

# **Studies of redox and exchange reactions of (seleno)cysteine peptides and model compounds**

*Dissertation*

zur Erlangung des akademischen Grades  
doctor rerum naturalium (Dr. rer. nat.)

vorgelegt der

Naturwissenschaftlichen Fakultät II – Chemie, Physik und Mathematik  
der Martin-Luther-Universität Halle-Wittenberg

von Herrn Dipl.-Chem. Alex Schneider  
geboren am 24.05.1979 in Tschita-46, Russland

Gutachter:

1. Prof. Dr. L.A. Wessjohann (Halle/Saale)
2. Prof. Dr. C. Jacob (Saarbrücken)

Halle (Saale), 05.04.2011

*Für meine Familie  
und meine Freunde*

**"Selenium has the narrowest band of any toxic chemical  
between what's safe and what's toxic,"**

said Joseph Skorupa,  
biologist for the U.S. Fish and Wildlife Service.

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## Acknowledgements/Danksagung

Für die interessante Themenstellung, die Betreuung der Arbeit und die stetige Unterstützung danke ich im besonderen **Herrn Prof. Dr. Ludger Wessjohann**.

Dem Korreferenten sei für die Übernahme des Korreferates gedankt.

Für die Unterstützung meiner elektrochemischen Arbeiten und die freundliche Aufnahme in Exeter (Großbritannien) und Saarbrücken danke ich **Herrn Prof. Dr. Claus Jacob**.

Eu gostaria de agradecer ao **Senhor Prof. Dr. Braga** pela acolhedora recepção no período em que estive em Santa Maria (Brasil). Também sou grato a ele por toda ajuda no laboratório e pelas discussões intelectuais referentes à Química de Selênio. O tempo que despendi no Brasil foi para mim uma experiência interessante e enriquecedora.

Besonderer Dank geht an **Herrn Dr. Wolfgang Brandt** für seine Unterstützung in Modeling und quantochemischen Berechnungen.

Des Weiteren möchte ich mich bei **Herrn Prof. Dr. Bernhard Westermann** für seine Unterstützung und Diskussions- und Hilfsbereitschaft bedanken.

Der gesamten Abteilung sei für die freundliche Zusammenarbeit gedankt.

**Frau Martina Lerbs, Frau Katharina Michels** und **Herrn Dr. Jürgen Schmidt** danke ich für die Aufnahme von ESI-MS-Spektren sowie **Frau Christne Kuhnt** für die Aufnahme von HPLC/MS-Chromatogrammen.

Bei **Frau Maritta Süße, Herrn Dr. Tilo Lübken** und **Frau Dr. Andrea Porzel** möchte ich mich für die Aufnahme und die Hilfe bei der Auswertung von zahlreichen NMR-Spektren bedanken.

**Frau Gudrun Hahn** und **Frau Anett Werner** danke ich für die Aufnahme von HPLC-Chromatogrammen.

Bei **Herrn Dr. Norbert Arnold** und **Frau Dr. Katrin Franke** möchte ich mich für die Hilfe bei der Auswahl verschiedener Trennungsmethoden bedanken.

**Herrn Dr. Muhammad Abbas, Herrn Dr. Oliver Kreye, Frau Angela Schaks, Herrn Dr. Kai Naumann, Herrn Tobias Draeger, Herrn Dr. Simon Rieping-Dörner, Frau Dr. Chrisiane Neuhaus, Herrn Matthäus Getlik, Herrn Martin Claudio Nin Brauer** danke ich für die freundliche Zusammenarbeit, der praktischen Unterstützung und den anregenden Diskussionen.

Ein besonderer Dank gilt meinem Freund **Dr. Andreas Wagner**. Ich möchte mich ebenso bei allen meinen **FreundenInnen** für ihren Optimismus und Beistand während all dieser Zeit bedanken.

**В заключении я хотел бы поблагодарить мою семью, в частности моих родителей и мою сестру, за их терпение и поддержку во всем.**

## Abbreviations

AA	Any of the 21 proteinogenic amino acids
Ac	Acetyl
Acm	Acetoamide
ACN	Acetonitrile
ADP	Adenosine diphosphate
All	Allyl
AMP	Adenosine monophosphate
Arg (R)	Arginine
Asp (D)	Aspartic acid
ATP	Adenosine 5'-triphosphate
Bn	Benzyl
Boc	<i>N</i> -tert-Butoxycarbonyl
Bzh	Benzhydryl
Cbz (Z)	Carbobenzyloxy
COSY	Correlation spectroscopy
Cys (C)	Cysteine
Dbs	Dibenzosuberyl
DCM	Dichloromethane
DEPBT	(3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one
DIAD	Diisopropyl azodicarboxylate
DIPEA	<i>N</i> -Ethyl-diisopropylamine
DMAD	Dimethyl acetylenedicarboxylate
DMAP	4-(Dimethylamino)pyridine
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethylsulfoxide
DmTrxR	Thioredoxin reductase in <i>Drosophila melanogaster</i>
Dpm	Diphenylmethyl
DTT	Dithiothreitol
ESI	Electrospray ionization
Et	Ethyl
FAD	Flavin adenine dinucleotide
Fmoc	9-Fluorenylmethyl carbamate
Glu (E)	Glutamic acid
Gly (G)	Glycine
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidized)
h	Hour(s)
HBTU	<i>O</i> -Benzotriazole- <i>N,N,N',N'</i> -tetramethyl-uronium-hexafluoro-phosphate
His (H)	Histidine
HMBC	Heteronuclear Multiple Bond Correlation
HMPA	Hexamethylphosphoramide
HOBt	Hydroxybenzotriazole
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear Single Quantum Correlation
LiHMDS	Lithium hexamethyldisilazide

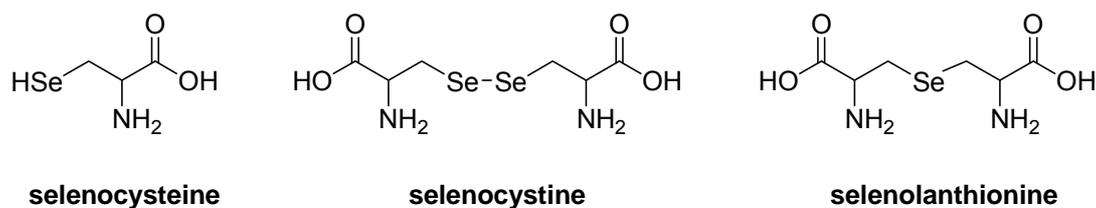
MCR	Multi component reaction
Me	Methyl
MeOH	Methanol
Mob (PMB)	<i>p</i> -Methoxybenzyl
MS	Mass spectrometry
NADP <sup>+</sup>	Nicotinamide adenine dinucleotide phosphate
NHE	Normal hydrogen electrode
NMR	Nuclear magnetic resonance
Npys	S-3-Nitro-2-pyridinesulfenyl
Ph	Phenyl
Pro (P)	Proline
py	Pyridine
r.t	Room temperature
RP	Reverse phase
Sec (U)	Selenocysteine
SECIS	SelenoCysteine Insertation Sequence
Ser (S)	Serine
SPE	Solid Phase Extraktion
SPPS	Solid Phase Peptide Synthesis
SSE	Standard silver reference electrode
-Su	Succinimide
TBHP	tert-Butylhydroperoxide
t-Bu	tert-Butyl
Tec	Tellurocysteine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropyl silane
TOF-MS	Time of flight mass spectrometry
Trt	Triphenylmethyl
Trx	Thioredoxin
TrxR	Thioredoxin reductase
-Ts	Tosyl
Tyr (Y)	Tyrosine
UV	Ultraviolet
Val (V)	Valine

# 1 Introduction\*

## 1.1 Selenium in chemistry and biochemistry

In 1817, the Swedish chemist Berzelius and his colleague Gahn have observed, during the study of a method to produce sulphuric acid, an impurity with a very intense scent. Berzelius thought it was Tellurium (Latin *tellus* meaning "earth"), but he later proved that it was a previously unknown element. He named it selenium (Se) after the Greek moon goddess Selene.(Berzelius, 1818a; Berzelius, 1818b) It ranks seventieth in abundance among the elements of the Earth's crust and constitutes approximately  $1 \times 10^{-5} - 8 \times 10^{-5}$  % of the elemental composition.(Chizhikov and Schastlivyi, 1968) Since many selenium compounds can be toxic in higher amount and the volatile members exhibit a characteristic garlicky smell, selenium research was quite unattractive for more than 100 years. In 1916, this element was detected for the first time in normal human tissue samples.(Gassmann, 1916) Interestingly, this finding had not attracted attention for more then 35 years. Only in 1954, Pinsent was the first one who reported that selenium is essential for the enzyme activity of "formic dehydrogenase" in *E. coli*.(Pinsent, 1954) The discovery of selenium as an essential rare element in mammals and birds by Klaus Schwarz and others,(Patterson et al., 1957; Schwarz and Foliz, 1957) and the knowledge of its incorporation into proteins at first recognized as element(Shum and Murphy, 1972) and later as selenocysteine (Figure 1.1.)(Cone et al., 1976; Forstrom et al., 1978) started the rapidly increasing interest in biological and medicinal selenium research. A highlight of this development was the unravelling of the amino acid sequence of glutathione peroxidase, by which selenocysteine was established as the 21<sup>st</sup> proteinogenic amino acid.(Bock et al., 1991; Gunzler et al., 1984) The next keystone was the discovery of Chambers et al. that selenocysteine (Sec) is encoded by the codon TGA (UGA), which is normally used as stop signal.(Chambers et al., 1986; Hatfield and Gladyshev, 2002; Zinoni et al., 1986) The reinterpretation of the stop codon as codon for selenocysteine is induced by a very special secondary structural motive of the mRNA, the so called SECIS-element (*Selenocysteine Insertation Sequence*) which in cooperation with a large protein cluster reprograms the ribosomal machine to incorporate Sec.(Baron et al., 1993; Bock and Stadtman, 1988; Heider and Bock, 1993; Stadtman, 1996) It became also quite clear that there are more factors involved in the biosynthesis of selenocysteine.(Allmang and Krol, 2006)

\* - adopted from review Wessjohann, L. A., Schneider, A., Abbas, M., and Brandt, W., *Selenium in chemistry and biochemistry in comparison to sulfur. Biol. Chem.* 388 (2007): 997-1006.

**Figure 1.1.**

Until today, many selenocysteine containing proteins have been discovered, mostly in mammals, bacteria, and fish. (Castellano et al., 2004; Gan et al., 2005; Jensen et al., 2005; Kryukov and Gladyshev, 2000; Kryukov et al., 2003; Martens and Suarez, 2003; Novoselov et al., 2006; Pedersen et al., 2006; Sanders et al., 1999; Fu et al., 2002) An excellent overview of human selenoproteins and their function was given by Gromer et al. (Gromer et al., 2005) For many of them, the enzymatic function is still unknown, whereas for others their function in redox processes is clearly established. (Angstwurm and Gaertner, 2006; Beckett and Arthur, 2005; Brandt and Wessjohann, 2005; Brown and Arthur, 2001; Flohe et al., 1973; Gladyshev and Hatfield, 1999; Gromer et al., 2005; Patching and Gardiner, 1999; Walker et al., 2004; Whanger, 2000) The most crucial question to be asked by a chemist working on selenoproteins to our opinion is: why does nature go through such lengths to have selenium, i.e. selenocysteine, in a certain position instead of its closest "relative" sulfur, i.e. cysteine.

## 1.2 Selenium vs. sulfur

When comparing some interesting general properties of selenium and sulfur (Table 1.1.) a functional advantage of selenium over sulfur in enzyme reactions becomes not immediately evident. Ion radii, redox potentials, and electronegativity are similar. The polarizability and thus the nucleophilicity of selenium appears to be somewhat higher (see e.g. Kang and Spears, 1990b). But is this enough to render Sec-enzymes so much more efficient than Cys-enzymes of the same type? Could a fivefold excess of easier accessible Cys-enzymes not make up the Sec-advantage, e.g. in nucleophilicity? Indeed, several organisms appear to be able to live with very few or without (yet known) Sec-proteins, and cysteine in many proteins can be tuned by folding or flanking amino acids to be more nucleophilic or to alter redox potentials. (Gromer et al., 2003; Johansson et al., 2006; Schneider et al., 2007) Thus the question remains: What is the evolutionary advantage of incorporating Sec by the complex and costly SECIS mechanism instead of using readily available Cys?

An important difference is the pKa of R-SH vs. R-SeH. This was studied and utilized, among others, by Moroder et al. and Rabenstein et al. (Moroder, 2005; Moroder et al., 2005; Rabenstein and Yeo, 1994; Rabenstein and Yeo, 1995; Rabenstein and Weaver, 1996) They studied S-S vs. S-Se vs. Se-Se-bridge formation in Cys/Sec peptides and revealed first valuable insights.

A detailed analysis based on model compounds and on thioredoxin reductases, which are important members of the Sec/Cys-enzymes, was performed by us using synthesis, electrochemistry, MS, NMR, molecular modelling, quantum mechanical calculations, site directed mutations, and activity assays. (Brandt and Wessjohann, 2005; Gromer et al., 2006; Schneider et al., 2007) The combined study allowed a detailed insight into the effect of neighbouring groups to pKa values of S-H vs. Se-H.

**Table 1.1. General properties of selenium and sulfur(Wessjohann et al., 2007)**

Property	Se	S
Electron configuration	[Ar] 3d <sup>10</sup> .4s <sup>2</sup> .4p <sup>4</sup>	[Ne] 3s <sup>2</sup> .3p <sup>4</sup>
Atomic radius (in pm, emp.)	115	100
Atomic radius (in pm, calc.)	103	88
Covalent radius (in pm, emp.)	116	102
Van der Waals radius (in pm)	190	180
Ion radius (Pauling) ox. state: (I)	66	53
(VI)	42	29
(-I)	232	219
(-II)	198	184
Bond length (in pm) of dichalcogen	232 (Se-Se)	205 (S-S)
Bond length (in pm) carbon-chalcogen	196 (C-Se)	180 (C-S)
Molecular volume (in cm <sup>3</sup> )	16.42	15.53
Diatomic bond energies (kJ mol <sup>-1</sup> )	332.6 (Se-Se)	425.3 (S-S)
	314.5 (Se-H)	344.3 (S-H)
	590.4 (Se-C)	714.1 (S-C)
	- 371.1 (Se-S) -	
Homolytic bond cleavage energies (kJ mol <sup>-1</sup> )		
HX-H	334.9	381.6
CH <sub>3</sub> X-CH <sub>3</sub>	234	307.9, 272
CH <sub>3</sub> X-XCH <sub>3</sub>	197.6*	204.7*, 273.6
Electron affinity (in kJ/mol)	195.0	200
Ionization energies (in kJ/mol) 1st	914.0	999.6
2 <sup>nd</sup>	2045	2252
3 <sup>rd</sup>	2973.7	3357
Redox potentials (V)		
$XO_4^{2-} + H_2O + 2e^- \Rightarrow XO_3^{2-} + 2OH^-$ (pH=7)	0.05*	-0.93*
$XO_3^{2-} + 6H^+ + 6e^- \Rightarrow X^{2-} + 3H_2O$ (pH<7)	0.276*	0.231*
$X + 2e^- \Rightarrow X^{2-}$	-0.924*	-0.476 (vs. SCE)
$RX - XR + 2e^- \Rightarrow 2RX^-$ $R = CH_2CH(NH_2)COOH$	-0.488 (vs. NHE)	-0.233 (vs. NHE)
Polarizability (in Å <sup>3</sup> )	3.8	2.9
Pauling electronegativity	2.55	2.58
pKa	5.2	8.3

\* calculated using quantum mechanical methods

Additional values may be taken from <http://www.webelements.com>

### 1.3 Biochemistry and function of selenocysteine

In recent years the number of detected and identified selenoproteins has grown substantially. There are more than 20 prokaryotic selenoproteins (Kryukov and Gladyshev, 2004) and even 25 human selenoproteins. (Kryukov et al., 2003) Table 1.2. shows several important selenocysteine-containing enzymes and their biological functions. (Roy et al., 2005)

**Table 1.2. Selenocysteine-containing enzymes and their biological functions**

Enzyme	Reaction
Formate dehydrogenases	$HCOOH \rightarrow CO_2 + 2H^+ + 2e^-$
NiFeSe-hydrogenases	$H_2 \rightarrow 2H^+ + 2e^-$
Glycine reductase	$Gly + 2e^- + 4H^+ + ADP + P_i \rightarrow acetate + NH_4^+ + ATP$
Selenophosphate synthetase	$HSe^- + ATP \rightarrow HSePO_3H_2 + AMP + P_i$
Glutathione peroxidases (GPx)	$H_2O_2 + 2GSH \rightarrow H_2O + GSSG$
Phospholipid-hydroperoxide-GPx	$ROOH + 2GSH \rightarrow ROH + H_2O + GSSG$
Type I iodothyronine deiodinase	$L-Thyroxine + 2e^- + H^+ \rightarrow 3,5,3'-triiodothyronine + I^-$
Thioredoxin reductase	$NADPH + Trx_{ox} \begin{matrix} S \\   \\ S \end{matrix} \rightarrow NADP^+ + Trx_{red} \begin{matrix} SH \\   \\ SH \end{matrix}$
Selenoprotein W	?
Selenoprotein P	<i>Antioxidant?</i>

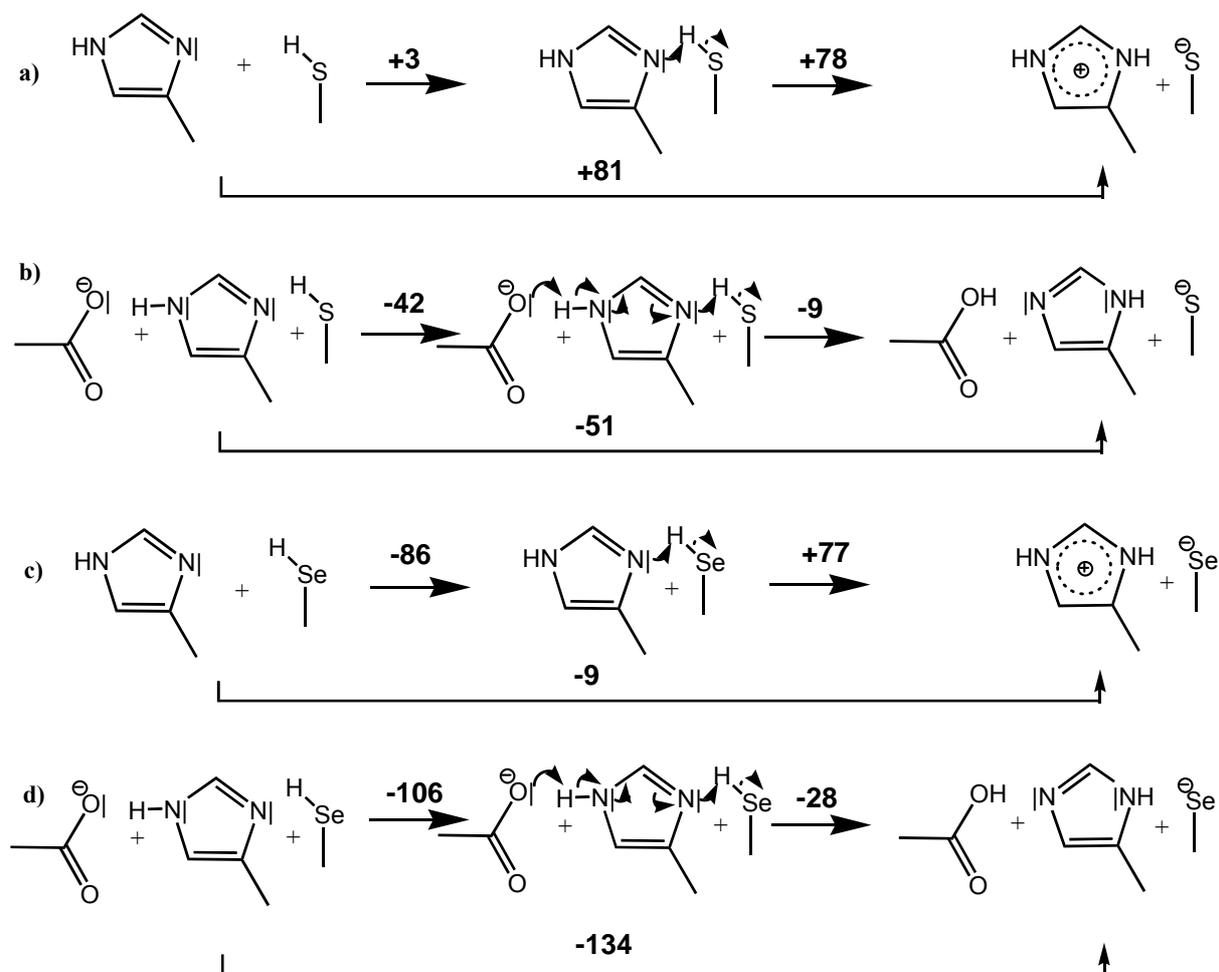
*GSH: reduced glutathione, ROOH: Lipid hydroperoxide, Trx: thioredoxin (a soluble reductive peptide)*

#### 1.3.1 Mammalian thioredoxin reductases

Mammalian thioredoxin reductases are so-called large thioredoxin-reductases (TrxRs) and contain a selenocysteine (Sec) residue located in a flexible C-terminal tail. (Gladyshev et al., 1996; Gromer et al., 1998; Kryukov et al., 2003; Tamura and Stadtman, 1996) The currently accepted model for the catalytic mechanism involves the transfer of electrons from the NADPH's reduced nicotinamide ring via a flavin to the N-terminal cysteines (Cys<sup>59</sup> and Cys<sup>64</sup>). Here the electrons are picked up by the other subunit of the C-terminal redox active site, formed by the sequentially adjacent Cys<sup>497</sup>Sec<sup>498</sup>-pair, which subsequently transfers them to the final substrate, e.g. thioredoxin. (Berggren et al., 1997; Berggren et al., 1999; Brandt

and Wessjohann, 2005; Fujiwara et al., 1999; Gasdaska et al., 1999; Gladyshev et al., 1998; Gromer et al., 2006; Hill et al., 1997; Sun et al., 1999; Zhong et al., 2000)

Very recently our group suggested, based on quantum mechanical calculations, that the formation of a selenolate anion is essential for a benefit over a sulfur analog; and that its formation is supported by a novel swapping triad (Glu-His-Sec/Glu-His-Cys, Figures 1.2. and 1.3.).(Brandt and Wessjohann, 2005; Gromer et al., 2006) The use of such a triad in redox processes is quite surprising since this activation principle is previously known from proteases to enhance the nucleophilicity of a serine oxygen or of a cysteine sulfur atom. However, similar to proteases the formation of an anion species (selenolate or thiolate, respectively.) is also supporting the initial redox process by increasing the reductive power (by generating a HOMO of higher energy). The quantum mechanical calculations did show that in the case of cysteine the formation of a thiolate anion is disfavored, if imidazole (the side chain of His) is the only available proton acceptor. Therefore, it is not as surprising that a catalytic triad (Cys-His-Glu/Asp) has an evolutionary advantage as it leads to the favored formation of a thiolate anion.(Brandt and Wessjohann, 2005; Gromer et al., 2006) Alternatively, the incorporation of selenocysteine in thioredoxin reductases leads to a similar advantage: formation of a selenolate anion. Compared to thiolate formation, this process is thermodynamically much more favored, even with imidazole alone acting as base (compare Figure 1.2a vs. 1.2c). If selenol is part of a triad (compare Figure 1.2b vs. 1.2d), the deprotonation is enhanced even more – or in other words: it can work under more acidic conditions compared to the sulfur case.



**Figure 1.2.** Thermodynamic values for the formation of a thiolate (a, b) or selenolate anion (c, d) based on DFT *ab initio* calculations (energies in kJ/mol). (a, c) values for catalytic diads, (b, d) values for catalytic triads. (Brandt and Wessjohann, 2005)

This advantage together with the different stabilities of a disulfide bond vs. a selenenylsulfide bond leads to a thermodynamically favored process for the formation of the active reduced form of the C-terminal Cys/Sec by 9.6 kJ/mol in comparison to a corresponding Sec→Cys mutated enzyme. This corresponds well with a ca. 100fold enhanced catalytic rate constant compared to a Sec498Cys mutant and demonstrates the advantage of selenocysteine over cysteine in pH-dependent redox processes. (Zhong and Holmgren, 2000)

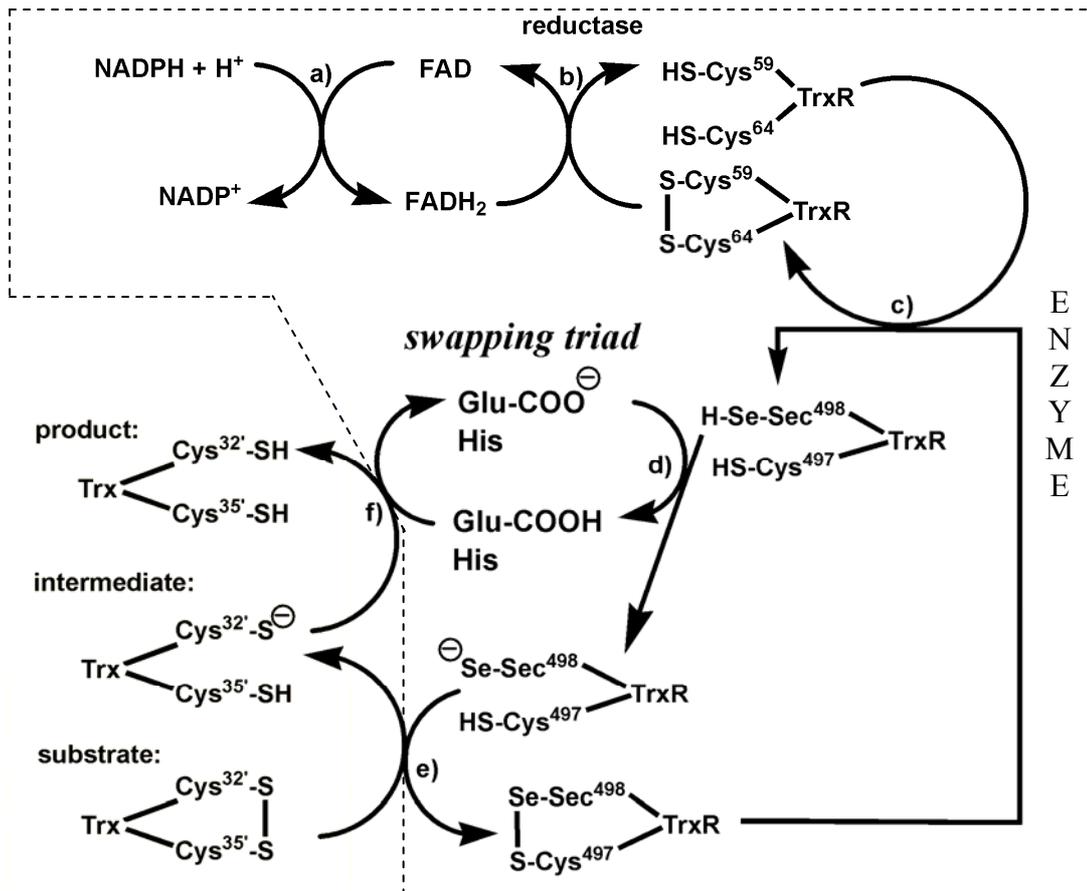
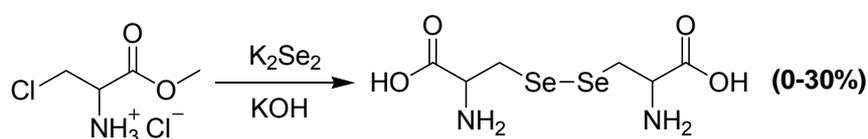


Figure 1.3. General catalytic mechanism in large thioredoxin reductases including a swapping catalytic triad that consists of Glu, His and  $\text{Sec}^{498}/\text{Cys}^{497}$

## 2 Synthesis of selenocysteine/selenocystine and its derivatives

### 2.1 Early synthetic methods\*

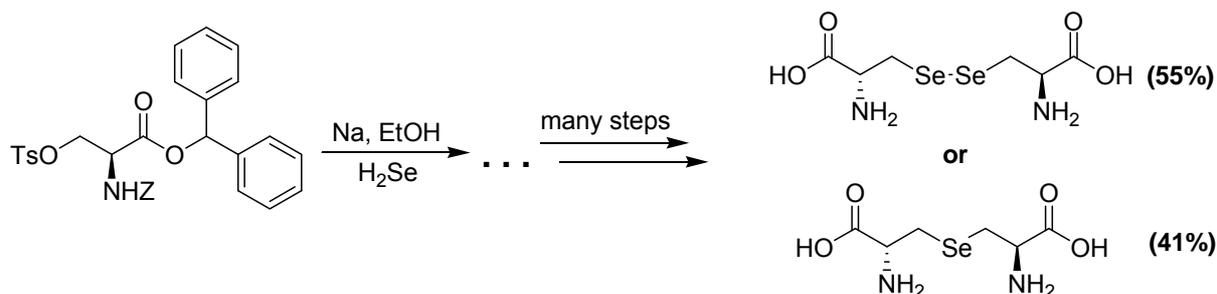
Fredga pioneered selenocysteine chemistry with his synthesis of DL- and meso-selenocystine and later also of D- and L-selenocystine (Scheme 2.1.).(Fredga, 1936) In this simple method, he mixed 3-chloroalanine methyl ester hydrochloride with aqueous potassium diselenide for 36 h at room temperature, but the yields are low and inconsistent (0-30%) and, therefore, this method never became popular.



**Scheme 2.1. The first synthesis of selenocystine reported by Fredga**

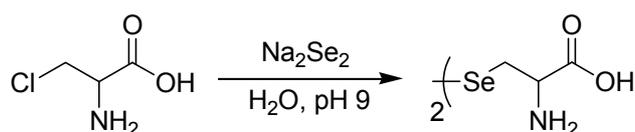
All methods for the synthesis of Sec published until 1973 were reviewed by Zdansky.(Zdansky, 1973) Unfortunately, most of them are tedious, time consuming and had low overall yields. Most methods for the synthesis of selenocysteine and its derivatives are based on the displacement of a serine hydroxyl group by various (organo) selenide anions. In most cases, the hydroxyl group is activated as tosylate. Walter introduced this approach and prepared selenocysteine derivatives in the relatively high yields.(Theodoropoulos et al., 1967) He used sodium benzyl selenolate as a nucleophilic agent and obtained Z- and Boc-protected benzyl-L-selenocysteine benzyl ester in 89% and 88% yield, respectively. Later he reported the analogous synthesis of L-selenocystine and L-selenolanthionine with an overall yield of 55% and 41%, respectively (Scheme 2.2.).(Roy et al., 1970) Besides of the moderate yield, these syntheses required an excess of hydrogen selenide for the preparation of one of the starting materials (sodium hydrogen selenide) and, therefore, they are impractical for labeling purposes and with respect to safety.

\* - adopted from article Wessjohann, L.A., Schneider, A., *Synthesis of selenocysteine and its derivatives with an emphasis on selenenylsulfide (-Se-S-) formation. Chem. Biodiv. 5, (2008): 375-388.*



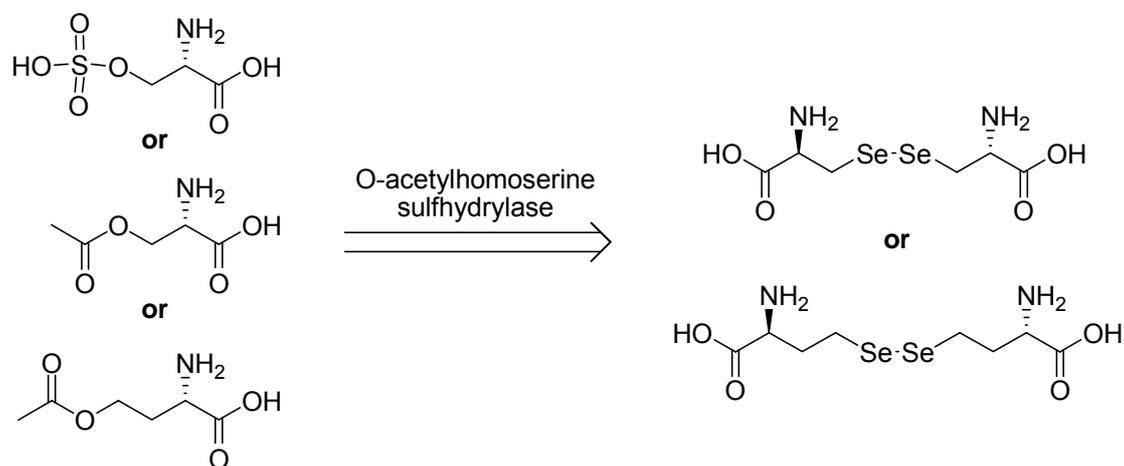
**Scheme 2.2.**

In 1985 Soda et al. obtained unprotected racemic selenocystine from  $\beta$ -chloroalanine with  $\text{Na}_2\text{Se}_2$  in water in 62% yield (Scheme 2.3). (Chocat et al., 1985a; Tanaka and Soda, 1987) This synthesis was similar to the method of Fredga (Fredga, 1936) and based on that of Klayman and Griffin for synthesis of dialkyl diselenides. (Klayman and Griffin, 1973) They also reported the formation of L-selenocystine from  $\beta$ -chloro-L-alanine, without mentioning any yield or experimental detail.



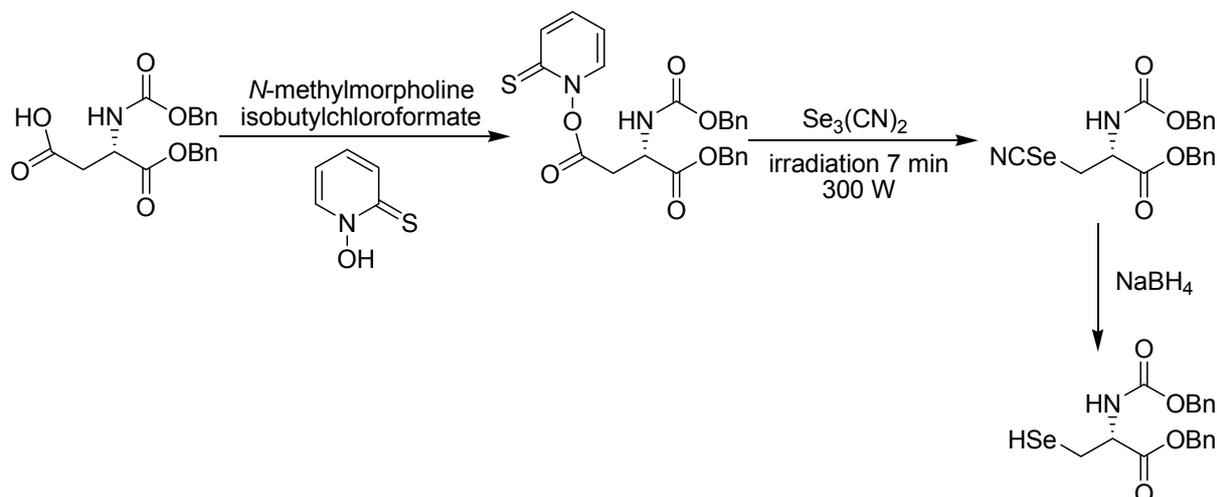
**Scheme 2.3. Soda's method**

At that time, Soda's group also reported the first biocatalytic synthesis of L-selenocystine and L-selenohomocystine (Scheme 2.4.). For this, L-O-acetyl-serine and -homoserine, and L-serine-O-sulfate were reacted with  $\text{Na}_2\text{Se}_2$  using O-acetylhomoserine sulfhydrylase (EC 4.2.99.10) as catalyst in good yields (50-60%). (Chocat et al., 1985b)



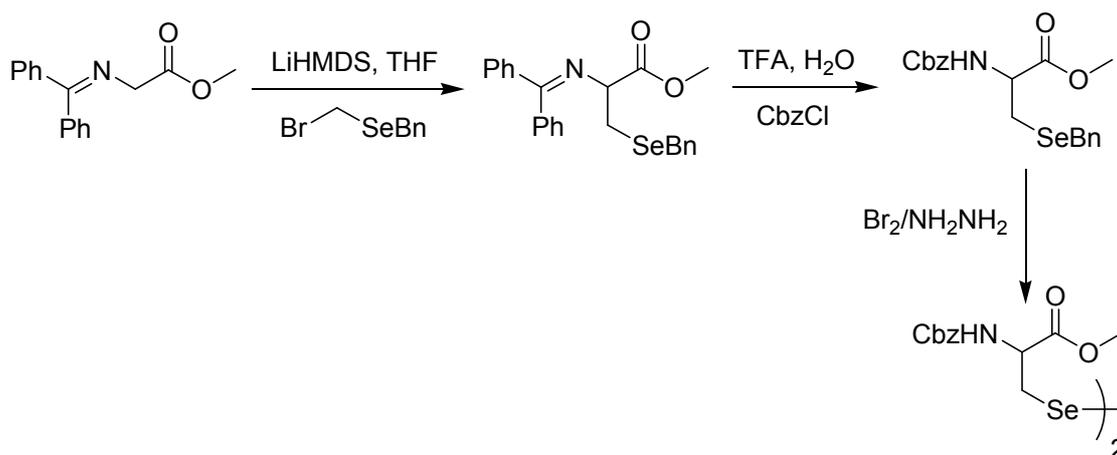
**Scheme 2.4. Biocatalytic synthesis of L-selenocystine and L-selenohomocystine**

Barton and co-workers reported a conceptually very different approach that involves a radical chain reaction (Scheme 2.5.). The (pyridine-2-thione)-ester is formed via a mixed anhydride from partially protected L-aspartic acid. (Barton et al., 1986) Irradiation, using triselenocyanide as a scavenger and selenocyanating reagent, gave the acylselenocyanate, which could be reduced to protected selenocyst(e)ine.



