SUPPORTING INFORMATION

Decacationic Pillar[5]arene: A New Scaffold for the Development of ^{129}Xe MRI Imaging Agents

P. U. Ashvin I. Fernando,[1] Yurii Shepelytskyi,[2] Paul T. Cesana,[1] Alanna Wade,[2] Vira Grynko,[2] Adriana M. Mendieta,[1] Lauren E. Seveney,[1] Joseph D. Brown,[1,3] Francis T. Hane,[2] Mitchell S. Albert,[2,4] Brenton DeBoef[1*]

[1] Department of Chemistry, University of Rhode Island, 140 Flagg Road, Kingston, Rhode Island 02881, United States

[2] U.S. Army Corps of Engineers, Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180, United States

[3] Department of Chemistry, Lakehead University, 955 Oliver Road, Thunder Bay, Ontario P7B 5E1, Canada

[4] United States Coast Guard Academy, 31 Mohegan Avenue, New London, Connecticut 06320, United States

[5] Thunder Bay Regional Research Institute, 980 Oliver Road, Thunder Bay, Ontario P7B 5E1, Canada

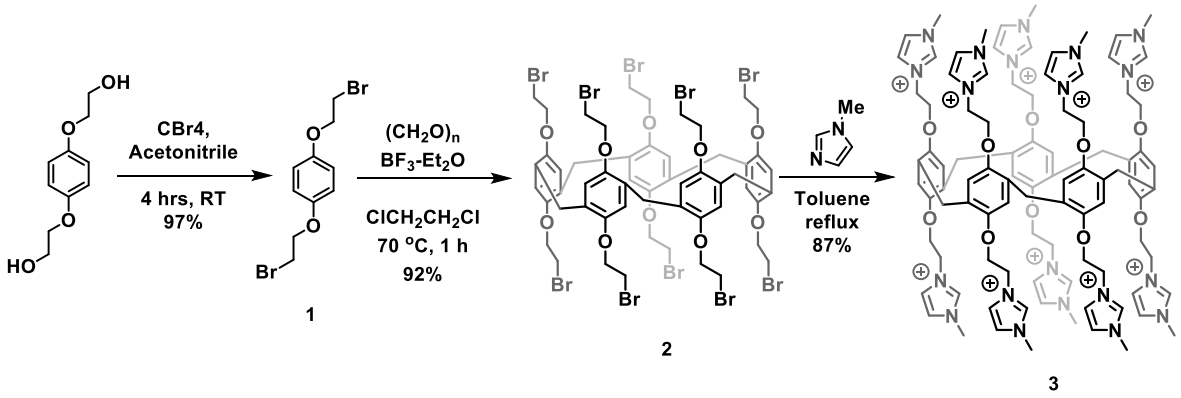
* Corresponding author: B. DeBoef
E-mail: bdeboef@uri.edu

Reagents

Substrates, including CBr_4, dry acetonitrile, dry 1,2-dichloroethane, boron trifluoride ethane, triphenyl-phosphine, and methyl imidazole, were purchased from Sigma-Aldrich, Fisher Scientific, and TCI chemicals. All reagents were stored under an inert atmosphere before use. Unless otherwise noted, all reactions were performed under N_2.

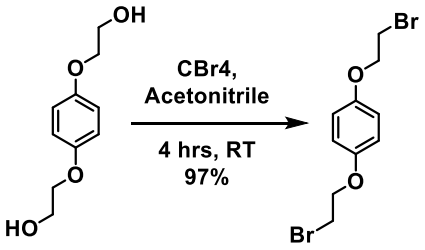
Instrumentation

NMR spectra were obtained using Bruker Avance 300 MHz and 400 MHz spectrometers. Low resolution mass spectrometry was performed using a Shimadzu LRMS-2020. High resolution mass spectrometry was performed using a Thermo Scientific LTQ Orbitrap XL™ instrument. Fluorescence spectra was obtained using Shimatzu Fluorimeter, and UV-Vis data was obtained via Shimatzu UV-Vis spectrometer.



Scheme S1: Synthetic route for the synthesis of Water soluble pillar[5]arene. The above molecule was synthesized according to the literature procedures,[1] with further optimizations and improved yields.

Synthesis of brominated monomer, 1



Scheme S2: Synthesis of 1. A solution of 1,4-bis(2-hydroxyethoxy)benzene (10.0 g, 50.4 mmol) and triphenylphosphine (31.5 g, 120mmol) in dry acetonitrile (250 mL) was cooled in an ice bath. Under vigorous stirring, carbon tetrabromide (39.8 g, 120 mmol) was slowly added in four portions. After each addition, the solution immediately turned bright yellow, and with stirring, returned back to its colorless form. On the final addition the solution stayed cloudy. The mixture was stirred at room temperature for 4 hours and 20 minutes. Then cold water (200 mL) was added to the reaction mixture, producing a white precipitate. The precipitate was collected, washed with another 200 mL of cold water, followed by methanol/water (3:2, 3 × 100 mL). The collected white solids were dried under vacuum

for 24 hours and used without further purification. (14.5 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.89 (s, 4H), 4.27 (t, J = 6.3 Hz, 4H), 3.64 (t, J = 6.3 Hz, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.5, 117.2, 68.6, 29.6.

