Supramolecular hosts that recognize methyllysines and disrupt the interaction between a modified histone tail and its epigenetic reader protein

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Supporting Information

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Experimental procedures — General, Titration conditions, Synthesis, FRET assay

General

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded at 500 MHz, 360 MHz or 300 MHz at 23 °C unless otherwise stated. Proton chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane, and are referenced to residual proton in the NMR solvent ($CHCl_3$ δ 7.26; DOH δ 4.79; Acetone-d5 δ 2.05). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, sext = sextet, m = multiplet and/or multiple resonances, br = broad), integration and coupling constant in Hertz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded at 125 MHz, 90 MHz or 75 MHz at 23 °C, unless otherwise stated. Carbon chemical shifts are reported in parts per million downfield from tetramethylsilane and are referenced to the carbon resonances of the solvent. Infrared (IR) spectra were obtained using a Perkin Elmer 1000 FT-IR spectrometer. Data are represented as follows: frequency of absorption (cm^{-1}), intensity of absorption (s = strong, m = medium, w = weak, br = broad). ESI-MS data were obtained on a Finnegan LCQ-Trap. Melting points were collected on a

Gallenkamp Melting Point apparatus. Peptide identity was confirmed by mass spectrometry on a Finnegan LCQ-Trap or Micromass Q-ToF II in ESI mode.

All chemicals were supplied by Sigma-Aldrich and used as received, unless otherwise indicated. *p*-sulfonatocalix[4]arene, (**1**) was purchased from TCI America. Fmoc-Lys(Me_3)-OH was purchased from GL Biochem. Dialysis was performed using Spectra/Por Float-A-Lyzer with a MWCO of 100-500 Da.

HPLC Purification:
All reactions purified by reverse phase HPLC were performed on a 250 mm x 22 mm preparative C18 Alltech Apollo 10 µm column on a Shimadzu HPLC detecting at 280 nm.

Microwave Conditions
Microwave reactions (where indicated) were performed on a Biotage Initiator microwave in a heavy walled glass microwave vial.

ITC Titrations
ITC titrations were performed on a Microcal VP-ITC (GE Healthcare). Titrations were carried out at 303 K in buffered H_2O (40 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) by titrating 1-10 mM solution of calixarene into a 0.07-0.14 mM solution of peptide. Binding curves were produced using the supplied Origin software and fit using a 1-sites binding model. For weak host-guest interactions N was fixed to 1.00 to produce a satisfactory fit.

NMR Titrations
All titrations were performed in 40 mM Na_2HPO_4/NaH_2PO_4 D_2O (pD 7.0 = pH 7.4) buffer. Receiving solutions (0.14-1 mM, peptide) were made by weighing peptide and dissolving it in buffer and using this solution to make titrant solutions (2-50 mM, calix[4]arene). Titrations were performed on 500 MHz or 360 MHz NMR at 23 °C by titrating calix[4]arene solution (via micropipette) into peptide in increasing amounts.

Experimental
Peptide Synthesis
Peptide synthesis was performed using standard Fmoc solid phase synthesis. All peptides were prepared with a C-terminal tyrosine to aid in purification as a UV-absorbent handle. All peptides were prepared acetylated at the N-terminus (except for H3K4 peptide which was prepared as the N-terminal primary amine as seen in nature), and C-terminal primary amide. Fmoc-NovaPEG Rink Amide resin (EMD Chemicals) was swollen in DCM overnight and was deprotected with 20% piperidine in DMF and washed with DMF. N^{α}-Fmoc and sidechain protected amino acids (5 eq., EMD Chemicals, ChemImpex and Advanced ChemTech) were activated and loaded onto the resin with HBTU (4.9 eq.) in 3 mL of DMF

with DIEA (10 eq.) and shaken for 45 minutes. Fmoc deprotection was performed using 20% piperidine in DMF (3 x 10 minutes). The N-terminus was acetylated using a 30:20:50 pyridine/acetic anhydride/DCM mixture for 2 hours. The peptide was cleaved from the resin with a 95:2.5:2.5 solution of TFA/H_2O/triisopropylsilane, and the resin was washed with additional TFA (3 x 5 mL). This TFA solution was concentrated *in vacuo* and peptide was precipitated with the addition of cold diethyl ether. After centrifugation and decanting, peptides were dried *in vacuo* overnight then purified by preparative RP-HPLC using a gradient of 0.1% TFA in H_2O and 0.1% TFA in acetonitrile (90:10 to 10:90, H_2O:Acetonitrile). Peptide identities were confirmed with ESI-MS.

Histone 3 Lysine 4 Peptide
H_2N-ARTKQTAY-C(O)NH_2: retention time: 25.3 min. ESI-MS: $[M+H]^+$ 938.6
Calculated: $[M+H]^+$ 938.1
H_2N-ARTK(me_3)QTAY-C(O)NH_2: retention time: 12.8 min. ESI-MS: $[M]^+$ 979.5
Calculated: $[M]^+$ 979.1

Histone 3 Lysine 9 Peptide
Ac-TARKSTGY-C(O)NH_2: retention time: 14.0 min. ESI-MS: $[M+H]^+$ 924.4
Calculated: $[M+H]^+$ 924.0
Ac-TARK(me_3)STGY-C(O)NH_2: retention time: 14.0 min. ESI-MS: $[M]^+$ 966.6
Calculated: $[M]^+$ 966.0

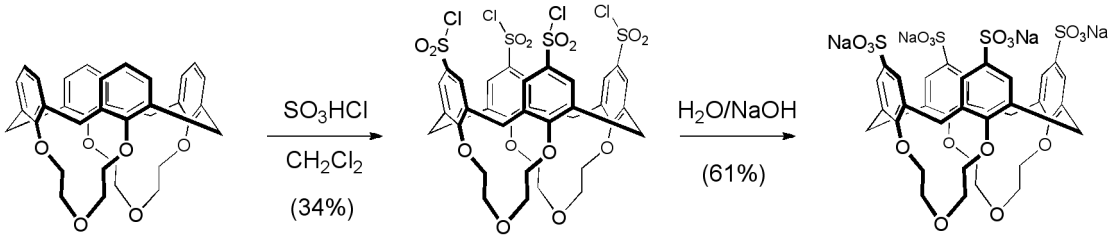
Histone 3 Lysine 27 Peptide
Ac-AARKSAPY-C(O)NH_2: retention time: 17.7 min. ESI-MS: $[M+H]^+$ 904.3
Calculated: $[M+H]^+$ 904.1
Ac-AARK(me_3)SAPY-C(O)NH_2: retention time: 23.1 min. ESI-MS: $[M]^+$ 946.4
Calculated: $[M]^+$ 946.1

Histone 3 Lysine 36 Peptide
Ac-GGVKKPHY-C(O)NH_2: retention time: 15.6 min. ESI-MS: $[M+H]^+$ 926.7
Calculated: $[M+H]^+$ 926.1
Ac-GGVK(me_3)KPHY-C(O)NH_2: retention time: 16.4 min. ESI-MS: $[M]^+$ 968.1
Calculated: $[M]^+$ 968.1

Calixarene Synthesis
All reactions were carried out under nitrogen unless otherwise indicated. **5**[1], **7**[1], **8**[1] and **10**[2] are known compounds.

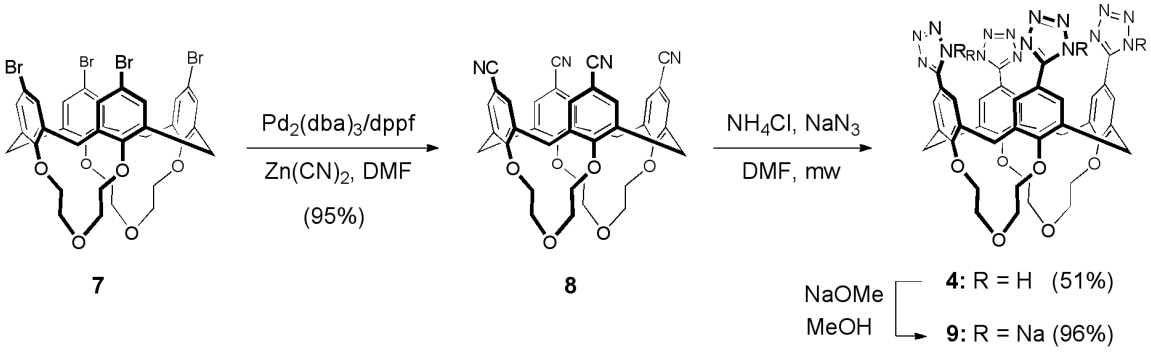
The rigidified biscrown calix[4]arene **5** was converted to its chlorosulfonylated product **6** upon reaction with chlorosulfonic acid. Large quantities of pure **6** proved difficult to isolate. Its poor solubility in most organic solvents precluded conventional chromatographic techniques, and yields were consistently below 35%. Nevertheless, the product was then hydrolyzed with sodium hydroxide and

purified by HPLC leaving the pure sulfonate salt **3** in excellent yield. The advanced biscrown-tetrabromocalix[4]arene **7**, available in one step from **5** was cyanated under palladium mediated conditions. Subjecting **8** to microwave irradiation in the presence of excess NH_4Cl and NaN_3 yielded **4** which was then converted to its sodium salt using NaOMe in 49% yield over 2 steps. Known chlorosulfonyl calix[4]arene **10** was hydrolyzed with water in the presence of pyridine and converted to its sodium salt. Low molecular weight impurities were dialysed out of the mixture leaving pure **2** in 61% overall yield.



