Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Evidence for an intrinsic binding force between dodecaborate dianions and receptors with hydrophobic binding pockets

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Contents

1. Analytical Details

NMR

All NMR spectra were measured on a *Bruker WB-300* NMR spectrometer (*Bruker BioSpin GmbH, Rheinstetten, Germany*) equipped with a temperature control unit and an AVANCE III unit. A 10 mm probe head allows measurements of larger using 10 mm NMR tubes as well as standard 5 mm NMR tubes.

Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS measurements were performed on a *Bruker Esquire-LC* ion trap mass spectrometer (*Bruker Daltonik, Bremen, Germany*) at the University of Bremen. Samples were dissolved in CH_3CN/CH_2Cl_2 (LCMS grade) at concentrations of approximately 10^{-6} mol L^{-1} and injected into the mass spectrometer via a syringe pump at a flow rate of 3 mL min^{-1}. Spectra were recorded for three to five minutes and averaged. Instrument parameters were set to:
Mode: negative (positive in experiments with oxidized TTF receptors)
Trap Drive: 100.4
Octopole RF Amplitude: 150.0 Vpp
Lens 2: 60.0 V
Capillary Exit: -133.8 V
Lens 1: 5.0 Volt
Dry Temperature: 300°C
Nebulizer: 5 psi
Dry Gas: 5 L/min
HV Capillary: 4000 V
HV End Plate Offset: -500 V
Fragmentation was induced by collision induced dissociation (CID) in an ion trap. Ions with a defined m/z-value were isolated and specifically activated by collisions with the background gas He at a pressure of 10^{-5} mbar. The collision is induced by applying an adjustable ac voltage to the ion trap end caps. Increase of the amplitude leads to higher excitation strength. Fragmentation of host-guest complexes under formation of $[B_{12}X_{12}]^{2-}$ in this study was observed at amplitudes around 0.4 V. Commonly, organic molecules start to fragment between 0.4 V and 0.5 V.

2. Substrates

The parent *closo*-dodecaborate $[B_{12}H_{12}]^{2-}$ was prepared according to the literature procedure from $NaBH_4$ and I_2 in diglyme.[1] Halogenation of $Na_2[B_{12}H_{12}]$ following literature methods yielded the corresponding perhalogenated dodecaborates $[B_{12}F_{12}]^{2-}$,[2] $[B_{12}Cl_{12}]^{2-}$,1 $[B_{12}Br_{12}]^{2-}$,[3] and $[B_{12}I_{12}]^{2-}$.[4] Tetraalkylmmonium salts were precipitated by adding the corresponding tetraalkylmmonium chlorides to aqueous solutions of the sodium salts of dodecaborates. TTF-containing receptors[5] **2a,b** and calix[4]azulene derivative[6] **3** were prepared as described before. α- and β-cyclodextrins **5a,b** were purchased from *Sigma-Aldrich*. D_2O was purchased from *Deutero GmbH*.

3. Mass-spectra of host-guest complexes of $[B_{12}X_{12}]^{2-}$ with hosts 2a,b

For (-)-ESI-MS measurements, B-clusters $[B_{12}X_{12}]^{2-}$ were taken as tetraalkylammonium or Na salts.

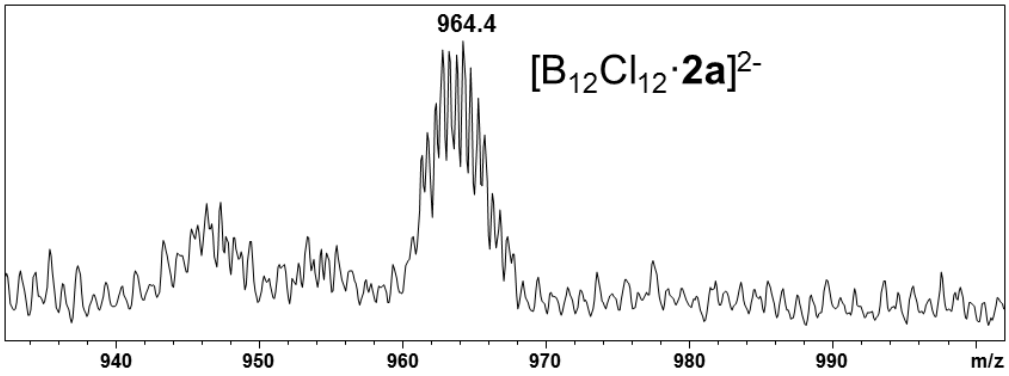

Figure S1. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}Cl_{12}]^{2-}$ and **2a**.

