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Regiospecific Alkylation of Histidine and Histamine at C-2

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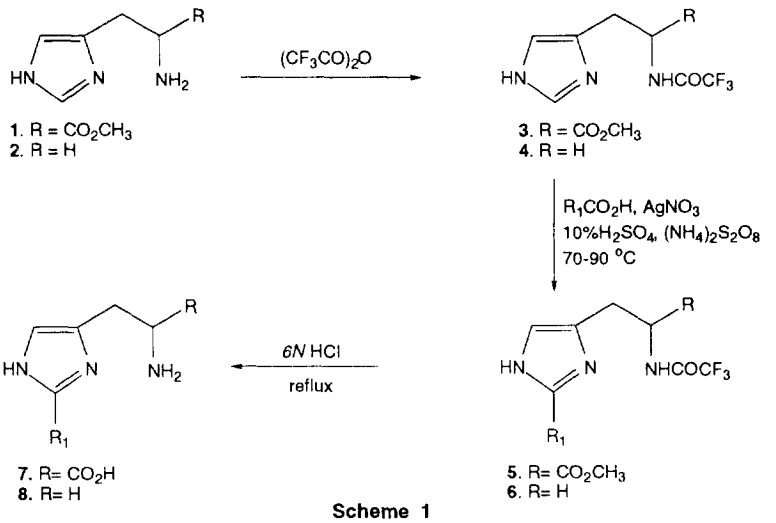
Abstract *: The first regiospecific synthesis of 2-alkyl-L-histidines and 2-alkylhistamines starting from fully protected L-histidine and histamine via silver catalyzed radical decarboxylative oxidation of alkylcarboxylic acids by ammonium persulfate in 10% H_2SO_4 is described.*

Five membered rings containing more than one heteroatom constitute one of the largest and most diverse groups of heterocyclic compounds. Among this class, the imidazole ring system has special biological importance, in large part because of its presence in the essential amino acid histidine and in histamine, the decarboxylation product of histidine. Among the functions of histidine are important structural and functional roles in peptides and proteins. For example, it is found in the active site of numerous enzymes, in which its major function appears to be the catalysis of proton transfer.[1] The numerous biological functions of histamine include its involvement in allergic manifestations such as asthama and urticaria,[2] and its role as a neurotransmitter in the central nervous system.

In part as a result of the importance of histidine in biology, ring-substituted histidines have received considerable attention as antimalarials,[3] antioxidants,[4] nurotoxins,[5] components of peptide hormones,[6] replacements of histidine in protein biosynthesis,[7] and as potential prodrugs for the corresponding histamines. Synthetic design of ring-alkylated bioimidazoles, such as histidine and histamine, depends on the targeted ring positions, and of these, the 2-position presents the greatest challenge. For example, as part of our research to develop new routes to ring-substituted imidazoles, including analogs of histidine and histamine, we have recently reported a general regiospecific syntheses for $N(1)^{\tau}$-alkylhistidines and histamines.[8] However, routes to 2-alkyl histidines and histamines are less straightforward. Thus, several racemic 2-substituted histidines have been obtained in low yield by tedious, multistep synthesis from simple imidazoles,[5, 9-11] and resolution of the final product becomes a formidable task.[9] The corresponding 2-alkylhistamines also require multistep syntheses which involve severe reaction conditions.[12] Direct introduction of the alkyl group on histidine by electrophilic substitution is not possible, because all electrophiles react preferentially at the 4-position of the imidazole ring. Finally, synthetic strategies involving nucleophilic substitution on the imidazole ring require protection of the imidazole ring NH group.

In considering alternative routes to 2-alkylimidazoles, we noted that radical reactions under carefully defined conditions have been found to produce methylated imidazoles, but in very low yields.[13] Additionally, Minisci *et al.*[14-17] also successfully alkylated, arylated, acylated, and alkenylated protonated pyridines, quinolines, and certain other heteroaromatic nitrogen compounds using radical reactions. Furthermore, they observed that the reaction occured specifically at the most electron deficient carbon atoms.

