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PEPTIDE SYNTHESIS WITH BENZISOXAZOLIUM SALTS—II

ACTIVATION CHEMISTRY OF 2-ETHYL-7-HYDROXYBENZISOXAZOLIUM FLUROBORATE; COUPLING CHEMISTRY OF 3-ACYLOXY-2-HYDROXY-N-ETHYLBENZAMIDES

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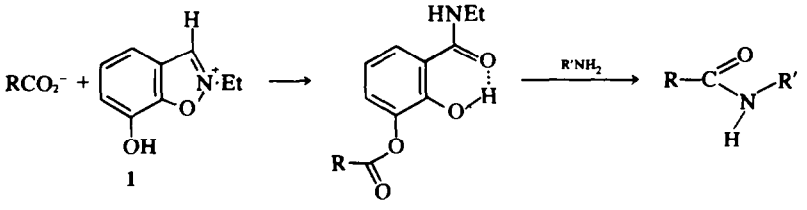
Abstract—Mechanistic aspects of the application of 2-ethyl-7-hydroxybenzisoxazolium fluoroborate, **1**, to peptide synthesis are presented. Optimal conditions are described for the formation of 3-acyloxy-2-hydroxy-N-ethylbenzamides, **2**, from **1** and peptide acids. Amines are found to react with esters, **2**, as their 2-oxyanion conjugate bases. Racemization in model systems is found to occur via oxazolones, and the low racemizing tendency of the esters, **2**, is shown to result from a unique internal buffering effect.

In the preceding paper,[1] we have outlined the reasoning which led us to synthesize the 2-ethyl-7-hydroxybenzisoxazolium cation, **1**, and the evidence which establishes the active esters, **2**, as products of the reactions of **1** with carboxylic acid anions. In the following paper,[2] we report results of the application of this reaction to the synthesis of simple amino acid derivatives. In this paper, we describe the known practical features of the activation sequence **1**→**2**, carried out with the model, carbobenzoxyglycine, and salient features of the coupling sequence, **2**→amide, carried out with a variety of peptide derivatives. Evidence relevant to the mechanisms of coupling and racemization for the esters **2** is described.



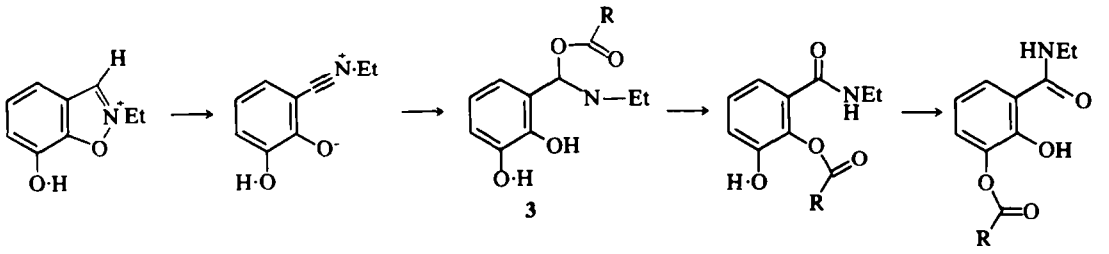
1 **2**

1. ACTIVATION CHEMISTRY

In the preceding paper,[1] we have established the structures of the products of reactions of **1** with carboxylate anions as **2** and have argued by analogy that the mechanism of the transformation is as shown below. We have also demonstrated that for the cases of hippurate and Z-Gly-L-PheOH, negligible oxazolone formation occurs during this transformation; i.e., acyl "leakage" from the imino-anhydride, **3**, is not a problem. Under optimum conditions, to be defined in this section, the conversion **1**→**2** has been found to proceed in high yield, even with very hindered acids.† Results of our investigations with a range of peptide acids are presented in the accompanying paper. In this section we report results with carbobenzoxyglycine, whose behavior is representative of most peptide acids.

Although a detailed kinetic analysis was not carried out, half times for decomposition of **1** at pH 4, 4·5, and 5 of 2·8, 1·5, and 1·0 minutes, respectively, were observed. The pseudo first order rate constants are not linear with hydroxide ion concentration, probably because of an intervention in this pH range of the O–H conjugate base of **1**. Since carboxylic acids are observed to be reactive only as their anions, and reactions run at pH values

†Although satisfactory elemental analyses were not obtained because of the tendency of the substance to retain traces of hydrocarbons, the product, m.p. 82–84°, resulting from reaction of **1** and carbobenzoxy-α-aminoisobutyric acid is formed in 86% yield and is characterizable by IR and NMR as the corresponding ester, **2**.



above 5 are attended by the formation of an inhomogeneous, yellow polymer, activation reactions are best conducted in the pH range of 4–5. Relatively low yields of active ester, contaminated with yellowish byproduct, were obtained in acetonitrile or DMF, combining **1** with the triethylamine salt of carbobenzoxyglycine. These results accord exactly with those observed for the 2-ethylbenzisoxazolium cation.[3,4]

Isotopic dilution experiments, using carbobenzoxyglycine-^{14}C, were carried out to determine exact yields for a variety of aqueous coupling conditions. Using an aqueous solution of the sodium salt of the acid, either unbuffered or with pH control by indicator or pH stat, gave yields in the range of 70–83% of active ester for pH values in the range 4·5–5·0. On the other hand, use of pyridine buffer (5% in water) consistently gave clean products with yields in the range of 90–95%.

Table 1. Reaction between Z-GlyOH and **1**

Product	Yield (%)
Active ester, **2**, R = ZGly	94·1
ZGlyOH	2·2
Neutral, unidentified material	1·6
Loss in workup to acidic extraction	0·9
Total	98·9%

The optimum activation procedure involves slow addition of finely powdered **1** to a chilled, vigorously stirred 0·2–1·0 M aqueous solution of an alkali metal salt of the acid, containing 5–7% of a pH 5 pyridine-pyridine hydrochloride buffer, overlayered with an organic solvent such as ethyl acetate. Addition of **1** in small portions appears to minimize formation of neutral yellow impurities. The results of a radiotracer study of an activation carried out in this manner are given in Table 1. Isotopic dilutions were carried out both for active ester and starting acid. Control experiments established that losses of active ester, **2**, into either HCl or $NaHCO_3$ extractions upon workup were less than 1%.

Unlike the aqueous activation reactions of the 2-ethylbenzisoazolium cation, which can give very high yields of active esters at carboxylate concentrations as low as 0·01 M,[4] **1** has proved to be considerably more concentration-sensitive. Pertinent data are given in Table 2; again, pyridine buffers were employed. It is clear that no solvent combination gives satisfactory activation results at carboxylate concentrations below 0·04 M, and that only acetonitrile–water or hexafluoroisopropanol–water mixtures are marginally acceptable in the range 0·1–0·04 M. This limitation is expected to be serious when dealing with highly insoluble peptide acids, and is compounded when dealing with substances of high molecular weight.

