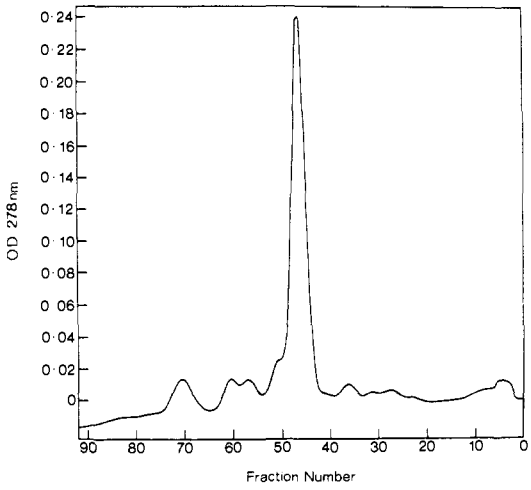

Figure 2. Chromatography of synthetic acyl carrier protein residues 65-74 on diethylaminoethylcellulose DE 52. For conditions see text.

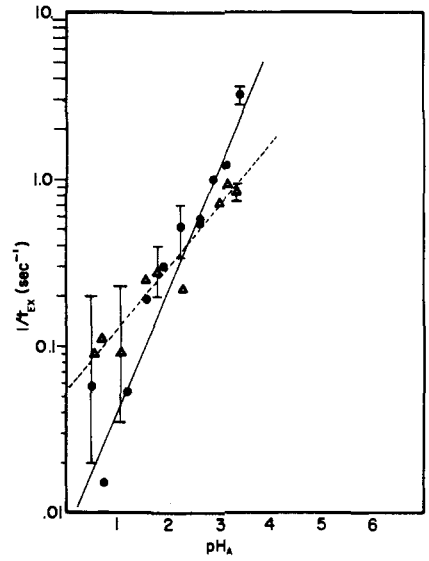

Figure 1. Exchange rate vs. apparent pH for asp-phe-OMe with and without La(III): solid circles, 0.1 *M* peptide, 0.5 *M* La(III); unfilled triangles; 0.1 *M* peptide. Linear regression lines are drawn through each set of points. Error bars based on an integral ratio accuracy of ±10% are given for some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

Table I. Amide Proton Spin-Lattice Relaxation Times Proton Counts, $T_{1,i}$ and $1/t_{ex}$ as a function of observed pH in 80% H_2O:20% D_2O Solutions[a]

pH_a	$1/T_{1,obsd}$ (sec^{-1})	P_{obsd}	$1/T_{1,i}$ (sec^{-1})	$1/t_{ex}$ (sec^{-1})
	Asp-Phe-OMe ≃ 0.1 *M*			
0.54	1.51 ± 0.02	0.94	1.42	0.091
0.70	1.42 ± 0.05	0.92	1.29	0.11
1.095	1.53 ± 0.07	0.94	1.44	0.092
1.57	1.56 ± 0.02	0.84	1.31	0.25
1.79	1.49 ± 0.04	0.81	1.20	0.28
2.30	1.56 ± 0.05	0.86	1.33	0.22
3.00	1.75 ± 0.05	0.57	0.99	0.75
3.13	1.88 ± 0.05	0.50	0.95	0.94
3.32	1.88 ± 0.14	0.56	1.05	0.83
	Asp-Phe-OMe ≃ 0.1 *M* La(III) = 0.5 *M*			
0.50	1,42 ± 0.05	0.96	1.36	0.057
0.75	1.51 ± 0.02	0.99	1.49	0.015
1.01	1.53 ± 0.04	1.00	1.53	0.000
1.20	1.78 ± 0.03	0.97	1.72	0.054
1.60	1.69 ± 0.03	0.89	1.51	0.19
1.92	1.88 ± 0.06	0.84	1.58	0.30
2.29	2.72 ± 0.05	0.77	1.75	0.52
2.66	3.03 ± 0.07	0.81	2.43	0.58
2.67	2.63 ± 0.09	0.80	2.12	0.53
2.90	4.46 ± 0.14	0.78	3.57	1.00
3.50	4.00 ± 0.14	0.70	2.77	1.20
3.40	6.54 ± 0.15	0.51	3.45	3.27

[a] Concentration of asp-phe-OMe: 25 ± 5 mg/ml, 30°C. The standard error in the regression coefficient $1/T_1$ is shown and we estimate the error in the integral ratio P to be ± 10%.

spectrometer at 100 MHz. The basic commercial instrument had previously been modified by us to incorporate a solvent "suppression-by-saturation" feature of our own design.[6] This allows the use of either normal FT or automatic T_1 (180-t-90° sequences) features of the instrument in up to 90% H_2O solvent systems. Our T_1 determinations were performed using at least ten points per T_1 determination followed by a regression analysis of the data taken from spectra. Determination of the proton count for the amide proton was performed by comparing the integral under the amide peaks to that of the 5 proton peak in the phenylalanine aromatic band. This, of course, requires that there is not differential Overhauser enhancement of the two signals due to direct interaction with the solvent. We checked for this by determining the ratios of the integrals at pH_a 1.32 ± 0.02 (at which acidity exchange effects are negligible) in samples containing 80, 70, 60, and 50% H_2O, respectively. A linear relationship was found which extrapolated to 0.23 at 100% H_2O. This indicates that either there are no Overhauser effects or that they are the same for amide and phenylalanine protons. In either case our method of determining the amide proton count is valid in this peptide.

