in **1** (vide supra) again implying little if any agostic interaction in the amine compound.

Further studies of the possible mechanism for CH bond activation at these metal centers and the possible interconversion of the chelate types are presently underway.

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Supplementary Material Available: Tables of positional parameters, general temperature factors, bond distances, and bond angles for **1** and **3a** (28 pages); listing of structure factor amplitudes for **1** and **3a** (36 pages). Ordering information is given on any current masthead page.

Two-Dimensional Coherence Transfer NMR Spectroscopy by Isotropic Mixing: Application to Protein NMR Assignments

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Homonuclear spin-locking with matched Hartmann-Hahn radio frequency fields to obtain long-range coherence transfer within spin systems has received much attention recently.[2-5] The 2D NMR experiments, often referred to as rotating frame, isotropic mixing, "TOCSY",[2] or "HOHAHA"[4] experiments, have considerable potential for identifying long amino acid side-chain systems in proteins (e.g., lysine, leucine, arginine, et al.). This is in contrast to the RELAY experiment,[6,7] where the mixing time must be tuned for optimal relayed intensity for different spin systems,[8,9] and generally only single-step relays can be obtained with good signal intensity for larger proteins due to relaxation limitations. As discussed below, however, there are still compelling reasons for performing RELAY experiments in conjunction with isotropic mixing experiments.

First we demonstrate the results of applying WALTZ-16[10] and MLEV-16[11] pulse sequences to obtain long-range coherence transfers (Figure 1). Each spectrum was obtained under identical conditions on our home-built 500-MHz spectrometer[12] using a 20-W pulse for both the preparation and 40-ms mixing pulses, corresponding to an applied radio frequency field of 16-20 kHz. Bax and Davis[3] have modified the MLEV-16 pulse sequence by incorporating a 180° refocusing pulse and straddling "purge" pulses to correct for pulse imperfections and to eliminate antiphase and dispersive magnetization. These errors do not appear to dominate isotropic mixing spectra when the applied radio frequency field is sufficiently strong; thus the purge pulses are not required for obtaining good quality spectra. Note also that the

