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Characterisation of Molecular and Supramolecular Systems by Electrospray Mass Spectrometry

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Summary: Electrospray mass spectrometry (ESMS) has been demonstrated to be a powerful technique for the characterisation – at both a molecular and a supramolecular level – of a series of highly-ordered catenanes based on interlocking rings composed of (i) macrocyclic polyethers containing either two π-electron rich 1,4-dioxybenzene or 1,5-dioxynaphthalene groups linked by 3,6,9-trioxaundecyl chains and (ii) tetracationic cyclophanes containing two π-electron deficient bipyridinium units linked by either *p*-xylyl or bitolyl residues.

The recently reported highly efficient template-directed syntheses[1,2] of catenanes and rotaxanes bear witness to the growing importance of molecular self-assembly processes[3] in the syntheses[4] of these novel classes of unnatural product.[5] We are finding, however, that, as the molecular assemblies and aggregates assume molecular weights in excess of *ca.* 3 kDa, the well-established soft-ionization mass spectrometric techniques, including fast atom bombardment mass spectrometry[6] (FABMS) have their limitations.

Electrospray ionization, developed as a mass spectrometric technique (ESMS) by Fenn *et al.* [7] has emerged recently as a powerful new method for the production of intact ions from complex high molecular weight species in solution. Although, to date, most of the applications relate to proteins and other biomolecules,[8] it is becoming increasingly clear that unnatural products in the mesomolecular weight range (2 - 5 kDa) and beyond are amenable[9] to direct analysis by ESMS.

Although catenanes $\mathbf{1}.4PF_6$ – $\mathbf{4}.4PF_6$ (Figure 1) have already been identified[2-5] by FABMS, the ability of

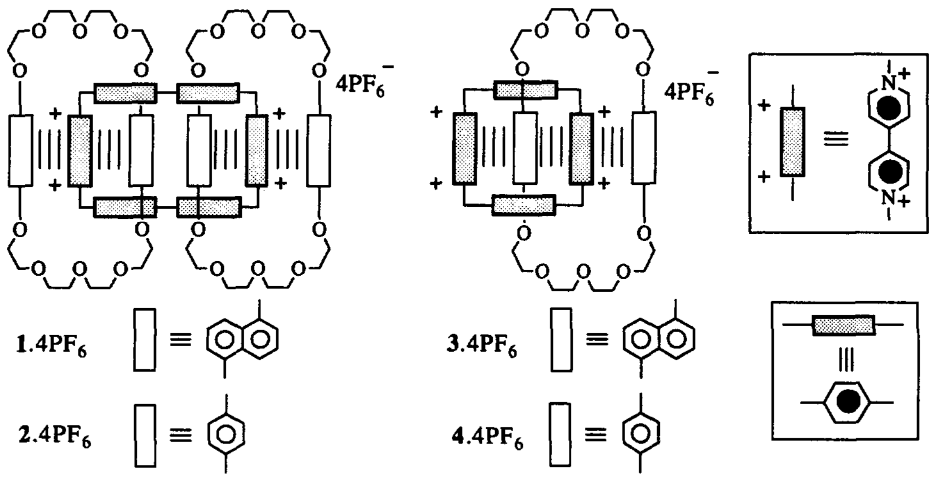

Figure 1 Diagrammatic representation of the catenanes $\mathbf{1}.4PF_6$ - $\mathbf{4}.4PF_6$

Table 1 The ESMS Characterisation[a] of Catenanes **1**.4PF$_6$ – **4**.PF$_6$

No. of PF$_6^-$ counterions lost from a monomeric species	No. of PF$_6^-$ counterions lost from a dimeric species	CATENANE			
		1.4PF$_6$ Mol.Wt. 2524.8	**2**.4PF$_6$ Mol.Wt. 2324.7	**3**.4PF$_6$ Mol.Wt. 1736.4	**4**.PF$_6$ Mol.Wt. 1636.4
1	2	2379.8(36)	2179.7(18)	1591.4(8.5)	1491.4(13)
–	3	1538.2(36)	1404.8(16)	1012.6(18)	946.0(17)
2	4	1117.4(100)	1017.4(100)	723.2(100)	673.2(100)
3	6	696.6(80)	629.9(5.5)	433.8(98)	400.5(88)
4	8	486.2(29)	–	–	264.1(9.5)

