erate. As the temperature raises, the a_2 radical state is more admixed, thereby allowing an increase in spin density at the β-pyrrole carbons. This could be responsible for the observed non-Curie law behavior of the CH_2 resonances in Fe(II) oxophlorin radical which experiences substantial spin density changes in going from the b_2 to a_2 radical state. The unusual limiting shifts of the meso proton resonances could be also explained in terms of the temperature-dependent a_2–b_2 mixing state.[12] Bearing in mind that the metal-free oxophlorin radical gives the ESR signal with the meso proton hyperfine coupling a_H = 4.6 G[3] which is translated into 334 ppm contact shift at 23 °C, the much smaller value of the observed shift for **3** could be also attributed to mixing of the two radical states and perhaps to metal and ligand (pyridine) effects as well. The small changes in the UV–visible spectra of **3** at varying temperatures appear to be in accordance with this b_2–a_2 mixing mechanism. The accidental near degenerate states of b_2 and a_2 radicals in **3** may allow the mixing of the two states through vibronic coupling and result in enhanced electron spin relaxation,[13] as the case for a_{1u} and a_{2u} radicals in Ru(II) or Co(III) porphyrin π-cation radicals. Full details of these and related studies will be published in the near future.[15]

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(13) The neutral radical state of **3** should demand enhanced electron spin relaxation, favorable for the unbroadened NMR spectrum. It has been suggested[14] that degenerate or near degenerate radicals may exhibit enhanced electron spin relaxation caused by modulation of the spin–orbit coupling between the electron spin and its orbital motion about the system and of the hyperfine interaction.

(14) Das, M. R.; Wagner, S. B.; Freed, J. *J. Chem. Phys.* **1970**, *52*, 5404–5417. McConnell, H. M. *J. Chem. Phys.* **1961**, *34*, 13–16.

(15) Morishima, I.; Fujii, H.; Shiro, Y.; Sano, S., to be submitted for publication.

trans-Diamminedichloroplatinum(II) Can Chelate d(GpTpG) via Both Guanines in a Similar Fashion as the Cis Isomer

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In contrast to the widely applied antitumor drug *cis*-$PtCl_2(NH_3)_2$ (cis-Pt),[1,2] the trans isomer (trans-Pt) exhibits marginal or no antineoplastic activity.[3,4] Both platinum compounds do react with DNA "in vitro" and "in vivo"[2,5,6] and for both isomers a kinetic preference for the nucleobase guanine is observed.[2,7,8] Since the antineoplastic activity of cis-Pt is ascribed to interactions with the cellular DNA,[1,2] it is tempting to ascribe the differences in biological activity between both isomers to the formation of different platinum–DNA adducts.

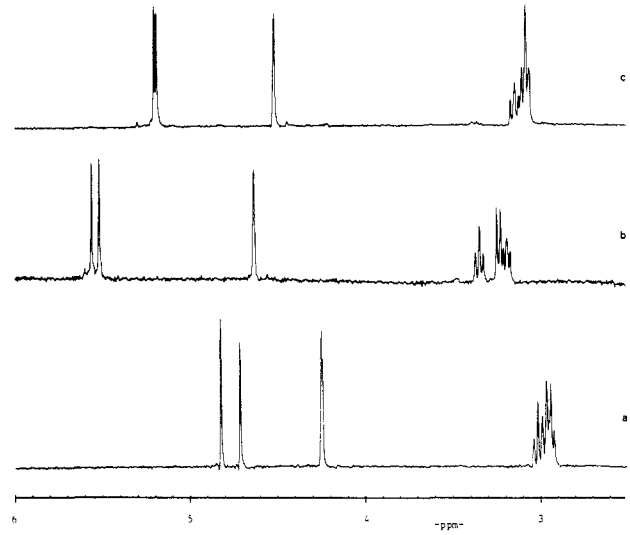

Figure 1. Downfield part of the 300-MHz NMR spectrum of unreacted d(GpTpG) (a), *trans*-$Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (b), and *cis*-$Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (c). The spectra were recorded at 300 K and pH′ 5.0 (pH′ denotes meter reading uncorrected for ^2H_2O). Chemical shifts are relative to tetramethylammonium chloride (TMA, 3.18 ppm downfield from DSS), which was added as an internal reference.

