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Silver(I)-mediated oxazoline formation: a mild route to 2,4-oxazoles in peptides

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We present a silver-promoted cyclization of peptide thioamides that enables site-specific insertion of oxazole and methyloxazole motifs through oxazoline intermediates. Demonstrated in di- and tetrapeptides, this mild, moisture-tolerant methodology delivers high-yield products, offering a robust and general strategy for constructing structurally diverse, conformationally constrained oxazole-containing peptidomimetics.

The incorporation of heterocyclic scaffolds into peptide frameworks is evolving as a strategic approach to influence secondary structure and improve pharmacokinetic properties in the development of therapeutic peptides.[1] It has been demonstrated that incorporating heterocyclic ring scaffolds, such as pyridines, thiazoles, oxazoles, and triazoles, can enhance peptide stability, membrane permeability, and resistance to enzymatic degradation by introducing conformational constraints and modulating electronic properties.[2–4] With the potential to bridge the gap between small molecules and biologics, heterocyclic peptides represent a critical frontier in next-generation therapeutics. While significant progress has been made towards developing new synthetic strategies tailored to these hybrid systems, there is continued interest in mild peptide-compatible methodologies that enable the efficient and site-selective incorporation of many heterocyclic scaffolds.[5–9] Synthesis of heterocyclic peptides remains a challenge due to complex regioselectivity, harsh reaction conditions, and the need for specialized protecting group strategies, thus underscoring the importance of continued methodological advancements in this field.

Oxazole heterocycles are privileged scaffolds in medicinal chemistry, valued for their ability to enhance drug-like properties through improved metabolic stability, hydrogen-bonding capacity, and structural rigidity.[10–12] Their structure imparts unique stability and supports favorable pharmacokinetics, bioactivity, toxicity profiles, and intermolecular interaction capabilities. Oxazoles, namely 2,4-disubstituted oxazoles or 5-methyl-2,4-disubstituted oxazoles, are ubiquitous motifs in natural products, prominently featured in bioactive metabolites from bacteria, fungi, cyanobacteria, and marine organisms. Their presence in plantazolicin A (an antibiotic),[13] rhizoxin (an antitumor agent),[14] muscoride A (an antimicrobial alkaloid),[15] and telomestatin (an anticancer agent)[16] underscores the significance of the oxazole motif in biological interactions. Further, the oxazole core serves as a key pharmacophore in small-molecule drug design, exemplified by oxaprozin (Daypro®, clinically approved nonsteroidal anti-inflammatory drug)[17] as well as numerous other therapeutics spanning antibacterial,[18] anticancer,[19–21] and antimalarial agents,[22,23] among others.

The synthesis of oxazoles typically requires harsh and/or anhydrous conditions, including use of extreme temperatures or strong acids, which limits functional group tolerance and complicates the preparation amongst sensitive functional groups commonly found in peptides (Fig. 1a).[24–28] Many traditional approaches also suffer from a narrow substrate scope, particularly with sterically hindered or electron-poor precursors, reducing their versatility.[25,29] Despite recent progress in oxazole synthesis,[30,31] such methods have limited applicability to peptidic systems. An alternative strategy involves the cyclization of β-hydroxyamides to oxazolines, converting linear precursors into cyclic structures while preserving the peptide backbone. However, these reaction conditions are often unstable, and robust methods to access oxazole peptidomimetics remain scarce.[32]

Thioamides, in which the carbonyl oxygen of an amide is replaced with a sulfur atom, are advantaged functional groups in chemical biology and coordination chemistry due to their enhanced reactivity and unique electronic properties. Compared to their amide counterparts, thioamides are more polarizable, have lower resonance stabilization, and display increased nucleophilicity and electrophilicity, making them versatile handles for metal coordination and synthetic modification.[33] A key feature of thioamides is their ability to coordinate soft metals such as silver(I), mercury(II), and copper(II)

