Synthesis of L-selenocystine, L-[^{77}Se]selenocystine and L-tellurocystine

Emily M. Stocking,[a] Jessie N. Schwarz,[a] Hans Senn,[b] Michael Salzmann[b] and Louis A. Silks*[,a]

[a] *Los Alamos National Laboratory, NIH National Stable Isotopes Resource, CST-4; MS C-345, Los Alamos, New Mexico 87545, USA*
[b] *Pharma Research New Technologies, Hoffmann-La Roche AG, CH-4002 Basel, Switzerland*

Synthetic routes for the synthesis of stable isotope labelled amino acids which contain either a selenium or a tellurium atom have been explored. L-Selenocystine, L-[^{77}Se]selenocystine and L-tellurocystine have been constructed in four steps from commercially available methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropionate. The sequence of reactions has been successfully scaled up giving significant quantities of the chalcogen based amino acids in fair to good overall yield.

Techniques for the biosynthetic incorporation of selenium and tellurium containing amino acids into biomacromolecules have been exploited to produce both heavy-atom derivatives and nuclear magnetic resonance probes. These chalcogen-based derivatives have played a significant role in the elucidation of both local and global structures of a variety of biomacromolecules.[1] Although the replacement of methionine by selenomethionine (Se-Met) in a protein was reported as early as 1957, it was not until 1990 that a selenium containing amino acid was successfully used as a 'heavy-atom' protein derivative by Hendrickson.[2] More recently, Odom and co-workers reported the incorporation of telluromethionine (Te-Met) into a protein (dihydrofolate reductase) to solve the crystallographic phase problem.[3] Odom's method has recently been used in the successful bioincorporation of Te-Met into the calcium binding protein annexin V.[4] Prompted by the increased demand for these compounds, we have initiated a programme to synthesize optically active and isotopically enriched selenium and tellurium containing amino acids. Thus far, we have focused our synthetic efforts on L-Se-Met, L-Te-Met, L-Te-tryptophan, L-Se-cystine and L-Te-cystine.

Chalcogen analogue replacement of cystine by L-Se-cystine residues in proteins can provide structural, mechanistic and functional information. Because of the pioneering work of Müller *et al.*, it is now a straightforward process to effect such a replacement.[5] Uniform replacement of cystine residues by L-Se-cystine may allow assignment of resonances as well as conformational analysis of unknown disulfide connectivities in protein structure by ^{1}H–^{77}Se correlated NMR experiments. Disulfide connectivities are important determinants in the folding pathways of proteins. Replacement of cystine by L-Se-cystine is likely to allow the identification of critical steps in the folding event. Moreover, replacement of active site cystine residues can give functional information based upon the differences in redox properties of the selenol and thiol functional groups.

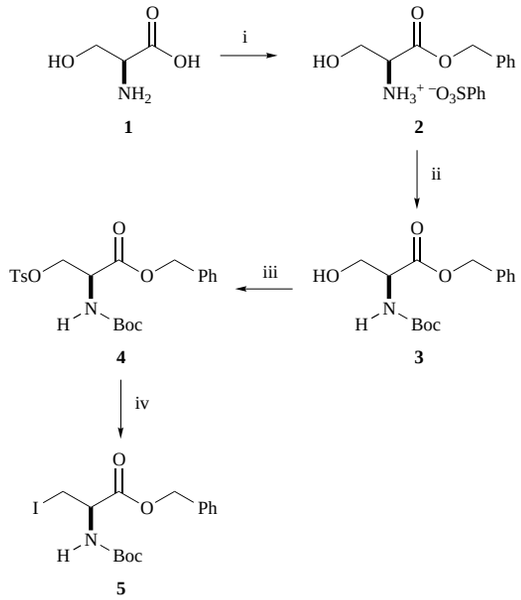
Since the ^{125}Te nucleus is NMR active (spin $\frac{1}{2}$), L-^{125}Te-cystine can also be used in high field NMR studies designed to elucidate structural and mechanistic aspects of proteins. L-Te-cystine has the added advantage of possessing a significant amount of electron density, which could provide a rational approach to heavy atom derivatization of biomolecules which contain cystine. The ditelluride bridge, which is more space-filling than any hitherto known disulfide or diselenide bridge, has the ability to function as a new biostructural engineering tool.

