Synthesis and Analysis of Pheomelanin Degradation Products. 2

Dilip G. Patil and Miles R. Chedekel*[1]

The Johns Hopkins University School of Hygiene and Public Health, Department of Environmental Health Sciences, Division of Environmental Chemistry, Baltimore, Maryland 21211

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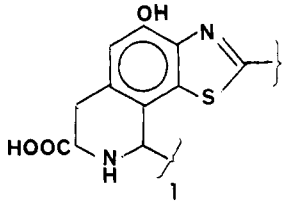
The syntheses of two of the HI degradation products, namely, α-amino-β-(4-hydroxy-7-benzothiazolyl)propionic acid (**4**) and α-amino-β-(3-hydroxy-4-aminophenyl)propionic acid (**5**), of the red-brown polymeric pheomelanin pigment are reported. These two amino acids along with the other major HI degradation products, **2**, **3**, and **6–9**, are useful for characterization and quantification of pheomelanin polymers, and an HPLC system for their separation and quantification is described.

Introduction

The melanin pigments occurring in the hair and skin of mammals are classified as either eumelanins or pheomelanins; eumelanins are black or dark brown, whereas pheomelanins are lighter red-brown or yellow pigments. Both are high-molecular-weight polymers of poorly defined structure.[2] Melanins catalyze several redox reactions,[3] and it has been suggested that such chemical reactivity may play an important role in the protective action of melanin in skin against ultraviolet light. However, pheomelanin as compared to eumelanin is exceptionally photolabile,[4] and it has been postulated that its photolysis contributes to the well-documented hypersensitivity to sunlight and susceptibility to chronic solar damage exhibited by fair-skinned individuals whose epidermis contains this pigment.[5]

The putative structure for the pheomelanin monomer **1**[6] was proposed on the basis of analysis of the chemical



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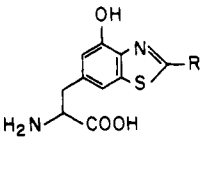
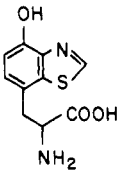
degradative products together with studies on the biosynthetic pathway of the pigment. Degradation of pheomelanin with hydriodic acid in a sealed tube at high temperatures afforded amino acids **2–7**[6] and isoquinolines **8** and **9**.[7] In order to have sufficient quantities of these degradation products for quantitative analysis and to place speculation about the structure of pheomelanin on firmer grounds, it was felt that proof of the structures of these amino acids by total synthesis should be sought. We have previously reported the synthesis of α-amino-β-(4-hydroxy-6-benzothiazolyl)propionic acid, **3**,[8] and herein we detail the synthesis of amino acid **4** and **5** and an HPLC system for separation and quantification of amino acids **3–6**.



2 R = CH_3
3 R = H



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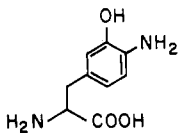

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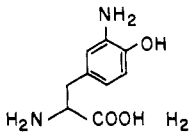

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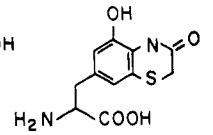

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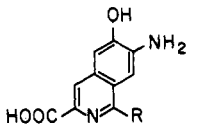

8 R = H
9 R = CH_3

Results and Discussion

Although a great deal of indirect evidence has been presented in support of the occurrence of pheomelanin in human skin, direct chemical or biochemical evidence is lacking.[9] In order to demonstrate the presence and to quantify pheomelanin in human skin, sufficient quantities of compounds **2–9** were needed. The total synthesis of these compounds will have the added benefit of providing sufficient quantities of compounds for development of standard chemical and/or biochemical analyses (e.g., radioimmunoassay).

Synthesis of α-Amino-β-(4-hydroxy-7-benzothiazolyl)propionic Acid (4). In this paper we report the first synthesis of amino acid **4**, via the NBS oxidation of 4-acetoxy-7-methylthiazole followed by displacement of the resulting benzyl bromide with diethyl acetamidomalonate and subsequent hydrolysis with hydrochloric acid (see Scheme I).

