Accepted Manuscript

Application of bis-2-(trimethylsilyl)ethyl diselenide to the synthesis of selenium-containing amino acid derivatives

Tsubasa Yonezawa, Masahito Yamaguchi, Masayuki Ninomiya, Mamoru Koketsu

PII: S0040-4020(17)30917-1

DOI: 10.1016/j.tet.2017.09.004

Reference: TET 28955

To appear in: *Tetrahedron*

Received Date: 1 August 2017

Revised Date: 30 August 2017

Accepted Date: 4 September 2017

Please cite this article as: Yonezawa T, Yamaguchi M, Ninomiya M, Koketsu M, Application of bis-2-(trimethylsilyl)ethyl diselenide to the synthesis of selenium-containing amino acid derivatives, *Tetrahedron* (2017), doi: 10.1016/j.tet.2017.09.004.

Graphical Abstract

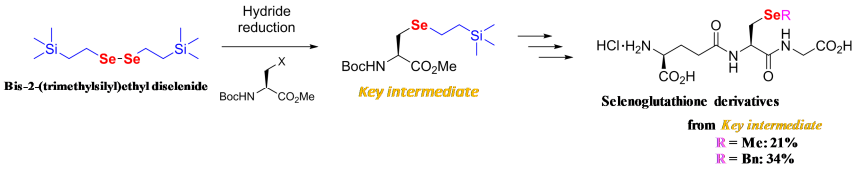
To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Application of bis-2-(trimethylsilyl)ethyl diselenide to the synthesis of selenium-containing amino acid derivatives

Leave this area blank for abstract info.

Tsubasa Yonezawa[a], Masahito Yamaguchi[a], Masayuki Ninomiya[a,b] and Mamoru Koketsu[a,b,*]

[a]*Department of Materials Science and Technology, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[b]*Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*



Tetrahedron
journal homepage: www.elsevier.com

Application of bis-2-(trimethylsilyl)ethyl diselenide to the synthesis of selenium-containing amino acid derivatives

Tsubasa Yonezawa[a], Masahito Yamaguchi[a], Masayuki Ninomiya[a,b] and Mamoru Koketsu[a,b,*]

[a]*Department of Materials Science and Technology, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*
[b]*Department of Chemistry and Biomolecular Science, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Selenation
Bis-2-(trimethylsilyl)ethyl diselenide
Selenium-containing amino acid
Selenocysteine
Selenoglutathione

ABSTRACT

Selenium-containing amino acids play a pivotal role as biomaterials for the synthesis of *Se*-dependent enzymes and repair proteins. Especially, selenocysteine and selenoglutathione are prominently involved in fundamental biological processes. In this study, a series of selenocysteine (Sec) and selenoglutathione (GSeH) derivatives were synthesized via 2-(trimethylsilyl)ethylselenation as a key step. Our findings suggested the relevance and application of a 2-(trimethylsilyl)ethylselenyl group to the *Se*-containing amino acid synthesis.

* Corresponding author. Tel./Fax: +81-58-293-2619; e-mail: koketsu@gifu-u.ac.jp

1. Introduction

Since the element selenium was firstly discovered from copper pyrites by the Swedish chemist Jöns Jacob Berzelius in 1817,[1] it had been predicted as a dangerous component causing livestock poisoning for over a century. In 1950s, Schwarz *et al.*, reported its ability to serve interchangeably with vitamin E in the prevention of vascular or muscular signs.[2] Subsequently, it was reported that the glutathione peroxidase (GPx) having *Se* in its catalytic center spares vitamin E by *Se*.[3] Owing to the discoveries of several *Se*-dependent GPx forms,[4-7] other selenoenzymes (iodothyronine deiodinases,[8-10] thioredoxin reductases,[11,12] and selenophosphate synthetase [7,13]), and specific selenoproteins (SelH, SelI, SelK, SelN, SelM, SelO, SelP, SelR, SelS, SelT, SelV, SelW, and Sep15),[13-16] the selenium is now recognized to be nutritionally essential for humans.

Selenocysteine (Sec) is often referred to as the 21st amino acid found in 25 human selenoenzymes and selenoproteins. The GPxs catalyze the reduction of harmful hydroperoxides through the catalytic triad formation of the Sec residue at the active site with tryptophan and glutamine.[16] The key role of Sec in the overall catalytic process is believed to arise from the powerful nucleophilicity of *Se*. In general, selenoproteins are involved in a variety of fundamental functions, most notably redox homeostasis.[17] With the exception of plasma selenoprotein P, selenoproteins contain a single Sec residue, which is incorporated by the co-translational modification of transfer RNA-bound serine at certain loci coded by specific uracil-guanine-adenine codons.[18,19]

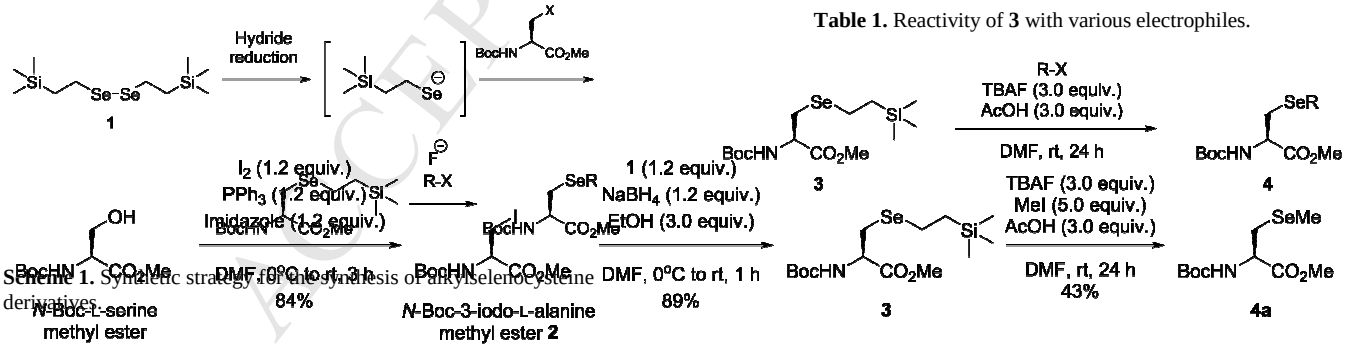
Organoselenium chemistry has attracted a great deal of attention due to the unique chemical behavior and potent pharmacological efficacy of *Se*-containing compounds. Our efforts have been directed to searching the possibilities of bis-2-(trimethylsilyl)ethyl (TSE) diselenide as a selenating agent in the synthesis of *Se*-containing biomolecules including sugars,[20,21] β-lactams,[22,23] and nucleosides.[24,25] Within the scope of our ongoing program, we would like to report the application of TSE diselenide to the *Se*-containing amino acid synthesis, particularly Sec and selenoglutathione (GSeH) analogues.

2. Results and discussion

For preparation of Sec derivatives, there are two general types of synthetic approaches: (1) those employing a Sec selenolate anion that attacks alkyl halides (RX), and (2) those using a substituted selenolate anion (RSe^-) that attacks β-halo alanine, *O*-tosyl serine, or serine-β-lactone, to form RSec. (1) One of the approaches utilizes selenocystine (dimeric Sec) as a precursor to possible building of structures.[26-28] By reference to a previous report,[29] Koide *et al.*, prepared *Se*-4-methoxybenzylselenocysteine through *in situ* formation of a Sec selenolate anion by reduction of selenocystine with $NaBH_4$ and reaction with 4-methoxybenzyl chloride.[30] (2) In the other, the substituted diselenide or selenol are needed to prepare with respect to each functionality. Gieselman *et al.*, reported the synthetic procedure which involved nucleophilic displacement of the *O*-tosyl moiety of l-serine derivatives by a 4-methoxybenzyl selenolate anion generated from 4-methoxybenzyl diselenide.[31] In this study, we therefore planned to use bis-2-(trimethylsilyl)ethyl (TSE) diselenide (TSE-Se-Se-TSE, **1**) as a selenating reagent which has two latent sites of reactivity. As outlined in Scheme 1, the initial step commences with *in situ* generation of a TSE selenolate anion ($TSE\text{-}Se^-$) from bis-TSE diselenide (**1**) by hydride reduction. This anion attack to β-halo alanine, resulting in introduction of TSE selenyl moiety onto amino acid skeleton. Second step involves *Se*-alkylation with alkyl halides using high specificity and affinity between Si and F. Our approach never goes through selenocystine or even offers to prepare the suitable diselenide or selenol.

