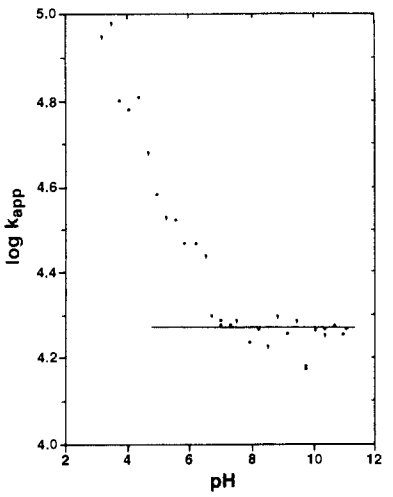

Figure 3. LRET rate constant dependence on pH. Since azide is an acid at low pH, tryptophan oxidation was accomplished with the dibromide radical, $Br_2^{\bullet-}$ formed in the same way as $N_3^{\bullet}$. The solute concentrations were peptide ca. 100 μM, Br^- 0.1 M, and phosphate buffer 5 mM, with all solutions saturated by N_2O and at 25 °C. The concentration of $Br_2^{\bullet-}$ generated by the pulse was 1 μM. The solution pH was adjusted up from pH 7 with N_2O-saturated NaOH, and down from pH 7 with N_2O-saturated HCl. The horizontal line drawn through the points is the average of the included rate constants.

are collected in Table I. As seen in Figure 2, the rate constants for the series TyrOH-$(Pro)_n$-TrpH, with the exception of the dipeptide TyrOH-TrpH, fall off exponentially with distance; the rate constants for the reversed series TrpH-$(Pro)_n$-TyrOH show the same dependence, but with a different apparent slope β. Assuming that each added proline separates the two aromatic amino acids by an additional 3.1 Å, we estimate β values of 0.23 $Å^{-1}$ and 0.37 $Å^{-1}$ for TyrOH-$(Pro)_n$-TrpH and TrpH-$(Pro)_n$-TyrOH, respectively. The result of this difference is a crossover in rate constants; while LRET is faster in TrpH-Pro-TyrOH than in TyrOH-Pro-TrpH, the reverse holds for the longest peptides. Our previous experiments suggest that the redox potential difference, $\Delta E'$, between the $Trp^{\bullet}$/TrpH and the $TyrO^{\bullet}$/TyrOH couples does not change with chain length,[4] eliminating this as a possible explanation of these unique results.

Although there is a net proton transfer in reaction 2, we had argued previously that this proton transfer cannot be rate determining at pH 7.0.[4] We now have additional data to support that kinetic argument; (i) in the TrpH-$(Pro)_n$-TyrOH series the observed rate constant decreases by a factor of approximately 300 from dipeptide to heptapeptide; and (ii) the electron transfer rate in TrpH-Pro-TyrOH is independent of pH from ca. 6.5 to 11 (Figure 3), although k_{app} does increase below 6.[12]

Might a "chain-end" effect explain the observed difference in β? For example, TyrOH and TrpH at the peptide chain ends interact electrostatically with the N-terminal ammonium and C-terminal carboxylate to affect electron transfer differentially. But there is no chain-end effect, for the LRET rate constants are unchanged with lysine addition to both the C- and N-terminal ends of TrpH-Pro-TyrOH and TyrOH-Pro-TrpH (Table I and Figure 2). Our previous conjecture on a correlation between the magnitude of β and the equilibrium redox potential difference, $\Delta E'$, is also inconsistent with these results. From equilibrium absorbances, such as those in Figure 1, we have estimated $\Delta E'$ for Lys-Tyr-Pro-Trp-Lys and Lys-Trp-Pro-Tyr-Lys of 41 and 61 mV, respectively; the same within experimental error.