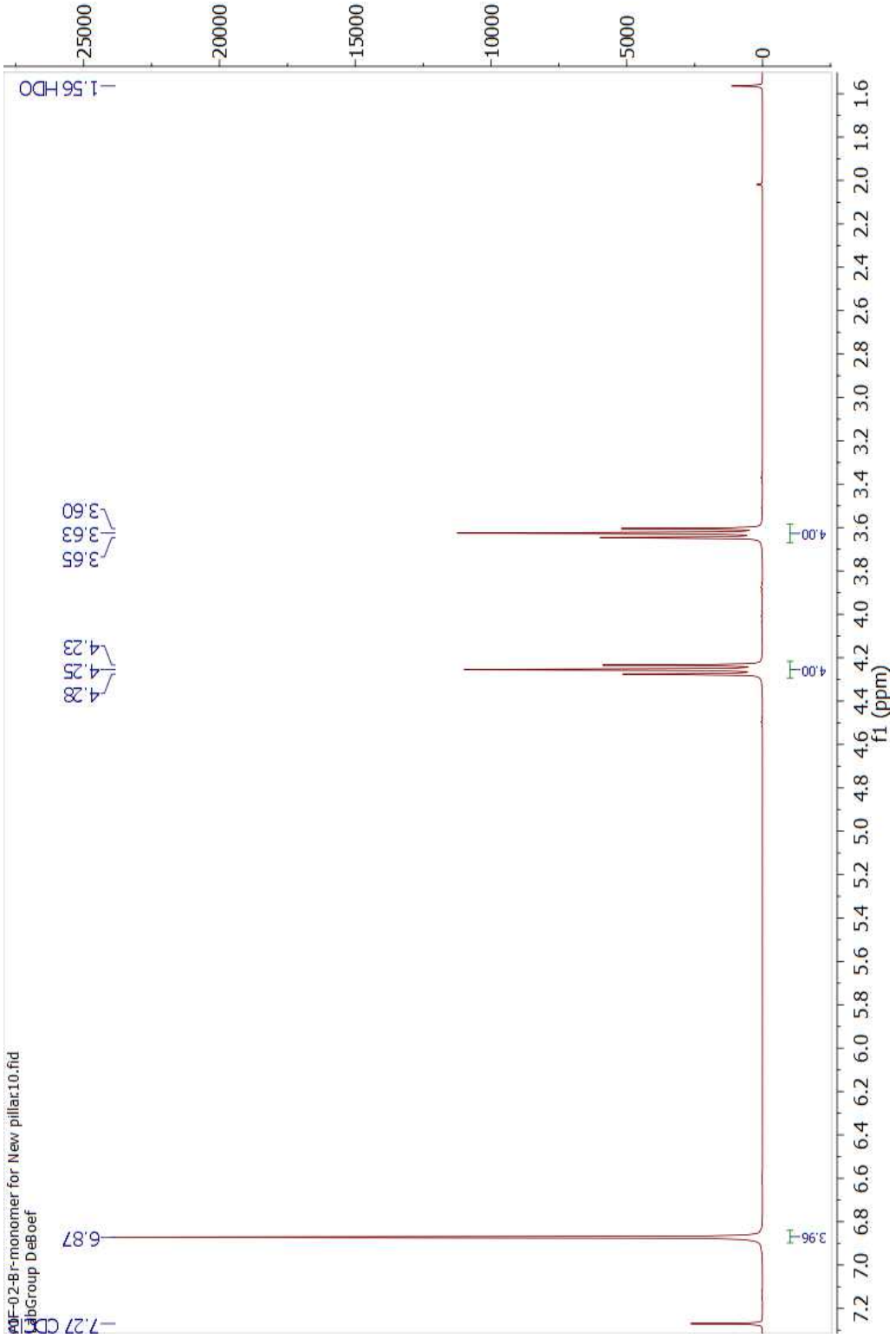


Figure S1: ^{1}H NMR of **1**

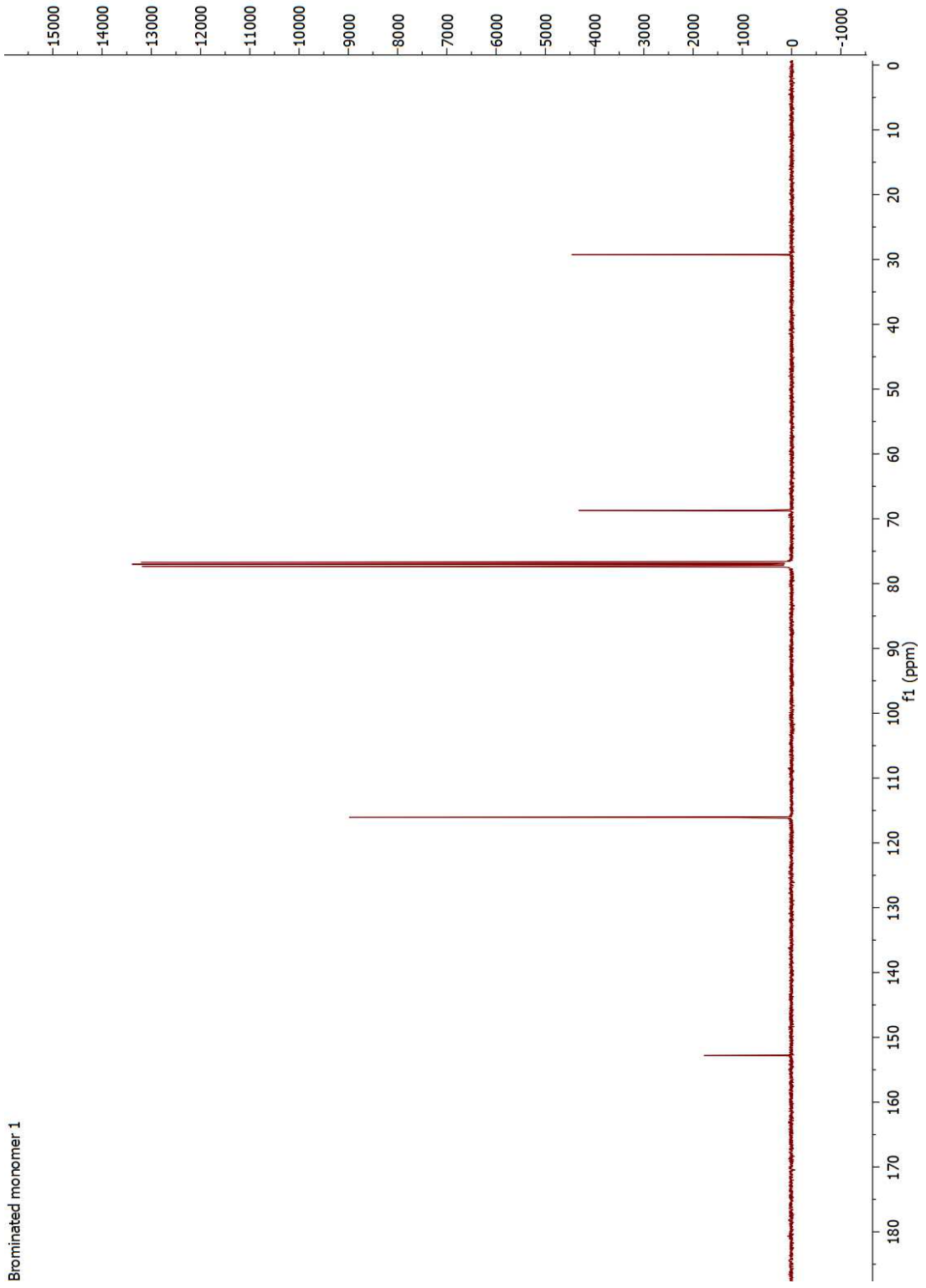
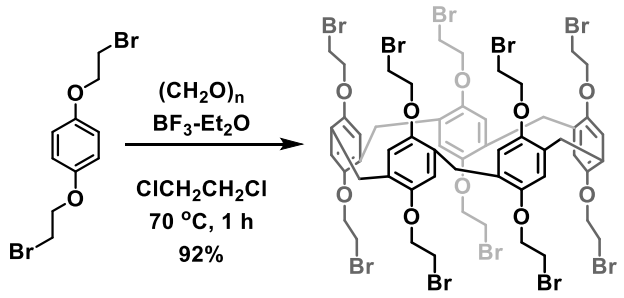


Figure S2: ^{13}C NMR of **1**

Synthesis of decabrominated pillar[5]arene, 2



Scheme S3: Synthesis of 2. A solution of **1** (3.37 g, 11.5 mmol) and paraformaldehyde (0.349 g, 11.5 mmol) in 1,2-dichloroethane (50 mL) was cooled in an ice bath. Boron trifluoride etherate (3.26 g, 23.0 mmol) was added to the solution, and the mixture was stirred at room temperature for 1 hour. The reaction mixture was then washed with water (2 × 50 mL), saturated $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL). The organic layer was dried with Na_2SO_4. The solvent was evaporated to provide a pure product (85% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 10H), 4.23 (t, J = 6.3, 5.1 Hz, 20H), 3.84 (s, 10H), 3.63 (t, J = 5.6 Hz, 20H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.7, 129.1, 116.1, 68.9, 30.7, 29.4.

