in vacuo and the residue shaken with a small quantity of water. The precipitate formed was isolated by filtration and recrystallized from ethanol to afford 0.21 g (48% of theory) of colorless crystals, mp >300 °C.

Derivative. Compound **11a** was warmed briefly in acetic anhydride. The reaction mixture was then evaporated to dryness and the residue crystallized with ethanol to afford the 4-acetamido derivative: mp 277–278 °C; NMR (Me_2SO-d_6) δ 2.37 (3 H, 6-CH_3), 2.72 and 2.87 (3 H + 3 H, acetyl CH_3's), 7.5–8.4 (5 H, Ph), 9.04 and 9.27 (1 H + 1 H, NH's).

4,6-Diamino-7-(ethoxycarbonyl)-2-phenyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (11c).[20] A portion of **5a** (1.0 g, 3.23 mmol) dissolved in 50 mL of glacial acetic acid was hydrogenated in the presence of 50 mg of 10% palladium-on-carbon at room temperature under 1 atm of hydrogen until the hydrogen uptake ceased. The mixture was stirred at room temperature for an additional 24 h. The catalyst was removed by vacuum filtration and the filtrate evaporated in vacuo. The residual oil was shaken with a small quantity of water, and the resulting suspension was adjusted to neutral pH by the addition of aqueous ammonia. The solid generated was collected by filtration and recrystallized from ethanol to give 0.86 g of colorless crystals (83% of theory): mp 258–262 °C; NMR (Me_2SO-d_6) δ 1.36 (3 H, t, CH_3CH_2), 4.28 (2 H, q, CH_3CH_2O), 7.5–8.4 (5 H, Ph).

7-Acetyl-4-amino-6-methyl-5*H*-pyrrolo[3,2-*d*]pyrimidine (12a). A portion of **3b** (400 mg, 1.5 mmol) and 250 mg of 10% palladium-on-carbon were mixed with 10 mL of glacial acetic acid and then attached to an atmospheric pressure hydrogenation apparatus. Following purging of oxygen the reaction mixture was heated to slightly less than 100 °C with a preheated oil bath and then stirred vigorously for 4 h at 90–100 °C. Hydrogen adsorption appeared to cease after 12 min. The reaction mixture was then permitted to cool to room temperature, and the catalyst was separated by vacuum filtration. Evaporation of the solvent in vacuo afforded a clear tan oil which crystallized when treated with a small amount of ethyl ether. The solid was isolated by filtration and dissolved in water, and the resultant solution was filtered. The addition of excess ammonia to the filtrate generated a precipitate which when it was filtered and dried was found to weigh 195 mg (69% of theory); mp 350–51 °C. The analytical sample was prepared by reprecipitation from a slightly acidic aqueous solution with excess ammonia: NMR (CD_3CO_2D) δ 2.63, 2.86 (3 H + 3 H, CH_3C=O and 6-CH_3), 8.52 (1 H, C^2H); mass spectrum (EI), *m/e* (relative, intensity) 191 (1.3), 190 (10.3, M^+), 175 (11.3), 95 (10.3), 69 (94.8),[27] 45 (100.0); precise mass (calcd/found) 190.085 456/190.085 318.

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Registry No. 2a, 30720-60-6; **2b**, 74366-57-7; **2c**, 74366-58-8; **2d**, 74366-59-9; **3a**, 74366-60-2; **3b**, 74366-61-3; **4a**, 74366-62-4; **4b**, 74366-63-5; **5a**, 74366-64-6; **5b**, 74366-65-7; **5c**, 74366-66-8; **6**, 23582-09-4; **7a**, 74366-67-9; **7b**, 74366-68-0; **7c**, 74366-69-1; **7d**, 74366-70-4; **7e**, 74366-71-5; **7f**, 74366-72-6; **7g**, 74366-73-7; **7h**, 74366-74-8; **8a**, 74366-75-9; **8b**, 74366-76-0; **8c**, 74366-77-1; **8d**, 74366-78-2; **9**, 74366-79-3; **10a**, 74366-80-6; **10b**, 74366-81-7; **10c**, 74366-82-8; **10d**, 74366-83-9; **11a**, 74366-84-0; **11a** 4-acetamido derivative, 74366-85-1; **11b**, 74366-86-2; **11b** 4-acetamido derivative, 74366-87-3; **11c**, 74366-88-4; **11d**, 74366-89-5; **11e**, 74366-90-8; **11f**, 74366-91-9; **12a**, 74366-92-0; **12b**, 74366-93-1; **12c**, 74366-94-2; **12d**, 74366-95-3; **12e**, 74366-96-4; 2,4-pentanedione, 123-54-6; 1-morpholino-1-cyclohexene, 670-80-4; 3-piperidino-3-pentene, 21086-43-1.

Supplementary Material Available: Tables containing physical properties and analytical data for compounds **2b-d** (Table II), **3**, **4**, **5** (Table III), **7a–h** (Table IV), **8a–d**, **9** (Table V), **10a–d** (Table VI), **11a–f**, and **12a–e** (Table VII) (7 pages). Ordering information is given on any current masthead page.

(27) The mass spectrum of this compound was obtained by using a sample of its trifluoroacetate salt which is reflected by this signal.

