acid afforded methyl ester **9**. At this juncture, the terminal olefin in **9** was transformed to aldehyde **10** by regioselective hydroboration and subsequent oxidation.

The isolated C_{21} stereocenter was next established through the (+)-dibutylnorephedrine-catalyzed[9] addition of diethylzinc to aldehyde **10** to provide carbinol **11** as an inseparable 10:1 mixture of diastereomers. Macrolactonization using the method of Yamaguchi[10] afforded macrocycle **3** in 77% yield accompanied by 8% of the readily separable C_{21} epimer. This 16-step reaction sequence provided **3** in 41% overall yield.

The synthesis of diene component **4** began with β-silyloxy acid **13**,[11] which was readily converted to aldehyde **14** (Scheme III). Selective formation of the (*E*)-vinyl iodide was accomplished using Takai's chromium reagent[12] in 6:1 dioxane/THF to provide a 9:1 mixture of olefin isomers, which were separated after the next step. Reduction of ester **15** to aldehyde **16** was followed by the introduction of the oxazolidinone moiety through phosphonium salt **17** to provide the desired *E* unsaturated imide (23:1 ratio). This six-step reaction sequence afforded diene **4** in 42% overall yield.

Fragments **3** and **4** were coupled using a palladium-catalyzed Stille reaction[13] to provide the triene **2** in 65% yield, along with 17% recovered **3**. The subsequent Lewis acid-mediated intramolecular Diels-Alder reaction proceeded with high selectivity to give the desired cycloadduct **18** in 71% yield (Scheme IV).

Removal of the extremely hindered oxazolidinone auxiliary was achieved using Damon's recently reported lithio mercaptide method.[14] Following deprotection and oxidation of **19**, the resulting thioester **20** was efficiently reduced to keto aldehyde **21** by Fukuyama's hydrosilylation procedure[15] using Lindlar's catalyst.

The final ring was then assembled through an intramolecular aldol reaction of **21** to provide a 12:1 mixture of aldol diastereomers **22** and **23**. Unfortunately, the major adduct **22** was inert to dehydration. However, the two diastereomers could be equilibrated via the sodium alkoxide to give a 1:2.5 mixture favoring **23**. Formation and elimination of the mesylate of this adduct provided the differentially protected aglycon. The silyl protecting groups at C_9 and C_{17} could be removed cleanly with HF/acetonitrile to provide the (+)-lepicidin aglycon **24**. The analytical properties of the synthetic material agreed in all respects with those of the natural aglycon[16] with the exception of the optical rotation, which was equal and opposite in sign. This synthesis thus confirms the absolute stereochemical assignment of the natural product previously determined by a combination of X-ray diffraction and degradation.[1]

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Supplementary Material Available: Experimental procedures for all reactions as well as spectral and analytical data for all synthetic intermediates (13 pages). Ordering information is given on any current masthead page.

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Four-Dimensional Heteronuclear Triple Resonance NMR Methods for the Assignment of Backbone Nuclei in Proteins

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Recently, Bax's group demonstrated that three-dimensional (3D) heteronuclear triple resonance NMR spectroscopy of proteins, uniformly enriched with ^{13}C and ^{15}N isotopes, allows sequential resonance assignments in larger proteins (>10 kDa). They proposed an elegant approach which involved recording a set of five or more 3D NMR spectra that correlate chemical shifts of backbone nuclei.[1-3] In order to resolve problems of overlap

