released to the bulk solution) on the liposome surface more easily than that in the DDPC system.

The blocks of polymerized MDPC lipids (tetramer, estimated by GPC) on the liposome surface are more mobile than those of DDPC (two dimensionally cross-linked).[7] On a fluid surface like the mixed liposome of DMPC and oligomerized MDPC, pores induced by the release of lysolecithin might be quickly closed by the neighboring lipids.

Such a system would be quite useful to promote understanding of the sophisticated and complicated processes in living bodies.

Acknowledgment. We thank Dr. Yoshihiko Nagata, Tosoh Corp., for a kind donation of the polymerizable lipids. We also thank Prof. Masahide Yamamoto and Dr. Shinzaburo Ito of this department for generously allowing us to use the fluorescence anisotropy depolarization equipment.

Direct Cleavage versus Transpeptidation in the Autodecomposition of Peptides Containing 2,4-Diaminobutanoic Acid (DABA) and 2,3-Diaminopropanoic Acid (DAPA) Residues. Specific Cleavage of DAPA-Containing Peptides[1,2]

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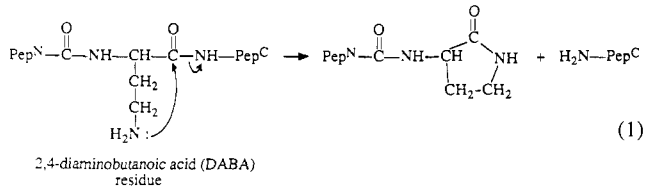
Abstract: Peptides containing 2,4-diaminobutanoic acid and 2,3-diaminopropanoic acid residues undergo transpeptidation by attack of their side-chain amino groups on the *N*-carbonyl (eq 2). Little or no direct cleavage by attack on the *C*-carbonyl (eq 1) is observed. The transpeptidation reactions of peptides containing 2,4-diaminobutanoic acid (DABA) or 2,3-diaminopropanoic acid (DAPA) residues reach an equilibrium in which the various peptides studied are about 70–80% transpeptidized; this extent of transpeptidation is in agreement with the equilibrium constants for other transamination reactions. The transpeptidation reaction is strongly catalyzed by phosphate and bicarbonate buffers, and the pH dependence of the reaction suggests that an unprotonated side-chain amino group is required for significant reactivity. The rate of the transpeptidation reaction is retarded by bulky substituents at the α-carbon of the residue at the amino-terminal side of the DAPA or DABA residue. The preference for transpeptidation over direct cleavage in the case of DABA residues can be explained by one or more of the following factors: (1) a preference for (*Z*)-amide (transpeptidation) over (*E*)-amide (direct cleavage); (2) greater ring strain in the tetrahedral intermediate for direct cleavage; (3) a steric effect resulting from unfavorable interactions in the possible transition states for direct cleavage (Scheme III). A stereoelectronic explanation is considered and rejected. Peptides containing *transpeptidized* DABA and DAPA residues (isoDABA and isoDAPA residues, respectively) undergo cleavage at the carboxy-terminal side of these residues on treatment with the Edman reagent followed by treatment with trifluoroacetic acid. Peptides can be induced to undergo direct cleavage at the carboxy-terminal side of *untranspeptidized* DAPA residues by treatment with the Edman reagent followed by heptafluorobutyric acid. The chemical and biological significance of these observations is discussed.

Although the 2,4-diaminobutanoic acid (DABA) residue does not occur naturally in most peptides, a number of investigators have been intrigued by the possibility that such a residue, synthetically produced from one of the standard amino acids by peptide modification, might serve as a site for specific cleavage of proteins, as shown in eq 1. In this paper, we shall refer to this

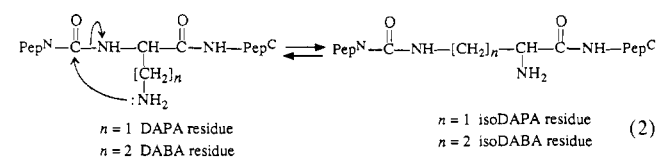


(1)

2,4-diaminobutanoic acid (DABA) residue

type of process as *direct cleavage*. For example, LeQuesne and Young[4] wrote, "It is tempting to suggest that under suitable conditions, α,γ-diaminobutyric acid [DABA] might serve as a source of instability in peptide chains". Similarly, Rudinger[5] suggested, "Selective peptide-bond fission based on the presence of amino groups in the γ-position of amino-acid chains might well find application in [structural] studies". Indeed, several groups have demonstrated that peptide-bond fission like that shown in eq 1 does occur in favorable cases.[6]

The reaction in eq 1, however, is not the only process by which nucleophilic side chains may become covalently involved with the peptide backbone. A side-chain amino group can in principle also attack the carbonyl group of the preceding residue (eq 2). In



n = 1 DAPA residue
n = 2 DABA residue

n = 1 isoDAPA residue
n = 2 isoDABA residue

(2)

(1) This paper is dedicated to Professor Donald S. Noyce following his retirement from the Department of Chemistry of the University of California, Berkeley.

(2) A preliminary account of this work has appeared: Blodgett, J. K.; Loudon, G. M. In *Peptides, Structure and Function: Proceedings of the Ninth American Peptide Symposium*; Deber, C. M.; Hruby, V. J.; Kopple, K. D., Eds.; Pierce: Rockford, IL, 1985; pp 371–374.

(3) Present address: Cortech, Inc., 6840 North Broadway, Suite F, Denver, CO 80221.

(4) LeQuesne, W. J.; Young, G. T. *J. Chem. Soc.* **1952**, 594–597.

(5) Poduska, K.; Katrukha, G. S.; Silaev, A. D.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1965**, *30*, 2410–2433.

(6) (a) Barrass, B. C.; Elmore, D. T. *J. Chem. Soc.* **1957**, 4830–4834. (b) Lipson, M. A.; Sondheimer, E. *J. Org. Chem.* **1964**, *29*, 2392–2394. (c) Katrukha, G. S.; Baratova, L. A.; Silaev, A. B. *Experientia* **1969**, *8*, 968–975.

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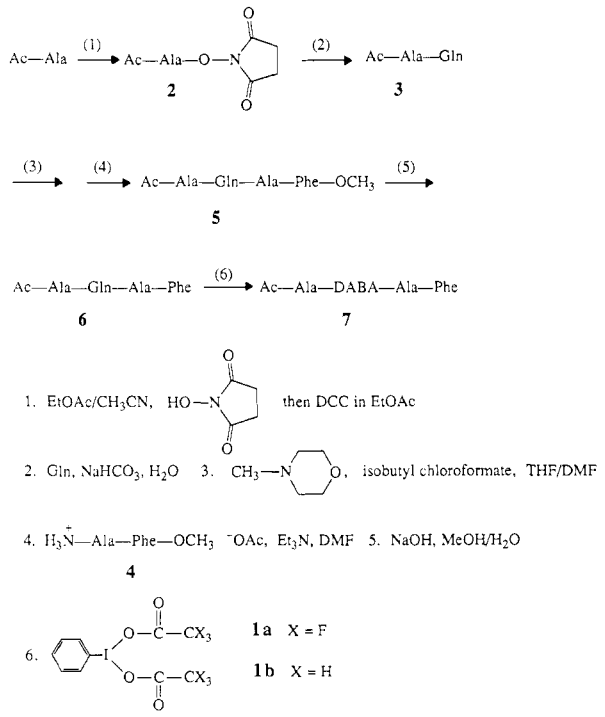
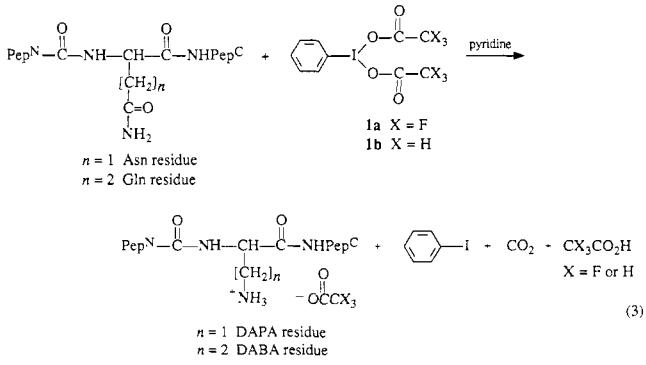
Scheme I. Preparation of Ac-Ala-DABA-Ala-Phe



this paper, we shall refer to this type of process as *transpeptidation*. Transpeptidation has also been observed in model peptides.[5]

Although both direct cleavage (eq 1) and transpeptidation (eq 2) have been observed experimentally, the cases in which these reactions were examined were designed to be favorable to the particular process under study. In no case have transpeptidation and direct cleavage been directly and systematically pitted against each other in peptides. This work was undertaken to determine which process (if any) is favored, and whether the resulting chemistry can be used in a specific peptide cleavage. Furthermore, although 2,3-diaminopropanoic acid (DAPA) residues presumably cannot undergo direct cleavage analogous to eq 1, it was also of interest to determine whether these residues can undergo transpeptidation reactions.

