

Supporting Information

for

A Cucurbit[8]uril Sponge

Vijayakumar Ramalingam, Sharon K. Kwee, Lisa M. Ryno, and Adam R. Urbach*

Department of Chemistry, Trinity University, San Antonio, TX, 78212

* to whom correspondence should be addressed: aurbach@trinity.edu

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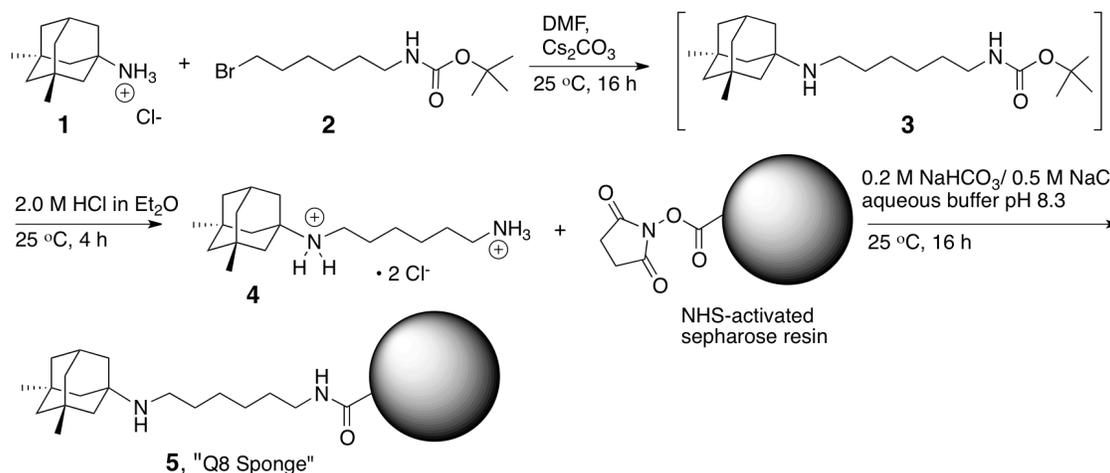
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Experimental Details

Materials. All reactions were carried out under nitrogen atmosphere unless otherwise noted. Glass vessels were dried at 100 °C and cooled under nitrogen before use. All reagents, chemicals and anhydrous solvents were purchased from commercial sources and used without any further purification unless otherwise indicated. ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded on a Varian instrument. Electrospray ionization mass spectrometry (ESI-MS) experiments were performed in pure water using a Thermo LCQ DECA XP plus mass spectrometer in positive ion mode. Isothermal titration calorimetry (ITC) experiments were carried out at 300 K on a VP-ITC calorimeter from Microcal, Inc. Cucurbit[6]uril and cucurbit[8]uril were synthesized from literature procedure as reported previously.¹ NHS-activated sepharose 4 fast flow resin suspended in 100% isopropanol was purchased from GE Healthcare Biosciences, 1-amino-3,5-dimethyladamantane hydrochloride (i.e., memantine hydrochloride, **1**) was from TCI America, 6-(boc-amino) hexyl bromide (**2**), anhydrous dimethylformamide (DMF), hydrochloric acid (2.0 M) in diethyl ether, and methyl viologen (MV) dichloride were from Sigma Aldrich, 35 % DCl in D₂O and neat D₂O were from Cambridge Isotope Laboratories, and H-Phe-Gly-Gly-OH was from Peptides International.

Synthesis of Q8 Sponge

Scheme S1:



Synthesis of *N*¹-(3,5-dimethyladamantyl)hexane-1,6-diamine hydrochloride (**4**).

In a 50 mL round bottom flask memantine hydrochloride (**1**) (2.75 mmol, 0.592 g) was added to anhydrous DMF (10 mL) and stirred magnetically under nitrogen atmosphere for 10 minutes at room temp. Cesium carbonate (4.12 mmol, 1.34 g) was added, and the mixture stirred vigorously for 30 minutes under nitrogen at room temp. Then, 6-(Boc-amino)hexyl bromide (**2**) (3.56 mmol, 1.03 g) was added, and the reaction stirred under nitrogen overnight (16 h) at room temp. The reaction mixture was then concentrated *in vacuo* to minimal volume. DCM (15 mL) was added, and the organic solution was washed with water (3 x 15 mL), dried over sodium sulfate, and concentrated *in vacuo* to obtain **3** as a white solid in 85% crude yield (confirmed by ¹H NMR and ESI-MS). To cleave the Boc protecting group, HCl (2M) in diethyl ether (4.5 mL) was added. The mixture stirred for 4 h at room temperature and was then concentrated *in vacuo* to obtain **4** as a gel (0.708 g, 82% crude yield). The gel (25 mg) was dissolved in water

