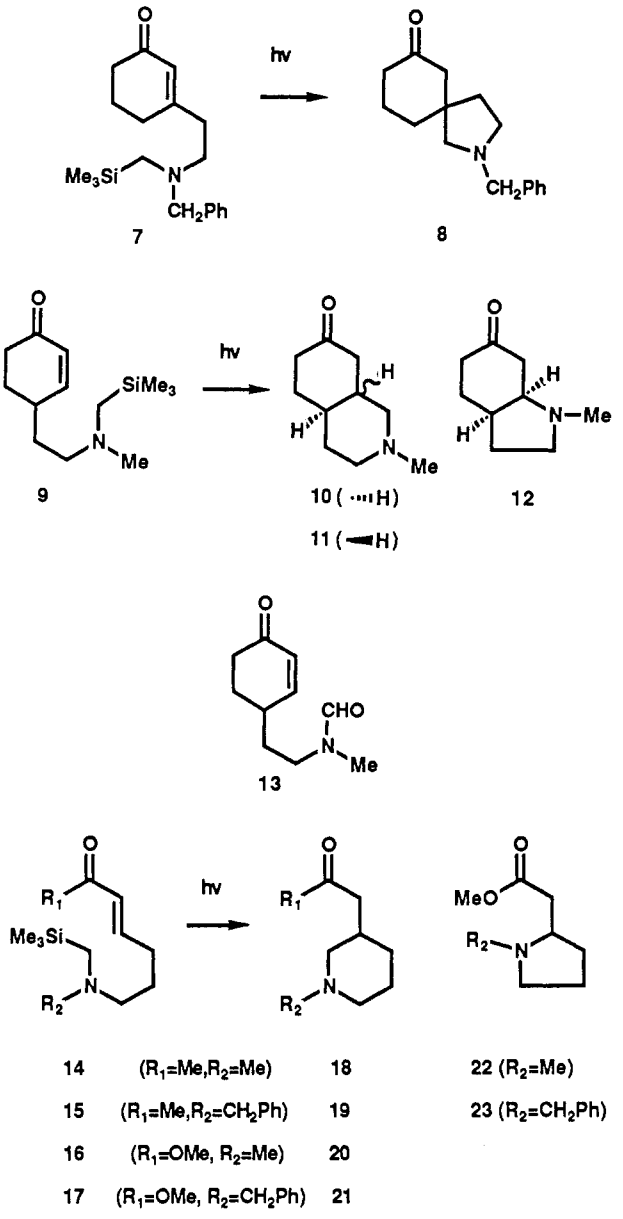
involves SET from the DCA anion radical to the α-keto radical **24** followed by enolate protonation.



A number of additional features of the reactions described above remain to be explored.[15] Encouragement to continue these efforts derives from the anticipated synthetic potential of these photocyclization processes.

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(14) (a) The rates of alkyl radical addition to mono-substituted alkenes depend on the nature of the alkene substituents. For example, the addition rates to CH_2=$CHCOCH_3$ are normally double those to CH_2=$CHCO_2CH_3$ (ref 14b). Importantly, simple α-amino alkyl radicals are electron rich (high-energy SOMO) and thus should add to electron poor olefins more efficiently. This should be contrasted to the chemistry of α-amido alkyl radicals which add intramolecularly to simple alkene groupings (ref 14c). (b) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *764*, 763 and references therein. (c) Hart, D. J.; Tasi, Y. M. *J. Am. Chem. Soc.* **1982**, *104*, 1430. Burnett, D. A.; Choi, J. K.; Hart, D. J.; Tsai, Y. M. *Ibid.* **1984**, *106*, 8201.

(15) For example, the comparative lack of and modest stereoselectivity of the respective direct and SET-sensitized photocyclizations of **9** needs to be addressed.

Line Narrowing of Amide Proton Resonances in 2D NMR Spectra of Proteins

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Structure determination of proteins by NMR relies on the determination of through-bond (COSY-type)[1] and through-space (NOESY-type)[2] connectivities.[3] Problems in applying this strategy to proteins larger than about 15 kD arise from the increased number of resonances and the increased line width of ^{1}H resonances, resulting in severe overlap in 2D NMR spectra. Moreover, the increase in ^{1}H line width causes partial cancellation of signal within COSY-type cross multiplets, severely affecting the sensitivity associated with this crucial step in the assignment procedure. Previous attempts to narrow proton resonances by applying random[4] or complete[5] deuteriation of the nonexchangeable protons resulted in a substantial line narrowing of the remaining protons. However, this approach offers only a modest narrowing of the relatively broad amide backbone resonances.