**Scheme 2.5. Barton's method**

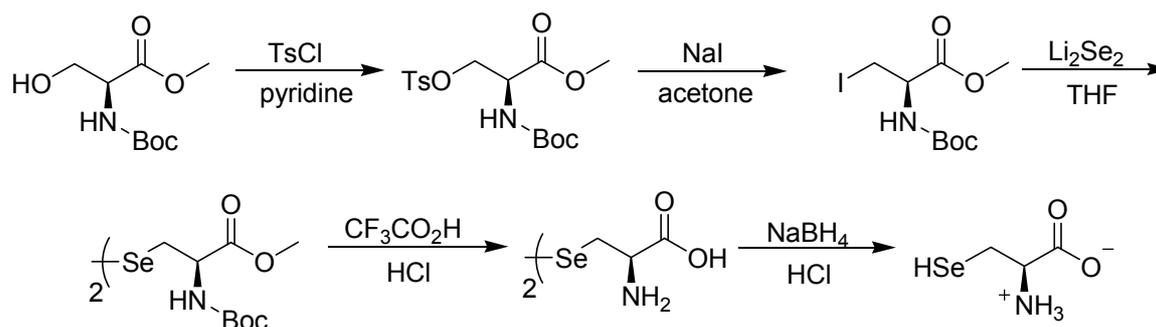
In yet another approach, Reich and co-workers offered an efficient synthesis of racemic selenocystine by alkylation of an O'Donnell glycine derivative with bromomethyl benzyl selenide under basic conditions (Scheme 2.6.). (Reich et al., 1986)



**Scheme 2.6.**

## 2.2 Recent synthetic methods\*

In 1997 Silks et al. reported the synthesis of enantiomerically pure L-selenocysteine and also L-tellurocysteine from suitably protected non-racemic  $\beta$ -haloalanines (Scheme 2.7.).(Stocking et al., 1997) Boc-protected serine methyl ester was converted into iodoalanine methyl ester via the tosylate. Reaction of the iodo compound with lithium diselenide or lithium ditelluride afforded protected selenocysteine or tellurocysteine, respectively. Deprotection of both the amino and carboxylate functionalities under acidic conditions, followed by purification, provided selenocysteine and tellurocysteine. Both can be reduced with sodium borohydride to obtain optically active selenocysteine (Sec) and tellurocysteine (Tec). Their overall yields from Boc-protected serine methyl ester (commercial available) were 47% and 14%, respectively. Unfortunately, the yields of this multistep synthesis did not transfer to scale-up procedures.

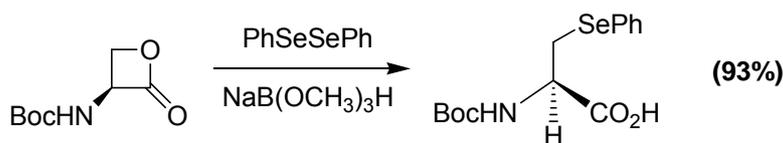


**Scheme 2.7. Silks's method of enantiomerically pure L-Sec and L-Tec**

At the same time, the group of Prof. Shirahama in Japan prepared *N*-Boc-protected L- and D-phenylselenoalanine from Vederas' serine- $\beta$ -lactone (Arnold et al., 1985; Arnold et al., 1987; Pansare et al., 1991) with sodium phenylselenolate prepared from diphenyl diselenide with sodium in THF-HMPA in 93% yield. (Sakai et al., 1997)

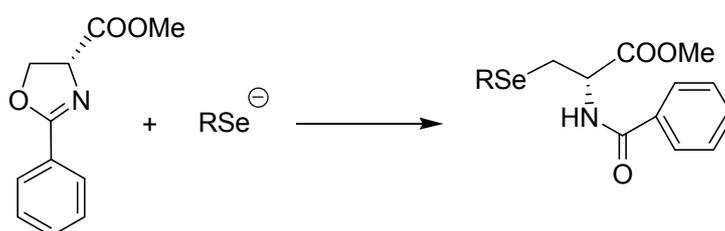
Later van der Donk and co-workers repeated this procedure with a small modification and obtained *N*-Boc-L-phenylselenocysteine in the same yield of 93% (Scheme 2.8.) with phenylselenolate anion generated from the reaction of diphenyl diselenide with sodium trimethoxyborohydride. (Okeley et al., 2000) Finally, the Boc-protected amino acid was converted into its better behaved Fmoc-derivate in 91% yield.

\* - adopted from article Wessjohann, L.A., Schneider, A., *Synthesis of selenocysteine and its derivatives with an emphasis on selenenylsulfide (-Se-S-) formation. Chem. Biodiv. 5, (2008): 375-388.*



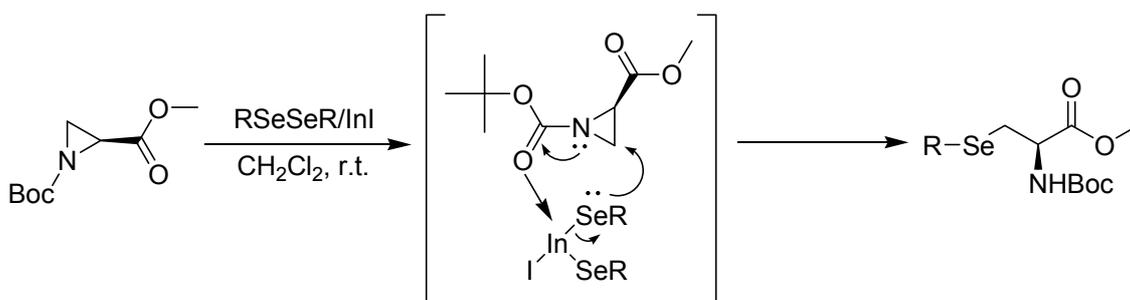
### Scheme 2.8. Van der Donk's method

As alternative to Vederas' serine- $\beta$ -lactone method, Braga reported the synthesis of selenocysteine derivatives by ring opening of 2-oxazolines in good yields (Scheme 2.9). (Braga et al., 2005)



### Scheme 2.9. Braga's method I

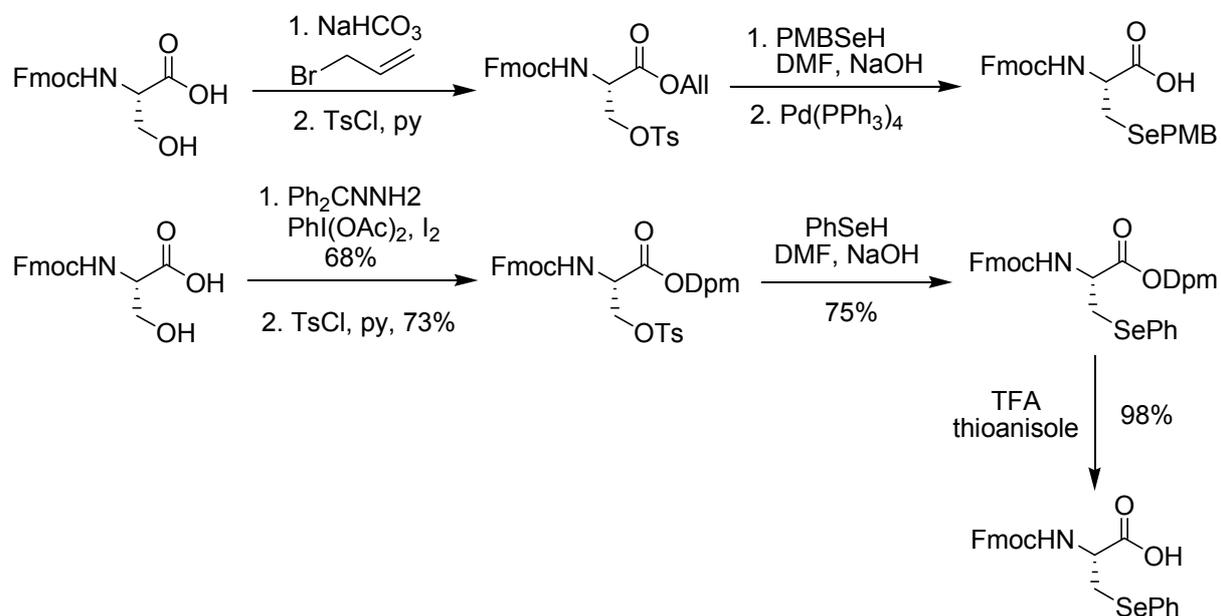
Later he developed a similar procedure starting from chiral *N*-Boc-aziridine using indium(I) iodide as reductant to produce selenides (Scheme 2.10). (Braga et al., 2006) The transition state proposed by Braga depicts the crucial importance of Boc-protection in this case.



### Scheme 2.10. Braga's method II

Later the group of van der Donk reported an alternative synthetic route to selenocysteine and Fmoc-phenylselenocysteine (Scheme 2.11). (Gieselman et al., 2001) They used three orthogonal protecting groups for the amino, carboxylate, and selenol functionalities. The carboxylate was protected as allyl ester, or as diphenylmethyl ester for the preparation of Fmoc-phenylselenocysteine. The four-step sequence provided Fmoc-phenylselenocysteine in

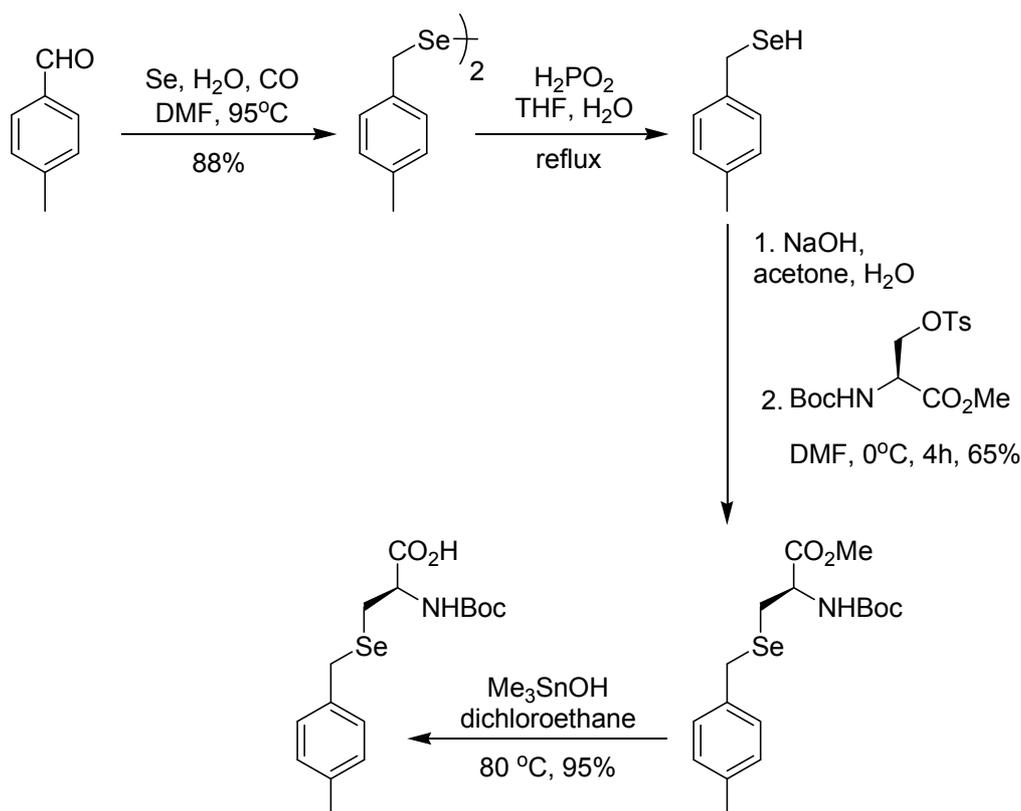
37 % overall yield on a 15 g scale and *N*-Fmoc-*Se*-(*p*-methoxybenzyl)-*Sec* in 61 % overall yield.



**Scheme 2.11. Van der Donk's alternative method (PMB = 4-methoxybenzyl, All = allyl, Dpm = diphenylmethyl)**

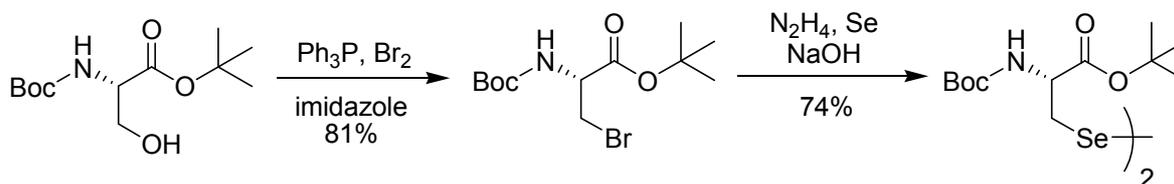
Fmoc is preferred to Boc to protect the amine, as its removal leads to less by-product formation, e.g. of dehydroalanine.

An efficient procedure similar to van der Donk's most recent method was developed by Dawson et al. to synthesize *N*-Boc-*Se*-(*p*-methylbenzyl)-*Sec* (Scheme 2.12.). (Metanis et al., 2006) The remarkable difference of this method was an unique preparation of (*p*-methylbenzyl)-diselenide, which was afforded employing elemental selenium under bubbling CO<sub>(g)</sub>. Me<sub>3</sub>SnOH was used to deprotect the methyl ester under mild conditions. This strategy led to the desired product in 45% overall yield.



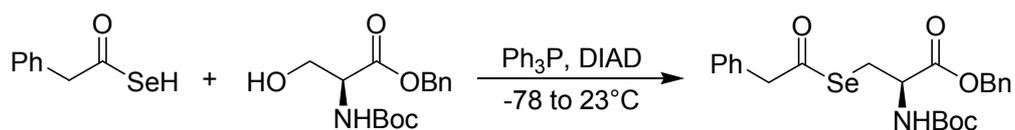
Scheme 2.12. Dawson's method

Lugtenburg et al. improved the nucleophilic displacement of the serine hydroxyl group by  $\text{M}_2\text{Se}_2$  (Scheme 2.13.).(Siebum et al., 2004) They activated the hydroxyl group with triphenylphosphane, bromine and imidazole, followed by  $\text{Na}_2\text{Se}_2$  prepared from the treatment of elemental selenium with hydrazine monohydrate and sodium hydroxide. The overall yield of this two step procedure to *N*-Boc-selenocysteine tert-butyl ester is 60%.



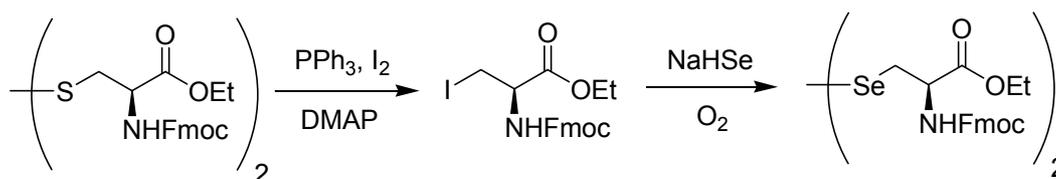
Scheme 2.13.

A similar, direct one-pot synthesis of a selenocysteine derivative under Mitsunobu conditions was achieved by nucleophilic displacement of a serine hydroxyl with an acyl protected selenol reagent by Knapp and Darout (Scheme 2.14.).(Knapp and Darout, 2005)



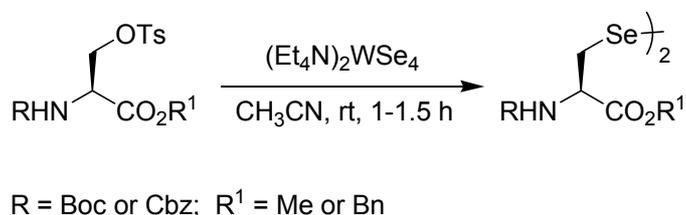
**Scheme 2.14.**

Iwaoka and coworkers have reported the two step conversion of disulfides to diselenides (cystine to selenocystine) via an iodide intermediate (Scheme 2.15.).(Iwaoka et al., 2006)



**Scheme 2.15. Iwaoka's method**

In 2003 Prof. Chandrasekaran's group introduced a new reagent for the synthesis of selenocysteine derivatives, a tetraselenotungstate  $[(Et_4N)_2WSe_4]$  that acted as Se-nucleophile (Scheme 2.16.).(Bhat et al., 2003) They produced Boc- and Cbz-protected selenocysteine methyl and benzyl ester from the corresponding serine tosylates in good yields (79-85%).



**Scheme 2.16.**

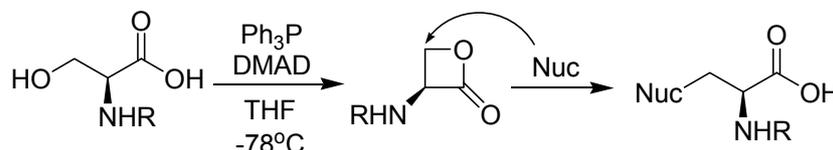
## 2.3 A new stereoselective synthesis of L-selenocysteine and its derivatives\*

### 2.3.1 Introduction – synthetic strategy

Within the scope of our study program of higher organochalcogenides, we decided to improve the synthetic route to selenocysteine Se-conjugates and tellurocysteine Te-conjugates with respect to the number of steps and scale-up. Our synthetic strategy is based on a previously reported, similar method for the generation of the unnatural amino acid (Se-phenyl)selenocysteine [(Ph)Sec] by ring opening of Boc-L-serine  $\beta$ -lactone (Scheme 2.8.).(Okeley et al., 2000)

Vederas and co-workers reported an efficient synthesis of  $\beta$ -lactones under Mitsunobu conditions (Scheme 2.17.).(Arnold et al., 1985; Arnold et al., 1987; Pansare et al., 1991) Interestingly, both procedures produce *N*-(*t*-Boc)- $\beta$ -lactone in high yield – 81% (with DMAD)(Arnold et al., 1987) and 67% (with DIAD)(Smith and Goodman, 2003) from *N*-(*t*-Boc)-D-serine, and 72% from *N*-(*t*-Boc)-L-serine.(Arnold et al., 1985)

Vederas's group has also shown, that serine  $\beta$ -lactone can be ring opened by nucleophiles to form  $\beta$ -substituted  $\alpha$ -amino acids (Scheme 2.17.).(Arnold et al., 1985)



**Scheme 2.17. Synthesis of  $\beta$ -substituted  $\alpha$ -amino acids via Vederas's  $\beta$ -lactone**

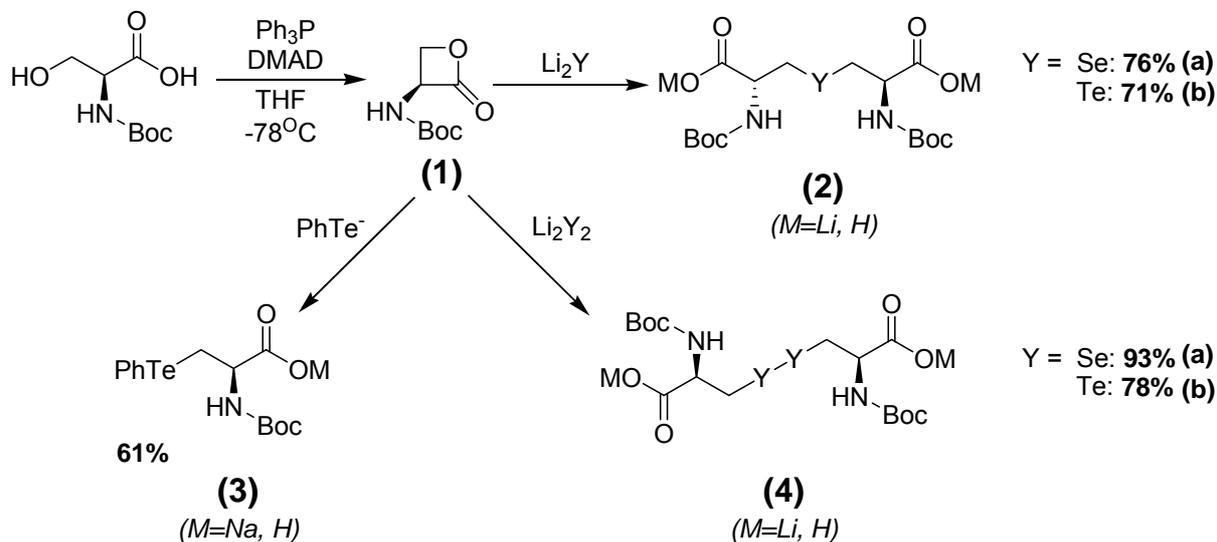
### 2.3.2 Synthesis

In order to demonstrate the wider scope of the  $\beta$ -lactone ring opening reaction, the possibility of transforming a serine  $\beta$ -lactone with several selenium and tellurium anions to the corresponding seleno- and tellurocysteine derivatives was investigated in a corporation with the Braga research group at the Federal University of Santa Maria, Brazil (Scheme 2.18.).

\* - adopted from article Schneider, A., Rodrigues, O. E. D., Paixao, M. W., Appelt, H. R., Braga, A. L., and Wessjohann, L. A., Stereoselective synthesis of Boc-protected L-seleno- and tellurolanthionine, L-seleno- and tellurocystine and derivatives. *Tetrahedron Lett.* 47, (2006): 1019-1021.

Boc-L-serine  $\beta$ -lactone (**1**) was synthesized by the ring closing reaction of Boc-protected L-serine with triphenylphosphine and DMAD in THF. The yield of this reaction was not higher than 44%. Vederas reported 81% yield (Arnold et al., 1987) and for a scale-up procedure 40% yield, but with the note it could be better in a smaller scale. (Pansare et al., 1991) The different yields might be explained by new methods for purification available nowadays compared to the 80-ties.

L-Selenolanthionine (**2a**) and L-tellurolanthionine (**2b**) are readily prepared by the attack of dilithium chalcogenides ( $\text{Li}_2\text{Se}$  and  $\text{Li}_2\text{Te}$ ) on the Boc-L-serine  $\beta$ -lactone (**1**). Furthermore, using dilithium dichalcogenides ( $\text{Li}_2\text{Se}_2$  and  $\text{Li}_2\text{Te}_2$ ), L-selenocystine (**4a**) and L-tellurocystine (**4b**) are obtained in good yields (Scheme 2.18.). Dilithium chalcogenides and dichalcogenides were available from the reaction of elemental selenium or tellurium with lithium triethyl-borohydride (super hydride) in a ratio 1:2 ( $\text{Li}_2\text{X}$ ) and 1:1 ( $\text{Li}_2\text{X}_2$ ), respectively. For the preparation of tellurocysteine conjugates, for example telluride **3**, the monoaryl- and monoalkyl telluride anions, produced by the reduction of the corresponding ditellurides with sodium borohydride, can be employed as nucleophiles. However, non-aromatic (R)-Tec-compounds are very sensitive, especially Te-cystine and Tec itself.



**Scheme 2.18. Stereoselective synthesis of seleno- and telluro-derivatives of L-cysteine, L-cystine, and L-lanthionine**

Thus, the most difficult task in the whole procedure is the purification. Selenium and tellurium compounds tend to contain elemental selenium/tellurium or mono-, di-, or oligochalcogenide impurities. From elemental Se and Te they can be cleaned to some extent

on RP-18 or similar material. Unfortunately, the non-aromatic Te compounds are air, light, base, and electrophile sensitive and decompose on prolonged exposure to silica.

Eventually, direct crystallization of the alkaline metal salts of the telluro- and selenocysteine derivatives **2** and **4** proved best with respect to purity, at the same time giving acceptable yields. The crystallization was carried out by the careful addition of 10-times the volume of cold hexane to a chloroform solution of the product. After 2 days at -20 °C the crystallization was completed. Ph-Tec-compound (**3**) can be crystallized as well, but it shows co-crystallization with diphenylditelluride. Free acids (**2-4**) (R = H) can be obtained by acidification with hydrochloric acid and rapid extraction.

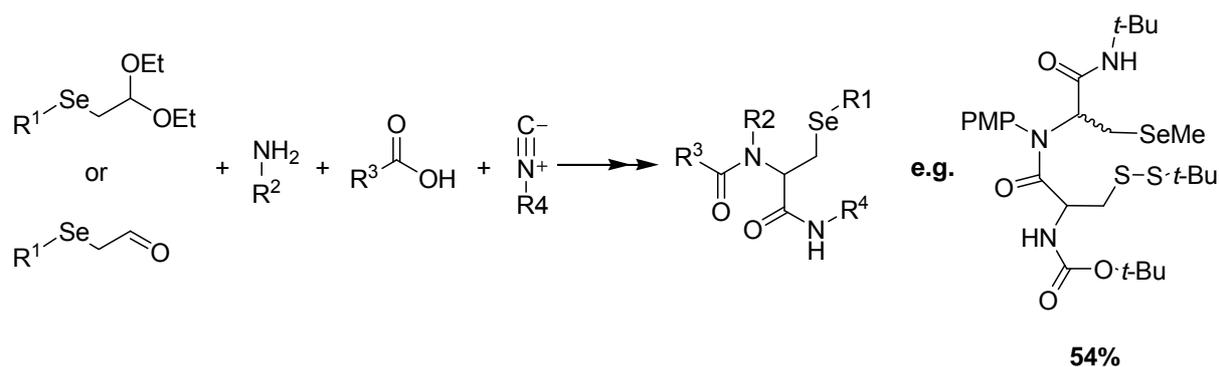
A typical problem using nucleophilic diselenide is the clean generation of the reagent, free of selenide (which will give Se-lanthionide, v.i.) or higher oligoselenides. The latter are less detrimental though, as reduction removes the excess of selenium, albeit as highly toxic and volatile H<sub>2</sub>Se. A major problem with selenium vs. sulfur in the synthesis of the amino acids is the ease of oxidation and elimination of selenium, giving undesired impurities, which are less evident or not observed in the sulfur series.

## 3 Synthesis of selenocysteine peptides

### 3.1 Introduction

Most of the literature related to the synthesis of selenocysteine/selenocystine peptides has been compiled by Moroder in a recent review. (Moroder, 2005) He discussed the syntheses of Sec-peptides by the Fmoc and Boc strategy. The article also covers the synthetic and biosynthetic incorporation of selenocysteine into peptides and proteins, e.g. by native chemical ligation, and gives some examples of site-specific replacement of cysteine residue(s) with selenocysteine(s). The biggest problem appears to be the clean preparation of Se-S-dichalcogenides, as this bridge easily disproportionates in model compounds to give a mixture of all three dichalcogenides.

In contrast to the classic peptide coupling approaches used so far, Wessjohann's group reported the first combinatorial one to produce a variety of selenocysteine analogs, especially small Sec-peptoids and peptides (Scheme 3.1). (Abbas et al., 2006) This conceptually totally different one pot method is fast and broadly applicable. It utilizes the Ugi four component reaction (Ugi-4CR) for the synthesis of model selenopeptoids and peptides under aqueous conditions. Selenopeptoids ( $R^2 \neq H$ ) are supposed to have a similar short-range environment and show similar redox, nucleophilicity, and ionization (pK) properties as a corresponding selenoprotein portion, but they are easier to synthesize and to study.



**Scheme 3.1.** Wessjohann's one pot synthesis of selenocysteine peptides and peptoids by Ugi multicomponent reaction in water.

Selenocysteine peptides can be synthesised by deprotection of  $R^1$  and  $R^2$ , or by directly using ammonium salts as amine component ( $R^2 = H$ ), and dipeptides and tripeptides ( $R^3$ ) as acid

component.(Abbas et al., 2006) Access to other selenium amide derivatives can be achieved by direct selenation(Bethke et al., 2003) or selenoalkylation.(Reich et al., 1986)

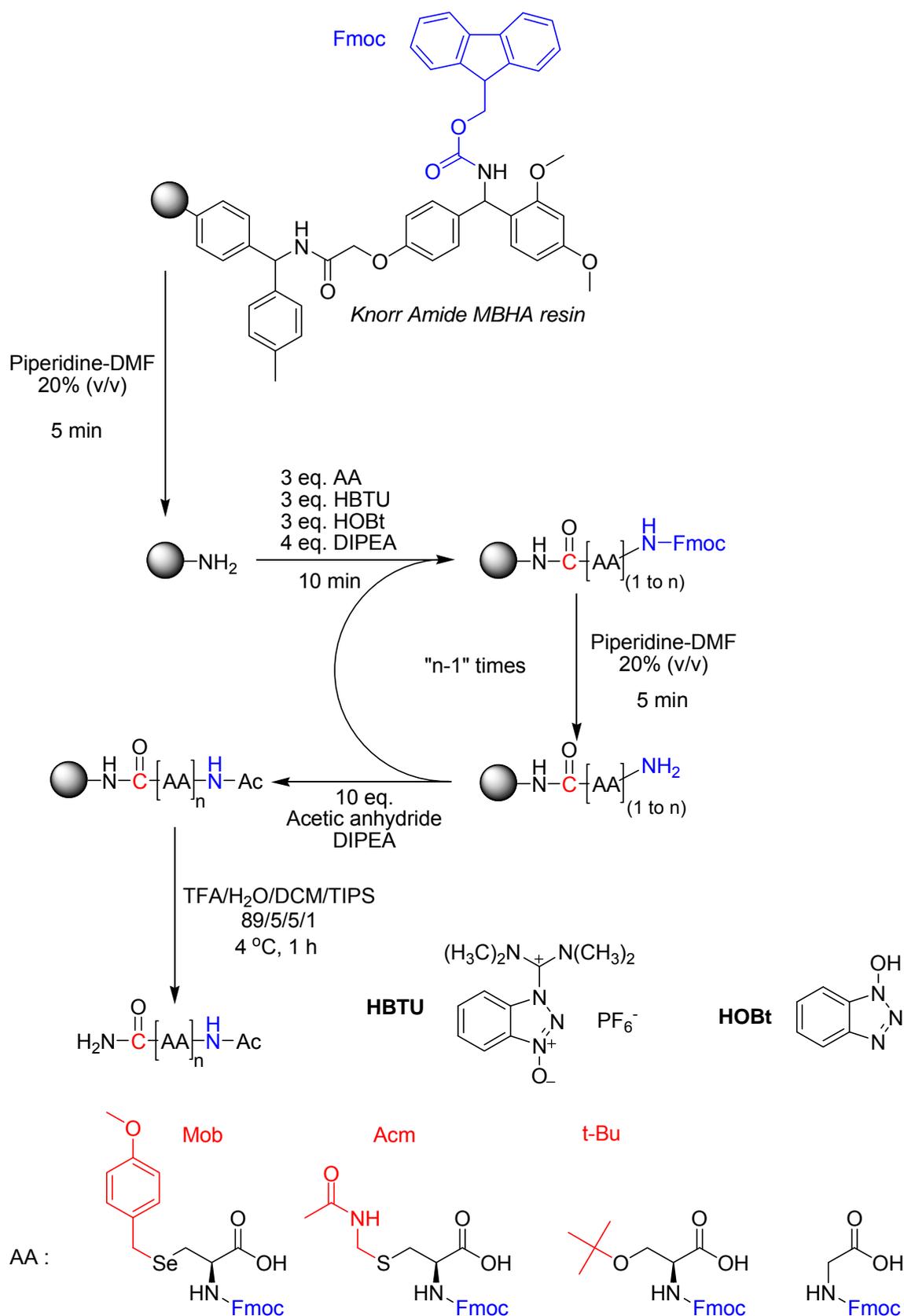
The method was also used to generate, in one pot, pept(o)ides with Sec and Cys in the same molecule (see example in Scheme 3.1.), which can be utilized for the selective formation of intramolecular Se-S-bridges by (oxidative) deprotection. This is discussed in detail in chapter 3.3.

### 3.2 Solid phase peptide synthesis of reduced selenocysteine peptides\*

For the further electrochemical investigation (see chapter 5.2) it was necessary to synthesize the amino acids sequences, which mimic and vary the crucial part of thioredoxin reductase enzymes (TrxRs). Using solid phase peptide synthesis (SPPS) the following protected amino acids sequences were synthesized: Ac-Gly-Cys(Mob)-Cys(Mob)-Gly-NH<sub>2</sub> (GCCG-Mob), Ac-Gly-Cys(Acm)-Cys(Acm)-Gly-NH<sub>2</sub> (GCCG-Acm), Ac-Gly-Cys(Mob)-Cys(Mob)-Ser-NH<sub>2</sub> (GCCS-Mob), Ac-Gly-Cys(Acm)-Cys(Acm)-Ser-NH<sub>2</sub> (GCCS-Acm), Ac-Ser-Cys(Mob)-Cys(Mob)-Gly-NH<sub>2</sub> (SCCG-Mob), Ac-Ser-Cys(Acm)-Cys(Acm)-Gly-NH<sub>2</sub> (SCCG-Acm), Ac-Ser-Cys(Mob)-Cys(Mob)-Ser-NH<sub>2</sub> (SCCS-Mob), Ac-Ser-Cys(Acm)-Cys(Acm)-Ser-NH<sub>2</sub> (SCCS-Acm), Ac-Gly-Cys(Mob)-Sec(Mob)-Gly-NH<sub>2</sub> (GCUG-Mob), Ac-Gly-Cys(Mob)-Sec(Mob)-Ser-NH<sub>2</sub> (GCUS-Mob), Ac-Ser-Cys(Mob)-Sec(Mob)-Gly-NH<sub>2</sub> (SCUG-Mob), Ac-Ser-Cys(Mob)-Sec(Mob)-Ser-NH<sub>2</sub> (SCUS-Mob).(Scheme 3.2.)

