Studies on the Synthesis of Histidine Peptides. I. N^{im}-Tosylhistidine Derivatives as Starting Materials[1,2]

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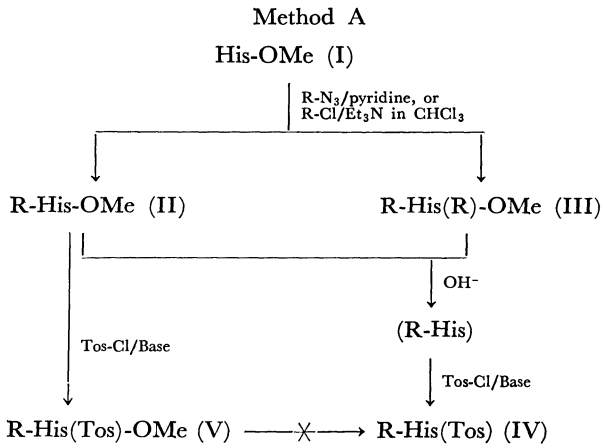
N^{α}-Nps-, Z(OMe)-, Z-, Boc-, and Aoc-derivatives of N^{im}-tosylhistidine were synthesized as materials for the syntheses of histidine-containing peptides, and the reactivity of the N^{im}-tosyl group was examined under several conditions commonly used for peptide syntheses. Although the N^{im}-tosyl group was somewhat more unstable than N^{im}-benzyl group, the use of the tosyl derivative was found to be advantageous in several points: the enhancement of the solubility of its peptide derivatives, the suppressive effect of racemization during its coupling reactions, and the high removability of the tosyl group under mild conditions.

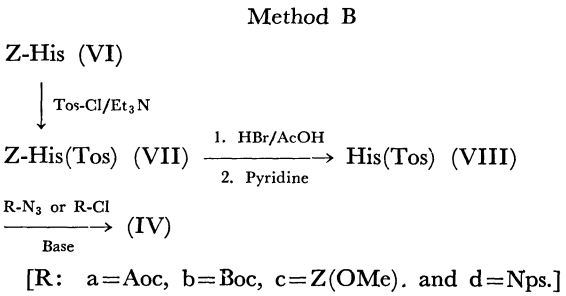
Although several different procedures for the incorporation of histidine residues into peptides have been published by many workers, those methods still have several problems.[3] Histidine-peptides can be synthesized without the protection of the imidazole group, but only limited, mild conditions have to be used for their coupling reactions because of the remaining presence of active hydrogens in the side chains. Further, the strict purification of the each intermediate is indispensable to obtain the final product in a reasonable purity. To improve the situation, the following N^{im}-protective groups have been proposed by many workers: benzyl,[4] benzyloxycarbonyl,[5] trityl,[6] adamantyloxycarbonyl,[7] 2,2,2-trifluoro-1-acylaminoethyl,[8] piperidinocarbonyl,[9] dinitrophenyl,[10] diphenylmethyl,[11] trinitrophenyl,[12] and *t*-butyloxycarbonyl.[13] These protective groups are each characteristic and may be useful in specific cases, but we cannot declare that all the problems involved in the synthesis of histidine peptides have been solved. Especially when histidine peptides are synthesized by the solid-phase method, the histidine residue is preferably incorporated into resin as a form of an N^{im}-protected derivative, which is highly soluble in methylene chloride or chloroform. Further, it may be desirable for the N^{im}-protecting group to be stable during successive coupling reactions and readily removable under mild conditions in the final stage. The present study will introduce a new N^{im}-protecting group which is useful for the relief of those problems.[1]

Originally, the tosyl group had been recognized as a stable amino-protecting group, and sodium treatment in liquid ammonia was the only practical procedure to remove the protective group from the peptide derivatives. Recently, however, the same group attached to the guanido group of arginine has been demonstrated to be removable[14] in anhydrous hydrogen fluoride (HF) at 0 °C.[15] Therefore, we checked the stability of the tosyl group attached to the imidazole moiety of histidine, and found that the group is also removable under the same conditions in anhydrous HF.[1] Thus, several derivatives of the N^{im}-tosylhistidine were synthesized in order to examine their applicability to the synthesis of histidine peptides.