New Histamine and Histidine Analogues via Transformations of the 2-Trifluoromethyl Group

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α-*N*-Benzoyl-2-(trifluoromethyl)histamine has been transformed, via a difluorodiazafulvene intermediate, into 2-carboxy- and 2-(carbomethoxy)histamine. 2-Carboxy-L-histidine was prepared by a similar route. 2-(Carbomethoxy)-L-histidine was prepared by methanolysis of α-*N*-(*tert*-butoxycarbonyl)-2-(trifluoromethyl)-L-histidine and acid hydrolysis of the intermediate ortho ester. 2-Cyanohistamine and 2-cyano-L-histidine are best prepared by ammonolysis of the corresponding α-*N*-(*tert*-butoxycarbonyl)-2-(trifluoromethyl)imidazoles and acid cleavage of the protecting group. During the hydrolysis of *N*-benzoyl protecting groups in hot, aqueous mineral acid, decarboxylation of 2-carboxyimidazoles occurs gradually; this side reaction is repressed by use of concentrated acid.

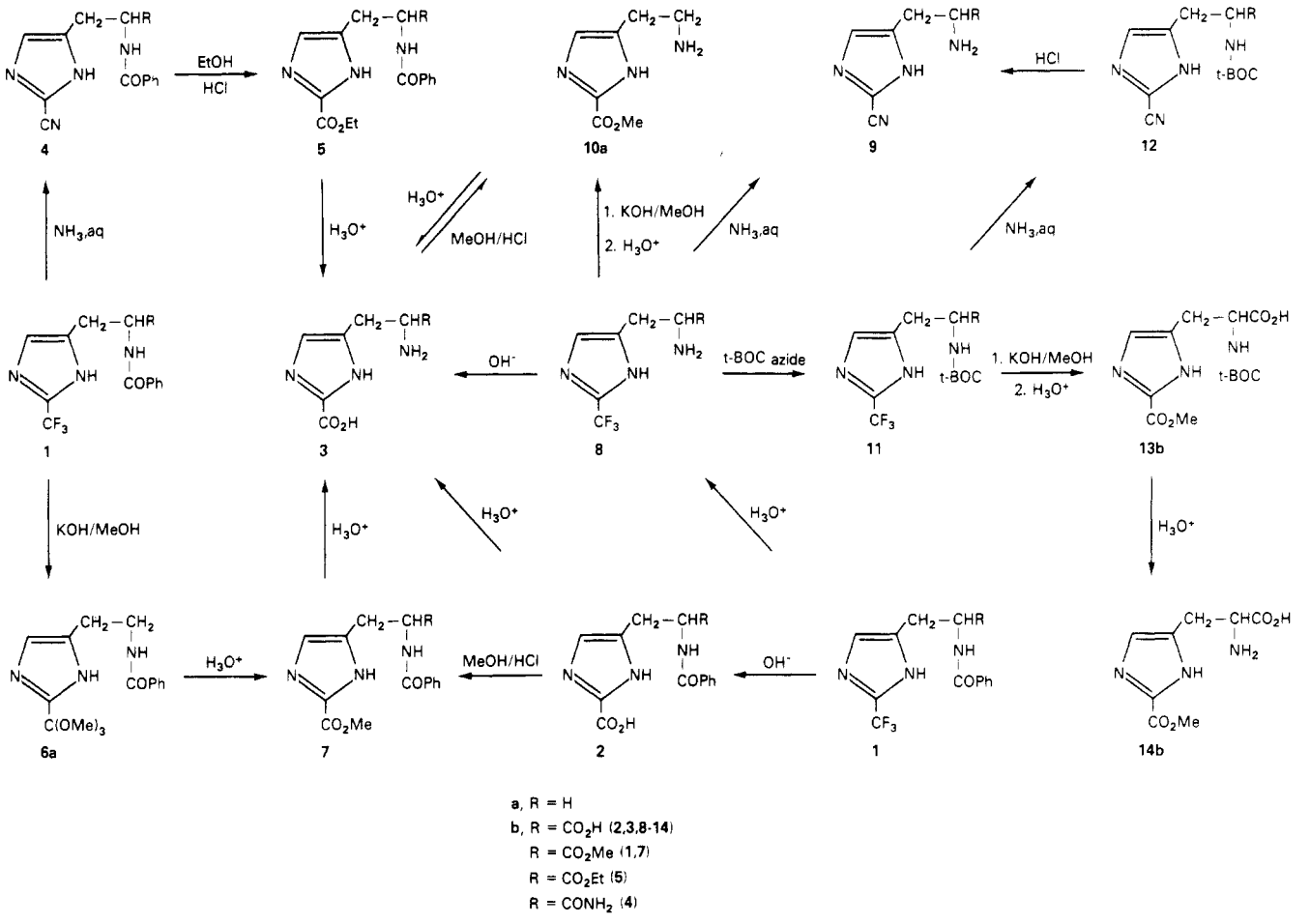
The histidine analogue 2-fluoro-L-histidine exhibits a wide range of interesting biological properties: the compound is incorporated into new protein at the expense of histidine—both in bacterial[2] and in mammalian[3] systems; it also shows antibacterial,[2,4] antiviral,[5] and antileukemic[6]

(1) Visiting Fellow, National Institutes of Health, 1977–1979.

(2) S. Naghshineh, K. L. Kirk, and L. A. Cohen, to be submitted for publication.

0022-3263/80/1945-3831$01.00/0

Scheme I



a, R = H
b, R = CO_2H (2,3,8-14)
R = CO_2Me (1,7)
R = CO_2Et (5)
R = $CONH_2$ (4)

activities. Furthermore, neither the 4-fluoro isomer[4,5] nor the other 2-halohistidines[2] have shown any comparable biological activities to date.