The imidazole ring contains a basic pyridine-like ring nitrogen whose behavior in an acidic environment parallels the heterocycles studied by Minisci *et al.*,[14-17] suggesting to us that a similar strategy might be successful to produce 2-alkyl bioimidazoles. We now report that chiral 2-alkyl-L-histidines (and histamines) are readily obtained by direct radical alkylation of suitably protected histidine and histamine, based on the modifications of Minisci's method for azaaromatics (***Scheme 1***). Of various groups used for blocking the side chain nitrogen (trifluoroacetyl, acetyl, *tert*-butyloxycarbonyl, benzyloxycarbonyl), the first was found to give the best results and could easily be removed by refluxing with dilute acid.



Scheme 1

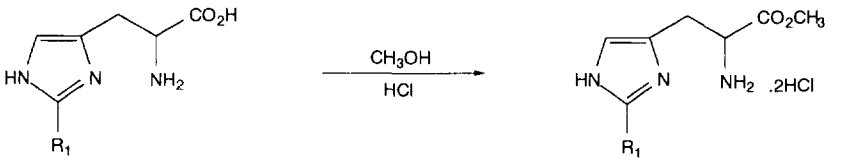
N-Trifluoroacetyl-L-histidine methyl ester (**3**) and *N*-trifluoroacetylhistamine (**4**) obtained by treatment of **1** and **2**, respectively, with trifluoroacetic anhydride, provided, on reaction with alkylcarboxylic acids in the presence of 10% H_2SO_4, silver nitrate and ammonium persulfate at 70-90 °C, the 2-alkyl derivatives **5** and **6** (***Scheme 1***). Yields range from 14-45%, increasing with the degree of branching in the alkyl group (***Table I***). A control experiment at room temperature with isobutyric acid gave **5c** [R_1= $CH(CH_3)_2$] in 32% yield. The alkylation is highly selective, with no alkylation observed at C-4(5). Ethyl, cyclohexyl and some functionalized alkyl groups are readily introduced in this manner. Although a crude product was obtained in low yield using acetic acid (R_1= CH_3), this was not further characterized. The method fails for more stable and less nucleophilic radicals such as aryl, heteroaryl and benzyl. Other methods for radical generation described by Minisci *et al.*[18] failed or gave significantly lower yields of product.

Table I: Radical Alkylation at C-2

	% yield	
R_1	**5**	**6**
a) C_2H_5	15	14
b) $CH_2(CH_2)_2CH_3$	17	20
c) $CH(CH_3)_2$	38	25
d) C_6H_{11}	39	39
e) $C(CH_3)_3$	45	38

The 2-alkyl-L-histidines (**7**) and 2-alkylhistamines (**8**) were obtained by the deprotection of **5** and **6** with refluxing solution of them in *6N* HCl. Evaporation of the solution produced the amine and amino acid dihydrochlorides. The free amino acids were obtained by elution with NH_4OH from a Dowex ion-exchange

column. Esterification of compounds **7** using anhydrous methanolic hydrogen chloride gave methyl ester derivatives **9**, that are suitable for peptide synthesis (***Scheme 2***).



7 **9**

Scheme 2

EXPERIMENTAL

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 (300 MHz) spectrometer. Mass spectra were provided by the Instrumentation Section of the Laboratory of Analytical Chemistry, NIDDK. Elemental analysis were performed by Atlantic Microlab, Norcross, GA or by Galbraith Laboratories, Knoxville, TN. Melting points were recorded on a Thomas-Hoover Capillary Melting Point Apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 MC Polarimeter. Chromatographic purification was performed with silica gel 60 (230-400 mesh). All TLC (silica gel) development was performed by use of 5% CH_3OH in $CHCl_3$. All reagents were obtained from commercial sources and were of analytical grade.

General method for the synthesis of α-N-trifluoroacetyl-2-alkyl-L-histidine methyl esters (5) and α-N-trifluoroacetyl-2-alkylhistamines (6). α-N-Trifluoroacetyl-L-histidine methyl ester (**3**, 1 mmol) or α-N-trifluoroacetylhistamine (**4**, 1 mmol), was added to a mixture of silver nitrate (0.6 mmol) and alkylcarboxylic acid (3 mmol) in 10% H_2SO_4 (20 mL), and the reaction mixture was heated at 70-90 °C. A freshly prepared solution of ammonium persulfate (3 mmol) in water (15 mL) was added dropwise over 15 minutes. The heating source was then removed and the reaction proceeded with evolution of carbon dioxide. After 15 minutes, the reaction was terminated by pouring it onto ice. The resulting mixture was made alkaline with 30% NH_4OH solution and extracted with ethyl actetae (3 x 50 mL). The combined extracts were washed with brine (2 x 10 mL) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford an oil, which on chromatography over silica [ethyl acetate:hexanes (6:4)] gave **5** or **6** in 14-45% yield (***Table I***).

