Synthesis of Novel Se-Substituted Selenocysteine Derivatives as Potential Kidney Selective Prodrugs of Biologically Active Selenol Compounds: Evaluation of Kinetics of β-Elimination Reactions in Rat Renal Cytosol

Ioanna Andreadou, Wiro M. P. B. Menge,† Jan N. M. Commandeur, Eduard A. Worthington,† and Nico P. E. Vermeulen*

Leiden Amsterdam Center for Drug Research (LACDR), Divisions of Molecular Toxicology and Medicinal Chemistry, Department of Pharmacochemistry, Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

Received October 11, 1995⊗

Eighteen Se-substituted selenocysteine derivatives were synthesized as potential kidney selective prodrugs which can be activated by renal cysteine conjugate β-lyase to selenium-containing chemoprotectants or antitumor agents. Selenocysteine derivatives with aliphatic and benzylic Se-substituents were synthesized by reducing selenocystine to selenocysteine followed by a reaction with the corresponding alkyl and benzyl halogenides. Selenocysteine derivatives with aromatic Se-substituents were synthesized by reaction of β-chloroalanine with substituted phenylselenol compounds, which were formed by reducing substituted diphenyl diselenides by $NaBH_4$. The enzyme kinetic parameters (apparent K_m and V_{max}) of the β-elimination reaction of the selenocysteine conjugates were studied in rat renal cytosol. The results suggest that Se-substituted L-selenocysteine conjugates are extremely good substrates for renal cysteine conjugate β-lyases as indicated by low apparent K_m and high V_{max} values. The benzyl-substituted Se-conjugates appeared to be better substrates than the phenyl- and alkyl-substituted Se-conjugates. Corresponding L-cysteine S-conjugates were too poor substrates to obtain proper enzyme kinetics. Recently, local activation of cysteine S-conjugates by renal cysteine conjugate β-lyases was proposed as a new strategy to target antitumor agents to the kidney. The present results show that Se-substituted selenocysteine conjugates may be more promising prodrugs because these compounds are much better substrates for β-lyase.

Introduction

During the last decade there has been a considerable interest in the identification of compounds with potential anticancer activity. Among these so-called antitumor agents are a number of sulfur- and selenium-containing compounds.[1] Compared to sulfur compounds, the corresponding selenium analogues appear to be much more active in cancer prevention.[2] A number of selenium compounds, such as sodium selenite, sodium selenate, and to a lesser extent selenocystine and selenomethionine, have been used succesfully as dietary supplements for suppression of tumor development in rodents.[1] Sulfur and selenium commonly occur in forms corresponding to oxidation states +6, +4, or −2. Both elements have similar covalent radii and possess the ability for multiple bonding. Replacement of oxygen and/or sulfur by selenium, another group VIB element, is known to modify the biological activities considerably.[3]

Very recently it was proposed to target thiol-containing antitumor compounds to the kidneys.[4,5] Thus *S*-(6-purinyl)-L-cysteine was used as a kidney selective prodrug which is bioactivated in the kidney by cysteine-*S*-conjugate β-lyases to the cytostatic agent 6-mercaptopurine. Because as yet no antitumor agents are available for the treatment of renal carcinomas, this may be a promising approach to target 6-mercaptopurine to the kidney. The kidney selectivity of *S*-(6-purinyl)-L-cysteine, like many other cysteine S-conjugates, is based on the fact that cysteine S-conjugates are actively taken up by kidney cells and the fact that the concentration of β-lyase in the kidney is relatively high.[6] Very recently, it was also shown that human renal carcinomas contain significant β-lyase activities which would favor targeting and local bioactivation of the prodrugs to agents with cytostatic and antitumor properties.[7]

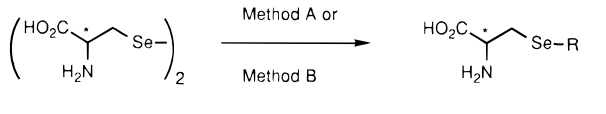
Another potentially important application of selenium compounds may be their use as chemoprotectors against toxic side effects of drugs, such as cytostatic agents. Thus sodium selenite and ebselen protect rats and mice from the nephrotoxicity of cisplatin without interfering with its antitumor activity.[8] It has been proposed that selenolmetabolites (R-SeH) which are formed upon glutathione-dependent (GSH) bioactivation of selenite and ebselen may react with covalently bound reactive aquated metabolites of cisplatin, thus resulting in the detoxication of cisplatin.[8,9] The fact that selenite and ebselen concentrate in the kidney and that GSH levels in the kidney are higher than in tumors may favor the protection against cisplatin in the kidney as well.[8]

On the basis of these findings, we decided to design, synthesize, and evaluate different classes of Se-substituted selenocysteine conjugates as a potentially new class of substrates for renal cysteine-*S*-conjugate β-lyases. Selenocysteine conjugates might be kidney selective chemoprotectors and/or antitumor agents by the generation of selenol compounds by renal β-lyases. Selenols possess more potent nucleophilic properties than the corresponding thiol compounds. It has also been found that selenols, such as 6-selenoguanine and (*p*-methoxyphenyl)selenol, possess antitumor and anticarcinogenic activities.[3] *Se*-Methylselenocysteine is an

* To whom correspondence should be addressed.
† Division of Medicinal Chemistry.
⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1996.

S0022-2623(95)00750-3 CCC: $12.00

Scheme 1. Synthetic Routes Followed for the Preparation of the Aliphatic and Substituted Benzylic Selenocysteine Derivatives[a]



1a

1; R = methyl, L-isomer
2; R = methyl, D-isomer
3; R = ethyl
4; R = n-propyl, L-isomer
5; R = n-propyl, D-isomer
6; R = i-propyl
7; R = n-butyl
8; R = benzyl
9; R = 4-methylbenzyl, L-isomer
10; R = 4-methylbenzyl, D-isomer
11; R = 4-methoxybenzyl
12; R = 4-chlorobenzyl
13; R = 3,4-dichlorobenzyl

[a] Method A: reduction with Na/NH_3. Method B: reduction with $NaBH_4$ and subsequent reaction of selenocysteine with the corresponding alkyl or benzyl halogenides, as described in the Experimental Section.

