Amino acids and peptides. Part 42.[1] Application of the 2-adamantyloxycarbonyl (2-Adoc) group to the protection of the imidazole function of histidine in peptide synthesis †,[2]

Yasuhiro Nishiyama, Noriyuki Shintomi, Yukihiro Kondo, Takako Izumi and Yoshio Okada *
Faculty of Pharmaceutical Sciences, Kobe-Gakuin University, Nishi-ku, Kobe 651-21, Japan

The N^{im}-2-adamantyloxycarbonyl (2-Adoc) group has been found to be both suitable for protection of the imidazole function of the histidine residue in peptide synthesis in terms of its stability to trifluoroacetic acid (TFA), tertiary amines and 1-hydroxybenzotriazole (HOBt), and in its reduction of the racemization rate during the coupling reaction. N^{im}-2-Adoc protection has also been applied successfully to the solid-phase synthesis of thyrotropin-releasing hormone which depends on *tert*-butoxycarbonyl (Boc)-chemistry.

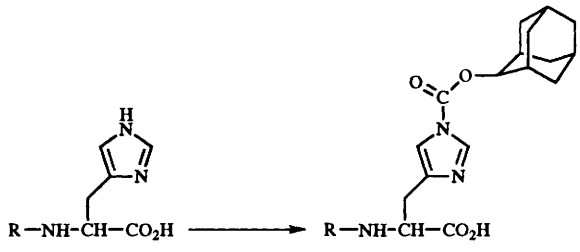
Introduction

Although a number of imidazole-protecting groups have been developed, the side-chain protection of histidine (His) has remained as one of the most serious problems in peptide synthesis.[3] Toluene-*p*-sulfonyl (Tos)[4,5] is probably the most popular protecting group in *tert*-butoxycarbonyl (Boc)-dependent peptide synthesis, but it has some disadvantages, such as susceptibility to 1-hydroxybenzotriazole (HOBt), an efficient coupling additive, and partial decomposition during removal of the N^{α}-Boc group by trifluoroacetic acid (TFA).[3,5-7] N^{im}-Dinitrophenyl (Dnp)[8] is also frequently used in Boc-chemistry, especially in solid-phase synthesis, but its main drawback is that N^{im}-Dnp is stable to anhydrous HF and trifluoromethanesulfonic acid (TFMSA), and it must be removed separately prior to final deprotection and cleavage from the resin. Jones and his co-workers[9] reported that the $N^{im}(\pi)$-locating protecting group could completely prevent side-chain induced racemization[10] during the coupling of His, and they recommended the use of π-benzyloxymethyl (Bom)[11] in Boc-chemistry and π-*tert*-butoxymethyl (Bum)[12] in fluoren-9-ylmethyloxycarbonyl (Fmoc)-chemistry. Unfortunately, the syntheses of Boc-His(Bom)-OH and Fmoc-His(Bum)-OH are relatively complex, and these derivatives are expensive. In addition, new side reactions caused by formaldehyde which was derived from the Bom group during HF treatment were reported.[13,14] From the viewpoint of the prevention of side-chain induced racemization, some of the τ-locating substituents, including Tos and Dnp, are actually effective in reducing the racemization as a result of their electron-withdrawing character.[3,15] Therefore, the π-nitrogen of the imidazole ring does not need to be protected in many syntheses of His-containing peptides.

Earlier, we reported the new ε-amino protecting group, 2-adamantyloxycarbonyl (2-Adoc),[16] which was stable to TFA, and cleavable by HF or 1 mol dm^{-3} TFMSA-thioanisole in TFA. We report herein the introduction of the 2-Adoc group to imidazole nitrogen, and the evaluation of N^{im}-2-Adoc group for peptide synthesis.

