Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Anion Binding by Biotin[6]uril in Water

Micke Lisbjerg, Bjarne Enrico Nielsen, Birgitte Olai Milhøj, Stephan Paul Albert Sauer and Michael Pittelkow*

Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark. E-mail: pittel@kiku.dk. Homepage: www.pittelkow.kiku.dk

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1 Apparatus

1.1 HRMS

HRMS analyses were performed on a Dionex UltiMate 3000 system coupled to an UltiMate 3000 diode array UV/Vis detector. The mobile phase solutions were 0.1 % formic acid in H_2O and 0.1 % formic acid in MeCN. The water used as eluent was purified by a Millipore system. The MS analysis (HRMS) was carried out on a Bruker MicrOTOF-QII-system with an ESI-source with the following settings: nebulizer 1.2 bar, dry gas 8.0 L min^{-1}, dry temperature 200 °C, capillary 3500 V, end plate offset -500 V, funnel 1 RF 400.0 Vpp, ISCID energy 0.0 eV, funnel 2 RF 600.0 Vpp, hexapole RF 800.0 Vpp, quadrupole ion energy 5.0 eV, low mass 300.00 *m*/z, collision energy 10.0 eV, collision RF 1600.0 Vpp, transfer time 160.0 µs, and pre puls storage 1.0 µs. The LC/MS data was processed using DataAnalysis v. 4.0 SP 5.

In the processing of HRMS measurements a sodium formate calibrant solution eluting in the first part of the HRMS-run was used to calibrate the system before each measurement.

1.2 NMR

^{1}H-NMR and ^{13}C-NMR spectra were recorded at 500 MHz and 126 MHz, respectively, on a Bruker Ultrashield Plus 500 spectrometer using residual non-deuterated solvent as the internal standard.

All NMR samples in D_2O were equipped with a DMSO-d_6 lock tube and the spectra were calibrated according to the non-deuterated DMSO signal (marked with * in the spectra). All D_2O samples contain 100 mM sodium phosphate buffer.

1.3 Isothermal Titration Calorimeter

A Microcal VP-ITC microcalorimeter was used for all titrations. The temperature was set at 30 °C and a stirring speed of 307 rpm was used. In total 56 injections of 5 μL of halide solution, with 240 s between each injection and an initial delay of 60 seconds, were used for all titrations.

1.4 Elemental analysis

Elemental analyses for C, H, N and Cl were performed with a CE Instrument: FLASH 1112 series EA, at the microanalytical laboratory, University of Copenhagen, Denmark.

1.5 Polarimeter

Optical rotation data was obtained on a Perkin Elmer 341 Polarimeter.

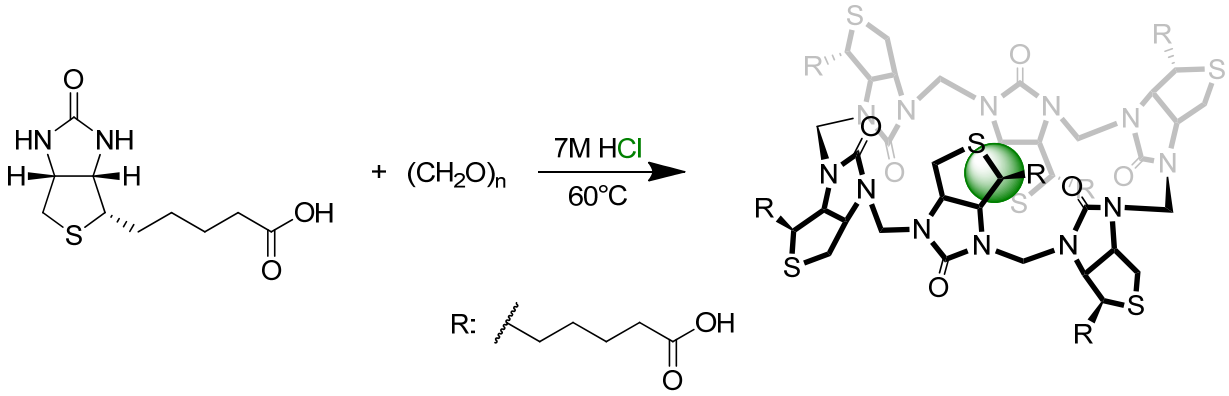
1.6 Diffractometer

All single-crystal X-ray diffraction data was collected at 122(1) K on a Bruker D8 Venture equipped with a IμS microfocus source, a KAPPA goniometer, a Oxford Cryosystems nitrogen cryostream cooling device and a PHOTON 100 CMOS, using MoKα radiation. The structures were solved using direct methods (SHELXS97) and refined using the SHELXL2013 software package.

1.7 Chemicals

Unless otherwise stated, all chemicals were purchased from commercial suppliers and used as received. Solvents were HPLC grade and used as received.

2 Biotin[6]uril-HCl:



Biotin (1.03 g ; 4.2 mmol) and para-formaldehyde (588 mg ; 19.5 mmol) were mixed in 7 M HCl (50 mL) and the heterogeneous solution was heated to 60 °C for 2 days. The solution was allowed to cool to 25 °C and water (80 mL) was added, followed by filtration. The filtrate was washed with water (4 × 20 mL). The product was dried in vacuum. Yield: 520 mg, 48 %.
M.p. > 150 °C (decomp.). $[\alpha]_D^{20}$ = -81.9 (c = 1, 0.1 M NaOH and 15 eq. NaCl).
The product was analysed by NMR in methanol-d_4 and under these conditions the carboxylic acids in Biotin[6]uril are esterified.
^{1}H-NMR (500 MHz, methanol-d_4) δ = 4.75 (s, 6H), 4.43 (s, 6H), 4.22 (dd, *J* = 9.3, 5.5 Hz, 6H), 3.40 (ddd, *J* = 11.5, 5.5, 3.1 Hz, 6H), 3.09 (dd, *J* = 12.9, 4.0 Hz, 6H), 2.98 (dd, *J* = 12.9, 6.2 Hz, 6H), 2.42 – 2.31 (m, 12H), 1.88 – 1.77 (m, 6H), 1.77 – 1.53 (m, 18H), 1.53 – 1.33 (m, 12H).
^{13}C-NMR (126 MHz, MeOD) δ = 175.84, 162.40, 63.88, 61.28, 55.18, 52.00, 51.33 (h, *J* = 22.1 Hz), 35.10, 34.63, 29.61, 29.05, 25.61.
HRMS (ESI$^+$) of the D_{18} ester: *m/z* $[M+H]^+$ calc. for $C_{72}H_{91}D_{18}N_{12}O_{18}S_6$: 1639.7496; found:1639.7431.
HRMS (ESI$^+$) of the acid: *m/z* $[M+H]^+$ calc. for $C_{66}H_{97}N_{12}O_{18}S_6$: 1537.5363; found:1537.5382.
Elemental Analysis for $C_{66}H_{97}N_{12}O_{18}S_6Cl$: [calc.] (found); C [50.35] (50.48), H [6.21] (6.09), N [10.68] (10.63), Cl [2.25] (1.90).

3 Chloride free Biotin[6]uril

Biotin[6]uril-HCl (116 mg ; 73.7µmol) was dissolved in 1 M NaOH (15 mL MilliQ), and $TlNO_3$ (29.7 mg ; 111.5 µmol) was added. The solution was stirred overnight at room temperature. The reaction mixture was filtered and acidified with conc. H_2SO_4. The product was filtered off and washed with water (3 × 10 mL). Yield: 76 mg 67.1%.
M.p. > 140 °C (decomp.). $[\alpha]_D^{20}$ = -85.6 (c = 1, 0.1 M NaOH).
^{1}H-NMR (500 MHz, D_2O): δ = 4.37 (s, 6H), 4.23 (s, 6H), 3.72 – 3.62 (m, 6H), 3.57 – 3.48 (m, 6H), 2.98 – 2.89 (m, 6H), 2.67 – 2.59 (m, 6H), 2.41 (dd, *J* = 13.0, 5.7 Hz, 6H), 1.64 (t, *J* = 7.0 Hz, 12H), 1.24 (s, 6H), 1.15 – 0.76 (m, 30H).
^{13}C-NMR (126 MHz, D_2O): δ = 182.75, 160.15, 60.96, 58.74, 53.07, 50.21, 45.80, 36.47, 32.62, 27.46, 26.47, 24.62.
Elemental Analysis for $C_{66}H_{96}N_{12}O_{18}S_6$: [calc+$H_2O$] (found+$H_2O$); C [50.95] (50.85), H [6.35] (6.22), N [10.80] (10.54).

