

Stereoselective and Divergent Construction of β -Thiolated/
Selenolated Amino Acids via Photoredox-Catalyzed Asymmetric
Giese Reaction

Hongli Yin, Mengjie Zheng, Huan Chen, Siyao Wang, Qingqing Zhou, Qiang Zhang, and Ping Wang*

Cite This: *J. Am. Chem. Soc.* 2020, 142, 14201–14209

Read Online

ACCESS |



Metrics & More

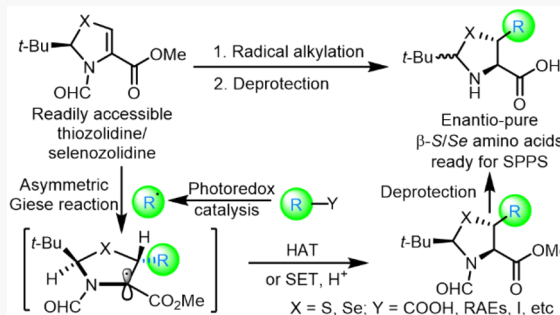


Article Recommendations



Supporting Information

ABSTRACT: Sulfur and selenium occupy a distinguished position in biology owing to their redox activities, high nucleophilicity, and acyl transfer capabilities. Thiolated/selenolated amino acids, including cysteine, selenocysteine, and their derivatives, play critical roles in regulating the conformation and function of proteins and serve as an important motif for peptide design and bioconjugation. Unfortunately, a general and concise method to attain enantiopure β -thiolated/selenolated amino acids remains an unsolved problem. Herein, we present a photoredox-catalyzed asymmetric method for the preparation of enantiopure β -thiolated/selenolated amino acids using a simple chiral auxiliary, which controls the diastereoselectivity of the key alkylation step and acts as an orthogonal protecting group in the subsequent peptide synthesis. Our protocol can be used to prepare a wide range of β -thiolated/selenolated amino acids on a gram scale, which would otherwise be difficult to obtain using conventional methods. The effect of our chemistry was further highlighted and validated through the preparation of a series of peptidyl thiol/selenol analogues, including cytochrome *c* oxidase subunit protein 7C and oxytocin.



INTRODUCTION

Cysteine (Cys) and its analogue selenocysteine (Sec) play irreplaceable roles in protein folding and stability,¹ enzymatic activity,² and redox regulation.³ The intricate design of free thiol and selenol is Nature's way of realizing these critical functions in biology. The significance of cysteine and selenocysteine is arguably more conspicuously presented in the domain of selective chemical protein modification,⁴ the construction of native peptide bonds (native chemical ligation, NCL),⁵ late-stage mutagenesis (to Ala and Ser),⁶ disulfide bond engineering,⁷ and the design of peptidyl ligands.⁸ As a case in point, the combination of the NCL–dechalcogenation strategy,⁹ one of the most efficient approaches used to tether two peptidyl segments, completely relies on the specific chemoselectivities exhibited by thiolated/selenolated proteinogenic amino acids (Figure 1A). After ligation and selective dechalcogenation, peptides bearing the corresponding native residues are produced. Moreover, disulfide bridges are crucial for the stability and activity of many important therapeutic peptides and proteins. Recent studies have suggested the use of thiolated amino acids as disulfide precursors can also enhance the stability and activity of peptides.¹⁰ In addition, Cys is a fundamental element for functional peptide design, thus fine-tuning the properties of synthetic peptide ligands could be achieved with thiolated amino acid preparation.¹¹ Nonetheless, the resultant β -thiolated/selenolated amino acids, in which the

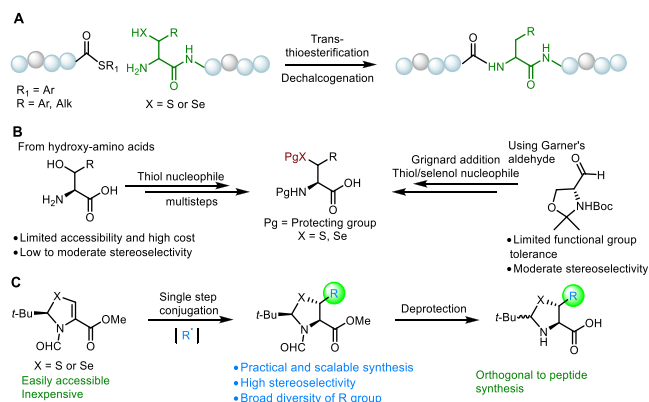


Figure 1. Development of a general method used for the preparation of enantiopure β -thio/selenolated amino acids. (A) NCL–dechalcogenation. (B) Representative methods for constructing thiolated/selenolated amino acids. (C) The photoredox-catalyzed asymmetric Giese reaction as a general solution to this synthetic problem.

Received: May 11, 2020

Published: July 27, 2020



ACS Publications

© 2020 American Chemical Society

14201

<https://dx.doi.org/10.1021/jacs.0c04994>
J. Am. Chem. Soc. 2020, 142, 14201–14209

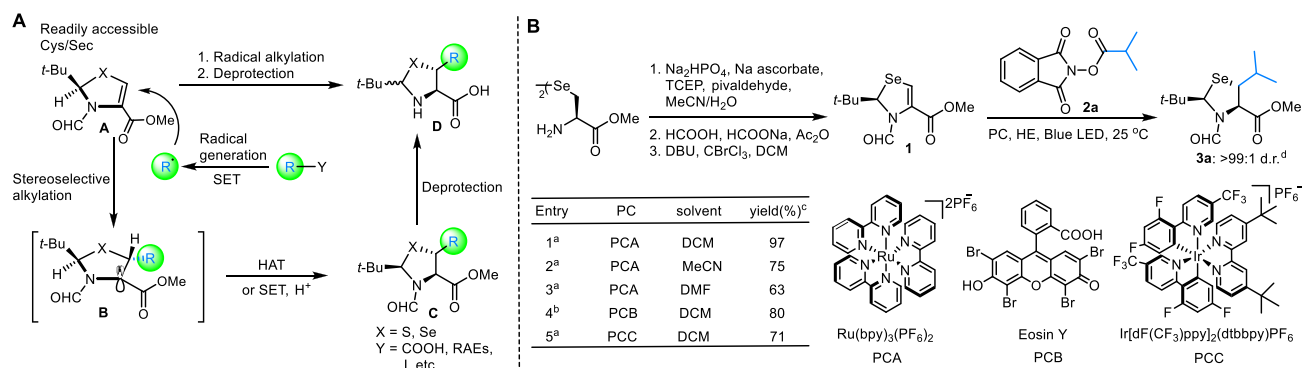


Figure 2. Design plan and optimization of the reaction conditions used in the preparation of β -selenolated Leu. (A) Symmetric Giese reaction via photoredox catalysis. (B) Standard conditions: PC (1 mol %), **2a** (1.2 equiv), HE (1.5 equiv), DIPEA (2.0 equiv), DCM, blue LED (10 W), 25 °C. Legend: (a) 18 h; (b) 48 h; (c) isolated yield; (d) diastereoselectivity determined by ¹H NMR and GC analysis of the crude reaction mixture. Abbreviations: PC, photoredox catalyst; HE, Hantzsch ester; DIPEA, *N,N*-diisopropylethylamine; DCM, dichloromethane; DMF, *N,N*-dimethylformamide.

β -carbon carries the thiol/selenol, are valuable precursors to bioactive peptides and proteins.

