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 $\simeq$ 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 $\simeq$ 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 $\simeq$0.1 *M* peptide and $\simeq$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 p*K* 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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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

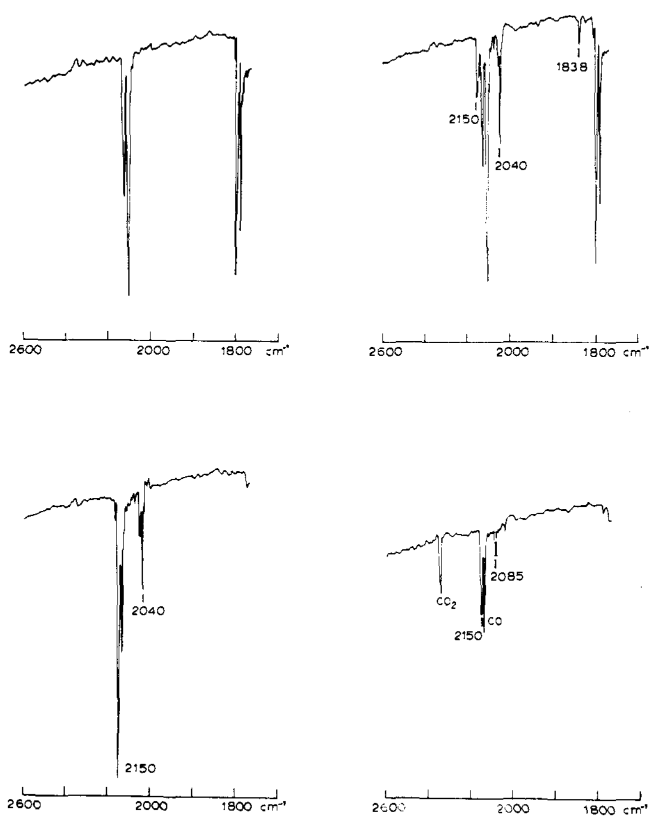
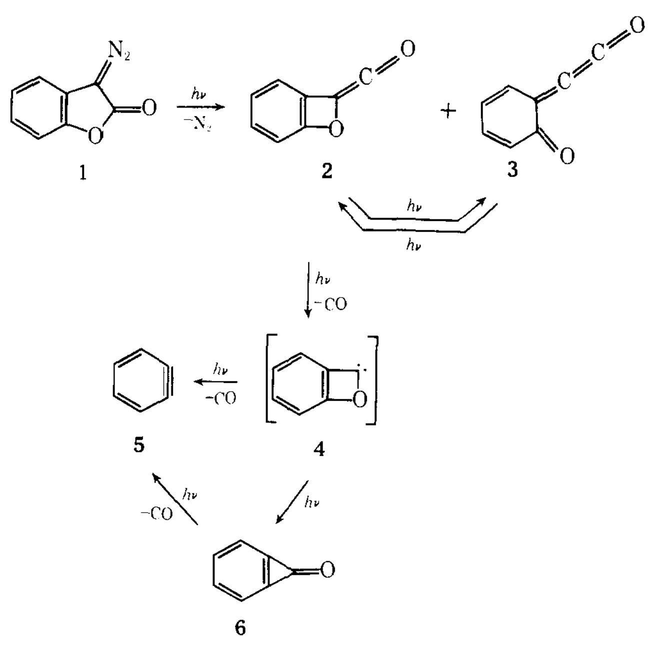


Figure 1. Infrared spectra of **1** matrix isolated in argon at 8 K before irradiation (upper left), after irradiation (>274 nm) for 1 min (upper right), after irradiation (>274 nm) for 10 min (lower left), and after irradiation with quartz optics for 524 min (lower right). Note the scale change at 2000 cm^{-1}.

in turn loses carbon monoxide giving benzyne (**5**) and a minor amount of a carbonyl compound (1838 cm^{-1}) which is decarbonylated by further irradiation. This minor component is probably **6**. The infrared absorption is identical with that observed in the photodecarbonylation of benzocyclobutenedione.[5] Benzyne was identified by comparison of infrared spectra.[5] The 3-diazobenzofuranone (**1**) is the best precursor for preparation of matrix isolated benzyne. In this experiment, we were able for the first time to see a carbon-carbon triple bond stretching frequency for benzyne at 2085 cm^{-1} (cf. 2083 and 2450 cm^{-1} calculated in Laing and Berry's[6] normal coordinate analysis of benzyne).



