Supporting Information

for

Binding trimethyllysine and other cationic guests in water with a series of indole-derived hosts: large differences in affinity from subtle changes in structure
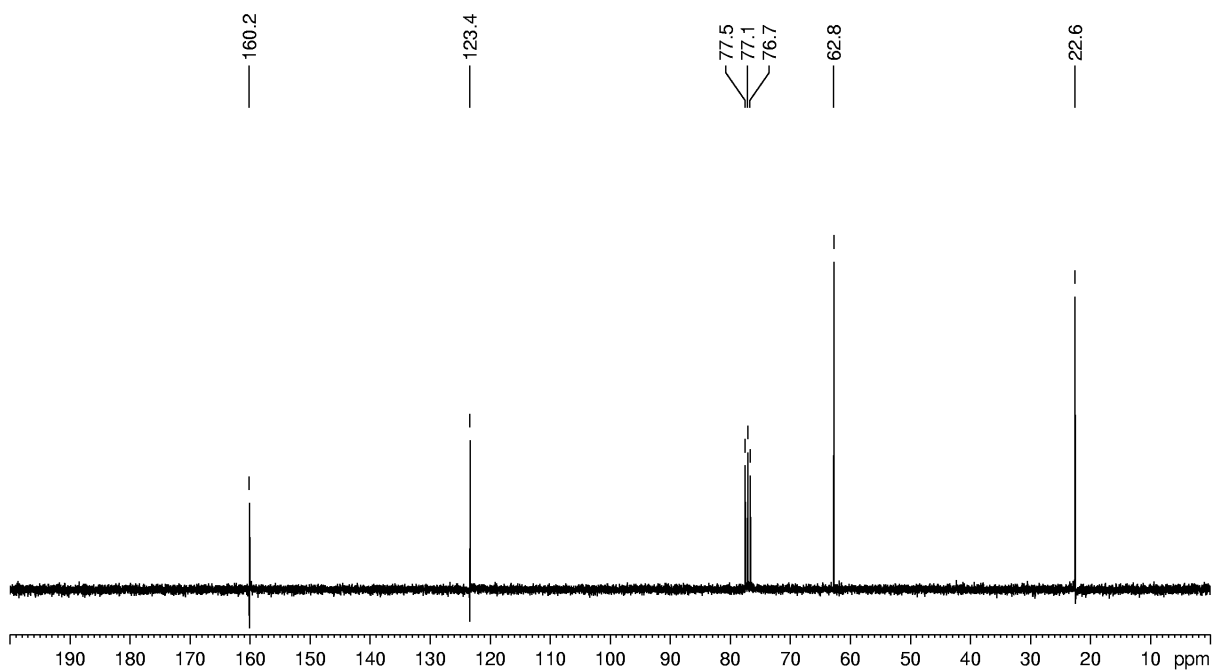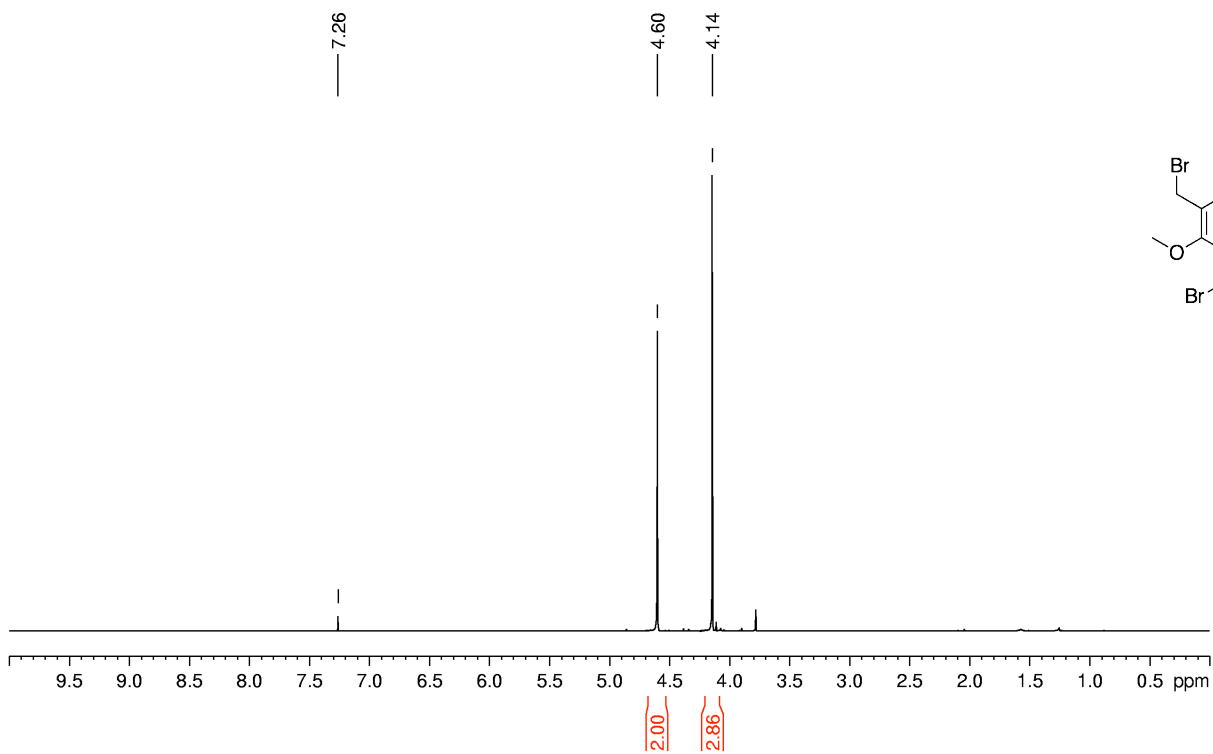
Amanda L. Whiting, and Fraser Hof*