Here, we demonstrate an alternative approach for obtaining line narrowing of the amide protons, based on the removal of the N–H heteronuclear dipolar relaxation mechanism. For both ^{14}N and ^{15}N labeled proteins, the heteronuclear dipolar coupling has a major effect on ^{1}H transverse relaxation; replacing ^{14}N by ^{15}N reduces the heteronuclear dipolar broadening contribution by only 26%.[6] This is to be contrasted with the case of small peptides, where scalar relaxation of the second kind[7] makes a major contribution to the line width of protons attached to ^{14}N.[8] Labeling with ^{15}N permits the efficient generation of heteronuclear zero- and double-quantum coherences,[9-12] which have relaxation rates that, to first order, are not affected by the heteronuclear dipolar coupling.[12,13] Hence, multiple quantum resonances can be significantly narrower than the corresponding amide ^{1}H resonances. The ^{15}N chemical shift contribution is easily removed from the multiple quantum frequency, yielding spectra that are similar in appearance to regular ^{1}H spectra, apart from line narrowing of the amide protons.

The new approach is demonstrated for the COSY experiment. The pulse scheme of this so-called pseudo-single quantum COSY (PS COSY) experiment is sketched in Figure 1. Because the pseudo-single quantum has to be present during the t_1 period, the C$^\alpha$H resonances have to be detected during t_2, necessitating the use of presaturation to suppress the H_2O resonance. To avoid excitation by the mixing pulse of H_2O that recovers during the evolution period, mixing is accomplished with a combination of selective and nonselective pulses.[14] Linearly frequency-dependent phase errors in the F_1 and F_2 dimensions, caused by the relatively long initial values of t_1 (8 ms) and t_2 (4 ms), can be calculated exactly and are easily corrected.[15]

Figure 2 shows a comparison of sections of the fingerprint (NH–C$^\alpha$H) regions of the COSY spectra of a staphylococcal nuclease/pdTp/calcium complex (18 kD). Both spectra have been recorded at 600 MHz ^{1}H frequency, with acquisition times of 74 and 100 ms in the t_1 and t_2 dimensions, respectively. Measuring times were 17 h per spectrum. Sine bell digital filtering (10° phase shifted) has been used in both dimensions, and data have been zero filled to give a digital resolution of 3.3 Hz in both dimensions. As expected, the sensitivity of the PS COSY experiment is im-

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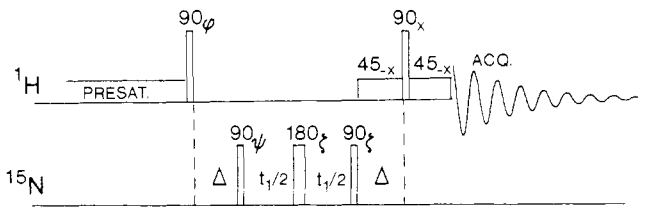

Figure 1. Pulse scheme of the PS COSY experiment. The duration of the delay, Δ, is set to 4 ms, slightly shorter than $1/(2J_{NH})$. The phase cycling used is as follows: $\phi = x, -x, \psi = 2x, 2(-x), \zeta = 4x, 4(-x)$, acquisition $x, -x, -x,x, -x,x,x,-x$. The phase ϕ is incremented by 90° for successive t_1 increments (TPPI).[16] The carrier frequency is positioned at the H_2O frequency, and presaturation of the H_2O resonance is essential. The low power pulses 45_{-x} are of duration Δ, and the rf power is adjusted such that they correspond to 45° pulses on resonance. Note that in general adjustment of the phase of the low power pulse relative to the high power pulse will be necessary because the change in power results in an unpredictable constant phase shift.

