DALTON
FULL PAPER

Synthesis with coordinated ligands: biomolecule attachment to cage amines

Paul S. Donnelly and Jack M. Harrowfield

Special Research Centre for Advanced Mineral and Materials Processing and Chemistry Department, University of Western Australia, Nedlands, WA 6907, Australia.
E-mail: jmh@chem.uwa.edu.au

Received 2nd November 2001, Accepted 15th January 2002
First published as an Advance Article on the web 25th February 2002

Conventional procedures can be used to obtain derivatives of carboxylic acid groups pendent to a cage amine complex. Polyamines (spermine, 323 tet) with both primary and secondary N-centres react predominantly at the former to give cage-substituted straight-chain amines carrying particularly high charges in aqueous solutions. Branched derivatives are obtained in reactions with tris(2-aminoethyl)amine (tren), though under the conditions associated with the use of an acyl chloride rather than an ester as the activated form of the reactant complex, poor yields of orthoamide species are obtained with this polyamine. Activation using the peptide-forming reagent EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) enables ready coupling of the cage to phenylalanine.

Introduction

The biological activity of cage amines and their derivatives, including their metal complexes,[1,2] has been shown to be diverse, ranging from the treatment of copper overload,[1] through application as an antihelminthic[3] and bacteriocide,[4] to binding to and cleavage of DNA.[5] The great kinetic inertness and thermodynamic stability of cage amine complexes provide considerable potential for their further applications such as in relaxivity enhancement agents[6] and, more importantly, as radiopharmaceuticals,[1,2,7,8] especially if convenient means of attachment of functional groups suitable for biomolecule interaction can be found. We have recently shown[9] that the cage amine 'diaminosarcophagine',1,8-diamino-3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane, $(NH_2)_2$sar, can be functionalised with up to four carboxymethyl substituents, and here we describe some reactions of the directly prepared Co(III) complexes, involving their linkage to molecules of potential or actual biological significance.

In choosing biomolecules for conjugation with an excellent metal-binding agent, both small peptides and proteins (*e.g.* antibodies) are obvious targets. The tumour binding peptides somatostatin and octreotide, for example, when bound to the ^{111}In complex of diethylenetriaminepentaacetic acid have found clinical use as imaging agents for neuroendocrine tumours.[10–12] Metal complex binding to monoclonal antibodies to provide reagents for radioimmunotherapy is of considerable current interest.[13] Perhaps less obvious targets are polyamines, though their biological influence on cell replication, seemingly largely a consequence of their ability to carry a positive charge, is well-recognised[14,15] and synthetic modifications to increase their charge should enhance their binding to bioreceptors.[16] In the context of the present work, amino acids and polyamines were considered equivalent in the sense that both could be used as nucleophiles to attack the carboxyl substituents on cage amine complexes, though here they are differentiated by the methods used to activate the carboxyl group prior to its attack. The polyamines investigated (Fig. 1) were spermine (4,9-diazadodecane-1,12-diamine, 343 tet), 323 tet (4,7-diazadecane-1,10-diamine) and tren (tris(2-aminoethyl)amine), while phenylalanine was chosen as a test amino acid because it is the terminal residue in the somatostatin analogue octreotide.[10]

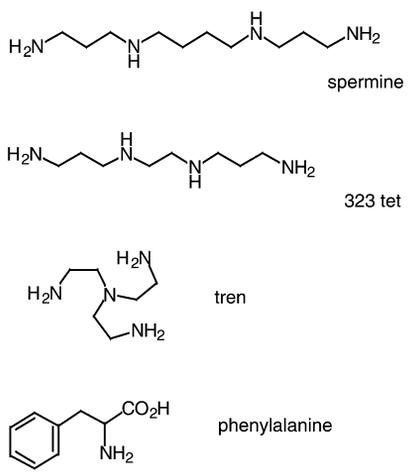


Fig. 1 Nucleophiles used to attack carboxymethylated cage amine complexes.

Experimental

Analytical and spectroscopic instrumentation

Nuclear magnetic resonance (NMR) spectra were acquired using either a Bruker AM 300 (^{1}H at 300 MHz and ^{13}C at 75.5 MHz) or a Bruker ARX 500 (^{1}H at 500.13 MHz and ^{13}C at 125.8 MHz) spectrometer. Chemical shifts for samples measured in D_2O are expressed relative to an internal standard of acetone (δ 2.22 for ^{1}H NMR and δ 30.89 for ^{13}C NMR spectra). Spectra in solvents other than D_2O were referenced to the residual solvent peak. Assignments were made with the aid of the DEPT technique.

UV-visible absorption spectra (200–800 nm) were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. Mass spectra were recorded using the electrospray technique (positive ion trap) on a VG Autospec instrument using a 1 : 1 mix of acetonitrile–water as a solvent. In the spectra reported, many dipositive ions are given as Co(II) species, though the operating resolution of the instrument was not such as to allow a distinction of this from the case of a Co(III) complex of a ligand anion formed by proton loss. Both situations may apply.[17]

 DOI: 10.1039/b110023h

Ion exchange chromatography was performed under gravity flow using H^+ Dowex 50W × 2 resin (200–400 mesh) or SP Sephadex C25 cation exchange resin (Na^+ form, 200–400 mesh). All evaporations were performed at 50 °C under reduced pressure (~20 mm Hg) using a Büchi rotary evaporator and a water aspirator.

The Schlenk technique using high purity argon or high purity nitrogen was employed wherever it was necessary to exclude oxygen or moisture from preparative mixtures. Deionised water was used in all preparations. All organic solvents were distilled prior to use. Acetonitrile was dried by distillation over CaH_2 and stored over 3 Å molecular sieves. DMSO, analytical grade, was stored over 4 Å sieves. All other reagents were purchased from standard commercial sources and were used as received.

Microanalyses for C, H and N were carried out by the Australian National University Microanalytical Service. All samples were thoroughly dried under vacuum (0.1 mm Hg) at 50 °C for 4 h prior to their analysis.

Preparative chemistry

$[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CO_2H)sar)]Cl_5$, $[Co(1,8\text{-}(NH_3)_2(3\text{-}CH_2CO_2H)sar)]Cl_5\cdot 2H_2O$ and $[Co((1,8\text{-}NH_2CH_2CO_2H)_2sar)]Cl_5$ were synthesised by previously reported methods.[9] In several instances, the compounds described ahead were isolated as perchlorates and were treated with due caution, though no untoward reactivity was noted. To simplify later discussion, each new compound is designated by the metal symbol (Co) and a number according to the following sequence of syntheses.

Co1: $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CO_2CH_3)sar)](CF_3SO_3)_5$. Under an atmosphere of dry nitrogen, $[Co((1\text{-}NH_3)(8\text{-}NH_2\text{-}CH_2CO_2H)sar)]Cl_5$ (0.50 g, 0.75 mmol) was added to anhydrous methanol (60 mL). Triflic (trifluoromethanesulfonic) acid (1.5 mL) was cautiously added, causing most of the orange complex to dissolve. The reaction mixture was heated at reflux for 17 h under N_2, then cooled and the methanol removed by evaporation under reduced pressure. The residue was chilled in an ice bath whilst diethyl ether (125 mL) was added to precipitate an orange solid. This was collected by filtration, washed well with diethyl ether and then recrystallised from CH_3CN–ether to give cobalt 1-ammonio-8-(methoxycarbonylmethylammonio)sarcophagine triflate, $[Co((1\text{-}NH_3)(8\text{-}NH_2\text{-}CH_2CO_2CH_3)sar)](CF_3SO_3)_5$, **Co1**, as orange needles (0.98 g, 0.73 mmol, 97%). (Found: C, 20.4; H, 3.3; N, 8.1; calcd for $CoC_{22}H_{40}N_8F_{15}O_{17}S_5\cdot(CF_3SO_3H)$: C, 20.59; H, 3.00; N, 8.35%). ^{1}H NMR (300 MHz; d_3-CH_3CN): δ 2.85, 12H, m, methylene cage protons; 3.40, 12H, m, methylene cage protons; 3.79, 3H, s, OCH_3; 3.95, s, 2H, CH_2CO_2H; 6.4, br s, NH.