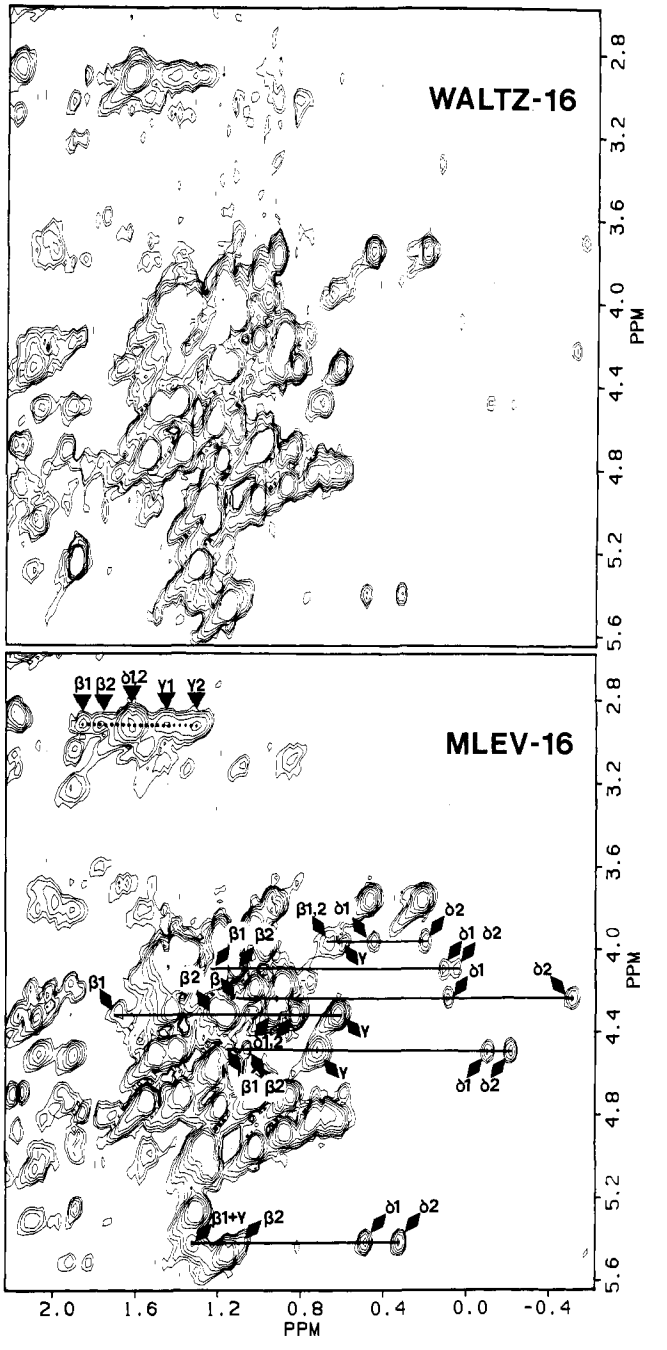

Figure 1. Selected regions of 2D isotropic mixing experiments using WALTZ-16 (above) and MLEV-16 (below) pulse cycling during the spin-locking period and performed off-resonance with 10-kHz spectral widths in both dimensions. 400 t_1 experiments were collected, zero-filled, and transformed, to generate a 1K × 1K matrix. The sample was 8 mM apoNeocarzinostatin (113 amino acids, MW 11500) in 100% D_2O; spectra were recorded at 45 °C. The six leucine and unique lysine side-chain spin systems are indicated.

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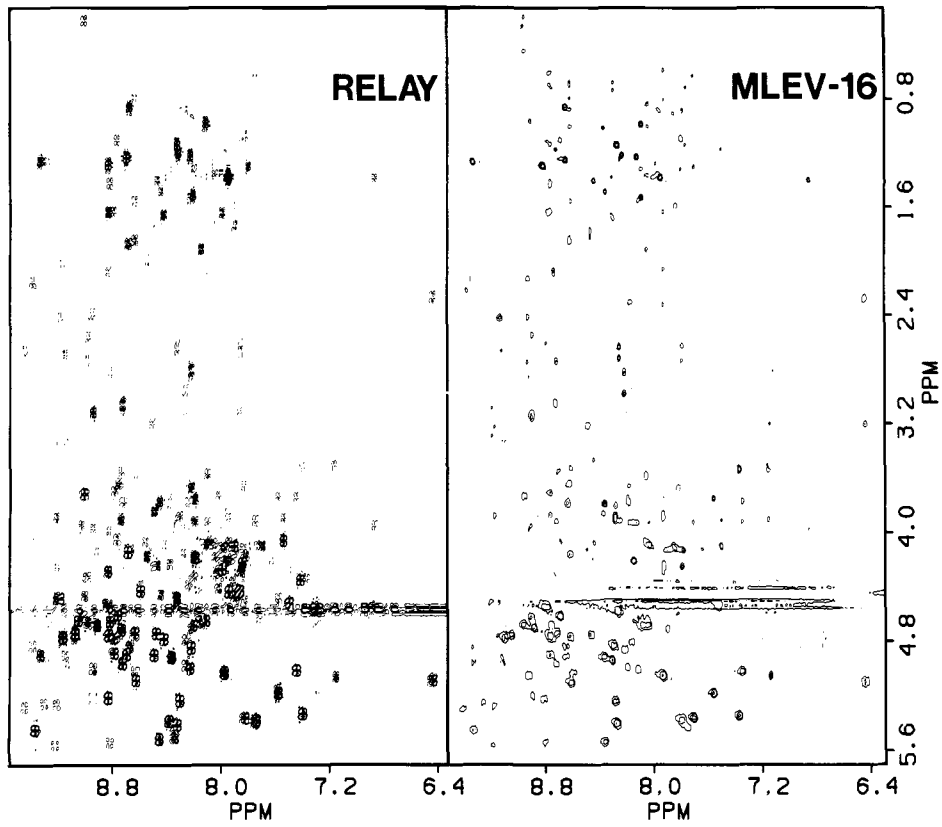

Figure 2. Regions containing NH–$C^{\alpha}H$, NH–$C^{\beta}H$, and NH–$C^{\gamma}H$ cross peaks of a phase-sensitive RELAY (left) and 2D MLEV-16 experiment (right). Both experiments were performed on-resonance with a spectral width of 6410 Hz; phase-sensitive data collection was obtained with TPPI[15] for the MLEV-16 experiment and with the hypercomplex method[16] for the RELAY experiment. Both single and double relays were seen in the MLEV-16 spectrum (see text).

