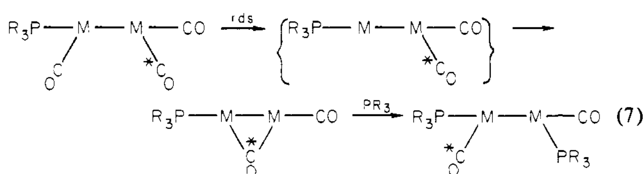
where only one PR_3 ligand is in an axial coordination site. The experiments carried out in these studies do not answer the question of whether an apical or axial phosphorus ligand is dissociated.[35] In either instance, intramolecular ligand rearrangements would accompany formation of the observed product. For example, the reaction of $Ir_4(CO)_8(PEt_3)_4$ with ^{13}CO did not result in any stereoselectivity of ^{13}CO incorporation in the trisubstituted cluster product (see ^{13}C NMR in Figure 2).

Activation parameters for PEt_3 dissociation in $Ir_4(CO)_8(PEt_3)_4$ were obtained from the temperature-dependent rate data provided in Tables VI and VII. The enthalpy (ΔH^*) and entropy (ΔS^*) of activation for Ir–PEt_3 bond disruption were determined to be 33.0 ± 2.7 kcal/mol and 19.1 ± 7.9 eu in C_2Cl_4.[32] The analogous parameters in the noninteracting heptane solvent were found to be 32.4 ± 2.2 kcal/mol and 18.7 ± 6.8 eu, respectively. Similar activation parameters were derived for P-n-Bu_3 dissociation in $Ir_4(CO)_8(P\text{-}n\text{-}Bu_3)_4$. Hence, these activation parameters, coupled with the small dependence of the rate on solvent, are consistent with a phosphine dissociation process.

Concluding Remarks. We have demonstrated that the spatial requirements of the phosphorus ligands render an important role in both phosphine and carbon monoxide ligand substitutional processes involving tetranuclear iridium cluster derivatives. The rate acceleration arising from sterically induced ligand dissociation observed herein in these Ir_4 species resembles that noted previously in mononuclear metal carbonyl phosphine complexes.[16,18] Concomitantly, it is recognized that electronic factors are influential to varying extents in these processes. However, this electronic effect may be akin to cis labilization in mononuclear metal complexes (a transition-state phenomenon),[36] as suggested by Sonnenberger and Atwood,[2] and would thus require loss of a ligand (CO) from a prior phosphine-substituted metal center. Hence, the rate-determining step (rds) in this latter mechanism (the skeletal sequence shown in reaction 7) does not necessitate the



presence of more than one metal center or, in other words, does not define cooperativity, i.e., metal atoms working together for a common purpose (ligand dissociation). On the other hand, steric acceleration arguments only require the ligand which is dissociated to be close proximity to the metal center which has been previously substituted.[37] Thus this steric mode of inducing ligand dissociation may better fit the definition for cooperativity by virtue of the cluster's framework dictating spatial confrontations between ligands.

Acknowledgment. The financial support of the National Science Foundation through Grant CHE 80-09233 is greatly appreciated.

Registry No. $Ir_4(CO)_9(PMe_3)_3$, 81616-62-8; $Ir_4(CO)_9(PPh_3)_3$, 19631-23-3; $Ir_4(CO)_9(P\text{-}i\text{-}Pr_3)_3$, 40919-64-0; $Ir_4(CO)_9(PEt_3)_3$, 28829-02-9; $Ir_4(CO)_9(P\text{-}n\text{-}Bu_3)_3$, 40919-66-2; $Ir_4(CO)_8(PMe_3)_4$, 78715-94-3; $Ir_4(CO)_8(PEt_3)_4$, 28829-04-1; $Ir_4(CO)_8(P\text{-}n\text{-}Bu_3)_4$, 28829-05-2; $Ir_4(CO)_8(P(POMe)_3)_4$, 81616-63-9; $Ir_4(CO)_8(P(OPh)_3)_4$, 81616-64-0; $Ir_4(CO)_8(PPh_2Me)_4$, 52390-85-9; $Ir_4(CO)_{12}$, 18827-81-1.

(35) Attempts at providing information in this regard are planned employing metal clusters containing mixed phosphine ligands.
(36) Lichtenberger, D. L.; Brown, T. L. *J. Am. Chem. Soc.* **1978**, *100*, 366 and other contributions to this series.
(37) The origin of the steric accelerations may be a ground-state property (e.g., as seen in the $Ir_4(CO)_8(PR_3)_4$ species), but it is often a transition-state property where the relief of interligand steric repulsions upon ligand dissociation allows the remaining metal–ligand bonds to attain values closer to their electronic equilibrium positions.

Peptide Formation in the Presence of a Metal Ion Protecting Group. 4. Synthesis of Penta- and Hexapeptide Sequences with the Pentaamminecobalt(III) Complex

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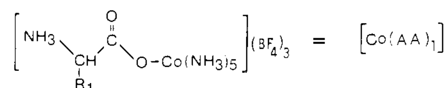
Contribution from the Department of Chemistry, Rutgers, The State University of New Jersey, New Brunswick, New Jersey 08903. *Received October 9, 1981*

Abstract: Kinetically inert metal ion protecting groups provide radically different approaches to the protection of the terminals and side chains of amino acids and peptides. Pentaamminecobalt(III) is a useful C-terminal protecting group for sequential peptide synthesis. This is demonstrated by the synthesis of a number of amino acid complexes of the type



where R = hydrophilic, hydrophobic, aliphatic, and aromatic amino acid side chains. The reaction of these complexes with Boc-amino acid active esters or Boc symmetric anhydrides results in the formation of $[Co(AA)_1(AA)_2Boc]$. The Boc group is removed with 95% trifluoroacetic acid to form $[Co(AA)_1(AA)_2]$, which is further used for sequential peptide synthesis. Alternatively, the $[(NH_3)_5Co^{III}–]$ group is selectively removed by rapid reduction with $NaBH_4$ or NaHS to form the N-protected peptide fragment, $[(AA)_1(AA)_2Boc]$, under extremely mild conditions. Tests for racemization of the amino acid directly bound to cobalt showed no detectable loss of chirality of $[(AA)_1]$. The detailed synthesis, monitoring, purification, and analysis of cobalt pentaammine derivatives of Leu-enkephalin (TyrGlyGlyPheLeu), Met-enkephalin (TyrGlyGlyPheMet), and the hexapeptide sequence HisGlyHisGlyHisGly are described and so is a general procedure for peptide synthesis with the cobalt(III) protecting group. The individual fragments of these peptide sequences are characterized by HPLC. The attractive features of the $[(NH_3)_5Co^{III}–]$ protecting group include its color, tripositive charge, ease of introduction, and removal. Such properties are invaluable in the purification of synthetic peptides.

During the development of synthetic peptide chemistry, many references were made to the potential use of metal ions in the protection and activation of amino acid derivatives.[1-4] One of the early uses of metal ion chelates in peptide synthetic chemistry

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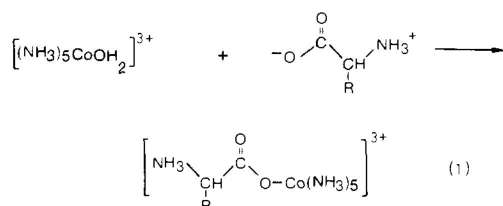
is in blocking the α-amino and carboxyl terminals of trifunctional amino acids so that their side chains can be selectively protected. A more ambitious role for metal ions in peptide synthesis was suggested by Collman[2] and Buckingham[3] on the reaction of *cis*-$[Co(en)_2L_2]$ (L is a solvent molecule) with amino acid esters in organic media. Their work showed that Co(III) can coordinate (and thus protect the amine terminal) and also activate the carboxyl moiety toward peptide formation. Although the synthetic aspect of this method was not competitive[19] with the current methodologies, a related reaction, namely the sequential hydrolysis of peptides with cobalt complexes, has shown promise and is still under current investigation.[4]

Our approach for the use of metal ions in peptide synthesis centers around limiting the metal ion to a protective role. Activation of amino acid derivatives is separately achieved by a variety of common organic reagents.[5] We have already shown[6] how specific Co(III) complexes have multiple advantages over a number of organic protecting groups. Furthermore, different metal ion complexes can perform different protecting roles in peptide synthesis (e.g., C- or N-terminal protection, side-chain protection, etc.).