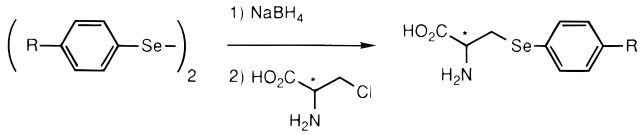
antitumor agent, and it has been shown that β-elimination is implicated in the mechanism of this activity.[10]

The enzyme kinetics of the β-elimination reactions of the new selenocysteine conjugates are evaluated using rat renal cytosol because this fraction probably will reflect the total activity of cytosolic β-eliminating enzymes in the kidney. Renal cytosol contains at least two cysteine conjugate β-lyase enzymes known to be active toward cysteine S-conjugates.[11,12] However, it is not yet known whether these enzymes are also the only enzymes active toward selenocysteine conjugates. The results of the present study indicate that the Se-substituted selenocysteine conjugates synthesized are β-eliminated in rat renal cytosol at a much higher rate than the corresponding sulfur analogues, which makes them a promising new class of kidney selective prodrugs for antitumor/anticarcinogenic and chemoprotective selenol compounds.

Results and Discussion

In the present study various new synthetical approaches are described which successfully lead to selenocysteine conjugates with a wide range of selenium-bound substituents. The synthetical pathways used are summarized in Schemes 1 and 2. The aliphatic and benzyl Se-substituents were introduced by reducing selenocystine to selenocysteine and subsequent reaction of selenocysteine with the corresponding alkyl or benzyl halogenides. The phenyl Se-substituted selenocysteine conjugates were synthesized by reducing the appropriately substituted diphenyl diselenides to the corresponding selenols and subsequent reaction with β-chloroalanine (Scheme 2). The nucleophilic substitution reactions shown in Scheme 2 are known to be stereoselective.[13,27,28] This stereoselectivity is supported by the fact that the D-selenocysteine conjugates synthesized were not β-eliminated at all by purified rat renal cysteine S-conjugate β-lyase, whereas the corresponding L-selenocysteine conjugates are very efficiently metabolized (data not shown/manuscript in preparation). It is also known that purified β-lyase only metabolizes L-cysteine S-conjugates but not D-cysteine S-conjugates.[12] Our observations suggest a similar stereoselectivity toward selenocysteine Se-conjugates, which can be

Scheme 2. Synthetic Route Followed for the Preparation of the *p*-Substituted Phenylselenocysteine Derivatives



14; R = -H
15; R = -CH_3, L-isomer
16; R = -CH_3, D-isomer
17; R = -OCH_3
18; R = -Cl

explained by the high structural resemblance between selenocysteine Se-conjugates and cysteine S-conjugates.

Both synthetical routes used gave good yields of optically pure Se-substituted selenocysteine conjugates. The structures of all 18 synthesized Se-substituted selenocysteine conjugates are shown in Schemes 1 and 2.

The specific β-elimination activities and the enzyme kinetic parameters determined with rat renal cytosol for the L-selenocysteine Se-conjugates, the D-selenocysteine Se-conjugates, and the L-cysteine S-conjugates are given in Table 1. As may be derived from Table 1, all synthesized selenocysteine conjugates are remarkably well transformed by rat renal cytosol via the β-elimination reaction as reflected by the high rate of pyruvate formation. Because upon β-elimination reactions of selenocysteine conjugates identical amounts of selenol compounds will be formed, these substrates are thus potentially good prodrugs for biologicaly active selenols. Formation of all anticipated selenols has been confirmed by GC−MS analysis after extraction of acidified enzymic incubation mixtures with ethyl acetate (data not shown). For one representative compound, *Se*-phenyl-L-selenocysteine, the stoichiometry of formation of selenol and pyruvic acid was quantified. Upon incubation of *Se*-phenyl-L-selenocysteine in cytosol, both phenylselenol and diphenyl diselenide were observed as metabolites upon GC−MS analysis of ethyl acetate extracts. Diphenyl diselenide was the major product formed. The formation of diphenyl diselenide can be explained by the rapid autoxidation of phenylselenol at the slightly basic (pH 8.6) conditions of the incubation.[30] Quantification of these organoselenocompounds revealed that the phenylselenol formed amounted to more than 90% of the amount of pyruvic acid formed. This result suggests that formation of pyruvic acid indeed is a good reflection of the amount of selenol formed upon β-elimination of selenocysteine conjugates.

It has been shown previously[14] that a number of structural requirements have to be met for L-cysteine S-conjugates to be substrates for cysteine-*S*-conjugate β-lyases. Structural requirements on the cysteine part are a L-configuration at the α-carbon atom, a primary amino group, a free carboxyl group, and an abstractable α-hydrogen atom. For the xenobiotic part of β-lyase substrates, it was shown that electron-withdrawing substituents on the sulfur atom were necessary for a high β-elimination activity. More detailed information with regard to the structure−activity relationships for β-elimination by this enzyme is lacking because of the small number of substrates which are as yet available. It has been suggested that the leaving group ability of thiolate anions and the abstractability of the C(α)-proton

Table 1. Specific Activities and Apparent Enzyme Kinetic Parameters for the β-Elimination of L-Selenocysteine Se-Conjugates, D-Selenocysteine Se-Conjugates, and L-Cysteine S-Conjugates by Rat Renal Cytosol[a]

no.	compound	specific activity (nmol/min·mg)	V_{max} (nmol/min·mg)	K_m (mM)	V_{max}/K_m (nmol/min·mg/mM)
1	*Se*-methyl-L-selenocysteine	7.1	8.3	0.6	13.2
2	*Se*-methyl-D-selenocysteine	4.0	ND	ND	ND
3	*Se*-ethyl-L-selenocysteine	11.0	14.4	0.6	21.8
4	*Se*-propyl-L-selenocysteine	8.0	12.3	0.5	25.7
5	*Se*-propyl-D-selenocysteine	3.3	ND	ND	ND
6	*Se*-butyl-L-selenocysteine	0.9	1.0	0.6	1.8
7	*Se*-isopropyl-L-selenocysteine	14.9	12.9	0.2	66.9
8	*Se*-benzyl-L-selenocysteine	9.8	9.5	0.1	70.0
9	*Se*-(4-methylbenzyl)-L-selenocysteine	13.1	13.5	0.2	70.2
10	*Se*-(4-methylbenzyl)-D-selenocysteine	4.9	ND	ND	ND
11	*Se*-(4-methoxybenzyl)-L-selenocysteine	10.2	14.5	0.3	44.9
12	*Se*-(4-chlorobenzyl)-L-selenocysteine	11.6	11.2	0.2	68.4
13	*Se*-(3,4-dichlorobenzyl)-L-selenocysteine	12.3	13.7	0.3	49.0
14	*Se*-phenyl-L-selenocysteine	13.8	20.2	0.5	44.2
15	*Se*-(4-methylphenyl)-L-selenocysteine	3.8	3.6	0.2	16.7
16	*Se*-(4-methylphenyl)-D-selenocysteine	0.1	ND	ND	ND
17	*Se*-(4-methoxyphenyl)-L-selenocysteine	4.3	6.2	0.4	14.0
18	*Se*-(4-chlorophenyl)-L-selenocysteine	5.2	7.3	0.4	16.7
19	*S*-ethyl-L-cysteine	0.1	ND	ND	ND
20	*S*-benzyl-L-cysteine	0.2	ND	ND	ND
21	*S*-(4-methylbenzyl)-L-cysteine	0.3	ND	ND	ND
22	*S*-(4-methoxybenzyl)-L-cysteine	0.1	ND	ND	ND
23	*S*-phenyl-L-cysteine	0.2	ND	ND	ND

