740 (1974); (b) L. T. J. Delbaere, M. N. G. James, N. Nakamura, and S. Masamune, *J. Am. Chem. Soc.*, **97**, 1973 (1975).
(14) M. J. S. Dewar, *J. Am. Chem. Soc.*, **74**, 3345 (1952); *Tetrahedron Suppl.*, **8**, 75 (1966); *Angew. Chem., Int. Ed. Engl.*, **10**, 761 (1971).
(15) R. Breslow, *Pure Appl. Chem.*, **28**, 111 (1971).
(16) D. Poppinger, Private communication.
(17) K. Kuchitsu, T. Fukuyama, and Y. Morino, *J. Mol. Struct.*, **1**, 463 (1968).
(18) N. L. Allinger and J. C. Tai, *Theor. Chim. Acta*, **12**, 29 (1968).
(19) For a review see G. Maier, *Angew. Chem., Int. Ed. Engl.*, **13**, 425 (1974).
(20) G. Maier and B. Hoppe, *Tetrahedron Lett.*, 861 (1973).
(21) S. Masamune, M. Suda, H. Ona, and L. M. Leichter, *J. Chem. Soc., Chem. Commun.*, 1268 (1972).
(22) The weak extinction coefficient ($\epsilon \sim 100$) observed for this band[20,21] would indicate that the transition is probably dipole forbidden.
(23) Queen Elizabeth II Fellow, 1973–1975.

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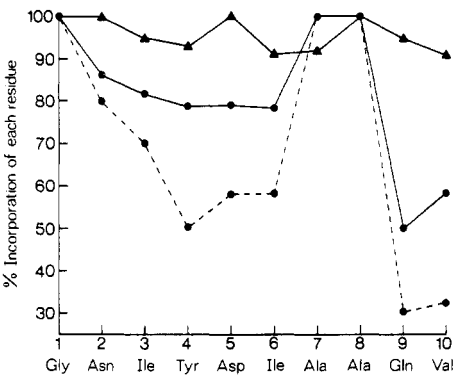

Figure 1. Amino acid incorporation during the solid-phase synthesis of acyl carrier protein, residues 65–74: ●----●, on polystyrene by an essentially standard procedure;[5] ●—● on polystyrene using double acylation and other modifications;[5] ▲—▲, present work on polydimethylacrylamide.

Polyamide Supports for Polypeptide Synthesis

Sir:

We report the preparation and use in peptide synthesis of a polyamide resin specifically designed to overcome problems inherent in the use of styrene-based polymers. The principles guiding the design of this resin have been described previously,[1] as have earlier experiments based on modification of commercial polyacrylamide.[2] In the synthesis of the stringent test sequence described below, these resins have given results clearly superior to those obtained elsewhere using the customary polystyrene support.

The cross-linked polydimethylacrylamide resin was prepared by persulfate-initiated emulsion copolymerization of a mixture of monomer, cross-linking agent, and functionalizing agent. Solutions containing freshly distilled dimethylacrylamide (5.47 g), *N,N'*-bisacryloylethylenediamine (1.1 g),[3] and ammonium persulfate (0.6 g) in water (50 ml), and cellulose acetate butyrate (3.3 g) and *N-tert*-butoxycarbonyl-β-alanyl-*N'*-acryloylhexamethylenediamine (1.35 g) in 1,2-dichloroethane (100 ml) were mixed at 52° under nitrogen and stirred at 500 rpm for 4.25 hr. The insoluble, washed resin was largely beaded but contained some amorphous polymer. The β-alanine content was 0.33 mmol/g. The resin swelled in dimethylformamide and acetic acid to approximately ten times its dry bed volume, rather more in water, but very much less in methylene chloride and other less polar organic solvents. These properties are the reverse of those of polystyrene based resins. Utility of the new resin in solid phase peptide synthesis is illustrated[4] with the decapeptide sequence residues 65–74 of acyl carrier protein. This difficult sequence has been studied previously in great detail on polystyrene,[5] but no satisfactory synthesis has hitherto been described.

The synthesis was initiated by cleavage of the butoxycarbonyl group and addition of a spacer-internal reference BOC-amino acid (leucine) as its symmetrical anhydride. The first amino acid of the sequence proper (glycine) was added as the substituted benzyl ester-activated ester derivative, Boc-Gly-$OCH_2C_6H_4CH_2CH_2COOC_6H_2Cl_3$ (2,4,5), simultaneously incorporating a labile peptide–polymer linkage. Subsequent amino acids were added as symmetrical anhydrides[6] or *p*-nitrophenyl esters (asparagine and glutamine). The side chains of aspartic acid and tyrosine were protected as the benzyl ester and 2,6-dichlorobenzyl ether,[7] respectively. One full synthetic cycle contained the following steps: (1) *t*-AmOH, 5 × 2 min; (2) AcOH, 5 × 2 min; (3) 1 *N* HCl–AcOH, 1 × 5 min, 1 × 25 min; (4) AcOH, 5 × 2 min; (5) *t*-AmOH, 5 × 2 min; (6) DMF, 10 × 2 min; (7) 10% NEt_3–DMF, 3 × 2 min; (8) DMF, 5 × 2 min; (9) coupling, sixfold excess of BOC-amino acid anhydride, DMF, 1 × 120 min; (10) DMF, 5 × 2 min. For activated ester couplings, steps 9 and 10 were replaced by: (9a) sixfold excess of 1-hydroxybenzotriazole, DMF, 1 × 5 min; (10a) DMF, 3 × 2 min; (11) coupling, sixfold excess of BOC-amino acid *p*-nitrophenyl ester, sixfold excess of 1-hydroxybenzotriazole, DMF, 1 × 240 min; (12) DMF, 5 × 2 min. Qualitative ninhydrin tests[8] for residual amine were performed during coupling steps 9 or 11 and compared with the fully deprotected resin at step 8. In all cases negative results were obtained after the reaction times indicated. Amino acid incorporations were estimated by hydrolysis of resin samples and amino acid analysis. The results are given in Figure 1 together with those obtained elsewhere[5] on polystyrene.

