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Rate Constants for Peptide *p*-Nitrophenyl Ester Coupling Reactions in Dimethylformamide. A Model for Steric Interactions in the Peptide Bond Forming Transition State[1]

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Rate constants are reported for 41 aminolysis reactions of N-protected amino acid *p*-nitrophenyl esters with amino acid ethyl or *tert*-butyl esters in DMF at 30°. With the exception of reactions involving proline esters as nucleophiles, all reactions yield rate constants which can be satisfactorily approximated as a product of two partial rate factors. A model which accounts for this observation is proposed and discussed, and generalizations to the behavior of other phenyl esters are considered.

The work described in this paper was initiated because rate constants for a number of aminolysis reactions of peptide esters of 3-acyloxy-2-hydroxy-*N*-ethylbenzamides were observed to fit the very simple rate law of eq 1, for

$$k_{A\text{-}B} = (k_{A\text{-}Gly})(k_{Gly\text{-}B})\left(\frac{1}{k_{GlyGly}}\right) \quad (1)$$

which $k_{A\text{-}B}$ is the second-order rate constant for the coupling of an active ester derived from a protected amino acid Z-A-OH with an amino acid ester, H-B-OEt.[2] This observation implies that activation energy changes for these reactions, which for the cases studied were largely sterically determined, must arise from independent effects of the substituents at the two amino acid sites, and suggests, moreover, that 400 rate constants for the possible dipeptide forming aminolyses can be estimated from only 39 measured rate constants. The *p*-nitrophenyl esters are the most widely used and easily studied of the peptide active esters, and for these reasons, we chose these esters for an investigation of the validity of eq 1. Although an aqueous medium as a solvent choice would permit comparison with the very extensive data available for aminolysis of simple *p*-nitrophenyl esters,[3] we chose DMF as a solvent which is more likely to be employed by the practicing peptide chemist. Previous studies had indicated that aminolyses in this solvent show first-order rate behavior with respect to amine.[1] It may be noted that recent studies of the aminolysis of phenyl esters in nonaqueous solvents have argued strongly that collapse of a reversibly formed tetrahedral intermediate is rate determining[4] and have established the potent catalytic capacity of hydrogen bond acceptors.[5]

Several earlier studies have considered the effects of peptide substituents on rates of peptide forming aminolysis reactions. Using 2,4,5-trichlorophenyl esters, Pless and Boissonnas established the half-times for reactions of 17 activated amino acids with benzylamine in dioxane, as well as half-times for the reaction of the trichlorophenyl ester of ZPheOH with 13 amino acid esters.[6] In an investigation directly pertinent to the present study, Khurgin and Dmitrieva measured hydrolysis and aminolysis rate constants for the *p*-nitrophenyl esters of 11 carbobenzoxy amino acids and noted a correlation in the nonhindered cases with σ^* values.[7,8]

Results

To obtain data to test the validity of eq 1, 30 rate constants were measured for the reactions of the *p*-nitrophenyl esters of carbobenzoxy derivatives of Gly, Ala, Leu, Pro, Val, and Phe with the ethyl esters of the first five of these amino aicds. Although this series does not provide examples of large inductive effects or special side-chain reactivity, it does span nearly all of the range of steric effects to be encountered in peptide synthesis, and it is expected that steric effects should provide the most interesting test cases for eq 1. Reactions were carried out in dimethylformamide at 30° under pseudo-first-order conditions at *ca.* 10^{-4} *M* active ester concentration, with at least a fourfold range of amine concentrations, between 0.002 and 0.1 *M*. Linear de-

Table I
Rate Constants for *p*-Nitrophenyl Ester Coupling Reactions in DMF

1. Z—A—O-*p*-NPh + H—B—OEt $\xrightarrow[30°]{\text{DMF}}$ HO-*p*-NPh + Z—A—B—OEt

A[a]	B[a]	k_2, M^{-1} min^{-1}[b]	k_2(calcd)[c]
Gly (1738-86-9)[d]	Gly (459-73-4)	26.3 (0.3)	
Ala (1168-87-2)	Gly	16.7 (1.2)	
Val (10512-93-3)	Gly	1.28 (0.01) 1.11 (0.07)	
Leu (1738-87-0)	Gly	11.2 (0.2) 11.2 (0.01)	
Pro (3304-59-4)	Gly	9.05 (0.1)	
Phe (2578-84-9)	Gly	14.1 (0.3)	
Asn (3256-57-3)	Gly	7.2 (0.3)	
Gly	Ala (3082-75-5)	6.06 (0.2)	
Ala	Ala	4.4 (0.2)	3.7
Val	Ala	0.26 (0.08)	0.28
Leu	Ala	2.16 (0.04)	2.5
Pro	Ala	1.31 (0.02)	2.0
Phe	Ala	2.5 (0.1)	3.1
Gly	Val (17431-03-7)	2.06 (0.02)	
Ala	Val	1.21 (0.04)	1.3
Val	Val	0.062 (0.002)	0.099
Leu	Val	0.56 (0.02)	0.88
Pro	Val	0.43 (0.05)	0.71
Phe	Val	0.62 (0.02)	1.10
Gly	Leu (2743-60-4)	2.84 (0.01)	
Ala	Leu	1.9 (0.1)	1.8
Val	Leu	0.119 (0.005)	0.14
Leu	Leu	1.05 (0.01)	1.2
Pro	Leu	0.670 (0.006)	0.97
Phe	Leu	1.3 (0.2)	1.5
Gly	Pro (5817-26-5)	6.87 (0.05)	
Ala	Pro	1.63 (0.02)	4.4
Val	Pro	0.155 (0.003)	0.33
Leu	Pro	0.51 (0.07)	3.0
Pro	Pro	0.135 (0.005)	2.4
Phe	Pro	1.39 (0.01)	
Gly	Phe (3081-24-1)	1.00 (0.02)	
Phe	Phe	0.46 (0.04)	0.54