(10 mL) and then purified by reversed phase preparative HPLC (Waters system with a Waters Novapak C18 column) using a gradient of 1%/minute acetonitrile in 0.1% aqueous TFA as eluent. The pure fractions, confirmed by analytical HPLC (Agilent system with a Waters Novapak C18 column) using the same elution gradient and by ESI-MS, and all pure fractions were combined and lyophilized to dryness to obtain **4** (10 mg, 40 %) as a transparent oil. ^1H NMR (500 MHz, D_2O) (Fig. S1): δ = 2.99 (4H, m), 2.26 (1H, s), 1.73 (2H, s), 1.65 (4H, m), 1.53 (4H, m), 1.39 (8H, m), 1.24 (2H, m), 0.90 (6H, s); ^{13}C NMR (125 MHz, D_2O) (Fig. S2): δ = 58.8, 48.9, 43.7, 41.1, 39.3, 36.5, 31.8, 29.4, 28.8, 26.4, 25.8, 25.3, 25.0; ESI-MS m/z [$\text{M}+\text{H}^+$] calculated 279.3; found 279.4 (Fig. S3).

Synthesis of N^1 -(3,5-dimethyladamantyl)hexane-1,6-diamine-coated sepharose resin (5**, i.e. the Q8 sponge).** NHS-activated sepharose resin suspended in 100% isopropanol (2 mL, approximately 70 mg, 36 μmol NHS groups based on the supplied manufacturer's literature) was added to a glass-fritted column, and the isopropyl alcohol was drained completely. Compound **4** (314 mg, 1 mmol) was dissolved in 2 mL of aqueous 0.2 M NaHCO_3 , 0.5 M NaCl , and the pH was adjusted to 8.0. This mixture was added to the isopropanol-free sepharose resin and stirred for 16 h at room temperature. The product **5** was obtained by washing with deionized water (20 mL, 10 times) and stored as an aqueous suspension at 4 $^\circ\text{C}$. This suspension can be stored stably for long periods (months) at 4 $^\circ\text{C}$. In most experiments, however, **5** was used as a dry powder, which should be used soon after drying because we have found the sponging capacity of the dry material to diminish significantly (>50%) over a period of days. The dry powder was obtained by washing the aqueous suspension with methanol

(3 x 20 mL) then diethyl ether (3 x 20 mL), then drying under reduced pressure for 15 minutes.

Quantitation of Q8-Binding Capacity of Sponge 5 by Isothermal Titration

Calorimetry. The approach taken here makes use of the known 1:1 binding stoichiometry of the Q8•MV complex to determine the concentration of free Q8 in a sample by experimentally determining the volume required to reach equivalence during the titration of a Q8 sample of unknown concentration with a standardized sample of MV.

A standard solution of MV (0.250 mM, determined by UV spectroscopy, $\epsilon_{257} = 20,400 \text{ M}^{-1} \text{ cm}^{-1}$) in sodium phosphate buffer (10 mM, pH 7.0) was prepared. A stock solution of Q8 in 10 mM sodium phosphate pH 7.0 was prepared to be approximately 50 μM based on the water and salt contained in a typical Q8 sample. The Q8 stock was placed in the sample cell and titrated with the MV standard at 27 °C using an injection schedule of 27 injections of 10 μL (following the throwaway 2 μL injection) with a separation of 250 s between injections. The data were fit to a “one-set-of-sites” binding model using the Origin software supplied with the instrument (the model is a simple binary equilibrium model that assumes non-interacting binding sites). The reaction stoichiometry (i.e., the ‘n’ value) calculated from this fit (i.e., the MV:Q8 ratio) was multiplied by the nominal cell concentration (i.e., 50 μM in this experiment) in order to determine the actual cell concentration. This method is valid only when the binding stoichiometry is known to be 1:1. A simple check of the method can be performed by inputting the new concentration and re-fitting the data to the same model; the fitted value for binding stoichiometry should now be exactly 1.0. (It is worth noting here that

this method is also useful for determining solubilities by determining the unknown concentration of a saturated solution.) For the representative batch described in the manuscript, the Q8 concentration was determined to be 56.0 μM .

