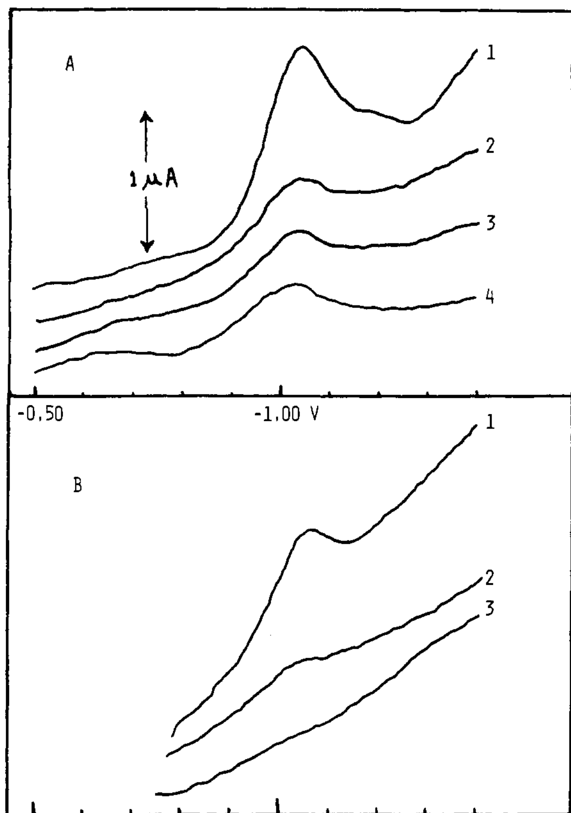
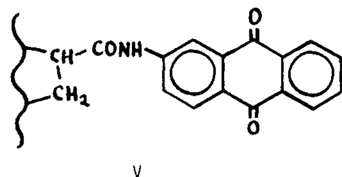


Figure 2. Alternating current voltammetry[12] of β-aminoanthraquinone adsorbed on acrylyl chloride treated oxide-free graphite, A, and untreated graphite with normal surface oxides, B (potential vs. Ag/AgCl(s); numbers indicate scan sequence).

hours. The sample was next immersed in a stirred solution of β-aminoanthraquinone, IV, in dry pyridine and finally washed with fresh pyridine and acetone. The successful preparation of surface derivative V was evidenced by the electrochemistry.



V

The sample was mounted in a kel-F holder which masked all but a single, edge-oriented surface. Voltammetric measurements were made using the graphite as working electrode in a three-electrode cell filled with 0.1 $\overline{\text{M}}$ tetrabutylammonium perchlorate in acetonitrile. In conventional cyclic voltammetry the faradaic current was poorly resolved from the very large capacitive component. Consequently, first-harmonic ac voltammetry was employed[12] using phase-selective rectification of the faradaic current.

Figure 2A shows the first four consecutive scans of freshly prepared sample (dc potential was scanned from −0.5 to −1.4 V). The ac current peaks correspond closely to the reversible half-wave potential for one-electron reduction of IV. (Measurements with an independent working electrode could detect no quinone free in solution before or after the experiment.) The peak diminishes significantly between the first and second cathodic cycles but remains constant for all successive scans. The change is due to the fact that a fraction of quinone is only physically adsorbed on the surface and is desorbed following reduction to the anion radical, as demonstrated by the following control experiment.

The exposed surface of the sample was renewed by brief-polishing on a ground glass plate. This process exposes fresh edge-oriented surface which immediately becomes covered with surface oxides, and results in complete disappearance of the cathodic peak. A freshly resurfaced sample was treated with the pyridine solution of IV, washed, and remounted as before. Figure 2B shows the first three cathodic cycles. Physically adsorbed IV is detected on the first scan and is completely removed by the third. These experiments demonstrate that the covalently bound acid chloride is required for the chemisorption of IV.

Our continuing efforts are directed at more detailed characterization of these new surface derivatives and at extending the variety of functional groups which may be incorporated by means of reactions at the oxide-free surface.

