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# Structure of the Carrier of the Cross-Polarization Magic Angle Spinning Carbon-13 Nuclear Magnetic Resonance Signal Assigned to 3,4-Dimethylenethiophene. Multiple Position-Labeling and Chemical Trapping in Annealed Glasses

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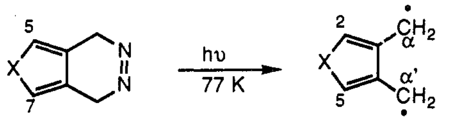
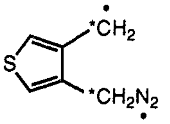
Cross-polarization magic angle spinning (CP MAS) $^{13}$C NMR spectra have been reported[1] for matrix-immobilized preparations of the isotopically labeled ESR-silent singlet biradical species[2] 3,4-dimethylenefuran-$\alpha,\alpha'$-$^{13}C_2$ (**1**, $\delta$ = 102 ppm) and 3,4-dimethylenethiophene-$\alpha,\alpha'$-$^{13}C_2$ (**2**, $\delta$ = 105 ppm) generated by UV irradiation of the precursor diazenes **3** and **4**.



**3**: X = O
**4**: X = S

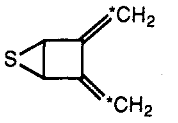
**1**: X = O
**2**: X = S

We give experimental support here for the assertion that such spectra from precursor **4** are associated with 3,4-dimethylenethiophene **2** and not with other plausible species, for example, the diazenyl biradical **5** or the bicyclic full-valence isomer **6**.



**5**

* = $^{13}$C



**6**

Photolysis at 77 K of the diazene precursor **4**-$\alpha,\alpha'$-$^{13}C_2$ in either 2-methyltetrahydrofuran (2-MTHF) or methanol generates an intense purple color. Simultaneously, the 66 ppm CP MAS $^{13}$C NMR resonance of the precursor decreases, and a peak at 105 ppm appears. The purple color and the 105 ppm resonance are associated with a transient species, since both disappear when the sample is annealed at 120 K and then reglassed for $^{13}$C NMR. Annealing also results in a decrease in the 66 ppm band, which is presumptive evidence of reaction of the diazene with the corresponding biradical in the softened matrix.

One alternative hypothesis for the 105 ppm resonance would ascribe it to the $^{13}CH_2$ group in the diazenyl biradical **5**. The chemical shift of the $N_2$-bearing $^{13}CH_2$ group would be similar to and therefore likely to be masked by that of the remaining unreacted diazene **4**-$\alpha,\alpha$-$^{13}C_2$. If **5** were stable at 77 K but unstable at 120 K, annealing the samples could complete the deazetation, but the biradical **2** so generated might not be detectable because of further reaction. These events would appear in the NMR spectrum of the reglassed samples as simultaneous disappearance of all of the 105 ppm band and part of the 66 ppm band, just as we observe. However, this interpretation is excluded by the following experiment.

By careful manipulation, it is possible to make samples of the thermally sensitive diazene **4**-$\alpha,\alpha$-$^{13}C_2$ in methanol containing 2 equiv of the efficient trapping agent fumaronitrile[2a,b] (Figure 1, spectrum A). Irradiation at 77 K generates the purple color and the 105 ppm $^{13}$C resonance of biradical **2**. A spectrum taken after several cycles of photolysis/annealing followed by a final photolysis is shown in Figure 1, spectrum B. A final annealing of this sample at 120 K followed by recooling to 77 K yields a spectrum (Figure 1, spectrum C) that shows the complete disappearance of the biradical band at 105 ppm and a dramatic growth of the band of the adduct **7** at 28 ppm. The difference spectrum (Figure 1, spectrum D; spectrum B–spectrum C) shows that the decrease in intensity of the 105 ppm band quantitatively[3,4] accounts for the growth in intensity of the 28 ppm band. Were the actual course of the reaction **5** + fumaronitrile → **7** + $N_2$, the increase in intensity of the 28 ppm band of **7** should have been *twice* that of the decrease in intensity of the 105 ppm band, and the band at 66 ppm also should have decreased a concomitant amount. That neither of these changes occurs indicates that the carrier of the 105 ppm band must have *two* $^{*}CH_2$ units and that the reaction observed is **2** → **7**.

