teristic of a 2,4-disubstituted pyridine derivative). The nmr spectrum of fraction b is in good agreement with the proposed structure. The absence of a significant proton resonance peak with a τ value in the range of 7.80–7.95 ppm, arising from the 4-methyl group on the pyridine ring, is evidence for essentially complete acylation at the 4 position. Fraction b was analyzed.

Anal. Calcd for $C_{10}H_{13}NO$: N, 8.58. Found: N, 8.85.

A sample of the ketone was converted to a yellow monopicrate, mp 122.8–124.2°.

Anal. Calcd for $C_{16}H_{16}N_4O_8$: N, 14.28. Found: N, 14.21.

The ir spectrum of the carbinol, fraction c, shows a very strong absorption band at 3.0 μ, characteristic of an O–H group. The nmr spectrum of this product is in good agreement with the assigned structure. The absence of a proton resonance peak at 7.80–7.95 ppm, attributable to the 4-methyl group on the pyridine ring, offers further support for essentially exclusive attack of the sodium amide at the 4-methyl group of 2,4-lutidine. The carbinol was analyzed.

Anal. Calcd for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 1036. Found: C, 75.68; H, 8.07; N, 10.18.

(3) Acylation of 2,4-Lutidine with Perfluorinated Esters. (a) Standard Addition Technique. This is the same as the general procedure used above for the other acylation reactions.

(b) Reverse Addition Technique. The acylation of the anion of 1 with an ester employing the reverse addition technique differs from the standard addition procedure in that the 2 equiv of the tar base anion is prepared from phenyllithium and 1 in a reaction vessel which is positioned above and connected to a second reaction vessel. In the bottom flask (three necked), which is equipped with a reflux condenser and a mechanical stirrer, is placed 1 equiv of the appropriate ester and 100 ml of anhydrous ether for every 0.1 mol of ester. The flask containing the ester is cooled to −5° with a salt and ice bath and the solution of the tar base anion is added dropwise. After addition of the anion the reaction mixture is allowed to warm to room temperature, stirred for 1 hr, and processed as with reactions employing the standard addition technique.

(4) The Acylation of 2,4-Lutidine at the 2-Methyl Group with Ethyl Trifluoroacetate Using the Reverse Addition Technique. Using phenyllithium (0.2 mol), 1 (21.4 g, 0.2 mol), and ethyl trifluoroacetate (14.2 g, 0.1 mol) there was obtained 8.48 g (39.6%) of 2,4-lutidine (bp 75° (42 mm)) by distillation. Upon extraction of the distillation residue with Skelly B there was obtained 18.5 g (91.2%) of 4-methyl-2-picolyl trifluoromethyl ketone (mp 130.4–131.8°) and 4.16 g of an intractable residue.

Registry No.—**1**, 108-47-4; **2**, 3197-57-7; **2** picrate, 3197-62-4; **3**, 51975-33-8; **3** picrate, 52920-03-3; **4** (n = 1), 52920-04-4; **4** (n = 1) copper salt, 52920-05-5; **4** (n = 2), 52920-06-6; **4** (n = 2) copper salt, 52920-07-7; **4** (n = 3), 52920-08-8; **4** (n = 3) copper salt, 52920-09-9; n-butyllithium, 109-72-8; phenyllithium, 591-51-5; sodium amide, 7782-92-5; phenylsodium, 1623-99-0; ethyl benzoate, 93-89-0; 2,4-dimethyl-6-phenylpyridine, 27068-65-1; ethyl propionate, 105-37-3; 2-methyl-4-(propionylmethyl)pyridine, 52920-10-2; 2-methyl-4-(propionylmethyl)pyridine picrate, 52920-11-3; 2-ethyl-1,3-bis(2-methyl-4-pyridyl)propanol-2, 52920-12-4; ethyl trifluoroacetate, 383-63-1; ethyl pentafluoropropionate, 426-65-3; ethyl heptafluorobutyrate, 356-27-4; 2-n-butyl-4,6-dimethylpyridine, 52919-93-4.