SPPS was performed on the Knorr Amide MBHA resin for Fmoc chemistry. Selenocysteine was synthesized from L-serine via  $\beta$ -lactone as described in the previous chapter (Scheme 2.18). All functional groups of amino acids were protected: selenol and thiol with *p*-methoxybenzyl (Mob) and/or acetamidomethyl (Acm), and alcohol as *tert*-butyl (*t*-Bu)-ether. All amino acid couplings were done with the HBTU/HOBt/DIPEA. Usually, pentafluorophenyl ester is used for the introduction of Fmoc-Sec and subsequent amino acids to avoid racemisation during coupling and formation of dehydroalanine or  $\beta$ -piperidylalanine containing side-products during subsequent chain elongation.(Besse and Moroder, 1997)

\* - adopted from article Schneider, A., Brandt, W., and Wessjohann, L.A., *The influence of pH and flanking serine on the redox potential of S-S and S-Se bridges of Cys-Cys and Cys-Sec-peptides. Biol. Chem.* 388, (2007): 1099-1101.



Scheme 3.2. Solid phase peptide synthesis of reduced selenocysteine tetrapeptides

We used Fmoc-Sec(Mob)-OH to introduce Sec during SPPS.(Koide et al., 1993b) Our model amino acids sequences, wherein selenocysteine was coupled to cysteine and glycine or serine, could be obtained with a reduced coupling time of 10 minutes per coupling. Moreover, 20 % piperidine in DMF was used to remove the *N*-terminal Fmoc groups faster, i.e. in 5 min. The resin cleavage was done with the cocktail TFA/H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>/TIPS (89/5/5/1, v/v) under mild conditions at 4 °C for 1 hour.(Besse and Moroder, 1997) All these operations were sufficiently fast and mild to avoid the formation of by-products. Therefore the protected tetrapeptides can be produced without using the time- and cost-consuming pentafluorophenyl method.

The *N*-terminus was protected with an acetyl group (Ac) and the *C*-terminus as amide (-CONH<sub>2</sub>) to obtain neutral species. The acetylation of the SCCG-Mob, SCCG-Acm, SCCS-Mob, SCCS-Acm, SCUG-Mob, and SCUS-Mob sequences was achieved on the resin with 10 equivalents acetic anhydride and DIPEA in DMF. GCCG-Mob, GCCG-Acm, GCCS-Mob, GCCS-Acm, GCUG-Mob, and GCUS-Mob were prepared using already acetyl protected glycine as last amino acid.

Mass spectra of reduced crude and Mob/Acm-protected tetrapeptides proved the correct formation of our model sequences. There are no peaks of  $\beta$ -elimination by-products and piperidine adducts. In case of selenocysteine peptides with serine, additional peaks, which belong to peptides with one and/or two protected hydroxyl groups, were found. The deprotection was not completed and gave a mixture of protected and unprotected peptides. That was also proved by HPLC, which showed peaks of the expected products. In case of cysteine peptides protected with *p*-methoxybenzyl (Mob) partly deprotected thiol groups were observed. This, however, was not problematic for our further research as the peptides were later completely deprotected, as a consequence of their oxidation in TFA. In case of the peptides – GCCG-Acm, GCCS-Acm, SCCG-Acm, SCCS-Acm, GCUG-Mob, each HPLC analysis showed a single peak of reduced and Mob/Acm-protected tetrapeptide and mass spectrometry gave the corresponding molecular ions.

**Table 3.1. Yields of Acm/Mob protected reduced tetrapeptides**

Peptide	Yield
GCCG-Acm	49 %
GCCS-Acm	34 %
SCCG-Acm	63 %
SCCS-Acm	64 %
GCUG-Mob	33 %
GCUS-Mob	10 %
SCUG-Mob	39 %
SCUS-Mob	15 %

In conclusion, all of the desirable model tetrapeptides were synthesized in good yields and with rapid, slightly modified solid phase peptide syntheses. It was not part of this study to optimize the yields of these reaction protocols (Table 3.1.), but it is already faster than previous methods and can be applied for the synthesis of libraries of selenenylsulfide peptides.

### 3.3 Oxidation of selenocysteine/cysteine and cysteine/cysteine peptides

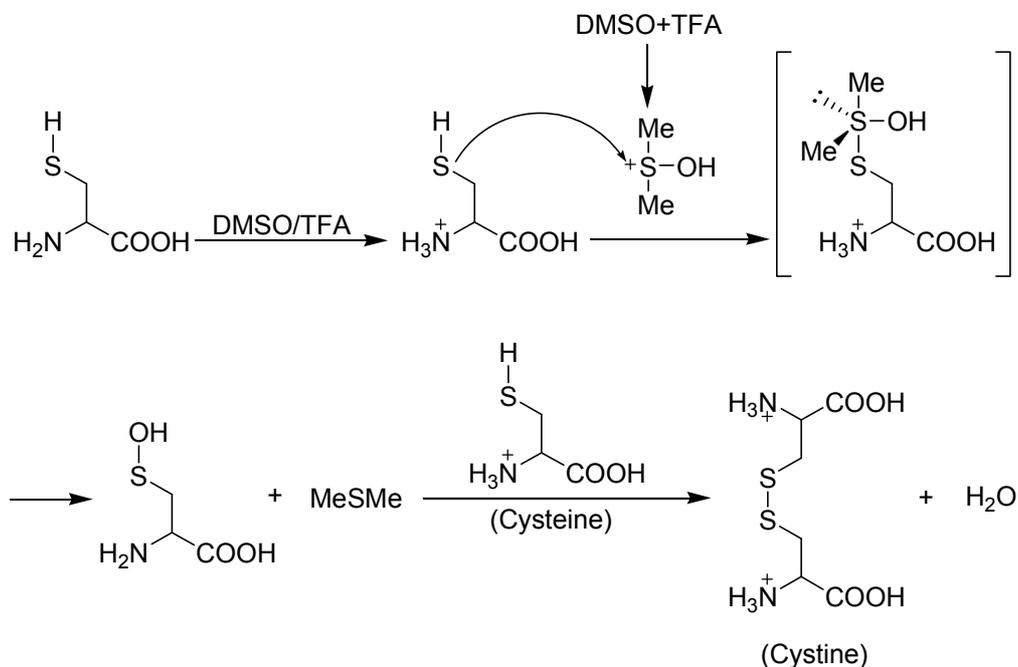
#### 3.3.1 Cysteine/cysteine peptides

##### 3.3.1.1 Introduction

Nowadays there are several standard procedures for disulfide bond formation, such as iodine oxidation in methanol (Kamber et al., 1980), thallium trifluoroacetate oxidation (Fujii et al., 1987) and DMSO/TFA oxidations (Otaka et al., 1991). Because of the high toxicity of thallium salts, we chose  $I_2/MeOH$  and DMSO/TFA oxidations to form the S-S-bond.

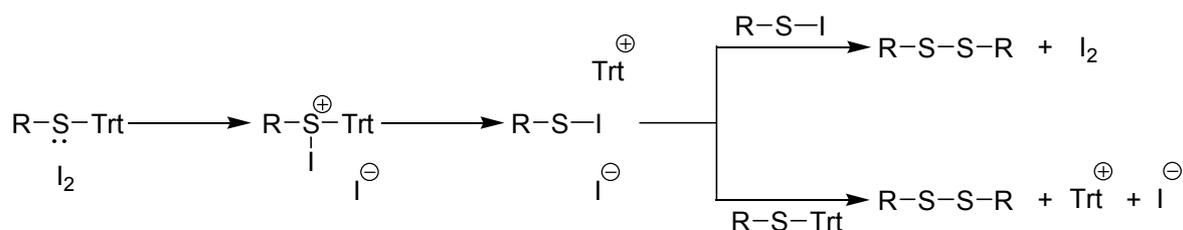
Wallace et al. were the first who used DMSO as an oxidant for the reaction of thiol to disulfide (Wallace and Mahon, 1965). Later the group of Professor Fujii from Japan reported the synthesis of cystine-containing peptides to form intramolecular (Otaka et al., 1991; Koide et al., 1993c) and intermolecular (Koide et al., 1993b) S-S bridges. He proposed also a reaction

mechanism and demonstrated this oxidation method for different protecting/leaving groups concerning the thiol function – Cys(Trt), Cys(Mob), Cys(Dbs), Cys(Bzh) (Scheme 3.3.).(Otaka et al., 1991)



**Scheme 3.3. Putative mechanism of the DMSO/TFA oxidation of cysteine to cystine**

Another common procedure of disulfide bond formation is oxidation by iodine.(Kamber et al., 1980) It is based on the simultaneous removal of the sulfhydryl protecting groups Cys(Acm)/Cys(Trt) and the disulfide bond formation. The mechanism of this oxidation is well known (Scheme 3.4.).(Kamber et al., 1980) The first interaction between iodine and sulfur leads to a charge-transfer complex, wherein sulfur acts as donor and iodine as acceptor. A charge separation leads to the iodosulfonium ion and the removal of the trityl carbenium ion gives the sulfenyl iodide. Finally the disulfide is afforded either by disproportionation of two sulfenyl iodides to the disulfide and iodine or by the attack of the electrophilic sulfur atom of R-S-I by the nucleophilic S-atom of R-S-Trt. The same results are obtained in case of S-acetamidomethyl(Acm)-cysteine peptides.



**Scheme 3.4.** Mechanism of the iodine oxidation of a trityl sulfide

### 3.3.1.2 Synthesis of oxidized cysteine/cysteine peptides

DMSO/TFA oxidation was tried for Mob-protected GCCG-Mob, SCCG-Mob, GCCS-Mob and SCCS-Mob. Unfortunately, it did not work on the Cys/Cys-peptides. MS and HPLC analyses showed mixtures of partly protected and unprotected tetrapeptides, but no signal of the desired 8-membered cyclodisulfides.

The cysteine-containing model tetrapeptides were also oxidised with iodine in methanol. The reaction of 10 equivalents of  $I_2$  per Ac<sub>m</sub>-group gave an unseparable mixture of side products. A smaller excess of  $I_2$  (5 equivalents) and reduction with aqueous sodium thiosulfate of the remaining iodine favoured cyclic disulfide tetrapeptides, as was detected by MS and HPLC analysis. Preparative HPLC purifications gave pure model peptides for further electrochemical investigations (see chapter 5.2) in acceptable to good yields (Table 3.2).

**Table 3.2.** Yields of oxidized AA-Cys-Cys-AA tetrapeptides containing disulfide bridges

Peptide	Yield
GCCG	46 %
GCCS	59 %
SCCG	34 %
SCCS	34 %

## 3.3.2 Selenocysteine/cysteine peptides

### 3.3.2.1 Introduction

Several works reported the oxidation of selenocysteine peptides to form Se-Se or Se-S bridges. (Besse and Moroder, 1997; Koide et al., 1993b; Koide et al., 1993a) All of them are based on the Pfitzner-Moffat like oxidation (Scheme 3.3.) of Sec-peptides with *p*-methoxybenzyl protecting group for the selenols with DMSO/TFA-reagent. The same

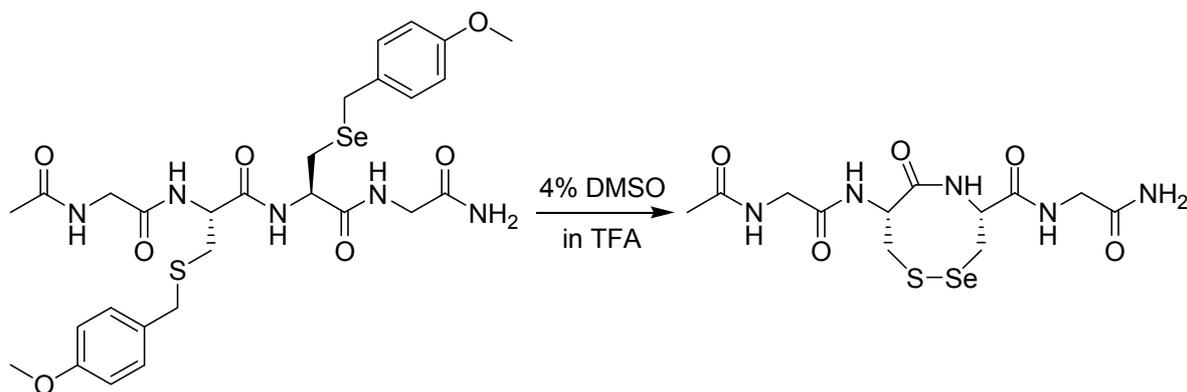
process was applied to produce selenenylsulfide (Se-S) bridges in peptides. Fujii was the first to report the synthesis of ring closed selenenylsulfide peptides through DMSO/TFA oxidation.(Koide et al., 1993a) He synthesized  $\alpha$ -rat atrial natriuretic peptide analogs [rANP(7-28)] with a 54-membered ring closed by formation of the Se-S bridge. Eventually, Moroder explored dichalcogene-formation involving selenium in more detail. He synthesised oxidized forms of monoselenocysteine analogs of glutaredoxine [10-17] Ac-Gly-Cys-Pro-Tyr-Sec-Val-Arg-Ala-NH<sub>2</sub> and closed the smaller 14-membered Se-S ring.(Besse and Moroder, 1997) He demonstrated that reaction at 4 °C for 30 min under high dilution ( $5 \times 10^{-4}$  M) gives only the monomeric cyclisation product and no dimer.

More difficult is the formation of an 8-membered ring as it would result from neighbouring Sec and Cys. However, exactly this combination is of biological importance, e.g. in thioredoxin reductases (TrxRs).(Birringer et al., 2002; Brandt and Wessjohann, 2005; Gromer et al., 2005; Jacob et al., 2003; Kryukov et al., 2003) The formation of eight-membered rings is hampered by the build-up of transannular and conformational strain and results in a considerable loss of entropy – while forming a bond of only some 200 kJ/mol bond energy.(Clayden et al., 2001; Biological Chemistry, special issue 10, 2007)

### 3.3.2.2 Synthesis of oxidized selenocysteine /cysteine peptides

In spite of the complexity of the formation of an 8-membered ring, we successfully used DMSO/TFA oxidation to form such 8-membered selenenylsulfide cyclic peptides, which mimic and vary the crucial part of TrxRs: Ac-Gly-Cys-Sec-Gly-NH<sub>2</sub> (GCUG), Ac-Gly-Cys-Sec-Ser-NH<sub>2</sub> (GCUS), Ac-Ser-Cys-Sec-Gly-NH<sub>2</sub> (SCUG), Ac-Ser-Cys-Sec-Ser-NH<sub>2</sub> (SCUS) (Scheme 3.5.). Since the flanking serine amino acids strongly influence the redox-behaviour of the Se-S-bridge, a protective group strategy was used with *p*-methoxybenzyl (Mob) for selenium and sulfur, and *tert*-butyl (*t*-Bu) for oxygen protection.

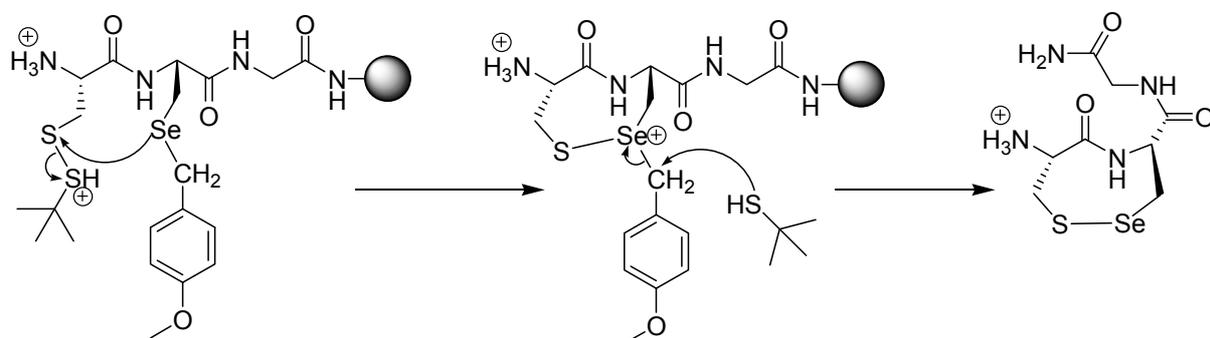
The desirable products were detected by MS and HPLC analysis and purified with preparative HPLC for further electrochemical investigations. Micro-HPLC coupled with an ion trap ESI-MS was used for the separation of monomeric and dimeric cyclic tetrapeptide and thus for the checking of the purity of the oxidation reaction. We observed that selenenylsulfide cyclic peptide easily forms the open-chain or cyclic dimers in concentrated solution or at basic pH.



**Scheme 3.5. Deprotective oxidation to 8-membered selenenylsulfide peptides**

Interestingly, the DMSO-TFA oxidation method applied on di-cystein-peptides did not give the corresponding 8-membered S-S cyclic peptides. One reason might be the higher nucleophilicity of selenium vs. sulfur.

Recently Prof. Hondal discovered that 8-membered cyclic selenenylsulfides can also be formed spontaneously during deprotection (Scheme 3.6.). (Harris et al., 2007) The octacycle was formed without the intermediacy of free selenols/thiols, when in the peptide Cys(*St*Bu)-Sec(Mob)-Gly-PAL-resin the thiol was protected by the (*S-t*-Bu) protecting group and the selenol by the *p*-methoxybenzyl group. The cyclic product was also formed when *S*-3-nitro-2-pyridinesulfonyl (Npys) was used as protecting group for the thiol instead of *S-t*-Bu. A mechanism was postulated in which the protonation of the sulfur atom of *S-t*-Bu or the pyridine ring nitrogen of Npys transforms these Cys-sulfur protective groups into leaving groups, followed by intramolecular attack of the selenide. The intermediate selenonium ion releases the *p*-methoxybenzyl residue which is scavenged by a nucleophile (*S-t*-Bu or water).



**Scheme 3.6. Hondal's method**

Finally, no details are given with respect to the *s-cis/s-trans* configuration of the amide bonds in any of these 8-membered rings. However, considering the linear precursors and the -S-Se-bond lengths, a conformationally flexible *s-trans* amide appears to be the most likely species.

## 4 Kinetic studies

### 4.1 NMR study of S-S/S<sup>-</sup> and Se-Se/Se<sup>-</sup> exchange reaction

#### 4.1.1 Introduction

The selenocystine compounds synthesized by traditional or by MCR-approaches can be studied versus the Cys-analogs by electrochemical and spectroscopic (MS, NMR) methods. There are several studies on the kinetics of the thiol-disulfide exchange reactions by NMR.(Guo et al., 1990; Keire and Rabenstein, 1989; Keire et al., 1992; Weaver and Rabenstein, 1995) Some reactions could be monitored by HPLC.(Rabenstein and Yeo, 1994; Rabenstein and Yeo, 1995; Rabenstein and Weaver, 1996; Siedler et al., 1993) Rabenstein et al. also investigated the kinetics of selenol/diselenide exchange reaction of selenocysteamine/selenocystamine by NMR spectroscopy (line shape analysis of exchange broadened resonances), and compared these results with the corresponding thiol/disulfide exchange reactions (Figure 4.1).(Pleasant et al., 1989)

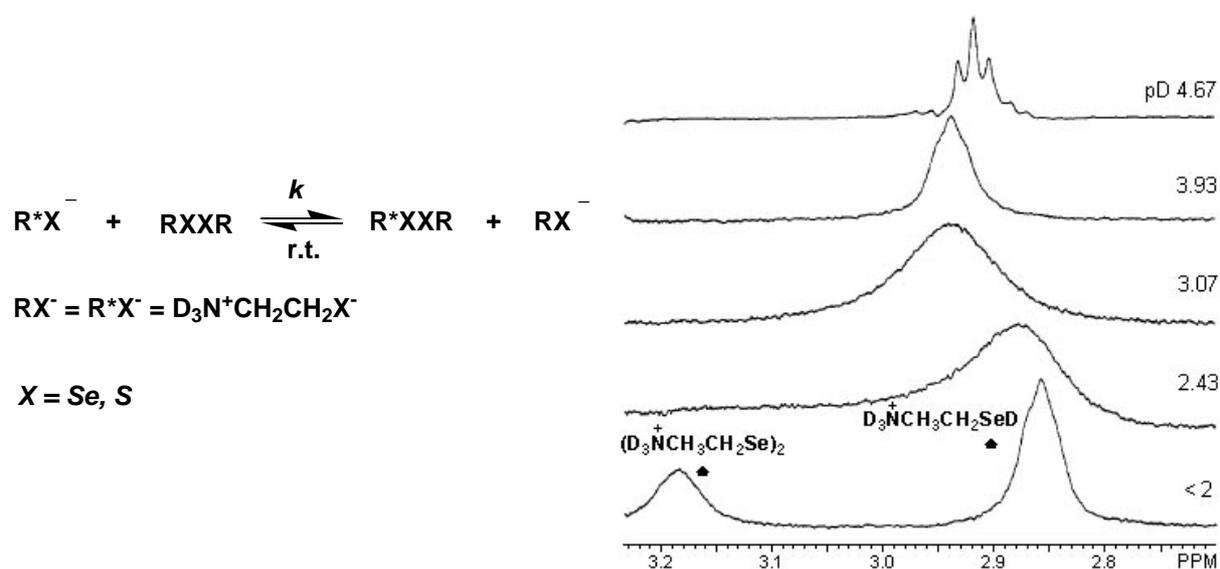


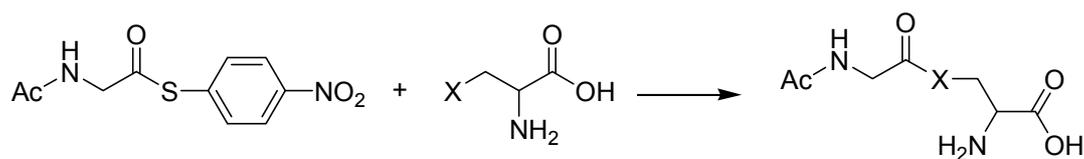
Figure 4.1. <sup>1</sup>H NMR spectra of a solution containing 2.9 mM selenocystamine and 14.2 mM selenocysteamine as a function of pD. Only the resonances for the methylene protons adjacent to the selenium are shown.(Pleasant et al., 1989)

The rate constants for X = Se are:  $k = 1,65 \times 10^7$  L/mol·s; and for X = S:  $k = 68,0$  L/mol·s, i.e. the selenocysteine/selenocystine exchange is approximately  $10^5$  times faster than the cysteine/cystine exchange at physiological pH.

In 1991 Rabenstein et al. investigated another exchange reaction – penicillamine/bis(penicillamine) selenide (RS<sup>-</sup>/RSSeSR) showing a  $10^8$  times faster reaction in comparison to the analogous reaction of 2-methyl-2-propanethiol with bis(*tert*-butylthio) selenide. (Rabenstein et al., 1991)

Comparison of the interchange reactions of RSH (organic thiols) reacting with RSSeSR (bis(alkylthio) selenides) either at sulfur or selenium has shown a rate difference of  $10^5$  in favor of selenium. (Kice and Slebockatilk, 1982)

Back and Moussa have measured the relative catalytic activity of ebselen, selenenamide, and diselenides in the BnSH-TBHP system. (Back and Moussa, 2003) Hondal studied the rate of the attack of cysteine and selenocysteine on a model thioester at different pH (pH range 5-8) (Scheme 4.2.). (Hondal, 2005)



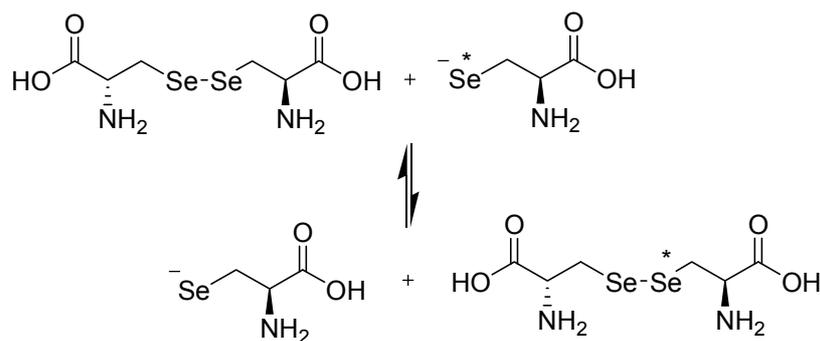
**X = Se, S**

#### **Scheme 4.2 Rate of attack on a model thioester with cysteine or selenocysteine (X=Se,S)**

Here it was shown that the rate of attack at pH 5 with X = Se is 1000 times faster than with X = S, whereas at pH 8 it is only 10 times faster relative to sulfur. Similar results were obtained with alkylation reactions of selenides vs. sulfides. (Kang and Spears, 1990a) The differential reactivity of acidic pH could be utilized for the selective labeling/sensing of selenols vs. thiols in vitro. (Maeda et al., 2006)

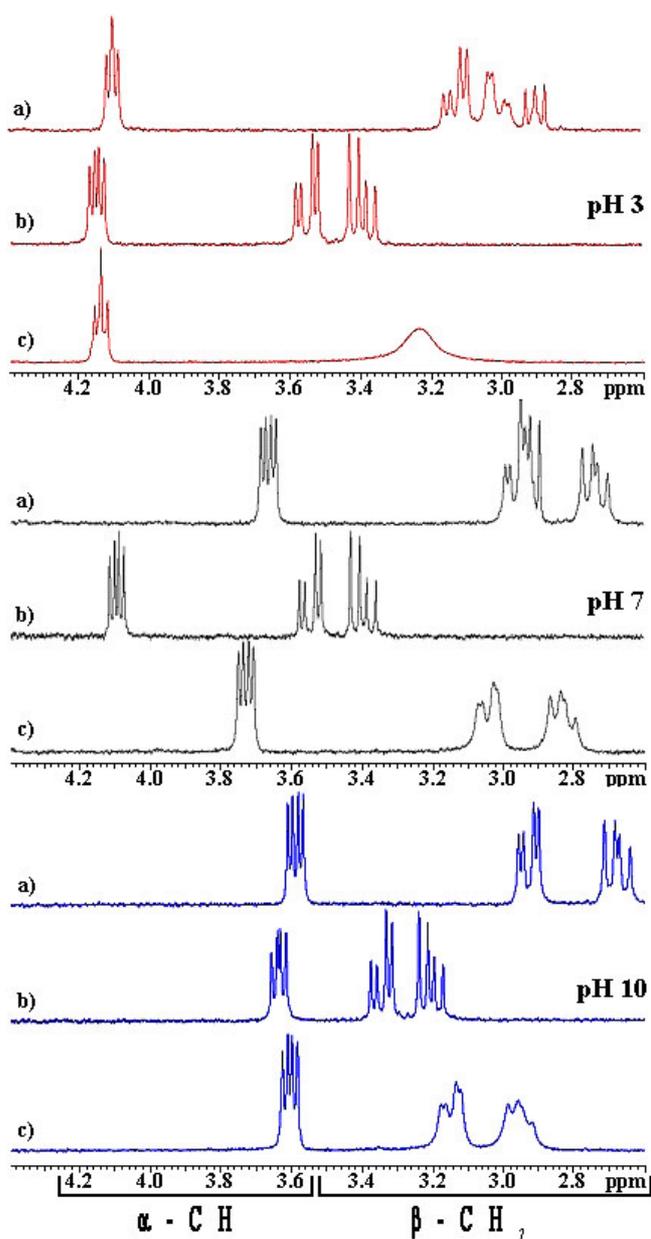
#### **4.1.2 NMR of exchange reaction selenocysteine/selenocystine**

The exchange reaction between selenocysteine and selenocystine in deuterated water at different pH was observed by NMR spectroscopy (Scheme 4.1.). The pH was adjusted by the addition of either DCl or NaOD.



**Scheme 4.1.** Exchange reaction between selenocysteine and selenocystine

Methylene and methine protons adjacent to the selenium were monitored at different pH-values (Figure 4.2.). Figure 4.2. shows the chemical shifts of selenol **4.2.a**, selenocystine **4.2.b** and a mixture of both **4.2.c** at broad pH range from 3 to 10. The exchange between selenocysteine and selenocystine is indicated by broad signals of the methylene and methine protons of the mixture **4.2.c**, especially at pH=7. It was impossible to make a line shape analysis in order to get kinetic data, due to the low solubility of selenocyst(e)ine at physiological pH.

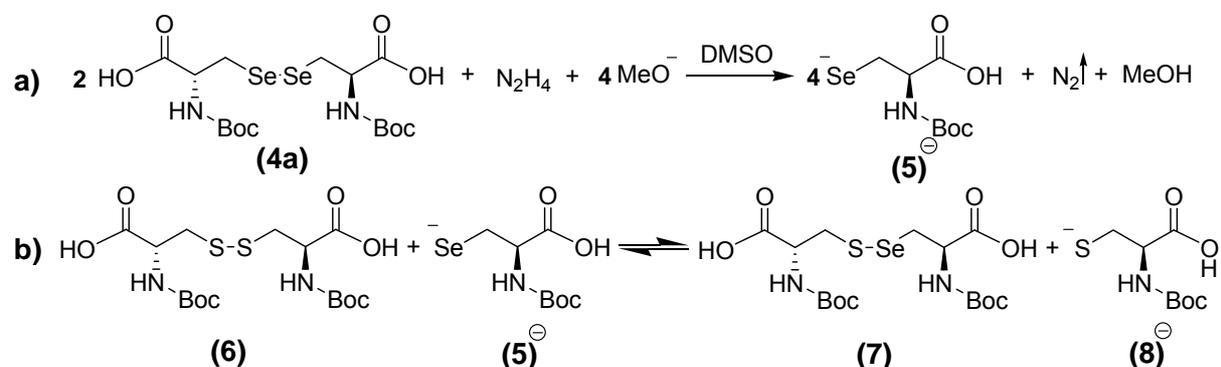


**Figure 4.2.**  $^1\text{H}$  NMR spectra of solutions containing (a) 10 mM selenocysteine, (b) 5 mM selenocystine, and (c) the mixture of both

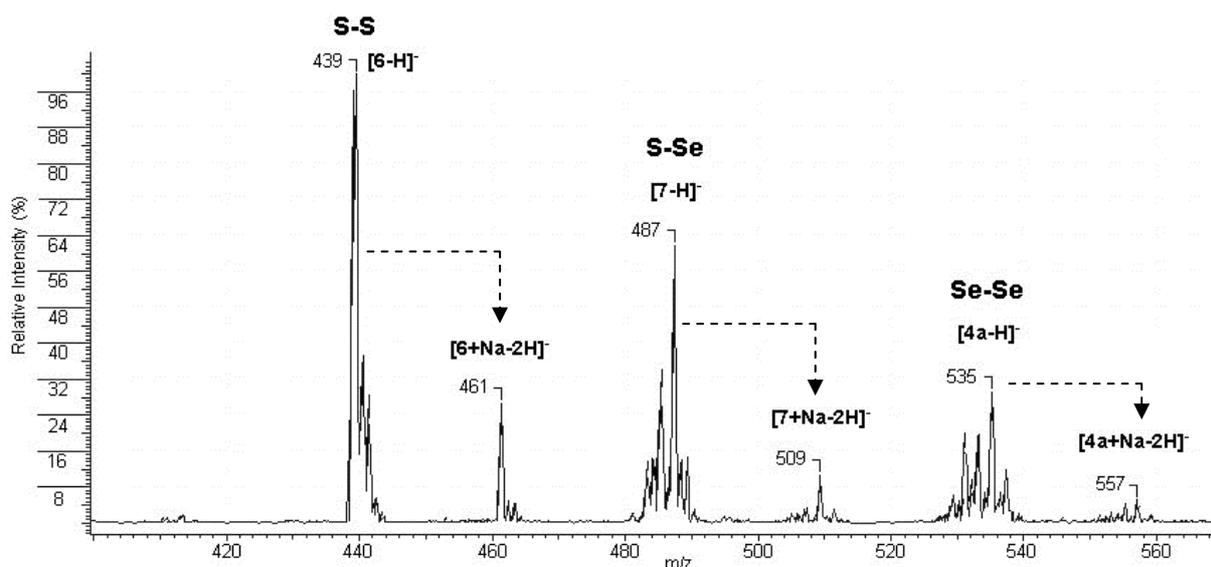
## 4.2 Mass spectrometry of Se-S exchange reaction

### 4.2.1 Equilibrium constant determination by mass spectrometry

In our laboratory we showed a possibility to determine equilibrium constants of exchange reactions with nucleophilic attack of selenolate/thiolate anions using mass spectrometric method. This was demonstrated with an example of the reaction of *N*-Boc protected cystine (**6**) (commercially available) with the *N*-Boc protected selenolate anion of selenocysteine (**5**) in the aprotic solvent DMSO (Figure 4.3.). Using an aprotic solvent simplifies the task of calculation, as it has no direct influence on the pH. *N*-Boc protected selenocysteine (**4a**) was prepared using the standard procedure with di-*tert*-butyl pyrocarbonate ( $\text{Boc}_2\text{O}$ ) under aqueous conditions in the presence of a base. The reduction of selenocysteine with hydrazine hydrate proceeded almost instantaneously at room temperature in DMSO upon addition of a concentrated ( $\sim 5$  M) solution of sodium methanolate in methanol to give a corresponding selenolate anion (**5**) (Scheme 4.3a). (Henriksen and Stuhr-Hansen, 1999) Thus we avoided the use of borohydride (e.g.  $\text{NaBH}_4$ ), that forms chalcogene-boron complexes, which diminish the nucleophilicity of the anion. (Wessjohann and Sinks, 1998) The selenolate ion **5**<sup>-</sup> reacts further with protected cystine **6** to provide selenenylsulfide **7** (Scheme 4.3b).



Scheme 4.3.



**Figure 4.3.** Mass spectrum of equilibrated Se-S exchange reaction Scheme 4.3b

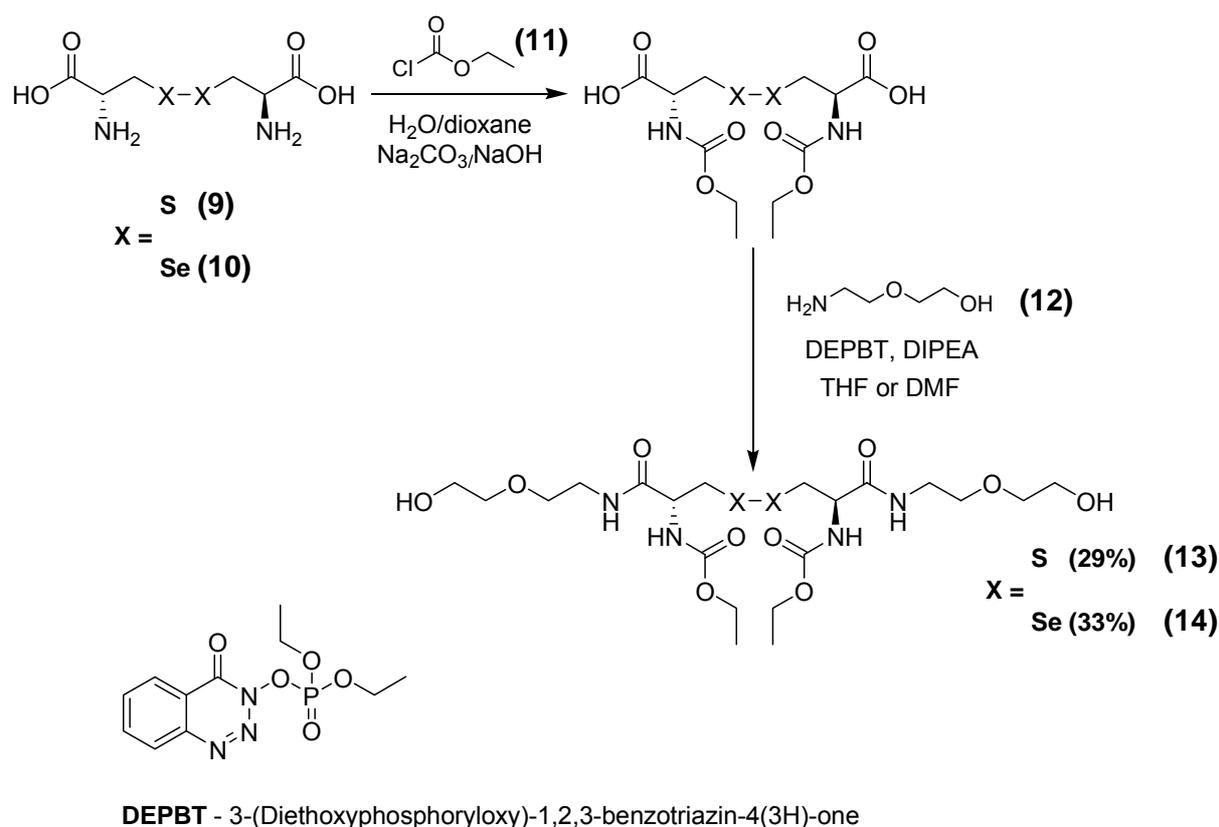
10-20 mg of the selenenylsulfide (7) were synthesized and, after purification by preparative HPLC, used as standard for the calibration of the mass spectrometer. The calibration for compound (7) was made with selenenylsulfide in MeOH with concentrations ranging from  $10^{-7}$  to  $10^{-4}$  g/mL. A linear dependence between concentration and relative peak intensity was observed between  $10^{-5}$  and  $10^{-4}$  g/mL. The coefficient of determination was  $R^2=0.9944$ . The exchange reaction was carried out in 0.7 mmol scale in DMSO. The reaction mixture was equilibrated for 1 h, then dissolved in 100 parts MeOH to slow down the exchange reaction and reach a concentration in the linear concentration range. Later it was observed that the equilibrium was reached already after 1 min. The relative peak intensity of the molecular ion of selenenylsulfide was measured and its equilibrium concentration was calculated. The equilibrium constant (Scheme 4.3b.) was calculated by the equation:

$$K_c = \frac{C[8^-] \times C[7]}{C[5^-] \times C[6]}$$

It results in  $K_c = 0.271$  in DMSO, i.e. at equilibrium ca. 2 times more disulfide is present than selenenylsulfide (Scheme 4.3b). Therewith it was illustrated to obtain the equilibrium constants of Se-S exchange reactions using mass spectrometry, which is also suitable for non UV-active substances not easily detectable by HPLC. Another advantage of this approach is that the detection limit of MS is noticeably lower than by NMR or HPLC. This allows now to investigate reactions showing a small equilibrium constant or having a low concentration of educts, or being fast.

