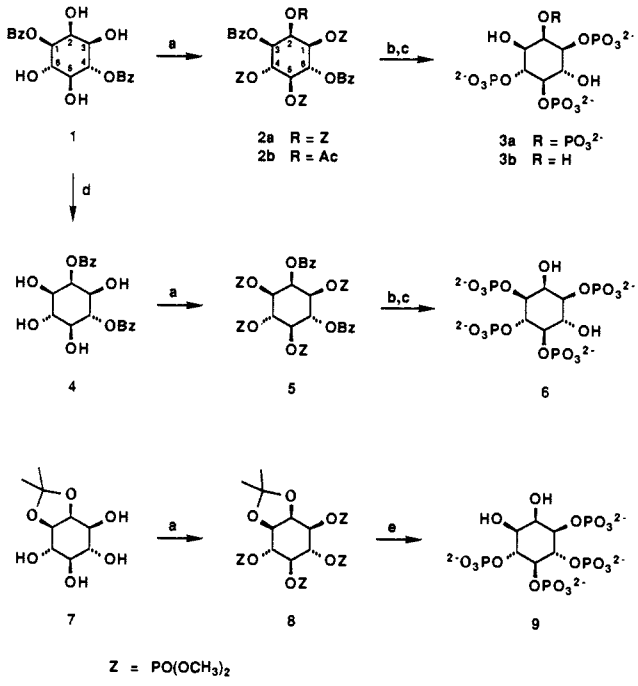
Scheme I[a]



[a] (a) excess $(CH_3O)_2PCl$, *i*-Pr_2NEt/DMF or CH_2Cl_2, 25 °C; then excess H_2O_2; except **1** → **2b**: 3.3 equiv of $(CH_3O)_2PCl$, 6 equiv of *i*-Pr_2NEt/DMF, −40 °C, then 2 equiv of AcCl, 0.13 equiv of DMAP, 25 °C, 30 min; then 10 equiv of H_2O_2, 25 °C; (b) 30% HBr/HOAc, 60 °C, 30 min; (c) LiOH or KOH, 60–80 °C, 1 h; (d) 6:4 pyridine-H_2O, 100 °C, 1 h; (e) 9 equiv of TMSBr, 25 °C, 30 min; then H_2O.

removed by ion exchange chromatography. This model study suggested that the above phosphorylation method has the potential to cleanly and efficiently convert all of the free hydroxyl groups in any appropriately protected inositol to phosphate monoesters.

Since the axial 2-hydroxyl group of *myo*-inositol derivatives is generally less reactive than equatorial ones,[21] preparation of **3b** by means of partial phosphitylation was investigated. When **1** was treated sequentially with (a) 3.3 equiv of dimethyl chlorophosphite and diisopropylethylamine at −40 °C, (b) acetyl chloride and dimethylaminopyridine,[22] and (c) hydrogen peroxide, the crude reaction mixture was shown by HPLC and ^{31}P NMR to contain the desired tris(dimethyl phosphate) (94%) along with about 4% of tetrakis(dimethyl phosphate) **2a** and approximately 2% of an unknown tris(dimethyl phosphate). No other products were detected. The 2-hydrogen of the major product showed no ^1H–^{31}P coupling, proving that the axial alcohol was the one which had not been phosphorylated. Demethylation of **2b** with hydrogen bromide in acetic acid and ester hydrolysis gave 1,4,5-IP_3 (**3b**) contaminated with about 5% bis(phosphates) in 88% yield.