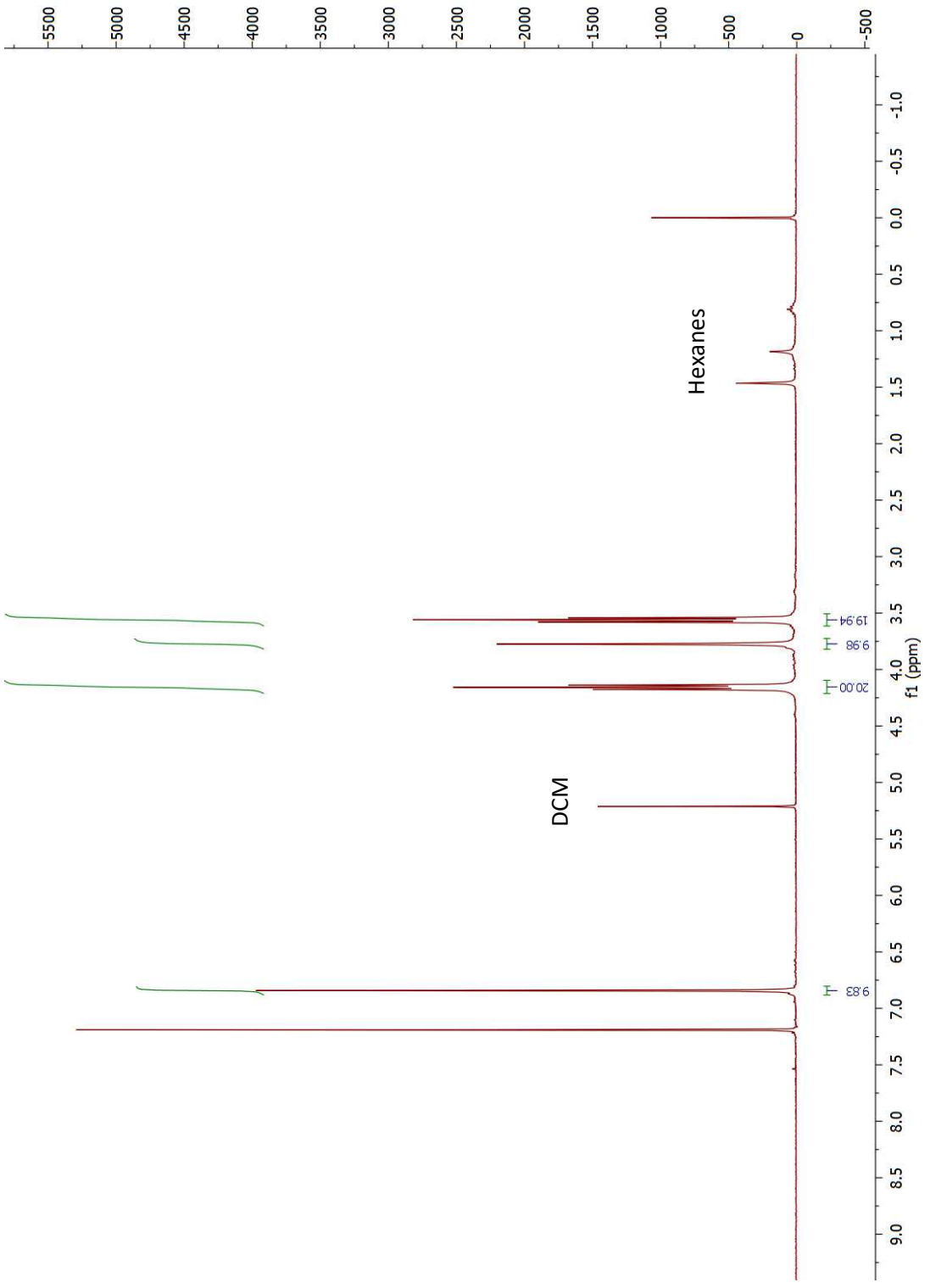


Figure S3: ^{1}H NMR of **2**

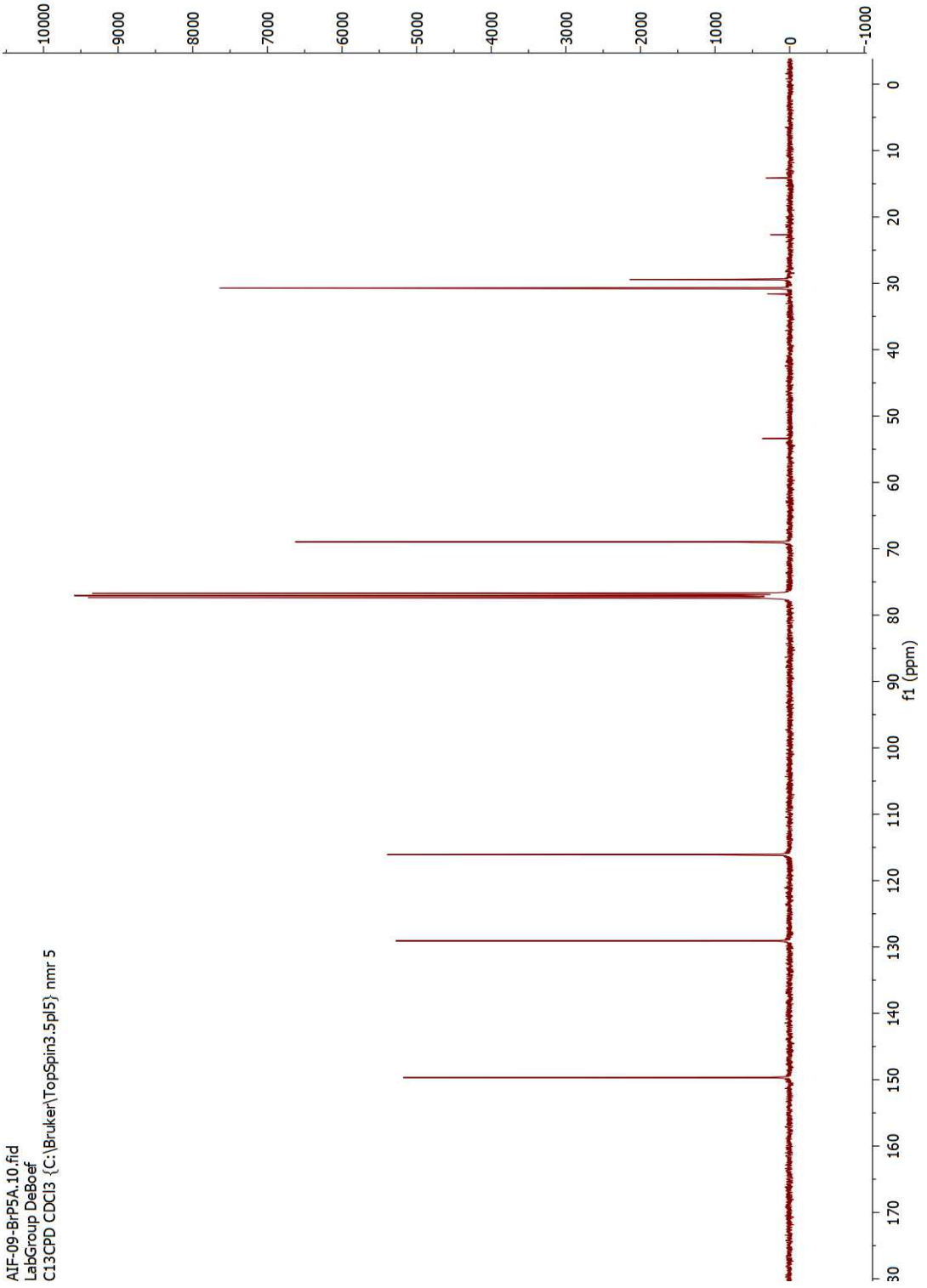
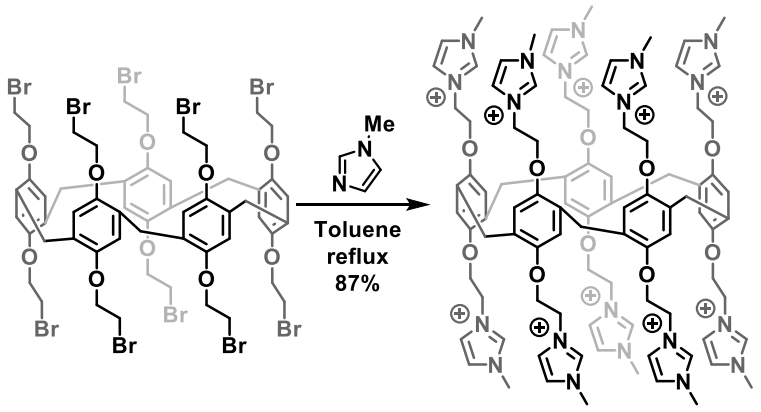


Figure S4: ^{13}C NMR of **2**

Synthesis of decamethyl imidazolium pillar[5]arene, 3



Scheme S4: Synthesis of 3. A mixture of **2** (1.68 g, 1.00 mmol) and *N*-methylimidazole (1.64 g, 20.0 mmol) in toluene (25 mL) was stirred in a 40 mL pressure tube at 120 ºC for 24 hours. After cooling, the solvent was removed by evaporation, and the residue was recrystallized from ethanol/diethyl ether (1:2) to give a white solid (2.1 g, 87%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.17 (br, 10H), 8.11 (s, 10H), 7.44 (br, 11H), 6.72 (br, 10H), 4.72 (br, 21H), 4.45 (br, 20H), 3.74 (br, 31H), 3.51 (br, 10H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 149.1, 137.9, 128.3, 123.7, 123.1, 114.7 66.9, 49.5, 36.3, 33.1, 29.2.