This paper describes our detailed results on the use of $[(NH_3)_5Co^{III}-]$ as a C-terminal protecting group. The synthesis, monitoring, purification, and analysis of cobalt peptides of Leu-enkephalin (TyrGlyGlyPheLeu), Met-enkephalin (TyrGlyGlyPheMet), and the hexapeptide sequence HisGlyHisGlyHisGly are described. The advantages of using Co(III) as a C-terminal protecting group over organic esters are pointed out.

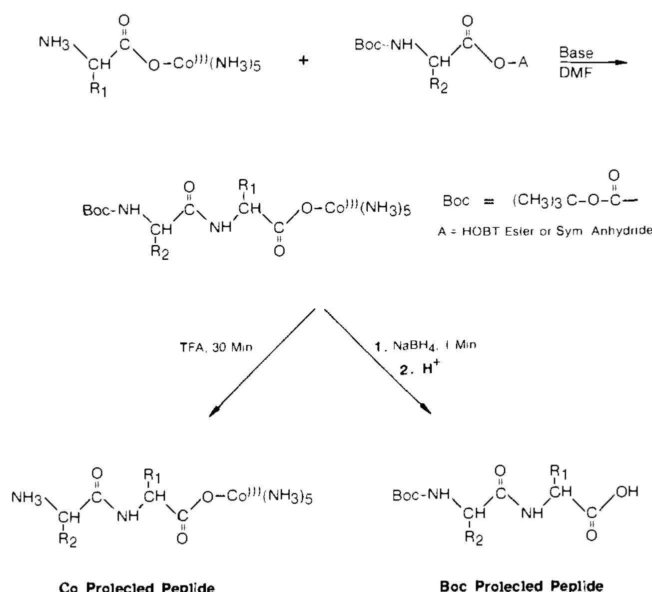
Results

A. Synthesis and Characterization of Cobalt(III)–Amino Acid Complexes. Some cobalt(III)–amino acid complexes have been synthesized previously as chloride and perchlorate salts.[7-10] The cobalt(III)–amino acid complexes reported here were synthesized starting with $[(NH_3)_5Co(OH_2)]^{3+}$ and the respective amino acid by using concentrated aqueous solutions of $[(NH_3)_5Co(OH_2)]^{3+}$ with an excess of saturated solutions of the amino acid at 80 °C (pH 3) for several hours (eq 1). In some cases the progress of

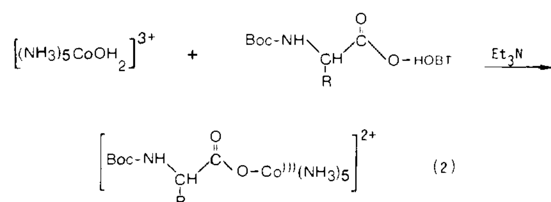


the reaction (eq 1) was monitored by HPLC. The cobalt–amino acid complexes (as fluoroborate salts) were characterized by their UV–vis spectra (λ_{max} 502–503 nm, ϵ 75–85 M^{-1} cm^{-1}), IR spectra, cobalt analysis, and amino acid analysis. Elemental analyses were obtained for some of the amino acid complexes.[6] With this method (eq 1) cobalt complexes of aspartic acid, glutamic acid (acidic), glycine, leucine, valine, alanine, phenylalanine, methionine, proline (neutral) and histidine, arginine, and lysine (basic amino acids) were synthesized.

Scheme I



For amino acids and derivatives that are not water soluble (e.g., tyrosine and N-protected amino acids), the method shown in eq 2 was used at room temperature. The cobalt complexes of Boc-glycine and Boc-*O*-benzyltyrosine were prepared by using the reaction in eq 2.



B. General Methods for the Synthesis of Pentaamminecobalt(III)–Peptides. 1. Formation of Cobalt–Peptide Complexes. Scheme I summarizes the general method for the synthesis of cobalt–peptide complexes. The Boc-amino acid is activated separately and is then added to a concentrated solution of the cobalt–amino acid or –peptide (dissolved in a minimum amount of DMF). The peptide formation was monitored quantitatively by following the disappearance of the band corresponding to the starting cobalt complex with HPLC[11] (using a water–methanol solvent system with 0.2% trifluoroacetic acid (TFA) with the pH adjusted to 2.5 with NaOH).

2. Purification of Cobalt–Peptide Complexes. The general scheme for the synthesis and purification of cobalt–peptide complexes is shown in Scheme II. After coupling the cobalt complex with Boc-amino acid, the N-protected cobalt peptides are purified as follows.

(1) Any excess Boc-amino acid, -active ester, or -symmetric anhydride was removed by extraction. The reaction mixture was added to ethyl acetate, and N-protected cobalt peptide was extracted from the organic layer with 5% aqueous acetic acid. *The cobalt–peptide derivative transfers completely to the aqueous layer (as judged by the pink color.)* The aqueous layer is then removed and extracted again with ethyl acetate to remove any organic impurities (excess Boc-amino acids, DCC, etc.). Quantitative evaluation of this extraction technique in the following syntheses showed <0.3% unreacted Boc-histidine contaminating the protected peptide, $[BocHisGlyCo(NH_3)_5]^{4+}$, after the extraction.

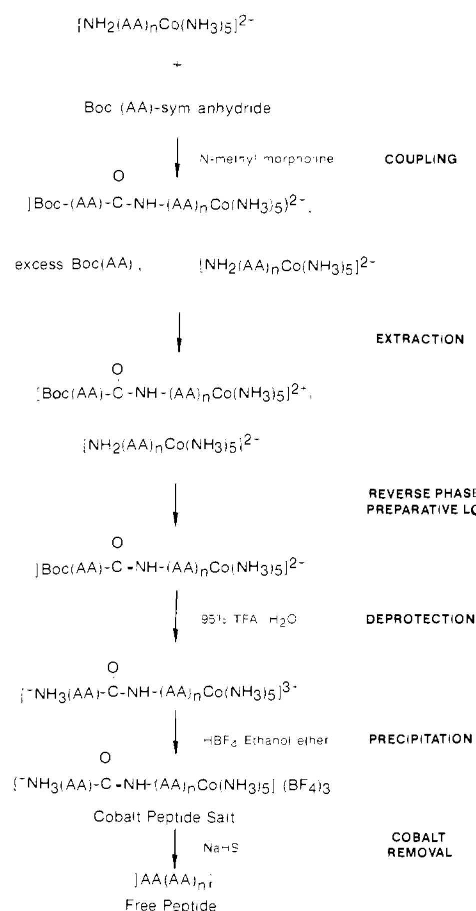
(2) Unreacted cobalt starting material was removed by using preparative octadecylsilane derivatized silica gel (C_{18}) columns