The study of these questions is particularly accessible since this laboratory has demonstrated previously[7] that DABA and DAPA residues in peptides can be formed quantitatively in an "acidic Hofmann rearrangement" from glutamine and asparagine residues, respectively, using *I,I*-bis(trifluoroacetoxy)iodobenzene (**1a**) or (diacetoxyiodo)benzene (**1b**) (eq 3).



Results and Discussion

Synthesis and Modification of Peptides. A number of N-acylated tetrapeptides were prepared by solution-phase methods for use in this study; a typical preparation is shown in Scheme I. Complete experimental details for the preparation of all peptides are available.[8] In all peptides, L-amino acids were used. The final rearrangement of Gln to DABA or Asn to DAPA was effected with either reagent **1a** or (diacetoxyiodo)benzene (**1b**). During the course of this study we and others[9] found the diacetoxy reagent **1b** superior to the bis(trifluoroacetoxy) reagent **1a** for peptide modification, although the latter is the better reagent for ordinary amides.[10]

The possible fates of a typical DABA-containing peptide, Ac-Ala-DABA-Ile-Gly, are shown in Scheme II. Two possible products, Ile-Gly (**8**) (from direct cleavage; eq 1) and Ac-Ala-isoDABA-Ile-Gly (**9**) (from transpeptidation; eq 2), as well as the starting peptide, can be directly observed and quantitated on the amino acid analyzer without prior hydrolysis. When treated with 0.25 M $NaHCO_3$ buffer at pH 9.74 (60 °C), this peptide gave the results shown in Figure 1. As Figure 1 shows, the transpeptidation reaction (eq 2) is the predominant process observed. The identification of the product was corroborated in two ways. First, it was dinitrophenylated with 1-fluoro-2,4-dinitro-

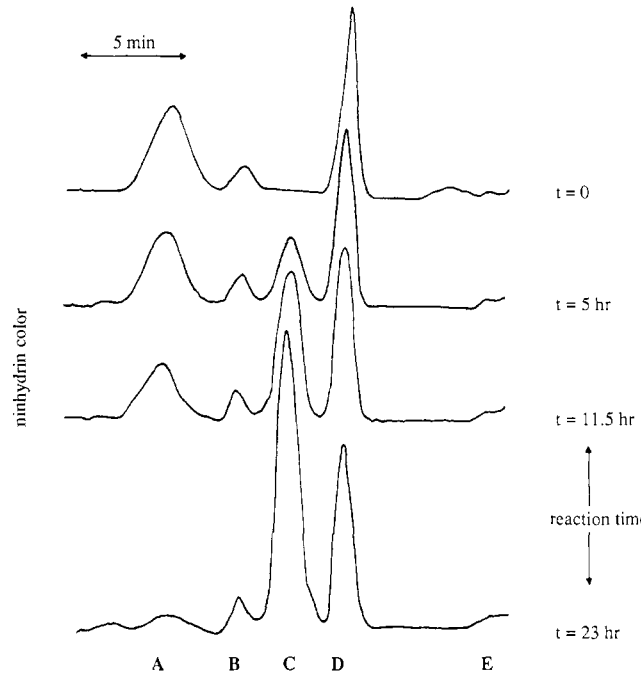
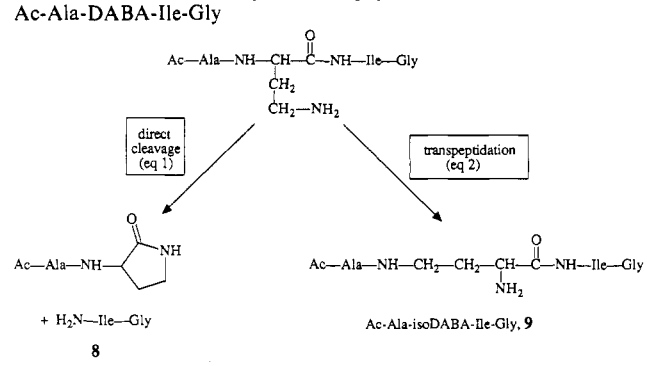


Figure 1. Time course of the amino acid analyzer trace in the incubation of Ac-Ala-DABA-Ile-Gly in 0.25 M $NaHCO_3$ buffer at pH 9.7, 60 °C. (A) Position of the starting peptide; (B) buffer-change artifact; (C) transpeptidation product Ac-Ala-isoDABA-Ile-Gly (**9**) (see Scheme II); (D) norleucine internal standard; (E) position of Ile-Gly (**8**) (see Scheme II).

Scheme II. Direct Cleavage vs Transpeptidation of Ac-Ala-DABA-Ile-Gly



(7) (a) Loudon, G. M.; Radhakrishna, A. S.; Almond, M. R.; Blodgett, J. K.; Boutin, R. H. *J. Org. Chem.* **1984**, *49*, 4272–4276. (b) Boutin, R. H.; Loudon, G. M. *J. Org. Chem.* **1984**, *49*, 4277–4284.

(8) Blodgett, J. K. Ph.D. Dissertation, Purdue University, 1986, pp 123–395.

(9) Holt, L. A.; Milligan, B. *Aust. J. Biol. Sci.* **1981**, *34*, 395–400.

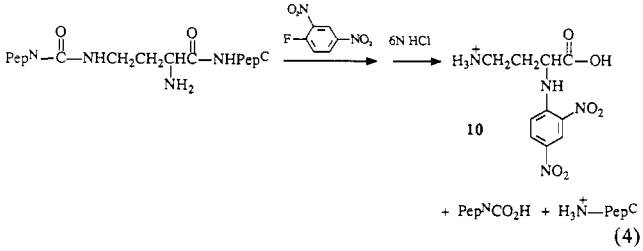
(10) Stimmel, J. B.; Blodgett, J. K.; Loudon, G. M., unpublished results.

Table I. Direct Cleavage as a Function of Peptide Structure

peptide	% liberation of carboxy-terminal dipeptide[a]		
	X = DABA	X = DAPA	X = Gln
Ac-Ala-X-Gly-Gly	14	3	15
Ac-Ala-X-Ala-Gly	10	0	8
Ac-Ala-X-Ile-Gly	8	0	0
Ac-Ala-X-Ala-Phe	10	0	5
Ac-Ala-X-Phe-Gly	7	0	3
Ac-Ala-X-Val-Gly	0	0	0
Ac-Ala-X-$NHCH_3$	44		

[a] 0.25 M bicarbonate buffer, pH 9.7, 60 °C, 4 days. The material not accounted for in the first two columns is either transpeptidation product (eq 2) or starting peptide.

benzene (Sanger reagent) and hydrolyzed to yield N^2-(2,4-dinitrophenyl)-2,4-diaminobutanoic acid (**10**) (eq 4).[11] Second,



(4)

the putative transpeptidation product **9** (Scheme II) was prepared by direct synthesis, and the product of the transpeptidation reaction was shown to be identical in all respects with compound **9**.

Several peptides were allowed to react at pH 9.7 ± 0.1, 0.25 M $NaHCO_3$ buffer, at 60 °C for 4 days. (This was determined to be greater than 10 half-lives for the transpeptidation reaction.) The peptide mixtures were examined directly for the presence of the dipeptide product **8** (Scheme II). The results of these experiments are given in Table I. The reaction mixtures showed excellent mass balance, with dipeptide, starting peptide, and transpeptidized peptide accounting for all of the material initially present. The data in Table I show that little direct cleavage occurs, and virtually none in excess of the control in which DABA is replaced by Gln. The small amount that does occur when X is either DABA or Gln is probably attributable to nucleophilic participation of either residue. That no direct cleavage is observed when X = DAPA indicates that little or no nonspecific peptide cleavage occurs under these conditions.

It seems clear that the transpeptidation reaction (eq 2) is a considerably faster reaction than direct cleavage (eq 1) in the cases studied. In order to elucidate the reasons for this observation, the transpeptidation reaction was studied more thoroughly.