In order to verify the apparently unique qualities of 2-fluorohistidine, we have developed new synthetic approaches[7] for a variety of 2-X-histidines and -histamines, including those with X = CF_3.[8] In the course of the latter work, we observed that simple 2-(trifluoromethyl)-imidazoles are readily transformed (via diazafulvene intermediates) into other 2-X-imidazoles, including those with X = $C(OR)_3$, COOH, COOR, and CN.[9] It was logical, therefore, to attempt the same conversions with 2-(trifluoromethyl)histamine, 2-(trifluoromethyl)-L-histidine, and their α-*N*-acyl derivatives, thus making available additional analogues for biological evaluation. In the 2-X-histidine series, α-*N*-Boc (Boc = *tert*-butoxycarbonyl) derivatives were also needed for the synthesis of peptides containing the histidine analogues. We were particularly interested in the 2-carboxy analogues, as a means of introducing excess negative charge on the imidazole ring, and in the 2-cyano series, since the latter function is quite small and highly electronegative.

The α-*N*-benzoyl groups of **1a** and **1b** (and the ester function of **1b**) can be removed by strong acid, without modification of the trifluoromethyl group, to give **8a** and **8b**, respectively (Scheme I).[8] It would seem logical, therefore, to effect further transformations of the trifluoromethyl group after deprotection of the side chain. Generally, such direct approaches proved unprofitable because the water-soluble materials could not be freed completely of inorganic byproducts.[10] Furthermore, the 2-cyano derivatives (**9a,b**) tended to form colored condensation proucts during concentration under neutral or alkaline conditions.

Synthesis of 2-X-Histamines. The preparation of 2-carboxyhistamine (**3a**) by alkaline hydrolysis of **8a** proved unsatisfactory due to difficulties in removal of salts from the zwitterionic product. Alternatively, alkaline hydrolysis of **1a** provided **2a**, and the latter compound was converted to **3a** by strong acid hydrolysis with an overall yield of 75%. Compound **2a** is very soluble in aqueous salt solutions; however, crystallization from water is readily effected after separation from sodium chloride. Prior to our recognition of this salt effect, alternative routes to **3a**

(3) (a) P. F. Torrence, R. M. Friedman, K. L. Kirk, L. A. Cohen, and C. R. Creveling, *Biochem. Pharmacol.*, **28**, 1565 (1979); (b) D. C. Klein, K. L. Kirk, J. L. Weller, T. Oka, A. Parfitt, and I. S. Owens, *Mol. Pharmacol.*, **12**, 720 (1976); (c) D. C. Klein, J. L. Weller, and K. L. Kirk, *ibid.*, **13**, 1105 (1977); (d) C. R. Creveling, K. L. Kirk, and L. A. Cohen, to be submitted for publication.

(4) (a) K. L. Kirk and L. A. Cohen, *ACS Symp. Ser.*, **No. 28**, 23 (1976); (b) P. A. Cohen and L. A. Cohen, work in progress.

(5) E. De Clercq, A. Billiau, V. G. Edy, K. L. Kirk, and L. A. Cohen, *Biochem. Biophys. Res. Commun.*, **82**, 840 (1978).

(6) (a) C. R. Creveling, K. L. Kirk, and B. Highman, *Res. Commun. Chem. Pathol. Pharmacol.*, **16**, 507 (1977); (b) C. R. Creveling, L. A. Cohen, B. Highman, and K. L. Kirk, *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **36**, 975 (1977).

(7) (a) K. L. Kirk, *J. Org. Chem.*, **43**, 4381 (1978); (b) L. A. Cohen, K. L. Kirk, and D. P. Davis, Abstracts, 178th National Meeting of the American Chemical Society, Washington, DC, Sept 1979, No. ORGN 122; work in progress.

(8) H. Kimoto, K. L. Kirk, and L. A. Cohen, *J. Org. Chem.*, **43**, 3403 (1978).

(9) H. Kimoto and L. A. Cohen, *J. Org. Chem.*, **44**, 2902 (1979).

(10) This strong tendency for complex formation is also evident from the fact that a number of the more polar compounds crystallize from water or alcohol as solvates.

were explored: (1) ammonolysis of **1a** to **4a**, ethanolysis of the cyano group to form **5a**, and acid hydrolysis of the latter compound to provide **3a** in an overall yield of 75%;[11] (2) **1a** was converted to **6a** by alkaline methanolysis, and the latter compound was hydrolyzed in strong acid to **3a** via the intermediate ester (**7a**). The ortho ester **6a** is not particularly stable and was obtained in only 45% yield after purification. Alkaline methanolysis of **8a** produced the corresponding ortho ester, which is readily hydrolyzed to **10a**; the 2-carbomethoxy derivative, prepared by this route, is burdensome to purify, and **10a** is preferably obtained by direct esterification of **3a**.

Debenzoylation of **2a**, **4a**, **5a**, or **7a** (and of parallel members of the histidine series) in hot hydrochloric acid (1–3 N) results in rapid decarboxylation via the imidazolium carboxylate zwitterion. In 1 N hydrochloric acid at 100 °C, **3b** decarboxylates with $k = 0.038\ h^{-1}$ ($t_{1/2} = 18.3$ h). No decarboxylation is observed, however, when hydrolysis is performed in concentrated hydrochloric acid.[12] In the latter medium, the concentration of kinetically active carboxylate ion is minimized.