Syntheses of Several N^{α}*-protected Derivatives of* N^{im}*-tosylhistidine.* The N^{im}-tosylhistidine derivatives were synthesized *via* two different procedures, shown in Schemes 1 and 2. One route started from the histidine methyl ester[16] (Method A), and the other route, from N^{α}-Z-histidine[5] (Method B). In Method A, the histidine methyl ester was acylated with acyl chlorides or azides, and the remaining unacylated histidine methyl ester was removed as completely as possible at this stage. The mono- and di-acylated histidine methyl esters thus obtained were saponified to obtain N^{α}-acyl-histidine, which was then tosylated using tosyl chloride in the saponification mixture (Scheme 1). If the saponification mixture is contaminated by the unreacted histidine ester, N^{α}, N^{im}-ditosylated histidine should be formed and its removal from the final product must be problem. Generally, the acylation of the



Scheme 1.



Scheme 2.

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histidine methyl ester gives a mixture of mono- and di-acyl esters, depending on the reaction conditions and on the reactivity of the reagents used. When two moles of Aoc- or Boc-chloride were used as the acylating reagent, the histidine methyl ester was acylated to form diacyl esters; in this stage, the unchanged histidine methyl ester was removed completely by simple washing with water, since the diacyl histidine ester is almost insoluble in water. When Aoc- or Boc-azide was used as the reagent, the N^{α}-acyl histidine ester was formed as the major product, even if two moles of the reagent were used; in this case, care must be taken to extract the product from the reaction mixture completely because the mono-acyl esters are somewhat soluble in water. In the present study, mono- and di-Boc-histidine esters were isolated as crystals in order to confirm their properties, as will be shown in the Experimental section.

In the case of the synthesis of the Nps-derivative, the formation of the mono-Nps-histidine methyl ester was predominant.[17] Although the N^{α}-Nps-histidine methyl ester has been reported to be a crystalline material, we could not crystallize it in the present study; thus, it was purified by silica-gel-column chromatography. The histidine benzyl ester[18] was also used as a starting material for the same purpose.

In one experiment, the N^{α}-Nps-histidine methyl ester was converted to the tosyl derivative, and then we attempted to obtain N^{α}-Nps-N^{im}-tosyl-histidine by saponification. However, we were unsuccessful because of the susceptibility of the N^{im}-tosyl group to caustic alkali.

In the case of the synthesis of the Z(OMe)-derivative using the azide method, there was no difficulty in washing out the unreacted histidine ester from the reaction mixture, since both the mono- and di-Z(OMe)-histidine esters were almost insoluble in water; the mixture of the mono- and di-acylated derivatives was directly saponified to obtain N^{α}-Z(OMe)-histidine, which was then further converted to the N^{im}-tosyl derivative, as has been shown before.

In Method B, N^{α}-Z-histidine[5] was tosylated with tosyl chloride; the product was treated with anhydrous hydrogen bromide in acetic acid to obtain N^{im}-tosyl-histidine, which was then treated with the respective acyl chlorides or azides to obtain N^{α}-protected-N^{im}-tosyl-histidines (Scheme 2). This procedure gave products identical with those obtained by Method A, but the yields were generally lower than those obtained by means of Method A. This may be attributed to the partial decomposition of the tosyl group during the HBr treatment and to the acylation reactions. The catalytic hydrogenolysis of the N^{α}-Z-N^{im}-tosyl-histidine with a palladium catalyst was attempted in order to obtain N^{im}-tosylhistidine, but the attempt was unsuccessful; during the reaction, the N^{im}-tosylhistidine formed was precipitated out over the catalyst and further hydrogenolysis was disturbed.

