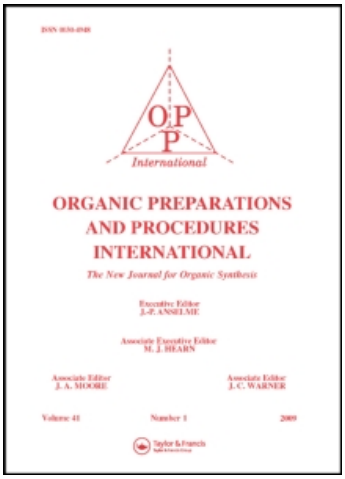

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ARGININE, HISTIDINE AND TRYPTOPHAN IN PEPTIDE SYNTHESIS.

THE IMIDAZOLE FUNCTION OF HISTIDINE†

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I. REACTIONS OF THE SIDE-CHAIN OF HISTIDINE IN PEPTIDE SYNTHESIS

The imidazole ring of histidine is often left without protection.[1-6] Yet, side-reactions and preparative difficulties should then be considered because neither the nucleophilicity nor the basic and catalytic properties of this moiety can be completely ignored.[4,5] The coupling of the N^{α}-protected histidine with the unsubstituted imidazole may proceed at a lower rate than that of other amino acids. Sometimes the yields are poor and in extreme cases there is no coupling at all.[1-3,7-10] There are two reasons for this. The basic character of imidazole makes N^{α}-protected histidine derivatives inner salts which are often sparingly soluble in solvents used in peptide-forming steps[8] and hinders the activation of the carboxyl group.[1] If this group is activated it can then intramolecularly acylate the nucleophilic imidazole (Fig. 1). This was demonstrated in the cases of azide and carbodiimide activation[11] where the imidazolide produced had a lower acylation potency than the parent compound.[2,11,12]

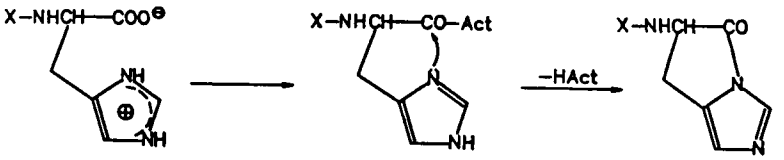


Figure 1.

During peptide bond formation, the unprotected imidazole of histidine can be acylated not only intramolecularly but intermolecularly as well.[2,4,13,14] The resulting N^{im}-acyl compounds possess also acylating properties.[2,4] Though their isolation is possible,[13] generally they are unstable.[2,4,14] In Merrifield's solid-phase peptide synthesis, no transfer of the N^{im}-acyl to the α-amino group is observed, because in trifluoroacetic acid-methylene chloride used in the next deprotection step to remove N^{α}-Boc, the α-amino functions are protonated and the imidazole groups are quantitatively deacylated.[2] A similar process of deacylation is assumed to take place during the removal of other N^{α}-protections with bases and thiols.[2] Deacylation is probably not significantly promoted by the milder acids used to remove N^{α}-2-(4-biphenylyl)-2-propoxycarbonyl and related groups.[2] Histidine also undergoes sulfonation in the course of sulfation of the Tyr residue within cholecystokinin peptides, but addition of water regenerated His quantitatively within 60 min.[15]

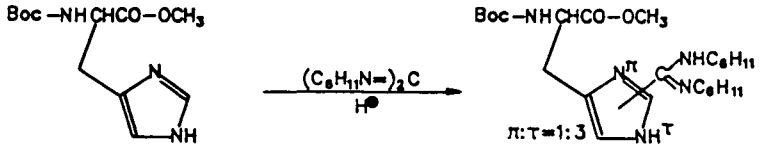


Figure 2.

Imidazole adds to dicyclohexylcarbodiimide to afford the amidine (Fig. 2). In the case of histidine derivatives, two isomers, π and τ are formed.[16] The reaction is catalysed by weak acids such as 1-hydroxybenzotriazole or triethylamine hydrochloride[16] and most likely by N-hydroxysuccinimide as well.[17] Amidines are produced first of all in the case of condensation of large peptide segments in DMF solution in the presence of an excess DCC and 1-hydroxybenzotriazole.[16,18,19] The imidazole system can be regenerated easily within simple derivatives by boiling in methanol

for two hours; addition of weak acid, e.g. an equivalent of pyridine hydrochloride or 2 N acetic acid accelerates the reaction.[16] This procedure, with extended reaction time, employed in synthetic ACTH of different species[18,20,21] led to active histidine polypeptides. However, it fails sometimes, as was the case with the porcine ACTH.[18] According to Ivanov et al.,[22] quantitative deamidination takes place during N^{α}-Boc group removal with trifluoroacetic acid or during final deprotection with hydrogen fluoride. On the other hand, according to Rink and Riniker,[16] neither trifluoroacetic acid nor concentrated hydrochloric acid, both used to take off temporary protecting groups, do not remove the amidine moiety to a high extent, and according to Yajima et al.,[18] hydrogen fluoride is also ineffective. Amidine was successfully removed from histidine residues within synthetic insulin segments by heating them in trifluoroethanol to 50-60°, possibly in the presence of hydrochloric or acetic acid.[19] Another procedure might involve the addition of a ten-fold excess of imidazole.[17] Total acidic hydrolysis of a peptide destroys the amidine.[16]

Alkylation of the histidine imidazole proceeds easily as was observed in the first attempt to anchor Boc-His(Bzl) to a chloromethylated polystyrene resin. The problem has been overcome by using a hydroxymethylated polymer to which the histidine derivative is condensed by means of DCC.[23] Alkylation of histidine, sometimes a major side-reaction, was also observed during esterification, with diazoalkanes, of ω-carboxyl groups of natural peptide fragments further used in the semi-synthesis of longer peptides. N^{im}-t-butoxycarbonylamino-2,2,2-trifluoroethyl proved to be the best protection against this side-reaction.[24]

In a given medium the imidazole moiety of histidine (pK_a 6.00) is implicated to function as an acid as well as a base (Fig. 3): on one side of the imidazole ring a proton is abstracted, while one accepted on the

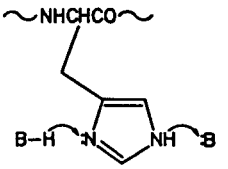


Figure 3.

other.[25] As a result, histidine can play the role of a general base and acid catalyst.[26] The catalytic effect of imidazole leads to enhanced O-acylation of the hydroxyl groups of serine, threonine and tyrosine in the course of building a peptide chain containing histidine.[6,12,27,28] Although the extent of the O-acylation can be minimized by adding pentachloro- or 2,4-dinitrophenol,[28] only the use of the N^{im}-substituted histidine suppresses this side-reaction.[12,29] During aminoacylation of histidine in a peptide segment being the anion at the C-terminus (Fig. 4), in addition to the expected peptide, a peptide of the double segment was formed[30] as probably also the result

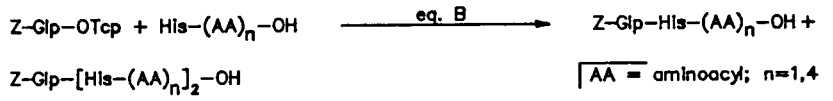


Figure 4.

of imidazole catalysis. Imidazole is a good catalyst in azide, ester and amide hydrolysis,[31-34] and thus it can catalyse cleavage of the chain in histidine-containing peptides (Fig. 5).[35]

The basic character of imidazole may complicate the isolation of histidine derivatives and peptides, especially of those with an

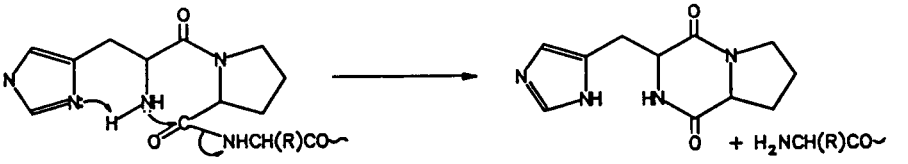


Figure 5.

unprotected carboxyl group or of those from a medium containing acids, e.g. 1-hydroxybenzotriazole.[36,37] Histidine easily forms complexes with metal ions.[26,38] Therefore, the final cleavage of some protecting groups with Zn-acetic acid leads consistently to problems in removing the last traces of zinc ions from the free peptides.[39,40] Histidine derivatives and peptides have also a tendency to bind salts, water and organic solvents[17,41,42] (Tables 6 and 8-13) and thus are somewhat difficult to characterize by elemental analysis and chromatography.[17]

The unprotonated imidazole function of the histidine residue is prone to photooxidation.[43-45] The product composition depends upon the environment of histidine in a protein and on reaction conditions. Under some conditions, aspartic acid may be a major end-product.[43] Aspartic acid is also obtained from histidine as a result of photoozonolysis at - 78° in yields greater than 80% (Fig. 6). On the basis of this reaction a quantitation of the histidine racemization attendant upon peptide synthesis was elaborated.[14]

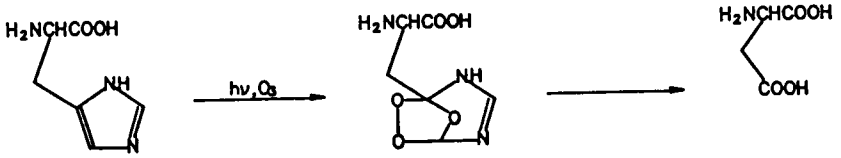


Figure 6.

At the beginning of the era of polypeptide synthesis in the 1960's, it was shown that the histidyl residue racemizes during incorporation into the peptide chain under conditions which have no effect on the configurational stability of other amino acids.[31,46,47] It is now known that the histidine imidazole moiety, both unprotected and in many cases protected, exerts a particularly strong influence on the configuration of the α-carbon of histidine in peptide synthesis. Comparative studies of racemiza-

tion rates under the influence of triethylamine, in the pentachlorophenyl ester series revealed that Z-His(Bzl)-OPcp racemizes 10,200 times faster than Z-Val-OPcp, 5,600 times faster than Z-Ala-OPcp and 11 times faster than Z-Cys(Bzl)-OPcp.[48] N^{im}-benzylhistidine (and S-benzyl cysteine as well) displays several orders of magnitude larger ease of racemization than other amino acids. Therefore, histidine appears to be unique being the fastest racemizing amino acid residue when the C-terminus is activated for coupling.[11,49] Because racemization is the main side-reaction in peptide synthesis,[50] this change of configuration induced by the imidazole ring at histidine α-carbon will be discussed in a separate section. In addition, the section will deal with methods determining racemization of histidine and with methods assigning the substituent in the imidazole ring. These latter are important for establishing the structure of histidine N^{im}-derivatives which enable the introduction of the amino acid into the peptide chain without racemization.