A sample of this Q8 stock solution (8.0 mL, 56.0 μM) was added to *freshly dried* Q8 sponge (2.1 mg), mixed by vortexing (5 minutes), and centrifuged (2 min, 14,000 rpm) to separate the solid resin from the supernatant (alternatively, the mixture may be filtered). A sample of the clear supernatant (or filtrate if filtered) was then titrated with the methyl viologen standard (0.250 mM) using the method detailed above, and a residual Q8 concentration of 22.5 μM was determined. The binding capacity of the sponge **5** was calculated by considering that a reduction in Q8 concentration of an 8.0 mL sample from 56.0 μM to 22.5 μM equals the removal of 268 nmol of Q8 from solution by 2.1 mg of the sponge, or 127 μmol Q8 per gram of sponge. The sample must be stored suspended in water and dried immediately before use in order to have this activity. If we stored the sample dry, then this same experiment yielded a sparging capacity of 41.3 μmol Q8 per gram of sponge.

Reversal of Peptide Dimerization. The dimerization and reversal of peptides was shown with the unprotected tripeptide phenylalanine-glycine-glycine (FGG). Dry Q8 (0.966 μmol) was added to a solution of FGG (966 μL , 2 mM in D_2O) to form a mixture containing a 1:2 ratio of Q8:FGG (i.e., $\text{Q8}\cdot(\text{FGG})_2$).² This ratio was confirmed by ^1H NMR spectroscopy. Dry sponge **5** (96 mg, 4.0 μmol) was added to the $\text{Q8}\cdot(\text{FGG})_2$ solution (800 μL), mixed for 10 minutes, and centrifuged (2 min, 14,000 rpm). The clear supernatant (500 μL) was removed, lyophilized to dryness and

resuspended in D₂O (500 μL). The ¹H NMR spectrum of this sample shows complete disappearance of the Q8 signals and return of the FGG signals to their unbound chemical shifts, which confirms the complete removal of Q8 and thus the reversal of FGG dimerization.

Separation of Q8 from Q6 using sponge 5. In a 1.5 mL microcentrifuge tube, Q6 (1.12 μmol) and Q8 (0.98 μmol) were mixed with 0.35 mL DCI solution (35% w/v in D₂O). This heterogenous mixture was sonicated to obtain a clear solution, which was then diluted with D₂O (1.0 mL). Sponge 5 (34 mg, 4.3 μmol) was added to this solution (1.35 mL), and the mixture was vortexed for 5 minutes, centrifuged (2 min, 14,000 rpm) and the clear supernatant removed by Pasteur pipette and filtered using through a cotton plug (stuffed into a separate Pasteur pipette). The filtrate was concentrated to dryness *in vacuo* (at room temp), redissolved in 0.6 mL D₂O, sonicated for 30 s, lyophilized to dryness, and resuspended in 0.6 mL DCI (35% w/v in D₂O). The ¹H NMR spectrum of this sample showed only resonances for Q6 (Fig. 4, main manuscript).

Recycling the Sponge. Described first is the protocol for removing Q8 from a solution of methyl viologen because this is the metric by which recyclability was measured. A stock solution of methyl viologen (2.0 mM) was prepared in deuterium oxide and quantified by UV spectroscopy ($\epsilon_{257} = 20,400 \text{ M}^{-1}\text{cm}^{-1}$). This solution was diluted to 1.0 mM with D₂O, and a sample (1.0 mL) was mixed with dry Q8 (1.0 μmol). The resulting mixture was vortexed 5 min, sonicated 1 min, and heated at 60 °C for 30

minutes to obtain Q8•MV (1.0 mM in D₂O) in 1:1 ratio as a colorless solution. The 1:1 ratio was confirmed by ¹H NMR spectroscopy, and binding was confirmed by observing the upfield perturbation of the chemical shifts of the viologen aromatic protons (Fig. S4b). In a microcentrifuge tube, 800 μL of this solution and dry Q8 sponge **5** (35 mg, 4.4 μmol) were added, vortexed for 10 minutes, and centrifuged (2 min, 14,000 rpm). The clear supernatant (500 μL) was carefully removed by pipet, lyophilized to dryness, and resuspended in D₂O (500 μL). The ¹H NMR spectrum of this sample a disappearance of the peaks corresponding to Q8, and a return of the chemical shifts of the MV aromatic protons to their original, unperturbed frequencies. Therefore, the experiment showed complete removal of Q8 (Fig. S4c).