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Studies on the Coupling Step in Solid Phase Peptide Synthesis. Further Competition Experiments and Attempts to Assess Formation of Ion Pairs[1]

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Competition experiments have been performed to study the possible influence of a number of α-amino protecting groups with urethane structure on the reactivity of amino acids in the coupling step under solid phase peptide synthesis conditions. No differences in reactivity could be detected, however, by this procedure, which we recently used for a similar study on the influence of amino acid side chains. A few additional experiments have been made with peptides instead of amino acids to gain insight into the prospects of fragment coupling. The data to be presented in the first half of this paper have been obtained by amino acid analysis of hydrolyzed peptide mixtures. Insolubilized hydrogen-bonded ion pairs are postulated to be formed on addition of an amino acid derivative in dichloromethane prior to the coupling in solid phase peptide synthesis. In the second half of this paper attempts have been made to determine the extent to which ion pairs are formed under different conditions. The influence of temperature, the concentration of soluble carboxyl component, and the nature of the solvent have been studied.

Peptide synthesis on a solid support, generally called solid phase peptide synthesis (SPPS), was introduced and pioneered by Merrifield[2,3] and is today a well-established technique which has been used for the preparation of many peptides. In this procedure synthesis takes place in a stepwise fashion starting from the carboxyl end, with the growing peptide attached by an ester bond to a polystyrene resin. Since the α-amino function must be protected, one cycle involves exposing the amino group and coupling to it the next amino acid. After a certain number of such cycles the peptide is stripped off the resin. Generally all protecting groups still remaining on the peptide are removed at the same time, leaving a crude free peptide which now has to be purified.

Table I
Competition Experiments with Different N^{α}-Protecting Groups

Expt no.[a]	N^{α}-protected amino acid used (incorporation, %)				Total incorporation (%)
1	Z(OMe)-Gly (41.7)	Z(OMe)-Phe (30.8)	Boc-Leu (23.9)	Boc-Val (4.0)	100.4
2	Bpoc-Gly (41.2)	Boc-Phe (29.0)	Bpoc-Leu (22.3)	Boc-Val (4.0)	96.5
3	Bhoc-Gly (40.6)	Bhoc-Phe (31.6)	Boc-Leu (22.2)	Boc-Val (4.1)	98.5
4	Ppoc-Gly (40.6)	Ppoc-Phe (31.8)	Boc-Leu (21.9)	Boc-Val (3.7)	98.0
5	Trt-Gly (19.3)	Boc-Phe (40.6)	Boc-Leu (27.2)	Boc-Val (7.9)	95.0
A[b]	Boc-Gly (39.7)	Boc-Phe (29.6)	Boc-Leu (24.8)	Boc-Val (4.8)	98.6
6	Z(OMe)-Gly (32.9)	Boc-Ala (27.2)	Z(OMe)-Phe (23.9)	Boc-Leu (17.9)	101.9
7	Bhoc-Gly (30.1)	Boc-Ala (26.8)	Bhoc-Phe (23.6)	Boc-Leu (14.8)	95.3
B[b]	Boc-Gly (32.3)	Boc-Ala (28.8)	Boc-Phe (24.0)	Boc-Leu (20.1)	105.2

[a] Expt 1–5 and A were performed with Ala-resin and 6, 7, and B with Val-resin. [b] See ref 4.

Competition experiments[4] were recently performed to obtain information on the reactivity of individual amino acids in the coupling step. Claims in the literature[5,6] of difficulties in the coupling of specific Boc-amino acids prompted us to try to arrange different Boc derivatives according to their reactivity in the coupling step under SPPS conditions. We considered competition experiments with a Boc-amino acid and a reference compound, but the number of amino acid analyses necessary in this approach caused us to select the present, less strict procedure with four competing components, although the significance in the figures obtained is partly lost. As expected, considerable differences in reactivity were found to exist. Those experiments have now been extended. Protecting groups themselves, *e.g.* on the α-amino function or even in side chains, could possibly influence the reactivity in different ways. Bulky groups could give rise to steric hindrance in the coupling step or to reduced penetration of the amino acid derivative into the interior of the resin. For this reason we have now conducted a series of experiments where the influence, if any, of different α-amino protecting groups has been investigated.

Peptides rather than amino acids have in a few cases been used for extension of the peptide chain in SPPS. For further references, see ref 7. Consequently, we have also been interested in seeing how a peptide would perform under competition conditions. This, on the other hand, has made necessary some further experiments which could be used for purposes of comparison.