MLEV-16 pulse sequence was superior to the WALTZ-16 sequence, which we have repeatedly observed. In addition, using the full transmitter power did not affect the probe, sample, or sample temperature, while it allowed the spectra shown to be acquired off-resonance without any noticeable resonance offset effects.

In the MLEV-16 spectrum the leucine $C^{\alpha}H$–$C^{\delta 1,2}H_3$ and $C^{\beta 1,2}H$–$C^{\delta 1,2}H_3$ cross peaks (the latter not shown) occur with good signal to noise ratios and make identification fairly straightforward. The identification of the lysine spin system is also clear when the COSY data are compared, but identification from the MLEV-16 data alone is unwise, as chemical shift arguments may need to be invoked for identifying the $C^{\delta 1,2}H$, $C^{\gamma 1,2}H$, and $C^{\beta 1,2}H$ resonances. This is a limitation of the isotropic mixing method, in that one cannot tell how many coherence transfer steps a cross peak corresponds to, since the evolution of the long-range coherence is complex and dependent upon several factors. A time-dependent study does not solve this problem, since double-relayed transfers in some cases can occur with shorter mixing times than will a single relay. This is most likely to be a problem in amino acids such as glutamate, glutamine, arginine, proline, and lysine, where some of the vicinal couplings can be quite weak. But these are the same residues which one hopes to identify by using isotropic mixing.

In our studies a combination of isotropic mixing, COSY, and RELAY experiments is used to identify direct couplings, single relays, and multiple relays. Figure 2 demonstrates the complexity of the results one obtains when performing these experiments on larger proteins; the multitude of cross peaks in the MLEV-16 spectrum presents many chances for misidentifying resonances. This is especially the case for threonine and alanine residues, as there are 18 alanine and 13 threonine spin systems to sort out in these spectra. Typically, alanine spin systems can be identified by a NH–$C^{\beta}H_3$ cross peak in a RELAY spectrum. In the MLEV-16 spectrum, many of the threonine NH–$C^{\gamma}H_3$ cross peaks are also observed, making the distinction between these two amino acid types difficult, if not impossible.

The RELAY spectrum also demonstrates a disadvantage of isotropic mixing experiments. For proteins where line broadening does not result in excessive signal cancellation due to unresolved antiphase components, the cross peak multiplet patterns—lost in the MLEV-16 experiment—are useful aids for spin system identification.[13] Antiphase cross peaks also aid in connecting cross peaks in the same spin system since the resonance chemical shifts are precisely defined between the positive and negative components. In addition the apparent J couplings in the related cross peaks will be the same, which helps in the case of overlapping resonances where the J couplings differ. Both of these phenomena have been useful in identifying spin systems in apoNCS. Lastly, RELAY experiments are useful since they can select for specific spin systems,[8,9,14] and additional cross peaks arise from at most single relays. Notable exceptions are the rare cases where strong coupling or relaxation effects lead to double relays, which are typically quite weak; these were not observed in our spectra.

The advantage of the isotropic mixing experiment is its ability to obtain both long-range coherence transfers and coherence transfers in spin systems with several strongly coupled spin partners. The many passive couplings in long aliphatic chains restrict the efficiency of relayed coherence transfer due to cosine modulation.[8] Complete identification of lysine and arginine side chains is important in NMR investigations of protein–DNA interactions, where such side chains are known to interact with DNA. Likewise, strong coherent transfers involving the entire sugar spin systems in nucleic acids are obtainable using long (80–200 ms) spin-locking times (data in preparation).

It appears that a combination of the above experiments will be invaluable for assigning protein NMR spectra; for example,

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the 12 valine spin systems in apoNCS were identified by using RELAY, and the 6 leucines were identified by using MLEV-16. If only the MLEV-16 experiment were performed, it could be difficult to discriminate between the valine $C^{\alpha}H-C^{\gamma}H_3$ and leucine $C^{\alpha}H-C^{\delta}H_3$ cross peaks, although the latter are usually (but not always) weaker. Performing both experiments usually overcomes the ambiguity.

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Registry No. Neocarzinostatin, 9014-02-2.

