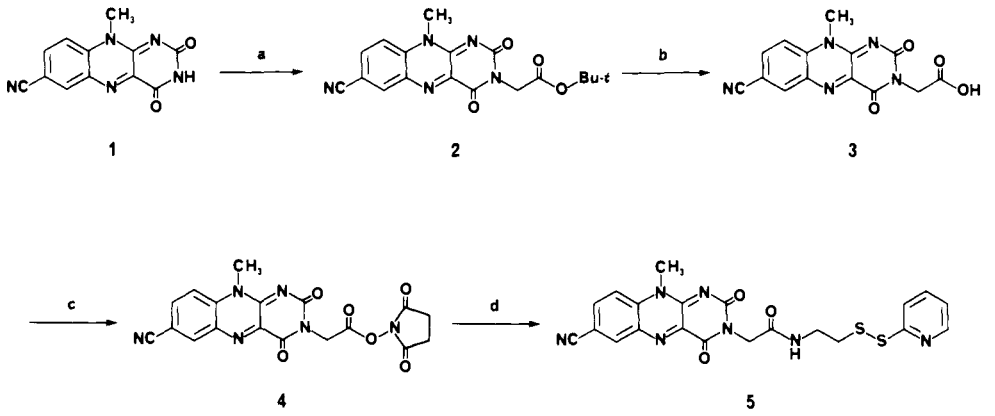
Scheme I[a]



[a] (a) *tert*-Butyl bromoacetate, K_2CO_3, DMF, 4 h (93%); (b) HCOOH, 12 h (90%); (c) *N,N'*-disuccinimidyl carbonate, pyridine, CH_3CN, 4 h (87%); (d) *o*-$(C_5H_4N)SSCH_2CH_2NH_3^+Cl^-$, $(C_2H_5)_3N$, $CHCl_3$, CH_3CN, 3 h (94%).

Table I. Aniline Hydroxylase Activity of Hemoglobin-Catalyst Systems[a]

	Fl-Hb^{3+}	Hb^{3+} + reductase	Hb^{3+}
K_m^{app}, mM	5.3 (5.5)[b]	5.3	5.6
k_{cat}^{app}, min^{-1}	0.169 (0.262)[b]	0.127	0.022
rel k_{cat}^{app}/K_m^{app}	100 (149)[b]	75	12

[a] Experiments were performed as described in Figure 1, except that the reaction mixture contained 0.375 mM NADPH and one of the hemoglobin systems. [b] Limiting K_m, k_{cat}, and k_{cat}/K_m.

Aniline hydroxylase activity of Fl-Hb^{3+} was examined (Figure 1), varying the aniline concentration at several fixed concentrations of NADPH.[12] The rate of hydroxylation of aniline was found to be dependent on the concentrations of both aniline and NADPH in a manner typical of the kinetics of two-substrate enzyme reactions. Thus, double-reciprocal plots of the rates for various concentrations of NADPH gave straight lines intersecting at a single point, clearly demonstrating that NADPH, as well as aniline, behaves as a substrate. Replots of the intercept and the slope of each line against the reciprocals of the NADPH concentrations gave the following values of k_{cat} and K_m for aniline and NADPH: k_{cat} = 0.262 min^{-1}, K_m(aniline) = 5.5 mM, and K_m(NADPH) = 0.22 mM, respectively. Microsomal cytochrome P-450 has been reported to carry out aniline hydroxylation[4c,13] with k_{cat} values ranging from 0.22 to 0.65 min^{-1}. The rate of the hydroxylase activity of Fl-Hb^{3+}, therefore, is comparable to that of microsomal cytochrome P-450.