Equilibrium in Transpeptidation. If we allow DABA- and DAPA-containing peptides to undergo transpeptidation, we find that the transpeptidation reaction comes to equilibrium. Indeed, this equilibrium can be approached from either direction. Figure 2, for example, shows the transpeptidation of both Ac-Ala-DAPA-Ile-Gly and Ac-Ala-isoDAPA-Ile-Gly as a function of time. Both progress curves are pseudo first order and take place with the same rate. The two curves approach, within experimental error, the same final value of percent transpeptidation, 82 ± 2%. Other DAPA-containing peptides approach the same degree of transpeptidation at equilibrium and show no significant variation of this quantity as a function of pH over the range 7.8–10. Corresponding peptides containing DABA and isoDABA residues show a small discrepancy (about 5%) in the apparent position of equilibrium, with the final values in the range 74–79%. The lack of perfect agreement is undoubtedly due to the small amount of direct cleavage that takes place (eq 1 and Table I).

It is interesting to compare the extent of transpeptidation with that predicted from other aminolysis reactions in the literature. Fresht and Requena[12] found that the equilibrium constants K_{eq}

(11) Biserte, G.; Holleman, J. W.; Holleman-Dehose, J.; Sautiere, P. *J. Chromatogr.* **1959**, *2*, 225–271.

(12) Fersht, A. R.; Requena, Y. *J. Am. Chem. Soc.* **1971**, *93*, 3499–3504.

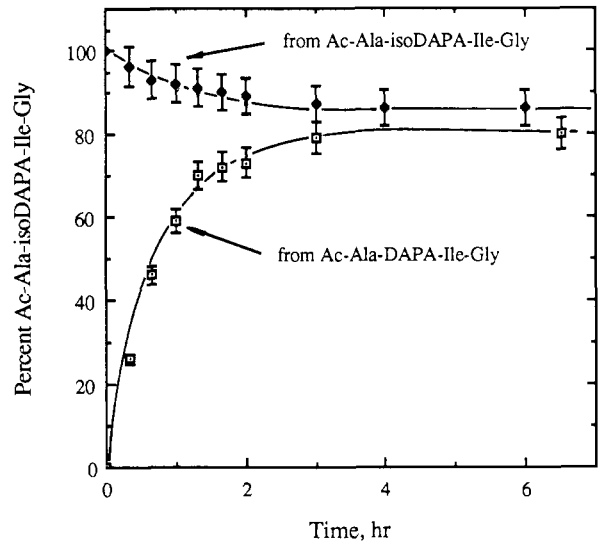


Figure 2. Approach to transpeptidation equilibrium from both Ac-Ala-DAPA-Ile-Gly (open symbols) and its transpeptidation product Ac-Ala-isoDAPA-Ile-Gly (closed symbols). Conditions: 60 °C; pH 7.8; 0.25 M sodium phosphate buffer. The lines in both curves are for a pseudo-first-order rate constant of 1.34 ± 0.11 h^{-1}, which was obtained by a nonlinear least-squares fit of the data described by the open symbols.

Table II. Conjugate-Acid pK_a Values of Amino Groups in DABA- and DAPA-Containing Tetrapeptides (26 ± 1 °C, Water)

X	pK_a of ammonium group in	
	Ac-Ala-X-Ile-Gly	Ac-Ala-isoX-Ile-Gly
DAPA	8.0	6.7
DABA	9.6	7.4

for aminolysis of formamide by amines of different conjugate-acid pK_a values (25 °C, 1 M ionic strength) were correlated by eq 5.

$$\log K_{eq} = 0.51pK_a \quad (5)$$

In order to relate our data to this work, we determined the pK_a values of the side-chain conjugate acids of the peptides Ac-Ala-X-Ile-Gly by titration (water). The values obtained are given in Table II. The most meaningful data for comparison are the results for DAPA- and isoDAPA-containing peptides at pH 10, since these results are uncomplicated by protonation of the relevant amino groups. The observed extent of transpeptidation, 82%, is in excellent agreement with that predicted by eq 2. Since our transpeptidation reactions were carried out at a different temperature and ionic strength from those in the literature, this comparison has only semiquantitative significance. Nevertheless, our data show that equilibria in *intramolecular* aminolysis of peptide amides and *intermolecular* aminolysis of formamides respond in about the same way to the conjugate-acid pK_a of amines.

Buffer Catalysis. One of the most significant observations is that the transpeptidation rates of DABA- and DAPA-containing peptides are strongly dependent on the nature of the buffer. This point is illustrated dramatically in Figure 3, in which the transpeptidation of Ac-Ala-DABA-Ile-Gly is compared in bicarbonate and triethylammonium acetate buffers at the same pH, temperature, and ionic strength. For certain peptides, an ammonium acetate buffer was also used, and the transpeptidation rates in the presence of this buffer were very similar to the rates in the presence of the tertiary amine buffers. This shows that the rates in the presence of tertiary amine buffers are not reduced significantly by some sort of steric effect in the buffer. The accelerating effect of bicarbonate buffers was observed for all DABA- and DAPA-containing peptides studied. Phosphate buffer also shows a qualitatively similar rate acceleration over amine buffers. To characterize further this buffer effect, the transpeptidation of Ac-Ala-DAPA-Ile-Gly was studied over a range of phosphate concentrations from 0.05 to 0.5 M (ionic strength

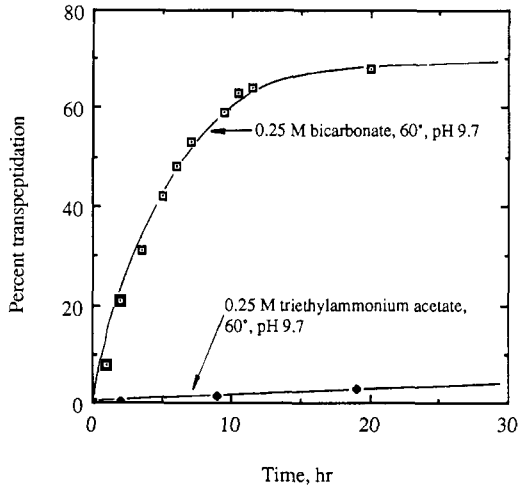


Figure 3. Comparison of the transpeptidation reaction of Ac-Ala-DABA-Ile-Gly at 60 °C and pH 9.7 in the presence of 0.25 M bicarbonate (open symbols) and 0.25 M triethylammonium acetate (closed symbols). The curves are drawn to indicate trends.

1.4, 60 °C, pH 7.75 ± 0.04). The pseudo-first-order rate constant k for transpeptidation was found to follow eq 6, where $[P_i]$ = total

$$k\ (h^{-1}) = (0.2 \pm 0.8) + (6.6 \pm 1.1)[P_i] \qquad (6)$$

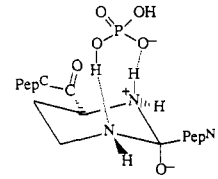
phosphate. Notice that the buffer-independent rate is zero within experimental error. There was no evidence of curvature in this buffer plot.

These observations show that transpeptidation is most strongly catalyzed by buffers that are capable of *bifunctional catalysis*. Such buffers are those that can both accept and donate a proton in a concerted fashion.[13] We shall return subsequently to the mechanistic significance of this catalysis.

Effect of pH. The transpeptidation reaction of DABA- and DAPA-containing peptides is strongly influenced by pH. The effect of pH is, of course, superimposed on the effect of buffer. Since the transpeptidation rate is immeasurably small in the absence of buffer, it was not possible to measure pH-independent transpeptidation rates across a useful pH range. However, the qualitative effect of pH can be seen by a comparison of the progress curves for transpeptidation of Ac-Ala-DABA-Ile-Gly and Ac-Ala-DAPA-Ile-Gly at pH values of about 10, 8, and 3 (Figure 4). The DABA-containing peptide undergoes transpeptidation at a significantly smaller rate at pH 8 (phosphate buffer) than at pH 10 (bicarbonate buffer). This observation is consistent with the requirement that the side-chain amino group be in its unprotonated (neutral) form. The pK_a of the side-chain ammonium group of protonated DABA residues is about 9.6 (Table II). On the other hand, that of protonated DAPA residues is 8.0. Thus, the fraction of unprotonated form of a DABA residue changes much more extensively over the range pH 8 to pH 10 than that of a DAPA residue. The transpeptidation rate at pH 3 for both types of peptides in the presence of citrate (a bifunctional buffer) is negligible.