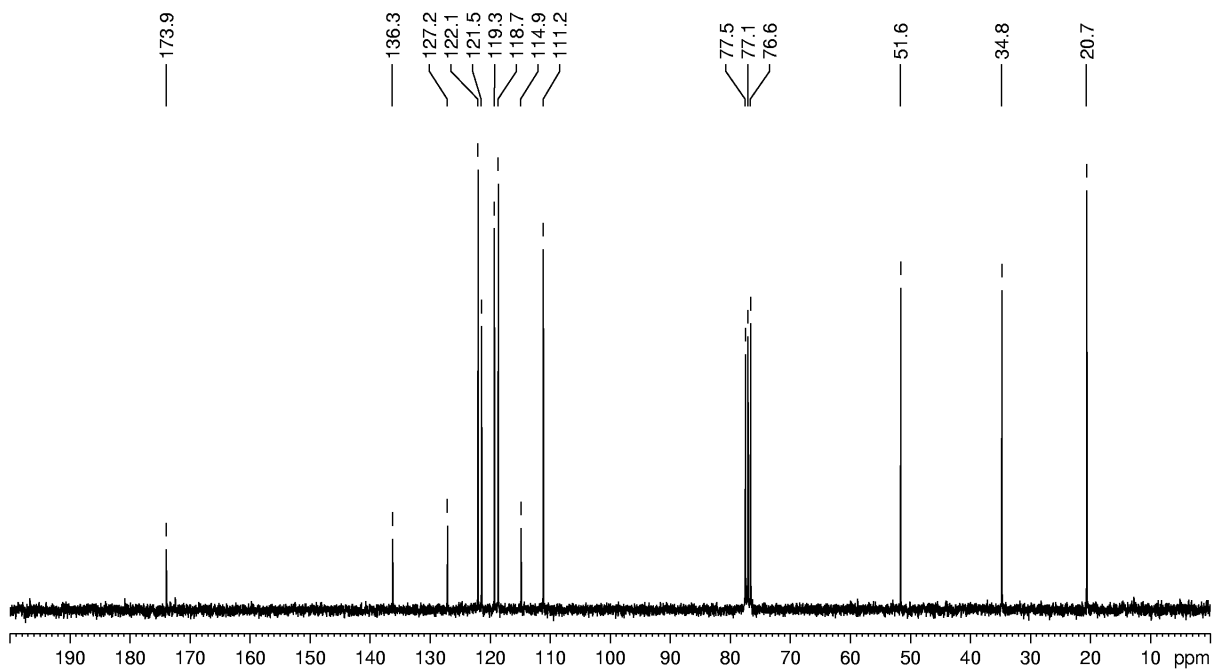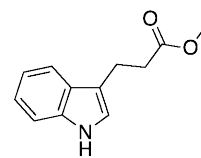### *Table of Contents for Supporting Information*
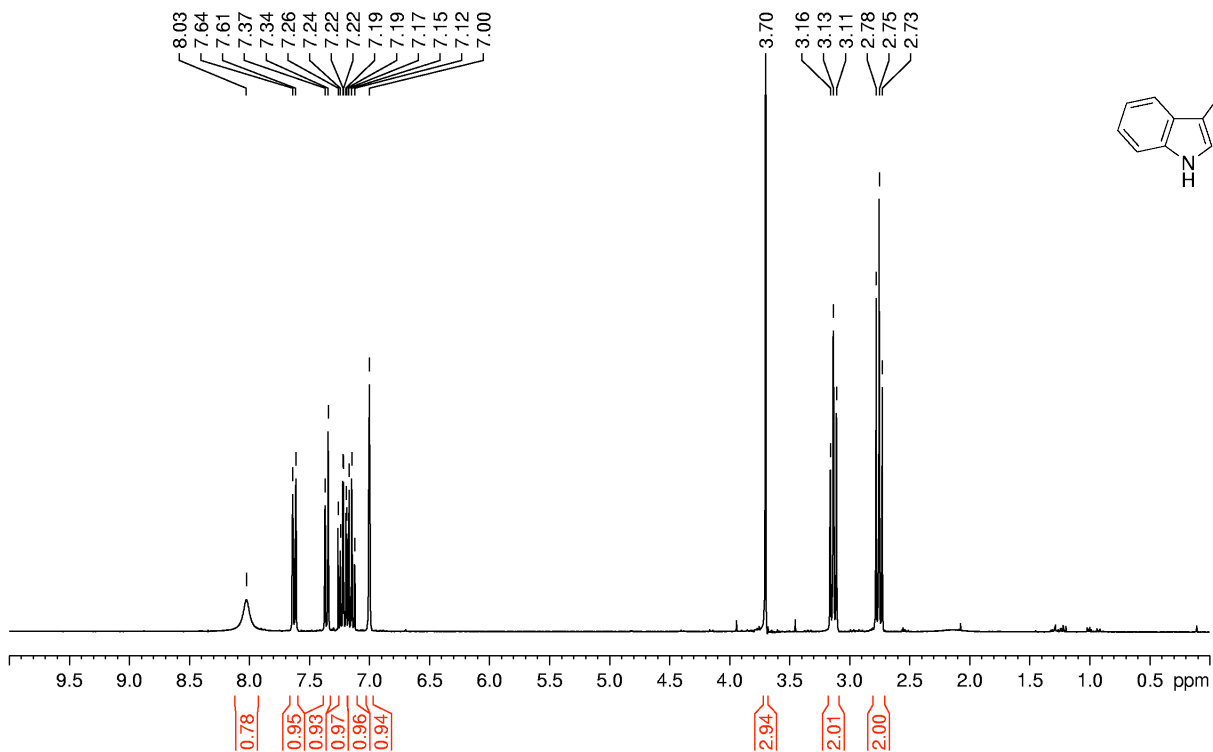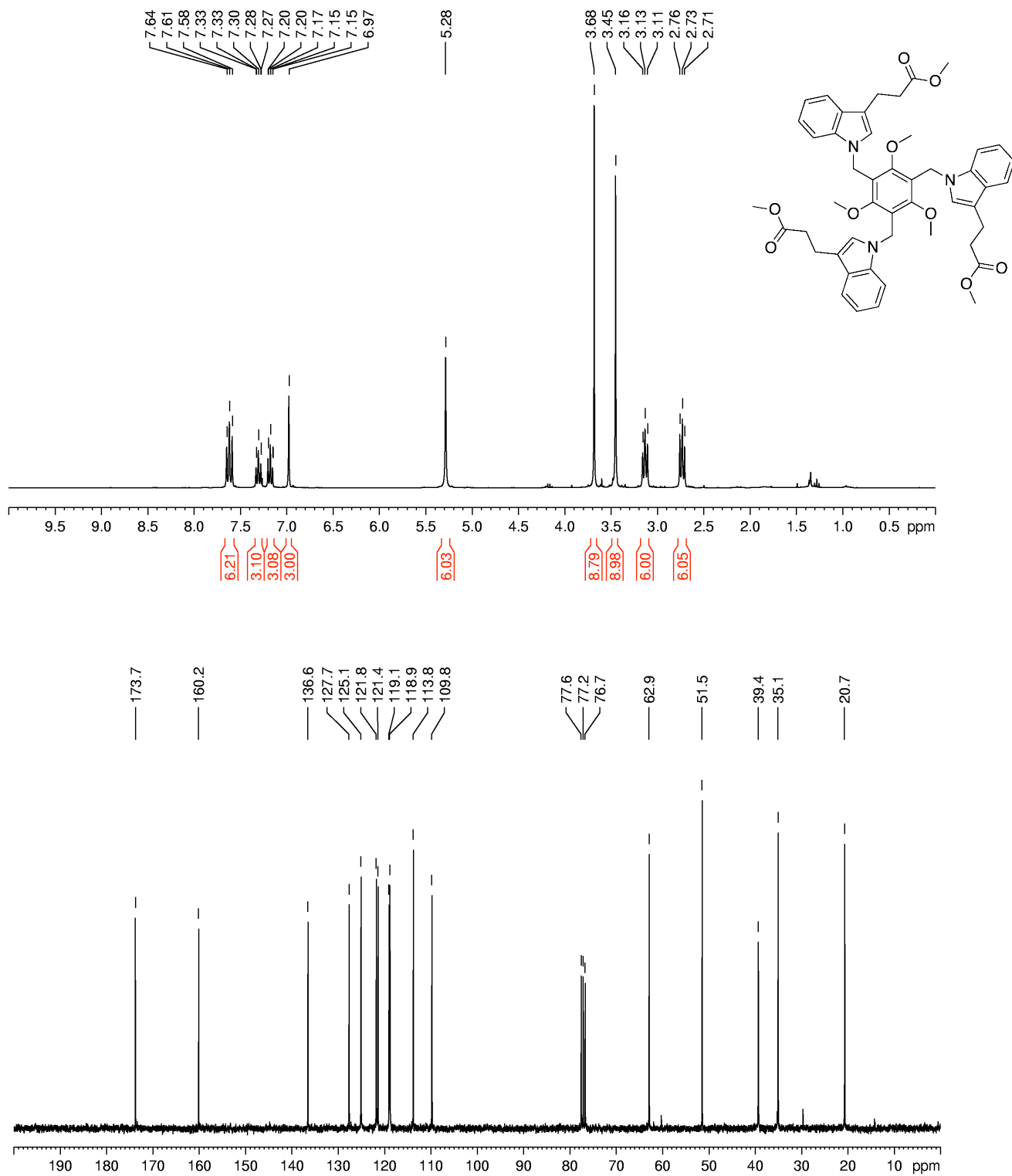
1. ^{1}H and ^{13}C NMR spectra

Compound 6 (1,3,5-tris(bromomethyl)-2,4,6-trimethoxybenzene)

Compound 7 (Methyl indole-3-propionate)

