^{1}H NMR Detection of Small-Molecules in Human Urine with a Deep Cavitand Synthetic Receptor

Daniel A. Ryan[a] and Julius Rebek Jr.*[a]

The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

jrebek@scripps.edu

Supporting Information

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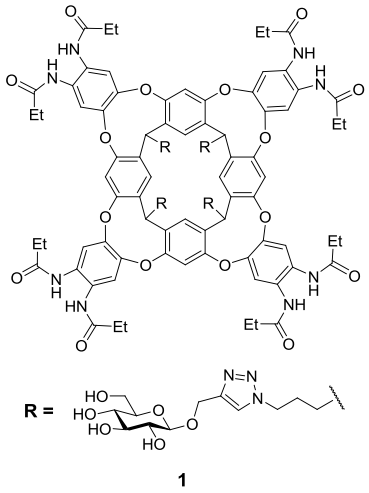
I. Instrumentation and Materials

Instrumentation. ^{1}H and ^{13}C NMR spectra were obtained at 600 MHz and 150 MHz on a Bruker DRX-600 spectrometer equipped with a 5 mm QNP probe; alternatively, some ^{13}C NMR spectra were obtained on a Bruker DRX-600 spectrometer equipped with a 5 mm cryoprobe. Spectra were recorded at 310 K unless otherwise stated. Chemical shifts are expressed in parts per million (δ scale) with respect to tetramethylsilane for chloroform-*d3* or dimethylsulfoxide-*d6*, or to sodium 3-(trimethylsilyl)propionate 2,2,3,3-*d4* for D_2O and biofluids analysis. Data are presented as follows: 1) chemical shift, 2) multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet and/or multiple resonances, b = broadened signal, app = apparent splitting pattern; otherwise Pople notation is used to describe spin systems with non-first order effects), 3) coupling constant (Hz), 4) integration, and 5) assignment. ^{1}H NMR assignments were generally supported by COSY, HMQC, and APT experiments. Mass spectrometry was performed at the Scripps Center for Metabolomics and Mass Spectrometry with an Agilent ESI-TOF instrument or an Applied Biosystems DE instrument for MALDI-TOF. All molecular modeling and semi-empirical calculations were performed using Spartan '04 Windows and graphically modeled using the PyMol visualization software (Schrödinger).

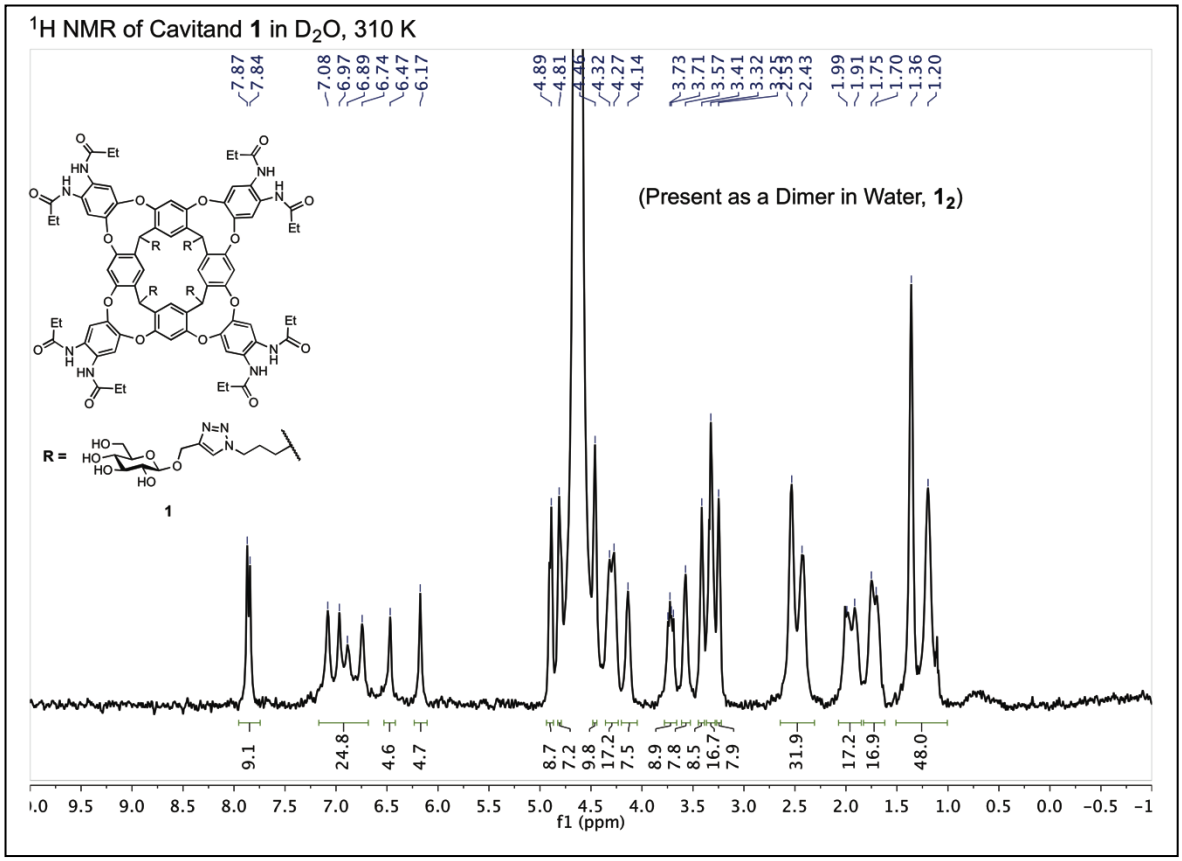
Materials. The carbohydrate-conjugated cavitand was synthesized and purified by preparative HPLC protocols as previously described.[1] Quinuclidine hydrochloride and amantadine hydrochloride were purchased from Sigma-Aldrich (St. Louis, USA). Freeze-dried pooled human urine (Kova-Trol III) was supplied by Hycor Biomedical and purchased through VWR. NMR solvents dimethylsulfoxide-d_6, acetone-d_6, and D_2O (100% *d*, ampules), as well as the NMR standard sodium 3-(trimethylsilyl)propionate 2,2,3,3-d_4 (TMSP), were purchased from Cambridge Isotope Laboratories, Inc. All other reagents and solvents were purchased from commercial suppliers and used without additional purification.

