Access to Any Site-Directed Isotopomer of Methionine, Selenomethionine, Cysteine, and Selenocysteine − Use of Simple, Efficient Modular Synthetic Reaction Schemes for Isotope Incorporation

Arjan H. G. Siebum,[a] Wei Sein Woo,[a] Jan Raap,[a] and Johan Lugtenburg*[a]

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Simple modular reaction schemes that allow access to any isotopomer of protected serine and homoserine have been worked out. These systems could be simply converted into cysteine, selenocysteine, homocysteine, homoselenocysteine, the essential amino acid methionine, and selenomethionine by Mitsunobu chemistry. These sulfur- and selenium-containing amino acids fulfil many essential roles in the living organism. In addition, homoserine could be converted in a few steps into optically active L-vinylglycine. As well as the stable isotopes ^{13}C, ^{15}N, ^{17}O, and ^{18}O, the radioactive isotopes of sulfur, selenium and carbon can also be easily introduced in a site-directed fashion. In view of the wide scope of the Mitsunobu reaction, we feel that many more important systems with the carbon skeleton of serine and homoserine should be preparable through this basic chemistry in any site-directed isotopically labeled form.

Introduction

Methionine (Met), cysteine (Cys), and selenocysteine (Sec) are the three proteinogenic amino acids possessing high-mass chalcogen atoms (S and Se) in their side chains (Figure 1). These amino acid residues are responsible for important protein functions that cannot be achieved by other amino acid residues. The sulfur atoms in methionine and cysteine, for example, coordinate to metal ions involved in many redox and catalytic processes of metaloproteins.[1,2]

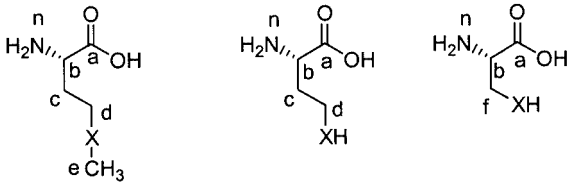


Figure 1. The structures of L-methionine (**1**), L-selenomethionine (**2**), L-homocysteine (**3**), L- homoselenocysteine (**4**), L-cysteine (**5**), and L-selenocysteine (**6**); the letters a, b, etc. indicate the source of the ^{13}C enriched starting materials: the "a" and "c" carbon atoms are derived from the carboxylic group of acetic acid and "b" and "d" from the methyl group of acetic acid, "e" originates from methyl iodide, and "f" from paraformaldehyde, while the source of ^{15}N, indicated by "n", is $^{15}NH_3$

[a] Leiden Institute of Chemistry, Gorleaus Laboratories, Leiden University,
P. O. Box 9502, 2300 RA Leiden, The Netherlands
Fax: (internat.) + 31-71-5274488
E-mail: Lugtenbu@chem.leidenuniv.nl

Cysteines are involved in the disulfide bridges that constitute an important element in protein structure and peptide hormones such as insulin. Cys also coordinates to metal ions in metaloproteins, forms covalent bonds with the haem group in the active site of the protein part of cytochrome C, and is the basis of the active site of hydrolytic enzymes such as papaine.[3]

A special case is selenocysteine, the 21st proteinogenic amino acid, which is translationally incorporated into proteins by a special mechanism.[4] Most human selenoproteins have homologues in which Sec has been replaced by Cys. These sulfur proteins, though, are poor catalysts in comparison with selenoproteins.[5] In the meantime, 18 different human selenoproteins have been described. Recently, it has been established that the human selenoproteonome consists of 25 selenoproteins.[6] Interestingly, the genome sequences of *H. influenzae* and *M. jannaschii* indicate that their genomes code for Sec-containing enzymes involved in the biosyntheses of selenocysteine.[7,8] Dietary selenium is an essential element in human nutrition, playing important roles in cancer prevention, immunology, aging, male reproduction, and other physiological processes.[6,9] Selenoproteins are thought to be responsible for most of the biomedical effects of dietary selenium, which is essential to mammals. The reduction of incidence of cancer by dietary supplementation with selenomethionine via *Se*-(methyl)selenocysteine has been reported.[10] Among the sulfur-containing amino acids, besides the effects discussed above, methionine deserves special mention. One of the nine essential amino acids that should be present in suf-

