which release of hydrogen to the zeolite seems to be more favorable. These data stress the importance of the binding of protonated species to the zeolite framework, as has already been demonstrated for carbenium ions.[22,23] The current model for rearrangement of carbonium ions gives emphasis to the rearrangement of nuclei (positively charged species) within electron clouds as opposed to the normal model for rearrangement of carbenium ions, which rearranges electrons (negatively charged species) to electron-deficient centers.

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Registry No. 1, 96-14-0; 1·H$^+$, 138093-65-9; 2-methylpentane, 107-83-5; methylcyclohexane, 108-87-2; methylcyclohexane conjugate acid, 138093-66-0; hydrogen, 1333-74-0.

Supplementary Material Available: Mass spectra for standard 2-methyl- and 3-methylpentane and for both after H–D exchange, ^{2}H NMR spectra for recovered methylcyclohexane and 3-methylpentane, and an X-ray diffractogram for zeolite Y (7 pages). Ordering information is given on any current masthead page.

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Homonuclear Deuterium Correlation Spectroscopy in the Rotating Frame

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High-resolution deuterium NMR has some attractive features in spite of poor sensitivity. Chief among these is the fact that each chemically distinct site in a perdeuterated molecule leads to exactly one resonance because of the small homonuclear ^{2}H–^{2}H couplings, which are invariably less than 0.5 Hz. In addition, the quadrupolar ^{2}H nucleus normally has higher effective relaxation rates than does ^{1}H, enabling faster pulse sequence repetition for signal averaging. In recent years, some interest has focused on NMR applications involving deuterium: polarization transfer from ^{2}H to ^{13}C is a significant example.[1,2] 2D homonuclear ^{2}H–^{2}H correlation is another important application,[3] performed with a COSY sequence optimized for small couplings.[4] While it is gratifying that correlation is at all possible in such cases of unresolved couplings, it is the purpose of this work to show that homonuclear deuterium correlation—*as well as relayed correlation*—can be performed with surprisingly high efficiency, far superior to the optimized COSY experiment.

The basis of our approach is coherence transfer under spin lock,[5,6,7] also known as isotropic mixing, TOCSY, or HOHAHA. *Net in-phase* transfer occurs under isotropic mixing (IM), in contrast to the *basic* INEPT[8] or DEPT[9] mechanism that leads to *anti-phase* transfer of coherence. While IM leads to a maximum transfer of 100% for a two-spin-$^1/_2$ system, which is equivalent to a spin permutation,[10] such is not the case for other systems. One of us (N.C.) has investigated[11,12] in detail the coherence transfer under isotropic mixing in several general spin-$^1/_2$ systems. The results for the spin-$^1/_2$ A_2X_2 system may be adapted to the two-spin-1 problem. It is possible to show by this means or by spin-1 commutator algebra that transverse magnetization of one of the coupled spins in this system goes over into the following observable terms under IM:

$$I_x \rightarrow \frac{1}{2}(1 + \cos 4\pi Jt)I_x + \frac{1}{2}(1 - \cos 4\pi Jt)S_x + \frac{1}{3}[(1 - \cos 2\pi Jt) - (1 - \cos 4\pi Jt)](I_x^2S_x - I_xS_x^2) + \frac{1}{6}\left(\sin 2\pi Jt + \frac{5}{2}\sin 4\pi Jt\right)(I_yS_z - I_zS_y) \quad (1)$$

Ignoring the anti-phase term, eq 1 predicts that the maximum in-phase coherence transfer efficiency is 77.78% and occurs at a mixing time $t = (4J)^{-1}$. This may be compared with the maximum efficiency for this system under the INEPT mechanism, which amounts to 66.67%[13,14] anti-phase, also for a duration of $(4J)^{-1}$. This result is easy to visualize since the $m_S = 0$ component of the I-spin "multiplet" does not evolve under weak coupling and therefore cannot be transferred: at most two-thirds of the magnetization in the I signal can be transferred under INEPT. Under isotropic mixing, on the other hand, the $m_S = 0$ component *does* evolve under the strong coupling induced by spin lock, leading to a higher maximum efficiency of transfer. Most significant, however, from the practical point of view is that the transfer under

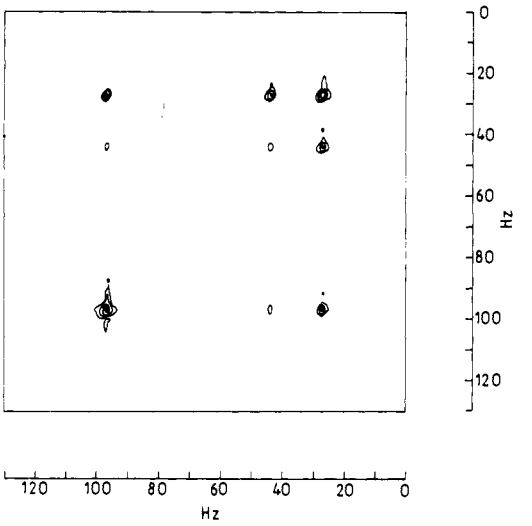

Figure 1. Two-dimensional ^{2}H–^{2}H magnitude mode TOCSY spectrum of pyridine-d_5 at 46.073 MHz, acquired in a double-buffered mode. Spectral width, 130 Hz, 64 experiments of 16 scans each; spin lock rf field strength, 1.5 kHz, the spin lock train being issued from the variable phase and amplitude channel of spectrometer modulator. Data processed with shifted sine bell window functions in both dimensions. Mixing time: 1.248 s. The experiments were performed on a Bruker MSL 300 P spectrometer with no hardware designed for high-resolution deuterium work. A 5-mm inverse probe was employed, using the X-nucleus decoupling coil for deuterium excitation, spin lock, and signal detection; field–frequency lock was *not* employed, and the experiments were performed without sample spinning.

