

Characterization of Hydrogen-bonded Aggregates in Chloroform  
by Electrospray Ionization Mass Spectrometry.

Xueheng Cheng, Quanyin Gao, Richard D. Smith\*

*Chemical Sciences Department and Environmental Molecular Sciences Laboratory  
Pacific Northwest Laboratories  
Richland, WA 99352*

Eric E. Simanek, Mathai Mammen, and George M. Whitesides\*

*Department of Chemistry  
Harvard University  
Cambridge, MA 02138*

Abstract

Molecular masses were calculated for seven hydrogen-bonded aggregates based on the cyanuric acid•melamine lattice using electrospray ionization mass spectrometry (ESI-MS). Negative ion spectra were obtained from chloroform solutions of these aggregates, with  $\text{Ph}_4\text{PCl}$  as the charge carrier. The ability to detect and characterize these high molecular weight aggregates demonstrates the potential of ESI-MS as an analytical tool for characterizing non-covalent assemblies generated by molecular self-assembly.

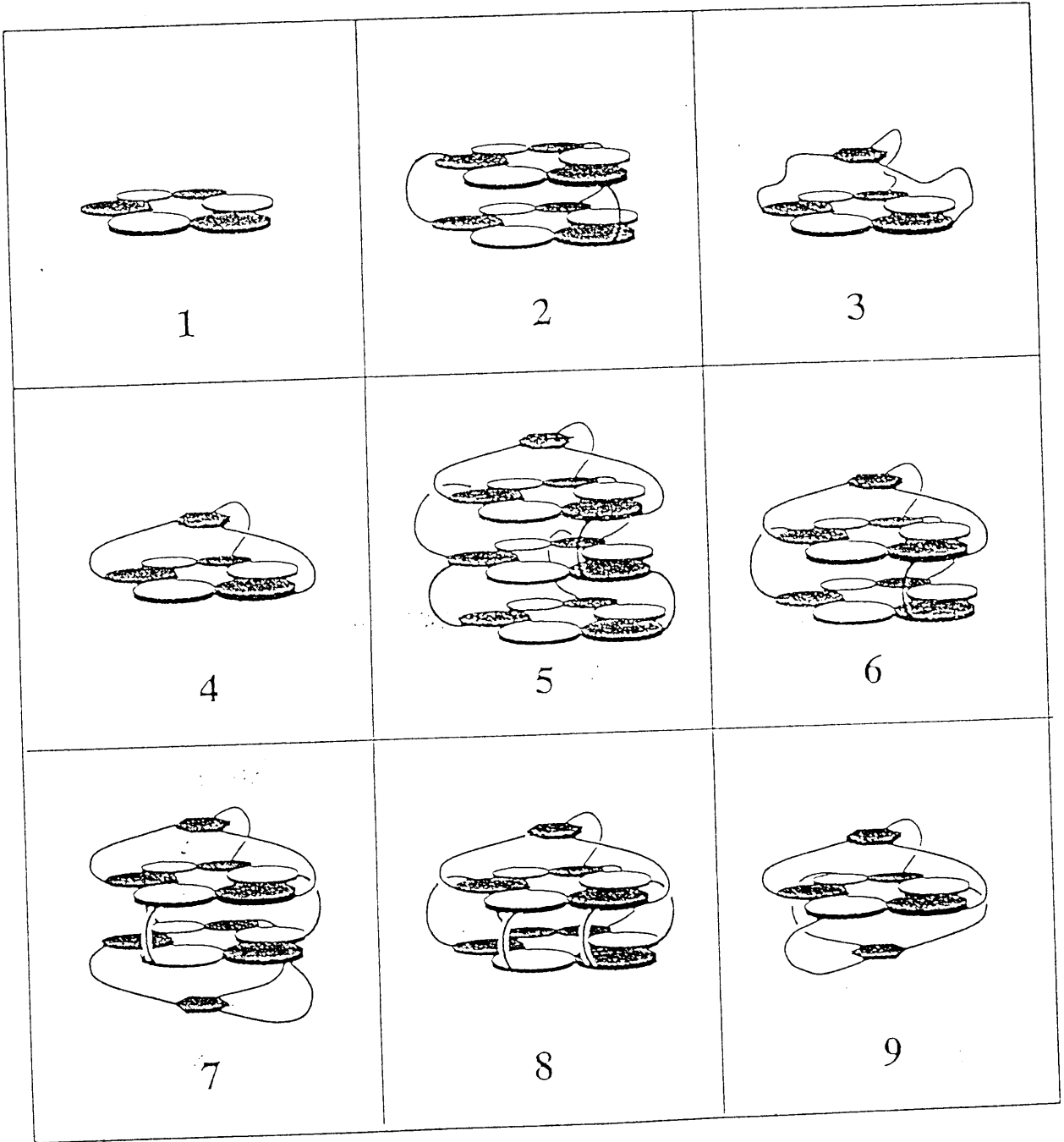
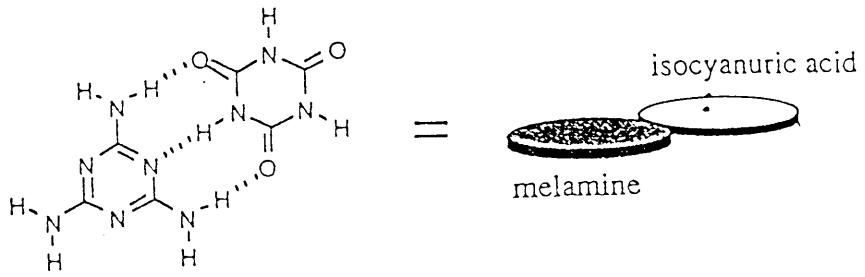
## Characterization of Hydrogen-bonded Aggregates in Chloroform by Electrospray Ionization Mass Spectrometry.