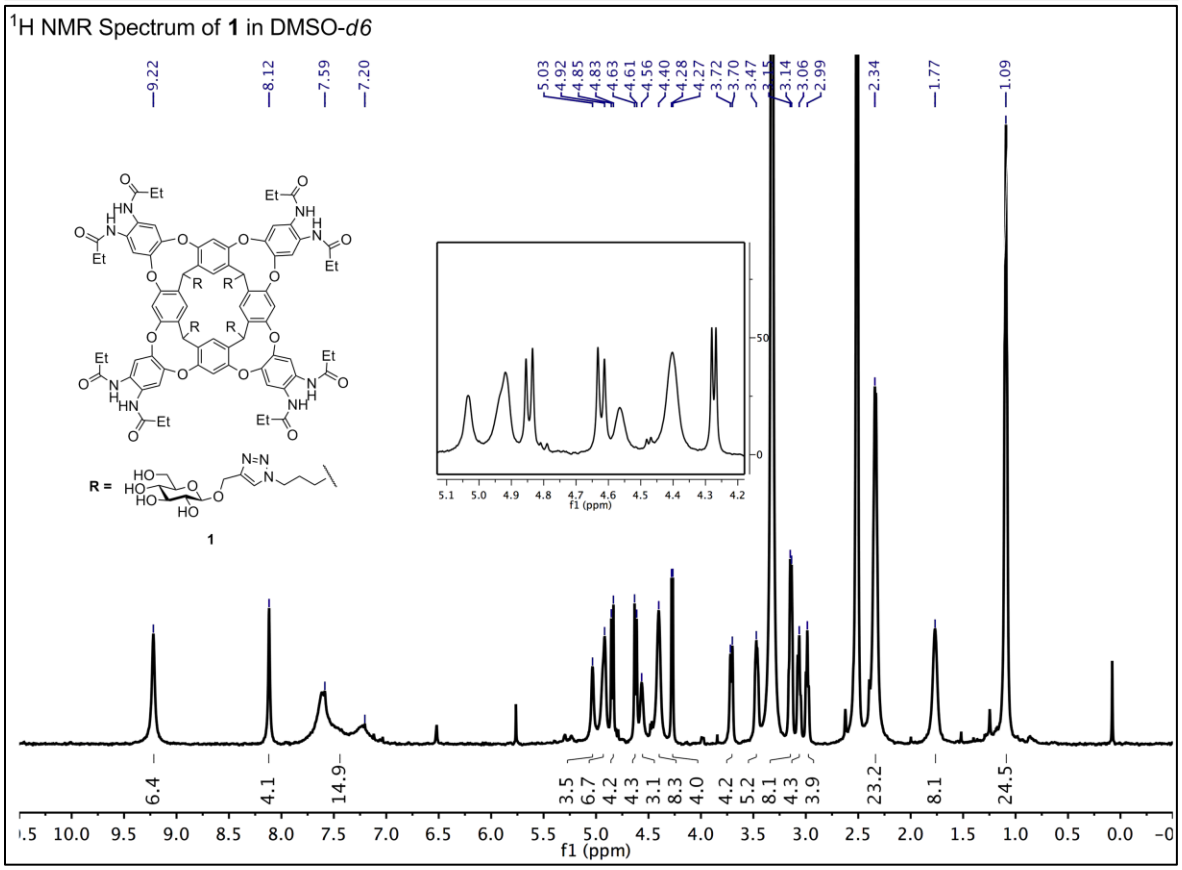
I. Spectroscopic and HPLC characterization of Cavitand 1.



Characterization of the cavitand **1** *in DMSO-d6:* A portion of cavitand **1** was lyophilized from deuterium oxide (2x) and characterized in DMSO-*d6*, which in this solvent provided a spectrum lacking the complex splitting patterns observed in water. ^{1}H NMR (600 MHz, DMSO-*d6*): δ = 9.22 (s, N*H*), 8.12 (s, 4H, *H*-triazole), 7.59 (broad s, 12H, Ar*H*), 7.20 (broad s, 4H, Ar*H*), 5.03 (app s, 4H, *H*O-C_2^{Glc}), 4.92 (m, 8H, *H*O-C_3^{Glc}, *H*O-C_4^{Glc}), 4.85-4.61 (ABq, *J* = 11.4 Hz, 8H, O-C*H*$_2$-$C^{triazole}$), 4.55 (s, 4H, *H*O-C_6^{Glc}), 4.40 (m, 8H, -C*H*$_2$-$N^{triazole}$), 4.28 (d, *J* = 7.76 Hz, 4H, $H1^{Glc}$), 3.71 (s, 4H, $H6^{Glc}$), 3.47 (s, 4H, $H6'^{Glc}$), 3.14 (app s, 8H, including $H5^{Glc}$), 3.06 (s, 4H), 2.99 (s, 4H, $H2^{Glc}$), 2.34 (m, 32H overlaps with solvent, -(O)CC*H*$_2$CH$_3$, -C*H*$_2$-$CH^{methine}$), 1.77 (broad s, 8H, -C*H*$_2$-), 1.09 (broad s, 24 H, -(O)CCH$_2$C*H*$_3$) ppm. ^{13}C NMR (150 MHz, DMSO-*d6*): 172.3, 157.7, 157.5, 153.9 (broad), 143.9, 127.9 (broad), 125.1, 124.1, 102.2, 77.0, 76.7, 73.4, 70.1, 61.5, 61.2, 48.9, 33.5 (broad), 29.3, 28.4, 9.7, 1.1 ppm. MS (MALDI-TOF)$^+$: m/z calcd. For $C_{124}H_{148}N_{20}O_{40}$ $[M+Na]^+$: 2580; found 2580.3.

Characterization of the cavitand dimer $\mathbf{1_2}$ *in* D_2O*:* ^{1}H NMR (600 MHz, D_2O): δ = 7.87 and 7.85 (2 app s, 8H, *H*-triazole), 7.08-6.74 (broad m, 24H, Ar*H*), 6.46 (broad s, 4H, ArH), 6.17 (broad s, 4H, ArH), 4.88 (app s, 8H, overlaps with solvent peak), 4.81 (app s, 8H, overlaps with solvent peak), 4.46 (broad s, H, overlaps with solvent peak), 4.31-4.28 (broad m, 8H), 4.13 (broad s, 8H, C*H*$_{methine}$), 3.72 (m, 8H), 3.57 (app s, 8H), 3.41 (app s, 8H), 3.32 (m, 16H), 3.24 (m, 8H), 2.53 and 2.41 (2 app s, broad, 32H, (O)CC*H*$_2$CH$_3$), 2.01 and 1.91 (2 app s, broad, 16H, -C*H*$_2$-), 1.74 and 1.70 (2 app s, broad, 16H, -C*H*$_2$-), 1.42-1.06 (m, 48H, (O)CCH$_2$C*H*$_3$) ppm; MS (MALDI-TOF)$^+$: m/z calcd. For $C_{248}H_{296}N_{40}O_{80}$ $[2M+H]^+$: 5015; found 5014.


