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Nucleophilic Reactivity of Peptides toward 2-Acyloxy-*N*-ethylbenzamides. The Utility of Free Peptides as Nucleophiles in Amide Bond Forming Reactions

D. S. Kemp,* Zmira W. Bernstein, and George N. McNeil

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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The free peptides (Gly-L-Leu-Gly)$_n$, n = 1, 2, 4, and 8, have been found to react slowly but cleanly with DMSO solutions of the *N*-ethylsalicylamide esters of Z(Gly-L-Leu-Gly)$_n$, n = 1, 2, and 4, to yield the sequence polymers, Z(Gly-L-Leu-Gly)$_n$OH, n = 2, 4, 8 and 16. The virtues and limitations of peptide synthesis using suspensions of peptides as nucleophiles are described.

The most commonly encountered amide-forming process in peptide synthesis involves reaction of an activated acyl derivative with a peptide derivative bearing a free N terminus and a blocked C terminus. In certain circumstances, it has been possible to obtain reasonable yields of clean products for coupling reactions in which the C-terminal blocking group of the nucleophilic component is reduced to a simple salt,[1] although difficulties can arise from insolubility and the necessary high basicity of the reaction medium. The simplest possible coupling situation would combine an N-blocked, C-activated peptide with a free, unblocked peptide as nucleophile. In this paper, we demonstrate that high yields of pure products can indeed be obtained with this procedure, provided that certain key conditions are met.

Two serious problems arise if one attempts to employ an amino acid or a free peptide as a reactive amine nucleophile. For all solvents of the aprotic type, the solubility of

$$\underset{\text{crystal}}{\underset{1}{NH_3^+\!\!-\!\!-\!\!-CO_2^-}} \overset{K_1}{\rightleftharpoons} \underset{\text{solution}}{\underset{2}{NH_3^+\!\!-\!\!-\!\!-CO_2^-}} \overset{K_2}{\rightleftharpoons} \underset{\text{solution}}{\underset{3}{NH_2\!\!-\!\!-\!\!-CO_2H}}$$

amino acids and peptides is low, presumably because the strong crystal lattice forces can be compensated for only by a solvent of high dielectric constant which can serve as both hydrogen bond donor and acceptor; on the other hand, in protic solvents, solvated material is present essentially exclusively as the zwitterion **2**, and the magnitude of this effect is essentially independent of chain length.[2]

We were led to attempt the present study through the conjecture that solubility in dipolar aprotic solvents should be lowest for amino acids and should converge to a value characteristic of the particular amide backbone as the peptide size is increased, and through the further conjecture that species larger than dipeptides should be present in solution in dipolar aprotic solvents as the neutral species **3** and not as the zwitterion **2**. If these conjectures are correct, then aminolysis of reactive acyl species should be possible using suspensions of free peptides in solvents such as DMF or DMSO and should occur with increasing ease as one changes the peptide size from small to medium. Should such a procedure be realizable, its mildness and simplicity might prove important advantages when designing coupling reactions between fragments in the 6–12 size range.

1. Results with Gly-L-Leu-Gly Peptides. Since we had previously prepared the peptide Z(Gly-L-Leu-Gly)$_2$OH and found this substance to be readily characterizable,[3] the coupling, Z-Gly-L-Leu-Gly-X with Gly-L-Leu-Gly, seemed an appropriate initial experiment. The active acyl derivative was chosen to be an *N*-ethylsalicylamide ester, despite

its rather low reactivity, since this species is readily available from the corresponding peptide acid, and it is not subject to any rapid decomposition reactions in neutral media.[4] In fact, when the tripeptide in the form of a fine crystalline precipitate was suspended and stirred in a DMSO solution containing 1 equiv of the *N*-ethylsalicylamide ester of Z-Gly-L-Leu-GlyOH for several hours, slow solution was observed, and after 48 hr at 22°, the mixture was homogeneous and no free peptide could be detected by tlc. Upon conventional work-up, a 88–92% yield of pure Z(Gly-L-Leu-Gly)$_2$OH was obtained. The product was obtained in higher yield and in a purer state than by alkaline saponification of the corresponding ethyl ester or by a 3-acyloxy-2-hydroxy-*N*-ethylbenzamide coupling with the tetramethylguanidine salt of Gly-L-Leu-Gly.[5] Investigation of DMF, hexamethylphosphoric triamide, sulfolane, *N*-methylpyrrolidone, or hexafluoroisopropyl alcohol revealed that use of any of these as solvent results in a reduced yield and longer reaction time than is required with DMSO, and all subsequent work was carried out in this solvent.