Since the hydroxyl groups of *myo*-inositol have generally been differentiated by means of cyclic ketals, an appropriately protected precursor for preparation of 1,3,4,5-IP_4 (**6**) has not been previously reported. Such a precursor, 2,4-dibenzoate **4**, can be conveniently prepared in modest yield by base-catalyzed isomerization of the readily available 1,4-dibenzoates.[23] When heated in refluxing aqueous pyridine, 1,4-dibenzoate **1** was converted with negligible hydrolysis to a mixture of **1**, 2,4-dibenzoate **4**, 1,6-dibenzoyl-*myo*-inositol, and an unidentified dibenzoate. The desired dibenzoate **4** was most easily purified by fractional crystallization of the mixture produced when this isomerization was carried to less than 50% conversion.[24] As shown in Scheme I, 1,3,4,5-IP_4 (**6**) was then prepared from dibenzoate **4** by the same series of phosphitylation, oxidation, and deprotection steps used to make 1,2,4,5-IP_4. A third naturally occurring inositol tetraphosphate, 1,4,5,6-IP_4 (**9**), was synthesized in a similar manner from 1,2-*O*-isopropylidene-*myo*-inositol (**7**).[18] In this case, however, demethylation with hydrogen bromide in acetic acid caused the cyclic ketal of tetrakis(dimethyl phosphate) **8** to be removed prematurely and some phosphate migration occurred. In contrast, when **8** was deprotected with bromotrimethylsilane, the cyclic ketal was retained until the reaction mixture was quenched with water. The ketal of the resulting tetrakis(dihydrogen phosphate) then underwent self-catalyzed hydrolysis, and pure 1,4,5,6-IP_4 (**9**) was isolated as the free acid by removal of the volatile byproducts.

In each of these syntheses, the general strategy was to avoid phosphate triester intermediates with free hydroxyl groups since these species are prone to undergo cyclization and migration. Phosphitylating agents, especially the previously unappreciated reagent dimethyl chlorophosphite, are convenient and versatile reagents for bypassing such intermediates. The validity of this approach was proven when careful ^{31}P NMR, ^1H NMR, and ion exchange HPLC[25] analysis of the final inositol poly(phosphates) detected no isomeric impurities. This methodology should be useful for the preparation of phosphate monoesters of other complex carbohydrates, nucleotides, and peptides.

Note Added in Proof. The synthesis of **6** has now been reported by deSolms, et al. (deSolms, S. J.; Vacca, J. P.; Huff, J. R. *Tetrahedron Lett.* **1987**, *28*, 4503), Ozaki et al. (Ozaki, S.; Kondo, Y.; Nakahira, H.; Yamaoka, S.; Watanabe, Y. *Tetrahedron Lett.* **1987**, *28*, 4691), and Billington and Baker (Billington, D. C.; Baker, R. *J. Chem. Soc., Chem. Commun.* **1987**, 1011).

Supplementary Material Available: Experimental procedures for the preparation and characterization of compounds **1–9** (7 pages). Ordering information is given on any current masthead page.

(21) (a) For a review, see: Shvets, V. I. *Russ. Chem. Rev.* **1974**, *43*, 488. (b) The regioselectivity reported for P(V) phosphorylation of a simpler system was very low: Krylova, V. N.; Lyutik, A. I.; Gornaeva, N. P.; Shvets, V. I. *J. Gen. Chem. USSR (Engl. Trans.)* **1981**, *51*, 183; *Zh. Obschch. Khim.* **1979**, *51*, 210.

(22) If this protection step was omitted, product **3b** was contaminated with at least one additional isomeric inositol phosphate indicating that phosphate migration had taken place.

(23) Base-catalyzed isomerization of inositol acetates, however, occurs with little selectivity: Angyal, S. J.; Melrose, G. J. H. *J. Chem. Soc.* **1965**, 6494.

(24) The unreacted starting dibenzoate (and presumably the other dibenzoates) can be recycled.

(25) Meek, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4162. Meek, J. L.; Nicoletti, F. *J. Chromatogr.* **1986**, *351*, 303.

An Exceptionally Simple Method of Preparing Matrix Isolated Biradicals, Biradicaloids, and Carbenes

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Biradicals, biradicaloids, and carbenes are common reactive intermediates in organic[2] thermal and photochemical transformations.[3] A common objective in the study of these species is their direct detection and characterization by spectroscopic methods. Recent years have witnessed ever increasingly so-

(1) Camille and Henry Dreyfuss Teacher Scholar.

(2) See: Gajewski, J. J. *Hydrocarbon Thermal Isomerizations*; Academic Press: New York, NY, 1981.

(3) See: *Tetrahedron* **1982**, *38*, 735-867.