Co2: $[Co((1,8\text{-}NH_2CH_2CO_2CH_3)_2sar)]Cl_5$. $[Co((1,8\text{-}NH_2\text{-}CH_2CO_2H)_2sar)]Cl_5$ (0.50 g, 0.75 mmol) and CF_3SO_3H (3 mL) in anhydrous methanol (100 mL) were heated at reflux under N_2 for 17 h. On cooling, the methanol was removed by evaporation under reduced pressure. The residue was chilled in an ice bath and diethyl ether (125 mL) added to give an orange precipitate, which was collected by filtration and washed with diethyl ether. The orange solid was dissolved in acetonitrile, the solution filtered, taken to dryness by evaporation under reduced pressure, and the residue then dried *in vacuo*. ^{1}H NMR (200 MHz): δ 2.68, 12H, m, methylene cage protons; 3.25, 12H, m, methylene cage protons; 3.46, 4H, s, CH_2CO; 3.68, 6H, s, OCH_3. ^{13}C NMR (75.5 MHz): δ 42.0 (OCH_3), 51.8, 51.9 (CH_2 cage), 53.2 (CH_2 cage cap), 58.9 (C cage) and 172.5 (ester carbonyl).

Aminolyses of Co1 and Co2 cations

In the following amide forming reactions, several bands were observed during the chromatographic separations. The desired amide was always the component that was present in the highest quantities. The other bands were the hydrolysed ester, unreacted ester and some products which were thought to be dimeric or oligomeric in nature, which were only present in very small amounts and therefore were not collected.

Co3: $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CONH_2CH_2CH_2NH_3)sar)](ClO_4)_5$. Under dry N_2, $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CO_2CH_3)\text{-}sar)](CF_3SO_3)_5\cdot CF_3SO_3H$ (0.80 g, 0.60 mmol) was dissolved in anhydrous DMSO (5 mL). 1,2-Ethanediamine (0.5 mL) was added, causing the orange solution to turn brown/red. The mixture was heated at 100 °C for 72 h, then cooled to room temperature, acidified with 1 M HCl, diluted with water to a total volume of 500 mL and applied to a column (30 cm × 2 cm diameter) of SP Sephadex (Na^+ form). Elution with 0.5 M sodium chloride resulted in the formation of four bands, the third being the major band (the first band proved to be the hydrolysed starting ester, the second was unreacted ester, whilst the fourth band was not characterised). The third-band eluate was absorbed on a column of H^+ form Dowex 50W × 2 (5 cm × 4 cm) which was washed with water (200 mL) and 1 M HCl (100 mL; to remove Na^+) and then eluted with 3 M HCl. The orange eluate was evaporated to dryness under reduced pressure. The orange residue was dissolved in the minimum amount of water and ethanol was added to the point of turbidity. Storing the mixture at 4 °C resulted in the precipitation of $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CONH_2CH_2CH_2NH_3)sar)]Cl_5$, **Co3** chloride, as a hygroscopic orange solid. This was collected by filtration, washed with ethanol and diethyl ether, then converted to the perchlorate by the following procedure: an aqueous solution of the chloride was shaken with excess acetate form Dowex 1 × 8 anion exchange resin, the resin filtered out, the solution taken to dryness, and perchloric acid added to a solution of the residue in the minimum volume of water. This resulted in the precipitation of $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CONH_2CH_2CH_2\text{-}NH_3)sar)](ClO_4)_5$, **Co3** perchlorate, as orange crystals (0.42 g, 0.44 mmol, 74%) (Found: C, 22.4; H, 5.0; N, 14.0; calcd for $CoC_{18}H_{44}N_{10}Cl_5O_{21}$, C, 22.22; H, 4.56; N, 14.40%). ^{1}H NMR (300 MHz): δ 2.90, 12H, m, cage CH_2; 3.10, 4H, t, $NH_2CH_2\text{-}CH_2NH_3$; 3.45, 12H, m, cage CH_2; 3.80, 2H, s, CH_2CON. ^{13}C NMR (75.5 MHz): δ 37.5, 40.0 ($NHCH_2CH_2NH_3$), 44.8 (NH_2CH_2CO), 52.6, 53.8, 55.0, 55.2 (cage CH_2), 56.6, 61.1 (C cage) and 175.5 (amide carbonyl). MS *m*/*z* 212 = $[CoC_{18}H_{42}\text{-}N_{10}O(CH_3CN)_4]^{3+}$.

Co4: $[Co\text{-}((1\text{-}NH_2)(8\text{-}NHCH_2CONH(CH_2)_2N(CH_2CH_2\text{-}NH_2))sar)]Cl_3\cdot 5HCl$. $[Co(1\text{-}(NH_3)(8\text{-}NH_2CH_2CO_2CH_3)sar)]\text{-}(CF_3SO_3)_5\cdot CF_3SO_3H$ (0.97 g, 0.72 mmol) was dissolved in anhydrous DMSO (8 mL) under N_2. Triethylamine (4 mL) was added and initally an attempt was made to react with a deficiency of tris(2-aminoethyl)amine (53 μL, 52 mg, 0.36 mmol). However, chromatographic monitoring (NaCl elution on Na^+ form SP Sephadex C25) showed reaction to be very slow under these conditions, so that after 96 h at 100 °C, excess tris(2-aminoethyl)amine (2 mL) was added and the reaction mixture was heated at 100 °C for a further 24 h. The reaction mixture was cooled, acidified using glacial acetic acid, diluted with water to a total volume of 500 mL, and the orange solution was applied to a column (30 cm × 2 cm diameter) of Na^+ form SP Sephadex C25. The column was washed with water (500 mL) and the orange compounds were eluted with sodium chloride solution (0.75 M). The major component of the chromatographic separation (there were five other minor bands) was then separately absorbed on a column of H^+ form Dowex 50W × 2 (4 cm × 4 cm). The column was washed with water (100 mL), 1 M HCl (150 mL), the orange compound eluted with 5 M HCl and the eluate was evaporated to dryness under reduced pressure. The orange residue was dissolved in water and **Co4** chloride, $[Co((1\text{-}NH_2)(8\text{-}NHCH_2CONH(CH_2)_2\text{-}N(CH_2CH_2NH_2))sar)]Cl_3\cdot 5HCl\cdot H_2O$, precipitated as an orange powder (0.18 g, 0.22 mmol, 60%) by the addition of ethanol (Found: C, 30.8; H, 6.8; N, 19.2; calcd for $CoC_{22}H_{54}N_{12}Cl_8O\cdot$

H_2O: C, 30.50; H, 6.86; N, 19.40%). ^{1}H NMR (300 MHz): δ 2.81–3.60, 36H, m, methylene protons from cage and tris-(2-aminoethyl)amine fragment; 3.9, 2H, s, CH_2CON. ^{13}C NMR (75.5 MHz): δ 34.5, 35.1, 44.6, 50.8, 50.9 (CH_2), 51.8, 54.2, 55.2 (CH_2 cage), 57.2, 62.0 (C cage), 169.0 (amide carbonyl). MS *m/z* 667 and 665 = $[CoC_{22}H_{53}N_{12}OCl_3]^+$; 318 = $[CoC_{22}H_{53}N_{12}OCl(CH_3CN)]^{2+}$; 279 = $[CoC_{22}H_{52}N_{12}O]^{2+}$; 227.6 = $[Co^{II}C_{22}H_{53}N_{12}O(CH_3CN)_3]^{3+}$ or $[CoC_{22}H_{54}N_{12}O(CH_3CN)_3 - H]^{3+}$.

