Amino acids and peptides. Part 45.[1] Development of a new N^{π}-protecting group of histidine, N^{π}-(1-adamantyloxymethyl)-histidine, and its evaluation for peptide synthesis†,[2]

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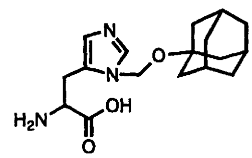
N^{π}-(1-Adamantyloxymethyl)histidine, His(N^{π}-1-Adom), is prepared and its properties are examined. The 1-Adom group can be easily removed by trifluoroacetic acid and it is stable to 20% piperidine–dimethylformamide and 1 mol dm^{-3} NaOH. His(N^{π}-1-Adom) derivatives can suppress racemization during coupling reactions. His(N^{π}-1-Adom) can be used in solid-phase peptide synthesis in combination with fluoren-9-ylmethoxycarbonyl as an N^{α}-protecting group. Thyrotropin-releasing hormone is successfully synthesized by using His(N^{π}-1-Adom).

Introduction

Various kinds of protecting groups for the imidazole nitrogen of histidine residues have been developed in peptide synthesis. It is well known that protecting groups on the π-nitrogen of the imidazole function are more effective than those on the τ-nitrogen in preventing racemization during peptide synthesis. Previously, N^{π}-benzyloxymethylhistidine, His(N^{π}-Bom), was developed.[3] The Bom group is stable to trifluoroacetic acid (TFA) and 1 mol dm^{-3} NaOH and is removable by hydrogenation over Pd catalyst or HF.[4] Therefore, His(N^{π}-Bom) can be used for peptide synthesis by *tert*-butoxycarbonyl (Boc) strategy in both solution and solid-phase methods. N^{π}-(*tert*-Butoxymethyl)histidine, His(N^{π}-Bum), was also developed in order to suppress racemization.[5] The Bum group can be removed by TFA and is stable under alkaline conditions. Therefore, His(N^{π}-Bum) is applied in peptide synthesis in combination with a fluoren-9-ylmethoxycarbonyl (Fmoc) group as the N^{α}-protecting group in a solid-phase method. However, it was reported that Fmoc-His(N^{π}-Bum)-OH had poor solubility in dichloromethane (DCM).[6] Under these circumstances, our studies were directed to the development of novel N^{π}-protecting groups with the objective of suppressing side-reactions, preventing racemization and increasing the solubility of His-containing peptide intermediates in organic solvents. Previously, it was reported that a 1-adamantyl ester group could be removed by TFA and is stable to 20% piperidine–dimethylformamide (DMF)[7] and that adamantyl ester derivatives exhibited high solubility in organic solvents.[8] These results provided us with an idea to design a novel N^{π}-protecting group.



Structure of H-His(N^{π}-1-Adom)-OH

In this paper, we describe the synthesis of His(N^{π}-1-Adom), an examination of its properties and its application to the synthesis of thyrotropin-releasing hormone (TRH).

According to Scheme 1, adamantan-1-ol (1-Ada-OH) reacted with dimethyl sulfoxide (DMSO) and acetic anhydride to give 1-adamantyloxymethyl methyl sulfide (1-Ada-OCH_2SCH_3),[9] which was converted to 1-adamantyloxymethyl chloride (1-Adom-Cl) by treating with sulfuryl dichloride. The 1-Adom-Cl is involatile and much easier to purify compared with Bum-Cl.[5] On the other hand, Z-His-OMe¶ was acetylated with acetic anhydride to give Z-His(N^{τ}-Ac)-OMe.[10] Z-His(N^{τ}-Ac)-OMe was reacted with 1-Adom-Cl, followed by treatment with $NaHCO_3$, to afford Z-His(N^{π}-1-Adom)-OMe in good yield. Z-His(N^{π}-1-Adom)-OMe was saponified with 1 mol dm^{-3} NaOH, followed by hydrogenation over Pd catalyst to afford H-His(N^{π}-1-Adom)-OH.

Next, stability and susceptibility of the 1-Adom group to various acids and bases were examined by measuring the regenerated His residue and the parent molecule, H-His(N^{π}-1-Adom)-OH, by an amino acid analyser, and the results are summarized in Table 1.

The N^{π}-1-Adom group was easily removed by TFA and was stable to 1 mol dm^{-3} NaOH and 20% piperidine–DMF at room temperature up to 48 h. Therefore, His(N^{π}-1-Adom) can be used for peptide synthesis in combination with an Fmoc group as the N^{α}-protecting group. Fmoc-His(N^{π}-1-Adom)-OH was prepared from His(N^{π}-1-Adom) and Fmoc-OSu (succinimidyl ester) in good yield and it is more soluble in organic solvents than is Fmoc-His(N^{π}-Bum)-OH.

Next, the efficiency of the N^{π}-1-Adom group in the prevention of side-chain-induced racemization was examined. Z-D-His(N^{π}-1-Adom)-OH was prepared by the same method as described above, and was coupled with H-L-Phe-OMe to give Z-D-His(N^{π}-1-Adom)-L-Phe-OMe. Z-D-His(N^{π}-1-Adom)-L-Phe-

† The customary L configuration for amino acid residues is omitted; only D isomers are indicated. Abbreviations used in this report for amino acids, peptides and their derivatives are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature: *Biochemistry*, 1966, **5**, 2485; 1967, **6**, 362; 1972, **11**, 1726. The following additional abbreviations are used: AcOEt, ethyl acetate; DMF, dimethylformamide; TFA, trifluoroacetic acid; Z, benzyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Fmoc, fluoren-9-ylmethoxycarbonyl; Fmoc-OSu, fluoren-9-ylmethyl *N*-succinnimidyl carbonate; 1-Adom, 1-adamantyloxymethyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DPPA, diphenylphosphoryl azide; NMM, *N*-methylmorpholine.

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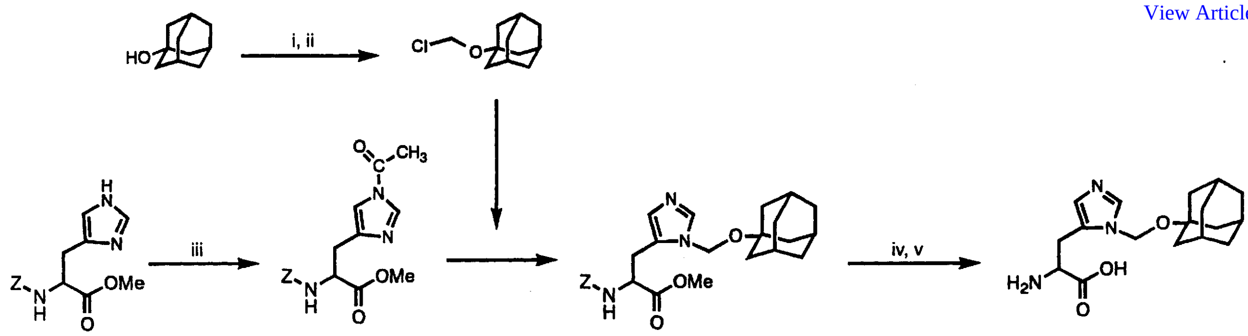
¶ Z = Benzyloxycarbonyl.



Scheme 1 Synthetic scheme for H-His(N$^{\pi}$-1-Adom)-OH. *Reagents:* i, DMSO, Ac_2O; ii, SO_2Cl_2; iii, Ac_2O; iv, NaOH; v, H_2/Pd.

Table 1 Stability and susceptibility of H-His(N$^{\pi}$-1-Adom)-OH

	Cleavage (%)						
Conditions	15 min	30 min	45 min	60 min	12 h	24 h	48 h
TFA (200 mol equiv., 5 mol equiv. anisole)	73.9	88.1	100	100			
25% HBr–AcOH (200 mol equiv.)	100						
0.1 mol dm^{-3} HCl (300 mol equiv.)	0	0	0	0	0	0	0
1 mol dm^{-3} NaOH (100 mol equiv.)	0	0	0	0	0	0	0
10% NH_2NH_2 (200 mol equiv.)	0	0	0	0	0	0	0
10% Et_3N–water + dioxane (50 mol equiv.)	0	0	0	0	0	0	0
10% NMM (50 mol equiv.)	0	0	0	0	0	0	0
20% Piperidine–DMF (200 mol equiv.)	0	0	0	0	0	0	0

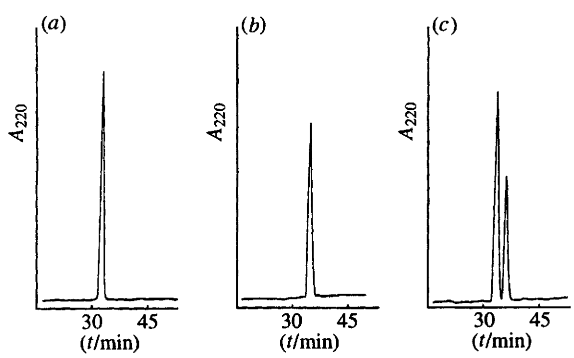


Fig. 1 HPLC profiles of (*a*) Z-D-His(N$^{\pi}$-1-Adom)-Phe-OMe, (*b*) Z-L-His(N$^{\pi}$-1-Adom)-Phe-OMe and (*c*) co-injection. Column and solvent system are described in Experimental section.

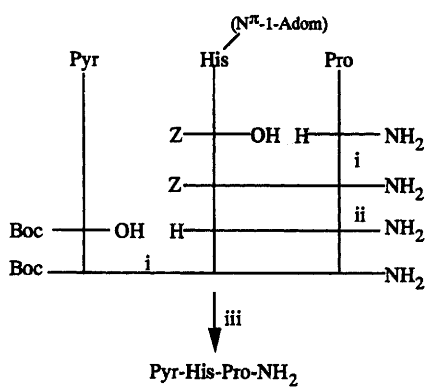


Fig. 2 Synthetic scheme for TRH: i, BOP (1.2 mol equiv.), HOBt (1.2 mol equiv.), NMM (1.8 mol equiv.); ii, H_2/Pd in MeOH (2 mol equiv. HCl); iii, TFA (2 mol equiv. thioanisole)

OMe was completely separated from Z-L-His(N$^{\pi}$-1-Adom)-L-Phe-OMe on HPLC as shown in Fig. 1. Therefore, this sequence was employed for a model study on racemization. Z-L-His(N$^{\pi}$-1-Adom)-OH was coupled with H-L-Phe-OMe by dicyclohexylcarbodiimide (DCC), DCC–*N*-hydroxybenzotriazole (HOBt), benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP),[11] 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-

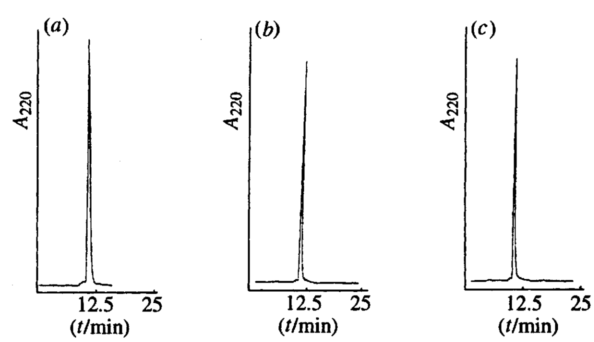


Fig. 3 HPLC profile of (*a*) synthetic TRH, (*b*) authentic TRH and (*c*) co-injection. Column and solvent system are described in the Experimental section.

tetramethyluronium hexafluorophosphate (HBTU)[12] or diphenylphosphoryl azide (DPPA)[13] and then the crude product was analysed by HPLC. The results summarized in Table 2 show that formation of the racemate was particularly low in all the coupling methods so far examined.

Finally, thyrotropin-releasing hormone (TRH) was synthesized by using Z-His(N$^{\pi}$-1-Adom)-OH as illustrated in Fig. 2. Z-His(N$^{\pi}$-1-Adom)-OH was coupled with H-Pro-NH_2 in the presence of BOP reagent to give Z-His(N$^{\pi}$-1-Adom)-Pro-NH_2. The Z group was removed by hydrogenation over Pd catalyst in the presence of 2 mol. equiv. of HCl (1 mol dm^{-3} HCl–1,4-dioxane). The resultant amine was coupled with Boc-Pyr-OH (pyroglutamic acid) by BOP reagent to afford Boc-Pyr-His(N$^{\pi}$-1-Adom)-Pro-NH_2. The protected tripeptide, purified by silica gel column chromatography, was treated with TFA to afford TRH. The synthetic TRH exhibited a single peak at the same position as authentic TRH without purification, as shown in Fig. 3.

Thus, we succeeded in developing H-His(N$^{\pi}$-1-Adom)-OH. The 1-Adom group was easily removed by TFA and was stable under alkaline conditions. The newly synthesized Fmoc-His(N$^{\pi}$-1-Adom)-OH exhibited high solubility in organic solvent, indicating that His(N$^{\pi}$-1-Adom) derivatives could be successfully employed in a solid-phase method.

Experimental

Mps were determined with a Yanagimoto micro apparatus and are uncorrected. On TLC (Kieselgel G, Merck), R_f1, R_f2 and R_f3 values refer to the systems $CHCl_3$–MeOH–AcOH (90:8:2); $CHCl_3$–MeOH–water (8:3:1, lower phase); and hexane–diethyl ether (15:1), respectively. Optical rotations were measured with an automatic DIP-360 polarimeter (Japan Spectroscopic Co. Ltd., Japan), and $[\alpha]_D$ values are in units of 10^{-1} deg cm^2 g^{-1}. ^1H (400, 500 MHz) and ^{13}C (100, 125 MHz) NMR spectra were recorded on either a Bruker DPX 400 or an ARX500 spectrometer. Chemical shift values are expressed as ppm downfield from tetramethylsilane used as an internal standard (δ-value). *J* Values are given in Hz. Attribution of ^{13}C signals was made also with the aid of a distortionless enhancement by polarisation transfer (DEPT) experiment, and multiplicities are indicated by the usual symbols. Mass spectra were measured with a Hitachi M-200 mass spectrometer using EI techniques. Amino acid compositions of acid hydrolysates (6 mol dm^{-3} HCl; 110 °C; 20 h) were determined with an amino acid analyser, K-202 SN (Kyowa Seimitsu Co.). On HPLC analysis, eluent A (0.05% aq. TFA) and eluent B (0.05% TFA in MeCN) were used. Light petroleum refers to that fraction with distillation range 30–60 °C.