### 4.2.2 Synthesis of a water soluble analogue of selenocystine

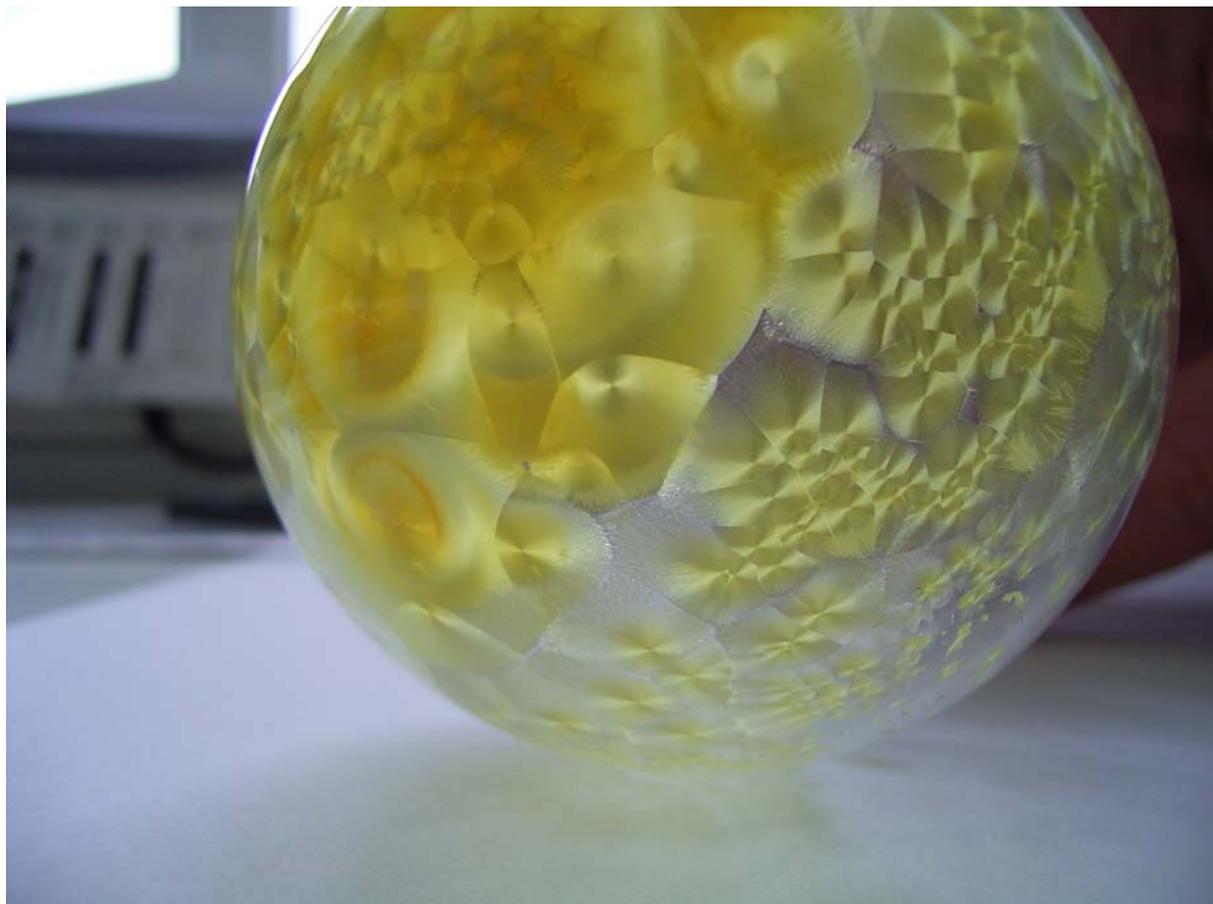
An almost neutral and water soluble amide analogue of selenocystine (**14**) was synthesized to extend our mass spectrometric investigations (Scheme 4.4.). This amide analogue is free of intrinsic acidic or basic groups. Thus it is more related to the natural setup found in selenoproteins. The purpose of the protection thus is to capture the pH-dependence of the chalcogen exchange, independent of possible charges build in the peptide. That gave us the possibility to monitor exchange reactions at different pH-values in a physiological milieu.



**Scheme 4.4.** Synthesis of water soluble analogue of (seleno)cystine

The amine group of cystine (**9**) or selenocystine (**10**) was protected as ethyl carbamate using a standard procedure: ethyl chloroformate (**11**) in water/dioxane under basic condition. The acid group was converted into a diethylene glycolamide using diethylene glycolamine (**12**) with an unprotected hydroxy group and DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one) as coupling reagent in DMF. The same results were obtained with THF as solvent. Ye and co-workers demonstrated that DEPBT can be used as an efficient coupling reagent for amide bond formation with remarkable resistance to racemization and without necessity to protect the hydroxy group of the amino component. (Ye et al., 2005)

Some side products and also their adducts with educts were observed by MS and HPLC. The target products (**13**) and (**14**) are very well soluble in water, but unfortunately all by-products are water soluble too and they can not be separated by recrystallization or precipitation using non-polar solvents. Therefore the crude mixture was roughly pre-cleaned by a RP-18 column with a MeOH/water gradient, followed by a final purification by preparative HPLC. Yellow crystals of diselenide (**14**) were obtained by evaporation of the solvent (Figure 4.4.).



**Figure 4.4.** Water soluble analogue of selenocystine (**14**)

Thus the water soluble and protected cystine and selenocystine analogues were obtained in very high purity (higher than 99%), and good overall yield of 25 % for cystine-derivate **13** and 31 % for selenocystine-derivate **14**.

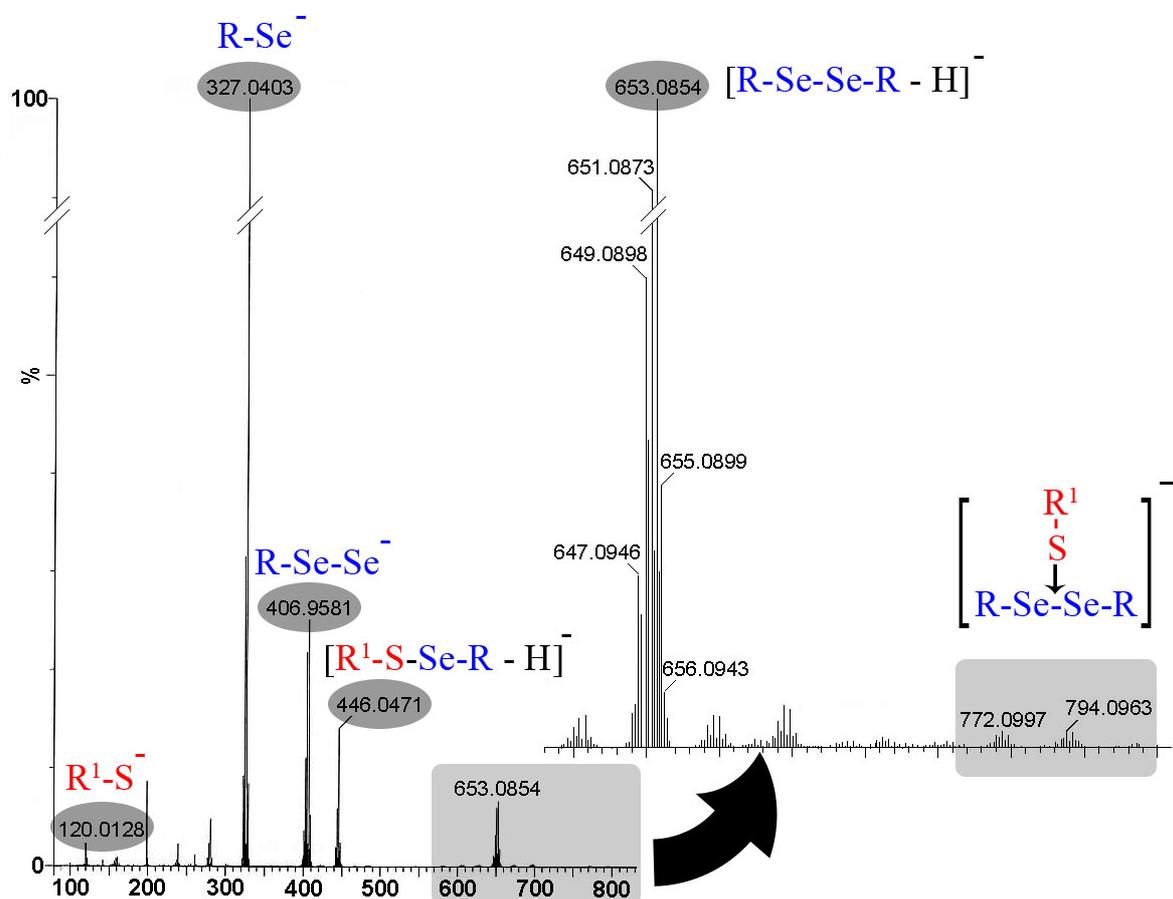
#### **4.2.3 Mechanistic elucidation of the Se-S exchange reaction**

The exchange reaction between the water soluble analogues of selenocystine **14** and cysteine **15** was monitored by the time-of-flight mass spectrometry (TOF-MS) to clarify the kinetics and potential mechanism of the Se-S exchange reaction (Scheme 4.5.).



proceeds by a  $S_N2$  mechanism and accordingly forms via a tri-chalcogen-intermediate [Se-Se...S] (**16**).

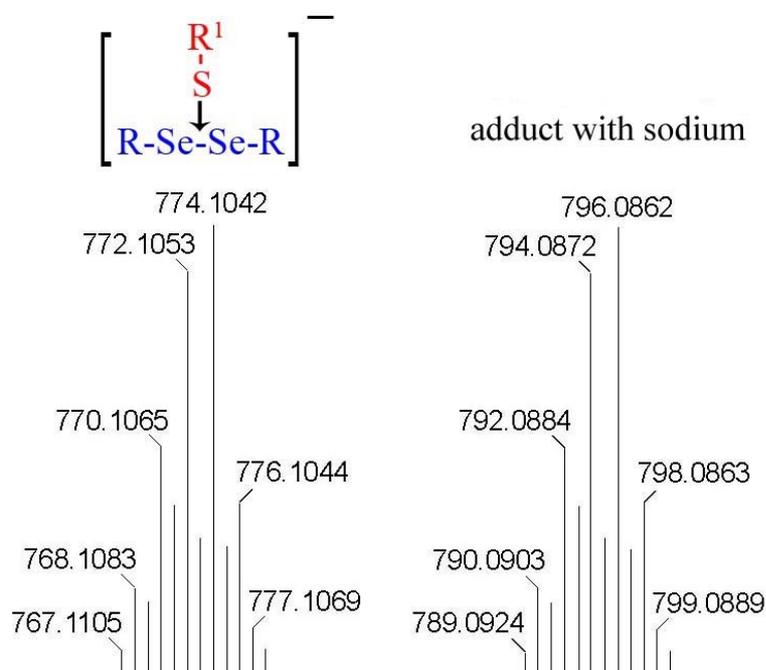
In the reaction mixture, the negative molecular ions of the educts  $[R-Se-Se-R - H]^- + R^1-S^-$  and of the products  $[R^1-S-Se-R - H]^- + R-Se^-$  with their fragments and adducts are present, based on the data of the mass spectrum in negative ion mode (Figure 4.5.). The fragmentation for each molecular ion was proved by MS/MS. In the range between 750 and 800  $m/z$  a small amount of a species with the mass of the proposed intermediate (**16**) was detected. All ions in that range were selectively collected for an extended time (few minutes) to get a good intensity of the peaks and a better resolution.



**Figure 4.5.** Mass spectrum in negative ion mode of equilibrated Se-S exchange reaction Scheme 4.5.

The mass spectrum of the putative trichalcogenide intermediate anion (**16**) was obtained with high resolution (Figure 4.6.). The isotopic distribution, which was determined by TOF-MS, corresponds to the distribution for substances with two selenium and one sulfur atom. The peak with higher intensity exhibits a  $m/z$  774.1042, which is very similar to the calculated  $m/z$

774.1038. We observed also the adduct with sodium in negative ion mode –  $m/z$  796.0862 (calc. 796.0857). That is due to one acid group of the intermediate anion, wherein hydrogen can be substituted by sodium in the mass spectrometer source.



**Figure 4.6. High resolution mass spectrum of the putative tri-chalcogen-intermediate**

MS/MS of the putative intermediate ion of  $m/z$  774.1042 was done to probe if this intermediate is a simple adduct of educts produced in the ionization source or not (Figure 4.7.). Fragments of the educts and products were obtained in this experiment. It demonstrates that fragmentation proceeds in two different paths “a” and “b” (see figure 4.7. above). According to the path “a”, the ions of the products of the Se-S exchange reaction are produced. The ions of the educts are formed by the path “b”. That means, there is a bond between the sulfur atom of cysteine and both or one selenium atom of the diselenide bridge of the selenocystine analogue. We do not exclude that the intermediate might also have a linear structure instead of a triangle.

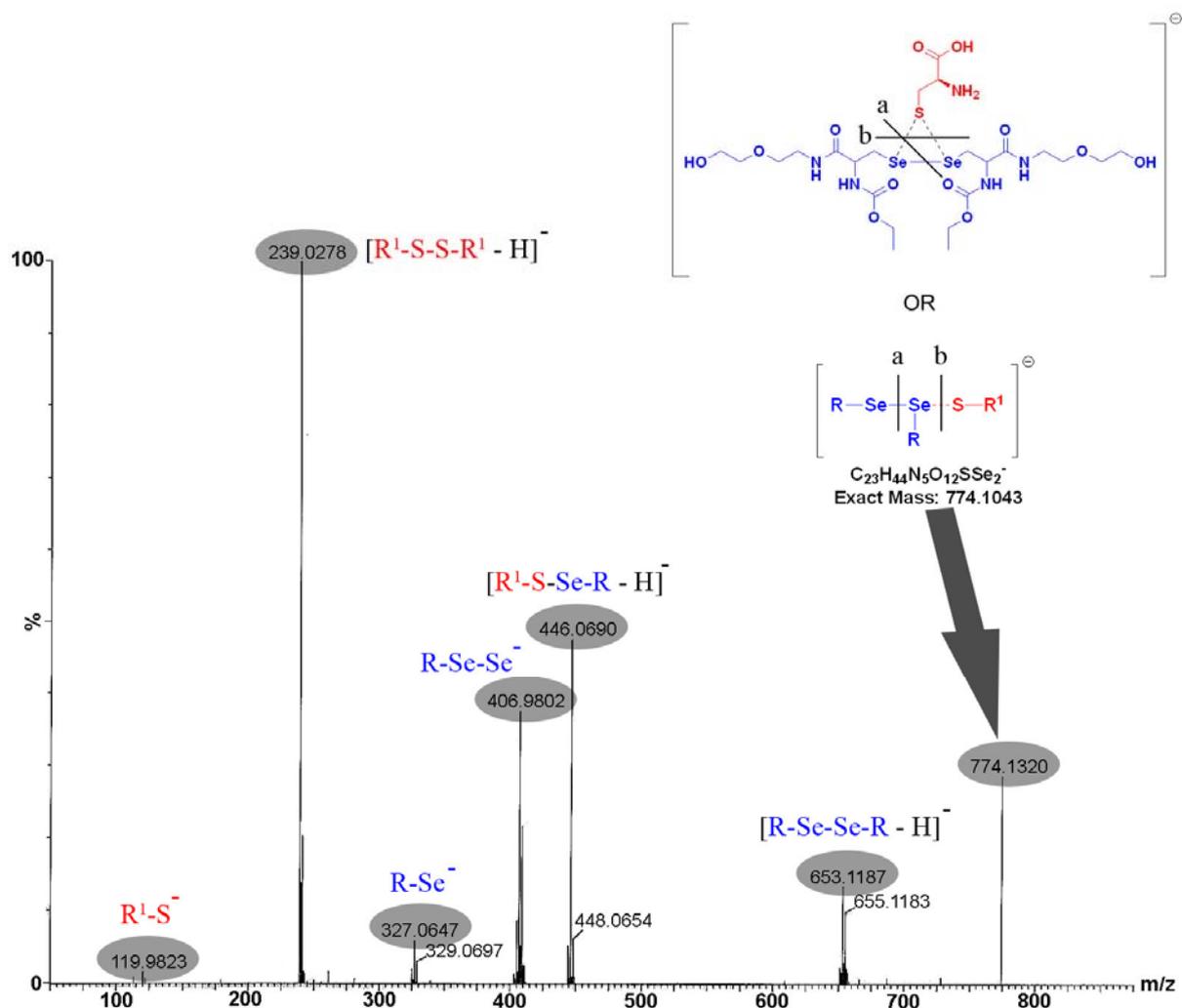


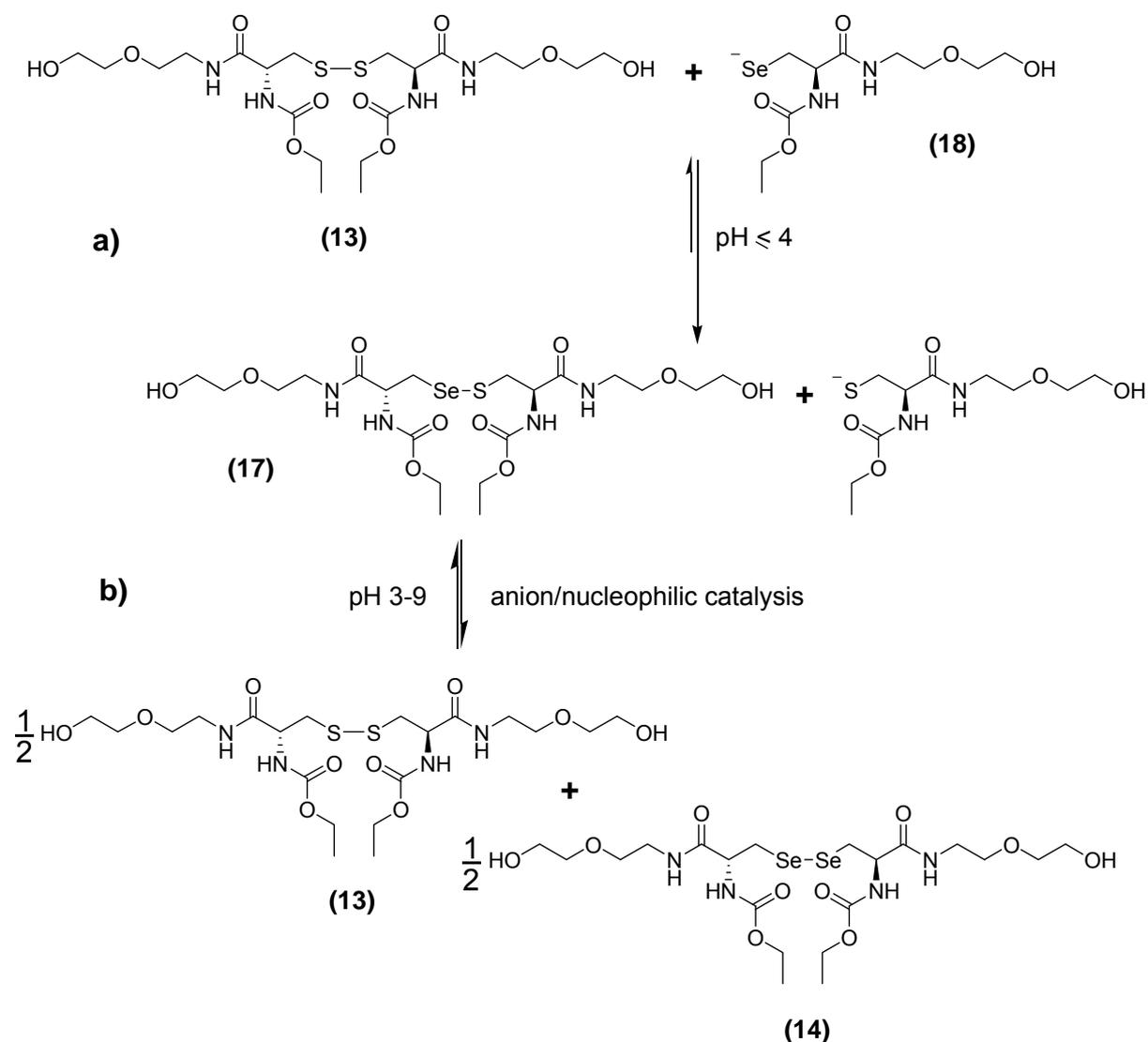
Figure 4.7. MS/MS of the [Se-Se...S] intermediate

It was indicated that during the Se-S exchange reaction under basic condition the tri-chalcogen-intermediate [Se-Se...S] was formed. Thus, it can be postulated that the exchange reaction between the selenocystine analogue and the thiolate anion of cysteine proceeds at least partially according to a  $S_N2$  mechanism, where the thiolate anion acts as a nucleophile.

### 4.3 HPLC investigation of Se-S exchange reaction

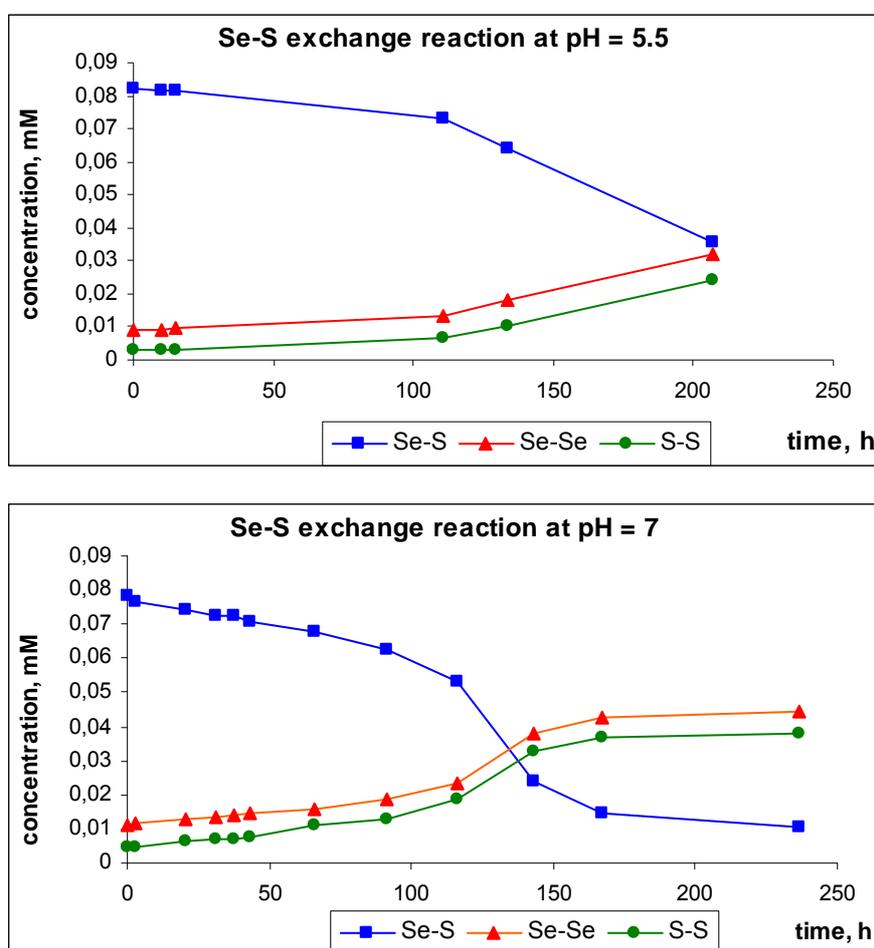
#### 4.3.1 Monitoring the disproportionation equilibrium of selenenylsulfide

The water soluble and protected selenenylsulfide **17** was synthesized by exchange reaction of the corresponding cysteine derivate **13** and the selenolate anion **18** at  $\text{pH} \leq 4$  (Scheme 4.6a) and purified by HPLC. As expected, the purified selenenylsulfide is unstable. It easily disproportionates to the disulfide (-S-S-) **13** and the diselenide (-Se-Se-) **14** in concentrated solution or at slightly basic pH. Catalysis by minute amounts of free chalcogenide needs to be avoided as much as possible.



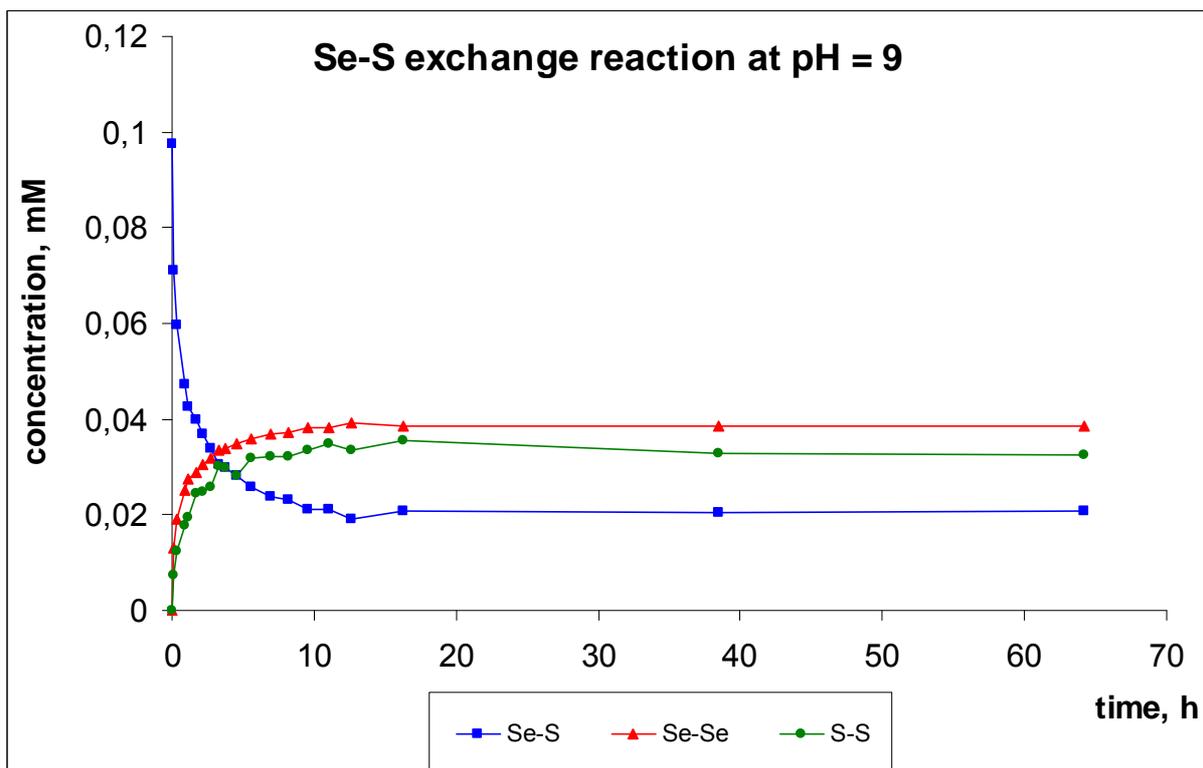
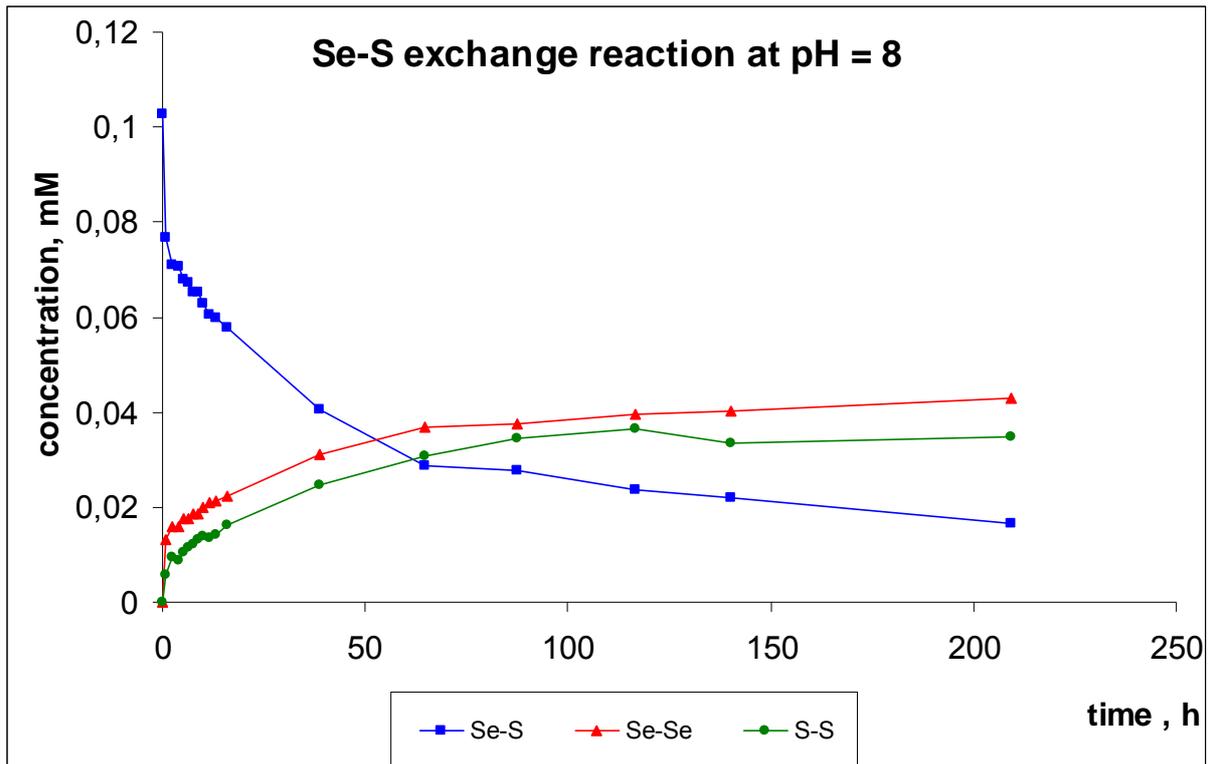
**Scheme 4.6.** Se-S exchange reaction at different pH-values (3-9)

This exchange reaction was investigated at pH-values between 3-9 and the disproportionation equilibrium monitored by HPLC (Schem 4.6b). A phosphate buffer (50 mM) was used for pH-setting. No peaks of the disulfide and the diselenide were observed within 10 days at pH 3, 4 and 5. This shows that the disproportionation reaction does not take place at a pH below 5. We detected products of disproportionation at pH 5.5 and 7, even though the reaction proceeds very slowly under these conditions (Graph 4.1.). There is a significant intrinsic pH shift from 7 up to 8.5 observed after 10 days, which leads to an increase of the reaction rate. The origin of the pH-change remained unclear (possible decomposition etc.). Thus HPLC results of disproportionation at pH 5.5 and 7 could not be mathematically described in order to obtain kinetic data. However, results at pH 8 and 9 allow us determine the rate and the equilibrium constants (Graph 4.2.).

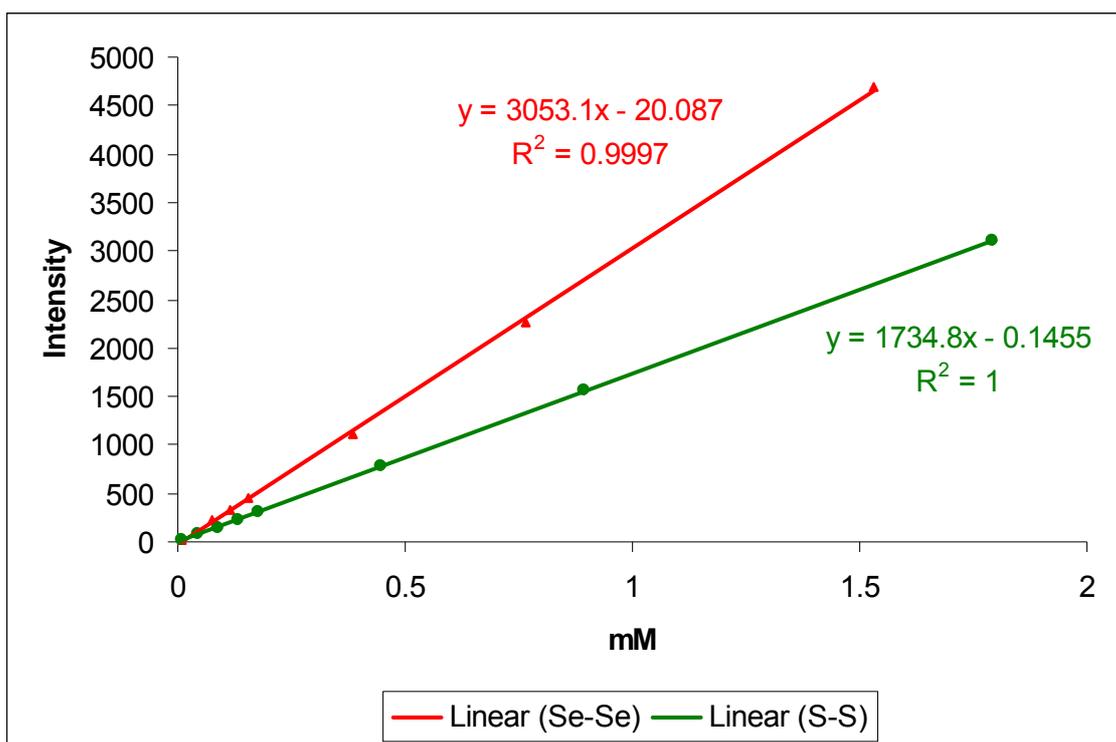


**Graph 4.1.**

The equilibrium is achieved at pH 8 after ca. 6 days and after 18 hours at pH 9 (Graph 4.2.).



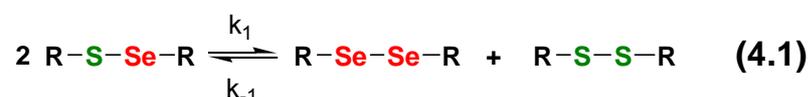
Graph 4.2.



