J. Am. Chem. Soc. 1986, 108, 341-343

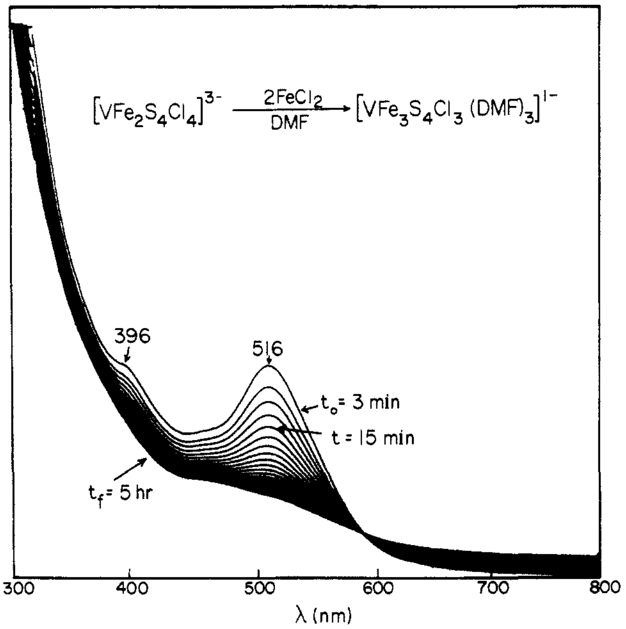

Figure 2. Spectrophotometric time course of the reaction of 3.2 mM $[VFe_2S_4Cl_4]^{3-}$ (**1**) and 6.9 mM $FeCl_2$ in DMF solution at ~25 °C.

with the pseudo-C_3 axis containing V(1) and S(3). Iron atoms occupy trigonally distorted FeS_3Cl sites. The near-Curie magnetic behavior of (Me_4N)(**4**)·2DMF (μ = 3.87 (8.0 K), 3.92 (100 K), 3.99 (200 K), 4.13 μ_B (300 K)) demonstrates a $S = {}^3/_2$ ground state, in common with isoelectronic Mo clusters.[3c,4f] Like those clusters, **4** is electronically delocalized. The average Fe–Cl distance (2.266 (8) Å) is longer than that in $[Fe_4S_4Cl_4]^{2-}$[10] (2.216 (2) Å) and **5**[4e] (2.236 (15) Å), indicating a mean Fe oxidation state $\lesssim$2.5+.[11] Analysis of the ^{57}Fe Mössbauer spectrum at 4.2 K gives $\delta_{1,2}$ = 0.42–0.43 mm/s[12] (two-site fit), vs. $\delta \approx$ 0.40 mm/s for $[Fe_4S_4Cl_4]^{2-}$,[13] also consistent with this oxidation state.[14] The mean V–O distance (2.130 (17) Å) is in the V(II,III)–O, but not the V(IV)–O, range for neutral oxygen ligands.[15] We conclude that the $[V^{4+} + 3Fe^{2+}]$ charge distribution does not apply and that the V atom is more reduced and the Fe atoms are fractionally more oxidized than in this formulation.

Assembly of cluster **4** proceeds via **1**. As seen in Figure 2, the spectrum of authentic **1**[6] (λ_{max} 396, 516 nm) in a 2.2$FeCl_2$/**1** system in DMF decays to an essentially featureless final spectrum (5 h) identical with that of isolated **4**. Cluster formation occurs by the reaction below, in which a cubane core is built from the linear $[VFe_2S_4]^{1+}$ core of **1** by Fe(II) insertion, rearrangement, and reduction (eq 1). A unique feature of **4** is the presence of

$$[VFe_2S_4Cl_4]^{3-} + 2FeCl_2 \rightarrow [VFe_3S_4Cl_3(DMF)_3]^{1-} + [FeCl_4]^{1-} + Cl^- \quad (1)$$