(1) (a) H. Jakubke and H. Jeschkeit, "Amino Acids, Peptides & Proteins", John Wiley & Sons, New York, 1977, p 52; (b) G. R. Pettit, "Synthetic Peptides", Vol. 1–5, Elsevier, Amsterdam, 1970–1980,
(2) J. P. Collman and E. Kimura, *J. Am. Chem. Soc.*, **89**, 6096 (1967).
(3) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.*, **89**, 4539, *ibid*, 2773 (1967).
(4) M. Rhee and C. Storm, *J. Inorg. Biochem.*, **11**, 17–24 (1979).
(5) M. Bodansky, Y. Klausner, and M. Ondetti, "Peptide Synthesis", John Wiley, New York, 1976.
(6) S. S. Isied and C. Kuehn, *J. Am. Chem. Soc.*, **100**, 6752–6754 (1978).
(7) (a) T. Yasui, J. Hikada, and Y. Shimura, *Bull. Chem. Soc. Jpn.*, **39**, 2417–24 (1966); (b) T. Yasui, J. Fujita, and Y. Shimura, *ibid.*, **42**, 2081–83 (1969); (c) T. Yasui, *ibid.*, **48**, 454–57 (1975); (d) T. Yasui and T. Ama, *ibid.*, **48**, 3171–74 (1975); (e) T. Yasui, *ibid.*, **49**, 472–76 (1976).
(8) J. H. Dunlop and R. Gillard, *Tetrahedron*, **23**, 349–352 (1967).
(9) C. Hawkins and P. Lawson, *Inorg. Chem.*, **9**, 6–11 (1970).
(10) J. Harrowfield, V. Norris, and A. Sargeson, *J. Am. Chem. Soc.*, **98**, 7282–7289 (1976).
(11) S. S. Isied, J. Lyon, A. Vassilian, and G. Worosila, *J. Liq. Chromatogr.*, **5**, 537–547 (1982).

Table I. Amino Acid Analysis for Leu-Enkephalin Fragments

	Leu	Phe	Gly	Tyr
$[LeuCo(NH_3)_5](BF_4)_3$	1.00 (1)			
$[PheLeuCo(NH_3)_5](BF_4)_3$	1.00 (1)	1.00 (1)		
$[GlyPheLeuCo(NH_3)_5](TFA)_3$	1.00	1.00	1.2 (1)	
$[GlyGlyPheLeuCo(NH_3)_5](TFA)_3$	1.00	1.00	2.16 (2)	
$[TyrGlyGlyPheLeuCo(NH_3)_5](TFA)_3$	1.00 (1)	0.95 (1)	2.00 (2)	0.89 (1)

Scheme II



with 0.2% sodium trifluoroacetate (NaTFA) (pH 2.5) in methanol–water or 0.4% isopropylammonium trifluoroacetate (pH 3.5) solvents. The N-protected cobalt–peptide retain on the column while the starting material was eluted. The N-protected cobalt complex was eluted by higher methanol concentrations in the above solvent system.

The Boc protecting group was removed from the cobalt–peptide complex by treating the complex with 95% trifluoroacetic acid (TFA) or 50/50 TFA–CH_2Cl_2 for 30 min at room temperature. The cobalt–peptide derivative was then precipitated as a fluoroborate or trifluoroacetate salt.

3. Removal of the $[(NH_3)_5Co^{III}–]$ Protecting Group. The $[(NH_3)_5Co^{III}–]$ protecting group was removed from the cobalt–peptide or cobalt-protected peptides in a few minutes at room temperature with mild reducing agents, e.g., $NaBH_4$, NaHS, or $(NH_4)_2S$. For the sulfide reagents, the black cobalt–sulfide solid was centrifuged and the peptide product was purified by chromatography (see Experimental Section).

C. Synthesis and Characterization of Leu-Enkephalin (Leu-Enk) and Met-Enkephalin (Met-Enk). With use of the above methods the fragments of $[Leu\text{-}Enk\text{-}Co(NH_3)_5]^{3+}$ were synthesized and characterized. Table I shows the amino acid analyses of the five synthesized fragments of $[Leu\text{-}Enk\text{-}Co(NH_3)_5]^{3+}$. Figure 1 shows a chromatogram for the resolution of all fragments of $[Leu\text{-}Enk\text{-}Co(NH_3)_5]^{3+}$, in a mixture prepared from the six pure components, with use of 0.2% TFA (pH 2.5) in methanol–water

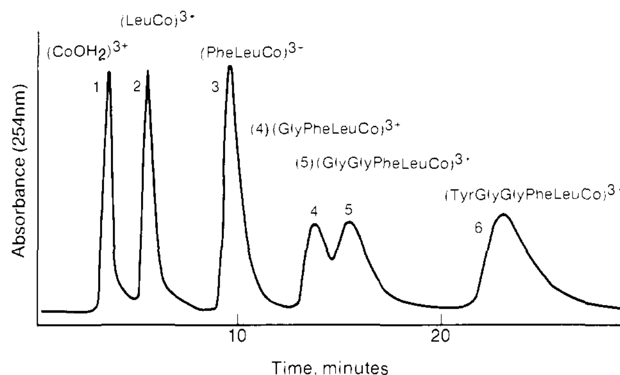


Figure 1. Elution profile of $[Leu\text{-}enkephalin\text{-}Co(NH_3)_5]^{3+}$ fragments. (This is a mixture prepared from the six pure fragments.) Chromatographic conditions: C_{18} column, flow = 2.0 mL/min, 40% MeOH–H_2O with 0.2% HTFA, pH 2.5 (adjusted with NaOH). Co = $[(NH_3)_5Co^{III}–]$.

Table II. Retention Times (min) of Cobalt(III) Derivatives of Leu-Enkephalin Fragments[a]

	free peptide[b]	cobalt–peptide[c] derivative
Leu		5.34
PheLeu	10.40	9.50
GlyPheLeu	12.95	13.70
GlyGlyPheLeu	12.15	15.30
TryGlyGlyPheLeu	15.54	22.97
standard Leu-enkephalin	15.62	

[a] Spherisorb ODS steel column (5μ), 40% methanol-water, 0.2% TFA, pH 2.5. [b] 1.5 mL/min. [c] 1.0 mL/min. λ = 254 nm.

solvent system on a C_{18}-derivatized silica gel column.

After removal of the $[(NH_3)_5Co^{III}–]$ protecting group, the synthesized Leu-enkephalin was compared to a standard sample with use of HPLC (Table II). Table II also shows the retention times of the synthesized Leu-enkephalin fragments, with and without the cobalt protecting group.

A separate analysis for the standard Leu-enkephalin and the synthesized sequence on an amino acid analyzer (0.35 M sodium citrate at 65 °C)[20] shows similar retention times for both compounds, using *o*-phthaldialdehyde derivatives and fluorescence detection.

Met-enkephalin (TyrGlyGlyPheMet) was synthesized and analyzed under conditions similar to those used for Leu-enkephalin. The retention times of the cobalt fragments on the waters RCM-C_{18} (10μ) column (at 2 mL/min) were as follows: 2.4 min, $[MetCo(NH_3)_5]^{3+}$; 4.24 min, $[PheMetCo(NH_3)_5]^{3+}$; 5.87 min, $[GlyGlyPheMetCo(NH_3)_5]^{3+}$; and 7.64 min, $[TyrGlyGlyPheMetCo(NH_3)_5]^{3+}$. Amino acid analysis of the synthesized Met-enkephalin was as follows: Met 1.00 (1), Gly 1.7 (2), Phe 0.92 (1), and Tyr 0.92 (1). The synthesized free Met-enkephalin had a similar retention time to that of the standard Met-enkephalin on a C_{18} reverse phase column (μ-Bondapak). The retention times of the synthesized and standard Met-enkephalin on an amino acid analyzer (using 0.35 M sodium citrate at 65 °C)[20] were identical.

