Stereoselective and divergent construction of β-thiolated/selenolated amino acids *via* photoredox-catalyzed asymmetric Giese reaction

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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 impact of our chemistry was further highlighted and validated through the preparation of a series of peptidyl thiol/selenol analogs, including cytochrome c oxidase subunit protein 7C and oxytocin.

INTRODUCTION

Cysteine (Cys) and its analog, selenocysteine (Sec) play irreplaceable roles in protein folding and stability,[1] enzymatic activity,[2] 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 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 (Fig. 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 acids preparation.[11] Nonetheless, the resultant β-thiolated/selenolated amino acids, in which the 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 for their widespread utilities is the limited accessibility of these pre-requisite enantiopure thiolated/selenolated amino acids. Currently, the majority of methods are indirect and confined to certain amino acid scaffolds despite the significant research efforts toward the preparation of β-thiolated/selenolated amino acids reported by multiple research groups.[12] Among the methods towards 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 (Fig. 1B). Unfortunately, modification of β-hydroxyl amino acids approach is extremely impractical which 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-thio/selenolated amino acids from readily accessible building blocks remains elusive.

Herein, we present a general and practical venue to access enantiopure β-thiolated/selenolated amino acids *via* asymmetric Giese reaction[14] (Fig. 1C). Our strategy starts from enantiopure selenozoline/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) furnishes thiazoline/selenozoline to produce β-substituted thiol/ selenolated amino acid framework. The chiral pivalaldehyde acetal of thiazoline/selenozoline controls the diastereoectivity 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-seleno/thiolated amino acids from a common precursor at will with high stereopurity and high yield. Products could be rapidly acquired on a multigram scale that are not easily accessible using conventional methods. Furthermore, the resulting thiazolino protected amino acids can be directly used in peptide synthesis after simple operation without complex protecting group manipulation.

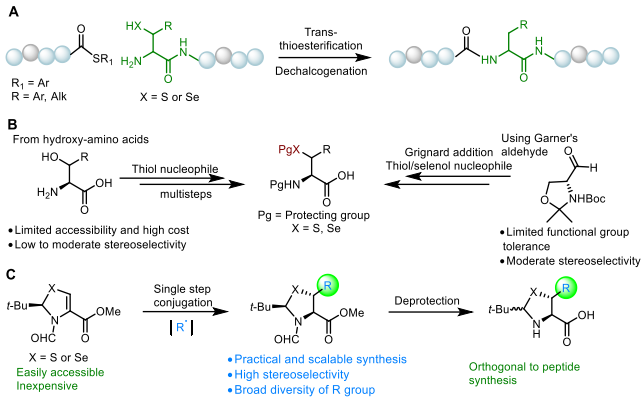


Figure 1. Development of a general method used for the preparation of enantiopure β-thio/selenolated amino acids. **A**. NCL-dechalcogenation. **B**. Representative methods for constructing thio/selenolated amino acids. **C**. The photoredox-catalyzed asymmetric Giese reaction as a general solution to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

RESULTS AND DISCUTION

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 centres",[15] chiral *S*, *N*-acetyl thiazoline served as a good starting point for the preparation of β-thiolated amino acids *via* Michael-type alkylation.[16] However, this transformation remains limited in synthesis due to its poor yield, 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 may lead to superior results. It is well established that convenient radical generation occurs from carboxylic acids,[17] redox-active esters (RAEs)[18] and alkyl halides[19] in the presence of 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 t-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 *trans*-product **C**, providing two new stereocenters in one step (Fig. 2A). Subsequent deprotection may furnish a broad family of β-thio/selenolated amino acids (**D**), which are suitable for direct peptide coupling.