Table 2. Variations of active ester yield with solvent and concentration of ZGlyOH

A. Water

Acid concentration (M)	Active ester yield (%)
0·25	89·5
0·10	74·8
0·01	55·5

B. THF–Water

Acid concentration (M)	THF–H_2O ratio (v/v)	Ester yield (%)
0·20	2·2:1	81·6
0·04	3·7:1	57·5

C. CH_3CN–Water

Acid concentration (M)	MeCN–H_2O ratio (v/v)	Ester yield (%)
0·20	2·2/1	90·0
0·04	3·3/1	81·8

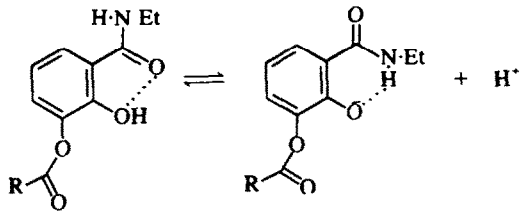
D. Other

Solvent	Acid conc (M)	Solv–H_2O ratio (v/v)	Ester yield (%)
DMF/H_2O	0·2	2:1	74·1
Hexafluoro isopropanol/H_2O	0·1	2:1	84·9

2. *Rates of aminolysis and hydrolysis of* 3 - *acyloxy* - 2 - *hydroxy* - N - *ethyl benzamides*, 2.

The esters, 2, were designed to undergo rapid aminolysis by an assisted mechanism; in this section we describe the optimum conditions for coupling with these esters and provide rate data which support an assisted aminolysis mechanism and allow an estimate of its efficiency.

An important initial issue is the acidity of the esters, 2. Approximate pK_a measurements in water by a UV method gave a value of 6·7 for 3 - acetoxy - 2 - hydroxy - N - ethylbenzamide, which may be compared with values of 8·1, 8·3, and 9·0 for the series, N-ethylsalicylamide, 3 - methoxy - 2 - hydroxy - N - ethylbenzamide, and 3 - hydroxy - 2 - methoxy - N - ethylbenzamide.* Because the phenol function of the neutral ester and the phenolate of its conjugate base are sterically shielded and strongly hydrogen bonded, the esters, 2, are expected to retain much of their acidity in nonhydrogen-bonding solvents, such as acetonitrile, DMF, and DMSO.[5] As a result, the conjugate base of 2 can be expected to be an important contributor to the chemistry of 2 under all likely aminolytic conditions.



As indicated in our preliminary reports,[6] it was quickly apparent that the coupling reaction rates of esters 2 with amines are markedly accelerated by the addition of strong bases, and the cleanness of aminolyses under these conditions prompted us to employ tetraalkyl ammonium salts of amino acids and peptides[7] as nucleophiles in dipolar aprotic solvents. However, this combination results in a rapid acid-base reaction and precipitation of one equivalent of neutral amino acid, and in order to achieve successful coupling reactions with a 1:1 ester–amine salt ratio, it is necessary to introduce an additional strongly basic species. Following the reaction of the ester, 2, of ZGlyOH with the tetramethylammonium salt of phenylalanine by isotopic dilution, we observed yields in DMSO of 70–80% with the bases, NaH, and the tetramethylammonium salts of hydroxide, N,N-dimethylglycinate, or α-aminoisobutyrate. In contrast, a 98% yield of ZGlyPheOH was observed with an equivalent of anhydrous tetramethylguanidine. This convenient, strong base has sufficient strength to dissolve many of the common amino acids in DMSO as their salts, and since our initial report[7b] of its value for this special problem, it has been found to have other uses in the peptide area.[8]

The most generally useful peptide coupling procedure which we have observed for the esters 2 involves their reaction with a tetralkylammonium or tetramethylguanidinium salt of an amino acid or free peptide in DMSO containing an equivalent of tetramethylguanidine (TMG). This highly unusual expedient of conducting peptide coupling reactions in the presence of a strong base requires a series of control experiments which test the extent to which the procedure is free from unwanted base-catalyzed side reactions such as racemization and nucleophilic participation. The simplest of these issues concerns the reaction between TMG and an ester, 2. Can one expect acylation of the TMG N–H function?

As a test of this point, the ZGly ester, 2, at 0·5 M concentration in DMSO was exposed to an equivalent of TMG at 20° for 40 minutes, at which time the mixture was quenched with acid and assayed by isotopic dilution; 100% recovery of ester was observed.

Two experiments imply that unlike simple tertiary amines, TMG reacts quantitatively with esters, 2, in CH_3CN, DMF, or DMSO to form salts. Thus, identical coupling rate constants are observed for the ZGly ester, 2, in DMF containing an equivalent of TMG, and for a DMF solution of the tetramethylammonium salt of the ester, prepared by combining the ester with one equivalent of tetramethylammonium phenylalaninate (quantitative recovery of precipitated phenylalanine observed). Moreover, the UV spectra in CH_3CN of the tetramethylammonium salt of the acetate ester, 2, is identical with that of the ester and an equivalent of TMG. The completeness of this acid-base reaction can be attributed largely to the delocalized character of the charge of the conjugate acid of TMG which is therefore able to retain its high base strength, even in aprotic solvents. The highly hindered character of the base presumably prevents its acylation; in none of the coupling reactions using TMG have we seen products derived from its action as a nucleophile.[9]

Rate constants for reactions of esters, 2, with amines are reported in Table 3, along with comparison data for analogous reactions of *p*-nitrophenyl esters.[10]

*The rather large effect of 1·4 pK_a units acidity increase which results when a 3-methoxy group is changed to a 3-acetoxy perhaps deserves comment. The Hammett *para* σ values for methoxy and acetoxy are −0·27 and +0·39;, the pK_a shifts from salicylamide are therefore in the expected direction, and the greater shift observed for acetoxy is well within the uncertainties expected for using *para* σ values to predict *ortho* inductive or field effects. There appears, therefore, to be no reason to invoke special stabilizing effects for the acetoxyl.

Table 3. Rates of aminolysis for esters, **2**

A. *Effect of solvent*
2, (R = ZGly) + GlyOEt T = 23°

Solvent	Rate constant (M^{-1} min^{-1})	Rate constant (M^{-1} min^{-1}) (0·1 M amine) ZGly*p*NP + GlyOEt
Dioxane	0·15	0·7
Acetonitrile	*ca* 0·8*	1·1
Pyridine	0·45	—
DMF	0·2	26
DMF + 1 eq $Et_4N^+BF_4^-$	0·15	—
DMSO	0·2	81

*rate measured by less accurate IR method

B. *Effect of added base*, T = 22–23°, DMF

Reagents	Base	Rate constant (M^{-1} min^{-1})
2, R = ZGly, GlyOEt	none	0·2
2, R = ZGly, GlyOEt	1 eq TMG	10·0
2, R = ZGlyPhe, GlyOEt	none	0·2
2, R = ZGlyPhe, GlyOEt	1 eq TMG	12·0

C. *Temperature effect*

Ester	Base	Solvent	Nucleophile	T	Rate constant (M^{-1} min^{-1})
1. **2**, R = ZGly	none	DMF	GlyOEt	3°	$8·9 \times 10^{-2}$
				22–23°	$2·1 \times 10^{-1}$
2. **2**, R = ZGly	1 eq TMG	DMSO–DMF 1:1	$GlyO^-Me_4^+N$	0–3°	15
		DMSO		22–23°	36
3. **2**, R = ZGlyPhe	1 eq TMG	DMSO	$GlyO^-Me_4^+N$	0–3°	15
				22–23°	36

D. *Steric and substitution effects*
1. Amino acid esters as nucleophiles, T = 22–23°, DMF
2, R = Z–A + TMG + B–OEt → Z–A–B–OEt

Ester, A	Amine, A	Rate constant (M^{-1} min^{-1})	Rate constant for Z–A–*p*NP + B–OEt (M^{-1} min^{-1})
Gly	Gly	10	26
L-Ala	*L*-Ala	1	4·8
L-Ala	*D*-Ala	0·6	—
L-Ala	*L*-Ala	0·5	1·2
L-Val	Gly	0·15	1·3
L-Val	*L*-Ala	0·06	0·26
L-Val	*L*-Val	0·03	0·06

2. Amino acid salts as nucleophiles, T = 22–23°, DMSO
2, R = Z–A + TMG + B–$O^-Me_4N^+$ → Z–A–B–$O^-Me_4N^+$

Ester, A	Amine, B	Rate constant (M^{-1} min^{-1})	Rate constant for Z–A–*p*NP + BOEt (DMF)
Gly	Gly	36	26
GlyPhe	Gly	36	19
Gly	Pro	0·08	6·9
Gly	Sarc	0·8	—
Gly	Phe	2·6	1·0
L-Val	*L*-Val	0·2	0·06
L-Val	*D*-Val	0·06	—

Several generalizations can be drawn from the limited data of the Table.