The benzoyl group of **4a** could not be removed without simultaneous hydrolysis of the cyano group; accordingly, repeated efforts were made to obtain **9a** by direct ammonolysis of **8a**. Regardless of the method of purification, the product could not be totally freed of inorganic residues. A more successful route to **9a** involved the reacylation of **8a** with Boc azide to form **11a**, ammonolysis of the latter compound to **12a**, and, finally, deblocking with hydrogen chloride in ethyl acetate. The use of α-*N*-(*tert*-butoxycarbonyl)histamine as the starting compound for the entire sequence was precluded because this blocking group would not have survived the acidic conditions involved in the introduction of the trifluoromethyl group.[8]

Synthesis of 2-X-Histidines. Transformations in the histidine series were similar to those for the histamines, and parallel difficulties were encountered in the separation of inorganic byproducts. Compound **2b** was obtained by alkaline hydrolysis of **1b** but was found to be very hygroscopic and difficult to purify; esterification of the crude diacid with methanolic hydrogen chloride was incomplete, and the process was completed by use of diazomethane to give **7b**; the purified diester was then converted into **3b** by strong acid hydrolysis. Reaction of **1b** with 5% aqueous ammonia led to the 2-cyanoimidazole, the side-chain ester function being simultaneously transformed to the amide (**4b**). This compound was converted into the diethyl ester **5b** with hydrogen chloride in ethanol, and the diester was hydrolyzed to **3b** with concentrated hydrochloric acid. The side-chain protecting groups of **4b** could not be removed without simultaneous hydrolysis of the cyano function, and repeated efforts were made to transform **8b** directly into **9b** by ammonolysis. As in the case of **8a**, various chromatographic procedures failed to provide a salt-free product. Accordingly, **8b** was converted into **11b** with Boc azide; the latter compound was subjected to ammonlysis to form **12b**, and the Boc function was cleaved with hydrogen chloride in ethyl acetate to provide **9b**.

The monoester **13b** is a useful intermediate for incorporation into synthetic peptides; however, this compound is not readily accessible by selective saponification of the diester or by selective esterification of the diacid. Accordingly, **11b** was subjected to alkaline methanolysis, and the intermediate ortho ester was hydrolyzed at pH 2 (25 °C); even under the relatively mild acid conditions, the Boc protecting group was cleaved, and **14b** was obtained.[13] The possibility of intercepting **13b** was not explored since the compound should be readily obtainable by reacylation of **14b**.

Instability of 2-Cyanoimidazoles. 2-Cyanoimidazole and 2-cyano-4-methylimidazole[9] show no unusual properties; on the other hand, 2-cyanohistamine and 2-cyanohistidine appear to undergo a condensation reaction involving the cyano and α-amino groups. Solid samples of **9a**, **9b**, **12a**, and **12b** turn dark brown before or during fusion;[14] initially colorless aqueous solutions of **9a** or **9b** (neutral or alkaline) become yellow-brown during concentration or upon storage of concentrated solutions for several days at ambient temperature. Darkening is accompanied by the appearance of a new chromophore (weak) at 300–330 nm, but no changes are observed for solutions made up at spectral concentration (even at 100 °C). Since **4a** and **4b** do not show such behavior, it would appear that the free α-amino group is involved; furthermore, the requirement of a solid state or a concentrated solution suggests the reaction to be inter- rather than intramolecular. Further study of this transformation is in progress.

Experimental Section[15]

α-*N*-Benzoyl-2-carboxyhistamine (2a). A suspension of 283 mg (1 mmol) of powdered α-*N*-benzoyl-2-trifluoromethylhistamine (**1a**)[8] in 25 mL of 0.5 N sodium hydroxide was stirred at ambient temperature. Solution occurred rapidly and a transient yellow-green color developed (possibly the diazafulvene intermediate). After 5 days, the solution (now almost colorless) was acidified to pH 2–3 and was evaporated to dryness under reduced pressure (bath temperature 40 °C). The residual solid was extracted three times with 30-mL portions of boiling ethanol; the combined extracts were chilled in ice, the solution was filtered to remove sodium chloride, and the filtrate was evaporated to dryness. The residual solid was dissolved in a small volume of water, and the solution was refrigerated; the product separated gradually as pellets of microcrystals (150 mg). Concentration and chilling of the mother liquor provided an additional 105 mg for a total yield of 90%. The product crystallized from water as microspheres: mp 157–159 °C; NMR (0.1 N NaOD in CD_3OD) δ 2.89 (2, t, $J = 7.0$ Hz, β-CH_2), 3.68 (2, t, $J = 7.0$ Hz, α-CH_2), 6.92 (1, s, H-4 or H-5), 7.4–7.8 (5, m, C_6H_5).

Anal. Calcd for $C_{13}H_{13}N_3O_3$·HCl (mol wt 295.7): C, 52.80; H, 4.77; N, 14.21; Cl, 11.99. Found: C, 53.04; H, 4.73; N, 14.26; Cl, 11.83.

2-Carboxyhistamine Dihydrochloride (3a). A solution of 259 mg (1 mmol) of **2a** in 20 mL of concentrated hydrochloric acid was heated on a steam bath for 15 h. The solution was chilled and was extracted with three 20-mL portions of ether. The aqueous layer was evaporated to dryness to give a colorless powder. This material was again extracted with ether (to remove last traces of benzoic acid); the residual solid crystallized from a small volume of cold 6 N hydrochloric acid as granules. Several crops were collected for a total yield of 190 mg (83%): the compound begins to foam (decarboxylation) at 157 °C, mp 168–170 °C; IR (KBr)

(11) Direct acid hydrolysis of **4a** to **3a** generates ammonium chloride, which is difficult to separate from the product.

(12) H. B. Burkett, K. L. Kirk, and L. A. Cohen, to be submitted for publication.

(13) Many of the Boc derivatives prepared in this study proved to be unusually susceptible to cleavage at low pH. This sensitivity may result from the strong electron-withdrawing effect of the 2-substituent, transmitted to the α-amino group through the imidazole ring and the side chain [cf. H. J. C. Yeh, K. L. Kirk, L. A. Cohen, and J. S. Cohen, *J. Chem. Soc., Perkin Trans.* 2, 928 (1975)] or through intramolecular catalysis by the somewhat acidic imidazole NH group.

(14) The Boc protecting group is lost during fusion.

