Formation of Nor-6 from Ascorbic Acid and the Cyclic Acetal of Maleic Aldehyde. L-Ascorbic acid (1) (35.2 g, 0.20 mol) was dissolved in 112 mL of distilled water that had been purged with nitrogen for 1 h. Freshly distilled 2,5-dimethoxy-2,5-dihydrofuran (Aldrich Chemical Co.) (39.0 g, 0.30 mol) was added at once and the reaction mixture was left stirring under a nitrogen atmosphere for 10 days. The yellow-colored solution was frozen and freeze-dried on a Virtis Freezemobile at 10–50 mtorr for 7 days to give a crude product (50.4 g). The yellow solid was purified with Norit the same way as described for **6**. The colorless, amorphous 2-(2-furyl)-3-keto-L-gulonolactone 3,6-hemiketal (17.6 g, 36.4%) was obtained: ^{1}H NMR (acetone-d_6) δ 7.60 (d, 1 H), δ 6.65 (m, 1 H), δ 6.53 (m, 1 H), δ 4.87 (s, 1 H), δ 4.62 (m, 1 H), δ 4.23 (m, 2 H); ^{13}C NMR (Me_2SO-d_6) 172.9, 150.2, 143.0, 110.5, 108.4, 106.9, 87.6, 77.6, 74.6, 73.5.

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Registry No. 1, 50-81-7; 3, 34218-22-9; 4, 22414-24-0; 6, 93135-89-8; nor-6, 93135-92-3; 8a, 93184-28-2; 8b, 93135-90-1; 8c, 93135-91-2; succinic anhydride, 108-30-5; succinimide, 123-56-8; *N*-methylsuccinimide, 1121-07-9; potassium succinimide, 50433-20-0; 2,5-dimethyl-2,5-dihydrofuran, 332-77-4.

Supplementary Material Available: Full NMR and mass spectral data of complex **8a** (Figures 2, 3, 4) and fractional coordinates for **8a** (Tables 1 and 2) (5 pages). Ordering information is given on any current masthead page.

Conversion of 2,3-Dihydropyrrolo[2,3-*b*]indoles to 2-(Alkylthio)-L-tryptophans

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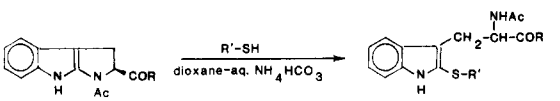
2,3-Dihydropyrrolo[2,3-*b*]indoles, easily derived from tryptophan derivatives by oxidation, are efficiently converted (48–85%) to 2-(alkylthio)tryptophan derivatives with thiols such as mercaptoethanol, ethanethiol, cysteine, or glutathione in ammonium bicarbonate buffered dioxane or dimethylformamide at 55 °C.

We have reported a novel conversion of *N*-acetyl-L-tryptophan esters, *N*-acetyl-L-tryptophanamide, and *N*-acetyltryptamine to the corresponding tricyclic 2,3-dihydropyrrolo[2,3-*b*]indoles by a one-step oxidation with aqueous *N*-bromosuccinimide at pH 9 or a preferable procedure using *tert*-butyl hypochlorite in triethylamine-buffered methylene chloride.[1,2] The tetracyclic system present in the sporidesmins, metabolites of *Pithomyces chartarum*,[3-5] was synthesized by *tert*-butyl hypochlorite oxidation of *N*-methyl-L-alanyl-L-tryptophan diketopiperazide.[2]

That these pyrroloindoles, themselves, can serve as useful intermediates is illustrated by their efficient conversion to 2-hydroxytryptophans by using sealed-tube hydrolysis with HCl. Crystalline 2-hydroxytryptophan, heretofore troublesome to prepare, is obtained by what is probably the most practical route at present.[6] This reaction, as well as the chlorination or acetoxylation of the 3a-position of 2,3-dihydropyrrolo[2,3-*b*]indoles[2] illustrate their use as reactive intermediates in the introduction of functional groups at the 2- or 3-position of the indole ring. In this paper, we extend the application of 2,3-dihydropyrrolo[2,3-*b*]indoles to the synthesis of 2-(alkylthio)-L-tryptophans.

