Studies on the Racemization and Coupling of N^{α},N^{im}-Protected Histidine Active Esters

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Racemization and coupling rate studies were carried out on N^{α} and N^{τ}-imidazole protected histidine active ester derivatives. The following pentachlorophenyl esters were studied: Z-His(Bzl)-OPcp, Z-His(Z)-OPcp, Z-His(Tos)-OPcp, and Boc-His(DNP)OPcp in THF solution using NEt_3 as a base. The racemization rate constants (k_r) decrease with increasing electron-withdrawing ability of the imidazole protecting group. Z-His(Bzl)-OPcp racemizes much faster than the Z, Tos, or DNP protected histidine and 10^3 times faster than Z-Ala-OPcp. Z-His(Bzl)-OPcp racemization is unusual since beyond 1 equiv of NEt_3 the racemization rate is practically independent of the base concentration. In addition Z-His(Bzl)-OPcp racemizes without an added base, and this autoracemization proceeds through intra- and intermolecular mechanisms. The racemizations of 2,4,5-trichlorophenyl esters of the above His derivatives were carried out in both THF and DMF, and in DMF the side-chain protecting group has no significant effect on the rate of racemization. Z-His(Bzl)-OTcp does not show autoracemization and k_r does depend on the base concentration. The His OPcp, OTcp, and ONp esters do not follow the general trend for other amino acids, that is the racemization rate is not parallel with the electron-withdrawing ability of the ester group. However, the rate increases with increasing solvent polarity. The coupling rate constants (k_c) of the above His active ester derivatives were determined in THF and DMF with H-Val-OMe. The basic imidazole side chain is sterically close to the NH group in the transition state and can intramolecularly catalyze the coupling procedure. If the imidazole side-chain basicity is decreased by Z, Tos, or DNP groups, this intramolecular catalysis is decreased and the coupling rate is slower. The coupling rate decreases as follows: OPcp > OTcp > ONp. In general, the coupling rates are larger in polar solvents; in DMF the coupling rates are 4 to 6 times faster than those in THF. The ratio of coupling and racemization rate constants, k_c/k_r, will allow us to estimate the relative extent of racemization during coupling. These values for pentachlorophenyl esters are Boc-His(DNP) 2400 > Z-His(Z) 1300 > Z-His(Tos) 640 > Z-His(Bzl) 10. The most extensive racemization is expected with Bzl and the least with DNP imidazole protection. The optical purity of these active esters was determined by converting them into the hydrazides; optically pure hydrazides were prepared from the methyl ester and used as standards. From Z-His(Z)-OX the hydrazides removed the imidazole protecting Z group. On the basis of the additivity principle we calculated the k_r and k_c values and they were in good agreement with the experimental values with the exception of the k_r value for Z-His(Bzl)-OTcp, where the calculated value is 115 times larger than the experimental value. This probably indicates deviation in the reaction mechanism for racemization.

Rapid racemization of histidine has been observed during peptide synthesis by several authors[1,2] and summarized by review articles.[3-5] The present paper reports a systematic investigation of coupling and racemization rates of N^{α}, Z, or Boc protected histidine active esters with different protecting groups on the N^{im} side chain.

In all the early works on *N*-imidazole protection, the position of the protecting group was unknown, and in most cases it was not investigated. The N^{im} protecting group can be at either the N^{π} or N^{τ} position.

All our protecting groups are assumed to be on the τ N. In several cases the location of N^{im} protecting group has been proven to be in the τ position.[3-5,8] Jones et al.[2] as well as Grønwald et al.[6] pointed out that the position of N^{im} protecting group seems to be very important for racemization during the coupling procedures. First Jones and his co-workers[7] prepared N^{α}-carbobenzoxy-N^{π}-phenacyl- as well as N^{τ}-phenacylhistidine to investigate racemization of the two derivatives. They observed that extensive racemization of the histidine occurred during DCC activation of the N^{τ}-phenacyl derivatives. A similar result was obtained with N^{τ}-benzylhistidine. However, N^{π}-phenacylhistidine showed much less racemization; 45% D enantiomer was detected during the synthesis of Z-His(N^{τ}-phenacyl)-Pro-NH_2 and less than 2% D form was obtained whent the N^{π}-phenacyl derivative was used for coupling with L-proline amide hydrochloride through the DCC method.

Table I. Autoracemization Rate Constants for Z-His(Bzl)-OPcp

C_E	obsd k_r (10^{-6} s^{-1})	calcd[a] k_r (10^{-6} s^{-1})
0.00		19
0.05	23	23
0.10	28	27
0.15	31	31
0.30	42	43

[a] From $k_r = 19 + 80C_E$.

I. Racemization of Histidine Pentachlorophenyl Ester Derivatives. Racemization of the active esters was carried out in THF solution in the presence of NEt_3 following the change of optical rotation, α, on a Cary 60 spectropolarimeter. IR spectra taken several times during racemization showed the presence of the active ester peak. Plots of the logarithm of the optical activity vs. time were linear, and the first-order racemization rate constants were found from the slopes. In most cases, k_r was measured for runs at several concentrations of NEt_3, for example, 7, 14,

(1) Windridge, G. C.; Jorgensen, E. C. *J. Am. Chem. Soc.* **1971**, *93*, 6318. (b) Syrier, J. L.; Beyerman, H. C. *Recl. Trav. Chim. Pays-Bas* **1974**, *93*, 117–120, 256–257.
(2) Brown, T.; Jones, J. H.; Richards, J. G. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1953.
(3) Kemp, D. S. In "The Peptides"; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 317.
(4) Kovacs, J. ref. 3, 1980; Vol. 2, p 529.
(5) Benoiton, N. L. ref 3, 1983; Vol. 5, 218.
(6) Gronwald, F. C.; Lundt, B. F.; Johansen, N. J. In "Peptides 1980", Proceedings of the 6th European Peptide Symposium, Helsingør. Denmark; Brunfeldt, K., Ed.; Scriptor: Copenhager, 1981; p 706.
(7) Jones, J. H.; Ramage, W. I. *J. Chem. Soc., Chem. Commun.* **1978**, 472.

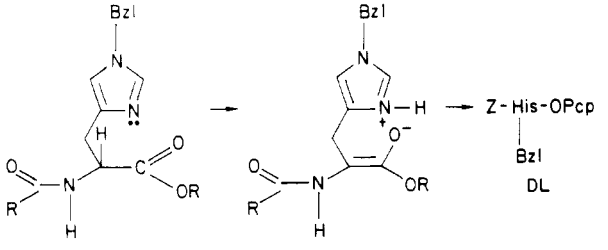
0022-3263/85/1950-1497$01.50/0

and 21 equiv. Except for Z-His(im-Bzl)-OPcp as discussed below, k_r was found to be proportional to the base concentration, C_B, and the values reported in Table III are for the second-order rate constants ($k_2 = k_1/C_B$).

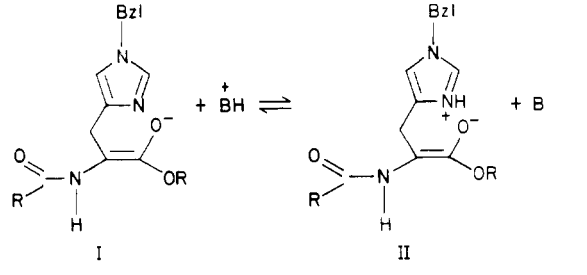
A. Unusual Behavior of Z-His(Bzl)-OPcp. The racemization of this ester is unusually fast. Also, in the range of base concentrations where we were working (≥7 equiv), the racemization rate constant was independent of the base concentration and therefore not second order. It was decided to investigate the rate at lower base concentrations. Two things were discovered: (1) at low base concentrations the rate constant does depend on C_B and (2) this ester racemizes even with no added base, i.e., it autoracemizes.

