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(15) (a) S. Danishefsky and T. Kitahara, *J. Am. Chem. Soc.*, **96**, 7807 (1974); (b) *J. Org. Chem.*, **40**, 538 (1975).
(16) (a) Cf. R. L. Kronenthal and E. I. Becker, *J. Am. Chem. Soc.*, **79**, 1095 (1957); (b) A. A. Petrov and K. B. Rall, *Zh. Obshch. Khim.*, **26**, 1588 (1956).
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(18) For a possibility of electronic deactivation of expoxidation by a neighboring lactone, see ref 6.
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(21) R. Hirschmann, N. G. Steinberg, and R. Walker, *J. Am. Chem. Soc.*, **84**, 1270 (1962).
(22) The hydroxy ester was not purified. Its existence could be inferred from the formation of a new substance (TLC analysis) which still exhibited a methyl ester resonance in its NMR spectrum and which cyclized to give **41**.
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(24) See E. Eliel, "The Stereochemistry of Carbon Compounds", McGraw-Hill, New York, N.Y., 1962.
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(26) We thank Professor Jon Clardy and Dr. James Springer of Iowa State University for these measurements. A copy of the computer printout of the crystal structure is available as Supplementary Material. This measurement also defines the stereochemistry of the anomeric methoxyl to be, as shown, in structures **46**, **47**, and **48**.
(27) Obviously, one cannot quantitatively define ground-state conformational populations from such measurement, but it would appear that conformation **53e** nicely accounts for the observed NMR data. The extrapolatibility of these results to the case of **52**, R = H, is unclear.
(28) We thank Professor P. A. Grieco of the University of Pittsburgh for comparing the two spectra and for a small reference sample of **54a**. The latter was valuable both in providing a mass spectrum and in demonstrating that our Wittig reactions on **50** were all unsuccessful. This was done by acetylating our reaction mixtures and finding no clear TLC spots corresponding to **54a**.
(29) For a discussion of the possible directing effect of a proximate Li^+ in these reactions, see S. Danishefsky, M. Tsai, and T. Kitahara, *J. Org. Chem.*, **42**, 394 (1977).
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(33) P. A. Grieco, J. A. Noguez, and Y. Masaki, *Tetrahedron Lett.*, 4213 (1975).
(34) S. M. Kupchan, R. Hemingway, D. Werner, and A. Karim, *J. Org. Chem.*, **34**, 3903 (1969).
(35) P. A. Grieco and K. Hiroi, *J. Chem. Soc., Chem. Commun.*, 1317 (1972).
(36) J. Schreiber, M. Haag, N. Hashimoto, and A. Eschenmoser, *Angew. Chem., Int. Ed. Engl.*, **10**, 330 (1971). The use of this reagent for lactone methylenation was first suggested to us by Professor Paul Dowd of the University of Pittsburgh. His suggestions are gratefully acknowledged.
(37) Cf. G. Kinast and L. F. Tietze, *Angew. Chem., Int. Ed. Engl.*, **15**, 239 (1976).
(38) Melting points are uncorrected. Combustion analyses were conducted by Galbraith Associates. Infrared spectra were obtained on Perken-Elmer Model 137 or 237 spectrophotometers. High-resolution mass spectra were obtained on a Varian Associates CH-5 instrument by direct insertion. NMR spectra were measured in the indicated solvents with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ) from the Me_4Si resonance. Pictures of all the infrared and NMR spectra described in this paper are found in the Ph.D. thesis of Paul F. Schuda, University of Pittsburgh, 1977. Pictures of the NMR spectra of bisnorvernolepin (**55**) and bisnorvernomenin (**56**) are available as Supplementary Material.
(39) Cf. Y. Kishi, M. Aratani, H. Tanino, T. Fukuyama, T. Goto, S. Inoue, S. Suguira, and H. Kakoi, *Chem. Commun.*, 64 (1972).
(40) The commercially available resin (100 g) was treated with 200 ml of dilute HCl. After filtration, the resin was washed with methanol, acetone, chloroform, benzene, and ether to remove soluble impurities. It was dried for 30 min by vacuum desiccation prior to use.
(41) (a) It is crucial that all the starting material, **42**, be dissolved prior to administration of the DIBAH and that the reducing agent be added in the manner indicated. Starting **42** is not easily separated from **43**. Also, higher reaction temperatures or the use of excessive reducing agent leads to formation of an orthoester diol.
(42) The NMR spectrum of a solution of homogeneous **43** indicates the presence of 10–20% of hemiacetal ring–chain tautomer. The chemical shifts given are for the major, hydroxy aldehyde component.
(43) (a) Curiously, if one adds at this stage the bulk suspension of Wittig reagent, this yield is sharply reduced. (b) This chromatography is complicated by the fact that triphenylphosphine oxide has only a slightly lower R_f value than the desired **52**. In some runs, some mixed fractions containing these two compounds were obtained, thereby necessitating partial rechromatography to reach the indicated yield of pure product.
(44) The acetic acid was prepared prior to use by treatment with potassium permanganate and distillation followed by treatment with triacetyl borate and distillation to remove traces of water.
(45) The NMR spectrum of this compound is available as Supplementary Material.

Peptide Synthesis Using the Four-Component Condensation (Ugi Reaction)

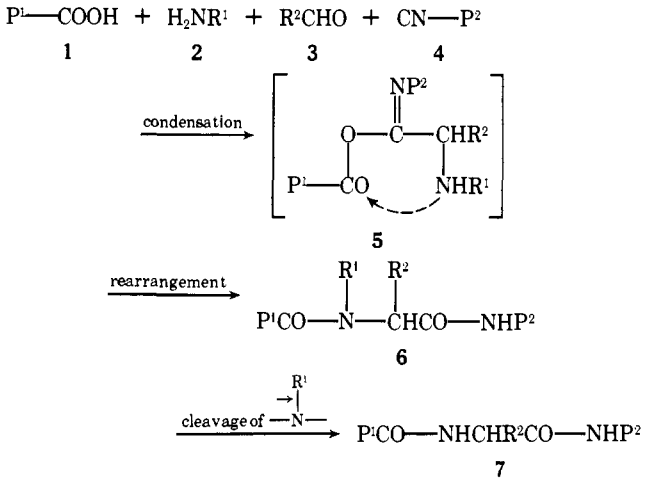
Michinori Waki and Johannes Meienhofer*

Contribution from the Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received February 28, 1977*

Abstract: Applications of the "four-component condensation" (4CC) or "Ugi reaction" to peptide fragment coupling were studied with syntheses of protected di-, tri-, and tetrapeptides. The most suitable solvents were alcohols, including methanol, 1-butanol, trifluoroethanol, and hexafluoro-2-propanol, as shown by syntheses of Ac-Gly-[*N*-Bzl-DL-Val]-Gly-OBu-*t* in 60–80% yields. The efficacy of several aldehydes in 4CC fragment condensation and subsequent cleavage of the auxiliary substituents was examined by syntheses of model dipeptide Pht-Gly-Gly-OR (R = H, Bu-*t*, Me) and tetrapeptide Z-Gly-Ala-Leu-Gly-OR (R = H, Bu-*t*) derivatives. 2-Nitrobenzaldehyde (photolytic cleavage), 2,4-dimethyloxybenzaldehyde, 1-*tert*-butyloxycarbonyl-3-formylindole (acidolytic cleavage), and 4-pyridinecarboxaldehyde (electrolytic cleavage) proved to be effective along with cyclohexyl isonitrile. As an example, Z-Gly-Ala-Leu-Gly-OBu-*t* was synthesized from Z-Gly-Ala-OH and H-Leu-Gly-OBu-*t* and shown to be indistinguishable from material prepared by conventional procedures.

