ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/231580456

Total synthesis of marine mercaptohistidines: Ovothiols A, B, and C

ARTICLE *in* THE JOURNAL OF ORGANIC CHEMISTRY · SEPTEMBER 1989

Impact Factor: 4.72 · DOI: 10.1021/jo00280a022

CITATIONS
25

READS
18

4 AUTHORS, INCLUDING:

Tod P Holler
University of Michigan
44 PUBLICATIONS **1,226** CITATIONS

SEE PROFILE

Available from: Tod P Holler
Retrieved on: 16 January 2016

Total Synthesis of Marine Mercaptohistidines: Ovothiols A, B, and C

Tod P. Holler, Fuqiang Ruan, Andreas Spaltenstein, and Paul B. Hopkins*

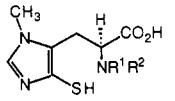
Department of Chemistry, University of Washington, Seattle, Washington 98195

Received March 3, 1989

Syntheses of ovothiols A and C in optically active form and ovothiols A and B in racemic form are reported. In all cases, synthesis of an S-protected mercaptoimidazole is followed by elaboration of an amino acid side chain.

Introduction

The ovothiols (1) are a family of 4-mercaptohistidine derivatives that are abundant in the eggs of marine invertebrates.[1] Unlike the 2-mercaptohistidine derivative ergothioneine, which has been known since early in this century, the ovothiols were first isolated only in the late 1970s. Obtained first as a chemical degradation product



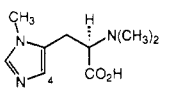
	R^1	R^2	Ovothiol
1a	H	H	A
1b	CH_3	H	B
1c	CH_3	CH_3	C

of adenochrome, a nonporphyrin iron-containing pigment found in the branchial heart of the common octopus,[2] ovothiols were subsequently isolated from the eggs of a variety of marine animals.[1,3] Most recently, the ovothiol substructure was identified in imbricatine, an alkaloid found in sea stars.[4] Also unlike ergothioneine, which exists primarily as a relatively unreactive thione tautomer, the ovothiols are quite reactive, for example, being oxidized more rapidly than glutathione by a variety of reagents.[5,6] It was recently suggested that the ovothiols might function as biological antioxidants,[7,8] scavenging reactive oxygen intermediates during early development of marine embryos. Some support for this hypothesis has been found.[9]

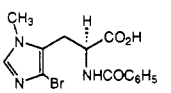
To unambiguously resolve a structural issue that had arisen in the course of assigning structures to the ovothiols,[10] we developed and reported in preliminary form syntheses of L-ovothiols A and C.[11] In the course of these studies, however, it became obvious that careful evaluation of the antioxidant activities of ovothiols would require quantities not easily accessible by our original synthesis and that, in many cases, racemates of ovothiols would suffice for these studies. For these reasons, we have developed a synthesis of racemic ovothiols applicable to all members of the family and practical for the preparation of gram quantities of ovothiols. Reported here are further details of both successful and unsuccessful efforts toward ovothiol synthesis.

Results and Discussion

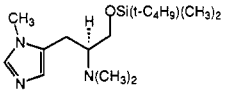
Our initial approach to synthesizing L-ovothiols was that of introducing the requisite methylation pattern and mercaptan function onto L-histidine, thus taking advantage of the naturally derived side-chain stereocenter. Methods for the controlled N-methylation of histidine are known,[12,13] reducing the problem to introduction of the mercaptan substituent at C-4 of the imidazole ring. Nitration of 2



2



3



4

afforded a 4-nitro derivative[14] that was reduced to an unstable amine, diazotized, and treated with potassium ethyl xanthate, affording only intractable products. The high stability of related imidazolediazonium ions has been previously overcome photochemically,[15] a modification that was in this case unproductive. Direct displacement of the nitro function using sodium *tert*-butanethiolate/DMF[16] and benzylmercaptan/trifluoroacetic acid likewise led to decomposition. The bromohistidine 3[17] failed to undergo direct displacement using sodium *tert*-butanethiolate/hexamethylphosphoric triamide at 135 °C[18] or palladium-catalyzed displacement using sodium *tert*-butanethiolate/catalytic tetrakis(triphenylphosphine)palladium in methanol,[19] both of these again returning starting material. One attempt to directly introduce the mercaptan by heating 2 in DMSO with elemental sulfur, a reaction precedented in the patent literature,[20] failed, affording

* Address correspondence to this author.

(1) Turner, E.; Klevit, R.; Hager, L. J.; Shapiro, B. M. *Biochemistry* **1987**, *26*, 4028.

(2) (a) Ito, S.; Nardi, G.; Prota, G. *J. Chem. Soc., Chem. Commun.* **1976**, 1042. (b) Ito, S.; Nardi, G.; Palumbo, A.; Prota, G. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2619.

(3) (a) Palumbo, A.; Misuraca, G.; d'Ischia, M.; Donaudy, F.; Prota, G. *Comp. Biochem. Physiol.* **1984**, *78b*, 81. (b) Rossi, F.; Nardi, G.; Palumbo, A.; Prota, G. *Comp. Biochem. Physiol.* **1985**, *80b*, 843. (c) Christophersen, C.; Anthoni, U. *Sulfur Rep.* **1986**, *4*, 365.

(4) Pathirana, C.; Andersen, R. J. *J. Am. Chem. Soc.* **1986**, *108*, 8288.

(5) Holler, T. P.; Hopkins, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 4837.

(6) Holler, T. P., unpublished results.

(7) Shapiro, B. M.; Turner, E. T. *Biofactors* **1988**, *1*, 85.

(8) Turner, E.; Klevit, R.; Hopkins, P. B.; Shapiro, B. M. *J. Biol. Chem.* **1986**, *261*, 13056.

(9) Turner, E.; Hager, L.; Shapiro, B. M. *Science* **1988**, *242*, 939.

(10) All ovothiols reported in the literature prior to 1986[2,3] are depicted, apparently incorrectly, as bearing a methyl substituent on the τ-nitrogen of the histidine nucleus (see: *Biochemistry* **1975**, *14*, 449). In the course of our studies, this error was discovered,[1,11] the π-methyl (here called 1-methyl) substituted pattern depicted in this paper being correct for ovothiols.

(11) Holler, T. P.; Spaltenstein, A.; Turner, E.; Kelvit, R. E.; Shapiro, B. M.; Hopkins, P. B. *J. Org. Chem.* **1987**, *52*, 4420.

(12) (a) Bowman, R. E.; Stroud, H. H. *J. Chem. Soc.* **1950**, 1342. (b) Reinhold, V. N.; Ishikawa, Y.; Melville, D. B. *J. Med. Chem.* **1968**, *11*, 258.

(13) Beyerman, H. C.; Maat, L.; von Zon, A. *Recl. Trav. Chim. Pays-Bas* **1972**, *91*, 246.

(14) (a) Tautz, W.; Teitel, S.; Brossi, A. *J. Med. Chem.* **1973**, *16*, 705. (b) Trout, G. E. *J. Med. Chem.* **1972**, *15*, 1259.

(15) (a) Kirk, K. L.; Cohen, L. A. *J. Am. Chem. Soc.* **1973**, *95*, 4619. (b) Kirk, K. L.; Nagai, W.; Cohen, L. A. *J. Am. Chem. Soc.* **1973**, *95*, 8389.

(16) Beck, J. R.; Yahner, J. A. *J. Org. Chem.* **1978**, *43*, 2048.

