oxidation of **5** and **6**), and nerolidol **14** (54%). Alternatively, treatment of **5** and **6** with trimethyl phosphite smoothly produced thiophosphate **17** (63.5%), Boc-*S*-methyl-Cys-Val-OMe (5%), and nerolidol (**14**) (28%). The peptidyl reaction products are accounted for by the inter- or intramolecular decomposition of the expected thiophosphonium reaction intermediate.[16] Of special relevance for structure identification of unknown prenylated proteins/peptides is that the isoprene unit can be cleaved from the peptide and identified while *simultaneously* tagging the cysteinyl residue with phosphite. Thus, the mild removal of an isoprenoid from cysteine recommends the further development of these methods and also suggests that allylic sulfides/sulfoxides might find utility in peptide chemistry as orthogonally removable thiol protecting groups.

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(17) All compounds described herein exhibited ^{1}H NMR, ^{13}C NMR, infrared, and mass spectra, as well as combustion analyses or high-resolution mass spectra, which were consistent with the assigned structures.

Complete Elimination of Spin Diffusion from Selected Resonances in Two-Dimensional Cross-Relaxation Spectra of Macromolecules by a Novel Pulse Sequence (SNOESY)

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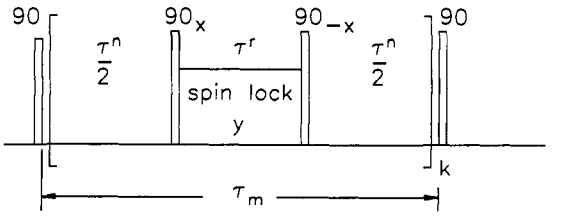
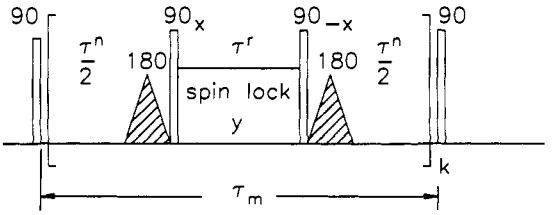
Spin diffusion represents a serious obstacle to the determination of highly accurate NMR solution structures. In ordinary NOESY (or ROESY) experiments, indirect magnetization transfer (spin diffusion) takes place simultaneously with direct magnetization transfer, but only the latter can be readily interpreted in terms of molecular geometry. The new method we report here (SNOESY) permits the evaluation of cross relaxation between a selected spin (or group of isolated spins) and *all* of its neighbors. Magnetization transfer between all other spin pairs is prevented, and thus spin diffusion is eliminated completely. Since longer mixing times can be used with SNOESY than with NOESY (or ROESY) without incurring complications from spin diffusion, SNOESY may permit the observation of pure, direct magnetization transfer between more distant spins and improve the quality of solution structures of macromolecules.

Several approaches have been taken to the spin diffusion problem. The amplitudes of spin-diffusion contributions can be diminished by using short cross-relaxation times in NOE spectroscopy.[1] Spin-diffusion effects can be evaluated by analysis of build-up curves,[2-4] by complete relaxation matrix analysis,[5] or by linear combinations of laboratory-frame and rotating-frame cross-relaxation data (DNOESY).[6] All of these approaches are passive, since spin diffusion takes place during the experiment and is removed only later during data processing and evaluation.

Scheme I



Scheme II



An appealing alternative approach is to suppress spin diffusion in real time during the experiment. Experiments have been described where one[7,8] or a few[8] particular cross-relaxation pathways have been eliminated from the complete cross-relaxation network. These experiments can be used to uncover particular spin-diffusion steps, but they selectively suppress only one pathway, and they fail to prevent cross relaxation through the many remaining pathways. The selective NOESY experiment proposed here, which is derived from methods reported earlier,[8,9] exploits the difference between cross-relaxation rates in the rotating frame (σ^r) and the laboratory frame (σ^n) in macromolecules (eq 1).[10]

$$\sigma^r = -2\sigma^n \tag{1}$$

In a normal NOESY (or ROESY) experiment, cross relaxation takes place simultaneously between all pairs of neighboring spins. Since many pathways are active, it is not easy to separate the contribution of a given direct cross-relaxation step. For example, cross relaxation between spins s and k can occur directly or through a number of indirect pathways (e.g., $s \rightarrow l \rightarrow k$). In the compensated experiment (Scheme I), magnetization is flipped rapidly between the rotating and laboratory frames during the mixing time, τ_m; all effective cross-relaxation rates are zero, and spin diffusion cannot take place. The effective cross-relaxation rate between spins k and l is given by the following:[11]

$$\sigma_{kl}^{eff} = \sigma_{kl}^{n}\frac{\tau^n}{\tau_m} + \sigma_{kl}^{r}\frac{\tau^r}{\tau_m} \tag{2}$$

where $\tau_m = \tau^n + \tau^r$. If $\tau^n = 2\tau^r$, eqs 1 and 2 yield $\sigma_{kl}^{eff} = 0$; thus, there is no cross relaxation and, consequently, no spin diffusion.

