Synthesis of L-(+)-Ergothioneine

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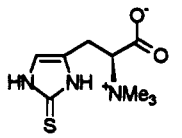
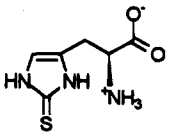
The first synthesis of L-(+)-ergothioneine (**1**), a rare natural amino acid, is described. The key step is the direct transformation of the imidazole derivative **11** into imidazole-2-thione **12**. This reaction consists of the cleavage and the re-formation of imidazole ring (ANRORC) with phenyl chlorothionoformate *via* a Bamberger-type intermediate. The conditions used are mild enough to preserve the asymmetric center at the α-carbon. The release of enantiomerically pure L-ergothioneine (**1**) from the ammonium derivative **15** was performed under acidic conditions to avoid the very easy racemization of the betaine function. An efficient and high-yield synthesis of 2-mercapto-L-histidine (**2**) which uses the new imidazole-2-thione formation reaction is also described.

L-(+)-Ergothioneine (**1**) is a natural amino acid discovered in the fungus *Claviceps purpurea*.[1] It is ubiquitously present in cells and tissues of most plants and mammalian species.[2] In human, L-ergothioneine is found in red blood cells, liver, kidney, brain, seminal fluid, and cataract-free lenses at concentrations which range from 100 μM to 2 mM.[3] However, it has been shown that ergothioneine is exclusively biosynthesized in fungi and mycobacteria. Hence, human populations would assimilate it through dietary intake. L-Ergothioneine has been proven to act as an antioxidant *in vivo*[4] and to afford protection from γ and UV radiation[5] as well as from singlet oxygen *in vitro*.[6] Recently, Arduini et al.[7] have shown that L-ergothioneine protects isolated perfused heart against the deleterious effect of post-ischemic reperfusion.

L-Ergothioneine is actually the only known naturally occurring compound with an imidazole-2-thione moiety.[8] It is a very hydrophilic amino acid whose thione group is under tautomeric thiol/thione equilibrium.[9] The thione form predominates largely at physiological pH, and this explains its stability toward oxidative dimerization in aerated aqueous solutions, a striking difference compared to biologically occurring alkylmercaptans such as cysteine or glutathione. In spite of its relatively simple structure, the synthesis of L-ergothioneine has proved to be difficult.[10] Two major problems must be resolved: (1) the preparation of the imidazole-2-thione moiety which remains a challenge due to the limited availability of the corresponding starting material;[11] (2) the very easy racemization of the asymmetric center during the formation of the betaine moiety due to the acidity of the α-carbon. All reported attempts to synthesize ergothioneine in the literature use 2-mercaptohistidine **2** as an intermediate. This intermediate represents in itself an interesting molecule. It has been used for the introduction of conformational constraints into peptides *via* thioether linkage[12] and for the preparation of the bactericidal products in combination with penicillanic acid and with cephalosporin derivatives.[13] Heath et al.[14] described the first synthesis of ergothioneine **1** from L-histidine methyl ester. Intermediate **2** was obtained by the degradation of the imidazole ring of L-histidine followed by cyclization of an α-amino ketone derivative with potassium thiocyanate. The transformation of **2** into ergothioneine afforded a partially racemized product (ee = 37%). Sunko and Wolf[15] have accomplished a formal synthesis of (±)-ergothioneine by preparing racemized 2-mercaptohistidine ((±)-**2**) in a new way in which the histidine chain was constructed from a preformed imidazole-2-thione system. Some other syntheses of intermediate **2** have been reported.[10,16] They were either too elaborate or had very poor overall yields.



1, L-ergothioneine



2, 2-mercapto-L-histidine

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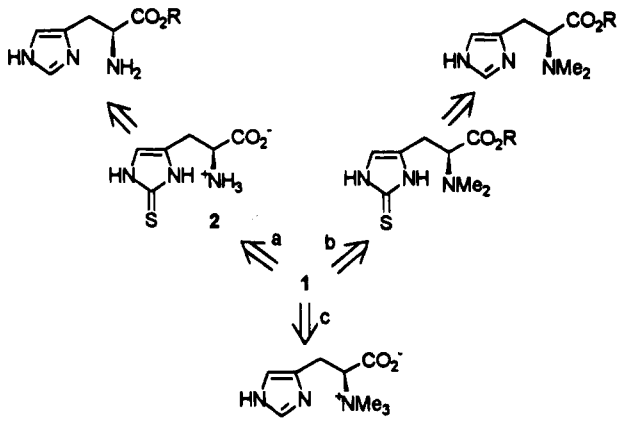
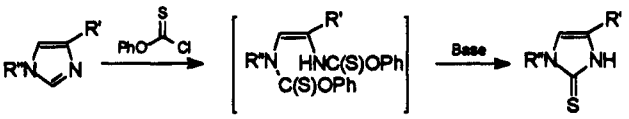


Figure 1.