Co5: $[Co((1,8\text{-}NH_3)_2(3\text{-}CH_2CONH(CH_2)_2N(CH_2CH_2NH_2)_2)sar)]Cl_3\cdot 5HCl$. A mixture of $[Co(1,8\text{-}(NH_3)_2(3\text{-}CH_2CO_2H)sar)]Cl_5\cdot 2H_2O$ (0.16 g, 0.24 mmol) and CF_3SO_3H (1 mL) in anhydrous methanol (30 mL) was heated at reflux under N_2 for 20 h. The methanol was removed by evaporation under reduced pressure. The syrupy residue was cooled in an ice bath whilst diethyl ether (125 mL) was gradually added. The resulting red precipitate was collected by filtration, washed with diethyl ether, then dissolved in acetonitrile, filtered and recrystallised by the addition of diethyl ether. This red solid was presumed to be the ester, $[Co(1,8\text{-}(NH_3)_2(3\text{-}CH_2CO_2CH_3)sar)]^{5+}$ and was used without further characterisation. The solid was dissolved in anhydrous DMSO (10 mL), tris(2-aminoethyl)amine (1 mL) added and the now dark purple reaction mixture was heated under N_2 at 90 °C for 20 h. The mixture was then diluted with water (200 mL) and applied to a column (30 cm × 2 cm diameter) of Na^+ form SP Sephadex C25 resin (pH ~10). The column was washed with ~10^{-3} M Na_2CO_3 and eluted with 0.05 M sodium citrate (pH 10) to give one major band and three other minor bands. The major band was collected and applied to a column (6 cm × 4 cm diameter) of H^+ Dowex 50W × 2. The column was washed with water (200 mL), 1 M HCl (150 mL) and then the red compound was eluted with 5 M HCl. The red eluate was evaporated to dryness under reduced pressure to give a residue which was dissolved in a small volume of 1 M HCl. Addition of ethanol gave $[Co((1,8\text{-}NH_3)_2(3\text{-}CH_2CONH(CH_2)_2N(CH_2CH_2NH_2)_2)sar)]Cl_3\cdot 5HCl\cdot 5H_2O$ as a red solid (0.10 g, 0.11 mmol, 46%) (Found: C, 28.6; H, 6.7; N, 18.0; Cl, 30.1; calcd for $CoC_{22}H_{57}N_{12}OCl_8\cdot 5H_2O$: C, 28.16; H, 7.20; N, 17.91; Cl, 30.22%). ^{1}H NMR (300 MHz) δ 2.72–3.95, complex m, 38H, methylene protons. ^{13}C NMR (75.5 MHz) δ 36.4, 36.8, 38.6, 52.4, 53.2, 53.4, 53.8, 54.7, 55.2, 55.7, 56.0, 56.2, 56.8, 57.7, 58.6, 62.5, 64.5, 64.3, 65.3 and 168.0. MS *m/z* 667 and 665 = $[CoC_{22}H_{53}N_{12}OCl_3]^+$; 317.7 = $[CoC_{22}H_{52}N_{12}O]^{2+}$; 279 = $[CoC_{22}H_{52}N_{12}O]^{2+}$; 227.7 = $[Co^{II}C_{22}H_{53}N_{12}O(CH_3CN)_3]^{3+}$.

Co6: $[Co((NHCH_2CONH(CH_2)_2N(CH_2CH_2NH_2)_2)sar)]Cl_3\cdot 8HCl$. A sample of the diester was prepared as previously described using $[Co((1,8\text{-}NH_2CH_2CO_2H)_2sar)]Cl_5$ (0.5 g, 0.75 mmol). The orange residue was dissolved in anhydrous DMSO (10 mL), tris(2-aminoethyl)amine (2 mL) was added and the now deep purple reaction mixture was heated at 90 °C for 20 h under N_2. Dilution with water (500 mL) gave an orange solution which was applied to a column (30 cm × 2 cm diameter) of Na^+ form SP Sephadex C25. The column was washed with ~10^{-3} M Na_2CO_3 and then eluted with 0.05–0.075 M sodium citrate (adjusted to pH ~10 with sodium carbonate) to give one major band. This orange band was absorbed to a column (10 cm × 4 cm diameter) of H^+ form Dowex 50W × 2. The column was washed with water (200 mL) and 1 M HCl (150 mL) before being eluted with 5 M HCl. The orange eluate was evaporated to dryness and the orange residue was dissolved in the minimum volume of 1 M HCl. This orange solution was added slowly to an ice-cooled mixture of ethanol–ether (60 : 40; 125 mL) to give $[Co((NHCH_2CONH(CH_2)_2N(CH_2CH_2NH_2)_2)sar)]Cl_3\cdot 8HCl\cdot CH_3CH_2OH\cdot 8H_2O$, **Co6**, as an orange powder (0.83 g, 0.60 mmol, 80%). (Found: C, 29.6; H, 7.5; N, 16.4; Cl, 27.7; calcd for $CoC_{30}H_{78}N_{16}Cl_{11}O_2\cdot 2C_2H_5OH\cdot 8H_2O$: C, 29.59; H, 7.74; N, 16.24; Cl, 28.25%. The presence of ethanol was confirmed (by NMR) on the solid submitted for analysis). ^{1}H NMR (300 MHz): δ 2.71–3.55, 48H, m, methylene protons; 3.79, 4H, AB quartet, CH_2CO. ^{13}C NMR (75.5 MHz): δ 32.5, 33.1, 42.7, 48.9, 49.0, 52.2, 52.5, 59.4 (CH_2) and 166.9 (amide carbonyl). MS *m/z* 217.5 = $[CoC_{30}H_{71}N_{16}O_2(CH_3CN)_3]^{4+}$; 227.5 = $[CoC_{30}H_{71}N_{16}O_2(CH_3CN)_4]^{4+}$; 248.3 = $[CoC_{30}H_{70}N_{16}O_2]^{3+}$; 262 = $[CoC_{30}H_{70}N_{16}O_2(CH_3CN)]^{3+}$; 275.5 = $[CoC_{30}H_{70}N_{16}O_2(CH_3CN)_2]^{3+}$; 372.5 = $[CoC_{30}H_{71}N_{16}O_2 - H]^{2+}$; 390.5 = $[CoC_{30}H_{72}N_{16}O_2Cl - H]^{2+}$; 408.5 = $[CoC_{30}H_{73}N_{16}O_2Cl_2 - H]^{2+}$; 427.5 = $[CoC_{30}H_{74}N_{16}O_2Cl_3 - H]^{2+}$; 444 = $[CoC_{30}H_{71}N_{16}O_2Cl_4 - H]^{2+}$.

Co7: $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CONH_2(CH_2)_3NH_2(CH_2)_2NH_2(CH_2)_3NH_3)sar)]Cl_9$. $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CO_2CH_3)sar)](CF_3SO_3)_5\cdot CF_3SO_3H$ (0.50 g, 0.37 mmol) was dissolved in anhydrous DMSO (7 mL), 323 tet (2 mL) added and the purple reaction mixture was heated at 90 °C under N_2 for 17 h. The reaction mixture was allowed to cool to room temperature and then diluted with water to a total volume of 500 mL, with the colour turning red/orange. This solution was applied to a column (30 cm × 2 cm diameter) of SP Sephadex C25 (Na^+ form) previously washed with ~10^{-3} Na_2CO_3. Elution with a 0.05–0.075 M sodium citrate solution (pH 10 with sodium carbonate) gave one major and four minor bands. The major band was absorbed on a column (5 cm × 4 cm diameter) of H^+ Dowex 50W × 2. The column was washed with water (200 mL) and 1 M HCl (200 mL) and orange material then eluted with 5 M HCl. The eluate was evaporated to dryness and the orange residue was dissolved in the minimum amount of 1 M HCl. Dropwise addition of this solution to an ice cooled ether–ethanol solution (50 : 50, 125 mL) gave an orange precipitate which was collected by filtration and washed with ethanol and ether to give $[Co((1\text{-}NH_3)(8\text{-}NH_2CH_2CONH_2(CH_2)_3NH_2(CH_2)_2NH_2(CH_2)_3NH_3)sar)]Cl_9\cdot 3H_2O$, **Co7** chloride, as an orange solid (0.25 g, 0.26 mmol, 72%). This solid proved to be hygroscopic and analyses were irreproducible (Found: C, 30.3; H, 7.0; N, 16.9; calcd for $CoC_{24}H_{62}N_{12}Cl_9O\cdot(H_2O)_3$, C: 29.81; H, 7.09; N, 17.38%). ^{1}H NMR (500 MHz): δ 1.70, 2H, m, $NH_2CH_2CH_2CH_2NH_3$; 1.91, 2H, m, $NH_2CH_2CH_2CH_2NH_2$; 2.70–3.41, 36H, m, methylene protons of cage and 323 tet moieties; 3.80, 2H, s, CH_2CO_2. ^{13}C NMR (125.8 MHz): δ 24.5, 26.3, 37.1, 37.3, 44.0, 44.4, 46.0, 46.6 (CH_2), 50.8, 51.8, 55.1 (CH_2 cage), 56.9, 61.6 (C cage), 167.2 (amide carbonyl). MS *m/z* 702 = $[CoC_{24}H_{55}N_{12}OCl(CH_3CN)_2 - H]^+$; 698 = $[CoC_{24}H_{56}N_{12}OCl_2(CH_3CN)]^+$.

