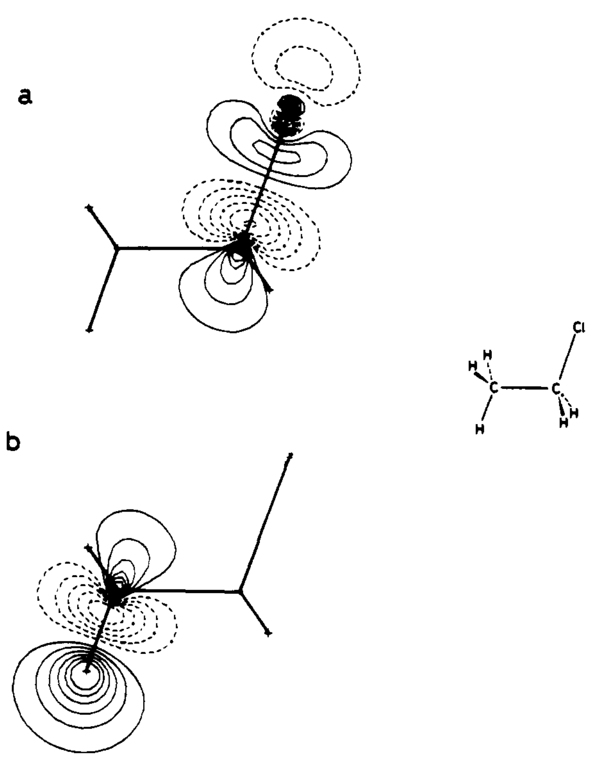

Figure 3. Unoccupied molecular hybrids of ethyl chloride participating in the delocalization interactions with hydride ion. (a) S_N2 and (b) E2.

itant mixing in of the lower-lying occupied MO's. The in-phase combination of these delocalized MO's at N_1 causes the resultant orbital to be localized almost perfectly on the site under attack. The orbital is of stronger s character relative to the lone-pair hybrid of ammonia.

Molecular hybrids of ethyl chloride are presented in Figure 3. They were calculated by placing a hydride ion on the C_α–Cl bond axis (in the S_N2 type model) and the C_β–H bond axis (in the E2 type model) at a distance which is 1.5 times the normal C–H and H–H bond lengths, away from C_α and H_β, respectively. The remaining six unoccupied orbitals have greatest amplitudes in other bond regions and do not contribute to the delocalization interaction in each case. The results of calculation suggest that the inversion at C_α in the S_N2 reaction and the breaking of the C_α–Cl bond in the E2 reaction should be ascribed to other sorts of interactions, probably to the exchange and polarization interactions.[5,6]

It was shown above that the one-electron orbitals participating virtually in the delocalization interaction are localized surprisingly well in the region of initial attacks. The stronger the localization is, the greater the delocalization stabilization would be. The present method will be of use to clarify the outcome of the delocalization interaction, particularly in the interactions of large systems with small reagents. A pair of hybrids, having the amplified lobes around the frontier of interaction, are determined with regard to each symmetry element of the composite reacting system.

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On CNDO/2-Predicted Charge Alternation

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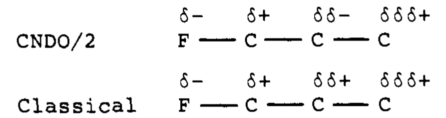


$$\text{CNDO/2}\quad \overset{\delta-}{F}-\overset{\delta+}{C}-\overset{\delta\delta-}{C}-\overset{\delta\delta\delta+}{C}$$

$$\text{Classical}\quad \overset{\delta-}{F}-\overset{\delta+}{C}-\overset{\delta\delta+}{C}-\overset{\delta\delta\delta+}{C}$$

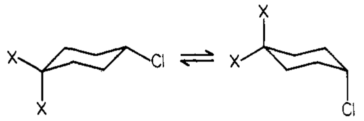
Figure 1. Calculated induced charges.