In approaching the synthetic problems presented by amino acid **4**, we first aimed at the synthesis of 4-methoxy-7-methylbenzothiazole (**13**) in a rather straightforward manner. To date, numerous methods have been devised for the preparation of substituted benzothiazoles. One such route, the cyclization of an arylthiourea by the action of molecular bromine, has proven, in our hands, to be a versatile method for the synthesis of 2-aminobenzothiazoles.[10] Treatment of 5-methyl-2-methoxyaniline (**10**)

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(3) (a) Gan, E. V.; Haberman, H. F.; Menon, I. A. *Biochim. Biophys. Acta* **1974**, *310*, 62–64. (b) Van Woert, M. H. *Life Sci.* **1967**, *6*, 2605–2612.
(4) Chedekel, M. R.; Post, P. W.; Deibel, R. M.; Kalus, M. *Photochem. Photobiol.* **1977**, *26*, 651–653.
(5) Agin, P. P.; Sayre, R. M.; Chedekel, M. R. *Photochem. Photobiol.* **1980**, *31*, 359–362.
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(7) Minale, L.; Fattorusso, E.; Cimino, G.; DeStefano, S.; Nicolaus, R. A. *Gazz. Chim. Ital.* **1970**, *100*, 880–887.
(8) Ismail, I. A.; Sharp, D. E.; Chedekel, M. R. *J. Org. Chem.* **1980**, *45*, 2243–2246.
(9) Chedekel, M. R. *Photochem. Photobiol.* **1982**, *35*, 881–855.

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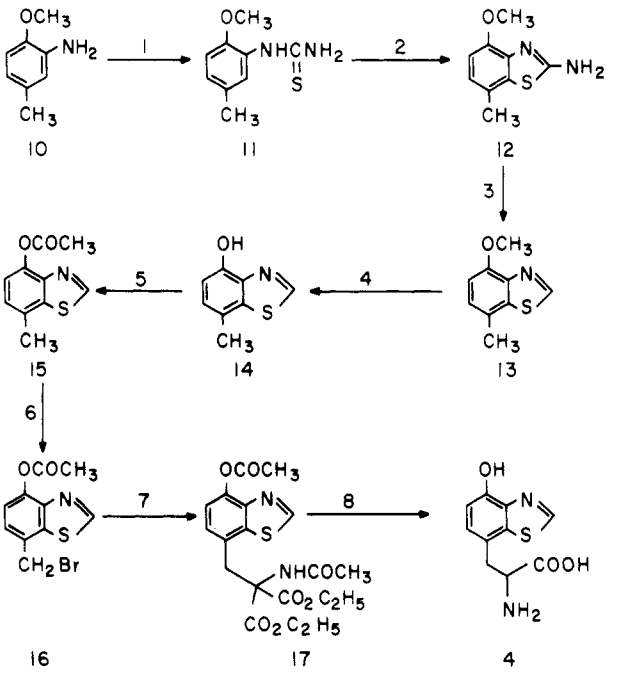
Scheme I



Reagents: (1) KSCN/HCl, 64% yield, (2) $Br_2/CHCl_3$, 93% yield, (3) $NaNO_2/H_3PO_3$, 83% yield, (4) concentrated HCl/heat (sealed tube), 94% yield, (5) Ac_2O/pyridine, 94% yield, (6) NBS/CCl_4, (7) diethyl acetamidomalonate/NaH/DMF, 96% yield from **15**, (8) 5 N HCl/heat, 92% yield.