Autoracemization. Evidently the benzyl protected imidazole side chain is basic enough to catalyze this autoracemization. Table I shows the observed values of the first-order racemization rate constant at zero concentration of NEt_3 and for various concentrations of the ester, C_E. It is possible for the autoracemization to proceed either by an intra- or an intermolecular mechanism, or both. If it were exclusively intramolecular, then k_r would be independent of C_E, but it is not. If it were only intermolecular, then k_r would be proportional to C_E, but it is not. However, k_r does vary linearly with C_E and this is consistent with the simultaneous operation of both mechanisms. From a graph of k_r vs. C_E, the intercept and slope of the straight line were evaluated, giving the equation $k_r = 19 + 80C_E$. Values of k_r (in units of 10^{-6} s^{-1}) calculated from this equation are included in Table I and are seen to agree very well with the observed values. The intercept, 19×10^{-6} s^{-1}, represents the intramolecular rate constant, and the slope, 80×10^{-6} M^{-1} s^{-1}, is the intermolecular rate constant.

The scheme below shows a possible explanation for the intramolecular autoracemization of this histidine ester derivative. Model studies show that N^π of the imidazole ring is sterically sufficiently close to the α-proton to remove it intramolecularly. The carbanion which forms first gives the more stable enolate, which is further stabilized intramolecularly by the protonated imidazole.



A possible explanation of the intermolecular autoracemization is shown. The enol form I, after α-hydrogen



abstraction by another histidine molecule B, can be in equilibrium with the protonated imidazole II. The protonated imidazole is sterically close to the negatively charged oxygen, and structure II could help to stabilize the enolate intramolecularly. When the τ-nitrogen was protected by the carbobenzoxy group, the rate of racemization dropped many times (see Table III); this can be explained by the foregoing scheme because the basicity of the N^π is decreased, and this shifts the equilibrium to the left (I) and decreases the stabilizing effect of II on the enolate. Similarly, the intramolecular racemization rate decreases due to decreased basicity of the π-nitrogen.

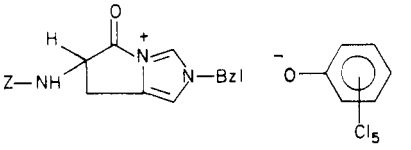
Table II. Dependence of the Racemization Rate of 0.05 M Z-His(Bzl)-OPcp on the Concentration of Triethylamine

C_B	total obsd k_r (10^{-6} s^{-1})	NEt_3 catalyzed k_r (10^{-6} s^{-1})	NEt_3 catalyzed k_r/C_B (10^{-6} M^{-1} s^{-1})
0	23	0	(6000)[a]
0.005	51	28	5600
0.015	110	87	5800
0.025	180	157	6300
0.05	240	217	4300
0.35	280	257	730
0.75	280	257	340

[a] Extrapolated.

Dependence of k_r on Base Concentration. In Table II are listed the experimental values of k_r for various concentrations, C_B, of NEt_3. Also listed are the values of the rate constant for the racemization produced solely by NEt_3, obtained by subtracting the autoracemization constant (23×10^{-6} s^{-1} for $C_E = 0.05$ M) from the total observed rate constant. This assumes that the different mechanisms are proceeding independently. The last column lists these rate constants divided by C_B. Above about 1 equiv of base k_r is unaffected by additional base and the reaction is first order. Below about half an equivalent of base, k_r/C_B is essentially constant at about 6000×10^{-6} M^{-1} s^{-1}, so that the base-catalyzed racemization is second order in this range.

This behavior of Z-His(Bzl)-OPcp in racemization is unique among amino acids. The fact that below 1 equiv of base, the rate of the racemization is second order, as expected from the behavior of the other amino acids so far investigated,[4] and the fact that more than 1 equiv of base does not increase the racemization rate in a practical sense seems to indicate that an intermediate species is formed from Z-His(Bzl)-OPcp by the base or with the base, which probably racemizes mainly by an intramolecular mechanism (independent of the presence of additional triethylamine). However, the IR spectra show the continued presence of the active ester. The intermediate cannot be the lactam below since its racemization necessarily could



not be an intramolecular first-order reaction. Veber[8] in 1975 isolated the cyclic acylimidazole from the azide, and the lactam, which is the racemizing species, can be detected by IR (1770 cm^{-1}, C=O). Veber suggested that a substituted imidazole in the π-position does not prevent cyclization and therefore racemization.

B. Effect of the Side-Chain Protecting Group on the Racemization Rate Constants. The effect on the rate of racemization of the different *N*-imidazole protecting groups, namely, Z, Bzl, Tos, and DNP, was investigated and the results are summarized in Table III. This effect

(8) Veber, D. F. In "Peptides: Chemistry, Structure and Biology", Proceedings of the 4th American Peptide Symposium; Walter, R., Meienhofer, J., Eds.; Ann Arbor Sci. Publ.: Ann Arbor, MI, pp 307–316.

Table III. Racemization Rate Constants of N^{α},N^{im}-Protected Histidine Active Ester Derivatives[a]

compound	solvent	10^{-6} k_r, M^{-1} s^{-1}
Z-His(im-Bzl)-OPcp	THF	800[a]
		4800[b]
Z-His(im-Bzl)-OTcp	THF	14[c]
	DMF	66
Z-His(im-Z)-OPcp	THF	2.3[d]
Z-His(im-Z)-OTcp	THF	3.8
	DMF	48
Z-His(im-Tos)-OPcp	THF	4.3[e]
Z-His(im-Tos)-OTcp	THF	5.0
	DMF	79
Boc-His(im-DNP)-OPcp	THF	1.1
Boc-His(im-DNP)-OTcp	THF	2.4
	DMF	150
Z-His(im-Z)-ONp	THF	3.0
Z-His(im-Tos)-ONp	THF	2.4

[a] For 7 equiv of base. [b] For 1 equiv of base. [c] Average of four experiments with 7, 14, 21, and 35 equiv of NEt_3. [d] Average of three experiments with 7, 14, and 35 equiv of NEt_3. [e] Average of three experiments with 14 equiv of NEt_3.

can be illustrated by comparing racemization rate constants of pentachlorophenyl esters of the histidine derivatives with other amino acid pentachlorophenyl esters included in Table III.

Two values of k_r are listed for Z-His(Bzl)-OPcp: 4800, which would be valid for comparisons for 1 equiv of base, which we used in coupling, and 800, which is valid for comparisons at 7 equiv of base. In any case, it is seen that the imidazole protecting group has a very significant effect on the racemization rate constants in THF.

The benzyl-protected histidine racemizes more rapidly than other amino acid pentachlorophenyl ester derivatives and also more rapidly than histidine OPcp esters protected by electron-withdrawing groups. The rate ratios using the 7-equiv value of k_r for Z-His(Bzl)OPcp are (Z-His(Bzl))/(Boc-His(DNP)) = 730, (Z-His(Bzl))/(Z-His(Z)) = 350, (Z-His(Bzl))/(Z-His(Tos)) = 190, (Z-His(Bzl))/(Z-Cys(Bzl)) = 1.9,[9] (Z-His(Bzl))/(Z-Glu(Bzl)) = 480,[4] and (Z-His(Bzl))/(Z-Ala) = 940.[4] In particular, it is interesting that the standard Z-Ala-OPcp racemizes almost 1000 times slower than Z-His(Bzl)OPcp.

II. Racemization of 2,4,5-Trichlorophenyl Esters. The kinetic study of the 2,4,5-trichlorophenyl ester of N^{α}, N^{im}-protected histidine was carried out in both THF and DMF. The racemization rate constants are listed in Table III.

