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Visible Light-Mediated Synthesis of Se–S Bond-Containing Peptides

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Abstract: A visible light-initiated method has been developed for preparation of Se–S bond-containing peptides. The method is based on generation of sulfur-centered radical employing organic dye. The protocol is tolerant to unprotected peptides with "sensitive" amino acids. The stability of Se–S bond is evaluated in buffers at different pH (3.0–10.0) and also in the presence of oxidants and reducing agents. Additionally, the ability of Se–S bond to serve as an oxidation sensitive linker in biocompatible materials has been confirmed.

Keywords: glutathione; peptide; photocatalysis; selenocysteine

The biological importance of Se–S bond is based on the fact that this bond is found in the active center of redox regulating enzyme, namely, thioredoxin reductase – one of the major components of the antioxidant system in mammalian cells.[1,2] Se–S bond- containing intermediate is formed in the catalytic cycle of the glutathione peroxidase–selenoenzyme that is responsible for reduction of H_2O_2 and other peroxides by glutathione.[3] Recently, low-molecular weight compounds with Se–S bond have been used as fluorescent probes for detection of reactive sulfur species (RSS) – H_2S and H_2S_2.[4,5] Selenosulfides have also been used as prodrugs for inhibition of protein tyrosine phosphatases.[6]

Compounds with Se–S bond are considered unstable thus the synthesis can be challenging.[7] The exchange reaction between diselenide and thiol, although theoretically possible, is unfavorable because the selenolate byproduct is a stronger nucleophile than thiol.[8] However, the reaction can be performed under suitable conditions.[7] For example, the Se–S bond-containing compound has been obtained by reacting diphenyl diselenide with silver trifluoromethylsulfide. This exchange reaction is favored due to the selenolate stabilization with silver atom.[9] Typically, Se–S bond is prepared by reaction between thiol and electrophilic selenyl species – selenyl halides[10,11] and organyl seleninic acids.[12–14] Benzeneselenol can also be utilized for the synthesis of related phenylselenyl sulfide by employing aryl or alkyl thiols and a catalytic amount of *t*BuOK,[15] whereas reaction of benzeneselenol with electrophilic *N*-phenyl-trifluoromethanesulfenamide[16] occurs in acidic conditions. Notably, various sugar-selenyl sulfides have been synthesized directly from sugar diselenides and glutathione in a phosphate buffer. Moreover, this efficient method has been extended from using glutathione as a thiol group-containing substrate to a protein – a single-cysteine mutant of subtilisin.[17] Another example of synthesis of Se–S bond-containing substrate obtained by direct reaction between diselenide (selenocystine) and thiol (penicillamine) occurs in the presence of Et_3N.[18] Se–S bond-containing cyclic dipeptides are obtained by treating –Se-benzhydryl and –S-trityl dipeptide with iodine.[19] Significantly, UV light has been employed for the exchange reaction between diaryl disulfide and dialkyl diselenide. The authors have also stated that Se–S bond is formed under UV light, while longer wavelength (>410 nm, visible light) reverses the reaction.[20]

Recently, we have reported an efficient method for the functionalization of Se–Se bond-containing peptides, based on the generation of a selenium radical via visible light-initiated reaction.[21] As a continuation of our research related to development of methods for modification of selenocysteine[22–23] (Sec) and cysteine[24] peptides, here we report a simple protocol for preparation of Se–S bond-containing peptides using visible light-initiated reaction.

The optimization of reaction conditions was performed using dipeptide dimer Boc-Sec-Gly-OBn **1a** and glutathione (GSH) (**2**) as model substrates. The