II. HISTIDINE RACEMIZATION IN PEPTIDE SYNTHESIS

The approach most frequently used in the investigation of histidine racemization in peptide synthesis or in chemical treatment of histidine-containing peptides is the total acidic hydrolysis of the peptides, followed by a quantitation of D-histidine in the hydrolysate. In one of these methods, the hydrolysate is reacted with the active form of glutamic acid or leucine to give glutamyl or leucyl dipeptides with the diastereoisomeric pair Glu-His/Glu-D-His or Leu-His/Leu-D-His among them. All these peptides are well-resolved in ion-exchange amino acid analyzers. If histidine peptide peaks are overlapping with peaks of other dipeptides, histidine has to be first isolated from the hydrolysate.[51-54] Separation of diastereoisomeric dipeptides by means of the amino acid analyzer allow-

ed the detection of 0.1% D-His.[55] When the isolated histidine fraction is reacted with L-phenylalanine or L-leucine N-carboxyanhydride and the formed diastereoisomeric dipeptides are resolved using reversed phase HPLC, then as little as 0.01% of D-His can be detected.[53,56] The amino acid hydrolysate can be directly chromatographed by reversed-phase HPLC applying an aqueous mobile phase containing L-proline coordinated to Cu^{++} as the chiral additive[57] or by GC (however, a prior transformation of amino acids into volatile derivatives is required) employing a chiral stationary phase.[58,59] Enzymic digestion of the hydrolysate with L-amino acid oxidase has also been elaborated in detail to achieve a reproducibility of ± 2%.[60]

Another degradative approach in the investigation of histidine racemization is the enzymic digestion of a peptide with aminopeptidase M.[58,61] Recently the configurational sequential analysis of peptides has been proposed, enabling the determination of the enantiomeric purity of the phenylthiohydantoins from Edman's degradation.[62]

In the third, more conservative approach to the investigation of histidine racemization, model peptides mainly dipeptides are used. Thus, in an ion-exchange amino acid analyzer, in addition to the aforementioned diastereoisomeric pairs of histidine dipeptides, the same pairs of histidyl dipeptides are resolved: His(Bzl)-Glu/D-His(Bzl)-Glu,[63,64] His-Ser/D-His-Ser and His-Ala/D-His-Ala.[55] By means of HPLC the following dipeptide pairs can be separated: Z(or Boc)-His-Phe-OCH_3/Z(or Boc)-D-His-Phe-OCH_3[65] and His-Leu/D-His-Leu.[66]

Finally, in some cases the detection of the diastereoisomer containing D-His directly in a synthesized oligopeptide may be successful mostly by HPLC.[53,58,67-70] Another example is ^1H NMR which enabled the demonstration of racemization at the histidine residue in one of the synthetic analogues of thyroliberin.[11]

Histidine N^{im}-derivatives can exist as τ and π isomers. Early work on the protection of imidazole nitrogens makes no distinction between substitution at N^{τ} and N^{π}, because this problem was not regarded as important for the racemization-free histidine incorporation into peptide chains. This was so much beyond the subject of consideration that in Wünsch's monograph, all histidine N^{im}-derivatives are depicted as the π-isomers.[1] At present, however, these compounds are known mainly as τ-isomers,[71] which results, in general, from a stronger nucleophilicity of this sterically less hindered position.[72] This assumption is not always valid. In the extreme case of the non-bulky, highly reactive alkylating agent, methyl mesylate, it has been reported that the second-order rate constant for first reaction at the π-nitrogen of N^{α}-acetylhistidine methylamide is significantly greater than that for the first reaction at the τ-nitrogen and the generalization that τ-substituted products are invariably predominant when histidine side-chains react with electrophiles has been contradicted.[26] The situation is further complicated by the fact that the yields of isolated products are often low: the mere fact that one product can easily be isolated in a pure state does not necessarily lead to the conclusion that it is the major component and thus the τ-derivative.[71] An excess acylating agent can lead to a mixture of the two isomers.[73,74] An alkylating agent can produce a mixture of the two isomers[75] and in addition, if used in excess, can afford the dialkyl derivative.[71] Therefore, methods to differentiate π and τ-derivatized histidines unambiguously are needed.

Various experimental approaches to this question can be found in the literature. IR spectroscopy and mass spectrometry have been used but tenuous correlations are involved and general criteria based on these techniques cannot be defined.[71] Both chemical degradation[76] and X-ray crystal-

lography[77] can be achieved only in special cases. An empirical rule based upon the coupling constant between the protons on the imidazole ring (CH) has been enunciated: compounds of the π-series should exhibit a cross-ring coupling constant 0.9-1.0 Hz whereas those with τ-orientation should give a value of 1.1-1.4 Hz.[78] However, only a modest number of examples, including only some of the types employed to N^{im}-histidine protection, have been used to derive this rule.[71] Moreover, it is not always possible to measure the constants with the necessary precision, even with advanced instrumentation, because the coupling constants are small, the ranges for the two substitution patterns abut each other and it often happens that some overlapping of the signals in the aromatic region, line broadening and/or solvent effects take place.[71,77,79] Both, π and τ-acylated histidines may be differentiated by ^{13}C chemical shifts of the carbon atoms of the imidazole ring and by ^{1}H chemical shift of the hydrogen-5 of the ring (Fig. 7).[73,80] The method, which is appropriate for substituents of N^{im}-CH_2-type, involves the measurement of nuclear Overhauser effects. If the substituent is at the π-position, the CH_2 signal is enhanced if the low-field adjacent proton between the heterocyclic nitrogens is irradiated. This is not observed if the high-field, more distant, ring proton is irradiated. If the substituent is at the τ-position, the CH_2 signal is enhanced irrespective of which the equidistant ring protons is irradiated.[71,79,81] In numerous instances, the simple dinstinction of N^{im}-structures can be achieved by conversion of a derivative of unknown orientation to one of the two known N^{im}-methylhistidines which is identified by means of amino acid analysis,[71,81] but HPLC or GC give equally good results.[71]

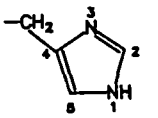


Figure 7.

Histidine racemization during peptide synthesis has been a subject of systematic investigations since the 1970's. At first, dependence of racemization of Boc-His, Z-His and their derivatives upon N^{im}-protecting groups, coupling reagents and conditions was examined. Tables 1 and 2 list the results for conventional and solid-phase peptide synthesis. As can be seen, even Z-His-N_3 racemizes, although to a small extent. Dicyclohexylcarbodiimide and N-ethyl-5-phenylisoxazolium-3'-sulfonate can cause extensive racemization. In the case of DCC, the addition of 1-hydroxybenzotriazole or N-hydroxysuccinimide suppresses the side-reaction. Under comparable conditions a remarkably higher racemization, sometimes almost quanitative, is observed for X-His(Bzl) than for other N^{im}-derivatives. The racemization seems to be dependent upon the basicity of a derivative. Derivatives protected with N^{im}-urethane groups or N^{im}-tosyl group racemize only to a small extent. The amount of racemization also depends on the choice of the tertiary amine and on polarity of the solvent. More racemization takes place during peptide synthesis on the solid phase than during that in solution.

In 1975, Veber ascertained that when solutions of either Glp-His-N_3.HCl or Boc-His-N_3.HCl in DMF are made neutral or basic by the addition of triethylamine in the absence of acylatable amine, the intermediate imidazolide, shown in Figure 8, is within 30 sec and reversibly formed. This is followed by a slower racemization at the histidine residue. The more basic the medium, the higher is the racemization. Boc-His treated with DCC

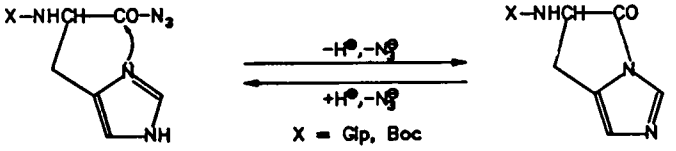


Figure 8.

TABLE 1. *Racemization of Histidine in Synthetic Peptides Obtained in Solution*

Derivative	Coupling Method	Solvent	D-His (%)	lit.
Z-His	N_3	DMF	0.8	55
Z-His(Z)	DCC	DMF	0.6	55
Z-His(Bzl)	DCC	DMF	1.8	55
Z-His(τ-Pac)	DCC	DMF	35	39
Z-His(π-Pac)	DCC	DMF	<2	39
Boc-His(Bzl)	DCC	CH_2Cl_2	1.8	63
Boc-His(Boc)	DCC	CH_2Cl_2	0.4	55
Z-His(Tos)	DCC	CH_2Cl_2	0.3	55
Boc-His(Bzl)	DCC, HOSu	CH_2Cl_2	<0.1	63
Boc-His(Bzl)	NEPIS	DMF	24	63
Z-His(Z)	NEPIS	CH_3CN	0.4	55
Boc-His	ClCOOiBu, NMM	DMF	<1	82
Boc-His(Bzl)	ClCOOiBu, NMM	DMF	7	82
Boc-His(Ztf)	ClCOOiBu, NMM	DMF	4	82
Boc-His(Tos)	ClCOOiBu, NMM	DMF	<1	82
Boc-His(Dnp)	ClCOOiBu, NMM	DMF	6	82
Boc-His(Boc)	ClCOOiBu, NMM	DMF	<1	82
Boc-His(Z)	ClCOOiBu, NMM	DMF	<1	82
Z-His(Z)	ClCOOiBu, NEt_3	THF	0.2	64
Z-His(Z)	ClCOOiBu, NMM	THF	<0.1	64
Boc-His(Boc)	ClCOOiBu, NMM	THF	0.3	64

TABLE 2. *Racemization of Histidine in Synthetic Peptides Obtained with Merrifield's Method*

Derivative	Coupling Method	Solvent	D-His (%)	lit.
Boc-His	DCC	DMF	5	63
Z-His	DCC	DMF	4.8	55
Boc-His(Bzl)	DCC	DMF	25	63
Z-His(Bzl)	DCC	DMF	37	55
Boc-His(Bzl)	DCC	CH_2Cl_2	11-12	63
Boc-His(Boc)	DCC	CH_2Cl_2	1.5	55
Boc-His(Ztf)	DCC	CH_2Cl_2	0.5	63
Boc-His(Tos)	DCC	CH_2Cl_2	0.5	83
Boc-His(Dnp)	DCC	CH_2Cl_2	0.6-1	63,83
Boc-His(Bzl)	DCC	DMF-CH_2Cl_2	48	84
Boc-His(π-Pac)	DCC	DMF-CH_2Cl_2	<2	84
Boc-His	DCC	DMF-CH_2Cl_2	2.3	83
Boc-His(Bzl)	DCC, HOSu	CH_2Cl_2	<0.1	63
Boc-His(Bzl)	DCC, HOBt	CH_2Cl_2	<0.3	64
Boc-His(Boc)	DCC, HOSu or HOBt	CH_2Cl_2	0.2	63
Boc-His(Bzl)	NEPIS	DMF	36	63
Boc-His(Bzl)	EEDQ	CH_2Cl_2	10	63
Boc-His(Bzl)	CDI	CH_2Cl_2	0.3	63
Boc-His(Bzl)-ONp	ONp	DMF	4.3	63

in dioxane produces also racemic Boc-His-imidazolide. Then, during peptide synthesis with the unprotected histidine imidazole, racemization is due to aminolysis of the racemic imidazolide.[11] This hypothesis coincides with the influence of the type and amount of the tertiary amine on the extent of the histidine racemization[31,46] as well as with the great tendency toward racemization of imidazolide as active species of the carboxyl group.[47] The observed effectiveness of N-methylimidazolide as acyl transfer catalyst would suggest a quaternary imidazolide formation. Therefore, simple substitution in the τ-position of the histidine imidazole does not necessarily prevent cyclization. Consequently, if the histidine residue is to be included into peptide chain without racemization, the nucleophilicity of the imidazole π-nitrogen has to be blocked.[11]

In 1978, Jones synthesized Z-His(π-Pac) and Z-His(τ-Pac) and according to the concept of Veber, condensed them with Pro-NH_2 with the aid of DCC under identical conditions.[39,84] π-Protected isomer led to optically pure dipeptide (less than 2% of D-His) whereas the τ-isomer suffered gross racemization (35% D-His). In an equimolar DMF solution of Z-His(τ-Pac) and dicyclohexyl- or diisopropylcarbodiimide at 0^{o}, there was no detectable reaction (TLC, NMR) over an hour period but there was a first-order loss of optical activity. After adding 1 equiv. Gly-OEt, the dipeptide and N-acylurea, racemized to the same extent, were isolated. These data have been rationalized as follows (Fig. 9). The loss of optical activity of N^{τ}-protected histidine derivatives results from intramolecular α-proton abstraction by the basic π-nitrogen atom. In the corresponding π-derivatives, π-nitrogen atom is not able to act as a base. Therefore such an intramolecular abstraction is impossible and these compounds do not racemize.[85] The process must be intramolecular because an added tertiary base does not increase the amount of racemization[85] (see ref.[86] also). A