labile ligands at *all* terminal binding sites. In acetonitrile with $Me_2PCH_2CH_2PMe_2$ (1 equiv) **4** is converted to black $(Me_4N)[VFe_3S_4Cl_3(dmpe)(DMF)]$ (47%) by substitution at the V site. In Me_2SO **4** is solvolyzed to $[VFe_3S_4Cl_3(Me_2SO)_3]^{1-}$, which undergoes stoichiometric stepwise thiolate (and areneoxide) substitution at the Fe sites, terminating with $[VFe_3S_4(SR)_3(Me_2SO)_3]^{1-}$ (R = *p*-tolyl: λ_{max} (ϵ_M) 433 (10 900); 12.9 (*m*-H), 11.6 (*p*-Me), −2.3 (*o*-H) ppm). With ethane-1,2-dithiolate (1 equiv) in acetonitrile, **4** yields black $(Me_4N)_2(Et_4N)_2[VFe_3S_4Cl_2(edt)]_2$ (42%). X-ray structural analysis has shown the existence of a centrosymmetric double-cubane anion whose subclusters contain distorted trigonal bipyramidal VS_3(edt) units and are linked by Fe–(μ_2-SR)–V bridges.[16]

The present work provides the only known route to VFe_3S_4 clusters. The final step in core assembly is the unique combination of trinuclear and mononuclear reactants, with the latter possibly extendable to metals other than Fe(II). The results above presage extensive manipulability of **4** by ligand substitution and redox reactions (E(1-/0) = +0.12 V vs. SCE). These matters, together with a more detailed evaluation of charge distribution and property comparisons with isoelectronic Mo clusters, will be the subjects of future reports. The only previously described V–Fe–S clusters are trinuclear[17] (V_2FeS_4, V_2FeS_3) and cubane-type[18] ($V_2Fe_2S_4$) organometallics unrelated to those described here.

Acknowledgment. This research was supported by NSF Grant CHE 81-06017. X-ray diffraction equipment was obtained by NIH Grant 1 S10 RR 02247. We thank Dr. G. C. Papaefthymiou for the Mössbauer spectral results.

Supplementary Material Available: Positional and thermal parameters for $(Me_4N)[VFe_3S_4Cl_3(DMF)_3]\cdot 2DMF$ (3 pages). Ordering information is given on any current masthead page.

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[10] Bobrik, M. A.; Hodgson, K. O.; Holm, R. H. *Inorg. Chem.* **1977**, *16*, 1851.

[11] In the series $[Fe_4S_4(SR)_4]^{1-,2-,3-}$, Fe–SR distances clearly increase as the mean oxidation state decreases: (a) Berg, J. M.; Holm, R. H. In "Metal Ions in Biology"; Spiro, T. G., Ed. Interscience: New York, 1982; Vol. 4, Chapter 1. (b) O'Sullivan, T.; Millar, M. *J. Am. Chem. Soc.* **1985**, *107*, 4096. Fe–Cl distances should behave similarly.

[12] Isomer shifts (δ) are referenced to Fe metal at 4.2 K; ΔE_{Q_1} = 1.12 mm/s, ΔE_{Q_2} = 1.36 mm/s.

[13] Kanatzidis, M. G.; Baenziger, N. C.; Coucouvanis, D.; Simopoulos, A.; Kostikas, A. *J. Am. Chem. Soc.* **1984**, *106*, 4500.

[14] An inverse linear relationship between isomer shift and (mean) oxidation state has been established for Fe–S complexes and clusters.[2b]

[15] (a) Seifert, H. J.; Uebach, J. *Z. Anorg. Allg. Chem.* **1981**, *479*, 32. (b) Cotton, F. A.; Duraj, S. A.; Extine, M. W.; Lewis, G. E.; Roth, W. J.; Schmulbach, C. D.; Schwotzer, W. *J. Chem. Soc., Chem. Commun.* **1983**, 1377. (c) Cotton, F. A.; Duraj, S. A.; Roth, W. J. *Inorg. Chem.* **1985**, *24*, 913.

[16] In the solid state the double cubanes $[Mo_2Fe_6S_8(SR)_6(R'_2cat)_2]^{4-}$, prepared by an entirely different method, have an analogous structure.[4b,g]

[17] Bolinger, C. M.; Rauchfuss, T. B.; Wilson, S. R. *J. Am. Chem. Soc.* **1982**, *104*, 7313.

[18] Rauchfuss, T. B.; Weatherill, T. D.; Wilson, S. R.; Zebrowski, J. P. *J. Am. Chem. Soc.* **1983**, *105*, 6508.

