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Supporting Information

A Simple Calixarene Recognizes Post-translationally Methylated Lysine

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General Experimental

^{1}H NMR experiments were carried out on a Bruker AV500 at 500 MHz. Compound **1** was purchased from TCI America. D_2O was purchased from Cambridge Isotopes Labs. Peptides were used as prepared by Sigma Genosys as C-terminal amides and N-terminal acetamides. Samples were >98% pure as confirmed by ^{1}H NMR. All amino acid and peptide NMR studies were carried out at 298 K in D_2O containing Na_2HPO_4/NaH_2PO_4 (40 mM) at a measured pD of 7.0 (pH 7.4). Concentrations of amino acids, peptides, and **1** were as listed in the footnote for Table 1. Concentrations of amino acids were held constant by their inclusion in both titrant and receiving phase. The limited availability of the peptides precluded their inclusion in titrant stocks, so the titrations were instead run with highly concentrated titrant solutions of **1** to ensure <5% overall dilution of peptide in the receiving solution during the titrations. NMR chemical shift data were fit to 1:1 binding isotherms to give K_{assoc} values using the spreadsheets made freely available by Prof. Sanderson of Durham University (http://www.dur.ac.uk/ j.m.sanderson/science/downloads.html).

Isothermal titration calorimetry was carried out using a MicroCal VP-ITC. Titrations were run at 303 K in H_2O (40 mM Na_2HPO_4/NaH_2PO_4, pH 7.4) using the concentrations listed in the footnote for Table 1. Data were fit to the manufacturer's included single-site binding model using Origin 7.0.

Molecular modeling was carried out in two stages: First, multiple different starting geometries were manually set up and minimizations were carried out at the semi-empirical level of theory using the RM1 parameter set as implemented in Spartan '06. Regardless of starting geometry, Lys(Me$_3$) consistently gave an "end-on" structure (with methyls buried in the binding pocket). Unmethylated lysine, arginine, and all methyl arginine derivatives gave collections of similar local minimum structures that were all "side-on," defined as having extensive engagement of side chain methylenes with the walls of the binding pocket. In the second stage, representative geometries of each complex were further minimized (HF/6-31G*) to produce the final structures depicted in Figure S4.
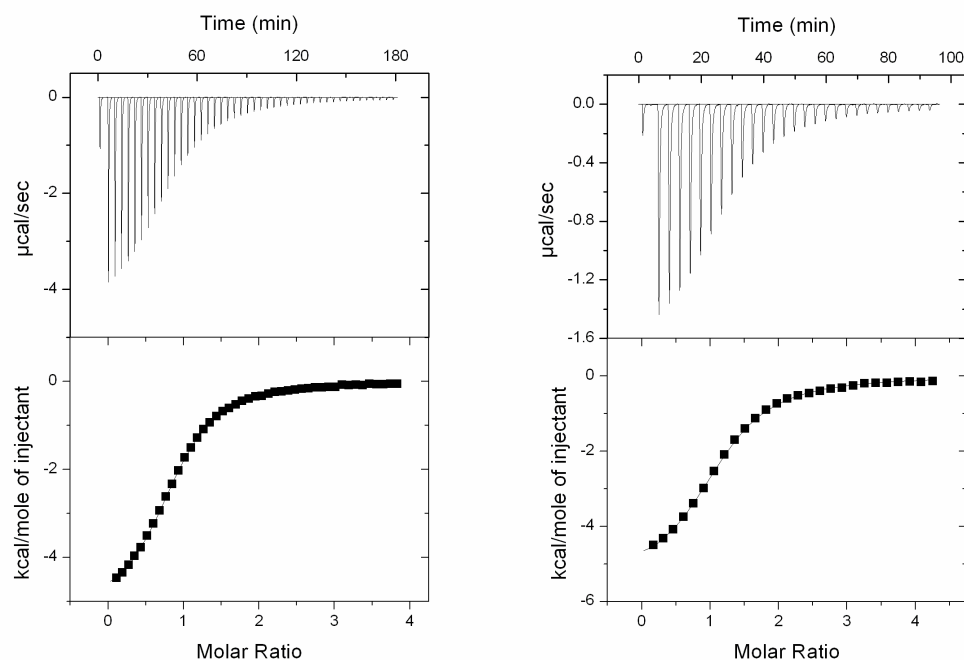
Supplementary binding data
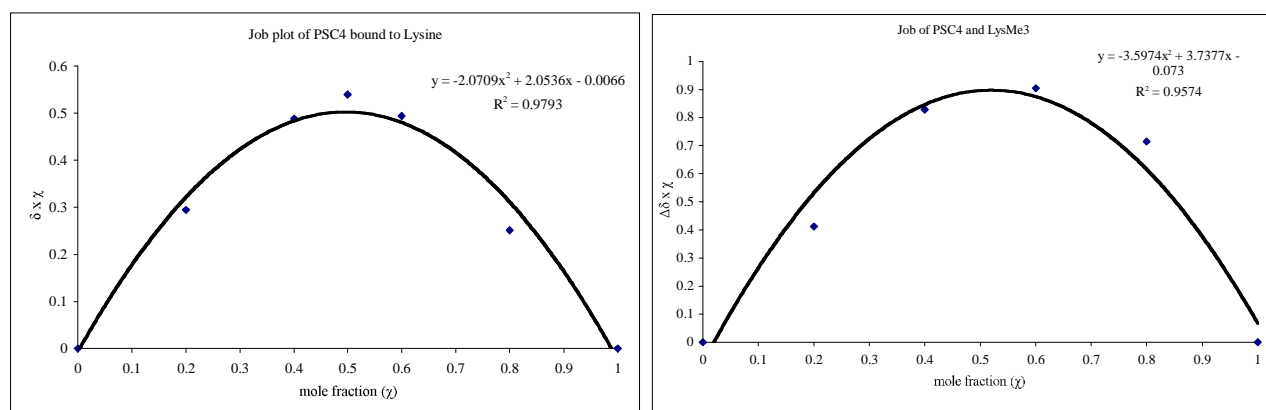
Figure S1. ^{1}H NMR Job plots for the binding of **1** to Lys (left) and Lys(Me$_3$) (right). Total concentration was held constant at 5 mM while the mole fraction of lysine derivative was varied from 0 to 1.