(15) Melting points are uncorrected. Microanalyses and mass spectral measurements were performed by the Microanalytical Services Section of this Laboratory, under the direction of Dr. David F. Johnson. Wherever possible, the identity and homogeneity of each compound were confirmed by UV, NMR, and mass spectra and by TLC. Conversions of trifluoromethyl group were monitored by UV spectra for completeness.

1754 cm^{-1} (C=O); NMR (D_2O) δ 3.20 (2, d, J = 6.0 Hz, β-CH_2), 3.34 (2, d, J = 6.0 Hz, α-CH_2), 7.39 (1, s, H-4 or H-5).

Anal. Calcd for $C_6H_9N_3O_2$·2HCl (mol wt 228.1): C, 31.61; H, 4.86; N, 18.42. Found: C, 31.50; H, 5.01; N, 18.21.

A similar procedure was used to prepare **3a** from **5a**, **7a**, or **10a**, ether extraction being omitted in the last case. The direct alkaline hydrolysis of **8a** also led to **3a**, but the product could not be totally freed of salts despite the use of several types of chromatography.

α-*N*-Benzoyl-2-(trimethoxymethyl)histamine (6a) and α-*N*-Benzoyl-2-(carbomethoxy)histamine (7a). To a solution of 1.0 g of powdered potassium hydroxide in 20 mL of methanol was added 142 mg (0.5 mmol) of **1a**, and the mixture was heated at reflux for 5 h. After addition of 0.2 g of sodium bicarbonate, the mixture was chilled in dry ice–acetone while 16 mL of 1 N hydrochloric acid was added slowly. Methanol was evaporated in vacuo and the aqueous solution (pH 8.4) was extracted with three 50-mL portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and evaporated to give a colorless, viscous oil (207 mg). Purification was effected by preparative TLC (250-μm Florisil) with ethyl acetate as developing solvent. The product (**6a**) crystallized from benzene as prisms: 72 mg (45% yield); mp 153–154 °C; NMR (CD_3OD) δ 2.94 (2, t, J = 6.6 Hz, β-CH_2), 3.70 (2, t, J = 6.6 Hz, α-CH_2), 3.16 (9, s, OCH_3's), 6.95 (1, s, H-4 or H-5), 7.3–7.7 (5, m, C_6H_5).

The NMR and mass spectra of freshly purified samples of **6a** showed no evidence of contamination with **7a**; upon storage at ambient temperature, however, crystalline **6a** was gradually transformed into **7a**, and no ortho ester remained after 1 week. The same transformation was effected by storage of a solution of **6a** in 1 N hydrochloric acid for 4 h at ambient temperature. The solution was evaporated to dryness, and **7a** crystallized from benzene as prisms: mp 148–149 °C; NMR (CD_3OD) δ 2.92 (2, t, J = 6.6 Hz, β-CH_2), 3.62 (2, t, J = 6.6 Hz, α-CH_2), 3.87 (3, s, OCH_3), 7.02 (1, s, H-4 or H-5), 7.3–7.8 (5, m, C_6H_5).

Anal. Calcd for $C_{14}H_{15}N_3O_3$ (mol wt 273.3): C, 61.53; H, 5.53; N, 15.38. Found: C, 61.33; H, 5.59; N, 15.08.

α-*N*-Benzoyl-2-cyanohistamine (4a). A suspension of powdered **1a** (142 mg, 0.5 mmol) in 50 mL of 5% aqueous ammonia was stirred at ambient temperature for 3 days. The clear solution was evaporated in vacuo, and the residual colorless powder was extracted with three 30-mL portions of warm tetrahydrofuran. The combined extracts were filtered and evaporated to give a crystalline residue. Recrystallization from tetrahydrofuran–benzene gave 97 mg of prisms, an additional 19 mg being recovered from the mother liquor (total yield, 97%): mp 167–168 °C; IR (KBr) 2227 cm^{-1} (CN); NMR (CD_3OD) δ 2.97 (2, t, J = 7.0 Hz, β-CH_2), 3.68 (2, t, J = 7.0 Hz, α-CH_2), 7.20 (1, s, H-4 or H-5), 7.4–7.8 (5, m, C_6H_5).

Anal. Calcd for $C_{13}H_{12}N_4O$ (mol wt 240.3): C, 64.98; H, 5.03; N, 23.32. Found: C, 65.10; H, 5.32; N, 23.44.

α-*N*-Benzoyl-2-carbethoxyhistamine (5a). Compound **1a** was converted into **4a** as described above. After evaporation of the tetrahydrofuran extracts, the crude residue was dissolved in 30 mL of ethanol containing 0.2 mL of water. The solution was saturated with hydrogen chloride and was heated at reflux for 3 h. The solution was chilled, a colorless precipitate was removed by filtration, and the filtrate was evaporated to give a crystalline residue. This material was dissolved in 50 mL of phosphate buffer (0.1 M, pH 7), and the solution was extracted with three 50-mL portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and evaporated to give 130 mg (91%) of a colorless powder, mp 193–195 °C. Recrystallization from ethanol–ethyl acetate gave **5a** as needles: mp 195–195.5 °C; NMR (CD_3OD) δ 1.36 (3, t, OCH_2CH_3), 2.92 (2, t, J = 7.0 Hz, β-CH_2), 3.64 (2, t, J = 7.0 Hz, α-CH_2), 4.34 (2, q, OCH_2CH_3), 7.02 (1, s, H-4 or 5), 7.3–7.8 (5, m, C_6H_5).

Anal. Calcd for $C_{15}H_{17}N_3O_3$ (mol wt 287.3): C, 62.70; H, 5.96; N, 14.63. Found: C, 62.97; H, 5.97; N, 14.87.