In SPPS dichloromethane is generally used as the reaction medium, with dimethylformamide (DMF) as an alternative if the amino acid derivative dissolves poorly. Both swell the resin satisfactorily. The difference in properties between the two solvents mentioned is considerable indeed, and one consequence of this will be emphasized in this paper.

The interaction between acetic acid and different amines in carbon tetrachloride and chloroform was studied by Barrow and Yerger[8] and reviewed recently by Davis.[9] Using infrared spectroscopy, different adducts, depending on the type of amine, solvent, and stoichiometry, were inferred, all of which were characterized by association *via* hydrogen bonds as ion pairs. Extrapolating these results to dichloromethane, strong interactions can be expected between the free amino terminus of the amino acid last coupled and the carboxyl group of the N-protected derivative after addition of the latter compound in the Merrifield procedure. To our knowledge this has not been considered so far, although solvents such as chloroform and dichloromethane have been used in peptide synthesis for many years. It may also explain the adsorption effect recently described by Esko and Karlsson[10] and later studied or used by Elliott, *et al.*,[11] and Losse and coworkers.[12] The latter part of this paper therefore deals with model experiments on noncovalent bonding of the carboxyl component to the amino group of an amino acid resin of Merrifield type. These experiments together with the competition experiments constitute our efforts so far toward attaining a better understanding of the coupling step in SPPS.

Table II
Competition Experiments on Fragment Coupling[a]

Expt. no.	Amino acid incorporation (%) Gly	Leu	Phe	Total incorporation (%)
8	79.1	18.8	21.1	99.1[b]
C	56.7		45.0	101.7
9A	74.7	21.6	21.7	96.5
9B[c]	77.4	22.3	22.2	99.7
D	58.3		41.0	99.3

[a] Z(OMe)-Leu-Phe[21] was used in these experiments together with Ala-resin (expt 8) and Val-resin (expt 9). [b] This value was obtained using the average found for Leu and Phe. [c] After hydrolysis for 72 hr. When not otherwise stated hydrolysis was for 24 hr.

Results and Discussion

Competition Experiments were carried out as described in the Experimental Section. Blocking groups tested and compared included the *tert*-butyloxycarbonyl[13] (Boc), *p*-methoxybenzyloxycarbonyl[14] [Z(OMe)], 2-(*p*-biphenyl)isopropyloxycarbonyl[15] (Bpoc), benzhydryloxycarbonyl[16] (Bhoc), 2-phenylisopropyloxycarbonyl[17] (Ppoc), and trityl[18] groups. Trityl amino acids are considered sterically hindered[19] (see Table I).

The experiments were performed with a polystyrene–*co*-1% divinylbenzene resin to which Boc-alanine or Boc-valine had been esterified according to Merrifield's original procedure. Prior to the coupling experiments, Boc was removed using 50% trifluoroacetic acid (TFA) in dichloromethane. A mixture of 1 equiv each calculated on the amount of amino acid resin of four different N^{α}-protected amino acids and the corresponding amount of dicyclohexylcarbodiimide (DCCI) were added and allowed to react with the resin for 2 hr. The resin was washed free from reactants and by-products, treated with 50% TFA as above, washed again, and dried. A resin sample was treated with HF,[20] the peptide mixture was extracted from the resin, and the solution was evaporated to dryness. A portion was hydrolyzed and then quantitatively analyzed for amino acids on an amino acid analyzer. The quantity found of the amino acid originally attached to the resin was arbitrarily set to 100 and the amount found of the competing amino acids was normalized accordingly. Total incorporation was obtained as the sum of the latter values. Assuming a relative experimental error of less than 3%, complete coupling reaction

Table III
Other Competition Experiments Performed

Expt no.	Protected amino acids used (incorporation, %)				Total incorporation (%)
10	Boc-Gly (33.8)	Boc-(NO_2)-Arg (27.4)	Boc-(Z)-Lys (18.6)	$(Boc)_2$-His (18.1)	97.9
11A	Boc-Gly[a] (94.9)	Boc-Ile[a] (2.6)			97.5
11B	Boc-Gly[a,b] (99.7)	Boc-Ile[a,b] (4.4)			104.1
E	Boc-Gly (68.6)	Boc-Ile[c] (29.7)			98.3

[a] Ten equivalents of each Boc derivative was used. [b] After hydrolysis for 72 hr. When not otherwise stated hydrolysis was for 24 hr. [c] See experiment 10A in ref 4; 3 equiv of Boc-Ile was used.

would give total incorporation values in the range 94–106. The results for different N^{α}-protecting groups are given in Table I.

