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Rapid, traceless, Ag(I)-promoted macrocyclization of peptides possessing an *N*-terminal thioamide

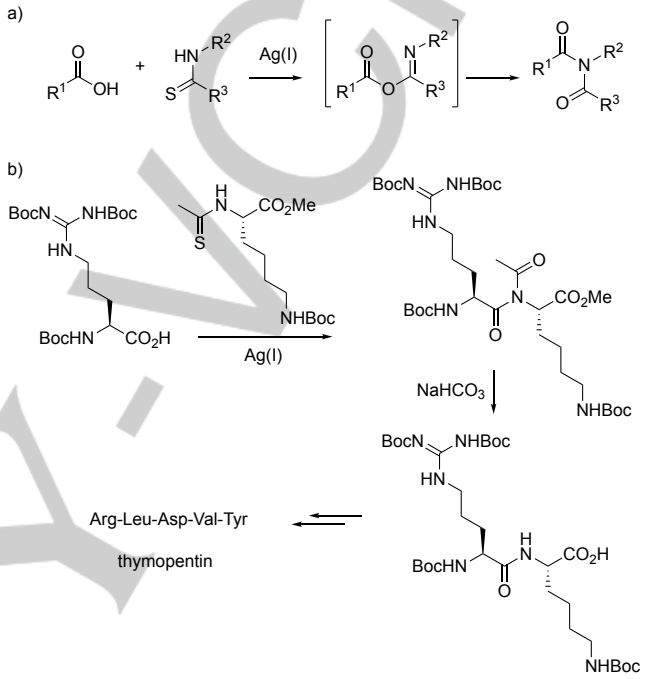
Varsha J. Thombare[a], Craig A. Hutton[a]*

Abstract: Peptide macrocyclization is often a slow process, plagued by epimerization and cyclodimerization. Herein we describe a new method for peptide macrocyclization employing the Ag(I)-promoted transformation of peptide thioamides. The Ag(I) has a dual function: chemselectively activating the thioamide and tethering the N-terminal thioamide to the C-terminal carboxylate. Extrusion of Ag_2S generates an isoimide intermediate, which undergoes acyl transfer to generate the native cyclic peptide, resulting in a rapid, traceless macrocylization process. Cyclic peptides are furnished in high yields within 1 hour, free of epimerization and cyclodimerization.

Cyclic peptides have significant therapeutic potential due to their conformational rigidity, high affinity for target proteins, stability to proteases and potential membrane permeability.[1-5] The straightforward capacity for structural modification and fine-tuning of cyclic peptides has led to predictions they will bridge the gap in drug design and development between the pharmacokinetic advantages of small organic molecules and the selectivity and potency of biologics. However, cyclization of linear peptides is frequently plagued by epimerization, cyclodimerization, and the formation of higher oligomers.[6-8] These issues originate with the π-character of amide bonds and their preferred transoid configurations, which leads to an extended peptide backbone in which the amino and carboxyl termini are not in close proximity. Head-to-tail cyclization is thus entropically disfavoured and notoriously slow. Efforts to overcome the intrinsic limitations of peptide head-to-tail cyclization include metal ion templating, [9-13] the use of 'capture' auxiliaries[14-19] and ring expansion/contraction approaches.[20-22]

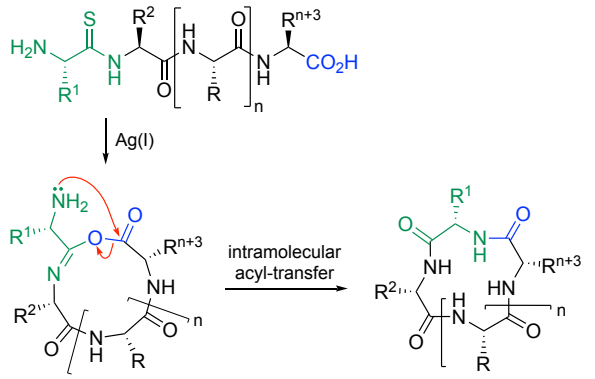
Our group has recently developed a new method for peptide synthesis through the Ag(I)-promoted reaction of thioamides with peptide C-terminal carboxylic acids, which has been exploited in the epimerization-free synthesis of peptides in the N→C direction (Scheme 1b).[23,24] This thioamide directed peptide synthesis proceeds through the generation of an isoimide intermediate, which can rearrange to imides via an O→N acyl transfer (Scheme 1a). Selective imide hydrolysis then generates the amide bond.[25]