Generally, N^{α}-acylated histidines which are obtained by the direct acylation of histidine are soluble in water, and troublesome procedures are necessary to remove any unchanged histidine from the reaction mixture. Therefore, the direct synthesis of N^{α}-acyl-N^{im}-tosyl-histidine from free histidine was concluded to be impractical.

The physical constants and analytical data of N^{α}-protected-N^{im}-tosylated histidine derivatives synthesized by Methods A and B are listed in Table 1.

Stability of the N^{im}*-Tosyl Group.* N^{α}-Protected-N^{im}-tosyl-histidines, such as N^{α}-Boc-, N^{α}-Z(OMe)-, and N^{α}-Nps-derivatives, were found to decompose gradually during storage at room temperature. This decomposition may be caused by the enhanced acidity of their own carboxyl groups resulting from the presence of the tosyl group in their side chains. Therefore, the storage of these compounds in the form of DCHA salts was considered to be practical.

The stability of the N^{im}-tosyl group was compared with that of other protective groups under various conditions commonly used for peptide synthesis; the data are summarized in Table 2. The tosyl group was removed easily in aqueous 1 N sodium hydroxide at room temperature, but 50% of this group survived in 25% aqueous ammonia at room temperature during

Table 1. Physicochemical properties of N^{α}-protected-N^{im}-Tos-histidine derivatives

		Mp (°C)	$[\alpha]_D$ (c 1)	Formula	Anal. % Calcd/Found			
					C	H	N	S
Aoc-L-His(Tos)-OH	IV-a	109—111 (dec.)	+10.0(f)	$C_{19}H_{25}N_3O_6S$	53.89 54.00	5.95 5.68	9.93 9.98	7.57 7.76
Boc-L-His(Tos)-OH	IV-b	123—115	+15.1(g)	$C_{18}H_{23}N_3O_6S$	52.81 52.83	5.66 5.55	10.27 10.32	7.81 7.58
Z(OMe)-L-His(Tos)-OH[a]	IV-c	179.5—180.5	+20.0(e)	$C_{34}H_{46}N_4O_7S$	62.37 62.11	7.08 7.21	8.56 8.44	4.90 4.85
Z-L-His(Tos)-OH[a]	VII	150—152 (dec.)	+19.1(e)	$C_{33}H_{44}N_4O_6S$	63.44 63.43	7.10 6.96	8.97 9.00	5.12 4.98
Nps-L-His(Tos)-OH	IV-d	141—142 (dec.)	+39.9(e)	$C_{19}H_{18}N_4O_6S_2$	49.35 49.62	3.92 3.74	12.12 12.21	13.84 13.39
Nps-L-His(Tos)-OMe	V-d	101—103	+34.1(e)	$C_{20}H_{20}N_4O_6S_2$	50.42 50.78	4.23 4.20	11.76 11.98	13.45 12.97
Nps-L-His(Tos)-OBzl		102—104	+24.8(e)	$C_{26}H_{24}N_4O_6S_2$	56.52 56.37	4.38 4.18	10.14 10.15	11.58 11.52

a)=DCHA salt (e)=DMF (f)=pyridine (g)=MeOH

Table 2. Behavior of N^{im}-protective groups under various conditions

N^{im}-protective group	H_2/Pd	Na/NH_3	1 M-NaOH[a]	25% aq. NH_3[a]	2 M-HBr in AcOH[a]	4 M-HCl in Dioxane[a]	Anhyd. CF_3CO_2H[b]	Anhyd. HF[c]	HOBT.[a]
Bzl[4]	B	A	S	S	S	S	S	S	S
Z[5]	A	A	A	A	B	B	B	A	B
Tri[6]		A	S		B	B	A		
Boc-TF[8]	S				A	A	A		
Tos	S	A	A	B	C	C	S	A	A

A: Removed. B: Slowly removed. C: Partially removed. S: Stable.
a) At room temp. 1 hr. b) At 15 °C 30 min. c) At 0 °C 1 hr.

one hour's treatment. From these facts, it was concluded that the N^{im}-tosyl group was little more stable than the N^{im}-Z group under alkaline conditions; the N^{im}-Z group is known to be removed not only in caustic alkali, but also in aqueous ammonia.[5] Only about 10% of the tosyl group was removed in 2 M HBr in acetic acid or in 4 M HCl in dioxane during one hour's treatment at roo mtemperature, while the complete removal of the group was observed during further storage overnight at room temperature.