Unfortunately, the synthesis of both Se- and Te-cysteine is complicated by the fact that they are readily oxidized in air to form either Se- or Te-cystine. To circumvent this problem, we have developed a short synthesis of L-Se-cystine, which can be reduced with sodium borohydride to form the optically active Se-cysteine. Previously reported syntheses of Se-cystine have been inefficient with respect to selenium incorporation.[6] Racemic Se-cystine has been constructed using chloroalanine and disodium diselenide in a 27% yield based on selenium.[7] Because of the high cost of the enriched ^{77}Se (US$32 mg^{-1}) and the quantity of material needed for bioincorporation (~500 mg), this method does not represent an efficient route to the target compounds. A subsequent report of the preparation of L-Se-cystine describes an improved overall yield of 55%.[8] This synthesis requires an excess of hydrogen selenide for the preparation of one of the starting materials (sodium hydrogen selenide) and, therefore, is impractical for labelling purposes. We report an improved two-step synthesis of L-Se-cystine, L-[^{77}Se]-Se-cystine and L-Te-cystine from elemental selenium or tellurium. The fact that near stoichiometric amounts of Se or Te are needed for the synthesis of the target compounds, as well as the reduced number of synthetic manipulations that are necessary, underscore the efficiency of our method. Moreover, to the best of our knowledge, this report represents the first chemical synthesis of L-Te-cystine.

Results and discussion

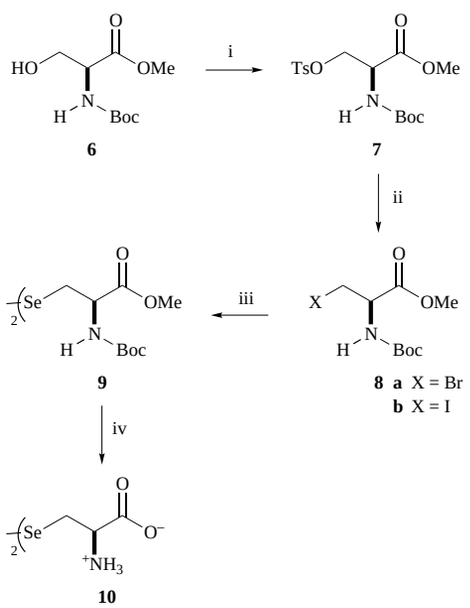
We have been interested in taking advantage of the versatility of suitably protected β-haloalanines for the synthesis of a wide variety of chiral amino acids. Pansare and Vederas pioneered the use of a serine derived β-lactone as an alanine β-cation synthon for the synthesis of a wide variety of substituted alanines.[9] Jackson *et al.*, have recently extended this work by promoting their use of zinc-based alanine β-anion synthons for the synthesis of a series of substituted alanines.[10] These 'synthons' are particularly useful because the transformation of a chiral compound to its target structure is often a more direct process than an asymmetric synthesis which employs chiral auxiliaries.[11] Because β-haloalanines have proven to be useful as β-cation, anion and radical synthons, we propose to exploit these chameleonic chemical properties in the synthesis of our target compounds.

The protected β-haloalanines can be conveniently constructed in optically active form as illustrated in Scheme 1. We have repeatedly reproduced this four step synthesis of the β-haloalanines, each time achieving similar yields to those reported. The main difficulty in carrying out this process is the instability of the iodide, which needed to be freshly crystallized before use. However, during the course of this



Scheme 1 *Reagents and conditions:* i, $PhCH_2OH$, $PhSO_3H$, PhH (44%); ii, di-*tert*-butyl pyrocarbonate (95%); iii, TsCl, C_5H_5N (85%); iv, NaI, acetone (80%)

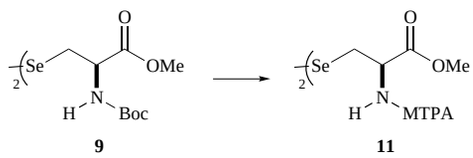
work methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropionate **6** became commercially available and we were pleased with the prospect of a four step synthesis of both L-Te-cystine and L-Se-cystine from readily available starting materials. By simply repeating the toluene-*p*-sulfonylation and subsequent substitution steps, gram quantities of the β-haloalanines were obtained (Scheme 2). As this work proceeded



