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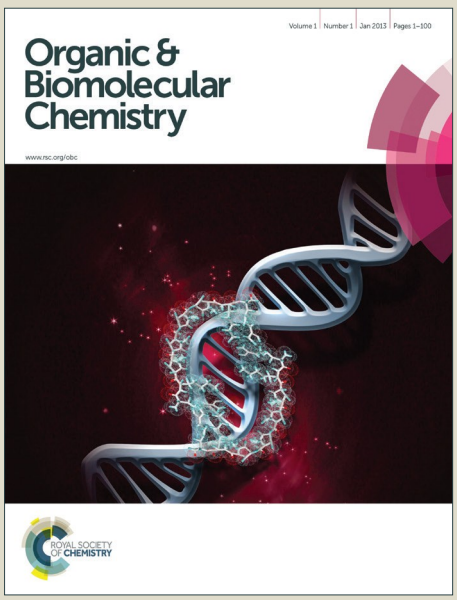

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REVIEW

Synthesis and Bioactivity of Antitubercular Peptides and Peptidomimetics: an Update

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Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), an infection that has been declared a global public health emergency by the World Health Organization. Current anti-TB therapies are limited in their efficacy and have failed to prevent the spread of TB, due to the long term drug compliance required and the genesis of multidrug-resistant strains (MDR). The number of chemotherapeutic agents currently available to treat MDR is limited, therefore there is a great need for new anti-TB drugs. Anti-TB peptides and peptidomimetics have emerged as an important and growing class of chemotherapeutic agents. This mini-review provides an update on peptides that exhibit very potent anti-TB activity, and their chemical syntheses, which could potentially be included in the pipeline for new anti-TB drug development.

1. Introduction

Mycobacterium tuberculosis (*Mtb*) is a Gram-positive pathogen responsible for most cases of tuberculosis (TB), which in 2013 alone resulted in 9 million new cases of TB and 1.5 million deaths.[1] Typical medicines used to treat tuberculosis are small organic molecules such as isoniazid, rifampicin, pyrazinamide and ethambutol, which are used as a first-line treatment option. However, if resistance to the first-line anti-TB drugs is observed, then second-line drugs are administered, which include aminoglycosides, D-cycloserine, ethionamide, protionamide, capreomycin (a cyclic pentapeptide), aminosalicylic acid and fluoroquinolones.[2] Usually, these drugs are effective if the patient complies with the long treatment period, but lack of compliance can lead to the development of multidrug resistant (MDR) or extremely drug resistant (XDR) *Mtb* strains, which are more difficult to treat. Bedaquiline is an anti-TB drug recently approved by the FDA for the treatment of MDR cases where other drugs have failed. However, bacterial resistance and poor patient outcome, has limited the application of bedaquiline as a second-line medication.[3,4] Therefore, due to the growing resistance to currently available anti-TB therapeutics, there is a pressing need for the development of new antitubercular agents. In particular, the discovery of molecules that aim for new biomolecular *Mtb* targets is important for the future development of drugs to treat MDR and XDR strains. The biomolecular targets inhibited by existing *Mtb* drugs and new targets are listed in Table 1 and Table 2 respectively.[5] The list is not exhaustive and only intends to illustrate the growing list of targets available to combat persistent *Mtb* and its mutants.

Table 1 Biomolecular targets inhibited by existing *Mtb* drugs

Anti-TB medicine	Target
Isoniazid	Enoyl-acyl-carrier protein-reductase
Rifampicin	DNA dependent RNA polymerase
Pyrazinamide	Fatty acid synthase
Ethambutol	Arabinosyltransferase
Ethionamide	β-ketoacyl ACP synthase
D-cycloserine	D-alanyl-D-alanine ligase
Aminoglycosides	Ribosome
Capreomycin	
Fluoroquinolone	DNA gyrase
Bedaquiline[6]	ATP synthase (new target)

Table 2 New biomolecular targets for anti-TB drug development.[5]

New Targets	
Deformylase	Mycothiol ligase
Maltosyltransferase	Mycolic acid cyclopropanation,
L,D-transpeptidase	Methionine aminopeptidase
Decaprenylphosphoryl-β-D-ribose 2′-epimerase	The proteasome complex
	Isocitrate lyase
ATP phosphoribosyl transferase	Cell wall lipid II or III

Naturally occurring peptides and their derivatives are an important and growing class of biopharmaceuticals with a current success rate approximately twice that of small molecule drugs.[7] This has been attributed to the advantages of peptides over their small organic counterparts, such as increased target affinity, and fewer off-target side-reactions and thus less side effects.

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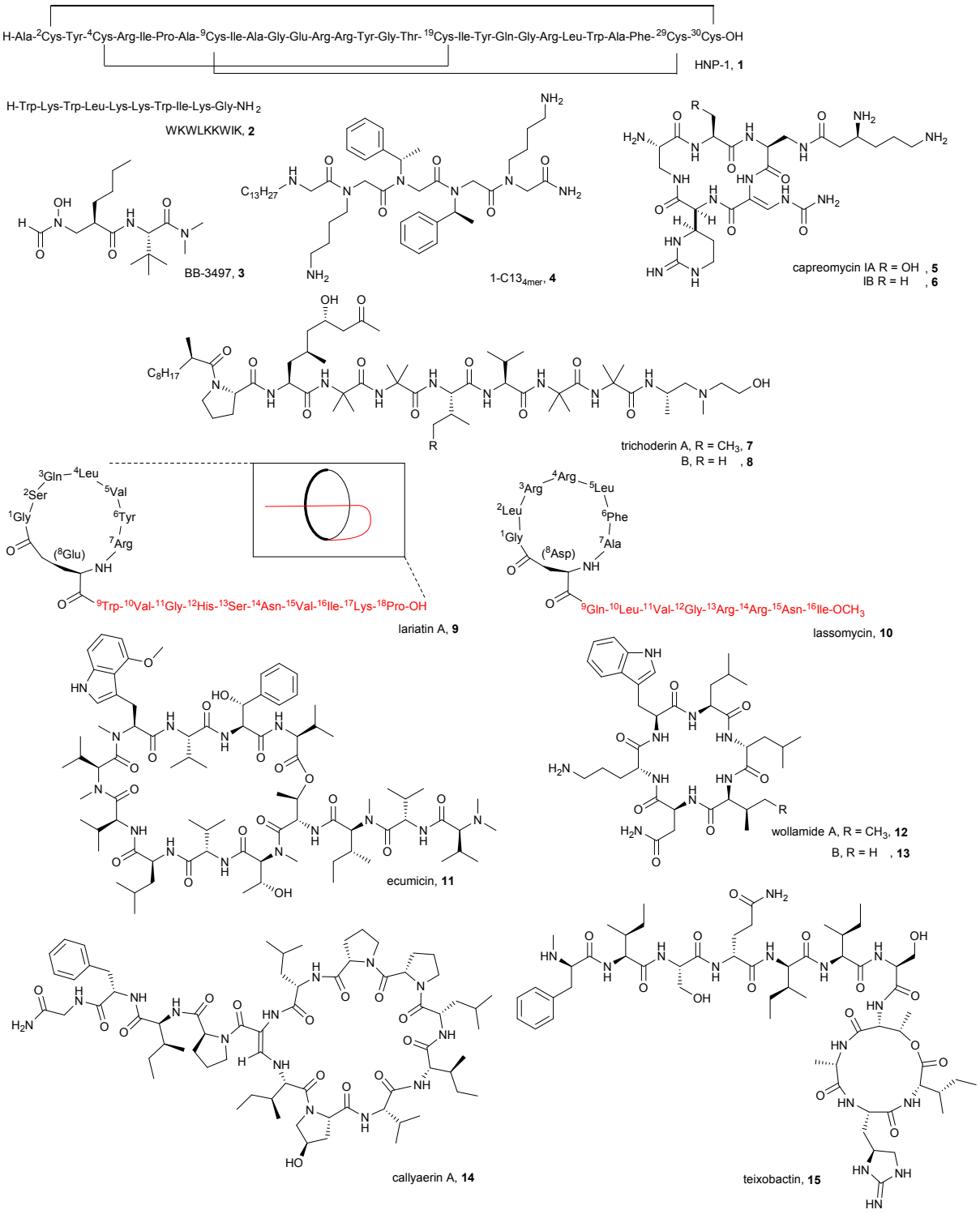


Fig 1. Structures of potent antitubercular peptides and peptidomimetics.

