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Synthesis of (*S*)-gizzerosine, a potent inducer of gizzard erosion in chicks

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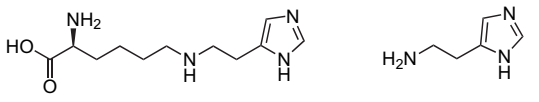
Abstract—(*S*)-Gizzerosine, a potent inducer of gizzard erosion in chicks, was synthesized using successive zinc-mediated and palladium-catalyzed coupling reactions as the key steps.

1. Introduction

Gizzerosine [(*S*)-2-amino-9-(4-imidazolyl)-7-azanonanoic acid (**1**) (Fig. 1)], generated during the heat treatment of brown fish meal, brings about gizzard erosion and ulceration in chicks, which cause a serious disease 'Black vomit'.[1,2] The structure and absolute stereochemistry of **1** were confirmed by the chemical synthesis of racemic[3–5] and optically active samples.[3,6] Biological studies of **1** showed it to be a potent agonist for the H_2-receptor of histamine (**2**),[7,8] causing 10 times more gastric secretions than **2**, and thereby, promoting acid indigestion and gizzard erosion. In addition, cAMP synthesis was enhanced by **1** 1000 times more than **2**.[9] On the other hand, **1** did not show any visible effects on rat stomach, though the gastric secretions being promoted.[2,10,11] This biological profile suggests **1** could be a possible drug candidate for gastric achlorhydria and osteoporosis. Since **1** can only be isolated in small amounts from fish meal (0.2 mg kg^{-1}),[2] synthetic supply is necessary for the standard of the quality control of fish meal and further biological studies. In a previous communication, we reported a facile and practical synthesis of (*S*)-**1**.[12,13] Herein, we describe in detail this synthesis and report the development of another.



(*S*)-gizzerosine (**1**) histamine (**2**)

Figure 1.

2. Results and discussion

2.1. Synthesis of Mori's intermediate (formal synthesis)

Mori et al. used enzymatic optical resolution of α-acylaminoadipic acid **3** as the key reaction and determined the absolute configuration to be *S* (Scheme 1).[6] We targeted the Mori's intermediates **4**[6,14] and **5** for the formal synthesis of **1** using (*S*)-serine as the starting material. (*S*)-Serine was first converted to the known iodide **6** in four steps.[15] Conversion of **6** to the aldehyde **5** via **9** using a zinc-mediated coupling reaction[16,17] with acrolein was attempted; however, 1,4-addition[18] did not occur and only the corresponding alanine derivative **10** (formed by quenching of the intermediate zinc iodide **7**) could be isolated. Next we aimed to prepare the alcohol **4** in a two-step procedure. The coupling reaction with allyl bromide[19] afforded the desired olefin **8**, but the use of propargyl bromide gave not alkyne **11** but allenyl compound **12**.[18] Hydroboration–oxidation of **8** under mildly basic conditions using sodium acetate[20] gave **4** without concomitant hydrolysis of the benzyl group, which was converted to (*S*)-gizzerosine **1** according to Mori's procedure.[6] The overall yield of **4** from (*S*)-serine was 58% over six steps (Scheme 1).

2.2. New synthesis using successive metal-mediated coupling reactions

2.2.1. Initial zinc-mediated coupling reaction with enol acetate. Whilst preparing Mori's intermediate **5**, we found that the coupling of the organozinc iodide **7** with 3-bromo-1-propenyl acetate (**13**)[21] afforded allylic acetate **14** as a 2:1 diastereomeric mixture at the 4-position in 98% yield, instead of enol acetate **15** (Scheme 2). Allylic acetate **14** was

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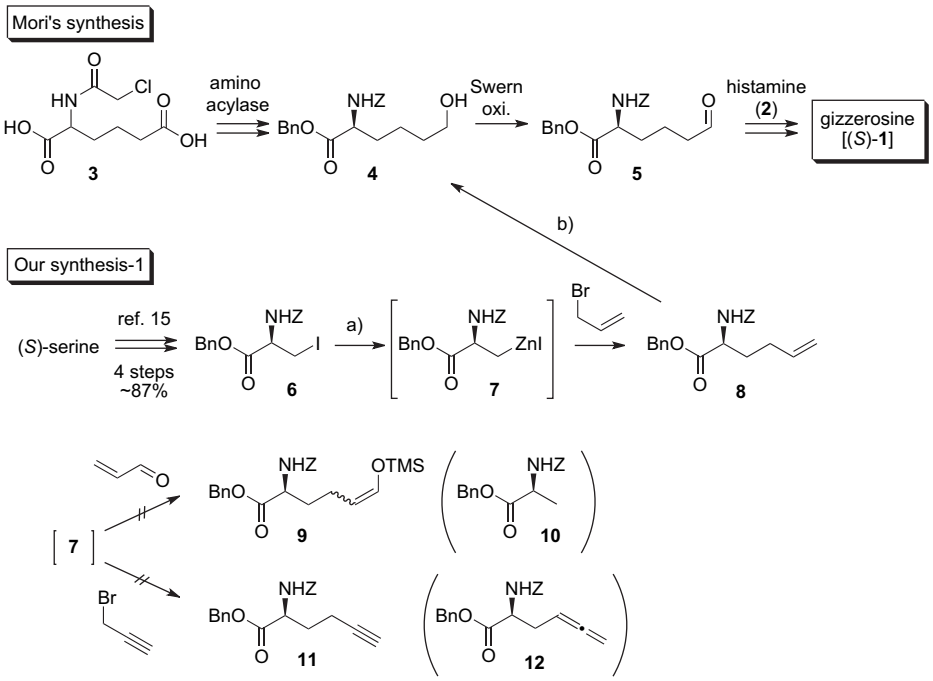
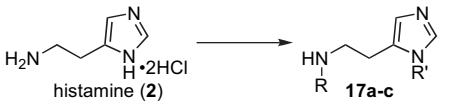