Cis-Pt preferently chelates neighboring purines,[9] and both GG and AG (but no GA) chelation has been reported in several studies.[9-12] To a smaller extent, also next-neighboring guanines (the so-called GNG chelates[13]) as well as guanines positioned in opposite strands (interstrand cross-links) can be bound by cis-Pt. Due to the stereochemistry of trans-Pt, this compound cannot chelate neighboring purines[14] but is thought to form "in vitro" mainly interstrand cross-links, long-range intrastrand cross-links, and monofunctionally bound adducts. Very recently, however, Pinto et al. reported to have indications for the formation of GNG chelates by trans-Pt, found in a "replication mapping" assay.[15] This prompted us to investigate the reaction of trans-Pt with the DNA trimer d(GpTpG) and to compare the product with the earlier investigated adduct of cis-Pt and d(GpTpG).

d(GpTpG) (disodium salt, to prevent the partial inactivation of the platinum compounds that occurs when the diammonium salt is used[16]) was synthesized via an improved phosphotriester method.[17] An equimolar reaction of cis- and trans-Pt with this trimer was performed at 37 °C for 2 weeks in the dark at room temperature (concentration about 4×10^{-6} M; pH 6–7). Gel permeation (Sephadex G25, Pharmacia, using the volatile salt triethylammonium bicarbonate TEAB as eluent) revealed in both cases only one well-defined major adduct. In the case of the trans isomer, also a variable but substantial amount of high molecular, UV-absorbing products was observed, which are most likely oligomers of d(GpTpG) cross-linked by trans-Pt. The major products for both isomers, however, appeared to be monomeric GNG chelates, in which the $Pt(NH_3)_2$ moiety is chelated via N7 to both terminal guanines, i.e., $Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7-

(1) Roberts, J. J.; Thomson, A. J. *Progr. Nucleic Acid Res. Mol. Biol.* **1979**, *22*, 71.

(2) Hacker, M. P.; Douple, D. P.; Krakoff, I. H., Eds. *Platinum Coordination Complexes in Cancer Chemotherapy*; Martinus Nijhoff Publishers: Boston, MA, 1984.

(3) Cleare, M. J.; Hoeschele, J. D. *Bioinorg. Chem.* **1973**, *2*, 187.

(4) Connors, T. A.; Jones, M.; Ross, W. C. J.; Braddock, P. D.; Khokhar, A. R.; Tobe, M. L. *Chem.-Biol. Interact.* **1972**, *5*, 415.

(5) Pinto, A. L.; Lippard, S. J. *Biochem. Biophys. Acta* **1985**, *780*, 167.

(6) Fichtinger-Schepman, A. M. J.; Reedijk, J.; Van Oosterom, A. T.; Lohman, P. H. M. *IARC, Scientific Publications*; in press.

(7) Martin, R. B. *A.C.S. Symp. Ser.* **1983**, *209*, 231.

(8) Eapen, S.; Green, M.; Ismail, I. M. *J. Inorg. Biochem.* **1985**, *24*, 233.

(9) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; den Hartog, J. H. J.; Lohman, P. H. M.; Reedijk, J. *Biochemistry* **1985**, *24*, 707.

(10) Dewan, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 7239.

(11) Eastman, A. *Biochemistry* **1983**, *22*, 3927.

(12) Johnson, N. P.; Mazard, A. M.; Escalier, J.; Macquet, J. P. *J. Am. Chem. Soc.* **1985**, *107*, 6376.

(13) Brouwer, J.; van de Putte, P.; Fichtinger-Schepman, A. M. J.; Reedijk, J. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7010.

(14) Lippard, S. J. *Science (Washington, D.C.)* **1982**, *218*, 1075.

(15) Pinto, A. L.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4616. After the submission of this manuscript, Prof Lippard (personal communication, 1986) has also made the observation that a species *trans*-$Pt(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] can be formed out of the trinucleotide d(GpTpG) and trans-Pt.

(16) Fichtinger-Schepman, A. M. J.; van der Veer, J. L.; Lohman, P. H. M.; Reedijk, J. *J. Inorg. Biochem.* **1984**, *21*, 103.

(17) Van der Marel, G. A.; van Boeckel, C. A. A.; Wille, G.; van Boom, J. H. *Tetrahedron Lett.* **1981**, 3887.