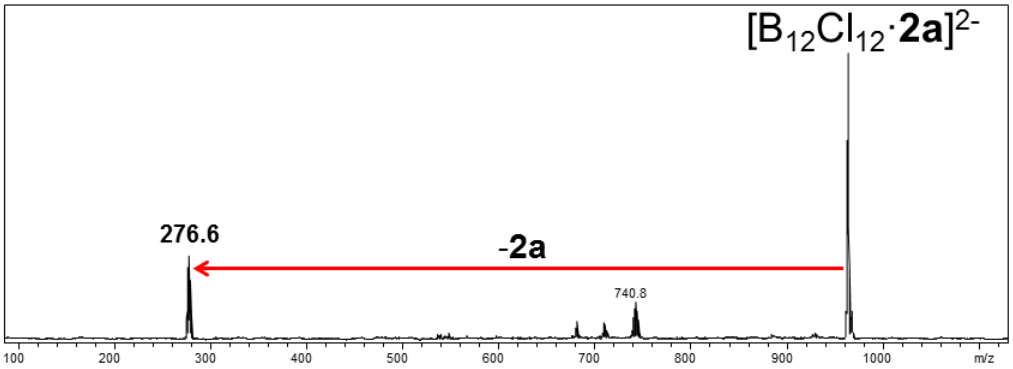

Figure S2. MS/MS spectrum after isolation and excitation of $[(B_{12}Cl_{12})\cdot\mathbf{2a}]^{2-}$. In addition to the dissociation of the host-guest complex, the formation of singly charged fragments with low intensity is observed. The driving force for such fragmentations is likely the charge separation.

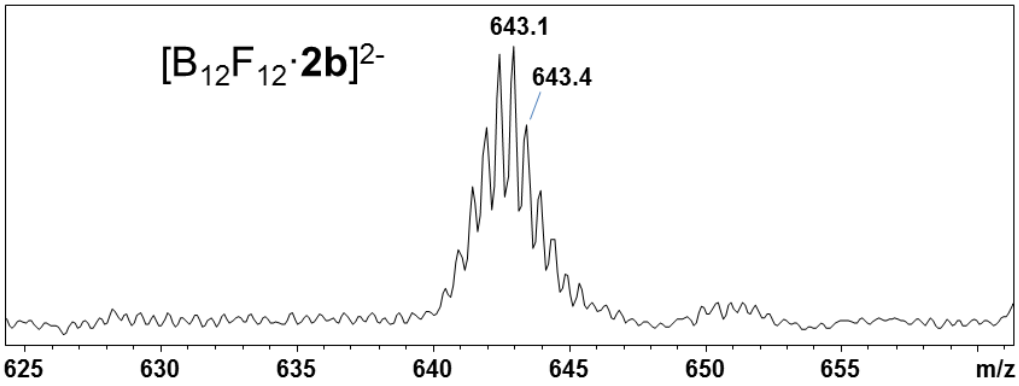

Figure S3. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}F_{12}]^{2-}$ and **2b**.

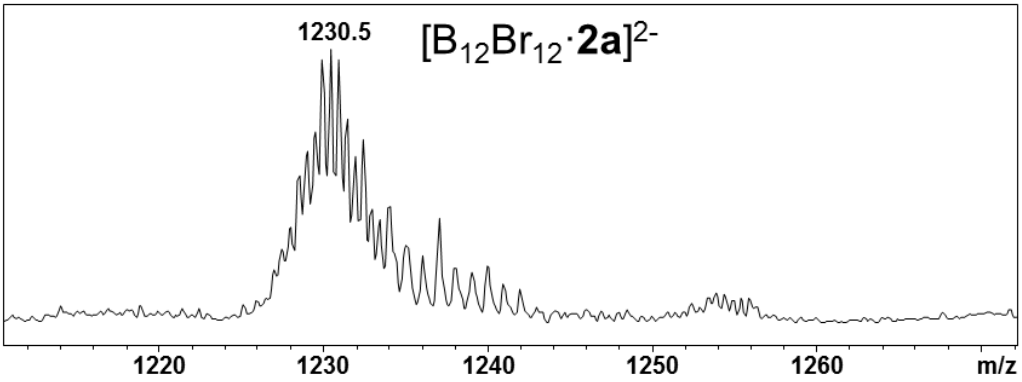

Figure S4. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}Br_{12}]^{2-}$ and **2a** (see also Figure S5).

