101*, 3617 – 3622.
[90] A. Kirschning, F. Hahn, *Angew. Chem.* **2012**, *124*, 4086 – 4096; *Angew. Chem. Int. Ed.* **2012**, *51*, 4012 – 4022.
[91] After the submission of this review, the first library of fully synthetic thiopeptide analogues was reported: X. Just-Baringo, P. Bruno, C. Pitart, J. Vila, F. Albericio, M. Álvarez, *J. Med. Chem.* **2014,** DOI: 10.1021/jm500062g.

 © 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim