^{13}C NMR Study of the Self-Association of Chloroquine, Amodiaquine, and Quinine

Leah B. Casabianca and Angel C. de Dios*

Department of Chemistry, Georgetown University, 37th and O Sts NW, Washington DC 20057

Received: June 17, 2004; In Final Form: July 31, 2004

Experimental and calculated ^{13}C NMR chemical shifts of quinoline ring carbons are used to investigate the self-association of the antimalarial drugs chloroquine, amodiaquine and quinine. The chemical shifts of each quinoline carbon in the monomer and dimer forms of each drug are extrapolated from plots of the observed chemical shifts at various concentrations. In the equation used to extrapolate the dimer and monomer chemical shift, a linear term is added to account for medium effects but is found to be unnecessary if an internal standard is used to correct for bulk susceptibility. The experimental changes in chemical shift are compared to changes in chemical shift calculated using the continuous set gauge transformation[1] method with the polarizable continuum model[2,3] (PCM-CSGT)[4] for several possible structures of the dimer. The deviations between calculated and experimental chemical shifts are used to select the best dimer structure for each drug.

Introduction

An important and well-studied class of antimalarial drugs contains the aromatic quinoline ring. Belonging to this class are the drugs chloroquine, amodiaquine, quinine, quinidine, and mefloquine. These drugs are believed to function by inhibiting the formation of hemozoin from heme in the digestive vacuole of *Plasmodium falciparum* (malaria parasite). When hemoglobin in red blood cells is digested by the parasite, heme (ferriprotoporphyrin IX) is released. Free heme is toxic to the parasite, but crystallizes as hemozoin, a reciprocal Fe−O41 dimer,[5] which is harmless to the parasite. Thus, it has been widely accepted that quinoline antimalarials bind with heme, preventing its crystallization. Several mechanisms are possible for drug−heme binding. These mechanisms have been investigated through the use of ^{1}H[6,7,8] and ^{13}C[9,10] solution NMR, and solid-state ^{13}C and ^{15}N NMR.[11] In solution, the drug−heme complex is formed through $\pi-\pi$ interactions,[6] whereas in the solid state a covalent complex has been recently suggested.[11]

The study of the self-association of these drugs is important to understanding the complexation of the drugs with heme. The interaction of these drugs among themselves, which presumably occurs through a $\pi-\pi$ mechanism, can give insight into the $\pi-\pi$ interactions between drugs and heme in solution. The examination of the $\pi-\pi$ interactions of antimalarial drugs has applications not only to the interaction of aminoquinoline antimalarials with heme but is also relevant to the study of $\pi-\pi$ interactions in general. The relative stability of perpendicular and parallel orientations in $\pi-\pi$ complexes has been studied in several recent reports.[12−15] For example, the most stable structures of the toluene dimer were shown to be ones in which the aromatic rings are stacked and slightly displaced.[12] On the other hand, for the benzene dimer, the T-shaped arrangement is slightly lower in energy.[13] In another set of papers,[14,15] $\pi-\pi$ complexes between amino acids were studied. Hydrogen bonding was also found to exert an influence on the relative stability of stacked and T-shaped structures. In these systems, structures in which the two aromatic rings are parallel usually appear to be more stable than T-shaped structures. However, when aprotic solvents are used, the T-shaped dimer becomes important. Hydrophobic solvents seem to facilitate hydrogen bonding between aromatic residues and favor the T-shaped dimer.[14,15]

The question of whether parallel or T-shaped structures are more stable is relevant to the study of antimalarial drug-heme complexes as well. In the solution structures of the complexes formed between heme and the drugs chloroquine, quinine, and quinidine, only diprotic quinine was found to have a structure in which the quinoline ring was almost parallel to the porphyrin ring of heme.[6] The other drug−heme complexes have angles between the two rings of roughly 25°.[6] The same effects that cause a T-shaped dimer to be favored in benzene and amino acid dimers may also be important in influencing the structure of drug-heme complexes.

Other aspects of $\pi-\pi$ interactions are also related to the study of the self-association of these drugs. First, the effects of substituents on the stability of $\pi-\pi$ interactions have recently been studied.[16] Antimalarial drugs are substituted quinoline rings, and a study of their self-association contributes to the base of knowledge regarding the effects of side chains on these aromatic interactions. Also, quinoline and structurally similar acridine antimalarial drugs are known to interact with nucleotide bases and intercalate into DNA.[17] The $\pi-\pi$ interactions of drug molecules with themselves are likewise presumably related to the interactions between the drugs and nucleotide bases.

Two studies have been carried out on the self-aggregation of chloroquine[18] and quinine.[19] In these reports, the authors use ^{1}H NMR chemical shifts and 2D NMR experiments to probe the nature of the complexes formed by the drugs in solution. In the first study,[18] the dimerization constant of chloroquine was determined from the change in chemical shift with concentration of the aromatic protons. Furthermore, NOESY experiments and T_1 relaxation times were employed to elucidate the geometry of the complex.[18] In the quinine study,[19] the authors examined the effects of temperature and concentration on the proton chemical shifts. They concluded from this information that quinine molecules aggregate as dimers and calculated the equilibrium constant for this association. Similar to the above, the structure of the dimer was also determined from NOESY

* To whom correspondence should be addressed. Telephone: (202) 687-0670. Fax: (202) 687-6209. E-mail: dediosa@georgetown.edu.

10.1021/jp047361b CCC: $27.50 © 2004 American Chemical Society
Published on Web 09/10/2004

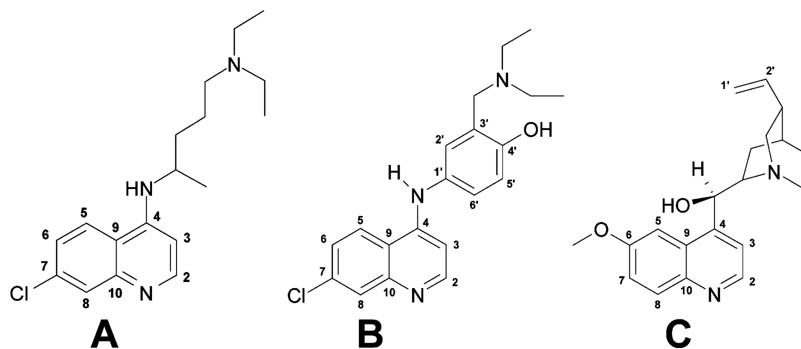

Figure 1. Structures of the antimalarial drugs (a) chloroquine, (b) amodiaquine, and (c) quinine. Quinoline carbons are numbered 2−10, and the other carbons appearing in the aromatic region of the spectra are numbered with primes.