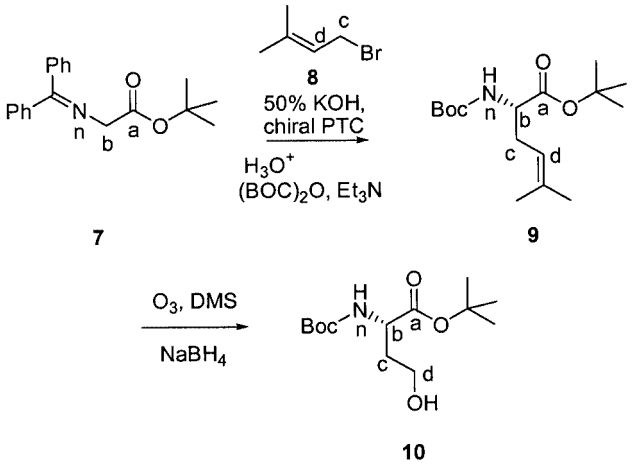
ficient quantity in food,[1] methionine reacts with ATP with formation of *S*-(adenosyl)methionine (SAM), which serves as the methyl donor in biological methylation. After the transfer of the methyl group, *S*-(adenosyl)homocysteine is formed, and hydrolyses to homocysteine, the essential reagent in the biosynthesis of cysteine.[1] Homocysteine can be reconverted into methionine by methylation by *N*5-methyl tetrafolate. Impairment of this regeneration process results in elevated plasma levels of homocysteine. It has been found that plasma levels of homocysteine are a causal risk factor for cardiovascular diseases of importance equal to that of hypercholesterimia, hypertension, and smoking.[11,12] Selenomethionine mimics almost all the roles of methionine. [^{75}Se]-Selenomethionine has a central place in medical diagnosis and therapy as a radiotracer in which temporal and spatial aspects of the interactions of various organs and tumors can be followed. Recently, the potential of [^{73}Se]-selenomethionine as a short-lived positron emitter for in vivo positron emission tomography (PET) has been discussed. This technique is the method of choice for measurement of the rate of protein synthesis in the brain as an important tool to understand the function of the human brain.[13] A similar role for [^{11}C]-methionine has also been discussed.[14] The substitution of Met residues by selenomethionine, constituting an isomorphous substitution of S by the heavy Se, is of great importance in X-ray crystallography of proteins.[15,16] Besides its properties as heavy atom in X-ray structural analysis, natural Se also contains 7.6% of the ^{77}Se isotope, a spin 1/2 nucleus with excellent properties for ^{77}Se spectroscopy.[17] In view of the importance of Cys, Sec, Met, and SeMet in various vital biological processes, access to the full set of ^{13}C and ^{15}N isotopomers of these amino acids is of crucial urgency. Their roles in nutrition can be followed in exquisite detail by mass spectrometry of these isotopomers, by analysis of the serum after administration in food. Site-directed incorporation into proteins can elucidate the structural factors relating to each carbon, hydrogen, nitrogen, and selenium atom with atomic resolution through ^{1}H, ^{13}C, ^{15}N and ^{77}Se NMR spectroscopy. Vibrational spectroscopy of a set of different site-directed isotopomers should provide the force fields of the various chemical bonds in the desired residue in a protein. Even more detailed knowledge should be obtainable by comparison of pairs of proteins in which a sulfur atom has been replaced by a selenium atom. Quantitative biosynthetic replacement has been achieved by use of methionine auxotrophe *E. coli* strains.[16] In the meantime, the chemical incorporation of cysteine, methionine (via homocysteine), selenocysteine, and selenomethionine (via homoselenocysteine) has also been achieved.[18−21] Two approaches for the synthesis of L-selenomethionine have been published,[22,23] and recently the preparation of L-selenocysteine and L-[^{77}Se]-selenocysteine has been published.[24] The syntheses for the sulfur analogues mentioned above cannot serve our purpose for the synthesis of the site-directed isotopomers, because they all start from starting materials with preformed carbon skeletons not amenable to simple stable ^{13}C and ^{15}N incorporation. For the preparation of the whole set of isotopomers of a system, the optimal method is to develop a modular synthetic approach involving a minimal number of steps in convergent fashion, each of them preferably in a high yield. These conditions and the use of relatively inexpensive isotopically enriched starting materials should provide the molecule in question in such a fashion that each atom derives in a unique way from the enriched starting material. Finally, the isotope-enriched L-amino acids should be prepared in the highest optical purity. Recently we published a synthetic scheme, based on the O'Donnell strategy,[25] to allow access to any site-directed isotopomer of L-leucine. A protected glycine derivative is treated with a primary alkyl halide in the presence of a chiral phase-transfer catalyst to form the L-form of leucine in high enantiomeric excess. For that study we also worked out a scheme to synthesize the protected glycine derivative in all its possible isotopomers. It is clear that for the preparation of the full set of isotopomers of methionine and selenomethionine we need to prepare the full set of isotopomers of homoserine, which can subsequently be converted into Met and SeMet via homocysteine and homoselenocysteine, respectively, followed by methylation with isotopically labeled methyl iodide. Similarly, the precursor for Cys and Sec, protected serine, would be prepared by the O'Donnell method. In this paper these schemes have been optimized for the preparation of Cys, Sec, Met, and SeMet with synthons in natural isotopic abundance. The starting materials on which these conversions are based are commercially available in any isotopically enriched form. In our schemes all atoms derive from well defined source. This approach has the advantage of a modular and efficient method, resulting in schemes in which any isotopomer is accessible, in high yield and in high enantiomeric excess (*ee*).

Results and Discussion

Synthesis

The central synthon in the preparation of L-methionine (**1**), L-selenomethionine (**2**), L-homocysteine (**3**), and L-homoselenocysteine (**4**) is the protected homoserine **10** (Scheme 1). The reactions shown in Scheme 1 and all further schemes were optimized with synthons of natural isotopic abundance. The starting materials − *tert*-butyl benzophenoneimine glycinate (**7**), dimethylallyl bromide (**8**), and the chiral cinchona-derived phase-transfer catalyst[26,27] − are all commercially available. In order to prepare homoserine **10**, **7** was dissolved in a toluene/chloroform mixture and treated with **8** in the presence of 0.01 equiv. chiral phase-transfer catalyst (PTC) and base (50% KOH) to yield the alkylated species in 90% yield.

Before treatment of the double bond of the side chain with O_3, the O_3-sensitive benzophenoneimine protecting group had to be removed with citric acid, and the resulting free amine was reprotected with the *N*-Boc group, which is stable to ozonolysis. The Boc group was introduced by treatment of the free amine with Boc-anhydride in the presence of triethylamine to give **9** in 92% yield over two steps.

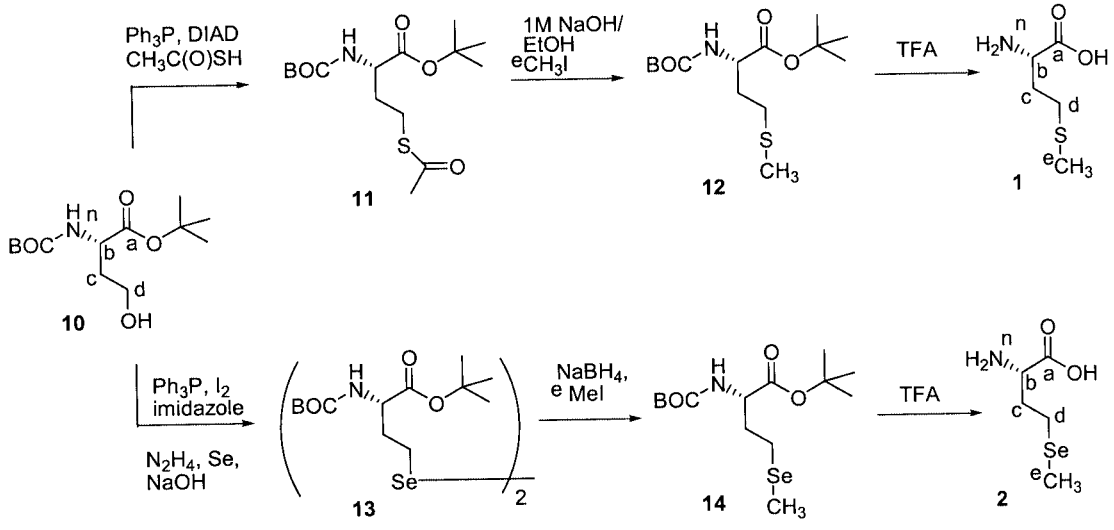


Scheme 1. Preparation of the protected homoserine **10** from the protected glycine derivative **7** and dimethylallyl bromide (**8**); the letters a, b, c, d, and n indicate the ^{13}C or ^{15}N source (see legend of Figure 1)

