ChemComm

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *Chem. Commun.*, 2021, **57**, 2081

Received 26th November 2020,
Accepted 22nd January 2021

DOI: 10.1039/d0cc07747j

rsc.li/chemcomm

Depsipeptide synthesis using a late-stage Ag(I)-promoted macrolactonisation of peptide thioamides†

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Macrolactonisation of peptides to generate cyclic depsipeptides is often challenging due to the low nucleophilicity of hydroxyl groups, epimerisation, cyclodimerisation, and potential acyl transfer reactions of the ester. Herein, we report a novel macrolactonisation strategy employing a Ag(I)-promoted conversion of peptide thioamides to isoimide intermediates, which undergo site-selective intramolecular acyl transfer to serine/threonine side chains to generate the macrolactone.

Cyclic peptides are of significant interest in medicinal chemistry due to their improved metabolic stability, increased conformational rigidity and membrane permeability, and overall higher affinity for their target proteins than their linear counterparts.[1–5] Cyclic depsipeptides contain one or more ester bond replacements of standard amide bonds, frequently through lactone formation by linking the C-terminus to serine or threonine (Ser/Thr) side chains, and often have remarkable biological activities.[6–14] Well known examples include teixobactin, which possesses potent activity against a range of Gram-positive pathogenic bacteria,[15] and romidepsin, an HDAC inhibitor with potent anti-cancer activity.[16] Several strategies have been employed for the synthesis of depsipeptides. Direct macrolactonisation of hydroxyacid-containing peptides is not a common approach to depsipeptides, as low yields are frequently encountered due to the low nucleophilicity of the hydroxyl group.[17–19] Early-stage ester bond formation with late stage macrolactamisation is an alternative approach to depsipeptides (see Fig. 1, upper);[20,21] however this strategy requires multiple orthogonal protecting groups. More importantly, premature acyl transfer can occur during the iterative deprotection steps, and the acidic conditions typically employed in the cleavage of depsipeptides from a solid support can result in side reactions of the ester group.[20]

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† Electronic supplementary information (ESI) available: Experimental procedures and characterisation data (NMR and MS spectra, HLPC traces). See DOI: 10.1039/d0cc07747j

Our group has developed a new method for amide bond synthesis through the Ag(I)-promoted reaction of thioamides with peptide C-terminal carboxylic acids.[22] Recently, we described a

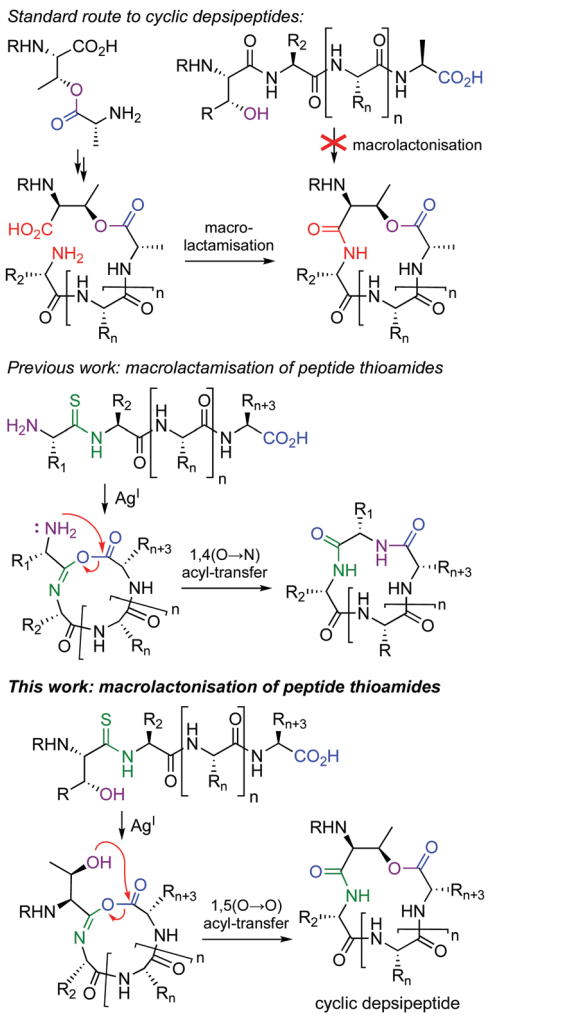


Fig. 1 Approaches to peptide macrocyclisation.

facile peptide macrocyclisation method that exploits the selective reactivity of peptide thioamides to generate isoimide intermediates, which undergo spontaneous intramolecular acyl transfer to furnish native cyclic peptides (Fig. 1, middle).[23] This new method enables the rapid cyclisation of peptides and overcomes the common drawbacks of standard macrolactamisation methods, such as epimerisation and cyclodimerisation.