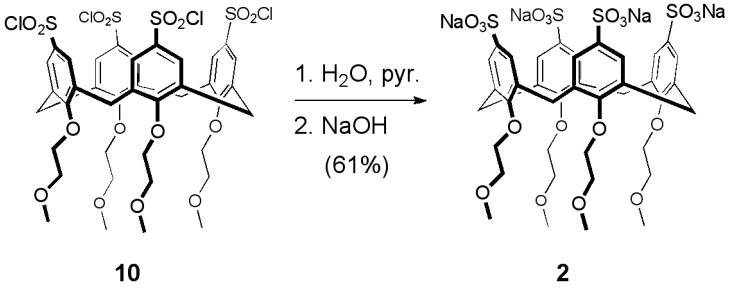
5 **6** **3**

Scheme 1: Synthesis of sulfonate functionalized biscrown-calix[4]arene **3**.



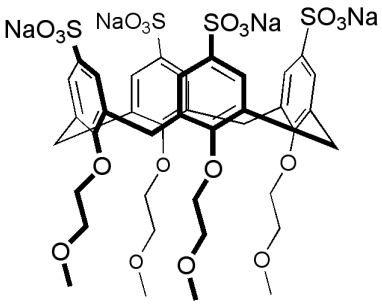
7 **8**

Scheme 2: Synthesis of tetrazole functionalized biscrown-calix[4]arene **4**.

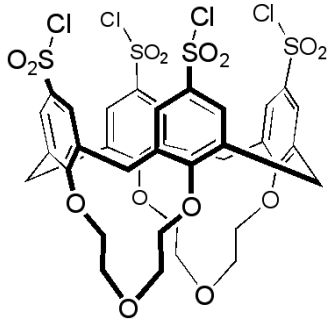


10 **2**

Scheme 3: Synthesis of sulfonate functionalized tetrakis(ethoxymethoxy) calix[4]arene **2**.

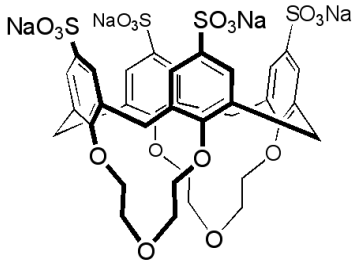


25,26,27,28-Tetrakis(ethoxymethoxy)-5,11,17,23-tetrakis(sulfonato)calix[4]arene (**2**). Chlorosulfonylated calix[4]arene **10** (70 mg, 0.067 mmol) was added to H_2O (1 ml) in pyridine (4 ml). The mixture was stirred at ambient temperature for 2h. All solvents were removed *in vacuo* and the resulting residue dissolved in minimal 10% NaOH. The mixture was subjected to aqueous dialysis for 24hr against pure H_2O. The water was removed *in vacuo* leaving pure **2** (44 mg, 61%) as a white solid. mp: 243-246°C (dec). IR (KBr pellet): 2925m, 1654w, 1467m, 1193s, 1118s, 1051s, 1036s, 655m, 620m; ^{1}H NMR (D_2O, 360 MHz): δ 3.41 (s, 12 H), 3.47 (d, 4 H, *J* = 13.5 Hz), 3.94 (t, 8 H, *J* = 4.8 Hz), 4.28 (t, 8 H, *J* = 4.3 Hz), 4.55 (d, 4 H, *J* = 13.4 Hz), 7.33 (s, 8 H); ^{13}C NMR (D_2O, 90 MHz): δ 30.6, 58.0, 72.0, 73.2, 126.0, 135.0, 137.0, 158.5. LR-ESI-MS: expected for $C_{40}H_{47}O_{20}S_4^-$ 975.2, found 975.8.

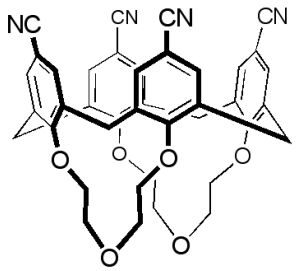


5,11,17,23-Tetrachlorosulfonyl-25,26,27,28-biscrown-3-calix[4]arene (**6**). Adapted from a previously reported procedure.[2] SO_3HCl (1.1 ml, 15.9 mmol) was cooled in an ethylene glycol/CO_2 bath under argon. **5** (200mg, 0.35 mmol) in 5 ml of dry CH_2Cl_2 was added over 1 h via syringe pump. The mixture was allowed to warm to ambient temperature and stirred for an additional 6 h. The reaction was quenched by pouring over 30 g of ice. A viscous brown precipitate immediately formed, was separated from the liquid phases and air-dried. It was then triturated in 1:1 MeOH:CH_2Cl_2 and the insolubles were filtered and air-dried affording **6** (67 mg, 34%) as a pale brown solid. mp: 238-242°C (dec). IR (KBr thin film): 2995m, 1695w, 1452w, 1373m, 1270m, 1222s, 1167s, 1050m, 887w, 612w, 551w; ^{1}H NMR (Acetone-d_6, 300 MHz): δ 3.85 (d, *J* =13.0 Hz, 2 H), 3.91 (d, *J* = 12.6 Hz, 2 H), 3.85 (td, 4 H, ^1J = 10.6 Hz, ^2J = 2.7 Hz), 4.25-4.50 (m, 8 H), 4.64-4.73 (m, 4 H), 4.85 (6, 2 H, *J* = 12.9 Hz), 5.51 (d, 2 H, *J* = 12.6 Hz), 7.99 (d,

4 H, *J* = 2.5 Hz), 8.05 (d, 4 H, *J* = 2.5 Hz) ; ^{13}C NMR (Acetone-d_6, 90 MHz): δ 30.4, 30.5, 74.1, 781, 128.8, 129.6, 130.0, 138.1, 140.1, 162.7. Poor solubility, low ionizability and reactivity precluded the acquisition of a mass spectrum.

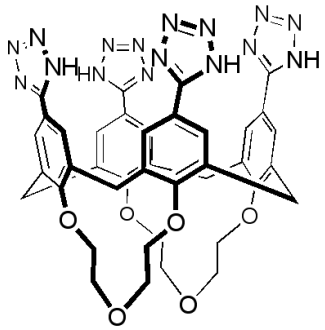


5,11,17,23-Tetrasulfonato-25,26,27,28-biscrown-3-calix[4]arene (**3**). A flask containing calixarene **6** (99mg, 0.10 mmol) in 2M NaOH (5 ml) was placed in an oil bath set to 50°C and stirred for 2h. All solvents were removed *in vacuo* and the crude product was purified by HPLC (C18 reverse phase column, 0.1% TFA in H_2O – 0.1% TFA in MeCN gradient). The solvents were removed *in vacuo* leaving pure **3** (59 mg, 61%) as a white solid. mp: 248-251°C (dec). IR (KBr pellet): 2927 w, 1686s, 1444s, 1395,w, 1211s, 1139s, 1058m, 845m, 804m, 726m, 667m, 631m; ^{1}H NMR (D_2O, 300 MHz): δ 3.50-3.62 (m, 4 H), 3.78-3.96 (m, 4 H), 4.28-4.54 (m, 12 H), 4.65 (d, 2 H, *J* = 12.6 Hz), 5.10 (d, 2 H, *J* = 12.5 Hz), 7.60 (m, 8H); ^{13}C NMR (D_2O, 90 MHz): δ 29.6, 30.2, 74.2, 76.2, 126.0, 126.7, 136.2, 136.3, 138.1, 157.8. LR-ESI-MS: expected for $C_{36}H_{35}O_{18}S_4^-$ 883.1, found 883.6.



5,11,17,23-Tetracyano-25,26,27,28-biscrown-3-calix[4]arene (**8**). Adapted from a previously reported procedure.[3] Calixarene **7** (250 mg, 0.37 mmol), $Pd_2(dba)_3$ [tris(dibenzylideneacetone)dipalladium(0)] (26.1 mg, 0.029 mmol), dppf (35 mg, 0.063 mmol), and $Zn(CN)_2$ (550 mg, 4.6 mmol) were added to an oven dried Schlenk tube. The vessel was evacuated and purged with N_2 three times. Anhydrous DMF (4 ml) was added and the mixture placed in an oil bath set to 145°C for 48h with vigorous stirring. The mixture was allowed to cool to ambient temperature and was transferred to a round bottom flask with EtOAc. All solvents were removed *in vacuo* and the crude black product was purified (SiO_2, 15% EtOAc in CH_2Cl_2) leaving 180 mg (95%) of a brown solid. ^{1}H NMR (Acetone-d_6, 300 MHz): δ 3.48 (d, 2 H, *J* = 12.4 Hz), 3.55 (d, 2 H, *J* = 12.6 Hz), 3.86 (td, 4 H, ^1J = 10.3 Hz, ^2J = 2.7 Hz), 4.24-4.42 (m, 4 H), 4.43-4.55 (m, 4 H), 4.67 (d, 2 H, *J* = 12.0 Hz), 5.28 (d, 2 H, *J* = 12.3 Hz), 7.72 (m, 8 H); ^{13}C NMR

(Acetone-d_6, 90 MHz): δ 29.3, 30.1, 74.1, 77.0, 108.0, 118.8, 132.9, 133.9, 136.2, 136.4, 159.3.
All spectral data match the literature.[1]