[a] For the determination of the specific activity for the β-elimination reactions by rat renal cytosolic β-lyase, incubations were performed with 0.2 mg of protein/mL for 10 min at 37 °C using substrate concentrations of 1 mM. ND: enzyme kinetic parameters could not be determined accurately due to too low of a turnover.

of the L-cysteine part of the substrate are rate-determing in this β-elimination reaction.[14]

The aim of the present study was to establish a qualitative substrate profile for rat renal cytosolic cysteine conjugate β-lyases in order to be able to more rationally design a number of structurally related substrates for these enzymes. Firstly, we replaced S by Se because selenols are better leaving groups than the corresponding thiols. We used five L-cysteine S-conjugates for comparison with the corresponding Se analogs. If we compare the specific β-elimination activities of β-lyase toward Se-substituted L-selenocysteine conjugates (compounds **1**−**18**, Table 1) with the corresponding L-cysteine S-conjugates (**19**−**23**, Table 1), we may conclude that the Se-conjugates are much better substrates for rat kidney cytosolic β-lyases than the corresponding S-analogues. We could not obtain appropriate enzyme kinetic parameters (V_{max} and K_m) with the S-substituted L-cysteine conjugates using cytosolic protein concentrations of 0.2 mg/mL and substrate concentrations from 0.05 up to 1 mM because the β-elimination activity was too low for accurate measurements.

Secondly, we synthesized a number of D-selenocysteine Se-conjugates for comparison with corresponding L-selenocysteine Se-conjugates. From the results in Table 1 it can be concluded that some of the D-isomers (**2**, **5**, and **10**) have relatively high β-elimination activities with rat kidney cytosolic β-lyases at a concentration 1 mM. However, the same conjugates showed a much lower β-elimination activity at concentrations of 0.5 mM and lower, as a consequence of which we could not determine accurately enzyme kinetic parameters (V_{max} and K_m). Preliminary experiments with the purified rat kidney 90−95 kDa β-lyase showed that D-isomers are not metabolized by β-lyase (data not shown). Therefore, the residual activity in cytosol can most likely be explained by the fact that the D-isomers of the selenocysteine conjugates investigated undergo oxidative deamination by renal D-amino acid oxidases followed by transamination by cytosolic transaminases to form the corresponding L-selenocysteine Se-conjugates which finally can be β-eliminated by β-lyase.[15] This mechanism was previously proposed to explain the nephrotoxicity of *S*-(1,2-dichlorovinyl)-D-cysteine.[15] At least three enzymes are taking part in this metabolic route, and for this reason we most likely could not obtain appropriate enzyme kinetic parameters.

To investigate which Se-bound substituents allow β-elimination reactions, we introduced different kinds of Se-substituents in the selenocysteine conjugates, including aliphatic, benzylic, and phenyl substituents, with and without various electron-withdrawing groups.

We selected apparent V_{max}/K_m ratios as turnover parameter for the substrates in order to characterize the catalytic efficiency of rat renal cytosolic β-lyases at low (potentially physiological) concentrations. It can be concluded from the data presented in Table 1 that most of the Se-substituted selenocysteine conjugates appear to be very good substrates for renal β-lyases. In the aliphatic series of L-selenocysteine conjugates, the addition of a methylene group in the Se-substituent increased the turnover until *Se*-propyl-L-selenocysteine (**4**, Table 1). Addition of one more methylene group (*Se*-butyl-L-selenocysteine, **6**, Table 1) resulted in a considerable decrease in the β-elimination of the Se-conjugate by rat renal cytosol. The isopropyl-L-selenocysteine (compound **7**, Table 1) appeared to be the best substrate in the aliphatic series of Se-conjugates. This might be due to steric properties resulting in a higher affinity (i.e., a lower apparent K_m) to the β-lyases.

In the series of *Se*-benzyl-substituted selenocysteine conjugates, all members (**8**, **9**, and **11**−**13**, Table 1) appeared to be very good substrates for β-lyases without large substituent effects. The electron-withdrawing substituents (4-chloro- and 3,4-dichloro substituents in compounds **12** and **13**, respectively, Table 1) did not seem to be essential for the β-lyase activity. Even

conjugates with electron-donating Se-substituents appeared to be good substrates.

In the series of *Se*-phenyl-substituted selenocysteine conjugates, *Se*-phenyl-L-selenocysteine (**14**, Table 1) appeared to be the most potent substrate, and *p*-substitution in the aromatic ring lowered this activity. Comparing the phenyl-substituted L-selenocysteines (**14**, **15**, **17**, and **18**, Table 1) with the corresponding *Se*-benzyl-substituted conjugates (**8**, **9**, and **11**−**13**, Table 1), it may be concluded that *Se*-benzyl substitution yields more potent substrates for β-lyases than corresponding phenyl substitution. The benzylic methylene group apparently contributes to a higher affinity (i.e., lower apparent K_m) in this series of conjugates, which might be due to lipophilic, steric, and/or electronic interactions between these substrates and renal β-lyases. However, because rat renal cytosol contains a mixture of at least two β-lyase-enzymes,[11,12] which most probably have different substrate selectivities, the observed structure−activity relationships in cytosol do not give information of the structure−activity relationships of the individual enzymes. In addition it cannot be ruled out that other pyridoxal-containing enzymes also contribute to the β-elimination of selenocysteine Se-conjugates.

We conclude that Se-substituted selenocysteine conjugates are much better substrates for renal cytosolic β-lyases than the corresponding S-substituted cysteine conjugates, that the electron-withdrawing character of the Se-substituent in L-selenocysteine conjugates is not the sole factor governing the β-elimination activity, and that steric and lipophilic factors probably also modulate the β-elimination activity of β-lyases. With this study several novel Se-containing substrates for rat kidney cytosolic β-lyase have been introduced, which due to their selenocysteine parts in the molecules may be actively taken up in mammalian kidney and subsequently may be bioactivated locally by renal β-lyases to selenols with potential antitumor and/or chemoprotective properties.