Bis-TSE diselenide (**1**) was prepared as published previously; following treatment of elemental selenium with sodium borohydride ($NaBH_4$), alkylation of the activated selenium with 2-(bromoethyl)trimethyl silane provided the desired selenating reagent (**1**) in 73% yield.[22,24] Under the Appel conditions,[32] *N*-[*tert*-butoxycarbonyl (Boc)]-3-iodo-L-alanine methyl ester (**2**) was prepared in 84 % yield starting from *N*-Boc-L-serine methyl ester (Scheme 2). It is noteworthy that treatment of **2** with bis-TSE diselenide (**1**, 1.2 equiv.) in the presence of $NaBH_4$ (1.2 equiv.) and EtOH (3.0 equiv.) in DMF for 1 h proceeded swimmingly to afford the requisite *Se*-TSE-selenocysteine (**3**) in 89% yield. The characteristic ^{77}Se NMR signal for the TSE selenyl group appeared at 177.8 ppm.[24] Subsequently, we attempted *Se*-methylation of **3** by means of iodomethane (MeI, 5.0 equiv.) and tetrabutylammonium fluoride (TBAF, 3.0 equiv.) at room temperature for 24 h, however, the reaction yielded an unexpected α-methylated compound as a major product. It was deemed that the basicity of TBAF was the cause of α-methylation. In order to suppress of the bothersome side effect of TBAF, AcOH was selected. Addition of AcOH (3.0 equiv.) brought the role switching of TBAF in the reaction progress, leading to facilitation of *Se*-methylation (**4a**, 43% yield). The ^{77}Se NMR signal arising from **3** significantly sifted to the higher magnetic side of 45.6 ppm in **4a**.

Table 1. Reactivity of **3** with various electrophiles.



Scheme 1. Synthetic strategy for the synthesis of alkylselenocysteine derivatives.

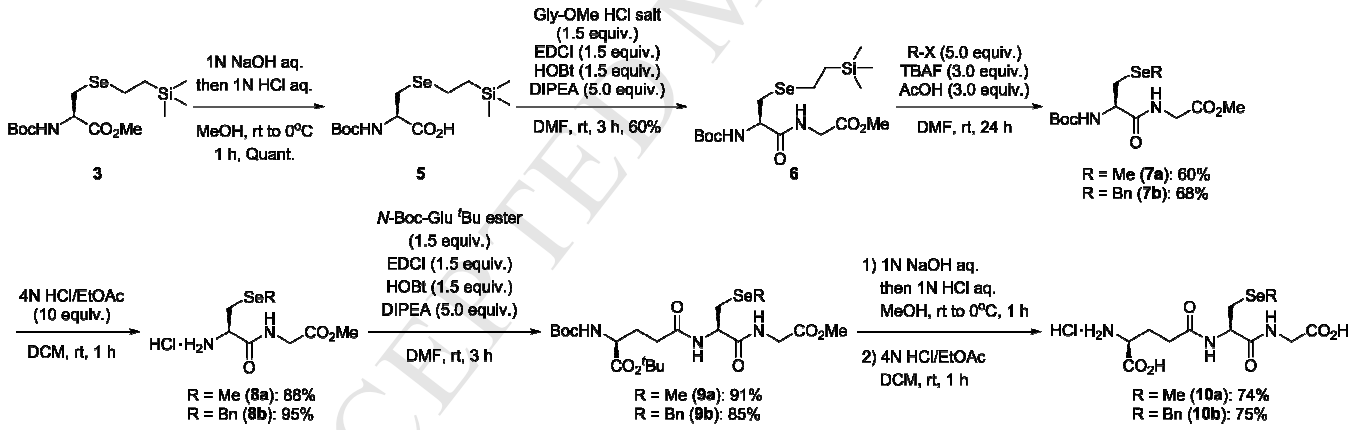
Scheme 2. Synthesis of *N*-Boc-*Se*-methyl-L-selenocysteine methyl ester (**4a**).

Having successfully established *Se*-methylation of **3**, we next probed the generality of this protocol with a variety of electrophiles. Results are summarized in Table 1. Although reactions with MeI and EtI furnished the corresponding *Se*-alkylated products (**4a**, 43% and **4b**, 48%, Entries 1 and 2), **3** did not react with *i*-PrI at all (Entry 3). Compared to these results, treatment with allyl and propargyl bromides offered easy access to

Entry	R-X (equiv.)	Yield (%)
1	MeI (5.0)	43 (**4a**)
2	EtI (10)	48 (**4b**)
3	*i*-PrI (3.0)	0 (**4c**)
4	CH_2=$CHCH_2Br$ (3.0)	81 (**4d**)
5	CH≡CCH_2Br (5.0)	78 (**4e**)
6	$MeOCH_2CH_2Br$ (3.0)	12 (**4f**)
7	N≡CCH_2Br (3.0)	34 (**4g**)
8	BnBr (2.0)	86 (**4h**)
9	*p*-MeBnBr (3.0)	73 (**4i**)
10	*o*-NO_2BnBr (3.0)	58 (**4j**)
11	*m*-NO_2BnBr (3.0)	46 (**4k**)
12	*p*-NO_2BnBr (3.0)	23 (**4l**)
13	*o*-ClBnBr (3.0)	58 (**4m**)
14	*m*-ClBnBr (3.0)	63 (**4n**)
15	*p*-ClBnBr (3.0)	59 (**4o**)
16	Me(C=O)CH_2Br (10)	68 (**4p**)
17	Ph(C=O)CH_2Br (3.0)	87 (**4q**)
18	MeO(C=O)CH_2Br (4.0)	55 (**4r**)
19	*tert*-BuO(C=O)CH_2Br (5.0)	50 (**4s**)
20	*o*-NO_2PhF (2.0)	13 (**4t**)
21	*p*-NO_2PhF (2.0)	0 (**4u**)
22	BzCl (3.0)	0 (**4v**)

the products in good yields (**4d**, 81% and **4e**, 78%, Entries 4 and 5). With 2-bromoethyl methyl ether and bromoacetonitrile, the yields were decreased considerably (Entries 6 and 7). In the category of benzyl bromides bearing various functional groups on the benzene ring, treatment with benzyl and *p*-methylbenzyl bromides gave *Se*-benzylated products (**4h** and **4i**) in 86% and 73% yields, respectively (Entries 8 and 9). With *o*-, *m*-, and *p*-nitro substituted benzyl bromides, the yields were affected by substitution patterns (Entries 10-12). In contrast, chloro-substituted positions have little influence on the reaction progress (Entries 13-15). When α-haloacyl reagents were used, the reactions worked well (Entries 16-19). To further explore the scope of our synthetic strategy, we tried nucleophilic aromatic substitution and acylation. Although the reactivity of **3** with nitrofluorobenzenes was low, the *Se*-arylated product (**4t**) was obtained in 13% yield only by the reaction with *o*-nitrofluorobenzene (Entry 20). The *Se*-benzoylation was unsuccessful (Entry 22), and the instability of the produced acyl selenide could explain it as a result of its hydrolysis.