2. X—A—O-*p*-NPh + H—B—O—Y $\xrightarrow[30°]{\text{DMF}}$ HO-*p*-NPh + XA—B—O—Y

X-A[a]	HB—O—Y[a]	k_2, M^{-1} min^{-1}	k_2(calcd)
BOCGly (3655-05-8)	GlyOEt	23.1 (0.2)	
BOCGly	AlaOEt	5.8 (0.3)	5.1
BOCLeu (3350-19-4)	GlyOEt	9.7 (0.1)	
BOCLeu	AlaOEt	1.9 (0.2)	2.1
ZGly	AlaO-*t*-Bu (15911-69-0)	8.43 (0.2)	
ZGly	LeuO-*t*-Bu (21691-53-2)	4.86 (0.05)	
ZAla	AlaO-*t*-Bu	6.7 (0.1)	5.4
ZAla	LeuO-*t*-Bu	3.5 (0.2)	3.1

[a] All amino acids have the L configuration. [b] The term in parentheses is the least-squares error in slope. [c] k_2(calcd) is obtained by applying eq 1 to the experimental rate constants observed for glycine couplings [d] Registry no. are in parentheses below compounds.

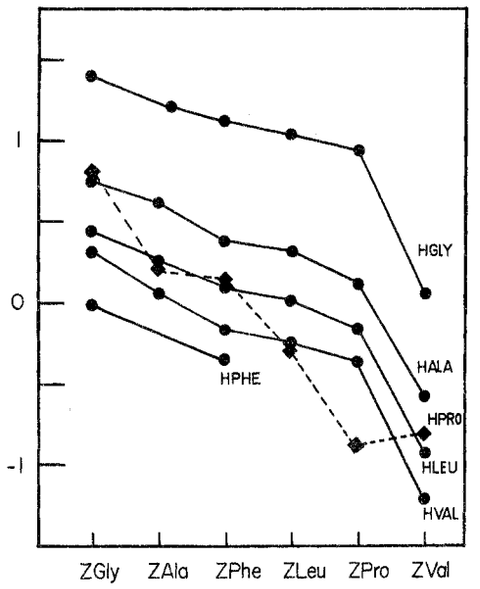


Figure 1. Log k_2, logs of second-order rate constants for coupling reactions of carbobenzoxyamino acid *p*-nitrophenyl esters with amino acid ethyl esters; data from Table I.

pendence of pseudo-first-order rate constant on amino concentration was noted in all cases, implying that the rates of these reactions are simply dependent on the products of amine and active ester concentrations. Data are presented in Table I.

Also included in the Table are comparisons of relative reactivities of Gly, Leu, and Ala derivatives bearing other blocking groups. In accord with the findings of Pless and Boissonnas,[6] the *tert*-butoxycarbonyl and benzyloxycarbonyl amino acid esters are found to be nearly identical in reactivity. A surprising finding is the significantly greater reactivity of the *tert*-butyl over the ethyl esters of Ala and Leu. A competition experiment was carried out in which equivalent amounts of HLeuOEt and HLeuO-*t*-Bu were allowed to react with the *p*-nitrophenyl ester of ZGlyOH in DMF. Cleavage of the neutral product mixture with trifluoroacetic acid gave ZGlyLeuOH in significant excess of the ZGlyLeuOEt formed, demonstrating that the effect is in fact real, and not an artifactive error of the kinetic procedure. Cases in which a more hindered derivative is more reactive are usually argued to arise from a relief of steric strain at the transition state, or from attractive London forces in a presumably polarizable transition state. It is difficult to argue for the former explanation in the case at hand.

Accompanying each entry of the table is an error estimate and a rate constant calculated from eq 1. It may be seen that with the exception of reactions of HProOEt, the success of the approximation is very good, and it may be noted that a still better fit would be possible by adjusting the partial rate factors for each amino acid. We have not chosen to do so, since the deviations from the present approximation should provide a measure of direct or indirect substituent–substituent interactions for the coupling transitions state.

A more obvious means of noting the magnitude of the proline anomaly is seen by the graph of Figure 1 which plots log k_2 for families of amino acids. The log of a rate constant which obeys eq 1 should be a simple sum of logs of partial rate factors, and families of such rate constants should show a simple additive increase or decrease as one amino acid is changed. As may be noted from the figure, exactly this behavior is observed for all amines but

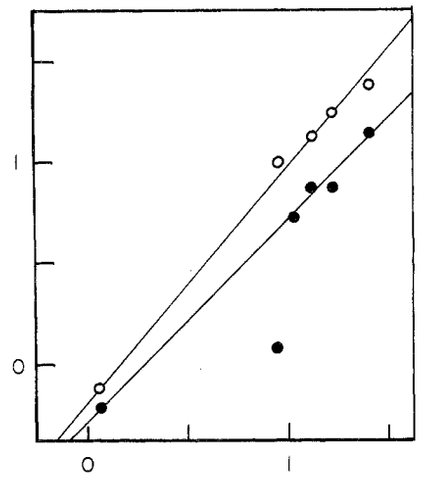


Figure 2. Open circles: logs of coupling rate constants for reaction of amino acid 2,4,5-trichlorophenyl esters with benzylamine in dioxane plotted against log k_2 for the corresponding reaction of a *p*-nitrophenyl ester with ethyl glycinate in DMF (ref 6). Closed circles: corresponding plot of log k_2 for reaction of the *p*-nitrophenyl ester with glycylglycine in water (ref 8).

