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Synthesis of Bridged Catechol–Homocysteine Derivatives as Potential Inhibitors of Catechol *O*-Methyltransferase

O. WILLIAM LEVER, JR.ˣ, CLIFTON HYMAN, and HELEN L. WHITE

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Abstract □ Catechol derivatives, covalently joined to homocysteine by sulfide or sulfonium linkages, were synthesized as potential catechol *O*-methyltransferase multisubstrate inhibitors which might bridge the enzymatic binding sites for the catechol substrate and the amino acid portion of the methyl donor *S*-adenosylmethionine. These compounds were found to be less effective inhibitors than the product inhibitor *S*-adenosylhomocysteine.

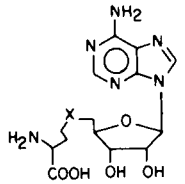
Keyphrases □ Catechol homocysteine derivatives - bridged, synthesis, inhibition of catechol *O*-methyltransferase □ Catechol *O*-methyltransferase - potential inhibitors, bridged catechol-homocysteine derivatives

Catechol *O*-methyltransferase (COMT; EC 2.1.1.6) catalyzes methyl transfer from *S*-adenosyl-L-methionine (SAM, I) to catechol substrates and plays an important role in the inactivation of catecholamines (1, 2). COMT inhibitors offer opportunities for control of catecholamine levels and have potential for treatment of disorders thought to arise from catecholamine deficiencies (3). For example, by decreasing the formation of *O*-methylated metabolites of levodopa and dopamine, COMT inhibitors may enhance the effectiveness of levodopa therapy for Parkinsonism (3–5).

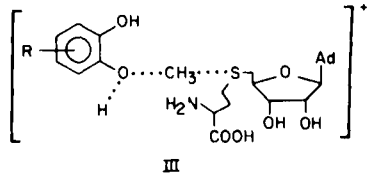
One approach to design of inhibitors of SAM-dependent methylases has focused on analogues of SAM or the product inhibitor *S*-adenosyl-L-homocysteine (SAH, II) (6–12). However, analogues of SAM and SAH may not be highly specific inhibitors for a single methylase since many methyltransferases utilize SAM as the methyl donor and are also subject to potent product inhibition by SAH (1). Although inhibitor specificity for COMT might be more readily achieved with catechol analogues, a number of potent *in vitro* inhibitors of this type are limited in clinical potential by poor *in vivo* activity and/or by unacceptable toxicity (3).

An intriguing alternative approach to COMT inhibitor design emanates from consideration of the proposed mechanism for methyl transfer by COMT. The mechanism appears to involve an S_N2-like nucleophilic attack by the catechol on the methyl group of SAM (13, 14) with a transition state which may be depicted as shown in III. This suggests that compounds such as IV and V, in which a catechol is covalently joined to homocysteine by sulfide or sulfonium linkages, might bridge the enzymatic binding sites for the catechol and for the amino acid portion of SAM and thus might function as multisubstrate (15), or rudimentary transition-state (16, 17), inhibitors of COMT.

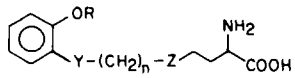
The direct transition state analogue IV (n = 1) would not be expected to be sufficiently stable to permit study in an aqueous environment. In an earlier study (18), this problem was circumvented by isosteric replacement of one of the catechol oxygen atoms by a methylene group (*cf.* VI, n = 1). The adducts thus obtained were stable, but were not potent COMT



I X = $\overset{+}{S}$Me [SAM]
II X = S [SAH]



III



IV Y = O; Z = $\overset{+}{S}$Me
V Y = O; Z = S
VI Y = CH_2; Z = S or $\overset{+}{S}$Me

Table I—Inhibition of Catechol-*O*-Methyltransferase [a]

