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Synthesis of selenocystine derivatives from cystine by applying the transformation reaction from disulfides to diselenides

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Abstract—A stepwise conversion of a disulfide (SS) to a diselenide (SeSe) bond through the corresponding iodide intermediate was implemented and was applied to the synthesis of selenocystamine and L-selenocystine derivatives from cystamine and L-cystine, respectively, in moderate yields.

1. Introduction

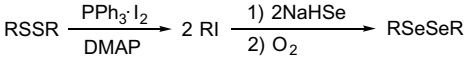
Selenocysteine (Sec)[1] is an interesting amino acid not only because it is a structural and chemical analog to cysteine (Cys), a common amino acid in living systems, but also because it has characteristic features as the active sites of several enzymes,[2] and as the 21st amino acid genetically coded in DNA.[3] Some efficient organic methodologies were developed for the synthesis of Sec derivatives starting from such compounds as serine,[4] β-chloroalanine,[5] and glycine.[6] However, no practical method to directly transform Cys to Sec derivatives has been reported.

Recently, point mutation of Cys to Sec in a polypeptide chain, that is, the replacement of the sulfur atoms of the Cys residues to selenium atoms, has attracted interest from a view point of oxidative refolding of proteins[7] as well as protein structure determination.[8] In the previous strategies, the Sec residues were incorporated into a polypeptide chain by chemical modification of the serine residues in natural enzymes[9] or by cysteine auxotrophic expression in the presence of Sec.[8,10] Native chemical ligation of polypeptides between the C-terminal thioester and the N-terminal Sec residue was also developed recently.[11]

We present here a novel chemical procedure to convert a disulfide (SS) bond to the heavier analog, that is, a diselenide (SeSe) bond, through the iodide intermediate. The reaction was successfully applied to the synthesis of selenocystamine and L-selenocystine ([L-Sec]$_2$), an oxidized dimer of L-Sec, derivatives starting from cystamine and L-cystine ([L-Cys]$_2$), respectively, in moderate yields. Our strategy will open the possibility for post-modification of the Cys residues in a polypeptide chain to Sec residues.

2. Results and discussion

Conversion of a C–S bond to a C–Se bond is not an easy task. The bond dissociation energy of the C–S linkage in diethyl sulfide (69.7 kcal/mol) is larger than that of the C–Se linkage in diethyl selenide (59.9 kcal/mol),[12] suggesting that the SN_2-type direct conversion by use of a selenide (HSe^-) or diselenide (Se_2^{2-}) anion would be difficult. However, this energetic disadvantage was recently overcome by Kreif et al. by the activation of the C–S linkage to the diphenyl sulfonium salt.[13] We employed an alternative pathway, which goes through the iodide intermediate (Scheme 1),[14] by exploiting the reaction of triphenylphosphine–iodine complex ($PPh_3{\cdot}I_2$) reported by Oae and Togo.[15]



Scheme 1.

Keywords: Selenocystine; Selenocystamine; Diselenides; Disulfides; Iodination.
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0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
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Table 1 summarizes the results of the transformation reactions from various disulfides to the corresponding diselenides. In the sequential reactions, the iodide intermediates were not isolated because the purification process usually caused significant loss due to instability or volatility.

We first applied the methodology to simple dialkyl disulfides. When di(*n*-butyl) disulfide (**1a**) was reacted with $PPh_3{\cdot}I_2$ complex in the presence of 4-dimethylaminopyridine (DMAP) as base, *n*-butyl iodide was obtained as a single product. Successive treatment of the iodide with the selenide ion (HSe^-), generated in methanol from selenium and sodium borohydride ($NaBH_4$),[16] and air oxygen afforded di(*n*-butyl) diselenide (**2a**) in 20% overall yield. Although the yield was low due to volatility of the iodide and the diselenide, the reaction proceeded clean, yielding the diselenide as an only selenium-containing product. Similarly, di(*s*-butyl) disulfide (**1b**) was transformed to the corresponding diselenide (**2b**) in a modest yield. However, when di(*t*-butyl) disulfide (**1c**) was applied to this transformation process, the corresponding iodide was not obtained. Thus, the methodology was applicable only for primary and secondary alkyl disulfides. As for the iodination mechanism, the iodide would be formed through the nucleophilic attack of an I^- ion at the carbon center of the C–S bond that may be activated with Ph_3P^+–I cation.[17]

The methodology was subsequently applied to cystamine derivatives (**3a**–**c**). The attempt to directly transform cystamine to selenocystamine was unsuccessful. Therefore, the amino groups were protected with benzoyl (Bz), benzyloxycarbonyl (Z) or 9-fluorenylmethoxycarbonyl (Fmoc) group. The latter two are commonly used protecting groups for amino acids and would be easily removed after the transformation to diselenides. When **3a** was employed through the transformation process from disulfides to diselenides, *N*,*N*′-dibenzoylselenocystamine (**4a**) was obtained in 32% yield. The yield was increased to 66% for **4b** and 55% for **4c**, which were protected with Z and Fmoc groups, respectively.