**Graph 4.3. Calibration of the disulfide and diselenide**

### 4.3.2 Deduction of kinetic equation

The calibrations of the disulfide and the diselenide were necessary for the further calculations (Graph 4.3.). The coefficients of determination  $R^2$  were 0.9997 for Se-Se and 1.0000 for S-S. The calibration showed that the Se-Se and S-S have different adsorption intensities. Its average ratio is about 1.75. The calibration of selenenylsulfide did not succeed, because of its instability. Therefore the concentration was calculated via the Se-Se concentration using stoichiometry, according to equation 4.1:

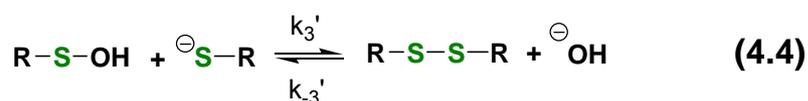
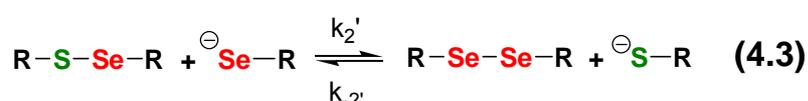
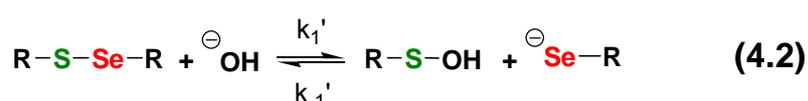


The concentration of S-S and Se-Se must be equal. The slight differences between the detected concentrations of Se-Se and S-S could either be measurement/calibration artefacts, e.g., because of interactions in the complex reaction mixture not relevant in the calibration of pure compound. Based on the HPLC results, more diselenide than disulfide was detected, especially at the start of the reaction (Table 4.1.). Because for the shift from parity of Se-Se and S-S concentration can be the intermediate involvement of hydroxide (or phosphate).

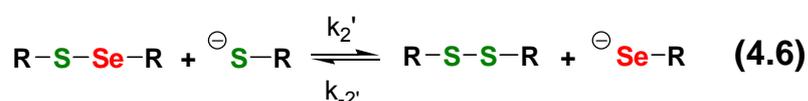
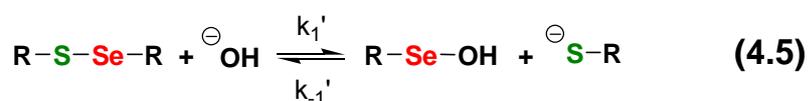
**Table 4.1. Experimental data of the disproportionation equilibrium Scheme 4.6b obtained by HPLC**

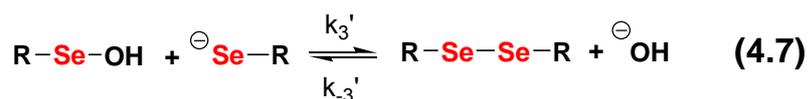
pH = 8				pH = 9			
<i>time</i>	<i>C (S-S)</i>	<i>C (Se-Se)</i>	$\frac{C (Se-Se)}{C (S-S)}$	<i>time</i>	<i>C (S-S)</i>	<i>C (Se-Se)</i>	$\frac{C (Se-Se)}{C (S-S)}$
0	0	0	-	0	0	0	-
0,70	0,0059	0,0131	2,22	0,10	0,0073	0,0132	1,81
2,56	0,0094	0,0158	1,68	0,36	0,0126	0,0190	1,51
4,14	0,0088	0,0160	1,82	0,89	0,0179	0,0252	1,41
5,18	0,0103	0,0175	1,70	1,15	0,0196	0,0275	1,40
6,22	0,0114	0,0177	1,55	1,68	0,0245	0,0287	1,17
7,52	0,0122	0,0187	1,53	2,21	0,0250	0,0303	1,21
8,82	0,0132	0,0187	1,42	2,74	0,0259	0,0318	1,23
10,13	0,0138	0,0199	1,44	3,27	0,0302	0,0334	1,11
11,69	0,0136	0,0211	1,55	3,79	0,0298	0,0338	1,13
13,25	0,0143	0,0214	1,50	4,57	0,0283	0,0347	1,23
16,16	0,0161	0,0224	1,39	5,61	0,0317	0,0359	1,13
38,88	0,0246	0,0310	1,26	6,91	0,0320	0,0368	1,15
64,57	0,0306	0,0370	1,21	8,21	0,0322	0,0373	1,16
87,40	0,0345	0,0375	1,09	9,52	0,0336	0,0381	1,13
116,49	0,0364	0,0395	1,09	11,08	0,0349	0,0382	1,09
140,25	0,0335	0,0403	1,20	12,64	0,0335	0,0391	1,17
209,39	0,0349	0,0430	1,23	16,32	0,0356	0,0384	1,08

This difference diminishes at the point of equilibrium and it proves that the reaction mechanism is more complicated as was supposed. It can be exemplified by the equations 4.2-4.4 or 4.5-4.7:



OR





Hydroxyl ions are strong nucleophiles. The selenolate anion is both a better nucleophilic and a better leaving group than the thiolate anion. Thus, the reaction 4.2 is faster than 4.5, and the reactions 4.3 and 4.7 proceed faster than 4.6 and 4.4, respectively. The following assumptions can be made:  $k_1' > k_2' > k_3'$  for eqs. 4.2-4.4 and  $k_3' > k_1' > k_2'$  for eqs. 4.5-4.7. Based on experimental data (Table 4.1.), more diselenide than disulfide was obtained. It is possible only in one case if the reaction mechanism is as in eqs. 4.2-4.4, otherwise the amount of diselenide and disulfide must be equal ( $k_3' \gg k_2'$  for eqs. 4.5-4.7). The difference between concentrations of diselenide and disulfide at pH 9 is less than at pH 8. It could be explain that at more basic pH the nucleophilicity of thiolate anion will be better, and therefore the reaction 4.4 will be faster. Also, this process is only relevant at the beginning of the reaction when no selenolate (or thiolate) as better catalyst is yet available. At a later stage that has changed.

For further calculation the disproportionation equilibrium was simplified to equation 4.1. The concentration of the selenenylsulfide is calculated via the concentration of the diselenide:

$$[\text{Se-S}] = [\text{Se-S}]_0 - 2 \times [\text{Se-Se}] \quad (4.8)$$

$[\text{Se-S}]_0$  = starting concentration at  $t = 0$ ; and  $[\text{Se-S}]$ ,  $[\text{Se-Se}]$  = concentration at any  $t$ .

The rate law of the second-order reaction is:

$$r = -\frac{1}{2} \frac{d[\text{Se-S}]}{dt} = \frac{dx}{dt} = k_1[\text{Se-S}]^2 - k_{-1}[\text{Se-Se}] \times [\text{S-S}] \quad (4.9)$$

At the equilibrium is  $dx/dt = 0$  and therefore

$$K_c = \frac{k_1}{k_{-1}} = \frac{[\text{Se-Se}]_{eq} [\text{S-S}]_{eq}}{[\text{Se-S}]_{eq}^2} \quad (4.10)$$

The integration of the differential equation 4.9 gives:

$$\ln \frac{x \left( \frac{1}{2} [Se - S]_0 - 2x_{eq} \right) + \frac{1}{2} [Se - S]_0 x_{eq}}{\frac{1}{2} [Se - S]_0 (x_{eq} - x)} = \frac{[Se - S]_0 \left( \frac{1}{2} [Se - S]_0 - x_{eq} \right)}{x_{eq}} k_1 t \quad (4.11)$$

wherein  $x = [Se - Se]$  and  $x_{eq} = [Se - Se]_{eq}$  = concentration of the diselenide at the equilibrium.

### 4.3.3 Calculation of kinetic data

Graph 4.4. is a representation of equation 4.11 with the data obtained from the measurements at pH = 8 and 9.

Straight lines in both cases (pH 8, 9) were obtained with good coefficients of determinations

$R^2$  and with slopes  $\frac{[Se - S]_0 \left( \frac{1}{2} [Se - S]_0 - x_{eq} \right)}{x_{eq}} k_1$ , therefore

for pH 8:

$$K_c = 3.86; \quad k_1 = 0.81; \quad k_{-1} = 0.21$$

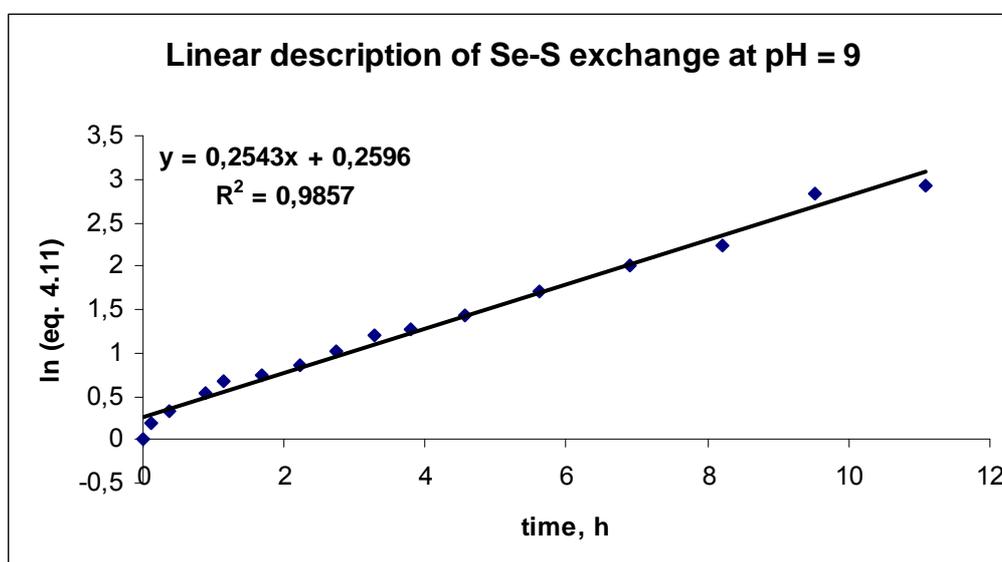
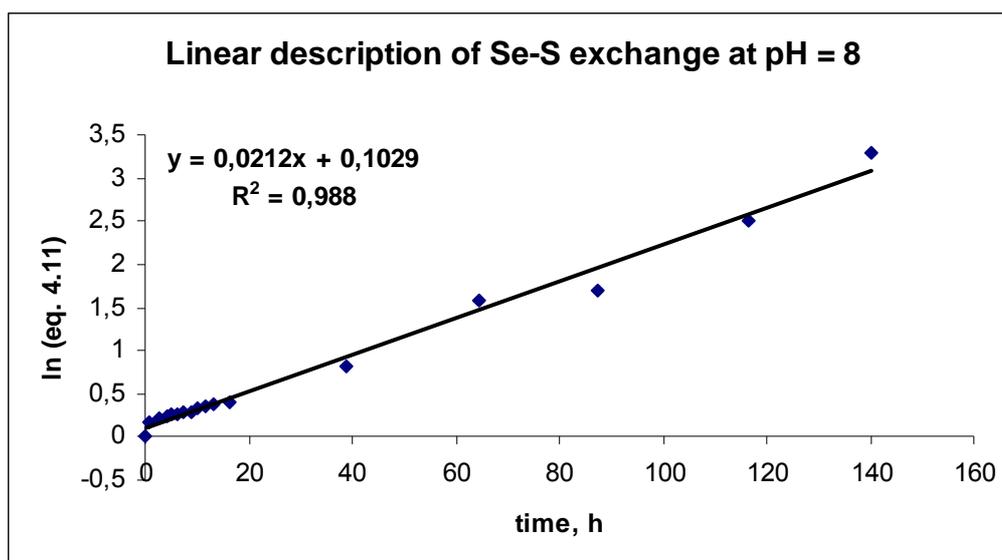
for pH 9:

$$K_c = 4.08; \quad k_1 = 10.54; \quad k_{-1} = 2.58,$$

i.e. at these pH-values, the equilibrium is shifted towards the homodimers by a factor of 4.

Thus, the ratio between rate constants at pH 8 and pH 9 is:

$$\frac{k_1^{pH=9}}{k_1^{pH=8}} = 13.$$



**Graph 4.4. Graphical representation of equation 4.11 at pH = 8 and 9**

These results indicate that the disproportionation is the favored route of the exchange reaction 4.1. (Scheme 4.6b). Selenenylsulfide is stable up to pH 6 and no fast exchange reaction is observed. Perhaps there is no sufficient concentration of  $\text{OH}^-$  at pH 6, which probably attacks the selenenylsulfide bridge at the sulfur atom to obtain sulfenic acid and the selenolate anion (equation 4.2.). Further the selenolate anion attacks another molecule of selenenylsulfide at the selenium atom to afford the diselenide and the sulfide anion (equation 4.3.), which react with sulfenic acid to give the disulfide (equation 4.4.).

The equilibrium constants at pH 8 and 9 are similar. The same result ( $1/K_c = 3,69$ ) was obtained during mass spectrometry investigation of Se-S exchange reaction between *N*-Boc cystine and *N*-Boc selenolate anion in DMSO (Scheme 4.3b). It means that equilibrium constant does not depend much on pH.

The rate constant of the disproportionation reaction 4.1 is 13 times faster at pH 9 as at pH 8. It could be concluded that there is no pH influence on the equilibrium, namely equilibrium concentrations of educts and products, but on the rate constant – i.e. the speed at which equilibrium is achieved.

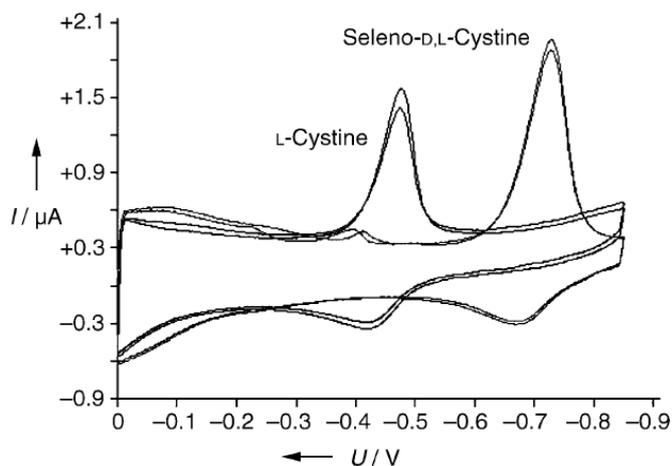
The results show that the reaction rate of the Se-S exchange reaction of (Se)cysteines increases with the pH, as would be expected from S-S-interchange studies.

## 5 Electrochemistry

### 5.1 Studies of the redox potential of diselenides and Se-S exchange kinetics

#### 5.1.1 Introduction

The strong adsorption of selenols on electrodes makes a direct measurement of redox potentials often impossible. Their determination thus usually utilizes indirect measurements based on exchange reactions with reference redox couples such as DTT ( $E_0 = -323\text{mV}$ ),  $\beta$ -mercaptoethanol ( $E_0 = -207\text{mV}$ ) and glutathione ( $E_0 = -205\text{mV}$ ). Nevertheless, Jacob et al. have demonstrated that useful results can be obtained for cysteine/cystine ( $E_0 = -233\text{mV}$  vs. NHE) and selenocysteine/selenocystine couples ( $E_0 = -488\text{mV}$  vs NHE) using cyclic voltammography with a dropping mercury working electrode (Figure 5.1.).(Jacob et al., 2003) The dropping electrode constantly produces fresh metal surface, and thus at least for the initial scan, adsorption phenomena can be distinguished.



**Figure 5.1.\*** Cyclic voltammograms of cystine and selenocystine

Moroder has completely reviewed works in this field.(Moroder, 2005; Moroder et al., 2005) In addition to Moroder's reviews, Jacob et al. and Wessjohann et al. continued showing possibilities to obtain redox potentials of diselenide and disulfide couples (i.e. pyridine, aniline, quinoline derivatives, and tetrapeptides of the C-terminal active center of thioredoxin

\* - Voltammograms of L-cystine (0.1 mM) and D,L-selenocystine (0.05 mM) were recorded in potassium hydrogen phosphate buffer (pH 7.0; 200 mM) at 25 °C and at a scan rate of 500 mV/s with a dropping mercury working electrode, a standard silver reference electrode (SSE), and a platinum counter electrode. The significant difference in redox potential of about 250 mV between selenocysteine/selenocystine (-710 mV versus SSE, i.e., -488 mV versus NHE) and cysteine/cystine (-455 mV versus SSE, -233 versus NHE) confers considerably more reducing properties to selenocysteine.

reductase) using direct measurements at a dropping mercury electrode. (Collins et al., 2005; Schneider et al., 2007)

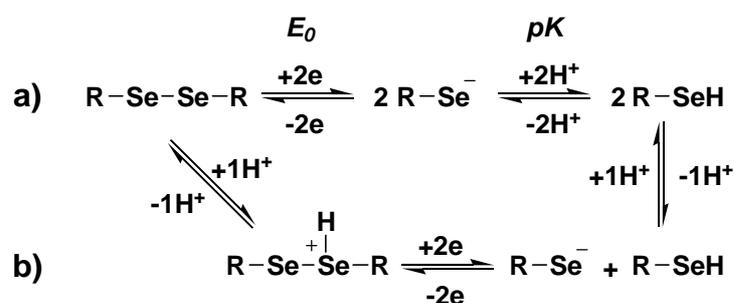
Koppenol et al. used a graphite working electrode with a glassy carbon counter electrode and an Ag/AgCl reference electrode to determine one-electron electrode potential of the  $NH_2CH(COOH)CH_2Se^\bullet + e \Rightarrow NH_2CH(COOH)CH_2Se^-$  couple ( $E^{0'}(\text{pH } 7) = 430 \text{ mV vs. NHE}$ ). (Nauser et al., 2006) They investigated also one- and two-electron transfers within the couple selenocystine / selenocysteine by an indirect method using benzyl viologen ( $BV^{2+}$ ) as oxidant.

### 5.1.2 Electrochemical investigation of selenocystine derivates

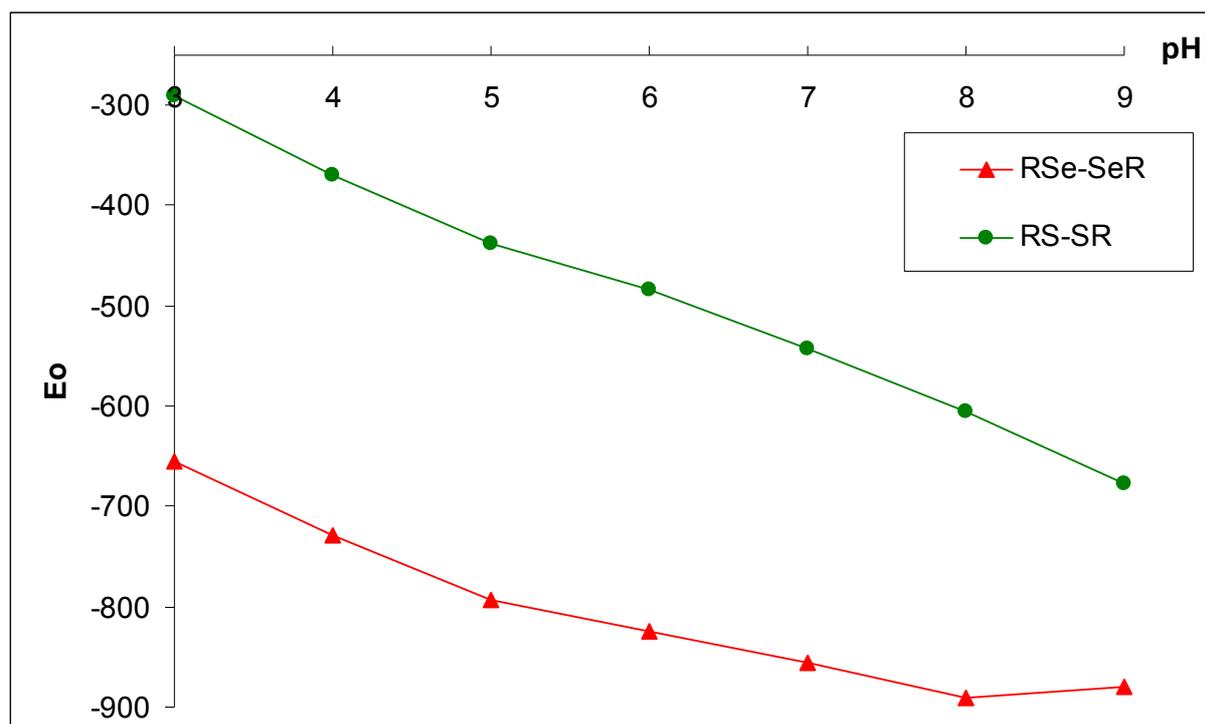
Investigations of potentials  $E_0'$  of selenocystine with different amino and carboxylate protecting groups in our laboratory show that protected selenocystine is more electronegative than its unprotected analogue (Table 5.1.).

Further investigations of  $E_0'$  dependence on the pH were made with the water soluble analogues of (selenol)cystine **13** and **14** (Scheme 4.4.). Phosphate buffer (50 mM) was used to set the pH. The results show that electropotentials of diselenide and disulfide of (Se)-cysteine analogues become more electronegative with increasing pH (Graph 5.1.).

This redox process can be described according to the following equations:



**Scheme 5.1.** Putative mechanism of redox process of diselenide (a)-protonation after reduction, (b)-protonation prior to reduction



**Graph 5.1.** Dependence of electric potentials of disulfide RS-SR (13) and diselenide RSe-SeR (14) on pH ( $C = 0.02$  mM; phosphate buffer = 50 mM; scan rate – 1000 mV/s; all electrochemical potentials are given versus SSE)

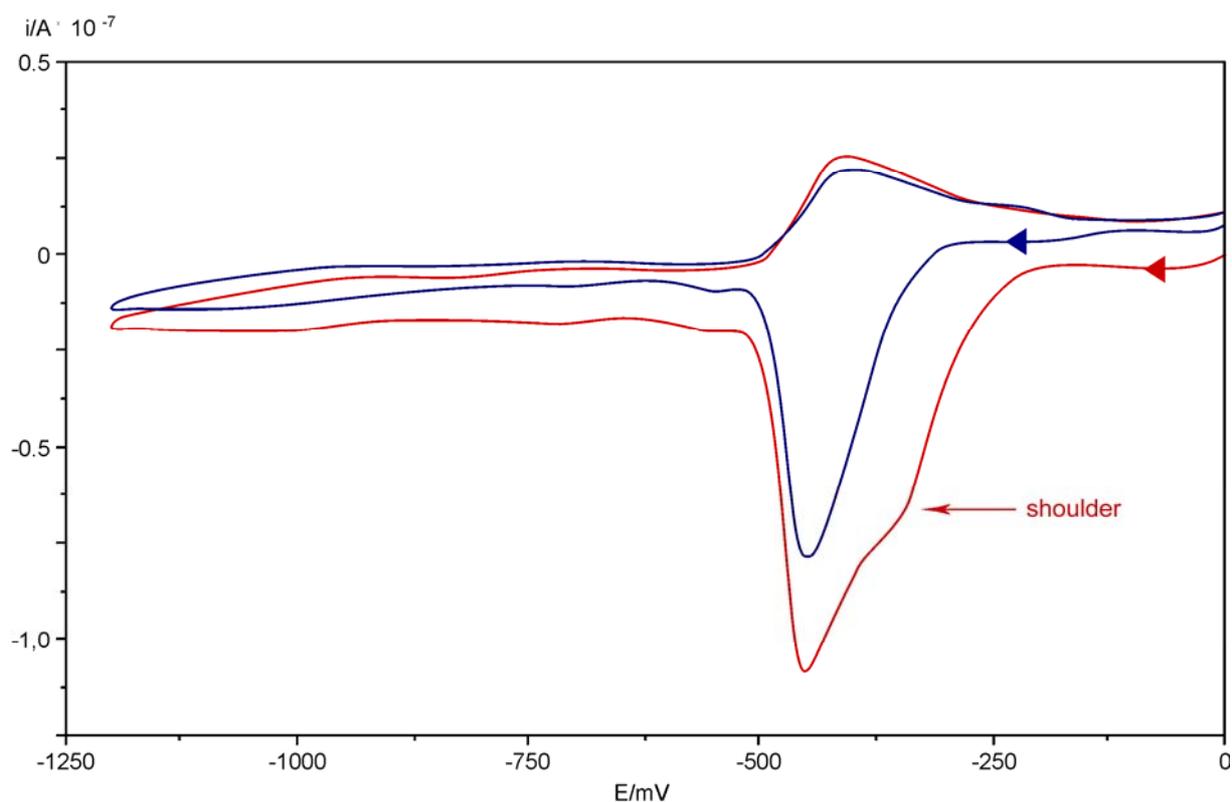
The diselenide will be reduced with 2 electrons to the selenolate anion, which is in equilibrium with its protonated form (Scheme 5.1a). Alternatively, the diselenide will be protonated first and then reduced with 2 electrons (Scheme 5.1b). At acidic pH, the latter equilibrium (Scheme 5.1a and 5.1b) shifts to the right, producing the selenol and thus the value of  $E'_0$  will be small. The dissociation of selenol with increasing pH shifts the equilibrium to the selenolate anion, resulting in a more negative value of  $E'_0$ .

Table 5.1. Experimental data of electric potentials of several synthesized diselenides

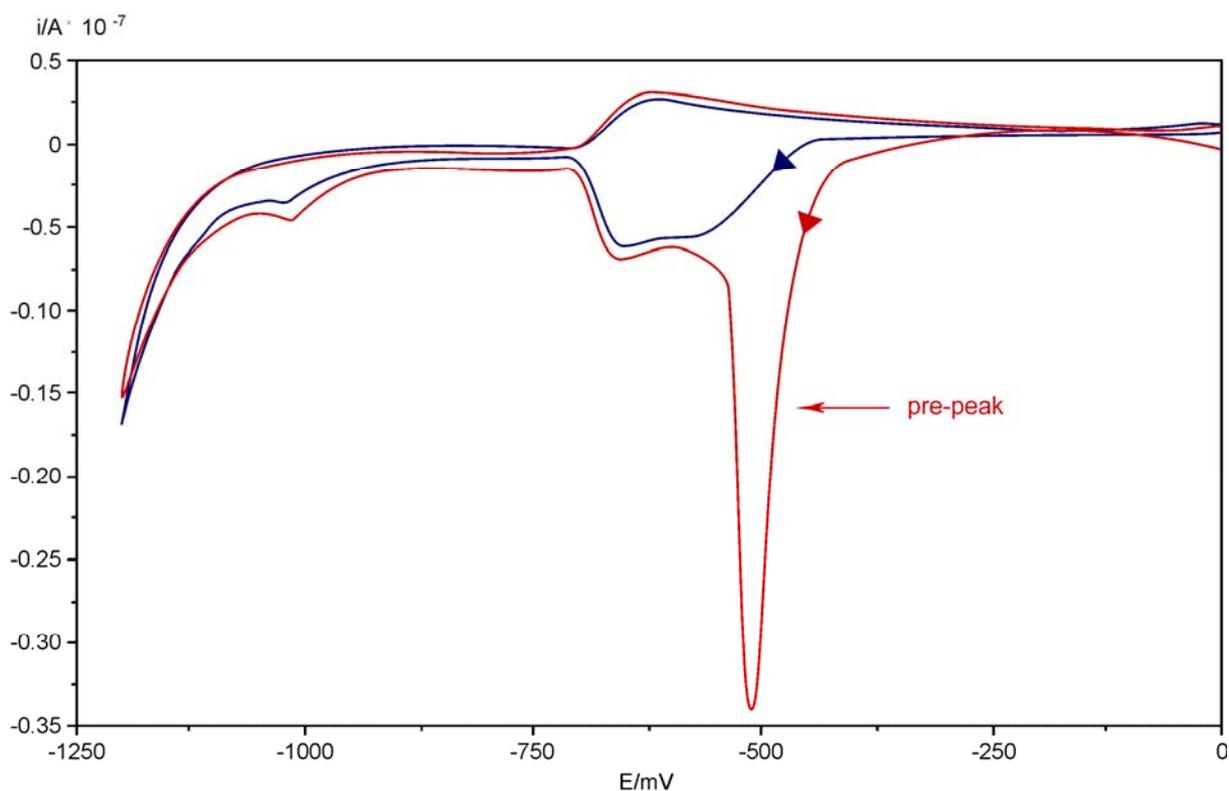
Compounds	$E'_0$ vs. NHE	$E'_0$ vs. SSE
 (10)	-477 mV <sup>1</sup> -488 mV <sup>2</sup>	-699 mV <sup>1</sup> -710 mV <sup>2</sup>
 (9)	-211 mV <sup>3</sup> -233 mV <sup>4</sup>	-433 mV <sup>3</sup> -455 mV <sup>4</sup>
 (4a)	-537 mV <sup>5</sup>	-759 mV <sup>5</sup>
 (6)	-267 mV <sup>5</sup>	-489 mV <sup>5</sup>
 (14)	-634 mV <sup>6</sup>	-856 mV <sup>6</sup>
 (13)	-321 mV <sup>6</sup>	-543 mV <sup>6</sup>

<sup>1</sup> – 0.04 mM in H<sub>2</sub>O, scan rate 500 mV/s<sup>2</sup> – 0.05 mM in potassium hydrogen phosphate buffer (pH 7.0; 200 mM), scan rate 500 mV/s<sup>3</sup> – 0.08 mM in H<sub>2</sub>O, scan rate 500 mV/s<sup>4</sup> – 0.1 mM in potassium hydrogen phosphate buffer (pH 7.0; 200 mM), scan rate 500 mV/s<sup>5</sup> – 0.06 mM in H<sub>2</sub>O, scan rate 500 mV/s<sup>6</sup> – 0.02 mM in potassium hydrogen phosphate buffer (pH 7.0; 50 mM), scan rate 1000 mV/s<sup>2</sup> and <sup>4</sup> – data taken from literature reference (Jacob et al., 2003)

From the cyclic voltammogram of the cystine derivate **13** it can be seen, that the reductive peak has a shoulder and it is more intensive as the oxidative peak (Figure 5.2.). This shoulder disappears with further redox-cycles (e.g. the 3<sup>rd</sup> scan – blue line in figure 5.2.). This proves that the shoulder is an adsorption peak. As is well known, in the case of weak reactant, the two peaks (adsorption and solution peaks) are not discernible and the reductive peak should be higher as for a simple reversible system and the oxidative peak should also be enhanced, but less so. It gives the best fit in our case with the cystine derivate **13** (Figure 5.2.). It means that the cystine derivate **13** adsorbs on the Hg electrode and this binding is weak.



**Figure 5.2.** Cyclic voltammogram of cystine derivate **13** (red line: 1<sup>st</sup> scan; blue line: 3<sup>rd</sup> scan; all electrochemical potentials are given versus SSE)



**Figure 5.3.** Cyclic voltammogram of selenocystine derivate **14** (red line: 1<sup>st</sup> scan; blue line: 3<sup>rd</sup> scan; all electrochemical potentials are given versus SSE)

The situation for the selenocystine derivate **14** is reversed (Figure 5.3.). At the first scan (red line) two reductive peaks were recorded, where the first is the high and sharp adsorption peak. At the third scan (blue line) the first peak moved close to the second and became its shoulder. The adsorption and solution peaks at the first scan are separated and it means the adsorption in this case is very strong. On the basis of the results, the selenocystine derivate is strongly adsorbed on the Hg-electrode.

It shows that the selenocystine derivate is stronger adsorbed on the electrode as its cysteine analogue. In case of selenenylsulfide we obtained already a mixture of electro potentials of the disproportionation products.

## 5.2 (Seleno)cysteine/(seleno)cysteine tetrapeptides

It has been shown that mutation of Sec to Cys in the C-terminal redox active site of mammalian TrxR leads to a 100-fold lower catalytic rate for Trx reduction (Table 5.2.).(Lee et al., 2000; Zhong et al., 2000; Zhong and Holmgren, 2000) In contrast to this, TrxR in *Drosophila melanogaster* (DmTrxR) has no Sec by nature, but it is otherwise structurally very similar to mammalian TrxR. Nevertheless, it still carries 50% activity compared to human

TrxR, i.e., it is almost unaffected by native substitution of Sec to Cys (Table 5.2.).(Kanzok et al., 2001) The only obvious difference to the mammalian form is the C-terminal tetrapeptide sequence, with its replacement of both glycine residues by serine, forming the sequence Ser-Cys-Cys-Ser-COOH. In addition, other mutational studies of the TrxR C-terminal tetrapeptide sequences suggest that the presence of an adjacent serine in selenium-free proteins might play a key role in achieving a catalytic activity similar to that found in related selenium containing isoenzymes.(Gromer et al., 2003) However, the corresponding Ser-Cys-Cys-Ser-COOH mutant of mammalian TrxR has less than 0.5% activity in comparison to the wild-type enzyme (Table 5.2.).(Johansson et al., 2006) It is also well known that Cys and Sec have very different pKa values and thus a different pH dependence. (Brandt and Wessjohann, 2005; Moroder, 2005)

Consequently, it can be concluded that not only are the flanking serine residues of functional importance for catalytic activity, but also the local pH in the protein's active site. In addition, folding in proteins can change redox properties. To elucidate the influence of Cys-Cys flanking serine as a function of both its pH and location (C- vs. N-terminal), but unaffected by distortion (protein folding) effects, a set of aa-Cys-Cys-aa, and aa-Cys-Sec-aa tetrapeptides was designed for electrochemical analysis (see chapter 3.2.2.). Different redox properties of tetrapeptides over a wide range of pH values (pH 3–8) should provide an explanation for the proposed importance of serine in the C-terminal redox active center.