a)

b)

c)

d)
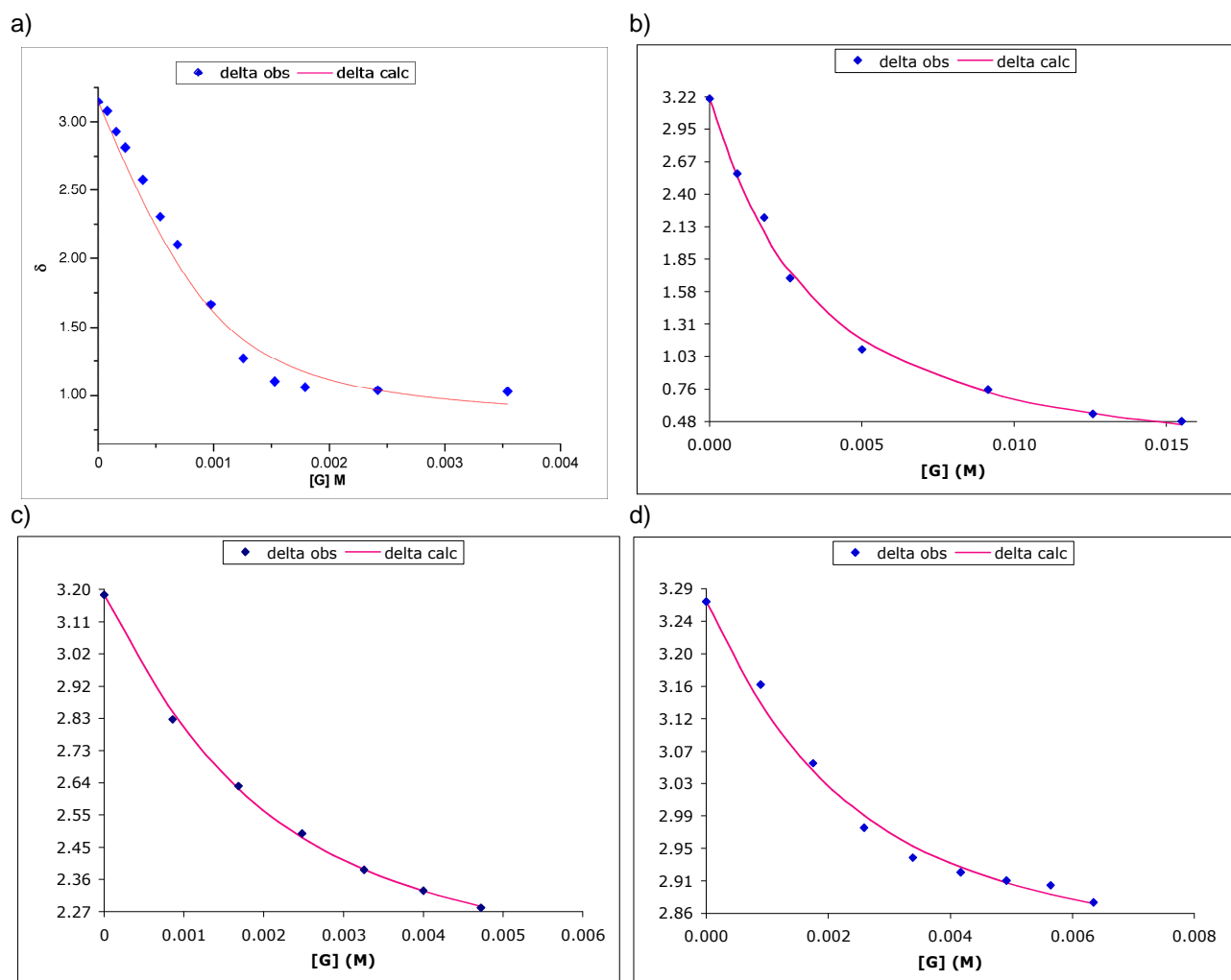
Figure S2. Exemplary ^{1}H NMR chemical shift data fit to 1:1 binding isotherms for the addition of **1** to a) Lys(Me$_3$), b) Arg, c) MMA, and d) aDMA. **[G]** = [**1**]