1-Adamantyloxymethyl methyl sulfide [9]

A mixture of DMSO (80 cm^3), Ac_2O (20 cm^3) and AcOH (10 cm^3) was stirred at room temperature for 6 h. To the above solution were added adamantan-1-ol (5.0 g, 33.0 mmol) and Ac_2O (40 cm^3) and the mixture was stirred at room temperature for 40 h. After addition of 3 mol dm^{-3} aq. NaOH (250 cm^3) to the above mixture, the oily material was extracted with hexane. The extract was washed successively with 1 mol dm^{-3} NaOH and water, and evaporated down. The residue, in 3.0 mol dm^{-3} aq. NaOH (100 cm^3), was stirred overnight and extracted with hexane. The extract was washed with water, dried over Na_2SO_4, and evaporated down. The residue was applied to a silica gel column (3 × 25 cm), equilibrated and eluted with hexane–diethyl ether (6:1). The eluent containing the desired sulfide was collected and concentrated to give 1-adamantyloxymethyl methyl sulfide as an oil (5.64 g, 80.9%), R_f3 0.40 (Found: C, 67.6; H, 9.45. Calc. for $C_{12}H_{20}OS$: C, 67.9; H, 9.49%); δ_H(400 MHz; $CDCl_3$) 1.5–1.8 (m, 6 H, adamantyl CH_2), 1.8–2.0 (m, 6 H, adamantyl CH_2), 2.15 (m, 3 H, adamantyl CH), 2.18 (s, 3 H, SCH_3) and 4.57 (s, 2 H, OCH_2S).

1-Adamantyloxymethyl chloride [14]

A solution of SO_2Cl_2 (2.88 g, 21.3 mmol) in CH_2Cl_2 (16 cm^3) was added dropwise to a solution of 1-adamantyloxymethyl methyl sulfide (3.48 g, 16.4 mmol) in CH_2Cl_2 (15 cm^3) over a period of 10 min. The reaction mixture was stirred for 20 min at room temperature. The solvent was removed under reduced pressure below 15 °C to give 1-adamantyloxymethyl chloride (3.29 g, quantitative), which was used without further purification; δ_H(400 MHz; $CDCl_3$) 1.5–1.8 (m, 6 H, adamantyl CH_2), 1.8–2.0 (m, 6 H, adamantyl CH_2), 2.15 (m, 3 H, adamantyl CH) and 4.61 (s, 2 H, OCH_2Cl).

Z-His-OMe [15]

To a mixture of H-His-OMe•2HCl [16] (6.05 g, 25.0 mmol) in $CHCl_3$ (50 cm^3) containing Et_3N (7.0 cm^3, 50.0 mmol) were added Z-Cl (6.7 cm^3, 30.0 mmol) and Et_3N (4.2 cm^3, 30.0 mmol) alternately at 0 °C during 20 min. The reaction mixture was stirred at room temperature for an additional 30 min. After removal of the solvent, the residue was dissolved in MeOH (60 cm^3) containing conc. NH_3 (2.0 cm^3) and the reaction mixture was stirred at room temperature for 1 h. After removal of the solvent, the residue was dissolved in 1 mol dm^{-3} HCl, which was washed with $CHCl_3$. The pH of the aqueous layer was adjusted with Na_2CO_3 to 8 using a pH meter. The oily material was extracted with AcOEt. The extract was dried over Na_2SO_4 and evaporated down. Light petroleum was added to the residue to afford crystals (7.0 g, 92.7%), mp 74–76 °C; $[\alpha]_D^{25}$ −15.2 (*c* 1.0, MeOH) {lit.,[17] mp 75–77 °C; $[\alpha]_D$ −12.1 (*c* 1, MeOH)}; δ_H(400 MHz; $CDCl_3$) 3.01–3.13 (m, 2 H, CH_2CH), 3.65 (s, 3 H, OCH_3), 4.57–4.61 (m, 1 H, $CHCH_2$), 5.07 (s, 2 H, $PhCH_2$), 6.27 (d, 1 H, *J* 6.0, CONH), 6.75 (s, 1 H, NH^{im}), 7.25–7.34 (m, 5 H, Ph), 7.48 (s, 1 H, 5^{im}-H) and 8.53 (s, 1 H, 2^{im}-H) (Found: C, 59.2; H, 5.52; N, 13.8. Calc. for $C_{15}H_{17}N_3O_4$: C, 59.4; H, 5.65; N, 13.9%).

Z-His(N^τ-Ac)-OMe

Z-His-OMe (5.02 g, 14.6 mmol) was dissolved in Ac_2O (17.4 cm^3). After 5 min, the solvent was removed *in vacuo*. The residue was triturated with dry diethyl ether. The precipitate was collected, dissolved in a small amount of $CHCl_3$ and retriturated with dry diethyl ether. The precipitate was collected and dried *in vacuo* over KOH pellets to give *Z-His(N^τ-Ac)-OMe* (4.13 g, 82%), mp 99–101 °C; $[\alpha]_D^{25}$ +35.0 (*c* 1.0, $CHCl_3$); R_f1 0.49, R_f2 0.75; MS (SIMS) *m/z* 346 (M + 1); δ_H(400 MHz; $CDCl_3$) 2.52 (s, 3 H, $COCH_3$), 3.05–3.13 (m, 2 H, CH_2CH), 3.72 (s, 3 H, OCH_3), 4.65–4.69 (m, 1 H, $CHCH_2$), 5.10 (s, 2 H, $PhCH_2O$), 6.07 (d, 1 H, *J* 8, CONH), 7.23 (s, 1 H, 5^{im}-H), 7.27–7.35 (m, 5 H, Ph) and 8.01 (s, 1 H, 2^{im}-H); δ_C(100 MHz; $CDCl_3$) 22.64 (p, CH_3CO), 30.09 (s, His β-C), 52.47 (p, OCH_3), 53.46 (t, His α-C), 66.92 (s, $PhCH_2O$), 113.78 (t, 5^{im}-C), 128.06, 128.08, 128.13 and 128.49 (t, 5 × C, Ph), 136.35 (t, 2^{im}-C), 139.53 (q, 4^{im}-C), 155.99 (q, $PhCH_2OCO$) and 166.15 and 171.87 (q, CO_2Me, $COCH_3$) (Found: C, 58.4; H, 5.4; N, 12.1. $C_{17}H_{19}N_3O_5{\cdot}1/4H_2O$ requires C, 58.4; H, 5.62; N, 12.0%).

