Registry No. *m*-Methylphenoxide, 20227-79-6; *m*-aminophenoxide, 68743-37-3; *m*-hydroxyphenoxide, 20217-24-7; *m*-methoxyphenoxide, 40529-20-2; *m*-fluorophenoxide, 32376-33-3; *m*-nitrophenoxide, 16554-54-4; *m*-cyanophenoxide, 18938-12-0; *m*-formylphenoxide, 38144-52-4; *m*-(trifluoromethyl)phenoxide, 72332-16-2; *p*-methylphenoxide, 22113-51-5; *p*-aminophenoxide, 19052-59-6; *p*-hydroxyphenoxide, 20217-26-9; *p*-methoxyphenoxide, 29368-59-0; *p*-fluorophenoxide, 32376-34-4; *p*-nitrophenoxide, 14609-74-6; *p*-cyanophenoxide, 14609-76-8; *p*-formylphenoxide, 18938-17-5; *p*-(trifluoromethyl)phenoxide, 72332-17-3; *m*-methylanilide, 37062-12-7; *m*-aminoanilide, 72611-39-3; *m*-hydroxyanilide, 72611-40-6; *m*-methoxyanilide, 72611-41-7; *m*-fluoroanilide, 72611-42-8; *m*-nitroanilide, 72611-43-9; *m*-cyanoanilide, 72611-44-0; *m*-formylanilide, 72611-45-1; *m*-(trifluoromethyl)anilide, 72611-46-2; *p*-methylanilide, 37062-13-8; *p*-aminoanilide, 72611-47-3; *p*-hydroxyanilide, 72611-48-4; *p*-methoxyanilide, 72611-49-5; *p*-fluoroanilide, 72611-50-8; *p*-nitroanilide, 934-70-3; *p*-cyanoanilide, 66365-37-5; *p*-formylanilide, 72611-51-9; *p*-(trifluoromethyl)anilide, 72611-52-0; *m*-methylbenzyl anion, 59305-38-3; *m*-aminobenzyl anion, 72611-53-1; *m*-hydroxybenzyl anion, 72611-54-2; *m*-methoxybenzyl anion, 72611-55-3; *m*-fluorobenzyl anion, 72611-56-4; *m*-nitrobenzyl anion, 72611-57-5; *m*-cyanobenzyl anion, 72611-58-6; *m*-formylbenzyl anion, 72611-59-7; *m*-(trifluoromethyl)benzyl anion, 72611-60-0; *p*-methylbenzyl anion, 59305-42-9; *p*-aminobenzyl anion, 72638-42-7; *p*-hydroxybenzyl anion, 72611-61-1; *p*-methoxybenzyl anion, 72611-62-2; *p*-fluorobenzyl anion, 72611-63-3; *p*-nitrobenzyl anion, 72409-67-7; *p*-cyanobenzyl anion, 18802-90-9; *p*-formylbenzyl anion, 72611-64-4; *p*-(trifluoromethyl)benzyl anion, 72611-65-5; *m*-methylphenol, 108-39-4; *m*-aminophenol, 591-27-5; *m*-hydroxyphenol, 108-46-3; *m*-methoxyphenol, 150-19-6; *m*-fluorophenol, 372-20-3; *m*-nitrophenol, 554-84-7; *m*-cyanophenol, 873-62-1; *m*-formylphenol, 100-83-4; *m*-(trifluoromethyl)phenol, 98-17-9; *p*-methylphenol, 106-44-5; *p*-aminophenol, 123-30-8; *p*-hydroxyphenol, 123-31-9; *p*-methoxyphenol, 150-76-5; *p*-fluorophenol, 371-41-5; *p*-nitrophenol, 100-02-7; *p*-cyanophenol, 767-00-0; *p*-formylphenol, 123-08-0; *p*-(trifluoromethyl)phenol, 402-45-9; *m*-methylaniline, 108-44-1; *m*-aminoaniline, 108-45-2; *m*-methoxyaniline, 536-90-3; *m*-fluoroaniline, 372-19-0; *m*-nitroaniline, 99-09-2; *m*-cyanoaniline, 2237-30-1; *m*-formylaniline, 1709-44-0; *m*-(trifluoromethyl)aniline, 98-16-8; *p*-methylaniline, 106-49-0; *p*-aminoaniline, 106-50-3; *p*-methoxyaniline, 104-94-9; *p*-fluoroaniline, 371-40-4; *p*-nitroaniline, 100-01-6; *p*-cyanoaniline, 873-74-5; *p*-formylaniline, 556-18-3; *p*-(trifluoromethyl)aniline, 455-14-1; *m*-methyltoluene, 108-38-3; *m*-methoxytoluene, 100-84-5; *m*-fluorotoluene, 352-70-5; *m*-nitrotoluene, 99-08-1; *m*-cyanotoluene, 620-22-4; *m*-formyltoluene, 620-23-5; *m*-(trifluoromethyl)toluene, 401-79-6; *p*-methyltoluene, 106-42-3; *p*-methoxytoluene, 104-93-8; *p*-fluorotoluene, 352-32-9; *p*-nitrotoluene, 99-99-0; *p*-cyanotoluene, 104-85-8; *p*-formyltoluene, 104-87-0; *p*-(trifluoromethyl)toluene, 6140-17-6.

Kinetic Studies in Peptide Chemistry. Coupling, Racemization, and Evaluation of Methods Useful for Shortening Coupling Time[1]

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Kinetic studies were carried out on N-protected methionine and glycylmethionine active esters to determine the racemization rate constants with triethylamine and the coupling rate constants with valine methyl ester. In contrast to cysteine dipeptide active esters, which racemize through an enolization mechanism, the methionine dipeptide active esters racemize through the usual 5(4*H*)-oxazolone route. The role of sulfur in the side chain is discussed. The time required for coupling a given percentage e.g., 99%) of the amine component can be significantly reduced by using an excess of the active ester. This method is evaluated quantitatively for second-order kinetics.

