Registry No.—3, 24978-13-0; 7, 24978-14-1.

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Solvent Modification in Merrifield Solid-Phase Peptide Synthesis

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Occasionally during the use of Merrifield solid-phase peptide synthesis from seemingly simple syntheses, steps occur where part of the peptide chain stops growing.[2-5] We encountered such a step at glutamine during the synthesis of the peptide H_2N-Ser-Arg-Phe-Gly-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Ser-Pro-Phe-Gly-Lys-COOH.[6] Although the use of trifluoroacetic acid[2] and double couplings[4] in two different solvents improved our synthesis somewhat, the use of a mixed solvent system of methylene chloride and dimethylformamide (DMF) gave the best results for our peptide. We interpret this to mean that our step was caused by a tertiary structure peculiar to this peptide, and we suggest that this solvent system may be generally useful for problems of this type.

Table I shows the results of several experiments using a variety of deblocking agents, coupling reagents, and reaction times. Each experiment was run in triplicate. Figure 1 is a graphic interpretation of Table I. Only the first five residues, HOOC-Lys-Gly-Phe-Pro-Ser-NH_2, could be completely coupled using methylene chloride as the solvent for dicyclocarbodiimide (DCCI), even when trifluoroacetic acid in methylene chloride was used for deblocking. Only 70% of the sixth amino acid, glutamine, could be added as an active ester in DMF within 6 hr. However, if 1.5 *M* of urea was added to DMF, glutamine could be added to an extent of 90% after 6 hr and the reaction was complete after 24 hr. If DMF ($^1/_3$ by volume) was added to the DCCI–methylene chloride couplings and allowed to react 6 hr, glycine (7th), glutamic acid (8th), and alanine (9th) could be coupled completely. If only DCCI–methylene chloride was used, just 50% of the chain continued to grow. Knowing this, amino acids 6 through 13 were coupled using DMF while it was not necessary for the coupling of the remaining three amino acids.

It should be mentioned that Merrifield,[2] while showing the usefulness of trifluoroacetic acid, actually used DCCI with DMF and methylene chloride as solvents in adding histidine while making bradykinin, since histidine was partly dissolved in DMF for the coupling. It appears that while deblocking with trifluoroacetic acid can overcome many of the chain-termination problems in peptide synthesis, the combination of deblocking with trifluoroacetic acid and coupling with DCCI in DMF and methylene chloride might prove more satisfactory.

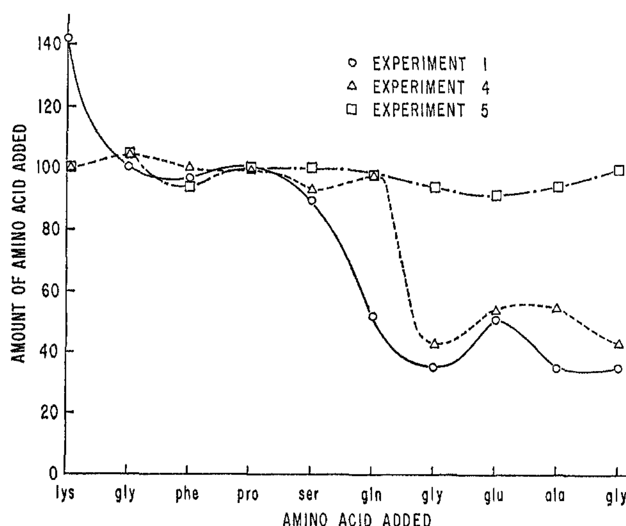

Figure 1.—Graphical interpretation of experiments 1, 4, and 5.

Experimental Section

Dry chloromethylated copolystyrene–2% divinylbenzene (20 g) (Biorad Beads S-X-2, 200–400 mesh, capacity 1.1 milliequiv/g) was mixed with 20 mm of both triethylamine and *e*,N-carbobenzoxy-*a*,N-*t*-butoxylysine in 80 ml of ethanol. The mixture was refluxed for 46 hr. The resin was washed in ethanol, methylene chloride, water and methanol and then dried. The resin contained 0.2 mmol of blocked lysine per gram of resin. The following cycle of deprotection, neutralization, and coupling was carried out on 1 g of resin with a total solution volume of 10 ml for each residue added: (1) three washes with the deprotecting solvent–acetic acid, propionic acid, or methylene chloride; (2) 30 min of reacting with the deprotecting agent—acetic acid and 1 *M* HCl, propionic acid and 0.8 *M* HCl, both with 1% by volume mercaptoethanol, or 50% trifluoroacetic acid in 50% methylene chloride with 5% by volume mercaptoethanol;[7] (3) three washes with the deprotecting solvent—acetic acid, propionic acid, or methylene chloride; (4) two washes with ethanol; (5) three washes with chloroform; (6) neutralization for 10 min with a mixture of 12.5% by volume of triethylamine and 87% by volume of chloroform; (7) three washed with chloroform; (8) three washes with methyl chloride if DCCI coupling or three washes with DMF if active ester coupling; (9) the coupling step depended upon the experiment and the amino acid being added as shown in Table I. It consists of one of the following procedures: (A) addition of 5 ml of methylene chloride containing 2.2 mmol of blocked amino acid and equilibration for 10 min, (B) addition of 5 ml of a solution of DMF and methylene chloride (60:40) containing 2.2 mmol of blocked amino acid with 10 min of equilibration time, or (C) addition of 10 ml of DMF containing 1.5 *M* urea with 4 mmol of the active ester of glutamine; (10) addition of 3 ml of DCCI (66 gm DCCI/400 ml of methylene chloride) followed by 2 ml of methylene chloride. This step is not performed for active ester additions. Coupling times are given in Table I.