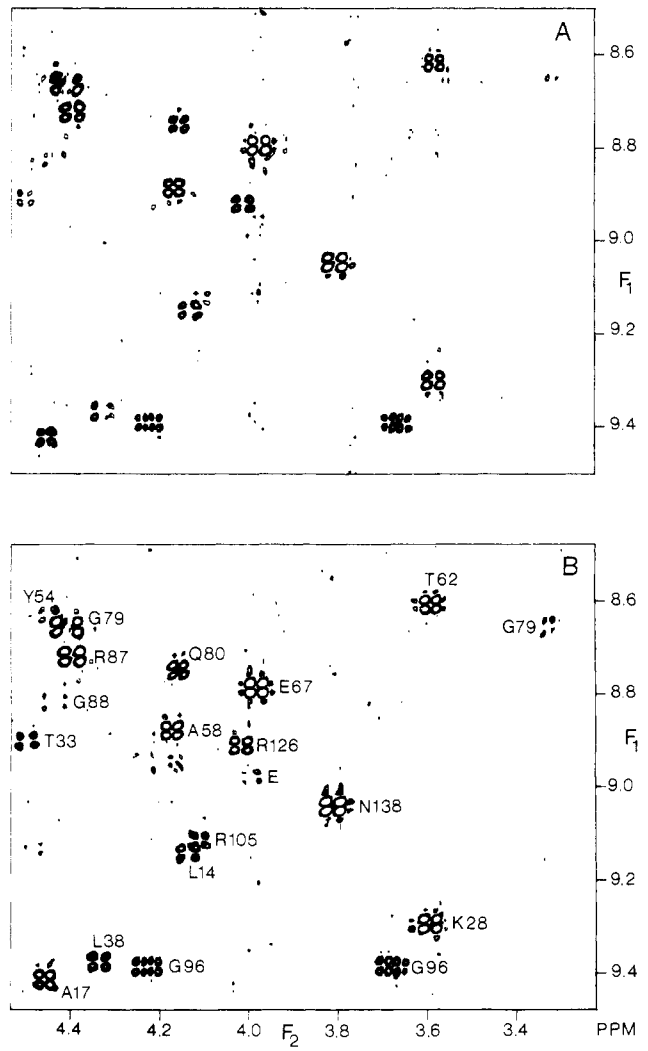

Figure 2. A comparison of two sections of the fingerprint (NH–C$^\alpha$H) region of a 1.5 mM staphylococcal nuclease/pdTp/calcium complex in 90%H_2O/10%D_2O, 100 mM NaCl, pH 6.5. Spectra are recorded at 600 MHz on a Bruker AM600 spectrometer. (A) Recorded with a regular COSY experiment (scheme of Figure 1a with omission of the 90° ^{15}N pulses; the remaining 180° ^{15}N pulse provides ^{15}N decoupling during t_1). (B) Recorded with the PS COSY scheme of Figure 1.

proved due to a decrease in cross peak cancellation. Comparison of individual F_1 and F_2 slices of the two spectra indicates an increase in signal-to-noise ratio by about a factor of 1.5. Because of the antiphase nature of the cross peaks and the strong digital filtering used, the difference in F_1 resolution is difficult to observe, although significant differences can be seen in the case of partially overlapping multiplets (Supplementary Material). About a dozen cross peaks (three in the region shown, Y54, G88, and E) are observed in the PS COSY spectrum and are too weak for detection in the regular COSY spectrum.

The ^{15}N–^1H dipolar interaction is of the same size as the ^1H–^1H dipolar coupling for two protons that are 0.22 nm apart. The effect of removal of this strong dipolar interaction on the 600 MHz NMR spectrum is partially offset by the fact that the NH multiple quantum coherence relaxation rate is increased by the ^{15}N chemical shift anisotropy. This is confirmed by a small difference in multiple quantum relaxation rates between data recorded at 500 and at 600 MHz.

As demonstrated here, removal of the strong heteronuclear dipolar broadening can increase the sensitivity and resolution of 2D NMR spectra of proteins. Use of the pseudo-single quantum idea is not restricted to the COSY experiment and is equally applicable to other types of homonuclear 2D experiments. Particularly promising appears the application to measurement of NH–CαH J couplings, currently under study in our laboratory. The pseudo-single quantum approach for in phase experiments gives a line narrowing of the F_1 amide resonances by about 30%; however, the effect on the final sensitivity and resolution of the 2D spectrum strongly depends on whether the F_1 resolution is limited for reasons other than relaxation. The PS COSY experiment is also directly applicable to proteins where only certain types of amino acids are labeled with ^{15}N, providing an effective means for editing the COSY spectrum, thus facilitating the assignment process.

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Supplementary Material Available: Comparisons of the fingerprint region COSY spectra recorded with conventional COSY and PS COSY schemes and F_1 traces taken through these regions (4 pages). Ordering information is given on any current masthead page.

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