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Abstract

Thioamides are important biophysical probes of peptide folding but are prone to α -C epimerization during Fmoc solid-phase peptide synthesis. The stereochemical integrity of thioamide-containing peptides can be dramatically improved by protecting the thioamide as a thioimide during synthesis. A drawback of this approach, however, is that once synthesis of the peptide is complete, regeneration of the thioamide requires the toxic, corrosive, and flammable gas H₂S. This work examines several approaches to supplant H₂S as a deprotection reagent in favor of a safer and more convenient alternative. Ultimately, a new application of the 4-azidobenzyl protecting group to thioamides was found to provide the most suitable means of both protection of α -C stereochemistry and conversion back to thioamide.

Disciplines

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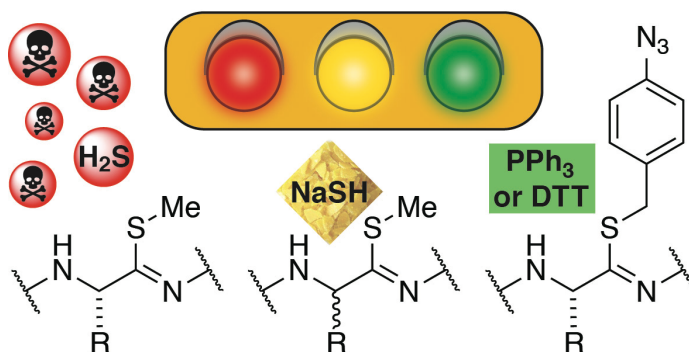
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Deprotection Strategies for Thioimides during Fmoc Solid-Phase Peptide Synthesis: A Safe Route to Thioamides

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ABSTRACT

Thioamides are important biophysical probes of peptide folding, but are prone to α -C epimerization during Fmoc solid-phase peptide synthesis. The stereochemical integrity of thioamide-containing peptides can be dramatically improved by protecting the thioamide as a thioimide during synthesis. A drawback of this approach, however, is that once synthesis of the peptide is complete, regeneration of the thioamide requires the toxic, corrosive, and flammable gas H_2S . This work examines several approaches to supplant H_2S as a deprotection reagent in favor of a safer and more convenient alternative. Ultimately, a new application of the 4-azidobenzyl protecting group to thioamides was found to provide the most suitable means of both protection of α -C stereochemistry and conversion back to thioamide.

INTRODUCTION

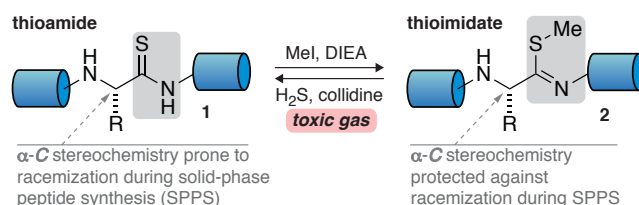
Thioamides mimic the shape and structure of peptide bonds but present different electronic and hydrogen-bonding properties—making thioamides important biophysical probes of peptide structure and function.^{1,2} For example, thioamides have been employed to interrogate hydrogen-bonding in α -helices and β -turn structures,³⁻⁵ as well as $n \rightarrow \pi^*$ interactions between peptide bonds across amino acids ($i \rightarrow i + 1$).⁶⁻⁸ Further, thioamides have been applied in photochemical isomerization schemes,^{9,10} and can serve

as quenchers of fluorescent probes to study peptide folding.¹¹⁻¹⁵ Moreover, thioamides are increasingly being discovered in natural products.² Thus, methods to incorporate thioamides into peptides via Fmoc solid-phase peptide synthesis (SPPS) are immensely valuable.

Thioacylating reagents have been developed that allow for the coupling of thioamide residues at seemingly any position in a peptide sequence.^{16, 17} Unfortunately, the stereochemical integrity of the resulting peptide is at risk because the α -C of the thioamide is susceptible to deprotonation and epimerization during Fmoc-deprotection of the *N*-terminal amine.¹⁸⁻²⁰ The stereochemical integrity of the thioamide amino acid therefore further degrades during subsequent coupling and Fmoc-deprotection of subsequent amino acids.²¹ This limitation in stereochemical stability constrains the peptide sequence space in which thioamide probes can be implemented.

We recently demonstrated a robust method to synthesize thioamide-containing peptides with high stereochemical integrity.¹⁸ The method relied upon protecting the thioamide (1) as a thioimidate (2), which significantly reduced epimerization at the α -C (Scheme 1). The thioimidate could then be converted back to the thioamide using H_2S after SPPS was complete.

