DALTON

# An electrospray mass spectrometry study of some metal-ion cage complexes

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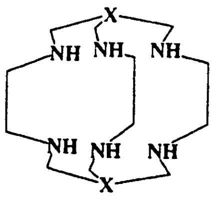
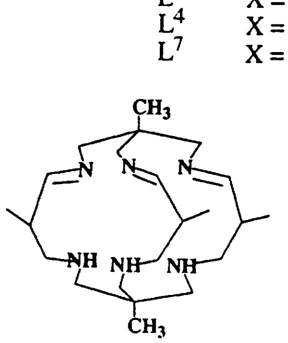
A series of cationic metal complexes of the bicyclic hexaamine cage compound *fac*-1,5,9,13,20-pentamethyl-3,7,11,15,18,22-hexaazabicyclo[7.7.7]tricosane have been examined by electrospray ionisation (ESI) mass spectrometry, along with metal complexes of related smaller and larger hexaamine cages. The ESI mass spectra are considerably simpler than the corresponding fast atom bombardment (FAB) mass spectra. The most abundant ion in the ESI mass spectra of divalent metal-ion cage complexes is the doubly charged molecular ion $[M(cage)]^{2+}$. For trivalent metal-ion complexes spectra obtained using a low-resolution quadrupole mass spectrometer suggested that the most abundant ion is of the type $[M(cage)^{3+} - H^+]^{2+}$. However, when the spectra of several of these cage complexes were obtained using a high-resolution sector instrument it can be shown that the most intense peaks are due to mixtures of these and other ions, $[M(cage)]^{2+}$, formed by reduction of the metal ion in the ion source. The ESI mass spectra of both di- and tri-valent metal-ion complexes also show the presence of ion pairs $[M(cage)^{x+} + anion^-]^{(x-1)+}$. In general ions arising from the free cage are not observed which makes the ESI technique suited for characterising the complex cations. However, varying the cone and skimmer potentials can alter the relative abundances of ions, and the degree to which reduction of the central ion occurs, so these parameters must be carefully controlled. The ESI mass spectra of analogous cobalt(III) complexes containing ammonia or ethane-1,2-diamine displayed more extensive fragmentation compared to those of the cobalt(III) cage complexes. This study demonstrates the potential of ESI mass spectrometry for the characterisation of metal cage complexes as a powerful adjunct to NMR spectroscopy and microanalysis.

Macropolycyclic or 'cage' compounds are three-dimensional complexing agents designed to encapsulate metal ions, other inorganic ions, or organic guest molecules.[1–3] By varying the size of the cavity within the cage or by selecting appropriate donor atoms it is possible to design molecules which display selectivity in their co-ordination behaviour. Sepulchrate (1,3,6,8,10,13,16,19-octaazabicyclo[6.6.6]icosane, sep) $L^1$ and sarcophagine (3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane, sar) $L^2$ are two examples of macrobicycles containing six secondary amines as donor atoms, and may be considered the parent molecules of one group of cage compounds.[4] The complexes $[CoL^1]^{3+}$ and $[CoL^3]^{3+}$ ($L^3$ = 1,8-dinitro derivative of $L^2$) were synthesized by reaction of formaldehyde and ammonia, or formaldehyde and nitromethane, with $[Co(en)_3]^{3+}$ (en = ethane-1,2-diamine). The compounds $L^2$ and $L^4$ (1,8-diamino derivative of $L^2$) have been used to complex many metal ions including both main-group and transition metals.[5,6] The resulting metal complexes commonly exhibit both thermodynamic and kinetic stability, even when the metal ion involved would normally form substitution-labile amine complexes.

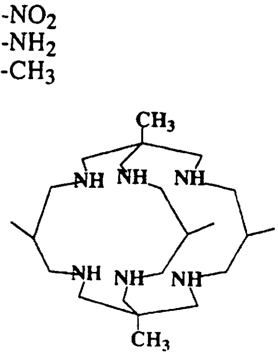
Recently, the synthesis of the cobalt(III) complexes of two new nitrogen-donor cage ligands 1,5,9,13,20-pentamethyl-3,7,11,15,18,22-hexaazabicyclo[7.7.7]tricosa-3,14,18-triene ($L^5$) and *fac*-1,5,9,13,20-pentamethyl-3,7,11,15,18,22-hexaazabicyclo-[7.7.7]tricosane ($L^6$) have been reported.[7] While the electronic spectra and redox chemistry of $[CoL^5]^{3+}$ may be considered typical for cobalt(III) amine/imine systems, the same is not true for $[CoL^6]^{3+}$ obtained by reduction of the former compound with $NaBH_4$. An X-ray crystallographic investigation of $[CoL^6][PF_6]_3 \cdot H_2O$ revealed that the Co–N bond distances varied from 2.010(4) to 2.032(4) Å, which are on average 0.05–0.06 Å longer than for typical cobalt(III) amine complexes, *e.g.* $[Co(NH_3)_6]^{3+}$ [1.965(1) Å][8] and $[Co(en)_3]^{3+}$ (1.964 ± 0.004 Å).[9]



$L^1$(sep) X = N
$L^2$(sar) X = C-H
$L^3$ X = C-$NO_2$
$L^4$ X = C-$NH_2$
$L^7$ X = C-$CH_3$



$L^5$



$L^6$

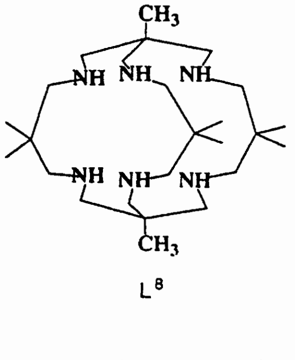
As a consequence $[CoL^6]^{3+}$ displays a number of unusual properties compared to other cobalt(III) hexaamine complexes, including $[CoL^2]^{3+}$, $[CoL^1]^{3+}$, $[Co(en)_3]^{3+}$ and $[Co(NH_3)_6]^{3+}$. The most obvious difference is in the colour of the compounds; $[CoL^6]^{3+}$ is pink in water whereas the complexes mentioned above are yellow-orange. This is due to significant shifts towards lower energy in the position of the ligand-field bands for the cobalt complex of the larger cage ligand. The electrochemical behaviour of cobalt(III) complexes of smaller- and larger-cavity ligands is also significantly different. For example, the $Co^{III}$–$Co^{II}$ redox couple is approximately 560 mV more positive for $[CoL^6]^{3+}$ than for the smaller-cage complex $[CoL^7]^{3+}$ ($L^7$ = 1,8-dimethyl derivative of $L^2$),[10] implying that the metal is considerably more destabilised in the oxidation state III when co-ordinated to the larger-cage ligand.

