

Article

Biohybrid -Se-S- Coupling Reactions of an Amino Acid Derived Seleninate

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Abstract: We describe the synthesis of the *N*-(2-seleninatoethyl) amide of *N*-Boc-phenylalanine, serving here as a peptide model, and its reductive coupling reactions under mild conditions with unprotected thiouridine and glutathione. Selenosulfide products such as these comprise reversibly conjugated bio-components, and can potentially find uses as probes of biological function, such as enzyme inhibitors, delivery systems, or structural mimics.

Keywords: click reaction; biomimetic; seleninic acid; thiol

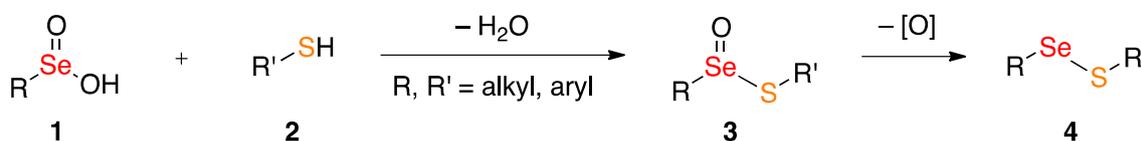
1. Introduction

Coupling reactions that join complex partners play a key role in biosynthesis as well as chemical synthesis. Chemists aspire to replicate in the laboratory the high efficiency and mild conditions achieved in Nature, and have developed a number of such reactions that succeed in aqueous solution at neutral pH and ambient temperature [1,2]. Unlike biosynthetic transformations, however, the chemical coupling reactions can embrace “unnatural” features such as uncommon bonds and unusual structures. The versatile azide-alkyne cycloaddition [3] exemplifies this feature: neither the azide nor the alkyne partner nor the triazole product would be considered biologically normal. Additionally, useful coupling reactions are often compatible with densely functionalized and generally unprotected partners incorporating, for example, peptide, nucleoside, carbohydrate, or oligomeric substructures [4,5]. The resulting biohybrid [6] products can feature useful biology-interactive properties such as (selected from among many recent examples) enzyme inhibitory activity [7–9], resistance to metabolic

degradation [10], scaffolding [11,12], imaging and labeling [13], diagnostic features [14,15], special compartmentalization behavior [16], and drug delivery [17–19].

For several years our research group has investigated the redox coupling [20] of seleninic acids (RSeO₂H, **1**, Scheme 1) with thiols **2** to give selenosulfides **4** [21–23]. A wide variety of biomimetic coupling partners can be assembled that incorporate the seleninic acid functionality as part of a carbohydrate, nucleoside, amino acid, or lipid framework [21]. The coupling reaction itself proceeds at room temperature in a wide variety of solvents, including water, alcohols, dioxane, THF, ethyl acetate, dichloromethane, acetonitrile, toluene, and DMF. The general mechanism of this reaction (Scheme 1) [20,24] proceeds initially through a thioseleninate intermediate **3**. Rapid reduction of **3** to the selenosulfide product **4** occurs in the presence of excess thiol (the byproduct is disulfide), or often with no obvious reducing agent, in which case the [O] from **3** “disappears” without reappearing as part of any isolable byproduct, even when close to 100% of the selenium and sulfur atoms are accounted for [21,23,24]. Despite this incomplete accounting, the conversion to selenosulfide **4** can be efficient for one-to-one mixtures of the two components. Air, moisture, buffers, and other additives do not seem to interfere [23]. The typical byproducts of the one-to-one coupling are the symmetrical disulfide and the symmetrical diselenide [23]. Exemplifying the potential of such couplings in bioorganic chemistry was the observation that a tyrosine phosphate mimic, in which a seleninate group (*i.e.*, Ar-CH₂SeO₂H) replaces phosphate (Ar-OPO₃H₂), effectively deactivated protein tyrosine phosphatase by forming a selenosulfide bond with the cysteine residue in the enzyme active site [22].

Scheme 1. The redox coupling reaction of seleninates and thiols.



In order to expand on these studies, we chose to construct the seleninate moiety as a peptide model, and to examine its redox coupling to the sulfur atom of two relatively complex partners: the unprotected 4-thiouridine (a nucleoside thioamide), and glutathione (a tripeptide with an active thiol).