Comparison of the rate of racemization with other amino acid trichlorophenyl esters in THF is given below and alanine was chosen as the standard:

Z—Cys(Bzl)	Z—Asp(OMe)	Z—His(Bzl)	Z—Phe
270	19	7.8	6.7
Z—Glu(OMe)	Z—His(Tos)	Z—His(Z)	Z—Ala
3.3	2.8	2.1	1

It is interesting that there is no significant effect of the side chain protecting group on the racemization rate in case of trichlorophenyl esters in THF or DMF solution with the exception of the τ-benzyl protected His. It is also interesting to note that there are little differences between the k_r values of the pentachlorophenyl and trichlorophenyl esters in THF solution, with exception of benzyl-protected His again. However, the Z-His(Bzl)-OTcp does not show measurable autoracemization in 24 h, and the second-order racemization rate constant does not depend on the base concentration.

Effect of Active Ester Group on Rate of Racemization. It was observed in this laboratory that the rates of racemization of amino acid active esters are parallel with the pK values of the corresponding phenols, with the exception of pentafluorophenyl ester; the more acidic the phenol the higher the racemization rate.[10] In general, it is parallel with the electron-withdrawing ability of the active ester[11] group. Table IV summarizes the effect of the active ester group on the racemization rate constants in THF: N^{α}-carbobenzoxy-L-histidine pentachlorophenyl esters were chosen as standards.

Table IV. Relative Racemization Rate Constants

Z-L-amino acid	-OPcp	-OTcp	-ONp
His(im-Tos)	1.0	1.2	0.56
His(im-Z)	1.0	1.7	1.3
His(im-Bzl)	1.0	0.02	

Table V. Coupling Rate Constants of N^{α},N^{im}-Protected-L-histidine Active Esters

compound	solvent	$k_c \times 10^{-2}$ M^{-1} s^{-1}	$t95$, h	$t99$, h
Z-His(im-Bzl)-OPcp	THF	0.86	4.7	25
Z-His(im-Bzl)-OTcp	THF	0.18	23	120
	DMF	1.2	3.4	18
Z-His(im-Z)-OPcp	THF	0.23	17	92
Z-His(im-Z)-OTcp	THF	0.12	34	180
	DMF	0.52	7.8	41
Z-His(im-Z)-ONp	THF	0.046	88	460
Z-His(im-Tos)-OPcp	THF	0.31	13	69
Z-His(im-Tos)-OTcp	THF	0.18	49	230
	DMF	1.0	4.1	21
Z-His(im-Tos)-ONp	THF	0.081	50	280
Boc-His(im-DNP)-OPcp	THF	0.26	16	81
Boc-His(im-DNP)-OTcp	THF	0.20	20	110
	DMF	0.77		

As seen from the table, the racemization rates of the histidine active esters decrease as follows for N^{im}-carbobenzoxyhistidine: trichlorophenyl > p-nitrophenyl > pentachlorophenyl; for N^{im}-tosylhistidine: trichlorophenyl > pentachlorophenyl > p-nitrophenyl. These results do not agree with the general trend mentioned above for other amino acids.[4]

N-protected amino acid active esters normally racemize about 10 times faster in DMF than in THF solution. It is clear from the data in Table III that there is a significant solvent effect on the racemization rate constants of histidine active esters as well. N^{α}-Carbobenzoxy-N^{im}-benzyl, N^{im}-tosyl-, and N^{im}-carbobenzoxy-L-histidine trichlorophenyl esters racemize 4.7, 16, and 13 times faster in DMF than in THF. However, the N^{im}-DNP-histidine ester racemizes 63 times faster in DMF than in THF.

III. Racemization of N^{α}-Carbobenzoxy-N^{im}-tosyl- and N^{im}-Carbobenzoxy-L-histidine p-Nitrophenyl Ester in THF. The rate constants of racemization are given in Table III. The rates of racemization are parallel with the rates of racemization of trichlorophenyl esters. Comparison of the rates of racemization with other amino acid p-nitrophenyl esters are given below with alanine chosen as standard:

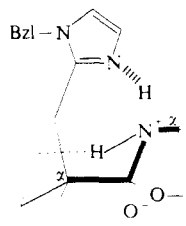
Z—Cys(Bzl) > Z—Asp(OMe) > Z—Phe ≃ Z—Met > Z—His(Z) ≃				
190	13	4.4	4.1	1.4
Z—Glu(OMe) > Z—His(Tos) > Z—Ala > Z—Val				
1.4	1.1	1	0.33	

(9) Kovacs, J.; Hsieh, Y. *J. Org. Chem.* **1982**, *47*, 4996 and previous papers reported in this publication.
(10) Kovacs, J.; Kisfalady, L. ; Cerprini, M. Q.; Johnson, R. H. *Tetrahedron* **1969**, *25*, 2555.
(11) Morawiec, J.; Konopinska, D.; Siemon, I. Z. *Rocz. Chem.* **1971**, *45*, 711.

IV. Coupling of N^{α},N^{im}-Protected-L-histidine Active Esters. The aminolysis rate constants were studied in THF with L-valine methyl ester which couples about 100 times slower than Gly due to the steric hinderance of the side chain. The coupling rate constants were determined by following the disappearance of the ester carbonyl absorption peak in the IR between 5.6–5.7 μm. The second-order rate constants for coupling, together with the time needed for 95% and 99% completion of the reactions are given in Table V.

Kemp[12] investigated the mechanism of aminolysis of *p*-nitrophenyl esters of Ala, Phe, Leu, Pro, and Val; the rate depends on the collapse of the reversibly formed tetrahedral intermediate. Three major changes are involved in the aminolysis, i.e., a C–N amide bond is formed while C–O ester and N–H amine bonds are broken. In the transition state, the acyl carbon and the amide nitrogen are tetrahedral, and the solvent is coordinated with the $-NH_2-$ as well as with the ester regions. Variation in rate constants reflect steric effects in the transition state. Conformational analysis led to three major conformations for the transition state. The first rotamer is an anti conformation about the developing C–N bonds, while the two remaining conformations are gauche rotamers. These conformers show the group interactions.

In the case of histidine, the side chain is also expected to influence the rate constants not only sterically but also electronically. This can be seen in the anti conformation for the transition state below:



Models clearly show that the imidazole N can be in the proper position sterically to act as a base. The proton is transferred intramolecularly from the amine to the imidazole nitrogen. The same favorable steric situations exist for the gauche conformations as well. This is an intramolecular base catalysis for coupling. When the basicity of imidazole is decreased, e.g., by protecting it with a benzyloxycarbonyl group, this intramolecular proton transfer is lessened, and this type of histidine active ester derivative couples more slowly.

A. Effect of the Side-Chain Protecting Groups on the Coupling Rate Constants. It is seen from the data presented in Table V that there is a noticeable effect of the imidazole protecting group on the coupling rate for the same N^{α}-protected active esters. The benzyl-protected Pcp ester, such as Z-His(im-Bzl)-OPcp, couples 3 times faster than the Z^{im}- or Tos^{im}-protected OPcp esters. This is explained by the intramolecular base catalysis for coupling. This is in contrast to racemization where the side-chain protecting benzyl group of histidine has a very significant effect on the rate. For comparison, the decreasing order of coupling rates for Z-amino acid pentachlorophenyl esters are listed below with Ala chosen as standard:

Cys(Bzl)	> His(Bzl)	> Ser ≃ Asp(OMe)	> Ala	> Pro	> Met
3.4	1.7	1.5	1	0.87	0.71

Trp	> His(Tos)	> Phe	> His(Z)	> Lys(Boc)	> Glu(OMe)	> Val
0.63	0.61	0.57	0.45	0.43	0.32	0.05

(12) Kemp, D. S.; Choong, S. L. H.; Pekaar, J. *J. Org. Chem.* **1974**, *39*, 3841.

Table VI. Coupling and Racemization Rate Ratios (k_c/k_r) of Histidine-Active Ester Derivatives in THF and DMF

	THF	DMF
Z-His(Bzl)-OPcp	10	
Z-His(Z)-OPcp	1300	
Z-His(Tos)OPcp	640	
Boc-His(DNP)-OPcp	2400	
Z-His(Bzl)-OTcp	50	130
Z-His(Z)-OTcp	310	110
Z-His(Tos)-OTcp	360	130
Boc-His(DNP)-OTcp	840	51
Z-His(Z)-ONp	150	
Z-His(Tos)-ONp	340	

It was concluded that, with the exception of Val, the amino acid side chain has a relatively small effect on the coupling rate.