Co8: $[Co((1,8\text{-}NHCH_2CONH(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2)sar)]Cl_3\cdot 9HCl$. A sample of the diester was prepared as above using $[Co((1,8\text{-}NH_2CH_2CO_2H)_2sar)]Cl_5$ (0.50 g, 0.75 mmol). The orange residue was dissolved in anhydrous DMSO (10 mL), 323 tet (2 mL) added and the now purple reaction mixture was heated at 90 °C N_2 for 48 h. The mixture was diluted with water and applied to a column (30 cm × 2 cm diameter) of Na^+ form SP Sephadex C25. The column was washed with ~10^{-3} M Na_2CO_3 and then eluted with 0.075 M sodium citrate (pH 10) to give one major band (and three other bands which were not collected). The major, orange band was absorbed to a column H^+ Dowex 50W × 2 (8 cm × 4 cm). The column was washed with water (200 mL) and 1 M HCl (200 mL), and then the complex was eluted with 5 M HCl. The orange eluate was evaporated to dryness and the residue was dissolved in the minimum amount of 1 M HCl. This orange solution was gradually added to cold ethanol–ether (60 : 40; 125 mL) to give $[Co(1,8\text{-}(NHCH_2CONH(CH_2)_3NH(CH_2)_2NH(CH_2)_3NH_2)_2sar)]Cl_3\cdot 9HCl\cdot CH_3CH_2OH\cdot 6H_2O$, **Co8** (0.61 g, 0.44 mmol, 58%) as an orange powder (Found: C, 31.3; H, 7.4; N, 16.0; Cl, 30.0; calcd for $CoC_{34}H_{87}N_{16}Cl_{12}O_2\cdot C_2H_5OH\cdot 6H_2O$: C, 31.09; H, 7.61; N, 16.11; Cl, 30.59%. The presence of ethanol was confirmed (NMR) on the solid submitted for analysis). ^{1}H NMR (300 MHz): δ 1.77, 4H, m, 2 × $CH_2CH_2CH_2$; 1.92, 4H, 2 × $CH_2CH_2CH_2$; 3.17, 48H, complex m, NCH_2; 3.75, 8H, AB quartet, 2 × CH_2CO. ^{13}C NMR (75.5 MHz):

δ 24.4, 26.3 $CH_2CH_2CH_2$, 36.5, 37.1, 43.6, 43.8, 44.6, 45.8, 46.4 NCH_2, 53.3 (CH_2 cage), 54.9 (CH_2 cage cap), 60.7 (C cage), 174.0 (amide carbonyl). MS *m/z* 436 = $[CoC_{34}H_{79}N_{16}O_2Cl_2]^{2+}$ and *m/z* 267 = $[CoC_{34}H_{78}N_{16}O_2]^{3+}$.

Co9: [Co((1-NH$_2$)(8-NHCH$_2$CONH(CH$_2$)$_3$NH(CH$_2$)$_4$NH(CH$_2$)$_3$NH$_2$)sar)]Cl$_3$·6HCl. [Co(1-(NH_3)(8-$NH_2CH_2CO_2CH_3$)sar)](CF_3SO_3)$_5$·CF_3SO_3H (0.97 g, 0.72 mmol) was dissolved in anhydrous DMSO (15 mL), spermine (1 mL) added and the purple reaction mixture was heated under N_2 at 80 °C for 16 h. The reaction mixture was allowed to cool to room temperature and then diluted with water (500 mL), resulting in the solution returning orange. The solution was applied to a column (40 cm × 2 cm diameter) of Na^+ form SP Sephadex C25. The column was washed with ~10^{-3} M Na_2CO_3 (pH ~10; 500 mL). Elution with 0.05 M sodium citrate (pH ~10) resulted in the rapid elution of two minor orange bands which were not collected. A third major orange band (~80%) was eluted with 0.060–0.075 M sodium citrate (pH 10). This orange band was absorbed on a column (8 cm × 2 cm) of H^+ form Dowex 50W × 2 which was washed with water and 1 M HCl, and then eluted with 5 M HCl. The orange eluate was taken to dryness and the residue was dissolved in the minimum amount of 1 M HCl, dropwise addition of this solution to a 60 : 40 ethanol–ether mixture (125 mL) giving an orange precipitate of [Co-((1-NH_2)-(8-$NHCH_2CONH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$)sar)]$Cl_3$·6HCl, **Co9** (Found: C, 30.5; H, 6.6; N, 15.9; calcd for $CoC_{26}H_{66}N_{12}Cl_9O·4H_2O$: C, 30.83; H, 7.36; N, 16.59%. This solid was hygroscopic and analyses were irreproducible). ^{1}H NMR (500 MHz): δ 1.78, 4H, m, $NCH_2CH_2CH_2CH_2NH_2$; 1.93, 2H, tt, $CH_2NH_2CH_2CH_2CH_2NH_2$; 2.11, 2H, tt, $CONH_2CH_2CH_2CH_2NH_2$; 2.90, 12H, m, methylene cage protons; 3.10, 10H, m, methylene protons of spermine moiety; 3.45, 2H, t, methylene protons from spermine; 3.50, 12H, m, methylene cage protons; 3.81, 2H, s, CH_2CO. ^{13}C NMR (125.8 MHz): δ 22.76, 23.71, 25.47, 36.26, 36.55, 43.57, 44.52, 45.12, 46.90, 46.99 (CH_2), 50.63, 50.99, 54.22 (CH_2 cage), 55.96, 60.60 (C cage), 168.24 (amide carbonyl). MS *m/z* 796 = $[CoC_{26}H_{64}N_{12}OCl_5 - H]^+$; 760 = $[CoC_{26}H_{64}N_{12}OCl_4 - H]^+$; 722 = $CoC_{26}H_{61}N_{12}OCl_3 - H]^+$; 686 = $[CoC_{26}H_{62}N_{12}OCl_2 - H]^+$; 345 = $[CoC_{26}H_{60}N_{12}OCl(CH_3CN)]^{2+}$; 325 = $[CoC_{26}H_{60}N_{12}OCl]^{2+}$; 307 = $[CoC_{26}H_{59}N_{12}O]^{2+}$; 232.3 = $[CoC_{26}H_{60}N_{12}O(CH_3CN)_2]^{3+}$.

Co10: [Co((1-NH$_2$)(8-NHCH$_2$C(NHCH$_2$CH$_2$)$_3$N)sar)]Cl$_3$·*x*HCl. Solid [Co((1-NH_3)(8-CH_2CO_2H)sar)]Cl_5 (0.20 g, 0.3 mmol) was added to a mixture of thionyl chloride (6 mL) and triflic acid (0.5 mL). The mixture was heated at reflux for 15 h. The thionyl chloride was removed *in vacuo* to give a brown syrupy residue which was dissolved in dimethylacetamide (5 mL). Triethylamine (3 mL) was added, followed by tris-(2-aminoethyl)amine (0.020 g, 0.13 mmol). The mixture was heated at 80 °C for 2 h, then allowed to cool to room temperature and an orange solid was collected by filtration. The solid was dissolved in water and applied to a column of SP Sephadex C25 (Na^+ form). The column was eluted using sodium citrate solutions increasing in concentration from 0.05–0.075 M (all adjusted to pH ~10 with Na_2CO_3). The separation proved to be quite difficult due to the number of components in the mixture. The only well-separated component was the third band, from which an orange solid, **Co10**, was recovered (*via* Dowex 50W × 2/HCl) in poor yield (20 mg). ^{1}H NMR (200 MHz): δ 2.84, 18H, m, CH_2; 3.13, 6H, t, CH_2; 3.45, 14H, m, CH_2. ^{13}C NMR (75.5 MHz): δ 34.6, 48.1, 49.4, 50.6, 52.7, 52.8, 53.0 (CH_2), 50.8, 54.2 and 58.6 (C).

Co11: [Co(1,8-(NHCH$_2$C(NHCH$_2$CH$_2$)$_3$N)$_2$sar)]Cl$_3$·*x*HCl. Thionyl chloride (15 mL) was added to [Co(($NH_2CH_2CO_2H$)$_2$sar)](CF_3SO_3)$_5$ (0.30 g, 0.24 mmol) and the mixture was heated under N_2 at reflux overnight. The thionyl chloride was removed *in vacuo*, the orange residue was dissolved in dimethylacetamide (10 mL) and tris(2-aminoethyl)amine (1.5 mL) was added, causing some immediate precipitation of an orange solid. The mixture was heated under N_2 at 80 °C for 1 h. After cooling, the solid was collected by filtration and then dissolved in water and subjected to chromatography as with **Co10**. Again, the separation proved to be difficult due to the number of components in the mixture, and the only material (**Co10**, 30 mg) readily isolated free of others was again in the third band. ^{1}H NMR (500 MHz): δ 2.85, 24H, m, CH_2; 3.05, 12H, t, CH_2; 3.38, 16H, CH_2. ^{13}C NMR (125.8 MHz): δ 38.4, 52.0, 53.2, 56.9 (CH_2), 51.7, 56.3 and 58.4 (C).