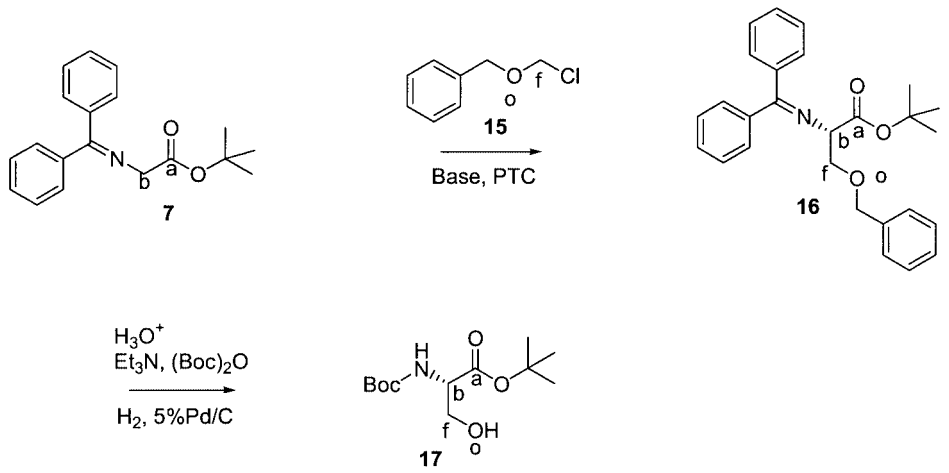
Subsequently, **9** was dissolved in dry dichloromethane and treated with ozone at −40 °C. The resulting ozonide could be reduced with dimethyl sulfide to provide the corresponding aldehyde, which was further reduced with $NaBH_4$ to give homoserine **10** in 78% yield. The preparation of all ^{13}C,^{15}N isotopomers of **7** as well as the full set of ^{13}C isotopomers of **8** is possible and has been reported by us in an earlier publication.[28] This means that all isotopomers of protected homoserine **10** are now accessible in a few steps, each in a high yield. We have also prepared **10** through alkylation of **7** with crotyl bromide, followed by the same conversions as described in Scheme 1. Compound **10** was obtained in good yield, but the synthesis and purification of dimethylallyl bromide (**8**) and its isotopomers proved to be more efficient than the preparation of isotopically enriched crotyl bromide. Scheme 2 describes the conversion of the protected homoserine into either Met (**1**) or SeMet (**2**). Mitsunobu treatment of **10** with thioacetic acid, triphenylphosphane, and diisopropyl diazodicarboxylate (DIAD) in dry THF gave **11** in 86% yield. Treatment of an ethanolic solution of **11** with aqueous NaOH in the presence of MeI gave immediate saponification of the thioester, followed by methyl thioether formation to give the required protected methionine **12** in near quantitative yield. Subsequent treatment of **12** with a 10% solution of trifluoroacetic acid in dichloromethane gave pure Met (**1**), which showed all the analytical characteristics of an authentic sample of commercial methionine after recrystallization. Carrying out the described reaction sequence with ^{13}C-enriched methyl iodide should also give incorporation of a stable ^{13}C isotope label in the SCH_3 group of methionine.

Treatment of **11** with NaOH in the absence of CH_3I gave the protected homocysteine, which upon deprotection with trifluoroacetic acid gave homocysteine (**3**), with all the characteristics of the authentic material. For the preparation of selenomethionine (**2**), **10** was treated with triphenylphosphane, iodine, and imidazole dissolved in dry dichloromethane to convert the alcohol function into the corresponding iodide in 86% yield. After this, the iodide was added to a DMF solution containing a mixture of elemental selenium, hydrazine monohydrate, and sodium hydroxide. The reaction between selenium and hydrazine in the presence of NaOH gives Na_2Se_2, which reacted twice with the iodide to give the *N*-Boc-protected homoselenocystine **13**. Treatment of **13** with $NaBH_4$ in the presence of methyl iodide resulted in the reduction of the diselenide bond, followed by methylation of the resulting selenide to give the protected selenomethionine. Deprotection with trifluoroacetic acid gave selenomethionine (**2**), while deprotection of **13** with trifluoroacetic and subsequent reduction with $NaBH_4$ gave homoselenocysteine. Both products show all the analytical characteristics as described in the literature.[29,30]

From the schemes presented above it is clear that the *N*-Boc-serine *tert*-butyl ester (**17**) is the ideal starting material for the preparation of the full set of isotopomers of cysteine and selenocysteine. For their preparations, the synthesis of



Scheme 2. Preparation of L-methionine (**1**) and L-selenomethionine (**2**) from L-homoserine (**10**)



Scheme 3. A short and efficient route to protected serine **17**

L-serine was optimized first (Scheme 3).[31] To prepare protected serine **17**, glycinate **7** was treated with benzyl chloromethyl ether under chiral phase-transfer conditions to give **16** in 70% yield. Benzyl chloromethyl ether can be prepared in good yield in a one-pot reaction from paraformaldehyde, benzyl alcohol, and HCl.[32] By this approach, ^{18}O and ^{17}O can be incorporated from isotopically enriched water and ^{13}C from isotopically enriched paraformaldehyde.[32]

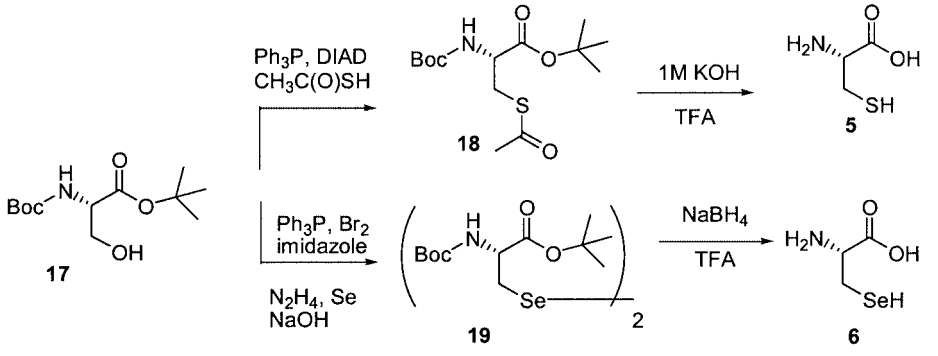
The *O*-benzyl protection has to be removed by catalytic reduction (Pd/C and hydrogen), but the benzophenoneimine protecting group is not stable under reducing conditions. It was therefore replaced with the *N*-Boc protecting group, by treatment of **16** first with citric acid, and then with Boc-anhydride and triethylamine to give *N*-Boc, *O*-benzylserine *tert*-butyl ester, which was finally converted into *N*-Boc-serine *tert*-butyl ester (**17**) by catalytic hydrogenation.

Scheme 4 depicts the conversion of the protected serine into Cys (**5**) and Sec (**6**). These reactions are essentially the same as discussed for Scheme 2. The Cys (**5**) and Sec (**6**) obtained in this way both show, after recrystallization, the same analytical characteristics as the authentic materials. In this way access is gained to the full set of ^{13}C and ^{15}N isotopomers for both Cys and Sec.