Scheme I



1	R = NH_2		
2	R = OCH_3		
3	R = NH_2	R' = Et	
4	R = NH_2	R' = CH_2CH_2OH	
5	R = NH_2	R' = CH_2-CH(NH_2)CO_2H	
6	R = NH_2	R' = CH_2-CH(NH-γ-Glu)CO-Gly	
7	R = OCH_3	R' = Et	
8	R = OCH_3	R' = CH_2CH_2OH	
9	R = OCH_3	R' = $CH_2CH(NH_2)CO_2H$	
10	R = OCH_3	R' = CH_2CH(NH-γ-Glu)CO-Gly	

Results

We find that exposure of the pyrroloindoles **1** or **2** derived from *N*-acetyl-L-tryptophanamide or from *N*-acetyl-L-tryptophan methyl ester, respectively, with 2.5–10-fold molar excesses of the thiols, ethanethiol, 2-mercaptoethanol, L-cysteine, or glutathione, in dioxane or dimethylformamide containing ammonium bicarbonate at 55 °C gives 2-(alkylthio)tryptophans. The reaction must be conducted under weakly basic conditions, since under acidic conditions (pH <6), pyrroloindoles undergo spon-

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0022-3263/84/1949-5069$01.50/0

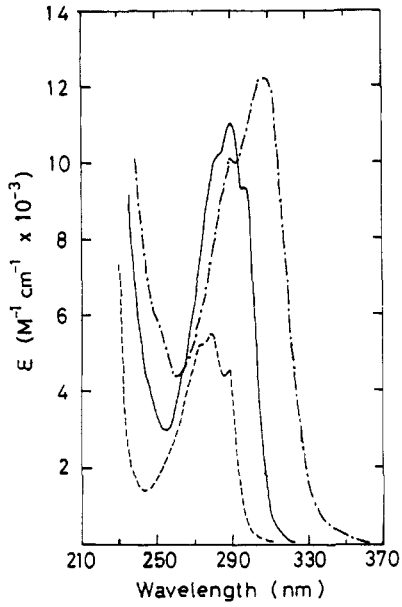


Figure 1. Ultraviolet absorption spectra of L-1-acetyl-2,3-dihydropyrrolo[2,3-*b*]indole-2-carboxamide (1) (-·-), N^{α}-acetyl-2-(ethylthio)-L-tryptophanamide (3) (—), and N^{α}-acetyl-L-tryptophanamide (- -) in methanol.

taneous hydrolysis to 2-hydroxytryptophan derivatives. The reaction in nonaqueous systems such as dioxane–triethylamine has so far been unsuccessful.

The following two general procedures were found to work most effectively in converting pyrroloindoles to 2-(alkylthio)tryptophans. A solution of **2** with excess thiol in a mixture of dioxane or dimethylformamide and 0.1–0.5 M ammonium bicarbonate was either (a) shaken at 55 °C for 2–5 h or (b) allowed to stand at room temperature for 2–3 days. The extent of the reaction can be followed by UV spectroscopy. In the case of the less soluble pyrroloindole (**1**), procedure b was unsuccessful. Shaking heterogeneous mixtures of **1** and thiols at 55 °C caused complete solubilization after periods of 4–12 h (depending upon the thiol) at which time the reactions were complete. When a volatile thiol such as ethanethiol was used, the reaction was carried out in a sealed tube.

The solutions were then lyophilized (ammonium bicarbonate is lost by volatilization) and the residues usually chromatographed on Sephadex G-25. The elution profile of a typical crude product exhibited at least 95% of its total UV absorbance (280 nm) in a single symmetrical peak. Material from this peak gave a single peak on HPLC and a UV spectrum (Figure 1) identical with a 2-(alkylthio)tryptophan.[7] Alternatively, extraction with ethyl acetate can be used to isolate the product from **1** and ethanethiol or 2-mercaptoethanol. Yields ranged from 48% to 85% for simple thiols and 61–80% for cysteine or glutathione.

Products were often amorphous solids which resisted crystallization and accordingly were analyzed after drying only. Some discrepancies between theoretical and actual compositions for C, H, and N will thus be noted which may be the result of traces of ammonium bicarbonate or disulfide impurities.

Discussion

Savige and Fontana showed[7] that L-3a-hydroxy-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indole-2-carboxylic acid, a product of peracetic acid oxidation of L-tryptophan, is converted upon reaction with thiols in an acidic medium to the corresponding 2-(alkylthio)-L-tryptophans. Nakagawa and co-workers demonstrated[8] a similar reaction with cyclized tryptamine derivatives prepared by dye-sensitized photooxygenation. The reactive tryptophan tricyclic compound[8,9] yields 2-(alkylthio)tryptophans possessing free α-amino and carboxyl groups, while our procedure produces N-acetylated derivatives of carboxyl-blocked tryptophans.