Optical Spectroscopic Studies of Heme Proteins at High Pressure

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Received May 31, 1985

There has been considerable interest in studying the physical and chemical behavior of small molecules at high static pressure by using diamond-anvil cells.[2] In contrast to the relatively rich chemistry now developing on small molecules at high densities, studies of metalloproteins have largely been limited to relatively low pressures (<7 kbar) using UV–vis absorption,[3,4] magnetic susceptibility,[5] or NMR spectroscopy.[6] Low-pressure studies[3,5] of a variety of oxidized heme proteins have conclusively shown evidence for spin-state changes for the iron site at pressures above 1 kbar. Optical absorption studies of reduced heme proteins, while not conclusive, have also been interpreted in terms of spin-state changes. Other changes within the heme pocket, most notably

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[1] (a) Isotopes and Structural Chemistry, INC-4, Los Alamos National Laboratory. (b) Chemistry Department, University of New Mexico.

[2] See, for example: Agnew, S. F.; Swanson, B. I.; Jones, L. H.; Mills, R. L. *J. Phys. Chem.* **1985**, *89*, 1678. Agnew, S. F.; Swanson, B. I.; Jones, L. H.; Mills, R. L.; Schiferl, D. *J. Phys. Chem.* **1983**, *87*, 5065. Nicol, M.; Yin, G. Z. *J. Phys.* **1984**, *45*, c8–163.

[3] Weber, G.; Drickamer, H. G. *Q. Rev. Biophys.* **1983**, *16*, 89.

[4] Ogunmola, G. B.; Zipp, A.; Chen, B.; Kauzmann, W. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1 and references therein.

[5] Messana, C.; Cerdonio, M.; Shenkin, P.; Noble, R. W.; Fermi, G.; Perutz, R. N.; Perutz, M. F. *Biochemistry* **1978**, *17*, 3652.

[6] Morishima, I.; Hara, M. *J. Biol. Chem.* **1983**, *258*, 14 428 and references therein.

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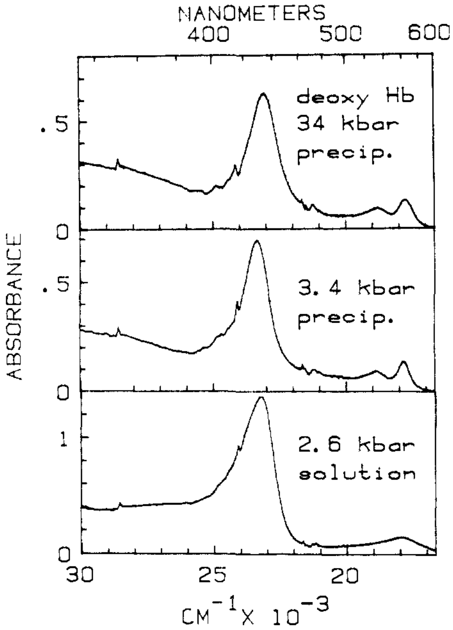

Figure 1. Absorption spectra of deoxyhemoglobin at the pressures indicated, all obtained at ambient temperature. The diamond-anvil cells and measurement techniques have been described[2] previously. The cell was loaded with a hemoglobin solution, prepared as described elsewhere,[9,10] which was concentrated and deoxygenated by means of a dry nitrogen stream and mixed with a small amount of dithionite during loading to retard oxygenation. Sample path length was approximately 85 μm.

in the proximal histidine in the β-chain of Hb via proton NMR, have also been detected.[6] The molecular bases for these changes and the behavior of the heme electronic states at higher pressures, however, remain open questions. We have used[7] both resonance Raman and absorption spectroscopy to address these problems in reduced heme proteins. Resonance Raman scattering is well suited for this application as it provides a structurally specific probe of the heme active site.

The absorption spectra and high-frequency region of the Raman spectra of deoxy-Hb and carbomonoxy-Hb at various pressures are displayed in Figures 1 and 2, respectively. At pressures above 3.4 kbar both deoxy and carbomonoxy-Hb formed a precipitate. This precipitation was reversible for CO-Hb but appeared to be irreversible (at least over the period of several days) for the deoxy species. This precipitation was easily visible under a microscope and for the deoxy-Hb was accompanied by a significant blue shift and sharpening of the Soret band (Figure 1). Deoxy-Mb displayed the same behavior at significantly higher pressure (ca. 7 kbar). Absorption and resonance Raman spectra were easily obtained for the precipitated proteins.