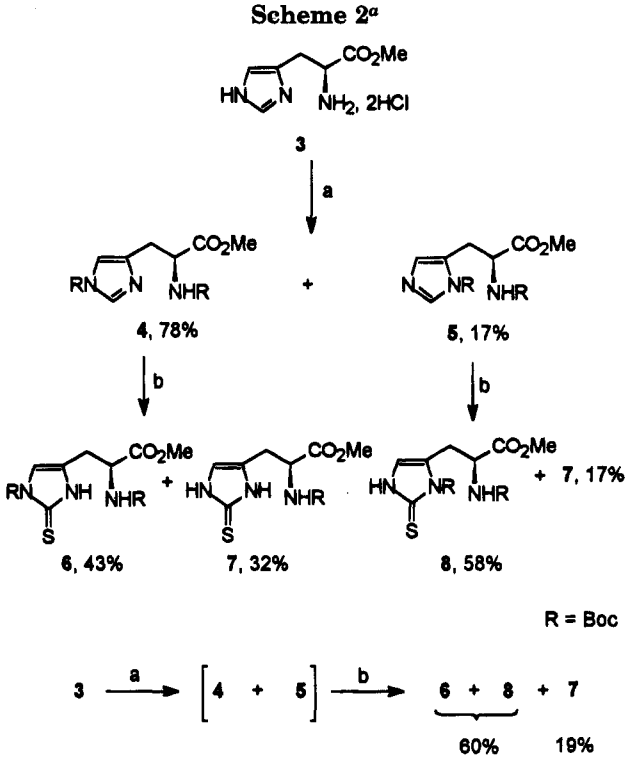
Scheme 1



As a part of our efforts to find an efficient approach for the synthesis of L-ergothioneine[17] and its analogs, we endeavored to develop a new and convenient methodology allowing the direct preparation of functionalized imidazole-2-thiones from the corresponding imidazoles.[18] This method consists of the sequential cleavage and reformation of the imidazole ring with phenyl chlorothionoformate *via* a Bamberger-type[19] intermediate: an ANRORC process[20] (addition of nucleophile, ring opening, ring closure) (Scheme 1).

The reaction can be carried out in two ways: method A is a "one-pot" procedure in which the corresponding imidazole is treated with phenyl chlorothionoformate in aqueous THF while being heated in the presence of a base; method B is a "two-step" procedure based on sequential ring-opening and ring-closure in different reaction media. The starting imidazoles can bear various functional groups. With this methodology in hand, three retrosynthetic strategies (Figure 1) could be considered for the preparation of L-ergothioneine (**1**). The most classic one (pathway a) starts from the synthesis of 2-mercapto-L-histidine (**2**) from which Heath's synthetic sequence is used to yield ergothioneine. This is followed by pathway b which consists of a partial methylation of the α-amino group before the construction of an imidazole-2-thione moiety, and the betaine function is finally produced. The most straightforward synthetic scheme (pathway c) was assumed to be the direct application of our sulfur introduction methodology with *N,N,N*-trimethylhistidine (hercynine). However, treatment of hercynine[21] with phenyl chlorothionoformate failed to afford the desired product. In this report, we describe a new and rapid synthesis of 2-mercapto-L-histidine (**2**) and then the first synthesis of enantiomerically pure L-ergothioneine (**1**) according to the second retrosynthetic pathway (pathway b).

Scheme 2[a]



[a] Reaction conditions: (a) Boc_2O, $NaHCO_3$, H_2O/THF, rt; (b) (i) ClC(S)OPh, $NaHCO_3$, H_2O/Et_2O, rt, (ii) TEA, MeOH, rt.

Results and Discussion

Synthesis of 2-Mercapto-L-histidine (2). Commercially available and optically active L-histidine methyl ester (**3**) was used as starting material. Attempts to convert **3** directly into its 2-mercapto analog with phenyl chlorothionoformate failed, probably because of the different reaction sites of the starting derivative. Consequently, the α-amino group and imidazole NH were both protected with a Boc group. Treatment of **3** with di-*tert*-butyl dicarbonate (Boc_2O) and $NaHCO_3$ in aqueous THF at room temperature gave a mixture of two isomers which were separated by chromatography on silica gel (Scheme 2). The first eluted product was the 1,4-disubstituted imidazole compound **4**[22] (78%) and the second was the 1,5-disubstituted one **5** (17%). The sulfur introduction reaction was performed respectively with **4** and **5** using method B. Treatment of **4** with 1.2 equiv of phenyl chlorothionoformate and $NaHCO_3$ in ether/water gave the cleavage products which were treated with triethylamine in methanol at room temperature. Di-Boc-mercaptohistidine derivative **6** was isolated (43%) along with mono-Boc-mercaptohistidine derivative **7** (32%). The latter was suggested to be the methanolyzed product of **6**. The same procedure used for **5** gave a similar result, and **8** (58%) and **7** (17%) were obtained. The mercaptohistidine compounds **6**, **7**, and **8** can also be prepared directly from **3** without isolation of the intermediate products **4** and **5** (Scheme 2). Thus, successive treatment of **3** with (1) di-*tert*-butyl dicarbonate in aqueous THF, (2) phenyl chlorothionoformate in ether/H_2O, and (3) triethylamine in methanol gave, after purification by chromatography, a 3:1 mixture of **6** and **8**, as shown by the ^1H NMR

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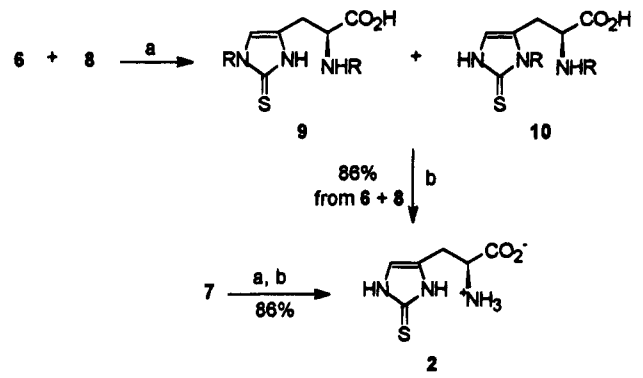
Scheme 3[a]



[a] Reaction conditions: (a) $LiOH·H_2O$, THF/H_2O, 0 °C to rt; (b) HCl, CH_2Cl_2, rt.

