

Solute–Solvent Interactions Probed by Intermolecular NOEs

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Nuclear Overhauser effects arising from the interactions of spins of solvent molecules with spins of a solute should reveal the “exposure” of solute spins to collisions with solvent. Such intermolecular NOEs could, therefore, provide information regarding conformation or structure of the solute. Determinations of solute–solvent NOEs of 1,3-di-*tert*-butylbenzene in solvents composed of perfluoro-*tert*-butyl alcohol, tetramethylsilane, and carbon tetrachloride have been carried out. A crude, but apparently reliable, method for prediction of intermolecular solvent–solute NOEs based on hard (noninteracting) spheres was developed. Comparison of experimental to predicted NOEs indicates that tetramethylsilane interacts with the solute according to the model. By contrast, intermolecular NOE data indicate attractive interactions between the solute and perfluoro-*tert*-butyl alcohol. All NOE results and the corresponding predictions confirm that proton H2 of the solute is protected by the flanking *tert*-butyl groups from interactions with solvent molecules.

Introduction

Intramolecular nuclear Overhauser effects (NOEs) are a familiar tool for obtaining stereochemical or regiochemical information in small molecule and biomolecular systems.¹ Intermolecular NOEs arise from dipolar interactions between spins on separate molecules and are routinely applied in studies of biological ligand–receptor complexes.^{2,3} Such NOEs have been employed to explore detergent proton–protein proton interactions,⁴ the positioning of peptides in lipid bilayers,⁵ and the interaction of several small organic molecules with proteins.⁶ Organometallic ion pairs, host–guest complexes, and other association phenomena involving small molecules have been examined by these experiments.^{7–9} Intermolecular NOEs have provided indications of selective solvent–solute interactions (“solvent sorting”) in mixed organic–water systems.^{10–12}

Theory for intermolecular interactions that produce dipolar relaxation has been available since the pioneering work of Blombergen.^{13,14} Most such efforts imagine that

the interacting solute and solvent molecules are hard spheres. The relaxation effect, and thus any NOE, depends on encounters of the spheres by mutual diffusion and the distance of their closest approach. Improvements to the basic theoretical models have included incorporation of rotational motion of the spheres as they approach one another,¹⁵ but the assumption that the solute and solvent are spherical remains a common one.

The purpose of the present work was to explore the abilities of solute–solvent NOEs to provide structural or conformational information. As part of these efforts, we attempted to develop a useful method for predicting solvent–solute NOEs. Given a reasonably reliable predictive method, significant deviations from expected behavior could signal particularly strong or long-lived interactions between solute and solvent partners. To test these ideas, we sought an experimental system in which the solvent–solute interactions are expected to be weak and where the solvent molecules could be regarded as spherical or nearly so. We desired a solute that would exhibit clear differences in the closest approach of solvent molecules to the spins of the solute. Thus, we have determined solvent spin–solute spin NOEs for solutions of 1,3-di-*tert*-butylbenzene (**1**) in perfluoro-*tert*-butyl alcohol (PFTB) and tetramethylsilane (TMS) or mixtures of these liquids with carbon tetrachloride. Molecular models suggest that proton H2 of **1** should be appreciably shielded from interactions with solvent molecules by virtue of the bulky *tert*-butyl groups next to it. The

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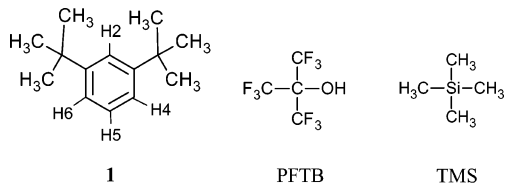
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remaining aromatic hydrogens (H4, H5, H6) and those of the *tert*-butyl groups should be much more accessible to direct interactions with solute molecules. Models of the solvents confirmed that these molecules are nearly spherical. We describe determination of solute proton–solvent spin NOEs for these systems and a procedure for predicting these NOEs that, in many instances, gives results that are very similar to the experimental results.