In contrast to these powerful applications derived from thiolated/selenolated amino acids, a major challenge to their widespread utility is the limited accessibility of these prerequisite enantiopure thiolated/selenolated amino acids. Currently, the majority of methods are indirect and are confined to certain amino acid scaffolds despite significant research efforts toward the preparation of β -thiolated/selenolated amino acids reported by multiple research groups.¹² Among the methods toward the synthesis of β -thiolated/selenolated amino acids, using thiol/selenol reagents and an electrophilic carbon to introduce S/Se is the most representative approach with enantiopure β -hydroxyl amino acids or Garner's aldehyde utilized to stereoselectively construct the C–S/Se bond (Figure 1B). Unfortunately, the modification of β -hydroxyl amino acids approach is extremely impractical, as it requires expensive and rare commercially available β -hydroxyl amino acids as the precursors (e.g., \$200/gram for β -hydroxy leucine) and it often leads to a diastereomeric mixture of β -thiol products. The latter tactic could achieve moderate diastereoselectivity, yet multistep manipulations are often required before incorporating β -thiol/selenol residues into solid-phase peptide synthesis.¹³ Therefore, a more general and concise protocol to high-value enantioselective β -thiolated/selenolated amino acids from readily accessible building blocks remains elusive.

Herein, we present a general and practical method to access enantiopure β -thiolated/selenolated amino acids via an asymmetric Giese reaction¹⁴ (Figure 1C). Our strategy starts from enantiopure selenazoline/thiazoline bearing a chiral pivalaldehyde acetal, which is easily prepared from L-Cys/Sec on a large scale. Visible-light photocatalysis generated alkyl radicals (including primary, secondary, and tertiary radicals) furnish thiazoline/selenazoline to produce the β -substituted thiolated/selenolated amino acid framework. The chiral pivalaldehyde acetal of thiazoline/selenazoline controls the diastereoselectivity of the radical addition reaction and acts as a “smart” protecting group, which is orthogonal to solid-phase peptide synthesis conditions and eliminates the need for protecting group manipulations. This strategy allows the concise stereoselective synthesis of a diverse range of β -selenolated/-thiolated amino acids from a common precursor at will with high stereopurity and high yield. Products can be

rapidly acquired on a multigram scale that are not easily accessible using conventional methods. Furthermore, the resulting selenazolidine/thiazolidine amino acids can be directly used in peptide synthesis after a simple operation without complex protecting-group manipulation.

RESULTS AND DISCUSSION

In contrast to the thiol/selenol nucleophiles used to generate the C–S/Se bond described in the existing methods, the stereoselective insertion of an alkylated group onto the β -carbon of Cys/Sec is superior. It has been reported that, using the concept of “self-regeneration of chirality centers”,¹⁵ chiral *S,N*-acetyl thiazoline served as a good starting point for the preparation of β -thiolated amino acids via a Michael-type alkylation.¹⁶ However, this transformation remains limited in synthesis due to its poor yield and low functional group tolerance and diversity, attributed to the severe β -elimination of the thiol group when alkyl metal reagents are employed in the reaction. The challenges notwithstanding, we envisioned that a radical 1,4-conjugate addition under mild photoredox conditions might lead to superior results. It is well-established that convenient radical generation occurs from carboxylic acids,¹⁷ redox-active esters (RAEs),¹⁸ and alkyl halides¹⁹ in the presence of a photoredox catalyst via single-electron transfer (SET). Mechanistically, production of alkyl radical via SET oxidation (carboxylic acids) or reduction (RAEs and alkyl halides) can lead to stereoselective radical addition from the reverse face of the bulky *tert*-butyl group in **A**, which generates radical intermediate **B**. **B** is reduced via H atom abstraction (HAT) or SET followed by a diastereoselective enolate protonation step to produce the *trans* product **C**, providing two new stereocenters in one step (Figure 2A). Subsequent deprotection may furnish a broad family of β -thiolated/selenolated amino acids (**D**), which are suitable for direct peptide coupling.

The importance of Se in biology causes selenolated amino acids to be in high demand; conversely, the sheer difficulty of handling labile Se during reactions prevents securing Se-amino acids from a large quantity. We began our exploration with the preparation of β -selenolated amino acids as more practical synthetic targets. The starting material, pivalaldehyde *N*,Se-acetal selenazoline **1**, was quickly prepared over three steps from the diselenide derivative of L-Sec methyl ester on a 20 g scale. An *N*-formyl group was used to protect the nitrogen

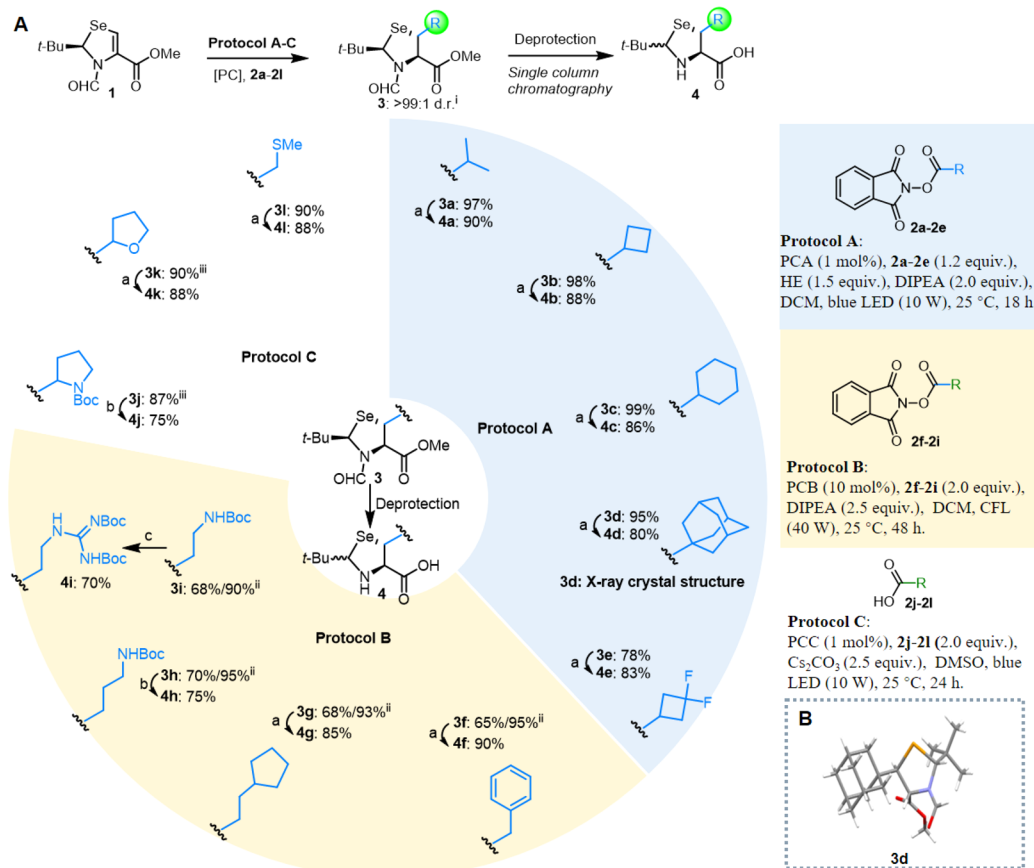


Figure 3. Asymmetric alkylation of selenazoline under photoredox conditions and their derivatization. (A) Scope of the β -selenolated amino acids. Deprotection conditions: (a) (1) LiOH (10.0 equiv), $\text{H}_2\text{O}/\text{MeOH}$ (1/3 v/v), 25 °C, 6 h, (2) 6 N HCl in dioxane, 50 °C, 8 h; (b) (1) LiOH (10.0 equiv), $\text{H}_2\text{O}/\text{MeOH}$ (1/3 v/v), 25 °C, 6 h, (2) 6 N HCl in dioxane, 50 °C, 8 h, (3) $(\text{Boc})_2\text{O}$ (1.0 equiv), THF/ H_2O (10/1 v/v), DIPEA (2.0 equiv), 25 °C, 1 h; (c) (1) LiOH (10.0 equiv), $\text{H}_2\text{O}/\text{MeOH}$ (1/3 v/v), 25 °C, 6 h, (2) 6 N HCl in dioxane, 50 °C, 8 h, (3) *N,N'*-bis-Boc-1-guanylpiprazole (1.0 equiv), DIPEA (2.0 equiv), MeOH, 25 °C, 3 h. (B) Absolute configuration of 3d as confirmed by X-ray crystallography. Legend: (i) diastereoselectivity determined by GC analysis of the crude reaction mixture; (ii) brsm, based on recovered starting material; (iii) mixture of diastereomers found.

