Synthesis of Functionalized L-Cysteine and L-Methionine by Reaction with Electron-Deficient Acetylenes

Boris A. Trofimov,* Anastasiya G. Mal'kina, Olesya A. Shemyakina, Valentina V. Nosyreva, Angela P. Borisova, Spartak S. Khutsishvili, Leonid B. Krivdin

A. E. Favorsky Irkutsk Institute of Chemistry, Siberian Branch, Russian Academy of Sciences,
1 Favorsky Str., 664033 Irkutsk, Russian Federation
Fax +7(3952)419346; E-mail: boris_trofimov@irioch.irk.ru

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Abstract: A novel families of nonconventionally functionalized amino acids have been synthesized in almost quantitative yields by chemo-, regio- and stereospecific addition of L-cysteine and L-methionine to electron-deficient functionalized acetylenes (cyanoacetylenes and methyl 4-hydroxy-4-methylpent-2-ynoate) under mild conditions (water, r.t., 2–5 h). Thus, for the first time, the uncommonly functionalized optically active amino acids composing of L-cysteine and L-methionine structural units along with cinnamic acid, dihydrofuranone, iminodihydrofuran, and other biologically important functionalities are readily available.

Key words: alkynes, amino acids, nucleophilic addition, cyclization

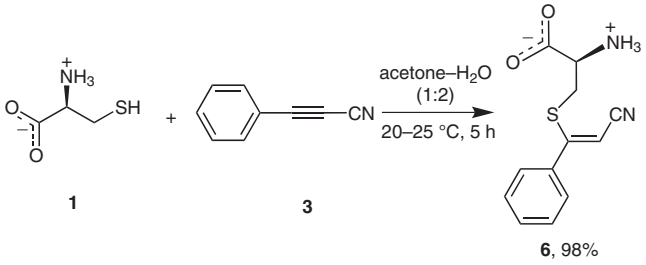
Advances in proteomics stimulate the interest to the synthesis of non-natural amino acids.[1] By employing non-natural amino acids, the activity of biological important peptides can be controlled.[1b,2] The demand for peptide-based structures is increasing rapidly in drug design.[1b,3] Cysteine and methionine are important amino acids for maintaining the integrity of cellular systems by controlling their redox state and detoxifying harmful compounds, including free radicals and active oxygen species.[4] They are the two principal sulfur amino acids found in mammals.[4,5] Cysteine proteases of the papain class, such as cathepsin B, L, K, and S, are intensively studied in medicinal chemistry. They relate to osteoporosis, cancer metastasis, rheumatoid arthritis, asthma, and infectious diseases.[6]

A number of cysteine derivatives, for example, *S*-methyl-, *S*-ethyl-, *S*-propyl-, *S*-allyl- and *N*-acetylcysteines, occurring in garlic, display significant enzymatic antioxidant protection and spare α-tocopherol in mice. Besides, they diminish fibronectin, TG, and cholesterol concentrations in plasma and liver. Hence, these compounds also possess protective effect for cardiovascular disease.[6,7]

Therefore, the search for new approaches to chemical modifications of cysteine and methionine (an essential amino acid) to their non-natural derivatives is a rewarding target of organic synthesis and corresponds to the modern trends in drug discoveries. Of particular interest are highly selective functionalizations, especially those which proceed under mild conditions to provide for isomeric purity of the products obtained.

Here we present our findings on modifications of L-cysteine (**1**) and L-methionine (**2**) in their addition reactions to electron-deficient acetylenes: 3-phenylprop-2-ynenitrile (**3**), α,β-acetylenic γ-hydroxy nitriles **4a–d** and methyl 4-hydroxy-4-methylpent-2-ynoate (**5**).

We have found that L-cysteine (**1**), under mild conditions [acetone–water (1:2), 20–25 °C, 5 h], adds smoothly to 3-phenylprop-2-ynenitrile (**3**) to quantitatively give the chemo-, regio- and stereospecifically adduct **6**, isolated in 98% yield (Scheme 1). Noteworthy, that the adduct **6** represents a combination of L-cysteine with a naturally occurring (*E*)-cinnamic acid derivative that contributes to its biological prospect.



Scheme 1 Modification of L-cysteine (**1**) with 3-phenylprop-2-ynenitrile (**3**)

To assign the configuration of adduct **6** relative to the double bond, we have performed nonempirical calculations (SOPPA,[8] aug-cc-pVTZ-J of Sauer et al.[9] for hydrogens, cc-pCVDZ of Dunning[10] for carbons[11]) of the one-bond olefinic ^{13}C-^{1}H spin-spin coupling constant, $^{1}J_{CH}$, and vicinal ^{13}C-^{1}H coupling involving *ipso* carbon of the phenyl group and the olefinic proton, $^{3}J_{CH}$, in both *Z*- and *E*-isomers of the model compound **7**.

