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Aprotic, Viscous Solvent Mixtures for Obtaining Large, Negative NOE Enhancements in Small Inorganic and Organic Molecules: Ideal Solvent Systems for Deducing Structures by NMR Techniques

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Summary: Solutions containing polymers of chlorotrifluoroethylene and deuterated NMR solvents permit the manipulation of the viscosity of NMR samples in order to maximize the nuclear Overhauser effect (NOE) interactions of small molecules. These aprotic solutions provide an inert, highly viscous medium in which reactive organometallic complexes show sufficiently large, negative NOE enhancements for structural elucidation by one- or two-dimensional NMR spectroscopy.

Pioneered by Wüthrich and others,[1] NMR techniques for three-dimensional structural determinations have been successful for macromolecular systems such as proteins and nucleic acids. The long rotational correlation times of these large molecules are favorable for large, negative NOE enhancement, which are a principal source of distance information.[2] Conversely, small molecules dissolved in common NMR solvents have short rotational correlation times. Structural determinations of small inorganic and organic molecules by NMR techniques are hampered by the small NOE enhancements and long T_1's associated with these correlation times.

In order to extend solution structural determinations to small, reactive organometallic complexes, we have sought to amplify NOE enhancements through the modification of rotational correlation times by inert, highly viscous solvent systems. Although we, and others, have had limited success in increasing the viscosity of NMR samples by the use of ethylene glycol-d_6 and D_2O/DMSO-d_6, these deuterated solvents have obvious shortcomings, including reactivity to protic-sensitive compounds, expense, high concentrations of proton impurities, and solvent–solute hydrogen-bonding interactions.[3] In searching for more general solvent systems, we have found that solutions of polymeric chlorotrifluoroethylene waxes[4] and NMR solvents mitigate these shortcomings and provide a medium in which reaction chemistry and NMR studies can be performed. A subsequent literature search revealed that the less viscous oils of chlorotrifluoroethylene

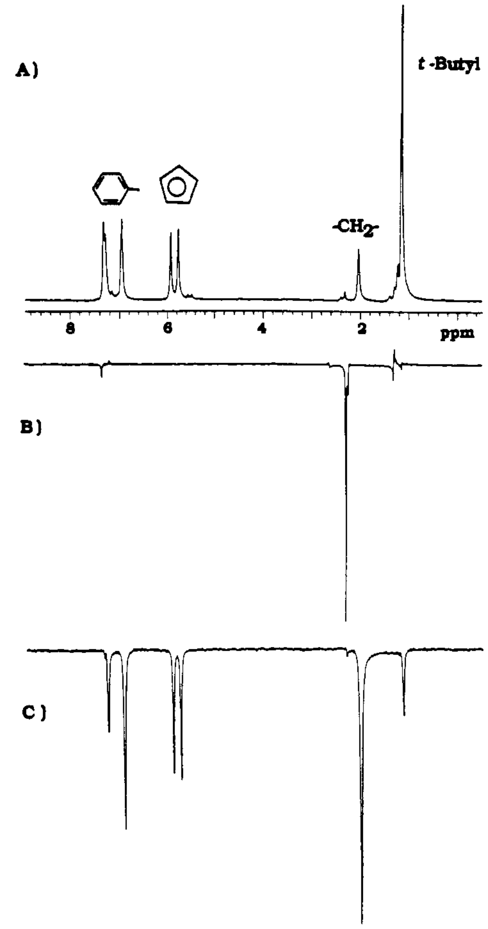
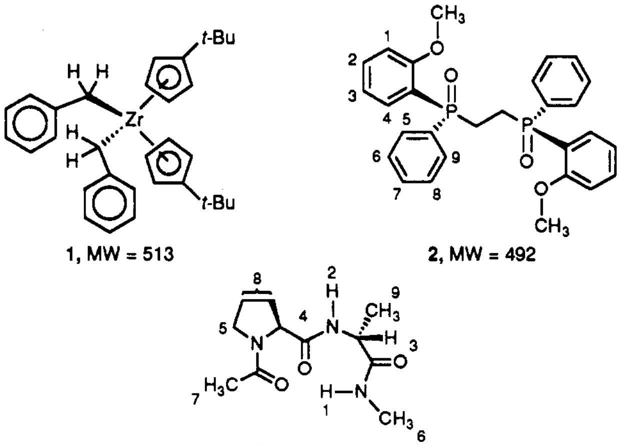


Figure 1. (A) 500-MHz ^1H NMR spectrum of 1 in 80% halocarbon wax and 20% benzene-d_6 at 10 °C. (B) Difference NOE spectrum of 1 in benzene-d_6 after irradiation of the benzylic protons. (C) Difference spectrum of 1 dissolved in 80% halocarbon wax and 20% benzene-d_6 after irradiation of benzylic protons.