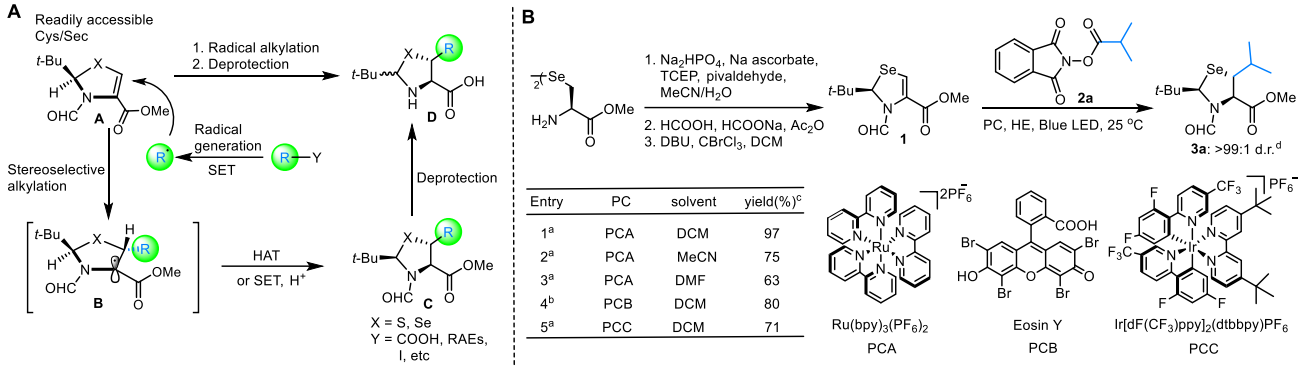
The importance of Se in biology renders selenolated amino-acids in the high demand, conversely, the sheer difficulty of handling labile *Se* during reactions prevents securing *Se*-amino acids from the large quantity. We began our exploration with the preparation of β-selenolated amino acids as a more practical synthetic targets. The starting material, pivalaldehyde *N*,*Se*-acetal selenozoline **1**, was quickly prepared over three steps from the diselenide derivative of L-Sec methyl ester on a 20-gram scale. The *N*-formyl group was used to protect the nitrogen atom as well as to improve the electrophilicity of the double bond.[20] Under irradiation of blue LED light (10 W), $Ru(bpy)_3(PF_6)_2$ (PCA, 1 mol%) catalyzed the coupling of **1** with *iso*-propyl *N*-hydroxyphthalimide ester **2a** (1.2 equiv.) provided 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 ^{1}H NMR and solvent switching (see supplementary information page 10 for details) (Fig. 2B). Screening of the solvent system suggested that dichloromethane (DCM) afforded the highest yield compared to MeCN and DMF; other photoredox catalysts such as Eosin Y (PCB) and $Ir[dF(CF_3)ppy]_2(dtbbpy)$- PF_6 (PCC) could also mediate same reaction with eroded yields. In all the case, only a single diastereoisomer *trans*-**3a** was obtained (>99:1 d.r.).

An ideal method is that 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 (Fig. 3A, Protocol A). Reaction of selenozolidine **1** with a series of cyclic carboxylate *N*-hydroxyphthalimide esters (cyclobutyl **2b**, cyclohexyl **2c** and adamantyl **2d**) and fluorinated cyclobutyl carboxylate ester **2e** yielded the desired products (**3b-3e**) in excellent yields and high diastereoselectivity on a gram scale. Notably, product **3d** bearing an adamantyl substituent was obtained with >90% yield, which suggested that generating sterically bulky quaternary centers could be accomplished. The absolute configuration of **3d** was confirmed *via* X-ray crystallography (Fig. 3B).

Coupling with selenozolidine **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-2i**) and DIPEA (2.5 equiv.) in DCM using PCB (Eosin Y), which gave the desired coupling products (**3f–3i**) in good yield at room temperature (Fig. 3A, Protocol B).[21]

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

We next sought to convert products **3** into target amino acids **4**, which might be used directly as SPPS-compatible amino acids. Desired products (**4a-4g, 4k** and **4l**) can be generated from their parent compounds after saponification of methyl ester and acidic removal of the N-formyl group over two steps (Fig. 3A. condition a). Substrates **4a** and **4l** represent β-seleno



Entry	PC	solvent	yield(%)[c]
1[a]	PCA	DCM	97
2[a]	PCA	MeCN	75
3[a]	PCA	DMF	63
4[b]	PCB	DCM	80
5[a]	PCC	DCM	71

Figure 2. Design plan and optimization of the reaction conditions used in the preparation of β-selenolated Leu. **A**. Asymmetric 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 ℃. [a]18 h. [b]48 h. [c]Isolated yield. [d]The diastereoselectivity was determined by ^{1}H NMR and GC analysis of the crude reaction mixture. PC, photoredox catalyst. HE, Hantzsch ester. DIPEA, *N*, *N*-diisopropylethylamine. DCM, dichloromethane. DMF, *N*, *N*-dimethylformamide.