In an attempt to define the scope of this procedure, we applied it to the couplings, (Gly-L-Leu-Gly)$_n$ + *N*-ethylsalicylamide ester of Z(Gly-L-Leu-Gly)$_n$, where n = 2, 4, and 8. The results obtained are shown in Table I. The products of the latter three reactions are amorphous by X-ray powder pattern and are highly insoluble materials. Satisfactory elemental analyses were observed for all, although the substances were usually obtained as hydrates which retained residual water tenaciously. An independent test of product character was available for the n = 4 and 8 cases through use of radiolabeled starting material. Assuming product homogeneity, one can estimate the molecular weight of the product from its specific activity, and these estimates are reported in the last column of Table I.

The solubilities of HGly-L-Leu-GlyOH and H(Gly-L-Leu-Gly)$_2$OH in DMSO at 22° were found to be 1.1 and 17 mg/ml (4.6×10^{-3} and 3.6×10^{-2} M), respectively. The poor result for an attempted coupling in DMF is directly attributable to poor solubility, for in this solvent, the solubility of Gly-L-Leu-Gly is only 0.07 mg/ml. From these results and the results of yield determinations by isotopic dilution, one can calculate "one-point" rate constants of 0.4 M^{-1} min^{-1} for the [3 + 3] coupling and 0.07 M^{-1} min^{-1} for the [6 + 6] coupling. The former is of the magnitude expected for an unhindered aminolysis reaction of a peptide *N*-ethylsalicylamide ester[6] and therefore supports the conjecture that dissolved Gly-L-Leu-Gly is largely present as the neutral **3** and not as **2**. The fivefold difference in rate constants probably exceeds the error in the determinations and provides evidence for the common view that rates of coupling reactions are slower for reactions of large peptide fragments.

2. Other Cases. The cases just considered are atypical in that the coupling reactions occur between a pair of glycine residues, and are therefore expected to be abnormally rapid. In an attempt to examine a more representative case, we prepared the tripeptide derivative, Boc-L-Ala-L-(γOBz)Glu-L-PheOH, converted it to its *N*-ethylsalicylamide ester, and combined the latter in DMSO solution with L-Ala-L(γOBz)Glu-L-Phe; after a reaction period of 70 hr, the desired N-protected hexapeptide was isolated in 90% yield. Further oligomerization attempts were thwarted by the extreme insolubility of the hexapeptide, obtained by treatment with trifluoroacetic acid.

Oligomers of the sequence HGly-L-Pro-L-AlaOH are of interest as collagen models,[7] and several such species have been reported.[8] Examination of this case provided an especially severe test of our method, since peptides of the form

Table I
Synthesis of Z(Gly-L-Leu-Gly)$_n$ Sequence Polymers

2-[Z-(Gly-L-Leu-Gly)$_n$O]-*N*-ethylbenzamide + H(Gly-L-Leu-Gly)$_n$OH $\xrightarrow[22°]{\text{DMSO}}$ Z(Gly-L-Leu-Gly)$_{2n}$OH

Product	Reaction time, days	Yield, %[a]	Mol wt Calcd	Mol wt Obsd
Z(Gly-L-Leu-Gly)$_2$OH	2	89 (91)		
Z(Gly-L-Leu-Gly)$_4$OH	2	98 (97)	1035[b]	1080
Z(Gly-L-Leu-Gly)$_8$OH	8	58 (79)	2024[b]	2160
Z(Gly-L-Leu-Gly)$_{16}$OH	8	51		

[a] Yields in parentheses were obtained by isotopic dilution. The N-terminal Gly of the free peptide was labeled. [b] Hydrate is formed on work-up.

Table II
Yields and Rate Constants for Ala-Gly Couplings

A. Coupling Reactions with HGly-L-Pro-L-AlaOH and *N*-Ethylsalicylamide Esters

Acyl species	Nucleophile	Reaction time, days	Yield, %
Z-L-Ala	HGly-L-Pro-L-AlaOH	3	98
Z-L-Pro-L-Ala	HGly-L-Pro-L-AlaOH	2.5	92

B. Coupling Reactions of Ala-*N*-ethylsalicylamide Esters with HGlyOEt

Acyl species	Nucleophile	k, M^{-1} min^{-1}
Z-L-Ala	GlyOEt	0.45
Z-L-Pro-L-Ala	GlyOEt	0.50
Z-Gly-L-Pro-L-Ala	GlyOEt	0.45