Chart I



1, MW = 513

2, MW = 492

3, MW = 241

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[1] (a) Wüthrich, K. *Acc. Chem. Res.* **1989**, *22*, 36. (b) Gordon, S. L.; Wüthrich, K. *J. Am. Chem. Soc.* **1978**, *100*, 7094. (c) Olejniczak, E. T.; Poulsen, F. M.; Dobson, C. M. *J. Am. Chem. Soc.* **1981**, *103*, 6574. (d) Zuidererweg, E. R. P.; Kaptein, R.; Wüthrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5837. (e) Weber, P. L.; Wemmer, D. E.; Reid, B. R. *Biochemistry* **1983**, *24*, 4553. (f) Wemmer, D. E.; Reid, B. R. *Annu. Rev. Phys. Chem.* **1986**, *36*, 105. (g) Wüthrich, K.; Wider, G.; Wagner, G.; Braun, W. *J. Mol. Biol.* **1982**, *155*, 311. (h) Wemmer, D. E.; Chou, S.-H.; Hare, D. R.; Reid, B. R. *Biochemistry* **1984**, *23*, 2262. (i) Weiss, M. A.; Patel, D. J.; Sauer, R. T.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 130. (j) Clore, G. M.; Lauble, H.; Frenkiel, T. A.; Gronenborn, M. *Eur. J. Biochem.* **1984**, *145*, 629. (k) Bruch, M. D.; Noggle, J. H.; Gierasch, L. M. *J. Am. Chem. Soc.* **1985**, *107*, 1400. (l) Kessler, H.; Bermel, W. In *Methods in Stereochemical Analysis*; Takeuchi, Y., Marchand, A. P., Eds.; VCH: Dusseldorf, Germany, 1986; Vol. 6. (m) Gronenborn, A. M.; Clore, G. M. *Prog. NMR Spectrosc.* **1985**, *17*, 1.

[2] (a) Noggle, J. H.; Schirmer, R. E. *The Nuclear Overhauser Effect*; Academic Press: New York, 1971. (b) Williamson, M. P.; Neuhaus, D. *The Nuclear Overhauser Effect in Structural and Conformational Analysis*; VCH: New York, 1989.

[3] (a) Landis, C. R.; Giovannetti, J. S. Unpublished results. (b) Giovannetti, J. S., Ph.D. Dissertation, University of Colorado, 1990. (c) Chin, Y.; Unger, S. W.; LaMar, G. N. *J. Magn. Reson.* **1986**, *67*, 346.

[4] Halocarbon Products Corp., 120 Dittman Ct., N. Augusta, SC 29841. Prior to dissolution of this wax in NMR solvent, the wax was heated to 90 °C in a Schlenk tube under dynamic vacuum for 60 min to release dissolved oxygen and water.

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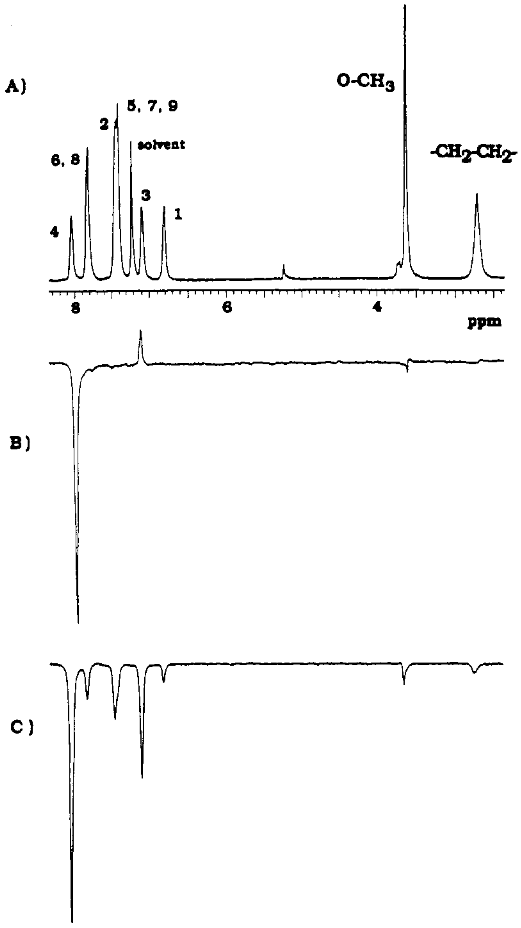