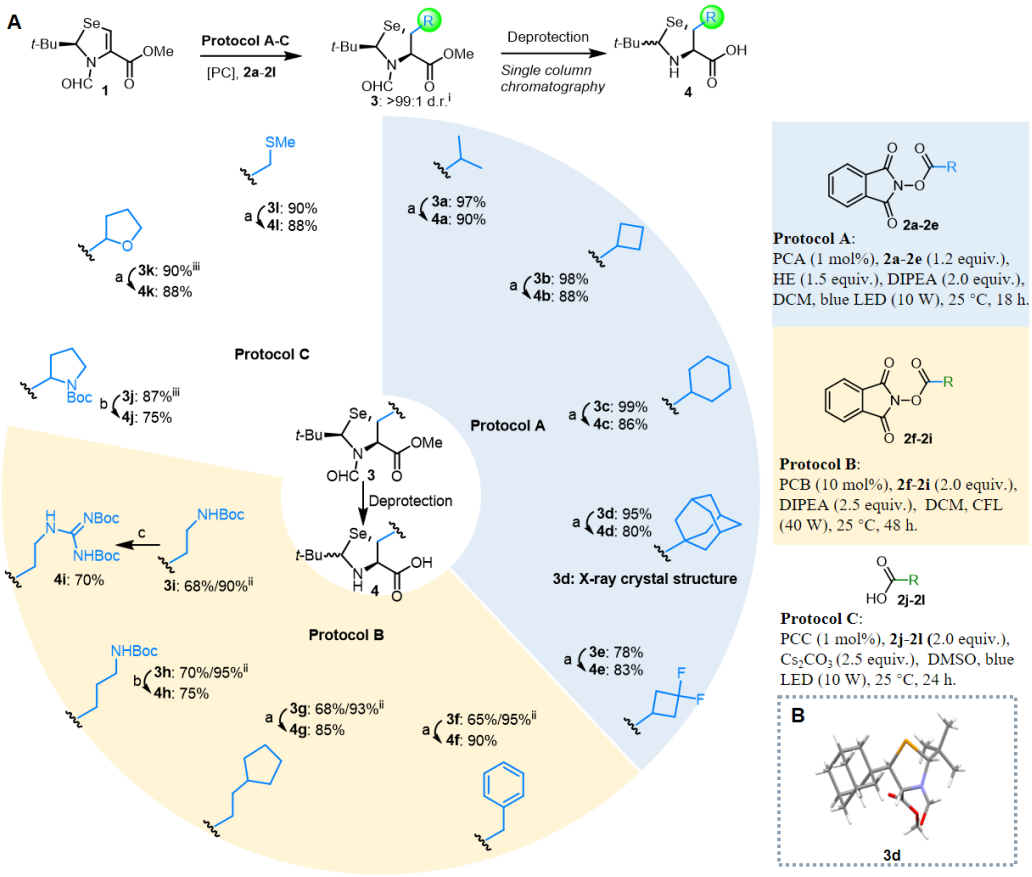


Figure 3. Asymmetric alkylation of selenozolidine under photoredox conditions and their derivatization. **A**. Scope of the β-selenolated amino acids. Deprotection conditions: a. 1) LiOH (10.0 equiv.), H_2O/MeOH (1:3 v/v), 25 ℃, 6 h; 2) 6 N HCl in dioxane, 50 ℃, 8 h. b. 1) LiOH (10.0 equiv.), H_2O/MeOH (1:3 v/v), 25 ℃, 6 h; 2) 6 N HCl in dioxane, 50 ℃, 8 h; 3) $(Boc)_2O$ (1.0 equiv.), THF/H_2O (10:1 v/v), DIPEA (2.0 equiv.), 25 ℃, 1 h. c. 1) LiOH (10.0 equiv.), H_2O/MeOH (1:3 v/v), 25 ℃, 6 h; 2) 6 N HCl in dioxane, 50 ℃, 8 h; 3) *N*, *N*'-bis-Boc-1-guanylpyrazole (1.0 equiv.), DIPEA (2.0 equiv.), MeOH, 25 ℃, 3 h. **B**. The absolute configuration of **3d** was confirmed by X-ray crystallography. [i]The diastereoselectivity was determined by GC analysis of the crude reaction mixture. [ii]brsm, based on recovered starting material. [iii]A mixture of diastereomers was found.