Results

Characterization of Intermolecular NOEs. The intensity of a solute proton NMR signal depends of the *z*-component of its corresponding magnetization prior to application of the pulse that produces the signal. Following inversion of a spin *X* associated with solvent molecules, the initial change in the solute signal intensity (the NOE) with time is described by eq 1.¹⁶

$$dH_z/dt = 2\sigma_{HX}X_z^0 \quad (1)$$

Here, H_z is proportional to the intensity of the signal of interest, X_z^0 is the *z*-component of the solvent spin magnetization when the system is at equilibrium, and σ_{HX} is the cross-relaxation rate due to the dipolar interactions of the solvent spin and a solute proton. If there are N_X solvent spins present and the gyromagnetic ratios of solvent spins and observed hydrogen spins are γ_X and γ_H , respectively, then $(X_z^0/N_X\gamma_X) = (H_z^0/\gamma_H)$ where H_z^0 is the *z*-component of the solute spin magnetization at equilibrium. According to eq 1, a plot of the intensity of the solute proton signal relative to its intensity in the absence of any perturbation of the solvent spins, as a function of time, is expected to be linear with an initial slope of $2\sigma_{HX}N_X\gamma_X/\gamma_H$. The behavior of H_z over longer times is described by a multiexponential function.⁸

Figure 1 shows the results of a typical intermolecular NOE experiment with 1,3-di-*tert*-butylbenzene. To evaluate the initial slopes of such data, changes in the intensities of the signals for H2, the *tert*-butyl protons, and protons H4–H6 were determined for a range of mixing times (t_{mix}). In most solvent systems, the signals for H4, H5, and H6 of **1** appeared as a single broad peak. However, in neat PFTB, signals for these spins are present as a second-order multiplet. In the latter case, variation of the intensity of the most intense signal of the multiplet was used in the data analysis. Observed NOE peak intensities were fit to the empirical function $A t_{mix} + B t_{mix}^2$ with the coefficient *A* being taken as the initial slope of the data. Investigations using synthetic data suggested that this procedure gives initial slopes that are reliable to better than 5%.

Table 1 presents values of σ_{HX} calculated from the initial slopes of $^1\text{H}\{^1\text{H}\}$ NOE experiments with the solute **1** dissolved in 100% TMS and in 50/50 v/v TMS/ CCl_4 .

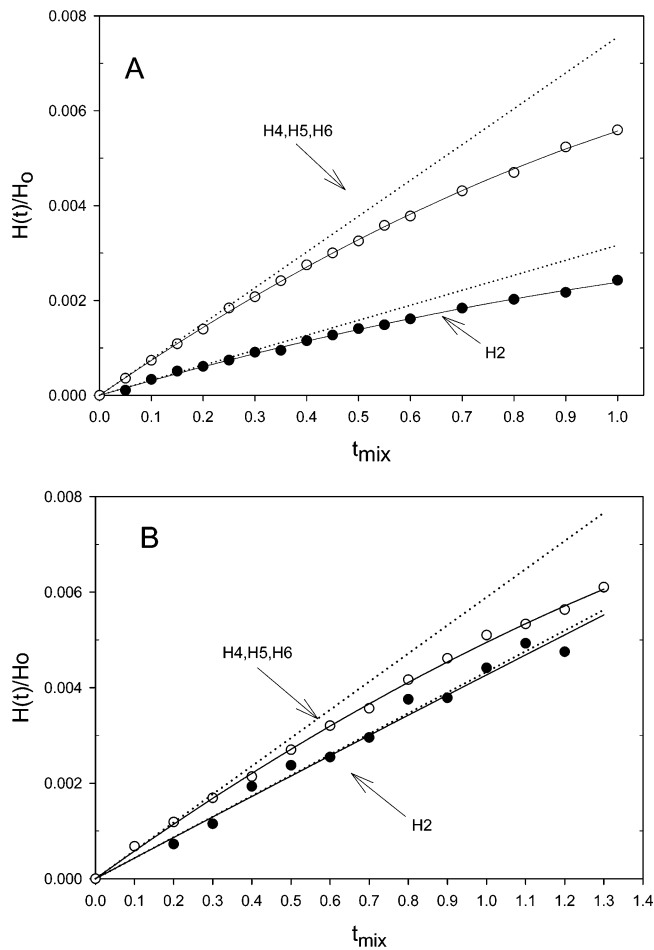


FIGURE 1. Typical results of aromatic proton–solvent proton $^1\text{H}\{^1\text{H}\}$ NOE experiments obtained in this work. Experimental signal intensities are represented by the points. Solid lines in each panel are calculated using the fitting function described in the text while the dotted lines are the initial slopes of the data indicated by the fitting function. Panel A shows NOEs due to the alcohol proton of the 100% PFTB system, the solvent where the largest deviations from the hard-sphere model described in the text were observed. Panel B shows NOEs from the solvent protons in the 50% TMS/50% CCl_4 solvent system where the experimental results agree with the predictions of the hard-sphere model.