References and Notes

(1) Yu. A. Zarafyanz et al., *Carbon*, 7, 127 (1967); S. S. Barton et al., *ibid.*, **10**, 395 (1972).
(2) The particular surface oxides obtained by this method are nonacidic; see, for example, H. P. Boehm and M. Voll, *Carbon*, **8**, 227, 741 (1970).
(3) G. R. Hennig, *Proc. Carbon Conf., 5th*, 143 (1961); J. M. Thomas and E. E. G. Hughes, *Carbon*, **1**, 209 (1964); B. McCarroll and D. W. McKee, *ibid.*, **9**, 301 (1970).
(4) Union Carbide VYB carbon yarn was used. Following treatment as described it was found to have a surface area of 240 m^2/g (B.E.T. method using N_2). This material consists of dense fibers, about 10μ in diameter. Deep grooves run parallel to the fiber axis but no significant pore structure is evident to 10^5X magnification by scanning electron microscopy.
(5) Decomposition occurred at room temperature contemporaneous with the addition of vinyl bromide. A trace of Br_2 was liberated as well.
(6) A. H. Fainberg and S. Winstein, *J. Am. Chem. Soc.*, **79**, 1603 (1957).
(7) R. F. Lane and A. T. Hubbard, *J. Phys. Chem.*, 77, 1401, 1411 (1973).
(8) R. W. Murray et al., *Anal. Chem.*, **47**, 1882 (1975); **48**, 1247 (1976); *J. Am. Chem. Soc.*, **98**, 7435 (1976).
(9) L. L. Miller et al., *J. Am. Chem. Soc.*, **97**, 3549 (1975); **98**, 8271; 8727 (1976).
(10) T. Kuwana et al., *Anal. Chem.*, **48**, 741 (1976).
(11) Generously provided by Mr. Y. Z. Leger of Union Carbide Corp.
(12) dc scans were made at 2 mV/s from positive to negative potentials with a 5-Hz ac modulation of 10-mV root mean square amplitude. Phase angle was optimized so as to diminish ac current in regions of no faradaic activity.
(13) While on leave from the University of München, K.C. wishes to thank the Deutsche Forschungsgemeinschaft for financial support.
(14) This work was supported by U.S. Army Research Office Grant DAHCO-75-0064-G.

Stephen Mazur,* Tomislav Matusinovic, Karl Cammann[13]
Department of Chemistry, University of Chicago
Chicago, Illinois 60637
Received January 25, 1977

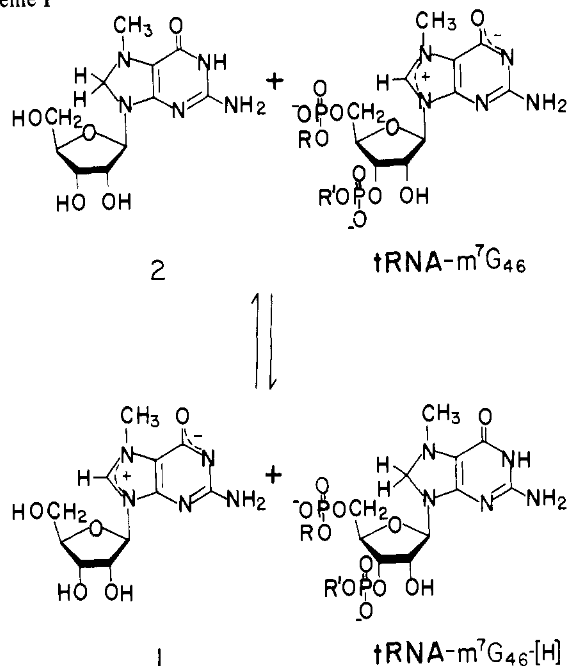
Isoenergetic Hydride Transfer. A Reversible, Phase Transition Mediated tRNA Modification

Sir:

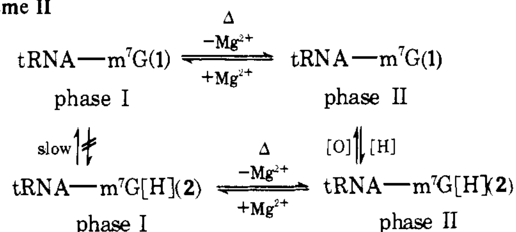
Transfer RNAs contain an abundance of modified nucleosides, the possible functions of which have been studied intensively for years.[1] In spite of such studies, and the availability of a detailed, three-dimensional model of one tRNA which illustrates the relative orientations and possible interactions of the modified nucleosides, the role of these species remains a central, unsolved problem. We wish to report a selective, reversible modification of tRNA by a novel, energetically degenerate process which is accompanied by a reversible alteration of the biochemical activity of the nucleic acid. The nature of the chemical and biochemical changes are in accord with predictions based on the x-ray crystallographic structure of yeast $tRNA^{Phe}$[2] and permit verification of the importance of a single tertiary interaction in the maintenance of structure and biochemical activity of tRNA.