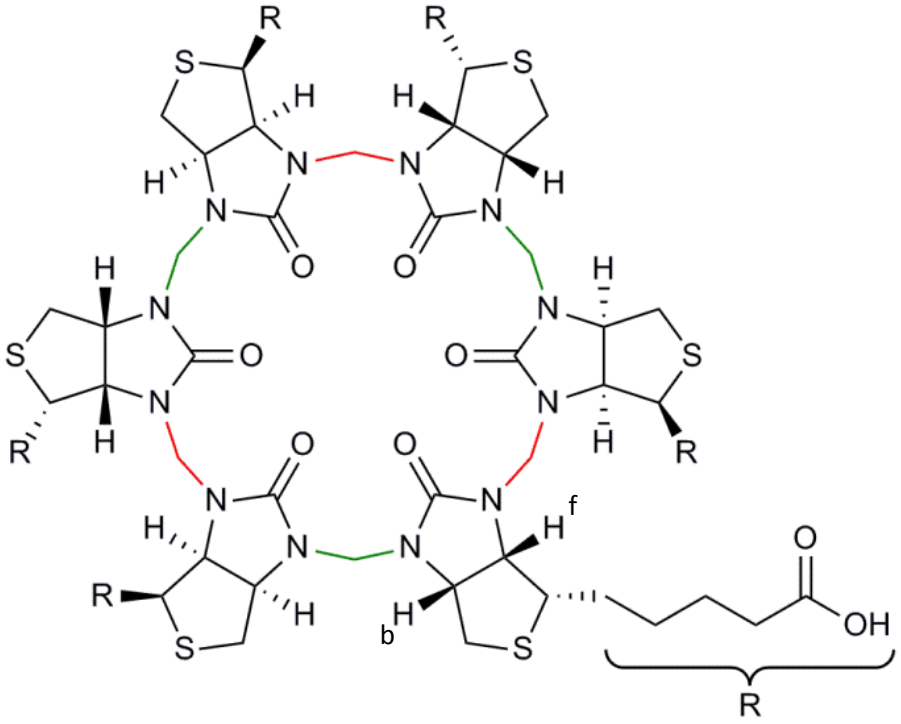


Figure S1: Schematic representation of Biotin[6]uril. The two hydrogens which are followed in the ^{1}H-NMR titration and Job plots are marked b and f.

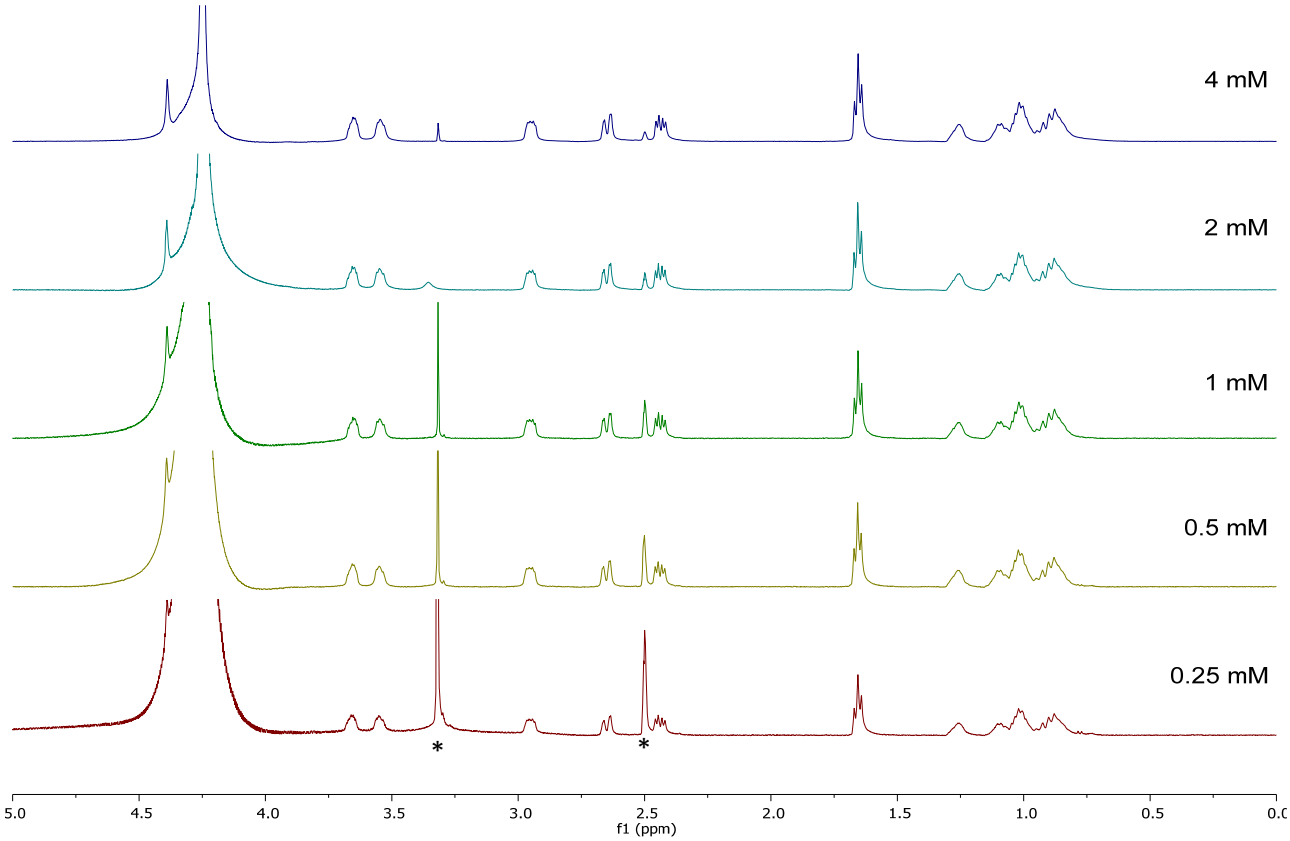


Figure S2: ^{1}H-NMR (500 MHz, D_2O) Biotin[6]uril, in 100 mM phosphate buffer pH = 7.5, at different concentrations. No aggregation is observed when diluting the sample. *External reference of DMSO-d6.

4 General Job Plot Method

A 2 mM solution of the corresponding $NaClO_4$ in 100 mM sodium phosphate buffer (D_2O) at pH 7.5 was mixed with a solution of 2 mM Biotin[6]uril in 100 mM sodium phosphate buffer (D_2O) at pH 7.5 in the ratios shown in Table S1 and each sample was analyzed by ^{1}H-NMR.

Table S1: Sample composition of Job plot.