HGly-ProX-OH are known to undergo formation of the diketopiperazine, Gly-Pro, under very mild conditions.[9] Although a coupling reaction between the *N*-ethylsalicylamide ester of Z-Gly-L-Pro-L-AlaOH and HGly-L-Pro-L-AlaOH was observed, it occurred anomalously slowly, and after 7 days at 22°, an isolated yield of only 54% of pure *Z* hexapeptide was observed. That this result is a peculiarity of this particular combination of active ester and nucleophile is shown by the data of Table II. The tripeptide nucleophile reacts satisfactorily with other C-terminal Ala esters, and the C-terminal tripeptide ester reacts at an expected rate with GlyOEt. (Although the rate constants of Table II, part B are inexact, being based on a "one-point" isotopic dilution for yield, they are sufficient to establish the important point that only the C-terminal amino acid affects the rate constant for reactions with GlyOEt.) The origin of the anomalous behavior of the [3 + 3] case remains unexplained; one possible explanation is unproductive association between the reactants, although it seems unlikely that a substantial effect of this kind could arise with molecules as small as tripeptides.

3. Summary. The above results establish that practical syntheses of large polypeptides can be achieved in DMSO solution using free peptides as nucleophiles. The nucleophile must be a tripeptide or larger, and it must be moderately soluble in DMSO; the activated acyl derivative must be stable in DMSO solution and must be sufficiently reactive to compensate for the low nucleophile concentration. It is clear that the *N*-ethylsalicylamide esters are too unreactive to be practical for peptide coupling reactions of average hindrance; moreover, they are expected to racemize to

the extent of several per cent under these reaction conditions.[10] It is also clear that without a means of controlling peptide solubility, the application of this procedure to a given coupling reaction must be attended by an unacceptable element of unpredictability. The importance of the successful synthesis of a octatetracontapeptide therefore hinges on the possibility of developing new stable, but satisfactorily activated acyl derivatives and side chain protective groups.

Experimental Section

All reagents and solvents were reagent grade. Amino acids were Calbiochem A grade. DMSO and tetramethylguanidine were distilled *in vacuo* from CaH_2. Optical rotations were carried out at 22°, using a Perkin-Elmer Model 141 polarimeter. Radioactive assays were carried out in dioxane-based counting solutions, using a Packard 3375 liquid scintillation spectrometer. Microanalyses were carried out by Scandinavian Microanalytical Laboratories. Unless otherwise indicated, analytical samples were dried for 24 hr at 65° (0.1 mm).

Z-Gly-L-Leu-GlyOH. The tetramethylammonium salt prepared by subjecting a solution of 6.07 g (46.2 mmol) of L-leucine in 42.1 g of 10% tetramethylammonium hydroxide to azeotropic distillation with benzene in a Dean-Stark apparatus was dissolved in 60 ml of DMSO containing 4.84 g (42 mmol) of tetramethylguanidine (TMG) and treated with 15.6 g (42 mmol) of 3-carbobenzoxyglycycloxy-2-hydroxy-*N*-ethylbenzamide under nitrogen. After 6 hr at 20°, the solvent was removed at 0.5 mm, and the residue was distributed between 100 ml each of 1 *N* HCl and ethyl acetate. The aqueous layer was extracted, and the combined organic phase was extracted with 0.5 *N* $NaHCO_3$. Acidification to pH 1 and extraction with ethyl acetate yielded an organic phase which was washed with 0.1 *N* HCl and water, dried, and evaporated. The solid was recrystallized from ethyl acetate to yield a first crop, 15.5 g, mp 143–145°, and a second crop, 0.66 g, mp 141–143°, $[\alpha]^{22}D$ −9.6° (*c* 2.1, EtOH), 97% (lit.[11] 143–144°, −9.5°). The resulting Z-Gly-L-LeuOH was converted to its oily 3-acyloxy-2-hydroxy-*N*-ethylbenzamide ester by reaction of its sodium salt in aqueous pyridine buffer with 11.6 g (46 mmol) of 2-ethyl-7-hydroxybenzisoxazolium fluoroborate, following the procedure previously reported for the ester of Z-Gly-L-PheOH. Addition of this ester in 80 ml of DMSO to a suspension of 3.14 g (42 mmol) of glycine in 25 ml of DMSO containing 9.17 g of TMG was followed 4 hr later by solvent removal and work-up as described above. Recrystallization from ethyl acetate–hexane yielded 13.24 g, mp 108.5–111°, and 0.27 g, mp 165–110°, 94%, $[\alpha]^{22}D$ − 15.2° (*c* 2.4, DMF) (lit.[12] 110°, −14.7°).