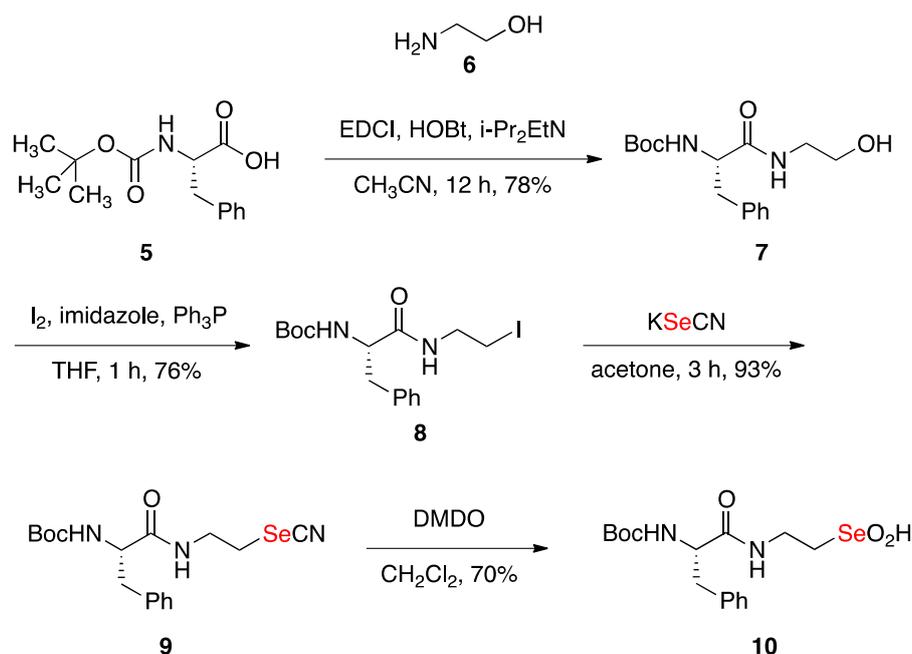
2. Results and Discussion

2.1. Synthesis of Seleninic Acid **10**

A greatly simplified “peptide” model compound was commercially available as *N*-(*tert*-butoxycarbonyl)-(*S*)-phenylalanine (**5**, Scheme 2). Standard amide formation with aminoethanol (**6**) mediated by the coupling reagent 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) gave the 2-hydroxyethylamide **7**. Conversion of **7** to the 2-iodoethylamide **8** occurred without incident, and then nucleophilic replacement of iodo by the selenocyanate anion led to **9**. Treatment of selenocyanate **9** with a slight excess of dimethyldioxirane (DMDO), our preferred reagent for the synthesis of seleninates [21], gave the stable seleninic acid **10**. Interestingly, in deuteriomethanol (CD₃OD) solution **10** reversibly forms its apparent diastereomeric seleninate trideuteriomethyl esters (1:1). Analogous behavior has been observed with nucleoside seleninic acids [25]. The structure of **10**

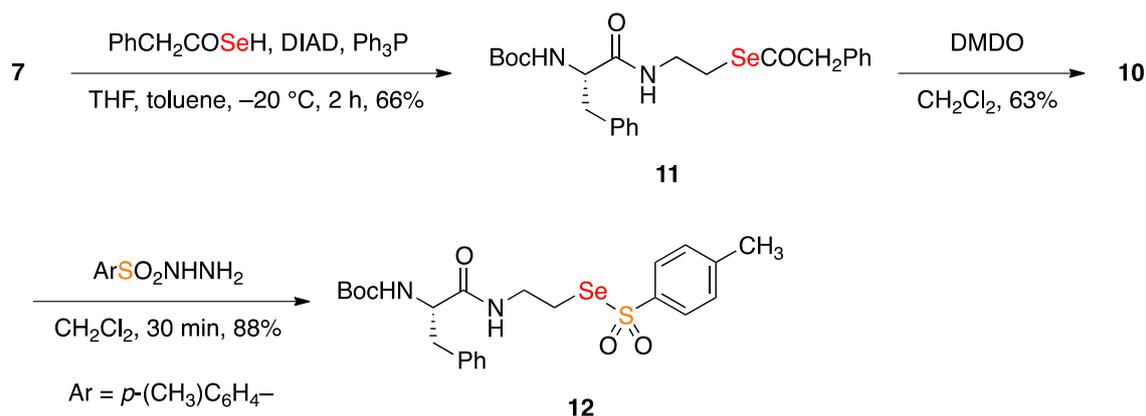
is thus initially assigned based on the ^1H -, ^{13}C -, and ^{77}Se - (1292.9 and 1294.1 ppm) NMR spectra of its trideuteriomethyl esters in deuteriomethanol solution, as well as the mass spectrum of the derived seleninate methyl ester with the appropriate Se isotope cluster centered on m/z 440 (the MNa^+ ion of the methyl ester). The ^{13}C -NMR spectra of **10** taken in acetone- d_6 or deuteriochloroform solution, however, showed the expected 12 signals for a single isomer of the seleninic acid (see Experimental).

Scheme 2. Synthesis of the seleninic acid **10** from *N*-(Boc)phenylalanine (**5**).



The structure of **10** was additionally confirmed by an independent preparation (Scheme 3). Thus, 2-hydroxyethylamide **7** was converted to the selenoester **11** by use of the selenocarboxylate Mitsunobu reaction [26]. Oxidation with DMDO as before gave **10** in similar overall yield and purity. Further structural confirmation of **10** based on the rapid and efficient redox coupling reaction of seleninates with *p*-toluenesulfonylhydrazide [27,28] was carried out, leading to the selenosulfonate **12**. This diagnostic reaction of seleninates could qualify in some respects as a potentially useful biohybrid coupling reaction, although the biomimetic sulfonylhydrazide partners are typically not as complex nor are they as readily available as the thiols.