5,11,17,23-Tetratetrazolyl-25,26,27,28-biscrown-3-calix[4]arene (**4**). Adapted from a previously reported procedure.[4] Calixarene **8** (50 mg, 0.075 mmol), NaN_3 (234 mg, 3.6 mmol), NH_4Cl (200 mg, 3.6 mmol) and DMF (1ml) were added to a microwave vial. The vessel was purged with argon, sealed, vortexed at high speed for 1 min and placed in a microwave reactor for 1 h at 110 °C. The mixture was transferred to separatory funnel with 50 ml of saturated $NaHCO_3$ and washed with 30 ml EtOAc. The aqueous layers were acidified to pH<1 with conc. HCl and extracted with EtOAc (3 x 30 ml). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting brown solid was triturated in CH_2Cl_2 and the insolubles were filtered and air-dried affording pure **4** (32 mg, 51%) as a pale brown solid. mp: 250-254°C (dec). IR(KBr thin film): 2920m, 2287w, 1660m, 1615m, 1556m, 1472m, 1455s, 1371m, 1223s, 1134m, 1082m, 1052m, 1019w, 917m, 853w, 821w, 590w, 567w; ^{1}H NMR (Acetone-d_6, 500 MHz): δ 3.57 (d, 2 H, J = 12.2 Hz), 3.65 (d, 2 H, J =12.3 Hz0, 3.88-3.98 (m, 4 H), 4.33-4.40 (m, 4 H), 4.41-4.54 (m, 8 H), 4.77 (d, 2 H, J = 12.1 Hz), 5.34 (d, 2 H, J = 12.1 Hz), 8.10 (s, 8 H); ^{13}C NMR (DMSO-d_6, 90 MHz): δ 29.2, 29.7, 34.3, 73.9, 76.8, 1191.8, 127.4, 128.3, 136.4, 157.8. LR-ESI-MS: expected for $C_{40}H_{35}O_6N_{16}^-$ 835.3, found 835.8. In order to gather binding data, **4** was converted to its sodium salt to promote water solubility by stirring with 4 eq. of NaOMe in MeOH and concentrating to dryness.

FRET Assay

The biosensor construct for the FRET assay was made by adapting procedures previously described.[5]

Protein expression

The genes encoding the histone-tail-containing molecular biosensor H3K27-MetBio3[5] and the *Paramecium bursaria chlorella* virus SET domain histone lysine methyltransferase (vSET)[6] were each cloned in to the L-

arabinose-inducible pBAD/HisB plasmids (Life Technologies). These plasmids were used to transform DH10B electro-competent *E. coli* using an electroporator set to discharge at a potential of 2 V. Immediately following electroporation, the bacteria were diluted with 1000 μL of LB media and allowed to recover at 37 °C for 20 minutes. After the short recovery time the bacteria were plated on LB-agar plates supplemented with ampicillin, and incubated overnight at 37 °C.

Five sterile 2L LB-culture flasks (4 for H3K27-MetBio3 and 1 for vSET) containing standard LB solution, 0.2% L-arabinose, and ampicillin at 100 μg/mL, were inoculated with a single colony picked from an LB-agar plate. The flasks were shaken at 250 rpm (New Brunswick Scientific) at 37 °C for 24h. Cultures are cooled to 4 °C for 2h and cells were pelleted by centrifugation at 8000 rpm for 15 minutes. The LB supernatant was decanted and the bacterial pellets re-suspended in 30 mL cold lysis-buffer (50 mM phosphate, 500 mM NaCl, 10 mM imidazole and 1× Complete protease inhibitor cocktail (Roche)). Resuspended cell pellets were lysed with a cell disruptor (Constant systems), and the cell debris pelleted by centrifugation at 14000 rpm for 45 minutes at 4 °C. A volume of 1 mL of Ni-NTA bead suspension (Amersham) was added to each of the supernatants, which were shaken on ice for 30 min. Proteins were purified following the manufacture's instructions. Protein concentrations were determined by absorbance spectroscopy using the previously reported[7] extinction coefficient of mCitrine (77,000 $M^{-1}cm^{-1}$) and the calculated extinction coefficient of vSET (18,575 $M^{-1}cm^{-1}$ at 280 nm).

Preparation of the methylated H3K27-MetBio3

The *in vitro* methylation of H3K27-MetBio3 was carried out in a total volume of 10 mL. The reaction contained 10 μM H3K27-MetBio3, the entire volume of purified vSET (about 1 mL), and 10 mM S-adenosyl methionine (SAM) in 50 mM phosphate buffer pH 8. All solutions were filtered through a 0.22 micron syringe filter to sterilize. The reaction was incubated overnight at 30 °C.

To purify the methylated H3K27-MetBio3 protein, the reaction mixture was separated by FPLC size-exclusion chromatography on an ÄKTA-design HPLC (Amersham), equipped with a superdex 75 16/60 column with a running buffer of 50 mM phosphate, pH 7.4. The fraction containing H3K27-MetBio3 was passed through the same column a second time to ensure complete removal of mTFP1-containing fragments produced by proteolysis. The protein containing fraction from the second pass through the column was concentrated by Millipore spin column with a nominal MWCO of 10,000 Da and diluted to 5 μM.

Binding assay with H3K27-MetBio3

For the FRET-based binding assay, the concentration of H3K27-MetBio3 was held constant at 1 μM. Fluorescence measurements were performed in black, flat-bottomed, 384 well plates (Corning), and acquired with a Safire2 microplate reader (Tecan). Calixarene solutions were prepared by serial dilution and added to microplate well that was brought to a final volume of 50 μL (30 μL buffer, 10 μL

H3K27-MetBio3, 10 μL analyte). For each microplate well, donor (494 nm, 5 nm bandwidth) and acceptor (526 nm, 5 nm bandwidth) fluorescence intensities were acquired using an excitation of 420 nm (10 nm bandwidth). Titrations were controlled by subtracting the intensities from identically prepared wells that contained the calixarene of interest and the unmethylated sensor that had been expressed and purified identically to the methylated sensor. This correction was performed for each concentration of calixarene.

Table 1: Calixarene stock solutions

Stock solutions	
[PSC] Stock	20000
10x dilution	2000
100x dilution	200

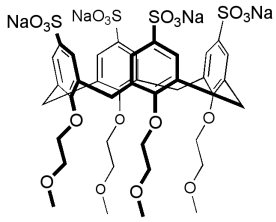
Table 2: Calixarene serial dilutions from stock solutions

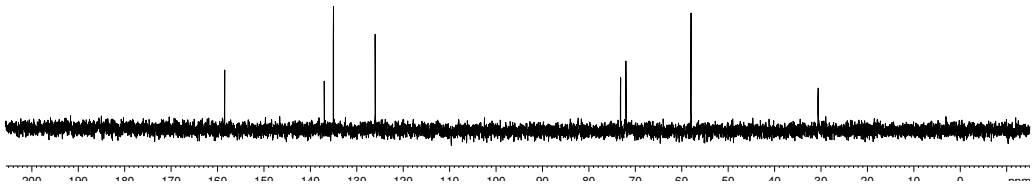
dilution series	[PSC]	buffer	stock
A	0	200	0
B	1.00	199	1.00
C	2.00	49.5	0.50
D	10.40	47.4	2.60
E	21.96	45.4	5.60
F	66.80	33.3	16.70
G	100.00	50	50.00
H	200.00	0	50.00
I	668	33.3	17
J	1,000	25	25
K	6,680	33.3	16.7
L	10,000	25	25