Scheme 1. Reversible protection of thioamide residue stereochemistry

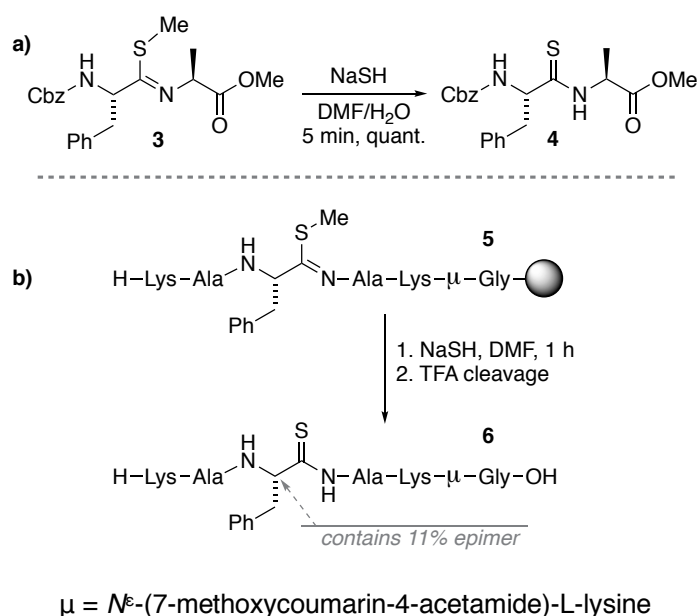


While this strategy can be easily implemented within normal SPPS methodology, the use of H_2S is problematic because it is highly toxic, corrosive, and flammable.^{22, 23} Additionally, it is a gaseous reagent which can be inconvenient to transfer and manipulate. We therefore sought to identify safer and more convenient conditions that involved solid or liquid reagents as opposed to gaseous ones.

RESULTS AND DISCUSSION

We initially investigated NaSH as an alternative to H₂S that can be easily weighed and transferred. We tested the potential for conversion of thioimide back to the thioamide using a model test dipeptide (**3**→**4**, Scheme 2a).

Scheme 2. Deprotection with NaSH.

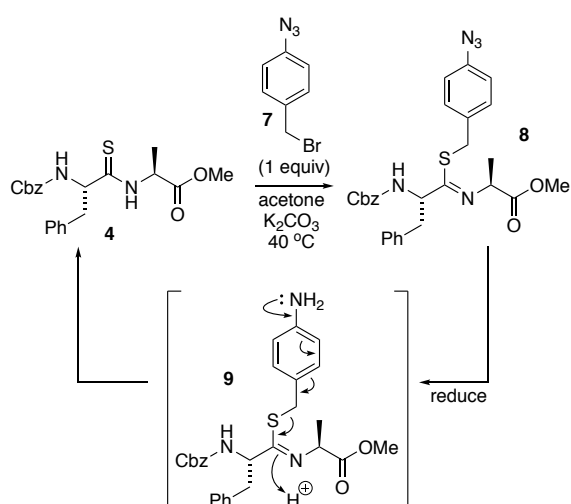


Our initial results were promising, as conversion to thioamide **4** was complete within minutes (Figure S4). Unfortunately, when we attempted the NaSH conditions with a thioimide peptide (**5**) on solid phase, we observed small amounts of epimerization of the final peptide (**5**→**6**, Scheme 2b, Figure S5), which negated the efficacy of the thioimide protection strategy. This result was likely due to the strongly alkaline nature of NaSH.²⁴ Additionally, because NaSH (and Na₂S) are hygroscopic and typically sold as hydrates, we surmised that small amounts of hydroxide may be responsible for a background epimerization of the liberated thioamide as well. Ultimately, we deemed NaSH as a non-ideal reagent for the purposes of thioimide deprotection for the following reasons: (i) NaSH is still a corrosive chemical. (ii) While NaSH is a solid, it readily generates H₂S. (iii) Solutions of NaSH in DMF are turbid and can clog fritted SPPS reaction vessels. (iv) Finally, while it may be possible to identify ‘on-resin’ conditions that would allow for the rate of conversion of thioimide back to thioamide to outcompete the rate of

background epimerization, such conditions would be sequence-dependent and introduce time-intensive optimization steps that would undermine the generality of this approach.

