

Electronic Supplementary Information

Aggregates of cucurbituril complexes in the gas phase

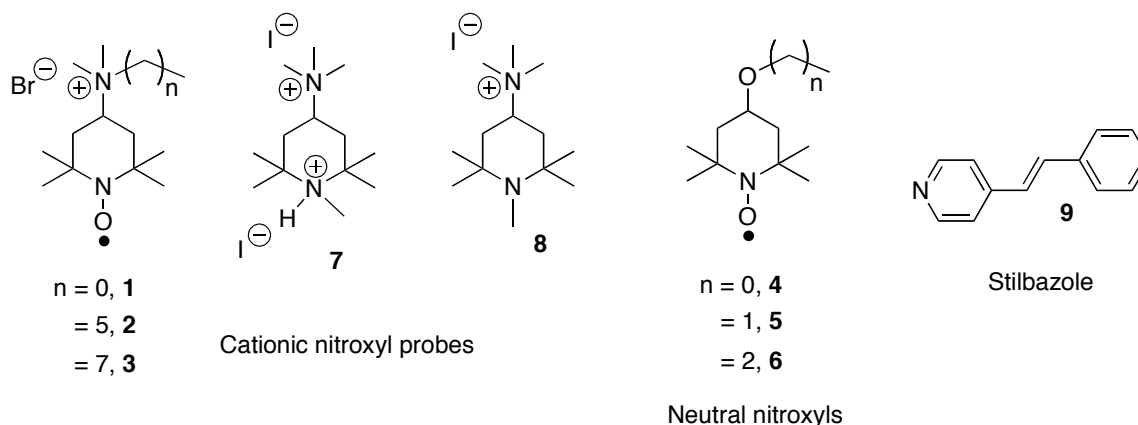
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Experimental

Materials and Methods

a) Materials, synthesis and sample preparation

Cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) were synthesized by following a published procedure.^{S1} The cationic nitroxyl probes and neutral nitroxyl derivatives (**1-7**) were synthesized according to published procedures.^{S2} Stilbazole, **9**, (Sigma-Aldrich), HBr, NaCl and KCl (Merck) were used without further purification. The samples were prepared by dissolving the cucurbituril, guest and additives in the desired amounts and allowed to equilibrate for 12 h.



References:

- (S1) Day, A.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem.* 2001, 66, 8094.
- (S2) N. Jayaraj, M. Porel, M. F. Ottaviani, M. V. S. N. Maddipatla, A. Modelli, J. P. Da Silva, B. R. Bhogala, B. Captain, S. Jockusch, N. J. Turro, V. Ramamurthy, *Langmuir*, 2009, 25, 13820.

b) Mass spectrometer details**ESI-Ion trap mass spectrometer**

The ESI-Ion trap mass spectrometric experiments were performed with a Bruker Daltonics HCT *ultra* mass spectrometer, equipped with a ESI source (Agilent) that utilized a nickel-coated glass capillary with an inner diameter of 0.6 mm. Ions were continuously generated by infusing the several aqueous solution samples into the source with a syringe pump (KdScientific, model 781100, USA) at flow rates of 4 μ L/min. The parameters were adjusted and are typically as follows: polarity: positive; capillary voltage: - 4.0 kV; capillary exit voltage: CE = 70 V; skimmer voltage: 25 V; temperature of drying gas: 300 °C. The experiments were carried out with a nebulizer gas pressure of 20 psi and a drying gas flow of 6 L/min. As discussed in the main text, the relative intensities of ions of interest could be significantly altered by changing the acceleration with the capillary exit voltage (CE 50-300V). The temperature of the drying gas (50-350 °C) does not change significantly the spectral distributions.

FT-ICR mass spectrometer

The FT-ICR mass spectrometer is a Bruker Apex Ultra (Billerica, U.S.A.) with a 7 Tesla actively shielded magnet from Magnex, Oxford U.K, equipped with an Apollo II dual source. Samples were analyzed by ESI, positive mode, with external calibration.

Additional Mass Spectra

The illustration of the aggregates is only a schematic representation. The actual configuration of the aggregates is not known.

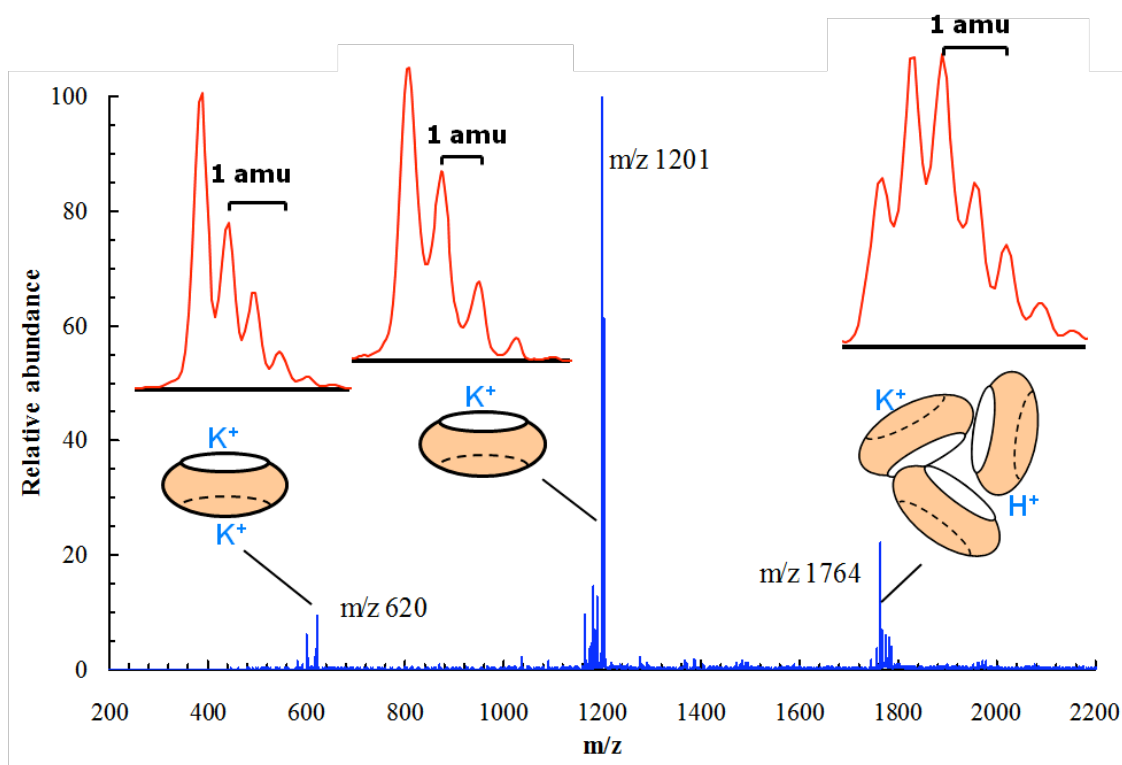


Figure S1: ESI-MS spectrum of a 70 μM aqueous solution of CB7 with KCl (40 μM) obtained under collision dissociation conditions (CE 300 V). Assignments: m/z 620: $[\text{CB7} + 2 \cdot \text{K}]^{2+}$; m/z 1201: $[\text{CB7} + \text{K}]^{+}$; m/z 1764: $[3 \cdot \text{CB7} + \text{H} + \text{K}]^{2+}$ (see Figures S7-S9). The insets show the isotope patterns. The simulation of the isotope distributions are in agreement with the experimentally observed profiles (within the mass resolution of the equipment).

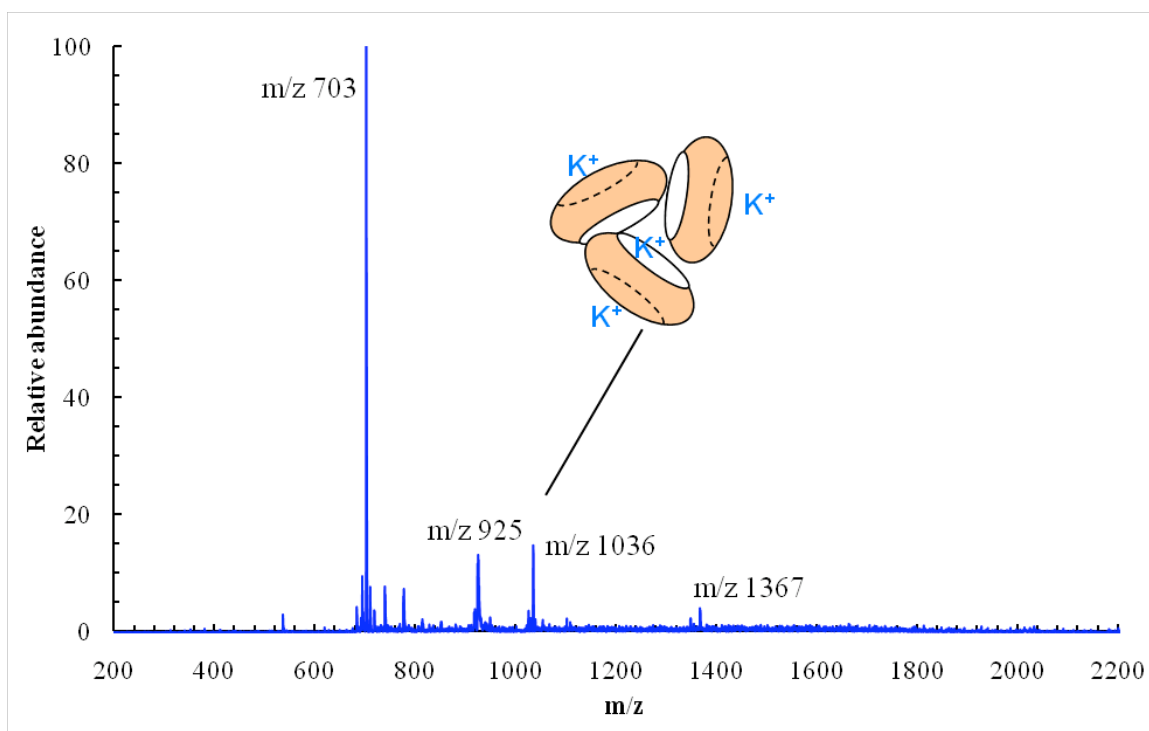


Figure S2: ESI-MS spectrum of a 10 μM aqueous solution of CB8 with KCl (100 μM) obtained under gentle ionization conditions (CE 70 V). Assignments: m/z 703: $[\text{CB8} + 2\cdot\text{K}]^{2+}$; m/z 925: $[2\cdot\text{CB8} + 3\cdot\text{K}]^{3+}$; m/z 1036: $[3\cdot\text{CB8} + 4\cdot\text{K}]^{4+}$; m/z 1367: $[\text{CB8} + \text{K}]^+$ (see Figures S10 and S11).

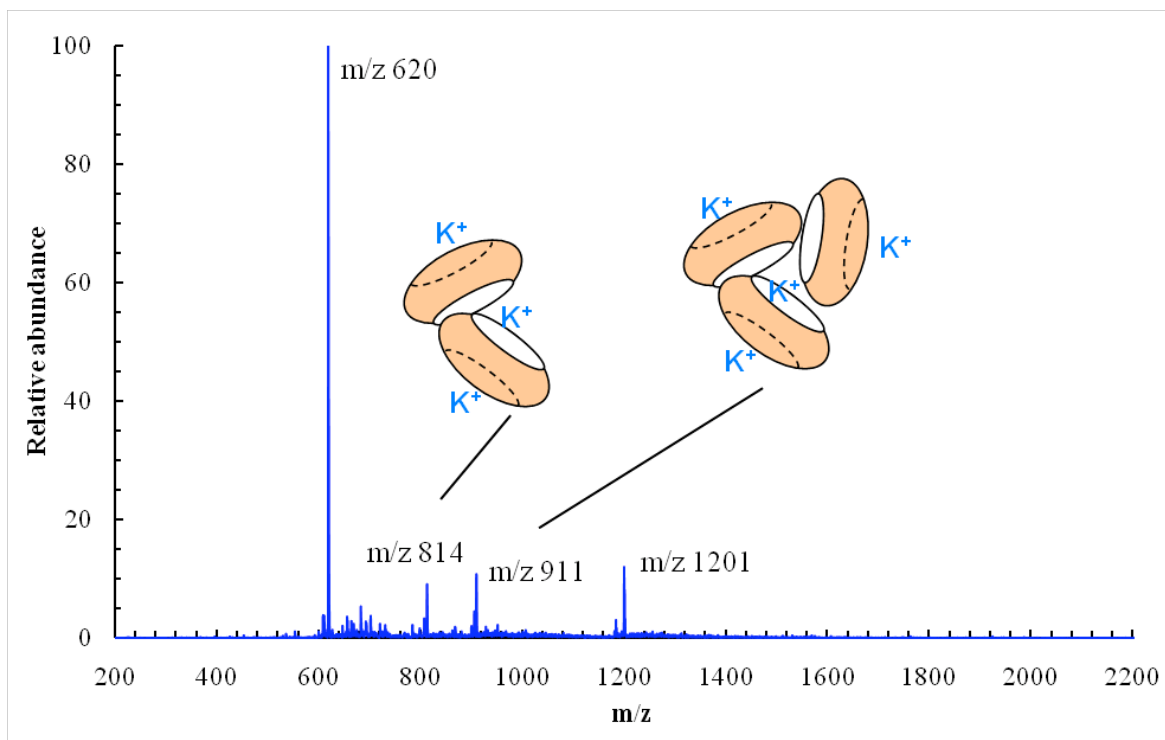


Figure S3: ESI-MS spectrum of a 10 μM aqueous solution of CB7 with KCl (100 μM) obtained under gentle ionization conditions (capillary exit 70 V). Assignments: m/z 620: $[\text{CB7} + 2\cdot\text{K}]^{2+}$; m/z 814: $[2\cdot\text{CB7} + 3\cdot\text{K}]^{3+}$; m/z 911 - $[3\cdot\text{CB7} + 4\cdot\text{K}]^{4+}$; m/z 1201: $[\text{CB7} + \text{K}]^+$ (see Figures S12 and S13).

