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TETRAHEDRON

Synthesis of Ring-Halogenated Histidines and Histamines [1]

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Abstract: Series of ring-halogenated histidines and histamines have been synthesized from the suitably protected parent bioimidazoles. The 5-X and 2,5-X_2- derivatives are obtained by direct electrophilic halogenation while 2-X derivatives (X= Br and I) are prepared by regiospecific electrophilic dehalogenation at C-5. © 1998 Elsevier Science Ltd. All rights reserved.

Ring-halogenated histidines[2] are an important class of compounds because of their presence in various enzymes[3] and biologically active peptides.[4] They are also found to possess important biological activities. For example, 2-iodo-L-hisitidine and 2-fluoro-L-histidine have been found to possess significant antimalarial activity against *Plasmodium falciparum* parasites resistant to chloroquine.[5] However, accessibility to this important class of compounds is restricted by the lack of efficient synthetic methodologies.

The first method for synthesizing iodohistidines was reported by Brunings,[6] and subsequently modified by several groups.[7] Surprisingly, all of these methods involve direct iodination of the histidine ring without any protection of amino and carboxyl group. This generally leads to a variety of complex and difficulty separable by-products in overall low yield.[8] Similar obstacles are encountered in the synthesis of other halohistidines, and syntheses of dibromo and dichlorohistidine are reported by reacting cuprous chloride/bromide with α-N-benzoyl-2,5-diaminohistidines under strong acidic conditions in low yields.[9] Furthermore, there is no synthetic method available for biologically important 2-halo-L-histidines and 2-halohistamines.

Our interest in halogenated histidines and histamines as antimalarials and components of peptides created a need for a general synthetic method for these compounds. Ideally, this method should be applicable to both mono and dihalobioimidazoles. Additionally, the method should be compatible to the synthesis of mixed dihalobioimidazoles and 2-halobioimidazoles.[10] Designing synthetic routes for ring-substituted bioimidazoles is a very tedious and challenging task. For example, as part of our research efforts to develop efficient routes to ring-substituted histidines and histamines, we have reported procedures for the regiospecific synthesis of N(1)$^{\tau}$-L-alkylhistidines and N(1)$^{\tau}$-alkylhistamines,[11] 2-alkyl-L-histidines and 2-alkylhistamines,[12] and more recently the first regiospecific synthesis of 1,2-dialkyl-L-histidines and 1,2-dialkylhistamines.[13]

Examination of available synthetic routes to halobioimidazoles clearly indicated that use of unprotected bioimidazoles as starting materials is the primary cause of over-consumption of halogenating reagent, low yields and multiplicity of products, possibly due to intramolecular participation of nucleophilic α-amino group. Furthermore, the unprotected carboxyl group of histidine during electrophilic halogenation leads to a lactone intermediate,[14,15] which on further halogenation could give a mixture of unseparable by-products.

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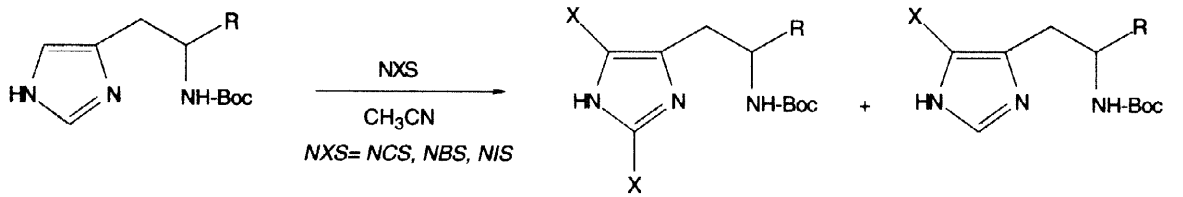
Finally, incomplete halogenation leads to difficult separations of mono and dihalobioimidazloes, in addition to unreacted starting material.

These problems could be alleviated by using suitably protected histidine and histamine as starting materials for halogenation reaction. Various amino-protecting groups were used during this study in our laboratory. Although, benzyloxycarbonyl, trifluoroacetyl and acetyl protecting-groups were also examined, the butyloxycarbonyl (BOC) was found to give the best results. In the case of histidine derivatives, methyl ester protection proved to be adequate for the carboxyl group. Both of these protecting groups could easily be removed by dilute acids at ambient temperature. We observed that milder *N*-halosuccinimides are better halogenating agents for bioimidazoles, and acetonitrile is the solvent of choice for these reactions. In all cases, halogenation reactions are carried out for 24 h at room temperature in the absence of light.

Electrophilic halogenation of commercially available **1** and **2** with one molar equivalent of *N*-halosuccinimide afforded a readily separable mixture of 2,5-dihalo derivatives (**3** and **4**) (10-25% yield) and 5-halo derivatives (**5** and **6**) in 70-75% yield (***Scheme 1***). The use of 2.5 equivalent of *N*-halosuccinimide gave 65-75% yield of 2,5-dihalo derivatives (**3** and **4**).



