Table I. Chemical Shifts, Peak Assignments, and Carbon–Carbon Coupling Constants for the C_{60} Portion of **1**

peak	chemical shift (ppm)	carbon type	cluster carbons	carbon type, $^1J_{CC}$ (Hz)
a	105.38	1	1, 2	3, 48
b	137.02	4	9, 10, 13, 14	3, 71; 7, 56
c	139.42	5	15, 16, 19, 20	2, 68; 6, 56; 9, 56
d	141.81	9	23, 24, 28, 29	5, 56; 10, not first order
e	142.32	10	33, 34, 38, 39	9, not first order; 11, 56[a]
f	142.48	14	43, 44, 47, 48	15, 56[a]
g	142.55	13	36, 41	8, 67; 12, 56
h	142.75	7	17, 18, 21, 22	4, 56; 6, 67; 8, 55
i	144.85	11	35, 37, 40, 42	6, 54; 10, 56; 12, 68
j	145.04	16	53, 54, 57, 58	12, 56; 15, 65
k	145.76	2[b]	7, 8, 11, 12	3, 57; 5, 68
l	145.77	6[b]	25, 27, 30, 32	5, 56; 7, 67; 11, 54
m	145.99	15	51, 52, 55, 56	14, 56; 16, 65; 17, 56
n	146.10	8	26, 31	7, 55; 13, 67
o	146.32	12	45, 46, 49, 50	11, 68; 13, 56; 16, 56
p	148.41	17	59, 60	15, 56
q	153.03	3	3, 4, 5, 6	1, 48; 2, 57; 4, 71

[a] No coupling was observed between peaks e and f, presumably due to small δ/J. [b] Peaks k and l are extremely close and may have the reversed assignment.

type 1, C–C connectivities provided assignments for carbon types 2–13 and 16. Types 5 and 7 were readily differentiated in that type 5 (peak c) couples with three full-intensity peaks (d, k, and l), while type 7 (peak h) couples with two full-intensity peaks (b and l) and a half-intensity peak (n). The coupling between carbon types 9 and 10 (peaks d and e) was not first order, and $^1J_{CC}$ could not be measured. Peaks e and f, corresponding to types 10 and 14, are very close, and the associated cross peaks are not visible.[8] Connectivities from carbon type 17 completed the assignments. The up–down pattern aided assignments in complicated regions.

Buckminsterfullerene contains one type of carbon and two types of carbon–carbon bonds. The ^{13}C NMR spectrum of C_{60} thus shows a single peak,[6] and C–C couplings corresponding to the two types of C–C bonds are not discernible. In contrast, derivative **1** shows 17 peaks and the associated couplings (Table I). The C–C coupling constants for the C_{60} portion of **1** fall into three groups, 48 Hz, 54–57 Hz, and 65–71 Hz. A plot of bond length versus $^1J_{CC}$ shows three distinct types of bonds (Figure 3).[9] The 48-Hz coupling corresponds to the bond between carbon types 1 and 3 and is comparable with the 47.7-Hz C(sp^2)–C(sp^3) coupling in benzyl alcohol.[10] The 54–57-Hz couplings correspond to fusions between five- and six-membered rings, and the 65–71-Hz couplings correspond to fusions between two six-membered rings (Figure 1b).[11] Considering carbon types 2 and 4–17, which have approximately equivalent geometries and symmetrical bonds,[1] the average coupling constants for these two ranges, 55.6 (2) and 67.2 (6) Hz, can be used to calculate the s character in the two types of bonds: 31.5% s for six–five ring fusions, and 34.0% s for six–six ring fusions.[12] The π-orbital accordingly has 3% s character.[13]

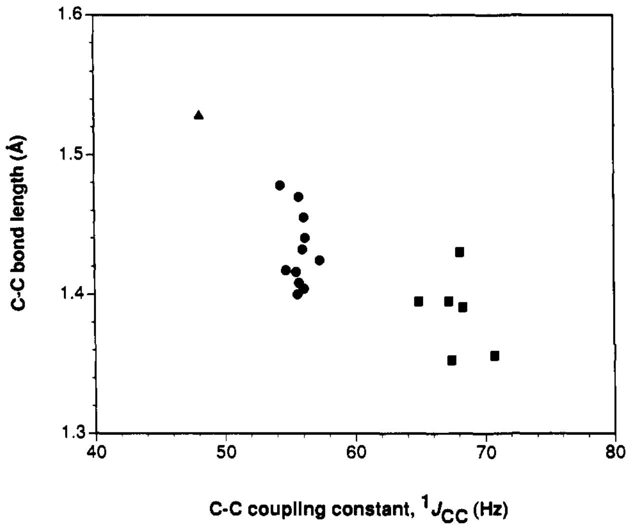

Figure 3. Plot of C–C bond length versus C–C coupling constant in **1** showing three groupings: C(type 1)–C(type 3) (▲), six–five ring fusions (●), and six–six ring fusions (■).

