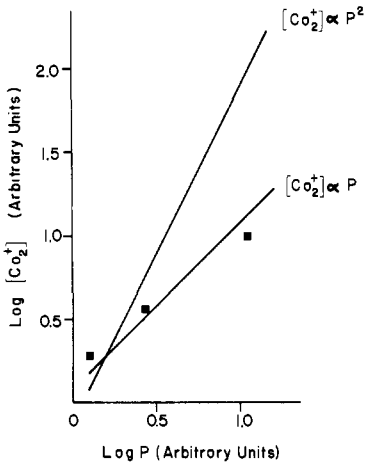

Figure 3. Dependence of Co_2^+ signal intensity on the sample number density in the photolysis region. Lines show the expected pressure dependences of products of direct unimolecular dissociation and ionization ($[Co_2^+] \propto P$) and of binary reaction ($[Co_2^+] \propto P^2$).

As shown in Figure 1, irradiation at 406 nm yielded Co^+ and Co_2^+ in a ratio of approximately 10:1. No other molecular ions were detected under these conditions, indicating maximum possible abundances of 0.3% relative to that of Co^+. Observed signal levels suggest that the yield of Co_2^+ from $Co_2(CO)_8$ in the laser focal volume exceeded 1% under the experimental conditions employed.

In light of the work on other organometallic systems,[6] the most likely mechanisms for the formation of Co^+ and Co_2^+ from $Co_2(CO)_8$ are as follows:[8]

$$Co_2(CO)_8 \rightarrow 2Co + 8CO$$

$$Co_2(CO)_8 \rightarrow Co_2 + 8CO$$

$$Co \rightarrow Co^+ + e^-$$

$$Co_2 \rightarrow Co_2^+ + e^-$$

Several experiments indicated that the Co_2^+ was not formed primarily via reaction of the abundant Co^+ ion with the parent or other molecular species.[9] Figure 2 shows the wavelength dependences between 400 and 410 nm of Co^+ and Co_2^+ signal intensities. The Co^+ spectrum contains a number of prominent peaks which can be assigned to atomic transitions of Co. These peaks are not observed in the Co_2^+ spectrum, indicating that the Co_2^+ ion yield is independent of Co^+ production. A second argument against an ion–molecule recombination mechanism for Co_2^+ production is provided by studies of the pressure dependence of Co_2^+ signal intensity, the results of which are shown in Figure 3. Variation of the $Co_2(CO)_8$ pressure in the photolysis region produced an approximately linear change in Co_2^+ signal strength, rather than the quadratic change which would be expected if a binary reaction were involved in Co_2^+ production. This pressure dependence also argues against a formation path in which neutral atomic and/or molecular recombinations are followed by ionization, which is in any case unlikely at the pressures employed in these experiments.[10] In conclusion, the Co_2^+ detected upon irradiation of $Co_2(CO)_8$ appears to be formed directly by decarbonylation of the parent compound and photoionization of the metal fragment rather than by combination reactions involving mononuclear species.

These results suggest that laser photodissociation of organometallic cluster compounds can provide an efficient method for producing ligand-free clusters in the gas phase. Given the diversity of known potential precursors,[5] this technique could provide specific access to a wide variety of bare transition-metal clusters.

Acknowledgment. We thank J. A. Welch for informative discussions and S. Buelow, O. Cheshnovski, and D. Worsnop for their help with this experiment.

(8) The multiphoton dissociation steps require five 406 nm photons to produce the metal atom or the metal dimer. Depending on the amount of excess energy retained by the metal fragment, at least three additional photons are required to produce the atomic cation and at least one photon is needed to ionize the dimer. These figures were calculated from the mass spectrometric appearance potential data of ref 8a, the atomic data of ref 8b, and the metal dimer dissociation energy data of ref 8c. (a) Winters, R. E.; Kiser, R. W. *J. Phys. Chem.* **1965**, *69*, 1618–1622. (b) Moore, C. E. *Natl. Bur. Stand. (U.S.) Circ.* **1952**, *No. 467*. (c) Blackborow, J. R.; Young, D. "Metal Vapour Synthesis in Organometallic Chemistry"; Springer-Verlag: Berlin, 1979; p 53.