Selenoglutathione (GSeH) appears to supply benefits by acting as a redox tripeptide substrate comprising glutamic acid (Glu), selenocysteine (Sec), and glycine (Gly).[33,34] In light of the above results, we shifted our focus to synthesize GSeH analogues from **3** as the starting point. Due to their structural complexity, their chemical synthesis is challenging. For conjugation of Gly with Sec, the methyl ester (**3**) was converted to the free carboxylate (**5**) under basic conditions (Scheme 3). Without further purification by silica gel column chromatography, Gly methyl ester was inserted into **5** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), and *N*,*N*-diisopropylethylamine (DIPEA), giving **6** in 60% yield with maintenance of the TSE selenyl moiety. Under slightly modified conditions of Table 1, *Se*-methylation and *Se*-benzylation actually succeeded in 60% for **7a** and 68% for **7b**. Acidic Boc-deprotection gave **8**, which was coupled with *N*-Boc-glutamic acid *tert*-butyl ester, to provide the tripeptide analogues (**9a**, 91% and **9b**, 85%). The final step was deprotection of the both amino and carboxylate functionalities which was effected in the same fashion, affording the targeted GSeH derivatives (**10a**, 74% and **10b**, 75% over two steps). The multistep sequence from **3** depicted in Scheme 3 allowed to construct the alkylselenyl Glu-Sec-Gly frameworks in overall yields of 21% for **10a** and 34% for **10b**.



Scheme 3. Synthesis of *Se*-alkylated selenoglutathione derivatives.

3. Conclusion

We have demonstrated a concise route for the preparation of Sec analogues by tuning the selenation step. Given the convenient methodology presented herein was easily applicable to the GSeH synthesis, it may be suited for the incorporation of alkylselenyl moieties into peptide scaffolds. Our findings highlight the great utility and versatility of a 2-(trimethylsilyl)ethylselenyl group. We believe that these results will contribute to the better understanding of physiological roles of selenopeptide-containing biomacromolecules.

4. Experimental section

4.1. General

All solvents and reagents were purchased from the suppliers and used without further purification. IR spectra were recorded on a JASCO FT/IR-460 Plus spectrophotometer. Optical rotations were measured with a JASCO P-2300. MS spectra were obtained using the Waters UPLC-MS system (Aquity UPLC XevoQTof). NMR spectra were recorded with JEOL JNM-ECS

400 and JNM-ECA 600 spectrometers with tetramethylsilane as an internal standard. ^{77}Se chemical sifts were expressed in δ values deshielded with respect to neat Me_2Se. Silica gel column chromatography (CC) was performed on silica gel N-60 (40-50 μm). Thin-layer chromatography (TLC) spots on plates pre-coated with silica gel 60 F_{254} were detected with a UV lamp (254 nm). Fractionations for all CCs were based on TLC analyses.

4.2. Synthesis of *N*-Boc-*Se*-[2-(trimethylsilyl)ethyl]-L-selenocysteine methyl ester (**3**)

After stirring of bis-2-(trimethylsilyl)ethyl diselenide (**1**, 3.6 mmol), $NaBH_4$ (3.6 mmol), and EtOH (9.0 mmol) in DMF (2 ml) at 0°C for 30 min, *N*-Boc-3-iodo-L-alanine methyl ester (**2**, 3.0 mmol) was added to the solution, and the reaction was continued at rt for an additional 1 h. The resultant solution was poured into distilled water, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hexane/EtOAc (15/1) to afford **3** (89% yield) as a yellow oil; $[\alpha]_D^{20}$ +18.8° (*c* 1.02, $CHCl_3$); IR (film): ν_{max} 3379, 2953, 1717 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.00 (s, 9H, TMS), 0.92 (dd, 2H, *J* = 7.1 and 11.2 Hz, $SeCH_2CH_2TMS$), 1.44 (s, 9H, ^{t}Bu), 2.62 (dt, 2H, *J* = 3.2 and 13.8 Hz, $SeCH_2CH_2TMS$), 2.99 (t, 2H, *J* = 4.6 Hz, H-β), 3.75 (s, 3H, CO_2Me), 4.63 (dt, 1H, *J* = 4.1 and 7.8 Hz, H-α), 5.37 (d, 1H, *J* = 7.8 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ -1.8, 18.7, 20.3, 26.0, 28.4, 52.6, 53.6, 80.2, 155.2, 171.8; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 177.8; HRESITOFMS: *m/z* 406.0906 $[M+Na]^+$ (calcd for $C_{14}H_{29}NO_4SeNa$, 406.0929).

4.3. Methylation of **3**

TBAF (1.0 M in THF, 0.9 mmol), AcOH (0.9 mmol), and MeI (1.5 mmol) were added to a solution of **3** (0.3 mmol) in DMF (0.5 ml). After stirring at rt for 24 h, the resultant solution was poured into distilled water, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hexane/EtOAc (10/1) to afford *N*-Boc-*Se*-methyl-L-selenocysteine methyl ester **4a** (43% yield) as a colorless oil; $[\alpha]_D^{20}$ +13.7° (*c* 1.40, $CHCl_3$); IR (film): ν_{max} 3376, 2978, 1747, 1715 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, ^{t}Bu), 2.00 (s, 3H, SeMe), 2.95 (d, 2H, *J* = 5.0 Hz, H-β), 3.74 (s, 3H, CO_2Me), 4.58 (d, 1H, *J* = 7.3 Hz, H-α), 5.35 (d, 1H, *J* = 4.0 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 5.31, 27.7, 28.4, 52.6, 53.4, 80.2, 155.2, 171.8; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 45.6; HRESITOFMS: *m/z* 320.0351 $[M+Na]^+$ (calcd for $C_{10}H_{19}NO_4SeNa$, 320.0377).

4.4. Various alkylation of **3**

Reaction parameters are given in Table 1. The operation procedures are exactly the same with the section of Methylation of **3**.

*4.4.1. N-Boc-Se-ethyl-L-selenocysteine methyl ester (**4b**)*

Colorless oil in 48% yield; $[\alpha]_D^{20}$ +10.6° (*c* 1.60, $CHCl_3$); IR (film): ν_{max} 3365, 2977, 1715, 1166 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (t, 3H, *J* = 7.3 Hz, SeEt), 1.44 (s, 9H, ^{t}Bu), 2.58 (dd, 2H, *J* = 7.8 and 15.6 Hz, SeEt), 2.99 (d, 2H, *J* = 5.0 Hz, H-β), 3.75 (s, 3H, CO_2Me), 4.60 (d, 1H, *J* = 8.2 Hz, H-α), 5.36 (d, 1H, *J* = 7.3 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.8, 18.4, 25.5, 28.4, 52.6, 53.5, 80.2, 155.2, 171.8; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 157.2; HRESITOFMS: *m/z* 334.0533 $[M+Na]^+$ (calcd for $C_{11}H_{21}NO_4SeNa$, 334.0555).