HProOEt. A statistical analysis is best applied to log k_2 values; the mean deviation of the 15 values which can be approximated by eq 1 is +0.052, the values ranging from −0.09 to +0.13. For the five HProOEt data, the mean deviation is +0.41, with a range of +0.09 to +0.94.[9] Clearly proline esters, unlike the other nucleophiles, show coupling rate constants which are very sensitive to interaction effects with substituents on the electrophilic partner.

Figure 1 also demonstrates an interesting, highly regular feature of these data. Whereas the substitution of a Val for a Gly causes a large rate change at both the C and N sites [av log (k_{Gly}/k_{Val}) = 1.40 (0.08) at C, 1.23 (0.09) at N], the substitution of Ala, or Leu for Gly causes a much larger rate change at the N than at the C site [*e.g.*, av log (k_{Gly}/k_{Ala}) = 0.18 (0.04) at C, 0.70 (0.09) at N].

Although the data of earlier workers are not extensive enough to permit test of eq 1, it is nonetheless interesting to compare where possible the effects of steric factors on rate constants as observed here for aminolysis of *p*-nitrophenyl esters in DMF with data observed for other solvents and esters. Figure 2 shows a log–log plot of the second-order rate constants observed by Pless and Boissonnas[6] for the reactions of 2,4,5-trichlorophenyl esters with benzylamine in dioxane as functions of the rate constants reported in Table I with ethyl glycinate as nucleophile. Also included is a similar plot of the data of Khurgin and Dmitrieva[8] for reactions of *p*-nitrophenyl esters with glycylglycine in water. Though the comparison data are not abundant, there appears to be a good linear correlation with nearly unit slope for rate constants resulting from structural changes of the active ester. It would therefore appear that similar steric effects attend phenolic ester couplings involving differing solvent or ester substitutions. Strikingly, the trichlorophenyl ester data imply that the opposite conclusion must be drawn for structural changes with the amine, for Figure 3 indicates that no significant correlation exists between the rate variations with amine substitution for reactions with 2,4,5-trichlorophenyl esters in dioxane and *p*-nitrophenyl esters in DMF.

Discussion

A theory or model which is proposed to rationalize the above observations must contend with several formidable uncertainties. Aminolysis of an ester involves three major

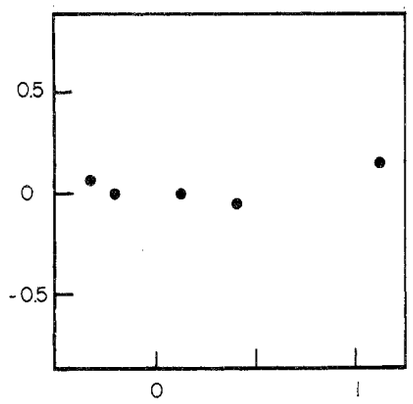
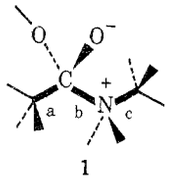


Figure 3. Log k_2 for the reaction of ZPhe trichlorophenyl ester in dioxane with amino acid methyl esters as functions of log k_2 for the reaction of ZPhe *p*-nitrophenyl ester in DMF with the corresponding amino acid ethyl esters (ref 6).

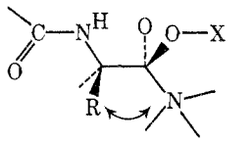
bond changes at the reaction site—a C–N amide bond is formed, C–O ester and N–H amine bonds are broken. Although the precise timing of these events remains obscure despite much careful investigation,[10] it is likely that the rate-determining transition state bears substantially tetrahedral substitution at both the acyl carbon and amide nitrogen atoms, and the solvent is coordinated with both the NH_2^+ and O–C–O–X regions. In principle, three rotamers are possible at each of three single bonds, resulting in 27 potential conformations for the rate-determining transition state.[11] A new center of asymmetry at the acyl carbon is unique to the transition state. (Although there is doubtless a preferred chirality at this center, none of the subsequent analysis of *p*-nitrophenyl ester results appears to offer insight into this preference, and in the ensuing discussion we



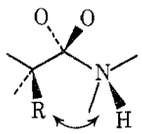
1

ignore it.) Clearly in a situation of this complexity, with relatively few incisive experimental findings, rigorous theories are uncalled for and at best one can hope to propose a plausible model which has heuristic value for new experiments. The model which is developed in the following discussion has proved very useful to us in rationalizing and predicting steric effects for a variety of intramolecular aminolysis reactions encountered during exploratory research with new types of peptide coupling reactions,[12] and for this reason, is developed here in etail.