Scheme 2 *Reagents and conditions:* i, TsCl (1.5 equiv.), C_5H_5N (4.0 equiv.), CH_2Cl_2 (71%); ii, NaI or NaBr acetone (85%); iii, Li_2Se_2 (1.0 equiv.), THF (85%); iv, 20% CF_3CO_2H–CH_2Cl_2 (v/v), then 6 M HCl, 75 °C, 12 h

we discovered that the iodides could be purified by careful sublimation. All attempts at purification by silica gel chromatography failed. Prolonged storage usually resulted in elimination of the iodide to give the protected anhydroalanine. Not surprisingly, the bromide **8a** was stable to silica gel chromatography and routine purifications could be performed.

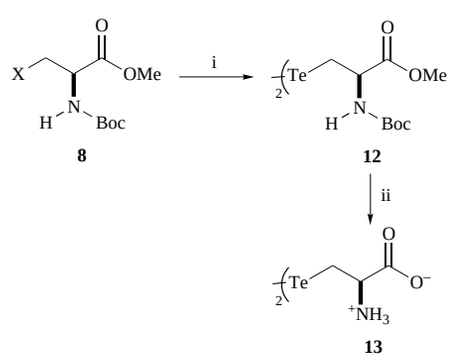
Treatment of selenium with 1 mol equiv. of Super-Hydride generates the dilithio diselenide. This was then added to a solution of the iodide **8b** at −78 °C. The protected L-Se-cystine was isolated in 89% yield. Using **8a** resulted in a significant decrease in yield (65%). Repeating the above protocol using **8b**, with sublimed 92% ^{77}Se, gave an 87% yield of the labelled amino acid **9**. The optical purity of **9** was evaluated by the following methods. *In situ* deprotection of the Boc protecting group with trifluoroacetic acid gave the amino ester, which was then coupled to Mosher's acid chloride, affording adduct **11**.



Scheme 3 *Reagents and conditions:* 10% CF_3CO_2H–CH_2Cl_2 (v/v) then *R*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, NEt_3 (74%)

Adduct analysis by ^1H and ^{77}Se NMR spectroscopy indicated that the diastereomeric excess was greater than 98%.[12] In addition, analysis *via* gas chromatography with a chiral stationary phase has been performed on **10**. Using this method, the sample was shown to be 99% optically pure (L-Se-cystine). The chemical purity of L-[^{77}Se]-Se-cystine was evaluated using supercritical fluid chromatography (SCL). The L-Se-cystine was first derivatized by dissolving it in acetonitrile, then adding MTBSTFA [*N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide]. We have compared this sample with the commercially available DL-Se-cystine from Sigma Chemical Co. The chromatogram showed very symmetrical peaks. The purity of L-[^{77}Se]-Se-cystine was indicated to be 78.0% and the commercial sample was 90.5% pure. In our L-[^{77}Se]-Se-cystine, there was one major impurity which amounted to approximately 15%. However, since the analysis was performed on the crude material, the sample ultimately contained a near quantitative amount of L-[^{77}Se]-Se-cystine (99%). The isotopic enrichment of L-[^{77}Se]-Se-cystine was determined by ion-spray mass spectrometry. We also examined the DL-Se-cystine from Sigma and, as expected, this was shown to contain the natural abundance ^{77}Se. The isotopic content of our L-[^{77}Se]-Se-cystine was determined to be 91 to 93% ^{77}Se.