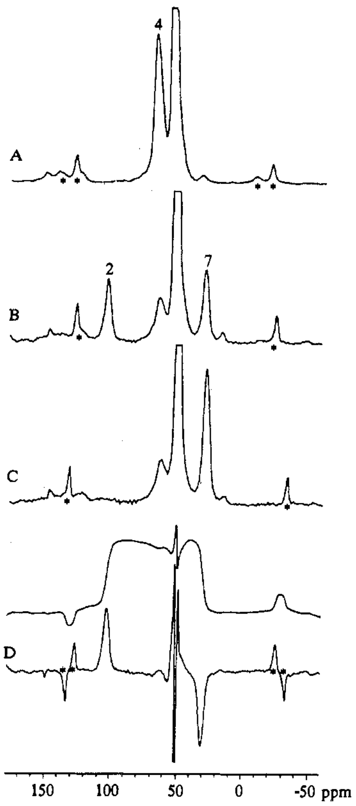
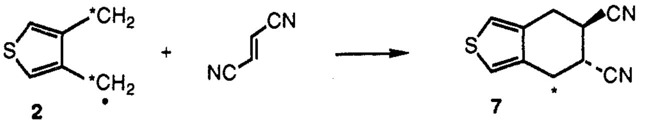


**Figure 1.** $^{13}$C CP MAS spectra at 25.16 MHz and 77 K demonstrating the trapping of **2** generated from **4**-$\alpha,\alpha$-$^{13}C_2$ by fumaronitrile in methanol. Spinning sidebands are marked by asterisks. (A) Spectrum before photolysis. A small impurity in the precursor is also seen at ~160 ppm. (B) Spectrum resulting from photolysis/annealing/photolysis. New peaks for **2**, formed in the final photolysis, and the trapping adduct **7**, produced in the prior annealing step, are indicated. A small unidentified photoproduct also appears at ~20 ppm. (C) Spectrum obtained after annealing the sample of spectrum B. (D) Spectrum B–spectrum C. That the net integral (drawn above the difference spectrum) is zero indicates quantitative conversion of **2** to **7** in the trapping reaction. The methanol difference line shape appears dispersive because the methanol crystallized upon refreezing, which caused the line to be narrower here than in the glassy state.



**2** **7**

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[1] (a) Zilm, K. W.; Merrill, R. A.; Greenberg, M. M.; Berson, J. A. *J. Am. Chem. Soc.* **1987**, *109*, 1567. (b) Zilm, K. W.; Merrill, R. A.; Webb, G. G.; Greenberg, M. M.; Berson, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1533.

[2] (a) Stone, K. J.; Greenberg, M. M.; Goodman, J. L.; Peters, K. S.; Berson, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 8088 and subsequent papers. (b) Berson, J. A. *Mol. Cryst. Liq. Cryst.* **1989**, *176*, 1 and references cited therein. (c) Lahti, P. M.; Ichimura, A. S.; Berson, J. A. *J. Org. Chem.* **1989**, *54*, 958. (d) Du, P.; Hrovat, D. A.; Borden, W. T. *J. Am. Chem. Soc.* **1986**, *108*, 8086.

[3] Although peak intensities in conventional solution-phase $^{13}$C NMR spectra may be distorted for a variety of reasons, peak intensities in solid-state $^{13}$C NMR spectra obtained with CP under the conditions used here are known to reflect atomic ratios accurately.[4] The present work illustrates the utility of this fact for quantitative monitoring of reactions of transient species in cold matrices.