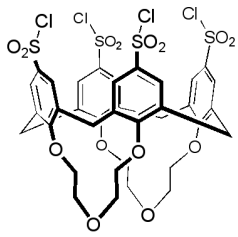
Table 3: An example of an assay

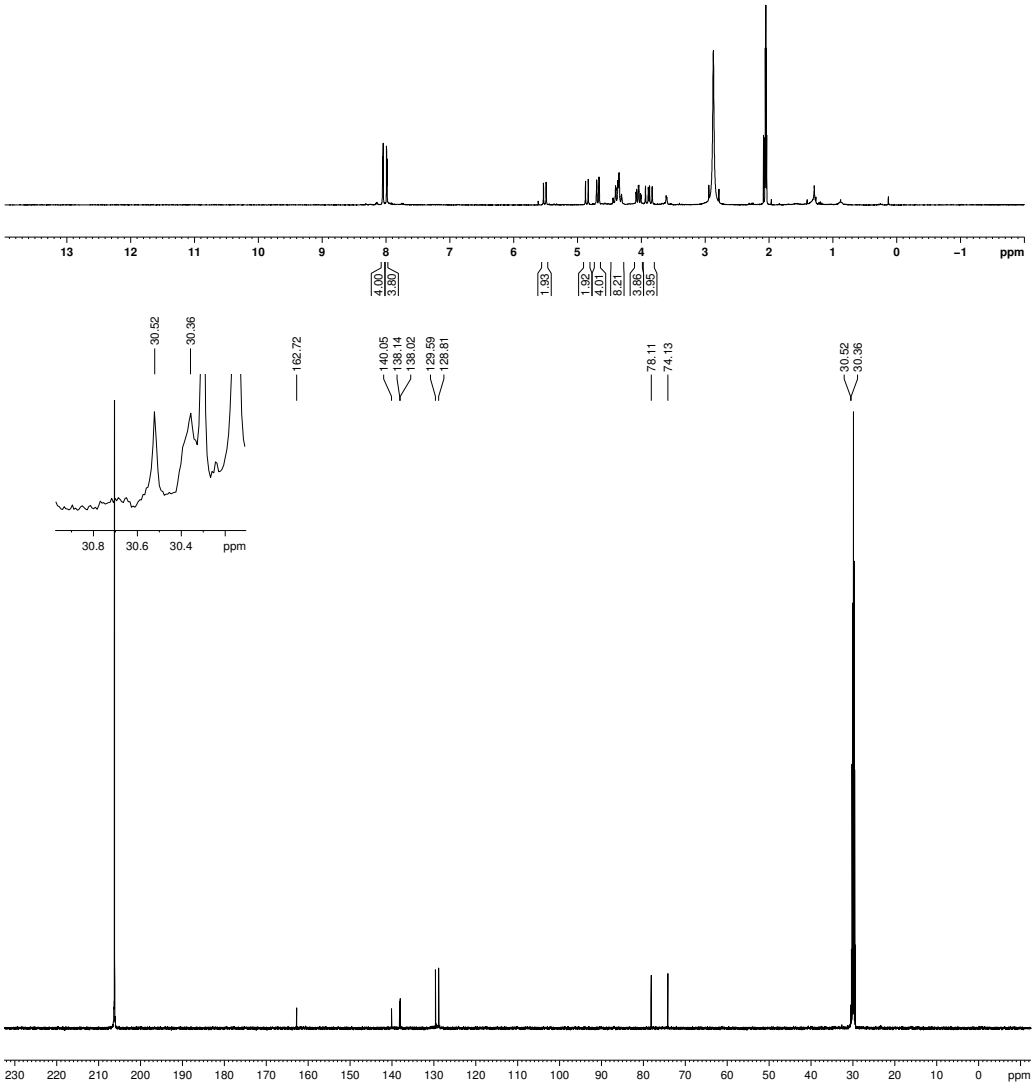
row	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
vPSC serial ds	**10**												**10**	**20**	**30**
serial ds used	A	B	C	D	E	F	G	H	I	J	K	L	vPSC stock	vPSC stock	vPSC stock
resultant [PSC]	0	0.20	0.40	2.08	4.39	13.36	20.00	40.00	134	200	1,336	2,000	4000	8000	12000
vMETBIO3 at 5uM	**10**														
vBuffer	**30**													**20**	**10**
total v	**50**														

Printouts of ^{1}H and ^{13}C spectra of new compounds





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4.00
3.80
1.93
1.92
4.01
8.21
3.86
3.95
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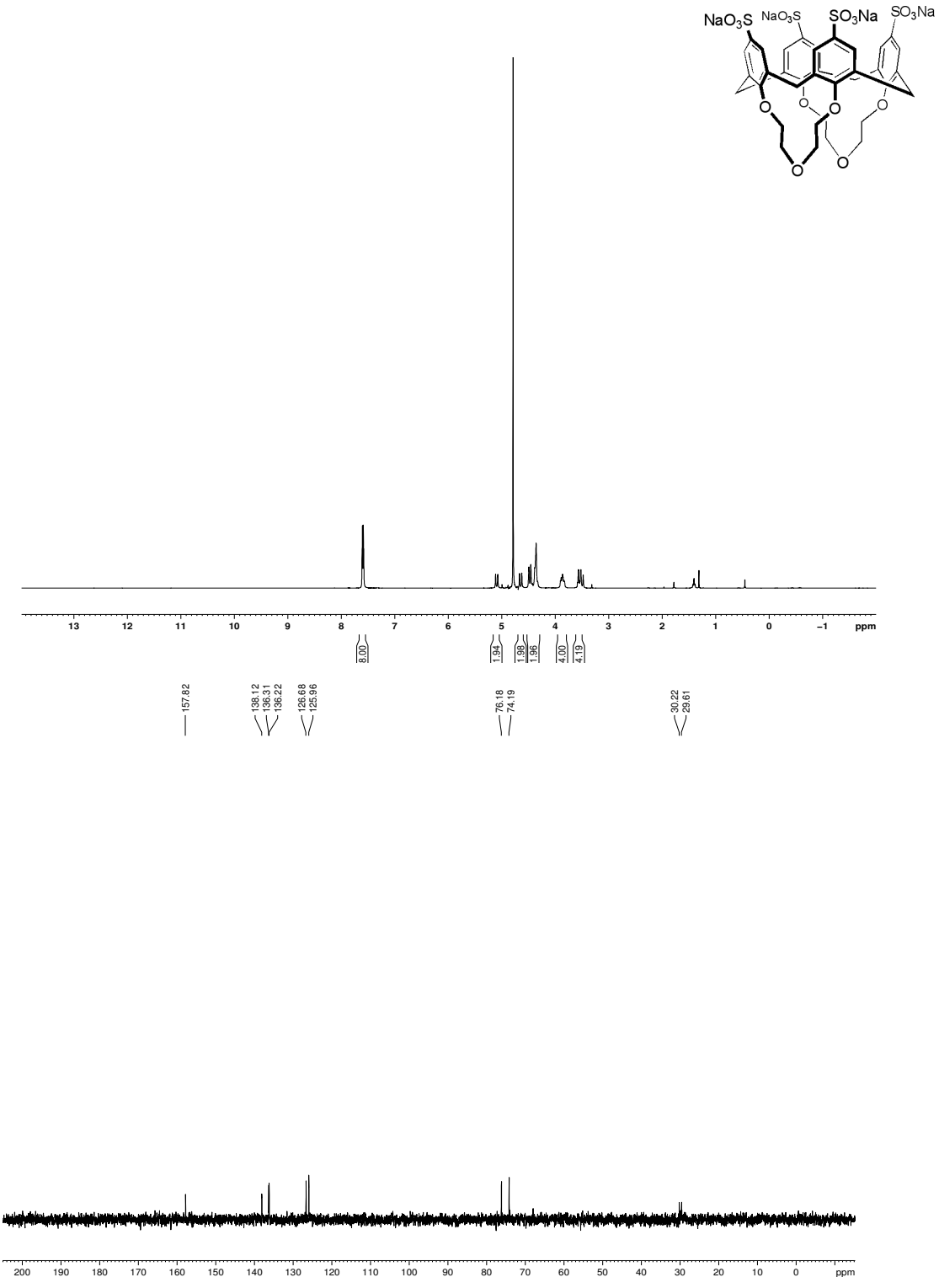
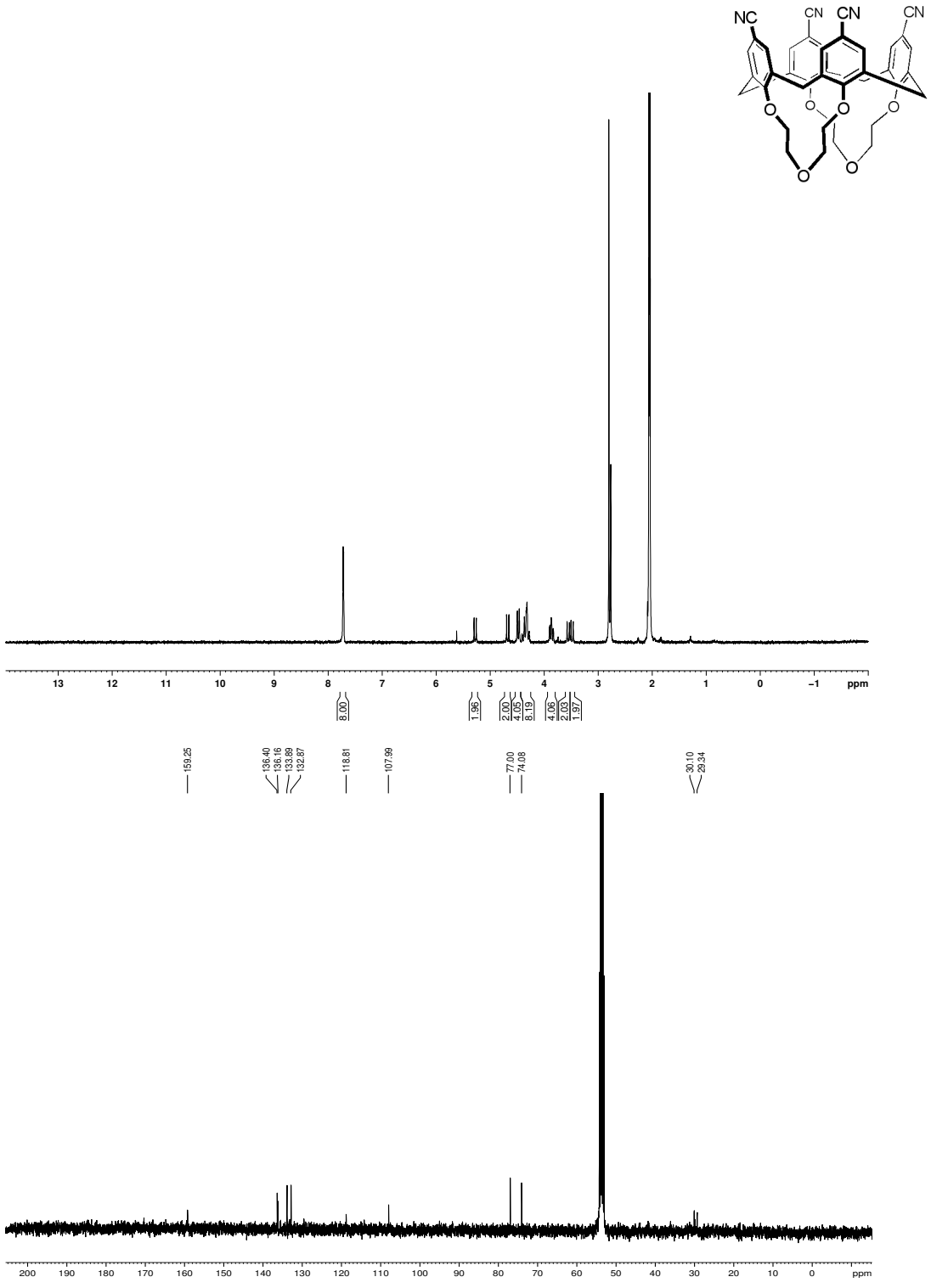
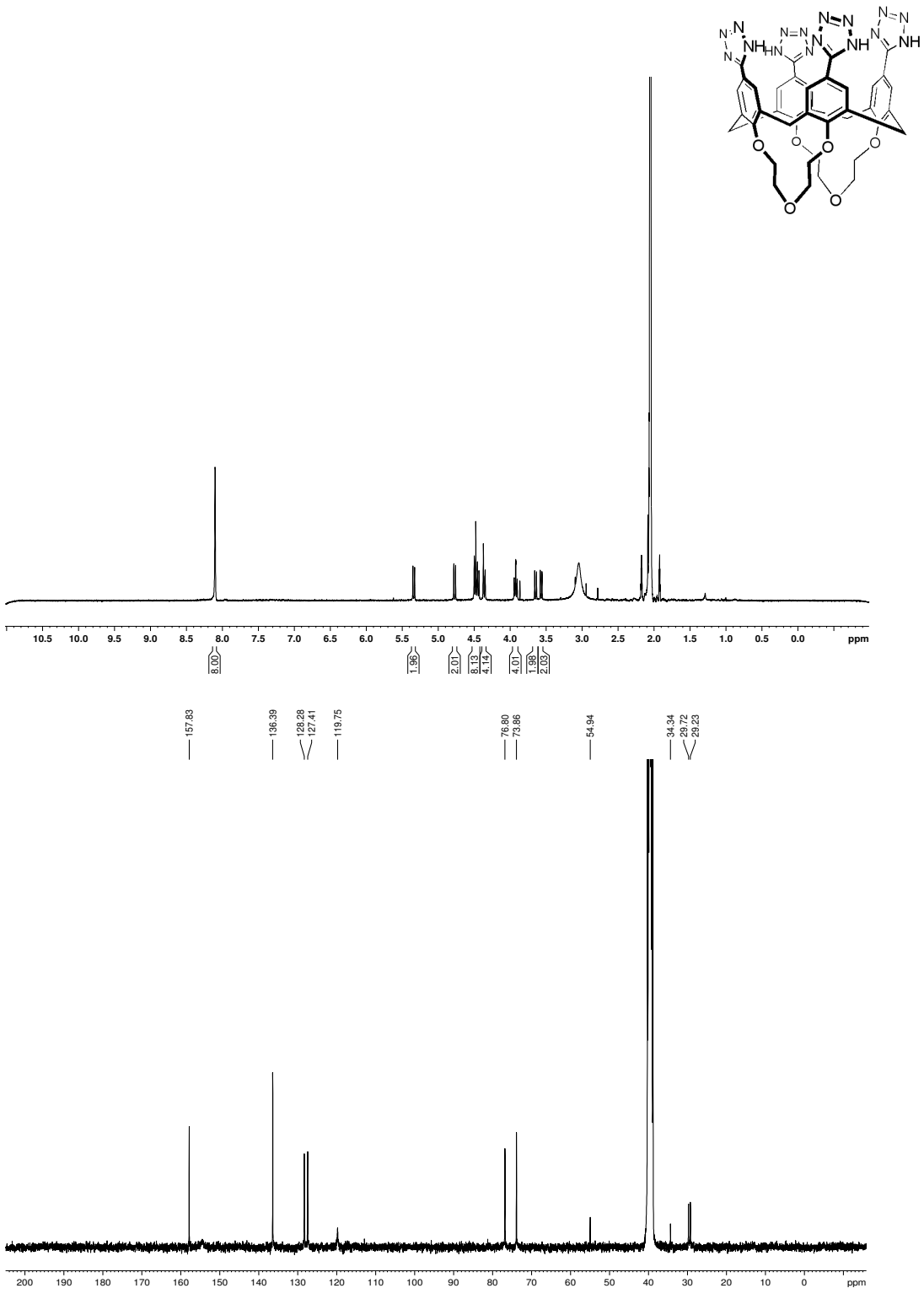
30.36
162.72
140.05
138.14
138.02
129.59
128.81
78.11
74.13
30.52
30.36
30.8 30.6 30.4 ppm
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