In the absence of other alternatives, we conclude that just the order of the TyrOH and TrpH residues in the asymmetric polypeptide chain may be responsible for the kinetic differences between the two peptide series. One possibility is based on the presumably limited number of orientations assumed by the TrpH indole side chain.[13] A hindered side chain would result in a geometric change when TrpH is moved between C- and N-terminal locations. Since Sakata et al.[14] have shown that electron-donor and -acceptor orientations can affect LRET rates, any geometric difference could result in "absolute" rate differences. However, insofar as the TrpH indole conformation should be largely insensitive to the number of prolines in the middle of the chain, this argument cannot explain the observed "relative" difference in β. Any other "absolute" effect is ruled out for the same reason. That the direction of electron transfer in both sets of peptides is from the TyrOH to the TrpH side chain is the basis of a second possible explanation. The difference in the magnitude of β may be due simply to the fact that net electron transfer is from N- to C-terminal in one peptide series and C- to N-terminal in the other. If this suggestion of a directional specificity is correct, then it would be reasonable for this LRET to be through-bond, not through-space. With respect to the low values of β reported here, we note the recent suggestion that through-bond processes should be less sensitive to distance than through-space processes.[15]

The observation of LRET in proteins leads to speculation about evolutionary pressures to direct electron transfer along specific paths and between specific protein groups. Currently, there are hints of such LRET specificity.[16] Our results are the first to suggest that molecular ordering might be one mechanism to establish a path specificity. The observation[14] that electron-donor and -acceptor orientations also affect the LRET rate suggests a second mechanism. The physiological significance of either is presently untested.

(12) Bobrowski and co-workers also observed a faster reaction at an unspecified acid pH (personal communication).

(13) Wells, G. B.; Mustafi, D.; Makinen, M. W. *J. Am. Chem. Soc.* **1990**, *112*, 2566–2574.

(14) Sakata, Y.; Nakashima, S.; Goto, Y.; Tatemitsu, H.; Misumi, S.; Asahi, T.; Hagihara, M.; Nishikawa, S.; Okada, T.; Mataga, N. *J. Am. Chem. Soc.* **1989**, *111*, 8979–8981.

(15) Falcetta, M. F.; Jordan, K. D.; McMurry, J. E.; Paddon-Row, M. N. *J. Am. Chem. Soc.* **1990**, *112*, 579–586.

(16) Steiner, J. P.; Faraggi, M.; Klapper, M. H.; Dorfman, L. M. *Biochemistry* **1985**, *24*, 2139–2146. Liang, N.; Pielak, G. J.; Mauk, A. G.; Smith, M.; Hoffman, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 1249–1252. Michel-Beyerle, M. E.; Plato, M.; Deisenhofer, J.; Michel, H.; Bixon, M.; Jortner, J. *Biochim. Biophys. Acta* **1988**, *932*, 52–70.

Kinetics of a 1,3-CH Carbene Insertion Reaction: *tert*-Butylchlorocarbene

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Last year, intensive activity was focused on the kinetics of *intramolecular* carbene reactions. This attention was largely restricted to the characteristic[1] 1,2-H[2] and 1,2-C[2f,3] shifts (or "insertions") that result in alkene formation. Much of this work depended on laser flash photolysis (LFP) of diazirine precursors to generate the carbenes that were subsequently monitored

(1) (a) Kirmse, W. *Carbene Chemistry*, 2nd ed.; Academic Press: New York, 1971; Chapter 12. (b) Jones, M., Jr., Moss, R. A., Eds. *Carbenes*; Wiley: New York, 1973; Vol. I, Chapter 1. (c) Regitz, M., Ed. *Methoden der Organischen Chemie* (Houben-Weyl); G. Thieme Verlag: Stuttgart, 1989; Vol. E19b.