7-Methylguanosine (**1**) is an unusual modified nucleoside in that it exists as a zwitterion at physiological pH. The nucleoside occurs naturally in ribosomal[3] and messenger[4] RNAs,

Scheme I



Scheme II



as well as in tRNA.[5] In *Escherichia coli*, e.g., approximately one-half of all tRNA isoacceptors contain 7-methylguanosine; in those tRNAs it resides exclusively at a single position in loop III.[5] X-ray crystallographic analysis of yeast $tRNA^{Phe}$ has indicated that the 7-methylguanosine moiety in position 46 forms a base triple with $cytidine_{13}$ and $guanosine_{22}$,[2] suggesting that this modification may serve to stabilize tRNA tertiary structure.[6] Selective chemical alteration of 7-methylguanosine would thus be of interest, in that it would permit the proposed function of this nucleoside to be tested experimentally.

Although numerous studies have employed chemically modified tRNAs,[7] successful alterations at a single site have been much less common and there is but one example of a selective, reversible tRNA modification.[8] The difficulty in effecting selective modifications derives both from the presence within a tRNA of several hundred chemically reactive functionalities and from the tertiary structure of the molecule, which renders inaccessible certain potentially reactive species. For example, 7-methylguanosine itself can be reduced with sodium borohydride, affording 8-hydro-7-methylguanosine (**2**) in quantitative yield.[6,9] Application of this reaction to 7-$methylguanosine_{46}$ in yeast $tRNA^{Phe}$ proved to be more difficult, however, since the remainder of the tRNA molecule (1) rendered the 7-methylguanosine moiety inaccessible to borohydride at moderate temperatures[6,10] and (2) contained other ribonucleosides known to react with $NaBH_4$, including N^4-acetylcytidine, dihydrouridine, 1-methyladenosine, and the Y-nucleoside.[6,10-12]

Our interest in the selective modification of the 7-methylguanosine moiety in $tRNA^{Phe}$ prompted us to reinvestigate the transformation of **1** to **2** in an effort to secure a reducing agent capable only of the reduction of **1**, as compared with the other components of $tRNA^{Phe}$. We had previously shown that reduced species **2** is itself a hydride transfer reagent, capable of the selective reduction of certain imines and immonium salts.[9b] Therefore, it seemed reasonable to anticipate that under appropriate conditions *nucleoside* **2** *would establish an equilibrium with 7-methylguanosine*$_{46}$ *in* $tRNA^{Phe}$ *via hydride transfer* (Scheme I) and the use of excess **2** would result in virtually complete reduction of 7-$methylguanosine_{46}$. Since the transfer of hydride from one 7-methylguanosine to another is an *energetically degenerate process*, this would represent *essentially the mildest method possible for effecting the tRNA modification of interest*; in fact **2** was shown not to react with any of the nucleosides in tRNA known to be affected by sodium borohydride. As anticipated, though, initial efforts to effect the reduction of 7-methylguanosine in *E. coli* $tRNA^{Phe}$ or in unfractionated *E. coli* tRNA at moderate temperatures were unsuccessful, owing to steric constraints imposed by the remainder of the tRNA molecules.[13]

Well-defined phase transitions have been shown to accompany the reversible conformational changes of tRNA which can be mediated, e.g., by variations in temperature, ionic strength, or Mg^{2+} concentration.[14] While 7-methylguanosine in native (phase I) tRNA could not be reduced with compound **2**, we anticipated that this transformation might be possible if the experimental conditions were adjusted to alter the phase of the substrate tRNA (and, therefore, the accessibility of the nucleoside of interest). In fact slow reduction of 7-methylguanosine was observed after incubation of the tRNA with nucleoside **2** under phase IV conditions; interestingly, *much more rapid transformation was noted when a more ordered phase II tRNA was employed.*[15] The reduced tRNA could be reoxidized easily while in phase II or IV via reaction with water or oxygen, or restored to phase I for subsequent biochemical measurements.[16] These experiments are summarized in Scheme II.