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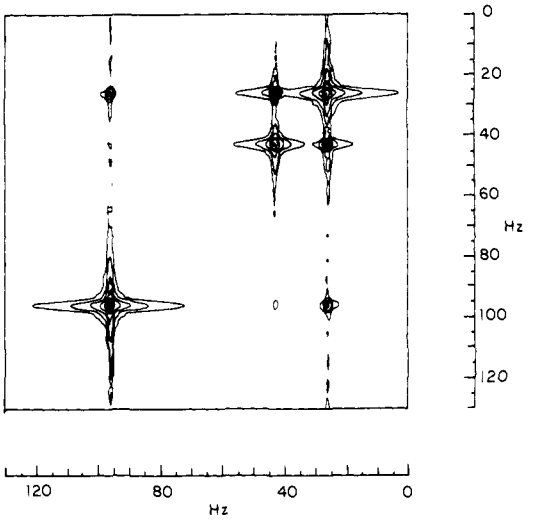

Figure 2. Two-dimensional ^2H-^2H magnitude mode optimized COSY spectrum of pyridine-d_5 under identical conditions. Optimal mixing time: 1.056 s.

spin lock is *in-phase*, while the transfer under INEPT, being *anti-phase*, would be partially cancelled out in the resulting unresolved multiplet.

These ideas were investigated on several perdeuterated molecules, including ethanol-d_6, THF-d_8, and pyridine-d_5. In each case, a neat sample was run; a COSY experiment optimized for small couplings was compared with a TOCSY experiment employing a WALTZ-16[15,16,17] mixing sequence, with the radio frequency (rf) field strength for mixing being in the range 1.5–2.5 kHz. It may be noted that the rf power employed during spin lock is typically between 0.5 and 1.0 W, which leads to no detectable heating at sensible repetition rates. The relative efficiencies of the laboratory-frame COSY experiment and the rotating-frame TOCSY experiment were quantified both with respect to the absolute intensities of the diagonal and cross peaks, as well as to the ratio of diagonal to cross peak intensities. In each case, both of these parameters were decidedly more favorable for the TOCSY experiment, typically by a factor of between 2 and 3 as expected from the theory outlined above.

Figure 1 shows the TOCSY spectrum of pyridine-d_5, for which the calculated magnitudes of the couplings[3,18] are as follows: J_{23} = 0.115 Hz, J_{34} = 0.181 Hz, and J_{24} = 0.045 Hz. While the data of Figure 1 were acquired in about 85 min, a run could in fact be performed in just about 10 min with no significant degradation of spectral quality. It is remarkable that the relay mechanism inherent in the spin lock experiment[5,6,7] results in a clear 2–4 cross-peak, although this coupling is less than 0.05 Hz! From the flip angle dependence of selective 1D pulsed spin lock experiments, we estimate that ROESY contributions to the cross-peak intensity under our conditions amount to less than 2%. This spectrum may be compared against the optimized COSY spectrum of Figure 2, displayed at a 4-fold intensity magnification with respect to Figure 1; in fact, both our optimized COSY and RELAY runs not only have much inferior sensitivity, but lead to a mere suspicion of the 2–4 cross-peak.

Given the fact that deuterium correlation spectroscopy exhibits no 2D spectral complexity owing to multiplet structure and given the further circumstance that isotropic mixing would be almost ideally behaved for this isotope, whose chemical shift range is just about 925 Hz on a 600-MHz machine, we believe that, the limited shift resolution notwithstanding, TOCSY studies on the perdeuterated species would be a most interesting possibility for structure elucidation of biomolecules, line widths permitting.

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Identification and Derivatization of (Oligosaccharyl)amines Obtained by Treatment of Asparagine-Linked Glycopeptides with N-GLYCANASE Enzyme

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The initial steps in the structural analysis of the asparagine (Asn)-linked oligosaccharides of a glycoprotein generally consist of chemical[1] or enzymatic[2] hydrolysis of the *N*-acetylglucosaminylasparagine bond between each oligosaccharide chain and the polypeptide, derivatization of the released oligosaccharide chains with a radioactive or fluorescent tag, and chromatographic fractionation of the labeled oligosaccharides. The purified oligosaccharides can be further characterized by ^1H NMR spectroscopy, mass spectroscopy, methylation analysis, and exoglycosidase digestions. Current methods for labeling the released oligosaccharides involve derivatization of the hemiacetal moiety at the reducing terminus of the sugar chain, for example, by reduction with sodium borotritide[1] or reductive amination with a chromophore such as 2-aminopyridine[3] (Scheme I, pathway b). We report here an alternative approach to labeling Asn-linked oligosaccharides that is based on our observation that treatment of glycopeptides with a peptide-N^4-(*N*-acetyl-β-glucosaminyl)-asparagine amidase (PNGase, EC 3.5.1.52) generates a set of (oligosaccharyl)amine intermediates that can be derivatized with electrophilic reagents (Scheme I, pathway a).

PNGase from almond emulsin (PNGase A) has been shown to be an amidase by ^1H NMR spectroscopic experiments.[4] The preferred PNGase for releasing Asn-linked oligosaccharides from glycoproteins is obtained from *Flavobacterium meningosepticum* (PNGase F, N-GLYCANASE enzyme).[5] PNGase F has a broader substrate specificity, a lower molecular weight (35 100 vs 68 000 Da), and a higher pH optimum (8.5 vs 5.0) than PNGase A.[6] It also seems likely that PNGase F would be a superior

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