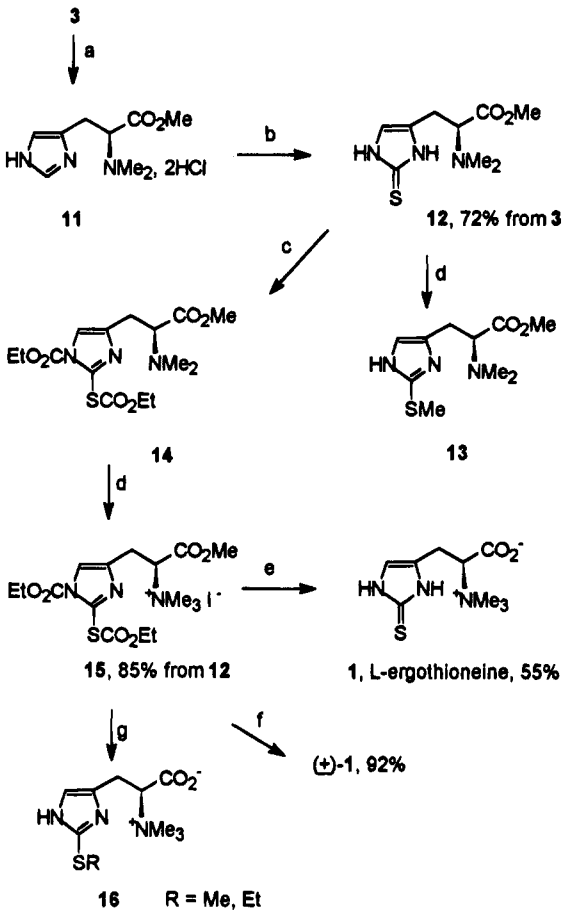
spectrum, in 60% yield, together with 19% of **7**. The 3:1 mixture of **6** and **8** was saponified with LiOH in THF/H_2O[23] at room temperature, and the Boc groups were removed by bubbling gaseous HCl in the CH_2Cl_2 solution of **9** and **10**[24] (Scheme 3). 2-Mercapto-L-histidine (**2**) was obtained after recrystallization from water in high yield (86%). Similarly, **7** was treated with LiOH and then gaseous HCl to give the desired 2-mercapto-L-histidine (**2**) in good yield (86%).

The 2-mercapto-L-histidine (**2**) thus obtained gave satisfactory analytical and physical data. The $[\alpha]_D$ was −10.2° (*c* 1.00, 1 N HCl) as compared with $[\alpha]_D$ −10.6° and $[\alpha]_D$ −9.5° (*c* 2.01, 1 N HCl) reported respectively by Ito[16b] and Ashley et al.[10a] This indicates that our synthetic product was enantiomerically pure and that the successive steps in our synthetic sequence, in particular the sulfur introduction reaction, caused no racemization.

Synthesis of L-Ergothioneine (1). As mentioned above, the unique synthesis of ergothioneine described in the literature had been accomplished *via* 2-mercapto-L-histidine (**2**). The overall yield was poor, and the final product was racemized to a considerable extent. The methylation step for the formation of betaine was responsible for this racemization.[14] In an original synthetic plan (Figure 1, pathway b), we envisaged avoiding use of 2-mercapto-L-histidine (**2**) as an intermediate. Accordingly, L-histidine methyl ester (**3**) was used as starting material. The generation of the imidazole-2-thione moiety could be performed on the substrate with the α-amino group dimethylated. This functionality has the advantage of being the precursor of the trimethylammonium function at a later stage.

The N,N-dimethylation of L-histidine methyl ester dihydrochloride (**3**) (Scheme 4) was performed with the reductive methylation procedure according to Reinhold et al.[21] Compound **3** was treated with formaldehyde in water and then hydrogenated for 5 h at room temperature in the presence of 10% Pd/C. Dimethylamino derivative **11** was obtained quantitatively and was subjected to the sulfur introduction reaction without further purification. The sulfur introduction reaction with **11** was first performed by application of method A with phenyl chlorothionoformate and $NaHCO_3$ in THF/H_2O at 80 °C for 18 h. 2-Mercaptohistidine derivative **12** was isolated in 49% yield. However, this product was

Scheme 4[a]



[a] Reaction conditions: (a) 37% aqueous CH_2O, H_2, 10% Pd/C, H_2O, rt; (b) (i) ClC(S)OPh, $NaHCO_3$, H_2O/Et_2O, rt, (ii) TEA, MeOH, rt; (c) $ClCO_2Et$, TEA, CH_2Cl_2, 10 °C; (d) MeI, THF, rt; (e) concd HCl, 75 equiv of $HSCH_2CH_2CO_2H$, 110 °C; (f) TEA, H_2O/MeOH, 60 °C; (g) 6 N HCl, 110 °C.

Table 1. Effects of Temperature on the Reaction of 11 with ClC(S)OPh[a]

entry	temp (°C)	time (h)	yield[b] (%)	product ee[c] (%)
1	80	24	52	20
2	60	29	52	66
3	40	41	46	90
4	30	114	46	86
5	20	114	41	>98

[a] Reaction carried out with 2.5 equiv of ClC(S)OPh and 8.0 equiv of $NaHCO_3$ in aqueous THF. [b] Isolated yields based on **11**. [c] Determined by 200-MHz ^1H NMR with chiral shift reagent Eu(tfc)$_3$.

partially racemized (ee = 20%) as determined by ^1H NMR mesurement in the presence of the chiral shift reagent Eu(tfc)$_3$. When performed at room temperature, the reaction yield was similar but the reaction time increased dramatically. Most importantly, the isolated product was almost enantiomerically pure. Table 1 details the extent of racemization as a function of temperature.