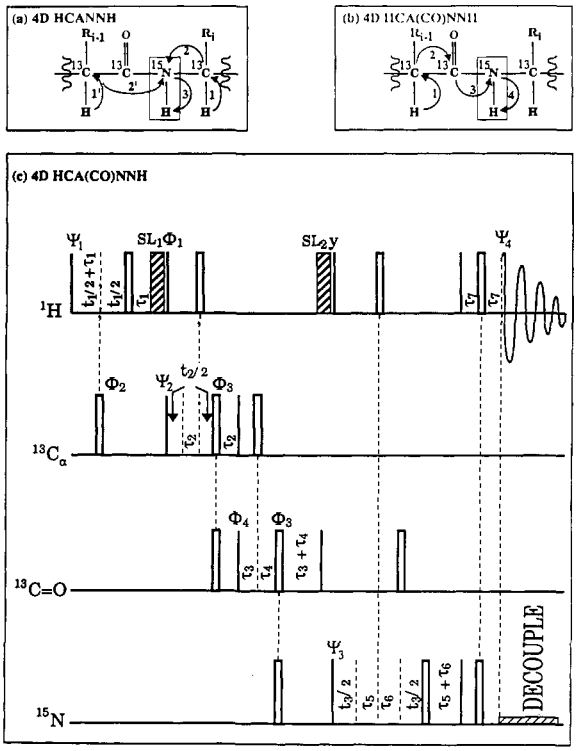

Figure 1. Schematic representation of the magnetization transfer pathways along the polypeptide chain for the HCANNH (a) and the HCA(CO)NNH (b) 4D NMR experiments. In the pulse sequence for the HCA(CO)NNH experiment (c), 90° pulses are depicted as narrow lines while open boxes represent 180° pulses. Cross-hatched boxes represent spin-lock pulses, SL_1 and SL_2, of 1 and 9 ms, respectively.[5,8] Typical values for the delays are as follows: $\tau_1 = 1.5$ ms, $\tau_2 = 1.7$ ms, $\tau_3 = 4.5$ ms, $\tau_4 = 9.5$ ms, $\tau_5 = 2.75$ ms, $\tau_6 = 11.0$ ms, and $\tau_7 = 2.25$ ms. The following phase cycling was employed: $\varphi_1 = 4(y),4(-y)$; $\varphi_2 = x,-x$; $\varphi_3 = 2(x),2(y),2(-x),2(-y)$; $\varphi_4 = 2(x),2(-x)$; $\psi_1 = x$; $\psi_2 = 8(x),8(-x)$; $\psi_3 = x,-x$; $\psi_4 = (x,-x,-x,x),2(-x,x,x,-x),(x,-x,-x,x)$. Unless indicated otherwise, the phase of the remaining pulses is kept at x. Quadrature detection during t_1, t_2, and t_3 was achieved by independently incrementing the phases of ψ_1, ψ_2, and ψ_3 in a States-TPPI manner.[9]

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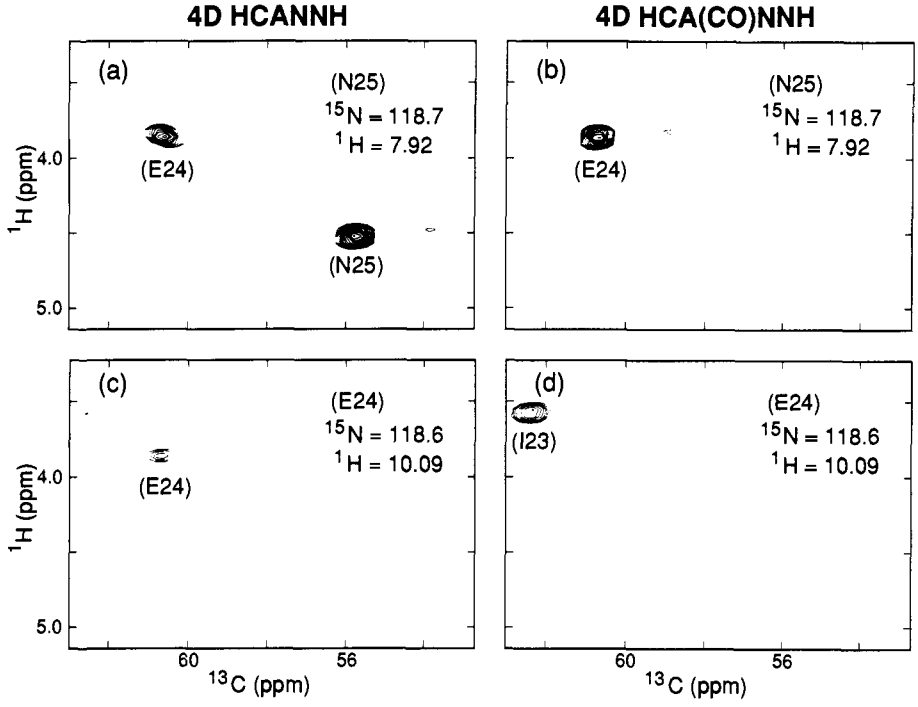