B. Effect of Active Ester Group on Coupling Rate. There is a noticeable effect of the active ester groups on coupling rate constants in THF as shown in Table V. This is expected as the active ester group is attached to the carbonyl carbon which is the reaction site. The coupling rate constants of the active esters decrease as follows: OPcp > OTcp > ONp. This is a general trend for most amino acids. This trend is valid for all imidazole-protected histidine derivatives investigated so far. There is no significant difference between the coupling rates of N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-DNP-histidine pentachlorophenyl ester and trichlorophenyl ester.

C. Effect of Solvent on the Coupling Rate. There is significant effect of the solvent on the coupling rate of trichlorophenyl esters. As seen in Table V, the coupling rates are 4 to 6 times faster in DMF than in THF. The phenyl esters in general couple much faster in dipolar aprotic solvent such as DMF. In contrast, the dicyclohexylcarbodiimide couplings are slower in polar solvent and the rate is so slow in DMF that it is not practical to use for peptide synthesis.[4]

V. Ratio of Coupling and Racemization Rate Constants. This ratio allows us to estimate the relative extent of racemization during coupling reactions. The larger this ratio, the smaller is the extent of racemization to be expected during coupling, provided the racemization rate measured for NEt_3 parallels that induced by an amine component during coupling. In laboratory synthesis of peptides an optimum condition is defined when the k_c/k_r value is ∞. The k_c/k_r values are listed in Table VI. For comparison, the decreasing order of k_c/k_r values of other α-benzyloxycarbonyl amino acids are given below:

for-OPcp esters	Pro	> Ala	> Trp	> Lys(Boc)	> His(Z) ≃ Met	> Phe	> His(Tos) >
	6100	4500	3700	1300	1300	900	800
	Glu(OMe)	> Val	> Asp(OMe)	> Ser	> Cys(Bzl)	> His(Bzl)	
	640	570	420	280	42	10	

for-ONP esters	Ala	> His(Tos)	> His(Z) ≃	Glu(OMe)	> Met	> Asp(OMe)	> Cys(Bzl)
	730	340	150	150	90	27	27

for-OTcp esters	Ala	> His(Tos)	> His(Z)	> Phe ≃	Glu(OMe)	> His(Bzl)	> Asp(OMe) >
	730	360	310	170	170	130	75
							Cys(Bzl)
							6.1

This order is not identical with the reverse order for racemization since these ratios also depend on the coupling rates. The order of these rate ratios will indicate the vulnerability of amino acids to racemization during coupling.

Preliminary investigation on Boc-His(Bzl)-OPfp·HOPfp indicates that this pentafluorophenyl active ester with a k_r value of 26×10^{-6} M^{-1} s^{-1} and a k_c value of 4.4×10^{-2} M^{-1} s^{-1} gives a k_2/k_r value of 1700. This indicates the

Table VII

	k_r(calcd)	k_r(exp)	k_c(calcd)	k_c(exp)
Z-His(Bzl)-OPcp	780	800	0.86	0.86
Z-His(Bzl)-OTcp	1600	14	0.46	0.18
Z-His(Z)-OPcp	2.3	2.3	0.31	0.31
Z-His(Z)-OTcp	5.8	3.8	0.16	0.12
Z-His(Z)-ONp	3.7	3.0	0.068	0.046
Z-His(Tos)-OPcp	4.2	4.2	0.27	0.27
Z-His(Tos)-OTcp	10	5.0	0.14	0.18
Z-His(Tos)-ONp	6.7	2.4	0.058	0.081

usefulness of this ester, even when the imidazole is protgected with Bzl, in practical peptide synthesis especially when it is used in excess relative to the amine component.[13]

VI. Determination of Optical Purity of the Active Ester Derivatives. It is important to investigate optical purity of monomer active esters since histidine may racemize during the activation of the C-terminal. This investigation was carried out by preparation of hydrazide from the methyl esters, assuming that this reaction proceeds without racemization.[14] The optically pure hydrazide was chosen as a standard, and physical constants, melting point, and specific rotation were compared with those of other hydrazides obtained from the active esters.

In case of Z-His(Bzl)-$NHNH_2$, the physical constants of the hydrazides prepared from the pentachlorophenolate salt, Z-His(Bzl)-$NHNH_3^+O^-$-C_6Cl_5. The hydrazide was obtained from the pentachlorophenolate salt by dissolving it in HCl, when the HOPcp precipitated out and the filtrate was neutralized with NaOH to obtain Z-His(Bzl)-$NHNH_2$, and its specific rotation was again identical with that of the standard.

From Z-His(Z)-OX only the Z-His-NH-NH_2 could be obtained. The carbobenzoxy group on the imidazole ring is not stable in the presence of NH_2–NH_2 and all three active esters gave the imidazole-unprotected hydrazide. Their optical purities, within experimental error, were identical with that of the standard. This clearly indicates the limited value of the Z group in the side-chain protection of histidine, since in coupling the amine nucleophile can similarly react with it, and it leads to serious side reaction as was pointed out in recent review articles.[15]

The Z-His(Tos)-$NHNH_2$s from Z-His(Tos) pentachlorophenyl, trichlorophenyl, and *p*-nitrophenyl esters were obtained without the loss of the Tos group, and their optical purities, within experimental error, were established.

VII. Additivity Principle for Prediction of Rate Constants of Racemization and Coupling of Histidine Derivatives. On the basis of the additivity principle,[16] we calculated the racemization and coupling rate constants and compared them with the experimental values.

Table VII shows the experimental and calculated k_r and k_c values. The experimental coupling rates of histidine active ester derivatives are in good agreement with theoretical values except for Z-His(Bzl)-OTcp, which is 3 times larger than the experimental value.

In the case of racemization rates, the experimental k_r values for Z-His(Z) active esters agree well with the theoretical values. Small deviations were found between the calculated and experimental values for Z-His(Tos) active ester, and very large deviations were found for Z-His(Bzl)-OTcp, which probably indicates deviation in reaction mechanism. Similarly, large deviations were observed in the case of glycylcysteine active ester derivatives and it was established that the racemization is not the expected 5(4*H*)-oxazolone but an enolization mechanism.[9]

Experimental Section

All melting points are uncorrected and were taken on a Thomas-Hoover melting point apparatus in open capillaries. The microanalysis was carried out by Schwarzkopf Microanalytical Laboratory, Woodside, NY. Infrared spectra were determined in potassium bromide pellets or barium fluoride or sodium chloride cells using Beckman IR-8 or Perkin-Elmer Model IR-283 spectrophotometer. All optical rotations and racemization studies were carried out on a Cary 60 spectropolarimeter, and all coupling studies were done on a Perkin-Elmer Model IR-283 spectrophotometer. The solvents used were of distilled reagent grade, unless otherwise indicated. THF was purified by distillation from sodium, after refluxing with NaOH for 96 h and $LiAlH_4$ for 48 h, and then stored over sodium metal under a nitrogen atmosphere. Triethylamine was stirred overnight with Z-Gly-ONP to remove the traces of primary and secondary amines and then distilled and stored over sodium under nitrogen atmosphere.

The following abbreviations are used: DCC = *N*,*N*′-dicyclohexylcarbodiimide, DCU = *N*,*N*-dicyclohexylurea, THF = tetrahydrofuran, DMF = *N*,*N*′-dimethylformamide, TFA = trifluoroacetic acid; Et_3N = triethylamine, EtOAc = ethyl acetate, Z = carbobenzoxy. Boc = *tert*-butyloxycarbonyl, Tos = *p*-tolylsulfonyl, HOAc = acetic acid, EtOH = ethanol. OPcp = pentachlorophenyl, OTcp = 2,4,5-trichlorophenyl, DNP = 2,4-dinitrophenyl, ONP = *p*-nitrophenyl.