Much more satisfactory results were obtained with the method B. Compound **11** was treated with phenyl chlorothionoformate and $NaHCO_3$ at room temperature for 16 h in ether/H_2O. The crude product obtained after evaporation of the solvents was then treated with triethylamine in methanol at room temperature. This procedure allowed the isolation of desired product **12** in 81% yield. It was slightly racemized (ee = 96%). The pure enantiomer of **12** was obtained by mixing the

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slightly racemized product with CH_2Cl_2 and filtration. In this way, the racemate, being insoluble in CH_2Cl_2, was eliminated. To further methylate the dimethylamino group to provide the trimethylammonium moiety of the future betaine function, it was necessary to protect the imidazole-2-thione system because direct methylation of **12** with various methylating agents led first to sulfur methylated product **13**. The protection of imidazole-2-thione was carried out with ethyl chloroformate and triethylamine in CH_2Cl_2 at 10 °C to give quantitatively protected product **14**. Methylation with MeI[25] at room temperature in anhydrous THF gave crystalline ammonium derivative **15** in 85% yield (Scheme 4). Other protecting groups, such as Boc, CO_2vinyl, CO_2Ph, *etc.*, could also be used, but our initial choice had the advantage of giving directly pure crystallized product. The realization of the last step which consists of the release of L-ergothioneine without racemization of the α-carbon proved to be very difficult. Under alkaline (LiOH, H_2O/THF, 0 °C; TEA, H_2O/MeOH, 60 °C[26] or rt; Na_2CO_3, H_2O, rt, respectively) or neutral conditions[27] [$(nBu_3Sn)_2O$, THF, rt, 21 days], the hydrolysis of **15** yielded racemized ergothioneine (±)-**1**. For instance, when **15** was treated with TEA in H_2O/MeOH at 60 °C for 45 h, (±)-**1** was isolated in 89% yield. This is in contradiction to the results reported by Konno et al.[26] who obtained optically active ergothioneine analogs in a saponification step under the same conditions. Even $NaHCO_3$ can cause racemization. Thus, a solution of **15** in D_2O in the presence of 1 N $NaHCO_3$ showed progessive H/D exchange at the α-carbon as monitored by ^1H NMR. These observations confirmed the very easy racemization of the α-trimethylammonio−carboxylic ester system. Hydrolysis under acidic conditions[28] to prepare L-ergothioneine was then investigated. Hydrolysis of **15** with 6 N HCl, after heating at reflux temperature, gave S-alkylated ergothioneine **16**. Addition of an appropriate mercaptan (β-mercaptopropionic acid, cysteine) or thiourea or its derivatives (imidazolidine-2-thione, *etc.*) as carbonium ion trap largely prevented this side reaction and allowed the isolation of L-ergothioneine **1** as the major product. For practical reasons, β-mercaptopropionic acid was chosen, because of its extractability in ether and moderate odor. Thus, when **15** was treated with concentrated HCl and β-mercaptopropionic acid (75 equiv) at reflux temperature for 26 h, enantiomerically pure L-ergothioneine **1** was isolated in 55% yield (Scheme 4).

Our synthetic ergothioneine gave correct analysis and behaved like the natural product[17] by ^1H NMR, ^{13}C NMR, mp, and TLC comparison. However, the magnitude of its optical rotation deserves some comments. Tanret[1] described an optical rotation of the isolated natural product $[\alpha]_D$ as +110° while Newton et al.[29] reported $[\alpha]_D$ as +116°. In our hand, when freshly recrystallized from 2:1 EtOH/H_2O, L-ergothioneine had an $[\alpha]^{25}_D$ of +109.5° (*c* 1.0, H_2O), and the elementary analysis corresponded to the dihydratated form. Upon drying *in vacuo* over P_2O_5, we obtained an anhydrous form according to the elementary analysis with an $[\alpha]^{25}_D$ of +126.6° (*c* 1.0, H_2O). It has previously been reported that the anhydrous ergothioneine took water when exposed to air.[1] We therefore assume that the hygroscopic properties of ergothioneine may alter the analytical results, which could explain the above discrepancies.

In summary, the efficient methodology developed in our laboratory for the direct preparation of imidazole-2-thiones from the corresponding imidazoles is of general synthetic importance. We have used this method to provide a simple and high overall yield synthesis of 2-mercapto-L-histidine (**2**). The synthesis of enantiomerically pure L-ergothioneine **1** has been achieved for the first time using this sulfur introduction reaction as a key step. The overall yield was good (34%), which enabled us to prepare L-ergothioneine **1** on a large scale.

Experimental Section

General. Tetrahydrofuran was distilled from sodium benzophenone ketyl under nitrogen. Methylene chloride was distilled from P_2O_5. Analytical thin-layer chromatography (TLC) was performed on 0.25 mm silica gel aluminum-supported plates. Preparative column chromatography was performed on silica gel 60 (0.040−0.063 mm). Melting points were uncorrected. Optical rotations were measured at 25 °C. ^1H and ^{13}C NMR spectra were recorded at 200 and 50 MHz, respectively. Chemical shifts were reported in ppm (δ) with $CHCl_3$, acetone, DMSO, or H_2O as internal standards. Mass spectra and high-resolution mass spectra (HRMS) were recorded on a Nermag R10-10B instrument. The ionization mode used in mass spectra was electron impact (EI) at 70 eV, chemical ionization (CI) in ammonia, or fast atom bombardment (FAB) on a glycerol matrix. Selected fragment ions were reported as their mass/charge ratio (*m/z*) followed by their relative intensities as compared to the base fragment in parentheses. Microanalysis were performed at Service de Microanalyse d'ICSN, Gif sur Yvette, France.