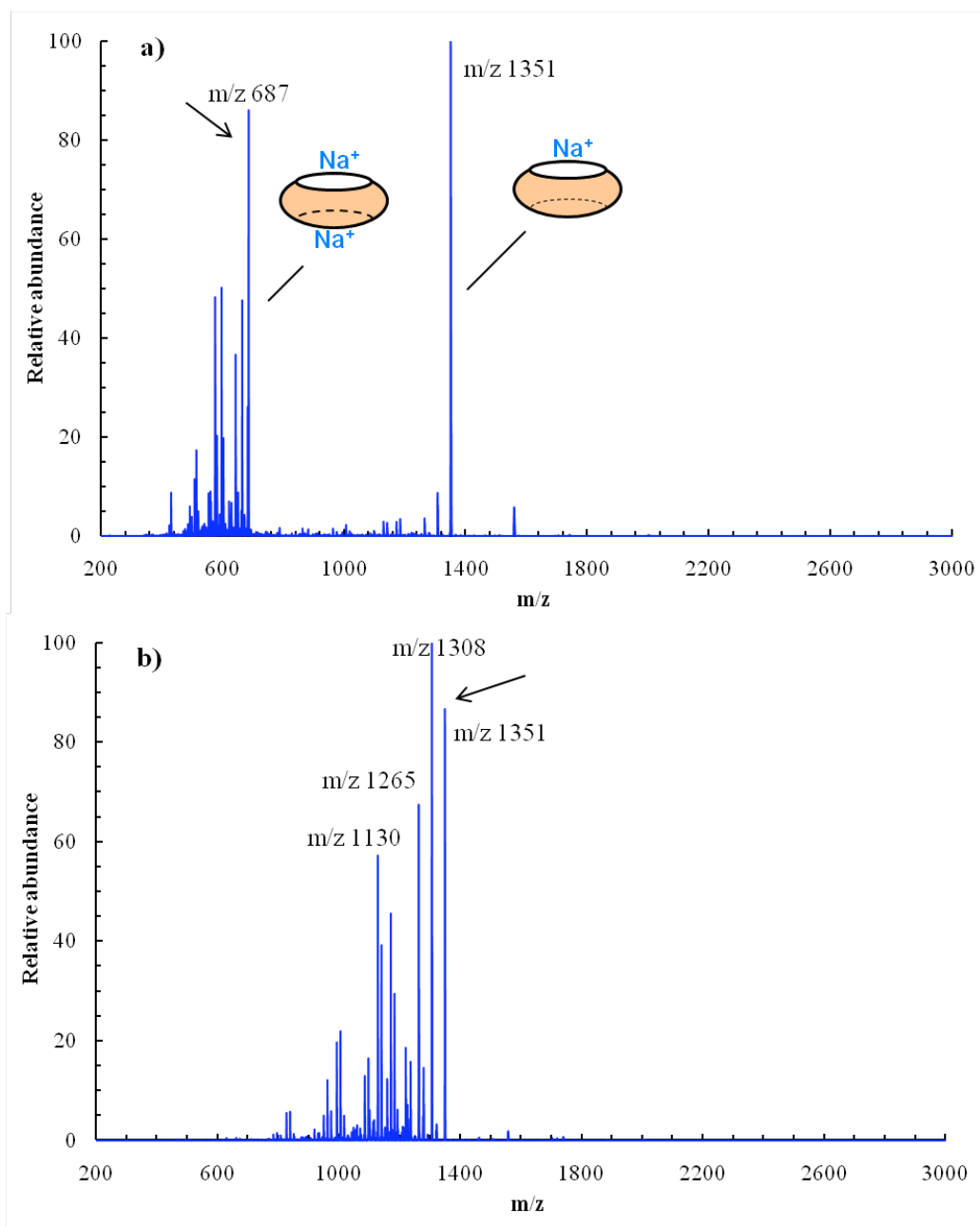


Figure S4: Consecutive fragmentations of m/z 687 (CB8) (see Figure 1). **a)** MS² 687; **b)** MS³, 687 → 1351. The arrow indicates the fragmented peak. Assignments: m/z 687: [CB8 + 2•Na]²⁺; m/z 1351: [CB8 + Na]⁺.

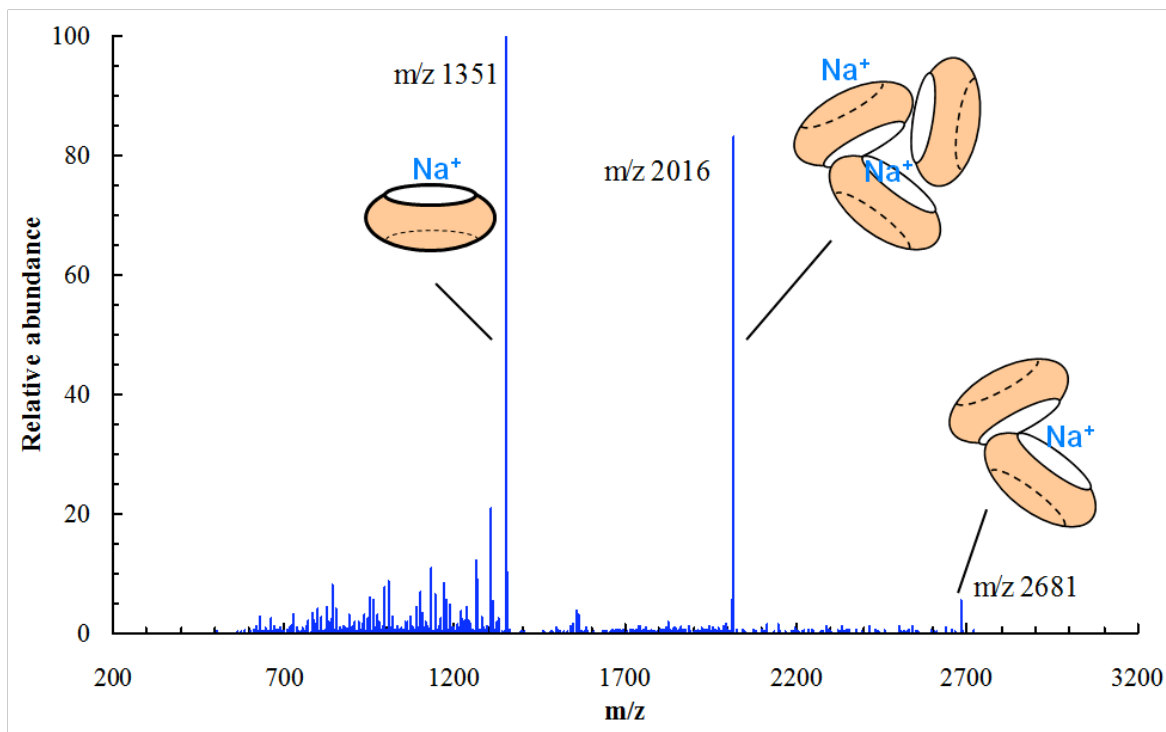


Figure S5: Fragmentation (MS^2) of m/z 2016 (CB8) (see Figure 1). The arrow indicates the fragmented peak. Assignments: m/z 1351: $[\text{CB8} + \text{Na}]^+$; m/z 2016: $[3 \cdot \text{CB8} + 2 \cdot \text{Na}]^{2+}$; m/z 2681: $[2 \cdot \text{CB8} + \text{Na}]^+$.

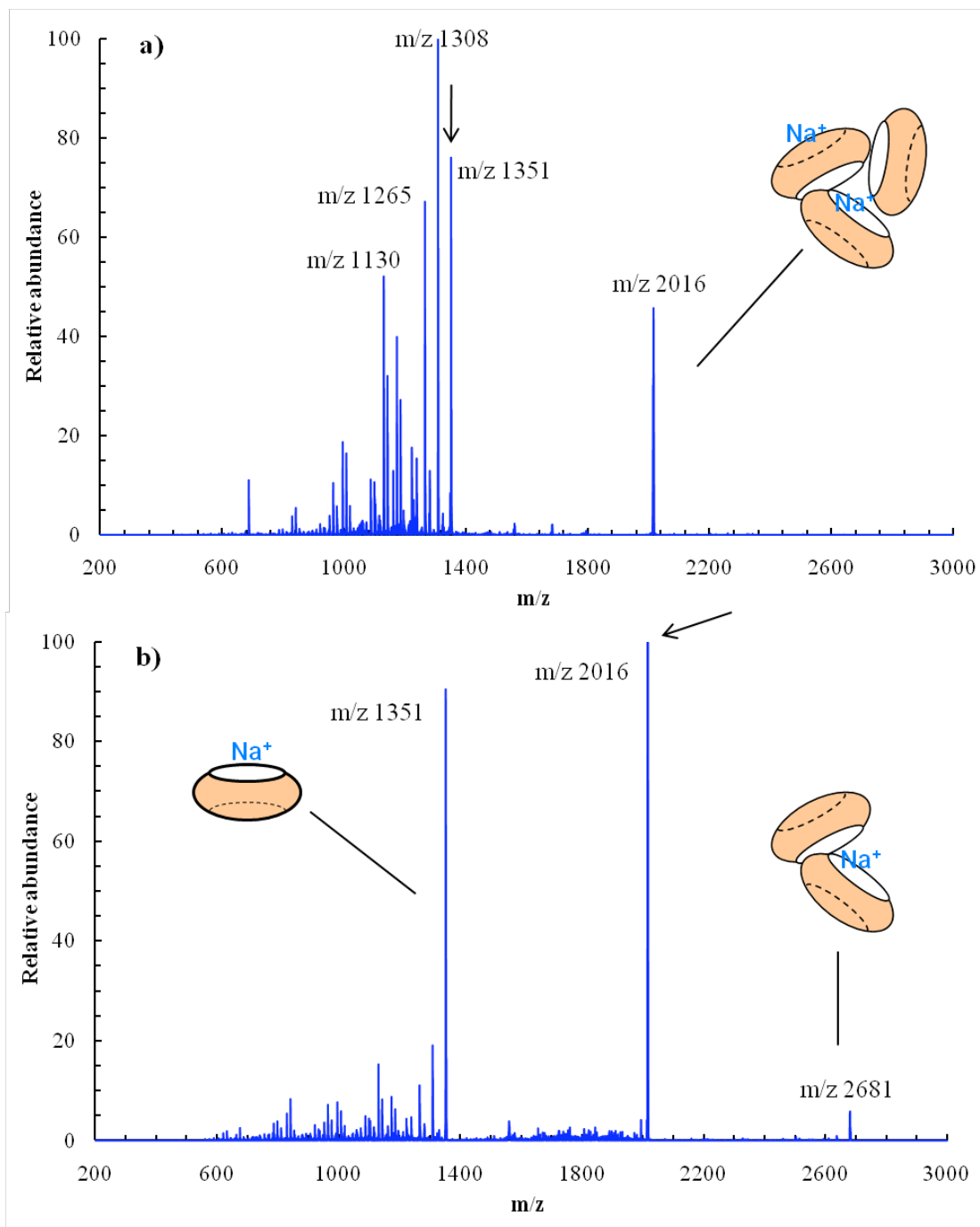


Figure S6: Consecutive fragmentations of m/z 1351 (CB8) (see Figure 1). **a)** MS² 1351; **b)** MS³, 1351 \rightarrow 2016. The arrow indicates the fragmented peak. . Assignments: m/z 1351: $[\text{CB8} + \text{Na}]^+$; m/z 2016: $[3 \cdot \text{CB8} + 2 \cdot \text{Na}]^{2+}$; m/z 2681: $[2 \cdot \text{CB8} + \text{Na}]^+$.

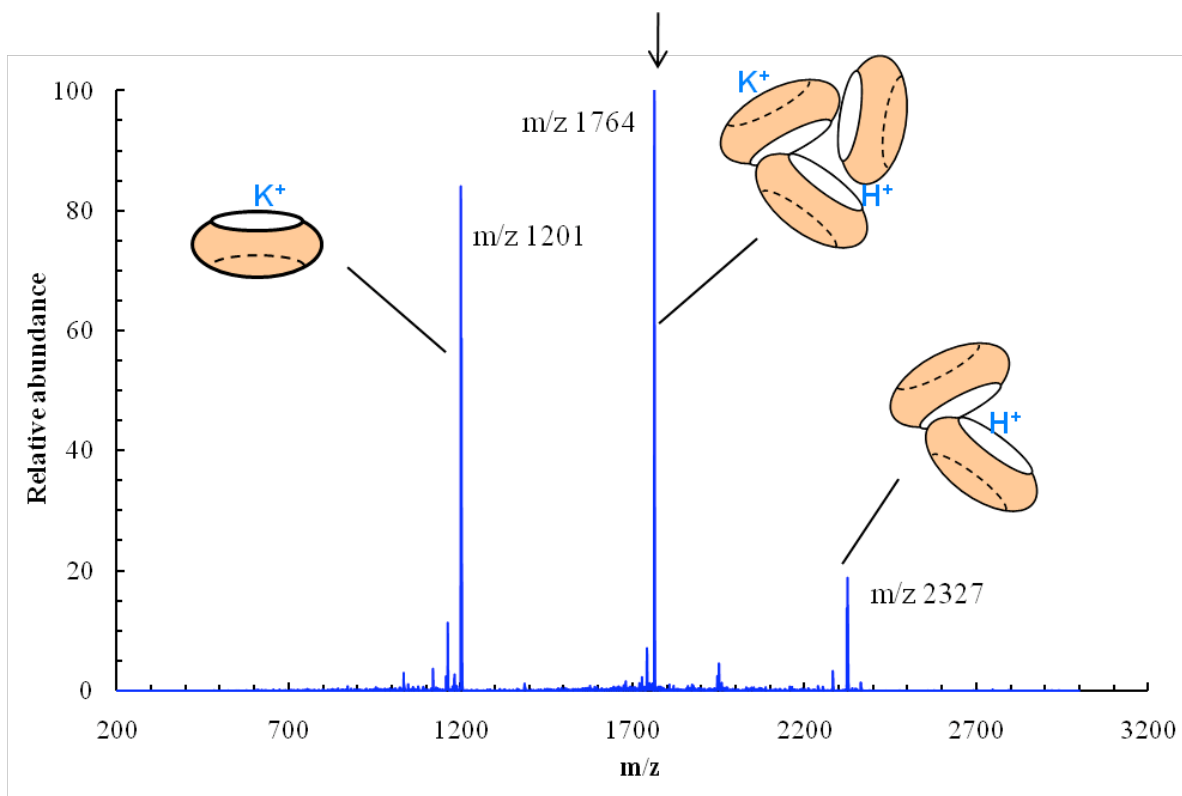


Figure S7: Fragmentation (MS^2) of m/z 1764 (CB7) (see Figure S1). The arrow indicates the fragmented peak. Assignments: m/z 1201: $[\text{CB7} + \text{Na}]^+$; m/z 1764: $[3\bullet\text{CB7} + \text{H} + \text{K}]^{2+}$; m/z 2327: $[2\bullet\text{CB7} + \text{H}]^+$.

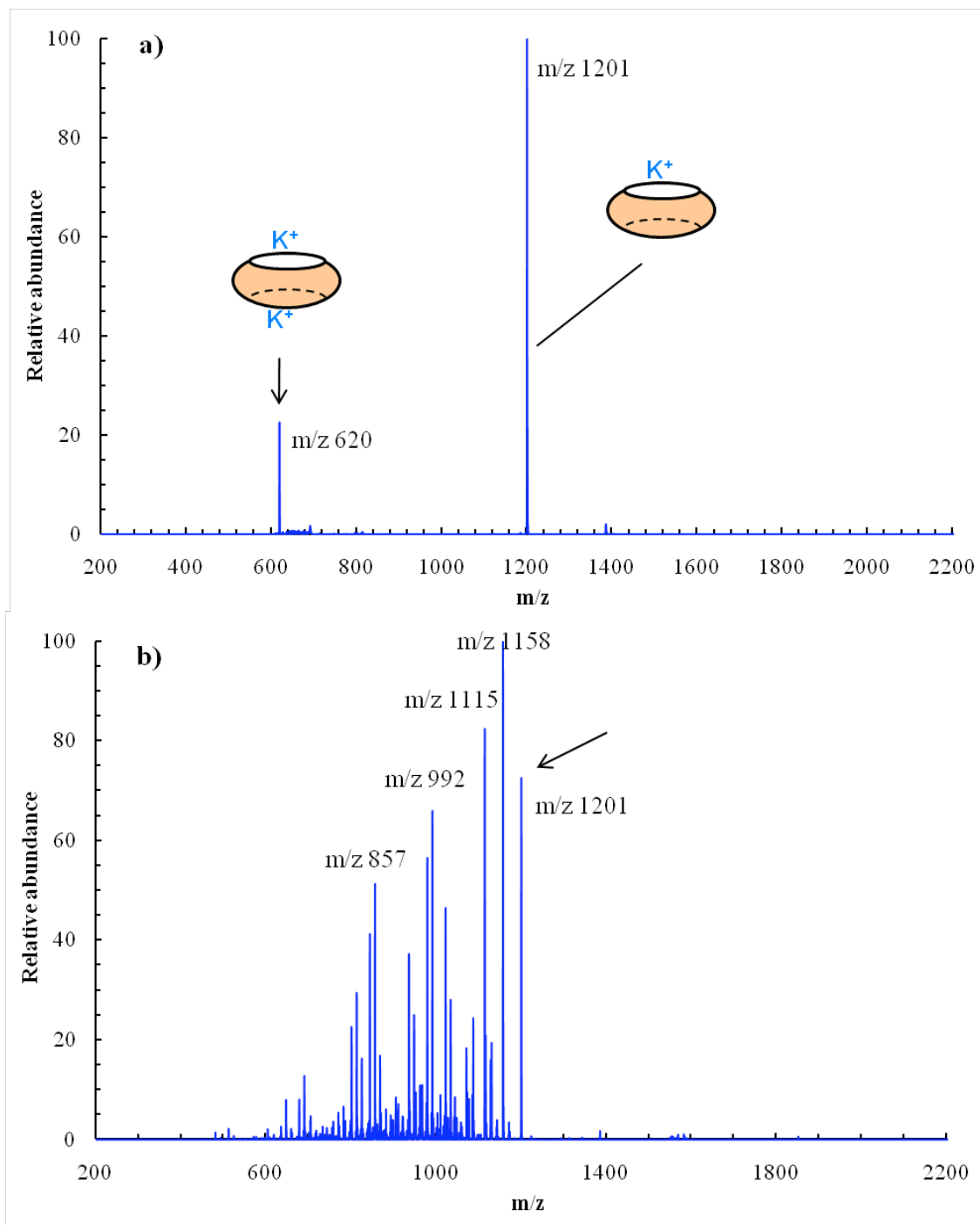


Figure S8: Consecutive fragmentations of m/z 620 (CB7) (see Figure S1). **a)** MS² 620; **b)** MS³, 620 → 1201. The arrow indicates the fragmented peak. Assignments: m/z 620: [CB7 + 2•K]⁺; m/z 1201: [CB7 + Na]⁺.