D. Synthesis of the Hexapeptide HisGlyHisGlyHisGly. Individual fragments of the cobalt–peptide $[GlyHisGlyHisGlyHisCo(NH_3)_5]^{6+}$ were synthesized by using the methodology described (Schemes I and II). The peptide coupling of these individual fragments was monitored quantitatively with use of HPLC. Amino acid analysis was also carried out on each of the

Table III. Quantitative Monitoring of Peptide Formation of Cobalt Fragments

product cobalt–peptide	% coupling	isolated yield %	amino acid His:Gly
$[HisGlyCo(NH_3)_5](BF_4)_4$	99	80	1.00:0.97
$[GlyHisGlyCo(NH_3)_5](BF_4)_4$	95	78	0.53:1.00
$[HisGlyHisGlyCo(NH_3)_5](BF_4)_5$	94	63	0.95:1.00
$[GlyHisGlyHisGlyCo(NH_3)_5](BF_4)_5$	99	80	0.65:1.00
$[HisGlyHisGlyHisGlyCo(NH_3)_5](BF_4)_6$	92	63	0.96:1.00

Table IV. Retention Times (min) of Cobalt Peptides and Free Peptides on HPLC[a]

	free peptide	cobalt-peptide derivative
Gly	1.40	1.46
HisGly	1.75	1.87
GlyHisGly	2.00	2.07
HisGlyHisGly	2.89	3.72
GlyHisGlyHisGly	3.57	4.20
HisGlyHisGlyHisGly	7.28	10.46

[a] Radial Pak C_{18} column (10μ), 2.0 mL/min with 0.4% isopropylammonium trifluoroacetate in water.

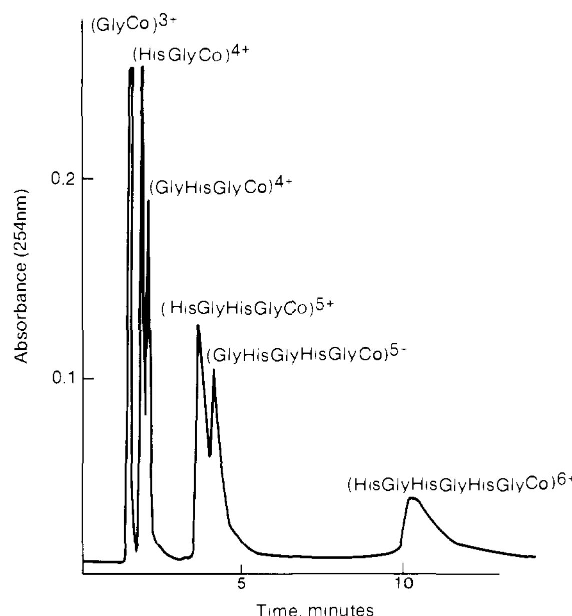


Figure 2. Elution profile of $[HisGlyHisGlyHisGlyCo(NH_3)_5]^{6+}$ fragments. (This is a mixture prepared from the six pure fragments.) Chromatographic conditions: C_{18} column, flow = 2.0 mL/min, 0.4% isopropylammonium trifluoroacetate in water, pH 3.5. Co = $[(NH_3)_5Co^{III}-]$.

purified fragments. Table III shows the percent coupling data, isolated yields, and amino acid analysis for the five individual fragments, beginning with $[GlyCo(NH_3)_5](BF_4)_3$. The coupling ranged from 92 to 99% for the five individual steps. The expected and observed amino acid analysis ratios verify the sequences synthesized. The isolated yields refer to the yield of cobalt–peptide complex after purification, precipitated as the fluoroborate or trifluoroacetate salt.

No significant racemization of histidine was observed in the synthesized hexapeptide, GlyHisGlyHisGlyHis. This was checked by subjecting a sample of free L-histidine and a sample of the hexapeptide to identical hydrolysis conditions and then treating the hydrolyzates with Boc-L-Leu-OSu.[12] Analysis of the dipeptides L-Leu-L-His and L-Leu-D-His formed from the standard L-His sample showed 7.3% L-Leu-D-His. The hexapeptide sample showed 6.2% L-Leu-D-His under the same conditions. Thus the histidine from the peptide undergoes almost equal racemization as the free L-histidine subjected to the same hydrolysis conditions.

Table IV shows the retention times of all cobalt–peptides and free peptide fragments on HPLC with 0.4% isopropylammonium trifluoroacetate in water as the eluent. Figure 2 shows an elution profile of the synthetic mixture of the pure cobalt–peptide fragments on HPLC. The fragments are eluted sequentially with the larger fragments retaining longer. The cobalt–hexapeptide fragment which is a 6+ ion at pH 3.5 is eluted at twice the retention time required to elute the pentapeptide fragment, thus making purification of this hexapeptide fragment possible with use of this solvent system.

Discussion

Inertness of the Co^{III}–Oxygen Bond in $[(NH_3)_5Co^{III}–O_2CR]$ Complexes. A large number of pentaamminecobalt(III)–amino acid complexes with hydrophobic, hydrophilic, and neutral amino acids have been synthesized.[6,7] The half-life for the carboxylate aquation from Co(III) has been determined for a number of similar Co(III) carboxylate complexes to be of the order of weeks in 0.1 M $HClO_4$,[13] and in organic media even slower aquation rates are expected. The rate of aquation was found to be insensitive to a variety of different substituents on the α carbon of the carboxylic acid. On the basis of these aquation rates, many cycles of peptide synthesis can be carried out on these Co(III) complexes without any measurable decomposition, especially in the presence of noncoordinating and weakly-coordinating anions such as BF_4^-, CF_3COO^-, $CF_3SO_3^-$, and ClO_4^-, which were used here. In the presence of Cl^- and other halide ions, aquation is expected to be more rapid.

Peptide Synthesis with the $[(NH_3)_5Co^{III}-]$ Protecting Group. The cobalt–peptides are synthesized with use of the $[(NH_3)_5Co^{III}$–amino acid] complexes (as BF_4^- salts) as the amine terminal and the Boc-protected amino acid as the carboxyl terminal. The carboxyl terminal is activated independently in CH_2Cl_2 to form an active ester or a symmetric anhyride by using the methods of organic peptide synthesis.[10] The two components are then mixed as concentrated solutions in DMF in the presence of mild bases (such as *N*-methylmorpholine), whereupon peptide formation proceeds very rapidly (ca. minutes) as monitored by HPLC (Scheme I). This is followed by purification, deprotection, and use in another cycle of peptide synthesis (Scheme II). In the examples we have studied so far, at the high concentrations used, rapid peptide formation and high yields of coupling (ca. 92–99%) are observed. Sequences including Gly, His, Phe, Leu, Met, and Tyr were synthesized with the $[(NH_3)_5Co^{III}-]$ protecting group. The yield of peptides obtained did not decrease as the number of residues increased in the series studied (Table III).

Racemization of the Amino Acid Bound to $[(NH_3)_5Co^{III}-]$.[12] In order for $[(NH_3)_5Co^{III}-]$ to be an acceptable protecting group for peptide synthesis, the chirality of the amino acid bound to it must be preserved. We have carried out a quantitative study[12] on the degree of racemization induced by the $[(NH_3)_5Co^{III}-]$ protecting group on the amino acid bound to cobalt in three representative cases: L-Leu, L-Phe, and L-His. The L-His-amino acid was chosen because of its known susceptibility to racemization.[1] No racemization (<0.3%) was induced by the $[(NH_3)_5Co^{III}-]$ group before and after introduction of cobalt onto the free amino acid in all three cases. It should be noted that in contrast some chelated complexes of Co(III) with amino acids[14]

(12) S. S. Isied, J. Lyon, and A. Vassilian, *Int. J. Pept. Protein Res.*, **19**, 354–360 (1982).
(13) F. Monacelli, F. Basolo, and R. G. Pearson, *J. Inorg. Nucl. Chem.*, **24**, 1241 (1962).
(14) (a) W. E. Keyes and J. I. Legg, *J. Am. Chem. Soc.*, **98**, 4970–75 (1976); (b) Y. N. Beloken, A. Melikian, T. Saleleva, V. Bakhutov, S. Vitt, and V. Belikov, *Tetrahedron*, **36**, 2327–35 (1980).
(15) E. Gross and J. Meinenhofer, Ed., "The Peptides: Analysis, Synthesis, Biology", Vol. I., Academic Press, New York, 1979.

are known to induce racemization of the amino acid bound to it, and these are therefore not suitable as protecting groups in peptide synthesis.