Recycling: The used sponge **5** (i.e., Q8 containing) (17.5 mg) was transferred carefully to a 50 mL glass container fitted with a medium porosity glass frit and stopcock with screw cap (i.e., a manual solid-phase peptide synthesis vessel. Alternatively, a fritted filter could be used), washed with water (20 mL, 3 times), and drained completely. A solution of memantine hydrochloride in D₂O (**1**, 20 mM, 4.0 mL) was added to the resin, and the mixture was stirred vigorously for 5 minutes and drained. This process was repeated ten times, and the resin was washed with excess water (10 x 20 mL) to remove any residual memantine hydrochloride. The sponge was tested again for its capacity to remove Q8 from a solution of Q8•MV complex as described above in this section (Fig. S4d).

References

1. Day, A.; Arnold, A. P.; Blanch, R. J.; Snushall, B. *J. Org. Chem.* **2001**, *66*, 8094-8100.
2. Heitmann, L. M.; Taylor, A. B.; Hart, P. J.; Urbach, A. R. *J. Am. Chem. Soc.* **2006**, *128*, 12574-12581.

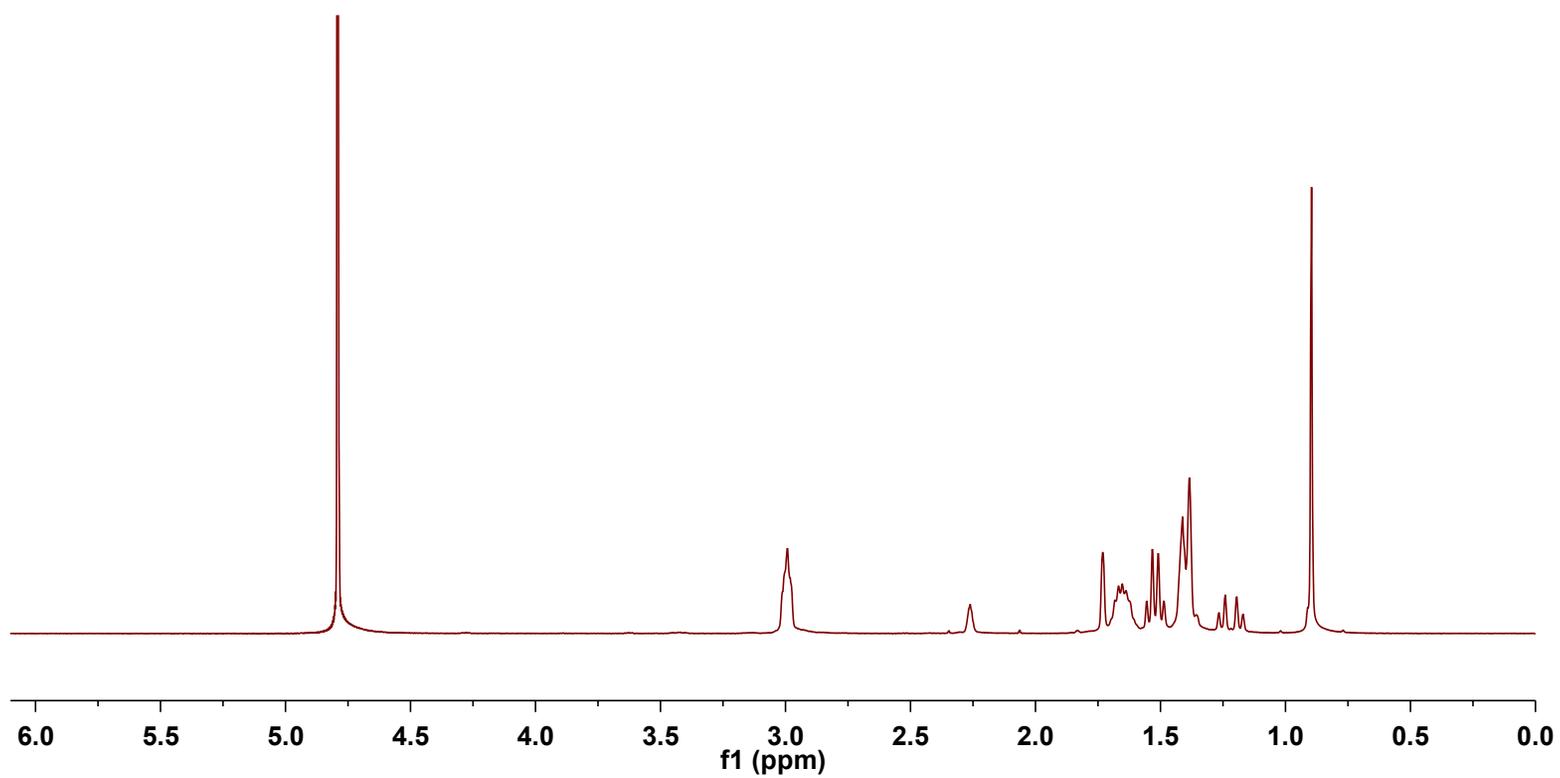
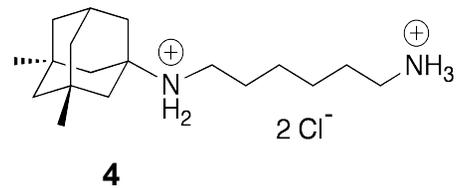