Attachment of amino acids using a peptide coupling agent

Co12 and Co13. [Co((1-NH_3)(8-$NH_2CH_2CO_2H$)sar)](CF_3SO_3)$_5$ (1.00 g, 0.85 mmol) was dissolved in anhydrous DMF (5 mL) under N_2. *N*-hydroxysuccinimide (0.146 g, 1.27 mmol) was added followed by diisopropylethylamine (0.55 g, 0.74 mL, 4.2 mmol) and the mixture was stirred for 10 min. L-Phenylalanine (0.21 g, 1.27 mmol) was added and the mixture was stirred for a further 10 min. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) (1.40 g, 7.3 mmol) was added and the mixture was stirred for 24 h. The mixture was then diluted with water to a total volume of 500 mL and applied to a column (30 cm × 2 cm diameter) of Na^+ form SP Sephadex C25. Elution with 0.05 M sodium citrate revealed one major broad band. This was collected and applied to a column (6 cm × 4 cm) of H^+ form Dowex 50W × 2. The column was washed with water (200 mL) and 1 M HCl (200 ml) and then eluted with 5 M HCl to provide two components, fraction 1, ~80% and fraction 2, ~20%.

The fraction 1 eluate was evaporated to dryness and the residue crystallised from water by the addition of ethanol to give [Co((1-NH_3)(8-Gly-Phe)sar)]Cl_5·$2H_2O$, **Co12**, as an orange powder, (0.49 g, 0.62 mmol, 73%) (Found: C, 38.1; H, 6.6; N, 15.7; calcd for $CoC_{25}H_{47}N_9O_3Cl_3·2H_2O = CoC_{25}H_{51}Cl_5N_9O_5$: C, 37.82; H, 6.47; N, 15.88%). ^{1}H NMR (500 MHz): δ 3.00, 26H, complex m, cage methylene and $CH_2C_6H_5$; 3.51, 2H, AB qt, CH_2CO_2; 4.82, t, O_2CCHCH_2; 7.29, 2H, m, ArH; 7.38, 2H, m, ArH; 7.45, m, 2H, ArH. ^{13}C NMR (125.8 MHz): δ 36.8 (CH_2Ar), 44.4 (CH_2N), 52.0, 53.2, 55.2, 55.3 (CH_2 cage), 54.0 (amino acid CH), 56.7, 61.1 (C cage), 28.1, 129.5, 129.9, 137.1 (ArC), 173.8, 176.1 (carboxyl). MS *m/z* 576 = $[CoC_{25}H_{43}N_9O_3]^+$; 329 = $[(CoC_{25}H_{44}N_9O_3)(CH_3CN)_2]^{2+}$. Visible spectrum, λ_{max} = 472 nm; ε = 138 M^{-1} cm^{-1}.

The second fraction eluate was treated similarly to give [Co((1-NH_3)(8-NH_2Gly-Phe-Phe)sar)]Cl_5·$5H_2O$, **Co13**, as an orange powder (0.13 g, 0.13 mmol, 15%) (Found: C, 40.4; H, 6.5; N, 14.6; calcd for $CoC_{34}H_{56}N_{10}O_4Cl_5·5H_2O = CoC_{34}H_{66}Cl_5N_{10}O_9$: C, 41.04; H, 6.68; N, 14.07%). ^{1}H NMR (200 MHz): δ 3.00, complex m, 30H, methylene protons; 7.20, 5H, m, ArH. ^{13}C NMR (75.5 MHz): δ 37.2 and 37.8 (CH_2Ar), 42.0 (CH_2N), 51.8, 52.1, 55.0 (CH_2 cage), 54.2 (amino acid CH), 56.4, 61.2 (C cage), 127.4, 127.6, 129.1, 129.3, 129.9, 136.5, 137.0 (ArC), 171.8, 173.0, 176.1 (carboxyl). MS *m/z* 723 = $[CoC_{34}H_{52}N_{10}O_4]^+$; 383 = $[(CoC_{34}H_{53}N_{10}O_4)(CH_3CN)]^{2+}$.

Results and discussion

Cage complex amides derived from polyamines

Structures deduced for the amides **Co3–Co11** are shown in Fig. 2. The reaction used to produce **Co3–Co9**, that between a carboxylic ester and an amine (Scheme 1), is a conventional method of amide formation,[18] though it was initially hoped that the proximity of the cationic centre to the ester unit might produce a significant enhancement of its reactivity.[19] This does not appear to be the case and is possibly advantageous in inhibiting multiple acylation of polyamines (and in avoiding reaction of [Co(1-NH_2)(8-$NHCH_2CO_2CH_3$)sar]$^{3+}$ with itself, even if the

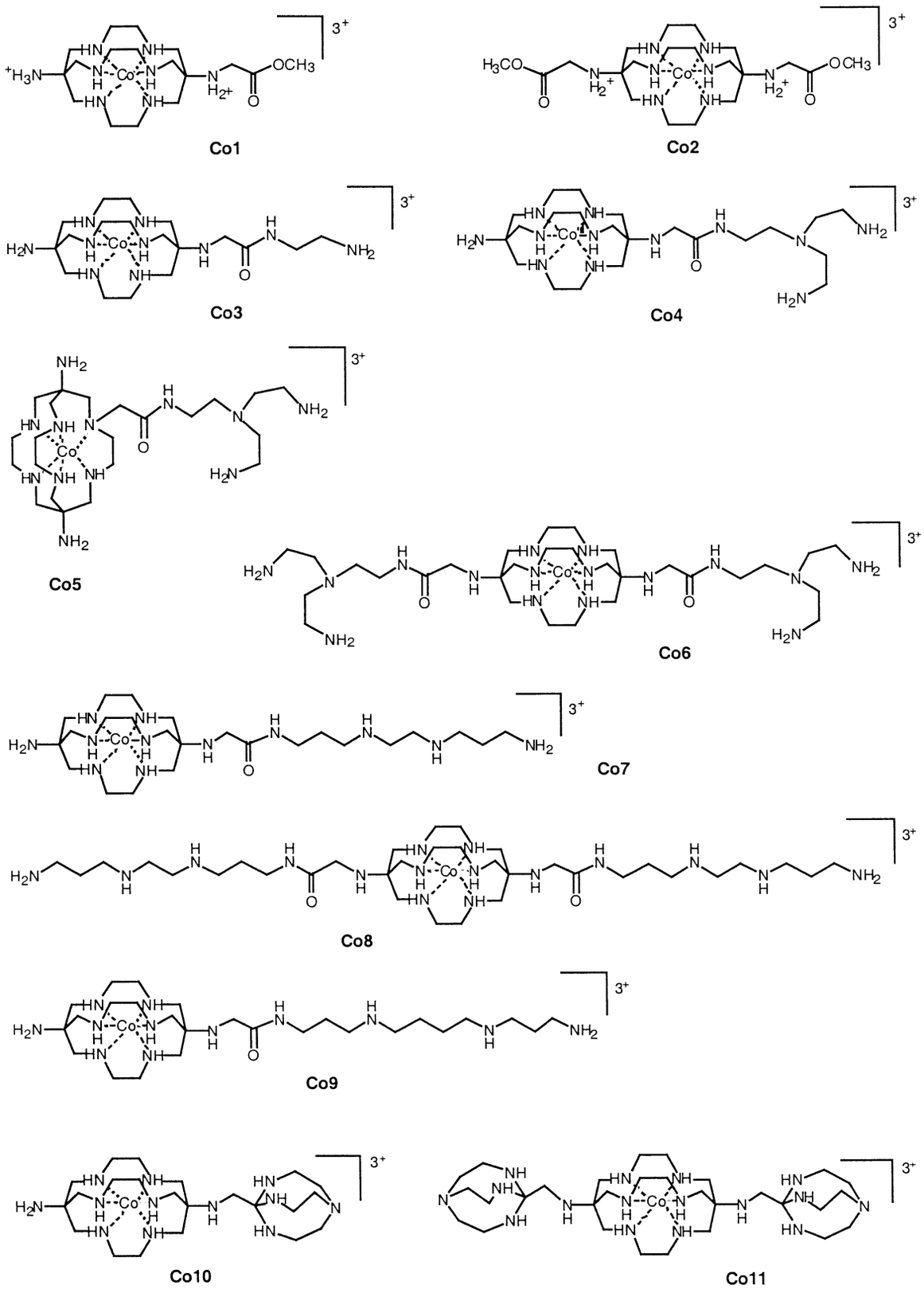
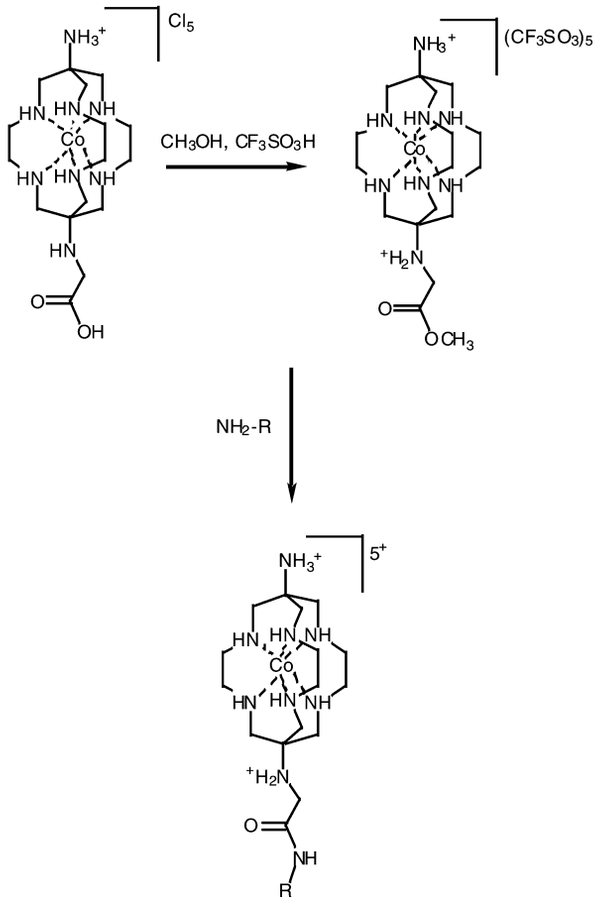