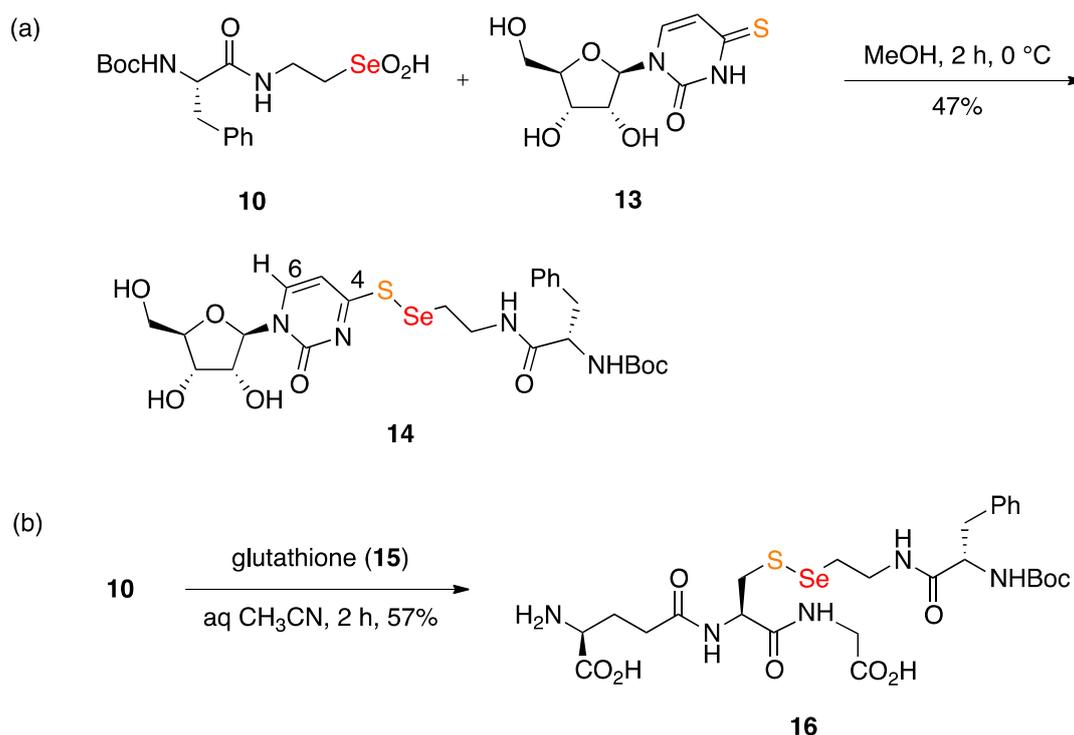
Scheme 3. Alternative synthesis of seleninate **10** and confirmation of the structure.



2.2. Coupling Reactions of Seleninic Acid **10**

The redox coupling reaction of seleninate **10** with commercial 4-thiouridine (**13**) was conveniently carried out in methanol solution, since both components easily dissolve (Scheme 4). Following our usual practice, **10** was added neat in one portion to a solution of **13** without any special precautions to keep the flask oxygen- or moisture-free. Chromatography of the product served to remove any disulfide and diselenide byproducts, and led to the modified nucleoside selenosulfide **14** in 47% yield. While selenosulfides can on occasion disproportionate to a mixture of the selenosulfide, diselenide, and disulfide, **14** was stable to storage. The 4-thioether structure for **14** is indicated by the pronounced downfield shift of H-6 to 8.52 ppm [29]. Sulfenylations of 4-thiouridine derivatives are known, and lead analogously to imidothioate *S*-derivatives (*i.e.*, disulfides) rather than *N*-substituted thioamides [29]. The coupled product **14** can be viewed as a reversibly modified nucleoside, since the Se-S bond can potentially be cleaved by reduction [30,31]; **14** can perhaps also serve as a simplified model for a peptide oligonucleotide hybrid.

Scheme 4. (a) Coupling reaction of seleninate **10** with 4-thiouridine. (b) Coupling reaction of **10** with glutathione.



The redox coupling reaction of seleninate **10** with commercial glutathione (GSH, **15**) was carried out in 1:1 acetonitrile/water, a solvent mixture that dissolved both reactants and the coupled product, selenosulfide **16** (Scheme 4). Reverse phase preparative HPLC served to purify **16**, which was characterized by its ¹H and ¹³C-NMR spectra, as well as its protonated molecular ion possessing the required Se isotope cluster. Purified **16**, as for **14**, did not disproportionate on storage. Other types of selenylations of the GSH thiol have been reported recently, among these GSH pseudoglycosylation with a glycosyl diselenide [32], selenylation of GSH with ebselen [33], selenylation with selenocysteine [34], and selenylation of GSH with various electrophilic selenoallyl species [35].