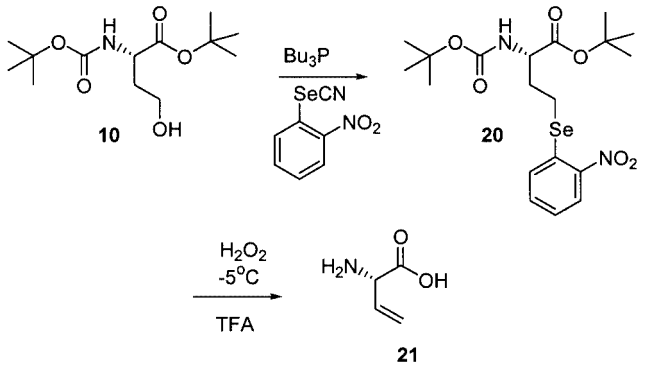
L-Vinylglycine (**21**) is a potent inhibitor of β-cystathionase in the methionine biosynthetic pathway. It is also an irreversible inhibitor for pyridoxal phosphate-dependent aminotransferases.[33] Again, the full set of isotopomers would be necessary for investigation of these vital biological processes at atomic resolution. Oxidation of the protected selenomethionine **14** with hydrogen peroxide at low temperature and subsequent $CH_3Se(O)H$ elimination should give the protected L-vinylglycine derivative. Unfortunately, this reaction gives **21** in only 20% yield. It is known from the literature, however, that formation of a terminal double bond by selenium oxide elimination can be accomplished efficiently in high yield by use of an *o*-nitrophenyl selenide.[34,35] By this approach, **20** was prepared in 82% yield by treatment of **10** with $(Bu)_3P$ in dry THF (Scheme 5), followed by addition of *o*-nitrophenyl selenocyanate. Subsequent treatment of **20** with aqueous H_2O_2 at −5 °C yielded the protected vinylglycine **21** in 79% yield. Deprotection with trifluoroacetic acid gave L-vinylglycine **21** with the same analytical characteristics as given in the literature. Protected vinylglycine can also serve as a starting material to form important novel chiral derivatives.

Discussion

From the Mitsunobu reaction of the phenyl selenocyanate shown in Scheme 5 it is clear that reagents of this type can easily convert primary alcohols into the corresponding



Scheme 4. Preparation of cysteine and selenocysteine



Scheme 5. Preparation of vinylglycine starting from protected homoserine

seleno and thio diethyl ethers. We feel that there should be no barrier to extension of these reactions to the preparation of the corresponding tellurium systems.[24] Our schemes also provide the building blocks for the preparation of cystathionine, an intermediate in the biosynthesis of cysteine (it is the thioether with the same carbon skeleton as Cys) and lanthionine (the thioether made of two Cys carbon skeletons), which occur in proteins in the skin[36−38] and are formed during the biosynthesis of certain antimicrobial compounds.[39,40]

Conclusion

In this paper we describe a modular and convergent strategy for the preparation of L-Ser, Cys, Sec, Met, SeMet, and vinylglycine in optically pure forms, in such a fashion that all the stable ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{77}Se and the radioactive isotopomers can be easily introduced in a site-directed manner, through the alkylation of a protected glycine derivative with alkyl halides in the presence of a chiral active phase-transfer catalyst. After the reaction, the optically active catalyst can be easily isolated, ready to be reused. By this method no difficult separation of the product from an optically active scaffold is needed.[41] The central step for the incorporation of S and Se is an efficient Mitsunobu reaction. From the scope of the Mitsunobu reaction it is to be expected that many important optically active α-amino acids with the serine and homoserine carbon skeleton should become easily available.

Experimental Section

General Remarks: ^1H NMR spectra were recorded with Jeol FX-200 and Bruker DPX-300 spectrometers, with tetramethylsilane (TMS: δ = 0 ppm) or water (H_2O: δ = 4.8 ppm) as internal standards. ^{13}C noise-decoupled NMR spectra were recorded on a Jeol FX-200 at 50.1 MHz or on a Bruker DPX-300 spectrometer at 75.5 MHz, with $CDCl_3$ (δ = 7 ppm), $(CD_3)_2CO$ (δ = 206 ppm), or TSP (δ = 0 ppm) as internal standards. All spectra were recorded in $CDCl_3$, except where noted otherwise. Column chromatography was performed on Merck silica gel 60 (0.040−0.063 mm 230−400 mesh), and spots on thin-layer chromatography were detected with UV-light, spraying with $KMnO_4$ solution, ninhydrin-staining (0.2% in ethanol) or staining with a mixture of 4,4′-methylenebis(*N*,*N*-dimethylaniline) and ninhydrin (TDM staining). Chiral HPLC was performed on a Chiralcel ODH column (25 cm) with hexane and 2-propanol (*i*PrOH) as the solvent system or on a Daicel crownpack CR(+) column (150 mm) with a $HClO_4$ solution as the solvent system. Dry diethyl ether (ether, Et_2O) was obtained by distillation from P_2O_5. Dry petroleum ether (PE) 40−60 and dry dichloromethane (DCM) were obtained by distillation from CaH_2. Dry tetrahydrofuran (THF) was obtained by drying over sodium benzophenone. Deaereated solvents were obtained by bubbling of a steady stream of argon or dry nitrogen through the liquid for one hour. All reagents were purchased from Aldrich Chemical Co., Acros Chimica, or Fluka.

***tert*-Butyl 2-(Benzhydrylideneamino)-5-methylhex-4-enoate:** Dimethylallyl bromide (**8**) (0.55 g, 1.1 equiv.), catalyst (102 mg, 0.05 equiv.), and aqueous KOH (50%, 10 equiv.) were added at 0 °C to a solution of **7** (1 g, 3.4 mmol) in toluene (10 mL). The mixture was stirred vigorously at 0 °C for 6 h and at room temp. overnight. It was then diluted with water (15 mL) and extracted with ethyl acetate (3 × 20 mL). After evaporation the crude product was purified by column chromatography (PE/Et_2O, 80:20). After the product had been collected, the column was eluted with DCM to recover the catalyst. The yield was 90% (1.11 g), *ee* > 90% (Chiralcel ODH, 25 cm, hexane/*i*PrOH, 99.9:0.1 v/v, flow 0.8 mL/min. Retention time (min): 15.26 (*S*), 16.47 (*R*). ^1H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.56 (s, 3 H, H6), 1.64 (d, $^4J_{H,H}$ = 0.75 Hz, 3 H, H6), 2.57 (m, 2 H, H3), 3.98 (1 H, dd $^3J_{H,H}$ = 5.62, $^3J_{H,H}$ = 7.54 Hz, H4), 5.05 (1 H, tq $^3J_{H,H}$ = 7.5, $^4J_{H,H}$ = 0.75 Hz, H4), 7.70−7.13 (m, 10 H, arom) ppm. ^{13}C NMR (75.5 MHz): δ = 17.74 (CH_3), 25.60 (CH_3), 27.85 (*t*Bu), 32.24 (C3), 66.22 (C2), 80.42 (Cq), 120.11 (C4), 127−140 (C, Phe), 136.55 (C5), 169.33 (CN), 171.03 (C1) ppm.