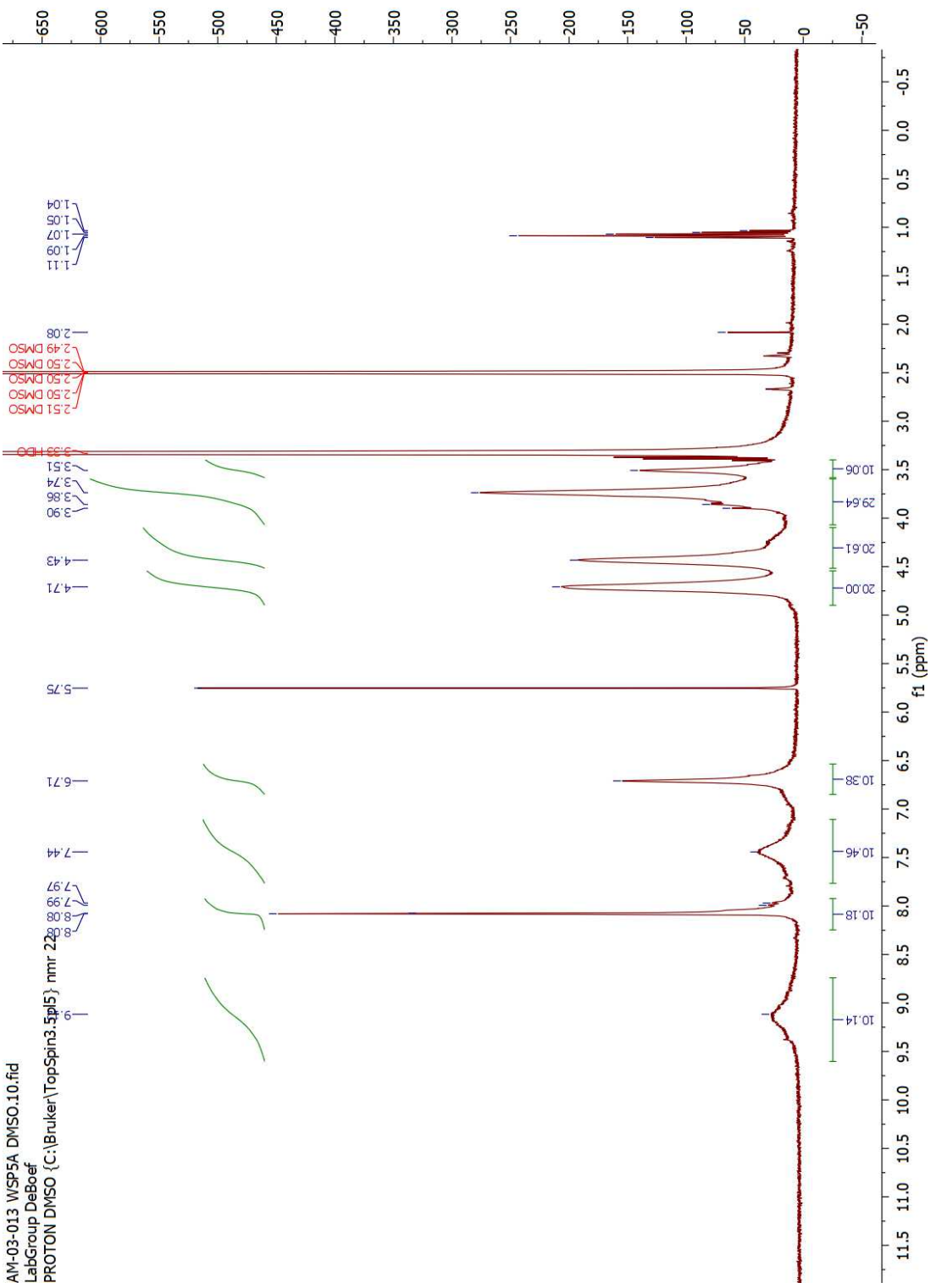


Figure S5: ^{1}H-NMR of **3**

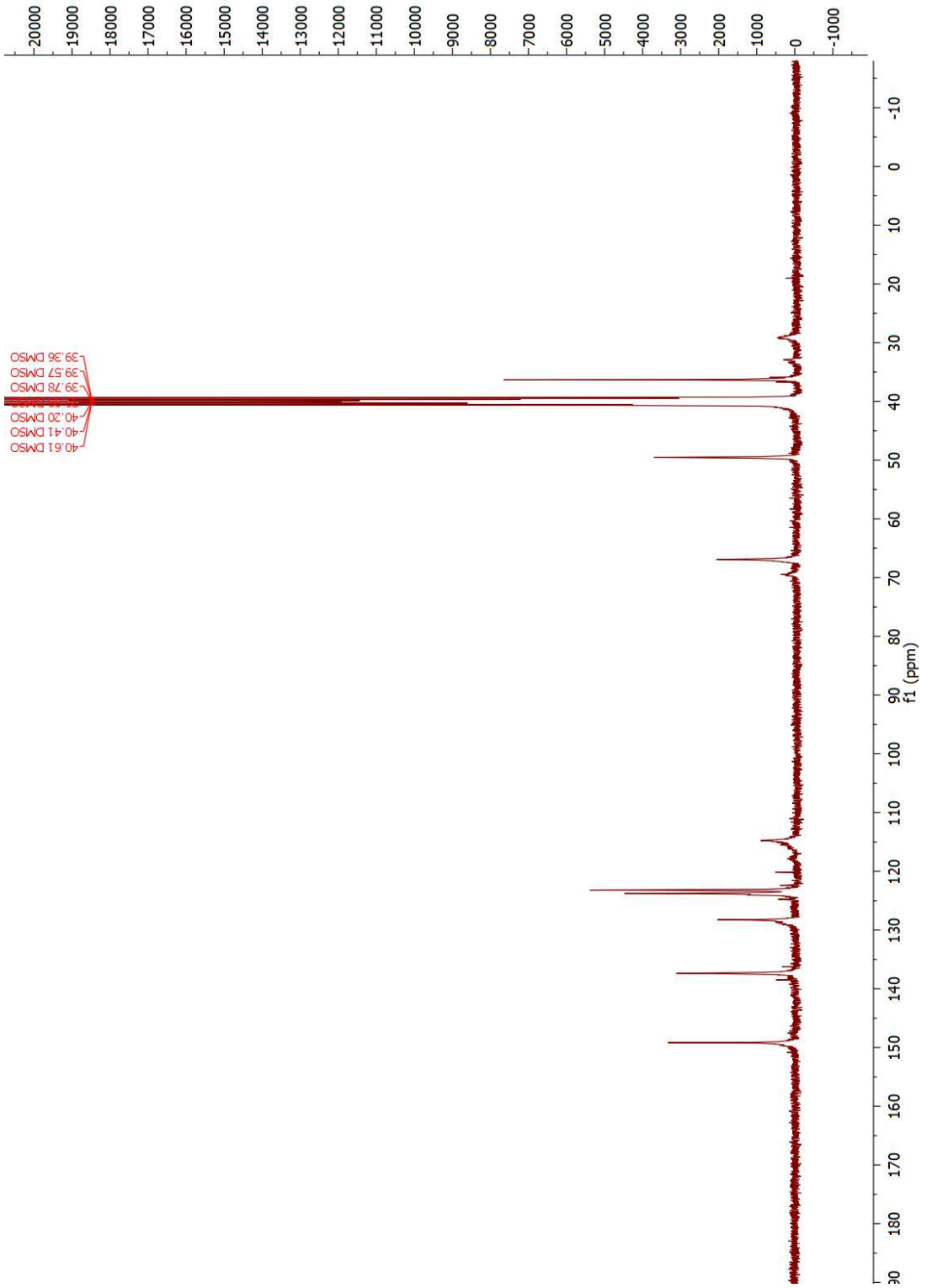


Figure S6: ^{13}C-NMR of **3**

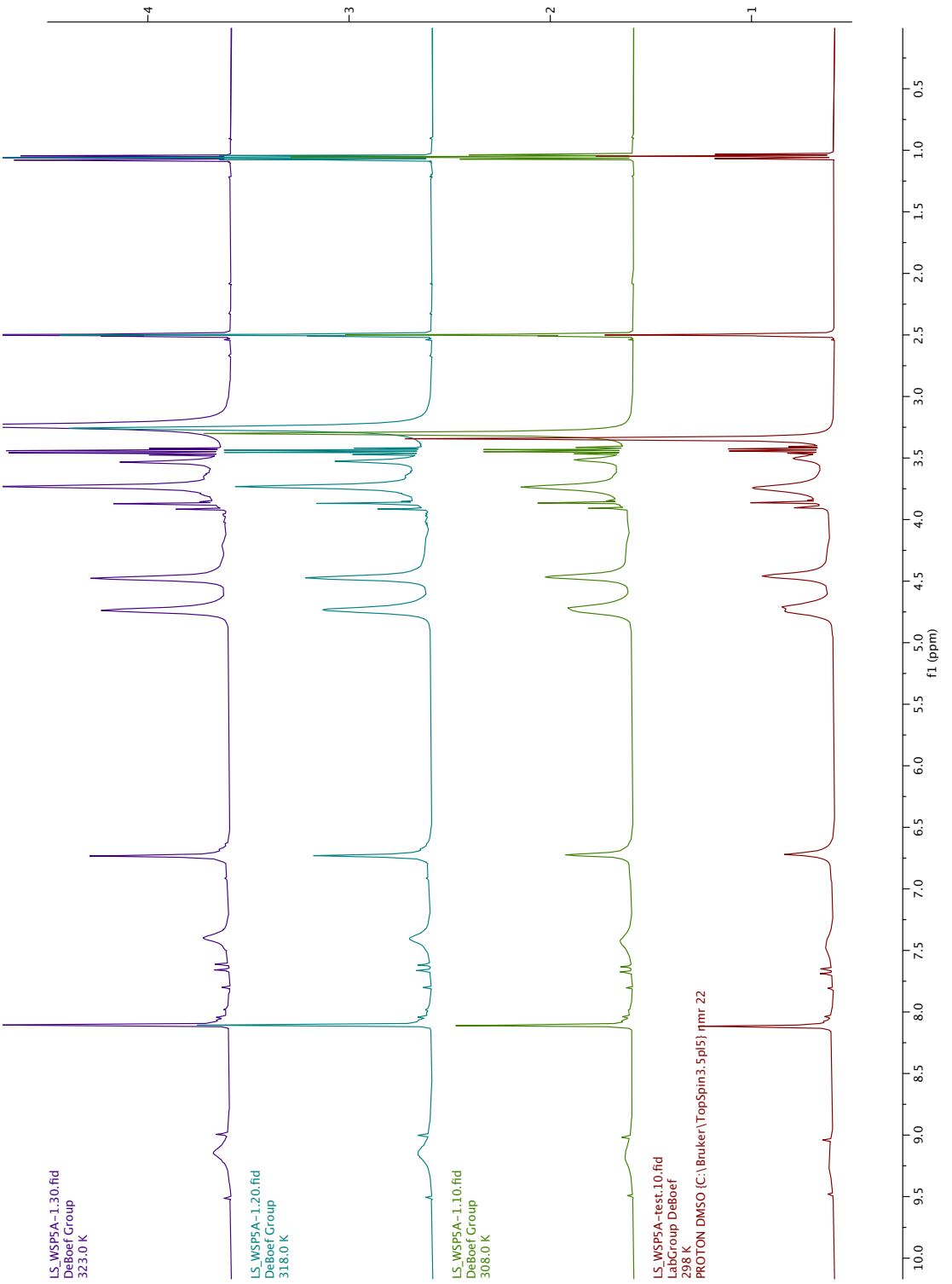


Figure S7: variable temperature ^{1}H NMR of **3**

Mass spectrometry of the water soluble P5A

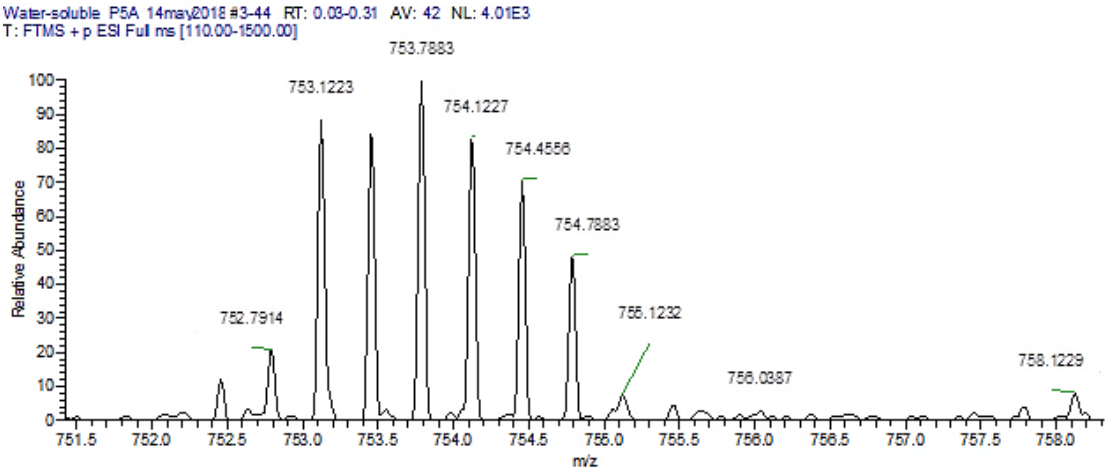


Figure S8: Mass spectrum of 3 for $[M - 3Br]^{3+}$ Calculated $C_{95}H_{121}O_{10}N_{20}Br_7$ 754.1270

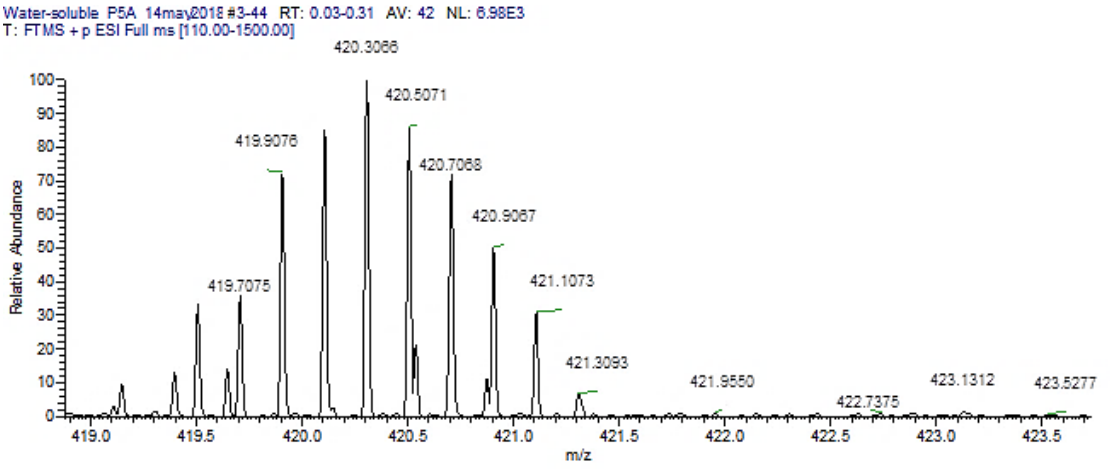