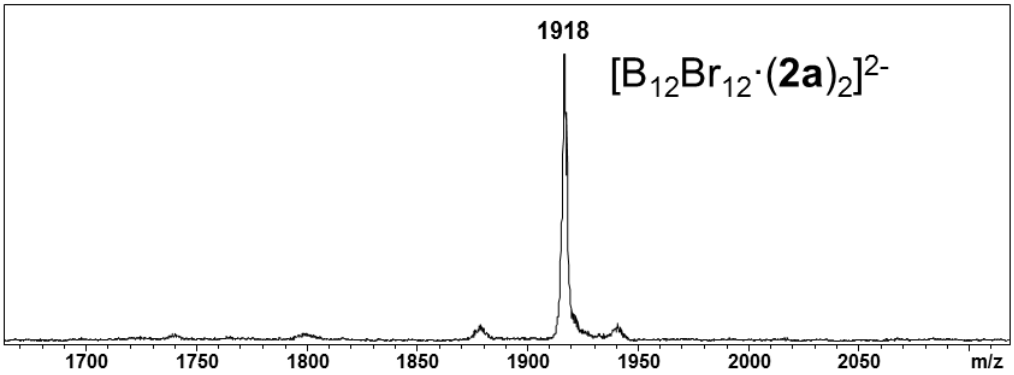

Figure S5. Higher mass range of the spectrum in Figure S4 showing the formation of the double decker complex with the brominated cluster $[B_{12}Br_{12}]^{2-}$ (corresponds to Figure 3c in the manuscript depicting the formation of the double decker complex with $[B_{12}Cl_{12}]^{2-}$).

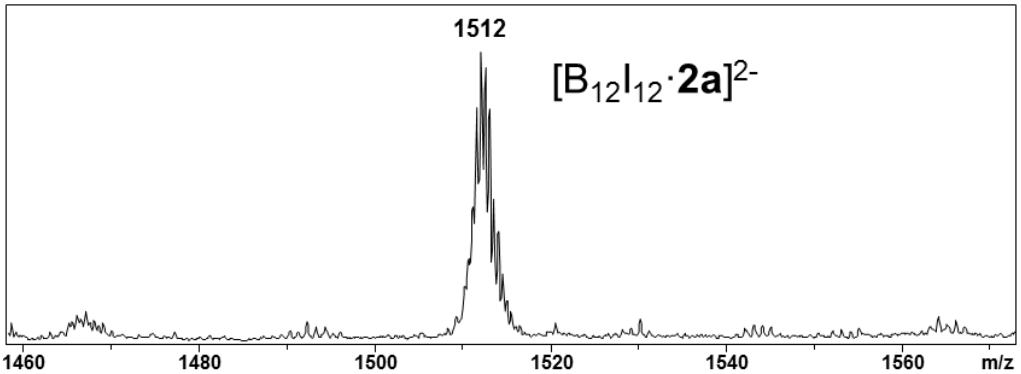

Figure S6. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}I_{12}]^{2-}$ and **2a.**

4. Mass-spectra of host-guest complexes of $[B_{12}X_{12}]^{2-}$ with cyclodextrins 5a,b

For (-)-ESI-MS measurements, B-clusters $[B_{12}X_{12}]^{2-}$ were taken as tetraalkylammonium or Na salts.

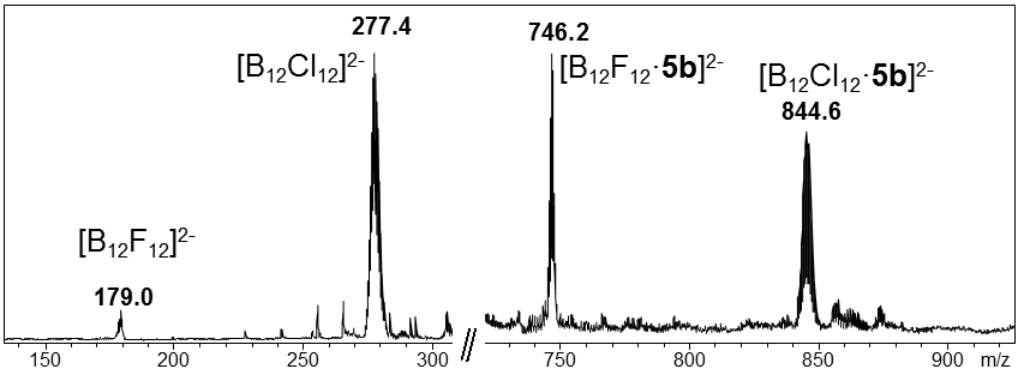

Figure S7. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}F_{12}]^{2-}$, $[B_{12}Cl_{12}]^{2-}$ and **5b.** Although the $[B_{12}F_{12}]^{2-}$ concentration was considerably lower than the $[B_{12}Cl_{12}]^{2-}$ concentration, as it can be seen from the signal intensities of the naked dianions, the complex intensity is higher for the fluorinated derivative.