α-N-Trifluoroacetyl-2-ethyl-L-histidine methyl ester (5a). Yield: 15%; oil; ^1H NMR ($CDCl_3$) δ 1.30 (t, 3H, CH_3, J= 7.6 Hz), 2.73 (q, 2H, CH_2, J= 7.6 Hz), 3.13 (m, 2H, CH_2), 3.68 (s, 3H, CH_3), 4.77 (m, 1H, CH), 6.72 (s, 1H, 4-H); analysis for $C_{11}H_{14}F_3N_3O_3$+0.1 $CH_3CO_2C_2H_5$ (302.1), calcd., C, 45.33; H, 4.93; N, 13.91; found, C, 45.66; H, 4.61; N, 13.63; MS(CI-NH_3) m/z 294 (M+1).

α-N-Trifluoroacetyl-2-butyl-L-histidine methyl ester (5b). Yield: 17%; oil; ^1H NMR ($CDCl_3$) δ 0.89 (t, 3H, CH_3, J= 7.1 Hz), 1.25 (m, 2H, CH_2), 1.7 (m, 2H, CH_2), 2.7 (t, 2H, CH_2, J= 7.1 Hz), 3.4 (m, 2H, CH_2), 3.7 (s, 3H, CH_3), 4.8 (t, 1H, CH), 6.9 (s, 1H, 4-H); ^{13}C NMR ($CDCl_3$) δ 13.4 (CH_3), 13.7 (CH_2), 22.0 (CH_2), 26.7 (CH_2), 29.7 (CH_2), 52.7 (CH_2), 53.1 (CH_3), 115.1 (C-4), 131.4 (C-5), 148.6 (C-2), 157.0 (CF_3), 170.0 (C=O); MS(CI-NH_3) m/z 322 (M+1); exact mass for $C_{13}H_{18}F_3N_3O_3$, calcd., 321.1294; found, 321.1300.

α-N-Trifluoroacetyl-2-isopropyl-L-histidine methyl ester (5c). Yield: 38%; oil; ^1H NMR ($CDCl_3$) δ 1.31 (d, 6H, 2 x CH_3, J= 7.2 Hz), 3.0 (m, 2H, 2 x CH), 3.16 (m, 1H, CH), 3.67 (s, 3H, CH_3), 4.76 (m, 1H, CH), 6.68 (s, 1H, 4-H), 8.72 (bs, 1H, NH), 9.65 (bs, 1H, NH); ^{13}C NMR (CD_3OD) δ 25.6 (CH_3), 32.5 (CH), 33.39 (CH_2), 56.39 (CH), 57.72 (CH_3), 118.4 (C-4), 138.7 (C-5), 158.1 (C-2), 160.9 (CF_3), 174.0 (C=O), 174.8

(C=O); analysis for $C_{12}H_{16}F_3N_3O_3$ (307.3), calcd., C, 46.91; H, 5.25; N, 13.68; found, C, 46.99; H, 5.28; N, 13.59; MS(CI-NH_3) m/z 308 (M+1).

α-N-Trifluoroacetyl-2-cyclohexyl-L-histidine methyl ester (5d). Yield: 39%; oil; ^{1}H NMR ($CDCl_3$) δ 1.6 (m, 10H, 5 x CH_2), 3.1 (m, 3H, CH, CH_2), 3.7 (s, 3H, CH_3), 4.1 (m, 1H, CH), 6.70 (s, 1H, 4-H); ^{13}C NMR ($CDCl_3$) δ 21.22 (CH_2), 26.0 (CH_2), 28.2 (CH_2), 31.5 (CH), 31.7 (CH_2), 52.8 (CH), 53.2 (CH_3), 113.4 (C-5), 134.2 (C-4), 153.1 (C-2), 157.3 (CF_3), 170.4 (C=O); analysis for $C_{15}H_{20}F_3N_3O_3$ (347.3), calcd., C, 51.87; H, 5.8; N, 12.09; found, C, 51.97; H, 5.83; N, 11.95; MS(CI-NH_3) m/z 348 (M+1).