The activity of Fl-Hb^{3+} was compared to that of ferric hemoglobin, Hb^{3+}, which was assayed in the presence and absence of 0.6 unit of NADPH-cytochrome P-450 reductase[14] (Table I). The apparent K_m values (K_m^{app}) for aniline observed in the three hemoglobin-catalyst systems with 0.375 mM NADPH were almost identical. In contrast the apparent k_{cat} (k_{cat}^{app}) of Hb^{3+} was markedly increased by reconstitution with the P-450 reductase, confirming results reported previously.[4] Nevertheless, the k_{cat}^{app} of Hb^{3+} in the reconstituted system was found to be still smaller than that of Fl-Hb^{3+}. This result demonstrates that the flavin introduced in Fl-Hb^{3+} can substitute for the reductase. The electron transfer between neighboring prosthetic groups in Fl-Hb^{3+} seems to proceed even more efficiently than that in the combined Hb^{3+} and reductase system. It is conceivable that Fl-Hb^{3+} acts in a fashion analogous to cytochrome P-450, and a detailed study of the mechanism of action of Fl-Hb^{3+} is proceeding.

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(12) Preliminary product analysis by HPLC showed that *p*-aminophenol is the dominant product over the ortho isomer (p/o > 4) in the hydroxylation of aniline and that the aminophenol is susceptible to further oxidation to benzoquinoimine in the reaction mixture. The procedure of Mieyal et al.[4a] employed for the kinetic assay is specific for para-hydroxylation products including *p*-benzoquinoimine.

(13) Lu, A. Y. H.; Jacobson, M.; Levin, W.; West, S. B.; Kuntzman, R. *Arch. Biochem. Biophys. 1972, 153*, 294-297.

(14) The reductase concentration of 0.6 unit/mL was sufficient to give a rate greater than 85% of the maximal rate under the conditions employed, i.e., the reaction mixture was essentially saturated with the reductase.

Separation of Cross-Relaxation and *J* Cross-Peaks in 2D Rotating-Frame NMR Spectroscopy

Horst Kessler,*† Christian Griesinger,‡ Rainer Kerssebaum,† Klaus Wagner,† and Richard R. Ernst*‡

Institut für Organische Chemie, Universität Frankfurt D-6000 Frankfurt, Germany
Laboratorium für Physikalische Chemie Eidgenössische Technische Hochschule CH-8092 Zürich, Switzerland

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Cross-relaxation rates measured with two-dimensional (2D) NOE spectroscopy (NOESY) have become one of the most important tools for the determination of molecular conformation in solution. However, in medium-size molecules cross-peak intensities often are close to zero, when the correlation time τ_c approaches the inverse of the Larmor frequency of the protons.[1] Then cross-relaxation in the rotating frame, measured in the CAMELSPIN experiment,[2,3] can be used because molecular reorientation rates are fast compared to the effective Larmor frequency in the rotating frame (only positive NOE's).[2] As has been shown before,[4] under conditions used for the rotating frame cross-relaxation experiment, also magnetization transfer between scalar coupled spins occurs (equivalent to isotropic mixing in the total correlation spectroscopy (TOCSY) experiment[4]), leading to so-called *J* cross-peaks. In this paper we demonstrate a method to suppress the coherent magnetization transfer through scalar coupling in rotating frame experiments, leaving more or less pure NOE cross-peaks in the spectrum.[5]

† Universität Frankfurt.
‡ Eidgenössiche Technische Hochschule.

(1) Noggle, J. H.; Shirmer, R. E. *The Nuclear Overhauser Effect*; Academic Press: New York, London, 1971.

(2) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1984**, *106*, 811.

(3) This experiment is sometimes called ROESY (rotating-frame Overhauser enhancement spectroscopy). Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *63*, 207.

(4) Braunschweiler, L.; Ernst, R. R. *J. Magn. Reson.* **1983**, *53*, 521.

(5) Part of: Griesinger, C. Ph.D. Thesis, Frankfurt, May 1986. Presented by H.K. at the 8th EENC, Spa, Belgium, June 1986.