atom as well as to improve the electrophilicity of the double bond.²⁰ Under irradiation of blue LED light (10 W), $\text{Ru}(\text{bpy})_3(\text{PF}_6)_2$ (PCA, 1 mol %) catalyzed the coupling of **1** with isopropyl *N*-hydroxyphthalimide ester **2a** (1.2 equiv) to provide the desired product, *trans*-**3a** (β -selenolated Leu), as a single diastereoisomer in the presence of Hantzsch ester (HE, 1.5 equiv) and DIPEA (2.0 equiv). It is worth noting that **3a** was obtained on a gram scale as a mixture of rotamers in near-quantitative yield (97%), as confirmed by variable-temperature ^1H NMR and solvent switching (see page 10 in the Supporting Information for details) (Figure 2B). Screening of the solvent system suggested that dichloromethane (DCM) afforded the highest yield in comparison to MeCN and DMF; other photoredox catalysts such as Eosin Y (PCB) and $\text{Ir}[\text{dF}(\text{CF}_3)\text{-ppy}]_2(\text{dtbbpy})\text{PF}_6$ (PCC) could also mediate the same reaction with eroded yields. In all cases, only the single diastereoisomer *trans*-**3a** was obtained (>99:1 dr).

An ideal method is one where an inexpensive or readily accessible starting material can be converted into a diverse family of members with higher values via a single-step reaction. Subsequently, a broad scope of carboxylic acids was evaluated, including primary, secondary, and tertiary carboxylic acids with various functionalities. RAEs derived from secondary and tertiary carboxylates were initially examined (Figure 3A, Protocol A). Reaction of selenazoline **1** with a series of cyclic

carboxylate *N*-hydroxyphthalimide esters (cyclobutyl, **2b**; cyclohexyl, **2c**; adamantyl, **2d**) and fluorinated cyclobutyl carboxylate ester **2e** yielded the desired products **3b–e** in excellent yields and high diastereoselectivity on a gram scale. Notably, product **3d** bearing an adamantyl substituent was obtained in >90% yield, which suggested that generating sterically bulky quaternary centers could be accomplished. The absolute configuration of **3d** was confirmed via X-ray crystallography (Figure 3B).

Coupling with selenazoline **1** and primary alkyl carboxylic acids could not be accessed effectively by PCA and PCC catalysis. After extensive investigations, the optimized conditions were obtained using a 40 W household compact fluorescent light (CFL) bulb to irradiate **1** in the presence of 2.0 equiv of RAEs (**2f–i**) and DIPEA (2.5 equiv) in DCM using PCB (Eosin Y), which gave the desired coupling products **3f–i** in good yield at room temperature (Figure 3A, Protocol B).²¹

Moreover, carboxylic acids bearing a stabilizing α -heteroatom (O, N, and S) were evaluated. Compounds **2j–l** were added to **1** in the presence of PCC directly, giving products **3j–l** in excellent yields (Figure 3A, Protocol C). In all cases, we were pleased to find that excellent diastereoselectivity was obtained under our reaction conditions.