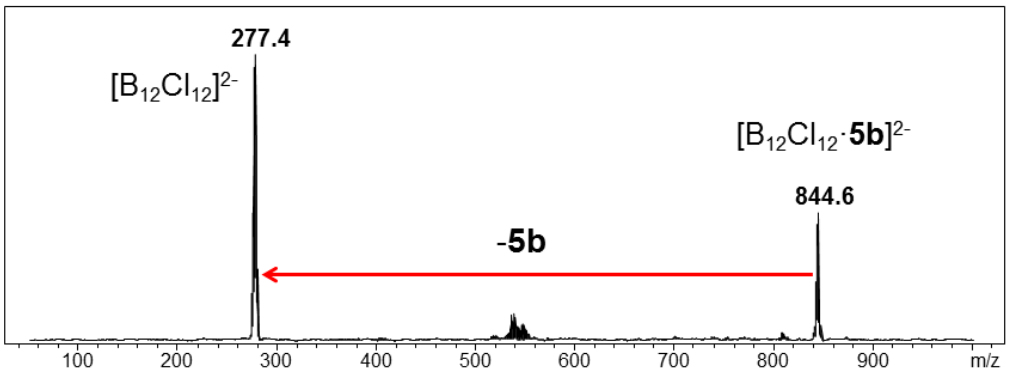

Figure S8. MS/MS spectrum after isolation and excitation of $\{B(_{12}Cl_{12})\cdot\mathbf{5b}]^{2-}$. Signals of the single charged species can be traced back to further fragmentation[7] of the $[B_{12}Cl_{12}]^{2-}$.

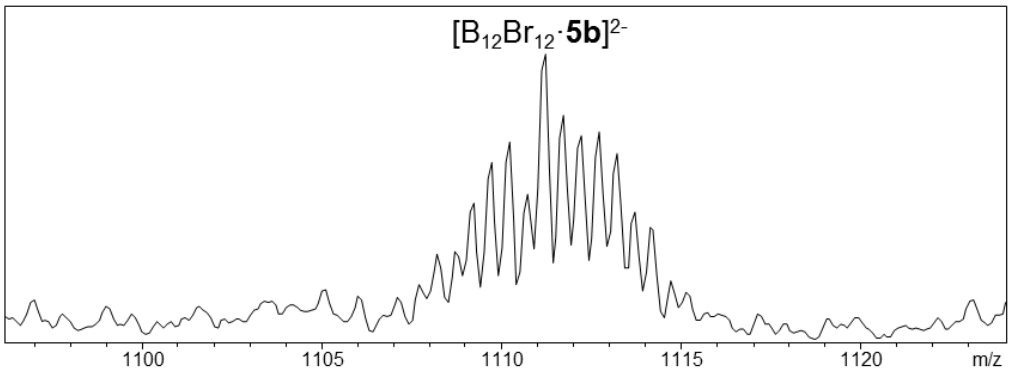

Figure S9. Partial (-)-ESI-MS spectrum after spraying of a solution containing $[B_{12}Br_{12}]^{2-}$ and **5b.** Note that the intensity is very low (signal to noise ratio) although similar concentrations as for $[B_{12}F_{12}]^{2-}$ and $[B_{12}Cl_{12}]^{2-}$ were used in this measurement.
Due to its weakness, the signal of $[(B_{12}I_{12})\cdot\mathbf{5b}]^{2-}$ complex was only slightly above the noise level.

5. Dissociation of $[\mathbf{5a}\cdot B_{12}Cl_{12}\cdot\mathbf{5b}]^{2-}$ complex

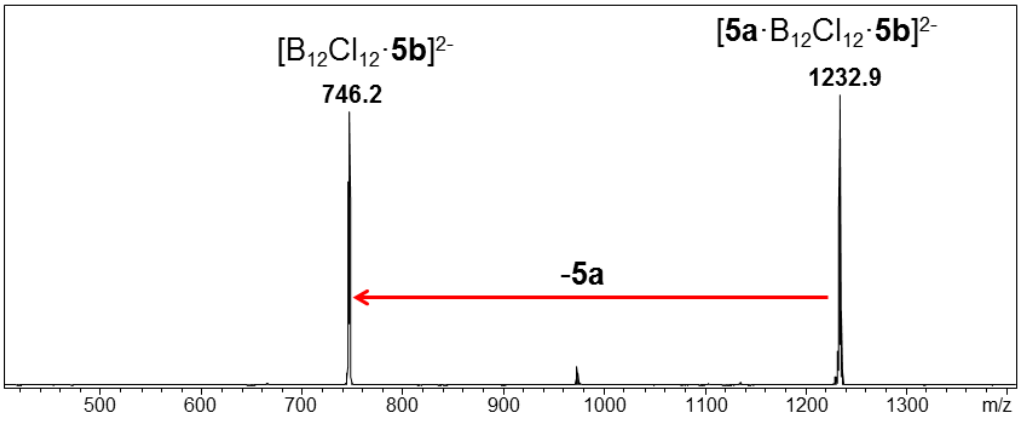

Figure S10. (-)-ESI MS spectrum after fragmentation of $[\mathbf{5a}\cdot B_{12}Cl_{12}\cdot\mathbf{5b}]^{2-}$ complex showing the exclusive formation of $[B_{12}Cl_{12}\cdot\mathbf{5b}]^{2-}$ with dissociation of α-CD **5a**.