Purification of Co^{III}–Peptide Complexes. The $[(NH_3)_5Co^{III}–]$ protecting group has advantages for the purification of peptides during solution peptide synthesis. The intermediates can be purified at any stage of the synthesis with two separation techniques. These are (a) extraction and (b) chromatography techniques. The protected cobalt peptide can be extracted from the organic layer to the aqueous layer, leaving unreacted protected amino acids, amino acid esters, or anhydrides in the organic phase. The color of the cobalt–peptide derivative is a visible indication of the completeness of the extraction. With this extraction, the Co(III) derivative is obtained in more than 99.5% purity as determined by amino acid analysis in several cases.

To remove any unreacted Co(III) from the N-protected product, we have used preparative reverse-phase C_{18} columns with two different solvent systems (0.2% NaTFA, pH 2.5, in methanol–water and 0.4% isopropylammonium trifluoroacetate in water). The Boc-protected cobalt–peptide retains significantly longer than the unprotected cobalt–peptide under these conditions.

Other chromatographic techniques have also been used to purify the cobalt–peptide complexes. Weak ion exchange resins such as Chelex 100 (a carboxylate resin) have been used to adsorb the cobalt(III)–peptide. The tripositive charge of the cobalt–peptide allows strong adsorption of the cobalt complex on the resin in neutral media while any unreacted free amino acid is washed off. Increasing the acidity of the eluent to 0.1 M TFA causes the cobalt–peptide to elute from the column free from any contaminating amino acids or Boc-amino acids. For this separation only a very short ion exchange column is used. In this way the Co(III) handles allow for the purification of the complex from unreacted amino acids and their derivatives.

Separation of Cobalt–Peptides by High-Pressure Liquid Chromatography (HPLC).[11] HPLC techniques were used to monitor peptide formation, as well as for the purification of the final product. In general the cobalt(III)–peptides are more separated from one another than the free peptides due to additional ion pairing effects imparted by cobalt (Tables II and IV). In 0.2% TFA the $[(NH_3)_5Co$–peptide$]$ complexes adsorb via their hydrophobic moiety and by ion pairing as CF_3COO^- salts to the C_{18} hydrocarbon chain of the derivatized silica gel. The selective elution of cobalt(III)–peptides can be qualitatively understood by using this principle. Figure 1 shows how the retention times of the Leu-Enkephalin fragments increase as the hydrophobicity of the peptide increases for a series of neutral amino acids with hydrophobic side chains. The difference in retention times of the tripeptide, $[GlyPheLeuCo(NH_3)_5]^{3+}$, and the tetrapeptide, $[GlyGlyPheLeuCo(NH_3)_5]^{3+}$, is small. But as a tyrosine fragment is added to form the pentapeptide fragment, a significant increase in retention time is observed.

For the Co(III) complexes of the glycine-histidine series, the hydrophobicity of the side chain is of minor importance in acidic media. However, as the number of histidine residues increases the net charge and ion pairing increase, resulting in an increase in retention time (Figure 2).

Removal of the $[(NH_3)_5Co^{III}]$ Protecting Group. The easy removal of the $[(NH_3)_5Co^{III}–]$ group by reduction (with borohydride or sulfide) makes it possible to selectively remove cobalt in the presence of the Boc protecting group, and thus readily form N-protected peptide fragments (Scheme II). This is one of the mildest known procedures for the removal of the C-terminal protecting group in the presence of Boc-N-protected peptides.

Metal Ion Protecting Groups in Peptide Synthesis. For the synthesis of polypeptides in solution, most methods currently available share in common the protection of the α-amino, α-carboxy, and reactive side chains with use of a wide variety of organic protecting groups.[1] As the number of peptide residues increases, these organic protecting groups modify the properties of the peptide derivative and render it less soluble in organic and polar solvents. This decrease in solubility in turn decreases the coupling rate of the peptide with other amino acid and peptide fragments. Therefore new protecting groups that confer high solubility on the peptide fragment are desired in order to make the synthesis of large polypeptides easier.

Inert metal complexes such as $[(NH_3)_5Co^{III}–]$ provide a versatile new class of protecting group. The variety of reactions that metal-coordinated amino acids and peptides undergo, including acid and base hydrolysis, redox reactions, nucleophilic substitution, and photochemical reactions, offers a large number of possibilities for their removal in the presence of other protecting groups. The color, charge, and high solubility of the metal–peptide derivatives are also extremely useful for the rapid synthesis and purification of these peptides. Furthermore, the solubility of the metal–peptide derivatives can be significantly altered by changing the counterion, thus introducing variation in solubility that can be exploited for precipitation and purification techniques. The solubility of these metallo-protected peptide derivatives in aqueous solution opens up new separation techniques not available to the peptides that are fully protected and only soluble in organic solvents.[5]

In conclusion, metal ion protecting groups provide radically different approaches to those currently in use in the protection of amino acids and peptides. It is through the investigation of such new methodologies that one can expect that the chemical synthesis of peptides will approach the efficiency of biological syntheses.

Experimental Section

Chemicals. $[(NH_3)_5Co^{III}(CO_3)]NO_3$[17] was prepared with use of literature methods and was converted to $[(NH_3)_5Co(OH)_2]^{3+}$ by acidification of a saturated solution with concentrated $HClO_4$, yielding a dark red crystalline solid. L-Amino acids (99.9% pure) were purchased from Aldrich and Tridom Chemical Co. Boc-amino acids and Leu- and Met-enkephalin standards were purchased from Bachem (Torrance, CA) and Peninsula Labs (San Carlos, CA). Solvents including DMF, CH_2Cl_2, THF, and ethyl acetate (EtAc) were all glass-distilled and kept dry with molecular sieves. Other solvents (ethanol, ether) were reagent grade and were used as supplied. Trifluoroacetic acid (HTFA) was purchased from Aldrich and Fisher Scientific. $His(Boc)_2$ was prepared according to the procedure of Schnabel[18] with use of di-*tert*-butyldicarbonate, $(Boc)_2O$, instead of Boc-fluoride. Dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBT) were purchased from Pierce Chemical Co. and Tridom Chemical Co.

Resins, Packings, and Solvents. Prep-Pak 500-C_{18} packing (37–50 μm) (Waters) was used for preparative reverse-phase chromatography. Gravity columns (20–30 cm in length and 1–2 cm in diameter) were used for the purification of cobalt–amino acids and -peptides. HPLC grade methanol and CH_3CN were purchased from JT Baker Co. Solvents were filtered through Metricel membrane filters (Gelman Instrument Co.). Bio-Gel P-2 resin (200–400 mesh) and Chelex 100 (200–400 mesh) were purchased from Bio Rad Labs.

Methods. HPLC was performed on a Waters Associates liquid chromatograph system equipped with a variable wavelength UV detector and a fixed wavelength detector so that it is possible to monitor at two different wavelengths simultaneously, when needed. A solvent system containing water–methanol with up to 0.2% HTFA was used for the elution of cobalt complexes of hydrophobic peptides. Cobalt complexes of hydrophilic peptides were separated with 0.4% aqueous isopropylammonium trifluoroacetate. The separations were done with use of Radial Pak C_{18} cartridges (10 μm octadecylsilane columns, 8 mm × 10 cm, Waters) in a Waters Radial Compression RCM 100 Module or Altex C_{18} columns (5 μm, 4.6 mm × 25 cm) with flow rates of 1–2 mL/min. UV–vis and IR spectra were obtained on Cary 118C and

(16) J. Kovacs in "The Peptides: Analysis, Synthesis, Biology", Vol. III, E. Gross, Ed., Academic Press, 1980, pp 485–538.

(17) F. Basolo and R. K. Murmann, *Inorg. Synth.*, **4**, 171 (1953).

(18) E. Schnabel, H. Herzog, P. Hoffmann, E. Kauke, and I. Ugi, *Justus Liebigs Ann. Chem.*, **716**, 175 (1968).