Our absorption data, as opposed to that of earlier workers, show clear evidence for a spin-state change in deoxy-Hb at 3.4 kbar. Both the blue shift of the heme Soret band and the presence of distinct α and β bands in the spectrum observed at 3.4 kbar are characteristic of low-spin heme. Earlier workers suggested[4,6] that this spin-state change is related to coordination of the distal histidine residue in the heme pocket, and we believe that this is indeed the case. Other than a red shift and broadening for the Soret band, the precipitation appears to have little effect on the heme absorption spectrum which remains characteristically low spin for CO-Hb. Further increases in pressure to 34 kbar produce interesting effects in the absorption spectra. The Soret and visible transitions of both deoxy-Hb and CO-Hb shift to lower energy as pressure is increased, although the shift of the Soret band is larger than either of the visible transitions for both proteins. A marked distinction occurs between deoxy-Hb and CO-Hb at pressures above 30 kbar where the former species maintains distinct α and β visible transitions and the latter does not. This, coupled with the Raman data (Figure 2), is a clear indication that the high-pressure behavior of CO-Hb and deoxy-Hb is distinctly different and strongly suggests that the extensive heme–CO interactions are maintained in CO-Hb at high pressure.

The resonance Raman spectra present a more detailed picture of the molecular effects of high pressure on the heme active site. Spectra obtained at ambient pressure in the diamond-anvil cell agree well with the published solution spectra of deoxy-Hb[8] and CO-Hb.[9] In the low-pressure region up to and including precipitation (1–3.4 kbar), CO-Hb exhibits extremely broad features in the high-frequency region (Figure 2). This results from a superposition of bands from liganded and unliganded (photolyzed) heme sites. In the 1560–1650-cm^{-1} region these modes overlap significantly and could not be resolved at our spectral resolution (10 cm^{-1}). Upon cooling to 180 K (2.6 kbar) or increasing the pressure to 34 kbar (room temperature) these high-frequency features sharpen dramatically. We attribute the above changes to photolysis of the CO group to form a mixture of deoxy-Hb and CO-Hb at low pressure; raising the pressure and/or lowering the temperature appears to suppress this photolability. Both CO-Hb and deoxy-Hb show sharp Raman features and good signal-to-noise at elevated pressures (34 kbar), in part by virtue of the better resonance condition produced by the red shift of the Soret band.

The spectra of deoxy-Hb above 3.4 kbar are indicative of low-spin heme. In particular, the spin-state sensitive modes, ν_2 and ν_3, occur[10] at frequencies characteristic of a low-spin species. As the pressure is increased to 34 kbar, nearly all of the high-frequency modes shift to higher frequency by 10–20 cm^{-1}. This effect is independent of the spin-state change at lower pressure and is evident in ν_4 (1357 cm^{-1} at 1 bar) as well. This mode is quite sensitive to changes in the heme π^* orbitals but is relatively insensitive[10] to changes in the iron spin state at ambient pressure.

The shifts of the high-frequency modes in CO-Hb with pressure are of the same magnitude as those observed for the deoxy species. However, the heme modes of CO-Hb appear at higher frequency than their deoxy Hb counterparts at 34 kbar, indicating a significant difference in their heme environments. We conclude that the CO still interacts significantly with the heme at high pressure. Again, we find no evidence for CO photolysis in CO-Hb at high pressures (34 kbar). This is surprising in view of the photolability[11] of CO at ambient pressure. It is likely that collapse of the distal pocket also occurs in Co-Hb and this either inhibits photolysis or enhances the already efficient geminate recombination of CO and heme.