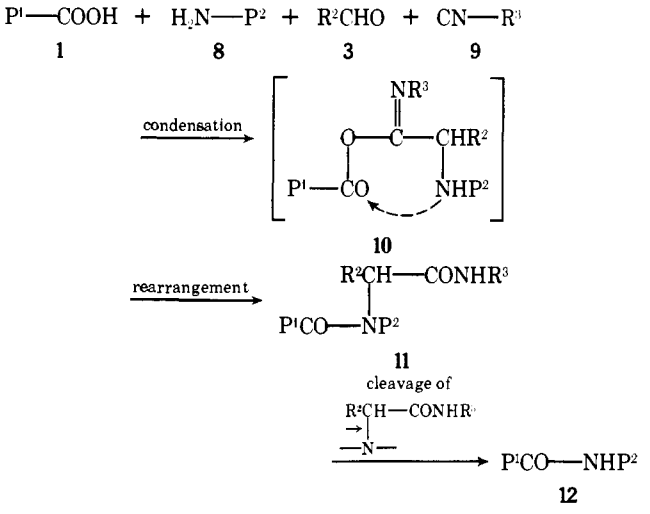
Present methods permit solution synthesis[1] of homogeneous peptides with up to 50 amino acid residues[2] but condensation of peptides of this size to obtain proteins with over 100 residues has not yet progressed beyond the pioneering stage. The remarkable ribonuclease *S*-protein synthesis by Hirschmann et al.[3] remains to be as yet the only preparation of a peptide of over 100 residues. This synthesis underscored the problem. The yield of the final coupling of a 44- with a 60-residue peptide was ca. 3%. Other efforts in enzyme synthesis have yet to be completed.[2,4,5] Difficulties arise from the second-order kinetics of peptide coupling and from the 100- to 1000-fold lower molar concentrations due to the increased

Scheme I. "4CC Synthesis"



P^1 : NH_2-protected amino acid, peptide
P^2 : COOH-protected amino acid, peptide
R^1: alkyl, aralkyl
R^2: alkyl, aralkyl, aryl
(numbers 1–18 represent structures; numbers 19–51 represent compounds)

Scheme II. "4CC Fragment Condensation"



R^2, R^3: alkyl, aralkyl, aryl

size and sharply decreased solubility in organic solvents of large protected peptides vs. small ones. Consequently the rate of peptide bond formation may become slower than irreversible intramolecular side reactions, racemization, and/or pseudo-first-order interactions with solvents. Stronger carboxyl activation would be of little avail and only increase the danger of racemization. If, instead, the activated carboxyl of one component and the free amino group of the other could be prelocked into close proximity the actual *peptide bond formation* would become a *concentration-independent* intramolecular reaction. The four-component condensation (4CC)[6] developed by Ugi et al.[7-11] provides such a mechanism for peptide fragment condensation.[12]

In the 4CC a carboxylic acid component, an amine, an aldehyde, and an isonitrile produce an unstable intermediate α adduct (structure **5** or **10**) which forms a stable peptide derivative (**6** or **11**) by a fast intramolecular rearrangement. This reaction may be utilized to synthesize tripeptide derivatives in one step ("*4CC synthesis*", Scheme I) or, alternatively, to couple two peptide intermediates ("*4CC fragment condensation*", Scheme II).[8]

In "4CC synthesis", Scheme I, an NH_2-protected amino acid or peptide (**1**), a suitable primary amine (**2**), an aldehyde (**3**), and an isonitrile derivative of a COOH-protected amino acid or peptide (**4**) form a new central amino acid residue in situ from the components **2** (nitrogen), **3** (α-carbon and amino acid side chain), and **4** (carboxyl carbon).[14] This approach requires (a) racemization-free synthesis of the isonitrile component (**4**), (b) stereoselective reaction to obtain the desired configuration at the central α carbon through asymmetric induction by suitable optically active primary amines (**2**), and (c) facile cleavage of the N-R^1 bond of structure **6** to produce the desired peptide, structure **7**.

In "4CC fragment condensation", Scheme II, an NH_2-protected amino acid or peptide (**1**) is coupled with a COOH-protected amino acid or peptide component (**8**) to produce a peptide in which the aldehyde (**3**) and isonitrile components do not contribute any of their atoms to the final product (**12**).[7] This strategy requires aldehydes (**3**) which provide high yields of product, structure **11**, from which the auxiliary moiety N(CHR^2CONHR^3) can be readily cleaved to afford the final product, structure **12**, under nondestructive conditions.

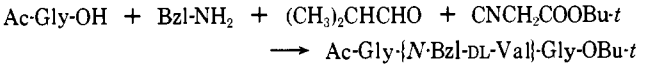
The 4CC has been described[8] as "easy to carry out and to proceed with excellent yield (often quantitative) under a wide variety of conditions ($t°$ = −80 °C to +80 °C in solvents such as benzene, methylene chloride, tetrahydrofuran, dimethylformamide, methanol, and water at ... 0.01-3.0 M initial component concentration)". Most experiments, however, have been conducted with simple model compounds. Ugi et al. have focused their studies on the solution of the stereoselectivity problem in "4CC synthesis" [8] with excellent results.[14,15]

We were interested in application of the 4CC to peptide fragment condensation[7] under reaction conditions commonly used in syntheses of biologically active peptides.[2] This paper reports results of exploratory studies on (1) the influence of solvents on the efficacy and product yield of "4CC synthesis" (Scheme I), (2) a potential formation of a central glycine residue in "4CC synthesis", and (3) the practicality of "4CC fragment condensation" (Scheme II).

Results and Discussion

A. Solvent Influence on 4CC Synthesis. The choice of organic solvents for peptide coupling narrows rapidly with increasing size of the protected intermediate components. Eventually, hexamethylphosphoramide, dimethyl sulfoxide, or even molten phenol[16] remain as last resort. It was, therefore, important to examine the compatibility and efficiency of 4CC in solvents of high dissolving power for protected peptides. In a standardized "4CC synthesis" (Scheme III) equivalent amounts of

Scheme III



acetylglycine, benzylamine, isobutyraldehyde, and *tert*-butyl 2-isocyanoacetate[17-19] were combined in different solvents at 0.5 M concentration and the yields of the ensuing model tripeptide N^{α}-acetylglycyl-DL-*N*-benzylvalylglycine *tert*-butyl ester[20] (**18**) were determined following isolation and crystallization. The results, 75% yield of **18** in CH_3OH, 72% in 1-butanol, 68% in 2,2,2-trifluoroethanol, 61% in 1,1,1,3,3,3-hexafluoro-2-propanol,[21] 51% in $CHCl_3$ or CH_2Cl_2, 49% in dioxane, 48% in tetrahydrofuran, and 20–30% in a wide range of solvents including CH_3CN, liquid NH_3, diethyl phosphite, dimethylacetamide, dimethylformamide, dimethyl sulfoxide, hexamethylphosphoramide, 1-methyl-2-pyrrolidone, phenol, pyridine, tetramethylenesulfone, and toluene, clearly indicated that good yields were obtained with alcohols as solvents. Similarly, 67% of crystalline N^{α}-phthalylglycyl-*N*-(α-cyclo-

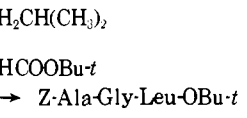
hexylcarbamoyl-2-nitrobenzyl)glycine *tert*-butyl ester (**23**) was obtained with methanol as a solvent, while the yield with dimethylformamide was 13%.

In our experience the choice of solvents in 4CC is narrow. To obtain good yields alcohols have to be used. Yields were poor with many other solvents including several (DMF, Me_2SO) of strong dissolving power for large protected peptides.[22] However, the good yields obtained with trifluoroethanol and hexafluoro-2-propanol in 4CC are a very encouraging result, since both alcohols are good solvents for otherwise poorly soluble protected peptides.[21]

The standardized "4CC synthesis", Scheme III, was also used for a brief study about the influence of different amine components (R^1) on the formation of Ac-Gly-{N·R^1-DL-Val}-Gly-OBu-*t* in methanol. Yields of crystalline tripeptide derivatives were 40% of compound **19** using 2-nitrobenzylamine,[23] 28% of **20** with 2,4-dimethyloxybenzylamine, and 45% of **21** with 4-aminomethylpyridine, while the use of 2-nitrophenylsulfenylamine[24] produced Nps-DL-Val-Gly-OBu-*t* (**22**) instead of the desired tripeptide derivative.

