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Total Synthesis of Microcin B17 via a Fragment Condensation Approach

Robert E. Thompson, Katrina A. Jolliffe,* and Richard J. Payne*

School of Chemistry, The University of Sydney, NSW 2006, Australia

kate.jolliffe@sydney.edu.au; richard.payne@sydney.edu.au

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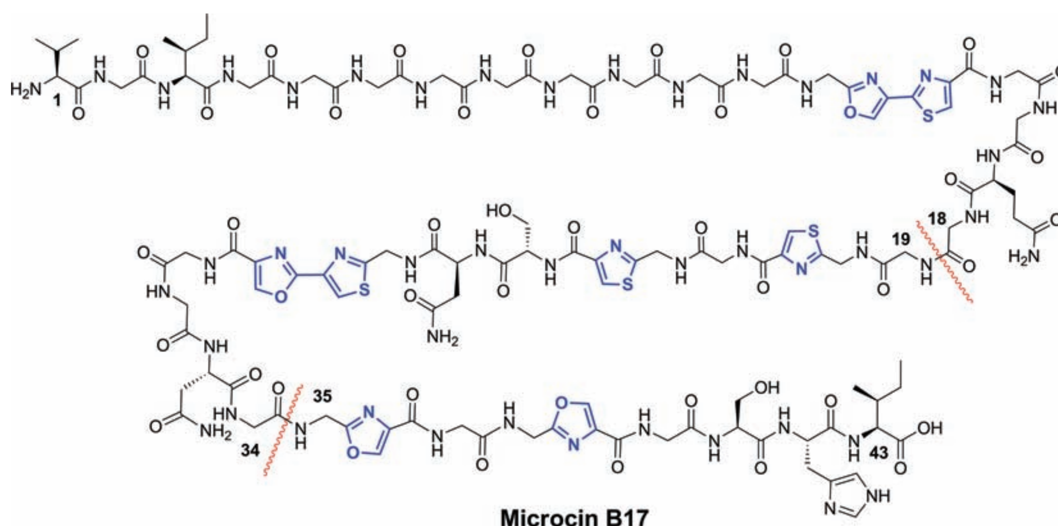
ABSTRACT



The total synthesis of the 43 amino acid antibacterial peptide Microcin B17 (MccB17) is described. The natural product was synthesized via a convergent approach from a heterocycle-derived peptide and peptide thioester fragments prepared via Fmoc-strategy solid phase peptide synthesis (SPPS). Final assembly was achieved in an efficient manner using two Ag(I)-assisted peptide ligation reactions to afford MccB17 in excellent overall yield.

Microcin B17 (MccB17, **1**) is a 43 amino acid antibacterial peptide secreted from various strains of the bacterium *Escherichia coli*.[1] MccB17 targets DNA gyrase, a member of the type II topoisomerase family of enzymes, essential for DNA replication in prokaryotic organisms.[2] MccB17 displays extensive post-translational modification, whereby specific serine and cysteine residues have undergone enzymatic dehydrative cyclization and dehydrogenation, affording several thiazole and oxazole heterocycles throughout the native peptide backbone.[3]

To date there has been only one total synthesis of this complex biomolecule. This work, reported by Jung and coworkers, utilized a linear solid-phase peptide synthesis (SPPS) approach, incorporating preformed oxazole and thiazole containing amino acids into the sequence.[4] Despite the successful assembly of the natural product, the practical length limitation of SPPS, coupled with the difficulties associated with preparing glycine rich sequences,[5] makes the routine preparation of MccB17 and analogues nontrivial by