1. Whereas the *p*-nitrophenyl ester aminolyses are strikingly accelerated by the polar solvents,[10] DMSO and DMF, the 3 - acyloxy - 2 - hydroxy - N - ethylbenzamides, **2**, aminolyze with rates that are nearly solvent independent.
2. The aminolysis of esters, **2**, show roughly a 2·4-fold rate increase for a 20° temperature change; essentially the same effect is seen for neutral and base-catalyzed couplings.
3. The coupling rate increases 50–60 fold when the ester, **2**, is converted to its anion. Amino acid salts are roughly 3·3 times more reactive than the corresponding esters.
4. Variations of rate constant with amino acid substituent roughly parallel those observed for *p*-nitrophenyl ester reactions, with the striking exception of proline as a nucleophile, which appears to be more than 200 times less reactive than expected by this analogy. The combination of TMG, ester, **2**, and amino acid ester is 2–4 times less reactive than amino ester and corresponding *p*-nitrophenyl ester, while, **2**, TMG, and amino acid anion react 1–3 times more rapidly. Although comparisons are possible in only four cases, the couplings with **2** anions appear to yield rate constants which can be approximated as products of partial rate factors for the acyl and amine components, as can *p*-nitrophenyl ester rate constants.[10]

Given the desirability of working with the rapidly reacting amino acid salts, natural solvent choices are water or alcohols, in which control of pH is expected to be easy. Unfortunately, a very striking failing of the esters **2** is their tendency to undergo hydrolysis at rates comparable to those of aminolysis. Fig 1 presents results obtained when the TMG salt of phenylalanine is allowed to react in solvent-water mixtures with the ester **2**, R = ZGly. Although the extent of hydrolysis can be diminished by lowering temperature or increasing reagent concentration, it cannot be eliminated, and as a result, peptide coupling reactions must be maintained in a nearly anhydrous state. The extent to which this result is atypical is revealed by the data of Table 4, which presents results of similar experiments with other activated species.

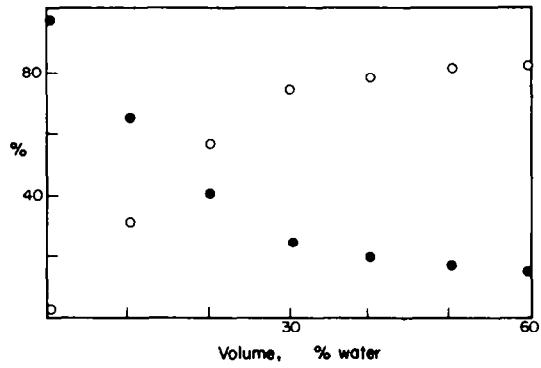


Fig 1. Reaction products observed for the combination of **2**, R = ZGly, 0·08*M*, with TMG and Phe, 0·09*M*, in DMSO-water mixtures. Closed circles correspond to yield of ZGlyPheOH, open circles to yield of ZGlyOH.

3. *Mechanisms of aminolysis and hydrolysis reactions of esters*, **2**

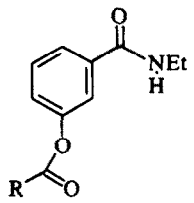
The magnitude of the catalytic effect on aminolysis exerted by the 2-oxyanion of the conjugate bases of **2** is the fundamental question with which a mechanistic discussion must begin. In an attempt to determine the intrinsic reactivity of

Table 4. Yields of coupling and hydrolysis products for aqueous peptide coupling reactions, 20 volume % H_2O

$$\underset{1\cdot0\ eq}{ZGly\text{-}Z} + \underset{1\cdot03\ eq}{PheO^-TMGH^+} \xrightarrow{H_2O} ZGlyPheO^- + ZGlyO^-$$

Solvent	T°	Active species	Reagent conc (M)	% Dipeptide	% $ZGlyO^-$
CH_3CN	0	Azide	0·02	60	1·2
CH_3CN	−15	Mixed anhydride	0·02	65	4·7
DMSO	20	*p*-nitrophenyl	0·08	98	0·9
			0·02	96	1·3
DMSO	20	N-hydroxysuccinimide	0·08	99	0·6
DMSO	20	Hydroxypiperidine	0·08	86	14
DMSO	20	2-mercaptopyridine	0·08	99	0·2
DMSO	20	8-hydroxyquinoline	0·08	97	0·6
DMSO	20	2-acyloxy-N-ethyl-benzamide	0·08	62	2·2
			0·02	28	1·6
DMSO	20	**2**, R = ZGly	0·08*	40	59
			0·02*	19	80
DMSO	20	5-nitro **2**, R = ZGly	0·08*	96	4

*One additional equivalent of TMG added.



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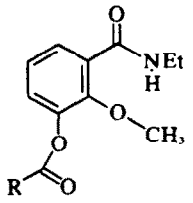

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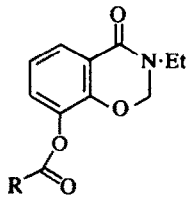

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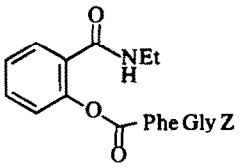
the 3 - acyloxy - N - ethylbenzamide system and of the neutral esters, **2**, we prepared and studied the esters **4**, **5**, and **6**. Rate data for these substances are reported in Table 5, for aminolysis in DMF with 0·1–0·6 M ethyl glycinate. The rate interpretations are complicated by two problems. As is frequently observed for aminolysis of phenyl esters, the rate dependence on concentration does not appear to be simple second order, but is better approximated by a sum of second order and third order terms (see experimental). On the other hand, the errors in assuming a simple second order rate expression are small, and the interpretation of the curvature observed in a plot of pseudo first order rate constant *vs* amine concentration is rendered difficult by medium effects resulting from the high amine concentrations used; for the rate comparisons required, the averaged rate constants provide sufficient information. Substance **6**, R = acetyl, was obtainable with difficulty (Experimental), and **6**, R = ZGly, could not be satisfactorily purified; consequently, model comparisons must be made between two series of acyl derivatives.