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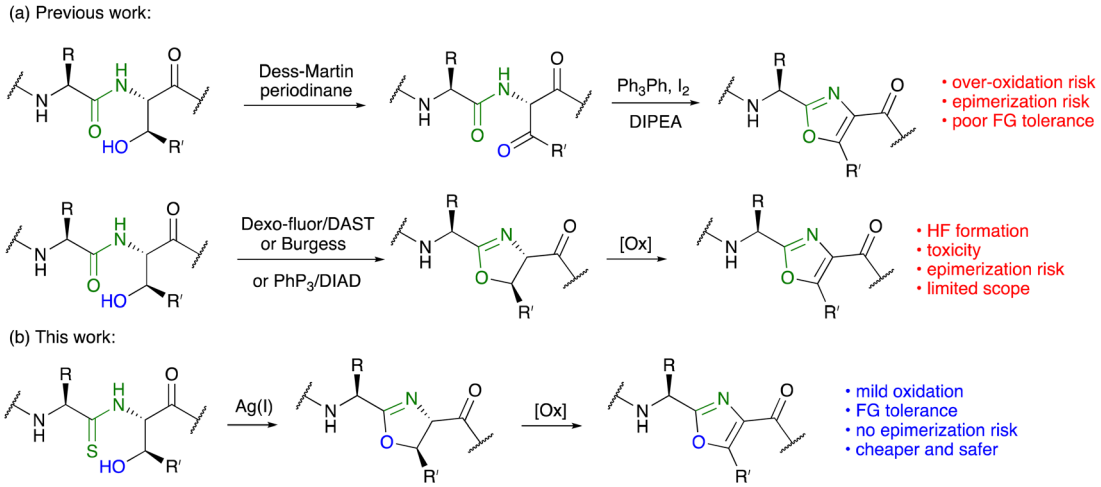


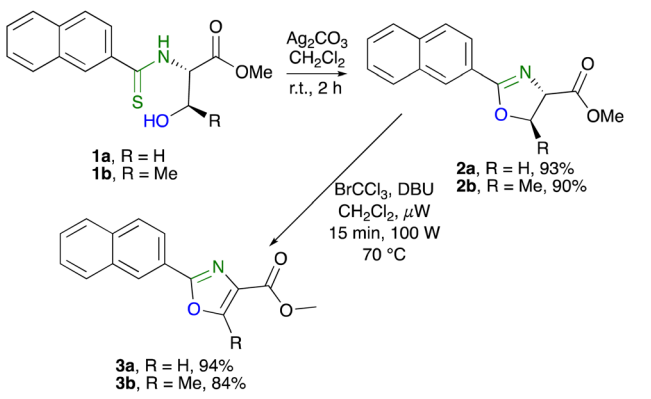
Fig. 1 (a) Current methods for incorporating 2,4-disubsituted oxazoles in peptides; (b) silver(I)-mediated β-hydroxyamide cyclization of thioamides.

through monodentate coordination at sulfur, which can activate the thioamide toward further transformation.[34] Hutton and coworkers have extensively explored this chemistry, highlighting how silver(I) coordination to thioamides enhances their electrophilicity, enabling regioselective transformations on peptide molecules under mild conditions.[35] Notably, silver–thioamide complexes have been utilized in peptide macrocyclization[36–39] and functionalization[40,41] strategies, demonstrating that silver-mediated activation offers a chemoselective and efficient route for functionalizing thioamide-containing substrates. Herein, we report a robust site-specific methodology to incorporate oxazoles and methyloxazoles into peptides *via* silver(I)-mediated β-hydroxyamide cyclization of thioamides adjacent to serine and threonine residues (Fig. 1b).

To investigate the feasibility of silver(I)-mediated oxazoline formation from thioamide starting materials, a simplified model system was developed to assess the nucleophilicity of serine (**1a**) and threonine (**1b**) hydroxyl side chains towards a neighboring thioamide in the presence of Ag_2CO_3. Based on prior research, Ag_2CO_3 was selected over other silver(I), mercury(I), and copper(II) salts.[35] The naphthyl thioamides **1a** and **1b** were treated with 1.5 equiv. of Ag_2CO_3 for 2 hours, affording the corresponding 2-oxazoline (**2a**) and 5-methyl-2-oxazoline (**2b**) in 93% and 90% isolated yield, respectively. Subsequent oxidation, using conditions adapted from the literature, furnished the corresponding oxazole (**3a**) and methyl oxazole (**3b**) in 94% and 84% isolated yield, respectively (Scheme 1).[42]