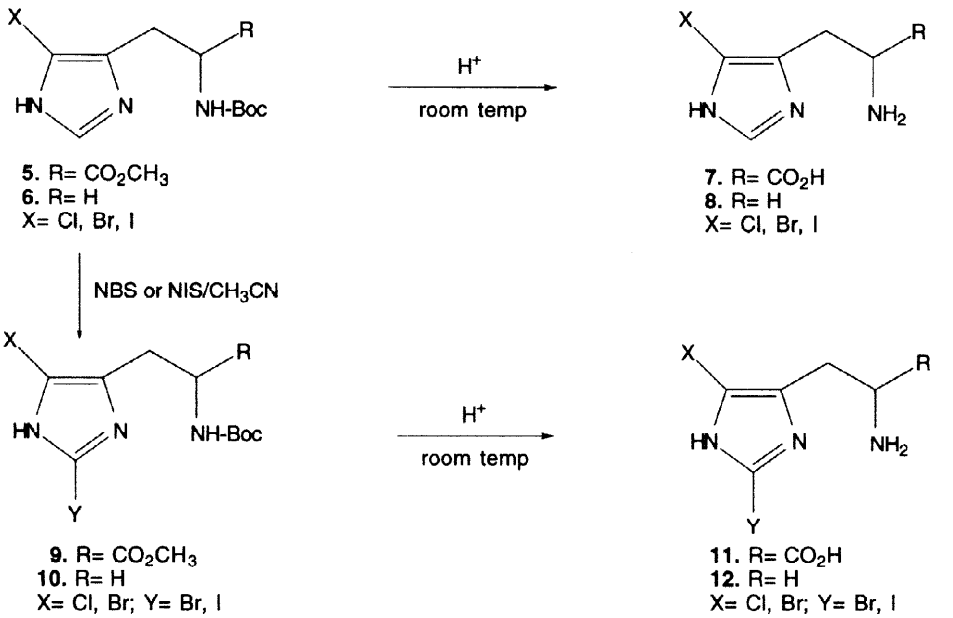
1. R= CO_2CH_3
2. R= H

3. R= CO_2CH_3
4. R= H
X= Cl, Br, I

5. R= CO_2CH_3
6. R= H
X= Cl, Br, I

Scheme 1

The monohalo derivatives **5** and **6** (X= Cl, Br) were further halogenated at C-2 to give mixed dihalogenated derivatives (**9** and **10**) in 60-90% yield (***Scheme 2***). However, attempts to obtain mixed haloderivatives with compounds **5** and **6** (X= I) proved to be unsuccessful. In these cases, the iodine present at C-5 of the imidazole ring was found to undergo electrophilic replacement by *N*-bromo and *N*-chlorosuccinimide prior to further substitution at C-2.



5. R= CO_2CH_3
6. R= H
X= Cl, Br, I

7. R= CO_2H
8. R= H
X= Cl, Br, I

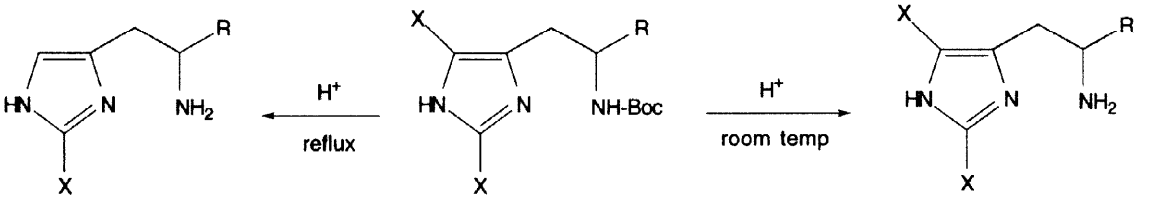
9. R= CO_2CH_3
10. R= H
X= Cl, Br; Y= Br, I

11. R= CO_2H
12. R= H
X= Cl, Br; Y= Br, I

Scheme 2

Protected halobioimidazoles (**3-6** and **9,10**) can be smoothly deprotected by reaction with *4N* H_2SO_4, *4N* HCl or 20% CF_3CO_2H for 2-72 h at ambient temperature. In the case of halohistidines the free amino acids were obtained by ion-exchange chromatography, whereas the histamine derivatives were directly obtained by

evaporation after acid hydrolysis (***Scheme 2 and 3***). It is important to note that all purification and separation of halogenated derivatives should be performed prior to deprotection.