As seen in Table I, a remarkably good agreement was obtained between expt 1–4 and A, performed with alanine attached to the resin. In our opinion this can only be due to complete noninterference by the protecting groups in the coupling step, which is then understood also to include penetration of the derivatives into the resin. Whether the protecting group has none, one, or two benzene rings in it does not seem to matter, as long as it is of urethane structure. Similarly the results of expt 6, 7, and B agree. In expt 5 the picture was different. As expected Trt-Gly coupled more poorly than other glycine derivatives studied, and in fact Boc-Phe and Boc-Leu showed higher reactivities. Since a protecting group of urethane structure does not seem to influence the coupling, it should be possible to use amino acid derivatives with different amino-protecting groups more liberally in the same synthesis. This may sometimes aid in the preparation of the protected amino acids.

Our interest in utilizing fragment condensation on a solid support was the reason for the experiments whose results are presented in Table II. In expt 8 1 equiv each of Boc-Gly and Z(OMe)-Leu-Phe[21] was allowed to react with Ala-resin for 2 hr in the presence of 2 equiv of DCCI. Experiment 9 was identical with expt 8, except that Val-resin was used. C and D were controls, performed with Boc-Phe instead of the dipeptide. Evidently, peptides show reduced reactivity in comparison with the C-terminal amino acid protected as Boc derivative. Preparative experiments have later demonstrated, however, that the remaining reactivity is high enough to secure a high yield of product as exemplified by synthesis of bradykinin using di- and tripeptide fragments.[7]

Experiment 10 in Table III presents data on the behavior of the three basic amino acids, Arg, Lys, and His, which all seem to couple well. Ala-resin was used in this experiment. Our attempts to also include Trp in the present work invariably resulted in very low "total incorporation," indicating loss of Trp due to decomposition.

Experiment 11, performed with 10 equiv of each amino acid derivative, serves to demonstrate in full magnitude the difference in reactivity between Boc-Gly and Boc-Ile. Since the Val-resin was used, an extended hydrolysis was necessary. Considering possible errors, we think it is safe to conclude that Boc-Gly is at least 20 times more reactive than Boc-Ile under the conditions used, which approximate those of SPPS. The reason for this difference in reactivity is, of course, the steric influence of the side chain of isoleucine.

Experiments on Carboxyl–Amino Group Interaction. As pointed out above, hydrogen-bonded ion pairs are known to be formed when acetic acid and an amine are mixed in carbon tetrachloride or chloroform. The rest of this paper will be devoted to model experiments to determine the extent of ion-pair formation under conditions related to SPPS.

Temperature, concentration, and the nature of the solvent are among the factors known to influence the stability of hydrogen bonds in solution. According to Pimentel and McClellan,[22] drastic effects are observed, as revealed by ir and Raman spectra, upon changes in temperature of 10–20° or upon variation of the concentration of the hydrogen bonding substances in an inert solvent. This study will illustrate the effect of changes in these three parameters on the system Boc-amino acid/polymer, where the polymer is of Merrifield type, a polystyrene matrix with a second amino acid with a free amino group attached *via* its carboxyl by an ester bond.

All following experiments were performed according to the same general scheme, the details of which are found in the Experimental Section. Boc-Phe was carefully equilibrated with Ala-resin under conditions specified with reference to solvent, temperature, and concentration of Boc-Phe. The solution was then filtered off and the resin washed twice with the same volume of fresh solvent. Dichloromethane was added, followed by DCCI, and reaction was allowed to proceed for 1 hr. The resin was carried through a normal washing procedure. Nonreacted amino groups were finally determined using the 2-hydroxy-1-naphthaldehyde procedure[23] developed in our department.