Figure S1. ^1H NMR (500 MHz) spectrum of **4** in D_2O , 25 °C

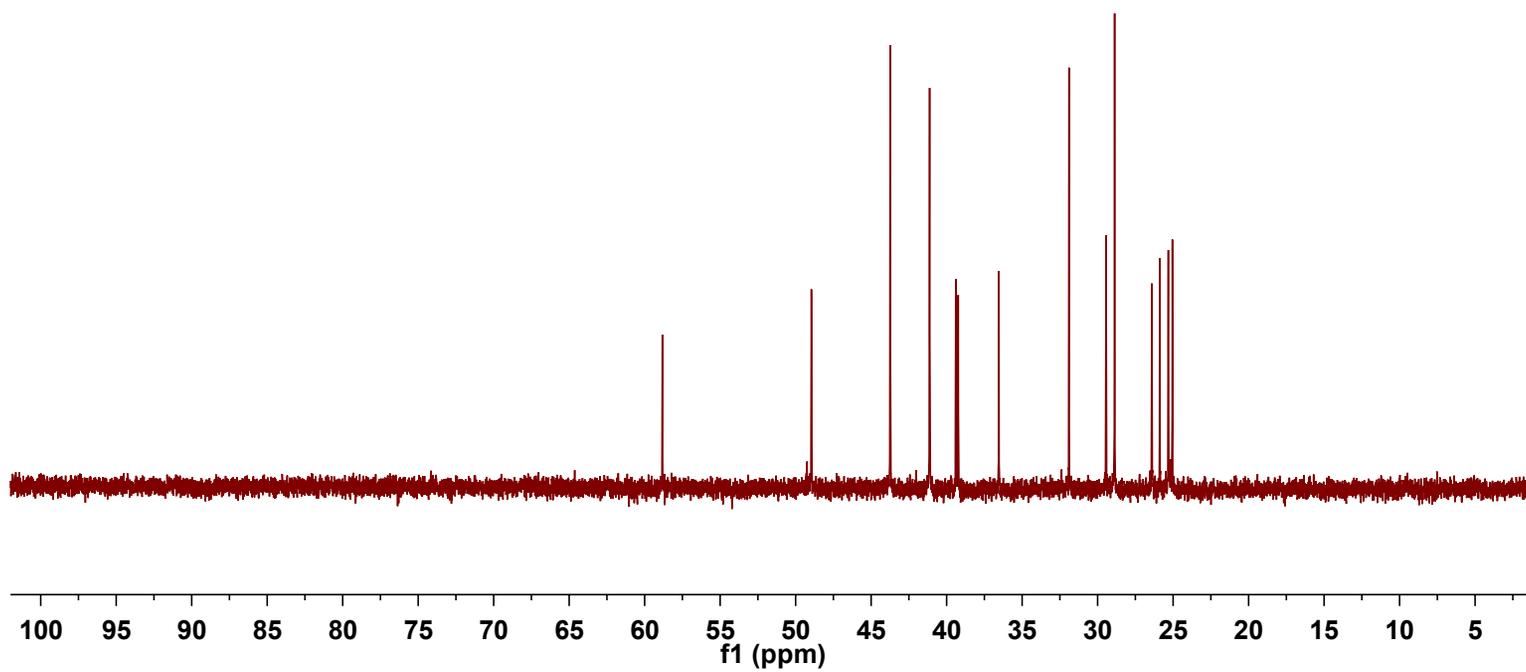
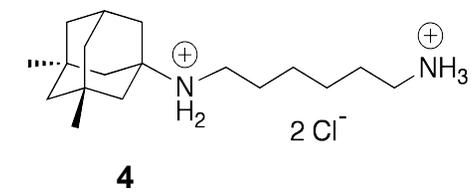