(9) The surprising efficiency of such processes was revealed by experiments in which products of the following reaction sequence were detected on microsecond timescales at sample pressures of 10^{-5} torr:

$$Cr(CO)_6 \rightarrow Cr^+ + 6CO + e^-$$

$$Cr^+ + Cr(CO)_6 \rightarrow Cr_2(CO)_n^+ + (6 - n)CO$$

(10) Such mechanisms have been employed to produce bare metal clusters under different experimental conditions. For example, see ref 3 and 4. Also see: Efremov, Yu. M.; Samoilova, A. N.; Kozhukhovsky, V. B.; Gurvich, L. V. *J. Mol. Spectrosc.* **1978**, *73*, 430–440.

Cobalt(III)-Mediated Peptide Synthesis. 1. Cobalt(III)-Activated Amino Acid Methyl Esters and the Synthesis of Dipeptides

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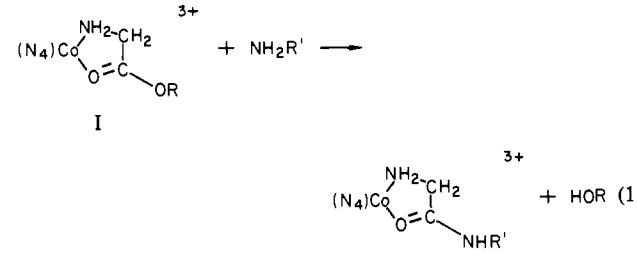
Previously we described the rapid aminolysis of Co(III)-chelated glycine esters (I) in aprotic solvents[1,2] [N_4 = $(en)_2$ or trien; R = CH_3, C_2H_5; R′ = H, CHR″CO_2Et] and suggested that reaction 1 might well provide a useful alternative to the active ester method for the synthesis of peptides. We have now extended this aspect to include coordinated amino acids other than glycine and herein report on a simple preparation for the Co(III) active ester, the coupling reaction and recovery of the dipeptide, and the degree of racemization at each stage. In the following communication[3] the preparation of two tetrapeptides and the synthesis of [Leu^5]enkephalin is described.



Treatment of $[Co(N_4)(AA)]I_2$[4] [7.5 mmol; N_4 = $(en)_2$ or trien;

(1) D. A. Buckingham, L. G. Marzilli, and A. M. Sargeson, *J. Am. Chem. Soc.*, **89**, 4539 (1967).

(2) D. A. Buckingham, J. Dekkers, and A. M. Sargeson, *J. Am. Chem. Soc.*, **95**, 4174 (1973).

(3) See D. R. Knighton, D. R. K. Harding, M. J. Friar, W. S. Hancock, G. D. Reynolds, C. R. Clark, R. F. Tasker, and D. A. Buckingham, *J. Am. Chem. Soc.*, following paper in this issue.

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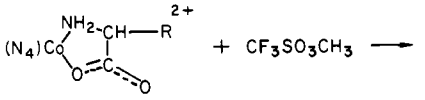
Table I. Couplings of AA'-OMe and $[Co(en)_2(AA\text{-}OMe)](CF_3SO_3)_3$ in Me_2SO and Recovered Yields

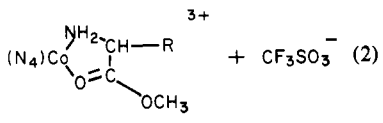
Co(III) amino acid ester CoAA-OMe	nucleophile AA'-OMe	base	reaction time, min	Co(III) dipeptide ester product CoAA-AA'-OMe	recovered yield,[a] %
Phe	His	Et_3N	5	Phe-His	90
Trp	Ala	Et_3N	5	Trp-Ala	91
Arg(NO_2)	Gly	Et_3N	5	Arg(NO_2)-Gly	85
Ala	Cys(Bzl)	*N*-MeMorph	30	Ala-Cys(Bzl)	85
Phe	Phe	Et_3N	5	Phe-Phe	73
Ala	Phe	Et_3N	5	Ala-Phe	83
Ser(Bzl)	His	NaOMe	5	Ser(Bzl)-His	83
Phe	Cys(Bzl)	*N*-MeMorph	30	Phe-Cys(Bzl)	72
Phe	Leu	NaOMe	5	Phe-Leu	88
Glu(OBzl)	His	NaOMe	5	Glu(OBzl)-His	76
Thr(Bzl)	Gly[b]	Et_3N	5	Thr(Bzl)-Gly[b]	89
Ala	Gly	Et_3N	5	Ala-Gly	89

[a] Estimated by AA spectroscopy. [b] Benzyl ester.

AA = Thr(Bzl), Ser(Bzl), Tyr(Bzl), Glu(OBzl), Lys(Z), Asp(OBzl), Phe, Leu, Ile, Ala, Gly, Trp, Arg(NO_2), Val, His(Bzl), Pro] in dry trimethyl phosphate (15 cm³) with methyl trifluoromethanesulfonate (30 mmol) for 15–90 min at 25–50 °C gives quantitative yields of $[Co(N_4)(AA\text{-}OMe)](CF_3SO_3)_3$ (III), reaction 2. These Co(III) amino acid methyl esters are readily



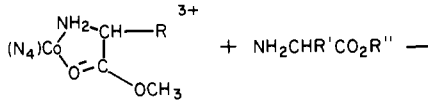
II



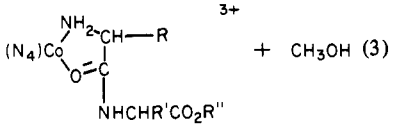
III

isolated as orange powders following trituration with Et_2O/MeOH. Some have been recrystallized from MeOH as ClO_4^- or I^- salts.[5]

The coupling (reaction 3) is rapid in dry Me_2SO, acetonitrile, or methanol.[6]



III



In a typical experiment Et_3N (3 mmol) was added to Ala-OMe·HCl (5 mmol) in dry Me_2SO (15 cm³). This solution was then added to $[Co(en)_2(Trp\text{-}OMe)](CF_3SO_3)_3$ (1.00 mmol) in dry Me_2SO (20 cm³). After 5 min the reaction was quenched by the addition of HOAc (100 μL). Chromatography on SP-C25 Sephadex (0.3–1.0 mol dm⁻³ of pyridinium acetate) and removal of eluant under reduced pressure at room temperature resulted in a 91% recovery of $[Co(en)_2(Trp\text{-}Ala\text{-}OMe)](OAc)_3$ (0.91 mmol). Similar conditions have been used to prepare a variety of dipeptide combinations (cf. Table I).[7-9]

Tritiation experiments indicate that racemization during coupling is minimal. Treatment of β-$[Co(trien)(Ala\text{-}OMe)](CF_3SO_3)_3$ (1.08 mmol) and Gly-Gly-OBzl·HTos (1.00 mmol) with Et_3N (1.50 mmol) in dry Me_2SO (5 cm³) in the presence of 5 mCi of ^3H_2O (1 μL) for 10 min gave, following chromatography, β-$[Co(trien)(Ala\text{-}Gly\text{-}Gly\text{-}OBzl)]^{3+}$ (0.875 mmol, measured activity 250 nCi) and β-$[Co(trien)(Ala)]^{2+}$ (0.202 mmol, measured activity 50 nCi). The tritium incorporations (corrected for quenching) represent maxima for racemization into the peptide (0.20%) and amino acid (0.16%) functions since other experiments suggested that slight residual activities remained in the trien moieties.[10]

The $Co(en)_2$ and Co(trien) moieties are asymmetric and some specificity is induced when optically active Co(III) units are used. Rate studies in acetonitrile[11] show that coupling to Δ- and Λ-$[Co(en)_2(AA\text{-}OMe)](CF_3SO_3)_3$ follows overall second-order kinetics with specific rate ratios k_Λ/k_Δ of 2.7, 1.0, 2.0, and 1.5 for the combinations CoAA-OMe:AA-OEt = Ala:Phe; Phe:Phe; Val:Phe; Ala:Val. Although not large, and of uncertain origin, these differences are mirrored in the relative amounts of Δ- and Λ-$[Co(en)_2(AA\text{-}AA'\text{-}OEt)]^{3+}$ ions recovered. Obviously, by choosing the appropriate Co(III) chirality some control can be exercised in avoiding couplings to mutarotated amino acid residues.

Removal of the dipeptide from the $Co(N_4)$ moiety can be achieved in several ways,[12] but we have found electrolytic reduction to be the cleanest and most efficient. For example, $[Co(en)_2(Ala\text{-}Gly\text{-}OMe)]^{3+}$ (438 μmol) in water (50 cm³) containing pyridinium acetate took 2 h at −1.0 V[13] vs. SCE (pH <5, adjusted with nitric acid) to reduce labile Co_{aq}^{2+}, enH_2^{2+}, and $^+H_2$-Ala-Gly-OMe. This solution (pH 5.5) was reduced in volume to 25 cm³ and H_2S bubbled in for 20 min to precipitate CoS which was removed by filtration. The filtrate was dried, diluted to 200 cm³, and loaded onto a Dowex 50W-X2 column, and the dipeptide ester was isolated by elution with 0.2 mol·dm⁻³ of pyridinium acetate. Spectrophotometric ninhydrin analysis showed a 96% recovery (420 μmol), and the dipeptide ester was pure by TLC.

