Table I. Nitrogen-Enhanced Negative Ion Mass Spectra of Benzene, Naphthalene, and Anthracene[a]

	Relative intensity			
m/e	Benzene[b]	Enhancement factors for benzene[c]	Naphthalene[d]	Anthracene[d]
12	1.5 ± 0.3		6.0	11.0
24	10.2 ± 1.9	1.3	7.1	17.0
25	19.3 ± 1.6	1.2	8.5	20.5
26	100.0	49.8	100.0	100.0
27	3.0 ± 0.3	49.5	2.0	2.0
36	2.3 ± 0.2	1.1	2.2	3.6
48	2.9 ± 0.1	1.0	1.0	2.2
49	3.1 ± 0.2	1.0	4.5	6.6
50	3.0 ± 0.2	3.0		7.3
60	0.4 ± 0.1	1.2		0.1
61				0.5
62				0.4
66				0.9
72	1.5 ± 0.2	1.2	0.8	4.7
73	1.0 ± 0.1	1.2	0.1	0.6
74	0.5 ± 0.1	1.0		2.0
79	0.2 ± 0.1	20.1		
84				0.6
96				2.0
97				1.8
121				0.9
127				2.3
128			2.8	2.8
129			0.7	0.7
145				1.4
177				1.0
178				3.5
179				2.4
180				1.0

[a] Intensities below 0.1 neglected because of the high gain factor and concurrent noise. [b] Sample pressure, $1.0 \pm 0.05 \times 10^{-6}$ torr; nitrogen pressure, $6.0 \pm 0.2 \times 10^{-5}$ torr. Average of ten spectra with average deviations. [c] Benzene, sample pressure, $1.0 \pm 0.05 \times 10^{-6}$; intensity = 1.0, compared with spectra taken under conditions of column 1,[b] which were recorded immediately after the reference spectra. Ratios of absolute intensities are averages of six spectra; deviations were less than 10%. [d] Sample pressure, $1.0 \pm 0.2 \times 10^{-6}$ torr; nitrogen pressure, $6.0 \pm 0.2 \times 10^{-5}$ torr. Averages of six spectra; average deviations were less than 10% of the relative intensity.

nique allows one to immediately distinguish the ions that are formed by process 1 or 2 as opposed to 3. High enhancement, as with *m/e* 26, clearly indicates a resonance capture process. Enhancement factors between 1.5 and 2.0 could indicate some resonance capture or collisional stabilization of the negative ion. Enhancement factors are not given for naphthalene or anthracene because these compounds were introduced through the solid probe and the spectra in the absence of an enhancing gas show variable relative intensities. In both cases, however, parent ion clusters were not observed immediately prior to and following the nitrogen-enhanced spectra recorded here, in which parent ions are prominent. In the presence of N_2 the molecule ions increased in relative and absolute intensity in the series $C_6H_6 < C_{10}H_8 < C_{14}H_{10}$, in accord with the increasing electron affinity in the series.[7] Ion–molecule reactions can still occur under these conditions, *e.g.*, *m/e* 79 in the spectrum of benzene.

Gas enhancement of negative ion mass spectra appears to offer a substantial improvement in the prospects for use of negative ion mass spectra as models for reduction processes and radiolysis reactions. If enhanced negative ion mass spectra are tabulated for these purposes, it would be useful if a specific gas and pressure were generally adopted.

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Solid-Phase Peptide Coupling

Sir:

Stepwise solid-phase peptide synthesis[1] has provided a rapid method for preparation in high yield of amino acid sequences of several peptide hormones.[2-6] In applying this approach to the total synthesis of the enzyme staphylococcal nuclease,[7] we have prepared protected peptide fragments by treating the *t*-butyloxycarbonyl (BOC) hydroxysuccinimide ester of glutamic acid or of aspartic acid with the peptide cleaved from the Merrifield polymer[8] and, in a special case, by hydrazinolysis of the protected peptide from the polymer as the hydrazide.[9] These fragments may be purified before coupling to give longer sequences.

We report here the efficient coupling of soluble protected di-, tri-, and tetrapeptides to the amino terminus of another peptide attached to the Merrifield polymer. To minimize racemization of the carboxyl component, N-ethyl-5-phenylisoxazolium-3′-sulfonate (NEPIS),[10] N,N′-dicyclohexylcarbodiimide (DCC) plus N-hydroxysuccinimide (HOSu),[11] and the azide coupling methods were applied.