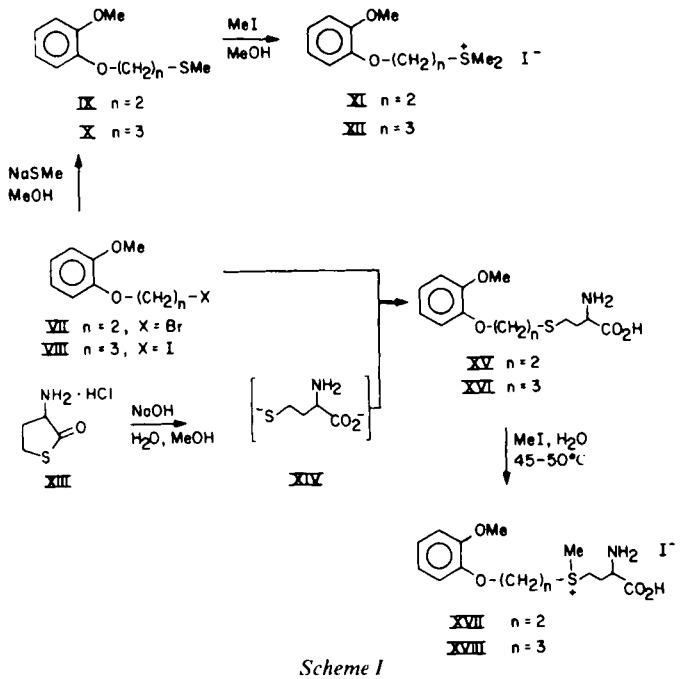
Compound	Percent Inhibition at 100 μM
XXIV	--[b]
XXV	14.9
XV	13.2
XVI	9.0
XVII	12.1
XVIII	5.6
IX	17.3
X	14.0
XI	5.9
XII	Stimulates[c]
XIX	4.7
II	72.2

[a] Rat liver COMT was incubated with 0.1 mM [^{14}C-methyl]*S*-adenosyl-L-methionine (0.3 Ci/mol), 0.25 mM 3,4-dihydroxybenzoic acid, 1 mM $MgCl_2$, 0.5 mM dithiothreitol, and 50 mM Tris-HCl buffer, pH 7.8, for 30 min at 37°C. [b] No significant inhibition observed. [c] Apparent stimulation of activity by 26.4% at 100 μM XII.

inhibitors (18), possibly because COMT is highly specific for the catechol functionality in substrates and in most inhibitors (2, 3). Retention of both catecholic oxygen atoms, for possible coordination at the catechol binding site, might facilitate multisubstrate binding. This could be accomplished by insertion of one or more additional methylenes in the briding chain. The bridging link would thereby be one or two units longer than required for an exact transition-state analogue (*cf.* III). However, a bridging length greater than that suggested by precise transition state mimicry led to increased potency in multisubstrate kinase inhibitors (19), and the expanded bridging chain in the present case might thus be an acceptable structural feature. Accordingly, sulfides and sulfonium salts of types IV and V were synthesized for *in vitro* evaluation as COMT inhibitors.

RESULTS AND DISCUSSION

Chemistry—The synthesis of the sulfides and sulfonium salts with *o*-methoxy substituents are outlined in Scheme I. The only salt obtained from reaction of iodide VIII with dimethyl sulfide was trimethylsulfonium iodide; none of the desired salt XII could be isolated. This type of reversion-ligand exchange process is well documented for sulfonium salts (20, 21), and has recently been adapted as a synthetic method for symmetrical and unsymmetrical organic sulfides (22). This problem was circumvented by preforming



Scheme I

sulfides IX and X followed by their alkylation with methyl iodide. The unsuitability of iodide VIII for direct formation of sulfonium salts was again noted in unsuccessful attempts to form bridged salt XVIII from VIII and methionine, although *S*-methyl methionine XIX was readily formed from methionine and methyl iodide under acidic (23) or neutral (24), *i.e.* zwitterionic amino acid, conditions. We then developed an alternative route to XVII and XVIII utilizing thiolactone XIII. Dianion XIV was generated from XIII by a method similar to those previously reported (25-27) and was alkylated with halides VII and VIII to provide sulfides XV and XVI. The resulting alkyl homocysteines were then alkylated with methyl iodide under neutral conditions (24), which led to specific methylation of the sulfide linkage in the presence of the unprotected amino acid moiety.