Experiments 14 and 20 above give the results for the two solvents normally used in SPPS. A high coupling yield was expected for dichloromethane.[10] These orientative experiments further demonstrate that carbon tetrachloride and benzene are even more efficient than dichloromethane in this context. At the other end of the table we find dioxane with about the same dielectric constant. Dioxane, however, has basic properties[9] and is not inert to proton donors.[22] Carbon tetrachloride and benzene have negligible acidity and basicity as well as low dielectric constants, *i.e.*, are more truly inert. It should be emphasized that all experiments in Table IV were performed under considerably more dilute conditions than normally used in preparative work.

The standard method for attachment of the first amino acid to the resin gives rise to some quaternary ammonium sites. To exclude their influence five extra experiments were performed with an Ala-resin without such sites, the results of which are given in parentheses (experiments 16 and 18–21). We interprete our results to mean that dimethylformamide and dioxane completely exclude association between the components.

The trend in the experiments of Table V was as expected.[10] By conducting experiments at a low enough temperature, dichloromethane can be brought to give results similar to those for carbon tetrachloride and benzene at room temperature.

Under concentration conditions more typical of those used in SPPS dichloromethane behaved approximately as carbon tetrachloride and benzene did at the low concentra-

tion used in Table IV. Extrapolating the results of Table IV, V, and VI, very strong association of Boc-Phe to Ala-resin can be envisaged at both high concentration and reduced temperature in dichloromethane.

Table IV
Innfluence of the Solvent on Carboxyl–Amino Group Interaction[a]

Expt. no.	Solvent	Dielectric constant (ε)[b]	Coupling yield[c] (%)
12	Carbon tetrachloride[d]	2.23	>99.5
13	Benzene[d]	2.27	>99.5
14	Dichloromethane[d]	9.08[e]	54–59
15	Chloroform[f]	4.81[e]	55
16	Tetrahydrofuran[f]	7.39	33 (39)[g]
17	Ethyl ether[d]	4.34[e]	28
18	HMPA[h]	30[e,i]	25 (13)[g]
19	Ethyl acetate[d]	6.02	18 (16)[g]
20	Dimethylformamide[g]	36.7	14 (0)[g]
21	Dioxane[f]	2.21	11 (0)[g]

[a] Performed at room temperature 23–25°. The resin used originally had 0.287 mmol of Ala/g. All experiments refer to a dilution of 57.1 ml/g of resin. [b] Values when not otherwise stated were taken from ref 9 and refer to 25°. [c] Determined according to ref 23. [d] "Pro analysi" quality. [e] Refers to 20°. [f] Filtered through a column of active aluminum oxide. [g] See discussion below. [h] Hexamethylphosphoramide. Kept over a molecular sieve, Linde 4A, for several weeks prior to use. [i] According to ref 24.

Table V
Influence of Temperature on Carboxyl–Amino Group Interaction[a]

Expt no.	Temp (°C)	Coupling yield (%)
22	37	30
23	23–25	54–59
24	4	95
25	−12	>99.5

[a] All experiments refer to dichloromethane. All conditions except temperature were the same as in Table IV.

Table VI
Influence of Concentration on Carboxyl–Amino Group Interaction[a]

Expt no.	Dilution (ml of solvent/g of resin)	Coupling yield (%)
26	57.1	54–59
27	28.6	73
28	14.3	93
29	7.1	>99.5

[a] All experiments refer to dichloromethane. All conditions were the same as in Table IV except dilution.

We do not want to make any definite statements about the stoichiometry. In the work of Barrow and Yerger mentioned above evidence was found not only for 1:1 adducts between acetic acid and amine but also for 2:1 adducts. Since excess of carboxyl component, normally Boc-amino acid, is always used in the SPPS procedure, it is possible that an amino group can bind more than one molecule of Boc derivative. Preliminary experiments simply involving repeated washing of the resin to recover material indicate that 6–7 additions of fresh dichloromethane[25] may be needed to remove the excess of Boc-amino acid used. After 10 washings about 0.6 equiv of Boc-amino acid had still not been recovered. No evidence for discrete adduct species was detected in this admittedly simple experiment. According to Elliott, *et al.*,[11] only the excess is removed by washing with fresh solvent.

Tables IV–VI provide fundamental data on the extent of association under different conditions between the components in the SPPS procedure. Some of the figures bear on the adsorption coupling method,[10] which has more recently proved useful in the preparation of two bradykinin analogs.[11,12a] The scope of this modified procedure, however, still remains to be determined.