B. Potential Formation of Central Glycine Residue. The "4CC synthesis", Scheme IV, forming a glycine residue with

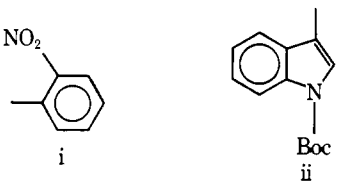
Scheme IV



the use of ammonia and formaldehyde would be ideal for coupling peptide fragments which are connected in a target sequence by glycine residues. The obvious advantages of this approach would be elimination of the need for both stereoselective induction and final cleavage from the product of undesired N substituents (R^1 in structure **6**). However, Ugi reported[9] that the use of ammonia can lead to irreversible side reactions, e.g., the Passerini reaction.[25] We wondered whether forcing conditions as the use of liquid ammonia as a solvent might succeed. In several attempts addition of paraformaldehyde or gaseous formaldehyde to a solution of benzyloxycarbonyl-L-alanine and *tert*-butyl 2-isocyano-4-methylvalerate[26] in refluxing liquid ammonia did not produce any detectable tripeptide (Z-Ala-Gly-Leu-OBu-*t*), but the formaldehyde was rapidly converted to hexamethylenetetraamine which precipitated from the solution. This approach was therefore abandoned.

However, the reported[11] successful use of formaldehyde along with benzylamine, phthalylglycine, and *tert*-butyl isocyanoacetate to form Pht-Gly-{*N*·Bzl-Gly}-Gly-OBu-*t* in 83% yield indicated that fragment condensation of a connecting glycine residue would still be feasible if a cleavable N substituent (R^1 in structure **6**) is used.[8,14,18,27] In preliminary experiments we obtained Z-Ala-{*N*·Bzl-Gly}-Leu-OBu-*t*, but it was contaminated with several unidentified side products and an evaluation of this approach will need further investigation. This strategy also requires preparation of isonitriles of the COOH-terminal peptide fragment[14] which may still present difficulties.[8,28,29]

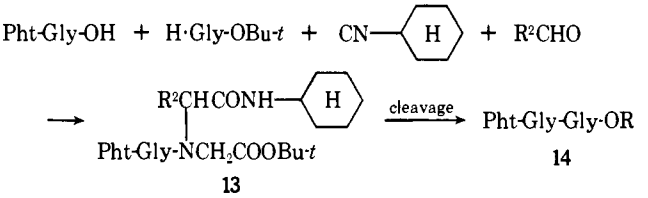
C. Peptide Fragment Condensation.[7] The "4CC fragment condensation" shown in Scheme II permits condensation of conventionally prepared peptide fragments without prior or additional modification, i.e., coupling of an NH_2-terminal N-protected peptide with a COOH-terminal carboxyl-protected peptide. This approach depends critically on the facile cleavage of the auxiliary N substituent (-CHR^2CONHR^3 in structure **11**) under mild conditions and without decomposition or racemization of the final product (structure **12**). This requires assistance of the cleavage reaction by the group R^2 of the aldehyde component (**3**). Use of 2-nitrobenzaldehyde[30] (R^2 = i) for potential photolytic cleavage[31] of **11** was reported[8] to be restricted to very simple model compounds. The 2,4-dimethyloxybenzyl and the 2,4,6-trimethyloxybenzyl groups may be removed from the amide nitrogen atom by treatment with trifluoroacetic acid.[32] Ugi et al.[8] proposed the use of *N*-*tert*-butyloxycarbonylindole-3-aldehyde (R^2 = ii) for acidolytic



cleavage of structure **11** → **12** using trifluoroacetic acid, and applied it to the synthesis of several dipeptides.

The usefulness of several aldehydes in "4CC fragment condensation" was assessed by the preparation of crystalline derivatives, structure **13**, of the model dipeptide Pht-Gly-Gly-OBu-*t* as shown in Scheme V (compounds **23–31**, Experimental Section). Methanol or methanol-dimethylformamide mixtures served as solvents. The use of 2-nitrobenzaldehyde yielded 67% of product **13** (R^2 = i); 3-nitrobenzaldehyde yielded 50%, 2,4-dimethyloxybenzaldehyde 34%, 3,5-dimethyloxybenzaldehyde 69%, 4-pyridinecarboxaldehyde 60%, *N*-*tert*-butoxycarbonyl-3-formylindole[33–35] 56%, and 3-formylindole 45% of respective products.[36] The results, as summarized in Table I, encouraged studies on tetrapeptides. The dipeptides Z-Gly-Ala-OH and H-Leu-Gly-OBu-*t* were coupled by ("4CC...) fragment condensation" with the aid of cyclohexyl isonitrile and three different aldehydes, each in methanol, to produce tetrapeptide derivatives (structure **15**, Scheme VI). Using 2-nitrobenzaldehyde, condensation proceeded to 71% completion within 15 h at 20 °C to provide Z-Gly-Ala-N^{α}-(α-cyclohexylcarbamoyl-2-nitrobenzyl)-

Scheme V



Scheme VI

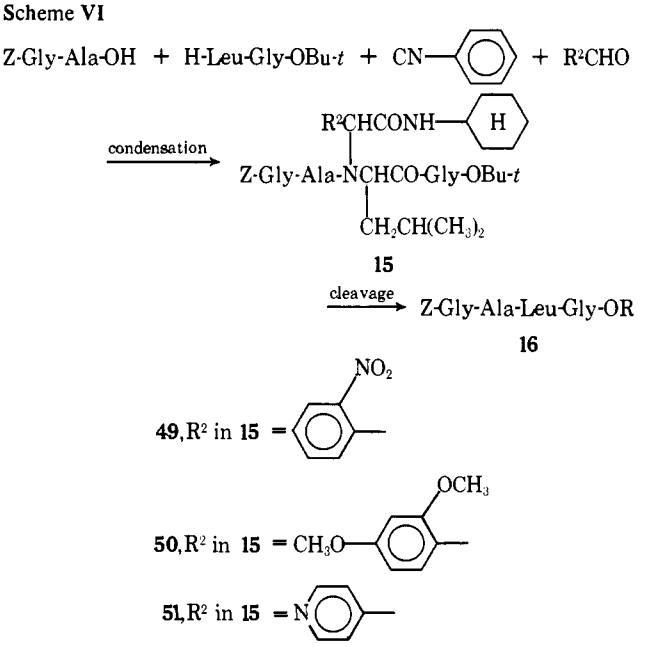
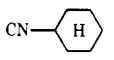
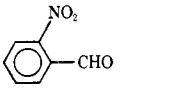
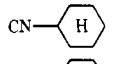
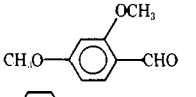
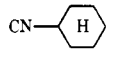
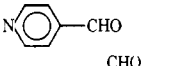
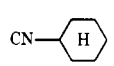
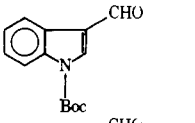
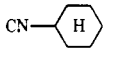
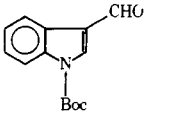
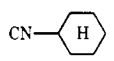
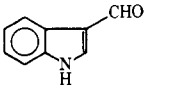
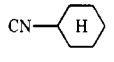
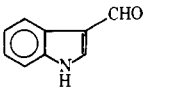
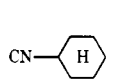
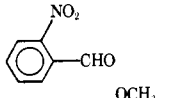
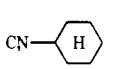
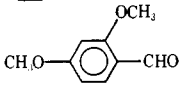
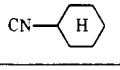
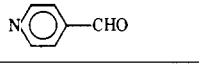


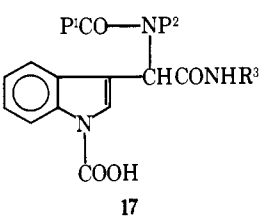
Table I. Peptide Fragment Coupling by Four-Component Condensation (Scheme II)