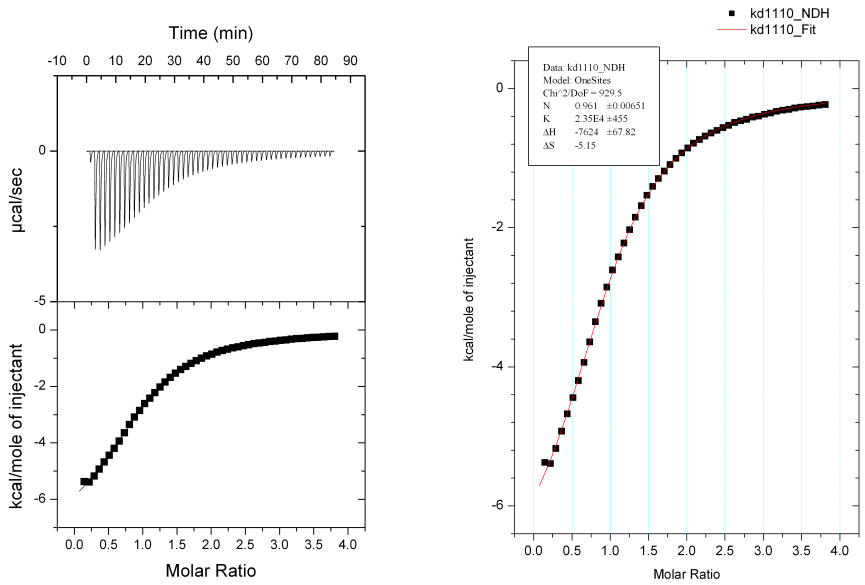






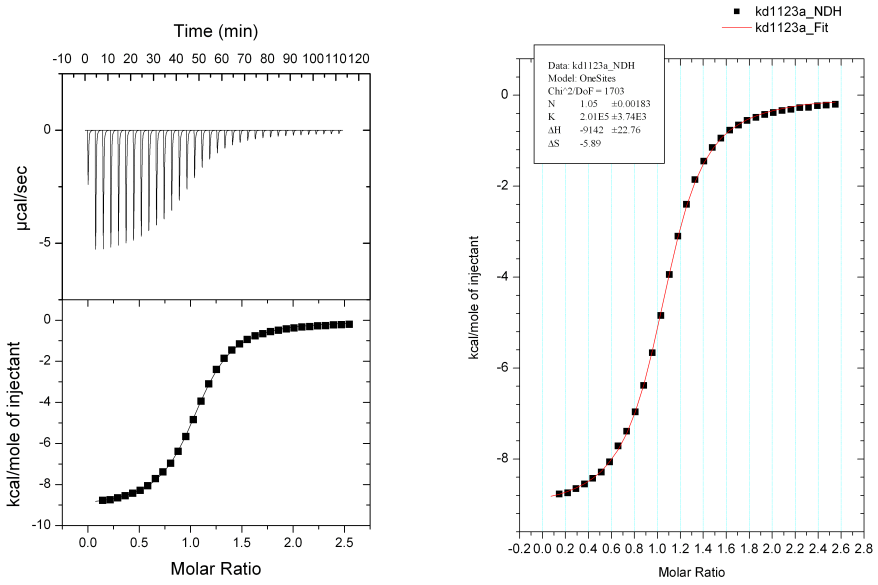
ITC titration and curve fits for each host-guest pair

Figure S1. ITC heats of binding and binding curve for host **1** into H3K4



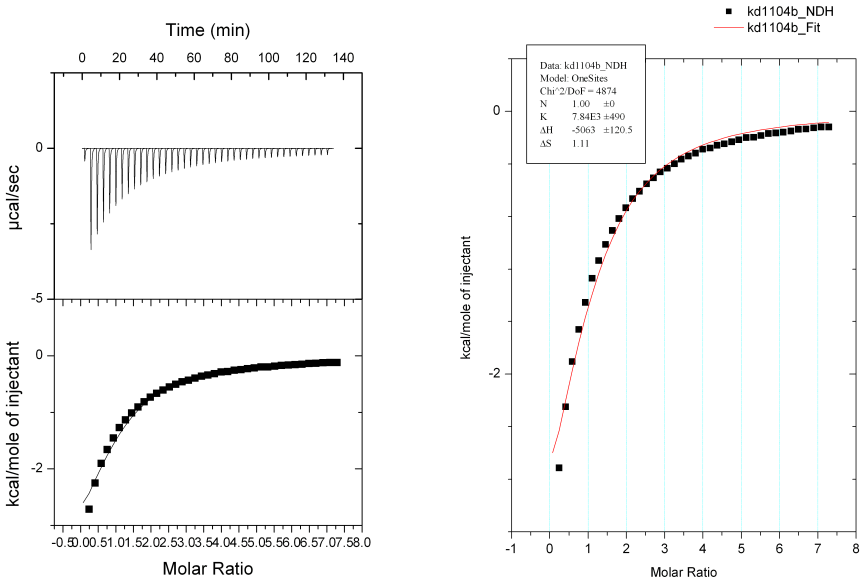
Replicate titration: N: 0.893 ± 0.0148; K: 1.96×10^4 ± 611; ΔH: -9036 ± 192.7; ΔS: -10.2