and relaxation rate data.[19] Though the precise structures differ slightly in their orientation, the dimers of both chloroquine and quinine were shown to be $\pi-\pi$ complexes with nearly parallel quinoline rings.[18,19]

In the present work, the self-associations of chloroquine, amodiaquine, and quinine (Figure 1) are studied by ^{13}C solution NMR spectroscopy. For each of the three drugs, observed chemical shifts of the quinoline carbons at various concentrations are used to extrapolate the chemical shift of each carbon in the monomer and dimer forms of the drug. On the theoretical front, the changes in chemical shift between the monomer and dimer are calculated using the continuous set gauge transformation[1] method with the polarizable continuum model[2,3] (PCM-CSGT)[4] for different possible structures of the dimer. The calculated and experimental changes in chemical shift are then compared to determine the best dimer structure for each drug. Ring current effects on the chemical shift of the quinoline carbons are also considered separately.

The carbon nucleus was chosen for this study rather than proton for two reasons. First, carbons are located farther from the periphery of the molecule than protons and are therefore less susceptible to influences arising from nonspecific collisions and medium effects. These effects likewise contribute to changes in chemical shift, and can complicate the analysis of chemical shift data. Second, calculations of carbon chemical shifts are more accurate than calculations of proton chemical shifts. In this investigation, the changes in chemical shift that are examined are due primarily to ring current effects. Ring current effects are expected to be the same order of magnitude for both proton and carbon. Thus, the nucleus for which absolute errors in predicted chemical shifts are known to be smaller should provide better results.

Experimental Details

Chloroquine diphosphate, amodiaquine dihydrochloride dihydrate, and quinine hydrochloride were obtained from Sigma-Aldrich Co. (St. Louis, MO) and were used without further purification. Methyl alcohol-*d* (99.5 atom % D) and deuterium oxide (99.9 atom % D) were also obtained from Sigma-Aldrich Co. and methanol-d_4 (99.8 atom % D) was from Cambridge Isotope Laboratories, Inc. (Andover, MA). Tetramethylsilane (TMS) was purchased from Cambridge Isotope Laboratories. Stock solutions were made of each drug by dissolving a known mass of drug in a 10 mL volumetric flask: chloroquine, 290 mM in D_2O; amodiaquine, 290 mM in CD_3OD; and quinine, 310 mM in CH_3OD. Ten successive dilutions were made of these stock solutions, with the lowest concentration of each near 10 mM. A 1-mL aliquot of each sample was transferred to a 5 mm (o.d.) NMR tube purchased from Wilmad Glass Co., Inc. (Buena, NJ). The actual concentrations of the dilute samples were determined by comparing NMR peak intensities to those of the most concentrated sample.

All NMR measurements were made on a Varian Unity Inova 500 MHz spectrometer using Varian VNMR version 5.1 software. The proton frequency of this spectrometer is 499.789 MHz while the carbon frequency is at 125.684 MHz. Sample and instrument temperatures were controlled at 298 K. For ^{13}C spectra, the 90° pulse width was 4.5 μs, and any residual methanol peak was presaturated. Amodiaquine and quinine chemical shifts were referenced to internal TMS. Carbon and proton peak assignments were made using DQF−COSY,[20] NOESY,[21] HMQC,[22] and HMBC[23] spectra that were obtained for the most concentrated sample of each drug. Two-dimensional spectra were taken in the phase-sensitive mode using the Haberkorn-States hypercomplex method.[24] For the 2D spectra, the 90° proton pulse was 11.1 μs and 512 increments were collected. A recycle delay of 13 s was used for the NOESY spectra.

Computational Details

Carbon chemical shifts were calculated for each drug in the monomer and in several possible structures of the dimer. Calculations were carried out using the hybrid functional of Becke and Lee, Yang, and Parr (B3LYP).[25,26] The basis set used was 6-31G**, which is a 6-31G[27] basis set with a set of p polarization functions on the hydrogen atoms and a set of d polarization functions on the heavy atoms. Solvent effects on the chemical shift were taken into account by utilizing the continuous set gauge transformation[1] method with the polarizable continuum model[2,3] (PCM-CSGT).[4] Corrections for basis set superposition error (BSSE) were calculated using the counterpoise method of Boys and Bernardi.[28] These corrections were found to be negligible and so were not included in the final calculations. Calculations were performed using the Gaussian98 program[29] on an SGI Origin 2000 workstation (Silicon Graphics, Inc.; Mountain View, CA) and on a PC cluster at the University of Illinois at Chicago.

Monomer structures for all drugs were first geometry optimized at the B3LYP[25,26]/6-31G[27] level. After the molecule was optimized, the aliphatic portion of each drug side chain was removed from the molecule for the NMR chemical shift calculations.

Figure 2 shows the relative orientation of the two molecules in each drug dimer, as well as the coordinate axes used. The origin is taken to be the center of the C9−C10 bond. Previous results[18,30] indicate that in the chloroquine dimer, the second molecule is rotated 180° about the *x* axis and 180° about the *y* axis with respect to the first molecule. In this structure, the side

