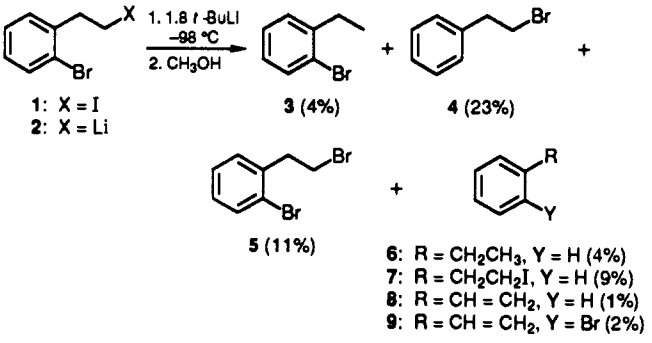
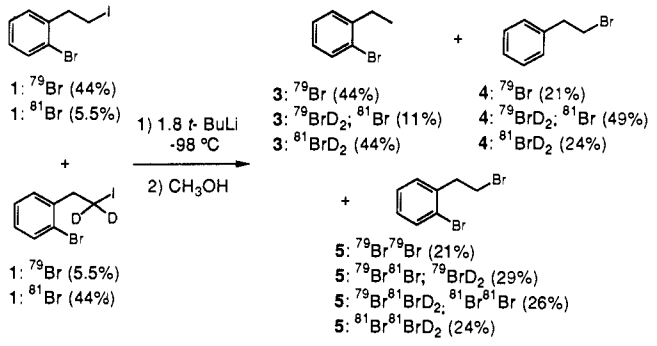
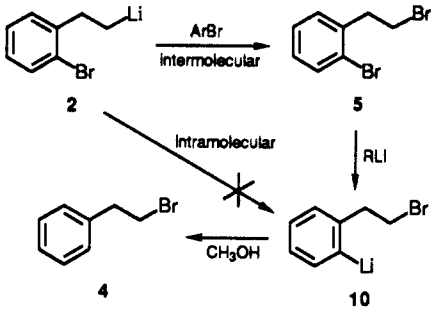


iment involving an equimolar mixture of **1**-^{79}Br and **1**-^{81}BrD$_2$ with minor amounts of **1**-^{81}Br and **1**-^{79}BrD$_2$ as shown. Analysis of the products by GC/MS shows **3** to be unscrambled, **4** to be fully scrambled, and **5** to be partially scrambled. When the reaction of labeled **1** is carried out in the presence of unlabeled lithium bromide, no incorporation of unlabeled bromide into **4** or **5** is observed.

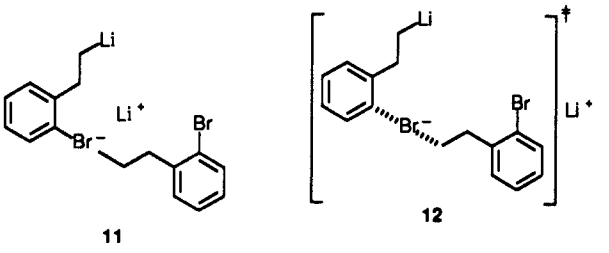


The significant result is that **2** is converted to **10** by an intermolecular process via **5**; the transfer of bromine from the aryl carbon to the methylene carbon apparently cannot proceed intramolecularly within the endocyclic restriction of a five-membered ring.



Four mechanisms have been suggested for the bromine–lithium exchange reaction: (1) a four-center process; (2) a stepwise process initiated by single electron transfer; (3) formation of an ate complex, and (4) an S_N2 reaction.[8] Reaction by the four-centered reaction should be possible intramolecularly for the conversion of **2** to **10**, so the present results do not support that mechanism in this case. Reaction by the pathway usually invoked for a single electron transfer which could be intermolecular would involve formation of an aromatic radical anion which expels bromide followed by bromide escape and capture by the alkyl radical of another molecule to give a radical anion that loses an electron to provide **5**. In this case incorporation of external bromide should be found in **4** and **5**. Since that was not observed, this version of the single electron transfer process is not consistent with our observations.[9]