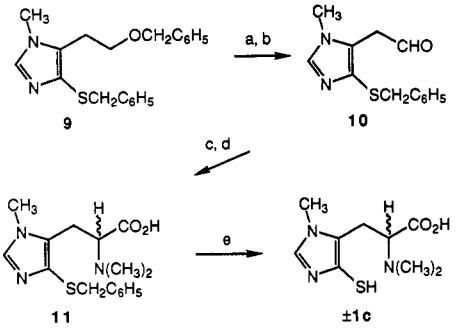
(17) Calo, V.; Ciminale, L. L.; Naso, F.; Todesco, P. E. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2567.

(18) Testaferri, L.; Tiecco, M.; Tingoli, M.; Bartole, D.; Massoli, A. *Tetrahedron* **1985**, *41*, 1373.

(19) Migata, T.; et al. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1385.

0022-3263/89/1954-4570$01.50/0 © 1989 American Chemical Society

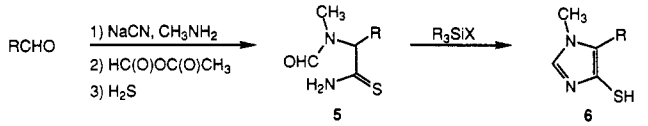
Scheme I



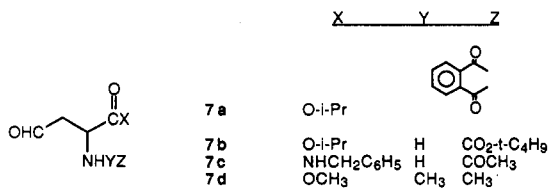
[a] $BF_3{\cdot}Et_2O$, $HS(CH_2)_3SH$. [b] ClCOCOCl, DMSO, $(i\text{-}Pr)_2NEt$. [c] $(CH_3)_2NH_2^+Cl^-$, NaCN. [d] HCl(aq). [e] Na, NH_3.

uncharacterized products. Approaches involving metalation of the imidazole ring of **4** followed by quenching with electrophilic sulfur reagents[21] were likewise abortive.

In contrast to the difficulties surrounding late introduction of the mercaptan function onto a preexisting imidazole ring, synthesis of the imidazole ring with the mercaptan in place proved straightforward. A new synthesis of 4-mercaptoimidazoles developed expressly for this purpose[22] involves conversion of an aldehyde to an α-(*N*-formylamino)thionoacetamide (**5**), followed by cyclization

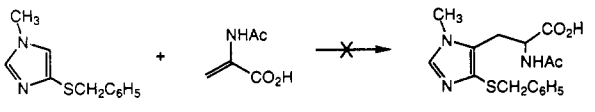


to the 4-mercaptoimidazole (**6**). Unfortunately, this synthesis failed when aldehyde **7** in a variety of protected forms (e.g., **7a–d**) was the starting material. It was evident



7a	O-i-Pr		
7b	O-i-Pr	H	CO_2-t-C_4H_9
7c	$NHCH_2C_6H_5$	H	$COCH_3$
7d	OCH_3	CH_3	CH_3

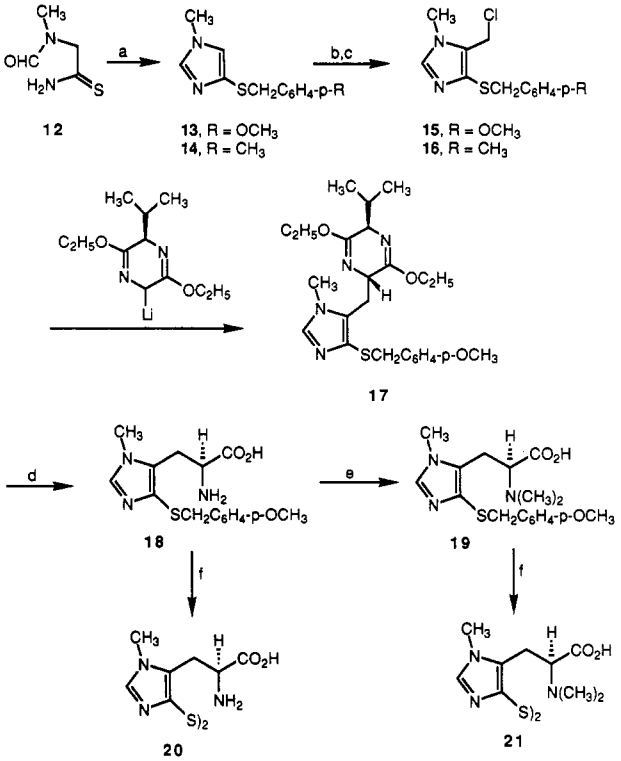
that functional group incompatibility was the heart of the problem; therefore, the amino acid side chain was deleted entirely, to be subsequently incorporated. It has been reported that tryptophan can be prepared by heating indole and α-acetamidoacrylic acid in acetic acid and acetic anhydride.[23] This method failed when the imidazole **8** was substituted for indole.



8

Success was at last achieved by the route shown in Scheme I. The protected mercaptoimidazole **9** was prepared (Scheme I).[22] Selective deprotection followed by oxidation gave aldehyde **10**, which was finally converted to the racemate of S-protected ovothiol C (**11**). Deprotection was achieved in modest and variable yield using

Scheme II[a]



[a] (i) $(CH_3)_3SiCl$, Et_3N; (ii) $CH_3OC_6H_4CH_2Cl$. [b] CH_2O, NaOAc. [c] $SOCl_2$. [d] HCl. [e] $NaBH_3CN$, CH_2O. [f] (i) $Hg(OCOCF_3)_2$, $HOCOCF_3$; (ii) H_2S; (iii) $CuCl_2$, O_2.

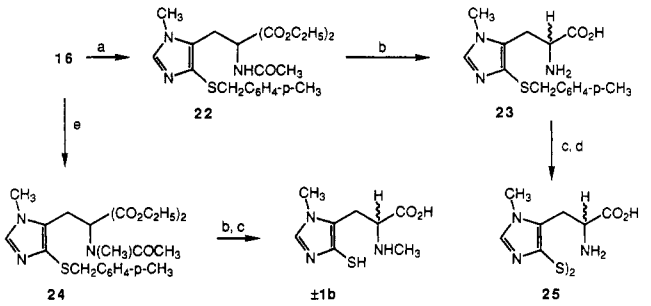
sodium in liquid ammonia.[24]

The racemate of ovothiol C prepared as shown in Scheme I was purified by the ion-exchange method originally employed in the isolation of ovothiol C from natural sources.[8] The synthetic product was indistinguishable from naturally derived material by UV and retention volume on ion-exchange HPLC. A 1:1 mixture of the synthetic and natural samples afforded a single set of resonances in the ^1H NMR spectrum at 500 MHz, verifying the assigned structure of ovothiol C. The synthesis shown in Scheme I is in principle applicable to syntheses of ovothiols A and B as their racemates. This has, however, not been attempted because at the time the latter synthesis was conducted, ovothiols A and B were unknown.