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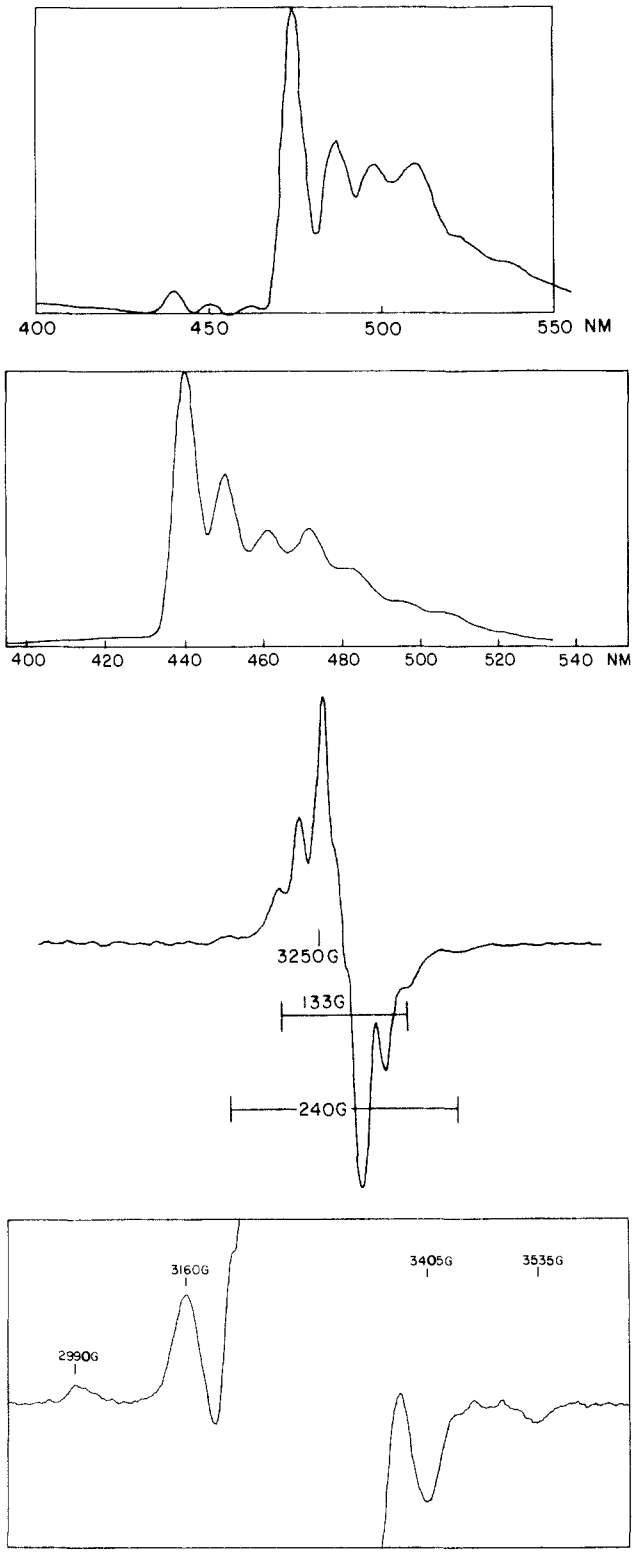


Figure 1. (a) The fluorescence spectrum produced by 254-nm photolysis of *m*-xylene in ethanol, λ excitation = 295 nm. (b) The fluorescence spectrum produced by 254-nm photolysis of **7** in ethanol, λ excitation = 295 nm. (c) The EPR spectrum of *m*-xylylene (**2**) produced by photolysis (unfiltered Hg–Xe) of **7** in ethanol. (d) The EPR spectrum of trimethylenemethane (**3**) produced by photolysis (unfiltered Hg–Xe) of **6** and **11** in methylcyclohexane.

phisticated time and spectral resolution of these species.[3] In fact one of the main limitations in the study of reactive intermediates is the availability of suitable photochemical precursors. In this communication we report the matrix isolation of *o*-xylylene (**1**), *m*-xylylene (**2**), trimethylenemethane (**3**), diphenylcarbene (**4**), and 1,8-naphthoquinodimethane (**5**) by using very simple and readily accessible precursors.

m-Xylylene (**2**) is a ground-state triplet biradical which was first prepared by extended 254-nm photolysis of an alkane matrix containing *m*-xylene at 77 K. The biradical can be detected by fluorescence spectroscopy (Figure 1a) which reveals that the *m*-xylylene emission (440 nm) is a minor impurity in the spectrum of the *m*-methylbenzyl radical (470 nm).[4] We now report that a much higher yield of *m*-xylylene (**2**) is realized by photolysis (254 nm, 5 RPR Rayonet bulbs) of an ethanol glass containing 0.003 M **7**. The fluorescence of **2** can also be realized by 254-nm photolysis of **7** in glassy 3-methylpentane and polycrystalline methanol at 77 K (Figure 1b). The biradical is the major product with this precursor. It is presumed that 254-nm photolysis of **7** leads to double homolytic bond cleavage to produce **2** and two chlorine atoms which can be expected to rapidly abstract hydrogen atoms from the matrix.