Experimental Section

Chemistry: General Methods. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer with tetramethylsilane or sodium 3-(trimethylsilyl)propionate as an internal standard. Melting points were measured on a Mettler FP-5 + FP-52 instrument and are uncorrected. β-Chloro-L-alanine and β-chloro-D-alanine were prepared from L-serine and D-serine, respectively, as described in the literature.[16] L-Selenocystine and D-selenocystine (**1a**) were prepared from β-chloro-L-alanine and β-chloro-D-alanine and disodium diselenide according to the literature.[13] *Se*-(4-Methoxybenzyl)-L-selenocysteine (**11**) was prepared as described in the literature.[17] *S*-Ethyl-L-cysteine (**19**) and *S*-benzyl-L-cysteine (**20**) were purchased from Sigma. *S*-(4-Methylbenzyl)-L-cysteine (**21**) was purchased from Advanced Chem. Tech., *S*-(4-methoxybenzyl)-L-cysteine (**22**) was purchased from Bachem Feinchemikalien AG, and *S*-phenyl-L-cysteine (**23**) was synthesized from phenylthiol and β-chloro-L-alanine according to the literature.[18] *N*-Acetyl-*S*-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine was prepared as described previously.[29]

***Se*-Methylselenocysteine [L-form (1) and D-form (2)] (Method A).** In a 100 mL cylinder-shaped condensation tube, 1.5 mmol (500 mg) of L-selenocystine or D-selenocystine was dissolved in 20 mL of liquid ammonia and kept at −70 °C under nitrogen. To this solution were added small pieces of metalic Na (±1.38 g, 6 mmol) until the color of the mixture remained blue. Then 1 drop of ethanol was added to remove the excess of sodium; 6 mmol (850 mg) of methyl iodide was added, and the cooling bath was removed in order to slowly evaporate the ammonia. When all the ammonia had evaporated, the residual liquid brown solid was dissolved in 1 N HCl, and the solution was concentrated in vacuum. The remaining solid was extracted with absolute ethanol. In this way, the product was separated from the inorganic salts. The crude product was recrystallized from ethanol and ether to give light yellow crystals.

***Se*-Methyl-L-selenocysteine:**[19,20] yield 33%; mp 166 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.05 (s, 3H, CH_3-Se), 3.07 (dd, 2H, C-CH_2-Se), 4.13 (t, 1H, N-CH-CO); ^{13}C-NMR (D_2O) δ (ppm) 6.03 (*C*H_3-Se), 25.65 (C-*C*H_2-Se), 54.56 (N-*C*H-C=O), 173.45 (CH-*C*O-OH).

***Se*-Methyl-D-selenocysteine:** yield 35%; mp 167−168 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.05 (s, 3H, CH_3-Se), 3−3.1 (dd, 2H, C-CH_2-Se), 4−4.1 (t, 1H, N-CH-CO); ^{13}C-NMR (D_2O) δ (ppm) 5.86 (*C*H_3-Se), 26.31 (C-*C*H_2-Se), 55.204 (N-*C*H-C=O), 173.8 (CH-*C*O-OH).

***Se*-Ethyl-L-selenocysteine (3) (Method B).** L-Selenocystine (1.5 mmol, 500 mg) was dissolved in 8 mL of 0.5 N NaOH and 2 mL of ethanol. At 0 °C, 0.4 g (15 mmol) $NaBH_4$ was added while stirring the reaction mixture. The mixture was allowed to reach room temperature, during which the color of the solution changed from yellow to colorless. After cooling again to 0 °C, 4 mL of 2 N NaOH and 6 mmol of ethyl iodide were added, and the mixture was stirred for 3 h at room temperature. Concentrated HCl was added until pH = 6−7, and the mixture was cooled to 0 °C. The product precipitated as a white solid and was recrystallized from ethanol and diethyl ether to give white crystals:[21] yield 30%; mp 202 °C; ^{1}H-NMR (D_2O) δ (ppm) 1.57 (t, 3H, CH_3-C), 2.8 (m, 2H, CH_2-Se), 3.07 (dd, 2H, Se-CH_2-C), 3.95 (t, 1H, N-CH-CO).

***Se*-*n*-Propylselenocysteine [L-form (4) and D-form (5)].** The same procedure was employed as for the preparation of *Se*-ethylselenocysteine (method B), using 6 mmol of *n*-propyl iodide instead of ethyl iodide. To obtain both the D- and L-isomers of the selenocysteine conjugates, 1.5 mmol of D- or L-selenocystine was used, respectively. The products were recrystallized from 50% ethanol and 50% water, giving white crystals. It was noticed that inorganic salts remained in the product after recrystallization. Changing the conditions of recrystallization did not prevent the cocrystallization of inorganic salts.[22]

***Se*-*n*-Propyl-L-selenocysteine:** yield 56%; mp 251 °C; ^{1}H-NMR (D_2O) δ (ppm) 0.95 (t, 3H, CH_3-C), 1.8 (m, 2H CH_2-C), 2.77 (m, 2H, C-CH_2-Se), 2.9 (dd, 2H, Se-CH_2), 3.8 (t, 1H, N-CH-CO); ^{13}C-NMR (D_2O) δ (ppm) 15.075 (*C*H_3-C), 24.64 (C-*C*H_2-C), 26.066 (C-*C*H_2-Se), 28.29 (Se- *C*H_2-C), 55.92 (N-*C*H-C=O), 178.2 (CH-*C*O-OH). Anal. Calcd ($C_{16}H_{13}NO_2Se$): C, 34.3; H, 6.24; N, 6.66. Found: C, 22.68; H, 4.66; N, 4.39.

***Se*-*n*-Propyl-D-selenocysteine:** yield 48%; mp 253 °C; ^{1}H-NMR (D_2O) δ (ppm) 0.98 (t, 3H, CH_3-C), 1.7 (m, 2H, CH_2-C), 2.65 (m, 2H, C-CH_2-Se), 3.05 (dd, 2H, Se-CH_2), 3.9 (t, 1H, N-CH-CO).

***Se*-Isopropyl-L-selenocysteine (7).** *Se*-Isopropylselenocysteine was synthesized according to method B, using 6 mmol of isopropyl iodide instead of ethyl iodide. The product was recrystallized from hot water, giving light yellow crystals. The product cocrystallized with a small amount of inorganic salts:[20] yield 67.7%; mp 207 °C; ^{1}H-NMR (D_2O) δ (ppm) 1.35 (t, 6H, CH_3-C-CH_3), 2.95 (dd, 2H, Se-CH_2-C), 3.2 (m, 1H, C-CH-C), 3.7 (t, 1H, N-CH-CO); ^{13}C-NMR (D_2O) δ (ppm) 24.118 (C-*C*H-C), 25.087 (*C*H_3-C), 25.148 (C-*C*H_3), 31.97 (Se-*C*H_2-C), 55.85 (N-*C*H-C=O), 175.09 (CH-*C*O-OH).