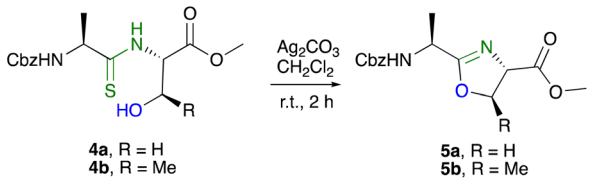
Upon successful silver-mediated generation and isolation of 2-oxazoline and 5-methyl-2-oxazoline derivatives in excellent yield, a Cbz-AX-OMe dipeptide model system was prepared in which alanine (A) was coupled to serine or threonine (X) to explore oxazole insertion within a peptide backbone. Thioamides **4a** and **4b** were treated with 1.0, 1.2, 1.5, 2.0, and 3.0 equivalents of Ag_2CO_3 to determine the minimal silver equivalents required to support oxazoline formation (Table 1). Using 1.5 equiv. of Ag_2CO_3 afforded the corresponding oxazolines **5a** and **5b** most optimally with 86% and 89% isolated yield, respectively (entry 3). Increasing to 2.0 and 3.0 equiv. resulted in a yield plateau at 85 to 87% (entries 4 and 5), while lower equivalents of Ag_2CO_3 resulted in incomplete conversion (entries 1 and 2). In all cases, only thioamide starting materials and oxazoline products were isolated by chromatography. No regeneration of amide products was observed. Subsequent reactions were carried out using 1.5 equiv. of Ag_2CO_3 to balance yield optimization with reagent economy.

The reaction mechanism was explored in detail for the Cbz-AS-OMe (**4a**) system using density-functional theory (DFT) calculations. Calculations were carried out using Gaussian 16 and postg programs with the LC-ωPBE functional and the XDM dispersion correction (see SI, page S3). Based on these calculations, a plausible reaction mechanism is shown in Scheme 2. To begin, two silver ions coordinate to the sulfur in a soft acid/soft base interaction, increasing the electrophilicity of the thiocarbonyl carbon. A carbonate counter-ion then deprotonates the hydroxyl side chain (blue), generating a nucleophilic oxyanion. Intramolecular cyclization occurs *via* nucleophilic attack of the oxyanion at the thiocarbonyl carbon producing



Scheme 1 Simplified model reaction: generation of oxazoline and methyloxazoline derivatives from thioamide.

Table 1 Optimization of silver-mediated generation of 2-oxazoline (X = serine) and 5-methyl-2-oxazoline (X = threonine) derivatives from Cbz-AX-OMe thioamides



4a, R = H
4b, R = Me

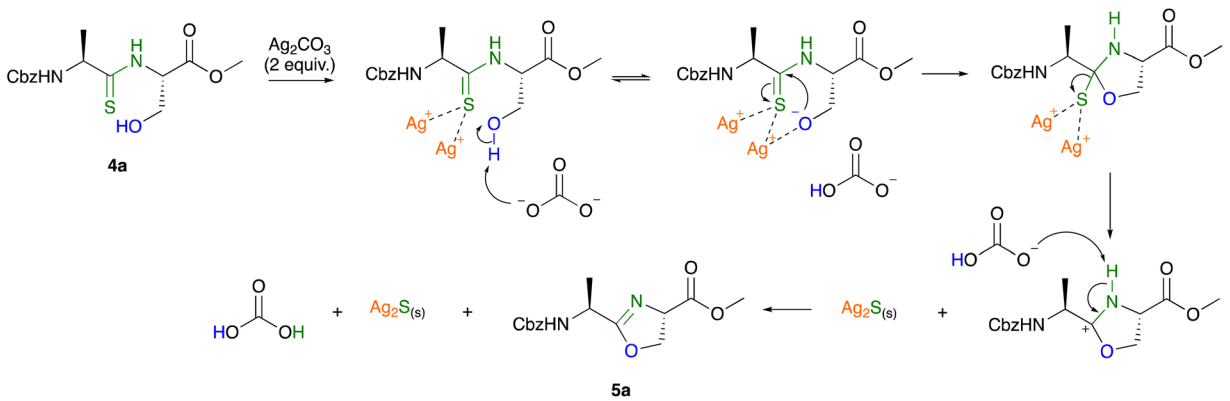
5a, R = H
5b, R = Me

Entry	Equiv. of Ag_2CO_3	Yield of **5a** [%]	Yield of **5b** [%]
1	1.0	44%	67%
2	1.2	76%	74%
3	1.5	86%	89%
4	2.0	85%	87%
5	3.0	87%	87%