Eighty-eight percent of the peptide chains were retained on the final resin, an average loss of 1.4% per cycle due to acidic cleavage of the benzyl ester in agreement with the results of Gutte and Merrifield.[9] The yield of peptide–polymer from 1 g of starting resin (not taking into account the 23 analytical samples removed during the course of the synthesis) was 1.27 g (theory 1.50 g). Cleavage of the peptide–resin (190 mg) with liquid HF in the presence of anisole for 1 hr at 0° gave40.8 mg of 0.1 *M* NH_4OH soluble product (theory 39.7 mg), actual peptide content 33.3 μmol (found: Asp, 2.11; Glu, 0.99; Gly, 1.10; Ala, 1.99; Val, 0.96; Ile, 1.96; Tyr, 0.90).[10] Chromatography of an aliquot equivalent to 15.6 μ*M* of this material on diethylaminoethylcellulose DE-52 using a linear gradient of 0.01–0.5 *M* ammonium bicarbonate, pH 8.1, gave the elution profile shown in Figure 2. Recovery of the main peak gave 7.48 μmol of peptide (48%) (found: Asp, 2.06; Glu, 1.02; Gly, 1.02; Ala, 1.97; Val, 0.97; Ile, 1.95; Tyr, 1.02). One-fifth of this material was rechromatographed under the same conditions and gave a single peak containing 0.82 μmol of peptide (55% recovery)[11] (found: Asp, 1.98; Glu, 1.03; Gly, 1.03; Ala, 1.98; Val, 1.00; Ile, 1.92; Tyr, 1.06). A single ninhydrin-reacting spot R_{Asp} 0.19 was obtained on electrophoresis at pH 6.5.

We draw attention to the following points relating to the use of the new solid support. (1) All the reactions are carried out in highly polar organic media in which both the peptide and polymer chains should be fully solvated.[1] (2) The coupling reactions appear to be very fast and a negative ninhydrin reaction is commonly obtained within a few minutes of the start. (3) Active ester couplings appear to be very sensitive to the presence of traces of formic acid which cause irreversible chain termination. Caution should therefore be exercised in the use of HCl–formic acid for deprotection.[12] We are now examining the use of dimethylacet-

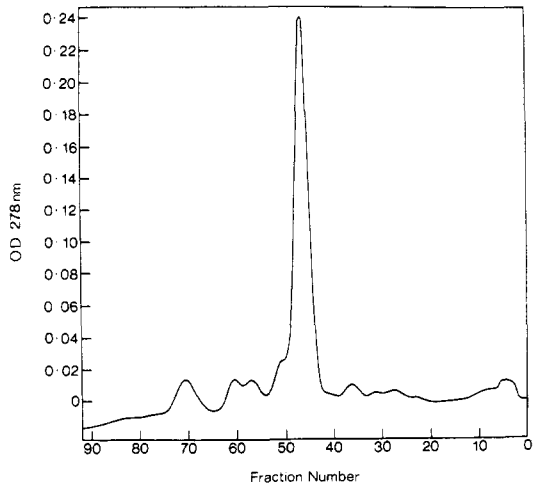

Figure 2. Chromatography of synthetic acyl carrier protein residues 65-74 on diethylaminoethylcellulose DE 52. For conditions see text.

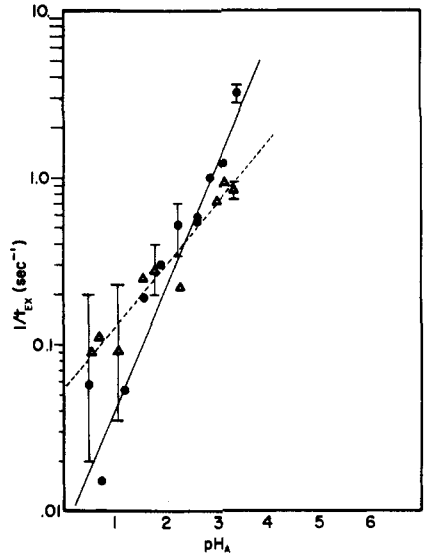

Figure 1. Exchange rate vs. apparent pH for asp-phe-OMe with and without La(III): solid circles, 0.1 *M* peptide, 0.5 *M* La(III); unfilled triangles; 0.1 *M* peptide. Linear regression lines are drawn through each set of points. Error bars based on an integral ratio accuracy of ±10% are given for some representative points.