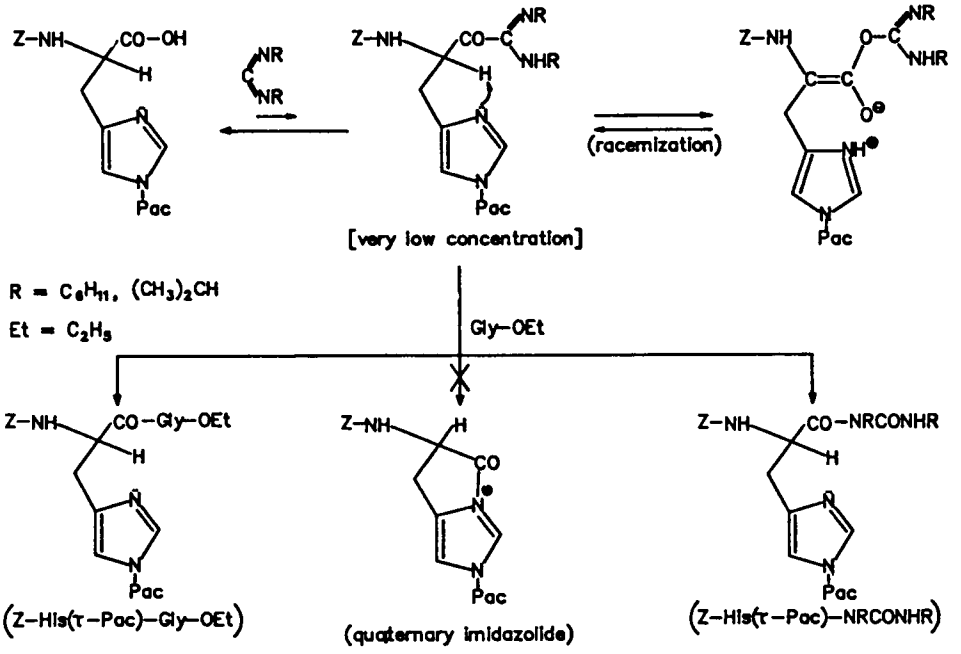


Figure 9.

behavior similar to that of N^{im}-phenacyl derivatives is also shown, during examination of optical stability, by Boc-His(τ-Bom), Boc-His(π-Bom) and their corresponding bromo homologues, all activated with diisopropylcarbodiimide.[40] Racemization could not be prevented by decreased basicity of the π-nitrogen atom, brought about by halogenation of the imidazole ring in the histidine derivative (Fig. 10).[40]

The mechanism closely related to that given by Jones[85] has been used to interpret the histidine racemization during deprotection by catalytic

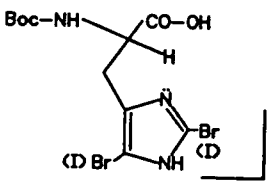


Figure 10.

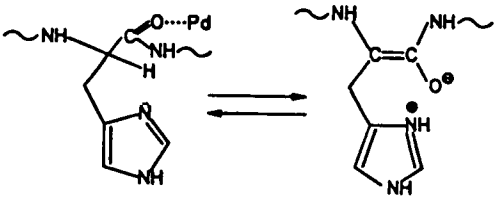


Figure 11.

transfer hydrogenation of an analog of the luteinizing hormone-releasing hormone.[87] The ratio of D-His^2/L-His^2 reaches 12/88 when ammonium formate in the presence of palladium acetate is used. The complexation of the CO groups of the peptide enhances the acidity of the histidine-residue α-hydrogen which is then able to protonate the imidazole N^{π} (Fig. 11). This results in stabilization of the enol form whose protonation in a subsequent step gives a mixture of L- and D-histidine. In the course of reduction by formic acid in the presence of palladium black, the histidine residue does not racemize appreciably (1% D-His). Formic acid protonates the imidazole group thus preventing abstaction of the α-hydrogen and, consequently, racemization cannot occur.

Kovacs's work[86] indicates that N^{α}-alkoxycarbonyl-N^{τ}-protected histidine active esters can racemize according to other mechanisms as well. Z-His(Bzl)-OPcp autoracemizes (Fig. 12) which, indeed, coincides with the

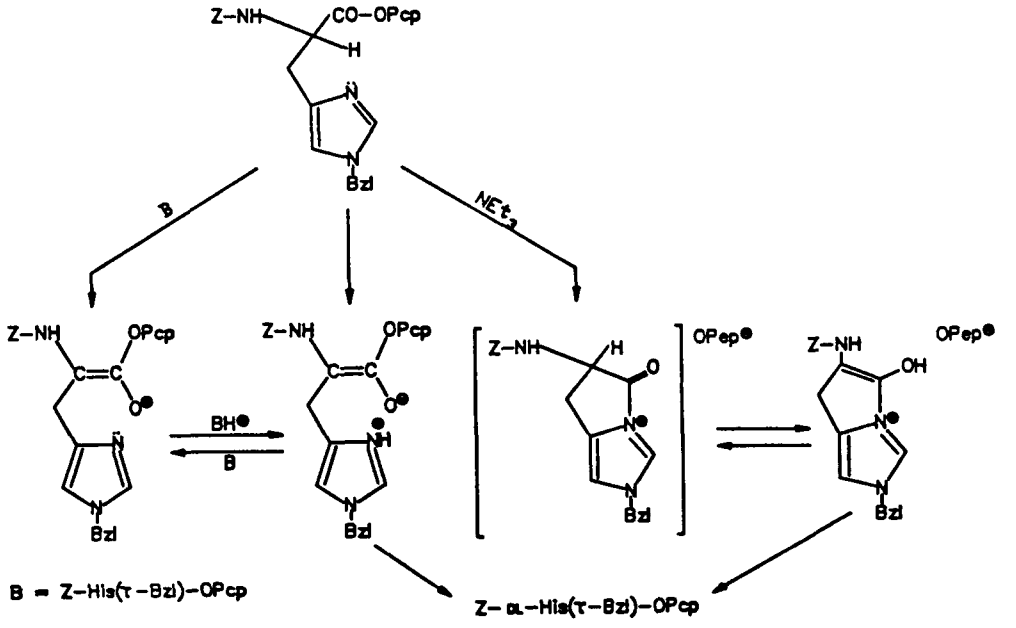


Figure 12.

above-discussed behavior of Z-His(τ-Pac)-CO(=NR)NHR [where R = C_6H_{11} or $(CH_3)_2CH$; Fig. 9]. However, kinetic data show that this process is not solely intramolecular but also intermolecular since the racemization of the ester depends upon the concentration of triethylamine (Fig. 12). Yet, this is valid only to about equivalent concentration of the base (Table 3) Above this value the racemization rate is more or less concentration independent. Below about 0.5 equiv of triethylamine, the reaction is second order as is the case for other amino acids. The fact that more than 1 equiv of base does not increase the racemization rate in practice seems to indicate that an intermediate is formed from Z-His(Bzl)-OPcp by the base or with the base; it probably racemizes mainly by an intramolecular mechanism independent of the presence of additional triethylamine. However, the IR spectra of basic solutions of Z-His(Bzl)-OPcp show the continued presence of the active ester and no other products.[86] Jones exluded the formation of the quaternary imidazolide in the instance of carbodiimide

TABLE 3. *Dependence of the Racemization Rate of 0.05 M Z-His(Bzl)-OPcp in Tetrahydrofuran on the Concentration of Triethylamine [86]*

$[NEt_3]$ (M)	total obsd k_r ($10^{-6}s^{-1}$)	NEt_3 catalyzed k_r ($10^{-6}s^{-1}$)	NEt_3 catalyzed $k_r/[NEt_3]$ ($10^{-6}M^{-1}s^{-1}$)	Reaction
0	23	0	6 000[a]	
0.005	51	28	5 600	second order
0.015	110	87	5 800	
0.025	180	157	6 300	
0.05	240	217	4 300	
0.35	280	257	730	first order
0.75	280	257	340	

a Extrapolated.

activation (Fig. 9).[85] However its formation, in undetectable quantities, from pentachlorophenyl-ester activation and its first-order racemization, depicted in Figure 12, may provide an explanation as suggested by Veber.[11]

Table 4 lists relative rate constants of the triethylamine-induced racemization of histidine active esters, which decrease as follows:

for N^{im}-benzyloxycarbonylhistidine: OTcp > ONp > OPcp

for N^{im}-tosylhistidine: OTcp > OPcp > ONp.

These results do not agree with the general trend to racemization of active esters of other amino acids, being parallel with the electron-withdrawing ability of the phenoxy group. These data draws attention to

TABLE 4. *Relative Racemization Rate Constants of Active Esters of Z-His(X) in Tetrahydrofuran [86]*

X	OPcp	OTcp	ONp
Tos	1.0	1.2	0.56
Z	1.0	1.7	1.30
Bzl	1.0	0.02	-

the trace amounts of racemization of Z-His(Bzl)-OTcp as compared with Z-His(Bzl)-OPcp. Z-His(Bzl)-OTcp does not show measurable autoracemization in 24 hrs, and the second-order racemization rate constant does not depend on the base concentration. As the only exception among the investigated active esters, this compound displays the discrepancy between the measured racemization rate constant (k_r) and the calculated one based on the additivity principle.[86,88] The calculated value is 115 times larger than the experimental value. This probably indicates deviation from the reaction mechanism for racemization of the ester. Another anomaly was

observed in the case of Boc-His(Dnp)-OTcp which racemizes 63 times faster in DMF than in THF whereas for N-protected amino acid active esters this coefficient amounts normally to about 10.[86] The data presented prove conclusively the difference in racemization mechanisms of histidine from those of all other amino acids and moreover, illustrates the variety and complexity of the mechanisms.

Table 5 gives the coupling and racemization rate ratios of histidine active esters. Provided that the racemization rate measured for triethyl-

TABLE 5. *Coupling and Racemization Rate Ratios of Histidine-Active Ester Derivatives in Tetrahydrofuran [86]*[a]

Z-Ala-ONp	730	Z-Ala-OPcp	6 100
Z-His(Tos)-ONp	340	Z-His(Z)-OPcp	1 300
Z-His(Z)-ONp	150	Z-His(Tos)-OPcp	640
Z-His(Bzl)-ONp	-	Z-His(Bzl)-OPcp	10
Z-Ala-OTcp	730	Boc-His(Dnp)-OTcp	840
Z-His(Tos)-OTcp	360	Boc-His(Dnp)-OPcp	2 400
Z-His(Z)-OTcp	310	Boc-His(Bzl)-OTfp.HOTfp	1 700[b,c]
Z-His(Bzl)-OTcp	50	Boc-His(Bzl)-OPfp.HOPfp	1 700[b,c]

a. Coupling with 1 equiv. Val-OCH_3; racemization in the presence of 7 equiv. NEt_3. b. 8 equiv. NEt_3. c. [49].

amine parallels that induced by an amine component during coupling, the larger this ratio, the smaller is the extent of racemization in peptide synthesis. One can conclude from these data that during incorporation of the histydyl residue into peptide chain, the configurational stability of the α-carbon atom is more pronounced for N^{im}-tosyl and N^{im}-urethane histidine derivatives than that for N^{im}-benzyl homologues.[86] The statement agrees with the amount of racemization during model peptide synthesis (Tables 1 and 2). Nevertheless it is worth noting that both 2,3,5,6-

tetrafluorophenyl and pentafluorophenyl ester prevent extensive racemization of Boc-His(Bzl). In contrast to the second compound which is apparently unstable in solution, the first one can be obtained in large quantities as an analytically pure species.[49]

Preventing the side-reactions discussed, including particularly that of racemization accompanying peptide syntheses with histidine, requires the appropriate protection of the imidazole, especially that of the π-nitrogen atom since its nucleophilicity and/or basicity are the cause of the racemization at this amino acid.