The use of a similar protocol for the construction of L-Te-cystine (Scheme 4) gave lower yields of the substitution prod-



Scheme 4 *Reagents and conditions:* i, Li_2Te_2 (1.1 equiv.), THF (56%); ii, 10% CF_3CO_2H–CH_2Cl_2 (v/v) then 20% CF_3CO_2H–H_2O (v/v), 50 °C, 12 h

uct. Erratic yields of **12** were obtained using **8b**, while the use of the **8a** gave somewhat less erratic yields of **12**. The most difficult step that we have attempted to optimize is the cleavage step. It was eventually found that more consistent yields could be obtained if the Boc group was cleaved with trifluoroacetic acid (TFA) instead of 6 M HCl. The methyl ester could subsequently be hydrolysed using aqueous TFA at 45–50 °C. Careful removal of the volatiles (no heating) gave an intense yellow oil. Neutralization with argonated 1.0 M NaOH with chilling resulting in a yellow solid **13**. Frequent filtrations through Celite were some-

Table 1 ^{1}H,[a] ^{13}C[a] and ^{77}Se[b] chemical shifts of L-cystine, L-selenocystine and L-tellurocystine in 1 M DCl at 298 K

	Chemical shift (ppm)						
	δ_H			δ_C			δ_{Se}
Compound	$^{1}H^{\alpha}$	$^{1}H^{\beta1}$[e]	$^{1}H^{\beta2}$[f]	$^{13}C^{\alpha}$	$^{13}C^{\beta}$	^{13}CO	^{77}Se
L-Cystine	4.38	3.32	3.17	51.5	36.1	170.2	—
L-Selenocystine[c]	4.34	3.48	3.34	53.0	26.6	170.2	294.8
L-^{77}[Se]Selenocystine[d]	4.34[h]	3.45[h]	3.32[h]	52.8	26.5[g]	170.1	294.3
L-Tellurocystine	4.20	3.63	3.44	47.5	0.70	170.1	—

[a] Relative to TMS. [b] Relative to $^{77}Se(CH_3)_2$. [c] ^{77}Se at natural abundance of 7%. [d] 92% enriched selenocystine. [e] Low field. [f] High field methylene proton. [g] Middle of multiplet, which arises due to strongly coupled spin system. [h] Determined from the [^{77}Se]-decoupled spectrum.

Table 2 $^{3}J(^{1}H^{\alpha},^{1}H^{\beta1,\beta2})$ and $^{2}J(^{1}H^{\beta1},^{1}H^{\beta2})$ coupling constants of L-cystine, L-selenocystine and L-tellurocystine in 1 M DCl at 298 K

	Coupling constant (J/Hz)[a]		
Compound	$^{3}J(^{1}H^{\alpha},^{1}H^{\beta1})$	$^{3}J(^{1}H^{\alpha},^{1}H^{\beta2})$	$^{2}J(^{1}H^{\beta1},^{1}H^{\beta2})$
L-Cystine	4.36	7.79	15.23
L-Selenocystine[b]	4.64	7.74	14.12
L-[^{77}Se]Selenocystine[c]	4.60[d]	7.70[d]	14.20[d]
L-Tellurocystine	6.47	6.78	13.10

[a] $^{1}H^{\beta1}$ is the low field, $^{1}H^{\beta2}$ the high field methylene proton. [b] ^{77}Se at natural abundance of 7%. [c] 92% enriched selenocystine. [d] Determined from the [^{77}Se]-decoupled spectrum.

times necessary to remove a grey or black precipitate which was assumed to be elemental tellurium. Alternatively, the residue could be quickly brought to pH ~11, filtered through Celite and acidified (pH ~4–5) with citric acid. The yellow solids were then collected, giving yields ranging from 40–45% (not optimized). The optical purity of **13**, as determined by reversed phase HPLC, was shown to be greater than 99%.

A summary of observed chemical shifts, as well as proton and carbon resonance assignments for the chalcogen containing series of L-cystine derivatives, is shown in Table 1. As expected, the α-protons resonate downfield with respect to the β-protons. An increased shielding effect is observed with the α-protons in the series of derivatives that begins with S and ends with Te. This shielding effect is reversed when one observes the β-protons in this same series of compounds. The β-carbon resonances also display a heavy atom shielding effect from S to Te. Table 2 shows the observed proton coupling constants for this series of amino acids.

Exploiting the versatile alanine β-cation synthons **8a** and **8b** has proven to be essential to the success of the improved syntheses of ^{77}Se labelled L-Se-cystine and L-Te-cystine reported herein. Moreover because L-serine can be produced biosynthetically in virtually any isotopic form (^{2}H, ^{13}C, ^{15}N) from labelled glycine, this work can be extended to produce β-cation synthons yielding any isotopomer of L-Se-cystine and L-Te-cystine. The potential to successfully synthesize such a large variety of isotopically labelled compounds should increase the number of biomacromolecules that can be investigated by spectroscopic and crystallographic means.