It has been reported that *N*-carbobenzoxy-*S*-benzyl-L-cysteine active esters racemize unusually fast[2] by the isomerization mechanism.[3] It has also been established that the *N*-carbobenzoxyglycyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester racemizes through enolization rather than the expected 5(4*H*)-oxazolone mechanism.[4,5] The deviation from the 5(4*H*)-oxazolone mechanism is amino acid side chain dependent, and therefore it is of interest to compare the effect of the side chain of the other sulfur-containing amino acid, namely methionine, on the rate and mechanism of racemization.

For practical synthetic purposes the coupling time is another important aspect which must be considered, since coupling reactions must be completed within a reasonable period of time. There are several ways of decreasing the coupling time. One is to choose a faster coupling active ester, avoiding such slow couplers as trichlorophenyl or *p*-nitrophenyl esters, which require days or even weeks to achieve 99% completion.[6] Another way is to increase the concentrations of the coupling species, and a third way is to change the ratio of their initial concentrations. Practical aspects of these methods will be illustrated in this paper. A quantitative evaluation of the last method will also be included.

In this work, four frequently used active esters were selected for the study. These were pentachlorophenyl, *p*-nitrophenyl, pentafluorophenyl, and *N*-succinimidoyl active esters of *N*-carbobenzyloxy- and *N*-*tert*-butyloxycarbonylmethionine, as well as the corresponding glycyl dipeptide active esters. Racemization was studied in anhydrous tetrahydrofuran solution in the presence of triethylamine. Coupling with an equimolar amount of L-valine methyl ester was followed by IR analysis in the same solvent. The results are given in Table I which contains the experimental as well as the calculated second-order rate constants for both racemization and coupling. The calculated values are obtained using the additivity principle published previously.[7]

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Table I. Experimental and Calculated Racemization[a] and Coupling[b] Rate Constants of *N*-Carbobenzoxy-, *N*-*tert*-Butyloxycarbonyl-, and *N*-Carbobenzoxyglycyl-L-methionine Active Esters[c]

compound	$10^6 k_r$	$10^2 k_c$	k_c/k_r
Z-Met-OPcp[17]	2.80 (3.00)	0.36 (0.27)	1286 (698)
Boc-Met-OPcp[18]	1.50 (0.898)	0.29 (0.26)	1940 (2865)
Z-Gly-Met-OPcp	312.5 (409.7)	1.84 (2.30)	59 (57)
Z-Met-ONp[19]	8.65 (6.24)	0.076 (0.06)	88 (97)
Boc-Met-ONp[20]	4.90 (1.44)	0.092 (0.056)	188 (394)
Z-Gly-Met-ONp	307.5 (655.6)	0.55 (0.506)	18 (7.8)
Z-Met-OPfp[21]	56.35 (58.53)	16.79 (10.0)	2980 (1724)
Boc-Met-OPfp[22]	24.70 (13.46)	11.10 (9.42)	4494 (7030)
Z-Gly-Met-OPfp	4710 (6146)	61 (85)	130 (138)
Z-Met-OSu[23]	82.1 (97.2)	2.59 (3.57)	315 (370)
Boc-Met-OSu[23]	47.10 (22.35)	2.20 (3.36)	467 (1500)

[a] Racemization with triethylamine in tetrahydrofuran solution. Two different concentrations of triethylamine were used. [b] Coupling with H-Val-OMe in tetrahydrofuran solution. [c] k_r and k_c values are in M^{-1} s^{-1}. Each value is the average of two experiments. Calculated values are in parentheses.

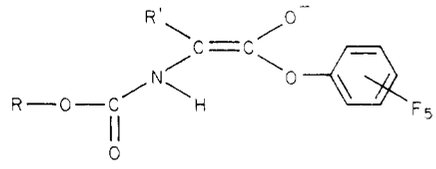
Rates of Racemization of *N*-Carbobenzoxy-L-methionine, *N*-*tert*-Butyloxycarbonyl-L-methionine, and *N*-Carbobenzoxyglycyl-L-methionine Active Esters. Data in Table I show that in general the *N*-*tert*-butyloxycarbonyl-L-methionine active esters racemize approximately half as fast as the corresponding *N*-carbobenzoxy derivatives. This has been observed previously for the racemization of other N-protected amino acid active esters.[7]

Racemization of the dipeptide active ester derivatives was followed by IR analysis, which permitted the detection of the presence of the 5(4*H*)-oxazolone in addition to the active ester as the racemization proceeded. The characteristic 5(4*H*)-oxazolone peak at 5.5 μm increased while the ester carbonyl peak at 5.6–5.7 μm decreased as the racemization proceeded, and the racemization rate reported was the rate of formation of the 5(4*H*)-oxazolone, since oxazolone racemizes much faster than it forms.[8,9]

Data in Table I also show that the racemization of the dipeptides is faster than the corresponding methionine active esters by two orders of magnitude. The relative rates of racemization of the various active esters of methionine and its dipeptides follow the usual trend of decreasing order of reactivity as given below, with the *p*-nitrophenyl ester chosen as standard (OSu, OPfp, ONp, OPcp): Z-Met-, 9.5, 6.5, 1, 0.32; Boc-Met-, 9.6, 5.0, 1, 0.31; Z-Gly-Met-, –, 15, 1, 1.