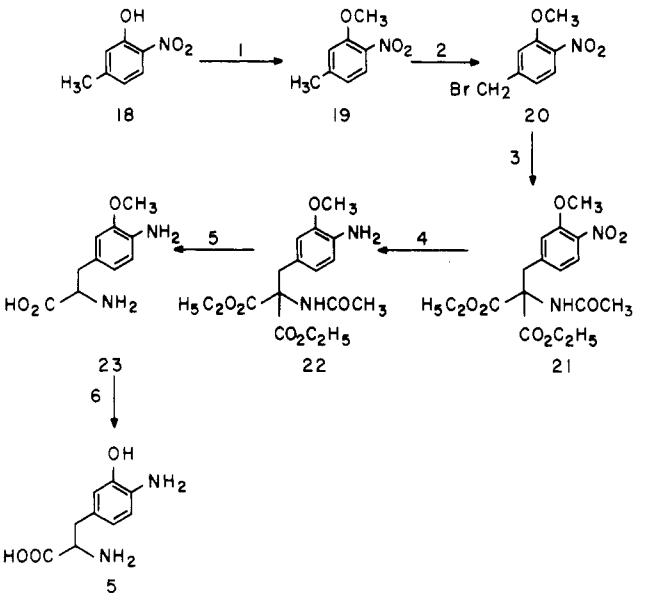
with potassium thiocyanate gave thiourea **11**, which upon treatment with bromine in chloroform afforded 2-amino-7-methyl-4-methoxybenzothiazole (**12**) in 93% yield. Reductive deamination of **12**, with sodium nitrite in 30% hypophosphorus acid gave 4-methoxy-7-methylbenzothiazole (**13**) in 83% yield. The structure was assigned on the basis of its spectral characteristics (NMR, IR, UV). Of special note is the presence of a singlet at 8.95 ppm in the proton NMR; this signal is characteristic of the proton in the 2-position of the benzothiazole nucleus.

NBS oxidation of **13** gave rise to a mixture of products containing the desired benzyl bromide along with ring-brominated and dibrominated species. In order to overcome this problem, we substituted the deactivating acetoxy group for the 4-methoxy substituent (see Scheme I). The 4-acetoxy group sufficiently deactivated the ring, and we were able to smoothly oxidize the resultant 4-acetoxy-7-methylbenzothiazole (**15**) to the corresponding benzyl bromide **16**, with NBS. However, even in this case, if stoichiometric amounts of NBS were employed, ring and dibromination complicated the workup. Thus NBS was used as the limiting reagent, and a nearly quantitative yield of a 70/30 mixture of **16** and **15** was obtained. While separation of this mixture at this stage was difficult, the mixture could be readily quantified by proton NMR and carried on to the next step.

The next reaction, i.e., displacement of the bromine by diethyl acetamidosodiomalonate, only effected benzyl bromide **16**, and the differences in R_f values between the resultant displacement product **17** and **15** were sufficiently large that separation by simple filtration chromatography over silica gel was possible. On the basis of the amount of recovered 4-acetoxy-7-methylbenzothiazole, the overall yield for conversion of **15** to **17** is 96%. Subsequent acid hydrolysis of **17** and ion-exchange chromatography of the crude reaction mixture afforded 92% yields of α-amino-β-(4-hydroxy-7-benzothiazolyl)propionic acid, **4**.

(10) Hugerschoff, A. *Chem. Ber.* **1903**, *36*, 3121.

Scheme II



Reagents: (1) $(CH_3)_2SO_4/K_2CO_3$, 92.8% yield, (2) NBS/CCl_4, (3) diethyl acetamidomalonate/NaH/DMF, 90% yield from **19**, (4) $SnCl_2 \cdot 2H_2O$/HCl, 88% yield, (5) 5 N HCl/heat, 85% yield, (6) 47% HI/heat (sealed tube), 95% yield.

Synthesis of α-Amino-β-(3-hydroxy-4-aminophenyl)propionic Acid (5). It was envisaged that the desired amino acid **5** could be conveniently synthesized from the known diethyl 1-acetamido-2-(3-methoxy-4-aminophenyl)-1,1-ethanedicarboxylate (**22**)[8] by acid hydrolysis and subsequent decarboxylation (see Scheme II). The procedure for synthesis of **22** involving the NBS oxidation of 3-methoxy-4-nitrotoluene (**19**) to 3-methoxy-4-nitrobenzyl bromide (**20**) followed by displacement of the bromine with diethyl acetamidosodiomalonate to yield diethyl 1-acetamido-2-(3-methoxy-4-nitrophenyl)-1,1-ethanedicarboxylate (**21**) has been previously reported.[8]