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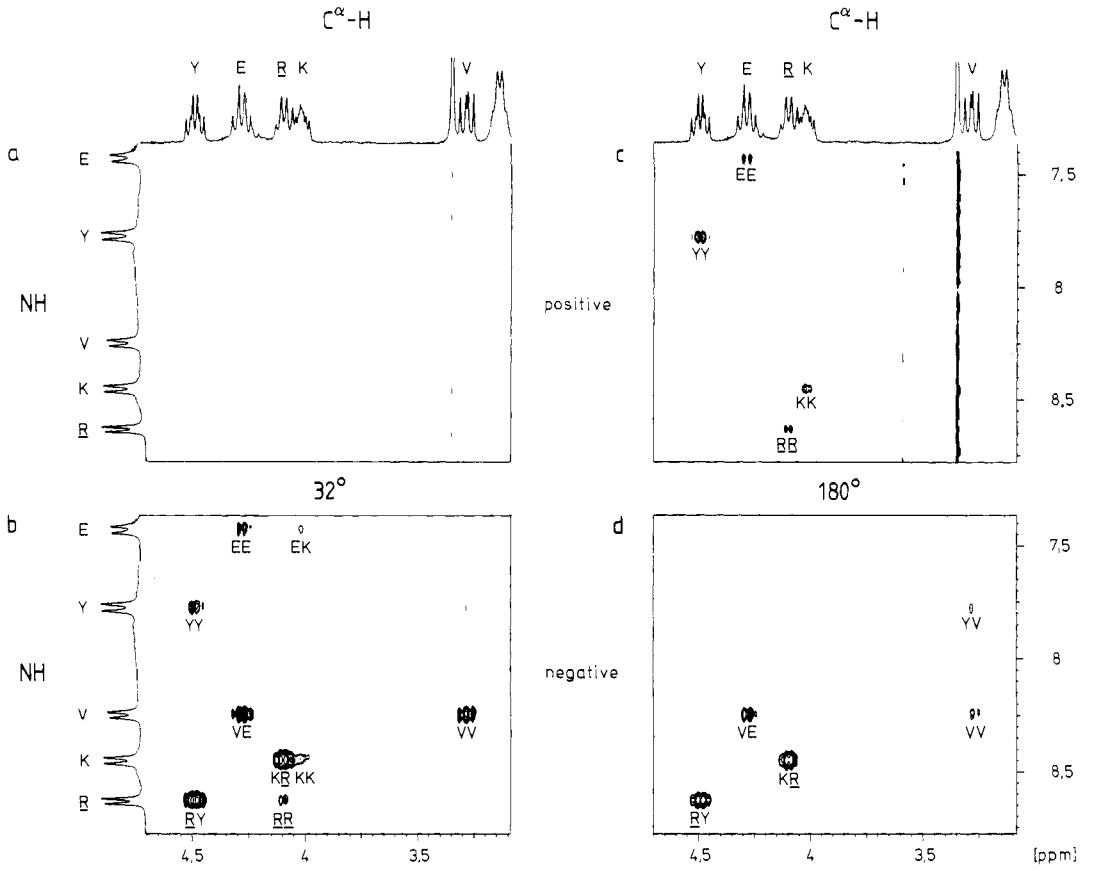

Figure 1. NH,H^{α} region of two rotating-frame NOE spectra of 20 mg of cyclo(-D-Arg(NO_2)-Lys(Z)-Glu(OBzl)-Val-Tyr-) in Me_2SO-d_6 recorded on a 300-MHz Bruker spectrometer with positive contour levels plotted in (a) and (c) and negative contour levels in (b) and (d). Spectra were obtained with the pulse sequence $90\text{-}t_1\text{-}(\beta\text{-}\tau\text{-})_n\text{-}t_2$ by using a mixing sequence $(\beta\tau)_n$ with $\beta = 32° \equiv 4.5$ μs, $\tau = 45$ μs, and $n = 5000$ for (a) and (b) and with $\beta = 180° \equiv 23.8$ μs, $\tau = 238$ μs, and $n = 945$ for (c) and (d). The total mixing time was 250 ms in both experiments. 656 t_1-values with 24 scans each were recorded; 4K data points in t_2; spectral width in both dimensions 2702 Hz. Zero filling to 2K in F1 and 8K in F2 and a squared cosine bell multiplication in both dimensions were applied. Cross-peaks are assigned by the one-letter IUPAC nomenclature of the amino acids neglecting the protecting groups (R = D-Arg(NO_2); K = Lys(Z); E = Glu(OBzl); V = Val Y = Tyr). The first letter assigns the NH signal in F1 and the second letter the H^{α} in F2.