The *o*-hydroxy sulfides XXIV and XXV were prepared by similar methods to those in Scheme I. Alkylation of *o*-benzyloxyphenol with 1,3-dibromopropane followed by reductive debenzylation provided the phenolic bromide XXII, which afforded sulfide XXV on treatment with dianion XIV. In the preparation of dimethylene analogue XXIV, protection of the phenol as the acid-labile tetrahydropyranyl group (*cf.* XXIII) was required to avoid intramolecular cyclization (28) of the unprotected phenolic bromide to benzodioxane XX during the reaction with dianion XIV. The free phenol was liberated during acidic workup.

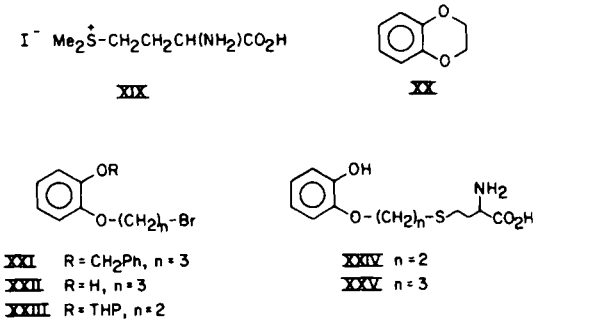
Enzyme Inhibition Studies—Sulfides and sulfonium salts prepared in this study were evaluated as COMT inhibitors by a radiochemical assay (29). The inhibition results listed in Table I indicate that these compounds are at least fourfold less effective inhibitors of COMT than the potent product inhibitor *S*-adenosylhomocysteine (II), and that the inhibition is not greatly affected by the type of bridging atom (sulfide or sulfonium), the length of the bridge (di- or trimethylene), or the nature of the *o*-substituent (hydroxy *versus* methoxy). Our goal was to enhance affinity and specificity of candidate COMT inhibitors by incorporation of portions of SAM (or SAH) into a catechol structure to increase the number of binding sites. Both the level and the narrow range of inhibition shown by the compounds in Table I, along with the results from the related study by Coward and co-workers (18), suggest that a multisubstrate approach to potentially clinically significant COMT inhibitors is likely to require a higher level of molecular complexity than that offered by bridged catechol-homocysteine structures. The additional presence of significant portions of the nucleoside moiety of SAM or SAH may be essential.

EXPERIMENTAL SECTION[1]

2-(2-Methoxyphenoxy)ethyl Bromide (VII)—A mixture of 2-methoxyphenol (124 g, 1.0 mol), 1,2-dibromoethane (379.7 g, 2.0 mol), and aqueous NaOH (48 g, 1.2 mol, in 300 mL of H_2O) was stirred overnight at 80°C. The product was extracted into $CHCl_3$ and the extracts were washed three times with 5% aqueous NaOH and then with water. The solvent was removed under reduced pressure, and the residue was distilled twice through a Vigreux column (15 cm). The fraction boiling at 125°C (0.25 mm) was dissolved in EtOH and the solution was cooled to provide crystals of VII as white needles (112.7 g, 49% yield), mp 45-46°C; ^{1}H-NMR ($CDCl_3$): δ 3.60 (t, 2, J = 7 Hz), 3.81 (s, 3), 4.30 (t, 2, J = 7 Hz), and 6.86 ppm (s, 4); UV (MeOH) λ_{max}: 271.5 (ε 2300) and 220.5 (ε 7400) nm; TLC (silica, $CHCl_3$): R_f 0.57.

Anal.—Calc. for $C_9H_{11}BrO_2$: C, 46.77; H, 4.80; Br, 34.58. Found: C, 46.75; H, 4.80; Br, 34.53.