Figure 1 shows the results of plotting the reciprocals of exchange lifetimes vs. pH for ≃0.1 *M* peptide and ≃0.1 *M* peptide in 0.5 *M* La(III) solutions. The equations $T_{1,i} = T_{1,obsd}/P$ and $t_{ex} = T_{1,obsd}/(1 - P)$ were used to analyze the data. $T_{1,i}$ is the intrinsic T_1 for the amide proton corrected for exchange and P is the ratio of the number of amide protons observed to the number that should be present in 80% H_2O based upon the five aromatic protons. The values of the data and derived parameters are given in Table I. The results can be interpreted quantitatively with good agreement using the data obtained by Molday et al.[7] from their studies of model compounds. On the basis of this the lack of acid catalyzed exchange is accounted for by the presence of the positively charged amino group. For asp-phe-OMe alone the slope of $1/t_{ex}$ vs. pH is 0.37 ± 0.04 based on a linear regression analysis while in the presence of La(III) the value becomes 0.74 ± 0.09. A completely base catalyzed exchange process would have a slope of 1.00.[3] However, the pK of the aspartyl carboxyl group in this peptide is 3.1 and therefore in the pH region in which we are observing the slopes may be smaller due to a competition of rate processes[7] and the expected onset of acid catalyzed exchange below pH 1. Our present hypothesis is that the La(III) binding to the aspartyl γ-carboxyl results in a net electron withdrawing effect on the amide nitrogen. This inductive effect is known to promote base catalysis.[3] The competitive displacement of the carboxyl proton of aspartic by the La(III) ion results in a slope of $1/t_{ex}$ vs. pH closer to 1 and in a further reduction of expected acid catalyzed exchange at low pH. It is possible that the effect of lanthanum ions in promoting amide exchange may prove useful as a tool in conformational investigations of peptides and proteins.

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References and Notes

(1) R. H. Mazur, J. M. Schlatter, and H. H. Goldkamp, *J. Am. Chem. Soc.*, **91**, 2684 (1969).
(2) C. D. Barry, J. A. Glasel, A. C. T. North, R. J. P. Williams, and A. V. Xavier, *Nature (London)*, **232**, 236 (1971).
(3) S. W. Englander, N. W. Downer, and H. Teitelbaum, *Annu. Rev. Biochem.*, **41**, 903 (1972).
(4) R. K. Gupta and A. G. Redfield, *Biochem. Biophys. Res. Commun.*, **41**, 273 (1970).
(5) T. P. Pitner, J. D. Glickson, J. Dadok, and G. Marshall, *Nature (London)*, **250**, 582 (1974).
(6) H. E. Bleich and J. A. Glasel, *J. Magn. Reson.*, **18**, 401 (1975).
(7) R. S. Molday, S. W. Englander, and R. G. Kallen, *Biochemistry*, **11**, 150 (1972).

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A Photochemical Method for the Introduction of Strained Multiple Bonds; Benzyne C≡C Stretch

Sir:

We wish to describe a photochemical method for the introduction of multiple bonds which is suitable for use in the synthesis of highly reactive molecules. The method is based on the 3-diazobutyrolactone part structure and is designed to build in strain energy in a series of steps such that introduction of the double bond occurs in a process involving strain relief in a high energy intermediate. Thermodynamic driving force is provided by the elimination of a small, stable molecule (nitrogen or carbon monoxide) at each step. The method is illustrated by facile synthesis of benzyne which permits observation of a carbon-carbon triple bond stretching frequency.

Irradiation of 3-diazobenzofuranone (**1**)[1] at low temperatures[2] gives two primary products, **2** (2150 cm^{-1}, Figure 1; λ_{max}^{2MeTHF} 255, 286, 293 nm, Figure 2)[2] and **3** (2040 cm^{-1}, Figure 1; λ_{max}^{2MeTHF} 462 nm, Figure 2).[2,3] The primary photoproducts readily interconvert photochemically with long wavelength (>350 nm) light favoring the ketene (**2**) and short wavelength light (254 nm) favoring **3**. Continued irradiation with short wavelength light decarbonylates the ketene, presumably giving the carbene (**4**). The carbene