This new approach has several attractive features. Firstly, the Co(III) moiety provides a sterically robust protecting group for the amino terminus. It can subsequently be easily removed by

(4) Some $[Co(N_4)(AA)]I_2$ complexes have been reported previously, but we have found the $N_4 = (en)_2$ series can best be synthesised via *trans*-$[Co(en)_2(Me_2SO)_2](ClO_4)_3$ and AA-OH in dimethyl sulfoxide, especially for side-chain protected amino acids. For $[Co(trien)(AA)]I_2$ very mild conditions can be used, and we have found that treatment of freshly prepared, concentrated, aqueous solutions of β-$[Co(trien)(OH)(H_2O)](ClO_4)_2$ with AA-OMe at pH 8–8.5, 25–30 °C, for 2–60 min affords excellent yields. Racemization of the amino acid during attachment to the Co(III) moiety is slight; D-amino acid oxidase analysis of Ala recovered from $[Co(en)_2(Ala)]I_2$ gave 0.2% racemate and from $[Co(trien)(Ala)]I_2$ gave 0.5%. For $[Co(trien)(Phe)]I_2$ no detectable racemate was found. ^3H experiments showed little proton exchange (<0.01%) during synthesis, and this suggests that the low level of racemization observed in the Co complexes occurs in the acid-catalyzed synthesis of the AA-OMe rather than in its attachment to cobalt. The preparative procedures and the racemization and proton exchange studies will be reported in detail elsewhere.

(5) All methyl ester complexes analyzed correctly for C, H, and N (e.g., Anal. Calcd for $[Co(en)_2(Thr(Bzl)\text{-}OMe)](CF_3SO_3)_3$: C, 26.86; H, 3.92; N, 8.24. Found: C, 26.95; H, 3.95; N, 8.23) and gave sharp ν_{CO} absorptions (1630 cm⁻¹) and clean ^1H NMR spectra in Me_2SO-d_6 (OMe singlet at 4.1 ppm). They are, however, extremely susceptible to hydrolysis, but we have stored them successfully for many months in the dark over P_2O_5.

(6) The major side product in these reactions is $[Co(N_4)(AA)]^{2+}$ which results primarily from hydrolysis of unreacted methyl ester complex upon quenching the reaction. It can easily be separated from the orange 3+ dipeptide complex by cation exchange chromatography (Dowex 50W-X2 or Sephadex SP-C25) and recovered as the iodide salt for subsequent realkylation.

(7) Preliminary experiments using HPLC suggest some chelated dipeptide combinations are prone to slow hydrolysis; hence the need for slight changes in reaction conditions and milder workup.

(8) Many of the dipeptide ester complexes have been crystallized as their iodide salts, and these give satisfactory C, H, and N analyses; ν_{CO} (ester) 1730 cm⁻¹; ν_{CO} (amide) 1625 cm⁻¹; ^1H NMR spectra in D_2O.

(9) D. A. Buckingham, C. E. Davis, D. M. Foster, and A. M. Sargeson, *J. Am. Chem. Soc.*, **92**, 5571 (1970).

(10) These experiments involved substantial exchange of ^3H into the amine functions of the trien ligands and extensive washing on the ion-exchange column was required to remove this. Co was determined spectrophotometrically, and ^3H incorporation was determined on a scintillation counter.

(11) D. A. Buckingham, C. R. Clark, and R. Tasker, unpublished studies. The second-order rate constants k_Δ and k_Λ relate to the addition reaction for formation of the amine–alcohol intermediate. See D. A. Buckingham, J. Dekkers, and A. M. Sargeson, *J. Am. Chem. Soc.*, **95**, 4174 (1973).

(12) Treatment with CN^- (warming) or $NaBH_4$ in neutral or alkaline conditions, or with zinc amalgam at pH 1–2, also results in rapid reduction to Co(II).