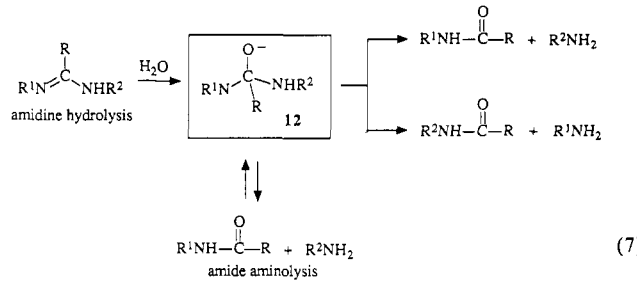
Comparison of DABA and DAPA Residues. The intrinsic tendencies toward transpeptidation of DAPA- vs DABA-containing peptides differ very little, as shown by the progress curves for transpeptidation of Ac-Ala-X-Ile-Gly (X = DABA or DAPA) at pH 9.7 (Figure 5A). The small differences that do exist are probably attributable to the greater amount of neutral side chain in the DAPA case (see Effect of pH). However, the effect of pH is such that at pH 7.8 a DAPA-containing peptide transpeptidizes much more rapidly than its DABA-containing analogue (Figure 5B). Thus, for a given neighboring sequence, there is a significant degree of selectivity for transpeptidation of DAPA-containing peptides over their DABA-containing analogues at pH 7.8.

Analysis of the Preference for Transpeptidation over Direct Cleavage. The observation that transpeptidation is strongly catalyzed by buffers capable of bifunctional catalysis, described above, suggests, by analogy with similar observations on other acyl-transfer reactions,[13] that the rate-determining step in the transpeptidation reaction is a diffusion-limited proton-transfer reaction either prior to or concurrent with breakdown of the tetrahedral intermediate. The transition state can thus be depicted by structure **11**. Cox and Jencks[13a] have shown that an eight-



11

membered ring (including the donated and accepted protons) provides the optimal atomic arrangement for such catalysis. Further evidence on the nature of the rate-limiting step comes from the hydrolysis of amidines. Amidine hydrolysis and amide aminolysis share a common intermediate, **12** (± proton transfers):



(7)

Perrin and coworkers[14] have shown that breakdown of this intermediate results in expulsion of the more basic amine component. The implication of these results for the present work is that the more basic amine (the side-chain amino group) is lost preferentially from the tetrahedral intermediate in either transpeptidation or direct cleavage. This implies, by microscopic reversibility, that attack of the amino group occurs prior to the rate-determining step in either pathway. Hence, we adopt as our model for the transition state the tetrahedral intermediate (plus one molecule of buffer catalyst). We discern three possible reasons for the predominance of transpeptidation.

(1) To the extent that breakdown of the tetrahedral intermediate has progressed toward final product, the transition state in the case of direct cleavage resembles an amide with the *E* configuration (i.e., a lactam); transpeptidation, on the other hand, affords an amide with the *Z* configuration. The *Z* configuration is generally the more stable configuration.

(2) The preference for transpeptidation may result from greater ring strain in the tetrahedral intermediate for direct cleavage, a five-membered ring, than in the tetrahedral intermediate for transpeptidation, a six-membered ring.[15] As a crude model, we can estimate that the heat of formation of a pyrrolidine ring is 5.96 kcal/mol greater than that of a *hypothetical* pyrrolidine with the same ring strain as a homologous piperidine.[16] Of course, the entropic loss associated with formation of a larger ring can offset up to 3 kcal/mol of this ring strain, so that the net free-energy cost for formation of a five-membered vs a six-membered ring at 60 °C is about 2.7 kcal/mol, which translates to a factor of about 60 in rate. Table I shows that this is about what is

(13) (a) Cox, M. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1981**, *103*, 580–587. (b) Lee, Y.-N.; Schmir, G. L. *J. Am. Chem. Soc.* **1979**, *101*, 6277–6282. (c) Cunningham, B. A.; Schmir, G. L. *J. Am. Chem. Soc.* **1967**, *89*, 917–922. (d) Pratt, R. F.; Surb, Y. S.; Shaskus, J. J. *J. Am. Chem. Soc.* **1983**, *105*, 1006–1014. (e) Indelicato, J. M.; Fisher, J. W.; Pasini, C. E. *J. Pharm. Sci.* **1986**, *75*, 304–306.

(14) Perrin, C. L.; Nunez, O. *J. Am. Chem. Soc.* **1987**, *109*, 522–527.

(15) We thank Professor Rich Gandour of Lousiana State University for suggesting this explanation.

(16) Cox, J. D.; Pilcher, G. *Thermochemistry of Organic and Organometallic Compounds*; Academic Press: New York, 1970; p 574.

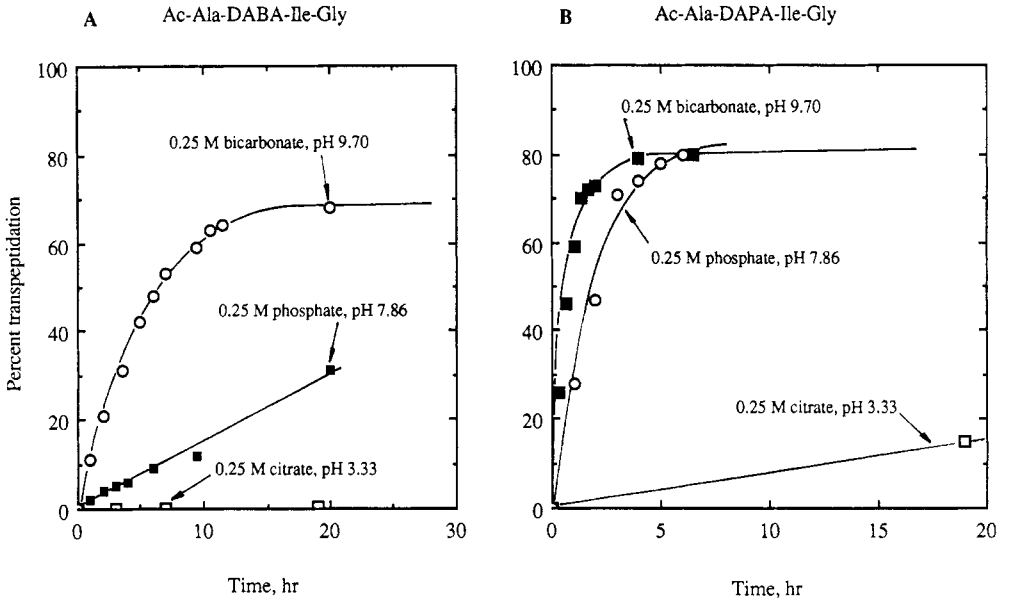


Figure 4. Effect of pH and buffer on the transpeptidation of Ac-Ala-DABA-Ile-Gly (panel A) and Ac-Ala-DAPA-Ile-Gly (panel B) at 60 °C. The curves are drawn to indicate trends.

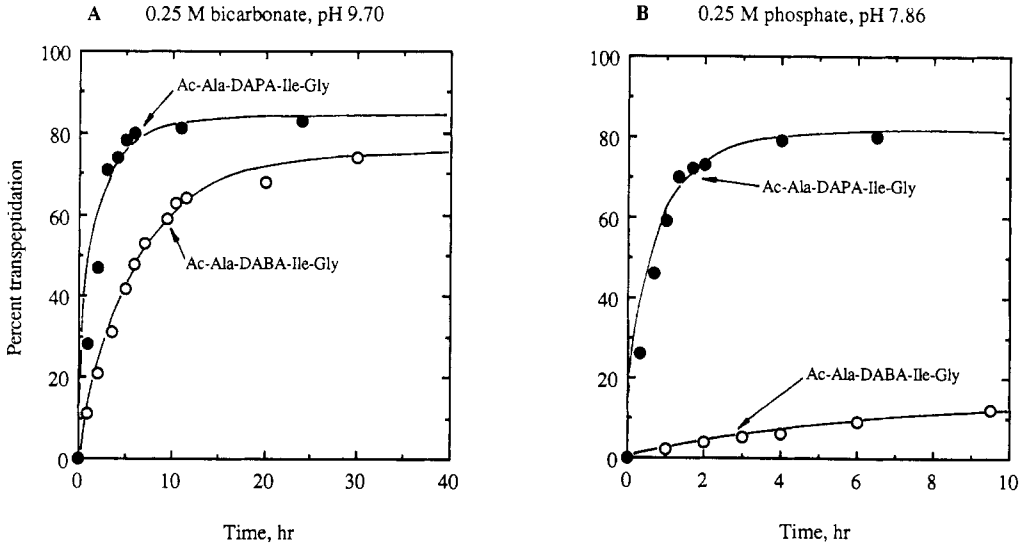
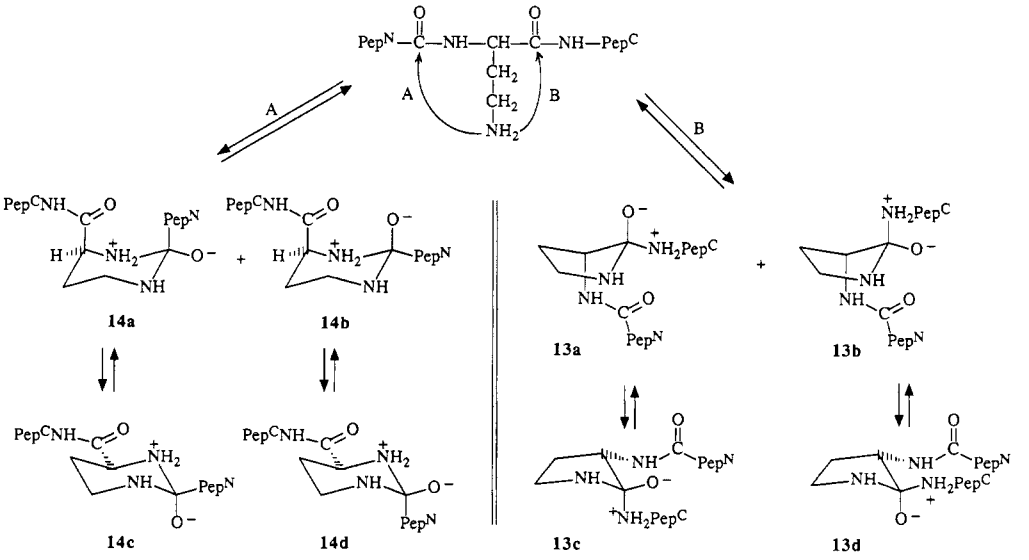