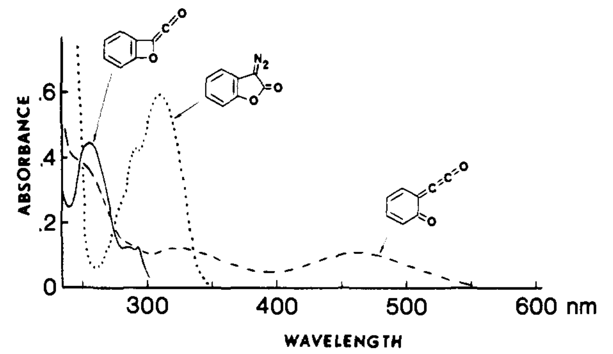


Figure 2. Dotted line, **1** in 2-MeTHF; dashed line, **3** (with some **2**) generated by photodecomposition of **1** with ultraviolet light (250-400 nm); solid line, **2** after bleaching of **3** away with visible light. All spectra and photochemistry at 77 K.

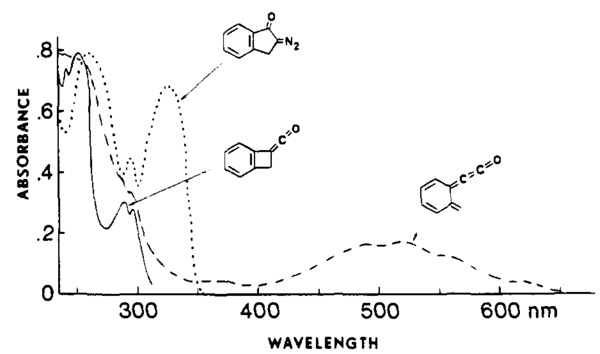
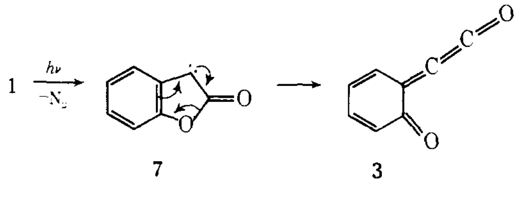
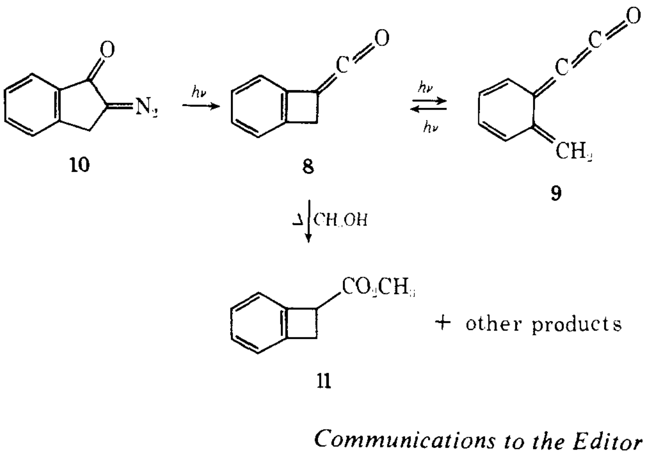


Figure 3. Dotted line, **10** in 2-MeTHF; dashed line, **9** (with some **8**) generated by photodecomposition of **10** with ultraviolet light (254 nm); solid line, **8** after bleaching of **9** away with visible light. All spectra and photochemistry at 77 K.

Formation of **3** as a primary product was unexpected. The formation of **3** can be viewed as a concerted reaction of the initial carbene (**7**).[7] The photochemical electrocyclic opening of the four-membered ring in **2** is analogous to that observed in the carbocyclic analog (**8**) which photointerconverts with **9** (vide infra).



Irradiation of 2-diazo-1-indanone (**10**) at low temperatures[2] gives ketene **8** as a primary product[8] as expected on



the basis of room temperature irradiations.[10,11] Irradiation of **8** gives **9** as a secondary product. A photostationary state is established between **8** and **9**. The ketene (**8**) has been trapped by warming in an argon matrix containing methanol. The product is identified as the known ester (**11**)[11] by infrared and mass spectrometry (ν_{CO} 1730 cm^{-1}; m/e 162). The spectra of **8** (2132 cm^{-1}; λ_{max}^{2MeTHF} 251, 289, 295 nm; Figure 3)[2] and **9** (2040 cm^{-1}; λ_{max}^{2MeTHF} 520 nm; Figure 3)[2] are quite similar to those of **2** and **3**. It is interesting to note that the absorption maximum for **9** lies to the red from that of **3**. The interconversions of **2** and **3** and of **8** and **9** constitute two novel photochromic systems since **2** and **8** are colorless while **3** and **9** are orange and purple-red, respectively.