The Raman and optical absorption spectra show evidence for three distinct processes as a function of pressure: (1) an iron spin-state change induced by the collapse of the distal heme pocket at 3.4 kbar, (2) protein structural changes which lead to precipitation, and (3) a subsequent perturbation of the heme electronic orbitals leading to increased intramolecular potentials within the porphyrin macrocycle as pressure is increased. While earlier studies[5] of oxidized heme proteins showed evidence for high- and low-spin equilibria over a narrow pressure range, we do not observe equilibrium mixtures of high- and low-spin forms. As noted above, the present results are consistent with the earlier suggestion that the spin-state change is accompanied by coordination of a histidine residue through collapse of the distal heme pocket. The structural change which leads to precipitation of deoxy-Hb, CO-Hb, and deoxy-Mb is also most likely related to the collapse of the distal heme pocket. It is significant that precipitation of deoxy-Mb occurs at higher pressure than that of deoxy-Hb or CO-Hb. More important is the distinct difference between deoxy-Hb and CO-Hb in terms of the reversibility of this precipitation. We suggest that

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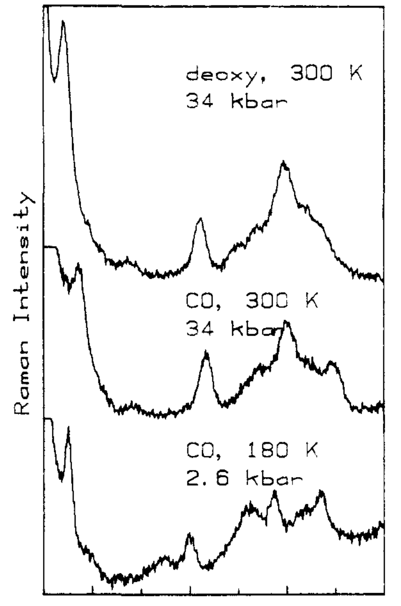

Figure 2. Resonance Raman spectra of deoxy-Hb prepared as in Figure 1 and CO-Hb, prepared by concentrating Hb solution with a stream of CO, at the pressures and temperatures noted. The apparatus has been described previously.[2,7] The 4579-Å line of a Spectra Physics 171 Ar ion laser operating at 50–100 mW was used as the excitation source. The spectral resolution was 10 cm^{-1} and 25–50 scans at 2 cm^{-1}/s were averaged. The large feature at 1335 cm^{-1} is the first-order Raman mode of diamond.

coordination of the distal histidine residue in deoxy-Hb at elevated pressures acts to prevent structural relaxation of the distal heme pocket when the pressure is released. Surprisingly, earlier studies of oxidized heme proteins showed no evidence of precipitation.

Acknowledgment. We are indebted to W. H. Woodruff for helpful comments. This work was performed under the auspices of the U.S. Department of Energy. The support of the NIH (GM33330) and Research Corporation to M.R.O. are also acknowledged.

Synthetic Approaches to Molecules with Sterically Hidden Functional Groups. 2. Bicyclo[8.8.2]eicosa-1(19),10(20),19-triene: The First Bicyclic Cumulatriene[1]

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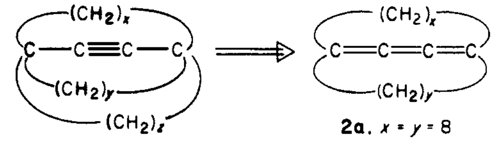
Received August 22, 1985

Recently we described a general method for the preparation of cumulatrienes from allenic phosphonates through a variant of the Horner–Emmons–Wittig reaction.[2] For some time, we have been exploring possible synthetic routes to tricyclic alkynes of structure **1**,[3] and retrosynthetic analysis suggested that [$\pi 6_s + \pi 4_s$] cycloaddition[4] of (*Z*)-1,3,5-hexatriene to bicyclic cumulatriene **2** might lead to the desired ring system. We now wish to report the successful synthesis of the title compound, **2a**.