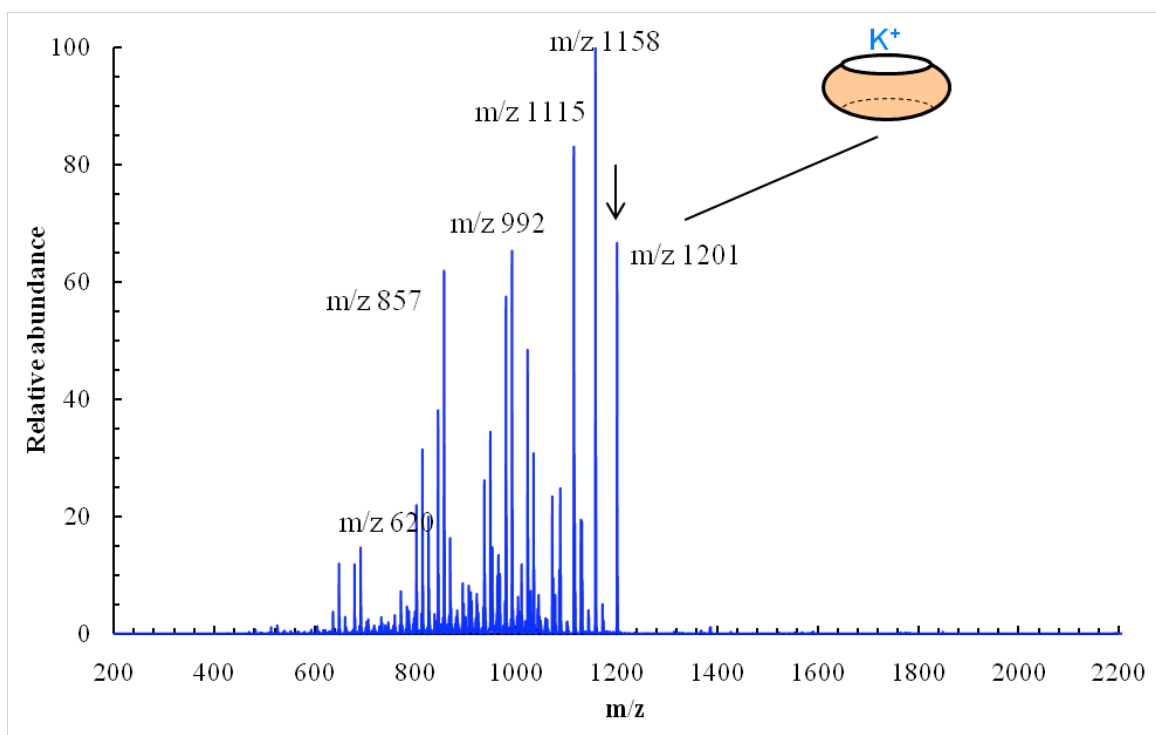


Figure S9: Fragmentation (MS^2) of m/z 1201 (CB7) (see Figure S1). The arrow indicates the fragmented peak. Assignment: m/z 1201: $[CB7 + K]^+$.

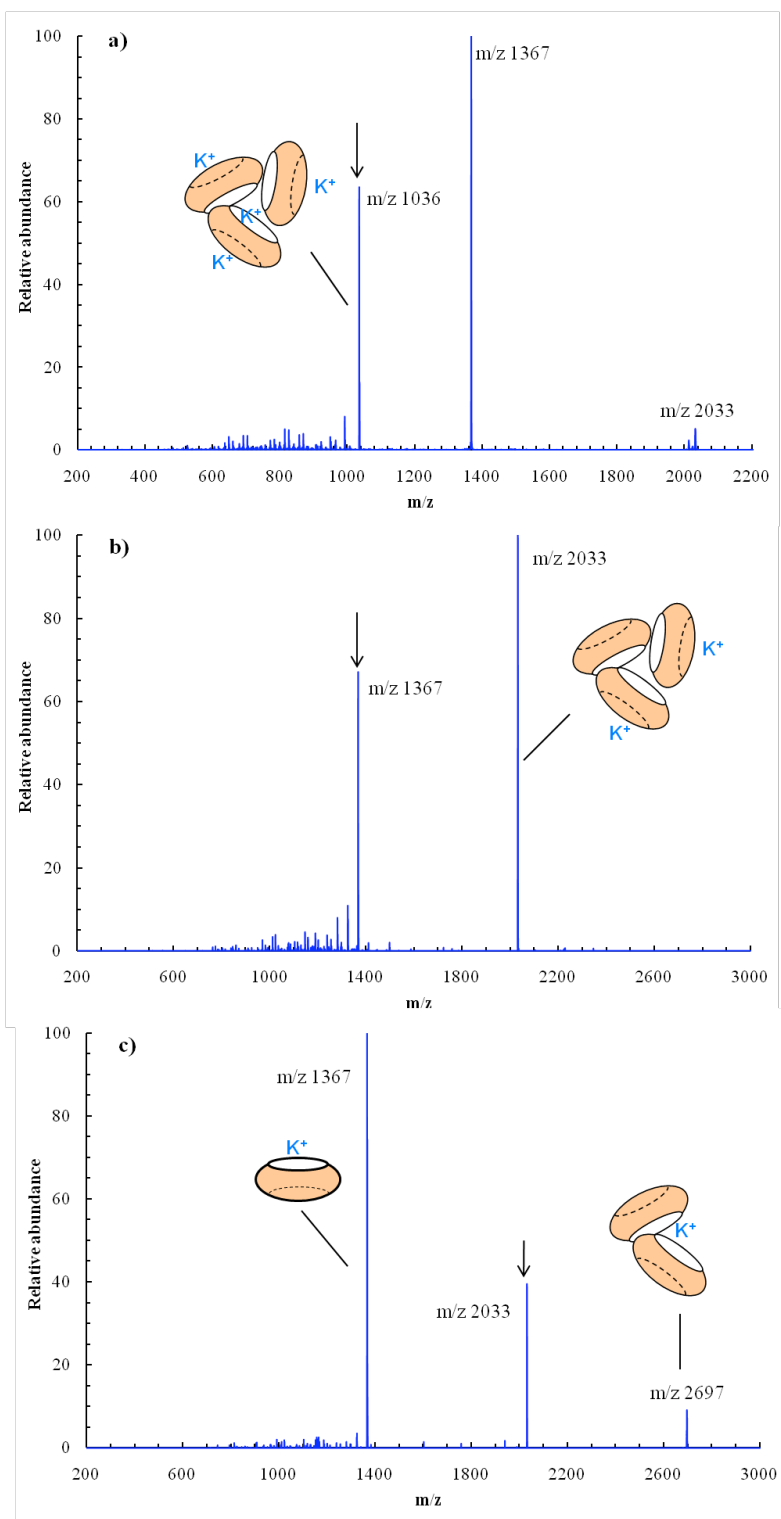


Figure S10: Consecutive fragmentations of m/z 1036 (CB8) (see Figure S2). **a)** MS² 1036; **b)** MS³, 1036 \rightarrow 2033; **c)** MS⁴, 1036 \rightarrow 1367 \rightarrow 2033. The arrow indicates the fragmented peak. Assignments: m/z 1036: $[3\cdot\text{CB8} + 4\cdot\text{K}]^{4+}$; m/z 1367: $[\text{CB8} + \text{K}]^+$; m/z 2033: $[3\cdot\text{CB8} + 2\cdot\text{K}]^{2+}$; m/z 2697: $[2\cdot\text{CB8} + \text{K}]^+$.

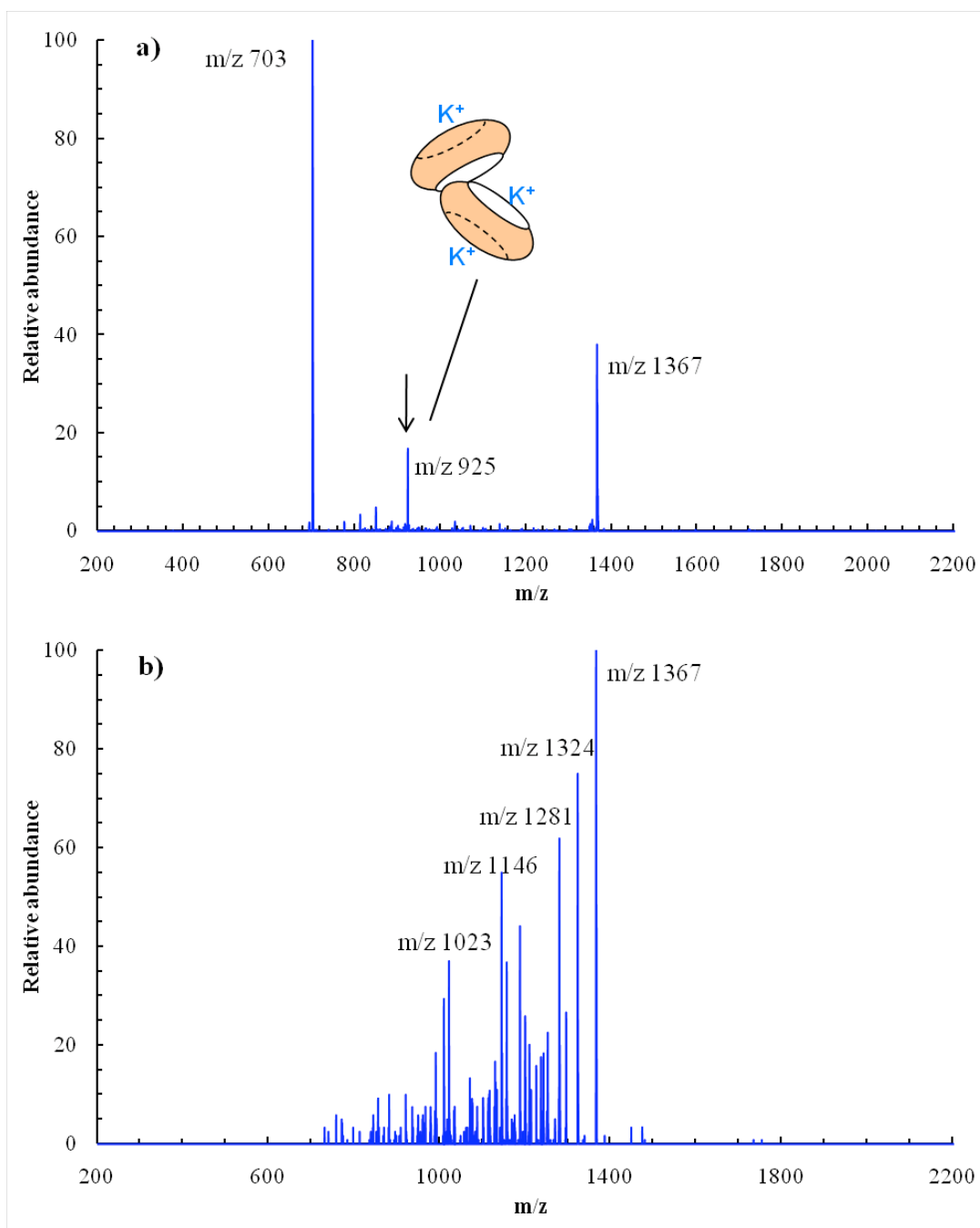


Figure S11: Consecutive fragmentations of m/z 925 (CB8) (See Figure S2). **a)** MS² 925; **b)** MS³, 925 → 1367. The arrow indicates the fragmented peak. Assignments: m/z 703: [CB8 + 2•K]²⁺; m/z 925: [2•CB8 + 3•K]³⁺; m/z 1367: [CB8 + K]⁺.

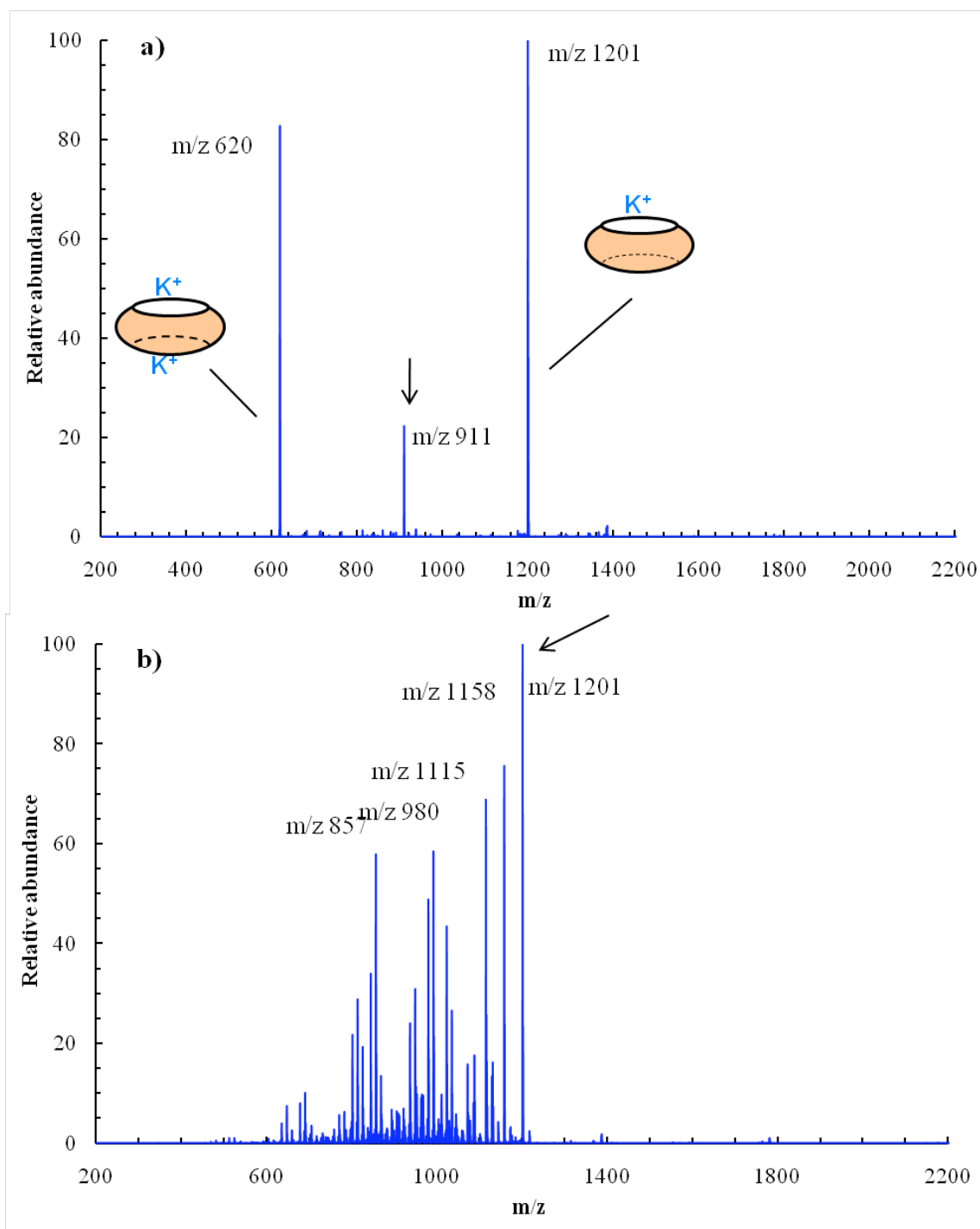


Figure S12: Consecutive fragmentations of m/z 911 (CB7) (see Figure S3). **a)** MS² 911; **b)** MS³, 911 → 1201. The arrow indicates the fragmented peak. Assignments: m/z 620: [CB7 + 2•K]²⁺; m/z 911: [3•CB7 + 4•K]⁴⁺; m/z 1201: [CB7 + K]⁺.