Sample no.	µL of Biotin[6]uril	µL of NaCl solution
1	50	450
2	100	400
3	150	350
4	200	300
5	250	250
6	300	200
7	350	150
8	400	100
9	450	50
10	500	0

Table S2: Job Plot data for $NaClO_4$ and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

Sample no.	χ(Biotin[6]uril)	δ(Biotin[6]uril) (Hz)	$\Delta\delta$(Biotin[6]uril) (Hz)	χ(Biotin[6]uril) Δδ
1	0.1	1887	59	5.9
2	0.2	1877	49	9.8
3	0.3	1876	48	14.4
4	0.4	1869	41	16.4
5	0.5	1865	37	18.5
6	0.6	1855	27	16.2
7	0.7	1848	20	14
8	0.8	1842	14	11.2
9	0.9	1834	6	5.4
10	1.0	1828	0	0

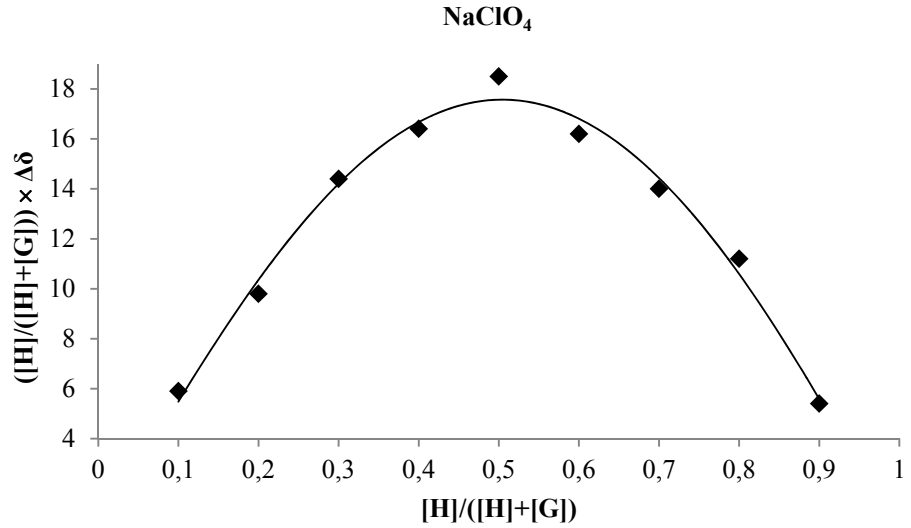


Figure S3: Job plot of $NaClO_4$ and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

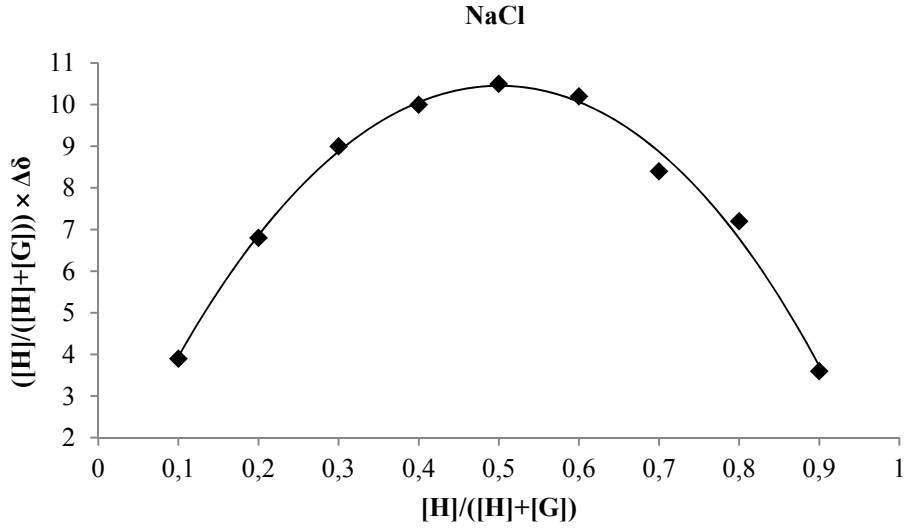


Figure S4: Job plot of NaCl and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

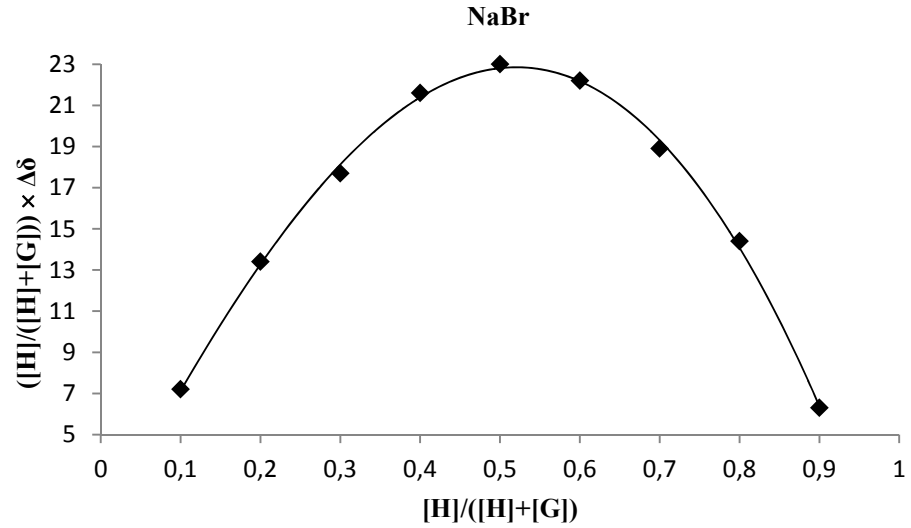


Figure S5: Job plot of NaBr and Biotin[6]uril. Proton f is followed, see figure S1 for proton notation.

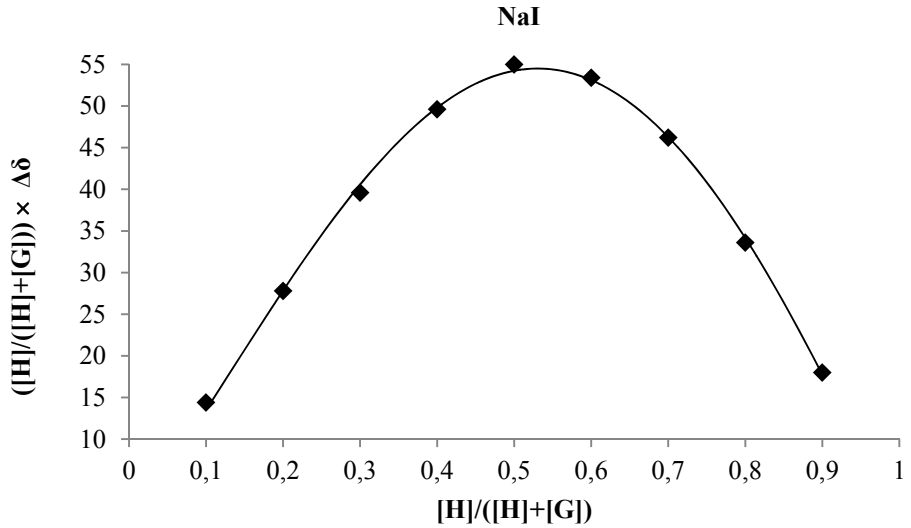


Figure S6: Job plot of NaI and Biotin[6]uril. Proton f is followed, see figure S1 for proton notation.