Periodically, 8 mg of deblocked peptide resin was dried and hydrolyzed with 1 ml of concentrated HCl and 1 ml of propionic acid for 2 hr at 130° in a sealed tube.[8] From preliminary results,

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(4) Private communication from Dr. Robert Colescott.
(5) Unpublished results of Professor A. B. Robinson while making portions of cytochrome c.
(6) This peptide was made for Dr. E. Eylar at The Salk Institute for Biological Studies, La Jolla, Calif.

(7) Mercaptoethanol is unstable in trifluoroacetic acid and another reducing agent is more advisable. Unpublished observations of J. Sharp and F. Westall.
(8) Unpublished procedure of J. Scotchler and R. Losier.

TABLE I

Expt no.	Deblocking agent[a]	Coupling agent[b]	Coupling time, hr	Amino acids added[c] 1 O-Lys	2 Gly	3 Phe	4 Pro	5[d] Ser	6 Gln	7 Gly	8 Glu	9 Ala	10 Gly	11[e] Trp	12[d] Ser	13 Gly	14 Phe	15 Arg	16[d] Ser
1	HOAc–HCl	DCCI–CH_2Cl_2	6	1.44	1.01	0.98	1.00	0.90		0.34	0.49	0.35	0.32	0.30	0.36	0.31	0.40		0.35
	1% $HO(CH_2)_2SH$	Active esters–DMF	6						0.53										
		DCCI–DMF, CH_2Cl_2 (3:7 ml)	6															0.40	
2	Propionic–HCl	DCCI–CH_2Cl_2	6	1.20	1.05	1.00	1.00	0.93		0.35	0.60	0.39	0.36	0.30	0.41	0.35	0.46		0.39
	1% $HO(CH_2)_2SH$	Active esters–DMF	6						0.58										
		DCCI–DMF, CH_2Cl_2 (3:7 ml)	6															0.35	
3	TFA–CH_2Cl_2	DCCI–CH_2Cl_2	6	1.11	1.02	0.98	1.00	0.92											
	5% $HO(CH_2)_2SH$	Active esters–DMF							0.70										
4	TFA–CH_2Cl_2	DCCI–CH_2Cl_2	6	1.00	1.05	1.00	1.00	0.93		0.42	0.53	0.53	0.42						
	5% $HO(CH_2)_2SH$	Active ester–DMF, urea	24						0.98										
5	TFA–CH_2Cl_2	DCCI–CH_2Cl_2	6	1.00	1.02	0.96	1.00	1.00											
	5% $HO(CH_2)_2SH$	DCCI–DMF, CH_2Cl_2 (3:7 ml)	6							0.93	0.88	0.94	1.00	0.90	0.94	1.02	1.00		0.96
		Active ester–DMF, urea	24						0.97									0.98	
6	TFA–CH_2Cl_2	DCCI–CH_2Cl_2	6	1.01	1.01	0.98	1.00	1.00											
		Active ester–DMF, urea	24						0.98										
		DMF–DCCI coupling followed by CH_2Cl_2–DCCI coupling	6							0.80									
7	TFA–CH_2Cl_2	DCCI–CH_2Cl_2		1.00	0.98	1.00	0.98	0.96											
	5% $HO(CH_2)_2SH$	Active esters–DMF																	
		DCCI–DMF, CH_2Cl_2 (3:7 ml)																	
		Active ester–DMF, urea							0.97										
		CH_2Cl_2–DCCI coupling followed by DMF–DCCI coupling	6							0.81									

[a] Deblocking agents: HOAc saturated with HCl–propionic acid saturated with HCl–trifluoroacetic acid, methylene chloride, mercaptoethanol (40:55:5 by volume). [b] Coupling agents: 1.5 mmol DCCI per 10 ml of methylene chloride, 1.5 mmol of active ester dissolved in DMF with 1% acetic acid, 1.5 mmol DCCI dissolved in 3.0 ml of DMF (pH 7) and 7 ml of methylene chloride, 1.5 mmol of active ester dissolved in DMF with 1% acetic acid and 1.5 *M* in urea. [c] As determined by amino acid analysis of 2 hr propionic acid–HCl (1:1) hydrolysis. Values given in this table are based on total addition of proline as 1.00. Error ±5%. [d] Serine values are adjusted to take into account serine destruction upon hydrolysis. Approximately 20% of the serine was destroyed during hydrolysis. [e] Tryptophan values were determined spectrophotometrically by the procedure of Patcharnik: A. Patcharnik, W. B. Lawson, and B. Witkop, *J. Amer. Chem. Soc.*, **80,** 4747 (1958).

it appears that all peptide bonds are routinely hydrolyzed. Amino acid analysis was performed with a Beckman amino acid analyzer which has an estimated accuracy of 5%.