(13) A potential of ca. −0.25 V (vs. SCE) is sufficient to reduce the Co(III) complex to Co_{aq}^{2+}, but we routinely use −1.0 V.

mild reduction to the labile Co(II) oxidation state. Secondly, Co(III) significantly activates the carboxyl group toward addition of an amino acid or peptide. A 10^4–10^6 enhancement in the rate of the coupling relative to the uncoordinated amino acid ester is involved,[11] and this allows for very short reaction times. Also the relatively mild reaction conditions suggest that more labile side-chain protecting groups may well be used. Further, the asymmetric nature of the $Co(N_4)$ group, its orange color (ϵ_{480} = 100–150 mol^{-1} dm^3 cm^{-1}), and its water solubility provide other features which differ from those usually encountered in peptide synthesis.

Cobalt(III)-Mediated Peptide Synthesis. 2. Synthesis of Tetrapeptides and [Leu^5]enkephalin

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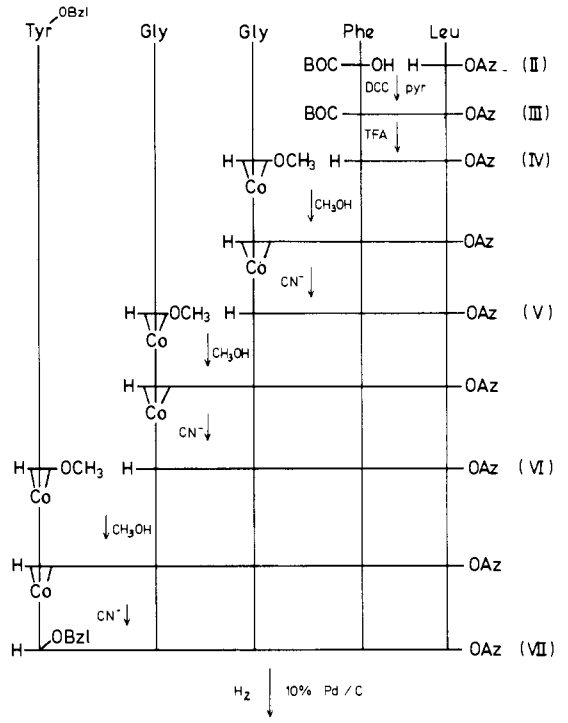
The preceding communication outlined the cobalt(III)-mediated synthesis of dipeptides.[1] We now report on the applicability of the method to larger peptides. The new feature here is the sequential removal of the cobalt(III) moiety, isolation of the growing peptide fragment, and its subsequent reuse to extend the amino acid chain. Two complementary isolation procedures are described. The first utilizes zinc amalgam reduction of $[Co(en)_2(peptide\text{-}OR)]^{3+}$ followed by isolation of the peptide-OR by gel filtration. The stepwise synthesis of Ala-Gly-Phe-Phe-OBzl and Leu-Ala-Gly-Gly-OEt starting from the C-terminal amino acid esters exemplifies this approach. The second method uses the lipophilic and chromogenic *p*-[*p*-(dimethylamino)phenylazo]benzyl C-terminal protecting group (Az-ester) recently devised in one of our laboratories.[2] Following displacement of the cobalt moiety with CN^-, the peptide ester intermediate is isolated by organic solvent extraction and silica gel chromatography.

In the synthesis of the tetrapeptide ester H-Ala-Gly-Phe-Phe-OBzl the following final coupling is representative of the first approach. To Gly-Phe-Phe-OBzl·CH_3COOH (15.8 μmol) in dry Me_2SO (0.2 cm^3) was added 1 equiv of Et_3N followed by $[Co(en)_2(Ala\text{-}OMe)](CF_3SO_3)_3$ (52.7 μmol), and the mixture was stirred for 20 min under anhydrous conditions (reaction 1).

$$\text{Gly-Phe-Phe-OBzl} + [Co(en)_2(\text{Ala-OMe})]^{3+} \rightarrow [Co(en)_2(\text{Ala-Gly-Phe-Phe-OBzl})]^{3+} + \text{MeOH} \quad (1)$$

Then HOAc (0.5 cm^3) and H_2O (4 cm^3) were added to quench the reaction, and the two orange cobalt(III) products[3] were separated by ion exchange chromatography (SP-C25 Sephadex, 8 cm × 1 cm). The coordinated ester hydrolysis product $[Co(en)_2(Ala)]^{2+}$ was eluted with 0.2 mol dm^{-3} of pyridinium acetate and the 3+ peptide-OR complex (reaction 1) with 1.0 mol dm^{-3} of pyridinium acetate. The latter eluate was reduced to a volume of 10 cm^3 under reduced pressure and freeze-dried when it yielded 12.4 mg (14 μmol, 88%) of $[Co(en)_2\text{Ala-Gly-Phe-Phe-OBzl}](CH_3COO)_3$.

