JOC The Journal of Organic Chemistry
Article
Cite This: J. Org. Chem. 2019, 84, 15309−15314
pubs.acs.org/joc

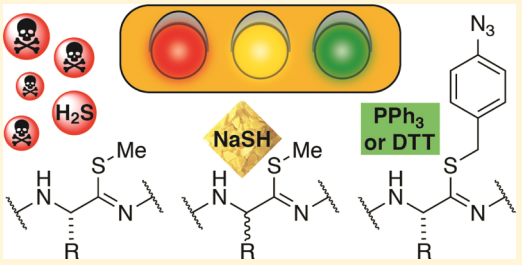
Deprotection Strategies for Thioimidates during Fmoc Solid-Phase Peptide Synthesis: A Safe Route to Thioamides

Luis A. Camacho, III, Yen H. Nguyen, John Turner, and Brett VanVeller*

Department of Chemistry, Iowa State University, Ames, Iowa 50011, United States

Supporting Information

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 thioimidate 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.



Downloaded via IOWA STATE UNIV on June 3, 2020 at 18:07:47 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

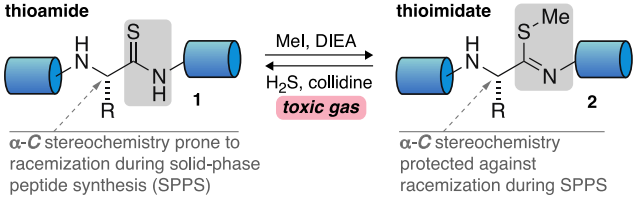
INTRODUCTION

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] Finally, 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 the 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.

To address this limitation, 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 H_2S, which can be easily weighed and transferred. We tested the potential for conversion of thioimidate back to the thioamide using a model dipeptide (**3** → **4**, Scheme 2a).

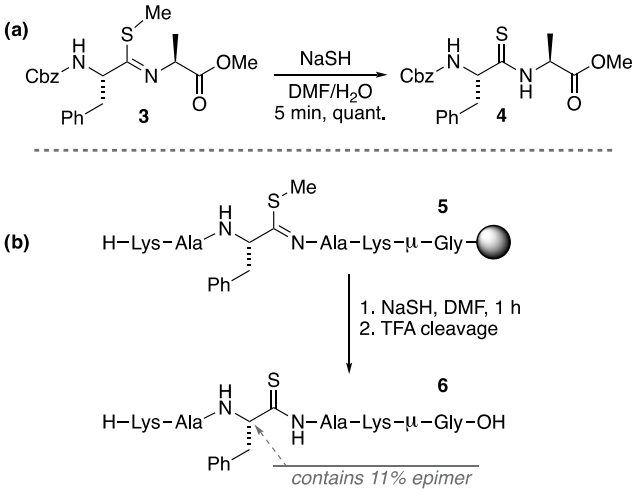
Our initial results were promising, as conversion to thioamide **4** was complete within minutes (Figure S4). Unfortunately, when we attempted the NaSH conditions with a thioimidate peptide (**5**) on solid phase, we observed small amounts of epimerization of the final peptide (**5** → **6**, Scheme 2b, Figure S5), which negated the efficacy of the thioimidate protection strategy. This result was likely due to the strongly alkaline nature of NaSH.[24] Additionally, because

Received: August 26, 2019
Published: November 14, 2019

© 2019 American Chemical Society

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314

Scheme 2. Deprotection with NaSH[a]



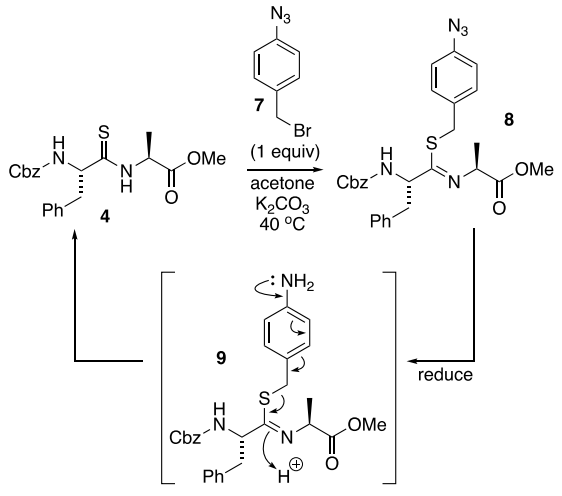
μ = N^{ε}-(7-methoxycoumarin-4-acetamide)-L-lysine