Compound 8

Compound 2
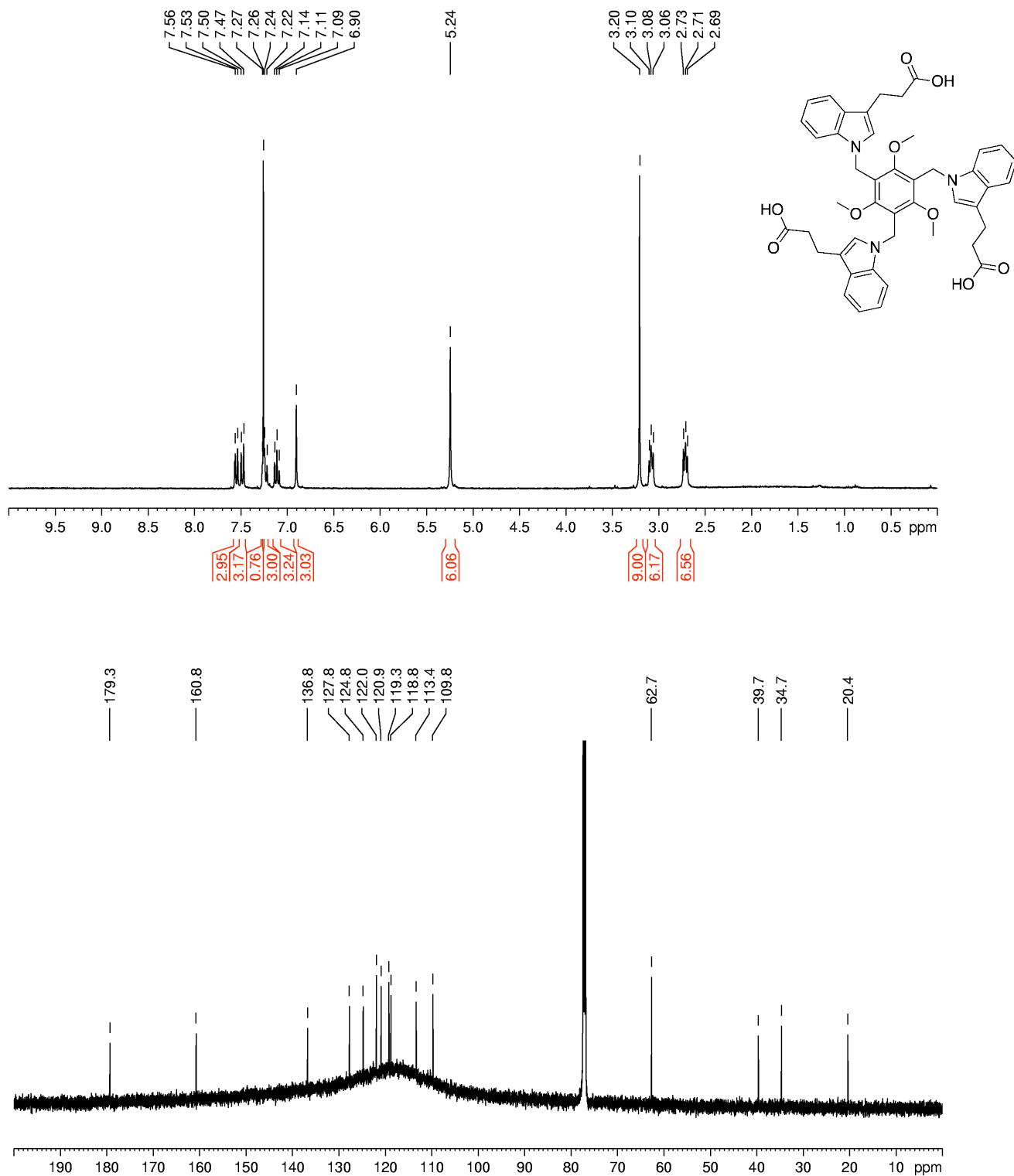
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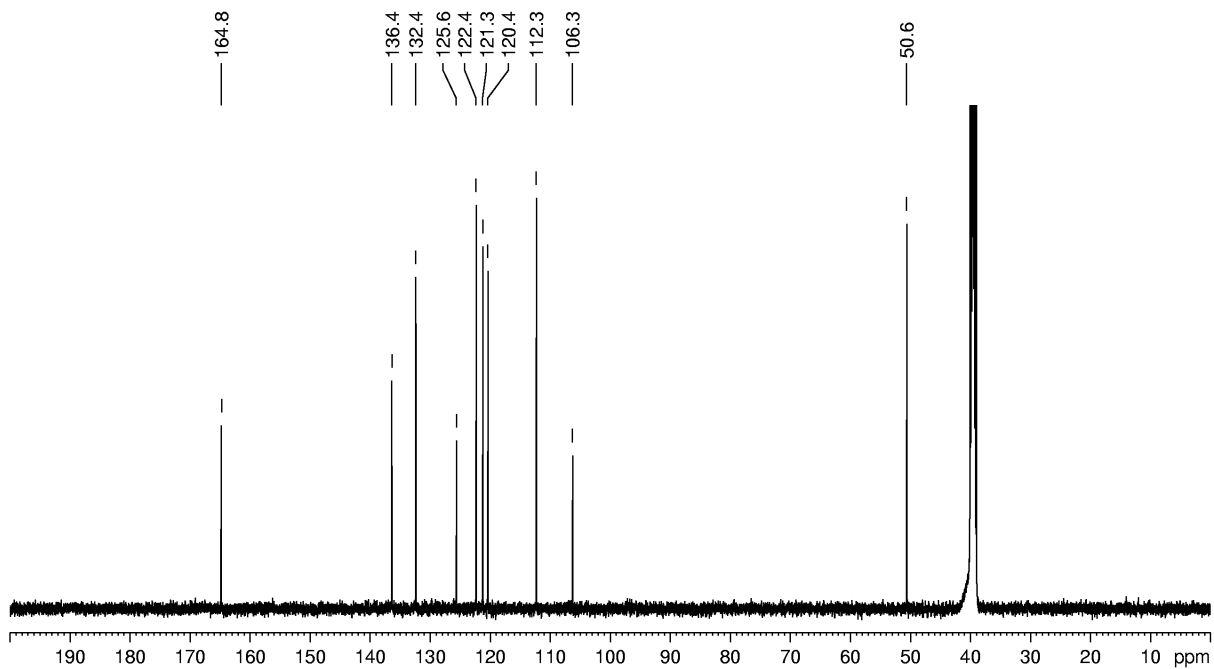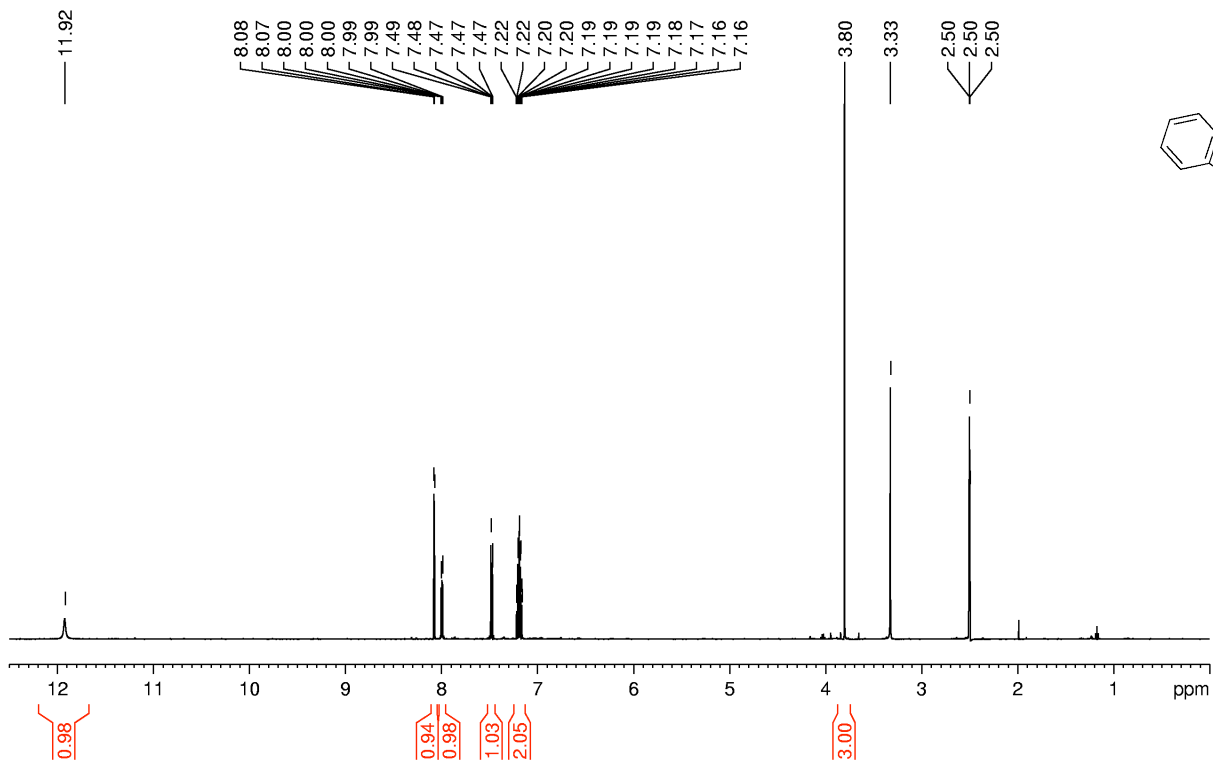
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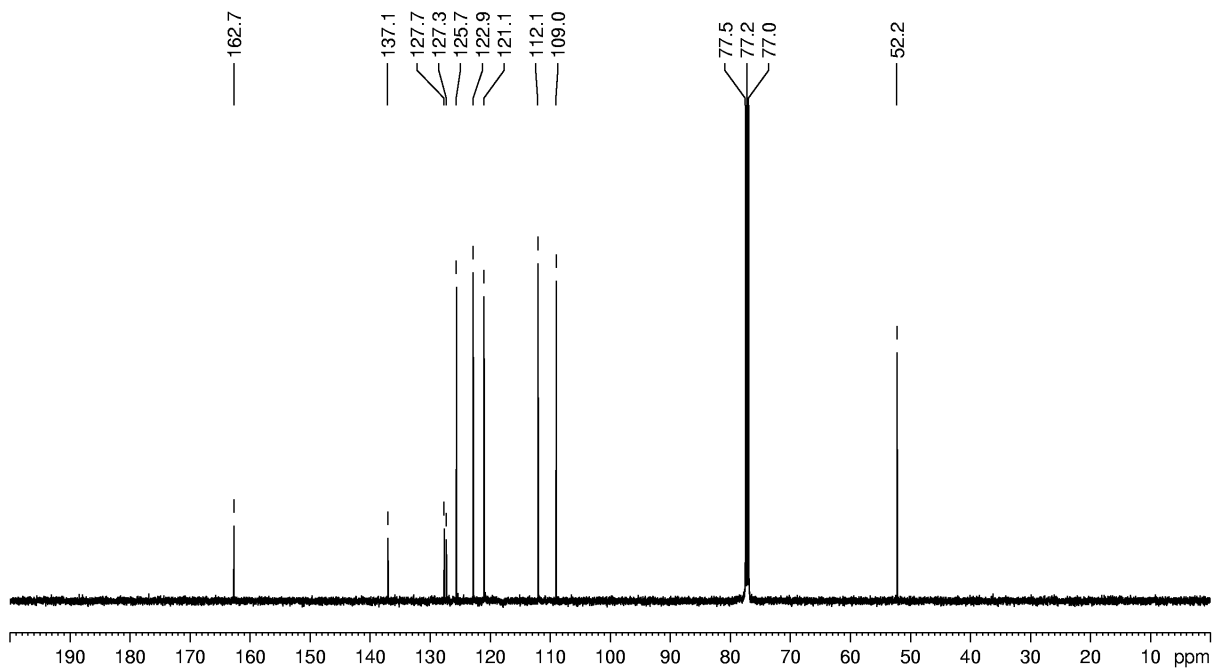