Results and discussion

First, Z-His(2-Adoc)-OH (Z, benzyloxycarbonyl) and Boc-His(2-Adoc)-OH were prepared from Z-His-OH[17] or Boc-His-OH[4] and 2-adamantyl chloroformate (2-Adoc-Cl) under the conditions of the Schotten–Baumann reaction as shown in Scheme 1. It is a valid assumption that the main product in the reaction of the histidine side-chain with equimolar amount of acylating reagent will be a less hindered nitrogen-, *i.e.* a τ-substituted one, therefore, it is predicted that the orientation of N^{im}-2-Adoc group is τ.[18] Furthermore, MeI treatment of Z-His(2-Adoc)-OH and Boc-His(2-Adoc)-OH followed by acid hydrolysis, according to a published procedure,[19] gave $N^{im}(\pi)$-methyl-His and His (*ca.* 90%). High recovery of unchanged His was presumably due to the electron-withdrawing effect of the 2-Adoc group. Since no τ-methyl isoform was detected, it seemed highly likely that N^{im}-2-Adoc was located on a τ-nitrogen.



R = Z or Boc

Scheme 1 *Reagents:* 2-Adoc-Cl, aq. NaOH–1,4-dioxane

The stability and susceptibility of the N^{im}-2-Adoc group under a variety of acidic and basic conditions were examined by measuring the Z-His or His regenerated from Z-His(2-Adoc)-OH with HPLC or an amino acid analyser, respectively. The results are summarized in Table 1 where a comparison is made

† 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. All amino acid residues except Gly are of L-configuration, when no specifications are given. The following additional abbreviations are used: AcOEt, ethyl acetate; 1-Adoc, 1-adamantyloxycarbonyl; 2-Adoc, 2-adamantyloxycarbonyl; 2-Adoc-Cl, 2-adamantyl chloroformate; Boc, *tert*-butoxycarbonyl; Boc_2O, di-*tert*-butyl dicarbonate; Bom, benzyloxymethyl; Bop, benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate; Bum, *tert*-butoxymethyl; CHA, cyclohexylamine; DCC, *N,N*-dicyclohexylcarbodiimide; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; Dnp, 2,4-dinitrophenyl; Fmoc, fluoren-9-ylmethyloxycarbonyl; Glp, pyroglutamic acid; HBTU, *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; MBHA, 4-methylbenzhydrylamine; NMM, *N*-methylmorpholine; -OPcp, pentachlorophenyl ester; -OSu, *N*-hydroxysuccinimidyl ester; TFA, trifluoroacetic acid; TFMSA, trifluoromethanesulfonic acid; Tos, toluene-*p*-sulfonyl; TRH, thyrotropin-releasing hormone; Z, benzyloxycarbonyl.

Table 1 Stability and susceptibility of N^{im}-2-Adoc group

	% Cleavage											
	2-Adoc						1-Adoc					
Conditions	Entry	10 min	30 min	60 min	120 min	24 h	Entry	10 min	30 min	60 min	120 min	24 h
7.6 mol dm^{-3} HCl in 1,4-dioxane	1	0	0	0	0	0	13	92	100	100	100	100
TFA	2	0	0	0	0	0	14	95	100	100	100	100
25% HBr in AcOH	3	100	100									
1 mol dm^{-3} TFMSA-thioanisole in TFA	4	100	100									
HF	5	100	100									
10% aq. $NaHCO_3$	6	0	0	0	0	0	15	0	0	0	0	0
10% DIEA in DMF	7	0	0	0	0	0	16	0	0	0	0	0
10% Et_3N in DMF	8	0	0	0	0	0	17	0	0	0	0	0
10% aq. Et_3N	9	64	92	94	95	100	18	58	89	95	95	100
20% piperidine in DMF	10	45	87	94	100	100	19	7	11	17	33	89
2 mol dm^{-3} aq. NaOH	11	100	100				20	100	100			
20% HOBt in DMF	12	0	0	0	0	0	21	0	0	0	0	0