Table 1 also records values of σ_{HX} calculated from the initial slopes of $^1\text{H}\{^1\text{H}\}$ and $^1\text{H}\{^{19}\text{F}\}$ experiments with the solute dissolved in 100% PFTB and in 50/50 v/v PFTB/ CCl_4 . Consistent with expectations based on the structure of the solute, it is clear that proton H2 of the solute experiences significantly reduced interactions with the spins of the solute molecules compared to the *tert*-butyl or the remaining aromatic protons.

Sample Properties. Intermolecular NOEs depend on the diffusive encounters of solute and solvent molecules. To aid in the interpretation of our results, the self-diffusion coefficients of the solute and solvent components were determined (Table 2). It may be noted that the diffusion coefficient obtained in this work for 100% tetramethylsilane agrees well with the value ($3.7 \times 10^5 \text{ cm}^2 \text{ s}^{-1}$) estimated from the data of Parkhurst and Jonas.¹⁷

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TABLE 1. 1,3-Di-*tert*-butylbenzene Solute–Solvent NOEs

solvent	$\sigma \times 10^3$ (H4, H5, H6)			$\sigma \times 10^3$ (H2)			$\sigma \times 10^3$ (<i>tert</i> -butyl)		
	obsd	calcd model 1	calcd model 2	obsd	calcd model 1	calcd model 2	obsd	calcd model 1 ^a	calcd model 2 ^a
100% TMS									
H–H ^b	8.4	7.2 (–16%)	7.0 (–20%)	7.3	5.3 (–38%)	5.2 (–40%)	7.6	6.9 (–10%)	6.7 (–13%)
50% TMS/50% CCl ₄									
H–H	5.7	5.9 (4%)	5.7 (0%)	4.4	4.1 (–7%)	4.0 (–10%)	5.0	5.6 (12%)	5.5 (10%)
100% PFTB									
H–H	7.6	1.4 (–543%)	1.6 (–475%)	3.2	0.9 (–356%)	1.0 (–320%)	0.83	1.3 (57%)	1.5 (81%)
H–F ^c	15.0	13.0 (–15%)	13.0 (–15%)	11.0	8.6 (–28%)	8.4 (–31%)	12.0	13.0 (8%)	12.0 (0%)
50% PFTB/50% CCl ₄ ^b									
H–H	2.1	0.63 (–333%)	0.66 (–318%)	0.84	0.43 (–95%)	0.44 (–91%)	0.36	0.60 (67%)	0.62 (72%)
H–F	6.6	5.4 (–22%)	5.3 (–24%)	4.1	3.8 (–8%)	3.6 (–14%)	5.1	5.3 (4%)	5.1 (0%)

^a Average of values calculated for 18 *tert*-butyl hydrogens. ^b Solute proton–solvent proton NOEs. ^c Solute proton–solvent fluorine NOEs.

TABLE 2. Diffusion Coefficients for the Samples Examined

solvent	$D_{\text{solute}} \times 10^6, \text{cm}^2 \text{s}^{-1}$	$D_{\text{solvent}} \times 10^6, \text{cm}^2 \text{s}^{-1}$
100% TMS	25.1	37.2
50% TMS/50% CCl ₄	12.9	20.0
100% PFTB	6.9	10.2
50% PFTB/50% CCl ₄	9.6	13.3