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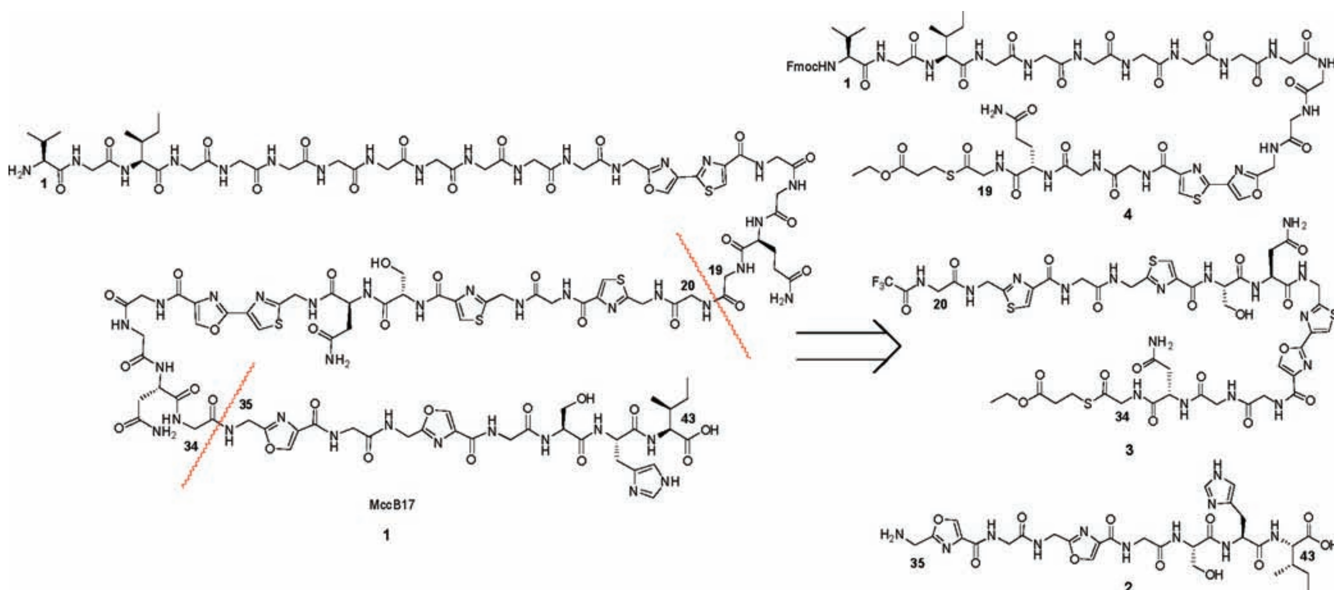
Scheme 1. Retrosynthesis of Microcin B17 (MccB17,**1**)



this strategy. In addition to this synthetic approach, MccB17 and several analogues have been prepared *via* biosynthetic means.[6] While this represents a powerful approach for accessing these post-translationally modified peptides, it cannot provide access to a comprehensive suite of analogues for detailed structure−activity studies. Given the limitations associated with these prior approaches, we sought to develop a more convergent route that would also be amenable to the synthesis of a library of MccB17 variants for biological testing in the future.

Ligation-based approaches for the synthesis of large polypeptides and proteins are of widespread interest as they allow rapid access to biologically and pharmaceutically relevant biomolecules.[7] We reasoned that a convergent ligation-based assembly of MccB17 would circumvent difficulties associated with the Fmoc-strategy SPPS of large peptides and, in addition, would provide convenient access to analogues unobtainable by biosynthetic means.[6,8] Our synthetic approach toward MccB17 involved disconnection at two junctions, namely Gly19-Gly20 and Gly34-Gly35 (Scheme 1). This provided target peptide **2** and peptide thioesters **3** and **4** that could be obtained through Fmoc-strategy SPPS. We envisaged the assembly *via* two cysteine-free direct aminolysis ligation reactions.[9]

Protecting groups on the *N*-termini of peptide thioesters **3** and **4**, specifically *N*-trifluoroacetamide and *N*-fluorenylmethylcarbamate (Fmoc) groups respectively, were incorporated to prevent polymerization of fragments during the ligation reactions.