We next considered using a different alkylating agent (**7**)²⁵ of the thioamide, instead of MeI, that could be cleaved via alternative conditions (Scheme 3). We hypothesized that the 4-azidobenzyl group in **8** would allow for an alternative mechanism of deprotection through reduction of the azide to an amine, followed by spontaneous aza-quinone methide elimination to give the liberated thioamide (**8**→**9**→**4**).²⁶

Scheme 3. Azido-benzyl protecting group.



We initially investigated the Staudinger reduction of the azide using PPh₃ and water as conditions for deprotection to give thioamide **4** (Table 1, entry 1).²⁷ While the reaction with PPh₃ consumed the starting material (**8**, Figure S6), subsequent hydrolysis of the aryl-iminophosphorane (Ar-N=PPh₃) was ineffective in neutral water as reported in literature.²⁸ Alternatively, weak acid conditions at this stage successfully catalyze hydrolysis of iminophosphorane to amine (Ar-N=PPh₂ → Ar-NH₂ + O=PPh₃) and cleanly liberated the thioamide (Table 1, entry 2, Figure S7).²⁶

Although the weak-acid conditions in entry 2 can be used with amide resins to deprotect the thioamide but leave the peptide still attached to resin, with so-called super-acid-sensitive resins (e.g., 2-chlorotrityl resin), the weak-acid conditions of entry 2 would cleave the peptide from the resin. Therefore, we sought to also identify neutral conditions for deprotection of the thioamide that would be compatible

with super-acid-sensitive resins. Thus, we investigated reduction of the aryl azide using dithiothreitol (DTT).^{26, 29} Accordingly, DTT gave the expected thioamide in excellent yield (Table 1, entry 3, Figure S8).

Table 1. Conditions for reductive release of thioamide (8→4)

| entry | conditions | yield of 4 (from 8) % |
|-------|---|--------------------------------------|
| 1 | PPh ₃ , THF, H ₂ O (1:1) | 0 |
| 2 | PPh ₃ , THF, H ₂ O (1:1) then AcOH (0.5 M) | quant. |
| 3 | dithiothreitol (DTT), DMF | 97 |

To determine if the azidobenzyl thioimide would be a viable protecting group during SPPS, we synthesized the short sequence F^(S)AKFG (**10**) from 2-chlorotrityl resin using established thioacylating agents (Figure 1).¹⁶ Thioamide-containing peptide **10** was protected as the thioimide **11** using conditions that are compatible with solid-phase methodology. (We surmise that the broadened peak shape for **11** is due to interconverting isomers of the thioimide (**12** ⇌ **13**). This kind of rotational isomerism is visible in the NMR spectra of model dimer **3** in Scheme 2 above (Figure S1)). From **11**, SPPS could in principle proceed normally to elongate the peptide, while the thioimide moiety in **11** guards against epimerization of the thioamide residue. Finally, once the sequence was complete, the thioamide could be cleanly restored without cleavage from the resin, as demonstrated by the conditions from entry 3 of Table 1 (Figure 1, **11**→**10**).

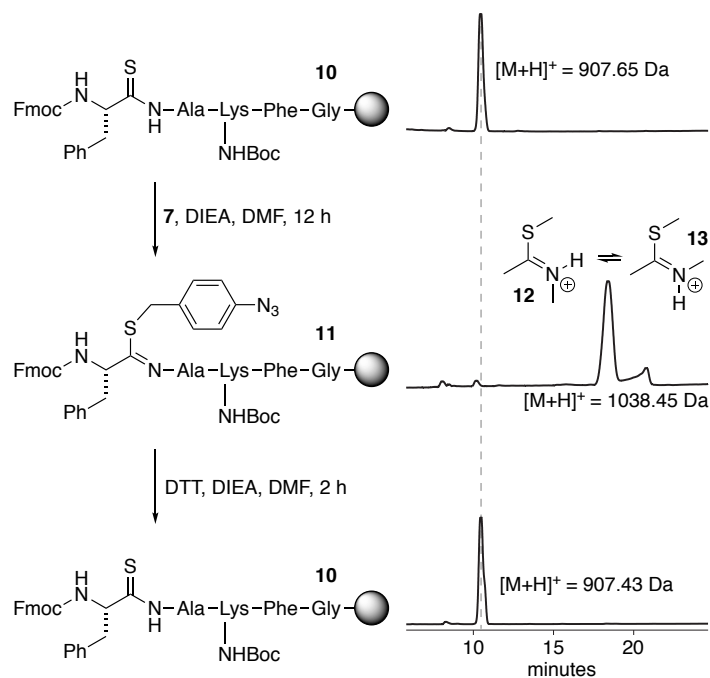


Figure 1. Demonstration of the interconversion between thioamide and thioimidate on-resin. The reaction can be performed using standard peptide-synthesis reaction vessels and was tracked using HPLC and ESI-MS.