Figure S3. Exemplary raw and integrated ITC data, and curves arising from fitting to the single site binding model. Left: data from titration of **1** into Lys(Me$_3$) ($N = 0.81$, K = 38,700 M^{-1}, $\Delta H = -21.7$ kJ/mol, T$\Delta S = 5.1$ kJ/mol). Right: data from titration of **1** into Arg-Lys(Me$_3$)-Ser-Thr ($N = 1.10$, K = 102,000 M^{-1}, $\Delta H = -22.9$ kJ/mol, T$\Delta S = 6.2$ kJ/mol).
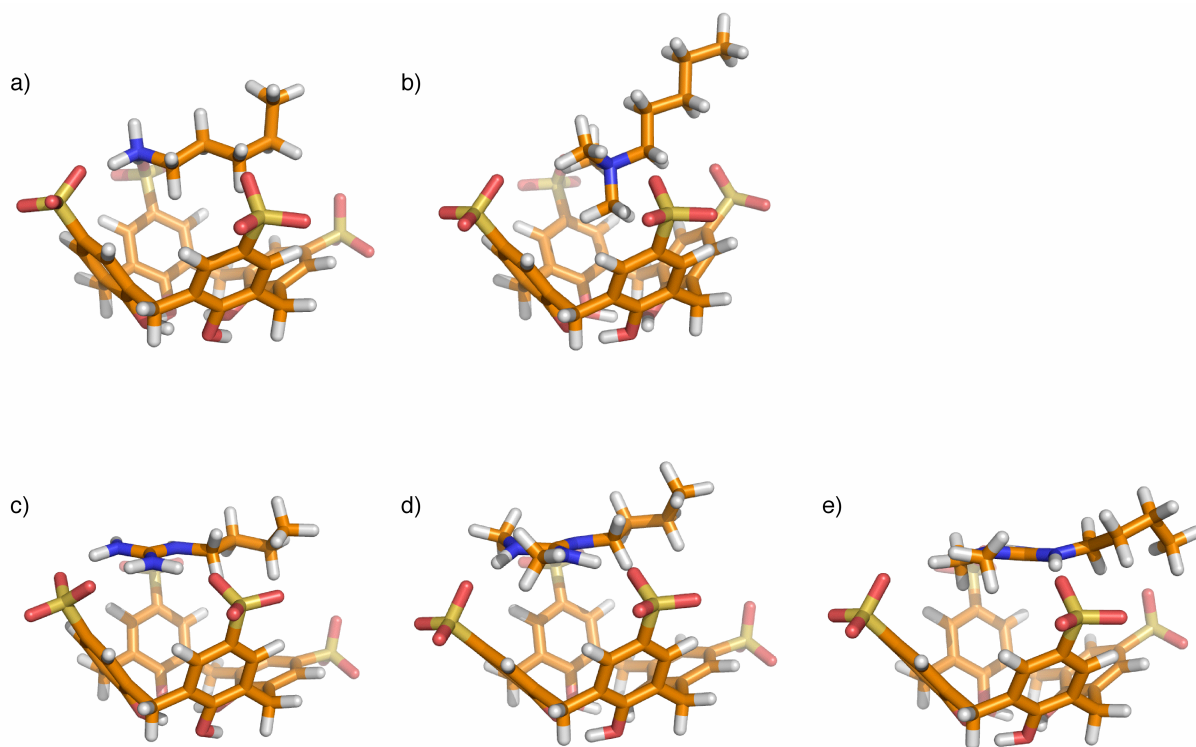
Figure S4. Energy-minimized structures (HF/6-31G*) of complexes between **1** and a) Lys, b) Lys(Me$_3$), c) Arg, d) sDMA and e) aDMA. All amino acid side chains have been simplified by truncation at Cα.