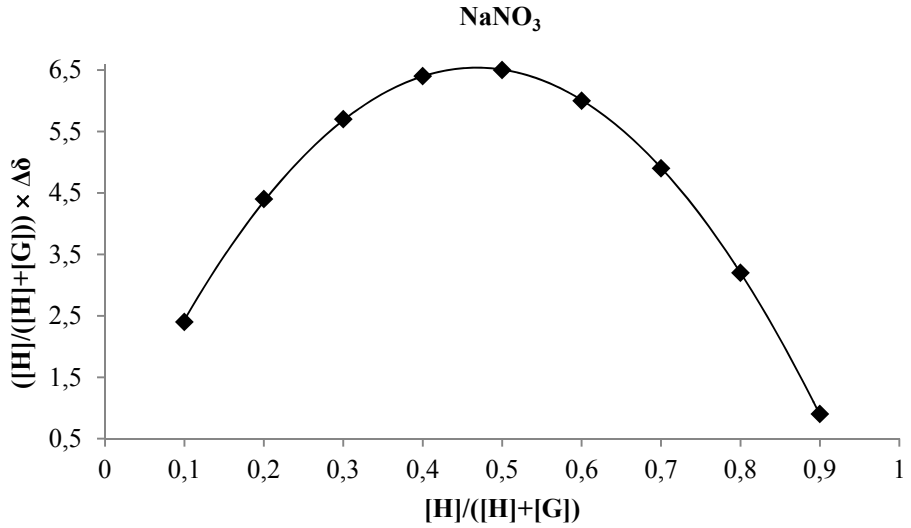


Figure S7: Job plot of $NaNO_3$ and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

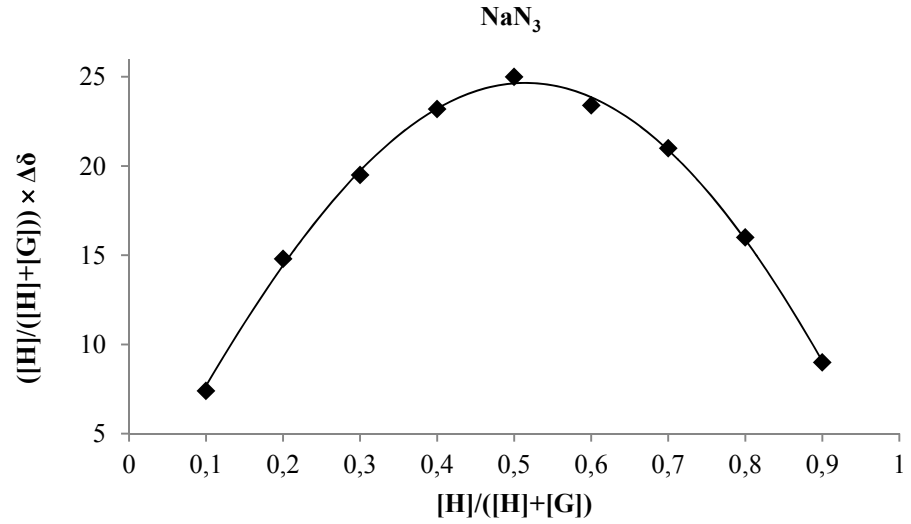


Figure S8: Job plot of NaN_3 and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

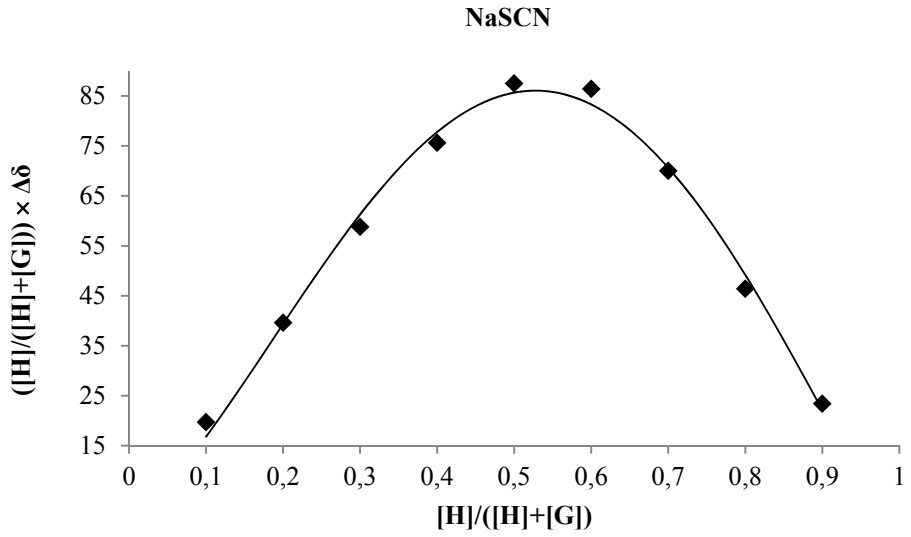


Figure S9: Job plot of NaSCN and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

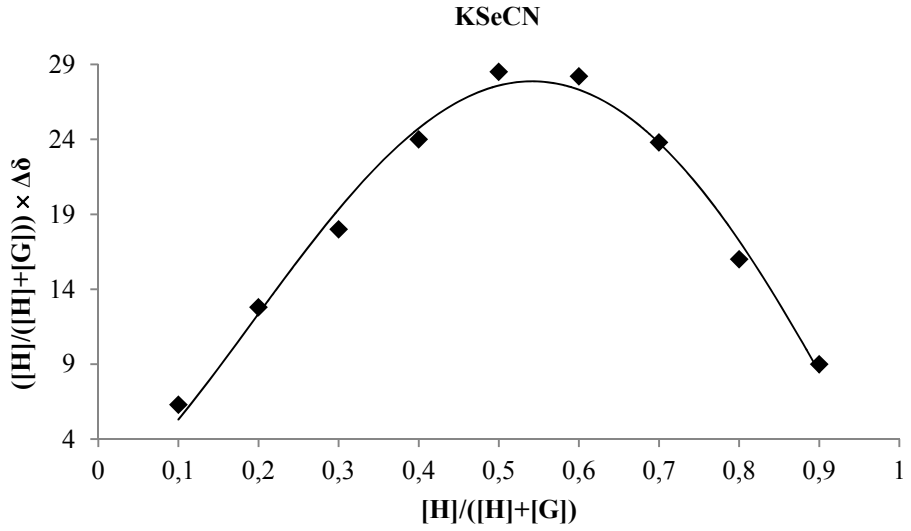


Figure S10: Job plot of KSeCN and Biotin[6]uril. Proton f is followed, see figure S1 for proton notation.

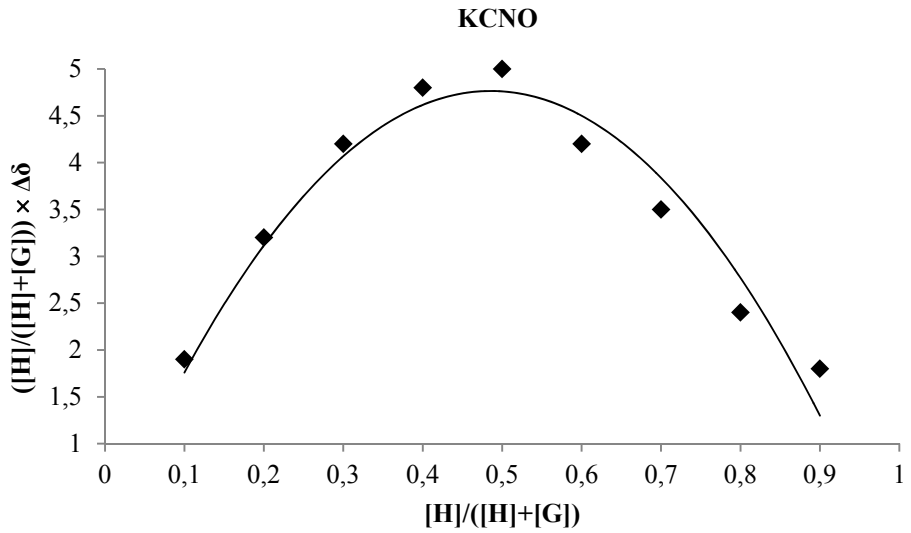


Figure S11: Job plot of KCNO and Biotin[6]uril. Proton b is followed, see figure S1 for proton notation.