We report the measurement of molecular weights of seven non-covalent aggregates based on the cyanuric acid•melamine (CA•M) lattice (Figure 1), obtained from negative ion spectra using electrospray ionization mass spectrometry (ESI-MS). These results are important for three reasons. First, our past assignments of the structures of these non-covalent assemblies has been based on inferences from a range of indirect techniques --  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopies, titration experiments, gel permeation chromatography (GPC), and vapor phase osmometry (VPO).<sup>1</sup> A more accurate determination of mass than that provided by GPC and VPO offers additional support the assigned stoichiometries and structures. Second, the ability to obtain mass spectra of non-covalent aggregates that are of limited stability -- those that form only in aprotic, nonpolar organic solvents -- provides a further demonstration of the value of ESI-MS as a technique with which to study molecular recognition and self-assembly in non-aqueous solutions. Third, the use of  $\text{Ph}_4\text{PCl}$  as an organic-soluble, charge carrier is undocumented to the best of our knowledge. These results also suggest that thermal dissociation experiments may be useful in determining the relative stabilities of noncovalent assemblies.

Table 1 lists recent studies that have successfully used ESI-MS to obtain masses of large, noncovalent assemblies. Our previous attempts to obtain mass spectra of these aggregates using other ionization techniques -- chemical (CI), electron (EI), fast-atom bombardment (FAB), ESI (in the absence of a charge carrier), and matrix assisted laser desorption (MALDI) were unsuccessful. Figure 1 summarizes the aggregates surveyed in this work, and Table 2 gives information about their masses. (The nomenclature used to represent these aggregates is that used in previous papers; detailed structures of the arms and connectors have been summarized.<sup>1</sup>) The aggregates are numbered in terms of increasing stability ( $1 < 2 \dots < 8 < 9$ ) as inferred from

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**Figure 1.** The aggregates (1 - 9) examined in this study are shown schematically. White disks correspond to isocyanuric acid groups. Darkened disks correspond to melamines. They are numbered in order of increasing stability (highest in stability is 9). Atomic details of these structures have been summarized.<sup>1</sup>



**Table 1:** Electrospray-ionization mass spectrometry has been used to characterize a variety of interactions. “Aggregate” describes the type of architecture studied. “Particles” refers to the number of molecules comprising the assembly.