Since the structure of this portion of **1** closely fits the soccer ball structure of buckminsterfullerene,[1] these hybridizations provide a good model for C_{60}.

Acknowledgment. J.M.H. is grateful to the National Science Foundation (Presidential Young Investigator Award, CHE-8857453), the Camille and Henry Dreyfus Foundation (New Faculty Grant), the Merck Sharp & Dohme Research Laboratories (postdoctoral fellowship for A.M.), the Shell Oil Company Foundation (Shell Faculty Fellowship), Xerox Corporation, Monsanto Company, and Hoffmann-La Roche for financial support. S.L. thanks Syntex for a fellowship. We thank James R. Heath for the preparation of ^{13}C-enriched soot and Joseph Lyssikatos for assistance with 1D spectra.

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(7) The O-bonded carbons in bisosmylated anthracene appear at 92.2 and 89.6 ppm. Wallis, J. M.; Kochi, J. K. *J. Am. Chem. Soc.* **1988**, *110*, 8207.

(8) Intensity decreases with decreasing δ/J. Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*; Delft University Press: Boston, 1982; p 164.

(9) A linear relationship between $^1J_{CC}$ and C–C bond lengths has been observed for benzo[*a*]pyrenes. Unkefer, C. J.; London, R. E.; Whaley, T. W.; Daub, G. H. *J. Am. Chem. Soc.* **1983**, *105*, 733.

(10) Ihrig, A. M.; Marshall, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 1756.

(11) In C_{70}, $^1J_{b,c} = {}^1J_{c,d} = 55$ Hz (six–five ring fusions), $^1J_{d,e} = 62$ Hz (six–six ring fusion), and $^1J_{a,b} = 68$ Hz (six–six ring fusion). Johnson has related the size of $^1J_{a,b}$ and $^1J_{d,e}$ to whether one or both of the bonded carbons belong to five-membered rings.[2] In C_{60} and in the C7–C60 portion of **1**, each of the carbons belongs to a five-membered ring, so six–six ring fusions in **1** and C_{60} are similar to bond a–b in C_{70}.

(12) $^1J_{C_xC_y} = [0.073(\% s_x)(\% s_y) - 17]$ Hz, $\% s_x = \% s_y$ for symmetrical bonds. Weigert, F. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 6021. Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: Philadelphia, 1978; p 57.

(13) POAV1 analysis of bond-equalized icosahedral C_{60} gives $s^{0.093}p$ hybridization for the π-orbital (8% s character). Haddon, R. C.; Brus, L. E.; Raghavachari, K. *Chem. Phys. Lett.* **1986**, *131*, 165.

Selective Modification of DNA Controlled by an Ionic Signal

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Received February 20, 1991

Oligonucleotide-directed alkylating agents[1] provide an exciting new method for site-specific derivatization of nucleic acids in vitro and in vivo.[2] The ultimate utility of this type of affinity technique is determined in part by the functional groups chosen for modifying the desired target. Selective modification by a compound of innate reactivity requires a slow rate of conversion in order to permit binding recognition to precede target derivatization. Consequently,

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(1) (a) Webb, T. R.; Matteucci, M. D. *J. Am. Chem. Soc.* **1986**, *108*, 2764–2765. (b) Vlassov, V. V.; Zarytova, V. F.; Kutiavin, I. V.; Mamaev, S. V.; Podyminogin, M. A. *Nucleic Acids Res.* **1986**, *14*, 4065–4076. (c) Vlassov, V. V.; Zarytova, V. F.; Kutyavin, I. V.; Mamaev, S. V. *FEBS Lett.* **1988**, *231*, 352–354. (d) Meyer, R. B.; Tabone, J. C.; Hurst, G. D.; Smith, T. M.; Gamper, H. *J. Am. Chem. Soc.* **1989**, *111*, 8517–8519. (e) Povsic, T. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 9428–9430.