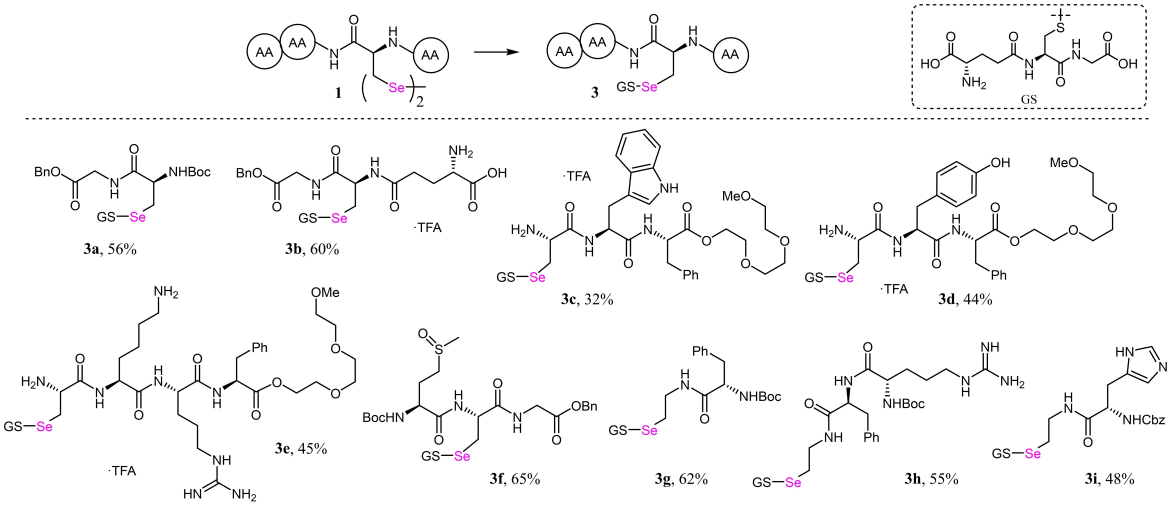
search for the most suitable photocatalyst was performed using 1 equiv. **1a**, 10 equiv. **2**, 0.02 equiv. transition metal catalyst or 0.05 equiv. organic dye in the mixture of acetonitrile and water (1:1), while irradiating the reaction mixture with blue LED light (max 460 nm, bright blue, x = 01440, y = 0.0395, > 50 000 lx) for 1 hour. The reaction performed without the catalyst showed only traces of the product **3a**. The highest selectivity and conversion of the starting materials was achieved employing Rose Bengal (RB). Other catalysts were: (i) less effective (bis[2-(4,6-difluorophenyl)pyridinato-C^2,*N*](picolinato)iridium(III) (FIrPic)); (ii) induced formation of the respective alkyl seleninic acid which subsequently led to deselenylation by elimination of H_2SeO_3 providing dehydroalanine (Dha) peptide[21] ($Ru(bpy)_3Cl_2$, 2,4,5,6-tetrakis(9*H*-carbazol-9-yl) isophthalonitrile (4-CzIPN), 9-mesityl-10-methylacridinium tetrafluoroborate, ethyl eosin, fluorescein), or (iii) failed to initiate the reaction (5-carboxytetramethylrhodamine). Performing the reaction in methanol and ethanol resulted in lower conversion of **1a** in the given time. The reaction performed in the day-light or in the dark did not lead to formation of **3a**, thus, confirming the necessity of LED_{460}. Other tested light sources (compact fluorescent lamp (CFL) – warm white (2291 K, x = 0.4915, y = 0.4105), red LED (max 660 nm, deep red, x = 0.5939, y = 0.2488)) were less efficient. The decrease of GSH amount (5 equiv.) did not result in full conversion of **1a**.

The presence of TEMPO did not prevent the formation of **3a**. Presumably, the generation of glutathionyl (GS•) radical under visible light irradiation, followed by the formation of Se–S bond or homocoupling reaction, proceeds much faster, than radical quenching with TEMPO.

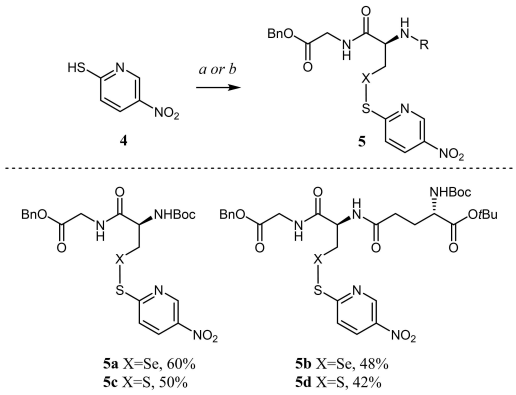
Next, the tolerance of amino acids with "sensitive" groups (Arg, Glu, Lys, His, Trp, Tyr, Met) was established (Scheme 1). Gratifyingly, selenocysteine and selenocystamine derivatives with Glu, Tyr, Arg, His, Lys showed excellent tolerance and provided the desired Se–S bond-containing products. Even Trp-containing product **3c** was isolated, albeit the yield was lower due to formation of side products that arise from Trp oxidation. A rapid oxidation of methionine has been reported under visible light irradiation in the presence of RB.[25] Thus, not surprisingly, Met sulfoxide-containing product **3f** was selectively obtained in good yield starting from Met-containing substrate.