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** and **3j**, a further Boc protection step was required to form β-selenolated Lys **4h** and **4j** (Fig. 3A. condition b). Guanidinylation was performed to yield β-selenolated Arg **4i** from ornithine **3i**, (Fig. 3A. condition c). For most cases, only a single purification was needed after these deprotection conditions, minimizing time-consuming isolation process.

We further expanded the reaction scope to a number of β-thiolated amino acids, which are listed in Figure 4. In line with

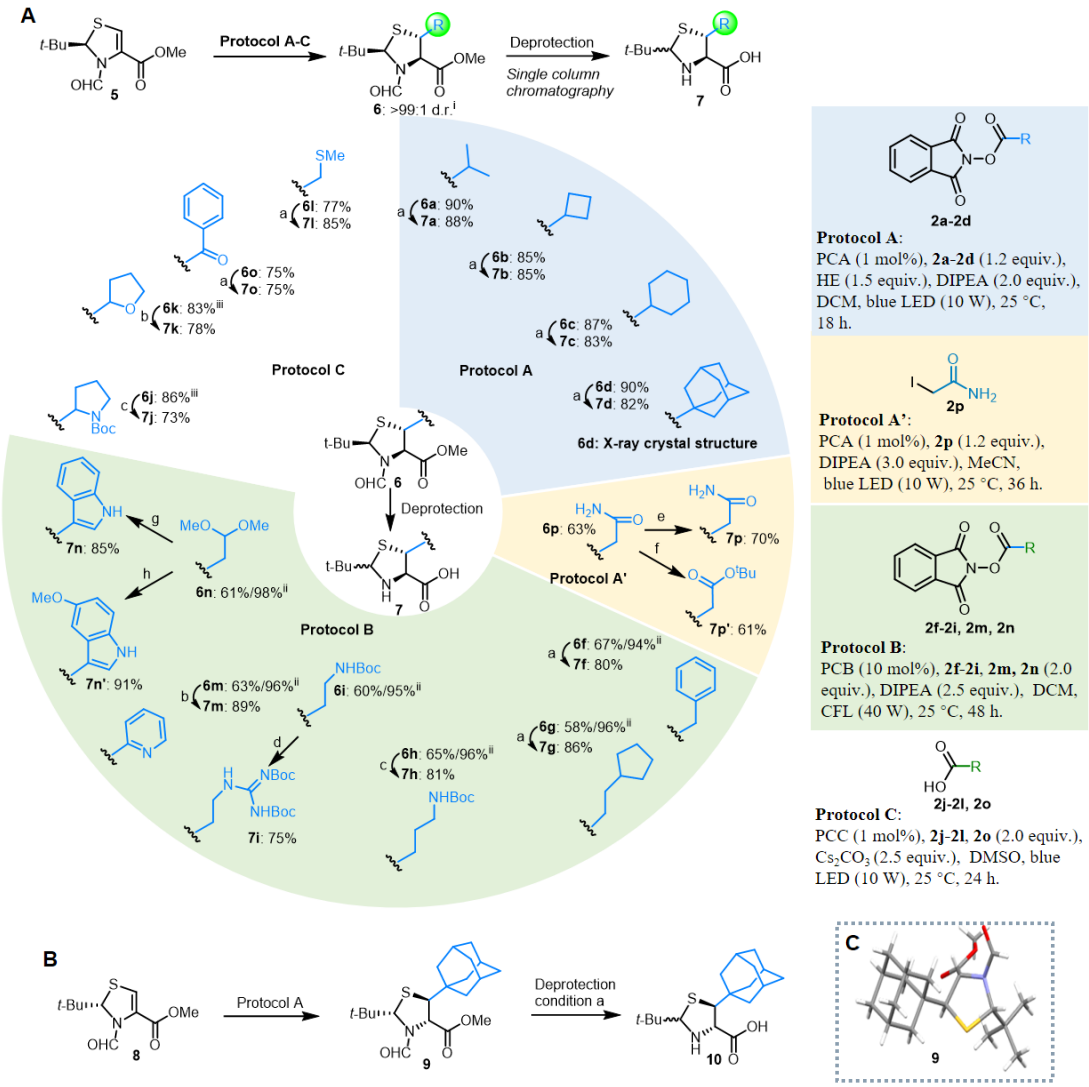
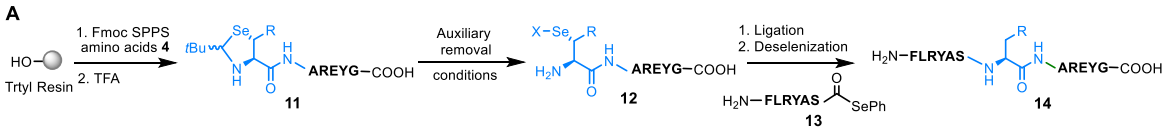
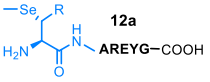
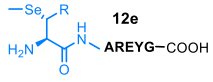
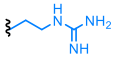
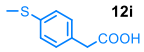
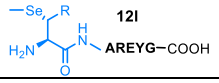