III. PROTECTING GROUPS FOR IMIDAZOLE FUNCTION OF HISTIDINE

At present, the following residues are used for the protection of the N^{im}-position of histidine:

1. alkyl groups
2. 2,4-dinitrophenyl
3. urethane groups
4. piperidinocarbonyl
5. arylsulfonyl groups
6. phenacyl groups
7. 1-alkoxycarbonylamino-2,2,2-trifluoroethyl groups
8. alkoxyalkyl groups.

1. Alkyl Groups

Alkyl groups belong to the earliest protections of the histidine imidazole and among them, the benzyl residue (Fig. 13) was up to the early 1970's the most popular.[1] The N^{im}-benzylhistidine, used in peptide synthesis is obtained, most probably as a mixture of both isomers,[89,90] by alkylation of histidine with benzyl bromide in the presence of liquid ammonia.[1] However, the commercial Boc-His(Bzl) (Sigma) proved to be the pure τ-isomer.[71] This protecting group is removed under forcing reductive conditions, either with sodium in liquid ammonia or

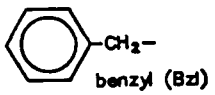


Figure 13.

catalytically, which can lead to side reactions.[1,87,91] The racemization of the N^{im}-benzylhistidine residue during peptide bond formation (Tables 1 and 2) resulted in a loss of importance of the benzyl group. At present, it is employed only sporadically[92,93] and not recommended[2,94] with the exception of Boc-His(Bzl)-OTfp.[49] Yet, three recently proposed methods for π-selective introduction of the benzyl group onto the histidine imidazole should be mentioned (Fig. 14).[74,89,95] Moreover, Boc-His(π-4Nb)-OCH_3, Boc-His(π-2Mb)-OCH_3, Boc-His(π-3Mb)-OCH_3, Boc-His(π-4Mb)-OCH_3 and from them dihydrochlorides of the corresponding π-alkylated histidine esters (method 2)[89] and Boc-His(π-3,4Dmb)-OCH_3 and 2HCl.His(π-3,4Dmb)-OCH_3 (method 3),[95]

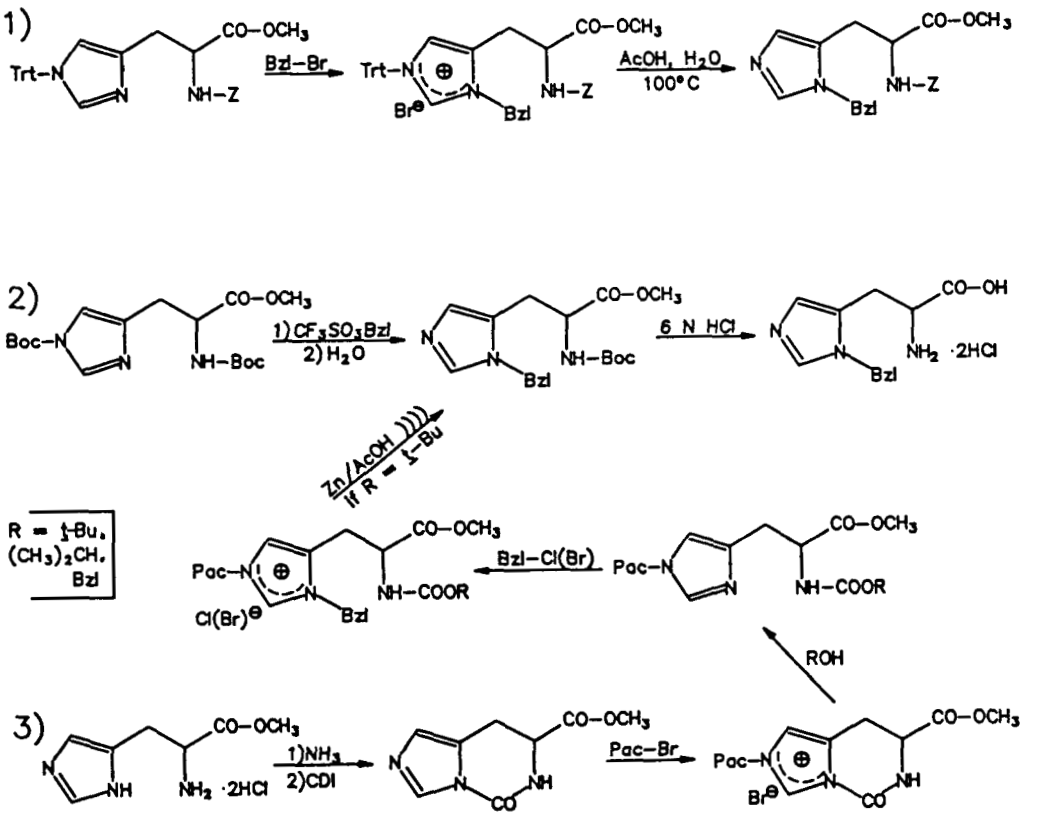


Figure 14.

TABLE 6. *N^{im}-Benzyl Derivatives of Histidine (1987-1988) [96]*

Compound	mp. (°C)	$[\alpha]_D^{20-27}$ (°)	lit.
Boc-His(π-Bzl)-OCH_3	glassy powder	-	74
	colorless gum	-	89
Boc-His(π-Bzl)	138-141	+ 4.86 (c 0.5, CH_3OH)	74
His(π-Bzl).2HCl.0.5H_2O	199-210[a,b]	+ 6.5 (c 2.0, H_2O)	89
	201-210[b]	+ 7.2 (c 2.04, H_2O)	95
His(π-Bzl)	288-231[b,c]	+ 6.04 (c 1.49, 1NHCl)	89
Boc-His(Bzl)-OPcp	131-132	- 2.25 (c 1.78, $CHCl_3$)	86
Boc-His(Bzl)-OTfp.HOTpf	110-110.5	-12 (c 1, THF)	49
Boc-His(Bzl)-OPfp.HOPfp	d	-	49
Z-His(Bzl)-OPcp	110-112	+ 8.23 (c 2.71, THF)	86
Z-His(Bzl)-OCH_3.HCl	48[a]	- 14.80 (c 2.77, CH_3OH)	86
Z-His(Bzl)-$NHNH_2$	148-149.5	- 7.59 (c 1.08, AcOH)	86
Fmoc-His(Bzl)	-	-	93
Boc-His(π-4Nb)-OCH_3	-	-	89
His(π-4Nb).2HCl	145-155[b]	+ 8.8 (c 2.2, CH_3OH)	89
Boc-His(π-2Mb)-OCH_3	colorless gum	-	89
His(π-2Mb).2HCl	226-228[b]	+ 5.7 (c 1.15, H_2O)	89
Boc-His(π-3Mb)-OCH_3	108-110	- 6.3 (c 2.05, CH_3OH)	89
His(π-3Mb).2HCl	220-225	+ 5.2 (c 2.04, CH_3OH)	89
Boc-His(π-4Mb)-OCH_3	colorless gum	-	89
His(π-4Mb).2HCl.1/3H_2O	125-128[b]	+ 6.5 (c 1.63, H_2O)	89
Boc-His(π-3,4Dmb)-OCH_3	colored gum	-	95
His(π-3,4Dmb).2HCl.0.5H_2O	83-110[a,e]	+ 0.55 (c 2.20, H_2O)	95

a. Hygroscopic solid. b. Decomposition. c. Sic. d. Analytically pure salt has not been obtained by recrystallization. e. Glass.

all of potential use in peptide synthesis, were obtained. No removal of π-benzyl or these derivatized π-benzyl groups was examined. Table 6 gives N^{im}-benzyl and related histidine derivatives both new and those resynthesized in 1975-1988.

In contrast to the benzyl group, N^{τ}-trityl group (Fig. 15), used as early as in the mid-50's has been gaining more and more acceptance and use since the mid-70's. This is due to its stability against nucleophiles, even hydrazine and thiols, and its removal by mild acidolysis under various conditions or by catalytic hydrogenolysis[67,68,97,98] and - as has been recenty documented - in most cases reduced susceptibility to racemization of His(τ-Trt) derivatives compared to those without Trt (Table 7).

TABLE 7. *Racemization of Histidine and τ-Trityl Histidine Derivatives in Model Peptide Synthesis (% D-His)*[67,68]

	A	B
Fmoc-His(τ-Trt)	0.2	0.1
Fmoc-His	1.1	0.7
Boc-His(τ-Trt)	0.3	0.5
Boc-His	0.7	0.4

A. Synthesis of His-Val-Val on solid-phase.
B. Synthesis of His-Val-Gly-Ala-Pro-NH_2 in solution.

The presence of N^{τ}-Trt has also prevented the formation of several minor by-products which occurred with an unprotected side-chain.[67,68] Therefore, Nτ-trityl has been suggested as protecting group with the α-Fmoc--ω-tBu strategy.[67,68,99-104] However, τ-Trt does not secure histidine against racemization which may occur under unfavorable conditions.[67,102,104] A system α-Boc--N^{τ}-Trt can be employed, although with some limitations, for

peptide synthesis in solution which was exemplified by preparation of a pentapeptide segment of a mast-cell-degranulating peptide[105] and of the human insulin B-chain and its analogue.[106] N^{τ}-trityl has been also exploited as a temporary protection for the regioselective introduction of π-Bzl, π-Pac and π-Bom into Z- and Boc-histidine methyl and benzyl esters (see Figures 14, 26 and 32). From the resultant intermediate imidazolium halides, the trityl group was removed selectively in the presence of N^{α}-Boc by means of an equivalent of silver acetate in aqueous acetic acid (see Figures 26 and 32).[39,40,107] The τ-trityl-protection can be simply and selectively introduced by treatment of the histidine under mild conditions with difunctional silane $(CH_3)_2SiCl_2$ followed by an equivalent of Trt-Cl (Fig. 15, route i).[108] Reaction of histidine with one equivalent of

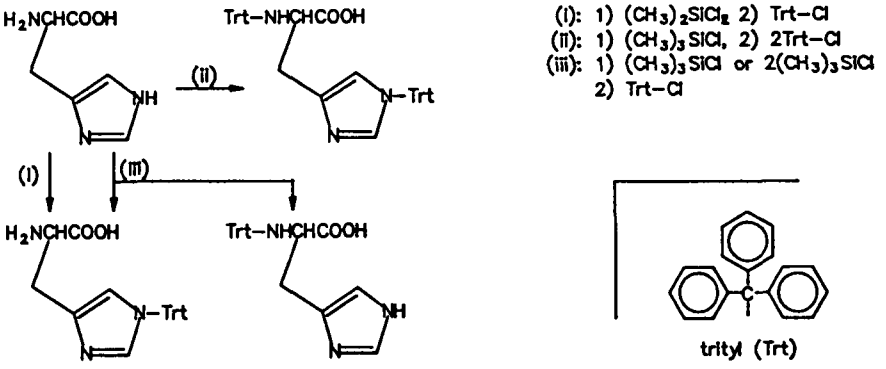


Figure 15.