(19) While this manuscript was in review, Wautier et al. reported a detailed investigation of a related complex, $[Co(tren)(NH_2CHRCOCH_3)]^{3+}$, for peptide synthesis. They concluded that racemization of the chelated amino acid acid is complete within seconds and is much faster than the coupling of this activated coordinated ester with another amino acid. This presumably makes the method of activation and protection at Co^{III} by chelation unsuitable for the synthesis of optically pure peptides. H. Wautier, D. Marchal, and J. Fastrez, *J. Chem. Soc., Dalton Trans.*, 2484–88 (1981); H. Wautier, V. Daffe, M. Smets, and J. Fastrez, *ibid.*, 2479–83 (1981).

(20) P. Johnson, *J. Chromatogr. Sci.*, **17**, 406 (1979).

Perkin-Elmer 283 spectrophotometers, respectively. Peptide hydrolyses were performed by dissolving a sample of cobalt peptide in 6 M HCl in a hydrolysis tube, evacuating, and leaving it at 110 °C for 24 h. The sample was then evaporated and diluted with citrate buffer and determined on an amino acid analyzer. Amino acid analysis was performed on a Glenco amino acid analyzer, and the separate amino acids and peptides were detected by fluorescence after reaction with *o*-phthaldialdehyde.

Synthesis of [$(NH_3)_5Co$–Amino Acid]$(BF_4)_3$ Complexes (Where Amino Acid = Gly, Ala, Val, Leu, Phe, Pro, His, Arg, Lys, Met). The pentaamminecobalt(III)–amino acid complexes were synthesized with use of a common procedure of which the preparation of [LeuCo$(NH_3)_5$]$(BF_4)_3$ is a representative example.

A. Synthesis of [LeuCo$(NH_3)_5$]$(BF_4)_3$. Four grams of [$(NH_3)_5$Co-(OH_2)]$(ClO_4)_3$ (8.7 mmol) was dissolved in 15 mL of water and added to 7 g of L-leucine (53 mmol) (not all of the leucine dissolved). The solution was heated with stirring for 6 h at 70 °C. The solution was then cooled to 0 °C, and the excess leucine was filtered off. $KHCO_3$ (2.8 g, (28 mmol) was added to the filtrate and the reaction mixture was stirred for 10 min. The precipitate ($KClO_4$) was filtered off and the filtrate was acidified with 48% HBF_4 and concentrated to 4.5 mL. Addition of ethanol (5 mL) and ether (150 mL) precipitated the desired pink product, which was filtered, chromatographed on a Bio Gel P-2, and eluted with water. Yield of [LeuCo$(NH_3)_5$]$(BF_4)_3$: 3.0 g, 70%. Anal. Calcd for [$CoC_6H_{28}N_6O_2$]$(BF_4)_3$·$2H_2O$: C, 12.60; H, 5.64; N, 14.70. Found: C, 12.19; H, 5.01; N, 13.93. The above procedure was used for the synthesis of all of the cobalt–amino acid complexes with the following changes. For the amino acid phenylalanine the reaction was carried out at 70 °C for 20 h. It was then treated as above to covert it to the BF_4 salt. This was then concentrated and applied to a Bio-Gel P-2 column (2 × 50 cm for every 4 mmol of cobalt complex). For the more water soluble amino acids, e.g., glycine and proline, direct precipitation from 48% HBF_4 and ethanol resulted in the precipitation of the pure cobalt–amino acid complex. Generally the isolated yield for the cobalt–amino acid complexes after chromatographic purification was between 60 and 80%. The title amino acid complexes were obtained in analytically pure form by using these precipitation and purification techniques.

Synthesis of [Boc(OBzl)TyrCo$(NH_3)_5$](TFA)$_3$. BocTyrOBzl (2.0 g, 5.39 mmol), DCC (1.1 g, 5.39 mmol), and HOBT (0.728 g, 5.39 mmol) were dissolved in CH_2Cl_2 with a minimum amount of DMF in order to dissolve the HOBT. The solution was stirred at 0 °C for 2 h. The resulting DCU was filtered off and the solution was concentrated to dryness by rotary evaporation. [$(NH_3)_5$Co$(OH)_2$]$(ClO_4)_3$ (7.73 g, 16.2 mmol) and 2 mL of tetramethylguanidine were added to the solid residue and this solution was stirred for 0.5 h. The solution was then added to 150 mL of ethyl acetate, to remove excess organic product, followed by 20 mL of 2% HOAc. The aqueous layer was separated and concentrated by rotary evaporation. The resulting residue was purified on a preparative reverse-phase C_{18} column. The residue was dissolved in 20% methanol-water (0.1% HTFA) and loaded on the column. The column was washed with 20% methanol–water (0.1% HTFA) in order to elute unreacted [$(NH_3)_5$Co(OH_2)]$^{3+}$. After that the remaining band was eluted with 60% methanol-water (0.1% HTFA) and concentrated to dryness by rotoevaporation. The resulting solid (yield 1.4 g, 35%) was dried under vacuum and the tyrosine content determined by amino acid analysis.

Synthesis of [BocGlyCo$(NH_3)_5$]$(BF_4)_2$. BocGly (456 mg, 2.6 mmol) was dissolved in 3 mL of CH_2Cl_2 at 0 °C. HOBT (597 mg, 3.9 mmol), dissolved in 2 mL of DMF, was added to the BocGly solution, and then DCC (536 mg, 2.6 mmol) in 2 mL of CH_2Cl_2 was added. After 5 min a white solid (DCU) began to form. This solution was stirred at 0 °C for 1 h and then at room temperature for 1 h. The solid was filtered off and the solution was concentrated to dryness by rotary evaporation. Then 0.46 g of [$(NH_3)_5$CoOH]$(ClO_4)_2$, dissolved in 2 mL of DMF, was added and the solution was stirred for 0.5 h at room temperature. The white solid DCU was filtered off. The [BocGlyCo$(NH_3)_5$]$(BF_4)_2$ was precipitated by the addition of 48% HBF_4, ethanol, and ether (0.10 g, 15%).

Synthesis of [$(NH_3)_5$Co(OH)]$(ClO_4)_2$. A solution of 0.7 mL of 10% NaOH was added to 0.6 g of [$(NH_3)_5$Co(OH_2)]$(ClO_4)_3$ (1.3 mmol) in 5 mL of water. The color of the solution changed from red to purplish red immediately. Ethanol was quickly added and the solution was cooled, filtered, and washed with ethanol and ether. Yield 0.46 g, 98%.

B. 1. Synthesis of [PheLeuCo$(NH_3)_5$](TFA)$_3$—Coupling by the Anhydride Method. Boc-L-Phe (1.59 g, 6.0 mmol) was dissolved in 5 mL of CH_2Cl_2 and cooled to 0 °C. DCC (0.619 g, 3.0 mmol) in 0.5 mL of CH_2Cl_2 at 0 °C was added and the solution was stirred at 0 °C for 2 h and then filtered to remove dicyclohexylurea (DCU). The filtrate was evaporated to dryness by rotary evaporation and the white solid was dissolved in 2.0 mL of DMF. Solid [LeuCo$(NH_3)_5$]$(BF_4)_3$ (0.536 g, 1.0 mmol) was added to this solution and the mixture was stirred to homogeneity. The coupling was initiated by the addition of *N*-methylmorpholine (0.42 mL, 3.8 mmol). The extent of coupling was monitored by the disappearance of the band corresponding to the starting material [LeuCo$(NH_3)_5$]$(BF_4)_3$ with use of HPLC. A sample of the reaction mixture withdrawn after 5 min showed that no starting material could be detected (within 1%). The solution was stirred at room temperature for 25 min, acidified with HTFA, and evaporated to dryness. The Boc group was removed by stirring the reaction solution with 6 mL of HTFA for 1 h. The excess HTFA was removed by rotary evaporation. EtAc (15 mL) was then added to the dry solid and the pink organic layer was extracted with 2 × 25 mL of aqueous 2% HAc. The pink aqueous layer containing the cobalt(III)–peptide was separated and purified by passing it through a small cation exchange column (Chelex 100). The pink solution was concentrated on top of the ion exchange column. The column was washed with water and then the cobalt complex was eluted with 0.3 M HTFA. The pink cobalt peptide solution was evaporated to dryness. Although the reaction was more than 99% complete (HPLC monitoring), the isolated yields after chromatographic manipulations varied between 59 and 83% for different preparations.

