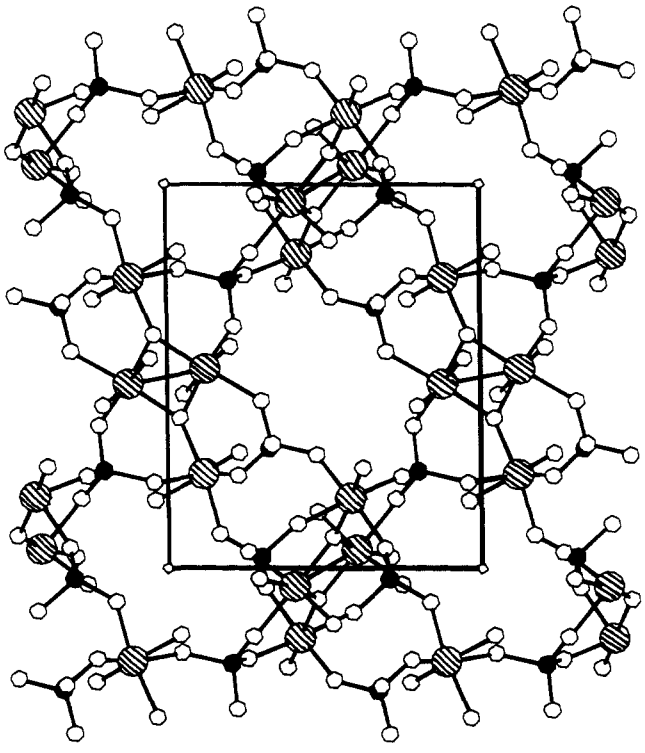

Figure 2. Ball-and-stick projection (as drawn by Chem-X[15]) of the unit cell contents of **1** down [001]. The solvate water molecules residing in the tunnels are not shown. Striped circles, Mo; filled circles, P; remaining circles, O.

1b), centered around the $\bar{1}$ site at $(0,^1/_2,^1/_2)$, Mo(2) has ordered trans molybdenyl and water oxygens, both of which are cis to three-coordinate O(10), and has a calculated[8] oxidation state of 5+. The Mo(3) pair also has disordered molybdenyl/water oxygen atoms, as with Mo(4) in **2** above, as well as a Mo–Mo single bond at 2.659 (5) Å. These tetramers are topologically quite similar to Fe_4 tetramers found in the mineral amarantite, $Fe_2(SO_4)_2O{\cdot}7H_2O$,[11] as well as in some synthetic compounds.[12]

The connection of these tetramers into a three-dimensional array by the phosphate groups is shown in Figure 2, which is a projection of the unit cell contents down [001] showing the tetramers connected into sheets, which lie in the (220) plane, by PO_4 and $PO_3(OH)$ groups. These sheets are connected by PO_4 groups into a 3D lattice. This connectivity generates tunnels parallel to [100], [010], and [001] which are filled with the waters of solvation and into which protrude the waters that are ligated to Mo.

Phosphate **1** is microporous toward water as demonstrated by the Brunauer type I absorption isotherm.[13] Both the absorption isotherm and the TGA data show the loss of 12 wt % H_2O, which is greater than 90% of the theoretical amount calculated from the crystal structure.[14] This 12 wt % corresponds to about 35 vol % internal void space, since the calculated density of **1** based on the X-ray data is near 3 g cm^{-3}, if one assumes that the density of the absorbed water is 1 g cm^{-3} or less. Since nearly half of the water in **1** is ligated to Mo, and since the powder X-ray diffraction pattern indicates rather small distortions of the lattice upon dehydration and the water absorption is totally reversible, one concludes that some of the Mo atoms in the tunnels of dehydrated **1** must possess a vacant coordination site.

Preliminary magnetic measurements on **1** show that $\mu_B = 2.6$ μ_B per four Mo atoms, consistent with the two metal–metal-bonded Mo atoms being diamagnetic and one unpaired electron on each of the other two d^1 Mo^{5+} atoms. Examination of the $\chi^{-1}(T)$ data above 100 °K shows it to be linear with $\theta = -102$ K, indicative of antiferromagnetic interactions, while below 100 °K the $\chi^{-1}(T)$ plot shows nonlinear behavior.