[a] $\mu = N^{\varepsilon}$-(7-methoxycoumarin-4-acetamide)-L-lysine.

NaSH (and Na_2S) 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 nonideal reagent for the purposes of thioimidate deprotection for the following reasons: (i) NaSH is still a corrosive chemical, (ii) while NaSH is a solid, it readily generates H_2S, (iii) solutions of NaSH in dimethylformamide (DMF) are turbid and can clog fritted SPPS reaction vessels, and (iv) finally, while it may be possible to identify 'on-resin' conditions that would allow for the rate of conversion of thioimidate 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.

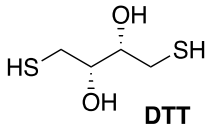
We next considered using a different alkylating agent (**7**)[25] of the thioamide, instead of MeI, which could be cleaved via alternative conditions (Scheme 3). We hypothesized that the 4-azidobenzyl group in **8** would allow for an alternative mechanism of deprotection through reduction of the azide 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 Strategy



We initially investigated the Staudinger reduction of the azide using PPh_3 and water as conditions for deprotection to give thioamide **4** (Table 1, entry 1).[27] While the reaction with PPh_3 consumed the starting material (**8**, Figure S6), subsequent hydrolysis of the aryl-iminophosphorane (Ar–N=PPh_3) was ineffective in neutral water, as reported in the literature.[28] Alternatively, weak-acid conditions at this stage successfully catalyzed the hydrolysis of iminophosphorane to amine (Ar–N=PPh_2 → Ar–NH_2 + O=PPh_3) and cleanly liberated the thioamide (Table 1, entry 2, Figure S7).[26]

Table 1. Conditions for Reductive Release of Thioamide (8 → 4)

entry	conditions	yield of **4** from **8** (%)
1	PPh_3, THF, H_2O (1:1)	0
2	PPh_3, THF, H_2O (1:1) then AcOH (0.5 M)	quant.
3	 DTT, DMF	97

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 the 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 and investigated neutral 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).

To determine if the azidobenzyl thioimidate 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 thioimidate **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 thioimidate (**12** ⇌ **13**). This kind of rotational isomerism is visible in the NMR spectrum of model dimer **3** in Scheme 2 above (Figure S1)). From **11**, SPPS could, in principle, proceed normally to elongate the peptide, while the thioimidate 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**).

The stability of azides during SPPS is well-documented.[30−34] However, we sought 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