If sequence I (given in Scheme I) is modified such that resonance s is selectively inverted each time the reference frame is

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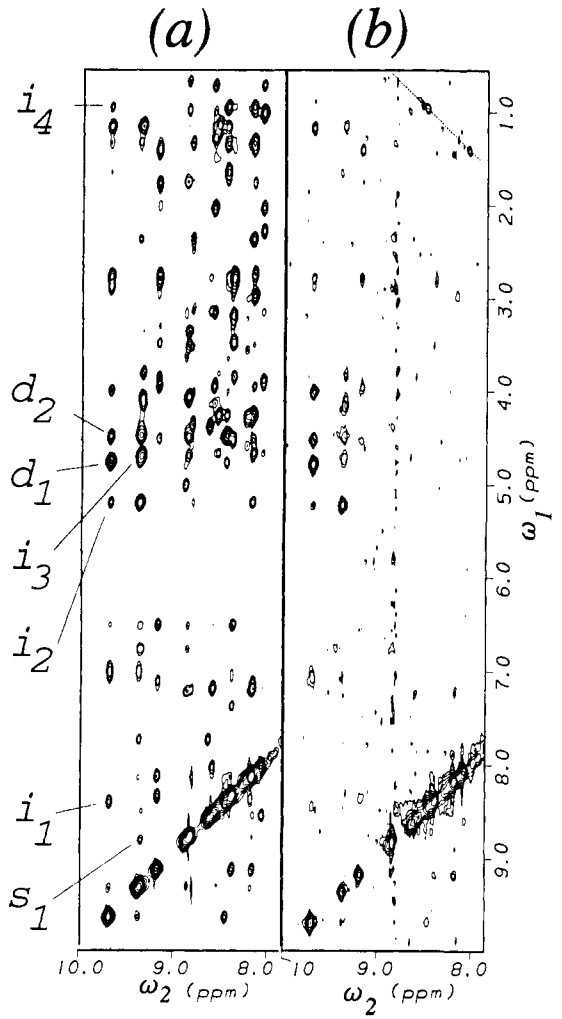

Figure 1. Cross-relaxation spectra (600 MHz) of ovomucoid third domain (OMTKY3) in ^2H_2O at pH* 4.1 and 25 °C. (a) Normal phase-sensitive NOESY spectrum;[15] (b) SNOESY spectrum obtained with pulse sequence II, with selective inversion of peaks in the 9.1–9.8 ppm range: 9.20 (Cys^{38} H^N), 9.37 (Ser^{26} H^N), 9.39 (Gly^{25} H^N), and 9.7 ppm (Tyr^{31} H^N).[16] Both spectra were base plane corrected[17] and scaled to a common intensity by reference to peak d_1, which is unaffected by spin diffusion as determined from cross-relaxation build-up curves.[4] Three classes of cross peaks are noted in the spectra: peaks d_1 and d_2 arise from direct magnetization transfer (d_1: Tyr^{31} H^N, Thr^{30} H^γ; d_2: Tyr^{31} H^N, Tyr^{31} H^α); peaks i_1–i_4 contain partial contributions from spin diffusion (i_1: Tyr^{31} H^N, Leu^{23} H^N; i_2: Tyr^{31} H^N, Cys^{24} H^α; i_3: Gly^{25} H^N, Gly^{25} H_1^α, Ser^{26} H^N, Gly^{25} H_1^α; i_4: Tyr^{31} H^N, Leu^{23} H^δ); and peak s_1 arises from pure spin diffusion (s_1: Ser^{26} H^N, Ser^{51} H^N). One should note significant reduction of S/N in spectrum b. S/N deterioration is intrinsic to the method due to the dissipative character of magnetization transfer in the rotating frame.

changed (sequence II, Scheme II), then the second term in eq 2 becomes negative for s and all its neighbors:

$$\sigma_{ks}^{\mathrm{eff}} = \sigma_{ks}^{\mathrm{n}}\frac{\tau^{\mathrm{n}}}{\tau_{\mathrm{m}}} - \sigma_{ks}^{\mathrm{r}}\frac{\tau^{\mathrm{r}}}{\tau_{\mathrm{m}}} \qquad (3)$$