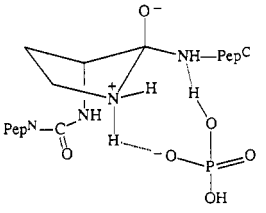
Figure 5. Comparison of the time course of transpeptidation of Ac-Ala-DAPA-Ile-Gly (closed symbols) and Ac-Ala-DABA-Ile-Gly (open symbols) at 60 °C and in the presence of 0.25 M bicarbonate buffer (panel A) and 0.25 M phosphate buffer (panel B). The curves are drawn to indicate trends.

Scheme III



observed; 0–10% of direct cleavage product is formed.

(3) The preference for transpeptidation may also be explained by a steric effect, as follows. Suppose the system is able to explore rapidly all possible tetrahedral intermediates, as shown in Scheme III. Let us make the crude assumption that gauche and/or 1,3-diaxial interactions in five-membered rings can be analyzed in the same way that they can in six-membered rings. Three of the tetrahedral intermediates for direct cleavage (species **13a**, **13c**, and **13d**) have gauche interactions between the two largest groups on the ring. The remaining species **13b** places both large groups in axial positions and therefore should have particularly unfavorable 1,3-diaxial interactions of these groups with ring hydrogens. To the extent that the tetrahedral intermediates resemble their respective transition states, these unfavorable interactions should retard the decomposition reactions by destabilizing the transition states. These nonbonded interactions may well be further aggravated by the presence of a buffer catalyst molecule bridging between the attacking and leaving nitrogen, as in **15**. On



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the other hand, there is one tetrahedral intermediate for transpeptidation (**14c**, Scheme III) in which the two largest groups are not involved in either 1,3-diaxial interactions or gauche interactions with each other. This intermediate should lead to the aminolysis transition state of lowest energy and, hence, to transpeptidation as the major reaction pathway.

It follows from this analysis that if a sterically less demanding leaving group is incorporated in the Pep^C position (eq 1 or Scheme III), a greater percentage of direct cleavage should be observed. That is, reaction pathways involving transition states resembling **13c** should become more important. In the first three entries of Table I, the proportion of direct cleavage is in the order Pep^C = Ile < Ala < Gly, which is in the same order as the steric requirements of the Pep^C side chain. The smallest Pep^C group in Table I is the *N*-methylamide group shown in the last entry; in this case, 44% direct cleavage (to liberate methylamine) is observed. The large amount of direct cleavage in this case could also be due to the greater basicity of methylamine; more basic amines tend to be expelled from tetrahedral intermediates,[14] a point discussed previously.

We also considered a stereoelectronic explanation for the results. Tetrahedral intermediate **14c** at first sight is the only intermediate in Scheme III that *both* lacks the unfavorable steric interactions described above *and* could achieve the antiperiplanar relationship of unshared pairs and leaving group required by stereoelectronic theory.[17] However, if we consider that the role of the buffer is to transfer a proton between the attacking and leaving nitrogen in **14c** as shown in Figure 6, then after removal of a proton from N^γ, the unshared pair thus liberated on this nitrogen is not antiperiplanar to the leaving group (N^α). An inversion at N^γ would be required to effect the alignment of this orbital antiperiplanar to the leaving nitrogen N^α. Such an inversion will likely have a standard free energy of activation of at least 6 kcal/mol,[18] much higher than the activation energy for diffusion away of the buffer. Hence, diffusional separation of the buffer molecule would occur prior to the inversion, the inversion itself would be rate-limiting, and transpeptidation would not show buffer catalysis, a consequence not in accord with the observations. Furthermore, Perrin and Nunez[14] have shown that stereoelectronic effects make a negligible contribution in the breakdown of tetrahedral intermediates in the hydrolysis reactions of amidines, the tetrahedral intermediates of which are formally similar to the ones involved in this work. Further difficulties with stereoelectronic theory have been discussed by Sinnott.[19]

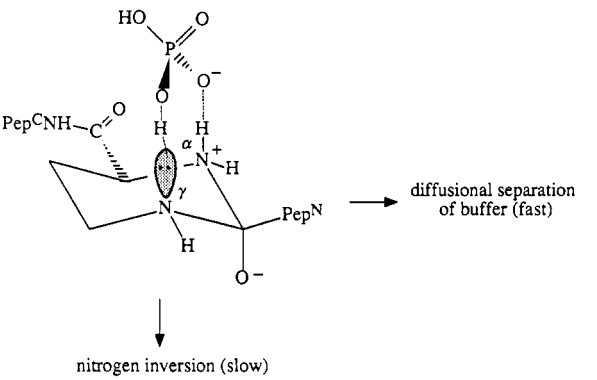


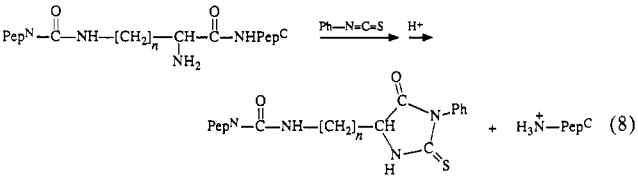
Figure 6. Tetrahedral intermediate **13d** (Scheme III) immediately following buffer-catalyzed proton transfer. The attacking nitrogen N^γ must undergo inversion before its electron pair can be oriented antiperiplanar to the leaving nitrogen N^α.

Table III. Edman Cleavage of Peptides at DABA and DAPA Residues following Transpeptidation at 60 °C[a]

peptide	% yield X = DABA	% yield X = DAPA
Ac-Ala-X-Gly-Gly	62	57
Ac-Ala-X-Ala-Gly	77	65
Ac-Ala-X-Ile-Gly	71	63
Ac-Ala-X-Ala-Phe	74	69
Ac-Ala-X-Phe-Gly	69	62
Ac-Ala-X-Val-Gly	77	65

[a] Prior to cleavage, transpeptidation was carried out for 38 h at 60 °C in 0.25 M sodium bicarbonate buffer, pH 9.7 ± 0.1.

Specific Cleavage of DABA- and DAPA-Containing Peptides. Although direct cleavage at DABA residues is not observed, it is possible to effect a specific cleavage of peptides at transpeptidized DABA and DAPA residues. As shown in eq 2, transpeptidation of a DABA- or DAPA-containing peptide gives a peptide with the corresponding isoDABA or isoDAPA residue. Such residues, because they contain α-amino groups, provide additional amino termini within the rearranged peptide. Since phenyl isothiocyanate (the Edman reagent) is the reagent of choice for cleaving peptides at their amino-terminal residues, it should in principle be possible to use this reagent to cleave any peptide at isoDABA and isoDAPA sites, as shown in eq 8. Accordingly,



Ac-Ala-isoDABA-Ile-Gly was treated with the Edman reagent followed by treatment with trifluoroacetic acid at room temperature. A 94% yield of the cleavage peptide Ile-Gly was formed. An analogous experiment on Ac-Ala-isoDAPA-Ile-Gly gave Ile-Gly in 83% yield. The manual Edman degradation is reported to occur in 60–80% yield when it is used to release amino-terminal residues in conventional peptides.[20] Unrearranged Asn- and Gln-containing peptides did not undergo this cleavage reaction, nor did rearranged DABA-containing peptides that had not been subjected to transpeptidation. DAPA-containing peptides that had not been subjected to transpeptidation did give 2–8% cleavage under these conditions. (See eq 9 and associated discussion for an explanation.) A number of other tetrapeptides were subjected

(17) Deslongchamps, P. *Stereoelectronic Effects in Organic Chemistry*; Pergamon: New York, 1983; Vol. 1, pp 101–162.
(18) Anet, F. A. L.; Yvari, I. *J. Am. Chem. Soc.* **1977**, *99*, 2794–2795.
(19) Sinott, M. L. *Adv. Phys. Org. Chem.* **1988**, *24*, 113–204.
(20) Konigsberg, W.; Hill, R. J. *J. Biol. Chem.* **1962**, *237*, 2547–2561.