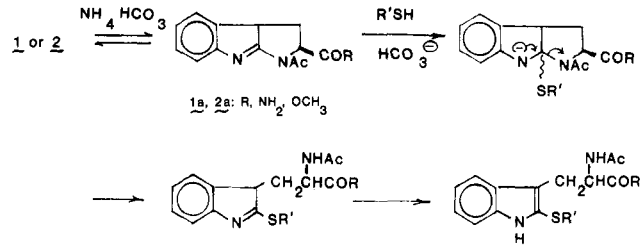
N-acetyl-2-[(2-nitro-4-carboxyphenyl)thio]-L-tryptophan methyl ester has been found[4] to be an excellent substrate for α-chymotrypsin. Likewise when esters **8** or **9** were exposed to α-chymotrypsin at pH 8, hydrolysis occurred. No starting material remained by HPLC analysis indicating that no racemization of the tryptophan moiety of **2**, **8**, or **9** occurred during the thiolysis reaction.

Tryptathione, 2-(*S*-cysteinyl)-L-tryptophan, is a constituent of phalloidin and related toxic peptides from the mushroom *Amanita phalloides*[11,12] and has been synthesized[13] from tryptophan derivatives by the in situ generation of sulfenyl chlorides from cysteine derivatives at low temperatures.

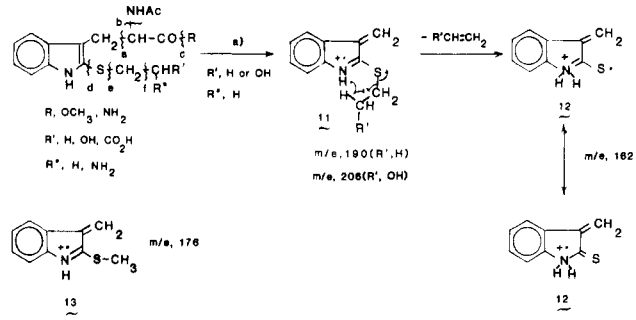
We find that tryptathione derivatives can easily be obtained in good yield by reacting **1** or **2** with L-cysteine. Even the more complex thiol, glutathione, gives good yields (63–68%).

The mechanism of this facile thiolysis has not been determined but a direct displacement seems unlikely while an addition–elimination process involving an intermediate pyrroloindolenine (**1a, 2a**) in equilibrium with the pyrroloindole seems more probable. Our choice of ammonium bicarbonate as a weak base may have been particularly

Scheme II



Scheme III



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fortuitous since it may serve also as a proton donor in one or more steps.

The electron-impact mass spectra of compounds **3**, **4**, **7**, and **8** show a significant peak at *m/e* 162 (see Experimental Section) from ion **12** evidently arising from a McLafferty rearrangement in the major fragment **11** resulting after the usual side chain cleavage (pathway a). Minor pathways with cleavage at b, c, and/or d are also evident (see Experimental Section).

Compounds **5** and **6**, the carboxamide and methyl ester derivatives of tryptathione, respectively, failed to exhibit a parent molecular ion either by chemical-ionization or electron-impact mass spectrometry but instead gave ions (CI) showing that β-elimination of $CH_2{=}C(NH_2)CO_2H$ (278, 293, respectively) followed by extrusion of sulfur (246, 261, respectively) were major thermal cleavage pathways. Electron-impact spectra of **5** and **6** show a major ion at *m/e* 161 which probably reflects cleavage of both side chains at a and e. The base peak (*m/e* 176) for ester **6** may arise from cleavage at a, then cleavage at f, with hydrogen transfer to give the ion **13**. The ion at *m/e* 186 for amide **5** may indicate that some cleavage at b ($-NH_2Ac$) and d has occurred.

Conclusion

The procedures described provide a new and simple synthetic route to 2-(alkylthio)-L-tryptophans previously obtainable only by the reaction of tryptophan derivatives with alkyl[14] or aryl[15] sulfenyl chlorides or by Savige and Fontana's method.