(2) For recent reviews, see: (a) Cohen, J. S., Ed. *Oligodeoxynucleotides*; CRC Press: Boca Raton, 1989. (b) Brakel, C. L., Ed. *Discoveries in Antisense Nucleic Acids*; Portfolio Publishing Company: The Woodlands, 1989. (c) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543–584. (d) Goodchild, J. *Bioconjugate Chem.* **1990**, *1*, 165–187.

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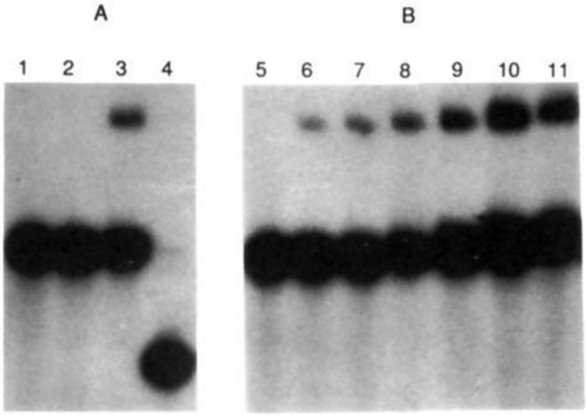


Figure 1. Autoradiograms of denaturing polyacrylamide (20%) gels used to identify the salt- and sequence-dependent cross-link of DNA. (A) Standard assays included 9 nM each **4** and **5** (radiolabeled) in 1 mM morpholinoethanesulfonate (MES), pH 7. Samples were incubated for 10 min at 25 °C before cross-linking was initiated by the addition of KF (final volume 10 μL). Reactions (25 °C) were quenched after 10 min with excess unlabeled **5** (19 μM) and then concentrated by ca. 50% under high vacuum. An equal volume of 80% formamide was added to fully denature the DNA. Finally, samples were separated by electrophoresis and detected by autoradiography. Lane 1: no KF. Lane 2: **4** was preincubated with 100 mM KF (30 min, 40 °C) prior to the standard procedure above using 250 mM KF. Lane 3: 250 mM KF. Lane 4: the noncomplementary sequence **6** was used to replace **5** under the standard conditions using 250 mM KF. (B) Samples were prepared as above and treated with the following concentrations of KF. Lane 5: none. Lane 6: 50 mM. Lane 7: 100 mM. Lane 8: 250 mM. Lane 9: 500 mM. Lane 10: 1000 mM. Lane 11: 2000 mM.

reaction times have often required 12–48 h.[1] If instead an appendage were designed with a latent but inducible activity, the equivalent of an extremely potent intermediate could be delivered to a single site precisely.[3] This report describes our successful development of a silyl phenol for use as a sequence-specific precursor of a highly reactive electrophile, an *o*-quinone methide. Alkylation of the desired DNA target was induced on command by altering the ionic nature of the reaction medium.

Organosilane compounds have often served as intermediates in the generation of quinone methides under aprotic conditions,[4] yet rarely has related chemistry been explored under protic[5] or physiological conditions. An aqueous environment would not necessarily preclude the use of a silyl group to protect a phenolic oxygen[6] or the use of aqueous fluoride ion to deprotect such a species.[7] Less than 10% of *tert*-butyldimethylsilyl-protected phenol **1** hydrolyzed in 50% aqueous acetonitrile/1 mM morpholinoethanesulfonate (MES), pH 7, overnight (22 °C). In the added presence of 100 mM KF, however, **1** converted to *p*-nitrophenol and 2-hydroxybenzyl alcohol (eq 1) with a half-life of ca. 300 min (^{1}H NMR).[8] The intermediacy of a quinone

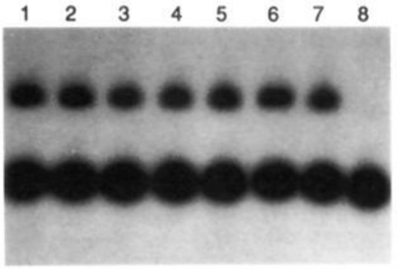
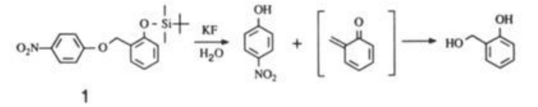