Methyl (*S*)-3-[1-(1,1-Dimethylethoxycarbonyl)-1*H*-imidazol-4-yl]-2-[(1,1-dimethylethoxycarbonyl)amino]propionate (4) and Methyl (*S*)-3-[1-(1,1-Dimethylethoxycarbonyl)-1*H*-imidazol-5-yl]-2-[(1,1-dimethylethoxycarbonyl)amino]propionate (5). To a mixture of L-histidine methyl ester dihydrochloride (**3**) (2.42 g, 10 mmol), H_2O (20 mL), and THF (40 mL) was added in small portions Na_2CO_3 (9.33 g, 88 mmol). Di-*tert*-butyl dicarbonate (10.0 g, 46 mmol) was then added. The mixture was stirred vigorously at room temperature for 1.5 h. The aqueous layer was separated from the organic layer and extracted with ethyl acetate (30 mL). The combined organic solutions were evaporated *in vacuo*, and the residue was purified directly by flash chromatography (50% EtOAc/cyclohexane) to give **4** (5.79 g, 78%) as a colorless solid [R_f 0.31 (50% EtOAc/cyclohexane), mp 105−107 °C (EtOAc/cyclohexane); $[\alpha]_D$ + 23.3° (*c* 1.00, CH_2Cl_2) (lit.[21] mp 85−88 °C, $[\alpha]^{25}_D$ +19.9° (*c* 1.16, $CHCl_3$)); IR ($CHCl_3$) 3435, 1750, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.57 (s, 9 H), 3.02 (d, *J* = 5.3 Hz, 2 H), 3.70 (s, 3 H), 4.55 (m, 1 H), 5.68 (br d, *J* = 8.1 Hz, 1 H), 7.11 (d, *J* = 1.0 Hz, 1H), 7.96 (d, *J* = 1.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.5, 30.4, 52.5, 53.4, 80.0, 85.9, 115.0, 137.4, 139.1, 147.4, 156.0, 172.9; MS (EI) *m/z* 369 (M^+, 4), 313 (23), 257 (30), 196 (20), 152 (36), 82 (31), 57 (100); HRMS calcd for $C_{17}H_{27}N_3O_6$ 369.1900, found 369.1917] and compound **5** (1.22 g, 17%) as a colorless oil [R_f 0.22 (50% EtOAc/cyclohexane); $[\alpha]_D$ −3.1° (*c* 1.30, CH_2Cl_2); IR ($CHCl_3$) 3440, 1750, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (s, 9 H), 1.57 (s, 9 H), 3.08 (dd, *J* = 8.8, 15.1 Hz, 1 H), 3.03 (dd, *J* = 5.1, 15.1 Hz, 1 H), 3.67 (s, 3 H), 4.56 (m, 1 H), 5.15 (d, *J* = 8.4 Hz, 1 H), 6.77 (s, 1 H), 7.97 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 28.1, 28.4, 29.0, 52.7, 52.9, 80.3, 86.3, 127.8, 131.4, 139.3, 148.3, 155.6, 172.9; MS (EI) *m/z* 369 (M^+, 4), 313 (7), 252 (58), 240 (2), 213 (8), 196 (30), 152 (100), 82 (45), 57 (99); HRMS calcd for $C_{17}H_{27}N_3O_6$ 369.1900, found 369.1895.

Methyl (*S*)-3-[1-(1,1-Dimethylethoxycarbonyl)-2-mercapto-1*H*-imidazol-4-yl]-2-[(1,1-dimethylethoxycarbonyl)amino]propionate (6) and Methyl (*S*)-2-[(1,1-dimethyl-

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ethoxycarbonyl)amino]-3-(2-mercapto-1*H*-imidazol-4-yl)propionate (7) from 4. To a mixture of **4** (5.30 g, 14.4 mmol) in ether (100 mL), distilled water (100 mL), and $NaHCO_3$ (7.24 g, 86.2 mmol) was added dropwise, under vigorous stirring, phenyl chlorothionoformate[30] (2.73 g, 16 mmol) at room temperature. The reaction mixture was stirred for 15 h, and the organic layer was separated. The aqueous layer was extracted with ether (50 mL). The combined organic solutions were washed with brine and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave a yellow oil which was then dissolved in methanol (45 mL) and treated with triethylamine (6.2 mL, 44.5 mmol) at room temperature under nitrogen for 22 h. The mixture was evaporated *in vacuo*, and the resulting residue was purified by flash chromatography (EtOAc/cyclohexane) to give **6** (2.49 g, 43%) as a white solid [R_f 0.28 (50% EtOAc/cyclohexane); mp 58−60 °C (EtOAc/cyclohexane); $[\alpha]_D$ −30.0° (*c* 1.01, MeOH); IR ($CHCl_3$) 3460, 3180, 1790, 1765, 1735, 1515, 1475 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.59 (s, 9 H), 2.83 (dd, J = 7.2, 15.4 Hz, 1 H), 3.03 (dd, J = 5.1, 15.4 Hz, 1 H), 3.75 (s, 3 H), 4.46 (m, 1 H), 5.26 (br s, 1 H), 6.93 (s, 1 H), 11.20 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 27.0, 28.0, 28.4, 52.5, 53.1, 80.7, 86.2, 114.4, 125.1, 147.5, 155.8, 165.1, 172.1; MS (EI) *m/z* 401 (M^+, 4), 357 (24), 301 (28), 245 (100), 201 (29), 184 (32), 114 (56); HRMS calcd for $C_{17}H_{27}N_3O_6S$ 401.1620, found 401.1595] and compound **7** as a white solid (1.40 g, 32%) [R_f 0.43 (EtOAc); mp 73−75 °C (EtOAc/cyclohexane); $[\alpha]_D$ −31.2° (*c* 1.05, EtOH); IR ($CHCl_3$) 3440, 3130, 1740, 1710, 1630, 1490 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.34 (s, 9 H), 2.63 (dd, J = 9.9, 14.7 Hz, 1 H), 2.78 (dd, J = 5.1, 14.7 Hz, 1 H), 3.60 (s, 3 H), 4.20 (m, 1 H), 6.50 (s, 1 H), 7.23 (d, J = 8.1 Hz, 1 H), 11.72 (br s, 1 H), 11.85 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 26.2, 28.1, 52.0, 52.8, 78.7, 113.3, 125.1, 155.7, 160.7, 172.5; MS (EI) *m/z* 301 (M^+, 28), 259 (19), 245 (30), 218 (16), 215 (5), 196 (61), 127 (83), 114 (66), 44 (100); HRMS calcd for $C_{12}H_{19}N_3O_4S$ 301.1097, found 301.1075.