Obviously, selenocystamine fragment can serve as a convenient linker. Furthermore, it has been demonstrated that in certain structures the selenoethyl moiety can be eliminated under reductive conditions.[26]

Notably, 5-nitropyridine-2-thiol (**4**) was also successfully employed in the visible light-mediated reaction providing the respective Se–S bond-containing products **5a**,**b** in good yields (Scheme 2). Therefore, a convenient and straightforward method is demonstrated for the preparation of 5-nitropyridin-2-yl)thio-Sec peptides as an alternative to the existing method.[27] This type of compounds has been reported to be used as electrophilic Se sources.[28] Furthermore, analogous S–S bond-containing products **5c**,**d** were synthesized employing Boc–Cys–Gly–OBn and Boc–Glu (O*t*Bu)–Cys–Gly–OBn. Although the preparation of asymmetrical disulfide may not be an easy task due to the formation of a mixture of symmetrical disulfides, the products **5c**,**d** were obtained in moderate yields and only negligible amounts of symmetrical disulfides were detected.



Scheme 1. Scope and limitation studies for the preparation of Se–S bond-containing compounds. Reaction conditions: diselenide (1 equiv.), GSH (10 equiv.), RB (0.1 equiv.), MeCN/H_2O, LED_{460}.



Scheme 2. Synthesis of (5-nitropyridin-2-yl)thio-Sec and Cys peptides. Reaction conditions: a) **1a** or **1b** (1 equiv.), **4** (5 equiv.), RB (0.1 equiv.), MeCN, LED_{460}; b) Cys peptide (1 equiv.), **4** (1 equiv.), RB (0.05 equiv.), MeCN, LED_{460}.

With the purpose to determine whether Se- or S-centered radical is formed via visible light-initiated reaction, Stern-Volmer analysis was performed (Figure 1). The photoluminescence quenching of RB was performed in a degassed mixture of acetonitrile/water (1:1). The quenching rate constant of RB with GSH was significantly higher ($8.8 \cdot 10^{-3}$ l/mol) than with **1a** ($0.12 \cdot 10^{-3}$ mol/l). Therefore, it allows the assumption that S-centered radical is rapidly formed under LED_{460} irradiation. Next, the GS radical reacts with the diselenide forming the desired Se–S bond-containing peptide **3a** or it undergoes the homocoupling reaction forming oxidized glutahione (GS-SG), therefore an excess of **2** is required for full consumption of **1a** (Scheme 3). Notably, both parts of the diselenide are utilized in the reaction.

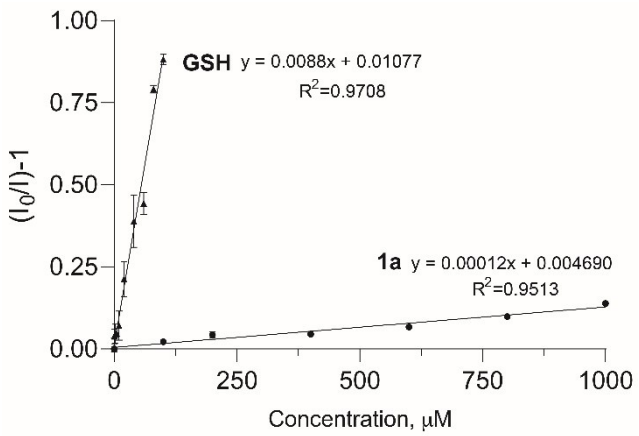
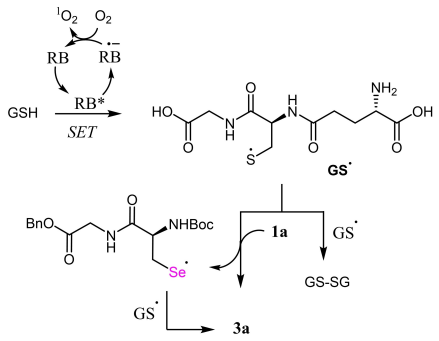


Figure 1. Photoluminescence quenching of RB with **1a** and GSH.