A typical procedure for the reduction of 7-methylguanosine moieties in tRNA under phase II conditions is illustrated for unfractionated *E. coli* tRNA.[17] A 0.5-mL incubation mixture containing 25 μmol of compound **2**[18] and 50 A_{260} units of tRNA was maintained at 50 °C for 5 min and then treated with 2 vol of cold ethanol. The precipitate was isolated by centrifugation, suspended in 1 mL of 0.5 M Tris HOAc, pH 5.5, and again precipitated with ethanol. Four precipitations effected complete separation of the reducing agent from the tRNA and the extent of reduction of the 7-methylguanosine moieties in tRNA was monitored both by the change in fluorescence and A_{310} values.[6a] A single sample of tRNA could be reduced and allowed to reoxidize several times, with no apparent net change in structure or biological activity after the final reoxidation.

Aminoacylation of the modified $tRNA^{Phe}$ with phenylalanine was assayed using the homologous aminoacyl-tRNA synthetase activity. Although Wintermeyer and Zachau reported that yeast $tRNA^{Phe}$ partially reduced at position 46 was aminoacylated at the same rate as untreated tRNA by yeast and *E. coli* phenylalanyl-tRNA synthetases,[12] in replicate experiments we found that *E. coli* $tRNA^{Phe}$ fully reduced at position 46 was aminoacylated *at a faster rate* than untreated $tRNA^{Phe}$; after reoxidation of the reduced tRNA, the treated and untreated $tRNA^{Phe}$s were activated at the same rate.[19,20] Photochemically induced cross-linking of 4-$thiouridine_8$ and $cytidine_{13}$ in *E. coli* $tRNA^{Phe}$ has been shown to be dependent on tRNA conformation,[21] so that disruption of a preexisting base triple might be expected to alter the rate of cross-linking. In fact the untreated tRNA was observed to undergo cross-linking almost 20% more quickly than tRNA containing **2** in the presence of 10 mM Mg^{2+},[22] in agreement with the observation that *E. coli* $tRNA_f^{Met}$ having 7-methylguanosine in position 47 underwent cross-linking almost twice as fast as the same species having adenosine in position 47.[6b]

Our interpretation of these results is that reduction of 7-methylguanosine disrupts the C_{13}-G_{22}-m^7G_{46} base triple and gives rise to a slightly less ordered tRNA structure. The concomitant, reversible change in the rate of tRNA aminoacylation and photoinduced cross-linking verifies the importance of 7-methylguanosine in maintenance of the tertiary structure of tRNA in solution.

Acknowledgment. We thank the National Science Foundation for financial support of this work.