Aggregate	Reference	Particles
Antibody and hapten	Suizdak <sup>a</sup>	2
Carbohydrate and cation	Suizdak <sup>b</sup>	2-4
Catenanes	Van Dorsselaer <sup>c</sup>	3-7
Catenanes	Fujita <sup>d</sup>	2
Cyclodextrins and guest	Camilleri <sup>e</sup>	2
Distamycin and DNA	Smith <sup>f</sup>	3
DNA duplex	Smith <sup>g</sup>	2
DNA quadreplex	Smith <sup>h</sup>	4
FK506 and FKBP	Ganem <sup>i</sup>	2
HIV protease and inhibitor	Ken <sup>j</sup>	3
Hydrogen-bonded aggregate	Lehn <sup>k</sup>	6
Leucine zippers	Ganem <sup>l</sup>	2
Supramolecular pigments	Kondo and Yoshida <sup>m</sup>	13
Polymetallic complexes	Lehn <sup>n</sup>	5

*a-n.* Reference 2 gives detailed citations to these studies.

Table 2. Aggregates surveyed.

Aggregate Number	Name	m/z	Species Observed	Assigned Formula	Molecular Weight	
					Formula	ESI-MS VPO
1	3M•3CA	1724.2	-----	C <sub>93</sub> H <sub>126</sub> N <sub>24</sub> O <sub>9</sub>	1724.2	----- 1700
2	6M•3bisCA	3635.5	-----	C <sub>192</sub> H <sub>246</sub> N <sub>54</sub> O <sub>21</sub>	3635.5	----- 3700
3	flex(M) <sub>3</sub> •3CA	3958.1	3•Cl <sup>-</sup>	C <sub>93</sub> H <sub>129</sub> N <sub>27</sub> O <sub>21</sub>	1961.2	3922.6 4000
4	hub(M) <sub>3</sub> •3CA	2768.1	4•Cl <sup>-</sup>	C <sub>135</sub> H <sub>168</sub> Br <sub>3</sub> N <sub>33</sub> O <sub>15</sub>	2732.8	2732.7 3200
5	hub(MMM) <sub>3</sub> •9CA	3252.4	5•Cl <sub>2</sub> <sup>=</sup>	C <sub>342</sub> H <sub>534</sub> N <sub>87</sub> O <sub>36</sub>	6440.6	6433.8 6500
6	hub(MM) <sub>3</sub> •6CA	2106.8	6•Cl <sub>2</sub> <sup>=</sup>	C <sub>201</sub> H <sub>262</sub> Br <sub>3</sub> N <sub>60</sub> O <sub>24</sub>	4142.4	4142.6 4200
7	2hub(M) <sub>3</sub> bisCA	2795.6	7•Cl <sub>2</sub> <sup>=</sup>	C <sub>276</sub> H <sub>318</sub> Br <sub>6</sub> N <sub>66</sub> O <sub>30</sub>	5519.9	5519.1 5400
8	hub(MM) <sub>3</sub> •3bisCA	2133.5	8•Cl <sub>2</sub> <sup>=</sup>	C <sub>207</sub> H <sub>244</sub> Br <sub>3</sub> N <sub>60</sub> O <sub>24</sub>	4196.4	4196.1 4500
9	hub(M) <sub>3</sub> •hub(CA) <sub>3</sub>	2085.5	9•Cl <sub>2</sub> <sup>=</sup>	C <sub>234</sub> H <sub>345</sub> N <sub>33</sub> O <sub>30</sub>	4100.6	4100.2 6500

titrations with methanol and GPC traces. In six of these nine cases, a peak corresponding to the expected mass is observed.

No peak corresponding to the ion with an  $m/z$  ratio of aggregates 1 or 2 is observed in concentrations up to 100mM; these complexes are the least stable that we have explored.

The stoichiometry of the parent ion observed for aggregate 3 is  $2\text{flex}(\text{M})_3 \cdot 6\text{CA} \cdot \text{Cl}^-$ . No ion was observed that corresponded to  $\text{flex}(\text{M})_3 \cdot 3\text{CA} \cdot \text{Cl}^-$ . It is unclear whether 3 exists as predicted ( $\text{flex}(\text{M})_3 \cdot 3\text{CA}$ ) and its stability under the conditions used for mass spectroscopy depends on the presence of an additional equivalent of  $\text{flex}(\text{M})_3 \cdot 3\text{CA}$  (yielding the observed  $2\text{flex}(\text{M})_3 \cdot 6\text{CA}$ ), or whether, instead, a structure incorporating two  $\text{flex}(\text{M})_3$  units exists in the gas phase.