Table I. Comparison of the e ⇄ a Conformational Free Energy Difference, $\Delta G°$, for **1** and **2** in $FCCl_3$

compound[a]	method	T, °C	$\Delta G°$, kcal/mol
1	^{1}H integration	−124	−0.37 ± 0.05
1-*2,2,6,6-d*$_4$	^{1}H integration	−125	−0.37 ± 0.04
1	^{19}F integration	−124	−0.41 ± 0.08
1-*2,2,6,6-d*$_4$	^{1}H bandwidth[b]	−124	−0.42 ± 0.10[c]
2	^{1}H integration	−86	−0.50 ± 0.03
2-*2,2,6,6-d*$_4$	^{1}H bandwidth[b]	−86	−0.52 ± 0.07[c]

[a] Deuterium substitution (*2,2,6,6-d*$_4$) has no measurable influence upon $\Delta G°$ but makes bandwidth measurement more accurate by eliminating long-range proton–proton coupling. [b] Extrapolated to −124 °C from a range of −35 to +66 °C. [c] 1% confidence limits. [d] Extrapolated to −86 °C from a range of −36 to +69 °C.

Much attention has been given the CNDO/2 prediction of alternation of induced charge in saturated molecules.[1] The CNDO/2 and the classical model[2] of the inductive effect are shown in Figure 1. Some experimental data have been interpreted as being consistent with alternation of induced charge[3] while other data have been interpreted as being inconsistent.[4] In order to provide a critical experimental test for the existence of induced-charge alternation, we studied the effect upon conformational enthalpies of net electrostatic interactions among induced charges. We report here the results of an experimental evaluation of the electrostatic component of the enthalpy difference between the two chair conformations of 4-chloro-1,1-bis(trifluoromethyl)cyclohexane (**1**).[5] The results are compared with those for 4-chloro-1,1-difluorocyclohexane (**2**).[6,7] Our results are not consistent with charge alternation.



1e $X = CF_3$ **1a**
2e $X = F$ **2a**
3e $X = H$ **3a**

For the equilibrium **1e** ⇄ **1a**, the electrostatic component of the conformational enthalpy change, ΔH_e, is given by eq 1.[8] A

$$\Delta H_e°(\mathbf{1}) = \Delta H°(\mathbf{1}) - \Delta H°(\mathbf{3}) \qquad (1)$$

similar relationship for the equilibrium **2e** ⇄ **2a** is given by eq 2. The conformational enthalpy change for chlorocyclohexane

$$\Delta H_e°(\mathbf{2}) = \Delta H°(\mathbf{2}) - \Delta H°(\mathbf{3}) \qquad (2)$$

(**3**) is taken from the literature[9] [$\Delta H°(\mathbf{3})$ = +0.45 ± 0.005

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[1] Pople, J. A.; Gordon, M. *J. Am. Chem. Soc.* **1967**, *89*, 4253–61.

[2] Ingold, C. "Structure and Mechanism in Organic Chemistry", 2nd ed.; Cornell University Press: Ithaca, N.Y., 1969.

[3] Williamson, K. L.; Mosser, S.; Stedman, D. E. *J. Am. Chem. Soc.* **1971**, *93*, 7208–13. Morishima, I.; Yoshikawa, K.; Okada, K.; Yonezawa, T.; Goto, K. *Ibid.* **1973**, *95*, 165–71. Davis, D. W.; Banna, M. S.; Shirley, D. A. *J. Chem. Phys.* **1974**, *60*, 237–45. Ernstbrunner, E. E.; Hudec, J. *J. Am. Chem. Soc.* **1974**, *96*, 7106–8. Eliel, E. L.; Rao, V. S.; Vierhapper, F. W.; Juaristi, G. Z. *Tetrahedron Lett.* **1975**, 4339–42. Eliel, E. L.; Vierhapper, F. W. *J. Org. Chem.* **1976**, *41*, 199–208. Lambert, J. B.; Netzel, D. A.; Sun, H.; Lilianstrom, K. K. *J. Am. Chem. Soc.* **1976**, *98*, 3778–83.

[4] Sardella, D. J. *J. Am. Chem. Soc.* **1972**, *94*, 5206–8.

[5] Samal, P. W. Ph.D. Thesis, Tufts University, 1976. The ^{1}H, ^{19}F, and ^{13}C NMR spectra and elemental analysis of **1** were consistent with the assigned structure.

[6] Giants, T. W. Ph.D. Thesis, Tufts University, 1971. The ^{1}H and ^{19}F NMR spectra and elemental analysis of **2** were consistent with the assigned structure.