				% condens	(compd no.)[a]	% cleavage	[reaction][b]	(compd no.)[a]
Formation of Model Dipeptide Pht-Gly-Gly-OR (Scheme V)								
Pht-Gly-OH	H-Gly-OBu-*t*			67	(**23**)	61	[$h\nu$]	(**35**)
Pht-Gly-OH	H-Gly-OBu-*t*			34	(**25**)	38	[H^+][c]	(**36C**)
Pht-Gly-OH	H-Gly-OBu-*t*			60	(**27**)	*d*	[e]	(ref 42)
Pht-Gly-OH	H-Gly-OBu-*t*			53	(**28**)	74	[H^+]	(**32** → **36A**)
Pht-Gly-OH	H-Gly-OMe			56	(**29**)	70	[H^+]	(**31B** → **37**)
Pht-Gly-OH	H-Gly-OBu-*t*			45	(**30**)	81	[H^+]	(**36B**)
Pht-Gly-OH	H-Gly-OMe			35	(**31**)	79	[H^+]	(**37**)
Formation of Tetrapeptide Z-Gly-Ala-Leu-Gly-OR (Scheme VI)								
Z-Gly-Ala-OH	H-Leu-Gly-OBu-*t*			71	(49)	78	[$h\nu$]	(**45**–4CC)
Z-Gly-Ala-OH	H-Leu-Gly-OBu-*t*			75	(**50**)	10	[H^+]	(**47**–4CC)
Z-Gly-Ala-OH	H-Leu-Gly-OBu-*t*			56	(**51**)			

[a] See Experimental Section. [b] $h\nu$, photolysis at 350 nm; H^+, acidolysis by CF_3COOH; e, electrolysis. [c] Acidolysis by anhydrous HF. [d] Analytical scale electrochemical reduction at controlled potential indicated complete cleavage.

Leu-Gly-OBu-*t* (**49**). Of the three tetrapeptide derivatives, two (**49** and **50**) were obtained in higher yields than the respective model dipeptide derivatives (see Table I), a result which encourages studies with larger fragments.

D. Cleavage of the Auxiliary Substituent N(CHR²CONHR³) (Scheme II). Cleavage of the N^{α}-(α-cyclohexylcarbamoyl-2-nitrobenzyl) substituent of **49** was obtained with satisfactory yield by photolysis at 350 nm (see Table I) and provided isolated crystalline Z-Gly-Ala-Leu-Gly-OBu-*t* (**45**-4CC) which was indistinguishable by several analytical criteria from authentic **45** prepared by conventional solution synthesis.[37] The successful photolytic removal of the N^{α}-(α-cyclohexylcarbamoyl-2-nitrobenzyl) group from **49** indicated that this procedure may be useful in peptide fragment condensation,[38] and not restricted[8] to "simple model 4CC products". Further studies on the usefulness of 2-nitrobenzaldehyde in "4CC fragment condensation" are clearly warranted. Cleavage of the N^{α}-(α-cyclohexylcarbamoyl-4-pyridylmethyl) group of the tetrapeptide derivative **51** remains to be examined. The N^{α}-(α-cyclohexylcarbamoyl-2,4-dimethyloxybenzyl) substituent was removed from **50** to a very small extent only (10%) by treatment with trifluoroacetic acid[32] (see Table I). Considerably improved cleavage will be required to render 2,4-dimethyloxybenzaldehyde suitable for "4CC fragment condensation". Thus the efficacy of a series of acids in cleaving the N^{α}-(α-cyclohexylcarbamoyl-2,4-dimethyloxybenzyl) group was examined using the model dipeptide, structure **13** (Scheme V). Treatment of compound **25** with anhydrous HF[40] resulted in 38% cleavage. This is still unsatisfactory, but it was superior to the 20–30% cleavage observed with several other acids, including HCOOH, HCl or HBr in HOAc, CH_3SO_3H, FSO_3H in CF_3COOH, and CF_3SO_3H in CF_3COOH, for 3 h at 20 °C. The mechanism of the acidolytic cleavage of peptide derivatives prepared by "4CC fragment condensation" with the aid of 1-*tert*-butyloxycarbonyl-3-formylindole has been discussed in detail by Ugi et al.[8] In brief, two successive CF_3COOH treatments with intermittent evaporation to dryness were required since the intermediate carbamic acid (structure **17**) resisted decarboxylation in CF_3COOH. Our



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experiments have corroborated these findings (see conversion of compounds **29** to **31** and **28** to **32**, Experimental Section). The results (see Table I) indicate that 1-*tert*-butyloxycarbonyl-3-formylindole may be one of the most promising al-

dehydes for "4CC fragment condensation" of larger peptides.

A preliminary analytical study of electrochemical cleavage[41] of the N^{α}-(α-cyclohexylcarbamoyl-4-pyridylmethyl) group of the dipeptide intermediate **27** under controlled potential[42] indicated quantitative reductive cleavage. Half-wave potentials of **27** were determined polarographically.[43] The results may warrant further studies and application of 4-pyridinecarboxaldehyde to "4CC fragment condensation" of larger peptides.

No cleavage was observed with common acidolytic or photolytic procedures when 3-nitrobenzaldehyde or 3,5-dimethyloxybenzaldehyde[44] were used in 4CC dipeptide formation (Scheme V). Attempts at reductive cleavage of **18** by catalytic hydrogenolysis in methanol or in liquid ammonia[45] failed.

E. Racemization. A central issue[8] for the ultimate practical utility of the "4CC fragment condensation" in syntheses of biologically active peptides is the absence or minimal occurrence of racemization at the COOH-terminal amino acid residue of the acid component (**1** in Scheme II). The reaction is expected to proceed with an exceedingly low relative rate of racemization.[8] Indeed, no racemate content was detected in gas chromatographic tests[46] of several model dipeptides prepared[8] by "4CC fragment condensation". Assessment of the configurational integrity of the two 4CC-synthetic tetrapeptide derivatives prepared in this study, i.e., Z-Gly-Ala-Leu-Gly-OBu-*t* (**45**-4CC) and Z-Gly-Ala-Leu-Gly-OH (**47**-4CC), by comparison with the corresponding conventional preparations (**45** and **47**) indicated very little if any racemization[47] but a more thorough evaluation will have to be made in further studies with the use of high-performance liquid chromatography.

Conclusion

Several model dipeptides and tetrapeptides have been synthesized by the fragment condensation strategy of the four-component condensation ("Ugi reaction"). Good results depended on the use of alcohols as solvents, but acceptable results were also obtained with alcohol-dimethylformamide mixtures. For the 4CC to become a useful routine procedure, two requirements must be met: (1) high-yield fragment *condensation*, and (2) high-yield and mild *cleavage* of auxiliary substituents. Both requirements depend critically on the choice of the aldehyde component. From the results of this study with small peptides, the most promising aldehydes appear to be (a) 1-*tert*-butyloxycarbonyl-3-formylindole (Ugi et al.[8]), affording 50–60% condensation and 70–75% cleavage (by CF_3COOH treatment); (b) 2-nitrobenzaldehyde, affording 65–70% condensation and 60–80% cleavage (by photolysis); (c) 4-pyridinecarboxaldehyde, affording 55–60% condensation and close to quantitative electrochemical cleavage (analytically). Less promising was the use of (d) 2,4-dimethyloxybenzaldehyde, affording 34–75% condensation and 10–38% cleavage (by acidolysis). The results of these preliminary studies warrant, in our opinion, a continuation and expansion of efforts to further develop a more routine use of the "4CC fragment condensation" in practical peptide synthesis. The method might offer the potential of resolving present shortcomings in the condensation of large peptide fragments, which will be a prerequisite for future syntheses of homogeneous proteins.