15. R= CO_2H
16. R= H
X= Br, I

3. R= CO_2CH_3
4. R= H
X= Cl, Br, I

13. R= CO_2H
14. R= H
X= Cl, Br, I

Scheme 3

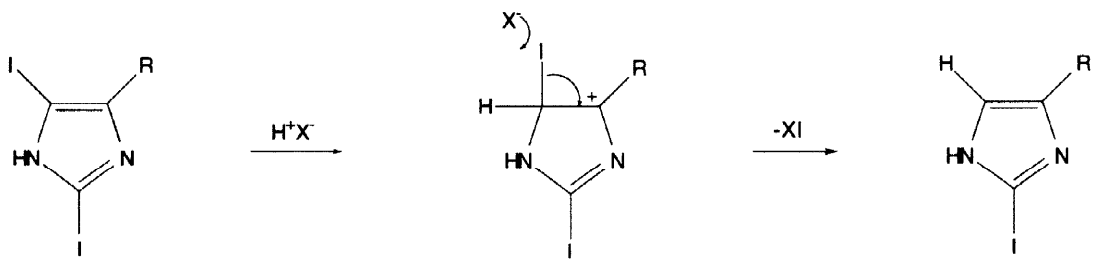


Fig. 1 : Electrophilic dehalogenation of dihalohistidine and histamine

Efforts to accelerate deprotection by the use of refluxing acid resulted in regiospecific electrophilic dehalogenation of bromine or iodine at C-5, with formation of previously unknown 2-bromo and 2-iodo derivatives **15** and **16** (X= Br, I) in 60-75% yield (***Figure 1, Scheme 3***). The reaction failed in the case of **3** and **4** (X= Cl), possibly due to the stability of carbon-chlorine bond. Regiospecific dehalogenation is observed in refluxing *1N* HCl or *1N* H_2SO_4 for 3 days. However, hydrochloric acid is preferred because of its volatility and ease of reaction work-up. For reasons unknown, residual traces of dihalo derivatives are not removed in most of the reactions. These traces are removed by crystallization of the final product. Use of more concentrated acid results in some dehalogenation at C-2 as well. This remarkable and very useful general route to 2-halobioimidazoles is consistent with earlier results on the regiospecificity of acid-catalyzed solvent isotope exchange in imidazoles.[16]

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 341 MC Polarimeter. However, enantiopurity of the final products was not examined. 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 di and mono N-(tert-butyloxycarbonyl)halohistidine methyl esters and N-(tert-butyloxycarbonyl)halohistamines 3-6

To a solution of N-(*tert*-butyloxycarbonyl)histidine methyl ester (**1**, 7.4 mmol) or N-(*tert*-butyloxycarbonyl)histamine (**2**, 7.4 mmol) in acetonitrile (50 mL) was added *N*-halosuccinimide (7.4 mmol), the reaction mixture stirred at room temperature in dark for 24 h under argon atmosphere. The solvent was removed and the

residue dissolved in EtOAc (100 mL). A saturated aqueous solution of sodium sulfide (10 mL) was added to the EtOAc and this stirred for 15 minutes. The organic layer was subsequently washed with saturated NaCl solution (2 x 15 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford a mixture of starting material, 5-halo and 2,5-halo derivatives. Purified by column chromatography using ethyl acetate:hexanes (60:40).

N-(tert-Butyloxycarbonyl)-2,5-dichloro-L-histidine methyl ester (3a). Yield: 13%; oil; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, 3 x CH_3), 2.93 (m, 1H, CH), 3.17 (m, 1H, CH), 3.78 (s, 3H, CO_2CH_3), 4.47 (m, 1H, CH), 5.36 (m, 1H, NH); MS (CI-NH_3) m/z 338 (M+); analysis for $C_{12}H_{17}Cl_2N_3O_4$ (338.2), calcd, C, 42.62; H, 5.07; N, 12.42; Cl, 20.97; found, C, 42.44; H, 5.23; N, 12.54; Cl, 21.12.

N-(tert-Butyloxycarbonyl)-2,5-dibromo-L-histidine methyl ester (3b). Yield: 14%; mp 65-67 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, 3 x CH_3), 2.94 (m, 1H, CH), 3.18 (m, 1H, CH), 3.79 (s, 3H, CO_2CH_3), 4.50 (m, 1H, CH), 5.37 (m, 1H, NH); MS (CI-NH_3) m/z 428 (M+1); analysis for $C_{12}H_{17}Br_2N_3O_4$ (427.1), calcd, C, 33.75; H, 4.01; N, 9.84; Br, 37.42; found, C, 33.49; H, 4.04; N, 9.56; Br, 37.20.

N-(tert-Butyloxycarbonyl)-2,5-diiodo-L-histidine methyl ester (3c). Yield: 25%; mp 86-88 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, 3 x CH_3), 2.97 (m, 1H, CH), 3.14 (m, 1H, CH), 3.77 (s, 3H, CO_2CH_3), 4.51 (m, 1H, CH), 5.40 (m, 1H, NH); MS(CI-NH_3) m/z 522 (M+1); analysis for $C_{12}H_{17}I_2N_3O_4$ (521.1), calcd, C, 27.66; H, 3.28; N, 8.06; I, 48.7; found, C, 28.0; H, 3.66; N, 8.14; I, 48.42.

N-Trifluoromethyl-2,5-diiodo-D-histidine methyl ester (3d). Yield: 90%; mp 64-65 °C; ^{1}H NMR ($CDCl_3$) δ 2.97 (m, 1H, CH), 3.14 (m, 1H, CH), 3.77 (s, 3H, CO_2CH_3), 4.51 (m, 1H, CH), 5.40 (m, 1H, NH); MS(CI-NH_3) m/z 518(M+1); analysis for $C_9H_8I_2F_3N_3O_3$ (517.0), calcd, C, 20.91; H, 1.56; N, 8.12; I, 49.09; found, C, 20.74; H, 1.69; N, 7.85; I, 49.22.

N-(tert-Butyloxycarbonyl)-2,5-dichlorohistamine (4a). Yield: 10%; mp 70-72 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, 3 x CH_3), 2.78 (t, 2H, CH_2, J= 5.9 Hz), 3.44 (m, 2H, CH_2), 4.91 (m, 1H, CH); MS(CI-NH_3) m/z 280 (M+); analysis for $C_{10}H_{15}Cl_2N_3O_2$ (280.2), calcd, C, 42.87; H, 5.39; N, 14.99; Cl, 25.31; found, C, 43.14; H, 5.33; N, 15.05; Cl, 25.44.