3. Experimental

3.1. General

Organic solvents used for reactions were of reagent grade and were used as received. Flash chromatography was performed by using silica gel (E. Merck 230–400 mesh) as the stationary phase. Silica gel 60 F₂₅₄ pre-coated plates were used for thin layer chromatography, and visualization was accomplished with UV light (254 nm) and iodine stain. Prep-scale reverse-phase chromatography was conducted with a Gilson 215 liquid handler/injector fitted with Gilson 333/334 binary HPLC pumps and UV/vis dual wavelength detector (model 156) and Trilution software. The chromatographies were carried out on a Waters XBridge Prep BEH 130 C18 5 μm OBD 19 × 100mm column (part # 186003587). The eluent was acetonitrile (HPLC grade) and Millipore water with 0.05% formic acid buffer. The analytical LC-MS analyses were conducted by using a Waters 2767 sample manager, consisting of a Waters 2525 binary gradient HPLC connected to a diode array detector and a Waters Micromass ZQ mass spectrometer with electro-spray ionization. The LC-MS samples were analyzed as solutions in water or acetonitrile, prepared at 0.15–0.20 mg/mL concentration. The LC-MS chromatography was carried out on an Atlantis-C18 column (4.6 × 50 mm; 5 μm) with linear gradients of 0.05% formic acid in acetonitrile and 0.05% formic acid water. High-resolution mass spectrometry (HRMS) was obtained on Waters LC-TOF mass spectrometer (model LCT-XE Premier) by using electrospray ionization in positive or negative mode. ¹H, ¹³C, and ⁷⁷Se NMR spectra were obtained on a Varian UNITY 400 or 500 instrument. Chemical shifts (δ) are reported in parts per million (ppm) and are referenced to the residual solvent signal. Coupling constants (*J*) are reported in hertz (Hz). The usual abbreviations are used to describe multiplicities: s (singlet), d (doublet), t (triplet), q (quartet), br (broad). NMR solvents and all other commercially available reagents were used as received and without any further purification.

(*S*)-*tert*-Butyl (1-((2-Hydroxyethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**7**) [36]. A solution of *N*-Boc-L-phenylalanine (**5**, 265.3 mg, 1.0 mmol) in of acetonitrile (8 mL) was stirred at 0 °C and then was treated sequentially with hydroxybenzotriazole (184 mg, 1.2 mmol), diisopropylethylamine (388 mg, 3.0 mmol) and f 2-aminoethanol (**6**, 79 mg, 1.3 mmol). After 15 min at 0 °C, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (230 mg, 1.2 mmol) was added and the reaction mixture was stirred for an additional 12 h at room temperature. The reaction was quenched with aqueous saturated sodium bicarbonate (10 mL). The reaction mixture was extracted with dichloromethane (3 × 10 mL). The combined organic extract was washed with brine, and then dried over sodium sulfate. The crude reaction product was purified by silica gel chromatography with 3% methanol in dichloromethane as the eluent to produce 240 mg (78%) of **7** as a colorless oil: ¹H-NMR (500 MHz, CDCl₃) δ 7.19–7.32 (m, 5 H), 6.62 (br s, 1 H), 5.30 (s, 1 H), 4.30–4.38 (m, 1 H), 3.50–3.63 (m, 2 H), 3.28–3.37 (m, 2 H), 3.00–3.09 (m, 2 H), 2.91 (br s, 1 H), 1.35 (s, 9 H); ¹³C-NMR (126 MHz, CDCl₃) δ 172.7, 156.0, 137.1, 129.6, 128.7, 127.0, 80.3, 61.5, 56.3, 42.4, 39.2, 28.5; ESI-MS *m/z* 309.2 MH⁺.

(*S*)-*tert*-Butyl (1-((2-Iodoethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**8**) [37]. A solution of **7** (240 mg, 0.785 mmol) in THF (6 mL) was combined with triphenylphosphine (411 mg, 1.57 mmol) and imidazole (106.8 mg, 1.57 mmol). After 5 min of stirring, iodine (398 mg, 1.57 mmol) was added and the reaction mixture was allowed to stir for an additional 1 h. The reaction mixture was

concentrated and dissolved in dichloromethane (20 mL), which solution was washed sequentially with 5% aqueous Na₂S₂O₃ (10 mL) and brine (10 mL), and then dried over sodium sulfate. Concentration and then chromatography with 3:7 ethyl acetate/hexanes as the eluent gave 248 mg (76%) of iodide **8** as a yellow solid: ¹H-NMR (500 MHz, CDCl₃) δ 7.20–7.36 (m, 5 H), 6.55 (br s, 1 H), 5.23 (br d, 1 H, *J* = 7.0 Hz), 4.29–4.38 (m, 1 H), 3.44–3.62 (m, 2 H), 3.02–3.18 (m, 4 H), 1.40 (s, 9 H); ¹³C-NMR (126 MHz, CDCl₃) δ 171.6, 155.7, 136.9, 129.5, 128.9, 127.2, 80.4, 56.1, 42.0, 38.9, 28.6, 4.3; ESI-MS *m/z* 419.1 MH⁺.

(*S*)-*tert*-Butyl (1-Oxo-3-phenyl-1-((2-selenocyanatoethyl)amino)propan-2-yl)carbamate (**9**). A solution of iodide **8** (232 mg, 0.555 mmol) in acetone (3 mL) was treated with potassium selenocyanate (120 mg, 0.832 mmol). The reaction mixture was stirred for 3 h, concentrated, and then partitioned between dichloromethane and aqueous ammonia chloride. The organic extract was dried over sodium sulfate, concentrated, and then chromatographed with 3% methanol in dichloromethane as the eluent to afford 204 mg (93%) of **9** as a white solid: ¹H-NMR (500 MHz, CDCl₃) δ 7.18–7.35 (m, 5 H), 6.55 (bs, 1 H), 5.10 (br d, 1 H, *J* = 7.0 Hz), 4.30–4.39 (m, 1 H), 3.65–3.74 (m, 1 H), 3.55–3.65 (m, 1 H), 3.10–3.17 (m, 1 H), 3.06 (app d, 2 H, *J* = 6.5 Hz), 2.94–3.03 (m, 1 H), 1.41 (s, 9 H); ¹³C-NMR (126 MHz, CDCl₃) δ 172.3, 155.7, 136.8, 129.5, 129.0, 127.4, 101.6, 80.7, 56.1, 40.1, 38.6, 29.1, 28.5; ⁷⁷Se NMR (95 MHz, CDCl₃) δ 199.7 (vs. PhSeSePh at 460.0 ppm as an external standard); ESI-MS *m/z* 420.0 MNa⁺.

