14. This is the first example of a controlled radical macrocyclization of an electrophilic radical.[13e] Further, the tin hydride promoted reductive removal of the nitrile group from a malononitrile is an unprecedented reaction of considerable generality. A forthcoming paper will address the scope of this reaction.

Iodomalononitriles are a readily available class of compounds that show great promise as versatile reagents in synthesis. The reactivity profile of substituted malononitrile radicals[5] goes well beyond that of existing electrophilic radicals. None of the transformations conducted in this paper have yet been accomplished with malonic ester radicals. Indeed, there are precious few bimolecular methods of any kind to form carbon–carbon bonds starting from unactivated di- and trisubstituted alkenes. Full details of the scope of the reactions reported herein and associated mechanistic studies will be reported in the near future.

Acknowledgment. We thank the National Institutes of Health (GM33378) and ICI, Americas, for funding of this work.

Supplementary Material Available: Representative procedures for each type of experiment reported, spectra for all products, and a model to assign the stereochemistry of **12** (9 pages). Ordering information is given on any current masthead page.

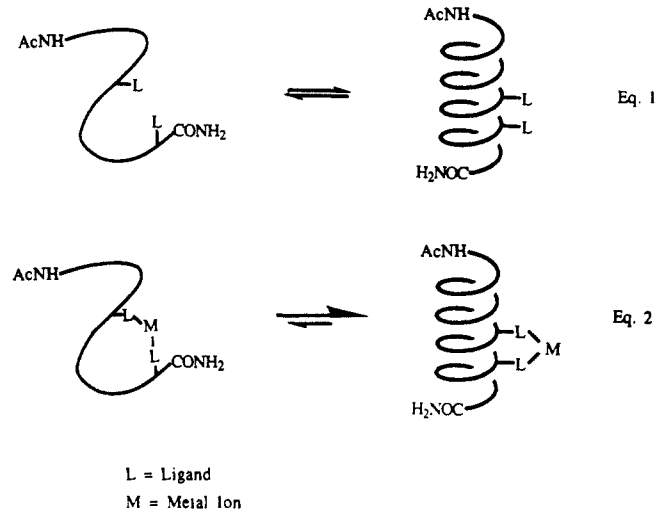

Figure 1. The coil-to-helix equilibrium of a peptide (eq 1) bearing two side chains capable of metal coordination should in theory be shifted to the right by simultaneous coordination to a single metal (eq 2), the result of reduction of entropy of the metal-coordinated coil conformation.

Metal Ion Enhanced Helicity in Synthetic Peptides Containing Unnatural, Metal-Ligating Residues

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The α-helical conformation adopted by 40% of all residues in proteins[1] is not, in isolation, energetically favored, as indicated by the existence of most short peptides in aqueous solution as random coils.[2] Protein helices and rare helical peptides apparently owe their existence to exogenous stabilizing factors.[3-5] Theory suggests that cross-links[6] stabilize the folded form of polypeptides by diminishing the entropy of the unfolded form relative to the acyclic counterpart (Figure 1).[7] We report here the use of metal ions as peptide side chain "cross-linking" agents.[8] The studies reveal that, for peptides containing metal-ligating residues, the position of the coil-to-helix equilibrium is strongly dependent on the number and spacing of ligating residues, tether length between backbone and ligand, and metal ion. In one remarkable case, an *11-residue* peptide is converted from random coil to ca. 80% helix content by addition of Cd^{2+} at 4 °C.