5 ^{1}H-NMR Titration

The ^{1}H-NMR (500 MHz, D_2O) titrations were carried out by the following general method.
A solution containing 1.91 mM of Biotin[6]uril in 100 mM sodium phosphate buffer at pH = 7.5 was added aliquots of the corresponding salt while keeping the Biotin[6]uril concentration an pH constant. The change in ^{1}H-NMR of either proton b or f (see figure S1 for notation) was plotted and fitted, using Origin 8.6.0 and the same procedure as previously reported.[1]

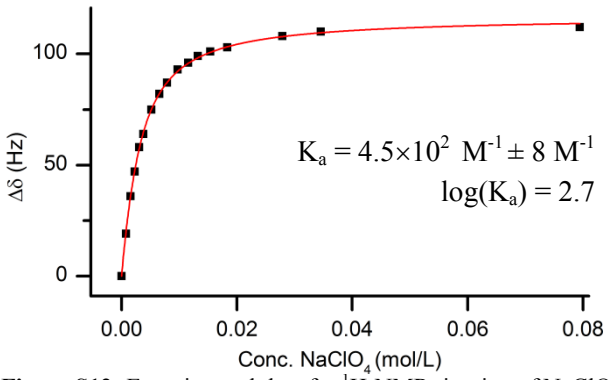


Figure S12: Experimental data for ^{1}H-NMR titration of $NaClO_4$ and Biotin[6]uril (1.91 mM). Black: Δδ of proton b. Red: fitted curve.

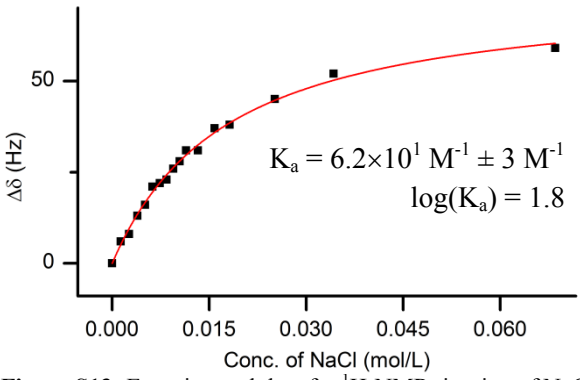


Figure S13: Experimental data for ^{1}H-NMR titration of NaCl and Biotin[6]uril (1.91 mM). Black: Δδ of proton f. Red: fitted curve.

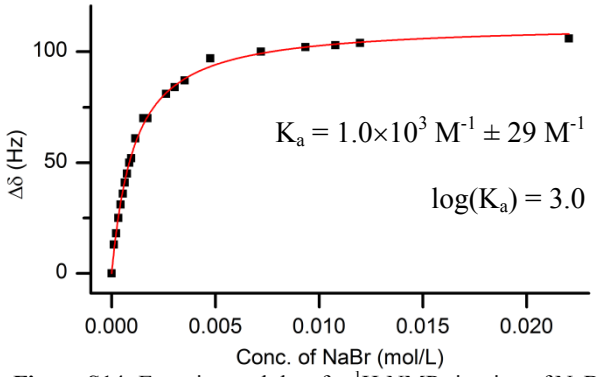


Figure S14: Experimental data for ^{1}H-NMR titration of NaBr and Biotin[6]uril (0.20 mM). Black: Δδ of proton f. Red: fitted curve.

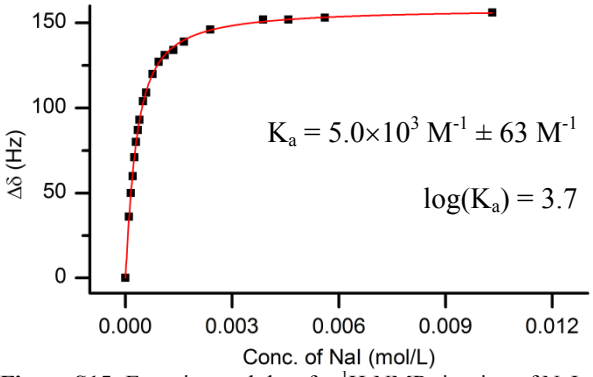


Figure S15: Experimental data for ^{1}H-NMR titration of NaI and Biotin[6]uril (0.20 mM). Black: Δδ of proton f. Red: fitted curve.

[1] M. Lisbjerg, B. M. Jessen, B. Rasmussen, B. E. Nielsen, A. Ø. Madsen, M. Pittelkow, *Chem. Sci.*, **2014**, *5*, 2647-2650.

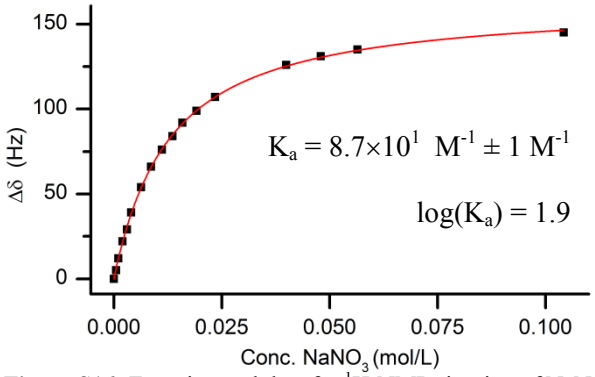


Figure S16: Experimental data for ^{1}H-NMR titration of $NaNO_3$ and Biotin[6]uril (2.01 mM). Black: Δδ of proton b. Red: fitted curve.

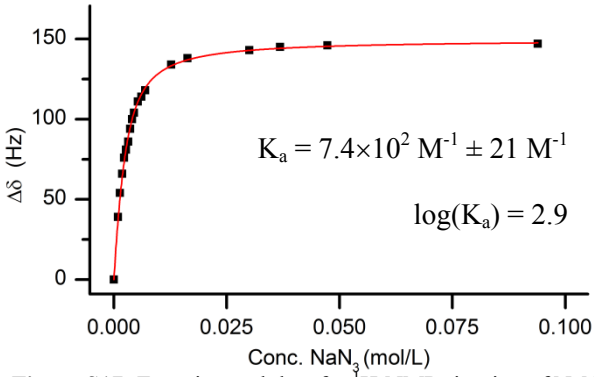


Figure S17: Experimental data for ^{1}H-NMR titration of NaN_3 and Biotin[6]uril (2.01 mM). Black: Δδ of proton b. Red: fitted curve.

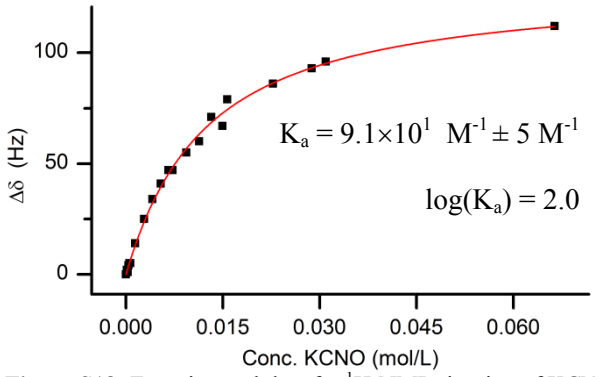


Figure S18: Experimental data for ^{1}H-NMR titration of KCNO and Biotin[6]uril (2.01 mM). Black: Δδ of proton b. Red: fitted curve.

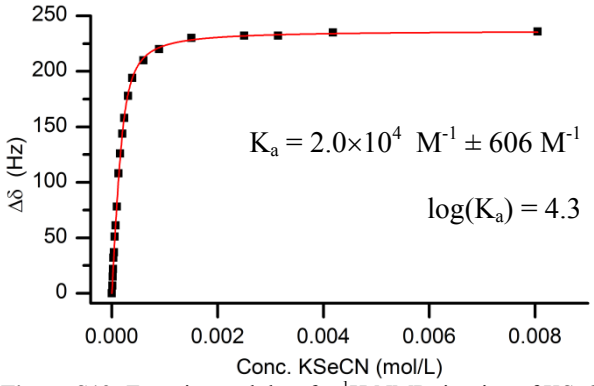


Figure S19: Experimental data for ^{1}H-NMR titration of KSeCN and Biotin[6]uril (0.20 mM). Black: Δδ of proton b. Red: fitted curve.

