that in the absence of H_2Se the regioselectivity of cycloadduct formation must have its origins in the relative efficiencies with which the two biradicals proceed to the cyclobutane adducts in competition with reversion to the ground-state enone and that the biradical **15** proceeds to products 3 times less efficiently than biradical **17**.

Our results imply that, at least for the reaction of **3** with EVE, the regiochemistry of enone cycloaddition is not governed by the orientation of the reactants in an exciplex precursor as has long been assumed. We are currently attempting to extend these results to other polar alkenes and other enones.

(*S*)-α-Amino-2,2′-bipyridine-6-propanoic Acid: A Versatile Amino Acid for de Novo Metalloprotein Design

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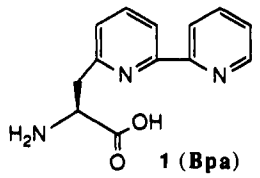
The de novo synthesis of metalloproteins[1] is limited by the number and diversity of *naturally* occurring metal-binding amino acids, particularly when compared to the wide variety of synthetic ligands which are available for selective complexation of metal ions in aqueous media.[2] Thus, a current objective is to expand the repertoire of protein building blocks through the design and construction of *synthetic* metal-binding amino acids that, when incorporated into a three-dimensional structural framework created by a polypeptide, may exhibit enhanced metal cation selectivities and a wider range of metal coordination geometries over those which are presently available.[3] Toward this goal, we report a preliminary evaluation of metal cation coordination to peptides which incorporate the unnatural amino acid (*S*)-α-amino-2,2′-bipyridine-6-propanoic acid (**1**), Bpa.[4]



1 (Bpa)

Ac-Bpa-Thr-Pro-D-Ala-Val-Xaa-NH_2

2: Xaa=Bpa

3: Xaa=Phe

The sequence of peptides studied was designed to include a central prolyl-D-amino acid dipeptide which has been shown to favor reverse turn (β-type II) formation in solution.[5] Hence, it was anticipated that the intramolecular metal-binding capabilities of **2** should be enhanced due to the proximity of the two bipyridyl side chains. In order to investigate the relative contributions of intra- and intermolecular coordination to metal binding, peptide **3** was also prepared.

Table I. Summary of Dissociation Constants for Peptides **2** and **3** with Divalent Metal Cations

metal ion	K_d,[a] M	
	2	**3**
Zn(II)	$9.6 \pm 0.6 \times 10^{-4}$ [b]	$2.7 \pm 0.3 \times 10^{-3}$
Co(II)	$1.1 \pm 0.2 \times 10^{-4}$ [b]	$1.1 \pm 0.1 \times 10^{-3}$
Cd(II)	$1.1 \pm 0.2 \times 10^{-4}$ [b]	$4.3 \pm 0.3 \times 10^{-3}$
Ni(II)	$1.1 \pm 0.2 \times 10^{-6}$	$5.0 \pm 0.5 \times 10^{-5}$
Cu(II)	$<1.0 \times 10^{-8}$ [c]	$0.8 \pm 0.2 \times 10^{-7}$

[a] Equilibrium binding constants were determined by UV spectroscopy at various metal cation concentrations at 25 °C in the presence of 200 mM NaCl. Dilute peptide solutions (<2 μM) were run in a 10-cm-path quartz cuvette; in all other cases a standard 1-cm-path cuvette was used. A minimum of three determinations were recorded in each case. [b] The peptide to metal stoichiometry was assumed to be 1:1. [c] Limiting value for K_d of **2** with Cu(II). The sensitivity of the UV method limits assessment at lower concentrations.

In the absence of metal ions, the electronic spectra of aqueous solutions of the peptides, **2** and **3**, show an absorption at 285 nm due to the bipyridyl moiety. Upon addition of divalent metal cations, this absorption decreases in intensity with the concomitant appearance of two new absorptions at around 240 and 310 nm.[6,7] Given that two well-defined isosbestic points are associated with this transformation, UV spectroscopy provides a convenient method for obtaining the dissociation constants,[8] as well as information concerning the stoichiometry of the metal-bound complexes.

As can be seen from Table I, the dissociation constants vary over 5 orders of magnitude, with the tightest binding being observed for Cu(II) and the weakest for Zn(II). The relative affinity for metal follows that predicted by the Irving-Williams order of stabilities; however, the magnitude is smaller than observed for parent complexes of 1,10-phenanthrolines and 2,2′-bipyridyl.[9] This diminished affinity is in part due to steric hindrance imposed by substitution at the 6-position of the 2,2′-bipyridyl unit of **1**.[10,11] The selectivity shown in Table I complements that reported for peptides containing unnatural oxygen-containing bidentate ligands[3b] or "zinc finger" proteins.[1e,12]

For binding of Cu(II) and Ni(II), the stoichiometry of binding was found to be 2:1 bipyridyl:metal for **2** and 1:1 for **3**. This corresponds, in each case, to a 1:1 metal ion/peptide complex. Comparison of circular dichroism titration studies of **2** and **3** with Cu(II) also provides valuable structural information and demonstrates that the metal-bound complexes are quite distinct (Figure 1). With peptide **2**, an intense, positive ellipticity at 315 nm (attributed to the bipyridyl chromophore) is observed; in contrast, a weak and broad ellipticity (310-330 nm) is seen with peptide **3**. These spectroscopic studies indicate that Cu(II) induces the

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(10) For comparison, the values for K_d for 2,2′-bipyridyl (bipy), 1,10-phenanthroline (phen), and 2-methyl-1,10-phenthroline (2-Mephen) with divalent metals M(II) are provided (see ref 9a for more examples). K_d = [M(II)][L]/[M(II)L]: bipy and Zn(II), $K_d = 7.4 \times 10^{-6}$ M; bipy and Ni(II), $K_d = 9.1 \times 10^{-8}$ M; bipy and Cu(II), $K_d = 4.7 \times 10^{-7}$ M; phen and Zn(II), $K_d = 4.0 \times 10^{-7}$ M; phen and Ni(II), $K_d = 2.0 \times 10^{-9}$ M; phen and Cu(II), $K_d = 3.8 \times 10^{-8}$ M; 2-Mephen and Zn(II), $K_d = 1.0 \times 10^{-5}$ M; 2-Mephen and Ni(II), $K_d = 1.1 \times 10^{-6}$ M; 2-Mephen and Cu(II), $K_d = 3.9 \times 10^{-8}$ M.