Isoimides have been exploited in peptide synthesis through inter- and intra-molecular trapping with nitrogen nucleophiles. Danishefsky and co-workers have developed couplings of amino (thio)acids mediated by isonitriles, wherein the intermediate isoimide (or thioFCMA) is trapped by an amino ester to generate a peptide bond.[26-29] Yudin and co-workers have generated aziridine- and oxadiazole-linked cyclic peptides via intramolecular trapping of isoimide intermediates.[30,31]



Scheme 1. Previous work: a) Ag(I)-promoted imide generation. b) N→C peptide synthesis employing Ag(I)-thioamide coupling

Herein we describe a facile peptide macrocyclization method that exploits the selective reactivity of peptide thioamides to generate isoimide intermediates, which undergo spontaneous intramolecular acyl transfer to furnish native cyclic peptides (Scheme 2). This new method enables the rapid cyclization of peptides and overcomes the common drawbacks of standard macrolactamization methods, such as epimerization and cyclodimerization.[32]



Scheme 2. This work: Ag(I)-promoted macrocyclization of peptide thioamides.

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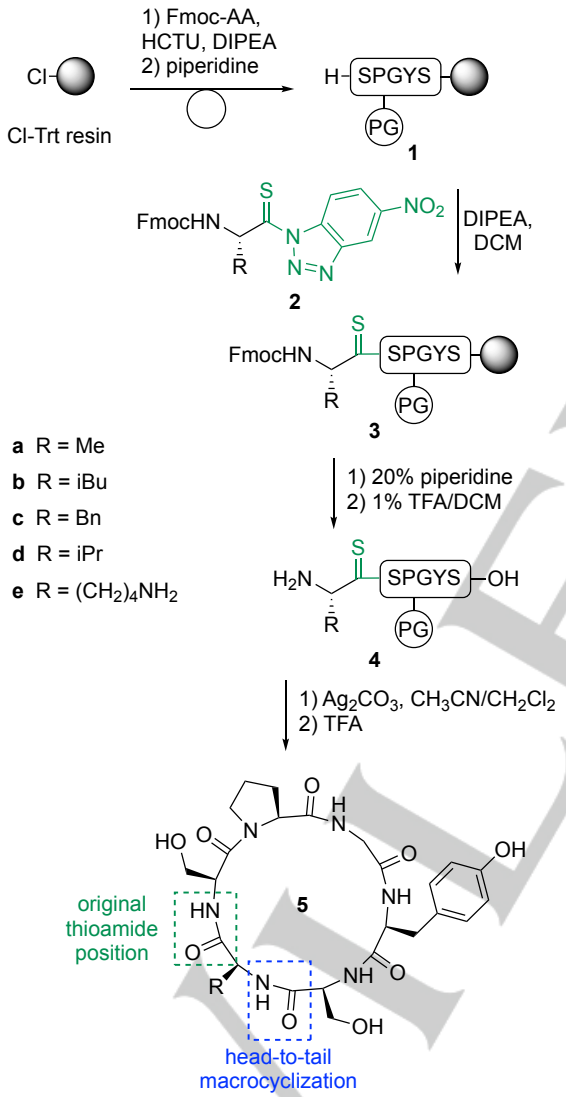
Our previous studies of intermolecular reactions of thioamides with peptide C-terminal carboxylates suggested that intramolecular reactions of peptides possessing a thioamide should generate a cyclic isoimide intermediate (Scheme 2).[23] If the thioamide functionality was located at the N-terminal peptide bond this would position the N-terminal amine as a pendant nucleophile to attack the carbonyl group and facilitate a 1,4-acyl transfer, generating an amide bond and thus completing a head-to-tail macrocyclization process. The alternative 1,3-acyl transfer to generate the imide would presumably be disfavoured by transannular conformational effects due to the cyclic nature of the isoimide positioning the C=N and C=O bonds in an anti orientation.[31]



Scheme 3. Ag(I)-promoted head-to-tail macrocyclization: model study.