Experimental

General

The ^{1}H, ^{13}C and ^{77}Se NMR spectra were recorded as $CDCl_3$ solutions on Bruker AM-300 or AM-500 spectrometers unless otherwise noted. ^{1}H chemical shifts are expressed in parts per million downfield relative to tetramethylsilane at 0.0 ppm; ^{13}C chemical shifts are referenced with respect to internal $CDCl_3$ (δ = 77.0 ppm); ^{77}Se chemical shifts are expressed in parts per million downfield relative to a 60% (v/v) solution of $(CH_3)_2Se$ in $CDCl_3$; ^{13} ^{125}Te chemical shifts are reported as values in parts per million relative to a 1.5 M solution of telluric acid which was referenced against neat dimethyl telluride. Positive chemical shifts denote resonances deshielded with respect to the reference. J Values are given in Hz. Accurate mass spectra were measured on a VG 70SQ GC–MS spectrometer. Microanalyses were performed by CST-4 staff on a Perkin-Elmer Series II CHNS/O Analyser #2400. Thin-layer chromatography was carried out on glass plates (silica gel 60 Å 250 μm thickness). Liquid chromatography separations were carried out on silica gel.[14] The columns were hand packed with silica gel 60 (230–400 mesh, Merck). Pressures used were usually between 5 and 8 psi (1 psi ≈ 6.894×10^3 Pa). Fractions were monitored by thin layer chromatography (TLC).

Elemental selenium (200 mesh), tellurium (200 mesh) and methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropionate were obtained from Aldrich Chemical Company and used without purification. Dichloromethane was distilled over calcium hydride. Tetrahydrofuran was distilled over potassium benzophenone ketal prior to use. Ether refers to diethyl ether.

The enantiomeric purity of the unprotected amino acids (except Te-cystine) was determined by gas chromatography (Hewlett-Packard 5890-0A GC) using a fused silica capillary column (25 meter) with a chiral stationary phase (Chirasil-Val MN 30504-12). The amino acids were derivatized to the *n*-propyl ester and the amino group was acetylated. Calibration samples of the D-, L- and DL-amino acids were prepared from commercially available amino acids. Enantiomeric excess (ee) was calculated from the integrated areas of the peaks of the D- and L-isomers. The GC was run using H_2 and a temperature gradient of 140 to 200 °C with increasing temperature of 1 °C min^{-1}. In addition, the enantiomeric purity of methyl ester Se-cystine was also determined using the Mosher's acid adduct. Using this method one resonance was observed in the ^{77}Se spectrum and there was one methoxy resonance observed in the ^{1}H spectrum. The optical purity of the Te-cystine could not be successfully determined using the same procedure as for Se-cystine and cystine. However, a reversed-phase HPLC procedure led to the separation of DL-cystine (and DL-Se-cystine) into two peaks, one for D,D- and L,L-cystine and the other for DL/LD-cystine. Modification of Te-cystine with diethyl ethoxymethylenemalonate worked well and an HPLC trace showed only one peak which indicated the ee was greater than 99%. The chemical purities were evaluated by using SCL (Instrument Lee Scientific Lee-SFC-6) which employs a mobile phase of CO_2 and a biphenyl-30 10 mm × 50 μm column and an oven temperature of 100 °C.

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(*p*-tolylsulfonyloxy)propionate 7

In a single-necked 500 cm^3, round-bottom flask fitted with a septum and containing a magnetic stirring bar was placed methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-hydroxypropionate (10.00 g, 45.61 mmol), 250 cm^3 of dichloromethane, pyridine (14.75 cm^3, 182.44 mmol) and toluene-*p*-sulfonyl chloride (13.04 g, 68.42 mmol). The mixture was stirred at ambient temperature for 48 h (or until complete by TLC analysis; 30% ethyl acetate–hexanes, v/v). Removal of the vol-