Solvent Size. The apparent molecular radii of the solvent molecules used in this work were estimated by the following method. A molecular model of the solvent molecule was constructed using standard bond lengths and angles. After minimization of the conformational energy, a van der Waals surface for the model was calculated using the Connolly method.¹⁸ The radius of the sphere “rolled” over the surface of the model in this calculations was 1.2 Å, corresponding to the van der Waals radius of a covalent hydrogen atom. Distances from the centers of the probing spheres to the central atom of the solvent molecule were calculated and averaged. Using this approach, it was estimated that the average radii of PFTB and of TMS are 2.87 ± 0.38 and 2.81 ± 0.42 Å, respectively. (These conclusions were essentially independent of the number of dots used to represent the molecular surface as long as their number exceeded 8000.) The hard-sphere radius for tetramethylsilane has been estimated by Parkhurst and Jonas to be 2.84 Å,¹⁷ in good agreement with the estimate from our calculation. The diffusion coefficient for a trace of TMS dissolved in PFTB is $1.17 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$, while the diffusion coefficient for PFTB in this system is $1.02 \times 10^{-5} \text{cm}^2 \text{s}^{-1}$. For spherical molecules, the diffusion coefficient is expected to scale with the molecular radius.¹⁹ Thus, the computed sizes of PFTB and TMS are consistent with experimental data for these two materials.

Prediction of Solvent–Solute NOEs. As in our previous work,¹⁶ we start with the theory for intermolecular relaxation due to Ayant et al.¹⁵ In their formulation, the solute proton of interest is considered to be located in a sphere of radius r_{H} . The solvent spin is similarly situated in a sphere of radius r_{X} . The intermolecular cross-relaxation rate σ_{HX} is given by

$$\sigma_{\text{HX}} = 1/10 \gamma_{\text{X}}^2 \gamma_{\text{H}}^2 \hbar^2 [6J_2(\omega_{\text{H}} + \omega_{\text{X}}) - J_2(\omega_{\text{H}} - \omega_{\text{X}})] \quad (2)$$

where J_2 is a spectral density function defined by these authors that depends on the sum of the diffusion coef-

ficients for the molecules containing the proton and the fluorine spins ($D = D_{\text{H}} + D_{\text{X}}$) and the distance of closest approach, r ($= r_{\text{H}} + r_{\text{X}}$).

Equation 2 is derived with the assumption that solvent molecules can approach the sphere representing the solute equivalently from all directions. In actual molecules, the structure of the solute will make solvent approaches from different directions nonequivalent. Some solvent approach paths will allow the solute hydrogen and solvent molecule to interact at their van der Waals contact distance, while other approaches will involve interactions at distances longer than this. To take into account the shape of the solute molecule as it interacts with solvent molecules, we used the empirical procedure previously described.¹⁶ This method assumes that the contribution to σ_{HX} of a single H-solvent spin interaction over a small element of the solute–solvent contact surface can be computed using the equations of Ayant et al.¹⁵ To find σ_{HX} for a molecular shape requires integrating over the surface of the molecule. To approximate the results of this process, we imagine a large number of equi-spaced rays extending in all directions from the center of a sphere of surrounding the hydrogen atom of interest. Each ray will intersect the surface of the solute molecule at a particular distance from the hydrogen and, at that distance, there will be a characteristic contribution to σ_{HX} . Summing the contributions associated with each ray is assumed to give the aggregate NOE for the hydrogen.

We used the Connolly algorithm to obtain a representation of the molecular surface of 1,3-di-*tert*-butylbenzene using the solvent radii indicated earlier. This procedure generates a collection of points that correspond to positions where a solvent sphere is able to make contact with the van der Waals surface of the solute. Each surface contact point represents a small portion of the molecular surface, typically about 0.005Å^2 in this work. The number of rays used in our procedure (~ 3000) was sufficiently high that there was a “Connolly surface dot” 0.1Å or closer to the ray extended from the hydrogen atom.

Two models for the solvent molecules were used in the calculations. Both retain the approximation that the solvent can be represented by a sphere. In the first model, it was assumed that all spins of a solvent molecule that contribute to dipolar relaxation are located at the center of the sphere. That is, the nine fluorine atoms of PFTB or the 12 hydrogens of TMS are assumed to be located at the center of the sphere that represents a solvent molecule. In the second model, at each solute–solvent interaction point we calculated the average of all possible

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interactions between individual solvent spins and the solute proton of interest for about 30 000 orientations of the solvent molecule. It was assumed that all orientations were equally probable; that is, there were no specific interactions between solute and solvent that would favor interaction of a particular solvent orientation with the solute. The two approaches gave very similar results (Table 1).