N^{α}-Carbobenzoxy-N^{im}-benzyl-L-histidine Pentachlorophenyl Ester. N^{α}-Carbobenzoxy-N^{im}-benzyl-L-histidine (3.80 g, 10 mmol) was suspended in CH_2Cl_2 (150 mL) and pentachlorophenol (2.66 g, 10 mmol) was added, the solution was cooled in ice bath, and DCC (2.06 g, 10 mmol) was added to it. The reaction mixture was stirred for 3 h in the ice bath and 2 h at room temperature and then filtered to remove DCU. The solution was concentrated to 10 mL under reduced pressure, and petroleum ether was added (3–5 mL); a small amount of precipitate formed, which was filtered out, and then more petroleum ether was added slowly. After refrigeration for 1 h the ester crystallized; it was filtered and washed with ether–petroleum ether (1:1) twice and dried overnight in vacuum at room temperature. The analytically pure sample was obtained by recrystallization from ethyl acetate and petroleum ether: yield 4.2 g (67%); mp 110–112 °C; $[\alpha]^{21}_D$ +8.23° (*c* 2.71, THF); IR (KBr) 5.6 μm (active ester); TLC showed one spot (ethyl acetate–benzene, 1:3). Anal. Calcd for $C_{27}H_{20}N_3O_4Cl_5$: C, 51.66; H, 3.21; N, 6.69; Cl, 28.24. Found: C, 51.82; H, 3.44; N, 6.64; Cl, 27.85.

N^{α},N^{im}-Dicarbobenzoxy-L-histidine Pentachlorophenyl Ester. N^{α},N^{im}-Dicarbobenzoxy-L-histidine (4.23 g, 10 mmol) was dissolved in EtOAc (100 mL) and pentachlorophenol (2.66 g, 10 mmol) was added to it. The solution was cooled to 5 °C, followed by the addition of DCC (2.06 g, 10 mmol). DCU was formed in 10 min, and the reaction mixture was left at 0 °C for 1.5 h. The DCU was filtered and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in EtOAc (15 mL) and filtered. The crystallization started after addition of pentane. Recrystallization from methylenechloride–pentane (2:1) afforded chromatographically pure compound: yield 3.25 g (48%); mp 123–125 °C; $[\alpha]^{21}_D$ +18.12° (*c* 3.15, DMF); TLC, 1 spot (EtOAc–benzene, 9:1); IR showed active ester peak at 5.6 μm (Nujol). Anal. Calcd for $C_{28}H_{20}N_3O_6Cl_5$: C, 50.06; H, 3.00; N, 6.26; Cl, 26.59. Found: C, 50.33; H, 3.22; N, 6.08; Cl, 26.06.

N^{α},N^{im}-Dicarbobenzoxy-L-histidine 2,4,5-Trichlorophenyl Ester. A modified procedure for the preparation of this active ester is outlined below; 2,4,5-trichlorophenol (3.96 g, 20 mmol) was added to a cold solution of *N*,N^{im}-dicarbobenzoxy-L-histidine (8.46 g, 20 mmol) in $CHCl_3$ (150 mL). The reaction mixture was cooled to 5 °C and then DCC (4.12 g, 20 mmol) was added. DCU was filtered after 20 min of stirring, and the solution was evap-

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orated to dryness under reduced pressure. The oily residue was triturated with petroleum ether at −10 °C when it crystallized. The product was filtered and washed with petroleum ether–ether (1:1). The crude product was dried overnight in vacuum over silica gel and then recrystallized from EtOAc and petroleum ether: mp 111–112.5 °C; $[\alpha]^{23}_D$ −6.56° (*c* 1.22, $CHCl_3$) [lit.[17] mp 111–112.5 °C; $[\alpha]_D$ −6.46°]. Anal. Calcd for $C_{28}H_{22}N_3Cl_3O_6$: C, 55.79; H, 3.58; N, 6.97; Cl, 17.63. Found: C, 55.89; H, 3.79; N, 7.10; Cl, 17.6.

N^{α},N^{im}-Dicarbobenzoxy-L-histidine *p*-Nitrophenyl Ester. N^{α},N^{im}-Dicarbobenzoxy-L-histidine (8.46 g, 20 mmol) and *p*-nitrophenol (4.17 g) were dissolved in EtOAc (70 mL) and DCC was added. The reaction mixture was stirred for 20 min and then filtered. Petroleum ether was added to the solution and cooled at −3 °C for 15 min, then more petroleum ether was added, and the crystalline ester was filtered and recrystallized from chloroform–petroleum ether (3:2). The product showed one spot on TLC (EtOAc–MeOH, 10:1): yield 8.6 g (76%); mp 106–107.5 °C; $[\alpha]^{23}_D$ −16.6° (*c* 2.048, THF); IR shows the characteristic active ester peak at 5.65 μm [lit.[18] mp 107–108 °C]. Anal. Calcd for $C_{28}H_{24}N_4O_8$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.37; H, 4.63; N, 10.05.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine Pentachlorophenyl Ester. To a cold solution of N^{α}-carbobenzoxy-N^{im}-tosyl-L-histidine (6.63 g, 15 mmol) and pentachlorophenol (3.98 g, 15 mmol) in CH_2Cl_2 (100 mL) was added DCC (3.09 g, 15 mmol) with stirring. DCU was formed immediately and the reaction mixture solidified in 40 min. It was diluted with CH_2Cl_2 (100 mL) and stirred for 10 min in an ice bath; then DCU was filtered. The solution was evaporated to dryness under reduced pressure and the residue redissolved to 20 mL CH_2Cl_2. After 20 min, a small amount of DCU was filtered out and the solution was evaporated to dryness again and redissolved in EtOAc. To the solution was added pentane to turbidity, and the solution was left at room temperature for 40 min and in the refrigerator for 0.5 h. The ester was filtered, washed with ether–petroleum ether (1:1) 3 times, and dried overnight in vacuum at room temperature. The crude product was crystallized once from THF and then from CH_2Cl_2: yield 5.30 g (51%); mp 149–151 °C; $[\alpha]^{19}_D$ +4.686° (*c* 2.134, THF): IR (KBr) 5.6 μm (active ester). Anal. Calcd for $C_{27}H_{20}N_3O_6Cl_5S$: C, 46.88; H, 2.91; N, 6.07. Found: C, 46.92; H, 2.92; N, 6.12.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine 2,4,5-Trichlorophenyl Ester. This compound was prepared from *N*-carbobenzoxy-N^{im}-tosyl-L-histidine (7.70 g, 17.4 mmol) and 2,4,5-trichlorophenol (3.43 g, 17.4 mmol) with DCC (3.58 g, 17.4 mmol) by using the same procedure described above. The crude product showed one spot on TLC (EtOAc–benzene, 9:1), recrystallized from CH_2Cl_2: yield 5.80 g (53%); mp 157–158 °C; $[\alpha]^{23}_D$ −3.56° (*c* 1.96, THF). Anal. Calcd for $C_{27}H_{22}N_3O_6Cl_3S$: C, 52.07; H, 3.56; Cl, 17.0. Found: C, 52.39; H, 3.63; Cl, 17.26.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine *p*-Nitrophenyl Ester. This compound was prepared from N^{α}-carbobenzoxy-N^{im}-tosyl-L-histidine (2.20 g, 5 mmol) in CH_2Cl_2 (70 mL), *p*-nitrophenol (0.7 g, 5 mmol), and DCC (1.03 g, 5 mmol) by the same procedure as described above. The solution was evaporated to one-half volume and filtered to remove more DCU, and petroleum ether–ether (1:1) was added to turbidity and then the solution was refrigerated for 5 h. The crystalline ester was filtered and washed with petroleum ether: yield 2.4 g (85%); mp 125–126 °C; $[\alpha]^{23}_D$ 3.97° (*c* 2.9, THF). Anal. Calcd for $C_{27}H_{24}N_4O_8S$: C, 57.44; H, 4.29. Found: C, 57.69; H, 4.46.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{im}-(2,4-dinitrophenyl)-L-histidine 2,4,5-Trichlorophenyl Ester. This ester was prepared from *N*-(*tert*-butyloxycarbonyl)-N^{im}-2,4-(dinitrophenyl)-L-histidine (4.21 g, 10 mmol), 2,4,5-trichlorophenol (1.99 g, 10 mmol) (50 mL), and DCC (2.06 g, 10 mmol) in EtOAc at −5 °C in the usual manner. The crude ester was solidified on trituration with petroleum ether–ether (1:1) (5.0 g), dried overnight in vacuum, dissolved in EtOAc (30 mL), and filtered. The solution was concentrated again and the residue solidified under petroleum ether–ether (1:1). The crystalline ester was filtered and the procedure was repeated twice to remove any insoluble material: yield 4.6 g (77%); mp 105–107 °C; $[\alpha]^{20}_{470}$ 8.49 (*c* 2.12, DMF); IR showed the characteristic active ester peak at 5.65 μm. Anal. Calcd for $C_{23}H_{20}O_8N_4Cl_3$: C, 45.98; H, 3.36; N, 11.66; Cl, 17.70. Found: C, 46.13; H, 3.64; N, 11.87; Cl, 17.52.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{im}-benzyl-L-histidine Pentachlorophenyl Ester. This ester was prepared from N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-benzyl-L-histidine (3.46 g, 10 mmol), pentachlorophenol (2.66 g, 10 mmol), and DCC (2.06 g, 10 mmol) in CH_2Cl_2 (70 mL) in the usual manner. The crude ester was dissolved in EtOAc (15 mL); more DCU was removed by filtration and precipitated with petroleum ether. The crystalline (white needle) product showed one spot on TLC (EtOAc–benzene, 4:1): mp 131–132 °C; $[\alpha]^{23}_D$ −2.25° (*c* 1.78, $CHCl_3$). Anal. Calcd for $C_{24}H_{22}N_3O_4Cl_5$: C, 48.55; H, 3.73; N, 7.08; Cl 29.87. Found: C, 49.11; H, 3.84; N, 7.26; Cl, 29.95.