Table 3. Potent antitubercular peptides and peptidomimetics discussed in this review.

Peptide	Source	MIC, μg/mL (strain)	Cytoxicity	Mechanism/Target	Ref.
HNP-1 (**1**)	Innate immunity	2.5 (H37Rv)	None	Microbial membrane disruption	12
WKWLKKWIK (**2**) (2013)	Peptide library	1.5 MIC_{90} (H37Rv)	Low against macrophage-like human THP-1 cells	Possibly microbial membrane disruption	13
BB-3497 (**3**) (2001)	Metalloenzyme inhibitor library[14]	0.25 MIC_{90} (H37Rv)	Not determined	Peptide deformylase	15
1-$C13_{4mer}$ (**4**) (2011)	Synthetic	5.3 (BCG) 5.5 (H37Rv)	None against Raw 264.7 and J774 mouse macrophage cell lines	Microbial membrane disruption	16
capreomycin (**5,6**) (1960)	*Streptomyces Capreolus*	2.0 (H37Rv)	Ototoxic	Ribosome	17
trichoderin A (**7**) (2010)	Marine sponge-derived fungus of	0.02 (BCG) 0.12 (H37Rv)	Not determined	ATP synthase	18,19
trichoderin B (**8**)	*Trichoderma sp.*	0.02 (BCG) 0.13 (H37Rv)	Not determined	ATP synthase	18,19
lariatin A (**9**) (2006)	*Rhodococcus jostii* K01-B0171	0.39 (H37Rv)	Not determined	Inhibits cell wall biosynthesis	20
lassomycin (**10**) (2014)	Collection of soil actinomycetes	0.78-1.56 (H37Rv)	None against NIH 3T3 and HepG2 cells	ATP-Dependent Protease ClpC1P1P2	21
ecumicin (**11**) (2014)	*Nonomuraea sp*. MJM5123	0.26 (H37Rv)	None against VERO cells	ClpC1 ATPase complex	22
wollamide A (**12**) (2014)	*Streptomyces nov. sp.* (MST-115088)	2.11 (BCG)	None against macrophages	Not determined	23
wollamide B (**13**)		2.29 (BCG)	None against macrophages	Not determined	23
callyaerin A (**14**) (2015)	Marine sponge (*Callyspongia aerizusa*)	2.0 MIC_{90}	None against THP-1 and MRC-5 cell lines	Not determined	24
teixobactin (**15**) (2015)	β-*proteobacterium Eleftheria terrae*	0.16 (H37Rv)	None against NIH/3T3 mouse embryonic fibroblast	Lipid II and III	25

MIC_{90} = minimal inhibitory concentration for 90% bacterial growth inhibition.

However, peptides containing only natural ribosomally synthesised amino acids have some disadvantages, such as poor *in vivo* stability and oral bioavailability, which limits their potential use as therapeutics. Peptide *in vivo* instability can be overcome by introducing unnatural amino acids (posttranslationally modified amino acids, D-amino acids, *N*-alkylated amino acids) or structural constraints (cyclic peptides and cyclic amino acids) into the peptide sequence. However, the design of peptides with favourable oral bioavailability remains challenging. Thus, based on the rich source of naturally occurring peptides and their wide range of biological activities, peptides represent a promising source of potential pharmaceutical agents to treat TB.

Recently, several comprehensive reviews on anti-TB peptides that were derived from natural sources have been reported.[8-11] However, this review focuses specifically on anti-TB peptides and peptidomimetics that exhibit high potency (Figure 1 and Table 3, **1-15**), with a particular emphasis on recently reported anti-TB peptides. In addition, analysis and advances of their chemical syntheses, where available, will also be discussed, with the objective of providing the reader with an insight as to what is involved when trying to generate peptide analogues for development as lead compounds with improved pharmacological properties.

2. Innate Immunity and Synthetic Peptides

The human neutrophil tetradecapeptide 1 (HNP-1, **1**) is one of the six human α-defensins which are part of the arsenal of innate immunity to combat infectious microorganisms. The main structural characteristic of α-defensins is a three-stranded β-sheet core stabilized by three intramolecular disulfides (Figure 1). Defensins are active against a broad range of bacteria, fungi, and viruses; they can also act as immuno-modulators and are known to neutralize secreted bacterial toxins.[26] It is thought that the bactericidal mechanism of defensins mainly involves microbial membrane disruption. Previous work has shown that HNP-1 (**1**) kills *Mtb* H37Rv *in vitro* and *in vivo* by primarily binding to the plasma membrane/cell wall while deoxyribonucleic acid (DNA) appears to be a secondary target.[12,27]

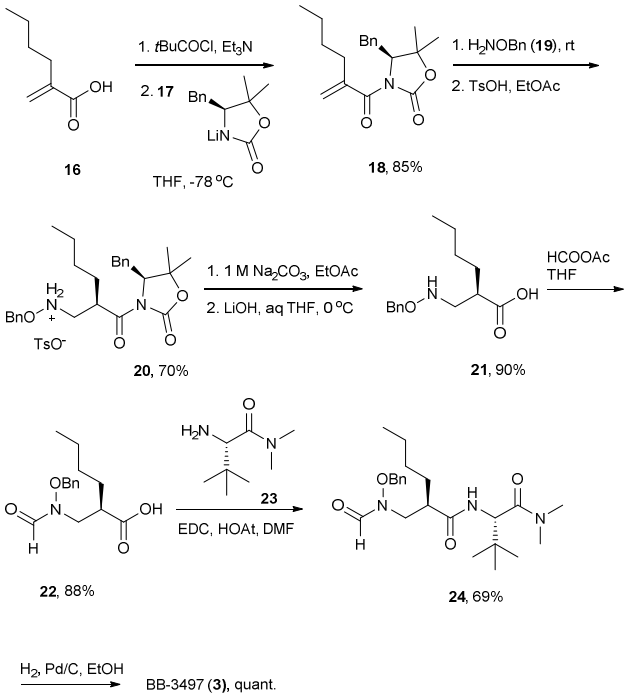
Importantly and interestingly, low plasma concentrations of HNP-1 (**1**) have been associated with the genesis of MDR.[28] The synthesis of HNP-1 (**1**) and analogues can be accomplished using conventional solid phase peptide synthesis (SPPS) protocols,[6,26] however, the regiospecific disulfide bond formation is synthetically challenging.[29] Moreover, the disadvantages of using large peptides containing solely natural amino acids for anti-TB treatment, such as HNP-1 (**1**), are their low plasma stability and high cost of production.