Fig. 2 A catalogue of cobalt-cage-complex/polyamine conjugates.

amino group is deactivated[9]), though it does mean that quite vigorous conditions involving a considerable excess of the polyamine were necessary for efficient amide formation. Where, as in the present cases, the polyamine is the less precious reagent, this need not be seen as a disadvantage, but obviously the simple esters cannot be seen as useful acylating agents for proteins which may be of low abundance. Nonetheless, both mono- and di-ester derivatives of the Co(III) cage amine complex are readily formed, and use of the triflate (trifluoromethanesulfonate), rather than sulfate,[9] salts provides materials of useful solubility in organic solvents.

Characterisation of the polyamine amides was generally quite straightforward on the basis of their NMR spectra. The product **Co3** of the reaction of the monoester **Co1** with ethane-1,2-diamine, for example, shows ten signals (though two near δ 55 are barely resolved) in its ^{13}C spectrum, as expected if rotation of the pendent arm is sufficiently rapid for the three arms of the cage to be rendered equivalent. Assuming



Scheme 1 Conversion of a pendent carboxymethyl substituent to ester and amide derivatives.

resonances due to the cage unit to be little shifted from those in simpler systems,[9] peaks at δ 37.5 and 40.0 can be assigned to the carbon atoms of the introduced $NHCH_2CH_2NH_3^+$ moiety, with the amide carbonyl resonance at δ 175.5. In the ^{1}H spectrum, assignment of "new" peaks is more difficult because of the overlap and complexity of the resonances but a triplet at δ 3.10 may be due to the methylene protons of $NHCH_2$-$CH_2NH_3^+$ and a singlet at δ 3.80 due to the methylene group protons of the original carboxymethyl entity, now carboxamidomethyl. An alternative formulation of the reaction product as a cyclic amidine species, though also consistent with all the mentioned measurements, was excluded on the basis of the observation of four and not three NH resonances in the ^{1}H spectrum recorded in $(CD_3)_2SO$. (See also discussion of the identification of **Co4** below.) The electrospray mass spectrum has a peak at *m*/*z* 212 which may be assigned to the expected tripositive cation with four molecules of the co-solvent acetonitrile, $[CoC_{18}H_{42}N_{10}O(CH_3CN)_4]^{3+}$. The synthesis of this compound shows that the carboxymethyl arm can be used as a site for extended functionalisation of the cage complex. The compound itself may be useful as an intermediate for even further reactions in which the new cage unit could act as a nucleophile which would possess enhanced reactivity when compared to the starting diaminosarcophagine ligand, as the introduced amine is more remote from the charged metal centre.[19] The new pendent arm could also coordinate to a metal ion to form dimetallic systems.

This success in forming an amide from the relatively simple amine led to the investigation of reactions involving the more complicated, potentially or actually biologically active tetramines, tren, 323 tet and spermine (343 tet) (Fig. 1). Complications were anticipated due to the presence of inequivalent N-centres in these amines, so that reactions involving tren were investigated first because, although it has two types of amine centre, only one can give stable amides.

Reaction of tren with the methyl ester (**Co1**) was first performed in a 0.3 : 1 stoichiometry in an attempt to obtain the molecule which would contain three cages linked in a tripodal arrangement. The fact that the amine was no longer in excess meant that triethylamine had to be added to neutralise the reaction mixture. Under these conditions, however, reaction proved to be extremely slow and so an excess of tren was added in an attempt to maximise the yields of the compound in which the two reactants were combined in a one to one ratio. Chromatographic separation of the reaction mixture yielded one major component which proved to be **Co4** (Fig. 2), where there is one tren unit attached to the cage *via* an amide linkage. The ^{13}C NMR spectrum of the molecule shows, presumably because of fortuitous isochronicity of two signals, eleven of the twelve signals that would be expected, again assuming rotation of the pendent group averages out any asymmetry due to the attachment of a substituent of at most twofold symmetry to a cage of threefold symmetry. This militates against any asymmetry in the pendent group that might result from formation of an (eight-membered) amidine ring, and since the chemical shift of the unsaturated, quaternary carbon (δ 169) is similar to that of the equivalent carbon in **Co3**, this is taken as combined justification for attributing both resonances to carboxamide carbon (and hence for defining both compounds as amides, as in Fig. 1).

Co4 has several sites where it could be protonated and it exhibits complicated speciation behaviour. The analysed solid was actually isolated from 1 M HCl in a procedure that gave a material that analysed well for the octachloride hydrate. The electrospray mass spectrum of **Co4** has peaks which can be attributed to the cation "flying" with overall charges of +1, +2 and +3. The major peak at *m*/*z* 665, is due to $[CoC_{22}H_{53}N_{12}OCl_3]^+$. The presence of the three chlorides is confirmed by the isotope pattern which results in a peak of similar intensity at *m*/*z* 666. Another relatively intense peak occurs at *m*/*z* 317.5 which is due to an overall 2+ cation with one molecule of solvent (acetonitrile), $[CoC_{22}H_{52}N_{12}OCl(CH_3CN)]^{2+}$. A minor peak is evident at *m*/*z* 279 which is due to $[CoC_{22}H_{52}N_{12}O]^{2+}$. There is also a peak at *m*/*z* 228 which could be due to either $[CoC_{22}H_{54}N_{12}O(CH_3CN)_3 - H]^{3+}$ or $[CoC_{22}H_{54}N_{12}O(CH_3CN)_3]^{3+}$ where the metal has undergone reduction to Co(II). Unfortunately, the mass resolution was not sufficient to distinguish these possibilities and both have been observed in electrospray mass spectra of other cobalt cage complexes.[17] It has been found that the reduction of the metal centre in these cobalt cage systems is dependent on the cone and skimmer potentials used.[17]

The 3-carboxymethyl cage complex was readily esterified using the same procedure as for **Co1**, and reaction of this ester with an excess of tren gave the constitutional isomer of **Co4**, **Co5**. The compound retained the red colour of the starting complex[9] and again was isolated as the octachloride salt. The electrospray mass spectrum was the same as that of **Co4** but the ^{13}C NMR spectrum reflected the asymmetry of the complex, exhibiting 20 distinct signals out of the 32 possible.

Reaction of the dimethyl ester, **Co2**, with an excess of tren and subsequent chromatography enabled the isolation of the diamide, **Co6**, in good yields. The ^{13}C NMR spectrum displays nine signals, reflecting effective D_3 symmetry of the molecule and seemingly excluding any cyclic amidine formation. The signal at δ 166.9 is thus assigned to carboxamide carbon.