***Se*-Butyl-L-selenocysteine (6).** *Se*-Butylselenocysteine was synthesized according to method B, using 6 mmol of *n*-butyl iodide instead of ethyl iodide. The product was recrystallized by 50% ethanol and 50% H_2O, giving white crystals[20]: yield 59.2%; mp 195 °C; ^{1}H-NMR (D_2O) δ (ppm) 0.85 (t, 3H, CH_3-C), 1.3 (m, 2H, C-CH_2-C), 1.55 (m, 2H, C-CH_2-C), 2.6 (m, 2H, CH_2-Se), 2.95 (dd, 2H, Se-CH_2-C), 3.5 (t, 1H, N-CH-CO); ^{13}C-NMR (D_2O) δ (ppm) 14.28 (*C*H_3-C), 23.74 (C-*C*H_2-C), 25.8 (C-*C*H_2-C), 27.55 (C-*C*H_2-Se), 33.36 (Se-*C*H_2-C), 56.25 (N-*C*H-C=O), 178.66 (CH-*C*O-OH). Anal. Calcd ($C_7H_{15}NO_2Se$): C, 37.51; H, 6.74; N, 6.25. Found: C, 36.45; H, 6.57; N, 6.05.

***Se*-Benzyl-L-selenocysteine (8).** *Se*-Benzylselenocysteine was synthesized according to method B, using 6 mmol of benzyl bromide instead of ethyl iodide. The product was recrystallized from hot water to give white crystals:[23] yield 55%; mp 171−172 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.78 (dd, 2H, C-CH_2-Se), 3.7 (s, 2H, Ar-CH_2-Se), 3.91 (t, 1H, N-CH-CO), 7.12 (m, 5H, Ar-H); ^{13}C-NMR (D_2O), δ (ppm) 27.8 (H-*C*H_2-Se), 31.6 (Ar-*C*H_2-Se), 56.9 (N-*C*H-CO), 130.1 (*p*-Ar-C), 131.77 (*o*-Ar-C), 133.37 (*m*-Ar-C), 139.8 (Ar-*C*-CH_2-Se), 182.32 (CH-*C*O-CO).

***Se*-(4-Methylbenzyl)selenocysteine [L-form (9) and D-form (10)].** *Se*-(4-Methylbenzyl)selenocysteine was synthesized according to method B, using 6 mmol of α-chloro-*p*-xylene instead of ethyl iodide. To obtain both the D- and L-isomers of the selenocysteine conjugates, 1.5 mmol of D- or L-selenocystine was used, respectively. The products were recrystallized by hot water to give white crystals.

***Se*-(4-Methylbenzyl)-L-selenocysteine:** yield 39%; mp 175 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.2 (s, 3H, Ar-CH_3), 2.7 (dd, 2H, C-CH_2-Se), 3.3 (t, 1H, N-CH-CO), 3.7 (s, 2H, Ar-CH_2-Se), 7.1 (m, 4H, Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 21.77 (Ar-*C*H_3), 28.23 (CH-*C*H_2-Se), 31.25 (Ar-*C*H_2-Se), 59.92 (N-*C*H-C=O), 130.28 (*o*-Ar-*C*), 130.81 (m-Ar-*C*), 138.35 (Ar-*C*-CH_2-Se), 138.348 (p-Ar-*C*), 182.42 (CH-*C*O-OH). Anal. Calcd ($C_{11}H_{15}NO_2Se$): C, 48.54; H, 5.55; N, 5.15. Found: C, 48.97; H, 5.63; N, 4.99.

***Se*-(4-Methylbenzyl)-D-selenocysteine:** yield 42%; mp 177 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.3 (s, 3H, Ar-CH_3), 2.7 (dd, 2H, C-CH_2-Se), 3.35 (t, 1H, N-CH-CO), 3.7 (s, 2H, Ar-CH_2-Se), 7.15 (m, 4H, Ar-H).

***Se*-(4-Chlorobenzyl)-L-selenocysteine (12).** *Se*-(4-Chlorobenzyl)-L-selenocysteine was synthesized according to method B, using 6 mmol of 4-chlorobenzyl chloride instead of ethyl iodide. The product was recrystallized from hot water giving white crystals: yield 40%; mp 168.5 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.7 (dd, 2H, C-CH_2-Se), 3.3 (t, 1H, N-CH-CO), 3.7 (s, 2H, Ar-CH_2-Se), 7.2 (s, 4H, Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 27.76 (CH-*C*H_2-Se), 31.57 (Ar-*C*H_2-Se), 56.94 (N-*C*H-C=O), 130.087 (*m*-Ar-*C*), 131.77 (*o*-Ar-*C*), 133.37 (Ar-*C*-CH_2-Se), 139.82 (*p*-Ar-*C*), 182.22 (CH-*C*O-OH). Anal. Calcd ($C_{10}H_{12}ClNO_2Se$): C, 41.3; H, 4.15; N, 4.68. Found: C, 41.05; H, 4.13; N, 4.79.

***Se*-(3,4-Dichlorobenzyl)-L-selenocysteine (13).** *Se*-(3,4-Dichlorobenzyl)selenocysteine was synthesized according to method B, using 6 mmol of 3,4-dichlorobenzyl chloride instead of ethyl iodide. The product was recrystallized from hot water giving white crystals: yield 45%; mp 159.5 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.7 (dd, 2H, C-CH_2-Se), 3.3 (t, 1H, N-CH-CO), 3.6 (s, 2H, Ar-CH_2-Se), 7.1 (d, 1H, m-Ar-H), 7.2−7.4 (m, 2H, *o*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 27.44 (CH-CH_2-Se), 32.304 (Ar-*C*H_2-Se), 57.056 (N-*C*H-C=O), 129.946 (*o*-Ar-*C*), 131.61 (*m*-Ar-*C*), 131.87 (*m*-Ar-*C*-Cl), 131.875 (Ar-*C*-CH_2-Se), 181.895 (CH-*C*O-OH). Anal. Calcd ($C_{10}H_{11}Cl_2NO_2Se$): C, 36.68; H, 3.45; N, 4.25. Found: C, 36.72; H, 3.39; N, 4.28.