In contrast to HNP-1 (**1**), a short synthetic nonapeptide, H-WKWLKKWIKG-NH_2 (**2**), was recently reported to exhibit broader antimicrobial activity and more potent anti-TB activity than (**1**).[13] Nonapeptide (**2**) was discovered by screening libraries of cationic antimicrobial peptides, which are typically inactive against *Mtb* due to the resistance conferred by the bacterial envelope, for activity against *Mtb*. Surprisingly, nonapeptide (**2**) exhibited a potent MIC_{90} value of 1.5 μg/mL. The synthesis of nonapeptide (**2**) was performed using standard Fmoc-SPPS, and due to its short peptide sequence, it is anticipated that large scale production of nonapeptide (**2**) can be achieved in a cost-efficient manner. However, given the all-L amino acid sequence for nonapeptide (**2**), it is also anticipated that peptide (**2**) might exhibit poor *in vivo* stability and therefore, further peptide modifications might be necessary to develop (**2**) into a potential lead anti-TB drug candidate.

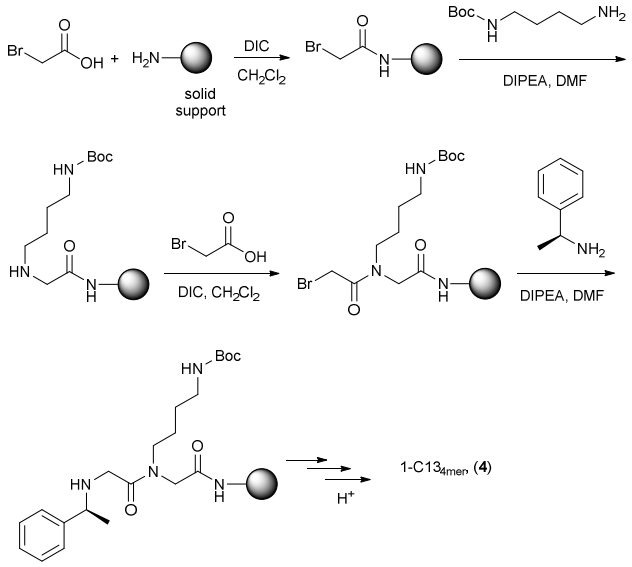
3. Peptidomimetics

BB-3497 (**3**) is a peptidomimetic that inhibits peptide deformylase (PDF), an essential bacterial metalloenzyme which deformylates the *N*-formylmethionine of newly synthesized proteins. Initially, it was shown that BB-3497 (**3**) displayed moderate bacteriostatic activity against several Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis*.[14] A few years later, BB-3497 (**3**) was found to exhibit potent activity against *Mtb* (MIC 0.25 μg/mL), a level of potency that is similar to front line anti-TB medicines, such as rifampicin and isoniazid.[15] In 2001, prior to the discovery of its anti-TB activity, the asymmetric synthesis of BB-3497 (**3**) was reported (Scheme 1).[30] The synthesis of **3** was initiated by treating carbonyl-activated acrylic acid **16** with oxazolidinone **17**, to provide homochiral acrylate **18**. Acrylate **18** then underwent an asymmetric Michael addition reaction with amine **19**, to yield the single Michael diastereomer **20**. The chiral auxiliary of **20** was then removed under basic conditions, and the resulting amino acid **21** was formylated to give formamide **22**. Formamide **22** was then coupled to *tert*-leucine **23**, to give *O*-benzyl protected dipeptide **24**, after which removal of the benzyl protecting group of **24** using catalytic hydrogenation provided the final product **3**. Since this initial synthesis of BB-3497 (**3**), no other synthetic strategies have been reported.

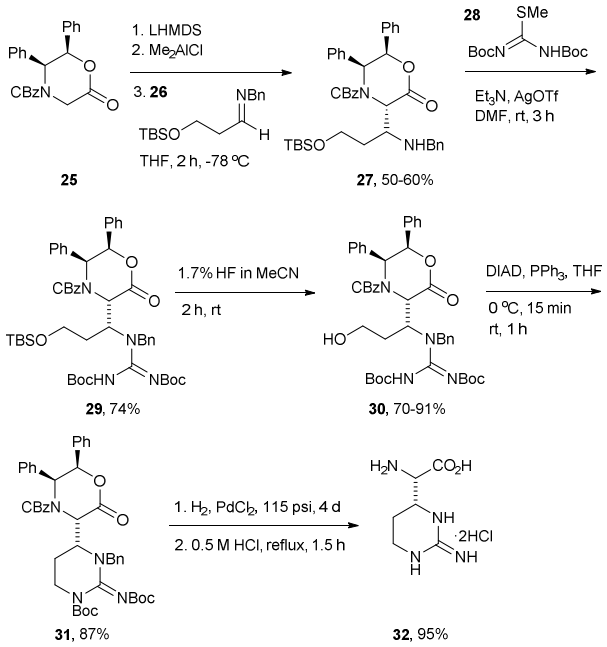
In order to provide further insight into the mechanism of action of BB-3497 (**3**), several extensive structure activity relationship studies (SAR) have been carried out.[31-33] A series of BB-3497 analogues where the *N*-formyl-hydroxylamine metal binding moiety was substituted by *N*-acetyl hydroxylamine, *N*-formylamine, hydrazide, amidoxime, *N*-hydroxyurea, thiol, carboxylic acid or phosphorous based chelants were synthesised and evaluated against *Escherichia coli* PDF.Nickel (PDF.Ni) enzyme and against *E. coli* (Gram-negative) and *Staphylococcus capitis* (Gram-positive). BB-3497 and an analogue containing the hydroxamic acid were strong PDF.Ni inhibitors (IC_{50} = 7 and 1 nM respectively), however, BB-3497 showed the strongest antimicrobial activity. The rest of the analogues were mainly inactive against the enzyme and had no antimicrobial activity. Therefore, it was concluded that the *N*-formyl-hydroxylamine metal binding moiety was crucial for enzyme inhibition and antibacterial activity.[33] BB-3497 derivatives with a methyl substituted methylene, two and no methylene units between the *N*-formyl hydroxylamine functionality and the n-butyl side-chain were also inactive against PDF.Ni and had no antimicrobial activity, thus showing that the optimal distance is one unsubstituted methylene unit.[32] In addition, BB-3497 analogues where the n-butyl group was substituted by side-chains such as alkyl, cycloalkyl, benzyl, etc., were prepared. The compounds containing n-butyl (BB-3497) and cyclopentylmethyl groups showed optimal enzyme inhibition and antibacterial activity. Interestingly, although less potent than BB-3497, most derivatives were still active against PDF, but showed a decrease or lack of antimicrobial activity. These differences were attributed to variation in compound lipophilicities.[32] Furthermore, it was revealed that substitutions of the *tert*-butyl and dimethyl amide moieties were well tolerated, and that these moieties can be investigated further to optimize the physicochemical properties of peptidomimetic **3**.[31]
Unfortunately, SAR studies of BB-3497 (**3**) have not been conducted with *Mtb*.



Scheme 1 Synthesis of BB-3497 (3)



Scheme 2 Submonomer solid phase peptoid synthesis of 1-C13$_{4mer}$ (**4**).