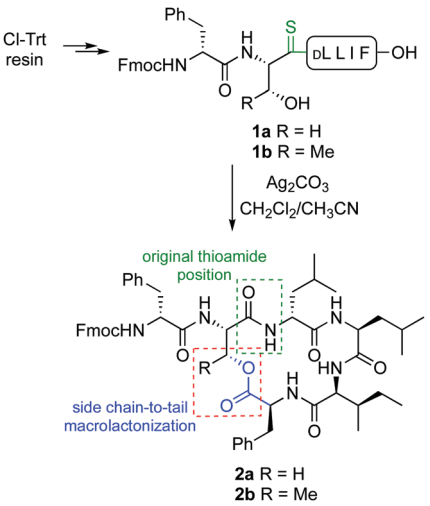
Herein, we describe the elaboration of the Ag(I)-promoted transformation of peptide thioamides that now enables an efficient, late stage macrolactonisation approach to cyclic depsipeptides. (Fig. 1, lower).

This process involves the synthesis of a linear peptide containing a single atom substitution – the incorporation of a thioamide linkage at the key Ser/Thr residue that is to undergo macrolactonisation. Ag(I)-promoted coupling of the thioamide and C-terminal carboxylate would generate a cyclic isoimide, which is then primed to undergo an intramolecular acyl transfer to the side chain hydroxyl group to facilitate formation of the macrolactone. In order to prevent acyl migration to the Ser/Thr amine group, our design incorporated at least one further residue to the N-terminal side of the key Ser/Thr residue.

In order to investigate the macrolactonisation process, peptide thioamides Fmoc-DPhe-Ser/Thr[S]-DLeu-Leu-Ile-Phe-OH **1a,b** were prepared using standard procedures.[23–28] Treatment of the peptide thioamide **1b** with Ag_2CO_3 in a variety of solvents was undertaken to optimise conversion to the macrolactone **2b** (Scheme 1). Use of DMF or ethyl acetate gave poor conversion to the macrocycle (Table 1, entries 1–3). Use of CH_2Cl_2 or CH_3CN gave reasonable yields of the macrocycle, with a 1 : 1 mixture of CH_2Cl_2 and CH_3CN giving improved yield (Table 1, entries 4–6). Use of 1.5 equivalents of Ag_2CO_3 was optimum (58% of macrolactone **2b** isolated, from **1b**), with equimolar or excess amounts resulted in reduced yields yield (Table 1, entries 6–8). Overall yield of the macrocycle **2b** was 25% from Cl-Trt resin (average of 90% per step). Applying the same reaction conditions to the serine-containing peptide **1a** generated the cyclic peptide **2a** in 30% overall yield from resin.

To demonstrate the scope of the Ag(I)-promoted thioamide macrolactonisation protocol, the strategy was applied to numerous depsipeptide natural products containing various ring sizes. Teixobactin was selected as an example of a depsipeptide containing a tetrapeptide core macrocycle,[20,21,29–32] with an ester linkage connecting the side-chain hydroxylisat group of D-threonine to the C-terminal carboxylic acid of isoleucine. Initially investigations toward teixobactin-like macrocycles were performed with the Lys-analogue in place of the natural L-*allo*-enduracididine residue, and with both Ser and Thr. The linear thiopeptides Fmoc-Ser(Bn)-DSer[S]-Ala-Lys(Cbz)-Ile-OH **3a** and Fmoc-Ser(Bn)-DThr[S]-Ala-Lys(Cbz)-Ile-OH **3b** were successfully synthesised by SPPS and underwent the Ag(I)-promoted macrolactonisation to provide the corresponding depsipeptides **4a** and **4b** in 35% and 34% yield, respectively (22% and 20% overall yield, respectively, from starting resin) (Scheme 2).

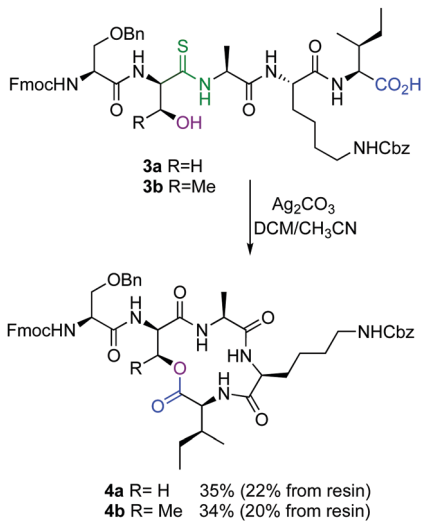
Further, the enduracididine-containing peptide Fmoc-Ser(Bn)-DThr[S]-Ala-End(Cbz)$_2$-Ile **5** was successfully converted to the teixobactin macrolactone **6** (Scheme 3). To investigate the possibility of an acyl transfer from the ester to the N-terminal amine,[20,21] deprotection of the Fmoc of the serine residue was effected by treatment with piperidine for 1 minute followed by quenching with 1 N HCl to pH 4 (Scheme 3). Macrocycle **7** was purified and analyzed by HPLC, with no acyl transfer observed. Close analogues of **7** have been converted to teixobactin through fragment coupling or Ser/Thr ligation.[21,29,30]