0002-7863/86/1508-3860$01.50/0

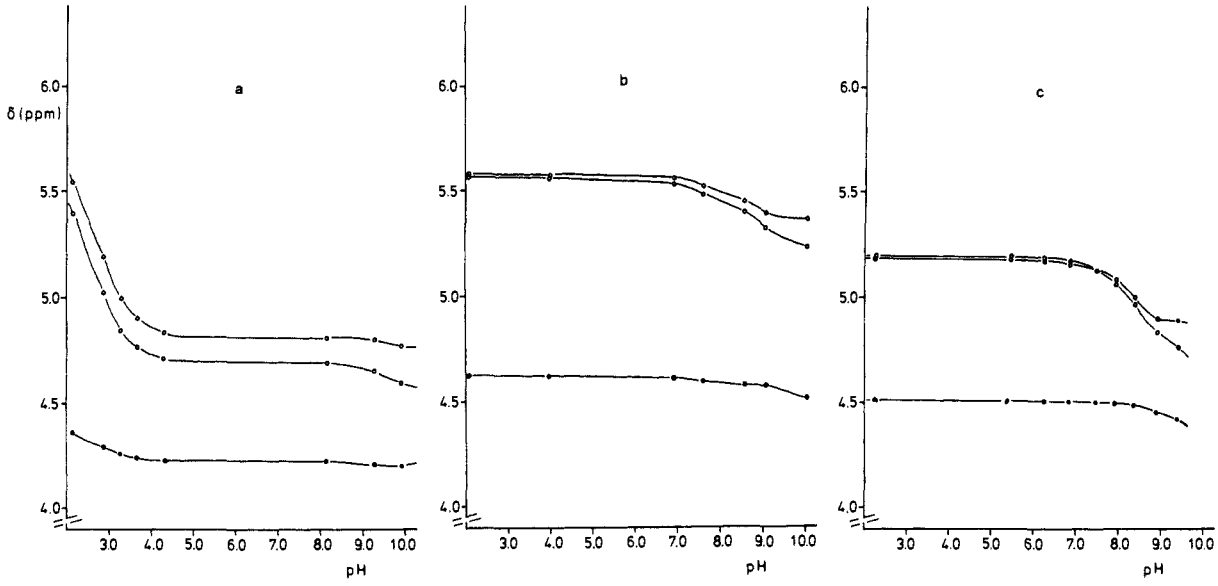

Figure 2. Plot of the chemical shifts vs. pH′ of the guanine H8 (O) and the thymine H6 (●) base protons of unreacted d(GpTpG) (a), *trans*-Pt-$(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (b), and *cis*-Pt$(NH_3)_2$[d(GpTpG)-*N*7(1),*N*7(3)] (c), recorded at 300 K.

(3)]. We have the following observations and arguments for this conclusion:

1. Proton NMR spectra showed only three base proton signals (see Figure 1), which is in agreement with the structure of these monomeric species. In both adducts, especially the guanine H8 base proton signals are shifted downfield. This is often seen in N7-platinated guanine residues in general,[18-21] including the above mentioned GNG adducts.[23-25] The larger downfield shifting of these signals in the trans compound compared with the cis adduct is assumed to originate from the larger shielding effect in the latter chelate. This effect is also observed in corresponding *cis*- and *trans*-bis(mononucleotide)platinum(II) compounds.[26]

2. For both compounds, N7-platination was ascertained by monitoring the pH′ dependence of the guanine H8 signals (see Figure 2). Compared to the unbound trimer, the pK_a' of the N1-deprotonation of the guanine is lowered in both trimer adducts from 10 to 8.5. Moreover, no N7-protonation effect—which is expected at pH 2—is observed, apparently as a result of platinum binding to this site.

3. Separation of a mixture of both adducts on size by gel permeation (Sephadex G25) showed that the cis-Pt adduct eluted just before the trans-Pt adduct. Intramolecular GNG chelation by cis-Pt has been established in the DNA trimer d(GpCpG).[23] Comparison of this adduct with the cis-Pt adduct of d(GpTpG) on the basis of NMR data[23,24] gave evidence for the same binding mode of the latter chelate. So, the elution behavior of the cis- and trans-Pt adduct when separated by means of gel permeation, which is based on size, gives a strong indication for a similar binding fashion of the trans isomer in d(GpTpG).