Figure S2. ITC heats of binding and binding curve for host **1** into H3K4me3



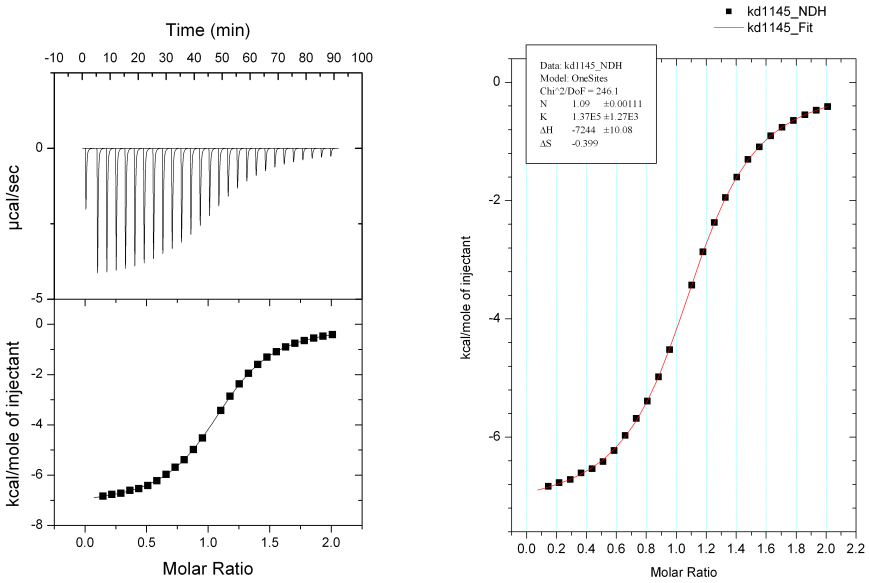
Replicate titration: N: 1.03 ± 0.00456; K: 1.96×10^5 ± 9.01×10^3; ΔH: -9328 ± 58.22; ΔS: -6.56

Figure S3. ITC heats of binding and binding curve for host **1** into H3K9



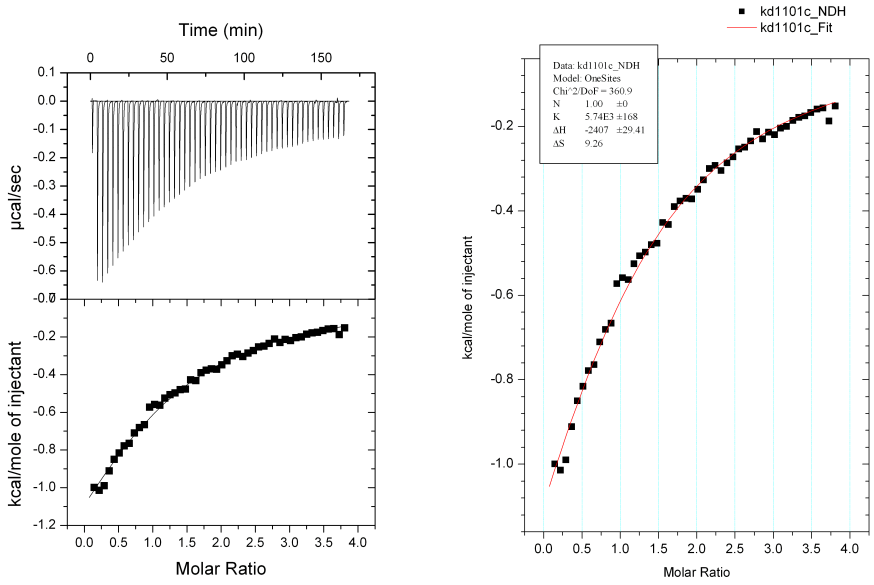
Replicate titration: N: 1.00 ± 0; K: $1.19 \times 10^4 \pm 1.16 \times 10^3$; ΔH: -4930 ± 158.2; ΔS: 2.38

Figure S4. ITC heats of binding and binding curve for host **1** into H3K9me3



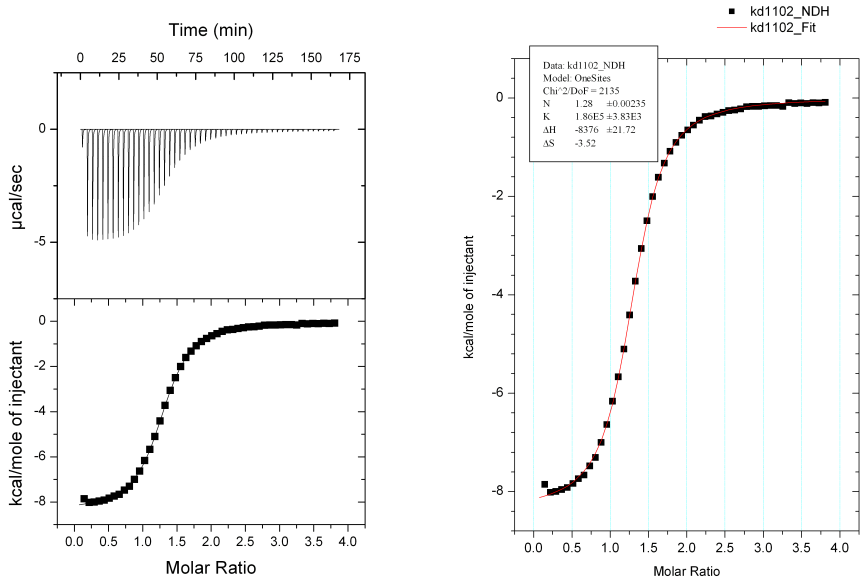
Replicate titration: N: 1.09 ± 0.000599; K: $1.41 \times 10^5 \pm 699$; ΔH: -7312 ± 5.623; ΔS: -0.562

Figure S5. ITC heats of binding and binding curve for host **1** into H3K27



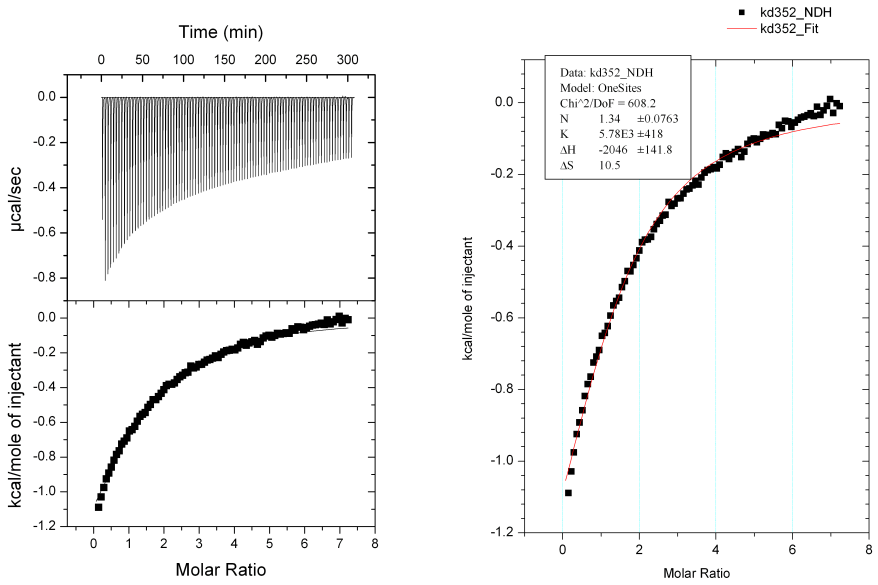
Replicate titration: N: 1.00 ± 0; K: 3.26x10^3 ± 112; ΔH: -3869 ± 66.6; ΔS: 3.31

Figure S6. ITC heats of binding and binding curve for host **1** into H3K27me3



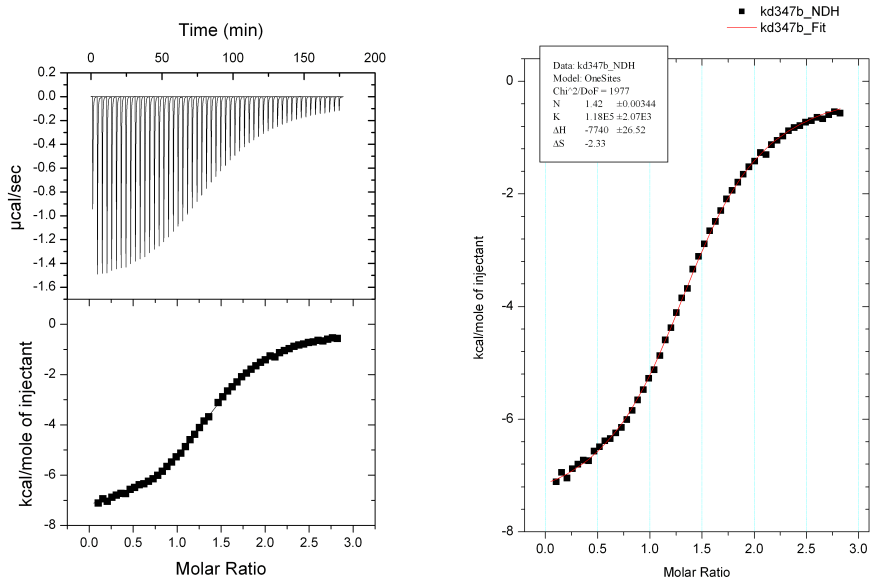
Replicate titration: N: 1.28 ± 0.00202; K: 1.85x10^5 ± 3.29x10^3; ΔH: -8486 ± 18.96; ΔS: -3.89