with those for the N^{im}-1-adamantyloxycarbonyl (1-Adoc)[20] group, the properties of which resemble those of the N^{im}-Boc group.[21] The N^{im}-2-Adoc group was stable to 7.6 mol dm^{-3} HCl in 1,4-dioxane and TFA at room temperature for up to 24 h, while the N^{im}-1-Adoc group was susceptible under the same conditions. The N^{im}-2-Adoc group was rapidly cleaved by anhydrous HF or 1 mol dm^{-3} TFMSA-thioanisole in TFA at 0 °C. N^{im}-2-Adoc and N^{im}-1-Adoc groups were stable to 10% *N*,*N*-diisopropylethylamine (DIEA) in *N*,*N*-dimethylformamide (DMF) and 10% Et_3N in DMF at room temperature for up to 24 h, but not stable to 20% piperidine in DMF, the deprotecting reagent for the N^{α}-Fmoc group. The N^{im}-2-Adoc group was fully stable to 20% HOBt in DMF at room temperature for up to 24 h. These results indicate that N^{im}-2-Adoc protection is suitable for N^{α}-Boc-dependent peptide synthesis and can be employed in combination with HOBt-mediated coupling methods.

Next, the efficiency of the N^{im}-2-Adoc group on the prevention of side-chain induced racemization was examined. Since, as shown in Fig. 1, Z-D-His(2-Adoc)-L-Phe-OMe was readily separated from Z-L-His(2-Adoc)-L-Phe-OMe by HPLC, this sequence was employed for a model study of racemization. Z-L-His(2-Adoc)-OH was coupled with H-L-Phe-OMe by *N*,*N*-dicyclohexylcarbodiimide (DCC), DCC–HOBt, benzotriazolyl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate (Bop),[22] *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)[23] or diphenylphosphoryl azide (DPPA),[24] after which the crude product was analysed by HPLC to determine the % D-L peptide. The results summarized in Table 2 show that the formation of the D-L peptide was very low with all the coupling methods so far examined. From a comparison with earlier reports,‡[9,15,25] it is concluded that the reducing effect of the 2-Adoc group on racemization is comparable to that of other electron-withdrawing N^{im}-protecting groups commonly used in Boc-chemistry. The racemization rate on DCC–HOBt coupling was lower than that on DCC alone. The racemization reducing effect of HOBt was previously demonstrated using Boc-His(Boc)-OH.[15] The aryl sulfonate-type protecting groups, including Tos, are not stable

Table 2 Racemization rate during the coupling of Z-His(2-Adoc)-OH and H-Phe-OMe

Coupling method	% D-L
DCC	1.5
DCC–HOBt	0.6
Bop	0.6
HBTU	1.2
DPPA	0.4

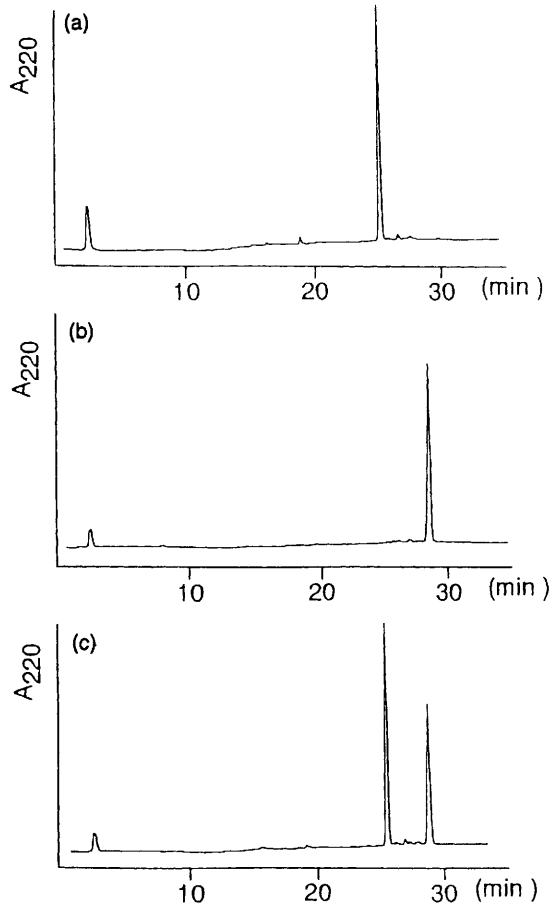


Fig. 1 HPLC profiles of (a) Z-L-His(2-Adoc)-L-Phe-OMe, (b) Z-D-His(2-Adoc)-L-Phe-OMe and (c) co-injection. Column and solvent system are described in Experimental section.