α-N-Trifluoroacetyl-2-tert-butyl-L-histidine methyl ester (5e). Yield: 45%; oil; ^{1}H NMR (CD_3COCD_3) δ 1.34 (s, 9H, 3 x CH_3), 3.1 (m, 3H, CH, CH_2), 3.64 (s, 3H, CH_3), 4.7 (m, 1H, CH), 6.83 (s, 1H, 4-H); ^{13}C NMR (CD_3COCD_3) δ 24.6 (CH_3), 32.4 (CH), 34.2 (CH_2), 36.35 (CH), 57.74 (CH_3), 118.2 (C-4), 138.9 (C-5), 160.5 (C-2), 161.33 (CF_3), 174.6 (C=O); MS(CI-NH_3) m/z 322 (M+1); exact mass for $C_{13}H_{18}F_3N_3O_3$, calcd., 321.1294; found, 312.1300.

α-N-Trifluoroacetyl-2-ethylhistamine (6a). Yield: 14%; mp 124-125 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, 3H, CH_3), 2.77 (m, 4H, 2 x CH_2), 3.58 (m, 2H, CH_2), 6.70 (s, 1H, 4-H); analysis for $C_9H_{12}F_3N_3O$ (235.2), calcd., C, 45.96; H, 5.14; N, 17.86; found, C, 46.12; H, 5.05; N, 17.77; MS(CI-NH_3) m/z 236 (M+1).

α-N-Trifluoroacetyl-2-butylhistamine (6b). Yield: 20%; mp 117-119 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, CH_3, J= 7.6 Hz), 1.44 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 2.68 (t, 2H, CH_2, J= 7.4 Hz), 2.77 (t, 2H, CH_2, J= 6.0 Hz), 3.60 (m, 2H, CH_2), 6.69 (s, 1H, 4-H); analysis for $C_{11}H_{16}F_3N_3O$ (263.3), calcd., C, 50.19; H, 6.13, N, 15.96; found, C, 50.10; H, 5.90; N, 15.70; MS(CI-NH_3) m/z 264 (M+1).

α-N-Trifluoroacetyl-2-isopropylhistamine (6c). Yield: 25%; mp 132-134 °C; ^{1}H NMR ($CDCl_3$) δ 1.9 (d, 6H, 2 x CH_3, J= 6.9 Hz), 3.1 (t, 2H, CH_2, J= 6.9 Hz), 3.30 (m, 1H, CH), 3.8 (t, 2H, CH_2, J= 6.9 Hz), 6.99 (s, 1H, 4-H); ^{13}C NMR ($CDCl_3$) δ 21.91 (CH_3), 21.99 (CH_3), 27.23 (CH), 28.45 (CH_2), 40.73 (CH_2), 116.86 (C-4), 134.7 (C-5), 154.93 (C-2), 158.0 (CF_3), 177.0 (C=O); analysis for $C_{10}H_{14}F_3N_3O$ (249.2), calcd., C, 48.19; H, 5.66; N, 16.85; found, C, 48.33; H, 5.75; N, 16.72; MS(CI-NH_3) m/z 250 (M+1).

α-N-Trifluoroacetyl-2-cyclohexylhistamine (6d). Yield: 39%; mp 110-111 °C; ^{1}H NMR ($CDCl_3$) δ 1.25-2.04 (m, 10H, 5 x CH_2), 2.66 (m, 1H, CH), 2.77 (t, 2H, CH_2, J= 5.9 Hz), 3.59 (m, 2H, CH_2), 6.69 (s, 1H, 4-H); analysis for $C_{13}H_{18}F_3N_3O$ (289.3), calcd., C, 53.97; H, 6.27; N, 14.52; found, C, 54.02; H, 6.25; N, 14.35; MS(CI-NH_3) m/z 290 (M+1).

α-N-Trifluoroacetyl-2-tert-butylhistamine (6e). Yield: 38%; mp 136-138 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 9H, 3 x CH_3), 2.78 (t, 2H, CH_2, J= 6.3 Hz), 3.6 (t, 2H, CH_2, J= 6.3 Hz), 6.7 (s, 1H, 4-H); ^{13}C NMR ($CDCl_3$) δ 25.35 (CH_3), 29.34 (C), 29.42 (CH_2), 40.19 (CH_2), 118.5 (C-4), 137.32 (C-5), 156.02 (C-2), 156.98 (CF_3); analysis for $C_{11}H_{16}F_3N_3O$ (263.3), calcd., C, 50.18; H, 6.12; N, 15.96; found, C, 50.25; H, 6.14; N, 15.81; MS(CI-NH_3) m/z 264 (M+1).