Scheme 1. Mori's synthesis and our formal synthesis of **1** using zinc-mediated coupling reaction: (a) i. Zn, $(CH_2Br)_2$, TMSCl, DMF, then **6**, ii. CuCN, LiCl, allyl bromide (quant.); (b) i. $BH_3 \cdot SMe_2$, THF, ii. 30% H_2O_2, NaOAc (68%).

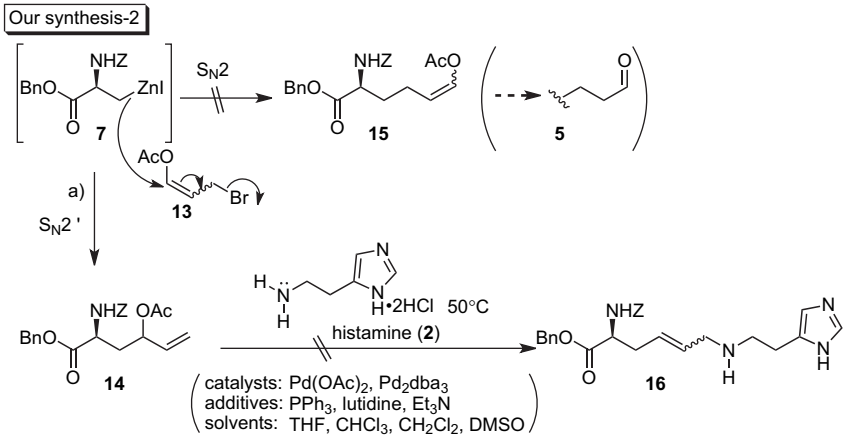
expected to be a good substrate for palladium-catalyzed coupling reactions with nitrogen nucleophiles,[22] but the coupling reaction with histamine (**2**) did not afford the desired product **16** under various conditions.

2.2.2. Protection of histamine. It was thought that this unreactivity was due to the insolubility of histamine itself and/or the poor nucleophilicity of the terminal amino group. Therefore, we prepared several histamine derivatives bearing electron-donating or electron-withdrawing protecting groups. Table 1 shows the protection of histamine (**2**) by nucleophilic substitution reactions. Bis-Boc product **17a** was the main product in entry 1. The primary amino group was selectively masked for benzyl carbamate **17b**[23] and trityl (Tr) ether **17c** but in low yields. Formation of benzyloxymethyl (BOM) ether and *m*-nitrobenzenesulfonamide (Ns) was observed only slightly by TLC. Since none of the product was formed in entry 6, benzyl derivatives were synthesized by reductive amination.

Table 1. Protection of histamine by nucleophilic substitution reactions

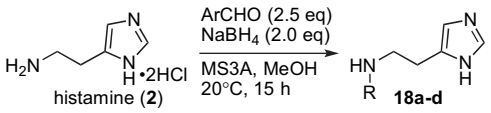


Entry	Conditions	Products	R	R′	Yields (%)
1	$(Boc)_2O$, Et_3N, MeOH	**17a**	Boc	Boc	83
2	ZCl, NaOMe, MeOH	**17b**	Z	H	36
3	TrCl, Et_3N, $CHCl_3$	**17c**	Tr	H	36
4	BOMCl, Et_3N, $CHCl_3$	—	BOM	H	Trace
5	NsCl, K_2CO_3, DMF	—	Ns	H	Trace
6	PMBCl, NaOMe, MeOH	—	PMB	H	—



Scheme 2. Preparation of **14** and trials of palladium-catalyzed coupling reaction with histamine: (a) CuCN, LiCl, **13**, DMF (98%).

Table 2. Protection of histamine by reductive amination reactions



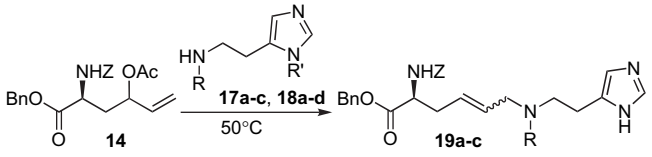
Entry	ArCHO	Products	R	Yields (%)
1	Benzaldehyde	**18a**	Benzyl	93
2	Anisaldehyde	**18b**	*p*-Methoxybenzyl	94
3	Piperonal	**18c**	Piperonyl	89
4	3,4-Dimethoxy-benzaldehyde	**18d**	3,4-Dimethoxybenzyl	10

As shown in Table 2, reductive amination of benzaldehyde, anisaldehyde, and piperonal with **2** afforded the desired compounds **18a**,[24] **18b**,[25] and **18c**,[25] respectively, in excellent yields.