Experimental Section

Acid hydrolyses of peptides were performed with 6 *N* HCl (110°, 24 hr, when not otherwise stated) in sealed evacuated tubes, and the amino acids were determined with a Biocal BC-200 or Durram D-500. Absorbance measurements were performed on a Coleman Hitachi 124 or Beckman Acta CIII to a precision of 0.001. Solvents were of standard quality when not otherwise stated. Amino acids used were of L configuration (except Gly). Resin refers to cross-linked polystyrene (1% divinylbenzene, Bio-Beads S·X-1).

Boc-Ala-resin. This was prepared like Boc-(NO$_2$)Arg-resin[26] from a chloromethylated resin with 0.75 mmol of Cl/g and after deblocking with 50% TFA/dichloromethane for 30 min gave on analysis[10] 0.287 mmol of Ala/g.

Boc-Val-resin. The same chloromethylated resin and the same procedure resulted in a product with 0.261 mmol of Val/g.

Boc-Ala-resin without Quaternary Sites. Chloromethylated resin with 1.75 mmol of Cl/g was converted to hydroxymethyl resin[27] and esterified with Boc-Ala accordingly,[27] giving a resin with 0.638 mmol of Ala/g.

Competition Experiments. A weighed sample of Boc-Ala- or Boc-Val-resin (about 300 mg) was reacted by rocking for 30 min with 3 ml of 50% TFA/dichloromethane in a 10-ml cylindrical glass vessel with a fritted disk filter, stopper, and stopcock. After washing with dichloromethane (3 × 2 min), neutralization with 10% triethylamine in the same solvent (10 min), and washing again with dichloromethane similarly, 1 equiv each (calculated on the amount of Ala or Val, bound to the resin) of generally four *different* protected amino acids was together added in 3 ml of dichloromethane; 10 min later, a corresponding amount (generally 4 equiv) of DCCI in a minimum of dichloromethane was added and coupling allowed to proceed for 2 hr. After washing with dichloromethane (3 × 2 min), the deprotection procedure was repeated, mainly to get rid of residual amino acids not covalently bound to the resin. An aliquot of dry resin was reacted with HF[20] (0°, 1 hr) and the peptide mixture was extracted from the resin with 8 × 5 ml of 10% HOAc. After evaporation of the solvents, the residue was hydrolyzed and analyzed for amino acids. 62–77% of the C-terminal amino acid could be accounted for. In expt 8 and 9 performed similarly, Z(OMe)-Leu-Phe[21] was allowed to compete with Boc-Gly.

Experiments on Carboxyl–Amino Group Interaction. A typical experiment was done as follows. A weighed amount of Boc-Ala-resin (~70 mg) was deprotected, washed, neutralized, and washed again as just described and allowed to equilibrate for 4 hr with 4 equiv of Boc-Phe in about 4 ml of solvent. The solution was filtered off, and the resin was washed twice for 2 min with the same volume of fresh solvent. Dichloromethane was added, followed by 2 equiv of DCCI in a minimal volume of the same solvent. After reaction for 1 hr, the resin was taken through a washing procedure including dichloromethane (2 × 2 min), absolute ethanol (2 × 2 min), and again dichloromethane (2 min). This was followed by determination of unreacted amino groups.[10]

Registry No.—Z(OMe)-Gly, 4596-54-7; Z(OMe)-Phe, 23234-86-8; Boc-Leu, 13139-15-6; Boc-Val, 13734-41-3; Bpoc-Gly, 23650-19-3; Boc-Phe, 13734-34-4; Bpoc-Leu, 18634-99-6; Bhoc-Gly, 3312-84-3; Bhoc-Phe, 3312-91-2; Ppoc-Gly, 52950-77-3; Ppoc-Phe, 57499-65-1; Trt-Gly, 52950-78-4; Boc-Gly, 4530-20-5; Boc-Ala, 15761-38-3; Z(OMe)-Leu-Phe, 14565-51-6; Boc-(NO$_2$)-Arg, 2188-18-3; Boc-(Z)-Lys, 2389-45-9; (Boc)$_2$-His, 20866-46-0; Boc-Ile, 13139-16-7.

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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-