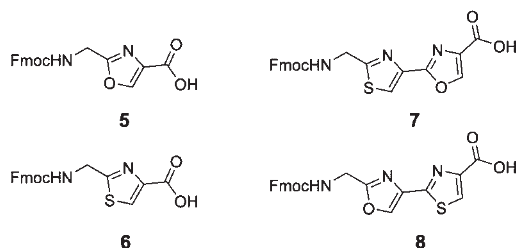


Figure 1. Fmoc-protected heterocyclic amino acids.

Preformed Fmoc-protected building blocks (**5**−**8**, Figure 1) were obtained using established procedures for oxazole and thiazole synthesis[10] and were incorporated directly into the Fmoc-strategy SPPS of fragments **2**−**4** (see Supporting Information for experimental details).

The preparation of *C*-terminal fragment **2** began from Fmoc-Ile-preloaded Wang resin (**9**, Scheme 2). Removal of the *N*-terminal Fmoc group using 20% piperidine in DMF was followed by coupling to Fmoc-His(Trt)-OH using benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium

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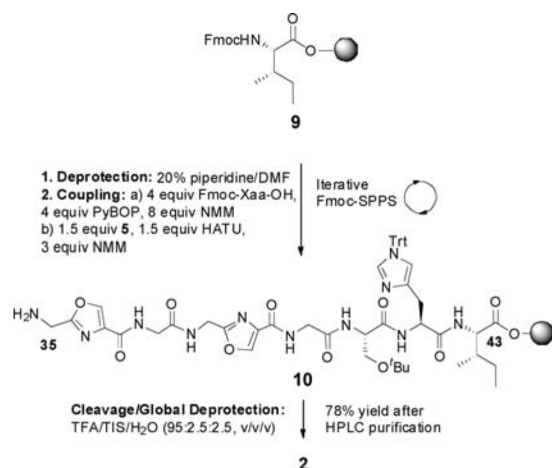
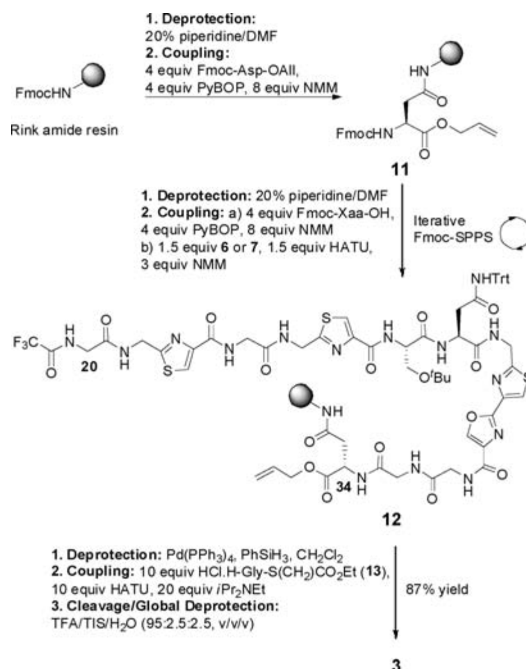
Scheme 2. Synthesis of Peptide Fragment **2** *via* Fmoc-SPPS



Scheme 3. Synthesis of Peptide Thioester **3** *via* Fmoc-SPPS



hexafluorophosphate (PyBOP) and *N*-methylmorpholine (NMM) in DMF. Assembly of the remaining peptide sequence was achieved using iterative Fmoc-strategy SPPS. Notably, only 1.5 equiv of Fmoc-protected oxazole building block **5** were coupled to the growing resin-bound peptide [using 2-(1*H*-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and NMM in DMF] to prevent excess waste of this precious building block. This coupling strategy was also applied to the incorporation of heterocyclic building blocks **6**, **7**, and **8** (see below). Acidolytic cleavage of the fully assembled peptide **10** from the resin using TFA/tri*iso*propylsilane (TIS)/H_2O (95/2.5/2.5, v/v/v) afforded MccB17(35−43) **2** in 78% yield after reversed-phase HPLC purification (see Supporting Information).