[a] Data are presented as *m/z* ratio and (relative abundance). Molecular weight and *m/z* values apply to the lowest mass component of any isotope distribution and are based on a scale in which ^{12}C = 12.000

the electrospray ionization technique to produce multiply-charged species means that it can be applied ultimately to the characterization of very much higher molecular weight compounds. Here, we demonstrate that electrospray ionization is not only applicable to providing molecular weight information, but also offers evidence for intermolecular association[10] between molecules in the case of some of the catenanes.

In many examples of electrospray ionization, positive ions are formed by protonation in an acidified aqueous solution. In the present case, catenanes **1**.4PF$_6$ - **4**.4PF$_6$ are already ionized so that representative cations can be formed[11] simply by the loss of one or more PF$_6^-$ counterions. The major peaks in all four spectra are formed at *m/z* ratios (Table I) consistent with this process, *e.g.* the base peak in each case corresponds formally to the loss of two PF$_6^-$ counterions (mol. wt. = 145 Da each) from a single catenane species. Thus, for **1**.4PF$_6$ (mol. wt. = 2524.8 Da), loss of two PF$_6^-$ counterions results in a molecular mass of 2234 Da with a net charge of two positive ions, *i.e.* a *m/z* ratio of 2234.8 / 2 = 1117.4 (Figure 2).

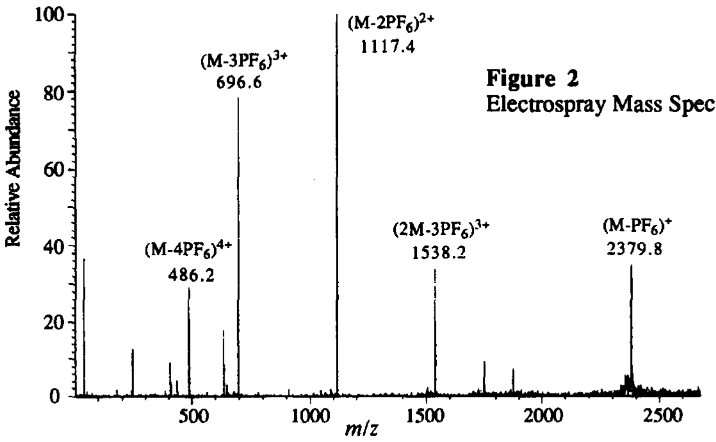

Figure 2
Electrospray Mass Spectrum of **1**.4PF$_6$

Intense peaks are also observed which correspond formally to the loss of one or three PF_6^- counterions from a single catenane species in every case. Two of the spectra also show peaks which correspond to the loss of all four PF_6^- counterions (Table I). In addition to these peaks, each spectrum contains a peak whose *m/z* ratio can only result from the loss of three PF_6^- counterions from an association of two catenane species. Thus, for compound $1.4PF_6$ (mol. wt. = 2524.8 Da), loss of three PF_6^- counterions from a dimer results in a molecular mass of 4614.6 Da with a net charge of three positive ions, *i.e.* a *m/z* ratio 4614.6 /3 = 1538.2 (Figure 2). Closer inspection of the peaks which represent the formal loss of one, two, or three PF_6^- counterions from a monomeric catenane species also reveals a more complex situation. A comparison of the relative intensities and mass separations of the isotopic components of these peaks with those predicted by theory (Figure 3) suggests that these ions arise largely from a dimeric form of the catenane species.