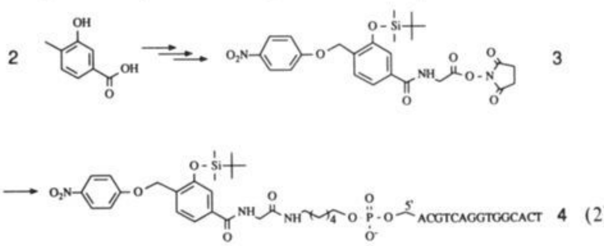
Figure 2. The addition of various salts induced cross-link formation between reagent and target. **4** and **5** (9 nM each) were combined in 1 mM MES, pH 7, and maintained at 25 °C for 10 min. Reaction (3 h) was initiated by the addition of 250 mM KF (lane 1), NaF (lane 2), CsF (lane 3), KCl (lane 4), KBr (lane 5), $KClO_4$ (lane 6), potassium phosphate, pH 7 (lane 7), and no salt (lane 8) to a final volume of 10 μL. Samples were further treated under the standard conditions described for Figure 1.

methide in this equation is suggested by the required loss of the silyl group prior to benzylic substitution. This simple model system then supported the use of a related derivative for selective alkylation of nucleic acids.



(1)

An appropriate silyl phenol was prepared and coupled to a sequence-directing oligonucleotide as depicted in eq 2.[8] Hybridization of the reactant **4** and its complementary target **5**, 5′-[^{32}P]d(AGTGCCACCTGACGTCTAAG), did not alone stimulate alkylation (lane 1, Figure 1). As desired, however, a high molecular weight derivative typical of a covalent cross-link structure was produced when an equivalent sample of **4** + **5** was treated with 250 mM KF (10 min) (lane 3). The product of this treatment appeared to contain the complete sequence of both strands since the electrophoretic properties of this material were identical with those of a related covalent duplex produced under complementary conditions (data not shown).[3f,g]



(2)

The fluoride-dependent activation above is fully consistent with the expected deprotection and decomposition of a silyl phenol such as **1** or **4**. The reactive intermediate responsible for alkylation of **5** appeared to form only transiently. When **4** was treated with 100 mM KF prior to addition of **5**, no high molecular weight species was evident (lane 2). Similarly, no products were detected when **4** was first incubated with a noncomplementary oligonucleotide, **6**, 5′-[^{32}P]d(CATGCGTTCCCGTG), and then activated by the addition of 250 mM KF (lane 4). Cross-linking therefore occurred only for a DNA sequence that bound tightly to **4** prior to initiation of reaction by added fluoride. After hybridization of **4** + **5**, the extent of cross-link formed in 10 min was clearly dependent on the concentration of KF added to the medium and reaction yields were as great as 30% (Cerenkov counting) (Figure 1B).

Other sources of fluoride also triggered selective modification of **5**. NaF and CsF, for example, were both effective in generating the cross-link species (Figure 2). Most interestingly, target

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(3) For example, see: (a) Summerton, J.; Bartlett, P. A. *J. Mol. Biol.* **1978**, *122*, 145–162. (b) Gamper, H. B.; Cimino, G. D.; Hearst, J. E. *J. Mol. Biol.* **1987**, *197*, 349–362. (c) Iverson, B. L.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4615–4619. (d) Pieles, U.; Sproat, B. S.; Cramer, F. *Nucleic Acids Res.* **1989**, *17*, 8967–8978. (e) Perrouault, L.; Asseline, U.; Rivalle, C.; Thuong, N. T.; Bisagni, E.; Giovannangeli, C.; Le Doan, T.; Helene, C. *Nature* **1990**, *344*, 358–360. (f) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1990**, *112*, 6397–6399. (g) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1991**, *113*, 5116–5117.

(4) For example: (a) Ramage, R.; Barron, C. A.; Bielecki, S.; Thomas, D. W. *Tetrahedron Lett.* **1987**, *28*, 4105–4108. (b) Mullen, D. G.; Barany, G. *J. Org. Chem.* **1988**, *53*, 5240–5248. (c) Angle, S. R.; Turnbull, K. D. *J. Am. Chem. Soc.* **1990**, *112*, 3698–3700.

(5) Karabelas, K.; Moore, H. W. *J. Am. Chem. Soc.* **1990**, *112*, 5372–5373.