Figure 2. Comparison of spectra, run under identical conditions, from the two different 4D NMR experiments for a 1.1 mM sample of (^{13}C,^{15}N) labeled human ubiquitin in 90% H_2O/10% D_2O. Shown are specific sequential planes ($f_1 = {}^1H_\alpha$ and $f_2 = {}^{13}C_\alpha$) at particular chemical shifts ($f_3 = {}^{15}N$ and $f_4 = {}^1H_N$). For each experiment, a total of $32(t_1) \times 8(t_2) \times 8(t_3) \times 512(t_4)$ complex points were accumulated in ~90 h, using a recycle delay of 1.2 s, to give acquisition times of 17.8, 4.8, 7.4, and 63.4 ms in t_1, t_2, t_3, and t_4, respectively. Some resonances were aliased in the f_2 and f_3 dimensions, but this did not introduce any ambiguity. Processing in the t_2 and t_3 dimensions was achieved using a two-dimensional maximum entropy algorithm[10] to give a final spectrum of $f_1(64) \times f_2(64) \times f_3(64) \times f_4(512)$ real points; each data set took ~14 h to process on 16 transputers in a Meiko Computing Surface.[11] The maximum entropy data processing gave a 3–4-fold increase in digital resolution in both f_2 and f_3. Comparison of chemical shifts independently determined from either 4D NMR spectrum shows that the $^{13}C_\alpha$ and ^{15}N chemical shifts could be determined with a precision of ±0.06 and ±0.03 ppm, respectively. Furthermore, the chemical shifts determined from 4D data processed with the maximum entropy algorithm differed by less than ±0.05 and ±0.03 ppm (for $^{13}C_\alpha$ and ^{15}N) when compared with more highly digitized 2D NMR spectra processed using a conventional Fourier transform.

of pairs of $^1H_\alpha$–$^{13}C_\alpha$ cross peaks, they more recently suggested a four-dimensional (4D) NMR experiment called HCACON;[4] this experiment gave the combined information that was previously obtained from two of the 3D NMR spectra. In this paper, we report two new 4D NMR experiments which together allow a complete and much simpler sequential assignment strategy. Intraresidue (and many sequential interresidue) connections are obtained from a 4D version of a 3D NMR experiment called H(CA)NNH.[5] Because we also measure the $^{13}C_\alpha$ chemical shift, we name our 4D sequence HCANNH. Sequential assignments are based on a novel complementary 4D NMR experiment which we call HCA(CO)NNH, where the magnetization is specifically transferred via the ^{13}CO group, whose chemical shift is not measured.

The 4D HCANNH experiment is a straightforward extension of the 3D H(CA)NNH experiment of Kay et al.;[5] by also allowing the magnetization to evolve according to the $^{13}C_\alpha$ chemical shift, we can correlate the $^1H_\alpha$, $^{13}C_\alpha$, ^{15}N, and ^1H_N chemical shifts within each residue in one experiment (Figure 1a). Interresidue cross peaks are also sometimes observed as a result of transfer of magnetization from the $^{13}C_\alpha$ spin of residue $(i-1)$ to the ^{15}N spin of residue (i) via an interresidue $^2J_{C_\alpha N}$ (~7 Hz) coupling (Figure 1a).[5] Although this interresidue cross peak is often too weak to be observed, when both are present one may not be able to identify which is which.[3,5]

In the 4D HCA(CO)NNH experiment, this interresidue correlation is detected specifically (Figure 1b). The pulse sequence (see Figure 1c) has been optimized so as to reduce the number of 180° pulses and delays.[5] Because most of the magnetization transfer steps rely on relatively large J couplings ($J_{HC} \approx 147$ Hz, $J_{C_\alpha C'} \approx 55$ Hz, $J_{C'N} \approx 15$ Hz, and $J_{NH} \approx 94$ Hz), the sensitivity of this experiment is very good even though it involves four magnetization transfer steps. The two 4D experiments provide the correlated chemical shifts of ^1H_N and ^{15}N with $^{13}C_\alpha$ and $^1H_\alpha$ as well as ^1H_N and ^{15}N with $^{13}C_{\alpha-1}$ and $^1H_{\alpha-1}$.

We demonstrate the methods here on uniformly (>90%) (^{13}C,^{15}N) labeled ubiquitin,[6] a 76-residue protein, but we are also successfully applying them to a truncated form of $p21^{Ha\text{-}ras}$ (166 residues, 18.9 kDa).[7] As an illustration of the assignment strategy, Figure 2 shows a comparison of identical f_2f_1 planes ($^{13}C_\alpha$,$^1H_\alpha$) at fixed f_3f_4 chemical shifts (^{15}N,^1H_N) from the two 4D NMR spectra. In the HCANNH spectrum (Figure 2a), two correlations are observed to the amide proton of N25, one from the $^1H_\alpha$ proton of N25 (intraresidue) and the other from the $^1H_{\alpha-1}$ proton of E24 (interresidue). In the corresponding f_2f_1 plane from the HCA(CO)NNH spectrum, *only* the interresidue correlation is observed (Figure 2b). Although the interresidue cross peak is often weaker in the HCANNH spectrum (Figure 2a), as expected from the

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relative magnitudes of the $^1J_{C_\alpha N}$ and $^2J_{C_\alpha N}$ couplings,[5] the specific detection of this cross peak in the HCA(CO)NNH spectrum allows its unambiguous identification. In the next step, the cross peak due to the intraresidue correlation for E24, in the HCANNH spectrum, is located by searching at the $^1H_\alpha$ and $^{13}C_\alpha$ chemical shifts determined for the previous interresidue correlation; the cross peak is found in the f_2f_1 plane shown in Figure 2c. In this case, the next interresidue correlation to I23 is missing from the HCANNH spectrum. However, this correlation is easily detected in the corresponding f_2f_1 plane of the HCA(CO)NNH spectrum, allowing one to proceed with the sequential assignment (Figure 2d). The comparisons shown in Figure 2 indicate that the detection of the interresidue connectivity in the HCA(CO)NNH experiment is at least as sensitive as the detection of the intraresidue connectivity in the HCANNH experiment; we have found this to be true in all cases in ubiquitin.