HGly-L-Leu-GlyOH. Hydrogenation in methanol–water solution at 1 atm for 10 hr of Z-Gly-L-Leu-GlyOH over 5% Pd/C gave, after filtration through Celite, washing of catalyst with hot water, concentration, and crystallization from water–EtOH, 92% tripeptide, mp 222–224°, $[\alpha]^{22}D$ −44.6° (*c* 2.0, H_2O) (lit.[13] 214–215°, −43.3°).

2-(Z-Gly-L-Leu-GlyO)-*N*-ethylsalicylamide. A solution of 2.79 g (7.38 mmol) of Z-Gly-L-Leu-GlyOH in 7.0 ml of 1 *N* NaOH, 5 ml of water, 0.7 ml of pyridine, and 15 ml of ethyl acetate was chilled and stirred vigorously as 2.3 g (9.8 mmol) of powdered 2-ethylbenzisoxazolium fluoroborate was added. Ethyl acetate (20 ml) was added, and after 3 min this was followed by 10 ml of 3 *N* HCl. After 15 min, the precipitated ester was collected and washed with 10 ml each of ethyl acetate, 1 *N* HCl, water, and ethyl acetate, and the organic filtrate was extracted with acid, $NaHCO_3$, and water, dried, and evaporated. Recrystallization from acetonitrile yielded 3.32 g, mp 170.5–171.5°, and 0.27 g, mp 169.0–70.0°, total 92.8%, $[\alpha]^{22}D$ −15.7° (*c* 1.5, DMF). *Anal.* Calcd for $C_{26}H_3N_4O_7$: C, 61.58; H, 6.51; N, 10.64. Found: C, 61.38; H, 6.52; N, 10.52.

Z(Gly-L-Leu-Gly)$_2$OH. To a solution of 2.63 g (5.00 mmol) of 2-(Z-Gly-L-Leu-GlyO)-*N*-ethylbenzamide in 42 ml of dry DMSO was added 1.23 g (5.01 mmol) of HGly-L-Leu-GlyOH as a finely powdered solid. The mixture was stirred magnetically for 45 hr at 22° in a flask equipped with a drying tube. At this time, all solid had dissolved, and the ninhydrin-positive peptide spot had disappeared by tlc. Removal of the solvent at 0.1 mm left an oil which was taken up in ethyl acetate and extracted with 10 ml of 1 *N* HCl. The aqueous extract was back extracted with six 10-ml portions of ethyl acetate. The combined organic extracts were washed with brine and dried briefly over $MgSO_4$. Recrystallization of the residue after evaporation and drying yielded 2.96 g (97.5%) of product, mp 161–165°, $[\alpha]^{22}D$ −20.7° (*c* 1.5, DMF), identical in all respects with a sample prepared by saponification of Z(Gly-L-Leu-Gly)$_2$OEt (lit.[3] 158–159°, −20.2°, −20.7°).

H(Gly-L-Leu-Gly)$_2$OH. Hydrogenation of Z(Gly-L-Leu-Gly)$_2$OH was carried out as described above, with periodic injections of sufficient water to dissolve product. The crude white solid obtained on work-up was triturated with ethanol and collected, 84%, mp 239–240°, $[\alpha]D$ −34.5° (*c* 1.2, HOAc). *Anal.* Calcd for $C_{20}H_{36}N_6O_7 \cdot 0.5H_2O$: C, 49.87; H, 7.76; N, 17.45. Found: C, 50.29; H, 7.69; N, 17.32.

2-[Z(Gly-L-Leu-Gly)$_2$O]-*N*-ethhthylbenzamide. A solution of 1.83 g (3.0 mmol) of Z(Gly-L-Leu-Gly)$_2$OH in 27 ml of water and 1.3 ml of pyridine was brought to pH 4.5 with 3 *N* HCl, overlayered with 30 ml of ethyl acetate, and treated with 0.8 g (3.3 mmol) of 2-ethylbenzisoxazolium fluoroborate. After 30 min at 22°, the mixture was worked up in the usual way. Recrystallization from acetonitrile gave 1.80 g (80%) of ester, mp 172–174°, $[\alpha]^{22}D$ −19.9° (*c* 1.5, DMF). *Anal.* Calcd for $C_{37}H_{51}N_7O_{10}$: C, 58.95; H, 6.82; N, 13.01. Found: C, 58.80; H, 7.08; N, 12.91.