Co6 obviously possesses many potential sites for protonation and may exist with a very high charge. The material isolated from 1 M HCl analysed as an undecachloride, suggesting all amino sites must be protonated, at least in the solid state. The electrospray mass spectrum has ions which show the cation in the +2, +3 and +4 state. The most abundant ion, which is at *m*/*z* 227.5, can be attributed to the tetrapositive cation associated with four molecules of the co-solvent acetonitrile,

$[CoC_{30}H_{71}N_{16}O_2(CH_3CN)_4]^{4+}$. Another ion which corresponds to the tetrapositive cation (plus $3CH_3CN$) is observed at *m*/*z* 217.5. The tripositive cation is also present in different forms with the "bare cation", $[CoC_{30}H_{70}N_{16}O_2]^{3+}$ (72% relative abundance) having a slightly higher abundance than the acetonitrile monosolvate, $[CoC_{30}H_{70}N_{16}O_2(CH_3CN)]^{3+}$ (69%) and a considerably higher abundance than the trisolvate (31%). A molecular ion which is due to either $[Co^{III}C_{30}H_{71}N_{16}O_2 - H]^{2+}$ or $[Co^{II}C_{30}H_{70}N_{16}O_2]^{2+}$ is also seen. This particular cation can also be observed associated with up to four chlorides, perhaps due to the strong anion binding ability of these highly charged cations.

Similar reactions allowed the attachment of the linear tetramine 323 tet to form both the mono- (**Co7**) and di-substituted (**Co8**) derivatives in which the 323 tet unit is attached *via* a formerly primary amine. **Co7** was isolated from 1 M HCl as a nonachloride. If each of the five non-coordinated amine sites were protonated this material would have a charge of +8. The other chloride could be due to the crystallisation of "HCl" in some form in the solid (*e.g.* $H_5O_2^+Cl^-$ in *trans*-$[Co(en)_2Cl_2]Cl{\cdot}HCl{\cdot}2H_2O$[20]) or the protonation of the amide. Under the isolation conditions the former would be more likely.[21] This is also the case for **Co8**.

The ^{13}C NMR spectra of **Co7** and **Co8** show eight signals due to all the inequivalent carbon atoms of the 323 tet moiety, and although it might be argued that cyclic amidine formation could be favoured in these systems (relative to those from tren), signals at δ 167.2 (**Co7**) and 174.0 (**Co8**) are again taken to be those of carboxamide carbon. Here, this proposition was confirmed by conversion of the chloride salts to the triflates, which allowed ^1H NMR spectra to be acquired in d_3-acetonitrile. These spectra showed a resonance due to an amide proton at δ 7.8 as well as other resonances δ 6.7, 6.9, 7.2 and 7.4 for the different amino groups present. This also showed that the conjugates contained a secondary amide and that the reaction had occurred on an originally primary amine site. Evidence for some reaction leading possibly to amides derived from secondary N atoms was only obtained when reaction temperatures above 90 °C were employed. The chromatographic separation of these conjugates on ion exchange resin was best achieved with sodium citrate solutions adjusted to pH 10 with sodium carbonate to minimise their potentially high charge.

The electrospray mass spectrum of **Co7** showed peaks at *m*/*z* 702 due to $[CoC_{24}H_{55}N_{12}OCl(CH_3CN)_2 - H]^+$ (or $[CoC_{24}H_{54}N_{12}OCl(CH_3CN)_2]^+$) and at *m*/*z* 698 due to $[CoC_{24}H_{56}N_{12}OCl_2(CH_3CN)]^+$. The electrospray mass spectrum of **Co8** has peaks at *m*/*z* 267 due to $[CoC_{34}H_{78}N_{16}O_2]^{3+}$ and *m*/*z* 436 due to $[CoC_{34}H_{79}N_{16}O_2Cl_2]^{2+}$.

The reaction of the biogenic amine spermine with the monoester **Co1** enabled the isolation of a molecule, **Co9**, in which spermine was attached to the cage, again in very high yields. The ^1H NMR spectrum of **Co9** has signals for the methylene protons of the cage in the usual region. The spermine moiety gives rise to signals at δ 1.78, 1.93, 2.11, 3.10 and 3.45 whilst the methylene protons adjacent to the amide carbon give a signal at δ 3.81. The ^1H NMR spectrum of the triflate salt in d_3-acetonitrile showed a single resonance at δ 7.93 due to the amide proton as well as resonances for the amino protons present which confirmed that the spermine moiety was attached *via* a formerly primary amine centre. Careful control of the temperature during the reaction was again required, to ensure that there was specificity in the site of reaction. The ^{13}C NMR spectrum has nine signals for the ten carbon atoms of the spermine fragment, the signal at δ 22.8 being due to two carbon atoms that have nearly identical chemical shifts. The amide carbon gives a signal at δ 168.2. The major peak in the electrospray mass spectrum is at *m*/*z* 232.3, which corresponds to $[CoC_{26}H_{60}N_{12}O(CH_3CN)_2]^{3+}$. There are also several peaks due to various chloride adducts which involve up to five chlorides. This again highlights the strong anion binding ability of these protonated cobalt cage polyamine conjugates.

The "dangling" polyamine tails of these compounds have potential to bind cations to form bi- or multi-metal complexes and preliminary investigations have shown that it is possible to form the Cu(II) complexes of **Co5**, **Co6** and **Co7**. The solution electrospray mass spectra have shown that the molecules are involved in strong solution interactions with chloride anions. The complexes also possess a reversible redox couple due to the presence of the encapsulated cobalt. This suggests that the polyamine conjugates could act as redox active receptor molecules[22] capable of detecting the presence of both cations and anions.

With the intention of converting the carboxymethyl-cage complexes into more reactive acylating agents, they were treated with thionyl chloride in the hope of producing the chlorocarbonylmethyl cages. Whatever the products here, the reactivity was certainly enhanced but this led to very complicated product mixtures being obtained on treatment with polyamines. In the case of reactions with tren, the mono- and bis- derivatives **Co10** and **Co11** could be isolated, in low yields, as powders. Significantly, these materials showed no resonances in their ^{13}C NMR spectra attributable to carbonyl carbon and, given the seemingly high symmetry of **Co11** in particular, they have been assigned orthoamide structures (Fig. 2). Given that orthoamides are normally acid-sensitive,[23] the retention of this form during chromatographic treatment of the reaction products may be associated with the reduction of basicity due to the proximity of the Co(III) centre. Their formation must be associated in some way with the particular reaction conditions but clearly they do not compare usefully with the ester pathway for generation of one particular product.

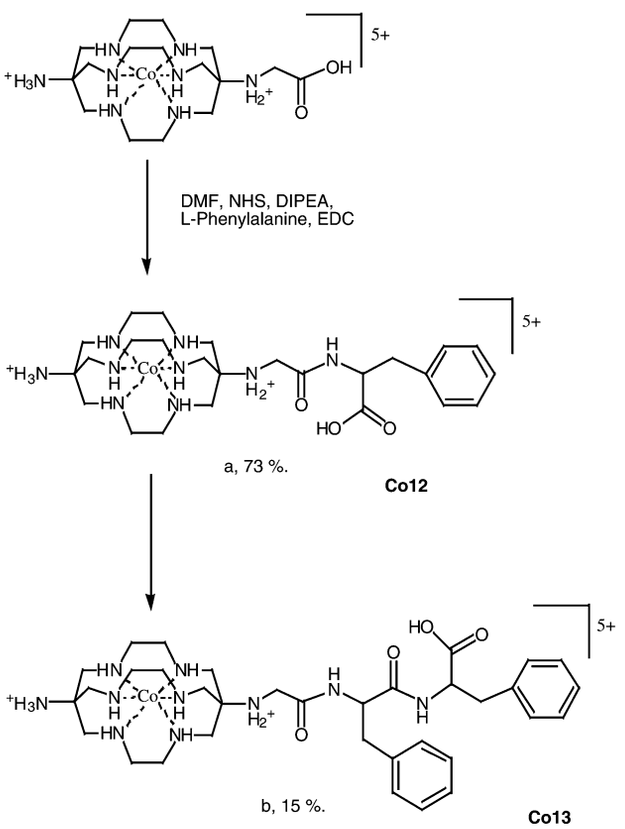
Cage complex amides derived from phenylalanine

Reaction of the monoester complex **Co1** with L-phenylalanine in dimethylformamide in the presence of EDC, diisopropylamine and *N*-hydroxysuccinimide gave coupled products in high yields, with the use of both EDC and phenylalanine in excess in fact resulting in extended coupling (Scheme 2). Since the complex ion reactant can be regarded as a substituted glycine, the major product, **Co12**, is a dipeptide, (subst)Gly-Phe, and the minor product, **Co13**, is a tripeptide, (subst)Gly-Phe-Phe. There would seem to be no special handicap to extending the incorporation of the cage unit to longer peptides.