Methyl (*S*)-3-[3-(1,1-Dimethylethoxycarbonyl)-2-mercapto-1*H*-imidazol-4-yl]-2-[(1,1-dimethylethoxycarbonyl)amino]propionate (8) and Methyl (*S*)-3-(2-Mercapto-1*H*-imidazol-4-yl)-2-[(1,1-dimethylethoxycarbonyl)amino]propionate (7) from 5. Di-Boc-histidine **5** (1.22 g, 3.3 mmol) was subjected to the above sulfur introduction procedure to yield **8** as a white solid (0.77 g, 58%) [R_f 0.20 (50% EtOAc/hexane); mp 57−59 °C (EtOAc/cyclohexane); $[\alpha]_D$ −56.5° (*c* 1.42, EtOH); IR ($CHCl_3$) 3440, 3140, 1745, 1735, 1500, 1465 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (s, 9 H), 1.61 (s, 9 H), 2.98 (dd, J = 9.0, 15.7 Hz, 1 H), 3.20 (dd, J = 4.8, 15.7 Hz, 1 H), 3.70 (s, 3 H), 4.45 (m, 1 H), 5.20 (d, J = 8.3 Hz, 1 H), 6.47 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 28.0, 28.2, 29.2, 52.8, 52.9, 80.7, 87.5, 114.8, 126.1, 148.7, 155.9, 164.9, 172.6; MS (EI) *m/z* 457 [(M + 56), 7], 357 (22), 301 (31), 245 (100), 201 (32), 184 (23), 114 (42), 57 (45)] and the already described **7** (0.17 g, 17%).

6, 7, and 8 Directly from 3. To a mixture of L-histidine methyl ester dihydrochloride (**3**) (2.42 g, 10 mmol), H_2O (10 mL), and THF (20 mL) was added in small portions $NaHCO_3$ (4.20 g, 50 mmol). Di-*tert*-butyl dicarbonate (4.80 g, 22 mmol) was then added. After 2 h of stirring at room temperature, phenyl chlorothionoformate (1.90 g, 11 mmol) was added dropwise. The reaction mixture was stirred vigorously at room temperature for 4 h. The aqueous layer was separated and extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with brine and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave a yellow oil which was treated in methanol (30 mL) with triethylamine (3.12 g, 31 mmol) at room temperature for 16 h. The reaction mixture was evaporated *in vacuo* to dryness, and the residue was purified by flash chromatography (EtOAc/cyclohexane) to give a 3:1 mixture of **6** and **8** as a colorless oil (2.41 g, 60%) and **7** (0.56 g, 19%).

2-Mercapto-L-histidine (2). (A) From 6 and 8. To an ice-cooled solution of the above 3:1 mixture of **6** and **8** (2.05 g, 5.10 mmol) in THF (15 mL) was added a cold solution of $LiOH{\cdot}H_2O$ (500 mg, 11.9 mmol). The resulting mixture was stirred at room tempearture until the reaction was complete (18 h) and was then acidified with 1 N aqueous HCl to pH 4−5. The mixture was extracted with ethyl acetate (2 ± 75 mL). The combined organic layers were washed with brine and dried ($MgSO_4$). After evaporation of the solvent, the crude acid was taken up in CH_2Cl_2 (20 mL) and gaseous HCl was bubbled in for 15 min at room temperature. After 1 h, the solvent was removed *in vacuo* and the residue was dissolved in water (25 mL). The pH of the solution was adjusted to 5−6 with a dilute aqueous solution of ammonia (7%). The 2-mercapto-L-histidine (**2**) started to crystallize. After 14 h at 4 °C, filtration and washing with cold water gave the pure desired product (0.62 g) as a white solid. The filtrate was concentrated to about 5 mL and cooled at 4 °C overnight to afford an additional 0.20 g of **2** (total yield: 0.82 g, 86%): mp 311 °C (H_2O, dec); $[\alpha]_D$ −10.2° (*c* 1.0, 1 N HCl) [lit. mp > 280 °C; $[\alpha]_D$ −10.6° (*c* 1.0, 1 N HCl)[16b]; mp > 310 °C, $[\alpha]_D$ −9.5° (*c* 2.01, 1 N HCl)[10a]; IR (KBr) 3210, 3150, 1650, 1625, 1505, 1495 cm^{-1}; ^1H NMR (D_2O + DCl) δ 2.98 (dd, J = 7.3, 15.8 Hz, 1 H), 3.09 (dd, J = 5.9, 15.8 Hz, 1 H), 4.13 (dd, J = 5.9, 7.3 Hz, 1 H), 6.69 (s, 1 H); ^{13}C NMR (D_2O + DCl) δ 27.8, 54.4, 118.8, 126.0, 159.3, 173.2; MS (FAB) *m/z* 188 (MH^+). Anal. Calcd for $C_6H_9N_3O_2S$: C, 38.49; H, 4.84; N, 22.44; S, 17.13. Found: C, 38.63; H, 4.82; N, 22.48; S, 16.93.

(b) From 7. Compound **7** (602 mg, 2 mmol) was saponified and deprotected according to the procedure described above for **6** and **8** using ethyl acetate as solvent instead of CH_2Cl_2, affording 2-mercapto-L-histidine (**2**) (320 mg, 86%). The latter was equally enantiomerically pure according to its $[\alpha]_D$ −9.7° (*c* 1.02, 1 N HCl).