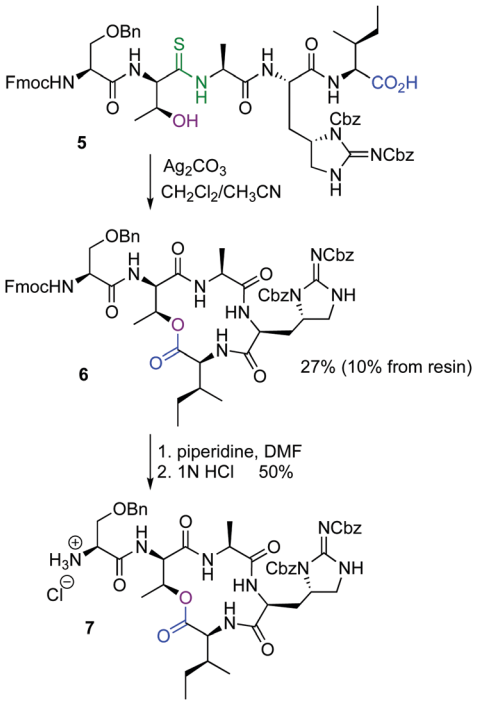
Scheme 1

Table 1 Optimisation of macrolactonisation of **1b**

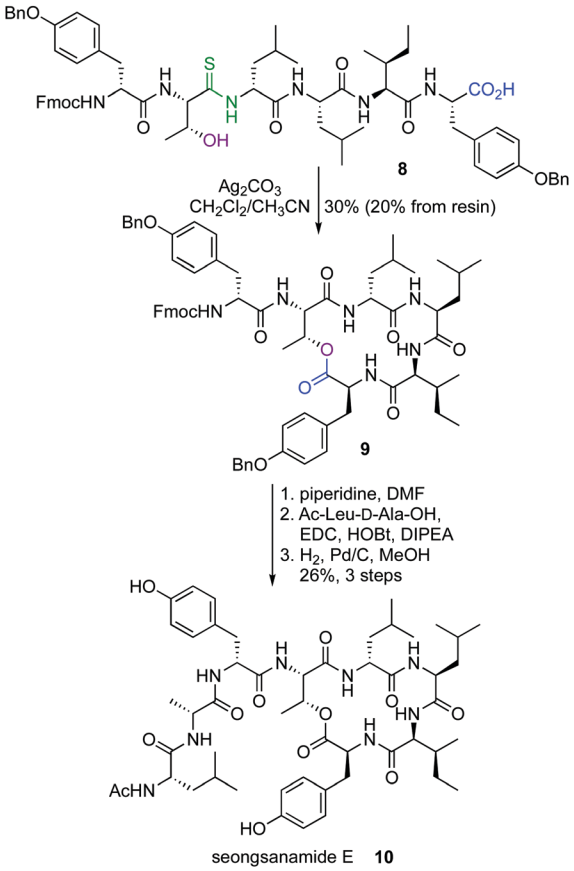
Solvent	Equiv. Ag_2CO_3	% Yield **2b**
EtOAc	1.5	8
DMF	1.5	4
DMF/Et_3N	1.5	2
DCM	1.5	50
CH_3CN	1.5	55
DCM/CH_3CN	1.5	58
DCM/CH_3CN	2.0	42
DCM/CH_3CN	1.0	48