6. Mass-spectra showing that complexes with hosts 3 and 4 were not observed

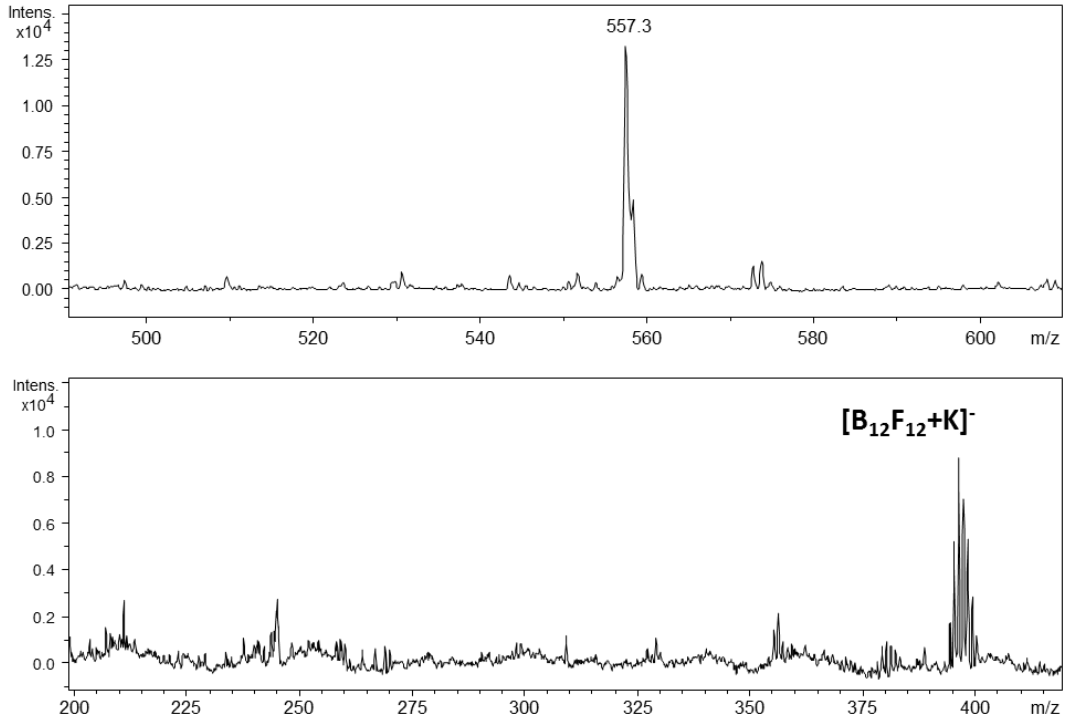

Figure S11. Partial ESI-MS spectra (+ top, - bottom) from a solution containing $Cs_2[B_{12}F_{12}]$ and host **3a**. Host **3a** was detected in the positive mode as $[M+Cs]^+$. Formation of the negatively charged host-guest complex $[B_{12}F_{12}{\cdot}\mathbf{3a}]^{2-}$ (m/z=391) was not observed.