These results show that microporous transition-metal oxides, with coordinatively unsaturated metals and very large internal void volumes, can be easily prepared.

Supplementary Material Available: Tables of experimental crystallographic details, positional parameters and B(eq) values, U values, intramolecular distances and bond angles, and the water absorption isotherm data (9 pages). Ordering information is given on any current masthead page.

(10) The Mo(3) and Mo(4) pairs both have Mo–Mo single bonds near 2.6 Å consistent with a d^1 configuration (Mo^{5+}). The water content of **1** observed by TGA requires nearly half of the disordered terminal O atoms on Mo(3) and Mo(4) to be water molecules.

(11) Süsse, P. *Z. Kristallogr.* **1968**, *127*, 261.

(12) Gorun, S. M.; Lippard, S. J. *Inorg. Chem.* **1988**, *27*, 149 and references therein.

(13) Ruthven, D. M. *Principles of Adsorption and Adsorption Processes*; Wiley: New York, 1984; p 49. Absorption data has been deposited as supplementary material.

(14) Possible reasons for removal of less than the theoretical amount of water could be incomplete occupation of the water solvate sites in the crystal (the solvate waters had rather large thermal parameters) or, since water loss starts at just slightly above room temperature according to TGA data, the compound may have lost some water during the isolation and workup.

(15) Chem-X, designed and distributed by Chemical Design Ltd., Mahwah, NJ.

Specific Deuteration Strategy for Enhancing Direct Nuclear Overhauser Effects in High Molecular Weight Complexes

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Proton NMR studies of high molecular weight systems are plagued by several problems, including poor resolution and sensitivity. In particular, these problems greatly limit the ability to observe specific nuclear Overhauser effects (NOEs), thus severely hampering structure determination efforts. Poor resolution results from the presence of an enormous number of resonances and inherently large line widths; the latter also significantly reduce the sensitivity of the NMR experiments. Resolution can be greatly improved through the use of isotope-editing techniques[1] which allow selective detection of a subset of NMR resonances. Resonance line-width increases are due to more efficient ^1H–^1H dipolar relaxation as motional correlation times increase; this problem can be alleviated through judicious use of deuterium substitution.[2-7] Another very important benefit of deuterium substitution is the possibility of increasing the size of NOEs via elimination of undesirable magnetization transfer pathways. Such pathways allow magnetization leakage away from the nuclei of interest and also provide indirect magnetization transfer routes between two nuclei, thereby complicating the analysis of NOE data. Substantial improvements in spectral resolution in protein NMR studies through random fractional deuteration have been previously demonstrated.[2-5] However, a significant weakness in such schemes is that unless deuterium incorporation is close to 100%, multispin magnetization transfer pathways will still exist which can lead to erroneous interpretations of NOE measurements, especially if longer mixing times are employed to take advantage of the deuteration. Also, the sensitivity of the fractional deuteration method is far from optimal (except for NOEs between exchangeable protons), due to the low probability of two nuclear

(1) Griffey, R. H.; Redfield, A. G. *Q. Rev. Biophys.* **1987**, *19*, 51-82.

(2) Crespi, H. L.; Kosika, A. G.; Smith, U. H. *Biochem. Biophys. Res. Commun.* **1974**, *61*, 1407-1414.

(3) Kalbitzer, H. R.; Leberman, R.; Wittinghofer, A. *FEBS Lett.* **1985**, *180*, 40-42.

(4) LeMaster, D. M.; Richards, F. M. *Biochemistry* **1988**, *27*, 142-150.

(5) Markley, J. L.; Putter, I.; Jardetzky, O. *Science* **1968**, *161*, 1249-1251.

(6) Torchia, D. A.; Sparks, S. W.; Bax, A. *J. Am. Chem. Soc.* **1988**, *110*, 2321-2322.