N^{α}-Carbobenzoxy-N^{im}-benzyl-L-histidine Methyl Ester Hydrochloride. To 40 mL of methanol, previously cooled below −10 °C, were added thionyl chloride (0.80 mL, 11.13 mmol) and then Z-His(Bzl)-OH (3.80 g, 10 mmol). The resulting clear solution was stirred at −10 °C for 3 h and left at room temperature overnight. The reaction mixture was evaporated to dryness under reduced pressure and the residue was crystallized from anhydrous ether. The hygroscopic compound melted at 48 °C: yield 2.80 g (65%); $[\alpha]^{23}_D$ −14.80° (*c* 2.77, MeOH). It was then converted to free ester by treatment with Na_2CO_3 (aq) and recrystallized with pentane: mp 81–83 °C; $[\alpha]^{24}_D$ +6.25° (*c* 2.00, MeOH). Anal. Calcd for $C_{22}H_{23}N_3O_4$: C, 67.2; H, 5.89. Found: C, 67.32; H, 6.10.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine Methyl Ester. Diazomethane in ether was added to N^{α}-carbobenzoxy-N^{im}-tosyl-L-histidine (1.50 g, 3.38 mmol) in small portions until the reaction mixture became pale yellow and then filtered; the filtrate was evaporated to dryness under reduced pressure. The residue was triturated with ether–petroleum ether (1:3) and then left at −10 °C for 3 h. The crystalline methyl ester was filtered, washed with petroleum ether, and dried in high vacuum overnight: yield 1.20 g (78%); mp 72–74 °C; $[\alpha]^{23}_D$ 3.91° (*c* 1.15, DMF). Anal. Calcd for $C_{22}H_{23}N_3O_6S_1$: C, 57.76; H, 5.18. Found: C, 57.56; H, 5.18.

N^{α}-Carbobenzoxy-L-histidine Hydrazide. (a) From Z-His(Z)-OMe·HCl. N^{α},N^{im}-Dicarbobenzoxy-L-histidine methyl ester hydrochloride[19] (2.10 g, 4.43 mmol) was dissolved in cold methylene chloride (30 mL), and triethylamine (0.62 mL, 4.43 mmol) was added to the solution and stirred for 15 min at 5 °C. The solution was washed with water twice (30 mL) to remove Et_3N salt and dried over anhydrous sodium sulfate. The solution was evaporated to dryness and the residue was dissolved in absolute methanol (4 mL), and hydrazine hydrate (0.44 mL) was added to it. The solution was stirred for 5 h at room temperature, when crystalline material precipitated out; the product was filtered and washed with anhydrous ether. Recrystallization from hot EtOH furnished needle shape crystals: yield 0.9 g (52%); mp 172–174 °C; $[\alpha]^{20}_D$ −37.56° (*c* 2.05, 1 N HCl) (lit.[20] mp 171–173 °C, $[\alpha]^{23}_D$ +35.7° (*c* 2.0, 1 N HCl)). The specific rotation given in the literature is for the Z-D-histidine hydrazide.

(b) From Z-His(Z)-OPcp. N^{α},N^{im}-Dicarbobenzoxy-L-histidine pentachlorophenyl ester (0.672 g, 1 mmol) was suspended in EtOH (5 mL) and 95% $NH_2NH_2·H_2O$ (0.067 mL, 2 mmol) was added to it. The reaction mixture turned clear and then solidified in 10 min. The crystalline product was filtered, washed with ether, and then dried in high vacuum: yield 0.26 g (85%); mp 165–179 °C; $[\alpha]^{23}_D$ −36.8° (*c* 0.60, HCl). The crude product was recrystallized from EtOH: mp 171–172 °C; $[\alpha]^{23}_D$ −37.98° (*c* 2.08, 1 N HCl); IR was identical with the previous hydrazide.

(c) From Z-His(Z)-OTcp. The hydrazide was prepared by the same method as described above: yield 73%, mp 165–170 °C; $[\alpha]^{23}_D$ −37.57° (*c* 0.51, 1 N HCl); IR was identical with the previous hydrazide.

(d) From Z-His(Z)-ONp. The hydrazide was obtained by the same method as described above; yield 73%; mp 165–170 °C; $[\alpha]^{23}_D$ −37.57° (*c* 0.51, 1 N HCl); IR was identical with the previous hydrazide.

N^{α}-Carbobenzoxy-N^{im}-benzyl-L-histidine Hydrazide. (a) From Z-His(Bzl)-OMe·HCl. N^{α}-Carbobenzoxy-N^{im}-benzyl-L-

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histidine methyl ester hydrochloride (0.86 g, 2.0 mmol) was dissolved in $CHCl_3$ (10 mL) and TEA (0.28 mL, 2.0 mmol) was added to it with stirring in an ice bath for 0.5 h. The solution was washed with water 3 times, dried over anhydrous Na_2SO_4, and then evaporated to dryness. The dried oil was dissolved in MeOH (3 mL); then 85% $NH_2NH_2 \cdot H_2O$ (0.3 mL) was added and the solution was stirred at room temperature for 4 h. The crude product which precipitated out was filtered. Two recrystallizations from hot water afforded white crystalline hydrazide: yield 0.14 g (18%); mp 148–149.5 °C (crude mp 143–146.5 °C); $[\alpha]^{23}_D$ −7.59° (*c* 1.08, AcOH). Anal. Calcd for $C_{21}H_{23}N_5O_3$: C, 64.10; H, 5.89; N, 17.81. Found: C, 63.46; H, 5.96; N, 17.97.