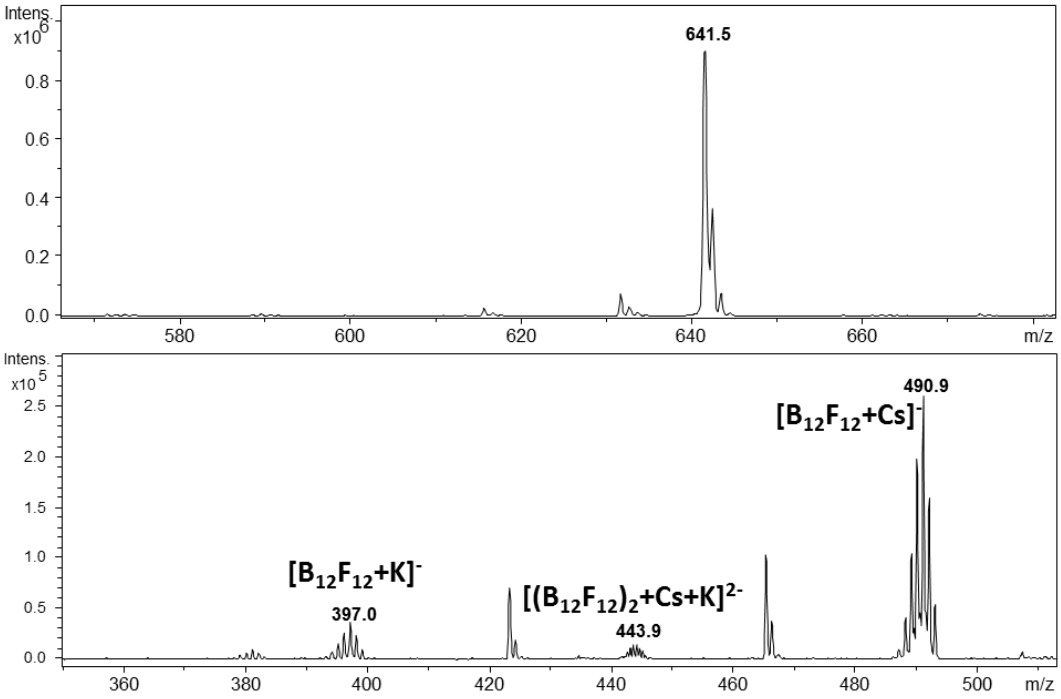

Figure S12. Partial ESI-MS spectra (+ top, - bottom) from a solution containing $Cs_2[B_{12}F_{12}]$ and host **3b**. Host **3b** was detected in the positive mode as $[M+Cs]^+$. Formation of the negatively charged host-guest complex $[B_{12}F_{12}{\cdot}\mathbf{3b}]^{2-}$ (m/z=433) was not observed.

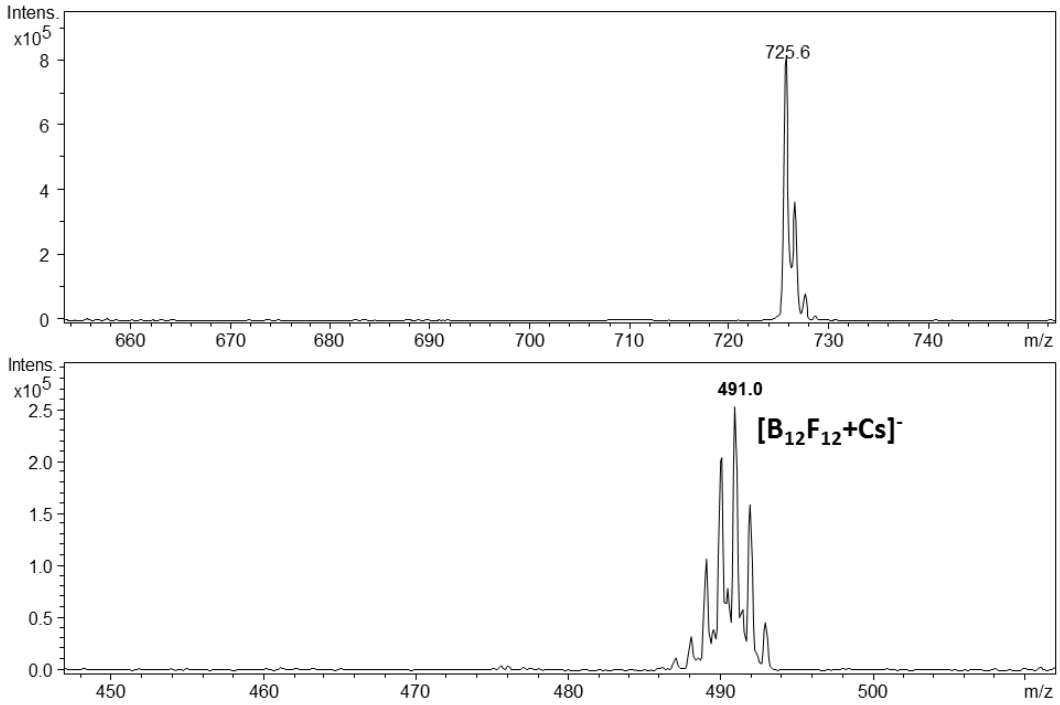

Figure S13. Partial ESI-MS spectra (+ top, - bottom) from a solution containing $Cs_2[B_{12}F_{12}]$ and host **3c**. Host **3c** was detected in the positive mode as $[M+Cs]^+$. Formation of the negatively charged host-guest complex $[B_{12}F_{12}\cdot\mathbf{3c}]^{2-}$ (m/z=475) was not observed.