Scheme 3 Synthesis of (2*S*,3*R*)-capreomycidine (**32**).

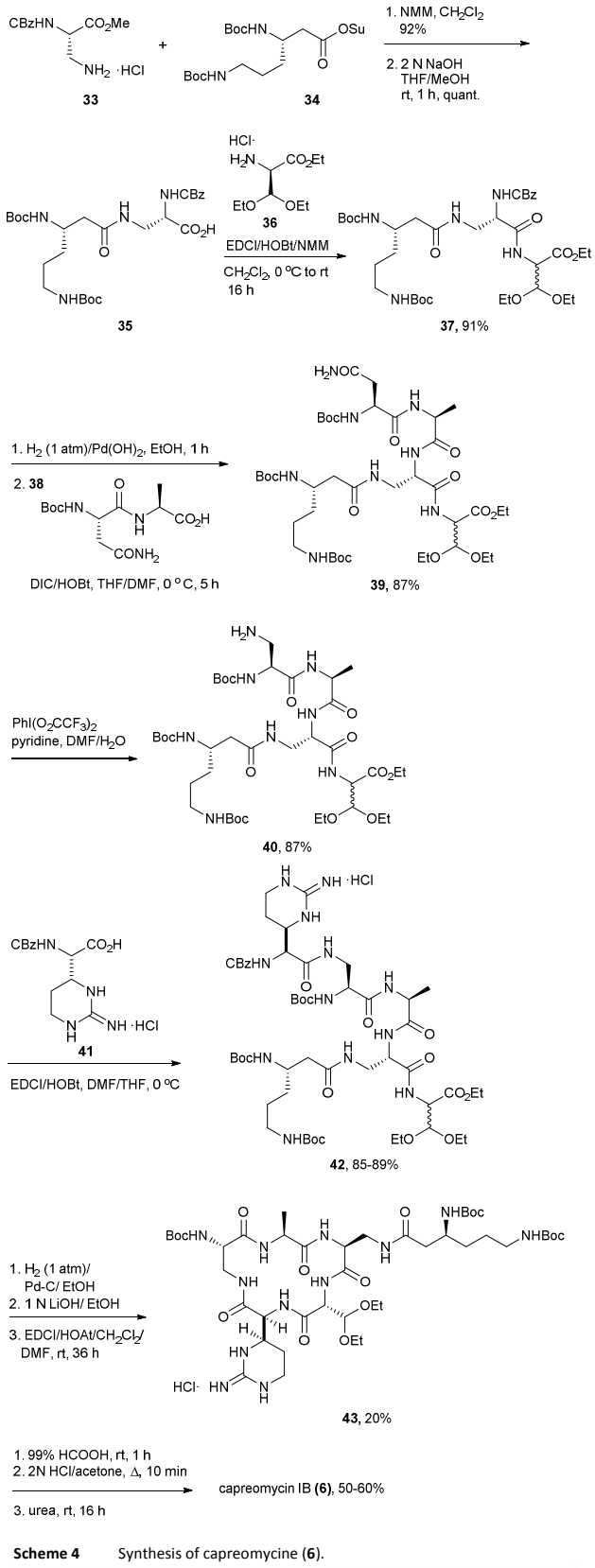
Peptoids are another class of peptidomimetics where the lateral side-chains are attached to the nitrogen atom of the amide bond instead of the α-carbon. This feature gives peptoids increased proteolytic stability.[34] A potent anti-TB 4-mer peptoid, named 1-C13$_{4mer}$ (**4**) has been reported in the literature.[16] This peptoid is composed of two *N*-lysine residues (*N*-(4-aminobutyl)glycine), two (*S*)-*N*-(1-phenylmethyl) glycine units and a *N*-(tridecyl)glycine residue at the *N*-terminus. This composition gives 1-C13$_{4mer}$ (**4**) surfactant-like character that could presumably direct its activity towards *Mtb* membrane disruption. The synthesis of **4** was easily accomplished by the submonomer solid phase method,[35] which consists of consecutive attachment of bromoacetic acid via DIC activation onto the solid support, followed by nucleophilic substitution of the corresponding side-chain-containing amino moiety (Scheme 2).

4. Peptides from Natural Sources

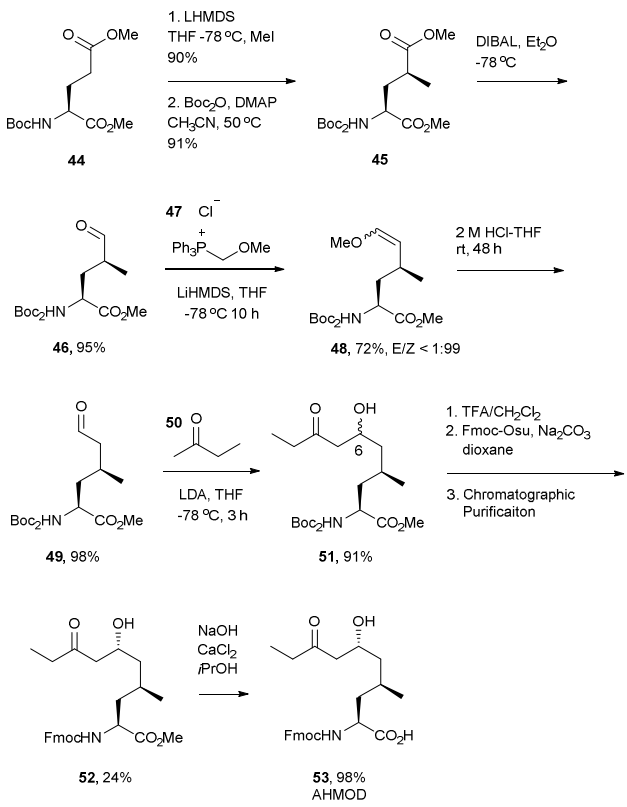
4.1 Capreomycin

Capreomycin is one of the few antitubercular peptide medicines that is currently used to treat cases of MDR TB. A family of four capreomycin peptides was first discovered in 1960, and both capreomycin IA (**5**) and IB (**6**) have been co-administered intramuscularly to treat cases of MDR TB for over 25 years. However, capreomycin usage is limited due to its renal and auditory toxicities.[1] The capreomycins contain two diaminopropanoic acid residues, the α,β-unsaturated amino acid ureido-dehydroalanine and the cyclic guanidino amino acid (2*S*,3*R*)-capreomycidine. Several early reports on the synthesis of capreomycin are found in the literature, however, most of these syntheses are low yielding,[36] which is attributed to the difficult stereospecific preparation of the key component capreomycidine. Therefore, capreomycin is commercially prepared by biosynthesis.[29]

One of the most recent successful chemical syntheses of capreomycidine and capreomycin IB (**6**) was reported in 2003.[37] In this account, the synthesis of capreomycidine was initiated by the enolate-aldimine reaction between chiral glycinate **25** and benzyl imine **26** (Scheme 3), which yielded the Mannich product **27** as a mixture of diastereomers, (3.3:1; (2*S*,3*R*):(2*S*,3*S*)) that were epimeric at the α-carbon. Guanidylation of **27** with isothiourea **28** only took place with the major diastereomer (2*S*,3*R*), to give **29** in 74% yield. Removal of the *tert*-butyldimethylsilyl protecting group generated alcohol **30**, which was followed by an intramolecular Mitsunobu cyclization reaction to yield compound **31**. Final protecting group removal gave (2*S*,3*R*)-capreomycidine (**32**) in a 20% overall yield.