Table IV. pH and Sequence Selectivity of Transpeptidation and Edman Cleavage

peptide	% yield			
	pH 10, 24 h		pH 8, 5 h	
	transpeptidation[a]	cleavage	transpeptidation[a]	cleavage
Ac-Ala-DABA-Ile-Gly	72	60	14[b]	13
Ac-Ala-DAPA-Ile-Gly	80	62	80	69
Ac-Val-DABA-Ile-Gly	6	5	0	0
Ac-Val-DAPA-Ile-Gly	42[c]	35	34	28
Ac-Gly-DABA-Ile-Gly	85	74	45	42
Ac-Gly-DAPA-Ile-Gly	89[c]	74	80	76

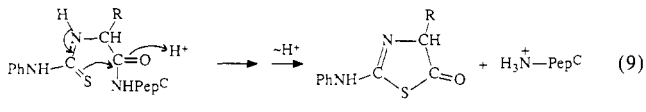
[a] The trnaspeptidation reaction was carried out at 60 °C at the indicated pH and time. [b] Inferred assuming 83% yield in the Edman cleavage as determined for Ac-Ala-isoDAPA-Ile-Gly. [c] Inferred assuming 94% yield in the Edman cleavage as determined for Ac-Ala-isoDAPA-Ile-Gly.

to transpeptidation at pH 9.74 (0.25 M bicarbonate buffer, 60 °C) for 38 h, followed by Edman degradation. The results are shown in Table III. The somewhat lower cleavage yield in the DAPA-containing peptides is consistent with the lower yield in the control peptides; the reason for this lower yield is not clear at this time. The data in this table are significant in two ways. First, they show that transpeptidation and cleavage of DABA- and DAPA-containing residues can be used for specific peptide cleavage. Since DAPA and DABA residues can be formed from Asn and Gln residues, respectively (eq 3), these data show that a specific peptide cleavage at Asn and Gln residues is in principle possible. Second, transpeptidation followed by Edman cleavage can be used to estimate transpeptidation yields. (Observing the release of a dipeptide by amino acid analysis is more convenient than following the progress of transpeptidation.)

Sequence Selectivity of Transpeptidation. The sequence selectivity of transpeptidation was studied by allowing DABA- and DAPA-containing peptides to undergo transpeptidation under optimum conditions and then subjecting the resulting peptides to Edman cleavage. DABA-containing peptides were allowed to transpeptidize at pH 9.7 (0.25 M bicarbonate buffer) and 60 °C for 24 h and DAPA-containing peptides at pH 7.8 (0.25 M phosphate buffer) and 60 °C for 5 h. After Edman cleavage, the results shown in Table IV were obtained. The data in this table are probably accurate to within ±10%. As a control, the transpeptidation and cleavage of a lysine-containing peptide, Ac-Ala-Lys-Ala-Gly, was examined. No Ala-Gly was liberated from this peptide.

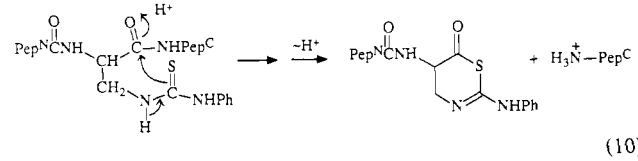
The data in Table IV indicate that transpeptidation is retarded by residues containing sterically demanding side chains. Thus, transpeptidation has the highest yield when the amino-terminal residue is glycine, whereas the yield is significantly reduced when the amino-terminal residue is valine. Independent studies in which the progress curves for transpeptidation were determined by direct observation of the transpeptidized product showed that the peptides containing both Gly and Ala in amino-terminal positions had come to transpeptidation equilibrium at pH 10 prior to the sampling time but that transpeptidation was still proceeding in the peptide containing amino-terminal valine. Hence, the effect of the valine is a kinetic effect, not an effect on the position of equilibrium. The data in Table IV show that there is some sequence selectivity, and the data also illustrate the more rapid transpeptidation of DAPA-containing peptides at pH 7.8. Unfortunately, the selectivity is not great enough to be of obvious practical value.

Direct Cleavage at DAPA Residues with the Edman Reagent. After an α-amino group reacts with the Edman reagent, peptide cleavage occurs by cyclization of the resulting thiourea adduct through formation of a five-membered ring (eq 9). It occurred



(9)

to us that cyclization through a six-membered ring might also occur and that such a cyclization might be useful in the cleavage of peptides at *untranspeptidized* DAPA residues (eq 10). Indeed,



(10)

the 2–8% cleavage yield of untranspeptidized DAPA-containing peptides in the Edman degradation noted previously indicates that such a process might be occurring and that more vigorous conditions might increase the yield. Accordingly, a number of DAPA-containing tetrapeptides were allowed to react with phenyl isothiocyanate, and the resulting adducts were treated with heptafluorobutyric acid at 60 °C for varying times. The cyclization reaction was monitored by following liberation of the C-terminal dipeptide. Optimization of the reaction showed that the highest cleavage yields occurred at a cyclization time of 10–12 h. These conditions are considerably more vigorous than those used in the usual Edman cyclization; see above. The results of these experiments are shown in Table V. It should be noticed that the identity of the residue at the *amino* side of the DAPA residue does not affect the yield (compare the third and seventh entries), although the yield does appear to be somewhat sensitive to the degree of hindrance at the carboxy side. As a control, several analogous DABA-containing peptides were subjected to the same cleavage conditions, and no cleavage was observed. It is also known that cleavage at Lys residues by a similar mechanism does not occur during the Edman degradation.[21]

Table V. Direct Edman Cleavage of DAPA-Containing Peptides[a]

peptide	% yield of C-terminal dipeptide
Ac-Ala-DAPA-Gly-Gly	72
Ac-Ala-DAPA-Ala-Gly	60
Ac-Ala-DAPA-Ile-Gly	52
Ac-Ala-DAPA-Ala-Phe	59
Ac-Ala-DAPA-Phe-Gly	60
Ac-Ala-DAPA-Val-Gly	51
Ac-Val-DAPA-Ile-Gly	59

[a] Each peptide was allowed to react with the Edman reagent and then treated with heptafluorobutyric acid at 60 °C for 10 h (see Experimental Section).

These data show that conversion of Asn residues to DAPA residues with (diacetoxyiodo)benzene, followed by treatment with the Edman reagent and cyclization, offers potential for use as a specific cleavage method for peptides at Asn residues. Efforts designed to increase both the yield and selectivity of these peptide modifications are in progress. Although Gln residues are converted into DABA residues by (diacetoxyiodo)benzene, we have shown that the resulting DABA residues do not undergo cleavage with the Edman reagent.

Significance of the Transpeptidation Reaction. The transpeptidation reaction studied in this paper has biological significance. Certain antibiotics contain DABA residues. It has been shown that one such antibiotic, polymixin M, loses its antibiotic activity when incubated at pH 10 (aqueous ammonia, 37 °C) for 3–5 days.[22] This result can be accounted for, at least in part, by the transpeptidation reaction studied here.[11] Our results suggest that incubation in phosphate or bicarbonate buffer would result in greater inactivation rates, even at lower pH values.

Of further significance is the fact that a number of β-lactam antibiotics are destroyed, and thus inactivated, by intramolecular aminolysis reactions very similar to the transpeptidation reaction reported here. These reactions are known to be strongly catalyzed by phosphate,[13e] evidently by a mechanism analogous to that reported here.

(21) Konigsberg, W. *Methods Enzymol.* **1967**, *11*, 76–93.
(22) (a) Katrukha, G. S.; Baratova, L. A.; Silaev, A. B.; Rudinger, J. *Experientia* **1968**, *24*, 540–541. (b) Silaev, A. B.; Baratova, L. A.; Katrukha, G. S. *J. Chromatogr.* **1966**, *24*, 61–67.