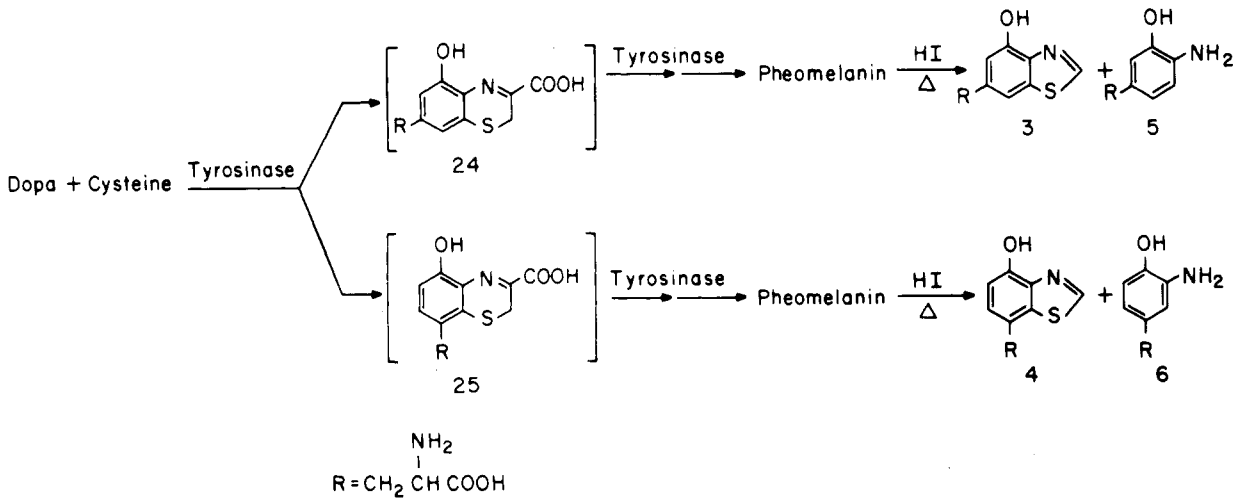
The above series of reactions was reinvestigated and several of the steps were modified to improve their convenience and/or yields. Reduction of **21** with stannous chloride in methanolic HCl gave the desired amine **22** in 88% yield. Subsequent acid hydrolysis with 5 N HCl and purification by ion-exchange chromatography afforded an 85% yield of α-amino-β-(3-methoxy-4-aminophenyl)-propionic acid (**23**). Finally, good yields of **5** are obtained by heating **23** in a sealed tube with 47% HI at 135 °C for 24 h. The structure of **5** was confirmed by UV, IR, and NMR analysis.

HPLC Analysis of Pheomelanin Degradation Products. Degradation of pheomelanin polymers with 47% HI in a sealed tube at 140 °C gives rise to the unique set of degradation products **2–9**, which can be used to identify and quantitate the presence of pheomelanin. The initial stages of pheomelanin biosynthesis involve formation of dihydrobenzothiazines **24** and **25** (see Scheme III),[11] which ultimately polymerize to afford the pheomelanin polymer. While the structures of the various pheomelanin monomer units may be questionable,[12] the substitution patterns of the various degradation products are indicative of their dihydrobenzothiazine origin, i.e., **24** vs. **25**. Indeed,

(11) Prota, G.; Crescenzi, S.; Misureae, G.; Nicolaus, R. A. *Experientia* **1970**, *15*, 1058–1059.

(12) Deibel, R. M.; Chedekel, M. R. *J. Am. Chem. Soc.* **1982**, *104*, 7306–7309.