References and Notes

(1) See, e.g., (a) D. B. Dunn and R. H. Hall in "Handbook of Biochemistry", H. A. Sober, Ed., Chemical Rubber Co, Cleveland, Ohio, 1968, p G-3 ff; (b) R. H. Hall, "The Modified Nucleosides in Nucleic Acid", Columbia University Press, New York, N.Y., 1971; (c) R. W. Chambers, *Prog. Nucleic Acid Res. Mol. Biol.*, **11**, 489 (1971); (d) S. Nishimura, *ibid.*, **12**, 49 (1972).
(2) (a) J. D. Robertus, J. E. Ladner, J. T. Finch, D. Rhodes, R. D. Brown, B. F. C. Clark, and A. Klug, *Nature*, **250**, 546 (1974); (b) S. H. Kim, F. L. Suddath, G. J. Quigley, A. McPherson, J. . Sussman, A. H.-J. Wang, N. C. Seeman, and A. Rich, *Science*, **185**, 435 (1974); (c) A. Klug, J. Ladner, and J. D. Robertus, *J. Mol. Biol.*, **89**, 571 (1974); (d) S. H. Kim, J. L. Sussman, F. L. Suddath, G. J. Quigley, A. McPherson, A. H.-J. Wang, N. C. Seeman, and A. Rich, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4970 (1974).
(3) (a) P. Fellner and F. Sanger, *Nature*, **219**, 236 (1968); (b) G. Attardi and F. Amaldi, *Ann. Rev. Biochem.*, **39**, 183 (1970).
(4) In certain eukaryotic mRNAs, 7-methylguanosine exists as part of an "mRNA cap" ($m^7GpppG_mC\cdots$) which is believed to enhance ribosomal binding and facilitate the initiation of mRNA translation. See, e.g., (a) R. P. Perry and D. E. Kelly, *Cell*, **1**, 37 (1974); (b) F. Rottman, A. J. Shatkin, and R. P. Perry, *ibid.*, **3**, 197 (1974); (c) C. M. Wei, A. Gerschowitz, and B. Moss, *ibid.*, **4**, 379 (1975); (d) R. Dasgupta, F. Harada, and P. Kaesberg, *J. Virology*, **18**, 260 (1976).
(5) B. G. Barrell and B. F. C. Clark, "Handbook of Nucleic Acid Sequences", Joynson-Bruvvers Ltd, Oxford, 1974.
(6) (a) T. Igo-Kemenes and H. G. Zachau, *Eur. J. Biochem.*, **18**, 292 (1971); (b) P. Delaney, J. Bierbaum, and J. Ofengand, *Arch. Biochem. Biophys.*, **161**, 260 (1974).
(7) A. Rich and U. L. RajBhandary, *Ann. Rev. Biochem.*, **45**, 805 (1976).
(8) M. Saneyoshi, *Biochem. Biophys. Res. Commun.*, **40**, 1501 (1970).
(9) (a) F. Pochon, Y. Pascal, P. Pitha, and A. M. Michelson, *Biochim. Biophys. Acta*, **213**, 273 (1970); (b) S. M. Hecht, B. L. Adams, and J. W. Kozarich, *J. Org. Chem.*, **41**, 2303 (1976).
(10) T. Igo-Kemenes and H. G. Zachau, *Eur. J. Biochem.*, **10**, 549 (1969).
(11) (a) P. Cerutti and N. Miller, *J. Mol. Biol.*, **26**, 55 (1967); (b) M. Molinaro, L. B. Sheiner, F. A. Neelon, and G. L. Cantoni, *J. Biol. Chem.*, **243**, 1277 (1968); (c) P. Cerutti, *Biochem. Biophys. Res. Commun.*, **30**, 434 (1968).
(12) It has recently been reported that partial, albeit selective modification of **1** in $tRNA^{Phe}$ can be achieved with $NaBH_4$ if the reaction is carried out at appropriate ionic strength: W. Wintermeyer and H. G. Zachau, *FEBS Lett.*, **58**, 306 (1975).
(13) This is in agreement with the observations of Zachau and his coworkers for yeast $tRNA^{Phe}$ and, since all of the sequenced *E. coli* tRNAs having 7-methylguanosine also have a C-G base pair analogous to C_{13}-G_{22} in $tRNA^{Phe}$, also suggests a generality among tRNA structures with respect to steric accessibility of the 7-methylguanosine moiety.
(14) (a) P. E. Cole, S. K. Yang, and D. M. Crothers, *Biochemistry*, **11**, 4358 (1972); (b) P. E. Cole and D. M. Crothers, *ibid.*, **11**, 4368 (1972); (c) S. K. Yang and D. M. Crothers, *ibid.*, **11**, 4375 (1972); (d) K. L. Wong, Y. P. Wong, and D. R. Kearns, *Biopolymers*, **14**, 749 (1975);(e) M. Caron and H. Dugas, *Nucleic Acids Res.*, **3**, 35 (1976).
(15) Phase IV tRNA is obtained at high temperature and is believed to correspond to randomly coiling single-stranded tRNA. Phase II tRNA, corresponding to a "clover leaf or close variant" structure, is obtained at moderate temperature in the presence of a high concentration of Na^+, but in the absence of Mg^{2+}.[14a-c]
(16) The reduced nucleoside in phase I tRNA underwent slow reoxidation to reafford 7-methylguanosine; the rate of reoxidation depended on the experimental conditions (pH, temperature, presence of oxygen, etc.) and paralleled observations at the nucleoside level.[9b]
(17) A similar procedure has been utilized successfully with purified $tRNA^{Phe}$.
(18) Prepared as described previously[9b] from $NaBH_4$ and 7-methylguanosine.
(19) A control was also run using $tRNA^{Tyr}$, which lacks 7-methylguanosine. Reduction with **2** had no effect on the biochemical activity of the tRNA.
(20) The difference between our results and those of Wintermeyer and Zachau[12] may simply reflect the use of different tRNA species. One may note, however, that the largest difference that we observed between the fully reduced and untreated *E. coli* $tRNA^{Phe}$s was 23% (±1.5–2.0%) after a 30-min incubation. Since Wintermeyer and Zachau[12] carried out their measurements with ±8% precision using yeast $tRNA^{Phe}$ which was only 60% reduced at position 46, they may not have been able to observe changes comparable to those reported here.
(21) A. Favre, A. Michelson, and M. Yaniv, *J. Mol. Biol.*, **58**, 367 (1971).
(22) After reoxidation, the tRNA sample which had been treated with **2** was again photocross-linked at the same rate as untreated tRNA.
(23) Career Development Awardee of the National Cancer Institute, 1975–1980; Alfred P. Sloan Research Fellow, 1975–1977; John Simon Guggenheim Fellow, 1977–1978.
(24) Fulbright-Hays Scholar on leave of absence from la Cattedra di Chimica Biologica, 2nd Medical School, University of Naples, Italy.
(25) American Cancer Society Postdoctoral Fellow, 1975–1976; National Institutes of Health Postdoctoral Fellow, 1976–1977.