These results may have some bearing on the evolution of lysine as the amine-containing amino acid residue. Lysine would not appear to offer a significant difference in basicity over a DABA residue. Although we can never definitively answer why lysine did evolve, we are in a position to suggest why DABA and DAPA residues did not. Had proteins containing DABA and DAPA residues ever evolved, they would probably have been rapidly transformed under the influence of the ubiquitous phosphate and bicarbonate buffers into complex mixtures containing isoDABA and isoDAPA residues. Because of their longer carbon side chains, lysine residues do not undergo this reaction and are thus stable toward transpeptidation under physiological conditions.[25]

Experimental Section

Reagents, peptides, and amino acid derivatives were purchased from Sigma, Aldrich, Vega Biochemicals, Nutri Biochemicals, Mann Research Biochemicals, or Pierce Chemical Co. Amino acid analyses were performed on a Beckman 119CL automated amino acid analyzer [ninhydrin detection; column 1, 6 × 195 mm, or column 2, 6 × 220 mm (both sulfonated polystyrene)] and are corrected to an internal standard. Retention times of unhydrolyzed peptides (RT_{AAA}), expressed in minutes, represent the time required for elution of a peptide (measured from the point of injection of the peptide sample) during a normal 92-min run. Proton nuclear magnetic resonance (NMR) spectra were recorded on a Varian Associates EM-360 (60 MHz), FT-80 (80 MHz), or FT-200 (200 MHz) spectrometer in the indicated solvents. Chemical shift values are relative to either added tetramethylsilane (TMS), the central peak in the $CH_1D_2SOCD_3$ (dimethyl sulfoxide, DMSO) pentuplet (residual proton signal, δ 2.50), or the central peak in the CH_1D_2COOD (acetic acid, AcOH) pentuplet (residual proton signal, δ 2.03) as the internal standard. All peak listings are expressed as ppm downfield from TMS. Integrations are reported relative to the resonance indicated by the single asterisk (*).

All gravity-flow column chromatography (ion exchange and gel filtration) employed glass columns. The anion-exchange resin DEAE-Sephadex was always used in the acetate (AcO^-) form. Thin-layer chromatography (TLC) was performed on Merck (no. 5775) silica gel plates. Titrations and pH-stat recordings were made on a Metrohm CombiTitrator E512/E473/E425 instrument. Optical rotations were taken on a Perkin-Elmer Model 241 polarimeter (1 mL, 1.0002 dm, jacketed cell). Melting points were taken on a Buchi (oil immersion) melting point apparatus and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory of the Purdue University Chemistry Department.

Abbreviations. Abbreviations used in this section are as follows: AAA, amino acid analysis; BAW, 1-butanol–acetic acid–water; Boc, *tert*-butoxycarbonyl; DABA, 2,4-diaminobutanoyl residue acylated on N^2 or 2,4-diaminobutanoic acid; DAPA, 2,3-diaminopropanoyl residue acylated on N^2 or 2,3-diaminopropanoic acid; DMF, *N*,*N*-dimethylformamide; Et_2O, diethyl ether; HFBA, 2,2,3,3,4,4,4-heptafluorobutanoic acid; norLeu, norleucine; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonate); TEA, triethylamine; TFA, trifluoroacetic acid; TLC, thin-layer chromatography.

Peptide Synthesis. Standard solution procedures were used for peptide synthesis, as shown in Scheme II. Because the synthesis of DABA- and DAPA-containing peptides is somewhat unusual, one example is described below. Complete synthetic procedures of all peptides used in this study are fully described elsewhere.[8]

***N*²-(*N*-Ac-L-Ala)-L-2,4-diaminobutanoyl-L-Ala-L-Phe (7).** The Gln-containing tetrapeptides prepared for this study were converted into their respective DABA-containing tetrapeptides by rearrangement of the carboxamide side chain of Gln with either compound **1a** or (diacetoxyiodo)benzene (**1b**).[23] A typical synthetic procedure is as follows. The tetrapeptide to be rearranged (1 equiv) was added to a stirred solution of compound **1a** (1.5 equiv) in DMF/H_2O (1:1 v/v, 7.5–10.0 mL of total solvent/mmol of peptide) at room temperature. (Compound **1a** is insoluble in H_2O and therefore requires initial dissolution in DMF. Subsequent slow addition of the required volume of H_2O does not precipitate the dissolved reagent.) After 15 min of reaction, pyridine (2 equiv) was added and stirring continued overnight at room temperature. The solvents were then removed in vacuo, and the resulting residue was dissolved in 5 mL of H_2O. The resulting turbid aqueous solution was extracted exhaustively with Et_2O to remove the iodobenzene byproduct and then evaporated briefly to remove residual Et_2O. The rearrangement product was then purified further as follows. The Et_2O-extracted aqueous reaction solution was basified with pyridine (10 equiv) and loaded onto a 2 × 26 cm DEAE-Sephadex column that had been washed with H_2O. The column was eluted with 0.72 L of H_2O, and fractions (80 × 9 mL) were collected. The rearrangement product was then located by TLC of column fractions using fluorescence quench, Cl_2/starch–KI stain,[24] and/or ninhydrin reactivity as indicators. Appropriate column fractions were combined and lyophilized twice.

Thus, compound **7** was prepared from *N*-Ac-L-Ala-L-Gln-L-Ala-L-Phe (**6**, 150 mg, 0.314 mmol), compound **1a** (202.5 mg, 0.471 mmol), and pyridine (49.7 mg, 0.628 mmol) in 2.5 mL of DMF/H_2O (1:1). (An additional 1 mL of DMF was added after 6 h of reaction to increase the solubility of **6**. The reaction mixture, however, remained very turbid.) After DEAE-Sephadex purification, fractions 10–20 (elution volume = 90–180 mL) were found to contain the desired peptide (**7**): yield 112.86 mg (0.251 mmol, 80%); mp 238–242 °C dec; $[\alpha]^{24}_D$ = −69.72° (*c* = 0.55, 0.1 M HCl); TLC, R_f = 0.26 (BAW, 4:1:1); NMR (80 MHz, AcOH-d_4) δ 1.34 and 1.37 (two overlapping doublets, 6 H, J_1 = 7.2 Hz, J_2 = 7.1 Hz)*, 2.04 (acetyl singlet overlapping with AcOH peaks), 2.14–2.47 (m, 1.82 H), 3.00–3.33 (one triplet and one multiplet overlapping to produce a multiplet, 4.09 H), 4.33–4.96 (two quartets and two triplets overlapping to produce an apparent multiplet, 4.00 H), 7.24 (s, 5.09 H); AAA Ala (1.86), DABA (1.07), Phe (0.97); RT_{AAA} = 44.5 min. Elemental Anal. Calcd for $C_{21}H_{31}N_5O_6 \cdot 0.5H_2O$: C, 55.02; H, 6.99; N, 15.28. Found: C, 54.97; H, 6.95; N, 15.33.

Studies of Peptide Cleavage and Transpeptidation. (A) Buffers. Cleavage/transpeptidation reactions were buffered with either bifunctional or monofunctional buffers.[13] Buffers of the former type were employed at 0.25 M concentration. Specific bifunctional buffers used were citrate at pH 3.32, phosphate at pH 8.07, bicarbonate at pH 10.02 and 10.71, and phosphate at pH 10.80. Specific monofunctional buffers used were PIPES (0.25 M) at pH 8.06, TEA/AcOH (0.25 M) at pH 10.89, and NH_4OH (0.1 M) at pH 11.15. A PIPES buffer was prepared by dissolving the appropriate amount of its disodium salt in H_2O and then adjusting the pH to the desired value with 6 M HCl. A TEA/AcOH buffer was prepared according to the Henderson–Hasselbalch equation and an NH_4OH buffer by dilution of concentrated (14.8 M) aqueous ammonia. (The pH values listed above for both bifunctional and monofunctional buffers are those of freshly prepared buffer solutions at room temperature.)

Following the preparation of these buffers, cleavage/transpeptidation reaction mixtures were prepared (see below) and their pH values determined. Reaction mixtures prepared with bifunctional buffers or PIPES displayed little variation in pH with temperature and were effectively buffered even at long reaction times (less than a 0.05 pH unit change after 4 days of reaction). Both the TEA/AcOH and NH_4OH buffers, however, were markedly temperature dependent and displayed ca. 0.6–0.8 pH unit changes after 2 days of reaction. In this paper, the indication of pH in a particular reaction refers to the *actual* value measured at 60 °C. The pH values cited here, because of variations with time and temperature, should be considered accurate only to ±0.1 unit.