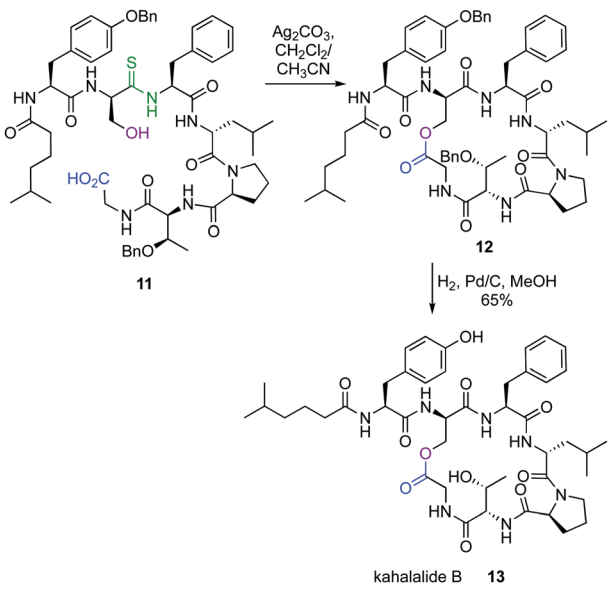
Scheme 2



Scheme 3



Scheme 4



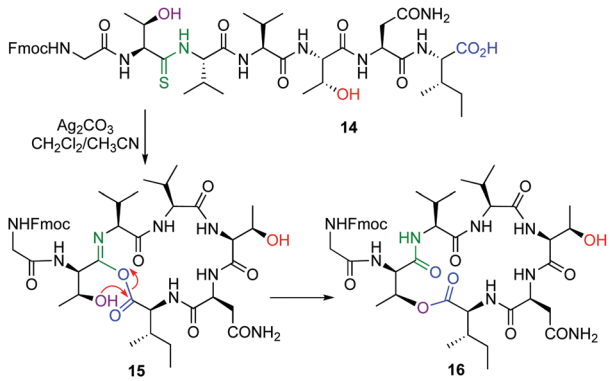
Scheme 5

Next, the total syntheses of depsipeptide natural products, seongsanamide E **10** and kahalalide B **13**, were investigated. Seonganamide E is a cyclic depsipeptide possessing five residues in the macrocycle, with ester formation between the hydroxyl group of threonine and the carboxylic acid of tyrosine.[33] Kahalalide B possesses six residues in the macrocycle, formed by ester linkage between the hydroxyl group of serine and the carboxylic acid of glycine.[34]

The linear thiopeptide Fmoc-DTyr(Bn)-Thr$^{[S]}$-DLeu-Leu-Ile-Tyr(Bn)-OH **8** was synthesised using our standard SPPS procedure. The linear thiopeptide **8** was treated with silver carbonate to afford the cyclic depsipeptide **9** in 20% an overall yield from starting resin. Following Fmoc deprotection of depsipeptide **9** coupling with Ac-Leu-DAla-OH using EDC/HOBt yielded benzyl protected seongsanamide E. Hydrogenolytic cleavage of the *O*-benzyl groups using Pd/H_2 afforded seongsanamide E **10** in 40% yield over two steps (Scheme 4), with the ^{1}H NMR spectrum of **10** identical to that of the natural product.[33]

Application of the lactonisation strategy to kahalalide B was also undertaken. The linear thiopeptide 5-MeHex-Tyr(Bn)-DSer$^{[S]}$-Phe-DLeu-Pro-Thr(Bn)-Gly-OH **11** was synthesised using our standard SPPS procedure, incorporating the N-terminal 5-methylhexanoyl group on resin. The thiopeptide was treated with silver carbonate to afford the depsipeptide **12**. Hydrogenolytic removal of the *O*-benzyl groups using Pd/H_2 afforded kahalalide B **13** in 10% an overall yield from starting resin (Scheme 5). Comparison of the ^{1}H NMR spectra of **13** with that of the natural product[34] and previously synthesised[35] material confirmed successful synthesis of the natural product.

In order to demonstrate the chemo- and regio-selectivity of the Ag(I)-promoted macrolactonisation protocol, a thiopeptide containing an additional threonine group was prepared. Accordingly, Fmoc-Gly-Thr$^{[S]}$-Val-Val-Thr-Asn-Ile-OH **14** was subjected to the standard Ag(I)-promoted macrolactonisation procedure, with the cyclic depsipeptide **16** possessing a



Scheme 6

6-residue macrocycle (Thr2–Ile7) the only cyclic product. No isomeric macrolactone from cyclisation across Thr5–Ile7 was detected (see Fig. S49, ESI†), indicating site-selective acyl transfer to the Thr2 alcohol from isoimide **15**, with no competing acyl transfer to Thr5 (Scheme 6). Thus, we suggest the macrolactonisation protocol does not require differential protection of multiple Ser/Thr residues.

In conclusion, this work described a new method for the macrolactonisation of peptides through a Ag(I)-promoted cyclisation of Ser/Thr-containing peptide thioamides. The selective reactivity of the thioamide group generates a cyclic isoimide intermediate, which undergoes spontaneous intramolecular acyl transfer to furnish the cyclic depsipeptide. This new method enables the preparation of a range of depsipeptide ring sizes and can overcome common drawbacks of standard macrolactonisation methods including limited reactivity of the Ser/Thr hydroxyl group, thereby requiring early-stage ester formation and multiple orthogonal protecting group strategies.

This work was supported by the Australian Research Council (DP180101804 and DP150100692). The support of the Bio21 Institute MSP and MSPF facilities is acknowledged. S. S. acknowledges an Albert Shimmins Fund Award and a MRS from The University of Melbourne.

Conflicts of interest

There are no conflicts to declare.

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