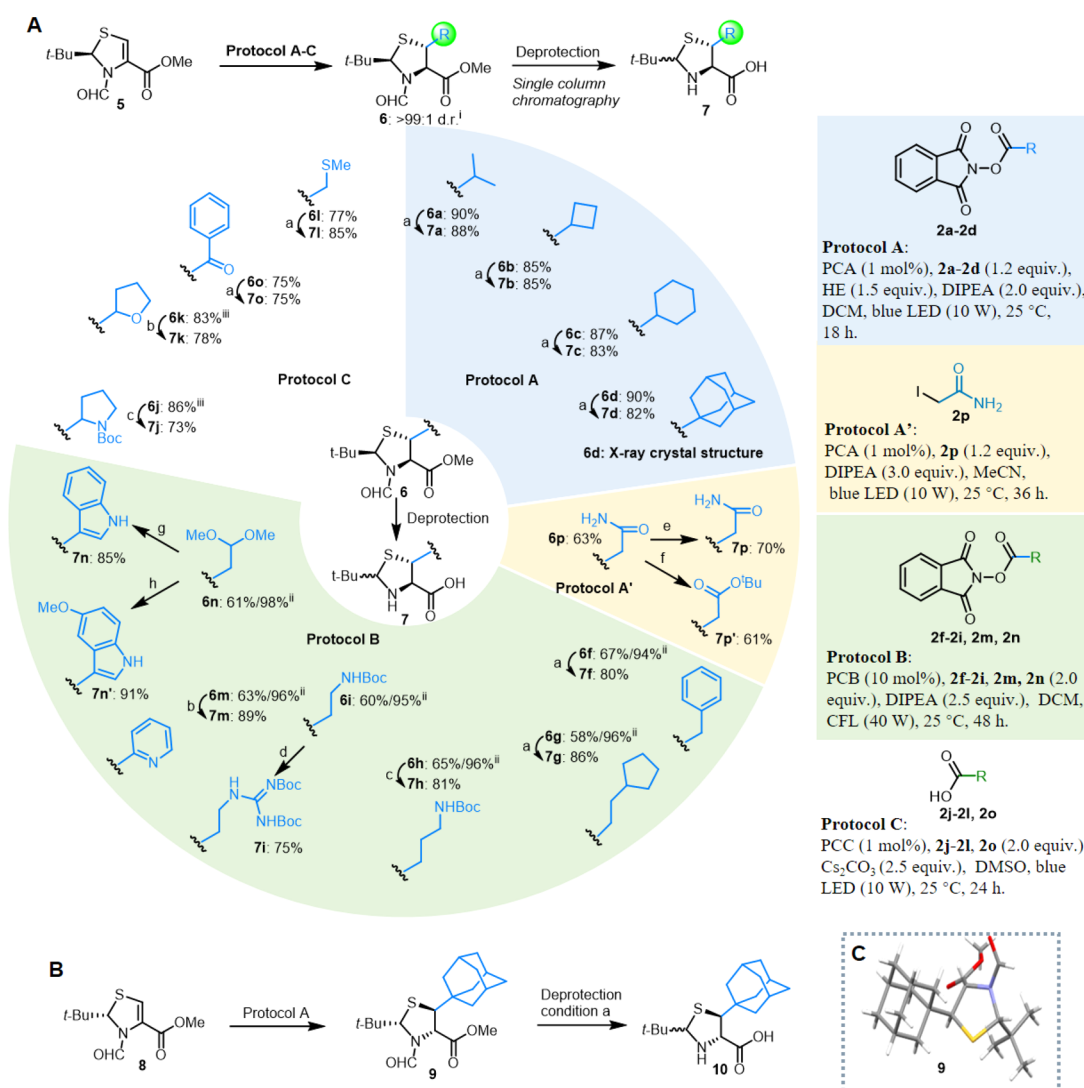


Figure 4. Asymmetric alkylation of thiazoline under photoredox conditions and derivatization. (A) Scope of β -thiolated amino acids. Deprotection conditions: (a) 6 N HCl, 90 °C, 8 h; (b) (1) 0.5 N HCl in MeOH, 25 °C, 12 h, (2) LiOH (10 equiv.), H₂O/MeOH (1/3 v/v), 25 °C, 6 h; (c) (1) 6 N HCl, 90 °C, 8 h, (2) (Boc)₂O (1.0 equiv.), DIPEA (2.0 equiv.), THF/H₂O (10/1 v/v), 25 °C, 1 h; (d) (1) 6 N HCl, 90 °C, 8 h, (2) *N,N'*-bis-Boc-guanylpiprazole (1.0 equiv.), DIPEA (2.0 equiv.), MeOH, 25 °C, 3 h; (e) (1) 33% HBr in AcOH, 80 °C, 1 h, (2) LiOH (10.0 equiv.), H₂O/MeOH (1/3 v/v), 25 °C, 6 h; (f) (1) 6 N HCl, 90 °C, 30 min, (2) *p*-TsOH (1.0 equiv.), isobutylene in DCM (50.0 equiv.), 40 °C, 12 h, (3) LiOH (10.0 equiv.), H₂O/MeOH (1/3 v/v), 25 °C, 6 h; (g) (1) PhNHNH₂ (2.0 equiv.), TFA/DCM (1/3 v/v), 25 °C, 30 min, 97%, (2) 0.5 N HCl in MeOH, rt, 12 h, (3) LiOH (10.0 equiv.), H₂O/MeOH (1/3 v/v), 25 °C, 6 h; (h) (1) *p*-MeO-PhNHNH₂ (2.0 equiv.), TFA/DCM (1/3 v/v), 97%, (2) 0.5 N HCl in MeOH, 25 °C, 12 h, (3) LiOH (10.0 equiv.), H₂O/MeOH (1/3 v/v), 25 °C, 6 h. (B) Synthesis of D- β -thiolated amino acids from D-cysteine. (C) Absolute configuration of **9** as confirmed by X-ray crystallography. Legend: (i) diastereoselectivity determined by GC analysis of the crude reaction mixture; (ii) brsm, based on recovered starting material; (iii) mixture of diastereomers found.

We next sought to convert products **3** into the target amino acids **4**, which might be used directly as SPPS-compatible amino acids. The desired products **4a–g,k,l** can be generated from their parent compounds after saponification of methyl ester and acidic removal of the *N*-formyl group over two steps (Figure 3A, conditions a). Substrates **4a,l** represent β -seleno Leu and Met, respectively. After acidic treatment, epimerization of the stereogenic center in the auxiliary was observed and the α,β -chiral center of the amino acids remained untouched. In the case of **3h,j**, a further Boc protection step was required to form β -selenolated Lys **4h,j** (Figure 3A, conditions b). Guanidinylation was performed to yield β -selenolated Arg **4i** from ornithine **3i** (Figure 3A, conditions c). For most cases, only a single purification was needed after these deprotection conditions, minimizing a time-consuming isolation process.

We further expanded the reaction scope to a number of β -thiolated amino acids, which are given in Figure 4. In line with the β -selenolated amino acid preparation, all of the RAEs (**2a–d,f–2i,m,n**) and carboxylic acids (**2j–l,o**) smoothly conjugated with thiazoline **5**, which was readily obtained from L-cysteine methyl ester hydrochloride (\$0.20/g) and pivalaldehyde (\$0.25/mL) over three steps on a 100 g scale (see page 4 in the Supporting Information for details). Products **6a–d,f–o** were acquired in high yields using Protocols A–C (Figure 4A). Notably, reaction of **5** with 2-iodoacetamide **2p** under photoredox dehalogenation conditions (Figure 4A, Protocol A') gave **6p** in 63% yield. Synthetically valuable handles such as the sulfide of methionine (**6l**), pyridine (**6m**), acetal (**6n**), ketone (**6o**), and amide (**6p**) were well tolerated, and the incorporation of an adamantyl nucleus (**6d**) offered a ready