***tert*-Butyl 2-Amino-5-methylhex-4-enoate:** The alkylated glycinate (1.11 g, 3.05 mmol) was dissolved in THF (10 mL) and stirred with citric acid solution (10%, 10 mL) for one night. TLC showed complete disappearance of the starting material, and the mixture was extracted with diethyl ether. The water layer was brought to pH 12 and extracted twice with ethyl acetate (30 mL) to give the product in 98% yield (0.6 g, 3.0 mmol) after evaporation. ^1H NMR (300 MHz): δ = 1.46 (s, 9 H, *t*Bu), 1.64 (s, 3 H, H6c), 1.72 (3 H, d $^4J_{H,H}$ = 1.0 Hz, H6t), 2.35 (m, 2 H, H3), 3.37 (dd, $^3J_{H,H}$ = 5.49, $^3J_{H,H}$ = 6.87 Hz, 1 H, H2), 5.10 (tq, $^3J_{H,H}$ = 6.87, $^4J_{H,H}$ = 1.0 Hz, 1 H, H4) ppm. ^{13}C NMR (75.5 MHz): δ = 17.19 (CH_3), 25.60 (CH_3), 27.73 (*t*Bu), 33.33 (C3), 54.76 (C2), 80.36 (*t*Bu), 119.00 (C4), 124.62 (C5), 174.61 (C1) ppm.

***tert*-Butyl 2-*tert*-Butoxycarbonylamino-5-methylhex-4-enoate (9):** The amino ester prepared above was dissolved in DMF (5 mL), triethylamine (0.3 g, 1 equiv., 0.42 mL) was added, followed by di-*tert*-butyl dicarbonate (0.71 mL, 1.1 equiv.), and the mixture was stirred until TLC showed completion of the reaction. The DMF was evaporated and ethyl acetate (25 mL) was added. The organic layer was washed thrice with $KHSO_4$ solution (pH 2, 25 mL), water, and brine, and was dried with $MgSO_4$. The crude product was purified over a short silica column (PE/Et_2O 80:20). The yield was 94% (0.85 g). ^1H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.45 (s, 9 H, *t*Bu), 1.61 (s, 3 H, H6c), 1.70 (d, $^4J_{H,H}$ = 1.0 Hz, 3 H, H6t), 2.45 (m, 2 H, H3), 4.21 (dd, $^3J_{H,H}$ = 5.6, $^3J_{H,H}$ = 13.5 Hz, 1 H, H2), 5.04 (m, 1 H, H4), 5.04 (s, 1 H, NH), ppm. ^{13}C NMR (75.5 MHz): δ = 18.64 (CH_3), 26.58 (CH_3), 28.69 (*t*Bu), 29.05 (*t*Bu), 31.94 (C3), 54.57 (C2), 80.14 (*t*Bu), 85.81 (*t*Bu), 118.80 (C4), 136.17 (C5), 155.91 (CO), 172.19 (C1) ppm.

***N*-Boc-homoserine *tert*-Butyl Ester (10):** A solution of **9** (0.84 g, 2.8 mmol) in DCM (150 mL) in a three-necked, round-bottomed flask fitted with a dropping funnel containing dimethyl sulfide (0.37 mL, 1.5 equiv.) in DCM (10 mL) was cooled to −50 °C in an acetone/dry ice bath. Ozone was passed through the solution until the liquid had a persistent blue color. The excess ozone was purged from the solution with a soft nitrogen stream, and subsequently the dimethyl sulfide was added dropwise while the temperature was kept below −40 °C. The reaction mixture was warmed to room temp. over two hours and the solvents were evaporated (careful, stench) in vacuo. The crude product was taken up in ethanol (60 mL) and the solution was cooled to 0 °C. $NaBH_4$ (0.21 g, 2 equiv.) was added, and the mixture was stirred for 4 h. Workup was accomplished by addition of HCl solution (1 M, 10 mL), followed by evaporation of the bulk of the ethanol. Diethyl ether (40 mL) was added, and the organic layer was extracted with $NaHCO_3$ solution, water, and brine, and the organic layers were subsequently dried with $MgSO_4$. The crude product was purified by column chromatography (gradient 20% to 40% Et_2O in PE). The yield was 78% (611 mg, 2.2 mmol). ^{1}H NMR (300 MHz): δ = 1.49 (s, 18 H, 2 × *t*Bu), 2.04 (m, 2 H, H3), 3.58 (m, 2 H, H4), 4.26 (m, 1 H, H2), 5.38 (d, $^3J_{H,H}$ = 7.8 Hz, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz): δ = 28.17 (*t*Bu), 27.87 (*t*Bu), 36.16 (C3), 50.93 (C2), 58.14 (C3), 80.12 (*t*Bu), 82.11 (*t*Bu), 156.40 (CO), 171.72 (C1) ppm.

***S*-Acetyl-*N*-boc-homocysteine *tert*-Butyl Ester (11):** DIAD (0.317 mL, 2 equiv.) was added at 0 °C under dry nitrogen to a solution of triphenylphosphane (418 mg, 1.6 mmol, 2 equiv.) in dry THF (10 mL) in a flame-dried round-bottomed flask, and the mixture was stirred until a white solid appeared. Stirring was continued for 10 min at 0 °C, after which **10** (0.22 g, 0.8 mmol) in dry THF (2 mL) was added. After the mixture had been stirred for 45 min, thioacetic acid (0.114 mL, 1.6 mmol) was added and stirring was continued for 3 h. Diethyl ether (30 mL) was added, and the mixture was extracted with water (25 mL), followed by drying of the organic layer over $MgSO_4$. After evaporation of the solvents, the resulting solid was taken up in DCM (3 mL) and flashed over silica, which was then rinsed with diethyl ether (50 mL). The product was concentrated in vacuo and was further purified by chromatography (PE/Et_2O, 85:15). The yield was 86% (0.23 g, 0.68 mmol). ^{1}H NMR (300 MHz): δ = 1.46 (s, 9 H, *t*Bu), 1.48 (s, 9 H, *t*Bu), 1.88 (m, 1 H, H3), 2.04 (m, 1 H, H3), 2.33 (s, 3 H, CH3), 2.90 (m, 2 H, H4), 4.22 (m, 1 H, H2), 5.14 (d, 1 H, NH) ppm. ^{13}C NMR (75.5 MHz): δ = 25.04 (C5), 27.93 (*t*Bu), 28.29 (*t*Bu), 30.54 (C4), 33.07 (C3), 53.31 (C2), 80.20 (*t*Bu), 82.28 (*t*Bu), 155.40 (CO), 171.09 (C1), 196.20 (SCO) ppm.

***N*-Boc-methionine *tert*-Butyl Ester (12):** Compound **11** (0.23 g, 0.68 mmol) was redissolved in ethanol (10 mL), and NaOH solution (2 M, 5 mL) was added to the stirred solution, which was kept under argon. The hydrolysis was followed by TLC. After 3 h, CH_3I (90 μL, 2 equiv.) was added, and the solution was stirred overnight. The solution was concentrated in vacuo to 4 mL, and ethyl acetate (50 mL) was added. The organic layer was washed with water (10 mL) and brine, and dried with $MgSO_4$. After evaporation the product was purified over a silica column (PE/Et_2O, 80:20), giving a 91% yield (190 mg). ^{1}H NMR (300 MHz): δ = 1.45 (s, 18 H, 2 × *t*Bu), 1.99 (m, 1 H, H3), 2.11 (s, 3 H, H5), 2.15 (m, 1 H, H3), 2.58 (m, 2 H, H4), 4.45 (1 H, H2), 5.25 (1 H, NH) ppm. ^{13}C NMR (75.5 MHz): δ = 15.36 (C5), 28.28 (6 × CH_3), 29.95 (C4), 31.72 (C3), 52.63 (C2), 80.35 (*t*Bu), 82.60 (*t*Bu), 155.58 (CO), 170.80 (C1) ppm.

