

to the protein through at least one nitrogen ligand.

FeMo protein from *C. pasteurianum*^{13,14} and FeMoco^{3,4,15} were isolated as previously described. ESE experiments were performed on a spectrometer described in detail elsewhere.¹⁶ Data were collected by using a two-dimensional three-pulse sequence. The echo intensity is sampled at time τ following the third $\pi/2$ pulse in a $\pi/2-\tau-\pi/2-t_1-\pi/2-\tau$ pulse sequence. The echo envelope is recorded for the evolution time t_1 for constant τ at a fixed magnetic field setting. Ligand hyperfine and quadrupolar couplings are observed as a modulation of the echo envelope. This modulation arises from forbidden transitions along the coupled electron-nuclear spin eigenstates which have been coherently excited by the strong microwave pulses.¹⁷

The time domain echo envelope pattern recorded at the $g = 3.7$ position in the EPR spectrum for the FeMo protein and FeMo cofactor are shown in Figure 1 (parts A and C, respectively). The cosine Fourier transform spectra, Figure 1 (parts B and D), facilitate identification of the couplings. Four transitions at 0.66, 1.45, 2.09, and 3.5 MHz are clearly identified in the spectrum of the protein. These frequencies arise from transitions among the nuclear quadrupole levels of an ^{14}N nucleus coordinated to the paramagnetic center. As seen in Figure 1D, these nitrogen quadrupole transitions are not observed in the spectrum of FeMoco isolated in NMF. The spectrum of FeMo protein prepared by using a phosphate buffer was identical with that obtained by using TRIS buffer, establishing that the transitions do not arise from a nitrogen atom in the buffer. Furthermore, allowing the FeMo protein to turnover under $^{15}\text{N}_2$ (with MgATP, Fe protein, and reductant present) did not change the modulation, eliminating an ephemeral albeit mechanistically significant nitrogen atom in the FeMoco unit as responsible for the nitrogen coupling. Finally, numerical simulations of the ESE spectrum and the frequency shifts of the peaks in the spectra recorded at a variety of different g values indicate that the Fermi-contact hyperfine interaction is 1.6 ± 0.1 MHz.¹⁸ A non-zero Fermi contact coupling can only arise from a covalently coordinated ligand. We conclude that the FeMo center is covalently bound to the protein by at least one nitrogenous ligand whose signature is clearly seen in the ESE experiment.

Mims and co-workers have tabulated ^{14}N coupling frequencies for some heme iron proteins and model iron complexes.¹⁹ In contrast to Cu(II) complexes where directly coordinated nitrogen atoms do not contribute to the echo envelope modulation,²⁰ quadrupole couplings from directly coordinated nitrogen atoms are observed in low-spin heme Fe(III) complexes. The lowest three frequencies in Figure 1B are not far from the three frequencies observed for the N_1 nitrogen in imidazole-heme-mercaptoethanol [or for the amine N in propylamine-heme-mercaptoethanol]. Moreover, these three frequencies are substantially different from those attributed to the remote N_3 nitrogen of imidazole ligands in either Cu(II) or low-spin Fe(III) heme complexes. These observations suggest that the spectrum in Figure 1B arises from the quadrupole transitions of a nitrogen atom directly coordinated to the FeMo center. It must be recognized, however, that in the absence of a chemical model for the system we are not able to exclude categorically the possibility that the modulation is due to a more remote nitrogen on a covalently coordinated nitrogenous ligand. This alternative interpretation is particularly important to consider since the details of the electronic structure of the FeMo

center as well as the effects of other, as yet, unidentified ligands on the nitrogen couplings are not yet known. We are presently investigating a variety of nitrogenous ligands on FeMoco as well as on model FeMo compounds to determine which ligand reproduces the experimental FeMo protein modulation spectrum.