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314

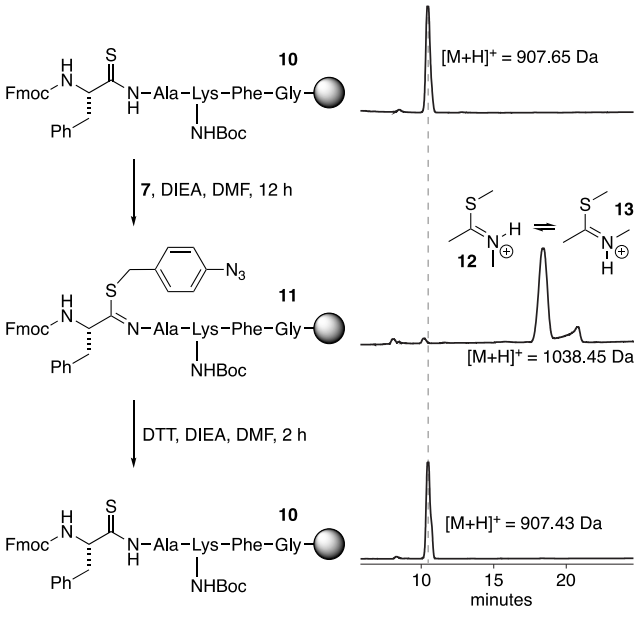


Figure 1. Demonstration of the interconversion between thioamide and thioimidate on-resin (Cl-Trt polystyrene). The reaction can be performed using standard peptide-synthesis reaction vessels and was tracked using high-performance liquid chromatography (HPLC) and electrospray ionization mass spectrometry (ESI-MS). As a precaution, *N*,*N*-diisopropylethylamine (DIEA) was added to the DTT step to ensure a basic environment and prevent cleavage from the so-called super-acid-sensitive chlorotrityl resin.

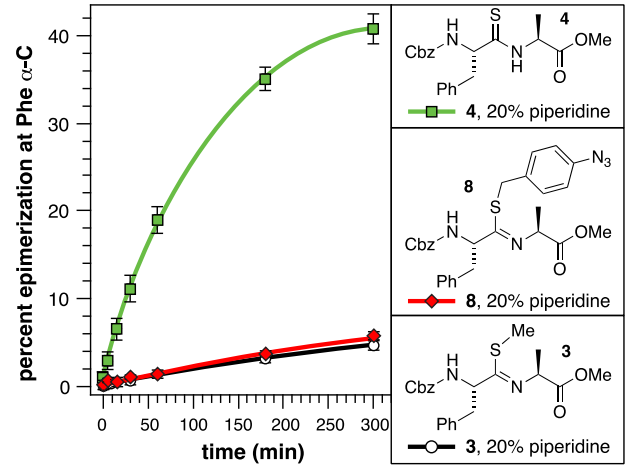


Figure 2. Epimeric stability of **8** and **3** with piperidine compared to **4**. Solutions of each compound (0.1 M) in DMF with 20% piperidine (v/v) were prepared and the total epimerization was tracked relative to 1,3,5-trimethoxybenzene as the internal standard.

thioimidate dipeptide and the thioimidate bearing the azidobenzyl group withstand epimerization to the same extent.

Finally, to confirm the utility of our approach, we synthesized peptides **16** and **17** through two different routes, with and without protection of the thioamide as a thioimidate, and compared the results (Figure 3). Without protection of the thioamide, the coupling of only two more residues leads to 13% epimerization of the phenylalanine-thioamide amino acid in **16**. Similarly, epimerization continues upon coupling two more residues (22% for **17**). In contrast, however, protection of the phenylalanine-thioamide amino acid as the thioimidate **11** followed by conversion back to the thioamide once couplings were complete lead to no detectable extent of epimerization (**11** → **16** and **11** → **17**). These results confirm that the 4-azidobenzyl protecting group for thioamides functions as anticipated to curtail epimerization of thioamide-containing peptides.

CONCLUSIONS

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 thioimidate 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. An initial version of this work was deposited in ChemRxiv on August 21, 2019.[35]

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, dichloromethane (DCM), and DMF were dried via Glass Contours solvent system.

NMR Spectroscopy. ^{1}H 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 ultra-performance liquid chromatography (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 the 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 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 ^{1}H NMR (400 MHz, Acetone-d_6) spectrum (Figure S1) was assigned where possible. Rotomeric isomerism about the thioimidate is signified by (*). HRMS (ESI-TOF) *m*/*z*: [M + H$^+$] calcd for $C_{28}H_{29}N_5O_4S$ 532.2013; found 532.2010. A UPLC-ESI LRMS indicates a single pure compound (Figure S3). ^{1}H 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,