7 **2**

The excitation spectra of **2** monitored at 440 and 460 nm are identical and centered at 295 nm in good agreement with the literature.[4] An excitation spectrum produced by monitoring at 470 nm had maxima at both 295 and 323 nm, the latter due to the presence of some monoradical.

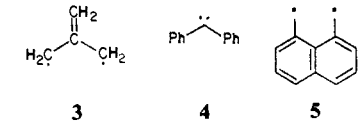
A spectrum identical with that of Figure 1b can also be produced by bombardment of **7** in 2-methyltetrahydrofuran (MTHF) with 2.5 MeV electrons. In this system electrons are ejected from MTHF by the ionizing radiation of a linear accelerator and are trapped by dichloride **7** to produce **2** and two chloride ions.

Direct photolysis of **7** at 77 K produced the EPR spectrum shown in Figure 1c, which except for the doublet impurity in the center of the spectrum is identical with that produced by other precursors.[4c,d] Of greater importance is that for the first time both fluorescence and EPR detection of **2** have been achieved from a common sample, thereby strengthening each individual spectroscopic assignment.

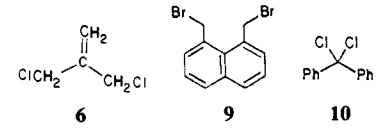
Positive results were also realized upon 254-nm photolysis of *o*-chloromethyl compound **8** which yielded a fluorescence spectrum very similar to that previously reported for *o*-xylylene (**1**).[4b,5]

8 **1**

Photolysis of **6**–**10** at 77 K gave no evidence by EPR spectroscopy for the formation of **3**–**5**. However photolysis (unfiltered 1000 W Hg–Xe) of 0.1 M alkene **11** and dichloride **6** in methylcyclohexane at 77 K produces the EPR spectrum of Figure 1d. This spectrum has zero field splitting parameters $|D/hc| = 0.025\ cm^{-1}$ and $|E/hc| = 0\ cm^{-1}$ identical with those of trimethylenemethane (**3**).[6] Photolysis of glassy matrices of **11**



3 **4** **5**



6 **9** **10**

containing either **9** or **10** produces the known EPR spectra of

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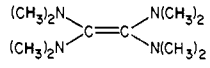
1,8-naphthoquinodimethane[7] (**5**) and diphenylcarbene[8] (**4**), respectively. The intensity of the diphenylcarbene EPR spectrum is much weaker than that obtained for the biradicals. These species were most likely produced by dissociative electron capture of **6–10**. Although **11** is the preferred reagent for matrix electron transfer and EPR spectroscopy, it is not useful in fluorescence spectroscopy due to its own strong emission.[9]



11

These preliminary results indicate that readily available dihalo compounds may be general precursors to biradicals, biradicaloids, and carbenes under matrix-isolation electron-transfer conditions. Applications of this approach to presently uncharacterized neutral intermediates and the utilization of other spectroscopic techniques are in progress.

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(7) (a) Pagni, R. M.; Burnett, M. N.; Dodd, J. R. *J. Am. Chem. Soc.* **1977**, *99*, 1972. (b) Platz, M. S. *J. Am. Chem. Soc.* **1979**, *101*, 3398.

(8) See: Trozzolo, A. M.; Wasserman, E. In *Carbenes*; Moss, R. A., Joes, M., Jr., Eds.; Wiley: New York, NY, 1975; Vol 2, p 185.

(9) Using the more sensitive technique of fluorescence spectrocopy, we have recently observed that photolysis of **10**, in the absence of **11**, yields a species having excitation and emission spectra identical with those reported for diphenylcarbene.[10]

(10) Trozzdo, A. M. *Acc. Chem. Res.* **1968**; *1*, 329.