Two general, preliminary points may be noted. First, it appears that the variations in rate constants observed in this study do reflect steric effects peculiar to the transition state, since what information is available implies that product stability shows a very different substituent pattern.[13] Second, there is more than adequate precedent in the quantitative behavior of other crowded systems to explain the range of effects observed in this study. To develop this point, one can note that two kinds of changes occur which affect the environment of a substituent R, attached at the carbon α to the acyl site as the *p*-nitrophenyl ester is converted into the transition state for aminolysis; first, a staggered 1,2 interaction is created between the R and acyl amino groups and the O or N atoms of the acyl carbon; second, a 1,3 interaction is created between the R or acylamino groups and the N–H functionality of the nucleophile.

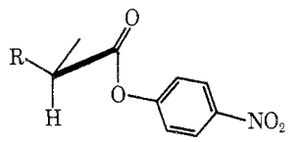


1,2 interaction



1,3 interaction

Despite the uncertainties in bond distances and structural features, one can find in the axial–equatorial energy differences for monosubstituted chair cyclohexanes a rough analogy for the new 1,3 interaction resulting from the conversion of **2** into **3**. For our aminolyses the change in free ener-



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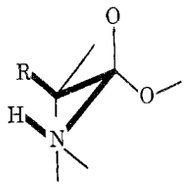

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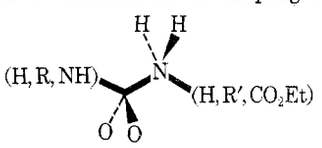
gy of activation, $\Delta(\Delta G^*)$, for the substitution of R = CH_3 for R = H is 0.25 kcal/mol, while that for the substitution of *i*-Pr for H is 1.9 kcal/mol. These may be compared with *A* values for Me and *i*-Pr of roughly 1.3 and 2.1 kcal/mol.[14,15] For substitution at the site α to the amino nucleophile, a change from R = H to R = Me leads to $\Delta(\Delta G^*)$ of 0.97 kcal/mol, which may be compared with an a–e interaction energy difference for *cis*-3-hydroxymethylcyclohexane of 2.1 kcal/mol.[15] Thus it is likely that even considering only 1,3 interactions, no special factors need be invoked to explain the magnitude of the rate differences seen in this study.

The peculiarities of the rate data which a model might seek to explain include (1) the success of eq 1 in predicting rate constants for most coupling reactions; (2) the failure of eq 1 for prediction of rate constants for reactions involving HProOEt; and (3) the differing magnitudes of substituent effects at C and N termini. In developing the model, we



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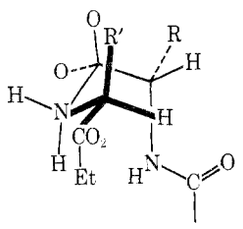
employ fact 3 to select among the conformational choices and show that the resulting conformations of lower energy lead to a prediction of facts 1 and 2.

Many of the 27 conformations of **1** can be readily seen to be impossibly crowded; inspection of the subclasses of anti and gauche rotamers about the developing C–N bond (bond b of **1**) allows the most simple analysis of this fact. Thus, the anti rotamer, **4**, allows minimal interaction be-



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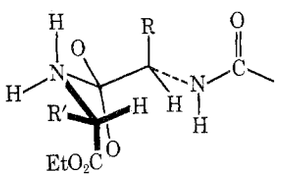

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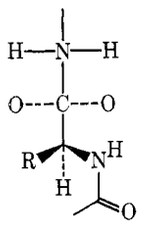
tween the bulky ends, and all nine conformers which maintain this anti relationship are therefore expected to be sufficiently close in energy to require more information before a stability ranking can be proposed for them. In contrast, the gauche C–N rotamers have very severe end group interactions unless the two proximate terminal groups are both hydrogens, as indicated in **5** and **6**. Although as will be seen, either **5** or **6** appears to be more crowded than the average of the nine anti C–N cases, the energy difference is probably small enough that **5** and **6** must be considered as possible contributors to product formation. There are thus 11 serious candidates for the conformations of the product forming transition state.

As the above structures indicate, in none of the 11 is a *direct* R–R′ interaction possible, and therefore *the conclusion that* **4, 5,** *and* **6** *are sterically favored also establishes the molecular basis for the validity of equation 1 for the nonproline nucleophiles.*

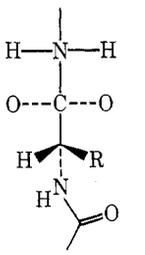
Explanation of the proline anomaly now rests on the results of an analysis of conformational preferences about the a and c bonds of the anti N–C conformation, **4**, and a consideration of **5** and **6**.

The C–C bond a of **1** has three possible rotamers, **7**, **8**, and **9**, which must differ in energy to the degree that the indicated pairs of interactions are different.[16]



1,3 NH—amido
1,3 NH—R

7



1,3 NH—R
1,2 O—amido

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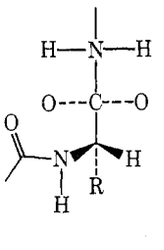
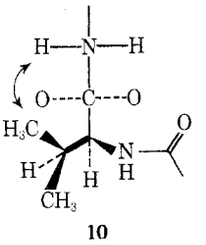