General method for the synthesis of 2-alkyl-L-histidine (7) and 2-alkylhistamines (8). A solution of **5** or **6** (1 mmol) in *6N* HCl (20 mL), was heated at reflux for 8 hours. The dihydrochloride salts of the 2-alkyl-L-histidines (7) and 2-alkylhistamines (**8**) were obtained directly by evaporation of the acidic hydrolysis solutions. A solution of the 2-alkyl-L-histidine dihydrochloride (7) in water was applied to an ion-exchange column (Dowex 50 x 2-200, H^+ form). The column was eluted with water until neutral to pH paper. The amino acid was then eluted with 10% NH_4OH solution. Evaporation of solvent gave the free crystalline amino acid **7**.

2-Ethyl-L-histidine dihydrochloride (7a). Yield: 95%; mp 216-218 °C (dec); ^{1}H NMR (D_2O) δ 1.22 (t, 3H, CH_3, J= 7.8 Hz), 2.84 (q, 2H, CH_2, J= 7.6 Hz), 3.12 (m, 2H, CH_2), 4.06 (t, 1H, CH, J= 6.7 Hz), 7.12 (s, 1H, 4-H); analysis for $C_8H_{13}N_3O_2.2HCl+H_2O$ (274.2), calcd., C, 35.05; H, 6.25; N, 15.33; Cl, 25.80; found, C, 34.88; H, 6.35; N, 15.38; Cl, 25.99; $[\alpha]_D^{20}$ -19.8° (c=1.6, *N* HCl).

2-Butyl-L-histidine (7b). Yield: 85%; mp 199-203 °C (dec); ^{1}H NMR (D_2O) δ 0.75 (t, 3H, CH_3, J= 7.4Hz), 1.17 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 2.61 (t, 2H, CH_2, J= 7.4 Hz), 3.00 (m, 2H, CH_2), 3.76 (m, 1H, CH), 6.82 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 13.91 (CH_3), 23.04 (CH_2), 26.44 (CH_2), 26.61 (CH_2), 30.36 (CH_2), 52.85 (CH), 118.65 (C-4), 127.53 (C-5), 149.72 (C-2), 169.86 (C=O); analysis for $C_{10}H_{17}N_3O_2$ (211.3), calcd., C, 56.85; H, 8.11; N, 19.89; found, C, 56.75; H, 7.98; N, 19.82; $[\alpha]_D^{20}$ -15.2° (c=3, CH_3OH).

2-Isopropyl-L-histidine dihydrochloride (7c). Yield: 88%; mp 218-219 °C (dec); ^{1}H NMR (D_2O) δ 1.29 (d, 6H, 2 x CH_3, J= 7 Hz), 3.14-3.16 (m, 3H, CH, CH_2), 4.2 (t, 2H, CH_2, J= 7.3 Hz), 7.2 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 20.6 (CH_3), 26.7 (CH), 28.7 (CH_2), 52.9 (CH), 118.75 (C-4), 127.9 (C-5), 154.6 (C-2), 170.1 (C=O); analysis for $C_9H_{15}N_3O_2.2HCl+0.75H_2O$ (283.7), calcd., C, 38.11; H, 6.57; N, 14.8; Cl, 24.99; found, C, 38.26; H, 6.33; N, 14.31; Cl, 25.11; $[\alpha]_D^{20}$ -23.1° (c= 1.2, *N* HCl).

2-Cyclohexyl-L-histidine dihydrochloride (7d). Yield: 90%; mp 231-232 °C (dec); ^{1}H NMR (D_2O) δ 1.0-2.1 (m, 10H, 5 x CH_2), 3.1 (m, 1H, CH), 3.5 (m, 2H, CH_2), 4.3 (m, 1H, CH), 7.3 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 20.6 (CH_2), 20.8 (CH_2), 20.87 (CH_2), 21.0 (CH_2), 25.8 (CH), 47.9 (CH), 113.8 (C-4), 121.2 (C-5), 148.0 (C-2), 166.5 (C=O); analysis for $C_{12}H_{19}N_3O_2.2HCl+1.5H_2O$ (337.2), calcd., C, 42.74; H, 7.17; N, 12.46; Cl, 21.02; found, C, 42.77; N, 7.05; N, 12.18; Cl, 21.10; $[\alpha]_D^{20}$ -20.2° (c=1.17, *N* HCl).