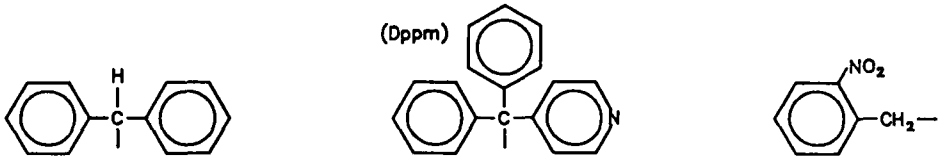
$(CH_3)_3SiCl$ (Fig. 15, route ii)[108] or three equivalents of that[109] and alkylation with two equivalents of Trt-Cl leads to N^{α},N^{τ}-ditritylhistidine[108,109] which may be used in peptide synthesis due to selective acidolytic removal of N^{α}-trityl.[67,68,99,104] Treatment of histidine with one or two equivalents of $(CH_3)_3SiCl$ followed by one equivalent of Trt-Cl gives a mixture of N^{τ}-trityl- and $N^{\alpha,\tau}$-ditritylhistidine (Fig. 15, route iii).[108]

TABLE 8. *N^{im}-Benzhydryl and N^{τ}-Trityl Derivatives of Histidine (1976-1988) [96]*

Compound	mp. (°C)	$[\alpha]_D^{20-25}$ (°)	lit.
His(τ-Trt)	218-220		108
	220-222[a]	- 2.1 (c 1, THF:H_2O=1:1)	108
	221	- 2.2 (c 1, TFH:H_2O=1:1)	99
Boc-His(τ-Trt)-OCH_3	foam	-	39
	oil	-	105
His(τ-Trt)-OCH_3	122	+ 7 (c 1, CH_3OH)[b]	105
Boc-His(τ-Trt)-OBzl	white crystals	-	107
Z-His(τ-Trt)-OCH_3	58-63[c]	+ 12.8 (c 1, $CHCl_3$)	39,71
Fmoc-His(τ-Trt)	133-134	+ 76.8 (c 1, $CHCl_3$)	99
	150[d]	+ 86.6 (c 5, $CHCl_3$)	68
Fmoc-His(τ-Trt)-OTcp	amorphous	-	67
Fmoc-His(τ-Trt)-OPcp	-	-	112
Trt-His(τ-Trt)	198	+ 3.6 (c 0.5, C_5H_5N)	108
	179-199	-	109
Trt-His(τ-Trt)-OTcp	amorphous	-	67
Boc-His(π-Bzh)-OCH_3	-	-	95
His(π-Bzh).2HCl.0.5H_2O	85-100[e]	+ 1.43 (c 2.03, H_2O)	95
Boc-His(Dppm)-OCH_3	oil	+ 2.0 (c 1.2, $CHCl_3$)	110
Boc-His(Dppm)-$NHNH_2$.Et_2O	92-93	+ 5.0 (c 1, CH_3OH)	110
Boc-His(Dppm).0.25Et_2O	foam	+ 10.0 (c 1.0, CH_3OH)	110
Z-His(Dppm)-OCH_3	-	+ 4.0 (c 1, CH_3OH)	110
Z-His(Dppm)	109-110	+ 10.5 (c 0.9, CH_3OH)	110

a. Analytical sample. b. $[\alpha]_{578}^{25}$. c. Crisp foam. d. Decomposition. e. Glass.

To improve the selectivity of N^{α}-Boc cleavage in the presence of N^{im}-protection, N^{im}-benzhydryl (Fig. 16) was suggested. It is resistant to 24 hours' treatment with 1 N HCl in acetic acid, but is removed within 10 min by formic acid.[97,98] Other protecting groups include N^{im}-diphenyl-4-pyridylmethyl and N^{im}-2-nitrobenzyl (Fig. 16) which are not acidolytically cleavable. The first undergoes a catalytic and electrolytic as well Zn-acetic acid reduction.[109,110] The N^{im}-2-nitrobenzyl residue is photolabile and in addition is slowly cleaved by catalytic hydrogenation.[111] Recently Boc-His(π-Bzh)-OCH_3 and 2HCl.His(π-Bzh)-OCH_3 have been reported.[95] However, the removal of the π-Bzh moiety was not examined.



benzhydryl (Bzh) diphenyl—4—pirydylmethyl 2—nitrobenzyl (2Nb)

Figure 16.

Table 8 presents N^{im}-benzhydryl, N^{τ}-trityl as well related histidine derivatives both new and those resynthesized in 1975-1988.

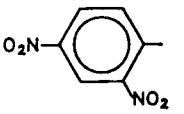
2. 2,4-Dinitrophenyl Group

Among histidine compounds with N^{im}-2,4,dinitrophenyl group (Fig. 17), Boc-His-(Dnp) is of fundamental importance for peptide synthesis, especially with Merrifield's method.[2,94,113] According to some investigators,[113] it is a typical histidine derivative for Merrifield's large scale peptide synthesis. The preparation of Z-His(Dnp) poses some difficulties.[10] N^{im}-Dnp stands up to strongly acidic media, even to total hydrolysis of peptides in 6 N HCl,[2,3,98] but is easily removed by thiolysis at pH 7-8 with mercaptans such as 2-mercaptoethanol or mercaptoacetic acid under

TABLE 9. *N^{im}-2,4-Dinitrophenyl Derivatives of Histidine (1974-1988) [96]*

Compound	mp. (°C)	$[\alpha]_D^{20-22}$ (°)	lit.
Boc-His(Dnp)-OTcp	105-107	+ 8.49 (c 2.12, DMF)[a]	86
	98	+ 1.8 (c 2, Dioxane)	123
Boc-His(Dnp)-OPcp	109	+ 5.8 (c 1, Dioxane)	124
Boc-His(Dnp).$(CH_3)_2CHOH$	-	-	93
His(Dnp)-OCH_3.2HCl.AcOEt	-	-	93
Z-His(τ-Dnp).HCl	203-205	- 7.8 (c 1, CH_3OH)	76

a. $[\alpha]_{470}^{20}$.

Figure 17. 2,4-dinitrophenyl (Dnp) 

non-aqueous or aqueous conditions[3,98,114,115] or with thiophenol in dimethylformamide.[2,82,94,116,117] It suffers from susceptibility to nucleophiles such as hydrazine and amino acid α-amino groups[3,86,98] and moreover, to photolytic degradation.[2,118] In some cases the dinitrophenyl group may not protect sufficiently against racemization during peptide synthesis in solution (Table 1). Therefore, at present, the masking moiety is used mainly in Merrifield's method[113,116,117,119-122] but only sparsely in peptide synthesis in solution.[120,125] Table 9 lists N^{im}-2,4-dinitrophenyl histidine derivatives both new and those resynthesized in 1974-1988.

3. Urethane Groups

The N^{im}-urethane protecting groups have a number of properties in common. Among them the most outstanding is the suppression of histidine racemization.[2,70] Yet, the urethane derivatives suffer from some shortcomings. First, they are reported to be unstable at room temperature and difficult to crystallize.[3,10,40,89,126] The problem may be solved by

careful preparation using pure and strong acylating agents,[126,127] and possibly by blocking the histidine carboxyl group with silyl ester.[127] Another approach is to transform the crude compounds into either dicyclohexylammonium salts or easily crystallized active esters such as 4-nitrophenyl.[3,126] However, yields of the salts may be poor and the esters unstable as well.[126] Second, N^{im}-urethane compounds, though each to a different degree and to lesser extent than N^{im}-acyl derivatives, are sensitive to nucleophiles even to reagents as mild as water or methanol.[2,65,128] Transfer of N^{im}-urethane moiety, resulting in acylation of α-amine groups has been pointed out in model investigations.[65,129] Although so far these reactions were not observed under peptide synthesis conditions, but with urethanes sufficiently stable during deprotection of α-amino group, the risk of an intermolecular $N^{im} \dashrightarrow N^{\alpha}$ shift accompanied by premature peptide chain termination in solid phase peptide synthesis needs to be considered.[2,3,8] Third, due to poor solubility of N^{im}-urethane-histidine derivatives they can adhere to resin beads and hence one can observe further side-reactions.[8] In strong and moderate anhydrous acids, any given N^{im}-urethane is slightly more stable than the corresponding N^{α}-urethane group,[130] but the N^{im}-urethane group can be selectively removed by hot weak acids.[2,3]

Among N^{im}-urethane derivatives, in Merrifield's peptide synthesis, until quite recenly, Boc-His(Z)[131,132] and Boc-His(Boc)[7,133,134] were most often used. N^{im}-Z (Fig. 18) stands up to repetitive N^{α}-Boc deprotection with trifluoroacetic acid[2] and possibly, protonation of the imidazole ring is responsible for the stability of the N^{im}-Z group in the acid.[3] N^{im}-Z is cleaved with hydrogen fluoride, 2 N HBr in acetic acid, hydrogen (catalytically), sodium in liquid ammonia, hydrazine, aqueous solutions of alkali, ammonia or amines,[2,3,86] and with trimethylsilyl triflate or

iodide.[127] Boc-His(Boc) has also been used for peptide synthesis in solution.[135] Boc-His(Boc)-OCH_3 is applied to the regioselective introduction of π-Bzl (Fig. 14),[89] π-Bom (Fig. 32)[40] and π-4BrBom,[40] and Z-His(Boc)-OCH_3[74] is employed to the same introduction of π-Bum (Fig. 33)[136]. N^{im}-Boc

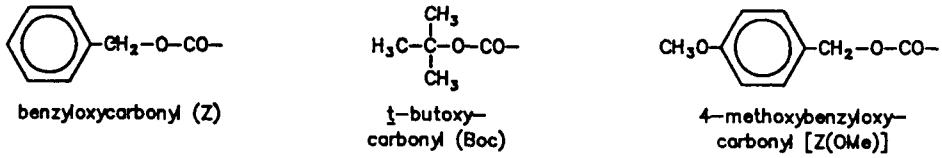
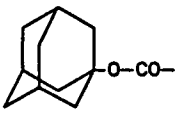


Figure 18.

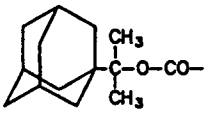
(Fig. 18) is removed with hydrogen fluoride, trifluoroacetic acid, mineral acids in organic solvents (but not with HCl in dioxane[130]), hydrazine hydrate, ammonia or NaOH in methanol.[2,3,8,128,137] Although the rate of acidolysis of N^{im}-Boc is slower than that of N^{α}-Boc,[2,3,130] the selective cleavage of the latter group occurs from Boc-His(Boc),[130] but not from a protected peptide.[7] In solid phase even N^{α}-2-(4-biphenylyl)-2-propoxycarbonyl group could not be eliminated selectively in the presence of N^{im}-Boc.[7] The importance of N^{im}-Boc protection, especially in connection with pentafluorophenyl-ester activation, seems to increase[70,138-141] as the importance of Fmoc-based solid phase peptide synthesis increases.[94,113] Fmoc-His(Boc) can be produced directly from Fmoc-His(Fmoc).[70] Z(OMe)-His[Z(OMe)]-ONp [for Z(OMe), see Fig. 18] was also obtained and used for incorporation of histidine into a tripeptide linked to a resin; both N^{α}- and N^{im}-protecting groups were removed by acidolysis.[8]

More suitable for storage than N^{im}-Boc compounds are N^{im}-adamatyloxycarbonyl derivatives (Fig. 19) whose susceptibility to acidolysis lies between that of N^{im}-Z and that of N^{im}-Boc.[3] The introduction of adamantyloxycarbonyl by means of Adc-F into Boc-His-OCH_3 or histidine-containing

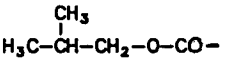
peptides affords a mixture of τ and π isomer.[73] Adc-His(Adc) is used in peptide synthesis in solution[142] and in solid phase.[2,94] N-1-(1-Adamantyl)-1-methylethoxycarbonyl (Fig. 19) was developed as α-amino protecting group of intermediate sensitivity to the proton attack between that of N^{α}-Boc and that of N^{α}-2-(4-biphenylyl)-2-propoxycarbonyl group.[143] Using Adpoc-F, Adpoc-His(Adpoc) was synthesized and the latter was used to produce thyroliberin; N^{im}-Adpoc served as a temporary protection. Both N^{α}- and N^{im}-Adpoc were removed within 18 min with 5% trifluoroacetic acid in methylene chloride.[144] By activation of Boc-His with 2 equivs. of isobutyl chloroformate, the mixed anhydride of Boc-His(Ioc) is easily formed.[82]



adamantyloxy-carbonyl (Adc)



1-(1-adamantyl)-1-methyl-ethoxycarbonyl (Adpoc)



Isobutoxycarbonyl (Ioc)

Figure 19.