2-(Carbomethoxy)histamine Dihydrochloride (10a). A solution of 182 mg (0.8 mmol) of **3a**·2HCl in 20 mL of methanol was saturated with hydrogen chloride, and the mixture was refluxed for 5 h. The solvent was evaporated to give a slightly yellowish powder. Crystallization was effected by addition of 2-propanol to a methanolic solution of the compound to give **10a**·2HCl as a colorless powder: mp 167–168 °C dec; IR (KBr) 1730 cm^{-1} (C=O); NMR (CD_3OD) δ 3.34 (4, m, α- and β-CH_2's), 4.12 (3, s, OCH_3), 7.76 (1, s, H-4 or -5).

Anal. Calcd for $C_7H_{11}N_3O_2$·2HCl (mol wt 242.1): C, 34.73; H, 5.41; N, 17.36. Found: C, 34.72; H, 5.41; N, 17.48.

Direct Conversion of 8a to 10a. A solution of **8a**·2HCl (252 mg, 1 mmol)[8] in 50 mL of methanol containing 2 g of powdered potassium hydroxide was refluxed for 3 days. The mixture was cooled and was acidified with concentrated hydrochloric acid. After addition of 10 mL of water, the solution was stored at ambient temperature for several days to hydrolyze the ortho ester and was then evaporated to dryness to give a reddish yellow gum. This material was extracted with 6 mL of a mixture of methanol and 2-propanol (1:1), and the extract was decolorized with charcoal. The filtrate was acidified with 0.2 mL of concentrated hydrochloric acid and was evaporated to dryness. A pale yellow powder was obtained (170 mg, 70%), whose IR, NMR, and mass spectra were identical with those of **10a** prepared by esterification of **3a**. Despite the additional steps involved in the preparation of **3a**, we consider the latter to be the preferred route for the preparation of **10a**.

Direct Conversion of 8a to 2-Cyanohistamine (9a). A solution of 126 mg (0.5 mmol) of **8a** in 60 mL of 5% aqueous ammonia was stored at ambient temperature for 3 days. The solvent was evaporated in vacuo, leaving a pale yellow solid. Purification was attempted by chromatography on Sephadex LH-20, with methanol as eluting solvent. A colorless solid was obtained which, on the basis of combustion analysis, still contained significant inorganic contaminants. Further efforts at purification—by crystallization, ion-exchange chromatography (Dowex 50-4X), or high-pressure liquid chromatography—failed to remove the inorganic residues, while UV, NMR, and mass spectra indicated **9a** to be the only imidazole derivative in the mixture. A dipicrate precipitated from an ethanol solution and was recrystallized from ethanol; mp 223–224 °C dec.

Anal. Calcd for $C_6H_8N_4$·$C_6H_3N_3O_7$·H_2O: C, 35.30; H, 2.63; N, 22.88. Found: C, 35.44; H, 2.63; N, 23.03.

α-*N*-(*tert*-Butoxycarbonyl)-2-(trifluoromethyl)histamine (11a). To a suspension of 756 mg (3 mmol) of **8a** in 20 mL of dry acetonitrile was added 0.5 g (3.5 mmol) of Boc azide and 1.8 mL of triethylamine. The mixture was kept at ca. 50 °C for 18 h, and the clear, yellow solution was evaporated to dryness. The crystalline residue was extracted twice with hexane (to remove excess azide), and the residue was dissolved in ethyl acetate. The solution was washed with water, dried (Na_2SO_4), and evaporated. The product recrystallized from methanol–water as colorless plates: 590 mg (75%); mp 135–136 °C.

Anal. Calcd for $C_{11}H_{16}F_3N_3O_2$ (mol wt 279.3): C, 47.31; H, 5.78; N, 15.05; F, 20.41. Found: C, 47.35; H, 5.84; N, 15.24; F, 19.99.

α-*N*-(*tert*-Butoxycarbonyl)-2-cyanohistamine (12a). A suspension of 279 mg (1 mmol) of **11a** in 50 mL of 5% aqueous ammonia was stored at ambient temperature for 3 days. The colorless solution was evaporated to dryness at reduced pressure (bath temperature 40 °C), and the residual material was extracted with three 30-mL portions of ethyl acetate. The combined extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue crystallized from methanol–water as granules: 165 mg (70%); mp 145–146 °C; IR (KBr) 2229 cm^{-1} (CN).

Anal. Calcd for $C_{11}H_{16}N_4O_2$ (mol wt 236.3): C, 55.91; H, 6.83; N, 23.72. Found: C, 55.84; H, 6.78; N, 23.63.

2-Cyanohistamine Dihydrochloride (9a). To a solution of 118 mg (0.5 mmol) of **12a** in 20 mL of dry ethyl acetate was added 1 mL of a saturated solution of hydrogen chloride in ethyl acetate. The mixture was stored for 24 h at ambient temperature. The solvent was decanted from a crystalline deposit; the crystals were washed with ether and dried in vacuo. This material is very hygroscopic and could not be transferred without becoming gummy; evolution of gas during the fusion of the compound suggested it to be the carbonic acid. The total product was dissolved in a small volume of water, and the solution was evaporated to dryness without heat. The residual solid crystallized from ethanol–acetone as plates: 92 mg (80%); the compound begins to sinter with darkening at 160 °C and melts to a brown liquid at 175–176 °C.

Anal. Calcd for $C_6H_8N_4$·2HCl·H_2O (mol wt 227.1): C, 31.73; H, 5.33; N, 24.67; Cl, 31.22. Found: C, 31.73; H, 5.20; N, 24.30; Cl, 31.52.