^{1}H NMR of Cavitand **1** in D_2O, 310 K
(Present as a Dimer in Water, $\mathbf{1_2}$)
R =
1
f1 (ppm)


^{1}H NMR Spectrum of **1** in DMSO-*d6*
R =
1
f1 (ppm)

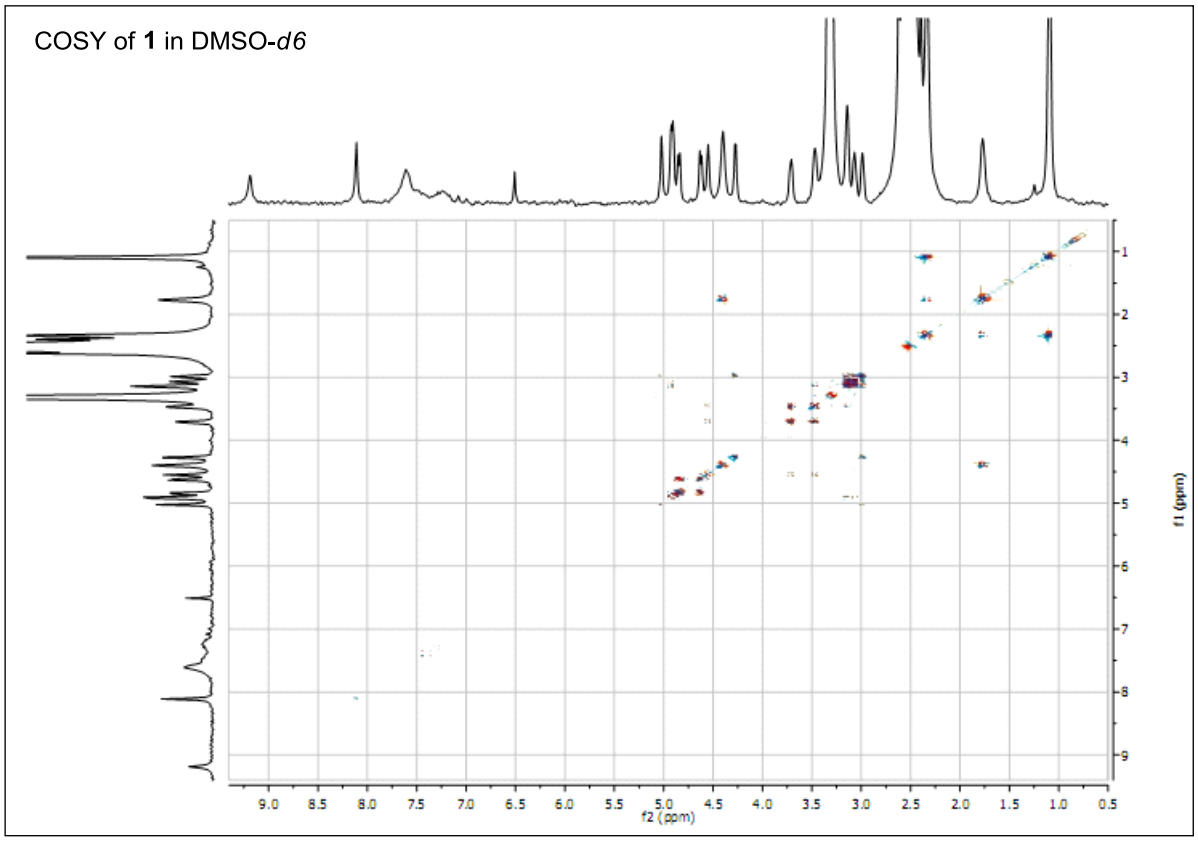
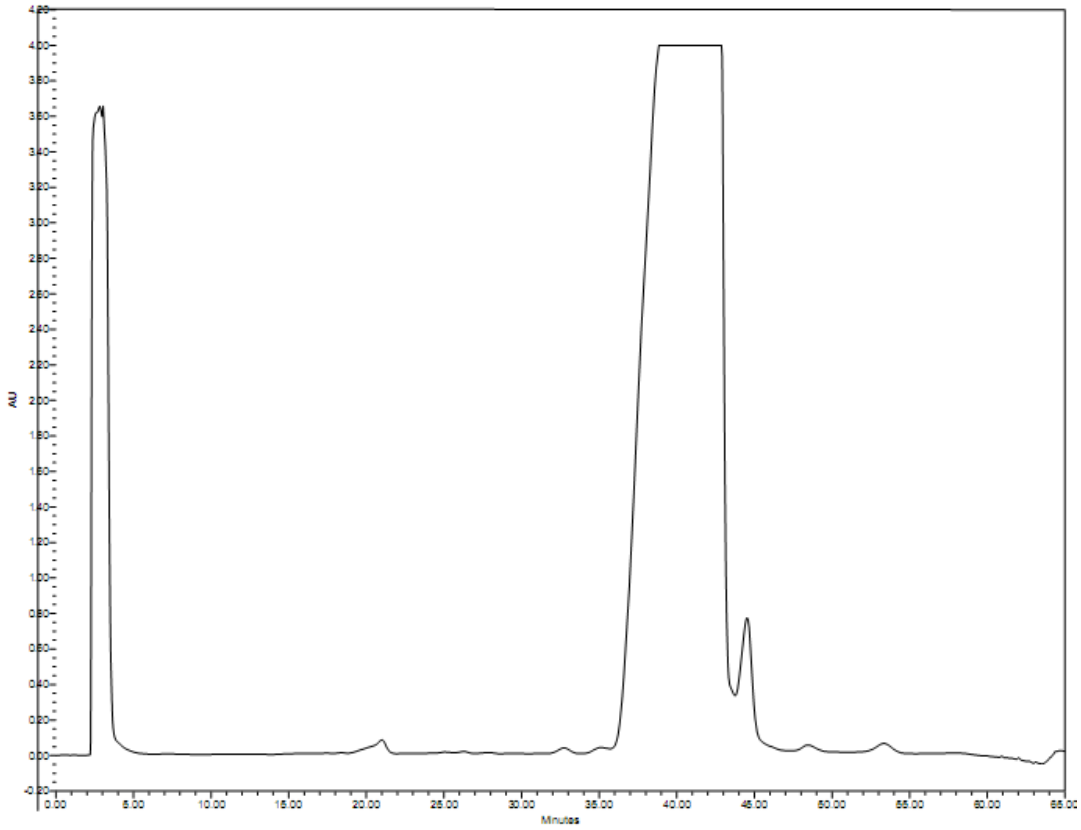


Figure S1. Preparative HPLC Chromatogram from purification of **1**.