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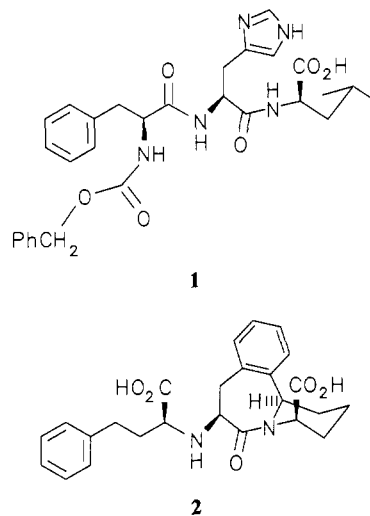
An Acyl-Iminium Ion Cyclization Route to a Novel Conformationally Restricted Dipeptide Mimic: Applications to Angiotensin-Converting Enzyme Inhibition

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The utility of inhibitors of angiotensin-converting enzyme^{1,2} as antihypertensive agents^{3,4} has been demonstrated clinically.⁵ Our approach to the design of ACE inhibitors was to mimic closely the three carboxy-terminus amino acids of the natural substrate, angiotensin I. The tripeptide fragment (*N*-benzyloxycarbonyl-Phe-His-Leu-OH; **1**) itself has modest affinity for ACE with a $K_M = 1 \times 10^{-5}$ M.⁶ Appropriate analyses led to the design of the tricyclic compound **2** as a lipophilic, conformationally restricted mimic of tripeptide **1**. Computer modeling confirmed a unique



minimum-energy conformation for **2** where the fused phenyl ring is oriented in close proximity to the terminal carboxyl group. Evaluation of inhibitor **2** containing this rigid tricyclic fragment provided useful insights into the binding requirements of ACE. The chemistry developed for the synthesis of **2** is generally useful for the synthesis of other conformationally restricted peptides. The desired optically active form of key tricyclic dipeptide intermediate **8** was prepared by using L-phenylalanine as the chiral template (Scheme I). *N*-Phthaloyl-L-phenylalanine **3** was coupled to racemic unsaturated amine **4**⁷ in 92% yield (EEDQ, CH_2Cl_2). Ozonolysis⁸ of the resulting diastereomeric amides **5** (O_3 , CH_2Cl_2 ,

(13) The FeMo protein was prepared by a modification of the procedure of Zumft and Mortenson (Zumft, W. G.; Mortenson, L. E. *Eur. J. Biochem.* **1973**, *35*, 401).

(14) The activity of the FeMo protein was 1800 ± 200 nmol of C_2H_4 formed/min/mg of protein.

(15) The activity of FeMoco as measured by the UW 45 Assay^{3,4} was 180 ± 20 nmol of C_2H_4 produced/min/nmol of Mo.

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1) $\text{O}_3, \text{CH}_3\text{OH}$
 2) CH_3SCH_3 , pyridine
 3) TFA, reflux, CH_2Cl_2

1) $\text{CF}_3\text{SO}_3\text{H}$
 2) Ph_2CN_2

1) H_2NNH_2

1) 9
 2) TFA
 3) LiOH

10a $\text{R} = \text{Et}, \text{R}' = \text{CHPh}_2$
 10b $\text{R} = \text{Et}, \text{R}' = \text{H}, \text{TFA salt}$
 10c $\text{R} = \text{R}' = \text{H}$



Phthalimido ester **7** was converted to free amine **8** (H_2NNH_2 , CH_3OH , Δ) which was then coupled to the 4-phenylbutyric ester side chain by $\text{S}_{\text{N}}2$ displacement of (*R*)-triflate¹⁰ **9** (CH_2Cl_2 , 1,8-bis(dimethylamino)naphthalene, 88% yield). Selective cleavage of the resulting benzhydryl ester **10a** (TFA, anisole, 25 °C, 91%) gave prodrug **10b** as a colorless analytically pure TFA salt ($[\alpha]_{\text{D}}^{20} = +25.5^\circ$, c 0.57, CH_3CN). Further hydrolysis with lithium hydroxide gave zwitterionic diacid **2** in 85% yield [mp 259–260 °C dec, $[\alpha]_{\text{D}}^{20} = +24^\circ$, 0.05, MeOH].

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- (12) A 3 mg/kg dose of **10b** caused half-maximal inhibition at 1 h post oral dosing, and significant inhibition was sustained for 4 h.

Fascination with chiral recognition by enzymes began in 1858 with Pasteur's report¹ of stereoselective fermentation of tartaric acid. Explanations of the process of chiral recognition later appeared, with notable contributions from Bergmann² in the 1930s, Ogston³ in 1948, and Hirschmann⁴ in 1960 as well as reviews by Popjak⁵ and Alworth⁶ in the early 1970s. Ogston³ proposed that chiral recognition requires only a three-point contact between enzyme and substrate. Alworth⁶ emphasized that chemical nonequivalence of enantiotopic groups is the critical factor for chiral recognition and clearly illustrated this for recognition of succinic acid.

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