Scheme 4 Synthesis of capreomycine (**6**).



Scheme 5 Synthesis of (2*S*)-amino-(6*R*)-hydroxy-(4*S*)-methyl-8-oxodecanoic acid (AHMOD) (**53**).

With the capreomycidine building block in hand, capreomycin IB (**6**) was then synthesized using solution phase methods (Scheme 4). Terminally protected diaminopropanoic acid **33** was coupled with lysine **34**, followed by hydrolysis of the methyl ester to give dipeptide **35**. Coupling of **35** with *R*-α-formylglycine diethylacetal ethyl ester **36** provided tripeptide **37** as a diastereomeric mixture (2.6:1 of *R*:*S* epimers). Tripeptide **37** was then coupled to dipeptide **38** to give pentapeptide **39** in 87% yield. Chemoselective Hofmann rearrangement facilitated the direct conversion of the primary amide of the asparagine residue in **39** into the primary amine in **40**. Coupling of **40** with a protected (2*S*,3*R*)-capreomycidine (**41**) gave hexapeptide **42**. After CBz removal and ethyl ester hydrolysis of hexapeptide **42**, intramolecular cyclisation using EDCI provided the cyclic peptide **43** in a low 20% yield. Interestingly, only the desired diastereomer was obtained after macrocyclization indicating that its linear precursor cyclizes more efficiently than the undesired diastereomer. Lastly, the Boc protecting group of **43** was removed and the acetal moiety was cleaved with acid, followed by reaction of the corresponding aldehyde intermediate with urea, to give capreomycin IB (**6**).

4.2 Trichoderins

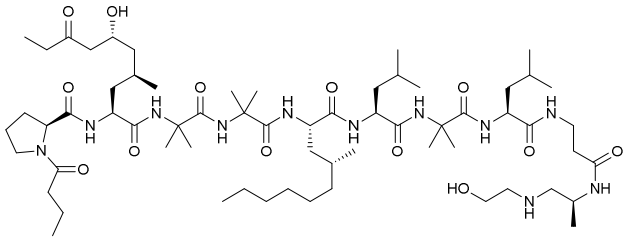


Figure 2 Structure of culicinin D (**54**).

Trichoderins (**7-8**) are peptaibols (peptides containing α-aminoisobutyric acid (Aib) and a *C*-terminal alcohol) which have shown to be very potent against dormant and live *Mtb* bacilli.[18] This anti-TB activity of trichoderins has been assigned to the inhibition of bacterial ATP synthase.[19] Trichoderins contain the unnatural amino acid 2-amino-hydroxy-4-methyl-8-oxodecanoic acid (AHMOD) and a (2*R*)-methyl octyl chain at the *N*-terminus. The synthesis of trichoderins is complex due to the presence of AHMOD, the *C*-terminal alcohol moiety and consecutive sterically hindered Aib residues. Synthetic protocols to prepare AHMOD can be found in the literature,[38] but most are lengthy, low yielding and difficult to scale up. However, an improved synthesis of a stereoisomer of this uncommon amino acid was recently reported (Scheme 5).[39] This synthesis began with the stereoselective methylation of *N*-Boc glutamic acid dimethyl ester **44**, to afford compound **45**, which is regioselectively reduced to aldehyde **46**. Conversion of **46** to the desired homologated aldehyde **49** proceeded via a Wittig reaction with the ylide of **47** via alkene **48** which was further hydrolysed to **49** under acidic conditions. The aldol reaction of **49** with the enolate of 2-butanone (**50**) gave the desired aldol products **51**, as an inseparable 1:1 mixture of C6 epimers. Removal of the Boc protecting group under conventional acidic conditions followed by protection of the crude amine with an Fmoc protecting group, facilitated the chromatographic separation of the desired diaestereomer **52** in a low overall 24% yield. The final methyl ester hydrolysis of **52** to give AHMOD **53**, proceeded in excellent yield (98%) under basic conditions that maintained the integrity of the Fmoc protecting group and the β-hydroxyketone moiety.

To the best of our knowledge the total synthesis of the trichoderins **7-8** has not been reported to date, but the preparation of the closely related compound culicinin D (**54**) has been accomplished via solid phase peptide synthesis (Figure 2).[38,40] During the synthesis of culicinin D (**54**), two problematic steps were encountered; these were the attachment of the alcohol unit to the solid support, and the difficult couplings of the consecutive Aib residues. These problems were minimized by attaching the amino group of the aminoalcohol building block to the resin instead of the alcohol, which then undergoes an intramolecular *O–N* acyl shift upon peptide synthesis completion, and by coupling the Aib residues with the powerful coupling agent fluoro-*N,N,N',N'*-tetra-methylformamidinium hexafluorophosphate.[40] However, the reported final overall yield of culicinin D is still low (6%), and

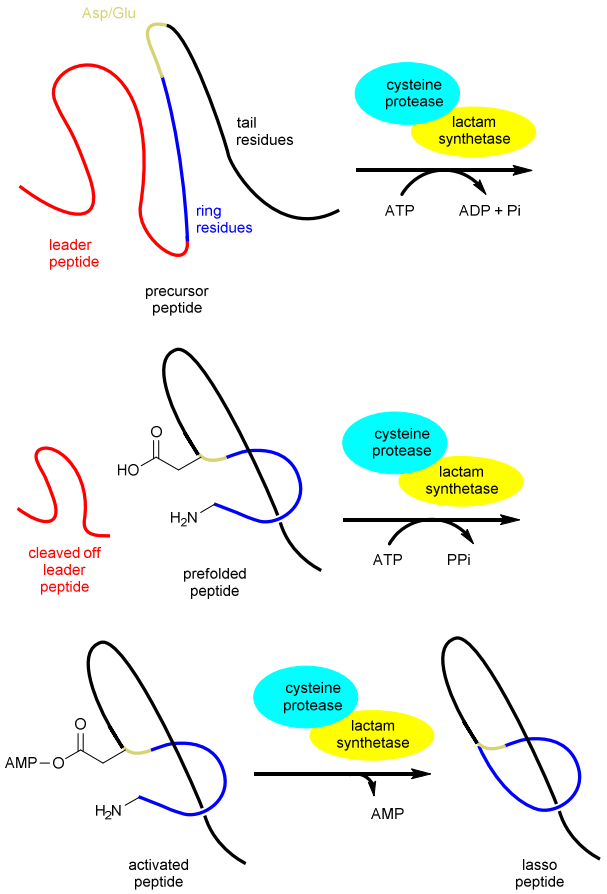
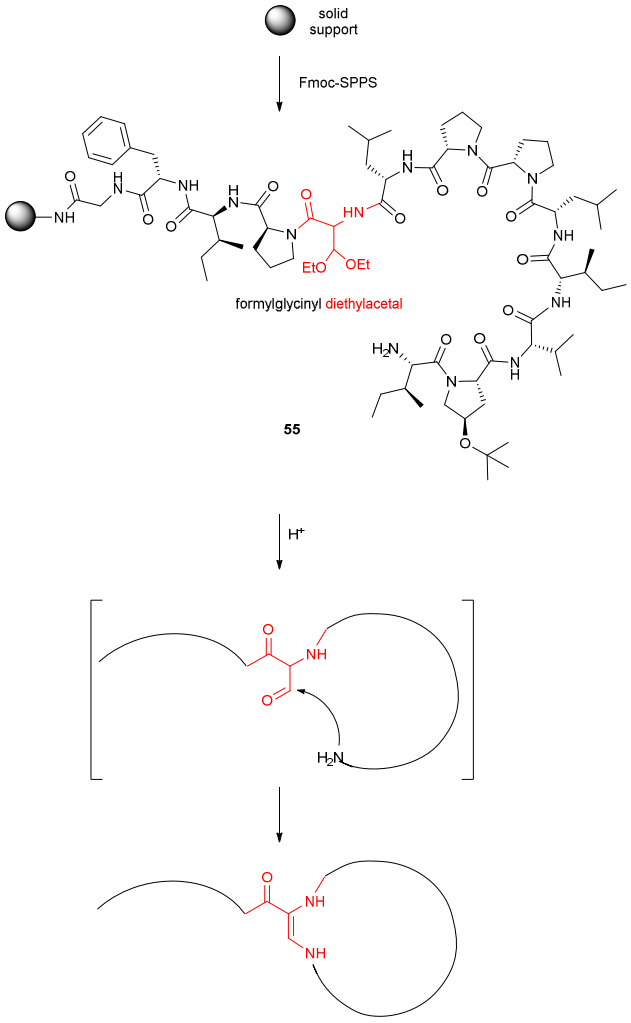