The stability of azides during SPPS is well-documented.³⁰⁻³⁴ However, we wanted to confirm that thioimidates related to **8** would display the same enantiomeric stability during SPPS that has previously been demonstrated for **3**.¹⁸ The Fmoc-deprotection step of the SPPS procedure requires piperidine and is chiefly responsible for epimerization of the α -C stereocenter of the thioamide amino acid.¹⁸⁻²⁰

As seen in Figure 2, the thioamide dipeptide **4** is very sensitive to epimerization with piperidine.

Conversely, both the methyl thioimidate dipeptide and the thioimidate bearing the azidobenzyl group withstand epimerization to the same extent.

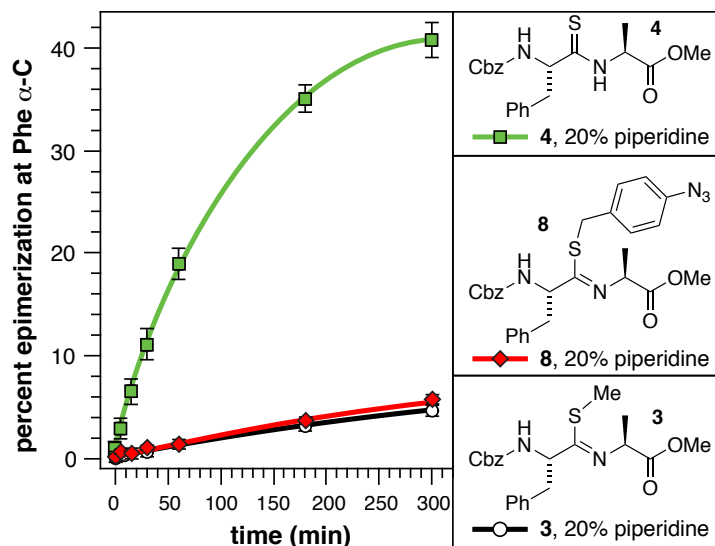


Figure 2. Epimeric stability of **8** and **3** with piperidine compared to **4**. Solutions of each compound (0.1 M) in DMF with 20% piperidine (v/v) were prepared and the total epimerization was tracked relative to 1,3,5-trimethoxybenzene as internal standard.

CONCLUSION

We report a new application of the 4-azidobenzyl protecting group to preserve the stereochemical integrity of thioamides during SPPS. Thioamides along the peptide backbone are prone to epimerization during the Fmoc-deprotection step of standard SPPS methodology. Protection of thioamides as thioimidates using the 4-azidobenzyl protecting group was shown to significantly curtail epimerization. Critically, the 4-azidobenzyl thioimide can be converted back to the thioamide once peptide synthesis is complete using relatively safe reagents (PPh_3 or DTT). These safer deprotection conditions supplant the use of the toxic, corrosive, and flammable gas H_2S as the preferred method to liberate alkyl-protected thioimidates. We anticipate that the methods described here can be immediately applied to SPPS methodologies for the implementation of thioamide biophysical probes in peptides.

EXPERIMENTAL SECTION

General Information. Silica gel (40 μm) was purchased from Grace Davison. All chemical reactants and reagents were purchased from commercial vendors and used without prior purification. Solvents were

purchased from Fisher Scientific, and THF, DCM, and DMF were dried via Glass Contours solvent system. *NMR Spectroscopy*: ^1H and ^{13}C NMR spectra for all compounds were acquired in deuterated solvents (as indicated) on a Bruker Spectrometer at the field strengths reported in the text. The chemical shift data are reported in units of δ (ppm) relative to residual solvent. *Liquid Chromatography*: Peptide analysis was accomplished on a Waters Acquity UPLC System with UV detection and a BEH C18 column 1.7 μm . UPLC solvents were water with 0.1% formic acid or acetonitrile with 0.1% formic acid. All flow rates were held constant at 0.450 mL/min. For larger peptides and final analysis, a Waters HPLC System was used. Detection was acquired using a Photodiode Array Detector and a SunFire C18 column 5 μm . HPLC solvents were water with 0.1% TFA and acetonitrile with 0.1% TFA. All flow rates were held constant at 1.00 mL/min. *Mass Spectrometry*: Masses of compounds were measured by LRMS (ESI) via a Waters Acquity QDa Detector. HRMS data was collected via an Agilent QTOF 6540 MSMS with ESI ionization. Chemical formulas found from HRMS data were obtained via Agilent MassHunter software equipped with the HRMS QTOF.