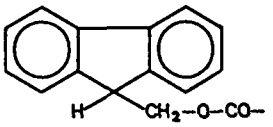
N^{im}-Ioc (Fig. 19) secures histidine against racemization; it is resistant to brief treatment with trifluoroacetic acid in methylene chloride or to catalytic hydrogenation and is removed by methanol in the presence of triethylamine or of pyridine hydrochloride, by alkali and nucleophiles. Applying the protection in peptide synthesis would require reaction conditions selected with great care.[65]

In Fmoc-chemistry solid phase peptide synthesis, Fmoc-His(Fmoc) (Fig. 20) is sometimes used.[70,93,94,101,145] In some instances, it fails because of low solubility.[101] Both Fmoc groups are removed simultaneously.[70] For Merrifield's solid phase peptide synthesis recently the N^{im}-2bromobenzyloxycarbonyl (Fig. 20) instead of N^{im}-Z has been elaborated and [Boc-

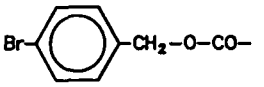
TABLE 10. *N^{im}-Urethane Derivatives of Histidine (1973-1988) [96]*

Compound	mp. (°C)	$[\alpha]_D^{20-25}$ (°)	lit.
Z-His(Z)	extra pure	-	127
Z-His(Z).EtOH		-	93
Z-His(Z)-OPcp	123-125	+ 18.12 (c 3.15, DMF)	86
Z-His(Z)-OTcp	111-112.5	- 6.56 (c 1.22, $CHCl_3$)	86
Z-His(Z)-ONp	106-107.5	- 16.6 (c 2.048, THF)	86
Boc-His(Boc)-OBzl	93-95	- 6.2 (c 1, Dioxane)	8
Boc-His(τ-Boc)-OCH_3	96[a]	+ 25.6 (c 1, CCl_4)	40,71
	85-88[b]	+ 19.9 (c 1.16, $CHCl_3$)	89
	127-128	-	137
Boc-His(Boc).DCHA	155-157	+ 18.8 (c 2.0, $CHCl_3$)	134
Boc-His(Boc).C_6H_6	82-82[c]	+ 12.5 (c 1.0, CH_3OH)	126
Boc-His(Boc).CCl_4	88-90[d]	+ 15.0 (c 1.0, CH_3OH)	126
Boc-His(Boc)-OSu	-	-	93
Z-His(Boc)	foam	-	10
Z-His(Boc)-OCH_3	-	-	136
Z-His(τ-Boc)-OCH_3	oil	-	74
Z-His(π-Boc)-OCH_3	oil	-	74
Fmoc-His(Boc)	-	-	70,113
Fmoc-His(Boc)-OPfp	-	-	141
Aoc-His(Aoc)-OCH_3	122-124	- 13.0 (c 2.2, C_5H_5N)	129
Z(OMe)-His[Z(OMe)]	oil	-	8
Z(OMe)-His[Z(OMe)]-ONp	110-111	- 9.5 (c 1, Dioxane)	8
Boc-His(Adc) τ:π = 2:1	-	-	73
Z-His(Adc).DCHA	153	+ 22.4 (c 2, CH_3OH)[e]	10
Adpoc-His(Adpoc)	113	+ 9.2 (c 0.5, DMF)	144
Boc-His(Ioc)	-	-	65
Z-His(Ioc)	-	-	65
Fmoc-His(Fmoc)	-	-	70,93
[Boc-His(2BrZ)]$_2$O	109-110	- 5.0 (c 1, CH_2Cl_2)	146

a. This derivative may be stored for short periods at 0°C. b. This derivative may be stored in a tightly closed bottle at room temperature for up to 9 months with no obvious deterioration. c. After half a year's and year's storage, the temperature rises to about 90°C and 100-110°C, respectively. d. Decomposition. After year's storage the temperature does not change. e. $[\alpha]_{578}^{26}$.



9–fluorenylmethoxycarbonyl (Fmoc)



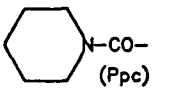
4–bromobenzyloxycarbonyl (2BrZ)

Figure 20.

His(2BrZ)]$_2$O has been synthesized.[146] Table 10 lists N^{im}-urethane-histidine derivatives reported during the 1973-1988 period.

4. Piperidinocarbonyl Group

The search for urethane-like protecting groups which are more resistant to nucleoleophilic attack led to the development of piperidinocarbonyl (Fig. 21). It is introduced into N^{α}-blocked histidine or its ester by means of Ppc-Cl.[1,3,71] Though from Z-His-OCH_3, a mixture of τ- and π-isomers of Z-His(Ppc)-OCH_3 is formed,[74] it is possible to get pure τ-isomer by crystallization [mp. 77-80°, $[\alpha]_D^{20}$ - 2.3 (c 1.04, CH_3OH)].[71] Fmoc-His-(Ppc) can be obtained directly from Fmoc-His(Fmoc).[70] The N^{im}-Ppc group is stable to acidolysis and catalytic hydrogenation but hydrazine and aqueous or methanolic alkali easily remove the group from the imidazole ring.[1,3] However, the increase in stability of the N^{im}-Ppc group to the nucleophilic attack is related to reduced resistance to histidine racemization. Very substantial racemization of the histidine residue took place when Fmoc-His(Ppc) as a preformed symmetric anhydride was used. Racemization was not measurable when the last reaction was carried out in the presence of 1-hydroxybenzotriazole.[70]



piperidinocarbonyl

Figure 21.

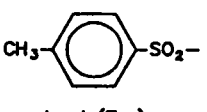
5. Arylsulfonyl Groups

So far, five N^{im}-arylsulfonyl protecting groups are known. Among them the tosyl (Fig. 22), probably in τ-orientation[71], is the oldest one.[1] Its main advantage is diminishing histidine racemization (Tables 1, 2 and 5).

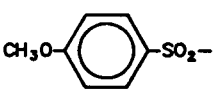
The N^{im}-tosyl group is effectively introduced by means of Tos-Cl in the presence of solid Na_2CO_3 in acetonitrile or chloroform-methanol.[147] N^{im}-tosyl derivatives are very susceptible to weak acids. The acidity of the molecule's own carboxyl group may be a reason for the gradual decomposition of N^{α}-protected N^{im}-tosylhistidines during storage at room temperature. Therefore, they are converted into dicyclohexylammonium salts for storage.[2,3,129] The fully protected peptide Boc-His(Tos)-Arg(Tos)-Gly-O<u>t</u>Bu decomposed after several days' storage even in the dry state.[148] Unmasking of the N^{im}-tosyl takes place under the influence of a host of reagents such as sodium in liquid ammonia,[129] hydrogen fluoride, trifluoromethanosulfonic acid, fluorosulfonic acid,[3] 2N HBr in acetic acid, HCl in organic solvents,[8,129] trifluoroacetic acid-dimethylsulfide,[149] 1 M trimethylsilyl triflate-thioanisole (1:1) in trifluoroacetic acid,[150,151] and alkali.[3,129] It is also removed by pyridine hydrochloride[82] or polyhydrogen fluoride,[152] acetic anhydride, acetic-formic anhydride or trifluoroacetic anhydride,[147] 1-hydroxybenzotriazole,[2,3,129] ammonia[129] and aqueous-methanolic triethylamine,[153] In model studies, lower levels of aminolytic transfer of the N^{im}-tosyl group onto an α-amino group than in the case of N^{im}-Z have been found.[129] Although the N^{im}-tosyl group does not interfere with catalytic hydrogenolysis of other groups, a small amount of the tosyl groups was reported to be cleaved during the removal of N-benzyloxycarbonyl moieties. This may be explained by a nucleophilic attack of the amino groups generated, during hydrogenolysis, on the N^{im}-tosyl protection.[129] Yet, it is worth noting, that treating Z-His(Tos)-OCH_3 with hydrazine, Z-His(Tos)-$NHNH_2$ can be obtained with a good yield.[86] It is probable that partial deprotection of the N^{im}-Tos group during N^{α}-Boc deprotection with trifluoroacetic acic occurs.[2,3,8,154,155] (see ref.[129] also). The ease of cleavage of N^{im}-tosyl group with acids, alkali and nuc-

leophiles imposes certain limitations on the range of compatibile reagents which will not cause the loss of the side chain protection.[2,22,156] Therefore the N^{im}-tosyl group is recommended[2,94] and applied[15,124,151,152,157-160] in Merrifield's solid phase peptide synthesis. However, it should be mentioned that in some sequences Boc-His(Tos) has stubbornly failed to couple. The use of Boc-His(Dnp) has in certain cases allowed the synthesis to continue.[122] In peptide synthesis in solution, the N^{im}-tosyl protection is employed only temporarily.[155,161,162]

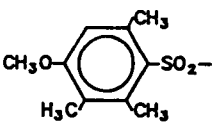
The lability of N^{im}-tosyl group in acidic media caused the search for blocking groups more stable under those conditions. N^{im}-4-methoxybenzenesulfonyl group (Fig. 22) was found to be cleaved neither by trifluoroacetic acid-anisole nor by 25% HBr in acetic acid. It also shows a higher resistance to methanesulfonic acid-anisole than N^{im}-Tos but is removed with hydrogen fluoride and trifluoroacetic acid in the presence of thioanisole, ethanedithiol, mercaptoethanol or, most easily in the presence of dimethylsulfide. N^{im}-4-Methoxybenzenesulfonyl moiety is also cleaved by 1-hydroxybenzotriazole, alkali and partially with hydrazine. Among others, Z(OMe)-His(Mbs)-ONp and Z-Glp-His-Pro-NH_2 were synthesized but N^{im}-protection proved to be partially cleavable in the presence of triethylamine. Therefore, this N^{im}-group is recommended for temporary protection.[148] The base lability of N^{im}-Tos and N^{im}-Mbs groups prompted the investigation of more base stable groups. N^{im}-4-Methoxy-2,3,6-trimethylbenzenesulfonyl (Fig. 22) was examined and found to be significantly more resistant to



tosyl (Tos)



4–methoxybenzene–sulfonyl (Mbs)



4–methoxy–2,3,6–trimethyl–benzenesulfonyl (Mtr)

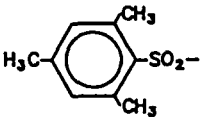
Figure 22.

TABLE 11. *N^{im}-Arylsulfonyl Derivatives of Histidine (1974-1988) [96]*

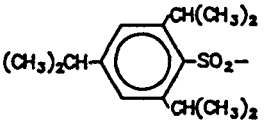
Compound	mp. (°C)	$[\alpha]_D^{19-26}$ (°)	lit.
Boc-His(Tos)-OTcp	140-141	- 11.9 (c 1.0, DMF)	154
Boc-His(Tos)-OPfp	-	-	148
Boc-His(Tos)-OSu	118-120	- 9.0 (c 1.0, DMF)	159
Aoc-His(Tos)	109-111[a]	+ 10.0 (c 1.0, C_5H_5N)	129
Z-His(Tos)	114	+ 13.7 (c 2.2, CH_3OH)[b]	10
		+ 17.4 (c 2.0, DMF)[b]	10
Z-His(τ-Tos)-OCH_3	72-74	+ 3.91 (c 1.15, DMF)	86
	79-80	+ 28.0 (c 1.0, $CHCl_3$)	71
Z-His(Tos)-$NHNH_2$	170-171	+ 4.62 (c 1.19, AcOH	86
Z-His(Tos)-OPcp	149-151	+ 4.686 (c 2.134, THF)	86
Z-His(Tos)-OTcp	157-158	- 3.56 (c 1.96, THF)	86
Z-His(Tos)-ONp	125-126	+ 3.97 (c 2.9, THF)	86
Z(OMe)-His(Tos).DCHA	179.5-180.5	+ 20.0 (c 1.0, DMF)	129
Fmoc-His(Tos)	110-115	- 13.1 (c 1.0, DMF)	163
Nps-His(Tos)	141-142[a]	+ 39.9 (c 1.0, DMF)	129
Nps-His(Tos)-OCH_3	101-103	+ 34.1 (c 1.0, DMF)	129
Nps-His(Tos)-OBzl	102-104	+ 24.8 (c 1.0, DMF)	129
Nps-His(Tos)-OSu	-	-	155
Tos-His(Tos)	183-185	+ 65.5 (c 5.0, EtOH)	13
Boc-His(Mts).DCHA	-	-	93

a. Decomposition. b. $[\alpha]^{25}_{578}$ + 13.9° (c 2.2, CH_3OH), + 18° (c 2.0, DMF).

aqueous-methanolic triethylamine than the above benzenesulfonyl-type protections. N^{im}-Mtr masking is removed by trifluoroacetic acid-dimethylsulfide or 1-hydroxybenzotriazole. It withstands conditions of N^{α}-Boc cleavage from small peptides, but was cleaved from a nonapeptide segment during N^{α}-Boc deprotection. Therefore, it is also proposed for transient protection.[153] Recently, N^{im}-mesitylenesulfonyl group (Fig. 23)[93] and N^{im}-2,4,6-triisopropylbenzenesulfonyl group (Fig. 23)[164] have been developed.