3-(2-Methoxyphenoxy)propyl Iodide (VIII)—3-(2-Methoxyphenoxy)propyl bromide (51% yield; bp 110-118°C, 0.3 mm Hg) was prepared from 2-methoxyphenol and 1,3-dibromopropane in ethanolic aqueous sodium hydroxide essentially as described for VII. The bromide (51.5 g, 0.21 mol) was



[1] Melting points were determined with a Buchi melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. ^{1}H-NMR spectra were determined with Varian T-60, XL-100, and CFT20 and Hitachi Perkin-Elmer R-24B spectrometers with tetramethylsilane as internal standard. COMT inhibition data were obtained by a radiochemical assay (29).

then refluxed with sodium iodide (44.4 g, 0.3 mol) in methyl ethyl ketone (350 mL) for 21 h. The mixture was filtered and the solvent removed under reduced pressure. The residue was dissolved in ether, and on addition of hexane there was deposited 31.4 g (51% yield) of VIII. A portion (6.9 g) of this product was chromatographed on silica gel (Et_2O) and then was recrystallized from ether–hexane to give 4.4 g of VIII as white needles, mp 39–41°C; ^{1}H-NMR ($CDCl_3$): δ 2.31 (quintet, 2, J = 6 Hz), 3.39 (t, 2, J = 6 Hz), 3.85 (s, 3), 4.08 (t, 2, J = 6 Hz), and 6.91 ppm (s, 4); UV (MeOH) λ_{max}: 273.5 (ε 2700, sh) and 224.5 (ε 7200) nm; TLC (silica, $CHCl_3$): R_f 0.58.

Anal.—Calc. for $C_{10}H_{13}IO_2$: C, 41.11; H, 4.49; I, 43.45. Found: C, 41.31; H, 4.53; I, 43.22.

2-(2-Methoxyphenoxy)-1-methylthioethane (IX)—A solution of bromide VII (23.1 g, 0.10 mol) in MeOH (60 mL) was added over a 0.5-h period to a cold (0°C) stirred solution of NaOMe (16.5 g, 0.30 mol) and methanethiol (25 g, 0.52 mol) in MeOH (125 mL). After 0.5 h, the mixture was concentrated under reduced pressure and the residue was extracted with ether. The extracts were concentrated and the residue was distilled (oven 95–115°C, 0.025–0.045 mm Hg) to give IX (18.7 g, 92% yield) as a colorless oil; ^{1}H-NMR ($CDCl_3$): δ 2.19 (s, 3), 2.89 (t, 2, J = 7 Hz), 3.83 (s, 3), 4.17 (t, 2, J = 7 Hz), and 6.88 ppm (s, 4); UV (MeOH) λ_{max}: 272.5 (ε 2600, sh) and 222.5 (ε 8200) nm; TLC (silica, $CHCl_3$): R_f 0.77.

Anal.—Calc. for $C_{10}H_{14}O_2S$: C, 60.57; H, 7.12; S, 16.17. Found: C, 60.52; H, 7.16; S, 16.11.

3-(2-Methoxyphenoxy)-1-methylthiopropane (X)—Sulfide X was prepared in 79% yield from iodide VIII by the same procedure as described above for IX. Sulfide X was isolated as a colorless oil, bp 155°C (oven), 0.32 mm Hg; ^{1}H-NMR ($CDCl_3$): δ 2.10 (m, 5), 2.72 (t, 2, J = 6 Hz), 3.85 (s, 3), 4.14 (t, 2, J = 6 Hz), and 6.92 ppm (s, 4); UV (MeOH) λ_{max}: 272.5 (ε 2400, sh) and 222.5 (ε 7500) nm; TLC [silica, $CHCl_3$–EtOAc (2:1)]: R_f 0.61.

Anal—Calc. for $C_{11}H_{16}O_2S$: C, 62.23; H, 7.60; S, 15.10. Found: C, 62.47; H, 7.67; S, 14.94.