Experimental Section

Methods and Instrumentation. Melting points are uncorrected. 1-Acetyl-2,3-dihydropyrrolo[2,3-*b*]indole-2-carboxamide (**1**) and methyl 1-acetyl-2,3-dihydropyrrolo[2,3-*b*]indole-2-carboxylate (**2**) were prepared by *tert*-butyl hypochlorite[16] oxidation of *N*-acetyl-L-tryptophanamide and *N*-acetyl-L-tryptophan methyl ester, respectively, as previously described.[2] L-Cysteine and glutathione were supplied by Nakarai Chemicals (Kyoto, Japan). Sephadex G-25 (fine) was obtained from Pharmacia Fine Chemicals and used to prepare a 3 cm × 55 cm column. Ultraviolet absorption spectra were obtained with a Hitachi 100-60 spectrophotometer equipped with a Hitachi recorder, Model 056. Optical rotations were measured with a Union Giken PM-71 high-sensitivity polarimeter. ^{1}H NMR spectra were obtained with a Varian Associates HR 220-MHz instrument in the solvent indicated with tetramethylsilane as an internal reference. Chemical shifts are expressed in δ units (ppm). Mass spectra were obtained with a V.G. Micromass Ltd. mass spectrometer, Model 7070F, either in the chemiionization mode with ammonia or in the electron-impact mode with 70 eV electrons. High-performance liquid chromatography (HPLC) was conducted with a Hitachi 638-30 chromatograph equipped with a UVILOG III UV detector (Kusano) and a Fudoh Kogyo Model R-31 recorder and using a TSK-gel ODS-120T column (4.6 mm × 25 cm) (Toyo Soda). The solvent system used was 0.02 M sodium acetate buffer (pH 4.5) (A)–80% methanol (B). Elution was conducted with the indicated linear gradient of B over 30 min. In the following preparations, aqueous ammonium bicarbonate solutions were prepared before use with deoxygenated water. Combustion analyses were performed by M. Shito, Service Center for Elemental Analysis of Organic Compounds, Kyushu University.

***N*-Acetyl-2-(ethylthio)-L-tryptophanamide (3).** (a) Ethanethiol (0.19 mL, 2.5 mmol) was added to a suspension of **1** (122 mg, 0.5 mmol) in a test tube containing a mixture of dioxane (3 mL) and 0.2 M NH_4HCO_3 (2 mL) and the tube was sealed and shaken at 55 °C overnight. The clear solution, which showed an ultraviolet spectrum (Figure 1) nearly identical with that of a 2-(alkylthio)tryptophan,[7] was diluted with water and lyophilized. The residue was chromatographed on a Sephadex column with 20% ethanol. Fractions (5.2 mL) containing pure **3** (monitored by UV) were pooled and lyophilized. Crystallization from ethyl acetate–petroleum ether gave 73 mg (48%) of **3**: mp 164–165 °C; $[\alpha]^{20}_D$ +19° (*c* 0.4, methanol). This eluted as a single peak on HPLC at 12 min (60 → 90% B).

Anal. Calcd for $C_{15}H_{19}N_3O_2S$: C, 58.99; H, 6.27; N, 13.76. Found: C, 58.53; H, 6.63; N, 13.27.

Mass spectra (CI), *m/e* 306 (P + 1); (EI), *m/e* 306 (3%), 305 (3%), 191 (32%), 190 (100%), 185 (12%), 162 (21%); ^{1}H NMR (CD_3OD) 7.62 (d, 7.5, 1, H_4), 7.27 (d, 7.5, 1, H_7), 7.09 (t of d 7, 1, 1, H_6), 7.00 (t of d (7, 1), 1, H_5), 4.66 (t, 7, 1, α-H), 3.40 (d of d (14, 6.5), 1, β-H), 3.19 (d of d (14, 7.5), 1, β-H), 2.83 (4 lines, 2 overlapping triplets, 7, 2, S–CH_2), 1.86 (s, 3, NAc), 1.21 (t, 7, 3, SCH $_2CH_3$). Also impurity peaks for EtOAc observed.

(b) A mixture of **1** (146 mg, 0.6 mmol), ethanethiol (0.45 mL, 6 mmol), dioxane (6 mL), and 0.5 M NH_4HCO_3 (2 mL) was shaken in a sealed tube at 55 °C overnight. The resulting solution was lyophilized, and the residue was dissolved in ethyl acetate with the aid of a small volume of ethanol, then extracted with 1 M HCl and then water (this effectively removes any unreacted **1** by converting it to water soluble *N*-acetyl-2-hydroxytryptophanamide), finally dried over Na_2SO_4, and evaporated. The residue was triturated with petroleum ether to afford 94 mg (51%) of crystalline **3**: mp 164–165 °C.

