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Supplementary Material Available: Listings giving full spectroscopic and analytical characterization of **5** and **7-12** and tables of X-ray crystallographic data, including thermal and positional parameters, bond lengths, and bond angles for **8** and **9** (49 pages). Ordering information is given on any current masthead page.

(14) All new compounds were spectroscopically characterized and furnished satisfactory elemental analyses (C, H, N ±0.4%) or high-resolution mass spectra. Details are provided in the supplementary material.

An Efficient Triple Resonance Experiment Using Carbon-13 Isotropic Mixing for Determining Sequence-Specific Resonance Assignments of Isotopically-Enriched Proteins

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Sequence-specific resonance assignments provide the basis for interpreting multidimensional NMR spectra and for determining 3D structures of proteins from these data.[1] A key step in this assignment procedure is the identification of amino acid spin systems which are sequential in the protein sequence. This is generally done using multidimensional nuclear Overhauser effect (NOE) spectroscopy (NOESY).[1,2] However, as NOEs arise between proton pairs separated by less than about 5 Å, NOESY cross peaks are observed not only between resonances of sequential residues but also for intraresidue and longer-range interactions. In proteins with severe chemical shift degeneracies, this extra information complicates the identification of sequential connections between amino acid spin systems. Several experiments have been described[3] which overcome this problem by using one- and two-bond scalar couplings to correlate backbone (e.g., $^{13}C^{\alpha}$ and H^{α}) resonances of residue $i-1$ with backbone (e.g., ^{15}N and H^{N}) resonances of residue i. However, these experiments cannot be used reliably for the common situation in which there are chemical shift degeneracies of both the $^{13}C^{\alpha}$ and H^{α} resonances between amino acid residues. Recently, the CBCANH[4] and CBCA(CO)NH[5] experiments have been described, which overcome this problem using correlations between the backbone ^{15}N and H^{N} resonances of residue i and the α and β resonances of residues $i-1$. While these experiments can provide data that is useful for determining sequential resonance assignments, the CBCANH[4] experiment relies on relatively small $^{2}J(^{13}C^{\alpha}_{i-1}-^{15}N_i)$ coupling constants for coherence transfer across the peptide bond and provides sequential cross peaks for only 50-75% of the spin systems in small (<15 kDa) proteins. Although more efficient sequential coherence transfer is obtained in the CBCA(CO)NH[5] experiment, this multiple relay experiment does not provide sequential connectivity information for peripheral side chain resonances beyond the C^{β} position.

In order to provide a more complete set of connections between spin systems of sequential amino acids, we have developed a powerful experiment called HC(C)(CO)NH-TOCSY. This triple resonance experiment uses carbon–carbon isotropic mixing to move magnetization from the peripheral side chain proton and carbon nuclei to the backbone C^{α} nucleus, through the carbonyl carbon, and to the backbone nitrogen and amide proton of the next residue in the sequence, resulting in selective detection of sequential magnetization transfer. A 3D ^{15}N-edited HC(C)(CO)NH-TO-