[2-(2-Methoxyphenoxy)ethyl]dimethylsulfonium Iodide (XI)—A solution of sulfide IX (2.97 g, 0.015 mol) and methyl iodide (2.74 g, 0.019 mol) in MeOH (10 mL) was stoppered and allowed to stand in the dark at ambient temperature for 17 h. Addition of ether precipitated sulfonium salt XI as an off-white powder, mp 100–101°C; ^{1}H-NMR (D_2O): δ 2.93 (s, 6), 3.73 (s, 3), 3.61 (t, 2, J = 6 Hz), 4.37 (t, 2, J = 6 Hz), and 6.93 ppm (m, 4); UV (MeOH) λ_{max}: 271.5 (ε 2500, sh) and 219.0 (ε 21,200) nm; TLC [silica, $CHCl_3$–MeOH–NH_4OH–H_2O (40:30:10:3)]: R_f 0.79.

Anal.—Calc. for $C_{11}H_{17}IO_2S$: C, 38.83; H, 5.04; I, 37.30; S, 9.43. Found: C, 38.83; H, 5.06; I, 37.33; S, 9.43.

[3-(2-Methoxyphenoxy)propyl]dimethylsulfonium Iodide (XII)—Sulfonium salt XII was prepared from sulfide X in 72% yield by the procedure described above for preparation of XI. Salt XII was isolated as an off-white microcrystalline solid, mp 98–99°C; ^{1}H-NMR (D_2O): δ 2.34 (br quintet, 2), 2.94 (s, 6), 3.50 (t, 2, J = 7 Hz), 3.87 (s, 3), 4.22 (t, 2, J = 6 Hz), and 7.06 ppm (br s, 4); UV (MeOH) λ_{max}: 271.5 (ε 4200, sh) and 219.5 (ε 23,900) nm; TLC [silica, $CHCl_3$–MeOH–H_2O, (40:30:3)]: R_f 0.78.

Anal.—Calc. for $C_{12}H_{19}IO_2S$: C, 40.68; H, 5.41; I, 35.82; S, 9.05. Found: C, 40.59; H, 5.41; I, 35.70; S, 9.01.

2-Amino-4-[2-(2-methoxyphenoxy)ethylthio]butyric Acid (XV)—DL-Homocysteinethiolactone hydrochloride XIII (12.24 g, 0.08 mol) was heated for 1.5 h at 65°C under nitrogen with a solution of NaOH (9.6 g, 0.24 mol) in 11% aqueous MeOH (88 mL). A solution of bromide VII (19.4 g, 0.084 mol) in MeOH (60 mL) was then added dropwise during a 0.5-h period. After an additional 3.5 h at 65°C, the mixture was concentrated under reduced pressure, the residue was dissolved in hot water, and the mixture was filtered. After cooling, the aqueous solution was extracted twice with ether and then adjusted to pH 7 with concentrated HCl. The resulting precipitate was collected and treated with EtOH. The mixture was filtered, and the filtrate was cooled to provide amino acid XV (11.7 g, 51% yield) as a white powder, mp 221–224°C; ^{1}H-NMR (Me_2SO-d_6): δ 1.92 (m, 2), 2.83 (t, 4, J = 7 Hz), 3.28 (br t, 1, J = 7 Hz), 3.72 (s, 3), 4.09 (t, 2, J = 7 Hz), and 6.90 ppm (s, 4); UV (MeOH) λ_{max}: 272 (ε 2600, sh) and 222.5 (ε 8000) nm; TLC (silica, MeOH): R_f 0.41.

Anal.—Calc. for $C_{13}H_{19}NO_4S$: C, 54.71; H, 6.71; N, 4.91; S, 11.24. Found: C, 54.47; H, 6.77; N, 4.84; S, 11.17.

2-Amino-4-[3-(2-methoxyphenoxy)propylthio]butyric Acid (XVI)—In the same manner as described for preparation of XV, the dianion of DL-homocysteine thiolactone was formed in aqueous methanol at 70°C. Iodide VIII was added as a warm oil and after 0.5 h at 70°C the solution was evaporated, the residue was washed with ether, treated with isopropyl alcohol, and the mixture was filtered. The filtrate was evaporated and the residue was dissolved in water. The pH was adjusted to 7.9 and the resulting precipitate was recrystallized from ethanol to give a 23% yield of XVI as a pale-yellow powder, mp 221–224°C; ^{1}H-NMR (Me_2SO-d_6): δ 1.95 (m, 4), 2.37–2.85 (m, 4), 3.32 (m, 1), 3.74 (s, 3), 4.01 (t, 2, J = 6 Hz), 4.6–6.8 (v br, 3), and 6.93 ppm (s, 4); UV (MeOH) λ_{max}: 272.5 (ε 2500, sh) and 222.5 (ε 7600) nm; TLC (silica, MeOH): R_f 0.54.