Under these conditions when $\tau^{\mathrm{n}} = 2\tau^{\mathrm{r}}$, one finds that $\sigma_{ks}^{\mathrm{eff}} = {}^4/_3\sigma_{ks}^{\mathrm{n}}$. Cross relaxation between the inverted resonance s and each of its neighbors is enabled, whereas direct cross relaxation between all other spins remains suppressed ($\sigma_{kl}^{\mathrm{eff}} = 0$).[12]

The direct cross-relaxation and spin-diffusion pathways of the small protein, turkey ovomucoid third domain (OMTKY3, 6062 Da), have been well documented.[13] When a long mixing time is used, most of the NOESY cross peaks (Figure 1a) contain contributions from spin diffusion. By contrast, all of the cross peaks in the SNOESY spectrum (Figure 1b) obtained with the same mixing time (199 ms) arise solely from direct magnetization transfer.[14] For example, peaks d_1 and d_2 (Figure 1a), which are due to pure, direct magnetization transfer, have the same amplitude in both spectra; peaks i_1–i_4, which have contributions from both direct and indirect cross relaxation in the NOESY spectrum, are reduced in amplitude in the SNOESY spectrum, and peak s_1 (Figure 1a), which arises from pure spin diffusion, is absent in the (9.1–9.8 ppm) SNOESY spectrum (Figure 1b). The above results are consistent with our previous analysis of cross relaxation in OMTKY3.[4,6]

The DNOESY[6] pulse sequence removes spin-diffusion effects over the entire spectrum but fails in regions where diagonal peaks overlap. By contrast, SNOESY fails only in regions where diagonal peaks *from spatially close neighbors* overlap. SNOESY requires that separate data sets be obtained for each spin. This requirement might be relaxed by a suitable heteronuclear edited mixing sequence with an ^{15}N- or ^{13}C-labeled protein.

Acknowledgment. This study was carried out at the National Magnetic Resonance Facility at Madison, WI, under support from NIH Grants LM04958 and RR02301. Equipment in the facility was purchased with funds from the University of Wisconsin, the NSF Biological Instrumentation Program (Grant DMB-8415048), the NIH Biomedical Research Technology Program (Grant RR02301), the NIH Shared Instrumentation Program (Grant RR02781), and the U. S. Department of Agriculture.

(12) G. Bodenhausen (4th Chianti Workshop on Magnetic Resonance: Nuclear and Electron Relaxation, San Miniato (Pisa), Italy, June 2–8, 1991) has proposed an elegant experiment in which direct cross relaxation (free of spin diffusion) is observed between a *single pair* of spins within a multispin system. By contrast, the experiment demonstrated here provides all direct cross relaxations from a selected spin (or group of spins).

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(14) When more than one spin is inverted, the spectrum can contain spin-diffusion cross peaks between pairs of inverted spins if they share a common cross-relaxation partner.

(15) Transients (32) were digitized with 2048 t_2 data points for each of 512 t_1 increments; τ_{m} = 199 ms.

(16) Transients (80) were digitized with 2048 t_2 data points for each of 512 t_1 increments: τ_{m} = 199 ms; τ^{r} = 22.1 ms; τ^{n} = 37.7 ms; the number of cycles, k = 3. A selective 180° Gaussian pulse (t_{p} = 3.23 ms) was applied over the 9.1–9.8 ppm range. Direct cross-relaxation cross peaks appear in both ω_1 and ω_2 only from resonances within this window.

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The Methyl Group at C(10) of 2,3-Oxidosqualene Is Crucial to the Correct Folding of This Substrate in the Cyclization–Rearrangement Step of Sterol Biosynthesis

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Recently, much new information has been obtained regarding the molecular details of the cyclization–rearrangement step in sterol biosynthesis from 2,3-oxidosqualene (**1**), including the nature of the protosterol intermediate (**2**) and the manner in which the stereochemistry at C(20) is rigorously controlled.[1-3] Described herein are new data and insights on the interesting question of how lanosterol synthase controls the stereochemistry of the ring system during the cyclization **1** → **2**, especially with regard to the generation of the high-energy B-ring twist-boat structure inherent in the trans-syn-trans geometry of the A/B/C ring portion of **2** (Chart I).

Studies carried out in this laboratory more than 20 years ago showed that the lanosterol synthase from hog liver converts the

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