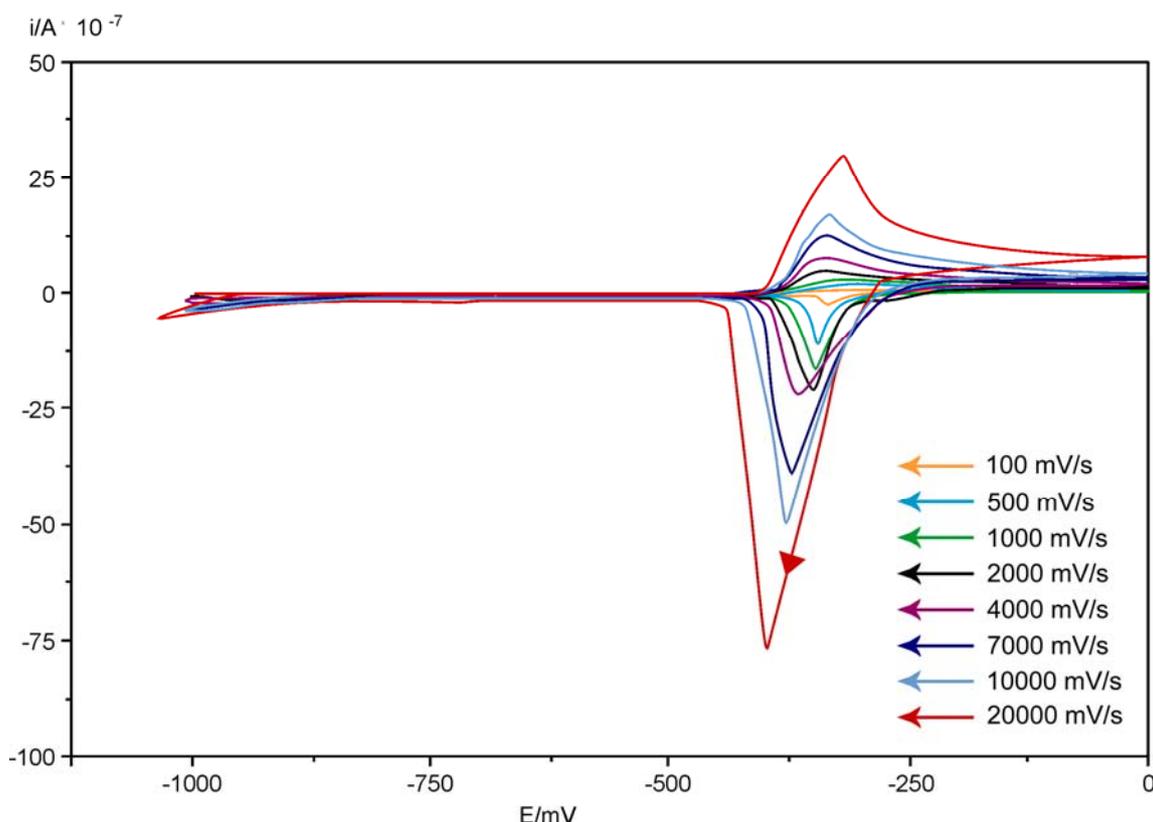
**Table 5.2. Catalytic activities of mutated and wild forms of TrxRs**

Active centre	Enzyme	$k_{cat}$ , %
-Gly-Cys-Sec-Gly	Mammalian enzyme ( <i>wild</i> )	100
-Gly-Cys-Cys-Gly	Mammalian enzyme	1
-Ser-Cys-Cys-Ser	<i>Drosophila melanogaster</i> ( <i>wild</i> )	50
-Gly-Cys-Cys-Ser	<i>Drosophila melanogaster</i>	45
-Gly-Cys-Cys-Gly	<i>Drosophila melanogaster</i>	5
-Ser-Cys-Cys-Ser	Mammalian enzyme	0.5

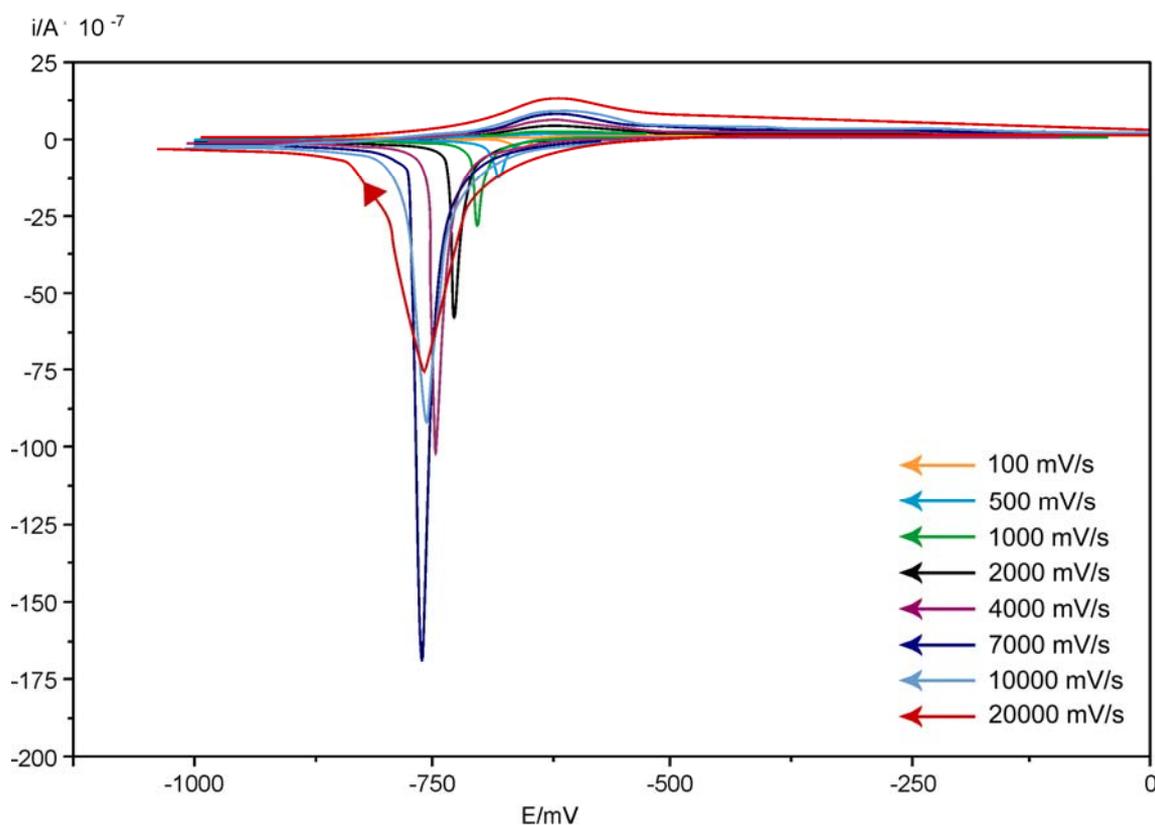
Redox potentials were measured using cyclic voltammetry with a dropping mercury working electrode vs. a standard silver electrode (SSE). The conditions for electrochemistry measurements were as reported by Jacob et al. (2003) for selenocystine and cystine. Although measurement of chalcogens on Hg is complicated by adsorption phenomena, the method is faster and superior to equilibrium titrations and carbon electrodes, and values are comparable in relative terms. pH values were adjusted using citric acid/phosphate buffer. Three parallel experiments for each pH value, scan rate, concentration and tetrapeptide were performed to obtain electrochemical data. Relative standard deviations were within 1–2.5%.

### 5.2.1 Study of adsorption

Surface processes namely adsorption on Hg were studied at two different pH values: pH 3 and 7. The cyclic voltammograms of model tetrapeptides GCCG and GCUG were obtained by variable sweep rates from 100 mV/s to max. 20'000 mV/s (Figure 5.4. and 5.5.).



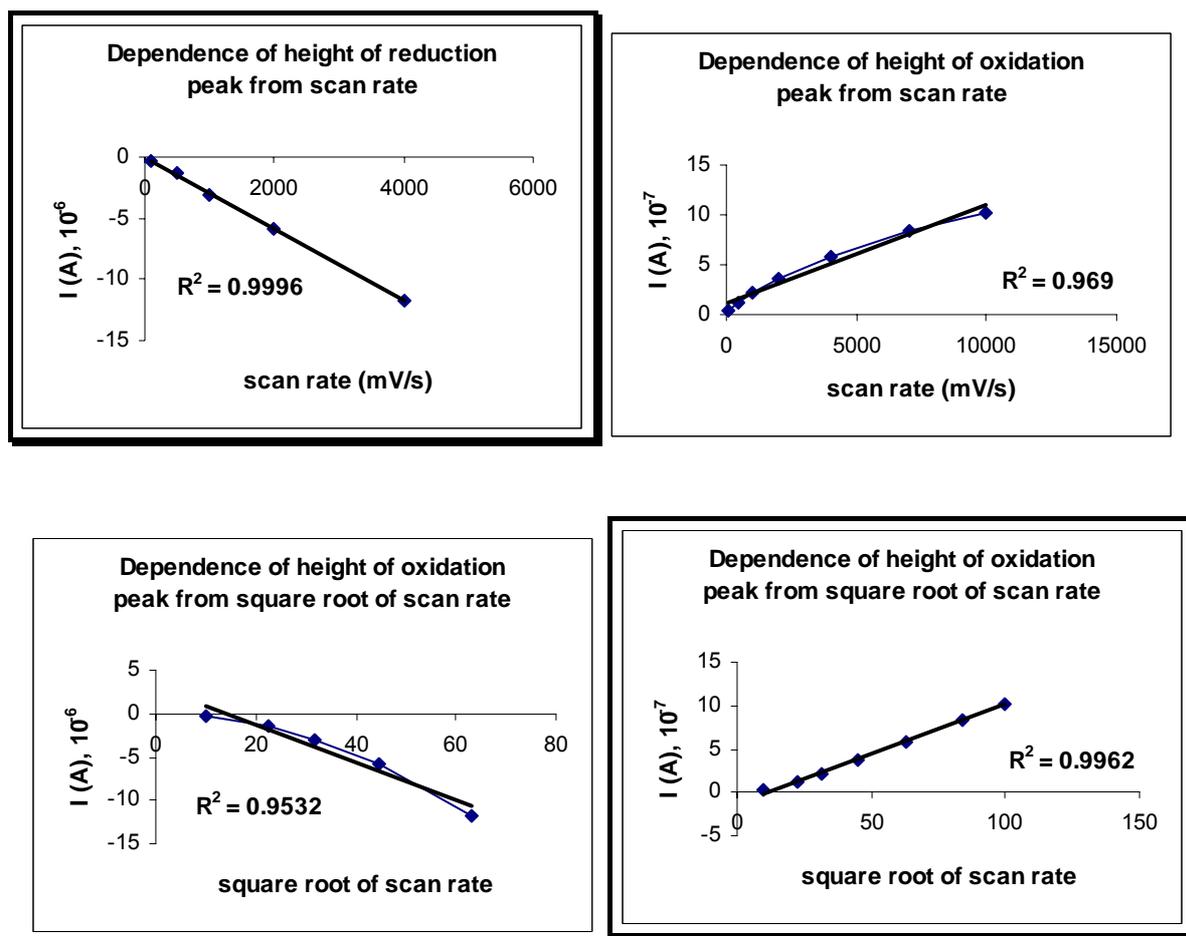
**Figure 5.4.** Cyclic voltammograms of GCCG at pH 3 with sweep rates of 100 to 20'000 mV/s (*all electrochemical potentials are given versus SSE*)



**Figure 5.5.** Cyclic voltammograms of GCUG at pH 7 with sweep rates of 100 to 20'000 mV/s (*all electrochemical potentials are given versus SSE*)

The presence of adsorption is usually obvious, if we have a pre-peak or post-peak (Figure 5.2. and 5.3.). But if the adsorption and diffusion processes occur simultaneously and give rise to charge transfer at the same potential, the presence of adsorption may not be readily apparent. The study of the total current  $I$  at the different scan rates is needed to detect the presence of adsorption in these cases. In the case of an adsorption, the peak current is proportional to the sweep rate  $v$ , and not to its square root,  $\sqrt{v}$ , as in the case of free species.

We plotted the cathodic and anodic peak current as a function of scan rate. Here the analysis of voltammogram of GCUG at pH 7 is presented as an example (Graph 5.2.). As can be seen from these graphs, the cathodic (reduction) peak current is proportional to the scan rate and the anodic (oxidation) peak current to its square root. That means, the cathodic peak is the reduction peak of adsorbed oxidant and the anodic peak is the oxidation peak of solution (non-adsorbed) species of reductant.



**Graph 5.2.** Study of adsorption of GCUG on a mercury electrode at pH=7

We made the same analyses for GCUG at pH 3 and GCCG at pH 3 and 7. For all these cases, the cathodic peak is the adsorption peak and the anodic peak is the oxidation peak of free species. Only in the case with GCCG at pH 3, we obtained both peaks (cathodic and anodic) as the adsorption peaks (Table 5.3.).

**Table 5.3.** Dependence of the anodic current of the oxidized model tetrapeptides on sweep rate at pH 3 and 7

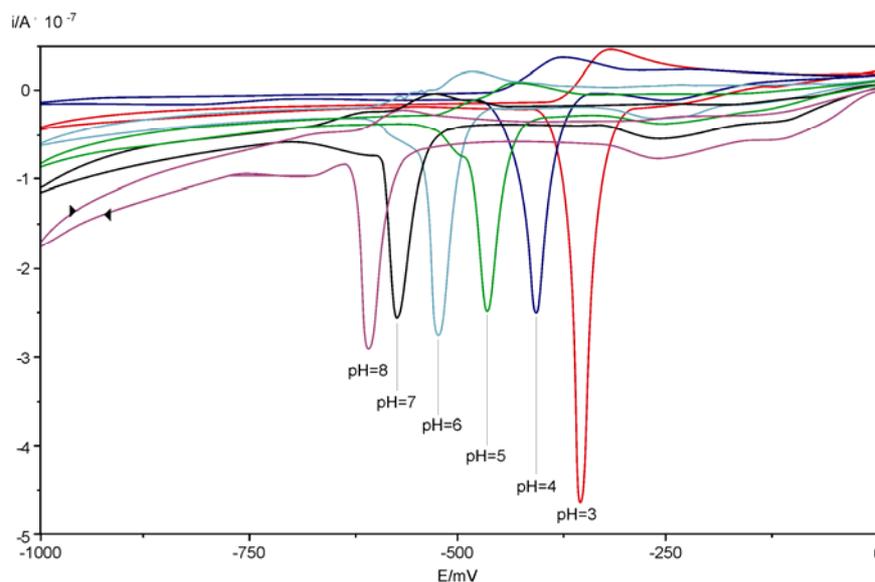
pH	Tetrapeptide	Proportional to	Hg-Absorption
3	GCCG	$\sim v$	+
	GCUG	$\sim \sqrt{v}$	-
7	GCCG	$\sim \sqrt{v}$	-
	GCUG	$\sim \sqrt{v}$	-

Therewith we have demonstrated that the reduction process of tetrapeptides proceeds after the adsorption of oxidant and gives non-adsorbed product in solution, with the exception of GCCG at pH 3. The adsorption of selenocysteine derivatives is stronger as with cysteine derivatives. A  $\Delta E$  test and adsorption study revealed that the reaction is ‘quasi-irreversible’, likely due to irreversible binding of most of the reduced SH or SeH to mercury.

### 5.2.2 Investigation of pH dependence on redox potential\*

By increasing the pH from 3 to 8, reductive and oxidative potentials of the model tetrapeptides (GCCG, GCCS, SCCG, SCCS, GCUG, GCUS, SCUG, SCUS) decrease, and move to more negative values (e.g. Figure 5.6. and 5.7.). Up to pH 6, the trend for reduction potentials of disulfide peptides with varied combinations of flanking Ser was almost identical, i.e., all Cys-Cys peptides with serine residues with respect to slope behaved similarly to each other and to a minor extent even to GCCG.\*\* Only under neutral and slightly basic conditions did the insertion of a polar serine neighbour induce a significant additional shift towards lower redox potential (Graph 5.3). With increasing pH above 7, reductive potentials reached almost the same value as for Cys-Sec peptides. Interestingly, the double serine derivative was less effective than the single serine forms.

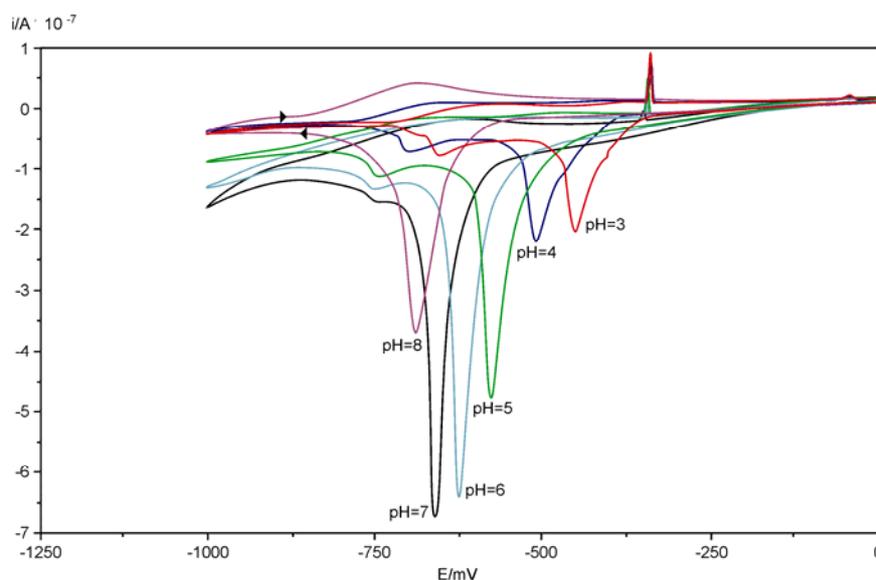
Measurements above pH 8 were not possible because of the decomposition of model tetrapeptides via  $\text{OH}^-$  interference with possible further oligomer formation.



**Figure 5.6.** Cyclic voltammograms of the disulfide GCCG (2  $\mu\text{M}$ ) at different pH (3–8), third cycle, scan rate 500 mV/s (all electrochemical potentials are given versus SSE)

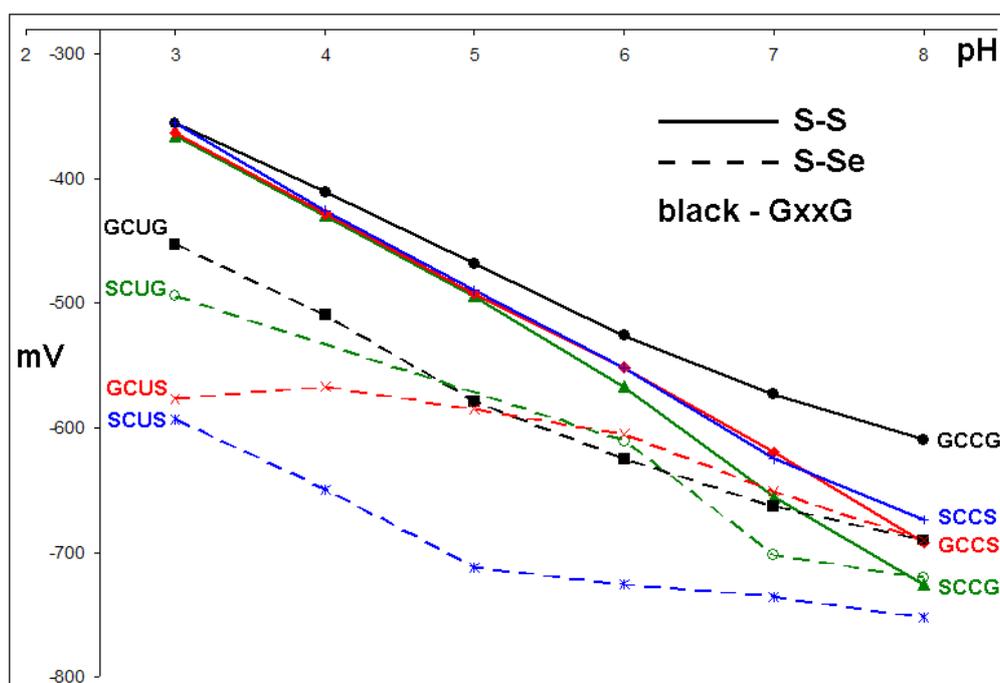
\* - adopted from article Schneider, A., Brandt, W., and Wessjohann, L.A., *The influence of pH and flanking serine on the redox potential of S-S and S-Se bridges of Cys-Cys and Cys-Sec-peptides*. *Biol. Chem.* 388, (2007): 1099-1101.

\*\* of course the absolute potentials vary, with the selenides always requiring more reducing power.



**Figure 5.7.** Cyclic voltammograms of the diselenide GCUG ( $4 \mu\text{M}$ ) at different pH (3–8), third cycle, scan rate  $500 \text{ mV/s}$  (all electrochemical potentials are given versus SSE)

With the introduction of selenium, i.e., in selenenylsulfide tetrapeptides, a general shift to lower redox potential occurred. The introduction of flanking serine(s) significantly shifted the potential towards even lower values. This also depended on the positioning of serine, but this time, as expected, with the lowest value for the double serine derivative. Most importantly, this is already evident at acidic pH.



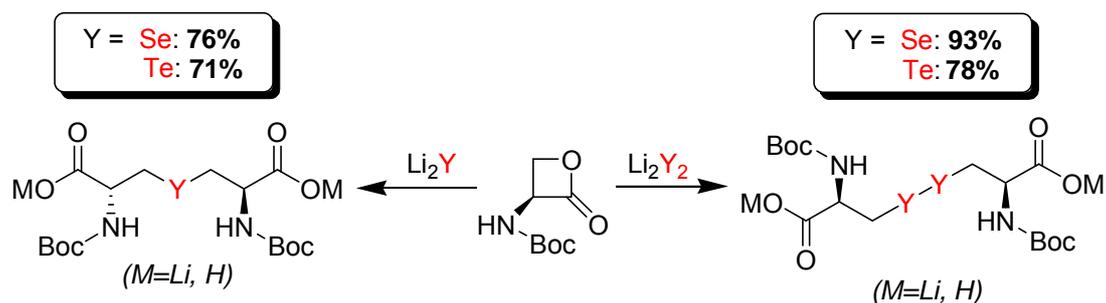
**Graph 5.3.** pH dependence of the reduction potentials of oxidized tetrapeptides (GCCG, SCCG, GCCS, SCCS, GCUG, SCUG, GCUS, SCUS)

In conclusion, the influence of flanking serine (hydrogen bond donor/acceptor) has no significant influence on cystine (S-S) bridges at acidic pH, but can significantly decrease the potential at basic pH, where a gain of more than 60 mV vs. glycine flanking can be achieved. Based on electrochemical experimental data, the most similar Cys-Cys tetrapeptide to GCUG at pH 7 is SCCG. Surprisingly, SCCG has a more negative reduction potential than SCCS and GCCS and is therefore likely to be catalytically more reactive. This result differs from the mutational studies on DmTrxRs performed by Gromer et al. (2003).

Our results clearly indicate that pH always has a significant and direct influence on the redox potential of both cysteines and selenocysteines, with more negative potentials at higher pH. A special flanking effect of serine (-OH) is not general. It can be observed only at certain pH values: the pH-dependent additional shift is significant for Cys-Cys (S-S) at neutral pH and even more so at basic pH. In contrast to disulfides, Cys-Sec (S-Se) shows serine-dependent shifts at even slightly acidic pH. In proteins the pH value experienced by the dichalcogen bridges obviously depends on the microenvironment within the protein's active site. Conformational and steric constraints, which are beyond the scope of this study focussing on electronic effects, may cause additional individual differences, as observed in the mutational studies. Also, in our experiments the C-terminal  $-\text{CO}_2\text{H}$  was protected to avoid an influence of its deprotonation status on the serine effect. In nature this is not so, and Iwaoka could show that flanking bases and acids strongly influence selenoxide redox reactivity (Iwaoka and Tomoda, 2000).

## 6 Summary

In the past decades organochalcogenides have been shown to be interesting compounds in biochemistry. Despite the increasing importance of the selenium and tellurium analogues of sulfur amino acids, there are very few methods available for the production of these compounds. To solve this problem, a short synthesis of Boc-protected L-selenolanthionine, L-tellurolanthionine, L-selenocystine, L-tellurocystine and L-tellurocysteine derivate was developed (Scheme 1).



**Scheme 1**

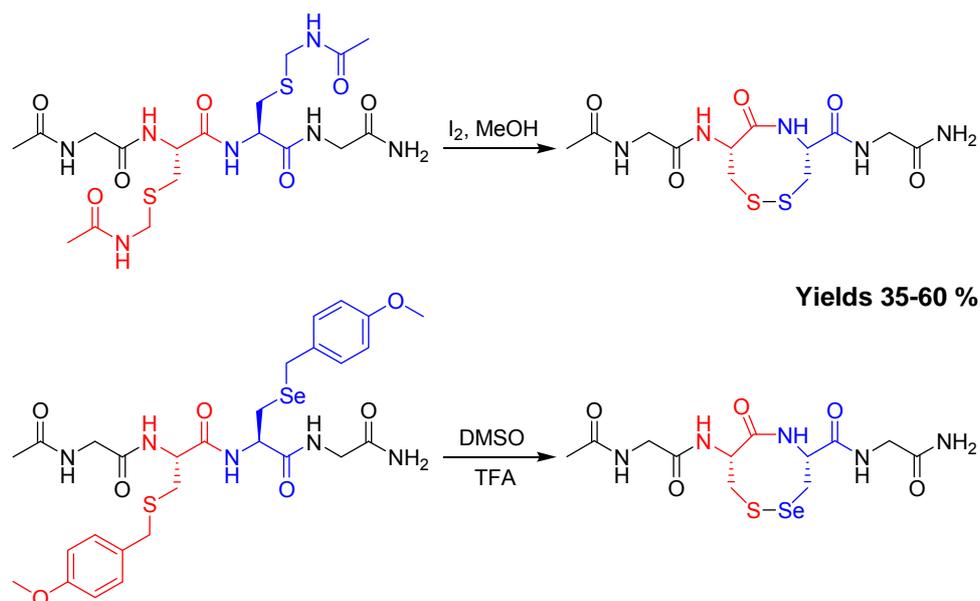
Investigation of potentials  $E_0'$  of selenocystine with different amino and carboxylate protecting groups has shown that protected selenocystine is more electronegative than its unprotected analog [e.g.,  $(\text{Boc-Sec-OH})_2$  has -549 mV vs. NHE and  $(\text{NH}_2\text{-Sec-OH})_2$  has -477 mV vs. NHE].

Using MS techniques, putative tri-chalcogen anionic intermediates  $[\text{Se-Se}\dots\text{S}]$  of the exchange reaction between the protected water-soluble analogs of selenocystine and the thiolate anion of cysteine could be detected. This indicates an  $\text{S}_{\text{N}}2$ -mechanism for this reaction. Further studies of Se-S exchange reactions using water-soluble analogs of selenocystine/cystine and selenenylsulfides have shown that exchange rate and equilibrium constants are strongly dependent on pH. As expected, exchange reactions were faster at more basic pH (e.g., with rate constants 13-times higher at pH 9 than at pH 8).

In selenocysteine (Sec, U)-containing proteins the selenenylsulfide bridge and its reduced thiol-selenol counterpart are the significant species. It was proposed that serine, a hydrogen donor/acceptor, as a flanking amino acid has an influence on the redox potential of S-S and S-Se bridges. To check the generality of this proposal, eight model tetrapeptides (GCCG, SCCG, GCCS, SCCS, GCUG, SCUG, GCUS, SCUS) were synthesized including the GCUG-sequence of human thioredoxin reductase and the SCCS-sequence of *Drosophila melanogaster*.

Using solid phase peptide synthesis (SPPS) the protected and reduced forms of model tetrapeptides were synthesized in good yields. SPPS was performed on the Knorr Amide MBHA resin for Fmoc chemistry. All amino acids have been coupled according to the HBTU/HOBt/DIPEA method.

The difficult formation of an 8-membered ring resulting from neighbouring Sec and Cys, like in thioredoxin reductases TrxR's, was successful with I<sub>2</sub>/MeOH and DMSO/TFA oxidation (Scheme 2).



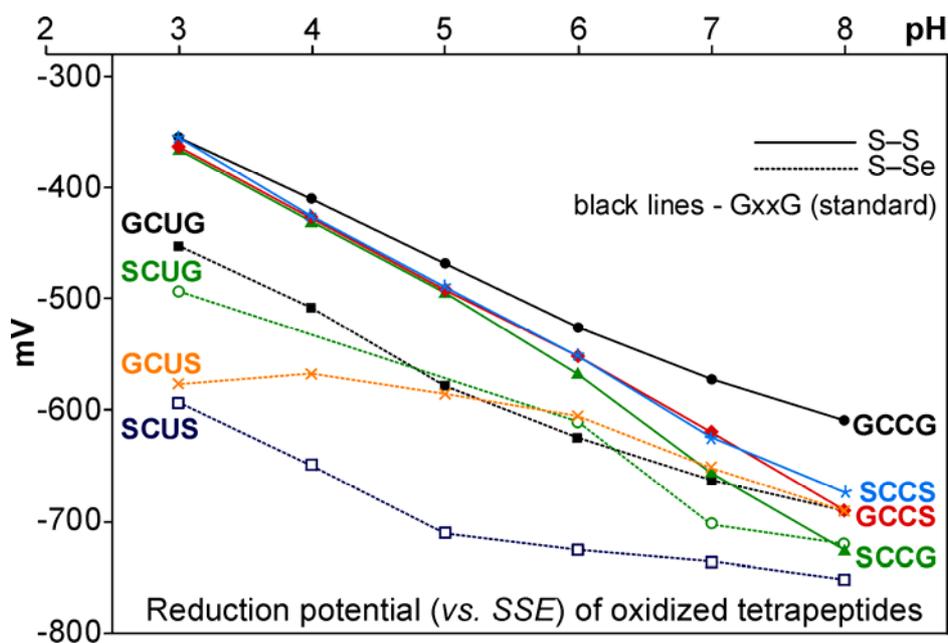
### Scheme 2

Investigations of  $E'_0$  dependence at varying pH-values showed the electric potentials of Se-Se, S-S and S-Se bridges of (Se)-cysteine analogues to become more electronegative with increasing pH.

The adsorption study of the model tetrapeptides done at different pH showed that the reduction process proceeds after the adsorption of the oxidant (disulfide/diselenide) to the electrode and (partly) results in non-adsorbed product (reductant: thiol/selenol) into the solution. An  $\Delta E$  test and the adsorption study confirmed that the reaction is 'quasi-irreversible', likely due to irreversible binding of most of the SH or SeH to mercury.

The redox potential of S-Se and S-S bridges of the model tetrapeptides strongly depends on the pH and of serine in its vicinity (Graph 1):

- (1.) higher pH gives better reducible dichalcogenides; and
- (2.) a significant influence of flanking serine on disulfide exists only at neutral and even more at basic pH.

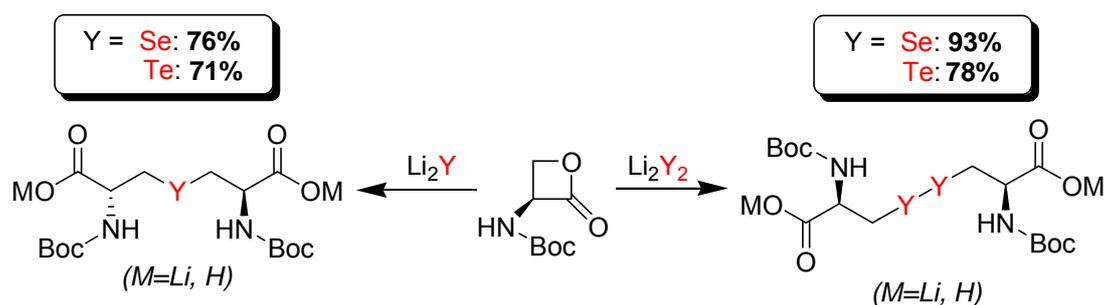


**Graph 1. pH dependence of the reduction potentials of oxidized tetrapeptides**

Such investigations, including the influence of serine as neighboring amino acid residues on the reactivity of the Se-S bridge at different pH values help to understand the altered reactivity of Cys and Sec, e.g., in mutated thioredoxin reductases.

## 7 Zusammenfassung

In den vergangenen Jahrzehnten wurde gezeigt, dass Organochalkogenide interessante Verbindungen in der Biochemie sind. Trotz der wachsenden Bedeutung von Selen- und Telluranaloga der schwefelhaltigen Aminosäuren, sind nur wenige Methoden für deren Synthese bekannt. Zunächst wurde daher eine einfache Synthese für L-Selenocystin, L-Tellurocystin und L-Tellurocystein Derivate entwickelt, um dieses Problem zu lösen (Schema 1).



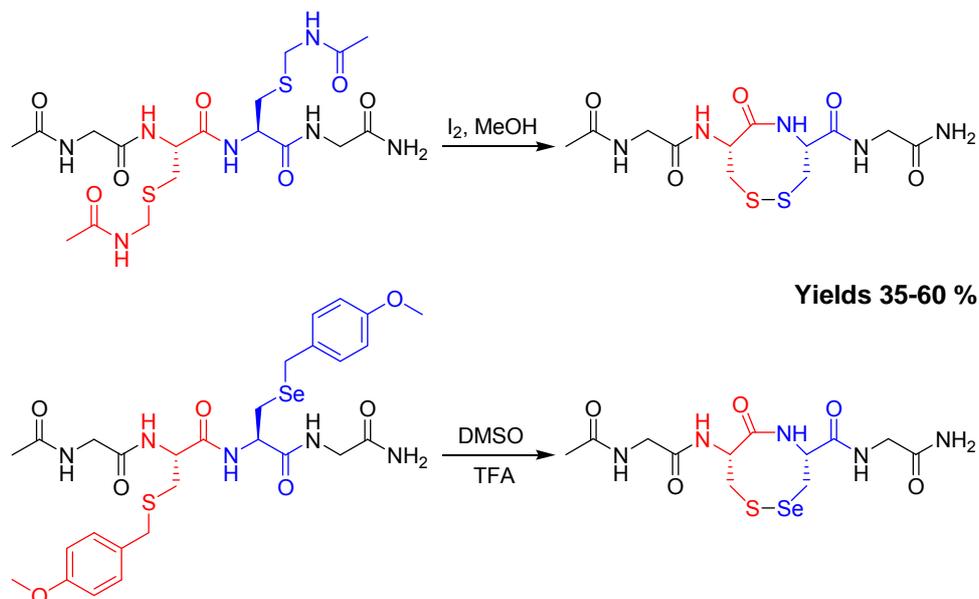
**Schema 1.**

Untersuchungen des Potentials  $E'_0$  von Selenocystin mit unterschiedlichen Amino- und Carbonsäureschutzgruppen zeigten, dass geschütztes Selenocystin elektronegativer als sein ungeschütztes Analogon ist [z.B.,  $E'_0((\text{Boc-Sec-OH})_2) = -549 \text{ mV}$  vs.  $E'_0((\text{NH}_2\text{-Sec-OH})_2) = -477 \text{ mV}$ ]. Mutmaßliche anionische Tri-chalkogen-Intermediate [Se-Se...S] der Austauschreaktion zwischen wasserlöslichen Analoga von Selenocystin mit dem Thiolat-anion von Cystein konnten mit Hilfe von MS Techniken detektiert werden. Dies deutet auf einen  $\text{S}_\text{N}2$ -Mechanismus hin. Weitere Studien der Se-S Austauschreaktionen der wasserlöslichen Analoga von Selenocystin/Cystin und Selenylsulfiden zeigten, dass die Austauschgeschwindigkeit und die Gleichgewichtskonstanten stark vom pH-Wert abhängen. Die Austauschgeschwindigkeiten waren schneller je basischer das Milieu war (z.B. erhöhte sich die Austauschgeschwindigkeit von pH = 8 zu pH = 9 um das 13-fache).

In Proteinen die Selenocystin (Sec, U) enthalten ist die Selenylsulfidbrücke und ihre reduzierte Thiol-Selenol Form die bedeutenden Spezies. Es wurde vorgeschlagen, dass Serin, ein Protonendonator/akzeptor, als benachbarte Aminosäure die Redoxpotentiale der S-S und Se-Se Brücken beeinflusst. Die acht Modeltetrapeptide GCCG, SCCG, GCCS, SCCS, GCUG, SCUG, GCUS und SCUS wurden synthetisiert um die Allgemeingültigkeit dieser Aussage zu überprüfen, unter ihnen GCUG, eine Sequenz der menschlichen Thioredoxin Reduktase, und SCCS, eine Sequenz der *Drosophila melanogaster*.

Die geschützten und reduzierten Modelltetrapeptide wurden mittels Festphasensynthese (SPPS) in guter Ausbeute hergestellt. SPPS wurde auf dem Knorr Amid MBHA Harz für Fmoc Chemie durchgeführt. Alle Aminosäuren wurden mit der HBTU/HOBt/DIPEA Methode gekoppelt.

Die schwierige Bildung eines 8-gliedrigen Ringes aus benachbarten Sec und Cys, wie in der Thioredoxin Reduktase TrxRs, gelang durch die Oxidation mit den Systemen I<sub>2</sub>/MeOH und DMSO/TFA (Schema 2).