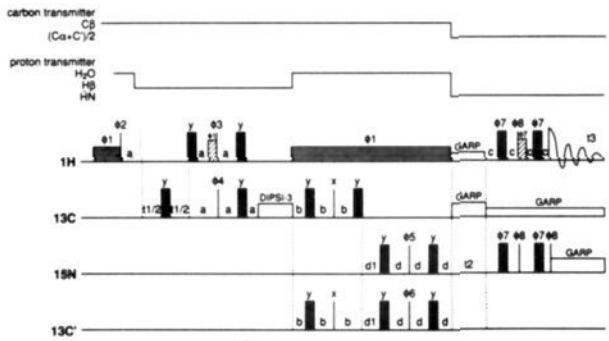

Figure 1. Pulse sequence of the 3D ^{15}N-edited HC(C)(CO)NH-TOCSY experiment. For pulses applied at transmitter frequencies corresponding to the center of the aliphatic (13C) or carbonyl (13C′) regions of the carbon-13 spectrum, the 90° and 180° pulse widths were adjusted to provide an excitation null at carbonyl and aliphatic resonance frequencies, respectively. Pulses were applied at the carbonyl resonance frequency by appropriate phase modulation through a waveform generator[6] while the synthesizer frequency was maintained at the $^{13}C^{\beta}$ carbon resonance frequency. Coherence transfer delays were tuned to $a = 1.5$ ms, $b = 3.2$ ms, $c = 2.7$ ms, $d_1 = 10.3$ ms, and $d = 13.5$ ms, respectively. Carbon-13 isotropic mixing was done using DIPSI-3.[7] During the evolution time t_2 the ^{1}H and ^{13}C spins were decoupled from ^{15}N, and during t_3 the aliphatic ^{13}C, carbonyl ^{13}C, and ^{15}N spins were decoupled from ^{1}H, using GARP.[8] GARP was also used to simultaneously decouple aliphatic and carbonyl ^{13}C spins from ^{15}N during the reverse-refocused INEPT transfer step that is between the t_2 and t_3 evolution times. These multipulse spin-lock and decoupling schemes were executed using waveform generators. Time proportional phase increments (TPPI) of 90° were used to obtain pure phase spectra. The pulse phases were cycled as follows: $\phi1 = 16(+x), 16(-x)$; $\phi2 = +x$ with TPPI(^{1}H); $\phi3 = 4(+y), 4(-y)$; $\phi4 = +x, -x$; $\phi5 = +x, +x, -x, -x$; $\phi6 = 8(+x), 8(-x)$; $\phi7 = +y$ with TPPI(^{15}N); $\phi8 = +x$ with TPPI(^{15}N); and the receiver phase as $+x, -x, -x, +x, -x, +x, +x, -x, -x, +x, +x, +x, -x, -x, -x, +x$, with TPPI($^{15}N$). Solvent suppression was done using appropriately placed 0.5-1.0-ms trim pulses (diagonally-hatched bars) and weak selective irradiation (horizontally-hatched bars) of the H_2O resonance as shown in the pulse sequence.

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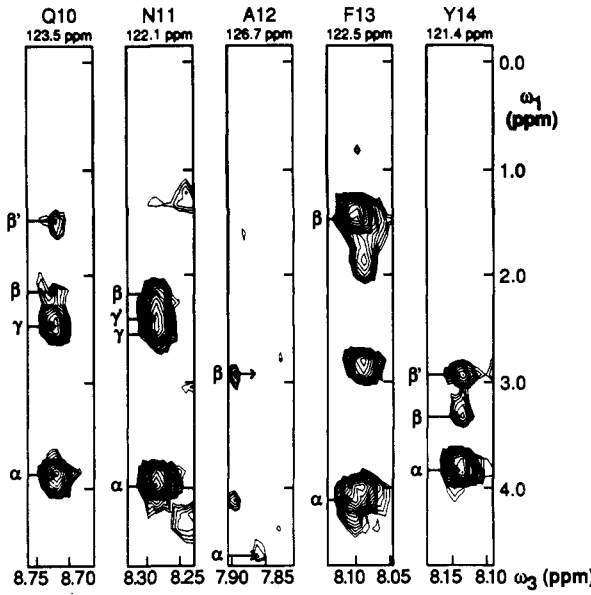

Figure 2. A panel showing strips from a 3D ^{15}N-edited HC(C)(CO)NH-TOCSY spectrum. These data were obtained using a 100% uniformly ^{13}C- and ^{15}N-enriched sample of a modified 8.2-kDa domain (called Z-Domain[9]) from the immunoglobulin-binding protein A of *Staphylococcus aureus* at a protein concentration of 2 mM in 10 mM K_2HPO_4, 0.2 mM NaN_3, pH 6.5, at a temperature of 30 °C. Shown in the figure are five representative $\omega_2 = {}^{15}N$ slices providing sequential connections for the polypeptide segment Gln-9–Tyr-14. Shown in each slice are 3D cross peaks between the side chain proton resonances of residue $i-1$ (ω_1 dimension), the ^{15}N resonance of residue i (ω_2 dimension), and the H^N resonance of residue i (ω_3 dimension). The slices themselves are labeled at the top with the ^{15}N chemical shift and the name of residue i. Each cross peak is labeled by an arrow with a tail at the ω_1 frequency of a side chain proton resonance of residue $i-1$ and a head at the ω_3 frequency of the backbone amide proton resonance of residue i. The sequential cross peak between $H^\alpha(11)$ and $H^N(12)$ was verified using a 2D version of the experiment recorded with a DIPSI-3 mixing time of 0 ms. This 3D data set was obtained using an isotropic mixing time of 24 ms and a total data collection time of ca. 40 spectrometer hours with a 500-MHz spectrometer. Data collection included 64 points in t_1 and t_2 and 1024 points in t_3, and the data were zero-filled prior to Fourier transformation, resulting in a final digital resolution of 66 Hz/point in ω_1, 18 Hz/point in ω_2, and 2.8 Hz/point in ω_3, respectively.