2-Carboxy-L-histidine Hydrochloride (3b).[16] A solution of 341 mg (1 mmol) of **1b**[8] in 50 mL of 0.1 N sodium hydroxide was stored at ambient temperature for 24 h. The solution was acidified to pH 2 with 6 N hydrochloric acid and was evaporated to dryness. Efforts to isolate **2b** free of salts were fruitless. Accordingly, a solution of the crude product in 50 mL of absolute methanol was saturated with hydrogen chloride, and the solution was stored at ambient temperature for 3 days. TLC indicated incomplete esterification. Sodium chloride was removed by filtration, and the filtrate was evaporated to dryness. The residual oil was dissolved in a mixture of ether and ethanol, and to the chilled solution was added a solution of diazomethane in ether until a yellow color persisted. The solution was stored at 0 °C for 10 min and at 25 °C for 30 min. The solvent was removed, leaving a yellow gum. Preparative TLC (1000-μm silica gel), with ethyl acetate as developing solvent, gave 120 mg (36%) of **7b** as a colorless oil: NMR (acetone-d_6) δ 3.28 (2, d, J = 6.0 Hz, β-CH_2), 3.68 (3, s, α-$COOCH_3$), 3.90 (3, s, 2-$COOCH_3$), 4.98 (1, q, J = 6.0, 7.2 Hz, α-CH), 7.30 (1, s, H-4 or H-5), 7.3–7.7 (5, m, C_6H_5), 8.00 (1, d, J = 7.2 Hz, α-NH). A solution of **7b** (90 mg) in 30 mL of concentration hydrochloric acid was heated on steam for 15 h. The reaction mixture was evaporated to a small volume, chilled, and extracted with three 50-mL portions of ether. The aqueous layer was evaporated to dryness and was again extracted with ether. The residual solid crystallized from water as prisms to give 65% of **3b**·HCl. Similarly, **5b** was hydrolyzed to **3b**·HCl: 72% yield; the compound begins to decarboxylate at 170 °C and melts to a clear liquid at 215–217 °C; IR (KBr) 1739 (COOH), 1639 (COO^-) cm^{-1}; NMR (D_2O) δ 3.40 (2, d, J = 7.2 Hz, β-CH_2), 4.37 (1, t, J = 7.2 Hz, α-CH), 7.46 (1, s, H-4 or H-5).

Anal. Calcd for $C_7H_9N_3O_4$·HCl·H_2O (mol wt 253.6): C, 33.15; H, 4.77; N, 36.57; Cl, 13.98. Found: C, 33.14; H, 4.85; N, 16.05; Cl, 13.88.

α-*N*-Benzoyl-2-cyano-L-histidine Amide (4b). A solution of 341 mg (1 mmol) of **1b** in 100 mL of 5% aqueous ammonia was stored at ambient temperature for 100 h. The solvent was removed in vacuo, and the residual solid was extracted with three 25-mL portions of ethanol. The combined extracts were evaporated to give a pale yellow powder. Chromatography on silica gel (ethyl acetate–tetrahydrofuran, 1:1) first gave 33 mg (12%) of α-*N*-benzoyl-2-cyanohistidine (identified by mass spectrum), followed by 205 mg (72%) of the amide **4b**. The latter material was recrystallized from ethyl acetate as fine plates: mp 192–193 °C; IR (KBR) 2237 cm^{-1} (CN); NMR (acetone-d_6) δ 3.32 (2, d, J = 6.9 Hz, β-CH_2), 5.06 (1, q, J = 6.9, 7.2 Hz, α-CH), 7.32 (1, s, H-4 or H-5), 7.3–7.7 (5, m, C_6H_5), 7.93 (1, d, J = 7.2 Hz, α-NH).

Anal. Calcd for $C_{14}H_{13}N_5O_2$ (mol wt 283.3): C, 59.35; H, 4.63; N, 24.72. Found: C, 59.23; H, 4.57; N, 24.59.

α-*N*-Benzoyl-2-carboxy-L-histidine Diethyl Ester (5b). A crude sample of **4b**, prepared from 1 mmol of **1b**, was dissolved in 50 mL of ethanol containing 0.30 mL of water. The solution was saturated with hydrogen chloride and was heated at reflux for 3 h. The solution was chilled, a colorless precipitate was removed, and the filtrate was evaporated to dryness. The residue was dissolved in 30 mL of phosphate buffer (0.1 M, pH 7), and the solution was extracted with three 30-mL portions of ethyl acetate. The combined extracts were dried (Na_2SO_4) and were evaporated to give 312 mg of a pale yellow powder. This material was purified by preparative TLC (1000-μm silica gel), with ethyl acetate as developing solvent, to give 276 mg (77%) of **5b** as a colorless gum which could not be crystallized: NMR (acetone-d_6) δ 1.16 (3, t, J = 7.2 Hz, OCH_2CH_3), 1.28 (3, t, J = 7.2 Hz, OCH_2CH_3), 3.27 (2, d, J = 6.0 Hz, β-CH_2), 4.09 (2, q, J = 7.2 Hz, OCH_2CH_3), 4.31 (2, q, J = 7.2 Hz, OCH_2CH_3), 4.94 (1, q, J = 6.0, 7.2 Hz, α-CH), 7.26 (1, s, H-4 or H-5), 7.3–7.7 (5, m, C_6H_5), 7.94 (1, d, J = 7.2 Hz, α-NH).

Anal. Calcd for $C_{18}H_{21}N_3O_5$ (mol wt 359.4): C, 60.16; H, 5.89; N, 11.69. Found: C, 59.96; H, 5.94; N, 11.46.