(7) Shon, K.; Opella, S. J. *J. Magn. Reson.* **1989**, *82*, 193-197.

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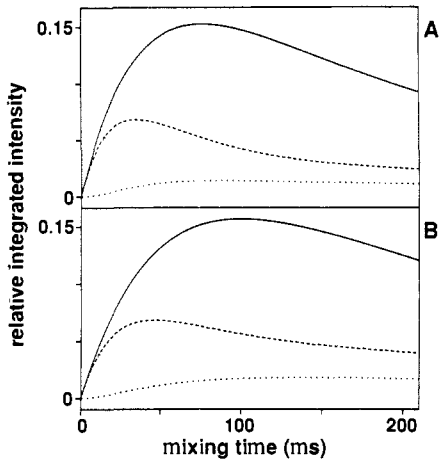

Figure 1. Simulated NOE mixing time behavior for the peptide obtained by a full relaxation matrix analysis,[13-15] using our program NOECAL. The calculations were done by assuming a 600-MHz resonance frequency and correlation times for isotropic tumbling and methyl group rotation of 25 and 0.01 ns, respectively. The data correspond to NOEs (integrated intensity) observed from the amide proton of G85 to the amide proton of either I84 (A) or G86 (B). In each panel, simulations for three situations are shown: (1) peptide deuterated at the two glycines and the isoleucine (solid line), (2) undeuterated peptide (long dashed line), and (3) undeuterated peptide for which the cross-relaxation rate between G85(NH) and I84(NH) (A) or G86(NH) (B) has been zeroed (short dashed line). The latter represents the spin-diffusion contribution to the observed NOE.

sites of interest in a particular molecule being simultaneously occupied by protons. Complete deuteration has been proposed as a method for enhancing NOEs in large biomolecules, but this will allow only NOEs between exchangeable protons to be observed.[6,7] In this communication we propose a strategy to improve the sensitivity of NOE measurements by specifically, and fully, deuterating sites carefully chosen to eliminate undesirable magnetization transfer pathways in the system of interest. In principle, the specific deuteration strategy provides a general method for enhancing NOEs between any pair of protons, and it results in optimal sensitivity since the nuclear sites of interest are fully protiated. We demonstrate the effectiveness of specific deuteration for obtaining structural data in studies of a peptide–antibody Fab' complex (MW 56 kDa).[8,9]

The strategy proposed to enhance direct NOEs requires some working model for the local structure, based on either preliminary experimental data or some other prior knowledge about the molecule of interest. The optimum deuteration scheme can be estimated either from a qualitative assessment of which protons are likely to be involved in undesirable magnetization transfer pathways or from quantitative simulations of the cross-relaxation behavior.

To illustrate the specific deuteration strategy, we focus on a complex of a 12 residue peptide antigen (MHKDFLEKIGGL, corresponding to residues 76–87 of the protein myohemerythrin) bound to its cognate Fab'.[10] The exchange rate is slow on the NMR time scale,[9] so NMR measurements can provide direct information on the peptide in its bound state. To determine the structure of the peptide in the antibody binding site, we are measuring NOEs to obtain estimates of some of the intrapeptide internuclear distances. Initial experiments revealed several amide–amide NOEs between adjacent residues in the peptide sequence. These observations suggest a working model in which the bound peptide has a helical conformation. Since this peptide adopts an α-helical conformation in the X-ray structure of the native protein, we used proton geometries derived from crystallographic coordinates[11] in calculations of the cross-relaxation behavior; possible cross-relaxation with the antibody was ignored.