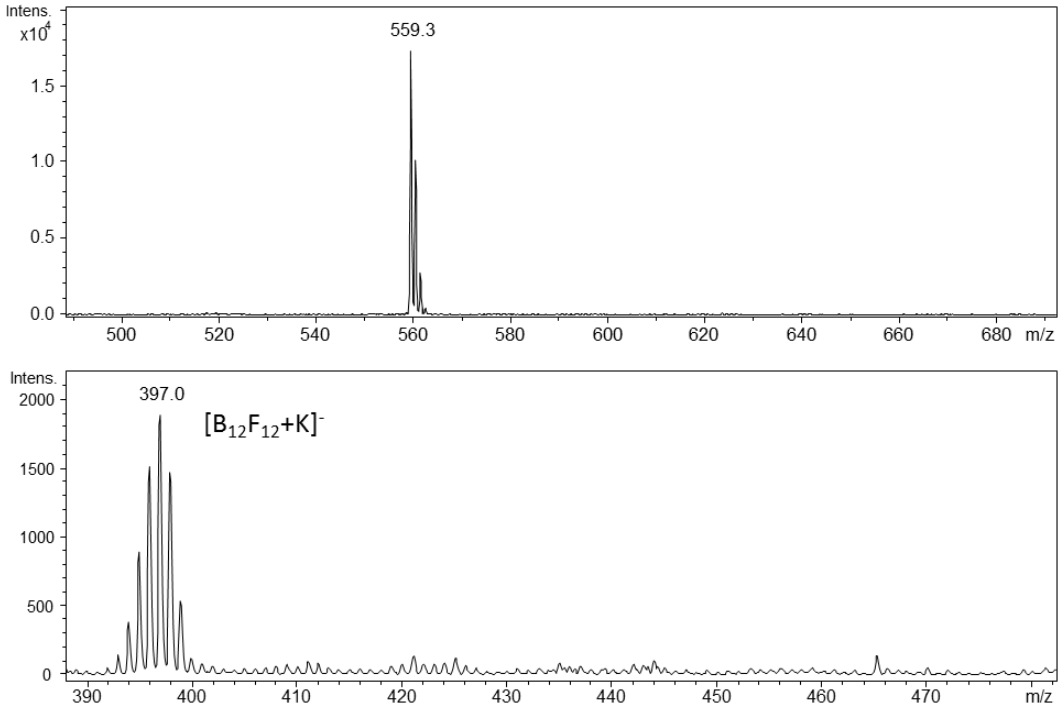

Figure S14. Partial ESI-MS spectra (+ top, - bottom) from a solution containing host **4** and $Cs_2[B_{12}F_{12}]$. Host **4** was detected in the positive mode as $[M-H]^+$ (see ref. 26 in the manuscript for details). Formation of the negatively charged host-guest complex $[B_{12}F_{12}\cdot\mathbf{4}]^{2-}$ (m/z=459) was not observed.

7. Complexation of Cyclodextrins with $[B_{12}F_{12}]^{2-}$ in solution.

Determination of the binding affinity between α-CD **5a** and β-CD **5b** with fluorinated dodecaborate **1a** was performed by means of NMR binding titrations. Dodecaborate **1a** was taken as a Na-salt $Na_2B_{12}F_{12}$, the chemical shift of ^{19}F-atoms of the guest for fitting of the binding data. In the binding experiments, the concentration of **1a** was kept constant, whereas the concentration of CD **5** was varied. Fitting of the binding data was performed as described before.[8]

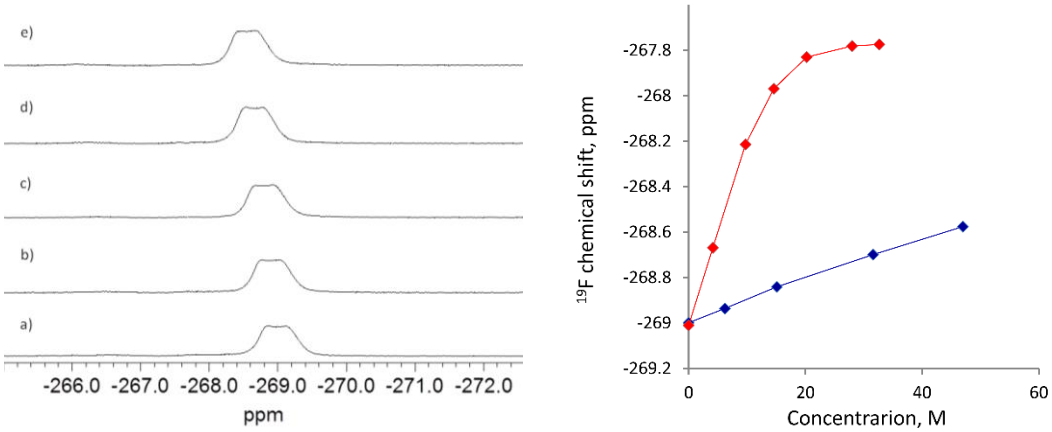

Figure S15. Left panel: Set of ^{19}F NMR spectra of $Na_2B_{12}F_{12}$ in D_2O measured upon variation of the β-CD concentration (7 T at 303.2 K). C $[Na_2B_{12}F_{12}]$ = 8.75 mM, C [β-CD] = a) 0 mM, b) 4.1 mM, c) 9.7 mM, d) 14.6 mM, e) 20.2 mM, f) 28.0 mM and g) 32.6 mM, respectively. Right panel: titration curves fort the complexes of $[B_{12}F_{12}]^{2-}$ with α-CD (blue diamonds) and β-CD (red diamonds).