***Se*-Phenyl-L-selenocysteine (14) (Method C).** Diphenyl diselenide (1.5 mmol) was dissolved in 8 mL of 0.5 N NaOH and 2 mL of ethanol. At 0 °C, 15 mmol (0.4 g) of $NaBH_4$ was added, and the mixture was allowed to reach room temperature. When the color changed from yellow to colorless, the reaction mixture was cooled to 0 °C, and subsequently 4 mL of 2 N NaOH and 7.88 mmol of β-chloroalanine were added. After stirring for 3 h at room temperature, the product was precipitated by adding concentrated HCl until pH = 7. The product was recrystallized from hot water giving white crystals:[24] yield 61%; mp 178.5 °C; ^{1}H-NMR (D_2O) δ (ppm) 3.2 (dd, 2H, C-CH_2-Se), 4.05 (t, 1H, N-CH-CO), 7.1 (m, 3H, *m*,*p*-Ar-H), 7.4 (m, 2H, *o*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 27.832 (CH-CH_2-Se), 53.57 (N-*C*H-C=O), 128.22 (*p*-Ar-C), 129.8 (*o*-Ar-C), 130.879 (*m*-Ar-C), 135.24 (Ar-C-Se), 171.249 (CH-*C*O-OH).

***Se*-(4-Methylphenyl)selenocysteine [L-form (15) and D-form (16)].** *Se*-(4-Methylphenyl)selenocysteine was synthesized according to method C, using 1.5 mmol of 4-methyldiphenyl diselenide instead of diphenyl diselenide and 6 mmol of β-chloroalanine (D- or L-form). After the mixture stirred for 3 h at room temperature, concentrated HCl was added until pH = 6−7. The products were precipitated as a white solid and recrystallized by hot water to give white crystals.

***Se*-(4-Methylphenyl)-L-selenocysteine:** yield 39%; mp 187 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.3 (s, 3H, CH_3-Ar), 3.05 (dd, 2H, Se-CH_2-C), 3.3 (t, 1H, N-CH-CO), 7−7.5 (m, 5H, *m*-,*o*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 21.586 (CH-*C*H_2-Se), 35.207 (CH_3-Ar), 57.105 (N-*C*H-CO), 126.718 (*o*-Ar-C), 131.565 (*o*-Ar-C), 134.63 (Ar-*C*-Se), 139.46 (*p*-Ar-C), 182.337 (CH-*C*O-OH). Anal. Calcd ($C_{10}H_{13}NO_2Se$): C, 46.52; H, 5.08; N, 5.43. Found: C, 46.40; H, 5.16; N, 5.25.

***Se*-(4-Methylphenyl)-D-selenocysteine:** yield 35%; mp 189 °C; ^{1}H-NMR (D_2O) δ (ppm) 2.25 (s, 3H, CH_3-Ar), 3.05 (dd, 2H, Se-CH_2-C), 3.3 (t, 1H, N-CH-CO), 7−7.5 (m, 4H, *m*-,*o*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 21.59 (CH-*C*H_2-Se), 35.22 (CH_3-Ar), 57.12 (N-*C*H-CO), 131.57 (*o*-Ar-C), 134.64 (*o*-Ar-C), 139.47 (Ar-C-Se), 182.34 (CH-*C*O-OH), 199.61 (*p*-Ar-C). Anal. Calcd ($C_{10}H_{13}NO_2Se$): C, 46.52; H, 5.08; N, 5.43. Found: C, 46.31; H, 5.12; N, 5.33.

***Se*-(4-Methoxyphenyl)-L-selenocysteine (17).** The same procedure as for the preparation of *Se*-(4-methylphenyl)-selenocysteine was employed using 1.5 mmol of 4-methoxydiphenyl diselenide and 6 mmol of β-chloro-L-alanine. The product was recrystallized from hot water to give white crystals: yield 41.5%; mp 173 °C; ^{1}H-NMR (D_2O) δ (ppm) 3.1 (dd, 2H, Se-CH_2-C), 3.3 (t, 1H, N-CH-CO), 3.7 (s, 3H, CH_3O-Ar), 6.75−7.5 (m, 4H, *m*-,*o*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 35.905 (CH-*C*H_2-Se), 56.87 (N-*C*H-C=O), 57.036 (Ar-O*C*H_3), 116.549 (*m*-Ar-*C*), 120.959 (*o*-Ar-*C*), 136.949 (Ar-*C*-Se), 160.146 (*p*-Ar-*C*), 182.333 (CH-*C*O-OH). Anal. Calcd ($C_{10}H_{13}NO_3Se$): C, 43.57; H, 4.98; N, 4.90. Found: C, 43.81; H, 4.78; N, 5.11.

***Se*-(4-Chlorophenyl)-L-selenocysteine (18).** The same procedure as for the preparation of *Se*-(4-methylphenyl)-selenocysteine was employed, using 1.5 mmol (571.5 mg) of 4-chlorodiphenyl diselenide. The product was recrystallized from hot water to give white crystals: yield 25.3%; mp 182 °C; ^{1}H-NMR (D_2O) δ (ppm) 3.1 (dd, 2H, Se-CH_2-C), 3.35 (t, 2H, C-CH-N), 7.15−7.55 (m, 4H, *o*-,*m*-Ar-H); ^{13}C-NMR (D_2O) δ (ppm) 35.145 (CH-*C*H_2-Se), 57.047 (N-*C*H-C=O), 129.052 (*m*-Ar-*C*), 130.685 (*o*-Ar-*C*), 134.307 (Ar-*C*-Se), 135.695 (*p*-Ar-*C*), 182.213 (CH-*C*O-OH). Anal. Calcd ($C_9H_{10}ClNO_2Se$): C, 38.77; H, 3.63; N, 4.96. Found: C, 38.80; H, 3.62; N, 5.03.

Biochemical Assays. Preparation of Rat Kidney Cytosolic Fraction. Male Wistar rats (160−180 g) obtained from Harlan-SD (Zeist, The Netherlands) were fed on a standard laboratory diet. Unfasted animals were sacrificed by decapitation, and the kidneys were isolated. After removal of the ligament, a 50% (w/v) homogenate was prepared in ice-cold potassium phosphate buffer (50 mM, pH 7.4) using a Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 4 °C for 30 min at 9000*g* to obtain the S_9 fraction. Subsequently, the S_9 fraction was centrifuged at 100000*g* for 60 min to give the cytosolic fraction. This fraction was dialyzed overnight against a 20-fold excess of potassium phosphate (50 mM, pH 7.4), lyophilized, and finally stored at −80 °C until use.[25]

Purified cysteine conjugate β-lyase was purified from rat renal cytosol as described by Yamauchi et al. [26] The purified protein has a molecular weight of ca. 90−95 kDa and is the major β-lyase in rat renal cytosol.[12]

Incubations. The lyophilized rat renal cytosolic fraction was reconstituted in a Tris-HCl buffer (pH 8.6, 50 mM; final protein concentration 0.2 mg/mL as determined with the Bio-Rad assay) and preincubated at 37 °C for 5 min. Because aromatic and benzylic selenocysteine conjugates had a lower solubility than their corresponding sulfur analogues, it required the use of an ultrasonic bath for prolonged times to prepare a stock solution of 1.5 mM of these substrates. Incubations were started by addition of substrate solution (in final concentrations varying from 1 to 0.05 mM) to give a final volume of 300 μL in the presence of 0.1 mM α-keto-γ-methiolbutyric acid (KMB). No decomposition was observed under these conditions. The mixtures were incubated at 37 °C for 10 min. The incubations were terminated by addition of 1 mL of *o*-phenyldiamine (OPD) solution (12 mM OPD in 3 M HCl).