1,3 NH—amido
1,2 O—R

9

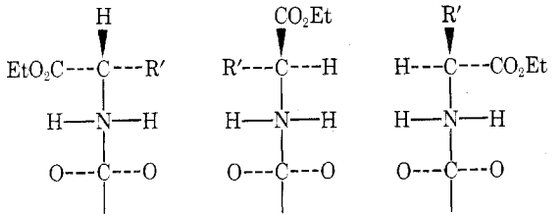
The three rotamers thus have two kinds of interactions involving alkyl or amido groups: 1,2 interactions with oxygen functions at the acyl carbon, and 1,3 interactions with hydrogens at the amino nitrogen. An *a priori* evaluation of the magnitude of these interactions is not possible, since the C–N bond b is likely of abnormal length, and the steric environment at the N–H and C–O sites may be altered by the presence of DMF molecules (the large solvent rate acceleration for this reaction should be recalled). However, the following argument can be based on the relative magnitude of the substituent effects. Of the two types of interactions, the 1,2-oxy interactions are expected to be large for both Me and *i*-Pr, while the 1,3-NH interaction must be large for *i*-Pr, but could be relatively small for Me if the N–C amide bond is long in the transition state. The observed change in free energy of activation with substitution at the acyl site is only 0.24 kcal/mol for H → Me, but becomes 1.8 kcal/mol for H → *i*-Pr. This pattern, therefore, supports the assertion that in the low energy rotamer, a large 1,2-oxy interaction is avoided, and the dominant energy change results from 1,3 interactions between the HN functions and amido or R groups. The rotamer **7** therefore appears to be the more stable if R ≠ H, and **7** and **9** must be the preferred rotamers for Gly (R = H). Avoidance of an overriding 1,2-oxy interaction presumably favors one rotamer of the three at the α–β alkyl bond of valine and thus forces this case (R = *i*-Pr) into conformation **10**, which has a significant 1,3 interaction.



10

The model predicts that substituents at the amino site must encounter an opposite interaction pattern. The 1,2 interaction now occurs between an alkyl group and N–H hydrogens and is expected to be small, while the 1,3 interactions between acyl C–O and alkyl or ester functions must be large.

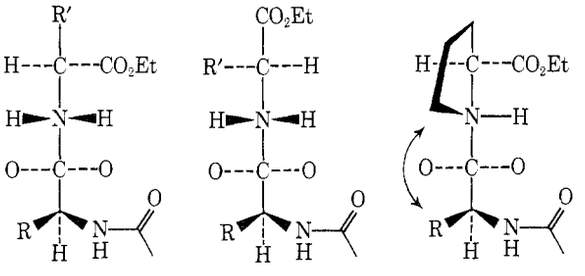
Unique among the amino acids, glycine can assume conformation **12**, R = H, which has no significant 1,3 interactions. All other amino acids are expected to assume conformations **12** and **13**, R ≠ H, which have only one 1,3 interac-



11 **12** **13**

tion of importance. The unusual, large rate constants for HGlyOEt are thus understandable. [For substitution at the amino site, $\Delta(\Delta G^*)$ = 0.9 kcal/mol for H → Me, and 1.5 kcal/mol for H → *i*-Pr.]

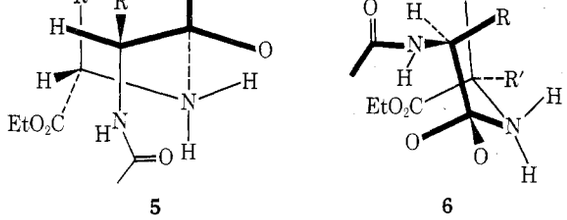
In **14** and **15** are summarized the overall structural features proposed by the model for the lowest energy conformers of the aminolysis transition state. From these the pro-



14 **15** **16**

line anomaly is readily rationalizable, for with HProOEt as a nucleophile, **14** necessarily becomes **16**, which now bears a new alkyl–alkyl interaction between R and the proline side chain resulting in direct steric interactions between the peptide substituents, and as a result, eq 1 cannot be obeyed. An equivalent deduction follows if the Pro side chain is considered as a part of **15**.

By a similar sort of analysis, one can show that for the two gauche conformations, **5** and **6**, the environment about



5 **6**

R in **5** has the 1,2 and 1,3 interactions of **9**, with an additional 1,3 interaction between bond and a C–H; similarly **6** is expected to be more crowded than **8**. A similar, more hindered situation obtains at R′, and it seems reasonable, as noted earlier, that neither conformation represents a major path for the reaction. Both **5** and **6** predict obedience to eq 1, but neither can be used to explain the proline deviations. Moreover, of the 9 rotamers theoretically possible at the a and c bonds of the trans N–C conformation, only two allow

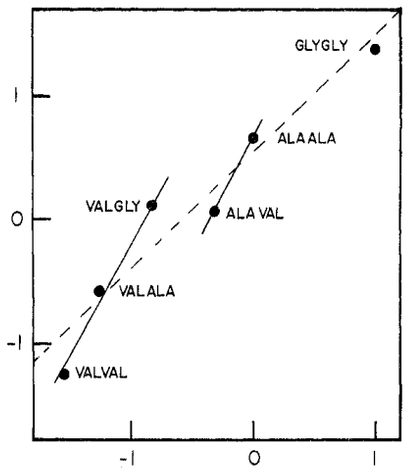


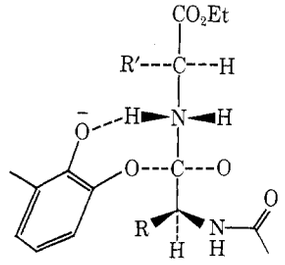
Figure 4. Log k_2 for the aminolysis reactions of esters of 2,3-dihydroxy-*N*-ethylbenzamide in DMSO as functions of log k_2 for the corresponding reaction of the *p*-nitrophenyl ester (ref 2).

the 1,3 R–C–C–N–X interaction which explains the proline result.