(6) Shirai, N.; Moriya, K.; Kawazoe, Y. *Tetrahedron* **1986**, *42*, 2211–2214.

(7) (a) Kendall, P. M.; Johnson, J. V.; Cook, C. E. *J. Org. Chem.* **1979**, *44*, 1421–1424. (b) Pogany, S. A.; Zentner, G. M.; Ringeisen, C. D. *Synthesis* **1987**, 718–719. (c) Sinhababu, A. K.; Kawase, M.; Borchardt, R. T. *Synthesis* **1988**, 710–711. (d) Myers, A. G.; Harrington, P. M.; Kuo, E. Y. *J. Am. Chem. Soc.* **1991**, *113*, 694–695.

(8) Experimental details on the syntheses and characterization of all compounds are described in the supplementary material.

modification was also induced by KCl, KBr, $KClO_4$, and potassium phosphate (Figure 2), but unlike the fluoride salts, these latter salts did not promote the related model transformation of **1** under comparable conditions. The nature of the duplex structure formed by **4** + **5** then likely stimulated a secondary salt dependent process (now under investigation) that also provided a suitable electrophile for selective alkylation.

Aqueous application of the silyl phenol described here illustrates an extremely intriguing principle by which a variety of new therapeutic and diagnostic reagents may be designed. DNA alkylation in this report was controlled by the ionic nature of the medium. Perhaps future compounds may be designed to decompose within a unique microenvironment established by a particular nucleotide sequence or conformation.[9]

Acknowledgment. Funds to initiate this project were kindly provided by the William J. and Florence M. Catacosinos Young Investigator Award for Cancer Research. Continuing research was supported in part by a seed grant from the Stony Brook Center for Biotechnology funded by the New York State Science and Technology Foundation.

Supplementary Material Available: Experimental details for the syntheses of **1**, **3**, and **4**, reaction of **1**, and preparation of oligonucleotides (6 pages). Ordering information is given on any current masthead page.

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(9) (a) Gupta, S. C.; Islam, N. B.; Whalen, D. L.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1987**, *52*, 3812–3815. (b) De Voss, J. J.; Hangeland, J. J.; Townsend, C. A. *J. Am. Chem. Soc.* **1990**, *112*, 4554–4556. (c) Lamm, G.; Pack, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9033–9036. (d) Lin, C. H.; Beale, J. M.; Hurley, L. H. *Biochemistry* **1991**, *30*, 3597–3602.

Electrochemical Detection of Fulleronium and Highly Reduced Fulleride (C_{60}^{5-}) Ions in Solution

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Received June 18, 1991

With the truncated icosahedral structure of buckminsterfullerene (C_{60}), as originally proposed by Smalley et al.,[1] now established by X-ray crystallography,[2] one of the important unresolved possibilities posed by this molecule is that C_{60}^{+} may explain the origin of the diffuse interstellar lines.[1,3] With this possibility in mind, the present work employs electrochemistry to probe the oxidation of C_{60} and its related fullerene, C_{70}. This study also describes the first electrochemical generation of C_{60}^{5-} in benzene and presents new electrochemical data on the stepwise electrogeneration of stable C_{60}^{n-} and C_{70}^{n-} (n = 1 to 4) ions in benzonitrile.[4a] Fulleride chemistry of this nature has recently acquired new importance with the advent of C_{60}-based superconducting materials.[5]

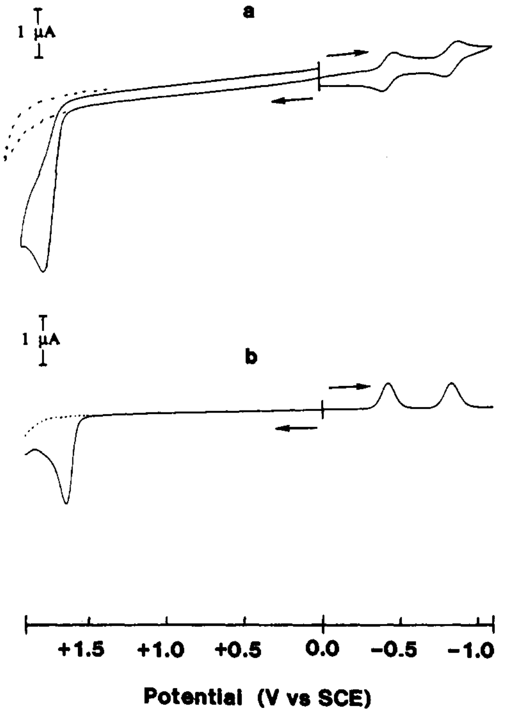

Figure 1. (a) Cyclic voltammogram (100 mV/s) and (b) differential pulse voltammogram (10 mV/s) of C_{70} in PhCN, 0.1 M $[(n\text{-Bu})_4N](PF_6)$. The dotted lines show the background current in the absence of C_{70}.