N-(tert-Butyloxycarbonyl)-2,5-dibromohistamine (4b). Yield: 18%; mp 112-114 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H, 3 x CH_3), 2.77 (t, 2H, CH_2, J= 6.0 Hz), 3.43 (m, 2H, CH_2), 4.90 (m, 1H, CH); MS(CI-NH_3) m/z 370 (M+1); analysis for $C_{10}H_{15}Br_2N_3O_2$ (369.0), calcd, C, 32.54; H, 4.09; N, 11.38; Br, 43.30; found, C, 32.77; H, 4.21; N, 11.43; Br, 43.65.

N-(tert-Butyloxycarbonyl)-2,5-diiodohistamine (4c). Yield: 25%; mp 82-84 °C; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, 3 x CH_3), 2.80 (t, 2H, CH_2, J= 5.7 Hz), 3.46 (m, 2H, CH_2), 4.86 (m, 1H, CH); MS(CI-NH_3) m/z 464 (M+1); analysis for $C_{10}H_{15}I_2N_3O_2$ (463.1), calcd, C, 25.94; H, 3.27; N, 9.07; I, 54.81; found, C, 25.85; H, 3.26; N, 9.02; I, 54.76.

N-(tert-Butyloxycarbonyl)-5-chloro-L-histidine methyl ester (5a). Yield: 66%; mp 155-156 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H, 3 x CH_3), 2.99 (m, 1H, CH), 3.15 (m, 1H, CH), 3.74 (s, 3H, CO_2CH_3), 4.48 (m, 1H, CH), 5.36 (m, 1H, NH), 7.45 (s, 1H, H-2); MS (CI-NH_3) m/z 304 (M+1); analysis for $C_{12}H_{18}ClN_3O_4$ (303.7), calcd, C, 47.45; H, 5.97; N, 13.83; Cl, 11.67; found, C, 47.52; H, 5.97; N, 13.78; Cl, 11.60.

N-(tert-Butyloxycarbonyl)-5-bromo-L-histidine methyl ester (5b). Yield: 73%; mp 158-159 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.99 (m, 1H, CH), 3.18 (m, 1H, CH), 3.76 (s, 3H, CO_2CH_3), 4.50 (m, 1H, CH), 5.36 (m, 1H, NH), 7.51 (s, 1H, 2-H); MS (CI-NH_3) m/z 348 (M+); analysis for $C_{12}H_{18}BrN_3O_4$(348.2), calcd, C, 41.39; H, 5.21; N, 12.07; Br, 22.95; found, C, 41.14; H, 5.16; N, 11.98; Br, 23.11.

N-(tert-Butyloxycarbonyl)-5-iodo-L-histidine methyl ester (5c). Yield: 69%; mp 140-142 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.98 (m, 1H, CH), 3.13 (m, 1H, CH), 3.76 (s, 3H, CO_2CH_3), 4.50 (m, 1H, CH), 5.42 (m, 1H, NH), 7.58 (s, 1H, 2-H); MS (CI-NH_3) m/z 396 (M+1); analysis for $C_{12}H_{18}IN_3O_4$ (395.2), calcd, C, 36.47; H, 4.59; N, 10.63; I, 32.11; found, C, 36.21; H, 4.44; N, 10.49; I, 32.20.

N-(tert-Butyloxycarbonyl)-5-chlorohistamine (6a). Yield: 76%; mp 49-51 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.82 (t, 2H, CH_2, J= 6.4 Hz), 3.42 (m, 2H, CH_2), 4.90 (m, 1H, NH), 7.42 (s, 1H, 2-H); MS(CI-

NH_3) m/z 246 (M+1); analysis for $C_{10}H_{16}ClN_3O_2$ (245.7), calcd, C, 48.88; H, 6.52; Cl, 14.46; N, 17.11; found, C, 48.65; H, 6.54; Cl, 14.50; N, 17.01.

N-(tert-Butyloxycarbonyl)-5-bromohistamine (6b). Yield: 70%; mp 50-51 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.80 (t, 2H, CH_2, J= 6.1Hz), 3.43 (m, 2H, CH_2), 4.83 (m, 1H, NH), 7.46 (s, 1H, 2-H); MS(CI-NH_3) m/z 292 (M+2); analysis for $C_{10}H_{16}BrN_3O_2$ (290.2), calcd, C, 41.38, H, 5.52; N, 14.48; Br, 27.59; found, C, 41.19; H, 5.57; N, 14.41; Br, 27.47.

N-(tert-Butyloxycarbonyl)-5-iodohistamine (6c). Yield: 71%; mp 57-59 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.79 (t, 2H, CH_2, J= 6.1 Hz), 3.43 (m, 2H, CH_2), 4.87 (m, 1H, NH), 7.56 (s, 1H, 2-H); MS(CI-NH_3) m/z 338 (M+1); analysis for $C_{10}H_{16}IN_3O_2$(337.2), calcd, C, 35.61; H, 4.75; N, 12.46; I, 37.69; found, C, 35.77; H, 4.71; N, 12.51; I, 37.47.