The metal ion can be readily extruded from $[CoL^6]^{3+}$ after it has been reduced to $Co^{II}$ using zinc under acidic conditions. The resulting free $L^6$ has been used to complex several metal ions from the first, second and third transition series, as well as some post-transition-series metal ions. The properties of these metal complexes are currently being explored, as well as those of the related expanded-cavity cage 1,5,5,9,13,13,20,20-octamethyl-3,7,11,15,18,22-hexaazabicyclo[7.7.7]tricosane ($L^8$) which has been synthesized by an analogous route to that of the corresponding pentamethyl compound.[11]

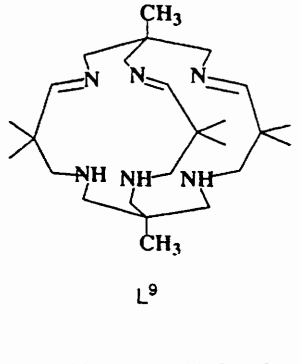
While the high effective symmetry and diamagnetism of some metal complexes of these cage ligands facilitates their characterisation by NMR spectroscopy, this is not always applicable since many complexes of interest contain paramagnetic metal ions which inhibit the observation and interpretation of NMR spectra. By identifying intermediates and by-products formed during the synthesis of $[CoL^5]^{3+}$ and $[CoL^9]^{3+}$, our understanding of the mechanisms for formation of these compounds has been improved.[11] Many intermediates and by-products have been shown by NMR spectroscopy to have low symmetry and/or complex structures. Their identification based on NMR spectra was sometimes ambiguous, and structure determination of crystalline samples was required.

Mass spectrometry, in general, is an adjunct to NMR spectroscopy for the characterisation of these molecules as it offers the opportunity for rapid determination of the mass of the parent ion once the patterns of complex fragmentation and ion formation are understood. For example, fast atom bombardment (FAB) mass spectrometry has been applied to the study of systems involving either naturally occurring or synthetic macrocycles and metal ions.[12–21] In general, these studies have involved crown ethers or related systems, and alkali or alkaline-earth metals. Some workers, however, have examined macrocylic systems containing more than one type of donor atom, or interactions between macrocycles and transition or post-transition metals.[12,13,15,18–21] This technique has also been used to determine the relative stability constants for a group of metal ions and the same ligand.[12–17] Other techniques have been used to explore the relative intrinsic affinities of metal ions for macrocycles in the gas phase.[22,23] However, there is usually considerable fragmentation of the complex in the process.

Electrospray ionisation (ESI) mass spectrometry is a powerful new technique which provides a relatively low-energy method for introducing a variety of polar, thermally labile molecules into the gas phase.[24] While a great deal of initial attention has been directed at the characterisation of large biomolecules such as proteins,[24,25] this technique has also been used to study inorganic systems[26–31] and has proven particularly useful for examining labile metal complexes.[32–34] More recently, it has been used to probe the complexation behaviour of several macrocycles towards a variety of metal ions in solution.[35] Results of a study of the ESI mass spectra of both smaller and larger polyamine cage complexes along with simpler amine complexes, in the presence of various anions, are reported here.



$L^8$



$L^9$

## Results and Discussion

Each of the metal cage complexes discussed in this paper have been characterised by microanalysis, visible spectroscopy, and wherever possible by NMR spectroscopy and electrochemical techniques.[4–7,11]

### General comments on spectra

Positive-ion ESI mass spectra of the metal-ion cage complexes were generally simple, usually containing two or three abundant ions which could easily be related to the structure of the complex. The simplicity of the spectra is attributed to the thermodynamic and kinetic stability of these metal complexes, as well as the relatively low energy of the electrospray ionisation process. A typical example of these spectra is that of $[MgL^2][CF_3SO_3]_2$ presented in Fig. 1. The doubly charged molecular ion is the base peak in the spectrum (*m*/*z* 153.9), which also contains a medium-intensity peak (*m*/*z* 457.5) arising from the ion pair $([MgL^2]^{2+} + CF_3SO_3^-)^+$. In all spectra of cage complexes there are no ions of significant intensity that could be attributed to free ligand, *i.e.* an ion formed by loss of the metal ion. In particular, cage complexes of both $Mn^{II}$ and $Mg^{II}$ clearly displayed peaks due to intact cage complexes, with little or no ligand loss or fragmentation. For such weak field and typically labile metal ions this result indicates quite remarkable stability. A further demonstration of the value of ESI mass spectrometry for characterising metal complexes of multidentate ligands was provided by the negative-ion spectra of ethylenedinitrilotetraacetate (edta) complexes of cobalt(II) and cobalt(III). The spectrum of $H[Co(H_2O)(Hedta)]$ contained one intense peak due to the ion $[H^+ + Co(edta)^{2-}]^-$, while for the cobalt(III) complex two significant peaks were seen at $m/z = 347.5$ and 303.4. The former peak, the more intense of the two, is assigned to the ion $[Co(edta)]^-$, while the latter is assigned to the same ion after it has lost one molecule of $CO_2$.

There were some consistent differences between the ESI mass spectra of di- and tri-valent metal-ion cage complexes, which are discussed in more depth in the relevant sections below. Cage complexes containing metal ions with more than one common isotope (Ni, Cu, Pt, Zn, Cd or Hg) showed isotopic distributions with relative intensities and *m*/*z* values consistent with the known isotopic composition of these complexes. Simple and

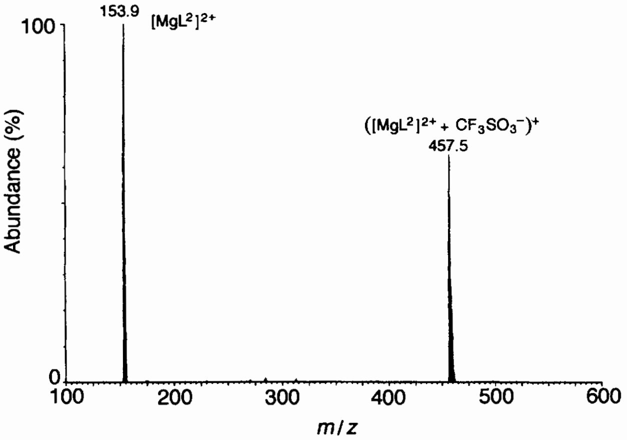


**Fig. 1** Positive-ion ESI mass spectrum of $[MgL^2][CF_3SO_3]_2$ obtained on a low-resolution quadrupole mass spectrometer