amide in place of dimethylformamide as a general solvent since traces of acetic acid are much less serious in this respect. (4) Incorporation of the labile peptide-polymer linkage with the first amino acid attached and under the same reaction conditions as subsequent couplings is advantageous and offers considerable flexibility. This feature can also be applied to polystyrene-based systems. (5) The excellent swellability of the resin in both water and polar organic solvents has suggested application to polynucleotide synthesis and to protein sequencing studies. Encouraging results have been obtained in both these areas.

References and Notes

(1) R. C. Sheppard, Proceedings of the 11th European Peptide Symposium, Vienna, 1971, North Holland Publishing Co., Amsterdam, 1973, p 111.
(2) E. Atherton and R. C. Sheppard, Proceedings of the 13th European Peptide Symposium, Israel, 1974, Wiley, New York, N.Y., 1975, p 123.
(3) Commercial polyacrylamide is usually cross-linked with methylenebisacrylamide (*N,N'*-bisacryloylmethylenediamine). In an extension of the work described in ref 2, we have prepared polyacrylamide cross-linked with *N,N'*-bisacryloylethylenediamine. This has given more reproducible results in peptide synthesis than the commercial resin, presumably because of the liberation of traces of formaldehyde or its equivalent from the latter.
(4) We have previously prepared other test sequences on alkylated polyacrylamides (e.g., the Merrifield–Dorman tetrapeptide Leu-Ala-Gly-Val, and bradykinin) but these are also easily synthesized on polystyrene.
(5) W. S. Hancock, D. J. Prescott, P. R. Vagelos, and G. R. Marshall, *J. Org. Chem.*, **38**, 774 (1973).
(6) F. Flor, Chr. Birr, and Th. Wieland, *Justus Liebigs Ann. Chem.*, 1601 (1973); H. Hagenmaier and F. Hartmut, *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1973 (1972).
(7) D. Yamashiro and C. H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).
(8) E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(9) B. Gutte and R. B. Merrifield, *J. Am. Chem. Soc.*, **91**, 501 (1969).
(10) Amino acid analyses are uncorrected for hydrolytic losses and are normalized to ten residues (cf. R. S. Hodges and R. B. Merrifield, *Int. J. Pept. Protein Res.*, **6**, 397 (1974)).
(11) It is evident that significant losses of this peptide occur on chromatography over and above the purification achieved.
(12) M. Ohno, S. Tsukamoto, S. Makisumi, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **45**, 2852 (1972).

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Catalysis of Amide Proton Exchange by Lanthanum Ions

Sir:

This communication reports studies stemming from our observation that a cation, tripositive lanthanum, increases base catalyzed amide proton exchange in a dipeptide. The method of study is via transfer of saturation NMR spectroscopy. The experiments were performed in a solvent of 80% H_2O and 20% D_2O, the deuterium being required for field locking. The dipeptide is a biologically active one, L-aspartyl-L-phenylalanine methyl ester, which has the property of tasting 200 times sweeter than sucrose.[1] We have been studying the conformation of the compound and analogs using the lanthanide ion probe technique,[2] and this observation of cation induced base catalysis, not previously reported for peptides, came about during that work.

Considerable work has gone into measurement of amide proton exchange as has been reviewed by Englander.[3] The rationale behind this approach is that amide proton exchange may be a sensitive indicator of polypeptide and protein conformation.

The transfer of saturation method[4] allows one to study amide proton exchange in cases where the characteristic lifetime, t_{ex}, for the exchange process is in the region from about 50 msec to 5 sec in systems where the observed amide proton spin lattice relaxation time, T_1, is of the order of 1 sec. Our method of performing the experiments allows us to conveniently study the phenomenon using a commercial Fourier transform NMR spectrometer without recourse to correlation spectroscopy.[5] In a paper to follow we will report additional measurements and comparison work with other ions and peptides.

L-Aspartyl-L-phenylalanine methyl ester was obtained through a gift from the Searle Chemical Corp. as a salt-free purified powder. It was checked for purity by thin layer chromatography in two solvent systems (butanol:acetic acid:water, 7:1:2 and 4:1:5). NMR samples were made up for standard 5-mm tubes by dissolving the solid peptide in solutions of stoichiometric ratios of H_2O:D_2O (99.8% isotopic purity) and adjusted to apparent pH's (pH_a read on a calibrated Radiometer Model 26 pH meter equipped with an Ingold "spaghetti" 3 mm combination electrode) with small amounts of HCl:DCl and NaOH:NaOD of the same proton to deuteron ratios. The pH's were measured before and after each NMR experiment. No attempt was made to outgas samples. $LaCl_3$ was used as the anhydrous salt labeled 99.99% obtained from Research Organic/Inorganic Chemical Corp., Sun Valley, Calif.

NMR experiments were performed on a JEOL-PFT-100