8. Cell cultures

The human malignant melanoma cell line RPMI-7951 (DSMZ no. ACC66) and the mouse fibroblast L929 were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig.

Cell culture conditions

All cells were grown as monolayers in cell culture flasks with 75 cm^2 surface and 250 mL volume (Greiner, Cat.-No.: 658175) and were cultured in Dulbecco's modified eagle's medium (DMEM) from *Sigma* with 10% fetal calf serum, 2 mM glutamine, 100 units/mL penicillin/steptomycin from *Biochrom AG* and 100 µg/mL streptomycin. All cultures were incubated in high moisture air, with 5% CO_2, at a temperature of 37°C. The medium was changed routinely 3 times a week.

Incubation and sample preparation for NMR measurements

a) Preparation of the incubation solution: For incubation, 70.5 mg of a white powder mixture containing 59.2 mg (0.148 mmol) of $Na_2B_{12}F_{12}$ and 10.9 mg (0.125 mmol) BF_4^- was dissolved in 125 mL DMEM. After addition of 197 mg (0.174 mmol) of β-cyclodextrin, the solution was sonicated for 10 minutes.
b) Cell culture flasks were taken out of the incubator, the old medium (20 mL) was removed and 20 mL of the incubation solution was added to each of 6 flasks. The cells were now incubated for 22 hours.
c) The remaining 5 mL of the incubation solution was used as reference for NMR studies.
d) After the incubation, the cell culture medium was removed (medium samples for NMR measurements were taken), cells were washed two times with 7 mL Hank's BSS (HBBS) from PAA *Laboratories GmbH* (Pasching, Austria).
e) After addition of 3 mL of a solution containing 1 mL Trypsin/EDTA and 9 mL HBBS, the cells were incubated for 5 minutes in the incubator.
f) 5mL of the standard cell culture medium were added and the cells were cleaved from the bottom of the flask. All cells (from 5 flasks) were filled in a 15 mL tube and centrifuge for 3 minutes at 800 rpm at 24°C. Cells from flask no. 6 were centrifuged separately and split afterward to three new cell culture flasks, each with 20 mL new cell culture medium.
g) After that step, the medium was removed and the cells were filled into a NMR tube.
h) Standard cell culture medium was added until measurement frame was reached. A capillary with D_2O was added for lock signal.

Toxicity tests

For toxicity tests, the human malignant melanoma cell line RPMI-7951 as well as the mouse fibroblast L929 were split into different categories: [A] cells were incubated with the standard cell culture medium (control samples); [B] cells were incubated with the ligand system solved in the standard cell culture medium; [C] cells were incubated with $Na_2B_{12}F_{12}$ solved in the standard cell culture medium; [D] cells were incubated with ligand-$Na_2B_{12}F_{12}$-complex solved in the standard cell culture medium. After incubation time and washing of the cells, these were split again (from one to three flasks). In all these cases, no notably differences could be observed in cell growing or form.

9. References

1. V. Geis, K. Guttsche, C. Knapp, H. Scherer and R. Uzun, *Dalton Trans.*, 2009, 2687-2694.
2. D. V. Peryshkov, A. A. Popov and S. H. Strauss, *J. Am. Chem. Soc.*, 2009, **131**, 18393-18403.
3. I. Tiritiris and T. Schleid, *Z. Anorg. Allg. Chem.*, 2004, **630**, 1555-1563.
4. W. H. Knoth, H. C. Miller, J. C. Sauer, J. H. Balthis, Y. T. Chia and E. L. Muetterties, *Inorg. Chem.*, 1964, **3**, 159-167.
5. M.-L. L. Watat, T. Dülcks, D. Kemken and V. A. Azov, *Tetrahedron Lett.*, 2014, **55**, 741–744.
6. T. D. Lash, J. A. El-Beck and D. A. Colby, *J. Org. Chem.*, 2009, **74**, 8830–8833.
7. J. Warneke, T. Dülcks, C. Knapp and D. Gabel, *Phys. Chem. Chem. Phys.*, 2011, **13**, 5712–5721.
8. M. H. Düker, H. Schäfer, M. Zeller and V. A. Azov, *J. Org. Chem*., 2013, **78**, 4905–4912.