Scheme III



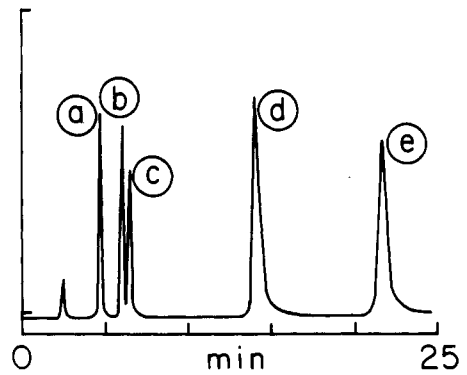


Figure 1. HPLC trace of a mixture of (a) Dopa, (b) 3-amino-4-hydroxyphenylalanine (**6**), (c) 4-amino-3-hydroxyphenylalanine (**5**), (d) amino acid **4**, (e) amino acid **3**.

the relative abundances of the various degradation products reveals information regarding the constitution of the pheomelanin polymer in question.

In a previous attempt to quantitate pheomelanin content in mixed pheomelanin and eumelanin copolymers, Ito and Jimbow reported on an HPLC separation of pheomelanin and eumelanin HI degradation products.[13] However, they were unable to separate the aminohydroxyphenylalanines **5** and **6**, thereby limiting the utility of their analysis. While we have not yet synthesized the two isoquinoline degradation products **8** and **9**, our HPLC system readily separates compounds **3–6** as well as Dopa and is enabling us to systematically reexamine the HI degradation of pheomelanin (see Figure 1). Indeed, under the reported HI degradation conditions[6] we find that **3** readily hydrolyzes to **5** and **4** to **6**. The rates of formation of **5** and **6** from **3** and **4**, respectively, are not the same and will need to be taken into account for quantitative analysis. These results, along with reexamination of the HI degradation of pheomelanins and photopheomelanins,[9] will be reported in a subsequent communication.

Experimental Section

Proton and carbon nuclear magnetic resonance spectra were recorded by using an IBM NR 80 NMR spectrometer, infrared spectra were obtained by using a Perkin-Elmer 298 infrared spectrometer, UV/vis spectra were recorded with a Cary Model 219 spectrophotometer, and fluorescence measurements were made with an Aminco SPF-500 spectrophotometer. High-performance liquid chromatography was performed on a Varian Associates Model 5060 liquid chromatograph equipped with a UV detector (280 nm). A 25 × 0.45 cm Zorbax O.D.S. reverse-bonded phase column (Dupont Instruments) was used for all analyses. Chromatograms were obtained under the following conditions: temperature, 30 °C; solvent, H_2O/0.2 M $NH_4H_2PO_4$, 0.005 N octanesulfonic acid sodium salt pH 1.5/acetonitrile (86/11/3); flow, 1 mL/min.

1-(2-Methoxy-5-methylphenyl)thiourea (11). A mixture of 2-methoxy-5-methylaniline (**10**; 20 g, 145 mmol), potassium thiocyanate (14.21 g, 145 mmol), and 20 mL of concentrated HCl in 150 mL of water was heated with stirring at reflux for 12 h. The mixture was allowed to cool to room temperature, whereupon it was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with water (2 × 10 mL) and brine (20 mL) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo to yield 26.5 g of crude thiourea **11**, which was purified by column chromatography over silica gel (1.5 × 40 cm column, eluting with hexane/ethyl acetate mixtures) to afford 18.1 g (64% yield) of pure **11**. An analytic sample of **11** was prepared by recrystallization from 95% ethanol: mp 122–123 °C; UV (EtOH) λ_{max} (ϵ) 290 nm (2846 M^{-1} cm^{-1}), 255 (1923); IR (KBr) 3440, 3130, 1620, 1300, 1120, 1030, 815, 715 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (3 H, s, Ar CH_3), 3.79 (3 H, s, OCH_3), 6.93 (2 H, dd, J = 6.7 Hz), 7.13 (1 H, s, Ar H). Anal. ($C_9H_{12}N_2OS$) C, H, N, S.[14]

4-Methoxy-7-methylbenzothiazole (13). To a cooled solution (10 ± 2 °C) of thiourea **11** (7.0 g, 36 mmol) in 25 mL of chloroform was introduced a cold solution of bromine (12.16 g, 76 mmol) during a 20-min period; the temperature was kept at 10 ± 2 °C during the addition. After addition, the reaction mixture was stirred for an additional 0.5 h at room temperature and finally heated at reflux for 20 min. The resultant yellow precipitate was collected by suction filtration and dried in vacuo (5 torr, 40 °C) to give a 93% yield of crude 2-amino-4-methoxy-7-methylbenzothiazole (**12**; 6.5 g, mp 164–165 °C). This compound was not further purified but rather carried on to the next step.