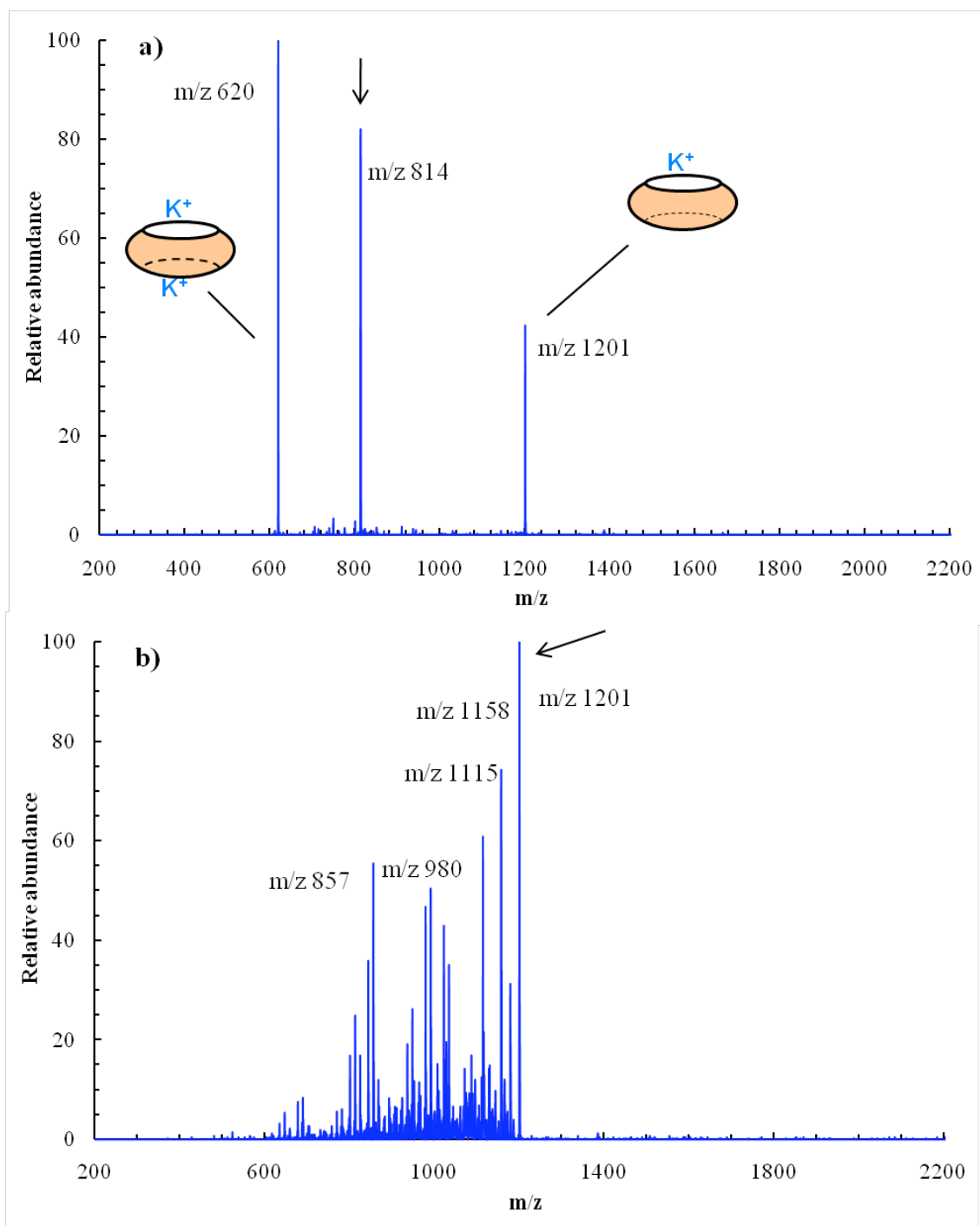


Figure S13: Consecutive fragmentations of m/z 814 (see Figure S3). **a)** MS² 814; **b)** MS³, 814 → 1201. The arrow indicates the fragmented peak. Assignments: m/z 620: [CB7 + 2•K]²⁺; m/z 814: [2•CB7 + 3•K]³⁺; m/z 1201: [CB7 + K]⁺.

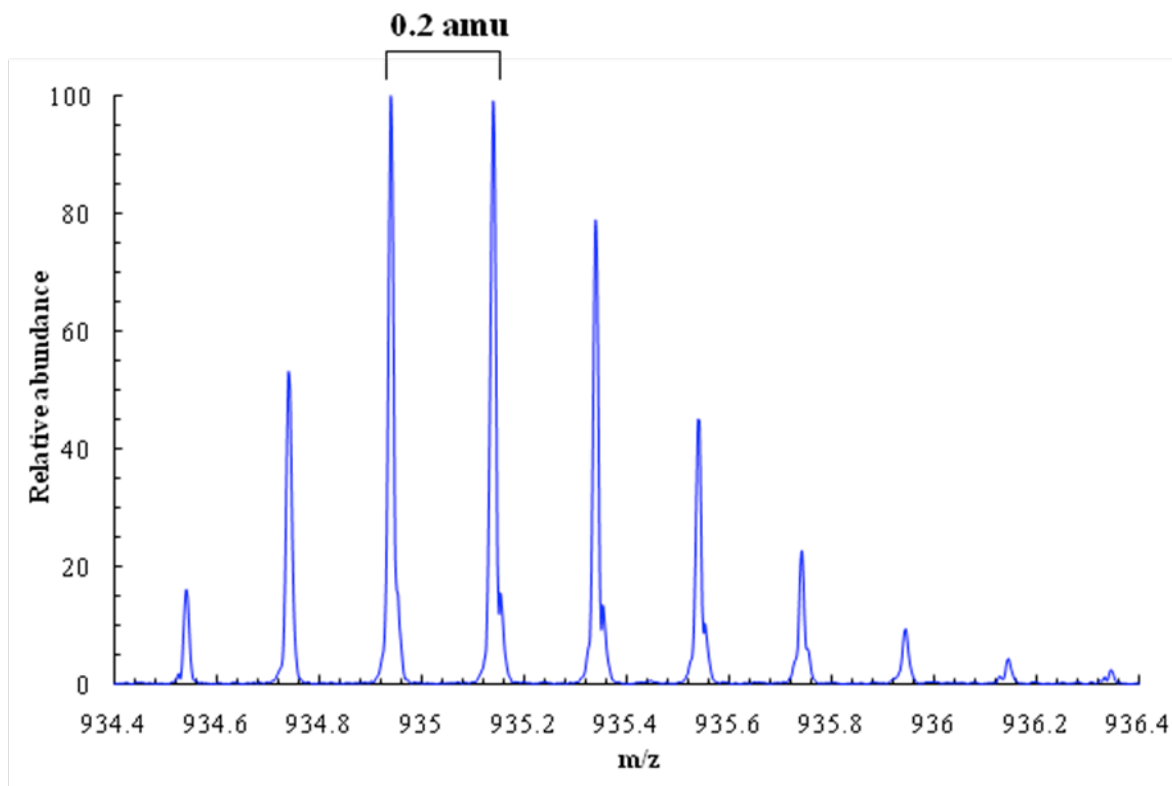


Figure S14: ESI-FT-ICR mass spectrum (full scan) of an aqueous solution of 1@CB8 (50 μM) with NaCl (50 μM) (CE 70 V) near m/z 935 ($[3\cdot\text{CB8}+3\cdot\mathbf{1}+2\cdot\text{Na}]^{5+}$). The isotope pattern shows a spacing of $\Delta m = 0.2$ amu, in agreement with a penta-charged ion.

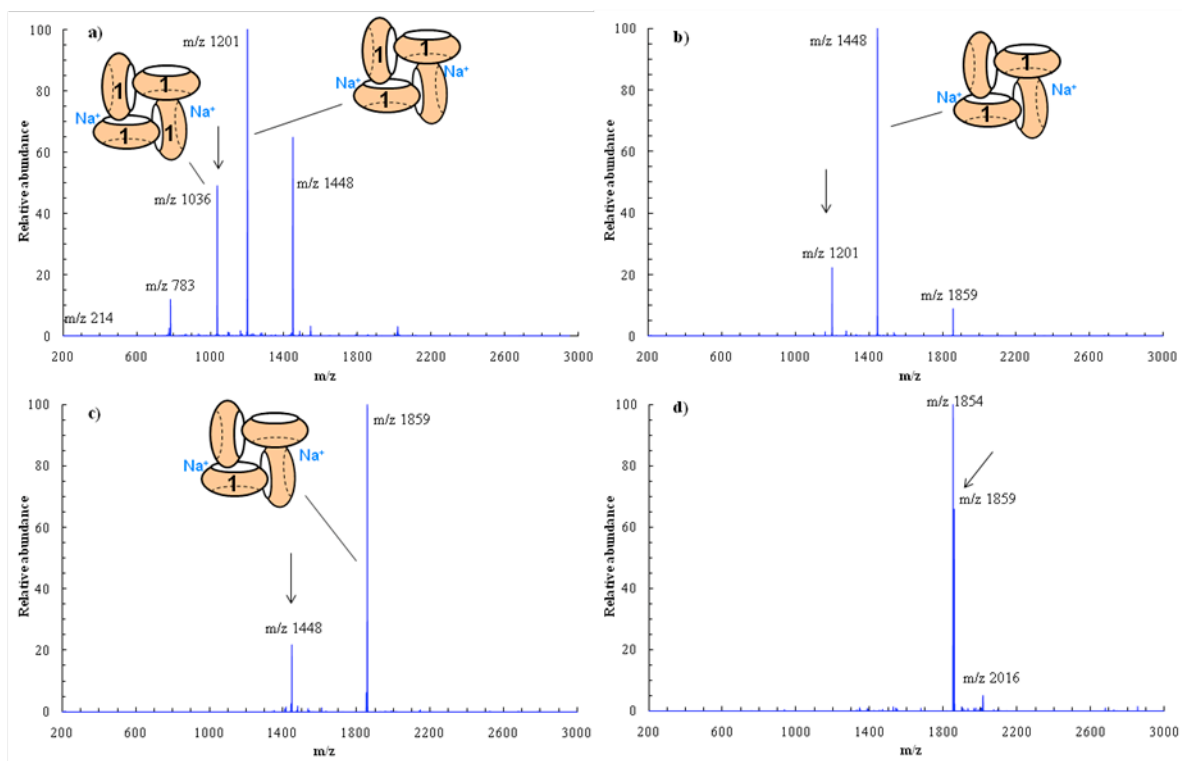


Figure S15: Consecutive fragmentations of m/z 1036 (**1**@CB8) (**a**) to **d**) in the presence of NaCl (see Figure 2). **a**) MS^2 1036; **b**) MS^3 , 1036 \rightarrow 1201; **c**) MS^4 , 1036 \rightarrow 1201 \rightarrow 1448; **d**) MS^5 1036 \rightarrow 1201 \rightarrow 1448 \rightarrow 1859. The arrows indicate the fragmented peak. Assignments: m/z 214: **1**; m/z 783: [CB8 + **1** + Na] $^{2+}$; m/z 1036: [4•CB8 + 4•**1** + 2•Na] $^{6+}$; m/z 1201: [4•CB8 + 3•**1** + 2•Na] $^{5+}$; m/z 1448: [4•CB8 + 2•**1** + 2•Na] $^{4+}$; m/z 1859: [4•CB8 + **1** + 2•Na] $^{3+}$; m/z 1854: [4•CB8 + **1**(-15) + 2•Na] $^{3+}$; m/z 2016: [3•CB8 + 2•Na] $^{2+}$.

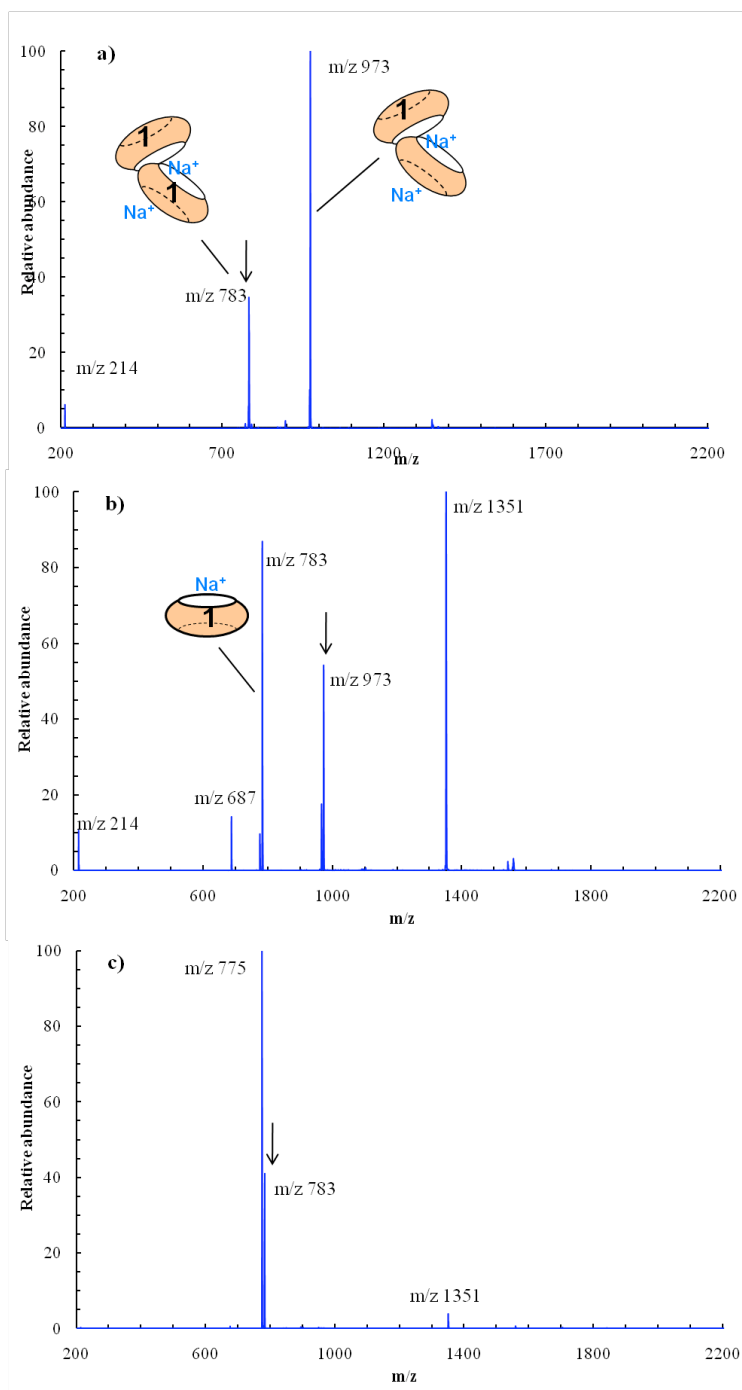


Figure S16: Consecutive fragmentations of m/z 783 (1@CB8) in the presence of NaCl (see Figure 2). **a)** MS² 783; **b)** MS³, 783 → 973; **c)** MS⁴, 783 → 973 → 783. The arrows indicate the fragmented peak. Assignments: m/z 214: **1**; m/z 214: **1**; m/z 687: [CB8 + 2•Na]²⁺; m/z 775: [CB8 + **1**(-15) + 2•Na]²⁺; m/z 783: [2•CB8 + 2•**1** + 2•Na]⁴⁺ or [CB8 + **1** + Na]²⁺; m/z 973: [2•CB8 + **1** + 2•Na]³⁺; m/z 1351: [CB8 + Na]⁺.