All racemized products were identified by coupling with glycine ether ester. The time required for each reaction could be estimated from the data of the aminolysis study with L-valine methyl ester. Glycine ethyl ester is expected to couple at least an order of magnitude faster than L-valine methyl ester.[7]

The racemization rate is parallel with the pK_a value of the corresponding phenol or in general with the electron-withdrawing ability of the ester group[10,11] with the exception of the pentafluorophenyl ester which racemizes 15 to 20 times faster than the pentachlorophenyl ester although the p*K* value of pentafluorophenol is almost the same as that of pentachlorophenol (5.5 and 5.2, respectively). A similar tendency was observed for other *N*-alkyloxycarbonylamino acid pentachlorophenyl and pentafluorophenyl esters.[2a,7,15] Sheppard proved that the electron-withdrawing ability of the pentafluorophenyl group is practically identical with that of the pentachlorophenyl group.[12] Based on Sheppard's investigation,[12] the fast racemization of the pentafluorophenyl ester cannot be explained by the electron-withdrawing ability of this group. We propose that the enolate **1**, which is the intermediate



1, R′ = amino acid side chain

in racemization, is stabilized by the very strong solvation due to the presence of the fluorinated ester group.

It is worthwhile to mention in connection with this proposal that the pentafluorophenyl esters have much higher solubility in most solvents than the pentachlorophenyl esters.

Comparison of the Rates of Racemization of the L-Methionine and L-Cysteine Active Ester Derivatives. *N*-Carbobenzoxy-*S*-benzyl-L-cysteine active esters are known to racemize unusually fast.[7] Comparison of the published values[7] of the second-order racemization rate constants of the N-protected L-cysteine active esters with those of the corresponding methionine active esters shows that the *N*-carbobenzoxy-*S*-benzyl-L-cysteine active esters racemize about 45 to 150 times faster than the corresponding methionine active esters, and the *N*-*tert*-butyloxycarbonyl-*S*-benzyl-L-cysteine active esters racemize about 8 to 26 times faster. Furthermore, it has been reported that *N*-carbobenzoxyglycyl-*S*-benzyl-L-cysteine *p*-nitrophenyl ester racemizes through α-hydrogen abstraction, and the deviation from the 5(4*H*)-oxazolone mechanism is amino acid side chain dependent,[4] while kinetic data and IR suggest that the *N*-carbobenzoxyglycyl-L-methionine active esters studied in this work racemize through the 5(4*H*)-oxazolone mechanism.

Barber and Jones[11] proposed that the unusually fast racemization of *N*-carbobenzoxy-*S*-benzyl-L-cysteine *p*-

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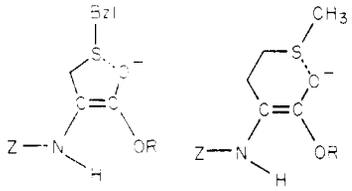
nitrophenyl ester can be attributed to the overlap of the p orbital of the α carbon with the d orbital of sulfur. However, model study suggests that such overlap would be even more favorable in the case of methionine which, in contrast to cysteine, behaves normally.

A reasonable explanation for the normal behavior of methionine may be the following: in the case of cysteine the d orbitals of sulfur overlap with the p orbital of the oxygen of the enolized carbonyl group and by this substantially increase the stability of the enolate of structure **2a**. Model studies show that this d–p orbital overlap is



2a 2b

also more favorable in the case of cysteine (**2a**) than methionine (**2b**). As a consequence, the cysteine active esters racemize much faster than the methionine active ester, and also the cysteine dipeptide active esters racemize through the enolization mechanism rather than the usual 5(4*H*)-oxazolone mechanism while the methionine dipeptide active esters behave normally.

Rates of Coupling of *N*-Carbobenzoxy-L-methionine, *N*-*tert*-Butyloxycarbonyl-L-methionine, and *N*-Carbobenzoxyglycyl-L-methionine Active Esters. The coupling rate constants are given in Table I. The results show that for the monomers there is no significant effect of the N-protecting groups on the rate of coupling since they are two atoms away from the reaction center. The rate is primarily affected by the active ester group which is directly attached to the reaction center. In general, the dipeptides couple four to seven times faster than the corresponding methionine derivatives. The decreasing order of reactivity for all the N-protected monomer and dipeptide active esters studied is (OPfp, OSu, OPcp, ONp): Z-Met-, 212, 33, 4.5, 1; Boc-Met-, 120, 24, 3.1, 1; Z-Gly-Met-, 111, –, 3.3, 1. The *p*-nitrophenyl ester is chosen as standard. Significant is the observation that the pentafluorophenyl esters couple at least two orders of magnitude faster than the corresponding *p*-nitrophenyl esters and 34 to 47 times faster than pentachlorophenyl ester. These results again fall into the previously observed general pattern that the pentafluorophenyl esters couple 20 to 73 times faster than the pentachlorophenyl esters.[2a,5,7,14,15] This large difference cannot be attributed to the electron-withdrawing ability of the pentafluorophenyl group.[12] Kisfaludy et al.[13] attributed this property to neighboring group participation by the fluorine atom in the ortho position which accelerates the collapse of the tetrahedral intermediate in the transition state. Furthermore, the solvation of the $^-$OPfp leaving group also contributes to the fast coupling rate. The above data demonstrate the unusual behavior of the pentafluorophenyl esters.