The intermolecular conversion of **2** to **10** is consistent with a transition structure that requires the carbons entering and leaving the bromine to be at a large bond angle. This disposition of the carbons would be expected for apical substituents in the 10-Br-2 transition structure of either an ate complex or an S_N2 reaction shown as **11** and **12**, respectively.[8,10,11] Further tests utilizing homologues and systems in which a defined large angle between the alkyllithium and the aryl bromide is enforced are under way. The present results can be taken to support a mechanism of bromine–lithium exchange for the conversion of **2** to **10** that proceeds via an ate complex or S_N2 process, to discount the four-center mechanism, and to make a radical mechanism unnecessary.[12] The generality of this conclusion will be tested by investigation of other systems.



Acknowledgment. We are grateful to the National Institutes of Health and National Science Foundation for financial support.

Supplementary Material Available: Experimental details of the syntheses and reactions of labeled **1** (10 pages). Ordering information is given on any current masthead page.

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(12) This analysis is for the monomeric unit of an organolithium reagent which probably exists as an aggregate. An intraaggregate reaction for **2** which would be intermolecular by the double-labeling criterion and could involve a formal seven-membered ring in which the carbons might not be fully apically arranged is possible but is discounted by our preliminary observation that the reaction is also intermolecular in (*o*-bromophenyl)-*n*-pentyl iodide, a system in which a monomeric unit could rearrange by an eight-membered ring. That result also reinforces the conclusion about the four-center mechanism based on **2**.

Secondary Structure Nucleation in Peptides. Transition Metal Ion Stabilized α-Helices†

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It is unusual for monomeric peptides less than 20 residues in length to adopt an α-helical conformation in aqueous solution.[1] Formation of α-helices in disordered polypeptides is a classical nucleation event, with the energetically unfavorable formation of the first turn being rate limiting.[1,2] A few studies have been aimed at promoting α-helix formation by introducing conformational constraints in peptides.[3] These approaches often require

† This paper is dedicated to the memory of Professor Emil T. Kaiser.

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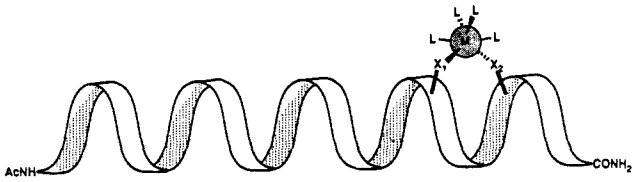

Ac-Ala-Glu-Ala-Ala-Ala-Lys-Glu-Ala-Ala-Ala-Lys-X_1-Ala-Ala-Ala-X_2-Ala-NH_2
Peptide A, X_1=Cys, X_2=His
Peptide B, X_1=X_2=His

Figure 1. Upper: Helix ribbon diagram illustrating metal ion complexation to the side chain of amino acid residues in positions i and $i + 4$ of an α-helix. Ac = acetyl. Lower: Sequences of peptides A and B.