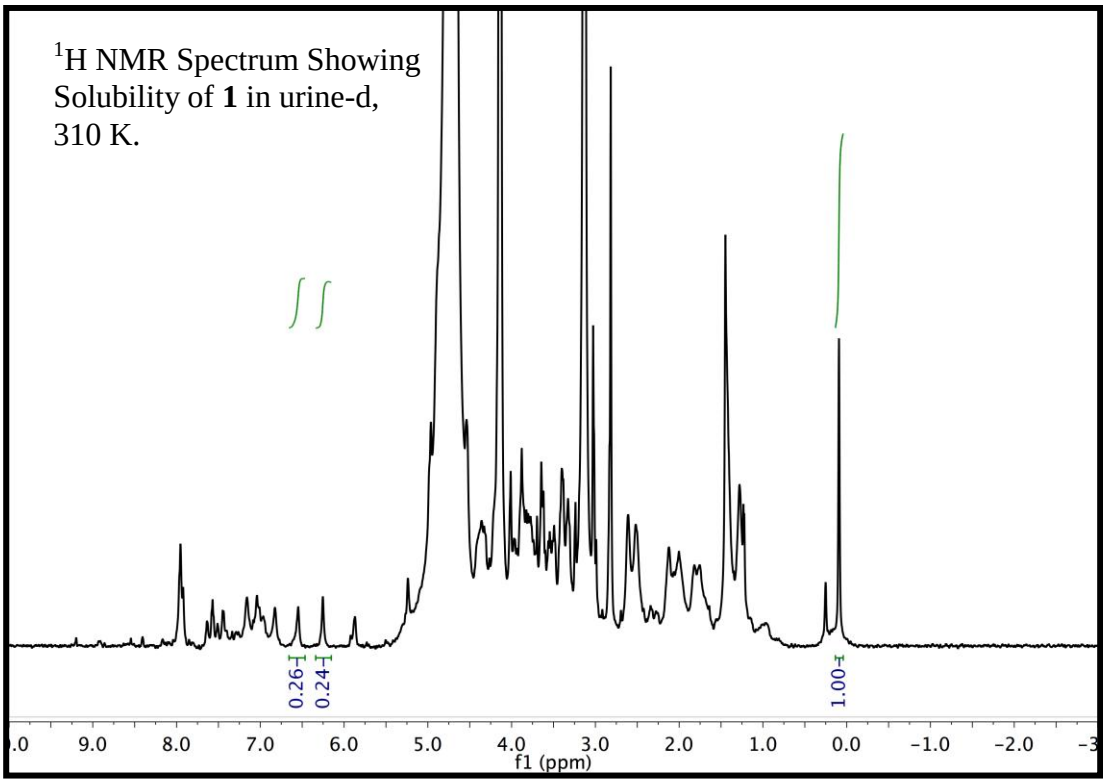
III. Solubility Determination and ^{1}H NMR Spectra of Cavitand 1 in Human Urine.

Preparation of External Standard for NMR Experiments. The external standard was prepared as previously described[1]: A ~10 mM solution of sodium 3-(trimethylsilyl)propionate 2,2,3,3-*d4* in deuterium oxide was prepared and syringe-injected into a Wilmad NMR capillary (#529-D) so that the height of the solution exceeds the height of the instrument receiver coil (approximately 75% of the tube volume). The capillary was carefully flame-sealed and then calibrated against 0.2 mM, 0.4 mM, and 0.8 mM standard solutions of adamantylamine hydrochloride using the following parameters: T_1 (longest) = 3.6 s (the external standard), d1 = 5 x T_1 = 18 s, p1 = pw90 = 16.5 μs, T = 310 K, with a zg pulse sequence. Wilmad PTFE Insert Holder (529-B) and positioning rod (529-C) were used to introduce the capillary to the NMR tube. The same external standard was used in all experiments with Kontes 240 precision NMR tubes rated for >400 MHz applications and variation of wall thickness of: od = 4.97 mm (-0.013 mm) and id = 4.20 mm (+0.013 mm). The external standard had an effective concentration of 0.54 mM at 310K.

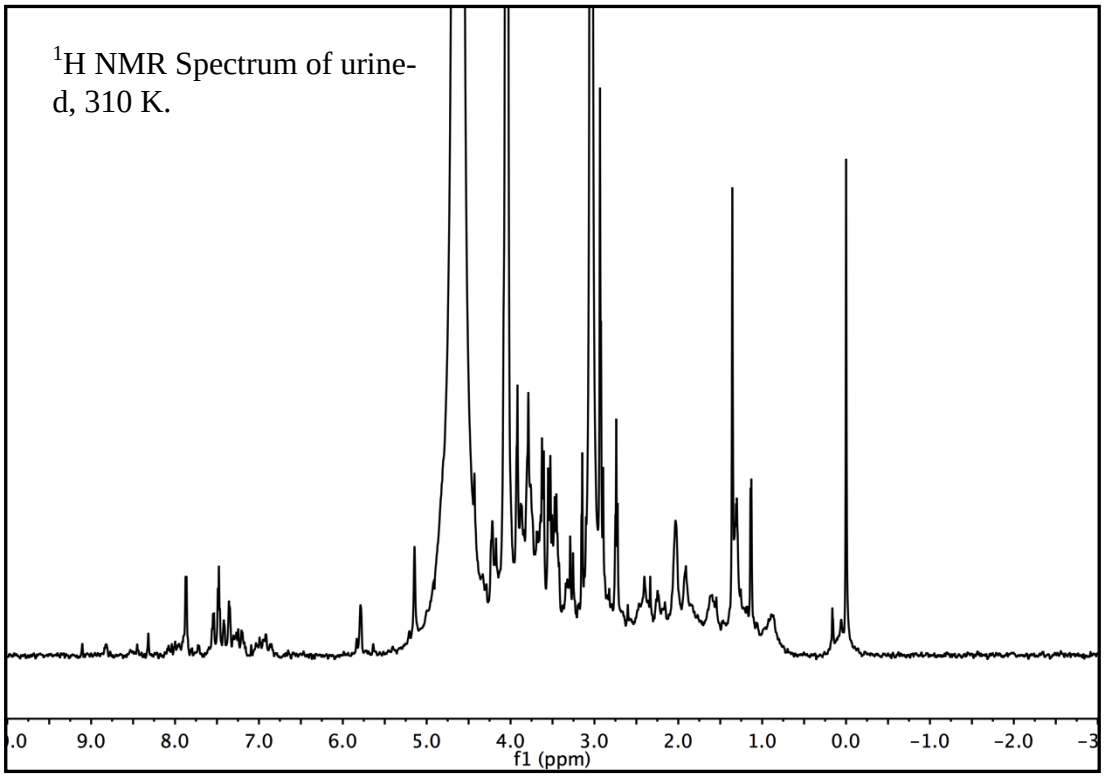
Standard	**ES Effective Concentration**
0.2 mM $AdNH_3Cl$	0.55 mM
0.4 mM $AdNH_3Cl$	0.53 mM
0.8 mM $AdNH_3Cl$	0.53 mM
Average	**0.54 mM**
St. Dev	0.012 mM

Solubility of Cavitand 1 in urine. Deuterium oxide (500 μL) was added to a dry mixture of cavitand **1** (0.6 mg, 0.235 μmol) and freeze-dried human urine powder (8.0 mg, taken from 110 mg freeze-dried powder from 15 mL total volume) in a Kontes 240 NMR tube. The resulting suspension was immersed in a 310 K water bath with periodic stirring using a Vortex Genie 2 and then the external standard was introduced using the Wilmad PTFE Insert Holder (529-B) and positioning rod (529-C). For spectrum acquisition, the sample was equilibrated at 310 K in the NMR instrument for 15 minutes and then a proton spectrum was acquired using the following parameters: T_1 (longest) = 3.6 s (the external standard), d1 = 5 x T_1 = 18 s, p1 = pw90 = 16.5 μs, ns = 233, without sample spinning. The same external standard was used in all experiments with a Kontes 240 precision NMR tubes. For spectrum processing, a Fourier Transform with a 4 Hz exponential function was applied, the spectrum was phased manually and baseline corrected.