It is interesting to attempt to generalize the model to other phenyl ester aminolyses. The marked insensitivity of the 2,4,5-trichlorophenyl ester rates to the steric environment of the amino component was noted above and would appear to require a very different steric situation at the amino but not the acyl side of the transition state. Possibilities include a longer C · · · O-ϕ bond, and attractive dispersion interactions between the ortho chlorine atom and the R or carboethoxyl groups. Similar halogen interactions must be invoked in simple acyclic systems to explain conformational preferences.[18] More information is needed before the intriguing features of this system can be placed in their proper perspective.

A second case of interest is provided by the aminolysis reactions of the 3-acyloxy-2-hydroxy-*N*-ethylbenzamides, which display a very similar steric pattern at both acyl and amino sites to that seen in this study for *p*-nitrophenyl esters,[2] and which appear on the basis of limited data to yield rate constants which obey eq 1.

Moreover, as indicated by Figure 4, the pattern of effects is similar to that found in the present study, although a somewhat greater sensitivity to acyl substitution, R, and a lesser sensitivity to R′ may be noted. Further evidence strongly supports a mechanism in which internal proton transfer or hydrogen bonding to the catechol monoanion occurs in the rate-determining transition state.[2] The above anti N–C model can be adapted to accommodate this special feature, and **17** or its acyl epimer is the result.

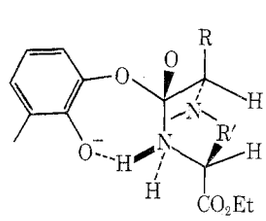


17

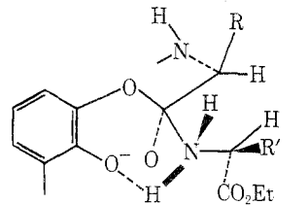
Molecular models imply that the introduction of the catechol ester functionality significantly increases the crowding of one quadrant of **17**, although even approximate molecular analogies which would allow energy estimates for this environment are problematic. Since the catechol environment of **17** is remote from R or R′, increased crowding

by the catechol need not increase the steric effects of rendering R and R′ bulky, and greater hindrance of **17** over **14** or **15** is therefore not inconsistent with the similar spans of rate constants for the catechol and *p*-nitrophenyl esters. However, the experimental finding that HProO$^-$ reacts more than a hundred times more slowly than expected with 3-carbobenzoxyglycyloxy-2-hydroxy-*N*-ethylbenzamide appears to be inexplicable in terms of the model **17** or its acyl epimer. The magnitude of this rate discrepancy is such that it is likely that no hydrogen bonding occurs between the catechol oxy anion and the single proline NH at the transition state. The acyl epimer of **17**, which allows hydrogen bonding, is therefore excluded, and the more hindered, nonhydrogen bonded **17** would have to be the energetically preferred conformer. This result is unreasonable since the asymmetry at the acyl carbon which positions the catechol must be induced by the asymmetry of the proline function, which is the only center of chirality in the starting materials; there appears to be no factor which can be invoked to override the energetically favorable hydrogen bond.

Consideration of structures **5** and **6** permits a consistent rationalization, for these structures allow the catechol oxy anion an uncrowded environment; thus **5** becomes **18**; and **6**, **19**. Structure **18** cannot accommodate a proline methylene substitution, while **19** can only do so at the expense of a severe 1,3-dialkyl interaction which is augmented by a buttressing effect of the 1,3-O—CO_2Et interaction on the opposite side of the molecule.



18



19

Summary

Models have been discussed which while admittedly speculative, appear to account for the gross features and at least approximately, for the details of steric effects on rates of aminolyses of peptide phenyl esters by peptide amines. The major experimental finding of obedience to eq 1 requires an anti conformation about the forming C–N bond or one of two gauche conformations in which the conformations about the remaining bonds are fixes as in **5** or **6**. These rotamers can be independently assigned as the less hindered among the 27 possibilities. For the *p*-nitrophenyl ester case, the proline deviations and the differing pattern of steric effects for acyl and amine substituents are consistent with only two among the anti C–N rotamers as providing the major reaction pathway.

More data are needed with other phenyl esters before general conclusions can be drawn, but we stress that the present model, however crude and speculative, provides a first step toward a predictive scheme for substituent effects on peptide bond forming reactions. In subsequent discussions we will describe application of this model to rationalizing the strikingly different substituent rate effects which arise when the peptide bond forming aminolysis reaction is made intramolecular.

Experimental Section

Unless otherwise specified, reagents and solvents were reagent grade; amino acids were Calbiochem A grade. Carbobenzoxyamino acids[19] were prepared by literature procedures and were recrystallized to constant melting point, or converted into their dicyclohexylamine salts and purified to constant melting point. The *p*-nitrophenyl esters[19] of N-protected amino acids were prepared using dicyclohexylcarbodiimide and *p*-nitrophenol, following the procedure of Bodanszky and duVigneaud;[20] ethyl acetate was used as solvent except for ZAsnOH, for which DMF was substituted. *Amino acid ethyl ester hydrochlorides were prepared by the Boissonnas modification*[21] of Fischer esterification and were recrystallized to literature melting point. DMF for kinetic runs was obtained by distilling a 3:1 mixture of reagent grade DMF and toluene at 30 mm through a 55-cm spinning band column. The middle DMF fraction was collected, sealed, and stored in a desiccator over P_2O_5–KOH. Optical rotations were measured in a 1-dm microcell, using a Perkin-Elmer Model 141 polarimeter.