In the case of [L-Cys]$_2$, protection of the both amino and carboxyl groups was required. Disulfides **5a** and **5b** were synthesized from [L-Cys]$_2$ by the reaction with ZCl or FmocCl, respectively, in 1 M NaOH, followed by the esterification in ethanol in the presence of concentrated H_2SO_4. The obtained compounds were then successfully transformed to the corresponding [L-Sec]$_2$ derivatives (**6a** and **6b**, respectively) in moderate yields. Stereochemistry of the C_α atoms was retained through the transformation reaction because **6a** and **6b** showed similar Cotton effects in the CD spectra to **5a** and **5b**, respectively, and also because they were not contaminated with the diastereomeric isomers in the NMR spectra. Deprotection of **6a** by hydrolysis in 1 M NaOH–DMF at 40 °C followed by the treatment in HBr–acetic acid at room temperature, afforded [L-Sec]$_2$ in 62% yield. The total yield of [L-Sec]$_2$ was 34% from unprotected [L-Cys]$_2$ through **5a** and **6a**.

The transformation reaction from disulfides to diselenides reported here is useful not only for simple primary and secondary alkyl disulfides, but also for cystamine and cystine derivatives, which have amide, urethane and ester functional groups. This suggests that the protocol would be extended to the chemical conversion of Cys to Sec residues in a polypeptide chain and a protein.

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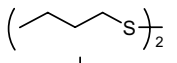
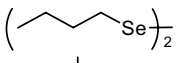
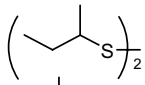
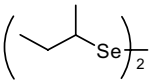
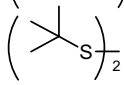
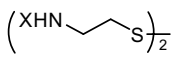
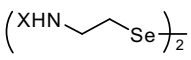
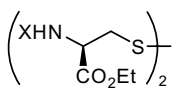
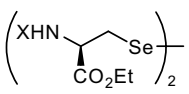
Table 1. Transformation reactions from disulfides to diselenides[a]

Reactants		Products		Yields[b] (%)
	**1a**		**2a**	20
	**1b**		**2b**	23
	**1c**			—[c]
	**3a** (X = Bz) **3b** (X = Z) **3c** (X = Fmoc)		**4a** (X = Bz) **4b** (X = Z) **4c** (X = Fmoc)	32 66 55
	**5a** (X = Z) **5b** (X = Fmoc)		**6a** (X = Z) **6b** (X = Fmoc)	66 75

[a] Reaction conditions from disulfides to iodides were RSSR–PPh_3–I_2–DMAP = 1:3:2:2 refluxed in benzene for 2 h. Reaction conditions from iodides to diselenides were NaHSe (2.5 equiv with respect to RSSR) in methanol at −4 °C for 16 h.

[b] Overall yields of isolated diselenides.

[c] *t*-Butyl iodide was not obtained.

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14. General procedure: To the suspension of $PPh_3{\cdot}I_2$ complex, prepared from PPh_3 (0.69 mmol) and I_2 (0.46 mmol) in benzene, were added disulfide **5b** (0.23 mmol) and DMAP (0.46 mmol), and the mixture was refluxed for 2 h. After extraction with ether, iodide was obtained as a mixture with triphenylphosphine sulfide and/or oxide. The crude product was dissolved in ether (or methanol), and the solution was added at −4 °C to a selenide solution prepared from Se (0.57 mmol) and excess $NaBH_4$ (~5 mmol) in methanol. The mixture was stirred at −4 °C for 30 min and then stored in a freezer overnight. After extraction with ether and purification by gel permeation chromatography, *N*,*N*′-bis(fluorenylmethoxycarbonyl)-L-selenocystine diethyl ester (**6b**) was obtained as a pale yellow solid in 75% yield; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.76 (4H, d, $J = 7.4$ Hz), 7.59 (4H, m), 7.39 (4H, t, $J = 7.4$ Hz), 7.32 (4H, t, $J = 7.4$ Hz), 5.76 (2H, d, $J = 7.2$ Hz), 4.69 (2H, m), 4.40 (4H, m), 4.23 (6H, m), 3.6–3.0 (4H, m), 1.29 (6H, t, $J = 7.1$ Hz); ^{13}C NMR ($CDCl_3$): δ 170.5, 155.7, 143.8, 143.7, 141.3, 127.8, 127.1, 125.1, 120.0, 67.2, 62.1, 54.4, 47.1, 32.3, 14.2; ^{77}Se NMR ($CDCl_3$): δ 298.1. Anal Calcd for $C_{40}H_{40}N_2O_8Se_2$: C, 57.56; H, 4.83; N, 3.36. Found: C, 57.44; H, 4.74; N, 3.49.
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