Figure S7. ITC heats of binding and binding curve for host **1** into H3K36



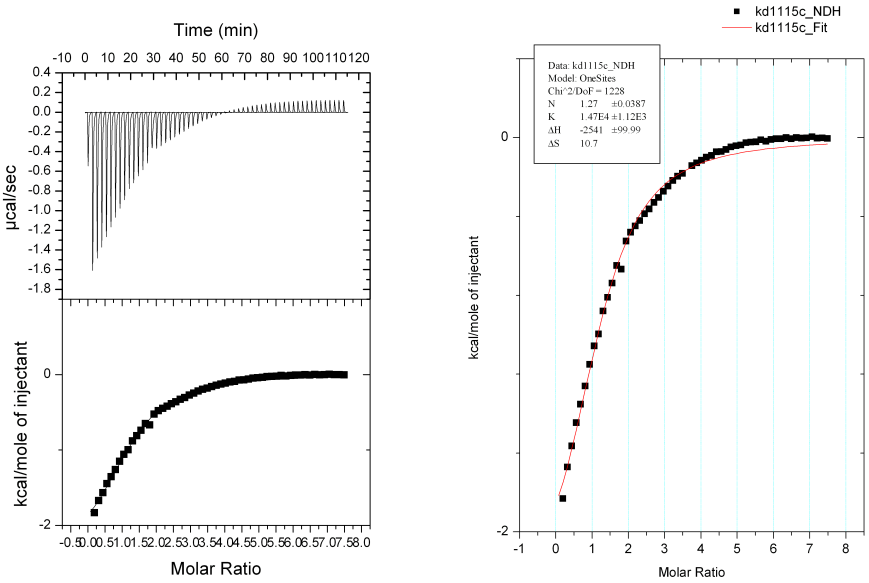
Replicate titration: N: 1.37 ± 0.0549; K: 9.91×10^3 ± 784; ΔH: -1748 ± 88.58; ΔS: 12.5

Figure S8. ITC heats of binding and binding curve for host **1** into H3K36me3



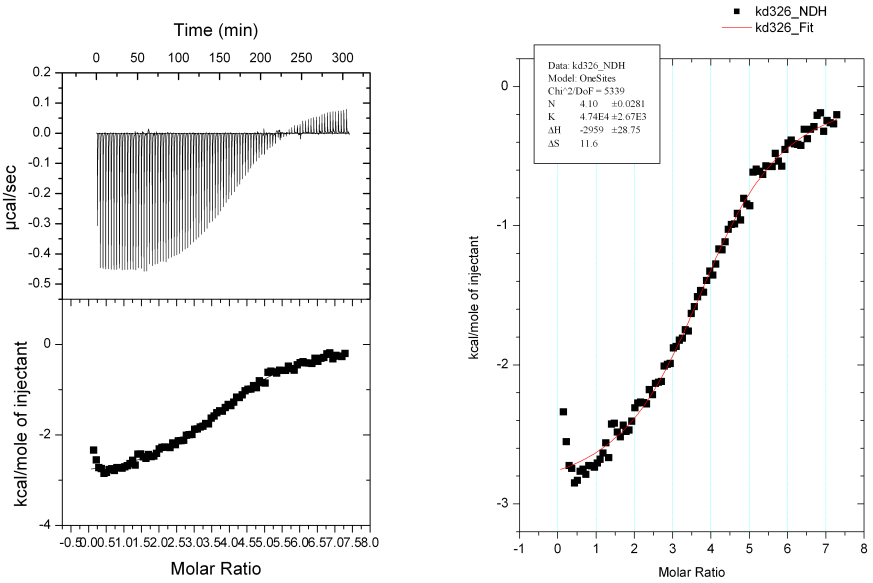
Replicate titration: N: 1.43 ± 0.00923; K: 1.01×10^5 ± 3.82×10^3; ΔH: -7887 ± 70.66; ΔS: -3.12

Figure S9. ITC heats of binding and binding curve for host **3** into H3K27me3



Replicate titration: N: 1.36 ± 0.0564; K: 8.91x10^3 ± 830; ΔH: -2438 ± 142.1; ΔS: 10.0

Figure S10. ITC heats of binding and binding curve for host **4** into H3K27me3



Replicate titration: N: 4.09 ± 0.0285; K: 5.14x10^4 ± 3.05 x10^3; ΔH: -3004 ± 29.58; ΔS: 11.6

Representative ^{1}H titration data as stacked plots

Figure S11. NMR titration of host **1** (50 mM) into H3K27me3 peptide (1 mM)

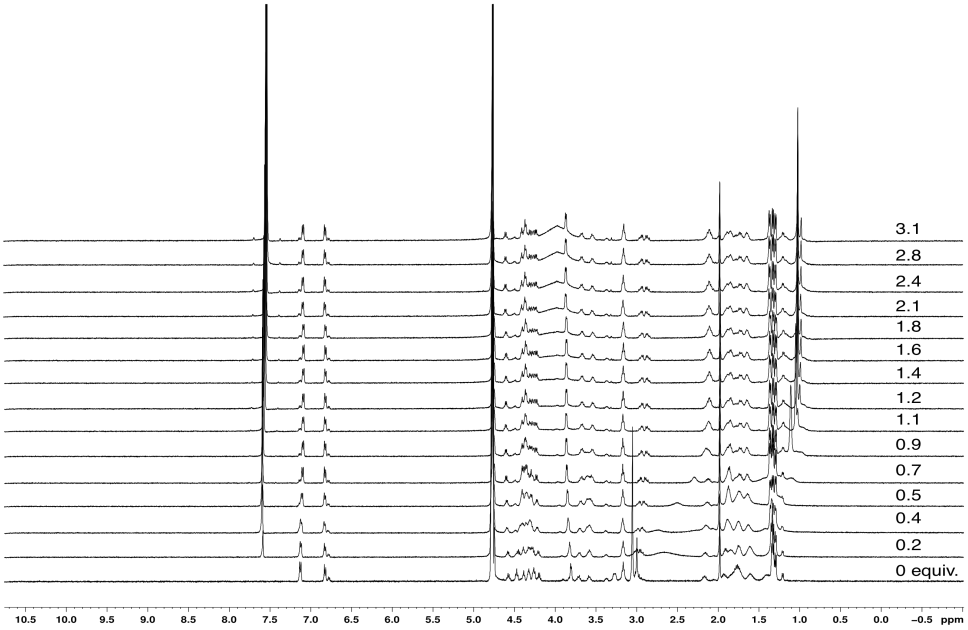


Figure S12. NMR titration of host **4** (2.2 mM) into H3K27me3 peptide (0.5 mM)

