Self-Assembly of an 11-Component Cylindrical Inorganic Architecture: Electrospray Mass Spectrometry and Thermodynamic Studies

Annie Marquis-Rigault,[†] Annick Dupont-Gervais,[‡] Paul N. W. Baxter,[†] Alain Van Dorsselaer,*[,‡] and Jean-Marie Lehn*[,†]

Laboratoire de Chimie Supramoléculaire and Laboratoire de Spectrométrie de Masse Bio-Organique, Université Louis Pasteur, Strasbourg, France

Received October 20, 1995[⊗]

Two hexaphenylhexaazatriphenylene $\mathbf{L^1}$ and three quaterpyridine $\mathbf{L^2}$ ligands are well-known to associate around six copper(I) ions to form a cylindrical inorganic cage **1**. Spectrophotometric and electrospray mass spectrometry (ESMS) titrations have been conducted to gain information on the thermodynamics and the formation pathway of the assembly **1**. From these data, the nature of intermediates present in solution was determined and the association constants of these species were calculated. It was also found that this assembling process occurs with positive cooperativity.

Introduction

Self-assembly processes have been devised for the generation of well-defined, discrete inorganic architectures,[1] such as double or triple helices,[2−7] cylindrical species,[8] and grid-type arrays.[9] The investigation of formation pathways and thermodynamic features of such spontaneous structure generation is of great interest for the design of self-assembling systems as well as within the general framework of self-organization phenomena.[1,10]

Earlier work based on spectrophotometric titration experiments has shown that trinuclear double-helical complexes, helicates, form with positive cooperativity.[11,12] Recently electrospray mass spectrometry (ESMS) was used to follow the assembly of a capped-trinuclear complex.[13] The present study combines the two approaches to gain insight into the self-assembly pathway and the formation thermodynamics of the 11-component cylindrical complex **1**[8] as well as into the general application of these methods to the investigation of self-assembly processes.

Complex **1** results from the association of two hexaphenylhexaazatriphenylene units (HAT) $\mathbf{L^1}$, three quaterpyridine moieties (qpy) $\mathbf{L^2}$, and six copper(I) cations[8] (Figure 1). Since each of the ligands is itself a good complexant for copper(I), the exclusive formation of **1** may be indicative of self-assembly with positive cooperativity. Such a process is generally quantified by graphical methods, assuming that the thermodynamic intermediates of formation are known, as well as their association constants.[14]

Experimental Section

ESMS Measurements. ESMS was performed on a VG BioQ triple-quadrupole mass spectrometer with a mass to charge (m/z) range of 4000 (VGBio Tech Ltd., Altrincham, U.K.). The accelerating cone voltage (V_c)[15] was set at 20 V to minimize fragmentation processes. Titration solutions were prepared from solutions of $\mathbf{L^1}$, $\mathbf{L^2}$, and $Cu(CH_3CN)_4BF_4$ with respective concentrations of 10^{-4}, 1.5×10^{-4}, and 3×10^{-4} M in CH_2Cl_2/CH_3CN (95/5, v/v) and infused into the mass spectrometer in a continuous flow at about 5 μL/min with a syringe pump.

Spectroscopic and Analytical Measurements. Electronic spectra in the UV−visible range were recorded in solution with a Varian Cary 3 spectrophotometer at 20 °C using quartz cells of 1 and 0.1 cm path lengths. In a typical experiment, a 10 mL solution containing two of the three components in CH_2Cl_2/CH_3CN (95/5, v/v) was titrated with the third one in the same solvent. The absorbances at 10 different wave lengths were recorded and transferred to the computer. Models for the distribution of species were fitted with the LETAGROP-SPEFO program;[16] the model giving the best fit was used to calculate the stability constants of the different complexes.

Results and Discussion

1. Initial Spectrophotometric Titrations. We first tried to follow the formation of **1** by spectrophotometric titration of ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ with $Cu(CH_3CN)_4BF_4$. The initially colorless solution changed through reddish to dark brown and finally turned deep purple. Analysis of the titration data, using the LETAGROP-SPEFO program,[16] which performs a nonlinear best fit of the spectrophotometric data, should have provided the thermodynamic parameters of the cylindrical complex formation. This program calculates the binding constant β_{hqm}

[†] Laboratoire Chimie Supramoléculaire, CNRS URA 422.
[‡] Laboratoire de Spectrométrie de Masse Bio-Organique, CNRS URA 31.
[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1996.