Anal.—Calc. for $C_{14}H_{21}NO_4S$: C, 56.16; H, 7.07; N, 4.68; S, 10.71. Found: C, 55.87; H, 7.12; N, 4.73; S, 10.79.

(3-Amino-3-carboxypropyl)[2-(2-methoxyphenoxy)ethyl]methylsulfonium Iodide (XVII)—Methyl iodide (1.88 g, 13.2 mmol) was added to a warm solution of sulfide XV (1.71 g, 6 mmol) in water (40 mL). The mixture was stirred at 50°C for 4 h followed by 12 h at 25°C. A cold condenser (~5°C) was used throughout. The residue, after evaporation under reduced pressure, was dissolved in 50% aqueous ethanol. The solution was filtered and cooled to provide salt XVII (1.00 g, 39% yield) as a pale yellow powder, mp 131–133°C; ^{1}H-NMR (D_2O): δ 2.34 (q, 2, J = 7 Hz), 2.99 (s, 3), 3.36–3.88 (m, 8), 3.77 (s, 3), 4.46 (t, 2, J = 6 Hz), and 6.96 ppm (m, 4); UV (MeOH) λ_{max}: 272 (ε 4900, sh) and 219 (ε 40,000) nm; TLC [silica, $CHCl_3$–MeOH–NH_4OH–H_2O (40:30:10:3)]: R_f 0.71.

Anal.—Calc. for $C_{14}H_{22}INO_4S$: C, 39.35; H, 5.19; I, 7.50; N, 3.28; S, 29.70. Found: C, 39.07; H, 5.20; I, 7.44; N, 3.22; S, 29.51.

(3-Amino-3-carboxypropyl)[3-(2-methoxyphenoxy)propyl]methylsulfonium Iodide (XVIII)—Methyl iodide (1.69 g, 12 mmol) was added to a warm (45°C) mixture of sulfide XVI and water (40 mL) in an apparatus protected with a cold water (~5°C) condenser. After 2 h at 45°C, an additional quantity (1.69 g, 12 mmol) of methyl iodide was added and stirring was continued for 3 h at 45°C and 14 h at ambient temperature. The mixture was filtered and evaporated, and the residue was dissolved in water. Ethanol was added, and the resulting solid was collected and dried to provide 2.77 g (63% yield) of salt XVIII as an off-white powder, mp 126–130°C; ^{1}H-NMR (D_2O): δ 2.39 (m, 4), 3.00 (s, 3), 3.58 (m, 4), 3.86 (s, 3), 3.91 (t, 1, J = 6 Hz), 4.22 (t, 2, J = 6 Hz), and 7.06 ppm (s, 4); UV (MeOH) λ_{max}: 298 (ε 190, sh), 272.5 (ε 2700), and 219.5 (ε 20,000) nm; TLC [silica, $CHCl_3$–MeOH–NH_4OH–H_2O (40:30:10:3)]: R_f 0.61.

Anal.—Calc. for $C_{15}H_{24}INO_4S$: C, 40.82; H, 5.48; I, 7.27; N, 3.17; S, 28.76. Found: C, 40.71; H, 5.52; I, 7.26; N, 3.14; S, 28.65.