2.2.3. Second palladium-catalyzed coupling reaction. The palladium-catalyzed coupling reactions of **14** with the histamine derivatives were examined under a variety of conditions (Table 3). The histamines with electron-withdrawing groups **17a** and **17b** did not react even in the presence of strong bases. Trityl derivative **17c** was also unreactive, probably due to its steric bulk. However, **18a** bearing an electron-donating benzyl group afforded the desired product **19a** in a yield of 50% when a mixture of PPh_3 (0.5 mol %) and Pd(II) (1 mol %) was used. Since increasing the amount of PPh_3 (1 mol %) gave multiple products, this mixture was subjected to further investigation. As expected, the more electron-donating derivatives **18b** and **18c** gave better yields of the coupling products **19b** and **19c**, respectively. The *E*/*Z* ratio of the products could not be ascertained due to overlappings in NMR spectra.

2.2.4. Total synthesis. We aimed to simultaneously carry out the deprotection of all three protecting groups and reduction of the chain double bond. Although the benzyl ester group and *N*-benzyloxycarbonyl group were easily deprotected and the double bond was reduced by hydrogenation, removal of the *N*-benzyl group of **19a** was unsuccessful (Table 4) and prolonged reaction times caused decomposition of gizzerosine framework. Additional experiments showed that gizzerosine itself was not stable under these reaction conditions. The *N*-PMB group (**19b**) was also largely inert to

Table 3. Palladium-catalyzed coupling reactions



Entry	Histamines	Catalysts	Additive	Solvents	Products	R	Yields (%)
1	**17a**	Pd_2dba_3	PPh_3, (base)[a]	THF	—	—	—
2	**17b**	Pd_2dba_3	PPh_3, (base)[b]	THF/DMF	—	—	—
3	**17c**	Pd_2dba_3	PPh_3	THF	—	—	—
4	**18a**	$Pd(OAc)_2$	PPh_3	THF[c]	—	—	—
5	**18a**	Pd_2dba_3	PPh_3	THF	**19a**	Bn	50
6	**18b**	Pd_2dba_3	PPh_3	THF	**19b**	PMB	92
7	**18c**	Pd_2dba_3	PPh_3	THF	**19c**	Piperonyl	71
8	**18d**	Pd_2dba_3	PPh_3	THF	—	—	—

[a] The following bases were used respectively: none, K_2CO_3, Et_3N, NaH, and KHMDS.
[b] The following bases were used respectively: none, NaH, and KHMDS.
[c] DMSO and MeCN were used respectively, besides THF.

Table 4. Hydrogenation and hydrolysis of **19a–c** toward the total synthesis

Entry	Substrate	R	Conditions	Yields (%)
1	**19a**	Bn	H_2, Pd/C, MeOH	—
2			H_2, $Pd(OH)_2$/C, THF/EtOH/H_2O	Trace
3			Raney Ni, MeOH/H_2O	—
4	**19b**	PMB	H_2, 5% Pd/C, THF/EtOH/H_2O	Trace
5			H_2, 5% Pd/C, 2 M HCl aq/EtOH	Trace
6			H_2, $Pd(OH)_2$/C, EtOH/H_2O	Trace
7			H_2, $Pd(OH)_2$, 2 M HCl aq/THF/EtOH	Trace
8	**19c**	Piperonyl	H_2, 10% Pd/C, THF/EtOH/H_2O	22
9			H_2, 10% Pd/C, $Pd(OH)_2$/C, THF/EtOH/H_2O	Decomp.
10			H_2, $Pd(OH)_2$/C, THF/EtOH/H_2O	Trace
11			H_2, $Pd(OH)_2$/C (excess), THF/EtOH/H_2O	47
12			Raney Ni, THF/EtOH/H_2O	Decomp.

hydrogenolysis. Selective removal under oxidative (CAN or DDQ), reductive (Li/NH_3), and acidic (2 M HCl aq) conditions also resulted in either decomposition or no reaction. On the other hand, the *N*-piperonyl group was readily removed, and finally, gizzerosine (*S*)-**1** was obtained from **19c** in 47% yield using excess amount of $Pd(OH)_2$/C. The overall yield was 29% in seven steps from (*S*)-serine.

3. Conclusion

We have developed new and facile synthesis of (*S*)-gizzerosine (**1**), a potent inducer of gizzard erosion, using successive zinc-mediated and palladium-catalyzed coupling reactions as the key steps. *N*-Piperonyl group was successfully applied as a new *N*-protecting group.