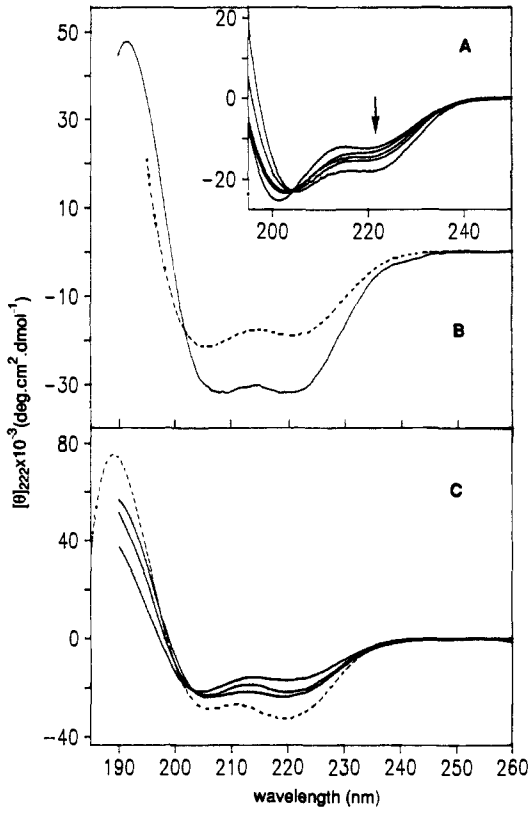

Figure 2. (A) CD spectrum of peptide A (5.9×10^{-6} M in 10 mM sodium borate, 0.5 mM mercaptoethanol, pH 8.0, 21 °C) in the presence of increasing amounts of $CdCl_2$. From top curve (arrow) to the bottom, $[Cd^{2+}] = 0$, 1.0×10^{-5}, 2.5×10^{-5}, 4.0×10^{-5}, and 1.5×10^{-4} M. (B) Peptide A (2.2×10^{-6} M) in water, pH 6.65, at 4 °C (dotted curve) and in 7.3×10^{-4} M $CdCl_2$ solution, pH 6.7, at 4 °C (solid curve). (C) Solid curves: peptide B (1.8×10^{-6} M in 5 mM sodium borate, pH 6.1, at 21 °C) in the presence of 0, 3.3×10^{-5}, and 6.6×10^{-5} M $CuSO_4$. Dashed curve: peptide B, 1.8×10^{-6} M in 5 mM sodium borate, 7.4×10^{-5} M $CuSO_4$, pH 6.4, at 0 °C.

considerable synthetic effort and will likely be difficult to apply to larger peptides and proteins. We report here a simple and versatile method for the stabilization of monomeric α-helices in water. The side chains of histidine and cysteine residues in positions i and $i + 4$ of a peptide can interact with transition-metal ions[4] to form a bidentate complex and concurrently fix the peptide backbone in an α-helical conformation. In this way, peptides of up to 75% α-helicity in water at room temperature and 90% α-helicity at 0–4 °C are obtained.

Peptides A and B (Figure 1) are similar to peptides designed by Marqusee and Baldwin to ensure high propensity for α-helix formation.[5] Peptides were synthesized by a solid-phase method

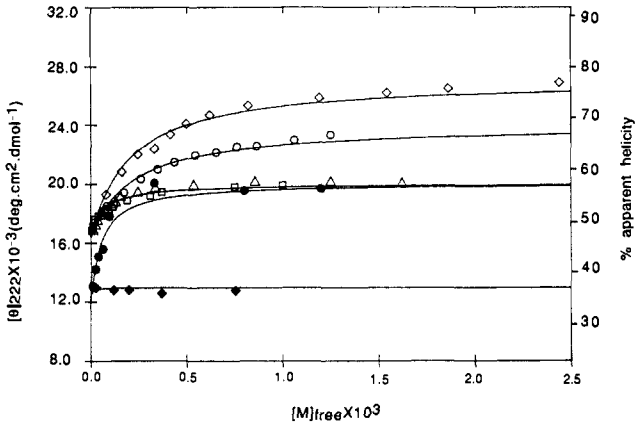

Figure 3. The dependence of mean residue molar ellipticity at 222 nm[9] of peptides A and B on the concentration of free metal ions. Peptide A (5.9×10^{-6} M in 10 mM sodium borate, 0.5 mM mercaptoethanol, 21 °C): (●) $CdCl_2$, pH 8.0, $K_d = 5.6 \times 10^{-5}$ M; (◆) $ZnCl_2$, pH 8.0. Peptide B (6.1×10^{-5} M in 5 mM sodium borate, 21 °C): (△) $ZnCl_2$, pH 7.5, $K_d = 7.5 \times 10^{-5}$ M; (□) $CuSO_4$, pH 5.3, $K_d = 6.6 \times 10^{-5}$ M; (○) $CdCl_2$, pH 7.5, $K_d = 2.2 \times 10^{-4}$ M; (◇) $NiCl_2$, pH 6.3, $K_d = 2.1 \times 10^{-4}$ M. The curves were fit to the data by using a nonlinear least-squares method.

using t-Boc chemistry[6] and purified by reversed-phase C_{18} HPLC.