*4.4.2. N-Boc-Se-allyl-L-selenocysteine methyl ester (**4d**)*

Colorless oil in 81% yield; $[\alpha]_D^{20}$ +6.7° (*c* 1.70, $CHCl_3$); IR (film): ν_{max} 3377, 3082, 2978, 1714, 1632, 1165 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, ^{t}Bu), 2.92 (d, 2H, *J* = 5.0 Hz, H-β), 3.19 (dd, 2H, *J* = 3.6 and 7.8 Hz, $SeCH_2CH{=}CH_2$), 3.76 (s, 3H, CO_2Me), 4.60 (d, 1H, *J* = 7.8 Hz, H-α), 5.04 (d, 2H, *J* = 10.1 Hz, $SeCH_2CH{=}CH_2$), 5.37 (d, 1H, *J* = 7.3 Hz, NH), 5.85 (dt, 1H, *J* = 10.1 and 27.0 Hz, $SeCH_2CH{=}CH_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2, 26.8, 28.4, 52.6, 53.4, 80.1, 117.1, 134.4, 155.1, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 160.2; HRESITOFMS: *m/z* 346.0524 $[M+Na]^+$ (calcd for $C_{12}H_{21}NO_4SeNa$, 346.0533).

*4.4.3. N-Boc-Se-propargyl-L-selenocysteine methyl ester (**4e**)*

Yellow oil in 78% yield; $[\alpha]_D^{20}$ +15.2° (*c* 1.32, $CHCl_3$); IR (film): ν_{max} 3377, 3292, 2978, 2114, 1744 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.40 (s, 9H, ^{t}Bu), 2.26 (s, 1H, $SeCH_2C{\equiv}CH$), 3.12-3.18 (m, 4H, H-β and $SeCH_2C{\equiv}CH$), 3.72 (s, 3H, CO_2Me), 4.62 (d, 1H, *J* = 6.9 Hz, H-α), 5.37 (d, 1H, *J* = 6.9 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 8.0, 26.7, 28.4, 52.6, 53.4, 72.1, 80.2, 80.3, 155.1, 171.6; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 219.7; HRESITOFMS: *m/z* 344.0355 $[M+Na]^+$ (calcd for $C_{12}H_{19}NO_4SeNa$, 344.0377).

*4.4.4. N-Boc-Se-methoxyethyl-L-selenocysteine methyl ester (**4f**)*

Colorless oil in 12% yield; $[\alpha]_D^{20}$ +8.91° (*c* 1.10, $CHCl_3$); IR (film): ν_{max} 3376, 2978, 1746, 1502, 1166 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, ^{t}Bu), 2.75 (dt, 2H, *J* = 2.8 and 6.4 Hz, $SeCH_2CH_2OMe$), 3.03 (t, 2H, *J* = 4.6 Hz, H-β), 3.37 (s, 3H, $SeCH_2CH_2OMe$), 3.62 (dt, 2H, *J* = 3.2 and 7.3 Hz, $SeCH_2CH_2OMe$), 3.75 (s, 3H, CO_2Me), 4.60 (d, 1H, *J* = 7.8 Hz, H-α), 5.58 (d, 1H, *J* = 7.3 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.4, 26.6, 28.5, 52.7, 53.8, 58.8, 72.8, 80.2, 155.4, 171.8; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 115.1; HRESITOFMS: *m/z* 364.0659 $[M+Na]^+$ (calcd for $C_{12}H_{23}NO_5SeNa$, 364.0639).

***4.4.5.** N-Boc-Se-cyanomethyl-L-selenocysteine methyl ester (**4g**)*

Colorless oil in 34% yield; $[\alpha]_D^{20}$ +10.8° (*c* 1.20, $CHCl_3$); IR (film): ν_{max} 3367, 2980, 2241, 1743, 1511 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, ^{t}Bu), 3.20-3.27 (m, 4H, H-β and $SeCH_2CN$), 3.79 (s, 3H, CO_2Me), 4.67 (d, 1H, *J* = 4.6 Hz, H-α), 5.37 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 3.2, 28.4, 53.0, 53.4, 80.7, 117.2, 155.2, 171.2; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 224.1; HRESITOFMS: *m/z* 345.0357 $[M+Na]^+$ (calcd for $C_{11}H_{18}N_2O_4SeNa$, 345.0329).

*4.4.6. N-Boc-Se-benzyl-L-selenocysteine methyl ester (**4h**)*

Yellow oil in 86% yield; $[\alpha]_D^{20}$ -2.0° (*c* 1.30, $CHCl_3$); IR (film): ν_{max} 3388, 2978, 1714, 1601, 1496 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, ^{t}Bu), 2.91 (s, 2H, H-β), 3.74 (s, 3H, CO_2Me), 3.80 (s, 2H, SeBn), 4.61 (d, 1H, *J* = 7.3 Hz, H-α), 5.29 (d, 1H, *J* = 6.9 Hz, NH), 7.21-7.29 (m, 5H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.0, 28.0, 28.4, 52.7, 53.4, 80.3, 127.1, 128.7, 129.0, 138.9, 155.2, 171.8; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 219.1; HRESITOFMS: *m/z* 396.0676 $[M+Na]^+$ (calcd for $C_{16}H_{23}NO_4SeNa$, 396.0690).

*4.4.7. N-Boc-Se-(p-methylbenzyl)-L-selenocysteine methyl ester (**4i**)*

White amorphous powder in 73% yield; $[\alpha]_D^{20}$ -3.51° (*c* 1.10, $CHCl_3$); IR (film): ν_{max} 3377, 2977, 1746, 1614, 1513 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, ^{t}Bu), 2.30 (s, 3H, $SeCH_2Ph\mathit{Me}$), 2.91 (s, 2H, H-β), 3.73 (s, 5H, $SeCH_2PhMe$ and CO_2Me), 4.60 (d, 1H, *J* = 4.1 Hz, H-α), 5.31 (d, 1H, *J* = 3.9 Hz,

NH), 7.07-7.17 (m, 4H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.2, 25.8, 27.7, 28.4, 52.6, 53.4, 80.1, 128.8, 129.3, 135.6, 136.6, 155.1, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 218.3; HRESITOFMS: *m/z* 410.0858 $[M+Na]^+$ (calcd for $C_{17}H_{25}NO_4SeNa$, 410.0846).

*4.4.8. N-Boc-Se-(o-nitrobenzyl)-L-selenocysteine methyl ester (**4j**)*

Yellow oil in 58% yield; $[\alpha]_D^{20}$ -25.8° (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3376, 2978, 1744, 1608, 1525, 1365 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, ^{t}Bu), 2.92-2.96 (m, 2H, H-β), 3.71 (s, 3H, CO_2Me), 4.19 (dd, 2H, *J* = 6.2 and 11.9 Hz, Se*CH*$_2$PhNO$_2$), 4.58 (d, 1H, *J* = 3.7 Hz, H-α), 5.36 (d, 1H, *J* = 3.7 Hz, NH), 7.37 (t, 2H, *J* = 3.9 Hz, Ar), 7.51 (dt, 1H, *J* = 0.7 and 3.9 Hz, Ar), 8.08 (d, 1H, *J* = 4.1 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.0, 26.6, 28.3, 52.7, 53.6, 80.3, 125.8, 128.2, 132.0, 133.4, 135.4, 147.8, 155.1, 171.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 236.2; HRESITOFMS: *m/z* 441.0563 $[M+Na]^+$ (calcd for $C_{16}H_{22}N_2O_6SeNa$, 441.0541).

*4.4.9. N-Boc-Se-(m-nitrobenzyl)-L-selenocysteine methyl ester (**4k**)*

Yellow oil in 46% yield; $[\alpha]_D^{20}$ -0.6° (*c* 1.20, $CHCl_3$); IR (film): ν_{max} 3390, 2978, 1745, 1581, 1529, 1365 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, ^{t}Bu), 2.88-2.92 (m, 2H, H-β), 3.73 (s, 3H, CO_2Me), 3.84 (s, 2H, Se*CH*$_2$PhNO$_2$), 4.59 (d, 1H, *J* = 3.7 Hz, H-α), 5.31 (d, 1H, *J* = 3.1 Hz, NH), 7.44 (t, 1H, *J* = 4.1 Hz, Ar), 7.61 (d, 1H, *J* = 3.9 Hz, Ar), 8.06 (dd, 1H, *J* = 0.5 and 4.1 Hz, Ar), 8.13 (s, 1H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.4, 26.8, 28.4, 52.8, 53.5, 80.4, 122.1, 123.8, 129.6, 135.1, 141.2, 148.4, 155.2, 171.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 233.6; HRESITOFMS: *m/z* 441.0569 $[M+Na]^+$ (calcd for $C_{16}H_{22}N_2O_6SeNa$, 441.0541).