Figure 3 Proposed biosynthesis of lasso peptides.[42]

further work is required in order to access larger amounts of this compound. Due to the structural similarity of trichoderins **7-8** and culicinin D (**54**), it is anticipated that similar challenges will be encountered during the synthesis of the trichoderins.

4.3 Lasso Peptides

Lariatin A (**9**) is a lasso peptide, in which the *C*-terminal segment (Trp9-Pro18) passes through the ring structure formed by the N-terminal segment (Gly1-Glu8) (see insert figure in the structure of **9** in Fig 1).[41] Lariatin A (**9**) was selective against mycobacteria with strong activity against M. tuberculosis. It is believed that Lariatin A (**9**) inhibits cell wall biosynthesis,[20] however, this mechanism of action has not been validated. Lassomycin (**10**) is another newly discovered lasso peptide, which showed potent activity (MIC 0.8–3 μg/mL), against a variety of M. tuberculosis strains, including MDR and XDR isolates.[21] It has been reported that lassomycin kills Mtb by targeting ATP-dependent protease ClpC1P1P2.[21]



Scheme 6 Proposed synthetic strategy to callyaerin A (**14**). The amino acid sequence of callyaerin A (**14**) has been substituted by curved lines (left) to emphasize the cyclisation that leads to the formation of the key enediamino group.

To date, the chemical synthesis of lasso peptides has not been reported, but their biosynthesis is well understood, thus allowing the engineering of novel structures.[34] Biosynthetically, lasso peptides are ribosomally synthesized and posttranslationally modified, and this process requires at least three genes which encode for a precursor peptide, a cysteine protease, and an ATP-dependent lactam synthetase (Figure 3). The precursor peptide is composed of an *N*-terminal leader peptide, which is fused to the core and tail of the lasso peptide sequence. It is hypothesised that the leader peptide acts as a chaperone to mediate pre-folding during maturation, because the threading cannot occur after ring formation. Thus, once the precursor peptide is folded, the *N*-terminal leader peptide is cleaved, and macrolactamization of the activated peptide generates the mature peptide with a lasso topology.[42]

4.4 Cyclic Peptides

Finally, the largest class of peptides with anti-TB activity are cyclic peptides. Ecumicin (**11**) is a newly discovered cyclotetradepsipeptide which has shown potent anti-TB activity against *Mtb* H37Rv and streptomycin, rifampicin and cycloserine resistant *Mtb* strains.[43] Ecumicin contains several unnatural amino acids in its sequence, all of which are commercially available or can be prepared from commercially available building blocks. For example *N*-methyl-4-methoxytryptophan can be synthesised from the readily available 4-methoxytryptophan using well known *N*-methylation techniques.[44] Although the chemical synthesis of ecumicin (**11**) has not been reported, it is anticipated that its synthesis can be accomplished based on previous reports of structurally similar compounds. Recently, the bactericidal mode of action of ecumicin (**11**) was attributed to decoupling of ClpC1-mediated ATP hydrolysis from ClpP1P2 proteolysis. ClpC1 is an *Mtb* essential hexameric ATPase, which associates and supports ATP-dependent protein degradation by ClpP, a compartmentalized protease complex found in many bacteria, mitochondria, and chloroplasts. The function of ClpC1 is to bind certain cell proteins and to unfold and translocate them into ClpP for degradation.[22] Thus, by inhibiting the action of ClpC1, ecumicin (**11**) stimulates ATPase activity.

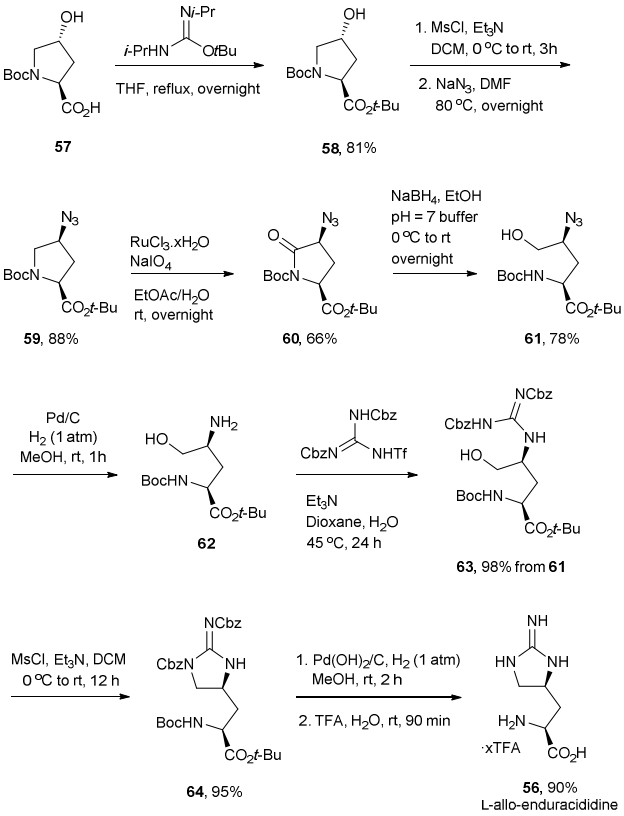
The wollamides **12-13** are cyclohexapeptides containing the non-natural amino acids D-ornithine (D-Orn), D-Leu, or allo-Ile. These peptides were active against *M. bovis*, BCG, but their activity against *Mtb* has not been reported.[23] Moreover, the simple structure of these wollamides when compared to other peptides described herein, suggests them to be potential drug lead candidates for future development of anti-TB agents.

Callyaerin A (**14**) is a naturally occurring cyclic peptide derived from the marine sponge *Callyspongia aerizusa*, for which the cyclic link occurs between the *N*-terminus and the side-chain of an α,β-dehydroamino acid,[45] namely (*Z*)-2,3-diaminoacrylic acid.[24] Callyaerin A (**14**) exhibited potent activity against *Mtb* with no observed cytotoxicity. It is anticipated that both the enediamino group and proline residues in the cyclic ring provides rigidity to the system, which might promote its bioactivity, whilst the hydrophobic residues in the linear chain might contribute to the bactericidal specificity of the peptide.[24] The chemical synthesis of the callyaerins has not

been reported to date, but one can propose that its synthesis could be accomplished by SPPS through intermediate **55** (Scheme 6), where a suitably protected α-formylglycine diethylacetal analogue (**36** in Scheme 4) is introduced as a suitable precursor to the enediamino group in **14** that is formed upon cyclization.