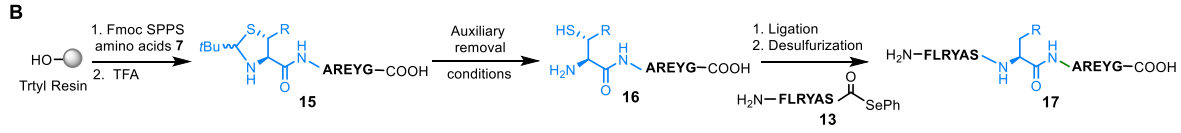
Figure 4. Asymmetric alkylation of thiozolidine under photoredox conditions and derivatization. **A**. Scope of β-thiolated amino acids. Deprotection conditions: a. 6 N HCl, 90 °C, 8 h. b. 1) 0.5 N HCl in MeOH, 25 °C, 12 h; 2) LiOH (10 equiv.), H_2O/MeOH (1:3 v/v), 25 °C, 6 h. c. 1) 6 N HCl, 90 °C, 8 h; 2) $(Boc)_2O$ (1.0 equiv.), DIPEA (2.0 equiv.), THF/H_2O (10:1 v/v), 25 °C, 1 h. d. 1) 6 N HCl, 90 °C, 8 h; 2) *N*,*N*'-bis-Boc-guanylpyrazole (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.), H_2O/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.), H_2O/MeOH (1:3 v/v), 25 °C, 6 h. g. 1) $PhNHNH_2$ (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.), H_2O/MeOH (1:3 v/v), 25 °C, 6 h. h. 1) *p*-MeO-$PhNHNH_2$ (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.), H_2O/MeOH (1:3 v/v), 25 °C, 6 h. **B**. Synthesis of D-β-thiolated amino acids from D-Cysteine. **C**. The absolute configuration of **9** was confirmed by X-ray crystallography. [i]The diastereoselectivity was determined by GC analysis of the crude reaction mixture. [ii]brsm, based on recovered starting material. [iii]A mixture of diastereomers was found.

the β-selenolated amino acids preparation, all the RAEs (**2a-2d**, **2f-2i**, **2m**, **2n**) and carboxylic acids (**2j-2l**, **2o**) smoothly conjugated with thiozolidine **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-gram scale (see supplementary information page 4 for details). Products (**6a-6d**, **6f-6o**) were acquired in high yields using Protocol A, B and C (Fig. 4A). Notably, reaction of **5** with 2-iodoacetamide **2p** under photoredox dehalogenation conditions (Fig. 4A, Protocol A') yielded **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 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**, exist as a mixture of rotamers as confirmed by variable temperature ^1H NMR and solvent switching.