Synthesis of 8. Compound **4** (500 mg, 1.25 mmol) was dissolved in acetone and *p*-azido-benzylbromide (265 mg, 1.25 mmol) and potassium carbonate (518 mg, 3.75 mmol) were added. The mixture was heated to 40 °C overnight and monitored via TLC until completion. The mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified via silica-gel chromatography (1:5 ethyl acetate:hexane) to yield the **8** as a yellow oil (398 mg, 60% yield). The ^1H NMR (400 MHz, Acetone- d_6) spectrum (Figure S1) was assigned where possible. Rotomeric isomerism about the thioimidate is signified by (*). ESI-HRMS [$\text{M}+\text{H}^+$] calc: 532.2013; Obs: 532.2010; Molecular Formula: $\text{C}_{28}\text{H}_{29}\text{N}_5\text{O}_4\text{S}$. A UPLC ESI LRMS indicates a single pure compound (Figure S3). ^1H NMR (400 MHz, Acetone- d_6) δ 1.05 (d, J = 6.6 Hz, 1.68H, H26), 1.21 (d, J = 6.8 Hz, 3H, H26), 3.02 (m, 2.27H, H14), 3.18 (dd, J = 5.9 Hz, 13.5 Hz, 0.99H), 3.61 (s, 2.5H, H30), 3.65 (s, 2.05H overlap, H30), 4.15 (d, J = 13.7 Hz, 1.56H), 4.36 (s, 1.58H, H23), 4.44 (q, J = 6.8 Hz, 0.94H, H25), 4.52 (q, J = 6.6 Hz, 0.57H, H25*), 4.94 (dd, J = 6.5 Hz, 14.88, 0.99H), 5.01 (H7, 2.1H, s), 5.09 (H7*, 1.8H, s), 6.52 (d, J = 8.5 Hz, NH, 0.89H), 6.89 (d, J = 7.1 Hz, NH*, 0.49H), 7.02 (m, 3H), 7.35 (m, 18H). ^{13}C NMR (100 MHz, acetone- d_6) δ 172.1, 172.0, 168.4, 165.2, 155.6, 155.2, 139.2, 138.3, 137.3, 137.1, 136.7, 136.3, 133.7, 130.9, 130.7, 129.9, 129.4, 128.5, 128.4,

128.3, 128.1, 127.8, 127.7, 127.7, 126.9, 126.5, 119.3, 118.7, 65.9, 59.6, 58.2, 55.8, 53.7, 51.4, 39.5, 38.7, 34.7, 32.0, 20.1, 18.9, 17.3, 13.8.

SPPS Procedures. Peptides were synthesized in the following manner: Fmoc-Gly-Chlorotrityl resin was swelled in DMF for 10 minutes in a ChemGlass Peptide Synthesis Vessel using nitrogen as an agitator. Solvents were removed from the vessel using vacuum.

Fmoc-deprotection steps were carried out using 20% piperidine in DMF for 1 minute followed by a treatment of fresh reagent solution for 2 minutes. The resin was then washed 5x for 30 seconds each with dry DMF.

Coupling steps were carried out by pre-activating 5.0 eq of Fmoc-Xaa-OH with 4.9 eq HATU and 10.0 eq of NMM (relative to moles of peptide on-resin) in DMF at an approximate concentration of 0.2 M concentration. Each coupling step was agitated with nitrogen gas for 5 min. Once complete, the resin was washed 3x for 30 seconds each with dry DMF.

Addition of the thioamide was achieved by dissolving 2 eq of a previously reported^{3,4} thioacylating agent (Fmoc-Xaa^(S)-Nbt) in dry DCM with DIEA (2 eq) at an approximate concentration of 0.1 M. This solution was agitated with the resin using nitrogen gas for 30 min. This procedure was repeated a second time. Following completion of the coupling steps, the resin was washed with dry DCM 3x for 1 min.

Thioamide protection was achieved with 0.4 M of **7** or 0.4 M Mel and 0.05 M DIEA in dry DMF. The resin was agitated for 6 h on rotary mixer and drained of solvent. Fresh reagent (0.4 M **7** or 0.4 M Mel and 0.05 M DIEA in dry DMF) was added and the resin was agitated for an additional 6 h on a rotary mixer. The reaction can be monitored by cleaving a small amount of resin with 20% HFIP/DCM and checking via UPLC.