Teixobactin (**15**) is an undecapeptide which comprises a cyclotetradepsipeptide containing the unnatural amino acid enduracididine. Teixobactin exhibits potent activity against *Mtb* (MIC 0.16 μg/mL), and unlike any known anti-TB agents, it inhibits the synthesis of important bacterial cell wall components, namely peptidoglycan and teichoic acid, by binding to their precursors, lipid II and III respectively.[25] The main target of teixobactin is lipid II (teixobactin binds to lipid II in a 2:1 ratio). Lipid II is a glycopeptide that stabilizes the exterior cell wall via saccharide polymerization and peptide side-chain cross-linking. This unique mechanism of action is believed to minimize the development of resistance, which makes teixobactin a promising lead for *Mtb* drug development. There are several antibacterial depsipeptides that are known to be either active against *Mtb* (i.e. lydiamicin A)[41] or to interact with lipid II (i.e katanosin B, plusbacin, enduracidin and ramoplanin),[46,47] although, the nature of this interaction remains elusive to date. However, given the structural similarity between teixobactin and these depsipeptides one can propose that the positively charged guanidine side chain plays an important role presumably by interacting with the phosphate moiety of the phospholipid.

To improve the potency and to gain further understanding of the biological properties of teixobactin (**15**), it is crucial to execute its total chemical synthesis. The biggest challenge to producing teixobactin is the synthesis of L-allo-enduracididine (**56**) (Scheme 7), an amino acid which is biochemically synthesized from arginine.[48] Some reports on the synthesis of **56** or analogues can be found in the literature but they either proceed with poor stereoselectivity or are difficult to scale up.[49,50] Recently, an improved (stereoselective-scalable) synthetic protocol of **56** was reported (Scheme 7).[51] The synthesis commenced with the protection of the carboxylate group of the *N*-Boc protected *trans*-hydroxyproline (**57**) to give *tert*-butyl ester **58**. This ester was treated with methanesulfonyl chloride and base to generate the corresponding mesylate which was substituted by an azido group to generate azidoproline **59**. Compound **59** was then oxidized to lactam **60**. Reductive ring opening of **60** was accomplished with $NaBH_4$ in a mixture of ethanol/buffer pH = 7 to yield **61**. In this step, the presence of the sterically hindered *tert*-butyl protecting group was advantageous, since it helped to inhibit lactone formation during the ring opening reaction. The azide of **61** was then reduced by Pd catalyzed hydrogenation to give the amino compound **62**, which was then smoothly guanidinylated to give **63** in excellent 98% yield. The key ring closure step was accomplished by nucleophilic substitution of the protected guanidine to the mesylated



Scheme 7 Recently reported synthesis of L-allo-enduracididine (**56**).

hydroxyl group of **63**, which generated **64**. Final protecting group removal gave enduracididine **56** in 30% overall yield.

With a range of synthetic strategies available for the preparation of enduracididine (**56**), it is anticipated that the synthesis of teixobactin can be accomplished using conventional SPPS. In addition, it is recommended that the cyclic tetradepsipeptide core of teixobactin (**15**) be constructed via macrolactamization rather than macrolactonization.[52]

5. Conclusions

In conclusion, there has been a significant increase in the number of peptides extracted from natural sources which have shown potent inhibitory activity against new *Mtb* biotargets, therefore they are considered to be important lead compounds for the development of new drugs to treat MDR and XDR cases of *Mtb*. However, most of these peptides contain complex unnatural amino acids and motifs, which as highlighted in this review, are chemically difficult to synthesise in a robust and efficient manner. Chemists around the globe have engaged in solving these problems in order to provide sufficient quantities of newly discovered anti-TB peptides or analogues with improved activity, in an effort to develop new drugs to fight the growing TB pandemic. Furthermore, structurally simpler peptides and peptidomimetics have also

emerged as promising lead compounds that can potentially be developed into new anti-TB drug candidates.

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The graphical abstract Image is adapted from a micrograph of Mycobacterium tuberculosis. Image courtesy of Dr Ray Butler and Janice Carr (Centres for Disease Control).