(2) (a) Stevens, I. D. R.; Liu, M. T. H.; Sundararajan, N.; Paike, N. *Tetrahedron Lett.* **1989**, *30*, 481. (b) Bonneau, R.; Liu, M. T. H.; Suresh, R. V. *J. Phys. Chem.* **1989**, *93*, 4802. (c) Liu, M. T. H.; Bonneau, R. *J. Am. Chem. Soc.* **1989**, *111*, 6873. (d) Jackson, J. E.; Soundararajan, N.; White, W.; Liu, M. T. H.; Bonneau, R.; Platz, M. S. *Ibid.* **1989**, *111*, 6874. (e) La Villa, J.; Goodman, J. L. *Ibid.* **1989**, *111*, 5973.

(3) (a) Ho, G.-J.; Krogh-Jespersen, K.; Moss, R. A.; Shen, S.; Sheridan, R. S.; Subramanian, R. *J. Am. Chem. Soc.* **1989**, *111*, 6875. (b) Liu, M. T. H.; Bonneau, R. *J. Phys. Chem.* **1989**, *93*, 7298. (c) Moss, R. A.; Ho, G.-J.; Shen, S.; Krogh-Jespersen, K. *J. Am. Chem. Soc.* **1990**, *112*, 1638. (d) Moss, R. A.; Ho, G.-J. *Tetrahedron Lett.* **1990**, *31*, 1225.

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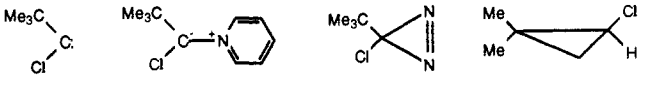
spectroscopically. Often, however, the carbenes were "invisible" (i.e., lacked a sufficiently intense UV absorption). Then, the elegant methodology of Platz et al. was applied, whereby the carbene was intercepted by pyridine to form an ylide, and the time-dependent absorption of the latter was used to indirectly monitor the kinetics of the carbene.[4]

The ylide technique has proven essential to the elucidation of the absolute kinetics of intramolecular carbene reactions, particularly the 1,2-H and 1,2-C shifts,[2,3] However, the third principal class of such reactions, 1,3-CH insertion,[1] is still unrepresented by a definitive study. Platz reported that *tert*-butylchlorocarbene (**1**) reacted with pyridine (pyr) in toluene to form ylide **2** (λ_{max} ~376 nm, $k \sim 2.4 \times 10^9$ M^{-1} s^{-1}) and that a (linear) correlation of the observed pseudo-first-order rate constants for the growth of **2** vs [pyr], extrapolated to [pyr] = 0, gave $k_0 \sim 1.1 \times 10^7$ s^{-1}, a rate constant that represented the *sum* of all first-order and pseudo-first-order decay channels of **1** in the absence of pyr.[4] These competitive pathways included *intermolecular* reactions of **1** with its diazirine precursor (**3**)[5] and with solvent, as well as *intramolecular* 1,3-CH insertion (to cyclopropane **4**) and 1,2-Me shift (to alkene **5**).



1 2 3 4

$Me_2C{=}CMeCl$ $Me_3CCCl{=}N{-}N{=}CClCMe_3$

5 6

Indeed, these results[4] are illustrative of a *general* restriction in the applicability of the ylide methodology: whenever a carbene's intramolecular reactions are "slow" ($k \sim 10^5$–10^6 s^{-1}), azine formation and other intermolecular reactions may contribute to k_0, and the ylide technique will not afford an accurate measure of the intramolecular kinetics. Azine formation is therefore a warning that the ylide method cannot be used in its simplest guise.