atiles *in vacuo* gave rise to a white solid. Addition of 150 cm^3 of ether was followed by stirring until a homogeneous suspension was obtained. Filtration, followed by concentration, afforded the crude **7**. The solids from the filtration were dissolved in dichloromethane and analysed by TLC for **7**. Crude **7** was then subjected to azeotropic removal of pyridine with heptane. Purification of **7** by flash chromatography afforded a 71% yield (12.11 g, 32.43 mmol), mp 74–75 °C; δ_H 1.44 (9H, s), 2.43 (3H, s), 3.68 (3H, s), 4.26 (1H, dd, *J* 10, 4), 4.37 (1H, dd, *J* 10, 3), 4.5 (1H, m), 5.34 (1H, d, *J* 8), 7.34 (2H, d, *J* 9), 7.75 (2H, d, *J* 9); δ_C 21.4, 28.0, 52.6, 52.8, 69.3, 80.1, 127.7, 129.7, 132.2, 145.0, 168.8, 182.8 (Found: C, 51.79; H, 6.23; N, 3.82. $C_{16}H_{23}NO_7S$ requires C, 51.46; H, 6.20; N, 3.75%).

Methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-bromopropionate 8a

In a single-necked 500 cm^3, round-bottom flask fitted with a septum and containing a magnetic stirring bar was placed **7** (5.00 g, 13.4 mmol), 250 cm^3 of HPLC grade acetone and 4.0 equiv. of NaBr (5.51 g, 53.55 mmol). The mixture was refluxed, under N_2, for 12 h (or until complete by TLC analysis; 30% ethyl acetate–hexanes, v/v). The reaction mixture was cooled to ambient temperature. Filtration, followed by removal of the volatiles *in vacuo*, gave rise to a yellow–white solid. Ethyl acetate (75 cm^3) was added and the solution was washed three times with saturated aqueous $Na_2S_2O_3$. The ethyl acetate solution was dried over Na_2SO_4 for 0.5 h with stirring. The resulting solution was then filtered and reduced to give the crude product **8**. Purification of **8** by flash chromatography afforded an 85% yield (3.30 g, 11.7 mmol), mp 55–56 °C; δ_H 1.29 (9H, s), 3.54 (1H, dd, *J* 10, 3), 3.63 (3H, s), 3.65 (1H, dd, *J* 10, 3), 4.57 (1H, m), 5.3 (1H, br d, *J* 8); δ_C 28.3, 34.0, 52.9, 53.9, 80.5, 154.9, 169.6.

Methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-iodopropionate 8b

The same procedure described for the preparation of **8a** was employed to prepare **8b** except that NaI was used. δ_H 1.45 (9H, s), 3.56 (2H, m), 3.79 (3H, s), 4.50 (1H, m), 5.4 (1H, br d, *J* 8); δ_C 7.53, 28.1, 52.8, 53.6, 80.2, 154.7, 169.7.

Dimethyl bis(*N*-*tert*-butoxycarbonyl)-L-selenocystine 9

In a 25 cm^3 three-necked round-bottom flask, under argon, previously flamed dried and purged with argon was placed elemental selenium (0.160 g, 2.03 mmol). To this was added freshly distilled THF (5 cm^3) followed by Super-Hydride (2.03 cm^3, 2.03 mmol). The suspension was heated to reflux and stirred for 0.5 h. In a separate 25 cm^3 three-necked round-bottom flask, previously flame dried and purged with argon, was placed **8b** (0.67 g, 2.03 mmol) under argon. To this was added 5 cm^3 of THF and the resulting solution was cooled to −78 °C. The diselenide solution was then transferred to the THF solution of **8b** *via* a cannula. The mixture was stirred at −78 °C for 20 min and then allowed to warm to ambient temperature (0.5 h). The solution was then stirred at ambient temperature (1 h) to ensure that the reaction was complete. The solution was then filtered through a pad of silica gel and the pad was washed with ~20% ethyl acetate–dichloromethane (v/v) to ensure the diselenide was removed. Purification of **9** by flash chromatography (50% ether–hexanes, v/v) afforded an 89% yield (0.51 g, 0.91 mmol) of a pale yellow solid, mp 92 °C; δ_H 1.38 (9H, s), 3.32 (1H, m), 3.69 (3H, s), 4.52 (1H, m), 5.39 (1H, br d, *J* 8); δ_C 28.2, 32.4, 52.4, 53.6, 80.0, 154.8, 171.1; δ_{Se} 306 (Calc. for $C_{18}H_{32}N_2O_8Se_2$: *M*, 564.0489. Found: M, 564.0495).