General method for the synthesis of mixed N-(tert-butyloxycarbonyl)halo-L-histidines (9) and mixed N-(tert-butyloxycarbonyl)halohistamines (10)

To a solution of **3** (1 mmol) or **4** (1 mmol) in acetonitrile (50 mL) was added *N*-halosuccinimide (2 mmol), the reaction mixture was stirred at room temperature in dark for 24 h under argon atmosphere. All solvent were removed and residue dissolved in ethyl acetate (150 mL). The saturated aqueous solution of sodium sulfide (15 mL) was added to organic solution, and mixture stirred for 15 minutes. The organic layer separated, and washed with NaCl solution (2 x 15 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford crude product. Purified by flash column chromatography using ethyl acetate: petroleum ether (1:1).

N-(tert-Butyloxycarbonyl)-5-chloro-2-bromo-L-histidine methyl ester (9a). Yield: 60%, oil; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H, 3 x CH_3), 2.95 (m, 1H, CH), 3.23 (m, 1H, CH), 3.80 (s, 3H, CO_2CH_3), 4.51 (m, 1H, CH), 5.40 (m, 1H, NH); MS(CI-NH_3) m/z 384 (M+1); analysis for $C_{12}H_{17}ClBrN_3O_4$ (382.6), calcd, C, 37.67; H, 4.48; N, 10.98; Br, 20.88; Cl, 9.27; found, C, 37.44; H, 4.42; N, 11.25; Br, 41.99 (total halogen calculated as bromine); Cl, 18.66 (total halogen calculated as chlorine).

N-(tert-Butyloxycarbonyl)-5-chloro-2-iodo-L-histidine methyl ester (9b). Yield: (71%); oil; ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9H, 3 x CH_3), 2.85 (m, 1H, CH), 3.20 (m, 1H, CH), 3.76 (s, 3H, CO_2CH_3), 4.48 (m, 1H, CH), 5.38 (m, 1H, NH); MS(CI-NH_3) m/z 430 (M+1); analysis for $C_{12}H_{17}ClIN_3O_4$ (429.6), calcd, C, 33.55; H, 3.99; N, 9.78; Cl, 8.25; I, 29.54; found, C, 33.15; H, 3.79; N, 9.66; Cl, 16.9 (total halogen calculated as chlorine); I, 59.6 (total halogen calculated as iodine).

N-(tert-Butyloxycarbonyl)-5-bromo-2-iodo-L-histidine methyl ester (9c). Yield: 88%; mp 120-122 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, 3 x CH_3), 2.95 (m, 1H, CH), 3.20 (m, 1H, CH), 3.78 (s, 3H, CO_2CH_3), 4.50 (m, 1H, CH), 5.35 (M, 1H, NH); MS(CI-NH_3) m/z 474 (M+); analysis for $C_{12}H_{17}BrIN_3O_4$ (474.1), calcd, C, 30.4; H, 3.61; N, 8.86; Br, 16.85; I, 26.77; found, C, 30.59; H, 3.68; N, 8.68; Br, 33.55 (total halogen calculated as bromine); I, 53.28 (total halogen calculated as iodine).

N-(tert-Butyloxycarbonyl)-5-chloro-2-iodohistamine (10a). Yield: 87%; mp 175-177 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, 3 x CH_3), 2.82 (t, 2H, CH_2, J= 5.9 Hz), 3.45 (m, 2H, CH_2), 4.89 (m, 1H, NH), MS(CI-NH_3) m/z 372 (M+1); analysis for $C_{10}H_{15}ClIN_3O_2$ (371.6), calcd, C, 32.32; H, 4.07; N, 11.31; Cl, 9.54; I, 34.15; found, C, 32.53; H, 4.07; N, 11.20; Cl, 19.02 (total halogen calculated as chlorine); I, 68.07 (total halogen calculated as iodine).

N-(tert-Butyloxycarbonyl)-5-bromo-2-iodohistamine (10b). Yield: 65%; mp 171-172 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, 3 x CH_3), 2.81 (t, 2H, CH_2, J= 5.9 Hz), 3.45 (m, 2H, CH_2), 4.89 (m, 1H, NH); MS(CI-NH_3) m/z 416 (M+1); analysis for $C_{10}H_{15}BrIN_3O_2$ (416.0), calcd, C, 28.86; H, 3.63; N, 10.10; Br, 19.20; I, 30.50; found, C, 28.67; H, 3.65 N, 9.88; Br, 38.64 (total halogen calculated as bromine); I, 61.45 (total halogen calcualted as iodine).

General method for the synthesis of halohistidines and halohistamines 7,8 and 11-14.

A mixture of protected mono/dihalohistidine or mono/dihalohistamine (1 mmol) in *4N* HCl or *4N* H_2SO_4 (10 mL) was stirred at room temperature for 72 h. In some cases, for deprotection of histamine derivative, trifluoroacetic acid (2 mL) in ethyl acetate (10 mL) at room temperature for 2 h was used. The

solvent was evaporated *in vacuo* to afford the salts of the halohistamine and halohistidine. A solution of the halohistidine dihydrochloride in water was applied to an ion-exchange column (Dowex 50 x 2-200, H^+ form). The column was eluted with water until the eluant was neutral to pH paper. The amino acid was then eluted with 10% NH_4OH. Evaporation of solvent gave the free crystalline amino acid. The dihydrochloride and trifluoroacetate salts of the halohistamine were obtained directly by evaporation after acid hydrolysis.