(1) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, Germany, 1995; Chapter 9.
(2) Lehn, J.-M.; Rigault, A.; Siegel, J.; Harrowfield, J.; Chevrier, B.; Moras, D. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2565.
(3) Koert, U.; Harding, M.; Lehn, J.-M. *Nature* **1990**, *346*, 339.
(4) Piguet, C.; Bernardinelli, G.; Williams, A. F. *Inorg. Chem.* **1989**, *28*, 2920.
(5) Krämer, R.; Lehn, J.-M.; De Cian, A.; Fischer, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 703.
(6) Constable, E. C. *Tetrahedron* **1992**, *48*, 10013.
(7) Potts, K. T.; Keshavars-K, M.; Tham, F. S.; Abruna, H. D.; Arana, C. R. *Inorg. Chem.* **1993**, *32*, 4422.
(8) Baxter, P. N. W.; Lehn, J.-M.; DeCian, A.; Fischer, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 69.
(9) Baxter, P. N. W.; Lehn, J.-M.; Fischer, J.; Youinou, M.-T. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2284.
(10) *Self-Organizing Systems*; Yates, F. E., Ed.; Plenum Press: New York, 1987.
(11) Pfeil, A.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1992**, 838.
(12) Garrett, T. M.; Koert, U.; Lehn, J.-M. *J. Phys Org. Chem.* **1992**, *5*, 529.
(13) Leize, E.; Van Dorsselaer, A.; Krämer, R.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1993**, 990.
(14) Perlmutter-Hayman, B. *Acc. Chem. Res.* **1986**, *19*, 90. Hill, T. L. *Cooperativity Theory in Biochemistry*; Springer Verlag: New York, 1985.
(15) Bruins, A. P.; Covey, T. R.; Henion, J. D. *Anal. Chem.* **1987**, *59*, 2642.
(16) Sillen, L. G.; Warnqvist, B. *Ark. Kemi* **1969**, *31*, 377.

0020-1669/96/1335-2307$12.00/0

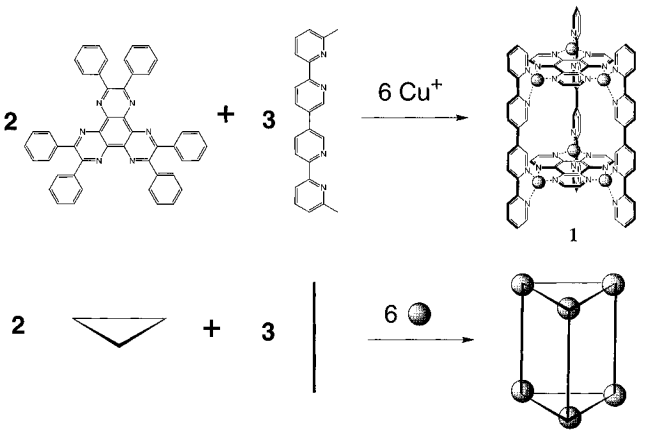

Figure 1. Top: Formation of the cylindrical complex **1**, from HAT $\mathbf{L^1}$ and qpy $\mathbf{L^2}$ units. For clarity, the methyl groups of qpy and the phenyl groups of HAT have been omitted in the structure of the complex. Bottom: Schematic representation of the complex and its subunits.

for each species $(\mathbf{L^1})_h(\mathbf{L^2})_q\mathbf{M}_m$ of a proposed chemical model, by iterative comparison of calculated with experimental absorbance values, searching for the global minimum of the error function. Models that do not fit the data are rejected; the one that fits the best should be closest to the real situation.

Unfortunately, due to the lack of information on the number and the stoichiometry of the intermediates, the analysis of this first titration led to unrealistic values for the association constants. To make use of the LETAGROP-SPEFO program, the determination of the thermodynamically significant species present in the solution, which may be intermediates in the formation pathway, is then a prerequirement.