Incubations and analysis with purified β-lyase were performed by the same procedures as described above, except that incubations were performed in the presence of 0.2 μg/mL purified β-lyase instead of 0.2 mg/mL cytosolic protein.

Derivatization with OPD and HPLC Analysis. Derivatization of incubation mixtures with OPD was performed directly in the incubation vials. After addition of OPD to the incubation mixtures, the caps of the incubation vials were closed and pierced with a needle before placing them in a preheated oven at 60 °C for 60 min. After proteins were precipitated by centrifugation, a 100 μL sample of the supernatant was analyzed on a C18 column (ChromSper C18; 5 mm particles, 100 × 3 mm) which was eluted isocratically at 0.4 mL/min of an eluent consisting of 45% methanol, 1% acetic acid, and 54% water. Detection was accomplished with a Perkin Elmer model 3000 fluorescent spectrometer equipped with a flow cell. Fluorescence was measured at $\lambda_{ex} = 336$ nm and $\lambda_{em} = 420$ nm. Calibration curves were constructed using pyruvate solutions in final concentrations ranging from 5 to 100 μM, incubating for 10 min at 37 °C, derivatizing with OPD, and analyzing the samples with the HPLC system as described above.[25]

Integrated peak areas of the calibration samples were plotted against pyruvic acid concentrations and fitted with linear regression. From these calibration curves, the concentrations in unknown samples were determined. All incubations were conducted in triplicate, each sample was analyzed three times, and ±SE was <10%. Data are presented as mean. Apparent K_m and V_{max} values were calculated by Lineweaver−Burk analysis.

Analysis of Selenol Compounds. For one representative selenocysteine conjugate, *Se*-phenyl-L-selenocysteine, the stoichiometry of formation of selenol and pyruvate was determined. To this end, *Se*-phenyl-L-selenocysteine (1 mM) was incubated in Tris-HCl buffer (pH 8.6) in the presence of rat renal cytosol (final concentration 3 mg/mL) and 0.1 mM KMB in a total volume of 2 mL. After 10 min at 37 °C, the enzymic reaction was terminated by the addition of 1 mL of 3 N HCl. For quantification of the formed metabolites by GC−MS, 50 μL of a solution of 0.2 mg/mL *N*-acetyl-*S*-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine was added as an internal standard. A portion of 0.3 mL was analyzed for pyruvic acid by the method described above. The remainder of the acidified incubation mixture was vortexed for 2 min with 2 mL of ethyl acetate. The ethyl acetate layer was separated and treated with 1 mL of ethereal diazomethane. After 15 min of derivatization, the mixture was heated at 40 °C for a short period in order to evaporate the excess of diazomethane, as demonstrated by the disappearance of the yellow color. To avoid evaporation of potentially volatile products, the remainder (±2 mL) was not further concentrated, and 1 μL was analyzed by GC−MS as described below.

For quantification of phenylselenol, a standard curve ranging from 25 to 200 μM was prepared by spiking 2 mL incubation mixtures without *Se*-phenyl-L-cysteine with known amounts of a freshly prepared solution of phenylselenol. The resulting mixtures were analyzed as described above. A stock solution of 10 mM phenylselenol was prepared by mixing 5 mL of 20 mM diphenyl diselenide in ethanol with 5 mL of 10 mg/mL sodium borohydride in 0.1 N NaOH. The yellow color of the diselenide disappeared instantaneously upon mixing if both solutions were deoxygenated by a nitrogen stream prior to mixing. Analysis of the stock solution by GC−MS revealed that reduction of diphenyl diselenide by this protocol was quantitative.

GC−MS analysis of methylated extracts was carried out on a HP 5890/MSD system equipped with a 25 m CP-Sil 19 capillary column obtained from Chrompack BV (The Netherlands). The operation temperatures were 280 °C (split injector) and 280 °C (ion source, electron impact ionization, electron energy of 70 eV). After starting at a temperature of 44 °C for 2 min, the column temperature was increased at a rate of 20 °C/min to a final temperature of 288 °C. Using these GC−MS conditions, the following retention times and mass spectra were obtained.

Methyl phenyl selenide: retention time, 6.38 min; mass spectrum *m/z* (rel intensity, selenium isotope, assignment) 172 (100, ^{80}Se, $M^{\bullet+}$), 157 (96, ^{80}Se, $M^{\bullet+} - CH_3$), 117 (10), 91 (26), 77 (26), 51 (25).

Diphenyl diselenide: retention time, 12.08 min; mass spectrum *m/z* (rel intensity, selenium isotope, assignment) 314 (24, 2 × ^{80}Se, $M^{\bullet+}$), 234 (13, ^{80}Se, $M^{\bullet+} - {}^{80}$Se), 157 (90, ^{80}Se, $C_6H_6Se^+$), 117 (19), 78 (68), 77 (100), 51 (51).

***N*-Acetyl-*S*-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine methyl ester:** retention time, 10.84 min; mass spectrum, see ref 29.

References

(1) Vadhanavikit, S.; Ip, C.; Ganther, H. E. Metabolites of sodium selenite and methylated selenium compounds administered at cancer chemoprevention levels in the rat. *Xenobiotica* **1993**, *23*, 731−745.

(2) Thompson, H. J.; Wilson, A.; Lu, J.; Singh, M.; Jiang, C.; Upadyaya, P.; Bayoumy, E.; Ip, C. Comparison of the effects of an organic and an inorganic form of selenium on a mammary carcinoma cell line. *Carcinogenesis* **1994**, *15*, 183−186.

(3) Bayoumy, K. E. Effects of organoselenium compounds on induction of mouse forestomach tumors by benzo(a)pyrene. *Cancer Res.* **1985**, *45*, 3631−3635.

(4) Hwang, I. Y.; Elfarra, A. Cysteine S-conjugates may act as kidney selective prodrugs: Formation of 6-mercaptopurine by the renal metabolism of S-(6-purinyl)-L-cysteine. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 448−453.