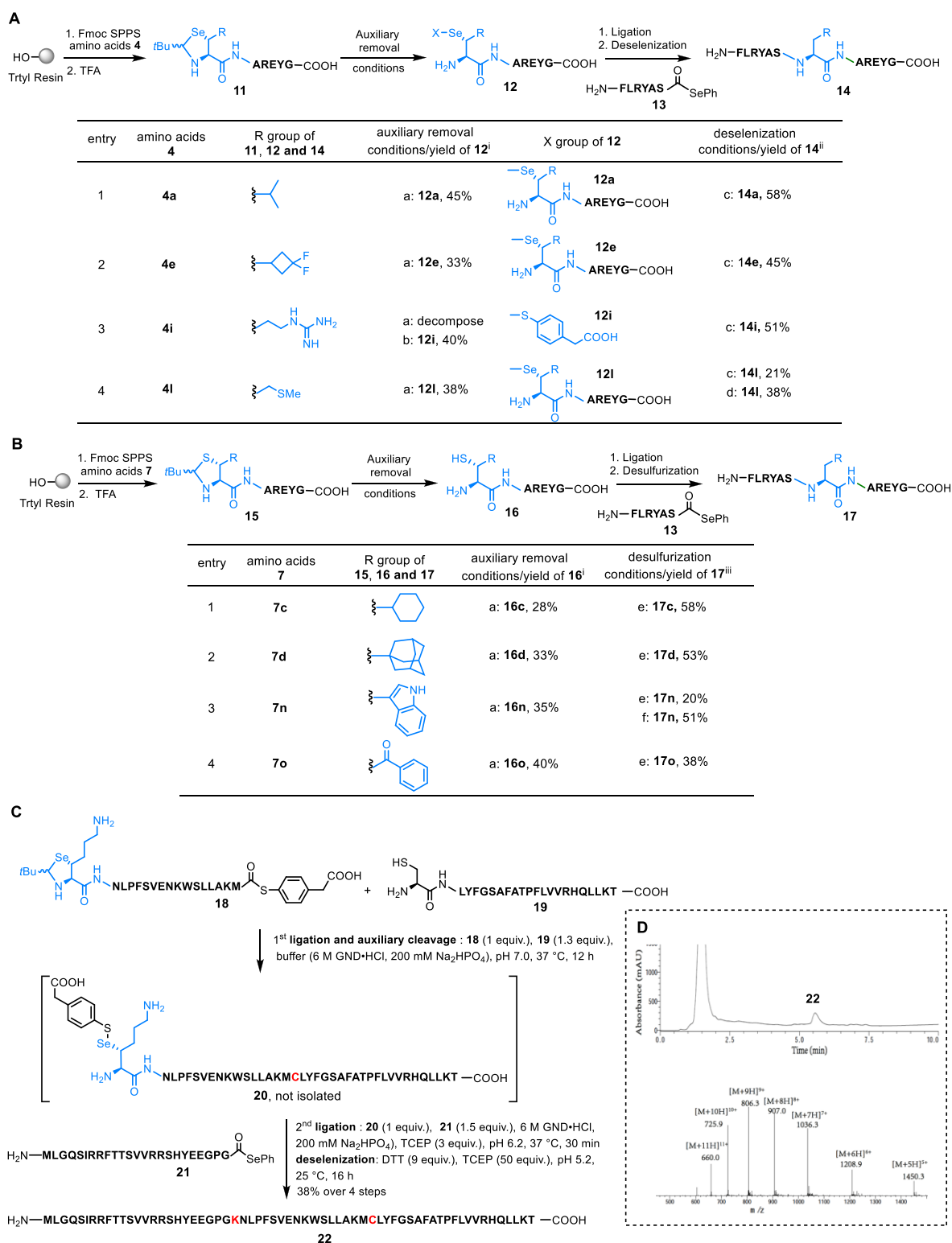


Figure 5. Exploiting the utility of β -thio/selenolated amino acids. (A) DSL-deselenization using β -selenolated amino acids. (B) NCL-desulfurization using β -thiolated amino acids. (C) One-pot synthesis of cytochrome *c* oxidase subunit 7C (**22**). (D) Crude HPLC trace of the final deselenization to give **22** and ESI mass spectrum of **22**. Conditions for auxiliary removal: (a) 6 M GND·HCl, 0.2 M Na₂HPO₄, 0.2 M MeONH₂, pH 4.0; (b) 6 M GND·HCl, 0.2 M Na₂HPO₄, MPAA (1.5 equiv), pH 6.8. Conditions for ligation, **13** (1.2 equiv), 6 M GND·HCl, 0.2 M Na₂HPO₄, TCEP (2.0 equiv), pH 6.2, 25 °C, 16 h. Conditions for deselenization: (c) 6 M GND·HCl, 0.2 M Na₂HPO₄, DTT (9.0 equiv), TCEP (50 equiv), pH 5.2, 25 °C, 16 h; (d) 6 M GND·HCl, 0.2 M Na₂HPO₄, DTT (250 equiv), TCEP (50 equiv), pH 5.2, 25 °C, 16 h. Conditions for desulfurization: (e) 6 M GND·HCl, 0.2 M Na₂HPO₄, 200 mM TCEP, 10 mM VA-044, 50 mM GSH, 37 °C, 16 h; (f) 6 M GND·HCl, 0.2 M Na₂HPO₄, 500 mM TCEP, 30 mM VA-044, 150 mM GSH, 37 °C, 16 h. Legend: (i) yield according to the original loading of the resin; (ii) yield of ligation and deselenization; (iii) yield of ligation and desulfurization. Abbreviations: GND, guanidine; MPAA, 4-mercaptophenylacetic acid; TCEP, tris(carboxyethyl)phosphine; VA-044, 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride; DTT, dithiothreitol; GSH, glutathione; Fmoc SPPS, Fmoc solid-phase peptide synthesis.

protocol to modify peptide distribution and lipophilicity/hydrophobicity. The structure and stereochemistry of **6d** were confirmed by X-ray crystallography. Pyridine-containing amino acids have significant effects on the biological properties of peptides and are often found in therapeutic peptides; therefore, substrate **6m** was used to demonstrate that the pyridine motif can be smoothly introduced. Compound **6a** exists as a mixture of rotamers, as confirmed by variable-temperature ^1H NMR and solvent switching.

In comparison with the delicate selenolated analogues, β -thiolated amino acids are more robust in nature. Thus, methods used for the deprotection of these compounds are more straightforward. The deprotection conditions used to yield β -thiolated amino acids are summarized in Figure 4A. Hydrolysis using 6 N HCl led to substrates **6a–d,f,g,o,l** directly in a single step (Figure 4A, conditions a), including β -thiolated Leu (**6a**) and Met (**6l**). Deformylation of **6k,m** was carried out using 0.5 N HCl followed by saponification to give **7k,m** in high yields (Figure 4A, conditions b). Similarly, acidic removal of the *N*-formyl group, followed by hydrolysis of the methyl ester and Boc group, gave the free amines from **6h,j**, which were reprotected with Boc to give β -thiol Lys **7h,j** in high yields, respectively (Figure 4A, conditions c). After acidolysis and guanidinylation, β -thiol Arg **7i** was produced in three steps starting from β -thiolated Orn **6i** (Figure 4A, conditions d). β -Thiol Trp analogues **7n,n'** were constructed via Fischer indole synthesis from γ -aldehyde dimethyl acetal **6n** (Figure 4A, conditions g and h). β -Thiol Gln **7p** and Glu **7p'** were obtained in good yields starting from **6p**, respectively. Selective deformylation in the presence of the side-chain amide moiety in **6p** was achieved using 33% HBr in acetic acid, and a subsequent saponification step provided β -thiol Gln **7p** in 70% yield (Figure 4A, conditions e). Maintenance of the methyl ester during acidic removal of the *N*-formyl group together with hydrolysis of the side-chain amide moiety of **6p** was possible using 6 N HCl at 90 °C for 30 min, leaving the methyl ester untouched. Functional group manipulations eventually produced β -thiol Glu **7p'** in good yield (Figure 4A, conditions f). Gram quantities of the β -thiolated amino acids could be easily prepared in the majority of these cases.

Mirror-image proteins have important therapeutic potential.²² Thus, we further explored the preparation of D- β -thiolated amino acids. With **8** (the enantiomer of **5**) as the starting material, an adamantyl radical was introduced in the same manner. Compound **9** (the enantiomer of **6d**) was obtained and subjected to acidolysis to produce **10** in high yield (Figure 4B). The absolute configuration of **9** was confirmed using X-ray crystallography (Figure 4C).

With these β -thiolated/selenolated amino acids in hand, we next explored their utility in peptide synthesis. Selenolated amino acids **4a,e,i,l** were introduced onto a solid-supported polypeptide N-terminus. After subsequent acidic cleavage from the resin and removal of the side-chain protecting groups using TFA, peptides **11a,e,i,l** were prepared, respectively (Figure 5A). In all cases, no double-coupling products were observed due to the steric bulkiness of the neighboring *t*-Bu group. Next, after deprotection of the auxiliary in the presence of excess MeONH_2 , diselenide dimer peptides **12a,e,l** were obtained in good yield after HPLC purification (Figure 5A, conditions a, entries 1, 2, and 4). Unfortunately, the deprotection of the pivalaldehyde acetal of β -selenolated Arg peptide (**11i**) failed in the presence of MeONH_2 due to the decomposition of peptide. As such, we opted for the employment of milder

conditions. Interestingly, we observed that this auxiliary was stable under NCL conditions in the presence of MPAA and TCEP, yet complete acetal removal of N-terminal selenolated amino acids was achieved in the presence of 1.5 equiv of MPAA in 6 M GND·HCl/0.2 M Na_2HPO_4 at pH 7.0, generating the MPAA-adduct peptide **12i** in good yield (Figure 5A, conditions b, entry 3). Mechanistic studies suggested that the selenazolidine ring was opened via the *in situ* produced disulfide of MPAA (see page 84 in the Supporting Information for details). To the best of our knowledge, the deprotection of selenazolidine described here has some of the mildest conditions reported to date. Meanwhile, one-pot diselenide-selenoester ligation (DSL)–deselenizations²³ between peptide selenoester **13** and selenolated peptides **12a,e,i,l** were conducted. Peptides **14a,e,i** were obtained according to a ligation and deselenization protocol in good yields (Figure 5A, conditions c). A low yield of **14l** was observed during deselenization. The deselenization proceeded well when excess amounts of DTT and TCEP were used (Figure 5A, conditions d). We further examined NCL–desulfurization using β -thiolated amino acids. Peptides **15c,d,n,o** were prepared via Fmoc-SPPS and TFA deprotection. Next, auxiliary removal was achieved successfully using an excess amount of MeONH_2 , providing thiolated peptides **16c,d,n,o** in good yields (Figure 5B, conditions a, entries 1–4). Ligation of peptide **16** with peptide **13** under NCL conditions, followed by one-pot desulfurization, yielded peptides **17c,d,o** smoothly (Figure 5B, conditions e). Though peptide **17n** was obtained in 20% yield after desulfurization, the reaction could be improved upon treatment of a large excess of TCEP and VA-044 (Figure 5B, conditions f). Therefore, this method not only significantly expanded the utility of NCL–dehalcogenation to generate native peptides but also provided a simple strategy to access peptides containing unnatural amino acids.^{6a,9f,24} For example, the synthesis of fluorinated peptide **14e** represents a general strategy to incorporate fluorinated amino acids into peptides, which opens up a new avenue to access fluorinated peptides.²⁵