The calculated values were compared with the experimental couplings in adduct **6** (Table 1). All the four nonrelativistic coupling contributions were taken into account, namely, Fermi contact, J_{FC}, spin-dipolar, J_{SD}, diamagnetic spin-orbital, J_{DSO}, and paramagnetic spin-orbital, J_{PSO}. All the calculations were carried out using the DALTON package[12] while geometrical optimizations were performed at the MP2/6-311G** level with the GAMESS code.[13]

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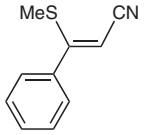
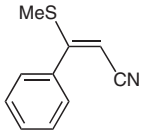
Table 1 Spin-Spin Coupling Constants of **7** Calculated at the SOPPA Level and Experimental Values for the Adduct **6**[a]



(*Z*)-**7**



(*E*)-**7**

Compound[b]	Coupling constant	J_{DSO}	J_{PSO}	J_{SD}	J_{FC}	J_{calc}	J_{exp}
(*E*)-**7**	$^1J_{CH}$	1.08	0.70	0.42	170.17	172.37	–
	$^3J_{CH}$	–0.31	0.16	0.01	10.38	10.24	–
(*Z*)-**7**	$^1J_{CH}$	1.01	0.53	0.42	176.41	178.13	178.0[c]
	$^3J_{CH}$	0.31	–0.15	–0.06	6.13	6.23	4.50[c]

[a] All couplings and their contributions are in Hz. Equilibrium MP2/6-311G** geometries were used throughout.
[b] Both isomers of **7** were adopted in their predominant conformations.
[c] Measured in compound **6**.

From Table 1, it follows that both couplings demonstrate the marked stereospecificity with respect to the configuration at the C=C bond in **6**, which allows one to unambiguously assign *Z*-configuration to adduct **6**. An additional evidence of the *Z*-configuration of adduct **6** has been gained from the 2D NOESY spectrum of **6** showing cross peak between olefinic proton (5.72 ppm) and those in the *ortho*-position of the phenyl group (7.47 ppm) (Figure 1).

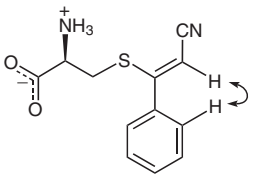


Figure 1 Cross-peak in the 2D (^{1}H, ^{1}H) NOESY NMR spectrum of the amino acid **6**

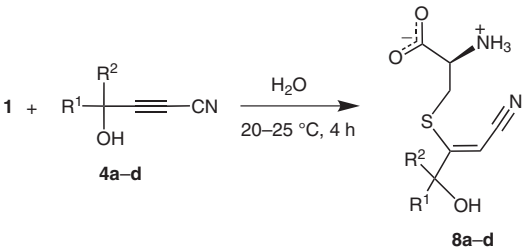
Zwitterionic form of amino acid **6** is supported by its IR (vaseline oil) spectrum where the broad strong absorption in the region of 3300–2500 cm^{-1} is observed. This absorption is commonly assigned to the $^+NH_3$ group.[14] Complementary, in the spectrum, the broad band at 2040 cm^{-1} is attributable to the same moiety. Carboxylate anion is spectrally manifested by broad absorption in the region of 1650–1558 cm^{-1} obviously overlapping with C=C bond stretching vibration (1592 cm^{-1}).

α,β-Acetylenic γ-hydroxy nitriles **4a–d** react with L-cysteine (**1**) under the same mild conditions (water, 20–25 °C, 4 h) in chemo-, regio- and stereospecific manner to form optically active amino acids **8a–d** of exclusive *Z*-configuration in 91–98% isolated yield (Table 2).

For the structure determination of amino acids **8a–d**, ^{1}H, ^{13}C, ^{1}H–^{1}H homonuclear, and ^{1}H–^{13}C heteronuclear 2D (NOESY and HMBC) NMR techniques have been employed.

In the ^{1}H and ^{13}C NMR spectra of **8a**,**c**,**d**, only single peaks of olefinic hydrogens (6.07–6.20 ppm) and carbons (100.2–101.1 ppm) are detected, thus indicating the configurational homogeneity of the compounds. For adduct **8b**, two sets of the olefinic hydrogen (6.13 and 6.14 ppm) and carbon signals are observed (100.6 and 100.8 ppm), that correspond to two diastereomers. The *Z*-configuration of adducts **8a–d** is explicitly supported by the 2D (^{1}H, ^{1}H) NOESY spectrum of the compound **8a**, where the cross-peak between signals of the olefin proton and methyl group protons is observed (Figure 2). The configurational assignment and substituent location for the compound **8a** were also based on the values of vicinal coupling constant $^3J_{CH}$ between olefinic proton and the carbon C-4 ($^3J_{C\text{-}4,H\text{-}2}$ = 4.6 Hz). Since the *trans*-vicinal $^3J_{CH}$ value is always larger than the corresponding *cis*-$^3J_{CH}$ value, the H-2 atom is located in the *cis*-position with respect to the C-4. Therefore, amino acid **8a** is the *Z*-isomer (Figure 2).