‡ In ref. 15, 0.3–0.9% racemization was observed on the DCC-coupling of Z-His(Tos)-OH with H-Ser(Bzl)-OBzl in CH_2Cl_2. More critical racemization during the coupling of N^{im}-Bzl derivative was reported in ref. 9 and ref. 15. In ref. 25, 0.3–0.7% racemization of His residue was observed during the solid-phase synthesis of ribonuclease A 115–124 decapeptide using N^{im}-Tos or Dnp protection.

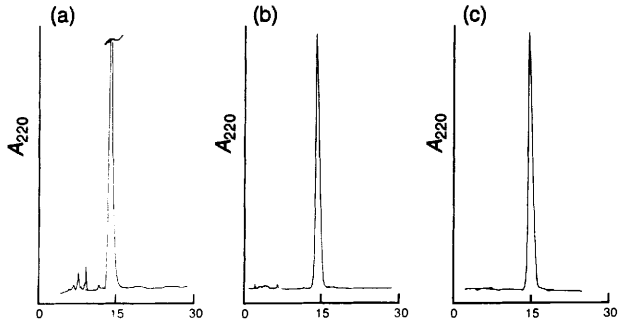
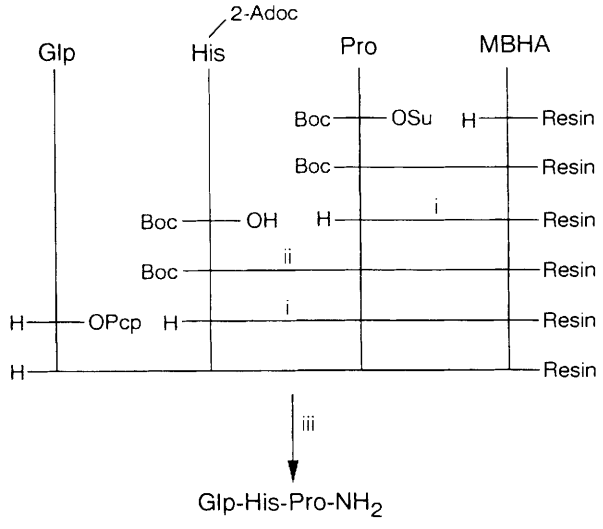


Fig. 2 HPLC profiles of synthetic and authentic TRH. (a), crude; (b), purified; (c), authentic. Column and solvent system are described in experimental section

to HOBt, therefore, these protections cannot be employed in combination with HOBt-mediated coupling methods.

Finally, thyrotropin-releasing hormone (TRH),[26] Glp-His-Pro-NH_2 (Glp, pyroglutamic acid) was synthesized by a solid-phase method using N^{im}-2-Adoc protection. The desired sequence was constructed on 4-methylbenzhydrylamine (MBHA) resin by active ester or DCC–HOBt coupling as shown in Scheme 2. The protected peptide resin thus obtained was



Scheme 2 *Reagents*: i, 50% TFA in CH_2Cl_2; ii, DCC–HOBt; iii, 1 mol dm^{-3} TFMSA–thioanisole in TFA containing *m*-cresol

treated with 1 mol dm^{-3} TFMSA–thioanisole in TFA, and the crude product was purified by preparative HPLC. The analytical HPLC profiles of the crude and purified products are shown in Fig. 2 in comparison with that of the authentic TRH purchased from Peptide Institute (Osaka, Japan). The high homogeneity of the crude product highlighted that the N^{im}-2-Adoc group was stable during the successive TFA-treatments, neutralization with *N*-methylmorpholine and HOBt-mediated coupling. The correct amino acid compositions were confirmed by amino acid analysis of the acid hydrolysate (6 mol dm^{-3} HCl, 110 °C, 18 h).