In order to investigate the proposed head-to-tail cyclization, peptide thioamides X[S]SPGYS **4** were prepared (X = A, L, F, V, K, Scheme 3). The pentapeptide SPGYS **1** was synthesized on chlorotrityl resin using a standard SPPS protocol. The thioamide-linked residue was then incorporated through treatment with benzotriazole-based thioacylating reagents **2**.[33-37] Following Fmoc-deprotection, the peptides **4**, containing a thioamide at the N-terminal backbone amide position, were cleaved from resin using 1% TFA in DCM.

The Ala1[S] peptide **4a** was dissolved in various solvents and Ag_2CO_3 was added. The reactions were monitored by LC/MS, with the cyclic peptide **5a** generated in good conversion in reactions performed in CH_2Cl_2 and CH_3CN, in the presence or absence of base (Table 1). Conversion to the cyclic peptide was negligible when the reaction was conducted in DMF/CH_3CN, with most of the linear peptide thioamide **4a** converted to the corresponding oxoamide. Accordingly, all subsequent cyclization reactions were performed in CH_2Cl_2/CH_3CN.

Table 1. Solvent effect for cyclization of peptide thioamide **4a** to **5a**.

Solvent	**5a** (%)[a]	linear peptide (%)[a]
CH_2Cl_2	86	1
CH_2Cl_2/DIPEA	85	2
CH_3CN	87	1
CH_3CN/DIPEA	84	2
DMF/CH_3CN	1	87
DMF/CH_3CN/DIPEA	1	88

[a] % conversion calculated by HPLC

The Ag(I)-promoted reaction of Ala1[S] peptide **4a** in CH_2Cl_2/CH_3CN was analysed by HPLC, which indicated clean conversion to the protected cyclic peptide as the major product (Figure 1). The crude cyclic peptide was treated with TFA to effect deprotection of side-chain protecting groups and the final cyclic peptide **5a** was purified by RP-HPLC (Figure 1). No attempt was made to optimise the SPPS of the peptide thioamide and only a single HPLC purification was performed after the final step. The cyclic hexapeptide was isolated in 31% yield from the starting resin, equating to an average of 93% yield for each step (over 16 steps).

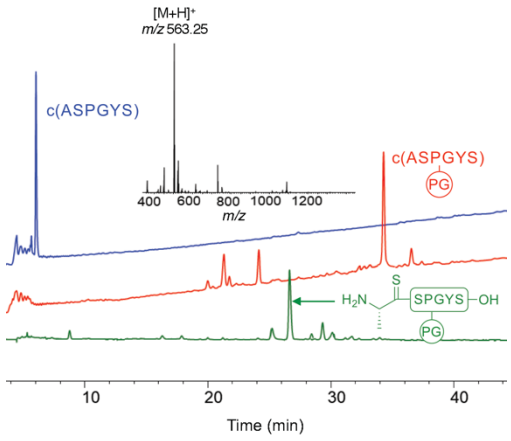


Figure 1. HPLC traces of the crude A[S]SPGYS linear peptide thioamide **4a** (green), the crude cyclic, protected peptide (red), and the purified deprotected peptide c(ASPGYS) **5a** (blue).

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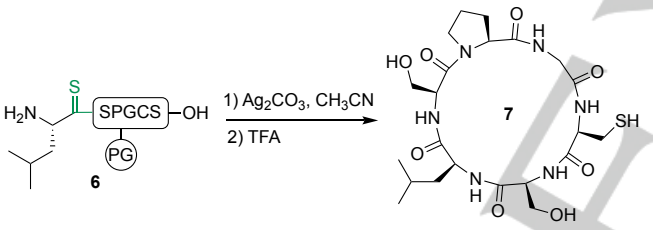
The scope of the cyclization protocol was investigated, employing peptide thioamides with a variety of N-terminal residues. Analogues **4b**–**e** all underwent efficient Ag(I)-promoted cyclization, providing cyclic hexapeptides **5b**–**e** in excellent overall yield after a single purification step (Table 2). The N-terminal valine peptide **4d** gave a slightly reduced yield, consistent with known effects of bulky, β-branched residues in other cyclization protocols.[7]

Table 2. Cyclic peptides **5** from linear peptides **4** with varying N-terminal thioamide-containing residues.