[7] Stolow, R. D.; Giants, T. W.; Roberts, J. D. *Tetrahedron Lett.* **1968**, 5777–80.

[8] Stolow, R. D. "Conformational Analysis, Scope and Present Limitations"; Chiurdoglu, G., Ed.; Academic Press: New York, 1971; pp 251–8.

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Table II. Electrostatic Component of the Conformational Enthalpy Change, $\Delta H_e°$, for 1 and 2

method	$\Delta H_e°$, kcal/mol 1e ⇄ 1a	 2e ⇄ 2a
SRME[a]	−1.05	−0.98
CNDO/2[a]	−0.18	−1.32
exptl[b]	−1.04 ± 0.07[c]	−1.13 ± 0.14[d]

[a] Calculated values of ΔE_e have been set equal to ΔH_e, a reasonable approximation. [b] In $FCCl_3$ solution. The gas-phase values would be expected to be somewhat more negative,[10] yielding an even larger discrepancy between the CNDO/2 result for 1 and experiment. [c] From eq 1, where $\Delta H°(1) = -0.59 \pm 0.06$ kcal/mol. Note: $\Delta S°(1) = -1.1 \pm 0.2$ eu; equilibrium constant (absolute temperature), 1.99 ± 0.04 (238 K), 1.74 ± 0.01 (263 K), 1.62 ± 0.01 (289 K), 1.49 ± 0.01 (308 K), 1.37 ± 0.01 (339 K). [d] From eq 2, where $\Delta H°(2) = -0.68 \pm 0.13$ kcal/mol. Note: $\Delta S°(2) = -0.8 \pm 0.5$ eu.

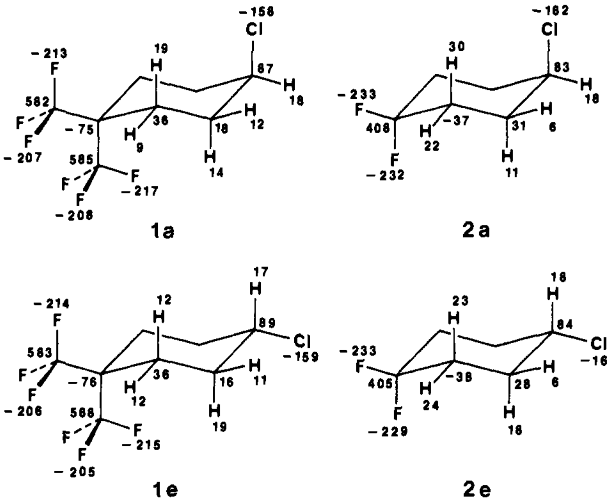

Figure 2. CNDO/2-computed charges for **1** and **2** (10^{-3} electron unit). Input geometries were optimized with Allinger's MM1-1973 molecular mechanics program.[15,16]

($FCCl_3$)]. The conformational enthalpy changes, $\Delta H°$, for **1** and **2** as their *2,2,6,6-d₄* derivatives in $FCCl_3$ solution were obtained by variable-temperature ^1H NMR bandwidth studies[8] at 100 MHz. These studies also yielded conformational free energy changes, $\Delta G°$, which were extrapolated to low temperature and compared with ^1H and ^{19}F NMR integration results for **1** and **2** (Table I). The four independent determinations of $\Delta G°$ for **1** are in excellent agreement with one another. For **1** and **2**, predominance of the chair conformation with chlorine axial is clearly attributable to intramolecular electrostatic interactions. The experimental values of $\Delta H_e°$ for **1** and **2** are listed in Table II. Our next task was to find a method of calculation that could successfully predict these values.