Z(Gly-L-Leu-Gly)$_4$OH. In the usual way, 1.063 g (2.25 mmol) of H(Gly-L-Leu-Gly)$_2$OH was suspended in 25 ml of DMSO containing 1.695 g of 2-[Z(Gly-L-Leu-Gly)$_2$O]-*N*-ethylbenzamide. After 48 hr of stirring, the clear solution was concentrated *in vacuo* and the residual oil was triturated with 10 × 15 ml of Et_2O which was discarded. The resulting powder was collected, washed with water and ether, and dried, wt 2.36 g (98.5%), mp 247.5–250°; for analysis the substance was dissolved in 1 *N* $NaHCO_3$, filtered, acidified, collected, and washed with water and EtOAc, mp 244°, $[\alpha]D$ −33.6° (*c* 1.0, HOAc). *Anal.* Calcd for $C_{48}H_{76}N_{12}O_{15} \cdot 0.5H_2O$: C, 53.86; H, 7.27; N, 15.71. Found: C, 53.96; H, 7.40; N, 15.33.

H(Gly-L-Leu-Gly)$_4$OH·HBr. A solution of 0.192 g of Z(Gly-L-Leu-Gly)$_4$OH in 10 ml of HOAc, saturated with HBr, was allowed to stand at 22° for 45 min and then treated with ether. The resulting gummy solid was washed with ether and recrystallized from methanol–ether to yield 0.479 g (98%) of product. Recrystallization gave mp 174°, $[\alpha]^{22}D$ −17.3° (*c* 0.7, MeOH). *Anal.* Calcd for $C_{40}H_{71}N_{12}BrO_{13} \cdot 1.5H_2O$: C, 46.40; H, 7.22; N, 16.24; Br, 7.73. Found: C, 46.39; H, 7.33; N, 15.79; Br, 8.21.

2-[Z(Gly-L-Leu-Gly)$_4$O]-*N*-ethylbenzamide. A 0.273-g (0.26 mmol) sample of Z(Gly-L-Leu-Gly)$_4$OH was dissolved in 0.25 ml of 1 *N* NaOH, 0.5 ml of pyridine, and 10 ml of water, and 15 ml EtOAc was added, followed by 85.2 mg (0.36 mmol) of 2-ethylbenzisoxazolium fluoroborate at 0° with stirring. A gel formed almost immediately which was stirred for 1 hr at 22°, filtered, and washed with EtOAc, 2 ml of 1 *N* HCl, water, 0.5 *N* $NaHCO_3$, EtOAc, and water. Drying yielded 0.25 g (81%), mp 236–238° dec. For analysis, the substance was precipitated several times from DMF with ether, mp 238–240°, $[\alpha]D$ −32.0° (*c* 0.9, HOAc). *Anal.* Calcd for $C_{57}H_{85}N_{13}O_{16} \cdot H_2O$: C, 55.81; H, 7.16; N, 14.85. Found: C, 55.81; H, 7.16; N, 14.45.

Z(Gly-L-Leu-Gly)$_8$OH. To the suspension obtained from 0.310 g (0.25 mmol) of the above HBr salt and 31 mg (0.30 mmol) of triethylamine in 8 ml of DMSO was added 2-[Z(Gly-L-Leu-Gly)$_4$O]-*N*-ethylbenzamide, 0.372 g (0.30 mmol). After 9 days at 22°, the mixture was triturated with ether, and the resulting solid was collected, washed with water, 1 *N* HCl, water, and EtOAc, and then dried to yield 0.496 g (82%). For analysis, the product was precipitated with ether from hexafluoroisopropyl alcohol, mp >250°, $[\alpha]D$ −40° (*c* 0.1, formic acid) or −55.9° (*c* 0.15, dichloroacetic acid). *Anal.* Calcd for $C_{88}H_{144}N_{24}O_{27} \cdot 3H_2O$: C, 52.20; H, 7.48; N, 16.61. Found: C, 52.33; H. 7.37; N, 16.54.