Experimental Section

Melting points are uncorrected. Microanalyses and other physicochemical measurements were carried out by the Physical Chemistry Department. Solvent systems for thin layer chromatography (silica gel G) were (A) $CHCl_3$–CH_3OH (96:4), (**B**) $CHCl_3$–CH_3OH (90:10), (C) $CHCl_3$–CH_3OH (5:1), (D) $CHCl_3$–CH_3OH–HOAc (95:5:1), (E) $CHCl_3$–CH_3OH–HOAc (90:10:2), (F) $CHCl_3$–CH_3OH–HOAc (83:15:2), (G) $CHCl_3$–2-propanol (90:10), (H) $CHCl_3$, (I) *n*-BuOH–HOAc–pyridine–H_2O (4:1:1:2).

Materials. D-Ala and amino acid derivatives were purchased from Bachem Inc., Marina Del Ray, Calif. Water-soluble carbodiimide and *N*-hydroxysuccinimide were obtained from Pierce Chemical Co., Rockford, Ill. Other chemicals and solvents were obtained mainly from Aldrich Chemical Co., Inc., Milwaukee, Wis. Benzylamine (Aldrich), isobutyraldehyde (Aldrich), and H-Gly-OBu-*t* (Bachem) were redistilled before use. 2-Nitrobenzylamine hydrochloride[23] and 2-nitrophenylsulfenylamine[24] were prepared by literature procedures. Free 2-nitrobenzylamine and 2,4-dimethyloxybenzylamine were obtained by extraction with ether from a suspension of their hydrochlorides in 1 M NaOH.

***N*$^{\alpha}$-Acetylglycyl-*N*$^{\alpha}$-(benzyl)-DL-valylglycine *tert*-Butyl Ester (18).** To a chilled solution of Ac-Gly-OH (58.6 mg, 0.5 mmol), benzylamine (53.6 mg, 0.5 mmol), and isobutyraldehyde (36.1 mg, 0.5 mmol) in CH_3OH (1 mL), *tert*-butyl 2-isocyanoacetate[17–19] (70.6 mg, 0.5 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C, kept for 15 h at 20 °C, and then evaporated to dryness in vacuo. The residual oil was extracted with $CHCl_3$, and the solution was washed successively with 0.5 M $NaHCO_3$, 0.5 M citric acid, and H_2O, and dried ($MgSO_4$). The filtered solution was concentrated in vacuo and the residual oil crystallized after addition of ether (174 mg). Recrystallization from CH_3OH–ether–petroleum ether yielded 157 mg (75%) of **18**, mp 160–161 °C (lit.[11] mp 158–161 °C), R_f 0.63 (A).

Compounds **19–22** were prepared as described above.

***N*$^{\alpha}$-Acetylglycyl-*N*$^{\alpha}$-(2-nitrobenzyl)-DL-valylglycine *tert*-Butyl Ester (19).** A 1-mmol scale reaction with 2-nitrobenzylamine[23] in CH_3OH (2 mL) for 1 h at 0 °C and 3 days at 20 °C yielded 187 mg (40%), mp 165–166 °C, R_f 0.45 (A), 0.79 (C). Anal. ($C_{22}H_{32}N_4O_7$) C, H, N.

***N*$^{\alpha}$-Acetylglycyl-*N*$^{\alpha}$-(2,4-dimethyloxybenzyl)-DL-valylglycine *tert*-Butyl Ester (20).** Reaction as for **19** with 2,4-dimethyloxybenzylamine yielded 135 mg (28%), mp 168–169 °C, R_f 0.57 (A). Anal. ($C_{24}H_{37}N_3O_7$) C, H, N.

***N*$^{\alpha}$-Acetylglycyl-*N*$^{\alpha}$-(4-pyridylmethyl)-DL-valylglycine *tert*-Butyl Ester (21).** A 1-mmol scale reaction with 4-aminoethylpyridine in CH_3OH (1 mL) for 1 h at 0 °C and 2 days at 20 °C (no acid wash in workup) yielded 188 mg (45%), mp 139–140 °C, R_f 0.21 (A). Anal. ($C_{21}H_{32}N_4O_5$) C, H, N.

***N*$^{\alpha}$-2-Nitrophenylsulfenyl-DL-valylglycine *tert*-Butyl Ester (22).** The same scale and reaction time as for **21** were applied using 2-nitrophenylsulfenylamine[24] in CH_3OH. The ensuing crude product (176 mg) was purified by column chromatography on silica gel 60 (18 × 2.4 cm, 0.2–0.5 mm, E. Merck) using $CHCl_3$ as an eluent. Evaporation of the main peak fractions followed by crystallization from $CHCl_3$–ether–petroleum ether yielded yellow crystals of **22** instead of the desired tripeptide derivative: yield 129 mg[49] (34%); mp 122–123 °C; R_f 0.87 (A), 0.28 (H). Anal. ($C_{17}H_{25}N_3O_5S$) C, H, N, S.

As a side product, di-2-nitrophenyl disulfide[50] was isolated from the faster eluting fractions by the above column chromatography: yield 18 mg;[49] mp 195.5–196.5 °C (lit.[35] mp 192–195 °C); R_f 0.96 (H). Anal. ($C_{12}H_8N_2O_4S_2$) C, H, N, S.

***N*$^{\alpha}$-Phthalylglycyl-*N*$^{\alpha}$-(α-cyclohexylcarbamoyl-2-nitrobenzyl)-glycine *tert*-Butyl Ester (23).** Cyclohexyl isonitrile (109 mg, 1 mmol) was added to a cooled (0 °C) stirred solution of Pht-Gly-OH (205 mg, 1 mmol), H-Gly-OBu-*t* (131 mg, 1 mmol), and 2-nitrobenzaldehyde (151 mg, 1 mmol) in CH_3OH (2 mL). After stirring for 1 h at 0 °C and 1 h at 20 °C, a white solid precipitated. Stirring was continued for 15 h at 20 °C. Workup as described for **18** afforded crystalline **23**; 389 mg (67%); mp 185.5–186 °C; R_f 0.89 (A). Anal. ($C_{30}H_{34}N_4O_8$) C, H, N.

Compounds **24–31** were prepared essentially as described above.

***N*$^{\alpha}$--Phthalylglycyl-*N*$^{\alpha}$-(α-cyclohexylcarbamoyl-3-nitrobenzyl)-glycine *tert*-Butyl Ester (24).** The reaction was started in CH_3OH (2.5 mL) using 3-nitrobenzaldehyde (2.5 mmol). After 2 h a large, white mass precipitated which was dissolved by the addition of CH_3OH (2.5 mL) and DMF (12 mL). Stirring was continued for 3 days at 20 °C. The product was recrystallized from $CHCl_3$–CH_3OH–ether to yield 726 mg (50%) of **24**, mp 203–204 °C, R_f 0.89 (A). Anal. ($C_{30}H_{34}N_4O_8$) C, H, N.

***N*$^{\alpha}$-Phthalylglycyl-*N*$^{\alpha}$-(α-cyclohexylcarbamoyl-2,4-dimethyloxybenzyl)glycine *tert*-Butyl Ester (25).** 2,4-Dimethyloxybenzaldehyde was used as an aldehyde. The reaction was carried out in 2.5-mmol scale in CH_3OH (5 mL) for 1 h at 0 °C, and then for 3 days at 20 °C. During the reaction, a yellow solid precipitated. Recrystallization from CH_3OH–ether produced colorless crystals: yield 504 mg (34%); mp