To further exhibit the practicality of this method, cytochrome *c* oxidase subunit protein 7C²⁶ (**22**) was prepared by a one-pot synthesis using the selenolated Lys building block **4h** as an example. As shown in Figure 5C, MPAA-thioester **18** and peptide **19** were combined under NCL conditions to yield peptide **20** via *in situ* production of MPAA disulfide from **18**. Without separation, the crude reaction mixture was directly combined with selenoester **21**, followed by an *in situ* chemoselective deselenization reaction using DTT and TCEP to give protein **22** in 38% isolated yield over four steps. Overall, we have successfully synthesized cytochrome *c* oxidase subunit 7C (**22**) via sequential one-pot NCL activation of the auxiliary and DSL–deselenization chemistry without any purification or solvent removal. The facile synthesis of **22** demonstrated the application of construction of β -selenolated amino acids, and the MPAA-mediated deprotection of selenazolidine could expand and enhance selenolated amino acid mediated ligations.

Disulfides have been extensively used to stabilize peptide conformations and confine structure rigidity. Current methods for disulfide bond engineering are limited to the replacement of the disulfide bond with thioether, selenoether, diselenide, and hydrocarbon bridges.²⁷ In order to further understand the effect of introducing cysteine analogues on protein stability, a series of oxytocin (a hormone stimulating parturition and lactation drug) analogues (**23a,d,h**) were prepared via SPPS

and subsequent oxidative folding, which contain various N-terminal β -thiolated amino acid residues (Figure 6A). The

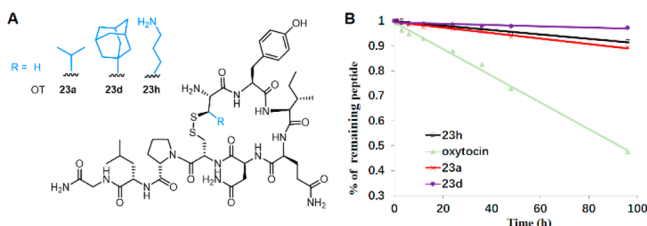


Figure 6. Thermal stability of synthetic oxytocin analogues. (A) Synthetic oxytocin analogues. (B) Thermal stability of oxytocin (OT) and 23a,d,h in water at 50 °C.

thermal stability of native oxytocin and its analogues were evaluated at 50 °C by LCMS analysis.²⁸ Native oxytocin degrades with a half-life of 3.9 days, whereas the synthetic analogues demonstrate significant stability (23a, 18.8 days; 23d, 68.6 days; 23h, 23.1 days) (Figure 6B). The introduction of β -thiolated amino acid residues is simple and straightforward; more importantly, our approach enables the construction of peptides bearing novel disulfide bridges with high stability and structural diversity.

This method is not without limitations. For example, the reaction is performed under photoredox conditions, and thus it will be difficult to introduce radical-sensitive groups (cyclopropyl methyl, disulfide). Furthermore, only the *trans* diastereomer is obtained, which is another limitation of this strategy. Considering the elegant work reported by Knowles and Miller,^{11b} a kinetic- and catalyst-controlled H atom transfer from B to C might serve as an alternative way to the other diastereomer (Figure 2A).

CONCLUSION

In summary, we have developed a practical and general strategy for the diastereoselective preparation of β -thiolated/selenolated amino acids via a photoredox-catalyzed asymmetric Giese reaction. In contrast to existing methods using nucleophilic and electrophilic thiol/selenol reagents to construct C–S/Se bonds, this method unprecedentedly combines the concept of “self-regeneration of chirality centers” with modern photoredox catalysis. Nevertheless, the described convenient approach allowed the scalable and diverse synthesis of proteinogenic amino acid analogues, including β -thiolated amino acids (Leu, Glu, Gln, Lys, Arg, Trp, Met), β -selenolated amino acids (Leu, Lys, Arg, Met), and a broad scope of unnatural β -thiolated/selenolated amino acids via a single-step asymmetric alkylation on a multigram scale. We believe that this methodology will not only significantly expand the utility of the ligation–dehalcogenation strategy but also allow the design and implementation of amino acids at will and the construction of disulfide-engineered peptides and proteins in academic and pharmaceutical settings.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/jacs.0c04994>.

Detailed experimental procedures and spectral data (PDF)

Crystallographic data (CIF)

Crystallographic data (CIF)

Crystallographic data (CIF)

Crystallographic data (CIF)

AUTHOR INFORMATION

Corresponding Author

Ping Wang – Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China; orcid.org/0000-0002-8640-1483; Email: wangp1@sjtu.edu.cn

Authors

Hongli Yin – Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

Mengjie Zheng – Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

Huan Chen – Department of Chemistry, University at Albany, State University of New York, Albany, New York 12222, United States; orcid.org/0000-0003-1682-8809

Siyao Wang – Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

Qingqing Zhou – Shanghai Key Laboratory for Molecular Engineering of Chiral Drugs, School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

Qiang Zhang – Department of Chemistry, University at Albany, State University of New York, Albany, New York 12222, United States; orcid.org/0000-0003-2787-1781

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/jacs.0c04994>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Financial support for this work came from the National Natural Science Foundation of China (91753102, 21672146, and 21907064) and the Shanghai Committee of Science and Technology (17JC1405300). Q.Z. thanks the National Science Foundation (CHE 1710174) and the National Institutes of Health (1R35 GM138336-01) for support.

REFERENCES

- (a) Mamathambika, B. S.; Bardwell, J. C. Disulfide-linked protein folding pathways. *Annu. Rev. Cell Dev. Biol.* **2008**, *24*, 211–235.
(b) Metanis, N.; Hilvert, D. Strategic use of non-native diselenide bridges to steer oxidative protein folding. *Angew. Chem., Int. Ed.* **2012**, *51*, 5585–5588.
(c) Reich, H. J.; Hondal, R. J. Why nature chose selenium. *ACS Chem. Biol.* **2016**, *11*, 821–841.
- Fass, D.; Thorpe, C. Chemistry and enzymology of disulfide cross-linking in proteins. *Chem. Rev.* **2018**, *118*, 1169–1198.