In a typical experiment, 106 mg (0.10 mmol) of *t*-BOC-γ-benzyl-L-glutamyl-ε-carbobenzoxy(Z)-L-lysyl-ε-Z-L-lysyl-O-benzyl-L-serine was activated with NEPIS (25.3 mg, 0.10 mmol) and 0.10 mmol of triethylamine in dimethylformamide, then added in fourfold excess to H_2N-L-leucyl-L-prolyl polymer (0.025 mmol, 83.3 mg) suspended in dimethylformamide. The reaction mixture was shaken at room temperature for 3 hr, and the resulting peptide polymer was washed with dimethylformamide, methanol, and ether to remove the soluble reactant, then was cleaved and deprotected with hydrogen bromide in trifluoroacetic acid at room temperature for 90 min. The polymer was removed by filtration and washed with trifluoroacetic acid and methylene chloride. The hexapeptide HBr·H_2N-Glu-Lys-Lys-Ser-Leu-Pro-OH was obtained from

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(2) A. Marglin and R. B. Merrifield, *J. Amer. Chem. Soc.*, **88**, 5051 (1966).
(3) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).
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(6) J. Meienhofer and Y. Sane, *ibid.*, **90**, 2966 (1968).
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(8) C. B. Anfinsen, D. Ontjes, M. Ohno, L. Corley, and A. Eastlake, *Proc. Nat. Acad. Sci. U. S.*, **58**, 1806 (1967).
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(10) R. B. Woodward and R. A. Olofson, *ibid.*, **83**, 1007 (1961).
(11) F. Weygand, D. Hoffmann, and E. Wünsch, *Z. Naturforsch.*, *B*, **21**, 426 (1966); J. E. Zimmerman and G. W. Anderson, *J. Amer. Chem. Soc.*, **89**, 7151 (1967).

(7) C. A. Coulson and A. Streitweiser, Jr., "Dictionary of π-Electron Calculations," W. H. Freeman, San Francisco, Calif., 1965.

the filtrate after evaporation upon trituration with ether (17.7 mg, 90%, mp 163–170° dec).

The product was characterized as follows: the NH_2-terminal residue was determined by the dansyl chloride method[12] to be glutamic acid, without a trace of the previous leucine amino terminus; thin layer chromatography gave a single spot (R_f 0.1),[13] and high-voltage electrophoresis gave single spots in pyridinium acetate buffer pH 3.6 (R_f 0.78) and at pH 6.5 (R_f 0.61), with L-lysine as reference (R_f 1.0); digestion with aminopeptidase M[14] (AP-M) was complete as judged by paper and thin layer chromatography and gave ratios upon amino acid analysis of Glu 0.96, Lys 1.66, Ser 1.00, Leu 1.0, Pro 0.86, confirmed by acid hydrolysis (6 *N* HCl, sealed evacuated tube, 110°, 20 hr) and consistent with 100% coupling efficiency without racemization at the carboxyl-terminal serine residue. Coupling conditions with NEPIS, in which reaction time was held to 2 hr with fourfold excess of soluble tetrapeptide and in which only twofold excess was used for 6- to 48-hr reaction time, gave incomplete reactions.

The same peptide coupling reaction was carried out with DCC plus hydroxysuccinimide (HOSu) as the coupling agent.[11] Complete coupling without detectable racemization was obtained in 2 hr at room temperature. Dansylation showed NH_2-terminal glutamic acid only and complete digestion with AP-M yielded the following amino acid ratios: Glu 1.06, Lys 1.99, Ser 1.01, Leu 1.0, Pro 0.98. Reaction for 4 hr at 0°, or reaction at room temperature using DCC without HOSu, gave a product in which dansyl analysis demonstrated a small proportion (<10%) of amino-terminal leucine and electrophoresis gave an additional minor spot.