2,4,6–trimethylbenzenesulfonyl (Mts)=mesitylenesulfonyl



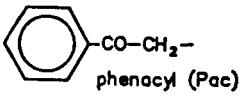
2,4,6–triisopropylbenzenesulfonyl (Tip)

Figure 23.

N^{im}-Arylsulfonyl histidine derivatives both new and those resynthetisized in 1974-1988 are listed in Table 11.

6. Phenacyl Groups

In comparison with N^{im}-protecting groups of the types so far discussed, those of N^{im}-phenacyl type (Fig. 24) are relatively new. Both N^{τ} and N^{π}-phenacyl derivatives were obtained.[39] For the unequivocal synthesis of the first type of compounds, either silver salts of N^{α}-protected histidine (Fig. 25)[39] or simultaneous masking of both nitrogen atoms, α and π, by means of carbodiimidazole



phenacyl (Pac)

Figure 24.

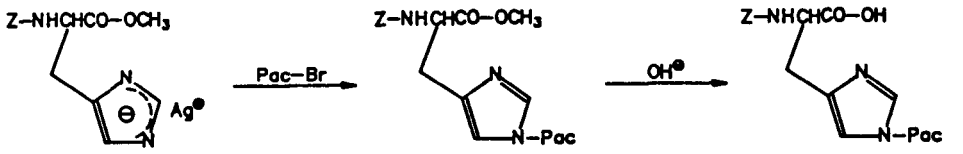


Figure 25.

were helpful. The N^{τ}-phenacyl group serves in the selective alkylation of nitrogen π (Fig. 14, # 3).[95] The phenacyl group was introduced at nitrogen π using the trityl group for protection of nitrogen-τ (Fig. 26).[39] π-Phenacyl group has been very recently used for a new histidine-peptide cyclisation method.[90]

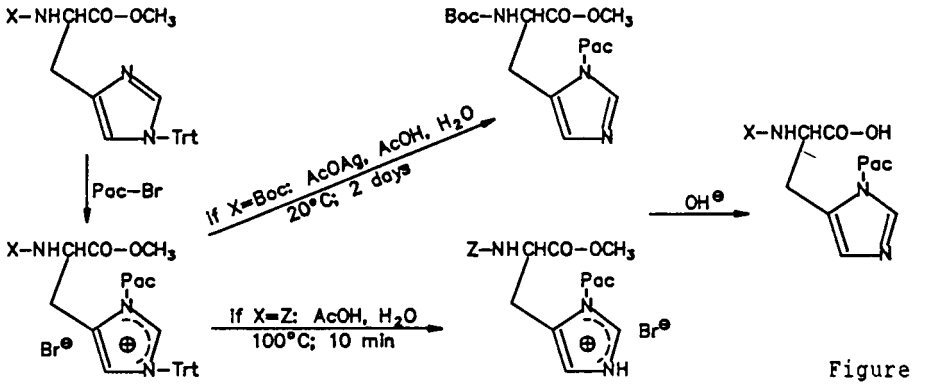
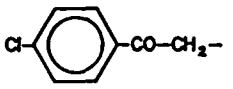


Figure 26.

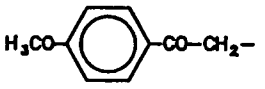
The phenacyl group withstands HBr in acetic acid, trifluoroacetic acid and trifluoromethanosulfonic acid but can be cleaved by photolysis, electrolytic reduction or better with Zn-acetic acid especially by ultrasonication.[39,95] None of these procedures seemed worth pursuing for peptides of any complexity.[39] The phenacyl group reacts with H_2-Pd(C) and reduction of CO moiety takes place, with hydrazine to give a complex mixture and with reactive acylating agents [illustrated by the reaction with $(Boc)_2O$; Fig. 27].[39] Some π-4-chloro- and π-4-methoxy-phenacylhistidine (Fig. 28) derivatives were prepared, but those do not offer advantages over the unsubstituted compounds.[40] N^{im}-Phenacylhistidine derivatives are shown in Table 12.

Figure 27.

Figure 28.



4–chlorophenacyl



4–methoxyphenacyl

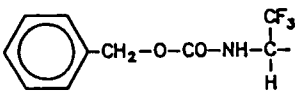
TABLE 12. *N^{im}-Phenacyl Derivatives of Histidine (1979-1987)*

Compound	mp. (°C)	$[\alpha]_D^{20-25}$ (°)	lit.
Boc-His$^+$(π-Pac,τ-Trt)-OCH_3.Br^-	177-179	- 9.2 (c 1, CH_3OH)	39
Boc-His(π-Pac)-OCH_3	139-141	- 9.3)c 1, CH_3OH)	39
Boc-His(π-Pac).0.5H_2O	181-183	+ 9.4 (c 1, CH_3OH)	39
Z-His$^+$(π-Pac,τ-Trt)-OCH_3.Br^-	111-116	- 3.2 (c 1, $CHCl_3$)	39
Z-His(π-Pac)-OCH_3.HBr	123-127	- 12.5 (c 1, CH_3OH)	39
Z-His(π-Pac)	205-208[a]	+ 10.9 (c 0.5, C_5H_5N)	39
		+ 2.8 (c 0.6, CH_3OH)	39
Boc-His(τ-Pac)-OCH_3	yellow foam	-	95
Z-His(τ-Pac)-OCH_3.HCl	205-210[a]	- 20.4 (c 1, CH_3OH)	39
Z-His(τ-Pac)-OCH_3.H_2O	67-70	+ 20.8 (c 1, $CHCl_3$)	39
	71-74	+ 21.2 (c 1.1, $CHCl_3$)	95
Z-His(τ-Pac).H_2O	119-123	+ 10.2 (c 1, CH_3OH)	39

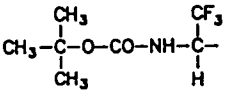
a. Decomposition.

7. 1-Alkoxycarbonylamino-2,2,2-trifluoroethyl Groups

Two of this type of protecting groups are known, Ztf and Btf (Fig. 29). The first is removed by HBr in acetic acid and hydrogen fluoride and is stable under conditions of saponification.[1] It was used in peptide synthesis including solid phase.[2,165,166] But currently, there are good reasons to abandon the practice: (i) marginal yields of solid phase couplings,[2,8,9] (ii) racemization of Boc-His(Ztf),[82] (iii) significant



1-benzyloxycarbonylamino-2,2,2-trifluoroethyl (Ztf)



1-t-butoxycarbonylamino-2,2,2-trifluoroethyl (Btf)

Figure 29.

Ztf-transfer (60%) on an α-amino group even against its acylation with mixed anhydride,[82] (iv) decomposition of N^{im}-Ztf-histidine derivatives in standard solvents employed in Merrifield's method,[167] N^{im}-Btf group was proposed as a protection compatible with α-Fmoc {Fmoc-His(Btf), mp. 143-145°, $[\alpha]_D^{23}$ + 14.7° (c 1, AcOEt)}.[168]

8. Alkoxyalkyl Groups

Like N^{im}-phenacyl blockage, this type of masking groups is relatively new. The first proposal was 1,1,1,3,3,3-hexafluoro-2-(4'-chlorophenoxymethoxy)propyl (HF-PA; Fig. 30). Z-His(HF-PA)-OSu has been used in thyro-

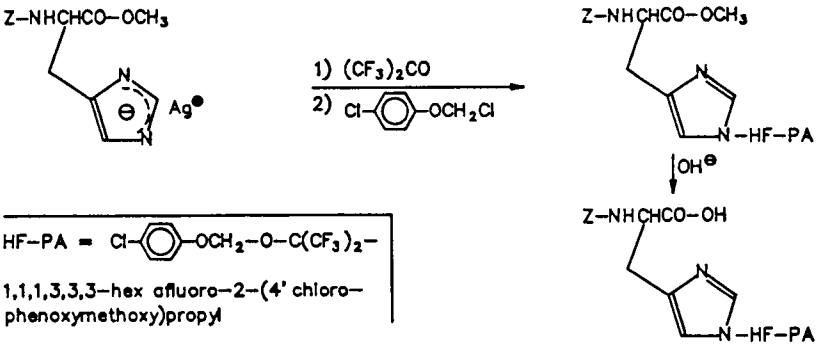


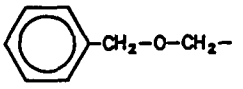
Figure 30.

liberin synthesis. N^{im}-HFPA is stable against alkali and catalytic hydrogenation and is removed by HCl in acetic acid.[169]

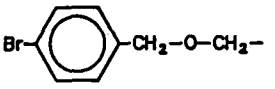
Next, benzyloxymethyl and 4-bromobenzyloxymethyl (Fig. 31), based on N-protection of pyrroles were applied to protect histidine N^{π}. The bromine substituent did not confer any additional advantages. Boc-His(π-Bom) can

be obtained (Fig. 32) with the help of N^{τ} temporary group which may be either trityl as in the case of Boc-His(π-Pac) (Fig. 26) or t-butoxycar-

Figure 31.



benzyloxymethyl (Bom)



4-bromobenzyloxymethyl (4BrBom)

bonyl; for comparison purposes, Boc-His(τ-Bom) was prepared as well by direct alkylation of Boc-His-OCH_3.[40]

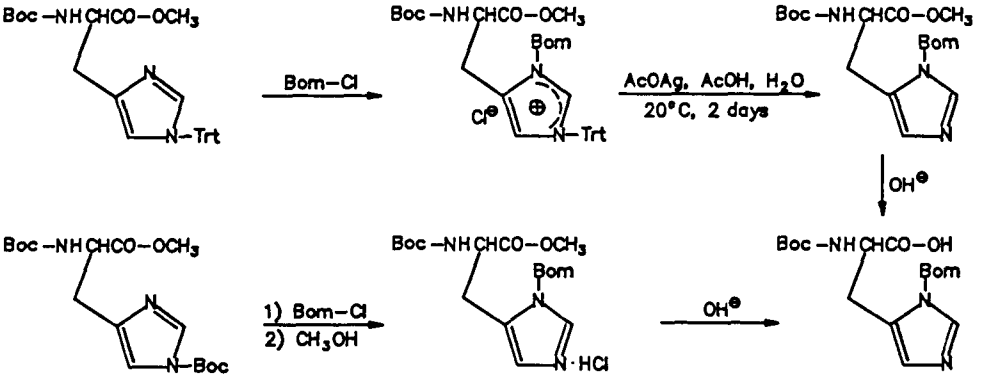


Figure 32.