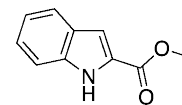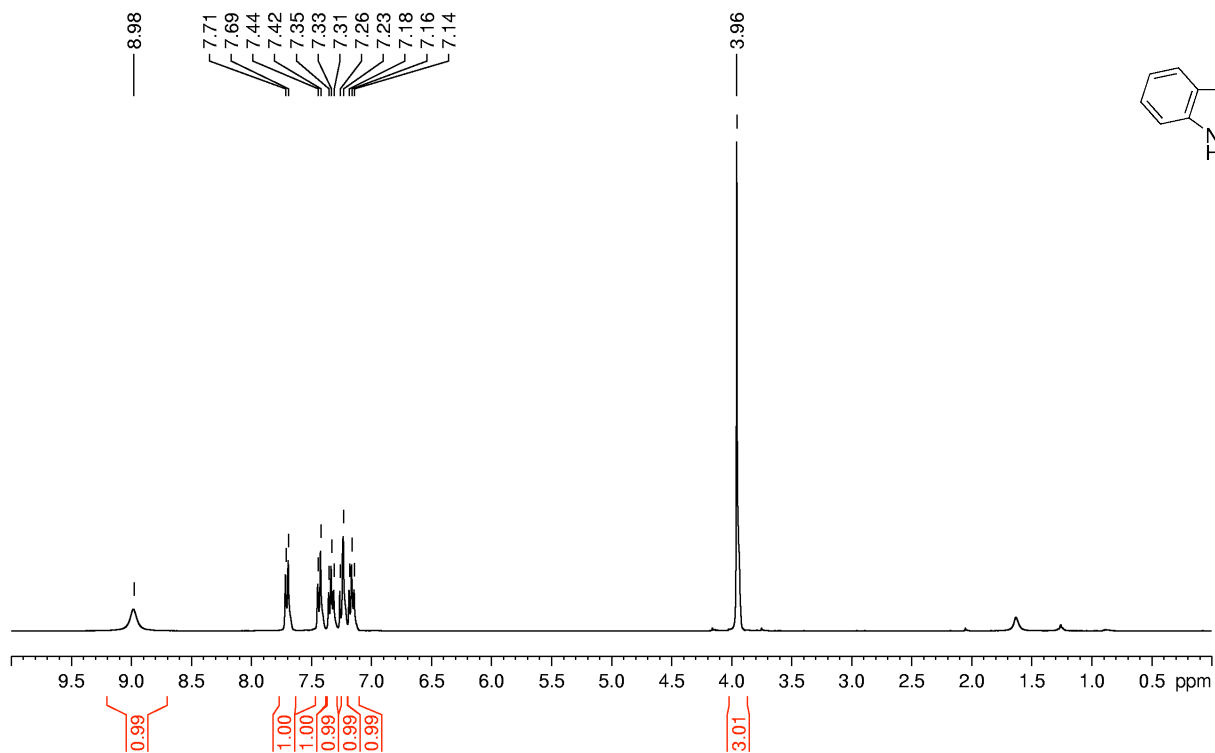
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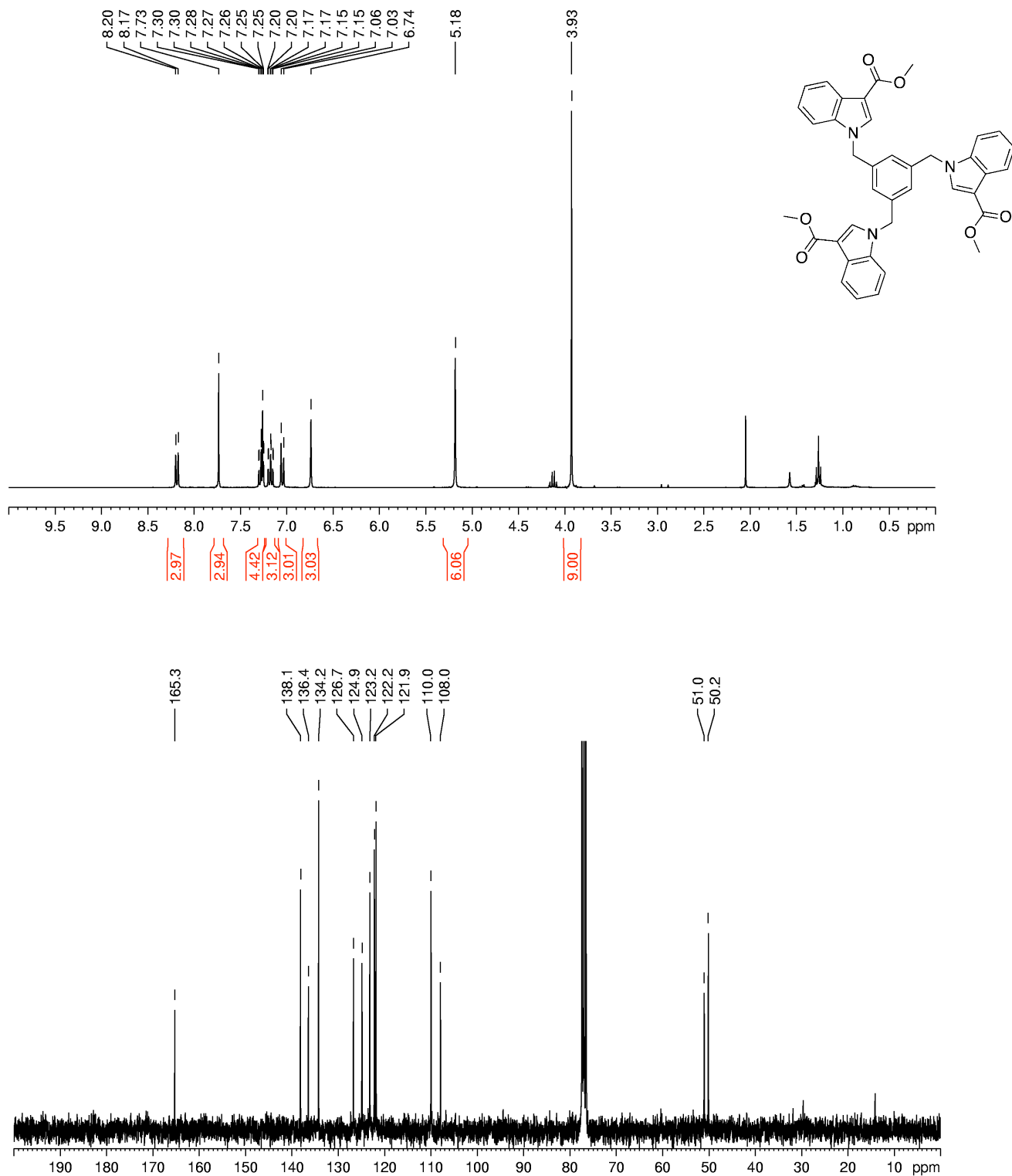
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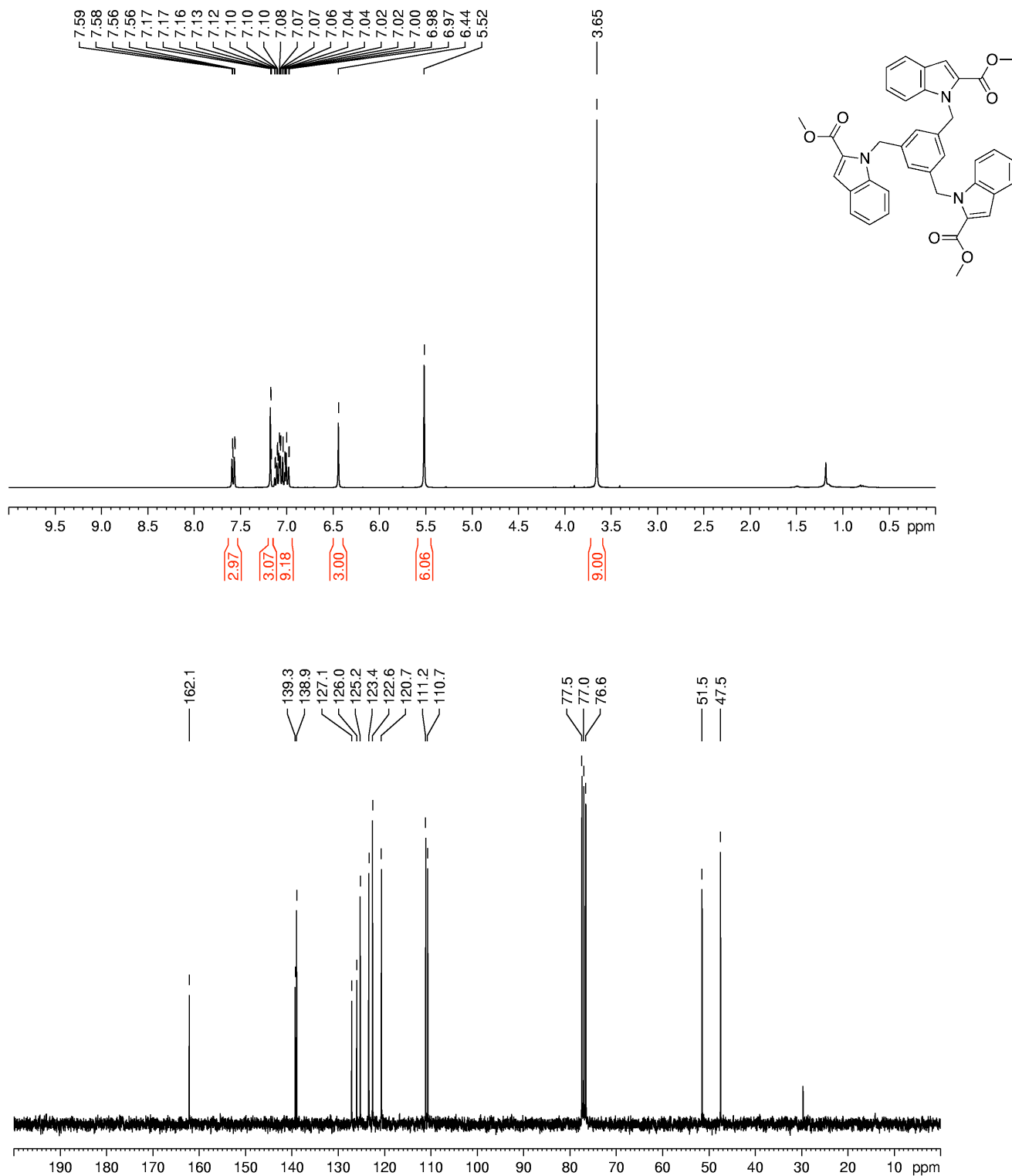