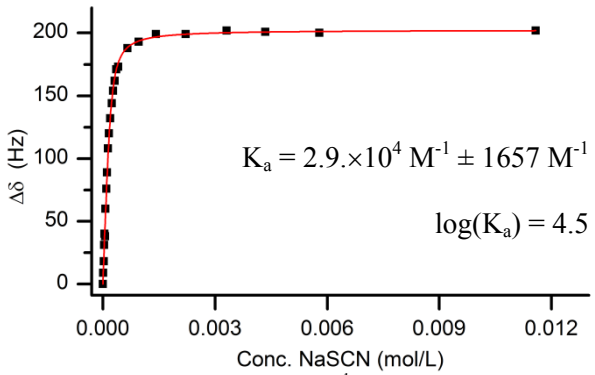


Figure S20: Experimental data for ^{1}H-NMR titration of NaSCN and Biotin[6]uril (0.20 mM). Black: Δδ of proton b. Red: fitted curve.

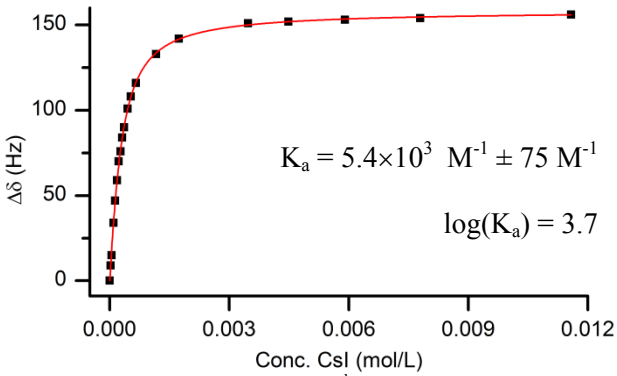


Figure S21: Experimental data for ^{1}H-NMR titration of CsI and Biotin[6]uril (0.20 mM). Black: Δδ of proton f. Red: fitted curve.

6 ITC Titrations

A 896 µM solution Biotin[6]uril in a sodium phosphate buffer pH = 7.5 was loaded into the cell of the ITC. The salts were dissolved in 100 mM phosphate buffer pH = 7.5 (for concentration see Table S3). The temperature was set at 30 °C and the stirr speed was 307 rpm. A total of 56 injections were done with a 240 s delay between each injection. Dilution experiments for the Biotin[6]uril and the salts were run under the same parameters, and subtracted from the raw data. The raw data was analyzed using Origin and fitted by the routines provided by MICROCAL. A low value c fitting procedure with reduced χ^2 was used to fit the data.[2]

Table S3: Concentration of samples for ITC

$NaClO_4$	NaCl	NaBr	NaI	$NaNO_3$	NaN_3	KCNO	KSeCN	NaSCN
89.9 mM	418 mM	74.4 mM	21.8 mM	401 mM	75.7 mM	140 mM	14.4 mM	12.0 mM

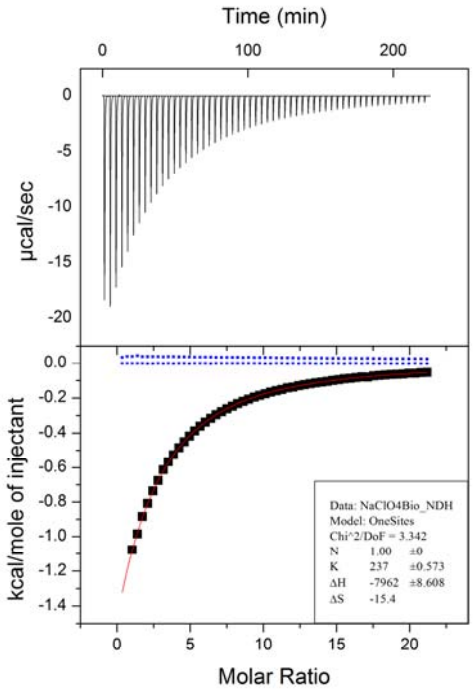


Figure S22: Data for the titration of Biotin[6]uril with $NaClO_4$ at pH 7.5. Fitted curve (red), dilution subtracted (blue).

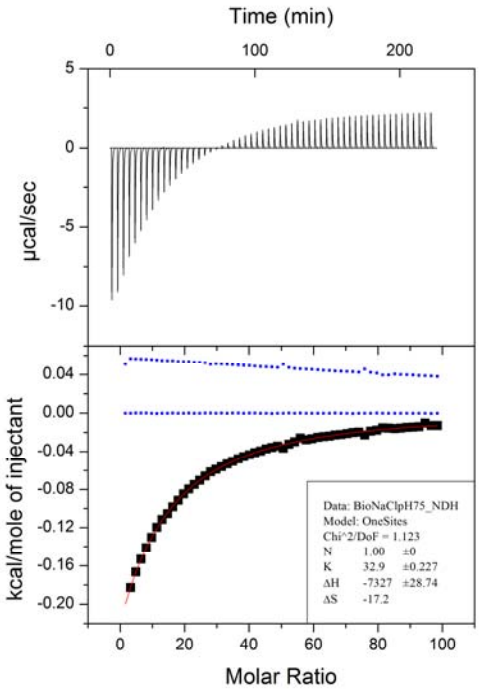


Figure S23: Data for the titration of Biotin[6]uril with NaCl at pH 7.5. Fitted curve (red), dilution subtracted (blue).

[2] *J. Am. Chem. Soc.*, **2003**, *125* (48), 14859–14866.

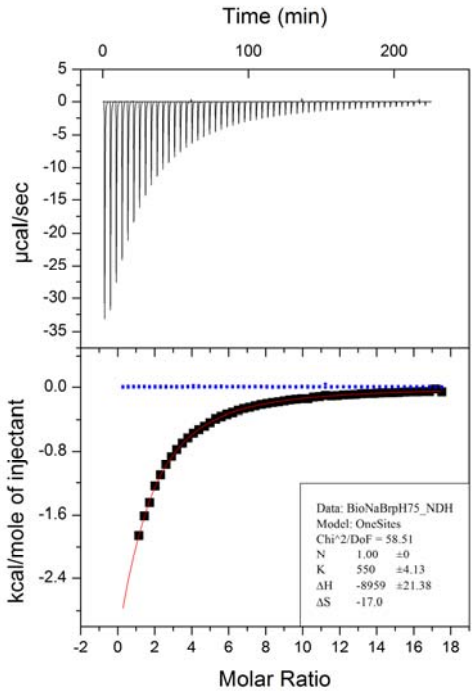


Figure S24: Data: for the titration of Biotin[6]uril with NaBr at pH 7.5. Fitted curve (red), dilution subtracted (blue).