Product Determination. For most reactions studied, products were isolated in at least 80% yield and characterized from reactions in DMF at 0.05 *M* reagent concentrations. The Z-protected ethyl esters of the following dipeptides were characterized by comparison of melting point and in most cases $[\alpha]_D$ with literature values: GlyGly, AlaGly, ValGly, LeuGly, GlyAla, AlaAla, ValAla, LeuAla, PheAla, AsnAla, ValVal, ValLeu, and LeuLeu. The following dipeptides were characterized as hydrazides, obtained by hydrazinolysis of the ethyl esters: ProGly, GlyVal, LeuVal, ProVal, GlyLeu, AlaLeu, ProLeu. The dipeptides with C-terminal proline residues were isolated in high yield as oils. The following new substances were prepared by the above coupling procedure and characterized.

Ethyl *tert*-Butoxycarbonyl-L-leucylglycinate. Needles from ether–petroleum ether: mp 83–84°, $[\alpha]^{22}_D$ −25.8 (1.6, EtOH). *Anal.* Calcd for $C_{15}H_{28}N_2O_5$: C, 56.94; H, 8.92; N, 8.85. Found: C, 56.98; H, 8.98; N, 8.90.

Benzyloxycarbonyl-L-prolyl-L-alanine Hydrazide. Crystals from ethanol–ether: mp 142–143°, $[\alpha]^{22}_D$ −12.7 (0.5, EtOH). *Anal.* Calcd for $C_{26}H_{22}N_4O_4$: C, 57.47; H, 6.63; N, 16.76. Found: C, 57.69; H, 6.66; N, 16.20.

Ethyl Benzyloxycarbonyl-L-asparaginyl-L-alaninate. Needles from ethanol–ether: mp 183–184°, $[\alpha]^{22}_D$ −38.2 (0.2, DMF). *Anal.* Calcd for $C_{17}H_{23}N_3O_6$: C, 55.88; H, 6.35; N, 11.50. Found: C, 55.79; H, 6.44; N, 11.47.

Ethyl *tert*-Butoxycarbonyl-L-leucyl-L-alaninate. Needles from ether–petroleum ether: mp 111–112°, $[\alpha]^{22}_D$ −40.2 (1.0, EtOH). *Anal.* Calcd for $C_{16}H_{30}N_2O_5$: C, 58.16; H, 9.15; N, 8.48. Found: C, 58.13; H, 9.02; N, 8.44.

Ethyl Benzyloxycarbonyl-L-alanyl-L-valinate. Needles from ethyl acetate–petroleum ether: mp 82–83, $[\alpha]^{22}_D$ −29.9 (1.1, EtOH). *Anal.* Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 8.00. Found: C, 61.89; H, 7.50; N, 8.00.

Kinetic Procedure. Within 2 days of a kinetic run, samples of amino acid ethyl or *tert*-butyl esters were liberated from their salts with 33% NaOH solution, extracted, dried over K_2CO_3, and distilled *in vacuo*, then stored at 0° until immediately before use. Ethyl glycinate was distilled before use. For rate measurements, stock solutions of amino acid ethyl or *tert*-butyl esters (0.1–0.3 *M*) and N-blocked amino acid *p*-nitrophenyl ester (*ca.* 10^{-3} *M*) were prepared in dry DMF. Volumes of amine stock solution were pipetted into four 25-ml volumetric flasks which were filled to 23 ml with DMF and brought to 30°. To initiate a run, 1.0 ml of *p*-nitrophenyl ester solution was added to the flask, which was filled with DMF to the mark, and the resulting solution was mixed and transferred to a 1-cm silica cuvette. Absorbance measurements were made at 325 nm in a Zeiss PMQ II spectrophotometer, equipped with a thermostated cell block maintained at 30 ± 0.1° and connected to a Hewlett-Packard 3440-3A digital voltmeter and H03571B-562A digital printer. Reactions were conducted at 0.003 to 0.1 *M* amine concentrations; in almost all cases four concentrations in the range 0.01 to 0.05 *M* were chosen. Reactions were followed to 2 to 2.5 half-lives, and infinity points were taken at 10 half-lives. Pseudo-first-order rate constants were obtained for each run at fixed amine concentration by a linear least-squares analysis of $\ln (A_\infty - A_t)$ *vs.* *t*. Second-order rate constants were obtained by a linear least-squares analysis of pseudo-first-order rate constants for reactions at different amine concentrations; in nearly all cases, four concentrations were used, but in two or three instances, five or three were employed. A value of 10% of the smallest pseudo-first-order rate constant was observed for the average zero intercept term, which presumably is attributable in part to aminolysis by traces of dimethylamine in the solvent.