4. An established method to digest platinated DNA and oligonucleotides enzymatically, followed by fast protein liquid chromatography (FPLC) separation,[9] applied to both platinated trimers resulted in *cis*- and *trans*-Pt$(NH_3)_2$(5′-dGMP-*N*7)-(dGuo-*N*7) together with 5′-TMP. This is expected for the above-proposed structures. It must be noted that the enzymatic digestion of the trans-Pt adduct appeared to be far more difficult, i.e., proceeded slower than the digestion of the cis-Pt adduct. This is probably a result of the more disrupted trimer structure of the trans-Pt adduct. The apparent existence of a N-type deoxyribose conformer, which is indicated by the H1′ doublet (see Figure 1b) and which is not observed in the unbound and in the cis-Pt bound trimer, supports this.

Finally, it should be noted that it appeared possible to construct both intrastrand platinum chelates with space-filling models (CPK), illustrating the flexibility of the d(GpTpG) structure.

Although the above-mentioned results clearly point toward mononuclear species, attention has also been given to the possibility of dinuclear interstrand chelation of two d(GpTpG) units by two *trans*-Pt$(NH_3)_2$ moieties. Due to DNA polarity, both parallel and antiparallel orientation of the two trimers would be possible. Parallel orientation in such a dinuclear species can simply be ruled out, since in that case the enzymatic digestion would have resulted in two platinum adducts in equal amounts: *trans*-Pt$(NH_3)_2$-(dGuo-*N*7)$_2$ and *trans*-Pt$(NH_3)_2$(5′-dGMP-*N*7)$_2$. This is not observed in the FPLC separations (vide supra). Moreover, the magnetic nonequivalence of the identical base protons in the two trans-Pt bound trimer units would lead to four H8 protons. This is also not observed (see Figure 1).

The antiparallel trimer orientation of such a dimeric species is rejected by the argument denoted in **3** and by the observation that the trans-Pt adduct was much more difficult to digest. This can be understood for a monomeric species (see **4**) but not for a dinuclear structure in which much more flexibility would be expected.

The above-mentioned results supply convincing evidence for the new trans-Pt GNG-chelation fashion, as proposed by Pinto et al. from biological experiments.[15] However, it has to be noted that in their study,[15] as well as in the present study, single-stranded DNA fragments are used, which may behave differently from double-stranded DNA fragments due to a more rigid structure of the latter. Nevertheless, these results indicate that similar GNG chelates can be formed by both cis- and trans-Pt. Although these chelates have different conformations, this observation will direct more attention to the GG and the AG chelates—which can be formed by cis-Pt only—as the crucial platinum–DNA lesions which might be responsible for the antineoplastic activity of cis-Pt.

Acknowledgment. This study was supported in part by the Netherlands Foundation of Chemical research (S.O.N.) with

(18) Marcelis, A. T. M.; Reedijk, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 121.
(19) Girault, J. P.; Chottard, J. C.; Guittet, E. R.; Lallemand, J. Y.; Huyn Dinh, T.; Igolen, J. *J. Biochem. Biophys. Res. Commun.* **1982**, *109*, 1157.
(20) Caradona, J. P.; Lippard, S. J.; Gait, M. J.; Singh, M. *J. Am. Chem. Soc.* **1982**, *104*, 5793.
(21) Den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; van der Marel, G. A.; Haasnoot, C. A. G.; Reedijk, J. *J. Am. Chem. Soc.* **1984**, *106*, 1528.
(22) Marcelis, A. T. M.; den Hartog, J. H. J.; van der Marel, G. A.; Wille, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *135*, 343.
(23) Marcelis, A. T. M.; den Hartog, J. H. J.; Reedijk, J. *J. Am. Chem. Soc.* **1982**, *104*, 2664.
(24) Van der Veer, J. L.; van den Elst, H.; den Hartog, J. H. J.; Reedijk, J., unpublished results.
(25) Den Hartog, J. H. J.; Altona, C.; van Boom, J. H.; Marcelis, A. T. M.; van der Marel, G. A.; Rinkel, L. J.; Wille-Hazeleger, G.; Reedijk, J. *Eur. J. Biochem.* **1983**, *134*, 485.
(26) Marcelis, A. T. M.; van Kralingen, C. G.; Reedijk, J. *J. Inorg. Biochem.* **1980**, *13*, 213.

financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.) through Grant 11-28-17. Financial support for the synthesis of d(GpTpG) was supplied by the Netherlands Organization for the Fight against Cancer. C. Erkelens is thanked for his assistance at the 300-MHz NMR facility of Leiden. Also F. J. Dijt, C. J. van Garderen, and Dr. G. W. Canters are thanked for the critical reading of the manuscript. The FPLC measurements were carried out at the Medical Biological Laboratory TNO (Rijswijk). Stimulating discussions with the group of Prof Dr. J. C. Chottard (Paris), made possible by the sponsorship of the French-Dutch cultural agreement, are gratefully acknowledged. We are indebted to Johnson Matthey Chemicals Ltd. (Reading, England) for their generous loan of K_2PtCl_4.

($\pi^* + \sigma^*$) Molecular Orbital Mixing in β-Chloro Ketones and β-Chloro Olefins

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We have been interested in molecular orbital mixing of distal functionalities as a potential means of photochemically activating a normally UV transparent functional group.[1] For example, a C–Cl bond remote from, but propitiously placed with respect to, a π chromophore may be expected to create a LUMO which is ($\pi^* + \sigma^*$) and therefore potentially C–Cl labile in the excited state. Such mixing is well-known in α(axial)-halo ketones and has been invoked to rationalize the spectral perturbations[2] and photoreactivity[3] characteristic of these compounds. This report describes theoretical and exprimental results from our study of ($\pi^* + \sigma^*$) mixing in β-halo ketones and β-halo olefins.

We initially calculated the degree of C—Cl/C=O mixing in the LUMO of the model substrate, 4-chloro-2-butanone, as a function of the dihedral angles, ϕ_1 and ϕ_2 (cf. Figure 1). Geometries were optimized by MNDO[4] and the wave functions calculated by using Gaussian 76[5] with an STO-3G basis set[6] (the relative degree of C–Cl involvement in the LUMO is represented by $(\sum C_i^2)^{1/2}$, where the $\{C_i\}$ run over the coefficients on Cl in this MO. It is evident from Figure 1 that C–Cl involvement is maximized when $\phi_1 = 90°$ and $\phi_2 = 180°$, and in this conformation, the extent of mixing is about half of that calculated for axial 2-chlorocyclohexanone (cf. Table I). Such mixing is not unique to the ketone and, for example, a ca. 10% greater interaction is calculated for the olefin analogue 4-chloro-2-methylbutene. Table I includes data for other α, β-, and γ-chloro ketones and two entries are noteworthy: (1) equatorial, but not axial, 3-chlorocyclohexanone has an appreciable σ^* component in its LUMO, an observation consistent with the enhanced UV and CD absorption characteristic of such substrates;[2,7] (2) the greatest mixing for a β-chlorine is calculated for the *exo*-6- and *anti*-7-

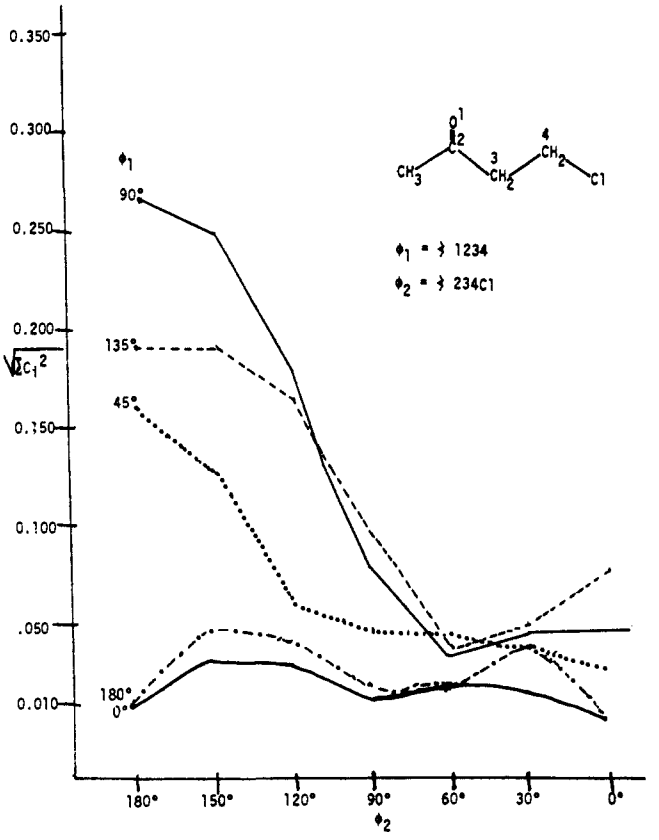

Figure 1. Relative amount of C–Cl involvement in the LUMO of 4-chloro-2-butanone as a function of the dihedral angles ϕ_1 and ϕ_2.