Delineation of α-Helical Domains in Deuteriated Staphylococcal Nuclease by 2D NOE NMR Spectroscopy

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Two-dimensional proton NMR spectroscopy is a powerful means of elucidating the three-dimensional structures of small proteins in solution.[1] However, because the number of proton signals and their line widths increase with molecular weight, 2D NMR spectra are difficult to interpret when the protein molecular weight exceeds 10000. Deuteriation of nonexchangeable protons diminishes these problems.[2,3a,b] It also prevents diffusion of magnetization from NH to CH protons, suggesting that resolution and sensitivity of d_{NN} connectivities will be significantly enhanced by protein deuteriation. We demonstrate that this is the case in an application of the deuteriation approach to liganded Staphylococcal nuclease,[4] an enzyme ternary complex having a MW of 18 kD. Three long sequences of d_{NN} connectivities, signatures

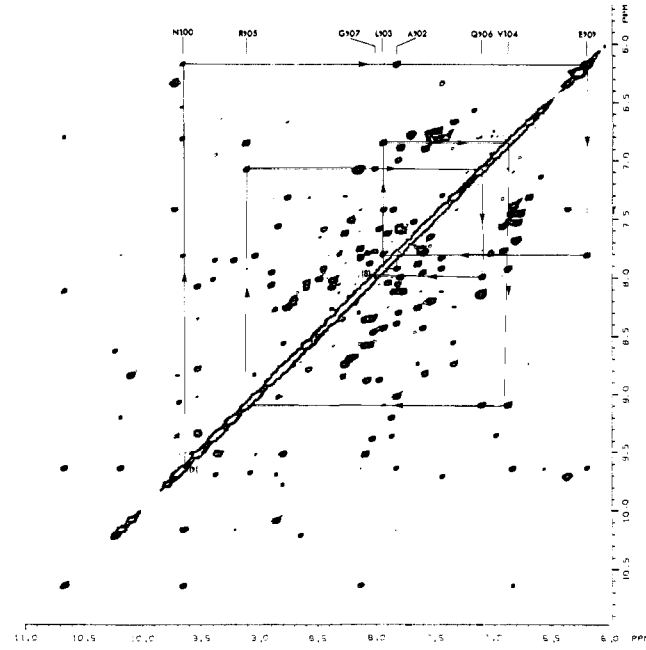

Figure 1. Downfield region of the 500 MHz absorption mode NOESY spectrum of deuteriated Nase. The sequence of d_{NN} connectivities traced by the solid line links the eight residues assigned at the top of the spectrum. The solution composition was as follows: H_2O, 90%; ^2H_2O, 10%; NaCl, 100 mM; borate buffer 50 mM, pH 7.7; Nase, 1.5 mM; pdTp, 5 mM; $CaCl_2$, 10 mM. NT 500 spectrometer settings were as follows: 90° pulse, 27 μs; recycle delay, 3 s; spectral window, 7400 Hz; mixing time, 0.15 s; 64 scans per t_1 values; 350 t_1 values; 36.5 °C. Water signal was suppressed by presaturation. Chemical shifts are referenced to HDO at 4.67 ppm.

of α-helices,[1,5-7] are clearly observed in the NOESY spectrum of the deuteriated protein.

Staphylococcal nuclease, Nase, is well characterized chemically and has been the subject of many structure-function studies.[8a-d] Recently, the Nase gene has been expressed in *Escherichia coli*,[9] a development that has given new impetus to these studies.[10a-f] We have prepared purified Nase from *E. coli*[10f,11] (provided by Professor John Gerlt) grown in defined media containing perdeuteriated amino acids (Merck). A comparison of ^1H NMR spectra of deuteriated and protiated Nase showed that 80–85% of the nonexchangeable hydrogens were deuteriated in the labeled protein. Spectrophotometric assays[12] showed that the protiated and deuteriated Nase samples had the same enzymatic activities.

The 6–11-ppm region of the NOESY spectrum of deuteriated Nase, Figure 1, shows many resolved intense cross-peaks. In

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(4) S. Nuclease, thymidine 3′,5′-bisphosphonate (pdTp), and Ca^{2+}.

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(11) Due to the construction of the expression vector, the sample obtained has a heptapeptide (M-D-P-T-V-Y-S) appended to the N-terminus of the protein. The heptapeptide extension has no effect on enzyme activity or stability.[10b]

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