2. Qualitative ESMS Studies. ESMS has been found to be a good method for determining the composition of the species present in a solution. Indeed, it has already been applied successfully to the characterization of various polynuclear metal complexes[17,18] occurring in thermodynamic equilibrium[13,19−21] in a wide range of concentrations. ES mass spectra are usually clear and easy to interpret because of the presence of several pseudomolecular peaks corresponding to successive loss of the counterions. Preserving the existing supramolecular associations when the solution is introduced into the mass spectrometer for analysis is obviously very important. It is possible to prevent alteration of the molecular species and of the distribution of the intermediates by controlling carefully their acceleration through the MS interface (accelerating cone voltage, V_c).[15]

(a) ESMS Titration of $\mathbf{L^1}$ and $\mathbf{L^2}$ by Cu(I). In a first series of experiments, solutions containing 2 equiv of $\mathbf{L^1}$, 3 equiv of $\mathbf{L^2}$, and increasing amounts of copper(I) (3, 6, and 12 equiv) were examined by ESMS. Measurements were performed once the thermodynamic equilibrium was assumed to be reached, that is once the spectra remained identical on a time course basis. Below 6 equiv of Cu(I), up to five thermodynamic intermediates were observed in addition to the final cylindrical complex **1** (Figure 2a and Table 1). When the stoichiometry was reached, i.e. 6 equiv of Cu(I) for 2 equiv of $\mathbf{L^1}$ and 3 equiv of $\mathbf{L^2}$, the spectrum exhibited only peaks corresponding to the different states of charge of the target species **1** (Figure 3). The small peaks at m/z = 752.7 and 1032.6 may be attributed to adducts of CH_3CN and H_2O with **1**. Interestingly, when an excess of Cu(I) was added (up to 12 equiv), the spectrum remained unchanged, indicating that the cage **1** is stable under these conditions.

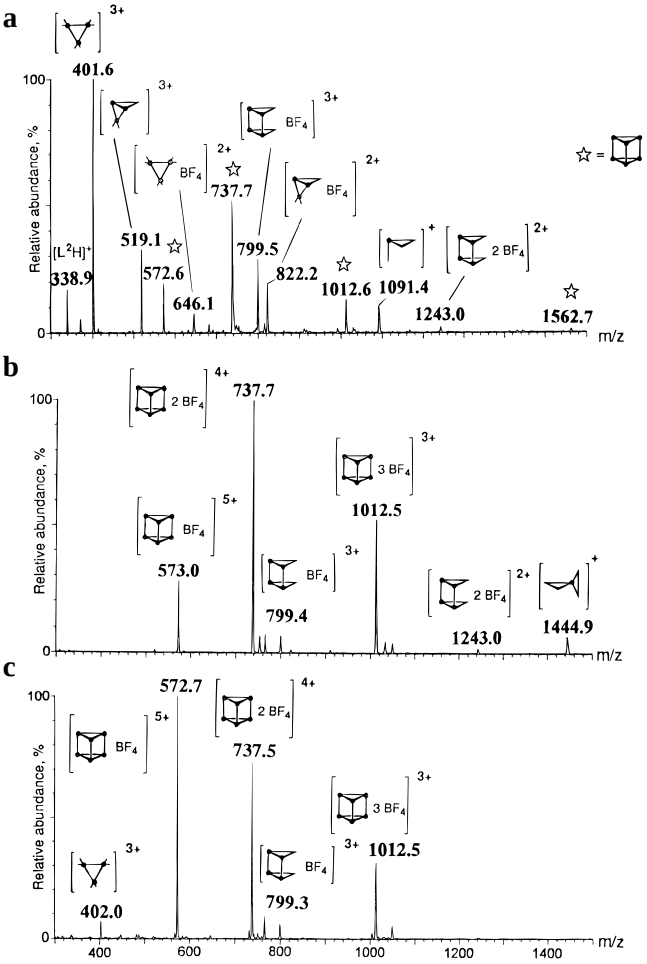

Figure 2. ES mass spectra of three solutions containing $\mathbf{L^1}$, $\mathbf{L^2}$, and $Cu(CH_3CN)_4BF_4$ in different ratios corresponding to the three types of titrations described (see text): (a) 2 equiv of $\mathbf{L^1}$, 3 equiv of $\mathbf{L^2}$, and 3 equiv of Cu(I); (b) 2 equiv of $\mathbf{L^1}$, 6 equiv of Cu(I); 1.5 equiv of $\mathbf{L^2}$; (c) 3 equiv of $\mathbf{L^2}$, 6 equiv of Cu(I), 1 equiv of $\mathbf{L^1}$. Stoichiometry would be reached for a solution containing 2 equiv of $\mathbf{L^1}$, 3 equiv of $\mathbf{L^2}$, and 6 equiv of Cu(I).