- (3) Jacob, C.; Giles, G. I.; Giles, N. M.; Sies, H. Sulfur and selenium: the role of oxidation state in protein structure and function. *Angew. Chem., Int. Ed.* **2003**, *42*, 4742–4758.
- (4) For classic examples, see: (a) Davie, E. A. C.; Mennen, S. M.; Xu, Y.; Miller, S. J. Asymmetric catalysis mediated by synthetic peptides. *Chem. Rev.* **2007**, *107*, 5759–5812. (b) Mousa, R.; Dardashti, R. N.; Metanis, N. Selenium and selenocysteine in protein chemistry. *Angew. Chem., Int. Ed.* **2017**, *56*, 15818–15827. An umpolung approach for the chemoselective arylation of selenocysteine in unprotected peptides: (c) Cohen, D. T.; Zhang, C. B.; Pentelute, L.; Buchwald, S. L. *J. Am. Chem. Soc.* **2015**, *137*, 9784–9787. (d) Cohen, D. T.; Zhang, C.; Fadzen, C. M.; Mijalis, A. J.; Hie, L.; Johnson, K. D.; Shriver, Z.; Plante, O.; Miller, S. J.; Buchwald, S. L.; Pentelute, B. L. A chemoselective strategy for late-stage functionalization of complex small molecules with polypeptides and proteins. *Nat. Chem.* **2019**, *11*, 78–85. (e) de-Gruyter, J. N.; Malins, L. R.; Baran, P. S. Residue-specific peptide modification: a chemist's guide. *Biochemistry* **2017**, *56*, 3863–3873. (f) Li, J.-B.; Tang, S.; Zheng, J.-S.; Tian, C.-L.; Liu, L. Removable backbone modification method for the chemical synthesis of membrane proteins. *Acc. Chem. Res.* **2017**, *50*, 1143–1153. (g) Jbara, M.; Maity, S. K.; Brik, A. Palladium in the chemical synthesis and modification of proteins. *Angew. Chem., Int. Ed.* **2017**, *56*, 10644–10655. (h) Zhang, Y.; Zhang, Q.; Wong, C.; Li, X. Chemoselective peptide cyclization and bicyclization directly on unprotected peptides. *J. Am. Chem. Soc.* **2019**, *141*, 12274–12279.
- (5) For selected examples, see: (a) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. H. Synthesis of proteins by native chemical ligation. *Science* **1994**, *266*, 776–779. (b) McGrath, N. A.; Raines, R. T. Chemoselectivity in chemical biology: acyl transfer reactions with sulfur and selenium. *Acc. Chem. Res.* **2011**, *44*, 752–761. (c) Bondalapati, S.; Jbara, M.; Brik, A. Expanding the chemical toolbox for the synthesis of large and uniquely modified proteins. *Nat. Chem.* **2016**, *8*, 407–418. (d) Burke, H.; McSweeney, M. L.; Scanlan, E. M. Exploring chemoselective S-to-N acyl transfer reactions in synthesis and chemical biology. *Nat. Commun.* **2017**, *8*, 1–16. (e) Flood, D. T.; Hintzen, J. C. J.; Bird, M. J.; Cistrone, P. A.; Chen, J. S.; Dawson, P. E. Leveraging the knorr pyrazole synthesis for the facile generation of thioester surrogates for use in native chemical ligation. *Angew. Chem., Int. Ed.* **2018**, *57*, 11634–11639. (f) Agouridas, V.; Mahdi, O. E.; Diemer, V.; Cargoët, M.; Monbaliu, J.-C. M.; Melnyk, O. Native chemical ligation and extended methods: mechanisms, catalysis, scope, and limitations. *Chem. Rev.* **2019**, *119*, 7328–7443. (g) Thompson, R. E.; Muir, T. W. Chemoenzymatic semisynthesis of proteins. *Chem. Rev.* **2020**, *120*, 3051–3126.
- (6) (a) Yan, L. Z.; Dawson, P. E. Synthesis of peptides and proteins without cysteine residues by native chemical ligation combined with desulfurization. *J. Am. Chem. Soc.* **2001**, *123*, 526–533. (b) Wan, Q.; Danishefsky, S. J. Free-radical-based, specific desulfurization of cysteine: a powerful advance in the synthesis of polypeptides and glycopolypeptides. *Angew. Chem., Int. Ed.* **2007**, *46*, 9248–9252. (c) Metanis, N.; Keinan, E.; Dawson, P. E. Traceless ligation of cysteine peptides using selective deselenization. *Angew. Chem., Int. Ed.* **2010**, *49*, 7049–7053. (d) Jin, K.; Li, T.; Chow, H. Y.; Liu, H.; Li, X. P-B desulfurization: an enabling method for protein chemical synthesis and site-specific deuteration. *Angew. Chem., Int. Ed.* **2017**, *56*, 14607–14611. (e) Malins, L. R.; Mitchell, N. J.; McGowan, S.; Payne, R. J. Oxidative deselenization of selenocysteine: applications for programmed ligation at serine. *Angew. Chem., Int. Ed.* **2015**, *54*, 12716–12721. (f) Dery, S.; Reddy, P. S.; Dery, L.; Mousa, R.; Dardashti, R. N.; Metanis, N. Insights into the deselenization of selenocysteine into alanine and serine. *Chem. Sci.* **2015**, *6*, 6207–6212.
- (7) Wu, C.; Leroux, J.-C.; Gauthier, M. A. Twin disulfides for orthogonal disulfide pairing and the directed folding of multicyclic peptides. *Nat. Chem.* **2012**, *4*, 1044–1049.
- (8) Hruby, V. J. Design in topographical space of peptide and peptidomimetic ligands that affect behavior: a chemist's glimpse at the mind-body problem. *Acc. Chem. Res.* **2001**, *34*, 389–397.
- (9) (a) Noisier, A. F. M.; Albericio, F. Advance in ligation techniques for peptide and protein synthesis. *Amino Acids, Pept. Proteins* **2014**, *39*, 1–20. (b) Moyal, T.; Hemantha, H. P.; Siman, P.; Refua, M.; Brik, A. Highly efficient one-pot ligation and desulfurization. *Chem. Sci.* **2013**, *4*, 2496–2501. (c) Reimann, O.; Smet-Nocca, C.; Hackenberger, C. P. R. Traceless purification and desulfurization of tau protein ligation products. *Angew. Chem., Int. Ed.* **2015**, *54*, 306–310. (d) Tang, S.; Liang, L.-J.; Si, Y.-Y.; Gao, S.; Wang, J.-X.; Liang, J.; Mei, Z.; Zheng, J.-S.; Liu, L. Practical chemical synthesis of atypical ubiquitin chains by using an isopeptide-linked Ub isomer. *Angew. Chem., Int. Ed.* **2017**, *56*, 13333–13337. (e) Chow, H. Y.; Zhang, Y.; Matheson, E.; Li, X. Ligation technologies for the synthesis of cyclic peptides. *Chem. Rev.* **2019**, *119*, 9971–10001. (f) Wang, S.; Thopate, Y. A.; Zhou, Q.; Wang, P. Chemical protein synthesis by native chemical ligation and variations thereof. *Chin. J. Chem.* **2019**, *37*, 1181–1193.
- (10) (a) Chen, S.; Gopalakrishnan, R.; Schaer, T.; Marger, F.; Hovius, R.; Bertrand, D.; Pojer, F.; Heinis, C. Dithiol amino acids can structurally shape and enhance the ligand-binding properties of polypeptides. *Nat. Chem.* **2014**, *6*, 1009–1016. (b) Murar, C. E.; Ninomiya, M.; Shimura, S.; Karakus, U.; Boyman, O.; Bode, J. W. Chemical synthesis of interleukin-2 and disulfide stabilizing analogues. *Angew. Chem., Int. Ed.* **2020**, *59*, 8425–8429.
- (11) (a) Guerin, D. J.; Miller, S. J. Asymmetric azidation-cycloaddition with open-chain peptide-based catalysts. A sequential enantioselective route to triazoles. *J. Am. Chem. Soc.* **2002**, *124*, 2134–2136. (b) Shin, N. Y.; Ryss, J. M.; Zhang, X.; Miller, S. J.; Knowles, R. R. Light-driven deracemization enabled by excited-state electron transfer. *Science* **2019**, *366*, 364–369.
- (12) For selected references, see: (a) Gracia-Vitoria, J.; Osante, I.; Cativiela, C. Stereoselective synthesis of modified cysteines. *Tetrahedron: Asymmetry* **2017**, *28*, 215–245. (b) Shang, S.; Tan, Z.; Dong, S.; Danishefsky, S. J. An advance in proline ligation. *J. Am. Chem. Soc.* **2011**, *133*, 10784–10786. (c) Townsend, S. D.; Tan, Z.; Dong, S.; Shang, S.; Brailsford, J. A.; Danishefsky, S. J. Advances in proline ligation. *J. Am. Chem. Soc.* **2012**, *134*, 3912–3916. (d) Wong, C. T. T.; Tung, C. L.; Li, X. Synthetic cysteine surrogates used in native chemical ligation. *Mol. Biosyst.* **2013**, *9*, 826–833. (e) Kulkarni, S. S.; Sayers, J.; Premjee, B.; Payne, R. J. Rapid and efficient protein synthesis through expansion of the native chemical ligation concept. *Nature Rev. Chem.* **2018**, *2*, 1–17.
- (13) An elegant enolate chemistry has been used to build thiol/selenol residues in three to five steps, but this method is limited to Asp, Asn, and Glu scaffolds. For selected reviews and references, see: (a) Thompson, R. E.; Chan, B.; Radom, L.; Jolliffe, K. A.; Payne, R. J. Chemoselective ligation-desulfurization at aspartate. *Angew. Chem., Int. Ed.* **2013**, *52*, 9723–9727. (b) Mitchell, N. J.; Sayers, J.; Kulkarni, S. S.; Clayton, D.; Goldys, A. M.; Ripoll-Rozada, J.; Pereira, P. J. B.; Chan, B.; Radom, L.; Payne, R. J. Accelerated protein synthesis via one-pot ligation-deselenization chemistry. *Chem.* **2017**, *2*, 703–715.
- (14) For selected references on the Giese reaction, see: (a) Giese, B.; Dupuis, J. Diastereoselective syntheses of C-glycopyranosides. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 622–623. (b) Giese, B. Formation of CC bonds by addition of free radicals to alkenes. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 753–764. (c) Jasperse, C. P.; Curran, D. P.; Fevig, T. L. Radical reactions in natural product synthesis. *Chem. Rev.* **1991**, *91*, 1237–1286. (d) Srikanth, G. S. C.; Castle, S. L. Advances in radical conjugate additions. *Tetrahedron* **2005**, *61*, 10377–10441.
- (15) (a) Polt, R.; Seebach, D. Stereoselective alkylation of glycine units in dipeptide derivatives: “chirality transfer” via a pivalaldehyde N,N-acetal center. *J. Am. Chem. Soc.* **1989**, *111*, 2622–2632. (b) Leonard, D. J.; Ward, J. W.; Clayden, J. Asymmetric α -arylation of amino acids. *Nature* **2018**, *562*, 105–109.
- (16) Jeanguenat, A.; Seebach, D. Stereoselective chain elongation at C-3 of cysteine through 2,3-dihydrothiazoles, without racemization. preparation of 2-amino-5-hydroxy-3- mercaptoalkanoic acid derivatives. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2291–2298.
- (17) For selected references, see: (a) Zuo, Z.; Cong, H.; Li, W.; Choi, J.; Fu, G. C.; MacMillan, D. W. C. Enantioselective