Conclusions

The 2-Adoc group can be easily introduced onto the τ-nitrogen in the imidazole ring of His by a one-step reaction of N^{α}-protected His-derivatives. The N^{im}-2-Adoc group is both stable to Boc-deprotecting conditions, and easily and rapidly removable by anhydrous HF or 1 mol dm^{-3} TFMSA–thioanisole in TFA. In addition, the N^{im}-2-Adoc group can effectively suppress the racemization of the His residue presumably as a result of its electron-withdrawing effect. Furthermore, steric hindrance of the adamantane moiety might contribute to the prevention of racemization as in the case of the N^{im}-trityl group.[27] Since N^{im}-2-Adoc is stable to HOBt, a HOBt-mediated coupling procedure can be employed in combination with this new protecting group. Solid-phase synthesis of TRH using N^{im}-2-Adoc protection was achieved successfully. The results obtained here show that the 2-Adoc group is both suitable for imidazole-protection in N^{α}-Boc-dependent peptide synthesis and promising for the synthesis of various His-containing peptides.

Experimental

Mps were determined with a Yanagimoto micro apparatus and are uncorrected. On TLC (Kieselgel G, Merck), R_f1 and R_f2 values refer to (1) $CHCl_3$–MeOH–water (8:3:1, lower phase), and (2) $CHCl_3$–MeOH–AcOH (90:8:2), 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}. Amino acid compositions of acid hydrolysates were determined with an automated amino acid analyser (K-101 AS or K-202 SN, Kyowa Seimitsu Co. Ltd., Japan). On the mobile-phase system for HPLC, A and B refer to water and MeCN, respectively, both containing 0.05% TFA.

2-Adoc-Cl was a generous gift from Watanabe Chemical Industries (Hiroshima, Japan). MBHA resin (0.24 mmol g^{-1}) was purchased from Kokusan Chemical Works (Japan). For solid-phase peptide synthesis, CH_2Cl_2 and DMF were of peptide synthesis grade (Kokusan Chemical Works), and MeOH was of HPLC grade (Merck). Boc-Pro-OSu[28] and Glp-OPcp[29] were prepared by published procedures. His(π-Me) (L-1-methylhistidine) and His(τ-Me) (L-3-methylhistidine) were purchased from Calbiochem (USA).

Z-His(2-Adoc)-OH·CHA

To an ice-cooled solution of Z-His-OH (8.7 g, 30 mmol) in 2 mol dm^{-3} aq. NaOH (30 cm^3) and 1,4-dioxane (20 cm^3) were added portions of 2-adamantyl chloroformate (8.4 g, 39 mmol) in 1,4-dioxane (10 cm^3). The reaction mixture was stirred with ice cooling for 2 h after which it was acidified with 6 mol dm^{-3} HCl, and then extracted with AcOEt. The extract was washed with water, dried (Na_2SO_4) and evaporated. To an ice-cooled solution of the residual oil in diethyl ether (100 cm^3) was added cyclohexylamine (CHA) (3.0 g, 30 mmol) in diethyl ether (100 cm^3). The resulting precipitate was filtered off and washed with diethyl ether; yield 12.9 g (75.9%), mp 150–153 °C (Found: C, 65.0; H, 7.4; N, 9.5. $C_{31}H_{42}N_4O_6 \cdot 0.5H_2O$ requires C, 64.7; H, 7.53; N, 9.73%).

Z-His(2-Adoc)-OH

To an ice-cooled suspension of Z-His(2-Adoc)-OH·CHA (1.0 g, 1.8 mmol) in AcOEt (100 cm^3) was added 10% aq. citric acid (20 cm^3), and the resultant biphasic solution was vigorously stirred for 30 min. The AcOEt layer was collected, washed with water, dried (Na_2SO_4) and evaporated to give an amorphous powder (690 mg, 81.9%), $[\alpha]_D^{27}$ +7.68 (*c* 1.0 in CH_2Cl_2); R_f1 0.73 (Found: C, 63.8; H, 6.3; N, 8.7. $C_{25}H_{29}N_3O_6 \cdot 0.2H_2O$ requires C, 63.7; H, 6.29; N, 8.92%).