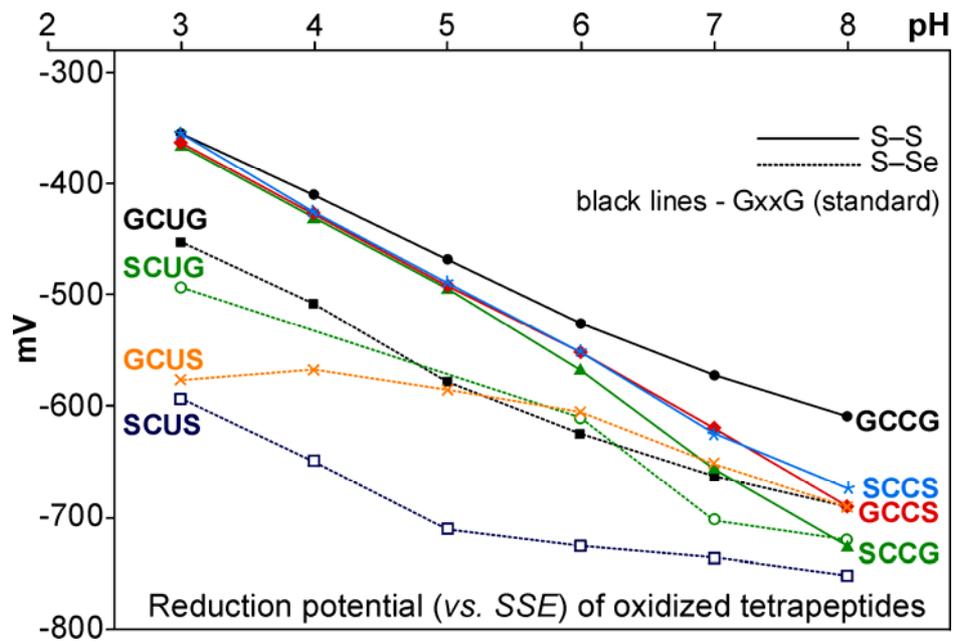
## Schema 2

Untersuchungen der pH-Wertabhängigkeit des  $E'_0$  zeigten, dass die elektrischen Potentiale der Se-Se, S-S und Se-S Brücken der (Se)-cystin Analoga, mit steigendem pH-Wert elektronegativer werden.

Die Absorptionsuntersuchung der Modelltetrapeptide bei unterschiedlichen pH-Werten zeigten, dass die Reduktion des Tetrapeptides nach der Adsorption des Oxidationsmittels (Diselenid/Disulfid) an die Elektrode abläuft und sie erzeugt nicht adsorbiertes Produkt (Thiol/Selenol) auch in der Lösung. Ein  $\Delta E$ -Test und die Absorptionsuntersuchung sagen aus, dass die Reaktion „quasi-irreversibel“ ist, wahrscheinlich durch die irreversible Bindung von Teilendes SH oder SeH an Quecksilber.

Das Redoxpotential der Se-Se und S-S-Brücken der Modelltetrapeptide ist abhängig von dem pH-Wert und der Nachbarschaft von Serin (Graph 1):

- (1.) höherer pH-Wert führt zu besser reduzierbaren Dichalkogeniden; und
- (2.) ein signifikanter Einfluss des benachbarten Serin auf das Disulfid existiert nur bei neutralem und basischem pH-Werten.



**Graph 1. pH-Wertabhängigkeit des Reduktionspotentials der oxidierten Tetrapeptide**

Solche Untersuchungen, der Einfluss von Serin als benachbarten Aminosäure auf die Reaktivität der Se-S Brücke bei unterschiedlichen pH-Werten mit eingeschlossen, hilft die modifizierte Reaktivität von Cys und Sec, z.B. in mutierter Thioredoxin Reduktase, zu verstehen.

## 8 Experimental part

### 8.1 Material and analytical methods (general remarks)

Commercially available chemicals and solvents were purchased from Aldrich, Fluka, Acros, and Merck. Amino acids and resins were bought from Novabiochem or Bachem. Tetrahydrofuran was distilled from Na/K-alloy under nitrogen.

**Column Chromatography** was performed on silica 60 (230-400 mesh, 0.040-0.063 mm) from Merck, LiChroprep RP-18 (40-63  $\mu\text{m}$ ) for liquid chromatography from Merck, Sephadex LH-20 (Pharmacia). Chromabond C18 SPE-cartridge (Macherey-Nagel) was used for purification of small amounts.

**Thin Layer Chromatography (TLC)** was performed on Merck silica gel 60 F254 plates (250  $\mu\text{m}$  layer thickness; aluminium sheets; particle size: 0.040-0.063 mm). Compounds were visualized by UV ( $\lambda=254\text{ nm}$ ), or by dipping the plates into a CerMOP-TLC reagent followed by heating. CerMOP-TLC reagent was prepared by dissolving 12.5 g molybdotophosphoric acid, 5.0 g  $\text{Ce}(\text{SO}_4)_2 \cdot \text{H}_2\text{O}$  and 30 mL of concentrated sulphuric acid in 470 mL of water.

**High Pressure Liquid Chromatography (HPLC)** was performed on a Knauer WellChrom K-2800 and Agilent 1100 with integrated photo diode array detector. For analytical runs a RP-18 column (YMC ODS-A 120, 5  $\mu\text{m}$ , 4.6 $\times$ 150 mm) at a flow rate of 1 mL/min was used. Preparative RP-HPLC separations were performed using a RP-18 column (YMC ODS-A 120, 5  $\mu\text{m}$ , 20 $\times$ 150 mm) at a flow rate of 20 mL/min. Substances were eluted with acetonitrile-water (ACN- $\text{H}_2\text{O}$ ) mixture as mobile phase, detection 210 nm.

**$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra** were recorded on a Varian Mercury 300 spectrometer at 300.22 and 75.50 MHz; Varian Mercury 400 spectrometer at 399.93 and 100.57 MHz; and Varian Inova 500 spectrometer at 499.80 and 125.69 MHz, respectively. All 2D spectra including COSY, HSQC, HMBC were recorded on a Varian Inova 500 using a 3 mm microsample inverse detection probe or Varian Mercury 400 spectrometers. The chemical shifts ( $\delta$ ) are given in ppm downfield from TMS ( $\delta=0\text{ ppm}$ ,  $^1\text{H}$ ) and  $\text{CDCl}_3$  ( $\delta=77.0\text{ ppm}$ ,  $^{13}\text{C}$ ), respectively. Coupling constants  $J$  values are given in Hz. For multiplets the following

abbreviation were used: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet or unresolved signal), br (broad). Deuterated solvents were purchased from Deutero GmbH.

**Optical rotations** were determined with a Jasco DIP-1000 digital polarimeter. The probes were measured in the 10 cm thermostatic cell with a sodium discharge lamp ( $\lambda = 589$  nm). Five parallel measurements were done.

**The ESI mass spectra** were performed on a SCIEX API-150EX instrument (Applied Biosystems, Concord, Ontario, Canada) combined with a HTS-PAL autosampler (CTC Analytics, Zwingen, Switzerland).

**The high resolution ESI mass spectra** were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an APOLLO electrospray ion source (Agilent, off axis spray). Nitrogen was used as drying gas at 150°C. The sample solutions were introduced continuously via a syringe pump with a flow rate of 120  $\mu\text{l h}^{-1}$ .

**Ion trap ESI-MS** experiments have been performed using a Finnigan LCQ DECA XP Max (Thermo Electron, San Jose, CA, USA) equipped with a standard electrospray interface. The MS system is coupled with a Hypersil Gold RP18-column (5  $\mu\text{m}$ , 1x150 mm, Thermo Finnigan). For the HPLC a gradient system was used starting from H<sub>2</sub>O:CH<sub>3</sub>CN 80:20 (each of them containing 0.2% HOAc) to 0:100 within 30 min, hold 10 min at 100% CH<sub>3</sub>CN. The flow rate was 70  $\mu\text{l min}^{-1}$ . 2  $\mu\text{l}$  was injected into HPLC-system. Detection: MS m/z 100-1000, DAD  $\lambda = 200$ -800 nm. The ESI mass spectrometric conditions were the following:

Sheath gas (N<sub>2</sub>): 30  
Spray Voltage: 5 kV  
Capillary Temp.: 260°C  
Capillary Voltage: 15 kV  
Tube Lens Offset: 40 V

**Quadrupole-time-of-flight (Q-TOF) ESI-MS/MS** measurements were performed on the Micromass Premier instrument (Waters, Manchester) equipped with a ESI ion source containing a stainless steel metal spray capillary (127  $\mu\text{m}$  inner diameter, 229  $\mu\text{m}$  outer diameter, 181 mm in length). The capillary voltage of 2.5 kV, source and desolvation

temperatures of 40 °C were applied as standard ESI operation conditions. The collision-induced dissociation (CID, argon collision gas with flow of 0.2 mL/min) was performed in the collision cell region; the collision energy was set to 2-10 eV for different ion species.

**Cyclic voltammetry** was performed on a 100B/W workstation (BAS) and on a high voltage potentiostat/galvanostat PGSTAT100 (AUTOLAB) coupled with a mercury drop electrode system (Metrohm 663 VA Stand) via IME663 interface. The VA Stand was operated in hanging mercury drop electrode (HMDE) mode. Voltammograms were recorded in citric acid-phosphate buffer (pH 3.0-8.0; 100-200 mM) at room temperature and at a varying scan rates of 100-20'000 mV/s with a dropping mercury working electrode, a standard silver reference electrode (SSE), and a platinum counterelectrode.

## 8.2 General procedures

### 8.2.1 Kaiser test

Prepare the following solutions:

1. Dissolve 5 g of ninhydrin in 100 mL ethanol.
2. Dissolve 80 g of liquified phenol in 20 mL of ethanol.
3. Add 2 mL of a 0.001 M aqueous solution of potassium cyanide to 98 mL pyridine.
4. Sample a few resin beads and wash several times with ethanol.
5. Transfer to a small glass tube and add 2 drops of each of the solutions 1-3 above.
6. Mix well and heat to 120°C for 4-6 minutes. A positive test (free primary amines) is indicated by blue resin beads.

### 8.2.2 General procedure I (GP I). Synthesis of tetrapeptides on solid phase

Solid phase peptide synthesis was performed on the Knorr Amide MBHA resin (0.81 mmol/g) for Fmoc chemistry (Table 8.1.). Peptide syntheses were done in 0.405 mmol scale. Selenocysteine was synthesized from L-serine via  $\beta$ -lactone. Functional groups were protected. Selenol and thiol were protected as p-methoxybenzyl (Mob) and/or acetamidomethyl (Acm), and alcohols as tert-butyl (t-Bu). All amino acids have been coupled using a 3-fold excess of reagents according to the HBTU/HOBt method with 4 eq. DIPEA in DMF (10 min). Removal of *N*-terminal Fmoc group was carried out with 20 % (v/v) piperidine in DMF (5 min). After each coupling and Fmoc-deprotection the Kaiser test was done (with part of resin). Acetylation for SCCG, SCCS, SCUG, SCUS sequences was achieved with 10 eq. acetic anhydride and DIPEA in DMF (10 min). Cleavage of tetrapeptides was achieved with TFA/H<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>/TIPS (89/5/5/1, v/v) at 4 °C (1 h). Finally, the peptides were crystallized from ether.

Table 8.1.

		GCCG/GCUG	GCCS/GCUS	SCCG/SCUG	SCCS/SCUS
1	Resin	Knorr Amide MBHA resin (0.81 mmol/g)			
2	Scale	0.405 mmol			
3	Pre-swelling	5 mL DMF, 1 h			
4	Fmoc-deprotection	5 mL Piperidine 20% in DMF, 5 min			
5	Wash	5 mL DMF, 2 min, 2 times			
6	Coupling  amino acid (AA) sequence	3 eq (AA, HBTU, HOBt) + 4 eq DIPEA, 5 mL DMF, 1 h  (2.5 eq in case of Fmoc-Cys(Mob)-OH)			
		<b>1</b>	<b>3</b>	<b>1</b>	<b>3</b>
		<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>
		<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>	<b>2 or 5 or 6</b>
		<b>4</b>	<b>4</b>	<b>3</b>	<b>3</b>
7	Wash	5 mL DMF, 2 min, 2 times			
8	Repeat	Steps 4, 5, 6,7 with corresponding amino acids			
9	Acetylation	-	-	10 eq acetic anhydride, 10 eq DIPEA, 5 mL DMF, 10 min	
10	wash	5 mL DMF, 2 min, 3 times 5 mL DCM, 2 min, 3 times 5 mL MeOH, 2 min, 3 times			
11	Drying	1 h in vacuo			
12	Cleavage	10 mL of 95% TFA, 2.5% TIPS, 2.5% H <sub>2</sub> O, 4°C, 1 h			
<b>1</b> – Gly(Fmoc)		<b>4</b> – Gly(Ac)			
<b>2</b> – Fmoc-Cys(Mob)-OH		<b>5</b> – Fmoc-Cys(Acm)-OH			
<b>3</b> – Fmoc-Ser(t-Bu)-OH		<b>6</b> – Fmoc-Sec(Mob)-OH			

### 8.2.3 General procedure II (GP II) for iodine oxidation

Oxidation of Cys-Cys peptides to intramolecular cystine was realized with iodine in methanol:

1. Dissolve the Cys-Acm peptide in 50% aqueous MeOH (1-10 mg/mL) under a blanket of nitrogen.
2. Add 1M HCl (0.1 mL/mg) followed immediately by a 0.1 M I<sub>2</sub> in 50% aqueous MeOH.
3. After 30 minutes of vigorous stirring, quench the iodine by adding 1 M aqueous sodium thiosulfate drop-wise until the mixture is colourless, and concentrate by evaporation under reduced pressure to approximately one third of original volume. Purify on C18 SPE-cartridge followed by preparative RP-HPLC with linear gradients of solvents A and B (A = acetonitrile containing 0,1% TFA; B = water containing 0,1% TFA; gradient: t = 0 min - A/B (2:98 v/v) -> t = 25 min - A/B (10:90 v/v); 10 ml/min, 210 nm).

Yields were calculated as follows: a 1 ml portion of the purified fraction was evaporated until dryness and weighed; the amount was multiplied by total volume of collected fraction of product. The solvent-free product has to be discarded because upon concentrating dichalcogenide interchange leads to polymer formation.

### 8.2.4 General procedure III (GP III) for DMSO/TFA oxidation

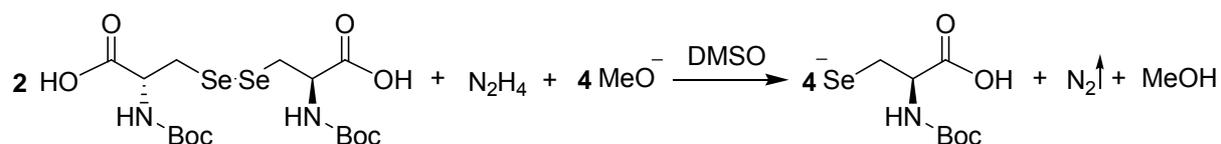
Intramolecular selenenylsulfide bridges were obtained by treatment of Cys-Sec peptides with DMSO in TFA:

1. Dissolve 0.037 mmol of reduced Mob-protected tetrapeptide in 70 mL ice cold TFA (0°C).
2. Add drop-wise a mixture of 3 mL DMSO and 8 mL TFA (final concentration of peptide is  $4.5 \times 10^{-4}$  M) and stir 30 min at 0°C.
3. Remove TFA in vacuum and add to rest of DMSO ether to precipitate oxidized peptide.
4. Purification by preparative RP-HPLC with linear gradients of solvents A and B (A = acetonitrile containing 0,1% TFA; B = water containing 0,1% TFA; gradient: t = 0 min - A/B (2:98 v/v) -> t = 25 min - A/B (10:90 v/v); 10 ml/min, 210 nm).

Yields were calculated as follows: a 1 ml portion of the purified fraction was evaporated until dryness and weighed; the amount was multiplied by total volume of collected fraction of product. The solvent-free product has to be discarded because upon concentrating dichalcogenide interchange leads to polymer formation.

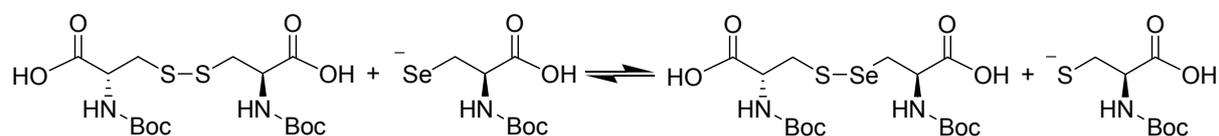
### 8.2.5 Determination of equilibrium constant of Se-S exchange reaction

#### Preparation of 1 M sodium selenolate solution



To a stirred solution containing 0.35 mmol Boc-protected selenocystine and 0.19 mmol (1.1 eq.) hydrazine hydrate in 6 mL DMSO was added dropwise 0.14 g (0.15 mL) 25% methanolic sodium methanolate until the yellow colour disappeared.

#### MS investigation

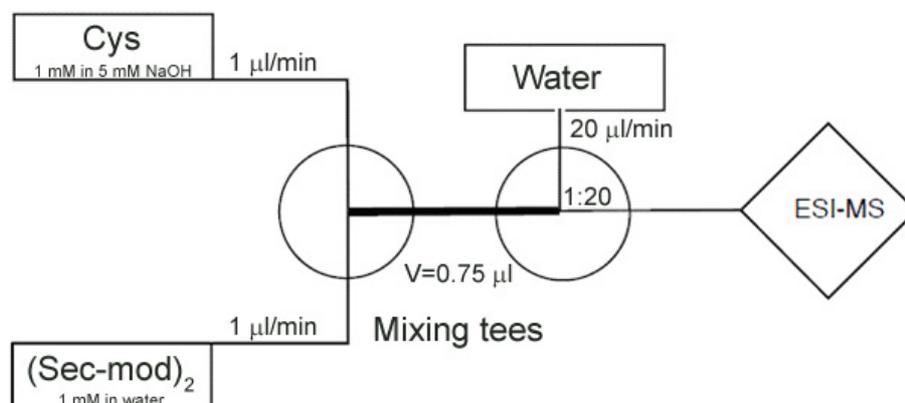


0.7 mmol sodium selenolate was added to 0.7 mmol Boc-protected cystine in 10 mL DMSO and stirred 1 hour at room temperature. 10  $\mu\text{L}$  of reaction mixture was diluted with methanol to 1 mL (1:100) and analysed by ESI-MS.

### 8.2.6 Mechanistic elucidation of Se-S exchange reactions

MS continuous-flow experiments were performed using two syringes containing solutions of the different reactants, mixing them before entering into the ionization source (Graph 8.1.). For this, a microreactor (Techlab, Peek mixing tee) was connected to the ESI spray capillary via second mixing tee which was attached between the microreactor and the ionization source to reduce the final sample concentration entering the mass spectrometer (see graphic below). First reactant was cysteine anion **15** (Cys) with 1 mM concentration in 5 mM NaOH water solution. Second reactant was water soluble analogues of selenocystine **14** (Sec-mod)<sub>2</sub> with

1 mM concentration in water. Flow rates of reactans were 1  $\mu\text{l}/\text{min}$ . The reaction volume was 0.75  $\mu\text{l}$ . The reaction time was 45 sec. The exchange reaction was monitored by Quadrupole-time-of-flight (Q-TOF) ESI-MS/MS.



**Graph 8.1. MS continuous-flow experiment**

### 8.2.7 Electrochemical analysis

The solutions of investigated compounds were prepared and degassed. Metrohm measuring vessel 10-90 mL was used for analyses. The measurements were carried out under nitrogen. The results were worked up with software GPES version 4.9.

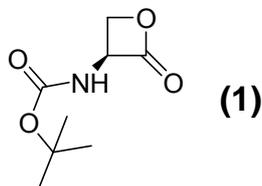
### 8.2.8 Buffer preparation

Phosphate (50 mM, pH=7) and citric acid-phosphate (pH 3-8) buffers were used for electrochemical investigations.

pH	0.2 M $\text{Na}_2\text{HPO}_4$ / mL	0.1 M citric acid / mL
3.0	20.55	79.45
4.0	38.55	61.45
5.0	51.50	48.50
6.0	63.15	36.85
7.0	82.35	17.65
8.0	97.25	2.75

### 8.3 Syntheses

#### *N*-(tert-butoxycarbonyl)-L-serine $\beta$ -lactone (**1**) (Arnold et al., 1987)

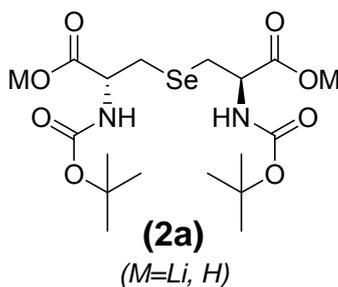


To triphenylphosphine (6.40 g, 24.4 mmol, dried in vacuo for 72 h over P<sub>4</sub>O<sub>10</sub>) in anhydrous THF (100 mL) at -78 °C under argon, dimethylazodicarboxylate (3.57 g, 24.4 mmol) was added dropwise over 10 min followed by a solution of *N*-(tert-butoxycarbonyl)-L-serine (5.0 g, 24.4 mmol) in THF (100 mL), added dropwise over 30 min. The mixture was stirred at -78 °C for 20 min before it is slowly warmed to room temperature within 2.5 h. The solvent was removed in vacuo, and the residual pale yellow syrup was purified by flash column chromatography on silica 60 (hexane/ethyl acetate, 4:1) to give *N*-(tert-butoxycarbonyl)-L-serine  $\beta$ -lactone (2.02 g, 44 %), a white solid after recrystallization from (CH<sub>3</sub>OH/hexane):

$[\alpha]_D$  -24.7 (22 °C, c 0.5, CH<sub>3</sub>CN);

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 399.9 MHz, ppm)  $\delta$  1.46 (s, 3 $\times$ CH<sub>3</sub>), 4.38–4.50 (m, CH<sub>A</sub>H<sub>B</sub>), 5.11 (br m, CH), 5.25 (br m, NH);

HRMS (ESI, [M+Na]<sup>+</sup>) calcd for C<sub>8</sub>H<sub>13</sub>NO<sub>4</sub>Na<sup>+</sup> 210.0737, found 210.0738.

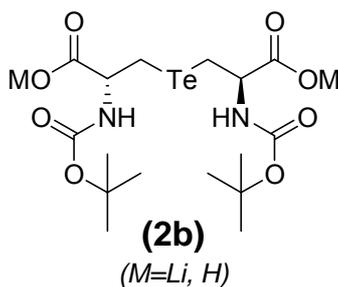
***t*-Boc-protected L-selenolanthionine 2a dilithium salt**

To a suspension of elemental selenium (41.08 mg, 0.52 mmol) in freshly distilled THF (3 mL), under argon super-hydride (1.04 ml of 1 M solution of lithium triethylborohydride in tetrahydrofuran, 1.04 mmol) was added. The resulting solution was heated to reflux and stirred for 15 min under argon. 6 mL of dry and degassed THF solution of *N*-(*t*-Boc)-L-serine  $\beta$ -lactone **1** (175 mg, 0.94 mmol) was added drop-wise over 10 min and stirred at 50 °C overnight to ensure that the reaction was complete. The solution can be filtered through a pad of reverse phase silica gel (RP-18) in order to remove rests of elemental selenium. The dilithium salt of product was crystallized from chloroform/hexane, 167 mg (0.357 mmol, 76%) yield.

**<sup>1</sup>H NMR** (CD<sub>3</sub>OD, 399.9 MHz, ppm)  $\delta$  1.45 (s, 3 $\times$ CH<sub>3</sub>), 2.93 (dd, *J* = 8.1, 12.7 Hz, CH<sub>A</sub>H<sub>B</sub>), 3.07 (dd, *J* = 4.3, 12.7 Hz, CH<sub>A</sub>CH<sub>B</sub>), 4.35 (br m, CH);

**<sup>13</sup>C NMR** (CD<sub>3</sub>OD, 100.6 MHz, ppm)  $\delta$  27.0, 28.7, 55.6, 80.7, 157.8, 174.4;

**HRMS** (ESI, [M-H]<sup>-</sup>) *m/z* calcd 455.0938, found 455.0940.

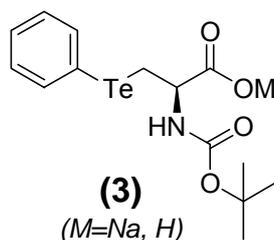
***t*-Boc-protected L-tellurolanthionine 2b**

To a suspension of elemental tellurium (70.18 mg, 0.55 mmol) in freshly distilled THF (3 mL), under argon super-hydride (1.1 ml of 1 M solution of lithium triethylborohydride in tetrahydrofuran, 1.1 mmol) was added. The resulting solution was heated to reflux and stirred for 15 min under argon. 6 mL of dry and degassed THF solution of *N*-(*t*-Boc)-L-serine  $\beta$ -lactone **1** (187 mg, 1 mmol) was added drop-wise over 10 min and stirred at 50 °C overnight to ensure that the reaction was complete. The solution can be filtered through a pad of reverse phase silica gel (RP-18) in order to remove rests of elemental tellurium. The dilithium salt of product was crystallized from chloroform/hexane, 183 mg (0.355 mmol, 71%) yield.

Isolation of *N*-Boc-amino acid **2b** (*M* = H): To 2 mL stirred THF solution of the reaction mixture or crystallized material, 2 mL degassed water and hydrochloric acid was added dropwise to reach pH 1–2, followed by 2 mL CHCl<sub>3</sub>. The immediately separated organic layer was washed under argon with 2 mL HCl-acidified degassed water, dried over MgSO<sub>4</sub>, concentrated in vacuo, and immediately measured. All processes were performed rapidly and strictly under argon.

<sup>1</sup>H NMR (pyridine-d<sub>5</sub>, 499.8 MHz, ppm)  $\delta$  1.51 (s, 3 $\times$ CH<sub>3</sub>), 3.54 (dd, *J* = 7.9, 12.0 Hz, CH<sub>A</sub>H<sub>B</sub>), 3.72 (dd, *J* = 5.6, 12.0 Hz, CH<sub>A</sub>CH<sub>B</sub>), 5.21 (br m, CH), 8.22 (d, *J* = 8.2, NH);

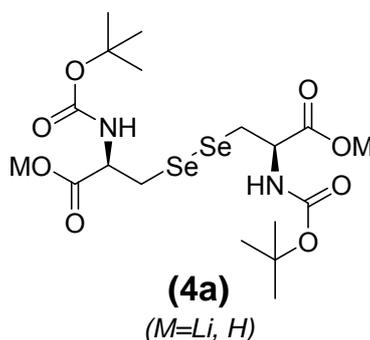
HRMS (ESI, [M-H]<sup>-</sup>) *m/z* calcd 505.0835, found 505.0844.

***t*-Boc-protected phenyltellurocysteine 3 sodium salt**

Diphenyl ditelluride (225 mg, 0.55 mmol) was dissolved in 3 mL ethanol. To this NaBH<sub>4</sub> (52 mg, 1.38 mmol) was added. The resulting solution was heated to reflux and stirred for 15 min under argon. 4 mL of dry and degassed THF solution of *N*-(*t*-Boc)-L-serine β-lactone **1** (187 mg, 1 mmol) was added drop-wise over 10 min and stirred at 50 °C overnight to ensure that the reaction was complete. The solution can be filtered through a pad of reverse phase silica gel (RP-18). The sodium salt of product was crystallized from chloroform/hexane, 254 mg (0.61 mmol, 61%) yield.

**<sup>1</sup>H NMR** (CDCl<sub>3</sub>, 399.9 MHz, ppm) δ 1.42 (s, 3×CH<sub>3</sub>), 3.26 (dd, *J* = 5.8, 12.6 Hz, CH<sub>A</sub>H<sub>B</sub>), 3.34 (dd, *J* = 5.1, 12.6 Hz, CH<sub>A</sub>CH<sub>B</sub>), 4.71 (br m, CH), 5.27 (d, *J* = 7.3, NH), 7.25 (br m, 3H, m, *p*-Ar-H), 7.79 (br m, 2H, *o*-Ar-H);

**HRMS** (ESI, [M-H]<sup>-</sup>) *m/z* calcd 394.0304, found 394.0311.

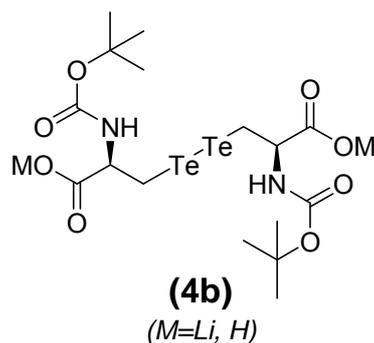
***t*-Boc-protected L-selenocystine 4a dilithium salt**

To a suspension of elemental selenium (79 mg, 1 mmol) in freshly distilled THF (3 mL), under argon super-hydride (1.05 ml of 1 M solution of lithium triethylborohydride in tetrahydrofuran, 1.05 mmol) was added. The resulting solution was heated to reflux and stirred for 15 min under argon. 6 mL of dry and degassed THF solution of *N*-(*t*-Boc)-L-serine  $\beta$ -lactone **1** (187 mg, 1 mmol) was added drop-wise over 10 min and stirred at 50 °C overnight to ensure that the reaction was complete. The solution can be filtered through a pad of reverse phase silica gel (RP-18) in order to remove rests of elemental selenium. The dilithium salt of the product was crystallized from chloroform/hexane, 253 mg (0.463 mmol, 93%).

<sup>1</sup>H NMR (CD<sub>3</sub>OD, 399.9 MHz, ppm)  $\delta$  1.45 (s, 3 $\times$ CH<sub>3</sub>), 3.20 (dd, *J* = 9.1, 12.6 Hz, CH<sub>A</sub>H<sub>B</sub>), 3.44 (dd, *J* = 4.8, 12.6 Hz, CH<sub>A</sub>CH<sub>B</sub>), 4.40 (br m, CH);

<sup>77</sup>Se NMR (CD<sub>3</sub>OD, 95.3 MHz, ppm)  $\delta$  307.9;

HRMS (ESI, [M-H]<sup>-</sup>) *m/z* calcd 535.0103, found 535.0110.

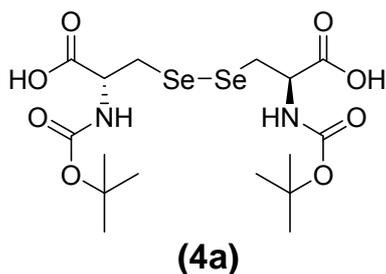
***t*-Boc-protected L-tellurocystine **4b****

To a suspension of elemental tellurium (140.4 mg, 1.1 mmol) in freshly distilled THF (3 mL), under argon super-hydride (1.1 ml of 1 M solution of lithium triethylborohydride in tetrahydrofuran, 1.1 mmol) was added. The resulting solution was heated to reflux and stirred for 15 min under argon. 6 mL of dry and degassed THF solution of *N*-(*t*-Boc)-L-serine  $\beta$ -lactone (187 mg, 1 mmol) was added drop-wise over 10 min and stirred at 50 °C overnight to ensure that the reaction was complete. The solution can be filtered through a pad of reverse phase silica gel (RP-18) in order to remove rests of elemental tellurium. The dilithium salt of product was crystallized from chloroform/hexane, 251 mg (0.39 mmol, 78%) yield.

Isolation of *N*-Boc-amino acid **4b** (*M* = H): To 2 mL stirred THF solution of the reaction mixture or crystallized material, 2 mL degassed water and hydrochloric acid was added dropwise to reach pH 1–2, followed by 2 mL CHCl<sub>3</sub>. The immediately separated organic layer was washed under argon with 2 mL HCl-acidified degassed water, dried over MgSO<sub>4</sub>, concentrated in vacuo, and immediately measured. All processes were performed rapidly and strictly under argon.

<sup>1</sup>H NMR (pyridine-d<sub>5</sub>, 499.8 MHz, ppm)  $\delta$  1.53 (s, 3 $\times$ CH<sub>3</sub>), 3.98 (dd, *J* = 8.5, 11.4 Hz, CH<sub>A</sub>H<sub>B</sub>), 4.27 (dd, *J* = 6.1, 11.4 Hz, CH<sub>A</sub>CH<sub>B</sub>), 5.12 (br m, CH), 8.31 (d, *J* = 7.6, NH);

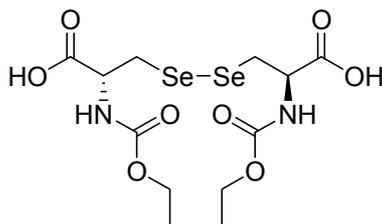
HRMS (ESI, [M-Li]<sup>-</sup>) *m/z* calcd 634.9897, found 634.9891.

***t*-Boc protected L-selenocystine 4a (alternative synthesis)**

Commercially available selenocystine (434.2 mg, 1.3 mmol) was dissolved in the mixture of 6 mL 1N aqueous NaOH-solution and 5 mL dioxane, and cooled in an ice bath. Tert-butyloxycarbonyl anhydride (Boc<sub>2</sub>O) 2.5 eq. (720 mg, 3.3 mmol) was added. The reaction mixture was stirred at room temperature during 1 hour, then concentrated in vacuo until 4-5 mL and diluted with water. The aqueous solution was covered with a layer of ca. 5-10 mL ethyl acetate and acidified dropwise with 1N HCl under stirring until pH 2-3. The separated aqueous phase was extracted more 3 times with ethyl acetate. The combined organic phases were washed with ca. 20 mL water and dried over MgSO<sub>4</sub>. After the evaporation of the solvent, the product was dried in the high-low vacuo overnight to give a yellow solid in 553 mg (79%).

<sup>77</sup>Se NMR (D<sub>2</sub>O+NaOD, 95.4 MHz, ppm) δ 307.3; (DMSO-d<sub>6</sub>, 95.4 MHz, ppm) δ 314.8;

HRMS (ESI, [M-H]<sup>-</sup>) m/z calcd 535.0103, found 535.0109.

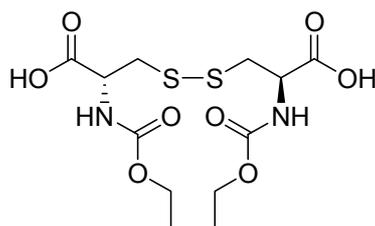
**L-selenocystine ethyl formate**

2.94 mL ethyl chloroformate (30.69 mmol) in 20 mL dioxane was added drop-wise at 0°C to selenocystine (3.44 g, 10.23 mmol) dissolved in 40 mL of water solution of Na<sub>2</sub>CO<sub>3</sub> (5%), 2 mL dioxane and 15 mL NaOH (1N). The reaction mixture was allowed to warm to room temperature and stirred overnight to ensure that the reaction was complete. The solution was acidified with 1N HCl to pH 1-2 and then extracted 3 times with ethyl acetate. The combined organic layers were dried over the MgSO<sub>4</sub> and concentrated in vacuo to give the product, 4.7 g (95%) yield as an orange oil.

**<sup>1</sup>H NMR** (pyridine-d<sub>5</sub>, 300.2 MHz, ppm) δ 1.12 (t, J = 7.0 Hz, 2×CH<sub>3</sub>), 3.81 (dd, J = 8.5, 12.4 Hz, CH<sub>A</sub>H<sub>B</sub>), 4.06 (dd, J = 5.3, 12.4 Hz, CH<sub>A</sub>H<sub>B</sub>), 4.15-4.23 (m, 2×CH<sub>2</sub>), 5.23-5.29 (m, 2×CH);

**<sup>13</sup>C NMR** (pyridine-d<sub>5</sub>, 75.5 MHz, ppm) δ 14.9, 33.5, 56.2, 60.9, 157.3, 174.1;

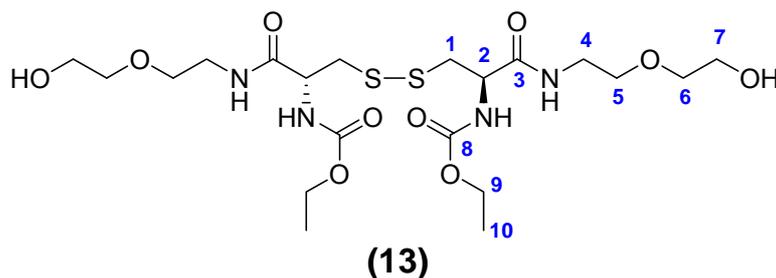
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 502.9442, found 502.9445.

**L-cystine ethyl formate**

2.35 mL ethyl chloroformate (24.5 mmol) in 20 mL dioxane was added dropwise at 0°C to cystine (2.45 g, 10.2 mmol) dissolved in 40 mL of water solution of Na<sub>2</sub>CO<sub>3</sub> (5%) and 13 mL NaOH (2N). The reaction mixture was allowed to warm to room temperature and stirred overnight to ensure that the reaction was complete. The solution was acidified with 1N HCl to pH 2 and then extracted 3 times with ethyl acetate. The combined organic layers were dried over the MgSO<sub>4</sub> and concentrated in vacuo to give the product, 3.4 g (87%) yield as a light brown oil.