Figure S9: Mass spectrum of 3 for $[M - 5Br]^{5+}$ Calculated $C_{95}H_{121}O_{10}N_{20}Br_5$ 420.1090

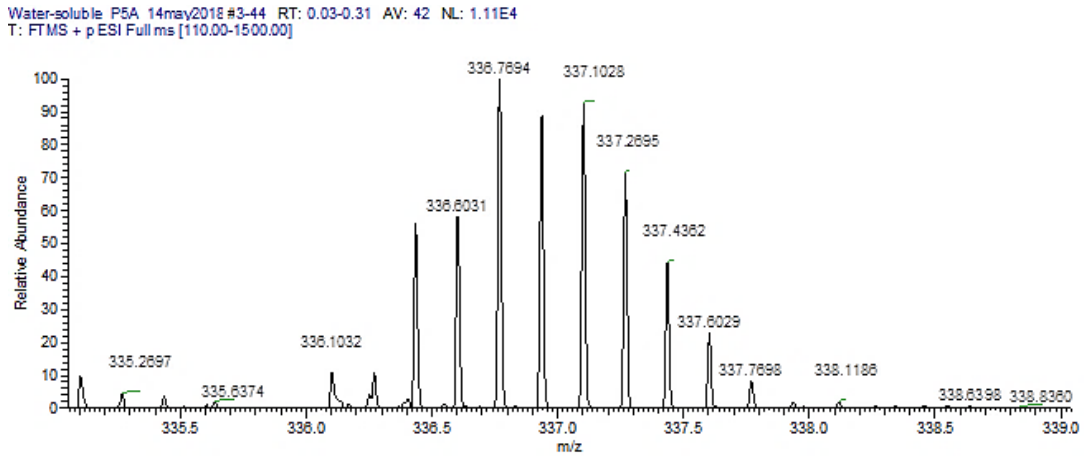


Figure S10: Mass spectrum of 3 for $[M - 6Br]^{6+}$ Calculated $C_{95}H_{121}O_{10}N_{20}Br_4$ 336.9378

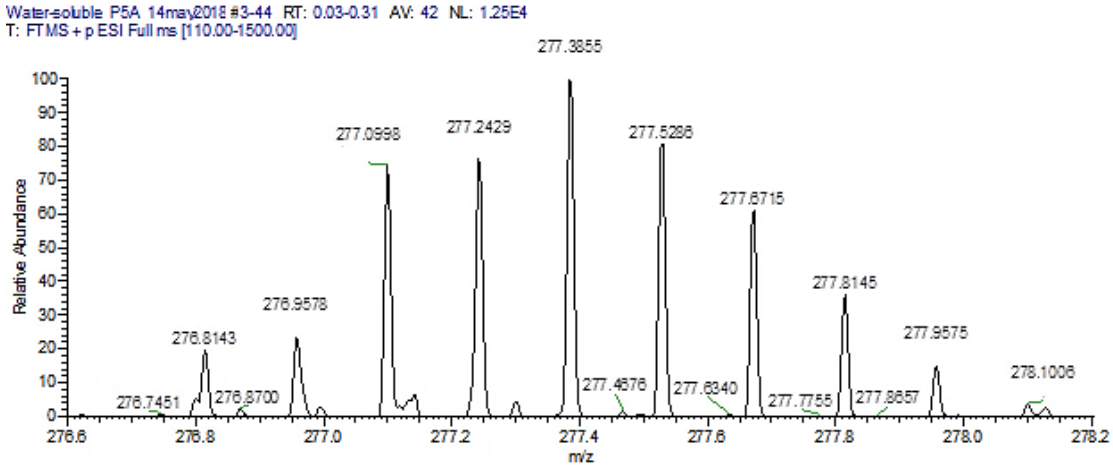


Figure S11: Mass spectrum of 3 for $[M - 7Br]^{7+}$ Calculated $C_{95}H_{121}O_{10}N_{20}Br_3$ 277.2442

Aqueous Xenon Solutions for Fluorescence Quenching Experiments.