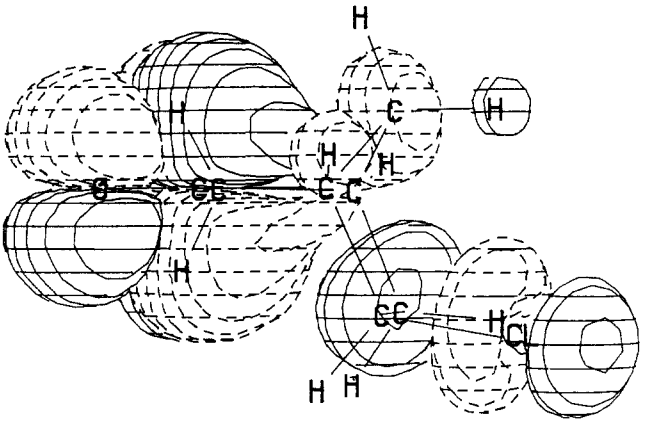

Figure 2. Contour plot of the LUMO of *exo*-6-chloro-2-norbornanone demonstrating both the π^* and σ^* components in this MO.

Table I. Relative ($\pi^* + \sigma^*$) Mixing in Representative Chloro Ketones[a]

chloro-1-cyclohexanone	orbital mixing	chloro-2-norbornanone	orbital mixing
2(a)-	1.00	1-	0.41
2(e)-	0.59	*exo*-3-	0.90
3(a)-	0.05	*endo*-3-	0.78
3(e)-	0.36	4-	0.19
4(a)-	0.04	*exo*-6-	0.53
4(e)-	0.32	*endo*-6-	0.06
		anti-7-	0.50
		syn-7-	0.13
		exo-5-	0.08
		endo-5-	0.13

[a] Orbital mixing values are $(\sum C_i^2)^{1/2}$ for C–Cl in the LUMO, normalized to 2(a)-chlorocyclohexanone. 4-Chloro-2-butanone: $\phi_1 = 90°$; $\phi_2 = 180°$; orbital mixing 0.51.

(1) Presented, in part, at the 190th National ACS Meeting, Sept 8–13, 1985, Chicago, IL, ORGN 31. Organic Photochemistry. 66. Part 65: Morrison, H.; Koviak, C. *Tetrahedron Lett.*, in press.

(2) (a) Allinger, N. L.; Tai, J. C.; Miller, M. A. *J. Am. Chem. Soc.* **1966**, *88*, 4495–4499. (b) Levin, C. C.; Hoffmann, R.; Hehre, W. J.; Hudec, J. *J. Chem. Soc. Perkin Trans. 2* **1973**, 210–220.

(3) Zimmerman, H. E. *Acc. Chem. Res.* **1982**, *10*, 312–317. Purohit, P. C.; Sonawane, H. R. *Tetrahedron* **1981**, *37*, 873–877 and references therein.

(4) Dewar, M. J. S.; Thiel, W. J. *J. Am. Chem. Soc.* **1977**, *99*, 4899–4907; *Quantum Chemistry Program Exchange*; No. 438.

(5) Binkley, J. S.; Whiteside, R. A.; Hariharan, P. C.; Seeger, R.; Pople, J. A.; Hehre, W. J.; Newton, M. D. *Quantum Chemistry Program Exchange*; No. 368.

(6) Hehre, W. J.; Stewart, R. F.; Pople, J. A. *J. Chem. Phys.* **1969**, *51*, 2657–2664. Newton, M. D.; Lathan, W. A.; Hehre, W. J.; Pople, J. A. *Ibid.* **1970**, *52*, 4064–4072.

0002-7863/86/1508-3862$01.50/0