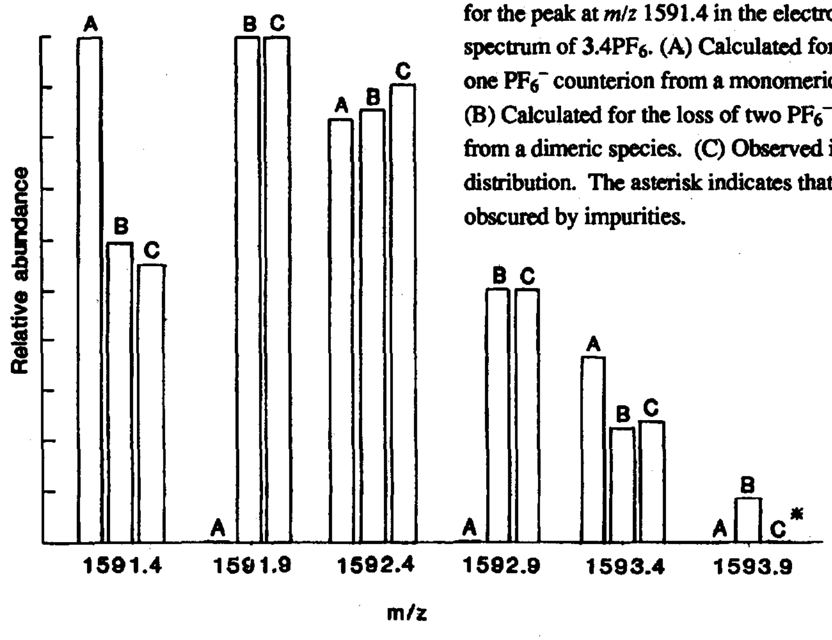

Figure 3
Theoretical and experimental isotope distributions for the peak at *m/z* 1591.4 in the electrospray mass spectrum of $3.4PF_6$. (A) Calculated for the loss of one PF_6^- counterion from a monomeric species. (B) Calculated for the loss of two PF_6^- counterions from a dimeric species. (C) Observed isotope distribution. The asterisk indicates that the peak is obscured by impurities.

Although the basis for the dimerisation is assumed to be intermolecular π-π stacking interactions[12], they may be supplemented by electrostatic interactions involving the PF_6^- counterions and the tetracationic catenanes. The spectra show evidence for the loss of two, three, four and six PF_6^- counterions from a dimeric form of each catenane. There is no evidence for peaks which correspond to the loss of five or seven PF_6^- counterions. Two spectra, however, contain peaks arising from the loss of all eight PF_6^- counterions.[13]

The use of ESMS confirms the expected structures of $1.4PF_6$ – $4.PF_6$. The higher molecular weight species give particularly intense spectra, a feature which offers promise for the analysis of more complex higher molecular weight analogues. In addition, there is the exciting prospect of being able to obtain structural information with the electrospray technique at the supramolecular level.

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10 The use of ESMS to detect and identify noncovalent receptor-ligand complexes of charged macromolecules with their specific molecular ligands has been described recently. See: Ganem, B.; Li, Y.-T.; Henion, J.D. *J. Am. Chem. Soc.* **1991**, *113*, 6294 and 7818.

11 Mass spectra were recorded using a Kratos Concept 1H double focussing magnetic sector instrument operating at 4 kV accelerating voltage fitted with an electrospray ion source. The spraying capillary was maintained at 3 kV above the endplate potential during analyses (Gallagher, R.T.; Chapman, J.R.; Mann, M. *Rapid Commun. Mass Spectrom.* **1990**, *4*, 369). Solvent delivery to the spraying capillary employed a Harvard Model 22 syringe pump operating at a flow rate of 3 μL min^{-1}. Solutions of samples (5 pmol μL^{-1} in MeOH-MeCN, 1:1) were introduced into the solvent flow *via* a Rheodyne Model 7120 injection valve with a 20 μL sample loop. Calibration of the instrument mass scale used a solution of CsI in MeOH-H_2O (1:1).

12 The fact that dimeric associations are evident in the ESMS of all four catenanes is perhaps hardly surprising, given the demonstrated abilities of **2**.4PF_6, **3**.4PF_6, and **4**.4PF_6 to enter into continuous π-π stacking interactions in one of the crystallographic directions in the solid state structures.

13 The loss of one PF_6^- counterion from a dimeric species would result in every case in a *m/z* ratio in excess of 3000 which is well above the recorded *m/z* range.

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