Table 5. Aminolysis rate constants for model systems DMF 30° GlyOEt, 0·1–0·6 M

Substance	Average k_2 (M^{-1} min^{-1})
4a R = Acetyl	$1{\cdot}5 \times 10^{-3}$
4b R = ZGly	$4{\cdot}6 \times 10^{-3}$
5 R = ZGly	$1{\cdot}5 \times 10^{-2}$
6 R = Acetyl	$1{\cdot}3 \times 10^{-2}$
2 R = ZGly, neutral T = 20–23°	$2{\cdot}0 \times 10^{-1}$
2 R = ZGly + 1 eq TMG T = 20–23°	10·0

The basis for any rate comparisons must be the system, **4**, which models the classical inductive and resonance activation of the esters, **2**, but which lacks any effects attributable to a 2-oxy grouping. The 2-oxygen function is expected to introduce three effects: both its steric effect and its conjugative interaction with the amide carboxyl should reduce the reactivity; while any special catalytic effect should increase the reactivity. That steric or conjugative effects can be dominant in related systems is shown by **7** and **8**; the latter reacts four times more slowly with ethyl glycinate in DMF.[6]



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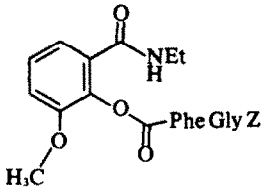

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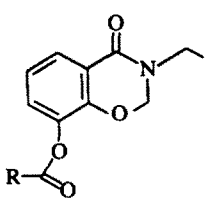
In contrast, comparison of rate data for **4a** and **5** reveal that the latter is three times more reactive, and in this less hindered case, the special catalytic effects of the methoxy must outweigh its steric or conjugative effects. One could argue that **5** should be still more reactive, were it not for the steric effect of the methoxy which interferes with conjugation between the ring and amide functionality. That such effects are small is implied by the comparison between **4a** and **6**; the latter substance is roughly nine times more reactive. The structure **6** is necessarily nearly planar and has the same orientation of oxygen lone pairs as the H-bonded, stable form of the neutral ester, **2**. Although **6** necessarily lacks whatever effects may arise from the chelated H-bond of **2**, it nonetheless is the best model for the reactivity of **2** which we have been able to conceive. Under the assumption that the ZGly derivative of **6** would react three times faster than the acetyl, we estimate the aminolytic rate constant for the neutral ester, **2**, to be *ca* 4×10^{-2} M^{-1} min^{-1}, which is significantly slower than that observed for **2** in the absence of TMG. We regard it as likely that much if not all of the reactivity displayed by the esters **2** under neutral coupling conditions can therefore be attributed to the presence of small amounts of the reactive conjugate base of **2**. It should be noted that the conjugate base is sufficiently reactive that the presence of only 2% of it in equilibrium with the



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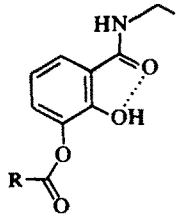

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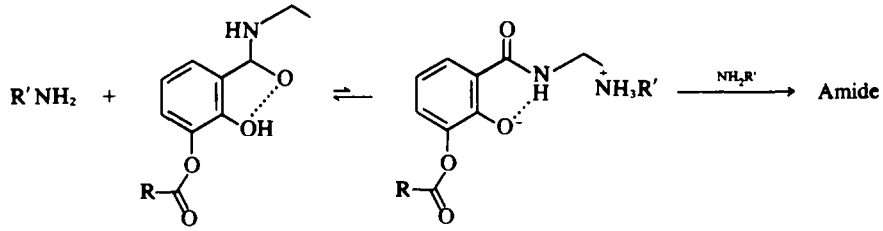


neutral ester would result in the rate constant observed for **2** under neutral coupling conditions.

Since the presence of an equivalent of TMG, **2** is quantitatively converted to its conjugate base, the rate constant observed with TMG can be unambiguously assigned to aminolysis of this species. From the above estimates, the conjugate base of **2** is seen to be more than 250 times more reactive than predicted by the model **6**, and more than 2000 times more reactive than predicted by **4**. Neither model takes into account the inductive and resonance deactivation expected from substitution of an oxyanion on the benzene function, so that the true catalytic effect of the oxyanion must be larger than either of these estimates.

Although it is usual to regard the catalytic effect of internal bases on phenyl ester aminolyses as resulting from general base catalysis of a rate determining attack of amine at the active ester carbonyl,[11,12] we are reluctant to do so in this instance. It is simple to show that in a mechanism of this kind, general base catalysis can only result in rate acceleration if the proton acceptor is a stronger base than the proton donor.[10,13] The pertinent pK_a difference is difficult to estimate, since it involves the intimate ion pair, and not the more usual solvent-separated species, but it seems likely that the pK_a difference between **2** and an ammonium ion of an amino acid ester is small and quite possibly in an unfavorable direction. Yet it is clear that even very weak proton acceptor atoms can have an observable catalytic effect on aminolysis reactions; the comparison of **4** and **6** provides an example, and other examples have been cited and assigned to a microscopic solvation effect.[14] The two viable mechanistic proposals with possible testable features are Jencks' suggestion that phenyl ester aminolysis may proceed by reversible carbonyl addition, followed by a rate-determining, general base catalyzed proton loss[13] (which may be concerted with loss of phenolate anion), and the view that proton acceptors can stabilize acyl transfer transition states by formation of a hydrogen bonded network which facilitates proton transfer from amine to leaving group but which does not involve complete proton transfer to catalyst. Such a mechanism would become a likely possibility if it could be shown that weak bases which are good H-bond acceptors are effective intramolecular catalysts for phenyl ester aminolysis. The extraordinary accelerating effect of the hydrogen bond acceptors DMSO and DMF on *p*-nitrophenyl ester aminolysis may be relevant to this proposal.

As a final point, we note that many of the features of the coupling rates given in Tables 3 and 4 can be rationalized in terms of the catalytic involvement of the 2-oxyanion. The striking absence of a solvent effect on rates of neutral couplings of **2**, as compared with *p*-nitrophenyl ester couplings, can be interpreted as resulting from the differing polarities of transition states which are structurally distinct (it may be noted that the reacting ester function of the transition state of **2** in which the oxyanion is hydrogen bonded to the proton of the amine necessarily must lose the *s*-trans conformation which can be retained in normal phenyl ester transition states.) Alternatively, the solvent in the *p*-nitrophenyl ester cases can be assumed largely to fulfill the role of an obligatory hydrogen bond acceptor, and the absence of a solvent effect in the case of esters **2** can be attributed to a swamping of the solvent role by a more effective intramolecular acceptor species. The recent observation by Lloyd and Young[15] that a decrease of rate constant with solvent polarity can be observed with 2-pyridinethiol esters, as well as similar observations in other systems,[10] raises the possibility that the absence of a solvent rate effect with **2** may be the result of a fortuitous cancellation of influences, and the issue is certainly clouded by the pK_a changes expected with solvent variation.

The observation that *p*-nitrophenyl acetate, in water, reacts 50 times more rapidly with glycine anion than with ethyl glycinate[11] contrasts with the 3·3 fold variation noted for the **2** anion; this lesser selectivity can be attributed to electrostatic destabilization of a transition state bearing a double negative charge. The anomalously slow reaction of proline with the **2** anion must reflect the inability of the secondary amine NH to accomodate the geometric requirements of the catechol mono anion. Finally, the anomalously rapid hydrolysis of esters **2** can be understood if hydrolysis proceeds by a general catalyzed, rate determining attack of water at the ester carbonyl. General catalysis of hydrolysis is expected to yield large rate accelerations since in this case the pertinent proton transfer is highly favorable.[13]

The above three effects must be regarded as

negative dividends of the special catalytic aminolysis mechanism available to the esters, 2. At the same time one can anticipate that the thousand fold rate enhancement of aminolysis over that expected from the intrinsic electronic activation of 2 should result in a relative freedom from reactions which usually compete with aminolysis but which are not expected to be catalyzed by a 2-oxyanion. We turn to the first such reaction, racemization, in the following section; others involving the side chain functionalities of amino acids are reported in the accompanying paper.