Figure 2 shows the mass spectrum of aggregate 4 ( $\text{hub}(\text{M})_3 \cdot 3\text{CA}$ ). This aggregate appears as the major ion (due to association with  $\text{Cl}^-$ ) at  $m/z$  2767.  $\text{Hub}(\text{M})_3 \cdot \text{Cl}^-$  appears at  $m/z$  2128. No peaks corresponding to the  $\text{hub}(\text{M})_3$  associated with more than three molecules of CA are observed. The peak at  $m/z$  2554 corresponds to the trismelamine and two molecules of CA. It is unlikely that this species is present in significant concentrations in solution: a range of techniques offer no evidence that such a species is present. Peaks with  $m/z$  greater than  $m/z$  2767 correspond to non-specific aggregates comprising molecules of CA and molecule(s) of  $\text{hub}(\text{M})_3$ . A majority of the peaks in the spectrum are assigned in Table 3.

In an effort to determine whether the ion at  $m/z$  2554 corresponded to a species present in solution or was the result of dissociation during ionization, we attempted collision-induced dissociation (CID) experiments. CID of the selected  $\text{Cl}^-$ -bound 4 resulted in the appearance of two products,  $\text{CA} \cdot \text{Cl}^-$  and  $\text{Hub}(\text{M})_3 \cdot \text{Cl}^-$ . No other species were observed. This observation is consistent with our belief that the aggregate is highly stable only when the components are in 1:3

Figure 2. The mass spectrum of **4** using electrospray ionization.