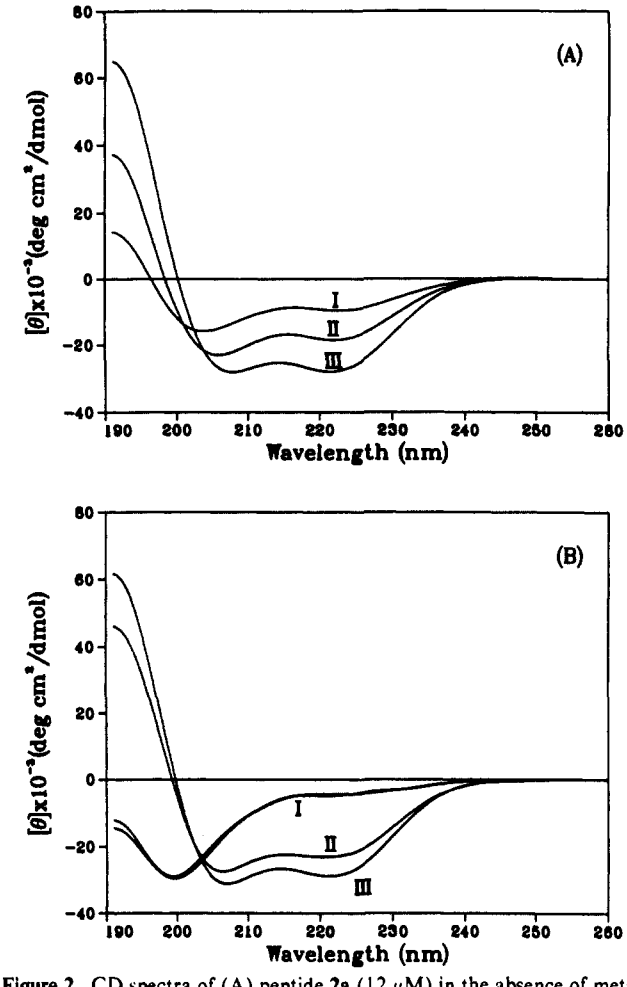

Figure 2. CD spectra of (A) peptide **2a** (12 μM) in the absence of metal ions (II) and in the presence of Cd^{2+} (III) or Ni^{2+} (I) (200 μM in metals) at 25 °C and (B) peptide **5a** (36 μM) in the absence of metals at 4 and 25 °C (I) and in the presence of Cd^{2+} (200 μM) at 25 (II) and 4 °C (III).

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Table I. Summary of Changes in $-[\theta]_{222}$ on Addition of Metal Ions to Peptides **1a–d**, **2a–d**, **3**, and **4c**

peptide	$-[\theta]_{222}$,[a] deg cm²/dmol	change in $-[\theta]_{222}$ with additive,[b] %				
		Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
1a	20 000	−45	−57	−17	−41	−26
1b	14 200	−5	−14	50	−1	4
1c	15 500	31	−5	0	3	−22
1d	13 800	26	−43	48	−8	−16
2a	18 600	−30	−48	−34	−34	50
2b	18 300	50	35	32	28	11
2c	18 400	26	−7	56	22	43
2d	17 800	33	23	18	23	24
3	17 500	−2	−5	0	−3	−8
4c	19 300	−10	−10	−20	−15	−13

[a] Peptide, 6–14 μM, pH 7.9 (200 mM aqueous sodium borate), 25 °C. [b] Metal ion, 200 μM.

Peptides **1** and **2** were designed to probe the structural optimum for helix stabilization by simultaneous chelation of two aminodiacetic acid bearing side chains. These peptides were expected

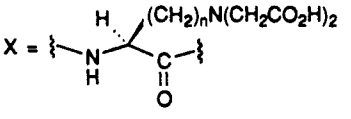
1a-d: Ac-XAla$_2$X(Ala$_4$GluLys)$_3$-NH$_2$

2a-d: Ac-XAla$_3$X(Ala$_4$GluLys)$_3$-NH$_2$

3: Ac-Ala$_3$(Ala$_4$GluLys)$_3$-NH$_2$

4c: Ac-Ala$_4$X(Ala$_4$GluLys)$_3$-NH$_2$

5a: Ac-XAla$_3$XAla$_4$GluLys-NH$_2$



a n = 1 **c** n = 3

b n = 2 **d** n = 4

to exhibit partial helicity in the *absence* of metal ions,[4,5,9] assuring the observability of small changes in equilibrium position. Circular dichroism (CD) spectra (190–260 nm) of peptides **1a–d** and **2a–d**[10] recorded with and without metal ions at 25 °C were characteristic of α-helix/coil equilibria[11] (Figure 2A), with one minimum between 202 and 207 nm and a second at 222 nm.[12] Reported in Table I is the metal ion induced percentage change in $-[\theta]_{222}$ observed on addition of metal ions; this roughly approximates the percentage change in helix content.

Nearly three-quarters of the 40 metal ion/peptide (**1a–d**, **2a–d**) combinations (Table I) significantly influenced the position of the coil-to-helix equilibrium, two-thirds of these increasing and the remainder decreasing helix content. Especially helix-stabilizing were the combinations **2c**/Cu^{2+}, **2a**/Cd^{2+} (Figure 2A), **1b**/Cu^{2+}, and **2b**/Co^{2+}; maximal destabilization was observed for **1a**/Ni^{2+}. The $i, i+4$ spacing (**2a–d**), indicated by models to accommodate simultaneous coordination of a single metal ion in the α-helical conformation more readily than the $i, i+3$ spacing, enhanced the helix content in 15 of 20 cases; the $i, i+3$ spacing (**1a–d**) led to enhancement of only four of 20. With one exception, all metal ions tested diminished the helicity of peptides **1a** and **2a**, suggesting that optimal metal ion chelation with this short tether is actually disruptive of the helical form. The exception was peptide **2a** in combination with the *largest* metal ion, Cd^{2+}.