4. Racemization behavior of peptide esters, 2

We summarize in Table 6 the important features of the racemization observed during peptide bond formation with esters, 2.

The salient features of these data include the large temperature effect on racemization, the very favorable racemate levels observed at high reagent

Table 6. Racemization during aminolysis with 2

A. *Neutral couplings*—Temperature effect
0·125 M Ester, 1·1–1·3 eq GlyOEt, DMF, 24 h

T°	2, R = Gly–L–Phe % Rac	2, R = B_z–L–Leu % Rac
22	0·072, 0·073	0·67, 0·83
5	0·020, 0·026	—
−22	0·0047	—

B. *Couplings in the presence of added base*

1. Active ester with Et_3N in the absence of nucleophile DMF, 0·4 M Et_3N, 12 h, 20–25°
Percent racemized: 2, R = ZGly–L–Phe, 3·5%
2, R = B_z–L–Leu, 25·1%

2. Effect of reagent concentration of racemization during aminolysis DMF, 22°, 2, R = ZGly–L–Phe, TMG, GlyOEt

Concentration (M)	% Racemization Found	Calc	
a. *Stoichiometry* 1:1:1			
0·5	0·062	0·049	% Rac calc for
0·125	0·12	0·17	$\frac{k_{rac}}{k_{couple}} = 2{\cdot}5 \times 10^{-5}$
0·05	0·36	0·38	(see Experimental)
0·005	5·3	2·6	
b. *Stoichiometry* 1:1·1:1·1			
1·0	0·048		
0·5	0·058		
0·125	0·087		
0·05	0·15, 0·18		

3. *Racemization during coupling with amino acid salts*
22° in DMSO, 0·3 M; 0–5° in 1:1 DMF–DMSO, 0·3 M

Conditions	% Racemized 2, R = ZGly–L–Phe	2, R = B_z–L–Leu
2 eq $GlyO^-Me_4N^+$		
0–5°	0·009, 0·010	0·14
22°	—	1·1, 3·3
1 eq TMG + $GlyO^-Me_4N^+$		
0–5°	0·023	0·24
22°	—	3·7, 4·0
2 eq TMG + Gly		
0–5°	0·027	0·24
22°	0·38–1·0	9·9

concentrations and low temperatures, and the effect of concentration on racemate formation.

This latter point requires comment. The fraction of product racemized during a peptide coupling reaction in which the product is chirally stable must be derivable from a ratio of rates (1).

(1) Instantaneous fraction of product racemized =

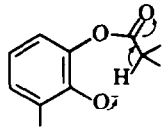
$$\frac{\text{racemization rate}}{\text{racemization rate} + \text{coupling rate}}$$

If both the racemization and coupling rates are determined by bimolecular processes involving the amine and the acylating agent, then the fraction racemized must be independent of concentration. Elsewhere, we have presented evidence that a concentration dependence is in fact observed for couplings with *p*-nitrophenyl esters, and DCC + N-hydroxysuccinimide or N-hydroxybenzotriazole;[10,17] we have also noted that at least in polar solvents with phenyl esters, racemization is a specific, not a general catalyzed process,[6] and as a result, racemization rate should be ideally a function of the square root of the products of amine and ester concentrations, and the racemization observed should therefore show a concentration dependence. It may be noted that the effect for esters, **2**, in the presence of TMG, is a factor of six for a ten-fold concentration change (0·5 → 0·05) which is larger than that observed for other systems. (For *p*-nitrophenyl, 2 fold for 0·2 → 0·04; for DCC–HOSu, 3 fold for 0·2 → 0·01; for DCC–HBT, 2 fold for 0·3 → 0·03).

Although the concentration data must be regarded as approximate, since the dilute reactions are very sensitive to traces of moisture, and quantitative conversions to tripeptide are not observed, nevertheless, an approximate agreement is observed between the experimental data and values obtained from the mathematical models discussed in the Experimental. The data are consistent with a ratio of first order racemization rate constant for the anion of **2** to second order coupling rate constant of **2** with ethyl glycinate of $2{\cdot}5 \times 10^{-5}$ M. Since the latter rate constant is reported (Table 3) to be 12 M^{-1} min^{-1}, the racemization rate constant for the anion of **2**, R = ZGly–L–Phe, in DMF at 22° is calculated to be 3×10^{-4} min^{-1}. This value may be compared with the estimate of 1×10^{-4} min^{-1} for the rate constant for the triethylamine-catalyzed racemization of the ester (Table 6, B.1.).

5. *The mechanism of racemization of esters*, **2**

We have reported elsewhere[18] that no kinetic isotope effect is observed for racemization of esters **2** when deuterium replaces the α-hydrogen of ZGly-L-Phe or Bz-L-Leu. This result rules out a significant contribution to racemization by a simple enolization mechanism or by the unique catechol mechanism, **9**. The remaining likely possibility involves oxazolones.



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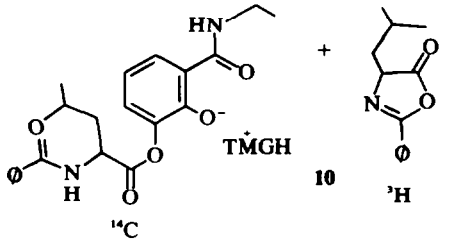
The necessity of oxazolones as intermediates in the racemization of **2**, R = B_z-L-Leu, is established by the following experiments. A solution containing an equivalent each of **2**, R = ^{14}C Bz-L-Leu, 2 - phenyl - ^{3}H - isobutyloxazol - 5 - one, **10**, and TMG were equilibrated for 90 min at 20° in DMSO. Separate isotopic dilutions were then carried out for oxazolone and active ester, and the $^{14}C/^{3}H$ ratios were determined for each, with the results shown below.

Clearly, an equilibrium is established between the oxazolone and the active ester, and the tritium incorporated into the active ester is of the approximate magnitude expected for the racemization observed under these conditions. A complication which results in the low tritium recovery is the existence of a TMG catalyzed process which destroys oxazolones, probably by polymerization. For example, when the oxazolone is allowed to stand for 90 min with 1 equiv of TMG in DMSO, and the mixture is then quenched with ethyl glycinate, less than 0·1% of peptide is formed, and only 30% of peptide is observed when 10 equiv of ethyl glycinate and 1 equiv of TMG were added concurrently to oxazolone.

In DMF, in which the base catalyzed decomposition is slower, a value of 0·01 M is obtained for the equilibrium constant: [oxazolone][phenolate]/[2



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$\xrightarrow[90\text{ min}]{\text{DMSO}}$ $\xrightarrow{H^+}$ Ester + Oxazolone

Fraction of initial activity

	Ester	Oxazolone
^{14}C	58%	14%
^{3}H	13%	18%

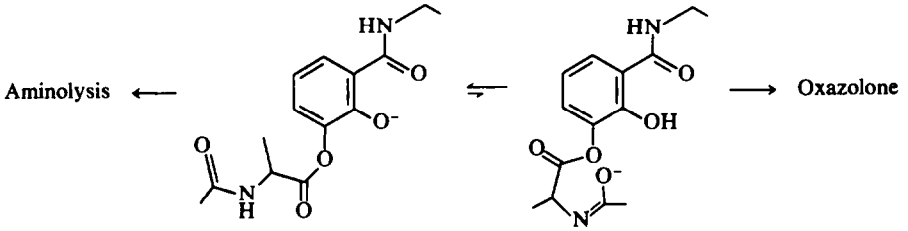
anion, R = BzLeu]. Because of the intervention of nonaqueous acid-base chemistry, it is difficult to relate this value to values reported by Goodman,[19] Young[20] *et al* for *p*-nitrophenylester–oxazolone equilibria under less basic conditions, but it seems likely that the esters **2** are a less activated system in an equilibrium sense.