Histidine residues were placed near the carboxyl termini of the peptides to maximize benefits from charge–helix dipole interactions.[7] Figure 2A shows the circular dichroism (CD)[8] spectrum of peptide A at 21 °C and in the presence of increasing amounts of $CdCl_2$. An enhancement in the helix content of the peptide is noted by the increase in the 222-nm minima. An isodichroic point occurs at 204 nm, which is characteristic of helix–coil transitions. Interaction of cadmium ion with histidine and cysteine side chains provides sufficient stabilization energy to induce up to 90% α-helicity at 4 °C ($[\theta]_{222} = -31\,500$).[9] In contrast, peptide A in the absence of metal ion is 54% α-helical ($[\theta]_{222} = -18\,800$) at the same temperature (figure 2B). Analogously, peptide B, with a pair of histidine residues, displays ~90% α-helicity ($[\theta]_{222} = -31\,100$) in the presence of Cu^{2+} ions at 0 °C (Figure 2C).

Metal ion selectivity is an expected consequence of site-specific metal–ligand interaction. The extent of helical induction, to a first approximation, depends on the affinity of metal ion toward the ligands employed, and on the compatibility of metal ion geometry and coordination sphere with the α-helical conformation. As shown in Figure 3, both Cu^{2+} and Zn^{2+} bind peptide B with similar affinities and increase the helical content to the same extent. On the other hand, Ni^{2+} exhibits a similar binding constant with respect to Cd^{2+} but shows higher helical induction. Strikingly, addition of K_2PdCl_4 and K_2PtCl_4 destabilizes the helical conformation, which is either the result of their unfavorable square-planar coordination geometry or, most likely, due to nonspecific binding to the peptide backbone.[4,10] It is also in-

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(9) Both peptides are >99% pure as judged by analytical HPLC and have been characterized by amino acid analysis and FAB MS. All reported CD data have an uncertainty of ±2–5%. For 100% helix, $[\theta]_{222} = -35\,000$ and is based on several CD measurements for both peptides in the presence and absence of metal ions in various TFE/H_2O mixtures at −10 to 20 °C. Also see ref 5 and see: (a) Lyu, P. C.; Marky, L. A.; Kallenbach, N. R. *J. Am. Chem. Soc.* **1989**, *111*, 2733. (b) Shoemaker, K. R.; Kim, P. S.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Nature* **1987**, *326*, 563.

teresting to note that while peptide B displays considerable helical induction in the presence of Zn^{2+}, peptide A is Cd^{2+} selective and addition of Zn^{2+} has no effect on the helical content. In addition, helicity is independent of concentration of added NaF up to 250 mM for both peptide A (2.5 μM in 5 mM sodium borate, pH 8.0) and peptide B (2.0 μM in 5 mM sodium borate, pH 6.1). A and B show CD spectra independent of the peptide concentration in the presence and the absence of metal ions in the measured range of 0.5–70 μM, consistent with intramolecular helical structures.[11] Nonligated metal coordination sites are most likely occupied by water molecules, and addition of external ligands such as 5-nitro-1,10-phenanthroline or mercaptoethanol does not affect the stability of the helical conformation.

Support for the metal ion complexation site comes from NMR studies. Both of the histidine 2-H and 4-H resonances in peptide B (2.5 mM in D_2O, pH 6.6) occurring at δ 7.87 and 7.74 and δ 6.89 and 6.87 show upfield shifts upon addition of Zn^{2+} to δ 7.75 and 7.71 and δ 6.87 and 6.67, respectively. Similar results are obtained for peptide A (2.5 mM in D_2O, pH 6.5) in the absence (δ 7.91 and 6.95) and the presence of Cd^{2+} (δ 7.71 and 6.91). Of the 17 backbone amide protons in peptide A (3.0 mM, $CdCl_2$ 0.3 M in H_2O, pH 5.1), 11 have been sequentially assigned by using COSY and NOESY spectra.[12] Interestingly, amide resonances for N-terminal amino acid residues exhibit $^3J_{HN\alpha} < 5$ Hz, which is further evidence that helical structure extends to the N-terminus.