(*S*)-2-(2-((*tert*-Butoxycarbonyl)amino)-3-phenylpropanamido)ethaneseleninic acid (**10**). Dimethyldioxirane (DMDO) was added to a stirred solution of selenocyanate **9** (111 mg, 0.28 mmol) in dichloromethane (7 mL) until starting material was consumed according to TLC analysis (total 1.05 mL of a 0.4 M solution of DMDO in moist acetone). The reaction mixture was concentrated and then chromatographed on silica gel with 8% methanol in dichloromethane as the eluent to give 77.3 mg (70%) of **10** as a colorless oil: ¹H-NMR (500 MHz, CD₃OD, as the two diastereomeric seleninate—CD₃ esters) δ 7.21–7.32 (m, 5 H), 4.22–4.31 (m, 1 H), 3.46–3.64 (m, 2 H), 3.04–3.12 (m, 2 H), 2.83–2.91 (m, 2 H), 1.38 (s, 9 H); ¹³C-NMR (126 MHz, CD₃OD, as the two diastereomeric seleninate—CD₃ esters) δ 174.3 and 174.2, 156.4, 137.4, 129.3, 128.3, 126.7 and 126.6, 79.6, 57.2 and 57.0, 56.1 and 56.2, 38.0, 33.2 and 33.3, 27.5; ¹³C-NMR (126 MHz, acetone-*d*₆) δ 173.3, 155.7, 138.2, 129.7, 128.5, 126.6, 78.8, 58.2, 56.2, 38.4, 33.7, 28.0; ¹³C-NMR (126 MHz, CDCl₃) δ 173.9, 155.9, 136.9, 129.6, 128.9, 127.2, 80.5, 56.3, 55.9, 38.9, 33.6, 28.6; ⁷⁷Se NMR (95 MHz, CD₃OD, as the diastereomeric seleninate esters, SeO₂CD₃) δ 1294.1, 1292.9 (vs. PhSeSePh at 460.0 ppm as an external standard); ESI-MS *m/z* 440.4 as the seleninate methyl ester·Na⁺.

(*S*)-Se-(2-(2-((*tert*-Butoxycarbonyl)amino)-3-phenylpropanamido)ethyl) 2-phenylethaneselenoate (**11**). A solution of triphenylphosphine (126 mg, 0.48 mmol) in tetrahydrofuran (1.5 mL) was stirred at −20 °C. Diisopropyl azodicarboxylate (97.1 mg, 0.48 mmol) was added dropwise and the reaction mixture was maintained at −20 °C until the white phosphonium intermediate formed. The reaction mixture was then cooled to −50 °C, and a solution of alcohol **7** (74.0 mg, 0.24 mmol) in tetrahydrofuran (2 mL) was added dropwise. After 5 min of stirring, a toluene solution (2 mL) of (2-phenyl)-selenoacetic acid [prepared by heating at reflux a 2 mL toluene solution of 100 mg (0.734 mmol) of phenyl acetic acid and 117 mg of Woollins's reagent for 2 h] was added by cannula and the reaction mixture was allowed to warm from −50 to 23 °C, and then was stirred for an additional 2 h. The solution was

concentrated and then chromatographed with 3:7 ethyl acetate/hexanes as the eluent to afford 77 mg (66%) of **11** as a colorless oil: $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 7.39–7.30 (m, 4 H), 7.30–7.25 (m, 4 H), 7.17 (d, 2 H, $J = 7.0$ Hz), 6.24 (bs, 1 H), 5.18 (bs, 1 H), 4.30 (bs, 1 H), 3.83 (s, 2 H), 3.33–3.47 (m, 2 H), 3.01 (br d, 2 H, $J = 6.0$ Hz), 2.77–2.92 (m, 2 H), 1.41 (s, 9 H); $^{13}\text{C-NMR}$ (126 MHz, CD_3OD) δ 200.7, 171.4, 155.8, 136.9, 132.9, 130.2, 129.6, 129.5, 129.0, 128.9, 128.8, 128.0, 127.1, 80.4, 56.2, 54.3, 40.1, 39.0, 28.5, 25.1; $^{77}\text{Se NMR}$ (95 MHz, CDCl_3) δ 547.6 (vs. PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 513.1 MNa^+ .

(*S*)-2-(2-((*tert*-Butoxycarbonyl)amino)-3-phenylpropanamido)ethaneseleninic acid (**10**) by oxidation of **11**. Dimethyldioxirane was added to a stirred solution of selenoester **11** (43.4 mg, 0.089 mmol) in dichloromethane (1 mL) until starting material was consumed according to TLC analysis (total 0.488 mL of 0.4 M solution of DMDO in moist acetone). The reaction mixture was concentrated and then chromatographed on silica with 8% methanol in dichloromethane as the eluent to give 22.6 mg (63%) of **10** as a colorless oil that spectroscopically matched **10** as prepared above.