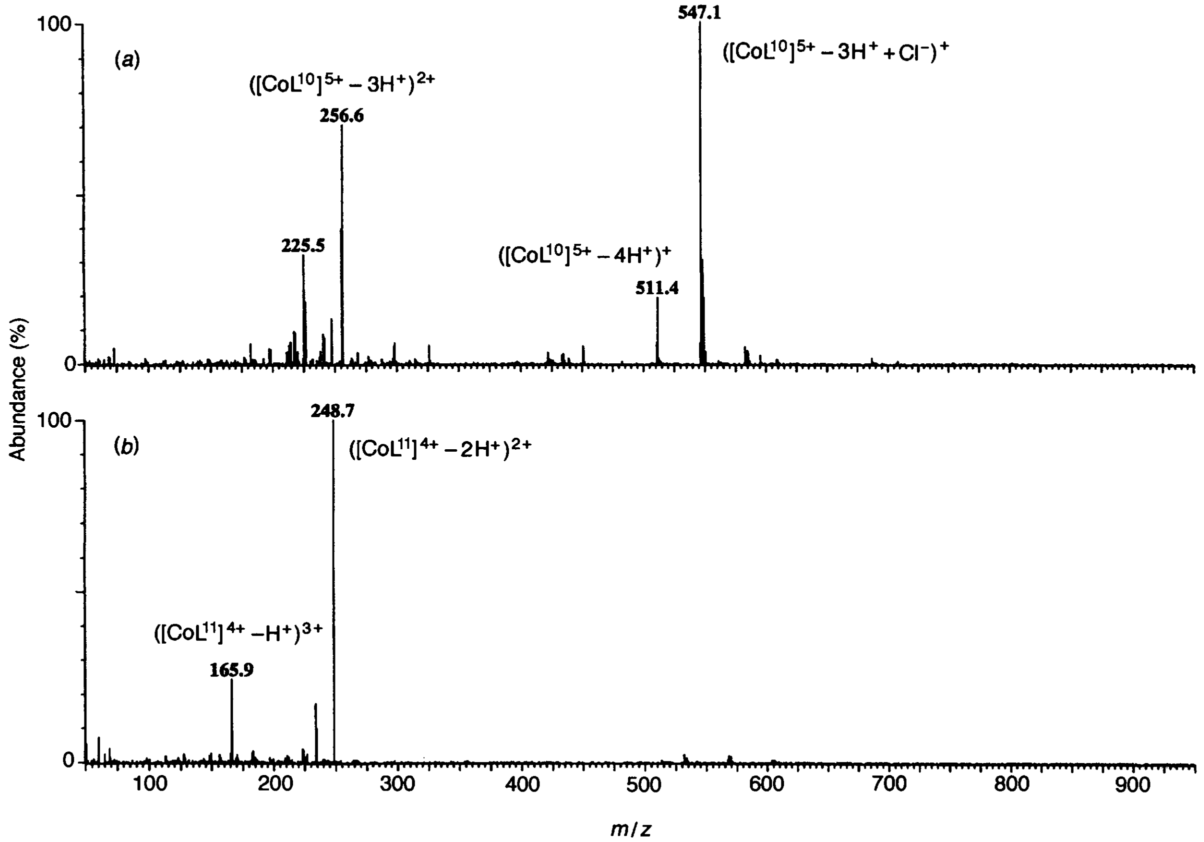
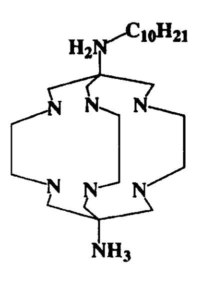
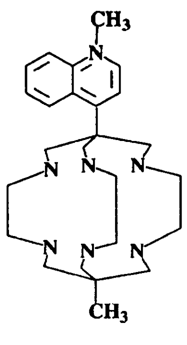


**Fig. 2** Positive-ion ESI mass spectra of (*a*) $[CoL^{10}]Cl_5$ and (*b*) $[CoL^{11}]Cl_4$ obtained on a low-resolution quadrupole mass spectrometer



$L^{10}$



$L^{11}$

readily interpretable spectra were also obtained for metal complexes of ligands containing appended organic moieties. For example, Fig. 2 illustrates the ESI mass spectra of two cobalt(III) complexes of small cage ligands, $L^{10}$ and $L^{11}$, featuring appended decyl and quinoline groups, respectively. There are no ions of significant intensity in either spectrum in which these organic groups have undergone fragmentation. Furthermore, the most abundant ions are similar to those observed in the spectra of other cobalt(III) complexes.

### Comparison of ESI and FAB mass spectra

The ESI and FAB mass spectra of $[CuL^6][CF_3SO_3]_2$ are shown together in Fig. 3. In general, we have found that FAB mass spectra are considerably more complex than the corresponding ESI mass spectra of the same compounds. This may be due to more extensive fragmentation of the cage ligand, and reactions of, or with, the glycerol matrix in FAB. Furthermore, the characteristic ions observed in the ESI mass spectra of metal-ion cage complexes are often absent from the corresponding FAB mass spectra. For example, the FAB mass spectrum of $[CuL^6][CF_3SO_3]_2$ does not show the ion $[CuL^6]^{2+}$, whereas this is the most abundant ion in the ESI mass spectrum, at $m/z$ = 229.6. In comparison, the ESI mass spectrum shows a weak intensity peak due to the ion $([CuL^6]^{2+} - H^+)^+$. However, intense peaks at $m/z = 457.6$ and 459.6 in the FAB mass spectrum are due to this ion containing the two different isotopes of copper. Surprisingly, the former peak, which contains the most abundant isotope of copper, is the less intense of the two. This is most likely a result of overlap of the peak due to the ion containing the heavier copper isotope with other peaks due to ions formed by reaction of, or with, the matrix.

### ESI mass spectra of simple amine complexes

In order to investigate the stabilities of the complex ions in the mass spectrometer we have obtained the ESI mass spectra of en and amine complexes of the kinetically inert cobalt(III) ion, and compared these to spectra of cobalt(III) cage complexes. The thermodynamic and kinetic stability of $[Co(en)_3]^{3+}$ is expected to be significantly less than that of the cobalt(III) cage complexes in solution, while that of $[Co(NH_3)_6]^{3+}$ would be even less. The positive-ion ESI mass spectrum of $[Co(en)_3][CF_3SO_3]_3$ (Fig. 4) shows a number of ions in addition to those found in the spectra of cobalt(III) cage complexes. For example, ions with $m/z$ values of 537.4, 387.4 and 119.1 can be assigned to $\{[Co(en)_3]^{3+} + 2\,CF_3SO_3^-\}^+$, $\{[Co(en)_3]^{3+} + CF_3SO_3^- - H^+\}^+$ and $\{[Co(en)_3]^{3+} - H^+\}^{2+}$, respectively. However, the most abundant ion in the spectrum, $m/z$ = 327.3, and the ion at $m/z = 177.2$, are due to related species, $\{[Co(en)_3]^{3+} - en - H^+ + CF_3SO_3^-\}^+$ and $\{[Co(en)_3]^{3+} - en - 2\,H^+\}^+$, respectively, in which one en ligand has dissociated from the cobalt complex. This is supported by the presence of a moderately abundant ion at $m/z = 61.1$ which most probably arises from protonated en.