Z-His(N^π-1-Adom)-OMe

A solution of Z-His(N^τ-Ac)-OMe (3.45 g, 10 mmol) and 1-Adom-Cl (3.0 g, 15 mmol) in CH_2Cl_2 (20 cm^3) was stirred at room temperature for 4 h. After removal of the solvent, the residue was triturated with dry diethyl ether to give Z-His(N^π-1-Adom)-OMe•HCl, mp 88–90 °C; $[\alpha]_D^{25}$ −27.1 (*c* 1.0, MeOH); R_f1 0.24, R_f2 0.53; MS (SIMS) *m/z* 467 (M^+); δ_H(400 MHz; $CDCl_3$) 1.5–1.7 (m, 6 H, CH_2, adamantyl), 1.7–1.9 (m, 6 H, CH_2, adamantyl), 2.0–2.3 (m, 3 H, CH, adamantyl), 3.1–3.3 (m, 2 H, CH_2CH), 3.74 (s, 3 H, OCH_3), 4.5–4.9 (m, 1 H, $CHCH_2$), 5.04 (s, 2 H, $PhCH_2O$), 5.70 (s, 2 H, NCH_2O), 6.33 (d, 1 H, *J* 7, CONH), 7.23 (s, 1 H, 5^{im}-H), 7.29 (s, 5 H, Ph) and 9.49 (s, 1 H, 2^{im}-H).

A solution of Z-His(N^π-1-Adom)-OMe obtained above, in $CHCl_3$ (50 cm^3), was treated successively with 5% aq. $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated down. The residue, in $CHCl_3$ (5 ml), was applied to a silica gel column (3 × 25 cm), equilibrated and eluted with $CHCl_3$–MeOH (30:1). The eluent containing the desired product was collected and the solvent was removed *in vacuo* to give *Z-His(N^π-1-Adom)-OMe* as an oily material (3.9 g, 83.4%), $[\alpha]_D^{25}$ −2.2 (*c* 1.0, MeOH); R_f1 0.24, R_f2 0.53; MS (SIMS) *m/z* 467 (M^+); δ_H(400 MHz; $CDCl_3$) 1.5–1.7 (m, 6 H, CH_2, adamantyl), 1.7–1.9 (m, 6 H, CH_2, adamantyl), 2.0–2.3 (m, 3 H, CH, adamantyl), 3.1–3.3 (m, 2 H, CH_2CH), 3.71 (s, 3 H, OCH_3), 4.5–4.9 (m, 1 H, $CHCH_2$), 5.06 (s, 2 H, $PhCH_2O$), 5.25 (s, 2 H, NCH_2O), 6.33 (d, 1 H, *J* 9.0, NH), 6.81 (s, 1 H, 5^{im}-H), 7.29 (s, 5 H, Ph) and 7.48 (s, 1 H, 2^{im}-H) (Found: C, 65.0; H, 7.0; N, 8.8. $C_{26}H_{33}N_3O_5{\cdot}2/3H_2O$ requires C, 65.1; H, 7.21; N, 8.76%).

Z-His(N^π-1-Adom)-OH

A solution of Z-His(N^π-1-Adom)-OMe (3.40 g, 7.52 mmol) in MeOH (24 cm^3) containing 1 mol dm^{-3} aq. NaOH (7.6 cm^3) was stirred at room temperature for 30 min. After removal of the solvents below 15 °C, the residue was dissolved in water (100 cm^3). The pH of the solution was adjusted with 1 mol dm^{-3} HCl to 7.0–7.5 (pH 7.2 is the most preferable) using a pH meter to give the *title acid* as a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo* (2.83 g, 83.0%), mp 96–98 °C; $[\alpha]_D^{25}$ −2.7 (*c* 1.0, MeOH); R_f2 0.44; MS (SIMS) *m*/*z* 454 (M^+ + 1); δ_H(400 MHz; $CDCl_3$) 1.53–1.69 (m, 12 H, adamantyl), 2.12 (s, 3 H, CH, adamantyl), 3.24–3.37 (m, 2 H, CH_2CH), 4.38–4.41 (m, 1 H, $CHCH_2$), 5.10 (s, 2 H, PhCH_2O), 5.37 (s, 2 H, NCH_2O), 6.14 (d, 1 H, *J* 4.15, CONH), 6.99 (s, 1 H, 5^{im}-H), 7.26–7.35 (m, 5 H, Ph) and 8.17 (s, 1 H, 2^{im}-H); δ_C(100 MHz; $CDCl_3$) 26.6 (s, His β-C), 30.5 (t, 3 × C, adamantyl), 36.0 and 41.5 (s, 6 × C, adamantyl), 55.0 (t, His α-C), 66.7 (s, Z), 68.7 (s, NCH_2O), 76.0 (q, adamantyl), 121.2 (t, 5^{im}-C), 127.9, 128.1 and 128.5 (t, 5 × C, Ph), 129.7 (q, 4^{im}-C), 134.4 (t, 2^{im}-C), 136.5 (q, Ph), 156.0 (q, CO, Z) and 173.3 (q, CO_2H) (Found: C, 63.2; H, 6.8; N, 8.9. $C_{25}H_{31}N_3O_5$·6/5H_2O requires C, 63.2; H, 7.09; N, 8.84%).