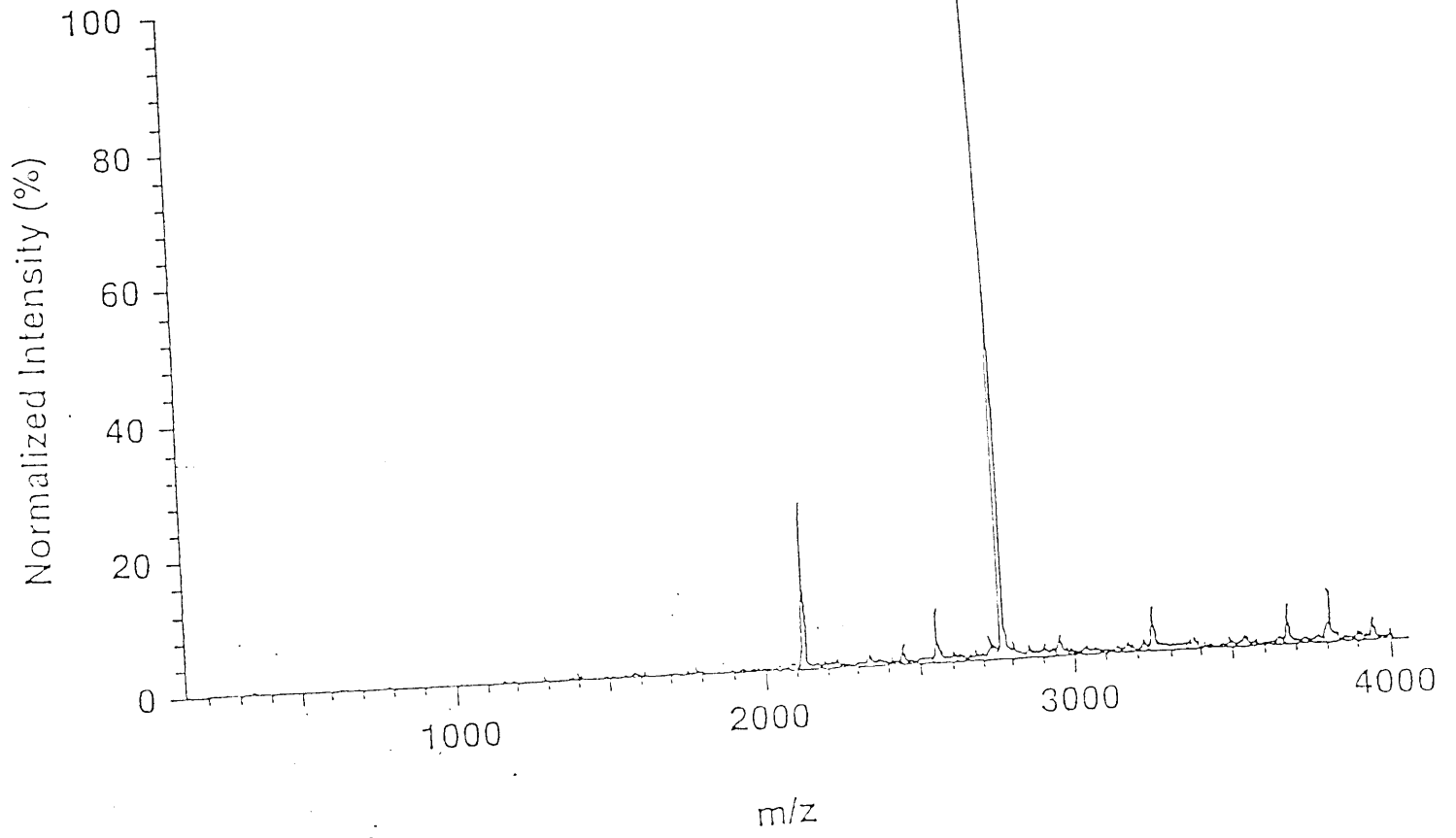


Table 3. Assignment of the major and minor peaks in the mass spectrum of 4. All peaks with a normalized intensity greater than 2% can be identified. The peak at  $m/z$  3679 is not labeled in Figure 2.

Ion ( $m/z$ )	Composition	Normalized Intensity (%)
2128.9	hub(M) <sub>3</sub> •Cl-	25
2555.0	hub(M) <sub>3</sub> •CA <sub>2</sub> •Cl-	10
2768.1	hub(M) <sub>3</sub> •CA <sub>3</sub> •Cl-	100
2955.3	(hub(M) <sub>3</sub> ) <sub>2</sub> •CA <sub>6</sub> •Ph <sub>4</sub> PCl•Cl-	5
3145.0	(hub(M) <sub>3</sub> ) <sub>2</sub> •CA <sub>6</sub> •2Ph <sub>4</sub> PCl•2Cl-	5
3253.2	(hub(M) <sub>3</sub> ) <sub>4</sub> •CA <sub>6</sub> •3Cl-	4
3679.7	(hub(M) <sub>3</sub> ) <sub>4</sub> •CA <sub>12</sub> •3Cl-	3
3813.5	(hub(M) <sub>3</sub> ) <sub>3</sub> •CA <sub>6</sub> •2Cl-	5

stoichiometry. We suggest that the peak at 2554 is from *non-specific* association. Additional work to examine the nature of this "aggregate" and other large species observed in the mass spectrum is underway.