The positive-ion mass spectrum of $[Co(NH_3)_6]Cl_3$ showed a very intense peak at $m/z = 116.7$. This could be assigned to the

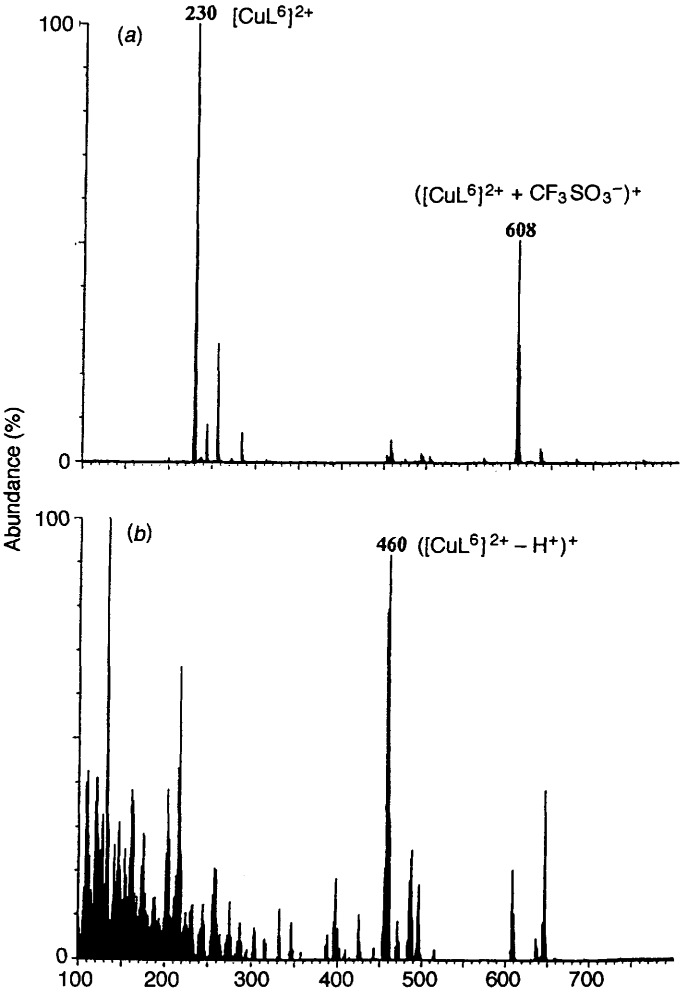


**Fig. 3** Mass spectra of $[CuL^6][CF_3SO_3]_2$: (*a*) positive-ion ESI, (*b*) FAB. Both spectra were acquired on a low-resolution quadrupole mass spectrometer

ion $\{[Co(NH_3)_6]^{3+} + HCl + Cl^-\}^{2+}$. However, we have not seen previously any evidence for the corresponding species in which a molecule of HCl and a chloride ion are associated with the initial complex. No other peaks in this spectrum, which occupied a relatively small *m*/*z* range due to the low molecular weights of both complex cation and anion, could be assigned implying that considerable fragmentation had occurred. The mass spectra of both $[Co(NH_3)_6][CF_3SO_3]_3$ and $[Co(NH_3)_6][PF_6]_3$ contained a number of peaks at low *m*/*z*, of medium to strong intensity, which have not been assigned. However, at higher *m*/*z* values some weaker peaks were found that could be assigned to species directly analogous to those found in the spectra of cobalt(III) cage complexes. For example, both spectra showed peaks at *m*/*z* values which supported their assignment to $\{[Co(NH_3)_6]^{3+} + 2\,\text{anion}^-\}^+$. The spectrum of $[Co(NH_3)_6][PF_6]_3$ also contained a weak peak attributable to $\{[Co(NH_3)_6]^{3+} + PF_6^- - H^+\}^+$, and a stronger peak due to a related species which had lost one amine ligand, $\{[Co(NH_3)_6]^{3+} + PF_6^- - H^+ - NH_3\}^+$. Another peak in this spectrum was assigned to the corresponding species in which two amine ligands have been lost. The major ions in the spectrum of $[Co(NH_3)_6][CF_3SO_3]_3$ were analogous to those observed for the hexafluorophosphate salt. The ion $[Co(NH_3)_6]^{3+}$ was not observed in the mass spectrum of either compound. It appears therefore that useful and interpretable mass spectra can be obtained for cobalt(III) complexes, provided the metal is complexed by chelating ligands.

### Divalent metal-ion complexes

Ions observed in the positive-ion ESI mass spectra of divalent metal-ion cage complexes, obtained at low resolution, are reported in Table 1, along with their assignments. The most abundant ions observed were the doubly charged ions $[M(\text{cage})]^{2+}$. In addition, most spectra also contained a peak due to a singly charged ion pair $[M(\text{cage})^{2+} + \text{anion}^-]^+$. The intensity of this peak was dependent on the relative concentrations and identities of the metal ion and anion. In nearly all cases there was excellent agreement between the *m*/*z* values observed and those calculated for their assigned ions. There were only two exceptions to this. The observed *m*/*z* values for the ions $[CdL^6]^{2+}$ and $[HgL^6]^{2+}$ were 254.3 and 298.2, respectively. These are significantly less than the expected values of 255.1 and 299.2, calculated using the masses of the most abundant isotopes of each of the elements present. Instead, the observed values are in closer agreement with those calculated using the average atomic weights of each of the elements present, 254.4 and 298.6, respectively. This arises because the large number of isotopes that both cadmium and mercury have are not resolved on the quadrupole instrument since both these ions are doubly charged and the spacing of the peaks is half that for a singly charged species. The spectra (not shown) of both complexes were then obtained at high resolution, which enabled complete resolution of each of the peak clusters due to the $[CdL^6]^{2+}$ and $[HgL^6]^{2+}$ ions, and showed that the most intense peak in each case did coincide exactly with the theoretically predicted mass for the most abundant isotope.