Comparison of the Ratios of the Coupling and Racemization Rate Constants. Data in Table I show that the decreasing order and the magnitude of racemization rate constants are not the same as the decreasing order and magnitude of coupling rate constants. With the assumption that the racemization induced by the coupling amine parallels that measured for triethylamine, the comparison of the k_c/k_r ratios will have significant practical values in peptide synthesis: a larger k_c/k_r value will indicate a higher degree of optical purity of the coupling product. A comparison of the k_c/k_r values of the various active esters studied is (OPfp, OPcp, OSu, ONp): Z-Met-, 34, 14.6, 3.6, 1; Boc-Met-, 24, 10.3, 2.5, 1; Z-Gly-Met-, 7.1, 3.3, –, 1. These values indicate that the *p*-nitrophenyl ester, one of the most frequently used active esters in peptide synthesis, would produce more of the undesired diastereomer under these conditions than the other esters studied. These results suggest that for coupling pentafluorophenyl active ester should be used whenever possible. The higher k_c/k_r values of the *N*-*tert*-butyloxycarbonyl derivatives, as shown in Table I, indicate that this N-protecting group should be favored over the *N*-carbobenzoxy group.

Table II. Calculated 99.9% Coupling Times (Hours) for H-Val-OMe in THF with Various Active Esters

	initial ester concn, M (initial valine concn, M)		
	0.06 (0.06)	0.12 (0.12)	0.12 (0.06)
Boc-Met-OSu	210	105	1.3
Z-Met-OSu	178	89	1.1
Boc-Met-OPfp	42	21	0.26
Z-Met-OPfp	27	14	0.17
Z-Gly-Met-OPfp	7.6	3.8	0.05
Boc-Met-ONp	5100	2600	32
Z-Met-ONp	5800	2900	36
Z-Gly-Met-ONp	840	420	5.2
Boc-Met-OPcp	1600	800	9.9
Z-Met-OPcp	1300	640	8.0
Z-Gly-Met-OPcp	250	130	1.6

The considerably lower k_c/k_r values for methionine dipeptides predict that they will give products of lower optical purity than the corresponding monomer active esters. This is in contrast with the corresponding cysteine derivatives which behave abnormally. The cysteine dipeptides give higher k_c/k_r values than those of the corresponding monomers.[7]

Methods To Reduce Coupling Time. The serious consequence of using slow-coupling active esters can be realized from Table II. Under the same experimental conditions, if the coupling is carried out at an initial concentration of 0.06 M, the 99.9% coupling times for Boc-Met-OPfp and Boc-Met-ONp are 42 and 5100 h, respectively. This clearly indicates the disadvantage of the slower coupling active esters.

For practical synthetic purposes, the coupling reaction must be fast enough to be essentially completed within a reasonable time. Aside from using a faster coupling active ester, it is possible to reduce coupling times significantly by varying the concentrations of the reacting species.[6]

In the case where the concentrations of two reactants, A and B, are the same ($C_A = C_B = C$), the second-order rate expression

$$\text{rate} = kC_AC_B \tag{1}$$

becomes $-dC/dt = kC^2$, and this integrates to $kt = 1/C - 1/C_0$, where C_0 is the initial concentration. If we let x be the fraction of reaction completed, then C will equal $(1 - x)C_0$ when t is t_x, and we have

$$t_x = x/[(1 - x)kC_0] \tag{2}$$

Thus, it is a well-known general characteristic of second-order reactions between two reactants at *equal* concentrations that *the time required to complete any given fraction of the reaction is inversely proportional to the initial concentration.* For example, the half-life is $1/(kC_0)$, the time required for 99% completion is $99/(kC_0)$, and the time for 99.9% is $999/(kC_0)$. Table II shows the 99.9% coupling times for H-Val-OMe with various active esters

as calculated from their rate constants (Table I). It is seen that the required times are twice as large for $C_0 = 0.06$ M as they are for $C_0 = 0.12$ M (for example, 210 h compared to 105 h for Boc-Met-OSu).

An even more effective way to reduce the coupling time of one reactant is to use an excess concentration of the other. We have previously illustrated this for some coupling reactions.[6] The striking effect on reaction times caused by changing the ratios of the initial concentrations of the reactants is analyzed below.

It is easy to see why there is such a large effect. We let C_0 now be the initial concentration of the limiting reagent A, and nC_0 be the initial concentration of the excess reagent B. Then n is the ratio of initial concentrations, and $n > 1$. In our reactions, A is H-Val-OMe, and B is one of the active esters. We now compare a given coupling reaction carried out under two conditions, first with equal initial concentrations of A and B ($n = 1$), and second with the same C_0 but with B initially twice as concentrated as A ($n = 2$). At the beginning of the two reactions, the rate, kC_AC_B, is twice as fast for $n = 2$ as for $n = 1$. But later, at comparable stages of completion of the coupling of A, the two rates become increasingly disparate. For example, after 99% of A has been coupled, $C_A = 0.01C_0$. For $n = 1$, $C_B = 0.01C_0$ at this point. But for $n = 2$, $C_B = 1.01C_0$, or 101 times as large as for $n = 1$, and consequently the rate at this point is 101 times as fast. In a similar way we find that at 99.9% completion, the reaction with $n = 2$ is proceeding 1001 times as fast as with $n = 1$. So in the later stages of our coupling reactions, this scavenger effect of an excess of active ester can enormously accelerate the coupling rate of the valine. The overall effect on reaction times can be calculated from the equations given below.

When $n \neq 1$, the second-order rate equation (eq 1) integrates to

$$kt = [\ln (C_B/nC_A)]/[(n - 1)C_0] \quad (3)$$

Now, when $t = t_x$, then $C_A = (1 - x)C_0$, and $C_B = (n - x)C_0$. Therefore,

$$t_x = \frac{\ln [(n - x)/n(1 - x)]}{(n - 1)kC_0} \quad (4)$$

This equation allows us to calculate the time required for the reaction of any fraction (x) of the limiting reactant (A) for any ratio (n) of initial concentrations, assuming a given k and C_0. For example, for $n = 2$, the 99% completion time is $(\ln 50.5)/(kC_0)$ and the 99.9% completion time is $(\ln 500.5)/(kC_0)$.