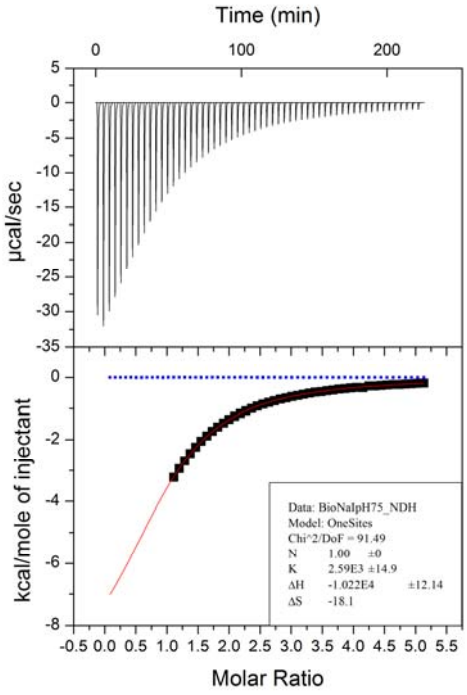


Figure S25: Data for the titration of Biotin[6]uril with NaI at pH 7.5. Fitted curve (red), dilution subtracted (blue).[3]

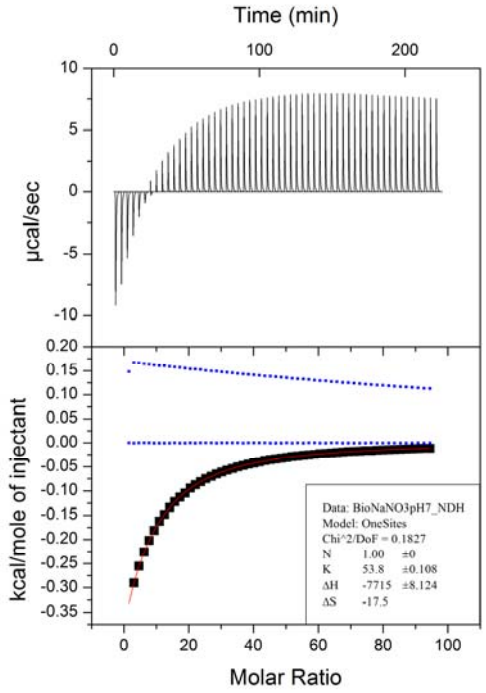


Figure S26: Data for the titration of Biotin[6]uril with $NaNO_3$ at pH 7.5. Fitted curve (red), dilution subtracted (blue).

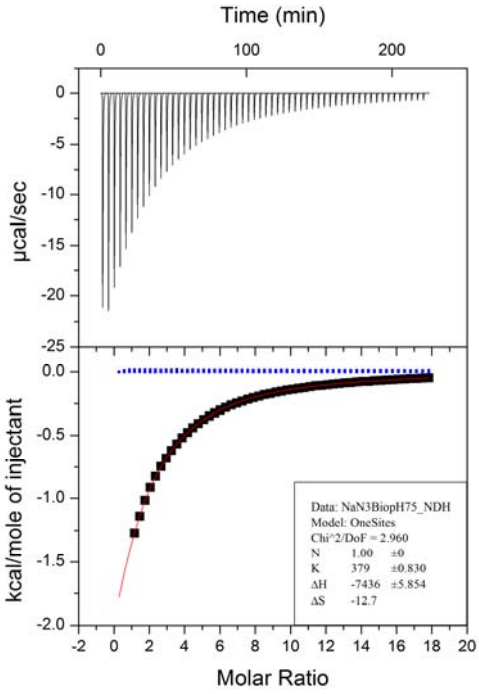


Figure S27: Data for the titration of Biotin[6]uril with NaN_3 at pH 7.5. Fitted curve (red), dilution subtracted (blue).

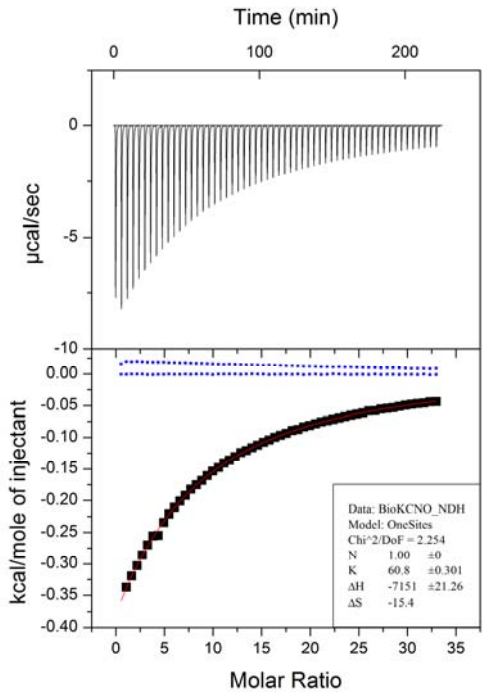


Figure S28: Data for the titration of Biotin[6]uril with KCNO at pH 7.5. Fitted curve (red), dilution subtracted (blue).

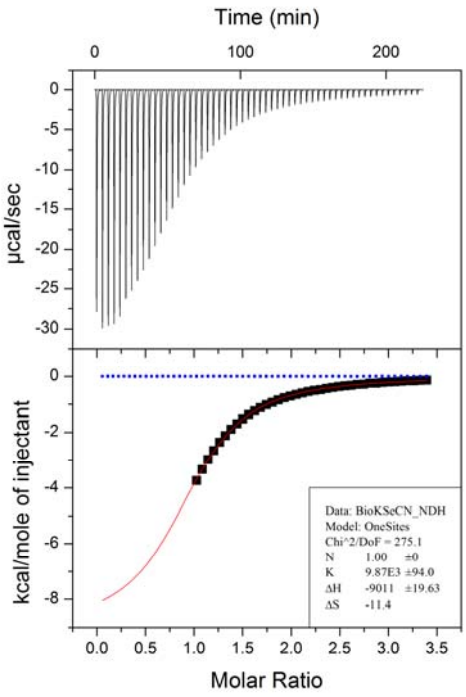


Figure S29: Data for the titration of Biotin[6]uril with KSeCN at pH 7.5. Fitted curve (red), dilution subtracted (blue).[3]

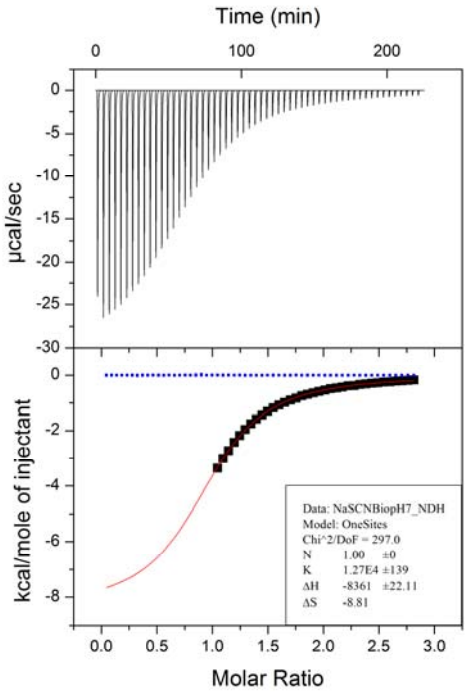


Figure S30: Data for the titration of Biotin[6]uril with NaSCN at pH 7.5. Fitted curve (red), dilution subtracted (blue).[3]

[3] The data was also fitted with all points below 1 eq. included together with N = 1. This gave similar values in K_a but with higher χ^2. Therefore a low value c was also employed here.

7 HRMS

All high resolution mass spectrometry samples were prepared from a stock solution of Biotin[6]uril (94.9 μM) in a $CH_3CO_2NH_4$ buffer (45.9 mM, pH = 7.6). 5 μL of each sample was injected directly in to the MS.

	Measured mass (m/z)	Calculated mass (m/z)	Error (ppm)	Conc. of template in sample (mM)
No template	1535.5193	1535.5212	1.2	-
$NaClO_4$	1635.4791	1635.4775	1.0	82
NaCl	1571.4933	1571.4933	2.9	100
NaBr	1615.4432	1615.4473	2.5	36
NaI	1663.4294	1663.4224	2.4	19
KSeCN	1642.4456	1642.4493	1.8	37
NaSCN	1594.4999	1594.5041	2.6	72

It was not possible to obtain the excat mass of Biotin[6]uril with the NO_3^-, N_3^-, CNO^-, under the same condition as used for the other templates.

8 Crystal Structure

The H_2O containing crystal of Biotin[6]uril, was produced for single crystal x-ray diffraction by slow evaporation of an ethanol solution containing the Biotin[6]uril-HCl complex.