Compared with delicate selenolated analogs, 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 Fig 4A. Hydrolysis using 6 N HCl led to



entry	amino acids **4**	R group of **11**, **12** and **14**	auxiliary removal conditions/yield of **12**[i]	X group of **12**	deselenization conditions/yield of **14**[ii]
1	**4a**		a: **12a**, 45%	 **12a**	c: **14a,** 58%
2	**4e**		a: **12e**, 33%	 **12e**	c: **14e,** 45%
3	**4i**		a: decompose b: **12i**, 40%	 **12i**	c: **14i,** 51%
4	**4l**		a: **12l**, 38%	 **12l**	c: **14l**, 21% d: **14l**, 38%



entry	amino acids **7**	R group of **15**, **16** and **17**	auxiliary removal conditions/yield of **16**[i]	desulfurization conditions/yield of **17**[iii]
1	**7c**		a: **16c**, 28%	e: **17c,** 58%
2	**7d**		a: **16d**, 33%	e: **17d,** 53%
3	**7n**		a: **16n**, 35%	e: **17n**, 20% f: **17n,** 51%
4	**7o**		a: **16o**, 40%	e: **17o**, 38%

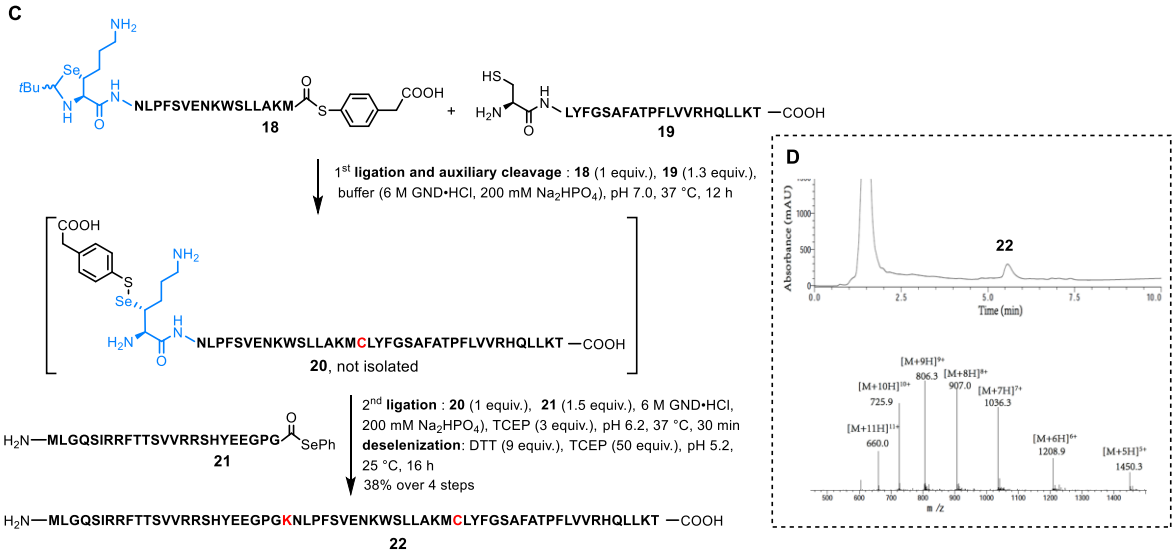


Figure 5. Exploiting the utility of β-thio/selenolated amino acids. **A**, DSL-deselenization using β-selenolated amino acids. **B**, NCL-desulfurization using β-thiolated amino acids. **C**, One-pot synthesis of cytochrome c oxidase subunit 7C **22**. **D**, Crude HPLC trace of final deselenization to give **22** and ESI mass spectra of **22**. Conditions for auxiliary removal: a. 6 M GND•HCl, 0.2 M Na_2HPO_4, 0.2 M $MeONH_2$, pH 4.0. b. 6 M GND•HCl, 0.2 M Na_2HPO_4, MPAA (1.5 equiv.), pH 6.8. Ligation: **13** (1.2 equiv.), 6 M GND•HCl, 0.2 M Na_2HPO_4, TCEP (2.0 equiv.), pH 6.2, 25 ℃, 16 h. Conditions for deselenization: c. 6 M GND•HCl, 0.2 M Na_2HPO_4, DTT (9.0 equiv.), TCEP (50 equiv.), pH 5.2, 25 °C, 16 h. d. 6 M GND•HCl, 0.2 M Na_2HPO_4, DTT (250 equiv.), TCEP (50 equiv.), pH 5.2, 25 ℃, 16 h. Conditions for desulfurization: e. 6 M GND•HCl, 0.2 M Na_2HPO_4, 200 mM TCEP, 10 mM VA-044, 50 mM GSH, 37 ℃, 16 h. f. 6 M GND•HCl, 0.2 M Na_2HPO_4, 500 mM TCEP, 30 mM VA-044, 150 mM GSH, 37 ℃, 16 h. [i]Yield according to the original