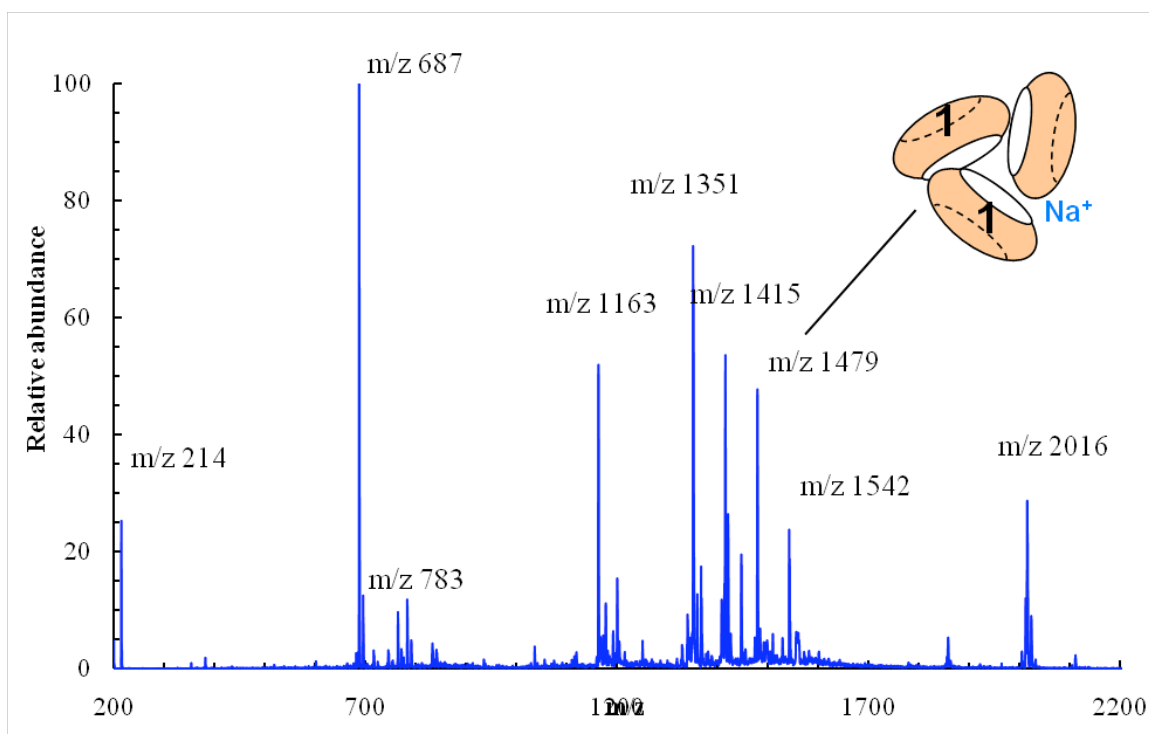


Figure S17: ESI-MS spectrum of a $\sim 50 \mu\text{M}$ aqueous solution of **1**@CB8 complexes with NaCl ($\sim 100 \mu\text{M}$) under collisional dissociation conditions (CE 300 V). Assignments: m/z 214: **1**; 687: $[\text{CB8} + 2 \cdot \text{Na}]^{2+}$; m/z 1163: $[3 \cdot \text{CB8} + 3 \cdot \text{1} + \text{Na}]^{4+}$; m/z 1351: $[\text{CB8} + \text{Na}]^{+}$; m/z 1415: $[3 \cdot \text{CB8} + \text{1} + 2 \cdot \text{Na}]^{3+}$; m/z 1479: $[3 \cdot \text{CB8} + 2 \cdot \text{1} + \text{Na}]^{3+}$; m/z 1542: $[\text{CB8} + \text{1}]^{+}$; m/z 2016: $[3 \cdot \text{CB8} + 2 \cdot \text{Na}]^{2+}$.

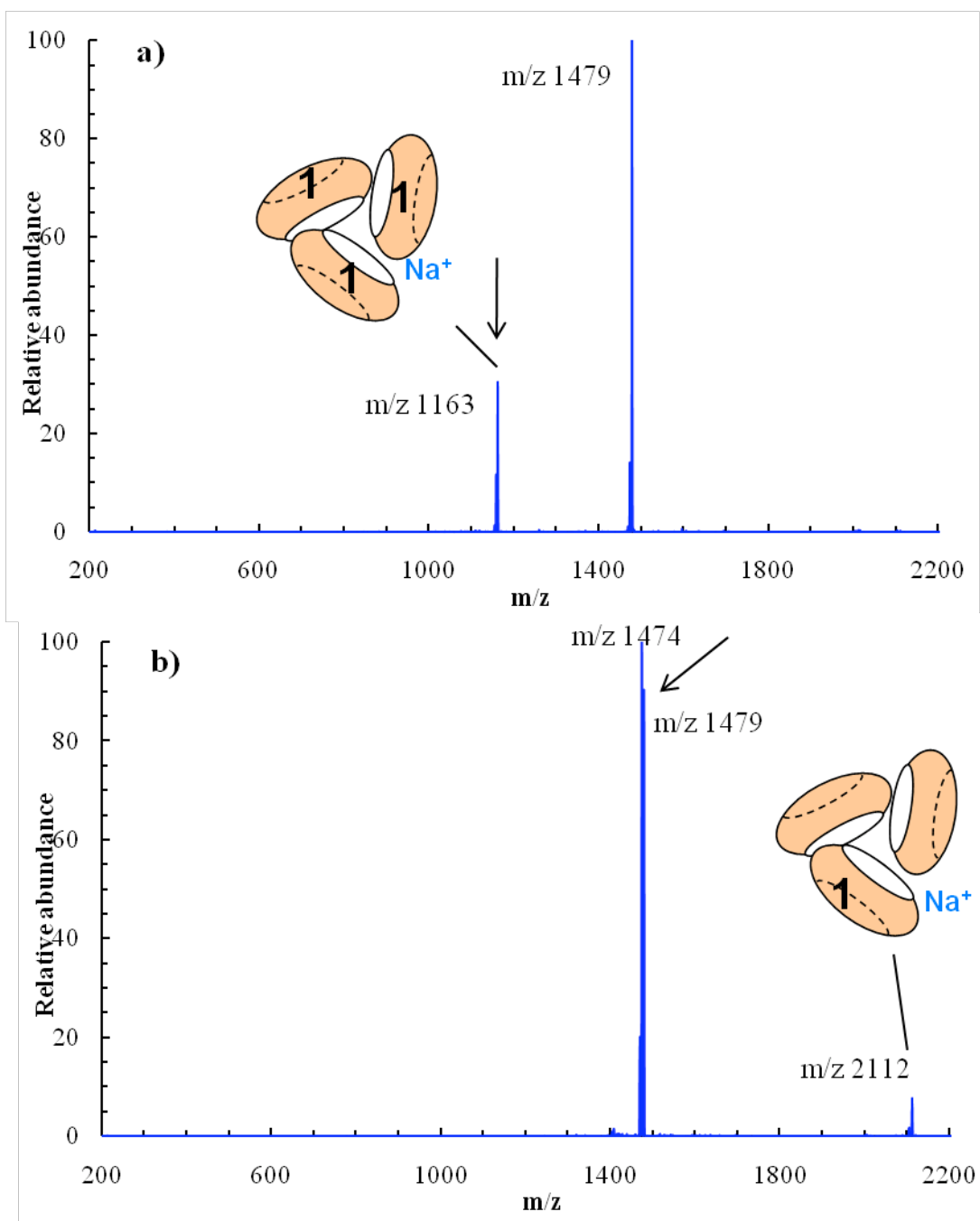


Figure S18: Consecutive fragmentations of m/z 1163 (**1**@CB8) (see Figure S17). **a)** MS² 1163; **b)** MS³, 1163 → 1479: The arrows indicate the fragmented peak. Assignments: m/z 1163 - [3•CB8 + 3•**1** + Na]⁴⁺; m/z 1479 - [3•CB8 + 2•**1** + Na]³⁺; m/z 1474 - [3•CB8 + 2•**1**(-15) + Na]³⁺; m/z 2112 - [3•CB8 + **1** + Na]²⁺.

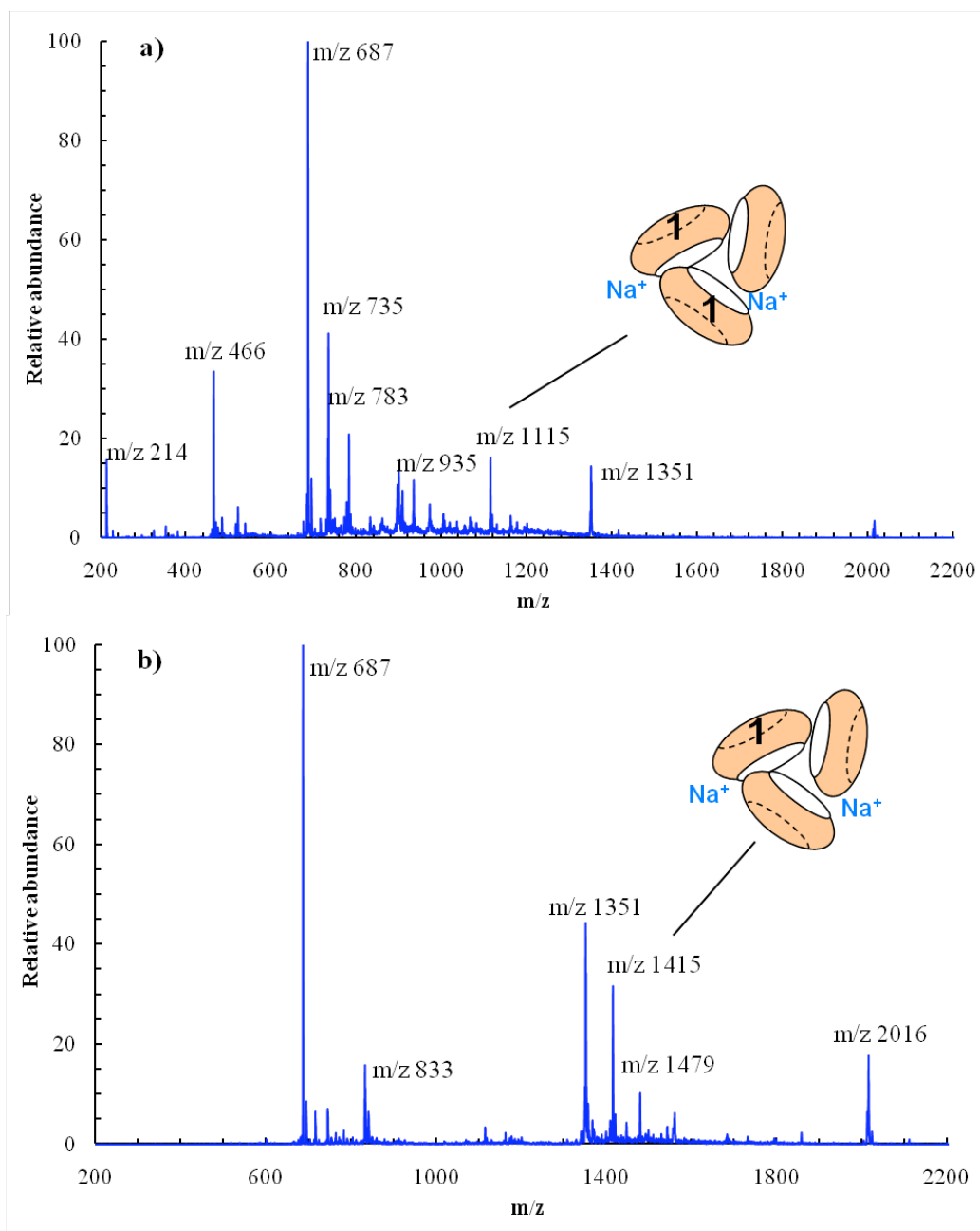


Figure S19: ESI-MS spectrum of an aqueous solution of **1** and CB8 in a ratio 1:5 with NaCl ($\sim 100 \mu\text{M}$) with CE 70 V (**a**) and 300 V (**b**). Assignments: m/z 214: **1**; m/z 466: $[\text{CB8} + 3\cdot\text{Na}]^{3+}$; m/z 687: $[\text{CB8} + 2\cdot\text{Na}]^{2+}$; m/z 735: $[2\cdot\text{CB8} + \mathbf{1} + 3\cdot\text{Na}]^{4+}$; m/z 783: $[\text{CB8} + \mathbf{1} + \text{Na}]^{2+}$; m/z 833: $[\text{CB8}(\text{NaCl})_5 + 2\cdot\text{Na}]^{2+}$; m/z 935: $[3\cdot\text{CB8} + 3\cdot\mathbf{1} + 2\cdot\text{Na}]^{5+}$; m/z 1115: $[3\cdot\text{CB8} + 2\cdot\mathbf{1} + 2\cdot\text{Na}]^{4+}$; m/z 1351: $[\text{CB8} + \text{Na}]^{+}$; 1415: $[3\cdot\text{CB8} + \mathbf{1} + 2\cdot\text{Na}]^{3+}$; m/z 1479: $[3\cdot\text{CB8} + 2\cdot\mathbf{1} + \text{Na}]^{3+}$; 2016: $[3\cdot\text{CB8} + 2\cdot\text{Na}]^{4+}$.

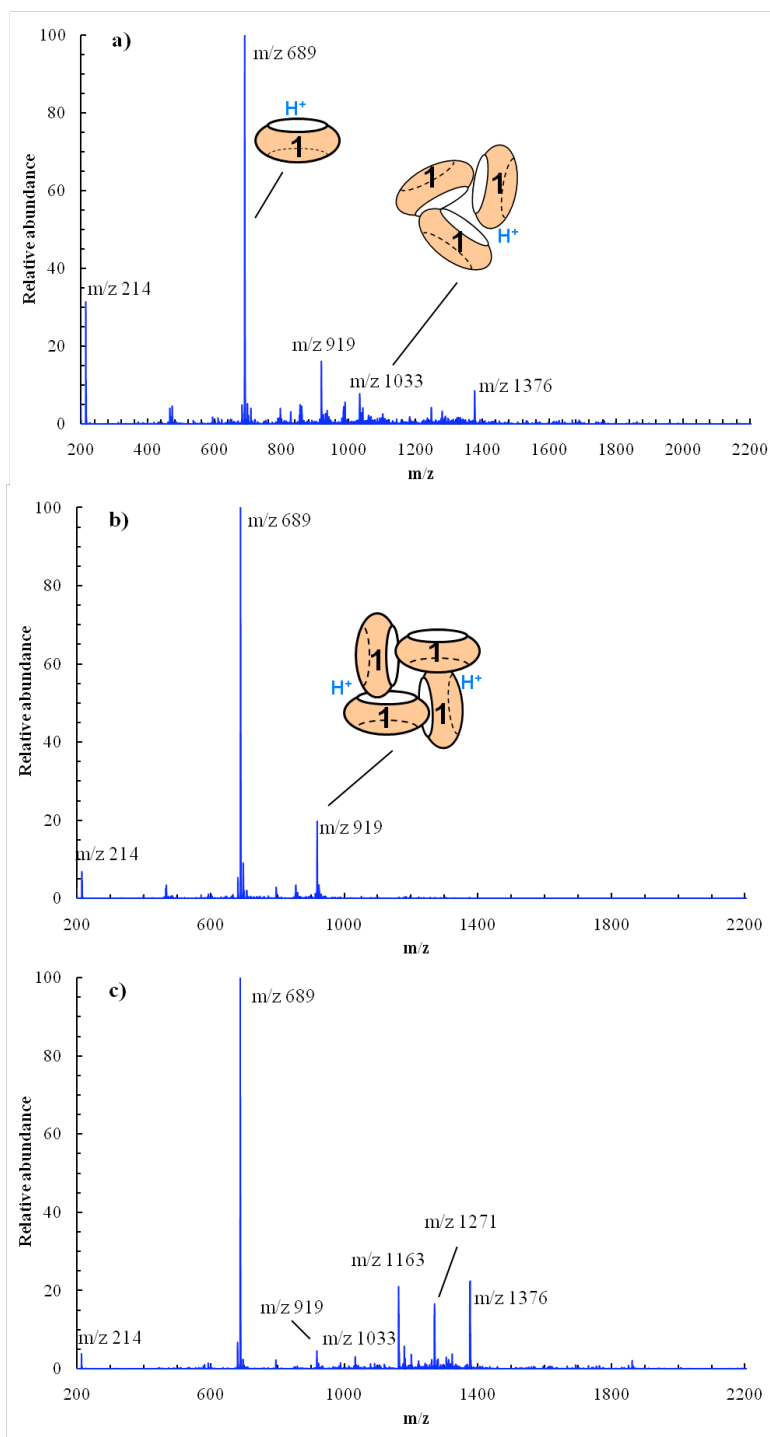


Figure S20: ESI-MS spectra of **1**@CB7. **a)** 50 μ M, CE 70 V; **b)** 500 μ M, CE 70 V; **c)** 500 μ M, CE 300 V. Assignments: m/z 214: **1**; m/z 689: $[CB7 + \mathbf{1} + H]^+$; 919: $[4 \cdot CB7 + 4 \cdot \mathbf{1} + 2 \cdot H]^{6+}$; m/z 1033: $[3 \cdot CB7 + 3 \cdot \mathbf{1} + H]^{4+}$; m/z 1163: $[CB7 + H]^+$; m/z 1271: $[4 \cdot CB7 + 2 \cdot \mathbf{1} + 2 \cdot H]^{4+}$; m/z 1376: $[CB7 + \mathbf{1}]^+$.