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 407.0553, found 407.0558.

This product was used as intermediate for further reaction. The end product is fully characterized.

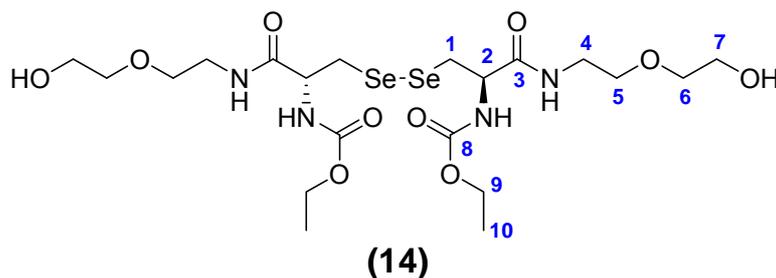
**Di 2-(2-aminoethoxy)ethanol L-cystine 13 ethyl formate**

The mixture of L-cystine ethyl formate (2.83 g, 7.4 mmol), DEPBT (8.68 g, 29 mmol, 4 eq.), DIPEA (5 mL, 29 mmol, 4 eq.) in 20 mL DMF was cooled to 0°C. 2-(2-Aminoethoxy)-ethanol (2.1 mL, 29 mmol, 4 eq.) in 5 mL DMF was added slowly dropwise to reaction mixture and stirred at room temperature overnight. The yellow-brown-orange solution was quenched with a saturated solution of NH<sub>4</sub>Cl and concentrated in vacuo till dryness. Water was added to the solid mixture and the obtained heterogeneous solution was filtrated through glass filter N4 to separate a white precipitation. The water solution was concentrated and purified with the RP-18 column (140 g) using different fractions: H<sub>2</sub>O, H<sub>2</sub>O:MeOH (10:1; 4:1; 2:1; 1:1; 1:2; 1:4) and MeOH. The product was identified in the fraction of H<sub>2</sub>O:MeOH (1:1). This fraction was concentrated and purified with the Chromabond C18 SPE-cartridge (1g) with the following purification by preparative RP18-HPLC (isocratic flow of acetonitrile/water = 11/89 v/v). After evaporation of solvent, the product was obtained as a white solid in 1.18 g (29%) yield.

**<sup>1</sup>H NMR** (D<sub>2</sub>O, 399.9 MHz, ppm) δ 1.25 (t, J = 7.0 Hz, 2×C<sup>10</sup>H<sub>3</sub>), 2.96 (dd, J = 9.0, 14.0 Hz, C<sup>1</sup>H<sub>A</sub>H<sub>B</sub>), 3.21 (dd, J = 4.1, 14.0 Hz, C<sup>1</sup>H<sub>A</sub>H<sub>B</sub>), 3.43-3.46 (m, 2×C<sup>4</sup>H<sub>2</sub>), 3.62-3.66 (m, 2×C<sup>5</sup>H<sub>2</sub>, 2×C<sup>6</sup>H<sub>2</sub>), 3.71-3.74 (m, 2×C<sup>7</sup>H<sub>2</sub>), 4.14 (q, J = 7.0 Hz, 2×C<sup>9</sup>H<sub>2</sub>), 4.34-4.47 (m, 2×C<sup>2</sup>H);

**<sup>13</sup>C NMR** (D<sub>2</sub>O, 100.6 MHz, ppm) δ 13.9 (C<sup>10</sup>), 39.1 (C<sup>1</sup>), 39.3 (C<sup>4</sup>), 54.2 (C<sup>2</sup>), 60.5 (C<sup>7</sup>), 62.4 (C<sup>9</sup>), 68.8 (C<sup>5</sup>), 71.6 (C<sup>6</sup>), 158.2 (C<sup>8</sup>), 172.9 (C<sup>3</sup>);

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 581.1922, found 581.1924.

**Di 2-(2-aminoethoxy)ethanol L-selenocystine 14 ethyl formate**

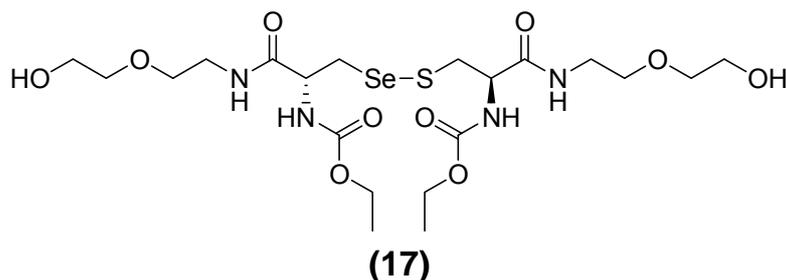
The mixture of L-selenocystine ethyl formate (3.78 g, 7.9 mmol), DEPBT (10 g, 33.4 mmol, 4.2 eq.), DIPEA (4 mL, 23.4 mmol, 3 eq.) in 15 mL DMF was cooled to 0°C. 2-(2-Aminoethoxy)-ethanol (3.95 mL, 55 mmol, 6.9 eq.) in 10 mL DMF was added slowly dropwise to reaction mixture and stirred at room temperature overnight. The green-brown solution was quenched with a saturated solution of NH<sub>4</sub>Cl and concentrated in vacuo till dryness. Water was added to the solid mixture and the obtained heterogeneous solution was filtrated through glass filter N4 to separate a green precipitation. The water solution was concentrated and purified with the RP-18 column (140 g) using different fractions: H<sub>2</sub>O, H<sub>2</sub>O:MeOH (10:1; 4:1; 2:1; 1:1; 1:2; 1:4) and MeOH. The product was identified in the fraction of H<sub>2</sub>O:MeOH (1:1). This fraction was concentrated and purified with the Chromabond C18 SPE-cartridge (1g) with the following purification by preparative RP18-HPLC (isocratic flow of acetonitrile/water = 15/85 v/v). After evaporation of solvent, the product was obtained as a yellow solid in 1.55 g (30%) yield.

**<sup>1</sup>H NMR** (D<sub>2</sub>O, 399.9 MHz, ppm) δ 1.25 (t, J = 7.2 Hz, 2×C<sup>10</sup>H<sub>3</sub>), 3.14 (dd, J = 9.0, 12.9 Hz, C<sup>1</sup>H<sub>A</sub>H<sub>B</sub>), 3.38 (dd, J = 4.7, 12.9 Hz, C<sup>1</sup>H<sub>A</sub>H<sub>B</sub>), 3.43-3.46 (m, 2×C<sup>4</sup>H<sub>2</sub>), 3.62-3.66 (m, 2×C<sup>5</sup>H<sub>2</sub>, 2×C<sup>6</sup>H<sub>2</sub>), 3.71-3.74 (m, 2×C<sup>7</sup>H<sub>2</sub>), 4.13 (q, J = 7.0 Hz, 2×C<sup>9</sup>H<sub>2</sub>), 4.39-4.42 (m, 2×C<sup>2</sup>H);

**<sup>13</sup>C NMR** (D<sub>2</sub>O, 100.6 MHz, ppm) δ 13.9 (C<sup>10</sup>), 29.8 (C<sup>1</sup>), 39.3 (C<sup>4</sup>), 55.6 (C<sup>2</sup>), 60.5 (C<sup>7</sup>), 62.3 (C<sup>9</sup>), 68.8 (C<sup>5</sup>), 71.7 (C<sup>6</sup>), 158.1 (C<sup>8</sup>), 173.1 (C<sup>3</sup>);

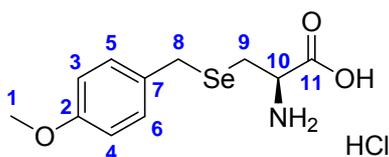
**<sup>77</sup>Se NMR** (D<sub>2</sub>O, 76.3 MHz, ppm) δ 289.6;

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 677.0811, found 677.0816.

**Di 2-(2-aminoethoxy)ethanol selenenylsulfide L-cystine ethyl formate 17**

Diselenide **14** (652 mg, 1 mmol) was dissolved in 3 mL of ethanol. The solution was degassed and kept under argon atmosphere. Sodium borohydride (304 mg, 8 mmol) was added portionwise and the reaction mixture was stirred until the yellow colour disappeared. Then disulfide **13** (558 mg, 1 mmol) was added. After 1 hour the solution was concentrated in vacuo and purified. Pre-purification was achieved with the Chromabond C18 SPE-cartridge (1g) with different fractions of H<sub>2</sub>O:MeOH. The fraction H<sub>2</sub>O:MeOH (1:1) containing the mixed chalcogenides was purified additionally by HPLC (isocratic flow of acetonitrile/water = 12/88 v/v). After evaporation of solvent the product was obtained as a white solid in 48 mg (8%) yield. The purified selenenylsulfide is very unstable. It easily disproportionates to the disulfide (-S-S-) **13** and the diselenide (-Se-Se-) **14**.

**ESI-MS** ([M+Na]<sup>+</sup>) m/z calcd 629.1, found 629.3.

**p-Methoxybenzyl-L-selenocysteine (8)****(8)**

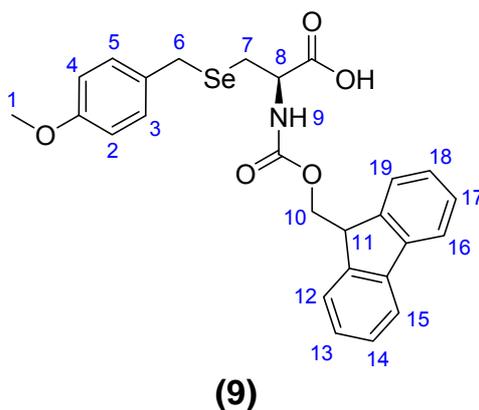
4.56 g (120 mmol) of NaBH<sub>4</sub> (8 eq.) was added portionwise to an ice-cooled solution of 5 g (15 mmol) selenocysteine in 60 mL NaOH (0.5 N). The reaction mixture was stirred at room temperature until the yellow colour disappeared. Then the solution was cooled in an ice bath and 25 mL NaOH (2N) was added, followed by dropwise addition of 4.9 mL (36 mmol, 2.4 eq.) p-methoxybenzyl chloride. After vigorous stirring at 4°C during 3 hours, the mixture was acidified with concentrated HCl to form a precipitate of crude **8**. The product was collected by filtration, washed with ether, and recrystallized from hot water. After drying in vacuo overnight, the product **8** was obtained as a light orange solid in 5.5 g (57%) yield.

**<sup>1</sup>H NMR** (D<sub>2</sub>O+CD<sub>3</sub>OD+DCl, 399.9 MHz, ppm) δ 3.03 (dd, J = 7.2, 14.1 Hz, C<sup>9</sup>H<sub>A</sub>H<sub>B</sub>), 3.09 (dd, J = 4.9, 14.1 Hz, C<sup>9</sup>H<sub>A</sub>H<sub>B</sub>), 3.83 (s, C<sup>1</sup>H<sub>3</sub>), 3.93 (s-like, C<sup>8</sup>H<sub>2</sub>), 4.26 (dd, J = 4.9, 7.2 Hz, C<sup>10</sup>H), 6.94 (d, J = 8.6 Hz, C<sup>3</sup>H, C<sup>4</sup>H), 7.35 (d, J = 8.6 Hz, C<sup>5</sup>H, C<sup>6</sup>H);

**<sup>13</sup>C NMR** (D<sub>2</sub>O+CD<sub>3</sub>OD+DCl, 100.6 MHz, ppm) δ 21.7 (C<sup>9</sup>), 27.1 (C<sup>8</sup>), 52.7 (C<sup>10</sup>), 55.4 (C<sup>1</sup>), 114.2 (C<sup>3,4</sup>), 130.3 (C<sup>5,6</sup>), 130.9 (C<sup>7</sup>), 158.2 (C<sup>2</sup>), 170.0 (C<sup>11</sup>);

**<sup>77</sup>Se NMR** (D<sub>2</sub>O+CD<sub>3</sub>OD+DCl, 76.3 MHz, ppm) δ 212.6;

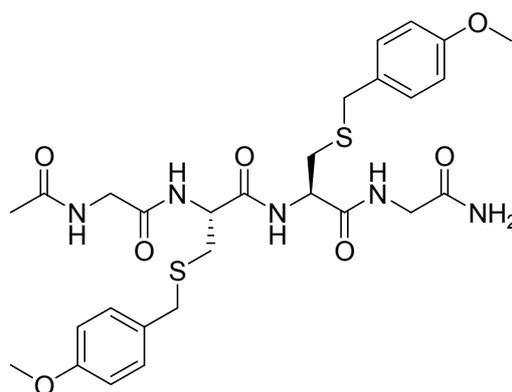
**ESI-MS** ([M-Cl]<sup>+</sup>) m/z calcd 290.0, found 290.2.

**Fmoc-Se-(p-methoxybenzyl)-selenocysteine (9)**

5.5 g (16.9 mmol) protected selenocysteine salt **8** was suspended in 70 mL Na<sub>2</sub>CO<sub>3</sub> (10% aqueous solution) and the mixture was cooled in an ice bath. A solution of 5.7 g (16.9 mmol) Fmoc-OSu in 45 mL dioxane was added. The resulting mixture was stirred at room temperature overnight. Water was added and the solution was extracted two times with ether. The aqueous phase was acidified with concentrated HCl to pH 2 and extracted 3 times with ethyl acetate. The combined organic phases were washed successively 2 times with 1N HCl and 2 times with water, and then dried over MgSO<sub>4</sub>. After removal of the solvent by evaporation, recrystallisation with n-hexane was done. Product **9** was dried in vacuo overnight to give a light yellow solid in 5.9 g (68%) yield.

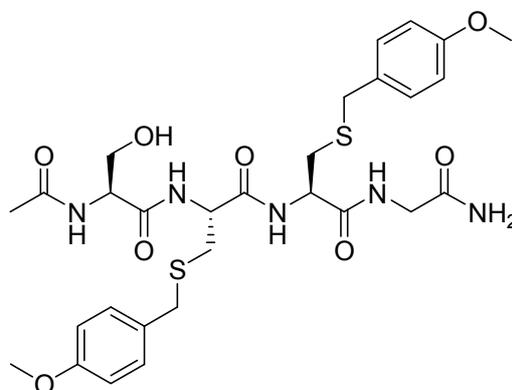
<sup>1</sup>H NMR (CDCl<sub>3</sub>, 300.2 MHz, ppm) δ 2.96 (d, J = 4.8 Hz, C<sup>7</sup>H<sub>2</sub>), 3.76 (s, C<sup>1</sup>H<sub>3</sub>), 3.77 (s-like, C<sup>6</sup>H<sub>2</sub>), 4.24 (t, J = 6.8 Hz, C<sup>11</sup>H), 4.43 (d, J = 6.8, C<sup>10</sup>H<sub>2</sub>), 4.65 (br m, C<sup>8</sup>H), 5.49 (d, J = 8.2 Hz, N<sup>9</sup>H), 6.81 (d, J = 8.6 Hz, C<sup>2</sup>H, C<sup>4</sup>H), 7.19 (d, J = 8.6 Hz, C<sup>3</sup>H, C<sup>5</sup>H), 7.28-7.42 (m, C<sup>13</sup>H, C<sup>14</sup>H, C<sup>17</sup>H, C<sup>18</sup>H), 7.59 (d-like, C<sup>12</sup>H, C<sup>19</sup>H), 7.76 (d, J = 7.3 Hz, C<sup>15</sup>H, C<sup>16</sup>H);

**ESI-MS** ([M+Na]<sup>+</sup>) m/z calcd 534.1, found 534.0.

**Synthesis of p-methoxybenzyl protected Cys-Cys tetrapeptide****Ac-Gly-Cys(Mob)-Cys(Mob)-Gly-NH<sub>2</sub>****(GCCG/Mob)**

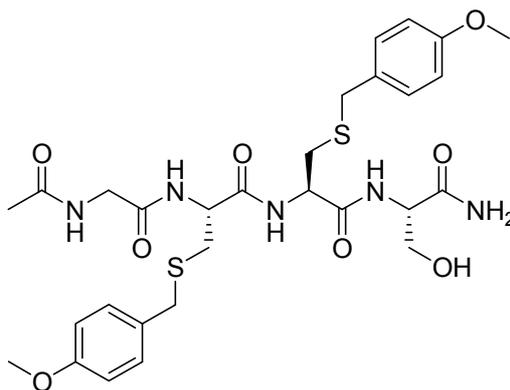
The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 152.9 mg (61%) yield.

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 642.2027, found 642.2020.

**Ac-Ser-Cys(Mob)-Cys(Mob)-Gly-NH<sub>2</sub>****(SCCG/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.81 mmol scale. The product was dried in vacuo overnight to give a white solid in 373.1 mg (71%) yield.

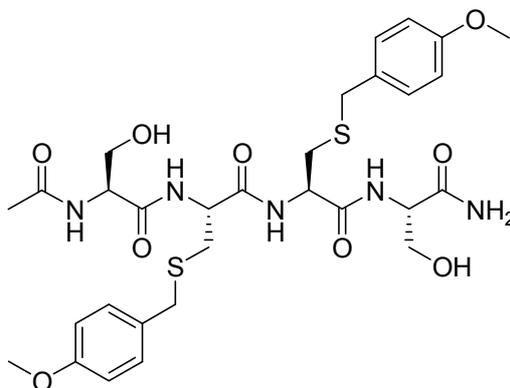
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 672.2132, found 672.2142.



**Ac-Gly-Cys(Mob)-Cys(Mob)-Ser-NH<sub>2</sub>**  
**(GCCS/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 141.7 mg (54%) yield.

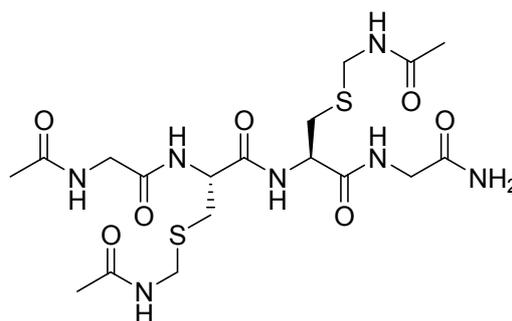
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 672.2132, found 672.2122.



**Ac-Ser-Cys(Mob)-Cys(Mob)-Ser-NH<sub>2</sub>**  
**(SCCS/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 189.7 mg (69%) yield.

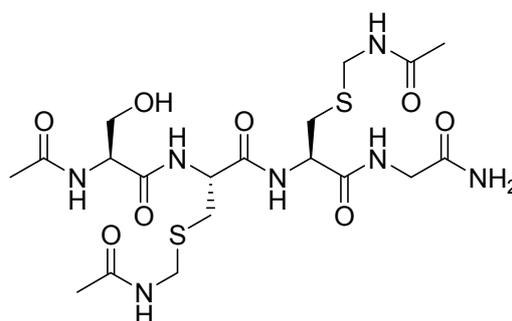
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 702.2238, found 702.2235.

**Synthesis of acetamidomethyl protected Cys-Cys tetrapeptide**

**Ac-Gly-Cys(Acm)-Cys(Acm)-Gly-NH<sub>2</sub>**  
**(GCCG/Acm)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 104.5 mg (49%) yield.

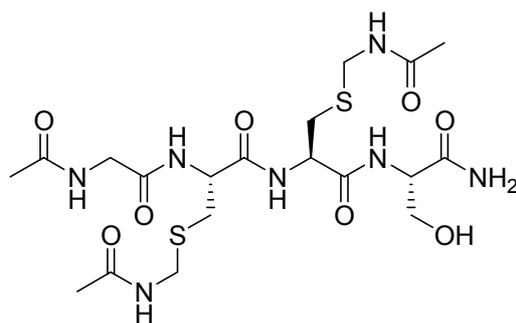
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 544.1619, found 544.1613.



**Ac-Ser-Cys(Acm)-Cys(Acm)-Gly-NH<sub>2</sub>**  
**(SCCG/Acm)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 139.5 mg (63%) yield.

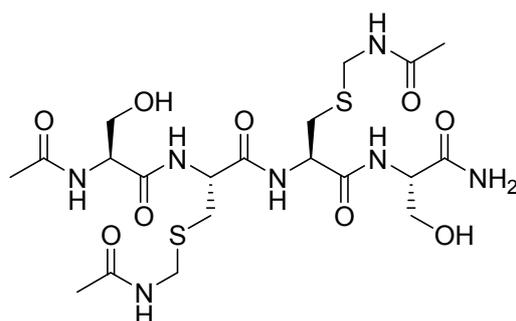
**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 574.1724, found 574.1728.



**Ac-Gly-Cys(Acm)-Cys(Acm)-Ser-NH<sub>2</sub>**  
**(GCCS/Acm)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 76.8 mg (34%) yield.

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 574.1724, found 574.1724.

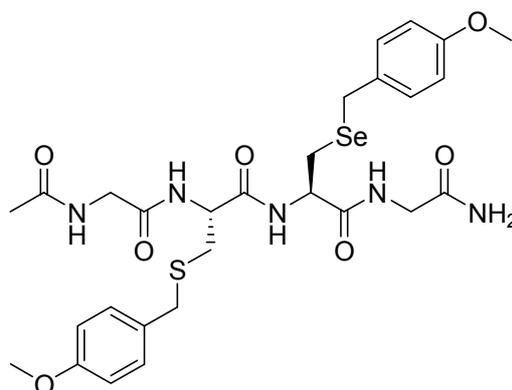


**Ac-Ser-Cys(Acm)-Cys(Acm)-Ser-NH<sub>2</sub>**  
**(SCCS/Acm)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 151 mg (64%) yield.

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 604.1830, found 604.1827.

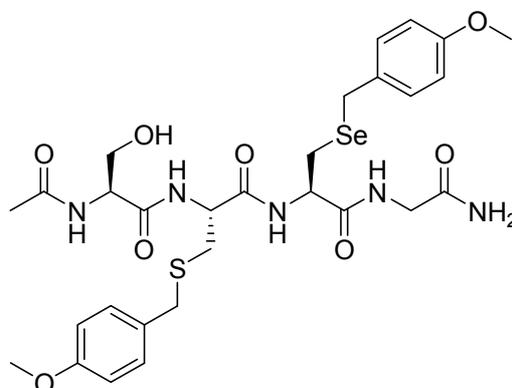
### Synthesis of p-methoxybenzyl protected Cys-Sec tetrapeptide



**Ac-Gly-Cys(Mob)-Sec(Mob)-Gly-NH<sub>2</sub>**  
**(GCUG/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 88.3 mg (33%) yield.

**HRMS** (ESI, [M+H]<sup>+</sup>) m/z calcd 668.1652, found 668.1665.



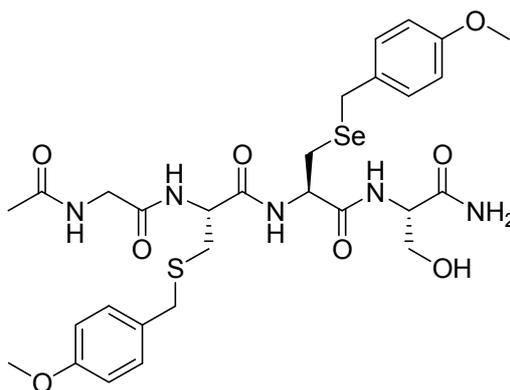
**Ac-Ser-Cys(Mob)-Sec(Mob)-Gly-NH<sub>2</sub>**  
**(SCUG/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 108.6 mg (39%) yield. HPLC (gradient 30% ACN in water to 70% of ACN in 20 min) shows 2 peaks: the product tr(1) = 9.1 min and alcohol protected peptide tr(2) = 14.2 min (1:1). The deprotection was not complete and gave a mixture of protected and unprotected peptides. This, however, was not

problematic as the peptides were later completely deprotected, as a consequence of their oxidation in TFA.

**HRMS** (ESI,  $[M(\text{tr}(1))+\text{H}]^+$ )  $m/z$  calcd 698.1757, found 698.1761;

**HRMS** (ESI,  $[M(\text{tr}(2))+\text{Na}]^+$ )  $m/z$  calcd 776.2203, found 776.2195.

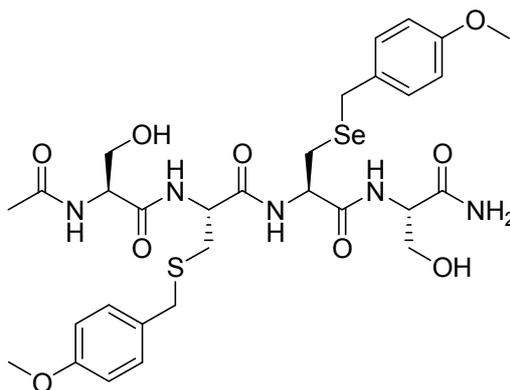


**Ac-Gly-Cys(Mob)-Sec(Mob)-Ser-NH<sub>2</sub>**  
**(GCUS/Mob)**

The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 29.2 mg (10%) yield. HPLC (gradient 30% ACN in water to 70% of ACN in 20 min) shows 2 peaks: the product  $\text{tr}(1) = 8.9$  min and alcohol protected peptide  $\text{tr}(2) = 13.0$  min (1:1). The deprotection was not complete and gave a mixture of protected and unprotected peptides. This, however, was not problematic as the peptides were later completely deprotected, as a consequence of their oxidation in TFA.

**HRMS** (ESI,  $[M(\text{tr}(1))+\text{H}]^+$ )  $m/z$  calcd 698.1757, found 698.1769;

**HRMS** (ESI,  $[M(\text{tr}(2))+\text{Na}]^+$ )  $m/z$  calcd 776.2203, found 776.2206.



***Ac-Ser-Cys(Mob)-Sec(Mob)-Ser-NH<sub>2</sub>***  
**(SCUS/Mob)**

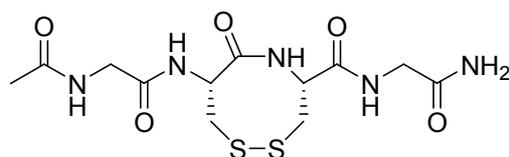
The synthesis was performed according to the general procedure I (GP I) in 0.405 mmol scale. The product was dried in vacuo overnight to give a white solid in 43.4 mg (15%) yield. HPLC (gradient 30% ACN in water to 70% of ACN in 20 min) shows 2 peaks: the product  $tr(1) = 8.6$  min and alcohol protected peptides  $tr(2) = 12.5$  min,  $tr(3) = 13.6$  min,  $tr(4) = 18.1$  min (2:1:2:1). The deprotection was not complete and gave a mixture of protected and unprotected peptides. This, however, was not problematic as the peptides were later completely deprotected, as a consequence of their oxidation in TFA.

**HRMS** (ESI,  $[M(tr(1))+H]^+$ )  $m/z$  calcd 728.1863, found 728.1868;

**HRMS** (ESI,  $[M(tr(2))+Na]^+$ )  $m/z$  calcd 806.2308, found 806.2309;

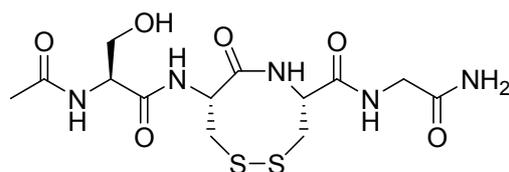
**HRMS** (ESI,  $[M(tr(3))+Na]^+$ )  $m/z$  calcd 806.2308, found 806.2303;

**HRMS** (ESI,  $[M(tr(4))+Na]^+$ )  $m/z$  calcd 862.2934, found 862.2929.

**Synthesis of oxidized Cys-Cys tetrapeptide****Ac-Gly-Cys-Cys-Gly-NH<sub>2</sub>****(GCCG)**

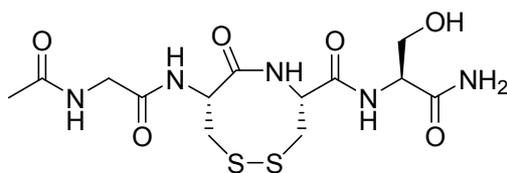
The synthesis was performed according to the general procedure II (GP II). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 43.4 mg (45%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 400.0720, found 400.0721.

**Ac-Ser-Cys-Cys-Gly-NH<sub>2</sub>****(SCCG)**

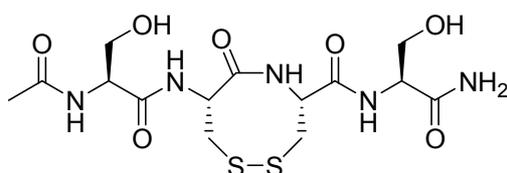
The synthesis was performed according to the general procedure II (GP II). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 44.5 mg (34%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 430.0825, found 430.0821.

**Ac-Gly-Cys-Cys-Ser-NH<sub>2</sub>****(GCCS)**

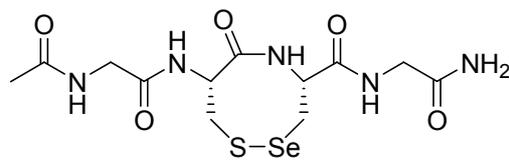
The synthesis was performed according to the general procedure II (GP II). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 41.4 mg (59%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 430.0825, found 430.0831.

**Ac-Ser-Cys-Cys-Ser-NH<sub>2</sub>****(SCCS)**

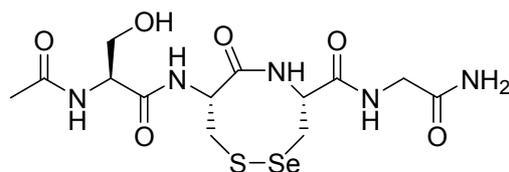
The synthesis was performed according to the general procedure II (GP II). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 50.8 mg (34%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 460.0931, found 460.0933.

**Synthesis of oxidized Cys-Sec tetrapeptide****Ac-Gly-Cys-Sec-Gly-NH<sub>2</sub>****(GcUG)**

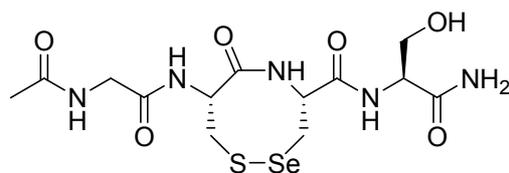
The synthesis was performed according to the general procedure III (GP III). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 28.6 mg (36%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 448.0164, found 448.0167.

**Ac-Ser-Cys-Sec-Gly-NH<sub>2</sub>****(ScUG)**

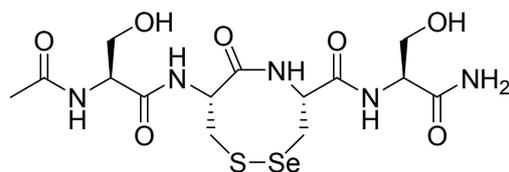
The synthesis was performed according to the general procedure III (GP III). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 51.2 mg (53%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 478.0270, found 478.0270.

**Ac-Gly-Cys-Sec-Ser-NH<sub>2</sub>****(GCUS)**

The synthesis was performed according to the general procedure III (GP III). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 8.2 mg (36%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 478.0270, found 478.0270.

**Ac-Ser-Cys-Sec-Ser-NH<sub>2</sub>****(SCUS)**

The synthesis was performed according to the general procedure III (GP III). After HPLC purification the product was kept in the deluted solution to avoid the dimerisation. The yield was calculated to be 15.9 mg (38%).

**HRMS** (ESI, [M+Na]<sup>+</sup>) m/z calcd 508.0376, found 508.0377.

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# Curriculum Vitae

## Alex Schneider

geboren am 24.05.1979

in Tschita-46, Russland

### Schulbildung / Universitätsausbildung

1986-1989		Grundschule, Tschita-46, Russland
1989-1995		Gymnasium, Kremenschuk, Ukraine (mit Auszeichnung)
1995-2000		Lomonossow Universität, Moskau; Fakultät für Chemie
	Abschluss	Chemiker (Diplom in Analytischer Chemie)
2000-2002		Promotion
		Lomonossow Universität, Moskau; Fakultät für Chemie
	Hauptfach	Analytische Chemie
	Abschluss	Kein Abschluss, da 2002 Umsiedlung nach Deutschland
2004-2007		Promotion
		Leibniz Institut für Pflanzenbiochemie, Halle (Saale)
	Hauptfach	Organische/Analytische Chemie
	Abschluss	2008

### *Tätigkeit während des Studiums*

06.1996-07.2001	Chromatographieforschungsgruppe der Lomonossow Universität, Moskau Konzentrierung, Identifizierung und quantitative Bestimmung von Verunreinigungen in pharmazeutischen Präparaten; Erarbeitung von neuen Methoden bzw. Optimierung alter analytischer Verfahren unter Verwendung von z.B. HPLC, GC, GC/MS; Erarbeitung neuer Projekte einschließlich Budgetplanung und juristischer Begleitung
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### *Wissenschaftliche Erfahrung*

10.1996-06.1997	Bestimmung von Dioxinen in Wasser- und Bodenproben mittels GC
01.1998-06.1998	Synthese pharmazeutischer Substanzen am Institut für Organische Chemie der Lomonossow Universität, Moskau
06.1998-08.1998	Forschungspraxis: Institut für Analytische Chemie an der Universität Leipzig (Prof. Dr. W. Engewald)
06.1999-08.1999	Forschungspraxis: Forensic Science Unit, Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, Scotland (Dr. A. Hunt, Dr. J. W. Thorpe)
01.1997-08.2000	Bestimmung von Explosivstoffen in organischen Extrakten und Wasser (forensische Untersuchungen)
09.2000-06.2001	Analyse und Vergleich pharmazeutischer Präparate für Novartis Pharma Services Inc., Moskau
01.2004-06.2004	Wissenschaftliches Praktikum (Organische&Kombinatorische Chemie) bei Merck KGaA, Darmstadt
08.2004-12.2004	Wissenschaftlicher Aufenthalt während des DAAD-Austauschprojekts in der Universität in Santa Maria, Brasilien

**Berufliche Tätigkeit**

07.2001-08.2002

Verkäufer für Industriewaren IKEA MOS

08.2002-12.2002

Verkaufsleiter IKEA MOS

Planung der Abteilung, Einstellung und Ausbildung des Personals, Organisation der Verkaufsabläufe, Beaufsichtigung und Kontrolle der Warenlieferungen, Marktforschung, Inventur

05.2006-jetzt

Geschäftsführer der Firma IDrug GmbH, einem auf dem Gebiet der analytischen Chemie spezialisiertem Dienstleister der Pharmaindustrie (Bekämpfung von Medikamentenfälschungen; Qualitätskontrolle von Wettbewerbern/Generika)

**Publikationen**

- Braga, A.L., Lüttke, D.S., Schneider, P.H., Vargas, F., Schneider, A., Wessjohann, L.A. and Paixao, M.W. Catalytic enantioselective aryl transfer: asymmetric addition of boronic acids to aldehydes using pyrrolidinylmethanols as ligands. *Tetrahedron Lett.* 46, (2005): 7827-7830.
- Schneider, A., Rodrigues, O.E.D., Paixao, M.W., Appelt, H.R., Braga, A.L., and Wessjohann, L.A., Stereoselective synthesis of Boc-protected L-seleno- and tellurolanthionine, L-seleno- and tellurocystine and derivatives. *Tetrahedron Lett.* 47, (2006): 1019-1021.
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**Sprachen**

- Russisch (Muttersprache, fließend in Wort und Schrift)
- Deutsch (fließend in Wort und Schrift)
- Englisch (gute Kenntnisse in Wort und Schrift)
- Portugiesisch (Grundkenntnisse)

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 Alex Schneider

**Erklärung**

Hiermit erkläre ich an Eides Statt, dass ich die vorliegende Arbeit selbständig und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe. Diese Arbeit wurde bisher an keiner anderen Institution zur Erlangung eines akademischen Grades vorgelegt.

Halle (Saale), den

(Alex Schneider)