Encouraged that the assignment of ovothiol structures was correct, we sought a synthesis of ovothiols in optically active form. Such a synthesis, using the method of Schollkopf,[25] is outlined in Scheme II. Cyclization of the thionoamide **12** followed by in situ protection as the *p*-methoxybenzyl thioether gave heterocycle **13**. Model experiments suggested that this protecting group would be more conveniently removed than benzyl at the required stage. Hydroxymethylation[26] of **13** followed by treatment with excess thionyl chloride afforded the hydrochloride of **15**, which was in turn alkylated by using the indicated anion to form **17** as a 5:1 mixture of diastereoisomers at the new chiral center. The major diastereoisomer was isolated by column chromatography and was hydrolyzed to yield the amino acid **18**. As anticipated, removal of the *p*-methoxybenzyl protecting group proceeded smoothly[27]

(20) *Jpn. Kokai Tokkyo Koho* JP 57 88, 166; *Chem. Abstr.* **1983**, *98*, 34581*d*.
(21) Iddon, B. *Heterocycles* **1985**, *23*, 417.
(22) Spaltenstein, A.; Holler, T. P.; Hopkins, P. B. *J. Org. Chem.* **1987**, *52*, 2977.
(23) Snyder, H. R.; MacDonald, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 1257.
(24) Corrie, J. E. T.; Hlubucek, J. R.; Lowe, G. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1421.
(25) (a) Schollkopf, U. *Top. Curr. Chem.* **1983**, *109*, 66. (b) Schollkopf, U. *Pure Appl. Chem.* **1983**, *55*, 1799.
(26) Masui, M.; Suda, K.; Inoue, M.; Izukura, K.; Yamauchi, M. *Chem. Pharm. Bull.* **1974**, *22*, 2359.

Scheme III[a]



[a] $CH_3CONHCH(CO_2Et)_2$, NaH. [b] HCl(aq). [c] (i) $Hg(OCOCF_3)_2$, $HOCOCF_3$; (ii) H_2S. [d] $CuCl_2$, O_2. [e] $CH_3CON(CH_3)CH(CO_2Et)_2$.

to yield the free thiol, which was not characterized but was instead converted directly to the more easily handled disulfide form by exposure of an aqueous solution containing a trace of copper(II) to the air. The resulting disulfide was purified on lipophilic Sephadex to afford **20**, the disulfide of ovothiol A. This material proved identical with the disulfide of naturally derived ovothiol A in the ^1H NMR spectrum (500 MHz), optical rotation,[3,10] and UV spectrum.

The disulfide of ovothiol C was prepared analogously by first methylating **18** as shown. The synthetic disulfide of ovothiol C (**21**) was again identical with natural material by 500-MHz ^1H NMR, optical rotation,[3,10] and UV analyses.

Multigram synthesis of racemic ovothiols could be accomplished most conveniently by modification of the L-ovothiol sythesis. For reasons that were not pursued, the *p*-methoxybenzyl protection group was incompatible with the alkylation of **15** using diethyl acetamidomalonate. The *p*-methylbenzyl group, in contrast, allowed alkylation using acetamidomalonates and could be cleaved easily. An unexpected further benefit of the *p*-methylbenzyl group was that it allowed purification of the precursor to **16**, the (hydroxymethyl)imidazole, by crysallization from the crude mixture, avoiding entirely any chromatography until quite late in the synthesis. Accordingly, **16** (Scheme III) was alkylated with diethyl acetamidomalonate to yield **22**, which was hydrolyzed to the amino acid **23** and in turn deprotected and oxidized to a mixture of diastereoisomeric disulfides of racemic ovothiol A (**25**). Simple modification of this approach afforded racemic ovothiol B. Because ovothiol B has been identified only as an *S*-carboxymethyl derivative,[1] it was not possible to compare this material with that from natural sources. For the case of ovothiol A racemate disulfide, it was demonstrated by combustion analysis and ^{19}F NMR spectroscopy that passage through an ion-exchange column removed the trifluoroacetate counterion.

In the course of manipulating samples of ovothiols, we have made observations of practical significance. In our experience, samples of S-protected ovothiols, for example, the *p*-methoxy- and *p*-methylbenzyl derivatives, are quite stable. In contrast, the free thiols undergo oxidation reactions the rates of which in solution are pH and metal-ion dependent. The free thiols in the solid state are likewise oxidation prone, although less so than in solution. Acidic aqueous solutions that are mixtures of thiol and disulfide forms show a single set of resonances in the ^1H NMR spectrum (500 MHz), presumably due to fast interchange. Furthermore, strongly basic solutions of ovothiol disulfides display new ^1H NMR resonances that are not eliminated on acidification, presumably indicative of some form of base-promoted decomposition. For experiments involving free thiol, we find that in situ reduction of aqueous disulfide, for example, using sodium borohydride or DTT, is a useful source of thiol. Ellman's reagent[28] is useful for the determination of ovothiols; UV analysis, using disulfide and a disulfide/sodium borohydride mixture as standards, can be used for determination of the disulfide/thiol ratio and total concentration.

Conclusion

Methods for the synthesis of both optically active and racemic ovothiols have been developed. In all cases, the syntheses involve elaboration of an amino acid function onto a preformed mercaptoimidazole moiety. These syntheses have confirmed the structures of ovothiols A and C by comparison with authentic samples (in the disulfide forms). The syntheses of the S-protected racemates are amenable to a multigram scale.

Experimental Section

General Methods. Unless otherwise specified, commercial chemicals were used as received. Tetrahydrofuran was distilled under argon from sodium benzophenone ketyl. Methylene chloride was distilled under argon from calcium hydride. Air- or water-sensitive reactions were conducted under a positive-pressure argon atmosphere. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 plates (0.25 mm): column chromatography was performed with Merck silica gel 60 (230–400 mesh). Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 257 grating infrared spectrophotometer; only major or diagnostic peaks are noted. Proton nuclear magnetic resonance (NMR) spectra were determined on a Varian CFT-20 (^1H 80 MHz). Varian VXR-300 (^1H 300 MHz), or Bruker WM-500 (^1H 500 MHz) spectrometer and, unless otherwise specified, are reported in parts per million (δ) downfield relative to internal tetramethylsilane. Coupling constants (J) are reported in hertz. Ultraviolet (UV) spectra were recorded on a Perkin-Elmer Model Lambda 3A spectrophotometer in double-beam mode. Mass spectra were measured on a Hewlett-Packard Model 5985 mass spectrometer, VG7070H double-focusing, or VG70SEQ mass spectrometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter and were recorded at ambient temperature as the D line of sodium. Combustion analyses were performed by Micanal of Tucson, AZ.

5-(2-Hydroxyethyl)-1-methyl-4-((phenylmethyl)thio)-imidazole. The *O,S*-dibenzylimidazole **9**, 2.90 g (8.6 mmol), and 2 mL of $BF_3{\cdot}Et_2O$ were added to 20 mL of propanethiol. The mixture was stirred at 45 °C for 10 h and diluted with 50 mL of water. After the mixture stirred at 45 °C for an additional 2 h, the volatiles were removed in vacuo and the residue was partitioned between CH_2Cl_2 and 5% aqueous NaOH. Reextraction of the aqueous layer with CH_2Cl_2, drying of the combined organic extracts ($MgSO_4$), and removal of the solvent in vacuo afforded an oil which was chromatographed on silica gel (5% CH_3OH/dichloromethane) to yield 1.65 g (77%) of the title compound as a colorless oil, which solidified on standing. A sample recrystallized from ethyl acetate gave colorless needles: mp 83–85 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 1.82 (1 H, br s, OH), 2.54 (2 H, t, J = 6 Hz, CH_2CH_2O), 3.41 (2 H, t, J = 6 Hz, CH_2CH_2O), 3.55 (3 H, s, CH_3N), 3.95 (2 H, s, SCH_2Ar), 7.07–7.20 (5 H, m, Ar), 7.41 (1 H, s, CH-2); IR (neat, NaCl) 3600 (OH) cm^{-1}; LRMS (ei) m/e 248 (M^+), 217, 215, 157, 159, 91 (100); HRMS (ei) calcd 248.0980, found 248.0972.