Figure 2. (A) 500-MHz ^{1}H NMR spectrum of 2 in 80% halocarbon wax and 20% benzene-d_6 at 10 °C. (B) Difference NOE spectrum of 2 in benzene-d_6 after irradiation of position 4. (C) Difference spectrum of 2 dissolved in 80% halocarbon wax and 20% benzene-d_6 after irradiation of position 4.

had been applied with mixed success for NOE studies of antibiotics having molecular weights around 1000.[5]

Results and Discussion

To illustrate the utility of this solvent system, we report a study of three compounds, bis(benzyl)bis(*tert*-butylcyclopentadienyl)zirconium(IV) (**1**), (*S*,*S*)-1,2-ethanediylbis[(*o*-methoxyphenyl)phenylphosphine oxide] (Dipampo, **2**), and a dipeptide, *N*-acetyl-L-prolyl-D-alanine methylamide (**3**). Structures and molecular weights are given in Chart I.

Figure 1A shows a 500-MHz ^{1}H NMR spectrum of **1** dissolved in 80% halocarbon wax (series 600)[4] and 20% benzene-d_6 (w/w) at 10 °C. Slight chemical shift differences and an increase in spectral line widths (from 3 to 14 Hz) are seen in this solvent mixture compared to those found in 100% benzene-d_6. Importantly, we observe that the solvent mixture provides an inert medium that does not degrade the highly reactive zirconium complex. Figure 1B shows the results of a one-dimensional steady-state NOE experiment for **1** dissolved in benzene-d_6 with irradiation of the benzylic protons. This NOE difference spectrum exhibits a positive NOE enhancement of less than 1% to the ortho protons of the benzene ring.

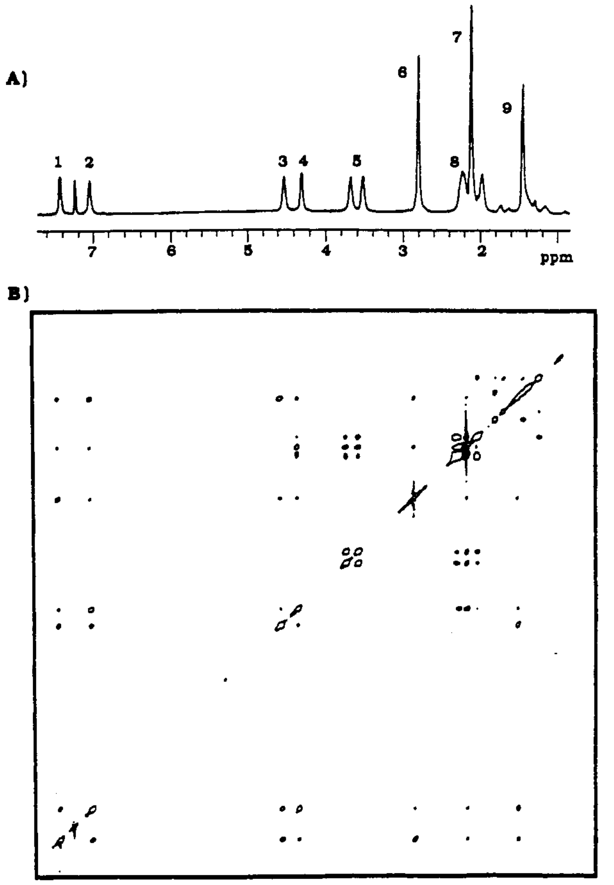

Figure 3. (A) 500-MHz ^{1}H NMR spectrum of **3** in 75% halocarbon wax and 25% chloroform-*d* at 10 °C. (B) Pure absorption phase 1K × 1K 2D NOE contour map of **2** taken with a 500-ms mixing time.

Dissolution of **1** in 80% halocarbon wax/20% benzene-d_6 (w/w) (Figure 1C) results in dramatically different enhancements upon selective saturation of the benzylic protons. The ortho protons on the benzene ring show a large, negative NOE enhancement of −42% along with negative NOE interactions, principally due to spin diffusion, to all other protons in the complex. These observations indicate lowering of molecular rotational correlation times into the slow tumbling NOE region.

In order to illustrate the application of this solvent system to NOE studies of smaller organophosphorus compounds, **2** was dissolved in 80% halocarbon and 20% chloroform-*d*. A 500-MHz ^{1}H NMR spectrum is shown in Figure 2A. Resonances in the spectrum are identified by the numbering scheme for the structure given in Chart I.