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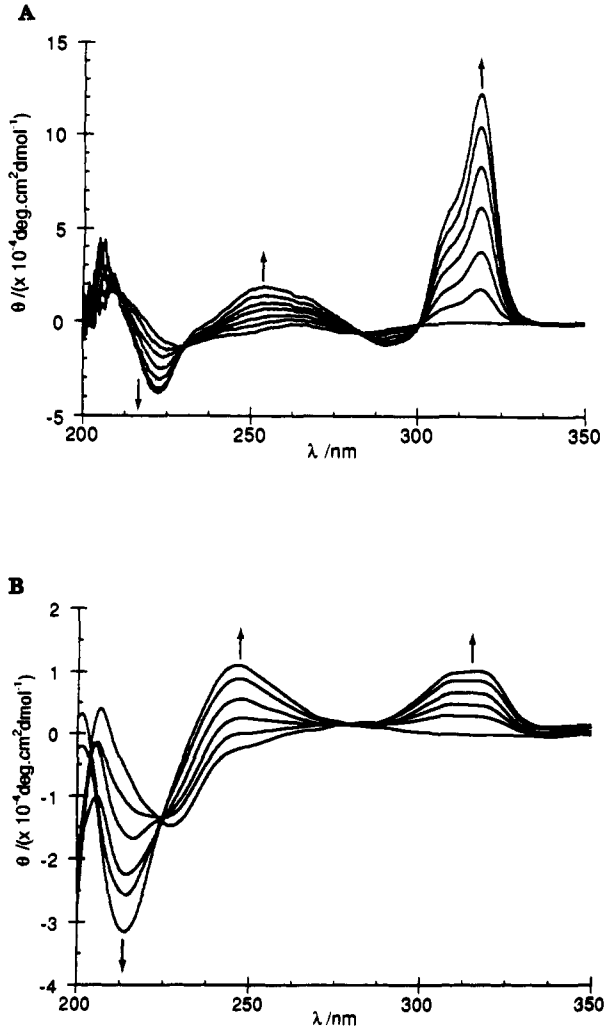

Figure 1. (A) Circular dichroism spectra of the titration of **2** (40 μM) with $CuCl_2$ (pH 6.6, 200 mM NaCl, 25 °C). $CuCl_2$ was added in 7.5-μM portions. (B) Circular dichroism spectra of the titration of **3** (40 μM) with $CuCl_2$ (pH 6.6, 200 mM NaCl, 25 °C). $CuCl_2$ was added in 7.5-μM portions.

formation of a chiral complex involving both bipyridyl ligands of **2**.

The coordination number of the metal in the peptide complexes was investigated through examination of the electronic spectra of the complex formed between **2** and Co(II).[13] Addition of Co(II) is accompanied by a weak absorption at 450 nm ($\epsilon_{max} <$ 100), which is suggestive of a five-coordinate complex. However, CD spectra of the Co(II) complex lacked the strong ellipticity observed with Cu(II) and this peptide. Thus, the conclusions from the Co(II) studies cannot provide general information regarding the coordination state of the other metal complexes, and in particular, alternative spectroscopic studies will be needed to establish the identity of the complex formed between **2** and Cu(II).

In conclusion, incorporation of the bipyridyl moiety into the polyamide framework of polypeptides should prove to be useful in de novo design of metalloproteins for both structural and functional roles, since it combines the wide scope of coordination chemistry available to this ligand with the versatility of protein biopolymers as templates for the assembly of organized three-dimensional structures.

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Registry No. 2, 136391-82-7; **3**, 136391-83-8; Zn(II), 23713-49-7; Co(II), 22541-53-3; Cd(II), 22537-48-0; Ni(II), 14701-22-5; Cu(II), 15158-11-9.

Supplementary Material Available: UV and CD spectra from titrations of **2** and **3** with $CuCl_2$ and of **2** with $CoCl_2$ and long-wavelength visible spectra from titration of **2** with $CoCl_2$ (5 pages). Ordering information is given on any current masthead page.

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Enantiofacial Protonation by Catalytic Antibodies

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Several enzymes generate enolates as reaction intermediates.[1-4] Strategies for efficient generation of various substituted enols in an antibody binding site should expand the scope of antibody catalysis. Herein, we report on catalytic antibodies which not only accelerate hydrolysis of enol esters but also influence enantiofacial protonation of the "enolate"[5] intermediate to afford an optically enriched α-substituted cyclohexanone.

Hapten **1** was utilized as the antigen for induction of antibodies (Chart I). Notable features within this structure include (a) the phosphonate moiety as a direct mimic of the tetrahedral transition state anticipated in the hydrolysis of the enol ester **2**[6] and (b) application of our abzyme–substrate destabilization principle[7] via the placement of the methyl group in the substrate adjacent to its original point on hapten **1**. Phosphonate **1** was synthesized using the Michaelis–Becker reaction[8] and conjugated to the protein keyhole limpet hemocyanin (KLH) for antibody induction.[9]

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