N^{π}-Bom prevents autoracemization of activated histidine derivatives. Boc-His(π-Bom) is crystalline, stable and in spite of zwitterionic character, freely soluble in a wide range of organic solvents, including non-polar ones.[40] Its crystal structure has been resolved offering the first proof of structure of N^{im}-modified histidine.[71,77] The protecting group is stable to the conditions of the routine operations of both Merrifield's solid phase peptide synthesis and a large number of classical peptide syntheses. It withstands 40% trifluoroacetic acid in methylene chloride, 1 N NaOH, amines, ammonia and 1-hydroxybenzotriazole but a slight reaction with excess hydrazine and some cleavage with HBr in acetic acid

was observed.[40] N^{π}-Bom can be removed with HBr in trifluoroacetic acid in the presence of anisole,[40] hydrogen fluoride[94] and with 1 M trimethylsilyl triflate in the presence of thioanisole or of diphenylsulfide in trifluoroacetic acid.[150,170] It is cleaved by hydrogenolysis[40,171,172] but this procedure suffers some limitations because of extentive reductive methylation (and detectable dimethylation) of the liberated amino groups by formaldehyde formed on cleavage of π-Bom protection.[172] π-Benzyloxycarbonyl was tested in many syntheses with the α-Boc--ω-Bzl methodology in solution,[29,40,42,58,107,123,173] in liquid phase,[171] and in solid phase.[101,158-160,174] In solid phase peptide syntheses, much better yields have been obtained with Fmoc-His(τ-Trt) than those with Fmoc-His(π-Bom). Unfortunately, Bom-protected imidazole can still be acylated and therefore can be the acyl-transfer catalyst.[101]

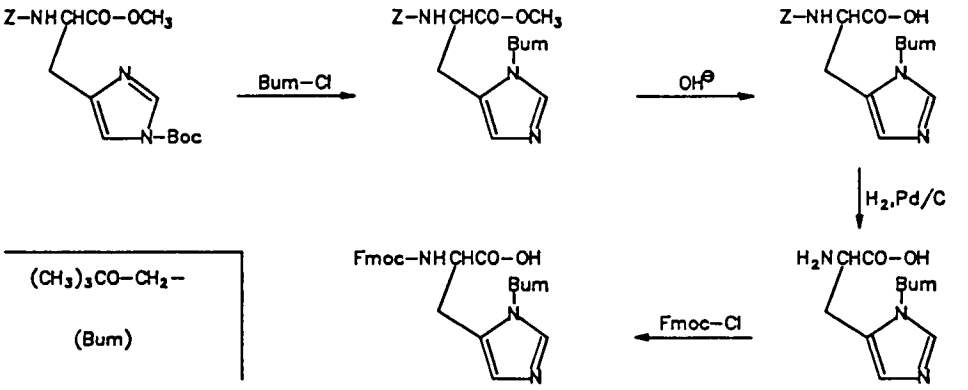


Figure 33.

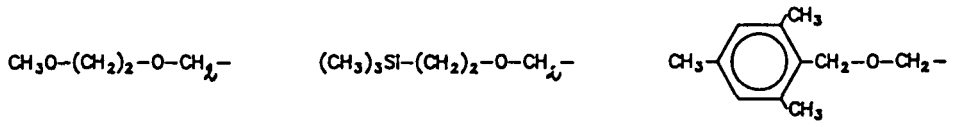
As a promising complement to the π-Bom group, the π-t-butoxymethyl group (Fig. 33), removable by trifluoroacetic acid and compatible with N^{α}-Z and N^{α}-Fmoc has been developed. Z- and Fmoc-His(π-Bum) were used in a tripeptide synthesis in solution.[136] The protection is recommended to solid phase peptide synthesis,[94,164] although with some limitations.[104] The

TABLE 13. *N^{im}-Alkoxyalkyl Derivatives of Histidine (1974-1988) [96]*

Compound	mp. (°C)	$[\alpha]_D^{18-20}$ (°)	lit.
Z-His(HF-PA)-OCH_3	56-58	-	169
Z-His(HF-PA)-OSu	oil	-	169
Boc-His^+(π-Bom,τ-Trt)-OCH_3.Cl^-	138-141	- 9.2 (c 0.6, CH_3OH)	40
Boc-His(π-Bom)-OCH_3[a]	103	- 10.8 (c 0.55, CH_3OH)	40
Boc-His(π-Bom).HCl[a]	152	- 19.1 (c 1.0, CH_3OH)	39
Boc-His(π-Bom)[a]	155	+ 6.9 (c 0.5, CH_3OH)	40
Boc-His(π-Bom).H_2O	108-110	+ 6.7 (c 0.5, CH_3OH)	40,77
Boc-His(π-4BrBom)	69-74	- 9.4 (c 1, CH_3OH)	175
Boc-His(τ-Bom)-OCH_3	oil	-	40
Boc-His(τ-Bom).0.3AcOEt	50[b]	+ 93.9 (c 1, $CHCl_3$)	40
Z-His(π-Bom)-OCH_3.HCl	142-145	- 27.8 (c 0.82, CH_3OH)	74
Z-His(π-Bom)-OCH_3	viscous oil	-	74
Z-His(π-Bom)	156-160	- 16.6 (c 2.0, DMF)	74
Fmoc-His(π-Bom)	-	-	101
Z-His(π-Bum)-OCH_3	-	-	71
Z-His(π-Bum)	186-187	- 10.7 (c 1, AcOH)	136
Z-His(τ-Bum)-OCH_3	-	-	71
Fmoc-His(π-Bum)	175-176	- 7.5 (c 1, AcOH)	136
Z-His(π-Mem)-OCH_3	oil	+ 5.2 (c 1.06, $CHCl_3$)	71
Z-His(τ-Mem)-OCH_3	oil	+ 19.9 (c 1.36, $CHCl_3$)	71
Z-His(π-Sem)-OCH_3	oil	+ 14.7 (c 1.07, $CHCl_3$)	71
Z-His(τ-Sem)-OCH_3	oil	+ 19.3 (c 1.53, $CHCl_3$)	71
Z-His(π-Tom)-OCH_3	-	-	71

a. Given in [96]. b. Crisp meringue.

newest attempted histidine N^{π}-protecting groups are: (2-methoxyethoxy)methyl, (2-trimethylsilylethoxy)methyl and (2,4,6-trimethylbenzyloxy)methyl



(2-methoxyethoxy)-methyl (Mem) | (2-trimethylsilylethoxy)-methyl (Sem) | (2-trimethylbenzyloxy)-methyl (Tom)

Figure 34.

(Fig. 34), introduced like N^{π}-t-butoxymethyl.[71] Deprotection of Sem group could be carried out using either 2-5 equivs of 1 M tetrabutylammonium fluoride in tetrahydrofuran between 45° and reflux temperature or with 3 N HCl at 60-90°.[176] Conditions for removing the Mem and Tom groups are not known.[71] Table 13 lists N^{im}-alkoxyalkyl-histidine derivatives.

IV. CONCLUSION

In peptide synthesis, histidine with its unprotected imidazole moiety may give side-reactions, the most particularly dangerous one being racemization. Some of these side-reaction could be minimized or avoided by using histidine with the imidazole function blocked. The eight types of groups masking this function are known but some of them never left the stage of model investigations. The same is also true for some individual proposals within applied types of protection. To warrant the retention of chirality of histidine during its incorporation into peptide chain, the N^{π}-protection was shown by Jones et al. to be necessary;[39,40,84,136] N^{τ}-blocking does not confer this protection. However, the masking of N^{π} requires somewhat more elaborate preparation[39,40,71,136] and sometimes the reagent introducing the protecting group is hardly available in a pure

state.[136,172] Yet, N^{π}-Bom, the most popular among these blocking groups, in spite of being commonly accepted for the histidine peptide synthesis in solution[29,40,42,58,107,121,173], does not prevent imidazole acylation.[101] Among the protecting groups known or supposed to be in τ-orientation, Trt, Tos, Dnp, Boc and Fmoc are presently used first of all and assumed to practically secure histidine against racemization in the course of activation and coupling. N^{τ}-masked histidine derivatives are more readily available than N^{π}-masked ones. Since all but Trt-protecting group of the above-mentioned τ-protections are sensitive to nucleophiles, they are not too stable under the conditions of peptide bond forming.[2,3] Therefore, recently, expectations have been directed to the easily introduced N^{τ}-trityl protection diminishing both racemization and by-product formation caused by the unmasked imidazole function.[67,68,99-103]

In Merrifield's solid phase peptide synthesis, N^{im}-Tos[15,113,151,152,157-160] and N^{im}-Dnp[113,116,117,119-122] are most often used. Presently, N^{π}-Bom is being tested[158,160,174] and N^{im}-2BrZ[146] and N^{im}-Tip[164] suggested. When solid phase synthesis is carried out using the α-Fmoc--ω-tBu strategy, N^{im}-Boc[70,138-141] and N^{im}-Fmoc[70,94,101,145] have been employed and N^{τ}-Trt[67,68,99-104] and N^{π}-Bum[94,164] is proposed.

In summary, the existing protecting groups for histidine imidazole function do not seem to be quite satisfactory. Therefore, in peptide synthesis in solution, histidine without the imidazole protection is still often incorporated by means of azide coupling in which racemization occurs to a relatively small extent (Table 1).[1-6,36,37,101,162,177] In the light of the main problem in the synthesis of histidine peptides, *viz.*, racemization it is important to note the potential of completely stereoselective enzymic incorporation of this amino acid into peptide chain.[178]

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ABBREVIATIONS

Ac = acetyl
Act = activation
Adc = adamantyloxycarbonyl
Adpoc = 1-(1-adamantyl)-1-methylethoxycarbonyl
Aoc = t-pentyloxycarbonyl
B = base
Boc = t-butoxycarbonyl
Bom = benzyloxymethyl
4BrBom = 4-bromobenzyloxymethyl
2BrZ = 2-bromobenzyloxycarbonyl
Btf = 1-t-butoxycarbonylamino-2,2,2-trifluoroethyl
Bum = t-butoxymethyl
Bzh = benzhydryl
Bzl = benzyl
CDI = carbonyldiimidazole
DCC = dicyclohexylcarbodiimide
DCHA = dicyclohexylamine
3,4Dmb = 3,4-dimethoxybenzyl
DMF = dimethylformamide
Dnp = 2,4-dinitrophenyl
Dppm = diphenyl-4-pyridylmethyl
EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
Fmoc = 9-fluorenylmethoxycarbonyl
HF-PA = 1,1,1,3,3,3-hexafluoro-2-(4'-chlorophenoxymethoxy)propyl
His(X) = τ-X, most probably
HOBt = 1-hydroxybenzotriazole
iBu = isobutyl
2Mb = 2-methoxybenzyl
3Mb = 3-methoxybenzyl
4MB = 4-methoxybenzyl
Mbs = 4-methoxybenzenesulfonyl
Mem = (2-methoxyethoxy)ethyl
Mtr = 4-methoxy-2,3,6-trimethylbenzenesulfonyl
Mts = 2,4,6-trimethylbenzenesulfonyl (mesitylenesulfonyl)
2Nb = 2-nitrobenzyl
4Nb = 4-nitrobenzyl
NEPIS = N-ethyl-5-phenylisoxazolium-3'-sulfonate
NMM = N-methylmorpholine
Nps = 2-nitrophenylsulfenyl
ONp = 4-nitrophenoxyl
OPcp = pentachlorophenoxyl
OPfp = pentafluorophenoxyl
OSu = succinimido-oxyl
OTcp = 2,4,5-trichlorophenoxyl
OTfp = 2,4,5,6-tetrafluorophenoxyl
Pac = phenacyl
Ppc = piperidinocarbonyl
Sem = (2-trimethylsilylethoxy)-methyl
tBu = t-butyl
THF = tetrahydrofuran
Tip = 2,4,6-triisopropylbenzenesulfonyl
Tom = (2,4,6-trimethylbenzyloxy)-methyl
Tos = tosyl
Trt = trityl
X = protecting group (general)
Z = benzyloxycarbonyl
Z(OMe) = 4-methoxybenzyloxycarbonyl
Ztf = 1-benzyloxycarbonylamino-2,2,2-trifluoroethyl

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