A 3.32 x 10^{-3} M aqueous solution of xenon was prepared according to the method of Dmochowski:[2] Deionized water (25 ml) was added to an acid-washed 50 mL round bottom flask. The flask was capped with a septum, bubbled with nitrogen for 20 minutes, and then evacuated through a 22-gauge needle for 5 minutes. After degassing, the needle was pushed to the bottom of the flask, and xenon was bubbled through the solution for 5 minutes. A 26-gauge needle was used to relieve excess pressure. Both needles were removed, and the solution was set aside. Next, a Chemware Tedlar™ gas sampling bag with a septum valve (6" x 6", Fisher Scientific) was connected by ¼" tubing and a three-way stopcock to both a xenon tank and a needle. The entire system was evacuated through the needle using a separate round bottom flask, its septa, and the house vacuum line. The Tedlar bag was filled with xenon, its nozzle closed, and the three-way stopcock was removed. The needle was placed directly onto the tubing connecting the Tedlar bag, and then pierced through the septum into the headspace of the round bottom flask that contained the xenon solution. The flask and attached Tedlar bag was placed in a 37 °C incubator for a few hours to equilibrate. This created a saturated solution of xenon; at 37 °C, that concentration is 3.32 x 10^{-3} M.[2,3]

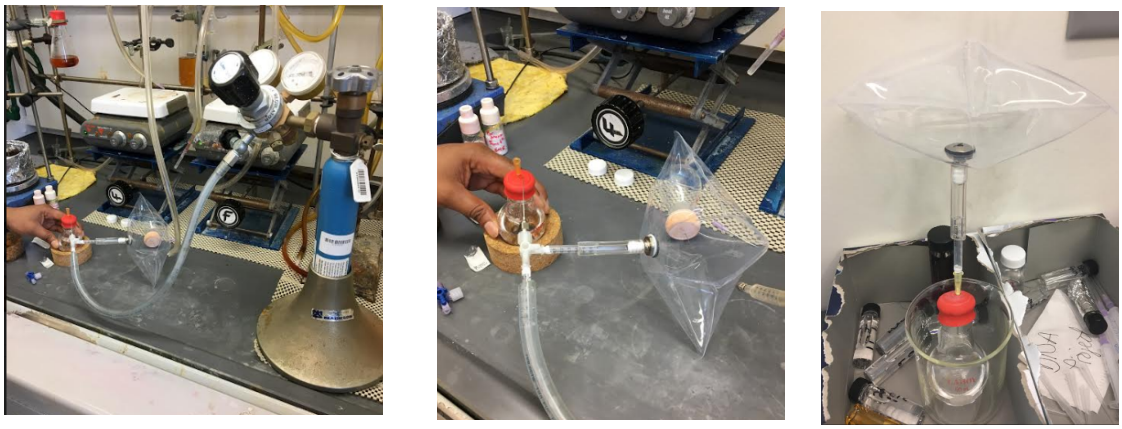


Figure S12: The experimental setup for obtaining the 3.32 x 10^{-3} M Xe solution in DI water. Photographs courtesy of P. U. Ashvin I. Fernando. Copyright 2020.

Fluorescence quenching of 3 with Xenon

A 5.1 x 10^{-4} M stock solution of P5A in deionized water was made using a 100 mL volumetric flask. The solution was then added into a 100 mL round bottom flask with a septum. The solution in the round bottom flask was gently bubbled with nitrogen for 5 minutes, and vacuum was applied for 5 minutes to degas the solution. The same dilution and degassing process was used to prepare a 1 x 10^{-3} M phosphate buffer solution. A stock solution of **3** was added to a reduced volume cuvette with a septum seal (1.5 mL, 1 cm path length, Starna Cells), and then phosphate buffer was added in order to dilute the **3** concentration to 15 μM at 1.5 mL. The solution in the cuvette was bubbled with nitrogen for 5 minutes and evacuated for 5 minutes in order to ensure degassing. Varying amounts of a 3.32 mM aqueous xenon solution (see above) was added so the xenon concentration varied from 0 to 9.8 x 10^{-4} M through the titration at 1.5 mL total volume, keeping the headspace above the solution to a minimum. The cuvette solution was allowed to equilibrate at 25 ^{o}C for 15 min in the fluorimeter before emission spectra were obtained (excitation at 292 nm, emission λ_{max} observed at 321 nm).

A saturated xenon measurement was made by directly bubbling xenon into a degassed solution of **3** in the cuvette for 5 minutes. The solution was allowed to equilibrate for at 25 ^{o}C in the fluorimeter before spectra were obtained.[2] All measurements were triplicated.

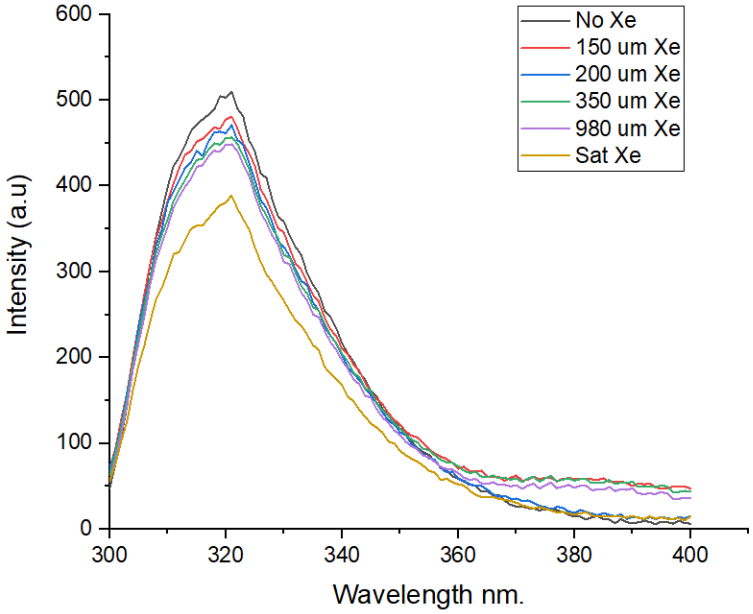
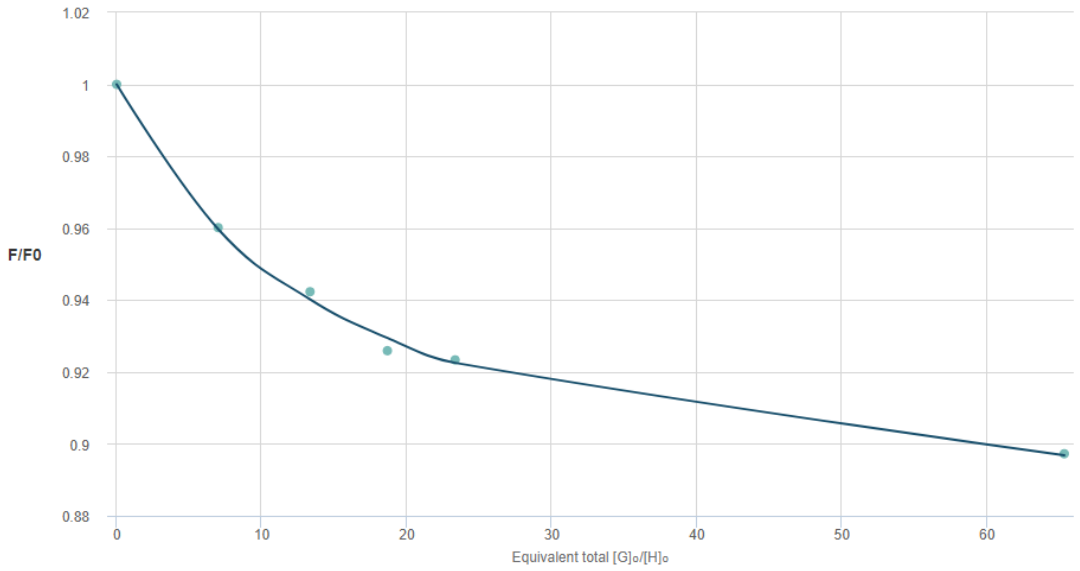