199-200 °C; R_f 0.81 (A). Anal. ($C_{32}H_{39}N_3O_8$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-3,5-dimethyloxybenzyl)glycine *tert*-Butyl Ester (26). Using 3,5-dimethyloxybenzaldehyde (2.5 mmol), reaction in CH_3OH for 1 h at 0 °C and then 15 h at 20 °C produced a white solid precipitate. Recrystallization from CH_3OH-ether yielded 1.02 g (69%) of **26**; mp 196.5-197.5 °C; R_f 0.90 (A), 0.97 (I). Anal. ($C_{32}H_{39}N_3O_8$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-4-pyridylmethyl)glycine *tert*-Butyl Ester (27). Reaction as for **24** with 4-pyridinecarboxaldehyde in CH_3OH (5 mL) produced a white solid precipitate. Washing with 0.5 M $NaHCO_3$ and H_2O, workup, and recrystallization from CH_3OH-ether produced colorless crystals: 805 mg (60%); mp 151-152 °C; R_f 0.50 (A). Anal. ($C_{29}H_{34}N_4O_6 \cdot {}^1/_2H_2O$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-1-*tert*-butyloxycarbonyl-3-indolylmethyl)glycine *tert*-Butyl Ester (28). The reaction was carried out in 2.5-mmol scale using 1-*tert*-butyloxycarbonyl-3-formylindole[33-35] as an aldehyde in CH_3OH (13 mL) and DMF (5 mL) for 1 h at 0 °C and then for 2 days at 20 °C. During the reaction, a white solid precipitated which was collected, washed with CH_3OH, and dried (925 mg). Recrystallization from $CHCl_3$-CH_3OH-ether produced a colorless powder: 894 mg[51] (53%); mp 189.5-190.5 °C; R_f 0.80 (A), 0.18 (H), 0.90 (I). Anal. ($C_{37}H_{44}N_4O_8$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-1-*tert*-butyloxycarbonyl-3-indolylmethyl)glycine Methyl Ester (29). Reaction of equimolar (2.5 mmol) amounts of Pht-Gly-OH, H-Gly-OMe·HCl, NEt_3, 1-*tert*-butyloxycarbonyl-3-formylindole, and cyclohexyl isonitrile in CH_3OH (13 mL) and DMF (5 mL) followed by workup as described for **23** afforded a colorless powder which was recrystallized from $CHCl_3$-CH_3OH-ether: yield 876 mg[51] (56%); mp 179-180 °C; R_f 0.89 (A), 0.11 (H). Anal. ($C_{34}H_{38}N_4O_8$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-3-indolylmethyl)glycine *tert*-Butyl Ester (30). The same scale and time of reaction as for **28** were applied using 3-formylindole as an aldehyde in CH_3OH (12 mL) and DMF (2 mL). A yellowish solid precipitated which was collected by filtration, washed with ether, and dried (680 mg). Recrystallization from $CHCl_3$-CH_3OH-ether afforded a colorless powder: 650 mg[51] (45%); mp 214-215.5 °C; R_f 0.64 (A), 0.05 (H). Anal. ($C_{32}H_{36}N_4O_6 \cdot {}^1/_2H_2O$) C, H, N.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-3-indolylmethyl)glycine Methyl Ester (31). The reaction was carried out as described for **29** using 3-formylindole (2.5 mmol) as an aldehyde in CH_3OH (12 mL) and DMF (1 mL) for 1 h at 0 °C, and then for 2 days at 20 °C. No precipitation occurred, but the solution became red during the reaction. After recrystallization from $CHCl_3$-CH_3OH-ether, the product was obtained as a colorless powder: yield 458 mg[51] (35%); mp 174-175 °C; R_f 0.64 (A), 0.06 (H), 0.89 (I). Anal. ($C_{29}H_{30}N_4O_6$) C, H, N.

When the protected dipeptide **29** was treated with CF_3COOH in the presence of anisole under N_2 for 1 h at 0 °C and chromatographed on a silica gel column using $CHCl_3$-CH_3OH (96:4) as an eluent, the same compound, **31B**, was obtained, yield 89%, mp 173-175 °C, undepressed mixture melting point with the above product, and indistinguishable from it by infrared spectrum and TLC, systems A and I.

N^{α}-Phthalylglycyl-N^{α}-(α-cyclohexylcarbamoyl-3-indolylmethyl)glycine (32). Compound **28** (135 mg, 0.2 mmol) was treated with CF_3COOH (4 mL) in the presence of anisole (0.4 mL) under N_2 for 1 h at 0 °C. Evaporation of CF_3COOH was followed by addition of H_2O. The resulting precipitate was filtered off, washed with H_2O, and dried (117 mg). Crystallization from CH_3OH-ether yielded 87 mg[51] of **32** (84%): mp 195-197 °C; R_f 0.68 (I), 0.25 (D). Anal. ($C_{28}H_{28}N_4O_6$) C, H, N.

Cleavage of Auxiliary Groups, N^{α}-Acetylglycyl-DL-valylglycine *tert*-Butyl Ester (33). The following photochemical cleavage was carried out as described by Wang.[39] Compound **19** (116 mg, 0.25 mmol) was dissolved in 250 mL of CH_3OH that had been deaerated by a stream of argon gas (2 mL/s) for 30 min inside a jacketed Pyrex tube (30 × 3.5 cm). The solution was further flushed with argon for 1 h with gentle magnetic stirring. The reaction vessel was then tightly stoppered and irradiated for 14 h at 3500 Å (16 × 24 W) in a Rayonet photochemical reaction chamber. The solvent was evaporated in vacuo to afford a dark brown solid which was dissolved in $CHCl_3$. The solution was applied to a column of silica gel (20 × 2.4 cm), which was eluted with $CHCl_3$-CH_3OH (96:4). The fractions containing material of R_f 0.26 (A) were combined. Evaporation yielded a powder which was washed with ether and recrystallized from CH_3OH-ether: 63 mg (76%); mp 181-182 °C; R_f 0.26 (A), 0.60 (C). Anal. ($C_{15}H_{27}N_3O_5$) C, H, N.

N^{α}-Acetylglycyl-DL-valylglycine (34). A. By Trifluoroacetic Acid Treatment of 20. Compound **20** (48 mg, 0.1 mmol) was treated with CF_3COOH (2 mL) in the presence of anisole (0.2 mL). The solution was kept for 15 h at 20 °C. Evaporation of CF_3COOH, trituration of the remaining solid with ether, and recrystallization from CH_3OH-ether yielded 17 mg[51] of **34** (62%): mp 186.5-188.5 °C with softening at around 181 °C; R_f 0.52 (I), 0.1 (E); identical infrared spectrum with that of an authentic sample obtained below, undepressed mixture melting point. Anal. ($C_{11}H_{19}N_3O_5 \cdot H_2O$) C, H, N. Yields were 55 and 60% after 2.5-h treatment with CF_3COOH at 0 and 20 °C, respectively.

B. By Trifluoroacetic Acid Treatment of 33. Compound **33** (33 mg, 0.1 mmol) was dissolved in CF_3COOH (2 mL), and kept for 30 min at 20 °C. Evaporation of CF_3COOH was followed by addition of ether. The resulting powder was filtered off and washed with ether: yield 26 mg[51] (95%); mp 186.5-187.5 °C; R_f 0.52 (I), 0.1 (E). Anal. ($C_{11}H_{19}N_3O_5$) C, H, N.

N^{α}-Phthalylglycylglycine *tert*-Butyl Ester (35).[52] Photolysis of **23** (145 mg, 0.25 mmol) and subsequent purification on a silica gel column as described for **33** afforded **35** which was recrystallized from $CHCl_3$-ether to provide a white powder which had the same R_f value on TLC as that of authentic material,[52] obtained in this work from Pht-Gly-OH and H-Gly-OBu-*t* by the DCCI method:[53] yield 48.2 mg (61%); mp 163.5-164 °C (lit.[52] mp 165-165.5 °C); no depression of mixture melting points with the authentic sample; R_f 0.78 (A).

N^{α}-Phthalylglycylglycine (36).[52,54] **A. By Trifluoroacetic Acid Treatment of 32.** Compound **32** (51.7 mg, 0.1 mmol) was treated with CF_3COOH (3 mL) for 15 h at 20 °C in the presence of anisole (0.1 mL) under N_2. During the reaction the solution became purple. After evaporation of CF_3COOH in vacuo, the residue was triturated thoroughly with ether. The resulting white solid was filtered off, washed well with ether, and dried. The ensuing powder had the same R_f values on TLC using several solvent systems as those of authentic material[52,54] obtained by treatment of Pht-Gly-Gly-OBu-*t* with CF_3COOH: yield 23 mg[51] (88%); mp 230-231 °C (lit.[52] mp 233-234 °C; lit.[54] mp 230-231 °C); no depression of mixture melting points with the authentic sample; R_f 0.60 (I), 0.19 (D), 0.41 (E); infrared spectrum identical with that of the authentic material.