A final important phenomenon was revealed with a study of the effect of differing amounts of TMG on the rate of racemization of **2**, R = Bz-L-Leu. The data of Fig 2 were obtained by combining this ester with varying amounts of TMG in DMF for 1 h. Ethyl glycinate and enough acid to bring the TMG concentration to 1 equiv were then added, and after a coupling period, the product was analyzed for racemic content. Remarkably, an increase in racemization rate with TMG quantity is observed only up to the point at which the base is used in excess and the free amine is expected to be present in the reaction mixture; no increase in racemization is observed with free TMG, and the system is therefore protected from accelerated racemization in the presence of strong bases. The same phenomenon is observed in DMSO and CH_3CN. This result must be viewed in the light of the above observation that ester, **2**, -oxazolone equilibrium is established under these conditions, and oxazolone is destroyed by excess TMG. Not only must this effect be invoked to explain the decrease in racemate actually observed, but it also makes

$$2 + \text{TMG} \longrightarrow \text{Salt} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Oxazolone} \xrightarrow[\text{TMG}]{k_2} \text{Decomposition}$$

difficult the exact interpretation of the racemization-leveling effect. No such ambiguity attends the data of Table 7, which reports polarimetric racemization data for a racemization experiment in DMSO. The first three entries of the Table establish that the racemization of **2** by an equivalent of TMG is a process whose rate is first order in the concentration of ester species, but does not depend on the product of ester and base concentrations. The last entry reveals that as suggested by Fig 2, the rate of racemization is independent of the concentration of excess TMG. All these results imply that rate of racemization (and therefore of oxazolone formation) is proportional to the concentration of the conjugate base of **2**.

This finding can be rationalized by the following model which assumes that all detectable racemization proceeds through unimolecular decomposition of the anion **12**, isomeric with and in equilibrium with the far more abundant anion **11**, which is the reactive aminolytic species.



11 **12**

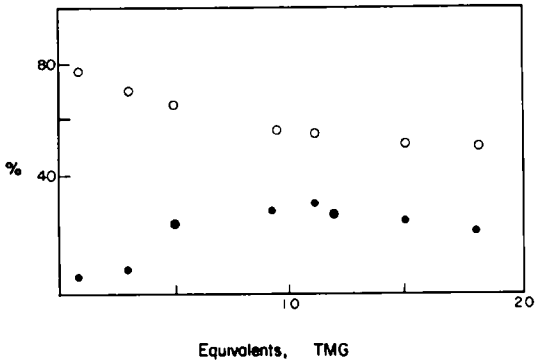


Fig 2. Racemization of **2**, R=Bz-L-Leu, in DMF, 22°, 1 h. Closed circles correspond to yield of Bz-DL-LeuGlyOEt after coupling with GlyOEt; open circles, to the total yield of Bz-L-LeuGlyOEt.

Table 7. Racemization of **2**, R = Bz-L-Leu, in the presence of TMG T = 23°, DMSO, rate followed polarimetrically

Reagent **2**	Concentrations (M) TMG	Half life of racemization (min)
0·06	0·06	82
0·2	0·2	90
0·4	0·4	85
0·04	0·06	85

The basicity-concentration relationships which link these species are shown in Fig 3. In region A, increasing medium basicity proportionately increases the concentrations of the catalytically reactive anion, **11**, and the racemizing anion, **12**. In

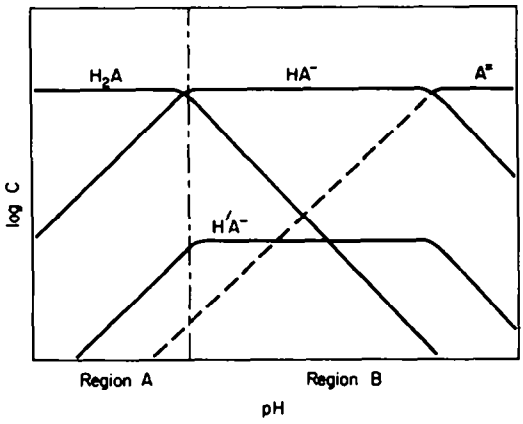
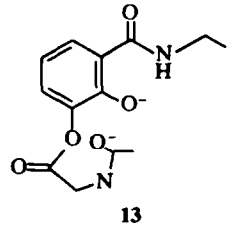


Fig 3. Concentration dependences of anions derived from esters, 2. H_2A = 2; HA^- = 11; $H'A^-$ = 12; $A^=$ = 13.

this region, increased basicity of medium will increase both coupling and racemization rates proportionately, with no change, at least to a first approximation, in their ratio. In region B, esters **2** are quantitatively converted to their anions **11**. Over a large pH span, the concentration of **11** is constant. Since **12** is an isomer of **11**, its concentration in this region is also constant; as a result the racemization rate and the ratio of racemization to coupling rates are all constant. Further pH increase does increase the concentration of the dianion, **13**, but this species is presumably an ineffective source of oxazolones, because of its intrinsically deactivated phenyl ester function and the energetic difficulties of reaching an oxazolone-generating transition state in which two negative charges appear in close proximity. It is clear why the esters, **2**, behave so satisfactorily under highly basic coupling conditions. Unlike normal phenolic esters, which would be expected to show roughly constant aminolysis rate but increased rate of racemization with added base, the esters **2** show an essentially constant rate ratio over a wide basicity range, because the two reacting species, **11** and **12**, are isomers and their equilibrium is therefore base-independent.

The 2-oxyanion of **11** thus serves two distinct and equally important functions—it selectively catalyzes the aminolysis of a thermodynamically deactivated phenolic ester, and it allows an internal buffering effect which disfavors racemization or other base-catalyzed processes which might otherwise operate at the acyl carbon. Although it cannot be claimed that all these effects were anticipated in the design of **2**, their uniqueness and efficacy is such that we urge that they be considered by those whose aim is the rational design of improved peptide coupling reagents.



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EXPERIMENTAL

All solvents and reagents were reagent grade. UV rate measurements were carried out using a Zeiss PMQ II spectrophotometer equipped with a thermostatted cell block. Spectroscopic characterizations and radioactive assays were carried out as described previously.[1,17] Tetramethylguanidine was distilled in vacuum from CaH_2.

3 - *Carbobenzoxyglycyloxy* - N - *ethylbenzamide*, **4b**. To a soln of 3 - hydroxy - N - ethylbenzamide[1] (1·65 g; 10 mM) and of carbobenzoxyglycine (2·1 g; 10 mM) in 4 ml MeOH and 20 ml dioxane, dicyclohexylcarbodiimide (2·3 g; 10 mM) was added. After 45 min the solution was filtered and evaporated, and the residue was dissolved in EtOAc which was filtered, washed with 3 N HCl, 0·5 N NaOH, and water, dried, and evaporated. Recrystallization (EtOAc) gave 1·85 g, 52%, m.p. 130–131°. (Found: C, 64·18; H, 5·86; N, 8·01; $C_{19}H_{20}N_2O_5$ requires: C, 64·03; H, 5·66; N, 7·86%).

