to the protein through at least one nitrogen ligand.

FeMo protein from C. pasteurianum<sup>13,14</sup> and FeMoco<sup>3,4,15</sup> were isolated as previously described. ESE experiments were performed on a spectrometer described in detail elsewhere.<sup>16</sup> Data were collected by using a two-dimensional three-pulse sequence. The echo intensity is sampled at time  $\tau$  following the third  $\pi/2$  pulse in a  $\pi/2 \cdot \tau \cdot \pi/2 \cdot t_1 \cdot \pi/2 \cdot \tau$  pulse sequence. The echo envelope is recorded for the evolution time  $t_1$  for constant  $\tau$  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.<sup>17</sup>

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 <sup>14</sup>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 <sup>15</sup>N<sub>2</sub> (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  $1.6 \pm 0.1$  MHz.<sup>18</sup> 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 <sup>14</sup>N coupling frequencies for some heme iron proteins and model iron complexes.<sup>19</sup> In contrast to Cu(II) complexes where directly coordinated nitrogen atoms do not contribute to the echo envelope modulation,<sup>20</sup> 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<sub>3</sub> 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 nitrogeneous ligand. This alternative interpretation is particularly important to consider since the details of the electronic structure of the FeMo

- (19) Peisach, J.; Mims, W. B.; Davis, J. L. J. Biol. Chem. 1979, 254, 12379.
- (20) Mims, W. B.; Peisach, J. J. Chem. Phys. 1978, 69, 4921.
  (21) Mims, W. B. J. Magn. Reson. 1984, 59, 291.

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.

Acknowledgment. We are grateful to W. B. Mims for many stimulating discussions.

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

Gary A. Flynn,\* Eugene L. Giroux, and Richard C. Dage

Merrell Dow Research Institute Cincinnati, Ohio 45215 Received August 10, 1987

The utility of inhibitors of angiotensin-converting enzyme<sup>1,2</sup> as antihypertensive agents<sup>3,4</sup> has been demonstrated clinically.<sup>5</sup> 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  $K_{\rm M} = 1 \times 10^{-5}$  M.<sup>6</sup> 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



miminum-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<sup>7</sup> in 92% yield (EEDQ, CH<sub>2</sub>Cl<sub>2</sub>). Ozonolysis<sup>8</sup> of the resulting diastereomeric amides 5 (O<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>).

- (3) Ondetti, M. A.; Cushman, D. W. J. Med. Chem. 1981, 24, 355-361.
  (4) Brunner, H. R.; Nussberger, J.; Waeber, B. J. Cardiovasc. Pharmacol. 1985, 1, suppl. 1.
- (5) Abrams, W. B.; Davies, R. O.; Ferguson, R. K. Fed. Proc. 1984, 43, 1314-1321.
- (6) Schweisfurth, H.; Dahlheim, H. Radioimmunoassay, Renin, Angiotensin (Symp. 1976), 1978, 71-75.

0002-7863/87/1509-7914\$01.50/0 © 1987 American Chemical Society

<sup>(13)</sup> 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).

<sup>(14)</sup> The activity of the FeMo protein was  $1800 \pm 200 \text{ nmol}$  of  $C_2H_4$  formed/min/mg of protein.

<sup>(15)</sup> The activity of FeMoco as measured by the UW 45 Assay<sup>3,4</sup> was 180  $\pm$  20 nmol of C<sub>2</sub>H<sub>4</sub> produced/min/nmol of Mo. (16) Thomann, H.; Tindall, P. Analytical Instrumentation, to be published.

 <sup>(16)</sup> Inomann, H.; Iindail, P. Analytical Instrumentation, to be published.
 (17) Mims, W. B Phys. Rev. 1972, B5, 2409; 1972, B6, 3543.
 (18) Jin, H.; Thomann, H.; Morgan, T. V.; Bare, R. E.; Stiefel, E. I., to

<sup>(18)</sup> Jin, H.; Ihomann, H.; Morgan, I. V.; Bare, R. E.; Stiefel, E. I., to be published.

<sup>(1)</sup> Wyvratt, M. J.; Patchett, A. A. Med. Chem. Revs. 1985, 5, 483-531.
(2) Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R.

J. Med. Chem. 1985, 28, 393-399.

Scheme I



CH<sub>3</sub>OH, -78 °C) followed by reduction with dimethyl sulfide (-78  $\rightarrow$  25 °C, 12 h), workup, and mild dehydration (TFA, CH<sub>2</sub>Cl<sub>2</sub>, reflux, 3 h) gave a 1:1 ratio of isomeric acylenamines 6a and 6b which were separated by preparative HPLC (50% EtOAc/hexane) in 70% overall yield. Cyclization of acylenamine 6a (CF<sub>3</sub>SO<sub>3</sub>H, CH<sub>2</sub>Cl<sub>2</sub>, 25 °C, 24 h) and re-esterification of the acidic product  $(Ph_2CN_2, CH_2Cl_2)$  gave the desired optically pure benzhydryl ester 7 (mp 156–157 °C,  $[a]_D^{20} = -88.1^\circ$ , 1.1, CHCl<sub>3</sub>) in 77% yield. The relative stereochemistry and conformation of 7 have been confirmed by X-ray crystallography.