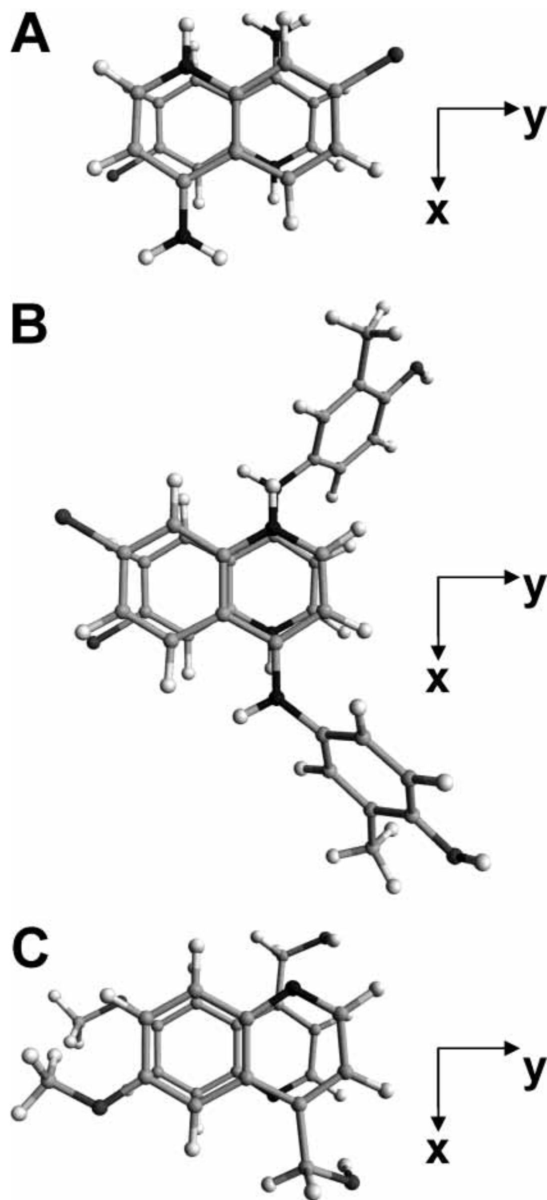

Figure 2. Dimer structure of each drug: (a) chloroquine, (b) amodiaquine, and (c) quinine, along with the coordinate axes used in the chemical shift calculations. The origin is defined to be the center of the C9−C10 bond in the molecule farther from the viewer. Structures (D) through (S) in Tables 2 and 3 are formed by offsetting the molecule that is closer to the viewer in either the *x* or *y* direction.

chain of one molecule is situated between the planes of the two rings and the side chain of the other molecule lies outside of the planes of the two rings. In quinine, previous results[19] indicate that the second molecule of the dimer is formed by rotating the monomer 180° about the *y* axis only. In this case, the side chains of both molecules lie outside the area between the planes of the two rings.

In the X-ray structure of amodiaquine,[31] the phenol ring is stacked above the quinoline ring, so that the phenol OH and quinoline Cl are superimposed. However, our NOESY data at the highest concentration suggest that the dimer structure of amodiaquine is similar to that of quinine. There are no cross-peaks observed between the phenol protons and those of the quinoline ring near the carbon that bears the chlorine atom. Thus, we assume that the dimer of amodiaquine is also formed between the two quinoline rings, without involving the phenol ring. Consequently, the amodiaquine dimer was similarly formed

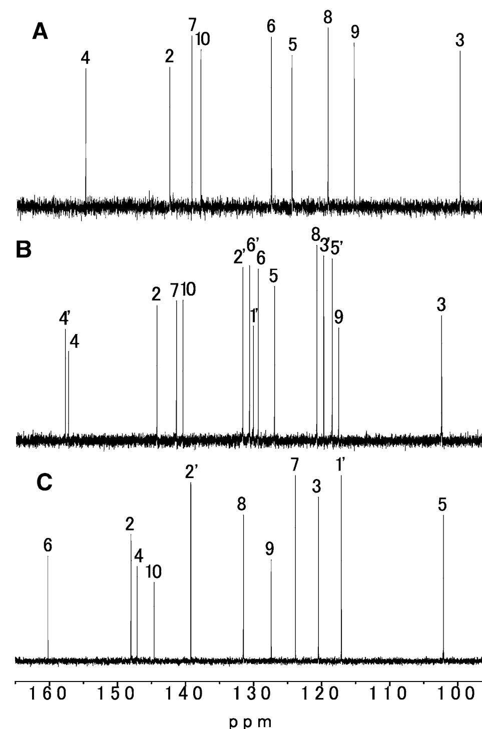

Figure 3. 1D ^{13}C NMR spectra of the most concentrated sample of each drug: (a) 290 mM chloroquine, (b) 290 mM amodiaquine, and (c) 310 mM quinine. Peak labels correspond to the numbering scheme of Figure 1. Assignments were made using DQF−COSY,[20] NOESY,[21] HMQC,[22] and HMBC[23] spectra. Only the aromatic region of each spectrum is shown.

by rotating the monomer 180° about the *y* axis to form the second monomer. However, because the side chain of amodiaquine contains a phenol ring that is not coplanar with the quinoline ring, a portion of each side chain will be between the planes of the two quinoline rings and a portion will be located outside of the planes of the two rings. In our structures, the majority of the side chain of both amodiaquine molecules is located between the planes of the two rings.

Chemical shifts were calculated for nineteen possible structures of each drug dimer. Three dimer structures with the quinoline rings directly eclipsed were considered, in which the distances between the quinoline rings are 3, 4, and 5 Å. These distances were chosen because in the crystal structure of chloroquine,[30] the distance between the two rings is between 3 and 4 Å. Calculations were also done with structures having the quinoline ring of the second monomer offset 0.5 Å in various combinations of the *x* and *y* directions.

Nucleus independent chemical shifts (NICS)[32] were also calculated using CSGT[1] with the B3LYP[25,26] functional and a 6-31G**[27] basis set. The NICS of each quinoline carbon was determined by calculating the shielding of a neutron placed at the position of each carbon of the second monomer in the drug dimer.[33] Solvent effects were not present in the NICS calculations.

Results

The 1D ^{13}C NMR spectra of 290 mM chloroquine, 290 mM amodiaquine, and 310 mM quinine are presented in Figure 3. Only the aromatic region is shown, with peak assignments corresponding to the numbering scheme in Figure 1. Spectra of 11 samples of each drug were taken with concentrations ranging from approximately 10 mM to approximately 300 mM in each case. The change in chemical shift with concentration

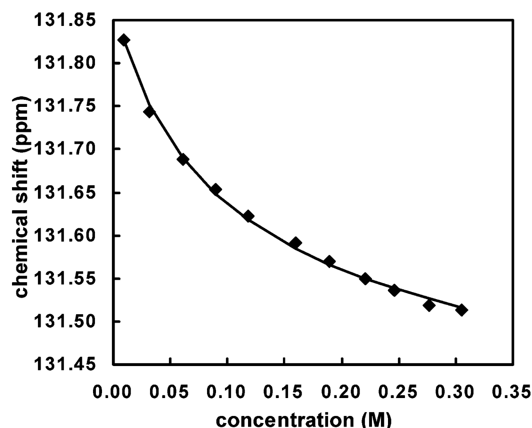

Figure 4. Chemical shift of quinine carbon 8 at various concentrations. Diamonds are experimental points and the solid line is the best-fit regression line given by eq 4.

is shown in Figure 4 for carbon 8 of quinine. Other quinoline carbons show a similar dependence of chemical shift on the concentration, some being deshielded, whereas most are shielded as the concentration increases.