Integration of the external standard with respect to the average of 2 resonances of the cavitand (those that were unobstructed by signals of the biofluid) provided a solubility of **1** of 70 μM at 310K (or 35 μM for $\mathbf{1}_2$), as shown below.



^{1}H NMR Spectrum Showing Solubility of **1** in urine-d, 310 K.



^{1}H NMR Spectrum of urine-d, 310 K.

IV. Binding Constant Determination for Cavitand 1 in Human Urine.

Previously, we determined the binding constant expression of cavitand **1**, depicted in eq (1), that fits to a dimeric state of the cavitand observed in aqueous media.[1] Table S1 lists the binding constant data for quinuclidine hydrochloride and amantadine hydrochloride in urine-d and in D_2O.

$$\left(\frac{1}{2}\right)\mathbf{1_2} + \mathbf{guest} \rightleftharpoons \mathbf{1 \bullet guest} \qquad (1)$$

$$K_a = \frac{[\mathbf{1 \bullet guest}]}{[\mathbf{1_2}]^{1/2} \times [\mathbf{guest}]}$$

Table S1. ^{1}H NMR Binding Constant Data for Encapsulation Complexes with Cavitand 1 at 310K (error estimated at +/- 15%).

Guest	Solvent	K_a ($mM^{-1/2}$)	K_a ($M^{-1/2}$)
Quinuclidine HCl	D_2O	0.19	6.0
Quinuclidine HCl	Urine-d	0.11	3.5
Amantadine HCl	D_2O	0.055	1.7
Amantadine HCl	Urine-d	0.037	1.2

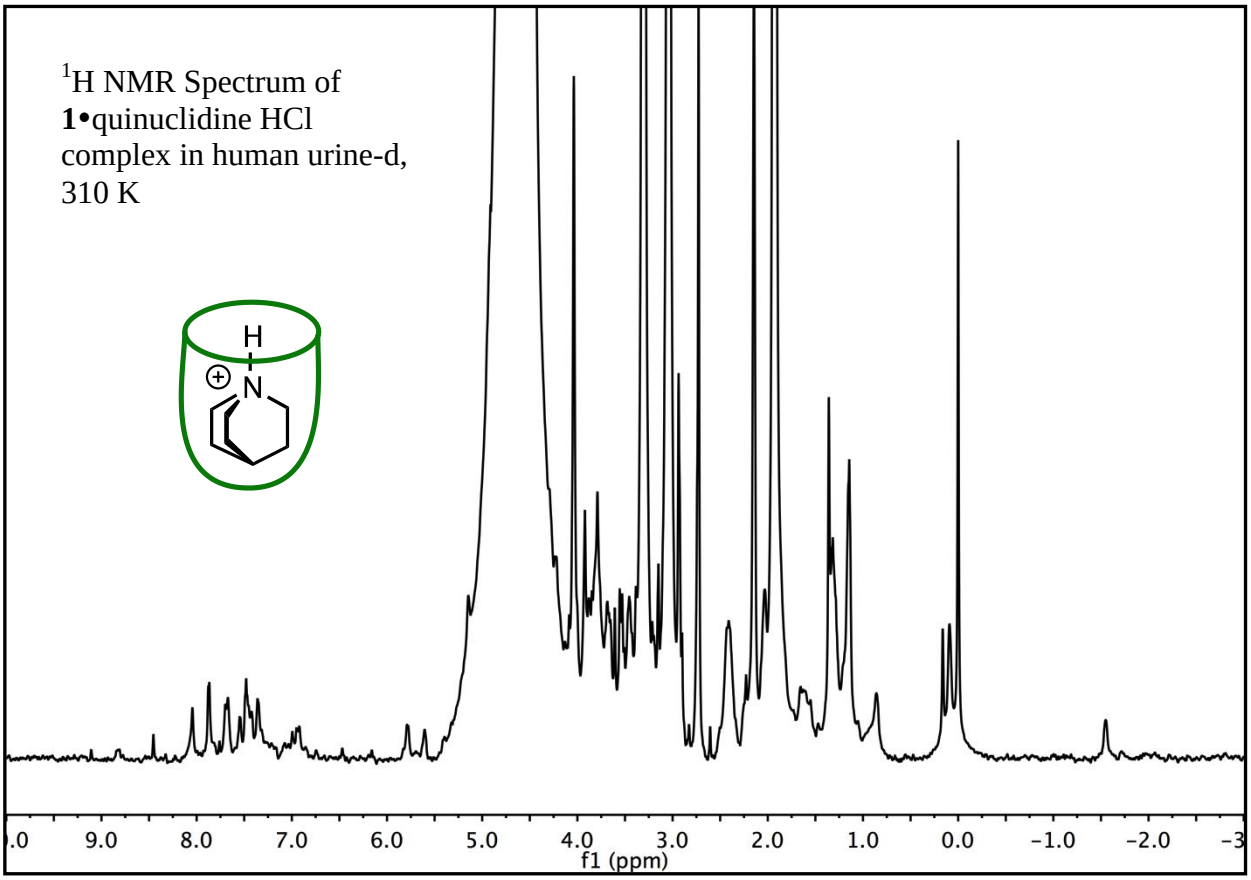
Procedure for Binding Constant Determinations in urine.