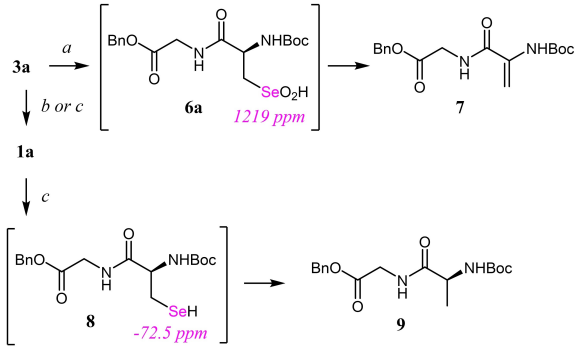
Next, the stability of Se–S bond-containing glutathione derivatives was evaluated. First, we examined the stability of **3a** under visible light irradiation. Prolonged irradiation (> 3 h) of **3a** and RB solution in MeCN/water with LED_{460} resulted in homolytic cleavage of Se–S bond, forming respective selenyl radical and GS radical. Selenyl radical is quickly oxidized to selenyl electrophile that reacts with H_2O_2 forming alkyl seleninic acid **6** and Boc–Dha–Gly–OBn **7** (Scheme 4).[21] H_2O_2 is produced from singlet oxygen reaction with water.

The reaction of **3a** with H_2O_2 led to fast oxidation to alkyl seleninic acid. Intermediate **6a** was detected by HRMS ([M+Na]=471.0633) and ^{77}Se NMR (δ 1219.8 ppm) spectroscopy. Besides, a small signal of selenonic acid **6b** (δ 1050.8 ppm) was also detected in the ^{77}Se NMR spectra (Figure S1). The alkyl seleninic acid **6a** is too unstable to be isolable, it delivers **7** via deselenylation.[21] The use of *t*BuOOH led to the formation of **7** in 2 h as well.

Reduction of **3a** with 3 equiv. 1,4-dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) provided diselenide **1a** and GSH in a short reaction time (30 min). Furthermore, increasing the amount of TCEP to 10 equiv. led to deselenylation and provided Boc-Ala-Gly-OBn **9** (Scheme 4). The reduction proceeds via formation of the respective selenol **8**



Scheme 3. Proposed mechanism for the formation of Se–S bond-containing substrates.



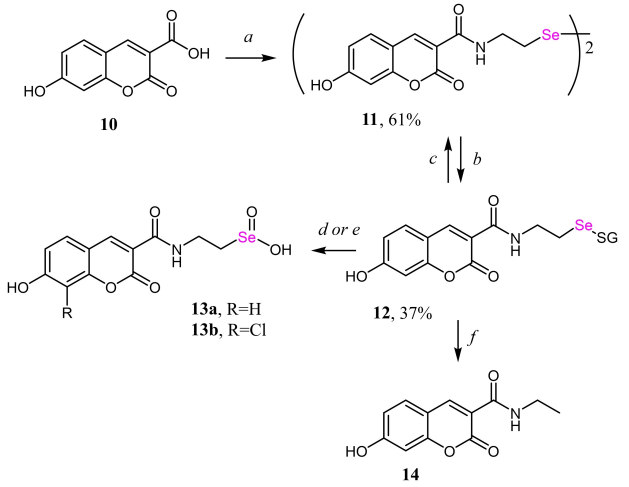
Scheme 4. Stability of **3a**. Reaction conditions: a) H_2O_2 or *t*BuOOH, MeCN/H_2O; b) DTT, MeCN/H_2O; c) TCEP, MeCN/H_2O.

that was detected by LC-MS ([M + Na] = 439.03) and ^{77}Se NMR spectroscopy (δ -72.5 ppm). The deselenylation proceeded smoothly yielding **9** and TCEP=Se ([M–H] = 328.9704).[29,30]

Further, derivative **12** was prepared starting with 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylic acid **10** (Scheme 5) to evaluate the possibility to utilize alkylselenylsulfide moiety as a cleavable linker for the introduction of fluorescent probe to thiol group-containing peptide,. Diselenide **11** was successfully employed in the visible light-initiated reaction with glutathione, thus, a mixture of DMSO/H_2O was used to ensure that the starting materials are fully dissolved. Luckily, the desired Se–S bond-containing product **12** was easily obtained in 37% yield. Next, the stability of **12** was established. A quick oxidation and formation of the respective seleninic acid **13 a** was observed in the presence of H_2O_2. Another oxidant – NaClO provided the respective chlorinated seleninic acid **13 b** ([M–H] = 377.9290) The use of DTT (10 equiv.) led to formation of diselenide **11**. Recently, it was demonstrated that a cyclic selenosulfide subjected to reductive conditions (TCEP or DDT), spontaneously eliminates selenoethyl moiety releasing ethylene molecule, besides, all reactions were very slow (up to 6 days).[26] We did not observe such transformation in the case of **12** – interaction with TCEP (10 equiv.) was very efficient providing ethyl amide **14** in 30 min.