(b) From Z-His(Bzl)-OPcp. Z-His(Bzl)-OPcp (0.336 g, 0.5 mmol) was suspended in MeOH and 95% $NH_2NH_2 \cdot H_2O$ (0.034 mL, 1 mmol) was added to it. The reaction mixture was stirred at room temperature when it turned to a clear solution and then started to crystallize in a few minutes. The crystalline product was filtered and washed with EtOH and then dried in high vacuum: yield, 0.21 g; mp 142–145 °C; $[\alpha]^{23}_D$ −6.4° (*c* 0.933, AcOH). The crude product gave a positive chlorine test. Analysis showed that 25.75% Cl is present. This indicated that the compound is the pentachlorophenol salt of the hydrazide. The crude salt (200 mg) dissolved in 1 N HCl when the pentachlorophenol precipitated out; it was filtered and washed with water: yield 70 mg (88%); mp 183–186 °C. The filtrate was neutralized with 1 N NaOH when the Z-His-(Bzl)-$NHNH_2$ precipitated out; yield 100 mg (85%); mp 145–147 °C; $[\alpha]^{23}_D$ −3.90 (*c* 1.1, AcOH). Recrystallization from water yielded 74 mg: mp 148–149 °C; $[\alpha]^{23}_D$ −7.5° (*c* 0.4, AcOH); IR is identical with that prepared from the methyl ester.

(c) From Z-His(Bzl)-OTcp. The hydrazide was obtained by the method described above. The compound gave a negative halogen test; it did not form a salt with trichlorophenol: yield 82% mp 147–149 °C; $[\alpha]^{28}_D$ +8.21° (*c* 1.95, AcOH); IR was identical with the previous hydrazide and mixed melting point was not depressed.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine Hydrazide. (a) From Z-His(Tos)-OMe. Z-His(Tos)-OMe (0.457 g, 1 mmol) was dissolved in MeOH (2 mL) and 95% $NH_2NH_2 \cdot H_2O$ (0.067 mL, 2 mmol) was added to it. The reaction mixture was left at room temperature overnight. The crude product was filtered and dried in high vacuum: yield 0.285 g (62%); mp 156–160 °C; $[\alpha]^{22}_D$ +4.62° (*c* 1.19, AcOH). Recrystallization from MeOH and H_2O afforded white crystalline hydrazide; mp 169–171 °C. Anal. Calcd for $C_{21}H_{23}N_5O_5S$: C, 55.13; H, 5.07. Found: C, 54.90; H, 5.33.

(b) From Z-His(Tos)-OPcp. N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidine pentachlorophenyl ester (0.692 g, 1 mmol) was suspended in MeOH (5 mL) and 95% $NH_2NH_2 \cdot H_2O$ (0.067 mL, 2 mmol) was added. The reaction mixture was worked up in the usual manner: yield 0.315 g (69%); mp 160–165 °C; $[\alpha]^{22}_D$ +4.25° (*c* 1.06, AcOH). The crude product was recrystallized from MeOH and H_2O: mp 170–171 °C; IR was identical with the previous hydrazide obtained from the methyl ester and hydrazine.

(c) From Z-His(Tos)-OTcp. The hydrazide was prepared by the same method as described above: yield 69%; mp 167–170 °C; $[\alpha]^{23}_D$ +4.67° (*c* 1.07, AcOH); IR was identical with the previous hydrazide.

(d) From Z-His(Tos)-ONP. The hydrazide was obtained by the same method as described above: yield 70%; mp 170–171 °C; $[\alpha]^{23}_D$ +4.62° (*c* 1.19, AcOH); IR was identical with the previous hydrazide.

N^{α}-Carbobenzoxy-N^{im}-benzyl-L-histidyl-L-valine Methyl Ester. (a) From Z-His(im-Bzl)-OPcp. A solution of Z-His-(im-Bzl)-OPcp (1.26 g, 2 mmol) and H-Val-OMe (0.26 mL, 2 mmol) in 15.4 mL of THF was left at room temperature for 5 h (calculated coupling time for 95% completion, t_{c95} = 4.7 h). The solution was then evaporated completely to dryness and the residue was triturated with petroleum ether in an ice bath. The crystalline crude product was filtered and washed with ether–petroleum ether (1:2) 3 times: yield, 0.90 g (91%); mp 78–82 °C; TLC, 2 spots (EtOAc–benzene, 1:1). The crude product was recrystallized from ether–petroleum ether (5:3): mp 95–96 °C; $[\alpha]^{21}_D$ +1.80° (*c* 1.11, DMF). Anal. Calcd for $C_{27}H_{32}N_4O_5$: C, 65.84; H, 6.55. Found: C, 65.56; H, 6.70.

(b) From Z-His(im-Bzl)-OTcp. The dipeptide methyl ester was obtained by using the same procedure described above. The coupling time was 24 h (calcd t_{c95} = 22 h). The crude product melted at 93–94 °C and the IR was identical with the previous compound; yield 89%.

N^{α},N^{im}-Dicarbobenzoxy-L-histidyl-L-valine Methyl Ester. (a) From Z-His(Z)-OPcp. A solution of Z-His(Z)-OPcp (1.34 g, 2 mmol) and H-Val-OMe (0.26 mL, 2 mmol) was left standing at room temperature for 18 h (calculated t_{c95} = 17.6 h). The isolation of the product was similar to that of the previous dipeptide: yield 0.80 g (75%); mp 103–106 °C. Recrystallization from ether–petroleum ether afforded chromatographically pure product: mp 116–117 °C; $[\alpha]^{23}_D$ +6.98° (*c* 1.29, DMF). Anal. Calcd for $C_{28}H_{32}O_7N_4$: C, 62.71; H, 6.01. Found: C, 62.71; H, 6.33.

(b) From Z-His(Z)-OTcp. The dipeptide methyl ester was prepared by the same method as described above. The coupling time was 40 h (calcd t_{c95} = 33.8 h): mp 101–104 °C yield 61%; IR was identical with the previous dipeptide methyl ester.

N^{α}-Carbobenzoxy-N^{im}-tosyl-L-histidyl-L-valine Methyl Ester. (a) From Z-His(Tos)-OPcp. A solution of Z-His(im-tosyl)-OPcp (1.38 g, 2 mmol) and H-Val-OMe (0.26 mL, 2 mmol) in THF (15.4 mL) was left standing at room temperature for 15 h (calcd $t_{c95°}$ = 13.2 h). The coupling product was worked up as described for Z-His(im-Bzl)-Val-OMe: yield 0.50 g (89%); mp 105–115 °C. The crude product was recrystallized from ether–petroleum ether (2:1): mp 104–105 °C; $[\alpha]^{27}_D$ +21.84° (*c* 1.03, DMF). Anal. Calcd for $C_{27}H_{32}O_7N_4S_1$: C, 58.26; H, 5.79. Found: C, 57.91; H, 6.01.

(b) From Z-His(Tos)-OTcp. The dipeptide methyl ester was obtained by the same method described above. The coupling time was 56 h (calcd for t_{c95} = 49 h): yield 82%; mp 104–106 °C; IR was identical with the previous compound.

N^{α}-(*tert*-Butyloxycarbonyl-N^{im}-(2,4-dinitrophenyl)-L-histidyl-L-valine Methyl Ester. (a) From Boc-His(DNP)-OPcp. Boc-His(DNP)-OPcp (0.67 g, 1.0 mmol) was dissolved in THF (7.7 mL, 1 mmol) and 0.13 mL of H-Val-OMe was added; then the solution was left at room temperature for 16 h (calcd t_{c95} = 15.5 h). The solution was evaporated to dryness; then the residue was triturated with ether–petroleum ether (1:4). The crude product was recrystallized from ether–petroleum ether (1:3): mp 76–78 °C dec; $[\alpha]^{27}_D$ +9.29° (*c* 1.13, DMF). Anal. Calcd for $C_{23}H_{30}N_6O_9$: C, 50.83; H, 5.66. Found: C, 50.53; H, 5.86.