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314

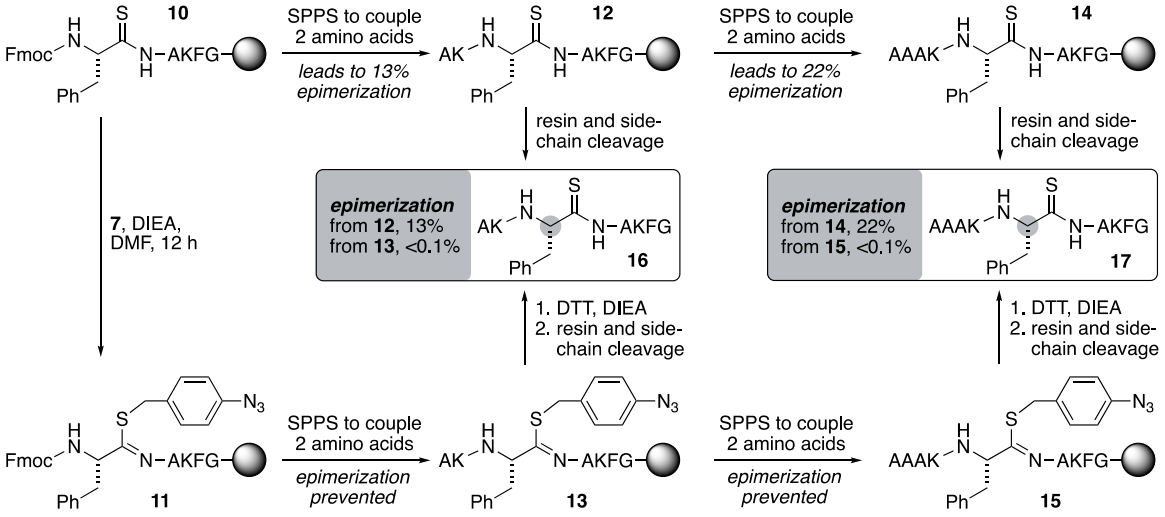


Figure 3. Epimerization of thioamide amino acid due to SPPS conditions. Resin and side-chain protecting group cleavage was achieved with trifluoroacetic acid (TFA). Percent epimerization was determined from peak areas of HPLC traces and compared to peptides containing authentic D-Phe.

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 = 7.1Hz, NH*, 0.49H), 7.02 (m, 3H), 7.35 (m, 18H). $^{13}C\{^{1}H\}$ NMR (100 MHz, acetone-d6) 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 min in a ChemGlass Peptide Synthesis Vessel using nitrogen as an agitator. Solvents were removed from the vessel using vacuum.

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

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

Addition of Thioamide. Addition of the thioamide was achieved by dissolving 2 equiv of a previously reported[3,4] thioacylating agent (Fmoc-Xaa$^{(S)}$-Nbt) in dry DCM with DIEA (2 equiv) 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 3× for 1 min.

Thioamide Protection. Thioamide protection 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 a 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 the 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 min. Post-TFA cleavage, the peptide solution is transferred to a conical tube and TFA was removed by blowing dry N_2 over the solution. Cold diethyl ether is added, forming a white precipitate, and the peptide was centrifuged at 10 000 rpm for 10 min. Waste diethyl ether is decanted, 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 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 the peptide that had been prepared with authentic D-phenylalanine (Figure S5).

Deprotection of 8 with PPh_3. Thioimidate **8** (5 mg, 0.01 mmol) and PPh_3 (2.5 mg, 0.01 mmol) were dissolved in 0.1 mL of THF/H_2O (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/H_2O (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 of 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 following the thioamide protection procedure from the SPPS procedures described above. The reaction can be monitored by cleaving a small amount of resin with 20% HFIP/DCM and analyzed using HPLC (Figures 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 μL 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.

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.9b02317.

Characterization of **8**, experimental details, NMR spectra, and LC traces (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: bvv@iastate.edu.

ORCID

Luis A. Camacho, III: 0000-0001-6329-1966

Brett VanVeller: 0000-0002-3792-0308

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the National Science Foundation (Grant no 1848261) for support of this research.

■ REFERENCES

(1) Choudhary, A.; Raines, R. T. An Evaluation of Peptide-Bond Isosteres. *ChemBioChem* **2011**, *12*, 1801−1807.