2-tert-Butyl-L-histidine dihydrochloride (7e). Yield: 90%; mp 201-202 °C (dec), ^{1}H NMR (D_2O) δ 1.4 (s, 9H, 3 x CH_3), 3.27 (m, 2H, CH_2), 4.3 (t, 1H, CH, J= 6.6 Hz), 7.3 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 26.6 (CH_3), 28.7 (CH), 34.14 (CH_2), 53.0 (CH), 118.8 (C-4), 128.1 (C-5), 156.9 (C-2), 170.12 (C=O); analysis for $C_{10}H_{17}N_3O_2.2HCl+1.3H_2O$ (307.6), calcd., C, 39.04; H, 7.07; N, 13.66; Cl, 23.05; found, C, 39.02; H, 7.03; N, 13.52; Cl, 23.04; $[\alpha]_D^{20}$ -17.8° (c=1.34, *N* HCl).

2-Ethylhistamine dihydrochloride (8a). Yield: 90%; mp 203-207 °C (dec) [lit.[12a] 209 °C (dec)]; ^{1}H NMR (D_2O) δ 1.29 (t, 3H, CH_3, J= 7.8 Hz), 2.88 (m, 4H, 2 x CH_2), 2.99 (t, 2H, CH_2, J= 6.6 Hz), 7.22 (s, 1H, 4-H); analysis for $C_7H_{13}N_3.2HCl$ (212.1), calcd., C, 39.64; H, 7.13; N, 19.81; Cl, 33.43; found, C, 39.77; H, 7.05; N, 19.88; Cl, 33.33; MS(CI-NH_3) m/z 140 (M+1).

2-Butylhistamine dihydrochloride (8b). Yield: 90%; mp 183-185 °C (dec); ^{1}H NMR (D_2O) δ 0.76 (t, 3H, CH_3, J= 7.5 Hz), 1.20 (m, 2H, CH_2), 1.59 (m, 2H, CH_2), 2.80 (t, H, CH_2, J= 7.6 Hz), 2.94 (t, 2H, CH_2, J= 7.4 Hz), 3.16 (t, 2H, CH_2, J= 7.5 Hz), 7.06 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 14.05 (CH_3), 19.2 (CH_2), 23.5 (CH_2), 26.8 (CH_2), 26.99 (CH_2), 33.5 (CH_2), 112.22 (C-4), 125.25 (C-5), 149.5 (C-2); analysis for $C_8H_{17}N_3.2HCl$ (228.2), calcd., C, 42.11; H, 8.39; N, 18.42; Cl, 31.08; found, C, 41.90; H, 8.15; N, 18.33; Cl, 31.20; MS(CI-NH_3) m/z 168 (M+1).

2-Isopropylhistamine dihydrochloride (8c). Yield: 75%; mp 125-127 °C (dec); ^{1}H NMR (D_2O) δ 1.4 (d, 6H, 2 x CH_3, J= 7.2 Hz), 3.12 (t, 2H, CH_2, J= 7.2 Hz), 3.3 (m, 3H, CH, CH_2), 7.24 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 22.4 (CH_3), 25.18 (CH), 29.4 (CH_2), 40.99 (CH_2), 119.9 (C-4), 130.49 (C-5), 156.1 (C-2); analysis for $C_8H_{15}N_3.2HCl+1.5H_2O$ (253.2), calcd., C, 37.95; H, 7.96; N, 15.59; Cl, 28.00; found, C, 38.09; H, 7.56; N, 15.7; Cl, 27.92; MS(CI-NH_3) m/z 154 (M+1)

2-Cyclohexylhistamine dihydrochloride (8d). Yield: 87%; mp 278-280 °C (dec) [lit.[19] mp 275 °C (dec)]; ^{1}H NMR (D_2O) δ 0.97-2.02 (m, 10H, 5 x CH_2), 3.02 (m, 1H, CH), 3.09 (t, 2H, CH_2, J= 7.5 Hz), 3.32 (t, 2H, CH_2, J= 7.2 Hz), 7,2 (s, 1H, 4-H); ^{13}C NMR (D_2O) δ 18.16 (CH_2), 20.81 (CH_2), 20.88 (CH_2), 26.0 (CH_2), 31.37 (CH_2), 33.98 (CH_2), 111.92 (C-4), 123.41 (C-5), 148.1 (C-2); analysis for $C_{11}H_{19}N_3.2HCl+0.5H_2O$ (275.2), calcd., C, 48.01; H, 8.05; N, 15.26; Cl, 25.76; found, C, 48.04; H, 7.88; N, 15.18; Cl, 25.84; MS(CI-NH_3) m/z 194 (M+1).