4. Experimental

4.1. General

The melting points measured by Yanaco MP-J3 micromelting point apparatus were uncorrected. Optical rotation values were measured by a Horiba Sepa-300 polarimeter. IR spectra were recorded by a Jasco FT-IR 4100 spectrometer (ATR, Zn–Se). ^{1}H and ^{13}C NMR spectra were recorded with Varian Inova 600 (150 MHz for ^{13}C), Inova 500 (500 MHz for ^{1}H and125 MHz for ^{13}C), and Gemini 2000 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers in $CDCl_3$ with tetramethylsilane (δ_H 0 ppm) and $CHCl_3$ (δ_C 77.00 ppm) or in D_2O with acetone (δ_H 2.22 ppm and δ_C 215.48 ppm) as internal standards. Mass spectra were recorded with a Jeol JMS-700 spectrometer using glycerol matrix. Merck silica gel 60 (63–212 μm) and Kanto silica gel 60N (spherical, neutral, 100–210 μm) were used for column chromatography.

4.1.1. Benzyl (*S*)-2-(benzyloxycarbonylamino)-5-hexenoate (8). A 500 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter, and a septum was placed under a nitrogen atmosphere. In the flask was placed zinc powder (15.2 g, 232 mmol) and 1,2-dibromoethane (1.00 ml, 11.6 mmol) in dry DMF (170 ml) was stirred at room temperature for 20 min. To the reaction mixture was added TMSCl (0.30 ml, 2.32 mol) and stirred at 60 °C for 30 min. Then to this mixture was added dropwise iodide **6** (MW: 439.24, 17.0 g, 38.7 mmol) in dry DMF (50 ml) and stirred at 60 °C for 20 min. A solution of CuCN (3.80 g, 42.5 mmol) and LiCl (3.60 g, 85.1 mmol) in dry DMF (40 ml) was added to the mixture at −55 °C. This was warmed to 0 °C and stirred for 10 min. Then the mixture was cooled to −55 °C again, allyl bromide (6.44 ml, 50.3 mmol) was added to this mixture. After the mixture was stirred at 0 °C for 2 h, unreacted zinc was filtrated through a Celite® pad and the filtrate was quenched with satd aq NH_4Cl soln. This mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc gave **8** (MW: 353.41, 13.7 g, 38.7 mmol, quantitative yield from **6**) as a colorless oil, which very slowly crystallized to form colorless needles; mp 37–38 °C, R_f 0.50 (hexane/EtOAc=3:1), $[\alpha]_D^{25}$ +1.80 (*c* 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.35 (s, 10H), 5.82–5.64 (m, 1H, H-5), 5.33 (pseudo d, 1H, *J*=8.1 Hz, NH), 5.17 (pseudo d, 2H, *J*=5.4 Hz), 5.12 (s, 2H), 5.02–4.92 (m, 2H), 4.50–4.40 (m, 1H, H-2), 2.20–1.70 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=172.38, 155.94, 136.85, 136.29, 135.33, 128.69, 128.60, 128.56, 128.37, 128.26, 128.18, 115.87, 67.12, 66.97, 53.45, 31.76, 29.18. IR: ν=3341 (m, N–H), 3064 (w), 3034 (w), 2953 (w), 1718 (s, C=O), 1641 (w), 1521 (m), 1454 (m), 1387 (w), 1343 (m), 1254 (m), 1211 (m), 1115 (w), 1047 (m), 1028 (m), 913 (m), 736 (m), 695 (s), 584 (w) cm^{-1}. FABMS: *m*/*z*=354 $(M+H)^+$, 310, 91 $(Bn)^+$. FABHRMS: calcd for $C_{21}H_{24}O_4N$ $(M+H)^+$ *m*/*z*=354.1705; found, 354.1709.

4.1.2. Benzyl (*S*)-2-(benzyloxycarbonylamino)-5-hydroxy-5-hexenoate (4). A 100 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter, and a septum was placed under a nitrogen atmosphere. To a solution of **8** (MW: 353.41, 1.70 g, 4.81 mmol) in dry THF (20 ml) was added dropwise $BH_3 \cdot SMe_2$ (2.0 M in THF, 1.20 ml, 2.41 mmol) at 0 °C and the mixture was stirred while the reaction temperature gradually raised to 20 °C. To this was again added dropwise $BH_3 \cdot SMe_2$ (1.20 ml, 2.41 mmol) and the mixture was further stirred at 20 °C for 12 h. The reaction mixture was cooled to 0 °C before being quenched with H_2O. To the resulting solution were added successively aq H_2O_2 (30%, 8.82 M, 1.8 ml, 16 mmol) and NaOAc (0.99 g, 12 mmol) in H_2O (3 ml), and the mixture was stirred at 20 °C for 12 h. The reaction mixture was extracted with EtOAc. The extract was washed with brine, dried with $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc gave **4** (MW: 371.43, 1.22 g, 3.28 mmol, 68.3%) as a colorless oil; R_f 0.27 (hexane/EtOAc=1:1), $[\alpha]_D^{26}$ −5.09 (*c* 1.06, $CHCl_3$) {lit.[6] $[\alpha]_D^{23}$ −3.9 (*c* 1.06, $CHCl_3$)}. ^{1}H NMR spectral data were in agreement with the published data.[14]