2. Preparation of [PheLeuCo$(NH_3)_5$]$(BF_4)_3$—Active Ester Method. To a solution of Boc-L-Phe (1.5 g, 5.6 mmol) in 4 mL of CH_2Cl_2 at 0 °C were added a solution of HOBT (0.87 g, 5.6 mmol) in 1.5 mL of DMF at 0 °C and a solution of DCC (1.18 g, 5.6 mmol) in 1.5 mL of CH_2Cl_2 at 0 °C. The formation of the active ester was continued for 1 h at 0 °C and then for 1 h at room temperature. The solution was then filtered and evaporated to dryness. DMF (2 mL) and then solid [LeuCo$(NH_3)_5$]$(BF_4)_3$ (1.0 g, 1.87 mmol) were added. Coupling was initiated by the addition of diisopropylethylamine (0.4 mL, 2.3 mmol) and the reaction was monitored by HPLC. A sample withdrawn from the reaction mixture after 10 min of reaction did not show the presence of [LeuCo$(NH_3)_5$]$^{2+}$ (within 1%). The reaction solution was stirred for 25 min at room temperature, evaporated to a viscous liquid, and treated with 2 × 15 mL of ether (to remove organics). The Boc group was removed by stirring the reaction mixture in 2 mL of CH_2Cl_2 and 4 mL of HTFA for 75 min at room temperature. The solution was evaporated to a viscous solution, dissolved in water, and purified as in method A with use of ion exchange (Chelex 100). Further purification was achieved by gel filtration (Bio-Gel P-2). Yield 1.04 g, 83%.

C. Synthesis of [GlyPheLeuCo$(NH_3)_5$](TFA)$_3$—Anhydride Method. Boc-glycine (0.461 g, 2.64 mmol) was dissolved in 2 mL of CH_2Cl_2 and cooled to 0 °C. DCC (0.272 g, 1.32 mmol) in 0.5 mL of CH_2Cl_2 at 0 °C was added and the solution was stirred for 2 h at 0° and then filtered to remove DCU. The filtrate was then rotoevaporated to dryness, and the white solid dissolved in 2 mL of DMF. To this solution was added [PheLeuCo$(NH_3)_5$]$(BF_4)_3$ (0.3 g, 0.44 mmol) and the mixture was stirred until dissolution. *N*-Methylmorpholine (0.1 mL, 0.9 mmol) was added and the progress of the coupling was monitored by HPLC. A sample withdrawn from the reaction mixture after 5 min showed the absence of the starting material, i.e., [PheLeuCo$(NH_3)_5$]$(BF_4)_3$. The reaction was left to proceed for an additional 15 min and then concentrated to a viscous liquid by rotary evaporation. The Boc group was removed by stirring the solution at room temperature for 1 h with 2 mL of HTFA. The solution was rotoevaporated to dryness, dissolved in 15 mL of EtAc, and extracted twice with 15 mL of 2% aqueous HOAc. The cobalt–tripeptide was further purified on a cation exchange resin (Chelex 100), eluted with 0.3 M HTFA, and evaporated to dryness at room temperature.

D. Synthesis of [GlyGlyPheLeuCo$(NH_3)_5$](TFA)$_3$—Anhydride Method. Boc-glycine (0.461 g, 2.64 mmol) was dissolved in 2 mL of CH_2Cl_2 and cooled to 0 °C. DCC (0.272 g, 1.32 mmol) in 0.5 mL of CH_2Cl_2 at 0 °C was added and the solution was stirred for 2 h at 0 °C. The solution was then filtered to remove the DCU. The filtrate was taken to dryness and the resulting white solid was dissolved in 2 mL of DMF. To this solution was added [GlyPheLeuCo$(NH_3)_5$](TFA)$_3$ (dissolved in 1.5 mL DMF) (from the previous experiment). *N*-Methylmorpholine (0.1 mL, 0.9 mmol) was added and the progress of the reaction was monitored by HPLC. A sample withdrawn after 10 min showed the absence of the starting material. The reaction mixture was left stirring for 30 min and then concentrated to a viscous liquid by rotary evaporation. The Boc group was removed by adding 2 mL of HTFA and stirring for 2 h. The solution was then evaporated to dryness at room temperature and dissolved in 15 mL of EtAc and extracted twice with 2% aqueous HOAc (15 mL). The aqueous layer was purified by ion exchange chromatography (Chelex 100) as described before. The yield of isolated cobalt–tetrapeptide complex after chromatography was 230 mg (55%).

E. Synthesis of [Leu-Enk-Co$(NH_3)_5$](TFA)$_3$. The cobalt–tetrapeptide [GlyGlyPheLeuCo$(NH_3)_5$](TFA)$_3$ (0.228 g, 0.22 mmol) was dissolved in 1.2 mL of water and the pH was adjusted to 8 with use of solid $NaHCO_3$. BocTyrOSu (OSu = *N*-hydroxysuccinamide) (160 mg, 0.44

mmol) in 1.2 mL of THF and 0.5 mL of dioxane was added to the cobalt–tetrapeptide and the reaction mixture was left stirring at room temperature for 24 h. (Later syntheses following this procedure showed the reaction to be complete after 75 min.) The solution was concentrated by rotary evaporation. The Boc group was removed by adding 2 mL of HTFA and stirring for 1.5 h. The solution was evaporated to dryness by rotary evaporation and further purified on a reverse-phase C_{18} column (20 cm × 2.5 cm) which was equilibrated with 20% methanol–water containing 2% HOAc. The resulting solution was evaporated to dryness. Yield of [Leu-Enk-Co$(NH_3)_5$](TFA)$_3$ was 26% (102 mg) (based on the weight of [PheLeuCo$(NH_3)_5$](TFA)$_3$).

F. Removal of the [$(NH_3)_5Co^{III}$–] Group. The [$(NH_3)_5Co^{III}$–] ion was removed by dissolving the cobalt peptide in aqueous 2% HOAc, adding 4 equiv of solid $NaBH_4$, and leaving the resulting solution set for 5 min at room temperature. The resulting black solution was treated with a few drops of 0.1 M HFTA and stirred. It turned colorless within 2 min. The free enkephalin peptide was compared to an authentic sample with use of HPLC and by comparing peptide retention times on an amino acid analyzer.[20]

G. Synthesis of [Met-Enk-Co$(NH_3)_5$](TFA)$_3$. The complex [MetCo$(NH_3)_5$](TFA)$_3$ was sequentially coupled to BocPhe, BocGly (twice), and BocTyrOSu with use of the same procedures described for the cobalt derivative of Leu-enkephalin, with the additional use of 0.2 mL of methyl ethyl sulfide as a scavenger during each Boc removal step. The [Met-Enk-Co$(NH_3)_5$]$^{3+}$ formed was puritied on a reverse-phase C_{18} column and eluted with 20% methanol–water containing 2% HOAc. The final isolated yield was 10% on the basis of [MetCo$(NH_3)_5$]$(BF_4)_3$. No effort was made to optimize the yield of [Met-Enk-Co$(NH_3)_5$](TFA)$_3$. The cobalt–peptide intermediates were characterized by HPLC and amino acid analysis.