H-His(N^π-1-Adom)-OH

Z-His(N^π-1-Adom)-OH (1.36 g, 3.00 mmol) in MeOH (20 cm^3) was hydrogenated over Pd catalyst for 6 h. After removal of Pd and the solvent, diethyl ether was added to the residue to give a precipitate, which was collected by filtration and dried over KOH pellets *in vacuo* to give the *title acid* (0.76 g, 80.0%), mp 228–229 °C (decomp.); $[\alpha]_D^{25}$ −8.3 (*c* 1.0, MeOH); R_f2 0.1; MS (SIMS) *m*/*z* 320 (M^+ + 1); δ_H(400 MHz; $CDCl_3$ + CD_3OD) 1.64–1.72 (m, 6 H, CH_2, adamantyl), 1.82–1.87 (m, 6 H, CH_2, adamantyl), 2.19 (m, 3 H, CH, adamantyl), 3.09–3.14 and 3.41–3.45 (m, 2 H, CH_2CH), 3.85–3.87 (m, 1 H, $CHCH_2$), 5.43–5.48 (m, 2 H, NCH_2O), 6.93 (s, 1 H, 5^{im}-H) and 7.68 (s, 1 H, 2^{im}-H) (Found: C, 63.7; H, 7.9; N, 13.2. $C_{17}H_{25}N_3O_3$ requires C, 63.9; H, 7.89; N, 13.2%).

Fmoc-His(N^π-1-Adom)-OH

To an ice-cooled solution of H-His(N^π-1-Adom)-OH (0.63 g, 2.0 mmol) in 10% aq. Na_2CO_3 (5 cm^3) was slowly added a solution of Fmoc-OSu (0.75 g, 2.2 mmol) in DMF (5 cm^3). The reaction mixture was stirred at room temperature for an additional 10 min. After dilution of the reaction mixture with water (50 cm^3), the diluted solution was washed successively with diethyl ether and AcOEt. The pH of the water layer was adjusted with 1 mol dm^{-3} HCl to 6 using a pH meter to afford a precipitate, which was collected by filtration and dried *in vacuo* over KOH pellets to yield the *title compound* (0.97 g, 90.9%), mp 160–161 °C; $[\alpha]_D^{25}$ +5.4 (*c* 1.0, MeOH); R_f2 0.50; δ_H(400 MHz; $CDCl_3$) 1.48–1.58 (m, 12 H, CH_2, adamantyl), 2.07 (s, 3 H, CH, adamantyl), 3.28–3.37 (m, 2 H, His β-CH_2), 4.19–4.22 (m, 1 H, 9-H, Fmoc), 4.28–4.44 (m, 2 H, CH_2O, Fmoc), 4.44 (m, 1 H, His α-CH), 5.37 (s, 2 H, OCH_2N), 6.28 (m, 1 H, CONH), 7.03 (s, 1 H, 5^{im}-H), 7.26–7.31 (m, 2 H, 2-, 7-H, Fmoc), 7.36–7.39 (m, 2 H, 3-, 6-H, Fmoc), 7.57–7.61 (m, 2 H, 1-, 8-H, Fmoc), 7.74–7.75 (m, 2 H, 4-, 5-H, Fmoc), 8.12 (s, 1 H, 2^{im}-H) and 10.05 (s, 1 H, COOH); δ_C(100 MHz; $CDCl_3$) 26.61 (s, His β-CH_2), 30.43 (t, 3 × C, adamantyl), 35.93 (s, 3 × C, adamantyl), 41.51 (s, 3 × C, adamantyl), 47.17 (t, 9-C, Fmoc), 55.00 (t, His α-C), 67.05 (s, CH_2O, Fmoc), 68.59 (s, CH_2N, Adom), 75.78 (q, C–O, adamantyl), 120.00 (t, 2 × C, 4-, 5-C, Fmoc), 121.92 (t, 5^{im}-C), 125.09 and 125.23 (t, 2 × C, 1-, 8-C, Fmoc), 127.09 and 127.14 (t, 2 × C, 2-, 7-C, Fmoc), 127.74 (t, 2 × C, 3-, 6-C, Fmoc), 129.61 (q, 4^{im}-C), 134.68 (t, 2^{im}-C), 141.29 (q, 2 × C, 4a-, 4b-C, Fmoc), 143.74 and 144.09 (q, 2 × C, 8a-, 9a-C, Fmoc), 156.04 (q, C=O, His) and 174.35 (q, C=O, Fmoc) (Found: C, 69.7; H, 6.5; N, 7.7%. $C_{32}H_{35}N_3O_5$·1/2H_2O requires C, 69.8; H, 6.59; N, 7.63%).