*4.4.10. N-Boc-Se-(p-nitrobenzyl)-L-selenocysteine methyl ester (**4l**)*

Yellow oil in 23% yield; $[\alpha]_D^{20}$ -4.21° (*c* 0.96, $CHCl_3$); IR (film): ν_{max} 3384, 2978, 1744, 1598, 1520, 1366 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, ^{t}Bu), 2.90-2.95 (m, 2H, H-β), 3.75 (s, 3H, CO_2Me), 3.85 (s, 2H, Se*CH*$_2$PhNO$_2$), 4.63 (d, 1H, *J* = 3.7 Hz, H-α), 5.31 (d, 1H, *J* = 3.2 Hz, NH), 7.45 (d, 2H, *J* = 4.4 Hz, Ar), 8.16 (d, 2H, *J* = 4.3 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.4, 26.8, 28.4, 52.8, 53.6, 80.5, 124.0, 129.8, 147.0, 147.0, 155.2, 171.5; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 237.5; HRESITOFMS: *m/z* 441.0560 $[M+Na]^+$ (calcd for $C_{16}H_{22}N_2O_6SeNa$, 441.0541).

*4.4.11. N-Boc-Se-(o-chlorobenzyl)-L-selenocysteine methyl ester (**4m**)*

Colorless oil in 58% yield; $[\alpha]_D^{20}$ -5.10° (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3370, 2978, 1745, 1500, 757 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, ^{t}Bu), 2.98 (dd, 2H, *J* = 3.0 and 4.4 Hz, H-β), 3.73 (s, 3H, CO_2Me), 3.88 (s, 2H, Se*CH*$_2$PhCl), 4.63 (d, 1H, *J* = 3.4 Hz, H-α), 5.33 (d, 1H, *J* = 3.7 Hz, NH), 7.16 (t, 1H, *J* = 1.6 Hz, Ar), 7.18 (t, 1H, *J* = 2.1 Hz, Ar), 7.27 (dd, 1H, *J* = 2.3 and 5.7 Hz, Ar), 7.34 (dd, 1H, *J* = 1.3 and 4.7 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.6, 26.5, 28.4, 52.7, 53.6, 80.3, 127.0, 128.5, 130.1, 130.7, 133.9, 136.9, 155.2, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 208.5; HRESITOFMS: *m/z* 430.0311 $[M+Na]^+$ (calcd for $C_{16}H_{22}ClNO_4SeNa$, 430.0300).

*4.4.12. N-Boc-Se-(m-chlorobenzyl)-L-selenocysteine methyl ester (**4n**)*

Colorless oil in 63% yield; $[\alpha]_D^{20}$ -3.70° (*c* 0.90, $CHCl_3$); IR (film): ν_{max} 3377, 2978, 1745, 1502, 784 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, ^{t}Bu), 2.91 (dd, 2H, *J* = 2.8 and 7.6 Hz, H-β), 3.75 (s, 3H, CO_2Me), 3.88 (s, 2H, Se*CH*$_2$PhCl), 4.62 (d, 1H, *J* = 3.7 Hz, H-α), 5.32 (d, 1H, *J* = 3.7 Hz, NH), 7.18 (m, 3H, Ar), 7.29 (s, 1H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.2, 27.2, 28.4, 52.7, 53.5, 80.4, 127.2, 127.3, 129.1, 129.9, 134.4, 141.0, 155.2, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 225.1; HRESITOFMS: *m/z* 430.0311 $[M+Na]^+$ (calcd for $C_{16}H_{22}ClNO_4SeNa$, 430.0300).

*4.4.13. N-Boc-Se-(p-chlorobenzyl)-L-selenocysteine methyl ester (**4o**)*

Colorless oil in 59% yield; $[\alpha]_D^{20}$ -4.20° (*c* 1.20, $CHCl_3$); IR (film): ν_{max} 3371, 2978, 1745, 1491, 776 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.46 (s, 9H, ^{t}Bu), 2.89 (dd, 2H, *J* = 5.5 and 9.2 Hz, H-β), 3.74 (s, 3H, CO_2Me), 3.75 (s, 2H, Se*CH*$_2$PhCl), 4.61 (d, 1H, *J* = 7.3 Hz, H-α), 5.31 (d, 1H, *J* = 10.0 Hz, NH), 7.20-7.26 (m, 4H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.1, 27.1, 28.4, 52.7, 53.5, 80.3, 128.8, 130.3, 132.8, 137.4, 155.2, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 225.1; HRESITOFMS: *m/z* 430.0305 $[M+Na]^+$ (calcd for $C_{16}H_{22}ClNO_4SeNa$, 430.0300).

*4.4.14. N-Boc-Se-(2-oxopropyl)-L-selenocysteine methyl ester (**4p**)*

Yellow oil in 68% yield; $[\alpha]_D^{20}$ +16.0° (*c* 1.20, $CHCl_3$); IR (film): ν_{max} 3365, 2978, 1745, 1701, 1513 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.45 (s, 9H, ^{t}Bu), 2.32 (s, 3H, SeCH$_2$(C=O)*Me*), 2.98-3.02 (m, 2H, H-β), 3.28 (s, 2H, Se*CH*$_2$(C=O)Me), 3.77 (s, 3H, CO_2Me), 4.59 (d, 1H, *J* = 5.5 Hz, H-α), 5.40 (d, 1H, *J* = 7.3 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.8, 27.7, 28.3, 32.4, 52.7, 53.4, 80.3, 155.2, 171.5, 203.6; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 163.3; HRESITOFMS: *m/z* 362.0455 $[M+Na]^+$ (calcd for $C_{12}H_{21}NO_5SeNa$, 362.0483).

*4.4.15. N-Boc-Se-(2-oxo-2-phenylethyl)-L-selenocysteine methyl ester (**4q**)*

Yellow oil in 87% yield; $[\alpha]_D^{20}$ +22.1° (*c* 1.00, $CHCl_3$); IR (film): ν_{max} 3365, 2978, 1744, 1668, 1597, 1511 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, ^{t}Bu), 3.04-3.09 (m, 2H, H-β), 3.73 (s, 3H, CO_2Me), 3.86 (s, 2H, Se*CH*$_2$(C=O)Ph), 4.63 (d, 1H, *J* = 5.5 Hz, H-α), 5.46 (d, 1H, *J* = 7.3 Hz, NH), 7.46 (t, 2H, *J* = 7.8 Hz, Ar), 7.57 (t, 1H, *J* = 7.8 Hz, Ar), 7.94 (d, 2H, *J* = 7.3 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.2, 27.5, 28.4, 52.7, 53.6, 80.2, 128.8, 133.5, 135.1, 155.2, 171.6, 195.0; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 179.1; HRESITOFMS: *m/z* 424.0639 $[M+Na]^+$ (calcd for $C_{17}H_{23}NO_5SeNa$, 424.0639).