2-tert-Butylhistamine dihydrochloride (8e). Yield: 92%; mp 225-228 °C (dec); ^{1}H NMR (D_2O) δ 1.32 (s, 9H, 3 x CH_3), 2.97 (t, 2H, CH_2, J= 7.2 Hz), 3.20 (t, 2H, CH_2, J= 7.2 Hz), 7.10 (s, 4-H); ^{13}C NMR (D_2O) δ 25.3 (CH_3), 30.65 (C), 35.53 (CH_2), 40.37 (CH_2), 119.22 (C-4), 130.81 (C-5), 158.6 (C-2); analysis for $C_9H_{17}N_3.2HCl+H_2O$ (258.2), calcd., C, 41.86; H, 8.19; N, 16.27; Cl, 27.46; found, C, 41.82; H, 7.94; N, 16.29; Cl, 27.54; MS(CI-NH_3) m/z 168 (M+1).

General procedure for the synthesis of (9). To a solution of 7 (1 mmol) in methanol (50 mL) cooled to 0 °C was bubbled HCl gas for 25 minutes. The solution was allowed to stand overnight at room temperature. Complete removal of solvent *in vacuo* afforded crystalline 2-alkyl-L-histidine methyl ester dihydrochlorides **9**.

2-Isopropyl-L-histidine methyl ester dihydrochloride (9a). Yield: 98%; mp 185-189 °C (dec); ^{1}H NMR (D_2O) δ 1.27 (d, 6H, 2 x CH_3, J= 7.0 Hz), 3.28 (m, 3H, CH_2, CH), 3.73 (s, 3H, CH_3), 4.35 (t, 1H, CH, J= 7.0 Hz), 7.15 (s, 1H, 4-H); analysis for $C_{10}H_{17}N_3O_2.2HCl+0.7H_2O$ (296.8), calcd., C, 40.46; H, 6.92; N, 14.15; found, C, 40.84; H, 6.70; N, 13.79; MS(CI-NH_3) m/z 212 (M+1); $[\alpha]_D^{20}$ +7.7° (c= 3.9, H_2O).

2-Cyclohexyl-L-histidine methyl ester dihydrochloride (9b). Yield: 99%; mp 158-162 °C (dec); ^{1}H NMR (D_2O) δ 1.1-2.0 (m, 10H, 5 x CH_2), 2.93 (m, 1H, CH), 3.29 (m, 2H, CH_2), 3.74 (s, 3H, CH_3), 4.35 (t, 1H, CH, J= 7.0 Hz), 7.16 (s, 1H, 4-H); analysis for $C_{13}H_{21}N_3O_2.2HCl$ (324.3), calcd., C, 48.16; H, 7.15; N, 12.96; Cl, 21.87; found, C, 47.94; H, 7.22; N, 12.81; Cl, 21.71; MS(CI-NH_3) m/z 252 (M+1); $[\alpha]_D^{20}$ +7.95° (c= 3.65, H_2O).

2-tert-Butyl-L-histidine methyl ester dihydrochloride (9c). Yield: 83%; mp 167-170 °C (dec); ^{1}H NMR (D_2O) δ 1.33 (s, 9H, 3 x CH_3), 3.28 (m, 2H, CH_2), 3.73 (s, 3H, CH_3), 4.36 (t, 1H, CH, J= 7.0 Hz), 7.16 (s, 1H, 4-H); analysis for $C_{11}H_{19}N_3O_2.2HCl+H_2O$ (316.2), calcd., C, 41.78; H, 7.33; N, 13.28; Cl, 22.42; found, C, 41.97; H, 7.37; N, 13.08; Cl, 22.18; MS(CI-NH_3) m/z 226 (M+1); $[\alpha]_D^{20}$ +10.9° (c= 3.5, H_2O).

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