Boc-His(2-Adoc)-OH·CHA

To an ice-cooled solution of Boc-His-OH [prepared from Boc-His(Boc)-OMe (25.0 g, 67.7 mmol) in the usual manner[4]] in 1 mol dm^{-3} aq. NaOH (136 cm^3) and 1,4-dioxane (50 cm^3) were added portions of 2-adamantyl chloroformate (17.4 g, 81.2

mmol) in 1,4-dioxane (100 cm^3). The reaction mixture was stirred and cooled with ice for 3 h, after which it was neutralized with citric acid and partially evaporated. The residual solution was acidified with citric acid, and then extracted with AcOEt. The extract was washed with water, dried (Na_2SO_4) and evaporated. To an ice-cooled solution of the residual oil in diethyl ether (200 cm^3) was added CHA (6.7 g, 67.7 mmol) in diethyl ether (100 cm^3) to afford a precipitate. This was filtered off and washed with THF–diethyl ether (1:4); yield 21.0 g (58.2 %), mp 137–141 °C, $[\alpha]_D^{26}$ +22.9 (*c* 1.0 in MeOH) (Found: C, 62.5; H, 8.46; N, 10.2. $C_{28}H_{44}N_4O_6 \cdot 0.2H_2O$ requires C, 62.7; H, 8.35; N, 10.45%)

Boc-His(2-Adoc)-OH

To an ice-cooled suspension of Boc-His(2-Adoc)-OH•CHA (2.4 g, 4.51 mmol) in AcOEt (150 cm^3) was added 10% aq. citric acid (30 cm^3), and the resultant biphasic solution was vigorously stirred for 30 min. The AcOEt layer was collected, washed with water, dried (Na_2SO_4) and evaporated. The oily residue was dissolved in small amount of 1,4-dioxane, and lyophilized to give a white powder (1.7 g, 80.6%), $[\alpha]_D^{26}$ +73.2 (*c* 1.0 in CH_2Cl_2), R_f1 0.75 (Found: C, 60.4; H, 7.17; N, 9.57. $C_{25}H_{29}N_3O_6$ requires C, 60.1; H, 7.21; N, 9.69%). This compound could be stored at room temperature for up to a month. (No change was observed on HPLC analysis.)

Methylation analysis of Z-His(2-Adoc)-OH and Boc-His-(2-Adoc)-OH

Z-His(2-Adoc)-OH (30.65 μmol) or Boc-His(2-Adoc)-OH (31.67 μmol) was dissolved in MeI (5 cm^3), and the solution stored at ambient temperature for 24 h. After removal of MeI, the residue was hydrolysed with 6 mol dm^{-3} HCl (1 cm^3) at 110 °C for 24 h. After removal of 6 mol dm^{-3} HCl, 0.1 mol dm^{-3} HCl (10 cm^3) was added to the residue, and 10 mm^3 of the resulting solution was applied to an amino acid analyser: 3.94 nmol (from Z derivative) or 4.04 nmol (from Boc derivative) of His(π-Me) (t_R 52.11 min) and 26.03 nmol (from Z derivative) or 27.36 nmol (from Boc derivative) of His (t_R 53.31 min) were detected. No His(τ-Me) (t_R 54.43 min) was detected in either cases.

Examination of stability and susceptibility of N^{im}-2-Adoc group

Z-His(2-Adoc)-OH or Z-His(1-Adoc)-OH (20 μmol) was dissolved in the test solution (1.0 cm^3), and aliquots of the solution were collected at 10, 30, 60, 120 min and 24 h. 0.5 mol dm^{-3} aq. $NaHCO_3$ or 1 mol dm^{-3} HCl was added to the ice-cooled solution to adjust it to pH 2. The extent of Z-His-OH (Entries 1, 2, 6–21 in Table 1) or His (Entries 3, 4 and 5 in Table 1) was determined by HPLC or on an amino acid analyser, respectively. The results are summarized in Table 1.