L-Methionine Trifluoroacetate (1): The protected methionine **12** (190 mg) was dissolved in DCM, and trifluoroacetic acid (TFA, 10%) was added. The mixture was stirred at room temp. for 1 night and then extracted with water to give (after evaporation) the methionine.TFA salt in 82% yield from **12** (135 mg, 0.51 mmol). Recrystallization of L-methionine from a water/ethanol mixture (pH 3.9) gave L-methionine in > 98% *ee* ($HClO_4$ (pH 1.5), 15% MeOH, flow 0.6 mL/min. Retention time (min): 3.31 (*R*), 6.39 (S). ^{1}H NMR (300 MHz, D_2O): δ = 2.13 (s, 3 H, H5), 2.20 (m, 2 H, H3), 2.70 (t, $^3J_{H,H}$ = 7.38 Hz, 2 H, H4), 4.20 (dd, $^3J_{H,H}$ = 6.3, $^3J_{H,H}$ = 6.5 Hz, 1 H, H2) ppm. ^{13}C NMR (75.5 MHz D_2O): δ = 14.69 (C5), 29.39 (C4), 29.76 (C3), 52.79 (C2), 172.79 (C1) ppm.

***tert*-Butyl 2-*tert*-Butoxycarbonylamino-4-iodobutyrate:** Triphenylphosphane (1.04 g, 4.0 mmol, 2 equiv.) was dissolved in dry dichloromethane (15 mL), and the solution was cooled to 0 °C and stirred under dry nitrogen while iodine (0.99 g, 1.99 equiv.) was added in portions. After 30 minutes, a mixture of **10** (0.55 g, 2.0 mmol) and imidazole (0.28 g, 2.05 equiv.) was slowly added to the dark purple solution. After one minute a white solid became visible. The reaction mixture was stirred for another two hours, while the temperature was maintained at 0 °C. Subsequently, the solids were filtered off and the solvent was evaporated. The resulting solid was redissolved in DCM (1 mL) and purified by column chromatography (PE/Et_2O, 90:10) to give the iodide in 86% yield (0.66 g, 1.74 mmol). ^{1}H NMR (300 MHz): δ = 1.45 (s, 9 H, *t*Bu) 1.48 (s, 9 H, *t*Bu), 2.17 (m, 1 H, H3), 2.35 (m, 1 H, H3), 3.43 (m, 2 H, H4), 4.24 (m, 1 H, H2), 5.13 (1 H, broad s, NH) ppm.

***N*-Boc-homoselenocystine Di-*tert*-Butyl Ester (13):** $N_2H_4 \cdot H_2O$ (100 μL, 1.1 equiv.) was added to a suspension of Se (0.15 g, 1.87 mmol, 1.1 equiv.) and NaOH (0.1 g) in DMF (5 mL). The mixture was stirred at 60 °C under argon for 3 h. Subsequently, the iodide prepared above (0.65 g, 1.70 mmol) was added dropwise, and the mixture was stirred for 3 h. TLC indicated complete disappearance of the starting material, and the solvent was evaporated in vacuo. The resulting oil was dissolved in diethyl ether (25 mL) and extracted with water (5 mL) and brine (5 mL), and was dried with $MgSO_4$ to give **13,** which was obtained in 71% yield (0.41 g) after column chromatography (PE/Et_2O, 85:15). ^{1}H NMR (300 MHz): δ = 1.45 (s, 18 H, 2 × *t*Bu) 1.48 (s, 18 H, 2 × *t*Bu), 1.96−2.32 (4 H, 2 × H3), 2.89 (m, 4 H, 2 × H4), 4.19 (m, 2 H, H1), 5.14 (2 H, broad s, NH) ppm.

***N*-Boc-selenomethionine *tert*-Butyl Ester (14):** Diselenide **13** (0.41 g, 0.60 mmol) was dissolved in EtOH (10 mL), the mixture was cooled to 0 °C, and $NaBH_4$ (50 mg, 1.33 mmol) was added. The mixture was stirred at 0 °C for 15 minutes, and CH_3I (0.18 mL, 2.9 mol) was added. After 30 minutes, HCl (1 M, 2 mL) was added, followed by water (20 mL), and the mixture was extracted thrice with EtOAc (25 mL). The collected organic fractions were dried with $MgSO_4$ and the solvent was evaporated in vacuo. The resulting oil was purified by column chromatography to give the title compound in 90% yield (0.38 g). ^{1}H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 1.97 (m, 1 H, H3) 1.99 (s, 3 H, H5), 2.16 (m, 1 H, H3), 2.53 (m, 2 H, H4), 4.25 (m, 1 H, H2), 5.19 (1 H, broad s, NH) ppm. ^{13}C NMR (75.5 MHz): δ = 4.06 (C5), 20.26 (C4), 28.35 (6 × CH_3), 33.56 (C3), 54.13 (C2), 79.69 (*t*Bu), 82.62 (cq), 155.24 (CO), 171.79 (C1) ppm.

Trifluoroacetate of Selenomethionine (2): The protected selenomethionine **14** was dissolved in TFA (10%, 15 mL) in dry DCM and the mixture was stirred for 3 h. When TLC indicated complete deprotection, the mixture was extracted twice with water (15 mL), and the collected water layers were evaporated under reduced pressure to give selenomethionine trifluoroacetic acid salt (0.31 g, 91% yield). ^{1}H NMR (200 MHz, D_2O, pH = 7): δ = 2.0 (s, 3 H, H5),

2.1−2.3 (m, 2 H, H3), 2.6 (t, $^3J_{H,H}$ = 7.14 Hz, 2 H, H4), 3.8 (m, 1 H, H2) ppm.

L-Homocysteine Trifluoroacetate (3): Compound **11** (0.5 mmol, 167 mg) was redissolved in deaereated ethanol (5 mL), and deaereated NaOH solution (1 M, 3 mL) was added to the stirred solution, which was subsequently kept under argon. After 6 h, the mixture was extracted twice with deaereated DCM (30 mL) and the collected organic layers were dried with $MgSO_4$ in an Erlenmeyer filled with argon. The solvent was then evaporated, and the product was redissolved in deareated TFA in DCM solution (10%, 10mL). After 12 h, the solvent and acid were extracted with deaereated water (2 × 10 mL), and the water layers were evaporated under reduced pressure. The yield of **3** (as the trifluoroacetate) was 73% (90 mg). ^{1}H NMR (200 MHz, D_2O): δ = 2.2 (m, 2 H, H3) 2.6 (m, 2 H, H4), 3.9 (m, 1 H, H2) ppm.