Now we demonstrate that a double extrapolation can suppress the importance of azine formation, allowing the extension of the ylide method to the determination of k_d, representing the intramolecular decay of **1** to **4** and **5**. Careful product studies then permit the partition of k_d into k_{ins} and k_{Me} for the 1,3-CH insertion and 1,2-Me migration, respectively. These represent the first absolute rate measurements of carbene 1,3-CH insertion and 1,2-methyl migration reactions. Moreover, the technique should be applicable to other "slow" intramolecular carbene reactions, enlarging the usefulness of the ylide kinetic methodology. Finally, the temperature dependence of the **4**/**5** distribution provides differential activation parameters that indicate an enthalpic advantage for the methyl migration that is opposed by entropic factors favoring CH insertion, further evidence that 1,2-carbenic rearrangements are strongly disfavored entropically.[3c] *tert*-Butylchlorocarbene (**1**) was generated in pyridine/isooctane solutions by LFP[6] of diazirine **3**.[5,7] The UV absorption of ylide **2** was readily detected at 376 nm.[4] We then selected five different initial concentrations of diazirine **3** (A_{362} = 0.800, 0.655, 0.553, 0.321, or 0.201). For each initial [**3**], we performed LFP experiments (293 K) at five different concentrations of pyr (0.31–1.6 mM in isooctane) and correlated the observed pseudo-first-order rate constants for the growth of ylide **2** with [pyr]. The resulting five correlations were linear and *parallel*, with slopes (k_f for ylide formation) of [(4.1–4.3) ± 0.2] × 10^9 M^{-1} s^{-1} (see Figure 1). Extrapolated to [pyr] = 0, these correlations gave five values for

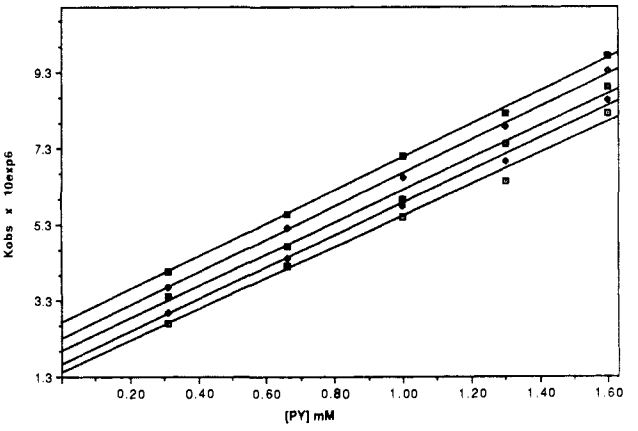


Figure 1. Pseudo-first-order rate constants (k_{obsd}, s^{-1}) for the LFP-initiated formation of ylide **2** from carbene **1** and pyridine vs [pyr] (mM). Each correlation was obtained at a different initial concentration of diazirine **3**; see text. The y intercepts (k_0), in order of decreasing [**3**], are 2.71 ± 0.10, 2.29 ± 0.13, 1.96 ± 0.02, 1.61 ± 0.15, and 1.41 ± 0.26 (all are ×10^6, s^{-1}). Correlation coefficients ranged from 0.997 to 0.999.

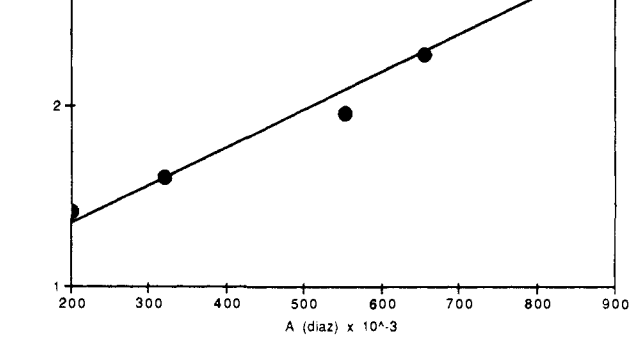


Figure 2. Extrapolated k_0 values (s^{-1}) for the formation of ylide **2** from carbene **1** and pyridine at [pyr] = 0 (see Figure 1) vs $1000A_{362}$, where A is the initial absorbance of diazirine **3** in the experiments used to obtain each k_0. The y intercept is k_d = (9.3 ± 1.1) × 10^5 s^{-1}; see text.

k_0 that decreased from 2.71 × 10^6 s^{-1} (for A_{362} = 0.800) to 1.41 × 10^6 s^{-1} (A_{362} = 0.201).