Since both the cage complex and the amino acid are chiral and the cage was used in its unresolved form, diastereomers would be expected in the products and, indeed, expansion of the ^{13}C NMR spectrum of **Co12** (Fig. 3) shows a doubling of all peaks consistent with the presence of L-Λ and L-Δ forms in similar concentrations. This is also observed for **Co13**. All aspects of both the ^1H and ^{13}C NMR spectra are completely consistent with the assigned structures.

Electrospray mass spectrometry (ESMS) has become one of the methods of choice in determining the purity of peptides,[24] and the technique is readily applied to cage complexes. The ESMS of **Co12** shows a peak at *m*/*z* 576 which corresponds to the unipositive cation, $[CoC_{25}H_{43}N_9O_3]^+$; and the major ion at *m*/*z* 329.5, due to the dipositive cation, $[CoC_{25}H_{44}N_9O_3(CH_3CN)_2]^{2+}$. For **Co13**, its tripeptide form is confirmed by the (most abundant) ion at *m*/*z* 383 corresponding to the dication acetonitrile adduct, $[(CoC_{34}H_{53}N_{10}O_4)(CH_3CN)]^{2+}$. Another peak occurs at *m*/*z* 723 which is due to the monopositive cation $[CoC_{34}H_{52}N_{10}O_4]^+$.

These cage–peptide conjugates retain the extraordinary properties of the parent molecule in that the metal complexes are still extremely resistant to decomplexation and they retain their integrity even in 5 M hydrochloric acid and in the presence of other metal ions at 50 °C for one hour, with no hydrolysis of



Scheme 2 Peptide coupling using an N(cage-complex-substituted)-glycine. NHS = *N*-Hydroxysuccinimide, DIPEA = *N*,*N*-diisopropyl-ethylamine, and EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbo-diimide.

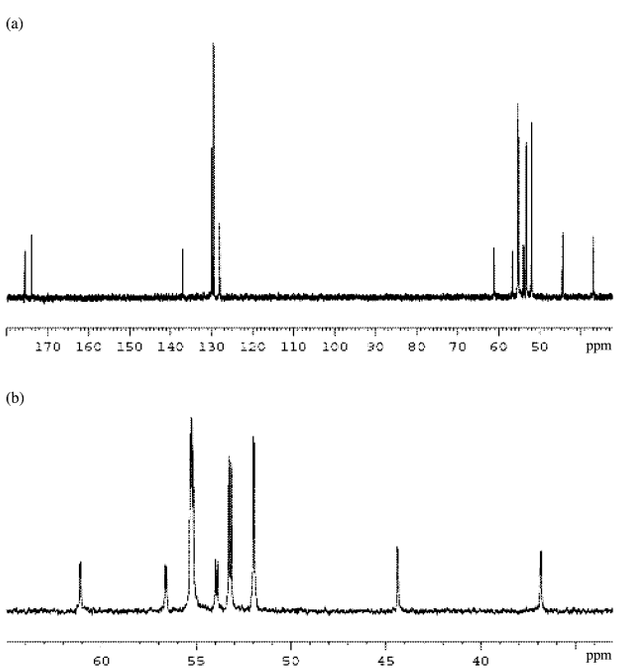


Fig. 3 (a) The 125.8 MHz, 298 K ^{13}C NMR spectrum of **Co12**. (b) An expansion showing the doubling of peaks assigned to the presence of diastereomers.

the peptide bonds. The electronic spectra show that the ligand field is not appreciably affected, as both compounds possess an absorption maximum at 472 nm.[9] This retention of characteristics of the parent ligand is important with respect to the potential medicinal applications of these new ligands.

The pendent arms of carboxymethylated sarcophagine ligands can be used as a point of further functionalisation. The cages can, of course, be readily obtained in their resolved forms[25] and this may add another dimension to their possible biological activity. The success of the EDC-coupling reaction shows that the introduced carboxymethyl arms could allow the further extension of the cage with peptide molecules such as the neuroendocrine tumour targeting octreotide or monoclonal antibodies. The grafting of peptides such as monoclonal antibodies onto the cage should be possible using this methodology. Such peptides would provide the specificity required if these new ligands are to be used for therapeutic or imaging applications.

References

1 A. M. Sargeson, *Pure Appl. Chem.*, 1986, **58**, 1511 and references therein.
2 A. M. Sargeson, *Coordin. Chem. Rev.*, 1996, **151**, 89.
3 C. A. Behm, I. I. Creaser, D. J. Maddalena, A. M. Sargeson and G. M. Snowdon, *Aust. J. Chem.*, 1995, **48**, 1009 and references therein.
4 W. E. Harper and J. M. Harrowfield, unpublished work.
5 S. Moghaddas, P. Hendry, R. J. Geue, C. Qin, A. M. T. Bygott, A. M. Sargeson and N. E. Dixon, *J. Chem. Soc., Dalton Trans.*, 2000, 2085.
6 L. S. Szczepaniak, A. M. Sargeson, I. I. Creaser, R. J. Geue, M. Tweedle and R. G. Bryant, *Bioconjugate Chem.*, 1992, **3**, 27.
7 N. Di Bartolo, A. M. Sargeson, T. M. Donlevy and S. V. Smith, *J. Chem. Soc., Dalton Trans.*, 2001, 2303.
8 D. Parker, in *Comprehensive Supramolecular Chemistry*, ed. D. N. Reinhoudt (J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle, executive eds.), Pergamon Press, Cambridge, 1996, vol. 10, ch. 17, p. 487.
9 P. S. Donnelly, J. M. Harrowfield, B. W. Skelton and A. H. White, *Inorg. Chem.*, 2000, **39**, 5817.
10 D. E. Reichert, J. S. Lewis and C. J. Anderson, *Coordin. Chem. Rev.*, 1999, **184**, 3.
11 C. J. Anderson and M. J. Welch, *Chem. Rev.*, 1999, **99**, 2219.
12 A. Heppeler, S. Froidevaux, H. R. Mäcke, E. Jermann, M. Béhé, P. Powell and M. Hennig, *Chem. Eur. J.*, 1999, **5**, 1974.
13 P. V. Bernhardt and P. C. Sharpe, *Inorg. Chem.*, 2000, **39**, 4123 and references therein.
14 R. J. Casero, Jr., *Polyamines: Regulation and Molecular Interaction*, R. G. Landes Company, Springer Verlag, New York, 1995.
15 G. Karigiannis and D. Papaioannou, *Eur. J. Org. Chem.*, 2000, 1841.
16 See, for example, (*a*) G. Brand, M. W. Hosseini and R. Ruppert, *Tetrahedron Lett.*, 1994, **35**, 8609; (*b*) M. Frugier, C. Florentz, M. W. Hosseini, J.-M. Lehn and R. Giegé, *Nucleic Acids Res.*, 1994, **22**, 2784.
17 S. F. Ralph, M. M. Sheil, L. A. Hick, R. J. Geue and A. M. Sargeson, *J. Chem. Soc., Dalton Trans.*, 1996, 4417.
18 I. L. Finar, *Organic Chemistry*, Longmans, London, 3rd edn., 1959, p. 189.
19 R. G. Wilkins, *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, VCH, Weinheim, 1991, ch. 6.
20 A. Nakahara, Y. Saito and H. Kuroya, *Bull. Chem. Soc. Jpn.*, 1952, **25**, 331.
21 B. C. Challis and J. A. Challis, in *Comprehensive Organic Chemistry*, ed. I. O. Sutherland, Pergamon Press, Oxford, 1979, vol. 2, ch. 9.9, p. 957.
22 P. D. Beer, P. A. Gale and G. Zeng Chen, *Coordin. Chem. Rev.*, 1999, **186**, 3.
23 See, for example, R. W. Alder, R. W. Mowlam, D. J. Vachon and G. R. Weismann, *J. Chem. Soc., Chem. Commun.*, 1992, 507.
24 R. D. Smith, L. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, 1990, **62**, 882.
25 R. J. Geue, T. W. Hambley, J. M. Harrowfield, A. M. Sargeson and M. R. Snow, *J. Am. Chem. Soc.*, 1984, **106**, 5478.