Dimethyl bis(*N*-*tert*-butoxycarbonyl)-L-[^{77}Se]selenocystine

The same procedure described for the preparation of **9** was used, however, the ^{77}Se (0.170 g, 2.21 mmol assuming 100% ^{77}Se) was sublimed prior to use. Yield 87% (0.54 g, 0.96 mmol); δ_H 1.38 (9H, s), 3.32 (1H, m), 3.69 (3H, s), 4.52 (1H, m), 5.39 (1H, br d, *J* 8); δ_C 28.0, 32.1 (dd, $^1J_{C-Se}$ 92, 17), 53.3, 53.5, 79.8, 154.7, 171.0.

L-Selenocystine 10

In a 100 cm^3 three-necked round-bottom flask, under argon, was placed **9** (0.440 g, 0.780 mmol). To this was added dichloromethane (50 cm^3) and the resulting solution was cooled to 0 °C. To this solution was slowly added 50 cm^3 of a mixture of dichloromethane–trifluoroacetic acid, 4:1, v/v and the mixture was stirred at ambient temperature for 3 h. The volatiles were removed *in vacuo* and the residue was evacuated till the pressure remained constant at 0.1 mmHg. The residue was then taken up in 6 M HCl, placed under argon and the temperature was brought to 75 °C for 12 h. Removal of the volatiles gave a 92% yield of the amino acid (0.239 g, 0.716 mmol). This material was determined by supercritical fluid chromatography (SCL) to be 87% pure. The product was crystallized from an aqueous solution by the addition of citric acid (pH 4–5) at 4 °C, mp 218 °C (decomp.); *m*/*z* 334.8 (M^+, calc. for $C_6H_{12}N_2O_4Se_2$: M, 335) (Found: C, 21.83; H, 3.77; N, 8.56. $C_6H_{12}N_2O_4Se_2$ requires C, 21.57; H, 3.62; N, 8.38%).

L-[^{77}Se]Selenocystine

The same procedure as outlined above was employed (0.540 g, 0.964 mmol assuming 100% ^{77}Se). Crude yield 0.406 g. This material was determined by SCL to contain 0.317 g of L-[^{77}Se]selenocystine; *m*/*z* 329.0 (M^+, calc. for $C_6H_{12}N_2O_4{}^{77}Se_2$: M, 329).

Dimethyl bis{*N*-[α-methoxy-α-(trifluoromethyl)phenylacetyl]}-L-selenocystine 11

In a 100 cm^3 three-necked round-bottom flask, under argon, was placed **9** (0.250 g, 0.443 mmol). To this was added dichloromethane (50 cm^3) and the resulting solution was cooled to 0 °C. To this solution was slowly added 50 cm^3 of a mixture of dichloromethane–trifluoroacetic acid, 4:1, v/v and the resulting mixture was stirred at ambient temperature for 3 h. The volatiles were removed *in vacuo* and the residue was brought to a constant pressure of 0.1 mmHg. The residue was then taken up in 20 cm^3 of dichloromethane and chilled to 0 °C. (*R*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.203 g, 0.802 mmol) was added, followed by triethylamine (0.18 g, 1.8 mmol). The reaction was stirred until the starting amine was consumed as indicated by TLC. Purification by flash chromatography gave the MTPA amide in 74% yield (0.260 g, 0.327 mmol). ^{1}H NMR spectroscopic examination of the α-methoxy region indicated only one resonance (de >98%). δ_H 3.17–3.18 (1H, m), 3.26–3.27 (1H, m), 3.42 (3 H, s), 3.68 (1H, q, *J* 3), 4.7–4.8 (1H, m), 7.30–7.49 (6H, m); δ_{Se} 308 (Calc. for $C_{28}H_{30}N_2O_8F_6Se_2$: *M*, 796.0237. Found: M, 796.0231).