***N*-Acetyl-L-2-[(2-hydroxyethyl)thio]tryptophanamide (4).** A heterogeneous mixture of **1** (122 mg, 0.5 mmol), 2-mercaptoethanol (0.35 mL, 5 mmol), dioxane (3.5 mL), and 0.5 M NH_4HCO_3 (2 mL) was shaken at 55 °C overnight. The resulting solution was diluted with water and lyophilized. The residue was chromatographed on a Sephadex column with 5% ethanol. Fractions containing pure product **4** which were well separated from impurity peaks were pooled and lyophilized. The fluffy powder (145 mg) was dissolved in 5 mL of ethyl acetate with the aid of a small volume of methanol and the solution was dired over Na_2SO_4. The residue, after evaporation, crystallized on trituration with petroleum ether. It gave a single peak on HPLC with a retention time of 6 min (60% → 90% B): yield, 137 mg (85%); mp 166–167 °C; $[\alpha]^{20}_D$ +26° (*c* 0.4, methanol).

Anal. Calcd for $C_{15}H_{19}N_3O_3S$: C, 56.06; H, 5.96; N, 13.07. Found: C, 55.53; H, 6.34; N, 12.63.

Mass spectra (CI), *m/e* 322 (P + 1); (EI), *m/e* 321 (3%), 303 (2%), 262 (2%), 206 (100%), 193 (11%), 185 (17%), 162 (83%); ^{1}H NMR (CD_3OD) 7.61 (d, 7, 1, H_4), 7.27 (d, 7, 1, H_7), 7.09 (t, 7, 1, H_6), 7.00 (t, 7, 1, H_5), 4.70 (t, 7, 1, α-H), 3.65 (t, 6.5, 2, CH_2OH), 3.39 (q, 14 and 6.5, 1β-H), 3.18 (q, 14 and 8, β-H), 2.95 (t, 6.5, 2, SCH_2), 1.86 (s, 3, NAc) .

***N*-Acetyl-L-2-(*S*-cysteinyl)tryptophanamide (5) Hydrochloride.** A mixture of **1** (122 mg, 0.5 mmol), L-cysteine (302 mg, 2.5 mmol), NH_4HCO_3 (316 mg, 4 mmol), dioxane (3 mL), and water (3 mL) was shaken at 55 °C for 7 h. Undesired L-cystine was removed by filtration and washed with water. The filtrate was lyophilized and the residue, dissolved in 3 mL of 0.1 M HCl, was chromatographed on a Sephadex column with water. Homogeneous fractions were pooled and lyophilized: yield, 160 mg (80%); retention time on HPLC, 20 min (20% → 60% B); $[\alpha]^{20}_D$ +49° (*c* 0.39, water).

Anal. Calcd for $C_{16}H_{20}N_4O_4S{\cdot}HCl$: C, 47.94; H, 5.28; N, 13.98. Found: C, 48.73; H, 5.54; N, 14.56.

Mass spectra (CI), *m/e* 330 (10%), 278 (26%), 261 (23%), 246 (100%), 244 (37%), 229 (44%); (EI), *m/e* 258 (10%), 214 (12%), 186 (39%), 161 (58%).

***N*-Acetyl-L-2-[(N_α-(γ-glutamyl)cysteinylglycyl-*S*]tryptophanamide (6) Hydrochloride.** A heterogeneous mixture of **1** (49 mg, 0.2 mmol), glutathione (307 mg, 1.0 mmol), dimethylformamide (2 mL), and 0.5 M NH_4HCO_3 (6 mL) was shaken at 55 °C overnight. The resulting solution was lyophilized. The residue was dissolved in water, acidified to pH 3 with 6 M HCl, and chromatographed on a Sephadex column with water. Homogeneous fractions were pooled and lyophilized: yield, 79 mg (63%); HPLC retention time, 14 min (20% → 60% B); $[\alpha]^{20}_D$ +28° (*c* 0.31, water).

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Anal. Calcd for $C_{23}H_{30}N_6O_8S$·HCl: C, 47.06; H, 5.32; N, 14.32. Found: C, 47.71; H, 5.47; N, 13.88.