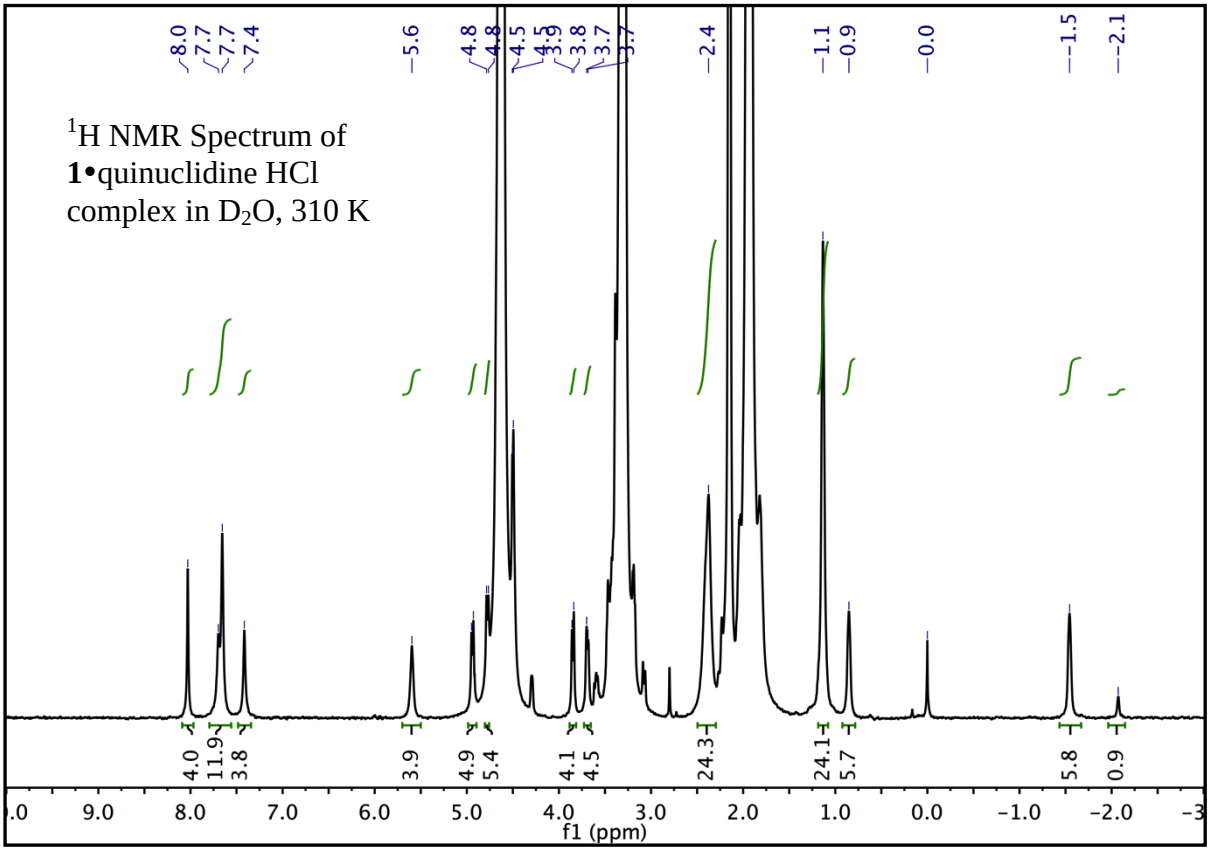
For a typical binding experiment, cavitand **1** (1.2 mg, 0.00047 mmol) was added to a one-dram vial, charged with deuterium oxide (1.0 mL, 99.96%-*d*), and stored in a 310 K water bath for 30 minutes with periodic agitation by a Vortex Genie. The saturated suspension of **1** thus obtained was filtered through a 0.45 μm PTFE syringe-filter (National Scientific) to provide a solution from which a 450 μL aliquot was syringe-transferred to a Kontes 240 NMR tube that contained freeze-dried human urine (8.0 mg, taken from 110 mg of freeze-dried powder from 15 mL of human urine).

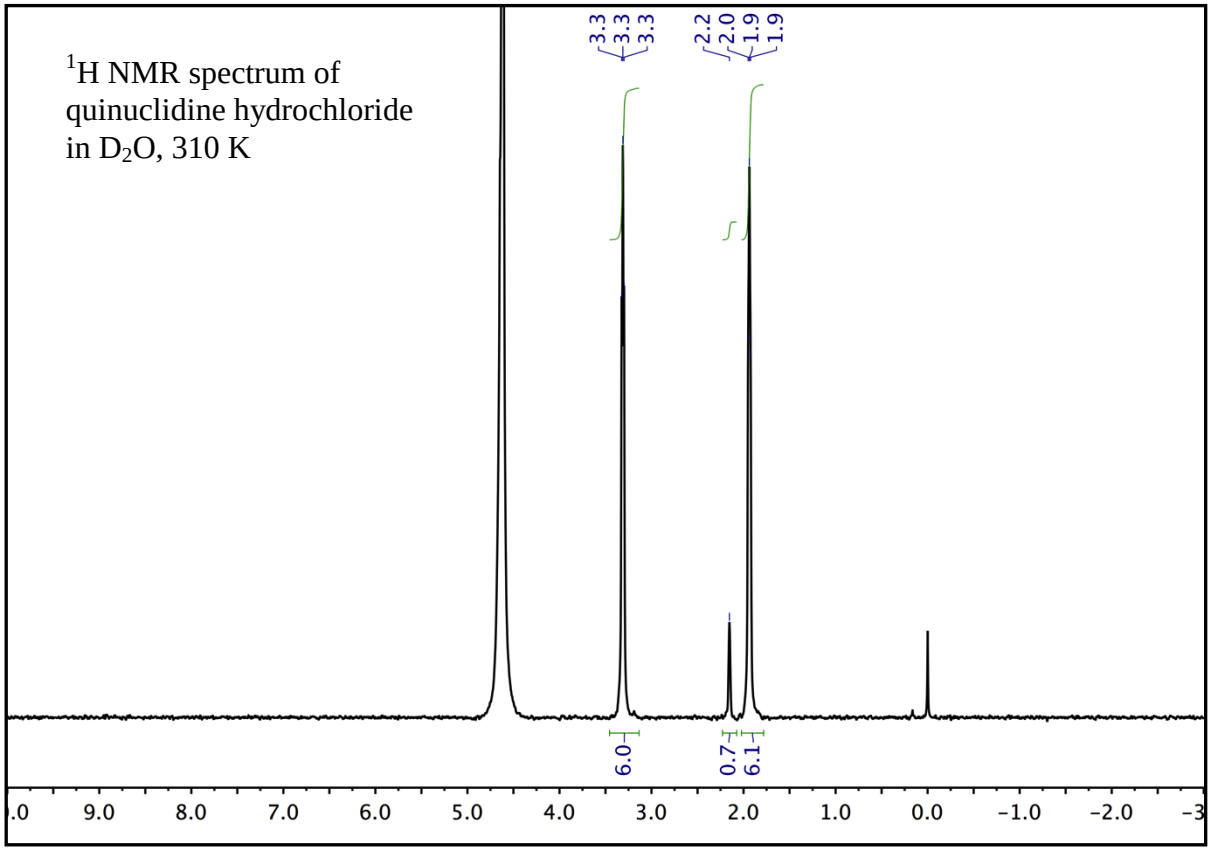
For binding constant determination, a 10 μL aliquot of a standard solution of guest was added by syringe to the solution of **1**, followed by introduction of the NMR external standard capillary, prepared as described earlier. Binding constants were obtained at 310 K following pre-equilibration at this temperature for 10 minutes in the NMR probe. The ^{1}H NMR acquisition parameters were: d1 = 5 x T1 (longest) = 18 seconds (the external standard, 0.54 mM); p1 = pw90 = 16.5 μs, and no sample spinning. The FID was processed by a Fourier Transform