1-Methyl-5-(2-oxoethyl)-4-((phenylmethyl)thio)imidazole (10). A solution of 0.757 g (6.0 mmol) of ClCOCOCl in 15 mL of CH_2Cl_2 was cooled to −60 °C and treated dropwise with 0.624 g (8.0 mmol) of dimethyl sulfoxide. After the mixture stirred for 0.5 h at −60 °C, a solution of 1.00 g (4.0 mmol) of 5-(2-hydroxyethyl)-1-methyl-4-((phenylmethyl)thio)imidazole in 2 mL of CH_2Cl_2 was added dropwise. The resulting mixture was stirred

(27) Nishimura, O.; Chieko, K.; Fujino, M. *Chem. Pharm. Bull.* **1978**, *26*, 1576.

(28) Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70.

at −50 to −55 °C for 0.75 h, and 1.78 g (14 mmol) of diisopropylethylamine were added. After stirring at −60 °C for 0.3 h, the mixture was allowed to warm to −20 °C over 0.5 h. Several grams of silica gel were added to the reaction mixture, and the resulting slurry was concentrated in vacuo (0 °C) and loaded onto a silica gel column. Chromatography with 5% CH_3OH/CH_2Cl_2 afforded 0.856 g (85%) of the title aldehyde **10** as a pale yellow oil: ^1H NMR (500 MHz, $CDCl_3$) δ 3.63 (3 H, s, CH_3N), 3.62 (2 H, d, J = 2 Hz, CH_2CO), 3.98 (2 H, s, SCH_2Ar), 7.13–7.29 (5 H, m, Ar), 7.68 (1 H, s, CH-2), 9.33 (1 H, t, J = 2 Hz, CHO); IR (neat, NaCl) 3068, 1728 (C=O) cm^{-1}; LRMS (ei) 246 (M^+), 218, 217, 185, 129, 128, 91 (100).

5-(2-Cyano-2-(dimethylamino)ethyl)-1-methyl-4-((phenylmethyl)thio)imidazole. The aldehyde **10**, 1.15 g (4.66 mmol), 1.6 g (19.6 mmol) of dimethylamine hydrochloride, and 1.7 g (35 mmol) of NaCN were dissolved in 6 mL of CH_3OH. The reaction was initiated by adding 2 mL of water, and the mixture was stirred at 25 °C for 4 h. Extraction with CH_2Cl_2, drying of the organic layer over $MgSO_4$, and removal of the volatiles in vacuo followed by chromatography on silica gel (10% CH_3OH/CH_2Cl_2) afforded the title cyanoamine, 0.83 g (60%), as a yellow oil: ^1H NMR (500 MHz, $CDCl_3$) δ 2.19 (6 H, s, $(CH_3)_2N$), 2.62–2.72 (2 H, m, CH_2), 2.86–2.89 (1 H, m, CHCN), 3.60 (3 H, s, CH_3N-1), 3.91 (1 H, d, J = 13 Hz, SCH_2Ar), 4.07 (1 H, d, J = 13 Hz, SCH_2Ar), 7.04–7.26 (5 H, m, Ar),7.49 (1 H, s, CH-2); IR ($CHCl_3$) 2225 (CN) cm^{-1}; LRMS (ei) m/e 300 (M^+), 273, 247, 217, 148, 124, 91 (100), 84.

DL-**Ovothiol C [(±)-1c].** 5-(2-Cyano-2-(dimethylamino)-ethyl)-1-methyl-4-((phenylmethyl)thio)imidazole, 0.766 g (max. 3.17 mmol), was heated under reflux in 12 N aqueous HCl for 18 h. The resulting mixture was evaporated to dryness in vacuo, and a 30-mg sample was kept for analytical purposes. The remaining crude, highly hygroscopic amino acid dihydrochloride was dissolved in 50 mL of liquid ammonia and treated with small, freshly cut pieces of Na at −33 °C until the deep blue color persisted for more than 10 s. The reaction was quenched immediately with ca. 1 g of solid NH_4OCOCH_3 and evaporated to dryness. One-tenth of this material was purified on Dowex 50X8, 200–400 mesh (0.5 M aqueous NH_4OCHO, pH 2.5, and then pH 4.5) to give 24.2 mg (34%) racemic ovothiol C as a pale yellow solid: ^1H NMR (500 MHz, D_2O) δ 3.10 (6 H, s $(CH_3)_2N$), 3.2 (1 H, dd, J = 9 and 15 Hz, CH_2), 3.35 (1 H, dd, J = 5 and 15 Hz, CH_2), 3.77 (3 H, s, CH_3N-1), 4.07 (1 H, dd, J = 5 and 9 Hz, CHN), 8.29 (1 H, s, CH-2); UV (H_2O) 234, 278 nm.

(Cyanomethyl)methylamine. NaCN (36.8 g, 0.75 mol) and CH_3NH_2·HCl (50.0 g, 0.74 mol) in 200 mL of water (0 °C) were treated dropwise with 37% aqueous H_2CO (56.4 mL, 0.75 mol, exothermic) over 0.5 h. After stirring for 1 h at 0 °C, the mixture was extracted with three 200-mL portions of CH_2Cl_2, dried ($MgSO_4$), and without concentration used directly in the next step. A small aliquot concentrated in vacuo gave the following spectroscopic data: ^1H NMR (80 MHz, $CDCl_3$) δ 2.55 (3 H, s, CH_3), 3.56 (2 H, s, CH_2); IR (neat) 3360 (NH), 2240 (CN) cm^{-1}.

***N*-(Cyanomethyl)-*N*-methylformamide.** Formic acid (95%, 750 mL, 19.9 mol) was cooled to 0 °C, treated with acetic anhydride (300 mL, 3.2 mol), and stirred 1 h at 25 °C. (Cyanomethyl)methylamine in CH_2Cl_2 prepared in the previous step was added dropwise to this mixture over 0.5 h at 0 °C. The ice bath was removed, and the mixture was allowed to stir 8 h at 25 °C. Cold water (1 L) was added, and the mixture was concentrated in vacuo to yellow oil, which was distilled (bp 90–105 °C, 0.45 mmHg) to yield the title compound as a pale yellow oil (46.0 g, 63.4% for two steps): ^1H NMR (80 MHz, $CDCl_3$) δ 2.98, 3.08 (3 H, 2 × s, ratio 1:4, CH_3), 4.17, 4.28 (2 H, 2 × s, ratio 1:4, CH_2), 8.02, 8.02 (1 H, 2 × s, ratio 1:4, CHO); IR (neat) 2245 (CN), 1670 (C=O); LRMS (ei) m/e 98 (M^+), 69 (M^+ − CHO); HRMS (ei) calcd 98.048, found 98.048.

2-(*N*-Formyl-*N*-methylamino)thionoacetamide (12). A solution of *N*-(cyanomethyl)-*N*-methylformamide (46.0 g, 0.47 mol) in 200 mL of absolute C_2H_5OH and 12.5 mL of triethylamine was treated with hydrogen sulfide at 25 °C for 4 h, at which time thin-layer chromatographic analysis indicated the reaction was complete. The mixture was concentrated in vacuo (bath temperature 35 °C) until crystallization commenced. After crystallization was complete, the mixture was filtered and the solid was washed with cold absolute C_2H_5OH to give the title compound (46.3 g, 74.7%) as a white crystalline solid, mp 89–91 °C. Concentration of the mother liquors produced a second crop (2.05 g, 3.3%) and a third crop (1.50 g, 2.4%) of white solid (combined yield 80.4%): ^1H NMR (300 MHz, D_2O) 2.68, 2.92 (3 H, 2 × s, ratio 1:1, CH_3), 4.20, 4.23 (2 H, 2 × s, ratio 1:1, CH_2), 7.92, 7.97 (1 H, 2 × s, ratio 1:1, CHO); IR ($CHCl_3$) 3400–3200 (NH_2), 1660 (C=O), 1225 (C=S); UV (H_2O) λ_{max} 265 nm (10 000 M^{-1} cm^{-1}); LRMS (ei) m/e 132 (100%, M^+); HRMS (ei) calcd 132.0357, found 132.0343.