The stereospecificity of the acyl-iminium ion induced electrophilic aromatic substitution may be a result of a preference for the equatorial orientation of the phthalimide moiety in the transition state. Curiously, the other acylenamine isomer was resistant to cyclization under similar conditions. It is believed that the concurrent methyl ester hydrolysis which is observed during the cyclization reaction is facilitated by through-space participation of the proximal aromatic ring. Further mechanistic considerations will be discussed elsewhere.

Phthalimido ester 7 was converted to free amine 8 ( $H_2NNH_2$ , CH<sub>3</sub>OH,  $\Delta$ ) which was then coupled to the 4-phenylbutyric ester side chain by  $S_N 2$  displacement of (R)-triflate<sup>10</sup> 9 (CH<sub>2</sub>Cl<sub>2</sub>, 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 ( $[\alpha]_D^{20} = +25.5^\circ$ , c 0.57, CH<sub>3</sub>CN). Further hydrolysis with lithium hydroxide gave zwitterionic diacid **2** in 85% yield [mp 259-260 °C dec,  $[\alpha]_D^{20} = +24^\circ$ , 0.05, MeOH].

Diacid 2, which inhibited rabbit lung ACE with a  $K_i$  of 1.2 ×  $10^{-11}$  M, is the most potent in vitro inhibitor of ACE we have examined.<sup>11</sup> Prodrug 10b was orally active in the conscious spontaneously hypertensive rat as determined by reduction of angiotensin I-induced increase in blood pressure.<sup>12</sup> The potency of inhibition observed for hindered diacid 2 suggests that the region of ACE which binds the terminal carboxyl group is uncluttered and that significant binding affinity, over that realized by other inhibitors, may be the result of hydrophobic interactions near this site.

We have demonstrated the utility of acyl-iminium ion chemistry in the preparation of a useful conformationally restricted dipeptide mimic. The key cyclization step occurs in excellent yield with a high degree of stereospecificity. The potent activity of the angiotensin-converting enzyme inhibitor 2 is a strong indication of the usefulness of this design approach. Further applications of this methodology, which may shed important insights into protein structure and function, are currently being explored in our laboratory.

Acknowledgment. We express gratitude to Douglas W. Beight for the preparation of precursors to compound 4. High field NMR studies provided by Dr. Michael Whalon and Edward Huber as well as analytical separations performed by Nicholas W. Brake were particularly helpful. Single-crystal X-ray structures were provided by Dr. John C. Huffman, Indiana University.

 (11) Bunning, P. Arzneim-Forsch./Drug Res. (II) 1984, 34, 1406-1410.
 (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.

## A "Siameso" Inhibitor: Chiral Recognition of a **Prochiral Bilaterally Symmetric Molecule by Carnitine** Acetyltransferase

William J. Colucci, Richard D. Gandour,\* and Frank R. Fronczek

> Department of Chemistry, Louisiana State University Baton Rouge, Louisiana 70803-1804

Paul S. Brady and Linda J. Brady

Department of Food Science and Nutrition University of Minnesota, St. Paul, Minnesota 55108 Received July 10, 1987

Fascination with chiral recognition by enzymes began in 1858 with Pasteur's report<sup>1</sup> of stereoselective fermentation of tartaric acid. Explanations of the process of chiral recognition later appeared, with notable contributions from Bergmann<sup>2</sup> in the 1930s, Ogston<sup>3</sup> in 1948, and Hirschmann<sup>4</sup> in 1960 as well as reviews by Popjak<sup>5</sup> and Alworth<sup>6</sup> in the early 1970s. Ogston<sup>3</sup> proposed that chiral recognition requires only a three-point contact between enzyme and substrate. Alworth<sup>6</sup> emphasized that chemical nonequivalence of enantiotopic groups is the critical factor for chiral recognition and clearly illustrated this for recognition of succinic acid.

0002-7863/87/1509-7915\$01.50/0 © 1987 American Chemical Society

<sup>(7)</sup> Amine 4 was prepared from 2,3-dichlorocyclohexene (Bergman, E. J. *Org. Chem.* 1963, 28, 2210) in 70% yield by displacement with potassium phthalimide and potassium iodide in DMF followed by dephthaloylation with hvdrazine.

<sup>(8)</sup> Marshall, J. A.; Flynn, G. A. Synth. Commun. 1977, 7, 417.

<sup>(9)</sup> Complete crystallographic data will be included in a forthcoming full paper

<sup>(10)</sup> Urbach, H.; Henning, R. Tetrahedron Lett. 1984, 25, 1143-1146.

<sup>(1)</sup> Pasteur, L. Compt. Rend. 1858, 46, 615-618.

<sup>(2)</sup> Bergmann, M.; Žervas, L.; Fruton, J. S.; Schneider, F.; Schleich, H. J. Biol. Chem. 1935, 109, 325-346. Bergmann, M.; Fruton, J. S. J. Biol. Chem. 1937, 117, 193-202.

Ogston, A. G. Nature (London) 1948, 162, 963.
 Hirschmann, H. J. Biol. Chem. 1960, 235, 2762-2767.

<sup>(5)</sup> Popjak, G. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1970; Vol. 2, Chapter 3.

<sup>(6)</sup> Alworth, W. L. Stereochemistry and Its Application to Biochemistry; Wiley-Interscience: New York, 1972.