Synthesis of peptide thioester fragment **3** was achieved using the side chain anchoring strategy.[11] This method has been shown to be an efficient route to peptide thioesters using Fmoc-strategy SPPS and, importantly, proceeds without epimerization of the resin bound amino acid. To this end, Rink amide resin was deprotected using 20% piperidine in DMF and Fmoc-Asp-OAll was subsequently immobilized using PyBOP and NMM in DMF to afford **11** (Scheme 3). Iterative Fmoc-strategy SPPS was then conducted as described for **2** to give resin bound **12**. Introduction of the *C*-terminal thioester moiety was initiated by treatment of **12** with $Pd(PPh_3)_4$ and phenylsilane to liberate the free *C*-terminal acid and was followed by coupling of HCl·H-Gly-$S(CH_2)_2CO_2Et$ (**13**) using HATU and *N*,*N*-diisopropylethylamine. Acidolytic cleavage from the resin then afforded the crude peptide thioester **3**. Poor solubility of this fragment hampered purification attempts by HPLC; however the highly efficient synthesis permitted isolation through precipitation in diethyl ether, to provide peptide thioester **3** in 87% yield and greater than 90% purity and, importantly, without epimerization of the penultimate asparagine residue (see Supporting Information).

Synthesis of the final fragment, *N*-terminal peptide thioester **4**, was performed on 2-chlorotrityl chloride resin (Scheme 4). Loading of Fmoc-Gly-OH was conducted in dichloromethane using *N*,*N*-diisopropylethylamine to furnish resin bound **14**. The desired sequence was subsequently assembled on the resin *via* Fmoc-strategy SPPS. Two 2,4-dimethoxybenzyl groups[12] were incorporated into the amide backbone to suppress the aggregating effects of the extended polyglycine sequences which we had encountered in our initial synthetic endeavors. Cleavage of the fully assembled protected resin-bound peptide **15** was achieved using hexafluoro-2-propanol/CH_2Cl_2 (4:1, v/v). The crude peptide was immediately subjected to the thioesterification procedure reported by Kajihara and co-workers, using PyBOP, ethyl 3-mercaptopropionate, and *N*,*N*-diisopropylethylamine in NMP.[13] Global deprotection and reversed-phase HPLC purification afforded the *N*-Fmoc protected peptide thioester **4** in 18% overall yield and 28 linear steps.

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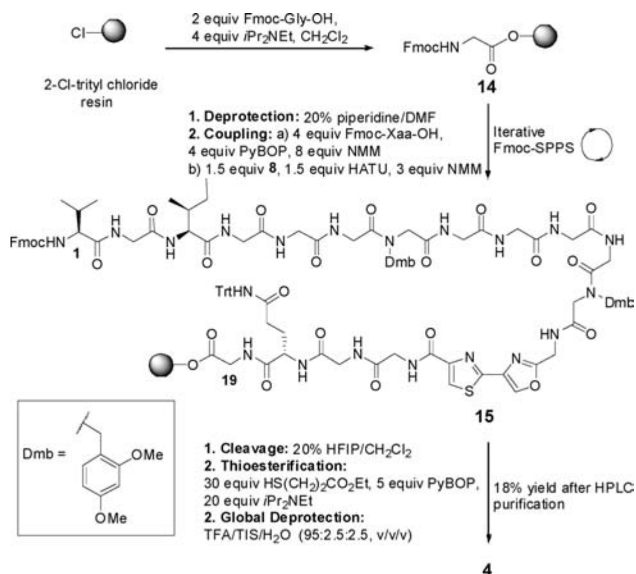
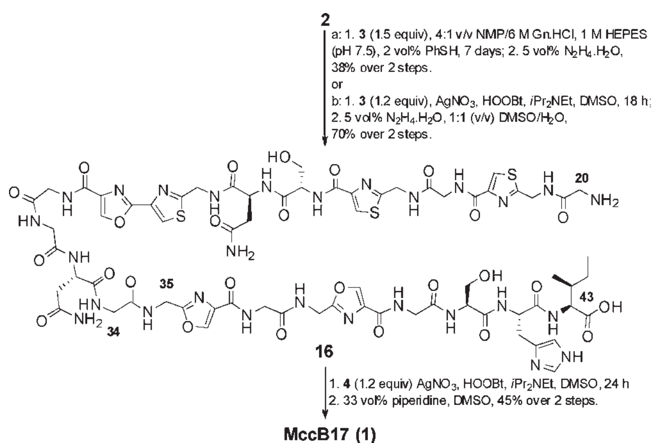
Scheme 4. Synthesis of Peptide Thioester **4** *via* Fmoc-SPPS