*4.4.16. N-Boc-Se-(2-methyl-2-oxoethyl)-L-selenocysteine methyl ester (**4r**)*

Colorless oil in 55% yield; $[\alpha]_D^{20}$ +7.60° (*c* 1.10, $CHCl_3$); IR (film): ν_{max} 3375, 2978, 1738, 1715, 1513 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 9H, ^{t}Bu), 3.05-3.10 (m, 2H, H-β), 3.18 (s, 2H, Se*CH*$_2$(C=O)OMe), 3.69 (s, 3H, SeCH$_2$(C=O)O*Me*),3.73 (s, 3H, CO_2Me), 4.61 (d, 1H, *J* = 6.9 Hz, H-α), 5.40 (d, 1H, *J* = 7.3 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.9, 27.4, 28.4, 52.6, 52.7, 53.5, 80.3, 155.2, 171.5, 171.7; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 184.5; HRESITOFMS: *m/z* 378.0439 $[M+Na]^+$ (calcd for $C_{12}H_{21}NO_6SeNa$, 378.0432).

*4.4.17. N-Boc-Se-(2-tert-butoxy-2-oxoethyl)-L-selenocysteine methyl ester (**4s**)*

Colorless oil in 50% yield; $[\alpha]_D^{20}$ +7.90° (*c* 1.20, $CHCl_3$); IR (film): ν_{max} 3367, 2979, 1717, 1512 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (s, 9H, ^{t}Bu), 1.45 (s, 9H, SeCH$_2$(C=O)O^t*Bu*), 3.10 (s, 2H, Se*CH*$_2$(C=O)O^{t}Bu), 3.09-3.13 (m, 2H, H-β), 3.75 (s, 3H, CO_2Me), 4.62 (d, 1H, *J* = 6.9 Hz, H-α), 5.45 (d, 1H, *J* = 7.8 Hz, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.8, 27.0, 28.0, 28.4, 52.7, 53.6, 81.8, 155.3, 170.5, 171.6; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 180.2; HRESITOFMS: *m/z* 420.0921 $[M+Na]^+$ (calcd for $C_{15}H_{27}NO_6SeNa$, 420.0901).

*4.4.18. N-Boc-Se-(o-nitrophenyl)-L-selenocysteine methyl ester (**4t**)*

Yellow oil in 13% yield; $[\alpha]_D^{20}$ +22.8° (*c* 0.77, $CHCl_3$); IR (film): ν_{max} 3383, 2979, 1712, 1567, 1514, 1333 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.42 (s, 9H, ^{t}Bu), 3.31-3.37 (m, 2H, H-β), 3.73 (s, 3H, CO_2Me), 4.70 (d, 1H, *J* = 6.9 Hz, H-α), 5.32 (d, 1H, *J* = 7.4 Hz, NH), 7.33 (t, 1H, *J* = 8.7 Hz, Ar), 7.51 (dt, 1H, *J* = 1.4 and 9.6 Hz, Ar), 7.62 (d, 1H, *J* = 7.8 Hz, Ar), 8.25 (dd, 1H, *J* = 1.4 and 8.7 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.7, 28.3, 52.9, 53.4, 80.2, 125.9, 126.6, 129.5, 132.0, 133.8, 143.0, 155.2, 171.2; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 324.3; HRESITOFMS: *m/z* 427.0363 $[M+Na]^+$ (calcd for $C_{15}H_{20}N_2O_6SeNa$, 427.0384).

4.5. Demethylation of **3**

1N NaOH aq. (3.9 mmol) was added to a solution of **3** (1.3 mmol) in MeOH (3 ml). After stirring at rt for 1 h, the resultant solution was diluted with iced $CHCl_3$, adjusted to pH 1 using 1N HCl, and partitioned with $CHCl_3$ twice. The organic layer was concentrated *in vacuo* to afford *N*-Boc-*Se*-[2-(trimethylsilyl)ethyl]-L-selenocysteine **5** (quantitative yield) as a yellow oil; $[\alpha]_D^{20}$ +12.3° (*c* 0.15, $CHCl_3$); IR (film): ν_{max} 3434, 2953, 1715 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 0.00 (s, 9H, TMS), 0.92 (dd, 2H, *J* = 7.8 and 11.4 Hz, $SeCH_2CH_2TMS$), 1.44 (s, 9H, ^{t}Bu), 2.65 (dt, 2H, *J* = 2.6 and 16.0 Hz, $SeCH_2CH_2TMS$), 2.96 (m, 2H, H-β), 4.64 (d, 1H, *J* = 6.9 Hz, H-α), 5.38 (d, 1H, *J* = 7.8 Hz, NH), 10.28 (br s, 1H, CO_2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ -1.7, 18.7, 20.5, 28.3, 28.4, 53.5, 80.6, 155.5, 171.6; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 178.6; HRESITOFMS: *m/z* 392.0777 $[M+Na]^+$ (calcd for $C_{13}H_{27}NO_4SiSeNa$, 392.0772).

4.6. Conjugation of Gly with **5**

Gly methyl ester HCl salt (2.0 mmol), EDCI (2.0 mmol), and HOBt (2.0 mmol) were added to a solution of **5** (1.4 mmol) in DMF (5 ml). After stirring at rt for 1 min, DIPEA (6.5 mmol) was added and the reaction mixture was stirred for 3 h. The resultant solution was poured into distilled water, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hexane/EtOAc (5/1) to afford *N*-Boc-*Se*-[2-(trimethylsilyl)ethyl]-L-selenocysteinyl-glycine methyl ester **6** (60% yield) as a white amorphous powder; $[\alpha]_D^{20}$ -4.29° (*c* 0.34, $CHCl_3$); IR (film): ν_{max} 3325, 2954, 1722 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 0.00 (s, 9H, TMS), 0.94 (dt, 2H, *J* = 3.7 and 11.9 Hz, $SeCH_2CH_2TMS$), 1.45 (s, 9H, ^{t}Bu), 2.60-2.66 (m, 2H, $SeCH_2CH_2TMS$), 2.93-2.97 (m, 2H, H-β of Sec), 3.75 (s, 3H, CO_2Me), 4.00-4.05 (m, 2H, CH_2 of Gly), 4.39 (d, 1H, *J* = 4.6 Hz, H-α of Sec), 5.37 (s, 1H, NH), 6.85 (s, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$): δ -1.7, 18.7, 20.4, 25.7, 28.4, 41.4, 52.6, 54.2, 80.6, 155.5, 170.1, 171.2; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 177.5; HRESITOFMS: *m/z* 463.1166 $[M+Na]^+$ (calcd for $C_{16}H_{32}N_2O_5SiSeNa$, 463.1143).

4.7. *Se*-Alkylation of **6**

TBAF (1.0 M in THF, 0.18 mmol), AcOH (0.18 mmol), and MeI (0.3 mmol) or BnBr (0.18 mmol) were added to a solution of **6** (0.06 mmol) in DMF (3 ml). After stirring at rt for 24 h, the resultant solution was poured into distilled water, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified using silica gel column chromatography eluted with *n*-hexane/EtOAc (2/1) to afford **7**.

*4.7.1. N-Boc-Se-methyl-L-selenocysteinyl-glycine methyl ester (**7a**)*

Yellow oil in 60% yield; $[\alpha]_D^{20}$ -9.50° (*c* 1.23, $CHCl_3$); IR (film): ν_{max} 3320, 2975, 1747 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.46 (s, 9H, ^{t}Bu), 2.05 (s, 3H, SeMe), 2.91-2.96 (m, 2H, H-β of Sec), 3.77 (s, 3H, CO_2Me), 4.04-4.08 (m, 2H, CH_2 of Gly), 4.41 (s, 1H, H-α of Sec), 5.48 (s, 1H, NH), 7.00 (s, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 5.3, 27.4, 28.4, 41.4, 52.5, 53.9, 80.6, 155.5, 170.0, 171.1; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 46.5; HRESITOFMS: *m/z* 377.0619 $[M+Na]^+$ (calcd for $C_{12}H_{22}N_2O_5SeNa$, 377.0592).