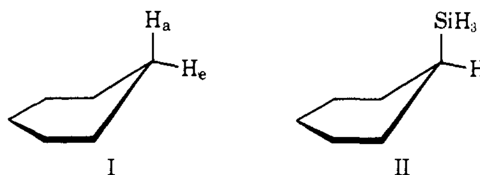
Sidney M. Hecht,*[23] Paolo Arcari,[24] Benjamin L. Adams[25]
Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139
Received January 10, 1977

Temperature Dependent Electron Spin Resonance Spectra of Cyclohexadienyl and Silyl-Substituted Cyclohexadienyl Radicals. On the Conformation of the Radicals[1]

Sir:

Although ESR spectra of a few relatively long-lived cyclohexadienyl radicals in solution have recently been reported from this[2] and other[3] laboratories, most cyclohexadienyl radicals have been too reactive to give good ESR spectra in solution. Therefore, no study on the temperature dependence of the ESR spectra has been made.[4]

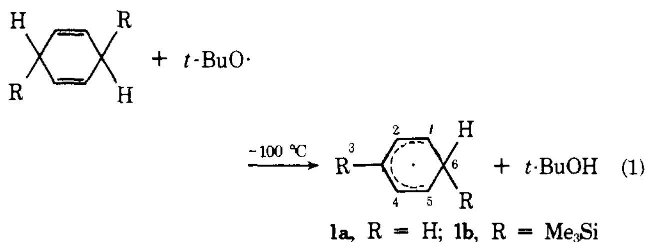
The equilibrium structure of the cyclohexadienyl radical is regarded as planar, but at the same time, the energy minimum is rather shallow so that the radical would be expected to vibrate between bent structures such as I.[4g] The INDO calcu-



lations of the parent cyclohexadienyl radical predicted that the magnitude of the hfcc of the methylene protons should decrease with increasing temperature due to the increasing out-of-plane deformation from the planar carbon framework.[4g]

Our recent studies on the ESR spectra of several 6,6-disilylcyclohexadienyl radicals showed that the silyl group in the radicals had a large ^{29}Si isotopic hfcc due to the enhanced σ-π conjugation that should be reflected in the preferred conformation of the radicals at low temperature.[2a] Consequently, it seemed very interesting and also very important to examine the temperature dependence of the ESR spectra of cyclohexadienyl radicals.

The cyclohexadienyl and silyl-substituted cyclohexadienyl radicals were generated by hydrogen abstraction from the corresponding 1,4-cyclohexadienes (eq 1).



Photolysis of a mixture of 1,4-cyclohexadiene, di-*tert*-butyl peroxide, and cyclopropane (ca. 1:1:1) in the cavity of an ESR spectrometer[5] gave a well-resolved spectrum of the cyclohexadienyl radical (**1a**) whose hfcc values agreed well with those of the literature:[4b] hfcc(gauss) (−100 °C) 2.65 ($H_{2,4}$), 9.13 ($H_{1,5}$), 13.56 (H_3), 48.1 (H_6). The peak-to-peak line width was less than 50 mG. Similarly, 2,6-bis(trimethylsilyl)cyclohexadienyl radical (**1b**) was generated: hfcc(gauss) (−100 °C) 2.15 ($H_{2,4}$), 8.00 ($H_{1,5}$), 35.9 (H_6).