NOE and J cross-peaks in the 2D rotating-frame experiment can be differentiated by their relative sign with respect to the diagonal peaks.[3] Nevertheless, it is very desirable to suppress J cross-peak contributions, because transfer via scalar coupling can be fast in comparison to transfer via dipolar cross-relaxation. Therefore strong deviations from the linear approximation of the build-up curve for the NOE cross-peak intensity can occur, and the build-up rates as well as the intensities of the cross-peaks for a given mixing time do not represent pure NOE effects. This effect is especially pronounced for geminal proton pairs whose NOE cross-peaks in conventional NOESY experiments are frequently used to calibrate the relation between cross-peak integrals and distances for interpreting further cross-peaks in the spectrum. Hence, an incomplete suppression of the J cross-peak contribution may invalidate the relation between initial build-up rate and internuclear distance. Furthermore, sequencial transfer via dipolar and scalar coupling can lead to additional cross-peaks which can be distinguished from pure NOE cross-peaks only by a second rotating-frame experiment with another transmitter offset.[6]

Mixing in the rotating frame can be achieved by a repetitive pulse sequence with the period $(\beta\text{-}\tau\text{-})$. The mean rf power is determined by β/τ, the duty ratio. In our experiments, we noticed a strong dependence of the amplitude of the J cross-peaks on the flip angle β for constant duty ratio β/τ. While for $\beta = \pi$ maximum intensity is obtained, it rapidly approaches zero for β significantly different from π. On the other hand, the NOE cross-peaks are virtually insensitive to the flip angle β. This immediately suggests a method for the suppression of J cross-peaks in rotating-frame NOE spectra: it is just necessary to apply a rapid sequence of small flip angle pulses for mixing.

As an example we show the NH,H^{α} region of two rotating-frame NOE spectra of the cyclic pentapeptide cyclo(-D-Arg-(NO_2)-Lys(Z)-Glu(OBzl)-Val-Tyr-) with positive and negative contour levels plotted separately. One is recorded with $\beta = 32°$ (Figure 1a,b), the other with $\beta = 180°$ (Figure 1c,d), and both with an average rf field strength $\gamma B_2 = 6$ kHz and a total mixing time of 250 ms. The sign of the cross-peak intensities must be interpreted relative to the sign of the diagonal peaks. The sign of the signals in the two spectra can be explained in the following way. Transfer through J-coupling contributes positive signal intensities while cross-relaxation leads to negative intensities. Cross-peaks between protons belonging to different spin systems are found to be negative in both spectra with almost equal intensities independent of the flip angle β. This indicates that the transfer via dipolar coupling is insensitive to the flip angle of the pulses in the mixing sequence. Cross-peaks between scalar coupled nuclei on the other hand contain contributions to the signal intensity due to transfer via scalar coupling *and* due to cross-relaxation, and the relative weight of the two contributions determines the sign and the amplitude of the cross-peaks. In the spectrum obtained with a 180° pulse sequence (Figure 1c), the positive J cross-peak contribution is dominant for the KK, EE, and YY cross-peaks, whereas in the spectrum with 32° pulses the negative NOE contribution prevails for all (NH,H^{α}) cross-peaks.

We tentatively suggest the following explanation for the different sensitivity of NOE and J cross-peaks on the flip angle. For achieving cross-relaxation between two spins, it is just necessary to lock the two spins by an effective field with a strong transverse component leading to a dominant contribution of transverse relaxation to the cross-relaxation rate. Only the average rf field

(6) Neuhaus, D.; Keeler, J. *J. Magn. Reson.* **1986**, *68*, 568.

strength is relevant, irrespective of the flip angle of the individual rf pulses. In addition, neither matching of the effective fields at the two spins nor exact transverse polarization is required.

On the other hand, coherent transfer through J-coupling is only effective when the chemical shift evolution is efficiently suppressed during the mixing period. This can be achieved by refocusing with a series of 180° pulses or in an even more sophisticated way with highly compensated composite π pulses, such as MLEV-17.[7] However, refocusing is not operative for pulses with small flip angles.

Further investigations concerning this useful effect are in progress. After finishing the experimental investigation we learned that equivalent observations have been made by Redwine and Wüthrich.[8]

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. C.G. was funded by a personal grant of the Fonds der Chemischen Industrie.