Methyl *N*-Acetyl-2-(ethylthio)-L-tryptophanoate (7). A solution of **2** (258 mg, 1.0 mmol), ethanethiol (0.75 mL, 10 mmol), dioxane (6 mL), and 0.2 M NH_4CO_3 (3 mL) was shaken in a sealed tube at 55 °C overnight and then lyophilized. The residue was chromatographed on a Sephadex column with 40% ethanol. Homogeneous fractions were pooled and lyophilized. Crystallization of the pasty residue (176 mg, 55%) was unsuccessful. The material was homogeneous by HPLC with a retention time of 19 min (60% → 90% B).

Mass spectra (CI), *m/e* 323 (6%), 322 (20%), 321 (100%, P + 1), 277 (9%), 261 (52%), 215 (5%); (EI), *m/e* 320 (4%), 261 (5%), 232 (3%), 190 (100%), 162 (19%), 157 (10%), 130 (27%), 117 (7%); ^{1}H NMR ($CDCl_3$) 8.59 (s, 1, $N_{ind}H$), 7.52 (d, 7.5, 1, H_4), 7.4-7.05 (m, 3, H_{5-7}), 6.23 (d, 7, 1, NHAc), 4.93 (5 lines, 2 overlapping triplets, 6, 1, α-H), 3.69 (s, 3, OCH_3), 3.39 (7 lines, *J* = 14, 6, and 7, 2 protons, β-H), 2.76 (q, 7, 2, SCH_2), 1.95 (s, 3, NAc), 1.25 (5 lines, 2 overlapping triplets), ca. 7, 3, SCH_2CH_3).

Methyl *N*-Acetyl-2-[(2-hydroxyethyl)thio]-L-tryptophanoate (8). A solution of **2** (103 mg, 0.4 mmol), 2-mercaptoethanol (0.28 mL, 4 mmol), dioxane (3.5 mL), and 0.5 M NH_4HCO_3 (2.0 mL) was shaken for 6 h at 55 °C and lyophilized. The residue was purified on a Sephadex column with 30% ethanol as described above and yielded 97 mg (72%) of pasty solid which was homogeneous on HPLC; retention time, 11 min (60% → 90% B). The ^{1}H NMR spectrum indicated some 30% 2-mercaptoethanol disulfide: mass spectra (CI), *m/e* 337 (P + 1, 100%), 261 (55%), 255 (19%); (EI), *m/e* 336 (3%), 304 (4%), 277 (3%), 245 (8%), 232 (3%), 206 (100%), 188 (8%), 162 (68%), 130 (17%), 128 (8%), 117 (9%); ^{1}H NMR ($CDCl_3$) 9.25 (s, 1, $N_{ind}H$, exchanges with CD_3OD), 7.48 (d, 7.5, 1, H_4), 7.27 (d, 7.5, 1, H_7), 7.20 (t, 7.5, 1, H_6), 7.11 (t, 7.5, 1, H_5), 6.27 (d, 7, 1, NHAc, slow exchange), 4.93 (q, 6, 1, α-H), 3.80 (t, 5.5, 2, CH_2OH), 3.68 (s, 3, OCH_3), 3.39 (7 lines, *J* = 14 and 6, 2 protons, β-H), 2.91 (5 lines, overlapping triplets, *J* = 5.5, ~3, SCH_2 and $(SCH_2CH_2OH)_2$ impurity), 1.94 (s, 3, NAc). Also a triplet (approximately 1 proton) noted at 3.91 (*J* = 6) corresponding to CH_2OH protons of impurity.

Methyl *N*-Acetyl-2-(*S*-cysteinyl)-L-tryptophanoate (9). A solution of **2** (103 mg, 0.4 mmol), L-cysteine (194 mg, 1.6 mmol), dioxane (3.5 mL), and 0.5 M NH_4CO_3 (4.5 mL) was shaken at 55 °C for 2 h and filtered to remove precipitated cystine. The filtrate was acidified with 6 M HCl and lyophilized. The residue was purified on a Sephadex column with water yielding 101 mg (61%) of fluffy powder; retention time on HPLC 28 min (20% → 60% B); $[\alpha]^{20}_D$ +14° (*c* 0.28, water).