B. By Trifluoroacetic Acid Treatment of 30. Treatment of **30** with CF_3COOH and workup as described above produced **36** in yields of 64% (4 h treatment at 0 °C), 80% (4 h at 20 °C), and 81% (15 h at 20 °C) that showed exactly the same properties as those of the authentic sample (e.g., melting point, no depression of mixture melting points, R_f values on TLC in several solvent systems (I, D, E), and infrared spectrum).

C. By Hydrogen Fluoride[40] Treatment of 25. Compound **25** (59.4 mg, 0.1 mmol) was treated with anhydrous HF (5 mL) in the presence of anisole (0.2 mL) for 3 h at 20 °C. Evaporation of HF under reduced pressure was followed by the addition of H_2O. The ensuing white solid was recrystallized from $CHCl_3$-CH_3OH-ether to afford crystalline **36**, 10 mg (38%): mp 229-231 °C; no depression of mixture melting point with the authentic sample; R_f 0.60 (I), 0.19 (D), 0.41 (E).

N^{α}-Phthalylglycylglycine Methyl Ester (37).[54] Treatment of **31** with CF_3COOH as described in **36A** produced **37** in yields of 60% (4 h treatment at 0 °C), 72% (4 h at 20 °C), and 79% (15 h at 20 °C) after recrystallization from CH_3OH-ether. The ensuing **37** (colorless needles[51]) had the same R_f values on TLC and infrared spectrum as those of authentic material obtained from Pht-Gly-OH and H-Gly-OMe with water-soluble carbodiimide:[55] mp 203-204.5 °C (lit.[54] 203-204 °C); no depression in mixture melting points with the authentic sample; R_f 0.51 (A), 0.71 (I).

Reference Compounds Prepared by Solution Synthesis, D-Alanine *tert*-Butyl Ester Hydrochloride (38). This compound was prepared from D-Ala following the procedure of Roeske[56] in 30% yield after recrystallization from EtOH-ether: mp 162-163 °C (lit.[57] mp 167 °C (cor) for L antipode); R_f 0.83 (I); $[\alpha]^{25}_D$ −0.95° (*c* 2, EtOH) [lit.[57] $[\alpha]^{20}_{546}$ +1.77° (*c* 2, EtOH) for L antipode]. Anal. ($C_7H_{16}NO_2Cl$) C, H, N.

N^{α}-Benzyloxycarbonylglycyl-L-alanine *tert*-Butyl Ester (39). DCCI[53] (5.16 g, 25 mmol) was added to a chilled solution of Z-Gly-OH (5.23 g, 25 mmol), H-L-Ala-OBu-*t*·HCl (4.54 g, 25 mmol), and NEt_3 (3.5 mL, 25 mmol) in THF (100 mL). The reaction mixture was stirred for 1 h at 0 °C and 15 h at 20 °C. The mixture was evap-

orated, and the residue was diluted with EtOAc. Dicyclohexylurea was filtered off and the solution was washed successively with 0.5 M $NaHCO_3$, 0.5 M citric acid, and H_2O, dried ($MgSO_4$), and concentrated in vacuo to leave a colorless oil, yield 8.35 g (99%), R_f 0.70 (A).

N^{α}-Benzyloxycarbonylglycyl-D-alanine *tert*-butyl ester (40) was prepared as described for **39** from H-D-Ala-OBu-*t*·HCl (8 mmol) to afford a colorless oil, 2.49 g (93%), R_f 0.70 (A).

N^{α}-Benzyloxycarbonylglycyl-L-alanine (41),[58,59] A solution of acyl dipeptide ester **39** (8.35 g, 24.8 mmol) in CF_3COOH (80 mL) was kept for 1 h at 0 °C. Evaporation of CF_3COOH was followed by the addition of ether and petroleum ether. The resulting solid was collected and recrystallized from EtOAc-petroleum ether: yield 4.85 g (70%); mp 129–130 °C; R_f 0.25 (A), 0.73 (I); $[\alpha]^{25}_D$ −9.55° (*c* 4, EtOH) (lit.[58] mp 133 °C, $[\alpha]^{20}_D$ −9.8° (*c* 4.4, EtOH); lit.[59] mp 130–131 °C; $[\alpha]^{25}_D$ −9.1° (*c* 4, EtOH)).

N^{α}-Benzyloxycarbonylglycyl-D-alanine (42)[60,61] was prepared in 66% yield from **40** as described for **41**: mp 128–129 °C; R_f 0.25 (A), 0.73 (**I**); $[\alpha]^{25}_D$ +9.51° (*c* 4, EtOH) (lit.[60] mp 119 °C; $[\alpha]^{23}_D$ +10.1° (*c* 2.9, EtOH); lit.[61] mp 135 °C; $[\alpha]^{23}_D$ +9.3° (*c* 5, EtOH)).

N^{α}-Benzyloxycarbonyl-L-leucylglycine *tert*-Butyl Ester (43). DCCI[53] (4.70 g, 22.8 mmol) was added to a cooled (−10 °C), stirred solution of Z-L-Leu-OH (6.05 g, 22.8 mmol), H-Gly-OBu-*t* (2.99 g, 22.8 mmol), and *N*-hydroxysuccinimide (2.62 g, 22.8 mmol) in THF (100 mL). Stirring was continued for 2 h at −10 °C and 15 h at 2 °C. Workup as described for **39** yielded an oil, 7.88 g (91%), R_f 0.79 (A).

L-Leucylglycine *tert*-Butyl Ester (44). Acyl dipeptide ester **43** (7.87 g, 20.8 mmol) was dissolved in 70 mL of CH_3OH and hydrogenated for 2 h in the presence of freshly prepared palladium black.[62] The filtrate was evaporated, the residue dissolved in EtOAc, and the solution washed with 0.5 M $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated to leave an oil, 3.70 g (73%), R_f 0.37 (A).

N^{α}-Benzyloxycarbonylglycyl-L-alanyl-L-leucylglycine *tert*-Butyl Ester (45). DCCI (206 mg, 1 mmol) was added to a cooled (−10 °C), stirred solution of **41** (280 mg, 1 mmol), compound **44** (244 mg, 1 mmol), and *N*-hydroxysuccinimide (115 mg, 1 mmol) in DMF (5 mL).[63] Stirring was continued for 2 h at −10 °C and for 24 h at 2 °C. The reaction mixture was then evaporated in vacuo, the residue triturated with EtOAc, the dicyclohexylurea removed by filtration, and the ensuing solution washed successively with 0.5 M $NaHCO_3$, 0.5 M citric acid, and H_2O, dried ($MgSO_4$), and evaporated to leave an oil (465 mg). Purification by silica gel column chromatography using $CHCl_3$–CH_3OH (96:4) as an eluent afforded an oil: 403 mg (80%); R_f 0.27 (A); $[\alpha]^{25}_D$ −43.07° (*c* 0.5, HOAc). Crystallization from ether–petroleum ether produced a white powder: 305 mg;[51] mp 152.5–153.5 °C with softening at around 114 °C; R_f 0.27 (A), 0.53 (**B**), 0.39 (G); $[\alpha]^{25}_D$ −43.11° (*c* 0.5, HOAc), −42.57° (*c* 0.5, CH_3OH), −31.89° (*c* 0.5, EtOH), and −22.85° (*c* 0.5, DMF). Anal. ($C_{25}H_{38}N_4O_7$) C, H, N.