(7) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.
(8) Redwine, O. D.; Wüthrich, K., personal communication.

From Carbohydrates to Optically Active Carbocycles I: Stereochemical Control in Sugar Hex-5-enyl Radical Cyclization

T. V. RajanBabu

Contribution No. 4226, Central Research and Development Department, Experimental Station, E328 E. I. du Pont de Nemours & Company Wilmington, Delaware 19898

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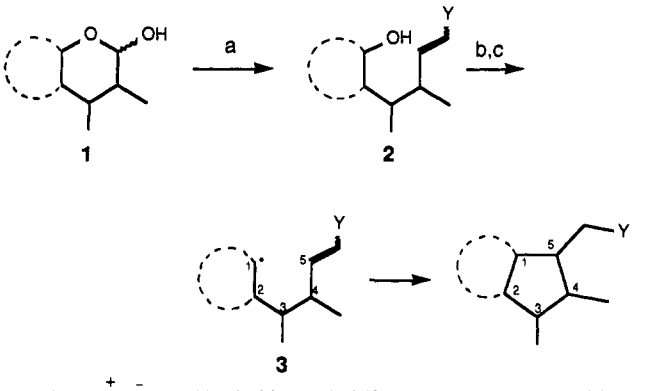
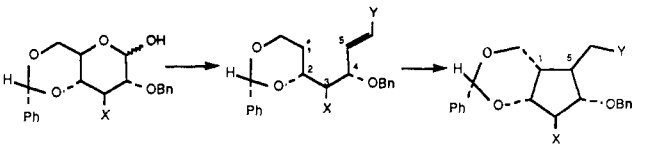
Recently, there has been growing synthetic interest in the use of free radical reactions to prepare five-membered ring compounds by cyclization of hex-5-enyl radicals.[1] Reaction conditions necessary for successful applications of this radical chain process have been delineated[2] based on the kinetic parameters for the prototypes of the primary steps, i.e., the generation, rearrangement, and subsequent trapping and regeneration of the radicals. The stereochemistry of products from variously substituted hex-5-enyl radicals also has been studied[3,4] and the process has been elegantly exploited in the syntheses of complex cyclopentanoid natural products.[5] However, for C-1 substituted hex-5-enyl and analogous cyclic radicals,[4] the stereoselectivity is generally not very high with regard to the newly formed 1,5-bond, although 1,5-cis products often predominate.[4c] In this paper, we wish to report an *unprecedented and exclusive 1,5-trans cyclization mode*[4e] that we discovered while developing a general synthetic strategy to transform readily available pyranose sugars to highly oxygenated

Scheme I[a]



[a] (a) $Ph_3\overset{+}{P}-\overset{-}{C}HY$; (b) (imidazolyl)$_2$C(S), CH_2ClCH_2Cl, Δ; (c) Bu_3SnH, AIBN, $PhCH_3$, Δ.

Scheme II



4 X=OBn

5a X=OBn Y=H
5b X=OBn Y=OMe

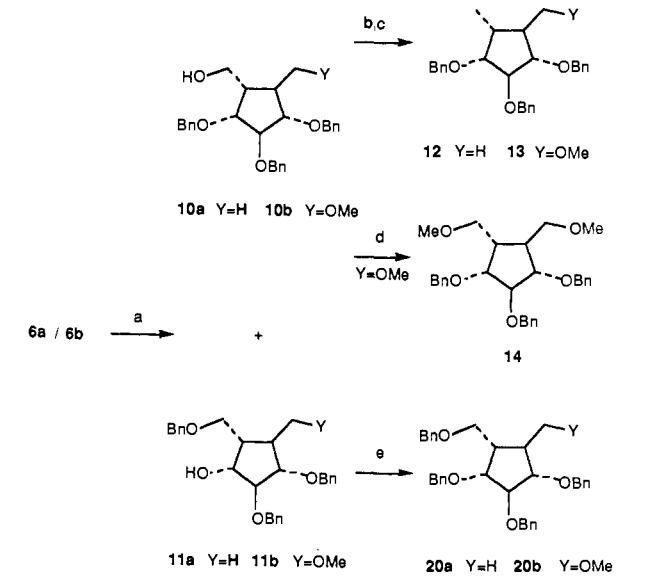
6a X=OBn Y=H
6b X=OBn Y=OMe

7 X=H

8 X=H Y=OMe

9 X=H Y=OMe

Scheme III[a]



[a] (a) LAH, $AlCl_3$; (b) NBS, Ph_3P; (c) Bu_3SnH; (d) NaH, MeI; (e) NaH, BnBr.

