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Racemization during the Synthesis of Histidine-containing Peptides

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Synopsis. A convenient test for racemization using an amino acid analyzer was developed and applied to examine the effects of several *im*-protecting groups and synthesis of histidyl peptides.

Urethane protected L-amino acid moieties are recognized to be free from racemization under the usual conditions of peptide synthesis. Jorgensen *et al.*, however, observed appreciable racemization of *t*-butoxycarbonyl-N^{im}-benzyl-L-histidine during the course of synthesis of an analog of angiotensin.[1] They separated a diastereomeric mixture of DL-His(Bzl)-L-Glu[2] by an amino acid analyzer, and applied the procedure to determine the extent of racemization during the course of coupling of Boc-L-His(Bzl) and L-Glu(OBzl)-OBzl or L-Glu(OBzl)-resin.[3] After the examination of seven DL-histidyl-dipeptides (DL-His-L-A where A=Asp, Glu, Val, Leu, Ile, Ser, and Ala), we found that His-Ser and His-Ala are also suitable systems for the amino acid analyzer detection of as little as 0.1% racemization.[4] Using the His-Ser system, we have examined the relationships between the extent of racemization and various *im*-protecting groups, as well as various coupling conditions.

The results for the conventional peptide synthesis are given in Table 1. With use of the DCC method, *im*-Z, *im*-Ts, and *im*-Boc on urethane protected L-histidines gave little racemization in CH_2Cl_2 or DMF (entries 1—3). However, *im*-Bzl led to a slightly higher level (entry 4). Insufficient masking of the basicity of the imidazole function by Bzl group seemed to be the main reason for the higher degree of racemization. Low levels of racemization (0.2—0.3%; entries 5 and 7) were observed in the usual MA method. The activation of Z-L-His(Z) under the strict conditions introduced by Anderson *et al.*[5] suppressed the racemization to below 0.1% (entry 6). Windridge and Jorgensen reported 24% racemization by Woodward's reagent during the course of coupling of Boc-L-His(Bzl) and L-Glu(OBzl)-OBzl using DMF as a solvent.[3] The present study showed only 0.4% racemization by this method (entry 8) in the coupling of Z-L-His(Z) and L-Ser(Bzl)-OBzl in CH_3CN. The azide method is considered free from racemization; however, Z-L-His-N_3 causes a slight degree of racemization (entry 9), which might be due to the basicity of free imidazole moiety.

The results of racemization during the solid-phase method are also given in Table 1. More appreciable racemization takes place in synthesis carried out by the solid-phase method than that by the conventional method. The remarkably high racemization (37%; entry 13) of Z-L-His(Bzl) coincides with the result of Windridge and Jorgensen.[3] The use of Z-L-His also causes appreciable racemization (4.8%). Racemization was reduced to 0.9% with Z-L-His(Ts). Lin *et al.* observed almost no racemization of Boc-L-His(Ts) in the synthesis of [L-His(Ts)^{119}, Ile^{120}]-ribonuclease decapeptide in contrast to 2.3% racemization of His^{119} when Boc-L-His was used.[6] Addition of either HOSu or HOBt with DCC resulted in an extensive reduction of racemization to 0.2% (entry 11). Weygand *et al.* reported the occurrence of less than 1% racemization with DCC plus HOSu.[7] König and Geiger also reported suppressed racemization by the DCC method in the presence of HOBt.[8]

Experimental

Preparation of Diastereomers (DL-His-L-A). Amino Acid Derivatives: Z-DL-His(Z)·EtOH (**1**) was prepared in a similar way to that for the L-isomer;[9] yield, 54%; mp 92—94 °C. L-Ser(Bzl)-OBzl·TsOH (**2**) was prepared following the general procedure by Izumiya and Makisumi;[10] yield, 72%; mp 120—122 °C; $[\alpha]_D^{20}$ −19.0° (*c* 2, MeOH).

*Z-DL-His(Z)-L-Ser(Bzl)-OBzl (**3**)*: IBCF (1 mmol) was added to a chilled solution (−15 °C) of **1** (1 mmol) and TEA (1 mmol) in THF (4 ml). After 10 min, a mixture of **2** (1 mmol), TEA (1 mmol) and THF (4 ml) was added to the chilled solution. The reaction mixture was allowed to stand at 0 °C for 1 h and at 20 °C for 19 h, and evaporated. The residue was dissolved in EtOAc and the solution was washed with 2% HCl, 4% $NaHCO_3$, and dried (Na_2SO_4). Evaporation of the filtrate afforded an oil (**3**).

DL-His-L-Ser: A part (0.5 mmol) of the crude oil (**3**) dissolved in a mixture (6 ml) of AcOH-MeOH-H_2O (6:3:1, v/v) was treated with H_2 in the presence of Pd black. The filtrate from the catalyst was evaporated, and the residue (DL-His-L-Ser) was used in an experiment with the analyzer.

Other diastereomers (DL-His-L-A) were prepared in the same manner as described above.

Separation of DL-His-L-A by the Amino Acid Analyzer: A part (1 μmol) of the residue (DL-His-L-A) was dissolved in 0.2 M citrate buffer (pH 2.2) and analyzed on a Hitachi amino acid analyzer: column, 0.6×10 cm; solvent, standard 0.2 M citrate buffer (pH 4.25); flow rate, 60 ml/h; jacket temperature, 55 °C. Pure diastereomers of His-Ser were prepared for the identification of peaks, and for the determination of color yields by ninhydrin reaction.