5-Chloro-L-histidine (7a). Yield: 97%; mp 215-225 °C (dec); ^1H NMR (D_2O) δ 3.04 (m, 2H, CH_2), 3.75 (t, 1H, CH, J= 5.85 Hz), 7.48 (s, 1H, 2-H); analysis for $C_6H_8ClN_3O_2$ (189.6), calcd, C, 38.01; H, 4.25; N, 22.16; Cl, 18.7; found, C, 38.21; H, 4.27; N, 22.26; Cl, 18.51; $[\alpha]_D^{25}$ -27° (c=1.8, H_2O).

5-Bromo-L-histidine (7b). Yield: 97%; mp 238-239 °C (dec); ^1H NMR (D_2O) δ 3.08 (m, 2H, CH_2), 3.82 (m, 1H, CH), 7.58 (s, 1H, 2-H); analysis for $C_6H_8BrN_3O_2$+0.3 H_2O (239.5), calcd, C, 30.09; H, 3.62; N, 17.54; Br, 33.37; found, C, 30.04, H, 3.60, N, 17.61; Br, 33.67; $[\alpha]_D^{25}$ -21° (c=2.7, H_2O).

5-Iodo-L-histidine (7c). Yield: 72%; mp 187-192 °C (dec); ^1H NMR (D_2O) δ 2.92 (m, 1H, CH_2), 3.06 (m, 1H, CH), 7.66 (s, 1H, 2-H); analysis for $C_6H_8IN_3O_2$+H_2O (299.1), calcd, C, 24.09; H, 3.37; N, 14.05; I, 42.43; found, C, 24.34; H, 3.05; N, 13.70; I, 42.31; $[\alpha]_D^{25}$ -18.5° (c=2.1, H_2O).

5-Chlorohistamine.2HCl (8a). Yield: 71%; mp 239-241 °C (dec); ^1H NMR (CD_3OD) δ 3.02 (m, 2H, CH_2), 3.16 (m, 2H, CH_2), 8.80 (s, 1H, 2-H); MS(CI-NH_3) m/z 146 (M+1); analysis for $C_5H_8ClN_3$.2HCl (218.5), calcd, C, 27.48; H, 4.61; N, 19.23; Cl, 48.67; found, C, 27.51; H, 4.56; N, 19.05; Cl, 48.80.

5-Bromohistamine.2CF_3CO_2H (8b). Yield: 85%; mp 104-108 °C (dec); ^1H NMR (CD_3OD) δ 3.1 (m, 2H, CH_2), 3.15(m, 2H, CH_2), 8.84 (s, 1H, 2-H); MS(CI-NH_3) m/z 190 (M+1); analysis for $C_5H_8BrN_3$.2CF_3CO_2H (418.1), calcd, C, 25.85; H, 2.41; N, 10.05; Br, 19.1; found, C, 25.98; H, 2.38; N, 9.99; Br, 19.25.

5-Iodohistamine.2CF_3CO_2H (8c). Yield: 91%; mp 110-115 °C (dec); ^1H NMR (CD_3OD) δ 2.82 (m, 2H, CH_2), 2.98 (m, 2H, CH_2), 8.5 (s, 1H, 2-H); ^{13}C NMR (CD_3OD) δ 24.6, 39.2, 72.8, 134.3, 139.0; MS(CI-NH_3) m/z 238 (M+1); analysis for $C_5H_8IN_3$.2CF_3CO_2H (465.1), calcd, C, 23.24; H, 2.16; N, 9.03; I, 27.28; found; C, 23.55; H, 2.22; N, 8.93; I, 27.35.

2-Bromo-5-chloro-L-histidine (11a). Yield: 79%; mp 208-210 °C (dec); ^1H NMR (D_2O) δ 2.98 (m, 2H, CH_2), 3.70 (m, 1H, CH); analysis for $C_6H_7BrClN_3O_2$ (268.5), calcd, C, 25.84; H, 2.63; N, 15.65; Br, 29.76; Cl, 13.2; found, C, 25.64; H, 3.0; N, 15.30; Cl, 26.8 (total halogen calculated as chlorine); Br, 59.9 (total halogen calculated as bromine); $[\alpha]_D^{25}$ -11.4° (c=1, H_2O).

5-Chloro-2-iodo-L-histidine (11b). Yield: 82%; mp 188-192 °C (dec); ^1H NMR (D_2O) δ 3.04 (m, 2H, CH_2), 3.75 (m, 1H, CH); ^{13}C NMR (DMF-d_7) δ 26.72, 54.66, 82.21, 127.26, 127.54, 172.63; analysis for $C_6H_7ClIN_3O_2$ (315.5), calcd, C, 22.84; H, 2.24; N, 13.32; Cl, 11.24; I, 40.22; found, C, 22.69; H, 2.44; N, 13.42; Cl, 22.41(tolal halogen calculated as chlorine); I, 80.20(total halogen calculated as iodine); $[\alpha]_D^{25}$ -6.3° (c =2.2, H_2O).