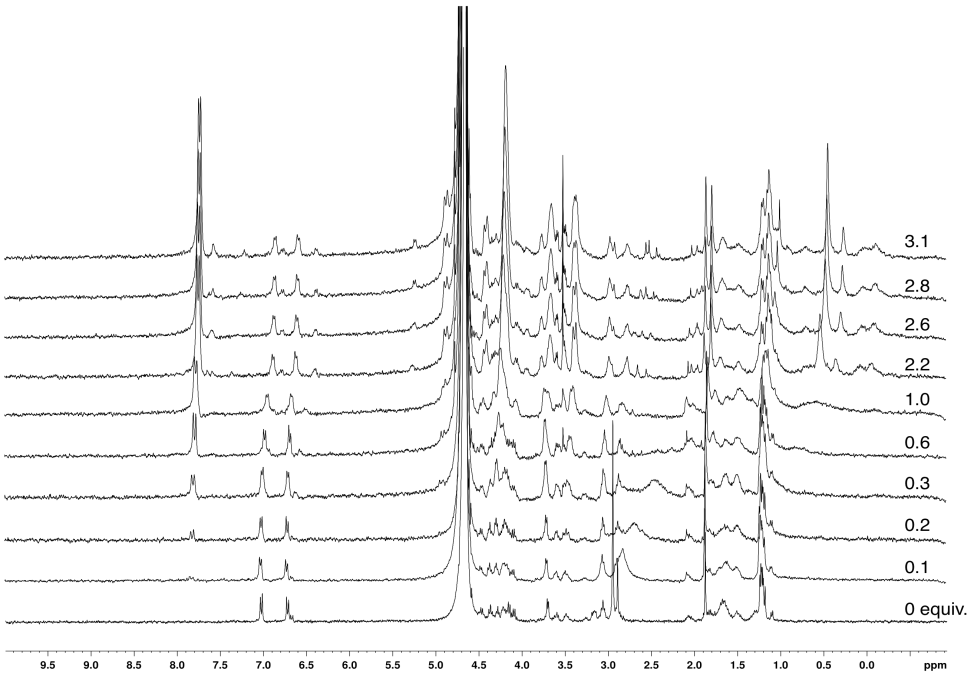
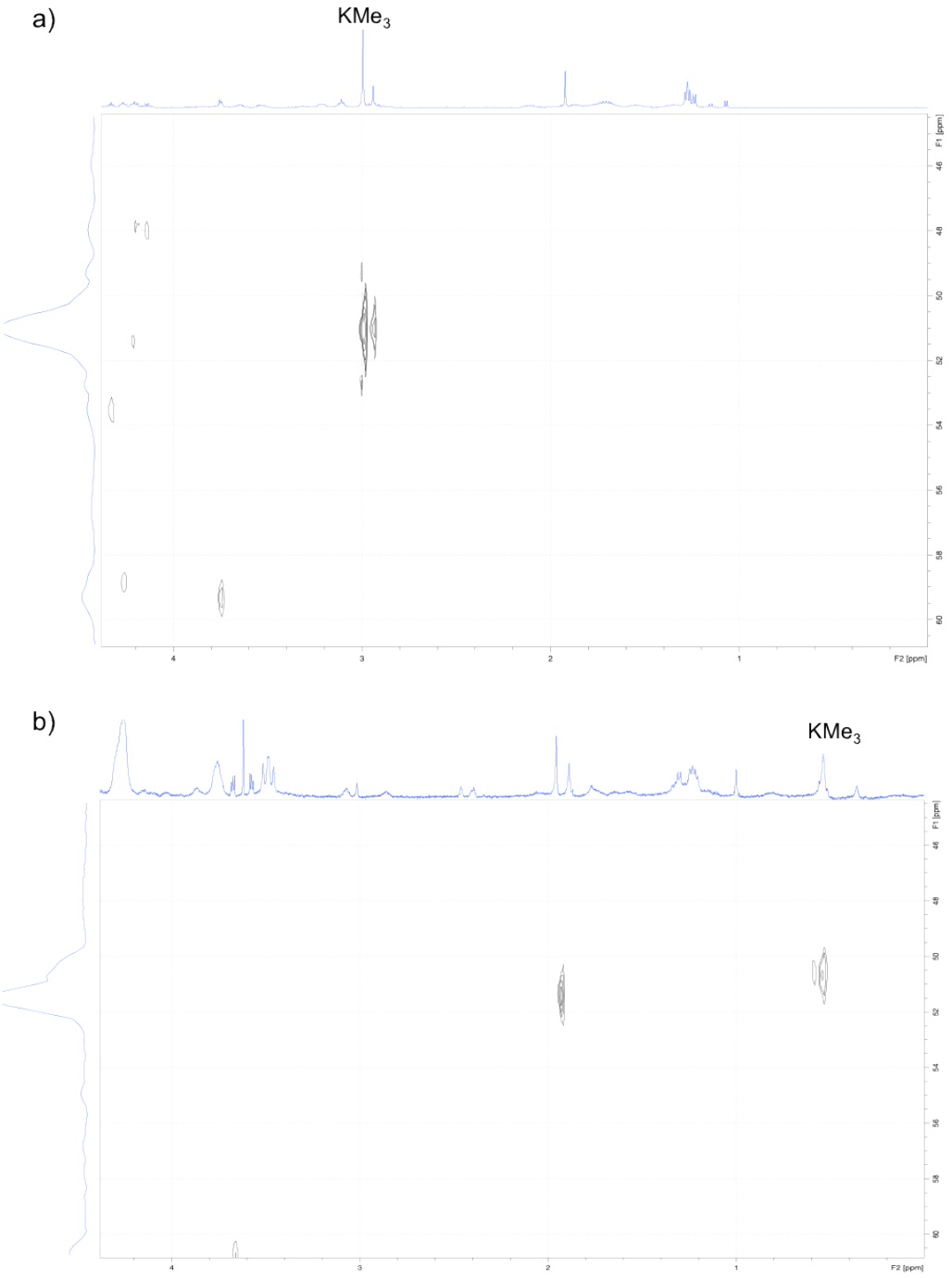


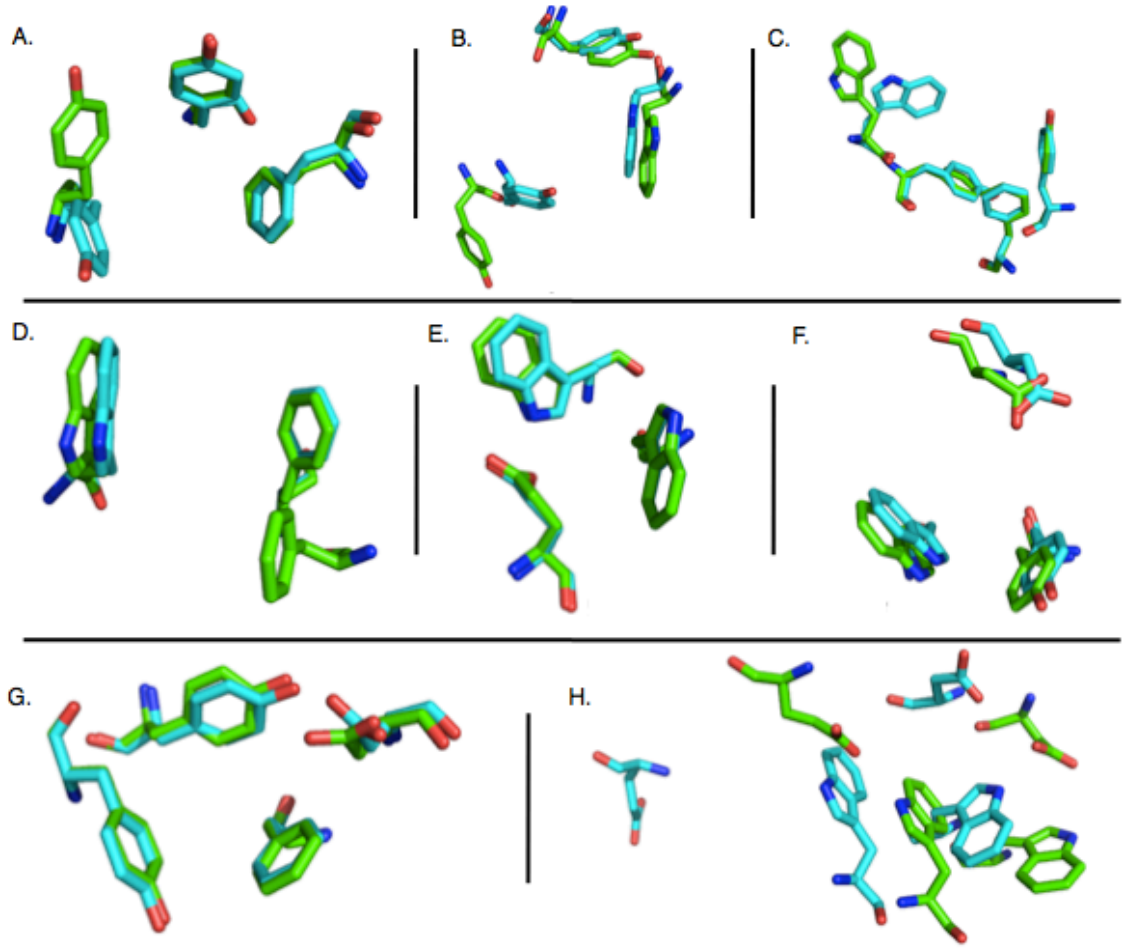
Fig S13. HSQC data tracking the upfield shift of the three methyl groups on K27. a) Blank H3K27me3 peptide. b) H3K27me3 peptide with 4 equivalents of host **4**. The ^{13}C chemical shift of $-^{+}N(\underline{C}H_3)_3$ is barely sensitive to binding (~51 ppm in both spectra), and allows assignment of ^{1}H shift of $-^{+}N(C\underline{H}_3)_3$ shifted upfield at 0.55 ppm in spectrum (b) due to binding inside the cavity of the calix[4]arene.



Overlay of free and bound structures of di- and trimethyllysine-binding proteins.

All structures containing di- or trimethyllysine were identified by searching the PDB. Subsequent searches were used to identify those examples where the structures of the same proteins were available in unbound states and X-ray crystal structures. The eight pairs of bound/unbound structures thus identified were each aligned using PyMol. The aromatic cage portions of these overlay structures are depicted in Figure S13.

Figure S14. PDB survey of trimethyllysine recognition domains for which structures of both empty and bound states are available (Teal = bound state, Green = unbound state).



A. PWWP domain (PDB id: 2X4W, 2X35) B. PHD finger (PDB id: 3LQI, 3LQH) C. EED domain (PDB id: 3JZN, 3JZG) D. PHD-type zinc finger (PDB id: 3O7A, 3O70) E. Chromodomain (PDB id: 2B2Y, 2B2W) F. PHD domain (PDB id: 2DX8, 2YYR) G. Tudor domain (PDB id: 3DB3, 3DB4) H. PHD domain (PDB id: 3N9M, 3N9L) — For this overlay only, larger-scale movements distal from the aromatic

cage cause a shift of the bound/unbound aromatic cages relative to each other; the cages themselves are aligned well with each other, supporting the hypothesis that cage residues move very little upon binding.

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