Crude **12** was dissolved in 100 mL of 30% hypophosphorous acid. The solution was cooled to −10 ± 2 °C and, with vigorous stirring, 15.0 g of sodium nitrite dissolved in the minimum amount of water was added dropwise such that the temperature remained below −10 °C. After addition was complete, the reaction was allowed to stand at ca. 5 °C for 48 h. The reaction mixture was then diluted with water (200 mL) and extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with 10% sodium hydroxide (2 × 10 mL), water (2 × 10 mL), and brine (20 mL) and dried over anhydrous sodium sulfate. The organic solvent was removed in vacuo to yield 6.00 g of crude **13**, which was purified by column chromatography over silica gel (100 g, 1.5 × 20 cm column, eluting with hexane/ethyl acetate mixtures) to afford 4.99 g (83% yield) of pure **13** as an oil: UV (EtOH): λ_{max} (ϵ) 306 nm (3181 M^{-1} cm^{-1}), 266 (3272), 257 (4090); IR (neat) 3065, 2840, 1495, 1455, 1325, 1265, 1050, 900, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.52 (3 H, s, Ar CH_3), 4.05 (3 H, s, OCH_3), 6.90 (1 H, d, J = 8.0

(13) Ito, S.; Jimbow, K. *J. Invest. Dermatol.* **1983**, *80*, 268–272.

(14) Satisfactory analytical data (±0.3% for C, H, N, and S) were obtained for all new compounds.

Hz, Ar H), 7.25 (1 H, d, J = 8.0 Hz, Ar H), 8.95 (1 H, s, N=CHS); ^{13}NMR ($CDCl_3$) 20.2, 55.9, 106.9, 123.4, 125.9, 135.8, 142.2, 151.5, 151.9 ppm.

4-Hydroxy-7-methylbenzothiazole (14). One gram (5.6 mmol) of pure **13** was heated at ca. 135 °C together with 10 mL of 47% HI in a sealed tube for 24 h. The product was obtained by removal of the solvent in vacuo to give 0.865 g (94% yield) of **14**: mp 154–156 °C; UV (EtOH) λ_{max} (ϵ) 316 (1760 M^{-1} cm^{-1}), 258 (2000); IR (KBr) 3285, 3150, 3090, 1595, 1505, 1455, 1395, 1100, 940, 832, 810 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.49 (3 H, s, Ar CH_3), 7.15 (1 H, d, J = 8.0 Hz, Ar H), 7.19 (1 H, d, J = 8.0 Hz, Ar H), 8.90 (1 H, s, N=CHS); ^{13}C NMR ($CDCl_3$) 20.4, 110.8, 122.6, 127.0, 134.7, 142.0, 148.7, 152.1 ppm. Anal. (C_8H_7NOS) C, H, N, S.

4-Acetoxy-7-methylbenzothiazole (15). A 1.10-g sample (6.6 mmol) of **14** was dissolved in a mixture of acetic anhydride (20 mL) and pyridine (0.5 mL) and stirred at room temperature for 12 h. Excess acetic anhydride was removed by concentration in vacuo to furnish **15** as colorless crystals (1.30 g, 94% yield), mp 69–71 °C; an analytical sample was prepared by crystallization from hexane: UV (EtOH) λ_{max} (ϵ) 292 (2906 M^{-1} cm^{-1}); IR (KBr) 1760, 1500, 1370, 1205, 1170, 1010, 860, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.45 (3 H, s, OCH_3), 2.56 (3 H, s, Ar CH), 7.27 (2 H, s, Ar H), 9.05 (1 H, s, N=CHS); ^{13}C NMR ($CDCl_3$) 169.0, 153.6, 145.3, 142.7, 136.0, 129.5, 125.8, 119.0, 29.0, 20.8 ppm. Anal. ($C_{10}H_9NO_2S$) C, H, N, S.