4.1.3. Benzyl (2*S*,4*RS*)-4-acetoxy-2-(benzyloxycarbonylamino)-5-hexenoate (14). A 100 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter and a septum was placed under a nitrogen atmosphere. The flask was charged with zinc powder (5.36 g, 82.0 mmol) and 1,2-dibromoethane (0.353 ml, 4.10 mmol) in dry DMF (20 ml) and stirred at room temperature for 20 min. To the reaction mixture was added TMSCl (104 μml, 822 μmol) and stirred at 60 °C for 30 min. Then to this mixture was added dropwise iodide **6** (MW: 439.24, 6.00 g, 13.7 mmol) in dry DMF (10 ml) and stirred at 60 °C for 20 min. A solution of CuCN (1.23 g, 13.7 mmol) and LiCl (1.16 g, 27.4 mmol) in dry DMF (10 ml) was added to the mixture at −55 °C. After being warmed to 0 °C and stirred for 10 min, the mixture was cooled to −55 °C again, and then bromide **13** (MW: 179.01, 2.94 g, 16.4 mmol) in dry THF (7 ml) was added. After the mixture was stirred for 2 h, unreacted zinc was filtrated through Celite® pad and the filtrate was quenched with satd aq NH_4Cl soln. This mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc gave **14** (a 2:1 diastereomeric mixture, MW: 411.45, 5.52 g, 13.4 mmol, 98% from **6**) as a colorless oil; R_f 0.28 (hexane/EtOAc=4:1),

$[\alpha]_D^{24}$ +16.9 (*c* 1.27, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ=7.35 (s, 10H), 5.81–5.64 (m, 1H, H-5), 5.50 (d, 1H, *J*=8.0 Hz, NH), 5.38–5.06 (m, 7H), 4.59–4.48 (m, 1H, H-2), 2.20 (t, 2H, *J*=6.0 Hz, H-3), 1.93 (s, 3H, Me). ^{13}C NMR (125 MHz, $CDCl_3$): δ=171.71, 170.12, 155.72 (NCO), 136.09, 135.35, 135.15, 128.66, 128.64, 128.57, 128.53, 128.51, 128.40, 128.23, 128.20, 128.13, 128.10, 117.71, 117.55, 70.80, 70.78, 67.43, 67.29, 67.13, 67.09, 51.05, 50.95, 36.67, 36.12, 21.08, 20.89. IR: ν=3450–3200 (br, m), 3033 (w), 2947 (w), 1719 (s), 1523 (m), 1455 (m), 1372 (m), 1341 (m), 1229 (s), 1047 (m), 738 (m), 696 (m) cm^{-1}. FABMS: *m*/*z*=412 $(M+H)^+$, 352 $(M+H-AcOH)^+$, 308, 91 $(Bn)^+$. FABHRMS: calcd for $C_{23}H_{26}O_6N$ $(M+H)^+$ *m*/*z*=412.7601; found, 412.1767.

4.1.4. N^{α},N^{τ}-Bis(*tert*-butoxycarbonyl)histamine (17a). A 100 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter, and a septum was placed under a nitrogen atmosphere. The flask was charged with histamine·2HCl (**2**·2HCl, MW: 184.07, 1.00 g, 5.43 mmol) and Et_3N (1.65 g, 16.3 mmol) in dry MeOH (20 ml) at room temperature and stirred for 30 min. After to this being added di-*tert*-butyl dicarbonate (Boc_2O, 2.37 g, 10.9 mmol), the mixture was stirred for 30 min. The reaction mixture was quenched with water and concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with $CHCl_3$/EtOH (30:1) gave **17a** (MW: 311.38, 1.41 g, 4.53 mmol, 83%) as a white powder; mp 129.5–130.5 °C, R_f 0.36 ($CHCl_3$/EtOH=30:1). ^1H NMR (300 MHz, $CDCl_3$): δ=8.00 (s, 1H), 6.82 (s, 1H), 4.99 (br s, 1H), 3.42 (t, 2H, *J*=6.3 Hz), 2.74 (t, 2H, *J*=6.6 Hz), 1.61 (s, 9H), 1.44 (s, 9H). IR: ν=3258 (m), 3130 (w), 2980 (m), 2933 (m), 1736 (s), 1700 (s), 1536 (m), 1391 (s), 1365 (s), 1274 (s), 1255 (s), 1155 (s), 1009 (s), 846 (m), 777 (m), 750 (m) cm^{-1}. FABMS: *m*/*z*=312 $(M+H)^+$, 256 $(M+H-t\text{-}Bu)^+$, 200 $(M+H-t\text{-}Bu_2)^+$, 156 $(M+H-Boc)^+$, 112 $(M+H-Boc_2)^+$, 57. FABHRMS: calcd for $C_{15}H_{26}O_4N_3$ $(M+H)^+$ *m*/*z*=312.1923; found, 312.1927.

4.1.5. N^{α}-(Benzyloxycarbonyl)histamine (17b). A 10 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, a Dimroth condenser, and a septum was charged with **2**·2HCl (30.0 mg, 0.163 mmol) and NaOMe (17.6 mg, 0.326 mmol) in MeOH (800 µl) and stirred at 50 °C for 2 h. Then the mixture was cooled to room temperature and then were added Na_2CO_3 (25.9 mg, 0.245 mmol), benzyl chloroformate (27.9 µl, 0.196 mmol), and H_2O (100 µl). This was stirred for 3 h. To the reaction mixture was added H_2O and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with $CHCl_3$/MeOH (1:1) gave **17b** (MW: 245.28, 14.2 mg, 59.1 µmol, 36%); R_f 0.63 ($CHCl_3$/MeOH=1:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.54 (s, 1H, NCH=N), 7.40–7.25 (m, 6H), 6.80 (s, 1H), 5.38 (s, 2H, $ArCH_2$), 3.37–3.45 (m, 2H), 2.72–2.82 (m, 2H). FABMS: *m*/*z*=246 $(M+H)^+$, 136, 91 $(Bn)^+$.