Examination of stability and susceptibility of H-His(N^π-1-Adom)-OH to acids and bases

H-His(N^π-1-Adom)-OH (6.4 mg, 0.02 mmol) was dissolved in an acid or a base (Table 1) at room temperature. Samples for amino acid analysis were prepared as follows. (1) In the case of acidic solution: 0.01 cm^3 of each solution was diluted with water or 0.02–0.5 mol dm^{-3} aq. Na_2CO_3 to adjust the pH to ~2. This solution (0.01–0.02 cm^3) was injected into the amino acid analyser and the amount of regenerated His residue and intact H-His(N^π-1-Adom)-OH was measured as a function of the time. (2) In the case of basic solution: 0.01 cm^3 of each solution was diluted with 0.1–1 mol dm^{-3} aq. HCl (0.09 cm^3) to adjust the pH to ~2 using pH test paper. This solution (0.01–0.02 cm^3) was used for amino acid analysis.

Z-His(N^π-1-Adom)-Phe-OMe

Z-His(N^π-1-Adom)-OH (200 mg, 0.44 mmol), H-Phe-OMe·HCl (125 mg, 0.57 mmol) and HOBt (87.5 mg, 0.57 mmol) were dissolved in DMF (5 cm^3) containing Et_3N (0.08 cm^3, 0.57 mmol). BOP reagent (250 mg, 0.57 mmol) and Et_3N (0.08 cm^3, 0.57 mmol) were added to the above cold solution and the reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed successively with 10% aq. citric acid, 5% aq. Na_2CO_3 and water, dried over Na_2SO_4, and evaporated down. Light petroleum was added to the residue to afford *Z-His*(*N^π*-1-*Adom*)-*Phe-OMe* as an amorphous powder (221 mg, 80%); $[\alpha]_D^{25}$ −5.6 (*c* 1.0, MeOH); R_f1 0.61, R_f2 0.80; HPLC, Column: Cosmosil Pack 5C 18-AR (4.6 × 250 mm), eluent: A:B, 69:31 to 55:45 for 50 min and to 69:31 for 5 min, t_R 34.476 min (Found: C, 65.4; H, 7.0; N, 7.9%. $C_{35}H_{42}N_4O_6$·1.5H_2O requires C, 65.4; H, 6.92; N, 8.25%).

Z-D-His(N^τ-Ac)-OMe

The *title compound* was prepared in 84.3% yield by the procedure for the synthesis of Z-His(N^τ-Ac)-OMe, mp 98–100 °C; $[\alpha]_D^{25}$ −37.1 (*c* 1.0, $CHCl_3$); R_f1 0.60 (Found: C, 59.1; H, 5.5; N, 12.2. $C_{17}H_{19}N_3O_5$ requires C, 58.8; H, 5.48; N, 12.1%).

Z-D-His(N^π-1-Adom)-OMe

The *title compound* was prepared in 84.0% yield by the procedure for the synthesis of Z-His(N^π-1-Adom)-OMe, oily material; $[\alpha]_D^{25}$ +2.8 (*c* 1.0, MeOH); R_f1 0.24, R_f2 0.53 (Found: C, 63.5; H, 6.8; N, 8.6. $C_{26}H_{33}N_3O_5$·1.5H_2O requires C, 63.2; H, 6.68; N, 8.50%).

Z-D-His(N^π-1-Adom)-OH

The *title compound* was prepared in 68.8% yield by the procedure for the synthesis of Z-His(N^π-1-Adom)-OH, mp 60–63 °C; $[\alpha]_D^{25}$ +2.2 (*c* 1.0, MeOH); R_f2 0.44 (Found: C, 60.45; H, 7.1; N, 8.5. $C_{25}H_{31}N_3O_5$·2.5H_2O requires C, 60.2; H, 7.22; N, 8.45%).

Z-D-His(N^π-1-Adom)-Phe-OMe

The *title compound* was prepared by the same method as in the case of the synthesis of its stereoisomer (L-L), as an amorphous powder (190 mg, 70.4%), $[\alpha]_D^{25}$ +9.6 (*c* 1.0, MeOH); R_f1 0.61, R_f2 0.80. HPLC, same conditions as in the case of L-L compound, t_R 32.970 min (Found: C, 65.5; H, 6.7; N, 8.5. $C_{35}H_{42}N_4O_6$·1.5H_2O requires C, 65.4; H, 6.92; N, 8.25%).