(L-Methionine)methylsulfonium Iodide (XIX)—In a manner similar to that described for the preparation of the methyl sulfonium salt from racemic methionine (24), a mixture of L-methionine (3.7 g, 0.025 mol), water (20 mL), and methyl iodide (3.9 g, 0.027 mol) was stirred at 40°C for 18 h. The mixture was taken to dryness under reduced pressure, and the residue was triturated with ethanol. The resulting solid was recrystallized from aqueous ethanol, and the product was washed with hot methanol to provide 5.0 g (68% yield) of XIX as a white microcrystalline solid, mp 185°C (dec.) [the mp for the corresponding salt from racemic methionine has been reported as 150°C (dec.) (23, 24) and 156–157°C (dec.) (30); we are unaware of a specific mp citation for the optically active form, although an elemental analysis has been reported previously (31)]; ^{1}H-NMR (D_2O): δ 2.28–2.50 (m, 2), 2.98 (s, 6), 3.42–3.60 (m, 2), and 3.91 ppm (t, 1, J = 7 Hz); UV (MeOH) λ_{max}: 217.5 nm (ε 61,800); $[\alpha]_D^{20}$ 17.70°; TLC [silica, $CHCl_3$–MeOH–NH_4OH–H_2O (40:30:10:3)]: R_f 0.63.

Anal.—Calc. for $C_6H_{14}INO_2S$: C, 24.75; H, 4.85; I, 43.59; N, 4.81; S, 11.01. Found: C, 24.78; H, 4.89; I, 43.59; N, 4.80; S, 11.00.

1-(2-Benzyloxyphenoxy)-3-bromopropane (XXI)—To a stirred solution of 2-benzyloxyphenol (30.0 g, 0.15 mol) and 1,3-dibromopropane (60.5 g, 0.30 mol) in ethanol (100 mL) was added a solution of NaOH (6 g, 0.15 mol) in water (150 mL). The mixture was refluxed for 9 h, allowed to stand overnight, and then extracted with $CHCl_3$. The organic layer was washed with aqueous NaOH and then with water. The extract was dried (Na_2SO_4) and concentrated, and the residue was distilled to provide an oil (bp 157°C, 0.002 mm Hg) which was crystallized with ether–hexane to give 20.5 g (42% yield) of the bromide as a white powder, mp 29–30°C; ^{1}H-NMR ($CDCl_3$): δ 2.30 (quintet, 2, J = 6 Hz), 3.60 (t, 2, J = 6 Hz), 4.15 (t, 2, J = 6 Hz), 5.10 (s, 2), 6.92 (s, 4), and 7.40 ppm (m, 5); UV (MeOH) λ_{max}: 275 nm (ε 2750); TLC [silica, $CHCl_3$–hexane (1:1)]: R_f 0.44.

Anal.—Calc. for $C_{16}H_{17}BrO_2$: C, 59.81; H, 5.33; Br, 24.89. Found: C, 60.01; H, 5.39; Br, 24.70.

3-(2-Hydroxyphenoxy)propyl Bromide (XXII)—A solution of the benzyloxy compound XXI (44.9 g, 0.14 mol) in ethanol (100 mL) was hydrogenated at 40 psi over 20% $Pd(OH)_2$ on charcoal until hydrogen uptake ceased. The catalyst was removed by filtration, and the solution was evaporated under reduced pressure to provide XXII as an oil, which was used directly in the preparation of sulfide XXV. In another experiment the oil crystallized to give long off-white prisms, mp 52–54°C [lit. (28) mp 57–58°C]; ^{1}H-NMR ($CDCl_3$): δ 2.27 (quintet, 2, J = 6 Hz), 3.52 (t, 2, J = 6 Hz), 4.13 (t, 2, J = 6 Hz), 5.46 (br s, 1), and 6.84 ppm (m, 4).

2-(2-(2-Tetrahydropyranyloxy)phenoxy)ethyl Bromide (XXIII)—A mixture of 2-(2-tetrahydropyranyloxy)phenol (19.4 g, 0.10 mol), NaOH (4.4 g, 0.11 mol), 1,2-dibromoethane (75.1 g, 0.40 mol), and 20% aqueous EtOH (100 mL) was heated at 80°C for 4.5 h. The solvent was removed under reduced pressure, and the residue was treated with $CHCl_3$. The extract was washed

with 5% aqueous NaOH, then water, and concentrated under reduced pressure. Distillation of the residue gave 10.2 g (34% yield) of XXIII as a colorless oil, bp 105–130°C, 0.2 mm Hg; ^{1}H-HMR ($CDCl_3$): δ 1.3–2.3 (m, 6), 3.56 (t, 2, J = 6 Hz), 3.90 (m, 2), 4.25 (t, 2, J = 6 Hz), 5.37 (br s, 1), and 6.2–7.2 ppm (m, 4).