The spectrum of the copper complex of $L^6$ contained a weak ion assigned to $([CuL^6]^{2+} - H^+)^+$, formed by loss of a proton to water in the surrounding medium. This ion was observed at *m*/*z* 458.8, in reasonable agreement with the expected value of 458.3. We excluded the possibility that this is the reduced ion $[CuL^6]^+$ (expected *m*/*z* is 459.3) by high-resolution measurements which gave *m*/*z* 458.32, in exact agreement with the expected value for $([CuL^6]^{2+} - H^+)^+$. In some cases peaks were present in the spectra of other divalent metal-ion cage complexes at *m*/*z* values consistent with the assignment to singly charged ions formed by loss of a proton from the parent ion, *i.e.* $[M(\text{cage})^{2+} - H^+]^+$. However, these always had very low abundances (<5%). These ions were more prevalent in the spectra of trivalent metal-ion cage complexes (see below).

### Trivalent metal-ion complexes

The positive-ion ESI mass spectra of a number of trivalent metal-ion cage complexes were initially obtained using the low-resolution VG Biotech Quattro mass spectrometer. The most abundant ions present in the spectra of the majority of these complexes were at relatively low *m*/*z*, and were initially assigned to doubly charged ions $[M(\text{cage})^{3+} - H^+]^{2+}$. An alternative assignment, to dipositive metal-ion complexes formed through reduction within the mass spectrometer, *i.e.* $[M(\text{cage})]^{2+}$, was initially rejected since it had been reported by other workers[28] that reduction of cationic complexes does not occur in an electrospray source due to the positive potential required (4 kV) to generate positive ions. However, the *m*/*z* of the experimentally observed ions, given the low resolution of the quadrupole instrument, could also be consistent with the latter assignment. Consequently, the spectra of these complexes were also obtained at a sufficiently high enough resolution (5000) to resolve clearly these possibilities. In general the spectra of complexes obtained using this instrument were very similar to that obtained earlier, with the most abundant ion the same in most cases. Table 2 presents the mass spectral data obtained at high resolution for trivalent metal-ion cage complexes, together with their assignments, and the 3+/2+ redox potentials for these complexes. For three of the complexes examined, $[CrL^6]^{3+}$, $[CoL^2]^{3+}$ and $[CoL^5]^{3+}$, there was little evidence from the mass spectrum that reduction was occurring to a significant extent.

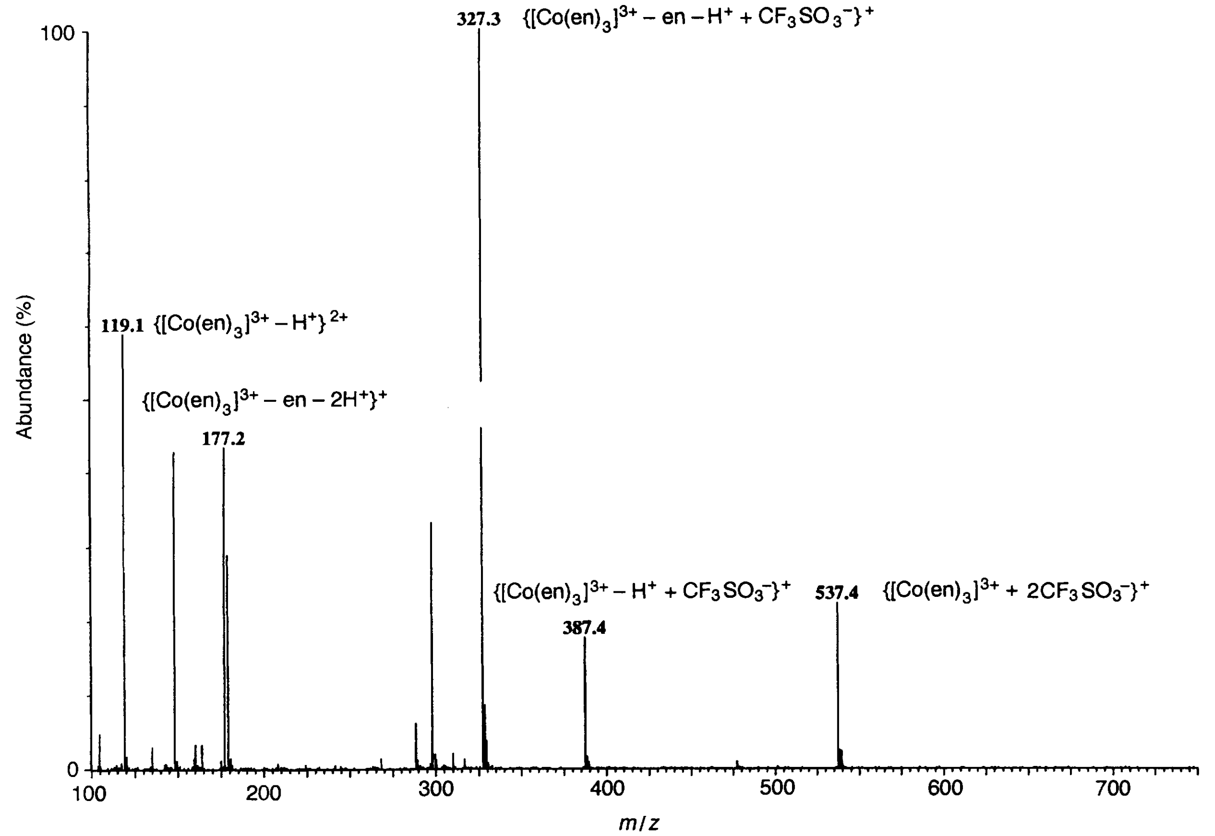


**Fig. 4** Positive-ion ESI mass spectrum of [Co(en)$_3$][CF$_3$SO$_3$]$_3$ obtained on a low-resolution quadrupole mass spectrometer