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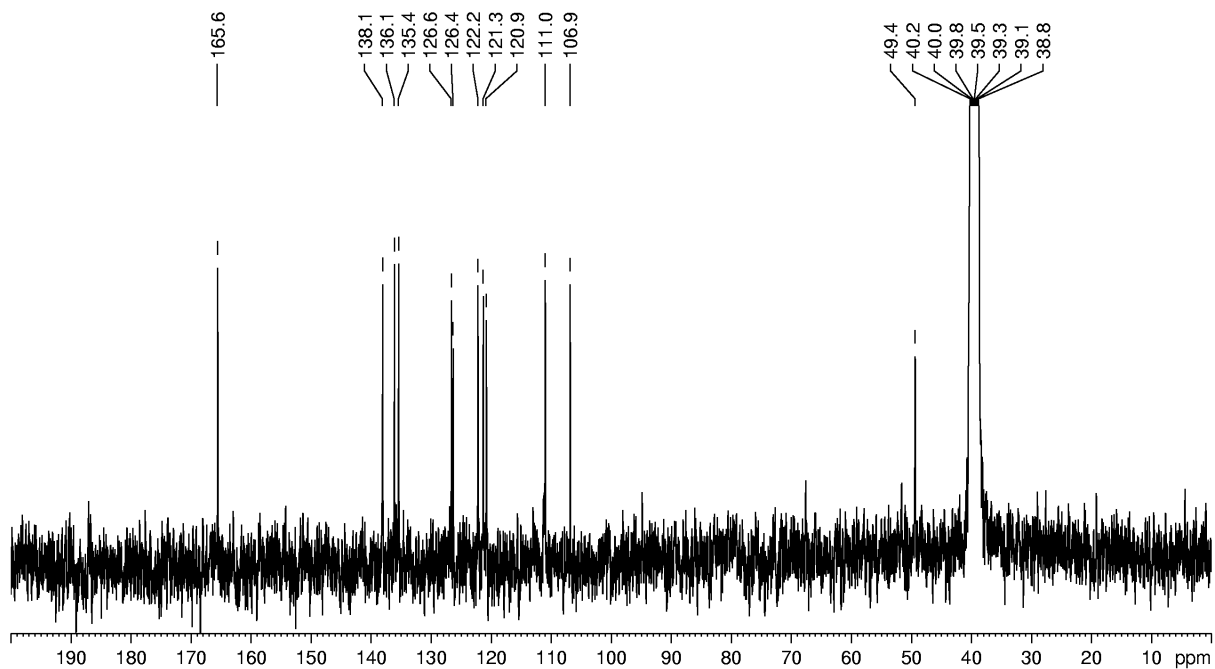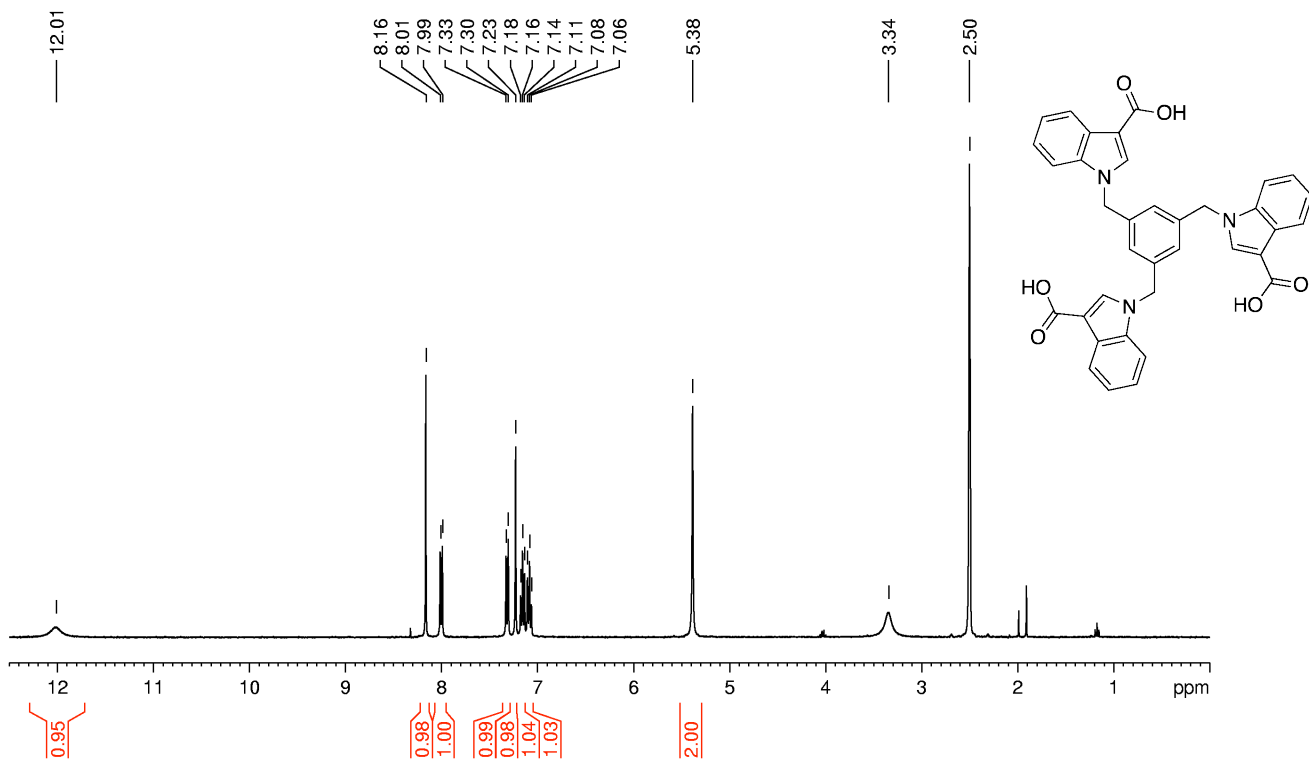
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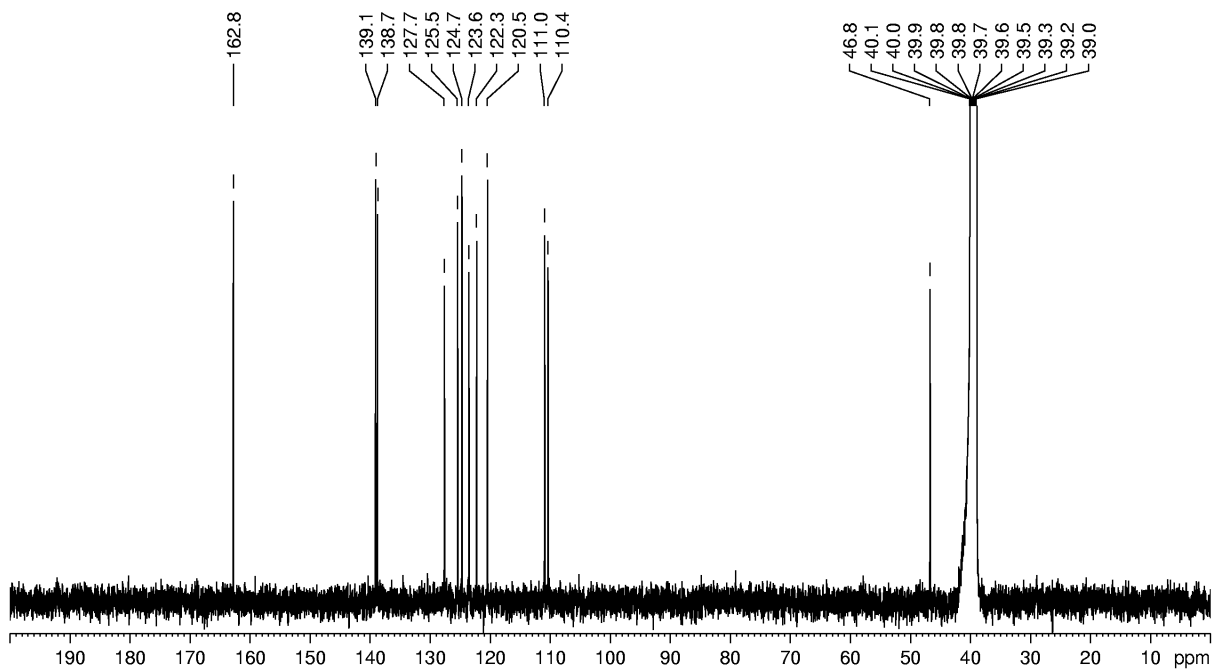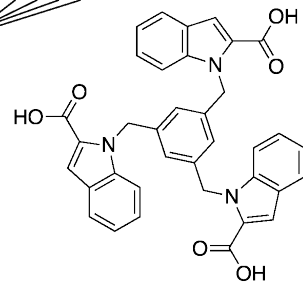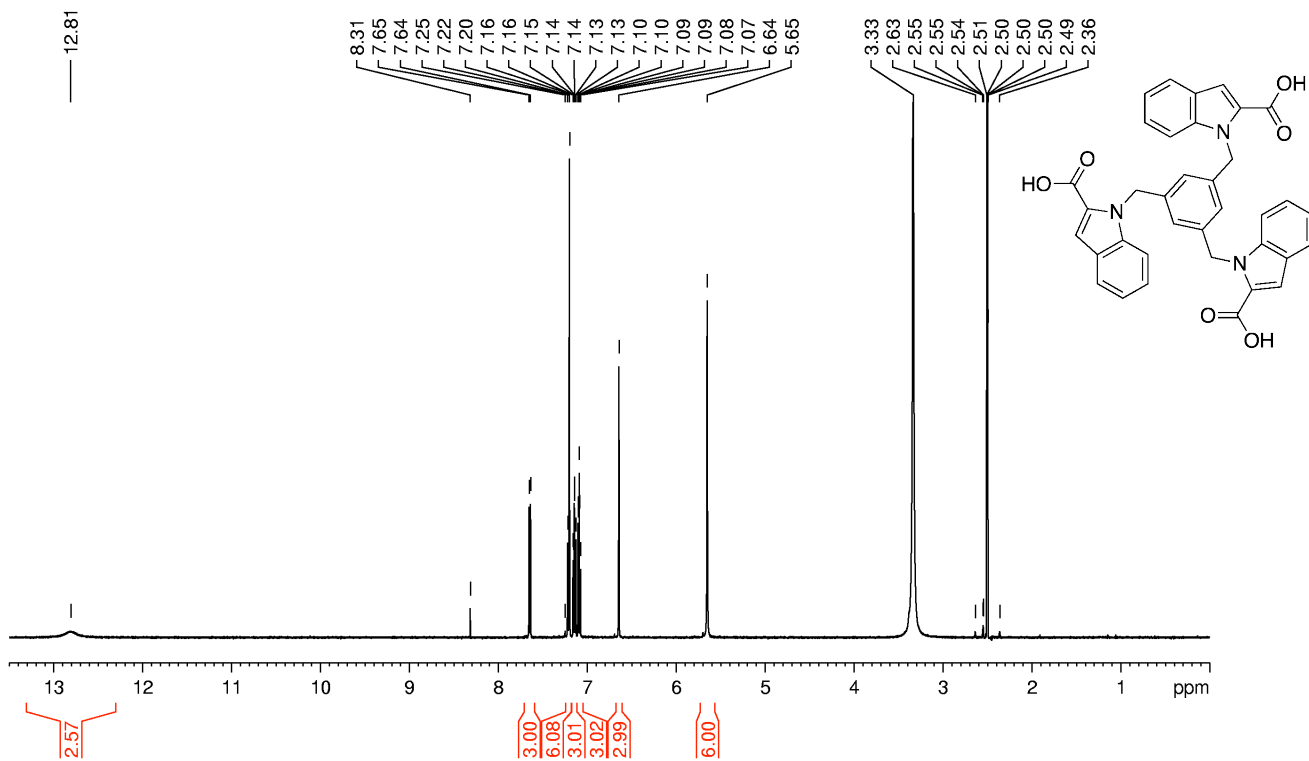