[4] Alemany, L. B.; Grant, D. M.; Pugmire, R. J.; Alger, T. D.; Zilm, K. W. *J. Am. Chem. Soc.* **1983**, *105*, 2133.

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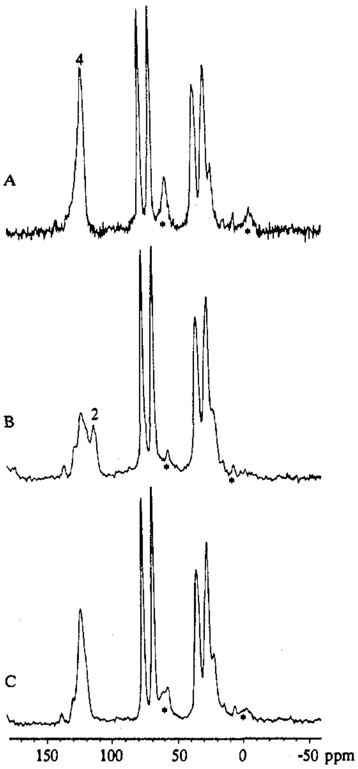

**Figure 2.** $^{13}C$ CP MAS spectra following the photolysis and annealing of **4**-5,7-$^{13}C_2$ in a 2-MTHF glass at 77 K. Prominent spinning sidebands are indicated by asterisks. (A) Spectrum before photolysis. The other peaks in this spectrum are for the 2-MTHF and its spinning sidebands. (B) Spectrum after photolysis, showing the appearance of **2**. (C) Spectrum after annealing.

In a second alternative account of the properties of 3,4-dimethylenethiophene, one might hypothesize a mixture of **2** and the bicyclic full-valence isomer **6**. The biradical **2** then could be responsible for the purple color and the extremely fast chemical reactions, and species **6** could be the carrier of the $^{13}C$ NMR spectrum of $\alpha,\alpha'$-$^{13}C$-labeled material. This scheme is made untenable by the $^{13}C$ NMR spectrum of 3,4-dimethylenethiophene generated from a different diazene precursor, **4**-*5,7*-$^{13}C_2$, in which the thiophene ring carbons adjacent to sulfur are isotopically enriched by a synthesis described in the supplementary material. The $^{13}C$ NMR spectrum of this precursor in diethyl ether or 2-methyltetrahydrofuran (2-MTHF) glass at 77 K shows resonances at 119 ppm (Figure 2, spectrum A). Brief irradiation under the usual conditions generates the purple color, and a new band appears near 114.9 ppm (Figure 2, spectrum B). The bridgehead carbon resonances of the bicyclic Kekulé isomer **6** should occur near 40–45 ppm (see supplementary material), but no bands can be discerned in that region in glassed solvents 2-MTHF, MeOH, or most convincingly $Et_2O$, where the region 20–50 ppm is blank. Thermal annealing causes the disappearance of both the color and the 114.9 ppm band (Figure 2, spectrum C). These observations are fully consistent with the behavior expected of the biradical **2**-2,5-$^{13}C_2$ and cannot be ascribed to the bicyclic compound **6**.

The present experiments show that singlet biradical **2** is the authentic carrier of the transient $^{13}C$ NMR spectra produced by irradiation of **4** immobilized in rigid matrices and also is the species directly involved in the cycloaddition chemistry observed in softened matrices.

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**Supplementary Material Available:** Additional $^{13}C$ CP MAS matrix spectra, details of the synthesis of **4**-*5,7*-$d_2$, and estimates of the chemical shift of **6** (10 pages). Ordering information is given on any current masthead page.