Table 2 Functionalization of L-Cysteine (**1**) with α,β-Acetylenic γ-Hydroxy Nitriles **4**



Product	R^1	R^2	Isolated yield (%)
8a	Me	Me	98
8b	Me	Et	96
8c	$(CH_2)_4$		91
8d	$(CH_2)_5$		94

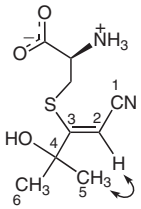


Figure 2 Cross-peaks in the 2D (^{1}H, ^{1}H) NOESY and HMBS NMR spectra of the amino acid **8a**

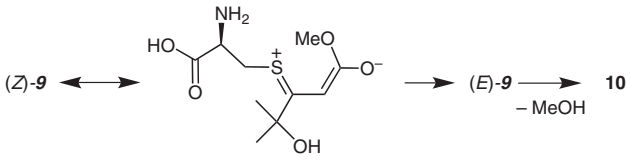
Z-Stereospecificity of the additions (Scheme 1 and Table 2) expectedly resulted from the known *trans*-mode of nucleophilic attack of acetylenes.[15]

The reaction between L-cysteine (**1**) and methyl 4-hydroxy-4-methylpent-2-ynoate (**5**) [acetone–water (1:2), 20–25 °C, 5 h] affords a mixture (2:1, ^{1}H NMR) of the primary adduct (*Z*)-**9** and dihydrofuranone **10**, originated from lactonization of adduct **9** (after its $Z \rightarrow E$-isomerization). Total yield of the mixture is above 95% (Scheme 2).

In this case, $Z \rightarrow E$-isomerization may be facilitated by the resonance redistribution of the double bond electron density (Scheme 3).

Upon standing the mixture of **9** + **10** at ambient temperature, the component ratio **9**:**10** gradually changes and reached 1:2 after 5 months (^{1}H NMR). From this mixture, the amino acid **10** is isolated in 60% yield, the isolated yield of the adduct **9** (*E*/*Z* = 1:5, ^{1}H NMR) is 30%.

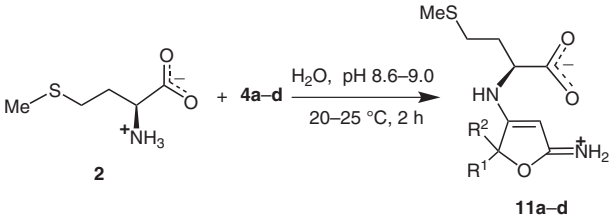
The dihydrofuran moiety is known to be a potent structure of numerous biological active compounds (vitamin C,[16] penicillic, tetronic acids and their thiol analogues,[17] anti-AIDS drugs, such as d4T [1-(2′,3′-dideoxy-γ-D-glycero-pent-2-enofuranosyl)thymine] and AZT {1-[4-azido-5-(hydroxymethyl)tetrahydrofuran-2-yl]-5-methylpyrimidine-2,4-(1*H*,3*H*)-dione}[18]). The dihydrofuran structure occurs in cardenolides[19] (cardioactive steroid lactones), in dysidiolide – the only known natural inhibitor of a protein phosphatase cdc25A[20] – as well as in a great variety of other natural molecules such as sesquiterpenes[21] and pulvinic acid derivatives,[22] etc. In strigol and its analogues, the dihydrofuranone part is primarily responsible for the germination of seeds.[23] Therefore, the combination of the dihydrofuran and cysteine entities is of particular promise for drug discovery.