2. Aqueous host conformations
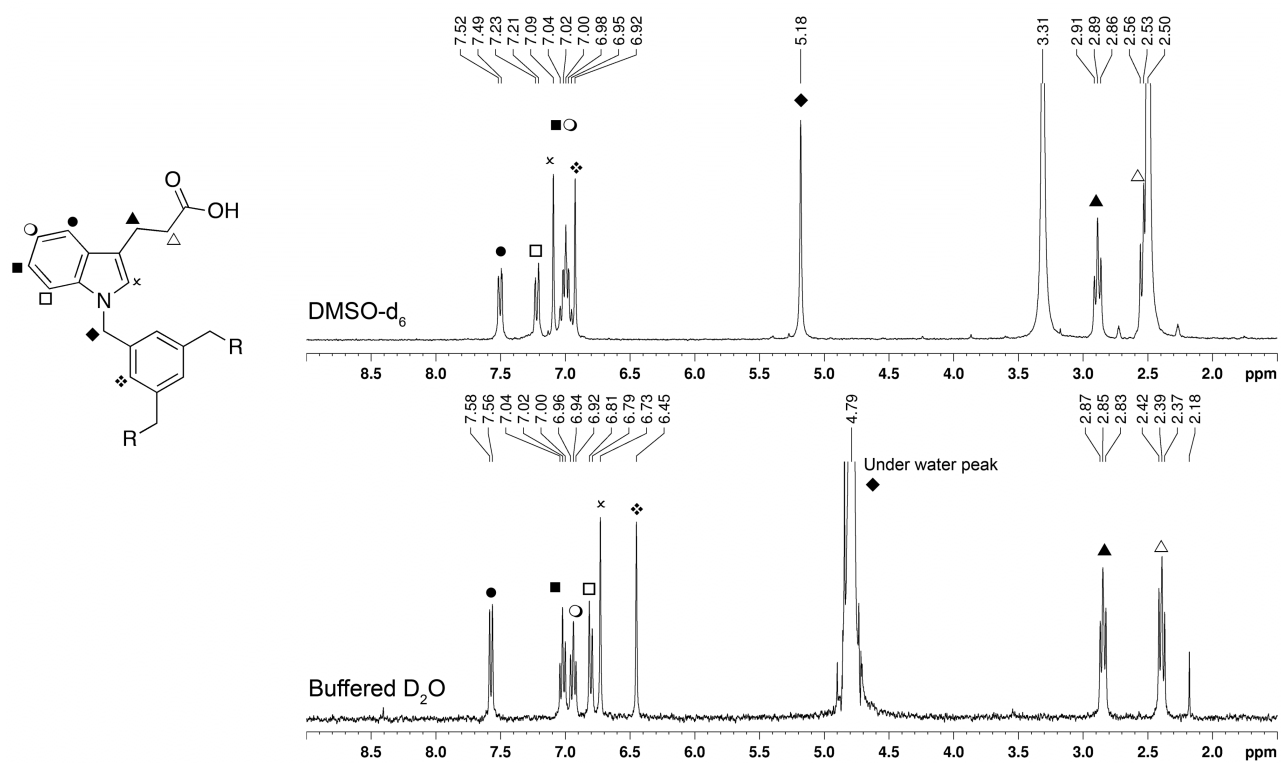
Figure S1. Host **1** chemical shift changes from DMSO-d_6 to 50 mM phosphate buffered D_2O.
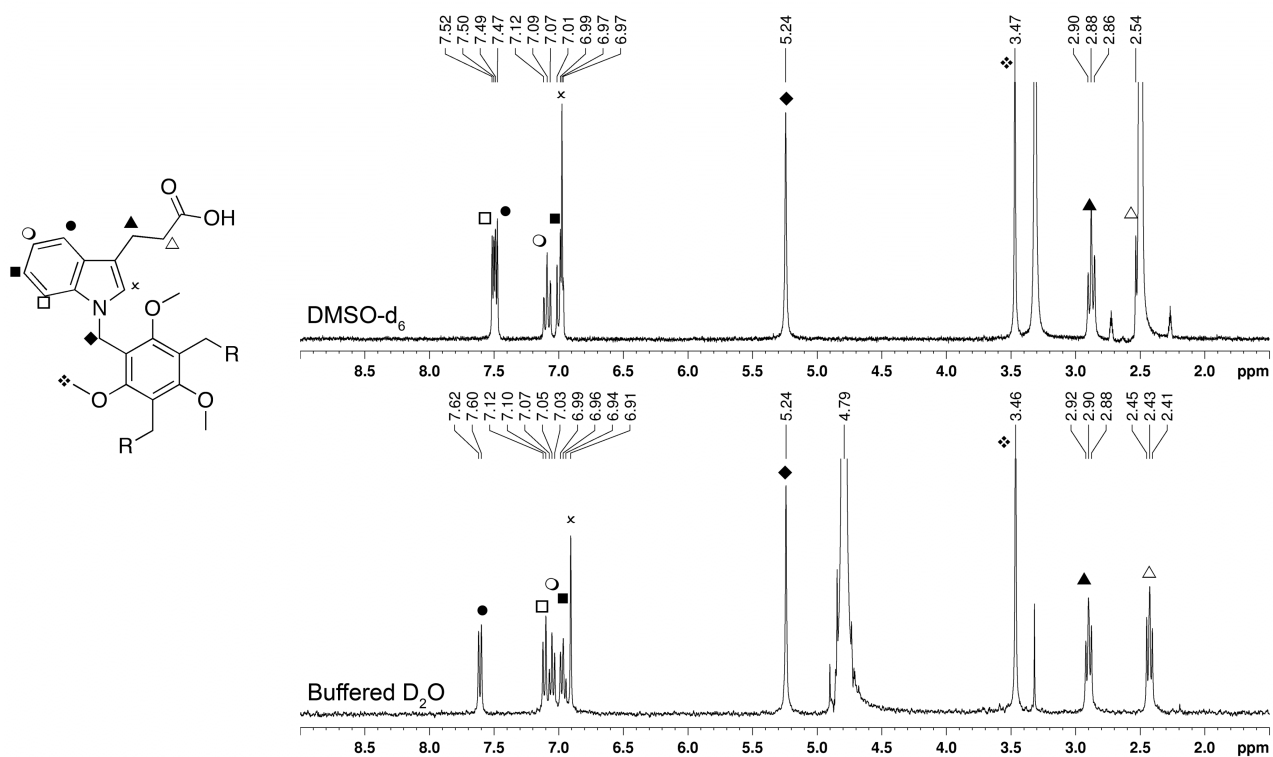
Figure S2. Host **2** chemical shift changes from DMSO-d_6 to 50 mM phosphate buffered D_2O.