**Table 1** The ESI mass spectral data for divalent metal-ion cage complexes*

| Complex | Major ions (*m/z*, abundance in %) |
|---|---|
| [MnL$^6$][CF$_3$SO$_3$]$_2$ | ([MnL$^6$]$^{2+}$ + CF$_3$SO$_3^-$)$^+$ (600.2, 83), [MnL$^6$]$^{2+}$ (225.7, 100) |
| [CuL$^6$][CF$_3$SO$_3$]$_2$ | ([CuL$^6$]$^{2+}$ + CF$_3$SO$_3^-$)$^+$ (608.2, 51), ([CuL$^6$]$^{2+}$ − H$^+$)$^+$ (458.8, 5), [CuL$^6$]$^{2+}$ (229.6, 100) |
| [ZnL$^6$][ClO$_4$]$_2$ | ([ZnL$^6$]$^{2+}$ + ClO$_4^-$)$^+$ (559.3, 28), [ZnL$^6$]$^{2+}$ (230.6, 100) |
| [ZnL$^6$][PF$_6$]$_2$ | ([ZnL$^6$]$^2$ + PF$_6^-$)$^+$ (605.1, 4), [ZnL$^6$]$^{2+}$ (230.5, 100) |
| [CdL$^6$]Cl$_2$ | ([CdL$^6$]$^{2+}$ + Cl$^-$)$^+$ (545.0, 16), [CdL$^6$]$^{2+}$ (254.3, 100) |
| [HgL$^6$]Cl$_2$ | ([HgL$^6$]$^{2+}$ + Cl$^-$)$^+$ (633.1, 28), [HgL$^6$]$^{2+}$ (298.2, 100) |
| [NiL$^6$][ClO$_4$]$_2$ | ([NiL$^6$]$^{2+}$ + ClO$_4^-$)$^+$ (553.2, 36), [NiL$^6$]$^{2+}$ (227.5, 100) |
| [CoL$^8$][PF$_6$]$_2$ | [CoL$^8$]$^{2+}$ (248.9, 100) |
| [CoL$^8$][CF$_3$SO$_3$]$_2$ | ([CoL$^8$]$^{2+}$ + CF$_3$SO$_3^-$)$^+$ (646.6, 31), [CoL$^8$]$^{2+}$ (248.6, 100) |
| [CuL$^4$][NO$_3$]$_2$ | ([CuL$^4$]$^{2+}$ + NO$_3^-$)$^+$ (439.5, 8), [CuL$^4$]$^{2+}$ (188.8, 100) |
| [MgL$^2$][CF$_3$SO$_3$]$_2$ | ([MgL$^2$]$^{2+}$ + CF$_3$SO$_3^-$) (457.5, 50), [MgL$^2$]$^{2+}$ (153.9, 100) |

* Positive-ion spectra obtained using the quadrupole mass spectrometer, skimmer potential = 50 V; percentages refer to the height relative to the most intense peak in each spectrum, masses to the most abundant isotopic species for each metal.

However, it must be pointed out that each of these spectra were obtained after the cone and skimmer potentials were optimised to provide the best overall spectrum. As will be shown below, by varying these two potentials it is possible with at least some complexes to produce significant quantities of ions corresponding to complexes in which the metal ion has been reduced. The high-resolution mass spectra of both [CoL$^6$]$^{3+}$ and [CoL$^9$]$^{3+}$ showed ions containing cobalt(II), which differ by *m/z* 0.5 from ions in which a proton had been lost from the initial metal complex. These differences were not resolved on the quadrupole instrument which was scanned with a resolution window of 1 *m/z* unit. It is noteworthy that it was the two complexes with the most positive 3+/2+ redox potentials which showed mass spectra containing reduced metal ions.

The most likely origin of the ions [M(cage)$^{3+}$ − H$^+$]$^{2+}$, seen in the spectrum of each of the trivalent metal-ion cage complexes examined, is loss of a proton attached to one of the secondary nitrogen atoms to water in the surrounding medium. For complexes containing tripositive metal ions these protons are weakly acidic [p$K_a$ 13–14 for cobalt(III)], and undergo deuterium exchange rapidly on dissolution in D$_2$O. The lower abundance of the corresponding ions [M(cage)$^{2+}$ − H$^+$]$^+$ in the ESI mass spectra of divalent metal-ion cage complexes is attributed to the lower acidity of co-ordinated amines. For example, they do not exchange protons bonded to secondary nitrogen atoms readily in neutral to basic solution.

Fig. 5 illustrates the positive-ion ESI mass spectrum of [CoL$^6$]$^{3+}$ obtained at high resolution under two different sets of operating conditions. Fig. 5(*a*) presents the spectrum obtained using cone and skimmer potentials chosen to optimise the intensity of the peak due to doubly charged ions at *m/z* 227.17, while Fig. 5(*b*) was obtained using different parameters that resulted in the most intense peak in the spectrum now being that due to a singly charged ion at *m/z* 453.31. It has been reported that by varying the skimmer potential it is possible to alter the extent of collisional activation of ions in the intermediate-pressure region of the ion source of the mass spectrometer.[28] This can result in significant levels of fragmentation at high skimmer potentials, whereas intact ions (and often solvated ions and ion pairs) are observed at low skimmer potentials.

**Table 2** The ESI mass spectral data for trivalent metal-ion cage complexes[a]

| Complex | Major ions ($m/z$, abundance in %) | $M^{3+/2+}$ Redox potential[b] |
|---|---|---|
| $[CoL^6]Cl_3$[c] | $([CoL^6]^{2+} + Cl^-)^+$ (490.30, 5), $([CoL^6]^{3+} - H^+ + Cl^-)^+$ (489.30, 7), $([CoL^6]^{3+} - 2\ H^+)^+$ (453.31, 7), $[CoL^6]^{2+}$ (227.67, 52), $([CoL^6]^{3+} - H^+)^{2+}$ (227.17, 100), $([CoL^6]^{2+} - 2\ H)^{2+}$ (226.66, 16) | +0.08 |
| $[CrL^6]Cl_3$ | $([CrL^6]^{3+} - H^+ + Cl^-)^+$ (482.29, 5), $([CrL^6]^{3+} - 2\ H^+)^+$ (446.31, 11), $([CrL^6]^{3+} - H^+]^{2+}$ (223.67, 100) | −1.27 |
| $[CoL^5][PF_6]_3$ | $([CoL^5]^{3+} + 2\ PF_6^-)^+$ (739.39, 21), $([CoL^5]^{3+} - H^+ + PF_6^-)^+$ (593.25, 39), $([CoL^5]^{3+} - 2\ H^+)^+$ (447.28, 7), $([CoL^5]^{3+} - H^+)^{2+}$ (224.14, 100) | −0.16 |
| $[CoL^9][PF_6]_3$ | $([CoL^9]^{3+} + 2\ PF_6^-)^+$ (781.28, 4), $([CoL^9]^{2+} + PF_6^-)^+$ (636.27, 11), $([CoL^9]^{3+} - H^+ + PF_6^-)^+$ (635.29, 12), $([CoL^9]^{2+} - H^+)^+$ (490.33, 4), $([CoL^9]^{3+} - 2\ H^+)^+$ (489.30, 4), $[CoL^9]^{2+}$ (245.67, 100), $([CoL^9]^{3+} - H^+)^{2+}$ (245.15, 20), $([CoL^9]^{2+} - 2\ H)^{2+}$ (244.66, 7) | +0.40 |
| $[CoL^9][CF_3SO_3]_3$ | $([CoL^9]^{3+} + 2\ CF_3SO_3^-)^+$ (789.28 100), $([CoL^9]^{2+} + CF_3SO_3^-)^+$ (640.28, 99), $([CoL^9]^{3+} - H^+ + CF_3SO_3^-)^+$ (639.27, 80), $([CoL^9]^{3+} - 2\ H^+)^+$ (489.31, 62), $[CoL^9]^{2+}$ (245.67, 64), $([CoL^9]^{3+} - H^+)^{2+}$ (245.16,11), $([CoL^9]^{2+} - 2\ H)^{2+}$ (244.66, 5) | +0.40 |
| $[CoL^2]Cl_3$ | $([CoL^2]^{3+} - H^+ + Cl^-)^+$ (377.17, 41), $([CoL^2]^{3+} - 2\ H^+)^+$ (341.20, 100), $([CoL^2]^{3+} - H^+)^{2+}$ (171.10, 16) | −0.40 |