Calculation of the electrostatic component of the conformational energy, E_e, by the approximate method of Jeans has been discredited.[8] We report here results obtained by use of an alternative method for approximation of E_e, which requires assignment of point charges at each nucleus in the molecule followed by summation of all pairwise Coulombic interactions, eq 3, where q_i and q_j are point charges on atoms i and j and r_{ij} is the distance between them.[10]

$$E_e = \sum E_{ij} = \sum (q_i q_j / r_{ij}) \qquad (3)$$

A major problem with this method is the need for computation of a suitable set of point charges. Here we compare use of charges calculated by an approximate molecular orbital theory (CNDO/2)[1,11,12] with charges estimated by the semiclassical theory of Smith, Ree, Magee, and Eyring (SRME).[13] The CNDO/2-computed charges for **1** and **2** are shown in Figure 2. They show the expected alternation in sign illustrated in Figure 1. However, by design, **1** has its CNDO/2-computed fractional negative charge on carbon-1 whereas **2** has CNDO/2-computed fractional negative charges on carbon-2 and carbon-6. All other carbons and all hydrogens in **1** and **2** have fractional positive CNDO/2-computed charges. All of the SRME-computed fractional charges on carbons and hydrogens in both **1** and **2** are positive, without exception (Figure 3).

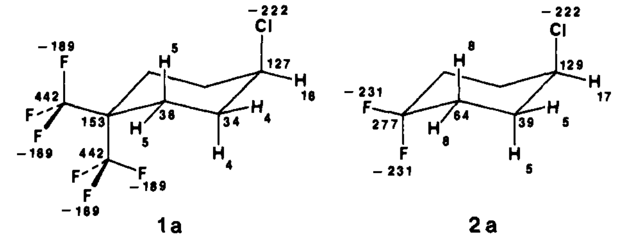

Figure 3. SRME-computed charges for **1** and **2** (10^{-3} electron unit). These charges are independent of conformation. Original SRME parameters are used; the C–H bond moment was taken as zero.[13]

Table III. Some of the Pairwise Electrostatic Interactions (E_{ij}) Calculated for 4-Chloro-1,1-bis(trifluoromethyl)cyclohexane (1), Using SRME Charges

interaction	E_{ij} of 1a	E_{ij} of 1e	ΔE_{ij}	
C-1· · ·Cl	−2.88	−2.39	−0.49	−0.53
F_3C*trans*· · ·Cl	+1.03	+0.95	+0.08	
F_3C*cis*· · ·Cl	+1.29	+1.41	−0.12	
C-2· · ·Cl	−0.85	−0.68	−0.17	−0.34
C-6· · ·Cl	−0.85	−0.68	−0.17	
H-2*cis*· · ·Cl	−0.13	−0.08	−0.05	−0.10
H-6*cis*· · ·Cl	−0.13	−0.08	−0.05	

Table IV. Some of the Pairwise Electrostatic Interactions (E_{ij}) Calculated for 4-Chloro-1,1-difluorocyclohexane (2), Using SRME Charges

interaction	E_{ij} of 2a	E_{ij} of 2e	ΔE_{ij}	
C-1· · ·Cl	−5.26	−4.42	−0.84	−0.16
F*trans*· · ·Cl	+3.51	+2.87	+0.64	
F*cis*· · ·Cl	+3.54	+3.50	+0.04	
C-2· · ·Cl	−1.44	−1.14	−0.30	−0.60
C-6· · ·Cl	−1.44	−1.14	−0.30	
H-2*cis*· · ·Cl	−0.20	−0.12	−0.08	−0.16
H-6*cis*· · ·Cl	−0.20	−0.12	−0.08	

Values of the electrostatic component of the conformational energy difference (ΔH_e in Table II) were computed by using eq 3 and the charges in Figures 2 and 3.[14] Interatomic distances were obtained from geometries optimized with Allinger's MM1-1973 molecular mechanics program.[15,16] Whereas the SRME-computed ΔH_e values show reasonable agreement with experimental values in Table II, the CNDO/2-computed ΔH_e value for **1** shows relatively poor agreement. Furthermore, for **1** the chief reason for poor agreement is that the CNDO/2-computed fractional charge on C-1 is negative rather than positive.

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(9) Höfner, D.; Lesko, S. A.; Binsch, G. *Org. Magn. Reson.* **1978**, *11*, 179–96.

(10) Abraham, R. J.; Rossetti, Z. L. *J. Chem. Soc., Perkin Trans. 2* **1973**, 582–7.

(11) Pople, J. A.; Beveridge, D. L. "Approximate Molecular Orbital Theory"; McGraw-Hill: New York, 1970; Chapter 4.

(12) Program CNINDO-DYNAM, used here, is QCPE Program No. 274.