Scheme 3 Isomerization and cyclization of adduct **9**

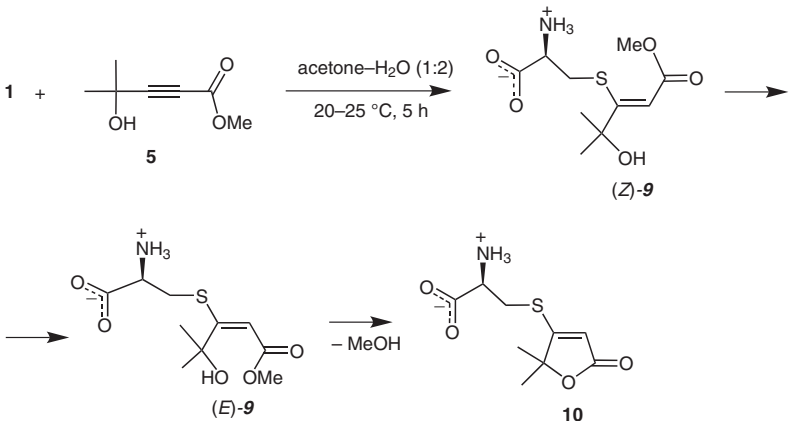
A similar combination of iminodihydrofuran cycle with an essential amino acid moiety in one molecule is attained by the addition of L-methionine (**2**) to α,β-acetylenic γ-hydroxy nitriles **4a**–**d**. As in the former cases, the reaction proceeds under very mild conditions (water, pH ~ 8.6–9.0, 20–25 °C, 2 h), but unlike the results with L-cysteine (**1**) it ends up directly with the iminodihydrofuran-modified methionines **11a**–**d** in 80–96% yields (Table 3).

Table 3 Modification of L-Methionine (**2**) with α,β-Acetylenic γ-Hydroxy Nitriles **4**

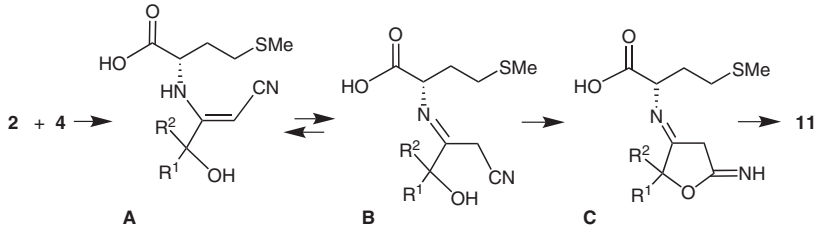


Product	R^1	R^2	Isolated yield (%)
11a	Me	Me	96
11b	Me	Et	96
11c	$(CH_2)_4$		93
11d	$(CH_2)_5$		80

Obviously, the primary *Z*-adducts **A** isomerize prototropically to imines **B** capable of cyclizing to iminotetrahydrofurans **C** which are finally transformed to amino acids **11a**–**d** (Scheme 4).



Scheme 2 Reaction of **1** with **5**



Scheme 4 Formation of **11**

The unusual zwitterionic structure of amino acids **11a–d** follows from glycine addition to α,β-acetylenic γ-hydroxy nitrile **4a**, where the positive charge transfer to iminodihydrofuran cycle is unambiguously shown by the single-crystal X-ray analysis.[24]

In summary, a novel approach to chemical modification of L-cysteine and L-methionine (an essential amino acid) to their non-natural derivatives has been developed. The approach comprises the chemo-, regio-, and stereospecific addition of L-cysteine and L-methionine to electron-deficient functionalized acetylenes such as cyanoacetylenes and methyl 4-hydroxy-4-methylpent-2-ynoate under mild conditions (aqueous media, ambient temperature). The methodology allowed us to prepare in almost quantitative yields novel families of optically active nonconventionally functionalized amino acids incorporated L-cysteine and L-methionine structural units along with cinnamic acid, dihydrofuranone, iminodihydrofuran, and other biologically important functionalities. Such non-natural amino acids are potential pharmaceuticals and promising building blocks and auxiliaries for drug design, proteomics, and controlled peptide modification.

All melting points were taken on a Kofler micro hot stage. Optical rotations were measured on a Polamat A polarimeter at 25 °C. IR spectra were measured on Bruker IFS-25 in KBr pellets and vaseline oil. The ^{1}H, ^{13}C, NOESY, and HMBC NMR spectra were recorded on a Bruker DPX-400 spectrometer in D_2O. L-Cysteine (**1**) and L-methionine (**2**) are commercial products. 3-Phenylprop-2-ynenitrile (**3**),[25] α,β-acetylenic γ-hydroxy nitriles **4a–d**,[26] and methyl 4-hydroxy-4-methylpent-2-ynoate (**5**)[27] were prepared according to published procedures.

(2*R*)-2-Ammonio-3-{[(*Z*)-2-cyano-1-phenylethenyl]sulfanyl}propanoate (6)

A solution of 3-phenylprop-2-ynenitrile (**3**; 0.127 g, 1 mmol) in acetone (1 mL) was added to solution of cysteine (**1**; 0.121 g, 1 mmol) in H_2O (2 mL). After stirring the mixture at 20–25 °C for 5 h, the stirring was stopped, and the solvents were evaporated. The residue was washed with Et_2O (5 × 0.5 mL) and dried in vacuo; yield: 0.243 g (98%); white microcrystalline powder; mp 152–153 °C; $[\alpha]_D^{25}$ –176.0 (*c* 0.01, 0.1 N HCl).