4-(((4-Methoxyphenyl)methyl)thio)-1-methylimidazole (13). 2-(*N*-Formyl-*N*-methylamino)thionoacetamide (**12**, 10.0 g, 75.6 mmol) suspended in 250 mL of CH_2Cl_2 and 60 mL (0.43 mol) of triethylamine at 25 °C was treated with chlorotrimethylsilane (40 mL, 0.32 mol). The resulting heterogeneous mixture was stirred mechanically at 25 °C for 5 h and concentrated in vacuo to a solid, which was taken up in absolute ethanol and filtered to remove triethylamine hydrochloride. The filtrate was cooled to 0 °C, and ca. 0.25 g of $NaBH_4$ was added. To this was added 4-methoxybenzyl chloride in sequential portions of 5, 2, and 2 mL (total 9.0 mL, 66.4 mmol). When the reaction was complete [TLC analysis, silica gel, 70:25:5, CH_2Cl_2:MeOH:NH_4OH(conc)], the mixture was concentrated to a brown oily solid, which was chromatographed on silica gel (CH_2Cl_2) to yield the title compound as an orange oil (11.9 g, 67%). A small aliquot further purified by bulb-to-bulb distillation (175 °C, 0.1 mmHg) gave the following spectroscopic data: ^1H NMR (300 MHz, $CDCl_3$) δ 3.61 (3 H, s, NCH_3), 3.79 (3 H, s, OCH_3), 4.00 (2 H, s, SCH_2), 6.71 (1 H, d, J = 3 Hz, imidazole (Im) H-5), 6.98 (2 H, d, J = 9 Hz, ArH), 7.14 (2 H, d, J = 9 Hz, ArH), 7.43 (1 H, br, Im H-2); IR (neat) 3130, 3110, 3030 (Ar H), 3000, 2960 (CH_3), 1610, 1510 (Ar) cm^{-1}; LRMS (ei) m/e 234 (M^+), 201 (M^+ − SH), 121 (100%, $C_8H_9O^+$); HRMS (ei) calcd 234.083, found 234.083.

5-(Hydroxymethyl)-4-(((4-methoxyphenyl)methyl)-thio)-1-methylimidazole. A solution of protected mercaptoimidazole **13**, 1.4 g (0.6 mmol), 12 g of sodium acetate, and 1.2 mL of acetic acid in 10 mL (33 mmol) of 37% aqueous formaldehyde was heated under reflux for 3.5 h, cooled to 25 °C, diluted with water, and brought to pH 9 with K_2CO_3. The mixture was extracted with CH_2Cl_2, and the organic extracts were dried ($MgSO_4$), concentrated in vacuo, and chromatographed on silica gel (5% $MeOH/CH_2Cl_2$) to yield the title compound (1.21 g, 76%) as a yellow oily solid. Recrystallization from ethyl acetate gave colorless prisms: mp 103–104 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 1.16 (1 H, br s, OH), 3.61 (3 H, s, NCH_3), 3.75 (3 H, s, OCH_3), 3.84 (2 H, s, CH_2S), 4.25 (2 H, s, OCH_2), 6.71 (2 H, d, J = 8 Hz, ArH), 6.97 (2 H, d, J = 8 Hz, ArH), 7.44 (1 H, s, N=CH—N); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.2 (q, NCH_3), 39.6 (t, CH_2S), 52.8 (t, CH_2O), 55.1 (q, OCH_3), 113.3 (d, *o*-anisyl), 129.8 (d, *m*-anisyl), 130.5 (s, Im C4 and C5), 135.3 (s, *p*-anisyl), 138.4 (d, Im C2), 158.2 (s, anisyl C1); IR ($CHCl_3$) 3550 (OH),3000, 2960 (CH), 1610, 1510 (Ar) cm^{-1}; LRMS (ei) m/e 264 (M^+), 231 (M^+ − SH), 121 (100%, $C_8H_9O^+$); HRMS (ei) calcd 264.093, found 264.094. Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.1; H, 6.1; N, 10.6. Found: C, 58.7; H, 6.2; N 10.4.

5-(Chloromethyl)-4-(((4-methoxyphenyl)methyl)thio)-1-methylimidazole Hydrochloride (15·HCl). To approximately 20 mL of freshly distilled $SOCl_2$ (0 °C) was added 1.52 g (5.75 mmol) of the previous product in small portions over 10 min. The mixture was stirred for 0.5 h, the ice bath was removed, and the mixture was stirred for an additional 0.5 h at 25 °C. The mixture was sequentially concentrated in vacuo, dissolved in 20 mL of THF, concentrated in vacuo, dissolved in 20 mL of THF, and concentrated in vacuo. The resulting white solid was suspended in THF and isolated by filtration. Drying in vacuo produced the title compound (1.71 g, 93%) as a white solid: mp 137–141 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 3.77 (3 H, s, OCH_3), 4.0 (3 H, s, NCH_3), 4.24, 4.3 (4 H, 2 × s, $ArCH_2Cl$ and $ArCH_2S$), 6.78 (2 H, d, J = 9 Hz, ArH), 7.14 (2 H, d, J = 9 Hz, ArH), 9.95 (1 H, s, Im H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 31.7 (t, $ArCH_2S$), 55.3 (q, OCH_3), 114.0 (d, *o*-anisyl), 124.9 (s, Im C-5), 127.9 (s, Im C-4), 133.1 (s, *p*-anisyl), 138.4 (d, Im C-2), 159.0 (s, anisyl C-1); IR ($CHCl_3$) 2970 (CH_3), 3500–2100 (br, N–H), 1610, 1510 (Ar) cm^{-1}. Anal. Calcd for $C_{13}H_{16}Cl_2N_2OS$: C, 48.9; H,5.1; N, 8.8; Cl, 22.2. Found: C, 48.6; H, 5.1; N, 8.8; Cl, 21.8.

Bislactim 17. A cold (−78 °C) solution of (3*R*)-2,5-diethoxy-3,6-dihydro-3-isopropylpiperazine, 8.5 g (40 mmol), in 150 mL of THF was treated with 29.2 mL of *n*-butyllithium (40 mmol,

1.37 M in hexanes) and stirred for 0.25 h. The (chloromethyl)-imidazole hydrochloride **15** was added to the mixture in small portions over 5 min, and the resulting mixture was stirred for 4.5 h at −78 °C and warmed over a period of 1 h to −30 °C, and finally rapidly to 25 °C. The mixture was concentrated in vacuo to a black oil. This oil was partitioned between ether and water. The ether layer was dried ($MgSO_4$) and concentrated in vacuo to yield an oil, which was chromatographed on silica gel (2% MeOH/CH_2Cl_2) to give the title compound as an orange oil (4.9 g, 70%) along with 1.0 g of material that was primarily the diastereoisomer. ^1H NMR (500 MHz, $CDCl_3$) δ 0.65, 0.98 (6 H, 2 × d, J = 7 Hz, $CH(CH_3)_2$), 1.20, 1.28 (6 H, 2 × t, J = 7 Hz, OCH_2CH_3), 2.20 (1 H, m, $CH(CH_3)_2$), 2.59 (1 H, dd, J = 8, 15 Hz, Im CH_2CH), 2.99 (1 H, dd, J = 5, 15 Hz, Im CH_2CH), 3.54 (3 H, s, NCH_3), 3.64 (1 H, t, J = 3 Hz, i-PrCH), 3.74 (3 H, s, OCH_3) 3.93 (2 H, app d, J = 3 Hz, $ArCH_2$), 4.0–4.2 (5 H, m, OCH_2 and Im CH_2CH), 6.73 (2 H, d, J = 9 Hz, ArH), 7.07 (2 H, d, J = 9 Hz, ArH), 7.40 (1 H, s, Im H-2); IR ($CHCl_3$) 2960 (C–H), 1685 (O—C=N), 1610, 1510 (Ar), 1380, 1365 ($CH(CH_3)_2$) cm^{-1}; LRMS (ei) m/e 458 (M^+), 429 ($M^+ - CH_2CH_3$), 415 (100%, $M^+ - CH(CH_3)_2$), 247 (98%, Im CH_2^+); HRMS (ei) calcd 458.235, found 458.233.