Figure S2. ^{13}C NMR (125 MHz) spectrum of **4** in D_2O , 25 °C

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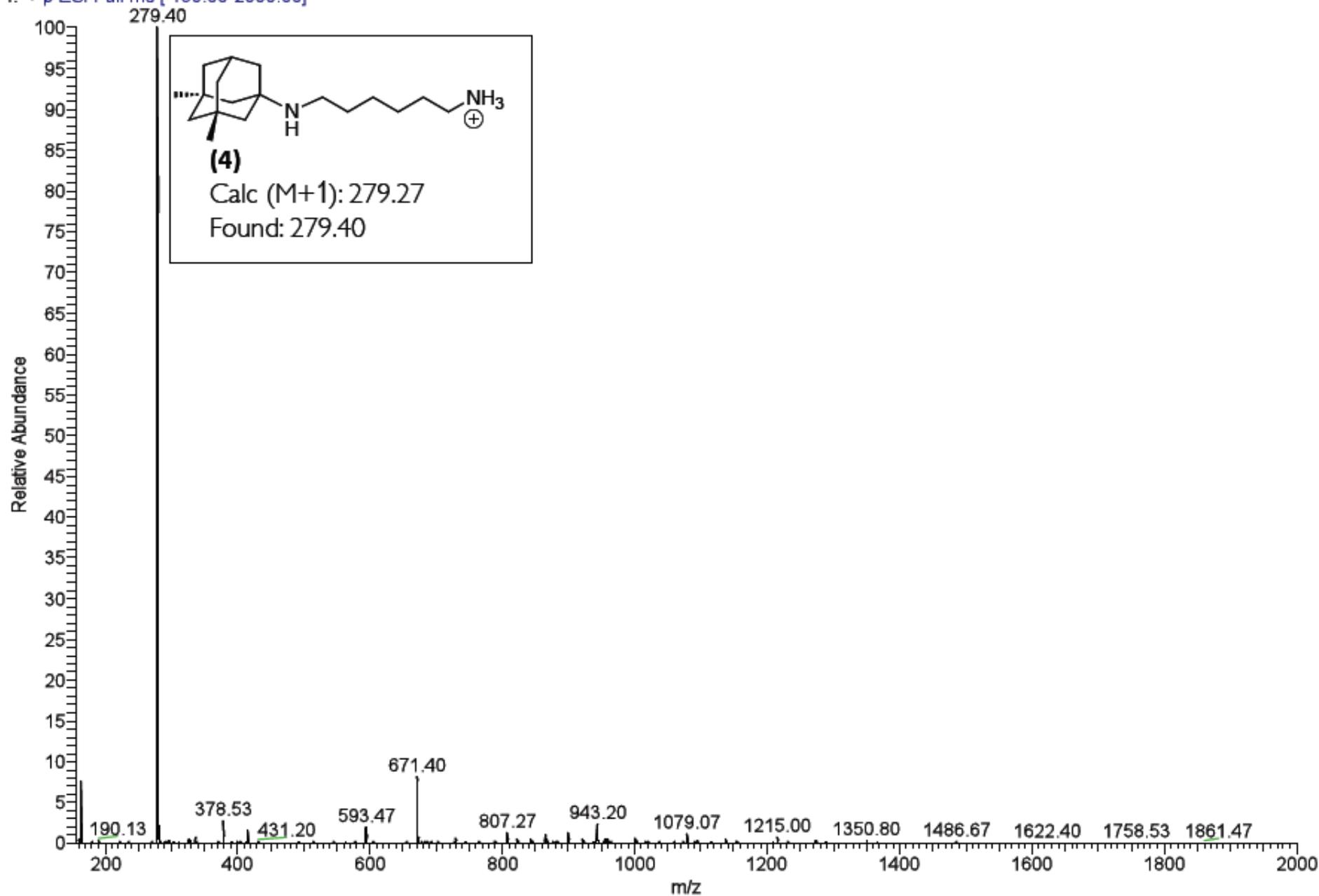