5-Bromo-2-iodo-L-histidine (11c). Yield: 88%; mp 188-195 °C (dec); ^1H NMR (DMSO-d_6) δ 2.9 (m, 2H, CH_2), 3.44 (t, 1H, CH, J= 6.9 Hz); ^{13}C NMR (DMF-d_7) δ 27.50, 54.72, 83.33, 114.21, 130.43, 172.83; analysis for $C_6H_7BrIN_3O_2$+0.9 H_2O (376.2), calcd, C, 19.15; H, 2.35; N, 11.17; Br, 21.24; I, 34.73; found, C, 19.19; H, 2.46; N, 11.53; Br, 21.6; I, 34.44; $[\alpha]_D^{25}$ -4.4° (c=1.6, H_2O).

5-Chloro-2-iodohistamine.2CF_3CO_2H (12a). Yield: 77%; mp 203-205 °C (dec); ^1H NMR (D_2O) δ 2.95 (t, 2H, CH_2, J= 7.5 Hz), 3.15 (t, 2H, CH_2, J= 7.2 Hz); MS(CI-NH_3) m/z 272 (M+1); analysis for $C_5H_7ClIN_3$.2CF_3CO_2H (499.5), calcd, C, 21.64; H, 1.82; N, 8.41; Cl, 7.7; I, 25.4; found, C, 21.54; H, 2.14; N, 8.22; Cl, 15.37 (total halogen calculated as chlorine); I, 50.75 (total halogen calculated as iodine).

5-Bromo-2-iodohistamine.2CF_3CO_2H (12b). Yield: 89%; mp 207-209 °C (dec); ^1H NMR (D_2O) δ 2.91 (t, 2H, CH_2, J= 7.4 Hz), 3.12 (t, 2H, CH_2, J= 7.1 Hz); MS(CI-NH_3) m/z 318 (M+1); exact mass, calcd, 314.8868, found, 314.8874; analysis for $C_5H_7BrIN_3$.2CF_3CO_2H (543.9), calcd, C, 19.87; H, 1.67; N, 8.77; Br, 14.69; I,

23.33; found, C, 20.04; H, 1.75; N, 8.57; Br, 29.2 (total halogen calculated as bromine); I, 46.88 (total halogen calculated as iodine).

2,5-Dichloro-L-histidine (13a). Yield: 95%; mp 254-256 °C (dec); ^{1}H NMR (D_2O) δ 2.90 (m, 2H, CH_2), 3.68 (m, 1H, CH); ^{13}C NMR (DMF-d_7) δ 27.01, 54.81, 124.89, 125.19, 128.62, 173.17; analysis for $C_6H_7Cl_2N_3O_2$+0.5 H_2O (233.1), calcd, C 30.92; H, 3.46; N, 18.03; Cl, 30.61; found, C, 30.90; H, 3.47; N, 18.42; Cl, 30.61; $[\alpha]_D^{25}$ -29° (c=1, H_2O).

2,5-Dibromo-L-histidine (13b). Yield: 83%; mp 196-202 °C (dec); ^{1}H NMR (D_2O) δ 3.01 (m, 2H, CH_2), 3.78 (m, 1H, CH); analysis for $C_6H_7Br_2N_3O_2$+0.7 H_2O (325.6), calcd, C, 22.13; H, 2.60; N, 12.90; Br, 49.08; found, C, 22.04; H, 2.59; N, 13.27; Br, 48.8; $[\alpha]_D^{25}$ -8.7° (c=1, H_2O).

2,5-Diiodo-L-histidine (13c). Yield: 77%; mp: 180-190 °C (dec); ^{1}H NMR (D_2O) δ 2.83 (m, 1H, CH), 3.04 (m, 1H, CH), 3.68 (m, 1H, CH); analysis for $C_6H_7I_2N_3O_2$ (407), calcd, C, 17.71; H, 1.73; N, 10.33; I, 62.37; found, C, 17.45; H, 1.85; N, 10.29; I, 62.59; $[\alpha]_D^{25}$ -14.3° (c=1.3, H_2O).

2,5-Diiodo-D-histidine (13d). Yield: 93%; mp 182-184 °C (dec); ^{1}H NMR (D_2O) δ 2.94 (m, 1H, CH), 3.12 (m, 1H, CH), 3.81 (m, 1H, CH); ^{13}C NMR (CD_3OD) δ 26.29, 52.62, 82.30, 119.47, 134.27, 171.30; analysis for $C_6H_7I_2N_3O_2$ (407.0), calcd, C, 17.71; H, 1.73; N, 10.33; I, 62.37; found, C, 17.5; H, 1.74; N, 10.05; I, 62.55; $[\alpha]_D^{25}$ +14.4° (c=1.3, H_2O).

2,5-Dichlorohistamine.2HCl (14a). Yield: 95%; mp 215-218 °C (dec); ^{1}H NMR (D_2O) δ 2.87 (t, 2H, CH_2, J= 7.1 Hz), 3.13 (t, 2H, CH_2, J= 7.0 Hz); MS(CI-NH_3) m/z 180 (M+1); analysis for $C_5H_7Cl_2N_3$.2HCl (253.0), calcd, C, 23.74; H, 3.58; N, 16.61; Cl, 56.06; found, C, 23.67; H, 3.73; N, 16.89; Cl, 55.87.