The high number of intermediate species observed in this mode of titration was not compatible with the use of the LETAGROP-SPEFO program for analyzing the spectrophotometric titrations. Therefore, a reversed titration was performed.

(b) ESMS Titration of $\mathbf{L^1}$ and Cu(I) by $\mathbf{L^2}$. In a second series of experiments, a solution containing 2 equiv of $\mathbf{L^1}$ and 6 equiv of Cu(I) was titrated with the quaterpyridine ligand $\mathbf{L^2}$. In this case, the ES mass spectra were simpler (Figure 2b and Table 1) and showed that, before the final stoichiometry was reached, only two intermediates were detected, besides the final cage **1**. These two intermediates were a mononuclear complex (2:0:1)[22] and the incomplete cylindrical complex (2:2:4) (Figure 2b and Table 1), where the numbers in parentheses (h:q:m) represent the composition in HAT (h), quaterpyridine (q), and metal cations (m). As in the case of the first titration experiment, once the final stoichiometry (h:q:m = 2:3:6) was reached, the

(17) Katta, V.; Chowdury, S. K.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 5348.
(18) Bitsch, F.; Hegy, G.; Dietrich-Buchecker, C.; Leize, E.; Sauvage, J.-P.; Van Dorsselaer, A. *New J. Chem.* **1994**, *18*, 801.
(19) Hopfgartner, G.; Piguet, C.; Henion, J. D.; Williams, A. F. *Helv. Chim. Acta* **1993**, *76*, 1759.
(20) Piguet, C.; Rivara-Minten, E.; Hopfgartner, G.; Bünzli, J. -C. G. *Helv. Chim. Acta* **1995**, *78*, 1541.
(21) Leize, E.; Jaffrezic, A.; Van Dorsselaer, A. *J. Mass Spectrom.*, submitted.
(22) Moucheron, C.; Dietrich-Buchecker, C. O.; Sauvage, J. -P.; Van Dorsselaer, A. *J. Chem. Soc., Dalton Trans.* **1994**, 885.

Table 1. Symbolism and Expected Multiply-Charged Peaks of the Species Encountered in the ES Mass Spectra

(h:q:m)	Symbolism	Formula	Expected m/z
1		$[(L^1)_2(L^2)_3Cu_6(BF_4)_5]^+$	m/z = 3212.1
		$[(L^1)_2(L^2)_3Cu_6(BF_4)_4]^{2+}$	m/z = 1562.7
		$[(L^1)_2(L^2)_3Cu_6(BF_4)_3]^{3+}$	m/z = 1012.8
		$[(L^1)_2(L^2)_3Cu_6(BF_4)_2]^{4+}$	m/z = 737.9
		$[(L^1)_2(L^2)_3Cu_6(BF_4)]^{5+}$	m/z = 572.9
		$[(L^1)_2(L^2)_3Cu_6]^{6+}$	m/z = 463.0
(2:0:1)		$[(L^1)_2Cu]^+$	m/z = 1445,1
(2:1:2)		$[(L^1)_2(L^2)Cu_2(BF_4)]^+$	m/z = 1934.1
		$[(L^1)_2(L^2)Cu_2]^{2+}$	m/z = 923.6
(2:2:4)		$[(L^1)_2(L^2)_2Cu_4(BF_4)_3]^+$	m/z = 2573.0
		$[(L^1)_2(L^2)_2Cu_4(BF_4)_2]^{2+}$	m/z = 1243.1
		$[(L^1)_2(L^2)_2Cu_4(BF_4)]^{3+}$	m/z = 799.8
		$[(L^1)_2(L^2)_2Cu_4]^{4+}$	m/z = 578.2
(1:2:3)		$[(L^1)(L^2)_2Cu_3(BF_4)_2]^+$	m/z = 1731.9
		$[(L^1)(L^2)_2Cu_3(BF_4)]^{2+}$	m/z = 822.5
		$[(L^1)(L^2)_2Cu_3]^{3+}$	m/z = 519.4
(1:3:4)		$[(L^1)(L^2)_3Cu_4(BF_4)_3]^+$	m/z = 2220.6
		$[(L^1)(L^2)_3Cu_4(BF_4)_2]^{2+}$	m/z = 1066.9
		$[(L^1)(L^2)_3Cu_4(BF_4)]^{3+}$	m/z = 682.3
		$[(L^1)(L^2)_3Cu_4]^{4+}$	m/z = 490.0
(1:1:1)		$[(L^1)(L^2)Cu]^+$	m/z = 1092.8
(0:3:3)		$[(L^2)_3Cu_3(BF_4)_2]^+$	m/z = 1379.5
		$[(L^2)_3Cu_3(BF_4)]^{2+}$	m/z = 646.3
		$[(L^2)_3Cu_3]^{3+}$	m/z = 402.0