Discussion

The solute 1,3-di-*tert*-butylbenzene was chosen for this work because the solvent exposures of the various hydrogens of the molecule are clearly different. The experimental results and the corresponding predicted NOEs (Table 1) are qualitatively consistent with H2 of the solute being protected from solvent interactions. Examination of Connolly surfaces indicated that it is impossible for a PFTB or TMS molecule to make van der Waals contact (~ 1.2 Å) with this hydrogen, although interactions at distances greater than ~ 1.4 Å are feasible. It is unfortunate that overlapping chemical shifts made it impossible to determine the separate NOEs for protons H5 and the H4, H6 pair since these are predicted to be shielded somewhat differently from solvent interactions.

A defect of our method for predicting the solute–solvent NOEs is that the solute molecule is assumed to be a conformationally rigid structure. The protection of proton H2 from solvent should vary with rotation and vibration of the *tert*-butyl groups of **1**. Calculations of the NOEs were done using the methods outlined above for conformations of **1** in which one, both, or neither of the *tert*-butyl groups were rotated so that two methyl groups provided maximum shielding of H2 from solvent. It was found that the differences in the calculated NOEs for these conformations were small, typically a few percent.

If we assume a maximum uncertainty of 10% in experimental results and a maximum uncertainty of 5% the calculations, most of the predicted solute–solvent NOEs are in reasonable agreement with experimental results for the 100% TMS and 50% TMS/50% CCl₄ solvent systems (Table 1), indicating that our relatively crude method for calculating the solvent–solute NOEs in these systems does well in predicting the observed NOEs, including the protection of proton H2. Significant deviations of observed σ_{HX} values from calculated σ_{HX} values thus may signal a breakdown in the assumptions used for the calculations, in particular the assumption that solute–solvent interactions are “non-sticky” collisions between hard spheres.

The NOEs that arise between the OH proton of PFTB and the aromatic protons of the solute are appreciably larger than expected, while the NOEs between this proton and the *tert*-butyl protons are smaller than predicted. Perfluoro-*tert*-butyl alcohol is a fairly strong acid, probably with a $\text{p}K_{\text{a}} < 10$.²⁰ Compound **1** is expected to be a reasonably strong π -base. Our observations suggest the formation of long-lived solute–PFTB complexes in which PFTB molecules are hydrogen-bonded to the aromatic ring. If such complexes persist longer than the time τ ($= r^2/D$, ~ 0.03 – 0.1 ns) that characterizes the spectral density for diffusive encounters, dipolar interactions of solvent OH, and to a lesser extent, solvent fluorine with the aromatic protons will be modulated to

TABLE 3. Proton Chemical Shifts^a

solvent	H2	H4, H6	H5	<i>tert</i> -butyl
100% TMS	7.389	7.106	7.106	1.303
50% TMS/CCl ₄	7.338	7.095	7.095	1.313
100% PFTB	7.633	7.299	7.235	1.337
50% PFTB/CCl ₄	7.472	7.182	7.182	1.332

^a In ppm, relative to TMS. In some systems, the shifts for H4, H6, and H5 were not resolved except in 100% PFTB.

some extent by rotational motion of the complex, rather than diffusion. Given a PFTB–solute interaction that persists as long as the estimated rotational correlation time of **1** (~ 0.2 ns), σ_{HX} could be as large as 200×10^{-3} , depending on the internuclear distance characteristic of a particular interaction. The observed $^1\text{H}\{^1\text{H}\}$ and $^1\text{H}\{^{19}\text{F}\}$ NOEs in solutions of **1** containing PFTB are thus not incompatible with formation of long-lived solute–solvent complexes.

The presence of unusual solvent–solute interactions in the PFTB systems may also be indicated by the shielding parameters for the protons of **1** (Table 3). The aromatic protons are appreciably deshielded when **1** is dissolved in 100% PFTB or the PFTB/CCl₄ mixture.