(*S*)-(4-Methoxyphenyl)methyl-L-ovothiol A (18). To 375 mg (0.84 mmol) of bislactim ether **17** was added 10.1 mL (2.5 mmol) of 0.25 M aqueous HCl, and the suspension was stirred overnight. The mixture was concentrated in vacuo and dissolved in 10 mL of concentrated aqueous HCl, heated at reflux for 0.75 h, concentrated in vacuo, diluted with water, and concentrated again in vacuo. The resulting material was chromatographed on silica gel [70:25:5 CH_2Cl_2:CH_3OH:NH_4OH(conc)] to yield the title compound (157 mg, 58%) as a yellow solid, which was triturated in 3 mL of ethanol and filtered to give a white solid: ^1H NMR (500 MHz, D_2O/DCl) 2.57 (1 H, dd, J = 7, 16 Hz, β-H), 2.64 (1 H, dd, J = 8, 16 Hz, β-H), 3.44 (3 H, s, OCH_3), 3.45 (3 H, s, NCH_3), 3.53 (1 H, dd, J = 7 Hz, α-H), 3.65 (2 H, m, $ArCH_2S$), 6.54 (2 H, d, J = 9 Hz, ArH), 6.70 (2 H, d, J = 9 Hz, ArH), 8.44 (1 H, s, Im H-2); ^{13}C NMR (75 MHz, D_2O/DCl, t-BuOH ref) 24.8 (5, C-β), 35.7 (q, NCH_3), 40.8 (t, $ArCH_2S$), 53.4 (d, C-α), 56.8 (q, OCH_3), 115.5 (d, o-anisyl), 124.2 (s, Im C5), 130.6 (s, Im C4), 131.6 (d, m-anisyl), 135.9 (s, p-anisyl), 138.2 (d, Im C2), 159.6 (s, anisyl C1), 172.2 (s, CO_2H). Anal. Calcd for $C_{15}H_{19}N_3S{\cdot}CH_3OH$: C, 54.4; H, 6.5; N, 11.9; S, 9.1. Found: C, 54.4; H, 6.2; N, 12.2; S, 8.7.

(*S*)-(4-Methoxyphenyl)methyl-L-ovothiol C (19). A mixture of 140 mg (1.0 mmol) of NaOAc·$3H_2O$, 60 μL (1.0 mmol) of HOAc, 0.30 mL (94.0 mmol) of 37% aqueous formaldehyde, and 0.60 mL of water was added to 50 mg (0.13 mmol) of (*S*)-(4-methoxyphenyl)methyl-L-ovothiol A, and the mixture was stirred at 25 °C until the solution became homogeneous. The solution was treated sequentially with 20-, 10-, and 10-mg portions of $NaBH_3CN$, stirred for 5 min, concentrated in vacuo, redissolved in 5 mL of water, and reconcentrated in vacuo. Chromatography on silica gel (70:25:5 CH_3OH:CH_2Cl_2:conc aqueous NH_3) afforded the title compound, 49 mg (89%) as an oily solid: mp 193–197 °C (dec); ^1H NMR (300 MHz, D_2O) δ 2.29 (1 H, dd, J = 2, 6 Hz, β-H), 2.54 (1 H, dd, J = 2, 4 Hz, β-H), 2.70 (6 H, s, $N(CH_3)_2$), 3.23 (1 H, dd, J = 4, 6 Hz, α-H), 3.50 (3 H, s, NCH_3), 3.74 (3 H, s, OCH_3), 3.76 (2 H, m, J = 5 Hz, $ArCH_2S$), 6.78 (2 H, d, J = 8 Hz, ArH), 6.87 (2 H, d, J = 8 Hz, ArH), 7.63 (1 H, s, Im H-2).

L-Ovothiol A (1a). A mixture of 25 mg (0.078 mmol) of (*S*)-(4-methoxyphenyl)methyl-L-ovothiol A (**18**) and 0.5 mL of anisole was cooled to −5 °C and treated with 5 mL of trifluoroacetic acid. Mercury(II) trifluoroacetate, 34 mg (0.080 mmol), was added, and the mixture was stirred 1 h. The mixture was concentrated in vacuo to an oil, taken up in water, and washed with ether. H_2S was bubbled into the aqueous layer for 0.6 h; the suspension was filtered through Celite and concentrated in vacuo to give 32 mg of the title compound as a pale green glass which was used in the following experiment. ^1H NMR (500 MHz, D_2O) δ 3.49 (1 H, dd, J = 7, 15 Hz, β-H), 3.57 (1 H, dd, J = 7, 15 Hz, β-H), 3.91 (3 H, s, NCH_3), 4.56 (1 H, t, J = 7 Hz, α-H), 865 (1 H, s, Im H-2); UV (H_2O) λ_{max} 237, 278 (shoulder) nm.

L-Ovothiol A Disulfide (20). The product of the previous reaction in 2 mL of water was treated with ca. 3 mg of $CuCl_2{\cdot}2H_2O$. This solution was stored exposed to the air at 5 °C for 12 h, warmed to 25 °C, passed through a column of CHELEX ion-exchange resin, and concentrated in vacuo. The resulting material was dissolved in 80% aqueous ethanol and chromatographed on Sephadex LH-20 (25–100 μm, 80% aqueous ethanol) to yield the title compound as a glassy solid in 94% yield from **17** (based on quantitative UV). ^1H NMR (500 MHz, D_2O) δ 2.29 (1 H, dd, J = 8, 15 Hz, β-H), 3.54 (1 H, dd, J = 6, 15 Hz, β-H), 3.34 (1 H, t, J = 7 Hz, α-H), 3.77 (3 H, s, NCH_3), 7.84 (1 H, s, Im H-2); optical rotation $[\alpha]^{20}_D$ +77° [c = 6.5 mg/mL, 0.1 M HCl(aq)]; UV [0.1 M HCl(aq)] λ_{max} 257 nm (ε 5900 M^{-1} cm^{-1}).

L-Ovothiol C (1c). The conversion of **19** to **1c** (30 mg) was carried out as described above for the conversion of **18** to **1a**. The product was used without further purification in the following step. ^1H NMR (300 MHz, D_2O) δ 3.02 (6 H, s, $N(CH_3)_2$), 3.39 (1 H, dd, J = 11, 15 Hz, β-H), 3.51 (1 H, dd, J = 4, 15 Hz, β-H), 3.89 (3 H, s, NCH_3), 4.17 (1 H, dd, J = 4, 11 Hz, α-H), 8.85 (1 H, s, Im H-2); UV (H_2O) λ_{max} 234, 277 (shoulder) nm.