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Registry No.—Ethyl *tert*-butoxycarbonyl-L-leucylglycinate, 51220-76-9; benzyloxycarbonyl-L-prolyl-L-alanine hydrazide, 52895-37-1; ethyl benzyloxycarbonyl-L-asparaginyl-L-alaninate, 52928-60-6; ethyl *tert*-butoxycarbonyl-L-leucyl-L-alaninate, 52895-38-2; ethyl benzyloxycarbonyl-L-alanyl-L-valinate, 52895-36-0.

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(10) See discussion and references in ref 3, **4**, and 5; also A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, **92,** 5442 (1970), and W. P. Jencks, *ibid.*, **94,** 4733 (1972).
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(12) D. S. Kemp and F. Vellaccio, unpublished observations.
(13) For example, valyl and isoleucyl peptides are known to hydrolyze more slowly than average, and heats of hydrolysis, though varying significantly, do not appear to reflect simple steric trends. Pertinent references include R. L. Hill in *Advan. Protein Chem.*, **20,** 37 (1965); M. Rawitscher, I. Wadsö, and J. M. Sturtevant, *J. Amer. Chem. Soc.*, **83,** 3180 (1961); and J. P. Greenstein and M. Winitz, "The Chemistry of the Amino Acids," Vol. 1, Wiley, New York, N.Y., 1961, pp 558 ff.
(14) J. A. Hirsch in "Topics in Stereochemistry," N. Allinger and E. Eliel, Ed., Interscience, New York, N.Y., Vol. 1.
(15) E. Eliel, N. Allinger, S. Angyal, and G. Morrison, "Conformational Analysis," Interscience, New York, N.Y., 1965, pp 44–66.
(16) Each of the conformers **7, 8,** and **9,** has six pertinent interactions; however, three of these occur identically, and in each case an additional interaction involves the α-H and is expected to be small. The remaining two are listed below the structures.
(17) Models suggest that this 1,3 alkyl–alkyl interaction should be most severe when both alkyl functions are relatively rigid, as is the case when both are derived from proline side chains; it may be noted that the deviation from eq 1 in the Pro-Pro case is very large.
(18) See ref 15, pp 13–19.
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Carbon-13 Nuclear Magnetic Resonance Spectra of Branched-Chain Sugars. Configurational Assignment of the Branching Carbon Atom of Methyl Branched-Chain Sugars[1]

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Carbon-13 nmr spectra of α and β anomers of branched-chain sugars, having the branched-chain group (methyl) at the 2 and 4 carbons and epimeric at the branching carbon atom, are reported and discussed.

The identification of a relatively large number of branched-chain sugars as the glycoside component of antibiotics,[2] the discovery that cell walls of some aquatic plants contain a high percentage of the branched-chain sugar apiose,[3] the isolation of branched-chain sugar nucleotides from the microorganism *Azobacter vinelandi*,[4] and the observed cytostatic and virostatic activity of nucleosides with branched-chain sugars[5-7] are all responsible for the rapid development of the synthetic chemistry of branched-chain sugars in recent years.

However, the determination of the configuration of a branching carbon atom in branched-chain sugars was notoriously difficult, since a simple and reliable method was not available.[8]

In late 1972 carbon-13 nmr spectroscopy was applied, for the first time, to the configurational assignment of quaternary carbon atoms in branched-chain sugars having the 1,3-dithian-2-yl and 2-methyl-1,3-dithian-2-yl residues as the branched chains.[20-23]

Using the observation on methylcyclohexanes[24,25] that the carbon-13 chemical shift of an axial methyl group is ~6 ppm upfield relative to that of an equatorial methyl group, we have unequivocally determined the configuration of the branching-carbon atom in a number of branched-chain sugars having the branched chain (methyl group) at the 4-carbon atom.[26] Since the influence of the configuration of the branching-carbon atom and the anomeric configuration upon the carbon-13 resonances of other carbon atoms of a branched-chain sugar was not thus far studied and since the methyl group is the most frequent branched chain in naturally occurring branched-chain sugars, a detailed analysis of carbon-13 nmr spectra of α and β forms of branched-chain sugars epimeric at the branching carbon atom seemed appropriate. The following branched-chain sugars were studied by carbon-13 nmr spectroscopy: methyl 4-*C*-methyl-3-*O*-methyl-6-*O*-triphenylmethyl-α-D-galactopyranoside (**1**), methyl 4-*C*-methyl-3-*O*-methyl-2-*O*-methylsulfonyl-6-*O*-triphenylmethyl-α-D-galactopyranoside (**2**), methyl 4-*C*-methyl-2,3-di-*O*-methyl-6-*O*-triphenylmethyl-α-D-galactopyranoside (**3**), methyl 4-*C*-methyl-3-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (**4**), methyl 4-*C*-methyl-3-*O*-methyl-2-*O*-methylsulfonyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (**5**), methyl 4-*C*-methyl-2,3-di-*O*-methyl-6-*O*-triphenylmethyl-α-D-glucopyranoside (**6**), methyl 4-*C*-methyl-2,3-di-*O*-methyl-6-*O*-triphenylmethyl-β-D-galactopyranoside (**7**), methyl 4-*C*-methyl-2,3-di-*O*-methyl-6-*O*-triphenylmethyl-β-D-glucopyranoside (**8**), methyl 4,6-*O*-benzylidene-2-deoxy-2-*C*-methyl-3-*O*-methyl-α-D-glucopyranoside (**9**), methyl 4,6-*O*-benzylidene-2-deoxy-2-*C*-methyl-3-*O*-meth-