Registry No.—DMF, 68-12-2; methylene chloride, 75-09-2.

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Synthesis of α,α-Dinitro-N′-fluorodiimide N-Oxides[1]

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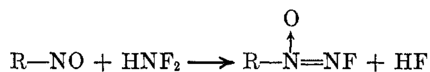
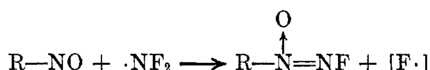
Syntheses of N′-fluorodiimide N-oxides have been reported by reactions of tetrafluorohydrazine[2-6] or di-





fluoramine[3,7] with nitroso compounds. Pseudonitroles gave α-nitro-N′-fluorodiimide N-oxides,[3,7] but α,α-dinitro-N′-fluorodiimide N-oxides have not been prepared directly; α,α-dinitro nitroso compounds are unknown.

In the present work, 1,1-dinitrobutyl-N′-fluorodiimide N-oxide was isolated from the reaction of the sodium salt of 1,1-dinitrobutane with tetrafluorohydrazine in methanol. The product was identified by analysis, and ir and nmr spectra. Most significantly, the ^{19}F signal, −125 ppm from trifluoroacetic acid, was in the region reported for other N′-fluorodiimide N-oxides. The mechanism for this reaction may involve 1,1-dinitro-1-nitrosobutane as a transient intermediate. The nitrosating agent may be nitrous acid resulting from the Neff reaction of the starting material; 1,1-dinitrobutane was also formed. An acid source is the abstraction of hydrogen from the solvent to give difluoramine which is readily dehydrofluorinated.

Preliminary work on this reaction was done with the salt of 1,1-dinitroethane, but the product was such a sensitive explosive that characterization could not be completed. The salt of nitroform did not react under these conditions. Sodium 2-propanenitronate on the other hand yielded only the coupling product, 2,3-dimethyl-2,3-dinitrobutane, as reported by Freeman.[8]

Experimental Section

Caution. Explosion shielding and remote manipulation are required for the N_2F_4 reaction and for product isolation.

1,1-Dinitrobutyl-N′-fluorodiimide N-Oxide.—A Fischer–Porter aerosol tube containing a solution of 14.8 g (0.10 mol) of 1,1-dinitrobutane and 0.10 mol of sodium methoxide in 45 ml of methanol was evacuated at liquid nitrogen temperature and filled with nitrogen several times. The tube was charged with 0.2 mol of tetrafluorohydrazine and the mixture was stirred for 20 hr at ambient temperature. The excess tetrafluorohydrazine was removed and most of the solvent was removed under vacuum. Methylene chloride (50 ml) was added and the solution was filtered and distilled to give 6.5 g of liquid, bp 46° (0.35 mm), which contained some 1,1-dinitrobutane. Chromatography with a 2 × 38 cm column of neutral active alumina and methylene chloride resulted in retention of the 1,1-dinitrobutane on the column as a bright yellow complex. Distillation of the eluent gave 1.3 g (6.2% yield) of 1,1-dinitro-1-butyl-N′-fluorodiimide N-oxide, bp 34–35° (0.15 mm).

Anal. Calcd for $C_4H_7N_4FO_5$: C, 22.86; H, 3.33; N, 26.7; F, 9.05. Found: C, 23.20; H, 3.17; N, 26.63; F, 9.0.

The proton nmr spectrum consisted of a triplet (J = 8 Hz) at δ 1.12 for CH_3, a multiplet at δ 1.9 for CH_3CH_2, and a triplet (J = 8 Hz) at δ 3.12 for the other methylene. The fluorine spectrum consisted of a broadened singlet at −125 ppm from external trifluoroacetic acid. The infrared spectrum consisted of bands at 3.42 (m), 3.53 (m), 6.4 (vs), 6.9 (m), 7.01 (m), 7.3 (m), 7.54 (s), 9.05 (w), 10.8 (w), 11.7 (m), 12.4 (m), and 13.2 μ (m).

Registry No.—1,1-Dinitrobutyl-N′-fluoridiimide N-oxide, 24903-89-7.

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Radical Anions Produced by Electrochemical Reduction of 1,3 Diketones[1]

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The half-life of radicals obtained by electrolysis of enolized 1,3 diketones is short because of rapid coupling reactions.[2] In DMSO as solvent, the reduction of the enolate anion of a 1,3 diketone causes decomposition *via* cleavage reactions.[2b] This latter fact appears to be inconsistent with one aspect of the pioneering work of Bauld and coworkers on the electron spin resonance (esr) spectra of dianion radicals.[3-6] These workers reported[6] esr data for the dianion radical formed by the electrochemical reduction of the dibenzoylmethide ion in DMF. On the basis of our observations of the electrochemical behavior of 1,3 diketones, we suggest that

(1) This work was supported by Grant No. GP-8350, National Science Foundation. Support for computing was provided by the National Science Foundation and the Wisconsin Alumni Research Foundation.

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