Peptide cleavage. Upon completion of the sequence, 30:70 TFA/DCM is used to cleave the peptide for 15 minutes. Post TFA cleavage, peptide solution is transferred to a conical tube and TFA was removed by blowing dry N₂ over the solution. Cold diethyl ether is added, forming a white precipitate and the peptide was centrifuged at 10,000 rpm for 10 minutes. Waste diethyl ether is decanted and fresh cold diethyl ether is added and the mixture was centrifuged again. The white precipitate was analyzed by HPLC and MS.

Deprotection of 3 with NaSH. Thioimidate **3** was dissolved in degassed DMF (0.1 M) and was added to a solution of NaSH in degassed water such that the final solution was 20% water and 1 M NaSH. The reaction was monitored by UPLC (Figure S4) using 1,3,5-trimethoxybenzene as an internal standard.

Deprotection of 5 with NaSH. Peptide **5** on resin was agitated with 0.4 mL of 0.3 M NaSH hydrate in DMF for 1 h. The reaction was analyzed by HPLC-MS following cleavage from the resin as described above and compared to peptide that had been prepared with authentic D-phenylalanine (Figure S5).

Deprotection of 8 with PPh₃. Thioimidate **8** (5 mg, 0.01 mmol) and PPh₃ (2.5 mg, 0.01 mmol) were dissolved in 0.1 mL THF:H₂O (1:1) and phenanthrene was added as an internal standard at 0.004 M. After 30 min the reaction was analyzed by ESI-LRMS and no product (**4**) was detected. Instead, a prominent mass peak corresponding to the iminophosphorane reduction product (Figure S6) was detected as described. Hydrolysis of the iminophosphorane was achieved by adding 0.1 mL of 1 M AcOH in THF:H₂O (1:1) to the reaction. After 5 min, UPLC analysis of the reaction (Figure S7) revealed that **4** was produced in quantitative yield.

Deprotection of 8 with DTT. Thioimidate **8** (50 mg, 0.1 mmol) was dissolved in 1 mL DMF and phenanthrene was added as an internal standard at 0.004 M. Dithiothreitol (DTT) (72 mg, 0.45 mmol) was added and the reaction was monitored by TLC for 1 h until completion. After 1 h the reaction was analyzed by UPLC (Figure S8) to reveal that **4** was produced in 97% yield.

Thioamide protection (10→11) was achieved with 0.4 M of **7** or 0.4 M MeI and 0.05 M DIEA in dry DMF. The resin was agitated for 6 h on rotary mixer and drained of solvent. Fresh reagent (0.4 M **7** or 0.4 M MeI and 0.05 M DIEA in dry DMF) was added and the resin was agitated for an additional 6 h on a rotary mixer. The reaction can be monitored by cleaving a small amount of resin with 20% HFIP/DCM and checking via HPLC (Figure S10 and S11).

Azide reduction of 11 to regenerate the thioamide 10. was achieved by placing resin in a fritted funnel with 0.5 M DTT and 0.1 M DIEA in dry DMF for 2 h on a rotary mixer. The reaction progress was monitored by cleaving the peptide (with side chain protection still intact) using 20% HFIP/DCM (Figure S12).

Determination of epimerization (Figure 2). Each compound (**3**, **4**, or **8**) was dissolved in 0.1 mL of dry DMF at a concentration of 0.1 M along with an equimolar amount of 1,3,5-trimethoxybenzene as an internal standard. To each vial was then added 0.2 mL of a solution of 60% piperidine in dry DMF to a final volume of 0.3 mL, where the final concentration of compounds **3**, **4**, or **8** and 1,3,5-trimethoxybenzene was 0.03 M and the final concentration of piperidine was 20%. The reactions were left to stir and 10 μ L aliquots were taken at each timepoint and diluted to 1 mL with MeOH in a UPLC vial. Each diluted aliquot was then injected into the UPLC and peak areas were used to determine epimeric percentages in the reaction.

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Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Choudhary, A.; Raines, R. T., An Evaluation of Peptide-Bond Isosteres. *ChemBioChem* **2011**, *12*, 1801-7.
- (2) Mahanta, N.; Szantai-Kis, D. M.; Petersson, E. J.; Mitchell, D. A., Biosynthesis and Chemical Applications of Thioamides. *ACS Chem Biol* **2019**, *14*, 142-163.