α-*N*-(*tert*-Butoxycarbonyl)-2-(trifluoromethyl)-L-histidine (11b). To a suspension of 888 mg (3 mmol) of **8b** in 40 mL of dry acetonitrile was added 0.5 g (3.5 mmol) of Boc azide and 2.4 mL of triethylamine. The mixture was maintained at ca. 50 °C for 24 h, and the clear, yellow solution was evaporated to dryness at a bath temperature of 30–35 °C. The solid residue was extracted twice with hexane, residual hexane was removed in vacuo, and the remaining material was extracted with three 20-mL portions of ethyl acetate. The combined extracts were concentrated, and the residual syrup was dried in vacuo. The yellow amorphous solid was dissolved in 20 mL of water, and the solution was adjusted to pH 3 and was refrigerated overnight. The product separated as almost colorless granules, mp 188–189 °C. Recrystallization from methanol–water gave 746 mg (77%) of **11b**, mp 193–195 °C with gas evolution.

Anal. Calcd for $C_{12}H_{16}N_3O_4F_3$ (mol wt 323.3): C, 44.59; H, 4.99; N, 13.00; F, 17.63. Found: C, 44.60; H, 5.02; N, 12.76; F, 17.52.

α-*N*-(*tert*-Butoxycarbonyl)-2-cyano-L-histidine (12b). A solution of 646 mg (2 mmol) of **11b** in 100 mL of 5% aqueous ammonia was stored at ambient temperature for 4 days. The solution was evaporated in vacuo, and the residual oil was dissolved in 10 mL of water. The solution was chilled in ice and was adjusted to pH 2. The solvent was evaporated slowly under a stream of air, and colorless crystals separated. The product was recrystallized from methanol–water as a hemihydrate: 416 mg (72%); mp 190 °C dec; IR (KBr) 2225 cm^{-1} (CN).

Anal. Calcd for $C_{12}H_{16}N_4O_4 \cdot {}^1/_2H_2O$ (mol wt 289.3): C, 49.82; H, 5.92; N, 19.37. Found: C, 49.86; H, 5.93; N, 19.52.

This material was dried overnight at 70–80 °C in vacuo.

Anal. Calcd for $C_{12}H_{16}N_4O_4$ (mol wt 280.3): C, 51.42; H, 5.75; N, 19.99. Found: C, 51.51; H, 5.80; N, 19.94.

2-Cyano-L-histidine Dihydrochloride (9b). The Boc protecting group was removed from **12b** by following the procedure for **12a**. The product crystallized from water–acetone as a fine powder which begins to turn yellow at 150 °C, darkens progressively, and fails to melt below 300 °C: IR (KBr) 2252 (CN), 1739 (COOH) cm^{-1}.

Anal. Calcd for $C_7H_8N_4O_2$·2HCl·$2H_2O$ (mol wt 289.1): C, 29.08; H, 4.88; N, 19.38; Cl, 24.52. Found: C, 29.18; H, 5.03; N, 19.68; Cl, 24.82.

2-(Carbomethoxy)-L-histidine (14b). To a solution of 323 mg (1 mmol) of **11b** in 20 mL of methanol was added 1 g of potassium hydroxide, and the mixture was heated at reflux for 18 h. The solution was evaporated to dryness, and the residual material was dissolved in 20 mL of water. The solution was acidified to pH 2 with dilute hydrochloric acid and was stored at ambient temperature for 2 days. The solvent was removed by lyophilization, and the residual solid was extracted with two 20-mL portions of warm methanol. The solution was concentrated to ca. 5 mL; the remaining solvent was allowed to evaporate in air and **14b** separated as colorless plates (150 mg, 65%). The compound begins to foam at 190 °C and becomes a clear, light brown melt at 217 °C.

Anal. Calcd for $C_8H_{11}N_3O_4$·H_2O (mol wt 231.2): C, 41.56; H, 5.67; N, 18.18. Found: C, 41.68; H, 5.60; N, 18.25.

Decarboxylation of 2-Carboxy-L-histidine. A solution of 1.2 mg of **3b** in 125 mL of 1 N hydrochloric acid was heated on a steam bath, and the decrease in UV absorption at 250 nm was monitored. The decarboxylation followed first-order kinetics with $t_{1/2}$ = 18.3 h and k = 0.038 h^{-1}. The product was identified as histidine by TLC on silica gel (development with *n*-butanol–ethyl acetate–acetic acid–water, 1:1:1:1).

Registry No. **1a**, 66675-24-9; **1b**, 66675-26-1; **2a**, 74419-63-9; **2a**·HCl, 74419-64-0; **3a**·2HCl, 74419-65-1; **3b**, 74419-66-2; **3b**·HCl, 74419-67-3; **4a**, 74419-68-4; **4b**, 74419-69-5; **5a**, 74419-70-8; **5b**, 74419-71-9; **6a**, 74419-72-0; **7a**, 74419-73-1; **7b**, 74419-74-2; **8a**, 74419-75-3; **8a**·2HCl, 66675-25-0; **8b**, 74419-76-4; **9a**·2HCl, 74419-77-5; **9a**·dipicrate, 74419-79-7; **9b**·2HCl, 74419-80-0; **10a**·2HCl, 74419-81-1; **11a**, 74419-82-2; **11b**, 74419-83-3; **12a**, 74419-84-4; **12b**, 74419-85-5; **14b**, 74419-86-6; Boc azide, 1070-19-5; α-*N*-benzoyl-2-cyanohistidine, 74419-87-7; histidine, 71-00-1.

(16) The conversion of L-histidine into 2-(trifluoromethyl)-L-histidine[8] was found to occur without measurable racemization.[17] Of the transformations described herein, none is likely to labilize the chiral center and we assume optical purity to have been maintained throughout.

(17) P. E. Hare and E. Gil-Av, *Science*, **204**, 1226 (1979). We thank Dr. Gil-Av for evaluating the optical purity of **8a** by chiral chromatography.