CSY pulse sequence is shown in Figure 1, and representative slices from a 3D spectrum recorded on an 8.2-kDa protein at 2 mM protein concentration are shown in Figure 2. A 2D spectrum exhibiting many connections between peripheral side chain protons and sequential backbone amide protons is presented in the supplementary material (Figure S1).

In HC(C)(CO)NH-TOCSY, the coherence transfer pathway depends on a series of one-bond scalar coupling constants that are all relatively independent of the protein conformation. Excellent 2D spectra for an 8.2-kDa protein were obtained using total collection times of 12–24 spectrometer hours on samples of 1–3 mM protein concentration on our 500-MHz NMR instrument. Cross peaks between Gly $C^\alpha H_{i-1}$ and $H^N{}_i$ resonances were observed to have phase shifts of 180° relative to other cross peaks in the spectra. Detailed analysis indicates that most of the cross peaks are sequential correlations from H^α, H^β, H^γ, H^δ resonances to the backbone ^{15}N and H^N resonances of the next amino acid in the sequence. For some asparagine and glutamine residues, cross peaks are also observed between aliphatic and side chain amide protons, uniquely identifying these spin systems. In fact, at appropriate isotropic mixing times the transfer from side chain aliphatic groups to these side chain amide protons is preferred over sequential magnetization transfer, attenuating sequential cross peaks in Asn-X or Gln-X dipeptide sequences. Aside from the cross peaks observed to these side chain amides, the carbonyl filter is highly selective for sequential connections and no intraresidue or long-range correlations involving backbone amide protons are observed.

The HC(C)(CO)NH-TOCSY pulse sequence of Figure 1 can be modified by introducing constant-time ^{13}C frequency labeling prior to the isotropic carbon-13 mixing period to generate 2D spectra which correlate peripheral side chain ^{13}C resonances with the backbone ^{15}N and H^N resonances of the next amino acid in the protein sequence. With this modification, the pulse sequence can also be run as a 4D-NMR experiment.

For small proteins HC(C)(CO)NH-TOCSY is quite efficient, is highly amenable to automated analysis by computer software, and provides significant advantages over conventional NOESY[2] or previously described triple-resonance[3-5] experiments for establishing sequential connections between spin systems of amino acid residues. By removing the coherence transfer step from $^{13}C^\alpha$ to $^{13}C'$ nuclei, the pulse sequence can also be modified into a related HC(C)NH-TOCSY experiment[10] which has intraresidue cross peaks from the side chain proton and carbon resonance of residue i to the backbone nitrogen and amide proton resonances of the same residue. Analyzed together, the HC(C)(CO)NH-TOCSY and the complementary HC(C)NH-TOCSY[10] experiments provide all of the information needed to determine sequence-specific resonance assignments of most backbone and side chain resonances in small proteins.

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Supplementary Material Available: Figure S1 depicting a 2D HC(C)(CO)NH-TOCSY spectrum using the pulse scheme outlined in Figure 1 (2 pages). Ordering information is given on any current masthead page.

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Total Synthesis of Westiellamide

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A considerable number of biologically active lipophilic cyclopeptides from marine organisms and fungi have been characterized in recent years.[1,2] Intensive structural and synthetic studies are addressing the use of naturally occurring and synthetic cyclic peptides in membrane transport and as models for hormone– and drug–receptor interactions.[3]

As a part of our program for the development of peptide mimetics, we have recently embarked on the total synthesis of westiellamide (**1**), a cyclic hexapeptide isolated by Moore et al.[4] from the terrestrial cyanophyte *Westiellopsis prolifica*. Westiellamide is identical to the earlier identified cycloxazoline[5]

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