The observed chemical shift at each concentration is assumed to be a weighted average of the chemical shift of the monomer and aggregate,[18,19] according to the following equation:

$$\delta_{\mathrm{obs}} = ([\mathrm{M}]\delta_{\mathrm{m}} + N[\mathrm{A}]\delta_{\mathrm{a}})/C_{\mathrm{t}} \quad (1)$$

where δ_{obs} is the observed chemical shift, δ_m is the chemical shift of the monomer, δ_a is the chemical shift of the aggregate, N is the number of monomers in the aggregate, and [M] and [A] are the concentrations of drug that are present as monomer and aggregate, respectively, at a total drug concentration C_t. Using a logarithmic fit[34] (not shown), it was determined that each of the drugs in this study aggregate as a dimer. The above equation then becomes

$$\delta_{\mathrm{obs}} = ([\mathrm{M}]\delta_{\mathrm{m}} + 2[\mathrm{D}]\delta_{\mathrm{d}})/C_{t} \quad (2)$$

where [D] is the concentration of drug that is present as the dimer and δ_d is the chemical shift of the dimer. Using this equation and the equation for *K,* the equilibrium constant of association, which is

$$K = [\mathrm{D}]/[\mathrm{M}]^2 \quad (3)$$

the observed chemical shift can be related to K, the chemical shift of the monomer and dimer, and the total concentration by the following equation:

$$\delta_{\mathrm{obs}} = \left[\left(\frac{-1 + \sqrt{1 + 8KCt}}{4K}\right)/C_{\mathrm{t}}\right](\delta_{\mathrm{m}} - \delta_{\mathrm{d}}) + \delta_{\mathrm{d}} \quad (4)$$

This equation takes into account the effect of dimerization on the chemical shift, but neglects other medium effects that may also contribute to a change in chemical shift. These effects, for example, bulk susceptibility and nonspecific collisions, are concentration dependent and would be expected to contribute a linear term to the above equation. To include these effects, a linear term, Y, is added to eq 4 to give

$$\delta_{\mathrm{obs}} = \left[\left(\frac{-1 + \sqrt{1 + 8KCt}}{4K}\right)/C_{\mathrm{t}}\right](\delta_{\mathrm{m}} - \delta_{\mathrm{d}}) + \delta_{\mathrm{d}} + \mathrm{Y}^{*}Ct \quad (5)$$

Equations 4 and 5 were used to fit the experimental data in order to extrapolate the dimer and monomer chemical shift of the quinoline carbons in each drug. Origin software version 5.0 (Microcal Software, Inc.; Northampton, MA) was used to perform the fitting. This program uses an algorithm similar to the Levenberg−Marquardt algorithm to find the values of δ_m and δ_d that minimize the difference between experimental and predicted values of δ_{obs}. In our analysis, K was assigned the same value for all carbons, instead of being taken as an adjustable parameter in the regression analysis. This leaves δ_m and δ_d as the only adjustable parameters in Equation 4, and δ_m, δ_d, and Y as the only adjustable parameters in Equation 5. The values of K for chloroquine and quinine were taken from proton data in refs 18 and 19, respectively. These values are 4.52 for chloroquine[18] and 4.759 for quinine.[19] In the case of amodiaquine, the value used for K is 3.48. This K was obtained from our carbon data, and was determined by fitting the data of each quinoline carbon using eq 4 with K as an adjustable parameter. The K given by each carbon differed slightly, and the value given by carbon 6 was chosen as the K to be used. This value was chosen because it was the one closest to the K values for chloroquine and quinine. The chemical shifts, δ_m and δ_d, are found to be not especially sensitive to the value of K used.

The linear term, Y, in eq 5 takes into account both bulk susceptibility as well as other medium effects such as collisions. Bulk susceptibility is not separable from the other effects. For amodiaquine and quinine, bulk susceptibility effects are removed by referencing chemical shifts to internal TMS, which presumably experiences the same effects on chemical shift due to bulk susceptibility as do the drugs. Chloroquine, however, is not soluble in methanol. Hence, the experiment was done in D_2O, preventing the use of TMS as an internal standard. For amodiaquine and quinine, the dimer and monomer chemical shifts produced using eq 4 were in better agreement with calculated changes in chemical shift (see below) than the values produced using eq 5. Thus, when bulk susceptibility has been taken into account in the experiment, a better agreement with calculations is achieved using an equation without an additional linear term. This reveals that collisions and other effects that have a linear dependence on chemical shift are not as significant as bulk susceptibility effects. On the other hand, the changes in chemical shift for chloroquine determined from eq 5 were in better agreement with calculated changes than values determined using eq 4. In the case of chloroquine, a linear term is needed in the regression equation in order to take into account bulk susceptibility in addition to other medium effects. Equations 4 and 5 do not represent the true experimental situation, as there are other effects present such as hydrogen bonding and possible interactions with counterions, which are not taken into account by these equations. However, it is hoped that these effects are small, allowing our application of eqs 4 and 5 to these systems. The best-fit line for quinine carbon 8 using eq 4 is shown as a solid line in Figure 4. The excellent agreement between experimental chemical shifts and the best-fit line indicates that the self-association among these drugs is described by a simple dimerization.

Table 1 presents experimental chemical shifts of quinoline carbons of each drug. The first two columns contain observed chemical shifts in the most- and least-concentrated sample of each drug. The third and fourth columns contain the extrapolated dimer and monomer chemical shifts, calculated using eq 4 for amodiaquine and quinine and using eq 5 for chloroquine. In each case the difference between δ_d and δ_m is the same sign as the difference between the experimental chemical shift at the highest and lowest concentrations.

TABLE 1: Experimental Chemical Shifts

Chloroquine				
	observed chemical shift			
carbon	290 mM	13 mM	δ_d	δ_m
2	142.14	142.25	142.13	142.26
3	98.65	98.53	98.85	98.49
4	154.71	155.64	154.81	155.76
5	123.83	124.18	123.73	124.24
6	126.91	127.40	127.11	127.45
7	138.82	139.40	139.14	139.46
8	118.44	119.26	118.41	119.37
9	114.52	115.44	114.47	115.58
10	137.45	138.38	137.40	138.51

Amodiaquine				
	observed chemical shift			
carbon	290 mM	12 mM	δ_d	δ_m
2	144.05	144.38	143.71	144.40
3	101.66	101.64	101.69	101.66
4	157.22	157.70	156.72	157.89
5	126.55	126.25	126.89	126.24
6	128.96	129.23	128.68	129.31
7	141.16	141.49	140.81	141.62
8	120.23	120.65	119.80	120.69
9	116.99	117.28	116.70	117.36
10	140.19	140.75	139.62	140.82