- (3) Miwa, J. H.; Pallivathucal, L.; Gowda, S.; Lee, K. E., Conformational Stability of Helical Peptides Containing a Thioamide Linkage. *Org Lett* **2002**, *4*, 4655-7.
- (4) Newberry, R. W.; VanVeller, B.; Raines, R. T., Thioamides in the Collagen Triple Helix. *Chem. Commun.* **2015**, *51*, 9624-7.
- (5) Walters, C. R.; Szantai-Kis, D. M.; Zhang, Y.; Reinert, Z. E.; Horne, W. S.; Chenoweth, D. M.; Petersson, E. J., The Effects of Thioamide Backbone Substitution on Protein Stability: A Study in Alpha-Helical, Beta-Sheet, and Polyproline II Helical Contexts. *Chem Sci* **2017**, *8*, 2868-2877.
- (6) Newberry, R. W.; Raines, R. T., The N \rightarrow Pi* Interaction. *Accounts of chemical research* **2017**, *50*, 1838-1846.
- (7) Newberry, R. W.; VanVeller, B.; Guzei, I. A.; Raines, R. T., N \rightarrow Pi* Interactions of Amides and Thioamides: Implications for Protein Stability. *J. Am. Chem. Soc.* **2013**, *135*, 7843-6.
- (8) Choudhary, A.; Gandla, D.; Krow, G. R.; Raines, R. T., Nature of Amide Carbonyl--Carbonyl Interactions in Proteins. *J Am Chem Soc* **2009**, *131*, 7244-6.
- (9) Wildemann, D.; Schiene-Fischer, C.; Aumuller, T.; Bachmann, A.; Kiefhaber, T.; Lucke, C.; Fischer, G., A Nearly Isosteric Photosensitive Amide-Backbone Substitution Allows Enzyme Activity Switching in Ribonuclease S. *J Am Chem Soc* **2007**, *129*, 4910-8.
- (10) Huang, Y.; Cong, Z.; Yang, L.; Dong, S., A Photoswitchable Thiopeptide Bond Facilitates the Conformation-Activity Correlation Study of Insect Kinin. *J Pept Sci* **2008**, *14*, 1062-8.
- (11) Goldberg, J. M.; Batjargal, S.; Petersson, E. J., Thioamides as Fluorescence Quenching Probes: Minimalist Chromophores to Monitor Protein Dynamics. *J Am Chem Soc* **2010**, *132*, 14718-20.
- (12) Petersson, E. J.; Goldberg, J. M.; Wissner, R. F., On the Use of Thioamides as Fluorescence Quenching Probes for Tracking Protein Folding and Stability. *Phys Chem Chem Phys* **2014**, *16*, 6827-37.
- (13) Goldberg, J. M.; Wissner, R. F.; Klein, A. M.; Petersson, E. J., Thioamide Quenching of Intrinsic Protein Fluorescence. *Chem. Commun.* **2012**, *48*, 1550-2.
- (14) Goldberg, J. M.; Batjargal, S.; Chen, B. S.; Petersson, E. J., Thioamide Quenching of Fluorescent Probes through Photoinduced Electron Transfer: Mechanistic Studies and Applications. *J Am Chem Soc* **2013**, *135*, 18651-8.
- (15) Walters, C. R.; Ferrie, J. J.; Petersson, E. J., Dithioamide Substitutions in Proteins: Effects on Thermostability, Peptide Binding, and Fluorescence Quenching in Calmodulin. *Chem. Commun.* **2018**, *54*, 1766-1769.
- (16) Shalaby, M. A.; Grote, C. W.; Rapoport, H., Thiopeptide Synthesis. Alpha-Amino Thionoacid Derivatives of Nitrobenzotriazole as Thioacylating Agents. *J Org Chem* **1996**, *61*, 9045-9048.
- (17) Yang, J.; Wang, C.; Xu, S.; Zhao, J., Ynamide-Mediated Thiopeptide Synthesis. *Angew Chem Int Ed Engl* **2019**, *58*, 1382-1386.
- (18) Camacho III, L.; Lampkin, B. J.; VanVeller, B., A Bottom-up Approach to Preserve Thioamide Residue Stereochemistry During Fmoc Solid-Phase Peptide Synthesis. *Org. Lett.* **2019**, *21*, 10.1021/acs.orglett.9b02598.
- (19) Szantai-Kis, D. M.; Walters, C. R.; Barrett, T. M.; Hoang, E. M.; Petersson, E. J., Thieme Chemistry Journals Awardees - Where Are They Now? Improved Fmoc Deprotection Methods for the Synthesis of Thioamide-Containing Peptides and Proteins. *Synlett* **2017**, *28*, 1789-1794.
- (20) Mukherjee, S.; Chatterjee, J., Suppressing the Epimerization of Endothioamide Peptides During Fmoc/T-Bu-Based Solid Phase Peptide Synthesis. *J Pept Sci* **2016**, *22*, 664-672.
- (21) Reiner, A.; Wildemann, D.; Fischer, G.; Kiefhaber, T., Effect of Thiopeptide Bonds on Alpha-Helix Structure and Stability. *J Am Chem Soc* **2008**, *130*, 8079-84.
- (22) Hemminki, K.; Niemi, M. L., Community Study of Spontaneous Abortions: Relation to Occupation and Air Pollution by Sulfur Dioxide, Hydrogen Sulfide, and Carbon Disulfide. *International archives of occupational and environmental health* **1982**, *51*, 55-63.
- (23) Lindenmann, J.; Matzi, V.; Neuboeck, N.; Ratzenhofer-Komenda, B.; Maier, A.; Smolle-Juettner, F. M., Severe Hydrogen Sulphide Poisoning Treated with 4-Dimethylaminophenol and Hyperbaric Oxygen. *Diving and hyperbaric medicine* **2010**, *40*, 213-7.
- (24) Sodium Hydrogensulfide; MSDS [Online]; Tessengerlo Kerly: Phoenix, AZ, Aug 6, 2018. <https://www.tkinet.com/en/Documents/Sodium%20hydrosulfide%20solution%20SDS.pdf> (accessed Aug 15, 2019)