(B) Cleavage/Transpeptidation Reactions. In order to assess the progress of cleavage (eq 1), transpeptidation (eq 2), and nonspecific cleavage in the model peptides prepared for this study, reaction mixtures were prepared by a standard methodology. A typical procedure is as follows. A tetrapeptide sample of known weight (6–10 μmol) was placed in a 12-mL Pyrex centrifuge tube and then dissolved in an appropriate buffer such that 2.95 mL of solution was produced. A 0.05-mL aliquot of an internal standard solution (either L-norLeu or L-Pro) was then added to produce a final volume of 3.0 mL (final solution concentration = 2.00–3.33 nmol/μL in starting peptide). The tube was fitted with a rubber septum and mixed well on a Vortex mixer. It was then placed in a stirring oil bath (60 °C unless indicated otherwise) and the exact reaction concentration determined by either (a) removal of a 0.1-mL reaction mixture aliquot and subsequent peptide bond hydrolysis/amino acid analysis or (b) removal and subsequent analysis of a diluted (see below) 0.1-mL reaction mixture aliquot as an *unhydrolyzed sample* on the amino acid analyzer. (The latter method required a standard solution of the particular peptide at known concentration.) Whether determined directly by amino acid analysis or by analysis of an unhydrolyzed reaction mixture aliquot, the exact concentration of starting material in the reaction served as the basis for calculation of cleavage or transpeptidation product required for 100% completion of reaction.

Reaction progress was determined as follows. As a function of time after the reaction tube was placed in the oil bath, 0.1-mL reaction aliquots were removed and quenched by dilution to ca. 0.3 mL with 0.2 mL of cold (4 °C) 0.067 M sodium citrate (diluting) buffer at pH 2.2. A 0.1-mL aliquot of this solution was then placed on the amino acid analyzer, and the reaction product(s) (a dipeptide produced by direct cleavage and/or an isomeric tetrapeptide produced by transpeptidation) were quantitated by peak integration using color values that were ob-

(23) Almond, M. R.; Stimmel, J. B.; Thompson, E. A.; Loudon, G. M. *Org. Synth.* **1987**, *66*, 132–140.

(24) Rydon, H. N.; Smith, P. W. G. *Nature* **1952**, *169*, 922–923.

tained from recently run standards (see Figure 1). Analyzer runs were all performed in standard sodium citrate buffers. The accuracy of all integrations is ±5%. Dilution or evaporation errors were corrected by the presence of the internal standard. The percent completion of reaction at any time was determined by dividing the quantitated amount of reaction product(s) (in nanomoles) by the amount required for 100% reaction (in nanomoles, determined by complete acid hydrolysis followed by amino acid analysis or by analysis of the unhydrolyzed sample). In Table I, results are expressed as the percent completion of direct cleavage rounded off to the nearest integral value.

(C) Manual Edman Degradation Procedure. Manual Edman degradation of DAPA- and DABA-containing tetrapeptides, used for cleavage of peptides containing transpeptidized DABA or DAPA residues or for direct cleavage of DAPA-containing peptides, was carried out with a modification of the method of Konigsberg and Hill.[20] The general procedure was as follows. A suitable quantity of peptide (0.1–1.0 μmol) was removed from a transpeptidation reaction mixture or a stock solution of a particular peptide, placed in a 12-mL Pyrex centrigue tube, and lyophilized once. The lyophilizate was dissolved in 2 mL of a buffer prepared by comining 60 mL of *N*-ethylmorpholine (Aldrich), 1.5 mL of AcOH, and 500 mL of 95% EtOH per liter. This coupling buffer was prepared fresh for each set of degradation experiments because it is somewhat unstable and decomposes to give nonvolatile products upon standing. An aliquot (100 μL) of Boc-L-Asp, which served as an internal standard, was then added followed by 100 μL of phenyl isothiocyanate (Aldrich). The reaction tube was fitted with a rubber septum and heated at 37 °C for 2.5 h in a stirred oil bath with periodic mixing on a Vortex mixer. After being cooled to room temperature, the reaction mixture was extracted with five 2-mL volumes of benzene (spectral grade). The benzene washings were discarded, and the aqueous phase was concentrated overnight in vacuo with NaOH (solid). The resulting yellow-white residue, which contained the phenylthiocarbamyl (PTC) adduct, was then treated with 2 mL of anhydrous TFA for 2 h at room temperature to effect cyclization and cleavage. When this step was carried out at 60 °C, 2 mL of HFBA (Aldrich) was used, and the reaction was run in vacuo in a sealed Pyrex tube. The TFA (or HFBA) was removed in vacuo, and the resulting concentrate was then dissolved in an appropriate volume of 0.067 M sodium citrate (diluting) buffer at pH 2.2. (Extensive degassing on a water aspirator was required before high vacuum could be used in order to prevent severe bumping of TFA.) After being filtered through a 0.2-μm cellulose acetate membrane filter, an aliquot of the diluted reaction concentrate was subjected to amino acid analysis and the dipeptide reaction product quantitated by peak integration using color values that were obtained from recently run standards. The accuracy of all integrations is ±5%. Errors resulting from dilution or bumping of the solution were corrected by the presence of the internal standard L-Asp, which was produced in situ from Boc-L-Asp during the TFA (or HFBA) treatment. The percent completion of cleavage was determined by dividing the quantitated amount of cleavage product (in nanomoles) by the amount required for 100% cleavage (in nanomoles, determined from the exact concentration of the transpeptidation reaction mixture or stock solution of the particular peptide).

Note Added in Proof. Weber and Miller[25] have previously suggested that lysine, rather than DABA or DAPA, evolved because of the transpeptidation reactions discussed in this paper.

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Registry No. 1a, 2712-78-9; **1b**, 3240-34-4; **2**, 56186-36-8; **3**, 121574-43-4; **4**, 121574-44-5; **5**, 121574-45-6; **6**, 105995-30-0; **7**, 121574-46-7; **8**, 868-28-0; **9**, 121574-64-9; Ac-Ala-OH, 97-69-8; Ac-Ala-Gln-Gly-Gly-OH, 105995-28-6; Ac-Ala-Gln-Ala-Gly-OH, 91861-95-9; Ac-Ala-Gln-Ile-Gly-OH, 105995-27-5; Ac-Ala-Gln-Phe-Gly-OH, 105995-29-7; Ac-Ala-Gln-Val-Gly-OH, 121574-47-8; Ac-Ala-DABA-Gly-Gly-OH, 121574-48-9; Ac-Ala-DAPA-Gly-Gly-OH, 121574-49-0; Ac-Ala-DABA-Ala-Gly-OH, 121574-50-3; Ac-Ala-DAPA-Ala-Gly-OH, 121574-51-4; Ac-Ala-DABA-Ile-Gly-OH, 121574-52-5; Ac-Ala-DAPA-Ile-Gly-OH, 121574-53-6; Ac-Ala-DAPA-Ala-Phe-OH, 121574-54-7; Ac-Ala-DABA-Phe-Gly-OH, 121574-55-8; Ac-Ala-DAPA-Phe-Gly-OH, 121574-56-9; Ac-Ala-DABA-Val-Gly-OH, 121574-57-0; Ac-Ala-DAPA-Val-Gly-OH, 121574-58-1; Ac-Ala-DABA-NHMe, 121574-59-2; Ac-Val-DABA-Ile-Gly-OH, 121574-60-5; Ac-Val-DAPA-Ile-Gly-OH, 121574-61-6; Ac-Gly-DABA-Ile-Gly-OH, 121574-62-7; Ac-Gly-DAPA-Ile-Gly-OH, 121574-63-8; H-Gly-Gly-OH, 556-50-3; H-Ala-Gly-OH, 687-69-4; H-Ala-Phe-OH, 3061-90-3; H-Phe-Gly-OH, 721-90-4; Ac-Ala-isoDAPA-Ile-Gly-OH, 121574-65-0; Ac-Ala-isoDABA-Gly-Gly-OH, 121574-66-1; Ac-Ala-isoDAPA-Gly-Gly-OH, 121574-67-2; Ac-Ala-isoDABA-Ala-Gly-OH, 121574-68-3; Ac-Ala-isoDAPA-Ala-Gly-OH, 121574-69-4; Ac-Ala-isoDABA-Ala-Phe-OH, 121574-70-7; Ac-Ala-isoDAPA-Ala-Phe-OH, 121574-71-8; Ac-Ala-isoDABA-Phe-Gly-OH, 121574-72-9; Ac-Ala-isoDAPA-Phe-Gly-OH, 121574-73-0; Ac-Ala-isoDABA-Val-Gly-OH, 121574-74-1; Ac-Ala-isoDAPA-Val-Gly-OH, 121574-75-2; Ac-Val-isoDABA-Ile-Gly-OH, 121574-76-3; Ac-Val-isoDAPA-Ile-Gly-OH, 121574-77-4; Ac-Gly-isoDABA-Ile-Gly-OH, 121574-78-5; Ac-Gly-isoDAPA-Ile-Gly-OH, 121574-79-6; H-Val-Gly-OH, 686-43-1; H-Gln-OH, 56-85-9.

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