The principal *intermolecular* pathway for the decay of **1** is reaction with **3** to produce azine **6** (see ref 5 and below), so that we next correlated the extrapolated k_0 for each initial [**3**] with the corresponding A_{362} (representing [**3**]). This correlation (Figure 2) was also linear (r = 0.986), with an intercept at A_{362} (or [**3**]) = 0 of (9.3 ± 1.1) × 10^5 s^{-1}, which we take as k_d for the *intermolecular* decay of carbene **1** to products **4** and **5**.

Photolysis of diazirine **3** in $CDCl_3$ (A_{352} = 1.0, 200-W Xe UV lamp, Pyrex filter, 25 °C, 2 h) gave a product mixture that was examined by 200-MHz ^1H NMR spectroscopy. The diazirine (δ 0.93) was gone and replaced by **4**[5,8] (Me's at δ 1.22 and 1.06), **5**[9] (Me's at δ 2.06, 1.80, and 1.70), and azine **6** (δ 1.32; m/e 237, 239, 241 MH^+). These were the only major products. Importantly, the percent intramolecular reaction [(**4** + **5**)/(**4** + **5** + **6**)] increased from 36 to 84% as the [diazirine] was reduced from A_{362} = 1.2 to A_{362} = 0.21. This behavior is in accord with the extrapolation shown in Figure 2. Similar photolyses of **3** in *n*-octane (A_{362} = 1.2) were then carried out at nine different temperatures, 251 ≤ T ≤ 328 K,[10] and the resultant **4**/**5** ratios (ranging from 0.85 ± 0.02 to 4.24 ± 0.02, respectively) were determined by SE-30 capillary GC using a calibrated flame-ionization detector. An Arrhenius correlation of ln **4**/**5** vs $1/T$ (not shown) gave ΔE_a [=E_a (CH insertion) − E_a (Me shift)] =

(4) Jackson, J. E.; Soundararajan, N.; Platz, M. S.; Liu, M. T. H. *J. Am. Chem. Soc.* **1988**, *110*, 5595.
(5) Moss, R. A.; Munjal, R. C. *J. Chem. Soc., Chem. Commun.* **1978**, 775.
(6) For a description of our LFP system, see: Moss, R. A.; Shen, S.; Hadel, L. M.; Kmiecik-Ławrynowicz, G. Włostowska, J.; Krogh-Jespersen, K. *J. Am. Chem. Soc.* **1987**, *109*, 4341.
(7) Graham, W. H. *J. Am. Chem. Soc.* **1965**, *87*, 4396. Diazirine **3** was distilled at 25 °C/1 mmHg from a decane solution, through a cold trap (−40 °C), into isooctane, $CDCl_3$, or some other desired solvent.
(8) Closs, G. L.; Closs, L. E. *J. Am. Chem. Soc.* **1960**, *82*, 5723.
(9) Frey, H. M.; Liu, M. T. H. *J. Chem. Soc. A* **1970**, 1916.
(10) The half-time for *thermal* decomposition of **3** at *358 K* is ~6 h.

3.4 ± 0.3 kcal/mol and ln (A_{ins}/A_{Me}) = 6.8 ± 0.5, with r = 0.979, 99.9% confidence level, for nine points. From the ratio of the preexponential factors, $\Delta S^{\ddagger}_{ins} - \Delta S^{\ddagger}_{Me}$ = 13.5 eu. If we correct for a 3-fold statistical advantage of insertion (nine CH) over methyl migration (three Me's) in **1**, $\Delta\Delta S^{\ddagger} \sim 11$ eu.[11]

The GC-determined product ratio of **4**/**5** at 293 K (which we take as k_{ins}/k_{Me}) is 2.92. Recalling that k_d ($=k_{ins} + k_{Me}$) = 9.3 × 10^5 s^{-1} at 293 K (see above), we obtain k_{ins} = 6.9 × 10^5 s^{-1} and k_{Me} = 2.4 × 10^5 s^{-1} for the competitive 1,3-CH insertion and 1,2-Me shift reactions of *tert*-butylchlorocarbene at 20 °C. We estimate errors of 10–15% in these values.