***N,N*-Dimethyl-L-histidine Methyl Ester (11).** To a solution of L-(+)-histidine methyl ester dihydrochloride (**3**) (18.16 g, 75 mmol) in 150 mL of deionized water was added an aqueous solution of formaldehyde (37%, 12.29 g, 150 mmol). The mixture was hydrogenated under a pressure of 5 atm of hydrogen in the presence of 10% Pd/C (1.0 g) for 5 h at room temperature. The catalyst was filtered off and then rinsed with water; the filtrate was evaporated to dryness *in vacuo* to give the expected product **11** as a colorless oil (20.3 g), which was directly used in the next step without further purification: ^1H NMR (D_2O) δ 2.91 (s, 6 H), 3.50 (m, 2 H), 3.72 (s, 3 H), 4.48 (dd, J = 5.5, 9.0 Hz, 1 H), 7.38 (s, 1 H), 8.61 (s, 1 H).

Methyl (*S*)-3-(2-Mercapto-1*H*-imidazol-4-yl)-2-dimethylamino)propionate (12). Method A. To a solution of *N,N*-dimethyl-L-histidine methyl ester dihydrochloride (**11**) (0.54 g, 2 mmol) in 10 mL of water was added slowly sodium bicarbonate (1.34 g, 16 mmol), followed by 10 mL of THF. Phenyl chlorothionoformate (0.69 mL, 5 mmol) was then added dropwise over 15 min at room temperature. The reaction mixture was stirred vigorously at the temperature and for the duration indicated in Table 1. After evaporation *in vacuo* to dryness, the crude product was directly chromatographed on silica gel (EtOAc/cyclohexane) to give the title compound. The ee was determined by ^1H NMR using a solution of 3 mg of product dissolved in 1 mL of $CDCl_3$ in the presence of 10 mg of $Eu(tfc)_3$.

Method B. To a solution of *N,N*-dimethyl-L-histidine methyl ester dihydrochloride (**11**) (21.5 g, 80 mmol) in 320 mL of water was added slowly sodium bicarbonate (53.8 g, 0.64 mol), followed by 320 mL of ether. Phenyl chlorothionoformate (28.8 mL, 208 mmol) was then added dropwise over 30 min. The reaction mixture was stirred vigorously at room temperature for 5 h. The organic layer was separated. The aqueous layer was extracted with ether (50 mL). The combined organic solutions were washed with brine and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave a yellow oil which was then dissolved in methanol (300 mL) and treated with triethylamine (34.5 mL, 248 mmol) at room temperature under nitrogen for 18 h. The solvent was evaporated *in vacuo*, and the residue was added to 150 mL of ethyl acetate and cooled at 4 °C overnight. The precipitate was filtered and rinsed with cold ethyl acetate to afford the first portion of **12** (8.16 g). The filtrate was evaporated to dryness *in vacuo* and purified by chromatography on silica gel (EtOAc/cyclohexane) to give the

(30) Phenyl chlorothionoformate is commercially available. It can be easily prepared in bulk amount by treatment of thiophosgene with phenol: (a) Rivier, H. *Bull. Soc. Chim. Fr.* **1906**, *35*, 837. (b) Kenji, T. JP Patent 03193759, 1991; *Chem. Abstr.* **1992**, *116*, 58982.

second portion of desired **12** (6.64 g). The total yield was 14.80 g (81%). It was mixed with 370 mL of CH_2Cl_2 and stirred at room temperature for 5 min. The insoluble solid was filtered. The filtrate was evaporated *in vacuo* to dryness to afford the enantiomerically pure **12** (13.3 g, 72%) as a white solid: R_f 0.12 (EtOAc); mp 170–171 °C (EtOAc); $[\alpha]_D$ +31.2° (*c* 1.0, MeOH); IR ($CHCl_3$) 3460, 3155, 1735, 1635, 1480 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.38 (s, 6 H), 2.81 (d, J = 7.0 Hz, 2 H), 3.46 (t, J = 7.0 Hz, 1 H), 3.70 (s, 3 H), 6.49 (s, 1 H), 10.77 (br s, 1 H), 11.26 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 24.3, 41.7, 51.9, 66.6, 113.1, 127.7, 159.4, 171.5; MS (EI) *m/z* 229 (M^+, 25), 170 (8), 116 (100). Anal. Calcd for $C_9H_{15}N_3O_2S$: C, 47.14; H, 6.59; N, 18.32; S, 13.99. Found: C, 47.26; H, 6.35; N, 18.39; S, 13.85.

Methyl (*S*)-3-[1-(Ethoxycarbonyl)-2-[(ethoxycarbonyl)-thio]-1*H*-imidazol-4-yl)]-2-(dimethylamino)propionate (14). Triethylamine (5.85 mL, 42.0 mmol) was added to a solution of methyl (*S*)-3-(2-mercapto-1*H*-imidazol-4-yl)-2-(dimethylamino)propionate (**12**) (3.85 g, 16.8 mmol) in 80 mL of methylene chloride at 10 °C. Ethyl chloroformate (3.5 mL, 37.0 mmol) was added dropwise at this temperature. At the end of the addition, a precipitate of triethylamine hydrochloride appeared. The mixture was stirred for 0.5 h at 10 °C and then treated with 50 mL of water. The organic layer was separated, washed with water, and dried ($MgSO_4$). Evaporation of the solvent *in vacuo* gave the title compound **14** as a colorless oil (6.19 g, 99%) which was essentially pure as observed by TLC and ^1H NMR. An analytical sample was obtained by chromatography (50% EtOAc/cyclohexane): R_f 0.32 (EtOAc); $[\alpha]_D$ −5.8° (*c* 1.10, CH_2Cl_2); IR ($CHCl_3$) 1770, 1745 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (t, J = 7.2 Hz, 3 H), 1.40 (t, J = 7.2 Hz, 3 H), 2.33 (s, 6 H), 2.83 (dd, J = 6.5, 14.6 Hz, 1 H), 2.98 (dd, J = 8.5, 14.6 Hz, 1 H), 3.65 (dd, J = 6.5, 8.5 Hz, 1 H), 3.68 (s, 3 H), 4.28 (q, J = 7.2 Hz, 2 H), 4.42 (q, J = 7.2 Hz, 2H), 7.44 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.4, 28.0, 42.0, 51.6, 64.9, 65.2, 66.9, 120.2, 135.2, 140.7, 148.6, 167.4, 172.3; MS (EI) *m/z* 373 (M^+, 17), 314 (39), 116 (100); HRMS calcd for $C_{15}H_{23}N_3O_6S$ 373.1308, found 373.1319.