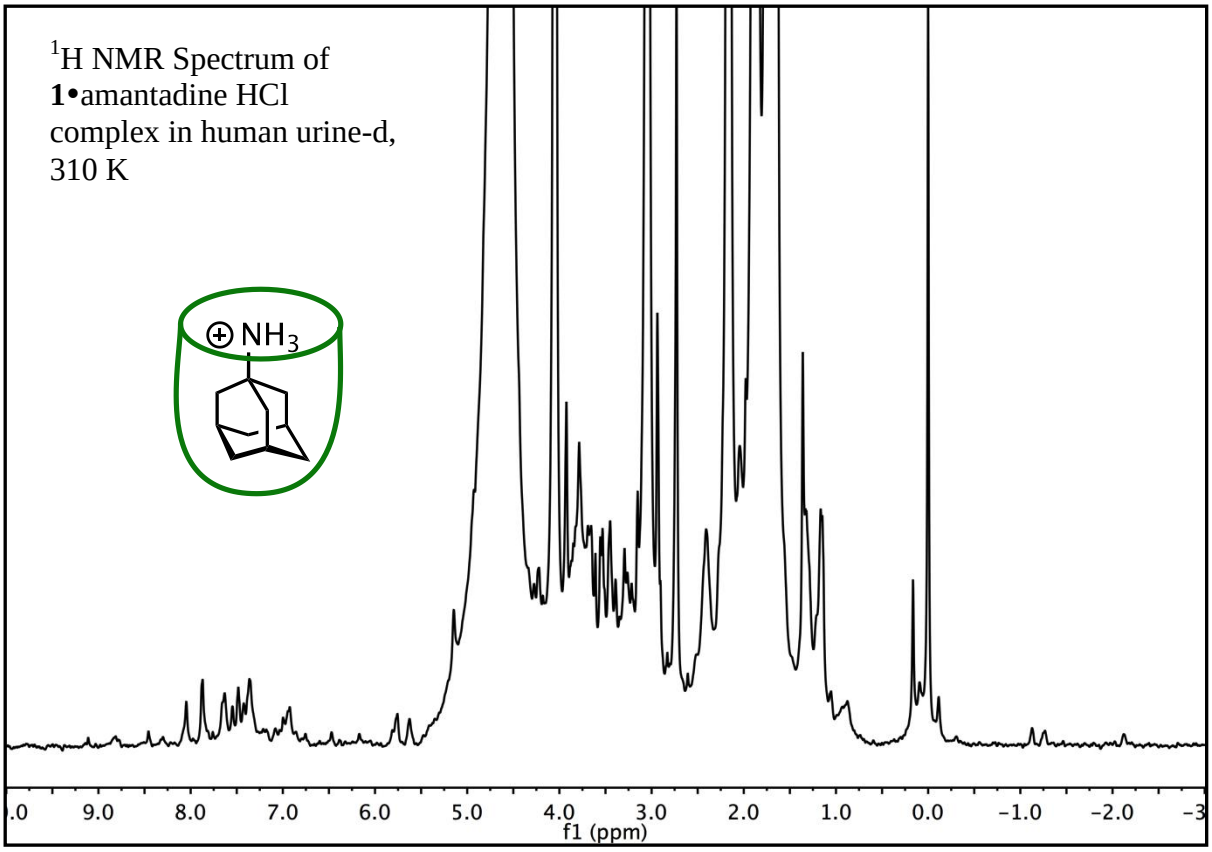
applying a 6 Hz exponential line-broadening function, followed by manual phasing, baseline correction, and integration. The equilibrium concentrations of cavitand and of the host-guest complex were determined directly from integration of their respective signals and comparison to the integral for the external concentration standard with an apparent concentration of 0.54 mM; the equilibrium concentration of free guest was determined from the total concentration of guest present from titration less the concentration of host-guest complex determined as above. The resulting K value is the average from at least 3 data points within 5 to 95% extent of complex formation.

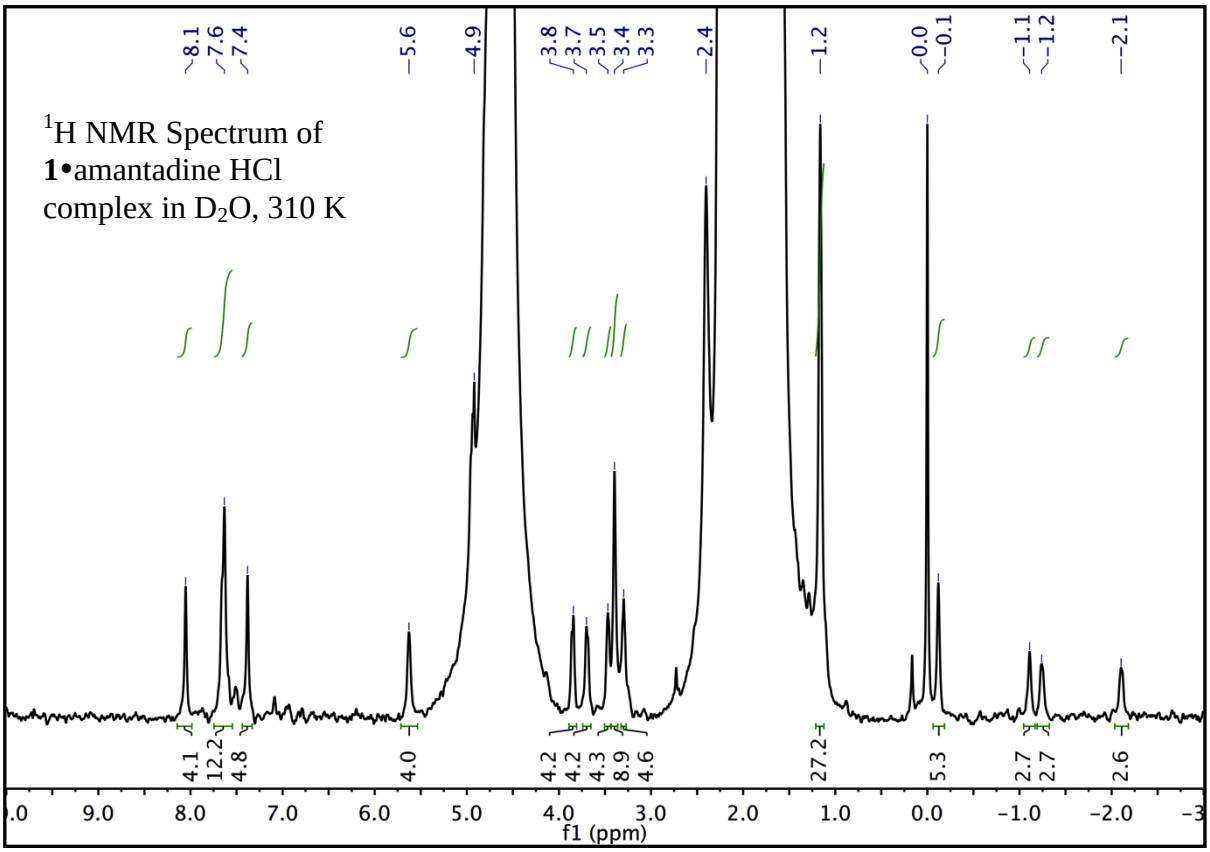
VI. ^{1}H NMR Spectra of Urine Caviplexes and Reference Spectra of Complexes and Guests in D_2O.