The 1,3-CH insertion and 1,2-Me migration of **1** are "slow" intramolecular carbene reactions. Thus, reported rate constants at ambient temperatures for 1,2-H shifts range from ≥10^8 s^{-1} for Me_2CHCCl[2f] to (1–3) × 10^6 s^{-1} for MeCCl,[2c,e] and the rate constant for the 1,2-C shift of cyclopropylchlorocarbene to chlorocyclobutene is variously reported as 3.8 × 10^5 s^{-1} [2f,3b] or (8–9) × 10^5 s^{-1}.[3a,c] More importantly, the excess of 1,3-CH insertion (to **4**) over 1,2-Me migration (to **5**), which increasingly obtains above −15 °C, is entropically controlled. Although Me migration is favored over 1,3-CH insertion by $\Delta\Delta H^{\ddagger} \sim 2.8$ kcal/mol, this is more than compensated by the favorable differential entropy of activation attending the insertion (13.5 eu ~ 4 kcal/mol at 293 K).

Our extrapolative methodology is not sufficiently precise to produce absolute activation parameters for these reactions, but the observed entropic disadvantage of 1,2-Me migration is consonant with the very unfavorable entropy of activation (~−20 eu) that attends the 1,2-C shift of cyclopropylchlorocarbene.[12] We are continuing our studies of intramolecular carbene reactions.

Acknowledgment. We are grateful to Professor H. D. Roth for a helpful discussion and to the National Science Foundation for financial support.

(11) Although there are nine equivalent C–H bonds and three equivalent C–Me bonds in **1**, conformations of the carbene where insertion or methyl migration are likely to occur present a 1:1 weighting of pathways.

(12) See ref 3c for a discussion of unfavorable activation entropy in a carbene 1,2-rearrangement.

Simplification of DNA Proton Nuclear Magnetic Resonance Spectra by Homonuclear Hartmann–Hahn Edited Two-Dimensional Nuclear Overhauser Enhancement Spectroscopy

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Two-dimensional ^{1}H NMR spectra of DNA fragments in solution supply a wealth of structural information. The first step in establishing the molecular structure using NMR spectroscopy is the assignment of protons to a particular nucleotide residue. Sequential assignment procedures using a combination of NOESY[1] and COSY[2] (as well as other coherence transfer experiments) have been developed which allow straightforward assignment of the base and most of the sugar protons in short DNA oligonucleotides which adopt a right-handed A- or B-DNA type structure.[3] However, the sequential assignment technique can fail for unusual DNA structures where some of the bases are syn rather than anti such as Z-DNA[4] and some drug–DNA complexes,[5] and in more complicated structures containing non-Watson–Crick base pairs such as DNA triplexes.[6] Spectral overlap in the base–H1′ and base–H2′,H2″ region of the NOESY spectra may also limit assignments in these and in longer B-DNA duplexes.

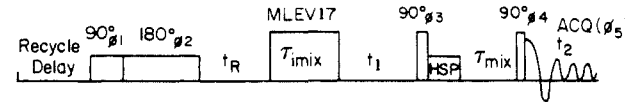
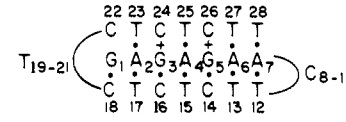