Z-His(2-Adoc)-Phe-OMe

To an ice–salt-cooled solution of Z-His(2-Adoc)-OH (500 mg, 1.07 mmol) and H-Phe-OMe•HCl (231 mg, 1.07 mmol) in DMF (20 cm^3) were added portions of DPPA (354 mg, 1.28 mmol) in DMF (5 cm^3). Et_3N (300 mm^3, 2.14 mmol) was added to the above solution, and the reaction mixture was stirred at room temperature overnight. After removal of the solvent from the mixture the residue was extracted with AcOEt. The extract was washed with 5% aq. $NaHCO_3$, 5% aq. citric acid and water, dried (Na_2SO_4) and evaporated. Chromatography on silica gel with $CHCl_3$ as the eluent yielded a colourless amorphous powder (520 mg, 77.3%), $[\alpha]_D^{26}$ +38.1 (*c* 1.0 in CH_2Cl_2), R_f2 0.56, t_R 25.1 min [μBondasphere C18 (3.9 × 150 mm); A:B 80:20 for 5 min, 80:20 to 20:80 for 15 min, and 20:80 for 10 min (1.0 cm^3 min^{-1})] (Found: C 66.2; H, 6.5; N, 8.7. $C_{35}H_{40}N_4O_7 \cdot 0.25H_2O$ requires C, 66.4; H, 6.45; N, 8.85%). Amino acid compositions of the acid hydrolysate (6 mol dm^{-3} HCl, 110 °C, 18 h) were His:Phe 0.94:1.00 (average recovery 61%).

Z-D-His(2-Adoc)-OH

The title compound was prepared from Z-D-His-OH (500 mg, 1.73 mmol) and 2-Adoc-Cl (450 mg, 2.08 mmol) in a similar manner to that described for the L-isomer; yield 680 mg (84.1%), $[\alpha]_D^{25}$ −7.8 (*c* 1.0 in CH_2Cl_2); R_f1 0.73.

Z-D-His(2-Adoc)-L-Phe-OMe

The title compound was prepared from Z-D-His(2-Adoc)-OH (500 mg, 1.07 mmol) and H-Phe-OMe•HCl (231 mg, 1.07 mmol) in a similar manner to that described for the L-L isomer; yield 550 mg (81.8%), $[\alpha]_D^{26}$ −6.15 (*c* 1.0 in CH_2Cl_2), R_f2 0.56, t_R 28.5 min [μBondasphere C18 (3.9 × 150 mm); A:B 80:20 for 5 min, 80:20 to 20:80 in 15 min, and 20:80 for 10 min (1.0 cm^3 min^{-1})]. Amino acid compositions of the acid hydrolysate were His:Phe 0.96:1.00 (average recovery 77%).

Racemization analysis during the coupling of Z-His(2-Adoc)-OH

To an ice-cooled solution of Z-His(2-Adoc)-OH (550 mg, 1.07 mmol), H-Phe-OMe•HCl (231 mg, 1.07 mmol), and Et_3N (150 mm^3, 1.07 mmol) in DMF (20 cm^3) were added (1) DCC (264 mg, 1.28 mmol), (2) DCC (264 mg, 1.28 mmol) and HOBt (196 mg, 1.28 mmol), (3) Bop (566 mg, 1.28 mmol) and Et_3N (180 mm^3, 1.28 mmol), (4) HBTU (485 mg, 1.28 mmol) and Et_3N (180 mm^3, 1.28 mmol), or (5) DPPA (352 mg, 1.28 mmol) and Et_3N (180 mm^3, 1.28 mmol). The reaction mixtures were stirred at 4 °C [(1) and (2)] or at room temperature [(3), (4) and (5)] overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 5% aq. $NaHCO_3$, 5% aq. citric acid and water, dried (Na_2SO_4) and evaporated. The amorphous residue for each was dissolved in MeCN, and analysed by HPLC [μBondasphere C18 (3.9 × 150 mm); A:B 80:20 for 5 min, 80:20 to 20:80 in 15 min, and 20:80 for 10 min (1.0 cm^3 min^{-1})] to determine the % of D-L peptide [=peak area of D-L × 100/(peak area of D-L + peak area of L-L)]. The results are shown in Table 2.