Anal. Calcd for $C_{17}H_{21}N_3O_5S$·HCl: C, 49.10; H, 5.33; N, 10.10. Found: C, 50.26; H, 5.59; N, 10.88.

Mass spectra (CI), *m/e* 345 (3%), 307 (16%), 293 (84%), 275 (22%), 261 (100%); (EI), *m/e* 306 (7%), 258 (22%), 216 (18%), 187 (17%), 176 (100%), 162 (40%), 161 (54%). ^{1}H NMR (CD_3OD): strong solvent impurities at δ 3.23 and 4.95 obscure some signals; nevertheless sharp singlets at 1.89 (NDAc) and 3.52 (CO_2CH_3) and an 8-line ABX spectrum) (δ_A = 2.94, δ_B = 3.21, J_{AB} = 14, J_{AX} = 8.5, J_{BX} = 7), most probably for the β-CH_2 of the tryptophyl side chain, can be discerned. A spectrum in CD_3CN containing ca. 10% CD_3OD exhibits impurity signals at 1.93 and 3.0 but again, sharp singlets at 1.80 and 3.46 are seen and a triplet (*J* = 7) at 4.60 for the tryptophyl α-H is evident. Both spectra show the usual aromatic pattern. While complicated by the poor solubility of **9** (ca. 10 mg/mL), these spectra are consistent with a single diastereomer only of **9** and strongly suggest that no racemization of **2** or **9** occurs during the thiolysis reaction.

Methyl *N*-Acetyl-2-[N_α-(γ-glutamyl)cysteinylglycyl-*S*]-L-tryptophanoate (10). The reaction was conducted with **2** (103 mg, 0.4 mmol), glutathione (492 mg, 1.6 mmol), dioxane (3.5 mL), and 0.5 M NH_4HCO_3 (4.5 mL) at 55 °C for 6 h. After lyophilization, the residue was dissolved in 1 M HCl (4 mL) and fractionated on a Sephadex column with water giving 164 mg (68%) of fluffy powder: retention time on HPLC, 24 min (20% → 60% B); $[\alpha]^{20}_D$ +17° (*c* 0.29, water).

Anal. Calcd for $C_{24}H_{31}N_5O_9S$·HCl: C, 47.88; H, 5.36; N, 11.63. Found: C, 48.71; H, 5.54; N, 11.88.

The reaction was also carried out at room temperature to give the same compound and was complete after 3 days.

Enzymatic Hydrolyses of 8 and 9. Compound **8** or **9** and α-chymotrypsin in a ratio of 20 to one were stirred at pH 8 (0.1 M NH_4HCO_3) for 4 h at 37 °C, then lyophilized, and analyzed by HPLC (20% → 60% B) to reveal a single major peak with a retention time of 4.5 or 16 min, respectively. Compounds **8** and **9** had retention times of 11 and 28 min, respectively, under these conditions.

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Registry No. **1**, 21018-89-3; **2**, 25690-48-6; **3**, 92818-08-1; **4**, 92818-09-2; **5**·HCl, 92843-98-6; **6**·HCl, 92818-10-5; **7**, 92818-11-6; **8**, 92818-12-7; **9**·HCl, 92818-14-9; **10**·HCl, 92818-13-8; ethanethiol, 75-08-1; 2-mercaptoethanol, 60-24-2; L-cysteine, 52-90-4; glutathione, 70-18-8.

Synthesis of Protected Aminocyclohexanediols

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As the model study for the synthesis of aminocyclitols and amino sugars, 2-cyclohexen-1-ol (**1**) was converted to five of the seven possible (1,2,3)-aminocyclohexanediols in protected form (**4a**, **6a**, **11a**, **13a**, and **18a**). Two flexible new approaches were employed: (1) the preparation and iodocyclization of the unsaturated carbonimidothioate **2**, and (2) the preparation, rearrangement, and subsequent iodocyclization of unsaturated carbonimidate **8**. The alkene functionalization reactions allow a high measure of regio- and stereochemical control over the placement of the eventual amino and hydroxy groups.

The vicinal amino alcohol functionality is the principle structural characteristic of aminocyclitols and amino sugars.[1] In many of these compounds a second hydroxy group flanks the amino alcohol. In connection with our interest in the synthesis of aminocyclitol antibiotics,[2] we have developed methods for the regio- and stereospecific introduction of the cis, vicinal amino alcohol functionality.[3-5] We now report the optimization of these procedures

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