[a] Positive-ion spectra obtained using the high-resolution magnetic sector mass spectrometer; percentages refer to height relative to the most intense peak in each spectrum, masses to the most abundant isotopic species for each metal. [b] Relative to the normal hydrogen electrode. Values reported are from refs. 5, 7 and 11. [c] Data taken from Fig. 6(*a*).

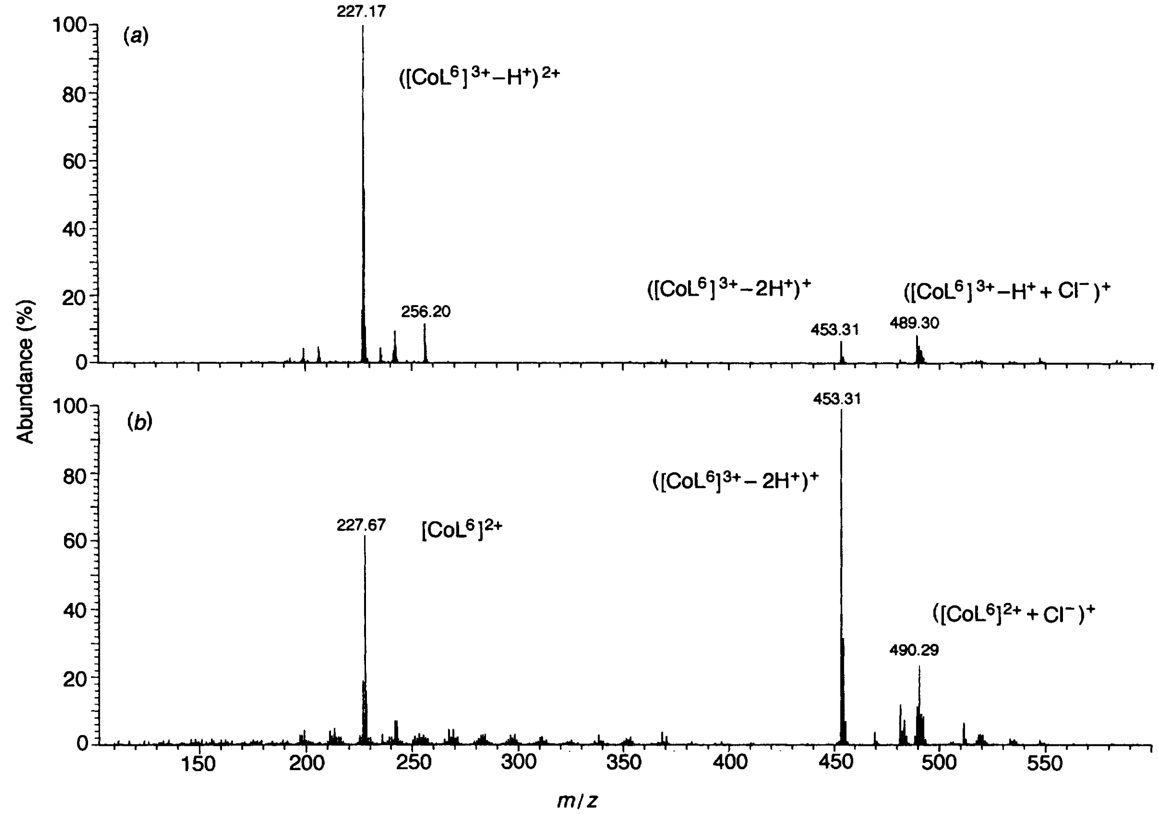


**Fig. 5** Positive-ion ESI mass spectra of $[CoL^6]Cl_3$ obtained on a high-resolution magnetic sector mass spectrometer: (*a*) cone potential = 9.0 V, skimmer potential = 18 V; (*b*) cone potential = 24.0 V, skimmer potential = 33 V

The behaviour observed here is similar to that observed for $[M + H^+]^+$ and $[M + 2\ H^+]^{2+}$ of peptides,[36] where it was noted that the optimum skimmer potential for doubly charged $[M + 2\ H^+]^{2+}$ ions was generally lower than for singly charged $[M + H^+]^+$ ions. This results from a combination of focusing differences and probably reflects an increasing propensity of doubly charged ions to fragment at higher potentials and energies.

Fig. 6 illustrates the effect of variations in skimmer and cone potentials on the relative amounts of the $[CoL^6]^{2+}$ and $([CoL^6]^{3+} - H^+)^{2+}$ ions in the spectrum of $[CoL^6]Cl_3$. Clearly the choice of instrumental conditions can have a major influence on the relative amounts of these ions in the mass spectrometer source, and we are currently investigating these phenomena in more detail for a greater range of trivalent complexes. Fig. 6(*c*) and 6(*d*) illustrate the theoretical spectra expected for $[CoL^6]^{2+}$ and $([CoL^6]^{3+} - H^+)^{2+}$, respectively. In each case there is an intense peak at the *m/z* corresponding to the monoisotopic species, as well as two peaks of weaker intensity at higher *m/z* due to the same ions but containing one or two $^{13}C$ atoms. By comparing these spectra with those obtained experimentally, it can be seen that the conditions employed to obtain Fig. 6(*a*) result in the $([CoL^6]^{3+} - H^+)^{2+}$ ion being more abundant than the $[CoL^6]^{2+}$ ion by a factor of approximately 2 : 1. However, at higher cone and skimmer potentials [Fig. 6(*b*)] the reduced ion, *i.e.* $[CoL^6]^{2+}$, is more abundant. Comparison of the absolute intensities indicates that the intensity of the peak arising from loss of a proton is reduced at the higher potentials rather than the reduced species increasing. These trends are mirrored elsewhere in the spectrum. For example, the relative amounts of the ions $([CoL^6]^{3+} - H^+ + Cl^-)^+$ and $([CoL^6]^{2+} + Cl^-)^+$ are seen to vary in the same manner. In the spectrum obtained using the lower