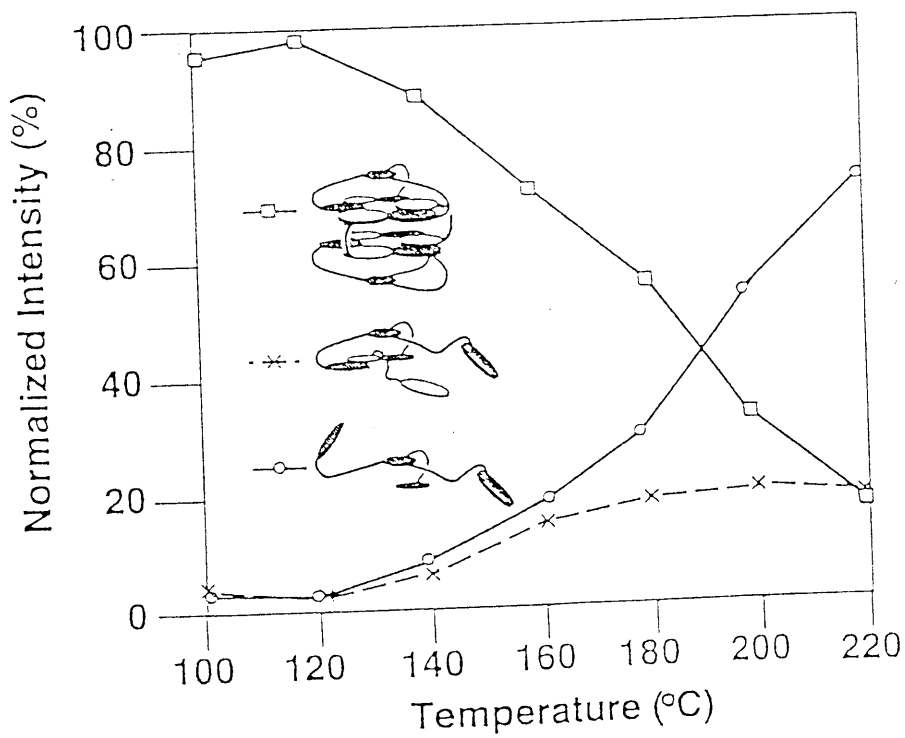
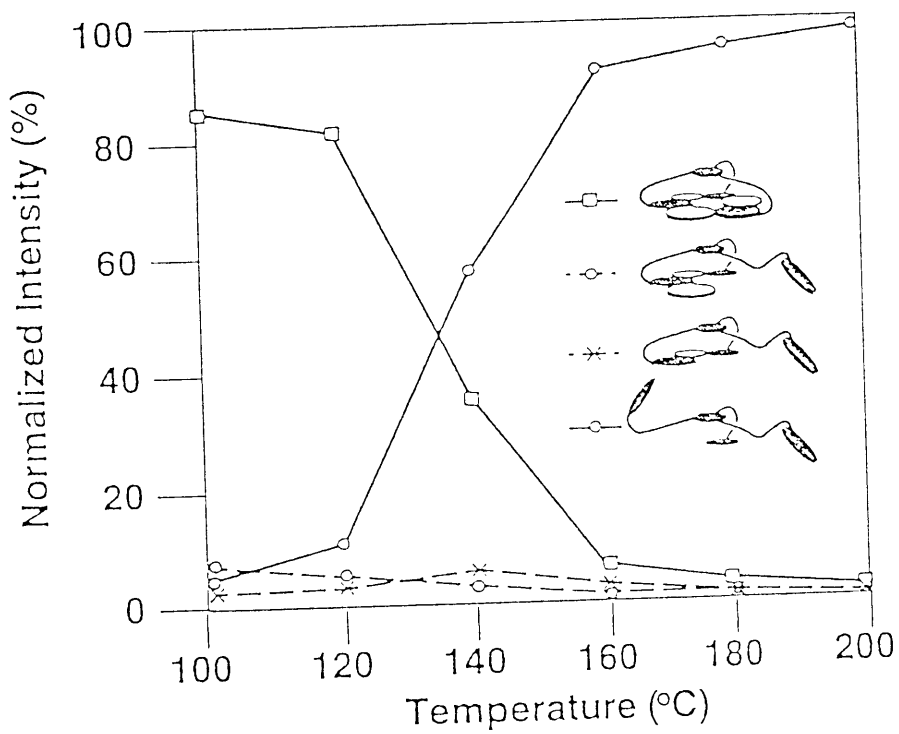
Aggregates 5 - 9 all yielded ions corresponding to the structures proposed previously. A summary of all ions is shown in Table 2.

**Thermal Dissociation.** Thermal dissociation experiments were performed on 4 and 7 to determine whether ESI-MS could be used to determine the relative stabilities of these aggregates. The thermal dissociation curves shown in Figure 3 are obtained by recording the mass spectra of an aggregate while heating the interface capillary over a range of temperatures. Aggregates of higher stability should give molecular ions at higher temperatures than aggregates which are less stable. The difference in crossing temperatures (the temperature at which the molecular ion appears at 50% its initial intensity) for 4 and 7 is approximately 50° C. While the magnitude of the temperature differences between temperatures are dependent in part on the calibration of the instrument and the design of the experiment, respectively, the relative values are as predicted.

**Charge Carriers.** Obtaining molecular weights of hydrogen-bonded aggregates using mass spectrometry has been problematic due primarily to the sensitivity of these aggregates to polar and protic solvents. Recent work by Lehn -- in which he covalently attaches a crown ether to individual subunits -- allows him to obtain molecular weights in the presence of the appropriate cation.<sup>2k</sup> The use of chloroform-soluble Ph<sub>4</sub>PCl *may* be a more general solution to this problem. Ph<sub>4</sub>PCl produces matrix peaks that are useful for calibration. Lehn's strategy, however, has produced peaks for aggregates that are very similar in structure to 1: his technique is more sensitive, but requires synthetic effort.