Scheme 5. Ligation-Based Assembly of MccB17 (**1**)



With the peptide and peptide thioester fragments (**2**–**4**) now in hand, attention turned to the ligation-based assembly of the natural product. The ligation between peptide **2** and peptide thioester **3** was first investigated using the direct aminolysis ligation reaction recently reported by Wong and co-workers.[9c] To this end, **2** and **3** were reacted in 4:1 v/v NMP/6 M Gn·HCl with 1 M HEPES at pH 7.5 with the addition of thiophenol to facilitate thioester exchange to the more reactive thiophenylester (Scheme 5). After 7 days the ligation reaction was treated with aqueous hydrazine to facilitate deprotection of the *N*-terminal trifluoroacetamide moiety, furnishing MccB17(20–43) **16** in 38% yield over the two steps. Owing to the modest yield obtained, we chose to investigate an alternative coupling strategy. The presence of a nonepimerizable *C*-terminal glycine residue permitted the use of a Ag(I)-promoted ligation reaction.[9a,b,14] As such, coupling of **2** and **3** in the presence of $AgNO_3$, 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (HOOBt), and *N*,*N*-diisopropylethylamine in DMSO was performed (Scheme 5). Gratifyingly after 18 h, a near-quantitative conversion to the desired product was achieved, as assessed by LC-MS analysis. At this stage, *in situ* hydrazinolysis of the *N*-terminal trifluoroacetamide moiety and subsequent HPLC purification provided MccB17(20–43) **16** in 70% yield over two steps. Given the success of the silver ion mediated formation of **16**, we chose to employ the same methodology for the crucial final ligation. To this end, peptide **16** and thioester **4** were treated with $AgNO_3$, HOOBt, and *N*,*N*-diisopropylethylamine in DMSO (Scheme 5). After 24 h the reaction had proceeded to completion as assessed by LC-MS analysis. At this point, *in situ* deprotection of the *N*-terminal Fmoc-carbamate moiety afforded MccB17 (**1**) in 45% yield over two steps after reversed-phase HPLC purification. Pleasingly, the spectroscopic data obtained for synthetic MccB17 were consistent with those reported for both isolated[15] and previously synthesized[4] MccB17 (see Supporting Information). Specifically, ^{1}H NMR, HSQC, HMBC, and mass spectral analysis of synthetic **1** correlated fully.

In summary, we have completed an efficient total synthesis of the 43 amino acid post-translationally modified antibacterial peptide MccB17. Key features in our approach include the use of Fmoc-SPPS to rapidly assemble three key peptide and peptide thioester fragments which, under Ag(I)-mediated ligation conditions, were effectively assembled to the target molecule. Given the efficient and convergent nature of the synthetic route described, it is anticipated that it will prove amenable to the preparation of libraries of MccB17 analogues that can be evaluated for antibacterial activity. Moreover, it may also find utility in the total synthesis of related peptide-based natural products and associated analogues, work toward which is currently underway in our laboratories and will be reported in due course.

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Supporting Information Available. Experimental procedures and characterization for all compounds. ^{1}H and ^{13}C NMR spectra and HPLC and mass spectral data for novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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