IR (vaseline oil, KBr): 3300–2500 with maxima at 3141, 3028, 2964, 2923, 2848 ($^+NH_3$, C=CH, CH), 2214 (CN), 2040 ($^+NH_3$), 1635 (COO^-), 1613, 1592 cm^{-1} (SC=C).

^{1}H NMR (400.13 MHz, D_2O): δ = 2.99–3.05 (dd, *J* = 8.04, 12.96 Hz, 1 H, SCH_2), 3.10 (dd, *J* = 4.40, 12.96 Hz, 1 H, SCH_2), 3.66 (dd, *J* = 4.40, 8.04 Hz, 1 H, CH), 5.70 (s, 1 H, =CH), 7.38–7.48 (m, 5 H, Ar).

^{13}C NMR (100.62 MHz, D_2O): δ = 32.9 (CH_2), 53.4 (CH), 98.3 (=CH), 117.4 (CN), 128.2 (C-3,5), 128.9 (C-2,6), 131.0 (C-4), 134.6 (C-1), 161.0 (SC=), 170.8 (COO^-).

Anal. Calcd for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28; S, 12.91. Found: C, 57.98; H, 5.10; N, 11.05; S, 13.12.

Amino Acids 8a–d; General Procedure

To a solution of cysteine (**1**; 1 mmol) in H_2O (4 mL), the appropriate α,β-acetylenic γ-hydroxy nitrile **4a–d** (1 mmol) was added. The mixture was stirred at 20–25 °C for 4 h and the H_2O was evaporated. The residue was washed with Et_2O (5 × 0.5 mL) and dried in vacuo to give the amino acids **8a–d**.

(2*R*)-2-Ammonio-3-{[(*Z*)-2-cyano-1-(1-hydroxy-1-methylethyl)ethenyl]sulfanyl}propanoate (8a)

Yield: 0.225 g (98%); white microcrystalline powder; mp 102–104 °C; $[\alpha]_D^{25}$ –14.2 (*c* 0.01, H_2O).

IR (vaseline oil, KBr): 3500–2500 with maxima at 3379, 3264, 3059, 2977, 2930, 2868, 2725, 2602 (OH, $^+NH_3$, C=CH, CH), 2209 (CN), 2064 ($^+NH_3$), 1616 (COO^-), 1580 cm^{-1} (SC=C).

^{1}H NMR (400.13 MHz, D_2O): δ = 1.36 (s, 3 H, CH_3), 1.37 (s, 3 H, CH_3), 3.45 (dd, *J* = 7.09, 13.69 Hz, 1 H, SCH_2), 3.63 (dd, *J* = 7.09, 13.69 Hz, 1 H, SCH_2), 3.94 (dd, *J* = 4.40, 7.09 Hz, 1 H, CH), 6.15 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 27.7 (CH_3), 35.9 (CH_2), 54.0 (CH), 75.2 [$C(CH_3)_2$], 100.2 (=CH), 117.5 (CN), 170.0 (SC=), 171.5 (COO^-).

Anal. Calcd for $C_9H_{14}N_2O_3S$: C, 46.94; H, 6.13; N, 12.16; S, 13.92. Found: C, 46.95; H, 6.37; N, 11.95; S, 13.63.

(2*R*)-2-Ammonio-3-{[(*Z*)-2-cyano-1-(1-hydroxy-1-methylpropyl)ethenyl]sulfanyl}propanoate (8b)

Yield: 0.234 g (96%); pale yellow microcrystalline powder; mp 129–130 °C; $[\alpha]_D^{25}$ –18.7 (*c* 0.01, H_2O).

IR (vaseline oil, KBr): 3450–2500 with maxima at 3195, 3045, 2972, 2930, 2630 (OH, $^+NH_3$, C=CH, CH), 2212 (CN), 2075 ($^+NH_3$), 1625 (COO^-), 1580 cm^{-1} (SC=C).

^{1}H NMR (400.13 MHz, D_2O): δ = 0.75–0.79 (m, 3 H, CH_2CH_3), 1.37, 1.38 (s, 3 H, CH_3), 1.71–1.73 (m, 2 H, CH_2CH_3), 3.50–3.72 (m, 2 H, SCH_2), 3.96–3.99 (m, 1 H, CH), 6.13 and 6.14 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 7.4 (CH_2CH_3), 25.6, 25.7 (CH_3), 32.8, 35.8, 35.9 (CH_2), 54.1 (CH), 78.2 [$C(CH_3)_2$], 100.6, 100.8 (=CH), 117.7 (CN), 169.5 (SC=), 171.7 (COO^-).

The doubling of all the NMR signals resulted from the two diastereomers.

Anal. Calcd for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.47; S, 13.12. Found: C, 48.91; H, 6.65; N, 11.64; S, 12.98.