L-Homoselenocystine Trifluoroacetate (4): The diselenide **13** (0.3 g, 0.44 mmol) was dissolved in TFA in DCM solution (10%, 10 mL), and the mixture was stirred for 18 h without stopper. The solvent and TFA were evaporated in vacuo. The resulting oily solid was taken up in water (5 mL) and washed twice with DCM (5 mL) to give, after evaporation of the water layer, the title compound in 79% yield (208 mg). ^{1}H NMR (300 MHz, D_2O): δ = 1.72−1.91 (m, 2 × 2 H, H3), 2.42 (m, 2 × 2 H, H4), 3.62 (d, $^3J_{H,H}$ = 6.45 Hz, 2 × 1 H, H1) ppm.

***tert*-Butyl 2-(Benzhydrylidene)amino-3-(benzyloxy)propionate (16):** *tert*-butyl *N*-(Benzophenoneimine)glycinate (**7**) (200 mg, 0.67 mmol) was dissolved in dry DCM (3 mL). The solution was cooled to −5 °C, and benzyl chloromethyl ether (1.5 equiv., 70μL), *O*-allyl *N*-methylanthracenyl cinchonidium bromide (20%, 80 mg), and BEMP (2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine, 0.58 mL, 3 equiv.) were added. The mixture was stirred vigorously at this temperature for 18 h. TLC indicated almost complete disappearance of the starting material, and water (5 mL) and DCM (20 mL) were added. The organic layer was washed with water (5 mL) and dried with $MgSO_4$, and the solvents were evaporated. The crude product was purified over a silica column (PE/ethyl acetate, 95:5), giving **16** in 70% yield (193 mg). *ee* > 81% (Chiralcel ODH, 25 cm, hexane/*i*PrOH, 99.5:0.5 v/v, flow 0.8 mL/min. Retention times (min): 16.45 (*R*), 18.81 (*S*). ^{1}H NMR (300 MHz): δ = 1.36 (s, 9 H, *t*Bu), 3.79 (dd, $^2J_{H,H}$ = 9.64, $^3J_{H,H}$ = 7.27 Hz, 1 H, H-3), 3.90 (dd, $^2J_{H,H}$ = 9.64, $^3J_{H,H}$ = 4.76 Hz, 1 H, H-3), 4.23 (dd, $^3J_{H,H}$ = 7.27, $^3J_{H,H}$ = 4.76 Hz, 1 H, H-2), 4.51 (d, $^2J_{H,H}$ = 12.34 Hz, 1 H, OCH_2), 4.52 (d, $^2J_{H,H}$ = 12.34 Hz, 1 H, OCH_2), 7.21−7.66 (m, 15 H, 3 × Phe) ppm. ^{13}C NMR (75.5 MHz): δ = 28.01 (*t*Bu), 66.51 (C2), 71.55 (C3), 73.18 (C4), 81.23 (*t*Bu), 125.64−139.68 (arom.), 169.42 (C=N), 171.22 (C1) ppm.

***O*-Benzyl-*N*-boc-serine *tert*-Butyl Ester:** The *O*-benzyl-protected serine ester **16** was dissolved in THF (5 mL), and the mixture was stirred with citric acid solution (10%, 5 mL) for one night. TLC showed complete disappearance of the starting material, and the mixture was washed twice with diethyl ether (15 mL). The water layer was brought to pH 13 and extracted twice with ethyl acetate (30 mL) to give the product in 95% yield (112 mg) after evaporation. The amino ester prepared above was dissolved in DMF (5 mL), and triethylamine (136 μL, 2 equiv.) was added, followed by di-*tert*-butyl dicarbonate (153 μL, 1.5 equiv.), and the mixture was stirred until TLC showed completion of the reaction. The DMF was evaporated, and ethyl acetate (25 mL) was added. The organic layer was washed with $KHSO_4$ solution (pH 2, 2 × 15 mL) and water, and dried with $MgSO_4$. The crude product was purified over a short silica column (PE/Et_2O, 85:15). The yield was 82% (128 mg). ^{1}H NMR (200 MHz): δ = 1.5 (s, 18 H, 2 × *t*Bu), 3.7 (m, 1 H, Hβ), 3.9 (m, 1 H, Hβ), 4.3 (m, 1 H, Hα), 4.4−4.6 (m, 2 H, OCH_2,), 5.4 (broad s, 1 H, NH), 7.2−7.4 (m, 5 H, Phe) ppm.

***N*-Boc-serine *tert*-Butyl Ester (17):** The *N*-Boc-protected serine derivative (128 mg, 0.36 mmol) was dissolved in a mixture of deaereated methanol and acetic acid (97:3 v/v, 10 mL), to which a catalytic amount of 10% Pd/C was added. Hydrogen was bubbled through at normal pressure for 18 h. The solution was flushed with nitrogen gas, and water (10 mL) and ethyl acetate (50 mL) were added. The organic layer was extracted twice with water (5 mL) and was evaporated in vacuo. The crude product was purified by column chromatography (PE/Et_2O, 80:20), giving **17** in a yield of 79% (75 mg, 0.29 mmol). ^{1}H NMR (200 MHz): δ = 1.45 (s, 9 H, *t*Bu), 1.49 (s, 9 H, *t*Bu), 3.9 (m, 2 H, H3), 4.3 (1 H, broad s, H2), 5.4 (1 H, broad s, NH) ppm.

***S*-Acetyl-*N*-boc-cysteine *tert*-Butyl Ester (18):** DIAD (0.317 mL, 2 equiv.) was added at 0 °C under a dry nitrogen to a solution of triphenylphosphane (0.44 g, 2.0 equiv.) in dry THF (10 mL) in a flame-dried round-bottomed flask, and the mixture was stirred until a white solid appeared. Stirring was continued for 10 min at 0 °C, after which **17** (0.22 g 0.84 mmol) in dry THF (2 mL) was added. After 45 min stirring, thioacetic acid (0.120 mL, 1.68 mmol) was added, and stirring was continued for 3 h. Diethyl ether (30 mL) was added to the reaction mixture, the mixture was extracted with water (15 mL), and the organic layer was dried with $MgSO_4$. After evaporation of the solvents, the resulting solid was taken up in DCM (5 mL) and flashed over a glass filter filled with silica, which was then rinsed with diethyl ether (50 mL). After evaporation of the ether, the product was further purified by chromatography (PE/Et_2O, 85:15). The yield was 76% (0.2 g). ^{1}H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 2.35 (s, 3 H, H4), 3.34 (m, 2 H, H3), 4.50 (broad s, 1 H, H2), 5.21 (1 H, broad s, NH) ppm.