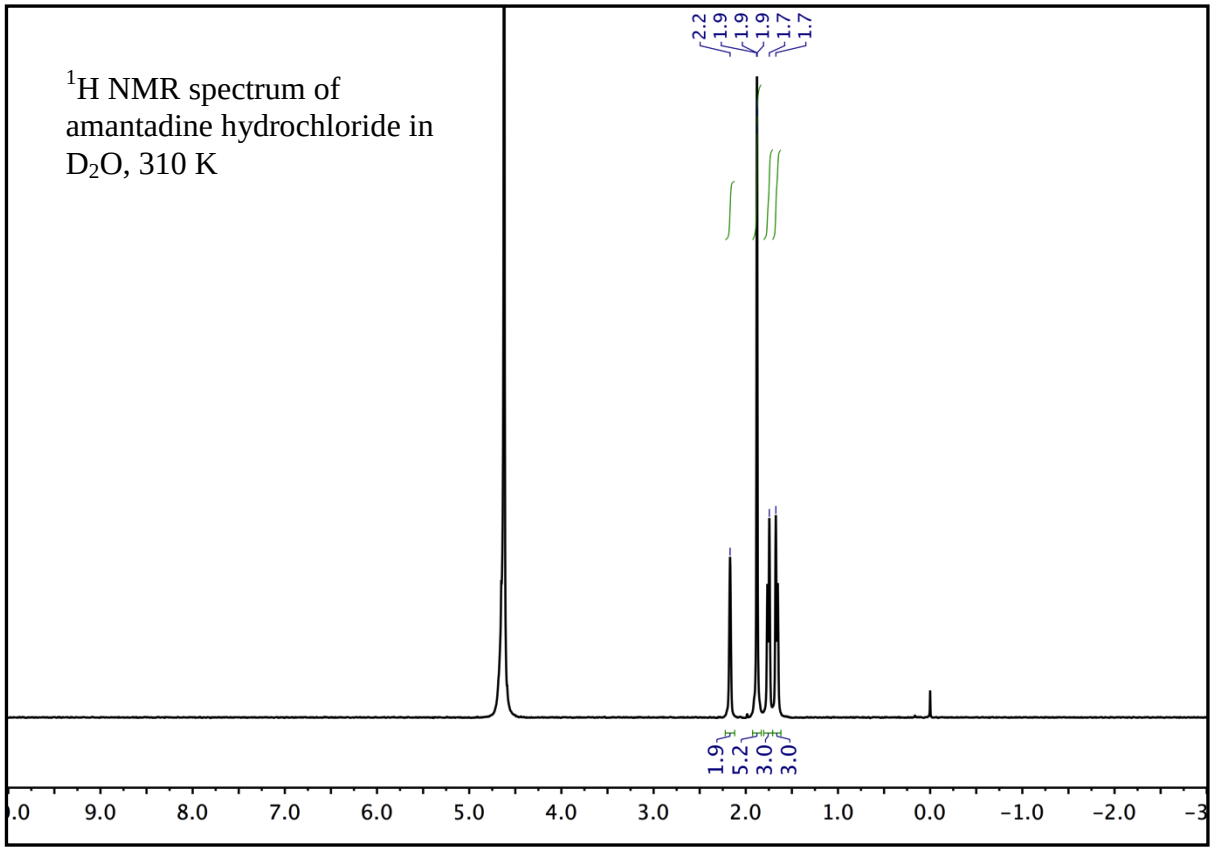



^{1}H NMR Spectrum of
1•quinuclidine HCl
complex in D_2O, 310 K
8.0
7.7
7.7
7.4
5.6
4.8
4.8
4.5
4.5
3.9
3.8
3.7
3.7
2.4
1.1
0.9
0.0
1.5
2.1
4.0
11.9
3.8
3.9
4.9
5.4
4.1
4.5
24.3
24.1
5.7
5.8
0.9
f1 (ppm)


^{1}H NMR spectrum of
quinuclidine hydrochloride
in D_2O, 310 K
3.3
3.3
3.3
2.2
2.0
1.9
1.9
6.0
0.7
6.1

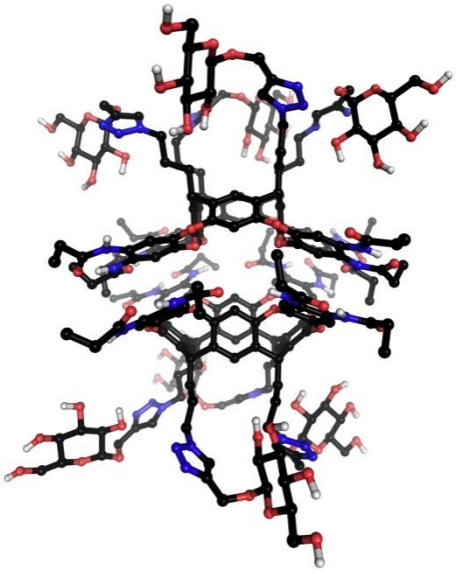

^{1}H NMR Spectrum of
1•amantadine HCl
complex in human urine-d,
310 K
⊕NH3
f1 (ppm)


^{1}H NMR Spectrum of
1•amantadine HCl
complex in D_2O, 310 K
8.1
7.6
7.4
5.6
4.9
3.8
3.7
3.5
3.4
3.3
2.4
1.2
0.0
-0.1
-1.1
-1.2
2.1
4.1
12.2
4.8
4.0
4.2
4.2
4.3
8.9
4.6
27.2
5.3
2.7
2.7
2.6
f1 (ppm)



VII. Model of Cavitand Dimer.

Figure S1. Model of Dimeric Velcrand $\mathbf{1_2}$ (MMFF minimized, with distance constraints for packing of the monomers from reported crystal structures of analogous dimers).[2]



V. References

(1) Ryan, D. A.; Rebek Jr., J. *J. Am. Chem. Soc.* **2011**, *133*, 19653.
(2) Pirondini, L.; Stendardo, A. G.; Geremia, S.; Campagnolo, M.; Samorì, P.; Rabe, J. P.; Fokkens, R.; Dalcanale, E. *Angew. Chem. Int. Ed.* **2003**, *42*, 1384.