2-Amino-4-[2-(2-hydroxyphenoxy)ethylthio]butyric Acid (XXIV)—A mixture of DL-homocysteinethiolactone hydrochloride (XIII, 4.59 g, 0.03 mol) and NaOH (3.6 g, 0.09 mol) in 20% aqueous MeOH (45 mL) was stirred under nitrogen at 25°C for 18 h, then heated at 65°C for 2 h. Bromide XXIII (9.90 g, 0.033 mol) was then added, and after 3 h at 65°C and 17 h at room temperature, the mixture was concentrated, the residue was treated with isopropyl alcohol, and the mixture was filtered. The filtrate was evaporated, and the residue was dissolved in water. The resulting aqueous solution was washed twice with ether. The aqueous phase was adjusted to pH 8 (1 M HCl), and the precipitate which formed was collected and then suspended in water. The aqueous solution was adjusted to pH 6 (1 M HCl), and the mixture was stirred for 0.75 h. The deprotected product, precipitated by adjustment to pH 7, was washed with a small amount of absolute EtOH followed by ether. This afforded 3.31 g (41% yield) of sulfide XXIV as a white powder, mp 206–209°C; ^{1}H-NMR (Me_2SO-d_6): δ 1.9 (m, 2), 2.68 (t, 2, J = 7 Hz), 2.83 (t, 2, J = 7 Hz), 3.33 (t, 1, J = 6 Hz), 4.05 (t, 2, J = 7 Hz), 6.5–7.0 (m, 4), and 7.9 ppm (v br, 4); UV (MeOH) λ_{max}: 274 (ε 2800, sh), and 215 (ε 7200) nm; TLC (silica, MeOH): R_f 0.51.

Anal.—Calc. for $C_{12}H_{17}NO_4S$: C, 52.25; H, 6.39; N, 5.08; S, 11.62. Found: C, 52.24; H, 6.34; N, 5.05; S, 11.67.

2-Amino-4-[3-(2-hydroxyphenoxy)propylthiobutyric Acid (XXV)—A mixture of DL-homocysteinethiolactone hydrochloride (XIII; 12.24 g, 0.08 mol) and NaOH (9.6 g, 0.24 mol) in 20% aqueous MeOH (60 mL) was stirred under nitrogen at 25°C for 18 h, then heated at 65°C for 2 h. Bromide XXII (15.0 g, 0.065 mol) was added, followed by a solution of NaOH (5.2 g, 0.13 mol) in 20 mL of 50% aqueous MeOH. After the addition of a second portion of XXII (15.0 g, 0.065 mol), the mixture was stirred at 25°C for 18 h and the solvent was removed under reduced pressure. The residue was dissolved in water, and the solution was extracted three times with $CHCl_3$. The aqueous phase was brought to pH ~2 with 6 M HCl and extracted twice with $CHCl_3$. The aqueous phase was brought to pH 7 with 1 M NaOH, and product XXV was collected as a white powder (8.93 g, 39% yield), mp 218–221°C; ^{1}H-NMR (Me_2SO-d_6): δ 1.90 (m, 4), 2.4–2.8 (m, 4), 3.25 (t, 1, J = 6 Hz), 3.96 (t, 2, J = 6 Hz), 6.55–6.95 (m, 4), and 7.9 ppm (v br, 3); UV (MeOH) λ_{max}: 274.5 (ε 2900), 279 (ε 2500), and 215 (ε 6800) nm; TLC (silica, MeOH): R_f 0.51.

Anal.—Calc. for $C_{13}H_{19}NO_4S$: C, 54.71; H, 6.71; N, 4.91; S, 11.24. Found: C, 54.56; H, 6.78; N, 4.91; S, 11.22.

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