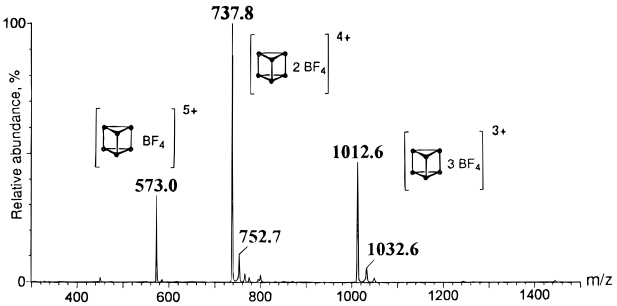

Figure 3. ES mass spectrum of a solution containing stoichiometric amounts of $\mathbf{L^1}$ (2 equiv), $\mathbf{L^2}$ (3 equiv), and $Cu(CH_3CN)_4BF_4$ (6 equiv). Exactly the same ES mass spectrum was obtained independently of the titration performed, i.e of the order of introduction of the different species in the solution. The only species detected is the final cage **1**.

ES mass spectrum displayed only complex **1** (Figure 3 and Table 1). With an excess of quaterpyridine, new species were generated that were identical to those obtained during the first titration before reaching stoichiometry in Cu(I) (Figure 2a and Table 1).

Since only two intermediates were observed in this second titration, they could serve as basis for the appplication of the LETAGROP-SPEFO program. On the basis of the (2:2:4) and (2:0:1) species identified, a minimal model for the formation of the cylindrical complex **1** was built (Figure 4). In this proposed minimal mechanism of the formation of **1**, we have introduced a necessary intermediate (2:1:2), although it was only detected as traces in the ES mass spectra, perhaps because of its short lifetime or a particular low stability in the interface of the ES source. Obviously the mass measurements could only provide the (*h*:*q*:*m*) composition of the intermediates. Possible structures are displayed in Figure 4. Several isomers, with identical masses, may be written for the (2:1:2) and (2:2:4) species. The composition of (0:3:3) was ascertained unambiguously both by the simultaneous presence of two states of charge,

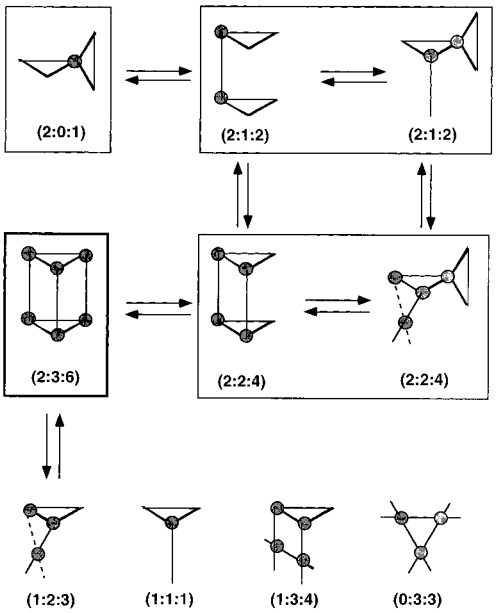

Figure 4. Proposed mechanistic pathway for the formation of the cylindrical complex 1. The numbers in parentheses (*h*:*q*:*m*) represent the composition in HAT $\mathbf{L^1}$ (*h*), quaterpyridine $\mathbf{L^2}$ (*q*), and copper(I) cations (*m*). The last row represents the species detected by ESMS in the presence of excess of quaterpyridine $\mathbf{L^2}$.

