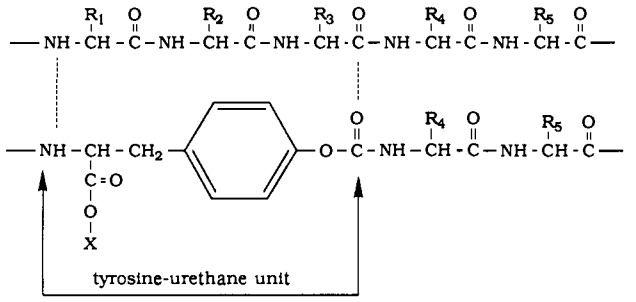

Figure 1. Overlap of the tyrosine–urethane unit with a regular tripeptide. The tyrosine–urethane unit is nine atoms long, corresponding (at least in a formal sense) to the length of a tripeptide segment.

Scheme I

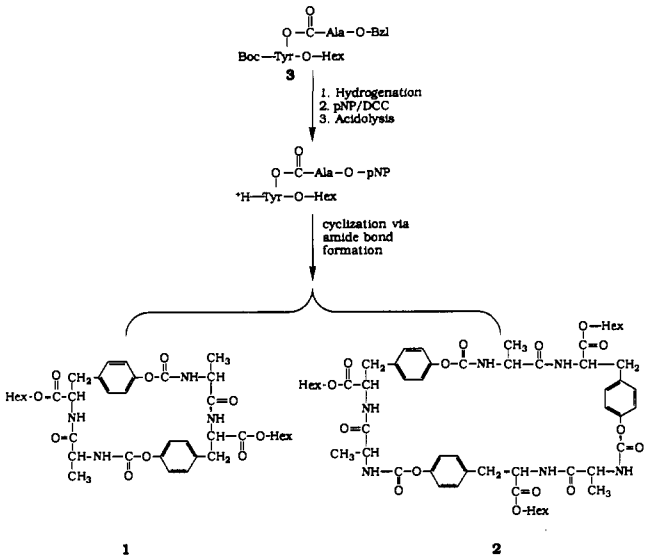

Table I. Metal Binding Studies[a]

peptide derivative	metal salt	picrate ions solubilized, mol/L	molar ratio of [metal]/[peptide][b]
none	Li picrate	~0	na[c]
none	Na picrate	~0	na[c]
none	K picrate	~0	na[c]
3	Li picrate	2.142×10^{-4}	0.026/1
3	Na picrate	1.075×10^{-4}	0.013/1
3	K picrate	3.055×10^{-5}	0.003/1
1	Li picrate	5.271×10^{-3}	0.636/1
1	Na picrate	2.100×10^{-4}	0.025/1
1	K picrate	5.666×10^{-5}	0.007/1

[a] 3 or 1 was dissolved in anhydrous chloroform, and an excess amount of either Li picrate, Na picrate, or K picrate was added. After stirring for 24 h, the excess of undissolved alkali picrate was removed and the amount of picrate ions present in the organic phase was determined by UV spectroscopy (see supplementary material for additional details). [b] Molar ratio of complexed metal ions to total peptide. [c] Not applicable.

cyclization promoter. Obviously, the Ala residue in **1** can be replaced by other amino acids.

The potential application of **1** as a cryptand was explored by examining the ion binding specificity of **1** toward alkali-metal ions (Li, Na, K) in a "phase-transfer" experiment[14] (Table I). Alkali picrates are virtually insoluble in chloroform. In the presence of peptides **3** or **1**, however, various amounts of metal ions were solubilized in the organic phase. The data reported in Table I were obtained after a uniform interval of 24 h, at which time the solubilization process had reached equilibrium.

When the linear pseudo*di*peptide **3** was used as the complexing agent, about 8 times more Li^+ ions than K^+ were solubilized, indicating a low degree of selectivity for Li^+ over K^+ ions. In absolute terms, however, only very small amounts of alkali ions were transferred into the chloroform phase, as indicated by the very low ratio of metal ions to peptide (see Table I). On the other hand, the macrocyclic pseudopeptide **1** solubilized about 0.6 molar equiv of Li^+ ions, which was 25 times higher than the amount of Na^+ and 91 times higher than the amount of K^+ ions. Thus, **1** is an effective and selective phase-transfer agent for Li^+ ions.

Since the structure of **1** can be readily modified by changing the C-terminus protecting groups or by replacing Ala by other L- or D-amino acids, our synthetic approach gives rise to a family of new macrocyclic pseudopeptides that may be used as synthetic building blocks in numerous bioorganic applications.

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Supplementary Material Available: Experimental details for the synthesis and characterization of **1** (6 pages). Ordering information is given on any current masthead page.

Physical Characterization of a Totally Synthetic 2[4Fe-4S] Clostridial Ferredoxin

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The 2[4Fe-4S] ferredoxins (Fd) serve as electron carriers in fermentative and photosynthetic pathways. In order to determine the influence of specific amino acids on the electron transferring properties of bacterial ferredoxins, Rabinowitz and co-workers have used semisynthetic procedures to substitute or delete specific amino acids near the N-terminus of native ferredoxins.[1-3] In this paper we report the first totally synthetic 2[4Fe-4S] ferredoxin, which, along with future synthetic variants, will be used to determine the effects of specific amino acid residues throughout the protein upon both its equilibrium and dynamic properties. The apoprotein was synthesized via standard tBOC procedures on polyvinyl (PAM) resins based on principles outlined by Merrifield.[4]

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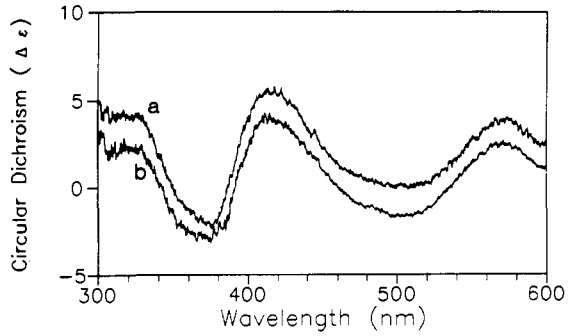

Figure 1. Circular dichroic spectra of 33 μM (a) *C. pasteurianum* Fd and (b) synthetic Fd in 0.1 M NaCl and 50 mM Tris, pH 8. Spectra are offset 0.1 deg/(M cm).