The tripeptide *t*-BOC-L-Leu-L-Ala-L-Tyr-OH (0.19 mmol, 89 mg) in dimethylformamide was activated with equimolar NEPIS and triethylamine at 0° and added in fourfold excess to H_2N-(ε-trifluoroacetyl-L-lysyl)$_5$ polymer, shaken for 2 days at room temperature, washed, and cleaved with HBr–TFA to yield the partially protected octapeptide H_2N-Leu-Ala-Tyr-(ε-TFA-Lys)$_5$-OH as the HBr salt. Thin layer chromatography gave a single spot in three systems.[15] After removal of the ε-TFA groups with piperidine at 0° for 1 hr, electrophoresis gave a single spot with R_f 1.35 (Lys, R_f 1.0) at pH 3.6; dansyl analysis showed only leucine as the amino terminus. Digestion with aminopeptidase M gave Leu 1.09, Ala 0.94, Tyr 1.01, Lys 5.0. For comparison, the same octapeptide product was prepared from *t*-BOC-L-Leu-L-Ala-L-Tyr-$NHNH_2$ (0.10 mmol, 48 mg) by conversion at −15° with 4 equiv of HCl in dioxane and 4.4 equiv of freshly prepared $NaNO_2$ to the azide and prompt addition to the penta-ε-TFA-lysyl polymer at 0–4° for 20 hr. The crude product obtained upon cleavage gave R_f values identical with those above in chromatographic and electrophoretic systems, but contained about one-third unreacted pentalysine as estimated from amino acid ratios after acid hydrolyses and enzyme digestions.

(12) W. R. Gray in "Methods in Enzymology," Vol. XI, C. H. W. Hirs, Ed., Academic Press, New York, N. Y., 1967, p 139.

(13) 1-Butanol–acetic acid–pyridine–water (4:1:1:2).

(14) 2% AP-M by weight in 0.07 *M* phosphate buffer, pH 7.8; volume 0.3 ml; 37° overnight. (AP-M obtained from Henley and Co., New York, N. Y.).

(15) (a) R_f 0.86;[13] (b) 1-butanol–acetic acid–water (4:1:5), R_f 0.71; (c) pyridine–water (80:20), R_f 0.67.

Although several model peptides have been coupled successfully to peptide polymers, our preliminary experience in coupling sequences of the staphylococcal nuclease indicates that refinement of conditions may be required when using longer peptides or when coupling certain pairs of carboxyl- and amino-terminal residues. For example, coupling the hydrophobic octapeptide or tetrapeptide sequences (both containing COOH-terminal valine) corresponding to residues 32–39 or 36–39 of staphylococcal nuclease[7] to the nonapeptide polymer containing residues 40–48, with NH_2-terminal aspartic acid, gave only 20–30% coupling with either NEPIS or DCC. The aspartic acid residue may offer special difficulty. Thus, the dipeptide Z-L-Phe-L-Tyr-OH was coupled readily with DCC-HOSu to Leu-Pro polymer (aminopeptidase digestion gave Phe 1.03, Tyr 0.98, Leu 1.0, Pro 1.03; dansyl showed all Phe; thin layer chromatography and electrophoresis systems all gave a single spot), but under identical conditions was coupled only to the extent of 40% to the β-benzyl-L-aspartyl-terminal nonapeptide polymer.

Nevertheless, the modification of the solid-phase synthetic approach described here offers the advantages of fewer steps in a long sequence and of convenience in substituting residues in structural analog studies.

Furthermore, coupling peptide fragments rather than individual amino acid monomers should give a major advantage in the purification of the polypeptide product. The peptides with incomplete sequences that accumulate from less than quantitative coupling at any step might be removed if they differ from the completed peptide by the several residues constituting any fragment rather than by single residues.

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On the Mechanism of Oxidative Decarboxylation. The Potassium Persulfate Promoted Decarboxylation of Substituted Phenylacetic Acids

Sir:

Conflicting opinion on the detailed steps of the mechanism for the anodic oxidation of the salts of carboxylic acids has raised the question as to whether a stepwise process (A) is involved in the electrochemical reaction[1,2] or whether the rate-determining step is a concerted process[3] (B).

$$RCO_2^- \longrightarrow [RCO_2\text{---e (anode)}]^{\ddagger} \longrightarrow RCO_2\cdot + e \quad (A)$$
$$RCO_2\cdot \longrightarrow R\cdot + CO_2$$
$$RCO_2^- \longrightarrow [R\text{---}CO_2\text{---e (anode)}]^{\ddagger} \longrightarrow R\cdot + CO_2 + e \quad (B)$$

Mechanistically analogous reactions carried out in homogeneous solution which formally lead to carboxylate radicals have been discussed in terms of one-bond or multibond scission. The classic work of

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(2) B. E. Conway, "Theory and Principles of Electrode Processes," The Ronald Press, New York, N. Y., 1965, pp 136, 166 ff, 244 ff.

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