Diethyl 1-Acetamido-2-(4-acetoxy-7-benzothiazolyl)-1,1-ethanedicarboxylate (17). A mixture of **15** (1.01 g, 4.8 mmol), NBS (0.800 g, 4.5 mmol), and dibenzoyl peroxide (10 mg, 4×10^{-3} mmol) in anhydrous CCl_4 (50 mL) was heated at reflux under argon for 8 h. At the end of the reflux period the reaction was allowed to cool to room temperature, washed free of succinimide with water (3 × 20 mL), and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo gave a yellow oil (1.75 g) that contained a 70/30 mixture of **16**/**15**. This mixture was not further purified but rather carried on to the next step.

The above yellow oil (1.75 g), potassium carbonate (1.62 g, 11.7 mmol), and diethyl acetamidomalonate (1.50 g, 6.88 mmol) were heated at reflux under argon in dry acetone (50 mL) for 24 h. At the end of the reflux the reaction was allowed to cool to room temperature, diluted with water (100 mL), and extracted with $CHCl_3$ (3 × 40 mL). The combined organic layers were washed with water (2 × 20 mL) and brine (20 mL) and dried over anhydrous sodium sulfate. Removal of the solvent in vacuo yielded 2.2 g of a gummy residue, which was chromatographed over silica gel (75 g, 1.0 × 20 cm column). Hexane (100 mL) eluted **15** (300 mg), while 10% ethyl acetate in hexane (100 mL) furnished pure **17** (1.36 g, 96% yield based on recovered **15**). An analytic sample was prepared by crystallization from ethyl acetate/hexane: mp 132–133 °C; UV (EtOH) λ_{max} (ϵ) 298 nm (1380 M^{-1} cm^{-1}), 290 (1571), 254 (3642); IR (KBr) 3410, 3185, 2985, 1770, 1735, 1675, 1490, 1205, 1050, 1010, 933, 800 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (6 H, t, J = 6.4 Hz, CH_2CH_3), 1.97 (3 H, s, CO_2CH_3), 2.44 (3 H, s, $NHCOCH_3$), 3.95 (2 H, s, Ar CH_2), 4.26 (4 H, q, J = 6.4 Hz, CH_2CH_3), 7.19 (1 H, d, J = 7.2 Hz, Ar H), 7.06 (1 H, d, J = 7.2 Hz, Ar H), 8.93 (1 H, s, N=CHS). Anal. ($C_{19}H_{22}N_2O_7S$) C, H, N, S.

α-Amino-β-(4-hydroxy-7-benzothiazolyl)propionic Acid (4). A mixture of **17** (1.00 g, 2.36 mmol) and 5 N HCl (30 mL) was heated at reflux under argon for 18 h. After cooling to room temperature, the solvent was removed in vacuo and the residue purified by ion-exchange chromatography; the crude residue was taken up in the minimum amount of 0.5 N HCl and loaded onto a 2.5 × 22 cm column of Dowex-50W (100 g, 8% cross-linking, 100–200 mesh), the column was then thoroughly washed with 0.5 N HCl (500 mL), and the product, **4**, was eluted with 3 N HCl (ca. 200 mL). Removal of the solvent in vacuo afforded 600 mg (92% yield) of the hydrochloride salt of **4**, which was purified by crystallization from water: mp 238–242(dec; UV (1 N HCl); λ_{max} (ϵ) 318 nm (1606 M^{-1} cm^{-1}), 280 (1960), 268 (1774); IR (KBr) 2820, 1750, 1600, 1500, 1450 cm^{-1}; ^{1}H NMR (DCl) δ 3.62 (2 H, d, J = 7.2 Hz, Ar CH_2), 4.57 (1 H, t, J = 7.2 Hz, CH_2CH), 7.22 (1 H, d, J = 8.0 Hz, Ar H), 7.58 (1 H, d, J = 8.0 Hz, Ar H), 10.24 (1 H, s, N=CHS); ^{13}C NMR (DCl) 34.4, 53.4, 115.0, 121.0, 132.0, 132.1, 133.9, 148.1, 160.8, 170.8 ppm. Anal. ($C_{10}H_{10}N_2O_3S$·HCl) C, H, N, S.