a tetrahedral intermediate. Silver sulfide (Ag_2S) then dissociates producing a carbocation intermediate. Finally, the bicarbonate ion deprotonates the secondary amine (green), neutralizing the carbocation resulting in the backbone inserted oxazoline (**5a**). The free-energy landscape (Fig. 2) supports this pathway. Silver coordination plays a significant role in the electrophilicity of the thioamide (**I**) and stabilization of the oxyanion in the transition state (**TS1**). Displayed in **TS1**, silver coordination draws the oxyanion towards the electrophilic carbon, reducing the distance from 3.678 Å to 2.175 Å and positions the nucleophile for intramolecular cyclization. Oxyanion generation is calculated to be the rate determining step, requiring a $\Delta G^{\ddagger}$ value of 12.7 kcal mol^{-1}, after which the cyclized tetrahedral intermediate (**II**) forms spontaneously with an overall free energy of −19.9 kcal mol^{-1}. Dissociation (and subsequent precipitation) of Ag_2S leads to the lower-energy carbocation (**III**). A bicarbonate counter-ion then deprotonates the secondary amine *via* a barrierless transition state (**TS2**), relieving the carbocation and producing the final oxazoline intermediate (**IV**). These results indicate that oxazoline formation requires more than one equivalent of Ag_2CO_3, consistent with the yields in Table 1, where 1.0 and 1.2 equiv. gave lower conversions, and 1.5 equiv. and above produced optimal yields, reflecting sufficient *in situ* silver ions to sustain the reaction. Experimentally, reactions generate a black insoluble Ag_2S precipitate, consistent with the proposed mechanism.

Applying the optimized reaction conditions (Table 1, entry 3), we investigated a series of dipeptide thioamides to evaluate the amino acid side chains influence on oxazoline formation at the thioamide residue. Cbz-ZS-OMe and Cbz-ZT-OMe dipeptide thioamides where prepared, where Z was varied to include bulky hydrophobic, basic, acidic, and cyclized side chains (Z = Phe, Lys(Boc), Glu(OtBu), Pro) coupled to serine or threonine to afford 2-oxazoline and 5-methyl-2-oxazoline derivatives, respectively (**5**, Fig. 3). Each thioamide dipeptide was treated with 1.5 equiv. of Ag_2CO_3 and conversion was monitored by TLC to completion. The resulting oxazolines and methyloxazolines (**5**) were isolated by flash chromatography and subsequently oxidized to the corresponding oxazoles and methyloxazoles (**6**, Fig. 3).

For the oxazole series, Ala (**5a**) and Phe (**5c**) analogues underwent oxazoline formation in 2 hours to give high isolated yields (86% and 84%, respectively). The bulkier residues, Pro (**5e**) and Lys(Boc) (**5g**), required 3 hours for full conversion and afforded moderate to good isolated yields (72% and 75%). Isoleucine dipeptides were explored in the reaction scope, giving 96% conversion to oxazoline products by NMR, but degraded on silica and could not be isolated in good yield. While other efforts were employed to purify Ile-oxazoline products, no successful isolation methods were established. Glu(OtBu) (**5i**) was the slowest to cyclize, requiring 5 hours and affording a 79% isolated yield. The methyloxazoline series showed similar trends: Ala (**5b**) and Phe (**5d**) produced the highest yields (89% and 80%, respectively) after 2 hours, while Lys(Boc) (**5h**) and Glu(OtBu) (**5j**) required 3 to 6 hours and produced 70% and 72% yields, respectively. Pro (**5f**) cyclized in 2 hours to give a 78% yield. In general, methyloxazolines were obtained in slightly lower yields than their oxazoline counterparts, with the exception of Pro. In all cases, high conversion to 2-oxazoline and 5-methyl-2-oxazoline derivatives was observed,



Scheme 2 Plausible reaction mechanism for the formation of backbone inserted oxazolines from dipeptides thioamides and neighboring nucleophilic hydroxyl amino acid side chains.