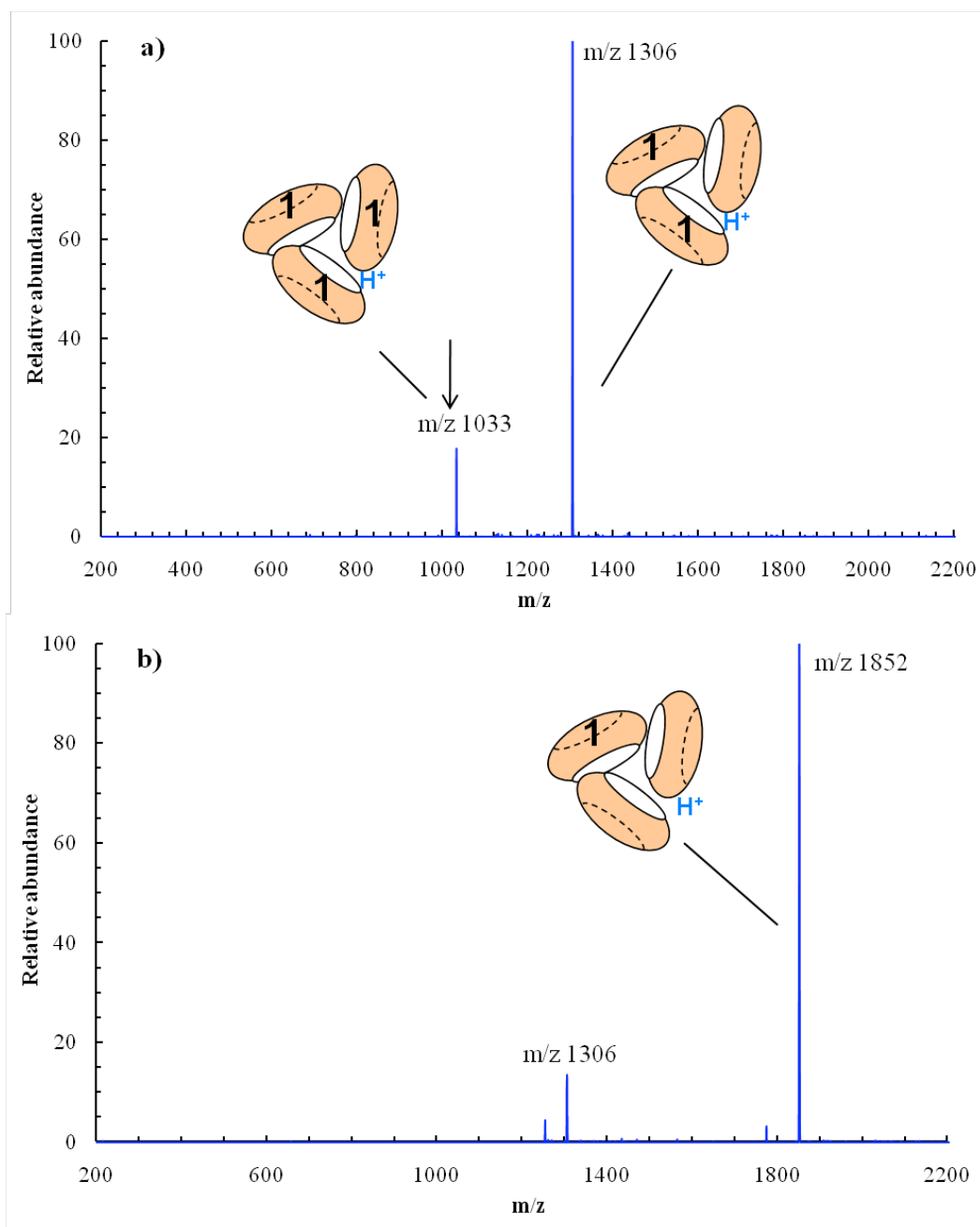


Figure S21: Consecutive fragmentations of m/z 1033 (**1**@CB7) (see Figure S20 **a**)). **a**) MS² 1033; **b**) MS³, 1033 → 1306. The arrows indicate the fragmented peak. Assignments: m/z 1033: [3•CB7 + 3•CAT1 + H]⁴⁺; m/z 1306: [3•CB7 + 2•**1** + H]³⁺; m/z 1852: [3•CB7 + **1** + H]²⁺.

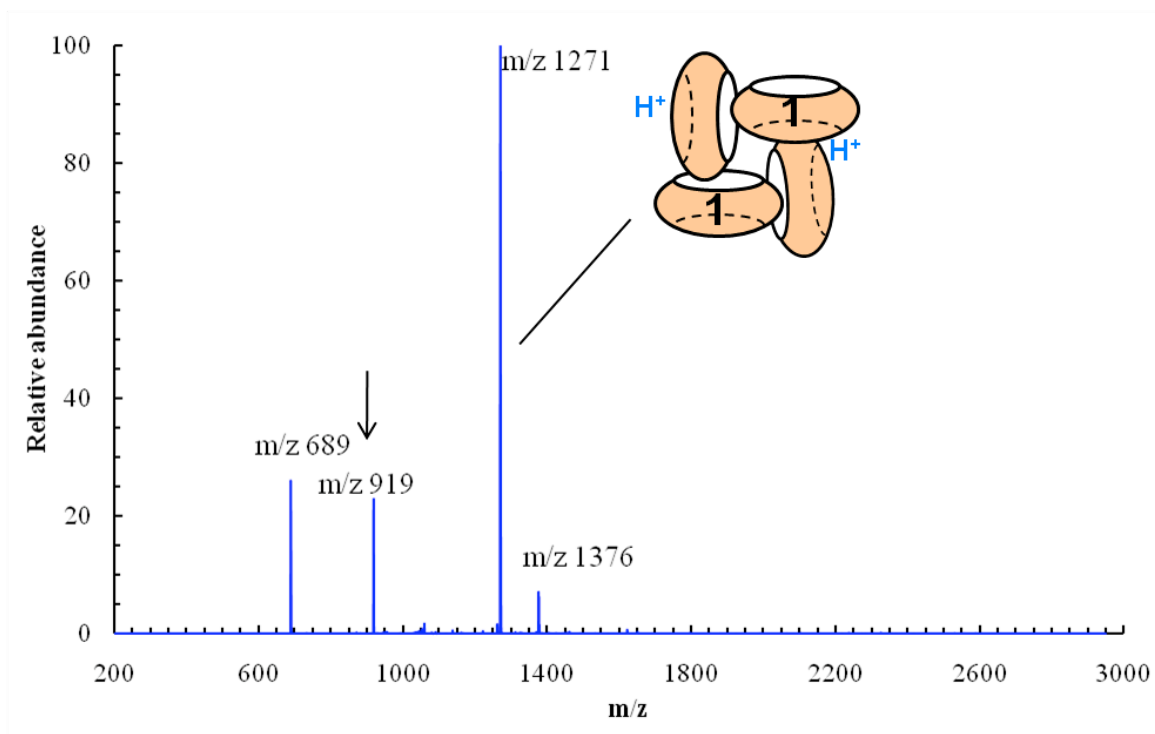


Figure S22: Fragmentation (MS^2) of m/z 919 (**1**@CB7) (see Figure S20 **a**) to **c**). The arrow indicates the fragmented peak. Assignments: m/z 689: $[CB7 + \mathbf{1} + H]^{2+}$; m/z 919: $[4 \cdot CB7 + 4 \cdot \mathbf{1} + 2 \cdot H]^{6+}$; m/z 1271: $[4 \cdot CB7 + 2 \cdot \mathbf{1} + 2 \cdot H]^{4+}$; m/z 1376 - $[CB7 + \mathbf{1}]^+$.

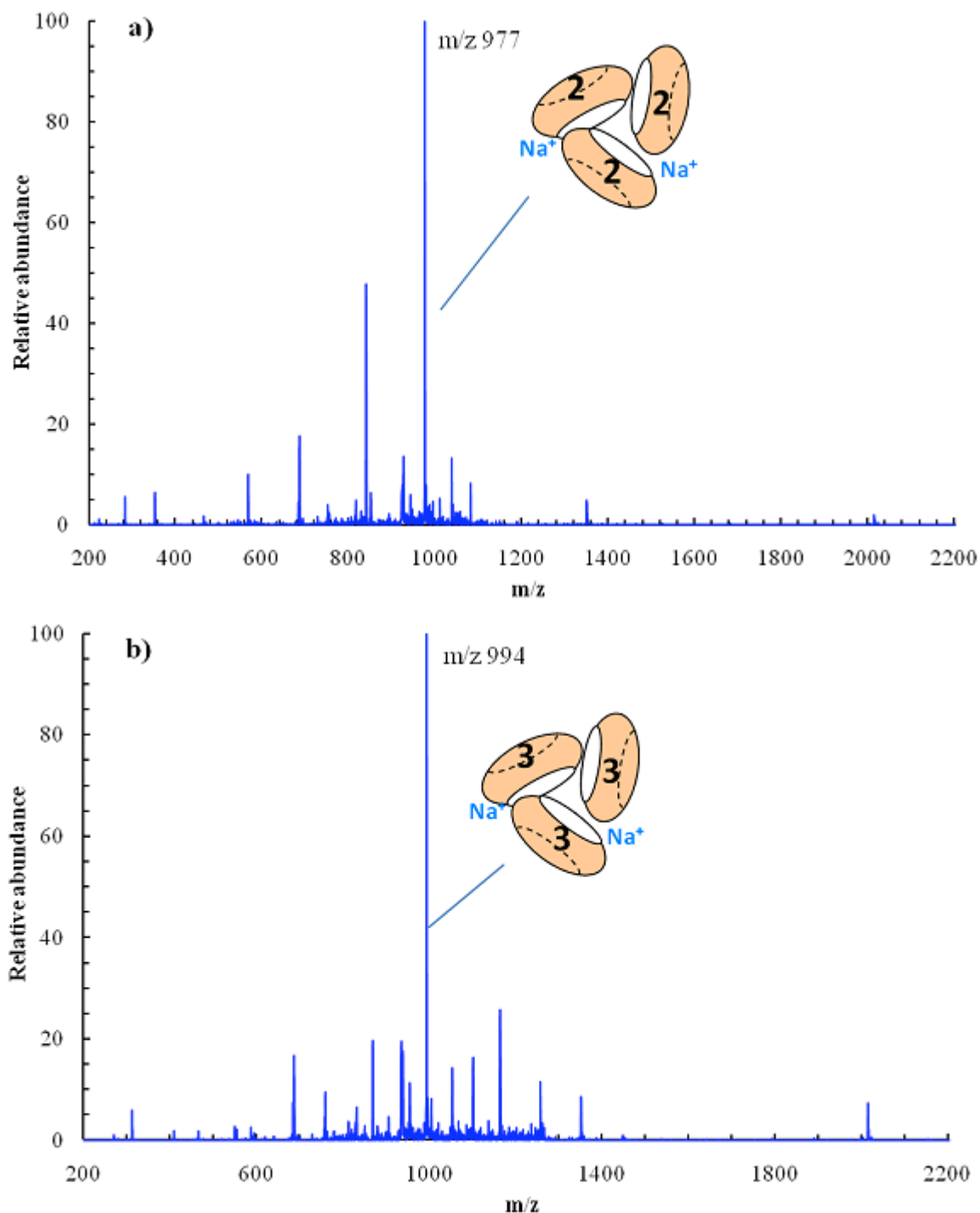


Figure S23: ESI-MS spectra of **2@CB8** (a) and **3@CB8** (b). The trimers of the complexes are: m/z 977: $[3\bullet\text{CB8} + 3\bullet\mathbf{2} + 2\bullet\text{Na}]^{5+}$ and m/z 994 - $[3\bullet\text{CB8} + 3\bullet\mathbf{3} + 2\bullet\text{Na}]^{5+}$, respectively.

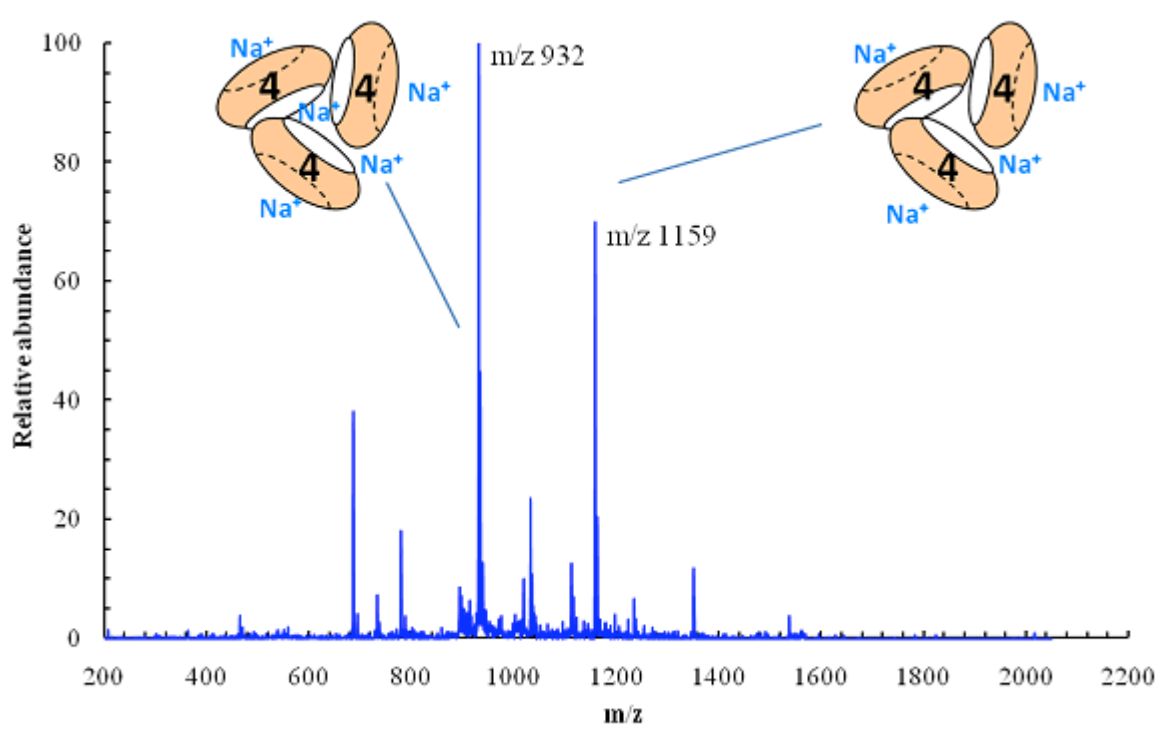


Figure S24: ESI-MS spectrum of a solution ($\sim 100 \mu\text{M}$) of **4@CB8** complexes. The ions at m/z 932 and 1159 were assigned to the trimers $[3\bullet\text{CB8} + 3\bullet\mathbf{4} + 5\bullet\text{Na}]^{5+}$ and $[3\bullet\text{CB8} + 3\bullet\mathbf{4} + 4\bullet\text{Na}]^{4+}$, respectively (see Figure S24).