Cyclic Peptide		HPLC rt (min)	Mass		Isolated Yield % [a]	Purity %[b]
			Calc.	Obs.		
c(ASPGYS)	**5a**	4.63	563.25	563.25	31	98
c(LSPGYS)	**5b**	7.73	605.29	605.29	29	96
c(FSPGYS)	**5c**	10.17	639.28	639.28	30	98
c(VSPGYS)	**5d**	5.93	591.28	591.26	19	99
c(KSPGYS)	**5e**	13.39	620.30	620.51	26	98
c(LSPGCS)	**7**	22.5	545.23	545.23	12	94

[a] Calculated from starting Cl-Trt resin

[b] Calculated by HPLC



Scheme 4. Ag(I)-promoted macrocyclization of peptide containing Cys residue.

The scope was further investigated with regard to incorporation of S-containing amino acid residues. Protected peptide thioamide L[s]SPGCS **6** was prepared using the methods described for peptides **4**, incorporating a Cys(Dpm) residue. The cyclization of **6** was slow under standard conditions (1.2 equiv. Ag_2CO_3), but proceeded rapidly in good yield in the presence of 2.5 equiv. Ag_2CO_3 (Scheme 4). Presumably, the Cys sulfur sequesters Ag(I), but excess Ag(I) sustains the cyclization reaction. The lower yield of cyclic peptide **7** reflects the more forcing deprotection conditions required for cleavage of the Dpm group.

With the model study demonstrating the successful general preparation of cyclic hexapeptides, the scope of the reaction with respect to ring size was investigated. Gramicidin S **8** and mahafacyclin B **9** were chosen as examples of naturally occurring cyclic peptides, containing 10 and 7 residues respectively. RGD cyclic peptide **10** and the monocyclic head-to-tail cyclized analogue of celogentin C **11** were chosen as examples of designed synthetic cyclic peptides, containing 5 and 8 residues, respectively. Linear peptides were synthesized using the standard SPPS protocol described for the hexapeptides. All Ag(I)-promoted macrocyclization reactions proceeded with high efficiency to give the cyclic peptides in excellent yield after a single purification (Figure 2). No evidence for epimerization of the C-terminal residue, or of formation of cyclodimers or higher oligomers, was observed in any reaction. These examples highlight the macrocyclization proceeds efficiently for a range of ring sizes and ligation sites, with Orn–Leu, Gly–Phe and Gln–Leu all generating the macrocyclic products in excellent yield. Cyclization at a D-Phe–Val site resulting in slightly lower yield, consistent with that observed for **5d**. The presence of a variety of protected side-chain functional groups, such as those in arginine, histidine, tryptophan, aspartate and glutamine residues – which could potentially coordinate to the Ag(I) or act as nucleophiles to intercept the isoimide intermediate – did not adversely affect the macrocyclization process.