As has been pointed out before, the N^{im}-tosyl group was readily cleaved in anhydrous HF at 0 °C within 30 min. When Nps-containing compounds weret reated by HF, the addition of skatole as a scavenger was effective, as will be shown in the second paper of this series.

One of the characteristics of the N^{im}-tosyl group was that it was almost unaffected by anhydrous trifluoroacetic acid during short treatment at room temperature; thus, Aoc-, Boc-, Z(OMe)-, and Nps-groups can be removed selectively from peptides containing the N^{im}-tosylhistidyl residue. This property was successfully used in the synthesis of Angiotensin II by the solid-phase procedure.[19]

The N^{im}-tosyl group did not interfere with the catalytic hydrogenolysis of other groups; however, a small amount of the tosyl group was found to be cleaved during the removal of *N*-benzyloxycarbonyl groups. This may be explained by a nucleophilic attack of the formed amino group during the hydrogenolysis. Bergmann and Zervas have pointed out that the N^{im}-acetyl group migrates to other amino groups under physiological conditions.[20] Later, Sakiyama *et al.*[4b] reported the same phenomenon with di-Z-histidine, in which the N^{im}-Z-group was observed to transfer to the amino group of the glycine methyl ester in dioxane at 50—60 °C. In the present study, although N^{α}-Boc-N^{im}-tosylhistidine was treated with the glycine methyl ester under the same condition, no migrated product was detectable within one hour in a reaction mixture. After six hours, however, the formation of the tosylglycine methyl ester was observed in a yield of more than 6.5%.

The N^{im}-tosyl group remained unchanged at room temperature for 24 hr. in the presence of two equivalents of triethylamine in dimethylformamide; this property should also be counted as an advantage of this protecting group in peptide synthesis.

Finally, it was noteworthy that the N^{im}-tosyl group was readily removed at room temperature in the presence of excess 1-hydroxybenzotriazole (HOBT), which is widely used as a convenient reagent for peptide synthesis.[21] During the reaction, the formation of 1-(tosyloxy)benzotriazole as the main product was confirmed. Those facts indicate that Geiger's procedure should always result in the formation of imidazole-free peptides, even if an N^{im}-tosyl derivative is used as the starting material. This procedure has also been successfully applied to the synthesis of histidine-peptides, as will be shown in the following report.

On the contrary, the N^{im}-Z group was found to be much more stable against the same reagent.

Experimental

The melting points were measured by the capillary method and were not corrected. Thin-layer chromatography (tlc) was carried out on Merck's Silica Gel G using the following solvent systems: (1) chloroform–methanol–acetic acid (95 : 5 : 3), and (2) *n*-butanol–acetic acid–water (4 : 1 : 1). The IR spectra were determined on a Shimadzu IR-27G Spectrometer, and the UV spectra, on a Hitachi ESP-2U spectrometer.

N^{τ}-Aoc-His-OMe (II-a) and N^{α},N^{im}-di-Aoc-His-OMe (III-a). Aoc-azide (15.7 g, 100 mmol) was added to a solution of His-OMe (I) in dry pyridine (20 ml), which had been isolated from His-OMe·2HCl (12 g, 50 mmol) following the procedure of Wünsch;[22] the mixture was then allowed to react for 3 days at 30 °C under stirring. The pyridine was removed under reduced pressure, the remaining oil was flushed with benzene, the residue was dissolved in ethyl acetate, and a small amount of an insoluble material was removed by filtration. *n*-Hexane was added to the filtrate until a part of the product precipitated out. After the mixture had been kept in a refrigerator for 5 hr, the crystals were collected by filtration and recrystallized from ethyl acetate to afford II-a; wt. 9.8 g (70%); mp 122—124 °C, $[\alpha]_D^{22}$ −13.0° (*c*, 2.2, pyridine). Found: C, 54.89; H, 7.58; N, 14.89%. Calcd for $C_{13}H_{21}O_4N_3$: C, 55.11; H, 7.47; N, 14.83%.