Figure S13: Fluorescence quenching phenomena when Xe was added in to a solution of **3** in phosphate buffer. Figure 4 in the manuscript shows the same data following Gaussian curve smoothing using Origin lab software.



http://app.supramolecular.org/bindfit/view/ef59fc0f-0786-4811-8258-0ad6bd25392c

Details

Time to fit	0.2020 s
SSR	1.8050e-5
Fitted datapoints	6
Fitted params	2

Parameters

Parameter (bounds)	Optimised	Error
K (0 → ∞)	4694.99 M^{-1}	± 11.0469 %

Quality of fit

Fit	RMS	Covariance
F/F0	1.7344e-3	2.8639e-3
Total	**1.7344e-3**	**2.8639e-3**

Figure S14: Binding calculations available at supramolecular.org

Xenon Occupancy Curve for 3

The fluorescence quenching of P5A is caused by the creation of a host guest complex between P5A and xenon in solution according to reaction (1):

$$(1) \quad P5A(sol'n) + Xe(sol'n) \leftrightharpoons Xe@P5A$$

The association constant, K_a, for this reaction is defined as,

$$K_a = \frac{[Xe@P5A]}{[Xe][P5A]}$$

and the occupancy of the P5A cage is defined as,

$$Occupancy = \frac{[Xe@P5A]}{[P5A]}$$

because the concentration of occupied P5A is equivalent to the concentration of xenon within the P5A. In order to determine the concentration of occupied P5A, an ICE table for reaction (1) must be developed (shown below), where Y represents the initial concentration of free xenon in solution.

	P5A	Xe	Xe@P5A
Initial	$15x10^{-6}$M	Y	0
Change	-X	-X	X
Equilibrium	$15x10^{-6}$M - X	Y-X	X

Table S1: ICE table depicting concentrations of xenon bound and unbound to P5A; used in order to determine occupancy curve for Xe.

Substituting the equilibrium concentrations shown above in the ICE table equivalent to the known K_a, $4.7x10^3M^{-1}$, the concentration of bound xenon, represented as X in the ICE table, can be determined.

$$K_a = \frac{[X]}{[Y-X][15x10^{-6}M]} = 4.7x10^3M^{-1}$$

The X values were determined for each xenon solution concentration titrated and shown in the table below. Then, they were graphed as a function of xenon solution concentration, and the occupancy curve was developed. The standard error for these data is ~5%.

[Xe] soln (M)	Occupancy
$1.05x10^{-4}$	0.318
$2.80x10^{-4}$	0.558
$3.50x10^{-4}$	0.613
$2.00x10^{-4}$	0.473
$9.80x10^{-4}$	0.818
$3.32x10^{-3}$	0.939

Table S2: X values for each xenon solution concentration titrated. Values were subsequently graphed shown in Fig. S15.

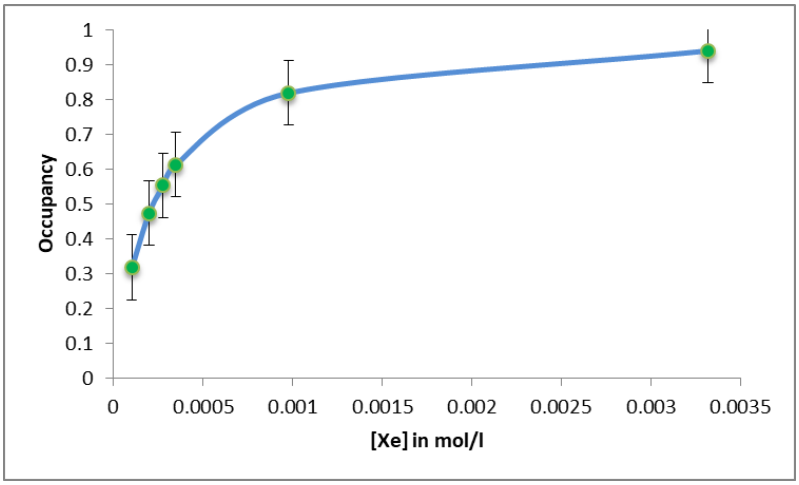


Figure S15: Occupancy curve for Xe in **3**

Dynamic Light Scattering (DLS)

A 100 μM sample of deca-imidazolium functionalized pillar[5]arene was prepared in ultrapure water (pH 6.998). The temperature was set to 25°C and the intensity distributions were analyzed. Measurements were averaged over three trials without disruption of the sample. DLS data was acquired on a Zetasizer Nano ZS90 system, using software from Malvern Instruments.

Control (without Xe)

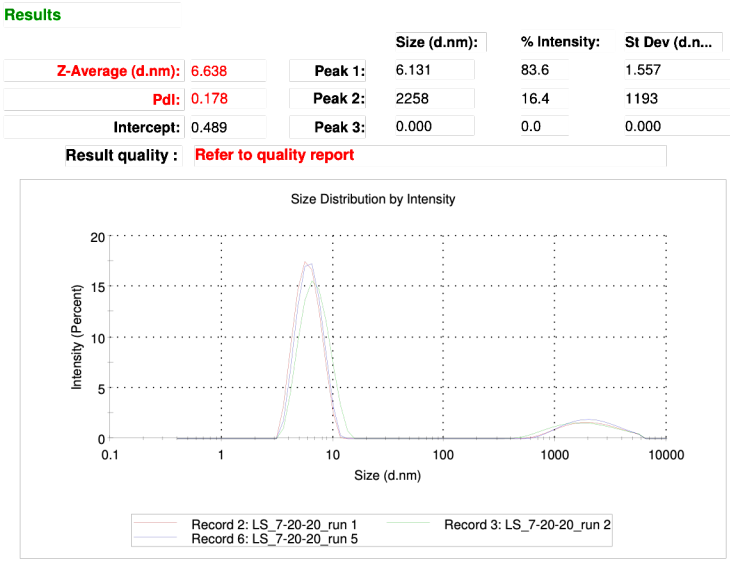


Figure S16: Size distribution by intensity of cationic WSP5A. Averaged DLS data of 100μM deca-cationic pillar[5]arene in ultrapure water.

Post-Xe

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	34.85	Peak 1:	4.203	60.0	1.270
PdI:	0.279	Peak 2:	4401	17.6	929.2
Intercept:	0.540	Peak 3:	499.4	14.3	177.6

Result quality : Refer to quality report

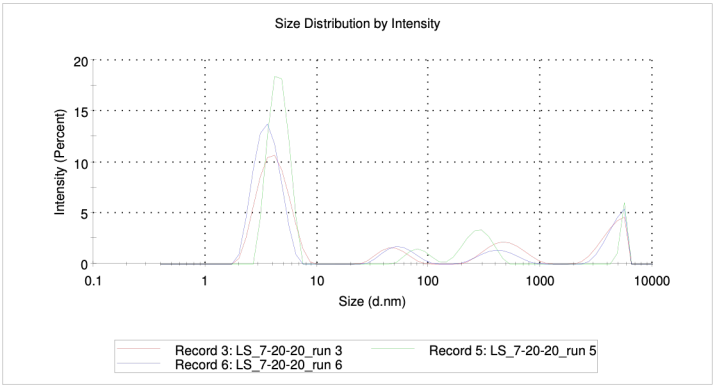


Figure S17: Size distribution by intensity of cationic WSP5A with ^{129}Xe gas. Averaged DLS data of 100μM deca-cationic pillar[5]arene in ultrapure water. ^{129}Xe was directly bubbled into the solution for ten minutes prior to any measurements.

Xenon NMR studies

Naturally abundant Xe gas was placed into a 1.0 L Tedlar bag and polarized to 60-80% via the spin exchange optical pumping (SEOP) technique using a Xemed polarizer (Xemed, Durham, NH, USA). It was then placed into a Tedlar bag which was immediately moved into a pressurized chamber within the bore of a Philips Achieva 3.0 T clinical MRI scanner to preserve its polarization. The pressure inside of the chamber was maintained between 20-45 kPa above atmospheric pressure using a pressure-sensitive ventilation device connected to a nitrogen (N_2) source, to facilitate the flow of HP ^{129}Xe gas from the Tedlar bag into the glass-fritted cell containing pillararene solution.