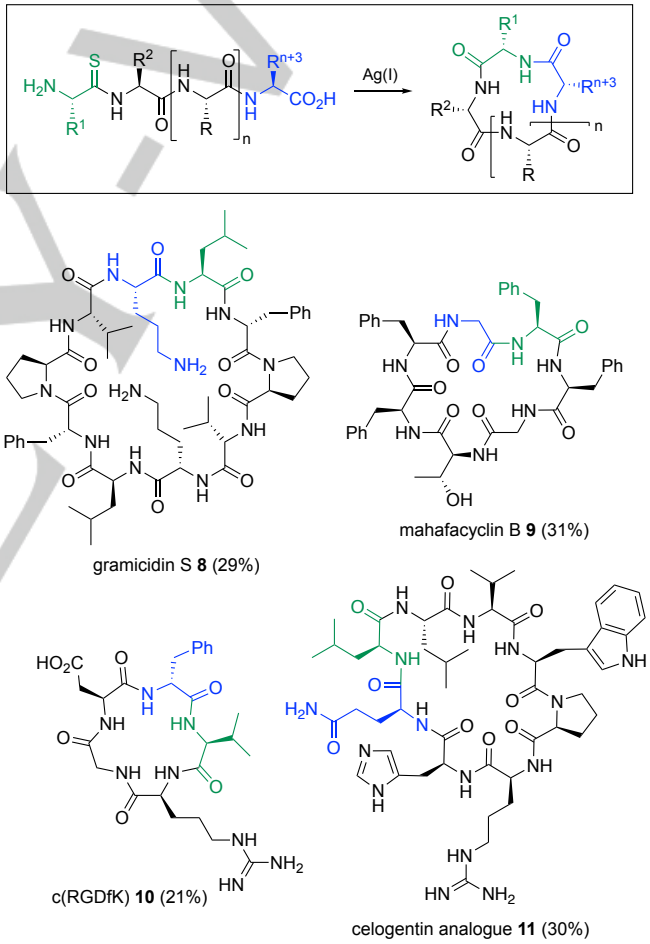


Figure 2. Diverse examples of cyclic peptides synthesized by Ag(I)-promoted thioamide cyclization (overall yields from starting resin shown in parentheses).

To directly compare the Ag(I)-promoted cyclization of peptide thioamides with a standard peptide macrocyclization process, the cyclization of thioamide-containing octapeptide **13** was performed in parallel with the HATU-promoted macrocyclization of octapeptide **12**. The standard macrocyclization of **12** with HATU required 16 hours to proceed to completion, generating the cyclic

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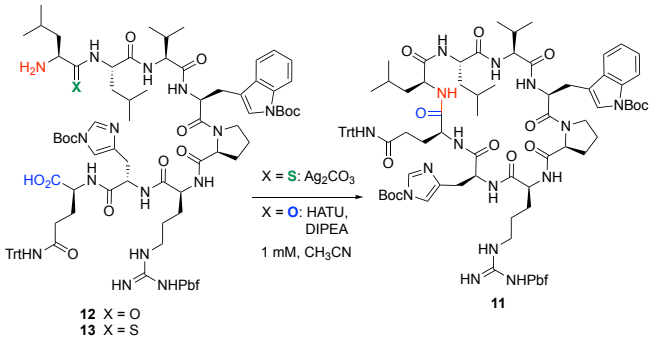
product **11** in 35% yield (HPLC). In contrast, the Ag(I)-promoted macrocyclization of the peptide thioamide **13** was complete in less than one hour, generating the product **11** in 83% yield (HPLC) (Figure 3).



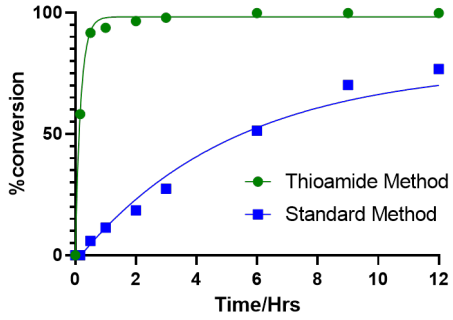


Figure 3. Comparison of rate of macrocyclization using standard HATU coupling (blue) and Ag(I)-promoted thioamide coupling (green) methods.

Our previous mechanistic studies of the intermolecular reaction showed that Ag(I) coordinates to both the carboxylate and the thioamide.[24] We propose that the same process in an intramolecular reaction results in a Ag(I)-templated cyclization (Figure 4), reminiscent of the metal ion-templated cyclization reactions of peptide thioesters.[10] Importantly, in this process the Ag(I) both chemoselectively activates the thioamide to nucleophilic attack by the carboxylate, and templates the cyclization by bringing the N- and C-termini in close proximity, thereby overcoming the entropic barrier to cyclization resulting in a fast reaction.

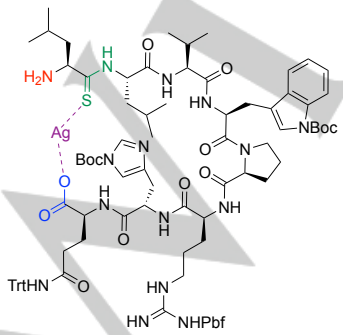


Figure 4. Ag(I) both chemoselectively activates the thioamide and templates the macrocyclization.

In conclusion, a single-atom (O→S) substitution of a linear peptide – through incorporation of a thioamide at the N-terminal backbone amide position – facilitates and rapid and traceless Ag(I)-promoted macrocyclization process. Cyclic peptides are furnished in excellent yield, free of epimerization and cyclodimerization.

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Keywords: macrocyclization • cyclic peptide • thioamide

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