$C_{66}H_{96}N_{12}O_{18}S_6$ 2(H_2O); M = 1571.92; Monoclinic; a = 12.2582(11) Å, b = 16.6588(13) Å, c = 20.6422(17) Å, α = 90°, β = 101.775°(3), γ = 90°; V = 4126.6(6) $Å^3$; T = 122 K; space group $P2_1$; Z = 2; μ(Mo-Kα) = 0.07 mm^{-1} ; 58643 reflections measured, 16755 independent reflections (Rint = 0.1066). The final R1 value was 0.080 [$F^2 > 2\sigma(F^2)$]. The final R1 value was 0.137 (all data). The final wR(F^2) (all data) value was 0.231. The goodness of fit on F^2 was 0.964

The structure contained disordered solvent molecules; these were removed using the solvent masking procedure as implemented in the olex2 software package. It was furthermore found that the unit cell contained two solvent accessible voids each covering a volume of 199 $Å^3$, each with an electron count corresponding to one water molecule.

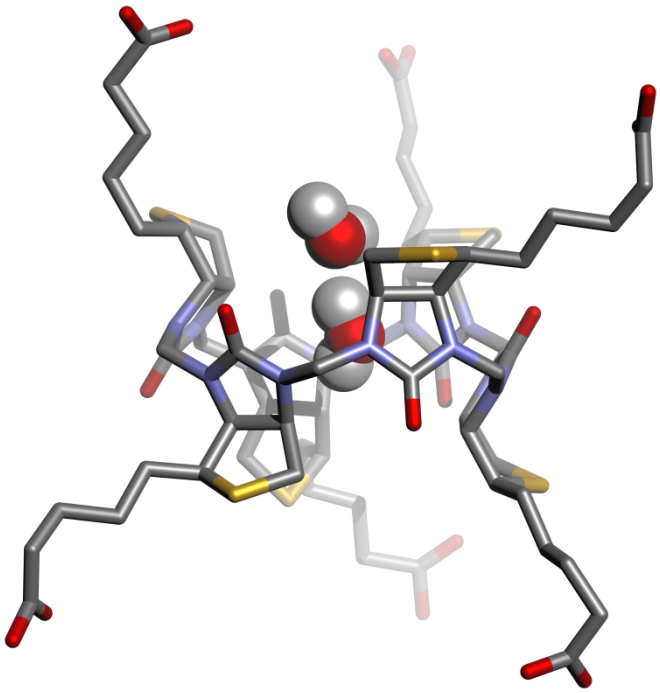


Figure S31: Structure of the Biotin[6]uril H_2O containing complex as seen from the side. Hydrogen atoms, disordered atoms and lattice solvent molecules have been omitted for clarity. Yellow: sulphur; Blue: nitrogen; Gray: carbon; Red: oxygen.

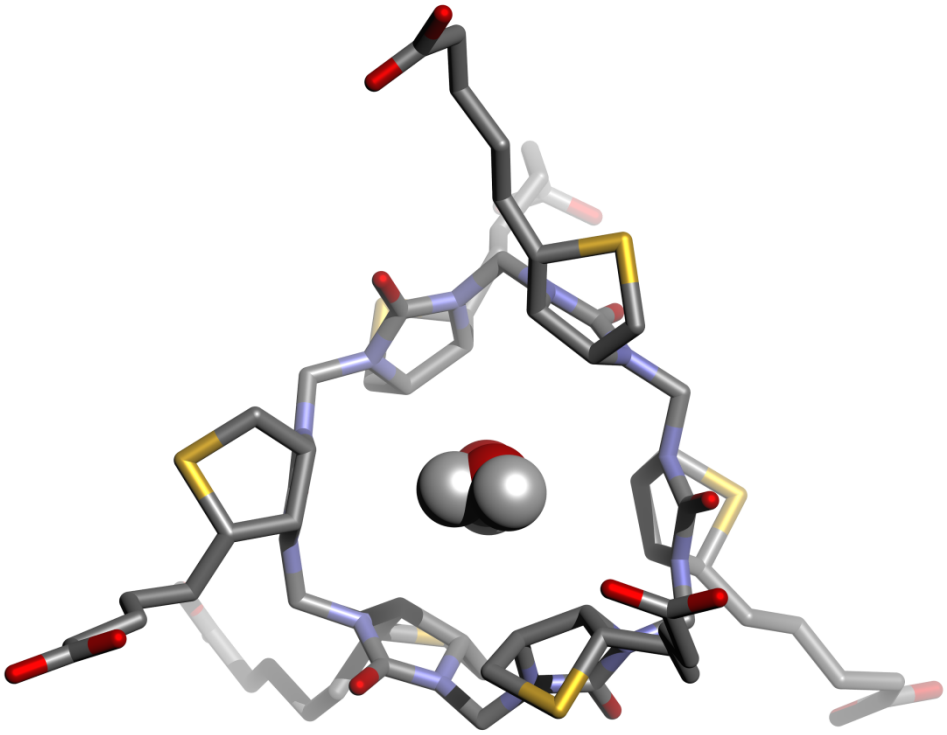


Figure S32: Structure of the Biotin[6]uril H_2O containing complexas seen from the top. Hydrogen atoms, disordered atoms and lattice solvent molecules have been omitted for clarity. Yellow: sulphur; Blue: nitrogen; Gray: carbon; Red: oxygen.

The crystal structure can be obtained free of charge at
http://www.ccdc.cam.ac.uk/Community/Requestastructure/Pages/DataRequest.aspx?
CCDC nr: 1029718

9 Internal volume of Biotin[6]uril

To calculate the volume of the crystal structures of Biotin[6]uril, the centre of the two circles made from the six C-H protons pointing in the cavity above and beneath the equator was used as the centre to calculate the radius of the cylinder. This was done with the "calculate centroid" function in Mercury CSD 3.3 RC5.

The radius was found using the average distance from the top centroid to the top six C-H and the bottom centroid to the bottom six C-H distances. As the height of the cylinder the distance between the two centroids were used.

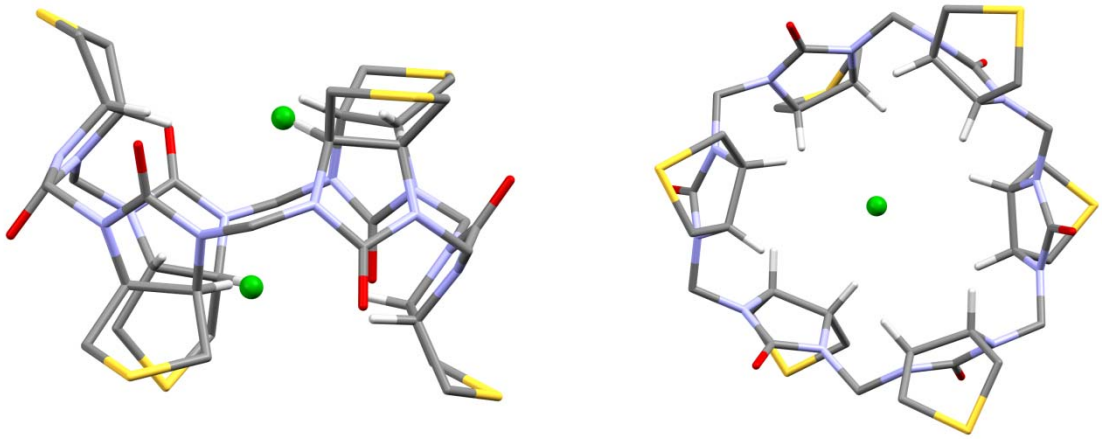


Figure S333: Schematic representation of Biotin[6]uril (side view (left) and topview (right)). Only the hydrogens used in the calculation are show, and the alkyl chains and non-binding hydrogens atines are removed for clarity. The centroids for the two circles above and below equator are marked with green. Yellow: sulphur; Blue: nitrogen; Gray: carbon; Red: oxygen.

Biotin[6]uril guest	Radius (Å)	Height (Å)	Volume (Å)
H_2O	2.76	3.59	85.79
NaI	2.81	3.74	92.97
EtOH	2.88	3.91	101.81