The filtrate of the crude crystals and mother liquor of the recrystallization were combined and concentrated under reduced pressure, and the residual oil was redissolved in ethyl acetate. The solution was washed well with water, dried over anhydrous sodium sulfate, and concentrated again to a residue, which was then used in the following reaction as N^{α}, N^{im}-di-Aoc-His-OMe without further purification.

Aoc-His(Tos) (IV-a via Method A). Compound II-a (9.5 g) and the oily residue obtained above were dissolved together in methanol (30 ml), after which the solution was treated with 1 N aqueous sodium hydroxide (100 ml) at room temperature. The saponification of the ester group and the

deacylation of the N^{im}-Aoc group were monitered by tlc using the (1) solvent. After the completion of the reactions had been confirmed by tlc, an aqueous solution of sodium carbonate (18 g in 300 ml) was added to the mixture and a solution of tosyl chloride (17.6 g, 90 mmol) in dioxane (50 ml) was stirred, drop by drop, into the mixture at room temperature. Stirring was continued for 4 hr; then water (500 ml) was added, and the mixture was washed three times with 100-ml portions of ether to remove the excess tosyl chloride. The aqueous phase was acidified to pH 2 with 0.5 M sulfuric acid, and the separated oily material was extracted with ethyl acetate. The extract was washed well with water, dried over anhydrous sodium sulfate, and concentrated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate–*n*-hexane to obtain needles; wt. 15.8 g (75% from I·2HCl). The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1.

Z-His(Tos)·DCHA (VII·DCHA). Tosyl chloride (9 g, 47 mmol) was added, portion by portion, to a solution of Z-His (VI) (10 g, 35 mmol)[5b] and sodium carbonate (7 g) in water (100 ml) over a 30-min period under vigorous stirring at 10—15 °C. Stirring was continued for an additional 4 hr at room temperature, and then the reaction mixture was washed twice with 50-ml portions of ether to remove the excess tosyl chloride. The aqueous phase was then acidified to pH 2 with 1 N sulfuric acid. The separated oil was extracted three times with 100-ml portions of ethyl acetate. The extracts were combined, and the combined solution was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was redissolved in ethyl acetate (100 ml), and dicyclohexylamine (5.5 g) was added to the solution; the crystalline materials thus formed were separated by filtration and recrystallized from methanol–ethyl acetate as needles; wt, 15.4 g (70.5%). The melting point, the $[\alpha]_D$ value, and the results of the elemental analysis of this compound are shown in Table 1.

Aoc-His(Tos) (IV-a via Method B). A suspension of VII·DCHA (10 g, 16 mmol) in 1 N sulfuric acid was vigorously shaken with ethyl acetate, and the extract was concentrated under reduced pressure. The residue was treated with 4 N HBr in acetic acid (20 ml) for 1 hr at room temperature. Then, the product was precipitated by the addition of dry ether (200 ml), and the precipitate was washed well by decantation with dry ether. After having been dried in a vacuum desiccator over sodium hydroxide, the solid mass was dissolved in methanol (100 ml), and the solution was neutralized with dry pyridine (6 ml) until precipitation occurred. The precipitates were collected by filtration and washed well with methanol; wt. 3.5 g (51.7%); mp 140—145 °C. This material (3.1 g, 10 mmol), which corresponds to VIII, was then dissolved in 5% aqueous sodium bicarbonate (50 ml). Aoc-azide (1.7 g, 10 mmol) was added to the solution, together with triethylamine (1.6 ml), and the mixture was stirred at room temperature for 2 days. Some insoluble materials thus formed were filtered off, and the filtrate was acidified to pH 2 with 1 N sulfuric acid. The separated oily material was extracted with ethyl acetate, and the extract was washed with water, dried over sodium sulfate, and concentrated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate–*n*-hexane; wt. 2.6 g (63%); mp 109.5—110.5 °C, $[\alpha]_D^{22}$ +10.3° (*c* 1, pyridine). The melting point showed no depression upon mixing with the same compound as synthesized by Method A.