In general, if we wish to compare t_x for any n with t_x for $n = 1$, we can define T as $t_x(n)/t_x(1)$ and find it from eq 4 and 2:

$$T = \frac{(1 - x)}{x(n - 1)} \ln \left[\frac{(n - x)}{n(1 - x)} \right] \quad (5)$$

The behavior of this relative reaction time is shown in Figure 1. The most dramatic reductions in reaction times occur when the reaction is carried close to completion (x near 1), where even small excesses have considerable scavenging effect. Thus, for $x = 0.999$, only a *1% excess* in the initial concentration of one reagent cuts the reaction time of the other to less than one-fourth of the value at equal concentrations.

As applied to our coupling reactions, the concentration effects described above are illustrated in Table II. In the three columns of numbers, the first shows the 99.9% coupling times for the various active esters with H-Val-OMe for initial concentrations both equal at 0.06 M. The second column shows that the effect of doubling both initial concentrations to 0.12 M is to cut the reaction times in half. The third column should be compared to the first and to the second. Compared to the first, it shows the dramatic reduction, by a factor of 999/ln 500.5 = 161, in the coupling time of valine caused by doubling the relative initial concentration of ester. Compared to the second, it shows that a still dramatic reduction, by a factor of 161/2 or about 80, is obtained by *reducing* the initial concentration of valine by half. Reaction time reductions of this magnitude can bring couplings that are unfeasible under equimolar conditions into the range of practicality.

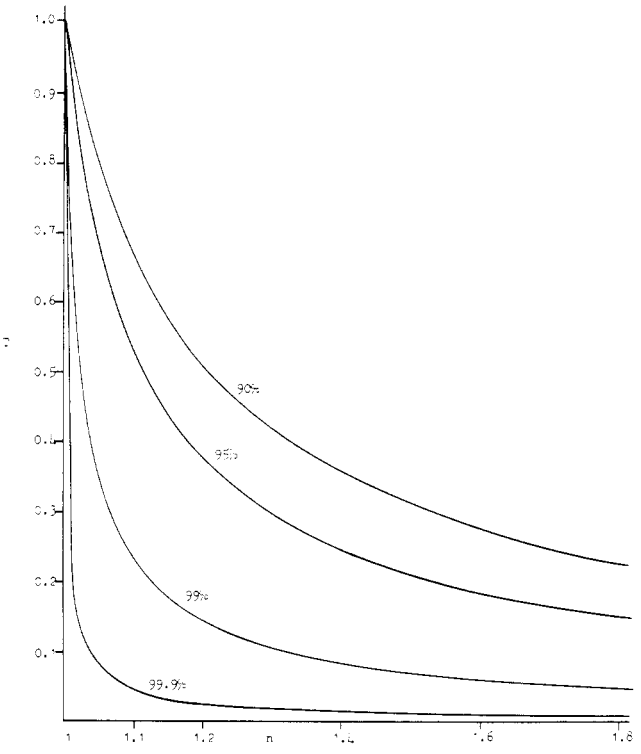


Figure 1. The effect of the ratio of initial concentrations, n, on the reaction time, T.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Optical rotations were determined on the Rudolph photoelectric polarimeter, Model 200S-340-8006. Infrared spectra and coupling kinetics were followed by the Perkin-Elmer 137 spectrophotometer. All kinetic studies were done in a constant temperature room (23 ± 1 °C), with no other thermostating used. Analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY. All solvents used were glass-distilled. For the kinetic studies, purified tetrahydrofuran[2a] was used. Tetrahydrofuran, ether, and triethylamine were stored over sodium and were redistilled before use. The valine methyl ester[16] was stored in the freezer and freshly redistilled before use.

The following N-protected methionine active esters used in the study were prepared according to the general procedure described earlier.[17] Departures from this procedure are given below. Crude products of the pentachlorophenyl and pentafluorophenyl esters were dissolved in ethyl acetate and precipitated by petroleum ether.

***N*-Carbobenzoxy-L-methionine Pentachlorophenyl Ester**:[17] $[\alpha]^{23}_D$ −17.25° (*c* 2, THF); $[\alpha]^{23}_D$ −3.3° (*c* 0.55, chloroform).

***N*-*tert*-Butyloxycarbonyl-L-methionine Pentachlorophenyl Ester**:[18] $[\alpha]^{23}_D$ −24.75° (*c* 2, THF).

(16) R. Schwyzer, B. Iselin, H. Kappelar, B. Riniker, W. Rittel, and H. Suber, *Helv. Chim. Acta*, **41**, 1273 (1958).
(17) J. Kovacs, M. Q. Ceprini, C. A. Dupraz, and G. N. Schmit, *J. Org. Chem.*, **32**, 3696 (1967).
(18) B. J. Johnson, *J. Pharm. Sci.*, **62** (6), 1019 (1973).

***N*-Carbobenzoxy-L-methionine *p*-Nitrophenyl Ester.**[19] After the reaction was completed the filtrate was evaporated to dryness in vacuo at 40 °C. The yellowish residue was dissolved in hot ethanol and the product crystallized on standing in the freezer for 2 h. The crystals were collected and washed several times with cold ethanol; $[\alpha]^{23}_D$ −23.25° (*c* 2, THF).

***N*-*tert*-Butyloxycarbonyl-L-methionine *p*-Nitrophenyl Ester.**[20] The same modification in procedure was used as for the carbobenzoxy analogue; $[\alpha]^{23}_D$ −25.3° (*c* 2, THF).

***N*-Carbobenzoxy-L-methionine Pentafluorophenyl Ester.**[21] The reaction time was 1 h; $[\alpha]^{23}_D$ −19.15° (*c* 2, THF).