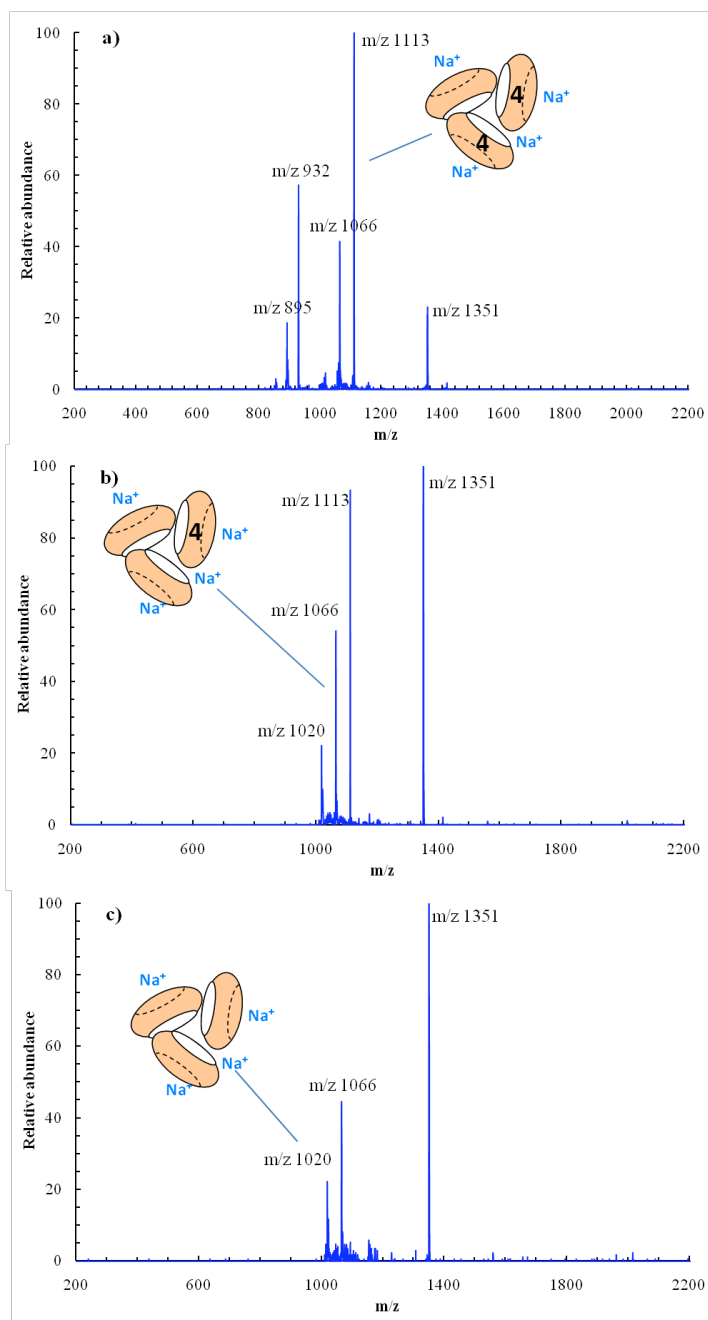


Figure S25: Consecutive fragmentations of m/z 932 (4@CB8) (see Figure S24). **a)** MS² 932; **b)** MS³ 932 → 1113; **c)** MS⁴ 932 → 1113 → 1066; The arrows indicate the fragmented peak. Assignments: m/z 895: [3•CB8 + 2•4 + 5•Na]⁵⁺; m/z 932: [3•CB8 + 3•4 + 5•Na]⁵⁺; m/z 1020: [3•CB8 + 4•Na]⁴⁺; m/z 1066: [3•CB8 + 1•4 + 4•Na]⁴⁺; m/z 1113: [3•CB8 + 2•4 + 4•Na]⁴⁺; m/z 1351 - [3•CB7 + 3•Na]³⁺ or [CB8 + Na]⁺.

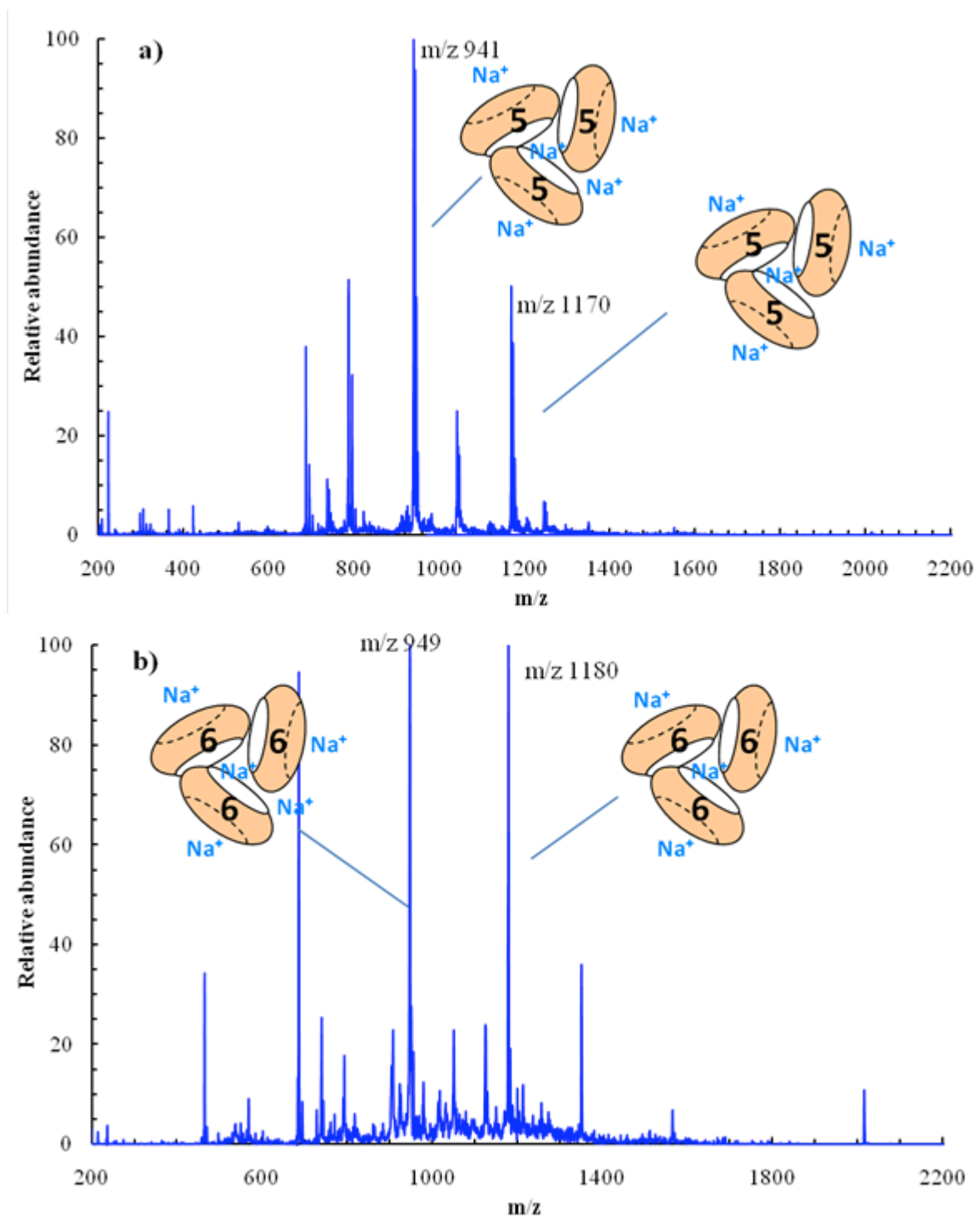


Figure S26: ESI-MS spectra of **5@CB8** (a) and **6@CB8** (b). The trimers of the complexes are: m/z 941: $[3 \cdot \text{CB8} + 3 \cdot \mathbf{5} + 5 \cdot \text{Na}]^{5+}$; m/z 1170: $[3 \cdot \text{CB8} + 3 \cdot \mathbf{5} + 4 \cdot \text{Na}]^{4+}$; m/z 949: $[3 \cdot \text{CB8} + 3 \cdot \mathbf{6} + 5 \cdot \text{Na}]^{5+}$; m/z 1180: $[3 \cdot \text{CB8} + 3 \cdot \mathbf{6} + 4 \cdot \text{Na}]^{4+}$.

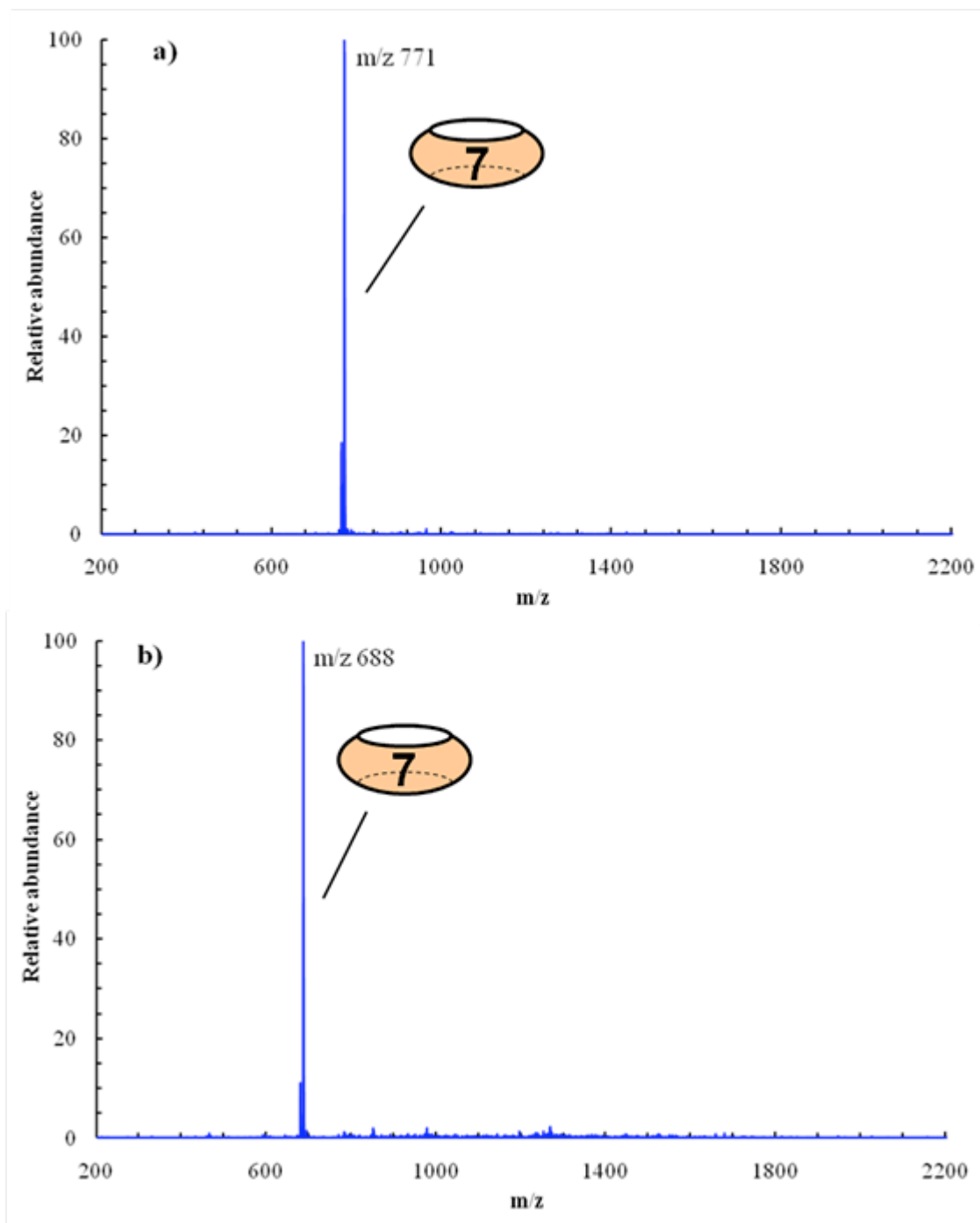


Figure S27: ESI-MS spectra of 7@CB8 (**a**) and 7@CB7 (**b**). Assignments: m/z 771: $[\text{CB8} + 7]^{2+}$; m/z 688 - $[\text{CB7} + 7]^{2+}$.

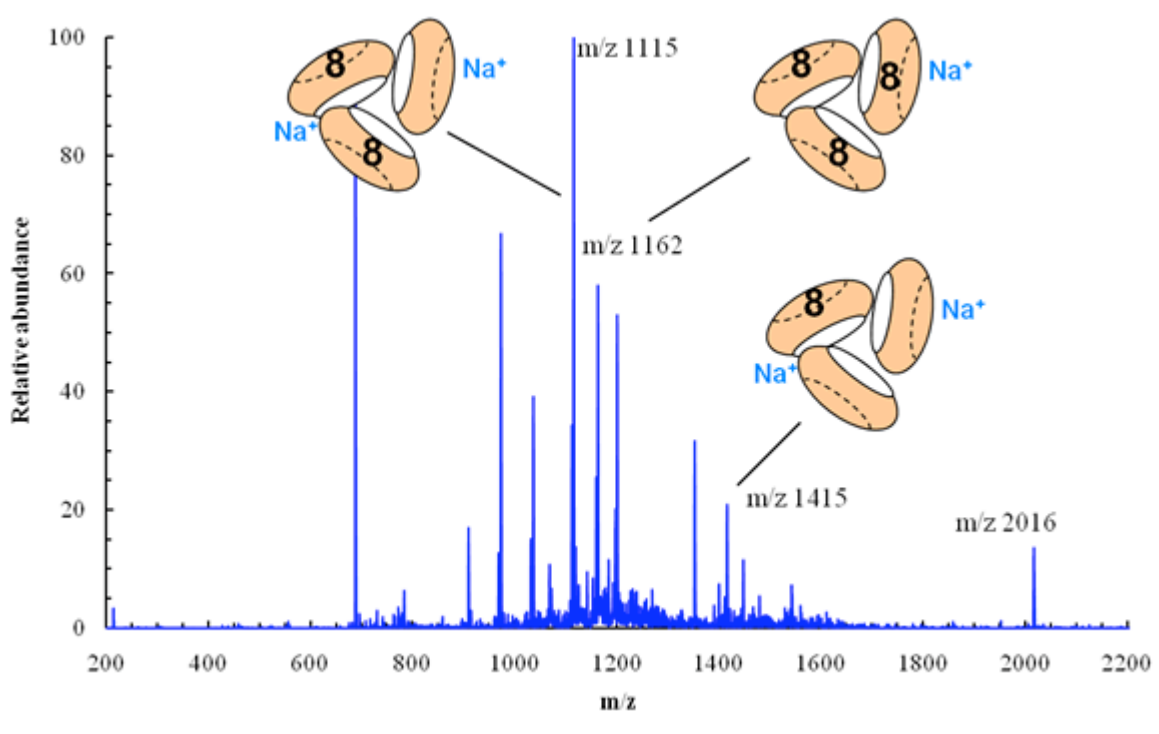


Figure S28: ESI-MS mass spectrum of an aqueous solution of 8@CB8 complexes (~ 100 μ M) prepared by addition of NaOH to the solution containing 7@CB8. The trimers are: m/z 1115: $[3\cdot\text{CB8} + 2\cdot\mathbf{8} + 2\cdot\text{Na}]^{4+}$; m/z 1162: $[3\cdot\text{CB8} + 3\cdot\mathbf{8} + \text{Na}]^{4+}$; m/z 1415: $[3\cdot\text{CB8} + \mathbf{8} + 2\cdot\text{Na}]^{3+}$; m/z 2016: $[3\cdot\text{CB8} + 2\cdot\text{Na}]^{2+}$.