Cyclic voltammograms of C_{60} and C_{70} in benzonitrile exhibit a single, irreversible oxidation at E_{ox} = +1.76 V vs SCE (+1.30 V vs Fc/Fc^{+}) at a scan rate of 0.1 V/s (Figure 1a for C_{70}).[6] Both oxidation peak currents rise well above the base line and are much larger than for any of the reversible one-electron reductions. This oxidation remains irreversible at scan rates up to 50 V/s and at temperatures down to −15 °C.

Differential pulse voltammograms also exhibit well-defined anodic peaks associated with these irreversible oxidations (Figure 1b). The peak current intensities are about 4 times that of each of the one-electron reductions. Rotating disk voltammograms show these oxidations to be diffusion controlled at rotation rates between 300 and 3000 rpm, and the ratios of maximum diffusion currents between the single oxidation and the first reduction are 4:1. Controlled-potential coulometry of both fullerenes at +1.90 V vs SCE gives $n = 3.9 \pm 0.1$ to confirm that four electrons are abstracted.[6] As expected, no significant current is recorded upon returning the potential to 0.00 V. Both oxidized solutions are orange, stable toward air, and EPR silent at −150 °C.[6] These data indicate that the oxidations of C_{60} and C_{70} proceed via overall four-electron transfers which are followed or accompanied by one or more chemical reactions to render the overall electrochemical oxidations irreversible. The behavior of C_{60} and C_{70} upon oxidation is consistent with that of other polyaromatic hydrocarbon molecules which often exhibit irreversible electrooxidations due to chemical reactions of the electrogenerated species.[7]

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(1) Kroto, H. W.; Heath, J. R.; O'Brien, S. C.; Curl, R. F.; Smalley, R. E. *Nature* **1985**, *318*, 162.

(2) Hawkins, J. M.; Meyer, A.; Lewis, T. A.; Loren, S.; Hollander, F. J. *Science* **1991**, *252*, 312.

(3) *Polycyclic Aromatic Hydrocarbons and Astrophysics*; Léger, A., d'Hendecourt, L., Boccara, N., Eds.; D. Reidel Publishing Company: Boston, 1987 and references therein.

(4) (a) Dubois, D.; Kadish, K. M.; Flanagan, S.; Haufler, R. E.; Chibante, L. P. F.; Wilson, L. J. *J. Am. Chem. Soc.* **1991**, *113*, 4364. (b) Dubois, D.; Kadish, K. M.; Flanagan, S.; Wilson, L. J., unpublished results.

(5) (a) Hebard, A. F.; Rosseinsky, M. J.; Haddon, R. C.; Murphy, D. W.; Glarum, S. H.; Palstra, T. T. M.; Ramirez, A. P.; Kortan, A. R. *Nature* **1991**, *350*, 600. (b) Rosseinsky, M. J.; Ramirez, A. P.; Glarum, S. H.; Murphy, D. W.; Haddon, R. C.; Hebard, A. F.; Palstra, T. T. M.; Kortan, A. R.; Zahurak, S. M.; Makhija, A. V. *Phys. Rev. Lett.* **1991**, *66*, 2830.

(6) Experimental conditions are as previously reported (ref 4). The stationary working electrode had a diameter of 1 mm. The reference electrode was used without the fritted-glass bridge in benzene to avoid additional potential drop between the reference and the working electrodes. Repeated oxidative scans passivated the working electrode, and thus it was cleaned with tissue paper before each scan to ensure reproducibility.

(7) (a) Yoshida, K. *Electrooxidation in Organic Chemistry*; John Wiley & Sons: New York, 1983. (b) Ross, S. D.; Finkelstein, M.; Rudd, E. J. *Anodic Oxidation*; Blomquist, A. T., Wasserman, H. H., Eds.; Academic Press: New York, 1975.

0002-7863/91/1513-7773$02.50/0