(*S*)-Se-(2-(2-((*tert*-Butoxycarbonyl)amino)-3-phenylpropanamido)ethyl) 4-methylbenzenesulfonoselenoate (**12**). A solution of seleninic acid **10** (22.5 mg, 0.056 mmol) in dichloromethane (3 mL) was added dropwise to a solution of *p*-toluenesulfonylhydrazide (11.3 mg, 0.061 mmol) in dichloromethane (1 mL). After 30 min, the reaction mixture was concentrated then and chromatographed on silica with 3:7 ethyl acetate/hexanes as the eluent to give 25.9 (88%) of selenosulfonate **12** as a yellow oil: $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 7.75 (d, 2 H, $J = 8.0$ Hz), 7.35 (d, 2 H, $J = 8.0$ Hz), 7.29 (t, 2 H, $J = 7.0$ Hz), 7.24 (t, 1 H, $J = 7.0$ Hz), 7.19 (d, 2 H, $J = 7.0$ Hz), 6.44 (br s, 1 H), 5.08 (br s, 1 H), 4.29–4.36 (m, 1 H), 3.55–3.66 (m, 2 H), 3.19–3.27 (m, 1 H), 3.11–3.19 (m, 1 H), 3.06 (br d, 2 H, $J = 6.5$ Hz), 2.45 (s, 3 H), 1.40 (s, 9 H); $^{13}\text{C-NMR}$ (126 MHz, CDCl_3) δ 171.9, 155.8, 145.2, 144.1, 136.9, 130.1, 129.5, 128.9, 127.3, 126.8, 82.3, 56.2, 39.4, 38.8, 33.0, 28.5, 21.9; $^{77}\text{Se NMR}$ (95 MHz, CDCl_3) δ 856.9 (vs. PhSeSePh at 460.0 ppm as an external standard); ESI-MS m/z 549.1 MNa^+ .

tert-Butyl (1-((2-(((1-((2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2-oxo-1,2-dihydropyrimidin-4-yl)thio)selenyl)ethyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**14**). A solution of 4-thiouridine (**13**, 11.9 mg, 0.0456 mmol) in methanol (0.5 mL) was stirred at 0 °C and then was treated with seleninic acid **10** (18.4 mg, 0.0456 mmol) in one portion. After 2 h at 0 °C, the reaction was concentrated and then chromatographed with 5% methanol in dichloromethane as the eluent to produce 13.9 mg (47%) of selenosulfide **14** as a colorless oil: R_f 0.28 (5% methanol in dichloromethane); $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ 8.52 (d, 1 H, $J = 7.2$ Hz), 7.27 (br s, 4 H), 7.21 (br s, 1 H), 6.89 (d, 1 H, $J = 7.2$ Hz), 5.87 (br s, 1 H), 4.27–4.32 (m, 1 H), 4.09–4.21 (m, 3 H), 3.81 and 3.97 (ABq, 2 H, $J = 10.0$ Hz), 3.70–3.78 (m, 1 H), 3.22–3.30 (m, 1 H), 3.07–3.15 (m, 2 H), 2.95–3.04 (m, 1 H), 2.86 (dd, 1 H, $J = 13.2, 10.0$ Hz), 1.31 (s, 9 H); $^{13}\text{C-NMR}$ (126 MHz, CD_3OD) δ 179.1, 174.7, 157.7, 156.1, 144.3, 138.8, 130.4, 129.5, 127.7, 103.6, 93.4, 85.9, 80.6, 76.6, 69.9, 61.1, 57.9, 39.4, 39.1, 33.5, 28.7; HRMS m/z 631.1367 MH^+ , calcd for $\text{C}_{25}\text{H}_{35}\text{N}_4\text{O}_8\text{SSe}$ 631.1341; NI-HRMS m/z 629.1191 MH^- , calcd for $\text{C}_{25}\text{H}_{33}\text{N}_4\text{O}_8\text{SSe}$ 629.1184.

(6*S*,14*R*,19*S*)-19-Amino-6-benzyl-14-((carboxymethyl)carbamoyl)-2,2-dimethyl-4,7,16-trioxo-3-oxa-12-thia-11-selena-5,8,15-triazaicosan-20-oic acid (**16**). A stirred solution of glutathione (**15**, 15.2 mg,

0.0496 mmol) in acetonitrile/water (1:1, 2 mL) was treated with seleninic acid **10** (20.0 mg, 0.0496 mmol) in one portion. After 2 h, the resulting solution was directly purified by reverse-phase chromatography (gradient 5–60% acetonitrile over 10 min) and then lyophilized to give 19.0 mg (57%) of **16** as a white amorphous powder showing a single peak at 3.28 min by HPLC analysis. ¹H-NMR (500 MHz, CD₃OD) δ 7.17–7.29 (m, 5 H), 4.68 (dd, 1 H, *J* = 9.5, 4.5 Hz), 4.20–4.28 (m, 1 H), 4.02 (t, 1 H, *J* = 6.5 Hz), 3.93 (br s, 2 H), 3.52–3.62 (m, 1 H), 3.42–3.52 (m, 1 H), 2.96–3.10 (m, 3 H), 2.80–2.96 (m, 3 H), 2.58 (t, 2 H, *J* = 6.5 Hz), 2.12–2.29 (m, 2 H), 1.35 (s, 9 H); ¹³C-NMR (126 MHz, CD₃OD) δ 173.3, 173.2, 171.7, 171.5, 170.4, 156.4, 137.4, 129.2, 128.3, 126.6, 79.5, 56.4, 53.7, 52.4, 40.7, 39.0, 38.3, 33.3, 31.2, 30.0, 27.5, 25.9; ESI-MS *m/z* 678.1 MH⁺.