Boc–His(Boc)–OMe (III-b). A solution of Boc-chloride in tetrahydrofuran, which had been prepared from *t*-butylalcohol (148 g) and phosgene (200 g) using an improved procedure described by Sakakibara *et al.*,[23] was stirred vigorously into a suspension of His–OMe·2HCl (I·2HCl) (120 g, 0.5 mol) and triethylamine (280 ml, 2 mol) in chloroform (800 ml) at 0—5 °C. Stirring was continued for an additional 2 hr at room temperature, and then chloroform (500 ml) was added to the reaction mixture. The chloroform solution was washed well with water, 1 N sulfuric acid, and a saturated sodium chloride solution successively, and then dried over anhydrous sodium sulfate. The dried solution was concentrated to dryness. A part of this material was crystallized from ether–*n*-hexane for analysis; mp 95—98 °C, $[\alpha]_D^{22}$ +13.0 °C (*c* 1, THF). Found: C, 54.95; H, 7.39; N, 11.71%. Calcd for $C_{17}H_{27}O_6N_3$: C, 55.27; H, 7.37; N, 11.38%.

Boc–His(Tos) (IV-b). The crude di-Boc-His-OMe obtained above was dissolved in methanol (340 ml), and aqueous 1 N sodium hydroxide (450 ml) was added to the solution at 10—15 °C. After 2 hr, a 10% solution of sodium carbonate (2 l) was added to the reaction mixture, and the whole mixture was washed well with ether. A solution of tosyl chloride (124 g, 0.65 mol) in dioxane (300 ml) was added to the washed solution under vigorous stirring at 10 °C. After the stirring had been continued for 3 hr at room temperature, the reaction mixture was washed well with ether, the aqueous phase was acidified to pH 2 with 1 N sulfuric acid, and the separated oily material was extracted with ethyl acetate. The extract was washed with a saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue was recrystallized from ethyl acetate to afford colorless needles; wt. 107 g (52% yield from I). The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1. The DCHA salt of Compound IV-b was prepared and then recrystallized from methanol–ethyl acetate; mp 160—162 °C, $[\alpha]_D^{22}$ +26.5° (*c* 1, MeOH). Found: C, 61.25; H, 7.95; N, 9.46; S, 5.38%. Calcd for $C_{30}H_{46}O_6N_4S$: C, 61.01; H, 7.79; N, 9.49; S, 5.13%.

N^{α}-Boc–His–OMe (II-b). This material was obtained much as in the case of N^{α}-Aoc–His–OMe (II-a), using 1.5 equivalents of Boc-azide as the reagent. The yield was 75%; mp 124—125 °C, $[\alpha]_D^{22}$ −13.5° (*c* 1, pyridine). Reported:[22] mp 124—125 °C, $[\alpha]_D$ −13.1° (*c* 2, pyridine).

Z(OMe)–His(Tos) (IV-c). Compound IV-c was synthesized *via* Method A (Scheme 1) in the same manner as IV-a, without any isolation of acylated histidine esters; it was then crystallized as the DCHA salt in an overall yield of 82%. The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1.