Considering a possible utilization of Se–S bond as a sensitive linker in biocompatible materials, the stability of **12** in phosphate-buffered saline (PBS, 20 mM) was established under various pH. The compound was stable in pH range 3.0–8.0 for 24 h, whereas a considerable amount of diselenide **11** was formed at pH 10.0 after 24 h (Table S1). Inspired by the good stability of a linker at physiological pH, absorption and emission spectra were collected for the solutions of **11**, **12** and **13 a** in PBS (pH = 7.4, c = 10 μM) (Figures S2–S4). The studied compounds showed absorbance maximum at 396–404 nm that is typical for the coumarin ring. Upon excitation at 350 nm the compounds' solutions emit light in blue region (Figure 2). Diselenide's **11** photoluminescence quantum yield is low (Φ = 1.6%), however, it is considerably higher in the case of **12** (Se–S, Φ = 29.5%). The highest emission quantum yield was observed after oxidation of **12** with hydrogen peroxide – the seleninic acid's **13 a** Φ is equal to 52.4%. The emission was characterized by CIE coordinates of pure blue light (x:0.1585, y:0.0160).

In conclusion, a simple protocol was developed for the synthesis of Se–S bond-containing peptides. A visible light-initiated reaction was employed for generation of sulfur-centered radical from unprotected glutathione that further reacted with protected and unprotected selenocysteine or selenocystamine peptides. Amino acids with sensitive groups (Arg, Lys, Trp, Tyr, His, Glu) showed tolerance under reaction conditions, although the products were obtained only in moderate yields. The use of Met-containing substrate provided the respective Met-sulfoxide. Notably, asymmetrical disulfides can be synthesized from 5-nitropyridine-2-thiol and Cys peptides. Fluorescent coumarin-selenocystamine conjugate with Se–S bond is stable under physiological conditions allowing to propose the Se–S bond as a valuable linker in biocompatible materials under oxidative stress conditions.



Scheme 5. Synthesis and stability of **12**. Reaction conditions: a) selenocystamine × 2HCl (1 equiv.), **10** (2.5 equiv.), *N*-methylmorpholine (NMM) (3 equiv.), 1-hydroxybenzotriazole (HOBt) (1 equiv.), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (3 equiv.), DMF; b) GSH (10 equiv.), RB (0.1 equiv.), DMSO/H_2O, LED_{460}; c) DTT, PBS; d) H_2O_2; e) NaClO, PBS; f) TCEP, PBS.

Experimental Section

Representative procedure for visible light-mediated Se-S bond formation. Rose Bengal (0.1 equiv.) was added to a solution of Sec-peptide **1** (1 equiv.) and L-glutathione **2** (10 equiv.) in

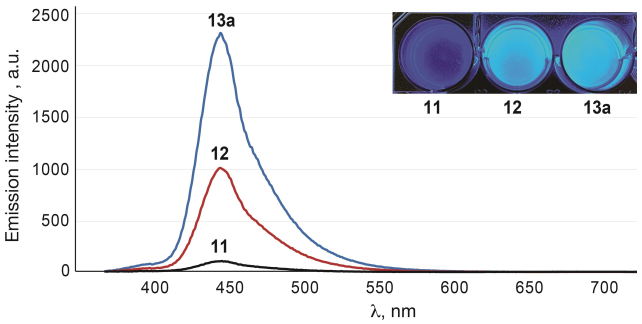


Figure 2. Emission spectra of **11**, **12**, **13 a** upon excitation at 350 nm (PBS, pH = 7.4).

mixture of MeCN/H_2O (1:1) and the mixture was irradiated by 36 W blue LEDs for 1 hour. After evaporation the residue was purified by reverse phase flash chromatography (C-18, MeCN/H_2O, 10–85%) to give the product **3**.

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