Saturation of the peak corresponding to position 4 on the anisoyl ring shows a positive NOE interaction of 11% to its neighboring proton (3). No NOE interactions to other protons are observed in Figure 2B. In contrast, when **2** is dissolved in 80% halocarbon and 20% chloroform-*d*, negative NOE interactions are observed to all protons in the compound upon irradiation of proton 4 (see Figure 2C). Distant protons in the molecule show perturbations due to spin diffusion, and the difference spectrum exhibits −29% NOE enhancement at proton 3.

To demonstrate the applicability of this solvent mixture to two-dimensional NMR examinations of very low molecular weight compounds, we conducted a series of NOESY experiments with **3** dissolved in 75% halocarbon/25% chloroform-*d* (w/w). Figure 3A depicts a one-dimensional spectrum of **3**, and Figure 3B shows a

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[5] (a) Williamson, M. P.; Williams, D. H. *J. Chem. Soc., Chem. Commun.* **1981**, 165. (b) Hyde, E.; Kalman, J. R.; Williams, D. H.; Reid, D. G.; Olsen, R. K. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1041. (c) Neuhaus, D.; Keeler, J. *J. Magn. Reson.* **1986**, *68*, 568.

[6] For spectroscopic studies of some related dipeptides, see: Boussard, G.; Marrand, M.; Aubry, A. *Biopolymers* **1979**, *18*, 1297.

contour plot of the two-dimensional NOESY spectrum with a 0.5-s mixing time at 10 °C. Although enhancements of NOE intensities are compromised by some loss of spectral resolution, distinct large NOE cross peaks are seen throughout the spectrum. Furthermore, we have demonstrated[7] that high-quality two-dimensional NOE time courses can be collected for the low-molecular-weight compounds **1** and **3**.

The significance of this work is the demonstration that solutions containing mixtures of perhalogenated polymers with common NMR solvents constitute viscous, aprotic media that extend the application of NMR methods for solution structure determination of compounds with molecular weights as low as 240. Full characterization of structures for compounds **1** and **3** in viscous solutions containing perhalogenated polymers is forthcoming.

Experimental Section

Chemicals. Chloroform-*d* and benzene-d_6 were obtained from Aldrich. Benzene-d_6 was dried over molecular sieves, freeze–pump–thawed, and stored in an inert-atmosphere glovebox. The halocarbon used in this experiment was a series 600 wax. This was a generous gift from Halocarbon Products Corp., 120 Dittman Ct., N. Augusta, SC 29841. Prior to dissolution of this wax in the NMR solvent, the wax was heated to 90 °C in a Schlenk tube under dynamic vacuum for 30 min to release dissolved oxygen.

Bis(benzyl)bis(*tert*-butylcyclopentadienyl)zirconium(IV) (**1**) was prepared by standard methods[8] and stored in a glovebox under an inert atmosphere. Dipampo (**2**) was a gift from Monsanto Corp., St. Louis, MO, and was used without any further purification. *N*-Acetyl-L-prolyl-D-alanine methylamide (**3**) was prepared by standard methods[9] and supplied to us by Dr. Samuel Gellman and Christopher Rito, University of Wisconsin, Madison, WI.

NMR Measurements. ^{1}H NMR spectra were obtained on a Varian VXR 500 spectrometer with a 5-mm ^{1}H/^{19}F probe. Each NMR sample contained 5 mg of each compound in a sealed tube which was degassed by six freeze–pump–thaw cycles.

Standard parameters for the steady-state experiment were as follows: temperature 10 °C, 5000-Hz sweep width, 16K data points, 75° pulse width, continous-wave irradiation time for a period of $5T_1$, and relaxation delay of $5T_1$. T_1 measurements prior to the steady-state experiment were determined by standard inversion recovery methods.[10] To obtain the difference spectra illustrated in this report, the following method was used. Two spectra were obtained, one spectrum with the irradiation set on-resonance and one spectrum with the set off-resonance. The difference spectra were obtained by subtracting the Fourier-transformed off-resonance spectra from the Fourier-transformed on-resonance spectrum. The resulting spectrum exhibited resonances which experience a NOE from the irradiated resonance. The percentage reported is defined as the difference between on- and off-resonance intensities of the peak during the steady-state experiment.

The two-dimensional NOESY experiment was performed by use of the standard Varian pulse sequence. A total of 256 experiments of 16 scans were performed, with collection of 1024 data points in F2. The spectral width in F2 was 3654 Hz. The mix time for the illustrated example was 0.5 s. Quadrature detection in the second dimension was accomplished through the method of States and Haberkorn.[11] Processing of the spectra was done by FELIX, obtained from Hare Corp.[12]

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(12) Hare Research Inc., Woodinville, WA.