$(0:3:3)^{3+}$ and $[(0:3:3) + BF_4]^{2+}$, and by the isotopic pattern of the peak $(0:3:3)^{3+}$ where the peaks are separated by 0.33 *m/z* unit (Figure 5).

Finally, in a third experiment designed to confirm the results of both previous ESMS titrations, 3 equiv $\mathbf{L^2}$ and 6 equiv Cu(I) were titrated by $\mathbf{L^1}$ (1, 2, and 4 equiv) (Figure 2c and Table 1). Only two intermediates were also observed: (0:3:3) and (2:2:4).

The formation pathways corresponding to the three different types of titration performed lead to the same final compound **1**, which was finally exclusively obtained.

It must be emphasized that the ESMS data provide crucial information on the dominant species present in solution, which are in equilibrium with species of all possible compositions and structures located on the energy hypersurface of this highly complex system.

In this way, a minimal set of intermediate species can be formulated, which then facilitates the calculation of equilibrium constants from analysis of the corresponding spectrophotometric titration curves.

3. Spectrophotometric Titration and Determination of the Thermodynamic Constants of the System. Using the same experimental conditions (solvent, concentration) as for the second ESMS experiments, a spectrophotometric titration of a solution containing 2 equiv of $\mathbf{L^1}$ and 6 equiv of Cu(I) with the quaterpyridine $\mathbf{L^2}$ was performed. Up to the final stoichiometry (3 equiv of $\mathbf{L^2}$), the spectra exhibited a sharp isosbestic point, which disappeared on addition of excess quaterpyridine.

This titration to the final stoichiometry was analyzed with the LETAGROP-SPEFO program on the basis of the above model (Figure 4). The global association constants of the following equilibria were determined, keeping in mind that before the final stoichiometry is reached, the metal is always present in excess in solution:

$$2\,\mathbf{L^1} + Cu^+ \rightleftharpoons \mathbf{L^1}_2Cu^+ \qquad \log\beta_{201} = 12.3$$

$$\mathbf{L^1}_2Cu^+ + \mathbf{L^2} + Cu^+ \rightleftharpoons \mathbf{L^1}_2\mathbf{L^2}Cu_2^{2+} \qquad \log\beta_{212} = 23.1$$

$$\mathbf{L^1}_2\mathbf{L^2}Cu_2^{2+} + \mathbf{L^2} + 2\,Cu^+ \rightleftharpoons \mathbf{L^1}_2\mathbf{L^2}_2Cu_4^{4+} \qquad \log\beta_{224} = 38.9$$

$$\mathbf{L^1}_2\mathbf{L^2}_2Cu_4^{4+} + \mathbf{L^2} + 2\,Cu^+ \rightleftharpoons \mathbf{L^1}_2\mathbf{L^2}_3Cu_6^{6+} \qquad \log\beta_{236} = 53.6$$