4.1.6. N^{α}-(Triphenylmethyl)histamine (17c). A 10 ml, round bottomed flask equipped with a magnetic stirrer bar, was charged with **2**·2HCl (15.0 mg, 81.5 µmol), Et_3N (24.9 µl, 179 µmol), and triphenylmethyl chloride (TrCl, 37.0 mg, 133 µmol) in $CHCl_3$ (600 µl). The mixture was stirred for 20 h at room temperature. Then this mixture was quenched with cold H_2O and extracted with $CHCl_3$. The combined organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with $CHCl_3$/EtOH (10:1) gave **17c** (MW: 353.49, 10.0 mg, 28.3 µmol, 35%); R_f 0.61 ($CHCl_3$/EtOH=10:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.40–7.10 (m, 16H), 6.50 (d, 1H, *J*=1.5 Hz), 2.72 (t, 2H, *J*=6.9 Hz), 2.39 (t, 2H, *J*=6.0 Hz).

4.1.7. N^{α}-Benzylhistamine (18a). A 100 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, a Dimroth condenser, and a septum was charged with **2**·2HCl (500 mg, 2.72 mmol) and NaOMe (293 mg, 5.43 mmol) in dry MeOH (20 ml) at room temperature. Then the mixture was stirred at 50 °C for 1 h. To the solution were added benzaldehyde (0.69 ml, 6.8 mmol) and MS3A (25 mg) at 0 °C and the mixture was stirred at 50 °C for 30 min. Then to the solution was added $NaBH_4$ (308 mg, 8.15 mmol) at −78 °C and the mixture was gradually warmed to room temperature. The reaction mixture was filtrated through a Celite® pad and concentrated in vacuo. The residue was diluted with water and extracted with $CHCl_3$. The combined extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was chromatographed on neutral silica gel. Elution with $CHCl_3$/EtOH (1:1) gave **18a** (MW: 202.13, 513 mg, 2.54 mmol, 93%) as a pale yellow oil; R_f 0.23 ($CHCl_3$/MeOH=30:1, 1% Et_3N). ^1H NMR (300 MHz, $CDCl_3$): δ=7.51 (d, 1H, *J*=0.9 Hz, NCH=N), 7.38–7.20 (m, 5H), 6.79 (d, 1H, *J*=0.6 Hz), 3.82 (s, 2H), 2.95 (pseudo t, 2H, *J*=6.0 Hz), 2.80 (pseudo t, 2H, *J*=6.0 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ=139.88, 134.32, 128.49, 128.13, 127.10, 53.81, 48.63, 26.11. IR: ν=3650–2200 (br s), 1567 (w), 1494 (m), 1453 (s), 1261 (w), 1104 (m), 938 (w), 820 (m), 733 (s), 697 (s), 662 (m), 624 (m) cm^{-1}. FABMS: *m*/*z*=202 $(M+H)^+$, 91 $(Bn)^+$. FABHRMS: calcd for $C_{12}H_{16}N_3$ $(M+H)^+$ *m*/*z*=202.1344; found, 202.1342.

4.1.8. N^{α}-(*p*-Methoxybenzyl)histamine (18b). In the same manner as described for **18a**, **18b** (MW: 231.29, 2.95 g, 12.8 mmol) was obtained from **2**·2HCl (2.50 g, 13.6 mmol) and *p*-methoxybenzylaldehyde (4.14 ml, 34.0 mmol) in a yield of 94% based on **2**·2HCl, as a pale yellow oil; R_f 0.29 ($CHCl_3$/MeOH=5:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.49 (s, 1H, NCH=N), 7.20 (pseudo d, 2H, *J*=8.7 Hz), 6.85 (pseudo d, 2H, *J*=8.7 Hz), 6.77 (s, 1H), 3.79 (s, 3H, OMe), 3.74 (s, 2H), 2.92 (pseudo t, 2H, *J*=7.2 Hz), 2.76 (pseudo t, 2H, *J*=7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ=158.80, 134.51, 132.15, 129.38, 113.89, 55.18, 53.10, 48.50, 26.26. IR: ν=3500–2200 (br s), 1611 (m), 1584 (w), 1509 (s), 1460 (m), 1441 (m), 1301 (w), 1244 (s), 1175 (m), 1104 (m), 1032 (m), 938 (w), 813 (s), 773 (w), 662 (w), 626 (w) cm^{-1}. FABMS: *m*/*z*=232 $(M+H)^+$, 121 $(PMB)^+$. FABHRMS: calcd for $C_{13}H_{18}O_3N_3$ $(M+H)^+$ *m*/*z*=232.1450; found, 232.1453.