4. Conclusions

Densely functionalized and unprotected coupling partners (4-thiuridine and glutathione) participate in the seleninic acid–thiol redox coupling reaction with the 2-seleninatoethyl amide **10** under mild and neutral conditions in protic solvents. The selenosulfide products **14** and **16**, while formed in modest yield, are stable and easily characterized. No obvious impediment prevents analogous coupling reactions between peptide-based seleninic acids and thiol-bearing peptides, proteins, or oligonucleotides, and such applications may now be contemplated.

Supplementary Materials

NMR spectra for compounds **8–12**, **14**, and **16**, and HPLC trace for **16**.

Supplementary materials can be accessed at: <http://www.mdpi.com/1420-3049/18/2/1963/s1>.

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References

1. Kolb, H.C.; Finn, M.G.; Sharpless, K.B. Click chemistry: Diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2004–2021.
2. Finn, M.G.; Fokin, V.V. Click chemistry: Function follows form. *Chem. Soc. Rev.* **2010**, *39*, 1231–1232.
3. Meldal, M.; Tornøe, C.W. Cu-Catalyzed azide-alkyne cycloaddition. *Chem. Rev.* **2008**, *108*, 2952–3015.
4. Sletten, E.M.; Bertozzi, C.R. Bioorthogonal chemistry: Fishing for selectivity in a sea of functionality. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 6974–6998.
5. Jewett, J.J.; Bertozzi, C.R. Cu-free click cycloaddition reactions in chemical biology. *Chem. Soc. Rev.* **2010**, *39*, 1272–1297.
6. Dirks, A.J.; Cornelissen, J.J.L.M.; van Delft, F.L.; van Hest, J.C.M.; Nolte, R.J.M.; Rowan, A.E.; Turjes, F.P.J.T. From (bio)molecules to biohybrid materials with the click chemistry approach. *QSAR Comb. Sci.* **2007**, *26*, 1200–1210.

7. Giffin, M.J.; Heaslet, H.; Brik, A.; Lin, Y.-C.; Cauvi, G.; Wong, C.-H.; McRee, D.E.; Elder, J.H.; Stout, C.D.; Torbett, B.E. A copper(I)-catalyzed 1,2,3-triazole azide-alkyne click compound is a potent inhibitor of a multidrug-resistant HIV-1 protease variant. *J. Med. Chem.* **2008**, *51*, 6263–6270.
8. Manetsch, R.; Krasinski, A.; Radic, Z.; Raushel, J.; Taylor, P.; Sharpless, K.B.; Kolb, H.C. *In situ* click chemistry: Enzyme inhibitors made to their own specifications. *J. Am. Chem. Soc.* **2004**, *126*, 12809–12818.
9. Colombano, G.; Travelli, C.; Galli, U.; Caldarelli, A.; Chini, M.G.; Canonico, P.L.; Sorba, G.; Bifulco, G.; Tron, G.C.; Genazzani, A.A. A novel potent nicotinamide phosphoribosyltransferase inhibitor synthesized via click chemistry. *J. Med. Chem.* **2010**, *53*, 616–623.
10. El-Sagheer, A.H.; Brown, T. Click nucleic acid ligation: Applications in biology and nanotechnology. *Acc. Chem. Res.* **2012**, *45*, 1258–1267.
11. Nimmo, C.M.; Shoichet, M.S. Regenerative biomaterials that “click”: Simple, aqueous-based protocols for hydrogel synthesis, surface immobilization, and 3D patterning. *Bioconjug. Chem.* **2011**, *22*, 2199–2209.
12. Azagarsamy, M.A.; Anseth, K.S. Bioorthogonal click chemistry: An indispensable tool to create multifaceted cell culture scaffolds. *ACS Macro Lett.* **2013**, *2*, 5–9.
13. Best, M.D. Click Chemistry and Bioorthogonal Reactions: Unprecedented selectivity in the labeling of biological molecules. *Biochemistry* **2009**, *48*, 6571–6584.
14. Canalle, L.A.; Vong, T.; Adams, P.H.H.M.; van Delft, F.L.; Raats, J.M.H.; Chivi, R.G.S.; van Hest, J.C.M. Clickable enzyme-linked immunosorbent assay. *Biomolecules* **2011**, *12*, 3692–3697.
15. Mindt, T.L.; Struthers, H.; Brnas, L.; Anguelov, T.; Schweinsberg, C.; Maes, V.; Tourwe, D.; Schibli, R. “Click to chelate”: Synthesis and installation of metal chelates into biomolecules in a single step. *J. Am. Chem. Soc.* **2006**, *128*, 15096–15097.
16. Cunningham, C.W.; Mukhopadhyay, A.; Lushington, G.H.; Blagg, B.S.J.; Prisinzano, T.E.; Krise, J.P. Uptake, distribution and diffusivity of reactive fluorophores in cells: Implications toward target identification. *Mol. Pharm.* **2010**, *7*, 1301–1310.
17. Liu, X.-M.; Quan, L.-D.; Tian, J.; Laquer, F.C.; Cibiroski, P.; Wang, D. Syntheses of click PEG-dexamethasone conjugates for the treatment of rheumatoid arthritis. *Biomacromolecules* **2010**, *11*, 2621–2628.
18. Kamphuis, M.M.J.; Johnston, A.P.R.; Such, G.K.; Dam, H.H.; Evans, R.A.; Scott, A.M.; Nice, E.C.; Heath, J.K.; Caruso, F. Targeting of cancer cells using click-functionalized polymer capsules. *J. Am. Chem. Soc.* **2010**, *132*, 15881–15883.
19. Ochs, C.J.; Such, G.K.; Yan, Y.; van Koerverden, M.P.; Caruso, F. Biodegradable click capsules with engineered drug-loaded multilayers. *ACS Nano* **2010**, *4*, 1653–1663.
20. Kice, J.L.; Lee, T.W.S. Oxidation-reduction reactions of organoselenium compounds. 1. Mechanism of the reaction between seleninic acids and thiols. *J. Am. Chem. Soc.* **1978**, *100*, 5094–5102.
21. Abdo, M.; Knapp, S. Biomimetic seleninates and selenonates. *J. Am. Chem. Soc.* **2008**, *130*, 9234–9235.
22. Abdo, M.; Liu, S.; Zhou, B.; Walls, C.D.; Wu, L.; Knapp, S.; Zhang, Y.-Y. Seleninate in place of phosphate: Irreversible inhibition of protein tyrosine phosphatases. *J. Am. Chem. Soc.* **2008**, *130*, 13196–13197.