(b) From Boc-His(DNP)-OTcp. The dipeptide methyl ester was obtained by the same method as described above. The coupling time was 24 h (calcd t_{c95} = 20 h): yield 93%; mp 76–78 °C dec; IR was identical with the previous compound.

Racemization Rate Studies on Active Esters. The kinetic studies were done in a constant-temperature room (23 ± 2 °C). The preparation and the storage of all solutions for these rate studies were carried out in a glovebag under a dry nitrogen atmosphere. The kinetics were followed on a Cary 60 spectropolarimeter. The solvents were absolute, and the purified triethylamine was stored over sodium. The kinetics were followed at 589 μm unless otherwise noted. The first reading was taken within 2 min of mixing of the reagents. The pseudo-first-order rate constants were obtained from the linear plots of ln α vs. time. The second-order rate constants were obtained by dividing the pseudo-first-order rate constants by the triethylamine concentration.

Aminolysis Rate Studies on Active Esters. THF solution of the active ester (0.13 M) was mixed with 1 equiv of L-valine methyl ester and the aminolysis was followed by the disappearance of the active ester carbonyl peak around 5.5–5.7 μm on a Perkin-Elmer Model 283 spectrophotometer with a time drive setting for fast reaction. A sealed 0.1-mm BaF_2 cell was used for the solution and a matched cell containing the solvent was in the reference beam. The second-order rate constants for the peptide bond formation were obtained from the linear plots of the reciprocal of concentration vs. time.

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Registry No. Z-His(im-Bzl)-OPcp, 66438-51-5; Z-His(im-

Bzl)-OTcp, 95485-15-7; Z-His(im-Z)-OPcp, 60666-46-8; Z-His(im-Z)-OTcp, 40917-51-9; Z-His(im-Tos)-OPcp, 95485-16-8; Z-His-(im-Tos)-OTcp, 95485-17-9; Boc-His(im-DNP)-OPcp, 42290-57-3; Boc-His(im-DNP)-OTcp, 95485-18-0; Z-His(im-Z)-ONP, 20531-27-5; Z-His(im-Tos)-ONP, 95485-19-1; Z-His(tos)-OMe, 95485-21-5; Z-His(Z)-OMe·HCl, 95485-27-1; H-Val-OMe, 4070-48-8; N^{α}-carbobenzoxy-N^{im}-benzyl-L-histidine methyl ester hydrochloride, 95485-20-4; N^{α}-carbobenzoxy-L-histidine hydrazide, 49706-31-2; N^{α}-carbobenzoxy-N^{im}-benzyl-L-histidine hydrazide, 26582-89-8; N^{α}-carbobenzoxy-N^{im}-tosyl-L-histidine hyrazide, 95485-22-6; N^{α}-carbobenzoxy-N^{im}-benzyl-L-histidyl-L-valine methyl ester, 95485-23-7; N^{α},N^{im}-dicarbobenzoxy-L-histidyl-L-valine methyl ester, 95485-24-8; N^{α}-carbobenzoxy-N^{im}-tosyl-L-histidyl-L-valine methyl ester, 95485-25-9; pentachlorophenol, 87-86-5; 2,4,5-trichlorophenol, 95-95-4; N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-benzyl-L-histidine pentachlorophenyl ester, 61266-04-4; N^{α}-(carbobenzoxy)-N^{im}-benzyl-L-histidine, 21929-66-8; N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-2,4-dinitrophenyl-L-histidyl-L-valine methyl ester, 95485-26-0; N^{α},N^{im}-dicarbobenzoxy-L-histidine hydrazide, 95514-76-4.

Ozonolysis of 1-Methylindenes. Solvent, Temperature, and Substituent Electronic Effects on the Ozonide Exo/Endo Ratio

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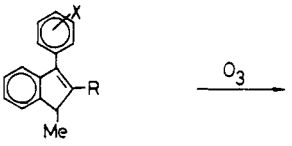
The ozonolyses of 1-methyl-3-aryl-, 1,2-dimethyl-3-aryl-, and 1-methyl-2,3-diarylindenes (**1a–e**, **4a–e**, **7a–e**, **7a′,b′,d′**) in various solvents at several temperatures have been undertaken. The data revealed the following. (a) The ozonolysis of indenes **1c**, **4c**, and **7c** in aprotic solvents yielded in each case a mixture of exo/endo ozonide isomers, the solvent-independent exo/endo ratio being 7:3, 3:2, and 3:7, respectively. In marked contrast, protic solvents exerted a significant influence on the ozonide stereochemistries. (b) The ozonolysis of indene **7c** in methanol at −70 °C afforded a novel methanol-participated product **14** as the major product, whereas the reaction at 20 °C lead to the production of a 72% yield of the expected ozonide isomers **8c**/**9c**. A similar trend was observed for **4c**. From **1c**, however, a more conventional methanol-participated product **10** was obtained along with ozonide isomers **2c**/**3c**. (c) The ozonide exo/endo ratios increased with the decrease of the reaction temperature. The exception was the ozonolysis of **4** in MeOH, in which the reverse trend was observed. (d) The substituent electronic effect can exert a significant influence on the ozonide composition.

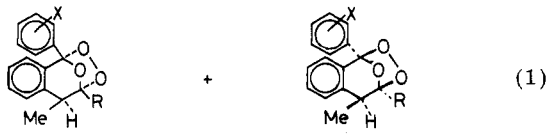
Recently we reported that (a) the ozonolysis of a series of 1-substituted indenes in CCl_4 at 20 °C affords in each case a corresponding mixture of exo/endo ozonide isomers and (b) the steric effects of 1- or 2-substituents play a significant role in determining the ozonide exo/endo ratio.[2] Since there are other factors which could affect the ozonide stereochemistry, we have consequently performed the ozonolysis of 1-methyl-3-aryl-, 1,2-dimethyl-3-aryl-, and 1-methyl-2,3-diarylindenes (**1a–e**, **4a–e**, **7a–e**, **7a′,b′,d′**) in various solvents, including protic ones, and at a series of temperatures. The product yields and ratios of the respective exo/endo isomeric ozonides from each reaction were determined. We anticipated that careful examination of the resulting data could provide further insight into the ozonolysis mechanism.[3,4]

Results

(I) Ozonolysis in Aprotic Solvents. The ozonolysis of 1-methyl-3-phenylindene (**1c**) in hexane, carbon tetrachloride, methylene chloride, acetone, and acetonitrile at 20 °C gave, in each case, a mixture of exo ozonide **2c** and the endo isomer **3c** in isolated yields of around 60%, the exo/endo isomer ratios averaging around 7:3 (Table I and IV). Similarly, the ozonide isomer ratios obtained from the ozonolyses of indenes **1a,b,d–e** in some aprotic solvents were found to vary little with the solvents (eq 1 and Table I). In this series the exo/endo ratio depended on the electronic nature of the substituent X, the ratio varying



1a, X = 4-OMe; R = H
1b, X = 4-Me; R = H
1c, X = R = H
1d, X = 4-Cl; R = H
1e, X = 3-Cl; R = H
4a, X = 4-OMe; R = Me
4b, X = 4-Me; R = Me
4c, X = H; R = Me
4d, X = 4-Cl; R = Me
4e, X = 3-Cl; R = Me



(1)

2a, X = 4-OMe; R = H
2b, X = 4-Me; R = H
2c, X = R = H
2d, X = 4-Cl; R = H
2e, X = 3-Cl; R = H
5a, X = 4-OMe; R = Me
5b, X = 4-Me; R = Me
5c, X = H; R = Me
5d, X = 4-Cl; R = Me
5e, X = 3-Cl; R = Me

3a, X = 4-OMe; R = H
3b, X = 4-Me; R = H
3c, X = R = H
3d, X = 4-Cl; R = H
3e, X = 3-Cl; R = H
6a, X = 4-OMe; R = Me
6b, X = 4-Me; R = Me
6c, X = H, R = Me
6d, X = 4-Cl; R = Me
6e, X = 3-Cl; R = Me

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