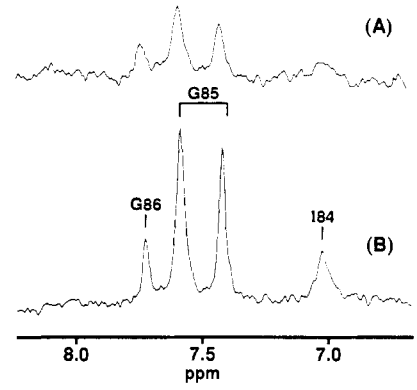

Figure 2. One-dimensional isotope-edited NOE spectra (600 MHz, mixing time = 90 ms) obtained from Fab' bound to the undeuterated (A) and specifically deuterated peptide (B). For the deuterated peptide, the NOEs are at their maximum intensity at approximately 90 ms; long mixing times can be employed since potential spin diffusion pathways are minimized. In both peptides, G85 was ^{15}N-labeled. The peptide in B is perdeuterated at the G85, G86, and I84 residues; the deuterated amino acids for G86 and I84 were obtained from MSD Isotopes and CIL, while the ^{15}N-labeled glycine (MSD) was deuterated by us.[16] The two spectra were acquired under identical conditions using a published pulse sequence[12] except that the final pulse was replaced by a $90°_x$–τ–$90°_{-x}$ composite pulse for water suppression; in these experiments, transfer of magnetization from the amide of G85 is monitored selectively. The spectra were acquired at 308 K for an approximately 0.5 mM complex in 90% H_2O/10% D_2O, pH 5, 0.1 M sodium deuterioacetate buffer solutions. The bound peptide concentrations were very similar (differing by less than 10%) in both solutions. Both spectra represent the averaging of 8192 transients. The free induction decays were apodized with a 6-Hz Lorentzian window function prior to Fourier transformation. The spectra have been plotted on a normalized scale such that at zero mixing time they would have the same integrated intensity; this eliminates differences in intensity caused by unequal sample concentrations and different spin–spin relaxation rates of the deuterated and undeuterated peptides during the isotope-editing part of the transient NOE experiment. An additional benefit of deuteration is that there will be a smaller loss of magnetization during the isotope-editing sequence.

As an example of the analysis of NOE behavior in the peptide, we consider the amide proton of G85 and examine its cross-relaxation with neighboring protons. The data shown in Figure 1 represents the transfer of magnetization from G85(NH) to I84-(NH) (A) and to G86(NH) (B) as a function of the mixing time. Results for undeuterated peptide and one in which the isoleucine and both glycines are perdeuterated at the carbon sites are shown; this deuteration scheme was chosen since it minimizes alternate magnetization transfer pathways from the amide protons. The magnitude of the NH–NH NOE is dramatically increased for the deuterated peptide.

To test the specific deuteration strategy, two peptides were synthesized with ^{15}N at the G85 residue. One peptide was undeuterated while the other was perdeuterated at isoleucine and both glycines. One-dimensional, transient NOE spectra were recorded by using an isotope-editing sequence[12] (Figure 2). The amide proton resonance of G85 is a doublet (at 7.58 and 7.42 ppm), due to the ^{15}N–^{1}H scalar coupling. The two resonances surrounding the G85(NH) doublet represent NOEs to amide protons assigned to I84 (7.02 ppm) and G86 (7.71 ppm).[9] The reverse NOEs, from I84(NH) and G86(NH) to G85(NH), are

(8) Tsang, P.; Fieser, T. M.; Ostresh, J. M.; Lerner, R. A.; Wright, P. E. *Pept. Res.* **1988**, *2*, 87–92.
(9) Tsang, P.; Fieser, T. M.; Lerner, R. A.; Houghten, R. A.; Wright, P. E. *Frontiers of NMR in Molecular Biology. UCLA Symposia on Molec. and Cellular Biology*; A. R. Liss: New York, 1990; Vol. 109, pp 63–73.
(10) Fab': fragment antigen binding region of antibody.
(11) Sheriff, S.; Smith, J. L.; Hendrickson, W. A. *J. Mol. Biol.* **1987**, *197*, 273–296.
(12) Rance, M.; Wright, P. E.; Messerle, B. A.; Field, L. D. *J. Am. Chem. Soc.* **1987**, *109*, 1591–1593.
(13) Olejniczak, E. T.; Gampe, R. T.; Fesik, S. W. *J. Magn. Reson.* **1986**, *67*, 28–41. (Note error in eq 3a.)
(14) Olejniczak, E. T. *J. Magn. Reson.* **1989**, *81*, 392–394.
(15) Keepers, J. W.; James, T. L. *J. Magn. Reson.* **1984**, *57*, 404–426.
(16) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; J. Wiley & Sons: New York, 1961; p 1831.