23. Abdo, M.; Knapp, S. Mechanism of a redox coupling of seleninic acid with thiol. *J. Org. Chem.* **2012**, *77*, 3433–3438.
24. Kice, J.L.; Purkiss, D.W. The induced decomposition of S-tert-butyl benzenethioseleninate. *J. Org. Chem.* **1987**, *52*, 3448–3451.
25. Abdo, M.; Zhang, Y.; Schramm, V.L.; Knapp, S. Electrophilic aromatic selenylation: New OPRT inhibitors. *Org. Lett.* **2010**, *12*, 2982–2985.
26. Knapp, S.; Darout, E. New reactions of selenocarboxylates. *Org. Lett.* **2005**, *7*, 203–205.
27. Back, T.G.; Collins, S.; Krishna, M.V. Reactions of sulfonylhydrazides with benzeneseleninic acid, selenium halides, and sulfur halides. A convenient preparation of selenosulfonates and thiosulfonates. *Can. J. Chem.* **1987**, *65*, 38–42.
28. Back, T.G.; Collins, S. A convenient synthesis of selenolsulfonates from the oxidation of sulfonylhydrazides with benzeneseleninic acid. *Tetrahedron Lett.* **1980**, *21*, 2213–2214.
29. Coleman, R.S.; Siedlicki, J.M. Synthesis of a 4-thio-2'-deoxyuridine containing oligonucleotide. Development of the thiocarbonyl group as a linker element. *J. Am. Chem. Soc.* **1992**, *114*, 9229–9230.
30. Nogueira, C.W.; Rocha, J.B.T. Diphenyl diselenide a Janus-faced molecule. *J. Braz. Chem. Soc.* **2010**, *21*, 2055–2071.
31. Flemer, S., Jr. Selenol protecting groups in organic chemistry: Special emphasis on selenocysteine Se-protection in solid phase peptide synthesis. *Molecules* **2011**, *15*, 3232–3251.
32. Boutureira, O.; Bernardes, G.J.L.; Fernandez-Gonzalez, M.; Anthony, D.C.; Davis, B.G. Selenenylsulfide-linked homogeneous glycopeptides and glycoproteins: Synthesis of human “hepatic Se metabolite A”. *Angew. Chem. Int. Ed. Engl.* **2012**, *51*, 1432–1436.
33. Sarma, B.K.; Mugesh, G. Antioxidant activity of the anti-inflammatory compound ebselen: A reversible cyclization pathway via selenenic and seleninic acid intermediates. *Chem. Eur. J.* **2008**, *14*, 10603–10614.
34. Liu, Q.; Wang, X.; Yang, X.; Liang, X.; Guo, Z. Fast cleavage of a diselenide induced by a platinum(II)-methionine complex and its biological implications. *J. Inorg. Biochem.* **2010**, *104*, 1178–1184.
35. Crich, D.; Karatholuvhu, M.; Krishnamurthy, V.; Hutton, T.K.; Brebion, F.; Subramanian, V. Dechalcogenative methods for the preparation of allylic sulfides. WO2008134058 (A1), 6 November 2008.
36. Salvadori, S.; Marastoni, M.; Balboni, G.; Sarto, G.P.; Tomatis, R. Synthesis and opioid activity of dermorphin tetrapeptides bearing D-methionine S-oxide at position 2. *J. Med. Chem.* **1986**, *29*, 889–894.
37. Higashiura, K.; Toyomaki, Y.; Ienaga, K. The chemical conversion of C-terminal glycines in peptides into taurine. *J. Chem. Soc. Chem. Commun.* **1989**, *1989*, 521–522.

Sample Availability: Samples of the compounds **7** and **10** are available from the authors.