7-*Acetoxy*-3-*ethyl*-*benzo*[1,3]-*oxazolin*-4-*one*, **6**. To a stirred soln of thiophosgene (1·8 g; 16 mM) in 20 ml CH_3CN at 0° was added a soln of **2** R = Acetyl (3·3 g; 15 mM) and Et_3N (4·5 g; 4·4 mM). After 1 h at 0° and 24 h at 20°, the solvent was removed, the residue taken up in $CHCl_3$, and the soln extracted with 1 N HCl, 0·5 N $NaHCO_3$, dried, and evaporated. The residue was chromatographed on silica gel to yield 600 mg of a product which was identified by NMR as the desired 7 - acetoxy - 3 - ethyl - 2 - thiobenzo[1,3]oxazolin - 4 - one, and which was used without further purification. ($CDCl_3$, δ: 1·3 (t, 3), 2·4 (s, 3), 4·5 (q, 2), 7–8 (m, 3).) The crude product was stirred in dioxane soln with 5 g dioxane-washed Raney nickel for 3 h. Filtration and evaporation yielded a residue which was dissolved in EtOAc, washed with H_2O, 3 N HCl, 0·5 N $NaHCO_3$, dried, and evaporated. The resulting residue was stirred with 20 ml 0·5 N ethanolic NaOH for 1 h, the solvent was evaporated and the residue dissolved in water which was then extracted with EtOAc. Acidification to pH 1, extraction with EtOAc, drying, and passage through a column of activated carbon gave 52 mg of a crystalline residue upon evaporation. Recrystallization from EtOAc gave needles of 7 - hydroxy - 3 - ethylbenzo[1,3]oxazolin - 4 - one, m.p. 164–5°, IR (Nujol) (cm^{-1}): 3130, 1645, 1585. (Found: C, 62·13; H, 5·82; N, 6·99. $C_{10}H_{11}NO_3$ requires: C, 62·17; H, 5·74; N, 7·25%).

The phenol (45 mg) was dissolved in a mixture of 37 mg pyridine and 48 mg Ac_2O and allowed to remain 24 h at 20°. Addition of EtOAc, extraction with 3 N HCl and 0·5 N $NaHCO_3$, drying, and evaporation gave 35 mg solid, recrystallized from EtOAc-cyclohexane, m.p. 65–66°. NMR ($CDCl_3$, δ): 1·20 (t, 3), 2·30 (s, 3), 3·59 (q, 2), 5·20 (s, 2), 7–8 (m, 3). (Found: C, 61·29; H, 5·70; N, 5·79; $C_{12}H_{13}NO_4$ requires: C, 61·28; H, 5·57; N, 5·95%).

3 - *Carbobenzoglycyloxy* - 2 - *methoxy* - N - *ethylbenzamide*, **5**. Treatment of **2**, R = ZGly in ether soln with CH_2N_2[1] overnight gave upon evaporation a residue which was recrystallized from EtOAc, m.p. 104·5–106°.

(Found: C, 62·29; H, 5·92; N, 7·03. $C_{20}H_{22}N_2O_6$ requires: C, 62·17; H, 5·74; N, 7·25%). Saponification yielded authentic 3 - hydroxy - 2 - methoxy - N - ethylbenzamide.[1]

3-Acetoxy-N-ethylbenzamide, **4a**. Acetylation of 3 - hydroxy - N - ethylbenzamide[1] was carried out as described for **6**. m.p. 71–73°. (Found: C, 63·59; H, 6·49; N, 6·70. $C_{11}H_{13}NO_3$ requires: C, 63·75; H, 6·32; N, 6·75%).

Isotopic dilution assay for formation of **2**, R = ZGly. A soln of 1·25 g (6·00 mM) 2-^{14}C ZGlyOH (744·9 dpm/mg) in 20 ml water containing 5 mM NaOH and 1 ml pyridine was brought to pH 5·0 (short range indicator paper) with 3 N HCl, overlayered with EtOAc, stirred, chilled to 0°, and treated portionwise with 1·65 g (6·6 mM) 2 - ethyl - 7 - hydroxybenzisoxazolium fluoroborate. The soln was stirred for 10 min, whereupon 223·9 mg ZGlyOH and 290·3 mg **2**, R = ZGly were added. After soln had occurred, the layers were separated, and the aqueous layer was extracted twice with EtOAc. The pooled organic phases were extracted with 3 N HCl, 0·5 N $NaHCO_3$, and water, dried, and evaporated. Two recrystallizations of the residue from EtOAc gave ester, m.p. 120–121°, 367·7 dpm/mg. One fifth of the mother liquors contained 10,280 dpm/mg; to the remainder was added 136·3 mg unlabeled ester; recrystallization gave ester, 154·0 dpm/mg. The HCl extracts were brought to pH 4 and extracted with EtOAc. The extract contained 8000 dpm. Acidification and extraction of the $NaHCO_3$ extracts, followed by drying, evaporation and recrystallization gave ZGlyOH, m.p. 119–120°, 83 dpm/mg. Isotopic dilution experiments yielded 99% recoveries of ester **2**, R = ZGly, when pure ester was subjected to the complete activation and work up procedure or to the $NaHCO_3$ extraction alone; 1·4% of activity was recovered from the 3 N HCl extracts.

The nonaqueous activation assays were carried out using 0·5 mM ZGlyOH, 0·5 ml 1·0 N NaOH, × ml of a buffer prepared by combining 20 ml pyridine and 60 ml 3 N HCl for 10 ml of total solvent. Only ester, **2**, was assayed.

Reaction of **2**, R = ZGly, *with TMG*. A soln of 742·8 mg (2 mM) **2**, R = ZGly, 450·2 dpm/mg in 5 ml DMSO, freshly distilled from CaH_2, was treated with 227 mg (2 mM) tetramethylguanidine, distilled from CaH_2. After 40 min at 20°, a solution of 514·7 mg **2**, R = ZGly in 20 ml EtOAc and 30 ml 3 N HCl were added. The organic phase was washed with H_2O, dried, and evaporated. Recrystallization yielded ester, m.p. 120–121°, 267·1 dpm/mg; 450·2 × (742·8/1257·5) = 266·0 dpm/mg.

Determination of pK_a values. For N-ethylsalicylamide, 3 - methoxy - 2 - hydroxy - N - ethylbenzamide, and 3 - hydroxy - 2 - methoxy - N - ethylbenzamide, the ϵ values were measured in aqueous soln containing 4% (v) CH_3CN at pH 1, pH 12, and a pH close to the pK_a value. Measurements were made at two wavelengths for each compound, buffers were phthalate-hydroxide (pH 6–8) or borate (pH 8–10), and pH was measured with a Radiometer 25-25SE-27 pH meter at 25°; pK_a values were calculated using the standard linear equation. The ester, **2**, R = acetyl was found to show an isospestic point at 304 nm for short time intervals up to pH 7·5; above this pH, hydroly.is becomes too rapid for accurate measurements. For this substance, ϵ measurements were made at pH 1 and at a series of pH values up to 7, at 328 and 338 nm, and the linear equation was solved graphically for pK_a.