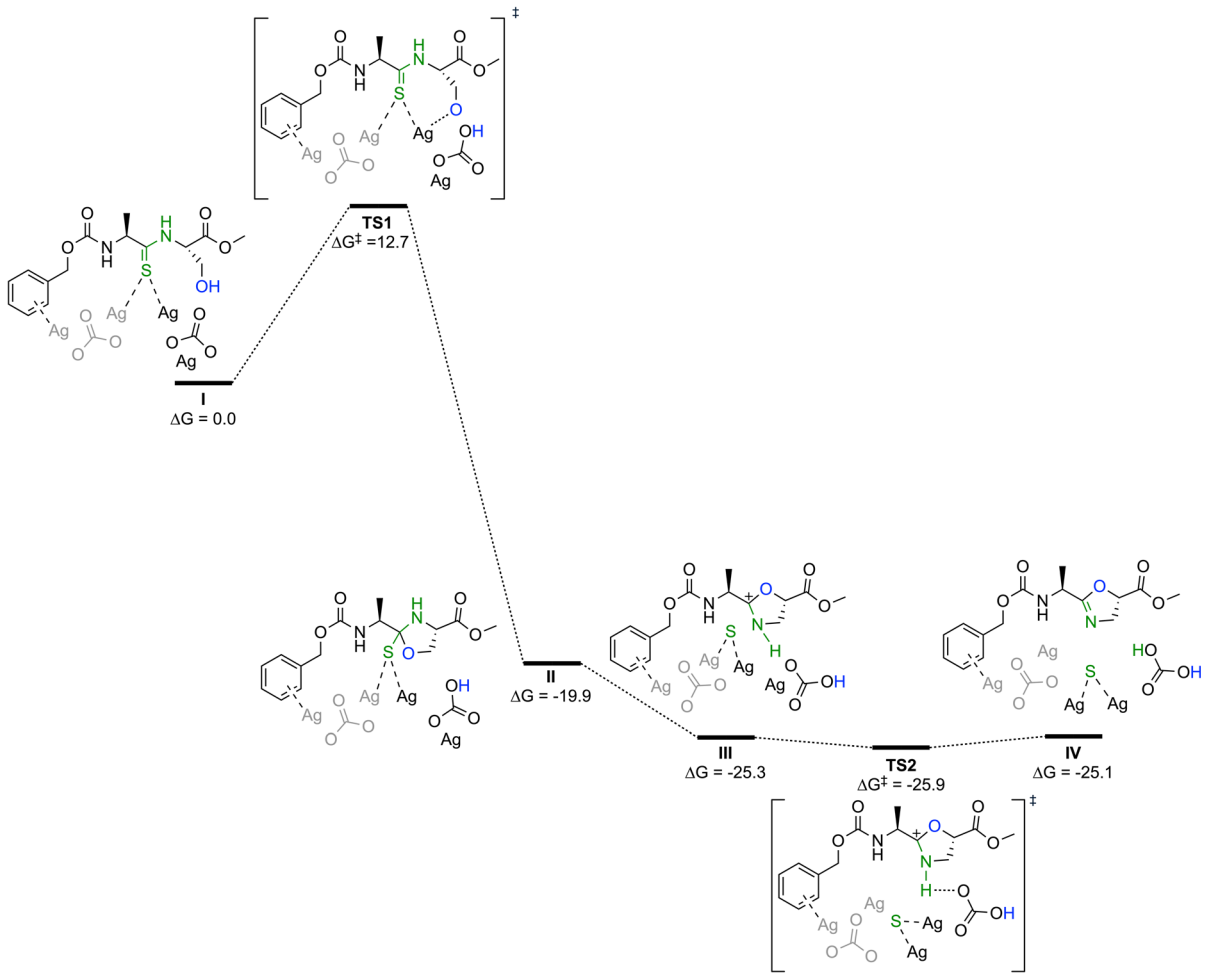


Fig. 2 Free energy landscape for **4a** to **5a** showing oxazoline formation *via* two transition states using two equiv. of Ag_2CO_2. Gibbs free energies (ΔG) are expressed in kilocalories per mole (kcal mol^{-1}). Note that the greater stability of **TS2**, relative to **III**, is due to the thermal free-energy correction and it is higher in electronic energy.

indicating that the amino acid side chains have little influence on reaction efficiency. Oxidation of these intermediates (**5**) to oxazoles and methyl oxazoles (**6**) proceeded in 68–86% yields for all analogues, consistent with literature findings.