(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*, 4655−4657.

(4) Newberry, R. W.; VanVeller, B.; Raines, R. T. Thioamides in the Collagen Triple Helix. *Chem. Commun.* **2015**, *51*, 9624−9627.

(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−>Pi* Interaction. *Acc. Chem. Res.* **2017**, *50*, 1838−1846.

(7) Newberry, R. W.; VanVeller, B.; Guzei, I. A.; Raines, R. T. N−>Pi* Interactions of Amides and Thioamides: Implications for Protein Stability. *J. Am. Chem. Soc.* **2013**, *135*, 7843−7846.

(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*, 7244−7246.

(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*, 4910−4918.

(10) Huang, Y.; Cong, Z.; Yang, L.; Dong, S. A Photoswitchable Thioxopeptide Bond Facilitates the Conformation-Activity Correlation Study of Insect Kinin. *J. Pept. Sci.* **2008**, *14*, 1062−1068.

(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*, 14718−14720.

(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*, 6827−6837.

(13) Goldberg, J. M.; Wissner, R. F.; Klein, A. M.; Petersson, E. J. Thioamide Quenching of Intrinsic Protein Fluorescence. *Chem. Commun.* **2012**, *48*, 1550−1552.

(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*, 18651−18658.

(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.* **2019**, *58*, 1382−1386.

(18) Camacho, L. A., III; Lampkin, B. J.; VanVeller, B. A Bottom-up Approach to Preserve Thioamide Residue Stereochemistry During Fmoc Solid-Phase Peptide Synthesis. *Org. Lett.* **2019**, *21*, 7015−7018.

(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 Thioxopeptide Bonds on Alpha-Helix Structure and Stability. *J. Am. Chem. Soc.* **2008**, *130*, 8079−8084.

(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. *Int. Arch. Occup. Environ. 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 Hyperb. Med.* **2010**, *40*, 213−217.

(24) Sodium Hydrogensulfide; MSDS [Online]; Tessenderlo Kerly: Phoenix, AZ, 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*, 6059−6065.

(26) Griffin, R. J.; Evers, E.; Davison, R.; Gibson, A. E.; Layton, D.; Irwin, W. J. The 4-Azidoberazyloxycarbonyl Function; Application as a Novel Protecting Group and Potential Prodrug Modification for Amines. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1205−1211.

(27) Leffler, J. E.; Temple, R. D. Staudinger Reaction between Triarylphosphines and Azides. A Study of Mechanism. *J. Am. Chem. Soc.* **1967**, *89*, 5235−5246.

(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. *Chem. Commun.* **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. Biophys. Res. Commun.* **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*, 14861−14867.

(31) Crause, P.; Fahrenholz, F. Affinities of Reactive Vasopressin Analogues for Bovine Antidiuretic Receptor. *Mol. Cell. Endocrinol.* **1982**, *28*, 529−541.

(32) Pothukanuri, S.; Winssinger, N. A Highly Efficient Azide-Based Protecting Group for Amines and Alcohols. *Org. Lett.* **2007**, *9*, 2223−2225.

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314

(33) Brase, S.; Gil, C.; Knepper, K.; Zimmermann, V. Organic Azides: An Exploding Diversity of a Unique Class of Compounds. *Angew. Chem., Int. Ed.* **2005**, *44*, 5188−5240.
(34) Erdmann, R. S.; Wennemers, H. Conformational Stability of Triazolyl Functionalized Collagen Triple Helices. *Bioorg. Med. Chem.* **2013**, *21*, 3565−3568.
(35) Camacho, L. A., III; Nguyen, N. H.; Turner, J.; VanVeller, B. Deprotection Strategies for Thioimidates during Fmoc Solid-Phase Peptide Synthesis: A Safe Route to Thioamides. 2019, ChemRxiv Preprint. https://chemrxiv.org/.

DOI: 10.1021/acs.joc.9b02317
J. Org. Chem. 2019, 84, 15309−15314