Quinine				
	observed chemical shift			
carbon	310 mM	9.5 mM	δ_d	δ_m
2	148.03	148.27	147.81	148.30
3	120.48	120.60	120.38	120.63
4	147.11	146.87	147.37	146.87
5	102.12	102.25	102.00	102.29
6	160.23	160.32	160.17	160.38
7	123.87	123.53	124.18	123.55
8	131.51	131.83	131.24	131.87
9	127.42	127.52	127.34	127.57
10	144.62	144.87	144.43	144.93

The experimental changes in chemical shift upon formation of the dimer, $\delta_d - \delta_m$, are shown in bold overlaid on the structure of each drug in Figure 5. In forming the dimer, most quinoline carbons of all drugs are shielded, in agreement with the expected ring current effects from the quinoline ring of the second monomer if the dimer is a stacked $\pi-\pi$ complex.[35] There are similarities in the chemical shift changes of chloroquine and amodiaquine. Carbons 4, 8, and 10 in the center of the quinoline ring experience a large shielding upon formation of the dimer in both drugs. Carbon 3 is deshielded in both chloroquine and amodiaquine, indicating that in the dimer this carbon may be located outside of the region directly above the second quinoline ring.[35] Quinine would not be expected to show the exact same trends as chloroquine and amodiaquine due to the different quinoline ring. It is notable that in each drug the changes in chemical shift are the same order of magnitude across all quinoline sites. This therefore rules out the possibility that these drugs are forming T-shaped dimers. If the dimer were T-shaped, carbons on one side of the quinoline ring would be closer to the π system of the other molecule than carbons on the other side of the quinoline ring. This would lead to drastically different changes in chemical shift between carbons on different sides of the quinoline ring, since carbons on opposite sides of the ring would be in different chemical environments.

The chemical shifts of each drug monomer and several dimer structures were calculated at the B3LYP[25,26] level of theory with a 6-31G**[27] basis set. The calculated changes in chemical shift are shown for the best dimer structure of each drug in Figure 5. Solvent effects are included in the calculations using the PCM-CSGT[4] method. It is found that it is necessary to include solvent in the calculations. When the change in chemical shift is calculated without solvent effects, the calculated values consistently overestimate the experimental values on one side of the quinoline ring and underestimate the experimental values on the other side of the ring (data not shown). This suggests that there is a dipole present in the monomer structure that is not accounted for in the calculations. When the dimer is formed, solvent molecules are presumably displaced from one face of the drug molecule. These solvent molecules contribute a dipole effect that is no longer present once the solvent molecules are replaced by the second drug molecule. When solvent effects are incorporated in the calculations, the discrepancy between calculation and experiment is significantly reduced.

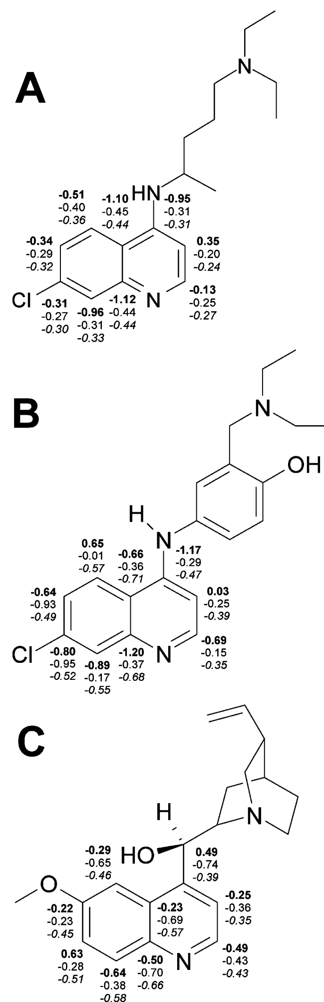

Figure 5. Experimental (bold), calculated (standard) and ring current (italicized) changes in chemical shift for each quinoline carbon of (a) chloroquine, (b) amodiaquine, and (c) quinine. Experimental changes are $\delta_d - \delta_m$ and calculated changes are $\sigma_m - \sigma_d$; thus, a positive number in each case means that the carbon is more deshielded in the dimer than in the monomer. Calculated and ring current changes in chemical shifts are those for dimer structure (O) for chloroquine, (B) for amodiaquine, and (P/Q) for quinine.

Table 2 contains the root-mean-square deviation (rmsd) between experimental and calculated changes in chemical shift for the quinoline carbons of the three drugs. It is observed that the deviations between calculated and experimental shifts of individual sites generally parallel that of the rmsd. It should be noted that large rmsds (greater than 1.0) are caused by one or

TABLE 2: Root Mean Square Deviation between Calculated and Experimental Changes in Chemical Shift

struct	distance	*x* offset	*y* offset	root mean square deviation		
				chloroquine	amodiaquine	quinine
A	3.0	0	0	1.92	2.71	3.91
B	4.0	0	0	0.60	0.57	1.20
C	5.0	0	0	0.49	0.59	0.58
D	4.0	+0.5	0	0.66	0.90	0.68
E	4.0	−0.5	0	0.67	0.63	0.67
F	4.0	0	+0.5	0.59	0.83	0.86
G	4.0	0	−0.5	0.66	0.83	0.86
H	4.0	+0.5	+0.5	0.68	0.70	0.73
I	4.0	+0.5	−0.5	0.53	0.70	0.73
J	4.0	−0.5	+0.5	0.60	1.85	1.02
K	4.0	−0.5	−0.5	1.29	1.85	1.02
L	5.0	+0.5	0	0.49	0.58	0.56
M	5.0	−0.5	0	0.51	0.60	0.58
N	5.0	0	+0.5	0.52	0.59	0.57
O	5.0	0	−0.5	0.48	0.59	0.57
P	5.0	+0.5	+0.5	0.51	0.59	0.56
Q	5.0	+0.5	−0.5	0.48	0.59	0.56
R	5.0	−0.5	+0.5	0.54	0.60	0.58
S	5.0	−0.5	−0.5	0.49	0.60	0.58