Racemization analysis during the coupling of Z-His(N^π-1-Adom)-OH

To an ice-cooled solution of Z-His(N^π-1-Adom)-OH (15 mg, 0.033 mmol), H-Phe-OMe·HCl (7.83 mg, 0.036 mmol) and Et_3N (5.0 × 10^{-6} dm^3, 0.036 mmol) in DMF (3 cm^3) were added (1) DCC (8.16 mg, 0.039 mmol), (2) DCC (8.16 mg, 0.039 mmol) and HOBt (6.06 mg, 0.039 mmol), (3) BOP (17.23 mg, 0.039 mmol) and Et_3N (5.5 × 10^{-6} dm^3, 0.039 mmol), (4) HBTU (14.8 mg, 0.039 mmol) or (5) DPPA (8.45 mg, 0.039 mmol) and Et_3N (5.1 × 10^{-6} dm^3, 0.039 mmol). The reaction mixtures were stirred at 4 °C overnight. After removal of the solvent, the residue of each was dissolved in MeCN, and analysed by HPLC [Column: Cosmosil Pack 5C 18-AR (4.6 × 250 mm), eluent: A:B = 69:31 to 55:45 for 50 min to 69:31 for 5 min, flow rate: 1.0 cm^3 min^{-1}] to determine the

Table 2 Racemization rate during the coupling of Z-His(N$^{\pi}$-1-Adom)-OH and H-Phe-OMe

Coupling method	D-L (%)
DCC	2.74
DCC–HOBt	0.55
BOP	0.60
HBTU	0.71
DPPA	0.50

percentage of D-L peptide [= peak area of D-L × 100/(peak area of D-L + peak area of L-L)]. The results are summarized in Table 2.

Synthesis of TRH

Z-His(N$^{\pi}$-1-Adom)-Pro-NH$_2$. To a solution of H-Pro-NH$_2$ (0.25 g, 2.2 mmol) and Z-His(N$^{\pi}$-1-Adom)-OH (0.79 g, 1.75 mmol) in DMF (2 cm^3) were added BOP (0.9 g, 1.8 mmol), HOBt (0.24 g, 1.8 mmol) and NMM (0.39 cm^3, 3.5 mmol). The reaction mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed successively with 5% aq. Na_2CO_3 and water. From the AcOEt solution, the desired compound was extracted with 0.25 mol dm^{-3} aq HCl (4 × 15 cm^3). The pH of the aq. solution was adjusted with Na_2CO_3 to 8 using pH test paper and oily material was extracted with AcOEt. The extract was washed with water, dried over Na_2SO_4 and evaporated down. Light petroleum was added to the residue to afford *crystals* (0.8 g, 83.0%), mp 93–95 °C; $[\alpha]_D^{25}$ −29.4 (*c* 1.0, MeOH) (Found: C, 64.3; H, 7.3; N, 12.4. $C_{30}H_{39}N_5O_5 \cdot 1/2H_2O$ requires C, 64.5; H, 7.22; N, 12.5%).

Boc-Pyr-His(N$^{\pi}$-1-Adom)-Pro-NH$_2$. To an ice-cooled solution of H-His(N$^{\pi}$-1-Adom)-Pro-NH$_2$·2HCl [prepared from Z-His(N$^{\pi}$-1-Adom)-Pro-NH$_2$ (0.21 g, 0.38 mmol) in MeOH by hydrogenation in the presence of Pd and 2 mol equiv. HCl] in DMF (2 cm^3) were added Boc-Pyr-OH (0.12 g, 0.55 mmol), BOP (0.27 g, 0.55 mmol), HOBt (0.081 g, 0.55 mmol) and NMM (0.2 cm^3, 1.67 mmol). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with $CHCl_3$. The extract was washed successively with 5% aq. Na_2CO_3 and water, dried over Na_2SO_4 and the solvent evaporated. The residue in $CHCl_3$ (3 cm^3) was applied to a silica gel column (1 × 40 cm), equilibrated and eluted with $CHCl_3$–MeOH (3:1) to give title compound (0.2 g, 54.0%); mp 157–158 °C; $[\alpha]_D^{25}$ −36.4 (*c* 0.5, MeOH); R_f1 0.60. Amino acid analysis: Glu, 1.00; His, 0.90; Pro, 1.10 (average recovery 85%) (Found: C, 59.9; H, 8.0; N, 13.1. $C_{32}H_{46}N_6O_7 \cdot H_2O$ requires C, 59.65; H, 7.51; N, 13.0%).

H-Pyr-His-Pro-NH$_2$·AcOH (TRH). Boc-Pyr-His(N$^{\pi}$-1-Adom)-Pro-NH$_2$ (54 mg, 0.085 mmol) was dissolved in anhydrous TFA (7.5 cm^3) containing thioanisole (0.02 cm^3, 0.17 mmol) and the reaction mixture was stirred at room temperature for 2 h. Dry diethyl ether was added to the solution to afford a precipitate, which was collected by filtration, washed with dry diethyl ether and dried *in vacuo*. The product as a solution in water (5 cm^3) was treated with Amberlite IRA-93ZU (acetate form) for 30 min and the water layer was lyophilized to give a fluffy powder (32.1 mg, 88.3%); $[\alpha]_D^{25}$ −62.2 (*c* 1.0, H_2O) {authentic sample: $[\alpha]_D^{25}$ −61.3 (*c* 1.0, in water)}. On analytical HPLC, synthetic TRH exhibited a single peak at the same position as authentic TRH as shown in Fig. 3. [Column: Cosmosil packed 5 C 18-AR (4.6 × 250 mm), eluent: isocratic A:B 90:10, flow rate: 0.5 cm^3 min^{-1}.]

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