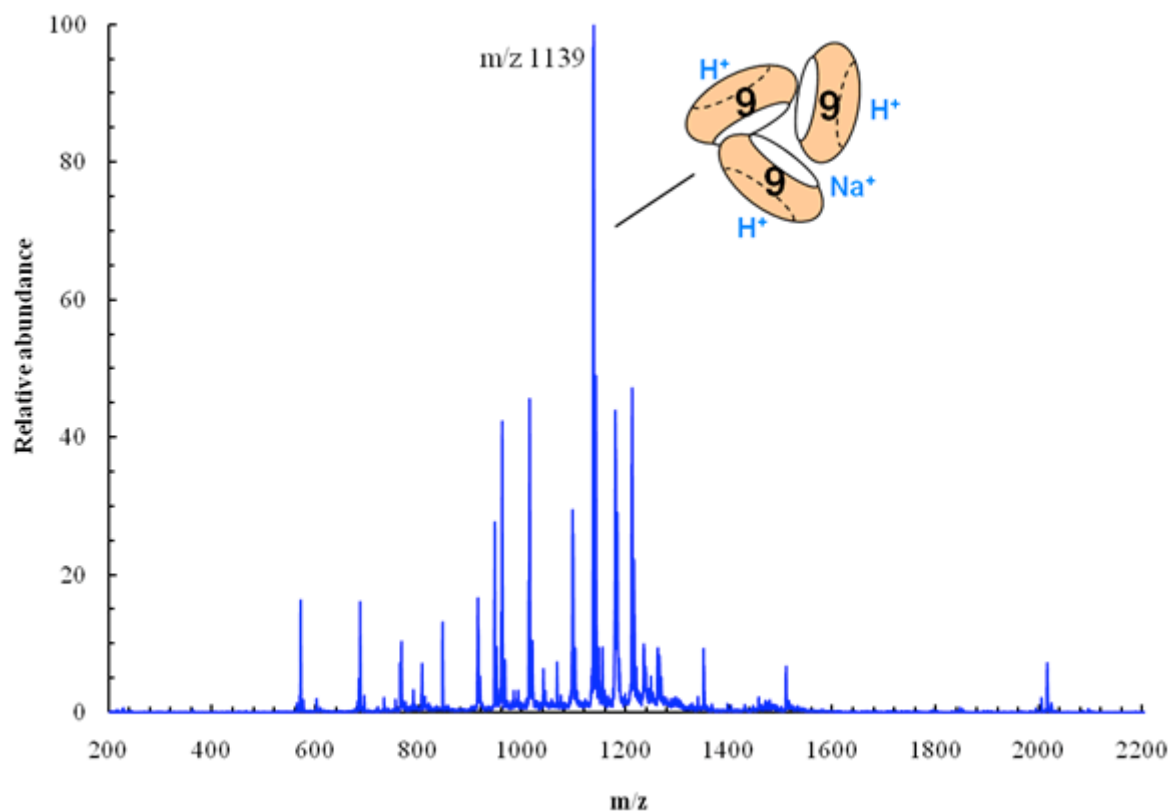


Figure S29: ESI-MS spectra of **9**@CB8. The trimer of the complexes is: m/z 1139: $[3 \cdot \text{CB8} + 3 \cdot \text{9} + 3 \cdot \text{H} + \text{Na}]^{4+}$.

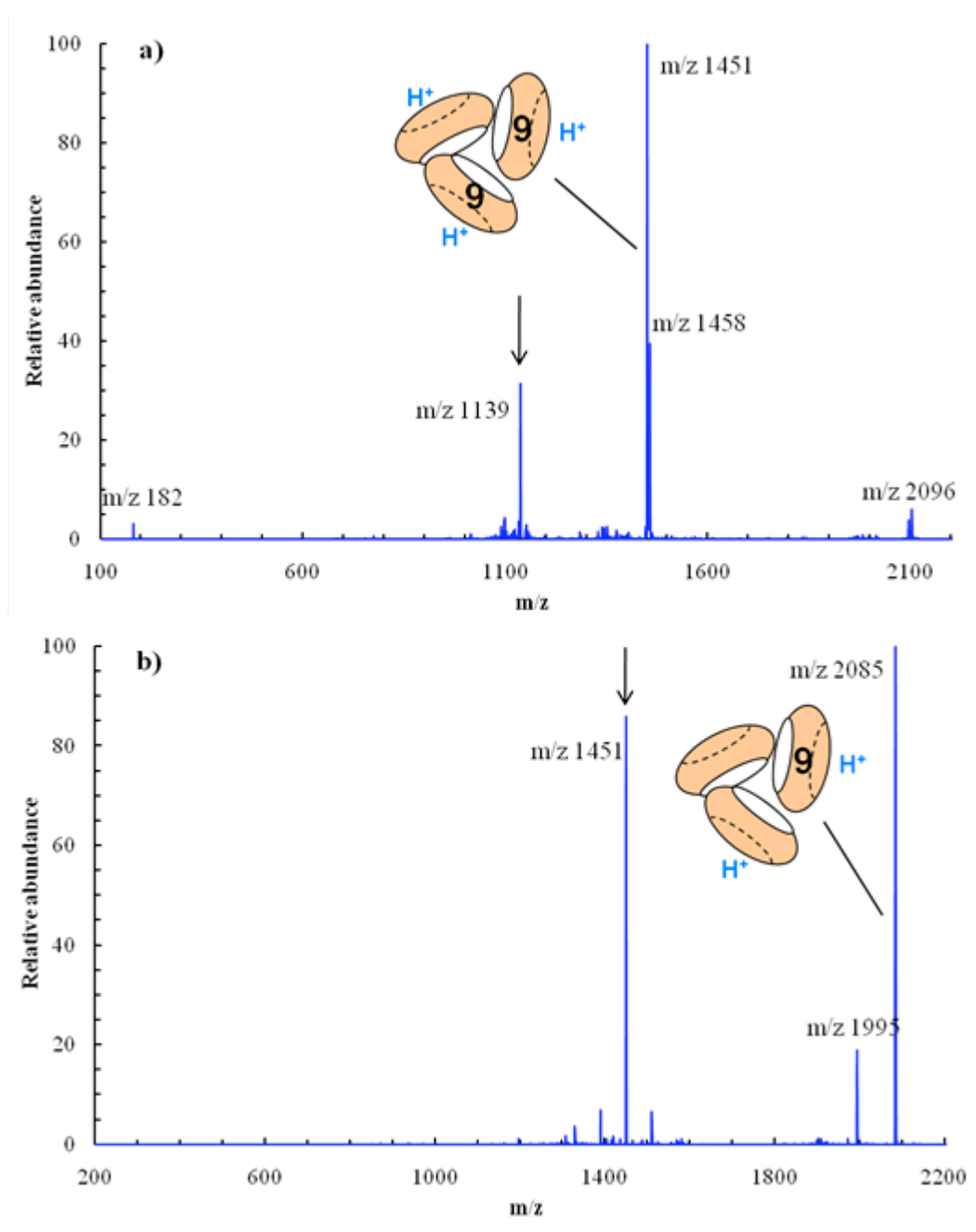


Figure S30: Consecutive fragmentations of m/z 1139 (**9**@CB8) (see Figure S29). **a)** MS² 1139; **b)** MS³ 1139 → 1451. The arrows indicate the fragmented peak. Assignments: m/z 182: [**9** + H]⁺; m/z 1139: [3•CB8 + 3•**9** + 3•H + Na]⁴⁺; m/z 1451: [3•CB8 + 2•**9** + 3•H]³⁺; m/z 1458: [3•CB8 + 2•**9** + 2•H + Na]³⁺; m/z 1995: [3•CB8 + 2•H]²⁺; m/z 2085 - [3•CB8 + **9** + 2•H]²⁺; m/z 2096 - [3•CB8 + **9** + H + Na]²⁺.

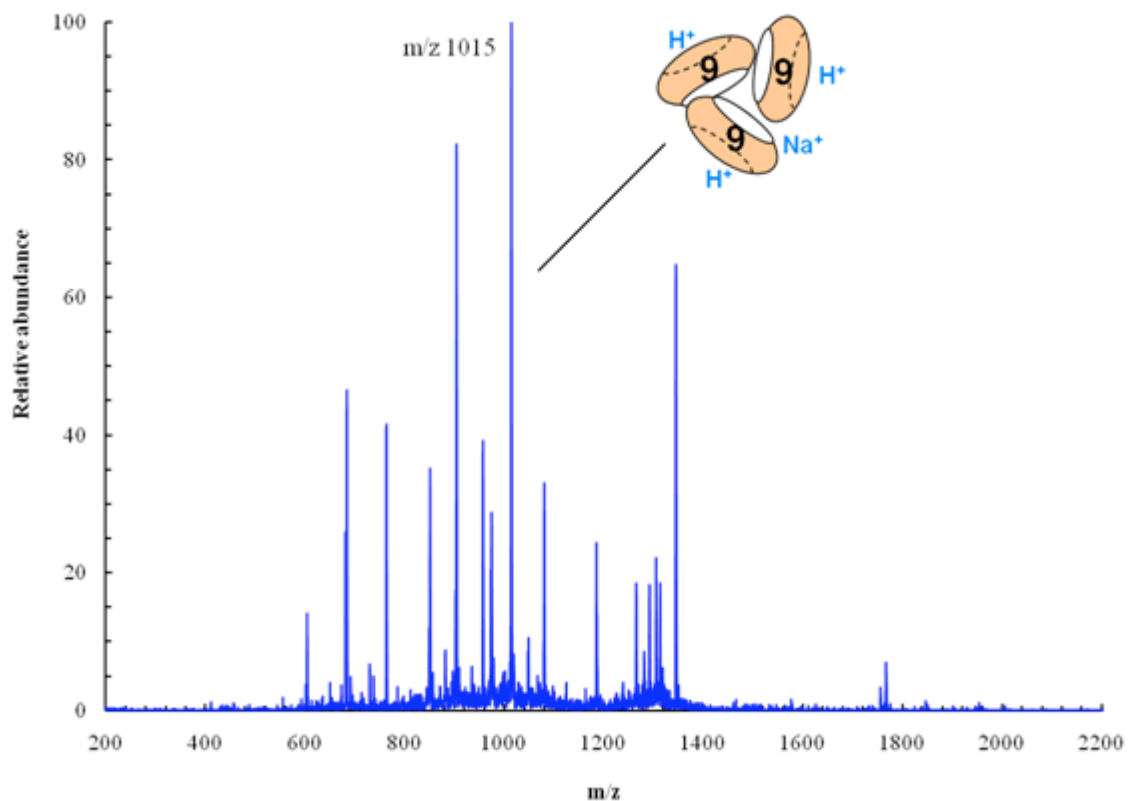


Figure S31: ESI-MS spectra of 9@CB7. The trimer of the complexes is: m/z 1015: $[3 \cdot \text{CB7} + 3 \cdot \mathbf{9} + 3 \cdot \text{H} + \text{Na}]^{4+}$ (see Figure S31).

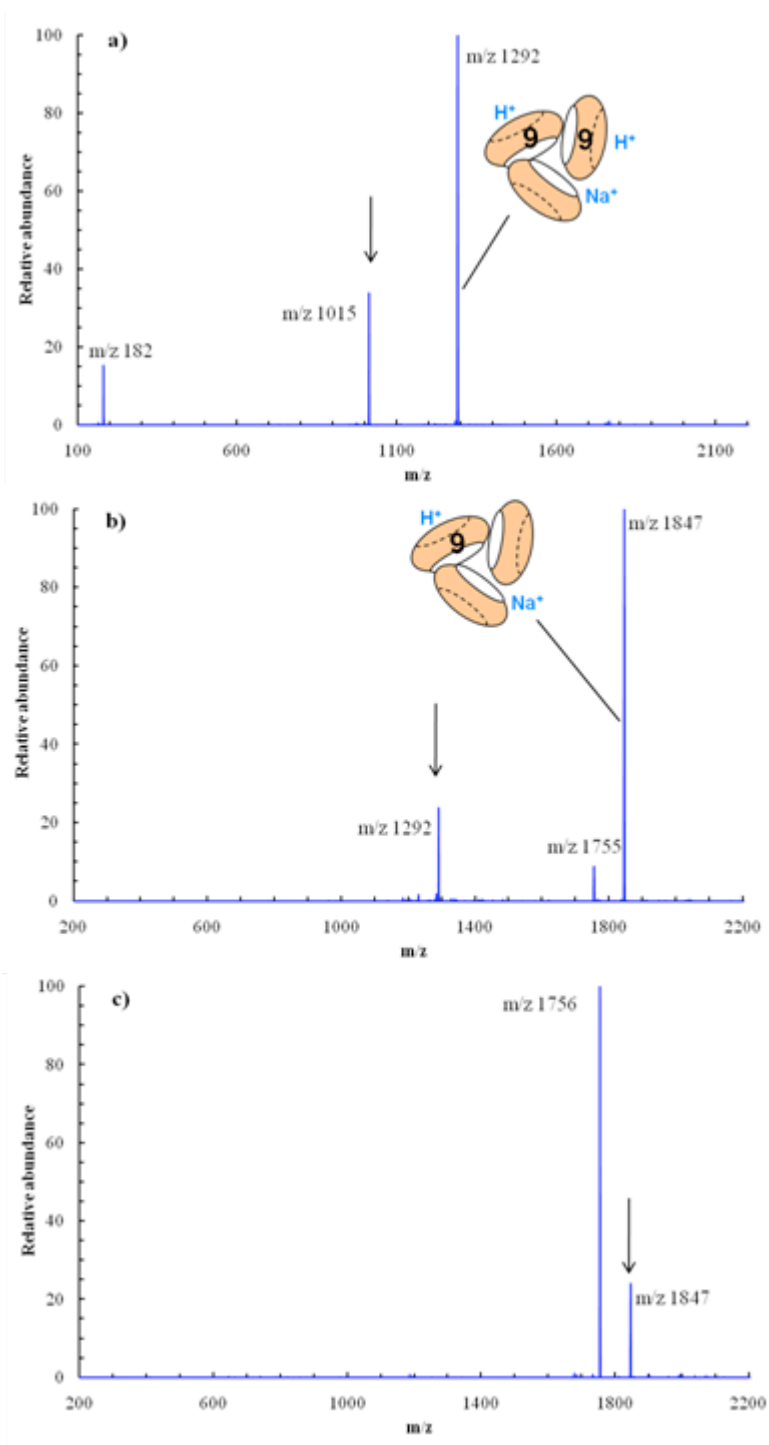


Figure S32: Consecutive fragmentations of m/z 1015 (see Figure S31). **a)** MS² 1015; **b)** MS³ 1015 → 1292; **c)** MS⁴ 1015 → 1292 → 1847; The arrows indicate the fragmented peak. Assignments: m/z 182: [9 + H]⁺; m/z 1015: [3•CB7 + 3•9 + 3•H + Na]⁴⁺; m/z 1292: [3•CB7 + 2•9 + 2•H + Na]³⁺; m/z 1847: [3•CB7 + 9 + H + Na]²⁺; m/z 1755: [3•CB7 + H + Na]²⁺.