The 55 amino acid sequence, which was confirmed immediately after synthesis, was that of the *Clostridium pasteurianum* Fd:[5]

```
1          11         21         31
AYKIADSCVS CGACASECPV NAISQGDSIF VIDADTCIDC

41         51
GNCANVCPVG APVQE.
```

The native apoprotein, prepared as previously described,[6] and synthetic apoprotein eluted to identical positions on high-resolution PAGE gels. In a modification of a previously reported protocol to reconstitute the iron–sulfur clusters,[6] the synthetic apoprotein was incubated anaerobically in a 20-fold molar excess of DTT and 50-fold molar excess of both $FeCl_3$ and Na_2S at 25 °C for 3 h. All synthetic holoprotein used throughout this study was purified by salt gradient chromatography.

The synthetic Fd differs significantly from previous [4Fe-4S]$^{2-/3-}$ analogues which contain 12 or fewer amino acids.[7] The major differences are that the apoprotein ligates two clusters and that one of the four cysteine residues which chelates each cluster is from a separate and distant segment of the polypeptide chain. Lastly, proper folding of the synthetic Fd may be critical in cluster formation since the synthetic peptide is synthesized from the C → N terminus, in contrast to the native protein which is synthesized in vivo from the N → C terminus.

The visible spectra of the oxidized native and synthetic ferredoxins were virtually identical (figure not shown). Similarly, the circular dichroic spectra of the oxidized native and synthetic holoprotein were identical as shown in Figure 1, indicating that the synthetic holoprotein is folded properly. The EPR spectra of reduced synthetic ferredoxin provides additional evidence for two spin-coupled [4Fe-4S] clusters (figure not shown). The principal *g* values determined for reduced synthetic Fd (1.89, 1.94, 2.05) are in excellent agreement with the *g* values (1.91, 1.94, 2.06) previously determined for reduced native *C. pasteurianum* Fd.[8] The partial reduction of the synthetic ferredoxin by *C. pasteurianum* hydrogenase at pH 8 and its full reduction by dithionite (see Figure 2) demonstrate its electron-transferring competence.

The reduction potential of both the synthetic and native Fd was −400 mV vs NHE at pH 8. Both reduction potentials were pH independent as determined directly at an edge pyrolytic graphite electrode[9] using square-wave voltammetry as previously described.[10] The reduction potential for previous analogue clusters, which are isoelectronic with Fd's, are characteristically 100 mV more negative than native Fd clusters. The difference between

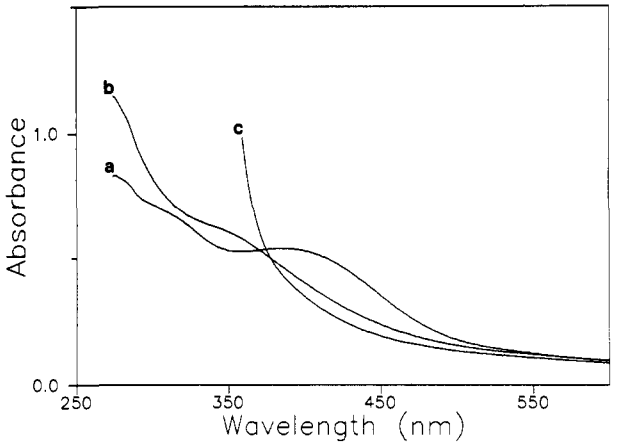

Figure 2. UV–visible spectra of 20 μM synthetic Fd in 0.5 M NaCl and 50 mM Tris, pH 8, where the Fd is (a) fully oxidized, (b) partially reduced by hydrogenase, and (c) fully reduced by dithionite.

the reduction potential of previous analogues and that of native clusters has been attributed to the protein matrix of native Fd's[11] and is further confirmed by this study.

We are presently using the above methods to examine ferredoxin variants, including a tyrosine-2 → histidine-2 variant which should result in a ferredoxin with a pH-dependent reduction potential as previously suggested.[10] While site-directed mutagenesis is a powerful tool for investigating the influence of the polypeptide on functionality, in this communication we have demonstrated for the first time that totally synthetic methods can be used successfully and with comparable ease to study a naturally occurring and biologically important metalloprotein.

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Dimolybdenum and Ditungsten Derivatives of the Trisilanol $[(c\text{-}C_6H_{11})_7Si_7O_9(OH)_3]$: $[(c\text{-}C_6H_{11})_7Si_7O_{12}]_2Mo_2$ (M≡M) and $[(c\text{-}C_6H_{11})_7Si_7O_{12}]_2W_2(\mu\text{-}H)(O\text{-}t\text{-}Bu)$

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The trisilanol $(c\text{-}C_6H_{11})_7Si_7O_9(OH)_3$, represented by I, is a potential source of a −3 siloxy ligand with interesting steric and electronic requirements.[2] Metal ions bound to I or a −1, −2, or

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