***N*-*tert*-Butyloxycarbonyl-L-methionine Pentafluorophenyl Ester.**[22] The reaction time was 1 h; $[\alpha]^{23}_D$ −28.25° (*c* 2, THF).

***N*-Carbobenzoxy-L-methionine *N*-Succinimidoyl Ester.**[23] THF was added to the ethyl acetate solution until all the *N*-hydroxysuccinimide was dissolved. The crude product was recrystallized from 2-propanol.

***N*-*tert*-Butyloxycarbonyl-L-methionine *N*-Succinimidoyl Ester.**[23] The same modification in procedure was used as for the carbobenzoxy analogue; $[\alpha]^{23}_D$ −21.5° (*c* 2, dioxane).

L-Methionine Pentachlorophenyl Ester Hydrochloride. *N*-*tert*-Butyloxycarbonyl-L-methionine pentachlorophenyl ester[18] (2.49 g, 5 mmol) was dissolved with stirring in a minimum amount of trifluoroacetic acid (TFA) under N_2. When all solid was dissolved, the TFA was removed under vacuum at 40 °C. The oily residue was dissolved in 5 mL of THF and then 1.05 mL (7.5 mmol) of 4.8 N HCl in ether solution was added dropwise with constant stirring. Crystallization occurred immediately. The solid was filtered and washed several times with ether and was recrystallized from THF–ether. The HCl salt was then dried overnight under vacuum at 40 °C: yield 2.1 g (97%); mp 168–169 °C; $[\alpha]^{23}_D$ +14.5° (*c* 2, DMF). IR showed the characteristic ammonium and ester carbonyl peaks at 3.4 and 5.6 μm (KBr), respectively. Anal. Calcd for $C_{11}H_{11}Cl_6NO_2S$: C, 30.44; H, 2.55. Found: C, 30.88; H, 3.16.

The above procedure was used for the preparation of other active ester hydrochloride salts described below unless stated otherwise.

L-Methionine Pentafluorophenyl Ester Hydrochloride: yield 85%, mp 130–130.5 °C, $[\alpha]^{23}_D$ +11.25° (*c* 2, DMF). IR showed the ester carbonyl peak at 5.55 μm (KBr).

Anal. Calcd for $C_{11}H_{11}ClF_5NO_2S$: C, 37.56; H, 3.15. Found: C, 37.85; H, 3.59.

Another attempt to prepare the hydrochloride salt was made using the same procedure except that HCl in methanol, instead of HCl in ether, was employed, but transesterification took place, producing the corresponding methyl ester in 12% yield with physical constants agreeing with those of the literature.[24]

L-Methionine *p*-Nitrophenyl Ester Hydrochloride. After the excess TFA was removed, 5 mL of ethyl acetate was added to dissolve the oily residue. The TFA salt was precipitated by adding ether. The crude pasty product was triturated with ethyl acetate at 40 °C when the TFA salt was obtained as a fine crystalline material. It was again dissolved in 5 mL of THF. Then 4.2 mL (19.75 mmol) of 4.8 N HCl in ether solution was added dropwise with stirring. Crystallization occurred immediately, and the solid was filtered, washed with ether, recrystallized from THF–ether, and dried as usual: yield 3.5 g (85%); mp 147.5–148.5 °C; $[\alpha]^{23}_D$ +1.75° (*c* 2, DMF). IR showed the ester carbonyl peak at 5.7 μm (KBr).

Anal. Calcd for $C_{11}H_{15}ClN_2O_4S$: C, 43.03; H, 4.93. Found: C, 42.41; H, 4.82.

N-Carbobenzoxyglycyl-L-methionine Pentachlorophenyl Ester. *N*-Carbobenzoxyglycine (1.254 g, 6 mmol) was dissolved in 200 mL of EtOAc, the solution was cooled to 0 °C, and 0.786 mL of isobutyl chloroformate (6 mmol) was added, followed by 0.669 mL of *N*-methylmorpholine (6 mmol). The reaction was carried out under N_2. L-Methionine pentachlorophenyl ester hydrochloride (2.604 g, 6 mmol) was added to the cloudy mixture which was kept at −5 to −10 °C, and then 0.669 mL of *N*-methylmorpholine in 50 mL of EtOAc was added dropwise over a period of 2 h. The morpholine salt was removed by filtration, and the filtrate was washed with water until neutral and then dried with anhydrous sodium sulfate and concentrated in vacuo. The product was precipitated with petroleum ether and the crude solid was recrystallized from EtOAc–petroleum ether. TLC showed one spot in benzene–EtOAc, 2:1 system: yield 3.3 g (93.5%); mp 138.5–139 °C; $[\alpha]^{23}_D$ −18.40° (*c* 2, THF). IR (KBr) showed the ester carbonyl peak at 5.62 μm, amide I at 6.03 μm, and amide II at 6.53 μm.

Anal. Calcd for $C_{21}H_{19}Cl_5N_2O_5S$: C, 42.84; H, 3.25; Cl, 30.11. Found: C, 42.46; H, 3.11; Cl, 30.06.

The above procedure was used for the preparation of the other active esters described below unless stated otherwise.

***N*-Carbobenzoxyglycyl-L-methionine *p*-Nitrophenyl Ester**: yield 87%; mp 97–98 °C; $[\alpha]^{23}_D$ −30.75° (*c* 2, THF). IR (KBr) showed the ester carbonyl peak at 5.65 μm.

Anal. Calcd for $C_{21}H_{23}N_3O_7S$: C, 54.66; H, 5.02. Found: C, 54.63; H, 4.96.