While the procedure that was used for the prediction of NOEs attempts to take into account the shape of the solute molecule, it employs a rough approximation for the solvent molecule, namely that this molecule can be represented by a sphere. Improvements in the prediction of intermolecular NOEs will likely have to come at the expense of using a better representation of the solvent. Some progress in this regard has been described.²¹

Summary. It is possible to estimate solvent spin–solute spin intermolecular NOEs reliably using an empirical procedure that is based on the van der Waals surface of the solute and the experimental diffusion coefficients for the solvent and solute. Experimental NOEs between 1,3-di-*tert*-butylbenzene and tetramethylsilane, a solvent molecule that should be well approximated as a sphere, are generally in good agreement with experiment. However, with perfluoro-*tert*-butyl alcohol $^1\text{H}\{^1\text{H}\}$ and $^1\text{H}\{^{19}\text{F}\}$ NOEs between solvent and solute do not agree well with predictions based on the encounters of hard spheres. In this case, it appears that an appreciable attractive interaction, possibly hydrogen bonding, between the solvent and the aromatic ring of the solute produce solvent–solute complexes that persist for ~ 0.2 ns or longer.

Experimental Section

1. Materials. Tetramethylsilane, carbon tetrachloride, perfluoro-*tert*-butyl alcohol, and 1,3-di-*tert*-butylbenzene were obtained commercially.

2. Preparation of NMR Samples. Samples for NMR spectroscopy were approximately 0.10–0.15 M in 1,3-di-*tert*-butylbenzene. The solute was weighed into 5 mm J. Young NMR tubes (Wilmad) and the appropriate volume of solvent added. Mixed solvent samples were prepared by addition of equal volumes of the solvents to the NMR tube. A sealed thin capillary tube containing acetone-*d*₆ (lock signal) was placed

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in the tube, and the samples were degassed by three to five freeze–thaw cycles before being sealed. Liquid volumes appeared to be additive. For samples containing TMS as a solvent, the sample tube consisted of 1.5 mm capillary attached to the end of a 5 mm J. Young tube. In these cases, the sample was inserted into a 5 mm NMR tube containing a mixture of 50% dimethyl sulfoxide-*d*₆ (lock signal) and 50% CCl₄.

All NMR spectra were run at a proton frequency of 500 MHz. All data presented in this paper were collected for samples at 25 °C. Temperatures are believed to have been stable to better than ±0.1 °C and accurate to better than ±0.5 °C.

Heteronuclear (¹H{¹⁹F}) and homonuclear (¹H{¹H}) solute proton–solvent spin NOEs were determined using the pulse sequences shown in the Supporting Information. A DPFGE sequence appended to the sequences prior to collection of the fid was used for suppression of the solvent signal.²² For both heteronuclear and homonuclear experiments, data were collected for 10–15 mixing times that ranged from 0.025 to 1.5 s. Typically, 16–64 scans were collected in order to average the effects of instrumental phase instabilities.

Diffusion coefficients were determined by the method of Wu et al.²³ using proton and fluorine signals of the sample and 8–12 values for the magnitude of the field gradient pulses that provide coherence defocusing and refocusing. A weak gradient was present during the mixing time to suppress possible effects of radiation damping. Field gradients were calibrated using a sample containing a Teflon plug of known dimensions²⁴ or by using the known diffusion coefficient of the HOD species in D₂O ($1.90 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at 25 °C).²⁵ Solute diffusion coefficients were determined using proton NMR signals of the solute methyl group. Experiments were run repetitively until three successive determinations of the diffusion coefficient agreed within ~2%.

Molecular modeling and dynamics calculations were done with SYBYL (Tripos Associates) and employed the Merck

(MMFF94) force field. Molecular surfaces were defined using the notions of Lee and Richards²⁸ as implemented in Connolly's algorithm (Quantum Chemistry Program Exchange program 429).¹⁸ The van der Waals radii used in the surface calculations for C, N, O were those given by Li and Nussinov.²⁹ The van der Waals radii used for H and F were 1.2 and 1.35 Å, respectively.³⁰ For simplicity, anisotropies in the atomic van der Waals radii, while certainly present in real molecules,^{31,32} were ignored for these calculations. The dependence of calculated NOEs on coordinate rotation and on the number of dots used to represent a molecular surface was examined.^{33,34} Variations of less than 1% in the calculated NOEs were observed when Cartesian coordinates were changed. Typically, molecular surfaces were represented by "Connolly dots" at a density of 200 dots per Å². Conclusions from calculations based on molecular surfaces were insensitive to the density of these dots as long as the number exceeded 100 dots per Å².

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Supporting Information Available: Pulse sequences used for the homonuclear and heteronuclear NOE experiments, a discussion of accelerating the heteronuclear ¹H{¹⁹F} NOE experiment, and other experimental considerations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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