Methyl (*S*)-3-[1-(Ethoxycarbonyl)-2-[(ethoxycarbonyl)-thio]-1*H*-imidazol-4-yl]-2-(trimethylammoniopropionate Iodide (15). To a solution of methyl (*S*)-3-[1-(ethoxycarbonyl)-2-[(ethoxycarbonyl)thio]-1*H*-imidazol-4-yl]-2-(dimethylamino)propionate (**14**) (6.19 g, 16.8 mmol) in 60 mL of anhydrous THF was added iodomethane (2.0 mL, 32.1 mmol). The reaction mixture was stirred at room temperature for 24 h. The precipitate was filtered and rinsed with THF to give the title product **15** as a white solid (7.36 g, 85%): mp 136 °C (THF, dec); $[\alpha]_D$ + 34.6° (*c* 1.0, MeOH); IR ($CHCl_3$) 1775, 1750, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, J = 7.2 Hz, 3 H), 1.38 (t, J = 7.2 Hz, 3 H), 3.31 (dd, J = 9.6, 14.36 Hz, 1 H), 3.62 (s, 9 H superimposed on dd, J = 4.5, 14.4 Hz, 1 H), 3.73 (s, 3 H), 4.25 (q, J = 7.2 Hz, 2 H), 4.40 (q, J = 7.2 Hz, 2H), 4.80 (dd, J = 4.5, 9.6 Hz, 1 H), 7.38 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.2, 14.4, 27.0, 53.8, 54.1, 65.5, 73.9, 122.2, 135.6, 136.1, 148.2, 166.9, 167.5. MS (FAB) *m/z* 388 (M^+ − I). Anal. Calcd for $C_{16}H_{26}IN_3O_6S$: C, 37.29; H, 5.09; N, 8.16; S, 6.22. Found: C, 37.25; H, 4.81; N, 8.02; S, 5.91.

L-(+)-Ergothioneine (1). Hydrochloric acid (35%, 100 mL) was added to a mixture of methyl (*S*)-3-[1-(ethoxycarbonyl)-2-[(ethoxycarbonyl)thio]-1*H*-imidazol-4-yl]-2-(trimethylammonio)propionate iodide (**15**) (2.05 g, 4.0 mmol) and β-mercaptopropionic acid (30 g, 283 mmol). The solution was stirred under reflux for 26 h. The solvent was evaporated *in vacuo*, and the residue was treated with 50 mL of deionized water. The excess β-mercaptopropionic acid was extracted with ether (3 × 50 mL). The aqueous solution, whose pH was brought to 6–7 with a dilute aqueous solution of ammonia (7%), was again evaporated to dryness *in vacuo*. The residue was purified by chromatography on silica gel (MeOH and then 95% MeOH/H_2O). The desired product was obtained as a colorless solid (725 mg). It was further purified by recrystallization and drying over P_2O_5 *in vacuo* to give 500 mg (55%) of pure ergothioneine **1**: mp 262 °C (EtOH/H_2O, dec); $[\alpha]_D$ 115.6° (*c* 1.0, H_2O) [lit. mp 290 °C; $[\alpha]_D$ +110° (H_2O);[1,31] mp 262–263 °C, $[\alpha]_D$ +116° (H_2O)[29,31]]; IR (KBr) 3420, 3080, 1645, 1615, 1490 cm^{-1}; ^1H NMR (D_2O) δ 3.10 (m, 2 H), 3.19 (s, 9 H), 3.83 (dd, J = 4.56, 10.98 Hz, 1 H), 6.70 (s, 1 H); ^{13}C NMR (D_2O) δ 25.4, 54.8, 79.8, 118.1, 126.7, 158.9, 173.1; MS (FAB) *m/z* 230 (M + H). Anal. Calcd for $C_9H_{15}N_3O_2S$: C, 47.14; H, 6.59; N, 18.32; S, 13.99. Found: C, 46.79; H, 6.44; N, 18.01; S, 14.01.

D,L-Ergothioneine ((±)-1). Methyl (*S*)-3-[1-(ethoxycarbonyl)-2-[(ethoxycarbonyl)thio]-1*H*-imidazol-4-yl]-2-(trimethylammonio)propionate iodide (**15**) (0.85 g, 1.65 mmol) was dissolved in 25 mL of triethylamine/water/methanol (1:4:6). The resulting mixture was heated at 60 °C for 45 h and then evaporated *in vacuo* to dryness. The residue was recrystallized with 67% EtOH/H_2O to the racemized ergothioneine (±)-**1** (0.35 g, 92%): mp 275 °C (H_2O, dec); spectral data were identical in all respects with those of the above enantiomer.

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(31) The concentration of sample for the measurement of $[\alpha]_D$ was not given.