- (25) Gjonaj, L.; Roelfes, G., Selective Chemical Modification of DNA with Alkoxy- and Benzyloxyamines. *Org Biomol Chem* **2015**, *13*, 6059-65.
- (26) Griffin, R. J.; Evers, E.; Davison, R.; Gibson, A. E.; Layton, D.; Irwin, W. J., The 4-Azidobenzoyloxycarbonyl Function; Application as a Novel Protecting Group and Potential Prodrug Modification for Amines. *J Chem Soc Perk T 1* **1996**, 1205-1211.
- (27) Leffler, J. E.; Temple, R. D., Staudinger Reaction between Triarylphosphines and Azides . A Study of Mechanism. *Journal of the American Chemical Society* **1967**, *89*, 5235-&.
- (28) Meguro, T.; Terashima, N.; Ito, H.; Koike, Y.; Kii, I.; Yoshida, S.; Hosoya, T., Staudinger Reaction Using 2,6-Dichlorophenyl Azide Derivatives for Robust Aza-Ylide Formation Applicable to Bioconjugation in Living Cells. *Chemical communications (Cambridge, England)* **2018**, *54*, 7904-7907.
- (29) Staros, J. V.; Bayley, H.; Standring, D. N.; Knowles, J. R., Reduction of Aryl Azides by Thiols - Implications for Use of Photoaffinity Reagents. *Biochem Bioph Res Co* **1978**, *80*, 568-572.
- (30) Fahrenholz, F.; Toth, G.; Crause, P.; Eggena, P.; Schwartz, I. L., [1,6-Alpha-Aminosuberic Acid, 3-(P-Azidophenylalanine), 8-Arginine] Vasopressin: A New Photoaffinity Label for Hydroosmotic Hormone Receptors. Characterization of the Ligand and Irreversible Stimulation of Hydroosmotic Water Flow in Toad Bladder by Photoaffinity Labeling. *J Biol Chem* **1983**, *258*, 14861-7.
- (31) Crause, P.; Fahrenholz, F., Affinities of Reactive Vasopressin Analogues for Bovine Antidiuretic Receptor. *Molecular and cellular endocrinology* **1982**, *28*, 529-41.
- (32) Pothukanuri, S.; Winssinger, N., A Highly Efficient Azide-Based Protecting Group for Amines and Alcohols. *Org Lett* **2007**, *9*, 2223-5.
- (33) Brase, S.; Gil, C.; Knepper, K.; Zimmermann, V., Organic Azides: An Exploding Diversity of a Unique Class of Compounds. *Angew Chem Int Ed Engl* **2005**, *44*, 5188-240.
- (34) Erdmann, R. S.; Wennemers, H., Conformational Stability of Triazolyl Functionalized Collagen Triple Helices. *Bioorg Med Chem* **2013**, *21*, 3565-8.