Scheme I



The peptide was quantitatively recovered from the complex following Hg/Zn reduction (reaction 2). To the complex (12.4 mg) in water (1.0 cm^3) adjusted to pH 1.0 (HCl_{aq}) was added zinc amalgam (2%, 5 cm^3) and the mixture was stirred vigorously for 5 min. The tetrapeptide was isolated from inorganic salts and ethylenediamine by gel filtration chromatography (Bio-Gel P-2, 70 × 2.5 cm, 2 mol dm^{-3} HOAc). It was shown to be homogeneous by thin-layer chromatography and amino acid analysis.[4] Yields of peptides by this procedure are generally good (>90%) but were sometimes lower,[5] and further experimentation in this area is being undertaken. Confirmation of the presence of benzyl and ethyl ester protecting groups following the reduction step is sometimes possible by high-resolution mass spectrometry.[6]

$$[Co(en)_2(\text{Ala-Gly-Phe-Phe-OBzl})]^{3+} \xrightarrow{Hg/Zn} Co_{aq}^{2+} + \text{H-Ala-Gly-Phe-Phe-OBzl} + 2enH_2^{2+} \quad (2)$$

The synthesis of [Leu^5]enkephalin is shown diagrammatically in Scheme I and is representative of the second approach. A conventional coupling was used for the first two amino acid units, Boc-Phe-OH and H-Leu-OAz.[7] The next coupling using the

(1) See C. R. Clark, R. F. Tasker, D. A. Buckingham, D. R. Knighton, D. R. K. Harding, and W. S. Hancock, *J. Am. Chem. Soc.*, preceding paper in this issue.

(2) G. D. Reynolds, D. R. K. Harding, and W. S. Hancock, *Int. J. Pept. Protein Chem.*, **17**, 231 (1981).

(3) The excess Δ,Λ-$[Co(en)_2(Ala\text{-}OMe)]^{3+}$ ion is rapidly hydrolyzed to $[Co(en)_2(Ala)]^{2+}$ during workup. Reaction rates in water: Δ form, $t_{1/2}$ = 76 s; Λ form, $t_{1/2}$ = 57 s [25 °C, I = 1.0 ($NaClO_4$)]. D. A. Buckingham, C. R. Clark, and R. F. Tasker, unpublished results.

(4) TLC, silica gel; methanol, R_f = 0.62; acetone, R_f = 0.55; 9:1 acetic acid–methanol, R_f = 0.22; 2:2:1 butanol–pyridine–water, R_f = 0.84. A sample hydrolyzed with 6 N HCl at 110 °C, 22 h, gave on amino acid analysis Ala 1.04, Gly 1.00, Phe 1.96.

(5) Very hydrophobic peptides such as Gly-Phe-Phe-OBzl are retarded when chromatographed on Bio-Gel P-2, presumably due to hydrophobic interactions between the solute and the support. The separation of such peptides from the other byproducts was difficult and resulted in lower yields (67% for Gly-Phe-Phe-OBzl).

(6) Ala-Gly-Phe-Phe-OBzl was not sufficiently volatile to give the characteristic parent ion by high-resolution mass spectroscopy. But Gly-Phe-Phe-OBzl gave *m/e* 459 and Ala-Gly-Gly-OEt gave *m/e* 231.

(7) Coupling times vary depending on the amino acid side chain and/or ester grouping. Generally more bulky side chains result in slower rates; e.g., coupling rates (mol^{-1} dm^3 s^{-1}) are 4.75 for $[Co(en)_2(\text{Gly-OMe})]^{3+}$ + Phe-OEt, 1.86 for $[Co(en)_2(\text{Val-OMe})]^{3+}$ + Phe-OEt (both in CH_3CN), 14 for $[Co(en)_2(\text{Gly-OC}_3H_7)]^{3+}$ + Gly-OEt, and 216 for $[Co(en)_2(\text{Gly-OC}_3H_7)]^{3+}$ + Val-OEt (both in Me_2SO) at 25 °C. Improved methods for the coupling of the more bulky amino acid residues will be described in detail later.

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