TABLE 3: Root Mean Square Deviation between Ring Current and Experimental Changes in Chemical Shift

struct	distance	*x* offset	*y* offset	root mean square deviation		
				chloroquine	amodiaquine	quinine
A	3.0	0	0	0.69	0.74	1.36
B	4.0	0	0	0.42	0.55	0.75
C	5.0	0	0	0.50	0.62	0.52
D	4.0	+0.5	0	0.42	0.52	0.71
E	4.0	−0.5	0	0.44	0.60	0.78
F	4.0	0	+0.5	0.48	0.55	0.75
G	4.0	0	−0.5	0.38	0.55	0.75
H	4.0	+0.5	+0.5	0.47	0.52	0.71
I	4.0	+0.5	−0.5	0.38	0.52	0.71
J	4.0	−0.5	+0.5	0.50	0.60	0.77
K	4.0	−0.5	−0.5	0.40	0.60	0.77
L	5.0	+0.5	0	0.50	0.61	0.50
M	5.0	−0.5	0	0.50	0.64	0.53
N	5.0	0	+0.5	0.52	0.62	0.52
O	5.0	0	−0.5	0.48	0.62	0.52
P	5.0	+0.5	+0.5	0.52	0.61	0.50
Q	5.0	+0.5	−0.5	0.48	0.61	0.50
R	5.0	−0.5	+0.5	0.53	0.64	0.53
S	5.0	−0.5	−0.5	0.49	0.64	0.53

two chemical shifts for which the calculated change in chemical shift is very far from experiment. On the other hand, in structures for which their respective rmsds are close to each other (within 0.03), the deviation between calculated and experiment for each site is not always lower in the structure that has a smaller rmsd. Thus, the rmsd can be taken only as a general measure of how well calculated values compare with experiment. Structures with a lower rmsd, therefore, are presumed to be closer to the true structure of the dimer than structures with much higher deviations between calculation and experiment.

From the first three rows of this table, the effect of increasing the distance between the quinoline rings can be seen. Large rmsds are present at 3 Å in all drugs, indicating that the quinoline rings in the dimers are not as close as 3 Å. The optimal distance between the two quinoline rings in both the chloroquine and quinine dimers is 5 Å. The distance between the two rings in the dimer of amodiaquine is between 4 and 5 Å, with a slight preference for 4 Å. These distances are slightly longer than the distance between the two ring planes found in the crystal structure of chloroquine, which is between 3.43 and 3.56 Å.[30]

In the remainder of Table 2, rmsds for dimer structures that have the second molecule of the dimer offset in either the *x* or *y* direction are presented. For all drugs, the rmsds between experiment and calculation have a narrower range at 5 Å than at 4 Å. This indicates that chemical shifts are less sensitive to changes in geometry when the two molecules are farther apart. This is due to orbital overlap and repulsion, which may affect the chemical shift when the quinoline rings of the two dimer molecules are offset. Of course, this is expected to be less significant when the two molecules are farther apart.

In chloroquine, the rmsds at 5 Å are always lower than the rmsds for the corresponding structures at 4 Å. Comparing the displaced structures to the structures in which the quinoline rings are eclipsed, a displacement can lead to either an increase or a decrease in rmsd for chloroquine at both 4 and 5 Å. The two structures for which the rmsd is lowest overall are structures (O) and (Q). The structure with the lowest rmsd at 4 Å separation is (I). This structure has the second molecule offset in the $+x$ and $-y$ directions, and is analogous to structure (Q). It is interesting to note that, in the crystal structure of chloroquine,[30] the quinoline ring of the second molecule is similarly displaced in the $+x$ and $-y$ directions.

For amodiaquine, the rmsds between calculated and experimental changes in chemical shifts are similar for the eclipsed structures at 4 and 5 Å. At 4 Å, offsetting the second molecule in any direction leads to a large increase in rmsd. At 5 Å, however, a displacement leads to only a small increase or decrease in rmsd. The structure of the amodiaquine dimer with the overall best agreement between calculation and experiment is structure (B), in which the two rings are separated by 4 Å and stacked directly on top of each other.

In quinine, structures with the quinoline rings 5 Å apart all have lower rmsds than structures with the quinoline rings 4 Å apart. At an intermolecular separation of 4 Å, a displacement leads to a decrease in rmsd. At 5 Å, a displacement leads to the same or to a slightly lower rmsd as compared to the eclipsed structure. Structures (L) and (P/Q) have the overall lowest rmsds of the structures considered (structures P and Q are equivalent in this case). Both of these structures have a $+x$ offset.

To examine more carefully the change in chemical shift caused by dimerization, ring current contributions to the total change in chemical shift were specifically calculated. As these are dimers of aromatic compounds, the change in chemical shift going from the monomer to the dimer is expected to be due mainly to ring current effects from the second monomer.[35] The portion of the total change in chemical shift that is due to ring current effects was calculated by finding the nucleus independent chemical shift (NICS)[32] of each quinoline carbon. This was done by calculating the chemical shift of a neutron placed at the position of that carbon in the dimer.[33] For all quinoline carbons at all dimer orientations of the three drugs, the calculated shielding is a positive number. This means that ring currents lead to a shielding effect, or a negative change in chemical shift, for all carbons. The NICS of each quinoline carbon are presented in italics in Figure 5. The calculated ring current contributions to the change in chemical shift are the same order of magnitude as the overall change in chemical shift for all drugs. This indicates that, as expected, ring current effects dominate the changes in chemical shift going from the monomer to the dimer of quinoline carbons in these systems. The root-mean-square deviations between the experimental and ring current changes in chemical shift are presented in Table 3.

For chloroquine, the ring current chemical shifts have a better agreement with experiment at 4 Å than at 5 Å, in both the eclipsed and displaced structures. This is opposite to what is

seen in the supermolecule calculations, where the structures with an intermolecular distance of 5 Å have lower rmsds. Similar to the supermolecule calculations, the structures that have the lowest rmsds are structures (G) and (I) and their analogues (O) and (Q). In amodiaquine, structures with an intermolecular distance of 4 Å have lower rmsds between ring current and experimental changes in chemical shift than do structures with an intermolecular distance of 5 Å. Comparing the displaced structures to the eclipsed structures at the same distance, displacement either leads to an increase or a decrease in rmsd. This was not the case in the supermolecule calculations at 4 Å, in which a displacement always leads to an increase in rmsd. In the case of quinine, rmsds between ring current and experimental changes in chemical shift are lower for the structures with an intermolecular distance of 5 Å than for the structures with an intermolecular distance of 4 Å. The structures with the overall lowest rmsd with experiment are structures (L) and (P/Q). These are the same structures for which the rmsds are lowest in the quinine supermolecule calculations.