The above studies indicate that unprecedented levels of helicity can be induced in short monomeric peptides by taking advantage of selective metal ion complexation. Detailed structural characterization of these peptides using 2-D NMR techniques is currently in progress. We are also utilizing unnatural amino acid side chains as potential ligands as well as studying the feasibility of stabilizing proteins by this approach.

Acknowledgment. We thank our colleagues R. Lerner, L. Walters, J. Dyson, P. Wright, B. Iverson, D. Hilvert, D. Rideout, C. Wong, and A. Satterthwait for helpful discussions and criticisms.

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Solution Structure of $(PPh_2Me)_2Fe(CO)(\eta^2\text{-}C(O)Me)I$. Direct DNMR Evidence for a Facile Alkyl ↔ η^2-Acyl Equilibrium

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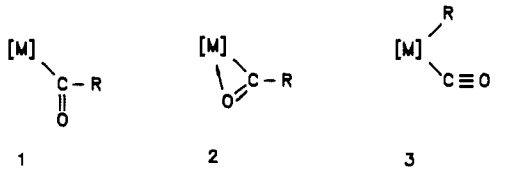
Transition-metal oxophilicity[1,2] and steric[3-6] factors have emerged as crucial parameters which determine the relative stability of mono- and bidentate acyl coordination modes **1** and **2** respectively. Although coordinatively saturated bidentate acyl



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structures have been *implicated* as intermediates in CO insertion chemistry,[7-12] little direct evidence[13] exists regarding the relative

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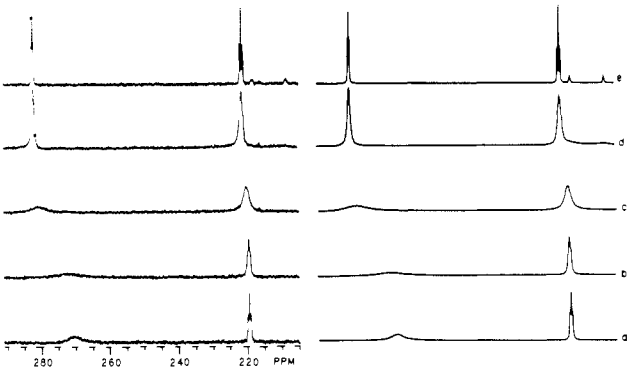
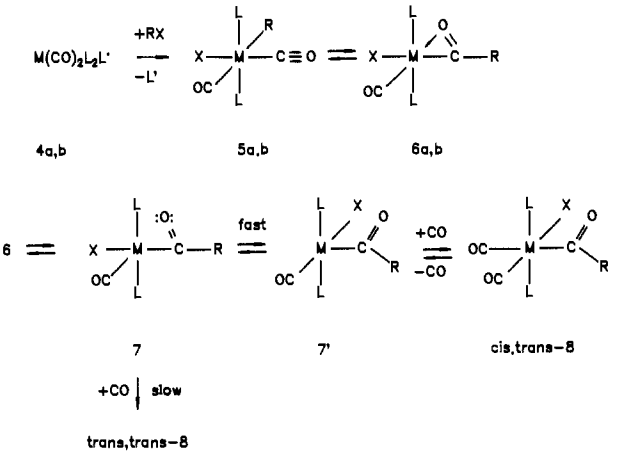


Figure 1. Variable-temperature ^{13}C NMR spectra in CD_2Cl_2 and DNMR simulations for **5b** ↔ **6b**: a, 309.5 K; b, 296.8 K; c, 270.0 K; d, 244.6 K; e, 220.0 K.

Scheme I[a]



[a] **a**: L = L' = PPh_3, M = Ru, R = Me.[13] **b**: L = PPh_2Me, L' = MeCN[4] or N_2,[6] M = Fe, R = Me.

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(13) In an early, insightful analysis of the solution IR spectra, Roper proposed that **5a** and **6a** (M = Ru, L = L' = PPh_3, R = Me) were in rapid equilibrium in solution; cf.: Roper, W. R.; Taylor, G. E.; Waters, J. M.; Wright, L. J. *J. Organomet. Chem.* **1979**, *182*, C46-C48.

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