Several observations support the model that in those equilibria in which helix content was enhanced, chelation of a single metal ion by a single peptide in both the helix and coil forms was important (e.g., Figure 1, Eq 2). Peptide **3**, which lacks ligand-bearing residues, showed only minor changes in the CD spectrum on addition of any metal ion. Peptide **4c**, containing only one ligand-bearing residue, exhibited a somewhat diminished helicity in the presence of all metal ions. Titration of peptide **2a** (12 μM) with Cd^{2+} revealed a maximal effect (within experimental error) on $-[\theta]_{222}$ at 1.0 equiv of Cd^{2+}; further addition (up to 16 equiv) afforded no additional change. Enhancement of helix content thus derives from species with a 1:1 stoichiometry (peptide **2a**:Cd^{2+}), likely with both ligands coordinated to metal ion in both coil and helical forms, and with a metal ion binding constant in the helical form of $>10^7$ M^{-1}. This high affinity for Cd^{2+}, as well as the observation that the helicity of both the metal ion free and Cd^{2+}-bound forms of peptide **2a** measured by CD were independent of peptide concentration in the range 10–100 μM, argues strongly against the involvement of peptide aggregation in these equilibria.

Especially remarkable was the observation that even peptides of negligible helix content could be forced into predominantly the helical conformation by this approach. The CD spectrum of peptide **5a**, a truncated analogue of **2a**, in the absence of metal ion (Figure 2B) was diagnostic for the random coil conformation and was essentially independent of temperature in the range 4–45 °C, indicative of insignificant (<5%) helix content. In contrast, addition of excess Cd^{2+} yielded a temperature-dependent CD spectrum indicative of the temperature-sensitive helix–coil equilibrium and with a high helix content (Figure 2B). We conservatively estimate that **5a**/Cd^{2+} is 82% helix at 4 °C;[13] we believe that this peptide displays the highest helical potential of any peptide this size or smaller yet discovered.[11]

The utility of high-affinity, unnatural, metal-binding ligands in stabilizing helical structure is thus demonstrated. The wide range of results of Table I emphasizes the critical role of ligand spacing and metal ion identity, no doubt reflecting the need to maximize entropy reduction of the unfolded state while simultaneously minimizing disruption of the folded state.[7] A deeper understanding of the structural and energetic origins of these phenomena awaits further studies.[11] It seems probable that a wider substructure search will yield even more efficient stabilizers of folded structure. Metal ion stabilized helices may prove useful as building blocks for the construction of macromolecular systems possessing metal ion dependent, defined three-dimensional structure.

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Supplementary Material Available: CD spectra from which data in Table I were derived (6 pages). Ordering information is given on any current masthead page.

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(10) Synthetic details will be reported elsewhere. Residues X in protected form were obtained by manipulation of commerically available, protected L-α-amino acids (n = 1, N; n = 2, Q; n = 3, ornithine; n = 4, K). For **1c,d** and **2c,d**, X (n = 3, 4) was incorporated by manual coupling (DCC) of X's and the intervening A's onto an 18-mer on an MBHA resin (automated synthesis, *t*-BOC protection). Syntheses of **1a,b** and **2a,b** were by sequential manual couplings (DCC/HOBt) to an MBHA-bound 17-mer (automated synthesis) as follows. **1a** and **2a**: (a) Boc-AXA-OH; (b) Boc-A-OH (**2a** only); (c) Ac-XA-OH. **1b** and **2b**: (a) Boc-XA-OH; (b) Boc-A-OH; (c) Boc-A-OH (**2b** only); (d) Boc-XA-OH; (e) Ac_2O. Peptides were cleaved with HF and purified by HPLC. FAB MS of each purified peptide afforded the predicted molecular ion. Peptide **1b** (free amino terminus) was sequenced, with satisfactory results. Peptide concentrations were determined by HPLC quantitation of alanine (phenyl isothiocyanate derivatization) after acid hydrolysis.

(11) Although this work was inspired by consideration of the α-helical conformation of peptides, further studies will be necessary to assign the α-helical conformation (rather than 3_{10}, π, etc.) to these substances. NMR experiments to resolve this issue are underway.

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(13) Helix content calculation assumed limiting molar ellipticities of −35 000 and 0, respectively, for helix and coil. For peptide **5a**, a value $[\theta]_{222}$ (helix) of −30 000 is probably more appropriate and the helix content correspondingly higher than we presently conservatively state. See: Chang, C. T.; Wu, C.-S. C.; Yang, J. T. *Anal. Biochem.* **1978**, *91*, 13.