N^{α}-Benzyloxycarbonylglycyl-D-alanyl-L-leucylglycine *tert*-Butyl Ester (46). Synthesis and workup as described for **45** using Z-Gly-D-Ala-OH (**42**) afforded an oil, 343 mg (68%), R_f 0.40 (A), which was crystallized from ether–petroleum ether: 240 mg;[51] mp 94–96 °C; R_f 0.40 (A), 0.60 (**B**), 0.46 (G); $[\alpha]^{25}_D$ −6.87° (*c* 0.25, HOAc), 0° (*c* 0.5, CH_3OH), +2.64° (*c* 0.5, EtOH), −12.44° (*c* 0.5, DMF). Anal. ($C_{25}H_{38}N_4O_7$) C, H, N.

N^{α}-Benzyloxycarbonylglycyl-L-alanyl-L-leucylglycine (47). Compound **45** (25.3 mg, 0.05 mmol) was treated with CF_3COOH (2 mL) for 30 min at 0 °C. Upon evaporation and addition of ether, a white solid was obtained which was recrystallized from CH_3OH–ether to afford a colorless powder: 18.4 mg[51] (82%); mp 228–230 °C dec; R_f 0.41 (F), 0.74 (**I**); $[\alpha]^{25}_D$ −36.36° (*c* 0.25, HOAc). Anal. ($C_{21}H_{30}N_4O_7 \cdot 1/2H_2O$) C, H, N.

N^{α}-Benzyloxycarbonylglycyl-D-alanyl-L-leucylglycine (48) was prepared as described for **47**, R_f 0.5 (F).

4CC Fragment Condensation. N^{α}-Benzyloxycarbonylglycylalanyl-N^{α}-(α-cyclohexylcarbamoyl-2-nitrobenzyl)leucylglycine *tert*-Butyl Ester (49). Cyclohexyl isonitrile (109 mg, 1 mmol) was added to a cooled (0 °C), stirred solution of Z-Gly-L-Ala-OH (**41**, 280 mg, 1 mmol), H-L-Leu-Gly-OBu-*t* (**44**, 244 mg, 1 mmol), and 2-nitrobenzaldehyde (151 mg, 1 mmol) in CH_3OH (1.5 mL). The reaction mixture was stirred for 1 h at 0 °C and 15 h at 20 °C. The solvent was then evaporated and the oily residue dissolved in EtOAc. The solution was washed successively with 0.5 M $NaHCO_3$, 0.5 M citric acid, and H_2O, dried ($MgSO_4$), and evaporated. The residue (670 mg) was purified by silica gel column (20 × 2.4 cm) chromatography with $CHCl_3$ as an eluent followed by $CHCl_3$–CH_3OH (96:4). The fractions containing material of R_f 0.48 (A) were combined and the solvent evaporated to afford **49** as an oil that was subjected to cleavage of the auxiliary group without further purification: yield of **49** 541 mg[49] (71%); R_f 0.48 (A); $[\alpha]^{25}_D$ +9.72° (*c* 1, $CHCl_3$). Anal. ($C_{39}H_{54}N_6O_{10} \cdot 1/2H_2O$) C, H, N.

N^{α}-Benzyloxycarbonylglycylalanyl-N^{α}-(α-cyclohexylcarbamoyl-2,4-dimethyloxybenzyl)leucylglycine *tert*-Butyl Ester (50). The above procedure was followed employing 2,4-dimethyloxybenzaldehyde (166 mg, 1 mmol) to give an oil: 584 mg[49] (75%); R_f 0.58 (A); $[\alpha]^{25}_D$ −42.35° (*c* 1, $CHCl_3$). Anal. ($C_{41}H_{59}N_5O_{10} \cdot 2H_2O$) C, H, N.

N^{α}-Benzyloxycarbonylglycylalanyl-N^{α}-(α-cyclohexylcarbamoyl-4-pyridylmethyl)leucylglycine *tert*-Butyl Ester (51). 4-Pyridinecarboxaldehyde was used under reaction conditions as described for **49**, Washing with 0.5 M $NaHCO_3$ and H_2O and workup provided an oil: 405 mg[51] (56%); R_f 0.36 (A); $[\alpha]^{25}_D$ −68.01° (*c* 1, $CHCl_3$). Anal. ($C_{38}H_{54}N_6O_8 \cdot 2.5H_2O$) C, H, N.

Z-Gly-Ala-Leu-Gly-OBu-*t* (45-4CC) by Photolysis of 49. Compound **49** (192 mg, 0.25 mmol) was irradiated as described for **33**, Product purification on a silica gel column (see **33**) yielded 99 mg (78%) of amorphous **45**, $[\alpha]^{25}_D$ −42.48° (*c* 0.25, HOAc). Crystallization from ether by dropwise addition of CH_3OH and *n*-hexane produced a white powder: 90 mg;[51] mp 152.5–154 °C; R_f 0.27 (A), 0.53 (**B**), 0.39 (G); $[\alpha]^{25}_D$ −44.99° (*c* 0.25, HOAc). Anal. ($C_{25}H_{38}N_4O_7$) C, H, N.

This material was indistinguishable from the above reference **45**, prepared by conventional methods, e.g., no depression in mixture melting points and identical IR.

Z-Gly-Ala-Leu-Gly-OH (47-4CC) by Trifluoroacetic Acid Treatment of 50. Compound **50** (156 mg, 0.2 mmol) was treated with CF_3COOH (4 mL) in the presence of anisole (0.4 mL) for 15 h at 20 °C. After evaporation of the solvent, the residual solid was dissolved in a mixture of DMF (2 mL) and $CHCl_3$ (1 mL). The solution was applied to a silica gel column (10 × 2.4 cm) and eluted with $CHCl_3$–CH_3OH–HOAc (83:15:2). The fractions containing material of R_f 0.41 (F) were combined. After evaporation of the solvent, ether was added to the residue. Recrystallization of the resulting solid (10 mg) from CH_3OH–ether yielded a white powder: 9 mg[51] (10%); mp 228–230 °C dec, no depression in mixture melting points with authentic **47**; R_f 0.41 (F), 0.74 (I); $[\alpha]^{25}_D$ −39.33° (*c* 0.25, HOAc); identical infrared spectrum with that of authentic **47**.

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(36) 4,4′-Dimethyloxybenzophenone, which is insoluble in methanol, ethanol, 1-butanol, and 2,2,2-trifluoroethanol, did not provide any product even when dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol.
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(43) Half-wave potentials of compound **27** in volts relative to the saturated calomel electrode were −0.78 (at pH 0.2 in 1 M HCl), −0.81 (pH 1, 0.1 M HCl), −0.87 (pH 2.25, 0.01 M HCl + 0.09 M KCl), −1.07 (pH 4.72, 0.1 M acetate buffer), and −1.19 (pH 7.0, 0.1 M phosphate buffer).
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Catalysis of the β Elimination of *O*-Phosphoserine and β-Chloroalanine by Pyridoxal and Zinc(II) Ion

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Abstract: The β-elimination reaction of *O*-phosphoserine and β-chloroalanine was investigated by proton NMR in deuterium oxide media at 30 ± 2 °C in the presence of pyridoxal and zinc ions. The reaction rate constants and relevant equilibrium constants were obtained for these systems. Catalysis by pyridoxal alone showed an increase in rate as pH was increased with a rate maximum in the region of pH ~9. Metal ion–pyridoxal catalysis was observed when zinc(II) was added to the amino acid–pyridoxal system. The observed catalysis is dependent on β-substituent electronegativity, and the rate-determining steps involve α-proton abstraction as well as β-substitution dissociation from the amino acid moiety. A new intermediate in the β-elimination reaction has been detected.

β elimination of electronegative substituents from α-amino acids is an interesting example of pyridoxal and metal ion catalysis. Gregerman and Christensen[1] have reported β elimination of chloride from β-chloroalanine, and Longenecker and Snell[2] have reported β elimination of phosphate from *O*-phosphothreonine and *O*-phosphoserine. The nonenzymatic reactions promoted by metal ions have mechanistic similarities to the corresponding enzymatic reactions. The mechanism involves the initial labilization of the α-hydrogen atom of the α-amino acid moiety following the condensation of α-amino acid and pyridoxal to form an aldimine Schiff base, **1**. β elimination then occurs when the β substituent is a reasonably