Nps–His–OMe (II-d). Nps-chloride (19 g, 0.1 mol) was added, portion by portion, to a suspension of I·2HCl (12 g, 50 mmol) and triethylamine (28 ml, 0.2 mol) in methanol (150 ml) at 5—10 °C over a 30-min period. The mixture was agitated vigorously during the reaction. After 2 hr, water (500 ml) was added to the reaction mixture, and then it was acidified to pH 2 with 1 N sulfuric acid. The mixture was washed well with three 200-ml portions of ethyl acetate; then excess sodium bicarbonate was added to the mixture, and an oily material thus separated was extracted with ethyl acetate. The extract was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure in a water bath below 40 °C. The oily residue was charged on the top of a silica-gel column (5×150 cm), using a mixture of $CHCl_3$–MeOH–pyridine (95 : 5 : 3), and the column was eluted with the same solvent system. The main fraction was collected and concentrated under reduced pressure to obtain Compound II-d as an oily material; 13.4 g (83%). Reported:[17] mp 134—136 °C.

Nps–His(Tos) (IV-d via Method A). The oily com-

pound, II-d (13.4 g), obtained above was dissolved in dioxane (125 ml) and then treated with aqueous 2 N sodium hydroxide (45 ml) over a period of 1 hr at 10—15 °C. After triethylamine (11 ml) had been added to the reaction mixture, a solution of tosyl chloride (11 g, 58 mmol) in dioxane (20 ml) was stirred into the mixture at 10 °C over a 45-min period. Stirring was continued for an additional 1 hr at 10—15 °C; then the mixture was neutralized to pH 7 with 1 N sulfuric acid, and the dioxane was removed by distillation under reduced pressure below 40 °C. Water (300 ml) was added to the residual solution, the solution was acidified to pH 2 with 1 N sulfuric acid, and an oil which separated was extracted with ethyl acetate. The ethyl acetate extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure at below 40 °C. The residual solid was washed with ether and recrystallized from ethyl acetate–*n*-hexane; wt. 14.4 g (75%). The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1.

Nps-His(Tos)-OMe (V-d). A solution of tosyl chloride (1.4 g, 7.4 mmol) in dioxane (5 ml) was stirred into a mixture of Compound II-d (2 g, 6.2 mmol) and triethylamine (1.4 ml) in dioxane (20 ml) at 5—10 °C over a 15-min period. Then the reaction mixture was diluted with water (50 ml), and the oil which separated was extracted with ethyl acetate. The extract was washed successively with 1 N sulfuric acid, water, aqueous 5% sodium bicarbonate, and water, and dried over anhydrous magnesium sulfate. The dried solution was concentrated to a residue under reduced pressure, and the solid was washed with ether and recrystalliyed from ethyl acetate; wt. 2.37 g (80.0%). The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1.

Nps-His(Tos)-OBzl. This compound was derived from His-OBzl-2Tos-OH[18] in a 72% yield in the same manner as has been described above. The melting point, the $[\alpha]_D$ value, and the results of elemental analysis are shown in Table 1.

Migration of the Tosyl Group from Boc-His(Tos) (VI-b) to Gly-OMe. Compound IV-b (4.1 g, 10 mmol) was added to a solution of the glycine methyl ester in absolute dioxane (40 ml); the free ester was then isolated from the hydrochloride (2.6 g, 20 mmol) using a procedure involving chloroform-ammonia.[22] The mixture was kept for 6 hr at 50—60 °C; then the solvent was distilled off under reduced pressure and the residue was redissolved in ethyl acetate (30 ml). The solution was shaken well with water (20 ml), washed further successively with 1 N sulfuric acid, aqueous 5% sodium bicarbonate, and water, and dried over anhydrous sodium sulfate. The dried solution was concentrated to an oil under reduced pressure, and the residue was dissolved in methanol. An aqueous 0.1 N sodium hydroxide solution (10 ml) was added to the solution, and the mixture was allowed to react for 1 hr at room temperature. Then, the methanol was removed by evaporation, and the aqueous layer was washed with ethyl acetate and acidified to pH 2 with 6 N sulfuric acid. The acidified solution was extracted well with ethyl acetate, and the extract was dried over anhydrous sodium sulfate and concentrated to a residue under reduced pressure; the residue was then recrystallized from ethyl acetate–petroleum ether; wt. 150 mg, mp 149—150 °C (reported value: 149—150 °C[24]). The IR spectrum of this material was identical with that of an authentic Tos-Gly. The melting point of a mixture of the product and the authentic Tos-Gly showed no depression. The presence of Bos-His in the water-layer was confirmed by tlc using the (2) solvent.