H(Gly-L-Leu-Gly)$_8$OH·HBr. The procedure given for the dodecapeptide hydrobromide was followed. Recrystallization from methanol–ether yielded an analytical sample, $[\alpha]D$ −32.1° (*c* 0.3, HOAc). *Anal.* Calcd for $C_{80}H_{139}N_{24}O_{25}Br \cdot 2H_2O$: C, 49.19; H, 7.39; N, 17.21; Br, 4.09. Found: C, 49.11; H, 7.68; N, 16.68; Br, 4.27.

2-[Z(Gly-L-Leu-Gly)$_8$O]-*N*-ethylbenzamide. To a solution of 57.0 mg (0.05 mmol) of Z(Gly-L-Leu-Gly)$_8$OH in 6 ml of water, 3 ml of acetonitrile, 0.03 ml of 1 *N* NaOH, and 1.5 ml of pyridine was added 15.3 mg of 2-ethylbenzisoxazolium fluoroborate. A gel formed, 15 ml of EtOAc was added, and the slurry was stirred for 30 min, whereupon 6 ml of 1 *N* HCl was added and the mixture was filtered. Washing of the solid with water, EtOAc, and ether followed by drying yielded 37.5 mg (61%) of ester. Using isoxazolium salt, tritiated in the 5 and 7 positions, 5.9×10^{-3} μCi/mmol, ester with specific activity 6.5×10^{-3} μCi/mmol was obtained. For analysis, the ester was triturated in boiling acetonitrile and then precipitated with ether from hexafluoroisopropyl alcohol, $[\alpha]D$ −63.5° (*c* 0.69, dichloroacetic acid). *Anal.* Calcd for $C_{97}H_{153}N_{25}O_{28}$·

Table III
Properties of Peptide Derivatives

A. Free Peptides

Registry no.	Peptide[a]	Preparation	Yield, %	[α]D, deg		C	H	N
51876-87-0	1. Ala (γOBz)-Glu-Phe	BOC derivative + TFA Crystallized from MeOH–H_2O	80	+18.2 (*c* 0.8, HOAc)				
						$C_{24}H_{29}N_3O_6$		
					Calcd	63.28	6.42	9.22
					Found	63.04	6.45	8.92
837-83-2	2. Gly-Pro-Ala	*Z* derivative + H_2/Pd	78	−181 (*c* 0.1, H_2O) Lit.[8] −177				

B. 2-Acyloxy-*N*-ethylbenzamide Esters

Registry no.	Acid	Purification	Yield, %	Mp, °C	[α]D, deg		C	H	N
51933-26-7	1. Boc-Ala(γOBz)-Glu-Phe	$CHCl_3$–hexane	87	97–98	−39 (*c* 0.6, HOAc)				
							$C_{38}H_{46}N_4O_9$		
						Calcd	64.93	6.60	7.97
						Found	64.83	6.50	7.78
51876-88-1	2. Boc-(Ala(γOBz)-Glu-Phe)$_2$	CH_3CN	64		−30.4 (*c* 0.3, HOAc)				
							$C_{62}H_{73}N_7O_{14}\cdot H_2O$		
						Calcd	64.28	6.54	8.47
						Found	64.11	6.44	8.39
52022-29-4	3. Z-Gly-Pro-Ala	CH_2Cl_2–petroleum ether	98	105–106	−102 (*c* 1, HOAc)				
							$C_{27}H_{34}N_4O_7$		
						Calcd	61.82	6.15	10.68
						Found	61.66	6.01	10.85
51876-89-2	4. Z-Pro-Ala	EtOAc	90	130–133	−119 (*c* 0.5, HOAc)				
							$C_{25}H_{29}N_3O_6$		
						Calcd	64.22	6.01	8.80