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Manganese(II) Gluconate. Redox Model for Photosynthetic Oxygen Evolution

Sir:

In spite of the importance and long-term interest in the oxygen-evolution step of photosystem II in green-plant photosynthesis,[1] there is neither agreement as to the electron-transfer mechanism nor to the manganese oxidation states and their number in the active group. One recent discussion proposes a two-quantum process with the manganese first oxidized from the +2 to the +3 state and then from +3 to the +4 state.[2] In support of this mechanism are studies of the photoactivation process[3] and of the functional sites of manganese within photosystem II.[4] Two earlier studies[5,6] provide a thorough review of the role of manganese and its photoactivation in photosystem II. An alternative four-step mechanism has been proposed[7] for the oxygen-evolution reaction, and has received experimental support from flash photolysis experiments.[8] The latter have led to the formulation of a number of alternative mechanistic models.[9] A recent investigation[10] concludes that there are three manganese atoms per water-oxidizing unit and that the three are equivalent. Almost coincidentally a three-manganese model system has been proposed[11] with a concerted reaction sequence that involves hydroperoxo and peroxo intermediates.

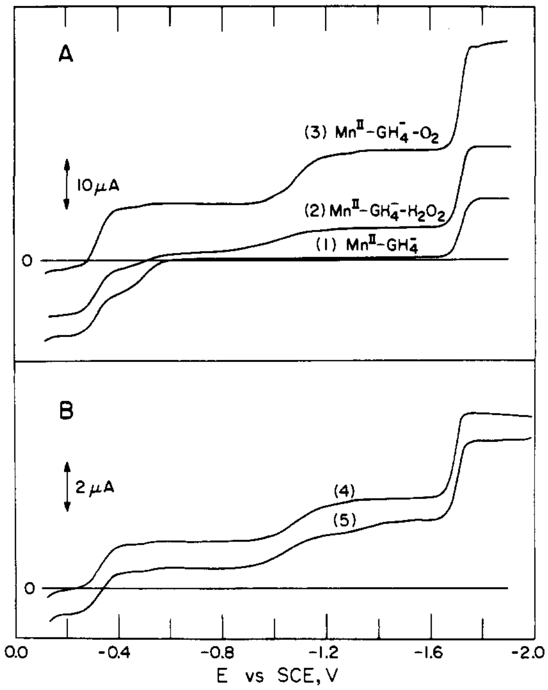

Figure 1. Polarograms of manganese in 0.1 *F* lithium gluconate (LiGH$_4$) and 0.3 *F* LiOH. A (1), 5 m*F* Mn^{II}(GH$_4$)$_2$ (Charles Pfizer Co.), colorless; (2), solution (1) plus 5 m*F* H$_2$O$_2$, brown, λ_{max}, 230 nm (ϵ, 8560), 462 (ϵ, 156), 500 (ϵ, 152); (3), solution (1) plus 1 atm of O$_2$ bubbled for 20 min, "cherry" red, λ_{max}, 273 nm (ϵ, 14300), 387 (ϵ, 2220), 515 (ϵ, 294). The latter solution also can be obtained from solution (1) by electrolysis at −0.2 V vs. SCE or by addition of K$_3$Fe(CN)$_6$ in a 2-to-1 mole ratio. Solutions are degassed with argon prior to recording their polarograms. B (4), 0.6 m*F* Mn^{II}(GH$_4$)$_2$ electrolyzed at −0.2 V vs. SCE with a Hg-pool electrode in a sealed cell (oxidation complete in about 30 min); (5), solution (4) after being degassed with argon for 2 hr. Similar results obtained for a 5 m*F* Mn^{II}(GH$_4$)$_2$ solution, but oxygen removal only 20% complete after 4 hr of argon degassing; 60% complete after 18 hr. Voltage scan-rate, 0.3 V min^{-1}; DME drop time, 4.0 sec. Curves represent the envelope of the maxima of the DME current oscillations, reducing current above zero axis and oxidizing current below. Increasing potentiometric promotion of oxidation to the left and of reduction to the right.

Because the conversion of water to oxygen is a four-electron process that requires two water molecules, the electron transfer mechanism for the photosynthetic manganese group is an intriguing and challenging question. The current belief is that manganese(II) is the oxidation state of the resting system and that it is converted by photooxidants to the +3 and/or +4 state.[10] These unresolved questions have prompted us to seek manganese complexes and solution conditions which result in oxidation-reduction chemistry that mimics that of the water-oxidizing species associated with photosystem II.

The manganese(II) gluconate complex[12] [Mn^{II}(GH$_3$)$_2$]$^{2-}$ (I) in basic media represents a system that undergoes oxidation-reduction chemistry which parallels much that is observed for the manganese group in photosystem II. It also represents a system that reversibly binds molecular oxygen. Figure 1A illustrates the polarographic behavior of I, of I in the presence of an equimolar amount of H$_2$O$_2$, and of I exposed to O$_2$ at 1 atm for 20 min prior to degassing with argon. Controlled potential electrolysis of I at −0.2 V vs. SCE results in a two-electron oxidation and a solution that gives curve (4) of Figure 1B. The effect of degassing with argon is indicated by the bottom curve of Figure 1B. Chemical oxidation of I with HgO or K$_3$Fe(CN)$_6$ yields a polarogram similar to curve (4); the titration stoi-