(2*R*)-2-Ammonio-3-{[(*Z*)-2-cyano-1-(1-hydroxycyclopentyl)ethenyl]sulfanyl}propanoate (8c)
Yield: 0.233 g (91%); pale yellow microcrystalline powder; mp 156–158 °C; $[\alpha]_D^{25}$ –11.5 (*c* 0.01, H_2O).

IR (vaseline oil, KBr): 3500–2500 with maxima at 3270, 3100, 3052, 2965, 2872, 2586 (OH, $^+NH_3$, C=CH, CH), 2215 (CN), 2070 ($^+NH_3$), 1616 (COO^-), 1584 cm^{-1} (SC=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.64–1.74, 1.94 (m, 8 H, CH_2-cyclopentyl), 3.52 (dd, *J* = 7.09, 13.94 Hz, 1 H, SCH_2), 3.65 (dd, *J* = 4.40, 13.94 Hz, 1 H, SCH_2), 3.96 (dd, *J* = 4.40, 7.09 Hz, 1 H, CH), 6.20 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 23.3, 23.4, 36.0, 38.7 (CH_2), 54.1 (CH), 85.8 (C-cyclopentyl), 100.8 (=CH), 117.6 (CN), 168.2 (SC=), 171.6 (COO^-).

Anal. Calcd for $C_{11}H_{16}N_2O_3S$: C, 51.55; H, 6.29; N, 10.93; S, 12.51. Found: C, 51.31; H, 6.39; N, 10.86; S, 12.39.

(2*R*)-2-Ammonio-3-{[(*Z*)-2-cyano-1-(1-hydroxycyclohexyl)ethenyl]sulfanyl}propanoate (8d)
Yield: 0.254 g (94%); pale yellow microcrystalline powder; mp 164–166 °C; $[\alpha]_D^{25}$ –15.6 (*c* 0.01, H_2O).

IR (vaseline oil, KBr): 3520–2500 with maxima at 3277, 3056, 2936, 2858, 2595 (OH, $^+NH_3$, C=CH, CH), 2211 (CN), 2043 ($^+NH_3$), 1616 (COO^-), 1584 cm^{-1} (SC=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.05–1.06, 1.40–1.46 and 1.58–1.65 (m, 10 H, CH_2-cyclohexyl), 3.40 (dd, *J* = 7.09, 13.94 Hz, 1 H, SCH_2), 3.50 (dd, *J* = 4.16, 13.94 Hz, 1 H, SCH_2), 3.83 (dd, *J* = 4.16, 7.09 Hz, 1 H, CH), 6.07 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 21.1, 24.5, 34.7 and 36.2 (CH_2), 54.1 (CH), 76.7 (C-cyclohexyl), 101.1 (=CH), 117.8 (CN), 170.6 (SC=), 171.6 (COO^-).

Anal. Calcd for $C_{12}H_{18}N_2O_3S$: C, 53.31; H, 6.71; N, 10.36; S, 11.86. Found: C, 53.08; H, 6.84; N, 10.28; S, 12.02.

Reaction of L-Cysteine (1) with Methyl 4-Hydroxy-4-methylpent-2-ynoate (5)
A solution of methyl 4-hydroxy-4-methyl-2-pentynoate (**5**; 0.142 g, 1 mmol) in acetone (1 mL) was added to solution of cysteine (**1**; 0.121 g, 1 mmol) in H_2O (2 mL). After stirring the mixture at 20–25 °C for 5 h, the stirring was stopped, and solvents were evaporated. The residue was washed with Et_2O (5 × 0.5 mL) to give 0.25 g (95%) of a mixture of amino acids **9** (0.166 g) and **10** (0.083 g), (2:1, ^1H NMR) The proportion changed to 1:1 in a month, and in 5 months the composition of the reaction mixture **9**:**10** changed to 1:2. The mixture was washed with EtOH (4 × 1 mL), the insoluble residue was dried in vacuo to give compound **10**. After evaporation of EtOH in vacuo, a mixture of *E*- and *Z*-isomers (1:5, ^1H NMR) of compound **9** was obtained.

(2*R*)-2-Ammonio-3-{[(*E*,*Z*)-1-(1-hydroxy-1-methylethyl)-3-methoxy-3-oxo-1-propenyl]sulfanyl}propanoate (9)
Yield: 0.079 g (30%); yellow microcrystalline powder; mp 96–98 °C.