Figure 3. Thermal dissociation curves of 4 and 7. The crossing temperature of 7 than that for 4 suggesting that 7 is more stable.

Figure 3. Thermal Dissociation Curves of 4 and 7.



The ability to obtain accurate masses for these non-covalent aggregates -- a representative sampling of those we have previously described -- from chloroform solutions using  $\text{Ph}_4\text{P}^+\text{Cl}^-$  suggests that ESI-MS may be applicable to the characterization of a variety of host-guest complexes. Utilizing mass spectrometry to obtain relative stabilities of aggregates may also be valuable for the comparison of aggregates that are not amenable to more traditional assays of stability. The aggregates for which it yields useful results in these studies are relatively stable; lower-stability aggregates (e.g. 1 - 3) give either unexpected results or no identifiable ions with the aggregates. The possible restriction to stable aggregates notwithstanding, ESI-MS should be welcome in the molecular recognition community as a new technique capable of determining stoichiometry, mass, or relative stabilities of large aggregates in organic solutions.

**Experimental.** Spectra were obtained on a Finnegan TSQ7000 triple quadrupole MS with a mass range of  $m/z$  4100 and typical resolution of 1  $m/z$ . Aggregates were dissolved in chloroform at 1mM with 5mM  $\text{Ph}_4\text{P}^+\text{Cl}^-$ . Analyses was done in negative ion mode.

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## References

1. Whitesides, G. M.; Simanek, E.E.; Mathias, J.P.; Seto, C.T.; Chin, D.N.; Mammen, M.; Gordon, D.M. *Acc. Chem. Res.* **1995**, *28*, 37.

2. (a) Suizdak, G.; Krebs, J.F.; Benkovic, S.J.; Dyson, H.J. *J. Am. Chem. Soc.* 1994, 116, 7937.
- (b) Suizdak, G.; Ichikawa, Y.; Caulfield, T.J.; Munoz, B.; Wong, C-H.; Nicolaou, K.C. *J. Am. Chem. Soc.* 1993, 115, 2877. (c) Bitsch, F.; Dietrich-Buchecker, C.O.; Khemiss, A-K.; Sauvage, J.P.; Dorselaer, A.V. *J. Am. Chem. Soc.* 1991, 113, 3024. (d) Fujita, M.; Ibukuro, F.; Hagihara, H.; Ogura, K. *Nature* 1994, 367, 720. (e) Haskins, N.J.; Saunders, M.R.; Camilleri, P. 1994, 423. (f) Gale, D. C.; Goodlett, D. R.; Light-Wahl, K. J.; Smith, R. D. *J. Am. Chem. Soc.* 1994, 116, 6027. (g) Light-Wahl, K.L.; Springer, D.L.; Winger, B.E.; Edmonds, C.G.; Camp, D.G. II; Thrall, B.D.; Smith, R.D. *J. Am. Chem. Soc.* 1993, 115, 803. (h) Goodlett, D.R.; Camp, D.G. II.; Hardin, C. C.; Corregan, M.; Smith, R.D. *Biol. Mass. Spec.* 1993, 22, 181. (i) Li, Y-T.; Hsieh, Y-L.; Henion, J.D.; Ocain, T.D.; Schiehser, G.A.; Ganem, B. *J. Am. Chem. Soc.* 1994, 116, 7487. (j) Baca, M; Kent, S. B. H. *J. Am. Chem. Soc.* 1992, 114, 3992.
- (k) Russell, K.C.; Leize, E.; Dorsselaer, A.V.; Lehn, J.M. *Angew. Chem. Int. Ed. Eng.* 1995, 34, 209. (l) Li Y-T.; Hsieh, Y-L.; Henion, J. D.; Senko, M. W.; McLafferty, F. W.; Ganem, B. *J. Am. Chem. Soc.* 1993, 115, 8409. (m) Kondo, T.; Ueda, M.; Yoshida, K.; Titani, K.; Isobe, M.; Goto, T. *J. Am. Chem. Soc.* 1994, 116, 7457. (n) Leize, E.; Dorsselaer, A.V.; Kramer, R.; Lehn, J-M. *J. Chem. Soc. Chem. Comm.* 1993, 990.