loading of the resin. [ii]Yield of ligation and deselenization. [iii]Yield of ligation and desulfurization. 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.

substrates (**6a** to **6d**, **6f**, **6g**, **6o** and **6l**) directly in a single step (Fig. 4A, condition a), including β-thiolated Leu (**6a**) and Met (**6l**). Deformylation of **6k** and **6m** was carried out using 0.5 N HCl followed by saponification to give **7k** and **7m** in high yields (Fig. 4A, condition b). Similarly, after acidic removal of *N*-formyl group, followed by hydrolysis of the methyl ester and Boc group gave the free amine from **6h** and **6j**, which were re-protected with Boc to give β-thiol Lys **7h** and **7j** in high yields, respectively (Fig. 4A, condition c). After acidolysis and guanidinylation, β-thiol Arg **7i** was produced in three steps starting from β-thiolated Orn **6i** (Fig. 4A, condition d). β-thiol Trp analogs (**7n** and **7n'**) were constructed *via* Fischer indole synthesis from γ-aldehyde dimethyl acetal **6n** (Fig. 4A, condition 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 saphonication step provided β-thiol Gln **7p** in 70% yield (Fig. 4A, condition e). Maintenance of the methylester during acidic removal of N-formyl group together with hydrolysis of side-chain amide moiety of **6p** was possible using 6 N HCl at 90 ℃ for 30 min, leaving the methyl ester remained untouched. Functional group manipulations eventually produced β-thiol Glu **7p**' in good yield (Fig. 4A, condition 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. Starting from **8** (the enantiomer of **5**), 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 (Fig. 4B). The absolute configuration of **9** was confirmed using X-ray crystallography (Fig. 4C).

With these β-thio/selenolated amino acids in hand, we next explored their utility in peptide synthesis. Selenolated amino acids **4a**, **4e**, **4i** and **4l** 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**, **11e**, **11i**, **11l** were prepared, respectively (Fig. 5A). In all cases, no double coupling products were observed due to the steric bulkiness of the neighboring *t*-Bu group. Next, deprotection of the auxiliary in the presence of excess $MeONH_2$, diselenide dimer peptides **12a**, **12e** and **12l** were obtained in good yield after HPLC purification (Fig. 5A, condition a, entry 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 the 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 (Fig. 5A, condition b, entry 3). Mechanistic studies suggested that the selenozolidine ring was opened *via* the *in situ* produced disulfide of MPAA (see supplementary information page 84 for details). To the best of our knowledge, the deprotection of selenozolidine described here is one of the mildest conditions reported to date. Meanwhile, one pot diselenide-selenoester ligation (DSL)-deselenization[23] between peptide selenoester **13** and selenolated peptides **12a**, **12e**, **12i** and **12l** were conducted. Peptides **14a**, **14e** and **14i** were obtained according to ligation and deselenization protocol in good yields (Fig. 5A, condition c). Low yield of **14l** was observed during deselenization. The deselenization proceeded well when excess equivalent DTT and TCEP were used (Fig. 5A, condition d). We further examined NCL-desulfurization using β-thiolated amino acids. Peptides **15c**, **15d**, **15n** and **15o** were prepared via Fmoc-SPPS and TFA deprotection. Next, auxiliary removal was achieved successfully using excess amount of $MeONH_2$, providing thiolated peptides (**16c**, **16d**, **16n** and **16o**) in good yields (Fig. 5B, condition a, entry 1-4). Ligation of peptide **16** with peptide **13** under NCL conditions, followed by one-pot desulfurization, yielded peptides **17c**, **17d** and **17o** smoothly (Fig. 5B, condition 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 (Fig. 5B, condition f). Therefore, this method not only significantly expanded the utility of NCL-dechalcogenation 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 7C[26] (**22**) was prepared by one-pot synthesis using selenolated Lys building block **4h** as an example. As shown in Fig 5C, MPAA-thioester **18** and peptide **19** were combined under NCL condition to yield peptide **20** *via in situ* production of MPAA disulfide from **18**. Without seperation, 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 sucessfully synthesized cytochrome c oxidase subunit 7C **22** *via* sequential one-pot NCL-activation of 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 selenozolidine could expand and enhance the selenolated amino acids-mediated ligations.