Figure S3. Electrospray ionization mass spectrometry (ESI-MS) data in positive ionization mode for compound **4**

Q8 sponge/ Recyclable Q8 sponge

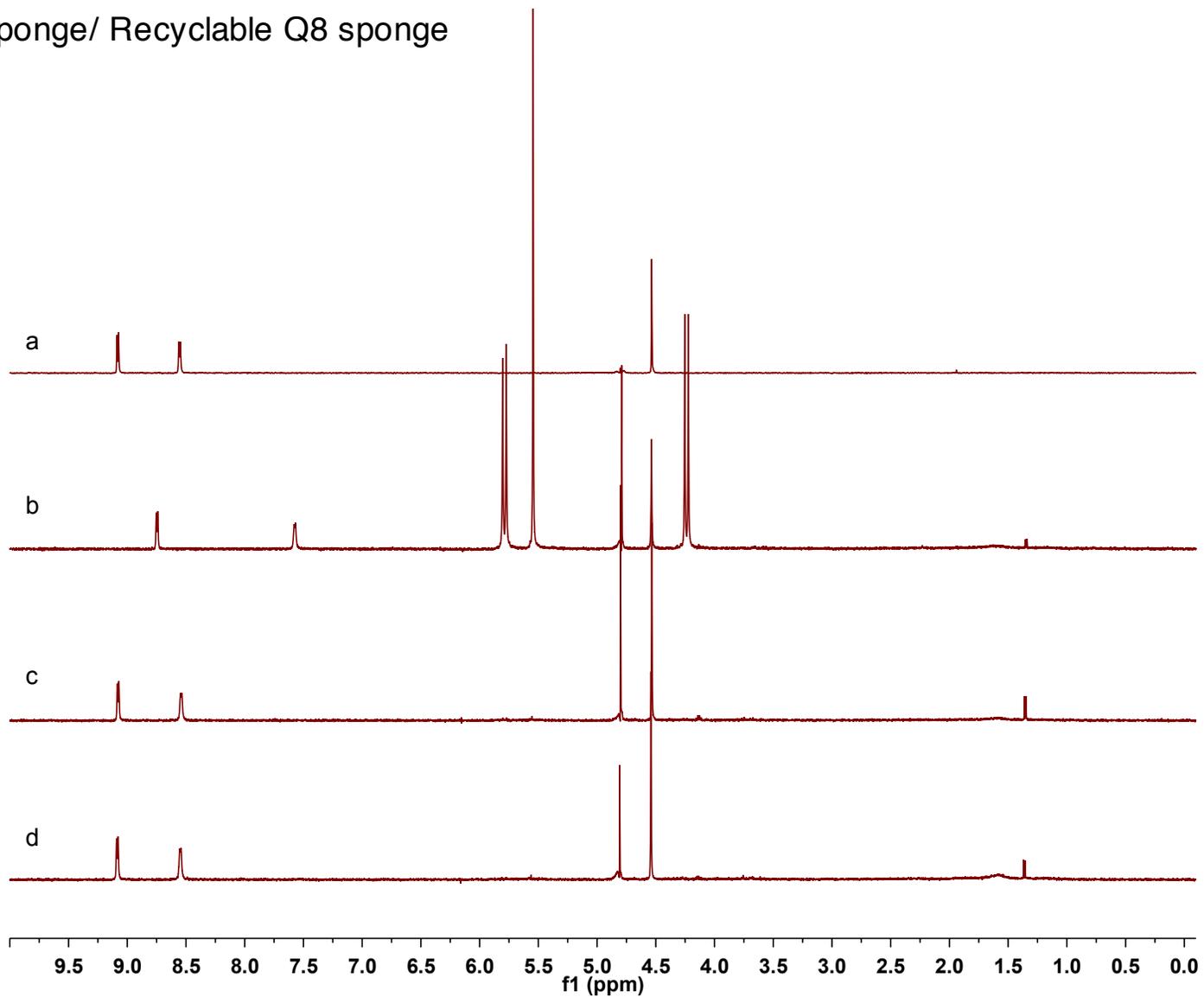


Figure S4. Overlay of ¹H NMR spectrum of a) methyl viologen (1 mM), b) Q8•MV (0.5 mM), c) Q8•MV (0.5 mM) + Q8 sponge (2.2 μmol), d) Q8•MV (0.5 mM) + Q8 recycled sponge (2.2 μmol) in D₂O, 25 °C.