Following polarization of ^{129}Xe gas, 2.5 mL of solution (10 mM) was transferred into a custom-made glass-fritted cell using a syringe. The cell containing the solution was then placed inside of a custom-made quadrature radio-frequency (RF) coil tuned to the Larmor frequency of ^{129}Xe (35.33 MHz) at 3.0 T. The Tedlar bag, already in the bore of the MRI, was then connected to the cell's inflow tube. Once connected, the pressure-stopper on the Tedlar bag was released to allow for the continuous flow of HP ^{129}Xe gas into the glass-fritted cell, which produced several microbubbles as it passed through the fine fritted disc, thereby dissolving into the solution. As HP ^{129}Xe gas continually flowed through the glass-fritted cell, ^{129}Xe nuclear magnetic resonance (NMR) spectral data was simultaneously obtained. The concentration of ^{129}Xe at any point during the spectral acquisition was between 1-5 mM24.

All ^{129}Xe NMR spectra were acquired using a Philips Achieva 3.0 T clinical MRI scanner. Scanner software was modified for the automatic measurement of hyperpolarized chemical ex-change saturation transfer (HyperCEST) depletion spectra. To saturate HP ^{129}Xe encapsulated within the pillararene molecules, a pre-pulse train consisted of 16- 30 ms sinusoidal pulses, with 0 ms pulse intervals and 1530^{O} flip angles. To acquire each depletion spectrum, a bunch of free induction decay (FID) spectra were collected at various chemical shift frequency offsets with TR = 4s. Each FID spectrum was

acquired using a selective 90-degree "spredrex" excitation pulse with a 9.95 ms duration and bandwidth of 1424 Hz (40.3 ppm at 3.0 T).

The data sampling number was 2048, which corresponds to the spectral resolution of 0.44 ppm. Saturation pre-pulse frequency was automatically adjusted to range from -110 ppm to 30 ppm, where 0 ppm represents dissolved-phase ^{129}Xe, with a predetermined step before each of the subsequent excitation pulses. The frequency changing step was equal to 2 ppm (70.6 Hz).

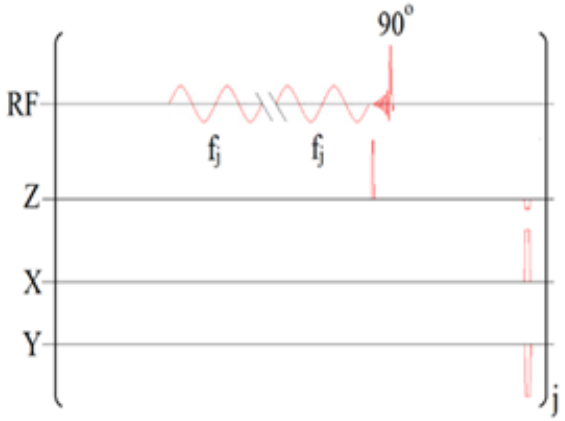


Figure S18: The pulse diagram shown above was used to acquire HyperCEST depletion spectra using saturation pre-pulse train including sinusoidal pulses. Here, fj represents the frequency of saturation pulses during FID acquisition number j. All spoiler gradients along x, y, z axis are also illustrated.

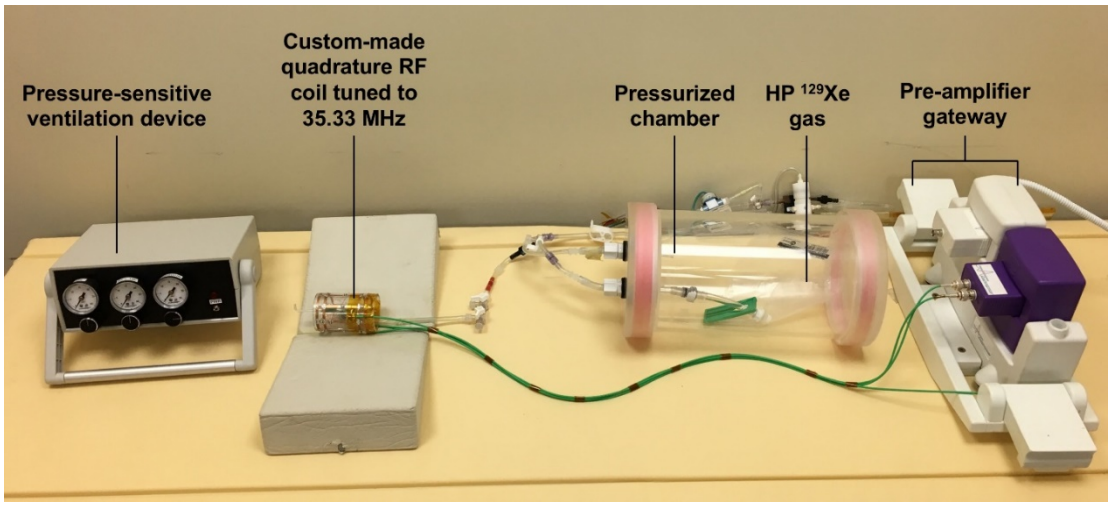


Figure S19: Experimental HyperCEST setup for *in vivo* studies. Above setup was used to obtain the Xe-spectra and to obtain the final depletion spectra.

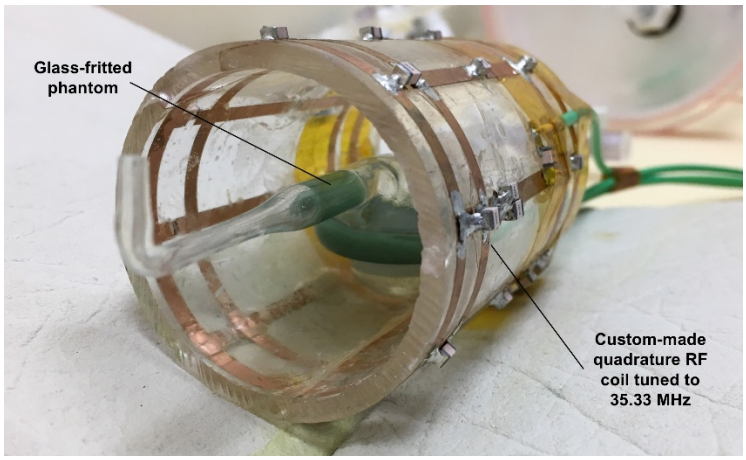


Figure S20: The image above illustrates the continuous flow of HP ^{129}Xe gas throughout the glass-fritted vessel. As depicted, once the flow of HP ^{129}Xe reaches the fine glass-fritted disc, numerous microbubbles are produced, thereby causing dissolution of HP ^{129}Xe into solution. The RF pulse is applied at the chemical shift frequency offset which corresponds to the ternary complex formed by the interaction of HP ^{129}Xe with the WSP5A solution.

HyperCEST depletion spectrum calculations and data analysis:

All obtained Xe spectra were analyzed using home-built MatLab script in MATLAB R2016b (The Mathworks, Inc, Natick, MA). The signal-to-noise ratio (SNR) was calculated for each spectrum, and hyperCEST depletion was calculated using the following equation:

$$Depletion_{f_j} = \frac{SNR_{f_j}^{ON} - SNR^{OFF}}{SNR^{OFF}}$$

where $SNR_{f_j}^{ON}$ corresponds to the SNR of the dissolved phase Xe when saturation pulses where applied at frequency f_j. SNR^{OFF} corresponds to the off-resonance SNR and was calculated as average SNR of the dissolved Xe peak for the first six saturation frequencies.

The obtained depletion spectrum was exported from MatLab to OriginPro 2016 software (OriginLab Corp., Northampton, MA), The spectrum was denoised using the low-pass parabolic Fast Fourier Transform filter. The pass frequency was equal to 0.063 Hz and the stop frequency was equal to the 0.132 Hz. Denoised spectrum was fitted using two Lorentzian peaks.

References

(1) Yao, Y.; Xue, M.; Chi, X.; Ma, Y.; He, J.; Abliz, Z.; Huang, F. A new water-soluble pillar[5]arene: synthesis and application in the preparation of gold nanoparticles. *Chem. Commun.* **2012**, *48* (52), 6505–6507.

(2) Hill, P. A.; Wei, Q.; Eckenhoff, R. G.; Dmochowski, I. J. Thermodynamics of xenon binding to cryptophane in water and human plasma. *J. Am. Chem. Soc.* **2007**, *129* (30), 9262–9263.

(3) Clever, H. L. *Solubility Data Series: Krypton, Xenon and Radon*; Elsevier, 2013; Vol. 2.