Incorporating CO into Poly(alkoxymethylene)acyl Ligands. Converting $(Ph_2PCH_2CH_2PPh_2)(CO)_2Co-COCH_3$ into *threo*-$(dppe)(CO)_2Co-COCH(OEt)CH(OEt)CH_3$

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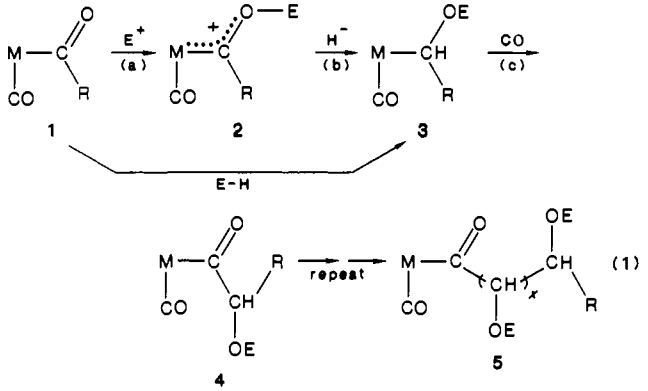
Small organic molecules (C_1-C_4) can be prepared from carbon monoxide with transition organometallic complexes as templates.[1,2] As a result of these studies, converting CO into poly(alkoxymethylene)acyl ligands **5** that retain an oxygen functionality on each carbon center[3] can be addressed. These acyl complexes could derive from repeating a three-step sequence of ligand reactions (eq 1): (a) electrophilic activation of an acyl ligand, (b) hydride

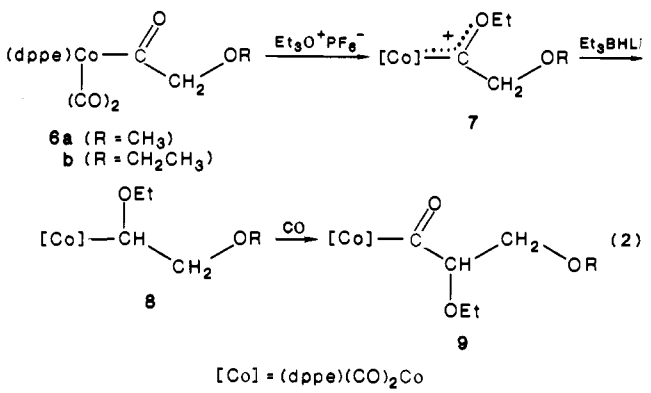


(1)

transfer to its oxycarbenoid derivative **2**, and (c) carbonylation of the resulting alkoxyalkyl group on **3**.[4] The first two steps, net reduction of the acyl ligand, are well established for alkoxycarbene complexes (E = CH_3,CH_2CH_3).[5] Carbonylating alkyl ligands bearing α-alkoxy substituents (step c), however, is relatively difficult[6] and only occurs in more labile organometallic systems.[7]

We now report coupling the acyl reduction (a and b) and carbonylation (c) steps by using the $(dppe)(CO)_2Co$ system (dppe = $Ph_2PCH_2CH_2PPh_2$).[8] These reactions moreover are stereoselective: complete diastereofacial selectivity at each emerging chiral center (step b) is observed in forming the cobalt diethoxybutanoyl **5** (x = 1, E = Et, R = CH_3).

Cobalt-alkoxyacetyl complexes **6a,b** were converted to their dialkoxypropionyl derivatives **9b** (eq 2) (overall yields 63-72%).



(2)

The starting **6a,b** were obtained (72-76% yields) after carbonylating (1 atm, 8 h) THF solutions containing $(dppe)(CO)_2Co-CH_2OR$, which were generated by alkylating $(dppe)(CO)_2Co^-Na^+$ with chloromethyl ethers.[9] Treating **6a,b** in CH_2Cl_2 successively with $Et_3O^+PF_6^-$, with $LiHBEt_3$ (-80 °C, 1 h), and with CO (1 atm at 22 °C, 10 h) affords **9a,b**. These result as air-stable, yellow crystals after combined column chromatography (alumina-CH_2Cl_2) and crystallization from CH_2Cl_2-ether-pentane. All acyl complexes reported herein are characterized by IR and ^1H, ^{13}C, and ^{31}P NMR spectroscopy and by acceptable elemental microanalyses.[10]

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(9) Experimental procedures resemble those used in preparing $(PMePh_2)_2(CO)_2$ cobalt-alkyl and -acyl complexes.[7d]

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