*4.7.2. N-Boc-Se-benzyl-L-selenocysteinyl-glycine methyl ester (**7b**)*

White amorphous powder in 68% yield; $[\alpha]_D^{20}$ +10.2° (*c* 0.19, $CHCl_3$); IR (film): ν_{max} 3323, 2957, 1718, 1495 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.46 (s, 9H, ^{t}Bu), 2.85-2.90 (m, 2H, H-β of Sec), 3.75 (s, 3H, CO_2Me), 3.81 (s, 2H, SeBn), 4.01-4.05 (m, 2H, CH_2 of Gly), 4.06 (s, 1H, H-α of Sec), 5.27 (d, 1H, *J* = 6.4 Hz, NH), 6.81 (s, 1H, NH), 7.22-7.30 (m, 5H, Ar); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.7, 28.0, 28.4, 41.4, 52.6, 54.0, 80.6, 127.1, 128.7, 129.1, 139.0, 155.5, 170.0, 171.1; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 221.8; HRESITOFMS: *m/z* 453.0910 $[M+Na]^+$ (calcd for $C_{18}H_{26}N_2O_5SeNa$, 453.0905).

4.8. Boc-Deprotection of **7**

4N HCl/EtOAc (3.0 mmol) was added to a solution of **7** (0.3 mmol) in DCM (3 ml) at 0°C. After stirring at rt for 1 h, the resultant solution was diluted with Et_2O (30 ml) and further decanted to afford **8**.

*4.8.1. N-Boc-Se-methyl-L-selenocysteinyl-glycine (**8a**)*

White amorphous powder in 88% yield; $[\alpha]_D^{20}$ +13.9° (*c* 0.68, MeOH); IR (film): ν_{max} 3434, 1635 cm^{-1}; ^1H NMR (600 MHz, D_2O): δ 2.02 (s, 3H, SeMe) , 2.98-3.03 (m, 2H, H-β of Sec), 3.71 (s, 3H, CO_2Me), 4.05 (s, 2H, CH_2 of Gly), 4.23 (t, 1H, *J* = 6.9 Hz, H-α of Sec); ^{13}C NMR (150 MHz, D_2O): δ 4.6, 24.2, 41.2, 52.4, 52.9, 169.3, 171.6; ^{77}Se NMR (113 MHz, D_2O): δ 42.1; HRESITOFMS: *m/z* 277.0079 $[M+Na]^+$ (calcd for $C_7H_{14}N_2O_3SeNa$, 277.0067).

*4.8.2. N-Boc-Se-benzyl-L-selenocysteinyl-glycine (**8b**)*

White amorphous powder in 95% yield; $[\alpha]_D^{20}$ +15.1° (*c* 0.79, MeOH); IR (film): ν_{max} 3435, 1633, 1495, 1454 cm^{-1}; ^1H NMR (600 MHz, D_2O): δ 2.93-2.97 (m, 2H, H-β of Sec), 3.60 (s, 3H, CO_2Me), 3.87 (d, 2H, *J* = 3.4 Hz, SeBn), 4.00 (d, 2H, *J* = 2.1 Hz, CH_2 of Gly), 4.09 (t, 1H, *J* = 6.2 Hz, H-α of Sec), 7.28 (dt, 1H, *J* = 4.1 and 13.2 Hz, Ar), 7.30-7.37 (m, 4H, Ar); ^{13}C NMR (150 MHz, D_2O): δ 22.5, 27.6, 41.2, 52.7, 52.9, 127.4, 128.9, 129.0, 138.9, 169.0, 171.5; ^{77}Se NMR (113 MHz, D_2O): δ 214.8; HRESITOFMS: *m/z* 353.0392 $[M+Na]^+$ (calcd for $C_{13}H_{18}N_2O_3SeNa$, 353.0380).

4.9. Conjugation of Glu with **8**

The operation procedures are exactly the same with the section of Conjugation of Gly with **5**.

*4.9.1. N-(Se-methyl-L-selenocysteinyl)-glycine methyl ester hydrochloride (**9a**)*

White amorphous powder in 91% yield; $[\alpha]_D^{20}$ -21.2° (*c* 0.67, $CHCl_3$); IR (film): ν_{max} 3433, 1644 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.44 (s, 9H, ^{t}Bu), 1.47 (s, 9H, ^{t}Bu), 2.00-2.05 (m, 2H, CH_2 of Glu), 2.04 (s, 3H, SeMe), 2.33-2.39 (m, 2H, CH_2 of Glu), 2.94 (d, 2H, *J* = 6.9 Hz, H-β of Sec), 3.75 (s, 3H, CO_2Me), 4.05 (t, 2H, *J* =5.5 Hz, CH_2 of Gly), 4.23 (d, 1H, *J* = 4.8 Hz, H-α of Sec), 4.74 (d, 1H, *J* = 6.9 Hz, CH of Glu), 5.42 (d, 1H, *J* = 8.3 Hz, NH), 7.14 (d, 1H, *J* = 7.6 Hz, NH), 7.41 (s, 1H, NH); ^{13}C

NMR (150 MHz, $CDCl_3$): δ 5.1, 26.7, 28.0, 28.3, 28.9, 32.4, 41.3, 52.4, 53.4, 53.5, 79.9, 82.2, 155.8, 170.0, 171.0, 171.5, 172.4; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 54.5; HRESITOFMS: *m/z* 562.1672 $[M+Na]^+$ (calcd for $C_{21}H_{37}N_3O_8SeNa$, 562.1644).

*4.9.2. N-(Se-benzyl-L-selenocysteinyl)-glycine methyl ester hydrochloride (**9b**)*

White amorphous powder in 85% yield; $[\alpha]_D^{20}$ -17.3° (*c* 0.81, $CHCl_3$); IR (film): ν_{max} 3434, 1644, 1453 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.43 (s, 9H, ^{t}Bu), 1.45 (s, 9H, ^{t}Bu), 1.99-2.04 (m, 2H, CH_2 of Glu), 2.27 (t, 2H, *J* = 7.6 Hz, CH_2 of Glu), 2.87-2.90 (m, 2H, H-β of Sec), 3.72 (s, 3H, CO_2Me), 3.80 (s, 2H, SeBn), 3.99-4.03 (m, 2H, CH_2 of Gly), 4.19 (d, 1H, *J* = 4.8 Hz, H-α of Sec), 4.69 (d, 1H, *J* = 6.9 Hz, CH of Glu), 5.41 (d, 1H, *J* = 8.3 Hz, NH), 6.83 (d, 1H, *J* = 7.6 Hz, NH), 7.21 (t, 1H, *J* = 6.9 Hz, Ar), 7.26-7.31 (m, 4H, Ar), 7.30 (s, 1H, NH); ^{13}C NMR (150 MHz, $CDCl_3$): δ 25.2, 27.9, 28.3, 28.3, 32.3, 41.2, 52.3, 52.6, 53.5, 79.8, 82.1, 126.9, 128.6, 128.9, 139.1, 155.8, 170.0, 171.0, 171.5, 172.4; ^{77}Se NMR (113 MHz, $CDCl_3$): δ 228.9; HRESITOFMS: *m/z* 638.1927 $[M+Na]^+$ (calcd for $C_{27}H_{41}N_3O_8SeNa$, 638.1957).

4.10. Full deprotection of **9**

The operation procedures are exactly the same with the sections of Demethylation of **3** and Boc-Deprotection of **7**.