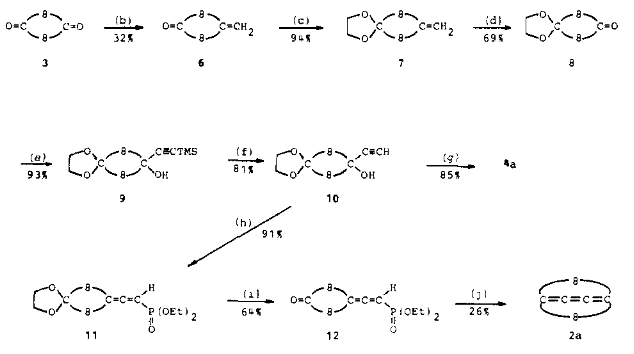


1

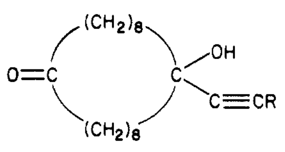
2a, x = y = 8

Our approach, which utilized 1,10-cyclooctadecanedione (**3**)[5] as starting material, is outlined in Scheme I. Initial attempts to prepare monoadduct **4** by the addition of one mole equivalent of acetylide to **3** led instead to diadduct **5**, with 41% of **3** recovered. Similar results were obtained during attempts to prepare the monoketal of **3**,[5] even though a 1:2:1 ratio of **3**/mono adduct/diadduct is statistically predicted in both cases.[6] Through some type of intramolecular interaction (dipole–dipole?), the reactivity of one carbonyl is *retarded* by the presence of the second.[7] However, once one of the groups suffered attack, the second is left "unprotected".

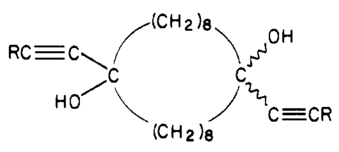
Scheme I^a



^{a}(a) 8 = $(CH_2)_8$; (b) 1.4 mol equiv of $Ph_3P{=}CH_2$ in THF, reflux 20 h; (c) 4.0 mol equiv of glycol, catalytic *p*-TsOH in benzene, reflux 105 min; (d) 1. CH_2Cl_2 solution, excess O_3 at −78 °C; 2. $(PhO)_3P$ workup; (e) 2.0 mol equiv of $Me_3SiC{\equiv}CLi$ (from $Me_3SiC{\equiv}CH$ and *n*-BuLi) in THF, 0 °C, 4 days; (f) 3.5 mol equiv of $AgNO_3$, 1.3 equiv of KCN in $EtOH/H_2O$ (2/1 v/v), 1 h at 25 °C; (g) THF solution, 9% (v/v) 1 N HCl, 2 days at 25 °C; (h) 1.3 mol equiv of $(EtO)_2PCl$, 2.0 mol equiv of pyridine in CH_2Cl_2, 48 h at 25 °C; (i) THF/H_2O (8/1 v/v), 1 equiv of HCl, 60 h at 25 °C; (j) 1.0 equiv of LDA, THF, 60 °C, 4.5 days.



4a, R = H
b, R = $Si(CH_3)_3$



5a, R = H
b, R = $Si(CH_3)_3$

Considerable exploratory work revealed that a Wittig reaction with **3** gave the highest yields of a monoadduct.[8] The resulting enone **6** was subjected to ketalization then ozonolysis to provide the desired monoketal **8**. Addition of trimethylsilyl acetylide and removal of the Me_4Si group gave **10**, which incidently provided **4a** upon hydrolysis. Treatment of propargyl alcohol **10** with chloro diethyl phosphite,[9] followed by gentle hydrolysis, provided allenic

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[1] Abstracted from: Hemling, T. C. Ph.D. Dissertation, University of Cincinnati, 1985. Parts of this work have been described: Joint Great Lakes and Central Regional Meeting of the American Chemical Society, Kalamazoo, MI, May 24, 1985. Abstr. 309. Hemling, T. C.; Macomber, R. S. *Abstr. Pap.—Am. Chem. Soc.* **1985**, *189th*, ORGN180.

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[7] However, the ^{13}C spectrum of **3** in CD_2Cl_2 solution at −90 °C showed no increase in multiplicity beyond the original five lines: δ 213.6, 42.3, 29.5, 28.2, 24.3.

[8] Unless otherwise noted, all compounds were purified by flash chromatography (Still, C. W.; Kahn, M.; Miltra, A. *J. Org. Chem.* **1978**, *43*, 2923) on 230–400 silica gel, providing products that were homogeneous by TLC and exhibited satisfactory spectral data (see supplementary material).

[9] As a leading reference, see: Macomber, R. S.; Krudy, G. A.; Seff, K.; Diaz-Miron, L. E. R. *J. Org. Chem.* **1983**, *48*, 1425.