observed in spectra (not shown) obtained for complexes containing peptides labeled selectively with ^{15}N at I84 or G86. It is clear from Figure 2 that deuteration greatly improved the sensitivity, due to a reduction in line width as previously noted,[2-5] but more significantly to an increase in the NOE via elimination of some competing relaxation pathways. In addition, the results from the deuterated peptide verify that the observed NH-NH NOEs do not arise from spin diffusion through the intervening $C^{\alpha}H$ proton.

In this communication we demonstrate the use of a specific deuteration strategy to enhance NOEs observed in high molecular weight systems. Random fractional deuteration will not adequately eliminate troublesome spin-diffusion pathways and also results in poor sensitivity compared to specific deuteration (except for NH-NH interactions). Extensive deuteration of a particular molecular fragment can be expensive and is usually unnecessary to achieve the desired goals. While the example provided here focused on amide-amide NOEs, the specific deuteration strategy is completely general and can be used to enhance NOEs between any pair of protons. The specific deuteration strategy is ideally suited for studies of synthetic peptides or other small molecules bound to protein receptors and should also be applicable to studies of proteins that are biosynthetically deuterated. The specificity of α and β deuteration will depend upon amino acid type, however, while specific deuteration at other side-chain positions will usually be independent of this factor and much less susceptible to problems of label scrambling.[17]

Through the use of a model structure for the system of interest, specific deuteration patterns may be designed to facilitate the observation of direct NOEs; experimental results subsequently obtained may then either lend support for the proposed model or indicate that modifications in it are required. Application of this strategy should render many high molecular weight systems more amenable to solution NMR studies.

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(17) LeMaster, D. M. *Q. Rev. Biophys.* **1990**, *23*, 133-174.

Reduction of Dinitrogen by a Zirconium Phosphine Complex To Form a Side-On-Bridging N_2 Ligand. Crystal Structure of $\{[(Pr^i_2PCH_2SiMe_2)_2N]ZrCl\}_2(\mu\text{-}\eta^2\text{:}\eta^2\text{-}N_2)$

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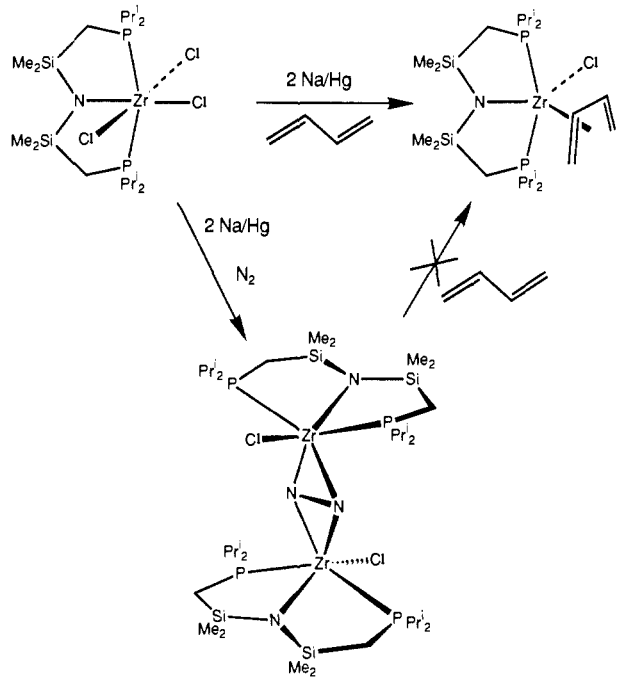
One of the milestones of modern inorganic chemistry was the discovery that dinitrogen (N≡N) could act as a ligand. Since the original report[1] by Allen and Senoff in 1965 on the preparation of $[Ru(NH_3)_5N_2]^{2+}$, numerous N_2 complexes have been synthesized and structurally characterized.[2] By far the most prevalent mode of coordination to a metal is end-on to one metal or end-on bridging to two metals. Activation of the coordinated N_2 is indicated by a lengthening of the N-N bond distance of 1.0975 Å for free N_2.[3] Typical N-N bond lengths are 1.03-1.16 Å for mononuclear compelxes and 1.12-1.33 Å for binuclear compounds. The side-on mode of N_2 coordination is extremely rare, and only a few polynuclear complexes displaying this mode of coordination have been crystallographically characterized.[4] Herein we report the synthesis and structure of only the second binuclear complex containing a *planar* side-on-bound bridging N_2 ligand, wherein dinitrogen has been irreversibly reduced to a $(N\text{-}N)^{4-}$ hydrazido ligand.[5]