In summary, the two 4D NMR experiments allow us to determine unambiguously intra- and interresidue connectivities between the backbone ^1H_N, ^{15}N, $^{13}C_\alpha$, and $^1H_\alpha$ nuclei. Because both experiments rely solely on one bond magnetization transfer steps, they are insensitive to the secondary structure of the protein. Our approach requires just two 4D NMR experiments, each of which can be recorded in a similar time to one or two of the (five or more) 3D NMR experiments used in the earlier approach. As with the 4D HCACON spectrum,[4] the higher dimensionality of our spectra reduces problems caused by overlap of $^1H_\alpha$-$^{13}C_\alpha$ cross peaks. Both of our 4D NMR experiments can be recorded on a protein sample dissolved in H_2O using an identical experimental set-up, which is a significant additional benefit. It avoids difficulties in comparing spectra with differing digital resolution or differing chemical shifts due to isotope effects. This will be particularly important for automated assignment procedures presently being developed.

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Copper(I) and Copper(II) Complexes of New Chelating Pterins

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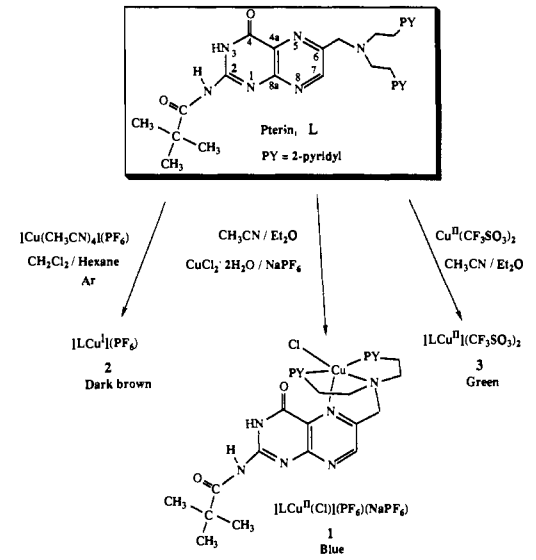
In this report, we describe a new class of pterin-derived ligands and copper ion complexes (Scheme I), designed to permit the controlled investigation of pterin/Cu/O_2 interactions and redox chemistry.[2-4] Such studies may be relevant to the active site

Scheme I



chemistry of a phenylalanine hydroxylase (PAH) from *Chromobacterium violaceum*, described recently by Benkovic and co-workers.[5,6] In mammalian systems, this is a non-heme iron, pterin, and dioxygen dependent enzyme which effects the hydroxylation of phenylalanine, giving tyrosine. Our current activities in bioinorganic copper–dioxygen chemistry[7] have interested us in PAH since, in this bacterial form, reaction of a reduced tetrahydropterin cofactor and O_2 occurs in the presence of a required copper ion cofactor. Since a 4a-hydroxypterin moiety can also be detected as a reaction product, the copper-mediated formation and/or reaction of a previously formed 4a-peroxypterin intermediate has been proposed.[5b,8]

To study the basic chemistry of such systems, we wanted to (1) synthetically modify pterins, placing a ligating group in the 6-position. Placement here would allow favorable chelation to the neighboring pterin N5 nitrogen and also allow proximity to the 4a-position where O_2/peroxide chemistry is suggested to occur.[5] EPR[9] and EXAFS[10] spectroscopic approaches implicate protein copper (as Cu(II)) coordination to N5, in addition to two or three protein-derived imidazole ligand donors. Substituents at the pterin 6-position should not inhibit the desired chemistry, since a variety of 6-substituted pterins (e.g., Me, $CH(OH)CH(OH)CH_3$, biopterin) act as substrates for PAH.[5] We also wanted to (2) use a nitrogen-containing chelate group, one that might mimic aspects of imidazole coordination but, more importantly, be known to effect Cu(I)/Cu(II) redox and O_2 chemistry,[6,7] and to (3) solubilize the pterin moiety (known to generally be very insoluble),[11]

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