Dimethyl bis(*N*-*tert*-butoxycarbonyl)-L-tellurocystine 12

In a 100 cm^3 three-necked round-bottom flask, under argon, previously flamed dried and purged with argon was placed elemental tellurium (1.1 equiv.; 0.497 g, 3.89 mmol). To this was added freshly distilled THF (25 cm^3) followed by Super-Hydride (1.1 equiv.; 3.89 cm^3, 3.89 mmol).[15] The suspension was stirred for 0.5 h during which time the solution became dark brown. In a separate 100 cm^3 three-necked round-bottom flask, under argon, previously flamed dried and purged with argon was placed **8b** (1.00 g, 3.54 mmol). To this was added 20 cm^3 of THF and the resulting solution was cooled to −78 °C. The ditelluride solution was then transferred to the THF solution of **8b** *via* a cannula. The mixture was stirred at −78 °C for 20 min and then allowed to warm to ambient temperature (0.5 h). The solution was then stirred at ambient temperature to ensure that the reaction was complete (1 h). The solution was filtered through a pad of silica gel and the pad was washed with dichloromethane–methanol to ensure complete removal of the

ditelluride. Purification of **12** by flash chromatography (30% ethyl acetate–hexanes, v/v) afforded a 56% yield (0.65 g, 0.99 mmol) of a red oil; δ_H 1.39 (9H, s), 3.49 (1H, m), 3.61 (1H, m), 3.70 (3H, s), 4.39 (1H, m), 5.36 (1H, br s); δ_C 7.19, 28.2, 52.5, 55.0, 80.1, 154.9, 171.4; δ_{Te} 132.2 (Calc. for $C_{18}H_{32}N_2O_8{}^{128}Te_2$: *M*, 660.0248. Found: M, 660.0248).

L-Tellurocystine 13

In a 500 cm^3 round-bottom flask was placed **12** (0.510 g, 0.773 mmol). To this was added dichloromethane (50 cm^3) and the resulting solution was cooled to 0 °C. To this solution was slowly added 50 cm^3 of a mixture of dichloromethane–trifluoroacetic acid, 4:1, v/v and the resulting solution was stirred at ambient temperature for 1 h. The solution was then reduced in volume *in vacuo* and 50 cm^3 of H_2O was added. The solution was chilled to 0 °C, then filtered through a pad of Celite. To the resulting yellow–orange solution was added 50 cm^3 of H_2O trifluoroacetic acid, 4:1, v/v. The solution was then placed under argon and warmed slowly to 50 °C. After 12 h the solution was reduced *in vacuo* to give an orange oil. The solution was then taken up in water and treated with 1 M NaOH until basic. During this process a black precipitate formed, which was removed by filtering through a pad of Celite. The resulting solution was treated with citric acid until the pH was ~4.0. The solution was again filtered through Celite and concentrated *in vacuo* to give an orange solid. The solid was then taken up in a minimal amount of H_2O and chilled to 4 °C. An orange solid formed, which was filtered and then dried, giving a 42% yield of L-Te-cystine (0.140 g, 0.312 mmol). The optical purity was determined on the diethyl ethoxymethylenemalonate adduct. Using HPLC fitted with a C18 reversed phase column there was one peak observed with a retention time (t_r) of 39.1 min. This was compared to D-cystine (35.8 min), L-cystine (36.3 min), D-Se-cystine (36.7 min) and L-Se-cystine (37.1 min). Mp 160 °C (decomp.); δ_{Te} 72.5 (NaOD), 159 (DCl, D_2O);[16] MS determined with ion spray with negatively charged ions (Calc. for $C_6H_{12}N_2O_4{}^{128}Te_2$: *M*, 432; found: M − H^-, 431) (Found: C, 16.13; H, 2.65; N, 5.91. $C_6H_{12}N_2O_4Te_2 \cdot H_2O$ requires C, 16.04; H, 2.69; N, 6.23%).

Acknowledgements

We gratefully acknowledge financial support from the National Stable Isotopes Resource, NIH Division of Research Resources (RR 02231) and a LDRD grant XAB2 from Los Alamos National Laboratory. And we thank Dr W. Walther and Dr W. Vetter for their help in the characterization of all new compounds.

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Paper 6/00180G
Received 4*th January* 1997
Accepted 4*th April* 1997