Scheme I



Previous work from our laboratory has shown that phosphine complexes of zirconium(IV) can bind 1,3-butadiene under reducing conditions. Thus, reaction of $ZrCl_3[N(SiMe_2CH_2PPr^i_2)_2]$ (**1**) with Na/Hg and 1,3-butadiene generates red $ZrCl(\eta^4\text{-}C_4H_6)[N(SiMe_2CH_2PPr^i_2)_2]$ (**2**), which can also be prepared[6] from magnesium butadiene ($Mg{\cdot}C_4H_6{\cdot}2THF$) and **1**. However, if reduction of **1** by Na/Hg is carried out just under N_2, a deep blue solution is obtained from which dark blue crystals of the formula $\{[(Pr^i_2PCH_2SiMe_2)_2N]ZrCl\}_2(N_2)$ (**3**), can be isolated in moderate yield.[7] Addition of 1,3-butadiene to the N_2 complex **1** does not

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(1) Allen, A. D.; Senoff, C. V. *J. Chem. Soc., Chem. Commun.* **1965**, 621.

(2) (a) Pelikan, P.; Boca, R. *Coord. Chem. Rev.* **1984**, *55*, 55. (b) Henderson, R. A.; Leigh, G. J.; Pickett, C. J. *Adv. Inorg. Chem. Radiochem.* **1983**, *27*, 197. (c) Dilworth, J. R.; Richards, R. L. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Stone, F. G. A., Abel, E. W., Eds.; Pergamon: Oxford, England, 1982; Vol. 8, Chapter 60, p 1073. (d) Chatt, J.; Dilworth, J. R.; Richards, R. L. *Chem. Rev.* **1978**, *78*, 589.

(3) *Tables of Interatomic Distances and Configurations in Molecules and Ions*; Chemical Society Special Publications; Sutton, L. E., Ed.; The Chemical Society: London, 1958; Vol. 11.

(4) (a) Evans, W. J.; Ulibarri, T. A.; Ziller, J. W. *J. Am. Chem. Soc.* **1988**, *110*, 6877. (b) Pez, G. P.; Apgar, P.; Crissey, R. K. *J. Am. Chem. Soc.* **1982**, *104*, 482. (c) Jonas, K.; Brauer, D. J.; Kruger, C.; Roberts, P. J.; Tsay, Y.-H. *J. Am. Chem. Soc.* **1976**, *98*, 74. (d) Kruger, C.; Tsay, Y.-H. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 998.

(5) Schrock, R. R.; Wesolek, A. H.; Wallace, K. C.; Dewan, J. C. *Inorg Chem.* **1988**, *27*, 2050.

(6) Fryzuk, M. D.; Haddad, T. S.; Rettig, S. J. *Organometallics* **1989**, *8*, 1723.

(7) Compound **3** was synthesized by stirring a toluene solution of $ZrCl_3[N(SiMe_2CH_2PPr^i_2)_2]$ (0.680 g, 1.15 mmol) with a 0.1% Na/Hg amalgam (0.106 g, 4.60 mmol) under 4 atm of N_2 for 2 days to give, after recrystallization from toluene, 0.270 g of product (44% yield). Satisfactory microanalysis and NMR spectral parameters were obtained for the dinitrogen complex **3** as detailed in the supplementary material.

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