4.1.9. N^{α}-Piperonylhistamine (18c). In the same manner as described for **18a**, **18c** (MW: 2456.28, 238 mg, 0.970 mmol) was obtained from **2**·2HCl (200 mg, 1.09 mmol) and

piperonal (408 mg, 2.72 mmol) in a yield of 89% based on **2**·2HCl, as a pale yellow oil; R_f 0.79 ($CHCl_3$/EtOH/28% NH_3 aq=3:3:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.54 (d, 1H, J=1.2 Hz, NCH=N), 6.82–6.79 (m, 2H), 6.78–6.72 (m, 2H), 5.95 (s, 2H, OCH_2O), 3.73 (s, 2H, $ArCH_2$), 2.92 (pseudo t, 2H, J=5.7 Hz), 2.79 (pseudo t, 2H, J=5.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ=147.69, 146.58, 133.62, 121.30, 108.63, 108.11, 100.89, 53.46, 48.41, 26.29. IR: ν=3500–2400 (br s), 1503 (m), 1489 (s), 1442 (m), 1246 (s), 1215 (m), 1103 (w), 1039 (m), 931 (w), 810 (w), 744 (s), 665 (w), 626 (w) cm^{-1}. FABMS: m/z=246 $(M+H)^+$, 135 $(piperonyl)^+$. FABHRMS: calcd for $C_{13}H_{16}O_2N_3$ $(M+H)^+$ m/z=246.1242; found, 246.1248.

4.1.10. N^{α}-(3,4-Methoxybenzyl)histamine (18d). In the same manner as described for **18a**, **18d** (MW: 245.28, 71.0 mg, 0.289 mmol, 11%) was obtained from **2**·2HCl (500 mg, 2.72 mmol) and 3,4-dimethoxybenzaldehyde (1.13 g, 6.79 mmol) in a yield of 11% based on **2**·2HCl, as a pale yellow oil; ^1H NMR (300 MHz, $CDCl_3$): δ=7.44 (s, 1H, NCH=N), 6.78 (s, 1H), 6.75 (s, 2H), 6.71 (s, 1H), 3.79 [s, 6H, $(OMe)_2$], 3.69 (s, 2H, $ArCH_2$), 2.86 (t, 2H, J=6.5 Hz), 2.73 (t, 2H, J=6.5 Hz).

4.1.11. Benzyl (2*S*,4*EZ*)-2-(benzyloxycarbonylamino)-6-(benzyl)[2-(4-imidazolyl)ethyl]amino-4-hexenoate (19a). In the similar manner as described for **19c**, **19a** (MW: 552.66, 671 mg, 1.22 mmol) was obtained from **14** (MW: 411.45, 1.00 g, 2.43 mmol) and N^{α}-benzylhistamine **18a** (587 mg, 2.91 mmol) in a yield of 50% based on **14**, as a yellow oil; R_f 0.29 ($CHCl_3$/MeOH=10:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.45 (s, 1H, NCH=N), 7.40–7.15 (m, 15H), 6.73 (s, 1H, NCH=C), 5.56–5.35 (m, 2H), 5.20–5.00 (m, 4H), 4.55–4.45 (m, 1H, H-2), 3.58 (s, 2H, NCH_2Ph), 3.00 (d, 2H, J=5.7 Hz, H-6), 2.80–2.40 (m, 6H). FABMS: m/z=553 $(M+H)^+$, 471, 279, 91 $(Bn)^+$. FABHRMS: calcd for $C_{33}H_{37}O_4N_4$ $(M+H)^+$ m/z=553.2815; found, 553.2819.

4.1.12. Benzyl (2*S*,4*EZ*)-2-(benzyloxycarbonylamino)-6-[2-(4-imidazolyl)ethyl](*p*-methoxybenzyl)amino-4-hexenoate (19b). In the similar manner as described for **19c**, **19b** (MW: 582.69, 262 mg, 449 μmol) was obtained from **14** (MW: 411.45, 200 mg, 0.486 mmol) and N^{α}-(*p*-methoxybenzyl)histamine **18b** (135 mg, 533 μmol) in a yield of 92% based on **14**, as a yellow oil; R_f 0.50 ($CHCl_3$/EtOH=10:1). ^1H NMR (300 MHz, $CDCl_3$): δ=7.46 (s, 1H, NCH=N), 7.36–7.28 (m, 10H), 7.16 (d, 2H, J=8.4 Hz), 6.83 (d, 2H, J=8.7 Hz), 6.74 (s, 1H, NCH=C), 5.58–5.36 (m, 3H), 5.20–5.00 (m, 4H), 4.54–4.45 (m, 1H), 3.78 (s, 3H, OMe), 3.52 (s, 2H), 2.99 (d, 2H, J=5.4 Hz), 2.68 (dd, 2H, J=16.5, 5.4 Hz), 2.56 (t, 2H, J=5.7 Hz), 2.49 (t, 2H, J=6.6 Hz). FABMS: m/z=583 $(M+H)^+$, 501, 230, 121 $(PMB)^+$, 91 $(Bn)^+$. FABHRMS: calcd for $C_{34}H_{39}O_5N_4$ $(M+H)^+$ m/z=583.2921; found, 583.2922.