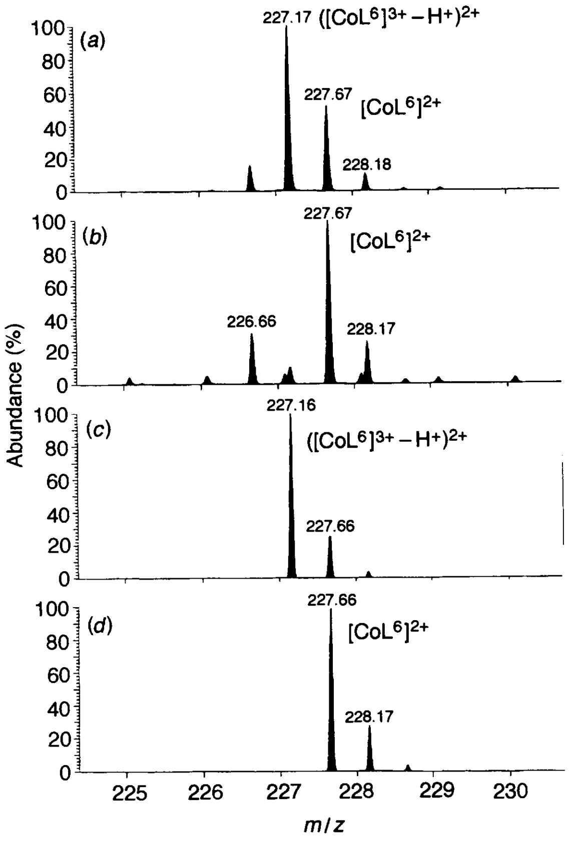


**Fig. 6** Expanded region of the positive-ion ESI mass spectra of $[CoL^6]Cl_3$ obtained a high-resolution magnetic sector mass spectrometer: (*a*) cone potential = 9.0 V, skimmer potential = 18 V; (*b*) cone potential = 24.0 V, skimmer potential = 33 V; (*c*) theoretical spectrum for the ion $[CoL^6]^{2+}$; (*d*) theoretical spectrum for the ion $([CoL^6]^{3+} - H^+)^{2+}$

cone and skimmer potentials [Fig. 6(*a*)] the peak due to the former ion was the more intense of the two, while the latter ion predominates under the conditions used to obtain the spectrum in Fig. 6(*b*). That the species due to proton loss dominates at lower cone/skimmer potentials may be due to the complex being solvated to a greater extent under these conditions thus enabling proton transfer to occur. This would be consistent with the observation that peaks due to association with neutral solvent molecules are often observed at lower cone/skimmer potentials. When higher potentials are employed proton transfer becomes less probable as the complexes are readily desolvated.

Several other peaks are present in Fig. 6(*a*) and 6(*b*). Of these, the majority are only of weak intensity, however, the ion at *m*/*z* 226.66 is more abundant than $([CoL^6]^{3+} - H^+)^{2+}$ at high skimmer and cone potentials. Since this peak must be due to a doubly charged ion, it is assigned to $([CoL^6]^{2+} - H_2)^{2+}$, *i.e.* loss of neutral $H_2$. Peaks due to analogous ions were also seen in high-resolution mass spectra of $[CoL^9]^{3+}$. The mechanism by which such ions could be formed is not clear.

The mass spectra of most cage complexes of trivalent metal ions also showed ions of the general types $[M(cage)^{3+} - 2\,H^+]^+$, $[M(cage)^{3+} + anion^- - H^+]^+$ and $[M(cage)^{3+} + 2\,anion^-]^+$. The intensities of these peaks were variable, depending on the identities of the metal complex and anion, as well as the instrument chosen to obtain the mass spectrum and the operating conditions selected. Small signals due to the triply charged ions $[Co(cage)]^{3+}$ were also sometimes seen in spectra obtained using the low-resolution mass spectrometer. In addition, the high-resolution spectra also showed the presence of ions $[M(cage)^{2+} + anion^-]^+$ for each of the complexes previously shown to undergo reduction in the mass spectrometer.

## Conclusion

Electrospray mass spectra are very useful for determining the molecular weights of metal-ion cage complexes at low skimmer potentials. The observed spectra are relatively simple, showing ions corresponding to the intact metal complex and some ion pairs. Reduction of the metal ion is evident with some cobalt(III) cage complexes, and fragmentation is negligible under the mild ionisation conditions.

## Experimental

The synthesis and characterisation of $[CoL^6]Cl_3$ and the precursor complex $[CoL^5]^{3+}$ have been reported.[7] Details of the synthesis and characterisation of several metal complexes of $L^6$, as well as of $[CoL^8]^{2+}$ and $[CoL^9]^{3+}$ will be published.[11] The complexes $[Co(NH_3)_6][CF_3SO_3]_3$, $[Co(NH_3)_6][PF_6]_3$ and $[Co(en)_3][CF_3SO_3]_3$ were prepared from solutions containing the corresponding chloride salts by addition of an excess of the appropriate anion.

Low-resolution ESI mass spectra of all complexes were obtained using a VG Biotech Quattro (Altrincham, UK) triple quadrupole mass spectrometer and water–methanol or -acetonitrile (1 : 1) solutions of the metal complexes. No differences were noted in either the abundance or type of ion(s) observed in the spectrum of $[CoL^6][CF_3SO_3]_3$ obtained using these two different solvent mixtures. The solvent stream was 50% aqueous acetonitrile, delivered by an ISCO (Lincoln, NE, USA) SFC-500 syringe pump at a flow rate of 5 μl $min^{-1}$, and 10 μl were injected for each analysis. Evaporation of droplets within the spectrometer was enhanced by both a concurrent and countercurrent flow of dry nitrogen gas. All spectra were recorded using a skimmer potential of 50 V unless otherwise indicated. At this potential there was little evidence for association of solvent molecules (water or acetonitrile) with ions produced by the mass spectrometer. A resolution of 0.5 (*m*/*z* peak width at half-height) was used for all experiments. The FAB mass spectra were obtained using the same instrument fitted with a 35 keV $Cs^+$-ion gun ($eV \approx 1.60 \times 10^{-19}$ J), and glycerol as the matrix.

High-resolution spectra were obtained on a VG AUTOSPEC magnetic sector-ToF mass spectrometer with 50% aqueous methanol as the solvent. Samples were introduced at a flow rate of 20 μl $min^{-1}$ *via* a Harvard syringe pump and 20 μl were injected for each analysis. A resolution of either 1000 or 2500 was used for all experiments.

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