L-Cysteine (5): Compound **18** (0.2 g) was dissolved in deaereated ethanol (10 mL), and deaereated NaOH solution (5 mL) was added to the solution, which was stirred under argon. The hydrolysis was followed by TLC. After 5 h the bulk of the ethanol was evaporated in vacuo (care was taken to keep the product under argon as much as possible) and the resulting solution was extracted twice with deaereated DCM (25 mL). The organic layers were collected and dried with $MgSO_4$, and the solution was filtered without vacuum suction, but with an argon blanket, created by a steady stream of argon coming from an inverted funnel suspended over the glass filter, covering the solution. TFA (5 mL) was added to the collected organic layers, and the mixture was allowed to stir under argon overnight. The reaction mixture was extracted twice with deaereated water (10 mL) to give cysteine TFA salt (0.133 g, 89%, 0.57 mmol) after evaporation of the water. ^{1}H NMR (300 MHz): δ = 2.58 (m, 2 H, H3), 3.78 (t, $^3J_{H,H}$ = 4.8 Hz, 1 H, H2) ppm. ^{13}C NMR (75.5 MHz): δ = 24.14 (C3), 54.70 (C2), 170.17 (C1) ppm.

***N*-Boc-selenocystine *tert*-Butyl Ester (19):** Triphenylphosphane (0.44 g, 2 equiv.) was dissolved in dry dichloromethane (5 mL), and the solution was cooled to 0 °C and stirred under dry nitrogen while bromine (86 μL) dissolved in DCM (2 mL) was added dropwise. After 20 min, a mixture of **17** (0.22 g, 0.84 mmol) and imidazole (0.12 g, 2 equiv.) dissolved in DCM (5 mL) was slowly added to the pale yellow solution. After one minute a white solid became visible. The reaction mixture was stirred for another two hours, while the temperature was maintained at 0 °C. Subsequently, the solids were filtered off and the solvent was evaporated. The re-

sulting solid was redissolved in DCM (1 mL) and purified by column chromatography (PE/Et_2O, 90:10) to give the bromide in 81% yield (0.22 g, 0.68 mmol). ^1H NMR (200 MHz): δ = 1.45 (s, 9 H, *t*Bu) 1.47 (s, 9 H, *t*Bu), 3.5 (m, 1 H, H3), 3. 6 (m, 1 H, H3), 4.5 (m, 1 H, H2), 5.2 (1 H, broad s, NH) ppm. A $N_2H_4 \cdot H_2O$ solution (40 μL) was added to a suspension of Se (62 mg, 1.1 equiv.) and pulverized NaOH (0.1 g) in DMF. The mixture was stirred under argon for 3 h at 60 °C. Subsequently the bromide (0.22 g, 0.68 mmol) was added dropwise and the mixture was stirred for 3 h. TLC indicated complete disappearance of the starting material, and the mixture was poured into HCl (1 M, 5 mL) and subsequently extracted with DCM (3 × 15 mL), after which the system was dried with $MgSO_4$ and the solvents were evaporated in vacuo to give **19** in 74% yield (161 mg, 0.25 mmol). ^1H NMR (300 MHz): δ = 1.44 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 3.34 (m, 2 H, H3), 4.50 (broad s, 1 H, H2), 5.21 (broad s, 1 H, NH) ppm.

L-Selenocysteine (6): $NaBH_4$ (30 mg, 3 equiv.) was added at 0 °C to a solution of the protected selenocystine **19** (0.26 g, 0.25 mmol) dissolved in ethanol (4 mL), and the reaction was allowed to proceed for 30 minutes at 0 °C. The solution was then concentrated to 1 mL in vacuo, deaereated water (5 mL) was added, and the solution was extracted twice with deaereated DCM (20 mL). The organic layers were collected and dried with $MgSO_4$, and the solution was filtered without vacuum suction, but with an argon blanket. TFA (2 mL) was added to the solution, and the whole was stirred overnight. The bulk of the acid and solvent were distilled off by use of a high vacuum pump equipped with a cold-trap, and the oily product was taken up in deaereated water (10 mL). Evaporation of the water in vacuo gave the selenocystine.TFA salt as an off-white solid in 85% yield (70 mg, 0.42 mmol). ^1H NMR (300 MHz, D_2O): δ = 3.05 (dd, $^3J_{H,H}$ = 5.55, $^2J_{H,H}$ = 14.17 Hz, 1 H, H-3), 3.14 (dd, $^3J_{H,H}$ = 4.41, $^2J_{H,H}$ = 14.17 Hz, 1 H, H-3), 4.41 (dd, $^3J_{H,H}$ = 5.55 Hz, $^3J_{HH}$ = 4.41 Hz, 1 H, H-21) ppm. ^{13}C NMR (75.5 MHz, D_2O): δ = 15.64 (C3), 54.14 (C2), 170.23 (C1) ppm.

***tert*-Butyl 2-*tert*-Butoxycarbonylamino-4-(2-nitrophenylselenyl)butanoate (20):** Bu_3P (95%, 93 mg, 1.2 equiv., 113 μL) dissolved in dry THF (3 mL) was added dropwise under dry nitrogen to a solution of **10** (100 mg, 0.36 mmol) and 2-nitrophenyl selenocyanate (0.25 g, 3 equiv.) in dry THF (5 mL) . After 45 minutes TLC indicated almost complete disappearance of the starting material, and the mixture was concentrated in vacuo and purified by column chromatography (PE/Et_2O, 90:10) to give **20** in 82% yield (137 mg, 0.30 mmol). ^1H NMR (300 MHz): δ = 1.45 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 2.05 (m, 1 H, H3), 2.20 (m, 1 H, H3), 2.93 (m, 2 H, H4) 4.27 (broad s, 1 H, H2), 5.19 (broad s, 1 H, NH) 7.32−8.30 (m, 4 H, Phe) ppm.

***N*-Boc-vinylglycine *tert*-Butyl Ester:** Compound **20** (137 mg) was dissolved in THF (10 mL), cooled to −5 °C, and treated with H_2O_2 (30%, 3 mL). After 30 minutes the solution was extracted thrice with diethyl ether (50 mL), and the collected organic layers were concentrated in vacuo. The crude product was purified by column chromatography (PE/Et_2O, 93:7) to give the desired product (61 mg, 79% yield). ^1H NMR (200 MHz): δ = 1.45 (s, 9 H, *t*Bu), 1.47 (s, 9 H, *t*Bu), 4.8 (broad s, 1 H, Hα), 5.2−5.4 (m, 3 H, Hγ + NH), 5.9 (m, 1 H, Hβ) ppm. ^{13}C NMR (50.1 MHz): δ = 27.9 (*t*Bu), 28.3 (*t*Bu), 56.3 (C2), 82.4 (Cq), 116.7 (C4), 133.2 (C3) ppm.

L-Vinylglycine (21): The protected vinylglycine (61 mg, 0.24 mmol) was dissolved in TFA in DCM solution (10%, 5 mL), and the mixture was stirred overnight. The acid and solvent were distilled off and the product was taken up in water. Evaporation of the water in vacuo gave pure vinylglycine trifluoroacetic acid salt in 94% yield (48 mg). ^1H NMR (200 MHz): δ = 4.6 (d,$^3J_{H,H}$ = 6 Hz, 1 H, H2), 5.6 (m, 2 H, H4), 6.1 (m, 1 H, H3) ppm.

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