2,5-Dibromohistamine.2CF_3CO_2H (14b). Yield: 88%; mp 214-215 °C (dec); ^{1}H NMR (D_2O) δ 2.88 (t, 2H, CH_2, J= 7.1 Hz), 3.14 (t, 2H, CH_2, J= 7.0 Hz); MS(CI-NH_3) m/z 270 (M+1); analysis for $C_5H_7Br_2N_3$ .2CF_3CO_2H (496.9), calcd, C, 21.75; H, 1.83; N, 8.45; Br, 32.16; found, C, 21.78; H, 2.09; N, 8.37; Br, 32.34.

2,5-Diiodohistamine.2CF_3CO_2H (14c). Yield: 96%; mp 73-75 °C (dec); ^{1}H NMR (CD_3OD) δ 2.79 (t, 2H, CH_2, J= 7.4 Hz), 3.0 (t, 2H, CH_2, J= 7.3 Hz); MS(CI-NH_3) m/z 364 (M+1); ^{13}C NMR (CD_3OD) δ 25.5, 39.8, 75.4, 87.7, 140.5; analysis for $C_5H_7I_2N_3$.2CF_3CO_2H (590.9), calcd, C, 18.29; H, 2.54; N, 7.11; I, 42.95; found, C, 18.10; H, 2.66; N, 7.24; I, 43.13.

General method for the synthesis of 2-halohistidines (15) and 2-halohistamines (16).

A solution of **3** or **4** (1 mmol) in *N* H_2SO_4 or *N* HCl (10 mL) was stirred with heating at 100 °C for 72 h under nitrogen atmosphere. The solvent was evaporated *in vacuo* to afford the salts of the 2-halohistamine and 2-halohistidine. A solution of the 2-halohistidine dihydrochloride in water was applied to an ion-exchange column (Dowex 50 x 2-200, H^+ form). The column was eluted with water until the eluant was neutral to pH paper. The amino acid was then eluted with 10% NH_4OH. Evaporation of solvent gave the free crystalline amino acid. The salts of the 2-halohistamine were obtained directly by evaporation of the acid hydrolysis solution. Crystallized from water/methanol.

2-Iodo-L-histidine (15a). Yield: 75%; mp 188-190 °C (dec); ^{1}H NMR (D_2O) δ 2.94 (m, 1H, CH), 3.07 (m, 1H, CH), 3.78 (m, 1H, CH), 6.93 (s, 1H, 5-H); analysis for $C_6H_8IN_3O_2$+0.5 H_2O (290.1), calcd, C, 24.84; H, 3.12; N, 14.48; I, 43.75; found, C, 24.51; H, 3.30; N, 14.09; I, 43.55; $[\alpha]_D^{25}$ -12.3° (c=1, H_2O).

2-Iodo-D-histidine (15b). Yield: 75%; mp 189-192 °C (dec); ^{1}H NMR (D_2O) δ 2.94 (m, 1H, CH), 3.09 (m, 1H, CH), 3.80 (m, 1H, CH), 6.93 (s, 1H, 5-H); ^{13}C NMR (CD_3OD) δ 29.96, 56.3, 85.0, 123.0, 139.0, 174.1; analysis for $C_6H_8IN_3O_2$+0.45H_2O(289.2), calcd, C, 24.92; H, 3.10; N, 14.53; I, 43.88; found, C, 25.29; H, 3.30; N, 14.32; I, 43.88; $[\alpha]_D^{25}$ +12.3° (c=1, H_2O).

2-Bromo-L-Histidine (15c). Yield: 61%; mp 222-227 °C (dec); ^{1}H NMR (D_2O) δ 2.73 (m, 1H, CH), 2.99 (m, 1H, CH), 3.65 (m, 1H, CH), 6.77 (s, 1H, 5-H); analysis for $C_6H_8BrN_3O_2$+H_2O (252.1), calcd, C, 28.58; H, 3.99; N, 16.67; Br, 31.70; found, C; 28.49; H, 4.10; N, 16.55; Br, 31.88; $[\alpha]_D^{25}$ -25° (c=1.1, H_2O).

2-Bromohistamine.2HCl (16a). Yield: 65%; mp 203-207 °C; ^{1}H NMR (CD_3OD) δ 2.79 (t, 2H, CH_2, J= 7.2 Hz), 3.1 (t, 2H, CH_2, J= 7.1 Hz), 6.93 (s, 1H, 5-H); MS(CI-NH_3) m/z 192 (M+1); analysis for $C_5H_{10}Cl_2BrN_3$ (262.96), calcd, C, 22.84; H, 3.83; N, 15.98; Cl, 26.96; Br, 30.39; found, C, 22.88; H, 3.78; N, 16.12; Cl, 26.76; Br, 30.42.

2-Iodohistamine.2HCl (16b). Yield: 66%, mp 217-224 °C (dec); ^{1}H NMR (D_2O) δ 3.15 (t, 2H, CH_2, J= 7.3 Hz), 3.31 (t, 2H, CH_2, J= 7.3 Hz), 7.41 (s, 1H, 5-H); MS(CI-NH_3) m/z 238 (M+1); analysis for $C_5H_{10}Cl_2IN_3$ (309.9), calcd, C, 19.35; H, 3.23; N, 13.55; Cl, 22.90; I, 40.97; found, C, 19.44; H, 3.29; N, 13.47; Cl, 22.81; I, 40.87.

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