(13) Smith, R. P.; Ree, T.; Magee, J. L.; Eyring, H. *J. Am. Chem. Soc.* **1951**, *73*, 2263–70. Smith, R. P.; Eyring, H. *Ibid.* **1952**, *74*, 229–34. Smith, R. P.; Mortensen, E. M. *Ibid.* **1956**, *78*, 3932–36.

(14) The 1,2 and 1,3 interactions were neglected. This is valid for SRME charges, which are independent of conformation. With CNDO/2 charges, when 1,3 interactions were included, the overall results were not improved (see ref 5, p 119).

(15) Allinger, N. L. *Adv. Phys. Org. Chem.* **1976**, *13*, 1–82.

(16) Program No. 318, QCPE, Indiana University, Bloomington, Ind.

We therefore conclude that CNDO/2-computed charges for **1** (Figure 2) do not correspond to the actual pattern of induced charges in the molecule.

Summaries of the most significant point charge interactions for **1** and **2**, using SRME charges, are given in Tables III and IV. In addition to the Cl···C-1 interaction[17] and the Cl···H-2*cis* interaction,[10,18] the attractive Cl···C-2 interaction makes an important contribution to stabilization of axial relative to equatorial chlorine in **1** and **2**.[19] However, we stress that calculation of the electrostatic component of the conformational energy by the charge interaction model should include *all* pairwise interactions in the molecule.[14]

In summary, an analysis of the electrostatic component of the conformational enthalpy change for **1** by the point charge interaction model leads us to conclude that the induced-charge alternation (Figure 1) predicted by CNDO/2 theory may be an artifact of the calculations rather than a molecular property. CNDO/2-computed charges are not consistent with experiment, whereas charges which conform to the classical model of the inductive effect (SRME charges[13]) give results which account reasonably well for the experimental data.

For **1** and **2**, we plan to compare the CNDO/2-predicted charges with MNDO-predicted charges.[20] We believe such a comparison will be of interest because for 1,1,1-trifluoroethane the CNDO/2-predicted negative charge on C-2 is −0.108 electron unit,[1] while the MNDO-predicted charge on C-2 is slightly positive (0.008 electron unit[21]).

Acknowledgment. We thank Dr. David Pensak for his kind assistance and Dr. Frank Bissett for running the ^{13}C NMR spectrum of **1**. The 100-MHz NMR spectrometer was purchased with matching funds from NSF Grant GP 8171.

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[17] Wood, G.; Woo, E. P.; Miskow, M. H. *Can. J. Chem.* **1969**, *47*, 429–31.

[18] Abraham, R. J.; Rossetti, Z. L. *Tetrahedron Lett.* **1972**, 4965–8.

[19] Nachtergaele, W. A.; Tavernier, D.; Anteunis, M. J. O. *Bull. Soc. Chim. Belg.* **1980**, *89*, 33–44 and references therein.

[20] (a) Dewar, M. J. S.; Rzepa, H. S. *J. Am. Chem. Soc.* **1978**, *100*, 58–67. (b) Dewar, M. J. S.; Ford, G. P. *Ibid.* **1979**, *101*, 5558–61.

[21] Professor M. J. S. Dewar kindly informed us of this unpublished MNDO result. Note that the corresponding SRME-computed charge on C-2 is significantly more positive, 0.102 electron unit.

Heme Asymmetry in Deuterohemin-Reconstituted Cytochrome *c* Peroxidase

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In the course of a comprehensive study of the proton NMR spectrum[1-4] of cytochrome *c* peroxidase (EC 1.11.1.5; CcP) we have observed that reconstituting the native apoprotein with deuterohemin IX (IB) doubles the number of certain assignable hyperfine-shifted proton resonances. This indicates the presence of heme asymmetry, which may be relevant to the function of this protein. CcP is a ferriheme enzyme isolated from baker's yeast by standard procedures.[5] Its function is to catalyze the oxidation of ferrocytochrome *c* by hydrogen peroxide and as such it plays an important role in cellular electron transport. Its enzymic cycle may involve ligand binding as well as electron transfer so that the observation of two sets of heme resonances presents a mechanism whereby the protein's reactivity may be subject to regulation.