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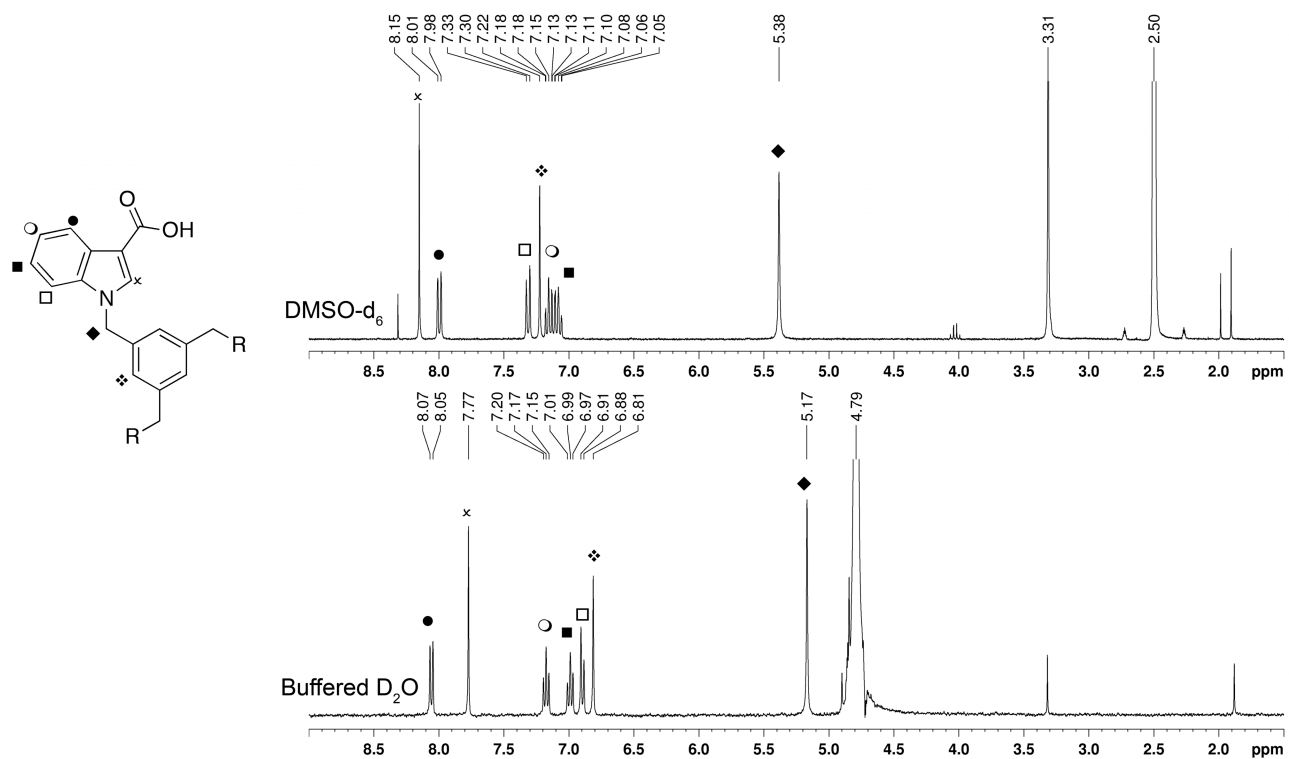
Figure S3. Host **3** chemical shift changes from DMSO-d_6 to 50 mM phosphate buffered D_2O.
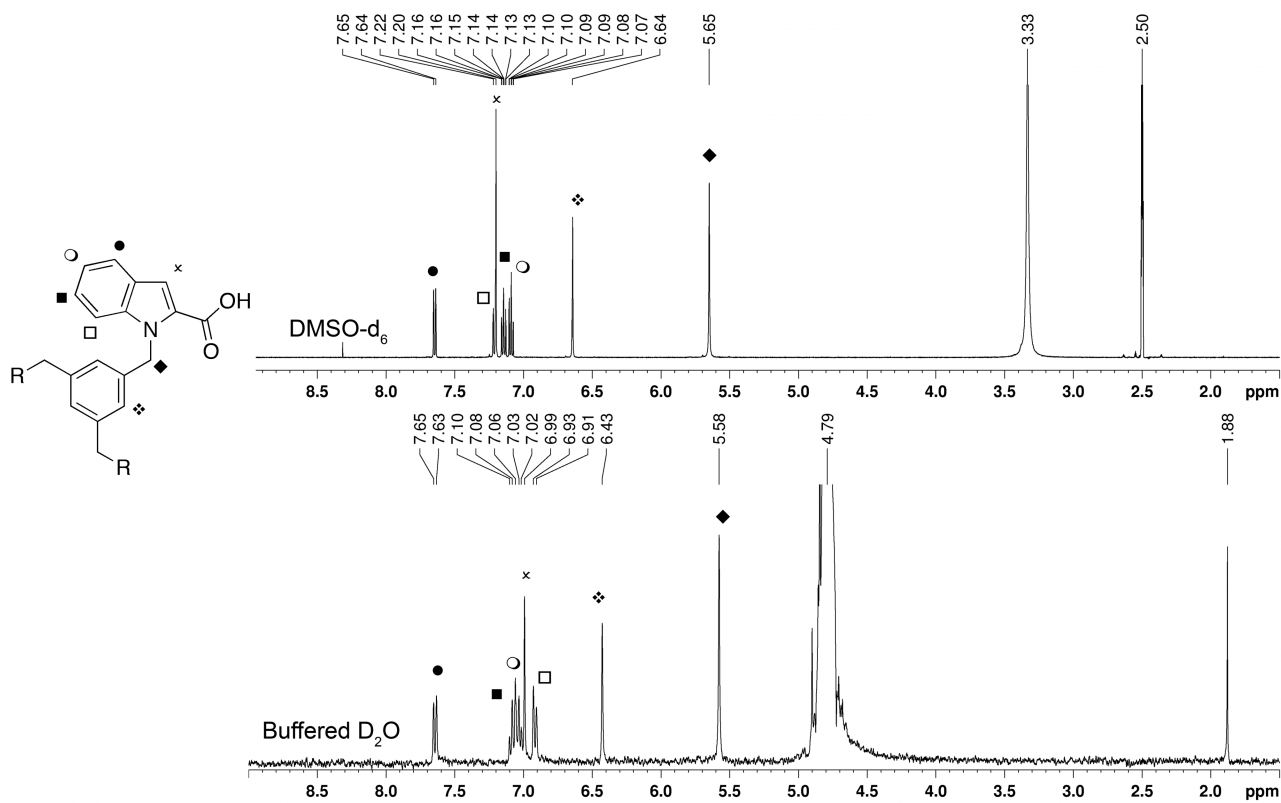
Figure S4. Host **4** chemical shift changes from DMSO-d_6 to 50 mM phosphate buffered D_2O.
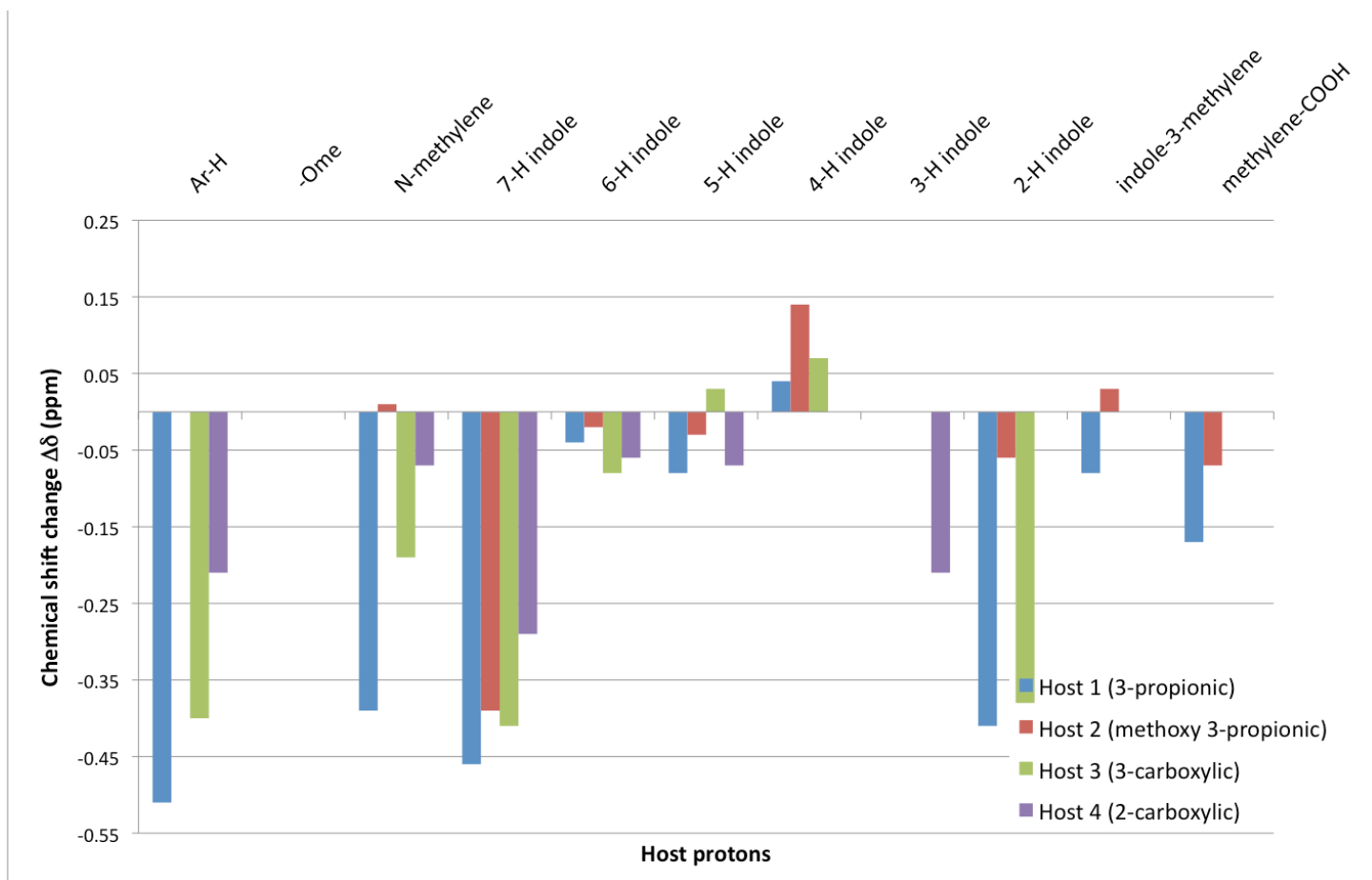
Figure S5. Overall comparison of all host chemical shifts from DMSO-d_6 (1 mM) to 50 mM phosphate buffered D$_2$O (1 mM, pH 7.0, pD 7.4).