4.1.13. Benzyl (2*S*,4*EZ*)-2-(benzyloxycarbonylamino)-6-[2-(4-imidazolyl)ethyl](piperonyl)amino-4-hexenoate (19c). A 20 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter, and a septum was placed under a nitrogen atmosphere. The flask was charged with **14** (MW: 411.45, 540 mg, 1.31 mmol), $Pd_2(dba)_3$ (60.0 mg, 65.6 μmol), and PPh_3 (18.2 mg, 69.4 μmol) in dry THF, and stirred for 15 min at room temperature. To the suspension was added N^{α}-piperonylhistamine **19c** (555 mg, 1.58 mmol), warmed up to 50 °C, and the mixture was stirred for 1 h. The reaction mixture was filtrated through a Celite® pad, added water, and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with $CHCl_3$/EtOH (20:1) gave **19c** (MW: 507.27, 550 mg, 0.922 mmol, 71% based on **14**) as a yellow oil; R_f 0.57 ($CHCl_3$/EtOH=30:1), $[\alpha]_D^{24}$ −0.703 (c 1.28, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ=7.47 (s, 1H, NCH=N), 7.40–7.25 (m, 10H), 6.75–6.65 (m, 4H), 5.91 (s, 2H, OCH_2O), 5.64–5.36 (m, 3H), 5.22–5.00 (m, 4H), 4.54–4.45 (m, 1H, H-2), 3.47 (d, 2H, NCH_2Ar), 2.98 (d, 2H, J=5.4 Hz, NCH_2C=), 2.78–2.40 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.79, 155.91, 147.76, 146.66, 136.19, 135.19, 134.30, 132.84, 131.73, 128.67, 128.56, 128.36, 128.23, 128.05, 127.07, 121.97, 109.19, 107.93, 100.86, 67.20, 66.93, 57.85, 54.78, 53.66, 52.56, 35.35, 23.18. IR: ν=3493–2100 (br s), 1717 (s), 1500 (s), 1487 (s), 1454 (m), 1440 (s), 1342 (w), 1244 (s), 1214 (s), 1187 (m), 1093 (w), 1038 (s), 975 (w), 930 (w), 810 (w), 749 (s), 696 (s), 665 (w) cm^{-1}. FABMS: m/z=597 $(M+H)^+$, 154, 136, 91 $(Bn)^+$, 73. FABHRMS: calcd for $C_{34}H_{37}O_6N_4$ $(M+H)^+$ m/z=597.2713; found, 597.2718.

4.1.14. (*S*)-2-Amino-9-(4-imidazolyl)-7-azanonanoic acid (gizzerosine, 1). A 30 ml, two-necked round bottomed flask equipped with a magnetic stirrer bar, an N_2 inlet adapter, and a septum was placed under a hydrogen atmosphere. The flask was charged with **19c** (MW: 596.67, 54.4 mg, 91.1 μmol) and $Pd(OH)_2$/C (110 mg) in 5 ml of EtOH/THF/H_2O (3:1:1). The mixture was stirred for 5 h at room temperature. Then this was filtrated through a Celite® pad and the filtrate was concentrated in vacuo. The resulting solid was purified through Sephadex® LH-20 column. Elution with MeOH/H_2O (1:1) gave gizzerosine **1** (MW: 240.30, 10.4 mg, 0.433 mmol, 47%) as a white solid. This solid was dissolved in 1 M HCl aq and stirred for 5 min. Then this solution was concentrated in vacuo. This resulting white amorphous solid was used for analysis; R_f 0.09 ($CHCl_3$/MeOH/28% NH_3 aq=3:3:1), mp 250–251 °C (dec) [lit.[6] 251–252 °C (dec)]; $[\alpha]_D^{22}$ +9.45 (c 0.555, H_2O) {lit.[6] $[\alpha]_D^{22}$ +10.3 (c 1.28, H_2O)}. ^1H NMR (300 MHz, D_2O): δ=8.30 (br s, 1H), 7.26 (br s, 1H), 3.75 (t, 1H, J=6.0 Hz), 3.37 (pseudo t, 2H, J=7.2 Hz), 3.11 (pseudo t, 4H, J=6.6 Hz), 1.89 (m, pseudo q, 2H, J=7.4 Hz), 1.74 (quint, 2H, J=7.5 Hz), 1.60–1.35 (m, 2H). ^{13}C NMR (75 Hz, D_2O): δ=175.08, 135.69, 131.45, 116.93, 54.99, 47.72, 46.86, 30.38, 25.65, 22.91, 22.04. IR: ν=3300–2300 (br s), 3116 (s), 2786 (s), 2450 (m), 1634 (s), 1601 (s), 1522 (s), 1462 (s), 1395 (s), 1348 (m), 1329 (m), 1235 (w), 1052 (w), 957 (w), 839 (w), 794 (w), 719 (w), 623 (m) cm^{-1}. FABMS: m/z=241 $(M+H)^+$, 207, 185, 93, 75, 57, 45. FABHRMS: calcd for $C_{11}H_{21}N_4O_2$ $(M+H)^+$ m/z=241.1665; found, 241.167. These spectral data are in good agreement with those reported.[6]

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