IR (KBr): 3500–2500 with maxima at 3264, 3080, 2979, 2943, 2875, 2548 (OH, $^+NH_3$, C=CH, CH), 2090 (NH_3^+), 1714, 1613 cm^{-1} (COO^-, C=O, SC=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.31 (s, 3 H, CH_3), 1.32 [s, 3 H, CH_3, (*Z*)-**9**], 1.34 (s, 3 H, CH_3), 1.35 [s, 3 H, CH_3, (*E*)-**9**], 3.08 [dd, *J* = 7.94, 14.06 Hz, 1 H, SCH_2, (*E*)-**10**], 3.19 [dd, *J* = 7.39, 16.18 Hz, 2 H, SCH_2, (*E*,*Z*)-**9**], 3.34 [dd, *J* = 3.79, 14.19 Hz, 1 H, SCH_2, (*Z*)-**9**], 3.64, 3.66 (s, 3 H, OCH_3), 3.85 [dd, *J* = 3.84, 7.55 Hz, 1 H, CH, (*Z*)-**9**], 3.94 [dd, *J* = 4.1, 8.36 Hz,1 H, CH, (*E*)-**9**], 6.44, 6.47 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 28.79, 28.82 (CH_3), 37.2, 37.6 (CH_2), 53.3, 53.8 (OCH_3), 54.8, 56.6 (CH), 76.4, 79.7 [$C(CH_3)_2$], 118.6, 121.4 (=CH), 161.3, 163.0, 168.8, 169.0 (C=O), 172.5, 172.9 (SC=).

Anal. Calcd for $C_{10}H_{17}NO_5S$: C, 45.61; H, 6.51; N, 5.32; S, 12.18. Found: C, 45.49; H, 6.59; N, 5.43; S, 12.25.

(2*R*)-2-Ammonio-3-[(2,2-dimethyl-5-oxo-2,5-dihydro-3-furanyl)sulfanyl]propanoate (10)
Yield: 0.139 g (60%); white microcrystalline powder; mp 183–185 °C; $[\alpha]_D^{25}$ +10.1 (*c* 0.01, H_2O).

IR (KBr): 3250–2500 with maxima at 3127, 3076, 2982, 2930, 2889, 2691, 2564, 2466 (OH, $^+NH_3$, C=CH, CH), 2207–2132 ($^+NH_3$), 1709, 1658, 1613 cm^{-1} (COO^-, C=O, SC=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.37 (s, 6 H, CH_3), 3.33 (dd, *J* = 7.68, 14.34 Hz, 1 H, SCH_2), 3.47 (dd, *J* = 4.16, 14.27 Hz,1 H, SCH_2), 3.92 (dd, *J* = 4.16, 7.36 Hz, 1 H, CH), 5.80 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 25.0, 25.1 (CH_3), 32.5 (CH_2), 52.5 (CH), 88.7 (CCH_3), 107.7 (=CH), 171.1, 173.0 (C=O), 177.2 (SC=).

Anal. Calcd for $C_9H_{13}NO_4S$: C, 49.74; H, 5.67; N, 6.06; S, 13.87. Found: C, 49.87; H, 5.57; N, 5.99; S, 13.78.

Amino Acids 11a–d; General Procedure
To a solution of methionine (**2**; 0.149 g, 1 mmol) and NaOH (0.01 g, 0.25 mmol) in H_2O (1 mL) was added the appropriate α,β-acetylenic γ-hydroxy nitrile **4a**–**d** (1 mmol). After stirring the mixture at 20–25 °C for 2 h, the stirring was stopped, and the H_2O was evaporated. The residue obtained was passed through neutral Al_2O_3 (2–3 cm, eluent: 50–70 mL of EtOH). The solvent was evaporated in vacuo to give amino acids **11a**–**d**.

(2*S*)-2-[(5-Iminio-2,2-dimethyl-2,5-dihydro-3-furanyl)amino]-4-(methylsulfanyl)butanoate (11a)
Yield: 0.248 g (96%); yellow microcrystalline powder; mp 138–141 °C; $[\alpha]_D^{25}$ –43.5 (*c* 0.01, H_2O).

IR (vaseline oil, KBr): 3450–2600 with maxima at 3215, 3059, 2979, 2923, 2875 (NH, =$^+NH_2$, C=CH, CH), 1687, 1617 cm^{-1} (COO^-, C=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.50 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 1.99 (s, 3 H, SCH_3), 1.94–2.11 (m, 2 H, SCH_2), 2.37–2.58 (m, 2 H, CH_2), 3.85 (dd, *J* = 4.99, 8.45 Hz, 1 H, CH), 4.91 (s, 1 H, = CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 14.0 (SCH_3), 23.4, 23.8 (CH_3), 29.6, 30.3 (CH_2), 59.7 (CH), 76.5 (CCH_3), 91.3 (=CH), 175.9, 176.9 (=CN, C=NH), 177.1 (COO^-).

Anal. Calcd for $C_{11}H_{18}N_2O_3S$: C, 51.14; H, 7.02; N, 10.84; S, 12.41. Found: C, 50.93; H, 7.21; N, 10.78; S, 12.64.