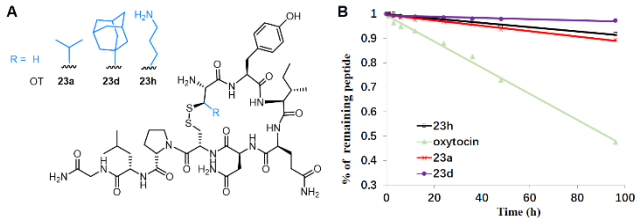


Figure 6. Thermal stability of synthetic oxytocin analogs**. A**. Synthetic oxytocin analogs. **B**. The thermal stability of oxytocin, **23a**, **23d** and **23h** in water at 50 ℃. OT, oxytocin.

Disulfides have been extensively used to stabilize peptides 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 impact of introducing cysteine analogs on protein stability, a series of oxytocin (a hormone stimulating parturition and lactation drug) analogs (**23a**, **23d**, and **23h**) were prepared *via* SPPS and subsequent oxidative folding, which contain various *N*-terminal β-thiolated amino acid residues (Fig. 6A). The thermal stability of native oxytocin and its analogs were evaluated at 50 °C by LCMS analysis.[28] Native oxytocin degraded with a half-life of 3.9 d, whereas synthetic analogs demonstrate significant stability (**23a**, 18.8 d; **23d**, 68.6 d; **23h**, 23.1 d) (Fig. 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 bridge with high stability and structural diversity.

This method is not without limitations. For example, reaction is performed under photoredox conditions, thus it will be difficult to introduce radical-sensitive groups (cyclopropyl methyl, disulfide). Furthermore, only *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 (Fig. 2A).

CONCLUSION

In summary, we have developed a practical and general strategy for diastereoselective preparation of β-thio/seleno-lated amino acids *via* photoredox-catalyzed asymmetric Giese reaction. In contrast to existing methods using nucleophilic and electrophilic thiol/selenol reagents to construct *C-S/Se* bond, this method unprecendently combines the concept of "self-regeneration of chirality centres" with modern photoredox catalysis. Nevertheless, the described convenient approach allowed the scalable and diverse synthesis of proteinogenic amino acid analogs 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-thio/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 ligation-dechalcogenation strategy, but also allow design and implementation of amino acids at will and construction of disulfide-engineered peptides and proteins in academic and pharmaceutical settings.

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

Detailed experimental procedures and spectral data

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Notes

There are no competing financial interests in this work

ACKNOWLEDGMENT

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

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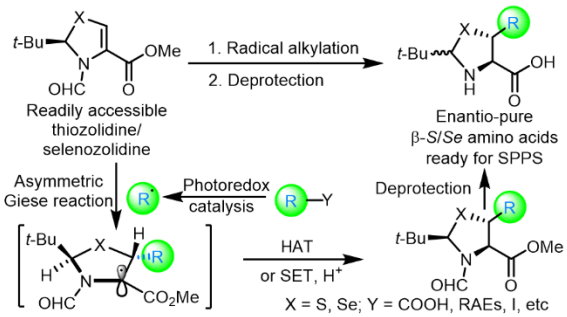
t-Bu
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1. Radical alkylation
2. Deprotection
Readily accessible
thiozolidine/
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Enantio-pure
β-S/Se amino acids
ready for SPPS
Asymmetric
Giese reaction
Photoredox
catalysis
Deprotection
HAT
or SET, H+
CO2Me
X = S, Se; Y = COOH, RAEs, I, etc