decarboxylative arylation of α -amino acids *via* the merger of photoredox and nickel catalysis. *J. Am. Chem. Soc.* **2016**, *138*, 1832–1835. (b) McCarver, S. J.; Qiao, J. X.; Carpenter, J.; Borzilleri, R. M.; Poss, M. A.; Eastgate, M. D.; Miller, M.; MacMillan, D. W. C. Decarboxylative peptide macrocyclization through photoredox catalysis. *Angew. Chem., Int. Ed.* **2017**, *56*, 728–732. (c) Bloom, S.; Liu, C.; Kölmel, D. K.; Qiao, J. X.; Zhang, Y.; Poss, M. A.; Ewing, W. R.; MacMillan, D. W. C. Decarboxylative alkylation for site-selective bioconjugation of native proteins *via* oxidation potentials. *Nat. Chem.* **2018**, *10*, 205–211.

(18) For selected examples, see: (a) Ni, S.; Garrido-Castro, A. F.; Merchant, R. R.; deGruyter, J. N.; Schmitt, D. C.; Mousseau, J. J.; Gallego, G. M.; Yang, S.; Collins, M. R.; Qiao, J. X.; Yeung, K.; Langley, D. R.; Poss, M. A.; Scola, P. M.; Qin, T.; Baran, P. S. A general amino acid synthesis enabled by innate radical cross-coupling. *Angew. Chem., Int. Ed.* **2018**, *57*, 14560–14565. (b) Pratsch, G.; Lackner, G. L.; Overman, L. E. Constructing quaternary carbons from N-(acyloxy)phthalimide precursors of tertiary radicals using visible-light photocatalysis. *J. Org. Chem.* **2015**, *80*, 6025–6036.

(19) Narayanam, J. M. R.; Tucker, J. W.; Stephenson, C. R. J. Electron-transfer photoredox catalysis: development of a tin-free reductive dehalogenation reaction. *J. Am. Chem. Soc.* **2009**, *131*, 8756–8757.

(20) Gracia-Vitoria, J.; Osante, I.; Cativiela, C.; Merino, P.; Tejero, T. Self-regeneration of chirality with L-cysteine through 1,3-dipolar cycloadditions between diazoalkanes and enantiomerically pure thiazolines: experimental and computational studies. *J. Org. Chem.* **2018**, *83*, 3960–3972.

(21) Schwarz, J.; König, B. Metal-free, visible-light-mediated, decarboxylative alkylation of biomass-derived compounds. *Green Chem.* **2016**, *18*, 4743–4749.

(22) (a) Wang, Z.; Xu, W.; Liu, L.; Zhu, T. F. A synthetic molecular system capable of mirror-image genetic replication and transcription. *Nat. Chem.* **2016**, *8*, 698–704. (b) Teng, P.; Ma, N.; Cerrato, D. C.; She, F.; Odom, T.; Wang, X.; Ming, L.-J.; van der Vaart, A.; Wojtas, L.; Xu, H.; Cai, J. Right-handed helical foldamers o-AApeptides. *J. Am. Chem. Soc.* **2017**, *139*, 7363–7369.

(23) Mitchell, N. J.; Malins, L. R.; Liu, X.; Thompson, R. E.; Chan, B.; Radom, L.; Payne, R. J. Rapid additive-free selenocystine–selenoester peptide ligation. *J. Am. Chem. Soc.* **2015**, *137*, 14011–14014.

(24) For applications of peptide ligation–desulfurization/deselenization reactions, see the selected reviews: (a) Ma, J.; Zeng, J.; Wan, Q. Postligation-desulfurization: a general approach for chemical protein synthesis. *Top. Curr. Chem.* **2014**, *363*, 57–102. (b) Kulkarni, S. S.; Sayers, J.; Premdjee, B.; Payne, R. J. Rapid and efficient protein synthesis through expansion of the native chemical ligation concept. *Nature Rev. Chem.* **2018**, *2*, 1–17.

(25) (a) Berger, A. A.; Völler, J.; Budisa, N.; Koksche, B. Deciphering the fluorine code-the many hats fluorine wears in a protein environment. *Acc. Chem. Res.* **2017**, *50*, 2093–2103. (b) Moschner, J.; Stulberg, V.; Fernandes, R.; Huhmann, S.; Leppkes, J.; Koksche, B. Approaches to obtaining fluorinated α -amino acids. *Chem. Rev.* **2019**, *119*, 10718–10801.

(26) Wikstrom, M. K. Proton pump coupled to cytochrome c oxidase in mitochondria. *Nature* **1977**, *266*, 271–273.

(27) Wang, Y.; Chou, D. H.-C. A thiol–ene coupling approach to native peptide stapling and macrocyclization. *Angew. Chem., Int. Ed.* **2015**, *54*, 10931–10934.

(28) de Araujo, A. D.; Mobli, M.; Castro, J.; Harrington, A. M.; Vetter, I.; Dekan, Z.; Muttenthaler, M.; Wan, J.; Lewis, R. J.; King, G. F.; Brierley, S. M.; Alewood, P. F. Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. *Nat. Commun.* **2014**, *5*, 1–12.