Discussion

In using rmsds between experimental and calculated changes in chemical shift to determine the dimer structure that is closest to the true structure of the dimer, there are several issues that one must bear in mind. First, the root-mean-square deviations are taken only as an overall measure of the difference between calculation and experiment. They are not very sensitive to individual carbon sites. Therefore, these rmsds must be employed with the stipulation that two rmsds should only be considered distinct if they differ by more than approximately 0.1. This prevents the designation of one correct structure for each dimer, because many structures have similar low rmsds in all cases. Second, the dimerization of these drugs in all likelihood is not described by a single correct dimer structure. Experimentally, what is observed in the NMR measurements is a time-averaged picture of the drug monomers and dimer. Although the mechanism is a simple dimerization, drug monomers are rapidly forming and re-forming the dimer in solution. The structure of each drug dimer, therefore, is presumably described by an average of several dimer structures that have similar, low rmsds between experimental and calculated changes in chemical shifts.

The information contained in the chemical shifts can, however, give insight into the characteristics of the dimers formed between these drugs. Using the root-mean-square deviations between calculated and experimental changes in chemical shift, and between ring current and experimental changes in chemical shift, some specifics relating to the dimer structure of each drug can be deduced. As was stated above, the rmsds cannot be used to single out one structure as the correct structure of each drug dimer. However, taking together the rmsds for both calculated and ring current chemical shifts at 4 and 5 Å, some dimer structures can be determined to be close to the correct dimer structure and other dimer structures can be ruled out. For chloroquine, the distance between the two monomers in the dimer is between 4 and 5 Å, and slightly closer to 5 Å. The second molecule is offset in the -y direction, and may also have some contribution from structures in which the second molecule is also offset in the $+x$ direction. For amodiaquine, the correct dimer structure has an intermolecular distance of between 4 and 5 Å, with a slight preference for 4 Å. The contribution to the dimer structure from structures with the rings displaced is probably not significant. For quinine, the dimer structure has an intermolecular distance of not less than 5 Å. The second molecule is offset in the $+x$ direction, and may have some contribution from structures that are also offset in the $-y$ direction.

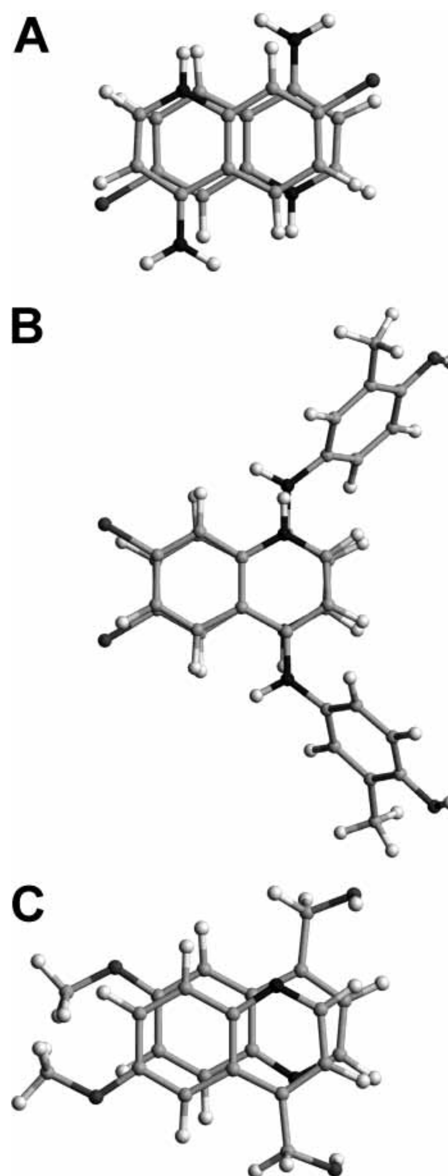

Figure 6. Best dimer structure for each drug. (a) chloroquine, structure (O); (b) amodiaquine, structure (B); and (c) quinine, structure (P/Q).

The calculated and ring current changes in chemical shift that are presented in Figure 5 are for the "best" structure of each drug dimer. This is the dimer structure for which the rmsd between calculated and experimental changes in chemical shift was the lowest out of all the structures considered here. The best structure for chloroquine is (O), for amodiaquine it is (B) and for quinine it is (P/Q). These structures are shown in Figure 6. For all drugs, the difference between calculated and experimental changes in chemical shift is large for carbon 4. This is the carbon that is attached to the side chain. The difference between calculated and experimental changes in chemical shift for carbon 4 may be due to the removal of the side chain in the chemical shift calculations. No change in the structure of the dimer is found to improve the calculated shielding at this site. In addition, carbons near the center of the quinoline ring also have large differences between calculated and experimental changes in chemical shift for both chloroquine and amodiaquine. As in carbon 4, improving the calculated shielding at these sites

was also not possible with the current level of theory employed. The agreement between calculation and experiment is not perfect, but the rmsds in Tables 2 and 3 indicate that the method used for the calculations is in general adequate. These rmsds are of the same order of magnitude as rmsds previously reported between experimental proton structural shifts and a model including electrostatic effects[36] and as rmsds between DFT calculations and an empirical model for proton chemical shifts.[37]

The best structure for the amodiaquine dimer has the quinoline rings of the two molecules stacked directly on top of each other. The best structures for the chloroquine and quinine dimers, on the other hand, have the two quinoline rings offset with respect to each other. It is well-known that in $\pi-\pi$ complexes the arrangement of the parallel dimer with the two aromatic rings offset with respect to each other is more stable than the dimer in which the two aromatic rings are eclipsed.[12,13,38] Thus, in amodiaquine there is some factor that accounts for the dimer structure having a large contribution from structures in which the two quinoline rings are stacked directly above each other. Amodiaquine has an aromatic side chain that is at an angle with respect to the quinoline ring. The side chain of amodiaquine, therefore, cannot be confined to the region either between the planes of the two quinoline rings or outside the planes of the two rings in the dimer. Consequently, when the two quinoline rings are offset, one of these rings is moved closer to the side chain of the other amodiaquine molecule. This may lead to steric interactions that destabilize the structures in which the quinoline rings are displaced. As a result, the dimer structure is perhaps forced to have the quinoline rings eclipsed. In contrast to ring current calculations, the large increases in rmsds when the two quinoline rings depart from an eclipsed conformation are seen only for the supermolecule calculations. Therefore, in this regard, the supermolecule calculations are slightly more selective. Chloroquine and quinine do not share the same situation as amodiaquine. The displaced structures of the chloroquine and quinine dimers do not have a large amount of steric hindrance, and their best dimer structures have the two quinoline rings displaced. Thus, the structure of each drug dimer is likely determined by a competition of the effects of stabilization from the displacement of the quinoline rings[38] and destabilization due to steric interference from the side chain.