L-Ovothiol C Disulfide (21). The product of the previous reaction was converted to the disulfide exactly as described for the air oxidation of **1a**, to afford the disulfide **21** as a glassy solid (88% from **19**, based on quantitative UV): ^1H NMR (500 MHz, D_2O) δ 2.57 (1 H, dd, J = 5, 13 Hz, β-H), 2.73 (6 H, s, $N(CH_3)_2$), 2.76 (1 H, dd, J = 11, 13 Hz, β-H), 3.33 (1 H, dd, J = 5, 11 Hz, α-H), 3.77 (3 H, s, NCH_3), 7.88 (1 H, s, Im H-2); optical rotation $[\alpha]^{20}_D$ +77° (c = 10 mg/mL, H_2O); UV [0.1 M HCl(aq)] λ_{max} 259 nm (ε 6800 M^{-1} cm^{-1}).

4-(((4-Methylphenyl)methyl)thio)-1-methylimidazole (14). Chlorotrimethylsilane (89 mgl, 0.70 mol) was added dropwise to a suspension of 2-(*N*-formyl-*N*-methylamino)thionoacetamide (20.0 g, 0.15 mol) in triethylamine (133 mL, 0.95 mol) and 0.45 L of CH_2Cl_2 over 1.5 h in an ice bath. During the addition, the reaction temperature (internal thermometer) was not allowed to exceed 5 °C. The resulting heterogeneous mixture was stirred mechanically at 20 °C for 5 h and concentrated in vacuo to a yellow solid which was taken up in absolute ethanol and filtered to remove triethylamine hydrochloride. The filtrate was cooled to 0 °C, and 1.0 g (0.026 mol) of $NaBH_4$ was added. To this was added 4-methylbenzyl chloride (21.0 mL, 0.16 mol). When the reaction was complete (~12 h, TLC analysis), the white solid was removed by filtration, and the filtrate was concentrated in vacuo to yield an orange solid which was used directly in the next step. A small amount of the title compound purified by silica gel chromatography gave the following spectroscopic data: ^1H NMR (300 MHz, $CDCl_3$) δ 2.28 (3 H, s, CH_3), 3.57 (3 H, s, NCH_3), 3.98 (2 H, s, SCH_2), 6.68 (1 H, d, J = 3 Hz, Im H-5), 7.04 (2 H, d, J = 8 Hz, ArH), 7.07 (2 H, d, J = 8 Hz, ArH), 7.40 (1 H, br s, Im H-2); IR ($CHCl_3$) 3130–3030 (Ar H), 2950 (CH_3), 1680, 1510 (Ar); LRMS (ei) m/e 218 (M^+), 219 ($M^+ + 1$).

5-(Hydroxymethyl)-1-methyl-4-(((4-methylphenyl)-methyl)thio)imidazole. A solution of S-protected imidazole **14** (crude), 61 g of sodium acetate, and 60 mL of acetic acid in 0.41 L (1.35 mol) of 37% aqueous H_2CO was heated under reflux for 7.5 h. The reaction mixture was cooled to 25 °C, diluted with 0.5 L of water, and brought to pH 9 with saturated aqueous K_2CO_3. The mixture was extracted with three portions of CH_2Cl_2, and the combined organic extracts were dried ($MgSO_4$), and concentrated in vacuo to afford a brown oil. The brown oil was suspended in 50 mL of water, concentrated in vacuo, resuspended in 50 mL of ethanol concentrated in vacuo, and resuspended in 50 mL of ethyl acetate and concentrated in vacuo. This three-step evaporation was repeated two additional times. The remaining brown oil was recrystallized from ethyl acetate to give white crystals (12.5 g, 33%), in two crops: mp 111–113 °C; ^1H NMR (300 MHz, $CDCl_3$) 1.70 (1 H, br s, OH), 2.29 (3 H, s, $ArCH_3$), 3.61 (3 H, s, NCH_3), 3.84 (2 H, s, SCH_2), 4.19 (2 H, s, OCH_2), 6.93 (2 H, d, J = 8 Hz, ArH), 7.05 (2 H, d, J = 8 Hz, ArH), 7.51 (1 H, s, Im H-2); IR ($CHCl_3$) 3550 (OH), 3000, 2960 (CH), 1630, 1510 (Ar); LRMS (ei) m/e 248 (M^+), 249 ($M^+ + 1$), 215 ($M^+ - SH$); HRMS (ei) calcd 248.098, found 248.099.

5-(Chloromethyl)-1-methyl-4-(((4-methylphenyl)-methyl)thio)imidazole Hydrochloride (16·HCl). A solution of 19.8 g (79.8 mmol) of the product of the previous step in 100 mL of CH_2Cl_2 was added dropwise to 60 mL of freshly distilled $SOCl_2$ at 0 °C over 40 min. The mixture was stirred for 0.5 h at 0 °C and stirred for an additional 0.5 h after the ice bath was removed. The mixture was concentrated in vacuo, dissolved in THF, concentrated in vacuo, dissolved in THF, and concentrated in vacuo to yield a pale yellow form, which was used directly in the next step. ^1H NMR (300 MHz, $CDCl_3$) 2.29 (3 H, s, $ArCH_3$),

4.03 (3 H, s, NCH_3), 4.21, 4.24 (4 H, 2 × s, $ArCH_2Cl$ and $ArCH_2S$), 7.06 (4 H, s, ArH), 10.09 (1 H, s, Im H); IR ($CHCl_3$) 2970 (CH_3), 3500–2100 (br, N–H), 1580, 1510 (Ar) cm^{-1}.

Diester 22. Diethyl acetamidomalonate (43.3 g, 0.20 mol) in 150 mL of dimethylformamide was added dropwise to a suspension of sodium hydride (5.74 g, 0.25 mol) in 33 mL of dimethylformamide with an ice bath to maintain the reaction temperature ≤10 °C. After the reaction mixture was slowly warmed to room temperature, a solution of the crude product of the previous step in 150 mL of dimethylformamide was added dropwise at room temperature while the internal temperature was maintained at ≤30 °C. The mixture was stirred for 4 h until complete by ^1H NMR analysis. The majority of the dimethylformamide was removed in vacuo to give a mixture of **22** and diethyl acetamidomalonate as a yellow solid, which was used directly in the next step. ^1H NMR (300 MHz, $CDCl_3$) δ 1.32 (6 H, t, J = 7 Hz, CH_2CH_3), 1.95 (3 H, s, $COCH_3$), 2.31 (3 H, s, $ArCH_3$), 3.35 (2 H, s, Im CH_2), 3.46 (3 H, s, NCH_3), 3.92 (2 H s, SCH_2), 4.28 (4 H, q, J = 7 Hz, CH_2CH_3), 7.02 (4 H, m, ArH), 7.43 (1 H, s, Im H2).

***S*-[(4-Methylphenyl)methyl]-DL-ovothiol A (23).** A mixture of crude diester **22** and 6 N HCl (600 mL) was heated at reflux for 12 h and cooled to 25 °C. The reaction mixture was concentrated in vacuo, diluted with water, and brought to pH 7 with aqueous ammonium hydroxide before loading on an Amberlite XAD-4 column (2.0 cm × 65 cm). The column was eluted with 5.5 L of H_2O, at which point all of the glycine had been eluted [^1H NMR or TLC analysis (SiO_2, 70:25:5 CH_2Cl_2:CH_3OH:conc aqueous NH_3, ninhydrin]. Elution sequentially with 1.0 L of 10% aqueous CH_3OH, 2.0 L of 20% aqueous CH_3OH, 2.0 L of 25% aqueous methanol, 1 L of 35% aqueous CH_3OH, and 4.0 L of 50% aqueous CH_3OH afforded fractions containing **23** (^1H NMR, TLC analysis), which were concentrated in vacuo to afford **23** as a yellow solid, which was triturated with ethanol and filtered to yield a while solid. Including some **23** obtained as a pale yellow solid by purification of the mother liquors on Amberlite XAD-2, the total yield of **23** is 14.5 g (59% for three steps): mp 207–210 °C (EtOH); ^1H NMR (500 MHz, D_2O/DCl) δ 2.28 (3 H, s, $ArCH_3$), 2.91 (1 H, dd, J = 8, 16 Hz, β-H), 2.99 (1 H, dd, J = 8, 16 Hz, β-H), 3.78 (3 H, s, NCH_3), 3.81 (1 H, t, J = 7 Hz, α-H), 4.00 (2 H, m, SCH_2) 6.99 (2 H, d, J = 8 Hz, ArH), 7.14 (2 H, d, J = 8 Hz, ArH), 8.77 (1 H, s, Im H2); HRMS (FABS, glycerol) calcd 306.127 (M + 1), found 306.129. Anal. Calcd for $C_{15}H_{19}N_3O_2S$: C, 55.7; H, 6.6; N, 13.0; S, 9.9 Found: C, 55.3; H, 6.2; N, 12.8; S, 9.6.