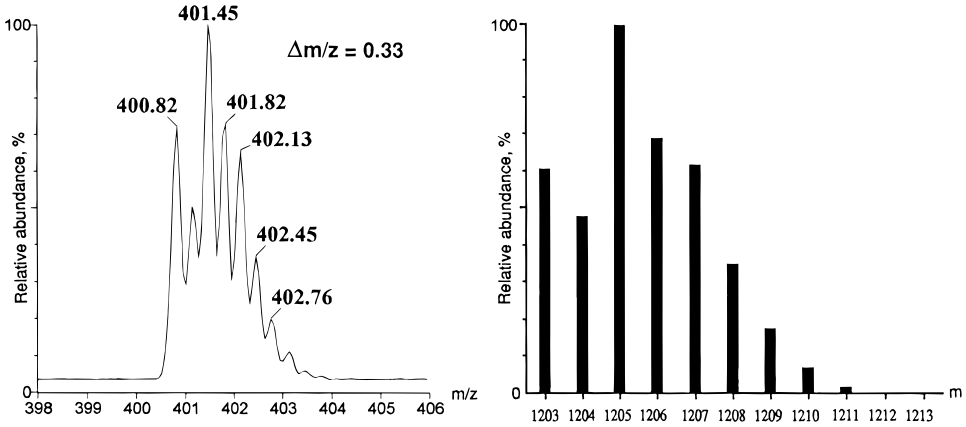

Figure 5. Isotopic patterns observed (left) and calculated (right) for the triply-charged ion of the (0:3:3) species. Isotopic peaks are separated by 0.3 *m/z* unit.

On the basis of these results, one may search for the presence of cooperativity in the formation of the closed cylindrical complex **1** using the minimal model of three predominant species along the assembly pathway.

In its simpler form, cooperativity is positive when the binding of the nth substrate yields a species presenting an enhanced binding for the $(n+1)$th substrate, i.e.

$$K_{n+1}/K_n > [n(t-n)]/[(n+1)(t-n+1)]$$

where n = number of occupied sites and t = total number of sites.

However, we cannot use this definition in our case, as the three global binding equilibria retained are not bimolecular, but we can estimate the amount of cooperativity by various graphical methods. Scatchard plots, where the ratio of the occupancy over the concentration in free substrate is plotted as a function of the occupancy, are particularly informative.

The site occupancy r is defined as the average number of quaterpyridine ligands bound per (2:0:1) complex [HAT/Cu(I)].

Then $r = ([\mathbf{L^2}]_0 - [\mathbf{L^2}])/[\mathbf{L^1}_2Cu^+]_0$, where $[\mathbf{L^2}]_0$ is the total amount of ligand added at any point of the titration, $[\mathbf{L^2}]$ is the concentration of free ligand, and $[\mathbf{L^1}_2Cu^+]_0$ is the initial concentration of the complex.

The Scatchard plot obtained (Figure 6) presents a downward concave curvature, indicating that the process of formation of **1** from the components considered occurs with a positive cooperativity.[14]

The degree of cooperativity is given by the Hill coefficient n_H, calculated at the maximum r_{max} of the plot: $n_H = t/(t - r_{max})$.

Substitution of the values of t (=3) and r_{max} (=0.54) leads to $n_H = 1.22$ for the Hill coefficient. This value greater than 1 measures a real, although weak, positive cooperativity for the formation of **1** along the pathway considered.

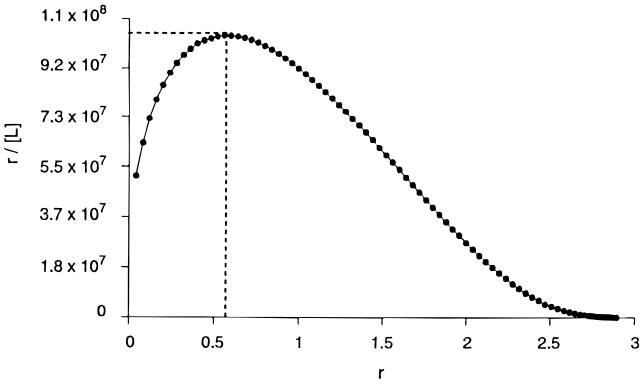

Figure 6. Scatchard plot for the formation of the cylindrical complex **1**, obtained from analysis of the titration data. [L] is concentration of ligand $\mathbf{L^2}$, mol·L^{-1}; $r_{max} = 0.54$ (see text).

Conclusions

The present results allowed the determination of the thermodynamic parameters for the formation of complex **1**. A positive cooperativity was found for this self-assembly process. The analysis was made on the basis of the predominant intermediates characterized by ESMS. Application to other processes of inorganic self-assembling is in progress. One may expect that the extension of the combined use of UV−visible spectrophotometry and ESMS to other systems will provide crucial information on the thermodynamics and the mechanism of self-assembly of complex supramolecular architectures in solution.

Acknowledgment. Financial support from BioAvenir (Rhône Poulenc Santé) is gratefully acknowledged by A.D.-G. and A.V.D., and P.N.W.B. thanks the Collège de France for a postdoctoral position.

IC951362O