***N*-Carbobenzoxyglycyl-L-methionine Pentafluorophenyl Ester.** The preparation procedure was the same as previous except for crystallization, when petroleum ether was added to the concentrated EtOAc solution until it turned slightly turbid and was left standing in the freezer. A white solid appeared after 1 week, and then more petroleum ether was added to complete the precipitation; crude yield 95.6%, mp 62–67 °C. The crude solid was dissolved in 3 mL of THF and purified on a silica gel column (50 g of silica gel; 30 × 2 cm column; eluant benzene–EtOAc, 2:1). The eluates were monitored by TLC. Fractions containing the dipeptide were combined and concentrated in vacuo at 40 °C. Petroleum ether was added as before. White crystals appeared after standing for 3 days in the freezer: yield 64%; mp 69.5–71 °C; $[\alpha]^{23}_D$ −13.75° (*c* 2, THF). IR (KBr) showed the ester carbonyl peak at 5.57 μm. The compound was not obtained in analytically pure form.

Anal. Calcd for $C_{21}H_{19}F_5N_2O_5S$: C, 49.8; H, 3.78. Found: C, 51.22; H, 4.25.

Racemization Rate Studies on Active Esters. The kinetic study was done in a room of constant temperature (23 ± 1 °C). All operations needed for preparation of the solutions for these rate studies were carried out under dry nitrogen. The concentration of the active esters in THF was 0.04 M. Racemization was initiated by adding 7 equiv of TEA per mole of active ester used and was repeated with 35 equiv of TEA except in the case of *N*-carbobenzoxyglycyl-L-methionine pentafluorophenyl ester where 2 and 7 equiv of TEA were used since the active ester racemized very fast and the data obtained were not reliable if the base concentration was high. All kinetics were followed at 589 nm. The first reading was taken within 5 min of mixing the reagents. Racemization was followed up to at least 50% of completion and the pseudo-first-order rate constant was obtained from the linear plots of log α vs. time. The second-order racemization rate constants listed in Table I were determined by dividing the pseudo-first-order rate constants by the base concentration.[2a]

Aminolysis Rate Studies on Active Esters. Calibration curves of the esters listed in Table I were obtained by measuring the net absorbances of the active ester peaks by the base-line method.[25] Standard solutions of 0.13 M, 0.1 M, and 0.08 M active esters in THF solution were used in each case. A THF solution which was 0.13 or 0.1 M active ester and 1 equiv of L-valine methyl ester per mole of active ester were used to study the aminolysis of all esters.

The coupling rate constants were determined from the measurement of the disappearance of the active ester carbonyl peak

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(21) L. Kisfaludy, M. Q. Ceprini, B. Rakoczy, and J. Kovacs, "Peptides, Proceedings of the Eighth European Peptide Symposium", Noordwijk, The Netherlands, September 1966, H. C. Beyerman, A. van de Linde, and W. Massen van den Brink, Eds., North-Holland Publishing Co., Amsterdam, 1967, p 25.
(22) L. Kisfaludy. M. Löw, O. Nyeki, T. Szirtes, and I. Schön, *Justus Liebigs Ann. Chem.*, **9**, 1421 (1973).
(23) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).
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(25) R. T. Conley, "Infrared Spectroscopy", Allyn and Bacon, Boston, 1966, p 210.

in the IR spectrum between 5.5 and 5.7 μm. A sealed 0.1-mm NaCl cell was used for the sample solution. A matched NaCl cell containing THF was in the reference beam. Aminolysis was followed up to about 40% of completion for all samples except for *N*-carbobenzoxyglycyl-L-methionine pentafluorophenyl ester. The second-order rate constants for the peptide bond formation listed in Table I were obtained from the linear plots of the reciprocal of concentration versus time.

***N*-Carbobenzoxy-DL-methionylglycine Ethyl Ester. From *N*-Carbobenzoxy-DL-methionine *p*-Nitrophenyl Ester.** To the solution from the racemization study (10 mL containing 0.1617 g of 0.4 mmol of *N*-carbobenzoxy-DL-methionine *p*-nitrophenyl active ester which was allowed to be 90% racemized) was added 0.05584 g (0.4 mmol) of glycine ethyl ester hydrochloride. The mixture was stirred for 8 h at room temperature, the ammonium salt was removed by filtration, and the filtrate was evaporated to dryness in vacuo at 40 °C. The residue was then dissolved in 30 mL of EtOAc, washed with 0.1 N HCl and then with water, dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo at 40 °C. The product was precipitated with petroleum ether and recrystallized from EtOAc–petroleum ether; yield 0.098 g (66%); mp 80–80.05 °C. TLC showed one spot in benzene–EtOAc, 2:1 system. IR (KBr) showed the ester carbonyl peak at 5.68 μm, amide I at 6.05 μm, and amide II at 6.5 μm.

Anal. Calcd for $C_{17}H_{24}N_2O_5S$: C, 55.42; H, 6.57. Found: C, 55.61; H, 6.66.

This compound was prepared previously by the azide coupling procedure[26] but a lower melting point (72–74 °C) was reported. This compound was also prepared according to the procedure described above from the racemate solutions of Z-Met-OPcp, Z-Met-OPcp, and Z-Met-OSu.

***N*-*tert*-Butyloxycarbonyl-DL-methionylglycine Ethyl Ester. From *N*-*tert*-Butyloxycarbonyl-DL-methionine Pentachlorophenyl Ester.** The preceding procedure was used: yield 67%; mp 94–95.5 °C. IR (KBr) showed the ester carbonyl peak at 5.7 μm, amide I at 6 μm, and amide II at 6.5 μm.

Anal. Calcd for $C_{14}H_{26}N_2O_5S$: C, 50.28; H, 7.84. Found: C, 50.58; H, 7.79.