(5) Elfarra, A.; Hwang, I. Y. Targeting of 6-mercaptopurine to the kidneys. Metabolism and kidney-selectivity of S-(6-purinyl)-L-cysteine analogs in rats. *Drug Metab. Dispos.* **1993**, *21*, 841−845.

(6) Commandeur, J. N. M.; Stijntjes, G. J.; Vermeulen, N. P. E. Enzymes and transport systems involved in the formation and disposition of glutathione S-conjugates. Role in bioactivation and detoxication mechanisms of xenobiotics. *Pharmacol. Rev.* **1995**, *47*, 271−330.

(7) Nelson, J. A.; Pan, B. F.; Swanson, D. A.; Elfarra, A. A. Cysteine conjugate beta-lyase activity in human renal carcinomas. *Cancer Biochem. Biophys.* **1995**, *14*, 257−263.

(8) Baldew, G. S.; Van der Hamer, C. J. A.; Los, G.; Vermeulen, N. P. E.; De Goeij, J.J. M.; McVie, J. G. Selenium-induced protection against cisplatin nephrotoxicity in mice and rats. *Cancer Res.* **1989**, *49*, 3020−3023.

(9) Vermeulen, N. P. E.; Baldew, G. S.; Los, G.; McVie, J. G.; De Goeij, J. J. M. Reduction of cisplatin nephrotoxicity by sodium selenite. Lack of interaction at the pharmacokinetic level of both compounds. *Drug Metab. Dispos.* **1993**, *21*, 30−36.

(10) Ip, C.; Hayes, C.; Budnick, R. M.; Ganther, H. E. Chemical form of selenium, critical metabolites and cancer prevention. *Cancer Res.* **1991**, *51*, 595−609.

(11) Abraham, D. G.; Patel, P. P.; Cooper, A. J. L. Isolation from rat kidney of a cytosolic high molecular weight cysteine-S-conjugate β-lyase with activity towards Leukotriene E_4. *J. Biol.Chem.* **1995**, *270*, 180−188.

(12) Stevens, J. L.; Robbins, J. D.; Byrd, R. A. A purified cysteine conjugate β-lyase from rat kidney cytosol. *J. Biol.Chem.* **1986**, *261*, 15529−15537.

(13) Tanaka, H.; Soda, K. Selenocysteine. *Methods Enzymol.* **1987**, *143*, 240−243.

(14) Stijntjes, G. J. Renal cysteine conjugate β-lyase. Ph.D. Thesis, Vrije Universiteit, Amsterdam, The Netherlands, 1993.

(15) Wolfgang, G. H. I.; Gandolfi, A. J.; Stevens, J. L.; Brendel, K. *In vitro* and *in vivo* nephrotoxicity of the L- and D-isomers of S-(1,2-dichlorovinyl)-cysteine. *Toxicology* **1989**, *58*, 33−42.

(16) Walsh, C. T.; Schonbrunn, A.; Abeles, R. H. Studies on the mechanism of action of D-amino acid oxidase. *J. Biol. Chem.* **1971**, *246*, 6855−6866.

(17) Koide, T.; Itoh, H.; Otaka, A.; Yasui, H.; Kuroda, M.; Esaki, N.; Soda, K.; Fujii, N. Synthetic study of selenocystine-containing peptides. *Chem. Pharm. Bull.* **1993**, *41*, 502−506.

(18) Nakayasu, K.; Tanaka, A. Cysteine derivates. *Jpn. Kokai Tokkyo Koho JP* **1985**, *60*, 258, 161 (*85*, 258, 161); *Chem. Abstr.* **1986**, *104*, P186853z.

(19) Foster, S. J.; Ganther, H. E. Synthesis of [^{75}Se]trimethylselenonium iodide from [^{75}Se] selenocystine. *Anal. Biochem.* **1984**, *137*, 205−209.

(20) Esaki, N.; Tanaka, H.; Miles, E. W.; Soda, K. Enzymatic synthesis of Se-substituted L-selenocysteine with tryptophan synthase. *FEBS Lett.* **1983**, *161*, 207−209.

(21) Esaki, N.; Nakamura, T.; Tanaka, H.; Suzuki, T.; Morino, Y.; Soda, K. Enzymatic synthesis of selenocysteine in liver. *Biochemistry* **1981**, *20*, 4492−4496.

(22) According to ^{1}H- and ^{13}C-NMR, no other organic contaminants were present. The elemental analyses gave the correct ratio between C, H, and N. Therefore, it was concluded that contaminants were inorganic salts which cocrystallized under the present conditions.

(23) Theodoropoulos, D.; Schwartz, I. L.; Walter, R. Synthesis of selenium-containing peptides. *Biochemistry* **1967**, *6*, 3927−3932.

(24) Sayuda, K.; Tanaka, H. Selenoaminoacids. *Jpn. Kokai Tokkyo Koho* **1979**, *79*, 52,033 (Cl. C12D13/06); *Chem. Abstr.* **1979**, *91*, 122166h.
(25) Stijntjes, G. J.; Te Koppele, J. M.; Vermeulen, N. P. E. High-performance liquid chromatography fluorescence assay of pyruvic acid to determine cysteine conjugate β-lyase activity: Application to S-1,2-dichlorovinyl-L-cysteine and S-2-benzothiazoyl-L-cysteine. *Anal. Biochem.* **1992**, *206*, 334−343.
(26) Yamauchi, A.; Stijntjes, G. J.; Commandeur, J. N. M.; Vermeulen, N. P. E. Purification of glutamine transaminase K/cysteine conjugate β-lyase from rat renal cytosol based on hydrophobic interaction HPLC and gel permeation FPLC. *Protein Expr. Purif.* **1993**, *44*, 552−562.
(27) Esaki, N.; Nakamura, T.; Tanaka, H.; Suzuki, T.; Morino, Y.; Soda, K. Enzymic synthesis of selenocysteine in the liver. *Biochemistry* **1981**, *20*, 4492−4496.
(28) Tamura,T.; Oikawa, T.; Ohtaka, A.; Fujii, N.; Esaki, N.; Soda, K. Synthesis and characterization of the selenium analog of glutathione disulfide. *Anal. Biochem.* **1993**, 151−154.
(29) Commandeur, J. N. M.; Brakenhoff, J. P. G.; de Kanter, F. J. J.; Vermeulen, N.P. E. Nephrotoxicity of mercapturic acids of three structurally related 2,2-difluoroethylenes in the rat. *Biochem. Pharmacol.* **1988**, *37*, 4495−4504.
(30) Foster, D. G. Selenophenol. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. No. 3, pp 771−773.

JM950750X