(1) For reviews, see: (a) Julia, M. *Pure Appl. Chem.* **1974**, *40*, 553. (b) Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073. (c) Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Vol. 1. (d) Stork, G. In *Current Trends in Organic Synthesis*; Nozaki, H., Ed.; Pergamon Press: Oxford, 1983. (e) Hart, D. J. *Science (Washington, D.C.)* **1984**, *223*, 883. (f) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 553. (g) See also: *Selectivity and Synthetic Applications of Radical Reactions*; Tetrahedron: Symposia-in-print No. 22; Giese, B., Ed.; Pergamon Press: Oxford, 1985; Vol. 41.

(2) (a) Chatgilialoglu, C.; Ingold, K. U.; Scaiano, J. C. *J. Am. Chem. Soc.* **1981**, *103*, 7739. (b) For a compilation of other relevant references, see also: Park, S.-U., Chung, S.-K.; Newcomb, M. *J. Am. Chem. Soc.* **1986**, *108*, 240.

(3) (a) Beckwith, A. L. J.; Blair, I.; Phillipou, G. *J. Am. Chem. Soc.* **1974**, *96*, 1613. (b) Beckwith, A. L. J.; Easton, C. J.; Serelis, A. K. *J. Chem. Soc., Chem. Commun.*, **1980**, 482 and references cited therein. (c) Beckwith, A. L. J.; Easton, C. J.; Lawrence, T.; Serelis, A. K. *Aust. J. Chem.* **1983**, *36*, 545. (d) For a theoretical study, see: Beckwith, A. L. J.; Schiesser, C. H. *Tetrahedron* **1985**, *41*, 3925. See ref 14 in this paper.

(4) (a) Beckwith, A. L. J.; Phillipou, G.; Serelis, A. K. *Tetrahedron Lett.* **1981**, *22*, 2811. (b) Wolff, S.; Agosta, W. C. *J. Chem. Res. Synop.* **1981**, 78. (c) For cyclization of 2-(but-3-enyl)cyclopentyl and cyclohexyl radicals the 1,5-cis/trans ratio is reported to be 8.3 and 3.5, respectively.[4a,b] It must be emphasized that Beckwith has recognized the relative importance of C-1 vs. C-4 substituent in these cyclizations. See ref 3c. (d) For a conformationally rigid system, see: Corey, E. J.; Pyne, S. G. *Tetrahedron Lett.* **1983**, *24*, 2821. (e) For a case of trans-selective (trans to cis ratio, 2/1) cyclization with an exceptionally bulky C-1 substituent, see: Bradney, M. A. M.; Forbes, A. D.; Wood, J. *J. Chem. Soc., Perkin Trans. 2* **1973**, 1655. (f) RajanBabu, T. V., unpublished results.

(5) (a) Burnett, D. A.; Choi, J-K.; Hart, D. J.; Tsai, Y.-M. *J. Am. Chem. Soc.* **1984**, *106*, 8201, see also ref 10b. (b) Curran, D. P.; Chen, M.-H. *Tetrahedron Lett.* **1985**, *26*, 4991. (c) Curran, D. P.; Rakiewicz, D. M. In ref 1g, p 3943. (d) Corey, E. J.; Shimoji, K.; Shih, C. *J. Am. Chem. Soc.* **1984**, *106*, 6425. (e) Reference 1d. (f) See also: Clive, D. L. J.; Beaulieu, P. L. *J. Chem. Soc., Chem. Commun.* **1983**, 307. Wujek, D. G.; Porter, N. A. In ref 1g, p 3973.

0002-7863/87/1509-0609$01.50/0