The methodology was further applied to tetrapeptide systems to demonstrate feasibility with increased complexity. Thioamides were installed within the peptide sequence using the established solid phase peptide synthesis (SPPS) method which enables site-specific thioamide incorporation *via* amino acid benzotriazolides.[43,44] Four tetrapeptides were efficiently synthesized using Fmoc-Phe-benzotriazolide and Fmoc-Ile-benzotriazolide, yielding thioamide-containing analogs Ac-Tyr-IleΨ{(C═S)NH}-Ser/Thr-Ala-NH_2 (**7a**/**b**) and Ac-Gly-PheΨ{(C═S)NH}-Ser/Thr-Ala-NH_2 (**7c**/**d**). Each peptide was treated with 3 equiv. Ag_2CO_3—a stoichiometry selected to ensure complete conversion after initial reactions stalled using only 1.5 equiv. This stalling is likely due to the ability of the silver ion to coordinate in a bidentate fashion with other amide carbonyls present in the extended peptide backbone, effectively sequestering the reagent and preventing cyclization of the thioamide. The formation of 2-oxazoline (**8a**/**c**) and 5-methyl-2-oxazolines (**8b**/**d**) were monitored by LC-MS (Fig. S2–5) for completion using peak area normalization (Scheme 3). Thioamide **7a** achieved 97% conversion to the desired 2-oxazoline tetrapeptide whereas its threonine-containing counterpart, **7b**, generated only 23% conversion of the desired 5-methyl-2-oxazolines (**8b**) with the rest remaining as uncyclized thioamide starting material. These systems required extended reaction times between 19 and 21 hours. Thioamides **7c** and **7d** progressed to 96% and 92% conversion of 2-oxazoline (**8c**) and 5-methyl-2-oxazolines (**8d**), respectively, in 2 to 6 hours. The excellent conversion to oxazolines demonstrates compatibility of this methodology with larger peptide systems.

In summary, we report a robust site-specific method for inserting oxazole and methyloxazole motifs *via* silver-mediated intracyclization of peptide thioamides. This mild, moisture-tolerant approach offers a peptide-compatible alternative to existing oxazole and methyloxazole synthesis methods, delivering high yields broad amino acid tolerance. Supported by DFT calculations, we elucidate the distinct role of silver(I) coordination in driving this transformation. Further investigations will focus on applying this methodology to solid-phase peptide synthesis, enabling iterative oxazole incorporation in mid-sized peptide sequences.