3. NMR titrations

Binding titrations in water were carried out in D_2O containing Na_2HPO_4/NaH_2PO_4 (total phosphate concentration of 50 mM) at pH 7.0 (pD 7.4). Acidity of buffered solutions (pD) in D_2O was measured using a saturated KCl pH electrode and corrected to pD by addition of 0.4 units to the measured pH reading.[1] All tri-acid hosts were prepared as described in Section 2 and converted to their tri-sodium salts with 3 equivalents NaOMe prior to titration. pH variation of each host in 50 mM phosphate buffered D_2O from pH 5 – 10 (pD 5.4 – 10.4) showed no pH-induced chemical shift changes of protons expected to be adjacent to or affected by the protonation state of the carboxylates. This suggests that the four hosts studied remain in their deprotonated state as three carboxylates at the pH used for the following titrations. At the least, we can conclude that no protonation/deprotonation equilibria exist within ≥2 log units on either side of the experimental pH.

All guests were commercially available and used as received, with the exception of n-butyl trimethylammonium iodide (BuNMe₃I). This was synthesized from N,N-dimethylbutylamine and methyl iodide using a published procedure.[2] Compound spectra were a match to known product.

A dimerization constant (K_{dimer}) for each host was determined from the concentration-dependent host chemical shift changes during a ^{1}H NMR dilution titration. The shift data was fitted to a dimerization model using HypNMR2006 to give the dimerization constant.[3] Concentration-dependent chemical shifts for each host upon the addition of guest were also determined by ^{1}H NMR titrations. The association constants (K_{assoc}) of the host-guest interactions were determined by non-linear curve fitting for a 1:1 binding event using HypNMR software. The pre-determined values for the host self-dimerization, where necessary, were included in the HypNMR calculations to isolate the strength of the host-guest interaction itself.

a) Dilution studies. A 10 – 15 mM solution of each host was prepared in buffered D_2O to create a stock solution. The stock solution was then added sequentially to an NMR tube originally containing 0.5 mL of blank, buffered D_2O in a "reverse" dilution titration. Additions continued until the NMR tube contained a total of 1.5 mL. The concentrations observed in these studies ranged from 15 to 0.2 mM. Representative data and curve fitting are presented in Figure S6. Non-linear curve fitting of the dilution data for each host in buffered D_2O solution was used to generate dimerization constants for each host using the HypNMR software package.[3] In the example shown in Figure S6, a dimerization constant (formation of H2) of 22 M^{-1} (log 1.3514) was determined for host **2** using the concentration-dependant chemical shifts of seven host proton signals.
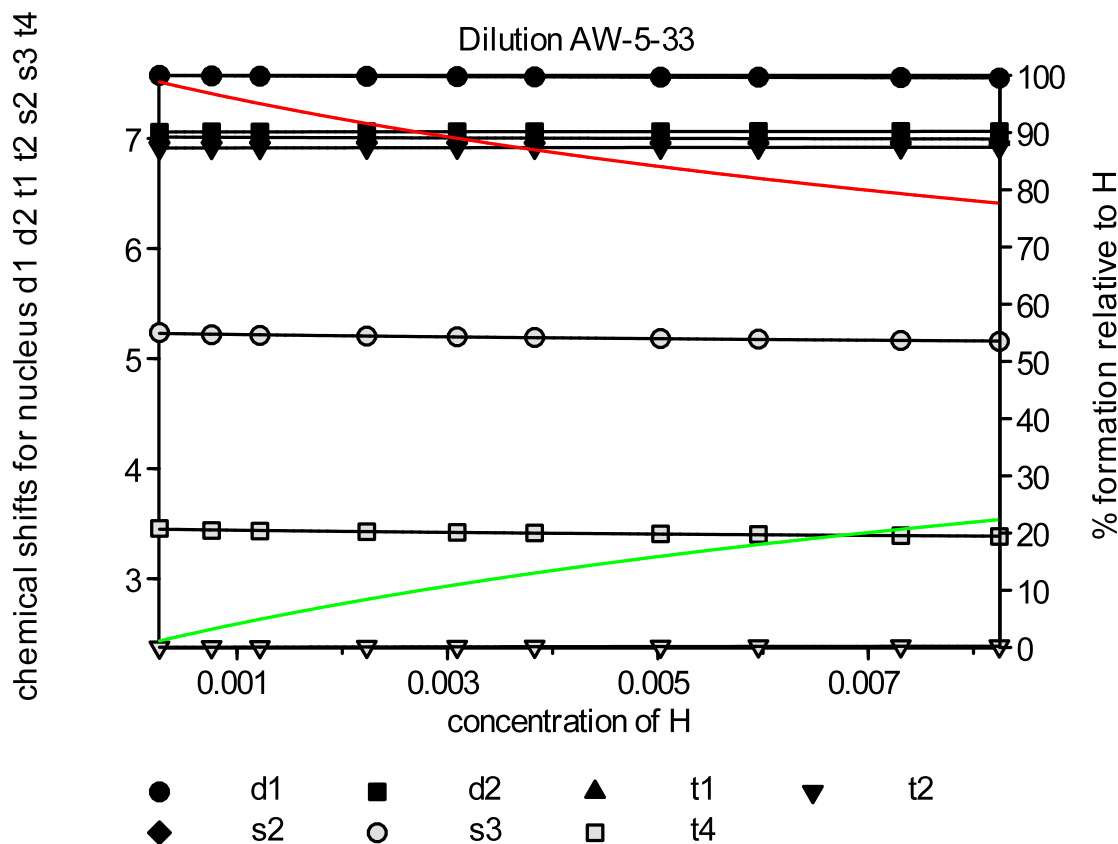
HypNMR2008
Refinement concluded at 4:40:36 PM on 10/27/2011
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Project title: Dilution AW-5-33

Converged in 1 iterations with sigma = 2.475903

	value	standard deviation	Comments
1 log beta(H2)	1.3514	0.1244	1.4(1)

Figure S6. Dilution curve results for host **2**. $K_{dimerization}$ determined using seven proton signals on host.

b) Guest binding. Host solutions (0.5 – 1 mM) were prepared fresh in buffered D_2O. A portion (0.5 mL) was removed to become the receiving solution in the NMR tube and a portion was used to make up guest solutions (2-250 mM) in order to ensure that the host concentration remained constant throughout the titration. The maximum concentration of guest solutions to be titrated was determined taking into account both guest solubility and buffer concentration. Guest species that could undergo a proton transfer (e.g. NMe_3H^+) were kept to concentrations under 50 mM in order to not overwhelm the buffer present. ^{1}H NMR binding titrations were carried out at 295 K on a Brüker AVANCE360 spectrometer at 360 MHz. Concentration-dependant chemical shift data of multiple signals from each titration were fit to a 1:1 (H:G) binding isotherm using HypNMR. To correct for the known strength of the host dimerization determined above, the H2 equilibrium constant is included in the calculation for each guest. Representative fitted data is presented in Figure S7. The final host-guest association constants were determined by an average of 2 – 4 titrations. In the example shown in Figure S7, an association constant of 38 M^{-1} (log 1.5849) for host **2** binding to acetylcholine chloride was determined

using the concentration-dependant chemical shifts of seven host proton signals. The dimerization constant of 22 M^{-1} (log 1.3514) determined above was included as a fixed value in this calculation.
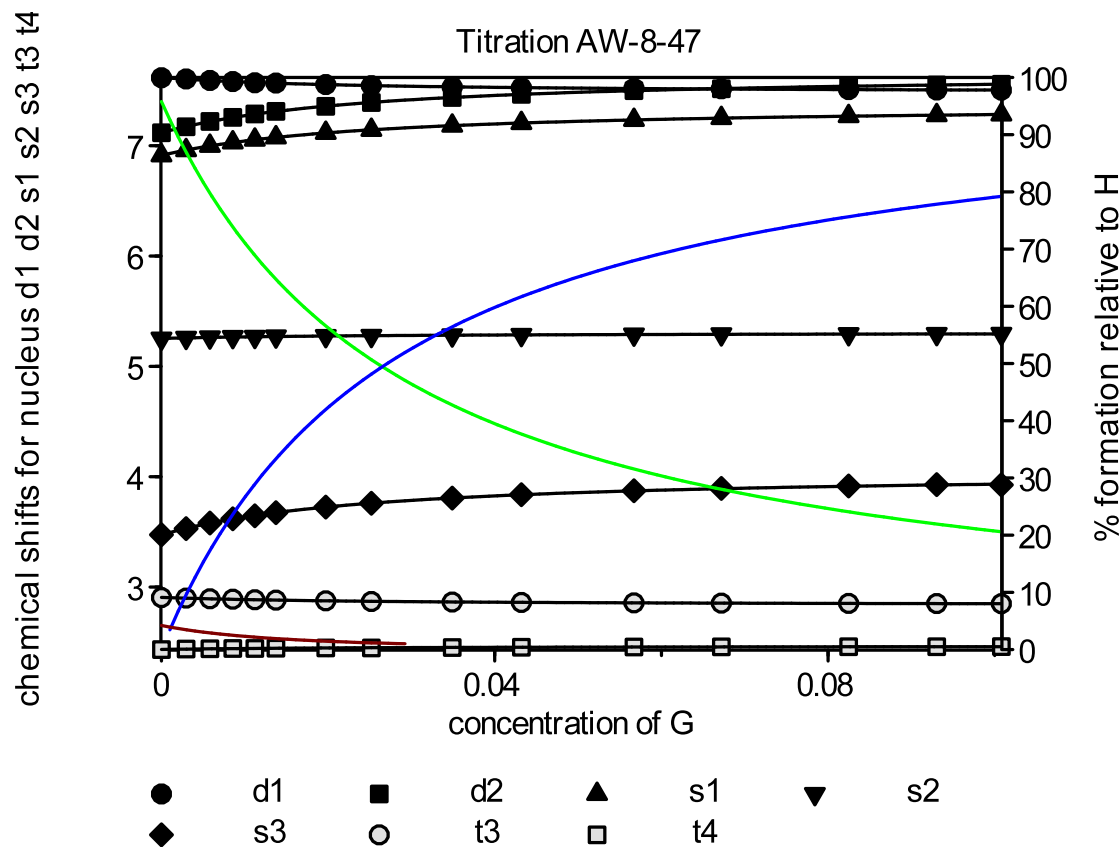
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Refinement concluded at 5:51:18 PM on 10/27/2011

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Project title: Titration AW-8-47

Converged in 9 iterations with sigma = 1.389723

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log beta(H2)	1.3514	fixed	

Figure S7. Titration curve results for host **2** being titrated by AChCl. Resulting K_{assoc} determined from seven proton signals on host that shifted during titration.

4. References

[1]   P. K. Glasoe, F. A. Long, *J. Phys. Chem.* **1960**, *64*, 188-190.
[2]   Y. J. Kim, M. T. Lek, M. P. Schramm, *Chem Commun.* **2011**, *47*, 9636-9638.
[3]   P. Gans, A. Sabatini, A. Vacca, HypNMR - http://www.hyperquad.co.uk/hypnmr.htm **2006**.