H. Synthesis of [HisGlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_3$. When [GlyCo$(NH_3)_5$]$(BF_4)_3$ is used as the starting reactant, the synthesis of this cobalt–hexapeptide derivative was carried out by stepwise elongation, using the symmetric anhydrides of BocGly and $(Boc)_2$His. $(Boc)_2$His (4.26 g, 12 mmol) was dissolved in dry CH_2Cl_2 at 5 °C and 1.23 g (6 mmol) of DCC was added. The solution was stirred for 1 h at 5 °C. The resulting DCU was filtered off and the CH_2Cl_2 was evaporated to dryness on a rotoevaporator. The symmetrical anhydride was then dissolved in a minimum amount of DMF (ca. 1–2 mL) and the solution was cooled to 5 °C. [GlyCo$(NH_3)_5$]$(BF_4)_3$ (0.942 g, 2 mmol) dissolved in the minimum amount of DMF (ca. 1 mL) was then added to the anhydride solution and the pH adusted to 8 by the addition of *N*-methylmorpholine. The reaction was left stirring at 5 °C and the progress of the peptide formation was monitored by HPLC. Aliquots (10 μL) were taken from the reaction solution at various times. The aliquot was added to 200 μL of 5% HAc and 5 μL of the resulting solution was injected into the HPLC. The ratio of the areas of the starting material (at $t = 0$) before the addition of *N*-methylmorpholine and at time t was taken to be directly related to the percent of unreacted starting material. When the reaction was judged to be complete (>99%) (~1 h in this case), the reaction mixture was dissolved in EtAc (15 mL) and the protected cobalt–peptide was extracted twice with equal volumes of 5% HOAc. The aqueous pink cobalt solution was then extracted extensively with EtAc to remove any excess Boc-amino acid or amino acid anhydride. The aqueous solution was concentrated to dryness by rotary evaporation. The N-protected cobalt complex was purified before removal of the Boc group by preparative reverse-phase C_{18} chromatography. The concentrated sample was dissolved in a 0.4% solution of isopropylammonium trifluoroacetate (IPA(TFA)). The unreacted cobalt–amino acid was eluted with 0.4% IPA(TFA) and the N-protected cobalt complex retained on the column. The protected cobalt–peptide was then removed with water and evaporated to dryness by rotary evaporation. The Boc group was removed by dissolving the complex in a minimum amount of water, adding a sufficient amount of HTFA to the mixture to make a 95% aqueous HTFA solution, and stirring the mixture for 1 h at 5 °C. The excess HTFA was removed by rotary evaporation and the complex was converted to the BF_4^- salt by adding 1 M HBF_4 and concentrating the solution to one fourth of its initial volume. The [HisGlyCo$(NH_3)_5$]$^{4+}$ was precipitated by adding this solution into 10× its volume of ethanol. The solid cobalt–peptide complex was cooled and collected by filtration. The isolated yield of [HisGlyCo$(NH_3)_5$]$(BF_4)_4$ was 80% for several preparations.

The cobalt–tripeptide complex, [GlyHisGlyCo$(NH_3)_5$]$(BF_4)_4$, was prepared by repeating the above procedure with use of 0.15 g of [HisGlyCo$(NH_3)_5$]$(BF_4)_4$ (0.213 mmol), 0.164 g of BocGly (0.94 mmol), and 0.096 g of DCC (0.47 mmol). The reaction was monitored by HPLC; greater than 95% coupling occurred. After extraction and purification on reverse-phase C_{18} and removal of the Boc group, precipitation resulted in the [GlyHisGlyCo$(NH_3)_5$]$(BF_4)_4$ salt (0.127 g, 78% yield).

The cobalt–tetrapeptide complex, [HisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$, was prepared in a manner similar to that described above for the dipeptide, but starting with 0.15 g of [GlyHisGlyCo$(NH_3)_5$]$(BF_4)_4$ (0.2 mmol), 0.42 g of $(Boc)_2$His (1.18 mmol), and 0.122 g of DCC (0.59 mmol). After purification, Boc removal, and precipitation, 0.15 g (63% yield) of [HisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$ was obtained.

[GlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$ was synthesized similarly with 0.10 g of [HisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$ (0.101 mmol) and the symmetric anhydride of Boc-glycine, 0.187 g of Boc-glycine (1.02 mmol) and 0.105 g of DCC (0.51 mmol). The yield was 0.0843 g, 79%.

In the same way [HisGlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_6$ was made from 0.060 g of [GlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$ (0.0575 mmol) and the symmetric anhydride of $(Boc)_2$histidine, 0.204 g of $(Boc)_2$His (0.575 mmol), and 0.053 g of DCC (0.258 mmol). The yield of the cobalt–hexapeptide complex was 0.046 g, 63%. The coupling times for the addition of glycine and histidine to the cobalt–peptides varies from 1 h (dipeptide) to 6 h (for the hexapeptide). These were determined by HPLC monitoring as described earlier.

Acknowledgment. This work is supported by Grant GM 263-24 from the US Public Health Service and by the Charles and Johanna Busch Memorial Fund (Rutgers). S. Isied is a recipient of a Public Health Service Research Career Development Award (5 K04 AM 00735).

Registry No. [GlyCo$(NH_3)_5$]$(BF_4)_3$, 68582-21-8; [AlaCo$(NH_3)_5$]$(BF_4)_3$, 81704-64-5; [ValCo$(NH_3)_5$]$(BF_4)_3$, 81688-35-9; [LeuCo$(NH_3)_5$]$(BF_4)_3$, 81688-38-2; [PheCo$(NH_3)_5$]$(BF_4)_3$, 81688-40-6; [ProCo$(NH_3)_5$]$(BF_4)_3$, 68582-23-0; [HisCo$(NH_3)_5$]$(BF_4)_3$, 81688-42-8; [ArgCo$(NH_3)_5$]$(BF_4)_3$, 81688-45-1; [LysCo$(NH_3)_5$]$(BF_4)_3$, 81738-82-1; [MetCo$(NH_3)_5$]$(BF_4)_3$, 81688-48-4; [Boc(OBz)TyrCo$(NH_3)_5$](TFA)$_3$, 81688-51-9; [BocGlyCo$(NH_3)_5$]$(BF_4)_2$, 68628-04-6; [PheLeuCo$(NH_3)_5$](TFA)$_3$, 81704-72-5; [PheLeuCo$(NH_3)_5$]$(BF_4)_3$, 81688-54-2; [GlyPheLeuCo$(NH_3)_5$](TFA)$_3$, 81688-57-5; [GlyGlyPheLeuCo$(NH_3)_5$](TFA)$_3$, 81688-60-0; [Leu-Enk-Co$(NH_3)_5$](TFA)$_3$, 81688-63-3; [Met-Enk-Co$(NH_3)_5$](TFA)$_3$, 81704-75-8; [MetCo$(NH_3)_5$](TFA)$_3$, 81688-65-5; [HisGlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_6$, 81704-77-0; [HisGlyCo$(NH_3)_5$]$(BF_4)_4$, 81688-68-8; [GlyHisGlyCo$(NH_3)_5$]$(BF_4)_4$, 81688-71-3; [HisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$, 81704-79-2; [GlyHisGlyHisGlyCo$(NH_3)_5$]$(BF_4)_5$, 81688-74-6; [$(NH_3)_5Co(OH_2)$]$(ClO_4)_3$, 13820-81-0; [$(NH_3)_5CoOH$]$(ClO_4)_2$, 18885-27-3; Boc-L-Phe, 13734-34-4; Boc-Gly, 4530-20-5; BocTyrOSu, 20866-56-2; $(Boc)_2$His, 20866-46-0; PheLeu, 3303-55-7; GlyPheLeu, 15373-56-5; GlyGlyPheLeu, 60254-83-3; TyrGlyGlyPheLeu, 58822-25-6; Leu-enkephalin, 58569-55-4; Gly, 56-40-6; HisGly, 2578-58-7; GlyHisGly, 7758-33-0; HisGlyHisGly, 81671-57-0; GlyHisGlyHisGly, 81671-58-1; HisGlyHisGlyHisGly, 81671-59-2.