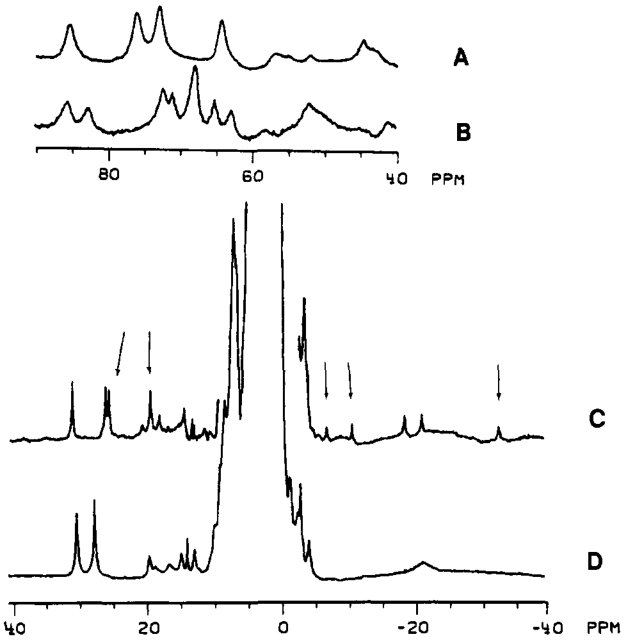
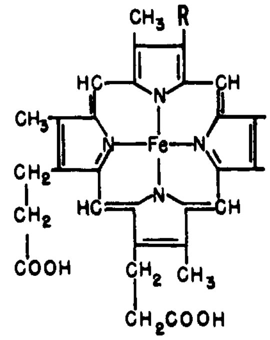


Figure 1. Downfield portion of the proton spectrum of the aquo forms of CcP (A) and deuterohemin-reconstituted CcP (B) showing double the number of peaks in the hemin methyl region between 60 and 90 ppm. C and D illustrate the effect of reconstitution upon the spectrum of the cyanide-ligated forms of the enzyme. D shows the spectrum of native CcP-CN with the two characteristic hemin methyl resonances between 26 and 31 ppm. In the deuterohemin CcP-CN spectrum (C) double the number of hemin methyl resonances indicates the heme heterogeneity. The upfield portion of spectrum C shows four instead of the expected two pyrrole 2,4 protons, further establishing this heterogeneity. Peaks comprising the minor fraction at 28 °C are indicated by arrows and include the tentatively assigned methine resonance at −5.6 ppm. All spectra were obtained on a Nicolet 360-MHz spectrometer. Samples were prepared in 0.1 M KNO$_3$ at pH 7.4, 25 °C. Shifts were referenced to residual water and are reported relative to external 2,2-dimethyl-2-silapentane-5-sulfonate.



IA, R = vinyl
B, R = H

The observation of two sets of hyperfine resonances appears to be related to dual sets of proton[6,7] and carbon[8] resonances which were observed for *C. thummi thummi* hemoglobin fractions and duplicity in the hyperfine resonances of both native and reconstituted sperm whale myoglobin.[9] Such previous spectral anomalies were interpreted as originating from two orientations of the heme group within the heme pocket. These orientations

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[1] Satterlee, J. D.; Erman, J. E. *Arch. Biochem. Biophys.* **1980**, *202*, 608.

[2] Satterlee, J. D.; Erman, J. E. *J. Biol. Chem.*, in press.

[3] Satterlee, J. D.; Erman, J. E. Proceedings of the Symposium on the Interaction between Iron and Proteins in Oxygen and Electron Transport, in press.

[4] Erman, J. E.; Satterlee, J. D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 2062.

[5] Yonetani, T.; Chance, B.; Kajiwara, S. *J. Biol. Chem.* **1966**, *241*, 2981.

[6] LaMar, G. N.; Smith, K.; Gersonde, K.; Sick, H.; Overkamp, M. *J. Biol. Chem.* **1980**, *255*, 66.

[7] LaMar, G. N.; Overkamp, M.; Sick, H.; Gersonde, K. *Biochemistry* **1978**, *17*, 353.

[8] Ribbing, W.; Krumpelmann, D.; Ruterjans, H. *FEBS Lett.* **1978**, *92*, 105.

[9] LaMar, G. N.; Budd, D. L.; Viscio, D. B.; Smith, K. M.; Langry, K. C. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5755.

0002-7863/81/1503-199$01.00/0