(2*S*)-2-[(2-Ethyl-5-iminio-2-methyl-2,5-dihydro-3-furanyl)amino]-4-(methylsulfanyl)butanoate (11b)
Yield: 0.261 g (96%); yellow microcrystalline powder; mp 160–163 °C; $[\alpha]_D^{25}$ –52.4 (*c* 0.01, EtOH).

IR (KBr): 3490–2670 with maxima at 3386, 3218, 3052, 2973, 2923 (NH, =$^+NH_2$, C=CH, CH), 1689, 1616 cm^{-1} (COO^-, C=C).

^1H NMR (400.13 MHz, D_2O): δ = 0.63–0.68 (m, 3 H, CH_3CH_2), 1.45, 1.47 (s, 3 H, CH_3), 1.96 (s, 3 H, SCH_3), 1.75–2.02 (m, 4 H, CH_2CH_3, SCH_2), 2.34–2.54 (m, 2 H, CH_2), 3.79–3.84 (m, 1 H, CH), 4.95 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 6.2, 6.3 (CH_2CH_3), 14.1, 14.2 (SCH_3), 22.7, 22.9 (CH_3), 29.7, 30.2, 30.3, 30.5 (CH_2), 59.8, 59.9 (CH), 78.2 (CCH_3), 94.2 (=CH), 175.8, 175.9, 176.5, 177.0, 177.1 (C=O, C=NH, =CN).

The doubling of all the NMR signals resulted from the two diastereomers.

Anal. Calcd for $C_{12}H_{20}N_2O_3S$: C, 52.92; H, 7.40; N, 10.29; S, 11.77. Found: C, 52.89; H, 7.64; N, 10.54; S, 11.94.

(2*S*)-2-[(2-Iminio-1-oxaspiro[4.4]non-3-en-4-yl)amino]-4-(methylsulfanyl)butanoate (11c)

Yield: 0.264 g (93%); yellow microcrystalline powder; mp 169–171 °C; $[\alpha]_D^{25}$ –28.0 (*c* 0.01, EtOH).

IR (KBr): 3450–2650 with maxima at 3216, 3052, 2962, 2930, 2875 (NH, $=^+NH_2$, C=CH, CH), 1681, 1612 cm^{-1} (COO^-, C=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.81 (m, 4 H, CH_2), 1.97 (s, 3 H, SCH_3), 1.92–2.07 (m, 6 H, SCH_2, CH_2), 2.38–2.54 (m, 2 H, CH_2), 3.86 (dd, *J* = 4.74, 8.70 Hz, 1 H, CH), 4.96 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 14.3 (SCH_3), 24.4, 24.5, 29.9, 30.6, 37.3, 37.9 (CH_2), 60.0 (CH), 78.2 (C-cyclopentyl), 101.4 (=CH), 174.9, 176.1, 177.3 (C=O, C=NH, =CN).

Anal. Calcd for $C_{13}H_{20}N_2O_3S$: C, 54.91; H, 7.09; N, 9.85; S, 11.27. Found: C, 54.69; H, 7.28; N, 9.57; S, 11.04.

(2*S*)-2-[(2-Iminio-1-oxaspiro[4.5]dec-3-en-4-yl)amino]-4-(methylsulfanyl)butanoate (11d)

Yield: 0.238 g (80%); yellow microcrystalline powder; mp 183–185 °C; $[\alpha]_D^{25}$ –21.1 (*c* 0.01, EtOH).

IR (KBr): 3490–2600 with maxima at 3216, 3032, 2934, 2861 (NH, $=^+NH_2$, C=CH, CH), 1684, 1614 cm^{-1} (COO^-, C=C).

^1H NMR (400.13 MHz, D_2O): δ = 1.26–1.29, 1.57–1.87 (m, 10 H, CH_2), 2.05 (s, 3 H, SCH_3), 2.01–2.17 (m, 2 H, SCH_2), 2.44–2.62 (m, 2 H, CH_2), 3.91 (dd, *J* = 4.74, 8.45 Hz, 1 H, CH), 4.99 (s, 1 H, =CH).

^{13}C NMR (100.62 MHz, D_2O): δ = 14.0 (SCH_3), 21.1, 21.2, 23.2, 29.5, 30.3, 32.5, 33.1 (CH_2), 59.6 (CH), 76.7 (C-cyclohexyl), 92.9 (=CH), 176.01, 176.69, 176.92 (C=O, C=NH, =CN).

Anal. Calcd for $C_{14}H_{22}N_2O_3S$: C, 56.35; H, 7.43; N, 9.39; S, 10.74. Found: C, 56.58; H, 7.26; N, 9.10; S, 10.58.

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