This compound was also prepared from the racemate solutions of Boc-Met-OPfp, Boc-Met-ONp, and Boc-Met-OSu.

***N*-Carbobenzoxyglycyl-DL-methionylglycine Ethyl Ester. From *N*-Carbobenzoxyglycyl-DL-methionine Pentachlorophenyl Ester.** The preceding procedure was used: yield 88%; mp 123–124 °C. IR (KBr) showed ester carbonyl peak at 5.7 μm, amide I at 6.1 μm, and amide II at 6.5 μm.

Anal. Calcd for $C_{19}H_{27}N_3O_6S$: C, 53.63; H, 6.40. Found: C, 52.79; H, 6.38.

This compound was also prepared from the racemate solutions of Z-Gly-Met-OPcp and Z-Gly-Met-ONp.

***N*-Carbobenzoxy-L-methionyl-L-valine Methyl Ester. From *N*-Carbobenzoxy-L-methionine Pentafluorophenyl Ester.** To the THF solution of *N*-carbobenzoxy-L-methionine pentafluorophenyl ester (0.04 M, 0.449 g, 1 mmol) in THF was added 0.13 mL (1 mmol) of L-valine methyl ester. The mixture was allowed to react until 90% completion (22 min) followed by evaporating to dryness in vacuo at 40 °C. The residue was dissolved in 30 mL of EtOAc and washed five times with water, and then the solution was dried with anhydrous sodium sulfate, filtered, and concentrated in vacuo at 40 °C. The product was precipitated with petroleum ether and recrystallized from EtOAc–petroleum ether: yield 0.3521 g (89%); mp 103.5 °C; $[\alpha]^{23}_D$ −5.0° (*c* 2, THF); $[\alpha]^{23}_D$ −26.5° (*c* 1, MeOH). The physical constants of the dipeptide derivative thus prepared agree with those of the literature.[27]

The preparation from the corresponding *N*-succinimidoyl, pentachlorophenyl, and *p*-nitrophenyl active esters followed the same procedure except that the coupling times for 90% completion were 2.4, 6.9, and 33 h, respectively.

***N*-*tert*-Butyloxycarbonyl-L-methionyl-L-valine Methyl Ester. From *N*-*tert*-Butyloxycarbonyl-L-methionine Pentafluorophenyl Ester.** The preceding procedure was used except that the reaction time for 90% completion was 34 min; yield 83%; mp 124.5–125 °C; $[\alpha]^{23}_D$ −15.25° (*c* 2, THF). IR (KBr) showed the ester carbonyl peak at 5.7 μm, amide I at 6.05 μm, and amide II at 6.55 μm.

Anal. Calcd for $C_{16}H_{30}N_2O_5S$: C, 53.02; H, 8.34. Found: C, 53.00; H, 8.22.

The preparation from the corresponding *N*-succinimidoyl, pentachlorophenyl, and *p*-nitrophenyl active esters followed the same procedure except that the coupling times for 90% completion were 2.8, 8.6, and 27.2 h, respectively.

***N*-Carbobenzoxyglycyl-L-methionyl-L-valine Methyl Ester. From *N*-Carbobenzoxyglycyl-L-methionine Pentachlorophenyl Ester.** The preceding procedure was used except that the reaction time for 90% completion was 3.4 h; yield 91%. After crystallization from EtOAc–petroleum ether: mp 123.5–124 °C; $[\alpha]^{23}_D$ −6.5° (*c* 2, THF).

Anal. Calcd for $C_{21}H_{31}N_3O_6S$: C, 55.61; H, 6.89. Found: C, 55.91; H, 6.91.

The preparation from the corresponding *p*-nitrophenyl and pentafluorophenyl active ester followed the same procedure except that the coupling times for 90% completion were 13.4 h and 6.1 min, respectively.

Registry No. (Z)-Met-OPcp, 4841-70-7; Boc-Met-OPcp, 33385-88-5; (Z)-Gly-Met-OPcp, 66438-53-7; (Z)-Met-ONp, 2483-42-3; Boc-Met-ONp, 2488-18-8; (Z)-Gly-Met-ONp, 66438-54-8; (Z)-Met-OPfp, 17543-51-0; Boc-Met-OPfp, 50903-61-2; (Z)-Gly-Met-OPfp, 66438-52-6; (Z)-Met-OSu, 3392-01-6; Boc-Met-OSu, 3845-64-5; L-methionine pentachlorophenyl ester hydrochloride, 72658-49-2; L-methionine pentafluorophenyl ester hydrochloride, 72658-50-5; L-methionine methyl ester hydrochloride, 2491-18-1; L-methionine *p*-nitrophenyl ester hydrochloride, 56979-49-8; *N*-(carbobenzoxy)-DL-methionylglycine ethyl ester, 15998-55-7; *N*-(carbobenzoxy)-DL-methionine *p*-nitrophenyl ester, 4108-24-1; *N*-[(*tert*-butyloxy)carbonyl]-DL-methionylglycine ethyl ester, 72658-51-6; *N*-[(*tert*-butyloxy)-carbonyl]-DL-methionine pentachlorophenyl ester, 72658-52-7; *N*-[(carbobenzoxy)glycyl]-DL-methionylglycine ethyl ester, 72658-53-8; *N*-[(carbobenzoxy)glycyl]-DL-methionine pentachlorophenyl ester, 72658-54-9; *N*-(carbobenzoxy)-L-methionyl-L-valine methyl ester, 33857-84-0; *N*-[(*tert*-butyloxy)carbonyl]-L-methionine-L-valine methyl ester, 72658-55-0; *N*-[(carbobenzoxy)glycyl]-L-methionyl-L-valine methyl ester, 72658-56-1.

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