Glp-His(2-Adoc)-Pro-MBHA resin

HCl•MBHA resin (833 mg, 0.2 mmol) was neutralized with 10% NMM in CH_2Cl_2 (5 cm^3; 1 min × 1, 10 min × 1), and then washed with CH_2Cl_2 (5 cm^3; 1 min × 10). Boc-Pro-OSu (199 mg, 0.4 mmol) and 0.5 mol dm^{-3} HOBt in DMF (0.8 cm^3) were added to the suspension of the above resin in CH_2Cl_2 (5 cm^3) which was then shaken for 1 h. The resin was then filtered off and washed with CH_2Cl_2 (5 cm^3; 1 min × 10). Boc-Pro-MBHA resin thus obtained was treated with 50% TFA in CH_2Cl_2 (5 cm^3; 1 min × 1, 30 min × 1), and washed with CH_2Cl_2 (5 cm^3; 1 min × 10). After neutralization and washing, Boc-His(2-Adoc)-OH (173 mg, 0.4 mmol), 0.5 mol dm^{-3} HOBt in DMF (0.8 cm^3) and DCC (83 mg, 0.4 mmol) were added to the suspension of the H-Pro-MBHA resin in CH_2Cl_2 (5 cm^3). After the mixture has been shaken for 1 h, the resin was filtered off and washed with CH_2Cl_2 (5 cm^3; 1 min × 3), MeOH–CH_2Cl_2 (1:1, 5 cm^3; 1 min × 3) and CH_2Cl_2 (5 cm^3; 1 min × 3). Boc-His(2-Adoc)-Pro-MBHA resin thus obtained was treated with 50% TFA in CH_2Cl_2, and then neutralized as described above. Glp-OPcp (151 mg, 0.4 mmol) and 0.5 mol dm^{-3} HOBt in DMF (0.8 cm^3) were added to a suspension of the H-His(2-Adoc)-Pro-MBHA resin in CH_2Cl_2 (5 cm^3). After the mixture had been shaken for 1 h, the resin was filtered off and washed with CH_2Cl_2 (5 cm^3; 1 min × 5), DMF–CH_2Cl_2 (1:1, 5 cm^3; 1 min × 3), MeOH–CH_2Cl_2 (1:1, 5 cm^3; 1 min × 3), MeOH (5 cm^3; 1 min × 3) and hexane (1 min × 3). The weight of the peptide resin after drying over KOH pellets *in vacuo* was 860 mg (96.6%).

Glp-His-Pro-NH_2

Glp-His(2-Adoc)-Pro-MBHA resin (860 mg) obtained above was suspended in 1 mol dm^{-3} TFMSA–thioanisole in TFA (10 cm^3) containing *m*-cresol (0.5 cm^3), and the suspension gently shaken at ice-bath temperature for 30 min and then at room temperature for 60 min. After removal of the resin by filtration, dry diethyl ether was added to the filtrate to afford a precipitate, which was collected by centrifugation, and washed with ice-cooled diethyl ether. To the solution of the above precipitate in 3% aq AcOH (5 cm^3) was added Amberlite IRA-45 (acetate form, 300 mg), and the resultant suspension was stored at room temperature for 30 min. After removal of the Amberlite resin, the filtrate was lyophilized to give a white solid (40 mg), and purified with preparative HPLC [YMC-D-ODS (20 × 250 mm), 2% aq. MeCN containing 0.05% TFA (10 cm^3/min)]; yield 28 mg (38.7% from the starting resin), $[\alpha]_D^{25}$ −61.3 (*c* 0.34 in water) [authentic sample (purchased from Peptide Institute) −61.2 (*c* 1.0 in water); lit.[29] −65.5 (*c* 1.0 in water)], t_R 15.54 min [98.3%; YMC-R-ODS (4.6 × 250 mm), A:B 98:2 for 20 min, and then 98:2 to 50:50 in 10 min (1.0 cm^3 min^{-1})]. Amino acid compositions of the acid hydrolysate were Glu:His:Pro 1.11:0.97:1.00 (average recovery 88.7%). The HPLC profile is shown in Fig. 2.

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