References

1 World Health Organization (WHO). Global tuberculosis report 2014. http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf).
2 A. Zumla, P. Nahid and S. T. Cole, *Nat. Rev. Drug Discov.*, 2013, **12**, 388-404.
3 S. K. Field, *Ther. Adv. Chronic Dis*., 2015, **6**, 170-184.
4 E. Segala, W. Sougakoff, A. Nevejans-Chauffour, V. Jarlier and S. Petrella, Antimicrob. Agents *Chemother*., 2012, **56**, 2326-2334.
5 G. Lamichhane, *Trends Mol. Med*., 2011, **17**, 25-33.
6 K. Hards, J. R. Robson, M. Berney, L. Shaw, D. Bald, A. Koul, K. Andries and G. M. Cook, *J. Antimicrob. Chemother*., 2014, **70**, 2028-2037.
7 D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.,* 2013, **81**, 136-147.
8 A. Padhi, M. Sengupta, S. Sengupta, K. H. Roehm and A. Sonawane, *Tuberculosis*, 2014, **94**, 363-373.
9 M. Abedinzadeh, M. Gaeini and S. Sardari, *J. Antimicrob. Chemother*., 2015, **70**, 1285-1289.
10 T. Teng, J. Liu and H. Wei, *Cell. Physiol. Biochem*., 2015, **35**, 452-466.
11 K. Mdluli, T. Kaneko and A. Upton*, Cold Spring Harbor Perspect. Med*., 2015, **5**, 1-24.
12 S. Sharma, I. Verma and G. K. Khuller, *Eur. Respir. J*., 2000, **16**, 112-117.
13 Ramón-García, R. Mikut, C. Ng, S. Ruden, R. Volkmer, M. Reischl, K. Hilpert and C. J. Thompson, *Antimicrob. Agents Chemother.*, 2013, **57**, 2295-2303.
14 J. M. Clements, R. P. Beckett, A. Brown, G. Catlin, M. Lobell, S. Palan, W. Thomas, M. Whittaker, S. Wood, S. Salama, P. J. Baker, H. F. Rodgers, V. Barynin, D. W. Rice and M. G. Hunter, *Antimicrob. Agents Chemother*., 2001, **45**, 563-570.
15 A. Sharma, S. Sharma, G. K. Khuller and A. J. Kanwar, *Int. J. Antimicrob. Agents*, 2009, **34**, 226-230.
16 R. Kapoor, P. R. Eimerman, J. W. Hardy, J. D. Cirillo, C. H. Contag and A. E. Barron, *Antimicrob. Agents Chemother*., 2011, **55**, 3058-3062.
17 N. Rastogi, V. Labrousse and K. S. Goh, *Curr. Microbiol.*, 1996, **33**, 167-175.
18 P. Pruksakorn, M. Arai, N. Kotoku, C. Vilchze, A. D. Baughn, P. Moodley, W. R. Jacobs Jr and M. Kobayashi, *Bioorg. Med. Chem. Lett*., 2010, **20**, 3658-3663.
19 P. Pruksakorn, M. Arai, L. Liu, P. Moodley, W. R. Jacobs Jr and M. Kobayashi, *Biol. Pharm. Bull*., 2011, **34**, 1287-1290.
20 M. Iwatsuki, R. Uchida, Y. Takakusagi, A. Matsumoto, C. L. Jiang, Y. Takahashi, M. Arai, S. Kobayashi, M. Matsumoto, J. Inokoshi, H. Tomoda and S. Omura, *J. Antibiot*., 2007, **60**, 357-363.
21 E. Gavrish, C. S. Sit, S. Cao, O. Kandror, A. Spoering, A. Peoples, L. Ling, A. Fetterman, D. Hughes, A. Bissell, H. Torrey, T. Akopian, A. Mueller, S. Epstein, A. Goldberg, J. Clardy and K. Lewis, *Chem. Biol*., 2014, 21, 509-518.
22 W. Gao, J. Y. Kim, J. R. Anderson, T. Akopian, S. Hong, Y. Y. Jin, O. Kandror, J. W. Kim, I. A. Lee, S. Y. Lee, J. B. McAlpine, S. Mulugeta, S. Sunoqrot, Y. Wang, S. H. Yang, T. M. Yoon, A. L. Goldberg, G. F. Pauli, J. W. Suh, S. G. Franzblau and S. Choa, *Antimicrob. Agents Chemother*., 2015, **59**, 880-889.
23 Z. G. Khalil, A. A. Salim, E. Lacey, A. Blumenthal and R. J. Capon, *Org. Lett*., 2014, **16**, 5120-5123.
24 G. Daletos, R. Kalscheuer, H. Koliwer-Brandl, R. Hartmann, N. J. de Voogd, V. Wray, W. Lin and P. Proksch, *J. Nat. Prod.*, 2015, **78**(8), 1910-1925.
25 L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen and K. Lewis, *Nature*, 2015, **517**, 455-459.
26 G. Wei, M. Pazgier, E. De Leeuw, M. Rajabi, J. Li, G. Zou, G. Jung, W. Yuan, W. Y. Lu, R. I. Lehrer and W. Lu, *J. Biol. Chem*., 2010, **285**, 16275-16285.
27 S. Sharma, I. Verma and G. K. Khuller, *Arch. Microbiol*., 1999, **171**, 338-342.
28 L. M. Zhu, C. H. Liu, P. Chen, A. G. Dai, C. X. Li, K. Xiao, Y. Chen, J. Cao and Y. R. Chen, *Int. J. Tuberc. Lung Dis*., 2011, **15**, 369-374.
29 F. Von Nussbaum, M. Brands, B. Hinzen, S. Weigand and D. Häbich, *Angew. Chem. Int. Ed*., 2006, **45**, 5072-5129.
30 L. M. Pratt, R. P. Beckett, S. J. Davies, S. B. Launchbury, A. Miller, Z. M. Spavold, R. S. Todd and M. Whittaker, *Bioorg. Med. Chem. Lett*., 2001, **11**, 2585-2588.
31 S. J. Davies, A. P. Ayscough, R. P. Beckett, J. M. Clements, S. Doel, L. M. Pratt, Z. M. Spavold, S. W. Thomas and M. Whittaker, *Bioorg. Med. Chem. Lett*., 2003, **13**, 2715-2718.
32 S. J. Davies, A. P. Ayscough, R. P. Beckett, R. A. Bragg, J. M. Clements, S. Doel, C. Grew, S. B. Launchbury, G. M. Perkins, L. M. Pratt, H. K. Smith, Z. M. Spavold, S. W. Thomas, R. S. Todd and M. Whittaker, *Bioorg. Med. Chem. Lett*., 2003, **13**, 2709-2713.
33 H. K. Smith, R. P. Beckett, J. M. Clements, S. Doel, S. P. East, S. B. Launchbury, L. M. Pratt, Z. M. Spavold, W. Thomas, R. S. Todd and M. Whittaker, *Bioorg. Med. Chem. Lett*., 2002, **12**, 3595-3599.
34 T. A. Knappe, F. Manzenrieder, C. Mas-Moruno, U. Linne, F. Sasse, H. Kessler, X. Xie and M. A. Marahiel, *Angew. Chem. Int. Ed.*, 2011, **50**, 8714-8717.
35 R. N. Zuckermann, J. M. Kerr, S. B. H. Kent and W. H. Moos, *J. Am. Chem. Soc.*, 1992, **114**, 10646-10647.
36 D. E. DeMong and R. M. Williams, *Tetrahedron Lett*., 2001, **42**, 3529-3532.
37 D. E. DeMong and R. M. Williams, *J. Am. Chem. Soc*., 2003, **125**, 8561-8565.
38 K. Y. Hung, P. W. R. Harris and M. A. Brimble, *Org. Lett*., 2012, **14**, 5784-5787.
39 K.-Y. Ko, S. Wagner, S.-H. Yang, D. P. Furkert and M. A. Brimble, *J. Org. Chem*., 2015, **80**(17), 8631-8636.
40 M. Stach, A. J. Weidkamp, S.-H. Yang, K.-y. Hung, D. P. Furkert, P. W. R. Harris, J. B. Smaill, A. V. Patterson and M. A. Brimble, *Eur. J. Org. Chem*., 2015, **28**, 6341-6350.
41 M. Iwatsuki, H. Tomoda, R. Uchida, H. Gouda, S. Hirono and S. Omura, *J. Am. Chem. Soc*., 2006, **128**, 7486-7491.
42 J. D. Hegemann, M. Zimmermann, X. Xie and M. A. Marahiel, *Acc. Chem. Res*., 2015, **48**, 1909-1919.
43 W. Gao, J. Y. Kim, S. N. Chen, S. H. Cho, J. Choi, B. U. Jaki, Y. Y. Jin, D. C. Lankin, J. E. Lee, S. Y. Lee, J. B. McAlpine, J. G. Napolitano, S. G. Franzblau, J. W. Suh and G. F. Pauli, *Org. Lett*., 2014, **16**, 6044-6047.
44 R. Roodbeen and K. J. Jensen, *Methods in Molecular Biology*, 2013, **1047**, 141-149.
45 D. Siodłak, Amino Acids, 2015, **47**, 1-17.

46 F. Von Nussbaum and R. D. Süssmuth, *Angew. Chem. Int. Ed*., 2015, **54**, 6684-6686.
47 X. Fang, K. Tiyanont, Y. Zhang, J. Wanner, D. Boger and S. Walker, *Mol. Biosyst*., 2006, **2**, 69-76.
48 K. Hatano, I. Nogami, E. Higashide and T. Kishi, *Agric. Biol. Chem*., 1984, **48**, 1503-1508.
49 L. Sanière, L. Leman, J. J. Bourguignon, P. Dauban and R. H. Dodd, *Tetrahedron*, 2004, **60**, 5889-5897.
50 D. E. Olson, J. Y. Su, D. A. Roberts and J. Du Bois, *J. Am. Chem. Soc*., 2014, **136**, 13506-13509.
51 W. Craig, J. Chen, D. Richardson, R. Thorpe and Y. Yuan, *Org. Lett*., 2015, **17**(18), 4620-4623.
52 L. M. De Leon Rodriguez, A. J. Weidkamp and M. A. Brimble, *Org. Biomol. Chem*., 2015, **13**, 6906-6921.