*4.10.1. Se-Methyl-selenoglutathione (**10a**)*

Yellow amorphous powder in 74% yield; $[\alpha]_D^{20}$ -17.2° (*c* 0.64, MeOH); IR (film): ν_{max} 3434, 1634 cm^{-1}; ^{1}H NMR (600 MHz, D_2O): δ 1.95 (s, 3H, SeMe), 2.13-2.18 (m, 2H, CH_2 of Glu), 2.51 (t, 2H, *J* = 6.8 Hz, CH_2 of Glu), 2.82-2.86 (m, 2H, H-β of Sec), 3.94-4.03 (m, 3H, CH_2 of Gly and CH of Glu), 4.50 (dd, 1H, *J* = 4.8 and 8.9 Hz, H-α of Sec); ^{13}C NMR (150 MHz, D_2O): δ 4.3, 25.3, 25.5, 30.9, 41.2, 52.8, 53.4, 171.8, 173.3, 174.3; ^{77}Se NMR (113 MHz, D_2O): δ 53.2; HRESITOFMS: *m/z* 392.0355 $[M+Na]^+$ (calcd for $C_{11}H_{19}N_3O_6SeNa$, 392.0337).

*4.10.2. Se-Benzyl-selenoglutathione (**10b**)*

Orange amorphous powder in 75% yield; $[\alpha]_D^{20}$ -18.7° (*c* 0.71, MeOH); IR (film): ν_{max} 3435, 1634 cm^{-1}; ^{1}H NMR (600 MHz, D_2O): δ 2.09-2.14 (m, 2H, CH_2 of Glu), 2.41 (t, 2H, *J* = 6.2 Hz, CH_2 of Glu), 2.76-2.81 (m, 2H, H-β of Sec), 3.64 (s, 2H, SeBn), 3.74-3.78 (m, 1H, CH of Glu), 3.87-3.90 (m, 2H, CH_2 of Gly), 4.30-4.34 (m, 1H, H-α of Sec), 7.20-7.27 (m, 5H, Ar); ^{13}C NMR (150 MHz, D_2O): δ 24.0, 25.5, 27.3, 30.9, 41.2, 52.8, 53.4, 127.1, 128.9, 139.4, 170.1, 171.7, 173.0, 174.0; ^{77}Se NMR (113 MHz, D_2O): δ 228.4; HRESITOFMS: *m/z* 468.0671 $[M+Na]^+$ (calcd for $C_{17}H_{23}N_3O_6SeNa$, 468.0650).

Acknowledgments

This work was supported by a Grant-in-Aid for Science Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 15K07856) to which we are grateful.

References and notes

1. Berzelius, J. J. *Afhandl. Fys. Kemi Mineral.*, **1818**, *6*, 42.
2. Schwarz, K.; Bieri, J. G.; Briggs, G. M.; Scott, M. L. *Proc. Soc. Exp. Biol. Med.*, **1957**, *95*, 621–625.
3. Rotruck, J. T.; Pope, A. L.; Ganther, H. E.; Swanson, A. B.; Hafeman, D. G.; Hoekstra, W. G. *Science*, **1973**, *179*, 588–590.
4. Ursini, F.; Maiorino, M.; Gregolin, C. *Biochim. Biophys. Acta*, **1985**, *839*, 62–70.
5. Takahashi, K.; Cohen, H. J. *Blood*, **1986**, *68*, 640–645.
6. Chu, F. F.; Doroshow, J. H.; Esworthy, R. S. *J. Biol. Chem.*, **1993**, *268*, 2571–2576.
7. Arthur, J. R. *Cell. Mol. Life Sci.*, **2000**, *57*, 1825–1835.
8. Arthur, J. R.; Nicol, F.; Beckett, G. J. *Biochem. J.*, **1990**, *272*, 537–540.
9. Davey, J. C.; Becker, K. B.; Schneider, M. J.; St. Germain, D. L.; Galton, V. A. *J. Biol. Chem.*, **1995**, *270*, 26786–26789.
10. Bianco, A. C.; Salvatore, D.; Gereben, B.; Berry, M. J.; Larsen, P. R. *Endocr. Rev.*, **2002**, *23*, 38–89.
11. Tamura, T.; Stadtman, T. C. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 1006–1011.
12. Arnér, E. S. J. *Biochim. Biophys. Acta*, **2009**, *1790*, 495–526.
13. Guimaraes, M. J.; Peterson, D.; Vicari, A.; Cocks, B. G.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Ferrick, D. A.; Kastelein, R. A.; Bazan, J. F.; Zlotnik, A. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 15086–15091.
14. Sunde, R. A. *Annu. Rev. Nutr.*, **1990**, *10*, 451–474.
15. Behne, D.; Kyriakopoulos, A. *Annu. Rev. Nutr.*, **2001**, *21*, 453–473.
16. Labunskyy, V. M.; Hatfield, D. L.; Gladyshev, V. N. *Physiol. Rev.*, **2014**, *94*, 739–777.
17. Papp, L. V.; Lu, J.; Holmgren, A.; Khanna, K. K. *Antioxid. Redox Signal.*, **2007**, *9*, 775–806.
18. Burk, R. F. *FASEB J.*, **1991**, *5*, 2274–2279.
19. Stadtman, T. C. *Annu. Rev. Biochem.*, **1996**, *65*, 83–100.
20. Kawai, Y.; Ando, H.; Ozeki, H.; Koketsu, M.; Ishihara, H. *Org. Lett.*, **2005**, *7*, 4653–4656.
21. Nanami, M.; Ando, H.; Kawai, Y.; Koketsu, M.; Ishihara, H. *Tetrahedron Lett.*, **2007**, *48*, 1113–1116.
22. Garud, D. R.; Ando, H.; Kawai, Y.; Ishihara, H.; Koketsu, M. *Org. Lett.*, **2007**, *9*, 4455–4458.
23. Ninomiya, M.; Garud, D. R.; Koketsu, M. *Coord. Chem. Rev.*, **2011**, *255*, 2968–2990.
24. Kogami, M.; Koketsu, M. *Org. Biomol. Chem.*, **2015**, *13*, 9405–9417.
25. Fukuno, S.; Ninomiya, M.; Koketsu, M. *Synlett*, **2017**, *28*, 831–834.
26. Braga, A. L.; Wessjohann, L. A.; Taube, P. S.; Galetto, F. Z.; de Andrade, F. M. *Synthesis*, **2010**, 3131–3137.
27. Phadnis, P. P.; Mugesh, G. *Org. Biomol. Chem.*, **2005**, *3*, 2476–2481
28. Muttenthaler, M.; Alewood, P. F. *J. Pept. Sci.*, **2008**, *14*, 1223–1239.
29. Oikawa, T.; Esaki, N.; Tanaka, H.; Soda, K. *Proc. Natl. Acad. Sci. USA*, **1991**, *88*, 3057–3059.
30. Koide, T.; Itoh, H.; Otaka, A.; Yasui, H.; Kuroda, M.; Esaki, N.; Soda, K.; Fujii, N. *Chem. Pharm Bull.*, **1993**, *41*, 502–506.
31. Gieselman, M. D.; Xie, L.; van der Donk, W. A. *Org. Lett.*, **2001**, *3*, 1331–1334.
32. Trost, B. M.; Rudd, M. T. *Org. Lett.*, **2003**, *5*, 4599–4602.
33. Beld, J.; Woycechowsky, K. J.; Hilvert, D. *Biochemistry*, **2007**, *46*, 5382–5390.
34. Yoshida, S.; Kumakura, F.; Komatsu, I.; Arai, K.; Onuma, Y.; Hojo, H.; Singh, B. G.; Priyadarsini, K. I.; Iwaoka, M. *Angew. Chem. Int. Ed. Engl.*, **2011**, *50*, 2125–2128.