Aminolytic rate constants for **4**, **5**, *and* **6**. Aminolyses were followed by UV spectrometry under pseudo first order conditions in distilled DMF at $2–3 \times 10^{-4}$ M active ester and 0·1–0·6 M GlyOEt concentrations; GlyOEt was distilled before use. The wavelengths of measurement for **4a**, **4b**, **5**, and **6** were 290, 292, 295, and 320 nm, respectively. For **4b** and **5**, the reaction was carried out preparatively and ZGlyGlyOEt isolated in satisfactory yield. The pseudo first order rate constants obtained from 3–5 aminolysis runs at different GlyOEt concentrations were detectably nonlinear in amine concentration, but gave an excellent fit to the equation $k_u[GlyOEt] + k_{gb}[GlyOEt]^2$. The rate constants k_u and k_{gb} obtained for **4a**, **4b**, **5**, and **6** are, respectively: $1·15 \times 10^{-3}$, $7·2 \times 10^{-4}$; $1·2 \times 10^{-3}$; $1·2 \times 10^{-2}$; $1·1 \times 10^{-3}$, $9·5 \times 10^{-2}$; $1·6 \times 10^{-2}$; $1·15 \times 10^{-3}$, $7·2 \times 10^{-4}$. A fit to an averaged second order rate constant gave respective errors in predicting the largest pseudo first order rate constant of 12, 38, 20, and 10% respectively. The significance of the larger third order contributions for ZGly esters is not known.

Rates of aminolysis of esters, **2**. Since the 2,3 - dihydroxy - N - ethylbenzamide formed in aminolysis rapidly oxidizes at UV concentrations, photometric assay of aminolysis gave irreproducible results, and an isotopic dilution assay was employed. Protected dipeptide esters and acids were prepared as described in the accompanying paper; DMF was purified by distillation at 40 mm from molecular sieves; DMSO was distilled from CaH_2; other solvents were spectro grade. All reactions with amino esters phenylalanine, proline, and sarcosine as nucleophiles were run under second order conditions. Equimolar amounts of the amino acid ester hydrochloride and TMG were combined in 10 ml DMF, the TMG HCl salt was filtered and washed with *ca* 2 ml DMF, and the filtrate was combined with a second equivalent of TMG and equilibrated to 23 ± 1° in a water bath. The soln was stirred with a micro magnet as a soln of an equivalent of ester **2** in DMF was added; DMF was added to volume. At appropriate times, aliquots were quenched in an aqueous HCl–EtOAc mixture and treated with a soln of pure peptide product. After acid-base extraction, the organic phase was dried and evaporated, and the residue was recrystallized to constant activity and counted. Reactions involving glycine or valine as nucleophiles were run under pseudo first order conditions with the tetramethylammonium salts of the amino acids in 10 to 40-fold excess. Between 8 and 16 pseudo first order rate constants were measured at differing nucleophile concentrations for each reaction. A plot of k_{obs}/[amine] *vs* [amine] gave k_2 as intercept and zero slope.

Hydrolysis and aminolysis of active esters. A soln of L-phenylalanine, TMG, organic solvent, and water was brought to 20° and mixed with a soln of ^{14}C labeled **2**, R = ZGly in the solvent. After the reaction was completed, aliquots were removed. The first was diluted with an aqueous soln of the sodium salt of ZGlyOH, 3–5 equivalent. The second was diluted with a similar soln containing ZGly-DL-PheOH. Extraction, acidification, recovery, and recrystallization (2–3 times) gave pure acids whose specific activities were assayed. The ZGly esters of *p*-nitrophenol, N-hydroxysuccinimide,[21] 8 - hydroxyquinoline,[22] and N-hydroxypiperidine,[23] and 2-mercaptopyridine[24] were prepared by literature procedures. All but the latter compound had literature properties; the thiopyridine ester showed m.p. 80–82° (lit 65–68°).[24] (Found: C, 59·43; H, 4·76; N, 9·07; S, 10·56. $C_{15}H_{14}NO_3S$ requires: C, 59·60; H, 4·67; N, 9·27; S, 10·58%). The acyl azide of ZGlyOH was prepared from the hydrazide by diazotization ($NaNO_2$) at −5° in a HCl,

HOAc, water mixture. Cold ether was added, and after 5 min, the ether was separated and washed at −5° with aq. NaHCO and water, and dried; CH_3CN was added, the ether was removed at −5° in vacuum, and the mixture was combined in the usual way with nucleophile. The ethyl carbonic anhydride of ZGlyOH was prepared at −15° in CH_3CN by addition of ethyl chloroformate to the Et_3N salt of ZGlyOH.

Racemization experiments. The racemization results of Table 6 were obtained by the method previously reported.

Racemization of 2, R = Bz-L-Leu,[17] *by TMG.* A soln of TMG and 2, R = Bz-L-Leu in dry DMF, CH_3CN, or DMSO was prepared. After 1 h, excess GlyOEt HCl and sufficient TMG to bring the GlyOEt to 1·1 eq were added. After 20 h, the mixture was worked up in the usual way. Polarimetric racemization runs were carried out in dry solvents and followed using a Perkin-Elmer model 141 Polarimeter.

Demonstration of oxazolone-active ester exchange. A soln of 159 mg (0·4 mM) 2, R = 7-^{14}C-benzoyl-L-Leucyl, $1{\cdot}93 \times 10^6$ dpm/mM, 41 mg (0·36 mM) TMG, and 93 mg (0·43 mM) 2 - phenyl - 4 - ^{3}H - isobutyl - oxazol - 5 - one, $2{\cdot}58 \times 10^6$ dpm/mM in 2 ml DMSO was allowed to remain at 20° for 90 min. Aliquots were quenched with ethereal solns of unlabeled water, drying, evaporating the ether extract, then extracting the residue with CCl_4; the ester was reisolated by recrystallizing the residue from CCl_4 extraction. A second experiment under identical conditions was diluted in separated aliquots with L and DL ester.

Derivation of rate ratio value for Table 6 B.2. Depending upon the relative rates of reaction of oxazolone with the anion of 2,3 - dihydroxy - N - ethylbenzamide and ethyl glycinate, expressions (2) or (3) must describe the formation of racemic, C′, and unracemized product, C, from the reaction of 2, R = ZGly-L-Phe, A, with ethyl glycinate, B, in the presence of TMG. The oxazolone is symbolized by D.

$$\text{(2)}\quad \begin{array}{l} A + B \xrightarrow{k_2} C \quad \dfrac{[C'_\infty]}{[A_0]} = \dfrac{k_1}{A_0 k_2} \exp\left(-\dfrac{k_1}{A_0 k_2}\right) \displaystyle\int_{k_1/A_0k_2}^{\infty} \frac{e^{-t}}{t}\,dt \\ \Big\downarrow k_1 \\ A' + B \xrightarrow{k_2} C' \end{array}$$

$$\text{(3)}\quad \begin{array}{l} A + B \xrightarrow{k_2} C \quad \dfrac{[C'_\infty]}{[A_0]} = \dfrac{k_1}{A_0 k_2} \ln\left\{1 + \dfrac{A_0 k_2}{k_1}\right\} \\ \Big\searrow k_1 \ \text{(slow)} \\ D + B \xrightarrow[\text{(fast)}]{k_3} C' \end{array}$$

At small ratios of k_1/A_0k_2, such as are observed for esters 2, the graphs of the two functions are identical. It is clearly not practical to obtain k_1/A_0k_2 as a simple function of C'_∞/A_0, the function of racemized product observed at the end of the reaction. To fit the data to these expressions, graphs of C'_∞/A_0 as functions of k_1/A_0k_2 were plotted, and values of k_1/A_0k_2 were obtained for each C'_∞/A_0 data point. Multiplication by A_0 gave estimates of k_1/k_2, which were averaged to $2{\cdot}5 \times 10^{-5}$.

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