## An Antibody-Catalyzed Cis–Trans Isomerization Reaction

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Biological systems are capable of synthesizing and screening tremendous chemical diversity to produce molecules with remarkable biological functions. For example, the immune system can generate more than $10^{12}$ different antibodies and identify and amplify those that bind a given ligand with high selectivity and affinity. Recently the principles and tools of organic chemistry have been used to exploit the remarkable machinery of the immune system for the generation of selective antibody catalysts.[1] One important approach that has emerged in the design of catalytic antibodies involves the induction of catalytic groups in antibody combining sites via mechanism-based hapten design.[2] We now report the application of this approach to cis–trans isomerization reactions of carbon–carbon double bonds, specifically the isomerization of the $\alpha,\beta$-unsaturated ketone **1**. Such reactions are important processes in chemical and biological systems; examples include the synthesis of vitamin D and the isomerization of retinal.[3]

One mechanistic model for antibody-catalyzed isomerization of **1** involves 1,4-nucleophilic addition of an active-site group to the enone, followed by rotation around the resulting $\alpha,\beta$-single bond and subsequent collapse of the intermediate to afford the isomerized product 3 (Scheme I). Consequently, not only must the antibody contain an active-site nucleophile or base, it must also accommodate the transition state for rotation about the $\alpha,\beta$-single bond. The disubstituted trans piperidinium hapten **4** was expected to afford an antibody combining site that fulfills both criteria. Molecular mechanics calculations suggest that the minimum energy conformation of trans hapten **4** places the nitrophenyl moieties in a geometry close to that for the perpendicular transition state required for enone isomerization.[4] In addition, the positively charged amino group should induce a complementary carboxylate in the antibody combining site[2a] which may be capable of reversible 1,4-nucleophilic addition to enone **1**. Alternatively, the carboxylate might act as a base to activate water for attack on enone **1**.

A 5:1 mixture of cis:trans hapten **4** was synthesized[5] via con-

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[1] (a) Schultz, P. G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 1283–1295. (b) Schultz, P. G.; Lerner, R. A.; Benkovic, S. J. *Chem. Eng. News* **1990**, *68*, 26–40. (c) Shokat, K. M.; Schultz, P. G. *Annu. Rev. Immunol.* **1990**, *8*, 335–363.

[2] (a) Shokat, K. M.; Leumann, C. J.; Sugasawara, R.; Schultz, P. G. *Nature* **1989**, *338*, 269–271. (b) Janda, K. D.; Weinhouse, M. I.; Schloeder, D. M.; Lerner, R. A.; Benkovic, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 1274.

[3] Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman and Co.: San Francisco, 1979.

[4] Molecular mechanics calculations were carried out with Biograf software (Mayo, S. L.; Olafson, B. D.; Goddard, W. A. Biodesign Inc., Pasadena, CA).

[5] To a solution of 4-nitroiodobenzene (10 mmol) in 30 mL of DMF at 20 °C were added hexamethylditin (10 mmol) and $(MeCN)_2PdCl_2$ (0.2 mmol). After 20 min, the reaction mixture was poured into 100 mL of $H_2O$ and the product extracted into 100 mL of diethyl ether. Evaporation of the ether layer afforded (4-nitrophenyl)trimethylstannane (9.1 mmol). Treatment of stannane (5 mmol) with glutaryl dichloride (2.5 mmol) and $(PPh_3)_2PdCl_2$ (0.2 mmol) in 20 mL of HMPA at 60 °C followed by aqueous workup, ether extraction, and silica gel chromatography (ethyl acetate/hexane) afforded bis-1,5(4-nitrophenyl)-1,5-pentanedione (1.9 mmol). Reductive amination of dione (1 mmol) with $NH_4OAc$ (1.5 mmol) and $NaCNBH_3$ (1 mmol) in 50 mL of MeOH at reflux afforded a 5:1 diastereomeric mixture of *cis*- and *trans*-2,6-bis(4-nitrophenyl)piperidine (**4**) as determined by HPLC and NMR. Monoalkylation of **4** (0.5 mmol) with 3-iodopropionic acid (0.5 mmol) in 20 mL of 1,2-dichloroethane at reflux afforded **5** (0.24 mmol) after purification by preparative TLC ($MeOH/CHCl_3/CH_3CO_2H$).

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