C. Peptide Acids and Esters

Registry no.	Product	Preparation	Yield, %	Mp, °C	[α]D, deg	Purification		C	H	N
51876-90-5	1.- Boc-Ala-(γOBz)Glu-PheOH	From Boc-Ala-(γOBz)Glu + Phe, method of ref 5	64	88–90	−11 (*c* 1, HOAc)	MeOH–water				
							$C_{29}H_{37}N_3O_8$			
							Calcd	62.69	6.71	7.56
							Found	62.19	6.69	7.42
51876-91-6	2. Boc(Ala-(γ-OBz)Glu-Phe)$_2$OH	Method of this paper	90 (crude)		−21 (*c* 1.5, HOAc)	Gel from MeOH				
							$C_{53}H_{64}N_6O_{13}\cdot 0.5H_2O$			
							Calcd	63.49	6.62	8.38
							Found	63.40	6.61	8.43
5891-41-8	3. Z-Gly-Pro-AlaOH	Z-Gly + Pro-Ala method of ref 5	88	147–149	−117 (−120) (*c* 1, MeOH)	EtOH–petroleum ether				
51876-92-7	4. Z-Ala-Gly-Pro-AlaOH	Method of this paper Z-Ala + Gly-Pro-Ala	48		−86.7 (3.2, HOAc)	EtOAc–ether				
							$C_{21}H_{28}N_4O_7\cdot 1.5H_2O$			
							Calcd	53.03	6.58	11.78
							Found	53.43	6.18	11.74
51876-93-8	5. Z-Pro-Ala-Gly-Pro-AlaOH	Method of this paper Z-Pro-Ala + Gly-Pro-Ala	92	166–170, 190	−133 (*c* 0.9, HOAc)	CH_3CN				
							$C_{26}H_{35}N_5O_8\cdot 0.5H_2O$			
							Calcd	56.30	6.55	12.63
							Found	56.31	6.31	12.53
51876-94-9	6. Z-Pro-Ala-Gly-OEt	See text	87	148–149	−95.4 (*c* 1, HOAc)	EtOAc–petroleum ether				
							$C_{20}H_{27}N_3O_6$			
							Calcd	59.24	6.72	10.36
							Found	59.25	6.60	10.31
51876-95-0	7. Z-Gly-Pro-Ala-Gly-OEt	See text	70	143–145	−98.4 (*c* 0.7, HOAc)	EtOAc–petroleum ether				
							$C_{22}H_{30}N_4O_7$			
							Calcd	57.12	6.55	12.12
							Found	57.21	6.36	12.08

[a] All amino acids have the L configuration.

H_2O: C, 54.55; H, 7.33; N, 16.40. Found: C, 53.91; H, 7.33; N, 16.40.

Z(Gly-L-Leu-Gly)$_{16}$OH. A suspension of 21.0 mg (9.8 μmol, of the above ester and 19.8 mg (9.6 μmol) of H(Gly-L-Leu-Gly)$_8$OH·HBr in 0.25 ml of DMSO containing 1.05 mg (10 μmol) of triethylamine was stirred for 24 hr, at which point a gel formed and a further 0.1 ml of solvent was added. After 8 days, the mixture was triturated with water, and the gel was collected, washed with water and ethyl acetate, and dried to yield 21.6 mg (57%). This extremely insoluble substance was triturated in a mortar with ether and dried *in vacuo*. *Anal.* Calcd for $C_{168}H_{280}N_{48}O_{51}\cdot 10H_2O$: C, 50.83; H, 7.63; N, 16.94. Found: C, 51.17; H, 7.84; N, 16.55.

Determination of Solubility and Yield by Isotopic Dilution. Example. A weighed sample of [1-^{14}C]-Gly-L-Leu-Gly, 0.74 μCi/mmol, was suspended in 2 ml of solvent in a sealed ampoule in a 30° bath. At 30-hr intervals, samples were filtered and aliquots of the filtrate were counted. In DMF, after 27, 41, and 75 hr, 455, 400, and 451 dpm/ml were observed corresponding to 6.8, 6.0, and 6.7 mg/ml.

Yields by isotopic dilution were determined by adding labeled product to the initial reaction mixture and recrystallizing the recovered product to constant activity.

Other Peptide Derivatives. Table III reports properties for other peptides prepared in this study. Detailed experimental procedures may be found in Z. Bernstein, Ph.D. Dissertation, Massachusetts Institute of Technology, 1971.

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Registry No.—Z-Gly-L-Leu-GlyOH, 16295-38-8; L-leucine, 61-90-5; 3-carbobenzoxyglycyloxy-2-hydroxy-*N*-ethylbenzamide, 16859-24-8; Z-Gly-L-LeuOH, 1421-69-8; 3-carbobenzoxyglycylleucyloxy-2-hydroxy-*N*-ethylbenzamide, 51876-76-7; glycine, 56-40-6; HGly-L-Leu-GlyOH, 2576-67-2; 2-(Z-Gly-L-Leu-Gly)-*N*-ethylsalicylamide, 51876-77-8; Z(Gly-L-Leu-Gly)$_2$OH, 51876-78-9; H(Gly-L-Leu-Gly)$_2$OH, 2576-71-8; 2-[Z(Gly-L-Leu-Gly)$_2$O]-*N*-ethylbenzamide, 51876-79-0; Z(Gly-L-Leu-Gly)$_4$OH, 51876-80-3; H(Gly-L-Leu-Gly)$_4$OH·HBr, 51876-81-4; 2-[Z(Gly-L-Leu-Gly)$_4$O]-*N*-ethylbenzamide, 51876-82-5; Z(Gly-L-Leu-Gly)$_8$OH, 51876-83-6; H(Gly-L-Leu-Gly)$_8$OH·HBr, 51876-84-7; 2-[Z(Gly-L-Leu-Gly)$_8$O]-*N*-ethylbenzamide, 51876-85-8; Z(Gly-L-Leu-Gly)$_{16}$OH, 51876-86-9; TFA, 76-05-1.