α-Amino-β-(3-methoxy-4-aminophenyl)propionic Acid (23). A mixture of **22**[15] (5.0 g, 14.1 mmol) and 5 N HCl (70 mL) was heated at reflux under argon for 8 h. After the mixture cooled to room temperature, the solvent was removed in vacuo and the residue purified by ion-exchange chromatography; the crude residue was taken up in the minimum amount of 0.5 N HCl and loaded onto a 2.5 × 22 cm column of Dowex-50W (100 g, 8% cross-linking, 100–200 mesh), the column was thoroughly washed with 0.5 N HCl (500 ml), and the product, **23**, was eluted by 2 N HCl (ca. 200 mL). Removal of the solvent in vacuo afforded 3.41 g (85% yield) of the dihydrochloride salt of **23**: mp 179–181 °C; UV (H_2O) λ_{max} (ϵ) 278 nm (2000 M^{-1} cm^{-1}), 272 (2200); IR (KBr) 3400, 3100, 2600, 1755, 1630, 1600, 1500, 1325, 1275, 1190 cm^{-1}; ^{1}H NMR (D_2O) δ 3.33 (2 H, d, J = 7.2 Hz, Ar CH_2), 3.97 (3 H, s, Ar CH_3), 4.37 (1 H, t, J = 7.2 Hz, CH_2CH), 6.99 (1 H, s, Ar H), 7.11 (1 H, d, J = 8.0 Hz, Ar H), 7.43 (1 H, d, J = 8.0 Hz, Ar H); ^{13}C NMR (D_2O) 36.4, 54.8, 57.8, 114.5, 119.0, 122.8, 125.3, 138.0, 153.7, 171.9 ppm. Anal. ($C_{10}H_{14}N_2O_3$·2HCl) C, H, N.

α-Amino-β-(3-hydroxy-4-aminophenyl)propionic Acid (5). One gram (3.5 mmol) of **23** was heated at 130–135 °C together with 10 mL of 47% HI in a sealed tube for 24 h. The pure hydrochloride salt of **5** (900 mg, 95% yield) was obtained by removal of the solvent in vacuo followed by ion-exchange chromatography (vide supra). Due to the hygroscopic nature of this compound it was not possible to prepare an analytic sample. **5**: UV (H_2O) λ_{max} (ϵ) 275 nm (1900 M^{-1} cm^{-1}); IR (KBr) 3400, 2600, 1745, 1630, 1500, 1440, 1325, 1290, 1200, 1120, 1060, 960, 815 cm^{-1}; ^{1}H NMR (D_2O) δ 3.23 (2 H, d, J = 7.2 Hz, Ar CH_2), 4.31 (1 H, t, J = 7.2 Hz, CH_2CH), 6.95 (1 H, d, J = 8.0 Hz, Ar H), 6.96 (1 H, s, Ar H), 7.43 (1 H, d, J = 8.0 Hz, Ar H); ^{13}C NMR (D_2O) 36.1, 54.8, 118.0, 118.3, 122.4, 125.5, 137.7, 151.0, 171.9 ppm.

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Registry No. **3**, 82962-82-1; **4**·HCl, 88686-27-5; **5**, 88686-28-6; **5**·2HCl, 21819-90-9; **6**, 3387-86-8; **10**, 120-71-8; **11**, 88686-29-7; **12**, 88686-30-0; **13**, 88686-31-1; **14**, 88686-32-2; **15**, 88686-33-3; **16**, 88686-34-4; **17**, 88686-35-5; **22**, 73368-44-2; **23**·2HCl, 88686-36-6; Dopa, 59-92-7; potassium thiocyanate, 333-20-0; diethyl acetamidomalonate, 1068-90-2.

(15) Compound **22** was prepared from 3-methoxy-4-nitrotoluene (**19**) as previously described.[8]