***S*-[(4-Methylphenyl)methyl]-DL-ovothiol B.** Alkylation of the hydrochloride salt of halide **16** (from 1.0 g, 4.0 mmol of the corresponding alcohol) with diethyl *N*-methylacetamidomalonate[29] (1.9 g, 8.1 mmol) was carried out exactly as described above for preparation of **22**. Hydrolysis of the resulting crude diester (which NMR analysis revealed to contain DMF and diethyl *N*-methylacetamidomalonate) to afford the title compound likewise paralleled the preparation of **23** (*S*)-(4-methylphenyl)methyl-DL-ovothiol B; ^1H NMR (500 MHz, D_2O/DCl) δ 1.83 (3 H, s, $ArCH_3$), 2.31 (3 H, s, $NHCH_3$), 2.50 (2 H, m, 2 × β-H), 2.89 (1 H, d, J = 7 Hz, α-H), 3.36 (3 H, s, NCH_3), 3.56 (2 H, m, SCH_2), 6.55 (2 H, d, J = 8 Hz, ArH), 6.71, (2 H, d, J = 8 Hz, ArH), 8.40 (1 H, s, Im H-2).

DL-Ovothiol B (±1b). The conversion of (*S*)-(4-methylphenyl)methyl-DL-ovothiol B to DL-ovothiol B (trifluoroacetate salt) was accomplished in 73% yield as described below for the preparation of ±**1a**. ^1H NMR (500 MHz, D_2O) δ 2.96 (3 H, s, $NHCH_3$), 3.43 (1 H, dd, J = 6, 15 Hz, H-β), 3.46 (1 H, dd, J = 6, 15 Hz, H-β), 3.89 (3 H, s, NCH_3), 4.14 (1 H, br s, H-α), 8.53 (1 H, s, Im H-2); UV (H_2O) λ_{max} 234, 278 (shoulder) nm.

DL-Ovothiol A (±1a). A mixture of 1.0 g (3.28 mmol) of (*S*)-(4-methylphenyl)methyl-DL-ovothiol A and 1.0 mL of anisole was cooled to 0 °C and treated with 33 mL of trifluoroacetic acid. Mercuric trifluoroacetate (1.5 g, 3.51 mmol) was added. After the mercuric trifluoroacetate was dissolved, the ice bath was removed and the reaction mixture was stirred at 25 °C for 1.5 h. The mixture was concentrated in vacuo to an oil, taken up in 20 mL of deionized water, and washed with ether (3 × 15 mL). Hydrogen sulfide was bubbled into the aqueous layer for 0.5 h, and the black suspension was filtered through Celite with deionized water. An aliquot of the aqueous filtrate was concentrated in vacuo to obtain the following spectral data for DL-ovothiol A: ^1H NMR (500 MHz, D_2O) δ 3.49 (1 H, dd, J = 7, 15 Hz, β-H), 3.55 (1 H, dd, J = 7, 15 Hz, β-H), 3.94 (3 H, s, NCH_3), 4.56 (1 H, dd, J = 7 and 7 Hz, α-H), 8.6 (1 H, s, Im H-2); UV (H_2O) λ_{max} 237, 278 (shoulder) nm; HRMS (FABS, glycerol) calcd 202.065 (M + 1), found 202.063.

DL-Ovothiol A Disulfide (25). Oxygen was bubbled into the aqueous solution of DL-ovothiol A obtained from the previous experiment containing ~3 mg of $CuCl_2$ at 0 °C for 3 h. After warming to 25 °C, this solution was passed through a column of Dowex AG 50WX8 (water as eluent, then 1 M aqueous NH_4OH), hydrogen form. The Ninhydrin-active fractions were combined and concentrated in vacuo to yield 0.55 g (76%) of the title compound as a solid (mixture of diastereoisomers): ^1H NMR (500 MHz, D_2O) δ 2.8 (2 H, m, β-H), 2.9 (2 H, m, β-H), 3.7 (6 H, s, CH_3), 3.75 (2 H, m, α-H), 7.8 (2 H, m, Im H-2); UV [0.1 M HCl(aq)] λ_{max} 264 nm; MS (FABS, glycerol) 401 (M^+ + H). Anal. Calcd for $C_{14}H_{20}N_6O_4S_2 \cdot 2H_2O$: C, 38.5; H, 5.5; N, 19.3; S, 14.7. Found: C, 39.0; H, 5.4; N, 19.7; S, 13.9.

Acknowledgment. We thank the Searle Scholars Program and the National Institutes of Health (Grants GM23910 and GM35466) for financial support, Lisa Hager for technical assistance, Drs. B. M. Shapiro, E. Turner, J. Heinicke for disclosure of results prior to publications, and Susan McKenzie for typing the manuscript. P.B.H. holds a Research Career Development Award (AG 00417) and is a Sloan Fellow.

Registry No. (L)-**1a**, 108418-13-9; (DL)-**1a**, 122174-26-9; (DL)-**1b**·2TFA, 122174-28-1; (DL)-**1b** (*S*-(4-methylphenyl)methyl), 122174-41-8; (L)-**1c**, 105496-34-2; (DL)-**1c**, 122174-29-2; **8** (5-(2-hydroxyethyl)), 122174-30-5; **8** (5-(2-cyano-2-(dimethylamino)-ethyl)), 122174-31-6; **9**, 108560-38-9; **10**, 122174-32-7; **11**·2HCl, 122174-33-8; **12**, 108591-63-5; **13**, 110117-68-5; **13** (5-hydroxymethyl), 110117-69-6; **14**, 122174-34-9; **14** (5-hydroxymethyl), 122174-40-7; **15**·HCl, 110117-70-9; **16**·HCl, 122174-35-0; **17** (diastereomer 1), 110117-72-1; **17** (diastereomer 2), 122211-78-3; **18**, 110117-73-2; **19**, 110117-74-3; **20**, 73491-33-5; **21**, 110117-75-4; **22**, 122174-36-1; **23**, 122174-37-2; **24**, 122174-38-3; **25**, 122174-39-4; MeNH(CH_2CN), 5616-32-0; OHCNMe(CH_2CN), 36801-48-6; 4-$MeOC_6H_4CH_2Cl$, 824-94-2; 4-$MeC_6H_4CH_2Cl$, 104-82-5; $AcNHCH(COOEt)_2$, 1068-90-2; $AcNMeCH(COOEt)_2$, 36295-64-4; (3*R*)-2,5-diethoxy-3,6-dihydro-3-isopropylpiperazine, 110117-71-0.

Supplementary Material Available: ^1H NMR spectra of selected intermediates and ovothiols (19 pages). Ordering information is given on any current masthead page.

(29) Hess, J. *Chem. Ber.* **1972**, *105*, 441.