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Photobenzidine Rearrangements. V. Mechanistic Aspects. Rearrangement of Mixtures of Different *N,N'*-Dimethylhydrazo Aromatics, and the Nature of the Excited State[1-3]

Jiin-Duey Cheng and Henry J. Shine*

Department of Chemistry, Texas Tech University, Lubbock, Texas 79409

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Quantum yields of rearrangement of *N,N'*-dimethyl-*p*-hydrazotoluene (**1a**) to the *o*-semidine (**2a**) under irradiation in cyclohexane solution at 298 nm were unaffected by the triplet quencher, 1,3-cyclohexadiene. None of **2a** could be detected in the products of irradiation at 335 ± 2.5 nm in the presence of the triplet sensitizer, xanthone. The rearrangement appears to occur in the singlet excited state. Irradiation of a mixture of **1a** and *N,N'*-dimethyl-*p*-hydrazobiphenyl (**1d**) at 350 nm led to the formation of a new hydrazo compound, 4-phenyl-*N,N',4'*-trimethylhydrazobenzene (**1e**). Irradiation of a mixture of *N,N'*-dimethyl-*p*-hydrazoanisole (**1b**) and *N,N'*-dimethylhydrazomesitylene (**1c**) at 300 nm also gave a new hydrazo compound, *N,N'*-2,4,6-pentamethyl-4'-methoxyhydrazobenzene (**1f**). Irradiation of **1f** at 300 nm led to the formation of **1b** and **1c**. Crossed rearrangement products (*i.e.*, crossed semidines) were not found. It is proposed that although radicals are probably involved in the formation of scission products (*N*-methylarylamines), the formation of *o*-semidines may be intramolecular, and the formation of new hydrazo compounds may involve biomolecular, four-center reactions.

In contrast with acid-catalyzed and thermal reactions of hydrazo aromatics,[4,5] little is known about photochemical ones. The few studies that have been made show that hydrazobenzene and ring-substituted hydrazobenzenes are dehydrogenated by irradiation in solution,[6-8] whereas *N,N'*-dimethylhydrazobenzenes rearrange.[6,9] No mechanistic details are known about these rearrangements, however, and the present paper describes our attempts to obtain some understanding of them. We have tried to find if the photochemical rearrangements are intra- or intermolecular and, also, whether they occur in the singlet or triplet excited state. For the former purpose we have carried out rearrangements of mixtures of hydrazo compounds and of one unsymmetrical hydrazo compound and have searched for "crossover" rearrangement products. For the latter purpose we have measured the fluorescence and phosphorescence characteristics of several of the hydrazo compounds, to establish singlet and triplet state characteristics, and have made quantum yield measurements for rearrangement of a representative compound, *N,N'*-dimethyl-*p*-hydrazotoluene (**1a**) in the absence and presence of triplet quenchers.

Results

Irradiation of Mixtures of Hydrazo Aromatics. A. *N,N'*-Dimethyl-*p*-hydrazotoluene (1a) and *N,N'*-Dimethyl-*p*-hydrazobiphenyl (1d). Although **1a** rearranges slowly when irradiated at 300 nm (19% yield after 10 hr),[9] it did not rearrange after 14 hr of irradiation at 350 nm in cyclohexane solution. On the other hand, **1d** rearranged quite readily under the latter conditions. When a mixture of **1a** and **1d** was irradiated in cyclohexane solution at 350 nm for 14 hr at room temperature, almost 90% of the **1a** and 65% of the **1d** were recovered. Rearrangement of **1d** to the *o*-semidine (**2d**) and scission to *N*-methyl-4-aminobiphenyl (**3d**) and 4-aminobiphenyl (**4d**) occurred also. At the same time a new hydrazo aromatic (**1e**) was formed, too (Scheme I).