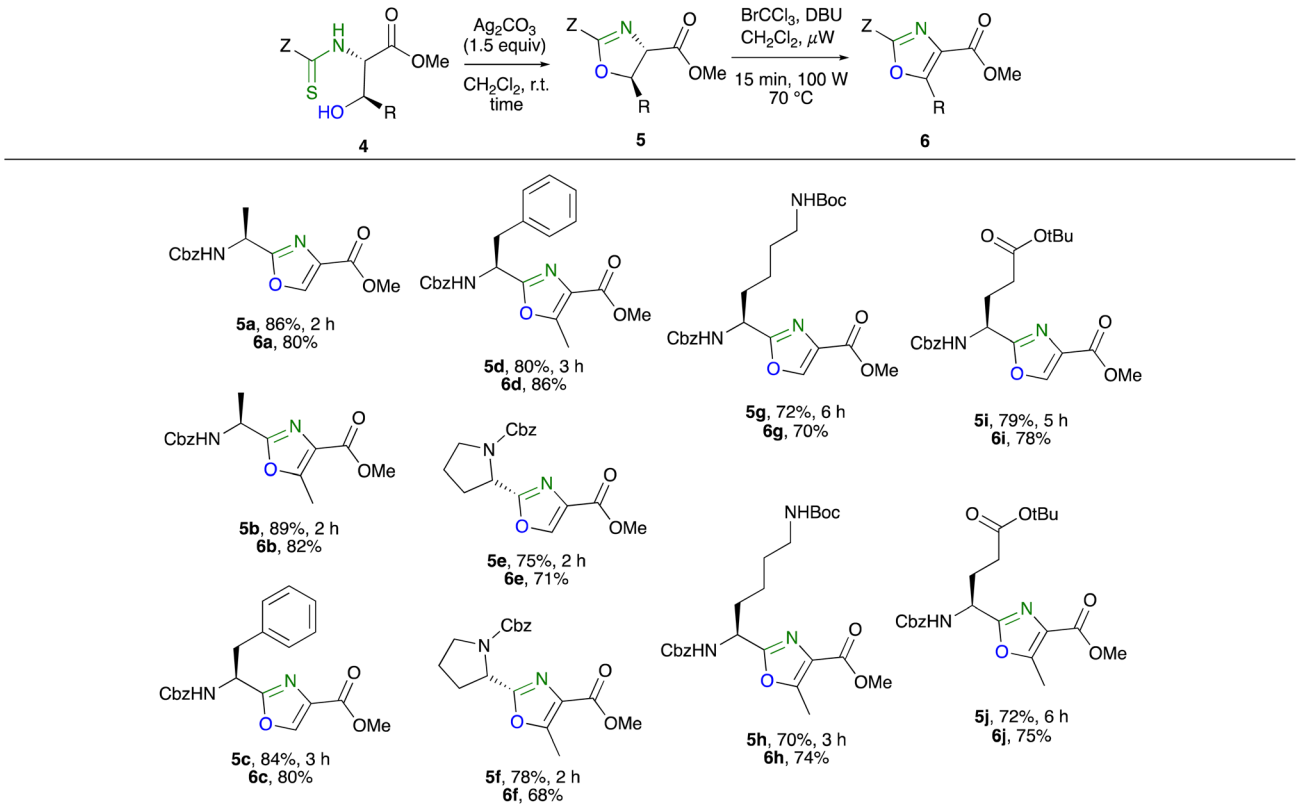
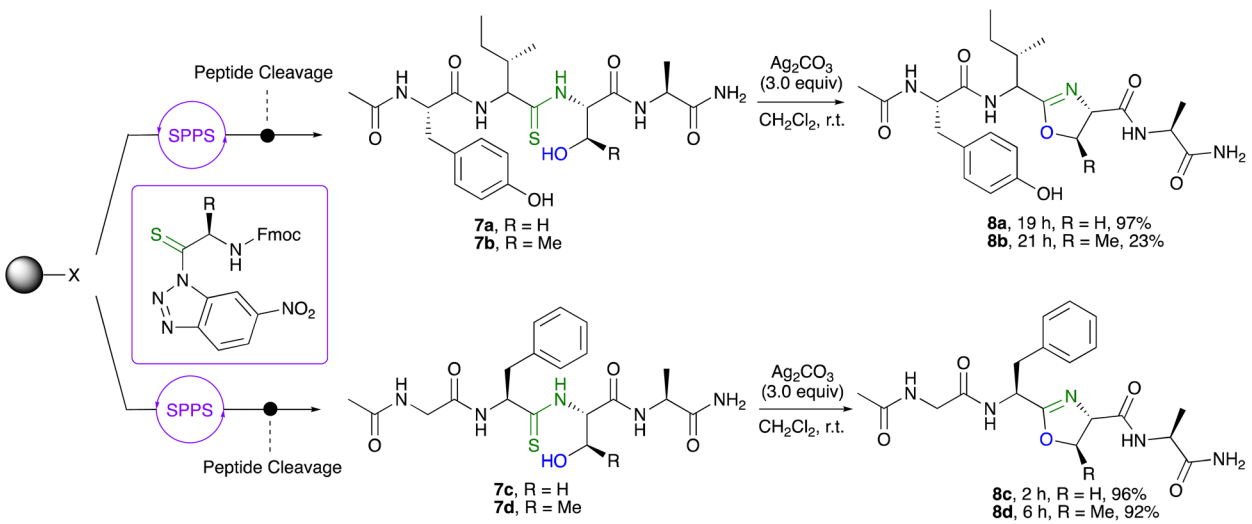


Fig. 3 Substrate scope of oxazoline and oxazole formation from Cbz-ZS-OMe and Cbz-ZT-OMe thioamide dipeptides with isolated yields and reaction times given.



Scheme 3 SPPS on amide resin using Fmoc-amino acids and benzotriazolides to afford the requisite thioamide starting materials, **7**, after cleavage. These were then subjected to silver(I)-mediated cyclization to afford the corresponding oxazolines, **8**, with conversion percentage to desired material given.

Author contributions

A. S. P.: methodology (lead); investigation (lead); validation (equal); writing – review and editing (equal). J. W. C.: methodology (supporting); validation (equal); writing – original draft preparation (lead); writing – review & editing (equal). K. M. C.: methodology (supporting), validation (equal); writing – review & editing (equal). Z. A. B: conceptualization (supporting). E. R. J.: resources, methodology (lead), validation (supporting), writing – review & editing (equal). C. L. C.: supervision (lead),

visualization, funding acquisition, writing – original draft preparation (supporting), writing – review & editing (equal).

Conflicts of interest

There are no conflicts to declare.

Data availability

Data for this article, including experimental procedures and characterization data, are available as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5sc06351e.

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