Diastereomers of His-Ser. Z-L(or D)-His(Z)-L-Ser(Bzl)-OBzl (**4**,**5**): Z-D-His(Z)·EtOH was prepared as described for **1**; yield, 71%; mp 93—95 °C; $[\alpha]_D^{23}$ −30.4° (*c* 2, EtOAc). Compounds **4** and **5** were prepared from Z-L(or D)-His(Z)·EtOH and **2** as described for **3**. Crude products were recrystallized from EtOAc-petroleum ether.

*L(or D)-His-L-Ser (**6**,**7**)*: Compounds **4** and **5** were hy-

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Table 1. Extent of recemization in conventional (entries 1—9) and solid-phase method (entries 10—14)

Entry	Coupling reagent	Acid component	Solvent	Reaction time (h)	Reaction temp (°C)	Extent of racemization (%)[a]
1	DCC	Z-L-His(Ts)	CH_2Cl_2	20	0, 20	0.3
2	DCC	Boc-L-His(Boc)	CH_2Cl_2	20	0, 20	0.4
3	DCC	Z-L-His(Z)	DMF	20	20	0.6
4	DCC	Z-L-His(Bzl)	DMF	20	20	1.8
5	IBCF	Z-L-His(Z)	THF	20	0, 20	0.2[b]
6	IBCF	Z-L-His(Z)	THF	20	0, 20	<0.1[c]
7	IBCF	Boc-L-His(Boc)	THF	20	0, 20	0.3[b]
8	Woodward's reagent	Z-L-His(Z)	CH_3CN	20	0, 20	0.4
9	Isopentyl nitrite	Z-L-His-$NHNH_2$	DMF	48	0	0.8
10	DCC	Boc-L-His(Boc)	CH_2Cl_2	4	20	1.5
11	DCC/HOSu or HOBt	Boc-L-His(Boc)	CH_2Cl_2	4	20	0.2
12	DCC	Z-L-His(Ts)	CH_2Cl_2	4	20	0.9
13	DCC	Z-L-His(Bzl)	DMF	4	20	37
14	DCC	Z-L-His	DMF	4	20	4.8

a) Defined as [100(D-L isomer)]/[(L-L isomer)+(D-L isomer)]. b) MA was prepared with TEA at −15 °C for 10 min and reacted at 0 °C for 1 h and at 20 °C for 19 h. c) MA was prepared with *N*-methylmorpholine at −15 °C for 1 min.

Table 2. Yields and physical constants of compounds **4**—**7**

Compounds[a]	Yield (%)	Mp (°C)	$[\alpha]_D^{20}$ (*c* 2)
Z-L-His(Z)-L-Ser(Bzl)-OBzl (**4**)	66	109—110	+23.5°[b]
Z-D-His(Z)-L-Ser(Bzl)-OBzl (**5**)	69	131—132	−32.7°[b]
L-His-L-Ser (**6**)[c]	69	192—193 (dec)	+4.1°[d]
D-His-L-Ser·H_2O (**7**)	67	197—198 (dec)	−6.0°[d]

a) Satisfactory elemental analyses were obtained for all compounds. b) Solvent, $CHCl_3$. c) G. Losse and G. Müller, *Chem. Ber.*, **94**, 2768 (1961), synthesized **6** from Trt-L-His(Trt)-L-Ser and afforded mp 138—141 °C. d) Solvent, 0.1 M NaOH.

drogenated as described for DL-His-L-Ser, and the crude products were recrystallized from water–EtOH. Melting points and other data of **4**—**7** are summarized in Table 2. Each dipeptide was subjected to analysis as described before; **6** was eluted at 85 ml of effluent volume, and **7** at 115 ml.

Detection of Racemization during the Conventional Method. Several acyl-L-histidines (1 mmol each) were coupled with **2** (1 mmol) by DCC,[11] IBCF,[5] Woodward's reagent,[12] and by the azide method.[13] The solvent of the reaction mixture was evaporated and the residue was worked up as described for **3**. The crude product was deblocked by a suitable method to afford His-Ser, a part (5 μmol) of which was subjected to analysis.

Detection of Racemization during the Solid-phase Method. Coupling was performed manually.[14] L-Ser(Bzl)-resin derived from Boc-L-Ser(Bzl)-resin (0.20 g, 0.06 mmol) was treated with four equivalents of DCC and a protected histidine (*e.g.*, Boc-L-His(Boc)). In some cases HOSu or HOBt (fourfold) was used together with DCC. The acyl-dipeptide-resin obtained was treated with HF (3 ml) at 0 °C for 1 h in the presence of anisole (0.3 ml). After removal of HF, the crude peptide was extracted with 1% AcOH, the extract being washed with ether and evaporated. The residue (His-Ser) was subjected to analysis.

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2) Abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used throughout. Additional abbreviations: DMF, *N*,*N*-dimethylformamide; HOBt, *N*-hydroxybenzotriazole; HOSu, *N*-hydroxysuccinimide; IBCF, isobutyl chloroformate; MA, mixed anhydride; OBzl, benzyl ester; TEA, triethylamine; EtOH, ethanol; THF, tetrahydrofuran; TsOH, *p*-toluenesulfonic acid.

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