Treatment of Boc-His(Tos)-Gly-OBzl with HOBT. HOBT (0.87 g, 6.4 mmol) was added to a solution of Boc-His(Tos)-Gly-OBzl[25] (1.8 g, 3.2 mmol) in tetrahydrofuran (30 ml), after which the mixture was kept for 1 hr at room temperature. The solution was concentrated to residue under reduced pressure, and the residue was taken in ethyl acetate (70 ml). The ethyl acetate solution was shaken with 1 N sulfuric acid, and the aqueous layer was separated and then neutralized with sodium carbonate to pH 8. The aqueous solution was extracted with two portions of ethyl acetate (100 ml), and the combined extract was washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. The dried solution was concentrated to a residue, which was subsequently recrystallized from ethyl acetate; wt. 10 g (78%); mp 138—140 °C, $[\alpha]_D^{22}$ −33° (*c* 1, THF). This material was confirmed to be Boc-His-Gly-OBzl by elemental analysis and by a study of its spectral data. Anal. Found: C, 59.72; H, 6.56; N, 13.66%. Calcd for $C_{20}H_{26}O_5N_4$: C, 59.69; H, 6.51; N, 13.92%. IR ν_{max}^{Nujol}: 3420, 3350, 3230, 1740, 1725, 1600, 1515, 1230, 1212, 1100 cm^{-1}. UV λ_{max}^{EtOH}: absence of 233 nm (ε 10530) [attributable to the N^{im}-tosyl group of Boc-His(Tos)-Gly-OBzl].

The original ethyl acetate layer, which was shaken with 1 N sulfuric acid to extract out the Boc-His-Gly-OBzl, was washed with aqueous sodium carbonate to remove the excess HOBT, after which the organic layer was washed further with water and then dried. The dried solution was concentrated under reduced pressure, and the residue was recrystallized from ethyl acetate; wt. 0.7 g (75.5%); mp 82—84 °C. Elemental analysis showed this material to be 1-(tosyloxy)-benzotriazole. Anal. Found: C, 54.19; H, 4.01; N, 14.46; S, 10.85%. Calcd for $C_{13}H_{11}O_3N_3S$: C, 53.98; H, 3.83; N, 14.53; S, 11.06%. The IR and UV spectra of this material were identical with those of an authentic sample.

1-(Tosyloxy)-benzotriazole. HOBT (2.7 g, 20 mmol) and Tos-chloride (3.8 g, 20 mmol) were dissolved together in tetrahydrofuran (50 ml), and then triethylamine (2.8 ml) was slowly added to the mixture. The materials thus precipitated were filtered off, and the filtrate was concentrated to a residue under reduced pressure. The residue was recrystallized from ethyl acetate; wt. 2.6 g (89.7%); mp 84—85 °C. IR ν_{max}^{Nujol}: 1617, 1592, 1400, 1395, 1200, 1186 cm^{-1}, absence of 3270 cm^{-1} (attributable to the *N-OH* band of HOBT). UV λ_{max}^{THF}: 236.5 nm (ε 17230).

Treatment of Boc-His(Z)-Gly-OBzl with HOBT. When Boc-His(Z)-Gly-OBzl (0.54 g, 1 mmol) was treated with HOBT as has been described above the starting material was recovered in an 89% yield under the same reaction conditions. Mp 107—108 °C, $[\alpha]_D^{22}$ +53° (*c* 1, THF). Found: C, 62.70; H, 6.28; N, 10.43%. Calcd for $C_{28}H_{32}O_7N_4$: C, 62.67; H, 6.01; N, 10.44%.

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2) Abbreviations used in this report are those recommended by IUPAC-IUB: *J. Biol. Chem.*, **247**, 977 (1972): Aoc, *t*-amyloxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine.

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