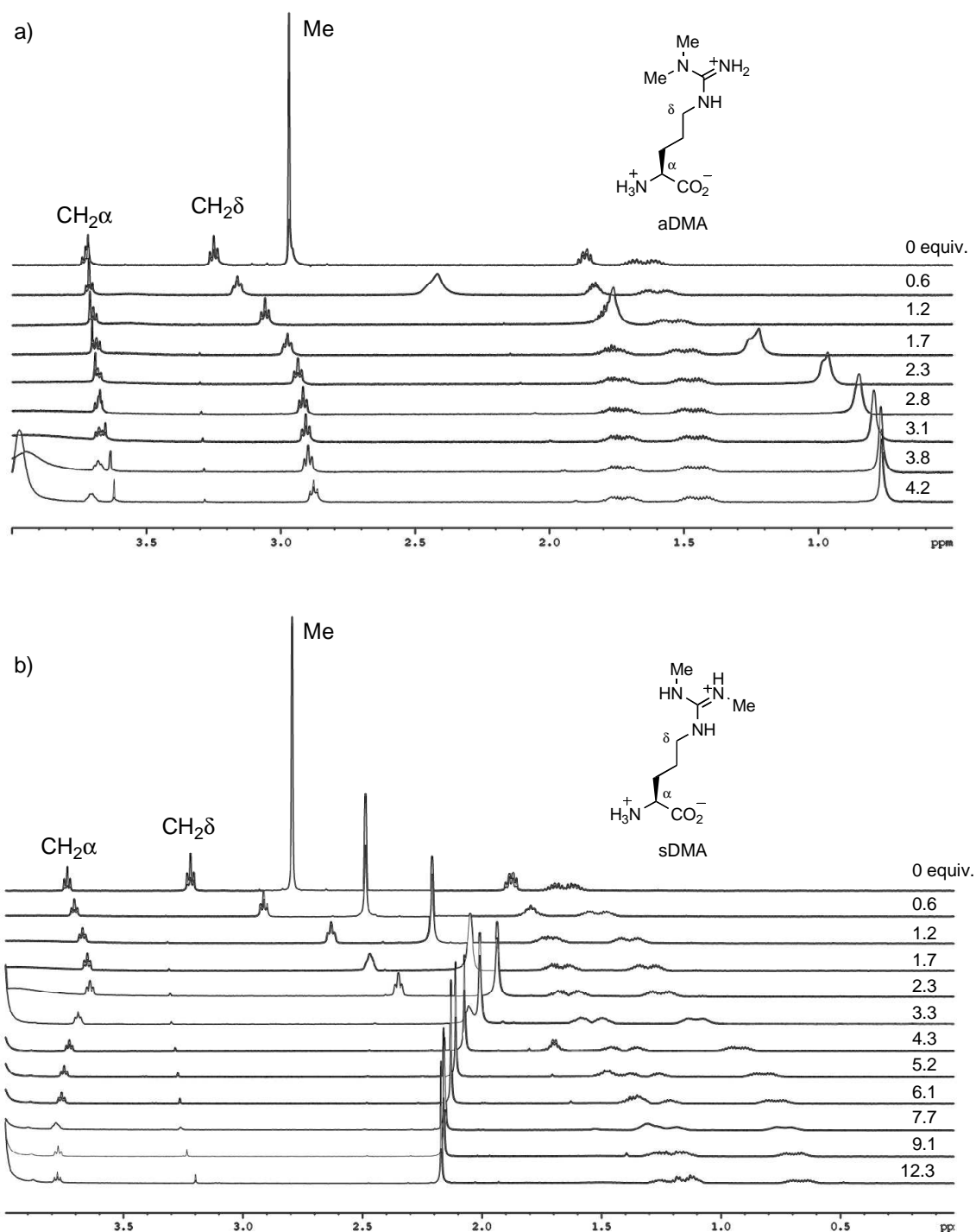
Figure S5. NMR stacked plots show the different chemical shift changes for aDMA (a) and sDMA (b) upon addition of **1**. Unlike all other sets of NMR data reported herein, the chemical shift changes for sDMA include signals that show smooth upfield shifts (e.g. CH$_2\delta$ and mixed CH$_2$ signals near 1.5) and others whose back-and-forth trends could not be fit to any simple 1:1, 2:1, or 1:2 binding isotherm (e.g. CH$_2\alpha$ and Me).