Conclusions

In the current work, changes in ^{13}C chemical shifts have been used to study the self-association of the drugs chloroquine, amodiaquine, and quinine. Experimental and calculated changes in chemical shifts were compared to determine the best structure of each drug dimer. Chemical shifts of the quinoline carbons in the monomer and dimer of each drug were extrapolated from observed chemical shifts over a range of concentrations. It was shown that bulk susceptibility and other medium effects that contribute linearly to the chemical shift can be included in the fitting equation. Compared to the effect of bulk susceptibility, other medium effects and nonspecific collisions were shown to be minor. Thus, a linear term does not need to be included in the fitting equation if the bulk susceptibility is taken into account through the use of an internal standard.

Calculations including solvent effects using the PCM-CSGT method[4] were found to give chemical shifts that are closer to experiment than calculations that do not include solvent effects. Even with solvent included in the calculations, calculated and experimental chemical shifts showed large differences for carbon 4 in all drugs. Large differences were also observed for carbons in the center of the chloroquine and amodiaquine rings. Ring currents were shown to contribute a shielding effect to the change in chemical shift going from the monomer to the dimer. Additionally, it was found that ring currents composed the majority of the overall change in chemical shift upon dimerization for these compounds.

The root-mean-square deviations of the differences between calculated and experimental changes in chemical shifts and between ring current and experimental changes in chemical shifts of the nine quinoline carbons for various dimer structures were compared. Although the rmsds are taken only as a vague indication of the relative difference between calculated and experimental changes in chemical shift for each drug, some information was gained by taking the structures with lower rmsds to be closer to the true dimer structure. Chloroquine and quinine were shown to have a preference for structures in which the two molecules are offset with respect to each other. On the other hand, the amodiaquine dimer was shown to have a large contribution from structures with the two quinoline rings eclipsed. The structure of the dimer, whether displaced or eclipsed, is possibly determined by a balancing act between the effects of a displaced quinoline ring structure having more favorable $\pi-\pi$ interactions[38] and a displacement that may lead to steric hindrance due to the drug side chain.

Acknowledgment. This work was produced as part of an undergraduate chemistry class, Chemistry-064, Introduction to Research Experimentation. The authors thank the following Chemistry-064 students for their help with this work: Leslie Claracay, Caitlyn Faller, Joshua Goulding, Michael O'Rourke, John Reppucci, and Eric Strauss. We also thank Prof. Cynthia Jameson for helpful discussions. Financial support was provided by the Georgetown University Chemistry Department.

References and Notes

(1) Keith, T. A.; Bader, R. F. W. *Chem. Phys. Lett.* **1993**, *210*, 223.
(2) Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
(3) Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239.
(4) Manalo, M. N.; de Dios, A. C.; Cammi, R. *J. Phys. Chem. A.* **2000**, *104*, 9600.
(5) Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. *Nature* **2000**, *404*, 307.
(6) Leed, A.; DuBay, K.; Ursos, L. M. B.; Sears, D. N.; de Dios, A. C.; Roepe, P. D. *Biochemistry* **2002**, *41*, 10245.
(7) Moreau, S.; Perly, B.; Biguet, J. *Biochimie* **1982**, *64*, 1015.
(8) Moreau, S.; Perly, B.; Chachaty, C.; Deleuze, C. *Biochim. Biophys. Acta* **1985**, *840*, 107.
(9) Constantinidis, I.; Satterlee, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 927.
(10) Constantinidis, I.; Satterlee, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 4391.
(11) de Dios, A. C.; Tycko, R.; Ursos, L. M. B.; Roepe, P. D. *J. Phys. Chem. A* **2003**, *107*, 5821.
(12) Gervasio, F. L.; Chelli, R.; Procacci, P.; Schettino, V. *J. Phys. Chem. A* **2002**, *106*, 2945.
(13) Hobza, P.; Selzle, H. L.; Schlag, E. W. *J. Phys. Chem.* **1996**, *100*, 18790.
(14) Gervasio, F. L.; Procacci, P.; Cardini, G.; Guarna, A.; Giolitti, A.; Schettino, V. *J. Phys. Chem. B* **2000**, *104*, 1108.
(15) Chelli, R.; Gervasio, F. L.; Procacci, P.; Schettino, V. *J. Am. Chem. Soc.* **2002**, *124*, 6133.
(16) Sinnokrot, M. O.; Sherrill, C. D. *J. Phys. Chem. A* **2003**, *107*, 8377.
(17) Bolte, J.; Demuynck, C.; Lhomme, M. F.; Lhomme, J.; Barbet, J.; Roques, B. P. *J. Am. Chem. Soc.* **1982**, *104*, 760.
(18) Marchettini, N.; Valensin, G.; Gaggelli, E. *Biophys. Chem.* **1990**, *36*, 65.
(19) Uccello-Barretta, G.; Di Bari, L.; Salvadori, P. *Magn. Reson. Chem.* **1992**, *30*, 1054.
(20) Rance, M.; Sørensen, O. W.; Bodenhausen, G.; Wagner, G.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 479.
(21) Kumar, A.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1.
(22) Sklenar, V.; Bax, A. *J. Magn. Reson.* **1987**, *71*, 379.
(23) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.

(24) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286.

(25) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(26) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(27) Hehre, W. J.; Ditchfield, R.; Pople, J. A. *J. Chem. Phys.* **1972**, *56*, 2257.

(28) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553.

(29) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.7; Gaussian, Inc.: Pittsburgh, PA, 1998.

(30) Karle, J. M.; Karle, I. L. *Acta Crystallogr. C.* **1988**, *44*, 1605.

(31) Yennawar, H. P.; Viswamitra, M. A. *Curr. Sci.* **1991**, *61*, 39.

(32) Schleyer, P. v. R.; Maeker, C.; Dransfeld, A.; Jiao, H.; Hommes, N. J. R. v. E. *J. Am. Chem. Soc.* **1996**, *118*, 6317.

(33) Wolinski, K. *J. Chem. Phys.* **1997**, *106*, 6061.

(34) Chachaty, C. *Prog. Nucl. Magn. Reson. Spectrosc.* **1987**, *19*, 183.

(35) Johnson, C. E.; Bovey, F. A. *J. Chem. Phys.* **1958**, *29*, 1012.

(36) Dejaegere, A.; Bryce, R. A.; Case, D. A. In *Modeling NMR Chemical Shifts: Gaining Insights into Structure and Environment;* ACS Symposium Series 732; Facelli, J. C., de Dios, A. C., Eds.; American Chemical Society: Washington, DC, 1999; pp 194−206.

(37) Sitkoff, D.; Case, D. A. *J. Am. Chem. Soc.* **1997**, *119*, 12262.

(38) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.