Figure 1. Pulse scheme of the HOENOE experiment. The selective 90° and 180° pulses, MLEV17[12b] isotropic mixing, and the nonselective 90° mixing pulses are applied with the carrier positioned in the center of the CH5 region. The nonselective 90° t_2 acquisition pulse can be positioned anywhere in the spectrum. The refocusing delay is $t_r = (2/\pi)t_{90}$, where t_{90} is the length of the selective 90° pulse. In order to minimize the required phase cycle extended by the necessity to apply the EXORCYCLE[8] for the selective excitation in the preparation period, a homospoil pulse is applied during the NOE mixing time. The phase cycle used is as follows, with all the elements phase cycled with CYCLOPS after eight scans: $\phi_1 = x$; $\Phi_2 = x, y, -x, -y$; $\phi_3 = -x, x, -x, x, x, -x, x, -x$; $\phi_4 = x$; $\phi_5 = x, x, x, x, -x, -x, -x, -x$. To obtain hypercomplex pure-phase 2D spectra,[16] the phase of ϕ_3 is changed by 90° for odd and even scans and the data are stored in two separate memory locations, resulting in a 32 × 2 step phase cycle.

Scheme I



In order to simplify the NOESY spectra of DNA oligonucleotides for assignment purposes, we present an experiment that enables one to selectively trace the NOE connectivities of cytidine H6 resonances. This approach substantially simplifies the analysis of a 2D NOE spectrum in the base to sugar proton regions and allows straightforward identification of corresponding resonances. The HOENOE (two-dimensional *HO*HAHA[7] *e*dited *NOE* spectroscopy) experiment is based on selective excitation of cytidine H6 protons via in-phase coherence transfer from the scalar-coupled cytidine H5 protons prior to the t_1 evolution period of a regular 2D NOE experiment.[8]

The pulse scheme of the HOENOE experiment is shown in Figure 1. During the preparation period a selective excitation pulse sequence is applied in the region of interest, which for our application is between 5.3 and 6.5 ppm where the CH5 as well as the sugar H1′ resonances appear. For simplicity, we used a selective spin echo excitation in which a selective 90° pulse is applied, followed by a selective 180° pulse and a short refocusing period t_r. This pulse scheme, with the 180° pulse phase cycled through EXORCYCLE,[9] gives a pure-phase, highly selective excitation of frequencies $\pm 1/3t_{90}$ from the carrier with an excitation profile that is approximately $(\sin \pi x)/\pi x$ for $-1 < x < 1$. For frequencies outside this range the excitation is negligible. The

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(1) (a) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95–117. (b) Kumar, A.; Ernst, R. R.; Wüthrich, K. *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1–6.

(2) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246.

(3) (a) Scheek, R. M.; Russo, N.; Boelens, R.; Kaptein, R. *J. Am. Chem. Soc.* **1983**, *105*, 2914–2916. (b) Hare, D. R.; Wemmer, D. E.; Chous, S.-H.; Drobny, G.; Reid, B. R. *J. Mol. Biol.* **1983**, *171*, 319–336. (c) Feigon, J.; Leupin, W.; Denny, W. A.; Kearns, D. R. *Biochemistry* **1983**, *22*, 5843–5951. (d) Chazin, W. J.; Wüthrich, K.; Rance, M.; Hyberts, S.; Denny, W. A.; Leupin, W. *J. Mol. Biol.* **1986**, *190*, 439–453.

(4) Feigon, J.; Wang, A. H.-J.; van der Marel, G. A.; van Boom, J. H.; Rich, A. *Nucleic Acids Res.* **1984**, *12*, 1243–1263.

(5) Gilbert, D. E.; van der Marel, G. A.; van Boom, J. H.; Feigon, J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3006–3010.

(6) (a) Rajagopal, P.; Feigon, J. *Nature* **1989**, *339*, 637–640. (b) Rajagopal, P.; Feigon, J. *Biochemistry* **1989**, *28*, 7859–7870.

(7) Davis, D. G.; Bax, A. *J. Am. Chem. Soc.* **1985**, *107*, 7197–7198.

(8) This pulse sequence is significantly different in application from the related nonselective TOCSY-NOESY experiment of Kessler et al. (*Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 564–566) in that it simplifies the spectrum rather than increasing the complexity.

(9) Bodenhausen, G.; Freeman, R.; Turner, D. L. *J. Magn. Reson.* **1977**, *27*, 511–514.

0002-7863/90/1512-5644$02.50/0